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

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TITLE RUNNING HEAD. Lamellarin D – NLS and DTPA conjugates.

ABSTRACT: 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**, **4** showed a decrease in activity potency in BJ skin fibroblasts, a normal cell culture. Cellular internalization was analyzed and nuclear distribution pattern was observed for **4**, which contains a nuclear localization signalling sequence.

KEYWORDS: Lamellarin D; PEG; DTPA; Nuclear localization signal; Dendrimer

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 to 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 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 x 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 ºC 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 ºC and centrifugation (10 min, 2000 r.p.m.). 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 (2h, *J* = 6.6 Hz, 8H); 1.76 and 1.83 (2h, *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 pre-activated for 15 min at r.t. with TCFH (63 mg, 23 mmol) and NEt_3 (32 µL, 23 mmol) in CH_2Cl_2 dry (15 mL). Compound **6** (53 mg, 0.08 mmol) and DMAP (9 mg, 0.08 mmol) in CH_2Cl_2 dry (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 ºC. IR (film) ν 1704, 1487, 1428, 1284, 1111 cm^{-1}. ^1H 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 ºC solution of **10d** (771 mg, 0.82 mmol), in MeOH-THF (100 mL, 80:20). The mixture was stirred at -78 ºC 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 ºC. 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 disc. 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 pre-swollen 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 one hour, 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 2h, the resin was washed with DMF and CH_2Cl_2, and the coupling was monitored using the Kaiser test. Re-couplings 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 x 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 ºC 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 ºC. IR (film) ν 1705, 1429, 1267, 1223 cm^{-1}. ^1H 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 ºC. 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 ºC. IR (film) ν 3415, 1679, 1429, 1271 cm^{-1}. ^1H 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 a 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 ºC. IR (film) ν 1760, 1710, 1486, 1430, 1274, 1255 cm^{-1}. ^1H 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)propylamino)-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*-butoxycarbonylaminoacetyl)-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 ºC. 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 ºC 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}. ^1H 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**, **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 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 bottom microwell dishes at 30 10^3 cells/cm^2. After 24 h, the culture medium was discarded and replaced by fresh DMEM medium containing either 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 ^oC.

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 bottom 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, U.S.A) 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, U.S.A), 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. Afterwards 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 ºC, 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. 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 1µM Lam-D, **1**, **2** or **4** at a Absence of compound was used as a negative control. The cells were then incubated for 12 h at 37 ºC. 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 ºC 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**[3] (**Scheme 1**). 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 mono-protected 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-, and di-conjugates 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 $BocNH\text{-}CH_2(CH_2OCH_2)_6CH_2COOH$ 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** (**Fig. 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, **Fig. 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** (**Fig. 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, pre-activation of **8** with TCFH (*30*) and NEt_3, followed by the addition of a solution of **6** and DMAP were required, affording **15** (**Fig.**

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 analogs (**1**, **2**, **4** and **15**) was evaluated against BJ human skin fibroblasts, and a panel of three human tumor 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%.

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 (**Fig.3**, positive control). Conjugate **4**, carrying the NLS signal, was localized to the nucleus in HT-29 and A-549 cells (**Fig. 3**, **j1**, **j2**, **k1** and **k2**), suggesting that its higher activity could correlate to subcellular co-localization 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 (**Fig. 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**, **4** in BJ skin fibroblasts as normal cells was used in the present study. In this cellular culture, no significative variation, or even less citotoxicity was obtained for conjugates **3**, **4** than the parent compound Lam-D. PEGylated conjugates **1** and **2** have much higher cytotoxicity to MDA-MB-231 cells than does 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 A-549 cancer cell line could correlate to co-localization of **4** in nuclear regions where GFP–Topo I accumulated (**Fig. 3k**). The NLS peptidic sequence is at least partly responsible for nuclear import of **4** in A-549 and HT-29 cell lines. Contrariwise, 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 strategies, 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 co-localization 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.

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References:

(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 Deliv. 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 Del. Rev. 56*, 1273-1289.

(4) Greenwald, R. B., Pendri, A., Conover, C. D., Zhao, H., Choe, Y. H., Martinez, 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 Del. 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. (2008) 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., Fernandez 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.
(21) Veronese, F. M., and Harris, J. M. (2008) Peptide and protein PEGylation III: advances in chemistry and clinical applications. *Adv. Drug Del. 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.

Footnotes:

[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 percent growth inhibition; *i*Pr, isopropyl; Lam, Lamellarin; MDA-MB-231, breast adenocarcinoma cell line, MOM, methoxymethyl; MSNT, 1-(mesitylene-2-sulfonyl)-3-nitro-*1H*-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(ethyleneglycol); 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.

[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.

[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'*-tetramethylchloroformamidyl chloride as activating agents.

[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 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.

Table 1. Cytotoxicity of compounds **1**, **2**, **4**, **15** in three human cancer cell lines.

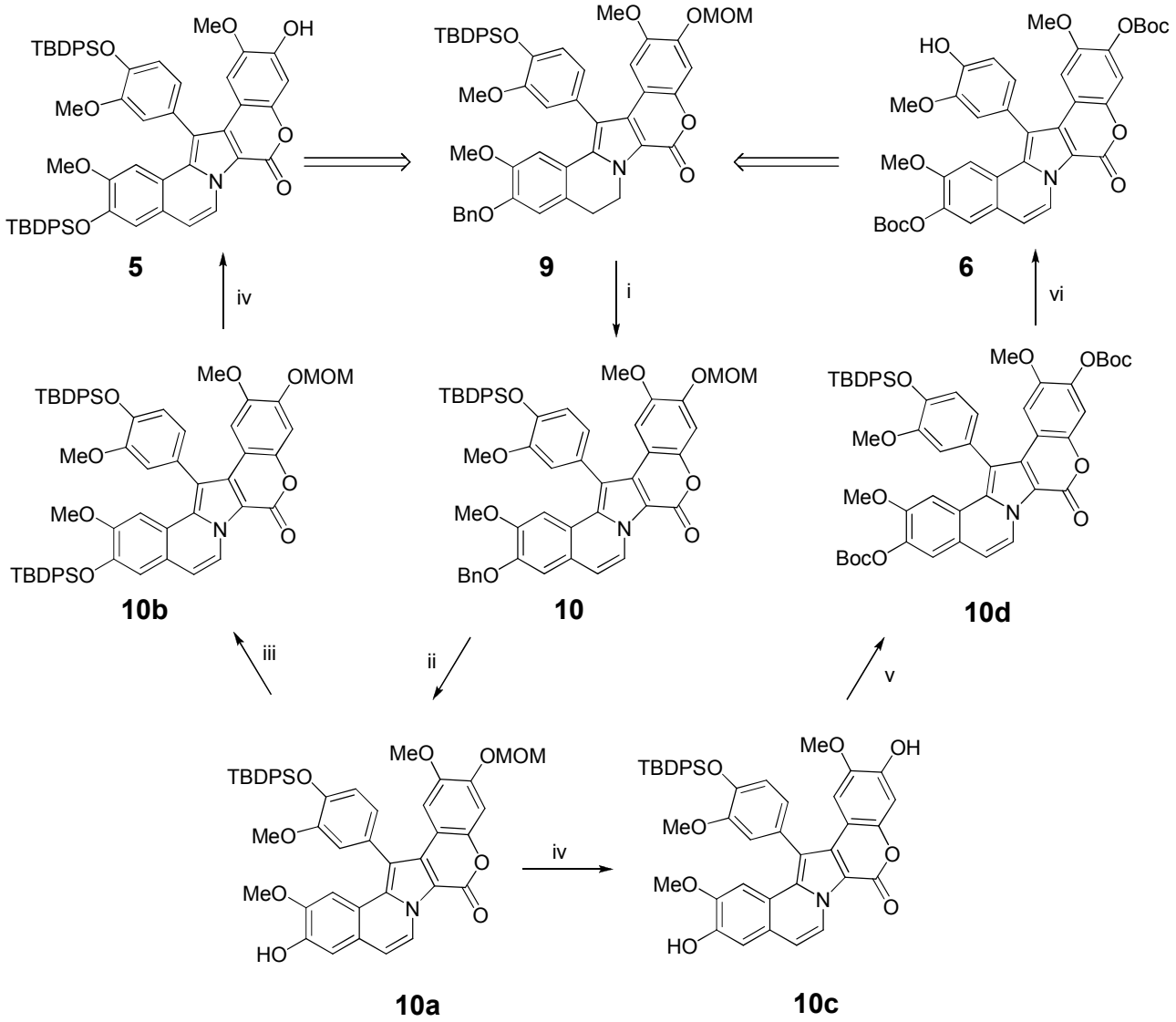
Compound	Cytotoxicity (M)			
	HT-29 Colon	A-549 Lung	MDA-MB-231 Breast	BJ Skin Fibroblast
Lam-D	$3.00\ 10^{-6}$	$1.22\ 10^{-7}$	$1.34\ 10^{-7}$	$6.37\ 10^{-9}$
1	$1.68\ 10^{-6}$	$8.86\ 10^{-8}$	$4.07\ 10^{-8}$	$6.51\ 10^{-9}$
2	$3.92\ 10^{-6}$	$2.20\ 10^{-7}$	$8.31\ 10^{-8}$	$1.54\ 10^{-8}$
4	$1.01\ 10^{-6}$	$4.79\ 10^{-8}$	$4.79\ 10^{-8}$	$3.14\ 10^{-8}$
15	$1.94\ 10^{-6}$	n.d.	$1.24\ 10^{-6}$	---

Table 2. Cellular internalization as measured by FACS.

Compound	Cellular Internalization (AU)				Cellular Internalization Quotient (CIQ)[a]			
	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%

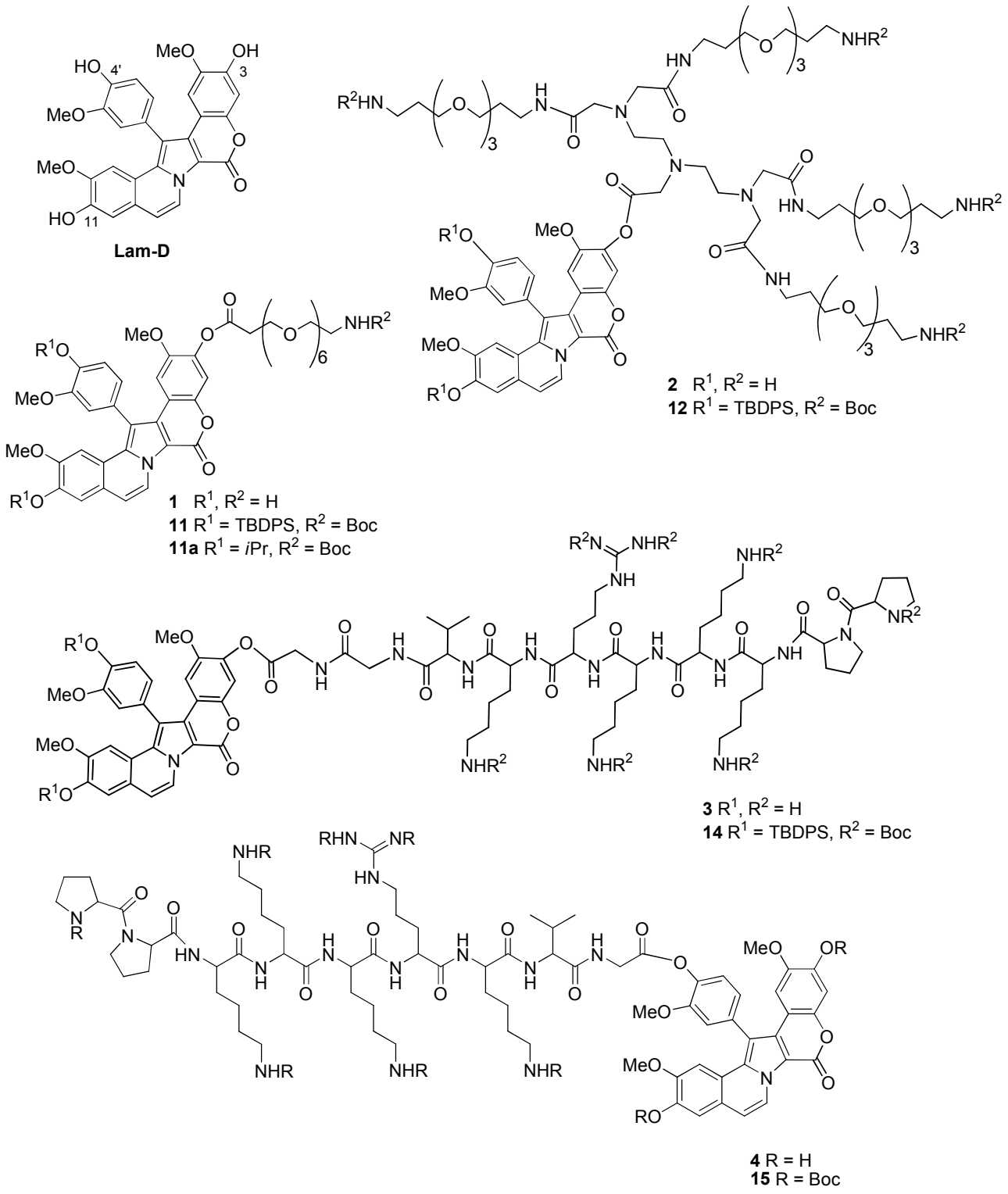
[a] CIQ was calculated in reference to the cellular uptake of Lam-D.

Scheme 1.



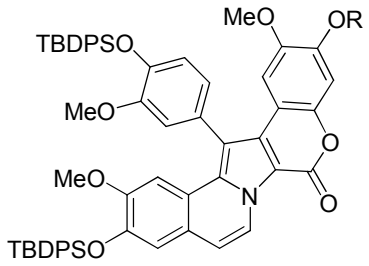
Reagents and conditions: i: DDQ, $CHCl_3$, MW, 120 ºC, 10 min (81%); ii: H_2, Pd-C, EtOAc, MeOH, r.t., 16 h (95%); iii: TBDPSCl, Im, DMAP, DMF, r.t., 24 h (58%); iv: Me_3SiI, CH_2Cl_2 r.t., 20 min (84% for **5**; quant. yield for **10c**); v: Boc_2O, DMAP, CH_2Cl_2 r.t., 16 h (quant.); vi: 1M TBAF in THF, MeOH, -78 ºC, 15 min (85%).

Figure 1.

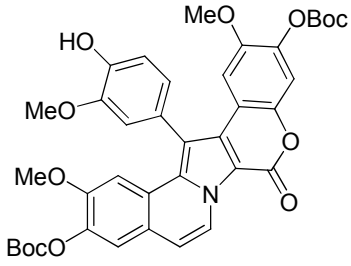


Structures of Lam-D conjugates

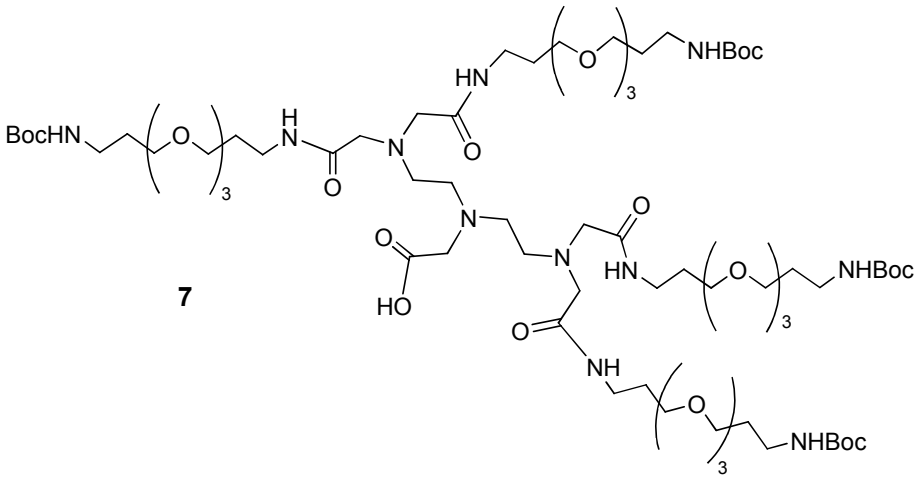
Figure 2.



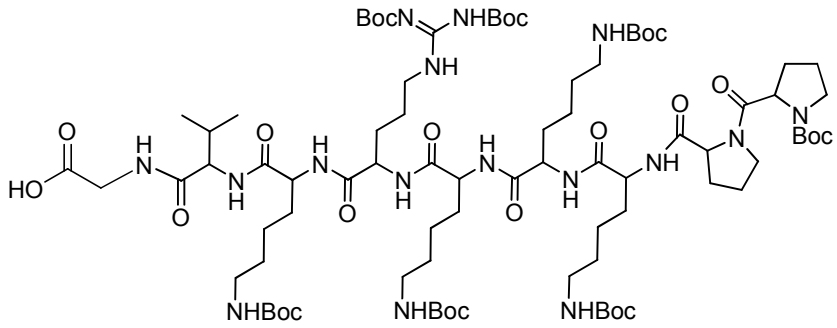
5: R = H
13: R = $COCH_2NHBoc$



6



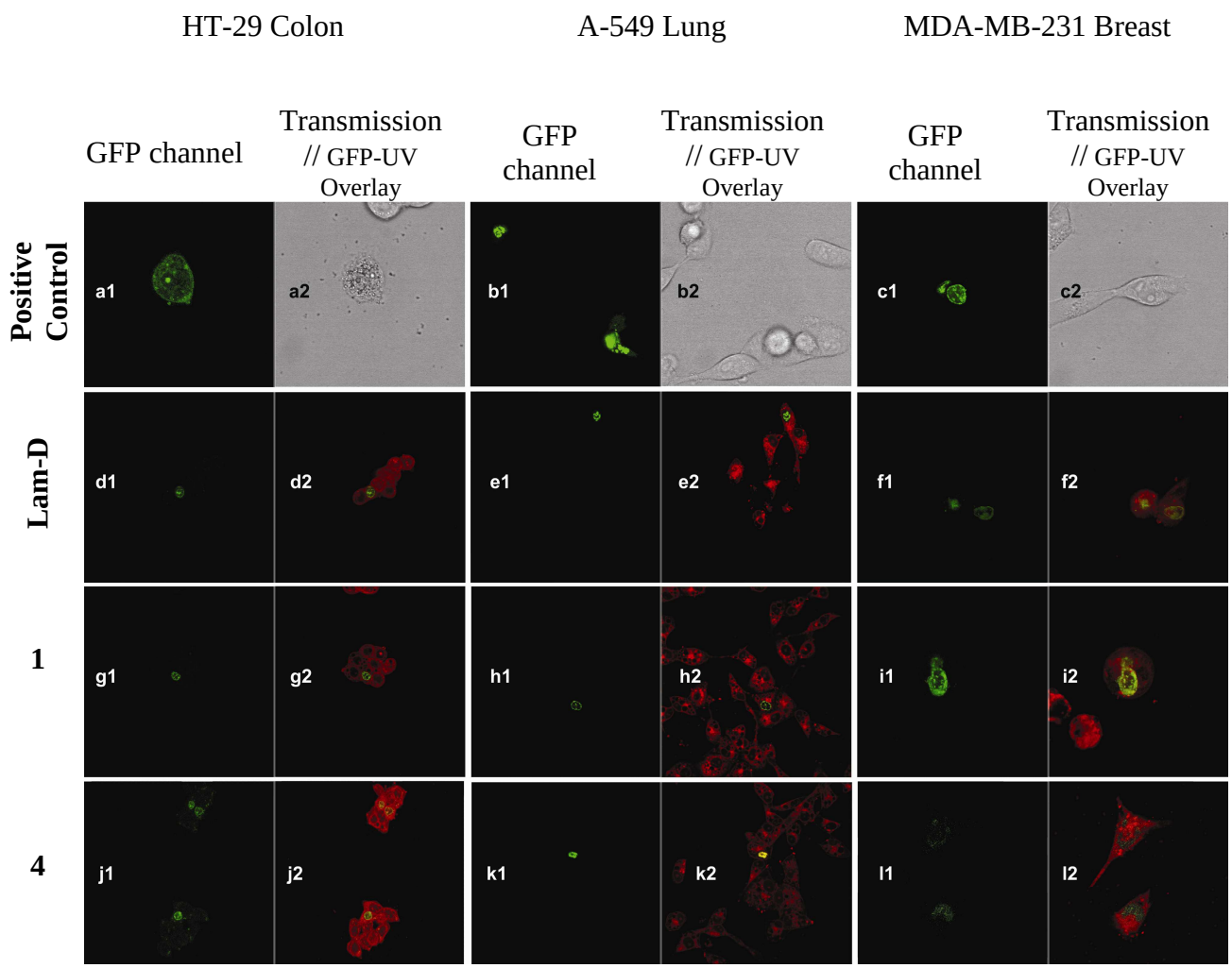
7



8

Building blocks for the synthesis

Figure 3. Internalization of Lam-D and conjugates **1** and **4** by Topo I-GFP transfected cells.



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.