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Novel Glycolipids Based on Cyclodextrins

STÉPHANE MOUTARD[1,2], BRUNO PERLY[1], PAUL GODÉ[2], GILLES DEMAILLY[2] and FLORENCE DJEDAÏNI-PILARD[2,*]

[1]*CEA/Saclay DSM/DRECAM, Service de Chimie Moléculaire, F-91191 Gif sur Yvette, Cedex, France;* [2]*Université de Picardie-Jules Verne, Laboratoire des Glucides, 33 rue St Leu, F-80039 Amiens, France*

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Abstract

Novel amphiphilic cyclodextrins have been prepared by grafting a phospholipid on a modified cyclodextrin through a spacing arm to combine the selectivity in size of cyclodextrins and the transport properties of phospholipids. Synthesis and full characterization by NMR and mass spectrometry have been performed. The aggregation process in water has been characterized by light scattering, DSC and ^{31}P NMR. This compound appears to assemble into large objects and displays a very low CMC. The detergent properties of the phospholipidyl-cyclodextrins have been evaluated.

Introduction

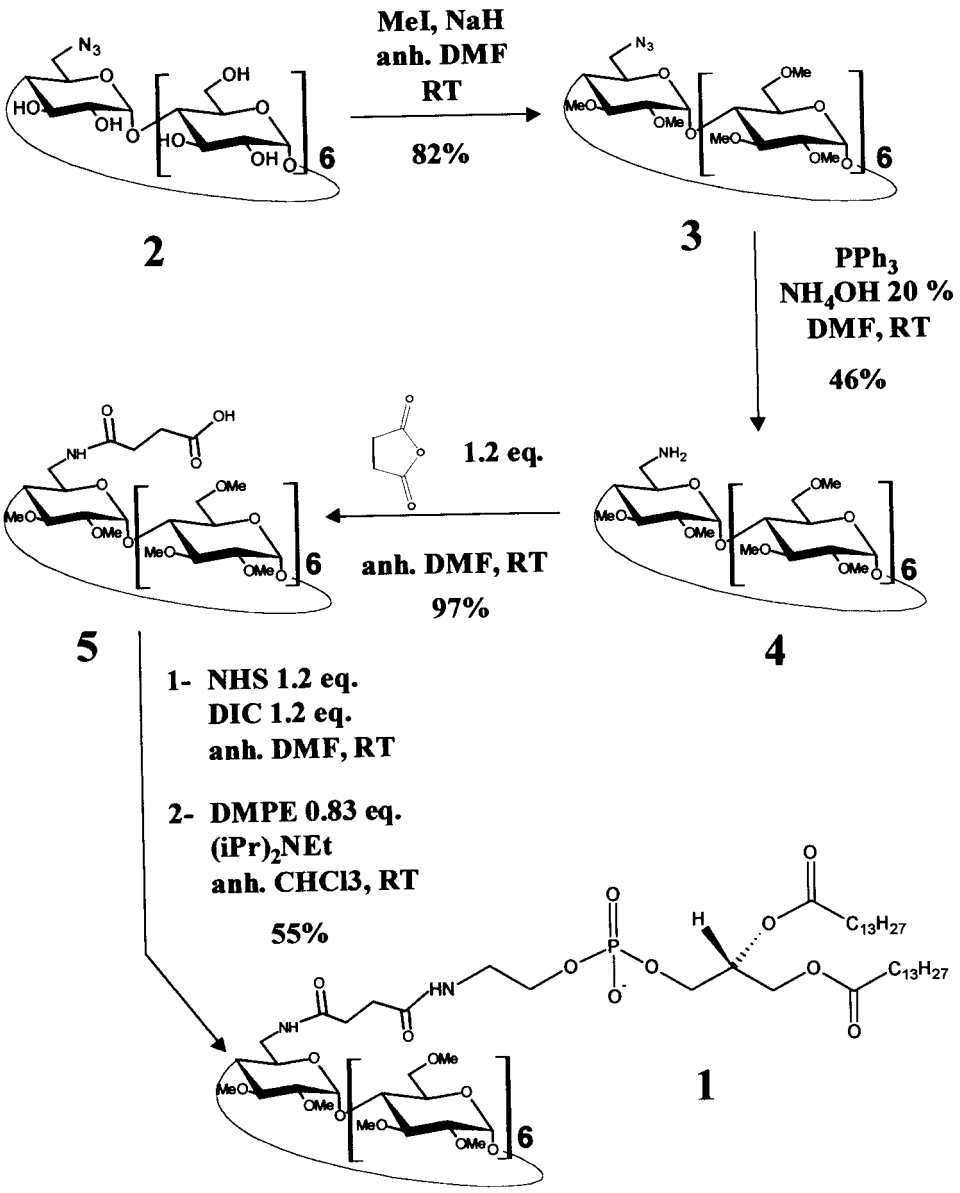
The continuing challenge of using cyclodextrins (CDs) for solubilization and drug targeting has led to the preparation of a wide variety of modified derivatives in order to improve the properties of these host molecules [1]. A possible approach would be to combine the size specificity of cyclodextrins with the transport properties of organized structures such as vesicles and micelles or to insert them in preformed lipidic matrix such as liposomes or analogs. Literature data dealing with the synthesis of amphiphilic derivatives of cyclodextrins points out properties for these compounds. Amphiphilic cyclodextrins bearing multiple hydrophobic chains on the primary face have been extensively investigated. [2, 3]. Another class of amphiphilic cyclodextrins has been obtained by grafting one single hydrophobic (aliphatic chains or steroids) moiety on the primary face of cyclodextrins [2, 4–6]. The properties of these new derivatives strongly depend on the structure of the adduct with a very specific effect of the methylation of the cyclodextrin core. Their properties have been investigated in details in absence and in presence of a synthetic phospholipidic matrix by Nuclear Magnetic Resonance (NMR), and very efficiently using deuterium NMR [7] and scattering techniques (light, X-rays, neutrons). It has been shown that a high diversity of structures such as liposomes, vesicles, micelles or even mixed phospholipid/cyclodextrin derivatives films [8] can be obtained depending on the structure of the molecule. It was also shown that the cavity of the CD moiety retains a full capacity to include external guests which is of considerable importance for potential applications [9]. The key-point in this study is that going from one type of structure to another is achieved by very small modifications of the cyclodextrin moiety (presence or absence of a spacing arm, methylation or not of the hydroxyl groups).

In the present approach, the steroid was replaced by a phospholipid (1,2-di-myristoyl-*sn*-glycero-3-phosphoethanolamine, DMPE) to mimic as closely as possible natural membranes. The objective is therefore to graft a phospholipid on a cyclodextrin to investigate the properties of these novel glyco-conjugates. We report here on the synthesis of 6^{I}-(1,2-ditetradecanoyl-*sn*-glycero-3-phosphoethanolamido-succinylamido)-6^{I}-deoxy-heptakis-2,3-*O*-methyl-hexakis-6-*O*-methyl-cyclomaltoheptaose, further named TRIMEB-Succ-DMPE **1** (Scheme 1), the structural characterization and aqueous solution behaviors of this compound. Since structurally it is a conjunction of an extremely hydrophobic part and of a large hydrophilic head-group through a spacer, strong cooperation in self-aggregation in water is expected. Surface tension measurements are used to evidence the self-assembly property of **1** in water. The structure of **1** self-aggregates is currently under investigation using scattering techniques. The detergent properties of **1** have been evaluated by ^{31}P NMR and light scattering.

Materials and methods

The starting β-cyclodextrin was a kind gift from Roquette Frères SA (Lestrem, France). DPME and DMPC were purchased from Sigma and Aventi Polar lipids and used without further purification. Deuterated solvents were from Eurisо-Top (France). Purification was achieved by semi-preparative High Performance Liquid Chromatography (HPLC). Characterization and structure determinations were achieved by NMR on Bruker instruments (DRX 500 for ^{1}H and ^{13}C operating at 500 MHz for proton and AC200 operating at 81

* Author for correspondence. E-mail: florence.pilard@sc.u-picardie.fr



Scheme 1.

MHz for ^{31}P). In all cases, the temperature was regulated to 25.0 ± 0.1 °C unless indicated otherwise. The molecular structures were further confirmed by High Resolution Mass Spectrometry (HR-MS) using electrospray infusion mode performed in positive and negative modes on a QTOF (Micromass UK) mass spectrometer. Dynamic light scattering experiments were achieved on an AMTEC 2000 goniometer fitted with an ionized argon laser source (Spectra Physics 2016) at a scattering angle of 90.4° and a wavelength of 514.5 nm.

Phases transition temperatures were determined by differential scanning calorimetry (DSC) using a Mettler FP85 microfurnace connected to a FP90 Central Processor with 40 μL aluminium crucibles. The heating rate is 5 °C/min. The apparatus is calibrated with indium, benzophenone and benzoic acid. All the products were freeze-dried before the study.

Surface tension (γ) was measured at 20 °C for each solution, obtained by dilution of a mother solution S_0 (100 μM) in the range $S_0/2$, $S_0/4$, $S_0/6$, $S_0/16$, $S_0/32$, $S_0/64$ and $S_0/128$, after attaining thermal and area equilibrium (more than 12 hours) using the Wilhelmy method (TD 2000 Prolabo tensiometer). The critical micelle concentration value (CMC) was performed using graph γ = f(log C), in witch C indicates the molar concentration of the solution.

Results and discussion

Synthesis

Phospholipidyl-cyclodextrins were obtained by formation of an amide linkage between a mono-6^I-amino-6^I-deoxy-heptakis-2,3-*O*-methyl-hexakis-6-*O*-methyl-cyclomaltoheptaose and a phospholipid: 1,2-di-myristoyl-

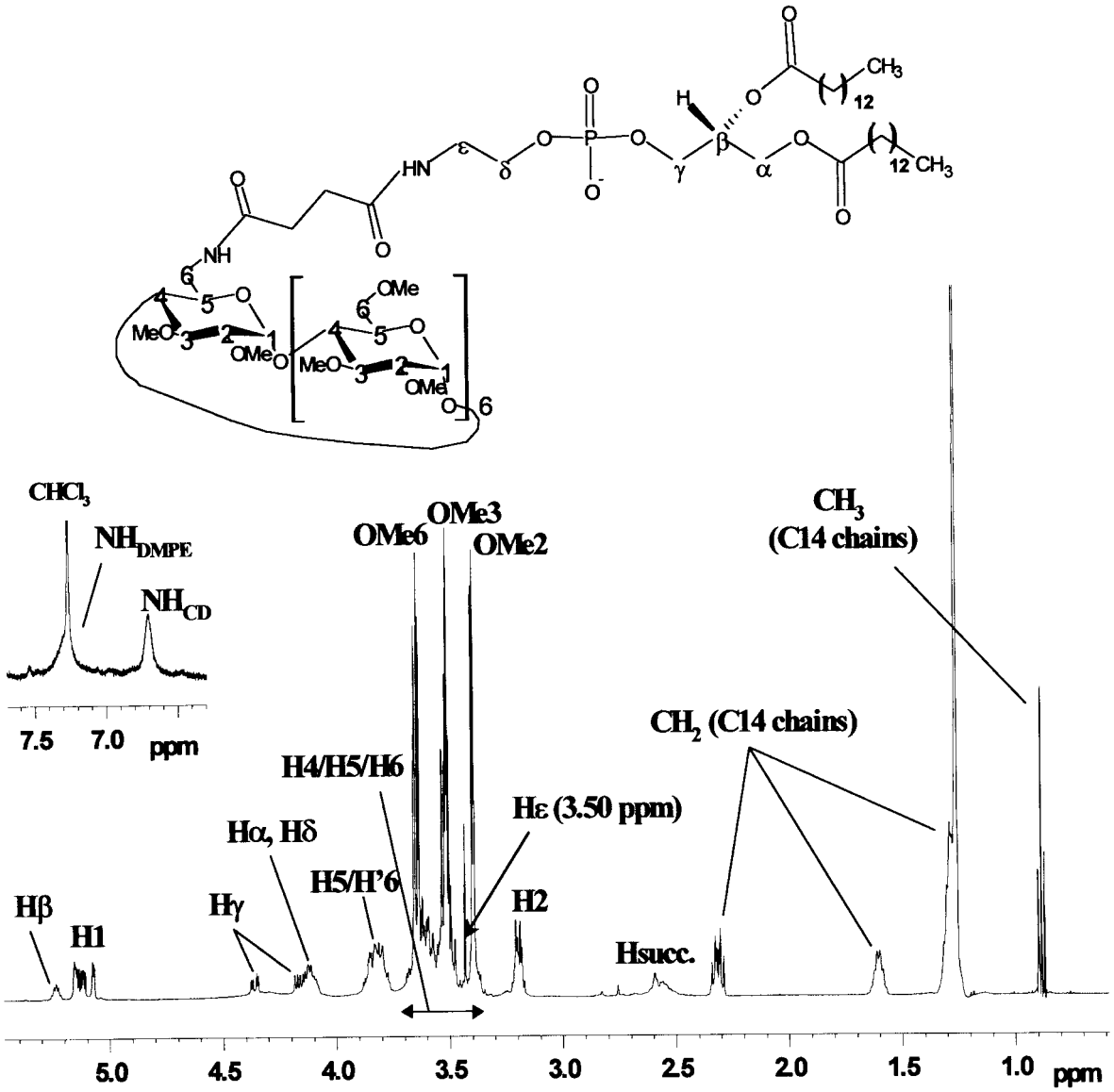


Figure 1. ^{1}H NMR spectrum (500 MHz, 25 °C, 5 × 10^{-3} M in $CDCl_3$) of compound **1**.

sn-glycero-3-phosphoethanolamine (DMPE) through a succinic acid spacer.

The synthetic pathway to TRIMEB-Succ-DPME **1** (Scheme 1) involved the preparation of 6^{I}-azido-6^{I}-deoxycylomaltoheptaose **2** [2]. Permethylation of **2** was achieved using standard conditions and afforded **3** in good yield. **3** was reduced smoothly in **4** as described elsewhere [2] and purified by ion-exchange chromatography. Nucleophilic addition [10] of **4** on succinic anhydride in DMF afforded **5**. Compound **5** was further reacted with DMPE under optimized coupling conditions using ESI-MS control since it was necessary to find the optimal balance between the best yield of coupling and the loss of one or both aliphatic chains in basic conditions. The main results will be described elsewhere. The final materiel was purified by HPLC (A: CH_2Cl_2/MeOH/H_2O/TFA 63/31/5.5/0.5; B: MeOH, A/B 60/40, Rt = 6.8 mn) and led to **1** with 55% yield after purification. The chemical structure of **1** was assessed using high resolution NMR and mass spectrometry [11]. The accurate data obtained by ESI-HRMS confirmed the molecular formula of **1** (found *m/z* = 2130.1682 $[M]^{-}$, calc 2130.1490 for $C_{99}H_{178}N_2O_{44}P$). The NMR analysis was performed in $CDCl_3$ (Figure 1).

The mono-substitution of the cyclodextrin derivative has been shown by digital integration of the NMR signals arising from the DMPE and cyclodextrin moieties. Since the ^{1}H and ^{13}C NMR spectra are relatively complex (Figure 1), a complete analysis was obtained from stepwise identification by COSY, RELAY, DEPT, HMQC and HMBC experiments. The DMPE and cyclodextrin moieties were sequenced using T-ROESY experiment [12] (Figure 2). This experiment confirms the attachment of DMPE to the mono-6-aminocyclodextrin derivative through the succinic acid spacer by evidencing dipolar interactions (indicating spatial proximities). Indeed in the partial T-ROESY contour plot, each amide proton yields dipolar cross peak arising from the neighboring methylene group of the succinic acid part.

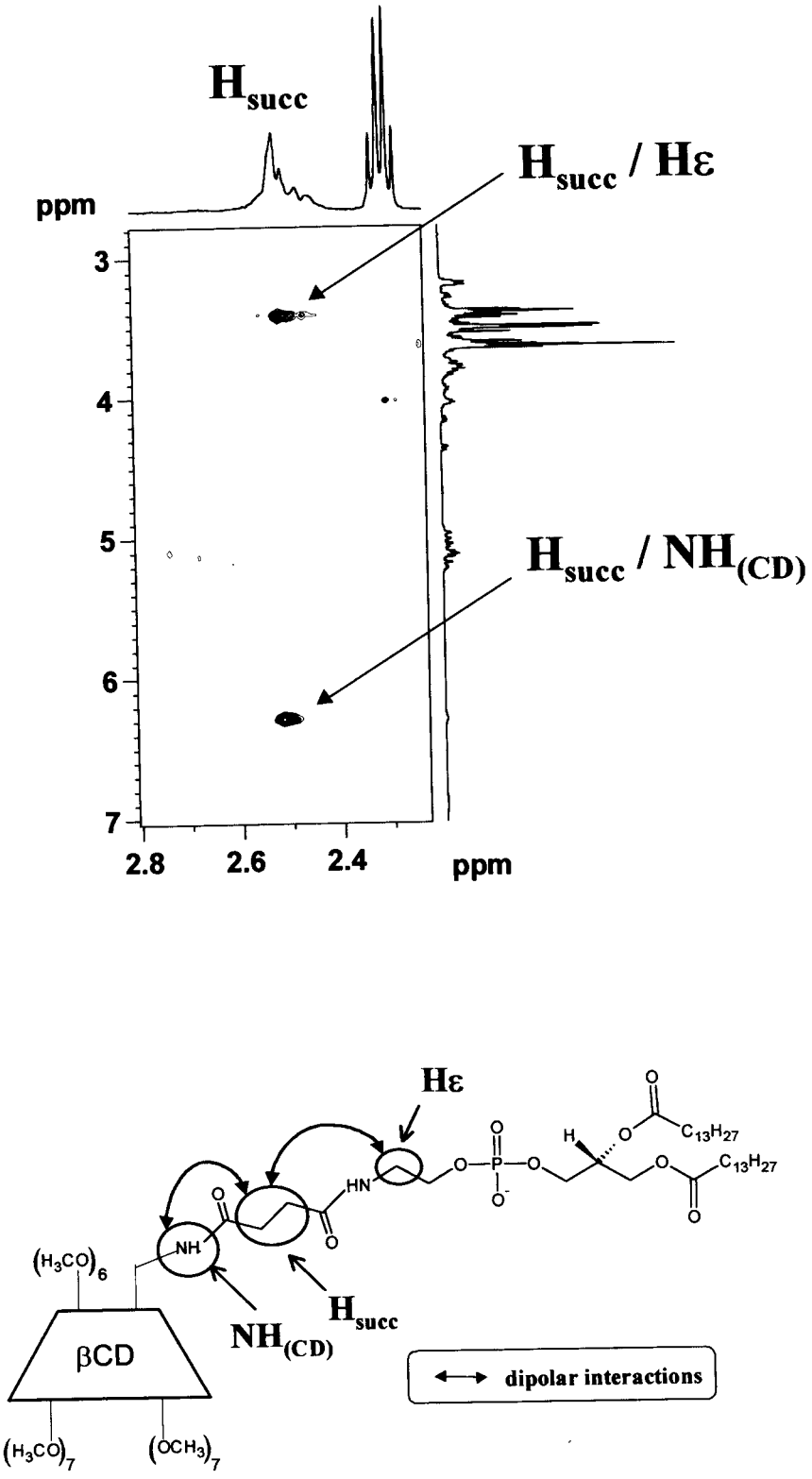


Figure 2. Partial contour plot of a T-ROESY experiment (500 MHz, $CDCl_3$, 25 °C, 300 ms of spin lock) performed on **1** (5×10^{-3} M).

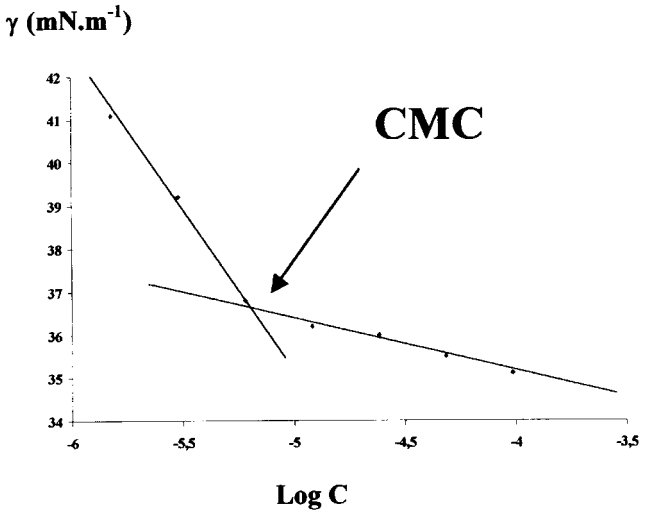


Figure 3. Surface tension of **1** solutions in pure water at 20 °C as a function of concentration.

*Properties of TRIMEB-Succ-DPME **1***

The combination of methylation and monosusbtitution by an hydrophobic unit is expected to afford highly water soluble derivative. TRIMEB-Succ-DPME **1** indeed shows a high solubility in water (up to 5×10^{-2} M) but it seems to be connected to the existence of an aggregation process as indicated by the ^{1}H-NMR spectrum **1** performed in D_2O which displays very broad signals (data not shown).

Surface tension was measured for a series of solutions of increasing concentrations in pure water at 20 °C. As displayed on Figure 3, surface tension levels off at 37 mN/m for an extremely low concentration of solute. The critical micellar concentration (CMC) was indeed found to be 7×10^{-6} M. This indicates a highly associative behavior for **1** as already described for other mono-substituted derivatives [2, 6]. The same measurements performed on the sodium salt in buffered solution (phosphate buffer 0.001 M) showed no significant variation of the CMC value.

A sample of TRIMEB-Succ-DMPE (0.2 mM in pure water and in buffered solution) was analyzed by light scattering and indicates the existence of polydisperse aggregates in the size range of 50 and 300 nm. Investigations by DSC in water show a broad phase transition between 1 and 10 °C. ^{31}P NMR of a concentrated sample of **1** in water (1 : 1, w : w) indeed shows major variation of the spectrum in this temperature range (studies under investigation).

*Inclusion properties of TRIMEB-Succ-DMPE **1***

The inclusion properties of **1** in water has been investigated using 4-*t*-butyl benzoic acid sodium salt (BuPhCOONa). This molecule has been shown to form inclusion complexes with natural β-cyclodextrin, per(2,6-di-*O*-methyl)-cyclomaltoheptaose (DIMEB) and per(2,3,6-tri-*O*-methyl)-cyclomaltoheptaose (TRIMEB) [8, 13]. To better understand the behavior of the model guest toward TRIMEB-Succ-DMPE **1**, we have investigated in a first step the inclusion of the former guest in TRIMEB. Inclusion in TRIMEB is evidenced by modifications of the ^{1}H-NMR spectrum of

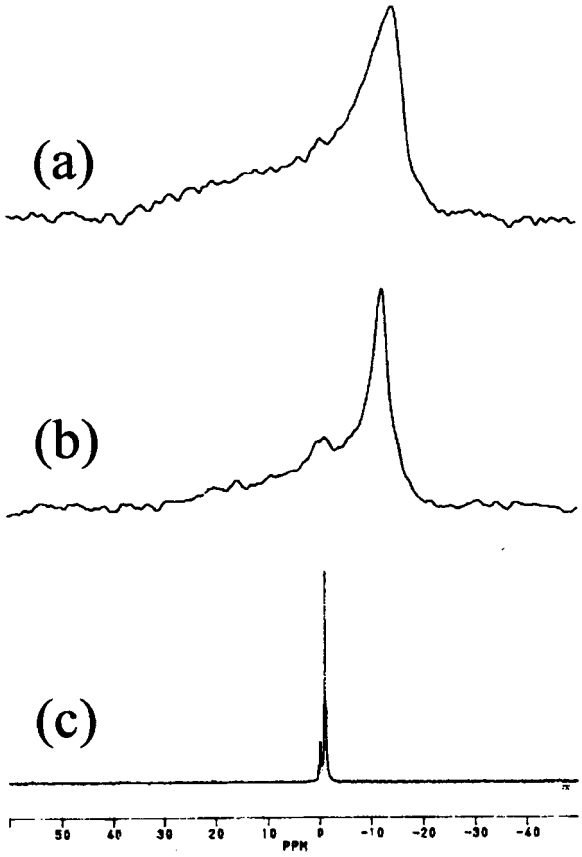


Figure 4. ^{31}P NMR spectra (81 MHz, 25 °C) of the mixed DMPC/TRIMEB-succ-DMPE phases. (a) Pure DMPC liposomes 15 mM in water (Tris 0.1 M, pH = 7); (b) a + TRIMEB-succ-DMPE 1 mM; (c) a + TRIMEB-succ-DMPE 2.5 mM.

the host molecule in the absence or in the presence of BuPhCOONa. Evidences for the inclusion, determination of the stoichiometry (1 : 1) and of the association constant K_c (1600 M^{-1} at 25 °C) were derived from chemical shifts variations in the ^{1}H NMR spectra in aqueous solutions at pH 7. A similar procedure was applied to demonstrate the preservation of the inclusion properties of the cyclodextrin moiety by TRIMEB-Succ-DMPE **1**.

Further NMR experiments were performed to investigate the geometry in solution of the inclusion complexes. The T-ROESY sequence is used to prove interactions between protons of host and guest molecules. Complex formation was demonstrated by the presence of the same strong dipolar cross peaks between protons located in the cavity (H3 and H5) of TRIMEB and **1** and aromatic protons of guest. At this stage, we can postulate that both inclusion complexes (BuPhCOONa/TRIMEB and BuPhCOONa/**1**) exhibit the same geometry in solution.

Detergent properties

TRIMEB-Succ-DMPE **1** exhibits a very high solubilization power for lipids such as DMPC (Figure 4).

This can be followed by visual observation and by ^{31}P NMR. The milky suspension of sample (a) is changed to a translucent solution in (b) an becomes completely clear in (c). By using ^{31}P NMR one can observe a typical aniso-

tropic bilayer spectrum in (a), a partial collapse of the later in (b) and a fully isotropic (sharp lines) in (c). Light scattering experiments performed on (c) indicate the formation of monodisperse mixed micelles (average diameter of 16 nm) between **1** and DMPC.

Conclusion

This new class of amphiphilic cyclodextrins presented here displays surprising self-organization properties close to phospholipids and further retains the ability of the cyclodextrin moiety in terms of inclusion of guest molecules. Further X-Ray diffusion experiments will be performed to characterize the structures observed in concentrated solutions. These molecules further present a considerable solubilization power for model membranes. The influences of both the nature and size of the polar head (cyclodextrin) and of the length of the fatty acid chains on the properties of these glyco-conjugates will be investigated by the synthesis of a rationalized series of phospholipidyl-CD's.

Acknowledgments

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References

1. A.R. Khan, P.F. Keith, J. Stine, and V.T. D'Souza: *Chem. Rev.* **98**, 5 (1998).
2. R. Auzely-Velty, B. Perly, O. Taché, Th. Zemb, Ph. Jehan, P. Guenot, J.P. Dalbiez, and F. Djedaïni-Pilard: *Carbohydr. Res.* **318**, 82–90 (1999), and references therein.
3. B.J. Ravoo, R. Darcy, A. Mazzaglia, D. Nolan, and K. Gafihey: *Chem. Commun.* 827–828 (2001).
4. N. Bellanger and B. Perly: *J. Mol. Struct.* **273**, 215–220 (1992).
5. J. Lin, C. Créminon, B. Perly, and F. Djedaïni-Pilard: *J. Chem. Soc Perkin Trans. 2*, 2639–2646 (1998), and references therein.
6. R. Auzély-Velty, F. Djedaïni-Pilard, S. Desert, B. Perly, and Th. Zemb: *Langmuir* **16**, 3727–3734 (2000), and references therein.
7. M. Roux, R. Auzély-Velty, F. Djedaïni-Pilard, and B. Perly: *Biophysical Journal* **82**, 813 (2002).
8. I. Javierre, M. Nedyalkov, J.J. Benattar, S. Weisse, R. Auzély-Velty, F. Djedaïni-Pilard, and B. Perly: *Journal of Colloid and Interface Science* **254**, 120–128 (2002).
9. R. Auzély-Velty, C. Péan, F. Djedaïni-Pilard, Th. Zemb, and B. Perly: *Langmuir* **17**, 504–510 (2001), and references therein.
10. C. Pean, C. Créminon, A. Wijkhuisen, J. Grassi, P. Guenot, Ph. Jéhan, J.P. Dalbiez, B. Perly, and Djedaïni-Pilard: *J. Chem. Soc Perkin Trans. 2*, 853–863 (2000), and references therein.
11. Characterization of **1**: Mp: 121 °C, $[\alpha]_D = +89.4$ (c = 1, $CHCl_3$), MS: found *m/z* = 2130.1682 $[M]^-$, calc 2130.1490 for $C_{99}H_{178}N_2O_{44}P$, IR: 2930 cm^{-1} ν(C–H) ; 1738 cm^{-1} ν(C=O esters); 1662 cm^{-1} ν(C=O amides); ^{1}H NMR (500.13 MHz, $CDCl_3$): δ(ppm); CD moiety: 6.71 (NH); 5.07–5.16 (7H, H1); 3.75–3.90 (H5, H6); 3.5–3.7 ($H4^{II-VII}$, H6′); 3.63–3.66 (Me6); 3.45–3.57 (H3); 3.50–3.55 (Me3); 3.43–3.39 (Me2); 3.39 ($H4^{I}$); 3.17–3.22 (7H, H2). Coupling constants: J(1–2) = 3.6 Hz; J(2–3) = 9.7 Hz; J(3–4) = 8.7 Hz; J(4–5) = 9.7 Hz; J(5–6) = 4.0 Hz; J(5–6′) = 1.6 Hz; J(6–6′) = −10.4 Hz. DMPE moiety: 7.29 (NH); 5.24 (m, 1H, Hβ); 4.36 (dd, 1H, Hγ); 4.17 (dd, 1H, Hγ'); 4.12 (Hα); 4.10 (Hδ); 3.50 (Hε); 2.32 (dt, 4H, H2α, H2β); 1.61 (ml, 4H, H3α, H3β); 1.26 (ml, 40H, H4α/H4β → H13α/H13β); 0.89 (t, 6H, H14α, H14β). Coupling constants: J(γ-γ') = 12.1 Hz; J(γ-β) = 3.7 Hz ; J(γ'-β) = 6.3 Hz; J(2α-3α) = J(2β-3β) = 7.7 Hz; J(13α-14α) = J(13β-14β) = 6.9 Hz. Succinic spacer: 2.5–2.6 ppm (Hb, Hc). ^{13}C NMR: δ(ppm); CD moiety: 102.0 – 99.5 (7s, Cl); 83.0 – 80.2 (C2, C3, C4); 73.9, 71.7 – 70.6 (C5); 71.4 – 72.3 (C6); 62.2 – 60.4 (Me6); 59.0 – 59.7 (Me3, Me2); 40.2 ($C6^{I}$). DMPE moiety: 173.8 (C1α, C1β); 70.5 (Cβ); 66.2 (Cδ); 64.9 (Cα); 62.9 (Cγ); 40.9 (Hε); 34.7, 34.9 (2s, C2α, C2β); 32.6 (C12α, C12β); 30.4 – 29.8 (C4α/C4β → C11α/C11β); 25.5 (C3α, C3β; 23.4 (C13α, C13β); 14.8 (C13α, C14β). Succinyl spacer: 174.1 (Cd); 173.2 (Ca); 31.4, 31.0 (Cb, Cc).
12. C. Pean, C. Créminon, A. Wijkhuisen, B. Perly, and F. Djedaïni-Pilard: *J. Chim. Phys.* **96**, 148 (1999).
13. G. Wenz: *Angew. Chem. Int. Ed. Engl.* **33**, 803, (1994).