UNIVERSITAT DE BARCELONA

FACULTAT DE FARMÀCIA

DEPARTAMENT DE FARMACOLOGIA I QUÍMICA TERAPÈUTICA

“Síntesi Total de la Lamel·larina D i Anàlegs de Cadena Oberta: Estudis d’Internalització Cel·lular i d’Inhibició de Topoisomerases”

Daniel Pla Queral

2009

INSTITUT DE RECERCA BIOMÈDICA –
PARC CIENTÍFIC DE BARCELONA

UNIVERSITAT DE BARCELONA

FACULTAT DE FARMÀCIA

DEPARTAMENT DE FARMACOLOGIA I QUÍMICA TERAPÈUTICA

"Síntesi Total de la Lamel·larina D i Anàlegs de Cadena Oberta: Estudis d'Internalització Cel·lular i d'Inhibició de Topoisomerases"

Memòria presentada per:

Daniel Pla Queral

Per a optar al títol de Doctor per la Universitat de Barcelona
Programa de doctorat: Química Orgànica a la Indústria Químico-farmacèutica.
Bienni 2005-2007

Dirigida per:

Dra. Mercedes Álvarez Domingo i Dr. Fernando Albericio Palomera

Barcelona, 2009

Agraeixo als meus mentors Fernando Albericio i Mercedes Álvarez la motivació i aprenentatge que m'han lliurat en el transcurs d'aquesta tesi.

Als meus pares i companys, gràcies pel vostre suport i estima.

El treball descrit en aquesta memòria ha estat realitzat a l'Institut de Recerca Biomèdica (IRB) situat al Parc Científic de Barcelona de la Universitat de Barcelona. Aquest ha gaudit dels ajuts que es detallen a continuació.

Projecte "Combiestrategias para el Descubrimiento de Nuevos Fármacos Peptídicos y/o Heterocíclicos" finançat per la Comissió de Ciència i Tecnologia (BQU 2003-00089, BQU 2006-03794). Grup de recerca reconegut per la Generalitat, Química Combinatòria per al desenvolupament de nous compostos (2005 SGR2005-00062). "Centro de Investigación Biomédica en Red, de Bioingeniería, Biomateriales y Nanomedicina" (CIBER-BBN 0074) finançat pel Ministerio de Sanidad y Consumo a través de l'Instituto de Salud Carlos III.
Conveni PharmaMar S.A. – Parc Científic de Barcelona amb el projecte "Síntesis de Nuevos Agentes Terapéuticos".

D. P. ha rebut una beca predoctoral de l'Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau de Barcelona (B-IR-2006-2), i una beca d'estada a Alemanya de la Generalitat de Catalunya (2007 BE-2 00340).

ÍNDEX

ÍNDEX D'ABREVIATURES

A-549: Línia cel·lular humana de càncer de pulmó
Ac: Acetil
Ar: Aril
AU: Unitats d'absorbància
Bn: Benzil
Boc: *tert*-Butoxicarbonil
q: Quadruplet
CI: Ionització química
CIQ: Quocient d'internalització cel·lular
d: Doblet
DDQ: 2,3-Dicloro-5,6-diciano-*p*-benzoquinona
DNA: Àcid desoxiribonucleic
DIPCDI: *N*,*N*′-Diisopropilcarbodiimida
DIPEA: Diisopropiletilamina
DMAP: 4-Dimetilaminopiridina
DMEM: Medi de cultiu d'Eagle modificat per Dulbecco.
DMF: *N*,*N*-Dimetilformamida
DMSO: Dimetilsulfòxid
DPE-Phos: (oxidi-2,1-fenilene)bis(difenilfosfina)
DTPA: Àcid dietilentriamina-*N*,*N*,*N*′,*N*′,*N*″-pentaacètic
EDC·HCl: Hidroclorur de *N*-(3-dimetilaminopropil)-*N*′-etilcarbodiimida
ES: Electroespray
Et: Etil
FAB: Bombardeig amb àtomos ràpids
FACS: Separador de cèl·lules activat per fluorescència
FBS: Sèrum fetal boví
FITC: Isotiocianat de fluoresceïna
GFP: Proteïna fluorescent verda
GI: Inhibició del creixement
PG: Grup protector
HeLa: Línia cel·lular humana de càncer de cèrvix
HMBC: Heterocorrelació a llarga distancia amb detecció inversa
HOAt: 1-Hidroxi-7-azabenzotriazole
HOBt: 1-Hidroxibenzotriazole
HRMS: Espectrometria de masses d'alta resolució
HPLC: Cromatografia líquida d'alta pressió
HSQC: Heterocorrelació vía detecció inversa
HT-29: Línia cel·lular humana de càncer de colon
IC: Concentració inhibitòria
IE: Impacte electrònic
Im: Imidazol
*i*Pr: Isopropil
IQ: Ionització química
IR: Espectroscòpia d'infrarroig
J: Constant d'acoblament
Lam: Lamel·larina
m: Multiplet

MALDI-TOF: Ionització per desorció laser assistida per matriu - Temps de vol

MDA-MB-231: Línia cel·lular humana de càncer de mama

Me: Metil

MOM: Metoximetil

MS: Espectroscòpia de masses

MSNT: 1-(Mesitilen-2-sulfonil)-3-nitro-*1H*-1,2,4-triazole

m/e: Massa/càrrega

MW: Microones

NLS: Senyal de localització nuclear

NMI: *N*-Metilimidazole

NBS: *N*-Bromosuccinimida

m-CPBA: Àcid metacloroperbenzoic

mp, M.p.: Punt de fusió

Pbf: (2,2,4,6,7-Pentametildihidrobenzofuran-5-sulfonyl)

PBS: Tampó fosfat salí

PEG: poli(etilenglicol)

Ph: Fenil

PS: Poliestirè

PyBOP: Hexafluorofosfat de (benzotriazol-1-iloxi)tripirrolidinofosfoni

RMN: Ressonància magnètica nuclear

r.t.: Temperatura ambient

s: Singlet

SAR: Relació estructura-activitat

SRB: Sulforhodamina B

t: Triplet

TBAF: Fluorur de tetrabutilamoni

*t*Bu: *tert*-Butil

TBDPS: *tert*-Butildifenilsilil

TCFH: Hexafluorofosfat de *N*,*N*,*N*′,*N*′-tetrametilcloroformamidini

TFFH: Hexafluorofosfat de *N*,*N*,*N*′,*N*′-tetrametilfluoroformamidini

Top, Topo: Topoisomerasa

Ts: *p*-Toluensulfonil

TFA: Àcid trifluoroacètic

THF: Tetrahidrofurà

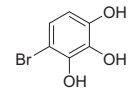
UV: Ultravioleta

WGA: Aglutinina de llavor de blat

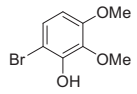
δ: Desplaçament químic

ÍNDEX DE FIGURES

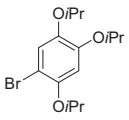
Compostos Precursors



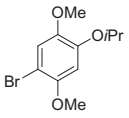
2a Eur. J. Org. Chem.



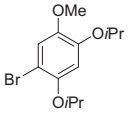
2b Eur. J. Org. Chem.



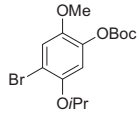
2d Eur. J. Org. Chem.



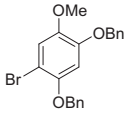
2e Eur. J. Org. Chem.



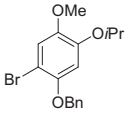
2f Eur. J. Org. Chem.



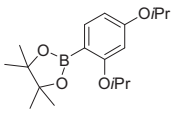
2g Eur. J. Org. Chem.



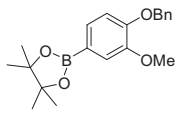
Sl Bioconjugate Chem. l



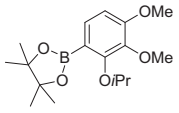
Sl Bioconjugate Chem. l



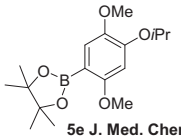
5c J. Med. Chem.



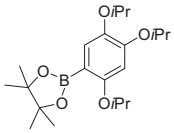
11a Bioconjugate Chem. l



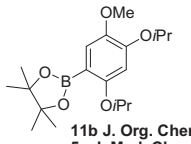
5d J. Med. Chem.



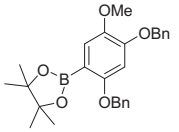
5e J. Med. Chem.



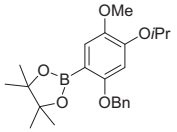
5b J. Med. Chem.



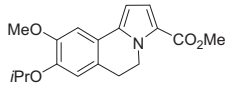
11b J. Org. Chem.
5a J. Med. Chem.



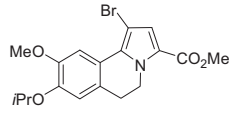
12b Bioconjugate Chem. l



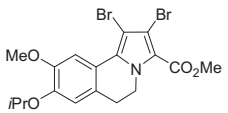
12a Bioconjugate Chem. l



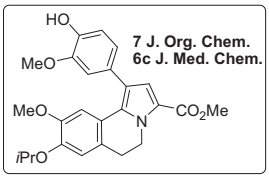
1 J. Org. Chem.
1 J. Med. Chem.
10 Bioconjugate Chem. l



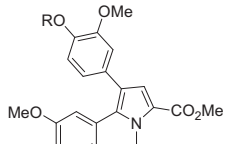
5 J. Org. Chem.
2 J. Med. Chem.



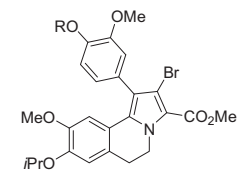
3 J. Med. Chem.



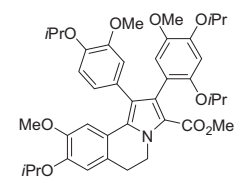
7 J. Org. Chem.
6c J. Med. Chem.



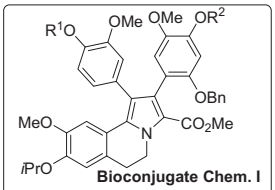
8 R = *i*Pr **J. Org. Chem.**
6d R = *i*Pr **J. Med. Chem.**
13a R = Bn **Bioconjugate Chem. l**



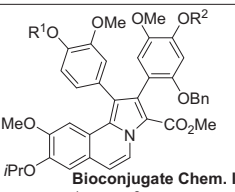
9 R = *i*Pr **J. Org. Chem.**
8d R = *i*Pr **J. Med. Chem.**
14a R = Bn **Bioconjugate Chem. l**



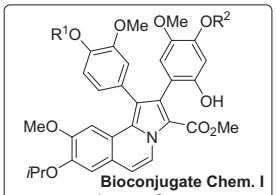
14 J. Org. Chem.
9d J. Med. Chem.



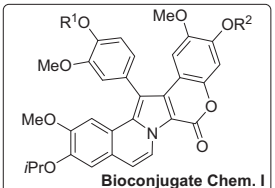
Bioconjugate Chem. l
15a R^1= *i*Pr; R^2 = Bn
15b R^1= Bn; R^2 = *i*Pr
15c R^1, R^2 = Bn



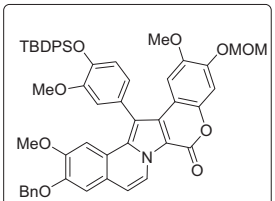
Bioconjugate Chem. l
16a R^1= *i*Pr; R^2 = Bn
16b R^1= Bn; R^2 = *i*Pr
16c R^1, R^2 = Bn



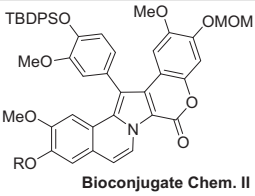
Bioconjugate Chem. l
17a R^1= *i*Pr; R^2 = H
17b R^1= H; R^2 = *i*Pr
17c R^1, R^2 = H



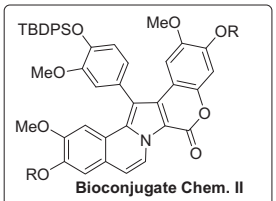
Bioconjugate Chem. l
18a R^1= *i*Pr; R^2 = H
18b R^1= H; R^2 = *i*Pr
18c R^1, R^2 = H



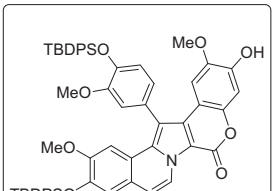
10 Bioconjugate Chem. II



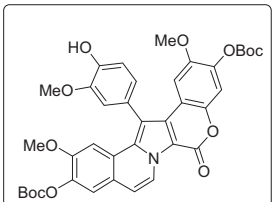
Bioconjugate Chem. II
10a R = H
10b R= TBDPS



Bioconjugate Chem. II
10c R^1 = H
10d R^1= TBDPS

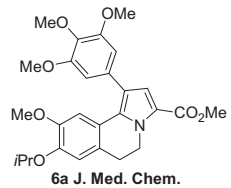


5 Bioconjugate Chem. II

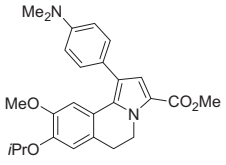


6 Bioconjugate Chem. II

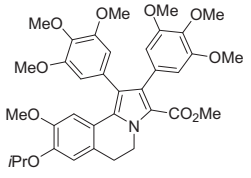
Anàlegs de Lamel·larina de Cadena Oberta



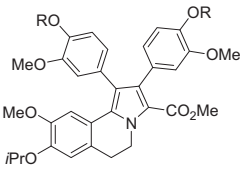
6a J. Med. Chem.



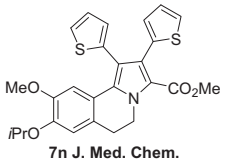
6l J. Med. Chem.



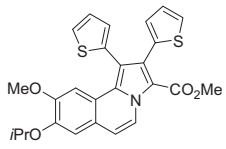
7a J. Med. Chem.



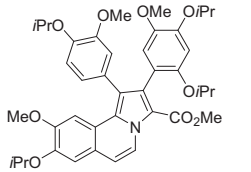
7c R = H **J. Med. Chem.**
7f R = Me **J. Med. Chem.**



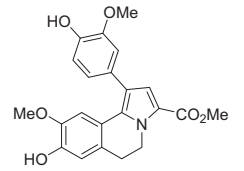
7n J. Med. Chem.



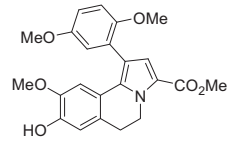
11n J. Med. Chem.



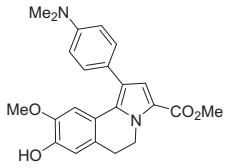
12d J. Med. Chem.



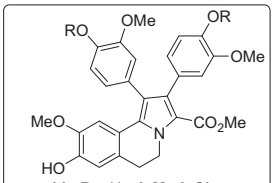
13c J. Med. Chem.



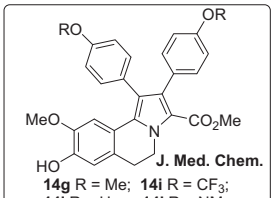
13e J. Med. Chem.



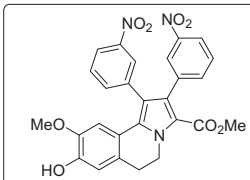
13l J. Med. Chem.



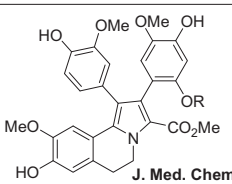
14c R = H **J. Med. Chem.**
14f R = Me **J. Med. Chem.**



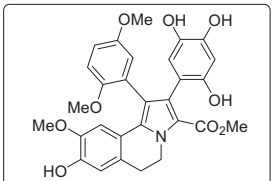
J. Med. Chem.
14g R = Me; **14i** R = CF_3;
14j R = H; **14l** R = NMe_2



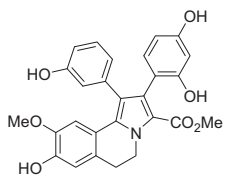
14m J. Med. Chem.



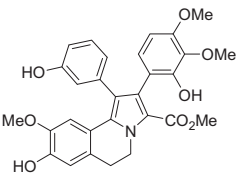
J. Med. Chem.
15d R = H; **15e** R = Me



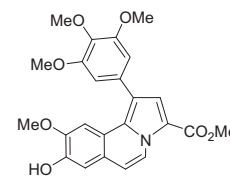
15g J. Med. Chem.



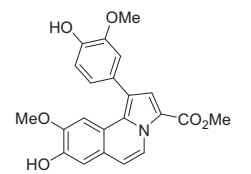
15h J. Med. Chem.



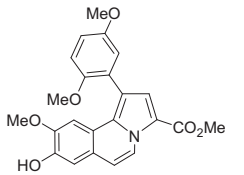
15i J. Med. Chem.



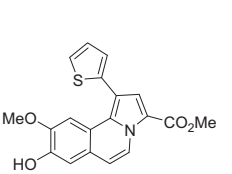
16a J. Med. Chem.



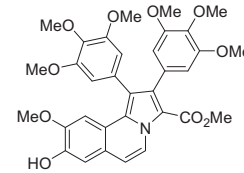
16c J. Med. Chem.



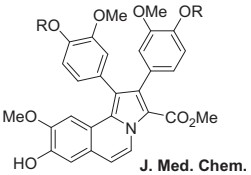
16e J. Med. Chem.



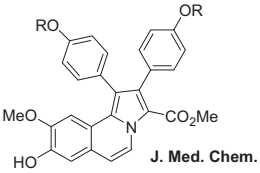
16n J. Med. Chem.



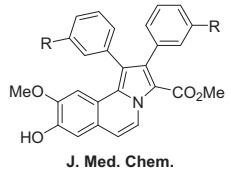
17a J. Med. Chem.



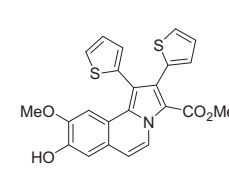
J. Med. Chem.
17c R = H; **17f** R = Me



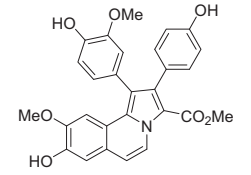
J. Med. Chem.
17g R = Me; **17i** R = CF_3; **17j** R = H



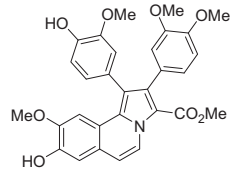
J. Med. Chem.
17k R = OH; **17m** R = NO_2



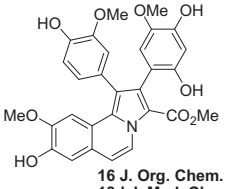
17n J. Med. Chem.



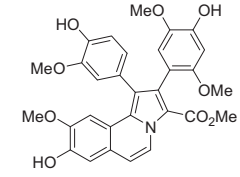
18a J. Med. Chem.



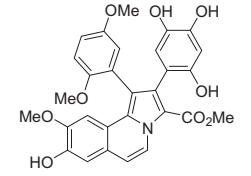
18b J. Med. Chem.



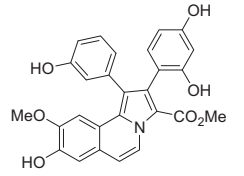
16 J. Org. Chem.
18d J. Med. Chem.



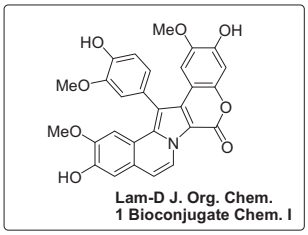
18e J. Med. Chem.



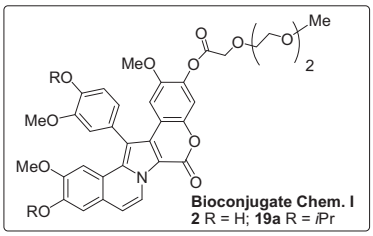
18g J. Med. Chem.



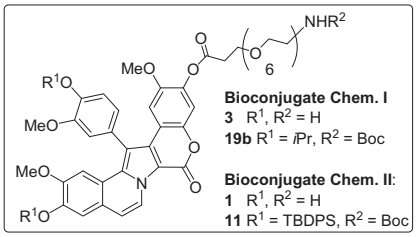
18h J. Med. Chem.



Lam-D J. Org. Chem.
1 Bioconjugate Chem. I

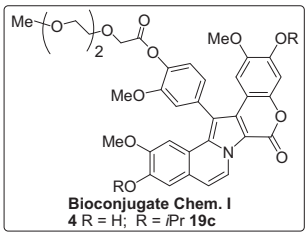


Bioconjugate Chem. I
2 R = H; **19a** R = *i*Pr

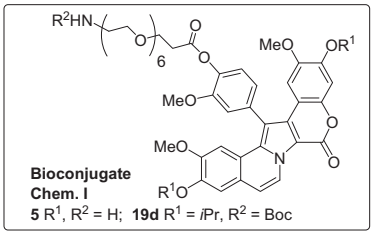


Bioconjugate Chem. I
3 R^1, R^2 = H
19b R^1 = *i*Pr, R^2 = Boc

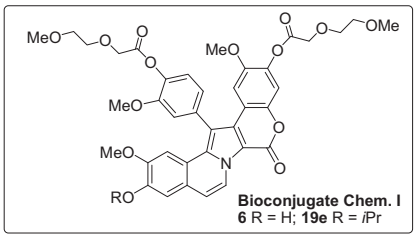
Bioconjugate Chem. II:
1 R^1, R^2 = H
11 R^1 = TBDPS, R^2 = Boc



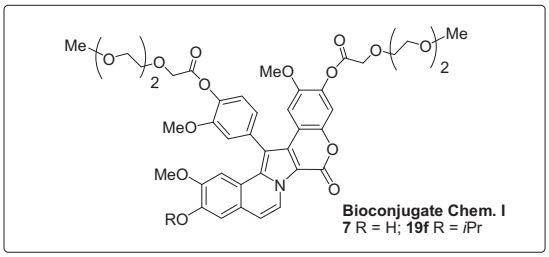
Bioconjugate Chem. I
4 R = H; R = *i*Pr **19c**



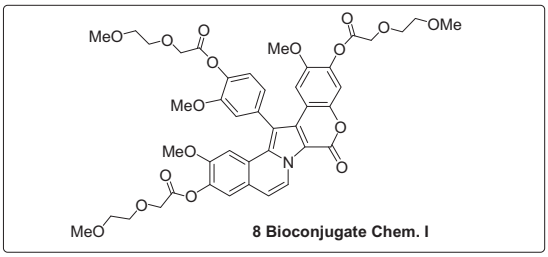
Bioconjugate Chem. I
5 R^1, R^2 = H; **19d** R^1 = *i*Pr, R^2 = Boc



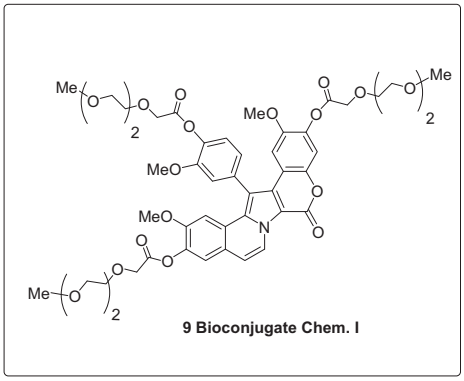
Bioconjugate Chem. I
6 R = H; **19e** R = *i*Pr



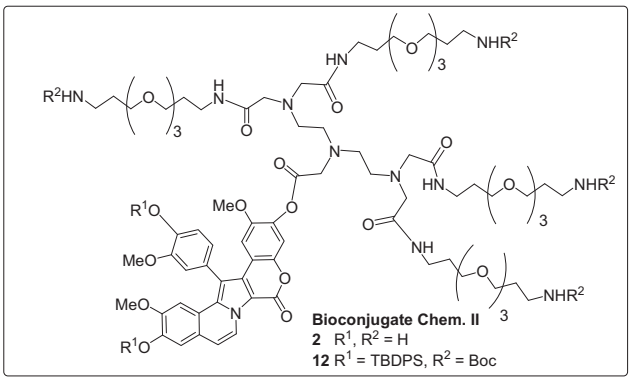
Bioconjugate Chem. I
7 R = H; **19f** R = *i*Pr



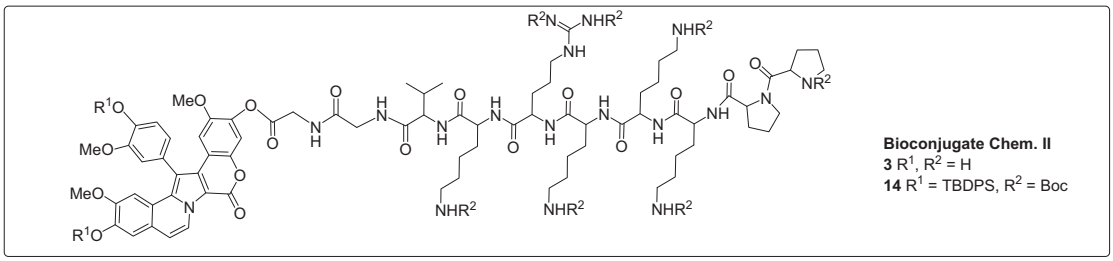
8 Bioconjugate Chem. I



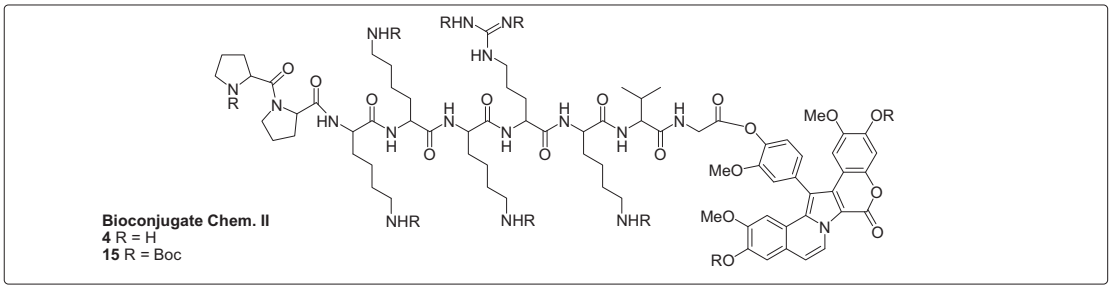
9 Bioconjugate Chem. I



Bioconjugate Chem. II
2 R^1, R^2 = H
12 R^1 = TBDPS, R^2 = Boc



Bioconjugate Chem. II
3 R^1, R^2 = H
14 R^1 = TBDPS, R^2 = Boc



Bioconjugate Chem. II
4 R = H
15 R = Boc

INTRODUCCIÓ I OBJECTIUS

INTRODUCCIÓ

La recerca farmacològica de nous agents terapèutics s'ha desenvolupat paral·lelament al progrés de les ciències biomèdiques en els darrers anys. Resulta interessant observar com fins a un 78% dels fàrmacs pel tractament del càncer prové d'origen natural, o són derivats d'aquestos.[1]

Els productes naturals d'origen marí tenen una trajectòria breu, en comparació amb d'altres provinents de diferents fonts. Tanmateix, no va ser fins a finals dels anys 70 quan veritablement es va accelerar el procés del descobriment de nous productes d'origen marí. L'èxit es deu a la gran diversitat de microorganismes, plantes i animals que es troben al mar, així com als darrers avenços tècnics que han fet accessibles les bioprospeccions al subsòl i medi marí. Tot plegat s'ha traduït en l'increment creixent d'alcaloides d'aquestes fonts marines amb estructures sense precedents. Estudis sobre relacions tròfiques presa-depredador i la defensa per l'espai vital, apunten que aquests productes són metabòlits secundaris, alhora que compleixen un paper de prevenció molt important contra competidors.[2] La gran diversitat present en medis marins i el fet que els oceans cobreixen un 70% de la superfície terrestre, fan apuntar que els nous descobriments sorgiran de les profunditats oceàniques.

Malgrat que els productes naturals presenten pesos moleculars elevats i són sovint citats com una excepció a les regles de Lipinski, hi mantenen generalment compatibilitat en termes de Log P i de nombre màxim 5 grups donadors de ponts d'hidrogen. D'aquesta manera, els compostos bioactius d'origen natural acostumen a presentar baixa hidrofobicitat i serà en la modulació d'interaccions associades a processos biològics[3] quan aquestes molècules mostraran el seu potencial. El seu origen natural els hi confereix avantatge i privilegi estructural a l'hora d'aprofitar la ressemblança amb intermedis biosintètics o metabòlits endògens. De manera anàloga, el disseny de síntesi d'estructures mimètiques als productes naturals serveix com a punt de

[1] Newman, D. J.; Cragg, G. M., Natural Products as Sources of New Drugs over the Last 25 Years. *J. Nat. Prod.* **2007**, *70*, 461-477.
[2] McClintock, J. B.; Baker, B. J.; Marine Chemical Ecology. CRC Press, Boca Raton, Florida, **2001**.
[3] Jou, D., Introducció a la termodinàmica de processos biològics, Institut d'Estudis Catalans, Barcelona, 1985

partida per explorar petites modificacions estructurals i posar de manifest les relacions estructura-activitat.[4]

Ziconotida i Ecteinascidina 743 són els únics darrers fàrmacs marins aprovats per al tractament de dolor crònic sever, i càncer de teixits tous, respectivament. D'altres com l'Aplidina i la Kahalalida F es troben en fase clínica. La Thiocoralina, les Variolines i les Lamel·larines es troben en fase de desenvolupament pre-clínic *(Fig. 1)*.

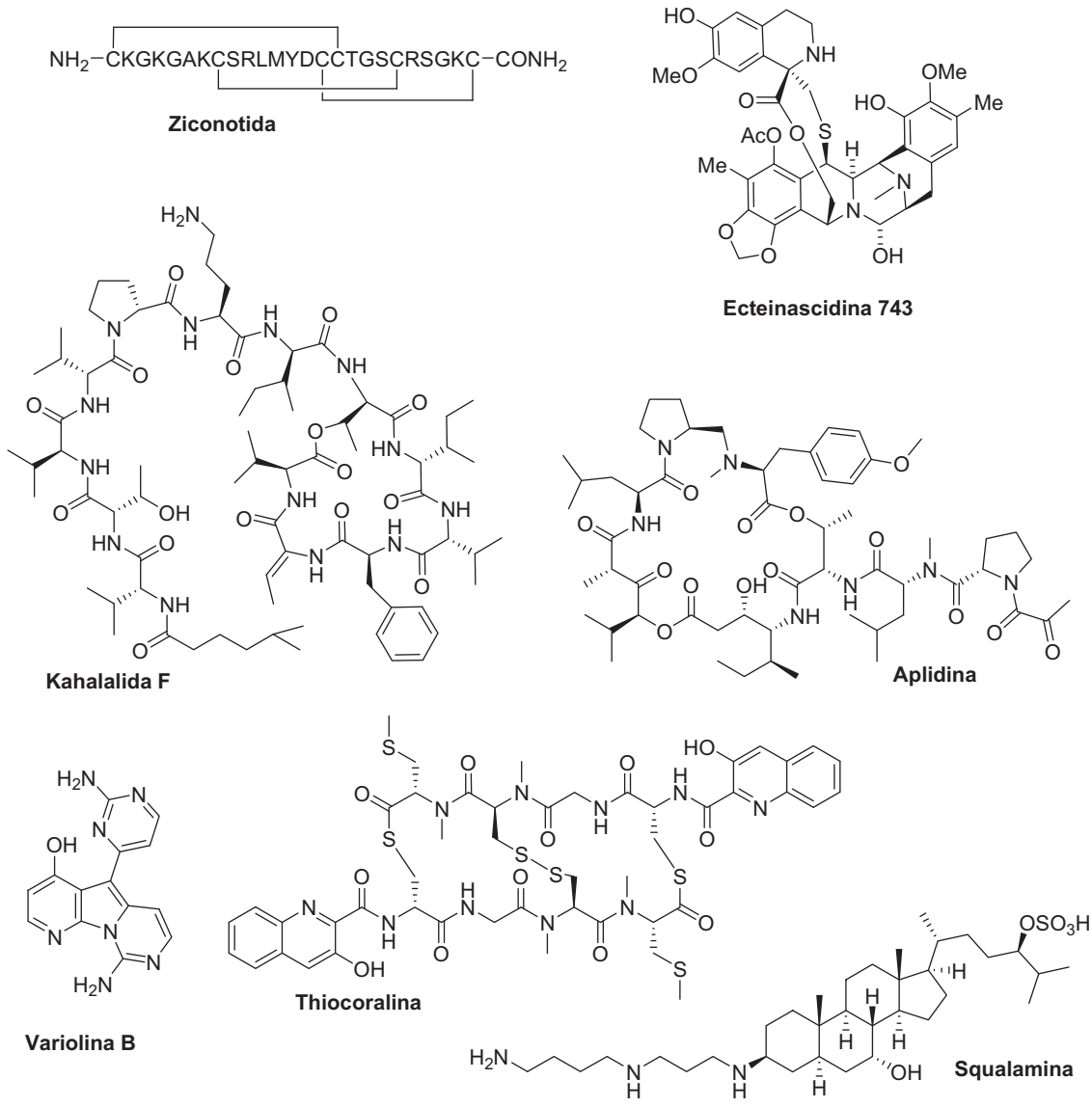


Fig. 1. Productes naturals d'origen marí aprovats com a fàrmacs i candidats a ser-ho.

[4] Tan, D. S., Diversity-oriented synthesis: exploring the intersections between chemistry and biology.

Les Lamel·larines són una gran família d'alcaloides marins amb activitat anticancerígena potencial que han estat aïllats de diversos organismes marins, principalment ascidians i esponges. El sistema 3,4-diarilpirrole és característic a totes les Lamel·larines. Les Lamel·larines pentacícliques que presenten un sistema poliheterocíclic amb un nucli pirrol, són els compostos més actius. D'entre aquestes ultimes se n'ha aïllat de totalment insaturades, i d'altres de saturades entre les posicions 8 i 9.

Fins a la data, la Lam-D i la Lam-H han estat identificades com a compostos cap de sèrie en la inhibició de topoisomerasa I i VIH-1 integrasa, respectivament—ambdós enzims nuclears són sobreexpressats en trastorns de desregulació. A més a més, ha estat descrita la eficàcia d'aquests compostos en el tractament de cèl·lules tumorals resistents a múltiples medicaments, sense flux cel·lular mediat de sortida, així com la seva activitat immunomoduladora i la selectivitat en línies cel·lulars de melanoma.

Prèviament a la realització d'aquesta tesi, es comptava amb la experiència en la síntesi en fase sòlida de Lamel·larines. En el grup de recerca s'havien sintetitzat les Lamel·larines L, O, Q i U *(Fig. 2)*.[5,6] Aquesta tesi parteix inicialment de la síntesi en solució de l'esquelet de construcció 5,6-dihydropyrrolo[2,1-*a*]isoquinolina,[7] que ha resultat robust i de gran utilitat per les posteriors síntesis.

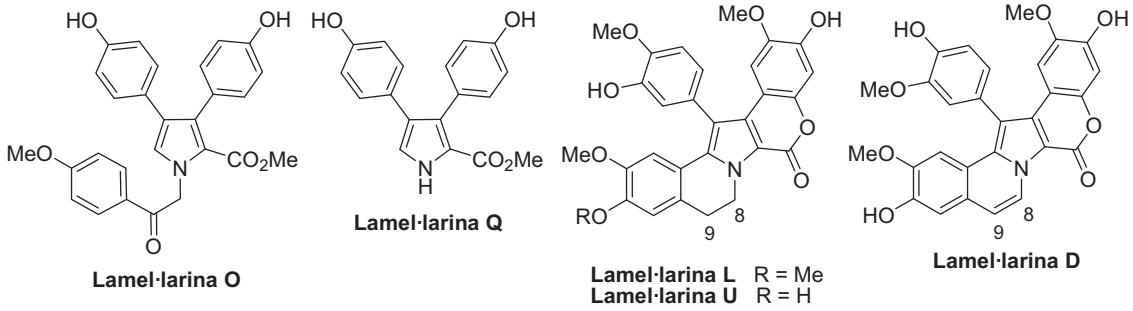


Fig. 2 Exemples de Lamel·larines sintetitzades al nostre grup de recerca.

El cap de sèrie destacat és la Lamel·larina D i presenta activitat antiproliferativa contra diverses línies tumorals cancerígenes en un rang de concentracions nanomolar

[5] Cironi, P.; Manzanares, I.; Albericio, F.; Álvarez, M., Solid-Phase Total Synthesis of the Pentacyclic System Lamellarins U and L. *Org. Lett.* **2003,** 5, 2959-2962.
[6] Marfil, M.; Albericio, F.; Álvarez, M., Solid-phase synthesis of lamellarins Q and O. *Tetrahedron* **2004,** 60, 8659-8668.
[7] Olsen, C.; Parera, N.; Albericio, F.; Álvarez, M., 5,6-Dihydropyrrolo[2,1-*a*]isoquinolines as scaffolds for synthesis of lamellarin analogues. *Tetrahedron Lett.* **2005,** 46, 2041-2044.

baix. La inhibició de la topoisomerasa I, i la disrupció del potencial transmembrana mitocondrial han estat descrits com a modes d'acció de la molècula, que acaba per desencadenar un mecanisme de mort cel·lular apoptòtica.[2] La Topoisomerasa I és una diana especialment interessant en quimioteràpia, ja que és un dels enzims implicats en la bioenginyeria de replicació cel·lular.

Les topoisomerases[8] són enzims que actuen sobre la topologia de l'ADN. La configuració de doble hèlix de l'ADN fa que sigui dificultosa la separació d'ambdues cadenes de nucleòtids, fet imprescindible quan d'altres enzims han de transcriure'n les seqüències. La topoisomerasa de tipus I és un enzim no depenent d'ATP, que actua formant un trencament transitori a una de les cadenes del dúplex d'ADN, relaxa les tensions i finalment en relliga les cadenes d'ADN. L'estabilització de l'intermedi fosfotirosil ADN-topoisomerasa per l'acció d'inhibidors, converteix a les topoisomerases en un agent que malmet l'ADN. Aquest és precisament un novedós mode d'acció per fàrmacs contra el càncer.[9,10]

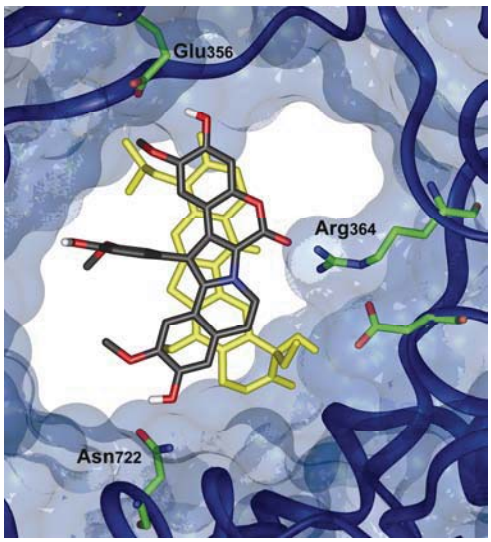


Fig. 3. Model cristal·logràfic de topoisomerasa-ADN-topotecan (entrada 1k4t Protein Data Bank) amb superposició de la Lamel·larina D a la regió activa.

El model cristal·logràfic del complex ternari d'ADN-topoisomerasa-topotecan,[11] un inhibidor de topoisomerasa derivat de la camptothecina, es va utilitzar com a base d'estudis de dinàmica molecular amb el consegüent reemplaç del fàrmac per la Lamel·larina D. Els resultats que se'n van derivar, resulten d'importància en la

[8] Corbett, K. D.; Berger, J. M., Structure, Molecular Mechanisms, and Evolutionary Relationships in DNA Topoisomerases. *Annu. Rev. Biophys. Biomol. Struct.* **2004,** 33, 95-118.
[9] Holden, J. A., DNA Topoisomerases as Anticancer Drug Targets: from the Laboratory to the Clinic. *Curr. Med. Chem.: Anti-Cancer Agents* **2001,** 1, 1-25.
[10] Cortés, F.; Pastor, N.; Mateos, S.; Domínguez, I., Topoisomerase Inhibitors as Therapeutic Weapons. *Expert Opin. Ther. Patents* **2007,** 17, 1-12.
[11] Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B.; Stewart, L., The Mechanism of Topoisomerase I Poisoning by a Camptothecin Analog. *Proc. Natl. Acad. Sci. USA* **2002,** 99, 15387-15392.

identificació dels grups fenòlics de les posicions 3 i 11 de la Lamel·larina D com a grups essencials en la inhibició la topoisomerasa (*Fig. 3*).[12,13]

Els complexos proteics, operen en rangs de forces motrius de l'ordre dels pN, i són talment màquines moleculars molt sofisticades que processen matèria, energia i informació. L'èxit dels últims anys en el coneixement detallat de mecanismes bioquímics, la identificació de noves dianes terapèutiques i l'aplicació de noves tecnologies biofísiques, ha permès obtenir alta sensibilitat i precisió en accedir a l'estudi de sistemes biològics complexos. Amb tot això es configura un espai interdisciplinari on es disposa de novedoses i millors aproximacions de com funciona l'enginyeria de la vida.

[12] Marco, E.; Laine, W.; Tardy, C.; Lansiaux, A.; Iwao, M.; Ishibashi, F.; Bailly, C.; Gago, F., Molecular Determinants of Topoisomerase I Poisoning by Lamellarins: Comparison with Camptothecin and Structure-Activity Relationships. *J. Med. Chem.* **2005,** 48, 3796-3807.
[13] Facompré, M.; Tardy, C.; Bal-Mayeu, C.; Colson, P.; Pérez, C.; Manzanares, I.; Cuevas, C.; Bailly, C., Lamellarin D: A Novel Potent Inhibitor of Topoisomerase I. *Cancer Res.* **2003,** 63, 7392-7399.

OBJECTIUS

L'objectiu inicial d'aquesta tesi doctoral, és la síntesi d'una quimioteca d'anàlegs de cadena oberta de les Lamel·larines pentacícliques. La eliminació de l'anell B del sistema pentacíclic per transformació de la lactona en un ester metílic va conduir a molècules més flexibles. Els components de la quimioteca es diferenciaran en els substituents dels anells aromàtics i el grau d'oxidació del sistema de pirrolo[2,1-*a*]isoquinolina.

El segon objectiu estretament lligat a l'anterior consisteix en la avaluació de la citotoxicitat dels compostos obtinguts i el posterior estudi de relacions estructura-activitat.

El perfil farmacològic de la Lamel·larina D, com a cap de sèrie amb millors bioactivitats, impulsa a desenvolupar una metodologia novedosa per la seva síntesi.

Degut a la falta de solubilitat del producte natural, el següent objectiu consistirà en la preparació de conjugats a fi de millorar-la, i d'augmentar-ne també la biodisponibilitat. Per comprovar aquest últim objectiu, es requerirà de tècniques per avaluar la internalització cel·lular de la Lamel·larina D i conjugats.

Finalment es planteja com a objectiu últim d'aquesta tesi, l'estudi del mecanisme d'acció de la Lamel·larina D i molècules anàlogues.

1 AVANÇOS RECENTS EN ALCALOIDES DEL GRUP DE LES LAMEL·LARINES: AÏLLAMENT, SÍNTESI I ACTIVITAT

AVANÇOS RECENTS EN ALCALOIDES DEL GRUP DE LES LAMEL·LARINES: AÏLLAMENT, SÍNTESI I ACTIVITAT.

Recent advances in Lamellarin alkaloids: isolation, synthesis and activity.

Daniel Pla,[1,2] Fernando Albericio,[1,2,3] and Mercedes Álvarez[1,2,4,*]

Anti-Cancer Agents in Medicinal Chemistry, **2008**, *8*, 746-760

[1] Institute for Research in Biomedicine, Barcelona Science Park, University of Barcelona, E-08028, Barcelona, Spain
[2] CIBER-BBN Networking Centre on Bioengineering, Biomaterials and Nanomedicine, E-08028 Barcelona, Spain;
[3] Department of Organic Chemistry, University of Barcelona, E-08028, Barcelona, Spain
[4] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, E-08028, Barcelona, Spain

Resum

La revisió de la literatura recent sobre les Lamel·larines descrita en aquest capítol remarca els avanços en aquest camp durant el període comprès entre 2004 fins desembre de 2007. La presentació dels resultats s'ha estructurat envers l'aïllament de fonts naturals, les darreres estratègies sintètiques i el mode d'acció d'aquests interessants compostos. L'abast recent en aquesta temàtica contempla la síntesi total dels productes naturals, la preparació dels corresponents anàlegs, estudis sobre el mecanismes d'acció, i relacions estructura-activitat. Ha estat en aquest any 2008 que s'ha descrit també una nova síntesi per la Lamel·larina U i G èter trimetílic per alquilació d'α-aminonitril,[1] així com nova metodologia sintètica per accedir a l'esquelet pentacíclic de les Lamel·larines via reacció de Grob entre 3-nitro-2-(trifluorometil)-2*H*-cromens i 1,3,3-trimetil-3,4-dihidroisoquinolines.[2] Aquesta ressenya clarament il·lustra la importància dels productes naturals en la descoberta de nous medicaments així com en el desenvolupament de noves metodologies sintètiques.

[1] Liermann, J. C.; Opatz, T., Synthesis of Lamellarin U and Lamellarin G Trimethyl Ether by Alkylation of a Deprotonated α-Aminonitrile. *J. Org. Chem.* **2008,** 73, 4526-4531.
[2] Korotaev, V. Y.; Sosnovskikh, V. Y.; Kutyashev, I. B.; Barkov, A. Y.; Shklyaev, Y. V., A facile route to the pentacyclic lamellarin skeleton via Grob reaction between 3-nitro-2-(trifluoromethyl)-2*H*-chromenes and 1,3,3-trimethyl-3,4-dihydroisoquinolines. *tetrahedron Lett.* **2008,** 49, 5376-5379.

Recent Advances in Lamellarin Alkaloids: Isolation, Synthesis and Activity

D. Pla[1,2], F. Albericio[1,2,3] and M. Álvarez [1,2,4,*]

[1]*Institute for Research in Biomedicine, Barcelona Science Park, University of Barcelona, E-08028, Barcelona, Spain;* [2]*CIBER-BBN Networking Centre on Bioengineering, Biomaterials and Nanomedicine, E-08028 Barcelona, Spain;* [3]*Department of Organic Chemistry, University of Barcelona, E-08028, Barcelona, Spain and* [4]*Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, E-08028, Barcelona, Spain*

Abstract: Lamellarins are a large family of marine alkaloids with potential anticancer activity that have been isolated from diverse marine organisms, mainly ascidians and sponges. All lamellarins feature a 3,4-diarylpyrrole system. Pentacyclic lamellarins, whose polyheterocyclic system has a pyrrole core, are the most active compounds. Some of these alkaloids are potently cytotoxic to various tumor cell lines. To date, Lam-D and Lam-H have been identified as lead compounds for the inhibition of topoisomerase I and HIV-1 integrase, respectively—nuclear enzymes which are over-expressed in deregulation disorders. Moreover, these compounds have been reported for their efficacy in treatment of multi-drug resistant (MDR) tumors cells without mediated drug efflux, as well as their immunomodulatory activity and selectivity towards melanoma cell lines. This article is an overview of recent literature on lamellarins, encompassing their isolation, recent synthetic strategies for their total synthesis, the preparation of their analogs, studies on their mechanisms of action, and their structure-activity relationships (SAR).

Key Words: Lamellarins, marine alkaloids, nitrogen heterocycles, cytotoxic agents, topoisomerase I.

INTRODUCTION

Lamellarins are a large family of marine alkaloids characterized by their unusual structures and important activities. From a structural perspective, two groups of lamellarins can be found. Members of the larger of the two groups possess a pentacyclic system of 6-oxobenzo[*b*]pyrano[3,4-*b*]pyrrolo[2,1-*a*] isoquinoline with a substituted phenyl ring at position 14. Pentacyclic lamellarins may be saturated (Table **1**) or unsaturated (Table **2**) between positions 8 and 9.

The second group of lamellarins, which are less structurally complex, are derivatives of methyl 3,4-bis(*p*-hydroxyphenyl)pyrrole-2-carboxylate, and which differ in their *N*-pyrrole substituent (Fig. (**1**)). Lamellarins O (Lam-O) and P (Lam-P) [1] contain a common *p*-methoxyacetophenone on their *N*-pyrrole, Lam-Q [2] has a un-substituted pyrrole, and Lam-R has an *N*-(*p*-hydroxyphenyl)pyrrole [2]. The bioactivities of these compounds are not significant.

Lamellarins can be biosynthesized from three molecules of tyrosine or DOPA [2, 3], similarly to several related marine alkaloid families such as lukianols [4], ningalins [5], polycitones [6] and purpurone [7] (Fig. (**1**)).

Several reviews on lamellarins have recently been published [8]. Herein is covered work related to their isolation, synthesis and activity that was published between 2004 and December 2007.

ISOLATION OF LAMELLARINS

Lamellarins were initially isolated from a prosobranch mollusk of the genus Lamellaria [9] and subsequently found in various organisms, mostly ascidians, which are prey of the former. More than thirty lamellarins have been isolated to date, but only few show interesting bioactive properties [3,9-16]. Venkateswarlu *et al.* [14] recently isolated from the Indian red colonial ascidian *Didemnum obscurum* three new lamellarin alkaloids (Lam-γ, Lam-α, and Lam-Є) plus eight known lamellarin alkaloids (Lam-M, Lam-K, Lam-K diacetate, Lam-K triacetate, Lam-U, Lam-I, Lam-C diacetate, and Lam-X triacetate). The same authors also described from the same ascidian four new lamellarin alkaloids (Lam-ζ, Lam-η, Lam-ϕ and Lam-χ) and seven known lamellarins (Lam-K, Lam-I, Lam-J, Lam-K triacetate, Lam-L triacetate, Lam-F and Lam-T diacetate) [15]. The structures of the lamellarins isolated by Venkateswarlu *et al.* [14, 15] were established using standard spectroscopic techniques, and the structure of Lam-K triacetate was confirmed by X-ray crystallographic analysis.

SYNTHESIS

Lamellarins are rather complex structural targets. Several approaches to their synthesis can be found in the literature. These fall into two main synthetic categories: (a) pyrrole formation as the cornerstone of the synthesis; and (b) transformation of a pre-existing pyrrole derivative through cross-coupling reactions.

a) Pyrrole Ring Formation

A highly efficient synthesis of Lam-K and Lam-L was described by Ruchirawat *et al.* [17]. The pyrrole ring was constructed *via* Michael addition followed by a ring-closing reaction of benzyldihydroisoquinoline derivatives with ethoxycarbonyl-β-nitrostyrenes (Fig. (**2**)). Formation of the pyrrole ring produces the dihydropyrrolo[2,1-*a*]isoquinoline in which all the phenol groups were protected as benzyl-ethers. Deprotection by hydrogenolysis followed by base-mediated lactonization gave the natural products. The same methodology was used to prepare several natural saturated and unsaturated lamellarins, as well as various analogs [18].

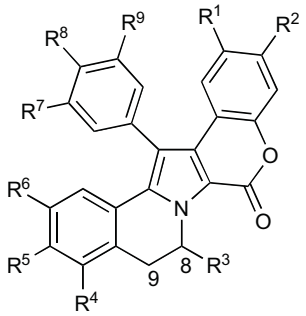
The same authors reported an elegant preparation of the lamellarin skeleton using a slightly different pyrrole ring formation from benzyldihydroisoquinoline and a phenacyl bromide [19]. They used polymer-supported reagents to simplify the work-up and obviate column chromatography. The pyrrole ring was constructed in one pot by quaternization of the isoquinoline followed by an aldol-type condensation. Subsequent intramolecular Friedel-Crafts transacylation, and finally, lactonization, afforded the lamellarin skeleton. As shown in Fig. (**3**), polymer-supported reagents were used for the following steps: selective monobromination of *ortho*-substituted acetophenones (Amberlyst A-26 Br_3-form and PVPHP); base-mediated pyrrole formation *via* condensation of benzyldihydroisoquinoline with either phenacyl bromide or α-nitrocinnamate (Amberlyst A-26 $NaCO_3^-$form); and a novel acid-mediated lactone formation *via* either Friedel-Crafts transacylation and lactonization, or *O*-debenzylation and lactonization (Amberlyst-15).

Several lamellarins and derivatives were obtained by solid-phase synthesis (SPS) on an appropriate solid support and under different cleavage conditions [20]. The lamellarin skeleton was

*Address correspondence to this author at the Institute for Research in Biomedicine, Barcelona Science Park, University of Barcelona, E-08028, Barcelona, Spain; E-mail: mercedes.alvarez@irbbarcelona.org

1871-5206/08 $55.00+.00

Table 1. Structure of Reduced Pentacyclic Lamellarins



Lamellarins	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	Ref.
Lam-A	OMe	OH	OH	OMe	OMe	OMe	OMe	OH	H	[1]
Lam-C	OMe	OH	H	OMe	OMe	OMe	OMe	OH	H	[1]
Lam-C sulf.	OMe	OSO_3Na	H	OMe	OMe	OMe	OMe	OH	H	[2]
Lam-E	OMe	OH	H	OH	OMe	OMe	OH	OMe	H	[3]
Lam-F	OMe	OH	H	OH	OMe	OMe	OMe	OMe	H	[3]
Lam-G	OH	OMe	H	H	OH	OMe	OH	OMe	H	[3]
Lam-G sulf.	OH	OMe	H	OSO_3Na	OH	OMe	OH	OMe	H	[2]
Lam-I	OMe	OH	H	OMe	OMe	OMe	OMe	OMe	H	[4]
Lam-J	OMe	OH	H	H	OH	OMe	OMe	OMe	H	[4]
Lam-K	OMe	OH	H	OH	OMe	OMe	OMe	OH	H	[4]
Lam-L	OMe	OH	H	H	OH	OMe	OH	OMe	H	[4]
L sulf.	OMe	OSO_3Na	H	H	OH	OMe	OH	OMe	H	[2]
Lam-S	OH	OH	H	H	OH	OMe	OH	OH	H	[2]
Lam-T	OMe	OH	H	OMe	OMe	OMe	OH	OMe	H	[5]
Lam-T sulf.	OMe	OSO_3Na	H	OMe	OMe	OMe	OMe	OH	H	[5]
Lam-U	OMe	OH	H	H	OMe	OMe	OH	OMe	H	[2]
Lam-U sulf.	OMe	OSO_3Na	H	H	OMe	OMe	OH	OMe	H	[5]
Lam-V	OMe	OH	OH	OMe	OMe	OMe	OH	OMe	H	[5]
Lam-V sulf.	OMe	OSO_3Na	OH	OMe	OMe	OMe	OH	OMe	H	[5]
Lam-Y	OMe	OH	H	H	OMe	OH	OH	OMe	H	[2]
Lam-Y sulf.	OMe	OSO_3Na	H	H	OMe	OH	OH	OMe	H	[5]
Lam-Z	OH	OMe	H	H	OH	OMe	OH	OH	H	[2]
Lam-β	OMe	OH	H	H	OH	OH	OH	OMe	H	[6]
Lam-γ	OMe	OH	H	OH	OMe	OMe	OH	OMe	OMe	[7]
Lam-χ triacetate	OMe	OAc	H	H	OAc	OMe	OMe	OAc	H	[8]
Dihydro- Lam-η	OMe	OH	H	H	OMe	OMe	OMe	OMe	H	[8]

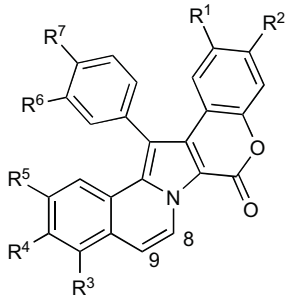
synthesized on solid phase through formation of the pentacyclic system from an open chain dihydroisoquinolinium salt by an intramolecular [3+2] cycloaddition [21]. The use of different Lewis acids as cleavage-deprotection reagents in SPS has been exploited for introducing diversity to produce analogs for screening (Fig. (**4**)).

A biomimetic synthesis of lamellarin and lukianol skeleton was developed by Steglich *et al*. [22]. It is based on formation of 3,4-diarylpyrrole-2,5-dicarboxylic acid from aryl pyruvic acids and 2-arylethylamines. The method has been used for the synthesis of ningalin B, Lam-G, Lam-K, lukianol A and a lukianol-lamellarin hybrid (Fig. **5**).

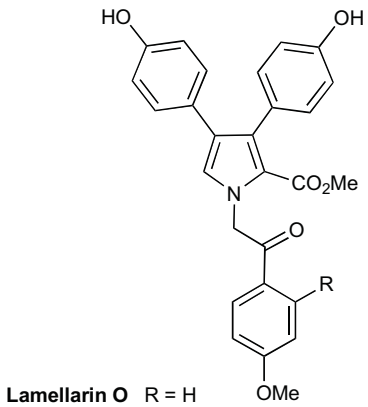
Lam-Q dimethyl ether was obtained by Raney Ni reduction of 3,4-diarylpyrrole-2-carboxylates (**1**) [23], which were obtained by cyclization of α-oxoketene-*N,S*-acetals in the presence of Vilsmeier reagent. Lam-Q dimethyl ether has been demonstrated to be a synthetic precursor of Lam-O dimethyl ether and lukianol A [24].

The total synthesis of Lam-α 20-sulfate [25] has been performed using a Hinsberg-type pyrrole synthesis and Suzuki-Miyaura cross-coupling as the key reactions. The synthesis featured an interesting combination of protecting groups for the phenols [26]. The pentacyclic system of lamellarins was obtained with two orthogonal protecting groups, isopropoxy (13-O*i*Pr) and benzyloxy (20-OBn). The 20-sulfate analog was prepared by a sequence comprising debenzylation of Lam-α 20-OBn, formation of the 2,2,2-trichloroethylsulfate of the resulting 20-OH, deprotection of Lam-α 13-O*i*Pr, and finally, reductive elimination of the 2,2,2-trichloroethyl sulfate protecting group (Fig. (**7**)).

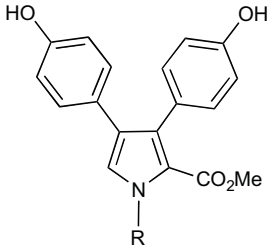
Table 2. Structure of Oxidized Pentacyclic Lamellarins



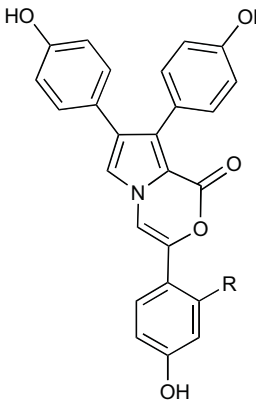
Lamellarins	R^1	R^2	R^3	R^4	R^5	R^6	R^7	Ref.
Lam-B	Ome	OH	OMe	OMe	OMe	OMe	OH	[1]
Lam-B sulf.	OMe	OSO_3Na	OMe	OMe	OMe	OMe	OH	[2]
Lam-D	OMe	OH	H	OH	OMe	OMe	OH	[1]
Lam-H	OH	OH	H	OH	OH	OH	OH	[3]
Lam-M	OMe	OH	OH	OMe	OMe	OMe	OH	[4]
Lam-N	OMe	OH	H	OH	OMe	OH	OMe	[4]
Lam-W	OMe	OH	OMe	OMe	OMe	OH	OMe	[5]
Lam-X	OMe	OH	OH	OMe	OMe	OH	OMe	[2]
Lam-α	OMe	OH	H	OMe	OMe	OH	OMe	[7]
Lam-α sulf.	OMe	OSO_3Na	H	OMe	OMe	OH	OMe	[9]
Lam-ζ	OMe	OH	OMe	OMe	OMe	OMe	OMe	[8]
Lam-*C*	OMe	OH	OH	OMe	OMe	OMe	OMe	[7]
Lam-η	OMe	OH	H	OMe	OMe	OMe	OMe	[8]
Lam-ϕ	OMe	OAc	OMe	OMe	OAc	OMe	OAc	[8]



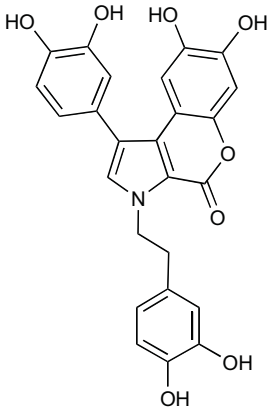
Lamellarin O R = H
Lamellarin P R = OH



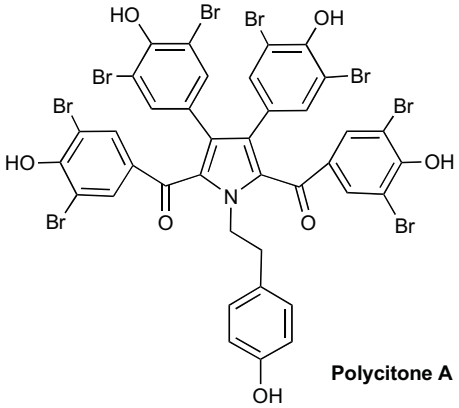
Lamellarin Q R = H
Lamellarin R R = *p*-(C_6H_5)OH



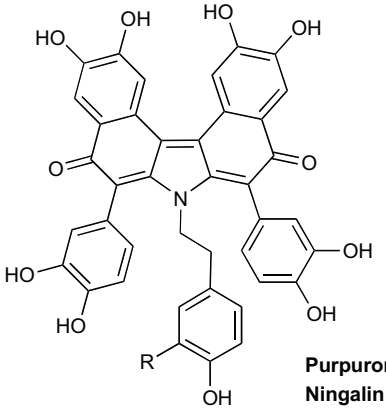
Lukianol A



Ningalin B



Polycitone A



Purpurone R = H
Ningalin D R = OH

Fig. (1). Structures of lamellarins O–R, lukianol A, ningalins B and D, polycitone A, and purpurone.

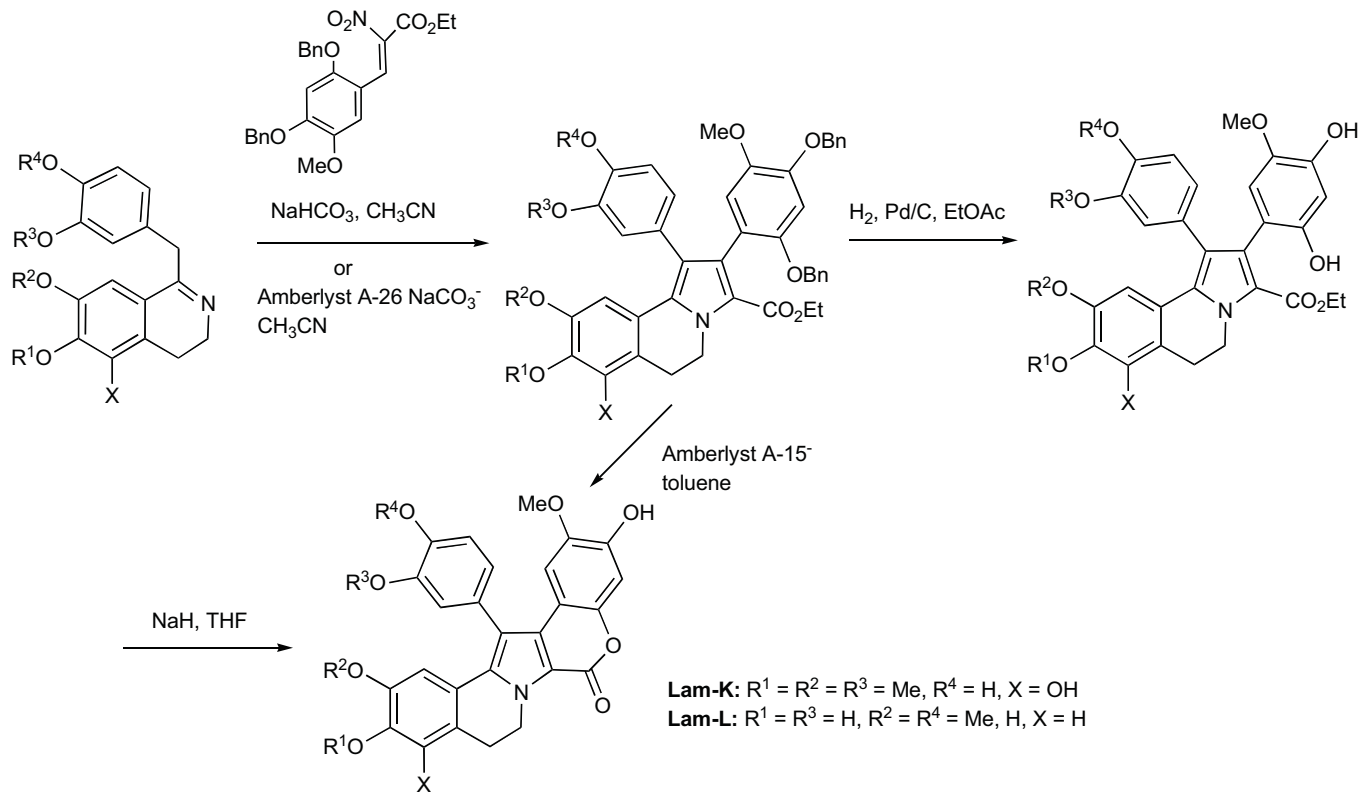


Fig. (2). Synthesis of lamellarins K and L.

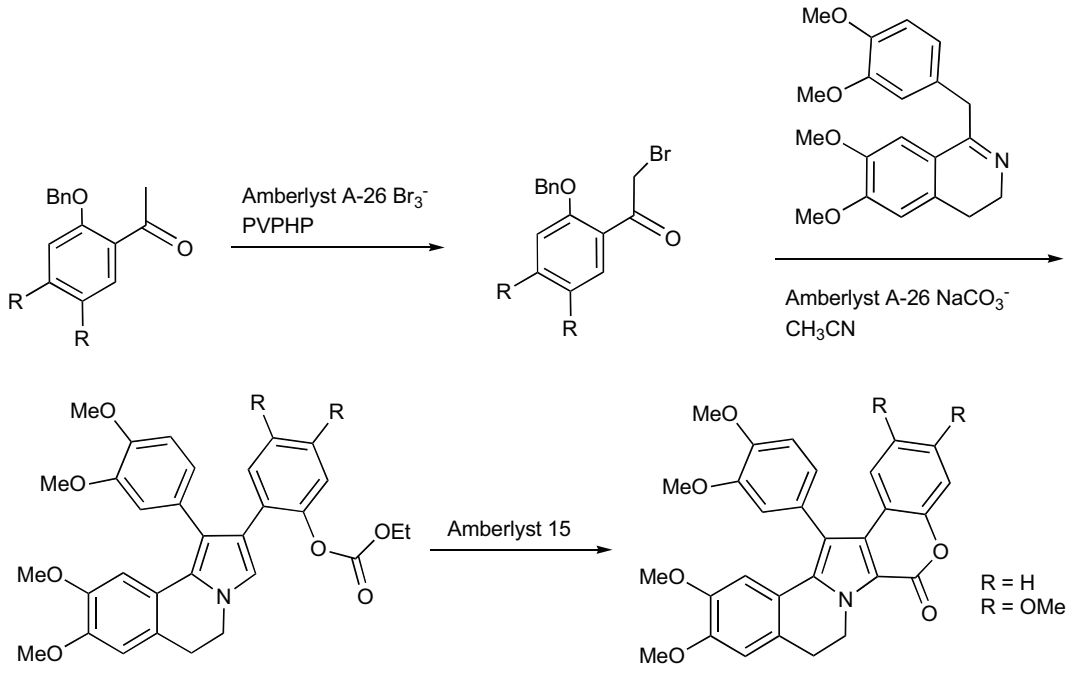


Fig. (3). Synthesis of the lamellarin skeleton with polymer-supported reagents.

A similar procedure employing the appropiate boronic acids for the cross-coupling reaction has been used for the total synthesis of Lam-D, Lam-L, and Lam-N [27].

The 1,2-diaryl-substituted pyrrolo[2,1-*a*]isoquinoline skeleton of lamellarins has been obtained by a new route *via* 1,5-dipolar electrocyclization of azomethine ylides (Fig. (**8**)) [28]. The reagents comprised the stilbenic amides **2** available from the acids obtained by condensation of substituted benzaldehydes with phenylacetic acid. Cyclization of the amide **2** using the Bischler-Napieralski procedure afforded 3,4-dihydroisoquinolines **3**. Subsequent reaction of the isoquinolines with ethyl bromoacetate gave the quaternary

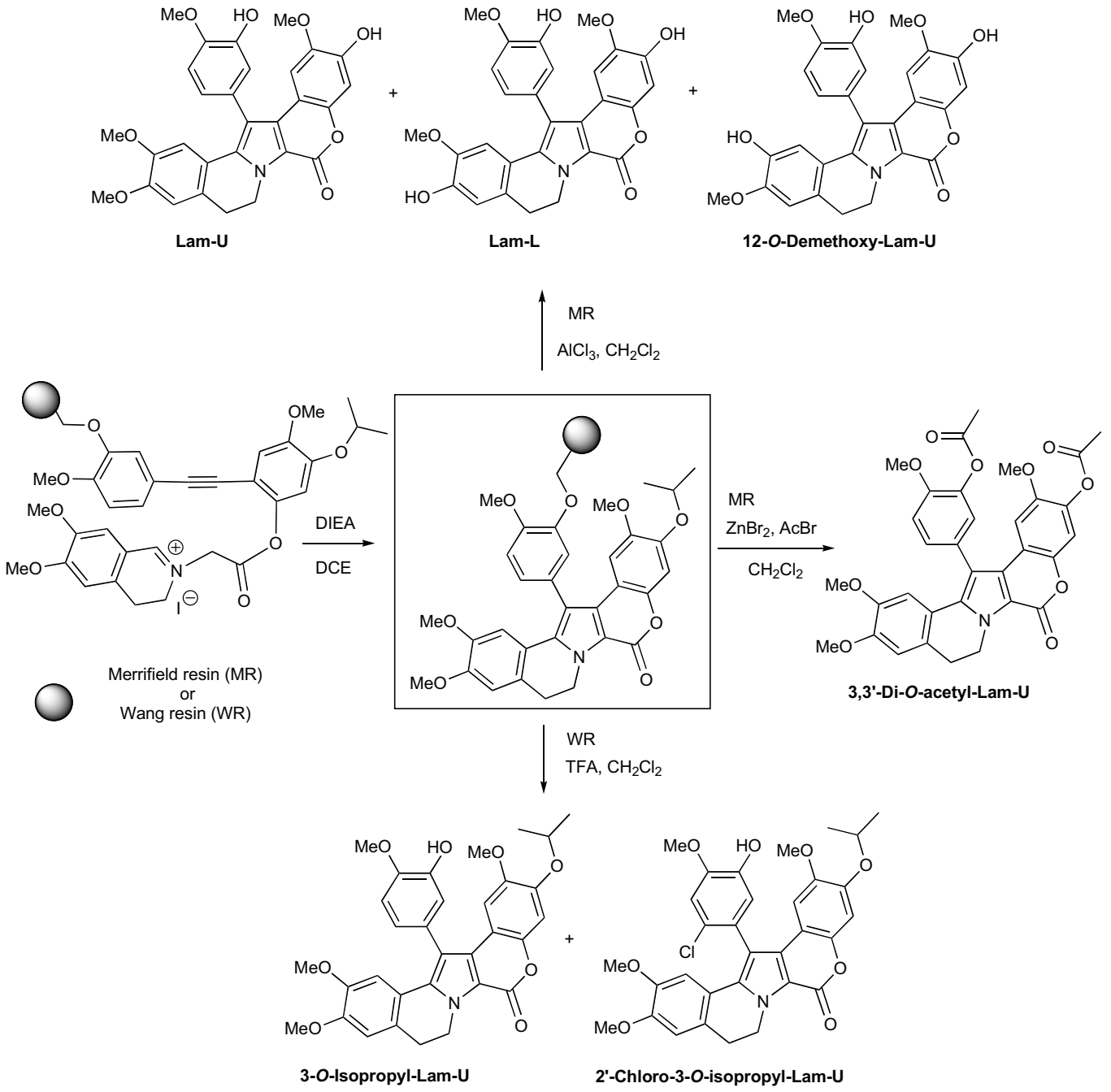


Fig. (4). Lewis acids for cleavage-deprotection in solid-phase synthesis.

salts, which upon treatment with triethylamine in dry ethanol afforded the pyrrole derivatives. Removal of the allylic protecting group with Pd-C and TsOH resulted in simultaneous formation of the pentacyclic lamellarin skeleton and lactonization. This elegant method was not used for the preparation of a natural product; it was only applied to the construction of the lamellarin skeleton.

A recently developed method for rapid access to the pyrroloisoquinoline core structures related to lamellarins is based on silver-catalyzed domino cycloisomerization-dipolar cycloaddition of alkynyl *N*-benzylideneglycinate **4** and acetylene mono- or di-carboxylate (Fig. (**9**)). Reactions conducted at 60ºC in toluene using 2,6-di-*ter*-butyl-4-methylpyridine (DTBMP) as base, and in the absence of oxidants, afforded optimal results (Fig. (**9**)). Several diversely substituted pyrroloisoquinolines were thus prepared [29].

b) Transformation of a Pyrrole Derivative Through Cross-Coupling Reactions

Lam-Q and Lam-O have been synthesized on Merrifield resin with *N*-protected 3,4-dibromopyrrole-2-carboxylate as scaffold (Fig. (**10**)) [30]. The process comprises incorporation of a substituted pyrrole ring onto a *p*-alkoxy iodo phenyl resin through a Negishi cross-coupling reaction, followed by Suzuki cross-coupling to introduce the second substituted phenyl ring, and finally, *N*-alkylation. A Lewis acid was used for the final cleavage. The beauty of this strategy is that diversity can be introduced at each step, including the final cleavage (by using the appropriate Lewis acid).

Lam-G trimethyl ether has been obtained by three successive halogenation/cross couplings of a pyrrole-2-carboxylate (Fig. (**11**))

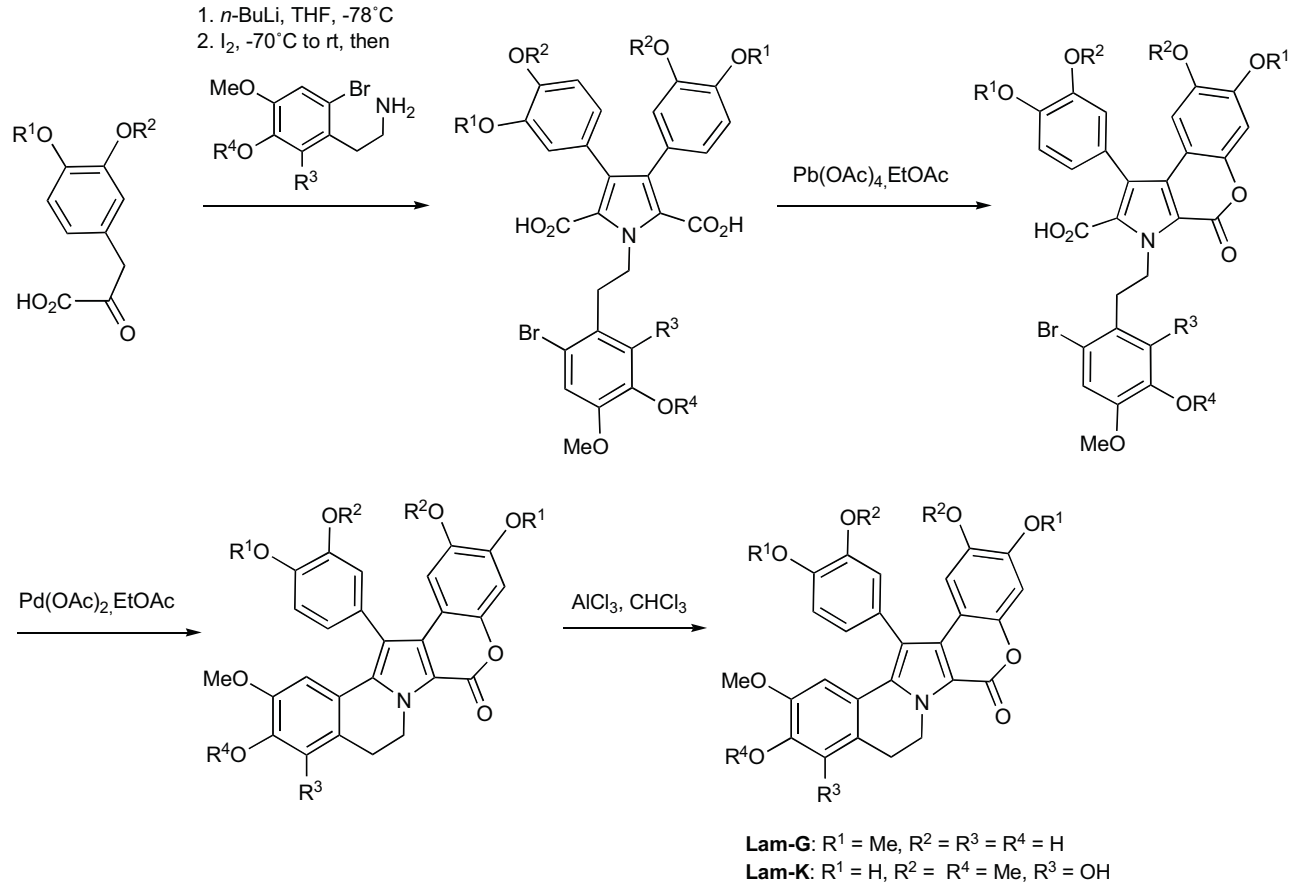


Fig. (5). Synthesis of lamellarins G and K.

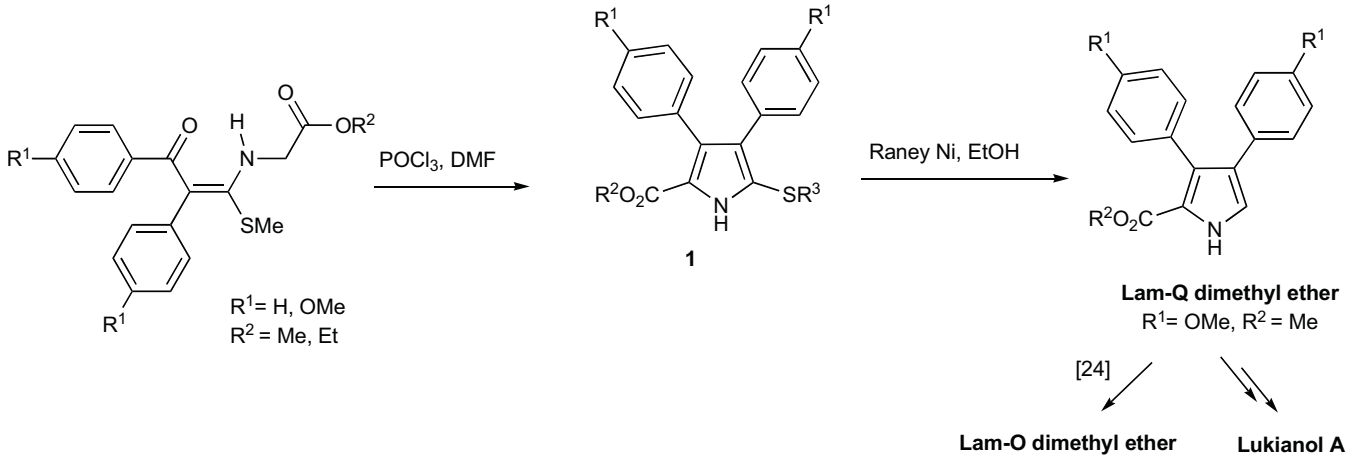


Fig. (6). Synthesis of lamellarin Q dimethyl ether.

[31]. Coupling of *N*-protected bromopyrrole **5** with boronic acid **6** gave the aryl pyrrole **7**. Treatment of **7** with an equimolar amount of NBS led to selective halogenation at position C5. The second coupling with boronic acid **8** under Suzuki conditions gave the diarylpyrrole **9**, which, owing to the quality of the tosyl functionality as a leaving group, readily underwent cyclization under basic conditions.

A total synthesis of Lam-D has been developed starting from two sequential and regioselective bromination and cross-coupling reactions of the scaffold **10** [32], followed by oxidation, deprotection of the phenol-groups and lactonization (Fig. (**12**)) [33].

The aforementioned strategy has been employed to prepare numerous open chain analogs of lamellarins containing the mono- and bis-aryl scaffolds **11** and **12**, respectively, and their corresponding oxidized derivatives **13** and **14** (see Table **5**) [34]. Other C4-C5-bisarylpyrrole-2-carboxylate simplified analogs were synthesized by Banwell *et al.* [35].

Lam-Q dimethyl ether and Lam-O have been synthesized from C3-C4-bisaryl pyrroles obtained by regioselective halogenation and Suzuki-Miyaura reaction of a 2-trichloroacetylpyrrole (Fig. (**13**)). [36].

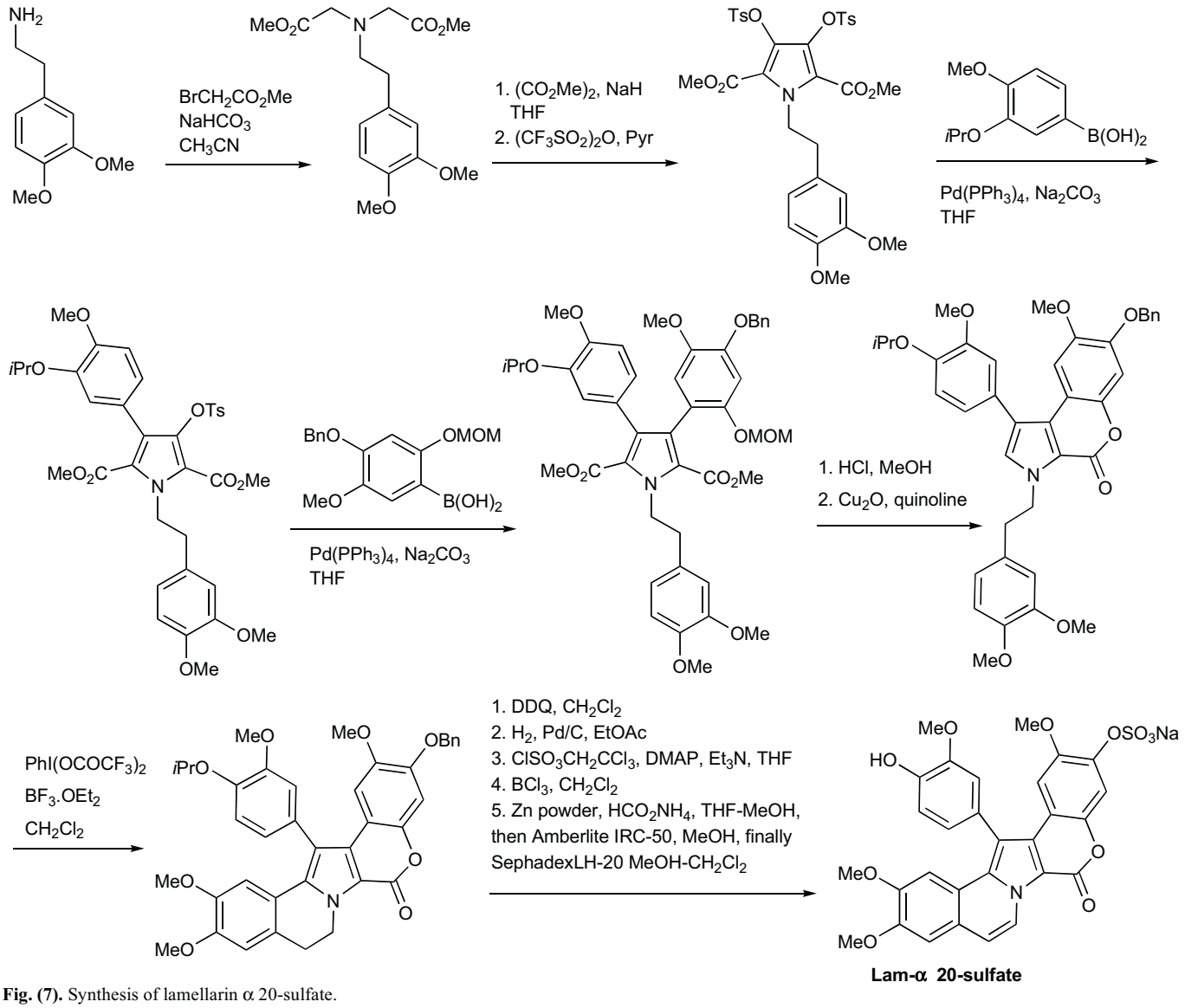


Fig. (7). Synthesis of lamellarin α 20-sulfate.

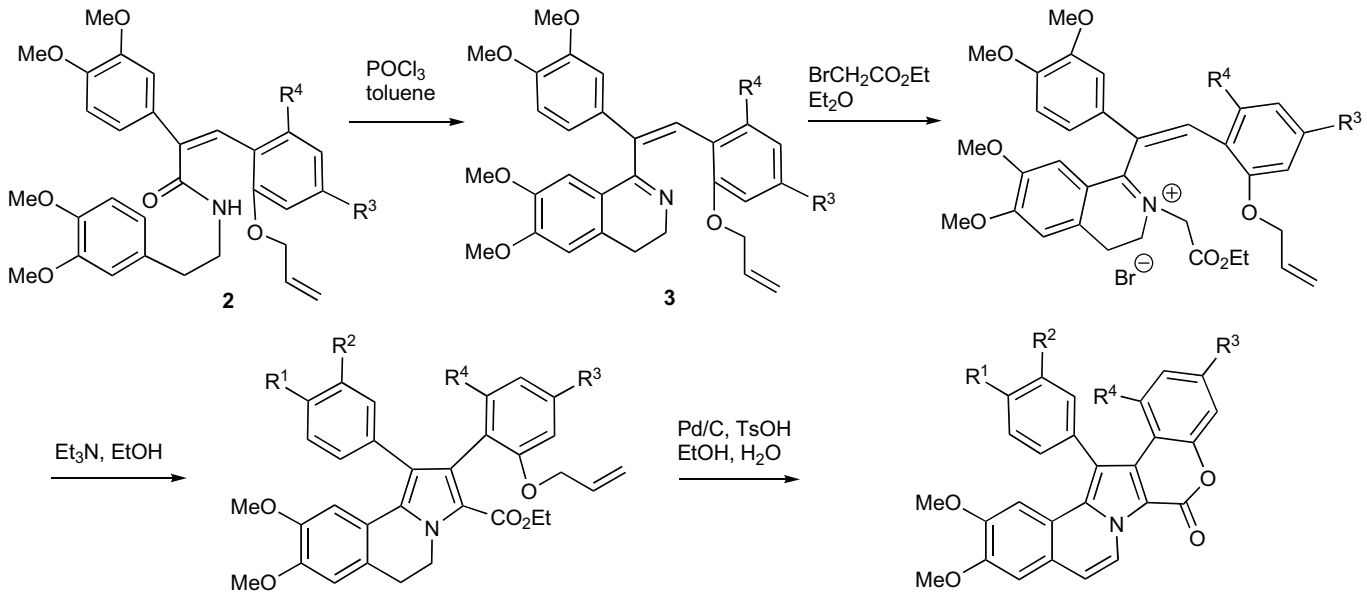


Fig. (8). Synthesis of the lamellarin skeleton by electrocyclization of azomethine ylides.

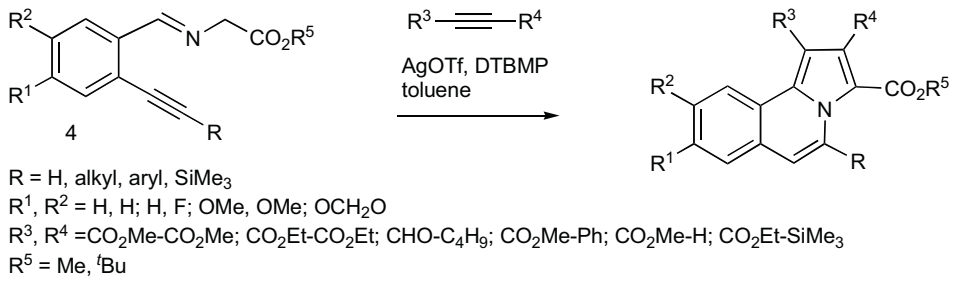


Fig. (9). Synthesis of pyrroloisoquinoline core structures of lamellarins.

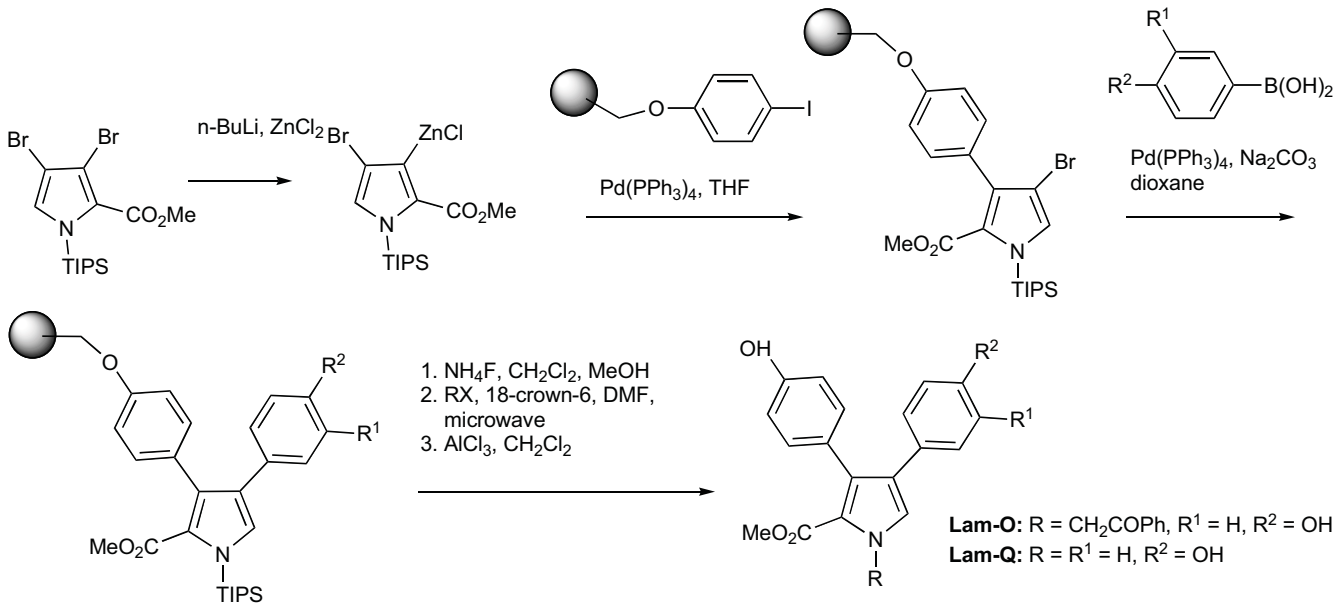


Fig. (10). Solid phase synthesis of lamellarins O and Q.

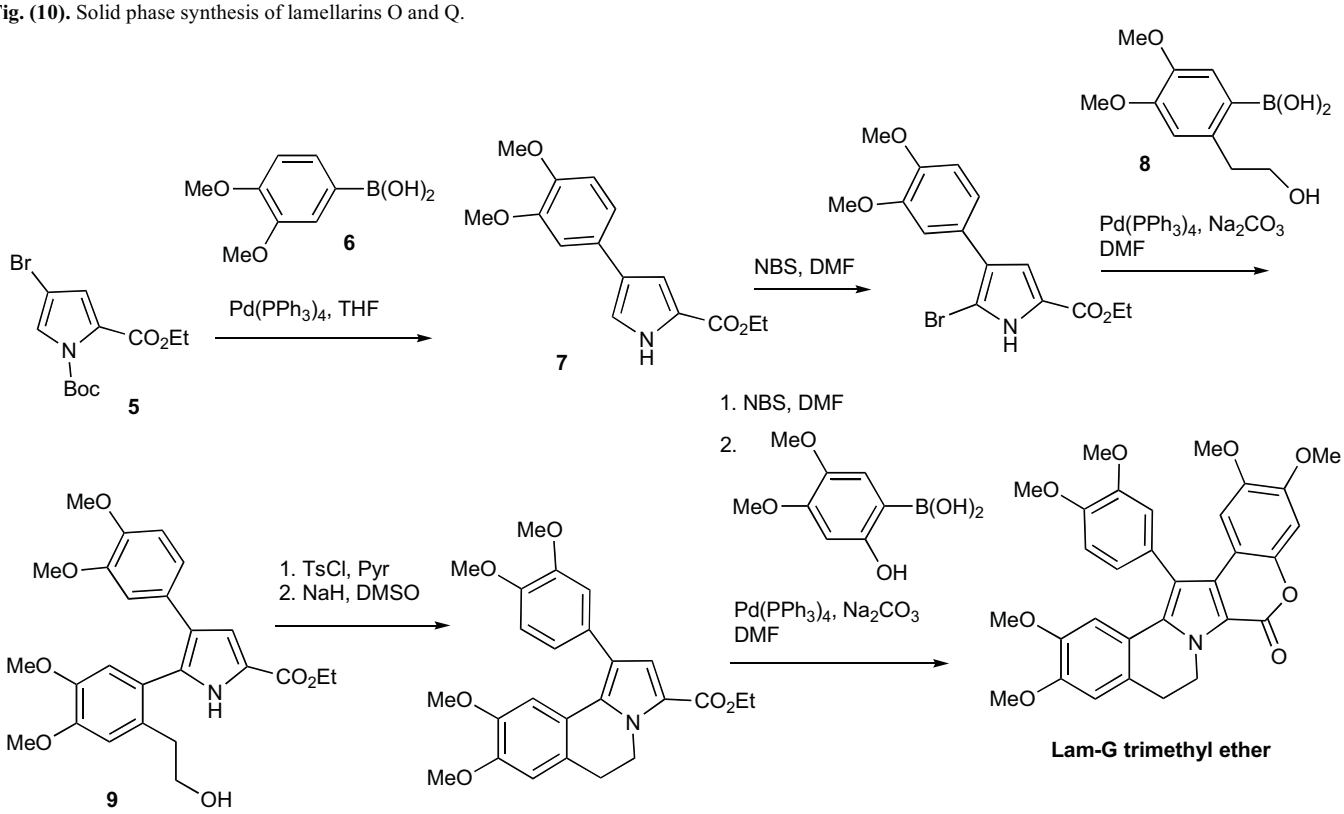


Fig. (11). Synthesis of lamellarin G trimethyl ether.

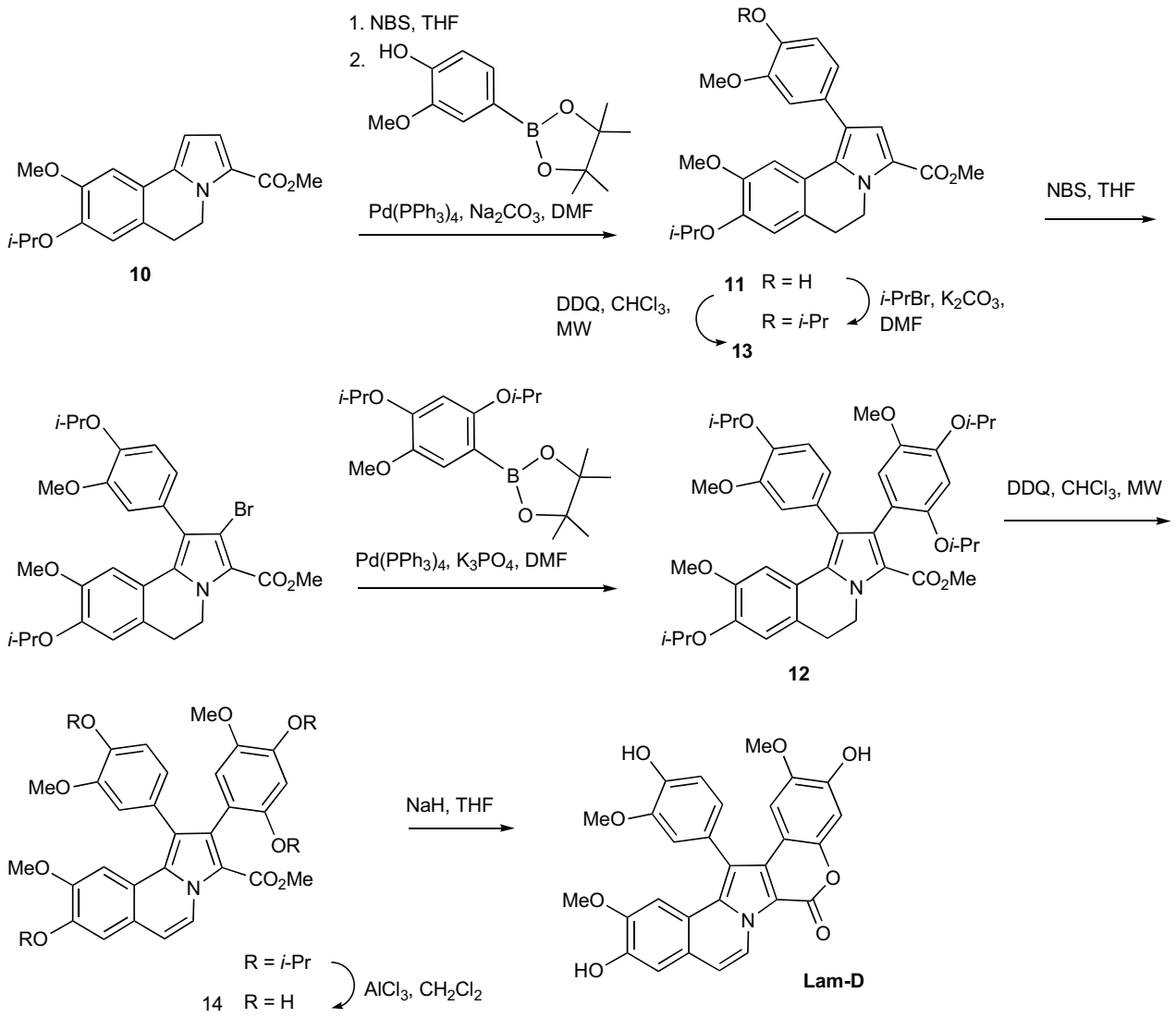


Fig. (12). Synthesis of lamellarin D.

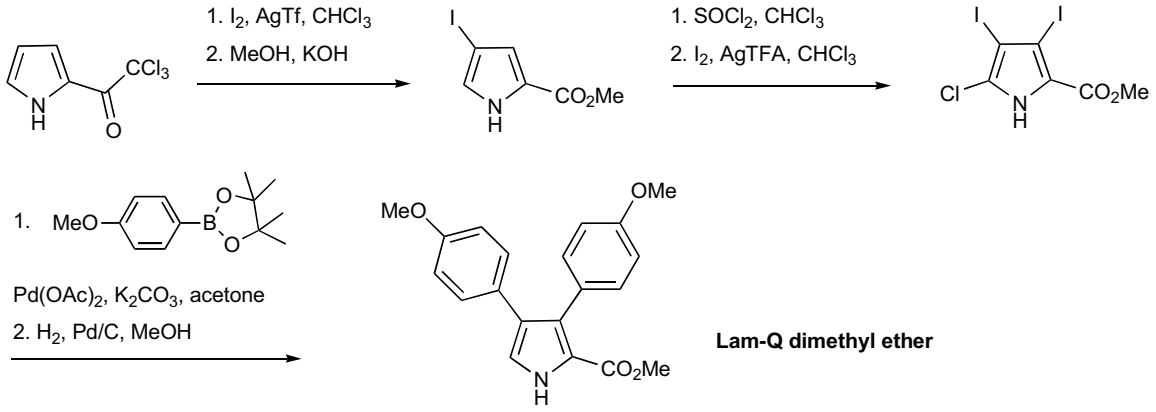


Fig. (13). Synthesis of lamellarin Q dimethyl ether.

ACTIVITY AND MECHANISM OF ACTION

Lamellarins and their derivatives are multi-drug resistance (MDR) reversal agents. As some of them are highly cytotoxic, they have been tested against various cancer cell lines. The results are summarized in Table **3**.

Lam-D, Lam-K and Lam-M are among the most cytotoxic molecules in the series. The best studied member is Lam-D, which is highly cytotoxic to a wide range of tumor cell lines, particularly human prostate cancer cells [15] and leukemia cells [37]. Several molecular targets have been described for Lam-D and other lamellarins.

Table 3. Cytotoxicity of Isolated Lamellarins to Various Culture Cell Lines

Isolated Compound	Culture Cell Line[a]	IC_{50} (M)[b]	Ref.
Lam-D	HeLa	$10.5\ 10^{-9}$	[39]
	CEM	$5\ 10^{-9}$	[37]
	CEM/C2	$7.2\ 10^{-7}$	
	DU145	$10.9\ 10^{-9}$ [c]	[8]
	MDA-MB-231	$2.5\ 10^{-7}$ [c]	[34]
	A549	$2\ 10^{-7}$ [c]	
	HT29	$5.1\ 10^{-6}$ [c]	
	XC	$1.24\ 10^{-8}$	[39]
	Vero Cells	$1.05\ 10^{-8}$	
	MDCK	$2.25\ 10^{-8}$	
Lam-F	COLO205	$9\ 10^{-9}$	[8]
Lam-H	HeLa	$>10^{-6}$	[39]
Lam-H hexaacetate	HeLa	$1.15\ 10^{-5}$	[39]
	CEM	$1.95\ 10^{-6}$	[37]
	CEM/C2	$6.93\ 10^{-6}$	
Lam-L triacetate	COLO205	$0.25\ 10^{-9}$	[8]
Lam-N	HeLa	$5\ 10^{-6}$	[9]
Lam-T	HeLa	$2.7\ 10^{-5}$ [d]	[9]
Lam-U sulf	HeLa	$1.4\ 10^{-4}$ [d]	[9]
Lam-V sulf	HeLa	$1.3\ 10^{-4}$ [d]	[9]
Lam-W	HeLa	$2.8\ 10^{-4}$ [d]	[9]
Lam-α	HeLa	$5.1\ 10^{-6}$ [d]	[49]
Lam-α sulf	HeLa	$2.74\ 10^{-4}$ [d]	[9]
Lam-ζ	COLO205	$5.6\ 10^{-9}$	[8]
Lam-χ	DU145	$2.99\ 10^{-6}$ [c]	[8]
Lam-χ triacetate	COLO205	$0.2\ 10^{-9}$	[8]
Lam-η	HeLa	$2.5\ 10^{-6}$	[39]
	CEM	$3.03\ 10^{-6}$	[37]
	CEM/C2	$5.55\ 10^{-6}$	
	COLO205	$1.78\ 10^{-7}$	[8]
Lam-dihydro η	COLO205	$5\ 10^{-8}$	[8]
Lam-ϕ	COLO205	$5.6\ 10^{-8}$	[8]

a: cervical cancer cells (HeLa); human leukemic lymphoid cells (CEM); human leukemia cells resistant to camptothecin (CEM/C2); human prostate carcinoma cells (DU145); Rous sarcoma virus transformed rat cell line (XC); monkey kidney epithelial cells (Vero cells); Madin-Darby canine kidney cells (MDCK); colon cancer cells (COLO205); human breast adenocarcinoma cancer cells (MDA-MB-231); human colon cancer cells (HT29); human lung cancer cells (A549); b: IC: inhibitory concentration (in most cases the values not comparable, because the assays were performed in different conditions); c: GI: growth inhibition; d) LD: lethal dose.

Molecular Structure-Activity Determinants

Lamellarins with a C8-C9 double bond are generally more cytotoxic than their corresponding saturated analogs. This is very clear in the case of Lam-D, which is considerably more cytotoxic than its synthetic saturated analog, Lam-501 (Fig. (**14**)), which has no effect on topoisomerase I. As such, Bailly *et al.* postulated that the planarity of the pentacyclic structure is important for cytotoxicity [38].

Ishibashi *et al.* synthesized several derivatives of Lam-D [39], then evaluated the compounds for cytotoxicity against a HeLa cell line to determine the SAR. Their results are summarized in Table **4**. Most of the derivatives with hydroxyl groups at both the C-3 and C-11 positions (**15a**, **15c**, **15f** and **15g**) showed quite high activity, with IC_{50} values of 10.5-70.0 nM. The low toxicity of **15b** might be partly due to its low solubility in the assay medium. The hydroxyl substituent at C-3 appears to be a prerequisite for activity, since the activity of **15d** (IC_{50}, 0.85 μM), which lacks the hydroxyl group, was markedly lower than that of **15a** (IC_{50}, $10.5\ 10^{-3}$ μM). The importance of the 3-hydroxyl group for bioactivity is also apparent when comparing the activity of **15e** (IC_{50}, 2.5 μM) with those of **15k** (IC_{50}, 5.7 μM) and **15l** (IC_{50} >100 μM). The hydroxyl group at C-11 might also be important for activity, since methylation of both hydroxyl groups at C-11 and C-4' of **15a** and **15e** leads to much lower activity. In contrast, **15g**, which has the 11-hydroxy group but lacks the 4'-hydroxyl group of **15a**, still maintains high activity. The presence of a hydroxyl group at C-4', and methoxy groups at C-3' and C-2, does not appear to affect activity, since the 4'-dehydroxy, 3'-demethoxy, and 2-demethoxy derivatives (**15g**, **15f**, and **15c**, respectively) displayed slightly lower activity than the parent compound. In conclusion, this study provided basic SAR on Lam-D: hydroxyl groups at the C-3 and C-11 positions of **15a** appear to be essential for cytotoxicity.

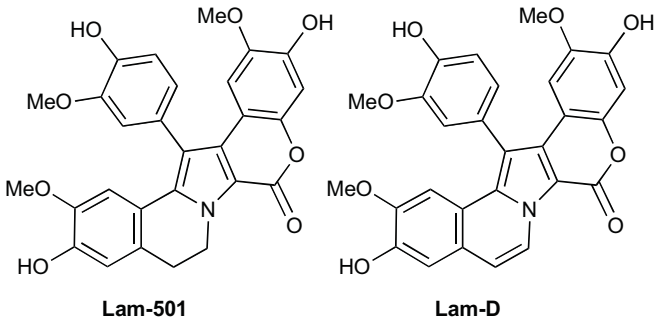


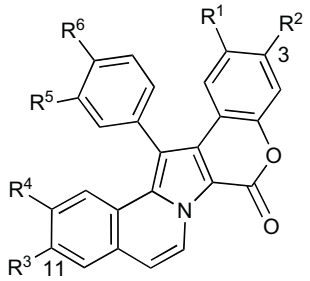
Fig. (14). The structures of lamellarin 501 and lamellarin D.

These results agree with those of a molecular dynamics study performed by Iwao *et al.* [37] to establish molecular interactions for the complex of Lam-D and the enzyme topoisomerase I. These researchers proposed that the guanidinium group of Arg^{364} maintains a close relationship with the lactone ring of the molecule. Moreover, they observed that direct hydrogen bonding interactions between the 3-OH oxygen and the Glu^{356} carboxylate oxygen, and between the 11-OH oxygen and the side chain amide nitrogen of Asn^{722}, were maintained throughout the entire simulation.

A library of open lactone analogs of Lam-D [34] was recently synthesized (Table **5**). The 45 members of the library all feature a methyl 8-hydroxy-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate scaffold, which differs from Lam-D primarily in that it lacks a lactone ring. The absence of the pyranone ring affords flexibility to the derivatives, and more importantly, greater solubility. Two series of compounds were prepared: derivatives of the 1-aryl-scaffold and of the 1,2-diaryl-scaffold. Members of both series feature either a single or double bond between C5 and C6 (which correspond to the C8 and C9 in lamellarins). The compounds from each series differ in their respective numbers and positions of the OH/OMe substituents on the aryl rings, and in the presence of functional groups such as NO_2, NMe_2, OCF_3 and heterocycles instead of aryl rings. All the compounds were tested for cytotoxicity against a panel of three human tumor cell lines: A-549 lung carcinoma, HT-29 colon carcinoma and MDA-MB-231 breast adenocarcinoma. The most active compounds are shown in Table **5**.

Structurally simplified analogs of Lam-D, in which the lactone ring was removed, and, in the case of derivatives **11** and **13**, an additional aryl group was removed, all had lower activity than Lam-D. These data reveal that the complete structure is crucial for biological activity, despite being less soluble in biological media than

Table 4. Cytotoxic Activity of Lamellarin Derivatives 15a-l on HeLa Cells [39]



Compound	R^1	R^2	R^3	R^4	R^5	R^6	IC_{50} (μM)
15a	OMe	OH	OH	OMe	OMe	OH	$10.5\ 10^{-3}$
15b	OH	OH	OH	OH	OH	OH	>100
15c	H	OH	OH	OMe	OMe	OH	$39.5\ 10^{-3}$
15d	OMe	H	OH	OMe	OMe	OH	0.85
15e	OMe	OH	OMe	OMe	OMe	OMe	2.5
15f	OMe	OH	OH	OMe	H	OH	$38.0\ 10^{-3}$
15g	OMe	OH	OH	OMe	OMe	H	$70.0\ 10^{-3}$
15h	H	H	OH	OMe	OMe	OH	4.0
15i	H	H	OH	OH	OH	OH	1.1
15j	OAc	OAc	OAc	OAc	OAc	OAc	11.0
15k	H	H	OMe	OMe	OMe	OMe	5.7
15l	-OCH_2O-		OMe	OMe	OMe	OMe	>100

simpler molecules. In a general overview, oxidized derivatives showed greater activity than the corresponding reduced analogs [34]. These data reveal that the complete structure is crucial for biological activity, despite being less soluble in biological media than simpler molecules. This fact can probably be attributed to the greater hydrogen bonding capacity of these analogs—namely, with active sites, as has been described for Lam-D [37]. The donor effect of the methoxy-substituents may explain why **12c** and **14b** were quite active despite not being able to act as hydrogen bond donors. Compounds **14g**, **14a**, **14h** and Lam-D have identical substituents on their scaffolds and at position 1 of their aryl rings. For these compounds, the greater the substitution of the aryl ring at position 2 of the scaffold, the lower the activity. The simplified analog **14a** maintained 63% of activity of Lam-D in HT29 cells, and **14g**, which has a hydroxy group at C4" (the same position as C-3 in Lam-D) was nearly as active. The open lactone compound **14h** may undergo lactonization in physiological conditions. Therefore, **14h** merits further study as a pharmacodynamic improvement on Lam-D, a validated lead compound.

Topoisomerases, the Initial Biomolecular Engines of Cell Growth

Topoisomerases, nuclear enzymes that can change the topology of DNA [40, 41], are amongst the most promising targets for inhibiting cellular proliferation. DNA topoisomerases are crucial in cellular replication; hence, they are especially attractive targets for cancer therapy. Interaction of a drug with a DNA topoisomerase can produce a stable, cytotoxic complex that inhibits post-cleavage DNA religation processes [42]. Indeed, this mode of action has been reported as a novel mechanism for many anticancer drugs [41].

Inhibition of topoisomerase I by Lam-D has been extensively studied in the past few years. Cancer cells are more susceptible to the DNA damage incurred, and thus are more likely to die. Hence, drugs targeted at topoisomerase I are selective for malignant cells. Like integrases, topoisomerases also cleave and join DNA, but *via* different pathways. The cytotoxicity of Lam-D is closely related to its inhibition of topoisomerase I. Interestingly, Vanhuyse *et al.* [43] reported that camptothecin-resistant P388CPT5 murine leukemia cells have a low relative index of resistance to Lam-D. Therefore, topoisomerase I is a privileged intracellular target for Lam-D.

P glycoprotein is the most common protein efflux pump in cells. Despite its recognition of CPT as a substrate and further mediated transport outside cell, investigations into the mechanism of action of Lam-D revealed that it is not sensitive to MDR-mediated drug efflux by P glycoprotein without active transporters to carry it out of the cell cytoplasm.

Although the pro-apoptotic effects of Lam-D could be understood as the final consequence of its stabilization of topoisomerase complexes, experiments [44] have revealed that it has other cellular targets. It has also been suggested that Lam-D induces apoptosis of leukemia cells by disrupting the mitochondrial transmembrane potential (MTP).

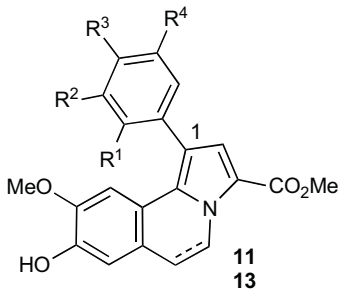
Using reliable real-time flow cytometry techniques and swelling of mitochondria isolated from leukemia cells, Bailly *et al.* showed that Lam-D directly induces MPT (mithocondrial permeability transition). Furthermore, they discovered that mitochondria are required to mediate Lam-D–induced nuclear apoptosis in a cell-free system [44].

In summary, Lam-D is rich in pharmacological potential which should be exploited for the development of treatments against chemoresistant cancer cells.

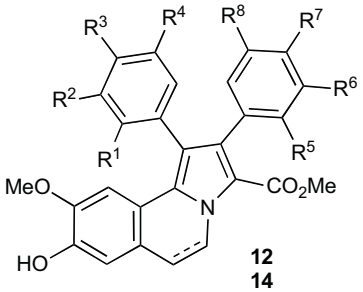
Docking of LAM-D with Topoisomerase I

Computational techniques have been used to elucidate the structural basis and the mode of interaction of the covalent complex formed by Lam-D, topoisomerase I and DNA [37, 38]. Staker *et al.*

Table 5. Cytotoxicity (GI_{50} μM) of Open-Lactone Lamellarin Analogs to Various Cancer Cell Lines [34]



11
13

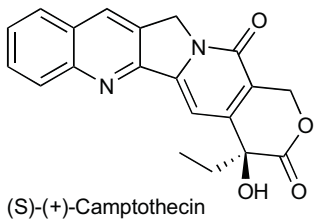


12
14

Cmpd.	Bd[a]	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	A-549[b]	HT-29[c]	MDA-MB-231[d]
Lam-D										0.20	5.1	0.25
11a	S	H	OMe	OH	H	--	--	--	--	14.2	18.0	22.3
11b	S	OMe	H	H	OMe	--	--	--	--	n.a.	n.a.	12.7
12a	S	H	OMe	OH	H	H	OMe	OH	H	14.3	n.a.	8.5
12b	S	H	OMe	OMe	H	H	OMe	OMe	H	11.2	n.a.	7.7
12c	S	H	H	OMe	H	H	H	OMe	H	9.2	10.3	14.4
12d	S	H	H	NMe_2	H	H	H	NMe_2	H	n.a.	n.a.	13.7
12e	S	H	NO_2	H	H	H	NO_2	H	H	18.0	11.3	10.1
12f	S	H	OMe	OH	H	OH	H	OH	OMe	5.0	17.1	3.1
12g	S	H	OMe	OH	H	OMe	H	OH	OMe	8.9	n.a.	7.6
12h	S	OMe	H	H	OMe	OH	H	OH	OH	13.7	8.4	10.5
12i	S	H	OH	H	H	OH	H	OH	H	n.a.	n.a.	19.0
12j	S	H	OH	H	H	OH	OMe	OMe	H	14.7	n.a.	15.7
13a	D	H	OMe	OH	H	--	--	--	--	10.9	23.9	11.2
13b	D	OMe	H	H	OMe	--	--	--	--	13.3	n.a.	19.9
13c	D	1-(2-thienyl)				--	--	--	--	n.a.	n.a.	26.3
14a	D	H	OMe	OH	H	H	OMe	OH	H	7.1	8.1	7.5
14b	D	H	H	OMe	H	H	H	OMe	H	n.a.	9.7	9.9
14c	D	H	H	OH	H	H	H	OH	H	3.5	9.8	4.1
14d	D	H	OH	H	H	H	OH	H	H	6.3	18.4	7.2
14e	D	H	NO_2	H	H	H	NO_2	H	H	n.a.	8.9	18.3
14f	D	1,2-*bis*(2-thienyl)								20.4	n.a.	19.7
14g	D	H	OMe	OH	H	H	H	OH	H	9.8	10.1.	15.0
14h	D	H	OMe	OH	H	OH	H	OH	OMe	0.45	7.9	0.71
14i	D	OMe	H	H	OMe	OH	H	OH	OH	4.7	7.1	3.2
14j	D	H	OH	H	H	OH	H	OH	H	20.8	n.a.	10.6

a: Bond C^5-C^6 S = single and D = double; b: human lung carcinoma cells (A-549); c: human colon carcinoma cells (HT-29); d: human breast adenocarcinoma cells (MDA-MB-231).

[45] published a 2.10 Å resolution crystal structure of human topoisomerase I covalently linked to double-stranded DNA (Protein Data Bank entry 1k4t) and the chemotherapy drug topotecan. It was used to model the Lam-D-mediated stabilization of topoisomerase I–DNA complex.



(S)-(+)-Camptothecin

Fig. (15). Structure of (+)-camptothecin.

Bailly *et al.* removed topotecan from the original structure to obtain a template on which to model the drug-free covalent complexes. The main difference between the two approaches [37, 38] remains in the exocyclic phenyl ring of Lam-D, which is rotated 180° relative to the conformation reported in a previous very similar proposal, such that the methoxy group at C13 is close to the 6-amino group of adenosine in the major groove.

Finally, to support the latter refined [37] model of the cleavable Top1-DNA complex stabilized by Lam-D, a quantitative estimation of the contribution to the free energy of binding of the crucial 20-

OH group was obtained through a set of precise, thermodynamic-integration free-energy simulations.

The inhibition of topoisomerase functionality alone probably does not result in cell death, but when the Lam-D stabilized ternary complex encounters a replication fork, the single DNA strand break is converted into a double DNA strand break which kills the cell.

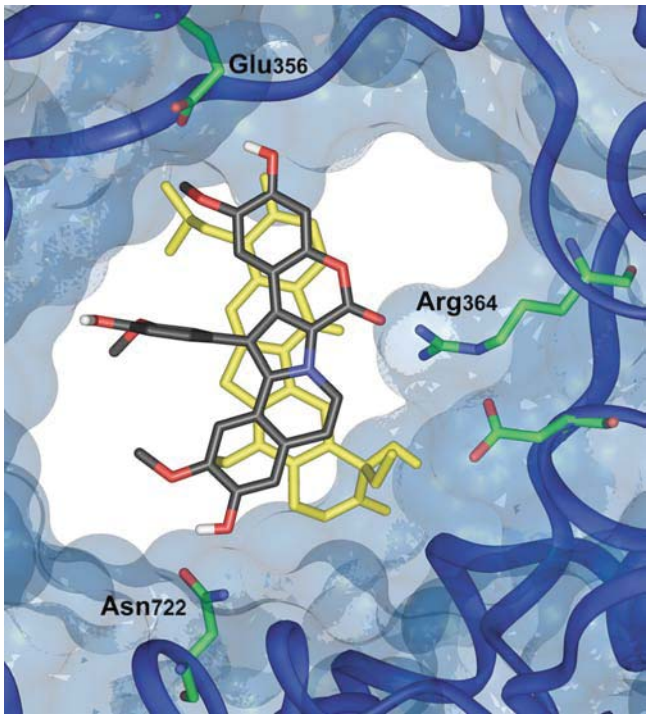


Fig. (16). Crystallographic model of topotecan–DNA–topoisomerase (Protein Data Bank entry 1k4t) featuring Lam-D superimposed in the active site [37].

Lam-D: Taking Control of Mitochondria

Mitochondria [46, 47] are subcellular organelles evolved from bacterial symbiosis and therefore contain their own genome. Cancer cells have unlimited replicative potential; are resistant to cell death stimuli; exhibit several mitochondrial disorders (*e.g.* dysfunction, and genetic instability with alterations such as mutations, deletions or translocations of the mitochondrial DNA [mtDNA]); and are highly glycolytic. The rapid and continuous growth of tumor cells is highly energy-dependent, and cancer cells often develop drug resistance, consequently becoming unaffected by pro-apoptotic signals. The dependency of cancer cells on glycolysis for ATP synthesis indicates that the mitochondrial engineering of the respiratory chain might be inefficient. The significance of mitochondria in mediating apoptosis has led to an interest in exploiting radio- and chemotherapeutic agents to trigger cancer cell death. To date, direct and specific targeting of mitochondria to obtain a persistent antitumor response has not been achieved, but there have been several encouraging cases in which some level of activity was reached. The vast majority of conventional anti-cancer drugs indirectly exploit mitochondria to exert cytotoxicity *via* multiple activation pathways that implicate p53 or death receptors.

mtDNA metabolism can also be targeted by topoisomerase inhibitors. Type I and type II topoisomerases have been identified in mitochondria, and have been shown to be inhibited there by known topoisomerase inhibitors.

Lam-D induces early disruption of the inner MPT through induction of pore opening [44]. This is considered as a predominant mechanism for mediating the release of pro-apoptotic molecules such as cytochrome c to the cytoplasm. Hence, agents that permeabilize cancer cell mitochondria may eliminate the resistance of these cells to apoptosis. Early studies have revealed that MPT pore opening precedes the proteolytic activation of caspase-3 in Lam-D mediated apoptosis. Furthermore, a greater gain in cell depolarization was observed in tumor cells (P388 leukemia, A549 lung cancer and MCF-7 breast cancer) rather than in non-tumor ones (NIH3T3 fibroblasts and H9C2 cardiomyocytes). The direct targeting of mitochondria by Lam-D is highly advantageous over classical anticancer drugs. Lam-D may be effective for treating cancers in which signal transducing systems are interrupted (*e.g.* those implying mutations of p53). Latest data suggest that mitochondrial reactive oxygen species generation is crucial for overriding the chemoresistance of non-small cell lung carcinoma cells [48].

Focus on HIV Integrase

Current HIV treatments comprise reverse transcriptase inhibition, which prevents single-stranded viral RNA genome form being translated into double stranded DNA, and protease inhibition, which blocks the production of mature infectious virions. Whereas drugs that target these two viral enzymes have been in use for more than ten years, inhibitors of the third HIV enzyme, integrase (IN), have yet to be developed. Integrase is a viral protein of 32 kDa responsible for the insertion of newly reverse-transcribed double-stranded viral DNA into the host genome [49]. An IN inhibitor could offer improvements in selectivity, despite the fact that the enzyme is only briefly active in the replication cycle of the virus. Integration of viral DNA into host cell chromosomal DNA to form a provirus is an essential step in the viral life cycle. IN is an ideal

Table 6. Inhibition of HIV-1 Integrase and of MCV Topoisomerase, and Cytotoxicity, of Several Lamellarin Sulfates [9, 50]

Compound	IC_{50} Integrase (μM)	IC_{50} MCV (μM)	LD_{50} Cytotoxicity (μM)
Lam-H [a]	1.3	0.23	5.7
Lam-N [b]	19	100	5
Lam-T [b]	24	100	27
Lam-U 20-sulfate [b]	25	500	145
Lam-V 20-sulfate [b]	51	500	130
Lam-W [b]	14	170	28
Lam-α [a]	> 1600	ND	5.1
Lam-α 20-sulfate [a, b]	22	≥ 170	274
Lam-α 13,20-disulfate [a]	49	70	29

Infection assays were performed using either (a) HeLa cells or (b) p4 – 2 cells.

target for drug design because it does not have any known cellular homologs in mammals, and therefore, the reactions that it catalyzes are unique. Moreover, IN is required for viral replication and mutations in key residues. During the past 15 years many IN inhibitors have been identified, some of which are highly selective against IN and block viral replication. IN inhibitors fall into two major classes: catechol-containing hydroxylated aromatics and diketoacid-containing aromatics.

The mechanisms by which small molecule inhibitors of recombinant HIV-1 IN act are unknown. Important structural motifs identified to date for HIV-integrase inhibitors are 1,2- and 1,4-diphenols, which can be oxidated to the corresponding quinones [50, 51].

Ridley *et al.* [51] reported that the sulfate group is critical for the anti-HIV-1 integrase activity of Lam-α 20-sulfate, because Lam-α showed no inhibition of HIV-1 integrase at concentrations up to 1.6 μM. HIV-1 integrase has been demonstrated to be a DNA manipulating enzyme and is a rarely exploited target. The low cytotoxicity of the sulfate compounds is interesting in the context of antiviral agents. Indeed, during a screening program aimed at identifying inhibitors of HIV-1 integrase. Reddy *et al.* [16] discovered that Lam-α 20-sulfate [25] strongly inhibited both terminal cleavage of integrase and strand transfer *in vitro*. However, they reported that the disulfate analog Lam-α 13,20-disulfate is less selective than Lam-α 20-sulfate, as they observed it to inhibit molluscum contagiosum virus (MCV) topoisomerase at roughly the same concentration as that used in the HIV-1 integrase assay.

Lam-H—which has six OH groups, all *ortho* to each other—exhibited very potent inhibition of HIV-1 integrase (IC_{50}, 1.3 μM), but unfortunately, was even more active in the non-selective MCV topoisomerase counterscreen (IC_{50}, 0.23 μM). It was very cytotoxic toward HeLa cells (LD_{50}, 5.7 μM).

This review clearly illustrates the importance of natural products in drug discovery as well as in the development of new synthetic methods. Since first being isolated from natural sources, lamellarins have been extensively screened against numerous therapeutic targets and have inspired novel synthetic strategies for natural targets and related analogs.

ACKNOWLEDGEMENTS

The work carried out in the laboratory of the authors was partially supported by CICYT (CTQ2006-03794/BQU), Instituto de Salud Carlos III (CB06-01-0074), the *Generalitat de Catalunya* (2005SGR 00662), the Institute for Research in Biomedicine, and the Barcelona Science Park.

ABBREVIATIONS

A549	=	Human lung cancer cells
CEM	=	Human leukemic lymphoid cells
CEM/C2	=	Human leukemia cells resistant to camptothecin
COLO205	=	Colon cancer cells
CPT	=	Campthotecin
DCE	=	Dichloroethane
DDQ	=	2,3-Dichloro-5,6-dicyanoquinone
DIEA	=	Diisopropylethylamine
DMF	=	Dimethylformamide
DMSO	=	Dimethylsulfoxide
DNA	=	Deoxyribonucleic acid
DOPA	=	3,4-Dihydoxyphenylalanine
DTBMP	=	2,6-Di-*tert*-butyl-4-methylpyridine
DU145	=	Human prostate carcinoma cells
GI	=	Growing inhibition
H9C2	=	Fetal rat heart cells
HeLa	=	Cervical cancer cells
HIV	=	Human immunodeficiency virus
HT	=	29 Human colon cancer cells
IC	=	Inhibitory concentration
IN	=	Integrase
Lam	=	Lamellarin
LD	=	Letal dose
MCV	=	Molluscum contagiosum virus
MCF-7	=	Human breast adenocarcinoma cells
MDA-MB-231	=	Human breast adenocarcinoma cancer cells
MDCK	=	Madin-Darby canine kidney cells
MDR	=	Multidrug resistant or resistance
MPT	=	Mithocondrial Permeability Transition
MR	=	Merrifield resin
MTP	=	Mitochondria transmembrane potential
NIH3T3	=	Mouse embryonic fibroblast cells
NBS	=	*N*-Bromosuccinimide
PVPHP	=	Polymer bound pyridine hydrobromide perbromide
SAR	=	Structure activity relationship
SPS	=	Solid-phase synthesis
Tf	=	Triflate
TFA	=	Trifluoroacetic acid
THF	=	Tetrahydrofurane
TIPS	=	Triisopropylsilyl
Top-1	=	Topoisomerase 1
Ts	=	Tosyl
XC	=	Sarcoma virus transformed rat cell line
WR	=	Wang resin

REFERENCES

[1] Urban, S.; Butlet, M. S.; Capon, R. J. *Aust. J. Chem.*, **1994**, *47*, 1919-1924.
[2] Urban, S.; Hobbs, L.; Hooper, J. N. A.; Capon, R. J. *Aust. J. Chem.*, **1995**, *48*, 1491-1494.
[3] Carroll, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.*, **1993**, *46*, 489-501.
[4] Yoshida, W. Y.; Lee, K. K.; Carroll, A. R.; Scheuer, P. J. *Helv. Chim. Acta*, **1992**, *75*, 1721-1725.
[5] Kang, H.; Fenical, W. *J. Org. Chem.*, **1997**, *62*, 3254-3262.
[6] Rudi, A.; Goldberg, D. I.; Frolow, Z. S. F.; Benayahu, Y.; Schleyer, M.; Kashman, Y. *J. Org. Chem.,* **1994**, *59*, 999-1003.
[7] Chan, G. W.; Francis, T.; Thureen, D. R.; Offen, P. H.; Pierce, N. J.; Westley, J. W.; Johnson, R. K. *J. Org. Chem.,* **1993**, *58*, 2544-2546.
[8] Recent revisions about lamellarin alkaloids: a) Bailly, C. *Curr. Med. Chem.: Anti-Cancer Agents,* **2004**, *4*, 363-378. b) Cironi, P.; Albericio, F.; Álvarez, M. *Progress in Heterocyclic Chemistry*, Gribble, G. W., Joule, J. A., Eds.; Pergamon: Oxford, U.K., **2004**; Vol. *16*, pp. 1-26; c) Handy, S. T.; Zhang, Y. *Org. Prep. Proc. Int.*, **2005**, *37*, 411-445; d) Fan, H.; Peng, J.; Hamann, M. T.; Hu, J.-F. *Chem. Rev.*, **2008**, *108*, 264-287.
[9] Andersen, R. J.; Faulkner, D. J.; Cun-heng, H.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc*., **1985**, *107*, 5492-5495.
[10] Davis, R. A.; Carroll, A. R.; Pierens, G. K.; Quinn, R. J. *J. Nat. Prod.,* **1999**, *62*, 419-424.
[11] Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.,* **1988**, *19*, 4570-4574.
[12] Reddy, M. V. R.; Faulkner, D. J.; Venkateswarlu, Y.; Rao, M. R. *Tetrahedron*, **1997**, *53*, 3457-3466.
[13] Ham, J.; Kang, H. *Bull. Korean Chem. Soc*., **2002**, *23*, 163-166.

[14] Krishnaiah, P. V.; Reddy, L. N.; Venkataramana, G.; Ravinder, K.; Srinivasulu, M.; Raju, T. V.; Ravikumar, K.; Chandrasekar, D.; Ramakrishna, S.; Venkateswarlu, Y. *J. Nat. Prod.,* **2004**, *67*, 1168-1171.

[15] Reddy, S. M.; Srinivasulu, M.; Satyanarayana, N.; Kondapi, A. K.;Venkateswarlu, Y. *Tetrahedron,* **2005**, *61*, 9242-9247.

[16] Reddy, R. M. V.; Rao, M. R.; Rhodes, D.; Hansen, M. S. T.; Rubins, K.; Bushman, F. D.; Venkateswarlu, Y.; Faulkner, D. V. *J. Med. Chem.*, **1999**, *42*, 1901-1907.

[17] Ploypradith, P.; Mahidol, C.; Sahakitpichan, P.; Wongbundit, S.; Ruchirawat, S. *Angew. Chem. Int. Ed.*, **2004**, *43*, 866-868.

[18] Ploypradith, P.; Petchmanee, T.; Sahakitpichan, P.; Litvinas, N. D.; Ruchirawat, S. *J. Org. Chem.*, **2006**, *71*, 9440-9448.

[19] Ploypradith, P.; Kagan, R. K.; Ruchirawat, S. *J. Org. Chem.*, **2005**, *70*, 5119-5125.

[20] Cironi, P.; Cuevas, C.; Albericio, F.; Álvarez, M. *Tetrahedron*, **2004**, *60*, 8669-8675.

[21] Cironi, P.; Manzanares, I.; Albericio, F.; Álvarez, M. *Org. Lett.*, **2003**, *5*, 2959-2962.

[22] Peschko, C.; Winklhofer, C.; Terpin, A.; Steglich, W. *Synthesis*, **2006**, 3048-3057.

[23] Mathew, P.; Asokan, C. V. *Tetrahedron Lett.*, **2005**, *46*, 475-478.

[24] Fürstner, A.; Weintritt, H.; Hupperts, A. *J. Org. Chem.,* **1995**, *60*, 6637-7741.

[25] Non systematic IUPAC numeration.

[26] Yamaguchi, T.; Fukuda, T.; Ishibashi, F.; Iwao, M. *Tetrahedron Lett.*, **2006**, *47*, 3755-3757.

[27] Fujikawa, N.; Ohta, T.; Yamaguchi, T.; Fukuda, T.; Ishibashi, F.; Iwao, M. *Tetrahedron*, **2006**, *62*, 594-604.

[28] Nyerges, M.; Töke, L. *Tetrahedron Lett.,* **2005**, *46*, 7531-7534.

[29] Su, S.; Porco, J. A. Jr. *J . Am. Chem. Soc.*, **2007**, *129*, 7744-7745.

[30] Marfil, M.; Albericio, F.; Álvarez, M. *Tetrahedron,* **2004**, *60*, 8659-8668.

[31] Handy, S. T.; Zhang, Y.; Bregman, H. *J. Org. Chem.*, **2004**, *69*, 2362-2366.

[32] Olsen, C.; Parera, N.; Albericio, F.; Álvarez, M. *Tetrahedron Lett.*, **2005**, *46*, 2041-2044.

[33] Pla, D.; Marchal, A.; Olsen, C.; Albericio, F.; Álvarez, M. *J. Org. Chem.*, **2005**, *70*, 8231-8234.

[34] Pla, D.; Marchal, A.; Olsen, C. A.; Francesch, A.; Cuevas, C.; Albericio, F.; Álvarez, M. *J. Med. Chem*., **2006**, *49*, 3257-3268.

[35] Banwell, M. G.; Hamel, E.; Hockless, D. C. R.; Verdier-Pinard, P.; Willis, A. C.; Wong, D. J. *Bioorg. Med. Chem.*, **2006**, *14*, 4627-4638.

[36] Smith, J. A.; Ng, S.; White, J. *Org. Biomol. Chem.*, **2006**, *4*, 2477-2482.

[37] Marco, E.; Laine, W.; Tardy, C.; Lansiaux, A.; Iwao, M.; Ishibashi, F.; Bailly, C.; Gago, F. *J. Med. Chem.*, **2005**, *48*, 3796-3807.

[38] Facompré, M.; Tardy, C.; Bal-Mayeu, C.; Colson, P.; Pérez, C.; Manzanares, I.; Cuevas, C.; Bailly, C. *Cancer Res*., **2003**, *63,* 7392-7399.

[39] Ishibashi, F.; Tanabe, S.; Oda, T.; Iwao, M. *J. Nat. Prod.*, **2002**, *65*, 500-504.

[40] a) Cortés, F.; Pastor, N.; Mateos, S.; Domínguez, I. *Expert Opin. Ther. Patents,* **2007**, *17*, 1-12. b) Corbett, K. D.; Berger, J. M. *Annu. Rev. Biophys. Biomol. Struct.,* **2004**, *33*, 95-118.

[41] Holden; J. A. *Curr. Med. Chem. – Anti-Cancer Agents*, **2001**, 1, 1-25.

[42] Dias, N.; Vezin, H.; Lansiaux, A.; Bailly, C. *Top*. *Curr*. *Chem*., **2005**, *253*, 89-108.

[43] Vanhuyse, M.; Kluza, J.; Tardy, C.; Otero, G.; Cuevas, C.; Bailly, C.; Lansiaux, A. *Cancer Lett.*, **2005**, *221*, 165-175.

[44] Kluza, J.; Gallego, M. A.; Loyens, A.; Beauvillain, J. C.; Fernández Sousa-Faro, J. M.; Cuevas, C.; Marchetti, P.; Bailly, C. *Cancer Res.*, **2006**, *66*, 3177-3187.

[45] Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B.; Stewart, L. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 15387-15392.

[46] Dykens, J. A.; Marroquin, L. D.; Will, Y. *Expert Rev. Mol. Diagn.*, **2007**, *7*, 161-175.

[47] Dias, N.; Bailly, C. *Biochem. Pharm.*, **2005**, *70*, 1-12.

[48] Gallego, M. A.; Ballot, C.; Kluza, J.; Hajji, N.; Martoriati, A.; Castera, L.; Cuevas, C.; Formstecher, P.; Joseph, B.; Kroemer, G.; Bailly, C.; Marchetti, P. *Oncogene*, **2007**, 1-12.

[49] Meadows, D. C.; Gervay-Hague, J. *Chem. Med. Chem.*, **2006**, *1*, 16-29.

[50] Lee, J. Y.; Yoon, K. J.; Lee, Y. S. *Bioorg. Med. Chem. Lett.,* **2003**, *13*, 4331-4334.

[51] Ridley, C. P.; Reddy, M. V. R.; Rocha, G.; Bushman, F. D.; Faulkner, D. J. *Bioorg*. *Med*. *Chem*., **2002**, *10*, 3285-3290.

Received: December 3, 2007 **Revised: April 11, 2008** **Accepted: May 5, 2008**

2 BROMACIÓ REGIOSELECTIVA DE FENOLS LLIURES I PROTEGITS

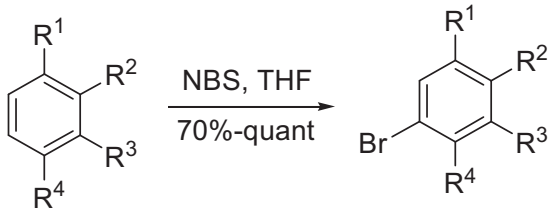
BROMACIÓ REGIOSELECTIVA DE FENOLS LLIURES I PROTEGITS.

Regioselective monobromination of free and protected phenols.

Daniel Pla,[a] Fernando Albericio,[a,b,*] and Mercedes Álvarez[a,c,*]

European Journal of Organic Chemistry, **2007**, 1921-1924

[a] Institute for Research in Biomedicine, Barcelona Science Park - University of Barcelona, Josep Samitier 1–5, 08028 Barcelona, Spain.
[b] Department of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain.
[c] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain



R^1	**R^2**	**R^3**	**R^4**
H	OH	OH	OH
H	OMe	OMe	OH
H	OMe	OMe	OH
OH	OH	H	OH
O*i*Pr	O*i*Pr	H	O*i*Pr
OMe	O*i*Pr	H	OMe
OMe	O*i*Pr	H	O*i*Pr
OMe	OBoc	H	O*i*Pr
OMe	OBoc	H	O*i*Pr

Resum

Es descriu un estudi comparatiu de les avantatges d'usar THF en comptes de DMF en reaccions de monobromació regioselectiva de polifenols altament activats, els seus èters, i els derivats de carbonat de *tert*-butil en condicions de reacció suaus. La bromació amb *N*-bromosuccinimida emprant dissolvents polars, proporciona una fàcil i ràpida aproximació als derivats de substitució en les posicions electrònicament més afavorides. Un control ajustat de la temperatura i temps de reacció curts va proporcionar els millors rendiments de producte bromat (de 70% a quantitatiu) quan es va emprar THF en comptes DMF.

DOI: 10.1002/ejoc.200600971

Regioselective Monobromination of Free and Protected Phenols

Daniel Pla,[a] Fernando Albericio,*[a,b] and Mercedes Álvarez*[a,c]

Keywords: *N*-Bromosuccinimide / Aromatic substitution / Phenols / Alkoxybenzenes / Bromobenzene / Regioselectivity

A comparative study of the advantages of using THF compared with DMF in regioselective monobromination reactions of highly activated polyphenols, their ethers, and their *tert*-butyl carbonate derivatives under mild conditions is described. Bromination with the common reagent *N*-bromosuccinimide in polar solvents provided an easy and fast approach to aromatic electrophilic substitution at the most electronically favored positions with respect to *O*-substituents. Tight control of the reaction temperature as well as short reaction times afforded better isolated yields (from 70 % to quantitative) of bromides when THF was used as the solvent instead of the classically used DMF.

Introduction

Chemical functionalization of substituted benzenes provides compounds capable of modulating the physico-chemical and/or biological activities of natural products. Saframycins,[1] renieramycins,[2] ningalins,[3] ecteinascidins,[2c,2d,4] and lamellarins are an important group of marine natural products[5] possessing as a common structural motif polyhydroxy- and polymethoxybenzenes. These privileged structures are characterized by their cytotoxic activity and potential utility for cancer chemotherapy. Many total syntheses are based on Pd^0-catalyzed cross-coupling reactions leading to C–C bond formation.[6] Sequential regioselective bromination and Suzuki cross-coupling reactions have been used in the total synthesis of lamellarin D and in the preparation of a library of lamellarin analogues.[7]

Bromo- and iodobenzenes have been extensively used in cross-coupling reactions as reagents or precursors of the organometallic required for each reaction. Thus, efficient methodologies for the preparation of OH/OMe-substituted halobenzenes are required. Bromination of trihydroxybenzenes and their ether derivatives has been achieved using diverse combinations of reagents and conditions, the most common of which is bromine in halogenated solvents (e.g., CCl_4 and $CHCl_3$[8]) or in AcOH.[9] Bromination reactions have also been carried out with bromine with catalysts such as CF_3CO_2Ag,[10] tetrabutylammonium bromide with V_2O_5/H_2O_2, supported reagent systems such as $NaClO_2$, NaBr, and $Mn(acac)_3$ with Montmorillonite K10 or moistened silica gel.[11] The harsh reaction conditions associated with most bromination methods, namely the use of bromine and the consequent formation of polyhalogenated byproducts, has led to the development of reagents such as pyridinium bromide perbromide[12] which can be used preformed or can be generated in situ by the addition of pyridine to bromine.[13] *N*-Bromosuccinimide (NBS) has previously been used for the bromination of the aforementioned class of trihydroxy and trialkoxy substrates, whether in CCl_4 or with the heterogeneous system CCl_4/SiO_2 as Lewis acid to promote nuclear bromination.[14] Aromatic electrophilic substitution of activated systems with NBS is generally favored in polar solvents such as DMF and CH_3CN.[15] Ionic liquids[16] such as 1-butyl-3-methylimidazolium hexafluorophosphate have recently been reported as the solvents of choice for these kinds of substrates. We report herein a new and mild procedure for the regioselective monobromination of benzenetriol, its methyl and isopropyl ethers, and its *tert*-butyl carbonate.

Results and Discussion

The bromination reactions of trioxybenzenes using freshly crystallized NBS in either DMF or THF were evaluated. We chose these two highly polar solvents as it is known that polar solvents drive the bromination reaction through the stabilization of bromocyclohexadienone-type intermediates.[17] DMF[18] as well as MeCN[15] have been described previously as good solvents for nuclear bromination reactions of aromatic systems with NBS. However, the bromination of a polyphenol with NBS in THF has only been reported once,[19] and has only rarely been used for the bromination of monosubstituted methoxy- or benzyloxyanilines.[20]

[a] Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona,
Josep Samitier 1–5, 08028 Barcelona, Spain
E-mail: malvarez@pcb.ub.es
[b] Department of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain
[c] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona,
08028 Barcelona, Spain
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

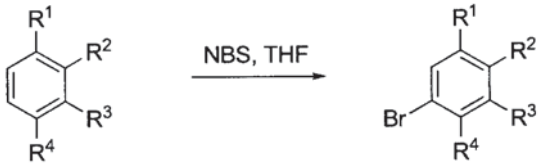
To the best of our knowledge, no comparative study on the role of solvent in polyphenols of benzenes has ever been carried out. The results of attempts to regioselectively brominate **1a**–**g** in THF and DMF are detailed in Table 1. Reaction progress was monitored by HPLC. Good-to-excellent yields of regioselectively monobrominated compounds were obtained. DMF and THF performed similarly in the bromination of the alkoxy-protected phenols, but THF gave higher yields with phenols (entries 1–4). Low-temperature reactions in THF probably allow more selectivity by avoiding polybromination and decomposition processes. Bromination of **1b** in DMF at room temperature (entry 3) gave a mixture of the starting material [retention time (t_r) = 3.6 min], **2b** (t_r = 6.2 min), a regioisomer (t_r = 6.6 min), and a dibromo derivative (t_r = 9.7 min). However, when this reaction was performed at –60 °C, the freezing point of DMF, **2b** was obtained in a yield of 28%. Although these reaction conditions were suitable for the monobromination of phenols with OH/OMe groups in positions 2 and 3 (entries 1–3), yields decreased dramatically with 1,2,4-trisubstituted phenols and methoxyphenols in both solvents. Bromination of 1,2,4-trihydroxybenzene (**1c**) in THF at –78 °C gave **2c** in 17% yield. The same reaction in DMF led to decomposition of the starting material (entry 4). In both cases (THF, DMF, entry 4) decomposition was observed during the purification process as a dark material adhered to the alumina pad. In contrast, excellent reaction yields were obtained for protected 1,2,4-trihydroxybenzenes. Bromination of **1d** in THF and in DMF afforded **2d** in 96 and 89% yields, respectively.

The regioselectivity of the process was directed through a combination of electronic and steric effects enhanced by temperature control. Thus, bromination of **1a** and **1b** occurred only at positions 4 and 6 (*ortho* with respect to R^4, double favored effect), respectively, with no reaction observed at the less hindered position 5 (*meta* with respect to R^4, only one favored effect). The regioselectivity was evaluated through a NOESY (nuclear Overhauser effect spectroscopy) experiment. Formation of 6-bromo-2,3-dimethoxyphenol (**2b**) instead of the 4-bromo derivative was demonstrated by the positive NOE between C3–OMe (singlet at δ = 3.83 ppm) and the proton at position 4 (doublet at δ = 6.40 ppm) of the benzene ring (see the Supporting Information). The *para* disposition of the aromatic protons of compounds **2d**–**g** was demonstrated by the presence of two singlets in their respective ^{1}H NMR spectra. Bromination of **1d**–**g** (entries 5–9) occurred only at position 5 (*ortho* with respect to R^4, double favored effect), with no reaction at position 3 (bis *ortho*). These results clearly indicate that electronic effects are more important than steric effects in determining the orientation of electrophilic substitution. For instance, bromination of **1f** occurred at position 5, *ortho* to the isopropyloxy group, which is more hindered than the methoxy group, but not at position 3, where steric hindrance was too great.

The reaction times of these reactions are related to the nature of the oxygenated substituents. Phenols (entries 1–4) required longer reaction times than methoxy or isopropyl ethers (entries 5–7). Boc-protected phenol **1g** required a higher temperature and longer reaction time (entry 9). Attempts to brominate **1g** at –78 °C were unsuccessful (entry 8), however, high yields and regioselectivity were obtained for this reaction in DMF and THF at 0 °C.

The preparation of **2g** is an example of a bromination of a diprotected 1,3-dihydroxy-4-methoxybenzene in which the two protecting groups can be selectively removed under controlled acidic conditions.[21]

Table 1. Bromination of phenols and phenoxy ethers.



1 2

Entry	Comp.	R^1	R^2	R^3	R^4	THF			DMF		
						t /min	*T* /°C	% yield[a]	*t* /min	*T* /°C	% yield[a]
1	**a**	H	OH	OH	OH	60	–78	92	45	0	39
2	**b**	H	OMe	OMe	OH	70	–78	70	45	–60	28
3	**b**	H	OMe	OMe	OH	45	25	67	45	25	36[b]
4	**c**	OH	OH	H	OH	60	–78	17	45	0	dec.[c]
5	**d**	O*i*Pr	O*i*Pr	H	O*i*Pr	15	–78	96	15	0	89
6	**e**	OMe	O*i*Pr	H	OMe	15	–78	95	15	0	95
7	**f**	OMe	O*i*Pr	H	O*i*Pr	10	–78	quant.[d]	15	0	96
8	**g**	OMe	OBoc	H	O*i*Pr	60	–78	n.r.[e]	–	–	–
9	**g**	OMe	OBoc	H	O*i*Pr	45	0	quant.[d]	45	0	quant.[d]

[a] Yields of isolated products unless specified. [b] Percentage of **2b** in the reaction crude, as measured by HPLC, which also contained 38% of a regioisomer and 5% of a dibromo-2,3-dimethoxyphenol. [c] A complex mixture of decomposition products was obtained. [d] quant. = quantitative yield. [e] n.r. = no reaction, the starting material **1g** was recovered.

In conclusion, an effective procedure for the regioselective monobromination of activated phenols, their ethers, and Boc derivatives has been developed. THF was found to be a better solvent than DMF for free phenol substrates, however, similar yields were obtained for both solvents in the reactions of methoxy and protected phenols.

Experimental Section

Reactants and solvents were purified according to literature procedures.[22] HPLC/MS spectra were recorded with a Waters Alliance LC/MS System consisting of a Waters ZQ mass detector, a photodiode array detector, and an Alliance HPLC system equipped with an XTerra C_{18} column (150×4.6 mm, 5 μm). ^{1}H and ^{13}C NMR spectra were recorded with a Varian Mercury 400 MHz and a Gemini 200 MHz spectrometer. The multiplicity of the carbon atoms was assigned through DEPT and gHSQC experiments and typical abbreviations for off-resonance decoupling are used: (s) singlet, (d) doublet, and (q) quartet. The same abbreviations were used for the multiplicity of signals in the ^{1}H NMR spectra, as well as (h) heptet and (br. s) broad singlet. Spectra were referenced to appropriate residual solvent peaks ([D$_6$]DMSO or $CDCl_3$). IR spectra were obtained on a Thermo Nicolet FT-IR spectrometer. HRMS: were recorded with a Bruker Autoflex high-resolution mass spectrometer.

General Procedure for the Bromination Process: Solid NBS (1 mmol) was added to a cooled solution of the aromatic compound (1 mmol) in THF or DMF (5 mL) and the reaction mixture was stirred at the indicated temperature until complete consumption of the starting material. The mixture was allowed to reach room temperature and the solvent evaporated under reduced pressure. The resulting residue was taken up in EtOAc, filtered through a pad of neutral alumina, and dried. Pure products were characterized as detailed below.

4-Bromo-1,2,3-trihydroxybenzene (2a):[7a] Starting from 1,2,3-trihydroxybenzene (3.86 g, 30.3 mmol), **2a** (5.69 g, 27.8 mmol, 92%) was obtained as a yellowish solid using THF as solvent. IR (film): $\tilde{\nu}$ = 3116, 2962, 1401, 1261, 1094, 1021, 800 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 6.26 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H), 6.72 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H), 6.83 (s, 1 H, OH), 7.09 (s, 1 H, OH), 7.34 (s, 1 H, OH) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 99.5 (s), 108.0 (d), 121.4 (d), 134.4 (s), 143.4 (s), 145.5 (s) ppm. MS (ESI-TOF): *m*/*z* (%) = 203 (100) [MBr^{79}], 205 (97) [MBr^{81}]. HRMS: calcd. for $C_6H_4O_3Br$: 202.9349; found 202.9346.

6-Bromo-2,3-dimethoxyphenol (2b):[23] Starting from 2,3-dimethoxyphenol (3.36 g, 21.8 mmol), **2b** (3.57 g, 15.3 mmol, 70%) was obtained as a yellowish oil using THF as solvent. IR (film): $\tilde{\nu}$ = 3440, 2941, 1466, 1429, 1204, 1168, 1089 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.83 (s, 3 H, OMe), 3.89 (s, 3 H, OMe), 6.13 (s, 1 H, OH), 6.40 (d, $^3J_{H,H}$ = 8.8 Hz, 1 H), 7.13 (d, $^3J_{H,H}$ = 8.8 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 56.0 (q, OMe), 61.1 (q, OMe), 100.2 (s), 105.1 (d), 126.7 (d), 136.3 (s), 146.7 (s), 151.9 (s) ppm. MS (ESI-TOF): *m*/*z* (%) = 255 (100) [MBr^{79}Na], 257 (98) [MBr^{81}]. HRMS: calcd. for $C_8H_9O_3NaBr$ 254.9627; found 254.9620.

1-Bromo-2,4,5-trihydroxybenzene (2c): Starting from 1,2,4-trihydroxybenzene (300 mg, 2.4 mmol), **2c** (83 mg, 0.4 mmol, 17%) was obtained as a yellowish oil using THF as solvent. IR (film): $\tilde{\nu}$ = 3334, 1457, 1289, 1133, 837 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 5.03 (br. s, OH), 5.36 (br. s, OH), 5.48 (br. s, OH), 6.97 (s, 1 H), 7.16 (s, 1 H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 98.3 (d), 104.8 (d), 115.3 (s), 141.8 (s), 144.4 (s), 149.4 (s) ppm. MS (ESI-TOF): *m*/*z* (%) = 203 (100) [MBr^{79}], 205 (97) [MBr^{81}].

1-Bromo-2,4,5-tris(isopropyloxy)benzene (2d): Starting from 1,2,4-tris(isopropyloxy)benzene (4.01 g, 15.9 mmol), **2d** (5.05 g, 15.3 mmol, 96%) was obtained as a yellowish oil using THF as solvent. IR (film): $\tilde{\nu}$ = 2976, 2932, 1489, 1384, 1200, 1109 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.30 (d, *J* = 6.4 Hz, 6 H, Me), 1.31 (d, *J* = 6.0 Hz, 6 H, Me), 1.34 (d, *J* = 6.0 Hz, 6 H, Me), 4.34 (h, *J* = 6.4 Hz, 1 H), 4.39 (h, *J* = 6.0 Hz, 1 H), 4.44 (h, *J* = 6.0 Hz, 1 H), 6.60 (s, 1 H), 7.02 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.1 (q), 22.2 (q), 72.6 (d), 73.3 (d), 73.4 (d), 105.2 (s), 109.1 (d), 123.2 (d), 144.3 (s), 149.0 (s), 149.4 (s) ppm. MS (ESI-TOF): *m*/*z* (%) = 353 (98) [MBr^{79}], 354 (23) [MBr^{79} + 1], 355 (100) [MBr^{81}], 356 (21) [MBr^{81} + 1]. HRMS: calcd. for $C_{15}H_{23}O_3NaBr$: 353.0728; found 353.0723.

1-Bromo-2,5-dimethoxy-4-isopropyloxybenzene (2e):[14e,24] Starting from 1,4-dimethoxy-2-isopropyloxybenzene (1.22 g, 6.21 mmol), **2e** (1.63 g, 5.92 mmol, 95%) was obtained as a yellowish oil using THF as solvent. IR (film): $\tilde{\nu}$ = 2975, 2934, 1501, 1382, 1201 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.33 (d, $^3J_{H,H}$ = 6.0 Hz, 6 H, Me), 3.78 (s, 3 H, OMe), 3.81 (s, 3 H, OMe), 4.48 (h, $^3J_{H,H}$ = 6.0 Hz, 1 H), 6.57 (s, 1 H), 7.03 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.0 (q, Me), 56.7 (q, OMe), 57.1 (q, OMe), 72.4 (d), 102.1 (s), 103.7 (d), 117.3 (d), 145.5 (s), 147.2 (s), 150.2 (s) ppm. MS (ESI-TOF): *m*/*z* (%) = 274 (95) [MBr^{79}], 275 (19) [MBr^{79} + 1], 276 (79) [MBr^{81}], 305 (5) [MBr^{81} + 1]. HRMS: calcd. for $C_{11}H_{16}O_3Br$: 274.0205; found 274.0277.

1-Bromo-2,4-bis(isopropyloxy)-5-methoxybenzene (2f): Starting from 1,3-bis(isopropyloxy)-4-methoxybenzene (1.56 g, 6.96 mmol), **2f** (2.11 g, 6.95 mmol, quant) was obtained as a yellowish oil using THF as solvent. IR (film): $\tilde{\nu}$ = 2977, 2934, 1496, 1386, 1211, 825 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.34 (d, $^3J_{H,H}$ = 6.4 Hz, 6 H, Me), 1.35 (d, $^3J_{H,H}$ = 6.0 Hz, 6 H, Me), 3.80 (s, 3 H, OMe), 4.44 (h, $^3J_{H,H}$ = 6.0 Hz, 1 H), 4.46 (h, $^3J_{H,H}$ = 6.4 Hz, 1 H), 6.60 (s, 1 H), 7.02 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.2 (q), 22.4 (q), 56.8 (q), 72.4 (d), 74.2 (d), 105.4 (s), 108.7 (d), 117.0 (d), 144.4 (s), 147.2 (s), 148.8 (s) ppm. MS (ESI-TOF): *m*/*z* (%) = 302 (92) [MBr^{79}], 303 (57) [MBr^{79} + 1], 304 (83) [MBr^{81}], 305 (47) [MBr^{81} + 1]. HRMS: calcd. for $C_{13}H_{20}O_3Br$: 303.0596; found 303.0590.

4-Bromo-5-isopropyloxy-2-methoxyphenyl *tert*-Butyl Carbonate (2g): Starting from 5-isopropyloxy-2-methoxyphenyl *tert*-butyl carbonate (0.312 g, 1.11 mmol), **2g** (0.400 g, 1.11 mmol, quant) was obtained as a yellowish oil using THF as solvent. IR (film): $\tilde{\nu}$ = 2978, 2935, 1764, 1502, 1139 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.32 (d, $^3J_{H,H}$ = 6.0 Hz, 6 H, Me), 1.52 (s, 9 H, Me), 3.78 (s, 3 H, OMe), 4.37 (h, $^3J_{H,H}$ = 6.0 Hz, 1 H), 6.76 (s, 1 H), 7.12 (s, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.1 (q), 27.6 (q), 56.6 (q), 73.5 (d), 83.6 (s), 110.5 (s), 111.9 (d), 116.6 (s), 117.2 (d), 139.5 (s), 145.9 (s), 148.3 (s) ppm. MS (ESI-TOF): *m*/*z* (%) = 383 (100) [MBr^{79}], 384 (11) [MBr^{79} + 1], 385 (97) [MBr^{81}], 386 (9) [MBr^{81} + 1]. HRMS: calcd. for $C_8H_9O_3NaBr$: 383.0470; found 383.0465.

Supporting Information (see also the footnote on the first page of this article): NMR spectra of the products.

Acknowledgments

This work was partially supported by the Comisión Interministerial de Ciencia y Tecnología (CICYT) (BQU 2003-00089), the Generalitat de Catalunya, and the Barcelona Science Park. D. P. thanks

the Fundación Mutua Madrileña (FMM) for a predoctoral fellowship.

[1] For reviews of saframycins, see: a) T. Arai, A. Kubo in *The Alkaloids* (Ed.: A. Brossi), Academic Press, New York, **1983**, vol. 21, chap. 3; b) W. A. Remers, *The Chemistry of Antitumor Antibiotics*, Wiley-Interscience, New York, **1988**, vol. 2, chapter 3; c) H. Irschik, W. Trowitsch-Kienast, K. Gerth, G. Höfle, H. Reichenbach, *J. Antibiot.* **1988**, *41*, 993–998; d) W. Trowitzsch-Kienast, H. Irschik, H. Reichenbach, V. Wray, G. Höfle, *Liebigs Ann. Chem.* **1988**, 475–481.

[2] a) K. L. Rinehart, T. G. Holt, N. L. Fregeau, P. A. Keifer, G. R. Wilson, T. J. Perun, R. Sakai, A. G. Thompson, J. G. Stroh, L. S. Shield, D. S. Seigler, *J. Nat. Prod.* **1990**, *53*, 771–792; b) K. L. Rinehart, T. G. Holt, N. L. Fregeau, J. G. Stroh, P. A. Keifer, F. Sun, L. H. Li, D. G. Martin, *J. Org. Chem.* **1990**, *55*, 4512–4515; c) A. E. Wright, D. A. Forleo, G. P. Gunawardana, S. P. Gunasekera, F. E. Koehn, O. J. McConnell, *J. Org. Chem.* **1990**, *55*, 4508–4512; d) Y. Guan, R. Sakai, K. L. Rinehart, A. H.-J. Wang, *J. Biomol. Struct. Dyn.* **1993**, *11*, 793–818.

[3] H. Kang, W. Fenical, *J. Org. Chem.* **1997**, *62*, 3254–3262.

[4] a) R. Sakai, K. L. Rinehart, Y. Guan, A. H. Wang, *J. Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 11456–11460; b) R. Sakai, E. A. Jares-Erijman, I. Manzanares, M. V. Silva Elipe, K. L. Rinehart, *J. Am. Chem. Soc.* **1996**, *118*, 9017–9023.

[5] For reviews of lamellarin alkaloids, see: a) P. Cironi, F. Albericio, M. Álvarez, *Prog. Heterocycl. Chem.* **2004**, *16*, 1–26; b) C. Bailly, *Curr. Med. Chem. Anti-Cancer Agents* **2004**, *4*, 363–378; c) S. T. Handy, Y. Zhang, *Org. Prep. Proced. Int.* **2005**, *37*, 411–445.

[6] a) J. Hassan, M. Sévignon, C. Gozzi, E. Schulz, M. Lemaire, *Chem. Rev.* **2002**, *102*, 1359–1469; b) K. C. Nicolaou, P. G. Bulger, D. Sarlah, *Angew. Chem. Int. Ed.* **2005**, *44*, 4442–4489; c) N. T. S. Phan, M. Van Der Sluys, C. W. Jones, *Adv. Synth. Catal.* **2006**, *348*, 609–679; d) I. Ozdemir, M. Yigit, E. Cetinkaya, B. Cetinkaya, *Appl. Organomet. Chem.* **2006**, *20*, 187–192.

[7] a) D. Pla, A. Marchal, C. A. Olsen, F. Albericio, M. Álvarez, *J. Org. Chem.* **2005**, *70*, 8231–8234; b) D. Pla, A. Marchal, C. A. Olsen, A. Francesch, C. Cuevas, F. Albericio, M. Álvarez, *J. Med. Chem.* **2006**, *49*, 3257–3268.

[8] a) A. Ballio, *Gazz. Chim. Ital.* **1951**, *81*, 782–785; b) G. K. Hughes, N. K. Matheson, A. T. Norman, E. Ritchie, *Aust. J. Sci. Res.* **1952**, *5*, 206–212; c) J. M. Bruce, F. K. Sutcliffe, *J. Chem. Soc.* **1955**, 4435–4439; d) Z. Getahun, L. Jurd, P. S. Chu, C. M. Lin, E. Hamel, *J. Med. Chem.* **1992**, *35*, 1058–1067; e) G. P. Crowther, *J. Org. Chem.* **1984**, *49*, 4657–4663; f) R. G. F. Giles, A. B. Hughes, M. V. Sargent, *J. Chem. Soc., Perkin Trans. 1* **1991**, 1581–1587; g) O. Arjona, M. Garranzo, J. Mahugo, E. Maroto, J. Plumet, B. Sáez, *Tetrahedron Lett.* **1997**, *38*, 7249–7252; h) T. Tseng, Y.-M. Tsheng, Y.-J. Lee, H.-L. Hsu, *J. Chin. Chem. Soc.* **2000**, *47*, 1165–1169.

[9] a) B. H. Alexander, T. A. Oda, R. T. Brown, S. I. Gertler, *J. Org. Chem.* **1958**, *23*, 1969–1970; b) R. G. F. Giles, C. A. Joll, M. V. Sargent, M. G. Tilbrook, *J. Chem. Soc., Perkin Trans. 1* **1999**, 3029–3038; c) V. Brizzi, M. Francioli, M. Brufani, L. Filocamo, G. Bruni, P. Massarelli, *Farmaco* **1999**, *54*, 713–720; d) S. Jinno, T. Okita, K. Inouye, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1029–1032; e) Z. Novák, G. Timári, A. Kotschy, *Tetrahedron* **2003**, *59*, 7509–7513.

[10] a) E. Brown, M. Loriot, J. P. Robin, *Tetrahedron Lett.* **1982**, *23*, 949–952; b) M. Loriot, J. P. Robin, E. Brown, *Tetrahedron* **1984**, *40*, 2529–2535; c) J. H. Rigby, U. S. M. Maharoof, M. E. Mateo, *J. Am. Chem. Soc.* **2000**, *122*, 6624–6628.

[11] a) M. Hirano, S. Yakabe, H. Monobe, T. Morimoto, *Synth. Commun.* **1998**, *28*, 669–676; b) M. Hirano, H. Monobe, S. Yakabe, T. Morimoto, *Synth. Commun.* **1998**, *28*, 1463–1470; c) U. Bora, G. Bose, M. K. Chaudhuri, S. S. Dhar, R. Gopinath, A. T. Khan, B. K. Patel, *Org. Lett.* **2000**, *2*, 247–249; d) M. Y. Park, S. G. Yang, V. Jadhav, Y. H. Kim, *Tetrahedron Lett.* **2004**, *45*, 4887–4889; e) H. Tajik, F. Shirini, P. Hassan-zadeh, H. R. Rashtabadi, *Synth. Commun.* **2005**, *35*, 1947–1952.

[12] a) E. C. Horning, J. A. Parker, *J. Am. Chem. Soc.* **1952**, *74*, 2107–2108; b) M. G. Banwell, J. M. Cameron, M. Corbett, J. R. Dupuche, E. Hamel, J. N. Lambert, C. M. Lin, M. F. Mackay, *Aust. J. Chem.* **1992**, *45*, 1967–1982.

[13] Y. Zhao, Y.-L. Ku, X. J. Hao, S. S. Lee, *Tetrahedron* **2000**, *56*, 8901–8913.

[14] a) D. Friedman, D. Ginsburg, *J. Org. Chem.* **1958**, *23*, 16–17; b) G. R. Pettit, M. P. Grealish, D. L. Herald, M. R. Boyd, E. Hamel, R. K. Pettit, *J. Med. Chem.* **2000**, *43*, 2731–2737; c) A. F. Barrero, E. J. Álvarez-Manzaneda, R. Chahboun, M. Cortés, V. Armstrong, *Tetrahedron* **1999**, *55*, 15181–15208; d) A. F. Barrero, E. J. Alvarez-Manzaneda, R. Chahboun, *Tetrahedron Lett.* **1997**, *38*, 2325–2328; e) T. Ishikawa, K. Shimooka, T. Narioka, S. Noguchi, T. Saito, A. Ishikawa, E. Yamazaki, T. Harayama, H. Seki, K. Yamaguchi, *J. Org. Chem.* **2000**, *65*, 9143–9151.

[15] a) T. Oberhauser, *J. Org. Chem.* **1997**, *62*, 4504–4506; b) S. Maiti, S. Sengupta, C. Giri, B. Achari, A. K. Banerjee, *Tetrahedron Lett.* **2001**, *42*, 2389–2391; c) M. C. Carreño, J. L. Garcia Ruano, G. Sanz, M. A. Toledo, A. Urbano, *J. Org. Chem.* **1995**, *60*, 5328–5331.

[16] a) R. Rajagopal, D. V. Jarikote, R. J. Lahoti, T. Daniel, K. V. Srinivasan, *Tetrahedron Lett.* **2003**, *44*, 1815–1817; b) J. S. Yadav, B. V. S. Reddy, P. S. R. Reddy, A. K. Basak, A. V. Narsaiah, *Adv. Synth. Catal.* **2004**, *346*, 77–82.

[17] Y. L. Chow, D. C. Zhao, C. I. Johansson, *Can. J. Chem.* **1988**, *66*, 2556–2564.

[18] a) N. Fujikawa, T. Ohta, T. Yamaguchi, T. Fukuda, F. Ishibashi, M. Iwao, *Tetrahedron* **2006**, *62*, 594–604; b) R. H. Mitchell, Y. H. Lai, R. V. Williams, *J. Org. Chem.* **1979**, *44*, 4733–4735; c) D. D. Weller, E. P. Stirchak, *J. Org. Chem.* **1983**, *48*, 4873–4879; d) P. J. Dijkstra, H. J. den Hertog, J. van Herden, S. Harkema, D. N. Reinhoudt, *J. Org. Chem.* **1988**, *53*, 374–382; e) J. M. Gnaim, P. M. Keehn, B. S. Green, *Tetrahedron Lett.* **1992**, *33*, 2883–2886.

[19] S. Jinno, N. Otsuka, T. Okita, K. Inouye, *Chem. Pharm. Bull.* **1999**, *47*, 1276–1283.

[20] a) L. F. Tietze, F. Haunert, T. Feuerstein, T. Herzig, *Eur. J. Org. Chem.* **2003**, 562–566; b) T. T. Howard, B. M. Lingerfelt, B. L. Purnell, A. E. Scott, C. A. Price, H. M. Townes, L. McNulty, H. L. Handl, K. Summerville, S. J. Hudson, J. P. Bowen, K. Kiakos, J. A. Hartley, M. Lee, *Bioorg. Med. Chem.* **2002**, *10*, 2941–2952; c) D. L. Boger, R. J. Wysocki, T. Ishizaki, *J. Am. Chem. Soc.* **1990**, *112*, 5230–5240.

[21] Elimination of the Boc-protecting group from 5-isopropyloxy-2-methoxyphenyl *tert*-butyl carbonate took place selectively and in excellent yield by refluxing with aq. 3 M HCl/dioxane (1:1) for 30 min, in accord with: M. M. Hansen, J. R. Riggs, *Tetrahedron Lett.* **1998**, *39*, 2705–2706.

[22] W. Armarego, C. Chai, *Purification of Laboratory Chemicals*, Elsevier, Amsterdam, **2003**.

[23] M. V. Sargent, *J. Chem. Soc., Perkin Trans. 1* **1987**, 2553–2563.

[24] Previously obtained in 93% yield from the same substrate by treatment with a mixture of NBS and SiO_2.

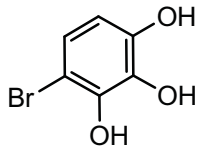
Received: November 6, 2006
Published Online: February 27, 2007

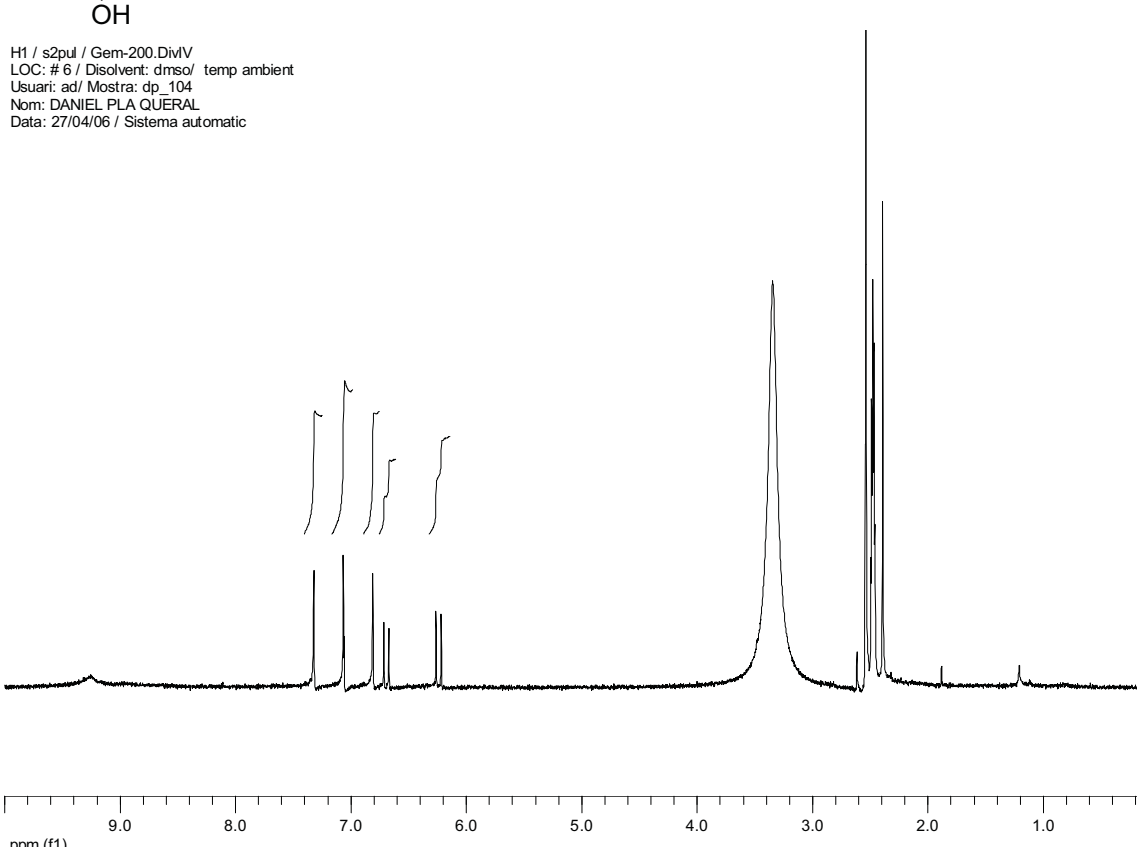
Eur. J. Org. Chem. **2007** · © WILEY-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, 2007 · ISSN 1434–193X

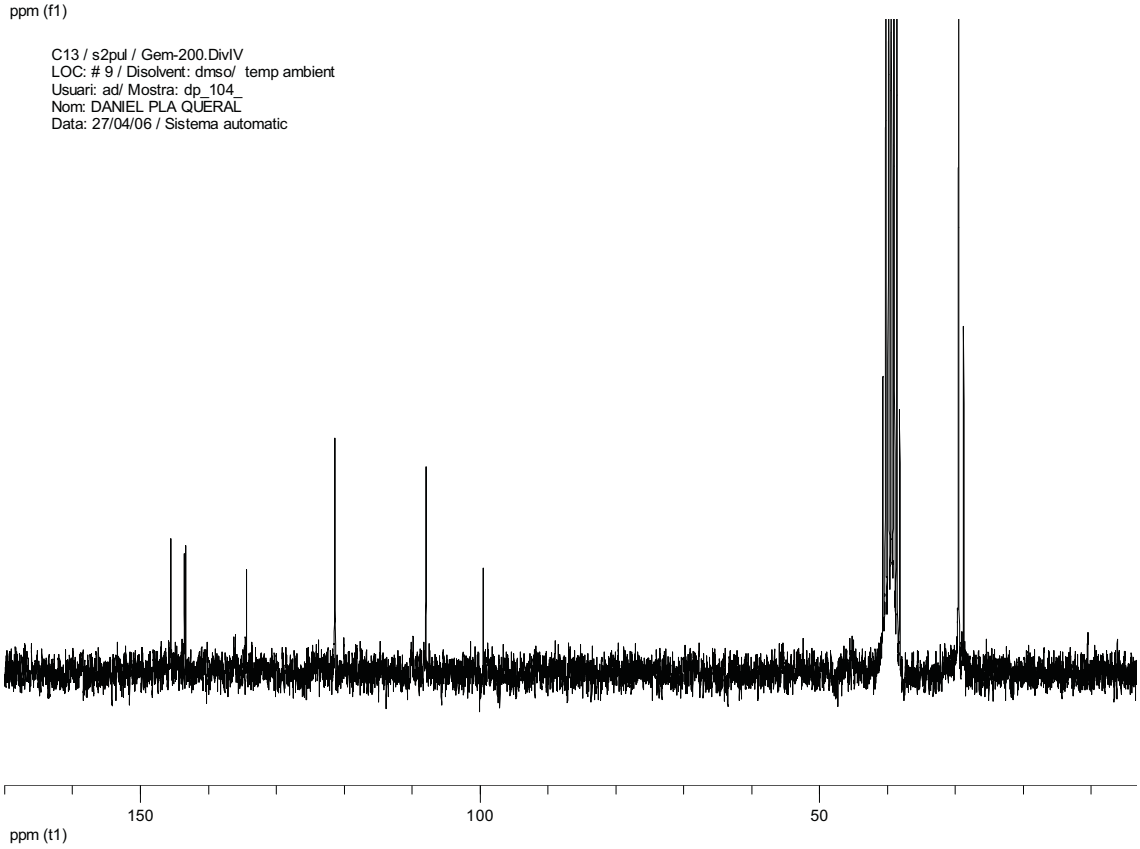
SUPPORTING INFORMATION

Title: Regioselective Monobromination of Free and Protected Phenols
Author(s): Daniel Pla, Fernando Albericio,* Mercedes Álvarez*
Ref. No.: O200600971

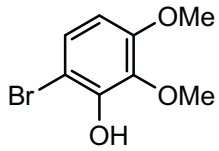
2a


OH
Br
OH
OH

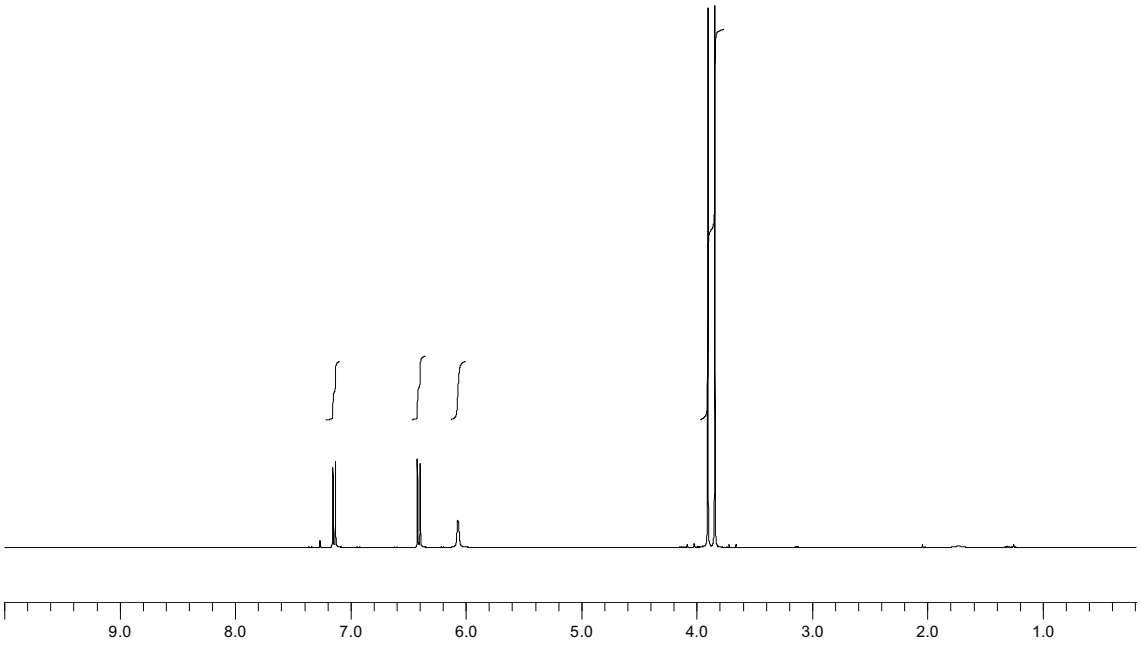

H1 / s2pul / Gem-200.DivIV
LOC: # 6 / Disolvent: dmso/ temp ambient
Usuari: ad/ Mostra: dp_104
Nom: DANIEL PLA QUERAL
Data: 27/04/06 / Sistema automatic
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)


C13 / s2pul / Gem-200.DivIV
LOC: # 9 / Disolvent: dmso/ temp ambient
Usuari: ad/ Mostra: dp_104_
Nom: DANIEL PLA QUERAL
Data: 27/04/06 / Sistema automatic
150
100
50
ppm (t1)

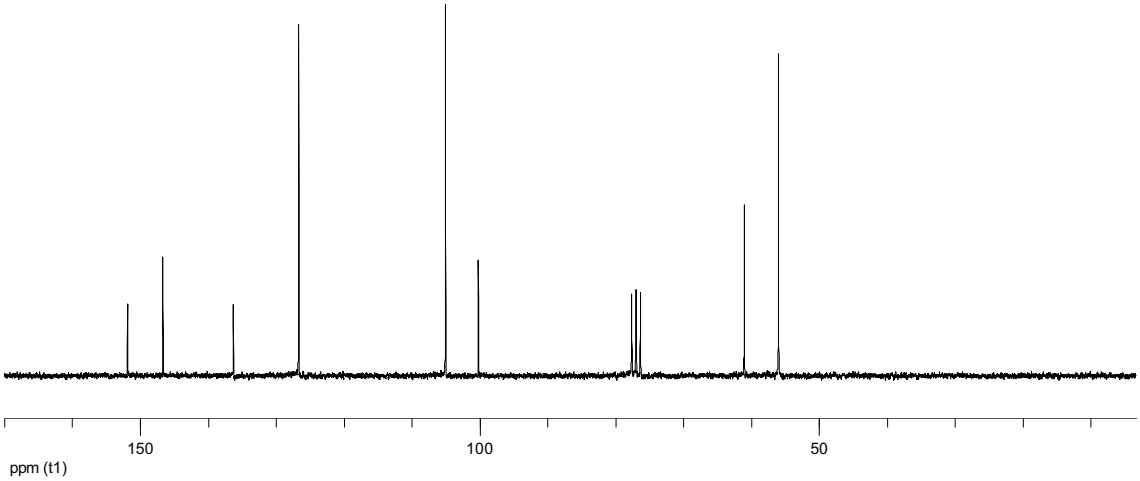
2b



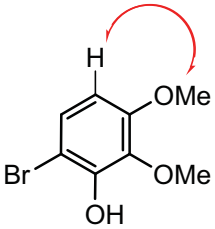
H1 / s2pul / Mercury-400_qui
cdcl3/Temp: 25C /N reg: M40006-4520
Usuari: ad / Mostra: dp90
Nom: DANIEL PLA QUERAL
Data:16/05/06 / Sist automatic

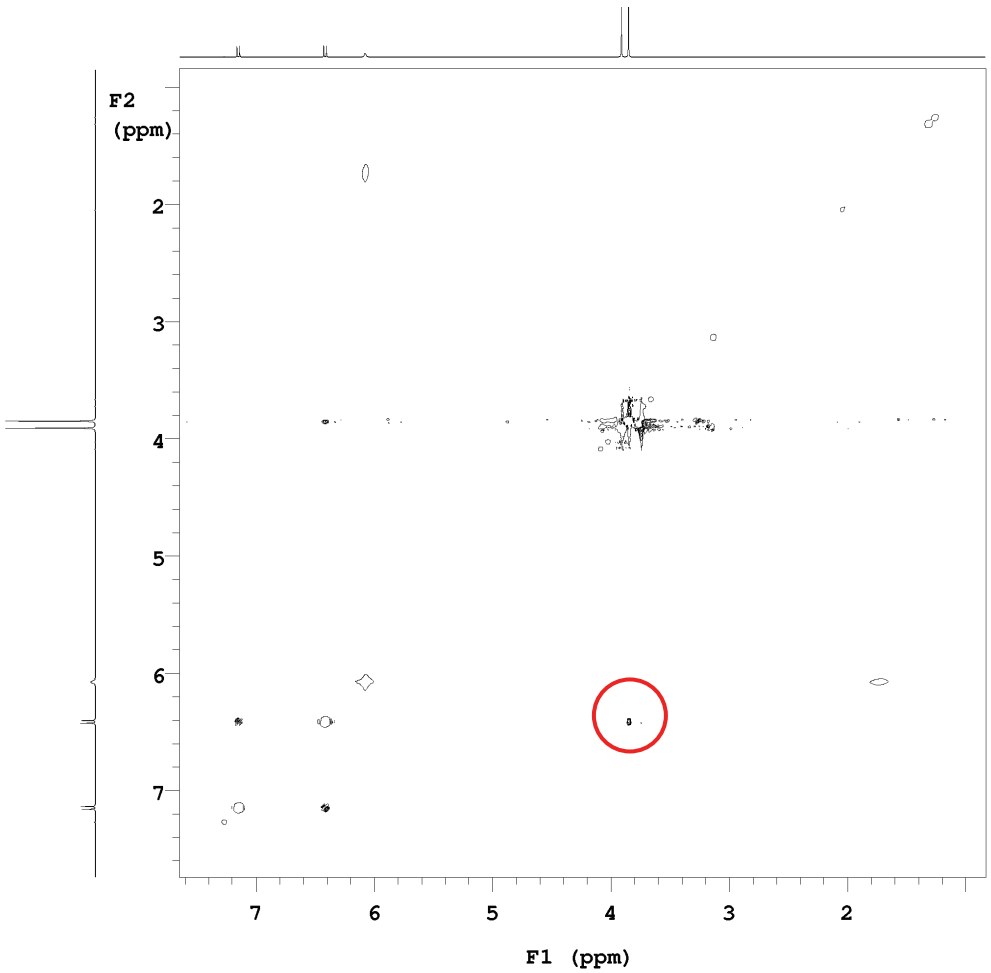


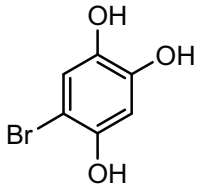
C13 / s2pul / Gem-200.DivIV
LOC: # 8 / Disolvent: cdcl3/ temp ambient
Usuari: ad/ Mostra: dp90
Nom: DANIEL PLA QUERAL
Data: 27/04/06 / Sistema automatic



^{1}H-^{1}H NOESY Spectrum
Positive NOE between the C^{3}-OMe (singlet at 3.83 ppm) and the proton on position 4 (doublet at 6.40 ppm) of the benzene ring.

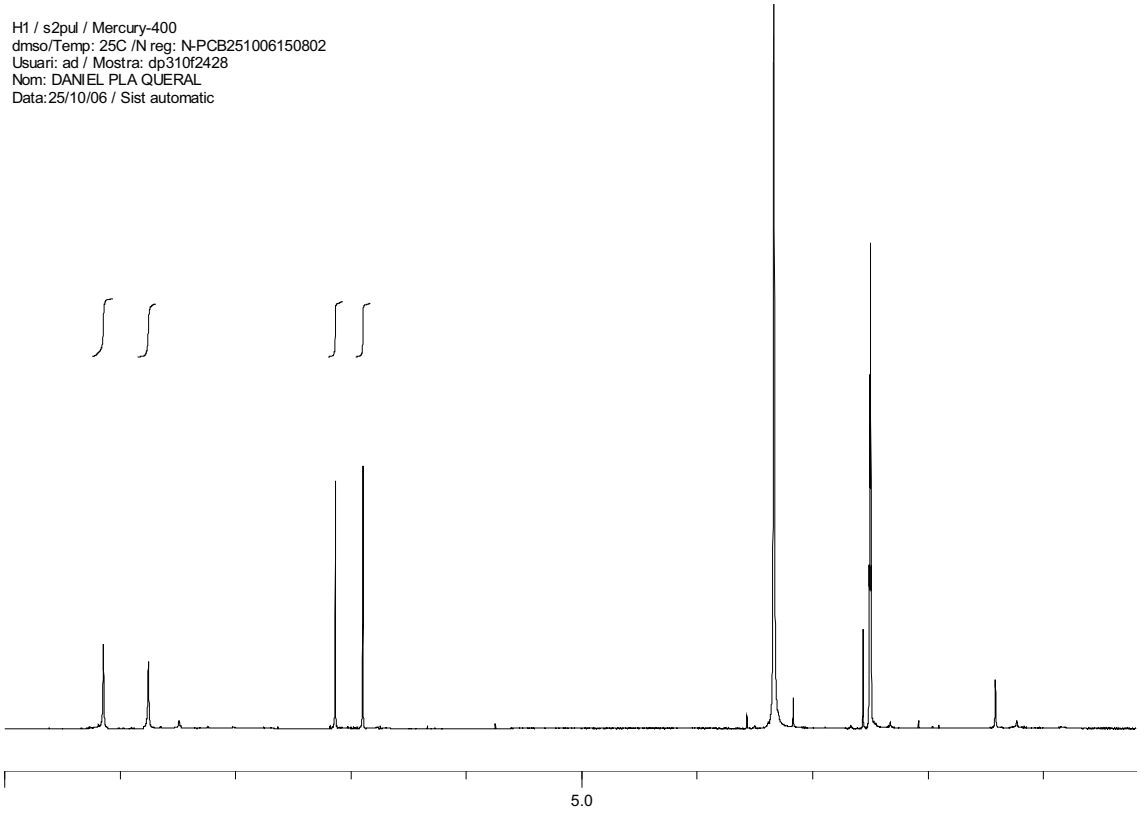




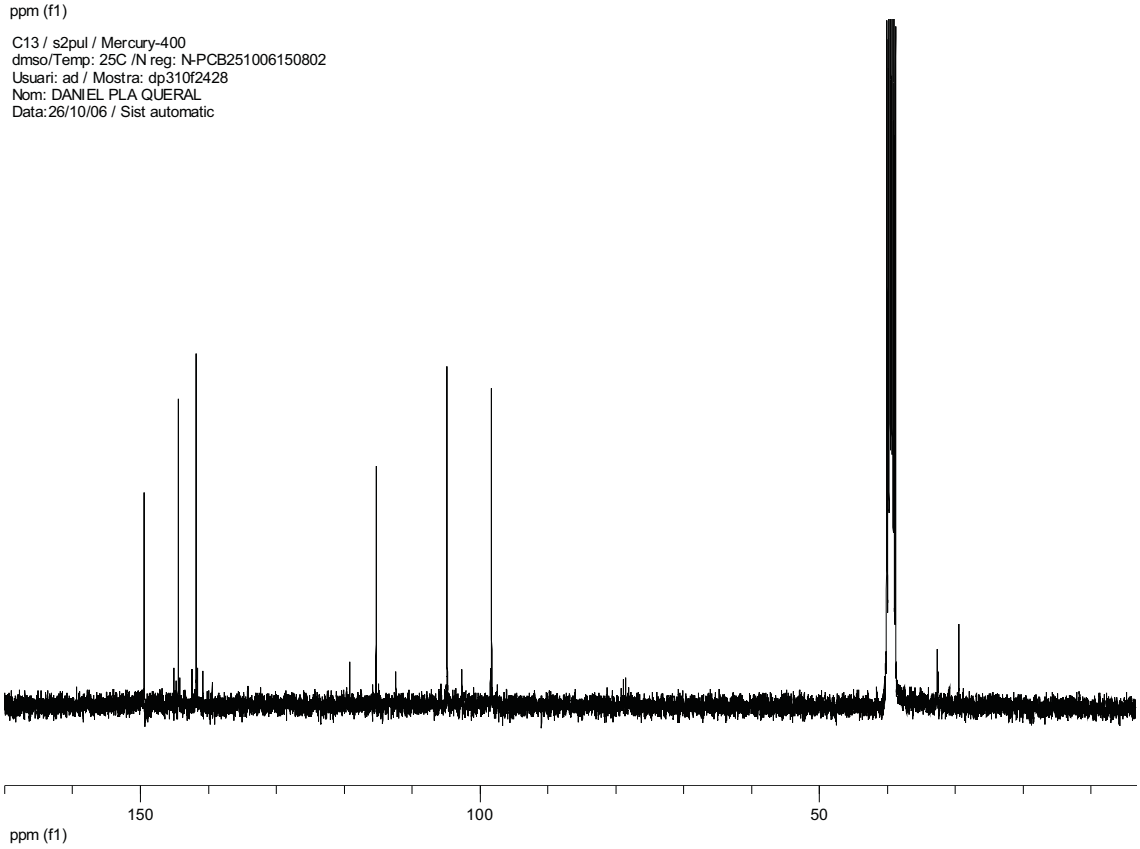


2c

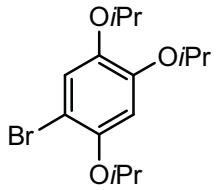
H1 / s2pul / Mercury-400
dmso/Temp: 25C /N reg: N-PCB251006150802
Usuari: ad / Mostra: dp310f2428
Nom: DANIEL PLA QUERAL
Data:25/10/06 / Sist automatic



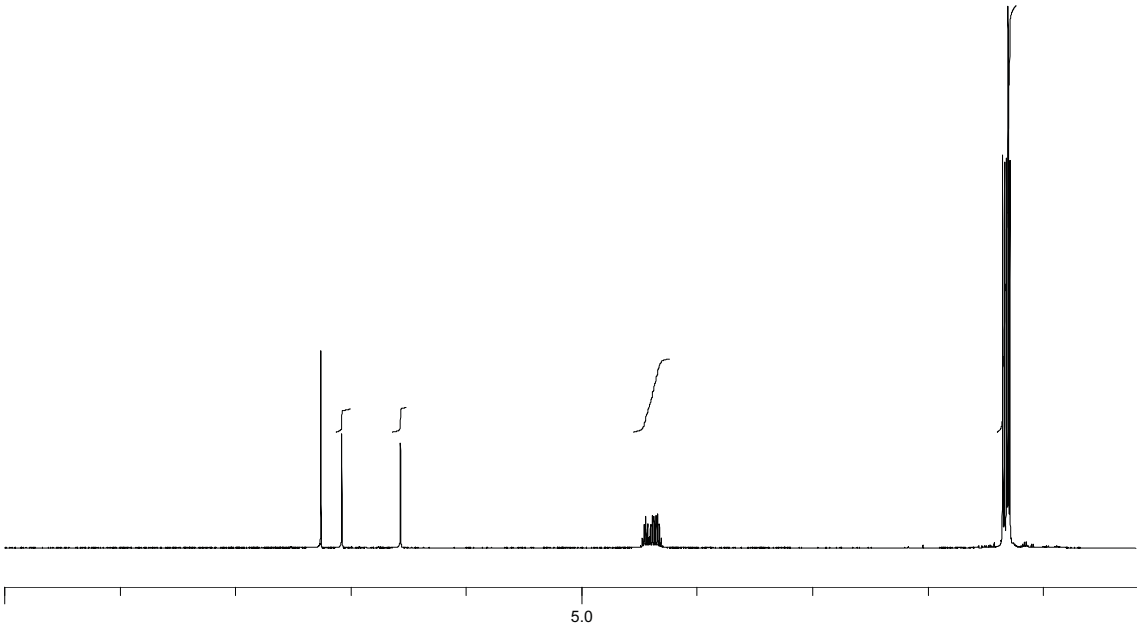
C13 / s2pul / Mercury-400
dmso/Temp: 25C /N reg: N-PCB251006150802
Usuari: ad / Mostra: dp310f2428
Nom: DANIEL PLA QUERAL
Data:26/10/06 / Sist automatic



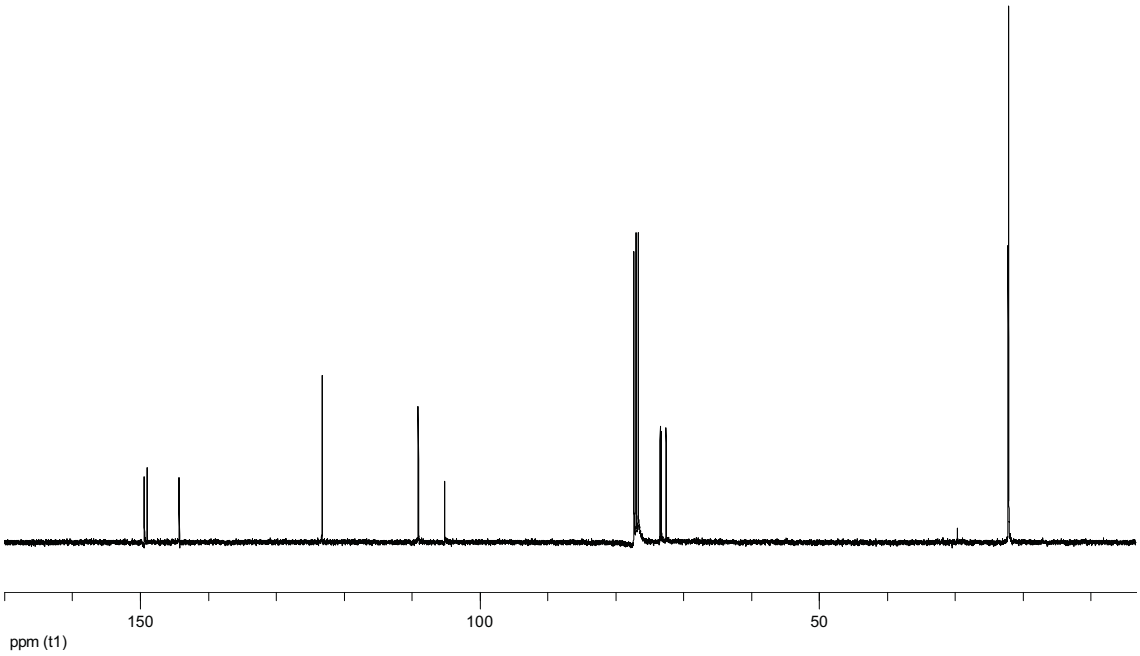
2d



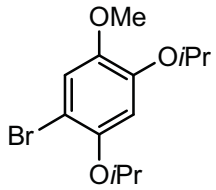
H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB210705170922
Usuari: ad / Mostra: dp103
Nom: DANIEL PLA QUERAL
Data:21/07/05 / Sist automatic



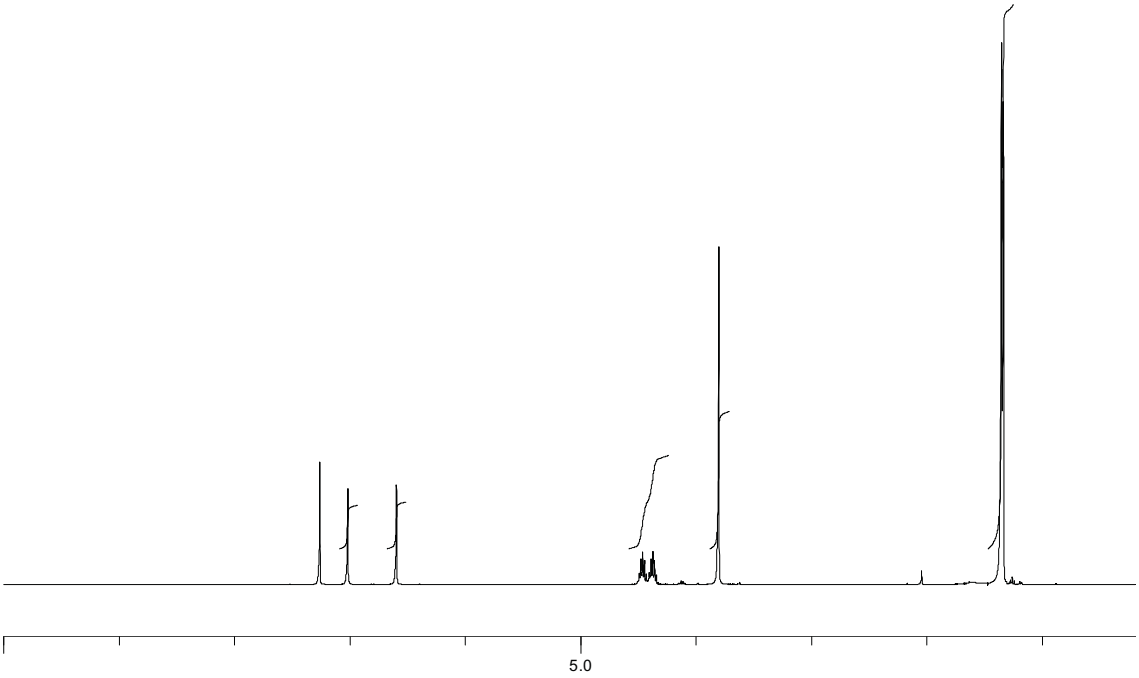
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB280705182951
Usuari: ad / Mostra: dp103
Nom: DANIEL PLA QUERAL
Data:29/07/05 / Sist automatic



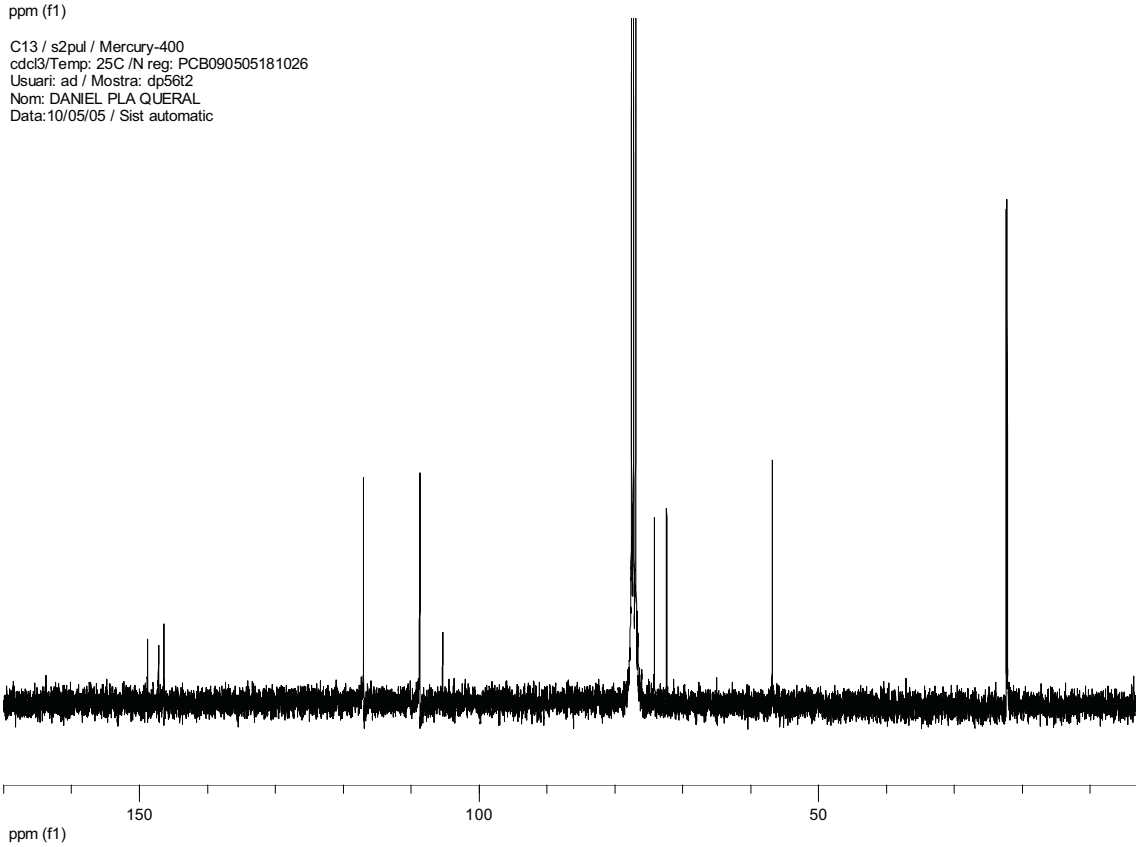
2e



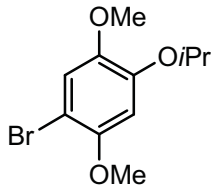
H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB0304052029
Usuari: ad / Mostra: dp_56
Nom: DANIEL PLA QUERAL
Data:03/04/05 / Sist automatic



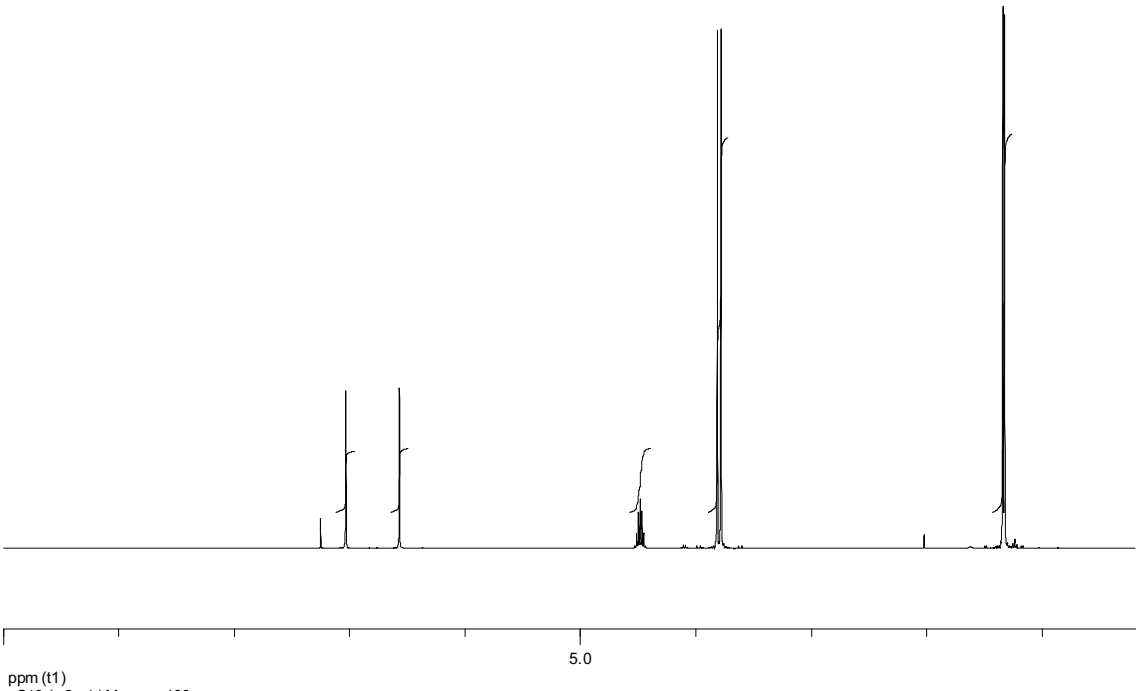
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB090505181026
Usuari: ad / Mostra: dp56t2
Nom: DANIEL PLA QUERAL
Data:10/05/05 / Sist automatic



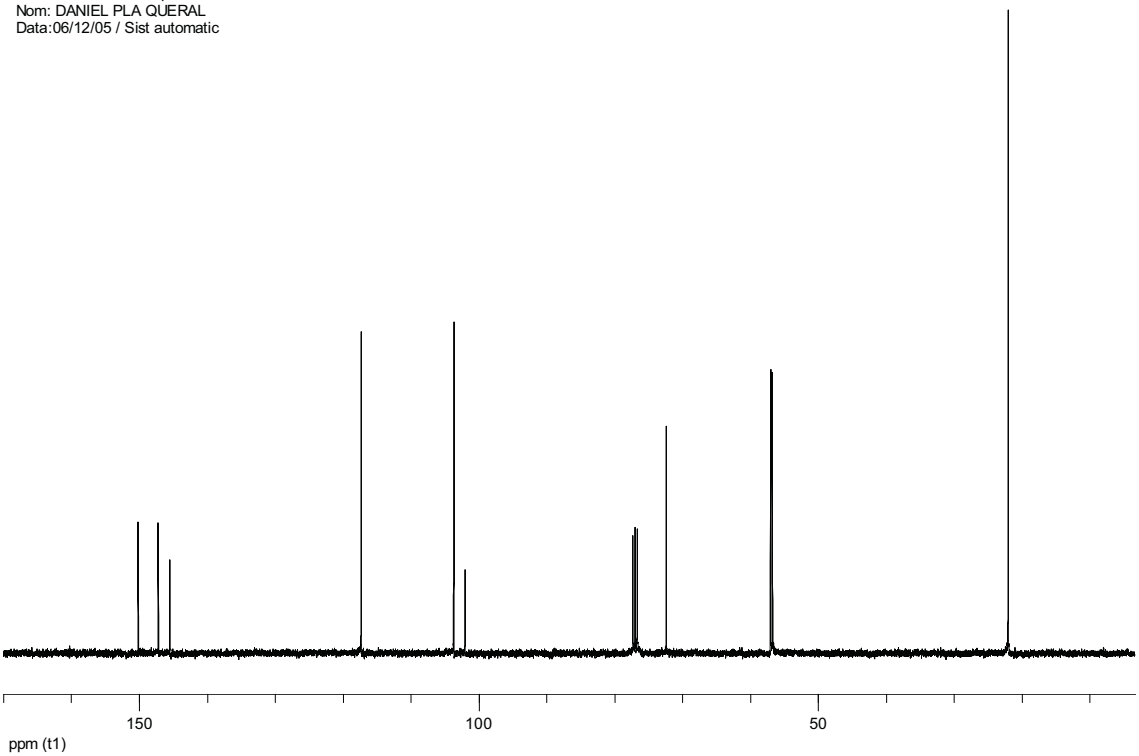
2f



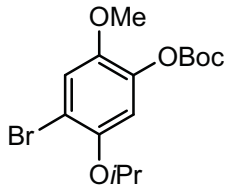
H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB061205163526
Usuari: ad / Mostra: dp177f913
Nom: DANIEL PLA QUERAL
Data:06/12/05 / Sist automatic



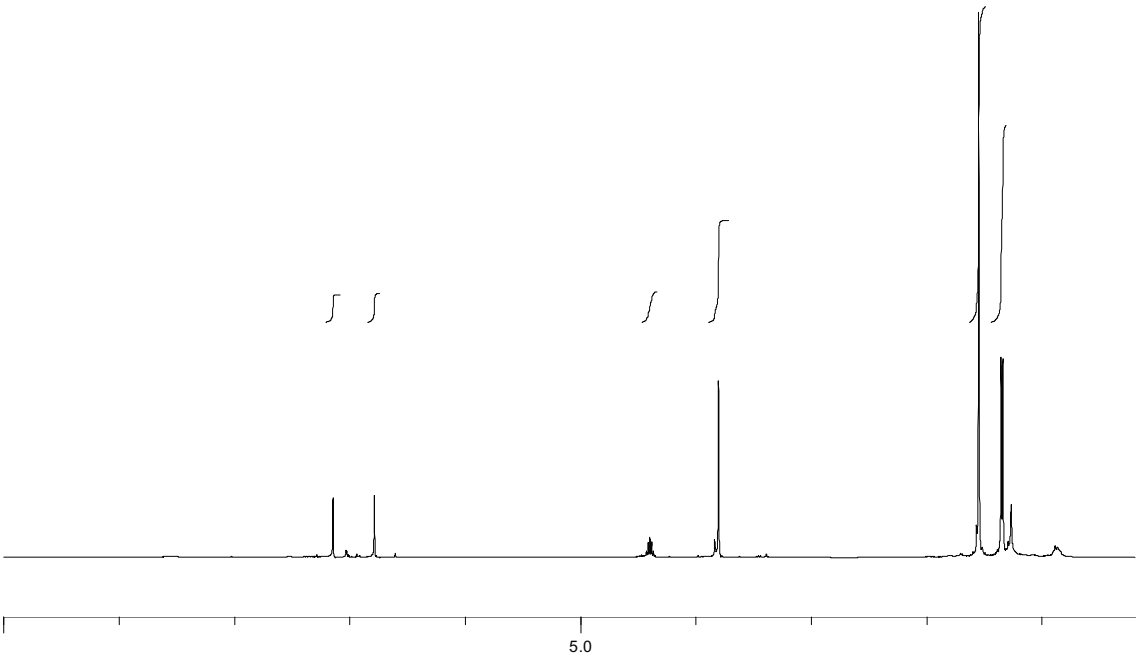
ppm (t1)
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB061205215616
Usuari: ad / Mostra: dp177f913
Nom: DANIEL PLA QUERAL
Data:06/12/05 / Sist automatic



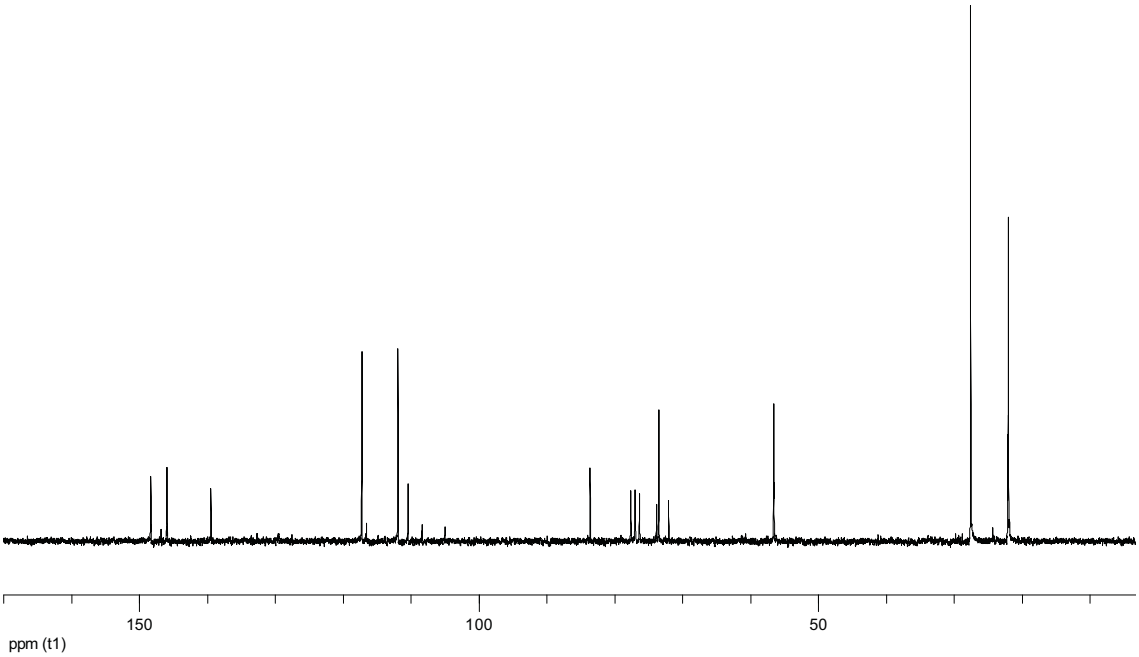
2g



H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: D-PCB290306145125
Usuari: ad / Mostra: dp218_3
Nom: DANIEL PLA QUERAL
Data:29/03/06 / Sist automatic



ppm (t1)
C13 / s2pul / Gem-200.DivIV
LOC: # 7 / Disolvent: cdcl3/ temp ambient
Usuari: ad/ Mostra: dp218_5
Nom: DANIEL PLA QUERAL
Data: 27/04/06 / Sistema automatic



ppm (t1)

3 SÍNTESI TOTAL DE LA LAMEL·LARINA D

SÍNTESI TOTAL DE LA LAMEL·LARINA D.

Modular total synthesis of Lamellarin D.

Daniel Pla, Antonio Marchal,[‖] Christian A. Olsen,[⇕] Fernando Albericio, [‡,*] Mercedes Álvarez [§,*]

Journal of Organic Chemistry, **2005**, *70*, 8231-823

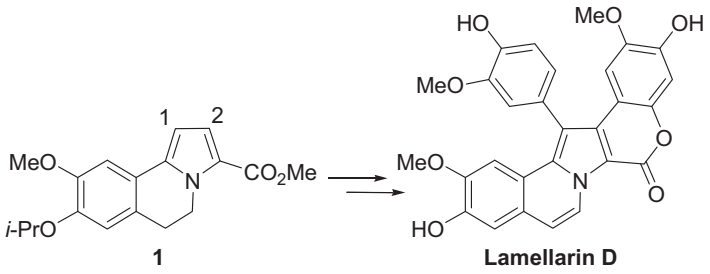
Biomedical Research Institute, Barcelona Scientific Park-University of Barcelona, 08028 Barcelona

[‖] Current address: Department of Inorganic and Organic Chemistry, University of Jaén, 23071 Jaén; amarchal@ujaen.es

[⇕] Current address: The Danish University of Pharmaceutical Sciences, Copenhagen, Denmark cao@dfuni.dk

[‡] Department of Organic Chemistry, University of Barcelona, 08028 Barcelona

[§] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona



1 **Lamellarin D**

Resum

En aquest capítol es descriu la síntesi total de la Lamel·larina D. La metodologia es fonamenta en la utilització seqüencial dels processos de bromació regioselectiva i acoblament creuat de Suzuki per la introducció dels grups aril a les posicions 1, i 2 del compost **1**. La oxidació amb 2,3-dicloro-5,6-diciano-*p*-benzoquinona (DDQ) i microones lliurà una pirroloisoquinolina, que seguida de la desprotecció dels grups fenol i subsegüent lactonització, donà la Lamel·larina D (18% en vuit etapes des d'**1**).

JOC*Note*

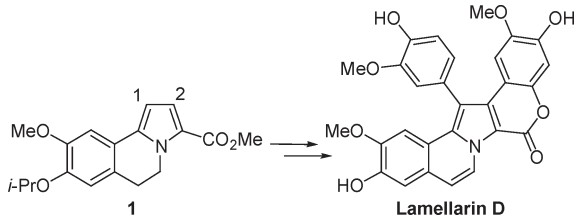
Modular Total Synthesis of Lamellarin D

Daniel Pla, Antonio Marchal,[†] Christian A. Olsen,[‡] Fernando Albericio,*[,§] and Mercedes Álvarez*[,||]

Biomedical Research Institute, Barcelona Scientific Park, University of Barcelona, 08028 Barcelona, Spain

albericio@pcb.ub.es; malvarez@pcb.ub.es

Received May 30, 2005



A modular total synthesis of lamellarin D, a marine alkaloid with potent cytotoxic as well as topoisomerase I inhibition properties, has been accomplished. A sequential and regioselective bromination/Suzuki cross-coupling procedure was applied for the introduction of aryl groups at positions 1 and 2 of scaffold **1**. Microwave-assisted 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) oxidation to yield pyrroloisoquinoline **15**, followed by phenol group deprotection and subsequent lactonization, gave lamellarin D (18% in eight steps from **1**).

Lamellarin D is a potent cytotoxic agent first isolated in 1985 by Faulkner and co-workers from the marine prosobranch mollusc *Lamellaria* sp.[1] Since then, a family of more than 30 lamellarins has been isolated from natural sources, and several synthetic strategies have been reported.[2] Recently, lamellarin D was identified as a potent inhibitor of topoisomerase I, thus providing some insight regarding its biological mechanism of action.[3] Furthermore, a structure−activity relationship (SAR) study with derivatives of lamellarin D afforded candidates for in vivo preclinical development of their antitumor activity.[4] The total synthesis of lamellarin D was achieved utilizing a modification of the strategy developed by Banwell and co-workers for the synthesis of lamellarin K.[5] This strategy allowed the preparation of several derivatives by acylation of the free phenolic sites.

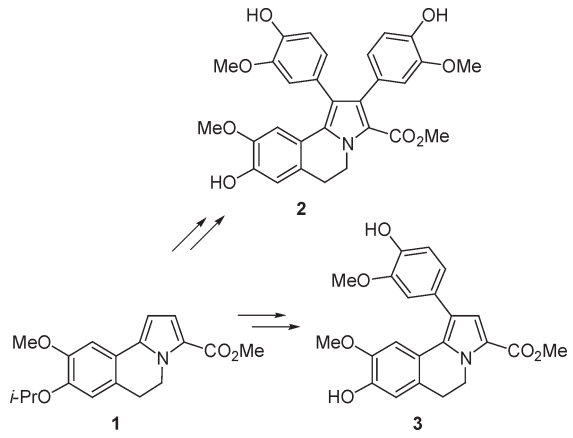


FIGURE 1. Structures of compounds **1**−**3**.

In addition, Ishibashi et al. described a synthetic route to lamellarin D[6] which they used for the preparation and biological evaluation of 10 derivatives that differ from the natural product in the substitution pattern of the nonheterocyclic aromatic rings. Important conclusions about the relation between substitution and activity were reached from that study.[7]

To prepare libraries of biologically active analogues of such natural products for lead discovery and/or optimization in medicinal chemistry, it is essential to have robust and versatile chemistries at hand. To that end, we have developed an efficient and highly convergent synthetic route to lamellarin D.

In a previous communication, we described the utility of methyl 5,6-dihydropyrrolo[2,1-*a*]isoquinoline-2-carboxylate **1** as a scaffold for the synthesis of open-chain lamellarin analogue **2**, which contains two methoxyphenols on the pyrrole ring, and for the simplified lamellarin analogue **3** (Figure 1).[8]

Derivative **2** differs from lamellarin D in lacking the lactone ring and the aromatization of the isoquinoline ring, structural modifications that afford conformational flexibility, whereas compound **3** lacks the entire aryl ring at position 2 of the scaffold. Compounds **2** and **3** were obtained using a synthetic procedure based on selective halogenation of the pyrrole ring followed by a Pd(0)-catalyzed Suzuki cross-coupling reaction.

Dibromination of the scaffold was accomplished in excellent yield using an excess of NBS in THF. Furthermore, regioselective monobromination was achieved in excellent yield by modifying the reaction time and the amount of NBS. Both compounds, **2** and **3**, proved to have cytotoxic effects on cancer cell lines; thus, the preparation and evaluation of a library of related compounds is in progress.

The total synthesis of lamellarin D described in the present paper is based on the findings described for preparation of **2** and **3**. This new protocol involves two

[†] Current address: Department of Inorganic and Organic Chemistry, University of Jaén, 23071 Jaén, Spain.

[‡] Current address: The Danish University of Pharmaceutical Sciences, Copenhagen, Denmark.

[§] Department of Organic Chemistry, University of Barcelona.

[||] Laboratory of Organic Chemistry, University of Barcelona.

(1) Andersen, R. J.; Faulkner, J.; Chun-heng, H.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 5493.

(2) For a recent review, see: Cironi, P.; Albericio, F.; Álvarez, M. In *Progress in Heterocyclic Chemistry*; Gribble, G. W., Joule, J. A., Eds.; Pergamon: Oxford, UK, 2004; Vol. 16, pp 1.

(3) Facompré, M.; Tardy, C.; Bal-Mahieu, C.; Colson, P.; Pérez, C.; Manzanares, I.; Cuevas, C.; Bailly, C. *Cancer Res.* **2003**, *63*, 7392.

(4) Tardy, C.; Facompré, M.; Laine, W.; Baldeyrou, B.; García-Gravalos, D.; Francesch, A.; Mateo, C.; Pastor, A.; Jiménez, J. A.; Manzanares, I.; Cuevas C.; Bailly, C. *Bioorg. Med. Chem.* **2004**, *12*, 1697.

(5) Banwell, M.; Flynn, B.; Hockless, D. *Chem. Commun*. **1997**, 259.

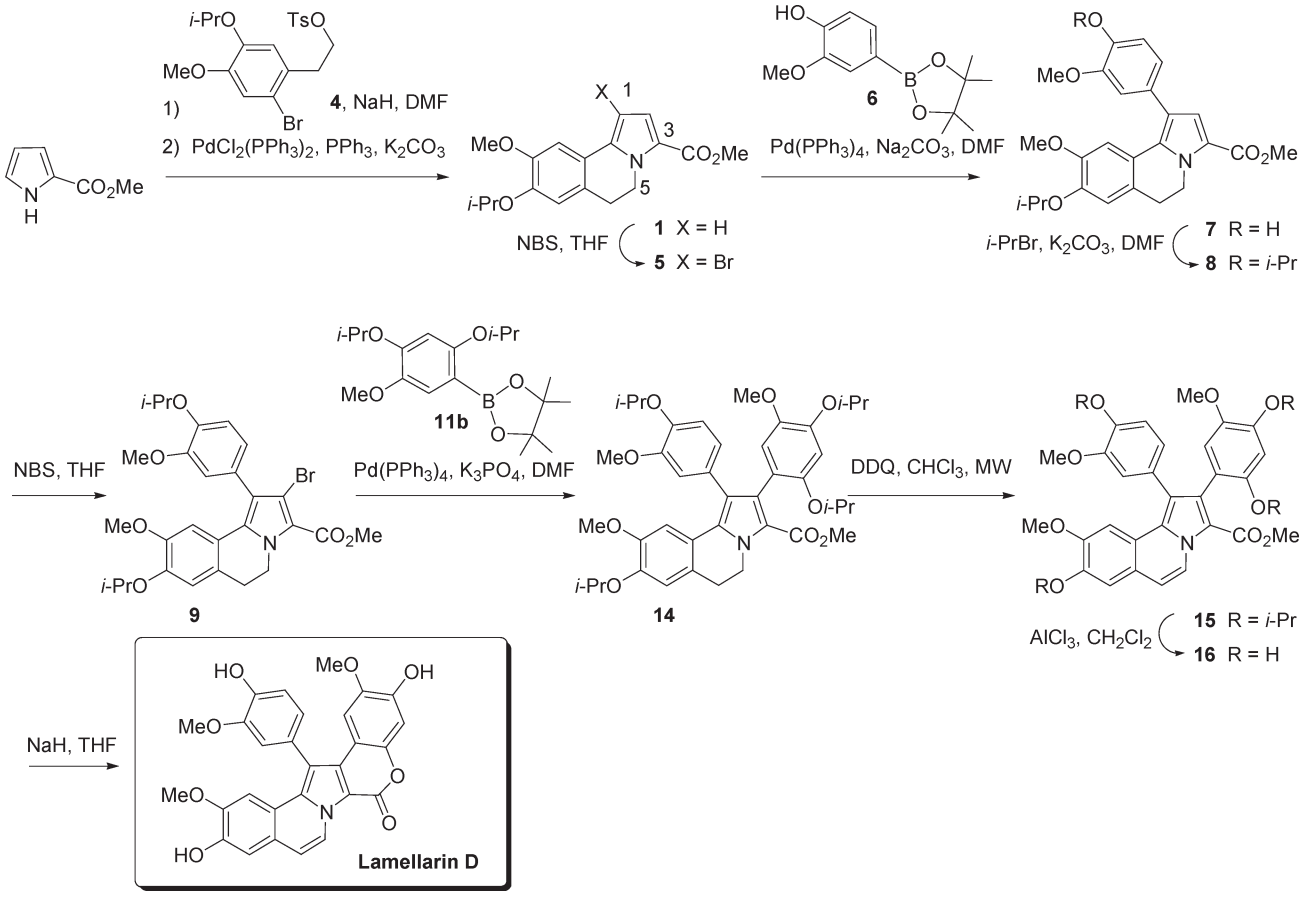
(6) Ishibashi, F.; Miyazaki, Y.; Iwao, M. *Tetrahedron* **1997**, *53*, 5951.

(7) Ishibashi, F.; Tanabe, S.; Oda, T.; Iwao M. *J. Nat. Prod*. **2002**, *65*, 500.

(8) Olsen, C. A.; Parera, N.; Albericio, F.; Álvarez, M. *Tetrahedron Lett*. **2005**, *46*, 2041.

10.1021/jo051083a CCC: $30.25
Published on Web 09/02/2005

SCHEME 1. Synthesis of Lamellarin D



sequential brominations and cross-coupling reactions using differently substituted arylboronic esters, followed by oxidation to give the aromatic isoquinoline ring, and lactonization to afford lamellarin D (Scheme 1). Key steps of this procedure are the regioselective bromination of the tricyclic system **8** in the presence of two highly activated benzene rings and the efficient preparation and introduction of building block **11b**.

N-Alkylation of methyl pyrrole-2-carboxylate[9] with tosylate **4**,[10] followed by Heck cyclization, gave scaffold **1**. Regioselective bromination of position 1 followed by Pd(0)-catalyzed cross-coupling with commercially available boronic ester **6** furnished compound **7**. Protection of the phenol as an isopropoxyether was achieved by reaction with 2-bromopropane in DMF using K_2CO_3 as a base.[11] The regioselective bromination of **8** to give compound **9** was achieved in 84% yield by treatment with NBS in THF at 70 °C for 1.5 h.[12] The electrophilic substitution occurs on the pentagonal heterocycle exclusively, and no traces of bromination on the activated benzene rings were observed in these conditions. Similar regioselectivity was also described for the synthesis of lamellarin G trimethyl ether.[13]

Preparation of the 2,4-diisopropoxy-5-methoxyphenylboranes **11a** and **11b** needed for the second Pd(0)-catalyzed cross-coupling reaction required optimization. Thus, the syntheses were attempted from bromobenzene derivative **10**[14] by employing bromine−lithium exchange followed by reaction with borolane **12a** or **12b**, respectively (Table 1, entries 1 and 2). The phenylboronic acid derivative **11a** was obtained in lower yield than the borolane **11b**, and its purification and manipulation were more tedious. Furthermore, boronic acid **11a** was inefficient to introduce the second aryl moiety through cross-coupling reaction with **9** using $Pd(PPh_3)_4$ and Na_2CO_3, as in the previous reaction for the preparation of **7**; thus, coupling with **11b** was investigated in detail. Preparation of **11b** was attempted by Pd(0)-catalyzed reaction between bromide **10** and pinacolborane **13** (entries 3 and

(9) Methyl pyrrole-2-carboxylate was obtained from pyrrole by acylation with trichloroacetyl chloride as described by Harbuck, J. W.; Rapoport, H. *J. Org. Chem.* **1972**, *37*, 3618. The 2-trichloroacetylpyrrole was treated with a solution of NaOMe in MeOH at 0 °C to furnish the methyl ester.

(10) Tosylate **4** was obtained from the 2-bromo-5-isopropoxy-4-methoxybenzaldehyde as detailed in Supporting Information. A Wittig reaction afforded the styrene, while anti-Markovnikov hydroboration and standard tosylation of the resulting 2-(2-bromo-5-isopropoxy-4-methoxyphenyl)ethanol gave **4**. Preparation of 2-(2-bromo-5-isopropoxy-4-methoxyphenyl) ethanol was described from the same benzaldehyde using a different procedure by: Treu, M.; Jordis, U. *Molecules* **2002**, *7*, 374.

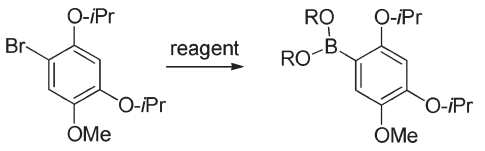
(11) ^{1}H NMR spectra of **7** and **8** differ only in the integrated area of the isopropoxy signals, whereas two OC*H*$(CH_3)_2$ signals can be observed at 71.4 and 71.6 ppm in the ^{13}C NMR.

(12) This reaction required extensive fine-tuning, as longer reaction times and/or higher temperature resulted in complex mixtures due to additional bromination. Furthermore, oxidation of the dihydroisoquinoline ring was observed by ^{1}H NMR. The reaction could be followed by HPLC.

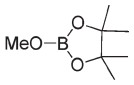
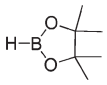
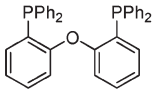
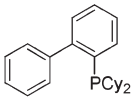
(13) Handy, S. T.; Zhang, Y.; Bregman, H. *J. Org. Chem.* **2004**, *69*, 2362.

(14) Obtained from 3-isopropoxy-4-methoxybenzaldehyde by Baeyer−Villiger reaction followed by protection of the resulting phenol and bromination; see Supporting Information for details.

TABLE 1. Preparation of Boronic Acid 11a and Boronic Ester 11b



10 **11a** R = H
11b R,R = Me_2C-CMe_2

entry	reagent	conditions	compound yield
1	$B(O\text{-}iPr)_3$ **12a**	*n*BuLi, THF -78 °C, 15 min	**11a** 20%
2	 **12b**	*n*BuLi, THF -78 °C, 15 min	**11b** 59%
3	 **13**	$Pd(OAc)_2$, Et_3N[a]  (DPEphos)	**11b** 80%
4	**13**	$Pd(OAc)_2$, Et_3N[a] 	**11b** 59%

[a] In refluxing dioxane; DPEphos = (oxydi-2,1-phenylene)bis-(diphenylphosphine).

4). $Pd(OAc)_2$ together with (DPEphos)[15] or biphenyl ligand [2-(dicyclohexylphosphino)biphenyl][16]afforded **11b** in good yields.[17,18]

The Suzuki−Miyaura cross-coupling reaction between **11b** and **9** was tested under several experimental conditions without satisfactory yields. The lack of reactivity of boronic ester **11b** could presumably be attributed to its steric hindrance. Ultimately, the problem was solved by using an excess of **11b**, $Pd(PPh_3)_4$ as the catalyst, and K_3PO_4 as a base, instead of K_2CO_3, affording the biaryl derivative **14** in high yield.[17] Handy and co-workers[13] also described the use of a total of 8 equiv of a boronic acid to lead to a 46% yield of the coupling product, in a related structure. Applying our improved protocol with 5 equiv of **11b**, of which 3 equiv were added at the beginning of the reaction and the last 2 equiv by syringe pump over 2.5 h, led to a yield of 87%.

The aromatization of dihydroisoquinoline **14** to give **15** was achieved using DDQ in $CHCl_3$ in a sealed tube with controlled microwave (MW) irradiation at 120 °C for 5 min.[19] Several experimental conditions with a simple analogue were tested for the optimization of this oxidation, and the MW-assisted DDQ method proved to be superior.[20] Cleavage of the four isopropoxyether protecting groups[21] in **15** with $AlCl_3$ followed by lactonization using NaH as a base afforded lamellarin D.

In conclusion, the preparation of lamellarin D has been accomplished in eight steps (18% overall yield) from scaffold **1**. The strategy is based on two consecutive, regioselective bromination−Suzuki cross-coupling steps for introducing the appropriate aryl groups in positions 1 and 2 of scaffold **1**. Synthesis of an *ortho*-substituted borolane (**11b**) and its coupling to compound **9** were optimized, and high yields were obtained in the remaining sequence leading to lamellarin D.

In addition to our previous report on open-chain lamellarins, the methodology reported herein can be exploited for the preparation of analogues of the natural product by utilizing the concept of diverted total synthesis (DTS).[22]

Experimental Section

2-(2,4-Diisopropoxy-5-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (11b). A mixture of **10** (1.59 g, 5.3 mmol), Et_3N (28.9 mL, 21 mmol), $Pd(OAc)_2$ (62.4 mg, 0.28 mmol), DPEphos (0.29 mg, 0.54 mmol), and **13** (2.36 mL, 15.76 mmol) in dioxane (5.3 mL) was heated at 100 °C for 13.5 h. After cooling to room temperature, the reaction mixture was quenched with saturated NH_4Cl, and the aqueous solution was extracted with Et_2O. The organic solution was dried, filtered, and concentrated. The resulting oil was purified by flash chromatography (SiO_2 previously deactivated with 5% Et_3N). Elution with hexane/AcOEt (70:30) afforded **11b** (1.47 g, 79%) as a brown syrup: IR (film) ν 2976, 2929, 1407, 1371, 1145, 1112, 1032. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.29 (d, 6H, J = 6.0 Hz, 2Me); 1.33 (s, 12H, 4Me); 1.35 (d, 6H, J = 6.0 Hz, 2Me); 3.84 (s, 3H, OMe); 4.24 (h, 1H, J = 6.0 Hz); 4.53 (h, J = 6.0 Hz, 1H); 6.51 (s, 1H); 7.14 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.9 (q, 2Me); 22.2 (q, 2Me); 24.8 (q, 4Me); 56.4 (q); 71.0 (d); 75.0 (d); 83.2 (s, C4, C5); 107.8

(15) Kranenburg, M.; van der Burgt, Y. E. M.; Kamer, P. C. J.; van Leeuwn, P. W. N. M.; Goubitz, K. Fraange, *J. Organometallics* **1995**, *14*, 3081.

(16) Wolfe, J. P.; Singer, R. A.; Bryant, H. Y.; Buchwald, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 9550.

(17) Other catalysts such as $PdCl_2(dppf)_2$ were inefficient for this transformation: Baudoin, O.; Guénard, D.; Guéritte, F. *J. Org. Chem.* **2000**, *65*, 9268.

(18) Reaction of **10** with bis(neopentyl glycolato)diboron catalyzed by $Pd(OAc)_2$ and DPEphos afforded the debrominated compound, 2,4-diisopropoxy-1-methoxybenzene, instead of the desired building block **11b**.

(19) A doublet at 9.24 ppm in the ^1H NMR spectrum, characteristic of H-5, verified the formation of **15**.

(20) Oxidation of methyl 1,2-bis(2-thienyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate using either (i) DDQ in $CHCl_3$ at reflux temperature, (ii) MnO_2 in refluxing toluene or pyridine, or (iii) Pd−C in toluene or decalin were unsuccessful (unpublished results).

(21) Protection of phenol groups as isopropoxyethers was previously demonstrated to be very efficient in the solid-phase synthesis of lamellarins: Cironi, P.; Manzanares, I.; Albericio, F.; Álvarez, M. *Org. Lett.* **2003**, *5*, 2959. Marfil, M.; Albericio, F.; Álvarez, M. *Tetrahedron* **2004**, *60*, 8659.

(22) For an example of DTS, see: Gaul, C.; Njadarson, J. T.; Shan, D.; Diorn, D. C.; Wu, K.-D.; Tong, W. T.; Huang, X.-Y.; Moore, M. A. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 11326 and references therein.

(d); 118.9 (d); 145.2 (s); 150.4 (s); 158.2 (s). MS(CI) 350 (M, 100); 351 (M + 1, 92); 352 (M + 2, 48); 353 (M + 3, 12).

Methyl 2-(2,4-diisopropoxy-5-methoxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (14). 11b (3 mL, 1.88 mmol, 0.63M in DMF), $Pd(PPh_3)_4$ (145 mg, 0.13 mmol), and 2 M K_3PO_4 (1.25 mL) were added to a solution of bromide **9** (350 mg, 0.63 mmol) in DMF (10 mL). The reaction mixture was stirred at 110 °C, and another portion of **11b** (5 mL, 1.25 mmol, 0.25 M in DMF) was added by syringe pump over 2.5 h. After 6 h of heating, the solvent was evaporated, and the residue was dissolved in AcOEt. The organic solution was washed with sodium diethyldithiocarbamate (0.02 M solution), brine, and water, dried, filtered, and concentrated to give a crude material that was purified by column chromatography on silica gel. Elution with hexane/AcOEt (60:40) gave **14** (383 mg, 87%) as a reddish oil: IR (film) ν 2975, 1693, 1438, 1254, 1208, 1111 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.31 (d, 12H, J = 6.0 Hz, 4Me); 1.36 (d, 12H, J = 6.0 Hz, 4Me); 3.00 (m, 2H, C5); 3.33 (s, 3H, OMe); 3.51 (s, 3H, OMe); 3.59 (s, 6H, OMe, CO_2Me) 4.09−4.14 (m, 1H, CH); 4.41−4.55 (m, 3H, CH); 4.64−4.69 (m, 2H, C6); 6.46 (s, 1H); 6.48 (s, 1H); 6.66 (br, 1H); 6.74−6.79 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.0 (q, Me); 22.1 (q, Me); 22.2 (q, Me); 29.0 (t, C6); 42.8 (t, C5); 50.8 (q, CO_2Me); 55.1 (q, OMe); 55.7 (q, OMe); 56.1 (q, OMe); 71.5 (d, OCH); 71.6 (d, OCH); 71.7 (d, OCH); 107.2 (d); 109.1 (d); 114.9 (d); 115.0 (d); 116.0 (d); 116.1 (d); 119.2 (s); 119.5 (s); 121.3 (s); 121.5 (s); 123.3 (d); 125.5 (s); 127.9 (s); 129.1 (s); 130.6 (s); 144.5 (s, C−OMe); 145.7 (s); 145.8 (s); 146.2 (s); 148.5 (s); 149.4 (s, C−OMe); 150.2 (s, C−OMe); 162.7 (s, C═O). MS (MALDI-TOF) 701 (M, 100), 702 (M + 1, 39), 703 (M + 2, 8). HRMS *m/z* calcd for $C_{41}H_{51}NO_9$ 701.3564, found 701.3558.

Methyl 2-(2,4-diisopropoxy-5-methoxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxypyrrolo-[2,1-*a*]isoquinoline-3-carboxylate (15). A mixture of **14** (411 mg, 0.59 mmol) and DDQ (173 mg, 0.76 mmol) in dry $CHCl_3$ (15 mL) was purged with Ar and irradiated with a microwave at 120 °C for 5 min in a sealed vessel. The organic solution was washed with 2 M NaOH, water, and brine. The solvent was removed to afford a crude residue, which was purified by flash chromatography on silica gel. Elution with hexane/AcOEt (55:45) gave **15** (336 mg, 82%) as a pale yellow oil: IR (film) ν 1684, 1211 cm^{-1}. ^1H NMR (acetone-d_6, 400 MHz) δ 1.25−1.35 (m, 24H); 3.38 and 3.40 (2d, 3H, OMe); 3.54 and 3.59 (2s, 3H, OMe); 3.60 and 3.62 (2s, 3H, OMe); 3.62 (s, OMe); 4.19−4.35 (m, 1H); 4.48−4.59 (m, 2H); 4.72 (h, 1H, J = 5.6 Hz); 6.56 (s, 1H); 6.60 (d, 1H, J = 8.8 Hz); 6.69−6.82 (m, 2H); 6.90−7.13 (m, 2H), 7.28 and 7.16 (2s, 1H); 7.29 (s, 1H); 9.24 (d, 1H, J = 7.6 Hz, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.8 (q, 2Me); 21.9 (q, 2Me); 22.1 (q, 2Me); 22.2 (q, 2Me); 50.7 (q, CO_2Me); 55.2 (q, OMe); 55.5 (q, OMe); 56.1 (q, OMe); 71.2 (d); 71.4 (d); 71.5, 71.6 (d); 71.8 (d); 105.6 (d), 106.6 (d); 107.0 (s); 110.7 (d); 111.7 (d); 113.1 (s); 115.4 (d), 115.6 (d); 115.9 (d), 116.1 (d); 118.2 (s); 119.4 (s); 119.8 (s); 123.3 (d); 129.4 (s); 131.6 (s); 144.3 (s); 146.0 (s); 146.2 (s); 147.3 (s); 149.5 (s); 149.9 (s); 150.2 (s); 162.9 (s, C═O). MS (MALDI-TOF) 699 (M, 100), 700 (M + 1, 59), 701 (M + 2, 17). HRMS *m/z* calcd for $C_{41}H_{49}NO_9$ 699.3407, found 699.3402.

Methyl 2-(2,4-dihydroxy-5-methoxyphenyl)-8-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]-isoquinoline-3-carboxylate (16). A solution of **15** (143 mg, 0.20 mmol) and $AlCl_3$ (148 mg, 1.06 mmol) in dry CH_2Cl_2 (1 mL) was stirred at room temperature for 2 h. The reaction mixture was then quenched with saturated NH_4Cl and washed with water and brine. The organic phase was dried, filtered, and concentrated, and the resulting crude material was purified by flash chromatography. Elution with hexane/AcOEt (gradient from 25:75 to pure AcOEt) gave **16** (69.1 mg, 64%) as a light brown solid: IR (film) ν 3426, 1679, 1380, 1268, 1242, 1210 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.51 (s, 3H, Me); 3.55 (s, 3H, Me); 3.66 (s, 3H, Me); 3.77 (s, 3H, Me); 5.43 (br, 1H, OH); 5.56 (s, 1H, OH); 5.61 (s, 1H, OH); 5.87 (s, OH); 6.33−6.92 (m, 3H); 6.92−7.27 (m, 5H); 9.19 (m, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 51.4 (q, Me); 55.4 (q, Me); 56.0 (q, Me); 56.4 (q, Me); 102.7 (d); 104.8 (d); 110.4 (d); 112.5 (d); 112.7 (s); 113.8 (d); 113.9 (s); 114.0 (d); 114.1 (d); 119.1 (s); 123.4 (d, C5); 124.2 (d); 124.5 (s); 131.3 (s); 140.2 (s); 144.8 (s); 145.8 (s); 146.1 (s); 146.6 (s); 146.7 (s); 148.6 (s); 162.3 (s, C═O). MS (MALDI-TOF) 531 (M, 100), 532 (M + 1, 38), 533 (M + 2, 11). HRMS *m/z* calcd for $C_{29}H_{25}NO_9$ 531.1529, found 531.1524.

Lamellarin D. A mixture of NaH (60% dispersion, 25.7 mg, 0.64 mmol) and **16** (33.9 mg, 0.06 mmol) in THF (3.5 mL) was stirred for 3 h at room temperature and for 1 h at 40 °C. The solvent was removed under reduced pressure, and AcOEt was added to the residue. The organic solution was washed with saturated NH_4Cl, water, and brine and then dried, filtered, and concentrated. The residue was purified by flash chromatography. Elution with AcOEt/MeOH (gradient from 100:0 to 80:20) furnished lamellarin D (23.7 mg, 75%) as a pinkish-white solid. The spectroscopic data were in accordance with previous reports.[1,6]

Acknowledgment. This work was partially supported by CICYT (BQU 2003-00089), Generalitat de Catalunya, Barcelona Science Park, and PharmaMar S. L., which is also gratefully acknowledged for performing the preliminary biological tests. A.M. thanks the Junta de Andalucía, UJA, and UB for financial support and staying facilities. Authors thank Dr. Carmen Cuevas for her encouragement to perform the present work.

Supporting Information Available: Materials and methods, experimental procedures, characterization data, and ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

JO051083A

SUPPLEMENTARY MATERIAL

for the

full communication

entitled

Modular Total Synthesis of Lamellarin D

authored by

Daniel Pla,[†] *Antonio Marchal,*[†,∥] *Christian A. Olsen,*[†,◦] *Fernando Albericio,*[†,‡,*] *Mercedes Álvarez*[†,§,*]

[†] Biomedical Research Institute, Barcelona Scientific Park-University of Barcelona, 08028 Barcelona; [∥] Current address: Department of Inorganic and Organic Chemistry, University of Jaén, 23071 Jaén; [◦] Current address: The Danish University of Pharmaceutical Sciences, Copenhagen, Denmark; [‡] Department of Organic Chemistry, University of Barcelona, 08028 Barcelona; [§] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona.

TABLE OF CONTENTS

EXPERIMENTAL SECTION

General

Tetrahydrofuran was freshly distilled from sodium/benzophenone. Triethylamine was distilled from calcium hydride prior to use and kept in potassium hydroxide. Dimethylsulfoxide was refluxed with calcium hydride, distilled under reduced pressure and kept over 4Å molecular sieves. Dichloromethane (99.99% anhydrous) and *N*,*N*-dimethylformamide (99.99% anhydrous) were purchased and kept over 4Å molecular sieves. *N*-Bromosuccinimide was recrystallized previous to use. All organic extracts were dried with anhydrous sodium sufate or magnesium sulfate. Microwave-assisted reactions were carried out in a laboratory microwave oven. The automatic syringe pump was used as specified for controlled addition of some reactants. Automatic flash chromatography was done in a medium pressure liquid cromatograph with commercial silica gel columns (47-60 μm). Analytical HPLC samples were run on a 4.6x150 mm C_{18} column (5 μm) with acetonitrile/water elution and photodiode array detection. Melting points were determined in open capillaries and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 MHz spectrometer. In addition, ^{1}H NMR, and gHSQC were recorded on a 500 MHz spectrometer. Spectra were referenced to appropriate residual solvent peaks (d_6-acetone, d_6-DMSO or $CDCl_3$). IR spectra were obtained on a FT-IR spectrometer. Vibration frequencies are expressed in cm^{-1}. HR-MS were performed on a high resolution mass spectometer by Unidad de Espectrometría de Masas (Universidad de Santiago de Compostela).

The following abbreviations are used: Ac, acetate; CI, chemical ionization; DEAD, diethyl azodicarboxylate; DDQ, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DPEphos (oxydi-2,1-phenylene)bis(diphenylphosphine); FAB, fast atom bombarding; EI, electronic impact; ESMS, electrospray mass spectrometry; ; gHSQC, gradient heteronuclear single quantum correlation; *m*-CPBA, *meta*-chloroperoxybenzoic acid; MALDI-TOF, matrix-assisted laser desorption/ionization - time of flight; M.p., melting point; NBS, *N*-bromosuccinimide; NMR, nuclear magnetic resonance; THF, tetrahydrofuran; Ts, *p*-toluenesulfonyl.

*Mercedes Álvarez. Tel.: +34 93 403 70 86; fax: +34 93 403 71 26; e-mail: malvarez@pcb.ub.es

3-Isopropoxy-4-methoxyphenol.[1] A solution of 3-isopropoxy-4-methoxybenzaldehyde (2.68 g, 13.8 mmol) in CH_2Cl_2 (20 mL) was added to a solution of *m*-CPBA (50% purity, 14.3 g, 41.4 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred at reflux for 16 h. The solids were then filtered off, and the solvent was removed *in vacuo*. NaOH (1.7 g) in EtOH/H_2O (50:50, 40 mL) was added to the residue, and after stirring for 2 h the solution was neutralized with 4N HCl. The aqueous solution was extracted with CH_2Cl_2 (3 × 60 mL), and the pooled organic layers were washed with water (3 × 75 mL), dried, filtered and concentrated. The residue was purified by flash chromatography on silica gel. Elution with hexane/CH_2Cl_2 (95:5, to 85:15) afforded 1.93 g (77%) of a white crystalline solid. M.p. 122.3-122.5 ºC; IR (film) ν 3433, 2977, 1508, 1129 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.36 (d, 6H, *J* = 6.4 Hz, 2Me); 3.80 (s, 3H, OMe); 4.48 (h, 1H, *J* = 6.4 Hz); 6.34 (dd, 1H, *J* = 8.8 and 2.8 Hz, H6); 6.48 (d, 1H, *J* = 2.8 Hz, H2); 6.74 (d, 1H, *J* = 8.8 Hz, H5). ^{13}C NMR ($CDCl_3$,) δ 22.0 (q, 2Me); 56.8 (q, OMe); 71.3 (d); 104.2 (d, C2); 106.4 (d, C6); 113.4 (d, C5); 144.6 (s); 148.3 (s); 149.9 (s). MS (CI) 182 (M, 7); 183 (M+1, 100); 184 (M+2, 11).

2,4-Diisopropoxy-1-methoxybenzene.[2] K_2CO_3 (4.03 g, 29.2 mmol) and 2-bromopropane (1.9 mL, 20.3 mmol) were added to a solution of 3-isopropoxy-4-methoxyphenol (2.65 g, 14.6 mmol) in dry DMF (20 mL). The mixture was microwaved at 80 ºC under Ar for 40 min. The mixture was then cooled to room temperature and the solvent was removed in vacuo. AcOEt was added and the organic solution was washed with 2M NaOH, saturated $NaHCO_3$, water and brine. The organic layer dried and concentrated gave 2.51 g (77%) of the titled compound as a yellowish oil. IR (film) ν 2976, 1508, 1129 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.31 (d, 6H, *J* = 6.0 Hz, 2Me); 1.36 (d, 6H, *J* = 6.4 Hz, 2Me); 3.80 (s, 3H, OMe); 4.42 (h, 1H, *J* = 6.4 Hz); 4.50 (h, 1H, *J* = 6.0 Hz); 6.42 (dd, 1H, *J* = 8.8 and 2.8 Hz, H5); 6.52 (d, 1H, *J* = 2.8 Hz, H3); 6.78 (d, 1H, *J* = 8.8 Hz, H6).

1-Bromo-2,4-diisopropoxy-5-methoxybenzene (**10**). A solution of 2,4-diisopropoxy-1-methoxybenzene (2.51 g, 11.2 mmol) in dry DMF (36 mL) was stirred and cooled to -75ºC. Solid NBS (1.99 g, 11.2 mmol) was added, the mixture was stirred for 1 h at the same temperature and allowed to reach room temperature. The solvent was evaporated and AcOEt was added. The organic solution was filtered through a pad of neutral alumina and concentrated. The residue was purified by flash chromatography on silica gel. Elution with hexane/AcOEt (gradient from 100:0 to 90:10) gave 2.88 g

---

[1] Testaferri, L.; Tiecco, M.; Tingoli, M.; Chianelli, D.; Montanucci, M. *Tetrahedron.* **1982,** *38*, 3687.

[2] Iinuma, M.; Iwashima, K.; Matsuura, S. *Chemical & Pharmaceutical Bulletin.* **1984**, 32, 4935.

(85%) of the compound **10** as a brown oil. IR (film) ν 2977, 2934, 1496, 1386, 1211, 825 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.34 (d, 6H, *J* = 6.4 Hz, 2Me); 1.35 (d, 6H, *J* = 6.0 Hz, 2Me); 3.80 (s, 3H, OMe); 4.44 (h, 1H, *J* = 6.0 Hz); 4.46 (h, 1H, *J* = 6.4 Hz); 6.60 (s, 1H); 7.02 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 22.2 (q, 2Me); 22.4 (q, 2Me); 56.8 (q, OMe); 72.4 (d); 74.2 (d) ; 105.4 (s); 108.7 (d); 117.0 (d); 144.4 (s); 147.2 (s); 148.8 (s). MS (CI) 302 (MBr^{79}, 92); 303 (MBr^{79}+1, 57); 304 (MBr^{81}, 83); 305 (MBr^{81}+1, 47).

2-Bromo-5-isopropoxy-4-methoxystyrene. A solution of methyltriphenylphosphonium bromide (24.6 g, 68.9 mmol) and DMSO (3.88 mL, 50.3 mmol) in freshly distilled THF was stirred and cooled with an ice-bath under Ar and NaH (60% dispersion, 6.04 g, 151.0 mmol) was added. After stirring for additional 30 min at room temperature 2-bromo-3-isopropoxy-4-methoxybenzaldehyde (13.74 g, 50.3 mmol) was added and the stirring was continued for 30 min. The solvent was removed under reduced pressure and AcOEt (200 mL) was added. The organic solution was washed with water, 1N HCl, saturated $NaHCO_3$, and brine, then dried, filtered and concentrated. The residue was treated with hexane, filtered and the solid was washed with AcOEt. The organic solutions were concentrated and the residue was purified by flash chromatography. Elution with hexane/CH_2Cl_2 (80:20) afforded the bromide, 10.98 g (81%) as a colorless syrup. IR (film) ν 1497, 1256, 1206, 1162, 912 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.37 (d, 6H, 3H, *J* = 6.1 Hz, 2Me); 3.85 (s, 3H, OMe); 4.55 (h, 1H, *J* = 6.1 Hz); 5. 25 (d, 1H, *J* = 10.8 Hz); 5.55 (d, 1H, *J* = 17.2 Hz); 6.98 (dd, 1H, *J* = 17.2 and 10.8 Hz); 7.01 (s, 1H); 7.09 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 22.0 (q, 2Me); 56.2 (q, OMe); 71.9 (d); 113.7 (d); 114.5 (t); 114.7 (s); 115.8 (d); 129.6 (s); 135.4 (d); 146.7 (s); 151.0 (s). MS (FAB) 270 (MBr^{79}, 54); 271 (MBr^{79}+1, 26); 272 (MBr^{81}, 57); 273 (MBr^{81}+1, 23).

2-(2-Bromo-5-isopropoxy-4-methoxyphenyl)ethanol.[3] A solution of 2-bromo-5-isopropoxy-4-methoxystyrene (6.03 g, 22 mmol) was dissolved in dry THF (30 mL), cooled on an ice-bath, and BH_3•SMe_2 (12 mL, 2.0 M solution in THF, 24.0 mmol) was added. The mixture was allowed to reach room temperature and stirred for 3 h. The mixture was cooled to 0 °C, and a solution of NaOH (11.27 g, 282 mmol) in EtOH/H_2O (65:35, 30 mL) was added during 1 h. H_2O_2 (30%, 8 mL, 70.6 mmol) was added and the mixture was stirred at room temperature for 3 h. The solvents were removed under

---

[3] Treu, M.; Jordis, U. *Molecules* **2002**, *7*, 374.

reduced pressure and AcOEt (200 mL) was added. The organic solution was washed with water and brine, then dried and concentrated. The residue was purified by flash chromatography. Elution with hexane/AcOEt (gradient from 90:10 to 80:20) gave 4.96 g (77%) of alcohol as a white solid. M.p. 63.7-65.6 ºC; IR (film) ν 3436, 1600, 1256, 1035, 842 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.35 (d, 6H, *J* = 6.4 Hz, 2Me), 1.48 (br, 1H, OH); 2.93 (t , 2H, *J* = 6.8 Hz), 3.83 (s, 3H), 3.86 (t, 2H, *J* = 6.8 Hz), 4.49 (h, 1H, *J* = 6.4 Hz), 6.82 (s, 1H), 7.03 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 22.0 (q, 2Me); 38.8 (t, C2); 56.2 (q, OMe); 62.3 (t, C1); 71.8 (d); 116.4 (d); 118.5 (d); 129.6 (s); 146.6 (s); 149.8 (s). MS (CI) 288 (MBr^{79}, 26); 289 (MBr^{79}+1, 11); 290 (MBr^{81}, 27); 291 (MBr^{81}+1, 7). HRMS *m/z* calcd for $C_{12}H_{18}O_3Br^+$ 289.0439, found 289.0430.

2-(2-Bromo-5-isopropoxy-4-methoxyphenyl)ethyl p-toluenesulfonate (**4**). A solution of TsCl (11.98 g, 62.25 mmol) in CH_2Cl_2 (100 mL) was added to a cooled solution of 2-(2-bromo-5-isopropoxy-4-methoxyphenyl)ethanol (15.0 g, 51.87 mmol), Et_3N (7.94 mL, 57.06 mmol) and DMAP (63.5 mg, 0.51 mmol) in CH_2Cl_2 (500 mL). The reaction mixture was allowed to reach room temperature and was stirred for 8 h. The solution was washed with water, 2N HCl, saturated $NaHCO_3$ and brine, then dried and concentrated. Hexane was added to the residue to yield **4** (19.31 g, 84 %) as a white solid isolated by filtration. IR (film) ν 1361; 1176; 1188 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.35 (d, 6H, *J* = 6.0 Hz, 2Me); 2.43 (s, 3H, Me); 2.99 (t, 2H, *J* = 7.2 Hz); 3.81 (s, 3H, OMe); 4.21 (t, 2H, *J* = 7.2 Hz); 4.45 (h, 1H, *J* = 6.0 Hz); 6.72 (s, 1H); 6.92 (s, 1H); 7.28 (d, 2H, *J* = 6.4 Hz); 7.69 (d, 2H, *J* = 6.4 Hz). ^{13}C NMR ($CDCl_3$) δ 21.6 (q); 22.0 (q, 2Me); 35.3 (t, C2); 56.2 (q, OMe); 69.1 (t, C1); 71.8 (d); 114.5 (s); 116.1 (d); 118.4 (d); 127.3 (s); 127.8 (d, 2CH); 129.7 (d, 2CH); 133.0 (s); 144.6 (s); 146.6 (s); 150.1 (s). MS (CI) 442 (MBr^{79}, 38); 443 (MBr^{79}+1, 14); 444 (MBr^{81}, 36); 445 (MBr^{81}+1, 10).

Methyl 1-[2-bromo-5-isopropoxy-4-methoxyphenethyl]-1H-pyrrole-2-carboxylate. Methyl 1*H*-pyrrole-2-carboxylate (3.38 g, 27.0 mmol) and NaH (60% dispersion, 988 mg, 24.7 mmol) were stirred in dry DMF (25 mL) on an ice-bath for 30 min. A solution of **4** (5.98 g, 13.5 mmol) in dry DMF (35 mL) was added and the mixture was stirred for 5 h at room temperature. The DMF was then evaporated under reduced pressure, and Et_2O was added. The organic solution was washed with 1N HCl, saturated $NaHCO_3$ and brine, then dried and concentrated. The residue was purified by flash chromatography. Elution with hexane/AcOEt (gradient from 95:5 to 90:10) gave 4.34 g (81%) of the title compound as a white solid. M.p. 63 ºC. IR (film) ν 1706, 1497, 1244, 1107, 739 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.27 (d, 6H, *J* = 6.0 Hz, 2Me); 3.08 (t, 2H, *J* = 6.8 Hz); 3.80 (s, 3H, OMe); 3.82 (s, 3H, OMe); 4.34 (h, 1H, *J* = 6.0 Hz, CH); 4.50 (t, 2H, *J* = 6.8 Hz); 5.99 (dd, 1H, *J* = 4.0 and 2.0 Hz, H3); 6.46 (s, 1H); 6.55 (t,

1H, *J* = 2.0 Hz, H5); 6.93 (dd, 1H, *J* = 4.0 and 2.0 Hz, H4); 7.00 (s, 1H). ^{13}NMR ($CDCl_3$) δ 22.0 (q, 2Me); 37.7 (t); 48.9 (t); 51.0 (q); 56.2 (q); 71.7 (d); 107.8 (d); 114.5 (s); 116.0 (d); 118.2 (d); 118.4 (d); 121.3 (s); 129.2 (d); 129.6 (s); 146.6 (s); 149.8 (s); 161.5 (s, C=O). MS (EI) 395 (MBr^{79}, 1); 397 (MBr^{81}, 1). HRMS *m/z* calcd for $C_{18}H_{22}NO_4Br$ 395.0732, found 395.0723.

Methyl 8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-a]isoquinoline-3-carboxylate (**1**). A mixture of methyl 1-[2-bromo-5-isopropoxy-4-methoxyphenethyl]-1*H*-pyrrole-2-carboxylate (307 mg, 0.78 mmol) in dry DMF (5 mL), K_2CO_3 (235 mg, 1.71 mmol), PPh_3 (163 mg, 0.62 mmol), and $PdCl_2(PPh_3)_2$ (218 mg, 0.31 mmol) was purged with Ar for 15 min. The reaction mixture was stirred for 20 h at 125 ºC. After cooling to room temperature, the DMF was removed in vacuo, and Et_2O (100 mL) was added. The organic solution was washed with water and brine, dried, filtered through a pad of celite and concentrated. The residue was purified by flash chromatography. Elution with hexane/AcOEt (gradient from 95:5 to 85:15) gave **1** (199 mg, 81%) as a white solid. M.p. 109 ºC. IR (film) ν 1699, 1489, 1434, 1248, 1130 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.39 (d, 6H, *J* = 6.0 Hz, 2Me); 2.98 (t, 2H, *J* = 6.8 Hz, H6); 3.82 (s, 3H, OMe); 3,88 (s, 3H, OMe); 4.56 (h, 1H, *J* = 6.0 Hz, OCH); 4.60 (t, 2H, *J* = 6.8 Hz, H5); 6.41 (d, 1H, *J* = 4.0 Hz, H1); 6.75 (s, 1H, H7); 7.00 (d, 1H, *J* = 4.0 Hz, H2); 7.05 (s, 1H, H10). ^{13}C NMR ($CDCl_3$) δ 22.1 (q); 28.4 (t); 42.2 (t); 50.9 (q); 56.2 (q); 71.6 (d); 103.4 (d); 107.7 (d); 115.3 (d); 118.4 (d); 121.2 (s); 121.3 (s); 124.5 (s); 136.4 (s); 147.0 (s); 149.6 (s); 161.7 (s, C=O). MS (EI) 315 (M, 56); 316 (M+1, 12); 317 (M+2, 4). HRMS *m/z* calcd for $C_{18}H_{21}NO_4$ 315.1471, found 315.1476.

Methyl 1-bromo-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-a]isoquinoline-3-carboxylate (**5**). A mixture of **1** (1.46 g, 4.64 mmol) and NBS (835 mg, 4.69 mmol) dissolved in THF (40 mL) was stirred at 70 °C under Ar for 3 h. After cooling to room temperature the solvent was removed in vacuo, and the resulting residue was purified by column chromatography on silica gel. Elution with hexane/AcOEt (95:5 and 90:10) afforded **5** (1.72 g, 94%) as white crystals. M.p. 113.5-119.4 ºC. IR (film) 1705, 1440, 1245, 1213, 1197, 1066, 758 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.41 (d, 6H, *J* = 6.0, 2Me); 2.94 (d, 2H, *J* = 6.8 Hz, H6); 3.81 (s, 3H, OMe); 3.91 (s, 3H, OMe); 4.61-4.54 (m, 3H, H5 and OCH); 6.76 (s, 1H, H2); 7.01 (s, 1H, H7); 7.97 (s, 1H, H10). ^{13}C NMR ($CDCl_3$) δ 22.1 (q); 29.0 (t); 42.6 (t); 51.2 (q); 56.1 (q); 71.5 (d); 92.5 (s); 108.8 (d); 114.9 (d); 120.4 (s); 120.5 (s); 121.0 (d); 125.7 (s); 131.5 (s); 147.0 (s); 149.0 (s); 160.9 (s, C=O). MS (CI) 393 (MBr^{79}, 84); 394 (MBr^{79}+1, 100); 395 (MBr^{81}, 82); 396 (MBr^{81}+1, 89). HRMS *m/z* calcd for $C_{18}H_{21}NO_4Br^+$ 395.0732, found 394.0645.

Methyl 1-(4-hydroxy-3-methoxyphenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-a]isoquinoline-3-carboxylate (**7**). A mixture of **5** (249 mg, 0.63 mmol), **6** (237 mg, 0.95 mmol), and 2M Na_2CO_3 (1.26 mL) in DMF (10 mL) was purged with Ar for 10 minutes. Then $Pd(PPh_3)_4$ (147 mg, 0.126 mmol) was added and Ar was bubbled through the mixture for 5 minutes. The temperature was raised to 125 °C and stirring was continued for 16 h. After cooling, the solvents were evaporated in vacuo, and Et_2O was added. The organic solution was washed with water and brine, dried, and concentrated. The residue was purified by flash chromatography. Elution with hexane/AcOEt (50:50 and 40:60) gave **7** (215 mg, 78%) as a light brown solid. M.p. 176-180.9 ºC; IR (film) ν 3435, 3023, 1698, 1464, 1440, 1239, 1195, 760 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.37 (d, 6H, *J* = 6.0 Hz, 2Me); 2.99 (t, 2H, *J* = 6.4 Hz, H6); 3.42 (s, 3H, OMe); 3.84 (s, 6H, 2OMe); 4.53 (h, 1H, *J* = 6.0 Hz, CH); 4.60 (t, 2H, *J* = 6.4 Hz, H5); 5.62 (br, 1H, OH); 6.74 (s, 1H, H2); 6.89 (s, 1H, H7); 6.92 (d, 1H, *J* = 8.0 Hz, H5'); 6.94 (d, 1H, *J* = 2.0 Hz, H2'); 6.96 (dd, 1H, *J* = 8.0 and 2.0 Hz, H6'); 6.97 (s, 1H, H10). ^{13}C NMR ($CDCl_3$) δ 22.1 (q, Me); 29.0 (t, C6); 42.5 (t, C5); 51.1 (q, Me); 55.5 (q, Me); 56.0 (q, Me); 71.4 (d, OCH); 109.2 (d,C7); 112.0 (d, C5'); 114.3 (d, C6'); 114.7 (d, C2); 119.2 (d, C10); 120.0 (s); 121.2 (s); 121.7 (s); 122.4 (d, C2'); 125.7 (s); 128.6 (s); 131.7 (s); 144.5 (s); 146.4 (s); 146.5 (s); 148.6 (s); 161.8 (s). MS (EI) 437 (M, 92); 438 (M+1, 47); 396 (56); 395 (100); 394 (47). HRMS *m/z* calcd for $C_{25}H_{27}NO_6$ 437.1838, found 437.1833.

Methyl 8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-a]isoquinoline-3-carboxylate (**8**). K_2CO_3 (271 mg, 1.96 mmol) and 2-bromopropane (1.2 mL, 12.83 mmol) were added to **7** (419 mg, 0.96 mmol) in dry DMF (6 mL). The mixture was stirred at 80 ºC under Ar for 17 h. The mixture was then cooled to room temperature and the solvent removed in vacuo. AcOEt was added and the organic solution was washed with water, saturated $NaHCO_3$ and brine. The organic layer was then dried, filtered and concentrated. The residue was purified by flash chromatography. Elution with hexane/AcOEt (50:50) gave **8** (379 mg, 83%) as a light brown solid. IR (film) ν 1700, 1464, 1440, 1423, 1192 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.37 (2d, 12H, *J* = 6.0 Hz, 4Me); 3.00 (t, 2H, *J* = 6.8 Hz, H6); 3.40 (s, 3H, OMe); 3.81 (s, 3H, OMe); 3.84 (s, 3H, OMe); 4.53 (h, 2H, *J* = 6.0 Hz, 2CH); 4.61 (t, 2H, *J* = 6.8 Hz, H5); 6.74 (s, 1H, H2); 6.90 (s, 1H, H7); 6.92 (br, 1H, H2'); 6.94-6.98 (m, 2H, H6' and H5'); 6.99 (s, 1H, H10). ^{13}C NMR ($CDCl_3$) δ 22.1 (2q, 4Me); 29.0 (t, C6); 42.6 (t, C5); 51.1 (q, OMe); 55.4 (q, OMe); 56.0 (q, OMe); 71.4 (d, OCH); 71.6 (d, OCH); 109.2 (d, C7); 113.3 (d, C2'); 114.7 (d, C2); 116.4 (d, C5'); 119.3 (d, C10); 120.1 (s); 121.2 (s); 121.7 (d, C6'); 125.7 (s); 129.8 (s); 131.7 (s); 146.1 (s); 146.5 (s); 148.6 (s); 150.4 (s); 161.8 (s, C=O). MS (CI) 479 (M, 78); 480 (M+1, 100); 481 (M+2, 40). HRMS *m/z* calcd for $C_{28}H_{33}NO_6$ 479.2308, found 479.2320.

Methyl 2-bromo-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-a]isoquinoline-3-carboxylate (**9**). Compound **8** (756 mg, 1.58 mmol) was dissolved in freshly distilled THF, and NBS (339 mg, 1.90 mmol) was added in one portion. The mixture was stirred under Ar for 1.5 h at 70 °C. The solvent was removed under reduced pressure and the residue was purified by flash chromatography. Elution with hexane/AcOEt (gradient from 75:25 to 50:50) gave **9** (741 mg, 84%) as a yellow solid. IR (film) ν 1694, 1463, 1440, 1111. ^{1}H NMR ($CDCl_3$) δ 1.35 (d, 6H, *J* = 8.0 Hz, 2Me); 1.38 (d, 6H, *J* = 8.0 Hz, 2Me); 2.98 (t, 2H, *J* = 6.0 Hz, H6); 3.30 (s, 3H, OMe); 3.81 (s, 3H, OMe); 3.90 (s, 3H, OMe); 4.54 (m, 4H, H5 and 2OCH); 6.57 (s, 1H, H7); 6.70 (s, 1H, H10); 6.87 (dd, 1H, *J* = 7.9 and 2.0 Hz, H6'); 6.88 (br, 1H, H2'); 6.96 (d, *J* = 7.9 Hz, 1H, H5'). ^{13}C NMR ($CDCl_3$) δ 22.1 (q, 4Me); 28.7 (t, C6); 43.5 (t, C5); 51.2 (q, OMe); 55.1 (q, OMe); 56.1 (q, OMe); 71.4 (d, 2H, OCH); 107.7 (s, C2); 109.1 (d, C7); 114.6 (d, C10); 114.8 (d, C6'); 115.7 (d, C5'); 118.5 (s); 120.2 (s, C10a); 122.6 (s, C1'); 123.4 (s, C1); 125.5 (s, C6a); 127.6 (s, C4'); 132.1 (s, C10b); 146.8 (s, C8); 148.6 (s, C9); 150.4 (s, C3'); 161.4 (s, C=O). MS (CI) 560 (MBr^{81}, 84); 559 (MBr^{79}+1); 558 (MBr^{79}). HRMS *m/z* calcd for $C_{28}H_{32}NO_6Br$ 557.1413, found 557.1429.

2-(2,4-Diisopropoxy-5-methoxyphenyl)-boronic acid (**11a**). *n*BuLi (10.3 mL, 0.4M in hexane, 4.12 mmol) was added to 1-bromo-2,5-diisopropoxy-4-methoxybenzene (832 mg, 2.74 mmol) in dry THF (20 mL) at -78 ºC under Ar and was stirred for 15 min. Triisopropyl borate (0.95 mL, 4.12 mmol) was added and the mixture was allowed to reach room temperature and stirred overnight. The solvent was evaporated under reduced pressure and the resulting crude was quenched with water at 0 ºC and 2M HCl. The aqueous solution was extracted with Et_2O. The organic solution dried and concentrated afforded a crude material which was purified by flash column chromatography. Elution with hexane/AcOEt (gradient from 85:15 to 80:20) gave 147 mg (20%) of **11a** as a brown syrup. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.38 (d, 6H, *J* = 6.0 Hz, 2Me); 1.39 (d, 6H, *J* = 6.4 Hz, 2Me); 3.85 (s, 3H, OMe); 4.55-4.58 (m, 2H); 5.81 (s, 1H); 6.53 (s, 1H).

SUPPLEMENTARY NMR SPECTRA

for the

full communication

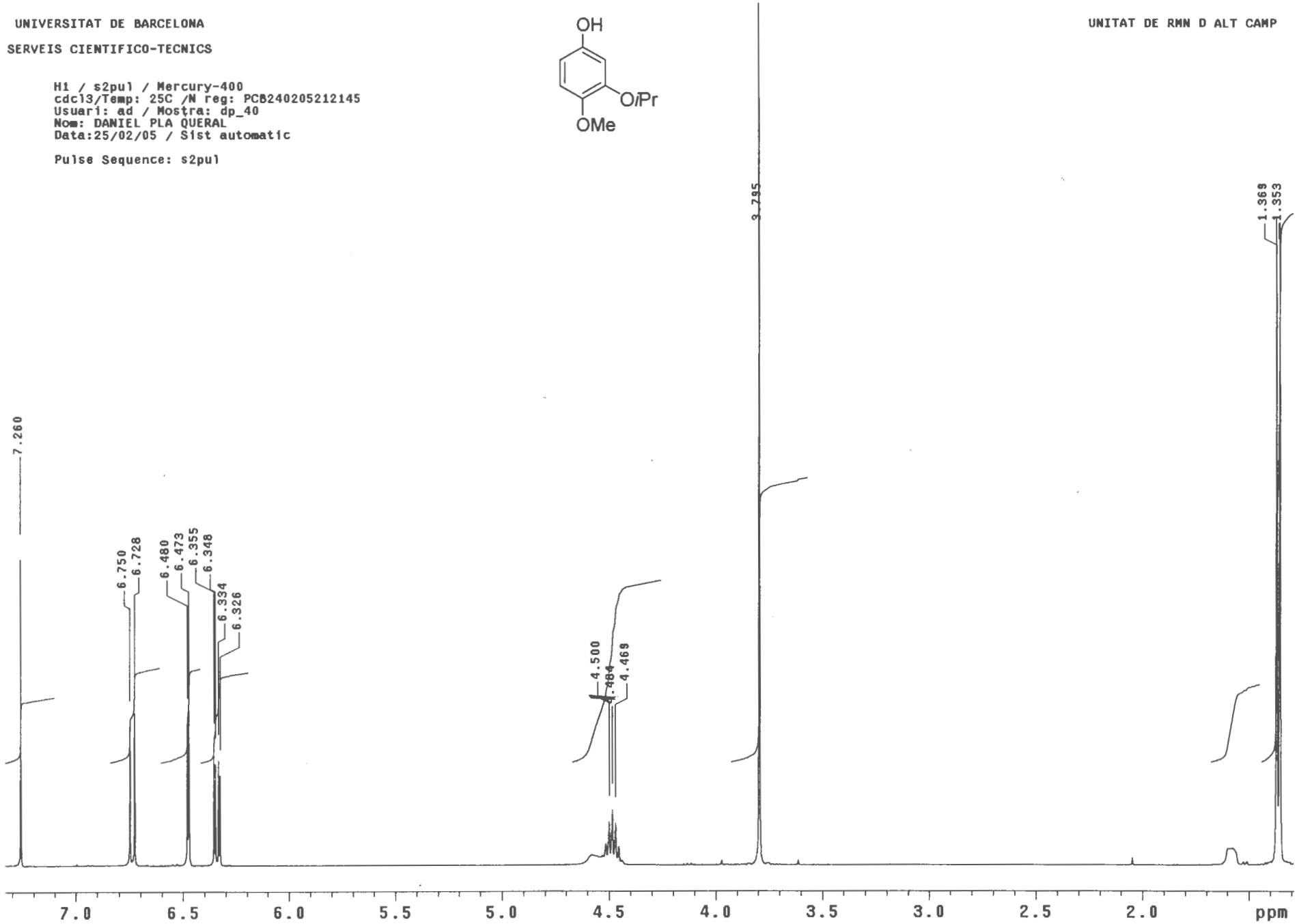
entitled

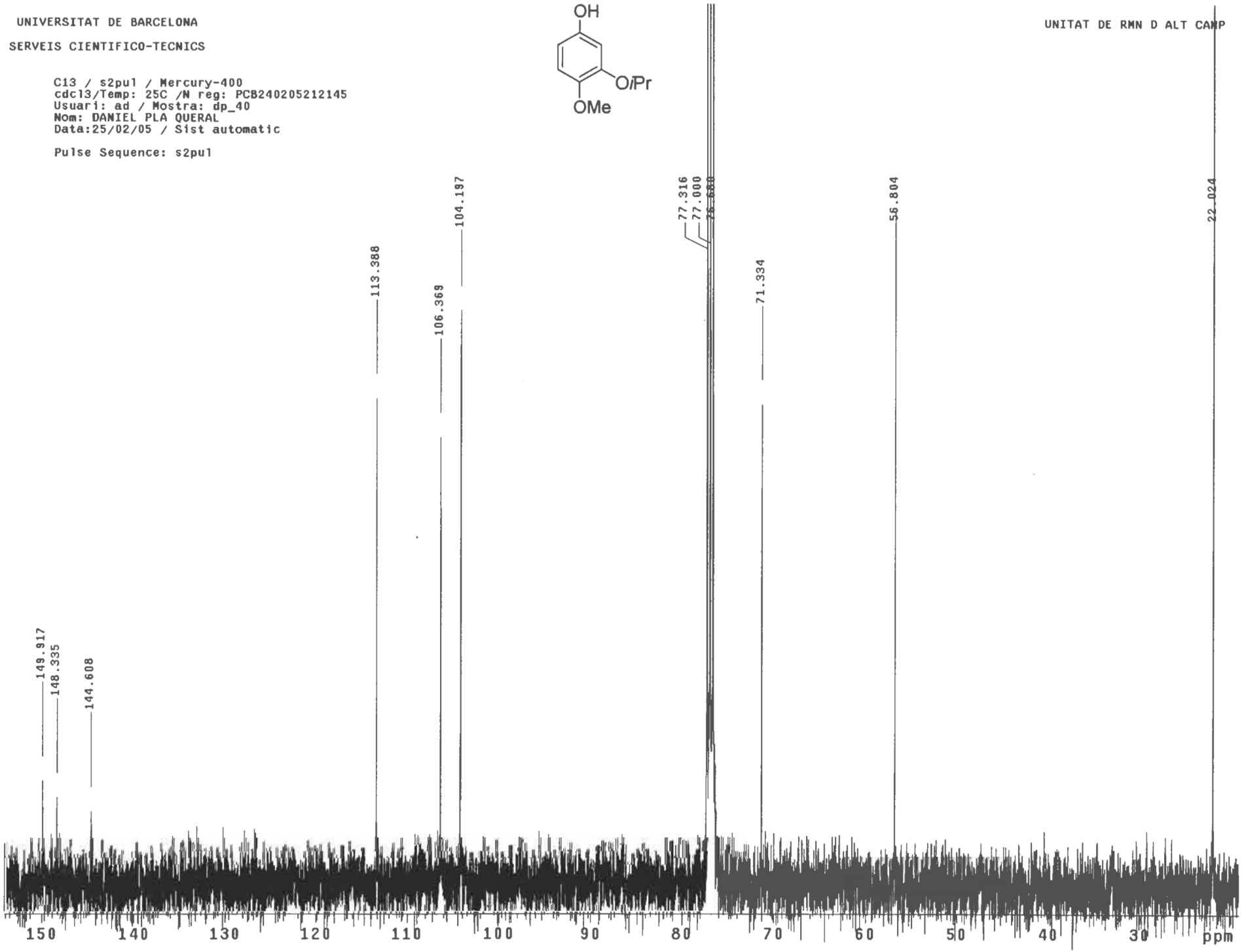
Modular Total Synthesis of Lamellarin D

authored by

Daniel Pla,[†] *Antonio Marchal,*[†,∥] *Christian A. Olsen,*[†,◦] *Fernando Albericio,*[†,‡,*] *Mercedes Álvarez*[†,§,*]

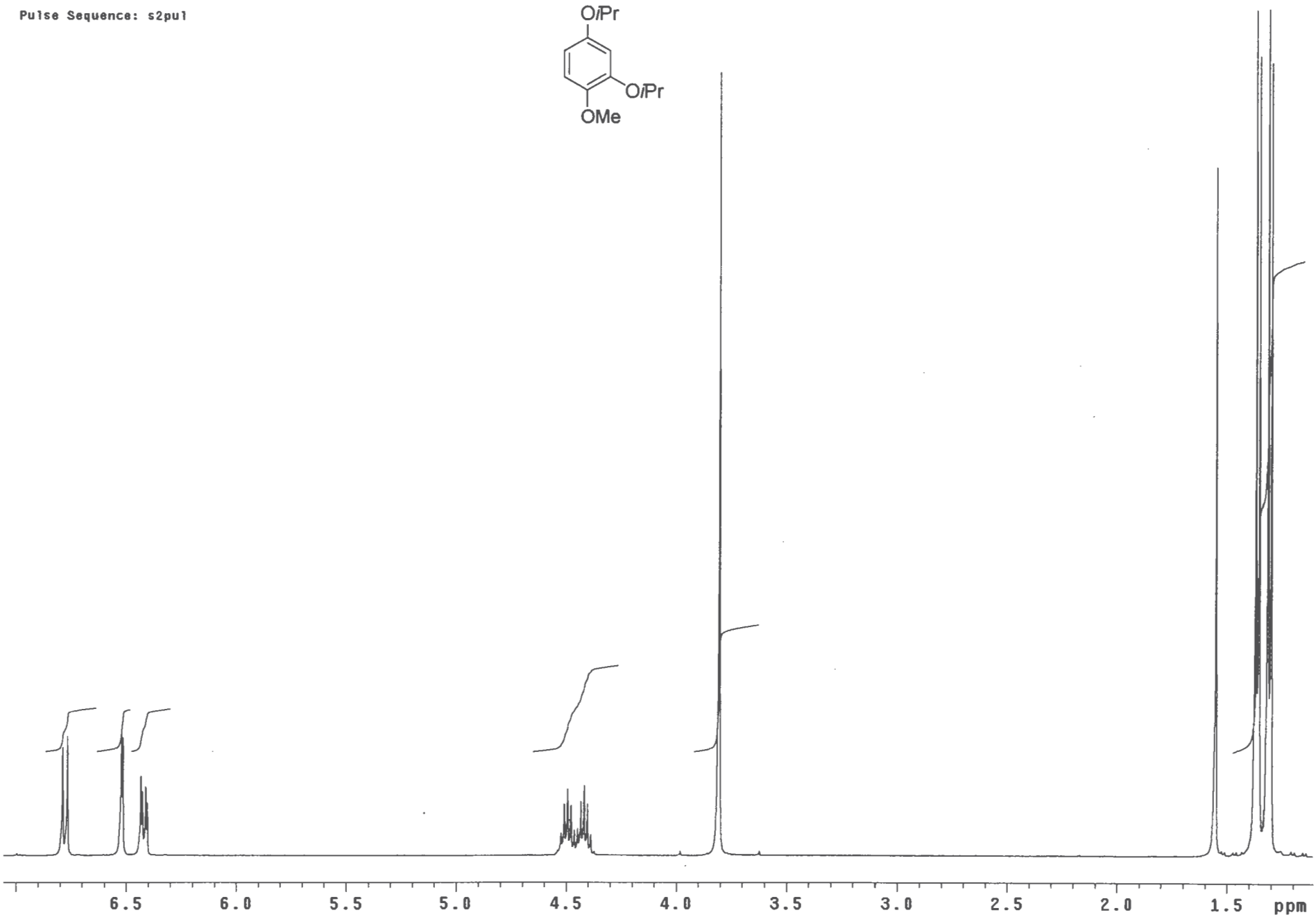
[†] Biomedical Research Institute, Barcelona Scientific Park-University of Barcelona, 08028 Barcelona; [∥] Current address: Department of Inorganic and Organic Chemistry, University of Jaén, 23071 Jaén; [◦] Current address: The Danish University of Pharmaceutical Sciences, Copenhagen, Denmark; [‡] Department of Organic Chemistry, University of Barcelona, 08028 Barcelona; [§] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona.


UNIVERSITAT DE BARCELONA
SERVEIS CIENTIFICO-TECNICS
H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB240205212145
Usuari: ad / Mostra: dp_40
Nom: DANIEL PLA QUERAL
Data:25/02/05 / Sist automatic
Pulse Sequence: s2pul
OH
OiPr
OMe
UNITAT DE RMN D ALT CAMP
7.260
6.750
6.728
6.480
6.473
6.355
6.348
6.334
6.326
4.500
4.484
4.469
3.795
1.368
1.353
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm


UNIVERSITAT DE BARCELONA
SERVEIS CIENTIFICO-TECNICS
UNITAT DE RMN D ALT CAMP
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB240205212145
Usuari: ad / Mostra: dp_40
Nom: DANIEL PLA QUERAL
Data:25/02/05 / Sist automatic
Pulse Sequence: s2pul
OH
OiPr
OMe
149.917
148.335
144.608
113.388
106.369
104.197
77.316
77.000
71.334
56.804
22.024
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

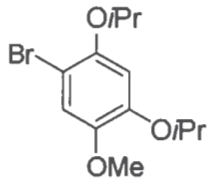
H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB210305061557
Usuari: ad / Mostra: dp_58
Nom: DANIEL PLA QUERAL
Data:21/03/05 / Sist automatic

Pulse Sequence: s2pul



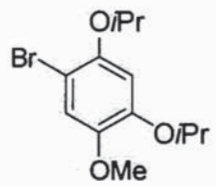
H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB030405202936
Usuari: ad / Mostra: dp_56_t2
Nom: DANIEL PLA QUERAL
Data:03/04/05 / Sist automatic

Pulse Sequence: s2pul



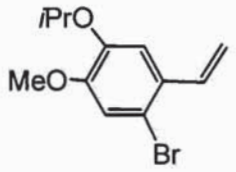
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB090505181026
Usuari: ad / Mostra: dp56t2
Nom: DANIEL PLA QUERAL
Data:10/05/05 / Sist automatic

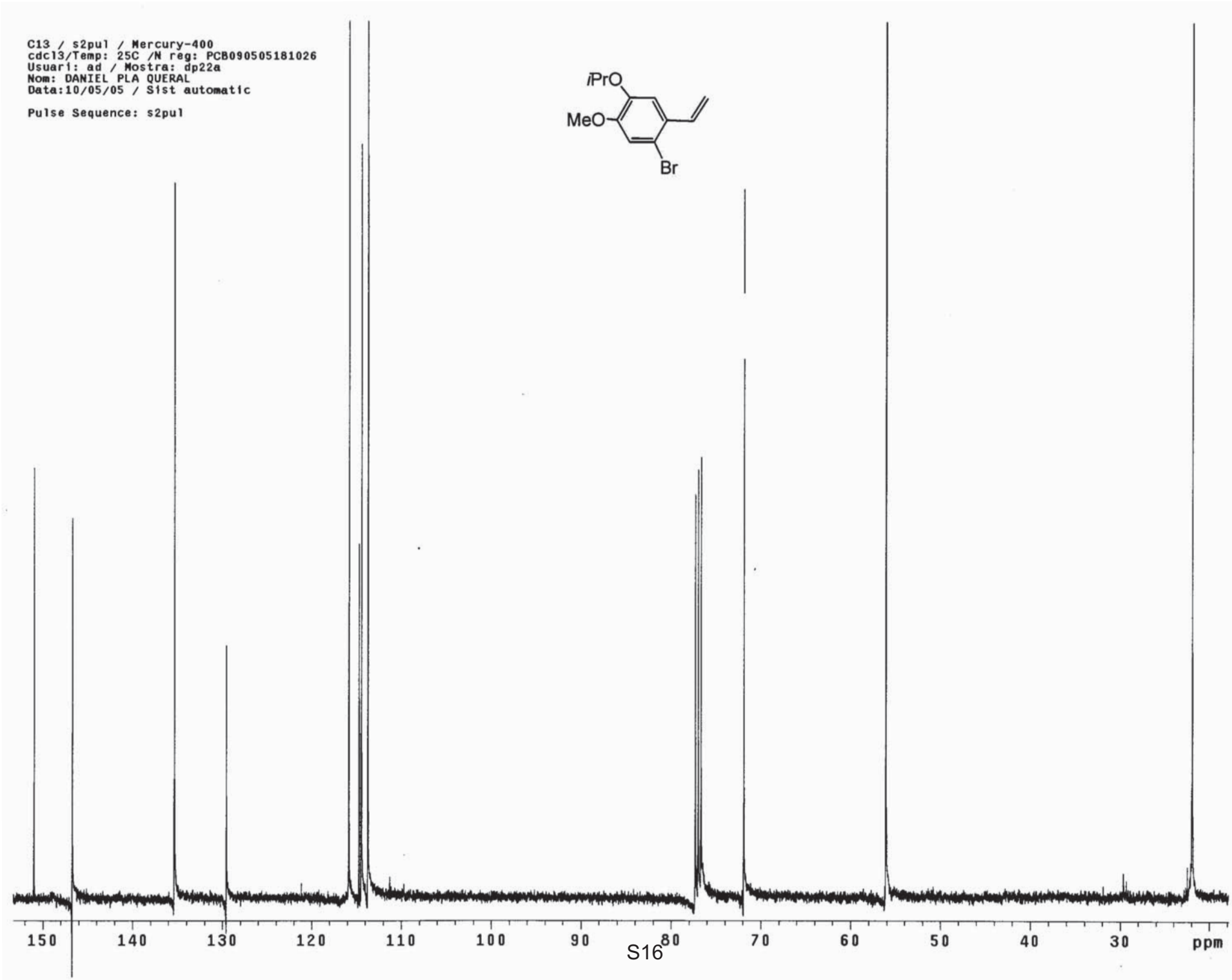
Pulse Sequence: s2pul



H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB0B1104103730
Usuari: ad / Mostra: dp_8_9pur
Nom: DANIEL PLA QUERAL
Data:08/11/04 / Sist automatic

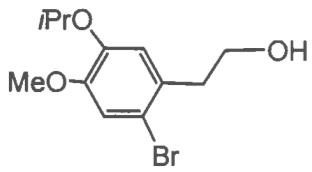
Pulse Sequence: s2pul

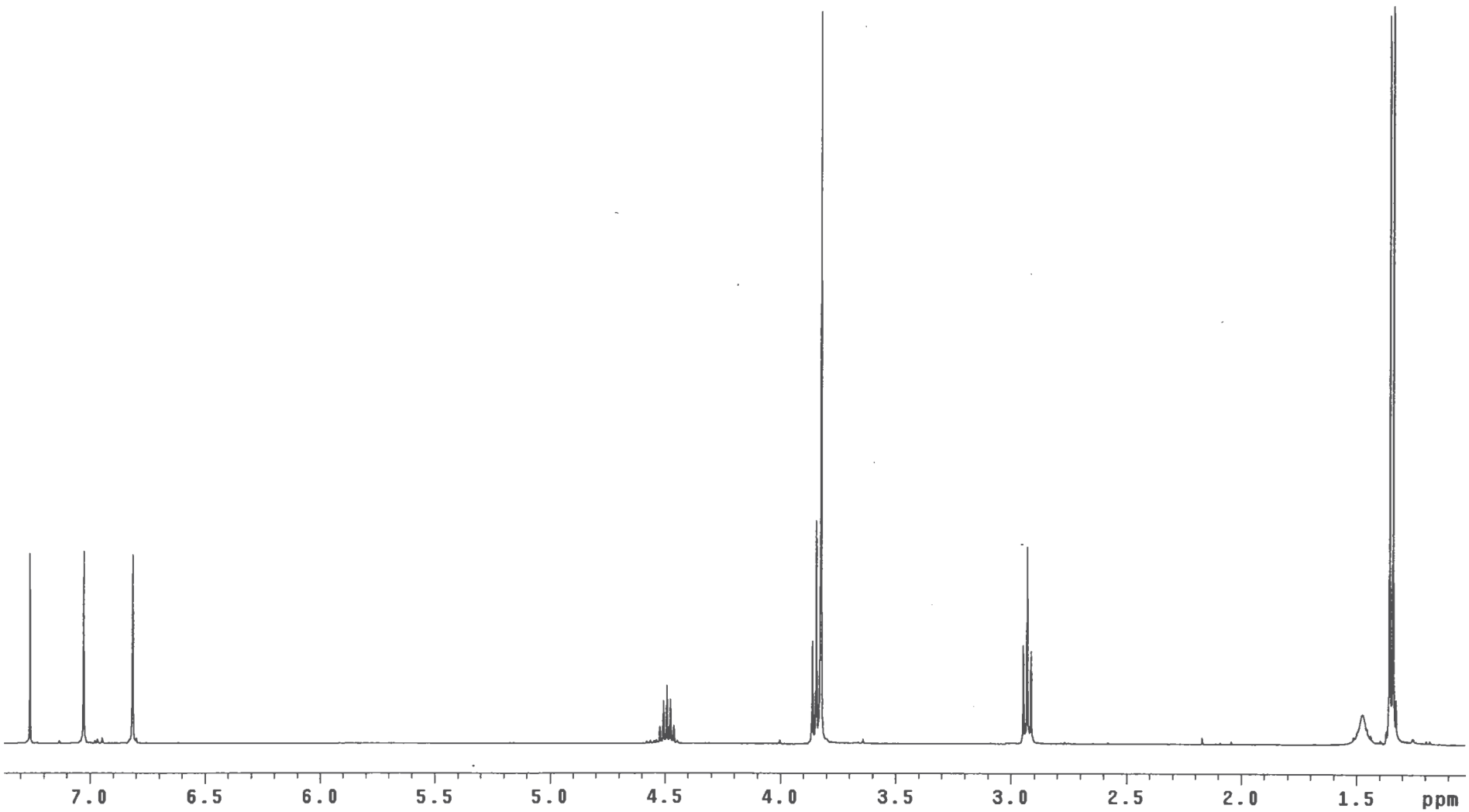



C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB090505181026
Usuari: ad / Mostra: dp22a
Nom: DANIEL PLA QUERAL
Data:10/05/05 / Sist automatic
Pulse Sequence: s2pul
iPrO
MeO
Br
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB171204152929
Usuari: ad / Mostra: dp_19antimar
Nom: DANIEL PLA QUERAL
Data:17/12/04 / Sist automatic

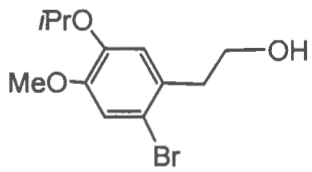
Pulse Sequence: s2pul

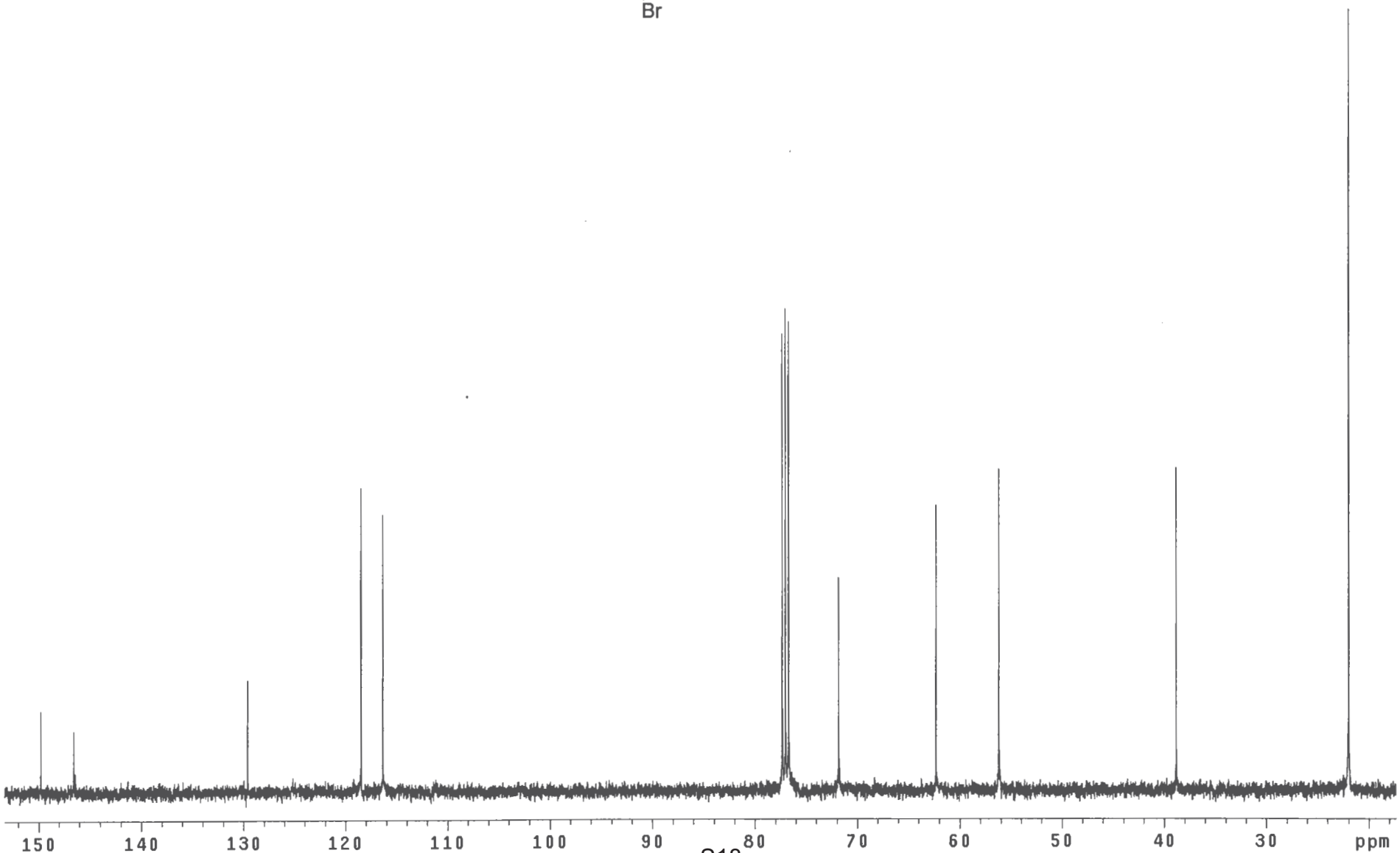


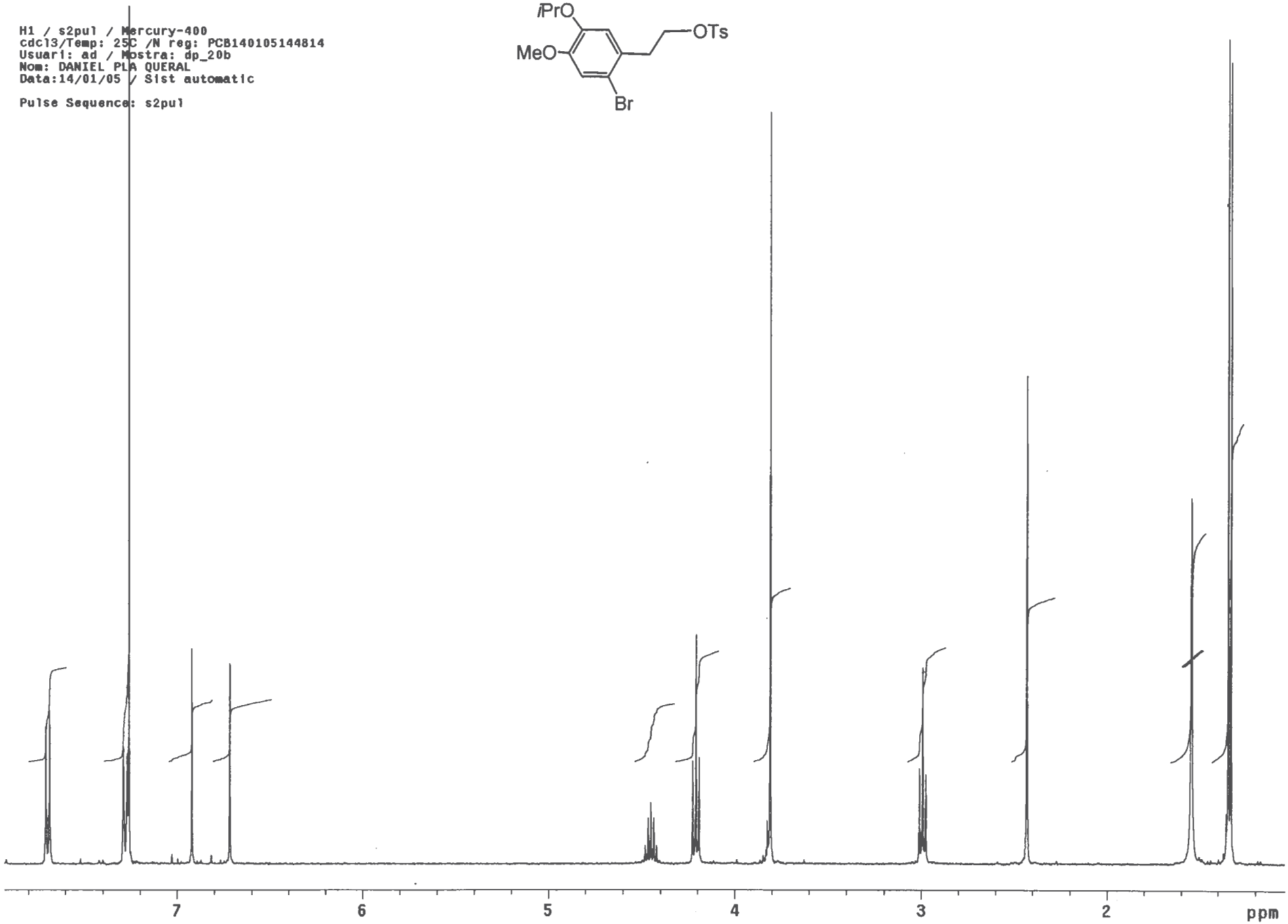


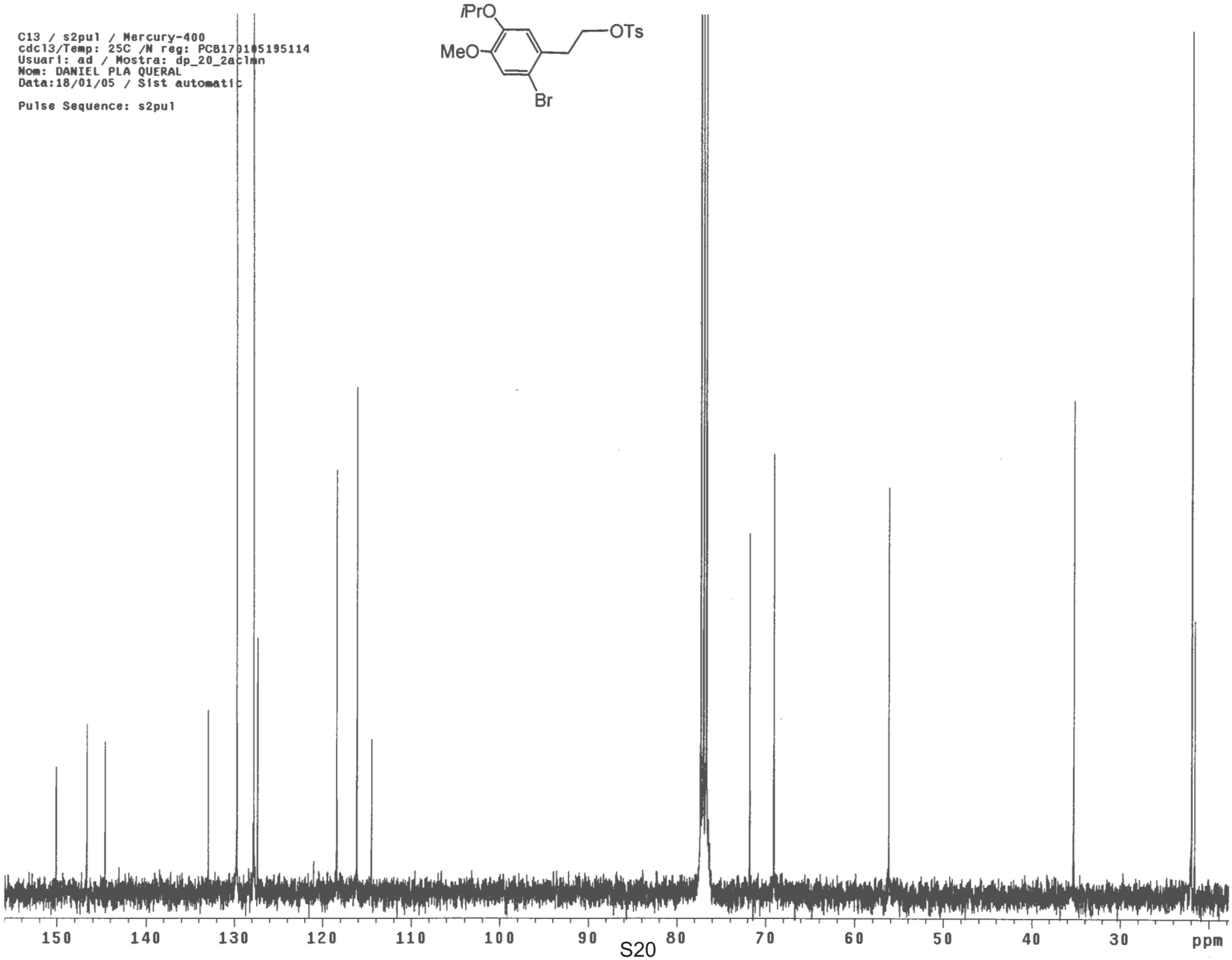
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB011204125655
Usuari: ad / Mostra: dp3ahidrobd
Nom: DANIEL PLA QUERAL
Data:01/12/04 / Sist automatic

Pulse Sequence: s2pul



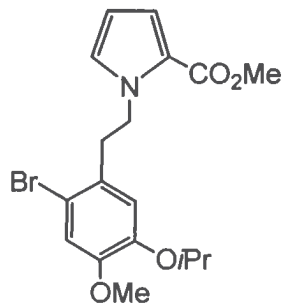



H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB140105144814
Usuari: ad / Mostra: dp_20b
Nom: DANIEL PLA QUERAL
Data:14/01/05 / Sist automatic
Pulse Sequence: s2pul
iPrO
MeO
OTs
Br
7
6
5
4
3
2
ppm


C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB170105195114
Usuari: ad / Mostra: dp_20_2aclmn
Nom: DANIEL PLA QUERAL
Data:18/01/05 / Sist automatic
Pulse Sequence: s2pul
iPrO
MeO
OTs
Br
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

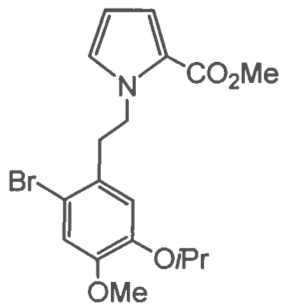
H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB150105235200
Usuari: ad / Mostra: dp_22b
Nom: DANIEL PLA QUERAL
Data:16/01/05 / Sist automatic

Pulse Sequence: s2pul



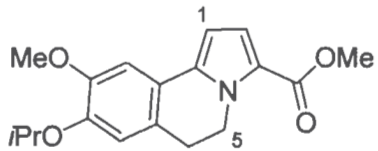
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB150105235200
Usuari: ad / Mostra: dp_22b
Nom: DANIEL PLA QUERAL
Data:16/01/05 / Sist automatic

Pulse Sequence: s2pul



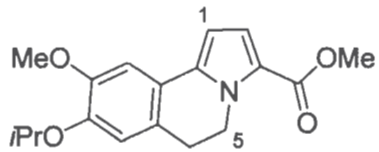
H1 / s2pul / Mercury-400_qui
cdcl3/Temp: 25C /N reg: M40005-1347
Usuari: ad / Mostra: dp_23b
Nom: DANIEL PLA QUERAL
Data:19/01/05 / Sist automatic

Pulse Sequence: s2pul



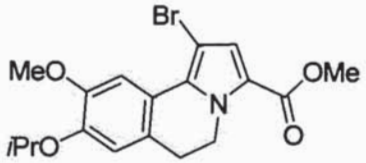
C13 / s2pul / Mercury-400_qui
cdcl3/Temp: 25C /N reg: M40005-1347
Usuari: ad / Mostra: dp_23b
Nom: DANIEL PLA QUERAL
Data:19/01/05 / Sist automatic

Pulse Sequence: s2pul



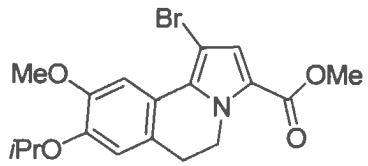
H1 / s2pul / Mercury-400_qui
cdcl3/Temp: 25C /N reg: M40005-1450
Usuari: ad / Mostra: dp_27b
Nom: DANIEL PLA QUERAL
Data:28/01/05 / Sist automatic

Pulse Sequence: s2pul



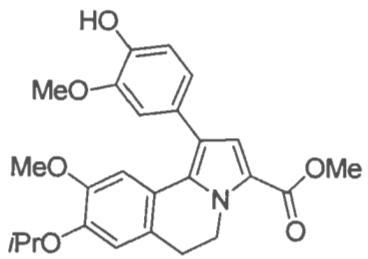
C13 / s2pul / Mercury-400_qui
cdcl3/Temp: 25C /N reg: M40005-1450
Usuari: ad / Mostra: dp_27b
Nom: DANIEL PLA QUERAL
Data:28/01/05 / Sist automatic

Pulse Sequence: s2pul



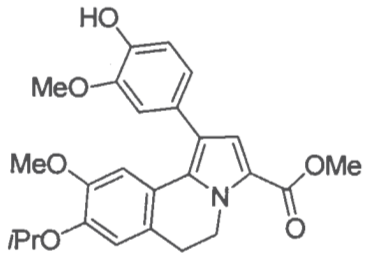
H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB040504105457
Usuari: ad / Mostra: cao49
Nom: PABLOGABRIEL CIRONI LOPEZ
Data:04/05/04 / Sist automatic

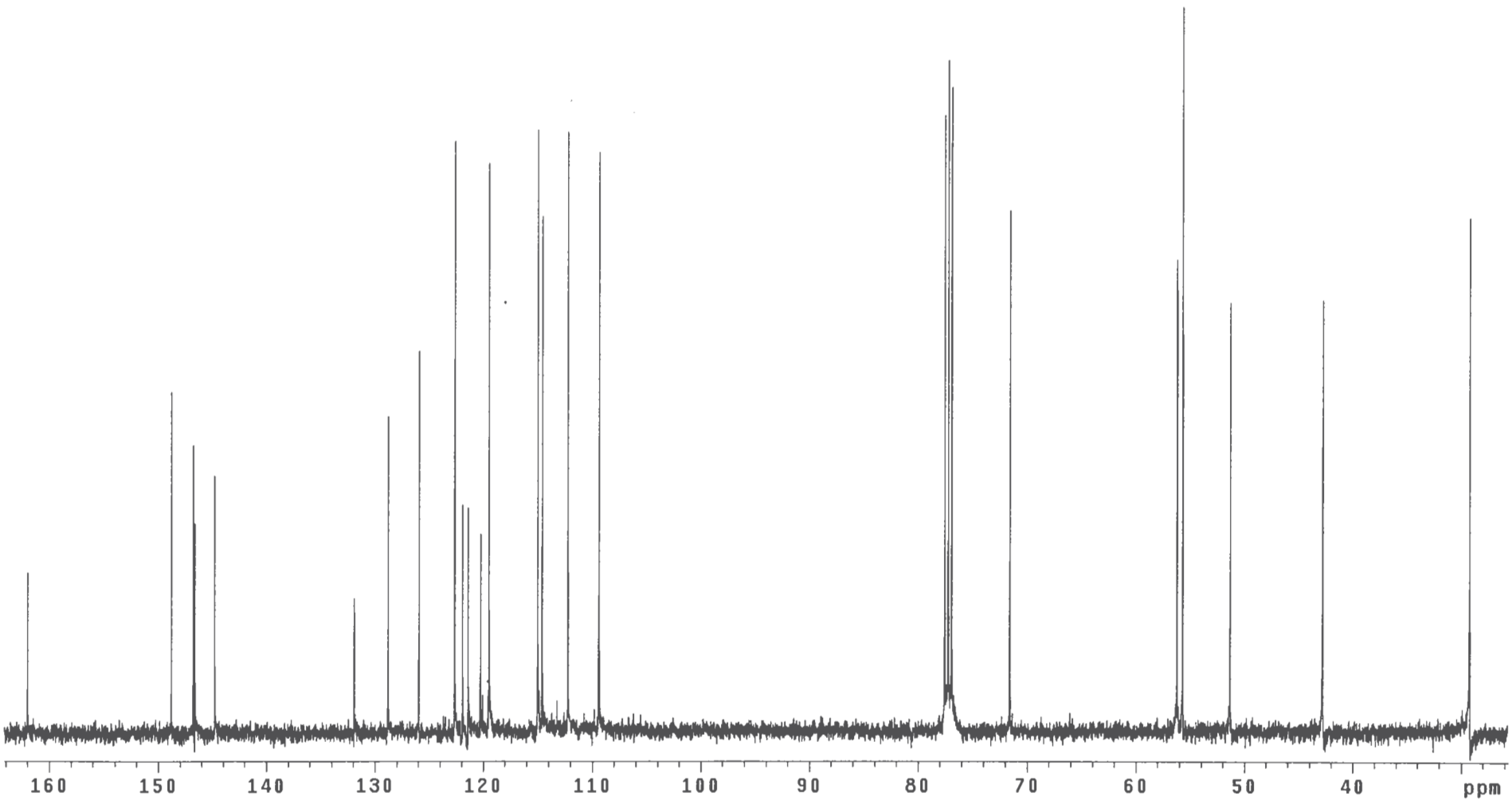
Pulse Sequence: s2pul



C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: M40004-2627
Usuari: ad / Mostra: cao49
Nom: PABLOGABRIEL CIRONI LOPEZ
Data:04/05/04 / Sist automatic

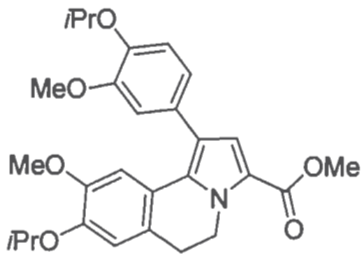
Pulse Sequence: s2pul





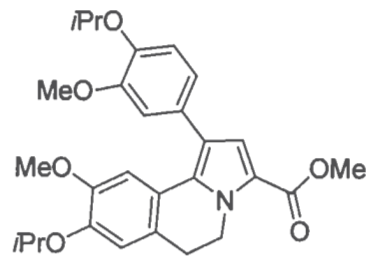
H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB090205114936
Usuari: ad / Mostra: dp_32pur
Nom: DANIEL PLA QUERAL
Data:09/02/05 / Sist automatic

Pulse Sequence: s2pul



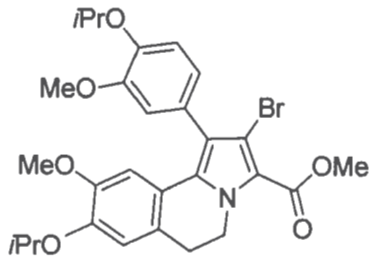
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: M40005-1806
Usuari: ad / Mostra: dp32pur
Nom: DANIEL PLA QUERAL
Data:12/02/05 / Sist automatic

Pulse Sequence: s2pul



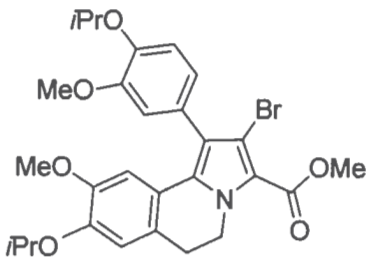
H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: M40005-1850
Usuari: ad / Mostra: dp34
Nom: DANIEL PLA QUERAL
Data:11/02/05 / Sist automatic

Pulse Sequence: s2pul



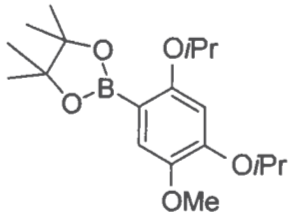
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: M40005-1850
Usuari: ad / Mostra: dp34
Nom: DANIEL PLA QUERAL
Data:11/02/05 / Sist automatic

Pulse Sequence: s2pul



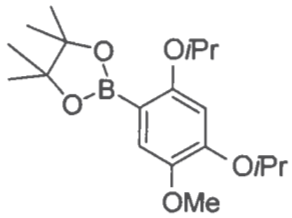
H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB080305175427
Usuari: ad / Mostra: dp_50b
Nom: DANIEL PLA QUERAL
Data:08/03/05 / Sist automatic

Pulse Sequence: s2pul



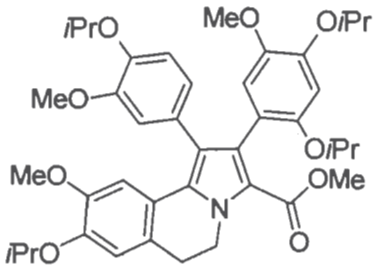
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB080305194610
Usuari: ad / Mostra: dp_50b
Nom: DANIEL PLA QUERAL
Data:08/03/05 / Sist automatic

Pulse Sequence: s2pul



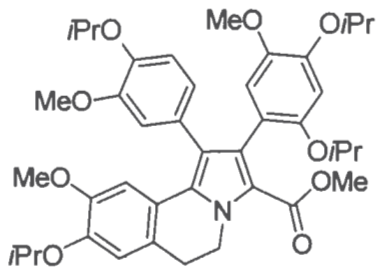
H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB110305180102
Usuari: ad / Mostra: dp_48c
Nom: DANIEL PLA QUERAL
Data:11/03/05 / Sist automatic

Pulse Sequence: s2pul



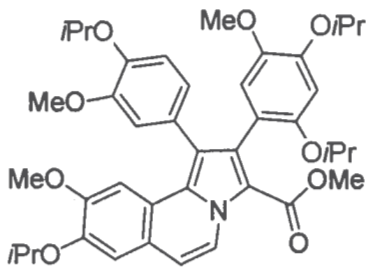
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: M40005-2107
Usuari: ad / Mostra: dp48c
Nom: DANIEL PLA QUERAL
Data:11/03/05 / Sist automatic

Pulse Sequence: s2pul



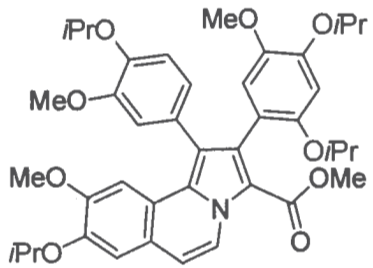
H1 / s2pul / Mercury-400
acetone/Temp: 25C /N reg: PCB050505174149
Usuari: ad / Mostra: dp5720mb23
Nom: DANIEL PLA QUERAL
Data:05/05/05 / Sist automatic

Pulse Sequence: s2pul



C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB150405191914
Usuari: ad / Mostra: dp_67b
Nom: DANIEL PLA QUERAL
Data:16/04/05 / Sist automatic

Pulse Sequence: s2pul

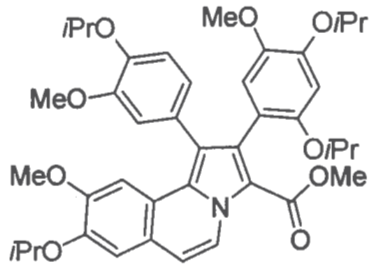


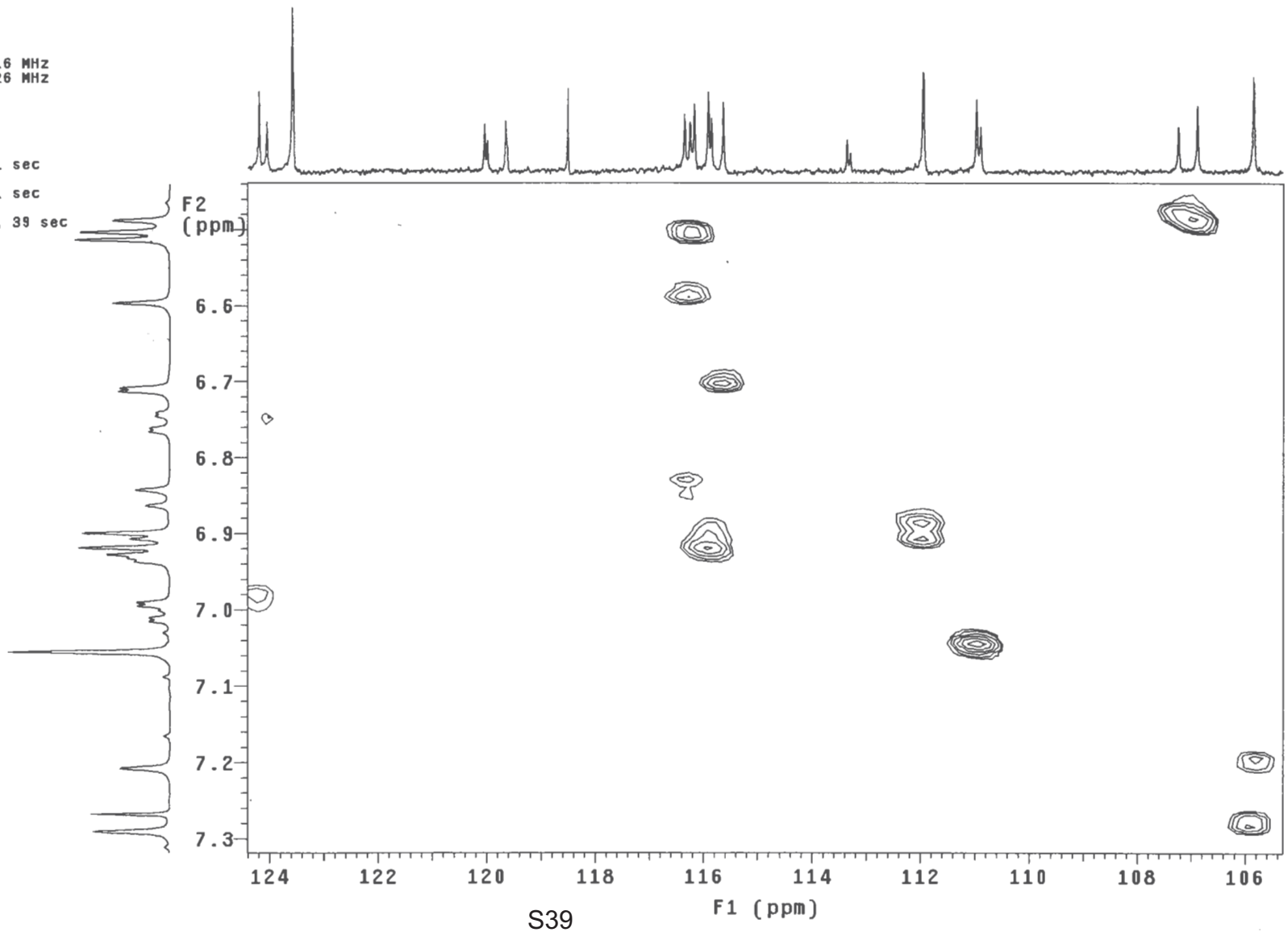
H1 / gHSQC / Mercury-400
cdcl3/Temp: 25C /N reg: PCB300305201722
Usuari: ad / Mostra: dp_5720m_b1
Nom: DANIEL PLA QUERAL
Data:31/03/05 / Sist automatic

Pulse Sequence: gHSQC

Solvent: cdcl3
Temp. 25.0 C / 298.1 K
File: MG0331-dp_5720m_b1-gHSQC
INOVA-500 "menhir"

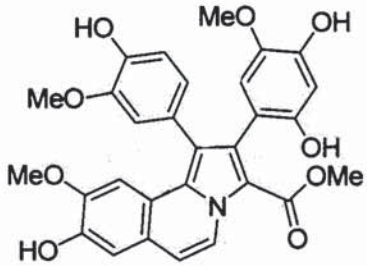
PULSE SEQUENCE: gHSQC
Relax. delay 1.000 sec
Acq. time 0.219 sec
Width 4672.9 Hz
2D Width 17094.0 Hz
16 repetitions
2 x 200 increments
OBSERVE H1, 400.1226116 MHz
DECOUPLE C13, 100.6184326 MHz
Power 45 dB
on during acquisition
off during delay
GARP-1 modulated
DATA PROCESSING
Gauss apodization 0.101 sec
F1 DATA PROCESSING
Gauss apodization 0.011 sec
FT size 2048 x 2048
Total time 2 hr, 18 min, 39 sec

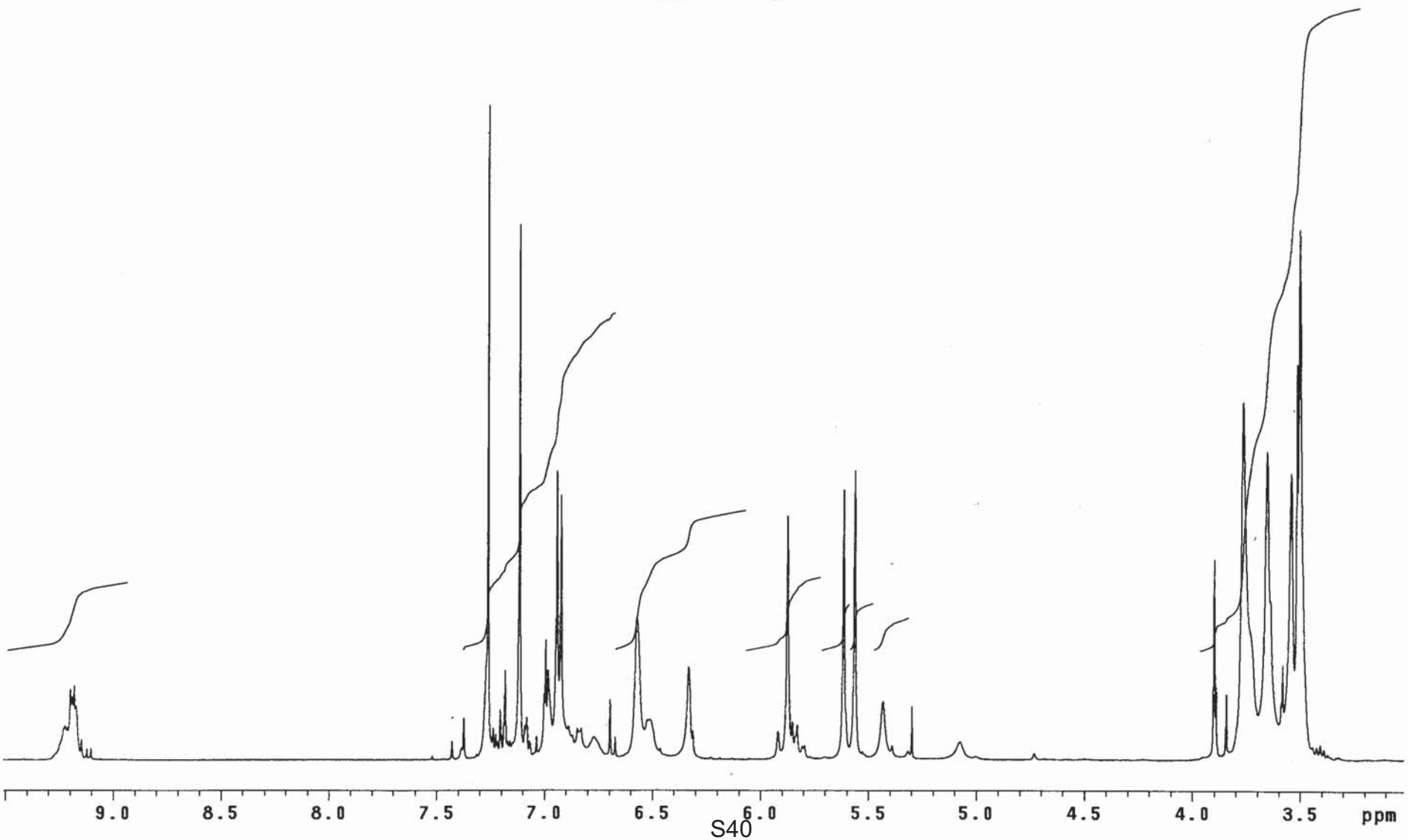




H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB170405163807
Usuari: ad / Mostra: dp_68c
Nom: DANIEL PLA QUERAL
Data:17/04/05 / Sist automatic

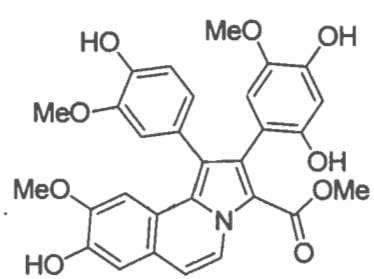
Pulse Sequence: s2pul





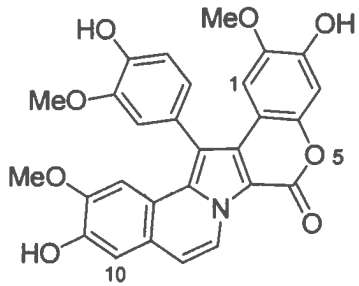
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: PCB170405183226
Usuari: ad / Mostra: dp_68c
Nom: DANIEL PLA QUERAL
Data:17/04/05 / Sist automatic

Pulse Sequence: s2pul



H1 / s2pul / Mercury-400
dmso/Temp: 25C /N reg: PCB220405180436
Usuari: ad / Mostra: dp69a
Nom: DANIEL PLA QUERAL
Data:22/04/05 / Sist automatic

Pulse Sequence: s2pul



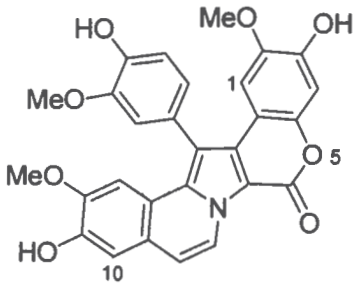
UNIVERSITAT DE BARCELONA

SERVEIS CIENTIFICO-TECNICS

UNITAT DE RMN D ALT CAMP

C13 / s2pul / Mercury-400
dmso/Temp: 25C /N reg: PCB220405180436
Usuari: ad / Mostra: dp69a
Nom: DANIEL PLA QUERAL
Data:23/04/05 / Sist automatic

Pulse Sequence: s2pul



4

SÍNTESI I ESTUDI DE LES RELACIONS ESTRUCTURA-ACTIVITAT D'ANÀLEGS CITOTÒXICS POTENTS DE LA LAMEL·LARINA D

SÍNTESI I ESTUDI DE LES RELACIONS ESTRUCTURA-ACTIVITAT D'ANÀLEGS CITOTÒXICS POTENTS DE LA LAMEL·LARINA D.

Synthesis and structure-activity relationship study of potent cytotoxic analogues of the marine alkaloid Lamellartin D.

Daniel Pla,[a] Antonio Marchal,[a‖] Christian A. Olsen,[a,⇕] Andrés Francesch,[b] Carmen Cuevas,[b] Fernando Albericio,[a, ‡,*] Mercedes Álvarez [a, §,*]

Journal of Medicinal Chemistry, **2006**, *49*, 3257-3268.

[a] Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Josep Samitier 1-5 , 08028 Barcelona, Spain.

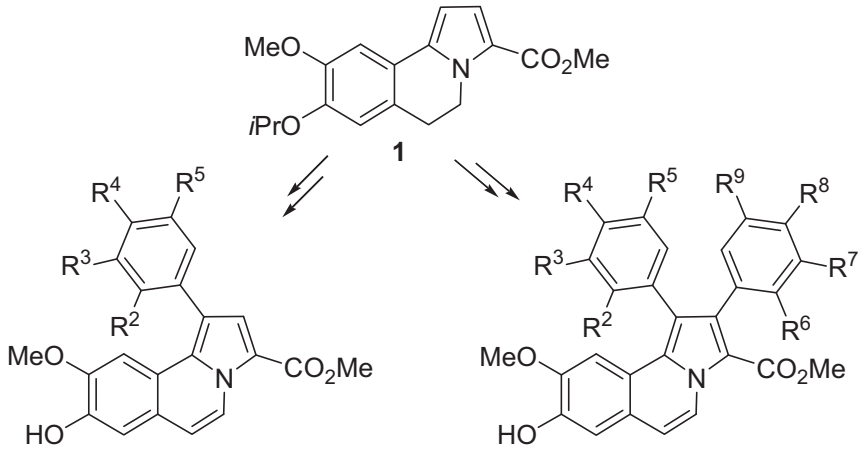
[b] Pharma Mar, Avda Reyes Católicos 1, E- 28770 Colmenar Viejo, Madrid, Spain.

[‖] Current address: Department of Inorganic and Organic Chemistry, University of Jaén, 23071 Jaén; amarchal@ujaen.es

[⇕] Current address: The Danish University of Pharmaceutical Sciences, Copenhagen, Denmark cao@dfuni.dk

[‡] Department of Organic Chemistry, University of Barcelona, 08028 Barcelona

[§] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona



Resum

En aquest capítol es descriu la preparació d'una quimioteca d'anàlegs de cadena oberta de la Lam-D a partir del compost tricíclic 5,6-dihidropirrolo[2,1-*a*]isoquinolina-3-carboxilat de metil (**1**). La introducció de diferents grups aryl mitjançant una química sequencial amb bromació regioselectiva seguida d'acoblaments creuats de Suzuki i catàlisi de Pd(0) ha permès la preparació de més de 40 anàlegs oberts de la Lam-D. Els compostos han estat obtinguts amb rendiment global de 24-44%. La seva activitat inhibidora del creixement cel·lular (GI_{50}) s'ha assajat en un panell de tres línies tumorals humanes, MDA-MB-231 (mama), A-549 (pulmó), i HT-29 (colon).Un estudi de relació estructura-activitat concluí que més d'un 75% dels anàlegs de cadena oberta analitzats mostraren citotoxicitats en un rang micromolar baix de GI_{50}.

Synthesis and Structure−Activity Relationship Study of Potent Cytotoxic Analogues of the Marine Alkaloid Lamellarin D

Daniel Pla,[†] Antonio Marchal,[†,§] Christian A. Olsen,[†,||] Andrés Francesch,[‡] Carmen Cuevas,[‡] Fernando Albericio,*[,†,⊥] and Mercedes Álvarez*[,†,○]

Institute for Research in Biomedicine, Barcelona Science Park, University of Barcelona, Josep Samitier 1-5, 08028 Barcelona, Spain, and Pharma Mar, Avda Reyes Católicos 1, E- 28770 Colmenar Viejo, Madrid, Spain

Received March 3, 2006

The marine alkaloid, Lamellarin D (Lam-D), has shown potent cytotoxicity in numerous cancer cell lines and was recently identified as a potent topoisomerase I inhibitor. A library of open lactone analogues of Lam-D was prepared from a methyl 5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate scaffold (**1**) by introducing various aryl groups through sequential and regioselective bromination, followed by Pd(0)-catalyzed Suzuki cross-coupling chemistry. The compounds were obtained in a 24−44% overall yield, and tested in a panel of three human tumor cell lines, MDA-MB-231 (breast), A-549 (lung), and HT-29 (colon), to evaluate their cytotoxic potential. From these data, the SAR study concluded that more than 75% of the open-chain Lam-D analogues tested showed cytotoxicity in a low micromolar GI_{50} range.

Introduction

In the search for new bioactive, small chemical molecules for research in chemical biology and medicinal chemistry, one must choose a starting point from the vast chemical space.[1] In this respect, natural products may serve as biologically prevalidated leads,[2,3] and indeed, more than 60% of the recently marketed drugs have been isolated from natural products or synthetic compounds based on natural products.[4] With the recent advances in natural products science, including the synthesis of complex libraries,[2,3] biosynthesis,[5] and isolation techniques,[6,7] the field has a promising future.[8] In particular, marine and microbial environments may serve as a source of new bioactive chemical compounds.[9]

Here, we used Lamellarin D (Lam-D, Figure 1), a potent cytotoxic agent against various tumor cells, as a lead. This marine alkaloid was first isolated from the marine prosobranch mollusc *Lamellaria* sp. in 1985 by Faulkner and co-workers.[10] Since then, a family of about 35 structurally related lamellarins has been isolated from natural sources, and several synthetic strategies have been devised for these natural products.[11,12] Of the family of lamellarins, Lam-D is one of the most potent lead candidates for anticancer chemotherapy. There is substantial evidence that Lam-D is an inhibitor of topoisomerase I[13] and a potent pro-apoptotic agent.[14] Recently, topoisomerase I binding studies have been elaborated further by comparing Lam-D and Camptothecin[15] (Figure 1) bound to the DNA-topoisomerase I complex using molecular dynamics simulations.[16] These also nicely correlate with structure−activity relationships (SAR) obtained with homologues of Lam-D with distinct OMe/OH substitution patterns on the pentacyclic framework.[16,17] Hence,

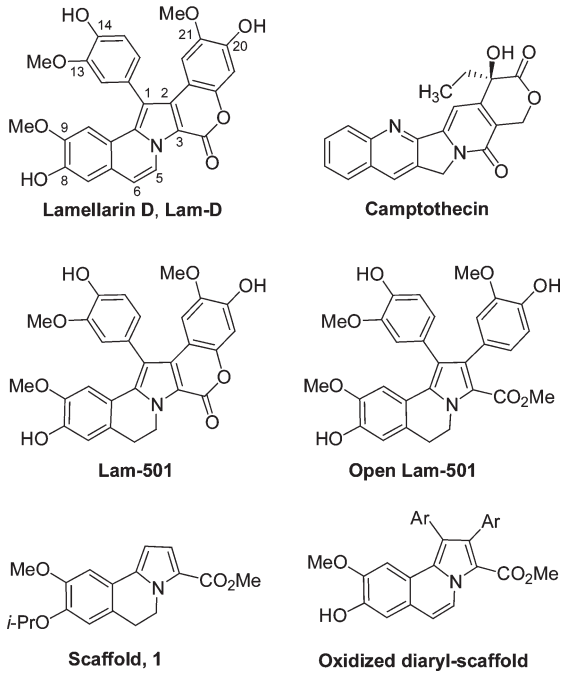


Figure 1. Structures of lamellarins, camptotecin, and scaffold **1**.

the 8-OH and 20-OH groups (Figure 1) are crucial for cytotoxic activity and also for topoisomerase I inhibition.

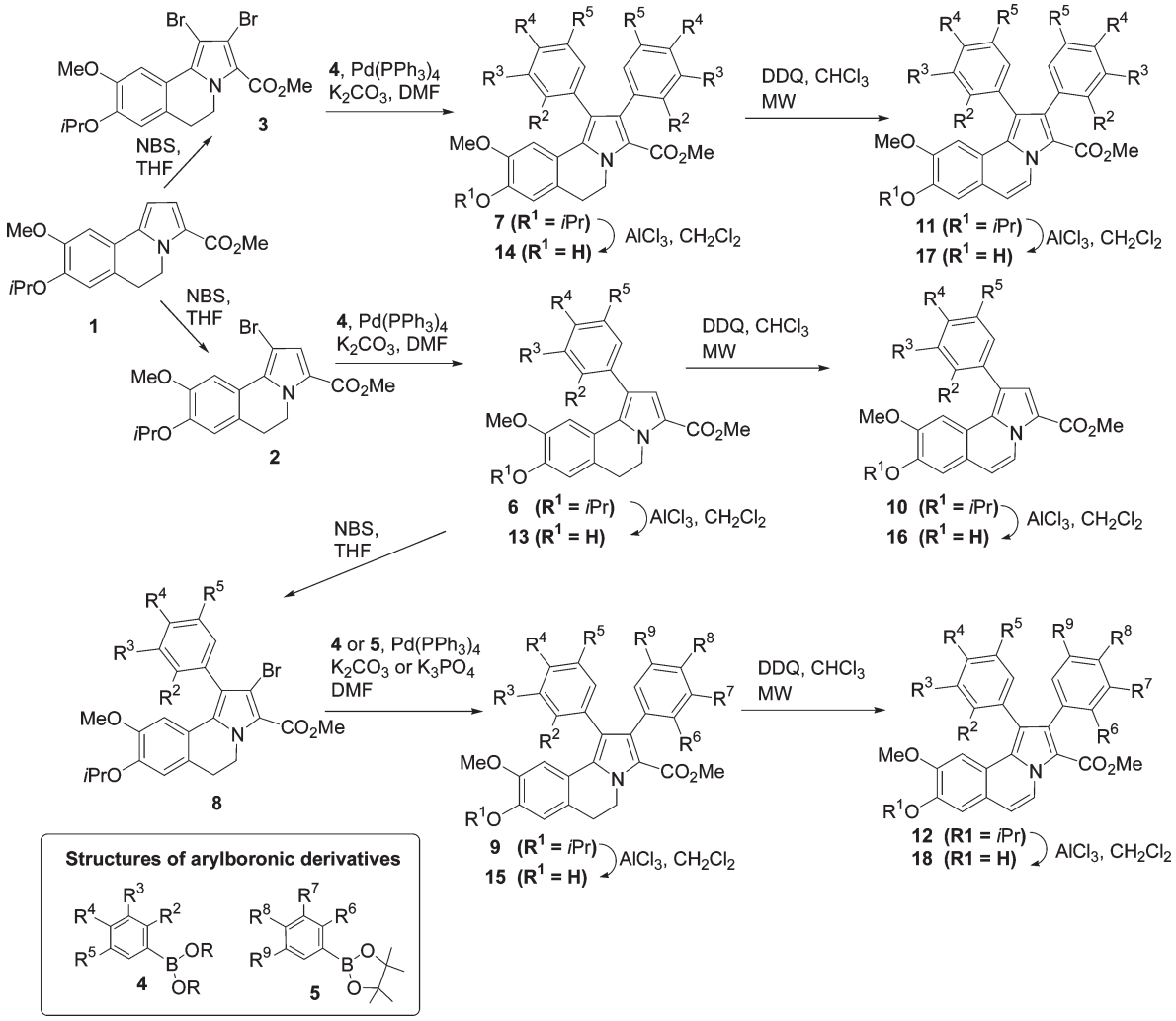
Moreover, the unsaturated C-5−C-6 motif of Lam-D compared to the saturated analogue (Lam-501, Figure 1) is important for potency,[13,18] a trend that was also observed with a range of Lam-D and Lam-501 derivatives in which the free phenolic sites were acylated.[18] Furthermore, the latter study afforded potent candidates for in vivo preclinical development of their antitumor activity. Interestingly, derivatization of the 8-OH and 20-OH groups with amino acids, thus preserving the hydrogen bonding capacity at these sites, affords potent compounds, whereas acylation with various carboxylic acids results in a considerable decrease in potency.[18]

We recently reported preliminary biological results showing that simplified tricyclic analogues of Lam-D lacking the lactone, such as open Lam-501 (Figure 1), retain some cytotoxic

* To whom correspondence should be addressed. Tel: (+34) 93403 7088. Fax: (+34) 93403 7126. E-mail: albericio@pcb.ub.es (F.A.); Tel: (+34) 93403 7086. Fax: (+34) 93403 7126. E-mail: malvarez@pcb.ub.es (M.A.).
† Barcelona Science Park.
‡ Pharma Mar.
⊥ Department of Organic Chemistry.
○ Laboratory of Organic Chemistry, Faculty of Pharmacy.
§ Current address. Department of Inorganic and Organic Chemistry, University of Jaén, 23071 Jaén, Spain. E-mail: amarchal@ujaen.es.
|| Current address. The Danish University of Pharmaceutical Sciences, Copenhagen, Denmark. E-mail: cao@dfuni.dk.

10.1021/jm0602458 CCC: $33.50
Published on Web 05/06/2006

Scheme 1. Synthesis of Open-Chain Lamellarin Analogues Library



activity.[19] This finding encouraged us to perform SAR studies using scaffold **1** by incorporating various aryl groups in positions 1 and 2, including their oxidized homologues (Figure 1).[20]

In addition to the initial achievements in the assembly of the pentacyclic lamellarin framework[21−23] and total synthesis of Lam-D,[21] pentacyclic and more simple lamellarins have been synthesized using solid-phase synthesis,[24−26] which should facilitate the preparation of compound libraries for biological evaluation. However, here, we found it more rational to prepare our library using the methyl 5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate scaffold **1** (Figure 1) and protocols developed for modular total synthesis of Lam-D[27] and tricyclic analogues.[19] While this study was in progress, another highly efficient synthesis of Lam-D and related analogues was published.[28]

Results and Discussion

Chemistry. The synthesis of an open-chain lamellarin analogue library was performed in solution starting from the methyl pyrrole-2-carboxylate by transformation into scaffold **1**.[19,27] The key steps in the process were the introduction of the aryl substituents at positions 1 and 2 of the scaffold using boron derivatives **4** and **5** as building blocks for the final structure. Following the procedure described for the total synthesis of Lam-D,[27] the synthetic strategy used consisted of the regioselective bromination of the scaffold followed by a Pd(0)-catalyzed Suzuki cross-coupling reaction, oxidation, and subsequent deprotection of all of the phenols present in each compound. The isopropyl ether was used as the protecting group for the phenols present in the final compounds and was maintained throughout the synthetic process.[29]

Three alternative ways were used to introduce the aryl groups on scaffold **1**, according to the final structure of the lamellarin analogues (Scheme 1). Monoaryl compounds **6** were prepared by regioselective bromination of scaffold **1** on position 1 to give bromo derivative **2**, which was used for Suzuki cross-coupling with boronic acids **4**. Diaryl derivatives **7** with the same substitution pattern in both aryl groups were obtained from dibromo scaffold **3** by simultaneous introduction of both aryl groups. Finally, for diarylated compounds **9**, with different substituents on the phenyl rings, we used two sequential regioselective bromination and cross-coupling reactions starting from scaffold **1** with monoaryl scaffolds **6** and bromides **8** as synthetic intermediates.[27]

An extensive range of aryl boronic derivatives **4** and **5** were used as building blocks (see Table 1 for the structures). Building blocks **4** are commercially available,[30] whereas ortho substituted borolanes **5** were obtained in good yields (52−81%) from the proper aryl bromide by Pd(0)-catalyzed cross-coupling borylation using the pinacolborane, as described in the Supporting Information.[27,31]

All of the Suzuki cross-coupling reactions between bromides **2**, **3**, and **8** and building blocks **4** were performed in DMF using $Pd(PPh_3)_4$ and K_2CO_3 as catalyst and base, respectively, with good yields. The phenolic group on position 4′ of **6c** (R^4 = OH)

Table 1. Substituents of Building Blocks **4** and **5** and Compounds **9**

4	R	R^2	R^3	R^4	R^5
a	H	H	OMe	OMe	OMe
b	H	H	H	OH	H
c	CMe_2CMe_2	H	OMe	OH	H
d		H	OMe	O*i*Pr	H
e	H	OMe	H	H	OMe
f	H	H	OMe	OMe	H
g	H	H	H	OMe	H
h	H	H	OMe	H	OMe
i	H	H	H	OCF_3	H
j	H	H	H	O*i*Pr	H
k	H	H	O*i*Pr	H	H
l	H	H	H	NMe_2	H
m	H	H	NO_2	H	H
n	H	2-thienyl			

5	R^6	R^7	R^8	R^9	%
a	O*i*Pr	H	O*i*Pr	OMe	80
b	O*i*Pr	H	O*i*Pr	O*i*Pr	52
c	O*i*Pr	H	O*i*Pr	H	64
d	O*i*Pr	OMe	OMe	H	61
e	OMe	H	O*i*Pr	OMe	81

9	scaffold 8	borolane	%
a	**8d**	**4b**	76
b	**8d**	**4f**	89
c	**8d**	**4a**	71
d	**8d**	**5a**	89
e	**8d**	**5e**	quant.
f	**8e**	**5c**	82
g	**8e**	**5b**	81
i	**8k**	**5d**	93

was protected as isopropoxyether by reaction with 2-bromopropane in basic conditions, thereby giving **6d**.[32] Generally, transformation of **6** into **8** was performed using *N*-bromosuccinimide (NBS) in tetrahydofuran (THF) with a careful control of the reaction time to obtain the desired mono and regiobromination, thereby avoiding the formation of complex mixtures.[33] Regioselective bromination of electron-rich systems, such as **6h**, **6l**, and **6n** using the same reaction conditions was unsuccessful because halogenation on the electron-rich aromatic ring could not be avoided with these compounds.[34] The Suzuki reaction conditions used to introduce the second aryl ring on **8** were basically the same as those when the boron derivatives **4** were used. However, with the more hindered borolanes **5**, several modification were required such as the slow addition of three equivalents[35] of **5** and the use of K_3PO_4 as the base to afford yields between 81% and quantitative for the second cross-coupling (see Experimental Section).[36] Compounds **9a**−**i** were prepared by the reaction of scaffolds **8** and the second building block **5**, as indicated in Table 1 and in the Experimental Section.

Optimization of oxidation was performed with the 2-thienyl derivative **4n**. Several experiments using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in $CHCl_3$ at reflux temperature, MnO_2 in refluxing toluene or pyridine,[37] or Pd-C in toluene or Decalin[38] afforded only traces of **10n**. The best reaction conditions were attained using DDQ in $CHCl_3$ as solvent in a sealed tube with microwave (MW) irradiation. The aromatization of dihydroisoquinolines **6**, **7**, and **9** to give the planar system of pyrrolo[2,1-*a*]isoquinoline present in compounds **10**−**12** was accomplished using the same protocol as that described in the Supporting Information.[39] The ^{1}H NMR was crucial for the control of the reaction because dihydroisoquinolines **6**−**9** have a characteristic ABXY spin system for the four protons of C^5H_2 and C^6H_2, whereas isoquinolines **10**−**12** hold an AB system in the aromatic area for the two protons C^5H and C^6H, the former being a significant signal.

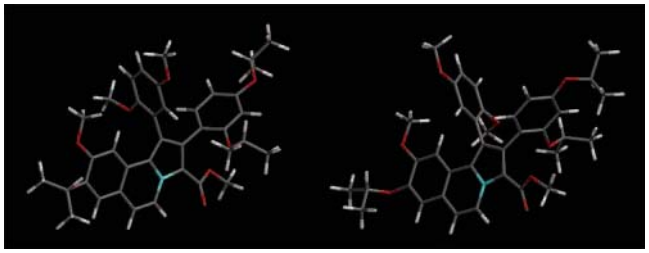


Figure 2. Minimized energy forms of the two rotamers of compound **12f**.

Compounds **9f**−**i** and **12f**−**i**, both with bulky substituents in the ortho position of the aryl rings, showed restricted rotation, and two conformers were observed by ^{1}H- and ^{13}C NMR. ^{1}H NMR experiments with **12f** at variable temperature showed the collapse of the signals at 75 °C (Figure 2 in the Supporting Information). For example, the coalescence of double doublets at 6.29 and 6.32 ppm[40] at 25 °C were easily observed (part a in Figure 1 of the Supporting Information) as a broad doublet at 6.31 ppm in the experiment at 75 °C (part c in Figure 1 of the Supporting Information), and the same occurred with the methoxy group signals. In the coalescence temperature, the signal of collapsed groups broadened and decreased in intensity. Figure 2 shows the minimized energy forms of the two rotamers of compound **12f**, calculated by the semiempirical method PM3.[41] The elimination of the bulky protecting groups led to the evanescence of the above-mentioned restricted rotation in all of the compounds.

All of the isopropoxy-protecting groups of dihydroisoquinolines **6**, **7**, and **9** and fully aromatic systems **10**−**12** were removed using $AlCl_3$ in CH_2Cl_2,[24−26,42] giving a good yield of valuable phenols **13**−**18**.[43,44] Despite the advantage of working with the protected phenol groups, the synthesis was performed without this protection in **4**, as demonstrated with the synthesis of **17c** and **15a**. Lamellarin analogues **13**−**18** were obtained as reddish oils or white solids, and their structures were confirmed by ^{1}H- and ^{13}C-NMR, using heteronuclear 2D correlations, such as HSQC, HMBC, and also MS and HRMS.

Biological Results. A panel of three human tumor cell lines was used to evaluate the cytotoxic potential of the Lam-D analogues: A-549 lung carcinoma NSCL, HT-29 colon carcinoma cells, and MDA-MB-231 breast adenocarcinoma.

A conventional colorimetric assay was set up to estimate GI_{50} values, that is, the drug concentration that causes 50% of cell growth inhibition after 72 h of continuous exposure to the test molecule. Lam-D was included in the test for comparison purposes. The results obtained are shown in Table 2.

More than 75% of the open-chain Lam-D analogues tested showed cytotoxicity in a low micromolar GI_{50} range. Molecular simplification of Lam-D by removing the lactone ring from all of the analogues and by the additional elimination of one aryl group in derivatives **13** and **16** produced a decrease in activity with respect to Lam-D. However, interestingly, these data provide crucial information about the importance of the full structure for the biological activity of the molecules despite their low solubility in the biological medium. In a general overview, the oxidized derivatives showed greater activity than the corresponding reduced analogues.[13] Derivatives with electron-withdrawing substituents such as nitro groups (i.e., **14m** and **17m**) decreased activity, and this decrease was dramatic with the introduction of a OCF_3 substituent as in **14i** and **17i**. The substitution pattern given by electron donor groups, such as

Table 2. In Vitro Cytotoxicity of the Open-Chain Analogues of Lam-D and Synthetic Intermediates[a]

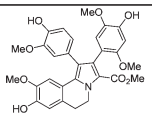
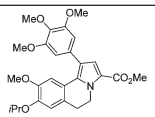
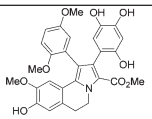
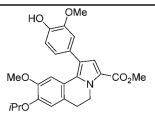
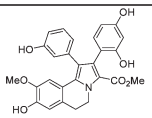
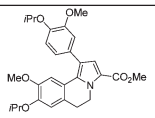
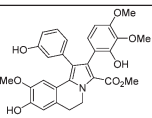
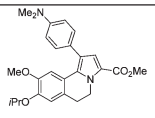
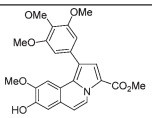
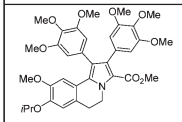
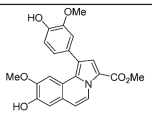
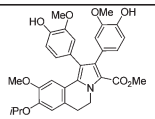
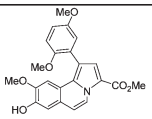
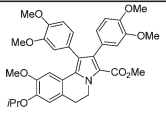
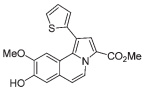
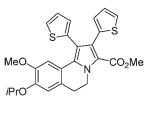
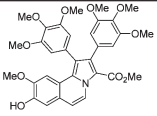
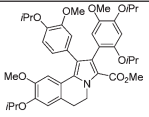
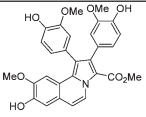
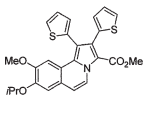
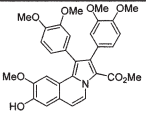
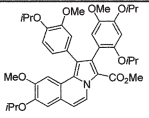
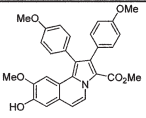
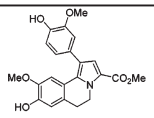
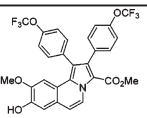
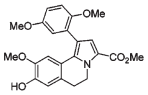
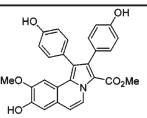
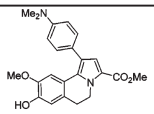
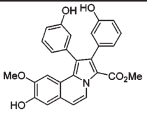
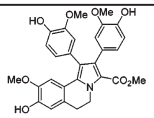
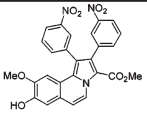
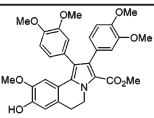
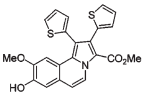
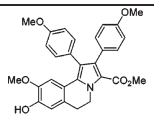
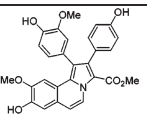
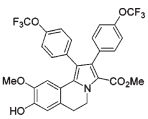
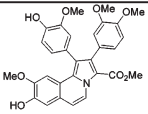
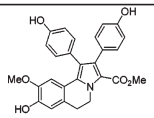
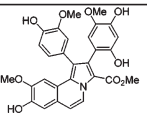
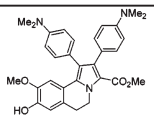
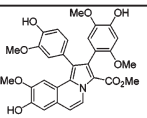
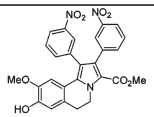
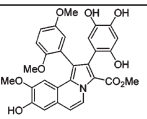
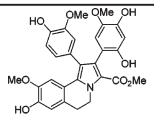
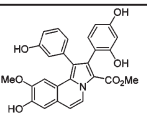
Compound		Cytotoxicity (GI_{50} μM)			Compound		Cytotoxicity (GI_{50} μM)		
		A-549	HT-29	MDA-MB-231			A-549	HT-29	MDA-MB-231
Lam-D		0.20	5.1	0.25		**15e**	8.9	n.a.	7.6
	**6a**	n.a.	n.a.	n.a.		**15g**	13.7	8.4	10.5
	**6c**	20.3	18.1	19.0		**15h**	n.a.	n.a.	19.0
	**6d**	n.a.	n.a.	n.a.		**15i**	14.7	n.a.	15.7
	**6l**	n.a.	n.a.	n.a.		**16a**	n.a.	n.a.	n.a.
	**7a**	67.9	34.0	n.a.		**16c**	10.9	23.9	11.2
	**7c**	14.7	6.9	7.1		**16e**	13.3	n.a.	19.9
	**7f**	0.81	1.0	0.98		**16n**	n.a.	n.a.	26.3
	**7n**	n.a.	n.a.	n.a.		**17a**	n.a.	n.a.	n.a.
	**9d**	n.a.	n.a.	n.a.		**17c**	7.1	8.1	7.5
	**11n**	n.a.	n.a.	n.a.		**17f**	n.a.	n.a.	n.a.
	**12d**	n.a.	13.6	n.a.		**17g**	n.a.	9.7	9.9

Table 2 (Continued)

Compound		Cytotoxicity (GI_{50} μM)			Compound		Cytotoxicity (GI_{50} μM)		
		A-549	HT-29	MDA-MB-231			A-549	HT-29	MDA-MB-231
	**13c**	14.2	18.0	22.3		**17i**	n.a.	n.a.	n.a.
	**13e**	n.a.	n.a.	12.7		**17j**	3.5	9.8	4.1
	**13l**	n.a.	n.a.	n.a.		**17k**	6.3	18.4	7.2
	**14c**	14.3	n.a.	8.5		**17m**	n.a.	8.9	18.3
	**14f**	11.2	n.a.	7.7		**17n**	20.4	n.a.	19.7
	**14g**	9.2	10.3	14.4		**18a**	9.8	10.1.	15.0
	**14i**	n.a.	n.a.	n.a.		**18b**	n.a.	n.a.	n.a.
	**14j**	n.a.	n.a.	n.a.		**18d**	0.45	7.9	0.71
	**14l**	n.a.	n.a.	13.7		**18e**	n.a.	n.a.	n.a.
	**14m**	18.0	11.3	10.1		**18g**	4.7	7.1	3.2
	**15d**	5.0	17.1	3.1		**18h**	20.8	n.a.	10.6

[a] n.a. = not active at 10 μg/mL.

O*i*Pr, NMe_2, OMe, and OH, was fundamental for activity. A comparison of **6c** and **6d** shows the importance of the free *p*-phenol on the aryl at position 1 of the scaffold. Although few O-protected phenol analogues, such as **6c**, **7a**, **7c**, and **7f**, presented cytotoxic activity, an important gain in activity was displayed by the same compounds with free OH functions. This

observation can probably be attributed to the additional capacity of these analogues to form hydrogen bonds with the active sites, as described for Lam-D.[13] Although the binding of these analogues with the same DNA−topI complex has not been demonstrated in the present work, other factors that could increase the activity are the solubility or the membrane-crossing issues. The donor effect of the methoxy substituents may explain why **14g** and **17g** were quite active, even without the possibility of acting as hydrogen-bond donors. Compounds **18a**, **17c**, **18e**, **18d**, and Lam-D had identical substituents on the scaffold and on the aryl at position 1 and afforded a gradation in activity potency with the increase upon the substitution of the aryl at position 2 of the scaffold. Except for **18e**, which was inactive, presumably due to lack of planarity by sterical hindrance. Simplified analogue **17c** maintained 63% of the activity of Lam-D in HT29 cells, and most of this behavior remained in the C4″-OH (same position as C-20 in Lam-D) group, as shown by **18a**. To our knowledge, open lactone compound **18d** may produce lactonization in a physiological environment. Therefore, **18d** must be considered for further study as a possible pharmacodynamic improvement for the validated Lam-D lead.

Conclusion

Here, we performed a SAR study using the marine alkaloid Lam-D. Efficient and convergent modular synthetic protocols were applied to the diverted total synthesis of more than 40 analogues of the natural product. This strategy allowed the introduction of structural elements that have not been previously studied in the lamellarin series. Thus, the SAR information provided in this study expands our knowledge about these compounds beyond substitutions on the core structure, which has already been provided by other groups.

Overall, our results are consistent with previous findings such as the critical importance of the cytotoxic activity of the planarity of the tricyclic isoquinoline motif. In addition, compounds with OH hydrogen-bond donors at C-8 and C-4″ were generally more potent than other analogues. Not surprisingly, compound **18d**, which showed the most resemblance to Lam-D, was the most potent compound against the three cell lines tested. This observation may be due to partial lactonization to give Lam-D under the assay conditions.

However, a remarkable retention of activity was observed for monoaryl analogues **13c** and **16c** against HT-29 colon carcinoma cells, toward which these compounds were only ca. 5-fold less potent than Lam-D. Furthermore, the moderate activity of compound **17n** against the A-549 and MDA-MB-231 cell lines (low micromolar) indicates that heterocyclic motifs may be included in a second-generation library. However, the hydrogen-bond donor at C-20 should be preserved in future library designs. On the basis of this work it is clear the importance of an extensive bioprospection of the natural sources to find lead candidates for constructing ponderous libraries.

Experimental Section

(A) General Procedures for Cross-Coupling Reactions. Synthesis of Monoaryl Derivatives 6. A solution of bromide **2** (1.0 mmol) in DMF (20 mL) was purged with Ar, and **4** (3.0 mmol), $Pd(PPh_3)_4$ (0.1 mmol), and 2 M K_2CO_3 (3.0 mmol) were added. The reaction mixture was stirred at 125 °C and followed by TLC until the starting material disappeared. The solvent was removed after cooling to room temperature, and the residue was dissolved in EtOAc. The organic solution was washed with water and brine, dried, and concentrated to give a crude material, which was later purified by column chromatography on silica gel. Elution with hexane/EtOAc (90:10 to 75:25) gave **6** (yield 32−92%).

(B) General Procedures for Cross-Coupling Reactions. Synthesis of Diaryl Derivatives 7. A solution of 1,2-dibromide **3** (189 mg, 0.4 mmol) in DMF (8 mL) was purged with Ar for 10 min, and **4** (2.4 mmol), $Pd(PPh_3)_4$ (46 mg, 0.04 mmol), and 2 M K_2CO_3 (2.4 mmol) were added. The reaction mixture was stirred at 125 °C and was then subjected to HPLC until the starting material disappeared or for a maximum 20 h. The solvent was removed after cooling to room temperature, and the residue was dissolved in EtOAc. The organic solution was washed with water and brine, dried, and concentrated to give a crude material, which was later purified by column chromatography on silica gel. Elution with hexane/EtOAc (75:25 to 40:60) gave **7** (yield 34−87%).

(C) General Procedure for the Regioselective Bromination of 6. NBS (1.20 mmol) was added in one portion to a solution of **6** (1.00 mmol) in THF (13 mL). The mixture was stirred at 70 °C under Ar for 90 min. The solvent was removed, and the residue was purified by flash chromatography. Elution with hexane/AcOEt (90:10 to 70:30) gave **8** (yield 84%, quantitative (quant)).

(D) General Procedures for Cross-Coupling Reactions. Synthesis of Diaryl Derivatives 9a−c. Arylboronic acids **4** (3.0 mmol), $Pd(PPh_3)_4$ (0.1 mmol), and 2 M K_2CO_3 (3.0 mmol) were added to a purged solution of bromide **8** (1.0 mmol) in DMF (20 mL). The reaction mixture was stirred at 125 °C for the time indicated for each compound (see Supporting Information). The solvent was removed, and the residue was dissolved in EtOAc. The organic solution was washed with water and brine, dried, and concentrated to give a crude material, which was later purified by column chromatography on silica gel. Elution with hexane/EtOAc (75:25 to 40:60) gave **9a−c** (yield 71−89%).

(E) General Procedures for Cross-Coupling Reactions. Synthesis of Diaryl Derivatives 9d−i. A solution of bromide **8** (1.0 eq) in DMF (20 mL) was purged with Ar for 10 min, and pinacol phenylboronate **5** (1.0 mmol), $Pd(PPh_3)_4$ (10%), and 2 M K_3PO_4 (3.0 mmol) were added. The reaction mixture was stirred at 115 °C, and another portion of boronate (2.0 mmol) was added dropwise using a syringe pump during the first hour of reaction. The solvent was removed, and the residue was dissolved in EtOAc. The organic solution was washed with water and brine, dried, and concentrated to give a crude material, which was later purified by column chromatography on silica gel. Elution with hexane/EtOAc (75:25 to 60:40) gave **9d−i** (yield 81%, quant).

(F) General Procedure for Oxidation. Synthesis of Compounds 10−12. A mixture of **6**, **7**, or **9** (1.0 mmol) and DDQ (1.3 mmol) in dry $CHCl_3$ (15 mL) was purged with Ar in a sealed vessel and microwaved at 120 °C for 10 min. The organic solution was washed with 2 M NaOH, water, and brine and then dried ($MgSO_4$), filtered, and evaporated in a vacuum. Washing with NaOH was avoided for products with free phenolic groups. Purification by column chromatography on silica gel eluting with hexane/AcOEt (85:15 to 60:40) gave **10−12** (yield 48−95%).

(G) General Method for Deprotection. Preparation of Compounds 13−18. Anhydrous $AlCl_3$ (1.3 mmol) for each isopropoxy ether was added to a solution of compound **6**, **7**, or **9−12** (1 mmol) in dry CH_2Cl_2 (1 mL). The mixture was sonicated for 10 min, quenched with sat. NH_4Cl, and then washed with water and brine. The aqueous solution was extracted with AcOEt. The organic extracts were dried and evaporated. The crude product was purified by flash chromatography to give the title compounds (yield 30−96%).

Methyl 8-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*] isoquinoline-3-carboxylate (13c). Following general procedure G and starting with **6c** (48 mg, 0.11 mmol), elution with hexane/AcOEt (80:20 to 70:30) gave a white solid (19 mg, 44%). Mp (MeCN) 205−207 °C. IR (film) ν 3424, 1696, 1439, 1246 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 2.98 (t, $J = 6.4$ Hz, 2H, H6); 3.47 (s, 3H, OMe); 3.84 (s, 3H, OMe); 3.86 (s, 3H, OMe); 4.59 (t, $J = 6.4$ Hz, 2H, H5); 5.62 (s, 1H, OH); 5.63 (s, 1H, OH); 6.78 (s, 1H); 6.85 (s, 1H); 6.91−6.97 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.9 (t); 42.5 (t); 51.1 (q); 55.6 (q); 56.0 (q); 108.1 (d); 112.0 (d); 113.8 (d); 114.3 (d); 119.1 (d); 120.0 (s); 120.5 (s); 121.5 (s); 122.4 (d); 126.8 (s); 128.6 (s); 131.7 (s);

144.5 (s); 144.9 (s); 145.0 (s); 146.4 (s); 161.7 (s). MS (MALDI-TOF) *m*/*z* 395 (M, 100); 396 (M + 1, 26). HRMS *m*/*z* calcd for $C_{22}H_{21}NO_6$, 395.1369; found, 395.1366.

Methyl 1-(2,5-Dimethoxyphenyl)-8-hydroxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (13e). Following general procedure G and starting with **6e** (17 mg, 0.04 mmol), elution with hexane/AcOEt (80:20 to 70:30) gave a white solid (12 mg, 76%). Mp (MeCN) 96−100 °C. IR (film) ν 3417, 1697, 1244 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.99 (t, J = 6.8 Hz, 2H, H6); 3.42 (s, 3H, OMe); 3.65 (s, 3H, OMe); 3.75 (s, 3H, OMe); 3.83 (s, 3H, OMe); 4.62 (t, J = 6.8 Hz, 2H, H5); 5.55 (s, 1H, OH); 6.67 (s, 1H); 6.75 (s, 1H); 6.85 (dd, J = 8.7, 2.8 Hz, 1H, H4′); 6.89 (d, J = 8.7 Hz, 1H, H3′); 6.90 (d, J = 2.8 Hz, 1H, H6′); 7.02 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.7 (t); 42.5 (t); 51.0 (q); 55.4 (q); 55.8 (q); 56.2 (q); 107.7 (d); 112.1 (d); 113.5 (d); 113.6 (d); 116.7 (s); 117.4 (d); 119.9 (d); 120.1 (s); 121.0 (s); 126.3 (s); 126.5 (s); 132.7 (s); 144.8 (s); 145.0 (s); 151.6 (s); 153.6 (s); 161.8 (s). MS (MALDI-TOF) *m*/*z* 409 (M, 100); 410 (M + 1, 43). MS (ESI-TOF) *m*/*z* 410 (M + 1, 100). HRMS *m*/*z* calcd for $C_{23}H_{24}NO_6$, 410.1598; found, 410.1598.

Methyl 1-(4-Dimethylaminophenyl)-8-hydroxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (13l). Following general procedure G and starting with **6l** (31 mg, 0.07 mmol), elution with hexane/AcOEt (80:20 to 70:30) gave a white solid (25 mg, 90%). Mp (MeCN) 169−170 °C. IR (film) ν 3441, 2925, 1693, 1439, 1194 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.95−2.96 (m, 8H, H6, NMe_2); 3.47 (s, 3H, OMe); 3.83 (s, 3H, OMe); 4.57 (t, J = 6.4 Hz, 2H, H5); 5.58 (bs, 1H, OH); 6.76 (s, 1H); 6.77 (d, J = 8.8 Hz, 2H); 6.90 (s, 1H); 6.95 (s, 1H); 7.31 (d, J = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.9 (t); 40.8 (q); 42.5 (t); 51.0 (q); 55.6 (q); 108.2 (d); 112.8 (2d); 113.7 (d); 119.2 (d); 119.9 (s); 120.9 (s); 121.7 (s); 124.7 (s); 126.7 (s); 130.1 (2d); 131.7 (s); 144.8 (s); 144.9 (s); 149.6 (s); 161.8 (s). MS (MALDI-TOF) *m*/*z* 392 (M, 100). MS (ESI-TOF) *m*/*z* 393 (M + 1, 100). HRMS *m*/*z* calcd for $C_{23}H_{25}N_2O_4$, 393.1809; found, 393.1809.

Methyl 1,2-Bis(3,5-dimethoxy-4-hydroxyphenyl)-8-hydroxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (14a, R^4 = OH). Following general procedure G and starting with **7a** (24 mg, 0.04 mmol) and an excess of $AlCl_3$ (0.8 mmol), upon elution with hexane/AcOEt (60:40 to 40:60), a yellowish solid (12 mg, 58%) was obtained. Mp (MeCN) 118−120 °C. IR (film) ν 3430,1689,1437,1210 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.02 (t, J = 6.4 Hz, 2H, H6); 3.40 (s, 3H, OMe); 3.64 (s, 3H, OMe); 3.68 (s, 6H, 2OMe); 3.71 (s, 6H, 2OMe); 4.59 (t, J = 6.4 Hz, 2H, H5); 5.41 (bs, 1H, OH); 5.43 (bs, 1H, OH); 5.58 (bs, 1H, OH); 6.39 (s, 2H); 6.40 (s, 2H); 6.67 (s, 1H); 6.78 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.9 (t); 42.9 (t); 50.9 (q); 55.5 (q); 56.2 (2q); 56.4 (2q); 107.8 (2d); 107.9 (2d); 108.3 (d); 113.7 (d); 117.8 (s); 120.2 (s); 121.4 (s); 126.3 (s); 126.5 (s); 126.9 (s); 131.2 (s); 132.7 (s); 133.3 (s); 133.5 (s); 144.9 (s); 146.0 (2s); 146.9 (2s); 162.4 (s). MS (MALDI-TOF) *m*/*z* 577 (M, 100); 578 (M + 1, 40). HRMS *m*/*z* calcd for $C_{31}H_{31}NO_{10}$, 577.1948; found, 577.1942.

Methyl 8-Hydroxy-1,2-bis(4-hydroxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (14c). Following general procedure G and starting with **7c** (28 mg, 0.05 mmol), elution with hexane/AcOEt (50:50 to AcOEt) gave a yellowish solid (15 mg, 60%). Mp (MeCN) 237−239 °C. IR (film) ν 3423, 1688, 1438, 1235, 1199 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.01 (t, J = 6.4 Hz, 2H, H6); 3.38 (s, 3H, OMe); 3.62 (s, 3H, OMe); 3.63 (s, 3H, OMe); 3.65 (s, 3H, OMe); 4.59 (t, J = 6.4 Hz, 2H, H5); 5.50 (bs, 1H, OH); 5.53 (bs, 1H, OH); 5.59 (bs, 1H, OH); 6.55 and 6.58 (2d, J = 1.6 Hz, 2H, H2′, H2″); 6.63 (s, 1H); 6.70 and 6.75 (2dd, J = 8.4, 1.6 Hz, 2H, H6′, H6″); 6.77 (s,1H); 6.78 and 6.83 (2d, J = 8.4 Hz, 2H, H5′, H5″). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 28.9 (t); 42.9 (t); 50.8 (q); 55.4 (q); 55.8 (q); 55.9 (q); 108.2 (d); 113.3 (d); 113.5 (d); 113.6 (d); 113.9 (d); 114.1 (d); 117.8 (s); 120.4 (s); 121.4 (s); 123.9 (d); 124.3 (d); 126.9 (s); 127.3 (s); 127.5 (s); 131.3 (s); 132.7 (s); 144.0 (s); 144.3 (s); 144.9 (s); 145.4 (2s); 146.3 (s); 162.5 (s). MS (MALDI-TOF) 517 (M, 100); 518 (M + 1, 15). HRMS *m*/*z* calcd for $C_{29}H_{27}NO_8$, 517.1737; found, 517.1731.

Methyl 1,2-Bis(3,4-dimethoxyphenyl)-8-hydroxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (14f). Following general procedure G and starting with **7f** (92.0 mg, 0.16 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave **14f** as a reddish oil (50.9 mg, 60%). IR (film) ν 3410, 1691, 1437, 1254 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.01 (t, J = 6.5 Hz, 2H, H6); 3.36 (s, 3H, OMe); 3.62 (s, 3H, OMe); 3.63 (s, 3H, CO_2Me); 3.67 (s, 3H, OMe); 3.83 (s, 3H, OMe); 3.84 (s, 3H, OMe); 4.59 (t, J = 6.5 Hz, 2H, H5); 5.67 (bs, 1H, OH); 6.62 (d, J = 1.6 Hz, 1H); 6.64 (d, J = 1.6 Hz, 1H); 6.66 (s, 1H); 6.71 (dd, J = 8.4 and 1.6 Hz, 1H); 6.72 (s 1H); 6.74 (dd, J = 8.4 and 1.6 Hz, 1H); 6.76 (d, J = 8.4 Hz, 1H); 6.77 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 28.8 (t); 42.8 (t); 50.8 (q); 55.3 (q); 55.5 (q); 55.6 (q); 55.7 (q); 55.8 (q); 108.2 (d); 109.9 (d); 110.9 (d); 113.7 (d); 114.1 (d); 114.3 (d); 117.7 (s); 120.2 (s); 122.9 (d); 123.4 (d); 123.4 (s); 126.8 (s); 127.8 (s); 128.0 (s); 131.2 (s); 132.5 (s); 141.6 (s); 144.8 (s); 147.3 (s); 147.5 (s); 147.5 (s); 148.5 (s); 162.3 (s). MS (MALDI-TOF) *m*/*z* 545 (M, 100). HRMS *m*/*z* calcd for $C_{31}H_{31}NO_8$, 545.2050; found, 545.2044.

Methyl 8-Hydroxy-9-methoxy-1,2-bis(4-methoxyphenyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (14 g). Following general procedure G and starting with **7g** (18.2 mg, 0.034 mmol), elution with hexane/AcOEt (80:20 to 50:50) gave **14g** (6.8 mg, 41%) as a reddish oil. IR (film) ν 2931, 1697 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.01 (t, J = 6.5 Hz, 2H, H6); 3.34 (s, 3H, OMe); 3.59 (s, 3H, CO_2Me); 3.76 (s, 3H, OMe); 3.77 (s, 3H, OMe); 4.59 (t, J = 6.5 Hz, 2H, H5); 5.52 (bs, 1H, OH); 6.50 (s, 1H); 6.74 (d, J = 9.0 Hz, 2H); 6.76 (s, 1H); 6.79 (d, J = 8.6 Hz, 2H); 7.03 (d, J = 9.0 Hz, 2H); 7.06 (d, J = 8.6 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.9 (t); 42.9 (t); 50.7 (q); 55.0 (q); 55.2 (q); 55.2 (q); 108.2 (d); 109.7 (d); 112.5 (2d); 113.6 (2d); 117.9 (s); 120.5 (s); 121.2 (s); 126.8 (s); 127.6 (s); 127.8 (s); 131.4 (s); 131.6 (2d); 132.3 (2d); 132.6 (s); 144.7 (s); 144.8 (s); 157.9 (s); 158.2 (s); 162.5 (s). MS (MALDI-TOF) *m*/*z* 485 (M). HRMS *m*/*z* calcd for $C_{29}H_{27}NO_6$, 485.1838; found, 485.1833.

Methyl 8-Hydroxy-9-methoxy-1,2-bis(4-trifluoromethoxyphenyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (14i). Following general procedure G and starting with **7i** (25.9 mg, 0.041 mmol), elution with hexane/AcOEt (85:15 to 65:35) gave **14i** (18.0 mg, 75%) as a reddish oil. IR (film) ν 2927, 1699 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.02 (t, J = 6.4 Hz, 2H, H6); 3.31 (s, 3H, OMe); 3.58 (s, 3H, CO_2Me); 4.61 (t, J = 6.4 Hz, 2H, H5); 5.57 (bs, 1H, OH); 6.33 (s, 1H); 6.79 (s, 1H); 7.04−7.06 (m, 2H); 7.10−7.17 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.8 (t); 42.9 (t); 50.8 (q); 54.9 (q); 107.9 (d); 113.8 (d); 118.2 (s); 119.5 (2d); 119.7 (q); 119.9 (q); 121.0 (s); 121.1 (2d); 121.7 (s); 127.0 (s); 131.3 (s); 131.6 (s); 131.8 (2d); 132.6 (2d); 133.8 (s); 134.3 (s), 144.9 (s); 145.1 (s); 147.8 (s); 147.9 (s); 161.9 (s). MS (MALDI-TOF) *m*/*z* 593 (M); 594 (M + 1). HRMS *m*/*z* calcd for $C_{29}H_{21}F_6NO_6$, 593.1273; found, 593.1268.

Methyl 8-Hydroxy-1,2-bis(4-hydroxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (14j). Following general procedure G and starting with **7j** (28.6 mg, 0.049 mmol), elution with hexane/AcOEt (60:40 to AcOEt) gave **14j** (15.0 mg, 67%) as a pale solid. Mp (MeCN) 190−195 °C. IR (film) ν 3194, 1683, 1436, 1267 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.92 (m, 2H, H6); 3.21 (s, 3H, OMe); 3.48 (s, 3H, CO_2Me); 4.43 (m, 2H, H5); 6.39 (s, 1H); 6.55 (d, J = 8.1 Hz, 2H); 6.67 (m, 3H); 6.85 (d, J = 8.2 Hz, 2H); 6.88 (d, J = 8.2 Hz, 2H); 9.16 (bs, 2H, OH); 9.31 (bs, 1H, OH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 27.8 (t); 42.4 (t); 50.4 (q); 54.5 (q); 108.8 (d); 113.8 (2d); 114.8 (d); 115.0 (2d); 117.0 (s); 118.8 (s); 120.7 (s); 125.5 (s); 125.6 (s); 126.3 (s); 130.8 (s); 131.2 (2d); 131.9 (2d); 132.2 (s); 145.6 (s); 145.7 (s); 155.4 (s); 155.9 (s); 161.5 (s). MS (MALDI-TOF) *m*/*z* 457 (M). HRMS *m*/*z* calcd for $C_{27}H_{23}NO_6$, 457.1525, found. 457.1520.

Methyl 8-Hydroxy-1,2-bis(3-hydroxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (14k). Following general procedure G and starting with **7k** (18.6 mg, 0.032 mmol), elution with hexane/AcOEt (60:40 to AcOEt) gave **14k** (12.3 mg, 85%) as a white solid. Mp (MeCN) 128−130 °C. IR

(film) ν 3299, 1680, 1440, 1202 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.94 (t, J = 6.5 Hz, 2H, H6); 3.20 (s, 3H, OMe); 3.49 (s, 3 H, CO_2Me); 4.43 (m, 2H, H5); 6.39 (s, 1H); 6.48−6.50 (m, 2H); 6.52−6.55 (m, 3H); 6.9−6.64 (m, 1H); 6.94−6.98 (t, J = 8.1 Hz, 1H); 7.06−7.10 (t, J = 8.4 Hz, 1H); 6.68 (s, 1H); 9.11 (bs, 1H, OH); 9.17 (bs, 1H, OH); 9.27 (bs, 1H, OH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.8 (t); 42.5 (t); 50.6 (q); 54.4 (q); 108.8 (d); 113.1 (d); 113.6 (d); 114.8 (d); 117.1 (d); 117.3 (d); 118.5 (s); 120.5 (s); 121.0 (d); 121.6 (d); 126.3 (s); 127.7 (d); 129.1 (d); 130.5 (s); 131.6 (s); 136.2 (s); 136.5 (s); 145.7 (s); 145.9 (s); 155.9 (s); 157.1 (s); 161.4 (s). MS (MALDI-TOF) m/z 457 (M). HRMS m/z calcd for $C_{27}H_{23}NO_6$, 457.1525; found, 457.1520.

Methyl 1,2-Bis(4-dimethylaminophenyl)-8-hydroxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (14l). Following general procedure G and starting with **11l** (5.4 mg, 0.0098 mmol), elution with hexane/AcOEt (80:20 to 50:50) gave **14l** (2.3 mg, 46%) as a white solid. Mp (MeCN) 245−247 °C. IR (film) ν cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.88 (s, 6H, NMe_2); 2.91 (s, 6H, NMe_2); 2.99 (t, J = 6.5 Hz, 2H, H6); 3.35 (s, 3H, OMe); 3.62 (s, 3H, CO_2Me); 4.56 (t, J = 6.5 Hz, 2H, H5); 5.48 (bs, 1H, OH); 6.54 (s, 1H); 6.59 (d, J = 8.8 Hz, 2H); 6.64 (d, J = 8.8 Hz, 2H); 6.74 (s, 1H); 7.01 (d, J = 8.8 Hz, 2H); 7.02 (d, J = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 28.9 (t); 40.5 (t); 40.5 (q); 40.7 (q); 50.7 (q); 55.2 (q); 108.3 (d); 111.4 (2d); 112.8 (2d); 113.4 (d); 117.6 (s); 120.8 (s); 121.7 (s); 121.9 (s); 123.9 (s); 126.7 (s); 131.2 (2d); 131.4 (s); 131.9 (2d); 144.4 (s); 144.6 (s); 146.6 (s); 149.8 (s); 161.1 (s). MS (MALDI-TOF) m/z 511 (M); 512 (M + 1). HRMS m/z calcd for $C_{31}H_{33}N_3O_4$, 511.2471, found. 511.2466.

Methyl 8-Hydroxy-9-methoxy-1,2-bis(3-nitrophenyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (14m). Following general procedure G and starting with **7m** (42.2 mg, 0.076 mmol), elution with hexane/AcOEt (80:20 to 50:50) gave **14m** (17.0 mg, 44%) as a reddish solid. Mp (MeCN) 241−243 °C. IR (film) ν 2926, 1701, 1540, 1439, 1350, 1227 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.06 (t, J = 6.5 Hz, 2H, H6); 3.29 (s, 3H, OMe); 3.59 (s, 3H, CO_2Me); 4.66 (t, J = 6.5 Hz, 2H, H5); 5.63 (bs, 1H, OH); 6.33 (s, 1H); 6.83 (s, 1H); 7.36−7.50 (m, 4H); 8.00−8.02 (m, 2H); 8.05−8.08 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 28.7 (t); 43.0 (t); 51.1 (q); 55.3 (q); 107.8 (d); 114.2 (d); 118.6 (s); 118.7 (s); 121.7 (d); 121.8 (d); 125.5 (d); 125.8 (d); 127.4 (s); 128.2 (d); 129.4 (d); 130.1 (s); 131.9 (s); 136.6 (d); 136.9 (s); 137.4 (d); 145.1 (s); 145.6 (s) 147.5 (s); 148.2 (s); 161.4 (s). MS (MALDI-TOF) m/z 515 (M); 516 (M + 1). HRMS m/z calcd for $C_{27}H_{21}N_3O_8$, 515.1329, found. 515.1323.

Methyl 2-(2,4-Dihydroxy-5-methoxyphenyl)-8-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (15d). Following general procedure G and starting with **9d** (48 mg, 0.07 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a reddish oil (26 mg, 70%). IR (film) ν 3419, 1686, 1439, 1246, 1197 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.95−3.15 (m, 2H, H6); 3.40 (s, 3H, OMe); 3.54 (s, 3H, OMe); 3.63 (s, 3H, OMe); 3.71 (s, 3H, OMe); 4.15−4.25 (m, 2H, H5); 5.54 (s, 3H, 3OH); 5.63 (s, 1H, OH); 6.31 (bs, 1H); 6.53 (bs, 1H, H2′); 6.56 (s, 1H); 6.72 (s, 1H); 6.75−6.79 (m, 2H); 6.82 (d, J = 8.0 Hz, 1H, H5′). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.8 (t); 43.2 (t); 51.5 (q); 55.5 (q); 55.9 (q); 56.4 (q); 102.8 (d); 108.5 (d); 113.4 (d); 113.8 (d); 114.0 (d); 114.3 (d); 118.9 (s); 120.0 (s); 122.5 (s); 123.8 (d); 126.8 (s); 126.9 (2s); 127.3 (s); 132.4 (s); 140.2 (s); 144.4 (s); 145.0 (s); 145.2 (s); 145.7 (s); 146.4 (s); 148.7 (s); 162.4 (s). MS (MALDI-TOF) 533 (M, 100); 534 (M + 1, 70); 535 (M + 2, 32). HRMS m/z calcd for $C_{29}H_{27}NO_9$, 533.1686; found, 533.1680.

Methyl 2-(2,5-Dimethoxy-4-hydroxyphenyl)-8-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (15e). Following general procedure G and starting with **9e** (67 mg, 0.10 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a brown solid (24 mg, 45%). Mp (MeCN) 140−145 °C. IR (film) ν 3423, 1688, 1265, 1196 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.02 (bs, 2H, H6); 3.38 (s, 3H, OMe); 3.57 (s, 3H, OMe); 3.60 (s, 6H, 2OMe); 3.63 (s, 3H, OMe); 4.57 (bs, 2H, H5); 5.53 (s, 1H, OH); 5.57 (s, 1H, OH); 5.59 (s, 1H, OH); 6.50 (s, 2H); 6.59 (s, 1H); 6.66 (s, 1H); 6.74−6.79 (m, 2H); 6.81 (d, J = 8.0 Hz, 1H, H5′). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.8 (t); 42.8 (t); 50.8 (q); 55.4 (q); 55.9 (q); 56.2 (q); 56.5 (q); 98.8 (d); 108.3 (d); 113.5 (d); 113.7 (d); 113.9 (d); 115.1 (d); 115.6 (s); 118.8 (s); 120.6 (s); 121.4 (s); 123.9 (d); 126.7 (s); 127.8 (s); 128.0 (s); 130.5 (s); 131.1 (s); 135.0 (s); 139.6 (s); 144.1 (s); 144.9 (s); 146.1 (s); 151.9 (s); 162.5 (s). MS (MALDI-TOF) 547 (M, 100); 548 (M + 1, 30). HRMS m/z calcd for $C_{30}H_{29}NO_9$, 547.1842; found, 547.1837.

Methyl 1-(2,5-Dimethoxyphenyl)-8-hydroxy-9-methoxy-2-(2,4,5-trihydroxyphenyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (15g). Following general procedure G and starting with **9g** (22 mg, 0.03 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a reddish oil (7 mg, 42%). IR (film) ν 3425, 1697, 1465, 1243 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.99 (t, J = 6.4 Hz, 2H, H6); 3.41 (s, 3H, OMe); 3.65 (s, 3H, OMe); 3.75 (s, 3H, OMe); 3.83 (s, 3H, OMe); 4.61 (t, J = 6.4 Hz, 2H, H5); 5.12 (bs, 1H, OH); 5.34 (bs, 1H, OH); 5.54 (bs, 1H, OH); 5.78 (bs, 1H, OH); 6.67 (s, 1H); 6.75 (s, 1H); 6.84 (dd, J = 8.9, 2.8 Hz, 1H, H4′); 6.88 (s, 1H); 6.89−6.92 (m, 2H); 7.02 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 30.9 (t); 42.5 (t); 51.0 (q); 55.4 (q); 55.8 (q); 56.2 (q); 107.6 (d); 112.2 (d); 113.5 (d); 113.6 (d); 116.7 (s); 117.4 (d); 119.9 (d); 120.1 (s); 121.1 (s); 126.3 (d); 126.5 (s); 132.7 (s); 144.8 (s); 145.0 (s); 149.8 (s); 150.3 (s); 151.6 (s); 153.6 (s); 161.8 (s). MS (MALDI-TOF) 533 (M, 100). HRMS m/z calcd for $C_{29}H_{27}NO_9$, 533.1686; found, 533.1684.

Methyl 8-Hydroxy-2-(2-hydroxy-4,5-dimethoxyphenyl)-1-(3-hydroxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (15i). Following general procedure G and starting with **9i** (23 mg, 0.04 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a reddish oil (16 mg, 85%). IR (film) ν 3405, 1684, 1437, 1196 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.01 (t, J = 6.8 Hz, 2H, H6); 3.36 (s, 3H, OMe); 3.60 (s, 3H, OMe); 3.81 (s, 3H, OMe); 3.85 (s, 3H, OMe); 4.46−4.71 (m, 2H, H5); 4.93 (bs, 1H, OH); 5.55 (bs, 1H, OH); 5.74 (bs, 1H, OH); 6.30 (d, J = 8.6 Hz, 1H); 6.53 (s, 1H); 6.60 (d, J = 8.6 Hz, 1H); 6.64−6.68 (m, 2H); 6.76 (s, 1H); 6.78 (d, J = 7.2 Hz, 1H); 7.11 (t, J = 7.2 Hz, 1H, H5′). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.8 (t); 43.0 (t); 51.0 (q); 55.3 (q); 55.6 (q); 60.9 (q); 102.9 (d); 108.4 (d); 113.5 (d); 113.7 (d); 115.8 (s); 117.6 (d); 118.9 (d); 120.3 (s); 121.6 (s); 123.6 (d); 126.1 (d); 126.8 (s); 127.0 (s); 129.3 (s); 131.6 (s); 135.3 (s); 137.2 (s); 144.8 (s); 144.9 (s); 147.5 (s); 151.4 (s); 155.5 (s); 162.3 (s). MS (MALDI-TOF) 517 (M, 100). HRMS m/z calcd for $C_{29}H_{27}NO_8$, 517.1737; found, 517.1731.

Methyl 8-Hydroxy-9-methoxy-1-(3,4,5-trimethoxyphenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (16a). Following general procedure G and starting with **10a** (23 mg, 0.05 mmol), elution with hexane/AcOEt (80:20 to 70:30) gave a yellowish solid (13 mg, 70%). Mp (MeCN) 212−213 °C. IR (film) ν 3409, 1678, 1207 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.57 (s, 3H, OMe); 3.86 (s, 6H, 2OMe); 3.91 (s, 3H, OMe); 3.92 (s, 3H, OMe); 5.82 (bs, 1H, OH); 6.75 (s, 2H, H2′, H6′); 6.94 (d, J = 7.6 Hz, 1H, H6); 7.14 (s, 1H); 7.31 (s, 1H); 7.42 (s, 1H); 9.22 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 51.2 (q); 55.5 (q); 56.2 (2q); 60.9 (q); 104.5 (d); 107.4 (2d); 110.5 (d); 112.5 (d); 114.1 (s); 118.1 (s); 119.4 (s); 121.8 (d); 123.5 (s); 124.1 (d); 130.8 (s); 132.9 (s); 137.2 (s); 146.0 (s); 146.7 (s); 153.3 (2s); 161.8 (s). MS (EI) m/z 393 (M, 100); 394 (M + 1, 12). MS (ESI-TOF) m/z 438 (M + 1, 100). HRMS m/z calcd for $C_{24}H_{24}NO_7$, 438.1547; found, 438.1547.

Methyl 8-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (16c). Following general procedure G and starting with **10d** (23 mg, 0.05 mmol), elution with hexane/AcOEt (80:20 to 70:30) gave a yellowish solid (13 mg, 70%). Mp (MeCN) 163−165 °C. IR (film) ν 1691, 1464, 1267, 1094 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.56 (s, 3H, OMe); 3.90 (s, 3H, OMe); 3.91 (s, 3H, OMe); 5.71 (bs, 1H, OH); 5.84 (bs, 1H, OH); 6.92 (d, J = 7.6 Hz, 1H, H6); 7.00 (d, J = 1.2 Hz, 1H, H2′); 7.03−7.05 (m, 2H, H5′, H6′); 7.12 (s, 1H); 7.33 (s, 1H); 7.39 (s, 1H); 9.21 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 51.1 (q); 55.5 (q); 56.0 (q); 104.5 (d); 110.5 (d); 112.3 (d); 113.0 (d); 114.0 (s); 114.3 (d); 116.2 (d); 118.1 (s); 119.6 (s);

122.2 (d); 123.5 (d); 124.0 (s); 129.1 (s); 130.9 (s); 144.9 (s); 145.9 (s); 146.5 (s); 146.7 (s); 161.8 (s). MS (EI) *m*/*z* 393 (M, 100); 394 (M + 1, 12). HRMS *m*/*z* calcd for $C_{27}H_{19}N_3O_8$, 393.1212; found, 393.1215.

Methyl 1-(2,5-Dimethoxyphenyl)-8-hydroxy-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (16e). Following general procedure G and starting with **10e** (26 mg, 0.06 mmol), elution with hexane/AcOEt (80:20 to 70:30) gave a yellow solid (14 mg, 57%). Mp (MeCN) 198−199 °C. IR (film) *ν*1690, 1465, 1206 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) *δ* 3.53 (s, 3H, OMe); 3.64 (s, 3H, OMe); 3.79 (s, 3H, OMe); 3.90 (s, 3H, OMe); 5.80 (s, 1H, OH); 6.93 (d, *J* = 7.6 Hz, 1H, H6); 6.94 (dd, *J* = 8.4, 2.8 Hz, 1H, H4′); 6.97 (d, *J* = 8.4 Hz, 1H, H3′); 6.99 (d, *J* = 2.8 Hz, 1H, H6′); 7.12 (s, 1H); 7.14 (s, 1H); 7.43 (s, 1H); 9.23 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* 51.1 (q); 55.4 (q); 55.8 (q); 56.3 (q); 104.4 (d); 110.2 (d); 112.1 (d); 112.4 (d); 113.4 (s); 114.0 (d); 114.3 (s); 117.9 (d); 120.0 (s); 122.4 (d); 123.6 (d); 124.0 (s); 127.0 (s); 131.5 (s); 145.8 (s); 146.8 (s); 152.2 (s); 153.6 (s); 161.8 (s). MS (MALDI-TOF) *m*/*z* 407 (M, 100); 408 (M + 1, 40). HRMS *m*/*z* calcd for $C_{23}H_{21}NO_6$, 407.1369; found, 407.1363.

Methyl 8-Hydroxy-9-methoxy-1-(2-thienyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (16n). Following general procedure G and starting with **6n** (15 mg, 0.04 mmol), elution with hexane/AcOEt (90:10) gave a white solid (6 mg, 45%). Mp (MeCN) 134−136 °C. IR (film) *ν* 3420, 1693, 1466, 1207 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) *δ* 3.62 (s, 3H, OMe); 3.91 (s, 3H, OMe); 5.82 (s, 1H, OH); 6.95 (d, *J* = 7.5 Hz, 1H, H6); 7.14 (s, 1H); 7.16 (s, 1H); 7.17 (d, *J* = 2.0 Hz, 1H); 7.34−7.35 (bd, 1H); 7.44 (dd, *J* = 4.1, 2.0 Hz, 1H); 7.48 (s, 1H); 9.22 (d, *J* = 7.5 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* 51.2 (q); 55.5 (q); 104.3 (d); 109.1 (s); 110.4 (d); 112.7 (d); 114.3 (s); 119.3 (s); 123.4 (d); 123.5 (d); 124.2 (s); 126.2 (d); 127.3 (d); 128.0 (d); 132.0 (s); 138.3 (s); 146.2 (s); 146.9 (s); 161.7 (s). MS (MALDI-TOF) *m*/*z* 353 (M, 100). MS (ESI-TOF) *m*/*z* 354 (M + 1, 100). HRMS *m*/*z* calcd for $C_{19}H_{16}NO_4S$, 354.0795; found, 354.0795.

Methyl 8-Hydroxy-9-methoxy-1,2-bis(3,4,5-trimethoxyphenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (17a). Following general procedure G and starting with **11a** (41 mg, 0.06 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a reddish oil (25 mg, 65%). IR (film) *ν* 3404, 1682, 1377, 1235 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) *δ* 3.51 (s, 3H, OMe); 3.69 (s, 6H, 2OMe); 3.70 (s, 6H, 2OMe); 3.72 (s, 3H, OMe); 3.84 (s, 3H, OMe); 3.85 (s, 3H, OMe); 5.82 (bs, 1H, OH); 6.46 (s, 2H); 6.53 (s, 2H); 6.95 (d, *J* = 7.6 Hz, 1H, H6); 7.14 (s, 1H); 7.15 (s, 1H); 9.30 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 50.9 (q); 55.3 (q); 56.0 (2q); 56.2 (2q); 60.88 (q); 60.92 (q); 104.8 (d); 108.3 (2d); 108.9 (2d); 110.4 (d); 111.8 (s); 112.4 (d); 118.2 (s); 119.0 (s); 123.6 (d); 124.4 (s); 126.9 (s); 130.5 (s); 131.7 (s); 135.3 (s); 136.8 (s); 137.1 (s); 146.0 (s); 146.7 (s); 152.0 (2s); 153.2 (2s); 162.4 (s). MS (MALDI-TOF) *m*/*z* 603 (M, 100); 604 (M + 1, 80). HRMS *m*/*z* calcd for $C_{33}H_{33}NO_{10}$, 603.2105; found, 603.2099.

Methyl 8-Hydroxy-1,2-bis(4-hydroxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (17c). Following general procedure G and starting with **11c** (46 mg, 0.08 mmol), a yellow solid (26 mg, 61%). Mp (MeCN) 235−237 °C. IR (film) *ν* 3415, 1680, 1376, 1211 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) *δ* 3.49 (s, 3H, OMe); 3.67 (s, 3H, OMe); 3.68 (s, 3H, OMe); 3.69 (s, 3H, OMe); 5.50 (bs, 1H, OH); 5.58 (bs, 1H, OH); 5.79 (bs, 1H, OH); 6.66 (d, *J* = 1.6 Hz, 2H, H2′, H2″); 6.72−6.77 (m, 2H, H6′, H6″); 6.80 (d, *J* = 8.0 Hz, 1H, H6); 6.91 and 6.92 (2d, *J* = 8.6 Hz, 2H, H5′, H5″); 7.12 (s, 1H); 7.13 (s, 1H); 9.30 (d, *J* = 8.0 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* 50.8 (q); 55.4 (q); 55.8 (q); 56.0 (q); 104.8 (d); 110.3 (d); 112.1 (d); 113.2 (d); 113.5 (d); 114.2 (d); 114.4 (d); 118.4 (s); 119.3 (s); 123.7 (d); 124.1 (d); 124.3 (s); 125.0 (d); 127.2 (s); 128.0 (s); 130.6 (s); 135.8 (s); 144.2 (s); 144.7 (s); 145.3 (s); 145.9 (s); 146.4 (s); 146.5 (s); 162.6 (s). MS (MALDI-TOF) 515 (M, 100); 516 (M + 1, 80). HRMS *m*/*z* calcd for $C_{29}H_{25}NO_8$, 515.1580; found, 515.1575.

Methyl 1,2-Bis(3,4-dimethoxyphenyl)-8-hydroxy-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (17f). Following general procedure G and starting with **11f** (22 mg, 0.04 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a yellowish oil (10 mg, 49%). IR (film) *ν* 3342, 1599 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) *δ* 3.47 (s, 3H, OMe); 3.68 (s, 3H, OMe); 3.70 (s, 3H, OMe); 3.71 (s, 3H, OMe); 3.87 (s, 3H, OMe); 3.88 (s, 3H, OMe); 5.79 (bs, 1H, OH); 6.70−6.78 (m, 4H); 6.85 (s, 2H); 6.92 (d, *J* = 7.6 Hz, 1H, H6); 7.11 (s, 1H); 7.12 (s, 1H); 9.29 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 50.7 (q); 55.3 (q); 55.6 (q); 55.7 (q); 55.8 (q); 55.9 (q); 104.8 (d); 109.9 (d); 110.3 (d); 111.1 (d); 111.9 (s); 112.1 (d); 114.2 (d); 115.0 (d), 118.3 (s); 119.3 (s), 123.2 (d); 123.7 (d); 124.2 (d); 124.3 (s); 127.8 (s); 128.7 (s); 130.6 (s); 135.7 (s); 145.9 (s); 146.5 (s); 147.5 (s); 147.5 (s); 148.0 (s); 148.9 (s); 162.6 (s). MS (MALDI-TOF) *m*/*z* 543 (M); 544 (M + 1). HRMS *m*/*z* calcd for $C_{31}H_{29}NO_8$, 543.1893; found, 543.1888.

Methyl 8-Hydroxy-9-methoxy-1,2-bis(4-methoxyphenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (17g). Following general procedure G and starting with **11g** (42 mg, 0.08 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a pale solid (27 mg, 71%). Mp (MeCN) 241−4 °C. IR (film) *ν* 2951, 1676 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) *δ* 3.28 (s, 3H, OMe); 3.54 (s, 3H, OMe); 3.71 (s, 3H, OMe); 3.74 (s, 3H, OMe); 6.77 (d, *J* = 8.6 Hz, 2H); 6.80 (s, 1H); 6.96 (d, *J* = 8.6 Hz, 2H); 7.08−7.11 (m, 3H); 7.11 (s, 1H); 7.19 (d, *J* = 8.5 Hz, 2H); 9.14 (d, *J* = 7.6 Hz, 1H, H5); 9.67 (bs, 1H, OH). ^{13}C NMR (DMSO-d_6, 100 MHz) *δ* 50.5 (q); 54.3 (q); 54.7 (q); 55.0 (q); 104.7 (d), 111.1 (d); 111.9 (d); 112.3 (2d); 113.8 (2d); 117.6 (s); 117.8 (s), 122.5 (s); 123.4 (d); 126.9 (s); 127.4 (s); 130.0 (s); 131.3 (2d); 132.8 (2d); 134.9 (s); 147.2 (s); 148.0 (s); 157.6 (s); 158.3 (s); 161.6 (s). MS (MALDI-TOF) *m*/*z* 483 (M). HRMS *m*/*z* calcd for $C_{29}H_{25}NO_6$, 483.1682, found. 483.1676.

Methyl 8-Hydroxy-9-methoxy-1,2-bis(4-trifluoromethoxyphenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (17i). Following general procedure G and starting with **11i** (29 mg, 0.05 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a reddish oil (8 mg, 30%). IR (film) *ν* 1727 cm^{-1}. ^1H NMR (Acetone-d_6, 400 MHz) *δ* 3.26 (s, 3H, OMe); 3.46 (s, 3H, OMe); 6.75 (s, 1H); 7.01 (d, *J* = 7.6 Hz, 1H, H6); 7.06 (d, *J* = 8.0 Hz, 2H); 7.09 (s, 1H); 7.23 (d, *J* = 8.7 Hz, 2H); 7.26 (d, *J* = 8.0 Hz, 2H); 7.36 (d, *J* = 8.7 Hz, 2H); 8.23 (bs, 1H, OH); 9.18 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR (Acetone-d_6, 100 MHz) *δ* 49.4 (q); 53.5 (q); 103.9 (d), 110.6 (d); 111.5 (q); 111.9 (d); 116.0 (q); 117.7 (s); 118.8 (2d); 120.6 (2d); 122.2 (s); 123.6 (s); 129.7 (d); 131.6 (s); 133.1 (2d); 133.5 (2d); 133.9 (s); 134.7 (s); 146.9 (s); 147.1 (s); 147.5 (s); 147.6 (s); 161.0 (s). MS (MALDI-TOF) *m*/*z* 591 (M). HRMS *m*/*z* calcd for $C_{29}H_{19}F_6NO_6$, 591.1117, found. 591.1111.

Methyl 8-Hydroxy-1,2-bis(4-hydroxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (17j). Following general procedure G and starting with **11j** (77 mg, 0.18 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a pale solid (32 mg, 53%). Mp (MeCN) 280−284 °C. IR (film) *ν* 3373, 1684 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) *δ* 3.29 (s, 3H, OMe); 3.54 (s, 3H, OMe); 6.58 (d, *J* = 8.5 Hz, 2H); 6.77 (d, *J* = 8.5 Hz, 2H); 6.90 (s, 1H); 6.95 (d, *J* = 8.5 Hz, 2H); 7.03 (s, 1H); 7.05−7.08 (m, 3H); 9.12 (d, *J* = 7.6 Hz, 1H, H5); 9.22 (bs, 1H, OH); 9.42 (bs, 1H, OH); 9.64 (bs, 1H, OH). ^{13}C NMR (DMSO-d_6, 100 MHz) *δ* 50.4 (q); 54.3 (q); 104.9 (d); 110.9 (d); 111.7 (d); 113.8 (2d); 115.2 (2d); 117.9 (s); 118.0 (s), 122.5 (s); 123.3 (d); 125.3 (s); 125.7 (s); 130.0 (s); 131.3 (2d); 132.7 (2d); 135.5 (s); 147.1 (s); 147.9 (s); 155.7 (s); 156.4 (s); 161.7 (s). MS (MALDI-TOF) *m*/*z* 455 (M, 100). HRMS *m*/*z* calcd for $C_{27}H_{21}NO_6$, 455.1369; found, 455.1363.

Methyl 8-Hydroxy-1,2-bis(3-hydroxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (17k, R^3 = OH). Following general procedure G and starting with **11k** (67 mg, 0.12 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a pale solid (24 mg, 46%). Mp (MeCN) 260−265 °C. IR (film) *ν* 3384, 1653 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) *δ* 3.29 (s, 3H, OMe); 3.53 (s, 3H, CO_2Me); 6.66−6.76 (m, 3H); 6.72 (t, *J* = 1.8 Hz, 1H); 6.70−6.72 (dd, *J* = 7.8, 1.9 Hz, 2H); 6.90 (s, 1H); 6.98−7.02 (dd, *J* = 8.7, 9.0 Hz, 1H); 7.10 (s, 1H); 7.11 (d, *J* = 7.6 Hz, 1H, H6); 7.19 (t, *J* = 8.0 Hz, 1H); 9.11 (d, *J* = 7.6 Hz, 1H, H5); 9.17 (bs, 1H, OH); 9.27 (bs, 1H, OH); 9.40 (bs, 1H, OH). ^{13}C NMR (DMS-d_6, 100 MHz) *δ* 50.6 (q); 54.3 (q); 104.9 (d), 111.1 (d); 112.1 (d); 113.4

(d); 114.1 (d); 117.2 (d); 117.7 (s); 117.8 (s); 118.2 (d); 121.1 (d); 122.3 (d); 122.4 (d); 123.4 (s); 127.7 (d); 129.3 (d); 129.5 (s); 134.9 (s); 136.1 (s); 136.7 (s); 147.2 (s); 148.1 (s); 155.8 (s); 157.2 (s); 161.6 (s). MS (MALDI-TOF) *m/z* 455 (M). HRMS *m/z* calcd for $C_{27}H_{21}NO_6$, 455.1369, found. 455.1363.

Methyl 8-Hydroxy-9-methoxy-1,2-bis(3-nitrophenyl)pyrrolo-[2,1-*a*]isoquinoline-3-carboxylate (17m). Following general procedure G and starting with **11m** (31 mg, 0.06 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a pale solid (23 mg, 82%). Mp (MeOH) = 185−190 °C. IR (film) ν 1689, 1537, 1379, 1348 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) 3.25 (s, 3H, OMe); 3.56 (s, 3H, CO_2Me); 6.76 (s, 1H); 7.19 (s, 1H); 7.25 (d, J = 7.6 Hz, 1H, H6); 7.53 (td, J = 7.6, 1.2 Hz, 1H); 7.67−7.72 (m, 2H); 7.85 (dt, J = 8.0, 1.2 Hz, 1H); 8.08 (d, J = 1.2 Hz, 1H); 8.07 (dt, J = 8.0, 1.2 Hz, 1H); 8.16−8.19 (m, 2H); 9.22 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 50.9 (q); 54.4 (q); 104.3 (d), 111.5 (d); 111.8 (s); 113.1 (d); 115.2 (s); 117.2 (s), 121.7 (d); 122.3 (d); 122.4 (d); 123.7 (s); 125.0 (d); 126.0 (d); 128.7 (d); 129.9 (d); 130.0 (s); 132.7 (s); 136.2 (s); 136.6 (s); 137.2 (d); 138.5 (d); 146.8 (s); 147.7 (s); 147.9 (s); 148.5 (s); 160.9 (s). MS (MALDI-TOF) *m/z* 513 (M); 514 (M + 1). HRMS *m/z* calcd for $C_{27}H_{19}N_3O_8$, 513.1172; found, 513.1167.

Methyl 8-Hydroxy-9-methoxy-1,2-bis(2-thienyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (17n). Following general procedure G and starting with **11n** (12 mg, 0.03 mmol), elution with hexane/AcOEt (90:10 to 75:25) gave a pale solid (5 mg, 40%). Mp (MeCN) 205−208 °C. IR (film) ν 3409, 1683, 1434, 1376, 1246 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.56 (s, 3H, OMe); 3.73 (s, 3H, OMe); 5.82 (bs, 1H, OH); 6.93−6.95 (m, 2H); 6.96 (d, J = 7.6 Hz, 1H, H6); 7.05 (dd, J = 3.4, 1.2 Hz, 1H); 7.08−7.11 (m, 2H); 7.13 (s, 1H); 7.26−7.28 (m, 1H); 7.39 (dd, J = 5.2, 1.2, 1H); 9.26 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 51.0 (q); 55.3 (q); 104.6 (d); 110.3 (d); 110.4 (s); 112.9 (d); 113.2 (s); 118.9 (s); 123.4 (d); 124.4 (s); 125.8 (d); 126.0 (d); 127.19 (d); 127.24 (d); 128.3 (d); 129.5 (s); 129.9 (d); 131.8 (s); 135.0 (s); 136.7 (s); 146.2 (s); 146.9 (s); 162.2 (s). MS (ESI) *m/z* 436 (M + 1, 100); 437 (M + 2, 65). MS (ESI-TOF) *m/z* 436 (M + 1, 100). HRMS *m/z* calcd for $C_{23}H_{18}NO_4S_2$, 436.0672; found, 436.0672.

Methyl 8-Hydroxy-2-(4-hydroxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (18a). Following general procedure G and starting with **12a** (82 mg, 0.14 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a pinkish solid (62 mg, 89%). Mp (MeCN) 260−262 °C. IR (film) ν 3364, 1653 cm^{-1}. ^1H NMR (MeOD-d_4, 400 MHz) δ 3.43 (s, 3H, OMe); 3.60 (s, 3H, OMe); 3.67 (s, 3H, OMe); 6.63 (d, J = 8.8 Hz, 2H, H3″, H5′); 6.69−6.72 (m, 2H, H2′, H6′); 6.81 (bd, J = 8.0 Hz, 1H, H5′); 6.88 (d, J = 7.6 Hz, 1H, H6); 6.98 (d, J = 8.8 Hz, 2H, H2″, H6″); 7.02 (s, 1H); 7.10 (s, 1H); 9.18 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR (MeOD-d_4, 100 MHz) δ 51.0 (q); 55.7 (q); 56.5 (q); 106.5 (d); 112.1 (d); 112.8 (s); 112.9 (d); 114.9 (2d); 116.3 (d); 116.8 (d); 120.1 (s); 120.2 (s); 124.3 (d); 125.6 (s); 126.0 (d); 128.2 (s); 128.9 (s); 132.2 (s); 132.9 (2d); 137.8 (s); 146.8 (s); 148.4 (s); 149.0 (s); 149.3 (s); 157.1 (s); 164.2 (s). MS (ESI-TOF) 486 (M + 1, 67); 486 (MNa^+, 100). HRMS *m/z* calcd for $C_{28}H_{23}NNaO_7^+$, 508.1367; found, 508.1367.

Methyl 2-(3,4-Dimethoxyphenyl)-8-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (18b). Following general procedure G and starting with **12b** (66 mg, 0.11 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a yellowish solid (38 mg, 67%). Mp (MeCN) 110−113 °C. IR (film) ν 3420, 1676 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.49 (s, 3H, OMe); 3.68 (s, 3H, OMe); 3.69 (s, 3H, OMe); 3.70 (s, 3H, OMe); 3.87 (s, 3H, OMe); 5.58 (bs, 1H, OH); 5.79 (bs, 1H, OH); 6.67 (d, J = 2.0 Hz, 1H); 6.72 (d, J = 1.6 Hz, 1H); 6.75 (d, J = 8.4 Hz, 1H); 6.78 (dd, J = 8.4, 2.0, 1H); 6.89 (dd, J = 8.0, 1.6 Hz, 1H); 6.92 (d, J = 7.6 Hz, 1H, H6); 6.93 (d, J = 8.0 Hz, 1H); 7.117 (s, 1H); 7.122 (s, 1H); 9.29 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 50.8 (q); 55.4 (q); 55.7 (2q); 56.0 (q); 104.8 (d); 109.9 (d); 110.3 (d); 111.9 (s); 112.1 (d); 114.2 (2d); 114.3 (d); 118.4 (s); 119.3 (s), 123.2 (d); 123.7 (d); 124.3 (s); 125.0 (d); 127.8 (s); 128.0 (s); 130.6 (s); 135.7 (s); 144.7 (s); 145.9 (s); 147.53 (s); 146.4 (s); 146.5 (s); 147.5 (s); 162.8 (s). MS (MALDI-TOF) 529 (M, 100). HRMS *m/z* calcd for $C_{30}H_{27}NO_8$, 529.1737; found, 529.1731.

Methyl 1-(3,5-Dimethoxy-4-hydroxyphenyl)-8-hydroxy-9-methoxy-2-(3,4,5-trimethoxyphenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (18c R^4 of 2-Ar = OH). Following general procedure G and starting with **12c** (80 mg, 0.12 mmol) and using an excess of $AlCl_3$ (0.32 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a yellowish solid (61 mg, 96%). Mp (MeCN) 163−166 °C. IR (film) ν 3421, 1678 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.49 (s, 3H, OMe); 3.69 (s, 3H, OMe); 3.70 (s, 3H, OMe); 3.72 (s, 6H, 2OMe); 5.44 (s, 1H, OH); 5.60 (s, 1H, OH); 5.80 (s, 1H, OH); 6.45 (s, 2H, H2″, H6″); 6.66 (d, J = 1.6 Hz, 1H, H2′); 6.91 (dd, J = 8.4, 1.6 Hz, 1H, H6′); 6.93 (d, J = 7.6 Hz, 1H, H6); 6.95 (d, J = 8.4 Hz, 1H, H5′); 7.13 (s, 2H, H7, H10); 9.30 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ. 50.8 (q); 55.4 (q); 56.0 (q); 56.2 (2q); 104.8 (d); 108.0 (2d); 110.3 (d); 111.7 (s); 112.2 (d); 114.20 (d); 114.23 (d), 118.3 (s); 119.2 (s), 123.6 (d); 124.3 (s); 125.0 (d); 126.2 (s); 128.1 (s); 130.6 (s); 133.4 (s); 135.7 (s); 144.7 (s); 145.90 (s); 145.93 (2s); 146.5 (s); 146.6 (s); 162.6 (s). MS (ESI-TOF) 514 (M, 26); 568 (M + Na, 100). HRMS *m/z* calcd for $C_{30}H_{27}NNaO_9^+$, 568.1578; found, 568.1578.

Methyl 2-(2,4-Dihydroxy-5-methoxyphenyl)-8-hydroxy-1-(4-hydropoxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (18d). Following general procedure G and starting with **12d** (97 mg, 0.14 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a light brown solid (63 mg, 86%). Mp (MeCN) 163−166 °C. IR (film) ν 3426, 1679 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.51 (s, 3H, OMe); 3.55 (s, 3H, OMe); 3.66 (s, 3H, OMe); 3.77 (s, 3H, OMe); 5.43 (br, 1H, OH); 5.56 (s, 1H, OH); 5.61 (s, 1H, OH); 5.87 (s, OH); 6.33−6.92 (m, 3H); 6.92−7.27 (m, 5H); 9.19 (m, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 51.4 (q); 55.4 (q); 56.0 (q); 56.4 (q); 102.7 (d); 104.8 (d); 110.4 (d); 112.5 (d); 112.7 (s); 113.8 (d); 113.9 (s); 114.0 (d); 114.1 (d); 119.1 (s); 123.4 (d); 124.2 (d); 124.5 (s); 131.3 (s); 140.2 (s); 144.8 (s); 145.8 (s); 146.1 (s); 146.6 (s); 146.7 (s); 148.6 (s); 162.3 (s). MS (MALDI-TOF) 531 (M, 100), 532 (M + 1, 38), 533 (M + 2, 11). HRMS *m/z* calcd for $C_{29}H_{25}NO_9$, 531.1529; found, 531.1524.

Methyl 2-(2,5-Dimethoxy-4-hydroxyphenyl)-8-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (18e). Following general procedure G and starting with **12e** (51 mg, 0.08 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a reddish solid (21 mg, 50%). Mp (MeCN) 149−151 °C. IR (film) ν 3389, 1681, 1438, 1206 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.50 (s, 3H, OMe); 3.61 (bs, 3H, OMe); 3.65 (2s, 6H, 2OMe); 3.70 (s, 3H, OMe); 5.56 (bs, 2H, 2OH); 5.78 (s, 1H, OH); 6.51 (s, 1H); 6.55 (bs, 1H); 6.72 (bs, 1H); 6.85−6.93 (m, 3H); 7.11 (s, 1H); 7.18 (s, 1H); 9.25 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 50.8 (q); 55.4 (q); 55.9 (q); 56.0 (q); 56.6 (q); 98.8 (d); 104.8 (d); 110.3 (d); 111.9 (d); 114.1 (d); 115.0 (d); 115.6 (d); 118.5 (s); 119.4 (s); 123.7 (d); 124.1 (s); 124.6 (d); 124.7 (s); 128.2 (s); 130.5 (s); 139.6 (s); 144.5 (s); 145.2 (s); 145.7 (2s); 146.5 (2s); 152.0 (s); 162.6 (s). MS (MALDI-TOF) 545 (M, 100); 546 (M + 1, 70). HRMS *m/z* calcd for $C_{30}H_{27}NO_9$, 545.1686; found, 545.1680.

Methyl 1-(2,5-Dimethoxyphenyl)-8-hydroxy-9-methoxy-2-(2,4,5-trihydroxyphenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (18 g). Following general procedure G and starting with **12g** (31 mg, 0.04 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a reddish solid (15 mg, 62%). Mp (MeCN) 149−150 °C. IR (film) ν 3418, 1690, 1466, 1208 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.53 (s, 3H, OMe); 3.65 (s, 3H, OMe); 3.79 (s, 3H, OMe); 3.90 (s, OMe); 6.91−6.99 (m, 5H, H6, H7, H3′, H4′, H6′); 7.12 (s, 1H, H3″); 7.14 (s, 1H); 7.43 (s, 1H, H6"); 9.22 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 51.1 (q); 53.4 (q); 55.8 (q); 56.3 (q); 104.4 (d); 107.2 (d); 110.2 (d); 112.1 (d); 112.4 (d); 113.4 (s); 114.0 (d); 114.3 (s); 117.9 (d); 120.0 (s); 122.4 (d); 123.6 (d); 123.9 (s); 127.0 (s); 130.8 (s); 131.5 (s); 145.8 (s); 146.8 (s); 152.2 (s); 152.8 (s); 153.4 (s); 153.6 (s); 153.7 (s); 161.8 (s). MS (MALDI-TOF) 531 (M, 100). HRMS *m/z* calcd for $C_{30}H_{27}NO_9$, 531.1529; found, 531.1527.

Methyl 2-(2,4-Dihydroxyphenyl)-8-hydroxy-1-(3-hydroxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (18h). Following general procedure G and starting with **12h** (27 mg, 0.04 mmol), elution with hexane/AcOEt (60:40 to 40:60) gave a yellow solid (8 mg, 40%). Mp (MeCN) 167−169 °C. IR (film) ν 3374, 1683, 1207 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.57 (s, 3H, OMe); 3.91 (s, 3H, OMe); 6.86−6.89 (m, 1H); 6.91 (d, J = 7.6 Hz, 1H, H6); 6.97−7.00 (m, 3H); 6.98−7.02 (m, 1H); 7.12 (s, 1H); 7.34 (t, J = 8.0 Hz, 1H, H5′); 7.35 (bs, 1H); 7.40 (s, 1H); 9.20 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 51.2 (q); 55.5 (q); 104.6 (d); 110.4 (d); 112.4 (d); 114.1 (d); 117.3 (d); 117.8 (s); 118.4 (s); 119.4 (s); 122.1 (d); 123.0 (d); 123.5 (d); 124.0 (d); 129.6 (d); 130.1 (s); 130.8 (s); 133.4 (d); 138.9 (s); 145.9 (s); 146.7 (s); 155.2 (s); 155.7 (s); 156.0 (s); 161.8 (s). MS (MALDI-TOF) 471 (M, 100). HRMS *m*/*z* calcd for $C_{27}H_{21}NO_7$, 471.1318; found, 471.1317.

Cell Growth Inhibition Assay. Screening. A colorimetric assay using sulforhodamine B (SRB) was adapted to perform a quantitative measurement of cell growth and viability, following a previously described method.[45] The cells were seeded in 96-well microtiter plates at 5×10^3 cells/well in aliquots of 195 μL of RPMI medium and allowed to attach to the plate surface by growing in a drug-free medium for 18 h. Afterward, samples were added in aliquots of 5 μL (dissolved in DMSO/H_2O, 3:7). After 72 h of exposure, the antitumor effect was measured by the SRB methodology. The cells were fixed by adding 50 μL of cold 50% (wt/vol) trichloroacetic acid (TCA) and incubated for 60 min at 4 °C. The plates were washed with deionized H_2O and dried; 100 μL of SRB solution (0.4 wt %/vol in 1% acetic acid) was added to each microtiter well and incubated for 10 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid. The plates were air-dried, and the bound stain was solubilized with Tris buffer. Optical densities were read on an automated spectrophotometer plate reader at a single wavelength of 490 nm. Data analyses were automatically generated by LIMS implementation. Using control OD values (C), test OD values (T), and time zero OD values (T_0), the drug concentration that causes 50% growth inhibition (GI_{50} value) was calculated from the equation, $100 \times [(T - T_0)/(C - T_0)] = 50$.

Acknowledgment. This work was partially supported by CICYT (BQU 2003-00089), Generalitat de Catalunya, and the Barcelona Science Park. PharmaMar S. L. is also gratefully acknowledged for performing the preliminary biological tests. A.M. thanks the Junta de Andalucía, UJA, and UB for financial support and for facilitating his stay.

Supporting Information Available: Experimental procedures and characterization by ^1H- and ^{13}C-NMR, HRMS, and HPLC analyses of synthesized compounds as well as ^1H NMR at variable temperature and gHSQC correlations of **12f**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dobson, C. M. Chemical Space and Biology. *Nature* **2004**, *432*, 824−828.
(2) Breinbauer, R.; Vetter, I. R.; Waldmann, H. From Protein Domains to Drug Candidates-Natural Products as Guiding Principles in the Design and Synthesis of Compound Libraries. *Angew. Chem., Int. Ed.* **2002**, *41*, 2878−2890.
(3) Balamurugan, R.; Dekker, F. J.; Waldmann, H. Design of Compound Libraries Based on Natural Product Scaffolds and Protein Structure Similarity Clustering (PSSC). *Mol. Biosyst.* **2005**, *1*, 36−45.
(4) Newman, D. J.; Cragg, G. M.; Snader, K. M. Natural Products as Sources of New Drugs over the Period 1981−2002. *J. Nat. Prod.* **2003**, *66*, 1022−1037.
(5) Clardy, J.; Walsh, C. Lessons from Natural Molecules. *Nature* **2004**, *432*, 829−837.
(6) Jaroszewski, J. W. Hyphenated NMR Methods in Natural Products Research, Part 1: Direct Hyphenation. *Planta Med.* **2005**, *71*, 691−700.
(7) Jaroszewski, J. W. Hyphenated NMR Methods in Natural Products Research, Part 2: HPLC−SPE-NMR and Other New Trends in NMR Hyphenation. *Planta Med.* **2005**, *71*, 795−802.
(8) Rouhi, A. M. Rediscovering Natural Products. *Chem. Eng. News.* **2003**, *81*, 77−91.
(9) König, G. M.; Kehraus, S.; Seibert, S. F.; Abdel-Lateff, A.; Müller, D. Natural Products from Marine Organisms and Their Associated Microbes. *ChemBioChem* **2006**, *7*, 229−238.
(10) Andersen, R. J.; Faulkner, D. J.; Cun-heng, H.; Van Duyne, G. D.; Clardy, J. Metabolites of the Marine Prosobranch Mollusc Lamellaria sp. *J. Am. Chem. Soc.* **1985**, *107*, 5492−5495.
(11) Cironi, P.; Albericio, F.; Álvarez, M. Lamellarins: Isolation, Activity and Synthesis. In *Progress in Heterocyclic Chemistry*; Gribble, G. W., Joule, J. A., Eds.; Pergamon: Oxford, U.K., 2004; Vol. 16, pp 1−26.
(12) Bailly, C. Lamellarins, from A to Z: A Family of Anticancer Marine Pyrrole Alkaloids. *Curr. Med. Chem.: Anti-Cancer Agents* **2004**, *4*, 363−378.
(13) Facompré, M.; Tardy, C.; Bal-Mahieu, C.; Colson, P.; Pérez, C.; Manzanares, I.; Cuevas, C.; Bailly, C. Lamellarin D: A Novel Inhibitor of Topoisomerase I. *Cancer Res.* **2003**, *63*, 7392−7399.
(14) Vanhuyse, M.; Kluza, J.; Tardy, C.; Otero, G.; Cuevas, C.; Bailly, C.; Lansiaux, A. Lamellarin D: A Novel Pro-Apoptotic Agent from Marine Origin Insensitive to P-Glycoprotein-Mediated Drug Efflux. *Cancer Lett.* **2005**, *221*, 165−175.
(15) Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B., Jr.; Stewart, L. The Mechanism of Topoisomerase I Poisoning by a Camptothecin Analog. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15387−15392.
(16) Marco, E.; Laine, W.; Tardy, C.; Lansiaux, A.; Iwao, M.; Ishibashi, F.; Bailly, C.; Gago, F. Molecular Determinants of Topoisomerase I Poisoning by Lamellarins: Comparison with Camptothecin and Structure−Activity Relationships. *J. Med. Chem.* **2005**, *48*, 3796−3807.
(17) Ishibashi, F.; Tanabe, S.; Oda, T.; Iwao M. Synthesis and Structure−Activity Relationship Study of Lamellarin Derivatives. *J. Nat. Prod.* **2002**, *65*, 500−504.
(18) Tardy, C.; Facompré, M.; Laine, W.; Baldeyrou, B.; García-Gravalos, D.; Francesch, A.; Mateo, C.; Pastor, A.; Jiménez, J. A.; Manzanares, I.; Cuevas C.; Bailly, C. Topoisomerase I-Mediated DNA Cleavage as a Guide to the Development of Antitumor Agents Derived from the Marine Alkaloid Lamellarin D: Triester Derivatives Incorporating Amino Acid Residues. *Bioorg. Med. Chem.* **2004**, *12*, 1697−1712.
(19) Olsen, C. A.; Parera, N.; Albericio, F.; Álvarez, M. 5,6-Dihydropyrrolo[2,1-*a*]isoquinolines as Scaffolds for Synthesis of Lamellarin Analogues. *Tetrahedron Lett.* **2005**, *46*, 2041−2044.
(20) For clarity, in this article, the numbering of the lamellarins and the scaffold is as in ref 15.
(21) Heim, A.; Terpin, A.; Steglich, W. Biomimetic Synthesis of Lamellarin G Trimethyl Ether. *Angew. Chem., Int. Ed.* **1997**, *36*, 155−156.
(22) Ishibashi, F.; Miyazaki, Y.; Iwao, M. Total Synthesis of Lamellarin D and H. The First Synthesis of Lamellarin-Class Marine Alkaloids. *Tetrahedron* **1997**, *53*, 5951−5962.
(23) Banwell, M.; Flynn, B.; Hockless, D. Convergent Total Synthesis of Lamellarin K. *Chem. Commun.* **1997**, 2259−2260.
(24) Cironi, P.; Manzanares, I.; Albericio, F.; Álvarez, M. Solid-Phase Total Synthesis of Pentacyclic System Lamellarins U and L. *Org. Lett.* **2003**, *5*, 2959−2962.
(25) Marfil, M.; Albericio, F.; Álvarez, M. Solid-Phase Synthesis of Lamellarins Q and O. *Tetrahedron* **2004**, *60*, 8659−8668.
(26) Cironi, P.; Cuevas, C.; Albericio, F.; Álvarez, M. Gaining Diversity in Solid-Phase Synthesis by Modulation of Cleavage Conditions from Hydroxymethyl-Based Supports. Application to Lamellarins. *Tetrahedron* **2004**, *60*, 8669−8675.
(27) Pla, D.; Marchal, A.; Olsen, C. A.; Albericio, F.; Álvarez, M. Modular Total Synthesis of Lamellarin D. *J. Org. Chem.* **2005**, *70*, 8231−8234.
(28) Fujikawa, N.; Ohta, T.; Yamaguchi, T.; Fukuda, T.; Ishibashi, F.; Iwao, M. Total Synthesis of Lamellarins D, L, and N. *Tetrahedron* **2006**, *62*, 594−604.
(29) An advantage of the protection was the increase in solubility of the compounds throughout the synthetic process as well as the prevention of undesired processes.
(30) Compound **4d** was not used as a building block. This entry is in the Table to introduce the substituents of compounds **6d**, **8d**, and **10d**.
(31) (a) Kranenburg, M.; van der Burgt, Y. E. M.; Kamer, P. C. J.; van Leeuwen, P. W. N. M.; Goubitz, K. Fraange. New Diphosphine Ligands Based on Heterocyclic Aromatics Inducing Very High Regioselectivity in Rhodium-Catalyzed Hydroformylation: Effect of the Bite Angle. *Organometallics* **1995**, *14*, 3081−3089. (b) Wolfe, J. P.; Singer, R. A.; Bryant, H. Y.; Buchwald, S. L. Highly Active Palladium Catalysts for Suzuki Coupling Reactions. *J. Am. Chem. Soc.* **1999**, *121*, 9550−9561.
(32) To obtain **2**, **3**, and **8**, the protection of the phenolic groups is crucial to avoid byproducts during bromination.

(33) Regioselectivity on the bromination of **6** to give **8** was easily checked by the absence of the singlet at 6.7 ppm, characteristic of H-2.
(34) A lower reaction time than that for the less electron-rich analogues or the lower reaction temperature did not improve the results.
(35) In a previous study on the preparation of Lam-D (ref 27), an excess of 6 equiv of boronate were used; however, the reduction of that amount to 3 equiv did not produce a significant change in the reaction yield.
(36) Alternatively, a more convergent synthesis of diarylated compound **9** with a range of substituted phenyl rings was attempted by a regioselective Suzuki cross-coupling reaction on the dibromo-scaffold **3.** However, our first studies using an equimolar amount of the boronic building block **4g** by the same reaction conditions as before produced 75% of a monoarylated bromide by HPLC-MS. Nevertheless, ^{1}H-NMR analyses evidenced the presence of an equimolecular amount of 1-aryl- and 2-aryl-bromides and, therefore, the absence of regioselectivity.
(37) Sotomayor, N.; Domínguez, E.; Lete. E. Oxidation Reactions of 2′-Functionalized 3-Aryltetrahydro and 3,4-Dihydroisoquinolines. *Tetrahedron* **1995**, *51*, 12721−12730.
(38) Bermejo, A.; Andreu, I.; Suvire, F.; Leonce, S.; Caignard, D. H.; Renard, P.; Pierre, A.; Enriz, R. D.; Cortes, D.; Cabedo, N. Syntheses and Antitumor Targeting G1 Phase of the Cell Cycle of Benzoyldihydroisoquinolines and Related 1-Substituted Isoquinolines. *J. Med. Chem.* **2002**, *45*, 5058−5068.
(39) It was not possible to oxidize scaffold **1**, **6l**, and **7l** using this procedure.
(40) Both double doublets were assigned by gHSQC to C5”-H. See the gHSQC of **12f** in the Supporting Information.
(41) Semiempirical method PM3 was used for the energy minimization of each rotamer.
(42) Mata, E. G. β-Lactams on Solid Support: Mild and Efficient Removal of Penicillin Derivatives from Merrifield Resin using Aluminum Chloride. *Tetrahedron Lett.* **1997**, *38*, 6335−6338.
(43) Concomitant demethylation of the 4-methoxy group occurred using an excess of 2.6 equiv of $AlCl_3$ when a rich electron-ring building block such as 3,4,5-trimethoxyphenyl was introduced to give, for instance, **14a** (R^4═OH) and **18c** (R^8═OH) with yields of 58 and 96%, respectively. This demethylation was avoided using 1.3 equiv of $AlCl_3$ in **16a** and **17a.**
(44) The letters and numbers assigned to compounds **13**−**18** are the same as those indicated in Table 1 and take into account the deprotection of the *i*PrO-groups (R^3, R^4, R^6, and R^8) to give OH.
(45) (a) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Waren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New Colorimetric Cytotoxicity Assay for Anticancer Drug Screening. *J. Natl. Cancer Inst*. **1990**, *82*, 1107−1112. (b) Faircloth, G. T.; Stewart, D.; Clement, J. J. A Simple Screening Procedure for the Quantitative Measurement of Cytotoxicity Assay. *J. Tissue Cult. Methods* **1988**, *11*, 201−205.

JM0602458

SUPPLEMENTARY MATERIAL

for the full communication entitled

Synthesis and Structure–Activity Relationship Study of Potent Cytotoxic Analogs of the Marine Alkaloid Lamellarin D

authored by

Daniel Pla,[a] *Antonio Marchal,*[a,‖] *Christian A. Olsen,*[a,⁂] *Andrés Francesch,*[b] *Carmen Cuevas,* [b] *Fernando Albericio,*[a,‡,*] *Mercedes Álvarez*[a,§,*]

[a] Biomedical Research Institute, Barcelona Scientific Park-University of Barcelona, E-08028 Barcelona; [b] Pharma Mar, Avda Reyes Católicos 1, E-28770 Colmenar Viejo, Madrid, Spain. [‖] Current address: Department of Inorganic and Organic Chemistry, University of Jaén, E-23071 Jaén; [⁂] Current address: The Danish University of Pharmaceutical Sciences, Copenhagen, Denmark; [‡] Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona; [§] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, E-08028 Barcelona.

[*] Mercedes Álvarez. Tel.: +34 93 403 70 86; fax: +34 93 403 71 26; e-mail: *malvarez@pcb.ub.es*

TABLE OF CONTENTS

EXPERIMENTAL SECTION

General Data

Reactants and solvents were purified accoding to *Purification of Laboratory Chemicals,* Armarego, W. and Chai C., Elsevier (2003). Melting points (M. p.) were determined in a Büchi Melting Point B540 in open capillaries and are uncorrected. Microwave-assisted reactions were carried out in a CEM Discover microwave. The automatic syringe pump was used as specified for controlled addition of some reactants. Automatic flash chromatography was done in an Isco Combiflash medium pressure liquid cromatograph with Redisep silica gel columns (47-60 μm). Reversed phase analytical HPLC was performed on a Waters Alliance separation module 2695 using a Waters Xterra MS C_{18} column (150 x 4.6 mm, 5 μm) and a Waters 996 PDA detector at 254 nm. A Branson ultrasound bath was used to perform sonication. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer and a Gemini 200 MHz spectrometer. Multiplicity of the carbons was assigned with DEPT and gHSQC experiments, although usual abbreviations according to off-resonance decoupling are used: (s) singlet, (d) doublet, (t) triplet, (q) quartet. The same abbreviations were used for the multiplicity of signals in H-NMR and also: (m) multiplet, (bs) broad singlet, (bd) broad doublet. Spectra were referenced to appropriate residual solvent peaks (d_6-acetone, d_6-DMSO, d_4-MeOH or $CDCl_3$). IR spectra were obtained on a Thermo Nicolet FT-IR spectrometer. HRMS were performed on a Bruker Autoflex high resolution mass spectometer by Unidad de Espectrometría de Masas (Universidad de Santiago de Compostela).

Methyl 8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (**1**). A mixture of methyl 1-[2-bromo-5-isopropoxy-4-methoxyphenethyl]-1*H*-pyrrole-2-carboxylate (307 mg, 0.78 mmol) in dry DMF (5 mL), K_2CO_3 (235 mg, 1.71 mmol), PPh_3 (163 mg, 0.62 mmol), and $PdCl_2(PPh_3)_2$ (218 mg, 0.31 mmol) was purged with Ar for 15 min. The reaction mixture was stirred for 20 h at 125 ºC. After cooling to room temperature, the DMF was removed in vacuo, and Et_2O (100 mL) was

---

added. The organic solution was washed with water and brine, dried, filtered through a pad of celite and concentrated. The residue was purified by flash chromatography. Elution with hexane/AcOEt (gradient from 95:5 to 85:15) gave **1** (199 mg, 81%) as a white solid. M.p. 109 ºC. IR (film) ν 1699, 1489, 1434, 1248, 1130 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.39 (d, 6H, *J* = 6.0 Hz, 2Me); 2.98 (t, 2H, *J* = 6.8 Hz, H6); 3.82 (s, 3H, OMe); 3,88 (s, 3H, OMe); 4.56 (h, 1H, *J* = 6.0 Hz, OCH); 4.60 (t, 2H, *J* = 6.8 Hz, H5); 6.41 (d, 1H, *J* = 4.0 Hz, H1); 6.75 (s, 1H); 7.00 (d, 1H, *J* = 4.0 Hz, H2); 7.05 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 22.1 (q); 28.4 (t); 42.2 (t); 50.9 (q); 56.2 (q); 71.6 (d); 103.4 (d); 107.7 (d); 115.3 (d); 118.4 (d); 121.2 (s); 121.3 (s); 124.5 (s); 136.4 (s); 147.0 (s); 149.6 (s); 161.7 (s, C=O). MS (EI) 315 (M, 56); 316 (M+1, 12); 317 (M+2, 4). HRMS *m/z* calcd for $C_{18}H_{21}NO_4$ 315.1471, found 315.1476.

Methyl 1-bromo-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (2). A solution of **1** (1.46 g, 4.64 mmol) and NBS (835 mg, 4.69 mmol) in THF (40 mL) was stirred at 70 °C under Ar for 3 h. After cooling to room temperature the solvent was removed in vacuo, and the resulting residue was purified by column chromatography on silica gel. Elution with hexane/AcOEt (95:5 and 90:10) afforded **5** (1.72 g, 94%) as white crystals. m.p. 113.5-119.4 ºC. IR (film) 1705, 1440, 1245, 1213, 1197, 1066, 758 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.41 (d, *J* = 6.0, 6H, 2Me); 2.94 (d, *J* = 6.8 Hz, 2H, H6); 3.81 (s, 3H, OMe); 3.91 (s, 3H, OMe); 4.61-4.54 (m, 3H, H5 and OCH); 6.76 (s, 1H, H2); 7.01 (s, 1H); 7.97 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 22.1 (q); 29.0 (t); 42.6 (t); 51.2 (q); 56.1 (q); 71.5 (d); 92.5 (s); 108.8 (d); 114.9 (d); 120.4 (s); 120.5 (s); 121.0 (d); 125.7 (s); 131.5 (s); 147.0 (s); 149.0 (s); 160.9 (s). MS (CI) 393 (MBr^{79}, 84); 394 (MBr^{79}+1, 100); 395 (MBr^{81}, 82); 396 (MBr^{81}+1, 89). HRMS *m/z* calcd for $C_{18}H_{21}NO_4Br^+$ 395.0732, found 394.0645.

Methyl 1,2-dibromo-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (3). Compound **1** (1.58 g, 5.00 mmol) was dissolved in THF (60 mL) and NBS (2.22 g, 15.0 mmol) was added in one portion. The mixture was stirred under Ar atmosphere for 8 hours at 60 °C. After cooling to room temperature the solvent was removed under reduced pressure and the resulting residue was subjected to column chromatography on silica gel. Elution with hexane/EtOAc (95:5 to 90:10) gave 1.420 g (60%) of compound **3** as white crystals. M. p. (MeCN) = 141.0-141.5 ºC. IR (film) ν 2975, 1698, 1440, 1389, 1251 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.39 (d, *J* = 6.0 Hz, 6H, 2Me); 2.94 (t, *J* = 6.8 Hz, 2H, H6); 3.90 (s, 3H, OMe); 3,91 (s, 3H,

OMe); 4.56 (h, *J* = 6.0 Hz, 1H, OCH); 4.60 (t, *J* = 6.8 Hz, 2H, H5); 6.76 (s, 1H); 7.99 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.2 (q); 29.0 (t); 43.9 (t); 51.6 (q); 56.3 (q); 71.6 (d); 97.9 (s); 109.3 (d); 109.4 (s); 114.8 (d); 119.4 (s); 119.6 (s); 126.2 (s); 131.9 (s); 147.6 (s); 149.1 (s); 160.7 (s). MS (ESI) 474 (M+1, 100); 475 (M+2, 45).

2-(2,4-Diisopropoxy-5-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (5a). Starting from 1-bromo-2,4-diisopropoxy-5-methoxybenzene (1.08 g, 3.6 mmol), a colourless syrup (1.00 g, 80%) was obtained. IR (film) ν 2976, 2929, 1407, 1371, 1145, 1112, 1032 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.29 (d, *J* = 6.0 Hz, 6H, 2Me); 1.33 (s, 12H, 4Me); 1.35 (d, *J* = 6.0 Hz, 6H, 2Me); 3.84 (s, 3H, OMe); 4.24 (h, *J* = 6.0 Hz, 1H); 4.53 (h, *J* = 6.0 Hz, 1H); 6.51 (s, 1H); 7.14 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.9 (2q); 22.2 (2q); 24.8 (4q); 56.4 (q); 71.0 (d); 75.0 (d); 83.2 (2s); 107.8 (d); 118.9 (d); 145.2 (s); 150.4 (s); 158.2 (s). MS (CI) 350 (M, 100); 351 (M+1, 92); 352 (M+2, 48); 353 (M+3, 12).

2-(2,4,5-Triisopropoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (5b). Starting from 1-bromo-2,4,5-triisopropoxybenzene (1.02 g, 3.1 mmol), a yellowish syrup (0.61 g, 52%) was obtained. IR (film) ν 2975, 2931, 1600, 1500, 1411, 1111 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.29 (d, *J* = 6.0 Hz, 6H, 2Me); 1.30 (d, *J* = 6.0 Hz, 6H, 2Me); 1.32 (d, *J* = 6.4 Hz, 6H, 2Me); 1.33 (s, 12H, 4Me); 4.23 (h, *J* = 6.0 Hz, 1H); 4.27 (h, *J* = 6.4 Hz, 1H); 4.46 (h, *J* = 6.0 Hz, 1H); 6.49 (s, 1H); 7.21 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.9 (2q); 22.0 (2q); 22.2 (2q); 24.8 (4q); 71.9 (d); 73.8 (d); 74.6 (d); 83.6 (2s); 108.0 (d); 127.0 (d); 143.2 (s); 145.3 (s); 159.0 (s). MS (CI) 378 (M, 100); 379 (M+1, 48); 380 (M+2, 11).

2-(2,4-Diisopropoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (5c). Starting from 1-bromo-2,4-diisopropoxybenzene (1.05 g, 3.9 mmol), a yellowish syrup (0.79 g, 64%) was obtained. IR (film) ν 2977, 2932, 1600, 1352; 1110 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.31 (d, *J* = 6.0 Hz, 6H, 2Me); 1.32 (s, 12H, 4Me); 1.33 (d, *J* = 6.0 Hz, 6H, 2Me); 4.40 (h, *J* = 6.0 Hz, 1H); 4.53 (m, 1H); 7.13 (dd, *J* = 8.8, 2.0 Hz, 1H); 7.37 (d, *J* = 8.8 Hz, 1H); 7.55 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.0 (2q); 22.1 (2q); 24.8 (4q); 69.7 (d); 72.6 (d); 83.0 (2s); 104.7 (d); 107.9 (d); 137.5 (d); 158.2 (s); 159.1 (s); 161.5 (s). MS (CI) 320 (M, 100); 321 (M+1, 55); 322 (M+2, 15).

2-(3,4-Dimethoxy-2-isopropoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (5d). Starting from 1-bromo-3,4-dimethoxy-2-isopropoxybenzene (1.06 g, 3.9 mmol), a yellowish syrup (0.76 g, 61%) was obtained. IR (film) ν 2976, 2932, 1592, 1354, 1094

cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.29 (d, *J* = 6.0 Hz, 6H, 2Me); 1.34 (s, 12H, 4Me); 3.84 (s, 3H, OMe); 3.86 (s, 3H, OMe); 4.46 (h, *J* = 6.0 Hz, 1H); 6.66 (d, *J* = 8.4 Hz, 1H); 7.41 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.4 (2q); 24.9 (4q); 55.8 (q); 60.6 (q); 76.4 (d); 83.3 (2s); 107.0 (d); 131.5 (d); 142.5 (s); 156.3 (s); 156.7 (s). MS (CI) 322 (M, 100); 323 (M+1, 35); 324 (M+2, 5).

2-(2,5-Dimethoxy-4-isopropoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (5e). Starting from 1-bromo-2,5-dimethoxy-4-isopropoxybenzene (0.69 g, 2.5 mmol), a colourless syrup (0.65 g, 81%) was obtained. IR (film) ν 2977, 2933, 1508, 1403, 1212 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.33 (s, 12H, 4Me); 1.37 (d, 6H, *J* = 6.0 Hz, 2Me); 3.78 (s, 3H, OMe); 3.84 (s, 3H, OMe); 4.58 (h, *J* = 6.0 Hz, 1H); 6.50 (s, 1H); 7.20 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.1 (2q); 24.9 (q); 56.7 (q); 57.3 (q); 71.4 (d); 83.2 (2s); 101.5 (d); 120.2 (d); 144.2 (s); 150.9 (s); 160.0 (s). MS (CI) 322 (M, 43); 323 (M+1, 100); 324 (M+2, 24).

Methyl 8-isopropoxy-9-methoxy-1-(3,4,5-trimethoxyphenyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (6a). Following the general procedure A for the cross-coupling reaction and starting from **2** (50 mg, 0.12 mmol), **6a** as a yellowish oil (46 mg, 75%) was obtained with 16 h of reaction time. IR (film) ν 1702, 1243, 1126 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.37 (d, *J* = 6.4 Hz, 6H, 2Me); 3.00 (t, *J* = 6.8 Hz, 2H, H6); 3.43 (s, 3H, OMe); 3.81 (s, 6H, 2OMe); 3.85 (s, 3H, OMe); 3.86 (s, 3H, OMe); 4.53 (h, 1H, *J* = 6.4 Hz, OCH); 4.61 (t, *J* = 6.8 Hz, 2H, H5); 6.67 (s, 2H, H2', H6'); 6.74 (s, 1H, H2); 6.89 (s, 1H); 7.00 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.1 (q); 29.0 (t); 42.6 (t); 51.2 (q); 55.4 (q); 56.2 (2q); 60.9 (q); 71.4 (d); 106.4 (2d); 109.2 (d); 114.8 (d); 119.0 (d); 120.2 (s); 120.9 (s); 121.7 (s); 125.8 (s); 131.7 (s); 132.2 (s); 136.8 (s); 146.7 (s); 148.6 (s); 153.2 (s); 161.7 (s). MS (MALDI-TOF) *m/z* 481 (M, 100); 482 (M+1, 70).

Methyl 1-(4-hydroxy-3-methoxyphenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (6c). Following the general procedure A for the cross-coupling reaction and starting from **2** (281 mg, 0.71 mmol), **6c** as a yellowish solid (288 mg, 92%) was obtained with 14 h of reaction time. M. p. 176-181 ºC; IR (film) ν 3435, 1698 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.37 (d, *J* = 6.0 Hz, 6H, 2Me); 2.99 (t, *J* = 6.4 Hz, 2H, H6); 3.42 (s, 3H, OMe); 3.84 (s, 6H, 2OMe); 4.53 (h, *J* = 6.0 Hz, 1H, OCH); 4.60 (t, *J* = 6.4 Hz, 2H, H5); 5.62 (br, 1H, OH); 6.74 (s, 1H, H2); 6.89 (s, 1H); 6.92 (d, *J* = 8.0 Hz, 1H, H5'); 6.94 (d, *J* = 2.0 Hz, 1H, H2'); 6.96

(dd, *J* = 8.0 and 2.0 Hz, 1H, H6'); 6.97 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.1 (q); 29.0 (t); 42.5 (t); 51.1 (q); 55.5 (q); 56.0 (q); 71.4 (d); 109.2 (d); 112.0 (d); 114.3 (d); 114.7 (d); 119.2 (d); 120.0 (s); 121.2 (s); 121.7 (s); 122.4 (d); 125.7 (s); 128.6 (s); 131.7 (s); 144.5 (s); 146.4 (s); 146.5 (s); 148.6 (s); 161.8 (s). MS (EI) 437 (M, 92); 438 (M+1, 47). HRMS *m/z* calcd for $C_{25}H_{27}NO_6$ 437.1838, found 437.1833.

Methyl 8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (6d). K_2CO_3 (271 mg, 1.96 mmol) and 2-bromopropane (1.2 mL, 12.83 mmol) were added to **6c** (419 mg, 0.96 mmol) in dry DMF (6 mL). The mixture was stirred at 80 ºC under Ar for 17 h. The mixture was then cooled to room temperature and the solvent removed in vacuo. AcOEt was added and the organic solution was washed with water, saturated $NaHCO_3$ and brine. The organic layer was then dried, filtered and concentrated. The residue was purified by flash chromatography. Elution with hexane/AcOEt (75:25) gave **6d** (379 mg, 83%) as a light brown solid. M. p. (MeCN) 177-180 ºC. IR (film) ν 1700, 1464, 1440, 1423, 1192 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.37 (d, *J* = 6.0 Hz, 12H, 4Me); 3.00 (t, *J* = 6.8 Hz, 2H, H6); 3.40 (s, 3H, OMe); 3.81 (s, 3H, OMe); 3.84 (s, 3H, OMe); 4.53 (h, *J* = 6.0 Hz, 2H, 2OCH); 4.61 (t, *J* = 6.8 Hz, 2H, H5); 6.74 (s, 1H, H2); 6.90 (s, 1H); 6.92 (bs, 1H, H2'); 6.94-6.98 (m, 2H, H6' and H5'); 6.99 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 22.1 (2q); 29.0 (t); 42.6 (t); 51.1 (q); 55.4 (q); 56.0 (q); 71.4 (d); 71.6 (d); 109.2 (d); 113.3 (d); 114.7 (d); 116.4 (d); 119.3 (d); 120.1 (s); 121.2 (s); 121.7 (d); 125.7 (s); 129.8 (s); 131.7 (s); 146.1 (s); 146.5 (s); 148.6 (s); 150.4 (s); 161.8 (s). MS (CI) 479 (M, 78); 480 (M+1, 100); 481 (M+2, 40). HRMS *m/z* calcd for $C_{28}H_{33}NO_6$ 479.2308, found 479.2320.

Methyl 1-(2,5-dimethoxyphenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (6e). Following the general procedure A for the cross-coupling reaction and starting from **2** (503 mg, 1.28 mmol), **6e** as a white solid (465 mg, 81%) of was obtained with 3 h of reaction time. M.p. (MeCN) 105-108 ºC. IR (film) ν 1699, 1465, 1241 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (d, *J* = 6.0 Hz, 6H, 2Me); 3.01 (t, *J* = 6.8 Hz, 2H, H6); 3.37 (s, 3H, OMe); 3.64 (s, 3H, OMe); 3.74 (s, 3H, OMe); 3.83 (s, 3H, OMe); 4.52 (h, *J* = 6.0 Hz, 1H, OCH); 4.63 (t, *J* = 6.8 Hz, 2H, H5); 6.71 (s, 1H); 6.72 (s, 1H); 6.84 (dd, *J* = 8.9, 3.0 Hz, 1H, H4'); 6.89 (d, *J* = 8.9 Hz, 1H, H3'); 6.90 (d, *J* = 3.0 Hz, 1H, H6'); 7.02 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.0 (q); 28.8 (t); 42.5 (t); 51.0 (q); 55.2 (q); 55.7 (q); 56.0 (q); 71.3 (d); 108.6 (d); 112.0 (d); 113.3 (d); 114.6 (d); 116.9 (s); 117.3 (d); 119.9 (d); 120.1 (s); 121.7 (s); 125.2 (s); 126.3

(s); 132.6 (s); 146.3 (s); 148.6 (s); 151.5 (s); 153.5 (s); 161.7 (s). MS (MALDI-TOF) *m/z* 451 (M, 100); 452 (M+1, 40).

Methyl 1-(3,5-dimethoxyphenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (6h). Following the general procedure A for the cross-coupling reaction and starting from **2** (505 mg, 1.28 mmol), **6h** as a white solid (483 mg, 84%) was obtained with 5 h of reaction time. M. p. (MeCN) 94–100 ºC. IR (film) ν 1700, 1591, 1242, 1194, 1148 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.38 (d, *J* = 6.0 Hz, 6H, 2Me); 2.99 (t, *J* = 6.4 Hz, 2H, H6); 3.44 (s, 3H, OMe); 3.76 (s, 6H, 2OMe); 3.84 (s, 3H, OMe); 4.54 (h, *J* = 6.0 Hz, 1H, OCH); 4.61 (t, *J* = 6.4 Hz, 2H, H5); 6.41 (t, *J* = 2.0 Hz, 1H, H4'); 6.62 (d, *J* = 2 Hz, 2H, H2', H6'); 6.74 (s, 1H); 6.95 (s, 1H); 7.00 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.1 (q); 28.8 (t); 42.5 (t); 51.1 (q); 55.4 (2q); 55.5 (q); 71.4 (d); 99.2 (d); 107.3 (2d); 109.5 (d); 114.7 (d); 119.1 (d); 120.2 (s); 121.0 (s); 121.7 (s); 125.8 (s); 131.8 (s); 138.5 (s); 146.6 (s); 148.5 (s); 160.8 (2s); 161.7 (s). MS (MALDI-TOF) *m/z* 451 (M, 100); 452 (M+1, 75); 453 (M+2, 30).

Methyl 8-isopropoxy-1-(3-isopropoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (6k). Following the general procedure A for the cross-coupling reaction and starting from **2** (504 mg, 1.28 mmol), **6k** as a white solid (512 mg, 89%) was obtained with 5 h of reaction time. M. p. (MeCN) 132-135 ºC. IR (film) ν 1701, 1597, 1240 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.31 (d, *J* = 6.0 Hz, 6H, 2Me); 1.37 (d, *J* = 6.0 Hz, 6H, 2Me) 3.00 (t, *J* = 6.4 Hz, 2H, H6); 3.41 (s, 3H, OMe); 3.84 (s, 3H, OMe); 4.47-4.55 (m, *J* = 6.0 Hz, 2H, 2OCH); 4.61 (t, *J* = 6.4 Hz, 2H, H5); 6.73 (s, 1H); 6.81 (ddd, *J* = 7.6, 2.8, 2.8 Hz, 1H); 6.88 (s, 1H); 6.98-6.99 (m, 1H, H2'); 7.00 (s, 1H); 7.01 (dm, *J* = 7.6 Hz, 1H); 7.25 (t, *J* = 7.6 Hz, 1H, H5'). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.0 (2q); 28.9 (t); 42.5 (t); 51.0 (q); 55.3 (q); 69.6 (d); 71.3 (d); 109.3 (d); 114.2 (d); 114.7 (d); 116.6 (d); 119.2 (d); 120.1 (s) 121.1 (s); 121.65 (d); 121.69 (s); 125.6 (s); 129.3 (d); 131.8 (s); 137.9 (s); 146.5 (s); 148.5 (s); 157.9 (s); 161.7 (s). MS (MALDI-TOF) *m/z* 449 (M, 100).

Methyl 1-(4-dimethylaminophenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (6l). Following the general procedure A for the cross-coupling reaction and starting from **2** (66 mg, 0.17 mmol), **6l** as yellowish solid (23 mg, 32%) was obtained with 16 h of reaction time. M. p. (MeCN) 187-190 ºC. IR (film) ν 1698, 1441, 1237, 1193 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.37 (d, *J* = 6.0 Hz, 6H, 2Me); 2.95 (s, 6H, NMe_2); 3.00 (t, *J* = 6.6 Hz, 2H,

H6); 3.42 (s, 3H, OMe); 3.83 (s, 3H, CO_2Me); 4.53 (h, *J* = 6.0 Hz, 1H, OCH); 4.60 (t, *J* = 6.6 Hz, 2H, H5); 6.73 (s, 1H); 6.76 (d, *J* = 8.8 Hz, 2H); 6.95 (s, 1H); 6.96 (s, 1H); 7.31 (d, *J* = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.1 (q); 29.0 (t); 40.8 (q); 42.6 (t); 51.0 (q); 55.4 (q); 71.3 (d); 109.2 (d); 112.7 (2d); 114.7 (d); 119.2 (d); 119.9 (s); 121.6 (s); 122.0 (s); 124.6 (s); 125.6 (s); 130.1 (2d); 131.7 (s); 146.3 (s); 148.5 (s); 149.6 (s); 161.9 (s). MS (MALDI-TOF) *m/z* 434 (M, 100); 435 (M+1, 70).

Methyl 8-isopropoxy-9-methoxy-1-(2-thienyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (6n). Following the general procedure A for the cross-coupling reaction and starting from **2** (46.4 mg, 0.12 mmol), **6n** as a yellowish oil (34 mg, 72%) was obtained with 16 h of reaction time. IR (film) ν 1702, 1248, 1193 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.37 (d, *J* = 6.0 Hz, 6H, 2Me); 2.99 (t, *J* = 6.8 Hz, 2H, H6); 3.47 (s, 3H, OMe); 3.84 (s, 3H, CO_2Me); 4.54 (h, *J* = 6.0 Hz, 1H, OCH); 4.61 (t, *J* = 6.8 Hz, 2H, H5); 6.74 (s, 1H); 6.97 (s, 1H); 7.02 (s, 1H); 7.05-7.07 (m, 2H); 7.30 (dd, *J* = 4.8, 1.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.1 (q); 28.8 (t); 42.6 (t); 51.1 (q); 55.3 (q); 71.4 (d); 108.9 (d); 113.3 (s); 114.7 (d); 120.2 (s); 120.4 (d); 120.8 (s); 125.2 (d); 125.7 (s); 126.6 (d); 127.2 (d); 132.9 (s); 138.1 (s); 146.8 (s); 148.7 (s); 161.6 (s). MS (MALDI-TOF) *m/z* 397 (M, 95); 398 (M+1, 40).

Methyl 8-isopropoxy-9-methoxy-1,2-bis(3,4,5-trimethoxyphenyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (7a). Following the general procedure B for the cross-coupling reaction a yellow oil (160 mg , 62%) was obtained with 16 h of reaction time. IR (film) ν 1691, 1580, 1237, 1125 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.37 (d, *J* = 6.4 Hz, 6H, 2Me); 3.04 (t, *J* = 6.4 Hz, 2H, H6); 3.35 (s, 3H, OMe); 3.62 (s, 6H, 2OMe); 3.66 (s, 9H, 3OMe); 3.78 (s, 3H, OMe); 3.82 (s, 3H, OMe); 4.53 (m, 1H); 4.61 (t, *J* = 6.4 Hz, 2H, H5); 6.37 (s, 2H); 6.38 (s, 2H); 6.72 (s, 1H); 6.74 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.1 (q); 29.0 (t); 43.0 (t); 51.0 (q); 55.2 (q); 56.0 (q); 56.1 (q); 60.8 (q); 71.4 (d); 108.1 (d); 108.3 (2d); 108.8 (d); 109.2 (d); 114.6 (d); 117.8 (s); 120.6 (s); 121.4 (s); 125.9 (s); 130.6 (s); 131.0 (s); 131.2 (s); 132.4 (s); 136.6 (s); 136.8 (s); 146.6 (s); 148.5 (s); 152.1 (s); 153.0 (s); 162.3 (s). MS (EI) *m/z* 648 (M+1, 32); 647 (M+, 100); 605 (77); 604 (42).

Methyl 1,2-bis(4-hydroxy-3-methoxyphenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (7c). Following the general procedure B for the cross-coupling reaction a white solid (195 mg , 87%) was obtained with 7 h of reaction time. M. p. (MeCN) 244-246ºC. IR (film) ν 3424, 1693, 1246 cm^{-1}.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35 (d, *J* = 6.0 Hz, 6H, 2Me); 3.02 (t, *J* = 6.4 Hz, 2H, H6); 3.32 (s, 3H, OMe); 3.60 (s, 3H, OMe); 3.61 (s, 3H, CO_2Me); 3.62 (s, 3H, OMe); 4.52 (h, *J* = 6.0 Hz, 1H, OCH); 4.60 (t, *J* = 6.4 Hz, 2H, H5); 5.72 (br, 1H, OH); 5.77 (br, 1H, OH); 6.56 and 6.58 (2d, *J* = 1.6 Hz, 2H, H2', H2"); 6.68 (s, 1H); 6.69 and 6.74 (2dd, *J* = 8.4, 1.6 Hz, 2H, H6', H6"); 6.73 (s, 1H); 6.77 and 6.83 (2d, *J* = 8.4 Hz, 2H, H5', H5"). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.1 (q); 29.0 (t); 43.0 (t); 50.8 (q); 55.2 (q); 55.8 (q); 55.9 (q); 71.4 (d); 109.2 (d); 113.3 (d); 113.5 (d); 113.8 (d); 114.1 (d); 114.6 (d); 117.8 (s); 121.0 (s); 121.6 (s); 123.9 (d); 124.3 (d); 125.8 (s); 127.3 (s); 127.4 (s); 131.3 (s); 132.7 (s); 144.0 (s); 144.3 (s); 145.3 (s); 146.3 (s); 146.4 (s); 148.5 (s); 162.5 (s). MS (MALDI-TOF) 559 (M, 100); 560 (M+1, 45).

Methyl 1,2-bis(3,4-dimethoxyphenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (7f). Following the general procedure B for the cross-coupling reaction a white solid (203 mg, 86%) was obtained with 5 h of reaction time . M. p. (MeCN) 233-235 ºC. IR (film) ν 1689 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (d, *J* = 6.1 Hz, 6H, 2Me); 3.03 (t, *J* = 6.4 Hz, 2H, H6); 3.31 (s, 3H, OMe); 3.62 (s, 3H, OMe); 3.63 (s, 3H, CO_2Me); 3.67 (s, 3H, OMe); 3.83 (s, 3H, OMe); 3.85 (s, 3H, OMe); 4.52 (h, *J* = 6.1 Hz, 1H, OCH); 4.61 (t, *J* = 6.4 Hz, 2H, H5); 6.64-6.77 (m, 8H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.1 (q); 29.0 (t); 43.9 (t); 50.9 (q); 55.2 (q); 55.6 (q); 55.7 (q); 55.8 (q); 55.9 (q); 71.4 (d); 109.3 (d); 110.0 (d); 111.0 (d); 114.2 (d); 114.4 (d); 114.6 (d); 117.9 (s); 121.0 (s); 121.5 (s); 123.0 (d); 123.5 (d); 125.8 (s); 127.9 (s); 128.1 (s); 131.3 (s); 132.7 (s); 146.5 (s); 147.5 (s); 147.6 (s); 147.7 (s); 148.5 (s); 148.7 (s); 162.4 (s). MS (MALDI-TOF) *m/z* 587 (M); 588 (M+1). HRMS *m/z* calcd for $C_{34}H_{37}NO_8$ 587.2514, found 587.2514.

Methyl 8-isopropoxy-9-methoxy-1,2-bis(4-methoxyphenyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (7g). Following the general procedure B for the cross-coupling reaction a white solid (154 mg , 73%) was obtained with 5 h of reaction time. M. p. (MeCN) 189-201 ºC. IR (film) ν1687 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (d, *J* = 6.1 Hz, 6H, 2Me); 3.02 (t, *J* = 6.4 Hz, 2H, H6); 3.28 (s, 3H, OMe); 3.59 (s, 3H, CO_2Me); 3.75 (s, 3H, OMe); 3.77 (s, 3H, OMe); 4.51 (h, *J* = 6.1 Hz, 1H, OCH); 4.60 (t, *J* = 6.4 Hz, 2H, H5); 6.55 (s, 1H); 6.72-6.79 (m, 1H, 5H); 7.02-7.06 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.1 (q); 29.1 (t); 43.0 (t); 50.8 (q); 55.0 (q); 55.1 (q); 55.2 (q); 71.3 (d); 109.2 (d); 112.5 (2d); 113.0 (2d); 113.7 (d); 114.6 (s); 121.2 (s); 121.5 (s);

125.8 (s); 127.7 (s); 127.8 (s); 131.4 (s); 131.6 (2d); 132.4 (2d); 132.8 (s); 146.3 (s); 148.5 (s); 157.9 (s); 158.3 (s); 162.5 (s). MS (MALDI-TOF) *m/z* 527 (M); 528 (M+1).

Methyl 8-isopropoxy-9-methoxy-1,2-bis(4-trifluoromethoxyphenyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (7i). Following the general procedure B for the cross-coupling reaction a white solid (171 mg , 67%) was obtained with 16 h of reaction time . M. p. (MeOH) 168-170 ºC. IR (film) ν 1697 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.37 (d, *J* = 6.1 Hz, 6H, 2Me); 3.04 (t, *J* = 6.4 Hz, 2H, H6); 3.25 (s, 3H, OMe); 3.58 (s, 3H, CO_2Me); 4.53 (h, *J* = 6.1 Hz, 1H, OCH); 4.63 (t, *J* = 6.4 Hz, 2H, H5); 6.39 (s, 1H); 6.74 (s, 1H); 7.03-7.17 (m, 8H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.1 (q); 28.9 (t); 43.0 (t); 50.8 (q); 54.7 (q); 71.4 (d); 108.9 (d); 114.7 (d); 115.2 (s); 119.6 (2d); 120.0 (q); 120.3 (q); 121.1 (2d); 125.9 (s); 130.8 (s), 131.4 (s); 131.6 (s); 131.8 (2d); 132.6 (2d); 133.9 (s); 146.7 (s); 147.9 (s); 148.0 (s); 148.6 (s); 162.0 (s). MS (MALDI-TOF) *m/z* 635 (M).

Methyl 1,2-bis(4-isopropoxyphenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (7j). Following the general procedure B for the cross-coupling reaction a white solid (190 mg, 82%) was obtained with 16 h of reaction time . M. p. (MeCN) 165-168 ºC. IR (film) ν 1686 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.28 (d, *J* = 6.1 Hz, 6H, 2Me); 1.30 (d, *J* = 6.1 Hz, 6H, 2Me); 1.36 (d, *J* = 6.1 Hz, 6H, 2Me); 3.01 (t, *J* = 6.5 Hz, 2H, H6); 3.30 (s, 3H, OMe); 3.59 (s, 3H, CO_2Me); 4.45-4.54 (m, 3OCH); 4.61 (t, *J* = 6.5 Hz, 2H, H5); 6.58 (s, 1H); 6.71 (d, *J* = 8.7 Hz, 2H); 6.73 (s, 1H); 6.77 (d, *J* = 8.7 Hz, 2H); 7.01 (d, *J* = 8.7 Hz, 2H); 7.04 (d, *J* = 8.7 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.8 (q); 22.0 (2q); 28.9 (t); 42.8 (t); 50.6 (q); 54.8 (q); 69.5 (d); 69.6 (d); 71.2 (d); 109.1 (d); 114.4 (2d); 114.5 (d); 115.7 (2d); 117.8 (s); 121.1 (s); 121.5 (s); 125.6 (s); 127.4 (s); 127.6 (s); 131.3 (s); 131.5 (2d); 132.2 (2d); 132.8 (s); 146.1 (s); 148.3 (s); 156.1 (s); 156.4 (s); 162.4 (s). MS (MALDI-TOF) *m/z* 583 (M); 584 (M+1).

Methyl 8-isopropoxy-1,2-bis(3-isopropoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (7k). Following the general procedure B for the cross-coupling reaction a white solid (193 mg, 83%) was obtained with 5 h of reaction time. M. p. (MeCN) 137-140 ºC. IR (film) ν 1693 cm^{-1}. IR (film) ν 2975 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.15 (d, *J* = 6.1 Hz, 6H, 2Me); 1.17 (d, *J* = 6.1 Hz, 6H, 2Me); 1.36 (d, *J* = 6.1 Hz, 6H, 2Me); 3.03 (t, *J* = 6.5 Hz, 2H, H6); 3.28 (s, 3H, OMe); 3.58 (s, 3H, CO_2Me); 4.27-4.37 (m, 2OCH); 4.51 (h, *J* = 6.1 Hz, 1H, OCH);

4.61 (t, *J* = 6.5 Hz, 2H, H5); 6.60 (s, 1H); 6.72 (s, 1H); 6.65-6.77 (m, 6H); 7.06-7.13 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.8 (q); 21.9 (q); 22.0 (q); 28.9 (t); 42.9 (t); 50.8 (q); 54.9 (q); 69.6 (d); 69.8 (d); 71.3 (d); 109.2 (d); 114.5 and 114.9 (2d); 115.0 (d); 117.7 (d); 118.1 (d); 120.8 (s); 121.6 (s); 123.2 (d); 123.5 (d); 125.7 (s); 127.9 (d); 129.1 (d); 131.2 (s); 132.7 (s); 136.5 (s); 136.7 (s); 146.2 (s); 148.4 (s); 156.7 (s); 157.6 (s); 162.4 (s). MS (MALDI-TOF) *m/z* 583 (M).

Methyl 1,2-bis(4-dimethylaminophenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (7l). Following the general procedure B for the cross-coupling reaction a white solid (158 mg, 71%) was obtained with 16 h of reaction time . M. p. (MeCN) 237-239 ºC. IR (film) ν 1686 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (d, *J* = 6.0 Hz, 6H, 2Me); 2.87 (s, 6H, NMe_2); 2.91 (s, 6H, NMe_2); 3.00 (t, *J* = 6.5 Hz, 2H, H6); 3.29 (s, 3H, OMe); 3.62 (s, 3H, CO_2Me); 4.50 (h, *J* = 6.0 Hz, 1H, OCH); 4.58 (t, *J* = 6.5 Hz, 2H, H5); 6.59 (d, *J* = 8.8 Hz, 2H); 6.60 (s, 1H); 6.64 (d, *J* = 8.8 Hz, 2H); 6.70 (s, 1H); 7.01 (d, *J* = 8.8 Hz, 2H); 7.03 (d, *J* = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.1 (q); 29.1 (t); 40.5 (t); 40.7 (q); 42.9 (q); 50.7 (q); 55.0 (q); 71.2 (d); 109.3 (d); 111.4 (2d); 112.8 (2d); 114.4 (d); 117.7 (s); 119.5 (s); 120.6 (s); 121.5 (s); 123.5 (s); 123.9 (s); 125.6 (s); 131.3 (2d); 131.4 (s); 131.9 (2d); 146.0 (s); 148.3 (s); 148.7 (s); 149.2 (s); 162.7 (s). MS (MALDI-TOF) *m/z* 553 (M); 554 (M+1).

Methyl 8-isopropoxy-9-methoxy-1,2-bis(3-nitrophenyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (7m). Following the general procedure B for the cross-coupling reaction a white solid (175 mg, 79%) was obtained with 5 h of reaction time. M. p. (MeCN) > 230 ºC. IR (film) ν 1698, 1534, 1437, 1348 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.37 (d, *J* = 6.1 Hz, 6H, 2Me); 3.07 (t, *J* = 6.5 Hz, 2H, H6); 3.22 (s, 3H, OMe); 3.58 (s, 3H, CO_2Me); 4.54 (h, *J* = 6.1 Hz, 1H, OCH); 4.67 (t, *J* = 6.5 Hz, 2H, H5); 6.36 (s, 1H); 6.78 (s, 1H); 7.35-7.50 (m, 4H); 8.00-8.07 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.0 (q); 28.9 (t); 43.1 (t); 51.1 (q); 55.3 (q); 71.4 (d); 109.0 (d); 114.8 (d); 117.6 (s); 118.0 (s); 121.7 (d); 121.8 (d); 125.5 (d); 125.8 (d); 126.5 (s); 128.2 (d); 129.4 (d); 130.2 (s); 133.5 (s); 134.6 (s); 135.0 (s); 136.7 (d); 136.8 (s); 137.4 (d); 142.9 (s); 143.7 (s) 144.6 (s); 148.8 (s); 161.5 (s). MS (MALDI-TOF) *m/z* 557 (M).

Methyl 8-isopropoxy-9-methoxy-1,2-bis(2-thienyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (7n). Following the general procedure B for the cross-coupling reaction and starting from **3** (93 mg, 0.22 mmol), a yellow oil (32 mg, 34%)

was obtained with 16 h of reaction time. IR (film) ν 1693, 1439, 1257 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.37 (d, *J* = 6.0 Hz, 6H, 2Me); 3.03 (t, *J* = 6.4 Hz, 2H, H6); 3.39 (s, 3H, OMe); 3.68 (s, 3H, OMe); 4.53 (h, *J* = 6.0 Hz, 1H, OCH); 4.62 (t, *J* = 6.4 Hz, 2H, H5); 6.71 (s, 1H); 6.73 (s, 1H); 6.86 (dd, *J* = 3.6, 1.2 Hz, 1H); 6.89-6.93 (m, 2H); 6.99 (dd, *J* = 5.2, 5.2 Hz, 1H); 7.22 (dd, *J* = 5.2, 1.2 Hz, 1H); 7.28 (dd, *J* = 5.2, 1.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.1 (q); 28.8 (t); 43.1 (t); 51.1 (q); 55.1 (q); 71.3 (d); 108.9 (d); 114.2 (s); 114.4 (d); 120.3 (s); 124.7 (s); 125.3 (d); 125.7 (s); 126.0 (d); 126.1 (s); 126.5 (d); 127.0 (d); 127.8 (d); 128.8 (d); 132.8 (s); 135.3 (s); 136.3 (s); 146.7 (s); 148.7 (s); 162.0 (s). MS (MALDI-TOF) *m/z* 420 (M, 100).

Methyl 2-bromo-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (8d). Following the general procedure C and starting from 6d (756 mg, 1.36 mmol), a yellow solid (741 mg, 84%) was obtained. M. p. (MeCN) 146-148 ºC. IR (film) ν 1694, 1463, 1440, 1111. ^1H NMR ($CDCl_3$) δ 1.35 (d, 6H, *J* = 6.0 Hz, 2Me); 1.38 (d, 6H, *J* = 6.0 Hz, 2Me); 2.98 (t, 2H, *J* = 6.0 Hz, H6); 3.30 (s, 3H, OMe); 3.81 (s, 3H, OMe); 3.90 (s, 3H, OMe); 4.54 (m, 4H, H5 and 2OCH); 6.57 (s, 1H); 6.70 (s, 1H); 6.87 (dd, 1H, *J* = 7.9 and 2.0 Hz, H6'); 6.88 (br, 1H, H2'); 6.96 (d, *J* = 7.9 Hz, 1H, H5'). ^{13}C NMR ($CDCl_3$) δ 22.1 (q); 28.7 (t); 43.5 (t); 51.2 (q); 55.1 (q); 56.1 (q); 71.4 (2d); 107.7 (s); 109.1 (d); 114.6 (d); 114.8 (d); 115.7 (d); 118.5 (s); 120.2 (s); 122.6 (s); 123.4 (d); 125.5 (s); 127.6 (s); 132.1 (s); 146.8 (s); 148.6 (s); 150.4 (s); 161.4 (s). MS (CI) 557 (MBr^{79}, 100); 558 (MBr^{79}+1, 23); 559 (MBr^{81}, 84). HRMS *m/z* calcd for $C_{28}H_{32}NO_6Br$ 557.1413, found 557.1429.

Methyl 2-bromo-1-(4,5-dimethoxyphenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (8e). Following the general procedure C and starting from **6e** (359 mg, 0.8 mmol), a yellow solid (368 mg, 87%) of was obtained. M. p. (MeCN) 161-162 ºC. IR (film) ν 1695, 1463, 1248, 1108. ^1H NMR ($CDCl_3$) δ 1.34 and 1.35 (2d, *J* = 6.4 Hz, 6H, 2Me); 2.99 (bs, 2H, H6); 3.30 (s, 3H, OMe); 3.67 (s, 3H, OMe); 3.75 (s, 3H, OMe); 3.90 (s, 3H, OMe); 4.48-4.75 (m, 3H, H5 and OCH); 6.55 (s, 1H); 6.70 (s, 1H); 6.81 (d, *J* = 2.8 Hz, 1H, H6'); 6.90-6.94 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 22.0 (q); 28.5 (t); 43.4 (t); 51.1 (q); 55.0 (q); 55.7 (q); 56.5 (q); 71.3 (d); 108.2 (s); 108.4 (d); 112.6 (d); 114.39 (d); 114.44 (d); 118.1 (d); 118.5 (s); 118.7 (s); 120.4 (s); 124.7 (s); 125.2 (s); 132.4 (s); 146.6 (s); 148.7 (s); 152.2 (s); 153.7 (s); 161.3 (s). MS (ES) 530 (MBr^{79},100); 531 (MBr^{79}+1, 26); 532 (MBr^{81},95); 533 (MBr^{81}+1, 17).

Methyl 2-bromo-8-isopropoxy-1-(3-isopropoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (8k). Following the general procedure C and starting from **6k** (392 mg, 0.74 mmol), a yellow solid (460 mg, quantitative) was obtained. IR (film) ν 1697, 1450, 1250. ^{1}H NMR ($CDCl_3$) δ 1.31 (d, *J* = 6.4 Hz, 6H, 2Me); 1.35 (d, *J* = 6.0 Hz, 6H, 2Me); 2.99 (t, *J* = 6.4 Hz, 2H, H6); 3.29 (s, 3H, OMe); 3.91 (s, 3H, OMe); 4.48-4.56 (m, 2H, 2OCH); 4.60 (t, *J* = 6.4 Hz, 2H, H5); 6.52 (s, 1H); 6.70 (s, 1H); 6.87-6.93 (m, 3H); 7.33 (td, *J* = 7.5, 1.3 Hz, 1H, H5'). ^{13}C NMR ($CDCl_3$) δ 22.0 (q); 22.1 (q); 28.7 (t); 43.5 (t); 51.2 (q); 55.0 (q); 69.7 (d); 71.4 (d); 107.3 (s); 109.1 (d); 114.5 (d); 115.5 (d); 118.1 (d); 118.5 (s); 120.0 (s); 122.7 (s); 123.2 (d); 125.5 (s); 129.6 (d); 132.0 (s); 136.1 (s); 146.8 (s); 148.6 (s); 158.0 (s); 161.3 (s). MS (ES) 528 (MBr^{79},100); 529 (MBr^{79}+1, 23); 530 (MBr^{81},87); 531 (MBr^{81}+1, 15).

Methyl 2-(4-hydroxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (9a). Following the general procedure D and starting from **8d** (155 mg, 0.28 mmol) and **4b**, a yellowish syrup (122 mg, 76%) was obtained with 15 h of reaction time. IR (film) ν 3441, 1686 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.31 (d, *J* = 6.0 Hz, 6H, 2Me); 1.36 (d, *J* = 6.0 Hz, 6H, 2Me); 3.03 (t, *J* = 6.4 Hz, 2H, H6); 3.31 (s, 3H, OMe); 3.58 (s, 3H, OMe); 3.61 (s, 3H, CO_2Me); 4.46 (h, *J* = 6.0 Hz, 1H, OCH); 4.52 (h, *J* = 6.0 Hz, 1H, OCH); 4.57-4.66 (m, 2H, H5); 5.01 (br, 1H, OH); 6.58 (d, *J* = 2.0 Hz, 1H, H2'); 6.65 (d, *J* = 8.8 Hz, 2H, H3", H5"); 6.69 (s, 1H); 6.71 (dd, *J* = 8.4, 2.0 Hz, 1H, H6'); 6.73 (s, 1H); 6.79 (d, *J* = 8.4 Hz, 1H, H5'); 6.97 (d, *J* = 8.8 Hz, 2H, H2", H6"). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.0 (q); 22.1 (q); 29.0 (t); 43.0 (t); 50.8 (q); 55.1 (q); 55.8 (q); 71.3 (d); 71.4 (d); 109.3 (d); 114.1 (d); 114.6 (d); 115.2 (d); 115.8 (d); 117.9 (s); 121.1 (s); 121.7 (s); 123.5 (d); 125.8 (s); 127.8 (s); 128.5 (s); 131.3 (s); 131.8 (d); 132.8 (s); 145.7 (s); 146.4 (s); 148.4 (s); 150.1 (s); 154.1 (s); 162.5 (s). MS (MALDI-TOF) 571 (M, 100), 572 (M+1, 83).

Methyl 2-(3,4-dimethoxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (9b). Following the general procedure D and starting from **8d** (150 mg, 0.27 mmol) and **4f**, a yellowish syrup (149 mg, 89%) of was obtained with 16 h of reaction time. IR (film) ν 1685 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.32 (d, *J* = 6.0 Hz, 6H, 2Me); 1.36 (d, *J* = 6.0 Hz, 6H, 2Me); 3.03 (t, *J* = 6.4 Hz, 2H, H6); 3.31 (s, 3H, OMe); 3.58 (s, 3H, OMe); 3.63 (s, 3H, CO_2Me); 3.65 (s, 3H, OMe); 3.85 (s, 3H, OMe); 4.45 (h, *J* = 6.0 Hz, 1H, OCH); 4.52 (h,

J = 6.0 Hz, 1H, OCH); 4.57-4.66 (m, 2H, H5); 6.62 (d, *J* = 2.0 Hz, 1H, H2'); 6.64 (d, *J* = 1.2 Hz, 1H, H2"); 6.70 (s, 1H); 6.70-6.73 (m, 3H, H6', H5", H6"); 6.73 (s, 1H); 6.79 (d, *J* = 8.0 Hz, 1H, H5'). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.0 (q); 22.1 (q); 29.0 (t); 43.0 (t); 50.9 (q); 55.1 (q); 55.6 (q); 55.7 (q); 55.8 (q); 71.3 (d); 71.4 (d); 109.2 (d); 110.0 (d); 114.2 (d); 114.6 (d); 115.1 (d); 116.0 (d); 117.8 (s); 121.0 (s); 121.6 (s); 123.1 (d); 123.5 (d); 125.8 (s); 127.9 (s); 128.7 (s); 131.3 (s); 132.7 (s); 145.8 (s); 146.4 (s); 147.3, 147.6 (s); 148.5 (s); 150.2 (s); 162.5 (s). MS (MALDI-TOF) 615 (M, 100), 616 (M+1, 73), 617 (M+2, 30).

Methyl 8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-2-(3,4,5-trimethoxyphenyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (9c). Following the general procedure D and starting from **8d** (156 mg, 0.28 mmol) and **4a**, a yellowish oil (128 mg, 71%) was obtained with 16 h of reaction time. IR (film) ν 1691 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.31 (d, *J* = 6.0 Hz, 6H, 2Me); 1.36 (d, *J* = 6.0 Hz, 6H, 2Me); 3.03 (t, *J* = 6.4 Hz, 2H, H6); 3.31 (s, 3H, OMe); 3.59 (s, 3H, OMe); 3.64 (s, 6H, 2OMe); 3.65 (s, 3H, CO_2Me); 3.82 (s, 3H, OMe); 4.45 (h, *J* = 6.0 Hz, 1H, OCH); 4.52 (h, *J* = 6.0 Hz, 1H, OCH); 4.57-4.66 (m, 2H, H5); 6.35 (s, 2H, H2", H6"); 6.62 (d, *J* = 2.0 Hz, 1H, H2'); 6.71 (s, 1H); 6.73 (dd, J = 8.0, 2.0 Hz, 1H, H6'); 6.74 (s, 1H); 6.81 (d, *J* = 8.0 Hz, 1H, H5'). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.9 (q); 22.1 (q); 29.0 (t); 43.0 (t); 50.9 (q); 55.1 (q); 55.9 (3q); 60.9 (q); 71.4 (d); 71.6 (d); 108.2 (2d); 109.3 (d); 114.6 (d); 115.1 (d); 116.3 (d); 117.7 (s); 120.9 (s); 121.4 (s); 123.5 (d); 125.7 (s); 128.7 (s); 130.7 (s); 131.3 (s); 132.6 (s); 136.5 (s); 145.8 (s); 146.5 (s); 148.5 (s); 150.4 (s); 151.9 (2s); 162.5 (s). MS (MALDI-TOF) 645 (M, 100), 646 (M+1, 45), 647 (M+2, 15).

Methyl 2-(2,4-diisopropoxy-5-methoxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (9d). Following the general procedure E and starting from **8d** (208 mg, 0.37 mmol) and **5a**, a reddish oil (235 mg, 89%) was obtained with 5 h of reaction time. IR (film) ν 1693. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.31 (d, 12H, *J* = 6.0 Hz, 4Me); 1.36 (d, 12H, *J* = 6.0 Hz, 4Me); 3.00 (m, 2H, H6); 3.33 (s, 3H, OMe); 3.51 (s, 3H, OMe); 3.59 (s, 6H, OMe, CO_2Me) 4.09-4.14 (m, 1H, OCH); 4.41-4.55 (m, 3H, OCH); 4.64-4.69 (m, 2H, H5); 6.46 (s, 1H); 6.48 (s, 1H); 6.66 (br, 1H); 6.74-6.79 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.0 (q); 22.1 (q); 22.2 (q); 29.0 (t); 42.8 (t); 50.8 (q); 55.1 (q); 55.7 (q); 56.1 (q); 71.5 (d); 71.6 (2d); 71.7 (d); 107.2 (d); 109.1 (d); 114.9 (d); 115.0 (d); 116.0 (d);

116.1 (d); 119.2 (s); 119.5 (s); 121.3 (s); 121.5 (s); 123.3 (d); 125.5 (s); 127.9 (s); 129.1 (s); 130.6 (s); 144.5 (s); 145.7 (s); 145.8 (s); 146.2 (s); 148.5 (s); 149.4 (s); 150.2 (s); 162.7 (s). MS (MALDI-TOF) 701 (M, 100), 702 (M+1, 39), 703 (M+2, 8). HRMS *m/z* calcd for $C_{41}H_{51}NO_9$ 701.3564, found 701.3558.

Methyl 2-(2,5-dimethoxy-4-isopropoxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (9e). Following the general procedure E and starting from **8d** (50 mg (0.09 mmol) and **5e**, a reddish oil (60 mg, quantitative) was obtained with 16 h of reaction time. IR (film) ν 1691, 1438, 1211 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 1.30 (d, *J* = 6.0 Hz, 6H, 2Me); 1.34 (d, *J* = 6.0 Hz, 6H, 2Me); 1.37 (d, *J* = 6.0 Hz, 6H, 2Me); 3.02 (t, *J* = 6.4 Hz, 2H, H6); 3.32 (s, 3H, OMe); 3.50 (s, 3H, OMe); 3.57 (s, 3H, OMe); 3.58 (s, 6H, 2OMe); 4.37-4.67 (m, 5H, H5, 3OCH); 6.45 (s, 1H); 6.55 (s, 1H); 6.63 (d, *J* = 2.8 Hz, 1H); 6.72-6.77 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.0 (q); 22.1 (q); 22.2 (q); 29.0 (t); 42.9 (t); 50.8 (q); 55.1 (q); 55.7 (q); 56.2 (q); 56.5 (q); 71.3 (d); 71.6 (d); 72.0 (d); 102.1 (d); 109.3 (d); 114.6 (2d); 116.2 (d); 116.7 (d); 117.4 (s); 118.8 (s); 121.3 (s); 121.6 (s); 123.1 (d); 125.7 (s); 129.1 (s); 131.1 (s); 144.0 (s); 145.5 (s); 146.3 (s); 146.4 (s); 148.4 (s); 150.1 (s); 151.3 (s); 162.5 (s). MS (MALDI-TOF) 673 (M, 100); 674 (M+1, 40).

Methyl 2-(2,4-diisopropoxyphenyl)-1-(2,5-dimethoxyphenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (9f). Following the general procedure E and starting from **8e** (102 mg, 0.19 mmol) and **5c**, a reddish oil (103 mg, 82%) of was obtained as a mixture of rotamers (6:4) with 16 h of reaction time. IR (film) ν 1695 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.18-1.27 (m, 6H, 2Me); 1.30 (d, *J* = 6.0 Hz, 6H, 2Me); 1.35 (d, *J* = 6.0 Hz, 6H, 2Me); 2.97-3.09 (m, 2H, H6); 3.29 and 3.30 (2s, 3H, OMe); 3.54 (s, 3H, OMe); 3.57 and 3.58 (2s, 3H, OMe); 3.63 and 3.64 (2s, 3H, OMe); 4.33 (h, *J* = 6.0 Hz, 1H, OCH); 4.42-4.54 (m, 2H, 2OCH); 4.61-4.73 (m, 2H, H5); 6.25 and 6.27 (2dd, *J* = 8.4, 2.4 Hz, 1H); 6.36 and 6.37 (2d, *J* = 2.4 Hz, 1H); 6.55 and 6.71 (2d, *J* = 3.2 Hz); 6.60 (s, 1H); 6.71 (s, 1H); 6.76-6.80 (m, 1H); 6.83 (d, *J* = 8.4 Hz, 1H); 6.88 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.0 (q); 22.1 (q); 24.5 and 24.8 (q); 28.8 (t); 42.8 (t); 50.6 (q); 55.0 (q); 55.5 (q); 55.9 (q); 69.7 (d); 70.0 (d); 71.3 and 71.4 (d); 102.9 and 103.0 (d); 105.8 and 106.2 (d); 108.1 (d); 111.6 (d); 113.4 and 113.9 (d); 114.9 (d); 117.6 and 118.4 (d); 122.1 (s); 125.0 (s);

126.0 (s); 128.3 (s); 129.2 (s); 131.3 and 131.4 (d); 145.9 (s); 148.7 (s); 151.8 (s); 152.4 (s); 153.3 (s); 156.6 (s); 157.4 (s); 162.7 (s). MS (MALDI-TOF) 643 (M, 100).

Methyl 1-(2,5-dimethoxyphenyl)-8-isopropoxy-9-methoxy-2-(2,4,5-triisopropoxyphenyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (9g). Following the general procedure E and starting from **8e** (104 mg (0.20 mmol) and **5b**, a reddish oil (112 mg, 81%) was obtained as a mixture of rotamers (1:1) with 16 h of reaction time. IR (film) ν 1699 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.29 (d, *J* = 6.4 Hz, 6H, 2Me); 1.31 (d, *J* = 6.0 Hz, 6H, 2Me); 1.33 (d, *J* = 6.4 Hz, 6H, 2Me); 1.36 (d, *J* = 6.0 Hz, 6H, 2Me); 3.00 (t, *J* = 6.4 Hz, 2H, H6); 3.36 (s, 3H, OMe); 3.64 (s, 3H, OMe); 3.79 (s, 3H, OMe); 3.83 (s, 3H, OMe); 4.30 (h, *J* = 6.0 Hz, 1H, OCH); 4.39-4.55 (m, 3H, 3OCH); 4.63 (t, *J* = 6.4 Hz, 2H, H5); 6.70 (s, 1H, H3"); 6.71 (s, 1H); 6.84 (dd, *J* = 8.8, 2.8 Hz, 1H, H4'); 6.86 (d, *J* = 8.8 Hz, 1H, H3'); 6.89 (s, 1H); 6.90 (d, *J* = 2.8 Hz, 1H, H6'); 7.02 (s, 1H, H6"). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.1 (q); 22.2 (2q); 22.3 (q); 28.9 (t); 42.8 (t); 51.0 (q); 55.3 (q); 55.8 (q); 56.1 (q); 70.4 (d); 71.4 (d); 71.5 (d); 73.4 (d); 106.7 and 107.5 (d); 108.6 (d); 112.1 (d); 113.4 (d); 114.7 (d); 117.0 (s); 117.4 (d); 120.0 and 120.6 (d); 120.1 (s); 121.7 (s); 125.2 (s); 126.4 (s); 132.7 (s); 142.7 (s); 146.4 (s); 148.6 (s); 150.4 (s); 151.5 (s); 153.3 (s); 153.6 (s); 161.8 (s). MS (MALDI-TOF) 702 (M, 100).

Methyl 2-(2,4-diisopropoxyphenyl)-8-isopropoxy-1-(3-isopropoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (9h). Following the general procedure E and starting from **8k** (163 mg, 0.31 mmol) and **5c**, a yellowish oil (188 mg, 95%) was obtained with 16 h of reaction time. IR (film) ν 1699 cm^{-1}. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.0 (q); 22.1 (2q); 22.2 (q); 28.7 and 29.0 (t); 42.6 (t); 51.1 (q); 55.3 (q); 69.7 (3d); 71.4 (d); 109.2 (d); 114.5 (d); 115.5 (d); 116.7 (d); 117.8 (s); 118.1 (d); 119.2 (d); 120.0 (s); 121.1 (s); 121.5 (s); 121.7 (d); 123.2 and 123.6 (d); 125.5 (s); 129.6 (d); 131.8 (s); 132.1 (s); 137.9 (s); 146.5 (s); 148.6 (s); 157.6 (s); 157.9 (s); 158.0 (s); 161.8 (s). MS (MALDI-TOF) 642 (M, 100).

Methyl 8-isopropoxy-2-(2-isopropoxy-4,5-dimethoxyphenyl)-1-(3-isopropoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (9i). Following the general procedure E and starting from **8k** (149 mg, 0.28 mmol) and **5d**, a reddish oil (170 mg, 93%) was obtained with 16 h of reaction time. IR (film) ν 1697 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.13 and 1.21 (2d, *J* = 6.4 Hz, 6H, 2Me); 1.35 and 1.36 (2d, *J* = 6.4 Hz, 12H, 4Me); 2.97-3.07 (m, 2H, H6); 3.30 (s, 3H, OMe);

3.62 (s, 3H, OMe); 3.78 (s, 3H, OMe); 3.79 (s, 3H, OMe); 4.17 (h, *J* = 6.4 Hz, 1H, OCH); 4.35 (h, *J* = 6.0 Hz, 1H, OCH); 4.51 (h, *J* = 6.4 Hz, 1H, OCH); 4.57-4.66 (m, 2H, H5); 6.45 (d, *J* = 8.4 Hz, 1H); 6.58 (s, 1H); 6.64-6.70 (m, 2H); 6.72-6.75 (m, 3H); 7.15 (t, *J* = 7.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.0 (q); 22.1 (q); 22.5 (q); 29.0 (t); 42.8 (t); 51.0 (q); 55.0 (q); 55.7 (q); 60.4 (q); 69.6 (d); 71.4 (d); 74.7 (d); 106.0 (d); 109.1 (d); 114.9 (d); 119.3 (s); 121.3 (d); 123.1 (d); 125.3 (d); 125.8 (d); 127.1 (s); 129.0 (d); 130.6 (s); 137.2 (s); 142.6 (s); 146.1 (s); 148.6 (s); 149.8 (s); 152.1 (s); 157.6 (s); 162.8 (s). MS (MALDI-TOF) 644 (M, 100).

Methyl 8-isopropoxy-9-methoxy-1-(3,4,5-trimethoxyphenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (10a). Following the general procedure F and starting from **6a** (25 mg, 0.05 mmol), a yellowish oil (21 mg, 85%) was obtained. IR (film) ν 1691, 1464, 1211 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (d, *J* = 6.0 Hz, 6H, 2Me); 3.51 (s, 3H, OMe); 3.85 (s, 6H, 2OMe); 3.90 (s, 3H, OMe); 3.92 (s, 3H, OMe); 4.67 (h, *J* = 6.0 Hz, 1H, OCH); 6.75 (s, 2H, H2', H6'); 6.95 (d, *J* = 7.6 Hz, 1H, H6); 7.06 (s, 1H); 7.33 (s, 1H); 7.43 (s, 1H); 9.24 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.9 (q); 51.2 (q); 55.4 (q); 56.2 (2q); 60.9 (q); 71.1 (d); 105.3 (d); 107.4 (2d); 110.5 (d); 112.5 (d); 114.0 (s); 118.3 (s); 119.6 (s); 121.9 (s); 123.3 (s); 123.4 (s); 130.7 (s); 132.8 (s); 137.1 (s); 147.7 (s); 150.1 (s); 153.2 (s); 161.8 (s). MS (MALDI-TOF) *m/z* 479 (M, 100).

Methyl 8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (10d). Following the general procedure F and starting from **6d** (77 mg, 0.16 mmol), a yellowish oil (55 mg, 72%) was obtained. IR (film) ν 1700, 1243 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (d, *J* = 6.0 Hz, 6H, 2Me); 1.42 (d, *J* = 6.0 Hz, 6H, 2Me); 3.49 (s, 3H, OMe); 3.84 (s, 3H, OMe); 3.91 (s, 3H, OMe); 4.58 (h, *J* = 6.0 Hz, 1H, OCH); 4.66 (h, *J* = 6.0 Hz, 1H, OCH); 6.92 (d, *J* = 7.6 Hz, 1H, H6); 7.00 (d, *J* = 8.8 Hz, 1H, H5'); 7.03-7.05 (m, 3H, H2, H2', H6'); 7.38 (s, 1H,); 7.42 (s, 1H); 9.23 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.9 (q); 22.0 (q); 51.1 (q); 55.3 (q); 55.9 (q); 71.1 (d); 71.7 (d); 105.3 (d); 110.5 (d); 112.3 (d); 113.9 (s); 114.2 (d); 116.2 (d); 118.2 (s); 119.8 (s); 122.3 (d); 122.6 (d); 123.2 (d); 123.3 (s); 130.3 (s); 130.7 (s); 146.4 (s); 147.6 (s); 150.0 (s); 150.4 (s); 161.8 (s). MS (MALDI-TOF) *m/z* 477 (M, 100).

Methyl 1-(2,5-dimethoxyphenyl)-8-isopropoxy-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (10e). Following the general procedure F and starting

from **6e** (33 mg, 0.07 mmol), a brownish oil (28 mg, 86%) was obtained. IR (film) ν 1690, 1505, 1466, 1213 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 1.42 and 1.43 (2d, *J* = 6.0 Hz, 6H, 2Me); 3.47 (s, 3H, OMe); 3.65 (s, 3H, OMe); 3.79 (s, 3H, OMe); 3.91 (s, 3H, OMe); 4.67 (h, *J* = 6.0 Hz, 1H, OCH); 6.92-7.00 (m, 4H); 7.04 (s, 1H); 7.16 (s, 1H); 7.44 (s, 1H); 9.25 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.9 and 22.0 (q); 51.1 (q); 55.3 (q); 55.8 (q); 56.2 (q); 71.0 (d); 105.2 (d); 110.2 (d); 112.0 (d); 112.4 (d); 113.6 (s); 113.9 (d); 114.2 (s); 117.9 (d); 120.2 (s); 122.5 (d); 123.27 (s); 123.34 (d); 126.9 (s); 131.5 (s); 147.6 (s); 150.1 (s); 152.1 (s); 153.6 (s); 161.8 (s). MS (MALDI-TOF) *m/z* 449 (M, 100).

Methyl 8-isopropoxy-9-methoxy-1-(2-thienyl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (10n). Following the general procedure F and starting from **6n** (34 mg, 0.09 mmol), a yellowish oil (16 mg, 48%) was obtained. IR (film) ν 1694, 1212 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 1.43 (d, *J* = 6.0 Hz, 6H, 2Me); 3.56 (s, 3H, OMe); 3.91 (s, 3H, OMe); 4.67 (h, *J* = 6.0 Hz, 1H, OCH); 6.96 (d, *J* = 7.6 Hz, 1H, H6); 7.06 (s, 1H); 7.16 (d, *J* = 2.4 Hz, 1H); 7.17 (s, 1H); 7.38-7.39 (bd, 1H); 7.42 (dd, *J* = 2.4, 2.4 Hz, 1H); 7.48 (s, 1H); 9.23 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.9 (q); 51.2 (q); 55.4 (q); 71.2 (d); 105.1 (d); 109.2 (s); 110.4 (d); 112.7 (d); 114.2 (s); 119.6 (s); 123.1 (d); 123.5 (s); 123.6 (d); 126.1 (d); 127.2 (d); 128.0 (d); 131.9 (s); 138.3 (s); 147.9 (s); 150.3 (s); 161.7 (s). MS (MALDI-TOF) *m/z* 395 (M, 100); 396 (M+1, 65).

Methyl 8-isopropoxy-9-methoxy-1,2-bis(3,4,5-trimethoxyphenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (11a). Following the general procedure F and starting from **7a** (43 mg (0.07 mmol), a reddish oil (41 mg, 95%) was obtained. IR (film) ν 1681, 1581, 1378, 1224 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 1.42 (d, *J* = 6.0 Hz, 6H, 2Me); 3.44 (s, 3H, OMe); 3.69 (2s, 12H, 4OMe); 3.71 (s, 3H, OMe); 3.82 (s, 3H, OMe); 3.85 (s, 3H, OMe); 4.63 (h, *J* = 6.0 Hz, 1H); 6.46 (s, 2H); 6.52 (s, 2H); 6.96 (d, *J* = 7.6 Hz, 1H, H6); 7.05 (s, 1H); 7.18 (s, 1H); 9.31 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.9 (q); 50.8 (q); 55.2 (q); 56.0 (2q); 56.2 (2q); 60.8 (q); 60.9 (q); 71.1 (d); 105.5 (d); 108.3 (2d); 108.8 (2d); 110.3 (d); 111.7 (s); 112.4 (d); 118.3 (s); 119.2 (s); 123.4 (d); 123.7 (s); 130.2 (s); 130.5 (s); 131.6 (s); 135.3 (s); 136.8 (s); 137.0 (s); 147.7 (s); 149.9 (s); 152.0 (2s); 153.1 (2s); 162.5 (s). MS (EI) *m/z* 645 (M, 100).

Methyl 1,2-bis(4-hydroxy-3-methoxyphenyl)-8-isopropoxy-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (11c). Following the general procedure F and starting

from **7c** (124 mg, 0.22 mmol), a yellowish solid (103 mg (84%) was obtained. M. p. (MeCN) > 250 ºC. IR (film) ν 3405, 1711, 1453, 1376 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.41 (d, *J* = 6.0 Hz, 6H, 2Me); 3.42 (s, 3H, OMe); 3.67 (s, 9H, 3OMe); 4.67 (h, *J* = 6.0 Hz, 1H, OCH); 5.61 (br, 1H, OH); 5.69 (br, 1H, OH); 6.64-6.67 (m, 2H, H2', H2"); 6.75 and 6.88 (2dd, *J* = 8.0, 1.6 Hz, 2H, H6', H6"); 6.79 (d, *J* = 7.6 Hz, 1H, H6); 6.90-6.95 (m, 2H, H5', H5"); 7.04 (s, 1H); 7.16 (s, 1H); 9.30 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR (MeOD-d_4, 100 MHz): δ 22.3 (q); 51.1 (q); 55.6 (q); 56.5 (q); 56.6 (q); 72.3 (d); 102.6 (d); 106.8 (d); 112.1 (d); 113.1 (d); 114.0 (d); 115.0 (d); 116.2 (d); 116.9 (d); 120.2 (s); 120.8 (s); 125.0 (s); 125.2 (d); 126.0 (d); 128.4 (s); 128.9 (s); 129.6 (s); 131.7 (s); 137.6 (s); 146.8 (s); 147.6 (s); 148.8 (s); 149.0 (s); 151.1 (s); 152.2 (s); 164.0 (s). MS (MALDI-TOF) *m/z* 557 (M, 100).

Methyl 1,2-bis(3,4-dimethoxyphenyl)-8-isopropoxy-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (11f). Following the general procedure F and starting from **7f** (72 mg, 0.12 mmol), a reddish solid (65 mg, 80%) was obtained. M. p. (MeCN) 85 ºC. IR (film) ν 1683 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.42 (d, *J* = 6.1 Hz, 6H, 2Me); 3.40 (s, 3H, OMe); 3.68 (s, 3H, CO_2Me); 3.69 (s, 3H, OMe); 3.70 (s, 3H, OMe); 3.86 (s, 3H, OMe); 3.87 (s, 3H, OMe); 4.67 (h, *J* = 6.0 Hz, 1H, OCH); 6.72-6.78 (m, 4H); 6.85 (bs, 2H, H2', H2"); 6.93 (d, *J* = 7.6 Hz, 1H, H6); 7.04 (s, 1H); 7.15 (s, 1H); 9.30 (d, 1H, *J* = 7.6 Hz, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ. 21.9 (q); 50.8 (q); 55.2 (q); 55.6 (q); 55.7 (q); 55.8 (q); 55.9 (q); 71.1 (d); 105.6 (d); 109.9 (d); 110.4 (d); 111.1 (d); 111.9 (s); 112.1 (d); 114.2 (d); 115.0 (d), 118.4 (s); 119.5 (s), 123.2 (d); 123.5 (d); 123.6 (s); 124.2 (d); 127.8 (s); 128.6 (s); 130.5 (s); 135.7 (s); 147.5 (s); 147.6 (s); 148.0 (s); 148.8 (s); 149.9 (s); 162.6 (s). MS (MALDI-TOF) *m/z* 585 (M).

Methyl 8-isopropoxy-9-methoxy-1,2-bis(4-methoxyphenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (11g). Following the general procedure F and starting from **7g** (78 mg, 0.15 mmol), a yellowish oil (61 mg, 80%) was obtained. IR (film) ν 2933, 1680 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 1.41 (d, *J* = 6.0 Hz, 6H, 2Me); 3.38 (s, 3H, OMe); 3.64 (s, 3H, CO_2Me); 3.77 (s, 3H, OMe); 3.78 (s, 3H, OMe); 4.65 (h, *J* = 6.0 Hz, 1H, OCH); 6.75 (d, *J* = 8.5 Hz, 2H); 6.87 (d, *J* = 8.5 Hz, 2H); 6.91 (d, *J* = 7.6 Hz, 1H, H6); 7.03 (s, 1H); 7.05 (s, 1H); 7.09 (d, *J* = 8.5 Hz, 2H); 7.18 (d, *J* = 8.5 Hz, 2H); 9.30 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.9 (q); 50.6 (q); 55.0 (q); 55.1 (q); 55.2 (q); 71.0 (d); 105.5 (d), 110.4 (d); 111.9 (d); 112.4 (2d); 113.7 (2d); 118.3 (s); 119.6 (s), 123.4 (s); 123.5 (d); 127.6 (s); 128.2 (s); 130.6 (s); 131.6 (2d);

133.0 (2d); 135.8 (s); 147.4 (s); 149.8 (s); 158.0 (s); 158.5 (s); 162.6 (s). MS (MALDI-TOF) *m/z* 525 (M).

Methyl 8-isopropoxy-9-methoxy-1,2-bis(4-trifluoromethoxyphenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (11i). Following the general procedure F and starting from **7i** (58 mg, 0.09 mmol), a yellowish solid (837 mg, 65%) was obtained. M. p. (MeOH) 165 - 170 ºC. IR (film) ν 2924, 1674 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.41 (d, *J* = 6.0 Hz, 6H, 2Me); 3.34 (s, 3H, OMe); 3.62 (s, 3H, CO_2Me); 4.66 (h, *J* = 6.1 Hz, 1H, OCH); 6.86 (s, 1H); 6.97 (d, *J* = 7.6 Hz, 1H, H6); 7.06 (s, 1H); 7.07 (d, *J* = 8.7 Hz, 2H); 7.18 (d, *J* = 8.7 Hz, 2H); 7.21 (d, *J* = 8.7 Hz, 2H); 7.30 (d, *J* = 8.7 Hz, 2H); 9.32 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ. 21.8 (q); 50.7 (q); 54.7 (q); 71.2 (d); 105.0 (d), 110.5 (d); 112.2 (q); 112.6 (d); 116.9 (q); 119.2 (s); 119.4 (2d); 121.1 (2d); 121.7 (s); 123.3 (s); 123.7 (d); 130.5 (s); 131.7 (2d); 133.3 (2d); 133.8 (s); 134.4 (s); 134.8 (s); 147.8 (s); 150.1 (s); 162.1 (s). MS (MALDI-TOF) *m/z* 633 (M); 634 (M+1).

Methyl 1,2-bis(4-isopropoxyphenyl)-8-isopropoxy-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (11j). Following the general procedure F and starting from **7j** (103 mg, 0.18 mmol), a yellowish solid (84 mg, 82%) was obtained. M. p. (MeCN) 158-160 ºC. IR (film) ν 2926, 1685 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.31 (d, *J* = 6.1 Hz, 12H, 4Me); 1.40 (d, *J* = 6.0 Hz, 6H, 2Me); 3.39 (s, 3H, OMe); 3.64 (s, 3H, CO_2Me); 4.47-4.55 (m, 2H); 4.64 (h, *J* = 6.1 Hz, 1H, OCH); 6.72 (d, *J* = 8.7 Hz, 2H); 6.85 (d, *J* = 8.7 Hz, 2H); 6.90 (d, *J* = 7.6 Hz, 1H, H6); 7.03 (s, 1H); 7.07 (s, 1H); 7.08 (d, *J* = 8.7 Hz, 2H); 7.15 (d, *J* = 8.7 Hz, 2H); 9.30 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ. 21.8 (q); 21.9 (q); 22.0 (q); 50.6 (q); 55.0 (q); 69.6 (d); 69.7 (d); 71.1 (d); 105.5 (d), 110.4 (d); 111.9 (d); 114.4 (2d); 115.8 (2d); 116.9 (s); 118.4 (s); 119.6 (s), 123.4 (s); 123.5 (d); 127.4 (s); 128.1 (s); 130.5 (s); 131.6 (2d); 133.0 (2d); 135.9 (s); 147.4 (s); 149.7 (s); 156.3 (s); 156.7 (s); 162.6 (s). MS (MALDI-TOF) *m/z* 581 (M); 582 (M+1).

Methyl 8-isopropoxy-1,2-bis(3-isopropoxyphenyl-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (11k). Following the general procedure F and starting from **7k** (102 mg, 0.18 mmol), a yellowish solid 76 mg (74%) was obtained. M. p. (MeCN) 145-147 ºC . IR (film) ν 2975, 1685 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.14-1.25 (m, 12H, 4Me); 1.41 (d, *J* = 6.1 Hz, 6H, 2Me); 3.39 (s, 3H, OMe); 3.64 (s, 3H, CO_2Me); 4.33-4.41 (m, 2H); 4.65 (h, *J* = 6.1 Hz, 1H, OCH); 6.71-6.81 (m, 5H); 6.90 (d,

J = 7.5 Hz, 1H); 6.94 (d, *J* = 7.6 Hz, 1H, H6); 7.08 (s, 1H); 7.11 (d, *J* = 7.6 Hz, 1H); 7.20 (s, 1H); 7.23 (t, *J* = 7.8 Hz, 1H); 9.32 (d, 1H, *J* = 7.6 Hz, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.5 (q); 21.8 (q); 22.0 (q); 50.6 (q); 55.0 (q); 69.6 (d); 69.8 (d); 71.0 (d); 105.5 (d), 110.3 (d); 112.1 (d); 115.2 (2d); 117.8 (d); 118.5 (s), 118.7 (d); 119.4 (s); 123.2 (d); 123.3 (d); 123.5 (s); 124.0 (d); 127.8 (d); 129.2 (d); 130.2 (s); 135.6 (s); 136.5 (s); 137.2 (s); 147.5 (s); 149.8 (s); 156.6 (s); 157.7 (s); 162.5 (s). MS (MALDI-TOF) *m/z* 581 (M).

Methyl 8-isopropoxy-9-methoxy-1,2-bis(3-nitrophenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (11m). Following the general procedure F and starting from **7m** (90 mg, 0.16 mmol), a yellowish solid (28 mg, 32%) was obtained. M. p. (MeCN) 215 - 220 ºC. IR (film) 2926, 1691, 1540, 1439, 1378, 1349, 1255, 1194 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (d, *J* = 6.1 Hz, 6H, 2Me); 3.32 (s, 3H, OMe); 3.63 (s, 3H, CO_2Me); 4.69 (h, *J* = 6.1 Hz, 1H, OCH); 6.86 (s, 1H); 7.06 (d, *J* = 7.5 Hz, 1H, H6); 7.10 (s, 1H); 7.41 (ddd, *J* = 7.6, 7.5, 1.3, 1H); 7.50 (dt, *J* = 7.6, 1.3, 1H); 7.6 (bm, 1H); 7.66 (dt, *J* = 7.5, 1.3 Hz, 1H); 8.08-8.10 (m, 2H); 8.14-8.16 (m, 2H); 9.37 (d, 1H, *J* = 7.6 Hz, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ. 21.8 (q); 51.0 (q); 55.1 (q); 71.2 (d); 104.8 (d), 110.6 (d); 112.7 (s); 113.3 (d); 115.5 (s); 118.6 (s), 121.9 (d); 122.3 (d); 123.2 (d); 124.0 (s); 125.4 (d); 126.4 (d); 128.1 (d); 129.4 (d); 130.5 (s); 133.1 (s); 136.5 (s); 136.6 (d); 137.4 (d); 138.0 (s); 147.3 (s); 148.2 (s); 148.3 (s); 150.3 (s); 161.6 (s). MS (MALDI-TOF) *m/z* 555 (M).

Methyl 8-isopropoxy-9-methoxy-1,2-bis(2-thienyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (11n). Following the general procedure F and starting from **7n** (30 mg, 0.12 mmol), **11n** as a yellowish oil (34 mg, 72%) was obtained. IR (film) ν 1684, 1373, 1245 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (d, *J* = 6.4 Hz, 6H, 2Me); 3.49 (s, 3H, OMe); 3.73 (s, 3H, OMe); 4.68 (h, *J* = 6.4 Hz, 1H, OCH); 6.93-6.94 (m, 2H); 6.97 (d, *J* = 7.6 Hz, 1H); 7.04-7.06 (m, 2H); 7.09 (dd, *J* = 5.2, 3.4 Hz, 1H); 7.15 (bs, 1H); 7.27 (dd, *J* = 3.8, 2.4 Hz, 1H); 7.38 (dd, *J* = 5.2, 1.2 Hz, 1H); 9.28 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.9 (q); 51.0 (q); 55.2 (q); 71.1 (d); 105.3 (d); 110.2 (d); 110.5 (s); 112.9 (d); 113.1 (s); 119.1 (s); 123.2 (d); 123.8 (s); 125.8 (d); 126.0 (d); 127.16 (d); 127.20 (d); 128.3 (d); 129.5 (s); 129.9 (d); 131.7 (s); 135.1 (s); 136.6 (s); 148.0 (s); 150.2 (s); 162.2 (s). MS (ESI) *m/z* 418 (M+1, 100); 419 (M+2, 45).

Methyl 2-(4-hydroxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (12a). Following the general procedure F and starting from **9a** (88 mg, 0.15 mmol), a yellowish syrup (78 mg, 89%) was obtained. IR (film) ν 3428, 2923, 1683 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.32 and 1.36 (2d, *J* = 6.0 Hz, 6H, 2Me); 1.41 (d, *J* = 6.0 Hz, 6H, 2Me); 3.41 (s, 3H, OMe); 3.49 and 3.651 (2s, 3H, OMe); 3.654 (s, 3H, OMe); 4.50 (h, *J* = 6.0 Hz, 1H, OCH); 4.66 (h, *J* = 6.0 Hz, 1H, OCH); 6.67 (d, *J* = 8.6 Hz, 2H, H3", H5"); 6.72 (d, *J* = 1.6 Hz, 1H, H2'); 6.83 (dd, *J* = 8.0, 1.6 Hz, 1H, H6'); 6.88 (d, *J* = 8.0 Hz, 1H, H5'); 6.92 (d, *J* = 7.6 Hz, 1H, H6); 7.038 (d, *J* = 8.6 Hz, 2H, H2", H6"); 7.039 (s, 1H); 7.19 (s, 1H); 9.30 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.7 (q); 21.9 (q); 22.2 (q); 50.7 (q); 55.1 (q); 55.8 (q); 71.2 (d); 71.4 (d); 105.6 (d); 110.4 (d); 112.0 (d); 114.0 (2d); 115.7 (d), 115.8 (d); 118.6 (s); 119.6 (s), 123.5 (d); 123.6 (s); 124.2 (d); 127.7 (s); 129.0 (s); 130.4 (s); 131.8 (2d); 135.9 (s); 146.0 (s); 147.5 (s); 149.8 (s); 150.2 (s); 154.2 (s); 162.7 (s). MS (MALDI-TOF) 569 (M, 100).

Methyl 2-(3,4-dimethoxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (12b). Following the general procedure F and starting from **9b** (98 mg, 0.16 mmol), a yellowish oil (64 mg, 66%) was obtained. IR (film) ν 2975, 1681 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.33 and 1.36 (2d, *J* = 6.0 Hz, 6H, 2Me); 1.42 (d, *J* = 6.0 Hz, 6H, 2Me); 3.41 (s, 3H, OMe); 3.66 (s, 3H, OMe); 3.680 (s, 3H, OMe); 3.683 (s, 3H, OMe); 3.86 (s, 3H, OMe); 4.50 (h, *J* = 6.0 Hz, 1H, OCH); 4.66 (h, *J* = 6.0 Hz, 1H, OCH); 6.72 (d, *J* = 1.8 Hz, 1H, H2"); 6.73 (d, *J* = 8.3 Hz, 1H, H5"); 6.75 (d, *J* = 1.9 Hz, 1H, H2'); 6.77 (dd, *J* = 8.3, 1.8, 1H, H6"); 6.83 (dd, *J* = 8.2, 1.9 Hz, 1H, H6'); 6.89 (d, *J* = 8.2 Hz, 1H, H5'); 6.93 (d, *J* = 7.6 Hz, 1H, H6); 7.04 (s, 1H); 7.19 (s, 1H); 9.31 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ. 21.8 (q); 21.9 (q); 22.2 (q); 50.8 (q); 55.2 (q); 55.68 (q); 55.69 (q); 55.9 (q); 71.1 (d); 71.5 (d); 105.6 (d); 109.9 (d); 110.4 (d); 111.9 (s); 112.1 (d); 114.2 (d); 115.7 (d), 116.0 (d); 118.5 (s); 119.6 (s), 123.3 (d); 123.5 (d); 123.6 (s); 124.1 (d); 127.8 (s); 129.1 (s); 130.5 (s); 135.7 (s); 146.1 (s); 147.51 (s); 147.53 (s); 147.6 (s); 149.9 (s); 150.3 (s); 162.6 (s). MS (MALDI-TOF) 613 (M, 100).

Methyl 8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-2-(3,4,5-trimethoxyphenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (12c). Following the general procedure F and starting from **9c** (98 mg, 0.15 mmol), a yellowish oil (81 mg, 83%) was obtained. IR (film) ν 2976, 1681 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.34

and 1.35 (2d, *J* = 6.0 Hz, 6H, 2Me); 1.42 (d, *J* = 6.0 Hz, 6H, 2Me); 3.41 (s, 3H, OMe); 3.66 (s, 3H, OMe); 3.67 (s, 6H, 2OMe); 3.71 (s, 3H, OMe); 3.84 (s, 3H, OMe); 4.50 (h, *J* = 6.0 Hz, 1H, OCH); 4.67 (h, *J* = 6.0 Hz, 1H, OCH); 6.43 (s, 2H, H2", H6"); 6.75 (d, *J* = 1.9 Hz, 1H, H2'); 6.85 (dd, *J* = 8.2, 1.9 Hz, 1H, H6'); 6.91 (d, *J* = 8.2 Hz, 1H, H5'); 6.94 (d, *J* = 7.6 Hz, 1H, H6); 7.05 (s, 1H); 7.22 (s, 1H); 9.31 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ. 21.8 (q); 21.9 (q); 22.1 (q); 50.8 (q); 55.2 (q); 55.95 (q); 55.97 (2q); 60.9 (q); 71.1 (d); 71.6 (d); 105.6 (d); 108.4 (2d); 110.4 (d); 111.7 (s); 112.3 (d); 115.7 (d); 116.2 (d), 118.3 (s); 119.5 (s), 123.4 (d); 123.6 (s); 124.1 (d); 129.2 (s); 130.5 (s); 130.6 (s); 135.7 (s); 136.7 (s); 146.2 (s); 147.7 (s); 149.9 (s); 150.5 (s); 151.9 (2s); 162.6 (s). MS (MALDI-TOF) 643 (M, 100).

Methyl 2-(2,4-diisopropoxy-5-methoxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (12d). Following the general procedure F and starting from **9d** (221 mg, 0.31 mmol), a pale yellow oil (208 mg, 95%) was obtained. IR (film) ν 1684, 1211 cm^{-1}. ^{1}H NMR (acetone-d_6, 400 MHz) δ 1.25-1.35 (m, 24H); 3.38 and 3.40 (2d, 3H, OMe); 3.54 and 3.59 (2s, 3H, OMe); 3.60 and 3.62 (2s, 3H, OMe); 3.62 (s, OMe); 4.19-4.35 (m, 1H); 4.48-4.59 (m, 2H); 4.72 (h, 1H, *J* = 5.6 Hz); 6.56 (s, 1H); 6.60 (d, 1H, *J* = 8.8 Hz); 6.69-6.82 (m, 2H); 6.90-7.13 (m, 2H), 7.28 and 7.16 (2s, 1H); 7.29 (s, 1H); 9.24 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.8 (q); 21.9 (q); 22.1 (q); 22.2 (q); 50.7 (q); 55.2 (q); 55.5 (q); 56.1 (q); 71.2 (d); 71.4 (d); 71.5 (d); 71.6 (d); 71.8 (d); 105.6 (d), 106.6 (d); 107.0 (s); 110.7 (d); 111.7 (d); 113.1 (s); 115.4 (d), 115.6 (d); 115.9 (d), 116.1 (d); 118.2 (s); 119.4 (s); 119.8 (s); 123.3 (d); 129.4 (s); 131.6 (s); 144.3 (s); 146.0 (s); 146.2 (s); 147.3 (s); 149.5 (s); 149.9 (s); 150.2 (s); 162.9 (s). MS (MALDI-TOF) 699 (M, 100), 700 (M+1, 59), 701 (M+2, 17). HRMS *m/z* calcd for $C_{41}H_{49}NO_9$ 699.3407, found 699.3402.

Methyl 2-(2,5-dimethoxy-4-isopropoxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (12e). Following the general procedure F and starting from **9e** (64 mg, 0.10 mmol), a reddish oil (52 mg, 77%) was obtained. IR (film) ν 1681, 1437, 1373, 1213 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.31-1.36 (m, 12H, 4Me); 1.41 (d, *J* = 6.0 Hz, 6H, 2Me); 3.42 (s, 3H, OMe); 3.54 (bs, 3H, OMe); 3.61 (s, 3H, OMe); 3.63 (s, 3H, OMe); 3.64 (s, 3H, OMe); 4.43-4.54 (m, 2H, 2OCH); 4.65 (h, *J* = 6.0 Hz, 1H, OCH); 6.46 (s, 1H); 6.60 (bs, 1H); 6.78 (bs, 1H); 6.86 (bs, 2H); 6.90 (d, *J* = 7.6 Hz, 1H, H6); 7.03 (s, 1H); 7.27 (s,

1H); 9.27 (d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.9 (q); 22.1 (q); 22.2 (q); 50.7 (q); 55.2 (q); 55.8 (q); 56.3 (q); 56.5 (q); 71.1 (d); 71.5 (d); 72.0 (d); 102.0 (d); 105.6 (d); 110.4 (d); 111.9 (d); 112.7 (s); 115.3 (d); 116.0 (d); 116.7 (d); 117.4 (s); 118.6 (s); 119.7 (s); 123.5 (d); 123.8 (d); 129.4 (s); 130.3 (s); 131.8 (s); 144.0 (s); 145.9 (s); 146.6 (s); 147.4 (s); 149.8 (s); 150.1 (s); 151.4 (s); 162.6 (s). MS (MALDI-TOF) 671 (M, 100).

Methyl 2-(2,4-diisopropoxyphenyl)-1-(2,5-dimethoxyphenyl)-8-isopropoxy-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (12f). Following the general procedure F and starting from **9f** (56 mg, 0.09 mmol), a yellowish oil (41 mg, 73%) was obtained as a mixture of rotamers (6.5:3.5). IR (film) ν 2924, 1699 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.13 and 1.17 (2d, *J* = 6.0 Hz, 6H, 2Me); 1.31 and 1.32 (2d, *J* = 6.0 Hz, 6H, 2Me); 1.41 and 1.42 (2d, *J* = 6.0 Hz, 6H, 2Me); 3.38 and 3.40 (2s, 3H, OMe); 3.41 and 3.58 (2s, 3H, OMe); 3.61 and 3.68 (2s, 3H, OMe); 3.64 (s, 3H, OMe); 4.29 and 4.36 (2h, *J* = 6.0 Hz, 1H, OCH); 4.48 (h, *J* = 6.0 Hz, 1H, OCH); 4.65 (h, *J* = 6.0 Hz, 1H, OCH); 6.29 and 6.32 (2dd, *J* = 8.4, 2.4 Hz, 1H); 6.38 and 6.39 (2d, *J* = 2.4 Hz, 1H); 6.66 (d, *J* = 3.0 Hz, 1H); 6.78 (dd, *J* = 9.0, 3.0 Hz, 1H); 6.86 (d, *J* = 9.0 Hz, 1H); 6.90 (d, *J* = 7.6 Hz, 1H, H6); 6.91 (d, *J* = 8.4 Hz, 1H); 7.02 and 7.03 (2s, 1H); 7.04 and 7.14 (2s, 1H); 9.25 and 9.28 (2d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.9 and 22.0 (q); 22.1 (q); 22.2 (q); 50.6 and 50.7 (q); 55.2 (q); 55.6 and 55.8 (q); 55.9 and 56.0 (q); 69.8 (d); 70.1 (d); 71.1 (d); 102.5 and 103.0 (d); 105.2 and 105.5 (d); 105.8 and 106.0 (d); 110.4 (d); 111.5 and 111.6 (d); 112.5 and 114.4 (d); 118.0 (d); 118.7 and 118.9 (d); 120.1 and 120.2 (s); 123.3 and 123.4 (s); 123.5 and 123.6 (d); 125.9 (s); 126.2 (s); 130.4 (s); 131.2 and 131.9 (d); 132.1 and 132.5 (s); 147.2 (s); 149.8 and 150.0 (s); 152.4 and 153.3 (s); 156.6 and 156.7 (s); 157.7 and 157.8 (s); 162.9 (s). MS (MALDI-TOF) 641 (M, 100).

Methyl 1-(2,5-dimethoxyphenyl)-8-isopropoxy-9-methoxy-2-(2,4,5-triisopropoxyphenyl)pyrrolo[2,1-*a*]isoquinoline-3-carboxylate (12g). Following the general procedure F and starting from **9g** (89 mg, 0.13 mmol) of, a yellowish oil (80 mg, 90%) was obtained as a mixture of rotamers (6.5:3.5). IR (film) ν 2925, 1696 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.06-1.12 (m, 12H, 4Me); 1.27-1.33 (m, 6H, 2Me); 1.38-1.44 (m, 6H, 2Me); 3.40 and 3.46 (2s, 3H, OMe); 3.59 and 3.67 (2s, 3H, OMe); 3.61 and 3.78 (2s, 3H, OMe); 3.63 and 3.90 (2s, 3H, OMe); 4.05-4.15 (m, 1H, OCH); 4.19 (h, *J* = 6.0 Hz, 1H, OCH); 4.43-4.50 (m, 1H, OCH); 4.60-4.69 (m, 1H, OCH); 6.46 and

6.47 (2s,1H, H3"); 6.64 and 7.43 (2s, 1H, H6"); 6.68 (d, *J* = 3.2 Hz, 1H, H6'); 6.77-6.90 (m, 3H, H6, H3', H4'); 7.02 and 7.16 (2s, 1H); 7.03 and 7.11 (2s, 1H); 9.24 and 9.27 (2d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.0 (q); 22.1 (q); 22.2 (q); 22.3 (q); 50.5 and 51.0 (q); 55.2 and 55.3 (q); 55.5 and 55.7 (q); 55.9 and 56.2 (q); 71.0 and 71.3 (d); 72.1 (d); 72.4 and 72.6 (d); 105.1 and 105.5 (d); 107.1 and 107.8 (d); 110.2 and 110.3 (d); 111.3 (s); 111.6 and 111.9 (d); 112.2 (d); 112.4 (d); 113.2 and 113.5 (s), 114.0 and 114.2 (s); 117.9 and 119.0 (d); 120.1 and 120.2 (s); 122.0 and 122.4 (d); 122.6 and 123.2 (s); 123.3 and 123.5 (d); 125.8 and 126.1 (s); 126.8 (s); 130.3 (s); 131.4 and 131.9 (s); 142.3 (s); 147.2 and 147.5 (s); 148.0 and 148.1 (s); 149.8 and 150.0 (s); 150.5 and 152.3 (s); 152.9 and 154.4 (s); 161.8 and 162.9 (s). MS (MALDI-TOF) 699 (M, 100).

Methyl 2-(2,4-diisopropoxyphenyl)-8-isopropoxy-1-(3-isopropoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (12h). Following the general procedure F and starting from **9h** (72 mg, 0.11 mmol), a yellowish oil (54 mg, 75%) was obtained as a mixture of rotamers (6:4). IR (film) ν 2975, 1699 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.34 (d, *J* = 6.0 Hz, 6H, 2Me); 1.37 (d, *J* = 6.0 Hz, 6H, 2Me); 1.42 (d, *J* = 6.0 Hz, 6H, 2Me); 1.43 (d, *J* = 6.0 Hz, 6H, 2Me); 3.49 and 3.51 (2s, 3H, OMe); 3.91 and 3.92 (2s, 3H, OMe); 4.53-4.60 (m, 2H, 2OCH); 4.64-4.70 (m, 2H, 2OCH); 6.90 and 6.91 (2dd, *J* = 8.0, 2.4 Hz, 1H, H5"); 6.93 and 7.63 (2d, *J* = 8.0 Hz, 1H, H6"); 6.95 (d, *J* = 7.6 Hz, 1H, H6); 6.98-7.02 (dm, *J* = 8.0 Hz, 1H, H4'); 7.04-7.07 (m, 2H, H2', H3"); 7.08-7.11 (dm, *J* = 8.0 Hz, 1H, H6'); 7.27 and 7.37 (2s, 1H); 7.36 (t, *J* = 8.0 Hz, 1H, H5'); 7.40 and 7.42 (2s, 1H); 9.23 and 9.24 (2d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.91 (q); 21.93 (q); 22.07 (q); 22.11 (q); 51.1 and 51.2 (q); 55.3 and 55.4 (q); 69.7 (d); 71.1 (d); 71.2 (d); 72.1 (d); 105.2 and 105.5 (d); 110.5 and 110.6 (d); 112.4 and 112.6 (d); 114.1 and 114.3 (s); 114.8 (d); 117.5 and 117.6 (d); 118.4 (s); 119.6 and 119.8 (s); 122.0 and 122.1 (d); 122.7 (d); 123.3 (d); 123.5 and 123.7 (s); 123.9 (d); 129.4 (d); 130.7 (s); 133.2 (d); 137.7 (s); 138.6 (s); 147.6 and 147.8 (s); 150.0 and 150.2 (s); 154.6 (s); 157.9 (s); 161.8 and 161.9 (s). MS (MALDI-TOF) 639 (M, 100).

Methyl 8-isopropoxy-2-(2-isopropoxy-4,5-dimethoxyphenyl)-1-(3-isopropoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (12i). Following the general procedure F and starting from **9i** (78 mg, 0.12 mmol), a yellowish oil (53 mg, 69%) was obtained as a mixture of rotamers (5.5:4.5). IR (film)

ν 2974, 1697 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88-1.01 (m, 6H, 2Me); 1.08-1.21 (m, 6H, 2Me); 1.33-1.45 (m, 6H, 2Me); 3.39 (s, 3H, OMe); 3.67 (s, 3H, OMe); 3.74 and 3.77 (2s, 3H, OMe); 3.81 (s, 3H, OMe); 4.06-4.20 (m, 1H, OCH); 4.32-4.50 (m, 1H, OCH); 4.65 (h, *J* = 6.0 Hz, 1H, OCH); 6.49 and 6.50 (2d, *J* = 8.4 Hz, 1H, H6"); 6.72 and 6.75 (2d, *J* = 8.4 Hz, 1H, H5"); 6.80 (d, *J* = 8.0 Hz, 1H, H4'); 6.85-6.89 (m, 1H, H2'); 6.91 (d, *J* = 7.6 Hz, 1H, H6); 6.95-6.99 (m, 1H, H6'); 7.04 (s, 1H); 7.07 and 7.08 (2s, 1H); 7.20 and 7.26 (2t, *J* = 8.0 Hz, 1H, H5'); 9.22 and 9.23 (2d, *J* = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.9 (q); 22.0 (q); 22.4 and 22.6 (q); 50.9 (q); 55.1 (q); 55.7 (q); 60.4 (q); 69.7 (d); 71.1 (d); 74.7 (d); 105.6 (d); 105.9 and 106.0 (d); 110.6 (d); 111.8 (d); 112.9 and 113.0 (s); 115.2 and 115.8 (d); 118.2 and 119.2 (d); 121.7 and 122.5 (s); 123.1 and 123.3 (d); 124.0 and 124.6 (d); 126.0 (d); 129.0 and 129.2 (d); 129.9 and 130.0 (s); 130.9 and 131.1 (s); 132.0 (s); 137.5 and 137.6 (s); 142.5 (s); 147.3 (s); 149.8 and 149.9 (s); 152.4 (s); 157.5 (s); 157.7 (s); 162.9 (s). MS (MALDI-TOF) 641 (M, 100).

Table 1. HPLC analytical results to support final compound purity.

compound	Gradient[d]	Solvent System[a]			
		SS1[b]		**SS2**[c]	
		retention time (min)	% purity	retention time (min)	% purity
13c	A	7.1	97.5	7.0	97.6
13e	A	9.2	96.7	9.2	96.3
13l	A	12.3	98.6	3.1	98.9
14a (R^4=OH)	B	10.6	98.1	10.5	97.7
14c	B	11.3	97.5	11.3	97.5
14f	A	9.0	98.8	8.8	98.6
14g	A	12.4	97.1	12.2	97.9
14i	C	12.0	98.9	12.0	97.5

14j	B	11.2	98.0	11.2	98.3
14k	B	11.6	97.8	11.6	99.1
14l	D	11.0	98.5	1.7	93.1
14m	D	9.3	98.3	9.3	97.9
15d	A	4.2	97.1	4.1	99.8
15e	B	10.7	99.0	10.5	96.9
15g	A	9.3	97.0	9.1	97.4
15i	B	11.8	96.4	11.7	97.2
16a	A	10.5	97.6	10.2	98.2
16c	A	8.3	97.7	8.3	97.8
16e	A	10.7	99.2	10.8	99.1
16n	A	9.5	98.4	9.5	97.9
17a	A	9.9	98.9	9.9	99.0
17c	A	7.1	100.0	7.0	100.0
17f	A	10.2	97.9	10.2	98.5
17g	A	14.0	98.1	14.0	98.7
17i	C	13.1	98.6	12.9	98.3
17j	E	10.5	97.9	10.4	99.3
17k	E	10.9	98.8	10.8	98.5
17m	D	10.3	97.1	10.3	97.1
17n	D	11.3	98.6	11.2	98.5
18a	A	7.2	98.2	7.1	98.0
18b	A	8.6	98.6	8.7	98.5
18c (R^4=OH)	E	9.8	97.8	9.8	97.2

18d	B	10.1	98.3	10.0	98.0
18e	B	11.5	96.8	11.5	97.2
18g	A	10.7	98.3	10.6	98.1
18h	A	8.4	94.3	8.3	99.2

a: Analysis were done in two solvent systems (SS), with a flow rate of 1 mL/min.

b: **SS1**: 20 μM NH_4AcO pH=5 (**X**) and MeCN (**Y**)

c: **SS2**: H_2O/0.045% TFA (**X**) and MeCN/0.036% TFA (**Y**)

d: A: 40 to 75% of eluent **Y** in 15 min; B: 20 to 70% of eluent **Y** in 15 min; C: 50 to 100% of eluent **Y** in 15 min; D: 50 to 80% of eluent **Y** in 15 min; E: 30 to 70% of eluent **Y** in 15 min.

Figure 1. gHSQC correlation of compound **12f**

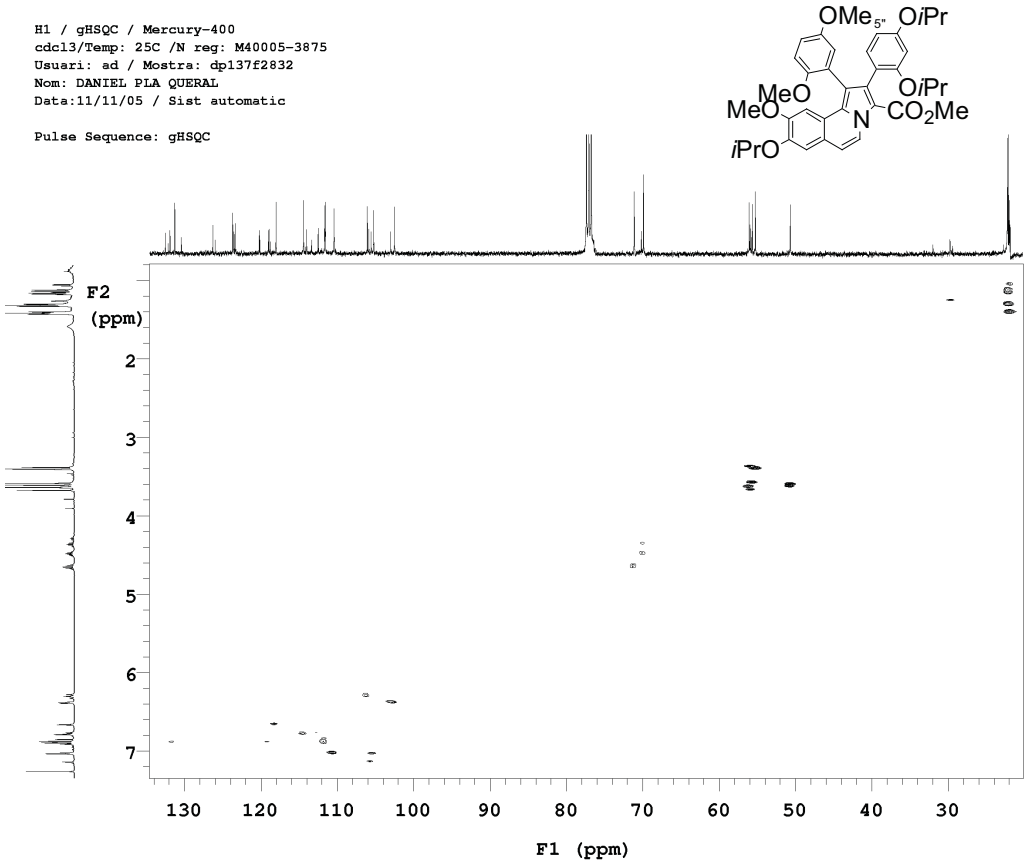
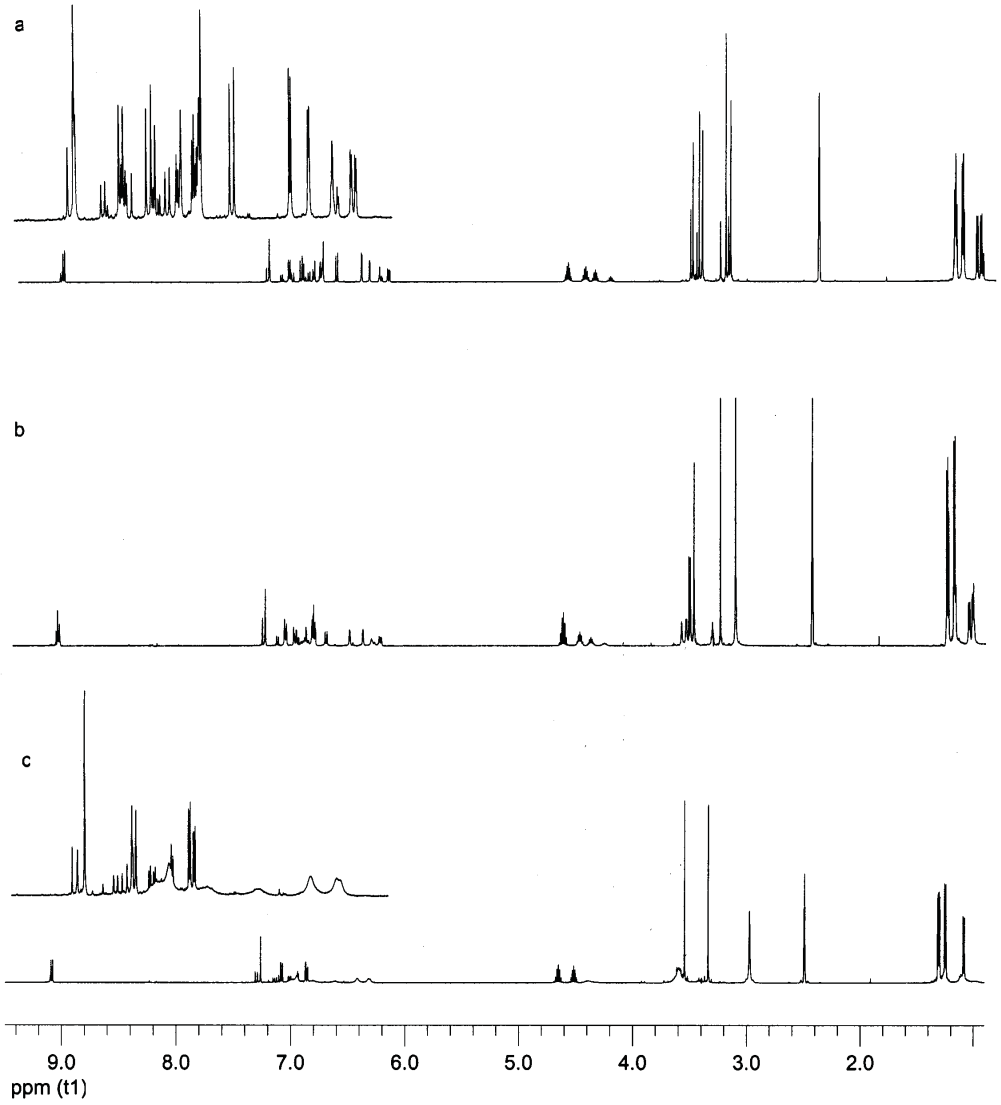


Figure 2. ^{1}H-NMR of compound **12f** at variable temperatures



5 BIOCONJUGATS DE LA LAMEL·LARINA D I: SÍNTESI I INTERNALITZACIÓ CEL·LULAR DE DERIVATS AMB POLIETILENGLICOL

BIOCONJUGATS DE LA LAMEL·LARINA D I: SÍNTESI I INTERNALITZACIÓ CEL·LULAR DE DERIVATS AMB POLIETILENGLICOL.

Lamellarin D Bioconjugates I: Synthesis and Cellular Internalization of PEG-Derivatives.

Daniel Pla,[a, b] Andrés Francesch,[c] Pilar Calvo,[c] Carmen Cuevas,[c] Rosa Aligué,[d]

Fernando Albericio,[a, b, ‡] Mercedes Álvarez,[a, b, §, *]

Bioconjugate Chemistry, **2009**, *20*, 1100-1111.

[a]Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain.

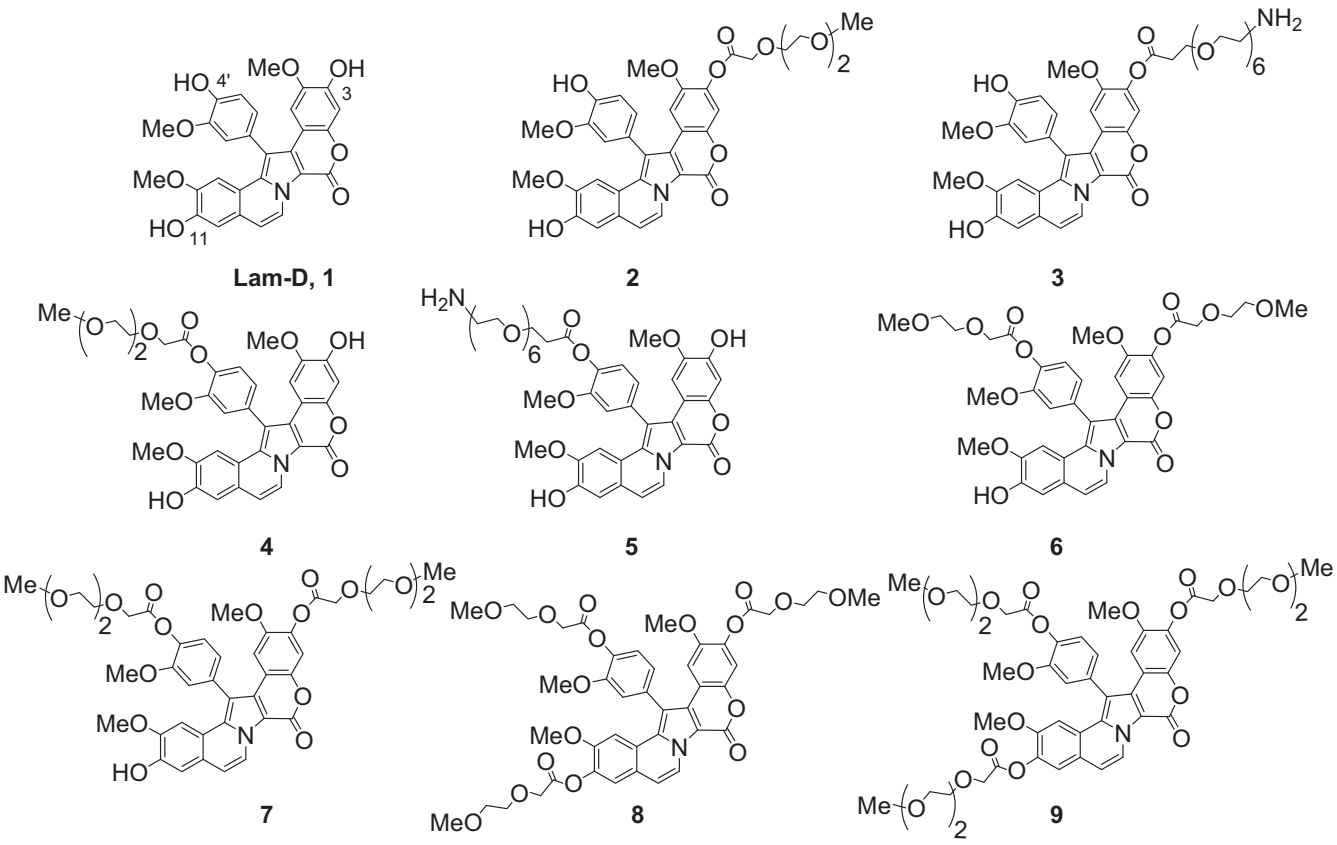
[b]CIBER-BBN Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Baldiri Reixac 10, E-08028 Barcelona, Spain.

[c]Pharma Mar S. A., Avda Reyes Católicos 1, E-28770 Colmenar Viejo, Madrid, Spain.

[d]Department of Cell Biology, Faculty of Medicine, University of Barcelona, Casanova 143, E-08036 Barcelona, Spain.

‡ Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona

§ Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, E-08028 Barcelona



Resum

En aquest capítol es descriu el disseny i la síntesi d'una sèrie de conjugats de la Lamel·larina D amb polietilenglicol (PEG). Mono-, di- i tri-PEG conjugats amb solubilitats millorades s'han obtingut amb rendiments compresos entre 18-57% global, partint dels corresponents precursors fenòlics protegits de la Lamel·larina D. Els conjugats **1-9** han estat assajats en un panell de tres línies cel·lulars tumorals humanes, MDA-MB-231 (mama), A-549 (pulmó), i HT-29 (colon), per tal d'avaluar el seu potencial citotòxic. La important millora aconseguida en la internalització cel·lular dels conjugats, es tradueix en un increment en la capacitat d'inhibició del creixement (GI_{50}) per més del 85% dels derivats en comparació a la Lamel·larina D. D'altra banda, s'ha determinat també un mecanisme de mortalitat cel·lular per apoptosi via marcatge de caspases-3,7, i parada del cicle cel·lular a la fase G2.

ARTICLES

Lamellarin D Bioconjugates I: Synthesis and Cellular Internalization of PEG-Derivatives

Daniel Pla,[†,‡] Andrés Francesch,[§] Pilar Calvo,[§] Carmen Cuevas,[§] Rosa Aligué,[||] Fernando Albericio,*[,⊥,†,‡] and Mercedes Álvarez*[,#,†,‡]

Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, and CIBER-BBN Networking Centre on Bioengineering, Biomaterials, and Nanomedicine, Baldiri Reixac 10, E-08028 Barcelona, Spain, Pharma Mar S. A., Avda de los Reyes 1, E-28770 Colmenar Viejo, Madrid, Spain, and Department of Cell Biology, Faculty of Medicine, University of Barcelona, Casanova 143, E-08036 Barcelona, Spain. Received November 24, 2008; Revised Manuscript Received March 24, 2009

Herein is reported the design and synthesis of poly(ethylene glycol) derivatives of Lamellarin D with the aim of modulating their physicochemical properties and improving the biological activity. Mono-, di-, and tri-PEG conjugates with improved solubility were obtained in 18−57% overall yields from the corresponding partially protected phenolic derivatives of Lamellarin D. Conjugates **1**−**9** were tested in a panel of three human tumor cell lines (MDA-MB-231 breast, A-549 lung, and HT-29 colon) to evaluate their cytotoxicity. Several compounds exhibited enhanced cellular internalization, and more than 85% of the derivatives showed a lower GI_{50} than Lam-D. Furthermore, cell cycle arrest at G2 phase and apoptotic cell-death pathways were determined for Lamellarin D and these derivatives.

INTRODUCTION

Compounds with a good therapeutic profile often do not reach the drug market because they show poor pharmacokinetics, mainly due to poor solubility. Attachment of well-defined, water-soluble, biocompatible, nontoxic, and non-immunogenic polymeric systems to drug candidates can overcome this limitation. Furthermore, these chemical modifications can significantly improve tumor targeting, in addition to therapeutic efficacy, by EPR[1] effect of the drug in tumor tissue. Advances in this field require robust and reliable chemical strategies for the preparation of such derivatives of key pharmacological lead compounds, because very often the polymeric backbone should be incorporated in the chemical structure in the middle of the synthetic strategy.

Lam-D (*1*−*6*) (**1**) is a marine alkaloid with a wide range of biological activities, such as antiproliferative activity against various tumor cell lines in the low nanomolar concentration. Lam-D inhibits the Topo I (*3*), thereby disrupting the potential of mitochondrial transmembrane (*4*), which promotes apoptosis (*7*). The conjugation of water-soluble biocompatible polymers to low molecular weight anticancer drugs has been reported to enhance the solubility of the drugs, enhance their accumulation in tumors, and prolong their retention in the circulatory system, thereby improving the drugs' pharmacodynamics and pharmacokinetics (*8, 9*). Notwithstanding, further controlled drug delivery by means of a readily hydrolyzable conjugation bond is required for the appropriate release of the bioactive drug (*10*). Therefore, modulation of biopolymers that bind drugs is of paramount importance for preclinical research and developments with new lead compounds (*11*). Recently, PEGylated compounds used in cancer therapy have been shown to promote the EPR effect (*12, 13*), raising the local concentration of drug accumulated in targeted solid tumors. It has been proposed that this effect is caused by vascular leakage in the tumor and a reduction in the tumor's lymphatic drainage (*14*).

Our group sought to modulate the physicochemical properties of Lam-D by creating a series of mono-, di-, and tri-PEG ester conjugates of this drug. Herein are described the syntheses using

* Corresponding authors. M.A. and F.A., Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain. Tel.: +34934037086; Fax: +34934037126. E-mail mercedes.alvarez@irbbarcelona.org.

† Barcelona Science Park-University of Barcelona.

‡ CIBER-BBN Networking Centre on Bioengineering, Biomaterials, and Nanomedicine.

§ Pharma Mar S. A.

|| Faculty of Medicine, University of Barcelona.

⊥ Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona.

Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, E-08028 Barcelona.

[1]Abbreviations: AU, absorbance units; A-549, lung carcinoma cell line; BJ, skin fibroblast cell line; Bn, benzyl; Boc, *tert*-butoxycarbonyl; CIQ, cellular internalization quotient; DDQ, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone; DMAP, 4-dimethylaminopyridine; DMEM, Dulbecco's modified Eagle's medium; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DPE-Phos, (oxidi-2,1-phenylene)bis(diphenylphosphine); EDC·HCl, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; EPR, enhance permeability and retention; FACS, fluorescence-activated cell sorting; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; HeLa, human cervix carcinoma cell line; HT-29, colon carcinoma cell line; GI_{50}, 50% growth inhibition; *i*Pr, isopropyl; Lam, Lamellarin; MDA-MB-231, breast adenocarcinoma cell line; MW, microwave; NBS, *N*-bromosuccinimide; PBS, phosphate buffered saline; PEG, poly(ethylene glycol); PEGylated, poly(ethylene glycol)ated; SAR, structure activity relationship; SRB, sulforhodamine B; WGA, wheat germ agglutinin; THF, tetrahydrofurane; Topo, topoisomerase.

10.1021/bc800503k CCC: $40.75
Published on Web 05/27/2009

modified synthetic strategies of these conjugates, as well as their biological activity.

EXPERIMENTAL PROCEDURES

General Procedures and Product Characterization. *A. General Procedures for Cross-Coupling Reactions.* A solution of bromide derivative **14** (1.0 mmol) in DMF (20 mL) was purged with Ar for 10 min, and boronate **12** (1.0 mmol), $Pd(PPh_3)_4$ (10%) and 2 M K_3PO_4 (3.0 mmol) were then added. The reaction mixture was stirred at 115 °C, and then another portion of boronate **12** (2.0 mmol) was added dropwise using a syringe pump during the first hour of reaction. The solvent was removed, and the residue was dissolved in EtOAc. The organic solution was washed with water and brine, dried, and concentrated to give a crude material, which was later purified by column chromatography on silica gel. Elution with hexane/EtOAc (75:25 to 60:40) gave **15a**−**c** (69−77% yield).

B. General Procedure for Oxidation. Synthesis of Compounds **16.** A mixture of **15** (1.0 mmol) and DDQ (1.0 mmol) in dry $CHCl_3$ (15 mL) was purged with Ar in a sealed vessel and heated in a microwave at 120 °C for 10 min. The organic solution was washed with water and brine and then dried ($MgSO_4$), filtered, and evaporated in a vacuum. The crude material was purified by column chromatography on silica gel. Elution with hexane/EtOAc (85:15 to 60:40) gave **16a**−**c** (65−93% yield).

C. General Method for Benzyloxy Removal. Pd/C (10%) was added to a solution of **16** (1.0 mmol) in MeOH (50 mL). The suspension was purged with H_2 and stirred for 16 h. The reaction mixture was filtered through a Celite pad, which was washed with CH_2Cl_2. The solvent was removed under vacuum to provide the compounds **17a**−**c** (yield 70−95%).

D. General Method for Lactonization. NaH (60% dispersion, 1.1 equiv) was washed twice with dry THF and a solution of **17** (1 mmol) was added in THF (60 mL). The mixture was stirred for 3 h at r.t. The solvent was then removed under reduced pressure, and EtOAc was added to the residue. The organic solution was washed with saturated aq. NH_4Cl, water, and brine, then dried and concentrated to provide the compounds **18a**−**c** (86% quant. yield).

E. General Method for Esterification. EDC·HCl (4 mmol × free OH) was added in one solid portion to a solution of DMAP (0.6 equiv in SM) PEG-COOH (4 mmol × free OH), and either Lam-D (*15*) or **18** (1 mmol) in dry CH_2Cl_2 (45 mL). The resulting solution was stirred at r.t. for 3 h. The reaction mixture was diluted with CH_2Cl_2 and washed with sat. aq. $NaHCO_3$ and brine. The organic phase was dried over anhydrous $MgSO_4$, and the solvent was removed under vacuum. The residue was directly purified on silica gel to provide compounds **8**, **9**, **19a**−**f** (40% quant. yield).

F. General Method for Isopropoxy Deprotection. Anhydrous $AlCl_3$ (1.3 equiv) was added to a solution of compound **19** (1 mmol) in dry CH_2Cl_2 (1 mL). The mixture was sonicated for 10 min, quenched with sat. NH_4Cl, and then washed with water and brine. Compounds **19b** and **19d** were treated only with brine, and the aqueous solution was extracted with EtOAc. The organic extracts were dried and evaporated. The crude product was purified by flash chromatography to give compounds **2**−**7** (yield 19−59%).

Methyl 1-(4-Benzyloxy-3-methoxyphenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (13a). A solution of methyl 1-bromo-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (543 mg, 1.4 mmol) in DMF (40 mL) was purged with Ar, and 2-(4-benzyloxy-3-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**11a**) (703 mg, 2.1 mmol), $Pd(PPh_3)_4$ (159 mg, 0.1 mmol), and 2 M K_2CO_3 (2.8 mL, 5.5 mmol) were added. The reaction mixture was stirred at 125 °C for 16 h. The solvent was removed after cooling to r.t., and the residue was dissolved in EtOAc. The organic solution was washed with water and brine, dried ($MgSO_4$), and concentrated to give a crude material, which was later purified by column chromatography on silica gel. Elution with hexane/EtOAc (90:10 to 75:25) gave **13a** (543 mg, 1.38 mmol) as a yellow oil (727 mg, quant.). IR (film) ν 1670, 1464, 1440, 1242. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.37 (d, J = 6.0 Hz, 6H); 2.98 (t, J = 6.4 Hz, 2H); 3.31 (s, 3H, OMe); 3.84 (s, 3H, OMe); 3.85 (s, 3H, OMe); 4.52 (h, J = 6.0 Hz, 1H, OCH); 4.60 (t, J = 6.4 Hz, 2H); 5.19 (s, 2H); 6.73 (s, 1H); 6.86 (s, 1H); 6.89 (s, 1H); 6.93 (dd, J = 8.0, 1.8 Hz, 1H); 6.96−7.00 (m, 2H); 7.31 (d, J = 7.2 Hz, 1H); 7.35 (dd, J = 7.2, 7.2 Hz, 2H); 7.44 (d, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 22.2 (2q); 29.0 (t); 42.6 (t); 51.1 (q); 55.4 (q); 56.1 (q); 71.0 (t); 71.4 (d); 109.1 (d); 113.1 (d); 114.1 (d); 114.7 (d); 119.2 (d); 121.1 (s); 121.47 (s); 121.54 (s); 125.6 (d); 127.1 (2d); 127.8 (d); 128.5 (2d); 129.8 (s); 137.2 (s); 146.5 (s); 146.8 (s); 148.5 (s); 149.5 (s); 161.7 (s). MS (ESI) 528 (M+1, 100); 529 (M+2, 37). HRMS *m/z* calcd for $C_{32}H_{34}NO_6$ 528.2381, found 528.2373.

Methyl 1-(4-Benzyloxy-3-methoxyphenyl)-2-bromo-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (14a). NBS (82 mg, 0.46 mmol) was added in one portion to a solution of **13a** (245 mg, 0.46 mmol) in THF (10 mL). The mixture was stirred at 70 °C under Ar for 90 min. The solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and filtered through a pad of neutral alumina. **14a** was obtained as a yellow oil (278 mg, 99%). IR (film) ν 1693, 1462, 1112 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35 (d, J = 6.0 Hz, 6H); 2.98 (t, J = 6.4 Hz, 2H); 3.20 (s, 3H, OMe); 3.86 (s, 3H, OMe); 3.90 (s, 3H, OMe); 4.51 (h, J = 6.0 Hz, 1H, OCH); 4.59 (t, J = 6.4 Hz, 2H); 5.20 (br, 2H); 6.54 (s, 1H); 6.70 (s, 1H); 6.85−7.00 (m, 3H); 7.28−7.47 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.0 (2q); 28.6 (t); 43.4 (t); 51.1 (q); 55.0 (q); 56.0 (q); 70.7 (t); 71.2 (d); 107.6 (s); 108.9 (d); 113.0 (s); 113.7 (d); 113.9 (s); 114.3 (d); 114.5 (d); 119.1 (s); 122.2 (s); 123.2 (d); 125.3 (s); 126.9 (2d); 127.6 (d); 128.4 (2d); 131.8 (s); 137.0 (s); 146.6 (s); 147.3 (s); 148.3 (s); 149.4 (s); 161.1 (s). MS (ESI) 606 (MBr^{79}+1, 100); 607 (MBr^{79}+2, 23); 608 (MBr^{81} + 1, 95). HRMS *m/z* calcd. for $C_{32}H_{33}NO_6Br$ 606.1486, found 606.1485.

Methyl 2-(2,4-Dibenzyloxy-5-methoxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (15a). Following the general procedure **A** and starting from **14b** (*16*) (674 mg, 1.21 mmol) and 2-(2,4-dibenzyloxy-5-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**12b**), a yellow oil (719 mg, 75%) was obtained. IR (film) ν 1691, 1254, 1212. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.31 (d, J = 6.2 Hz, 6H); 1.35 (d, J = 6.0 Hz, 6H); 3.03 (t, J = 6.4 Hz, 2H); 3.32 (s, 3H, OMe); 3.47 (s, 3H, OMe); 3.53 (s, 3H, OMe); 3.59 (s, 3H, OMe); 4.35−4.65 (m, 4H, 2OCH); 4.74 (s, 2H); 5.01 (s, 2H); 6.46 (s, 1H); 6.55 (s, 1H); 6.61 (br, 1H); 6.69−6.79 (m, 4H); 7.08−7.13 (m, 2H); 7.17−7.26 (m, 4H); 7.27−7.35 (m, 4H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 21.8 (2q); 22.0 (2q); 28.9 (t); 42.8 (t); 50.7 (q); 54.9 (q); 55.5 (q); 56.2 (q); 71.0 (t); 71.2 (d, t); 71.5 (d); 102.8 (d); 109.1 (d); 114.6 (2d); 115.8 (d); 115.9 (d); 118.2 (s); 118.7 (s); 121.1 (s); 121.5 (s); 122.9 (d); 125.4 (s); 126.5 (2d); 127.1 (2d); 127.5 (2d); 128.0 (2d); 128.2 (2d); 128.6 (s); 130.8 (s); 136.8 (s); 137.6 (s); 143.3 (s); 145.4 (s); 146.0 (s); 146.8 (s); 148.2 (s); 149.8 (s); 150.3 (s); 162.2 (s). MS (MALDI-TOF) 797 (M, 100), 798 (M+1, 54).

Methyl 2-(2-Benzyloxy-4-isopropoxy-5-methoxyphenyl)-1-(4-benzyloxy-3-methoxyphenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (15b). Following the general procedure **A** and starting from **14a** (277 mg, 0.46

mmol) and 2-(2-benzyloxy-4-isopropoxy-5-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**12a**), a yellow oil (282 mg, 77%) was obtained. IR (film) ν 1692, 1254, 1212. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.26 (d, J = 6.0 Hz, 6H); 1.36 (d, J = 6.0 Hz, 6H); 3.03 (t, J = 6.0 Hz, 2H); 3.21 (s, 3H, OMe); 3.55 (s, 3H, OMe); 3.57 (s, 6H, 2OMe); 4.37 (h, J = 6.0 Hz, 1H, OCH); 4.52 (h, J = 6.0 Hz, 1H, OCH); 4.63 (br, 2H); 4.82 (s, 2H); 5.12 (s, 2H); 6.46 (s, 1H); 6.55 (s, 1H); 6.66 (s, 1H); 6.72 (br, 4H); 7.16−7.41 (m, 10H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 21.9 (2q); 22.0 (2q); 28.0 and 28.9 (t); 42.2 and 42.8 (t); 50.7 (q); 55.0 (q); 55.5 (q); 56.2 (q); 70.5 (t); 71.1 (d); 71.3 (d); 71.5 (t); 104.5 (d); 109.0 (d); 113.3 (d); 114.3 (d); 114.4 (d); 116.1 (d); 118.2 (s); 118.7 (s); 121.0 (s); 121.4 (s); 122.9 (d); 125.4 (s); 126.5 (2d); 126.8 (2d); 127.1 (d); 127.5 (d); 127.7 (s); 128.0 (2d); 128.2 (2d); 130.8 (s); 137.0 (s); 137.7 (s); 144.0 (s); 145.9 (s); 146.0 (s); 146.1 (s); 148.1 (s); 148.8 (s); 150.2 (s); 162.2 (s). MS (ESI-TOF) 798 (M+1, 100), 799 (M+2, 39), 800 (M+3, 7). HRMS *m/z* calcd. for $C_{49}H_{51}NO_9$ 798.3636, found 798.3621.

Methyl 2-(2,4-Dibenzyloxy-5-methoxyphenyl)-1-(4-benzyloxy-3-methoxyphenyl)-8-isopropoxy-9-methoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-3-carboxylate (15c). Following the general procedure **A** and starting from **14a** (255 mg, 0.42 mmol) and 2-(2,4-dibenzyloxy-5-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**12b**), a reddish oil (246 mg, 69%) was obtained. IR (film) ν 1692, 1438, 1252, 1212. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.38 (d, J = 6.0 Hz, 6H); 3.04 (t, J = 6.0 Hz, 2H); 3.24 (s, 3H, OMe); 3.54 (s, 3H, OMe); 3.56 (s, 3H, OMe); 3.63 (s, 3H, OMe); 4.54 (h, J = 6.0 Hz, 1H, OCH); 4.64 (br, 2H); 4.77 (s, 2H); 5.05 (s, 2H); 5.14 (s, 2H); 6.50 (s, 1H); 6.59 (s, 1H); 6.67 (s, 1H); 6.71−6.77 (m, 4H); 7.11−7.14 (m, 2H); 7.21−7.42 (m, 13H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.0 (2q); 28.8 (t); 42.8 (t); 50.6 (q); 55.0 (q); 55.6 (q); 56.3 (q); 70.5 (t); 71.0 (t); 71.2 (d); 71.5 (t); 102.9 (d); 109.1 (d); 113.5 (d); 114.46 (d); 114.54 (d); 116.0 (d); 118.3 (s); 118.8 (s); 121.0 (s); 121.5 (s); 122.9 (d); 125.4 (s); 126.6 (2d); 126.8 (2d); 127.19 (d); 127.21 (2d); 127.6 (2d); 127.9 (s); 128.0 (2d); 128.3 (2d); 128.3 (2d); 128.6 (s); 130.8 (s); 136.9 (s); 137.1 (s); 137.7 (s); 143.4 (s); 146.1 (s); 146.3 (s); 146.9 (s); 148.3 (s); 149.0 (s); 150.4 (s); 162.2 (s). MS (ESI-TOF) 846 (M+1, 100), 847 (M+2, 49), 848 (M+3, 12). HRMS *m/z* calcd. for $C_{53}H_{52}NO_9$ 846.3636, found 846.3621.

Methyl 2-(2,4-Dibenzyloxy-5-methoxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (16a). Following the general procedure **B** and starting from **15a** (686 mg, 0.86 mmol), a yellow oil (637 mg, 93%) was obtained. IR (film) ν 1682, 1380, 1121 cm^{-1}. ^{1}H NMR ($CDCl_3$, 100 MHz) δ 1.32 (d, J = 6.0 Hz, 6H); 1.37 (d, J = 6.0 Hz, 6H); 3.42 (s, 6H, 2OMe); 3.59 (s, 3H, OMe); 3.63 (s, 3H, OMe); 4.49 (h, J = 6.0 Hz, 1H, OCH); 4.64 (h, J = 6.0 Hz, 1H, OCH); 4.78 (br, 2H); 5.03 (s, 2H); 6.48 (s, 1H); 6.54−6.73 (m, 2H); 6.79−6.94 (m, 3H); 7.03−7.11 (m, 3H); 7.15−7.20 (m, 3H); 7.23−7.34 (m, 6H); 9.31 (d, J = 7.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 21.7 (2q); 22.0 (2q); 50.6 (q); 54.9 (q); 55.5 (q); 56.3 (q); 70.9 (d); 71.1 (t); 71.2 (d); 71.3 (t); 102.5 (d); 105.4 (d); 110.3 (d); 111.7 (d); 112.7 (s); 115.2 (d); 115.6 (d); 115.7 (s); 115.9 (d); 118.0 (s); 118.4 (s); 119.5 (s); 123.2 (d); 123.6 (d); 126.4 (d); 127.1 (d); 127.2 (2d); 127.5 (2d); 128.0 (2d); 128.2 (2d); 129.0 (s); 130.0 (s); 131.6 (s); 136.8 (s); 137.5 (s); 143.1 (s); 145.7 (s); 147.0 (s); 147.2 (s); 149.6 (s); 149.9 (s); 150.3 (s); 162.3 (s). MS (MALDI-TOF) 795 (M, 100), 796 (M+1, 73).

Methyl 2-(2-Benzyloxy-4-isopropoxy-5-methoxyphenyl)-1-(4-benzyloxy-3-methoxyphenyl)-8-isopropoxy-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (16b). Following the general procedure **B** and starting from **15b** (1.06 g, 1.32 mmol), a yellow oil (685 mg, 65%) was obtained. IR (film) ν 1704, 1377, 1214 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 (2d, J = 6.0 Hz, 12H); 3.28 (s, 3H, OMe); 3.57 (br, 3H, OMe); 3.62 (s, 3H, OMe); 3.65 (br, 3H, OMe); 4.39 (h, J = 6.0 Hz, 1H, OCH); 4.65 (h, J = 6.0 Hz, 1H, OCH); 4.86 (br, 2H); 5.14−5.18 (m, 2H); 6.47 (br, 1H); 6.76−6.88 (m, 2H); 6.91 (d, J = 7.6 Hz, 1H); 7.03 (s, 1H); 7.14−7.21 (m, 3H); 7.27−7.49 (m, 10H); 9.31 (d, J = 7.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.9 (2q); 22.0 (2q); 50.7 (q); 55.2 (q); 55.6 (q); 56.4 (q); 70.6 (t); 70.8 (t); 71.0 (d); 71.5 (d); 104.3 (d); 105.5 (d); 110.3 (d); 111.8 (d); 112.8 (s); 113.3 and 113.6 (d); 115.2 (d); 116.2 (d); 118.5 (s); 119.6 (s); 123.4 (d); 123.7 (d); 127.0 (2d); 127.4 (s); 127.75 (2d); 128.2 (2d); 128.5 (2d); 128.6 (d); 128.7 (s); 128.8 (d); 129.2 (s); 130.2 (s); 131.9 (s); 137.2 (s); 137.7 (s); 144.1 (s); 146.6 (s); 147.4 (s); 149.7 (s); 150.4 (s); 162.6 (s). MS (ESI-TOF) 796 (M+1, 100), 797 (M+2, 54). HRMS *m/z* calcd. for $C_{49}H_{50}NO_9$ 796.3480, found 796.3480.

Methyl 2-(2,4-Dibenzyloxy-5-methoxyphenyl)-1-(4-benzyloxy-3-methoxyphenyl)-8-isopropoxy-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (16c). Following the general procedure **B** and starting from **15c** (244 mg, 0.29 mmol), a yellow oil (181 mg, 74%) was obtained. IR (film) ν 1681, 1374, 1212 cm^{-1}. ^{1}H NMR ($CDCl_3$, 100 MHz) δ 1.43 (d, J = 6.0 Hz, 6H); 3.30 (s, 3H, OMe); 3.52−3.65 (m, 9H, 3 OMe); 4.60−4.90 (m, 3H); 5.07 (s, 2H); 5.18−5.21 (m, 2H); 6.50 (s, 1H); 6.59 (br, 1H); 6.74 (br, 1H); 6.82−6.89 (m, 2H); 6.94 (d, J = 7.6 Hz, 1H); 7.06 (s, 1H); 7.09−7.15 (m, 2H); 7.20−7.23 (m, 4H); 7.30−7.46 (m, 10H); 9.33 (d, J = 7.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 21.9 (2q); 50.7 (q); 55.1 (q); 55.7 (q); 56.4 (q); 70.6 (t); 70.9 (d); 71.2 (t); 71.4 (t); 102.5 (d); 105.4 (d); 110.2 (d); 111.8 (d); 112.8 (s); 113.3 (d); 115.1 (d); 116.0 (d); 118.1 (s); 118.4 (s); 119.5 (s); 123.3 (d); 123.6 (d); 126.5 (d); 126.9 (2d); 127.2 (2d); 127.3 (2d); 127.7 (2d); 128.1 (2d); 128.3 (2d); 128.4 (2d); 129.0 (s); 130.1 (s); 131.7 (s); 136.9 (s); 137.1 (s); 137.5 (s); 143.2 (s); 146.5 (s); 147.1 (s); 147.3 (s); 149.0 (s); 149.6 (s); 150.4 (s); 162.4 (s). MS (MALDI-TOF) 844 (M+1, 100), 845 (M+1, 52), 846 (M+3, 8). HRMS *m/z* calcd. for $C_{53}H_{50}NO_9$ 844.3480, found 844.3467.

Methyl 2-(2,4-Dihydroxy-5-methoxyphenyl)-8-isopropoxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (17a). Following the general procedure **C** and starting from **16a** (637 mg, 0.80 mmol), a brown oil (433 mg, 88%) was obtained. IR (film) ν 1685, 1381, 1223 cm^{-1}. ^{1}H NMR ($CDCl_3$, 100 MHz) δ 1.32 and 1.35 (d, J = 6.0 Hz, 6H); 1.42 (d, J = 6.0 Hz, 6H); 3.43 (s, 3H, OMe); 3.54 (br, 3H, OMe); 3.60 (br, 3H, OMe); 3.75 (s, 3H, OMe); 4.50 (h, J = 6.0 Hz, 1H, OCH); 4.67 (h, J = 6.0 Hz, 1H, OCH); 6.33 and 6.45 (2br, 1H); 6.57 (s, 1H); 6.82−7.00 (m, 3H); 7.05 (s, 1H); 7.17 and 7.34 (2br, 1H); 7.26 (s, 1H); 9.23 (d, J = 7.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 21.6 (2q); 21.8 (2q); 51.0 (q); 54.8 (q); 55.5 (q); 56.2 (q); 70.8 (d); 71.1 (d); 102.5 (d); 105.3 (d); 110.1 (d); 112.1 (d); 112.4 (s); 112.8 (s); 114.1 (s); 115.0 (d); 115.6 (d); 119.0 (s); 119.1 (d); 122.9 (s); 123.2 (d); 123.4 (d); 128.4 (s); 130.2 (s); 130.6 (s); 139.8 (s); 145.5 (s); 145.7 (s); 147.3 (s); 148.2 (s); 149.5 (s); 162.1 (s); 129.0 (s). MS (MALDI-TOF) 615 (M, 100), 616 (M+1, 27).

Methyl 2-(2-Hydroxy-4-isopropoxy-5-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-8-isopropoxy-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (17b). Following the general procedure **C** and starting from **16b** (680 mg, 0.85 mmol), a brown oil (365 mg, 70%) was obtained. IR (film) ν 3440, 1684, 1381, 1218, 1121 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.32 (d, J = 6.0 Hz, 6H); 1.38 (d, J = 6.0 Hz, 6H); 3.41 (s, 3H, OMe); 3.48 and 3.55 (2s, 3H, OMe); 3.57 and 3.68 (2br, 3H, OMe); 3.72 (s, 3H, OMe); 4.50 (h, J = 6.0 Hz, 1H, OCH); 4.64 (h, J = 6.0 Hz, 1H, OCH); 5.82 (br, 1H, OH); 6.34 and 6.40 (2br, 1H); 6.52 (br, 1H); 6.73 (d, J = 7.2 Hz, 1H); 6.84−6.95 (m, 2H); 7.00 (s, 1H); 7.06 and 7.15 (2d, J = 2.4 Hz, 1H); 7.26 (br, 1H); 9.13 (d, J = 7.2 Hz, 1H). ^{13}C

NMR ($CDCl_3$, 50.3 MHz) δ 21.8 (2q); 22.0 (2q); 51.3 (q); 55.2 (q); 55.8 (q); 56.3 (q); 70.9 (d); 71.0 (d); 103.5 (d); 105.5 (d); 110.2 (d); 112.4 (d); 112.5 (s); 113.8 (s); 113.9 (d); 115.2 and 115.4 (d); 119.3 (s); 123.1 (d); 123.4 (d); 124.4 (s); 124.5 (d); 124.6 (s); 127.0 (s); 131.0 (s); 144.7 (s); 146.4 (s); 147.2 (s); 147.7 (s); 147.9 (s); 148.0 (s); 149.8 (s); 162.3 (s). MS (MALDI-TOF) 615 (M, 100), 616 (M+1, 16).

Methyl 2-(2,4-Dihydroxy-5-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-8-isopropoxy-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate (17c). Following the general procedure **C** and starting from **16c** (122 mg, 0.14 mmol), a yellow oil (80 mg, 95%) was obtained. IR (film) ν 3425, 1683, 1219, 1122 cm^{-1}. ^1H NMR (acetone-d_6, 400 MHz) δ 1.33 (d, J = 6.0 Hz, 6H); 3.38 (s, 3H, OMe); 3.58 (s, 3H, OMe); 3.62 (s, 3H, OMe); 3.73 (br, 3H, OMe); 4.70 (h, J = 6.0 Hz, 1H, OCH); 5.60 (s, 1H, OH); 6.37 (s, 1H); 6.57 (br, 1H); 6.82−6.88 (m, 2H); 7.07 (d, J = 7.5 Hz, 1H); 7.21 (br, 1H); 7.26 (s, 1H); 7.56 (s, 1H); 9.26 (d, J = 7.5 Hz, 1H). ^{13}C NMR (acetone-d_6, 50.3 MHz) δ 21.9 (2q); 51.3 (q); 55.3 (q); 55.9 (q); 56.4 (q); 71.0 (d); 102.5 (d); 105.5 (d); 110.2 (d); 112.5 (d); 112.6 (s); 113.3 (s); 113.9 (d); 114.0 (d); 114.3 (d); 114.4 (s); 119.3 (s); 123.2 (d); 123.5 (s); 124.4 (d); 127.2 (s); 130.1 (s); 131.1 (s); 140.0 (s); 144.6 (s); 145.7 (s); 146.4 (s); 147.7 (s); 148.4 (s); 149.9 (s); 162.2 (s). MS (ESI-TOF) 574 (M+1, 100), 575 (M+2, 23). HRMS m/z calcd. for $C_{32}H_{32}NO_9$ 574.2072, found 574.2055.

4′,11-Diisopropyl-Lam-D (18a). Following the general procedure **D** and starting from **17a** (433 mg, 0.70 mmol), a white solid (410 mg, quant.) was obtained. mp (MeCN) 218−220 °C. IR (film) ν 3359, 1699, 1432, 1258, 1157 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 1.44 (d, J = 6.0 Hz, 12H); 3.44 (s, 3H, OMe); 3.51 (s, 3H, OMe); 3.86 (s, 3H, OMe); 4.57−4.77 (m, 2H, 2OCH); 6.72 (s, 1H); 6.98 (br, 1H); 7.02 (s, 1H); 7.09 (s, 1H); 7.15 (s, 1H); 7.16−7.18 (m, 3H); 9.19 (d, J = 7.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 21.9 (4q); 55.1 (q); 55.5 (q); 56.2 (q); 71.2 (d); 71.8 (d); 103.5 (d); 104.6 (d); 105.6 (d); 107.7 (s); 109.8 (s); 110.3 (d); 110.8 (s); 112.2 (d); 115.0 (d); 116.9 (d); 118.9 (s); 123.1 (d); 123.9 (d); 124.7 (s); 128.8 (s); 129.3 (s); 134.3 (s); 143.2 (s); 146.2 (s); 146.9 (s); 147.1 (s); 148.4 (s); 150.0 (s); 151.3 (s); 155.4 (s). MS (MALDI-TOF) 583 (M, 100), 584 (M+1, 62).

3,11-Diisopropyl-Lam-D (18b). Following the general procedure **D** and starting from **17b** (365 mg, 0.59 mmol), a yellow solid (346 mg, quant. yield) was obtained. mp (MeCN) 130−132 °C. IR (film) 3435, 1704, 1430, 1223 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 1.35 (d, J = 6.0 Hz, 6H); 1.39 (d, J = 6.0 Hz, 6H); 3.42 (s, 3H, OMe); 3.44 (s, 3H, OMe); 3.85 (s, 3H, OMe); 4.49 (h, J = 6.0 Hz, 1H); 4.65 (h, J = 6.0 Hz, 1H); 6.73 (s, 1H); 6.87 (s, 1H); 6.94 (d, J = 7.2 Hz, 1H); 7.04 (s, 1H); 7.09 (s, 1H); 7.13−7.16 (m, 3H); 9.11 (d, J = 7.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 21.7 (2q); 21.8 (2q); 55.1 (q); 55.4 (q); 56.1 (q); 71.0 (d); 71.3 (d); 103.2 (d); 105.4 (d); 105.5 (d); 107.5 (s); 109.7 (s); 110.2 (d); 110.9 (s); 112.1 (d); 113.9 (d); 115.2 (d); 118.8 (s); 122.8 (d); 124.5 (d); 127.1 (s); 129.2 (s); 134.3 (s); 145.7 (s); 146.3 (s); 147.3 (s); 147.6 (s); 148.2 (s); 149.9 (s); 155.3 (s). MS (MALDI-TOF) 583 (M, 100), 584 (M+1, 77).

11-Isopropyl-Lam-D (18c). Following the general procedure **D** and starting from **17c** (80 mg, 0.14 mmol), a yellow solid (65 mg, 86%) was obtained. IR (film) ν 3312, 1710, 1432, 1264 cm^{-1}. ^1H NMR (acetone-d_6, 400 MHz) δ 1.31 (d, J = 6.0 Hz, 6H); 3.35 (s, 3H, OMe); 3.39 (s, 3H, OMe); 3.77 (s, 3H, OMe); 4.75 (h, J = 6.0 Hz, 1H); 6.73 (s, 1H); 6.87 (s, 1H); 7.01 (dd, J = 8.0, 1.7 Hz, 1H); 7.10 (d, J = 8.0 Hz, 1H); 7.145 (s, 1H); 7.153 (d, J = 1.7 Hz, 1H); 7.29 (d, J = 7.4 Hz, 1H); 7.44 (s, 1H); 9.05 (d, J = 7.4 Hz, 1H); 9.33 (s, 1H, OH); 9.83 (s, 1H, OH). ^{13}C NMR (acetone-d_6, 100 MHz) δ 21.4 (2q); 54.1 (q); 54.8 (q); 55.7 (q); 69.9 (d); 103.4 (d); 104.9 (s); 105.4 (d); 106.2 (d); 108.0 (s); 110.1 (d); 110.8 (d); 112.4 (s); 114.7 (d); 116.1 (s); 118.0 (d); 121.8 (s); 123.5 (d); 124.1 (d); 125.0 (s); 128.7 (s); 133.5 (s); 144.3 (s); 146.0 (s); 146.5 (s); 147.7 (s); 148.4 (s); 149.4 (s); 154.1 (s). MS (ESI-TOF) 542 (M+1, 100), 543 (M+2, 26). HRMS m/z calcd. for $C_{31}H_{28}NO_8$ 542.1809, found 542.1793.

4′,11-Diisopropyl-3-(2-(2-(2-methoxyethoxy)ethoxy)acetyl)-Lam-D (19a). Following the general procedure **E** and starting from **18a** (190 mg, 0.33 mmol), a yellow oil (204 mg, 84%) was obtained. IR (film) ν 1781, 1707, 1485, 1138 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.39 (d, J = 6.0 Hz, 12H); 3.35 (s, 3H, OMe); 3.39 (s, 3H, OMe); 3.40 (s, 3H, OMe); 3.52−3.54 (m, 2H); 3.62−3.65 (m, 2H); 3.68−3.71 (m, 2H); 3.78−3.81 (m, 2H); 3.83 (s, OMe); 4.40 (s, 2H); 4.56−4.67 (m, 2H); 6.820 (s, 1H); 6.93 (d, J = 7.2 Hz, 1H); 7.03 (s, 1H); 7.05 (s, 1H); 7.0−7.14 (m, 4H); 9.07 (d, J = 7.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.69 (q); 21.73 (q); 55.0 (q); 55.3 (q); 56.1 (q); 58.8 (q); 68.1 (t); 70.4 (t); 70.5 (t); 70.8 (t); 71.0 (d); 71.65 (d); 71.72 (t); 105.4 (d); 106.2 (d); 108.0 (s); 110.3 (d); 111.5 (s); 111.7 (d); 112.7 (d); 114.8 (d); 116.1 (s); 116.8 (d); 118.8 (s); 122.7 (d); 123.6 (d); 124.5 (s); 128.0 (s); 128.2 (s); 134.2 (s); 138.8 (s); 145.3 (s); 147.1 (s); 147.1 (s); 148.4 (s); 150.1 (s); 151.3 (s); 154.7 (s); 168.2 (s). MS (MALDI-TOF) 743 (M, 100), 744 (M+1, 90), 745 (M+2, 29).

3-[3-(2-(2-(2-(2-(2-(2-*tert*-Butoxycarbonylaminoethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propanoyl]-4′,11-diisopropyl-Lam-D (19b). Following the general procedure **E** and starting from **18a** (154 mg, 0.26 mmol), a brown oil (205 mg, 76%) was obtained. IR (film) ν 3355, 1709, 1256, 1111 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.42 (d, J = 6.0 Hz, 6H); 1.43−1.45 (m, 15H, O*i*Pr, *t*BuOC); 2.88 (t, J = 6.5 Hz, 2H); 3.31 (br, 4H, $2CH_2$); 3.40 (s, 3H, OMe); 3.43 (s, 3H, OMe); 3.51−3.54 (m, 4H, $2CH_2$); 3.61−3.66 (m, 16H); 3.75−3.80 (m, 2H, CH_2); 3.88 (s, 3H, OMe); 4.63 (h, J = 6.0 Hz, 1H); 4.70 (h, J = 6.0 Hz, 1H); 5.16 (br, 1H); 6.86 (s, 1H); 7.06 (d, J = 7.6 Hz, 1H); 7.10 (s, 1H); 7.12 (s, 1H); 7.15−7.19 (m, 4H); 9.23 (d, J = 7.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.66 (q); 21.69 (q); 28.2 (q); 34.5 (t); 40.1 (t); 54.9 (q); 55.3 (q); 56.0 (q); 66.2 (t); 69.9 (2t); 70.0 (2t); 70.2 (2t); 70.3 (7t); 71.0 (d); 71.6 (d); 105.4 (d); 106.2 (d); 110.3 (d); 111.4 (s); 111.5 (s); 111.8 (d); 111.9 (s); 112.7 (d); 114.7 (d); 115.9 (s); 116.8 (d); 118.8 (s); 122.7 (s); 122.8 (d); 123.6 (d); 124.5 (s); 128.2 (s); 139.3 (s); 147.1 (s); 147.2 (s); 148.4 (s); 150.1 (s); 151.3 (s); 154.8 (s); 156.0 (s); 169.2 (s); 174.7 (s). MS (MALDI-TOF) 919 ([M-Boc], 100), 920 ([M-Boc]+1, 55).

3,11-Diisopropyl-4′-(2-(2-(2-methoxyethoxy)ethoxy)acetyl)-Lam-D (19c). Following the general procedure **E** and starting from **18b** (104 mg, 0.18 mmol), a yellow oil (127 mg, 96%) was obtained. IR (film) ν 1781, 1704, 1113 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.31 (d, J = 6.0 Hz, 6H); 1.35 (d, J = 6.0 Hz, 6H); 3.32 (s, 3H, OMe); 3.40 (2s, 6H, 2OMe); 3.45−3.52 (m, 2H); 3.55−3.63 (m, 2H); 3.65−3.71 (m, 2H); 3.74 (s, 3H, OMe); 3.78−3.82 (m, 2H); 4.40−4.49 (m, 3H, OCH, CH_2); 4.61 (h, J = 6.0 Hz, 1H); 6.62 (s, 1H); 6.85 (s, 1H); 6.93 (d, J = 7.2 Hz, 1H); 7.00−7.03 (m, 2H); 7.16−7.19 (m, 2H); 7.22 (d, J = 8.0 Hz, 1H); 9.11 (d, J = 7.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.67 (q); 21.73 (q); 55.4 (q); 55.6 (q); 56.1 (q); 58.9 (q); 68.5 (t); 70.4 (t); 70.5 (t); 70.9 (t); 71.1 (d); 71.3 (d); 71.8 (t); 103.3 (d); 105.1 (d); 105.3 (d); 107.7 (s); 109.5 (s); 110.1 (s); 110.4 (d); 112.3 (d); 115.4 (d); 118.6 (s); 122.8 (d); 123.5 (d); 123.9 (d); 124.5 (s); 129.1 (s); 134.1 (s); 135.0 (s); 139.3 (s); 146.3 (s); 146.6 (s); 147.8 (s); 148.4 (s); 150.3 (s); 151.8 (s); 155.3 (s); 168.2 (s); 170.7 (s). MS (MALDI-TOF) 743 (M, 75), 744 (M+1, 100), 745 (M+2, 45).

4′-[3-(2-(2-(2-(2-(2-*tert*-Butoxycarbonylaminoethoxy)ethoxy)ethoxy)ethoxy)propanoyl]-3,11-diisopropyl-

Lam-D (19d). Following the general procedure **E** and starting from **18b** (82 mg, 0.14 mmol), a brown oil (143 mg, quant. yield) was obtained. IR (film) ν 3362, 1764, 1707, 1268, 1113 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.41 (d, $J = 6.0$ Hz, 6H); 1.42−1.44 (m, 15H, O*i*Pr, *t*BuOC); 2.94 (t, $J = 6.5$ Hz, 2H); 3.29−3.32 (m, 4H, $2CH_2$); 3.48 (s, 6H, 2OMe); 3.61−3.71 (m, 20H, $10CH_2$); 3.81 (s, 3H, OMe); 3.94 (t, $J = 6.6$ Hz, 2H, CH_2); 4.58 (h, $J = 6.0$ Hz, 1H); 4.70 (h, $J = 6.2$ Hz, 1H); 5.09 (br, 1H); 6.72 (s, 1H); 6.98 (s, 1H); 7.05 (d, $J = 7.4$ Hz, 1H); 7.11 (s, 1H); 7.13 (s, 1H); 7.21 (d, $J = 1.6$ Hz, 1H); 7.24 (dd, $J = 8.0$, 1.6 Hz, 1H); 7.30 (d, $J = 8.0$ Hz, 1H); 9.24 (d, $J = 7.4$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.6 (2q); 21.7 (2q); 28.3 (q); 34.6 (t); 40.2 (t); 55.4 (q); 55.5 (q); 56.1 (q); 66.3 (t); 70.0 (t); 70.1 (t); 70.2 (t); 70.3 (t); 70.4 (5t); 71.0 (t); 71.3 (t); 103.3 (d); 105.1 (d); 105.3 (d); 107.7 (s); 109.5 (s); 110.2 (s); 110.3 (d); 112.3 (d); 115.3 (d); 118.6 (s); 122.8 (d); 123.7 (d); 123.8 (d); 124.4 (s); 129.1 (s); 134.1 (s); 134.7 (s); 139.8 (s); 146.3 (s); 146.5 (s); 147.7 (s); 148.4 (s); 150.3 (s); 152.0 (s); 155.3 (s); 155.9 (s); 169.2 (s). MS (MALDI-TOF) 919 ([M-Boc], 100), 1018 (M, 35).

11-Isopropyl-3,4′-*bis*(2-(2-methoxyethoxy)acetyl)-Lam-D (19e). Following the general procedure **E** and starting from **18c** (32 mg, 0.06 mmol), a yellow solid (32 mg, 71%) was obtained. mp (MeCN) 173−175 °C. IR (film) ν 1781, 1707, 1274, 1116 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 1.44 (d, $J = 6.0$ Hz, 6H); 3.41 (s, 3H, OMe); 3.45 (s, 3H, OMe); 3.46 (s, 3H, OMe); 3.49 (s, 3H, OMe); 3.61−3.70 (m, 4H, $2CH_2$); 3.76−3.90 (m, 7H, OMe, $2CH_2$); 4.45 (s, 2H); 4.53 (s, 2H); 4.71 (h, $J = 6.0$ Hz, 1H); 6.81 (s, 1H); 7.04 (d, $J = 7.4$ Hz, 1H); 7.11 (s, 1H); 7.12 (s, 1H); 7.14 (s, 1H); 7.23−7.28 (m, 2H); 7.33 (d, $J = 8.0$ Hz, 1H); 9.19 (d, $J = 7.4$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 21.9 (2q); 55.6 (q); 55.8 (q); 56.2 (q); 59.0 (2q); 68.3 (t); 68.4 (t); 70.8 (t); 70.9 (t); 71.2 (d); 71.9 (2t); 105.3 (d); 106.1 (d); 108.3 (s); 110.3 (d); 110.8 (s); 111.9 (d); 112.9 (d); 115.3 (d); 115.9 (s); 118.7 (s); 122.8 (d); 123.7 (d); 123.8 (d); 124.5 (s); 128.1 (s); 134.1 (s); 134.8 (s); 138.9 (s); 139.5 (s); 145.3 (s); 147.4 (s); 148.6 (s); 150.4 (s); 152.0 (s); 154.8 (s); 168.2 (s). MS (ESI-TOF) 774 (M+1, 100), 775 (M+2, 31), 776 (M+3, 7). HRMS *m/z* calcd. for $C_{41}H_{44}NO_{14}$ 774.2756, found 774.2744.

11-Isopropyl-3,4′-*bis*(2-(2-(2-methoxyethoxy)ethoxy)acetyl)-Lam-D (19f). Following the general procedure **E** and starting from **18c** (32 mg, 0.06 mmol), a yellow solid (50 mg, 97%) was obtained. mp (MeCN) 125−127 °C. IR (film) ν 1708, 1464, 1275, 1114 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.44 (d, $J = 6.0$ Hz, 6H); 3.39 (s, 3H, OMe); 3.42 (s, 3H, OMe); 3.46 (s, 3H, OMe); 3.49 (s, 3H, OMe); 3.54−3.63 (m, 4H, $2CH_2$); 3.65−3.92 (m, 15H, OMe, $6CH_2$); 4.46 (s, 2H); 4.53 (s, 2H); 4.71 (h, $J = 6.0$ Hz, 1H); 6.82 (s, 1H); 7.06 (d, $J = 7.4$ Hz, 1H); 7.12 (s, 2H); 7.16 (s, 1H); 7.23−7.35 (m, 3H); 9.21 (d, $J = 7.4$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 21.9 (2q); 55.6 (q); 55.8 (q); 56.2 (q); 59.0 (2q); 68.3 (t); 68.4 (t); 70.5 (2t); 70.6 (t); 70.7 (t); 71.0 (2t); 71.2 (d); 71.9 (2t); 105.3 (d); 106.1 (d); 108.3 (s); 110.4 (d); 110.8 (s); 112.0 (d); 113.0 (d); 115.3 (d); 115.9 (s); 118.7 (s); 122.8 (d); 123.7 (2d); 124.5 (s); 124.6 (s); 128.1 (s); 134.2 (s); 134.8 (s); 139.0 (s); 139.5 (s); 145.4 (s); 147.4 (s); 148.6 (s); 150.5 (s); 152.0 (s); 154.9 (s); 168.2 (s). MS (ESI-TOF) 862 (M+1, 83), 863 (M+2, 20). HRMS *m/z* calcd. for $C_{45}H_{52}NO_{16}$ 862.3281, found 862.3266.

3,4′,11-*tris*(2-(2-Methoxyethoxy)acetyl)-Lam-D (8). Following the general procedure **E** and starting from Lam-D (*15*) (38 mg, 0.08 mmol), elution with hexane/THF (60:40 to 40:60) gave a white solid (26 mg, 40%). mp (MeCN) 148−149 °C. IR (film) ν 1779, 1707, 1117 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.405 (s, 3H, OMe); 3.410 (s, 3H, OMe); 3.44 (s, 3H, OMe); 3.45 (s, 3H, OMe); 3.46 (s, 3H, OMe); 3.61−3.64 (m, 4H, $2CH_2$); 3.66−3.68 (m, 2H, CH_2); 3.80−3.88 (m, 9H, OMe, $3CH_2$); 4.45 (s, 2H); 4.48 (s, 2H); 4.52 (s, 2H); 6.80 (s, 1H); 7.05 (d, $J =$ 7.6 Hz, 1H); 7.16 (s, 1H); 7.21 (s, 1H); 7.24 (d, $J = 1.6$ Hz, 1H); 7.25−7.28 (m, 1H); 7.33 (d, $J = 8.0$ Hz, 1H); 7.42 (s, 1H); 9.22 (d, $J = 7.6$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.7 (q); 55.8 (q); 56.3 (q); 58.99 (q); 59.02 (q); 59.03 (q) 68.32 (t); 68.34 (t); 68.4 (t); 70.9 (2t); 71.0 (t); 71.9 (2t); 72.0 (t); 106.1 (d); 106.4 (d); 109.1 (s); 112.1 (d); 112.3 (s); 112.8 (d); 115.1 (d); 115.8 (s); 120.7 (d); 123.2 (s); 123.6 (s); 123.8 (2d); 124.0 (d); 128.1 (s); 133.4 (s); 134.5 (s); 139.2 (s); 139.7 (s); 140.4 (s); 145.4 (s); 147.6 (s); 150.8 (s); 152.3 (s); 154.9 (s); 168.3 (s); 168.4 (s). MS (ESI-TOF) 848 (M+1, 100), 849 (M+2, 35). HRMS *m/z* calcd. for $C_{43}H_{46}NO_{17}$ 848.2760, found 848.2751.

3,4′,11-*tris*(2-(2-(2-Methoxyethoxy)ethoxy)acetyl)-Lam-D (9). Following the general procedure **E** and starting from Lam-D (*15*) (37 mg, 0.08 mmol), elution with hexane/THF (60:40 to 40:60) gave a pink solid (34 mg, 46%). mp (MeCN) 137−139 °C. IR (film) ν 1779, 1707, 1117 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.39 (s, 6H, 2OMe); 3.41 (s, 3H, OMe); 3.447 (s, 3H, OMe); 3.451 (s, 3H, OMe); 3.54−3.61 (m, 6H, $3CH_2$); 3.66−3.79 (m, 12H, $6CH_2$); 3.82−3.89 (m, 9H, OMe, $3CH_2$); 4.45 (s, 2H); 4.48 (s, 2H); 4.52 (s, 2H); 6.80 (s, 1H); 7.04 (d, $J = 7.6$ Hz, 1H); 7.15 (s, 1H); 7.21 (s, 1H); 7.24−7.27 (m, 2H); 7.32 (d, $J = 8.0$ Hz, 1H); 7.42 (s, 1H); 9.21 (d, $J = 7.6$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.7 (q); 55.8 (q); 56.3 (q); 59.0 (3q); 68.29 (t); 68.33 (t); 68.4 (t); 70.53 (2t); 70.60 (t); 70.62 (2t); 70.67 (t); 71.00 (t); 71.01 (t); 71.04 (t); 71.88 (2t); 71.90 (t); 106.1 (d); 106.4 (d); 109.1 (s); 112.1 (d); 112.3 (s); 112.8 (d); 115.1 (d); 115.8 (s); 120.6 (d); 123.1 (s); 123.6 (d); 123.7 (s); 123.8 (d); 124.0 (d); 128.1 (s); 133.4 (s); 134.5 (s); 139.2 (s); 139.7 (s); 140.4 (s); 145.4 (s); 147.6 (s); 150.8 (s); 152.3 (s); 154.9 (s); 168.3 (s); 168.5 (s). MS (ESI-TOF) 980 (M+1, 100), 981 (M+2, 62). HRMS *m/z* calcd. for $C_{49}H_{58}NO_{20}$ 980.3547, found 980.3529.

3-(2-(2-(2-Methoxyethoxy)ethoxy)acetyl)-Lam-D (2). Following the general procedure **F** and starting from **19a** (187 mg, 0.26 mmol), elution with hexane/THF (60:40 to 40:60) gave a yellow solid (73 mg, 44%). mp (MeCN) 163−164 °C. IR (film) ν 3284, 1786, 1684, 1424 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.38 (s, 3H, OMe); 3.42 (s, 3H, OMe); 3.50 (s, 3H, OMe); 3.54−3.58 (m, 2H); 3.64−3.69 (m, 2H); 3.70−3.75 (m, 2H); 3.80−3.84 (m, 2H); 3.91 (s, OMe); 4.43 (s, 2H); 6.04 (br, 1H, OH); 6.28 (br, 1H, OH); 6.82 (s, 1H); 6.88 (d, $J = 7.2$ Hz, 1H); 7.04 (s, 1H); 7.09 (s, 1H); 7.10−7.11 (m, 1H); 7.13 (s, 2H); 7.18 (d, $J = 7.6$ Hz, 1H); 9.02 (d, $J = 7.2$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.3 (q); 55.5 (q); 56.3 (q); 59.0 (q); 68.2 (t); 70.4 (t); 70.6 (t); 70.9 (t); 71.8 (t); 104.9 (d); 106.3 (d); 108.0 (s); 110.9 (d); 111.5 (s); 111.7 (d); 112.7 (d); 113.8 (d); 115.3 (d); 116.1 (s); 118.6 (s); 122.8 (d); 124.5 (d); 125.1 (s); 127.0 (s); 128.1 (s); 134.5 (s); 138.8 (s); 145.3 (s); 145.8 (s); 146.9 (s); 147.1 (s); 147.2 (s); 147.5 (s); 154.8 (s); 168.4 (s). MS (ESI-TOF) 660 (M+1, 100), 682 (M+Na, 80). HRMS *m/z* calcd. for $C_{35}H_{34}NO_{12}$ 660.2075, found 660.2062.

3-[3-(2-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propanoyl]-Lam-D (3). Following the general procedure **F** and starting from **19b** (140 mg, 0.14 mmol), elution with CH_2Cl_2/MeOH (95:5 to 90:10) gave a yellow oil (68 mg, 59%). IR (film) ν 3127, 1702, 1420, 1278 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 2.60 and 2.86 (2t, $J = 6.4$ Hz, 2H); 3.20 (br, 2H); 3.43 (s, 3H, OMe); 3.50 (s, 3H, OMe); 3.63−3.70 (m, 20H); 3.83−3.89 (m, 4H); 3.90 (s, 3H, OMe) 6.82 (s, 1H); 6.93 (d, $J = 7.5$ Hz, 1H); 7.01 (s, 1H); 7.10 (dd, $J = 8.0$, 1.6 Hz, 1H); 7.12 (2s, 2H); 7.22 (s, 1H); 7.24 (d, $J = 8.0$ Hz, 1H); 7.92 (br, 4H, NH_2, 2 OH); 9.10 (d, $J = 7.5$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 34.6 (t); 40.2 (t); 55.2 (q); 55.5 (q); 56.2 (q); 66.2 (t); 66.6 (t); 69.7 (t); 69.8 (t); 69.9 (t); 70.0 (2t); 70.1 (t); 70.2 (2t); 70.3 (t); 70.4 (t); 104.9 (d); 106.3 (d); 107.8 (s); 111.0 (d); 111.5 (s); 111.7 (d); 112.7 (d); 113.9 (d); 115.6 (d); 115.9 (s); 118.5 (s); 122.7 (d); 124.3 (d); 125.1 (s); 126.7 (s);

128.1 (s); 134.5 (s); 139.2 (s); 145.2 (s); 146.0 (s); 147.2 (s); 147.3 (s); 147.4 (s); 147.7 (s); 154.8 (s); 169.5 (s). MS (ESI-TOF) 835 (M+1, 100); 836 (M+2, 40), 837 (M+3, 8). HRMS *m*/*z* calcd. for $C_{43}H_{51}N_2O_{15}$ 835.3283, found 835.3277.

4′-(2-(2-(2-Methoxyethoxy)ethoxy)acetyl)-Lam-D (4). Following the general procedure **F** and starting from **19c** (111 mg, 0.15 mmol), elution with hexane/THF (60:40 to 40:60) gave a yellow solid (55 mg, 55%). mp (MeCN) 201−203 °C. IR (film) ν 3455, 1679, 1426 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.41 (s, 3H, OMe); 3.54 (s, 3H, OMe); 3.55 (s, 3H, OMe); 3.58−3.62 (m, 2H); 3.68−3.75 (m, 2H); 3.76−3.81 (m, 2H); 3.83 (s, OMe); 3.86−3.91 (m, 2H); 4.53 (s, 2H); 5.88 (br, 1H, OH); 5.98 (br, 1H, OH); 6.66 (s, 1H); 6.93−7.10 (m, 2H); 7.07 (s, 1H); 7.18 (s, 1H); 7.23−7.28 (m, 2H); 7.32 (d, J = 8.0 Hz, 1H); 9.17 (d, J = 7.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.8 (q); 55.9 (q); 56.3 (q); 59.1 (q); 68.5 (t); 70.6 (t); 70.7 (t); 71.1 (t); 71.9 (t); 103.6 (d); 104.4 (d); 104.7 (d); 109.6 (s); 110.0 (s); 110.8 (d); 115.5 (d); 118.6 (s); 123.3 (s); 123.7 (d); 124.0 (d); 125.5 (d); 125.6 (d); 128.2 (s); 129.3 (s); 134.3 (s); 135.4 (s); 135.8 (s); 139.5 (s); 143.5 (s); 146.4 (s); 146.9 (s); 147.0 (s); 147.2 (s); 152.1 (s); 168.5 (s). MS (ESI-TOF) 660 (M+1, 100), 682 (M+Na, 33). HRMS *m*/*z* calcd. for $C_{35}H_{34}NO_{12}$ 660.2075, found 660.2065.

4′-[3-(2-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propanoyl]-Lam-D (5). Following the general procedure **F** and starting from **19d** (130 mg, 0.13 mmol), elution with CH_2Cl_2/MeOH (95:15 to 85:15) gave a brown oil (61 mg, 57%). IR (film) ν 3133, 1700, 1425, 1277 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 2.60 and 2.95 (2t, J = 6.4 Hz, 2H); 3.16−3.24 (m, 2H); 3.43 and 3.47 (s, 3H, OMe); 3.52 (s, 3H, OMe); 3.62−3.77 (m, 20H); 3.83 (s, 3H, OMe); 3.89−3.96 (m, 4H); 6.65 (s, 1H); 6.95 (d, J = 7.4 Hz, 1H); 6.99 (s, 1H); 7.06 (s, 1H); 7.19−7.24 (m, 2H); 7.25 (d, J = 1.6 Hz, 1H); 7.26−7.31 (m, 1H); 9.10 (d, J = 7.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 34.7 (t); 40.2 (t); 55.6 (q); 55.8 (q); 56.3 (q); 66.4 (t); 66.7 (t); 69.7 (t); 69.8 (t); 69.9 (2t); 70.0 (2t); 70.1 (t); 70.2 (t); 70.3 (t); 70.4 (t); 103.7 (d); 104.6 (d); 104.8 (d); 107.7 (s); 109.4 and 109.9 (s); 111.0 (d); 112.3 (d); 115.5 (d); 118.4 (s); 123.0 (d); 123.9 (2d); 125.2 (s); 129.2 (s); 134.3 (s); 135.0 (s); 139.9 (s); 143.8 (s); 146.6 (s); 146.8 (s); 147.2 (s); 147.5 (s); 152.1 (s); 155.4 (s); 169.5 (s); 172.1 (s). MS (ESI-TOF) 835 (M+1, 100); 836 (M+2, 44), 837 (M+3, 23). HRMS *m*/*z* calcd. for $C_{43}H_{51}N_2O_{15}$ 835.3283, found 835.3277.

3,4′-*bis*(2-(2-Methoxyethoxy)acetyl)-Lam-D (6). Following the general procedure **F** and starting from **19e** (41 mg, 0.05 mmol), elution with hexane/THF (60:40 to 40:60) gave a yellow solid (19 mg, 48%). mp (MeCN) 162−164 °C. IR (film) ν 3170, 1780, 1681, 1425 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.40 (s, 3H, OMe); 3.44 (s, 3H, OMe); 3.45 (s, 3H, OMe); 3.56 (s, 3H, OMe); 3.61−3.64 (m, 2H); 3.66−3.68 (m, 2H); 3.80−3.82 (m, 2H); 3.83 (s, 3H, OMe); 3.85−3.88 (m, 2H); 4.45 (s, 2H); 4.52 (s, 2H); 6.00 (br, 1H, OH); 6.82 (s, 1H); 6.95−6.99 (m, 1H); 7.02 (d, J = 7.2 Hz, 1H); 7.06 (s, 1H); 7.14 (s, 1H); 7.18 (s, 1H); 7.24 (d, J = 2.0 Hz, 1H); 7.32 (d, J = 8.0 Hz, 1H); 9.17 (d, J = 7.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.76 (q); 55.80 (q); 56.3 (q); 58.99 (q); 59.04 (q); 68.3 (t); 68.4 (t); 70.9 (t); 71.0 (t); 71.9 (t); 72.0 (t); 104.7 (d); 106.2 (d); 108.4 (s); 110.7 (s); 110.9 (d); 112.0 (d); 113.0 (d); 115.3 (d); 116.0 (s); 118.6 (s); 123.1 (d); 123.8 (d); 125.2 (s); 125.5 (d); 128.1 (s); 134.4 (s); 134.9 (s); 139.1 (s); 139.6 (s); 145.5 (s); 147.0 (s); 147.3 (s); 147.5 (s); 152.2 (s); 154.9 (s); 168.3 (s); 168.4 (s). MS (ESI-TOF) 732 (M+1, 100), 733 (M+2, 32); 734 (M+3, 9). HRMS *m*/*z* calcd. for $C_{38}H_{38}NO_{14}$ 732.2287, found 732.2307.

3,4′-*bis*(2-(2-(2-Methoxyethoxy)ethoxy)acetyl)-Lam-D (7). Following the general procedure **F** and starting from **19f** (48 mg, 0.06 mmol), elution with hexane/THF (60:40 to 40:60) gave a white solid (8 mg, 19%). mp (MeCN) 151−152 °C. IR (film) ν 3204, 1780, 1681, 1425 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.39 (s, 3H, OMe); 3.41 (s, 3H, OMe); 3.44 (s, 3H, OMe); 3.55−3.58 (m, 5H, OMe, CH_2); 3.59−3.62 (m, 4H, $2CH_2$); 3.66−3.75 (m, 6H, $3CH_2$); 3.77−3.79 (m, 2H, CH_2); 3.83 (br, 3H, OMe); 3.87−3.90 (m, 2H, CH_2); 4.45 (s, 2H); 4.53 (s, 2H); 5.97 (br, 1H); 6.82 (s, 1H); 6.97−7.08 (m, 2H); 7.15 (s, 1H); 7.20 (s, 1H); 7.23−7.25 (m, 2H); 7.32 (d, J = 8.0 Hz, 1H); 9.20 (d, J = 7.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.76 (q); 55.82 (q); 56.3 (q); 59.1 (2q); 68.3 (t); 68.5 (t); 70.55 (t); 70.62 (t); 70.65 (t); 70.70 (t); 71.0 (t); 71.1 (t); 71.90 (t); 71.93 (t); 103.6 (s); 104.4 (s); 104.7 (d); 106.2 (d); 110.8 (s); 110.9 (d); 112.1 (d); 112.4 (s); 113.0 (d); 115.3 (d); 115.5 (s); 116.0 (s); 118.6 (s); 123.1 (s); 123.8 (d); 123.9 (d); 125.3 (s); 125.5 (d); 135.0 (s); 139.6 (s); 145.5 (s); 147.0 (s); 147.5 (s); 152.2 (s); 155.0 (s); 168.4 (s); 168.5 (s). MS (ESI-TOF) 820 (M+1, 100), 821 (M+2, 39); 822 (M+2, 14). HRMS *m*/*z* calcd. for $C_{42}H_{46}NO_{16}$ 820.2811, found 820.2797.

Cell Lines and Culture. Established human-derived cell lines used in this study were purchased from ATCC (American type Culture Collection): A-549, human lung carcinoma; BJ, Skin Fibroblast; HT-29, human colorectal adenocarcinoma; and MDA-MB-231, human breast adenocarcinoma. All cell lines were maintained in DMEM supplemented with 10% FBS and 100 units/mL penicillin and streptomycin at 37 °C and 5% CO_2.

GI_{50} Analysis. Triplicate cultures were incubated for 72 h in the presence or absence of test compounds **1**−**9** (at ten concentrations, typically ranging from 0.0026 to 10 μg/mL).

A colorimetric assay using SRB was adapted for a quantitative measurement of cell growth and viability, following a previously described method (*16*). Cells were plated in 96-well microtiter plates at a density of 5×10^3/well and incubated for 24 h. One plate from each different cell line was fixed, stained, and used for Tz reference (see next paragraph). The cells were then treated with vehicle alone (control) or the test compounds at the concentrations indicated. The treated cells were incubated for additional 72 h, and then assayed for cytotoxicity via colorimetric analysis.

The cells were washed twice with PBS, fixed for 15 min in 1% glutaraldehyde solution, rinsed twice in PBS, and stained in 0.4% SRB solution for 30 min at room temperature. The cells were then rinsed several times in 1% acetic acid solution and air-dried. SRB was then extracted in 10 mM trizma base solution, and the absorbance at 490 nm was then measured. Cell survival is expressed as a percentage of control cell growth.

Dose−response curves were obtained by using the NCI algorithm (*17*): Tz = number of control cells at time t_0, C = number of control cells at time t, and T = number of treated cells at time t.

If Tz < T < C (growth inhibition), then the result is 100 × ([T − Tz]/[C − Tz]).

If T < Tz (net cell death), then the result is 100 × ([T − Tz]/Tz).

After dose−curve generation, the results were expressed as GI_{50}.

General Treatments for Imaging. A-549, MDA-MB-231, and HT-29 cells were seeded onto MatTek (Ashland, USA) glass-bottomed microwell dishes at 30×10^3 cells/cm^2. After 24 h, the culture medium was discarded and then replaced by fresh DMEM medium containing compound **9** at 25 nM. A negative control (no compound) was also used. The cells were then incubated for 48 h at 37 °C.

The following dyes were used for colocalization experiments: WGA-FITC (Sigma, USA) and Mitotracker Red (Molecular Probes). Standard staining protocols were employed for live-cell imaging. Images were then subsequently acquired within the following 30 min.

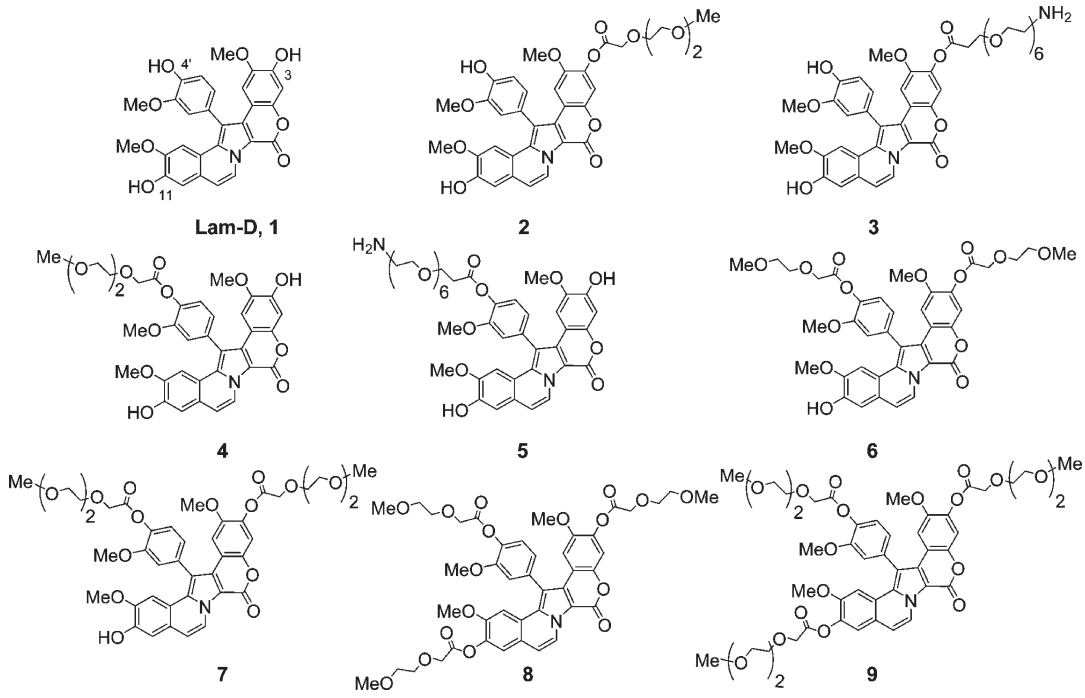


Figure 1. Structures of Lam-D 1 and PEG derivatives **2**−**9**.

Confocal Laser Scanning Microscopy. Confocal laser scanning microscopy was performed with a Leica TCS SPII spectral confocal laser scanning microscope (Leica Microsystems Heidelberg GmbH, Mannheim, Germany), using a 63× objective. FITC fluorescence was excited with an Ar laser at 488 nm. Lam-D and its derivatives were excited at 351 nm. Mitotracker was excited at 543 nm. The same microscope settings were maintained for each conjugate and concentration. To avoid crosstalk, two-fluorescence scanning was performed in a sequential mode.

Apoptosis Assay. The methodology used is based on fluorochrome inhibitors of caspases (FLICA; CaspaTag in situ caspase detection kits, Chemicon International, U.S. and Canada). The inhibitors are cell-permeable and non-cytotoxic. Once inside the cell, the inhibitor covalently binds to the active caspase. This kit uses a carboxyfluorescein-labeled fluoromethyl ketone peptide inhibitor of caspase-3 (FAM-DEVD-FMK), which fluoresces green. When added to a group of cells, the FAM-DEVD-FMK probe enters each cell and covalently binds to a reactive cysteine residue in the large subunit of the active caspase heterodimer, thereby inhibiting further enzymatic activity (*18*).

Cell cultures previously incubated with Lam-D for 48 h at a concentration of 25 nM were tested for apoptosis determination. After the latter drug treatments, 30 × FLICA reagent was added at 1:15 dilution in culture medium and then incubated for 1 h at 37 °C. The cells were then washed with PBS and subsequently analyzed by confocal scanning microscopy. The green fluorescence signal was a direct measure of the amount of active caspase-3/7 present in the cell at the time the reagent was added.

Uptake Measurements by FACS Flow Cytometry. 1×10^6 A-549, BJ, MDA-MB-231, and HT-29 cells were seeded onto 25 cm^2 cell culture flasks (Nalgene Nunc International, Naperville, USA) with 10 mL of DMEM. After 12 h, the culture medium was discarded and replaced by fresh DMEM medium containing compounds **1**−**9** at a concentration of 1 μM. A negative control (no compound) was also used. Cells were then incubated for 12 h at 37 °C. After incubation, cells were washed with 3 × PBS, detached with trypsin−EDTA 0.25%, and centrifuged at 1000× rpm. Finally, the medium was decanted, and the cellular pellet was resuspended in PBS and kept at 0 °C until measurements were performed. Fluorescence analysis was performed with a MoFlo cytometer (DakoCytomation, Colorado, USA), using the 351 nm excitation line of Ar laser (25 mW) and emission detection at 450 nm (tolerance range ±65 nm). CIQ is expressed as a percentage value in reference to Lam-D. It was calculated as a division of the fluorescence value obtained by the Lam-D fluorescence control under the same experimental conditions.

Cell Cycle Analysis. Cell cycle analysis were performed by DRAQ5 (Biostatus) staining to determine DNA content. DRAQ5 was added to the previous PBS cell culture suspensions (1 μL/4 $\times$ 10^5 cells), incubated for 15 min at 37 °C, and the cells were directly analyzed without any further treatment. Fluorescence analysis was performed with a MoFlo cytometer (DakoCytomation, Colorado, USA), using the 488 nm excitation line of an Ar laser (150 mW) and emission detection at 670 nm (tolerance range ±30 nm). Cell cycle profile was analyzed using *Cell Quest* software.

RESULTS

Lam-D contains three phenolic sites susceptible to modification. Despite previous SAR studies that highlighted the relevance of the phenolic residues at the C-3 and C-11 positions of Lam-D (*3, 19*), and their essential role for cytotoxicity and topoisomerase I inhibition (*3*), in this study, the free OH groups were chosen for attachment the PEG moiety. As esters readily hydrolyze under physiological conditions, our group envisioned that these derivatives would enable gradual release of the drug. Use of Lam-D derivatives having differently protected phenols allowed selective deprotection and further conjugation with PEG-carboxylic acids to enable synthesis of mono-PEG (**2**−**5**), di-PEG (**6**, **7**), and tri-PEG (**8**, **9**) conjugates (Figure 1).

The derivatives prepared contain monodisperse PEG backbones with OMe (**2**, **4**, **6**, **7**, **8**, and **9**) and NH_2 (**3**, **5**) terminal moieties and vary in their chain length. The PEGs used for derivatization contain one, two, or six sequentially linked units of ethylene glycol. Another source of diversity introduced in the PEG carriers was a spacer; acetyl (**2**, **4**, **6**, **7**, **8**, and **9**) or propanoyl (**3**, **5**) moieties were used.

Scheme 1

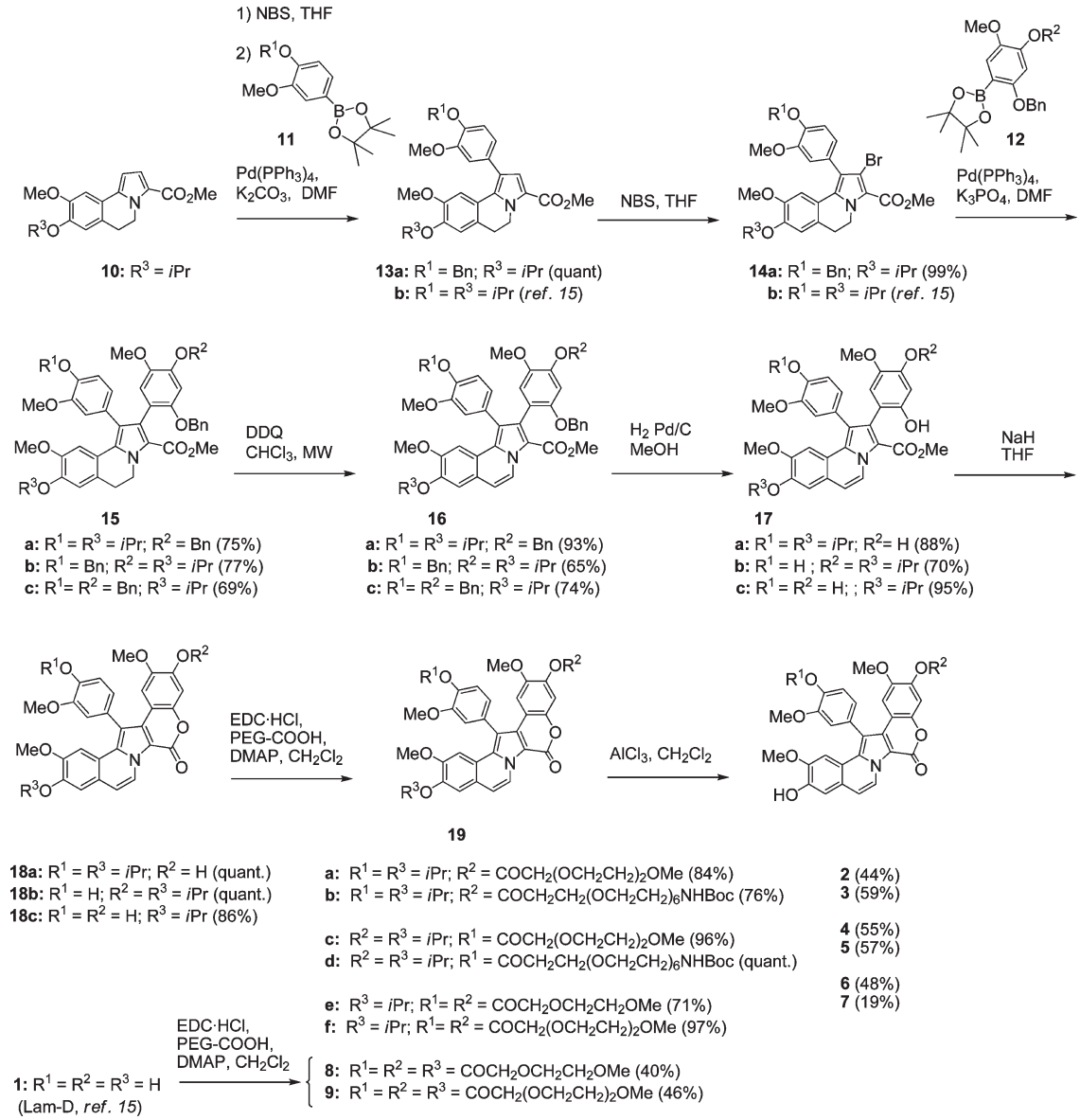


Table 1. Cytotoxicity of Compounds 1−9 in Three Human Cancer Cell Lines

	cytotoxicity (M)			
compound	HT-29 colon	A-549 lung	MDA-MB-231 breast	BJ skin fibroblast
Lam-D (**1**)	3.00×10^{-6}	1.22×10^{-7}	1.34×10^{-7}	6.37×10^{-9}
2	1.97×10^{-6}	7.13×10^{-8}	4.55×10^{-8}	6.28×10^{-7}
3	1.68×10^{-6}	8.86×10^{-8}	4.07×10^{-8}	6.51×10^{-9}
4	1.97×10^{-6}	5.46×10^{-8}	5.31×10^{-8}	2.90×10^{-6}
5	4.79×10^{-6}	3.47×10^{-7}	2.52×10^{-7}	3.51×10^{-6}
6	1.28×10^{-6}	6.29×10^{-8}	3.14×10^{-8}	1.81×10^{-9}
7	5.49×10^{-6}	5.37×10^{-8}	7.68×10^{-8}	3.17×10^{-9}
8	4.60×10^{-6}	7.31×10^{-8}	1.04×10^{-7}	4.90×10^{-8}
9	4.08×10^{-6}	4.69×10^{-8}	8.57×10^{-8}	2.67×10^{-8}

The methodology developed in our group for the total synthesis of Lam-D (*15*) was used for the preparation of conjugates **2**−**9**. The key steps of the synthesis comprise two successive processes of regioselective bromination and Pd(0)-catalyzed cross-coupling reactions starting from the scaffold **10**.

As shown in **Scheme 1**, several dialkoxy and trialkoxyphenylboronates **11a**,**b** and **12a**,**b** were required. These compounds were obtained with good yields by borylation of the corresponding aryl bromides with pinacolborane using $Pd(OAc)_2$ and DPE-Phos as catalysts (for details, see Supporting Information). The protected alkoxy-aryl bromides required were obtained by protection of commercially available phenols followed by mild bromination via NBS in THF at −78 °C (*20*).

Regioselective bromination of **10** (*21*) at position 1 using one equivalent of NBS in THF followed by Pd(0)-catalyzed cross-coupling reaction with the boronic ester **11a** or **11b** afforded the monoaryl scaffolds **13a** and **13b** (*15*), respectively, which were converted into the bromides **14a** and **14b** (*15*) by regioselective bromination at position 2. A second cross-

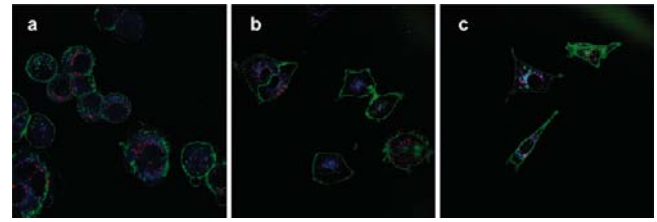
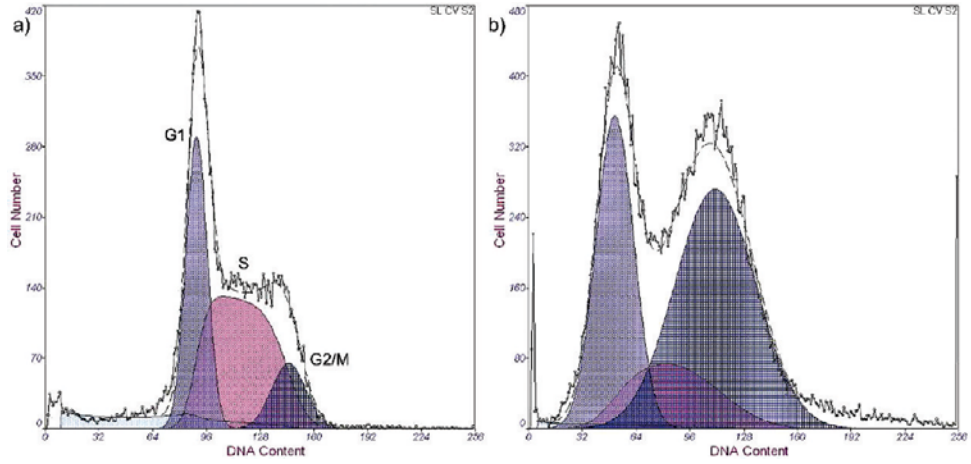


Figure 2. Cellular uptake of **9** and selective staining of mitochondria, Golgi apparatus, and cell membrane. The following cancer cell lines were used for the colocalization experiments. (a) HT-29, (b) A-549, and (c) MDA-MB-231.

Table 2. Cellular Internalization Uptake Measured by FACS

	cellular internalization uptake (AU)				cellular internalization quotient (CIQ)[a]			
compound	HT-29 colon	A-549 lung	MDA-MB-231 breast	BJ skin fibroblast	HT-29 colon	A-549 lung	MDA-MB-231 breast	BJ skin fibroblast
Lam-D (**1**)	82.7	328.5	443.8	259.4	100%[a]	100%[a]	100%[a]	100%[a]
2	41.1	463.1	266.6	321.0	50%	141%	60%	123.8%
3	88.0	393.0	527.5	333.4	106%	120%	119%	128.6%
4	59.8	500.6	364.8	501.5	72%	152%	82%	193.4%
5	84.4	373.7	328.1	371.4	102%	114%	74%	143.2%
6	90.3	407.2	535.0	317.7	109%	124%	121%	122.5%
7	49.3	379.3	432.1	279.3	60%	116%	97%	107.7%
8	78.7	532.6	414.8	352.0	95%	162%	94%	135.7%
9	143.3	514.8	529.7	371.0	173%	157%	119%	143.0%

[a] The CIQ has been calculated in reference to the cellular uptake of Lam-D.



c) Phase of Cell Cycle	control	Lam-D
G1 (%)	34.8	31.9
G2/M (%)	12.5	52.2
S (%)	52.6	15.9

Figure 3. Cell cycle progression of Lam-D in HT-29 cancer cell line. (a) HT-29 asynchronous colon cell line; (b) HT-29 with 1 μM Lam-D for 12 h.

coupling reaction, using the trialkoxyphenylboronates **12a** or **12b**, gave the diaryl derivatives **15a**−**c** (with the desired orthogonal protected phenol groups as *i*Pr/Bn ethers) in 45−64% yields from the scaffold **10**.

Tight control of the oxidation of **15** using 1 equiv of DDQ and MW irradiation at 120 °C for 10 min gave **16a**−**c** (65−93% yield), avoiding the complex mixtures obtained by simultaneous *O*-Bn cleavage using other conditions (*22*).

The *O*Bn protecting group was successfully removed to give **17a**−**c** in 70−95% yields by hydrogenolysis over Pd−C in methanolic solution. Lactonization in excellent yields (86% to quant.) was achieved by direct treatment of the methyl esters **17a**−**c** with sodium hydride in THF. Lactonization was needed before PEGylation to protect the phenol required in the lactone formation and to prevent undesired PEG removal caused by the instability of the ester bond under the basic reaction conditions.[2] Compounds **18a** and **18b**, having only one free phenol group (each of which at a distinct position), and **18c**, with two free phenol groups, were then further conjugated. The PEG-groups were successfully introduced by esterification of the free phenols of **18a**−**c** to give compounds **19a**−**f** using EDC·HCl with a catalytic amount of DMAP in CH_2Cl_2 (*23*). The same reaction conditions were used for the conjugation of Lam-D, obtained by deprotection of two isopropyl groups of **18c** with $AlCl_3$, to give the triester derivatives **8** and **9**. The isopropyl-protecting groups of **19a**−**f** were eliminated using $AlCl_3$ in CH_2Cl_2 with moderate to good yields, with concomitant removal of the *N*-Boc-protecting groups of **19b** and **19d**.

High-polarity solvents such as DMSO and PEG-400 were required to dissolve Lam-D (**1**), which is insoluble in most common solvents. The solubility of monoPEG compounds **2**−**5**, with a single conjugation of one phenolic residue, is much better to that of Lam-D.[3] Conjugate **3**, bearing six units of ethylene glycol and a NH_2 termination, showed 80-fold greater solubility in water than Lam-D. The solubility of conjugates **3**, **7**, and **9** -mono-, di-, and triester, respectively- in PEG-400 solvent was in accordance with the increase of PEG conjugations. Final compounds **2**−**9** were prepared in 18% to 57% yields from the corresponding phenolic Lam-D precursors.

Lastly, the conjugates were further evaluated biologically, and in vitro drug delivery assays were carried out.

Cytotoxicity and Cellular Uptake. The cytotoxicity of Lam-D (**1**) and its analogues (**2**−**9**) was evaluated against BJ human skin fibroblasts, and a panel of three human tumor cell lines: HT-29, A-549, and MDA-MB-231. A conventional colorimetric assay was used to estimate values of GI_{50} (i.e., the drug concentration that causes 50% of cell growth inhibition after 72 h of continuous exposure to the test molecule (*17*)). The results are shown in Table 1.

Conjugates **2**−**9** are all active against the cell lines: HT-29, the cytotoxicity of **2**−**9** is similar to that of Lam-D; in A-549 and MDA-MB-231 cell lines, the activity of **2**−**4** and **6**−**9** is 1.4−4.3-fold better than that of Lam-D. Furthermore, BJ skin fibroblasts were used in the present study to evaluate the effects of the drug and its conjugates in normal cells. In this non-tumoral cellular culture, conjugates **3**, **6**, and **7** maintained the same order of magnitude of cytotoxicities than Lam-D. Besides,

[2]Lactonization of methyl 2-(2,4-dihydroxy-5-methoxyphenyl)-8-hydroxy-1-(4-(2-(2-(2-methoxyethoxy)ethoxy)acetoxy)-3-methoxyphenyl)-9-methoxypyrrolo[2,1-*a*]isoquinoline-3-carboxylate did not provide compound **4**; rather, it led exclusively to formation of Lam-D.

[3]The conjugates are more soluble in water, EtOH, and PEG-400 than Lam-D. See Table SI1 in the Supporting Information.

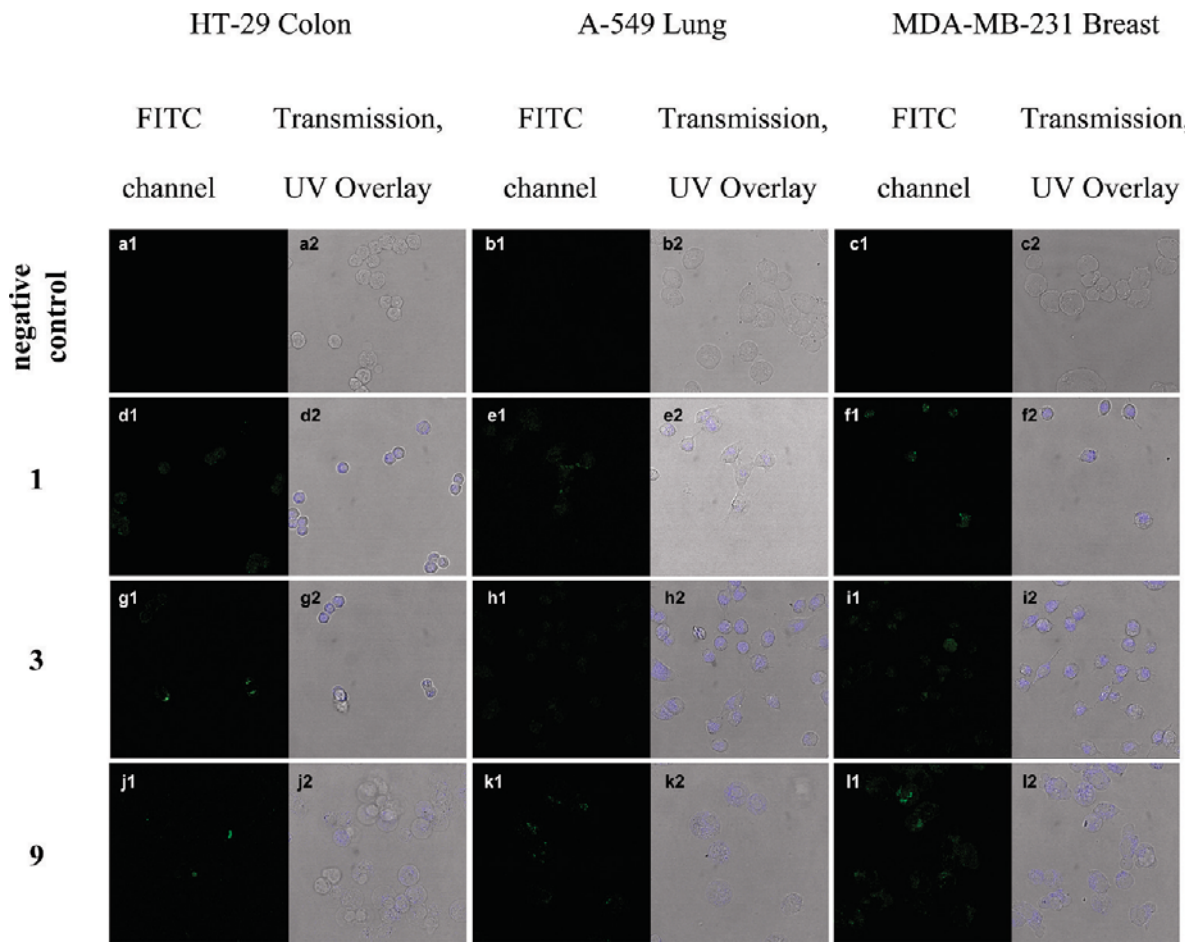
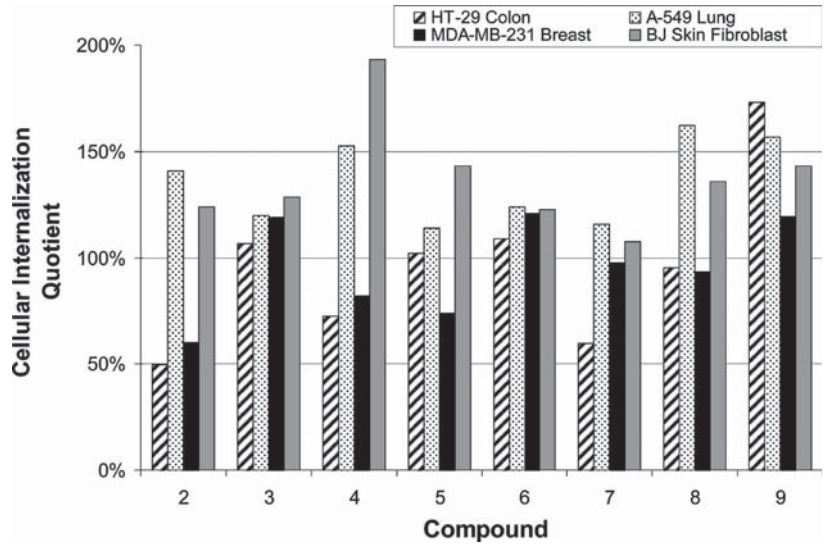


Figure 4. Detection of apoptosis by CaspaTag green fluorescence.

Chart 1. Cellular Internalization Quotient



conjugates **2**, **4**, **5**, **8**, and **9** showed a decrease of 4- to 500-fold in their cytotoxic activity for BJ skin fibroblasts.

The results of initial cellular internalization experiments in HeLa cells at different compound concentrations and exposure times were encouraging for Lam-D and **7** (see Figure SI1 and Figure SI2 in the Supporting Information). Compound **9** was selected as one of the best compounds of the series for colocalization experiments. Cell cultures were incubated with compound **9** for 48 h. WGA-FITC and Mitotracker were then employed for the selective staining of cell membrane-Golgi apparatus (green fluorescence) and mitochondria (red fluorescence), respectively. Likewise, UV fluorescence corresponding to **9** was observed in the cytoplasm;[4] however, no fluorescence was observed in the nuclear region of any of the cells for compound **9** (Figure 2).

[4]The Lam-D UV spectrum presents four characteristic absorption maxima: 237, 267, 356, and 382 nm. Excitation at 267 nm of **1** led to three emission maximum wavelengths: 319, 430, and 490 nm. The authors assumed that any minor deviation in the molar extinction

The internalization of compounds **1**−**9** was measured by FACS flow cytometry (see Table 2). Conjugates **3**, **6**, and **9** underwent the greatest cellular uptake in HT-29 and MDA-MB-231. Furthermore, conjugates **2**−**9** all had higher internalization in A-549 than did Lam-D. Results were normalized in function of Lam-D uptake; thus, the CIQ was calculated relative to 100% internalization for Lam-D (**1**). The comparative study showed that all the PEG conjugation motifs introduced enabled greater internalization in A-549 and BJ cell lines. For compounds **4**, **8**, and **9**, the ratio was greater than 150%. Although compounds **3**, **6**, and **9** exhibited greater internalization than did Lam-D (**1**) in HT-29 and MDA-MB-231, the differences observed for these cell lines were not as dramatic. Likewise, as observed in the results for **3**, monoconjugation enables greater uptake. The introduction of the PEG-NH_2 backbone at phenolic position 3 of Lam-D (compound **3**) led to better GI_{50} cytotoxicity and internalization than at position 4′ (compound **5**).

Effects of 1 on Cell Cycle Progression of HT-29 Cells. Lam-D was tested for effects on cell cycle progression in HT-29 tumor cells (Figure 3). Cells were treated with the product (see Experimental Section) and then analyzed by flow cytometry to determine if the cell cycle had been arrested at a specific phase. A control set of untreated cells was also used.

Compared to the control cells (Figure 3, panels **1a**,**c**), the cells treated with **1** (Figure 3, panels **1b**,**c**) showed an arrest in G2/M progression. Hence, it was concluded that Lam-D inhibits cell cycle progression. Furthermore, conjugates **3** and **9** provoked cell cycle arrest in a greater extent. The results showed one single peak corresponding to the G2/M phase (see Figure SI3 in the Supporting Information).

Induction of Apoptosis by 1, 3, and 9 in HT-29, A-549, and MDA-MB-231 Cell Lines. Many anticancer drugs induce cell cycle arrest and apoptosis. Apoptotic cells undergo characteristic morphological changes. Among these, the cell surface often bends and breaks up into membrane-enclosed fragments called apoptotic bodies. This process depends on a cascade of proteolytic enzymes called caspases (*24*).

To analyze whether Lam-D induces apoptosis, HT-29, A-549, and MDA-MB-231 cell lines were incubated with a single compound and then qualitatively measured for apoptosis (Figure 4, see Experimental Section). The assay consists of detecting active caspases (caspase-3/7) using a commercially available colorimetric test.

All cell lines showed a high level of apoptotic cell death after treatment with compounds **1**, **3**, and **9** (Figure 4d−l). A negative control of the assay was performed by analyzing untreated cells under the same experimental conditions (Figure 4a−c). Altogether, these results indicate that Lam-D and derivatives **3** and **9** induce cell cycle arrest and apoptosis.

DISCUSSION

A procedure for preparing PEGylated Lam-D pro-drugs has been described for the first time. One, two, or three phenol groups were selectively conjugated to the Lam-D in mild reaction conditions employing selective orthogonal protection (benzyloxy and isopropoxy groups).

The feasibility of the selective modification of the phenolic moieties of Lam-D has been demonstrated with readily available and inexpensive PEG-carboxylic acid as starting material. Conjugates functionalized with only a single phenolic residue of **2**−**5** (C-3 or C-4′ OH groups) are much more soluble than Lam-D in various solvents. Notwithstanding, di- and tri-PEG conjugates were also synthesized. Di- and tri-PEG conjugates—which imply greater steric hindrance—were also synthesized, thereby demonstrating the robustness of our method. The pH-labile ester conjugates were prepared in 18% to 57% yields from the corresponding Lam-D phenolic precursors.

Conjugates (**2**−**9**), screened against normal BJ skin fibroblasts and three human cancer cell lines, exhibited better internalization than Lam-D (**1**) did. Conjugation provided cytotoxicity amelioration for the cancerous cell lines. Indeed, the GI_{50} of conjugates **2**−**4**, **6**, **7**, and **9** (bearing mono-, di-, and tri-PEG backbones) in MDA-MB-231 and A549 cancer cell lines were 1 order of magnitude lower than that of Lam-D (**1**). Comparison of cytotoxic activity in BJ skin fibroblast between Lam-D and compounds **2**−**9** shown that bioconjugates are equal or less toxic than Lam-D. Compounds **2**, **4**, and **9** with higher cytotoxycity than Lam-D in A-549 and MDA-MB-231 tumor cell lines displayed less cytotoxyc-activity in the BJ normal cell line. Although these compounds **2**, **4**, and **9** possess better internalization in normal cells. These results indicate that these compounds have better affinity to components of tumor cell lines. Confocal microscopy revealed that Lam-D (**1**) and the conjugates (**2**−**9**) were internalized in the cytoplasmatic region. Furthermore, Lam-D induces cell cycle arrest at the G2 phase. Lam-D, and derivatives **3** and **9**, produce caspase-3,7-dependent apoptosis in all cell lines tested.

ACKNOWLEDGMENT

This study was partially supported by CICYT (Grant BQU 2006-03794), *Generalitat de Catalunya*, and the Barcelona Science Park. We gratefully the Networking Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN). This study was partially supported by the Industrial Consortium "NanoFarma" from the Spanish grants of CENIT program. We also thank Dr. J. Comas from Flow Cytometry Facility - SCT (UB) and M. Bosch from Confocal Microscopy Unit - SCT (UB) for their valuable discussions regarding this work. We also thank Ramon Eritja for recording Lam-D UV-emission spectra.

Supporting Information Available: Materials and methods, experimental procedures, characterization data of the precursor compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Pla, D., Albericio, F., and Álvarez, M. (2008) Recent advances in lamellarin alkaloids: isolation, synthesis and activity. *Anti-Cancer Agents Med. Chem. 8*, 746–760.

(2) Facompré, M., Tardy, C., Bal-Mayeu, C., Colson, P., Pérez, C., Manzanares, I., Cuevas, C., and Bailly, C. (2003) Lamellarin D: a novel potent inhibitor of topoisomerase I. *Cancer Res. 63*, 7392–7399.

(3) Marco, E., Laine, W., Tardy, C., Lansiaux, A., Iwao, M., Ishibashi, F., Bailly, C., and Gago, F. (2005) Molecular determinants of topoisomerase I poisoning by lamellarins: comparison with camptothecin and structure-activity relationships. *J. Med. Chem. 48*, 3796–3807.

(4) Kluza, J., Gallego, M. A., Loyens, A., Beauvillain, J. C., Fernández Sousa-Faro, J. M., Cuevas, C., Marchetti, P., and Bailly, C. (2006) Cancer cell mitochondria are direct proapoptotic targets for the marine antitumor drug lamellarin D. *Cancer Res. 66*, 3177–3187.

(5) Bailly, C. (2004) Lamellarins, from A to Z: a family of anticancer marine pyrrole alkaloids. *Curr. Med. Chem.: Anti-Cancer Agents 4*, 363–378.

(6) IUPAC nomenclature of Lam-D: 3,11-dihydroxy-2,12-dimethoxy-14-(4-hydroxy-3-methoxyphenyl)-6*H*[1]benzopyrano[4′,3′:4,5]-pyrrolo[2,1-*a*]isoquinoline-6-one.

(7) Vanhuyse, M., Kluza, J., Tardy, C., Otero, G., Cuevas, C., Bailly, C., and Lansiaux, A. (2005) Lamellarin D: a novel pro-

coefficients of **2**−**9** due to ester conjugation of **1** with aliphatic PEG backbones was not important.

apoptotic agent from marine origin insensitive to p-glycoprotein-mediated drug efflux. *Cancer Lett. 221*, 165–175.
(8) Iyer, A. K., Khaled, G., Fang, J., and Maeda, H. (2006) Exploiting the enhanced permeability and retention effect for tumor targeting. *Drug Discovery Today 11*, 812–818.
(9) Vicent, M. J., and Duncan, R. (2006) Polymer conjugates: nanosized medicines for treating cancer. *Trends Biotechnol. 24*, 39–47.
(10) Duncan, R. (2006) Polymer conjugates as anticancer nanomedicines. *Nat. Rev. Cancer 6*, 688–701.
(11) Pasut, G., and Veronese, F. M. (2007) Polymer−drug conjugation, recent achievements and general strategies. *Prog. Polym. Sci. 32*, 933–961.
(12) Torchilin, V. P. (2005) Lipid-core micelles for targeted drug delivery. *Curr. Drug Delivery 2*, 319–327.
(13) Lukyanov, A. N., and Torchilin, V. P. (2004) Micelles from lipid derivatives of water-soluble polymers as delivery systems for poorly soluble drugs. *Adv. Drug Delivery Rev. 56*, 1273–1289.
(14) Greenwald, R. B., Pendri, A., Conover, C. D., Zhao, H., Choe, Y. H., Martínez, A., Shum, K., and Guan, S. (1999) Drug delivery systems employing 1,4- or 1,6-elimination: poly(ethylene glycol) prodrugs of amine-containing compounds. *J. Med. Chem. 42*, 3657–3667.
(15) Pla, D., Marchal, A., Olsen, C., Albericio, F., and Álvarez, M. (2005) Modular total synthesis of lamellarin D. *J. Org. Chem. 70*, 8231–8234.
(16) Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Waren, J. T., Bokesch, H., Kenney, S., and Boyd, M. R. (1990) New colorimetric cytotoxicity assay for anticancer drug screening. *J. Natl. Cancer Inst. 82*, 1107–1112.
(17) Boyd, M. R., and Paull, K. D. (1995) Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. *Drug Dev. Res. 34*, 91–104.
(18) Rotonda, J., Nicholson, D. W., Fazil, K. M., Gallart, Y. G., Labelle, M., Peterson, E. P., Rasper, D. M., Ruel, R., Vaillancourt, J. P., Thornberry, N. A., and Becker, J. W. (1996) The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis. *Nat. Struct. Biol. 3*, 619–625.
(19) Pla, D., Marchal, A., Olsen, C. A., Francesch, A., Cuevas, C., Albericio, F., and Álvarez, M. (2006) Synthesis and structure-activity relationship study of potent cytotoxic analogues of the marine alkaloid lamellarin D. *J. Med. Chem. 49*, 3257–3268.
(20) Pla, D., Albericio, F., and Álvarez, M. (2007) Regioselective monobromination of free and protected phenols. *Eur. J. Org. Chem.* 1921–1924.
(21) Olsen, C., Parera, N., Albericio, F., and Álvarez, M. (2005) 5,6-Dihydropyrrolo[2,1-*a*]isoquinolines as scaffolds for synthesis of lamellarin analogues. *Tetrahedron Lett. 46*, 2041–2044.
(22) Complex mixtures were obtained using DDQ as oxidating agent and following Weissman, S. A., and Zewge, D. (2005) Recent advances in ether dealkylation. *Tetrahedron 61*, 7833–7863.
(23) Tardy, C., Facompré, M., Laine, W., Baldeyrou, B., García-Gravalos, D., Francesch, A., Mateo, C., Pastor, A., Jiménez, J. A., Manzanares, I., Cuevas, C., and Bailly, C. (2004) Topoisomerase I-mediated DNA cleavage as a guide to the development of antitumor agents derived from the marine alkaloid lamellarin D: triester derivatives incorporating amino acid residues. *Bioorg. Med. Chem. 12*, 1697–1712.
(24) Ekert, P. G., Silke, J., and Vaux, D. L. (1999) Caspase inhibitors. *Cell Death Differ. 6*, 1081–1086.

BC800503K

SUPPLEMENTARY MATERIAL

Lamellarin D Bioconjugates I: Synthesis and Cellular Internalization of PEG-Derivatives

*Daniel Pla,[a, b] Andrés Francesch,[c] Pilar Calvo,[c] Carmen Cuevas,[c] Rosa Aligué,[d] Fernando Albericio,[a, b, ‡, *] and Mercedes Álvarez,[a, b, §, *]*

[a]Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain. [b]CIBER-BBN Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Baldiri Reixac 10, E-08028 Barcelona, Spain.

[c]Pharma Mar S. A., Avda de los Reyes 1, E-28770 Colmenar Viejo, Madrid, Spain.

[d]Department of Cell Biology, Faculty of Medicine, University of Barcelona, Casanova 143, E-08036 Barcelona, Spain.

mercedes.alvarez@irbbarcelona.org

* Corresponding author: Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain Tel.: +34934037086; fax: +34934037126;

‡ Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona

§ Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, E-08028 Barcelona

TABLE OF CONTENTS

General Data:

Reagents and solvents were purified according to *Purification of Laboratory Chemicals,* Armarego, W. and Chai C., Elsevier (2003). Melting points (mp) were determined in a Büchi Melting Point B540 in open capillaries and are uncorrected. Automatic flash chromatography was done in an Isco Combiflash medium pressure liquid chromatograph with Redisep silica gel columns (47-60 μm). A Branson ultrasound bath was used to perform sonication. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer and a Gemini 200 MHz spectrometer. Multiplicity of the carbons was assigned with DEPT and gHSQC experiments, although the usual abbreviations according to off-resonance decoupling are used: (s) singlet, (d) doublet, (t) triplet, and (q) quartet. The same abbreviations were used for the multiplicity of signals in H-NMR and also: (m) multiplet, (bs) broad singlet, (bd) broad doublet. Spectra were referenced to appropriate residual solvent peaks (d_6-acetone, d_6-DMSO, d_4-MeOH or $CDCl_3$). IR spectra were obtained on a Thermo Nicolet FT-IR spectrometer. HRMS were performed on a Bruker Autoflex high resolution mass spectometer by Unidad de Espectrometría de Masas (Universidad de Santiago de Compostela) and by Servei d'Espectrometria de Masses (Universitat de Barcelona). Microwave-assisted reactions were carried out in a CEM Discover microwave. The automatic syringe pump was used as specified for controlled addition of some reactants. Reversed phase analytical HPLC was performed on a Waters Alliance separation module 2695 using a Waters Xterra MS C_{18} column (150 x 4.6 mm, 5 μm) and a Waters 996 PDA detector at 254 nm.

General Methods and Characterization of Synthesized Precursor Compounds:

2-(4-Benzyloxy-5-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (11a)

K_2CO_3 (2.49 g, 18.0 mmol) and BnBr (1.2 mL, 1.43 g/mL, 9.9 mmol) were added to a solution of 2-(4-hydroxy-5-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2.25 g, 9.0 mmol) in dry THF (25 mL) and DMF (0.5 mL). The mixture was stirred at 80 ºC under Ar for 16 h. The mixture was then cooled to r.t. and the solvent was removed *in vacuo*. EtOAc was added and the organic solution was washed with sat. aq. Na_2CO_3, water and brine. The organic layer was dried and concentrated, affording 3.06 g (quantitative yield) of the title compound as a white solid. mp (MeCN) 89-92 ºC. IR (film) ν 2977, 2931, 1598, 1410, 1354, 1138 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 1.33 (s, 12H); 3.92 (s, 3H, OMe); 5.182 (s, 2H); 6.88 (d, J = 8.0 Hz, 1H); 7.27-7.44 (m, 7H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 24.9 (4q); 56.0 (q); 70.6 (t); 83.6 (s); 112.9 (d); 117.1 (d); 127.1 (2d); 127.7 (d); 128.4 (2d); 128.9 (s); 136.9 (s); 148.9 (s); 150.7 (s). MS (ESI) 341 (M+1, 100); 342 (M+2, 10).

General Procedure for Bromination of Polialcoxybenzenes. Solid NBS (1 mmol) was added to a cooled solution (–78 ºC) of the aromatic compound (1 mmol) in THF (5 mL), and the reaction mixture was stirred until complete consumption of starting material. The reaction mixture was allowed to reach r.t., and the solvent was then evaporated under reduced pressure. CH_2Cl_2 was added to the remaining residue, and the resulting solution was filtered through a pad of neutral alumina. After solvent removal, pure products were obtained (99%-quant. yield).

1-Bromo-2,4-dibenzyloxy-5-methoxybenzene

Starting from 1,3-dibenzyloxy-4-methoxybenzene (7.2 g, 22.4 mmol), a white solid (9.0 g, quant. yield) was obtained. mp (MeCN) 105-106 ºC. IR (film) ν 2929, 1505, 1382, 1212, 843 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 3.84 (s, 3H, OMe); 4.99 (s, 2H); 5.07 (s,

2H); 6.59 (s, 1H); 7.08 (s, 1H); 7.31-7.41 (m, 10H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 56.7 (q); 71.5 (t); 72.2 (t); 103.1 (s); 104.1 (d); 116.7 (d); 127.2 (4d); 127.8 (d); 127.9 (d); 128.4 (2d); 128.5 (2d); 136.5 (s); 144.9 (s); 147.7 (s); 149.0 (s). MS (ESI) 399 (MBr^{79}+1, 97); 400 (MBr^{79}+2, 23); 401 (MBr^{81}+1, 100); 402 (MBr^{81}+2, 21).

1-Bromo-2-benzyloxy-4-isopropoxy-5-methoxybenzene

Starting from 1-benzyloxy-3-isopropoxy-4-methoxybenzene (11.3 g, 41.5 mmol), a white solid (14.4 g, 99%) was obtained. mp (MeCN) 73-75 ºC. IR (film) ν 2975, 2933, 1504, 1251, 844 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28 (d, *J* = 6.2 Hz, 6H); 3.80 (s, 3H, OMe); 4.39 (h, *J* = 6.2 Hz, 1H); 5.08 (s, 2H); 6.58 (s, 1H); 7.05 (s, 1H); 7.30-7.49 (m, 5H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 22.0 (2q); 56.7 (q); 72.1 (d); 72.3 (t); 103.2 (s); 106.0 (d); 117.0 (d); 127.3 (2d); 127.9 (d); 128.5 (2d); 145.8 (s); 147.0 (s); 149.0 (s). MS (ESI) 351 (MBr^{79}+1, 95); 352 (MBr^{79}+2, 15); 353 (MBr^{81}+1, 100); 354 (MBr^{81}+2, 12).

General Procedure for Pd (0) Catalyzed Borylations 3. A mixture of bromoalcoxybenzene (1 mmol), $Pd(OAc)_2$ (5%), DPEphos (0.1 mmol), and pinacolborane (3.0 mmol) in dry Et_3N-dioxane (83:17, 6 mL/mmol) was heated at 100 °C for 13.5 h. The cooled reaction mixture was quenched with saturated NH_4Cl, and the aq. solution was extracted with Et_2O. The organic solution was dried, filtered and concentrated. The resulting oil was purified by flash chromatography (SiO_2 previously deactivated with 5% Et_3N). Elution with hexane/EtOAc (70:30) afforded the title compounds **3a-3b** (62-88% yield).

2-(2-Benzyloxy-4-isopropoxy-5-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (12a)

Starting from 1-bromo-2-benzyloxy-4-isopropoxy-5-methoxybenzene (7.37 g, 21.0 mmol), a white solid (7.31 g, 88%) was obtained. mp (MeCN) 98-99 ºC. IR (film) ν

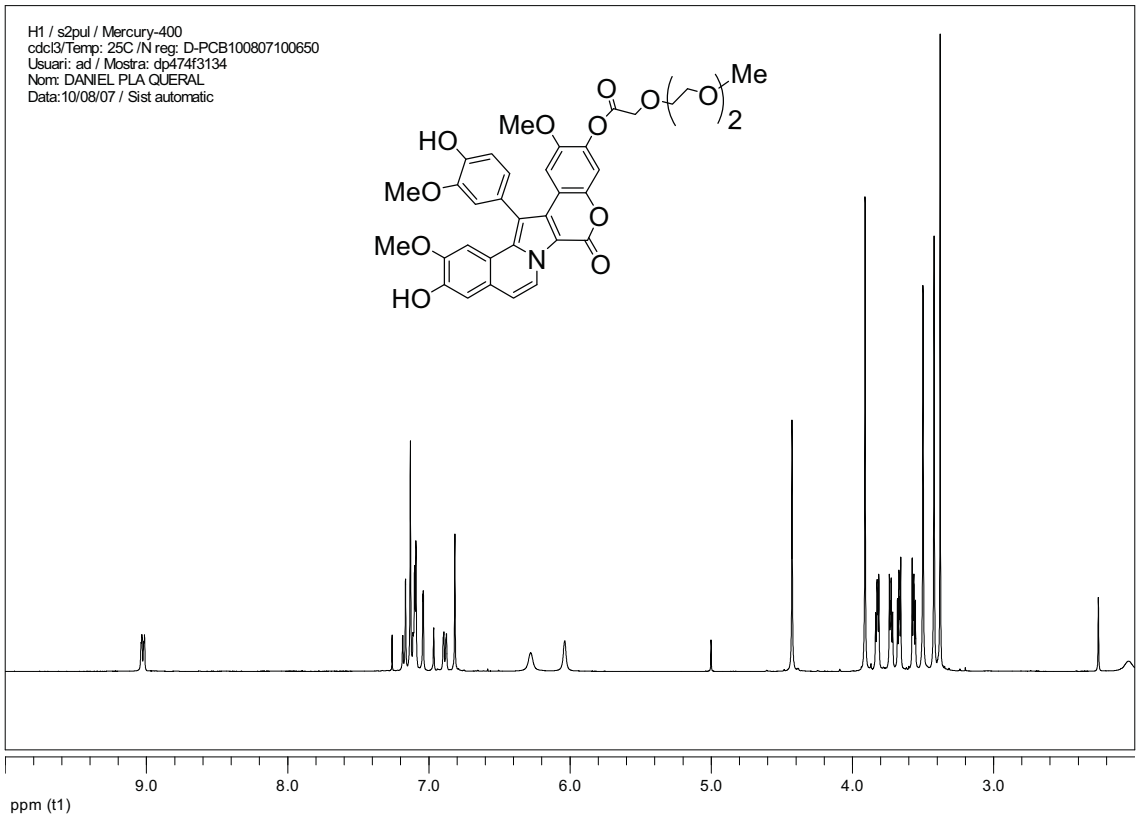
2979, 2931, 1601, 1409, 1139, 1040 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.33 (d, *J* = 6.0 Hz, 6H); 1.36 (s, 12H); 3.84 (s, 3H, OMe); 4.56 (h, *J* = 6.0 Hz, 1H); 5.07 (s, 2H); 6.59 (s, 1H); 7.28-7.40 (m, 4H); 7.61 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 21.7 (2q); 24.6 (4q); 56.2 (s); 70.8 (d); 71.7 (t); 82.8 (2s); 103.1 (d); 119.5 (d); 126.7 (2d); 127.0 (d); 127.7 (2d); 137.4 (s); 144.2 (s); 150.4 (s); 158.6 (s). MS (ESI) 399 (M+1, 100); 400 (M+2, 12).

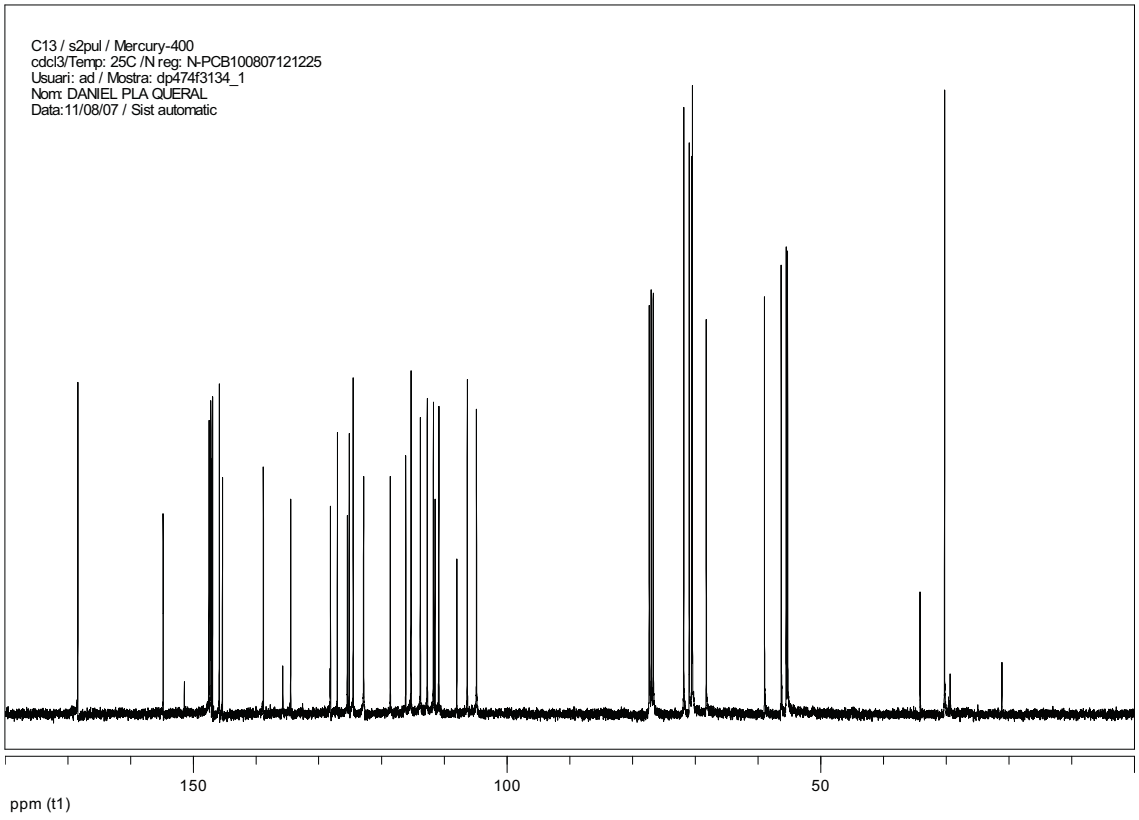
2-(2,4-Dibenzyloxy-5-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (12b)

Starting from 1-bromo-2,4-dibenzyloxy-5-methoxybenzene (1.00 g, 2.5 mmol), a yellow syrup (694 mg, 62%) was obtained. IR (film) ν 2925, 2853, 1596, 1433, 1272, 1134 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.33 (s, 12H); 1.35 (d, *J* = 6.0 Hz, 6H, 2Me); 3.89 (s, 3H, OMe); 4.95 (s, 2H); 5.15 (s, 2H); 6.54 (s, 1H); 7.22 (s, 1H); 7.30-7.39 (m, 8H); 7.50-7.54 (m, 2H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 25.0 (4q); 56.7 (q); 70.9 (t); 71.9 (t); 83.3 (s); 102.1 (d); 119.4 (d); 127.0 (2d); 127.2 (2d); 127.3 (d); 127.8 (d); 128.0 (2d); 128.5 (2d); 136.7 (s); 137.6 (s); 151.4 (s); 158.8 (s). MS (ESI) 447 (M+1, 100); 448 (M+2, 18).

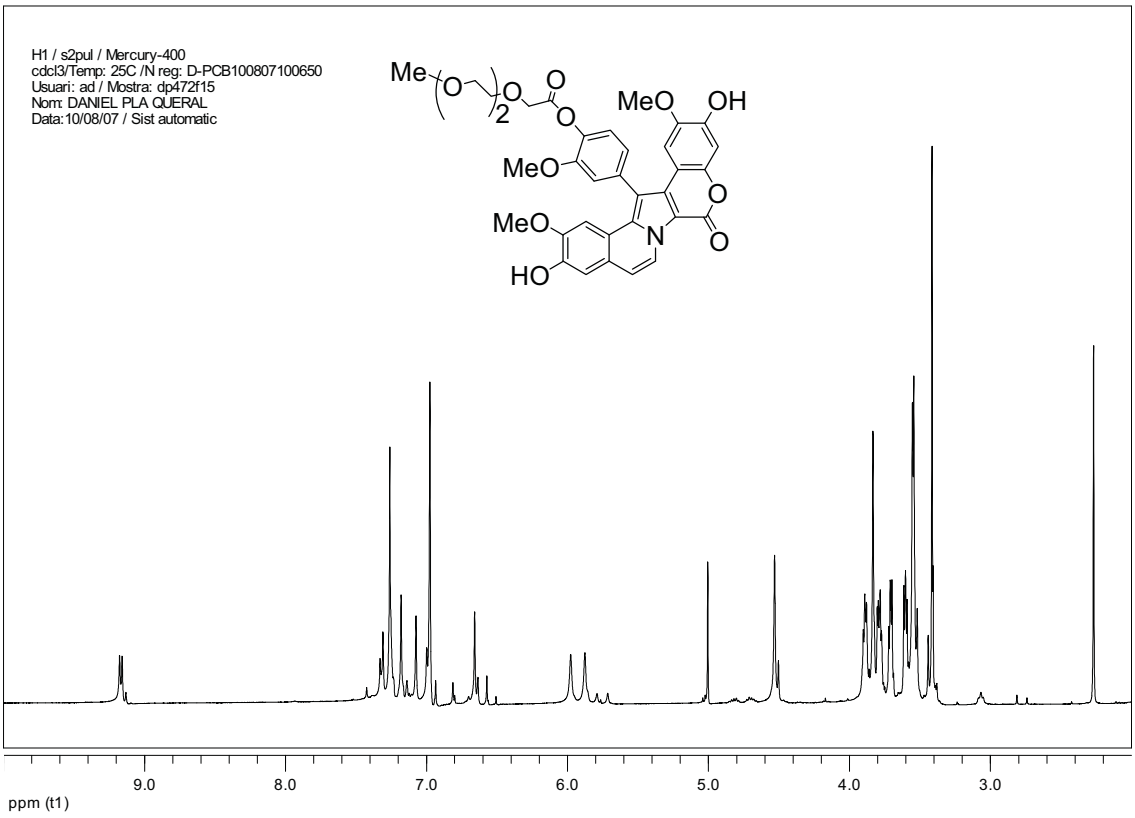
^{1}H, ^{13}C-NMR spectra of final compounds

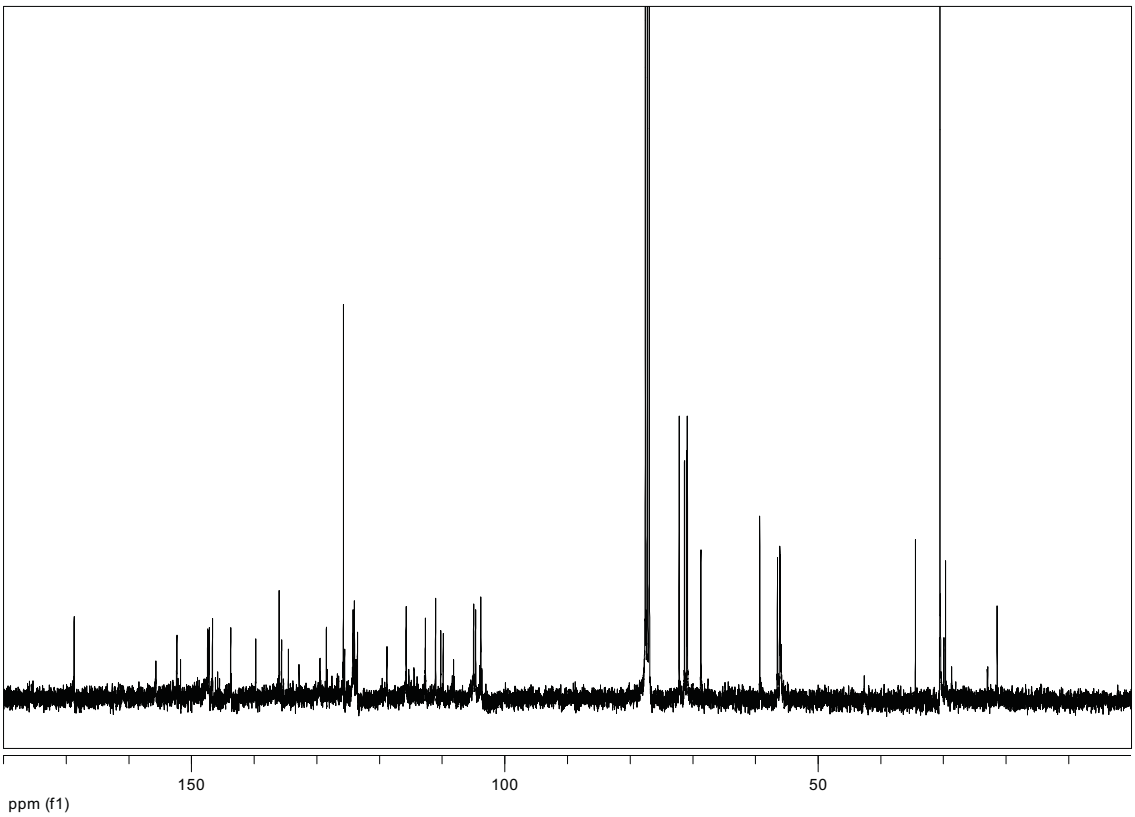
Compound **2**



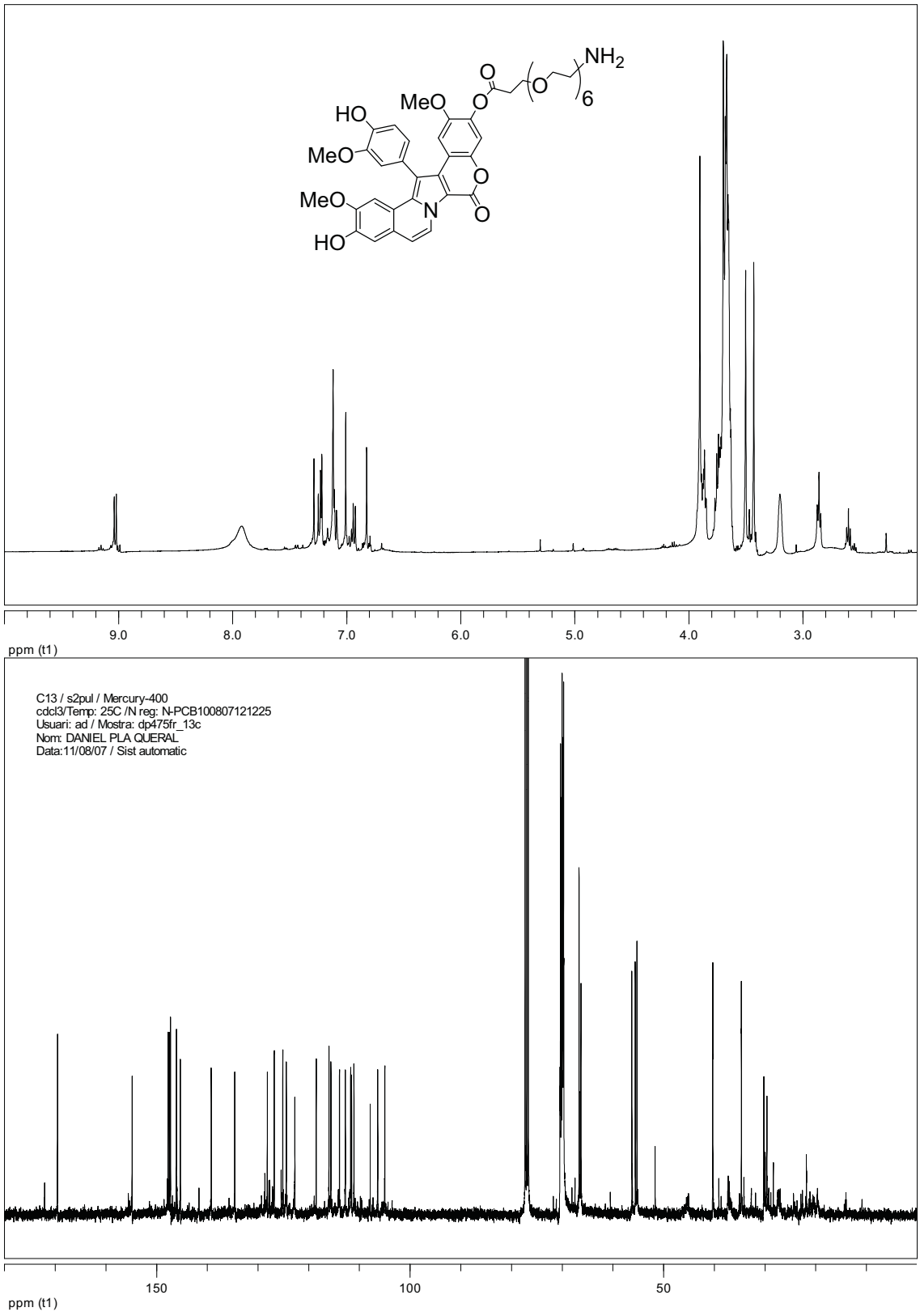


Compound **4**

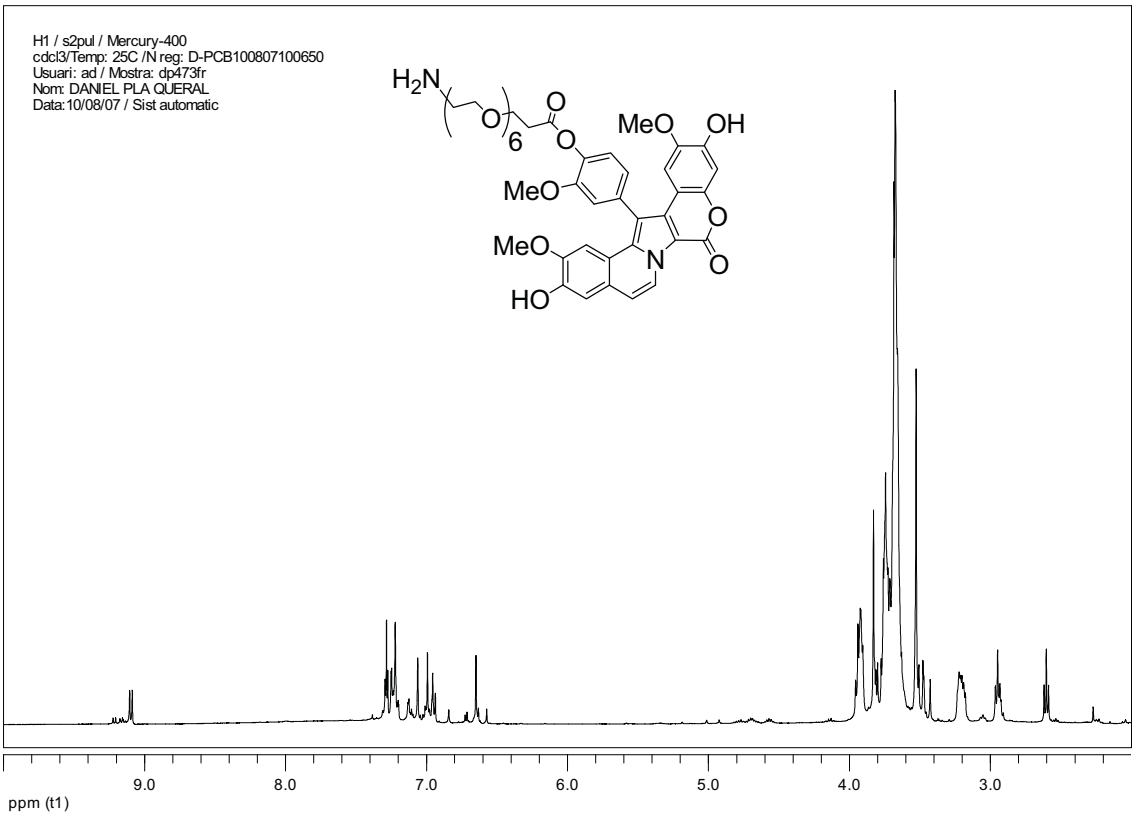

H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: D-PCB100807100650
Usuari: ad / Mostra: dp472f15
Nom: DANIEL PLA QUERAL
Data:10/08/07 / Sist automatic
Me
O
2
O
O
O
MeO
OH
MeO
O
MeO
N
O
HO
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)

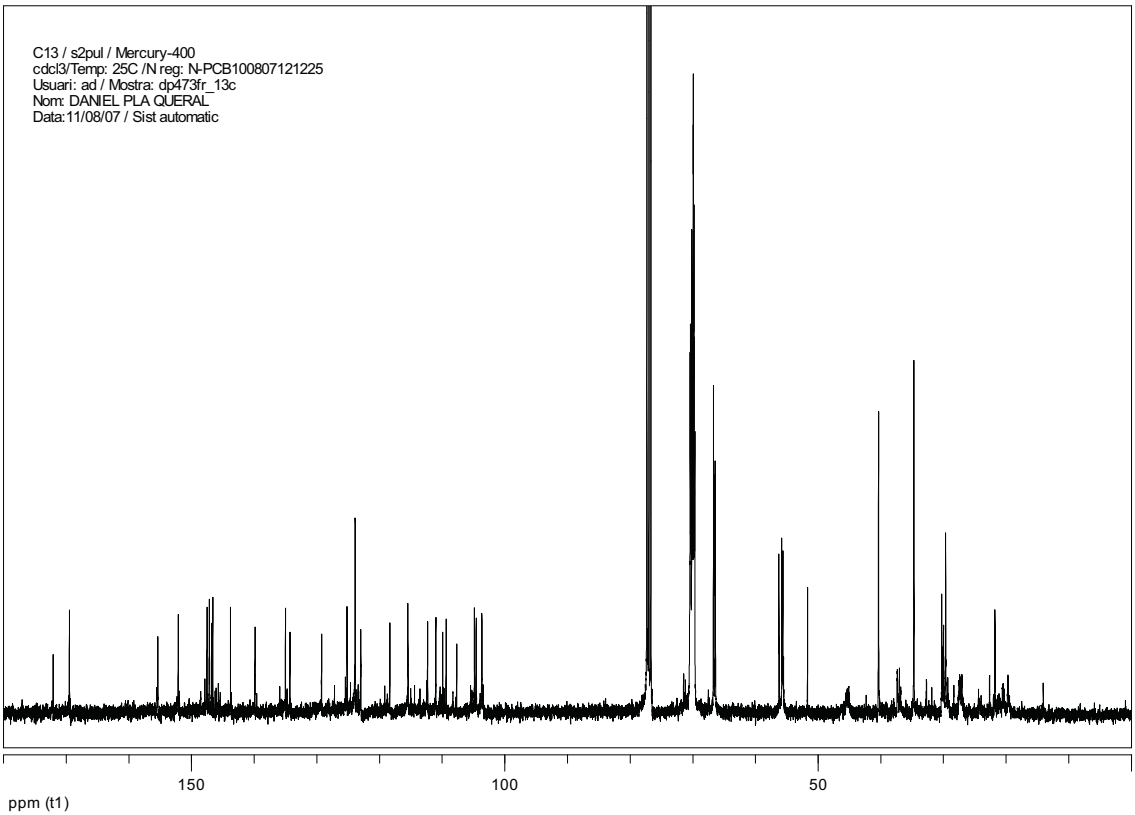

150
100
50
ppm (f1)

Compound **3**

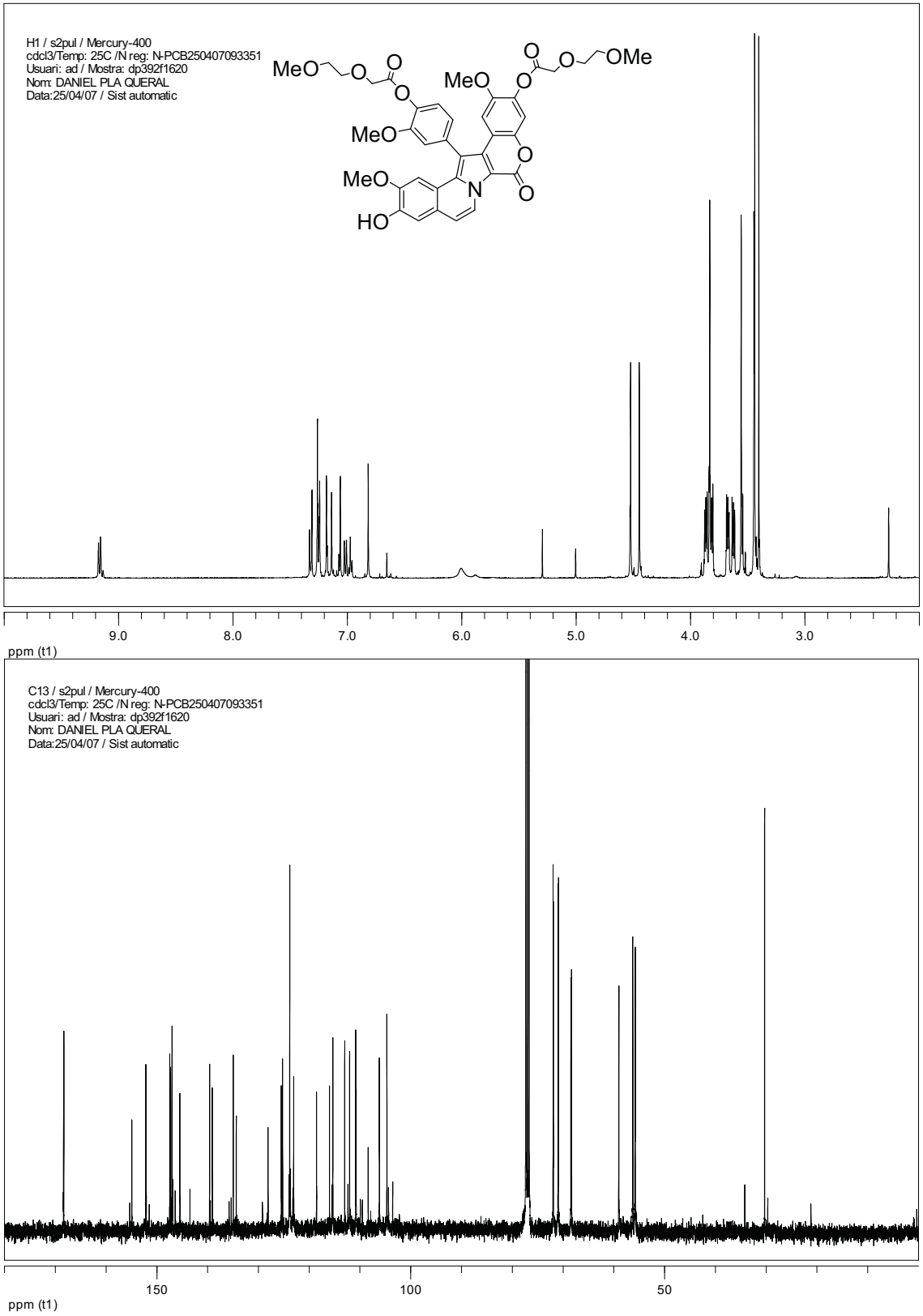

NH2
O
O
6
MeO
O
HO
MeO
O
MeO
N
O
HO
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: N-PCB100807121225
Usuari: ad / Mostra: dp475fr_13c
Nom: DANIEL PLA QUERAL
Data:11/08/07 / Sist automatic
150
100
50
ppm (t1)

Compound **5**

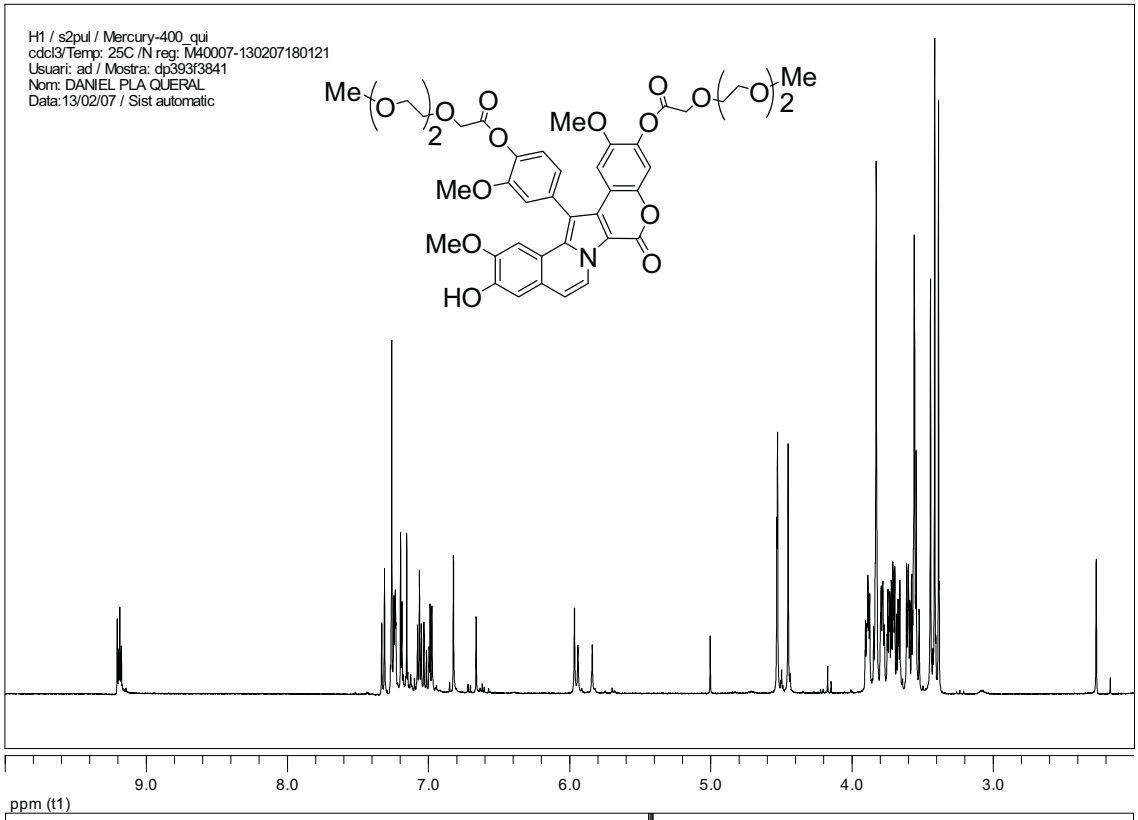

H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: D-PCB100807100650
Usuari: ad / Mostra: dp473fr
Nom: DANIEL PLA QUERAL
Data:10/08/07 / Sist automatic
H2N
O
6
O
O
MeO
OH
MeO
O
MeO
N
O
HO
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)

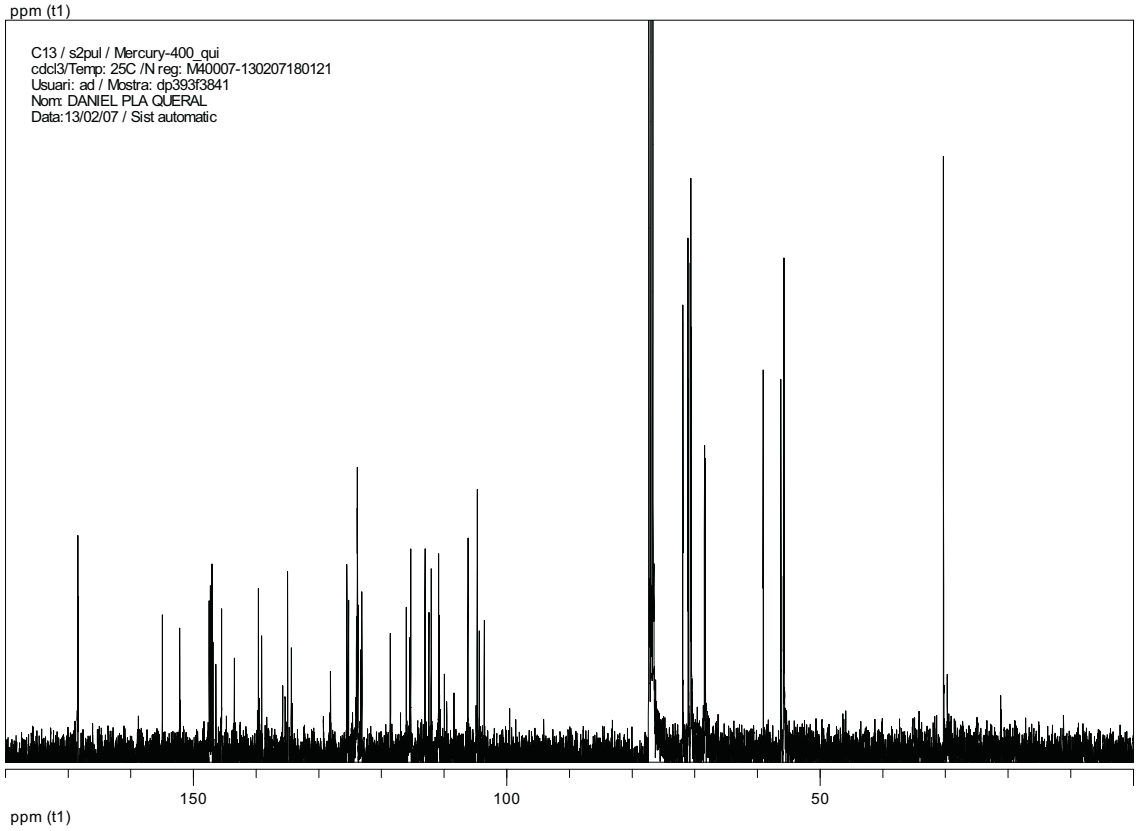

C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: N-PCB100807121225
Usuari: ad / Mostra: dp473fr_13c
Nom: DANIEL PLA QUERAL
Data:11/08/07 / Sist automatic
150
100
50
ppm (t1)

Compound **6**

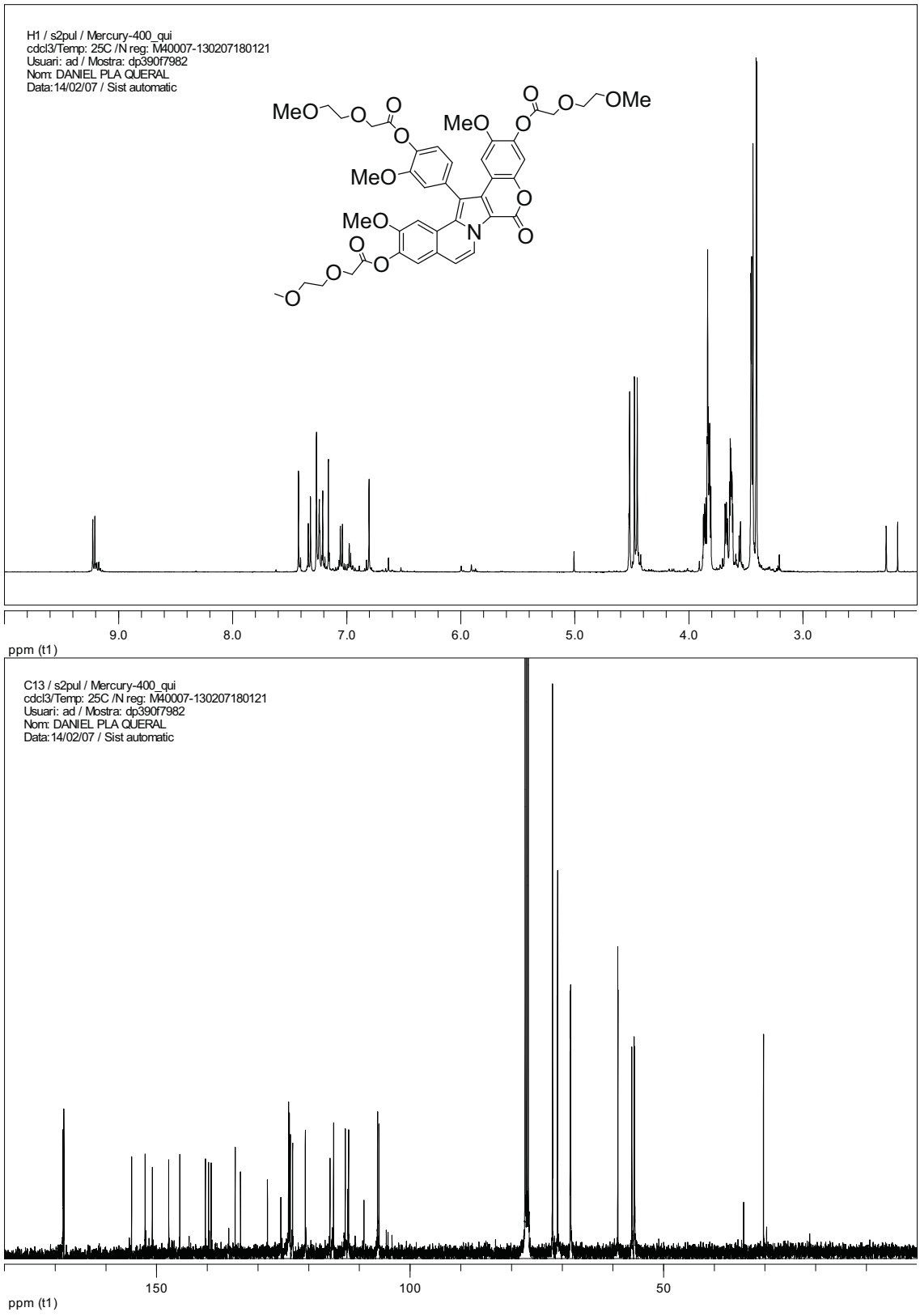

H1 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: N-PCB250407093351
Usuari: ad / Mostra: dp392f1620
Nom: DANIEL PLA QUERAL
Data:25/04/07 / Sist automatic
MeO
MeO
MeO
MeO
OMe
HO
N
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)
C13 / s2pul / Mercury-400
cdcl3/Temp: 25C /N reg: N-PCB250407093351
Usuari: ad / Mostra: dp392f1620
Nom: DANIEL PLA QUERAL
Data:25/04/07 / Sist automatic
150
100
50
ppm (t1)

Compound **7**


H1 / s2pul / Mercury-400_qui
cdcl3/Temp: 25C /N reg: M40007-130207180121
Usuari: ad / Mostra: dp393f3841
Nom: DANIEL PLA QUERAL
Data:13/02/07 / Sist automatic
Me
O
2
O
O
O
MeO
O
O
O
Me
2
MeO
MeO
N
O
O
HO
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)


C13 / s2pul / Mercury-400_qui
cdcl3/Temp: 25C /N reg: M40007-130207180121
Usuari: ad / Mostra: dp393f3841
Nom: DANIEL PLA QUERAL
Data:13/02/07 / Sist automatic
150
100
50
ppm (t1)

Compound **8**


H1 / s2pul / Mercury-400_qui
cdcl3/Temp: 25C /N reg: M40007-130207180121
Usuari: ad / Mostra: dp390f7982
Nom: DANIEL PLA QUERAL
Data:14/02/07 / Sist automatic
MeO
MeO
MeO
MeO
OMe
N
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)
C13 / s2pul / Mercury-400_qui
cdcl3/Temp: 25C /N reg: M40007-130207180121
Usuari: ad / Mostra: dp390f7982
Nom: DANIEL PLA QUERAL
Data:14/02/07 / Sist automatic
150
100
50
ppm (t1)

Compound **9**

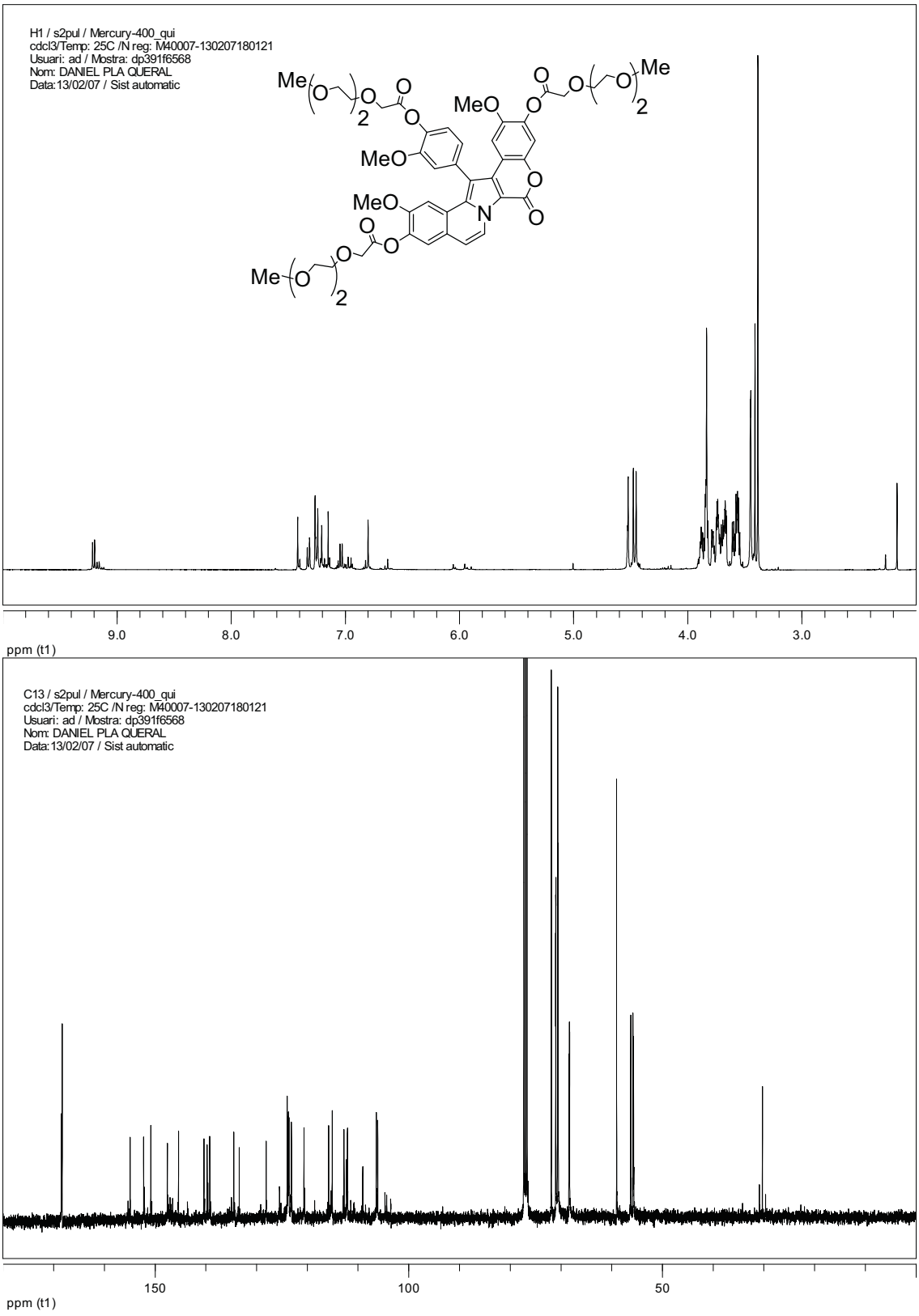
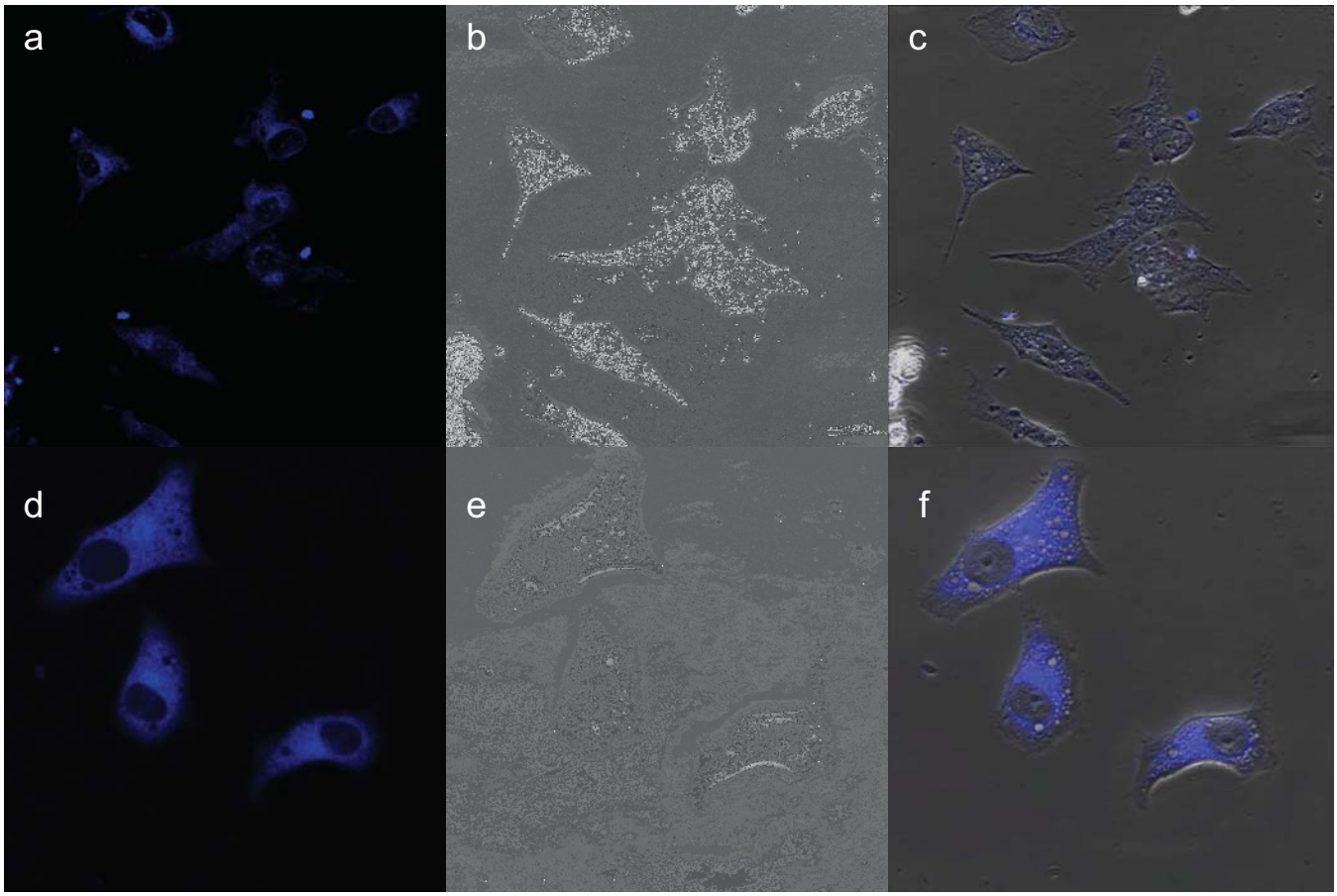

H1 / s2pul / Mercury-400_qui
cdcl3/Temp: 25C /N reg: M40007-130207180121
Usuari: ad / Mostra: dp391f6568
Nom: DANIEL PLA QUERAL
Data:13/02/07 / Sist automatic
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)
C13 / s2pul / Mercury-400_qui
cdcl3/Temp: 25C /N reg: M40007-130207180121
Usuari: ad / Mostra: dp391f6568
Nom: DANIEL PLA QUERAL
Data:13/02/07 / Sist automatic
150
100
50
ppm (t1)

Table SI1. Solubilities of compounds **1**, **3**, **7** and **9** in H_2O, PEG-400 and EtOH.

Compound	Solubility [a] (mg/mL)		
	H_2O	PEG-400	EtOH
1	< 0.23	14.3	< 0.25
3	18.8	6.8	3.4
7	2.9	14.7	12.3
9	7.2	39.8	10.4

[a] All trials were carried out at r.t. and atmospheric pressure using 0.8 to 2.6 mg of product.

Figure SI1. Uptake of test compounds by HeLa cells.



a-c) HeLa cells incubated with Lam-D at 1μM concentration for 24 h: **a)** UV image; **b)** transmission picture; and **c)** overlay picture. **d-f)** HeLa cells incubated with **7** at 1μM concentration for 24 h: **a)** UV image; **b)** transmission picture; and **c)** overlay picture.

Figure SI2. Uptake of Lam-D by HeLa cells.

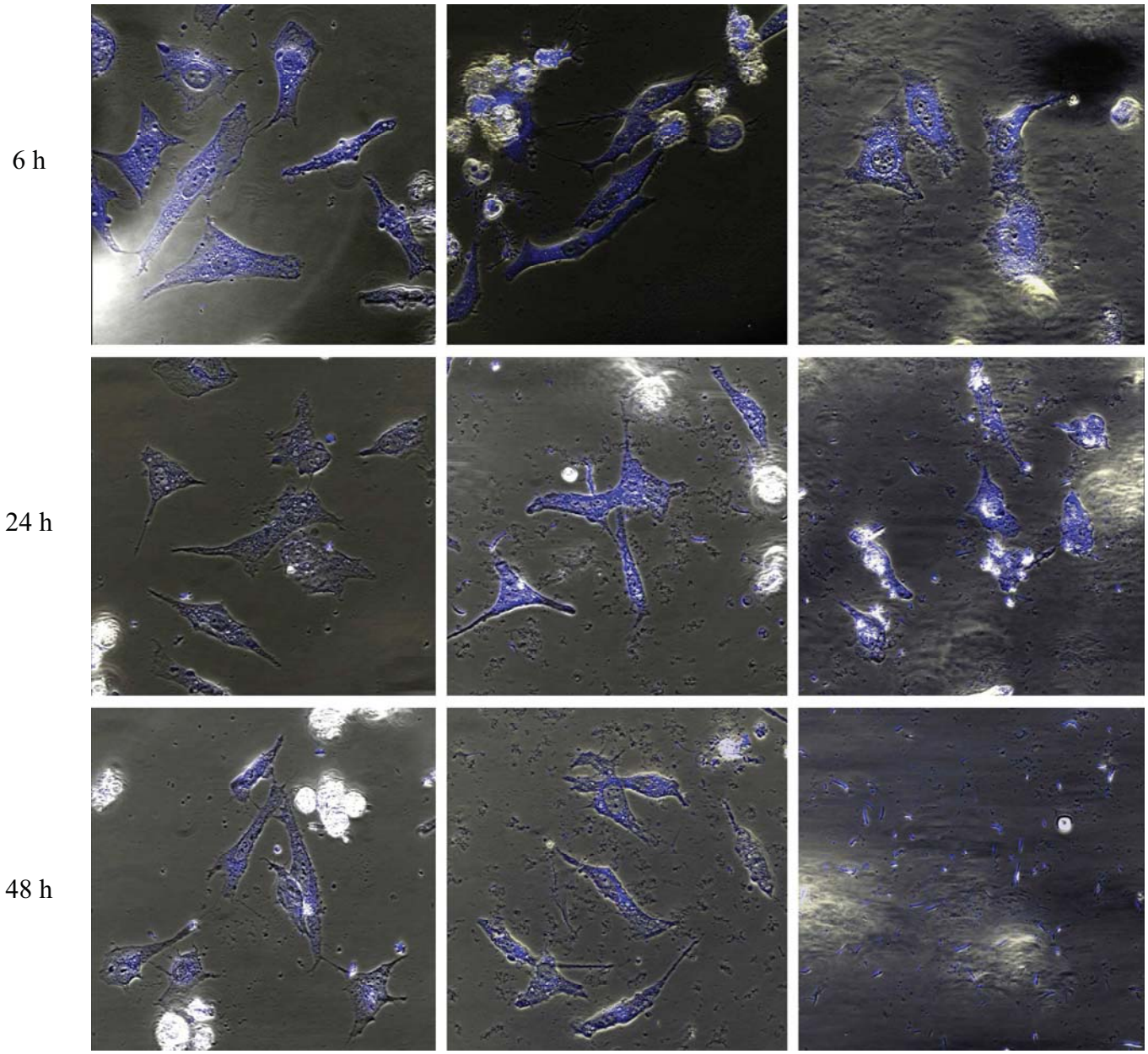
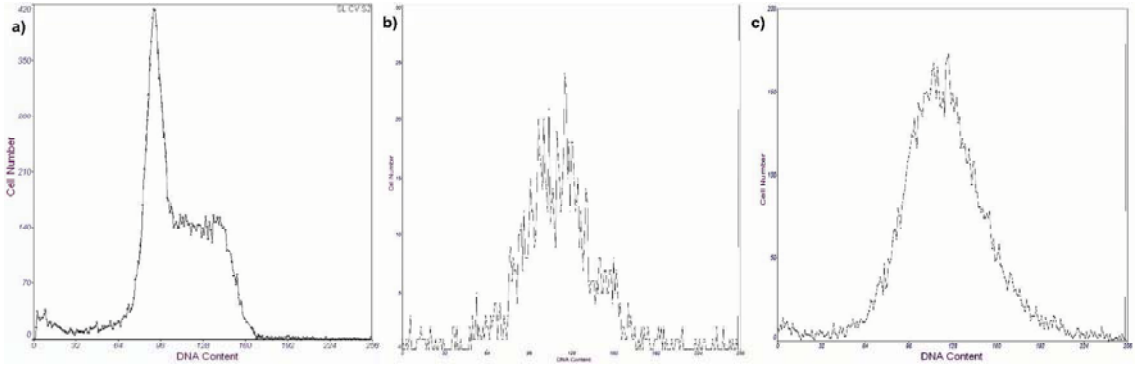


Figure SI3. Cell cycle progression of conjugates **3** and **9** in HT-29 cancer cell line



a) HT-29 asynchronous colon cell line.
b) HT-29 cell line treated with 1 μM conjugate **3** for 12 h.
c) HT-29 cell line treated with 1 μM conjugate **9** for 12 h.

6

BIOCONJUGATS DE LA LAMEL·LARINA D II: SÍNTESI I INTERNALITZACIÓ CEL·LULAR DE DERIVATS AMB DENDRÍMER I NLS

BIOCONJUGATS DE LA LAMEL·LARINA D II: SÍNTESI I INTERNALITZACIÓ CEL·LULAR DE DERIVATS AMB DENDRÍMER I NLS.

Lamellarin D Bioconjugates II: Synthesis and cellular internalization of Dendrimer- and NLS- Derivatives.

Daniel Pla,[a, b] Marc Martí,[a] Josep Farrera-Sinfreu,[a, c] Daniel Pulido,[c] Andrés Francesch,[d] Pilar Calvo,[d] Carmen Cuevas,[d] Miriam Royo,[c] Rosa Aligué,[e] Fernando Albericio,[a, b, ‡] Mercedes Álvarez,[a, b, §, *]

Bioconjugate Chemistry, 2009, *20*, 1112-1121.

[a]Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain;

[b]CIBER-BBN Networking Centre on Bioengineering, Biomaterials and Nanomedicine.

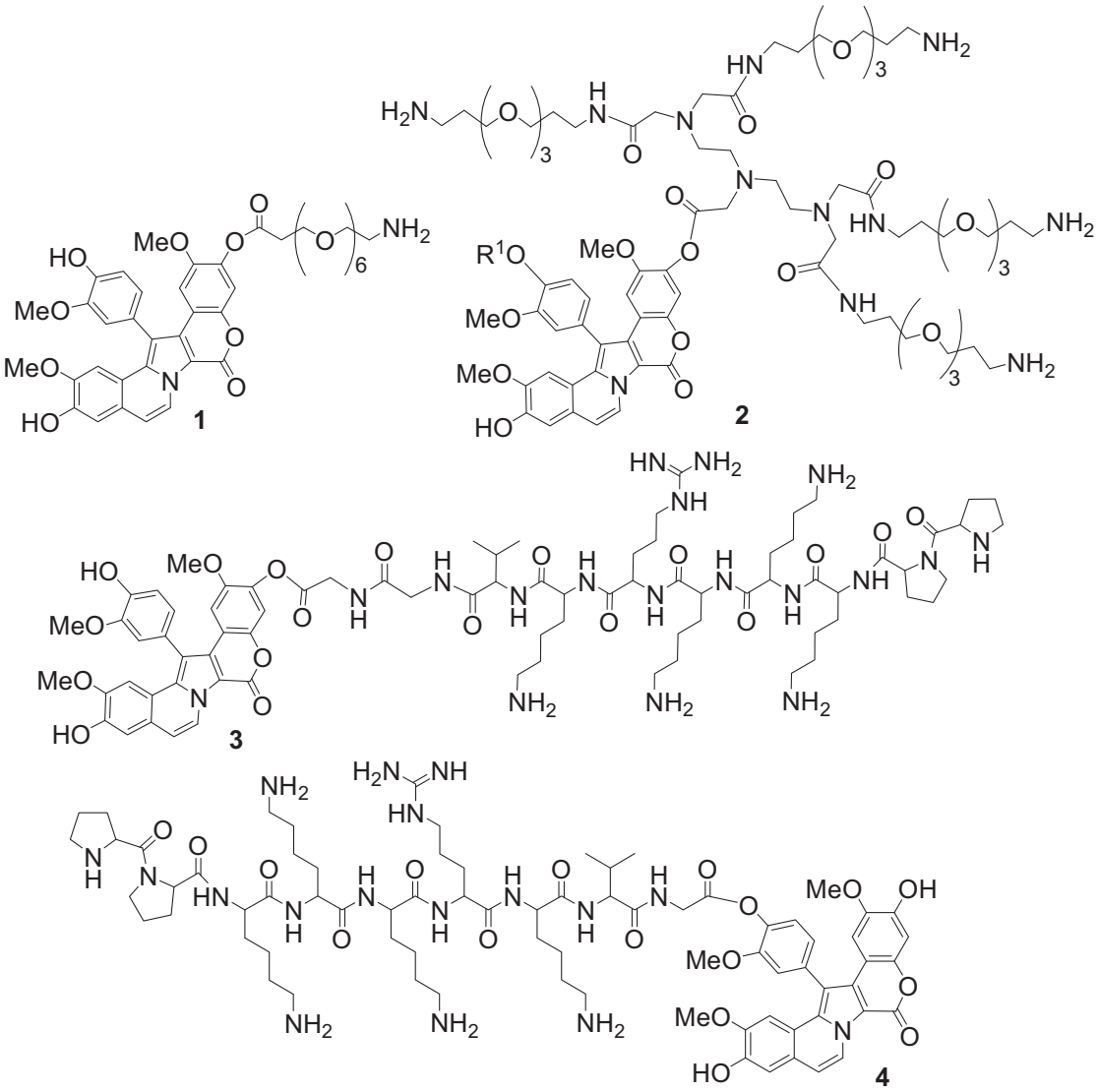
[c]Combinatorial Chemistry Unit - Barcelona Science Park (UQC-PCB).

[d]Pharma Mar S. A., Avda Reyes Católicos 1, E-28770 Colmenar Viejo, Madrid, Spain.

[e]Department of Cell Biology, Faculty of Medicine, University of Barcelona, Casanova 143, E-08036 Barcelona, Spain.

[‡]Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona;

[§]Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, E-08028 Barcelona.



Resum

En aquest apartat es descriu el disseny i la síntesi dels conjugats de Lamel·larina D amb seqüència peptídica de localització nuclear i estructures dendrimèriques de polietilenglicol. S'ha obtingut els conjugats **1-4** amb rendiments globals del 8-84% a partir de la Lamel·larina D protegida adequadament. Com a grups protectors dels intermedis sintètics es va emprar Boc i TBDPS i fou una elecció adequada per optimització de les condicions de desprotecció finals en una sola etapa. La conjugació ha portat una millora en la citotoxicitat i aquesta ha estat assajada en un panell de tres línies cel·lulars tumorals cancerígenes (MDA-MB-231 mama, A-549 pulmó, i HT-29 colon). S'ha analitzat el grau d'internalització i s'ha aconseguit interessants perfils de distribució nuclear per al compost **4**, que incorporava una llançadora peptídica amb senyal de localització nuclear.

Lamellarin D Bioconjugates II: Synthesis and Cellular Internalization of Dendrimer and Nuclear Location Signal Derivatives

Daniel Pla,[†,‡] Marc Martí,[†] Josep Farrera-Sinfreu,[†,§] Daniel Pulido,[‡,§] Andrés Francesch,[||] Pilar Calvo,[||] Carmen Cuevas,[||] Miriam Royo,[‡,§] Rosa Aligué,[⊥] Fernando Albericio,*[,#,†,‡] and Mercedes Álvarez*[,¶,†,‡]

Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain, CIBER-BBN Networking Centre on Bioengineering, Biomaterials, and Nanomedicine, Combinatorial Chemistry Unit - Barcelona Science Park (UQC-PCB), Pharma Mar S. A., Avda de los Reyes 1, E-28770 Colmenar Viejo, Madrid, Spain, and Department of Cell Biology, Faculty of Medicine, University of Barcelona, Casanova 143, E-08036 Barcelona, Spain. Received November 24, 2008; Revised Manuscript Received March 24, 2009

The design and synthesis of Lamellarin D conjugates with a nuclear localization signal peptide and a poly(ethylene glycol)-based dendrimer are described. Conjugates **1**−**4** were obtained in 8−84% overall yields from the corresponding protected Lamellarin D. Conjugates **1** and **4** are 1.4- to 3.3-fold more cytotoxic than the parent compound against three human tumor cell lines (MDA-MB-231 breast, A-549 lung, and HT-29 colon). Besides, conjugates **3** and **4** showed a decrease in activity potency in BJ skin fibroblasts, a normal cell culture. Cellular internalization was analyzed, and a nuclear distribution pattern was observed for **4**, which contains a nuclear localization signaling sequence.

INTRODUCTION

Actively mediated cellular delivery of biomolecules (*1*) has garnered great interest as a strategy for delivering cancer chemotherapeutics (*2*−*5*). Conjugates of a drug and a macromolecular vehicle such as NLS[1] peptidic sequences (*5*−*8*), PEG carriers (*9*), and dendrimers (*10*) may have better cellular internalization than the drug alone and, in some cases, may produce passive accumulation of the drug in tumors by the EPR effect (*11*). In addition, the therapeutic activity of these conjugates is associated with their capacity to release the drug at a specific subcellular target. Thus, the suitability of macromolecules as vehicles also extends to their propensity to deliver the drug to a predetermined intracellular location.

The marine alkaloid Lam-D (*12*−*15*) is a promising drug candidate due to its Topo I inhibition activity. Topoisomerases are nuclear enzymes crucial in cellular replication. They change the topology of DNA before and after the replication and transcription processes. Therefore, they are especially attractive targets for cancer therapy (*16*−*19*). Lam-D is limited by its insolubility in common solvent media, especially in water. Therefore, it has been used to investigate its conjugation to macromolecules. In the previous paper, we have described the first generation of Lam D-bioconjugates based on PEG esters such as **1** (*9*). In this paper, we describe a second generation of Lam-D conjugates (Figure 1) based on esterification with either a poly(ethylene glycol)-based dendrimer (in **2**) or NLS oligopeptide sequences (in **3** and **4**). The peptide NLS H-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-OH, which has been demonstrated (*20*) to shuttle compounds to the nucleus, was used in the present work.

The introduction of such oligomeric systems to Lam D demanded an integrated and robust synthetic scheme with a collection of suitable orthogonal protecting groups, in terms of selective removal and compatibility with the presence of other functional groups (*21*).

EXPERIMENTAL PROCEDURES

General Data. Reagents and solvents were purified according to *Purification of Laboratory Chemicals*, Armarego, W. and Chai C., Elsevier (2003). Melting points (m.p.) were determined

* Corresponding author. M.A. and F.A., Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain. Tel.: +34934037086; Fax: +34934037126; E-mail mercedes.alvarez@irbbarcelona.org.

† Barcelona Science Park-University of Barcelona.

‡ CIBER-BBN Networking Centre on Bioengineering, Biomaterials, and Nanomedicine.

§ Combinatorial Chemistry Unit - Barcelona Science Park (UQC-PCB).

|| Pharma Mar S. A.

⊥ Faculty of Medicine, University of Barcelona.

Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona.

¶ Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, E-08028 Barcelona.

[1] Abbreviations: AU, absorbance units; A-549, lung carcinoma cell line; Bn, benzyl; Boc, *tert*-butoxycarbonyl; Cbz, benzyloxycarbonyl; CIQ, cellular internalization quotient; DDQ, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DIPEA, diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMEM, Dulbecco's modified Eagle's medium; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DTPA, diethylenetriamine-*N*,*N*,*N*′,*N*′,*N*″-pentaacetic acid; EDC·HCl, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; EPR, enhance permeability and retention; FACS, fluorescence-activated cell sorting; FBS, fetal bovine serum; GFP, green fluorescent protein; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; HT-29, colon carcinoma cell line; GI_{50}, 50% growth inhibition; *i*Pr, isopropyl; Lam, Lamellarin; MDA-MB-231, breast adenocarcinoma cell line; MOM, methoxymethyl; MSNT, 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole; MW, microwave; NLS, nuclear location signal; NMI, *N*-methylimidazole; Pbf, (2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl); PBS, phosphate buffered saline; PEG, poly(ethylene glycol); PS, polystyrene; PyBOP, (benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate, SRB, sulforhodamine B; TBAF, tetrabutylammonium fluoride; TBDPS, *tert*-butyldiphenylsilyl; TBME, *tert*-butyl methyl ether; *t*Bu, *tert*-butyl; TCFH, *N*,*N*,*N*′,*N*′-tetramethylchloroformamidinium hexafluorophosphate; TFFH, *N*,*N*,*N*′,*N*′-tetramethylfluoroformamidinium hexafluorophosphate; TFA, trifluoroacetic acid; THF, tetrahydrofurane; Topo, Topoisomerase.

10.1021/bc800504t CCC: $40.75
Published on Web 05/27/2009

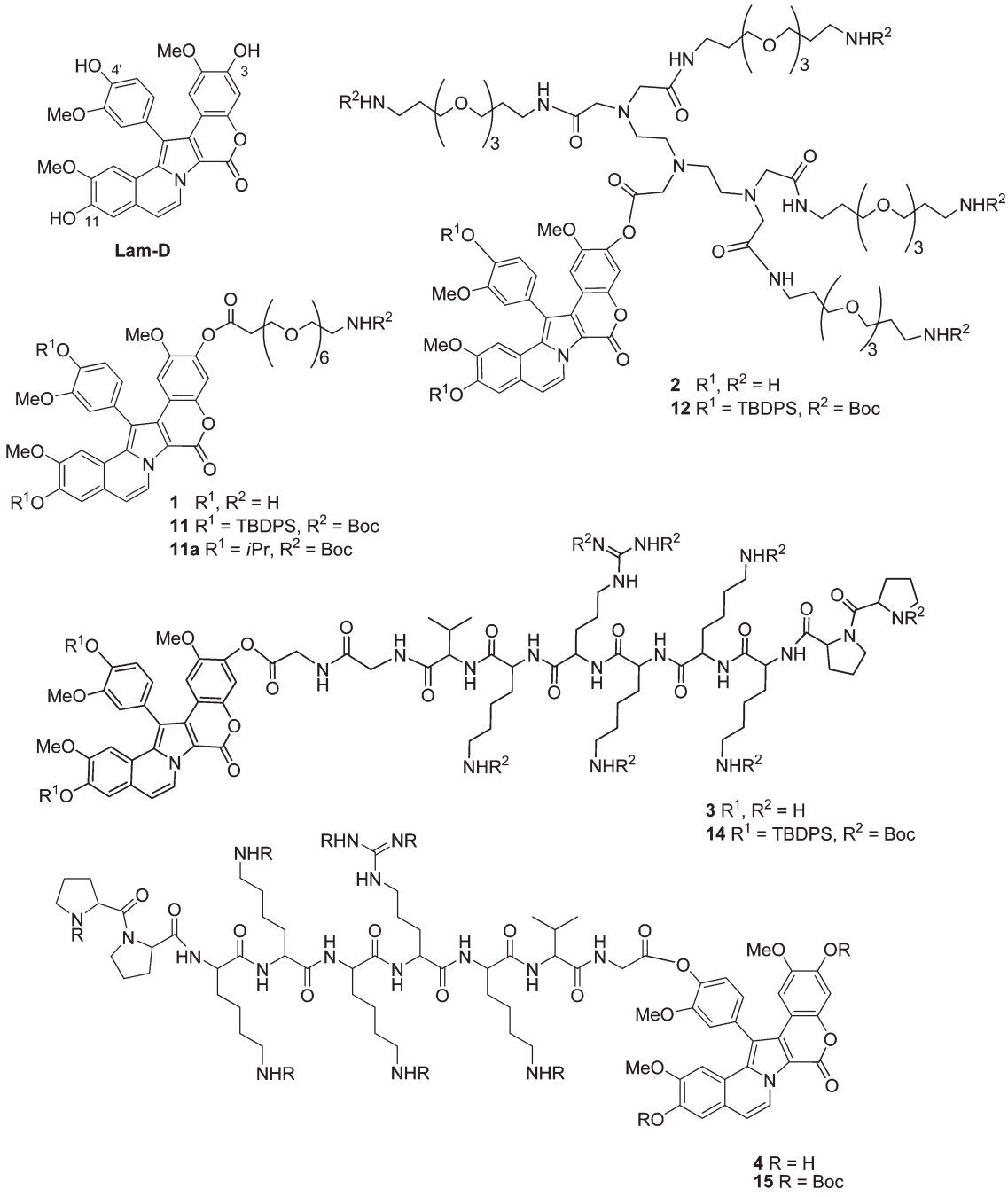


Figure 1. Structures of Lam-D conjugates.

in a Büchi Melting Point B540 in open capillaries and are uncorrected. Automatic flash chromatography was done in an Isco Combiflash medium-pressure liquid chromatograph with Redisep silica gel columns (47−60 μm). Sonication was performed in a Branson ultrasound bath. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer and a Gemini 200 MHz spectrometer. Multiplicity of the carbons was assigned with DEPT and gHSQC experiments, using standard abbreviations for off-resonance decoupling: (s) singlet, (d) doublet, (t) triplet, (q) quartet. The same abbreviations were also used for the multiplicity of signals in ^{1}H NMR, plus (m) multiplet, (bs) broad singlet, (bd) broad doublet. Spectra were referenced to appropriate residual solvent peaks (d_6-acetone, d_6-DMSO, d_4-MeOH, or $CDCl_3$). IR spectra were obtained on a Thermo Nicolet FT-IR spectrometer. HRMS were performed on a Bruker Autoflex high-resolution mass spectrometer by *Unidad de Espectrometría de Masas* (Universidad de Santiago de Compostela) and by *Servei d'Espectrometria de Masses* (Universitat de Barcelona). Microwave-assisted reactions were carried out in a CEM Discover microwave. The automatic syringe pump was used as specified for controlled addition of some reactants. Reversed-phase analytical HPLC was performed on a Waters Alliance separation module 2695 using a Waters Xterra MS C_{18} column (150 × 4.6 mm, 5 μm) and a Waters 996 PDA detector at 254 nm.

General Procedures. *A. General Method for Simultaneous Removal of TBDPSO and N-Boc.* HF (5 mL) at −196 °C was poured over solid **11**, **12**, or **14** (0.05−0.08 mmol). The solution was stirred for 1 min, and the solvent was immediately removed under vacuum at low temperature. MeCN was added to the crude, and the deprotected compound was precipitated by addition of MTBE, cooling to 0 °C and centrifugation (10 min, 2000 rpm). The residue was dried in vacuo to give the final Lam-D conjugates **1**−**3**.

B. General Method for MOMO Deprotection. Me_3SiI (142 μL, 3.00 mmol) was added at r.t. to a solution of **10a** or **10b** (1.00 mmol) in CH_2Cl_2 (225 mL), and the resulting orange solution was stirred at r.t. for 20 min. The solvent was removed in vacuo, and the residue was dissolved with EtOAc and then washed three times with sat NH_4Cl and brine. The organic phase was dried over anhydrous $MgSO_4$, and the solvent was removed in vacuo. Purification by column chromatography on silica gel by elution with hexane/EtOAc (80:20 to 60:40) gave the title compounds (84% quant yield).

C. General Method for Esterification. Synthesis of Conjugates. DMAP (0.6 mmol) and **5** (1 mmol) in dry CH_2Cl_2 (45 mL) were added to a solution of NHBocPEG$_6$-OH, or Boc-NLS-Gly-OH (4 mmol), and EDC·HCl (4 mmol) in dry CH_2Cl_2 (5 mL). The resulting solution was stirred at r.t. for 2 h. The reaction mixture was diluted with CH_2Cl_2 and washed with sat $NaHCO_3$ solution and brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed under vacuum, to provide the title compounds **11** and **13** (89% quant yield).

3-[3-(2-(2-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethoxy)-ethoxy)ethoxy)propanoyloxy]Lam-D (*22*) (1). Following the general procedure **A** and starting from **11** (24, 23, and 23 mg, 0.05 mmol), a yellow solid (35 mg, 84%) was obtained. The spectroscopic data are in accordance with previous reports (*9*).

3-[2-(Bis(2-(bis(2-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propylamino)-2-oxoethyl)amino)ethyl)amino)acetyl]Lam-D (*22*) (2). Following the general procedure **A** and starting from **12** (17 and 15 mg, 0.12 mmol), **2** as a yellow solid (8 mg, 36%) was obtained. ^{1}H NMR (D_2O, 400 MHz) δ 1.54 and 1.67 (2m, J = 6.6 Hz, 8H); 1.76 and 1.83 (2m, J = 6.6 Hz, 8H); 2.93 (2t, J = 7.2 Hz, 4H); 2.97 (2t, J = 7.2 Hz, 4H); 3.02−3.10 (m, 8H); 3.16−3.29 (m, 11H, OMe); 3.33−3.43 (m, 43H, OMe); 3.52−3.56 (m, 18H); 3.70 (br, 3H, OMe); 6.43 (s, 1H); 6.57 (br, 1H); 6.71 (br, 1H); 6.81−6.88 (m, 4H); 6.99 (br, 1H); 8.35 (br, 1H). ^{13}C NMR (D_2O, 100 MHz) δ 26.6 (t); 26.7 (t); 28.5 (t); 28.6 (t); 36.4 (t); 36.5 (t); 37.7 (t); 37.8 (t); 55.2 (q); 55.5 (q); 55.7 (q); 57.6 (t); 57.7 (t); 68.3 (t); 68.4 (t); 68.5 (t); 69.4 (t); 69.5 (t); 69.6 (t); 69.7 (t); 107.7 (d); 111.5 (d); 111.7 (d); 122.2 (d); 122.4 (d); 122.8 (d); 127.6 (d); 145.6 (d); 146.7 (d); 147.4 (s); 149.5 (s); 149.9 (s); 150.3 (s); 151.1 (s); 171.0 (s); 171.1 (s). MS (MALDI-TOF) 1684 (M+1, 100), 1685 (M+2, 93) 1686 (M+3, 49). HRMS *m/z* calcd for $C_{82}H_{131}N_{12}O_{25}$ 1683.9342, found 1683.9346. HPLC analysis: 6.9 min retention time (94% purity), with a gradient of 0% to 100% of eluent **B** over 15 min using the solvent system: H_2O/0.045% TFA (**A**) and MeCN/0.036% TFA (**B**).

3-[Gly-Gly-NLS]Lam-D (*22*) (3). Following the general procedure **A** and starting from **14** (28 and 17 mg, 0.16 mmol), **3** as a white solid (10 mg, 38%) was obtained. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.83−0.88 (m, 6H, 2CH$_3$); 1.22−1.36 (m, 10H, 5CH$_2$); 1.42−1.54 (m, 8H, 4CH$_2$); 1.56−1.62 (m, 8H, 4CH$_2$); 1.88−1.93 (m, 4H, 2CH$_2$); 2.05 (br, 2H, CH$_2$); 2.07 (br, 2H, CH$_2$); 2.33 (t, J = 1.8 Hz, 2H, CH$_2$); 2.55 (br, 1H, CH); 2.67 (t, J = 1.8 Hz, 2H, CH$_2$); 2.69−2.75 (m, 16H, 3CH$_2$); 3.36 (2s, 6H, 2OMe); 3.75 (s, 3H, OMe); 3.99−4.05 (m, 3H, CH); 4.14−4.25 (m, 7H, 3CH, 2CH$_2$); 4.58 (br, 1H, CH); 5.93 (br, 1H); 5.76 (br, 1H); 5.47 (br, 1H); 6.70 (s, 1H); 6.87 (s, 1H); 7.01 (dd, J = 8.0, 1.7 Hz, 1H); 7.10 (d, J = 8.0 Hz, 1H); 7.12−7.13 (m, 2H); 7.19 (s, 1H); 7.21 (br, 1H); 9.00 (d, J = 7.4 Hz, 1H, H8). MS (MALDI-TOF) 1575 (M+1, 100), 1576 (M+2, 92). HRMS *m/z* calcd. for $C_{77}H_{111}N_{18}O_{18}$ 1575.8324, found 1575.8319. HPLC analysis: 1.8 min retention time (96.7% purity), with 15 min isocratic MeCN/0.036% TFA.

4′-[Gly-NLS]Lam-D (*22*) (4). Peptide **8** (392 mg, 0.23 mmol) was preactivated for 15 min at r.t. with TCFH (63 mg, 23 mmol) and NEt_3 (32 μL, 23 mmol) in dry CH_2Cl_2 (15 mL). Compound **6** (53 mg, 0.08 mmol) and DMAP (9 mg, 0.08 mmol) in dry CH_2Cl_2 (5 mL) were then added. The resulting solution was stirred at r.t. for 120 h. The reaction mixture was diluted with CH_2Cl_2 and washed with sat $NaHCO_3$ solution and brine. The organic phase was dried over anhydrous $MgSO_4$, and the solvent was removed under vacuum. The residue was purified by flash chromatography: elution with CH_2Cl_2/MeOH (99:1 to 95:5) gave **15** (45% yield based on 40% transformation of **6**). The Boc protected compound **15** was then treated with 40% TFA in CH_2Cl_2 (10 mL) at r.t. for 1 h. The solvent was removed under reduced pressure, and the residue was purified by reverse-phase chromatography. Elution with H_2O/MeCN (60:40 to 40:60) gave **4** as a yellow solid (3 mg, 17%). ^{1}H NMR (D_2O, 500 MHz) δ 1.04 (d, J = 6.5 Hz, 3H, CH$_3$); 1.16 (d, J = 6.5 Hz, 3H, CH$_3$); 1.39−1.56 (m, 8H, 4CH$_2$); 1.66−1.77 (m, 10H, 5CH$_2$); 1.78−1.88 (m, 8H, 4CH$_2$); 2.00−2.12 (m, 4H, 2CH$_2$); 2.17 and 2.36 (2br, 2H, CH$_2$); 2.44 and 2.58 (2br, 2H, CH$_2$); 2.84 (br, 1H, CH); 2.96−3.05 (m, 10H, 5CH$_2$); 3.40 (br, 3H, OMe); 3.44 (s, 3H, OMe); 3.45 (s, 3H, OMe); 3.22 (t, J = 7.5 Hz, 1H, CH$_2$); 3.59 (t, J = 7.0 Hz, 1H, CH$_2$); 3.81 (br, 2H, CH$_2$); 4.23−4.42 (m, 5H, 5CH); 4.51 (t, J = 8.3 Hz, 1H, CH); 4.65 (dd, J = 9.0, 8.4 Hz, 1H, CH); 6.38 (s, 1H); 6.64−6.87 (m, 4H); 6.99 (br, 1H); 7.01 (br, 1H); 7.32 (br, 1H); 8.54 (br, 1H). MS (MALDI-TOF) 1518 (M+1, 100), 1519 (M+2, 82). HRMS *m/z* calcd. for $C_{75}H_{108}N_{17}O_{17}$ 1518.8109, found 1518.8114. HPLC analysis: 6.9 min retention time (95% purity), with a gradient of 50% to 100% of eluent **B** over 15 min using the solvent system: H_2O/0.045% TFA (**A**) and MeCN/0.036% TFA (**B**).

4′,11-Bis(*tert*-butyldiphenylsilyl)Lam-D (*22*) (5). Following the general procedure **B** and starting from **10b** (1.06 g, 1.04 mmol), **5** as a yellow solid (856 mg, 84%) was obtained. mp (MeCN) 278−280 °C. IR (film) ν 1704, 1487, 1428, 1284, 1111 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.15 (s, 9H); 1.17 (s, 9H); 3.18 (s, 3H, OMe); 3.47 (s, 3H, OMe); 3.64 (s, 3H, OMe); 5.89 (s, 1H); 6.68 (d, J = 7.4 Hz, 1H, H9); 6.69 (s, 1H); 6.91−6.93 (m, 3H); 6.95 (s, 1H); 7.05 (br, 1H); 7.06 (br, 1H); 7.37−7.48 (m, 10H); 7.71−7.79 (m, 10H); 9.04 (d, J = 7.4 Hz, 1H, H8). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.9 (s); 26.6 (q); 26.7 (q); 54.5 (q); 55.5 (q); 55.7 (q); 103.4 (d); 104.7 (d); 105.9 (d); 107.6 (s); 109.8 (s); 111.0 (s); 112.1 (d); 115.4 (d); 116.5 (d); 119.7 (s); 120.3 (d); 122.7 (d); 123.7 (d); 124.4 (s); 127.5 (d); 127.6 (d); 128.5 (s); 129.1 (s); 129.7 (d); 129.9 (d); 132.9 (s); 133.0 (s); 133.4 (s); 134.1 (s); 135.0 (d); 135.1 (d); 143.1 (s); 144.9 (s); 146.1 (s); 146.8 (s); 150.7 (s); 151.1 (s); 155.3 (s). MS (MALDI-TOF) 976 (M+1, 100), 977 (M+1, 57), 978 (M+2, 38).

3,11-Di-*tert*-butoxycarbonyl-Lam-D (*22*) (6). A solution of TBAF in THF (1 M, 1.64 mmol) was added to a −78 °C solution of **10d** (771 mg, 0.82 mmol), in MeOH-THF (100 mL, 80:20). The mixture was stirred at −78 °C for 15 min. Solvents were removed under vacuum, and the residue was dissolved in CH_2Cl_2. The organic solution was washed with water and brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed under vacuum. The residue was purified by flash chromatography: elution with hexane/EtOAc (60:40 to 40:60) gave **6** as a white solid (491 mg, 85%). mp (MeCN) 149−150 °C. IR (film) ν 1760, 1709, 1275, 1255 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.54 (s, 9H); 1.55 (s, 9H); 3.455 (s, 3H, OMe); 3.463 (s, 3H, OMe); 3.91 (s, 3H, OMe); 5.93 (br, 1H, OH); 6.83 (s, 1H); 6.97 (d, J = 7.4 Hz, 1H, H9); 7.11 (br, 1H); 7.15 (dd, J = 8.0, 1.8 Hz, 1H); 7.18 (br, 1H); 7.24−7.26 (m, 2H); 7.44 (br, 1H); 9.14 (d, J = 7.4 Hz, 1H, H8). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.5 (q); 55.3 (q); 55.6 (q); 56.3 (q); 83.89 (s); 83.95 (s); 106.4 (d); 106.6 (d); 108.8 (s); 111.8 (d); 112.6 (d); 113.0 (s); 113.5 (d); 115.4 (d); 115.7 (s); 120.3 (d); 123.0 (d); 123.6 (s); 123.8 (s); 124.0 (s); 124.4 (d); 128.1 (s); 128.3 (s); 133.6 (s); 140.0 (s); 141.0 (s); 145.4 (s); 145.9 (s); 147.7

(s); 147.9 (s); 150.9 (s); 151.0 (s); 151.1 (s); 155.0 (s). MS (MALDI-TOF) 699 (M, 56), 700 (M+1, 100).

Boc-Pro-Pro-Lys(Boc)-Lys(Boc)-Lys(Boc)-Arg(Boc$_2$)-Lys-(Boc)-Val-Gly-OH (8). The Boc protected peptide was synthesized manually on solid phase in a polypropylene syringe fitted with a porous polyethylene disk. Solvents and soluble reagents were removed by suction. Washings between deprotection, coupling, and subsequent deprotection steps were carried out with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min) using 10 mL solvent/g resin each time. Standard Fmoc/*t*Bu chemistry and chlorotrityl resin (0.5 g, 1.5 mmol/g) were used. The resin was preswollen in anhydrous CH_2Cl_2 and then in DMF. The first Fmoc-protected amino acid (Fmoc-L-Gly-OH) (155 mg, 0.7 equiv) was introduced in the presence of DIEA (635 μL, 5 equiv) in DMF. After 1 h, MeOH (0.5 mL) was added and the mixture was stirred for 30 min. The resin was then washed with DMF and CH_2Cl_2, and the synthesis continued as described below. The peptide was elongated through successive iterations of Fmoc removal and amino acid coupling. The Fmoc protecting group was removed with several treatments of 20% piperidine in DMF (1 × 1 min + 2 × 15 min). The resin was then washed with DMF and CH_2Cl_2. The corresponding Fmoc-protected amino acid (5 equiv) was introduced using DIPCDI (310 μL, 5 equiv) and HOBt (305 mg, 5 equiv) as coupling agents. After 2 h, the resin was washed with DMF and CH_2Cl_2, and the coupling was monitored using the Kaiser test. Recouplings were done when needed. Boc-L-Pro-OH (430 mg, 5 equiv) was used as a last amino acid. The peptide was finally cleaved from the resin using 3% TFA in CH_2Cl_2, (5 × 1 min). Washes were collected in a flask containing 50 mL of water. The CH_2Cl_2 was then evaporated under reduced pressure, MeCN (30 mL) was added to the aqueous solution, and the resulting mixture was then lyophilized. Peptide **8** (590 mg, 94%) was obtained as a white solid. HPLC analysis: 6.2 min retention time (92% purity), with a gradient of 50% to 100% of eluent **B** in 7 min using the solvent system: H_2O/0.045% TFA (**A**) and MeCN/0.036% TFA (**B**). HPLC ESI-MS calcd. for $C_{82}H_{144}N_{16}O_{24}$ $[M+H]^+$ 1738, found: $[M+2]^{2+}$/2, 870.

11-Benzyl-4′-*tert*-butyldiphenylsilyl-3-methoxymethyl-Lam-D (*22*) (10). A mixture of **9** (*27*) (1.54 g, 1.76 mmol) and DDQ (400 mg, 1.76 mmol) in dry $CHCl_3$ (25 mL) was purged with Ar in a sealed vessel and microwaved at 120 °C for 10 min. The organic solution was washed with water, and brine, dried over $MgSO_4$, filtered, and then concentrated in vacuo. Purification by column chromatography on silica gel by elution with hexane/EtOAc (80:20 to 60:40) gave **10** as a white solid (2.27 g, 81%). mp (MeCN) 144−145 °C. IR (film) ν 1705, 1429, 1267, 1223 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.17 (s, 9H); 3.43 (s, 3H, OMe); 3.46 (s, 3H, OMe); 3.50 (s, 3H, OMe); 3.65 (s, 3H, OMe); 5.23 (s, 2H); 5.24 (s, 2H); 6.77 (s, 1H); 6.92−6.96 (m, 3H); 7.06−7.08 (m, 2H); 7.19 (s, 1H); 7.25 (d, J = 5.4 Hz, 1H); 7.32−7.47 (m, 11H); 7.75−7.79 (m, 4H); 9.16 (d, J = 7.2 Hz, 1H, H8). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 19.9 (s); 26.7 (q); 55.2 (q); 55.5 (q); 55.7 (q); 56.3 (q); 70.7 (t); 95.4 (t); 105.1 (d); 105.5 (d); 105.6 (d); 109.4 (d); 111.3 (s); 111.4 (s); 112.3 (d); 115.4 (d); 119.3 (s); 120.3 (d); 123.1 (d); 123.7 (d); 124.5 (s); 127.2 (d); 127.7 (d); 128.0 (d); 128.4 (s); 128.5 (s); 128.6 (d); 128.9 (s); 129.9 (d); 133.3 (s); 134.1 (s); 135.1 (d); 136.2 (s); 145.0 (s); 146.0 (s); 146.2 (s); 146.6 (s); 149.0 (s); 149.5 (s); 151.2 (s); 155.3 (s). MS (MALDI-TOF) 871 (M, 18), 872 (M+1, 100).

4′-*tert*-Butyldiphenylsilyl-3-methoxymethyl-Lam-D (*22*) (10a). Pd/C (10%) was added to a solution of **10** (509 mg, 0.58 mmol) in MeOH/EtOAc (2:1, 58 mL), the mixture was purged with H_2, and the resulting suspension was stirred at r.t. for 16 h. The reaction mixture was filtered through a pad of Celite, which was then washed with CH_2Cl_2. The solvent was removed under vacuum to provide the **10a** as a brown solid (433 mg, 95%). mp (MeCN) 129−130 °C. IR (film) ν 3213, 1680, 1425, 1222 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.16 (s, 9H); 3.44 (s, 3H, OMe); 3.47 (s, 3H, OMe); 3.51 (s, 3H, OMe); 3.65 (s, 3H, OMe); 5.24 (s, 2H); 5.92 (br, 1H, OH); 6.77 (s, 1H); 6.93−6.94 (m, 2H); 6.98 (d, J = 7.4 Hz, 1H, H9); 7.05 (br, 1H); 7.13 (s, 1H); 7.16 (s, 1H); 7.25 (d, J = 5.6 Hz, 2H); 7.38−7.47 (m, 5H); 7.75−7.78 (m, 4H); 9.18 (d, J = 7.4 Hz, 1H, H8). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.8 (s); 26.7 (q); 55.3 (q); 55.5 (q); 55.7 (q); 56.3 (q); 95.5 (t); 105.0 (d); 105.3 (d); 105.6 (d); 110.8 (d); 111.2 (s); 111.5 (s); 112.4 (d); 115.4 (d); 118.8 (s); 120.4 (d); 123.3 (d); 123.7 (d); 125.3 (s); 127.7 (d); 128.6 (s); 129.0 (s); 129.96 (d); 129.98 (d); 133.37 (s); 133.43 (s); 134.4 (s); 135.2 (d); 135.3 (s); 145.1 (s); 146.1 (s); 146.3 (s); 146.66 (s); 146.69 (s); 146.8 (s); 151.3 (s); 155.4 (s). MS (MALDI-TOF) 781 (M, 60), 782 (M+1, 100).

4′,11-Bis(*tert*-butyldiphenylsilyl)-3-methoxymethyl-Lam-D (10b). TBDPSCl (389 μL, 1.07 g/mL, 1.47 mmol) was added to a solution of **10a** (768 mg, 0.98 mmol), imidazole (135 mg, 1.96 mmol), and DMAP (120 mg, 0.98 mmol) in dry DMF (30 mL). The mixture was stirred for 24 h under Ar. DMF was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2. The organic phase was washed with water and brine, and then dried over anhydrous $MgSO_4$. The solvent was removed under vacuum, and the residue was purified by flash chromatography. Elution with hexane/CH_2Cl_2 (40:60 to 20:80) gave **10b** (584 mg, 58%) as a brown oil. IR (film) ν 1679, 1426, 1113 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.13 (s, 9H); 1.15 (s, 9H); 3.17 (s, 3H, OMe); 3.42 (s, 3H, OMe); 3.49 (s, 3H, OMe); 3.61 (s, 3H, OMe); 5.21 (s, 2H); 6.71 (d, 1H, J = 7.4 Hz, H9); 6.74 (s, 1H); 6.90 (2s, 2H); 6.92 (s, 1H); 7.01−7.05 (m, 2H); 7.22 (br, 1H); 7.10−7.19 (m, 10H); 7.69−7.77 (m, 10H); 9.06 (d, J = 7.4 Hz, 1H, H8). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.9 (s); 26.6 (q); 26.7 (q); 54.5 (q); 55.4 (q); 55.7 (q); 56.3 (q); 95.4 (t); 105.1 (d); 105.5 (d); 105.9 (d); 107.9 (s); 111.3 (s); 111.4 (s); 112.3 (d); 115.3 (d); 116.5 (d); 119.7 (s); 120.3 (d); 122.7 (d); 123.6 (d); 124.4 (s); 127.4 (d); 127.6 (d); 127.7 (d); 127.8 (d); 128.4 (s); 128.8 (s); 129.5 (d); 129.7 (d); 130.0 (d); 132.9 (s); 133.0 (s); 133.4 (s); 134.7 (d); 135.0 (d); 135.1 (d); 144.9 (s); 145.9 (s); 146.2 (s); 146.5 (s); 150.7 (s); 151.1 (s); 155.3 (s). MS (ESI) 1020 (M+1, 100).

4′-*tert*-Butyldiphenylsilyl-Lam-D (*22*) (10c). Following the general procedure **B** and starting from **10a** (639 mg, 0.81 mmol), **10c** as a white solid (603 mg, quant) was obtained. mp (MeCN) 275−276 °C. IR (film) ν 3415, 1679, 1429, 1271 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.17 (s, 9H); 3.46 (s, 3H, OMe); 3.47 (s, 3H, OMe); 3.65 (s, 3H, OMe); 6.71 (s, 1H); 6.93−6.94 (m, 2H); 6.98−7.05 (m, 3H); 7.13 (s, 1H); 7.17 (s, 1H); 7.39−7.47 (m, 6H); 7.76−7.79 (m, 4H); 9.17 (d, J = 7.4 Hz, 1H, H8). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.9 (s); 26.7 (q); 55.3 (q); 55.5 (q); 55.7 (q); 103.6 (d); 104.7 (s); 104.8 (d); 105.0 (d); 107.8 (s); 109.9 (s); 110.8 (d); 110.9 (s); 112.3 (d); 115.5 (d); 118.8 (s); 120.4 (d); 123.4 (d); 123.8 (d); 125.4 (s); 127.7 (s); 127.9 (d); 129.3 (s); 130.0 (d); 133.4 (s); 133.5 (s); 135.2 (d); 143.2 (s); 145.1 (s); 146.2 (s); 146.7 (s); 146.8 (s); 147.0 (s); 151.3 (s); 155.5 (s). MS (ESI-TOF) 737 (M, 73), 738 (M+1, 100).

4′-*tert*-Butyldiphenylsilyl-3,11-bis(*tert*-butoxycarbonyl)-Lam-D (*22*) (10d). $(Boc)_2O$ (536 mg, 2.45 mmol) and DMAP (30 mg, 0.25 mmol) were added to a solution of **10c** (605 mg, 0.82 mmol) in CH_2Cl_2 (130 mL). The reaction mixture was stirred at r.t. under Ar for 16 h. The mixture was washed with sat $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness to give **10d** as a yellow solid (767 mg, quant). mp (MeCN) 188−189 °C. IR (film) ν 1760, 1710, 1486, 1430, 1274, 1255 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.15 (s, 9H); 1.56 (s, 9H); 1.57 (s, 9H); 3.40 (s, 3H, OMe); 3.42 (s, 3H, OMe); 3.64 (s, 3H, OMe);

6.83 (s, 1H); 6.90−6.93 (m, 2H); 7.01 (br, 1H); 7.04 (d, J = 7.4 Hz, 1H, H9); 7.23 (s, 1H); 7.25 (d, J = 5.8 Hz, 2H); 7.38−7.46 (m, 6H); 7.74−7.77 (m, 4H); 9.22 (d, J = 7.4 Hz, 1H, H8). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.9 (s); 26.7 (q); 27.6 (q); 55.3 (q); 55.6 (q); 55.7 (q); 83.96 (s); 84.02 (s); 106.5 (d); 106.7 (d); 108.9 (s); 111.9 (d); 112.7 (d); 113.1 (s); 115.0 (d); 115.8 (s); 120.3 (d); 120.5 (d); 123.3 (d); 123.4 (d); 123.7 (s); 123.9 (s); 127.7 (s); 127.9 (d); 128.3 (s); 130.0 (d); 133.4 (s); 133.6 (s); 135.1 (d); 140.0 (s); 141.0 (s); 145.3 (s); 145.5 (s); 147.7 (s); 150.8 (s); 151.0 (s); 151.2 (s); 151.5 (s); 155.1 (s). MS (MALDI-TOF) 938 (M+1, 100), 939 (M+2, 63).

4′,11-Bis(*tert*-butyldiphenylsilyl)-3-[3-(2-(2-(2-(2-(2-(2-*tert*-butoxycarbonylaminoethoxy)ethoxy)ethoxy)ethoxy)ethoxy)-ethoxy)propanoyl]Lam-D (*22*) (11). Following the general procedure **C** and starting from **5** (78 mg, 0.80 mmol), **11** as a yellow oil (112 mg, quant) was obtained. IR (film) ν 1710, 1486, 1429, 1283, 1159, 1114 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.04 (s, 9H); 1.06 (s, 9H); 1.36 (s, 9H); 2.81 (t, J = 6.5 Hz, 2H); 3.07 (s, 3H, OMe); 3.24 (br, 4H, $2CH_2$); 3.29 (s, 3H, OMe); 3.43−3.48 (m, 4H, $2CH_2$); 3.52−3.60 (m, 19H, $8CH_2$, OMe); 3.79 (t, J = 6.5 Hz, 2H); 5.05 (br, 1H); 6.68 (d, J = 7.4 Hz, 1H, H9); 6.75 (s, 1H); 6.81 (br, 2H); 6.85 (s, 1H); 6.91 (br, 1H); 6.95 (br, 1H); 7.03 (s, 1H); 7.24−7.38 (m, 10H); 7.61−7.68 (m, 10H); 9.00 (d, J = 7.4 Hz, 1H, H8). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.8 (s); 26.5 (q); 26.7 (q); 28.4 (q); 34.7 (t); 40.3 (t); 54.4 (q); 55.4 (q); 55.6 (q); 66.3 (t); 70.1 (t); 70.5 (t); 105.8 (d); 106.3 (d); 108.1 (s); 111.8 (s); 111.9 (d); 112.6 (d); 115.1 (d); 116.0 (s); 116.5 (d); 119.6 (s); 120.3 (d); 122.5 (d); 123.5 (d); 124.3 (s); 127.4 (s); 127.5 (d); 127.6 (d); 128.0 (s); 128.1 (s); 129.7 (d); 129.8 (d); 132.8 (s); 132.9 (s); 133.3 (s); 134.2 (s); 134.9 (d); 135.1 (d); 139.2 (s); 144.9 (s); 145.3 (s); 146.2 (s); 147.2 (s); 150.8 (s); 151.1 (s); 154.9 (s); 169.2 (s). MS (MALDI-TOF) 1311 (M+1-Boc, 100), 1433 (M+Na, 45).

4′,11-Bis(*tert*-butyldiphenylsilyl)-3-[2-(bis(2-(bis(2-(3-(2-(2-(3-*tert*-butoxycarbonylamino-propoxy)ethoxy)ethoxy)propyl-amino)-2-oxoethyl)amino)ethyl)amino)acetyl]-Lam-D (*22*) (12). A mixture of polystyrene solid supported DMAP (6.0 mg, 19 mmol) and **5** (31 mg, 32 mmol) in dry CH_2Cl_2 (2 mL) was added to a solution of **7** (51 mg, 32 mmol) and EDC·HCl (6 mg, 32 mmol) in dry CH_2Cl_2 (2 mL). The resulting solution was stirred at r.t. for 16 h. The reaction mixture was filtered, and the solvent was removed under vacuum. The residue was purified by flash chromatography with neutral alumina. Elution with CH_2Cl_2/MeOH (99:1 to 98:2) gave **12** as a yellow oil (22 mg, 26%). IR (film) ν 3323, 1665, 1548, 1428, 1275, 1112 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.11 (s, 9H); 1.13 (s, 9H); 1.41 (s, 18H); 1.42 (s, 18H); 1.61−1.77 (m, 16H); 2.34 (2t, 4H); 2.71−2.78 (m, 4H); 3.12 (s, 3H, OMe); 3.15−3.31 (m, 26H); 3.35 (s, 3H, OMe); 3.47−3.67 (m, 43H); 5.06 (br, 2H); 5.16 (br, 1H); 5.30 (br, 1H); 6.77 (d, J = 7.4 Hz, 1H, H9); 6.84 (s, 1H); 6.88 (br, 2H); 6.92 (s, 1H); 6.97 (br, 1H); 7.00 (br, 1H); 7.12 (s, 1H); 7.32−7.45 (m, 10H); 7.64−7.73 (m, 10H); 9.08 (d, J = 7.4 Hz, 1H, H8). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.4 (q); 19.8 (s); 26.6 (q); 26.7 (q); 27.3 (t); 28.4 (q); 29.4 (t); 29.7 (t); 35.3 (t); 37.0 (t); 38.4 (t); 39.5 (t); 54.5 (q); 55.4 (q); 55.7 (q); 57.2 (t); 59.0 (t); 69.3 (t); 69.4 (t); 70.1 (t); 70.4 (t); 70.5 (t); 105.9 (d); 106.4 (d); 108.2 (s); 112.0 (d); 112.9 (d); 115.2 (d); 116.2 (s); 116.6 (d); 119.7 (s); 120.4 (d); 123.5 (d); 124.4 (s); 127.6 (d); 127.7 (d); 128.0 (s); 128.1(s); 129.8 (d); 129.9 (d); 130.0 (s); 132.9 (s); 133.0 (s); 133.4 (s); 134.4 (s); 135.1 (d); 135.2 (d); 138.9 (s); 145.2 (s); 145.4 (s); 146.4 (s); 147.2 (s); 150.9 (s); 151.3 (s); 154.9 (s); 156.1 (s); 159.1 (s); 169.2 (s); 170.7 (s). MS (ESI-TOF) 820 ([M-Boc+3]/3, 100).

4′,11-Bis(*tert*-butyldiphenylsilyl-3-(*tert*-butoxycarbonyl-aminoacetyl)-Lam-D (*22*) (13). Following the general procedure **C** and starting from **5** (99 mg, 1.02 mmol), **13** was obtained as a yellow solid (103 mg, 89%). mp (MeCN) 197−199 °C. IR (film) ν 1711, 1509, 1486, 1429, 1283, 1158 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.14 (s, 9H); 1.15 (s, 9H); 1.49 (s, 9H); 3.17 (s, 3H, OMe); 3.37 (s, 3H, OMe); 3.62 (s, 3H, OMe); 4.23 (brd, J = 5.0 Hz, 2H); 5.13 (brt, J = 5.0 Hz, 1H); 6.73 (d, J = 7.4 Hz, 1H, H9); 6.85 (s, 1H); 6.91 (br, 2H); 6.93 (s, 1H); 7.02 (br, 1H); 7.05 (br, 1H); 7.12 (br, 1H); 7.36−7.48 (m, 10H); 7.70−7.77 (m, 10H); 9.05 (d, J = 7.4 Hz, 1H, H8). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.9 (s); 26.6 (q); 26.7 (q); 28.3 (q); 42.3 (t); 54.5 (q); 55.5 (q); 55.6 (q); 105.8 (d); 106.4 (d); 108.1 (s); 111.7 (d); 111.9 (s); 112.7 (d); 115.2 (d); 116.3 (s); 116.6 (d); 119.7 (s); 120.3 (d); 122.5 (d); 123.5 (d); 124.3 (s); 127.5 (s); 127.6 (d); 127.7 (d); 127.9 (s); 128.1 (s); 129.6 (s); 129.7 (d); 129.9 (d); 132.9 (s); 133.3 (s); 134.2 (s); 135.0 (d); 135.2 (d); 135.4 (s); 138.8 (s); 145.0 (s); 145.3 (s); 146.3 (s); 147.0 (s); 150.8 (s); 151.2 (s); 154.8 (s); 168.3 (s). MS (MALDI-TOF) 1132 (M, 100), 1133 (M+1, 79), 1134 (M+2, 37).

4′,11-Bis(*tert*-butyldiphenylsilyl)-3-[Gly-Gly-Boc-NLS]-Lam-D (*22*) (14). Lamellarin **13** (32 mg, 29 mmol) was treated with 40% TFA in CH_2Cl_2 (10 mL) at 0 °C for 10 min. The solvent was removed under reduced pressure, and the crude was used without further purification. A solution of this residue and DIPEA (4.9 μL, 29 mmol) in dry CH_2Cl_2 (3 mL) was added to a solution of **8** (50 mg, 29 mmol), HOBt (5 mg, 34 mmol), DIPEA (4.9 μL, 29 mmol), and EDC·HCl (7 mg, 34 mmol) in dry CH_2Cl_2 (2 mL). The resulting solution was stirred at r.t. for 4 h. The reaction mixture was diluted with CH_2Cl_2 and washed with sat $NaHCO_3$ solution and brine. The organic phase was dried over anhydrous $MgSO_4$, and the solvent was removed under vacuum. The residue was purified by flash chromatography with neutral alumina: elution with CH_2Cl_2/MeOH (98:2 to 95:5) gave **14** as a yellow oil (46 mg, 58%). IR (film) 3328, 1650, 1534, 1429, 1365, 1281, 1166 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.92−1.00 (m, 6H, $2CH_3$); 1.11 (s, 3H, CH_3); 1.13 (s, 3H, CH_3); 1.23−1.33 (m, 8H, $4CH_2$); 1.36−1.37 (m, 4H, $2CH_2$); 1.39 (s, 3H, CH_3); 1.41 (s, 18H, $6CH_3$); 1.44−1.50 (m, 4H, $2CH_2$); 1.46−1.50 (m, 8H, $4CH_2$); 1.84 (br, 4H, $2CH_2$); 2.07 (br, 4H, $2CH_2$); 2.43 (br, 4H, $2CH_2$); 2.71 (h, J = 6.8 Hz, 1H); 3.00 and 3.07 (2br, 8H, $4CH_2$); 3.14 (s, 3H, OMe); 3.34−3.41 (m, 2H, CH_2); 3.44 (s, 3H, OMe); 3.59 (s, 3H, OMe); 3.62−3.74 (m, 2H, CH_2); 3.92−4.23 (m, 5H, 5CH); 4.28 (br, 2H, CH_2); 4.47−4.53 (m, 2H, CH_2); 4.67−4.71 (m, 3H, 3CH); 4.77 (br, 1H); 4.86 (br, 1H); 6.67 (s, 1H); 6.71 (d, J = 7.4 Hz, 1H, H9); 6.80−6.89 (m, 2H); 6.91 (d, J = 1.8 Hz, 1H); 6.95−6.99 (m, 2H); 7.02 (d, J = 8.2 Hz, 1H); 7.32−7.47 (m, 10H); 7.56−7.75 (m, 10H); 8.2 (br, 1H); 8.3 (br, 1H); 8.4 (br, 1H); 8.5 (br, 1H); 9.07 (d, J = 7.4 Hz, 1H, H8). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.8 (s); 23.6 (t); 23.7 (t); 24.6 (t); 26.2 (t); 26.6 (q); 26.7 (q); 28.0 (q); 28.3 (q); 28.4 (q); 29.5 (t); 29.7 (t); 30.2 (t); 40.4 (t); 47.1 (t); 54.4 (q); 55.48 (q); 55.53 (t); 55.6 (q); 56.4 (d); 56.5 (d); 56.6 (d); 56.7 (t); 63.1 (d); 63.6 (d); 83.2 (s); 103.5 (d); 104.8 (d); 105.9 (d); 109.8 (s); 111.1 (s); 112.2 (d); 115.4 (d); 116.6 (d); 119.7 (s); 120.3 (d); 122.7 (s); 123.7 (d); 124.4 (s); 127.5 (d); 127.6 (d); 127.7 (s); 128.6 (s); 129.2 (s); 129.8 (d); 129.9 (d); 130.0 (s); 132.9 (s); 133.0 (s); 133.4 (s); 135.0 (d); 135.2 (d); 143.3 (s); 145.0 (s); 146.2 (s); 146.3 (s); 146.9 (s); 150.8 (s); 151.2 (s); 153.4 (s); 155.2 (s); 155.9 (s); 156.0 (s); 156.1 (s); 157.4 (s); 170.4 (s); 171.9 (s); 173.1 (s); 174.7 (s); 175.3 (s). MS (ESI) 884 ([M-Boc+3]/3, 24), 1326 ([M-Boc+2]/2, 77).

Cell Lines and Culture. Human-derived established cell lines used in this study were purchased from ATCC (American type Culture Collection): A-549, human lung carcinoma (ATCC no. CCL-185); BJ, skin fibroblast (ATCC no. CRL-2522); HT-29, human colorectal adenocarcinoma (ATCC no. HTB-38); and MDA-MB 231, human breast adenocarcinoma (ATCC no. HTB-26). All cell lines were maintained in DMEM supplemented

with 10% FBS and 100 units/mL penicillin and streptomycin at 37 °C and 5% CO_2.

GI_{50} Analysis. Triplicate cultures were incubated for 72 h in the presence or absence of test compounds Lam-D, **1**, **2**, **4**, and **15** (at ten concentrations, typically ranging from 0.0026 to 10 μg/mL).

A colorimetric assay using SRB was adapted for quantitative measurement of cell growth and viability, following a previously described method (*23*). Cells were plated in 96-well microtiter plates at a density of 5×10^3/well and incubated for 24 h. One plate from each different cell line was fixed, stained, and used for Tz reference (see next paragraph). The cells were then treated with vehicle alone (control) or compounds at the concentrations indicated. Treated cells were incubated for an additional 72 h, and then evaluated for cytotoxicity via colorimetric analysis. The cells were washed twice with PBS, fixed for 15 min in 1% glutaraldehyde solution, rinsed twice in PBS, and stained in 0.4% SRB solution for 30 min at room temperature. The cells were then rinsed several times in 1% acetic acid solution and air-dried. SRB was then extracted in 10 mM trizma base solution and the absorbance measured at 490 nm. Cell survival is expressed as a percentage of control cell growth.

Dose−response curves were obtained by using the NCI algorithm (*24*): Tz = number of control cells at time t_0; C = number of control cells at time t; and T = number of treated cells at time t.

If $Tz < T < C$ (growth inhibition), then the result is $100 \times ([T - Tz]/[C - Tz])$.

If $T < Tz$ (net cell death), then the result is $100 \times ([T - Tz]/Tz)$.

After dose−curve generation, the results were expressed as GI_{50} (the concentration that causes 50% cell growth inhibition, compared to control cultures).

General Treatments for Imaging. A-549, MDA-MB-231, and HT-29 cells were seeded onto MatTek (Ashland, USA) glass-bottomed microwell dishes at 30×10^3 cells/cm^2. After 24 h, the culture medium was discarded and replaced by fresh DMEM medium containing Lam-D, **1**, or **4** at 1 μM. Absence of compound was used as a negative control. Cells were then incubated for 12 h at 37 °C.

Topo I-GFP Visualization. Procedure for a single microwell dish transfection: A-549, MDA-MB-231, and HT-29 cells were seeded onto a MatTek (Ashland, USA) glass-bottomed microwell dish at 30×10^3 cells/cm^2. Culture medium was discarded after 24 h, cells were washed 3 × PBS, and 1400 μL of reduced serum media Opti-MEM I (Invitrogen, USA) were added. The preparation of DNA for transfection required previous dilution of 3 μL FuGENE (Roche Molecular Biochemicals, Indianapolis, IN) in 100 μL of reduced serum media Opti-MEM I (Invitrogen, USA), and further addition of 6.7 μL of the plasmid solution encoding green fluorescent protein GFP with full-length Topo I (*25*). The mixture was mixed thoroughly and incubated for 30 min at r.t., before addition to the dish. Samples were incubated at 37 °C and 5% CO_2 for 10 h. Afterward, medium was discarded, cells were washed three times with PBS, and new DMEM solution containing 1 μM Lam-D, **1**, or **4** was added. Absence of compound was used as a negative control. The cells were incubated for an additional 12 h at 37 °C and then analyzed by confocal microscopy.

Confocal Laser Scanning Microscopy. Confocal laser scanning microscopy was performed with a Leica TCS SPII microscope (Leica Microsystems Heidelberg GmbH, Mannheim, Germany), using a 63× objective. GFP fluorescence was excited with an Ar laser excitation at 488 nm. Lam-D and its derivatives were excited at 351 nm. The same microscope settings were used for each conjugate and concentration. To avoid crosstalk, the two-fluorescence scanning was performed in a sequential mode.

Uptake Measurements by Fluorescence-Activated Cell Sorting Flow Cytometry. 1×10^6 A-549, BJ, MDA-MB-231, and HT-29 cells were seeded onto 25 cm^2 cell culture flasks (Nalgene Nunc International, Naperville, USA) with 10 mL of DMEM. After 12 h, the culture medium was discarded and replaced by fresh DMEM medium containing compounds 1 μM Lam-D, **1**, **2**, or **4**. Absence of compound was used as a negative control. The cells were then incubated for 12 h at 37 °C. After incubation, the cells were washed three times with PBS, detached with trypsin−EDTA 0.25%, and centrifuged at 1000× rpm. Finally, the medium was decanted, and the cellular pellet was resuspended in PBS and kept at 0 °C until measurements were performed. Fluorescence analysis was performed with a MoFlo cytometer (DakoCytomation, Colorado, USA), using the 351 nm excitation line of an Ar laser (25 mW) and emission detection at 450 nm (tolerance range ±65 nm). CIQ is expressed as a percentage value in reference to Lam-D. It was calculated by dividing the fluorescence value of the test compound by the fluorescence value obtained with the Lam-D control under the same experimental conditions.

RESULTS

Orthogonal protecting groups had to be used for the three phenol groups of Lam-D and for the functional groups presented into the conjugation building blocks. *N*-Boc was used to protect the amino and guanidino groups of the oligomeric building blocks **7** and **8**. Synthesis of compounds **2**−**4** required the preparation of different building blocks: the protected Lam-D derivatives **5** and **6**, the DTPA-PEG dendrimer **7**, and the peptide **8** (Figure 2). Compounds **5** and **6** are the precursors for the conjugates at positions 3 and 4′, respectively, and contain two phenolic groups protected as either TBDPS ethers or Boc esters, respectively. In the protected peptide **8**, a glycine was introduced as spacer at the C-terminal position to avoid the steric hindrance caused by the C-terminal Val residue during coupling.

Synthesis of Protected Lamellarins 5 and 6. The protecting groups *Oi*Pr/*O*Bn used in earlier strategies (*9*) required harsh deprotection conditions incompatible with the synthesis of more complex Lam-D conjugates such as **4**.[2] The previously described lamellarin **9** (*26, 27*), for which three different and orthogonal protecting groups were employed (MOM, Bn, and TBDPS), was used as the precursor to Lam derivatives **5** and **6** (Scheme 1).[3] Lam **9** was prepared following Banwell's strategy for the total synthesis of Lam-K (*28*). Oxidation of **9** under MW irradiation using DDQ in $CHCl_3$ gave **10** with good yield. Compound **10** was subjected to changes of protecting groups (see Scheme 1). Catalytic hydrogenation of **10** over Pd−C in methanolic EtOAc gave **10a**, which was successfully converted into **10b** by TBDPS protection of the phenol. Removal of the MOM protecting group at position 3 of **10b** with trimethylsilyl iodide in $CHCl_2$ gave **5** in excellent yield. Moreover, compound **10a** was subjected to the latter conditions, which afforded the 4′-*O*TBDPS monoprotected Lam-D **10c** in quantitative yield. The free phenol groups of **10c** were protected as *tert*-butyl carbonates using Boc_2O, DMAP, and CH_2Cl_2 to give **10d** in quantitative yield. Finally, *O*TBDPS deprotection of **10d** with TBAF in MeOH gave the 4′-OH Lam-D intermediate **6** (85%).

As a side note, compounds **10a** and **10c** are privileged synthetic intermediates for the construction of additional mono-

[2]Conjugate **4** protected with *Oi*Pr on positions 3 and 11, and Boc-Lys, Boc-Pro and Pbf-Arg gave only mixtures on deprotection assays.

[3]The ester bonds of the protected Lam-D conjugates were labile; hence, to avoid problems with hydrolysis, we minimized the deprotection steps after condensation.

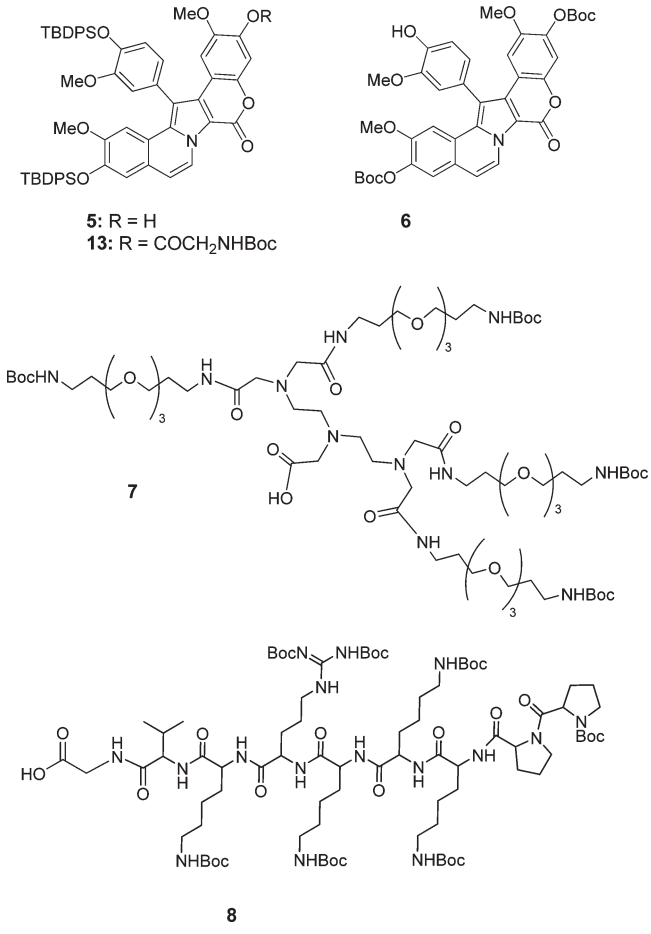


Figure 2. Building blocks for the synthesis. The UV emissions corresponding to Lam-D, **1**, and **4** are arbitrarily represented in red tones. The test compounds were seeded at a concentration of 1 μM and then incubated for 12 h.

and diconjugates at positions 11-OH and 3,11-OHs of Lam-D, respectively.

Synthesis of Poly(ethylene glycol)-Based Dendrimer 7. PEG-based dendrimer **7** was synthesized by coupling 1-(*tert*-butyloxycarbonylamino)-4,7,10-trioxa-13-tridecanamine to monobenzyl-protected diethylenetriamine pentaacetic acid with PyBOP and DIPEA. The tetracarboxylic moiety derivative was prepared from an orthogonally protected DTPA derivative (one benzyl and four *tert*-butyl protecting groups). The *t*Bu groups were eliminated using 4.0 M HCl in dioxane, and then, the benzyl group was eliminated by hydrogenolysis with Pd/C to render dendrimer **7** (*29*).

Synthesis of the Peptide NLS 8. The protected peptide **8** was synthesized on chlorotrityl resin following standard Fmoc/*t*Bu solid-phase chemistry, with 20% piperidine-DMF for the deprotection steps, and DIPCDI and HOBt as coupling reagents.

N-α-Fmoc-*N*-ω,*N*-ω′-bis-*tert*-butoxycarbonyl-L-arginine was used for the synthesis of the NLS peptide sequence. Attempts to use Fmoc-*N*-ω-Pbf-L-arginine resulted in harsher deprotection conditions and complex reaction crudes. The last amino acid used was Boc-L-Pro-OH (as the desired building block had to be completely protected). The peptide was cleaved from the resin using 3% TFA in CH_2Cl_2, and after solvent evaporation, it was lyophilized.

Esterification and Synthesis of Conjugates 1−4. To test the efficacy of the protecting scheme, the synthesis of bioconjugate **1** was repeated using the following strategy (*21*). An ester bond between **5** and BocNH-$CH_2(CH_2OCH_2)_6CH_2COOH$ was formed using EDC·HCl with a catalytic amount of DMAP to afford compound **11** (Figure 1) in quantitative yield. Compound **11** was considered a good model for the deprotection assays, because it contains the critical protecting groups and the ester functional group. Hence, it was used for the optimization of procedures and to test the success of the deprotection steps. Initial assays of sequential TBAF-TFA, two-step deprotection led to complex crude products. Furthermore, purification on SiO_2 gave low yields of **1**. The best results were obtained via simultaneous deprotection of both groups, using liquid HF at low temperature. Highly pure final product was obtained from the reaction crude by precipitation with MTBE. Notwithstanding, the scope of the reaction was limited to small amounts of starting material.[4] Compound **1** was synthesized in 84% overall yield from its precursor **5**. This is a major improvement over previous *Oi*Pr/*O*Bn strategies (45% yield, from 4′,11-diisopropyl-Lam-D) (*9*).

Formation of an ester bond between **5** and **7** (*29*) afforded compound **12** (Figure 1) in 26% yield, using EDC·HCl with a catalytic amount of PS solid supported DMAP in CH_2Cl_2. Deprotection of compound **12** using HF provided **2** in 36% yield.

Ester bond formation between **8** and protected lamellarin **5** or **6** using EDC·HCl was unsuccessful. The inaccessibility of the carboxylic acid in the *N*-Boc protected oligopeptide sequence **8** or steric hindrance of the free phenolic group in Lam-D building blocks **5** and **6** may have been decisive to the lack of reaction. Various attempts at ester bond formation between **8** and the scaffold **5** were also unsuccessful.[5] Therefore, taking advantage of the relatively easy amide bond formation (i.e., compared to ester bonds), an *N*-Boc-Gly-OH spacer was introduced at position 3 of **5** (affording **13** in 89% yield, Figure 2) for subsequent amide bond formation with **8**. *N*-Boc deprotection of **13** with 40% TFA followed by reaction with **8** in EDC·HCl, HOBt, and DIPEA as base gave **14** (Figure 1, 58% yield). Deprotection of **14** with HF under standard conditions afforded the NLS peptidic conjugate **3** in 38% yield.

The NLS conjugate at position 4′ of Lam-D could not be formed using the same conditions employed for ester bond formation between **6** and **8**.[6,7] Instead, preactivation of **8** with TCFH (*30*) and NEt_3 followed by the addition of a solution of **6** and DMAP were required, affording **15** (Figure 1) in 45% yield (relative to 40% transformation of **6**). Elimination of the nine Boc protecting groups with 40% TFA in CH_2Cl_2 gave compound **4** in 17% yield.

The ester bond of compound **3**, which has a double Gly spacer, is more susceptible to nucleophilic attack by nucleophiles from the medium than that of compound **4**, which has a single Gly spacer. Thus, the final conjugate **3** (derived from **14**) was water-labile. The rapid degradation of **3** made biological tests with this compound impossible.[8]

Cytotoxicity and Cellular Uptake. The cytotoxicity of Lam-D and its analogues (**1**, **2**, **4**, and **15**) was evaluated against BJ human skin fibroblasts, and a panel of three human tumor

[4]The yield for the deprotection was 84% working on a 20−30 mg scale. However, the procedure could not be scaled up. The stability **1** was studied in DMEM supplemented with 10% FBS and 100 units/mL penicillin and streptomycin at 37 °C. HPLC analysis indicates 97% of Lam-D liberation after 360 min of incubation.

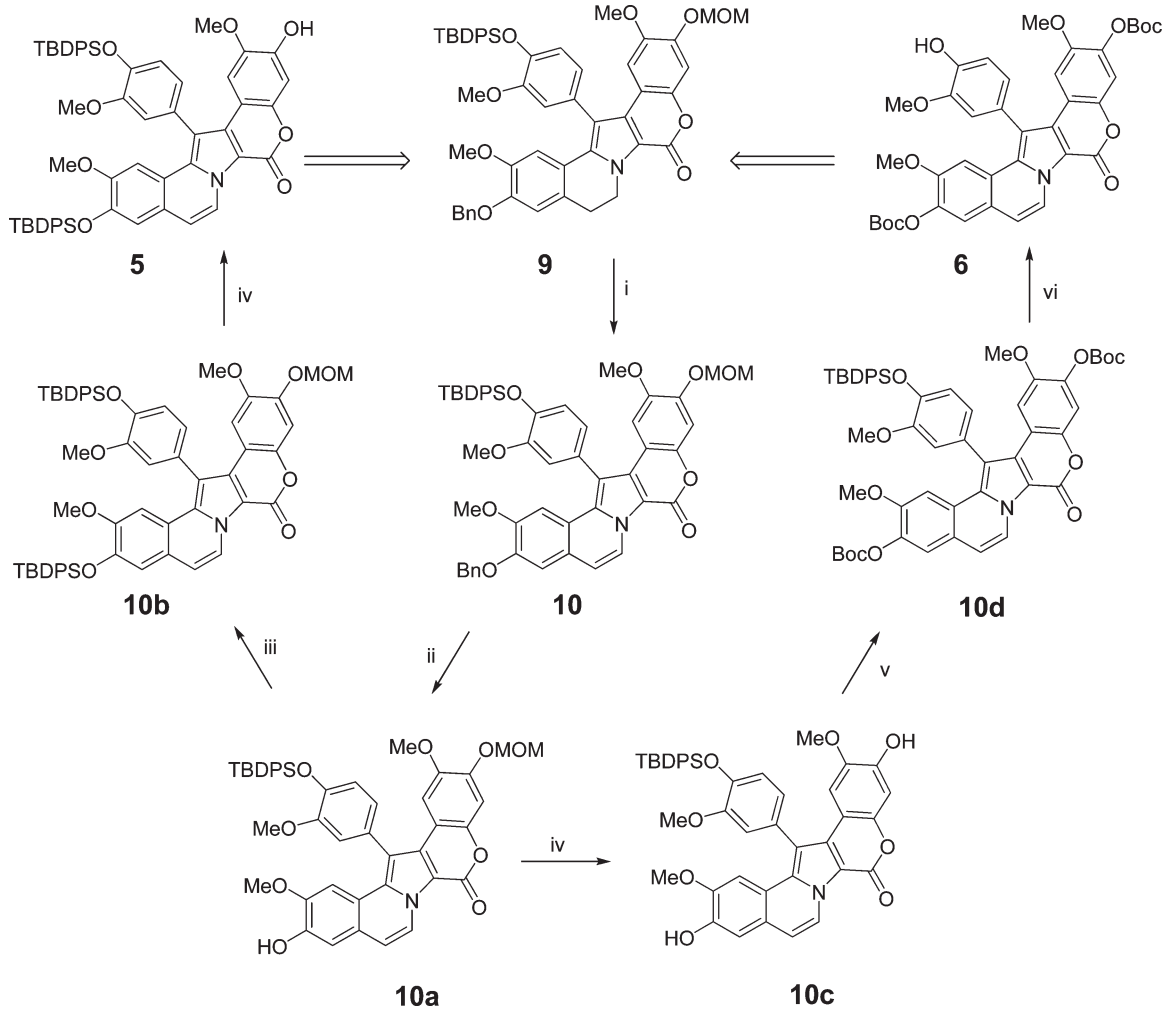
[5]Esterification of **5** was tested with EDC·HCl, TCFH, or *N*,*N*,*N*′,*N*′-tetramethylcholorformamidyl chloride as activating agent.

[6]*N*-Cbz-Gly-OH was anchored to **6** in quantitative yield. However, further Cbz deprotection could not be performed without concomitant hydrolysis of the conjugate ester bond.

[7]Other coupling reagents such as EDC·HCl, DIPCDI, TFFH, PyBOP with HOAt, and MSNT with NMI failed in ester bond formation.

[8]Compound **3** quickly hydrolyzes and liberates Lam-D into the medium, even on the time scale of an HPLC analysis.

Scheme 1[a]



[a] Reagents and conditions: (i) DDQ, $CHCl_3$, MW, 120 °C, 10 min (81%); (ii) H_2, Pd−C, EtOAc, MeOH, r.t., 16 h (95%); (iii) TBDPSCl, Im, DMAP, DMF, r.t., 24 h (58%); (iv) Me_3SiI, CH_2Cl_2 r.t., 20 min (84% for **5**; quant. yield for **10c**); (v) Boc_2O, DMAP, CH_2Cl_2 r.t., 16 h (quant.); (vi) 1M TBAF in THF, MeOH, −78 °C, 15 min (85%).

Table 1. Cytotoxicity of Compounds 1, 2, 4, and 15 in Three Human Cancer Cell Lines

	cytotoxicity (M)			
compound	HT-29 colon	A-549 lung	MDA-MB-231 breast	BJ skin fibroblast
Lam-D	3.00×10^{-6}	1.22×10^{-7}	1.34×10^{-7}	6.37×10^{-9}
1	1.68×10^{-6}	8.86×10^{-8}	4.07×10^{-8}	6.51×10^{-9}
2	3.92×10^{-6}	2.20×10^{-7}	8.31×10^{-8}	1.54×10^{-8}
4	1.01×10^{-6}	4.79×10^{-8}	4.79×10^{-8}	3.14×10^{-8}
15	1.94×10^{-6}	n.d.	1.24×10^{-6}	−

cell lines: A-549, HT-29, and MDA-MB-231 (Table 1). A conventional colorimetric assay was used to estimate values of GI_{50} (defined here as the drug concentration that causes 50% of cell growth inhibition after 72 h of continuous exposure to the test molecule). Lam-D was included for comparison. The results are shown in Table 1. The tested compounds in the tumor cell lines exhibited cytotoxicities from 4 μM to 40.7 nM, except for the Boc protected derivative **15**, which only had micromolar activity for the HT-29 and MDA-MB-231 cell cultures. BJ skin fibroblasts were used in the present study to evaluate the effects of the drug and its conjugates in normal cells. In this non-tumoral cellular culture, conjugate **1** citotoxicity was similar to that of Lam-D, or even 2.4−4.9-fold less for **2** and **4**.

FACS flow cytometry was used to measure cellular uptake quantification (*9*). The results are shown in Table 2. Interestingly, the cellular internalization quotient for the PEGylated derivatives **1** and **2** were higher than that of Lam-D in all cancer cell lines. Indeed, compound **4**, with an NLS sequence, was 10 times more active than Lam-D in A-549 and MDA-MB-231 cell lines and retained CIQ, despite having the highly charged NLS peptide. CIQ of conjugates in BJ cellular culture were from 76.8% to 128.6%.

Table 2. Cellular Internalization as Measured by FACS

	cellular internalization (AU)				cellular internalization quotient (CIQ)[a]			
compound	HT-29 colon	A-549 lung	MDA-MB-231 breast	BJ skin fibroblast	HT-29 colon	A-549 lung	MDA-MB-231 breast	BJ skin fibroblast
Lam-D	82.7	328.5	443.8	259.4	100%[a]	100%[a]	100%[a]	100%[a]
1	88.0	393.0	527.5	333.4	106%	120%	119%	128.6%
2	83.7	374.9	455.3	199.2	101%	114%	103%	76.8%
4	88.5	377.7	434.9	240.6	107%	115%	98%	92.8%

[a] CIQ was calculated in reference to the cellular uptake of Lam-D.

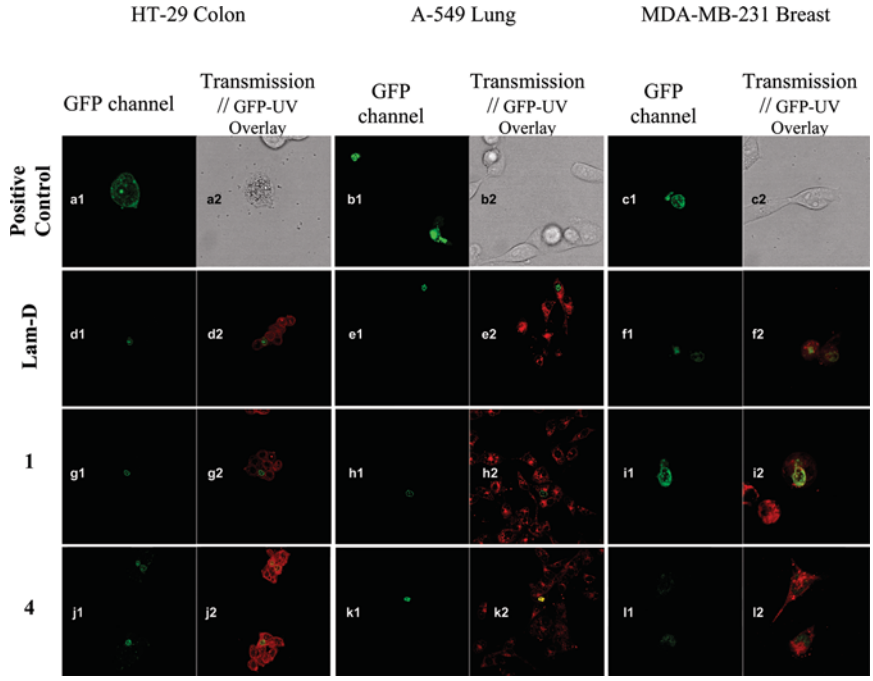


Figure 3. Internalization of Lam-D and conjugates **1** and **4** by Topo I-GFP transfected cells.

Cellular Distribution of Lam-D, 1 and 4. Tracking in GFP-Topo I Transfected Cell Cultures. Lam-D is a Topo I inhibitor. To determine whether Lam-D, **1**, and **4** localize to the same subcellular compartment as Topo I, a cellular localization assay was performed. A functional chimera of the green fluorescent protein EGFP with full-length Topo I (GFP-Topo I) was expressed in HT-29, A-549, and MDA-MD-231 cells, which were then treated with either Lam-D, **1**, or **4**. As described, GFP-Topo I was located in the nucleus (*25*) in all cell types (Figure 3, positive control). Conjugate **4**, carrying the NLS signal, was localized to the nucleus in HT-29 and A-549 cells (Figure 3, **j1**, **j2**, **k1**, and **k2**), suggesting that its higher activity could correlate to subcellular colocalization with its target, Topo I.

Interestingly, Lam-D and **1** showed both higher activity and greater nuclear localization in MDA-MB-231 cells than **4** did (Figure 3, **f1**, **f2**, **i1**, and **i2**).

DISCUSSION

We have described the synthesis of Lam-D conjugates with well-defined, water-soluble peptidic and dendritic systems as potential nontoxic drug delivery vehicles. Interestingly, the Lam-D conjugate containing a single backbone attached to a phenolic residue of Lam-D has very different solubility compared to Lam-D alone. Scaffold **10**, with three orthogonal protecting groups, has proven to be a good starting material, enabling synthesis of **5** and **6**, via protection group interchange, on a multigram scale and in good overall yield. New protected derivatives of Lam-D, which can be conjugated to one (C^{11}−OH, **10a**) or two (C^{3}−OH and C^{11}−OH, **10b**) phenol groups, have been isolated in good yields with this strategy. Compound **1** was synthesized in 84% overall yield from its precursor **5**; this constitutes an important improvement over previous strategies (45% yield, from 4′,11-diisopropyl-Lam-D) (*9*). Simultaneous removal of TBDPS*O* and *N*Boc with HF is a new and highly efficient deprotection scheme for compounds with labile ester bonds. The *O*Boc and TBDPS*O* protecting groups used in lamellarins **5** and **6** permitted optimization of the final deprotection conditions and represented adequate choices for total deprotection in the last step. Thus, single-step HF deprotection was the cleanest method for preparing Lam-D **1**-**3** (9−84% overall yields from **5**).

The new Lam-D conjugates reported here are excellent candidates for further biological evaluation. The evaluation of conjugates **3** and **4** in BJ skin fibroblasts as normal cells was used in the present study. In this cellular culture, no significant variation or even less cytotoxicity was obtained for conjugates **3** and **4** than the parent compound Lam-D. PEGylated conjugates **1** and **2** have much higher cytotoxicity to MDA-MB-231 cells than Lam-D alone. Surprisingly, compound **4** had nanomolar GI_{50} for MDA-MB-231 and A-549, representing 10-fold-lower GI_{50}, respectively, compared to Lam-D. These results in the A-549 cancer cell line could correlate to colocalization of **4** in nuclear regions where GFP−Topo I accumulated (Figure 3k). The NLS peptidic sequence is at least partly responsible for nuclear import of **4** in A-549 and HT-29 cell lines. In contrast, Lam-D and **1** were able to weakly reach the nucleus in only MDA-MB-231 cells. Altogether, we conclude that better cytotoxicity correlates with greater nuclear localization.

In summary, the use of a robust chemistry strategy, with a combination of solid-phase and solution strategies and a myriad of orthogonal and/or compatible protecting groups, has allowed the preparation of several Lam-D bioconjugates. All of them show some improved characteristics when compared with the parent lamellarin D. Particularly, **4**, which contains the NLS-peptide, shows a clearly improved cytotoxicity and a colocalization in the nucleus. To the best of our knowledge, **4** is one of the first examples of NLS peptide conjugation with small molecules.

Our results (*9*) indicate that Lam-D derivatives obtained through various chemical modifications may have markedly higher activity than the parent compound in certain tumor cell lines, increasing the selectivity between the tumor cell lines.

ACKNOWLEDGMENT

This study was partially supported by CICYT (Grant BQU 2006-03794 and BQU2005-00315), *Generalitat de Catalunya*, and the Barcelona Science Park. We gratefully acknowledge PharmaMar S.A. for performing the preliminary biological tests, and the Networking Centre on Bioengineering, Biomaterials and

Nanomedicine (CIBER-BBN). This study was partially supported by the Industrial Consortium "NanoFarma" from the Spanish grants of CENIT program. We thank Dr. J. Comas from Flow Cytometry Facility − SCT (UB) for valuable discussions regarding the present work. We also thank Prof. C. Mielke (Heinrich-Heine University, Medical School, Düsseldorf) for generous donation of a sample of GFP−Topo I plasmid.

LITERATURE CITED

(1) Vicent, M. J., and Duncan, R. (2006) Polymer conjugates: nanosized medicines for treating cancer. *Trends Biotechnol. 24*, 39–47.

(2) Torchilin, V. P. (2005) Lipid-core micelles for targeted drug delivery. *Curr. Drug Delivery 2*, 319–327.

(3) Lukyanov, A. N., and Torchilin, V. P. (2004) Micelles from lipid derivatives of water-soluble polymers as delivery systems for poorly soluble drugs. *Adv. Drug Delivery Rev. 56*, 1273–1289.

(4) Greenwald, R. B., Pendri, A., Conover, C. D., Zhao, H., Choe, Y. H., Martínez, A., Shum, K., and Guan, S. (1999) Drug delivery systems employing 1,4- or 1,6-elimination: poly(ethylene glycol) prodrugs of amine-containing compounds. *J. Med. Chem. 42*, 3657–3667.

(5) Nori, A., and Kopecek, J. (2005) Intracellular targeting of polymer-bound drugs for cancer chemotherapy. *Adv. Drug Delivery Rev. 57*, 609–636.

(6) de Koning, M. C., van der Marel, G. A., and Overhand, M. (2003) Synthetic developments towards PNA-peptide conjugates. *Curr. Opin. Chem. Biol. 7*, 734–740.

(7) Yoshikawa, T., Sugita, T., Mukai, Y., Yamanada, N., Nagano, K., Nabeshi, H., Yoshioka, Y., Nakagawa, S., Abe, Y., Kamada, H., Tsunoda, S.-i., and Tsutsumi, Y. (2008) Organelle-targeted delivery of biological macromolecules using the protein transduction domain: potential applications for peptide aptamer delivery into the nucleus. *J. Mol. Biol. 380*, 777–782.

(8) Boulanger, C., Di Giorgio, C., and Vierling, P. (2005) Synthesis of acridine-nuclear localization signal (NLS) conjugates and evaluation of their impact on lipoplex and polyplex-based transfection. *Eur. J. Med. Chem. 40*, 1295–1306.

(9) Pla, D., Francesch, A., Calvo, P., Cuevas, C., Aligué, R., Albericio, F., and Álvarez, M. (2009) Lamellarin D bioconjugates I: synthesis and cellular internalization of PEG-derivatives. *Bioconjugate Chem.* . the previous article in the same issue.

(10) Crespo, L., Sanclimens, G., Pons, M., Giralt, E., Royo, M., and Albericio, F. (2005) Peptide and amide bond-containing dendrimers. *Chem. Rev. 105*, 1663–1682.

(11) Maeda, H., Wu, J., Sawa, T., Matsumura, Y., and Hori, K. (2000) Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J. Control. Release 65*, 271–284.

(12) Pla, D., Albericio, F., and Álvarez, M. (2008) Recent advances in lamellarin alkaloids: isolation, synthesis and activity. *Anti-Cancer Agents Med. Chem. 8*, 746–760.

(13) Facompré, M., Tardy, C., Bal-Mayeu, C., Colson, P., Pérez, C., Manzanares, I., Cuevas, C., and Bailly, C. (2003) Lamellarin D: a novel potent inhibitor of topoisomerase I. *Cancer Res. 63*, 7392–7399.

(14) Marco, E., Laine, W., Tardy, C., Lansiaux, A., Iwao, M., Ishibashi, F., Bailly, C., and Gago, F. (2005) Molecular determinants of topoisomerase I poisoning by lamellarins: comparison with camptothecin and structure-activity relationships. *J. Med. Chem. 48*, 3796–3807.

(15) Kluza, J., Gallego, M. A., Loyens, A., Beauvillain, J. C., Fernández Sousa-Faro, J. M., Cuevas, C., Marchetti, P., and Bailly, C. (2006) Cancer cell mitochondria are direct proapoptotic targets for the marine antitumor drug lamellarin D. *Cancer Res. 66*, 3177–3187.

(16) Cortés, F., Pastor, N., Mateos, S., and Domínguez, I. (2007) Topoisomerase inhibitors as therapeutic weapons. *Expert Opin. Ther. Patents 17*, 1–12.

(17) Holden, J. A. (2001) DNA topoisomerases as anticancer drug targets: from the laboratory to the clinic. *Curr. Med. Chem.: Anti-Cancer Agents 1*, 1–25.

(18) Dias, N., Vezin, H., Lansiaux, A., and Bailly, C. (2005) Topoisomerase inhibitors of marine origin and their potential use as anticancer agents. *Top. Curr. Chem. 253*, 89–108.

(19) Pommier, Y. (2006) Topoisomerase I inhibitors: camptothecins and beyond. *Nat. Rev. Cancer 6*, 789–802.

(20) Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2007) *Molecular Biology of the Cell*, Garland Science, London.

(21) Veronese, F. M., and Harris, J. M. (2008) Peptide and protein PEGylation III: advances in chemistry and clinical applications. *Adv. Drug Delivery Rev. 60*, 1–2.

(22) IUPAC nomenclature of Lam-D: 3,11-dihydroxy-2,12-dimethoxy-14-(4-hydroxy-3-methoxyphenyl)-6*H*[1]benzopyrano-[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinoline-6-one.

(23) Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Waren, J. T., Bokesch, H., Kenney, S., and Boyd, M. R. (1990) New colorimetric cytotoxicity assay for anticancer drug screening. *J. Natl. Cancer Inst. 82*, 1107–1112.

(24) Boyd, M. R., and Paull, K. D. (1995) Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. *Drug Dev. Res. 34*, 91–104.

(25) Mielke, C., Kalfalah, F. M., Christensen, M. O., and Boege, F. (2007) Rapid and prolonged stalling of human DNA topoisomerase I in UVA-irradiated genomic areas. *DNA Repair 6*, 1757–1763.

(26) Cironi, P., Manzanares, I., Albericio, F., and Álvarez, M. (2003) Solid-phase total synthesis of the pentacyclic system lamellarins U and L. *Org. Lett. 5*, 2959–2962.

(27) Bailly, C., Francesch, A., Mateo, C., Jiménez, J. A., Pastor, A., and Cuevas, C. (2004) Pharma Mar, S. A. U., WO 2004/014917 A2, pp 220.

(28) Banwell, M., Flynn, B., and Hockless, D. (1997) Convergent total synthesis of Lamellarin K. *Chem. Commun.* 2259–2260.

(29) The synthesis of **7** is part of a dendrimer project and will be published in due time.

(30) Tulla-Puche, J., Torres, A., Calvo, P., Royo, M., and Albericio, F. (2008) *N*,*N*,*N*′,*N*′-Tetramethylchloroformamidinium hexafluorophosphate (TCFH), a powerful coupling reagent for bioconjugation. *Bioconjugate Chem. 19*, 1968–1971.

BC800504T

7 PINCES ÒPTIQUES PER A L'ESTUDI D'INHIBICIÓ DE TOPOISOMERASA

PINCES ÒPTIQUES PER A L'ESTUDI D'INHIBICIÓ DE TOPOISOMERASA.

Optical tweezers study of topoisomerase inhibition.

Daniel Pla[1,2,3,‖], Andy Sischka[3,‖], Fernando Albericio[1, 2, ‡], Mercedes Álvarez[1, 2, §], Xavier Fernàndez-Busquets[4,*] and Dario Anselmetti[3]

Small, **2009**, *5*, 1269-1272.

[1]Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain.

[2]CIBER-BBN Networking Centre on Bioengineering, Biomaterials and Nanomedicine.

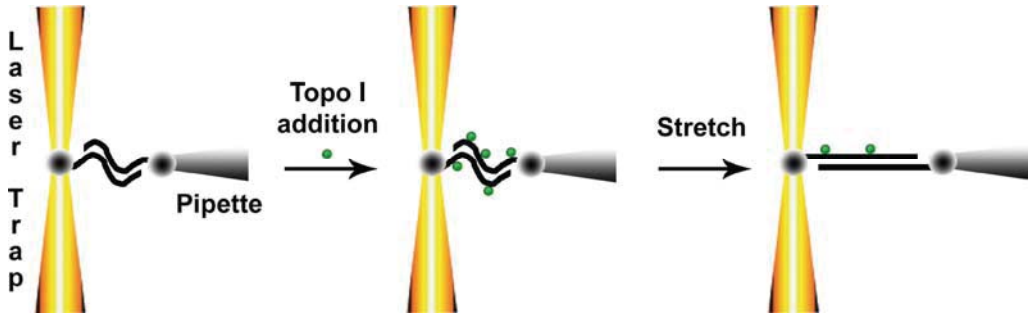
[3]Experimental Biophysics and Applied Nanoscience, Bielefeld University, D-33615 Bielefeld, Germany.

[4]Biomolecular Interactions Team, Nanobioengineering Group, Institute for Bioengineering of Catalonia, and Nanoscience and Nanotechnology Institute, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona.

‖ Authors contributed equally to the present work.

‡ Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona.

§ Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, E-08028 Barcelona.



Resum

La Topo I és una diana especialment interessant en quimioteràpia, és a dir, al complex de Topo I-DNA, el qual experimenta moviments mecànics essencials durant el trencament i relligament d'una sola cadena dins d'un dúplex d'ADN. Aquí es mostra l'ús de pinces òptiques en l'estudi de l'activitat de la topoisomerasa. El gran augment en la histèresi dels cicles de força és la evidencia de l'activitat tallant de l'enzim i correspon a la generació de dominis assimilables a monocadenes d'ADN a dintre de la doble cadena de DNA lineal. La recuperació posterior de l'empremta dactil·lar de força característica de la doble cadena de DNA, correspon a la relligació final de cadenes per part de la topoisomerasa. En contrast, la presència de Lamel·larina D, inhibidor d'aquesta, resulta en un gran augment en la força de histèresi degut a l'activitat tallant inicial de la topoisomerasa I, i una posterior manca de relligament. Aquests resultats posen de manifest el potencial de les pinces òptiques com a biosensors per l'estudi mecànic de l'ADN amb enzims modificants, i per la determinació dels seus inhibidors, i preveuen profundes implicacions en el descobriment de medicaments i nanotecnologia mèdica.

Optical-Tweezers Study of Topoisomerase Inhibition**

Daniel Pla, Andy Sischka, Fernando Albericio, Mercedes Álvarez, Xavier Fernàndez-Busquets, and Dario Anselmetti*

Topoisomerase is an especially interesting chemotherapy target, namely because of the topoisomerase-DNA complex, which undergoes mechanical motions essential to its function during the cleavage and religation of a single strand within a duplex DNA.[1–6] Here we show the use of optical tweezers to study topoisomerase activity, evidenced by a large increase in the hysteresis of the force cycles resulting from the generation of ssDNA-like domains inside dsλDNA, and subsequent strand religation indicated by recovery of the characteristic dsDNA force fingerprint. In contrast, the presence of the topoisomerase inhibitor Lamellarin D[7–9] results in a large increase in force hysteresis due to the initial nicking activity of Topo I and a subsequent absence of religation. These results highlight the potential of optical tweezers biosensors for the mechanistic study of DNA-modifying enzymes and for the screening of their inhibitors, and foresee profound implications in drug discovery and medical nanotechnology.

DNA-handling enzymes represent promising effectors of tumor proliferation as selective actuators against uncontrolled cellular growth. Topoisomerase (Topo) I from wheat germ[1] is an eukaryotic type IB Topo[2,3] that cleaves and rejoins one DNA strand through a covalent protein-DNA intermediate. The chemistry of this reaction involves nucleophilic attack of the phosphodiester backbone in duplex DNA by a tyrosine residue, which leads to a 3′-phosphotyrosyl linkage of the enzyme to one of the cleaved DNA strands.[2] A free rotation model of action has been described for type IB Topo, in which individual catalytic cycles can alter the DNA linkage number by multiple integral turns, thereby relieving torsional stress.[4] The distribution of cleavage sites in duplex DNA is non-random, but the principles governing cleavage site choice remain poorly understood. Topoisomerases are especially attractive targets for cancer therapy since their role in controlling DNA topology is crucial for correct cell division.[3,5,6] Topoisomerase-targeted drugs are more selective for malignant cells, which are more susceptible to the DNA damage inflicted.[6] Understanding the mechanism of action of the enzyme in the presence of its inhibitors is a requisite for the clinical development of therapeutic agents.

In this work, we used the widely known marine sponge alkaloid Lamellarin D (Lam-D, Figure 1)[7–9] to study Topo I inhibition. Lam-D belongs to a group of anticancer drugs which act by poisoning and stabilizing DNA-Topo I phosphotyrosyl intermediates,[8,10–12] a mechanism reported for numerous anticancer drugs.[3,5,6,12–14] It has been proposed that interaction of the drug with the enzyme results in a ternary complex that inhibits post-cleavage DNA religation.[13,15] In a previous structure-activity relationship study of Lam-D,[16] we prepared a library of open lactone analogues of the natural product (open chain Lam-D, OCLam-D, Figure 1). GI_{50} tested in a panel of three human tumor cell lines was significantly higher for OCLam-D when compared to Lam-D. In agreement with these data, AFM images of a plasmid DNA treated by Topo I in the presence of OCLam-D show all DNA molecules relaxed (Figure 2a,c,e), indicating poor enzyme inhibition. In contrast, Lam-D used at the same concentration is apparently able to block Topo I cleaving activity as indicated

[*] Dr. X. Fernàndez-Busquets
Biomolecular Interactions Team
Nanobioengineering Group
Institute for Bioengineering of Catalonia and
Nanoscience and Nanotechnology Institute
Barcelona Science Park-University of Barcelona
Baldiri Reixac 10, 08028 Barcelona (Spain)
E-mail: xfernandez_busquets@ub.edu

Dr. D. Pla, Prof. F. Albericio, Prof. M. Álvarez
Institute for Research in Biomedicine
Barcelona Science Park-University of Barcelona and
CIBER-BBN Networking Centre on Bioengineering, Biomaterials and Nanomedicine
Baldiri Reixac 10, 08028 Barcelona (Spain)

Dr. D. Pla, Dr. A. Sischka, Prof. D. Anselmetti
Experimental Biophysics and Applied Nanoscience
Bielefeld University
33615 Bielefeld (Germany)

Prof. F. Albericio
Department of Organic Chemistry
University of Barcelona
08028 Barcelona (Spain)

Prof. M. Álvarez
Laboratory of Organic Chemistry
Faculty of Pharmacy
University of Barcelona
08028 Barcelona (Spain)

[**] This work was supported by the Ministerio de Ciencia e Innovación (CTQ2006-03794/BQU, CSD2006-00012, BIO2008-01184), Instituto de Salud Carlos III (CB06-01-0074), and the Generalitat de Catalunya (2005-SGR00662, 2005-SGR00037), Spain. D.P. received a travel grant from the Generalitat de Catalunya (2007-BE-2-00340) and a Ph.D. fellowship from the Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau de Barcelona (B-IR-2006-2), Spain. A.S. and D.A. acknowledge financial support from the Collaborative Research Center SFB 613 from the Deutsche Forschungsgemeinschaft, Germany. D.P. and A.S. contributed equally to this work.

DOI: 10.1002/smll.200801322

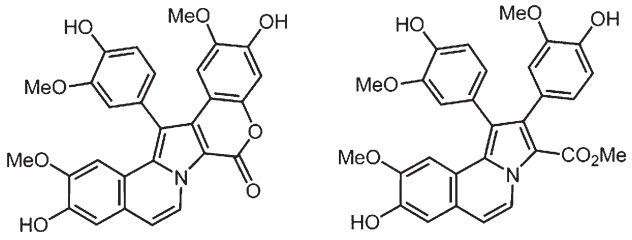


Figure 1. Chemical structures of Lamellarin D and of open chain Lamellarin D.

by the prevalence of supercoiled topoisomers (Figure 2b,d,f). However, AFM images do not permit to investigate the alternative inhibitory mechanism where Topo I would cleave but without releasing the newly generated DNA ends until completing the ligation step. To explore this scenario we have used an optical tweezers (OT) single molecule force spectroscopy (SMFS) approach.

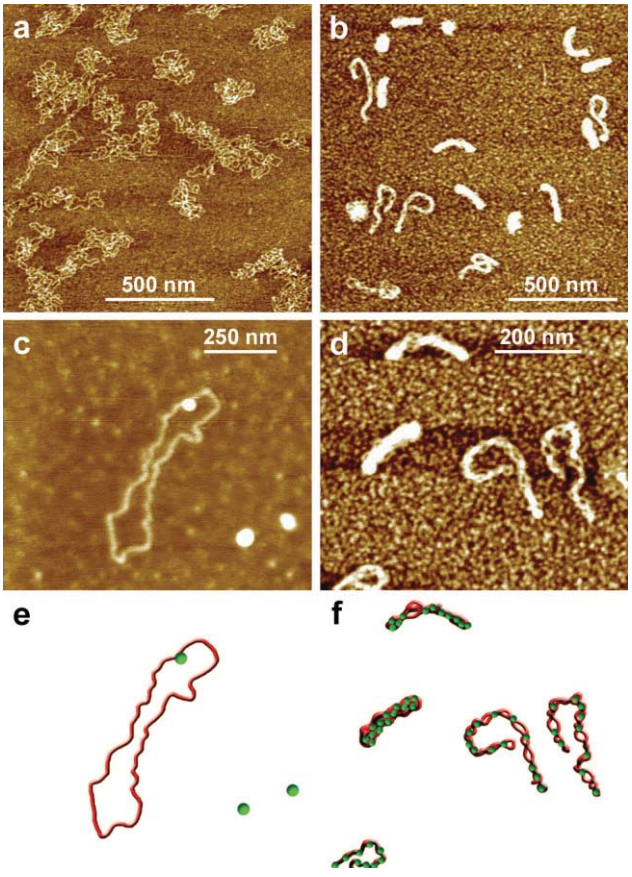


Figure 2. AFM imaging of Topo I-treated plasmid DNA. a,c) Supercoiled circular DNA treated by Topo I in the presence of OCLam-D, showing all DNA molecules relaxed as result of Topo I activity. b,d) Supercoiled circular DNA treated by Topo I in the presence of Lam-D. A similar number of molecules is shown in (a) and (b). The different apparent topoisomerase diameter and DNA width in (c) and (d) are the result of using AFM tips with different apex radii. e,f) Interpretive cartoon of the images from (c) and (d), respectively. Green dots represent topoisomerase molecules. Vertical color scale is 2 nm for all images.

Owing to their sub-piconewton force sensitivity, OT are a robust, powerful and highly sensitive biophysical tool which enables direct measurement of minute forces within biological engines such as Topo I. The lure of OT sensors resides in the fact that they provide biomolecular analysis of Topo I–DNA complexes at the smallest possible level, from which the enzymatic mechanism can be deduced as the sum of discrete phenomena. OT systems[17–19] have been successfully employed as a SMFS biosensor to measure the elastic responses of immobilized DNA molecules[20–22] and for the identification of molecular binding mechanisms.[23]

When a linear dsDNA molecule is pulled from the 5′ or 3′ ends, its elastic response reaches a characteristic force plateau. This was first attributed to its change in structure from the B-form to the overstretched S-form.[24] Based on data obtained at different ionic strengths, temperatures, and pH conditions,[25] a model was proposed whereby the overstretching plateau was assigned to a force-induced melting process ending in short dsDNA domains holding large ssDNA strands together.[25–27] In this overstretching transition plateau, further elongation results in an elastic response corresponding to a non-equilibrium process.[28] Partial melting of the DNA molecule can occur at forces below 150 pN and is observed as a deviation of the relaxation path from the stretching path that depends on kinetic effects as well as on the number and location of nicks on the dsDNA.[29] Therefore, the increase in hysteresis area can be used as a tight screening control of the nicking activity of Topo I.

Our OT setup employed a single linear dsDNA molecule immobilized between two streptavidin-coated microspheres through its 3′ ends.[21] We ran SMFS assays of this system alone and in the presence of Topo I (Figure 3a). Enzyme-induced changes in the DNA physical properties can be followed by plotting stretching-relaxing force cycles vs. DNA extension. Before Topo I addition, the individual dsDNA molecule held in the optical trap exhibits a stretch-retract profile that indicates the presence of strand breaks resulting from the DNA purification process (Figure 3b, black curve). The hysteresis curve of the 48.5-kbp λDNA reaches a plateau at ca. 62 pN at 17 μm, which marks the start of the melting process. The cleaving activity of Topo I was evidenced by a clear displacement of the stretching curve towards the ssDNA region (Figure 3b, red curve) as a result of the generation of single strand breaks that form ssDNA-like domains inside dsλDNA. At the end of the experiment, the ssDNA-like domains were repaired via Topo I ligating activity, although nicks in the resulting dsDNA persisted, as indicated by the large hysteresis area remaining between the stretching and relax curves (Figure 3b, green curve).

Figure 3c shows another typical experiment where the λDNA molecule held in the optical trap was cleaved by Topo I during the force cycle, as indicated by the sudden drop in the stretching curve (bold red, arrow). The formation of a higher level plateau (ca. 10 pN increase in force) and the total disappearance of hysteresis in stretching-relaxing cycles suggests that all nicks in the dsλDNA were removed (green curve). The commonly reported plateau at ca. 62 pN likely reflects disruption of base pair stacking in dsDNA molecules having at least one nick, but the plateau just above 70 pN

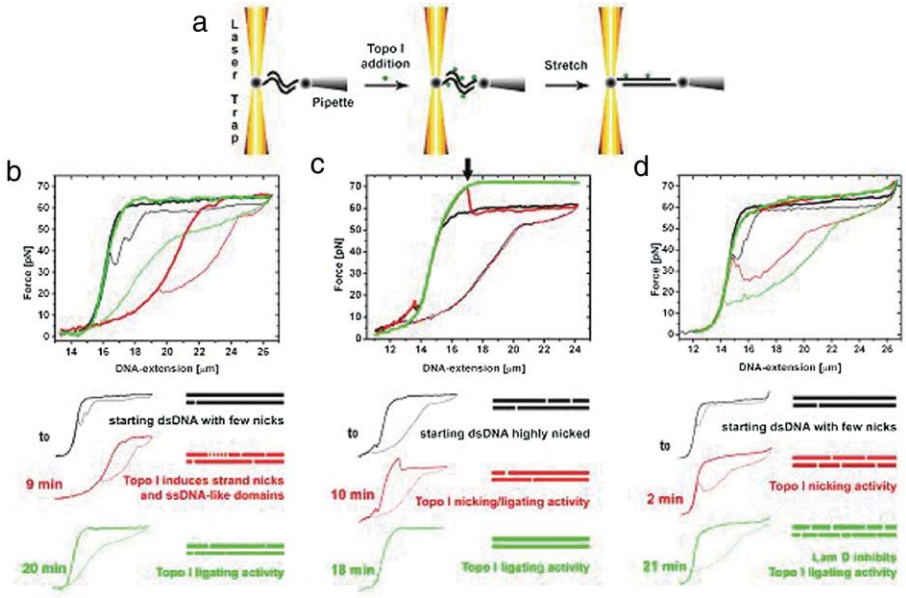


Figure 3. OT force assays of Topo I activity. a) Schematic of OT experiments, where each 3′ biotinylated end of the dsDNA is attached to one streptavidin-coated bead. b) OT force-stretching experiment starting from a dsλDNA molecule with few nicks, as indicated by the small hysteresis area (black curve). Stretching-relaxing cycles are indicated by half-bold half-thin plots. The cleaving activity of Topo I after 9 min of reaction generated an ssDNA-like domain, a heavily nicked dsDNA which exhibited the mechanical properties of ssDNA (red curve). After a further 11 min the ligating activity of Topo I removed the ssDNA-like domain (green curve). c) OT force-stretching experiment starting with highly nicked dsDNA, as indicated by the large hysteresis area (black curve). Topo I activity is evidenced by the presence of a higher level plateau that indicates total religation of the initial DNA nicks and a complete vanishing of the overstretching hysteresis (green curve, 18 min reaction time). The arrow indicates a drop in the stretching curve resulting from Topo I cleavage during the cycle. d) OT force assay of Topo I activity in the presence of Lam-D. Starting λDNA exhibited a small hysteresis area (black curve) corresponding to a dsDNA molecule with only few nicks. The cleaving activity of Topo I induced a large increase in hysteresis in the presence of Lam-D after the first two minutes of reaction (red curve). Stabilization by Lam-D of this intermediate complex resulted in inhibition of the religating activity of Topo I (green curve, acquired at 21 min reaction).

corresponds to an intact dsDNA molecule without a single nick. This complete recovery of a dsDNA was usually achieved by Topo I when ssDNA domains had not been generated. Under our experimental conditions, in the presence of ssDNA domains (Figure 3b) the DNA seems to be too damaged to be completely repaired by Topo I. Occasionally, an experiment was ended when both dsDNA strands had been cleaved, an outcome consistent with reports on the formation of a Topo I dimer-like complex which cleaves dsDNA.[30] In order to investigate the Topo I inhibition, experiments were performed as described above, except now in the presence of 10 μM Lam-D. The overall mechanic response of dsDNA molecules to this concentration of Lam-D showed a slightly tilted plateau deviation (Figure 3d), attributable to weak DNA intercalation of the drug, which agrees well with previously reported circular dichroism binding experiments.[10] This small effect of Lam-D enabled accurate SMFS analysis of the complex in the presence of the enzyme.

OT results always and reproducibly exhibited a large increase in hysteresis of the force cycles due to the initial nicking activity of Topo I (Figure 3d, red curve). The presence of Lam-D prevents the religation step and blocks enzyme turnover, as evidenced by the absence of a higher force plateau and by a large, non-vanishing hysteresis between the stretching and relaxing paths of the force cycle (Figure 3d, green curve). Taken together, the data presented here indicate that, upon Lam-D inhibition, Topo I keeps a non-covalent interaction with the 5′ end of the cleaved DNA strand sufficiently strong to prevent supercoil relaxation in solution.

Single molecule approaches should prove paramount for further elucidation of the mechanisms of action of potential topoisomerase inhibitors such as Lam-D,[8] namely, in enabling investigation of these molecules at pN sensitivity. We have shown that OT biosensor assays can be successfully applied to the study of the mechanism of Topo I inhibition by Lam-D at the single molecule level, representing a new approach to the study of the interaction between DNA binding enzymes and their inhibitors. We foresee that the use of OT-based screening to identify fundamental molecular mechanisms will have profound implications in biomolecular applications, drug discovery and medical nanotechnology.

Experimental Section

AFM imaging: Topo I activity assays were performed at 37 °C for 30 min in a 8-μl volume of reaction buffer (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mM (2S,3S)-1,4-bis-sulfanylbutane-2,3-diol) containing 1 μg of a 5-kbp plasmid DNA and 2.5 units of the enzyme (Sigma, Saint Louis, MI, USA). When required, 200 μM Lam-D or OCLam-D (synthesized[9,16]) was included. For AFM imaging, the sample was diluted 10 times with reaction buffer. Mica surfaces (Provac AG, Balzers, Liechtenstein) were silanized in an exsiccator with (3-aminopropyl)triethoxysilane (Fluka, Steinheim, Germany).[31] 5-μl diluted samples were deposited on the treated mica and let to adsorb for 15 min at room temperature, rinsed with deionized water (Millipore, Bedford, MA, USA) to remove weakly attached proteins, and finally dried under a gentle flow of nitrogen gas. Imaging was performed using non-contact aluminum-coated silicon nitride cantilevers with a spring constant of 40 Nm^{-1} and 300 kHz resonant frequency (Budget Sensors, Sofia, Bulgaria) on a Nanoscope IIIa AFM system equipped with a Multimode head and a type E piezoelectric scanner (Multimode, Veeco Instruments, Santa Barbara, CA, USA). The AFM was operated in tapping mode[32] at a scan line frequency of 1–2 Hz. Raw AFM images have been processed only for background removal (flattening) using the microscope manufacturer's image-processing software. Image analysis by Fast Fourier Transforms

was performed with the WSxM 2.0 SPM software (Nanotec, Madrid, Spain).

Optical tweezers assays: Force measurements and manipulation of individual dsλDNA molecules in the presence of Topo I and Lam-D were performed with a compact single beam OT system equipped with a fluid cell for liquid handling. This setup was integrated into a commercial inverted optical microscope as previously described,[21] and rebuilt for improved force resolution.[33] A maximal and linear force detection range of 90 pN combined with a force sensitivity of less than 0.2 pN allowed measurements of elastic properties of single molecules. Streptavidin-coated polystyrene microspheres (Spherotech, Libertyville, IL, USA) with a diameter of 3.28 μm were used in a diluted suspension of 5×10^{-4} % w/v for all experiments. λDNA (Promega Corp., WI, USA) was chemically modified[21] to ensure tethering to the beads. Beads, λDNA, and binding ligands were dissolved in 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA reaction buffer. λDNA was used at a concentration of 15 pM, and Lam-D was used at a final concentration of 10 μM. 5 units of Topo I were used for each run. All experiments were performed at 20 °C.

Keywords:

atomic force microscopy · DNA · lamellarin D · optical tweezers · topoisomerase

[1] W. S. Dynan, J. J. Jendrisak, D. A. Hager, R. R. Burgess, *J. Biol. Chem.* **1981**, *256*, 5860.
[2] J. J. Champoux, *Annu. Rev. Biochem.* **2001**, *70*, 369.
[3] K. D. Corbett, J. M. Berger, *Annu. Rev. Biophys. Biomol. Struct.* **2004**, *33*, 95.
[4] J. T. Stivers, T. K. Harris, A. S. Mildvan, *Biochemistry* **1997**, *36*, 5212.
[5] F. Cortés, N. Pastor, S. Mateos, I. Domínguez, *Expert Opin. Ther. Patents* **2007**, *17*, 1.
[6] J. A. Holden, *Curr. Med. Chem.: Anti-Cancer Agents* **2001**, *1*, 1.
[7] C. Bailly, *Curr. Med. Chem.: Anti-Cancer Agents* **2004**, *4*, 363.
[8] D. Pla, F. Albericio, M. Álvarez, *Anti-Cancer Agents Med. Chem.* **2008**, *8*, 746.
[9] D. Pla, A. Marchal, C. Olsen, F. Albericio, M. Álvarez, *J. Org. Chem.* **2005**, *70*, 8231.
[10] M. Facompré, C. Tardy, C. Bal-Mayeu, P. Colson, C. Pérez, I. Manzanares, C. Cuevas, C. Bailly, *Cancer Res.* **2003**, *63*, 7392.
[11] M. Vanhuyse, J. Kluza, C. Tardy, G. Otero, C. Cuevas, C. Bailly, A. Lansiaux, *Cancer Lett.* **2005**, *221*, 165.
[12] E. Marco, W. Laine, C. Tardy, A. Lansiaux, M. Iwao, F. Ishibashi, C. Bailly, F. Gago, *J. Med. Chem.* **2005**, *48*, 3796.
[13] N. Dias, H. Vezin, A. Lansiaux, C. Bailly, *Top. Curr. Chem.* **2005**, *253*, 89.
[14] Y. Pommier, *Nat Rev Cancer* **2006**, *6*, 789.
[15] B. L. Staker, K. Hjerrild, M. D. Feese, C. A. Behnke, A. B. Burgin, L. Stewart, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 15387.
[16] D. Pla, A. Marchal, C. A. Olsen, A. Francesch, C. Cuevas, F. Albericio, M. Alvarez, *J. Med. Chem.* **2006**, *49*, 3257.
[17] K. C. Neuman, T. Lionnet, J.-F. Allemand, *Annu. Rev. Mater. Res.* **2007**, *37*, 33.
[18] W. J. Greenleaf, M. T. Woodside, S. M. Block, *Annu. Rev. Biophys. Biomol. Struct.* **2007**, *36*, 171.
[19] M. J. McCauley, M. C. Williams, *Biopolymers* **2006**, *85*, 154.
[20] G. J. L. Wuite, R. J. Davenport, A. Rappaport, C. Bustamante, *Biophys. J.* **2000**, *79*, 1155.
[21] A. Sischka, R. Eckel, K. Toensing, R. Ros, D. Anselmetti, *Rev. Sci. Instrum.* **2003**, *74*, 4827.
[22] B. Taneja, B. Schnurr, A. Slesarev, J. F. Marko, A. Mondragon, *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 14670.
[23] A. Sischka, K. Toensing, R. Eckel, S. D. Wilking, N. Sewald, R. Ros, D. Anselmetti, *Biophys. J.* **2005**, *88*, 404.
[24] P. Cluzel, A. Lebrun, C. Heller, R. Lavery, J. L. Viovy, D. Chatenay, F. Caron, *Science* **1996**, *271*, 792.
[25] J. R. Wenner, M. C. Williams, I. Rouzina, V. A. Bloomfield, *Biophys. J.* **2002**, *82*, 3160.
[26] M. C. Williams, J. R. Wenner, I. Rouzina, V. A. Bloomfield, *Biophys. J.* **2001**, *80*, 874.
[27] M. C. Williams, I. Rouzina, V. A. Bloomfield, *Acc. Chem. Res.* **2002**, *35*, 159.
[28] M. Rief, H. Clausen-Schaumann, H. E. Gaub, *Nat. Struct. Mol. Biol.* **1999**, *6*, 346.
[29] R. Krautbauer, L. H. Pope, T. E. Schrader, S. Allen, H. E. Gaub, *FEBS letters* **2002**, *510*, 154.
[30] K. Søe, S. Hartung, F. Grosse, *Biochem. Biophys. Res. Comm.* **2006**, *349*, 178–185.
[31] Y. Lyubchenko, L. Shlyakhtenko, R. Harrington, P. Oden, S. Lindsay, *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 2137.
[32] C. Moller, M. Allen, V. Elings, A. Engel, D. J. Muller, *Biophys. J.* **1999**, *77*, 1150.
[33] A. Sischka, C. Kleimann, W. Hachmann, M. M. Schäfer, I. Seuffert, K. Tönsing, D. Anselmetti, *Rev. Sci. Instrum.* **2008**, *79*, 063702.

Received: September 9, 2008
Published online: March 16, 2009

8

DISCUSSIÓ GENERAL I CONCLUSIONS

DISCUSSIÓ GENERAL I CONCLUSIONS.

Nombroses han estat les Lamel·larines identificades durant les darreres dècades. Aquesta tesi comença amb una revisió dels avanços dels últims anys en aquest camp, que il·lustra la complexitat d'esforços esmerçats amb aquestes molècules. El guany amb informació biològica referent al mode d'acció d'aquestes molècules així com noves metodologies de síntesi que permeten la seva preparació, situa a aquestes molècules en un espai privilegiat de cara a futurs estudis. Per als membres més destacats de la família de les Lamel·larines (Lamel·larina D i H), se n'ha descrit inhibició de topoisomerasa I, inhibició d'integrases, disrupció del potencial transmembrana, i mecanismes de mort cel·lular per apoptosi.

Els procediments desenvolupats en aquesta tesi doctoral han contribuït al desenvolupament de rutes sintètiques per la construcció de la Lamel·larina D, el cap de sèrie amb activitat biològica més remarcada. S'ha desenvolupat una via nova per la síntesi total de la Lamel·larina D a partir de l'esquelet de construcció tricíclic 8-isopropoxi-9-methoxy-5,6-dihidropirrolo[2,1-*a*]isoquinolina-3-carboxilat de metil. La introducció regioselectiva i seqüencial dels grups arílics amb acoblaments creuats catalitzats per Pd(0), aromatització i desprotecció dels èters, lliurà els compostos finals amb rendiments del 18-61%.

Es va emprar àcids borònics i boronats com a elements de construcció per les síntesis. En aquest treball se'n descriu la seva preparació mitjançant reaccions de bromació regioselectiva de benzens altament activats en condicions suaus, i borilació catalitzada amb Pd(0). Els 7 sintons polialcoxiarílics requerits per una síntesi orientada en diversitat s'aïllaren amb rendiments del 43-80% (sobre 2 etapes).

Una quimioteca de 45 anàlegs de la Lamel·larina D ha estat sintetitzada amb rendiments globals del 18-44%. La eliminació de l'anell de lactona i les modificacions introduïdes en els patrons de substitució OMe / OH, han estat els elements de diversificació que s'ha introduït en els components de la quimioteca. Un grup de tres línies de cèl·lules tumorals humanes es va utilitzar per avaluar el potencial citotòxic dels productes: A-549 de càncer de pulmó NSCL, HT-29 carcinoma de còlon, i MDA-MB-231 adenocarcinoma de mama. Es va observar citotoxicitat en nombrosos compostos a concentracions micromolars baixes. L'estudi de les relacions estructura-activitat ha

posat de manifest la importància de la planaritat en el sistema tricíclic de pirroloisoquinolina, així com dels grups OH en les posicions C-8 i C-4" dels compostos.

La millora de les propietats de solubilitat de la Lamel·larina D es va iniciar amb la seva conjugació a diverses unitats de PEG. La síntesi d'aquestos s'ha portat a terme amb l'estratègia d'acoblaments creuats de Pd(0) i emprant benziloxi i isopropoxi com a grups protectors ortogonals dels fenols. Els conjugats mono-, di- i triester de Lamel·larina D han estat preparats amb rendiments globals de 18 a 57%, partint des dels corresponents precursors fenòlics de la Lam-D. Aquest és un procediment simple i eficaç per a la preparació de conjugacions de la Lamel·larina D amb potencial utilitat com a profàrmacs.

S'ha observat que la simple derivatització d'un sol fenol (el C-3 o C-4' OH) millora extraordinàriament les propietats de solubilitat de la Lam-D, i alguns di- i triester derivats mostraren una millora d'un ordre de magnitud en les GI_{50} per les línies cel·lulars cancerígenes MDA-MB-231 i A549. Estudis d'internalització cel·lular han revelat disposicions citoplasmàtiques dels productes per microscòpia confocal. La quantificació relativa dels conjugats envers la Lamel·larina D ha estat determinada per citometria de flux de fluorescència, i s'ha observat com les modificacions introduïdes han permès una millor internalització de tots els conjugats en la línia cel·lular A-549. A més a més, ha pogut ser determinat un mecanisme de mortalitat cel·lular per apoptosi via marcatge de caspases-3,7, i aturada del cicle cel·lular a la fase G2.

D'altra banda, l'accès al sistema pentacíclic de la Lamel·larina D també s'ha portat a terme seguint la metodologia inicialment descrita per Banwell i col·laboradors. Partint-se de la quaternització de la isoquinolina, seguida d'una cicloaddició sobre l'alquí i aromatització final per lliurar el sistema pentacíclic. Successius passos d'interconversió de grups protectors, i reaccions de condensació amb els corresponents derivats d'àcid carboxílic, ha possibilitat l'accès a conjugats de major complexitat estructural. Per la conjugació amb la Lamel·larina D es van seleccionar dendrímers nitrogentats i seqüències peptídiques de localització nuclear estructuralment ben definits i solubles en aigua. En aquest punt va ser de crucial importància una bona elecció dels grups protectors i la possibilitat d'optimitzar-ne la desprotecció final en un únic pas de reacció. Aquestes modificacions químiques han millorat significativament la solubilitat amb aigua dels productes, el seu alliberament cel·lular, i la seva citotoxicitat en les tres línies cel·lulars esmentades anteriorment. Estudis amb microscòpia confocal mostren

distribucions en regions nuclears per el conjugat a una seqüència peptídica de transport nuclear, amb co-localització a les zones amb presència de topoisomerasa. Aquest producte ha mostrat valors nanomolars en la potència inhibitòria del creixement cel·lular (GI_{50}) en les línies A-549 i MDA-MB-231, i representa un guanys d'un ordre de magnitud en comparació amb la Lamel·larina D.

Estudis sobre la inhibició de topoisomerasa portats a terme amb pinzes òptiques i microscòpia de forces atòmiques, aporten una perspectiva novedosa per la seva inhibició amb la Lamel·larina D. L'accès a resolució en forces del rang de pN que s'aconsegueix amb tecnologia de pinzes òptiques, ha permès registrar events d'escissió de monocadenes d'ADN i el seu posterior relligament com a part de la activitat enzimàtica, o bé, la falta de relligament en presència de la Lamel·larina D. Aquestes observacions concorden amb estudis de dinàmica molecular previs on s'indicava que en presència de la Lamel·larina D s'estabilitza el complex ternari intermedi ADN-topoisomerasa-Lamel·larina D.

Els resultats que se'n deriven emfatitzen el potencial de biosensors de pinzes òptiques per l'estudi mecanístic d'enzims ADN-modificants, i anticipen implicacions profundes en el descobriment de nous fàrmacs i nanotecnologia mèdica.

RESUM DE LA MEMÒRIA

Aquesta tesi comença amb la presentació de la família de Lamel·larines, uns productes naturals d'orígen marí amb interessants activitats biològiques. En el ***Capítol 1*** se'n descriuen els últims avanços quant al seu aïllament, nova metodologia sintètica i estudis sobre l'activitat biològica i el seu mode d'acció.

La consecució d'aquests mètodes sintètics ha obert la porta a la preparació del producte natural, i de molècules anàlogues. Així, en el ***Capítol 3*** es descriu la síntesi de la Lamel·larina D amb una metodologia d'acoblaments creuats successius de Suzuki amb catàlisi de Pd(0). Amb la preparació descrita al ***Capítol 2*** dels bromopolialcòxibenzens s'ha pogut realitzar la síntesi d'una quimioteca d'anàlegs sense lactona de la Lamel·larina D i amb la introducció de diferents patrons de substitució (es descriu al ***Capítol 4***). S'ha assajat l'activitat biològica i estudiat les relacions estructura-activitat d'aquests compostos, cosa que ha permès posar de manifest aquells grups estructuralment importants quant a activitat.

Al ***Capítol 5*** es descriu la síntesi d'uns conjugats amb polietilenglicol de la Lamel·larina D, i més enllà al ***Capítol 6*** es descriu la síntesi de conjugats estructuralment més complexos amb una seqüència peptídica de localització nuclear i un dendrímer. Amb els productes sintetitzats s'ha procedit a fer-ne una avaluació de la seva internalització i distribució cel·lulars, estudis co-localització dels productes amb marcatges selectius de membrana, mitocòndries i topoisomerasa.

Per l'estudi del mode d'acció de la Lamel·larina D com a inhibidor de la topoisomerasa, s'ha fet ús de biosensors de pinzes òptiques i microscòpia de forces atòmiques. Aquest treball es descriu al ***Capítol 7*** i cal destacar que aquestes novedoses tecnologies amb sensibilitats de pN i resolució nanomètrica es mostren d'elevada utilitat per a l'estudi de l'activitat de la topoisomerasa, i de la seva inhibició.