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Grau de Química

Treball Final de Grau

Synthesis and characterization of new coumarin-based caging groups.

Síntesi i caracterització de nous grups protectors fotolàbils basats en cumarina.

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There is a single light of science, and to brighten it anywhere is to brighten it everywhere.

Isaac Asimov

Abans de res, voldria agrair al Dr. Vicente Marchán Sancho la dedicació, orientació i atenció brindades durant tot aquest temps.
Als membres del grup de recerca, amb qui he compartit dia a dia la consecució d'aquest treball, gràcies per l'ajuda prestada. Especialment, a l'Albert, la Sara i l'Ana, companys de "batalla" amb una gran disposició i paciència.
Per últim, un agraïment no menys sincer a la meva família i amics, a aquells que em proporcionen la força, l'energia i els ànims per a seguir creixent com a persona i per a continuar formant-me. Gràcies per la comprensió i solidaritat amb aquest projecte i, sobretot, amb el temps sacrificat.
El recolzament de tots ha estat vital i és per això que aquest treball és, en part, vostre.
A tots, moltes gràcies.

REPORT

CONTENTS

1. SUMMARY

The use of light has enormous potential for controlling the outcome of molecular processes with high spatio-temporal precision. In this context, a promising approach consists of introducing photoremovable protecting groups (also referred to as caging groups) in key positions of the molecule whose activity has to be suppressed temporarily. Among photoremovable protecting groups described to date, dicyanocoumarin derivatives are particularly attractive since they exhibit a maximum absorption around 500 nm, which can be exploited to trigger uncaging with green light.

In this work, we have focused on the synthesis and characterization of three new dicyanocoumarin-caged model compounds with the aim of exploring how the uncaging process is affected both by the structure of the coumarin chromophore and by the nature of the leaving group.

Keywords: caged coumpounds, caging groups, coumarin, photoactivation.

2. RESUM

L'ús de la llum presenta un gran potencial a l'hora de regular processos moleculars amb una elevada precisió espai-temporal. En aquest context, una aproximació prometedora consisteix en introduir grups protectors fotolàbils en posicions clau de la molècula l'activitat de la qual es vol suprimir temporalment. Entre els grups protectors fotolàbils descrits fins al moment, els derivats de dicianocumarina són particularment atractius ja que presenten un màxim d'absorció al voltant de 500 nm, factor que pot ser explotat per desencadenar la desprotecció amb llum verda.

En aquest treball, ens hem centrat en la síntesi i la caracterització de tres nous models de compostos protegits amb dicianocumarina amb l'objectiu d'explorar com es veu afectat el procés de desprotecció tant per l'estructura del cromòfor de cumarina com per la naturalesa del grup sortint.

Paraules clau: molècules fotoactivables, grups protectors fotolàbils, cumarina, fotoactivació.

3. INTRODUCTION

3.1. PHOTOLABILE PROTECTING GROUPS AND CAGED COMPOUNDS

In recent years, the use of photoremovable protecting groups (PPGs) for the regulation of molecular processes with light has attracted great interest in many fields of Chemistry and Biology. Caged compounds are inert analogues of biologically active molecules whose activity has been disabled through the attachment of a protecting (caging) group via a photolabile chemical bond.[1] Hence, activation of these molecules can be precisely controlled by irradiation, typically with UV or visible light, in terms of time, place and dosage (Figure 1).[2] Furthermore, light can be considered as an ideal external trigger because it is non-invasive, does not cause sample contamination and its qualitative and quantitative properties can be precisely controlled.[3]

Depending on the final application, PPGs need to fulfill some of the following requirements:[4]

i) Uncaging should occur at wavelengths well above 365nm to minimize absorption by endogenous molecules and to avoid damage to the biological entity.
ii) The photoreaction should be fast, occur with high release efficiency and with no side-reactions.
iii) PPGs should be soluble in water (and the resulting caged compounds), they may show affinity to specific target components and/or pass through barriers.
iv) The photochemical byproducts must be biocompatible.

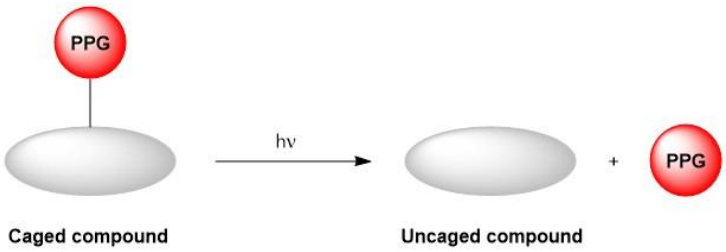


Figure 1. Schematic representation of light-triggered deprotection of a caged compound.

3.2. COUMARIN DERIVATIVES AS CAGING GROUPS

Numerous photoremovable protecting groups have been described to date.[4] Yet, most of them are photoactivated by UV light and only a few allow to cage multiple organic functionalities. An alternative to try to overcome these limitations can be found in the use of coumarin derivatives as caging groups.

So far, the coumarin chromophore has been extensively modified to shift its absorption maximum up to the limit between the UV and visible range. Even red-shifted absorptions up to the 500 nm have been reached upon appropriate substitutions. In particular, the introduction of an electron-donating group at the 7-position is known to cause a high bathochromic shift due to a large charge-transfer in the excited state. Further red-shifted absorption is possible by introduction of electron-withdrawing groups either at the 2- or 3-positions of the coumarin skeleton.[5] The modification of the carbonyl group of the lactone in the *N,N*-diethylamino(coumarin-4-yl)methyl platform has also been proved to be a valuable way to shift the wavelength of maximum absorption, thereby facilitating uncaging with blue and cyan light, which is more compatible with biological applications. Indeed, dicyanomethylenecoumarin derivatives, which are obtained by incorporating two nitrile groups at position 2 of the coumarin moiety, are particularly attractive since exhibit a maximum absorption around 500 nm (compound NdiEt-mcBA in Figure 2 and Table 1). In conclusion, the modified absorption spectra of such chromophores make coumarin derivatives very attractive compounds both to cage biologically relevant molecules, such as peptides, [6] oligonucleotides, [7] neurotransmitters or agrochemicals, but also to develop wavelength-selective systems in combination with known UV-absorbing caging groups.[3]

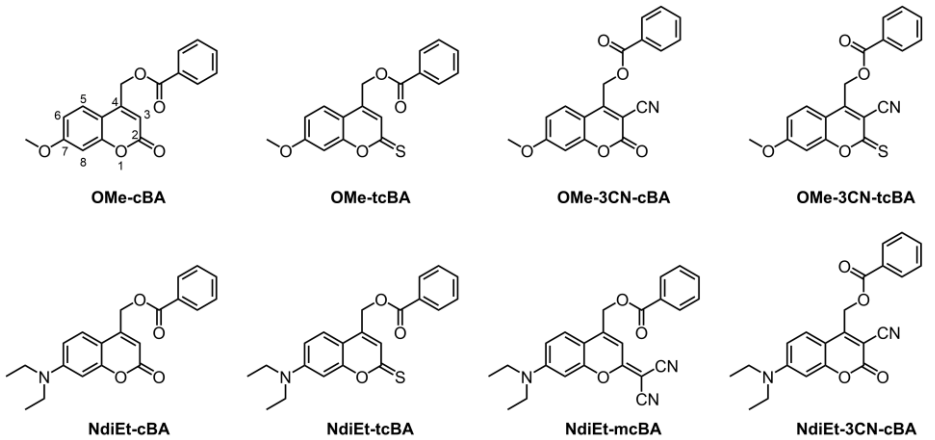


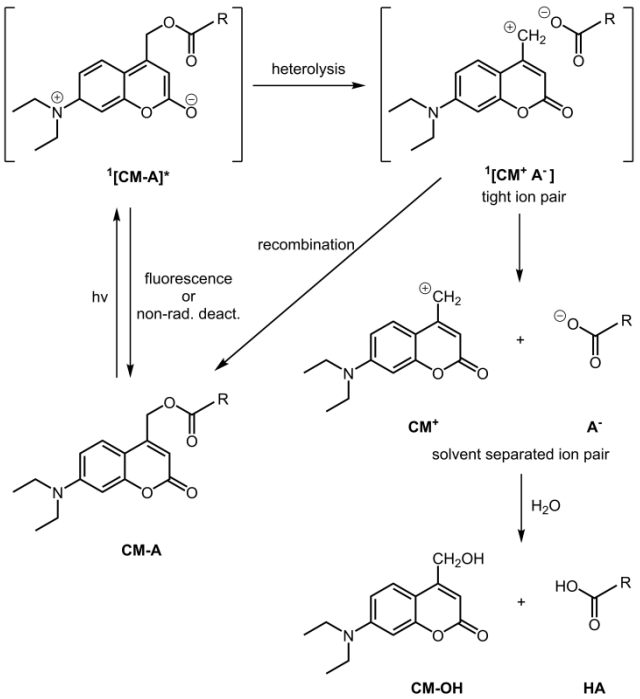
Figure 2. Structure of some coumarin derivatives.

Entry	Compound	λ_{max} [nm]
1	OMe-cBA	323
2	OMe-3CN-cBA	360
3	NdiEt-cBA	385
4	OMe-tcBA	398
5	OMe-3CN-tcBA	427
6	NdiEt-3CN-cBA	443
7	NdiEt-tcBA	472
8	NdiEt-mcBA	487

Table 1. Absorption maximum for different coumarin derivatives.[5]

(Coumarin-4-yl)methyl derivatives have been successfully applied to mask the biological activity of many functionalities via ester bond (CM-A). After absorption of a photon by CM-A, relaxation to the lowest excited singlet state, ^{1}[CM-A]*, occurs. Deactivation of ^{1}[CM-A]* by means of fluorescence and non-radiative processes competes with heterolytic bond cleavage forming a tight ion pair, ^{1}[CM^+ A^-], which can either recombine to lead back to ground-state CM-A or escape from the solvent cage and react with water to give (coumarin-4-yl)methyl alcohol (CM-OH) and the corresponding uncaged carboxilic acid (HA) (Scheme 1).[1],[4] It has been described that stabilization of the carbocation CM^+, which is a component of the primary intermediate of photocleavage, causes an increase of the photolysis rate constant since its

formation is favoured. Moreover, stabilization of the anion A^- can also accelerate the heterolytic bond cleavage. Furthermore, both factors retard the recombination reaction, which competes with cage escape and hydrolysis to give the desired products (CM-OH and uncaged HA). This, in turn, favours an increase in the efficiency of the competing product formation.[1]



Scheme 1. Mechanism for the photocleavage of (coumarin-4-yl)methyl esters.

In our research group we have focused on the use of dicyanocoumarin derivatives as caging groups of peptide carriers since they can be efficiently removed by irradiation with green light. Recently, the photophysical and photochemical properties of 4-(acetoxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)coumarin (**1**, Figure 3) have been studied. Taking into account that uncaging of this compound with green light (505 nm) was too slow, in this work we wanted to explore how structural modifications of the coumarin skeleton could be exploited to facilitate the uncaging process. First, we decided to introduce a methyl group at the C4 of the coumarin skeleton of 4-(acetoxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)coumarin

(**1**) since a secondary carbocation would be generated upon photolysis instead of a primary carbocation, which would facilitate the uncaging process by increasing the stability of the intermediate (Scheme 1). Secondly, the introduction of a better leaving group by replacement of acetate by benzoate would also facilitate the uncaging process. As shown in Figure 4 and Table 2, four dicyanocoumarin derivatives have been designed and synthesized in this work.

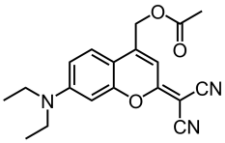


Figure 3. Structure of 4-(acetoxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)coumarin (**1**).

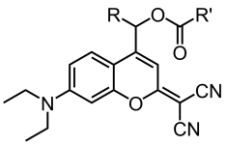


Figure 4. Structure of the target 2-(dicyanomethylene)-7-(*N,N*-diethylamino)coumarin derivatives.

Compound	R	R'
1	H	CH_3
2	CH_3	CH_3
3	H	Ph
4	CH_3	Ph

Table 2. Summary of 2-(dicyanomethylene)-7-(*N,N*-diethylamino)coumarin derivatives studied in this work.

4. OBJECTIVES

On the basis of these precedents, in this work we have focused on the synthesis and characterization of three new dicyanocoumarin derivatives, (**2**, **3** and **4)**. In addition, the uncaging of these compounds under green light irradiation together with that of coumarin **1** was carried out to study the influence of the stability of the carbocation intermediate and of the leaving group on the rate of the photolysis process.

The specific objectives of this work were:

1) Synthesis of 4-(benzoyloxymethyl)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)-2-thiocoumarin (**5**), which was necessary for the preparation of two dicyanocoumarin derivatives.

2) Synthesis and spectroscopic characterization of 4-(acetoxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**2**), 4-(benzoyloxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)coumarin (**3**) and 4-(benzoyloxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**4**).

3) Photolysis studies with dicyanocoumarin derivatives (**1**, **2**, **3** and **4**).

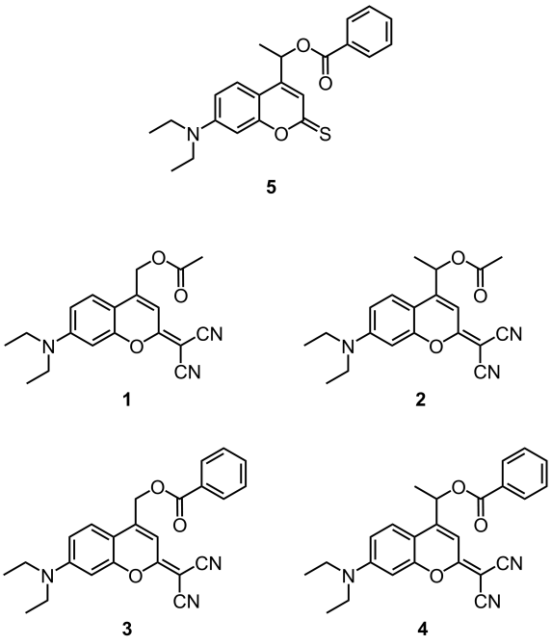
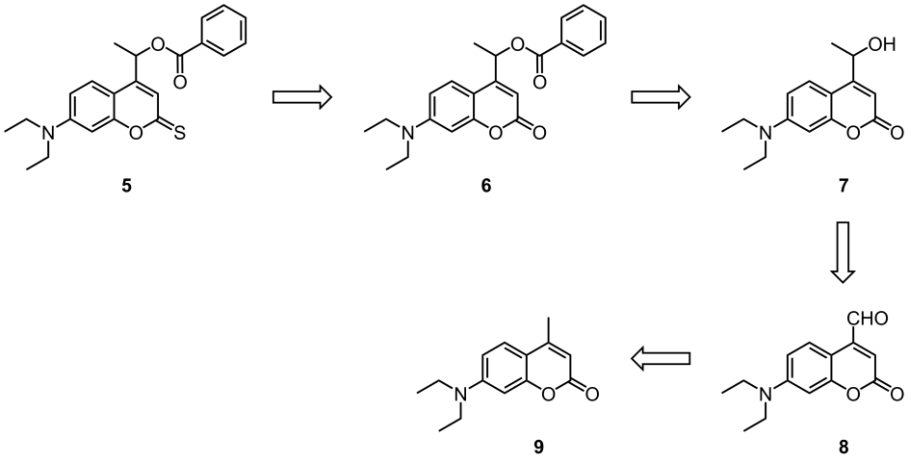


Figure 5.

5. RESULTS AND DISCUSSION

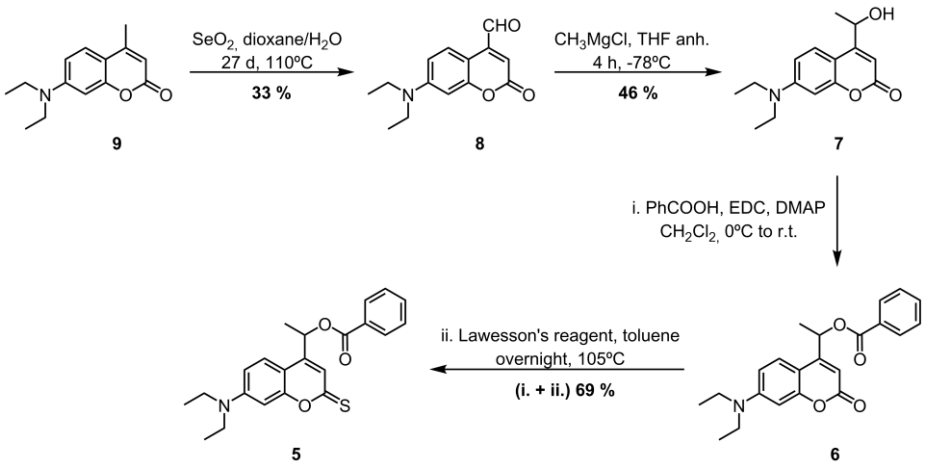
5.1. SYNTHESIS OF 4-(BENZOYLOXYMETHYL)-7-(*N,N*-DIETHYLAMINO)-4-(1-HYDROXYETH-1-YL)-2-THIOCOUMARIN (5)

As shown in Scheme 2, the synthesis of the thiocoumarin derivative (**5**), 4-(benzoyloxymethyl)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)-2-thiocoumarin, was planned through thionation of the carbonyl group of the lactone function of 4-(benzoyloxymethyl)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**6**) with Lawesson's reagent.[5] The benzoate derivative of coumarin can be obtained by esterification of the secondary alcohol function of 7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**7**), which can be prepared by methylation of the aldehyde function of 4-carbaldehyde-7-(*N,N*-diethylamino)coumarin (**8**) through Grignard reaction.[6] Compound **8** can be obtained from the commercially available 7-(*N,N*-diethylamino)-4-methylcoumarin (**9**) by oxidation of the allylic position with SeO_2.[8]



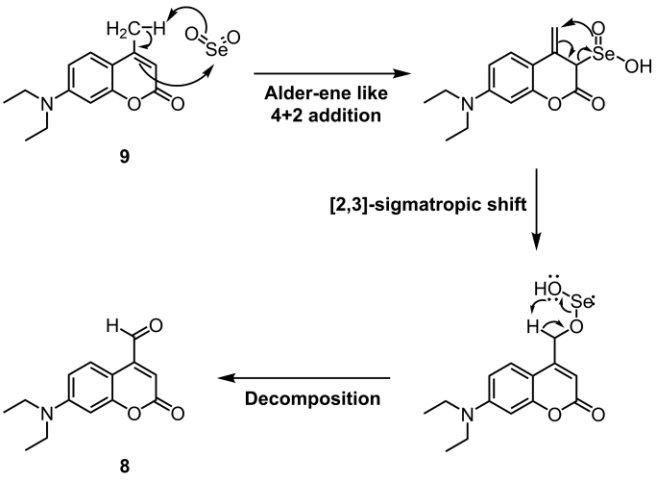
Scheme 2. Retrosynthetic analysis for the preparation of thiocoumarin **5** from **9**.

The synthesis route followed for the preparation of 4-(benzoyloxymethyl)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)-2-thiocoumarin (**5**) is shown in Scheme 3.



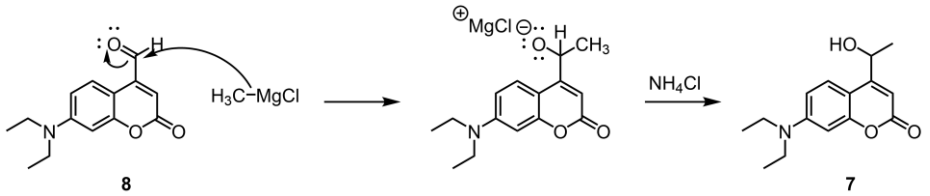
Scheme 3. Synthetic route followed for the preparation of **5**.

Starting with commercially available coumarin **9**, oxidation with SeO_2 in a dioxane/water mixture during 27 days at 110ºC conduced to the aldehyde **8**, which was isolated by silica column chromatography and characterized by ^{1}H-NMR.



Scheme 4. Mechanism for the oxidation of **9** with SeO_2.

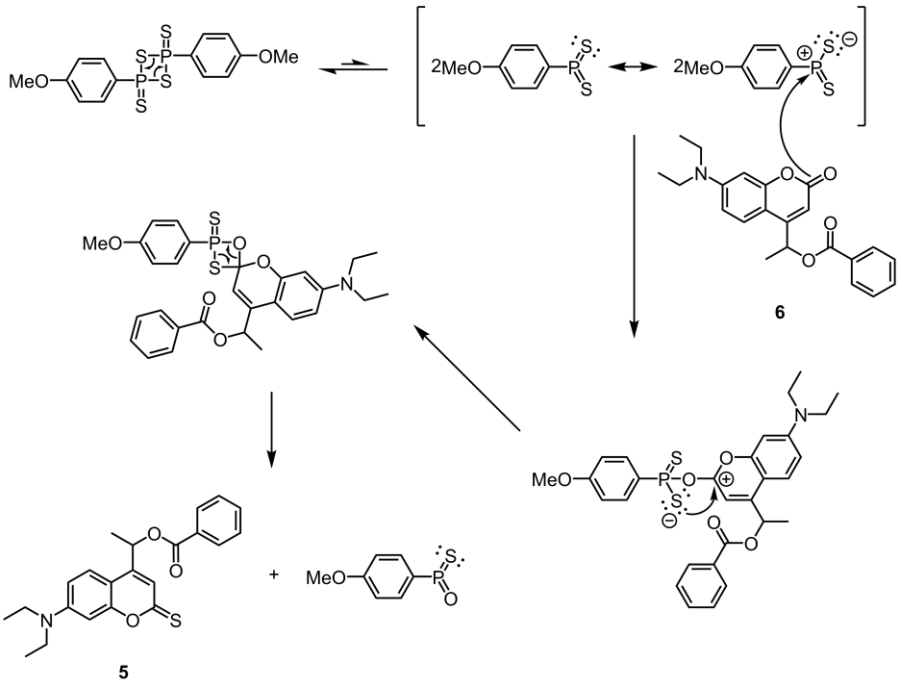
Next, the aldehyde group was reacted with CH_3MgCl in anhydrous THF for 4 hours at -78ºC to generate the coumarin alcohol derivative **7**, which was purified by silica column chromatography and characterized by ^{1}H-NMR.



Scheme 5. Mechanism for the Grignard reaction of **8**.

The following step involved protection of the secondary alcohol function of **7** by esterification with benzoic acid by using EDC as coupling reagent and DMAP as catalyst to give **6**, which was used without further purification in the next step.

Finally, 4-(benzoyloxymethyl)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)-2-thiocoumarin (**5**) was obtained by reaction of **6** with Lawesson's reagent overnight in toluene at 105ºC. According to the known higher reactivity of Lawesson's reagent for lactones than for esters, thionation occurred in the carbonyl group of the lactone rather than in the ester. Product **5** was isolated by silica column chromatography and fully characterized by ^{1}H-NMR and ^{13}C-NMR.



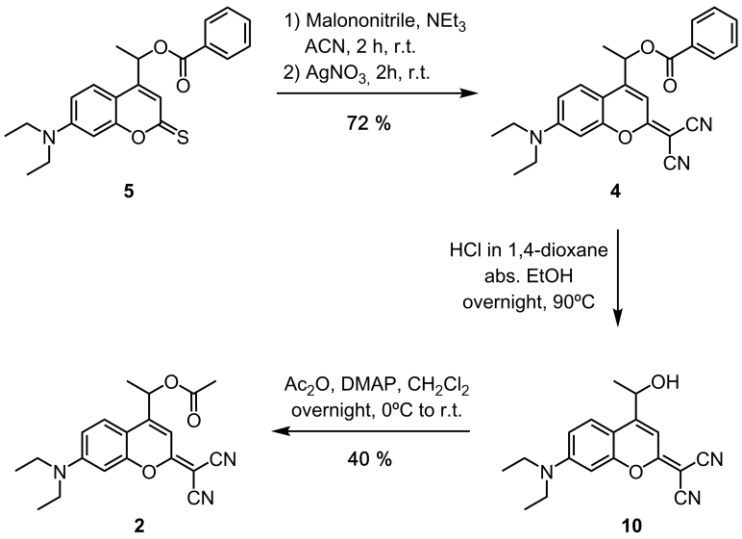
Scheme 6. Mechanism for the thionation of **6** by using Lawesson's reagent.

Lawesson's reagent has a four-membered ring of alternating phosphorus and sulphur atoms. Upon heating, the central four-membered ring opens to form two more reactive dithiophosphine ylides, which react with a carbonyl group to generate a thiaoxaphosphetane intermediate. Then, formation of a stable P=O bond forces the cycloreversion step.

5.2. SYNTHESIS OF 2-(DICYANOMETHYLENE)-7-(*N,N*-DIETHYLAMINO)COUMARIN DERIVATIVES

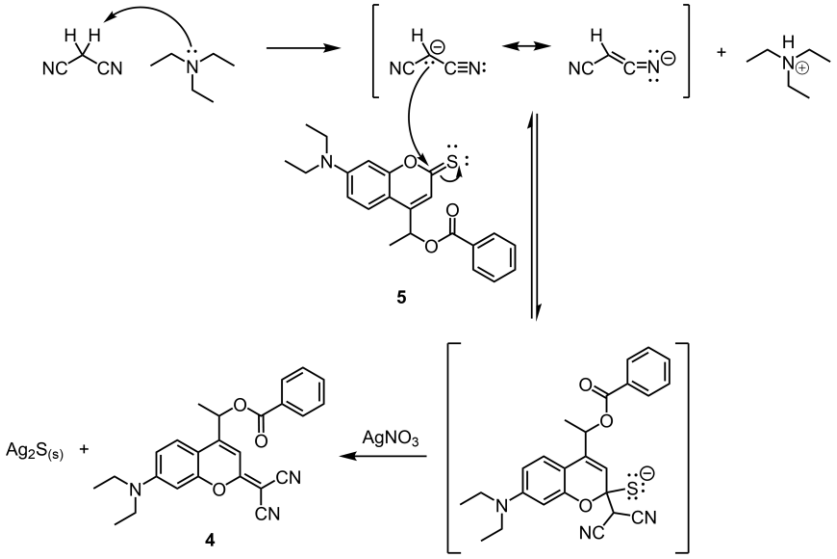
5.2.1. Synthesis of 2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin derivatives (2 and 4)

As shown in Scheme 7, condensation of malononitrile with the thiocarbonyl group of 4-(benzoyloxymethyl)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)-2-thiocoumarin (**5**) in the presence of silver nitrate afforded to 4-(benzoyloxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**4**).[5] The acetate derivative, 4-(acetoxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**2**), was obtained by esterification of the secondary alcohol function of compound **10**, which was obtained by acidic hydrolysis of **4**.



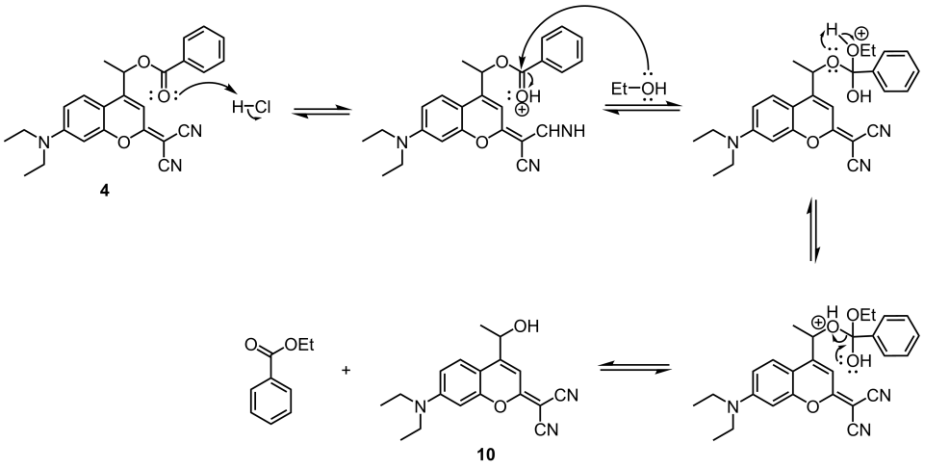
Scheme 7. Synthetic route followed for the preparation of **2** and **4** model caged compounds.

First, malononitrile was reacted with thiocoumarin **5** in anhydrous acetonitrile under an argon atmosphere and protected from light in the presence of triethylamine, which was used to deprotonate malononitrile. As shown in scheme 8, malononitrile carbanion acts as a nucleophile and attacks the thiocarbonyl group forming a tetrahedral intermediate. Finally, addition of $AgNO_3$ conduces to the formation of the target compound, 4-(benzoyloxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**4**), by facilitating the removal of the sulphur atom. Compound **4** was isolated by silica column chromatography and fully characterized by ^{1}H and ^{13}C NMR and ESI-MS.



Scheme 8. Proposed mechanism for the condensation of malononitrile with **5**.

Next, acidic hydrolysis of the ester was carried out by reaction of compound **4** with HCl in a 1,4-dioxane/ethanol mixture. We found that hydrolysis of **4** was very slow under the conditions previously used for the hydrolysis of **1**, so further optimization of this reaction will be required. However, a small amount of 2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**10**) could be isolated by silica column chromatography and used in the synthesis of **2**.

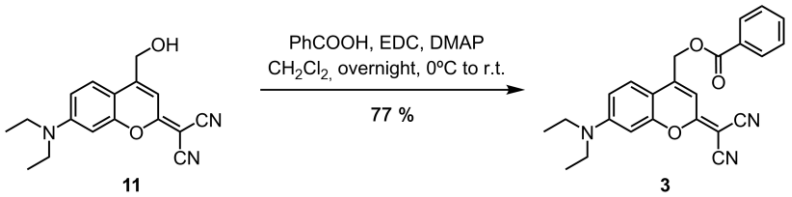


Scheme 9. Mechanism for the acidic hydrolysis of **4**.

Finally, acetylation of the secondary alcohol function of **10** by reaction with acetic anhydride and DMAP in DCM was carried out. The reaction mixture was stirred at room temperature overnight to give 4-(acetoxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**2**). The product was isolated by silica column chromatography and fully characterized by ^{1}H and ^{13}C NMR and ESI-MS.

5.2.2. Synthesis of 4-(benzoyloxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)coumarin (3)

As shown in Scheme 10, the synthesis of coumarin derivative **3** was performed through esterification of 2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(hydroxymethyl)coumarin (**11**) with benzoic acid by following the procedure previously used for the synthesis of **8**. Compound **3** was purified by silica column chromatography and characterized by ^{1}H and ^{13}C NMR.



Scheme 10. Synthesis of **3** model caged compound.

5.3. PHOTOLYSIS STUDIES OF DICYANOCOUMARIN MODEL CAGED COMPOUNDS (1-4)

Taking into account the shape of the absorption curve and the molar extinction coefficients of the dicyanocoumarin derivatives at their λ_{max} and at 505 nm,[9] we decided to evaluate if green light could be used to deprotect them, because it is less harmful to cells and penetrates deeper in tissues than UV or blue light. A 505 nm LED source was used for irradiation of solutions of the compounds at 37ºC, and the course of the uncaging process was monitored by reversed-phase HPLC-ESI MS. As shown in Figure 4, the concentration of the compounds decreased gradually with time upon irradiation, being very dependent on the structure of the coumarin chromophore and on the nature of the leaving group. Table 3 shows the percentage of the remaining caged compound after different times of irradiation with green light (505 nm).

	Irradiation time [min]				
Compound	**0**	**2**	**5**	**10**	**20**
1	100	85	60	5	0
2	100	28	2	0	0
3	100	55	20	0	0
4	100	15	0	0	0

Table 3. Percentage of the remaining caged compound upon irradiation with 505 nm light.

As expected, caged model compounds with a methyl group at the 4-position of the coumarin skeleton (**2** and **4**) showed a higher percentage of uncaging at shorter irradiation times in comparison with their unmethylated analogues (**1** and **3** respectively), which confirmed that photolysis can be accelerated by stabilization of the carbocation intermediate.

On the other hand, caged benzoic acid compounds (**3** and **4**) showed a higher percentage of photocleavage at shorter irradiation times when compared with their caged acetic acid analogues (**1** and **2** respectively). This, in turn, agrees with the predicted increase of the photocleavage rate in terms of stabilization of the leaving group (benzoate *vs* acetate).

Comparing the effect of the stabilization of the carbocation with that of the leaving group on the photolysis rate, one would say that the first prevails over the latter. Nevertheless, further studies should be done considering better leaving groups as well as other approaches for the stabilization of the carbocation intermediate to state this.

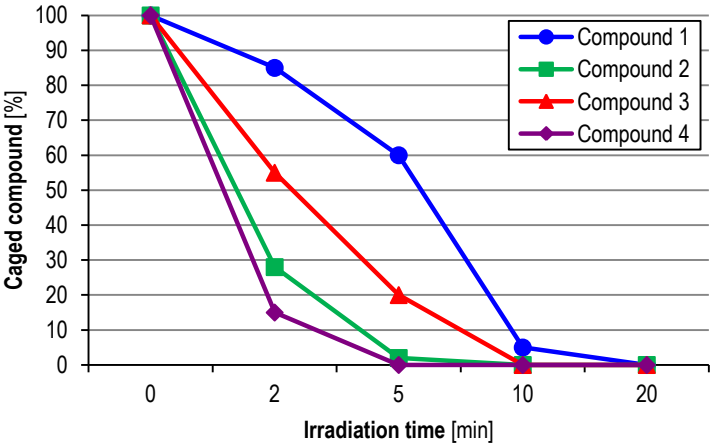


Figure 6. Graphical representation of the percentage of the remaining caged compounds upon irradiation with 505 nm light.

6. CONCLUSIONS

A thiocoumarin derivative, 4-(benzoyloxymethyl)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)-2-thiocoumarin (**5**), has been synthesized in 4 steps (overall yield: 10 %) and characterized by spectroscopic techniques. In addition, three new dicyanocoumarin-caged model compounds (**2**, **3** and **4**) have been synthesized and fully characterized by ^{1}H and ^{13}C NMR and ESI-MS. Photolysis studies with 4-(acetoxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)coumarin (**1**) and the three new coumarin derivatives synthesized in this work have demonstrated that stabilization of the carbocation intermediate by insertion of a methyl group at the 4-position of the coumarin skeleton has a positive effect on the uncaging process under green light irradiation. Similarly, the nature of the leaving group influences the rate of the photolysis process, being higher with benzoate than with acetate.

7. EXPERIMENTAL SECTION

7.1. MATERIALS AND METHODS

7.1.1. Reagents and solvents

All reagents were supplied by Sigma Aldrich, Alfa Aesar, Fluorochem or Acros.

The solvents and its quality and supplier are indicated in the following table:

Entry	Solvent	Quality	Supplier
1	DMF[(a)]	Synthesis quality	SDS
2	CH_2Cl_2	Synthesis quality	SDS
3	CH_2Cl_2	HPLC quality	Fisher
4	EtOH absolute	Synthesis quality	Panreac
5	MeOH	Synthesis quality	Carlo Erba
6	Acetonitrile[(b)]	HPLC quality	VWR
7	Toluene	Synthesis quality	Scharlau
8	H_2O	Mili-Q	-

(a) DMF was dried by standing over 4Å molecular sieves and bubbled with nitrogen to remove volatile amines.
(b) Acetonitrile was dried by standing over 4Å molecular sieves.

Table 4.

7.1.2. Nuclear magnetic resonance spectroscopy (NMR)

All NMR spectra were recorded at 25ºC on a Varian Mercury 400MHz, using $CDCl_3$ containing 0.03 % (v/v) of TMS. Coupling constants (*J*) are given in Hz and the following abbreviations were used to indicate multiplicities: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet.

7.1.3. Chromatographic techniques

7.1.3.1. High-performance liquid chromatography (HPLC)

Reversed-phase HPLC analyses were carried out on a Waters instrument equipped with a diode array detector. The detection was carried out within the following range: 200-800 nm.

A Jupiter 4u Proteo column (Phenomenex, 250 x 4.6 mm, 4 µm, 90 Å) was used at a constant flow (1 mL/min) using the following solvents: A (Formic acid 0.1 % in H_2O), B (Formic acid 0.1 % in ACN).

7.1.3.2. Thin layer chromatography (TLC)

TLC analyses were performed on aluminium plates coated with 0.2 µm thick layer of silica gel (60 F, 245 nm, Merck). TLC was visualized directly under an UV lamp (254 and 365 nm).

7.1.3.3. Column chromatography

Flash column chromatography was carried out with silica gel Chromatogel 60 Å (35-70 µm) from SDS.

7.1.4. Mass spectrometry

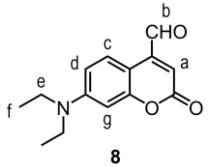
7.1.4.1. Electrospray ionization mass spectrometry (ESI-MS)

Electrospray ionization mass spectrometry analyses were carried out on a HPLC Waters 2695 equipped with a Micromass ZQ quadrupole analyzer and UV-Vis detector.

7.2. SYNTHESIS OF 4-(BENZOYLOXYMETHYL)-7-(*N,N*-DIETHYLAMINO)-4-(1-HYDROXYETH-1-YL)-2-THIOCOUMARIN (5)

7.2.1. Synthesis of 4-carbaldehyde-7-(*N,N*-diethylamino)coumarin (8)

7-(*N,N*-diethylamino)-4-methylcoumarin (**9**) (15 g, 64.9 mmol) was added to a solution of selenium dioxide (21.5 g, 194.5 mmol) in 1,4-dioxane (480 mL) and H_2O (16 mL). The reaction mixture was stirred for 27 days at 110ºC. After removal of the solvent under vacuum, the crude residue was dissolved in acetone, filtered through Celite and washed with acetone and methanol. Then, the solvent was removed under reduced pressure and the product was purified by column chromatography (silica gel, 50-100 % CH_2Cl_2 in hexane) to give 5.2 g (33 % yield) of a reddish-brown solid.

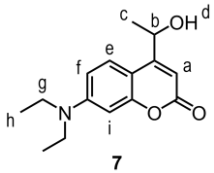


8

Reddish-brown solid.
^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.03 (s, 1H, H_b), 8.30 (d, J_{cd} = 9.2 Hz, 1H, H_c), 6.63 (dd, J_{dc} = 9.2 Hz, 1H, H_d), 6.52 (d, 1H, H_g), 6.45 (s, 1H, H_a), 3.43 (q, J_{ef} = 7.2 Hz, 4H, H_e), 1.22 (t, J_{fe} = 7.2 Hz, 6H, H_f).
R_f (5 % MeOH in CH_2Cl_2) = 0.56

7.2.2. Synthesis of 7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (7)

A solution of 4-carbaldehyde-7-(*N,N*-diethylamino)coumarin (**8**) (5.21 g, 21.2 mmol) in anhydrous THF (120 mL) was cooled at -78ºC under argon atmosphere. Then, a solution of CH_3MgCl in dry THF (15 mL, 45 mmol) was added dropwise in the dark and the reaction mixture was stirred for 90 minutes. After that, an additional amount of CH_3MgCl (4 mL, 12 mmol) was added to the mixture and it was stirred for 2 h. After addition of a saturated solution of NH_4Cl (100 mL), the mixture was brought to room temperature and the solvent was partially removed under reduced pressure. The solution was extracted five times with ethyl acetate (100 mL first and then 25 mL). The combined organic layers were dried over Na_2SO_4 and filtered. After removal of the solvent under vacuum, the product was purified by column chromatography (silica gel, 50-100 % CH_2Cl_2 in hexane and 0-2 % MeOH in CH_2Cl_2) to give 2.52 g (46 % yield) of a yellow solid.

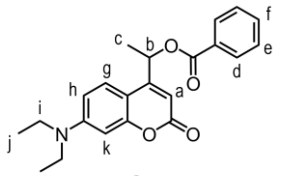


7

Yellow solid.
^1H NMR ($CDCl_3$, 400 MHz): δ 7.43 (d, J_{ef} = 9.0 Hz, 1H, H_e), 6.58 (dd, J_{fe} = 9.0 Hz, 1H, H_f), 6.51 (d, 1H, H_i), 6.27 (s,1H, H_a), 5.16 (s, 1H, H_d), 3.41 (q, J_{gh} = 6.8 Hz, 4H, H_g), 2.17 (t, 1H, H_b), 1.57 (d, J_{cb} = 6.8 Hz, 3H, H_c), 1.21 (t, 6H, H_h).
R_f (5 % MeOH in CH_2Cl_2) = 0.24
HPLC: R_t = 6.67 min (analytical gradient: 50 to 100 % in 30 min; A: 0.1 % formic acid in H_2O, B: 0.1 % formic acid in ACN).
MS (ESI): *m/z* calcd. for $C_{15}H_{19}NO_3$ $[M+H]^+$ 261.32; found 261.92

7.2.3. Synthesis of 4-(benzoyloxymethyl)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (6)

7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**7**) (2.52 g, 9.6 mmol), benzoic acid (1.4 g, 11.6 mmol), DMAP (1.3 g, 10.6 mmol) and EDC (1.6 g, 10.6 mmol) were cooled at 0ºC under an argon atmosphere and then dissolved in CH_2Cl_2 (40 mL, HPLC quality). The mixture was stirred at 0ºC for 10 min and 17 h at room temperature. After addition of CH_2Cl_2 (60 mL, synthesis quality), the solution was washed with saturated NH_4Cl (25 mL), 5 % aqueous $NaHCO_3$ (2 x 25 mL) and water (25 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under reduced pressure. An orange-brown oil was obtained which was used without further purification in the next step.

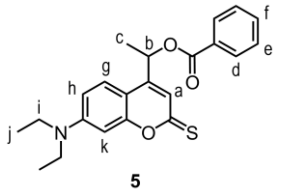


6

Orange-brown oil.
R_f (5 % MeOH in CH_2Cl_2) = 0.35

7.2.4. Synthesis of 4-(benzoyloxymethyl)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)-2-thiocoumarin (5)

Lawesson's reagent (2.5 g, 6.8 mmol) was added to a solution of 4-(benzoyloxymethyl)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**6**) crude in toluene (50 mL) under an argon atmosphere, and the mixture was stirred in the dark at 105ºC overnight. After removal of the solvent under vacuum the product was purified by column chromatography (silica gel, 50-65 % CH_2Cl_2 in hexane) to give 2.53 g (69 % yield for the two steps) of an orange solid.



5

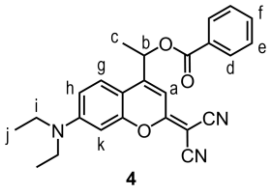
Orange solid.
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 (m, 2H, H_d), 7.60 (m, 1H, H_f), 7.55 (d, J_{gh} = 10.0 Hz, 1H, H_g), 7.48 (t, J = 7.6 Hz, 2H, H_e), 7.16 (s, 1H, H_a), 6.70-6.68 (m, 2H, H_h, H_k), 6.33 (q, J_{bc} = 6.8 Hz, 1H, H_b), 3.44 (q, J_{ji} = 7.2 Hz, 4H, H_i), 1.71 (d, J_{cb} = 6.8 Hz, 3H, H_c), 1.23 (t, J_{ji} = 7.2 Hz, 6H, H_j).
^{13}C NMR ($CDCl_3$, 101 MHz): δ 197.4, 165.5, 159.5, 150.9, 147.9, 133.5, 129.8, 129.5, 128.6, 124.9, 119.2, 110.4, 108.0, 97.6, 67.6, 45.0, 21.0, 12.4
R_f (20 % hexane in CH_2Cl_2) = 0.60

7.3. SYNTHESIS OF 2-(DICYANOMETHYLENE)-7-(*N,N*-DIETHYLAMINO)COUMARIN DERIVATIVES (2-4)

7.3.1. Synthesis of 4-(benzoyloxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (4)

A solution of malononitrile (52 mg, 0.79 mmol) and NEt_3 (0.2 mL, 1.54 mmol) in acetonitrile (2 mL, HPLC quality) was added to a solution of 4-(benzoyloxymethyl)-7-(*N,N*-diethylamino)-4-(hydroxyeth-1-yl)-2-thiocoumarin (**5**) (202 mg, 0.53 mmol) in acetonitrile (8 mL, HPLC quality) under argon. The reaction mixture was stirred in the dark for 2 h. Then, $AgNO_3$ (196 mg, 1.15 mmol) was added and stirring was kept for 2 hours. After filtration and elimination of the

solvent under reduced pressure, the product was purified by column chromatography (silica gel, 0-100 % CH_2Cl_2 in hexane) to give 158 mg (72 % yield) of an orange solid.

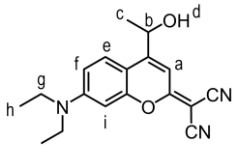


4

Orange solid.
^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09 (m, 2H, H_d), 7.61 (m, 1H, H_f), 7.54 (d, J_{gh} = 9.2 Hz, 1H, H_g), 7.49 (t, J = 7.6 Hz, 2H, H_e), 6.85 (s, 1H, H_a), 6.69 (dd, J_{hg} = 9.2 Hz, J_{hk} = 2.6 Hz, 1H, H_h), 6.61 (d, J_{kh} = 2.6 Hz, 1H, H_k), 6.33 (q, J_{bc} = 6.8 Hz, 1H, H_b), 3.45 (q, J_{ji} = 7.2 Hz, 4H, H_i), 1.73 (d, J_{cb} = 6.8 Hz, 3H, H_c), 1.24 (t, J_{ji} = 7.2 Hz, 6H, H_j).
^{13}C NMR ($CDCl_3$, 101 MHz): δ 172.1, 165.5, 155.4, 152.2, 151.6, 133.7, 129.8, 129.2, 128.7, 125.3, 114.7, 113.9, 110.7, 106.8, 104.6, 97.6, 67.9, 55.3, 45.0, 21.1, 12.5
R_f (CH_2Cl_2) = 0.63
HPLC: R_t = 21.7 min (analytical gradient: 50 to 100 % in 30 min; A: 0.1 % formic acid in H_2O, B: 0.1 % formic acid in ACN).
MS (ESI): *m/z* calcd. for $C_{25}H_{23}N_3O_3$ $[M+H]^+$ 413.47; found 413.92

7.3.2. Synthesis of 2-(dicyanomethylene)-7-(*N,N*-diethylamino)- 4-(1-hydroxyeth-1-yl)coumarin (10)

A solution of HCl in 1,4-dioxane (2.5 mL, 10 mmol) was added to a solution of 4-(benzoyloxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**4**) (272 mg, 0.66 mmol) in absolute EtOH. The reaction mixture was stirred at 90ºC overnight. Reversed-phase HPLC analysis revealed that the reaction was not complete under the hydrolysis conditions employed. After elimination of the solvent under vacuum, a small amount of the product was isolated by column chromatography (silica gel, 50-100 % CH_2Cl_2 in hexane and 0-3 % MeOH in CH_2Cl_2).

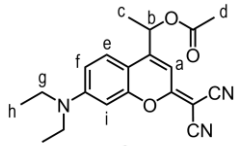


10

Yellow solid.
R_f (2.5 % MeOH in CH_2Cl_2) = 0.29
HPLC: R_t = 11.0 min (analytical gradient: 50 to 100 % in 30 min; A: 0.1 % formic acid in H_2O, B: 0.1 % formic acid in ACN).
MS (ESI): *m/z* calcd. for $C_{18}H_{19}N_3O_2$ $[M+H]^+$ 309.36; found 309.90

7.3.3. Synthesis of 4-(acetoxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (2)

Acetic anhydride (85 µL, 0.77 mmol) and DMAP (94 mg, 0.77 mmol) were added to a solution of 2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)coumarin (**10**) (47 mg, 0.15 mmol) in CH_2Cl_2 (4 mL, HPLC quality) under an argon atmosphere. The reaction mixture was stirred in the dark overnight. After removal of the solvent under reduced pressure, the product was purified by column chromatography (silica gel, 0-0.1 % MeOH in CH_2Cl_2) to give 21 mg (40 % yield) of an orange solid.

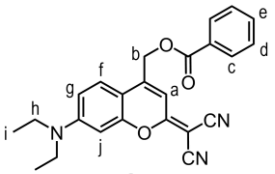


2

Orange solid.
^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.43 (d, J_{ef} = 9.2 Hz, 1H, H_e), 6.72 (s, 1H, H_a), 6.67 (dd, J_{fe} = 9.2 Hz, J_{fi} = 2.4 Hz, 1H, H_f), 6.60 (d, J_{if} = 2.4 Hz, 1H, H_i), 6.08 (q, J_{bc} = 6.8 Hz, 1H, H_b), 3.45 (q, J_{gh} = 7.2 Hz, 4H, H_g), 2.16 (s, 3H, H_d), 1.58 (d, J_{cb} = 6.8 Hz, 3H, H_c), 1.24 (t, J_{hg} = 7.2 Hz, 6H, H_h).
^{13}C NMR ($CDCl_3$, 101 MHz): δ 172.1, 169.9, 155.3, 152.3, 151.6, 125.2, 114.8, 113.9, 110.6, 106.7, 104.3, 97.6, 67.18, 55.2, 45.0, 21.1, 12.5
R_f (1 % MeOH in CH_2Cl_2) = 0.66
HPLC: R_t = 15.4 min (analytical gradient: 50 to 100 % in 30 min; A: 0.1 % formic acid in H_2O, B: 0.1 % formic acid in ACN).
MS (ESI): *m/z* calcd. for $C_{20}H_{21}N_3O_3$ $[M+H]^+$ 351.40; found 351.87

7.3.4. Synthesis of 4-(benzoyloxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)coumarin (3)

2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(hydroxymethyl)coumarin (**11**) (75 mg, 0.25 mmol), benzoic acid (37 mg, 0.31 mmol), DMAP (34 mg, 0.28 mmol) and EDC (43 mg, 0.28 mmol) were cooled at 0ºC under an argon atmosphere and then dissolved in CH_2Cl_2 (2.5 mL, HPLC quality). The mixture was stirred at 0ºC for 10 min and 4 h at room temperature. Extra benzoic acid (8 mg, 0.065 mmol), DMAP (6 mg, 0.05 mmol) and EDC (8 mg, 0.05 mmol) were added to the reaction mixture and it was stirred at room temperature overnight. The solvent was removed under vacuum and the product was purified by column chromatography (100 % CH_2Cl_2). 78 mg (77 % yield) of a yellow solid were obtained.



3

Yellow solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 (m, 2H, H_c), 7.62 (m, 1H, H_e), 7.49 (t, J = 7.6 Hz, 2H, H_d), 7.43 (d, J_{fg} = 9.2 Hz, 1H, H_f), 6.89 (s, 1H, H_a), 6.69 (dd, J_{gf} = 9.2 Hz, J_{gj} = 2.4 Hz, 1H, H_g), 6.62 (d, J_{jg} = 2.4 Hz, 1H, H_j), 5.47 (d, J_{hi} = 0.8 Hz, 2H, H_b), 3.46 (q, J_{hi} = 7.2 Hz, 4H, H_h), 1.24 (t, J_{ji} = 7.2 Hz, 6H, H_i).

^{13}C NMR ($CDCl_3$, 101 MHz): δ 171.8, 165.8, 155.1, 151.7, 146.0, 133.8, 129.8, 128.9, 128.8, 125.1, 114.5, 113.8, 110.7, 107.2, 106.4, 97.4, 61.7, 55.6, 45.0, 12.4

R_f (5 % MeOH in CH_2Cl_2) = 0.86

HPLC: R_t = 20.6 min (analytical gradient: 50 to 100 % in 30 min; A: 0.1 % formic acid in H_2O, B: 0.1 % formic acid in ACN).

MS (ESI): *m/z* calcd. for $C_{24}H_{21}N_3O_3$ $[M+H]^+$ 399.44; found 399.87

8. REFERENCES AND NOTES

1. Schmidt, R.; Geissler, D.; Hagen, V.; Bendig, J. Mechanism of Photocleavage of (Coumarin-4-yl)methyl Esters. *J. Phys. Chem. A.* **2007,** *111,* 5768-5774.
2. Mayer, G.; Heckel, A. Biologically Active Molecules with a "Light Switch". *Angew. Chem. Int. Ed.* **2006,** *45,* 4900-4921.
3. Hansen, M. J.; Velema, W. A.; Lerch, M. M.; Szymanski, W.; Feringa, B. L. Wavelength-selective cleavage of photoprotecting groups: strategies and applications in dynamic systems. *Chem. Soc. Rev.* **2015,** *44,* 3358-3377.
4. Klán, P.; Šolomek, T.; Bochet, C. G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J. Photoremovable Protecting Groups in Chemistry and Biology: Reaction Mechanisms and Efficacy. *Chem. Rev.* **2013,** *113,* 119-191.
5. Fournier, L.; Aujard, I.; Le Saux, T.; Maurin, S.; Beaupierre, S.; Baudin, J. B.; Jullien, L. Coumarinylmethyl Caging Groups with Redshifted Absorption. *Chem. Eur. J.* **2003,** *19,* 17494-17507.
6. Weis, S.; Shafiq, Z.; Gropeanu, R. A.; del Campo, A. Ethyl substituted coumarin-4-yl derivatives as photoremovable protecting groups for amino acids with improved stability for SPPS. *J. Photochem. Photobiol. A.* **2012,** *241,* 52-57.
7. Yamazoe, S.; Liu, Q.; McQuade, L. E.; Deiters, A.; Chen, J. K. Sequential Gene Silencing Using Wavelenght-Selective Caged Morpholino Oligonucleotides. *Angew. Chem. Int. Ed.* **2014,** *53,* 10114-10118.
8. Ito, K.; Maruyama, J. Studies on Stable Diazoalkanes as Potential Fluorogenic Reagents. 1. 7-Substituted 4-Diazomethylcoumarins. *Chem. Pharm. Bull.* **1983,** *31,* 3014-3023.
9. Gandioso, A, Departament de Química Inorgànica i Orgànica, Secció de Química Orgànica, Universitat de Barcelona. Tesi doctoral, en curs.

9. ACRONYMS

ACN: acetonitrile

Calcd.: calculated

DCC: *N,N*-dicyclohexylcarbodiimide

DCM: dichloromethane

DMAP: 4-dimethylaminopyridine

DMF: *N,N*-dimethylformamide

EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

ESI-MS: electrospray ionization mass spectrometry

HPLC: high-performance liquid chromatography

MS: mass spectrometry

NMR: nuclear magnetic resonance

PPG: photolabile protecting group

R_f: retention factor in TLC

R_t: retention time in HPLC

THF: tetrahydrofuran

TLC: thin layer chromatography

TMS: trimethylsilane

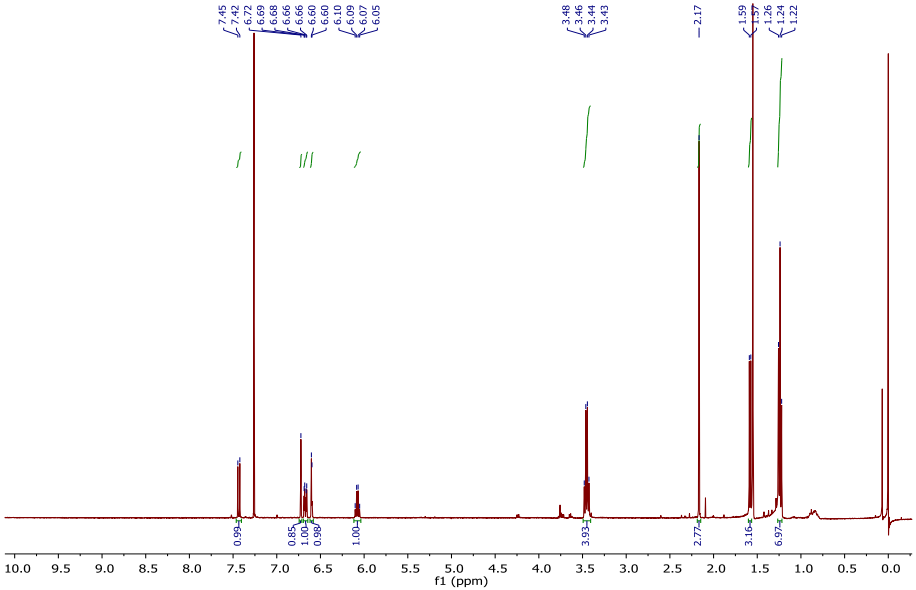
UV: ultraviolet

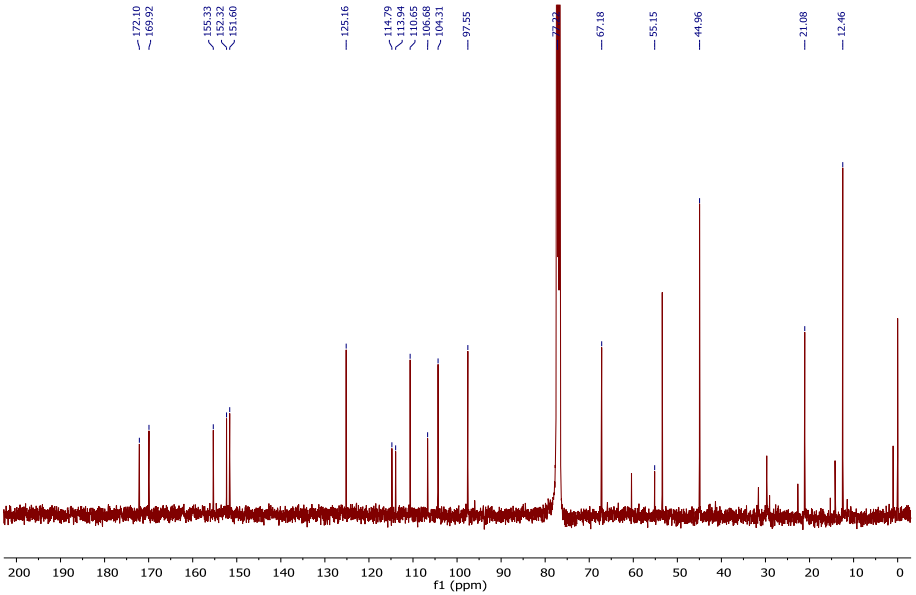
Vis: visible

APPENDICES

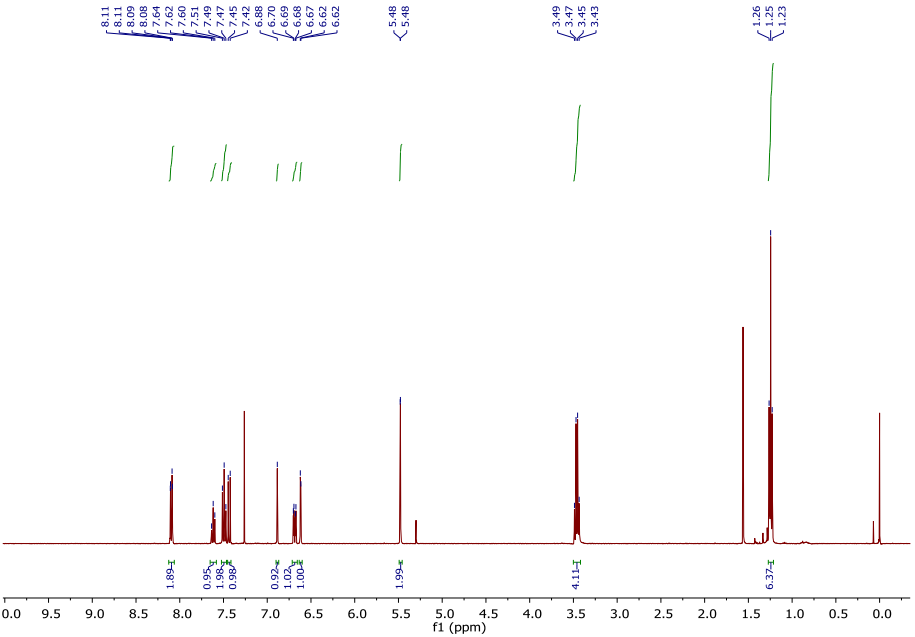
APPENDIX 1: ^{1}H AND ^{13}C NMR SPECTRA OF THE COMPOUNDS

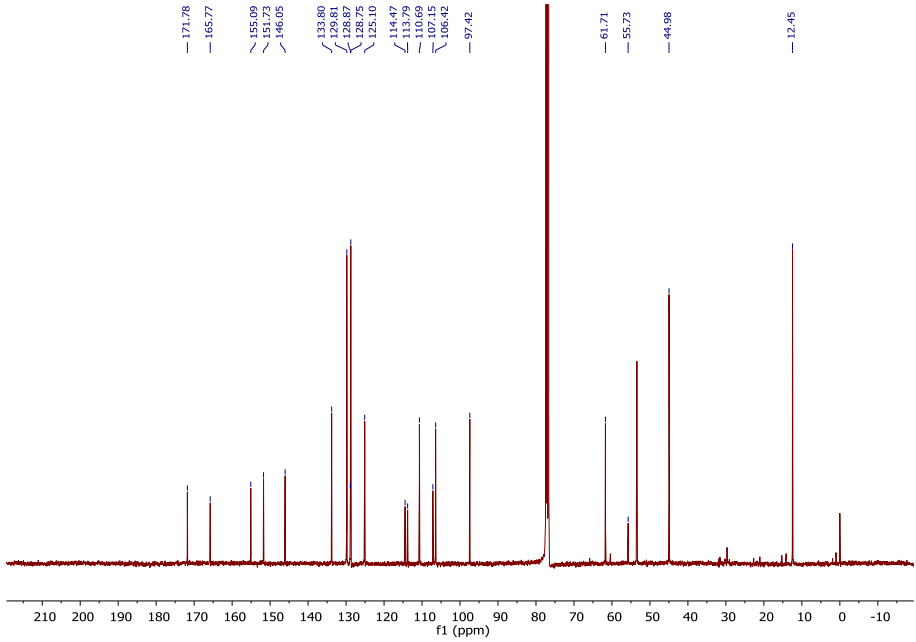
4-(acetoxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)-coumarin (2)



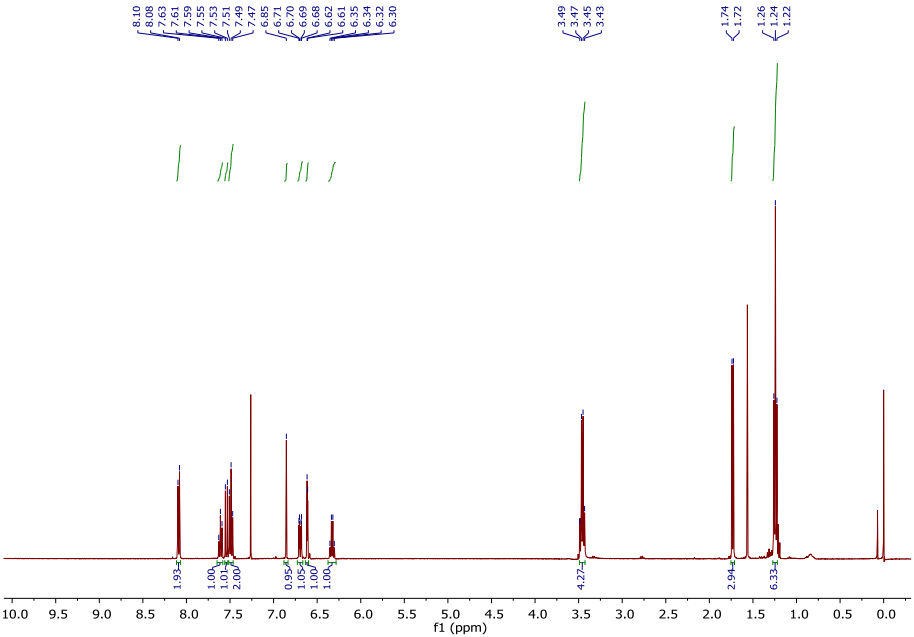


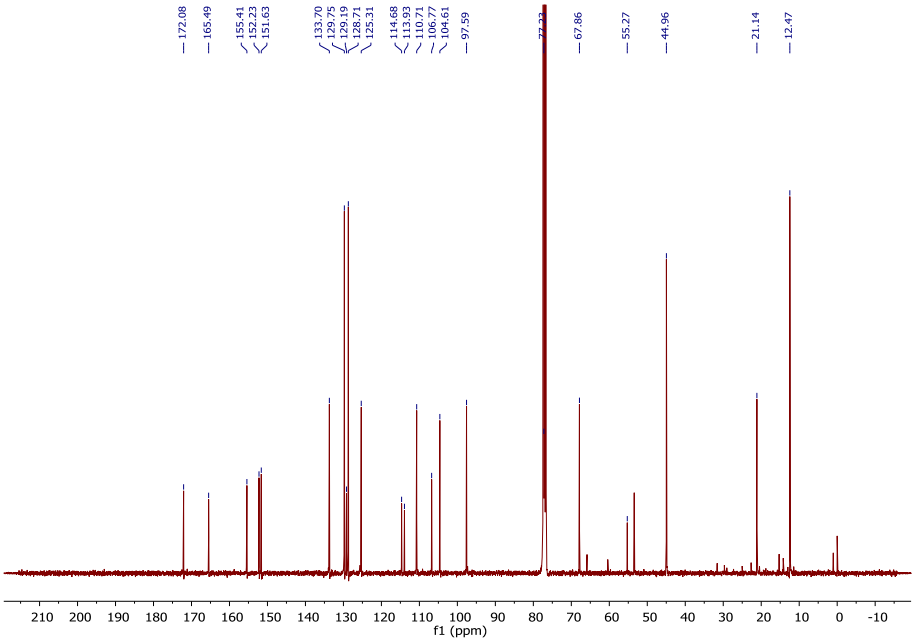
4-(benzoyloxymethyl)- 2-(dicyanomethylene)-7-(*N,N*-diethylamino)-coumarin (3)



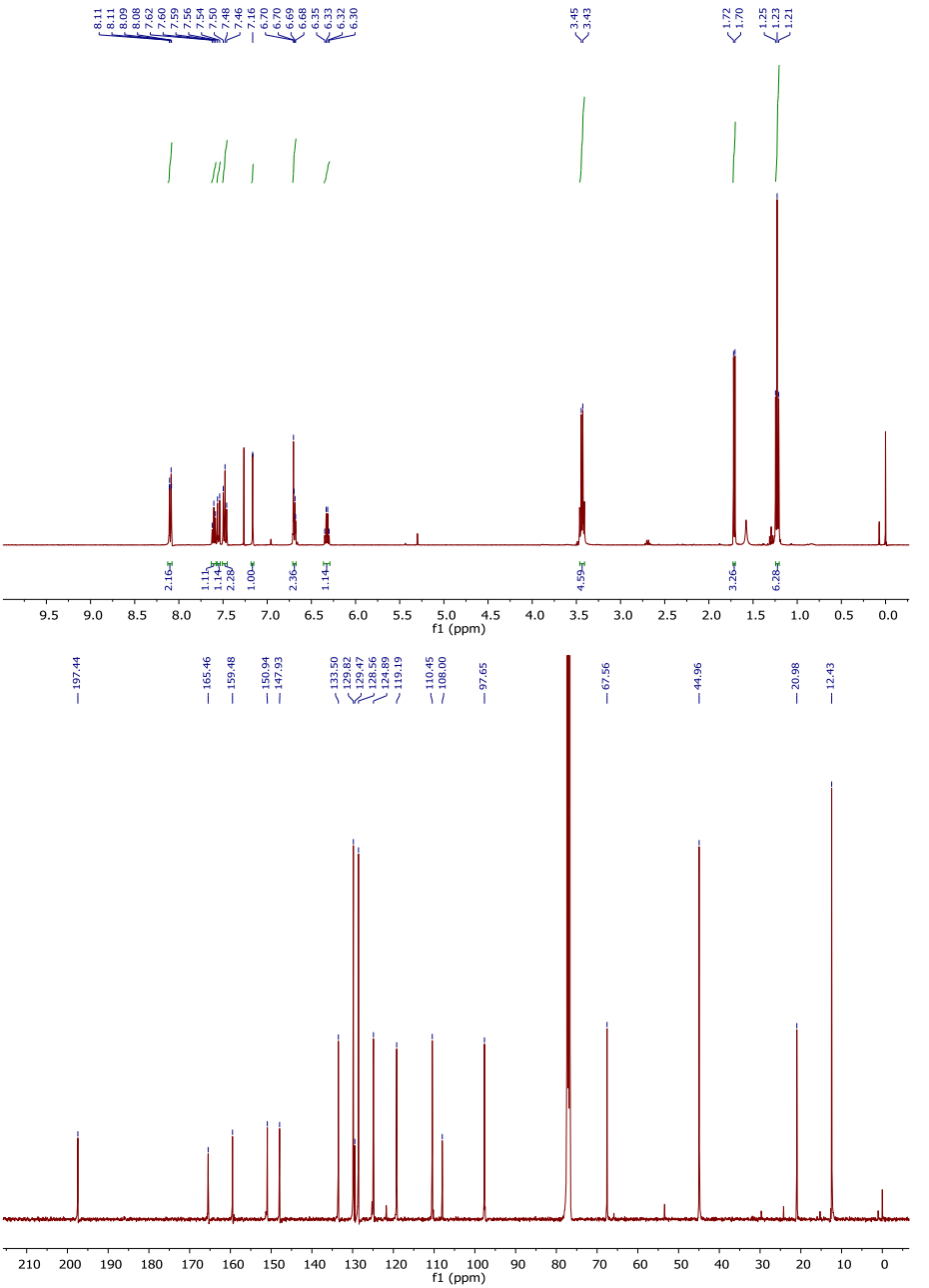


4-(benzoyloxymethyl)-2-(dicyanomethylene)-7-(*N,N*-diethylamino)-4-(1-hydroxyeth-1-yl)-coumarin (4)

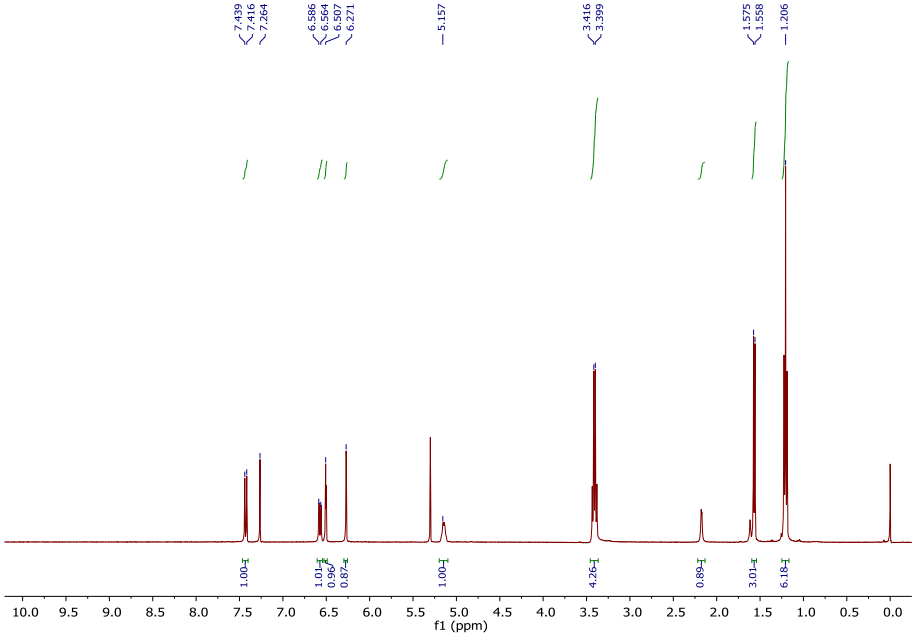




4-(benzoyloxymethyl)-7-(*N*,*N*-diethylamino)-4-(1-hydroxyeth-1-yl)-2-thiocoumarin (5)



7-(*N*,*N*-diethylamino)-4-(1-hydroxyeth-1-yl)-coumarin (7)



4-carbaldehyde-7-(*N*,*N*-diethylamino)coumarin (8)