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Studies on the topography of biomembranes: regioselective photolabelling in vesicles with the tandem use of cholesterol and a half-membrane phospholipidic probe

Wolfgang Hahn, Siw Bodil Fredriksen, Valérie Dollé, Yoichi Nakatani*, Guy Ourisson

Laboratoire de Chimie organique des Substances naturelles, CNRS et Université Louis Pasteur, Centre de Neurochimie, 5 rue Blaise Pascal, F-67084 Strasbourg, France

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Summary — The synthesis of two photosensitive phospholipids built to be incorporated into a monolayer of DMPC vesicles is described. Upon irradiation of bilayer systems containing them, they do label the DMPC chains, with very poor site-selectivity. Addition of cholesterol up to 33% mol improves dramatically the selectivity towards the penultimate methylene groups of the adjacent chain, as well as towards the C-25 methine at the end of the side-chain of cholesterol.

phospholipid / benzophenone / photoactivation / cholesterol / site-selectivity

Résumé — Étude sur la topographie des biomembranes. Deux phospholipides contenant un groupe benzophénone photoactivable et une chaîne myristoyle sont synthétisés. L'irradiation de vésicules de DMPC contenant ces sondes conduit à un marquage peu sélectif le long de la chaîne myristoyle. En présence de cholestérol, la sélectivité d'attaque du méthylène-13 de la chaîne est améliorée, et devient excellente aux fortes concentrations (33 % mol) de cholestérol, qui est alors attaqué en C-25, sur le CH en bout de chaîne latérale.

phospholipide / benzophénone / photoactivation / cholestérol / sélectivité

Introduction

The internal topography of biomembranes is difficult to study. It is an anisotropic medium, highly heterogeneous as its limits, the water/head-group interfaces. The detailed arrangement of the components of these universal cellular systems is in many cases deduced on reasonable grounds rather than defined experimentally. Yet, it would be extremely important to obtain direct evidence, for instance, on the internal topography of membrane-bound proteins. This problem has led to the classical attempts of Khorana [1] to develop photoactivable probes mimicking the phospholipid partners of biomembranes: an identical head-group was linked to one unchanged lipidic chain, whereas the second chain carried a photosensitive group. Similar attempts have been made later by Breslow [2] and by Lala [3]. In all these cases, the results have been very disappointing: the combination of the disorder of the lipidic chains of the probe and of the surrounding lipids led to unspecific labelling all across the membrane.

We have attempted to overcome this problem with a probe **1**, conceived to maintain, in its transmembrane conformation, the photosensitive benzophenone part-structure in the interlaminar space (fig 1). A similar reasoning had led Schreiber and Richards to synthesize a probe having similar structural characteristics [4]. The synthesis of these transmembrane probes was not easy [5], but the use of **1** became very promising once it had been realized that its transmembrane conformation could be made quasi-exclusive when the lipidic chains had been ordered by the addition of cholesterol, a normal constituent of eucaryotic membranes [6].

This finding led us to wonder whether the poor selectivity observed by our predecessors [1–3] with their 'half-probes' could not have been overcome by addition of cholesterol. We now show, with the novel 'half-probes' **2A** and **2B** (fig 1), similar to those of our predecessors, that this is indeed the case (we have already described briefly the results obtained with probe **2B** [7]).

Syntheses

The choice of probes to be synthesized was based on our previous experience with the transmembrane probe **1** (fig 1). We wanted to get phosphocholine derivatives with one myristoyl chain, and with a terminal benzophenone group on the second chain, long enough to place the carbonyl group of the benzophenone at the level of C-14 of the first chain in a fully extended conformation. We realized of course that this would necessarily place the terminal aromatic ring into the second leaflet of the double layer, but hoped this

* Correspondence and reprints

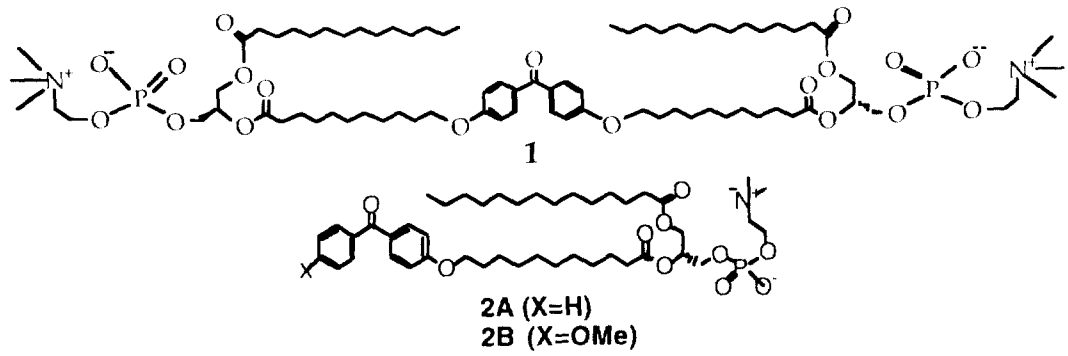


Fig 1. Structure of photosensitive phospholipidic transmembrane probe **1** and half-probes **2A** and **2B**.

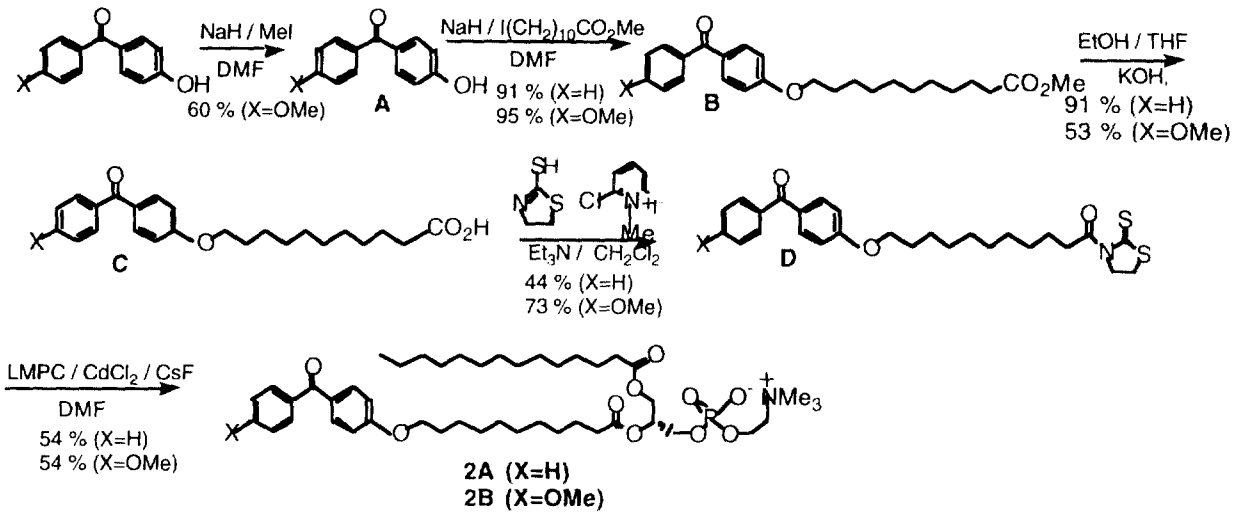


Fig 2. Synthesis of half-probes **2A** and **2B**.

intrusion would not be too detrimental to the selectivity, as the middle of the bilayer is anyway the most disordered and permissive part of the system. To maintain the closest possible analogy, from a photochemical point of view, with the transmembrane probe **1**, we initially selected as a goal the dialkoxy benzophenone **2B**; in this system, the 4′-methoxy group unavoidably introduces some perturbations in the bilayer, as it is somewhat hydrophilic; we therefore synthesized also the simpler probe **2A**, without methoxy substituent, even though this might have altered the photochemical properties of the system.

The syntheses of both probes followed the same procedure, already used in the transmembrane series (fig 2). The phenol (X = OH) was monomethylated with methyl iodide and sodium hydride. The 4-hydroxybenzophenones (4′-methoxy in the case of the **B** series) were then condensed with methyl 11-iodoundecanoate with the help of sodium hydride, and the resulting esters were hydrolyzed. The acids were converted into the corresponding thiazolidine-thiones, and these were treated with the lysomyristoylphosphocholine/cadmium chloride complex and cesium chloride in DMF. The intermediates and final products were all fully characterized.

The final phosphocholines were solids. They were easily dispersed into vesicles by addition to DMPC and water, and sonication. Differential microcalorimetry showed that, at the concentration used for the photochemical experiments of 5 mol%, they had only a small effect on the phase transition temperature T_m of these DMPC vesicles (T_m = 23.6 °C for **2A**, 22.9 °C for **2B**, vs 23.9 °C for DMPC [7]). This was confirmed by sizing of the vesicles and by permeation measurements: these probes are quite inconspicuous ones in DMPC membranes.

Photochemistry

In both series, we used DMPC vesicles containing variable amounts of cholesterol (up to 33% mol), and 5% mol of the corresponding probe. Vesicles were obtained by the freeze–thaw method or, especially when they contained cholesterol, by sonication. Photolysis was carried out with a 125 W medium-pressure mercury lamp in a quartz well above T_m.

Analysis of the products followed closely the degradative and analytical (GC) procedures developed for the transmembrane probe **1** and summarized in figure 3. The labelled cholesterols were characterized by NMR.

Results and discussion

In both **A** and **B** series, photolabelling was extensive: the photoexcited benzophenones are excellent reagents towards the closely packed alkyl chains of membranes. No terminal attack, on the less reactive methyl group,

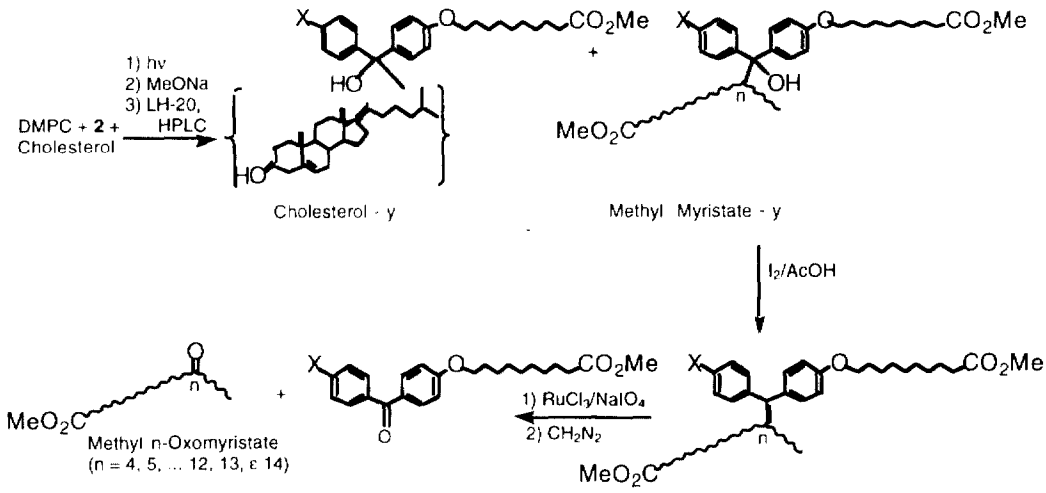


Fig 3. Scheme of chemical degradations for the determination of the functionalized positions in the photolabelled methyl myristates; X = H for probe **2A** and X = OMe for probe **2B**; y = probe fragment linked by photoactivation to cholesterol or methyl myristate.

was observed in the 4′-methoxy series A. In the non-methoxylated series A, with or without cholesterol, one of the minor products has not been fully identified.

In both series, in the absence of cholesterol, labelling was spread all along the myristoyl chain, at least as far from the middle of the double layer as C-4, ie, more than 10 Å away (fig 4). It has been shown that the photoexcited carbonyl group can attack any C–H bond located closer than about 3.1 Å from the oxygen [8]: the unselective labelling observed implies therefore considerable disorder of the probe and of the phospholipidic matrix. Addition of cholesterol, like in the case of the transmembrane probe **1**, induces progressively more selectivity; at 33% mol, the ω-1 position is by far the most labelled (fig 5). There is practically no difference in selectivity between the transmembrane probe and the probes **2A** and **2B**.

Like in the case of the transmembrane probe, cholesterol itself suffers attack, exclusively at the C-25 position, which is again a chemical demonstration of its anisotropic incorporation, perpendicular to the membrane. This was demonstrated as follows.

The functionalized cholesterols were isolated partially pure by HPLC from the fraction of cross-linked products isolated on a LH-20 column after transmethylation of the photolabelled products from the DMPC/**2B**/33% mol cholesterol system. Two fractions (approximately in the same amount) were obtained and their analysis was performed directly using CI-MS (NH_3) and NMR spectroscopies.

In the mass spectra of both fractions, some important peaks (m/z 795.6: $[M + H - H_2O]^+$; m/z 427.1, $[M - Ch(\text{cholesteryl moiety})]^+$ (base peak); m/z 369.5: $[M + H - H_2O - Ch]^+$ were observed. This shows clearly that cholesterol is linked with the probe **2B** (fig 6).

The 500 MHz ^1H NMR spectra showed that both fractions are composed of a diastereomeric mixture (A and B) of C-25 functionalized cholesterols: two peaks corresponding to the protons at C-18, C-19, C-21, C-26 and C-27 are observed (table I). Judging from the peak intensity, the ratio of the diastereomers A and B is 2 to 1 in the first fraction, and 1 to 3 in the second fraction. These ratios are in accordance with those obtained

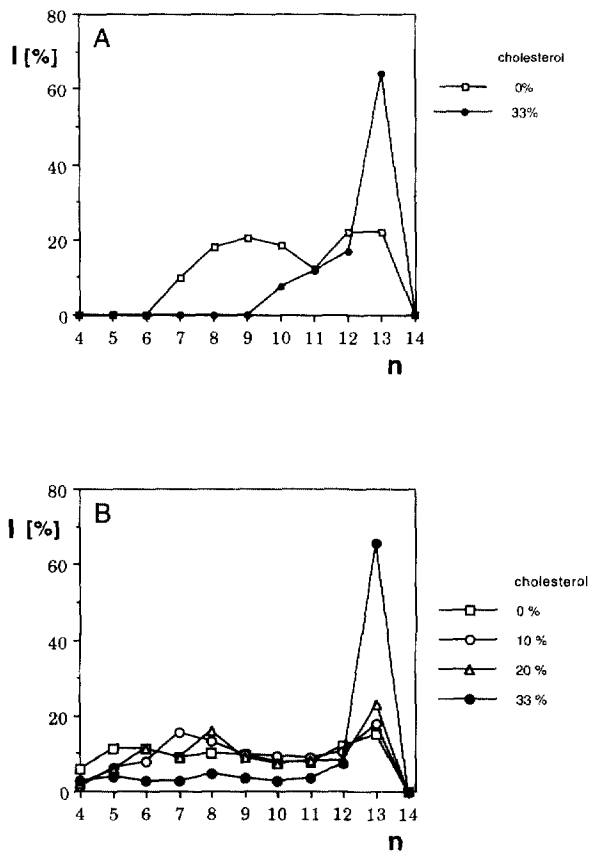


Fig 4. Effect of cholesterol (mol% indicated on the right) on the distribution of DMPC positions functionalized; **A**: in the case of probe **2A**, **B**: in the case of probe **2B**; I: percentage functionalization at carbon atom number n of the myristoyl chain, for example, n = 1 (carboxyl); n = 14 (C-14, position ω).

in the HPLC analysis (see the experimental part). For the determination of the functionalized position(s) on cholesterol, the assignment of the ^1H-NMR spectrum of the photolabelled cholesterol was achieved for the major

Table I. ^{1}H-NMR chemical shifts (ppm) observed in the diastereomeric mixture of C-25 functionalized cholesterols.

Position	*^{1}H NMR (δ ppm)*	*Approximate ratio of peak intensities*		*Assignment to diastereomers*
		First fraction	*Second fraction*	
C-18	0.66 (s)	1	3	B
	0.69 (s)	2	1	A
C-21	0.89 (d)	1	3	B
	0.94 (d)	2	1	A
C-19	1.00 (s)	1	3	B
	1.01 (s)	2	1	A
C-26, 27	1.09 (s), 1.10 (s)	1	3	B
	1.14 (s), 1.15 (s)	2	1	A

Table II. NMR characteristics of the C-25 functionalized cholesterol (C-3, C-6, different methyl groups on cholesterol and on the attached probe for diastereomer B).

Position	*^{13}C NMR (δ in ppm)*	*^{1}H NMR (δ in ppm)*	*Method used*
3 CH	70.9	3.55(m) [3.45(m)][a]	HMQC
6 CH	131	5.34(d) [5.27(d)]	HMQC
18 CH_3	12.2	0.66(s) [0.61(s)]	HMQC
19 CH_3	19.4	1.00(s) [0.94(s)]	HMQC
21 CH_3	18.8	0.89(d) [0.85(d)]	HMQC
26 CH_3	23.8	1.09(s) [0.79(d)]	HMQC, NOESY (with 2′CH_A)
27 CH_3	23.8	1.10(s) [0.80(d)]	HMQC, NOESY(with 2′CH_A)
28 C	83.5		HMBC (with CH_3-26, 27)
1′ C	139		HMBC (with 3′CH_B)
2′ CH_A	130	7.32	HMBC, NOESY, COSY
3′ CH_B	113	6.72	NOESY (with 11″CH_2), COSY
4′ C	157		HMBC (with 3′CH_A)
11″ CH_2	67.8	3.92	HMQC, NOESY
10″ CH_2	29.4	1.74	HMQC, NOESY, COSY
9′ CH_2	26.1	1.42	HMQC, NOESY, COSY
4″ CH_2	29.2	1.25	HMBC, NOESY, COSY
3″ CH_2	24.8	1.60	HMBC, NOESY, COSY
2″ CH_2	34.2	2.30	HMQC, NOESY, COSY
1″ CO	175		HMBC (with 2″CH_2 and CH_3O)
CH_3OCO	51.2	3.65	HMQC
CH_3OPh	52.0	3.78	HMQC

[a] [ ]: ^{1}H-NMR data for cholesterol itself.

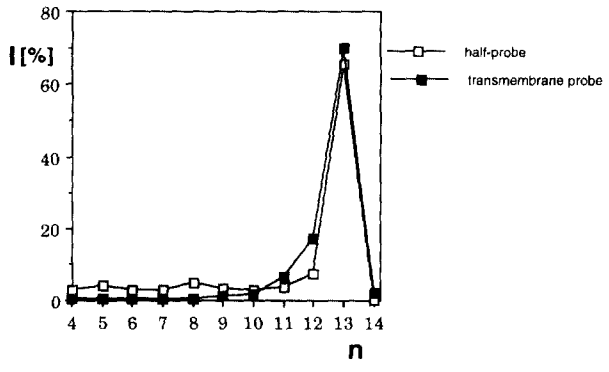


Fig 5. Comparison of the effect of 33 mol% cholesterol on the distribution of DMPC positions functionalized by irradiation of probes **1** and **2B**. *I* and *n* as for fig 4.

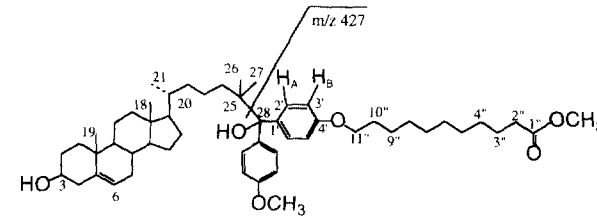


Fig 6. Structure of the functionalized cholesterol and the mass fragmentation for the base peak.

diastereomer *B* in the second fraction, using HMBC, HMQC, NOESY and ^{1}H-^{1}H COSY experiments, by comparison with cholesterol [6]. The methyl proton signals observed at about 0.80 ppm as two doublets for C-26 and C-27 in cholesterol were shifted about 0.29 ppm downfield and were observed as two singlets (table II).

A NOE was observed between the methyl protons (C-26 and C-27) and the H_A (C-2′) protons on the benzene ring. This indicates that the position at C-25 on the side-chain of cholesterol was functionalized and the benzene ring is located in the vicinity of the above two methyl groups. Furthermore, the HMBC spectrum

Table III. NMR chemical shifts (ppm) of methyl, methylene or benzene protons and connective carbons of the C-25 labelled cholesterol (for diastereomer B)[a].

CH_3, CH_2 or CH (benzene)	*Connective carbons*		
^1H	^{13}C	^{13}C-C-H	^{13}C-C-C-H, ^{13}C-C=C-H or ^{13}C-O-C-H
0.66	12.2(C-18)	42.4(C-13)	40.0(C-12), 56.1(C-17), 57.0(C-14)
0.89	19.3(C-21)	36.0(C-20)	56.1(C-17), 37.0(C-22)
1.00	19.5(C-19)	36.4(C-10)	37.4(C-1), 50.2(C-9), 141(C-5)
1.09	24.0(C-26, 27)	42.0(C-25)	24.0(C-26, 27), 38.5(C-24), 83.4(C-28)
2.30	34.2(2″CH_2)	24.8(3″CH_2)	29.1(4″CH_2), 174(1″CO)
3.65	51.2(CH_3OCO)		174(1″CO)
3.78	52.0(CH_3OPh)		158(4′C)
3.92	67.8(11″CH_2)	29.1(10″CH_2)	26.4(9″CH_2)
6.72	113(H_B)		139(1′C)
7.36	130(H_A)		157(4′C)

[a] δ in ppm (position).

indicates clearly cross-peaks between the two methyl proton signals (C-26, C-27) and the carbons C-25, C-26/C-27, C-24, C-28 (table III).

From the view-point of the reactivity or of their localisation, the C-20 or C-24 positions would also have been possible ones for photolabelling. The HPLC analysis showed that the second fraction is almost pure and is principaly composed of two compounds (diastereomers of C-25 functionalized cholesterol), but the first fraction contained, besides these diastereomers, another product (about 30%). In fact, in the ^{1}H-NMR spectrum of the first fraction, we have observed another peak at δ 0.68 ppm for the methyl protons of C-18 (about the same intensity as that of δ 0.66 ppm for the diastereomer **B** mentioned above). However, we could not determine the structure of this compound. From these results, we can conclude that the C-25 position is quite selectively functionalized (80% of the functionalized cholesterols) by irradiation of the DMPC/**2B**/33 mol% cholesterol vesicles. This value is almost as good as that obtained using the transmembrane probe **1** [6].

Conclusion

The half-probes described here are much more easily synthesized than their transmembrane prototype. They present very similar properties, even though the selectivities observed are slightly lower than with the transmembrane probe. For the ultimate goal of this work, which is the study of the topography of membrane-bound proteins, they would present the further advantage of being smaller than the transmembrane one; photolabelling would therefore add only a smaller lipophilic appendage to the already highly lipophilic protein, and therefore would probably ease the determination of the site(s) of attack.

Although the *intramolecular* functionalization of unactivated C–H bonds in sterols has been developed by Breslow [8] since the 1970s, there had been no such success in their *intermolecular* functionalization. The results described here, as well as our earlier ones, show that it is possible to achieve such selective functionalizations provided one works in a close-packed system like a membrane, and provided it is well ordered.

Experimental section

Materials

1-Myristoyl-*sn*-glycero-3-phosphocholine (LMPC) was purchased from Sigma or Sygena and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was purchased from Sygena or Fluka. They were shown to be pure by TLC. Cholesterol was from Aldrich and its purity was also checked by TLC. Other chemicals: 25 wt% NaOMe (sol in MeOH, Aldrich), $RuCl_3 \cdot xH_2O$ (Janssen), $NaIO_4$ (Fluka), methyl myristate (Fluka). Dimethyl tetradecanedioate was prepared from tetradecanedioic acid (Aldrich) with diazomethane. CH_2Cl_2 was distilled from CaH_2, NEt_3 was distilled from KOH, DMF was stored over 4 Å Linde molecular sieves and distilled. 2-Propanol and methanol were distilled from Mg, acetic acid was distilled from P_2O_5 and THF was distilled from Na. CsF and $CdCl_2$ were dried for 10 h at 140 °C in vacuo before use. All solvents were of analytical grade unless otherwise stated.

Analyses

Melting points were measured on a Reichert hot stage microscope and were uncorrected. UV spectra were measured on a Beckman DU 7 spectrophotometer, a Perkin Elmer DU-7 or a Unikon 820 spectrophotometer. NMR spectra were recorded on a Bruker SY (200 MHz) apparatus with $CHCl_3$ (δ = 7.26 ppm) or acetone (δ = 2.15 ppm) or DMSO (δ = 2.55 ppm) as internal standards for ^{1}H NMR, and on a Bruker AM 400 (400 MHz) or ARX (500 MHz) equipment with $CDCl_3$ (δ = 77.0 ppm), acetone-d_6 (δ = 205.7 ppm and 29.80 ppm) or DMSO-d_6 (δ = 39.7 ppm) as internal standard for ^{13}C NMR. The chemical shifts are reported in ppm downfield from TMS (°, *, Ÿ, ¢, # = interchangeable assignment). MS were measured on a VG Analytical ZAB-HF apparatus in the FAB mode, or on a Fisons TRIO 2000 mass spectrometer in the ammonia chemical ionisation (CI)-MS or electron impact (EI)-MS mode, coupled with a Carlo Erba Strumentatione HRGC or a Girdel 3000 gas chromatograph (GC). Microanalyses were performed by the Strasbourg Division of the Service Central de Microanalyse of CNRS. Differential scanning calorimetry (DSC) measurements were performed using the Perkin-Elmer DSC-2B calorimeter by heating at 1.0 K/min. TLC were run on pre-coated plates of silica gel 60F254 (Merck). In order to reveal the compounds, TLC plates were exposed to UV-light, left under an iodine atmosphere, or dipped in an ethanolic vanillin/sulfuric acid or in a Dittmer solution [9]. Purifications were performed on silica gel (40–63 μm, Merck) or Bio-Sil A (200–400 mesh,

Biorad) columns by medium pressure chromatography or on Sephadex LH-20 columns. Microanalyses were consistently very erratic (poorly crystallized products).

Syntheses

• *Non-methoxylated series A*

Mono-ester: 4-Hydroxybenzophenone (Janssen 99%, 3.04 g, 15.34 mol, 1 equiv) was dissolved in dry DMF (100 mL) and sodium hydride (55–60% dispersion in oil, 0.83 g, 17.40 mmol, 1.135 equiv) was added. The mixture was stirred at 50 °C for 30 min and then cooled (yellow solution). 11-Iodoundecanoic acid methyl ester (5.00 g, 15.34 mmol, 1 equiv), dissolved in 20 mL of dry DMF, was added and the reaction mixture was stirred at room temperature during 24 h. Ice-water was added under stirring and the mixture was neutralized with 1 N aqueous HCl. The product was filtered off and washed with water. 5.55 g (91%) of white crystals was obtained after drying under vacuum on P_2O_5 during 12 h. R_f 0.65 (hexane/AcOEt: 70:30); Mp 58–60 °C.

^{1}H NMR ($CDCl_3$) δ: 7.79–7.69 (4H, br, 2 CH-*A* and 2 CH-*C*), 7.52–7.42 (3H, br, 2 CH-*D* and CH-*E*), 6.90 (2H, d, J = 8.5 Hz, 2 CH-*B*), 3.98 (2H, t, J = 6.4 Hz, CH_2-*F*), 3.62 (3H, s, CO_2CH_3), 2.26 (2H, t, J = 7.3 Hz, CH_2-*H*), 1.76 (2H, m, CH_2CH_2O), 1.57 (2H, m, $CH_2CH_2CO_2Me$), 1.26 (12H, m, 6 CH_2-*G*).

^{13}C NMR ($CDCl_3$) δ: 195.7 (C-*a*), 174.5 (C-*m*), 163.1 (C-*e*), 138.6° (C-*b*), 132.8 (CH-*i*); 132.0° (C-*f*), 132.0* (2 CH-*c*), 129.9* (2 CH-*g*), 128.4 (2 CH-*h*), 114.3 (2 CH-*d*), 68.5 (CH_2-*j*), 51.6 (CH_3OOC), 34.3$^{\ddot{Y}}$, 29.7-29.4$^{\ddot{Y}}$, 26.2$^{\ddot{Y}}$ and 25.2$^{\ddot{Y}}$ (8 CH_2-*k* and CH_2-*l*).

Microanalysis: calc for $C_{25}H_{32}O_4$: C: 75.73%; H: 8.13%; found: C: 73.2%; H: 8.6%.

UV (CH_3CN): λ_{max} 195.5 nm (34300); 245 nm (12100); 286 nm (9300); 315 nm (1600); 355.5 nm (1400).

MS FAB: 397.3 (100, $[M + H]^+$); 199.0 (50, $[(CH_2)_{10}CO_2CH_3]^+$).

Mono-acid: A solution of the above ester (2.50 g, 6.30 mmol, 1 equiv) and 0.3 N ethanolic KOH (5% water, 50 mL, 13.56 mmol, 2.15 equiv) in THF (50 mL) was refluxed for 2 h. The solvents were evaporated, water was added and the solution was adjusted to pH 1 with 5% aqueous HCl. The precipitated acid was filtered off and washed with water, dried on P_2O_5 during 12 h. Yield 2.19 g (91%). R_f: 0.07 (hexane/AcOEt: 70:30); Mp 96–97 °C.

^{1}H NMR (DMSO-d_6) δ: 7.75–7.66 (4H, br, 2 CH-*A* and 2 CH-*C*), 7.65–7.50 (3H, br, 2 CH-*D* and CH-*E*), 7.07 (2H, d, J = 8.7 Hz, 2CH-*B*), 4.06 (2H, t, J = 6.4 Hz, CH_2-*F*), 2.16 (2H, t, J = 7.3 Hz, CH_2-*H*), 1.73 (2H, m, CH_2CH_2O), 1.45 (2H, m, $CH_2CH_2CO_2Me$), 1.25 (12H, m, 6 CH_2-*G*).

^{13}C NMR (DMSO-d_6) δ: 190.5 (C-*a*), 170.7 (C-*m*), 158.6 (C-*e*), 134.0° (C-*b*), 128.2 (CH-*i*), 125.2° (C-*f*), 127.9* (2 CH-*c*), 125.3* (2 CH-*g*), 124.4 (2 CH-*h*), 110.3 (2 CH-*d*), 64.0 (CH_2-*j*), 30.0$^{\ddot{Y}}$, 25.0–24.6$^{\ddot{Y}}$, 21.5$^{\ddot{Y}}$ and 20.7$^{\ddot{Y}}$ (8 CH_2-*k* and CH_2-*l*).

Microanalysis: calc for $C_{24}H_{30}O_4$: C: 75.36%; H: 7.91%; found: C: 75.1%; H: 8.2%.

MS FAB: 381.2 (35, $[M + H]^+$), 229.1 (65), 197.0 (35, $[PhCOPhO]^+$), 166.0 (100).

Thiazolidinethione: To a stirred suspension of the above acid (1.00 g, 2.61 mmol, 1 equiv), 2-mercaptothiazolidine (0.63 g, 5.23 mmol, 2 equiv) and 2-chloro-1-methylpyridinium iodide (1.00 g, 3.92 mmol, 1.5 equiv) in dry CH_2Cl_2 (50 mL) was added dropwise dry triethylamine (1.82 mL, 13.08 mmol, 5 equiv) at room temperature. The reaction mixture was refluxed for 5 h and then cooled. The solution was washed with 5% aqueous HCl (8 × 25 mL), with water (6 × 25 mL) and brine (1 × 25 mL). After drying ($MgSO_4$) and evaporation of the solvent, flash chromatography (hexane/AcOEt: 80:20) afforded the pure thiazolidinethione as yellow-light crystals. Yield 559 mg (44%). R_f: 0.42 (hexane/AcOEt: 70:30): Mp 70–72 °C.

^{1}H NMR ($CDCl_3$) δ: 7.93–7.71 (4H, br, 2CH-*A* and 2 CH-*C*), 7.59–7.45 (3H, br, 2 CH-*D* and CH-*E*), 6.93 (2H, d, J = 8.7 Hz, 2 CH-*B*), 4.56 (2H, t, J = 7.5 Hz, SCH_2), 4.02 (2H, t, J = 6.5 Hz, CH_2-*F*), 3.29–3.22 (4H, m, NCH_2 and CH_2-*H*), 1.81 (2H, m, CH_2CH_2O), 1.74 (2H, m, CH_2CH_2CO), 1.30 (12H, br s, 6 CH_2-*G*).

^{13}C NMR ($CDCl_3$) δ: 201.6 (C=S), 195.6 (C-*a*), 174.9 (C-*m*), 162.9 (C-*e*), 138.4° (C-*b*), 132.6 (CH-*i*), 131.9* (2 CH-*c*), 129.9° (C-*f*), 129.7* (2 CH-*g*), 128.2 (2 CH-*h*), 114.1 (2 CH-*d*), 68.3 (CH2-*j*), 56.1 (NCH_2), 38.5 (SCH_2), 29.6–29.1$^{\ddot{Y}}$, 28.3$^{\ddot{Y}}$, 26.0$^{\ddot{Y}}$ and 24.7$^{\ddot{Y}}$ (8 CH_2-*k* and CH_2-*l*).

Microanalysis: calc for $C_{27}H_{33}O_3S_2N$: C: 67.04%; H: 6.88%; found: C: 67.2%; H: 7.1%.

UV (CH_3CN): λ_{max} 197.5 nm (14900); 274 nm (9300); 379 nm (200); 381.5 nm (200); 385.5 nm (200).

MS FAB: 484.2 (20, $[M + H]^+$), 466.3 (10, $[M + H - H_2O]^+$), 365.2 (100, [M – thiazolidine]$^+$), 199.1 (25).

Probe 2A: Method A: All reagents were dried before use. The reaction was run under argon. To a suspension of LMPC.$CdCl_2$ (767 mg, 0.51 mmol, 2.5 equiv) and thiazolidinethione (100 mg, 0.20 mmol, 1 equiv) in dry DMF (30 mL) was added CsF (471.0 mg, 3.10 mmol, 15 equiv). The reaction mixture was stirred at room temperature in the dark for 4 days. DMF was distilled off, the residue was suspended in $CHCl_3$/MeOH/H_2O (4:5:1) and passed slowly through an ion exchange resin (IRC-50/A-21, 1/1, 30 mL bed volume). The fractions containing the product were collected and the solvents were evaporated. The product was isolated by chromatography (Bio-Sil A, $CHCl_3$/MeOH, gradient from 5/1 to 1/1). Yield 92.3 mg (54%), white powder. R_f: 0.51 ($CHCl_3$/CH_3OH/H_2O: 65:25:4).

UV (CH_3OH): λ_{max} = 285.5 nm (ε 6200), UV (CH_3CN): λ_{max} = 285.5 nm (ε 6215).

^{1}H NMR ($CDCl_3$) δ: 7.83–7.72 (4H, br, 2 CH-*A* and 2 CH-*C*), 7.56–7.43 (3H, br, 2 CH-*D* and CH-*E*), 6.94 (2H, d, J = 8.8 Hz, 2 CH-*B*), 5.20 (1H, br s, CH-*I*), 4.34 (4H, m, CH_2-*K* and CH-*N*), 4.12 (2H, m, CH_2-*L*), 4.06–3.99 (4H, m, CH_2-*F* and CH_2-*J*), 3.35 (9H, s, $3CH_3$-*M*), 2.31–2.27 (4H, m, CH_2-*H* and CH_2-*O*), 1.81 (2H, m, OCH_2CH_2), 1.57 (4H, br s, $CH_2CH_2CO_2$), 1.29–1.25 (32H, m, 6 CH_2-*G* and 10 CH_2-*P*), 0.87 (3H, m, CH_3-*Q*).

^{13}C NMR ($CDCl_3$) δ: 195.0 (C-*a*), 173.6$^{\#}$ (C-*m*), 173.3$^{\#}$ (C-*t*), 162.9 (C-*e*), 138.4° (C-*b*), 132.6 (CH-*i*), 131.8* (2 CH-*c*), 129.9° (C-*f*), 129.7* (2 CH-*g*), 128.2 (2 CH-*h*), 114.0 (2 CH-*d*), 70.0 (CH-*n*), 68.3$^{\phi}$, 66.2$^{\phi}$, 63.1$^{\phi}$, 63.0$^{\phi}$ and 59.4$^{\phi}$ (CH_2-*j*, CH_2-*o*, CH_2-*p*, CH_2-*q* and CH_2-*s*), 54.4 (3 CH_3-*r*), 34.2$^{\ddot{Y}}$, 31.9$^{\ddot{Y}}$, 29.7–29.2$^{\ddot{Y}}$, 26.1$^{\ddot{Y}}$, 24.9$^{\ddot{Y}}$ and 22.7$^{\ddot{Y}}$ (8 CH_2-*k*, CH_2-*l*, CH_2-*u* and CH_2-*v*), 14.1 (CH_3-*w*).

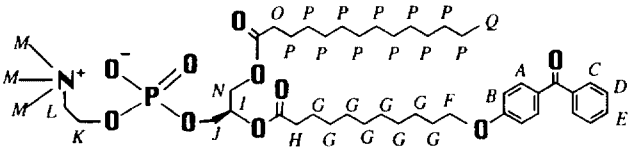
MS FAB: 854.4 (30, $[M + Na + H]^+$), 832.5 (100, $[M + H]^+$), 671.4 (10), 433.3 (30), 419.3 (50).

Microanalysis: calc for $C_{46}H_{74}O_{10}NP.2H_2O$: C: 63.64%; H: 9.05%; found: C: 63.0%; H: 9.2%.

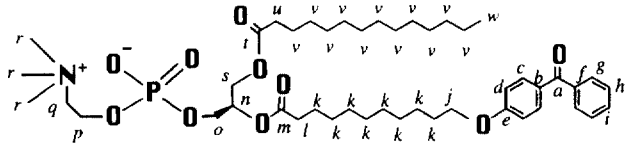
Phase transition temperature (T_m): T_m [DMPC (95% mol) + half-probe (5% mol)] = 22.85 °C; T_m [Half-probe (100% mol)] = 28.75 °C.

Method B: Chloroform (Carlo Erba) was dried as follows. After 1 h stirring over anhydrous $CaCl_2$, the solvent was

filtered off and distilled from fresh anhydrous $CaCl_2$, followed by a second distillation over diphosphorus pentoxide (P_2O_5). Dicyclohexylcarbodiimide (DCC, Aldrich) and 4-(dimethylamino)pyridine (DMAP, Aldrich) were dried in vacuo overnight. In a two-necked 100 mL flask, the core acid **2** (576.1 mg, 1.50 mmol) and LMPC **3** (243 mg, 0.50 mmol) were dried by repeated evaporation with toluene (3 × 10 mL) and then in vacuo at 30 °C overnight. Under argon, $CHCl_3$ (30.0 mL), DCC (309.00 mg, 1.50 mmol) and DMAP (183.00 mg, 1.50 mmol) were added. After 26 h, 30 mL of $CHCl_3$ were added and the crude mixture was applied onto a frit supporting a 2 cm layer of Celite (Fluka, previously washed with MeOH). After washing with 4 × 20 mL of $CHCl_3$, the filtrate was acidified with HCl (10% v/v) to pH 2. After extraction with $CHCl_3$/MeOH = 2:1 v/v (4 × 150 mL) the chloroform phase was washed 3× with a methanol/water solution (MeOH/H_2O = 1:1 v/v, pH 2) and evaporated to dryness. Flash chromatography on silica (40–63 μm, Merck, $CHCl_3$/MeOH = 9:1 v/v to eliminate the core acid, then $CHCl_3$/MeOH/H_2O = 70:26:4) yielded the half-probe **2A** as a transparent off-white solid (263.8 mg = 63%).



Arbitrary numbering of hydrogen atoms of the probe **2A**



Arbitrary numbering of carbon atoms of the probe **2A**

• *Methoxylated series B*

4-Hydroxy-4′-methoxybenzophenone: To a solution of 4,4′-dihydroxybenzophenone (2.50 g, 11.7 mmol) in dry DMF (75 mL) was added sodium hydride (50% dispersion in oil, 0.62 g, 12.9 mmol). The solution was stirred at room temperature for 20 min and then at 50 °C for 50 min. After cooling methyl iodide (1.66 g, 11.7 mmol), dissolved in 5 mL DMF, was slowly added and the reaction mixture was stirred at room temperature overnight. DMF was evaporated and the residue was dissolved in ether/ethyl acetate (ca 1:1). The organic solution was washed with water, dried ($MgSO_4$), and the solvents were evaporated. Flash chromatography (silica, $CHCl_3$/MeOH: 10:0.5) afforded the pure monomethoxy compound as white crystals. Yield 1.59 g (60%). Mp: 139–140 °C.

^{1}H NMR ($(CD_3)_2CO$) δ: 7.75 and 7.05 (4H, AB-syst, J = 8.9 Hz, 2 CH-*A* and 2 CH-*B*); 7.69 and 6.95 (4H, AB-syst, J = 8.8 Hz, 2 CH-*C* and 2 CH-*D*); 3.91 (3H, s, CH_3-E′).

^{13}C NMR ($(CD_3)_2CO$) δ: 191.0 (C-*a*), 163.0° (C-*e*), 162.0° (C-*i*), 132.8* (C-*b*), 132.4* (C-*f*), 130.3 (2 CH-*c* and 2 CH-*g*), 115.7$^{\ddot{Y}}$ (2 CH-*d*), 114.1$^{\ddot{Y}}$ (2 CH-*h*), 55.7 (CH_3-*j*).

Mono-ester: 4-Hydroxy-4′-methoxybenzophenone (1.55 g, 6.79 mmol) was dissolved in dry DMF (50 mL) and sodium hydride (50% dispersion in oil, 0.37 g, 7.71 mmol) was added. The mixture was stirred at 50 °C for 30 min and then cooled. 11-Iodoundecanoic acid methyl ester (2.22 g, 6.79 mmol), dissolved in 10 mL DMF, was added and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated, ice-water was added, and the crude product was filtered off. Recrystallisation from ethyl acetate/hexane (1:1.5) afforded the product as white crystals. Yield 2.75 g (95%). Mp 93–94 °C.

^{1}H NMR ($CDCl_3$) δ: 7.80–7.77 (4H, A_2B_2-syst, J = 4.8 Hz, 2 CH-*A* and 2 CH-*C*), 6.98–6.94 (4H, A_2B_2-syst, J = 6.7 Hz, 2 CH-*B* and 2 CH-*D*), 4.05 (2H, t, J = 6.5 Hz, CH_2-*E*), 3.89 (3H, s, CH_3-*E′*), 3.67 (3H, s, CO_2CH_3), 2.31 (2H, t, J = 7.5 Hz, CH_2-*G*), 1.82 (2H, m, CH_2CH_2O), 1.63 (2H, m, $CH_2CH_2CO_2Me$), 1.46 (2H, m, CH_2-*F*), 1.32 (10H, m, 5 CH_2-*F*).

^{13}C NMR ($CDCl_3$) δ: 194.0 (C-*a*), 174.2 (C-*n*), 162.4 (C-*e* and C-*i*), 132.2° (C-*b*), 132.1° (C-*f*), 130.5 (2 CH-*c* and 2 CH-*g*), 113.9* (2 CH-*d*), 113.4* (2 CH-*h*), 68.2 (CH_2-*k*), 55.4 (CH_3-*j*), 51.4 (CH_3OOC), 34.1$^{\ddot{Y}}$, 29.4$^{\ddot{Y}}$, 29.30$^{\ddot{Y}}$, 29.27$^{\ddot{Y}}$, 29.2$^{\ddot{Y}}$, 26.0$^{\ddot{Y}}$ and 25.0$^{\ddot{Y}}$ (8 CH_2-*l* and CH_2-*m*).

Mono-acid: A solution of the above ester (2.75 g, 6.45 mmol) and 0.3 N ethanolic KOH (5% water, 45 mL) in THF (45 mL) was refluxed for 6 h. The solvents were evaporated, water was added and the solution was adjusted to pH 1 with 1N aqueous HCl. The precipitated acid was filtered off and recrystallised from THF. Yield 1.41 g (53%). Mp 138–139 °C.

^{1}H NMR (DMSO-d_6) δ: 7.70–7.66 (4H, A_2B_2-syst, J = 6.3 Hz, 2 CH-*A* and 2 CH-*C*), 7.07–7.03 (4H, A_2B_2-syst, J = 7.1 Hz, 2 CH-*B* and 2 CH-*D*), 4.04 (2H, t, J = 6.5 Hz, CH_2-*E*), 3.84 (3H, s, CH_3-*E′*), 2.16 (2H, t, J = 7.5 Hz, CH_2-*G*), 1.74 (2H, m, CH_2CH_2O), 1.46 (2H, m, $CH_2CH_2CO_2H$), 1.40 (2H, m, CH_2-*F*), 1.24 (10H, m, 5 CH_2-*F*).

^{13}C NMR (DMSO-d_6) δ: 131.8° (C-*b*), 131.7° (C-*f*), 130.1 (2 CH-*c* and 2 CH-*g*), 127.5, 116.0 (2 CH-*d*), 113.7 (2 CH-*h*), 68.0 (CH_2-*k*), 39.9 (CH_3-*j*), 36.0$^{\ddot{Y}}$, 28.8$^{\ddot{Y}}$, 28.7$^{\ddot{Y}}$, 28.6$^{\ddot{Y}}$, 28.5$^{\ddot{Y}}$, 25.5$^{\ddot{Y}}$ and 24.4$^{\ddot{Y}}$ (8 CH_2-*l* and CH_2-*m*).

Thiazolidinethione: To a stirred suspension of the above acid (100 mg, 0.242 mmol), 2-mercaptothiazolidine (58 mg, 0.484 mmol) and 2-chloro-1-methylpyridinium iodide (93 mg, 0.363 mmol) in dry CH_2Cl_2 (3.5 mL) was slowly added dry triethylamine (0.17 mL, 123 mg, 1.21 mmol) at room temperature. The reaction mixture was refluxed for 5 h, then cooled and diluted with CH_2Cl_2 (10 mL). The solution was washed with 5% aqueous HCl (3 × 10 mL) and subsequently with water (3 × 10 mL). After drying (Na_2SO_4) and evaporation of the solvent, flash chromatography ($CHCl_3$/MeOH: 100:1) afforded the pure thiazolidinethione as yellow crystals. Yield 91 mg (73%). Mp 127–128 °C.

^{1}H NMR ($CDCl_3$) δ: 7.95–7.76 (4H, A_2B_2-syst, J = 5.0 Hz, 2 CH-*A* and 2 CH-*C*), 6.97–6.93 (4H, A_2B_2-syst, J = 6.5 Hz, 2 CH-*B* and 2 CH-*D*), 4.57 (2H, t, J = 7.5 Hz, SCH_2), 4.03 (2H, t, J = 6.6 Hz, CH_2-*E*), 3.29–3.22 (4H, m, NCH_2 and CH_2-*G*), 1.81 (2H, m, CH_2CH_2O), 1.68 (2H, m, CH_2CH_2CO), 1.47 (2H, m, CH_2-*F*), 1.32 (10H, br s, 5 CH_2-*F*).

^{13}C NMR ($CDCl_3$) δ: 202.0 (C=S), 195.0 (C-*a*), 175.0 (C-*n*), 162.8° (C-*e*), 162.7° (C-*i*), 132.22* (C-*b*), 132.19* (C-*f*), 130.1$^{\ddot{Y}}$ (2 CH-*c*), 130.0$^{\ddot{Y}}$ (2 CH-*g*), 114.0 (2 CH-*h*), 113.5 (2 CH-*d*), 68.3 (CH_2-*k*), 56.0 (CH_3-*j*), 55.5 (NCH_2), 38.5 (SCH_2), 29.5$^{\phi}$, 29.4$^{\phi}$, 29.3$^{\phi}$ and 29.1$^{\phi}$ (8 CH_2-*l* and CH_2-*m*).

Probe 2B: All compounds were dried before use. The reaction was run under argon. A suspension of LMPC (200 mg, 0.428 mmol) and $CdCl_2$ (142 mg, 0.772 mmol) in dry DMF (20 mL) was carefully heated until all material had dissolved. The solution was cooled to room temperature and the thiazolidinethione (87.9 mg, 0.171 mmol) and CsF (416 mg,

2.74 mmol) were added. The reaction mixture was stirred at room temperature in the dark for 4 days. DMF was distilled off, and the residue was suspended in $CHCl_3/MeOH/H_2O$ (4:5:0.5, ca 200 mL), and passed slowly through an ion exchange resin (IRC-50/A-21, 1:1, 30 mL bed volume). The fractions containing the product were collected and the solvents were evaporated. The product was isolated by chromatography (Bio-Sil A, $CHCl_3$/MeOH, gradient from 5:1 to 1:1). Yield of **2B** 79 mg (54%), white powder.

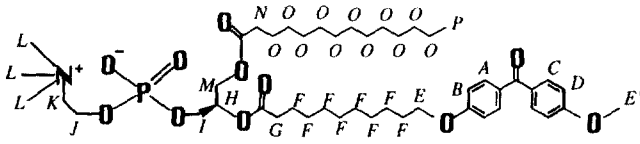
^1H NMR ($CDCl_3$) δ: 7.76–7.73 (4H, m, 2 CH-*A* and 2 CH-*C*), 6.94–6.91 (4H, m, 2 CH-*B* and 2 CH-*D*), 5.18 (1H, br s, CH-*H*), 4.40–4.30 (4H, m, CH_2-*J* and CH_2-*M*), 4.14–3.89 (4H, m, CH_2-*E* and CH_2-*I*), 3.86 (3H, s, CH_3-*E'*), 4.12 (2H, m, CH_2-*K*), 3.37 (9H, s, 3 CH_3-*L*), 2.30–2.24 (4H, m, CH_2-G and CH_2-N), 1.79 (2H, m, OCH_2CH_2), 1.57 (4H, br s, $CH_2CH_2CO_2$), 1.44 (2H, m, CH_2-*F*), 1.28–1.23 (32H, m, 6 CH_2-F and 10 CH_2-O), 0.85 (3H, t, J = 6,7 Hz, CH_3-P).

^{13}C NMR ($CDCl_3$) δ: 173.4* (C-*n*), 173.0* (C-*u*), 162.4 (C-*e* and C-*i*), 132.1 (2 CH-*c* and 2 CH-*g*), 113.9° (2 CH-*d*), 113.4° (2 CH-*h*), 70.6 (CH-*o*), 68.2$^{\phi}$, 66.3$^{\phi}$, 63.3$^{\phi}$, 62.9$^{\phi}$ and 59.3$^{\phi}$ (CH_2-*k*, CH_2-*p* , CH_2-*q*, CH_2-*r* and CH_2-*t*), 55.4 (CH_3-*j*), 54.5 (3 CH_3-*s*), 34.3$^{\Psi}$, 34.1$^{\Psi}$, 32.5$^{\Psi}$, 31.8$^{\Psi}$, 29.6$^{\Psi}$, 29.5$^{\Psi}$, 29.4$^{\Psi}$, 29.3$^{\Psi}$, 29.2$^{\Psi}$, 29.1$^{\Psi}$, 26.0$^{\Psi}$, 24.9$^{\Psi}$, 24.8$^{\Psi}$ and 22.6$^{\Psi}$ (8 CH_2-*l*, CH_2-*m*, CH_2-*v* and 11 CH_2-*w*), 14.0 (CH_3-*x*).

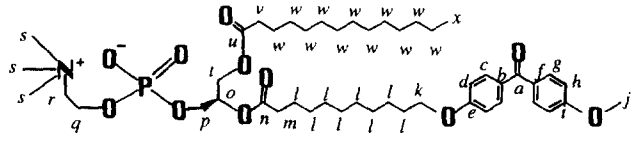
MS FAB: 862.4 (35, $[M + H]^+$), 678.4 (15, [M – phosphocholine moiety]$^+$).

Microanalysis: calc for $C_{47}H_{76}O_{11}NP$: C: 65.48%; H: 8.88%; Found: C: 61.9%; H: 9.2%.

UV (CH_3OH): λ_{max} 205.5 nm (9700), 223.5 nm (8300), 293.5 nm (10800), 375.5 nm (300), 379.5 nm (300); the phase transition temperatures (T_m): T_m[DMPC (95% mol) + half-probe (5% mol)] = 23.60 °C; T_m[Half-probe (100% mol)] = 46.32 °C.



Arbitrary numbering of hydrogen atoms of the probe **2B**



Arbitrary numbering of carbon atoms of the probe **2B**

• *Synthesis of methyl 13-oxomyristate*

This reference compound for gas chromatography – mass spectrometry analysis (GC/MS) was prepared according to the procedures described in the literature [10] with a slight modification. Methyl 13-oxomyristate:

IR (KBr) cm^{-1}: 2 916 and 2 840 ($\nu_{st,\ CH}$), 1 734 ($\nu_{st,\ C=O,\ ester}$), ($\nu_{st,\ C=O,\ ketone}$), 1 160–1 261 ($\nu_{st,\ C-O,\ ester}$).

^1H NMR ($CDCl_3$, 200 MHz) δ: 3.67 (3H, s, CH_3-*A*), 2.42 (2H, t, J = 7.4 Hz, CH_2-*G*), 2.30 (2H, t, J = 7.5 Hz, CH_2-*C*), 2.14 (3H, s, CH_3-*J*), 1.56 (4H, m, CH_2-*D* and CH_2-*F*), 1.27 (14H, m, 7 CH_2-*E*).

^{13}C NMR ($CDCl_3$, 400 MHz) δ: 207.6 (C-*h*), 173.3 (C-*b*), 52.0 (CH_3-*A*), 38.4 (CH_2-*g*), 34.1 (CH_2-*c*), 28–33 (7 CH_2-*e*), 27.4 (CH_3-*j*), 25.4 (CH_2-*d*), 23.6 (CH_2-*f*).

MS (FAB$^+$): 257.2 (100, $[M + H]^+$), 225.2 (93, $[M - OCH_3]^+$).

GC-MS (EI$^+$): 256.0 (1, $[M]^+$), 241.0 (1, $[M - CH_3]^+$), 225.0 (18, $[M - OCH_3]^+$), 199.0 (23, $[M - H_3CC{=}OCH_2]^+$), 166.9 (35, $[M - H_3CC{=}OCH_2 - OCH_3 - H]^+$), 148.9 (31), 124.9 (17, $[M - H_3CC{=}O - CH_2{-}CH_2 - COOCH_3 - H]^+$), 111.9 (17, $[M - H_3CC{=}O - CH_2{-}CH_2{-}CH_2 - COOCH_3]^+$), 97.9 (58, $[M - H_3CC{=}OCH_2 - CH_2{-}CH_2{-}CH_2 - COOCH_3]^+$), 82.9 (66, $M - H_3CC{=}OCH_2 - 2\ CH_2{-}CH_2 - COOCH_3 - H]^+$).

Vesicle preparation, photolysis, chemical degradations and analysis of the photolabelled compounds

• *Non-methoxylated series A*

Preparation of vesicles: Sample **A** [half-probe **2A** (6.72 mg, 8.08 μmol)/DMPC (104 mg, 153.5 μmol)] or sample **B** [halfprobe **2A** (19.3 mg, 23.2 μmol)/DMPC (194.85 mg, 288.0 μmol)/cholesterol (59.24 mg, 153.0 μmol)] was dissolved in $CHCl_3$ and the solvent was removed under reduced pressure. The homogenous milky film was dried overnight in vacuo and hydrated with 9 mL (sample **A**) or 50 mL (sample **B**) of distilled water (grade Milli-Q). For sample **A**, after gentle rotating the flask for 5 min at room temperature, then for 5 min at 40 °C, the homogenous white suspension was briefly vortexed (20 s) and submitted to ten freeze/thaw cycles. The vesicles obtained were sized by passing them at 40 °C seven times through a polycarbonate filter (pore size: 0.4 μm, Nucleopore) using a thermobarrel extruder (Lipex Biomembranes). The size distribution of vesicles was determined by the light scattering method on a Coulter N4-MD submicron particle analyzer. For sample **B**, the suspension obtained by hydration was sonicated at 40 °C for 45 min under argon using a Branson B-30 sonifier with a titanium tip using the continuous wave mode (power level 3) or the pulsed mode (power level 4, duty cycle = 30%). The suspension was then filtered at 40 °C on a polycarbonate filter (pore size: 0.4 μm, Nucleopore) using the thermobarrel extruder.

Photolysis: The vesicle suspension above obtained is placed in a photolysis vessel and the volume was adjusted to 80 mL (sample **A**) or 50 mL (sample **B**) with "Ultrapure" water. The photolysis was performed in a Pyrex well with a 125 W medium-pressure mercury lamp (Philipps HPK) at 40 °C (above T_m) under argon for about 4 h until the disappearance of the benzophenone absorption (UV, λ_{max} = 285 nm). The reaction mixture was lyophilized.

Isolation of photolabelled products as methyl esters: The white solid above obtained was dissolved in 50 mL (sample **A**) or 130 mL (sample **B**) of a 0.1 M NaOMe methanolic solution and stirred under argon overnight. After acidifying with 20 mL of 0.1 N HCl at 0 °C, the solvent was removed under reduced pressure, the residue was taken up in 20 mL (sample A) or 40 mL (sample B) of CH_2Cl_2, washed with brine (10 mL, sample A or 40 mL, sample B) and the aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL, sample **A** or 3 × 40 mL, sample **B**). The combined organic phases were again washed with brine (2 × 10 mL, sample **A** or 40 mL, sample **B**). After removal of the organic solvent, the residue was further dried by repeated azeotropic evaporation with 2-propanol (3 × 5 mL) to afford about 150 mg (sample **A**) or 152 mg (sample **B**) of an oily yellow-brown mixture of transmethylated compounds. The oily mixture (sample **A**) was diluted in a minimal amount of $CHCl_3$/MeOH (1:1) solution and chromatographed on a Sephadex LH-20 column (2 cm ∅ × 30 cm, fractions of 2 mL). Collection and evaporation of higher molecular weight fractions (eluted first) afforded a mixture (6.0 mg)

of photolabelled methyl myristates (MM*), which was confirmed by FAB^+-mass spectrometry (MS). For sample **B**, the photolabelled products (14 mg) were obtained by gel filtration of the oily transmethylated mixture in the same manner as above. Photolabelled methyl myristates (5.3 mg) and photolabelled cholesterols (3.6 mg) were then separated successively* on a silica-gel column (l = 32 cm, ∅ = 1.5 cm, eluent: CH_2Cl_2/EtOH = 100/2, fractions of 5 mL). Photolabelled methyl myristates: m/z 637.1 (21, $[M_{MM*}\text{-}H]^+$), 621.4 (18, $[M_{MM*} + H - H_2O]^+$), 423.6 (15), 397.2 (48, $[M_{MM*}$ + H-methyl myristate moiety$]^+$), 381.2 (17), 365.2 (24), 345.2 (37), 319.2 (13), 312.0 (50), 305 (36), and in addition small peaks derived from non-transmethylated photolabelled phospholipids (861.5, 806.7, 745.9, 671.4); photolabelled cholesterols (chol*): 779.4 (18), 765.4 (48, $[M_{chol*} - H_2O]^+$), 747.5 (12 $[M_{chol*} - 2H_2O]^+$), 733.5 (6), 705.4 (6), 534.9 (28), 423.3 (100), and in addition, small peaks derived from MM* (637.4, 621.3, 397.2, 381.2, 365.2).

The functionalized positions on the photolabelled methyl myristates were analyzed as described below.

Degradation of photolabelled fatty acid methyl esters: Chemical degradation of the photolabelled products was performed to afford compounds as keto-esters or di-esters for GC-MS analysis.

Dehydration of the tertiary alcohols: To a solution of the photolabelled compounds (6.0 mg, sample **A**) in 4 mL of acetic acid (distilled from P_2O_5), a small amount of iodine (Fluka) was added. After refluxing for 1 h, acetic acid was evaporated under reduced pressure. The oily residue was again taken to dryness with dioxane (3 × 5 mL) to afford the dehydrated compounds (6.0 mg). Dehydration of the photolabelled methyl myristates (5.3 mg, sample **B**) was performed in the same manner to afford the dehydrated derivatives (5.0 mg).

Oxidative cleavage of double bonds: To a solution of the dehydrated compounds (5.3 mg, sample **A** or 5.0 mg, sample **B**) in a mixture of CCl_4/CH_3CN = 1:1 (4 mL), an aqueous solution (4 mL) of $RuCl_3$,x H_2O (0.40 mg, 1.93 μmol) and $NaIO_4$ (77.40 mg, 362 μmol) was added. The two phase system was vigorously stirred for 8 h at room temperature. The aqueous phase was extracted with CH_2Cl_2 (3 × 2 mL, sample **A** or 3 × 4 mL, sample **B**). The combined organic solutions were washed with a sodium bisulfite solution (2 × 2 mL, sample **A** or 2 × 4 mL, sample **B**), then with 0.1 N HCl (3 × 2 mL, sample **A** or 2 × 4 mL, sample **B**) and dried overnight in vacuo to give 2.50 mg (sample **A**) or 3.3 mg (sample **B**).

Esterification of acid groups: The residue was dissolved in dry THF (10 mL) and diazomethane (ether solution, 2 mL) was added at 0 °C and the solution was stirred for 15 min at the same temperature. The excess of diazomethane was destroyed by acetic acid, and the solvents were evaporated under reduced pressure. The residue was again evaporated with dioxane (3 × 1 mL) and then dried in vacuo overnight to give the methyl n-oxo myristates and (possibly) dimethyl tetradecanedioate. They were analyzed by GC-MS.

GC and GC-MS analyses: GC analyses were perfomed by on-column injection (a- SE 30 capillary column, l = 25 m, $∅_{ext}$ = 0.32 mm, $∅_{film}$ = 0.2 μm, Spiral Dijon , Carlo Erba Strumentatione HRGC or b- DB 5 30 W fused silica capillary column, l = 30 m, $∅_{ext}$ = 0.32 mm, $∅_{film}$ = 0.1 μm, Ross type injector, GIRDEL 3000 gas chromatograph). The products were detected by flame ionisation and chromatograms were recorded on a Waters 746 or a Shimadzu C-R3A integration unit. Ethyl acetate and dichloromethane were used as solvents and the carrier gas was helium. Solutions (1 μL, concentration: 1 to 4 mg/mL) were loaded on the column. The column was operated with a thermal gradient (35 - 155 °C, 10 °C/min, then 155 - 280 °C, 2 °C/min). Other standard operating conditions were: heated inlet temperature: 300 °C; ion source temperature: 300 °C. Retention time (rT) of n-oxo-MM (n: position of oxo-group): 15.6 ($\omega - 7$), 15.7 ($\omega - 6$), 15.8 ($\omega - 5$), 15.9 ($\omega - 4$), 16.2 ($\omega - 3$), 16.3 ($\omega - 2$), 16.3 min ($\omega - 1$). Two authentic samples, methyl 13-oxotetradecanoate (rT: 16.3 min) and dimethyl tetradecanedioate (rT: 20.7 min) were used for comparison. Mass spectra were obtained at a constant accerating voltage of 3500 V with an electron energy of 70 eV. Relative intensity of GC peaks corresponding to each n-oxo MM isomer: for sample **A** (without cholesterol): $\omega - 13 \rightarrow w - 6$: 0%; $\omega - 7$, 9.7%; $\omega - 6$, 17.8%; $\omega - 5$, 20.3%; $\omega - 4$, 18.4%; $\omega - 3$, 12.0%; $\omega - 2$, 21.8%; $\omega - 1$, 21.8%; ω, 0%; for sample **B** (with 33 mol% cholesterol): $\omega - 13 \rightarrow \omega - 5$: 0%; $\omega - 4$, 7.4%; $\omega - 3$, 11.9%; $\omega - 2$, 16.8%; $\omega - 1$, 63.9%; ω, 0%. The identification of oxo-fatty acid methyl esters from the mass spectrum was performed as described previously [6]: the position of the carbonyl group of the oxo-MM isomers is determined from the mass spectrum by consideration of the α- and β-cleavages on either side of the carbonyl group as well as by the comparison with published mass spectra [11] or with the spectra of the authentic specimens cited above.

• *Methoxylated series B*

Preparation of small vesicles: The compositions of the lipid mixtures are shown in table IV. The dry lipid mixture was hydrated with 50 mL Ultrapure water (Millipore), and the suspensions were sonicated for 45 min in the dark with a Branson Sonifier B-30 (pulse mode, power level 6-7). The crude vesicle suspension was filtered through polycarbonate filters (Nucleopore, 0.8 and 0.4 μm, twice through each) to eliminate metal particles from the sonicator as well as multilamellar vesicles.

Photolysis: The filtered vesicle suspension was diluted with Ultrapure water to 80 mL. The photolysis was performed at 37 °C for 4 h, when UV showed that no unreacted benzophenone groups were present. The reaction mixture was lyophilized, and subsequently dried by repeated evaporations with 2-propanol and then in vacuo.

Isolation of photolabelled products as methyl esters: The crude reaction product was dissolved in 0.1 N NaOMe (65 mL), and the reaction mixture was stirred at room temperature overnight. After neutralization with 1 N HCl at 0 °C, the solvent was carefully evaporated. The residue was taken up in CH_2Cl_2 (20 mL) and the solution was washed with dilute NaCl solution. The aqueous phase was reextracted with CH_2Cl_2 (3 × 15 mL), the combined organic solutions were washed with NaCl solution (2 × 10 mL) and evaporated. The residue was dried by repeated evaporations with 2-propanol followed by drying in vacuo. The oily residue was dissolved in a small amount of $CHCl_3$/MeOH (1:1) and passed through a Sephadex LH-20 column (1 cm ∅ × 38 cm) using $CHCl_3$/MeOH (1:1) as eluent. The fractions collected are shown in table IV. Higher molecular weight photolabelled products [a mixture of photolabelled methyl myristates and photolabelled cholesterols] were eluted first and analyzed by FAB^+-MS (table V). Non-labelled compounds (methyl myristate, methyl 11-[4-(4-methoxybenzoyl)phenoxy]undecanoate, and cholesterol) were then eluted in the lower molecular weight fractions, and were subsequently quantified by GLC by comparison with standard samples of authentic methyl myristate and cholesterol.

* A mixture of photolabelled methyl myristates and photolabelled cholesterols (1.4 mg) was also obtained in the intermediate fractions.

Table IV. Composition of the vesicles prepared for the photolyses, together with the fractions obtained after photolysis, transmethylation and separation of products on a Sephadex LH-20 column.

Sample	*Composition of vesicles*			*Fractions obtained after separation of products*[b] *(mg)*
	Probe[a]	*DMPC*	*Cholesterol*	
A	4.76 mol%	95.24 mol%		*3.5*[c] MM*
	0.0116 mmol	0.232 mmol		97.4 MM
B	4.76 mol%	85.24 mol%	10 mol%	2.1 chol*
	0.0116 mmol	0.208 mmol	0.0244 mmol	*3.3* chol* + MM* 2.5
				104.1 chol + MM
C	4.76 mol%	75.24 mol%	20 mol%	3.1 chol*
	0.0116 mmol	0.183 mmol	0.0487 mmol	*3.5* chol* + MM* 2.0
				90.3 chol + MM
D	4.76 mol%	62.24 mol%	33 mol%	2.4 chol*
	0.0116 mmol	0.152 mmol	0.0804mmol	*3.3* chol* + MM* 3.0
				94.4 chol + MM

[a] The concentration of the probe in the photolysis reactions was ca $1.5 \cdot 10^{-4}$ M. [b] Fractions obtained by separation of photolabelled and unlabelled products by gel filtration on a Sephadex LH-20 column. The numbers underlined represent the weight of the fractions containing mainly photolabelled methyl myristates and photolabelled cholesterol. chol* = photolabelled cholesterols, MM* = photolabelled methyl myristates, chol = intact cholesterol, MM = intact methyl myristate. [c] Corresponds to 45% yield based on the initial probe concentration.

Table V. FAB-MS of the photolabelled products.

	Sample A (0 mol% cholesterol)[a]	*Sample B (10 mol% cholesterol)*	*Sample C (20 mol% cholesterol)*	*Sample D (33 mol% cholesterol)*
MM*[b]	678.3 (100)	651.2 (30) $[M1+H-H_2O]^+$	678.4 (100)	651.5 (30) $[M1+H-H_2O]^+$
	651.3 (15) $[M1+H-H_2O]^+$	427.3 (100) $[M2+H-chol]^+$	427.3 (10) $[M2+H-chol]^+$	427.3 (100) $[M+H-chol]^+$
	650.3 (15) $[M1-H_2O]^+$	or $[M3+H]^+$	or $[M3+H]^+$	or $[M3+H]^+$
	427.1 (15) $[M3+H]^+$			409.3 (12) $[M1+H-H_2O\ MM]^+$
Chol*				835.5 (30) $[M2+Na-H]^+$
		795.4 (7)$[M2+H-H_2O]^+$		795.6 (12) $[M2+H-H_2O]^+$
			678.5 (100)	777.5 (15) $[M2+H-2H_2O]^+$
		637.4 (65)	637.4 (<5)	637.4 (45)
				559.4 (30)
				515.3 (25)
				493.4 (<10)
				479.7 (15)
		453 (5)	453.1(10)	453.3 (85)
		427.3 (38) $[M2+H-chol]^+$	427.3 (35) $[M2+H-chol]^+$	427.3 (100) $[M2+H\ chol]^+$
		411.3 (20)	411.3 (45)	411.3 (55)
				395.2 (40)
				319.2 (50)
		409.3 (15) $[M2+H-H_2O-chol]^+$	409.3 (15) $[M2+H-H_2O-chol]^+$	409.3 (10) $[M2+H-H_2O-chol]^+$

[a] In this case, the MS results indicate, in accordance with the TLC analysis, that the dehydration of the photolabelled products has already taken place during work-up. [b] FAB-MS on the mixture of chol* and MM* for the samples B and C. *Abbreviation*: MM = myristyl moiety, chol = cholesteryl moiety, chol* = photolabelled cholesterols, MM* = photolabelled methyl myristates, M1 = mass of photolabelled myristates, M2 = mass of photolabelled cholesterols, M3 = mass of methyl 11-[4-(4-methoxybenzoyl)phenoxy]-undecanoate.

Degradation of photolabelled fatty acid methyl esters: Chemical degradation of the photolabelled products were performed to afford keto-esters or diesters for GC-MS analysis.

Dehydration of the tertiary alcohols: The mixture of photolabelled products (chol and MM) (half of the total amount obtained was used) was dehydrated by refluxing in dry acetic acid with a trace of iodine (ca 1 h, followed by TLC). The solvent was evaporated, and the residue was evaporated with dioxane. The elimination of water was confirmed by FAB^+-MS.

Oxidative cleavage of double bonds: The residue was dissolved in CCl_4/CH_3CN (1:1, 4 mL). An aqueous solution (2.5 mL) of $RuCl_3 \cdot xH_2O$ (0.5 mg, 0.003 mmol) and $NaIO_4$ (77.5 mg, 0.36 mmol) was added and the reaction mixture was stirred at room temperature overnight. The two phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 2 mL). The combined organic solutions were washed with dilute sodium bisulfite solution (2 × 2 mL) and then with 0.1 N HCl (2 × 2 mL). The solvents were evaporated and the residue was dried in vacuo.

Esterification of the acid groups: The residue was dissolved in dry THF (10 mL) and diazomethane (ether solu-

Table VI. Relative ratio of methyl *n*-oxo myristates and dimethyl tetradecanedioate determined by GC[a].

Oxo position in oxo-MM isomer	*RT (min)*[b]	*Sample A (0% chol)*	*Sample B (10% chol)*	*Sample C (20% chol)*	*Sample D (33% chol)*
4	27.8	5.8	1.0	2.0	2.9
5	28.2	11.3	6.1	5.9	4.0
6	30.4	11.3	7.8	11.4	2.7
7	30.5	8.8	15.8	8.9	2.9
8	30.9	10.2	13.4	16.1	4.8
9	31.3	9.6	9.7	8.9	3.4
10	31.7	7.9	9.5	7.5	2.9
11	31.9	7.8	9.0	8.3	3.6
12	34.1	12.1	10.7	8.2	7.3
13	35.2	15.2	18.0	23	65.4
14 (Dimethyl tetradecanedioate)					

[a] The identification of the various methyl *n*-oxo myristates (oxo-MM isomers) was performed by procedures described earlier [6]. [b] DB5 30W silica capillary column (J & W Scientific); Injection chamber 300 °C, oven 300 °C, column 150 °C.

tion, 1 mL) was added at 0 °C. The reaction mixture was stirred for 15 min at 0 °C. Excess diazomethane and solvent were evaporated at room temperature and the residue was dried in vacuo. The methyl oxo-myristates and (possibly) dimethyl tetradecanedioate were analyzed by GC-MS, and the results are given in table VI.

Upscaling the photochemical reaction and separating photolabelled products by a preparative silica-gel column chromatography: The concentration of the probe in the photochemical reaction (samples with 33% cholesterol) was increased with up to 50% compared to the reactions described above. A total amount of 56 mg of the probe was used in four different photolysis reactions. The solutions were thereafter combined, and subsequent procedures (work-up, transmethylation and separation of the photolabelled products and the unlabelled compounds) were performed as described above. Altogether, 39.5 mg containing the transmethylated photolabelled products (chol* and MM*) and side-products were obtained. This quantity allowed the use of a preparative silica-gel column for the separation of chol* and MM*. Using CH_2Cl_2/EtOH (100:3) as eluent, a fraction containing MM* (7 mg) and a fraction containing chol* (12.2 mg) were obtained, together with two fractions containing side-products. The pure MM* fraction was subjected to chemical degradation as described above.

Analysis of functionalized cholesterols

In order to purify the chol* fraction obtained, HPLC separation was performed at room temperature on a silica-gel column (CAPCELL PAK C_{18}, 4.6 mm id × 150 mm, Shiseido) using CH_3CN/MeOH as eluent with a gradient (t = 0: CH_3CN 100%; t = 35 min: MeOH: 100%). Other standard operating conditions: peak detection: UV spectrometer at 250 nm; elution rate: 1.0 mL/min. Retention time: chol* 7.0–9.5 min, which was collected in two fractions: the first eluted fraction (7.0–8.5 min, 0.4 mg) and the second one (8.5– 9.5 min, 0.4 mg). For the photolabelled fractions thus obtained, ^{1}H NMR spectra showed that the first fraction is a diasteteomeric mixture of C-25 functionalized cholesterols A and B [the ratio of A/B is about 2:1 from the peak intensities of C-18 at δ 0.69 (diastereomer A) and 0.66 ppm (diastereomer B)] along with an unidentified compound (C-18 at δ 0.68 ppm). The second fraction is a diastereomeric mixture of C-25 functionalized cholesterols A and B and the ratio of A/B is about 1:3. NMR data and CI–MS (NH_3) data: see "Results and discussion".

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