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Research paper

Synthesis of lipophosphoramidyl-cyclodextrins and their supramolecular properties

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ABSTRACT

The synthesis of lipophosphoramidyl-β-CD was obtained by an Atherton–Todd (AT) reaction that involved dioleylphosphite and either functionalized permethylated or native β-cyclodextrin. This AT reaction that produced dioleylphosphoramide by making use of the amino group grafted on cyclodextrin, was optimized for these cyclic oligosaccharides. These new amphiphilic compounds were fully characterized, and their self-assembling properties were investigated: the mean size diameter and polydispersity measured by Dynamic Light Scattering (DLS) were affected by the nature of the aqueous media and the temperature of storage. The encapsulation properties of these nanoparticles have been evaluated using carboxyfluorescein and scopolamine derivatives as model of guests.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides obtained by enzymatic degradation of starch. The most known CDs are composed of six (α-CD), seven (β-CD) or eight (γ-CD) α-(1→4) linked D-glucopyranosides. The hydrophobic, size-selective toroidal cavity is well known to incorporate a large range of hydrophobic molecules. This property is widely studied to solubilize drugs in aqueous media [1]. Indeed, due to the inclusion capacity of cyclodextrin's hydrophobic cavity and the transport properties of amphiphilic structures, amphiphilic cyclodextrins can be used to prepare new supramolecular assemblies such as beads, microparticles, nanoparticles or hydrogels. They can also be associated with preformed phospholipid membranes of liposomes.

According to a recent review, cyclodextrins still dominate supramolecular chemistry in water [2]. Per-substitution and mono-substitution of CD were realized to obtain pure compounds for transport of drugs [2]. Numerous chemical modifications have been carried out on CD by grafting alkyl chains of variable length to different positions in order to obtain amphiphilic derivatives able to form supramolecular aggregates considered as potential drug carriers [3]. For example, CDs have been modified with hydrophobic moieties (cholesterol moiety or phospholipidyl unit for example) to target membranes [4,5]. To simplify synthesis, aliphatic chains were also grafted directly on β-CD to produce amphiphilic compounds able to be incorporated in lipid bilayers [6]. Nevertheless, in most of the cases reported in literature, the cyclodextrin-based nanoparticles are only obtained in pure water [7–9] or are stabilized in biocompatible buffers by the presence of non-ionic surfactants (such as Tween 80), of detergents (Pluronic PE/F68) or emulsifier (Miglyol 821) [10–12].

The aim of this study was to develop the synthesis of novel non-ionic amphiphilic CDs able to generate, when formulated alone, stable nanoparticles in aqueous media (pure water and different buffers) and to estimate their encapsulation properties. Indeed, we have already prepared anionic amphiphilic cyclodextrin (I) (Scheme 1) by grafting a phospholipid on a modified CD [5]. This compound appeared to assemble into large objects and displayed a low critical aggregation concentration (7×10^{-6} M) in water and in buffered solutions. Unfortunately, these compounds exhibited too low chemical stability. On the other hand, neutral peptidolipidyl-CD derivatives (II) have been also reported [13]. These compounds were found to be poorly soluble in aqueous media, exhibiting a CMC (3×10^{-4} M) and able to spontaneously form nanosized aggregates. However, it was shown that the nanoparticles were not sterically stabilized and tented to merge into larger aggregates (250–350 nm). Based on these results we have designed new amphiphilic CD's (III) bearing a polarized function, named phosphoramide. This type of derivatives was

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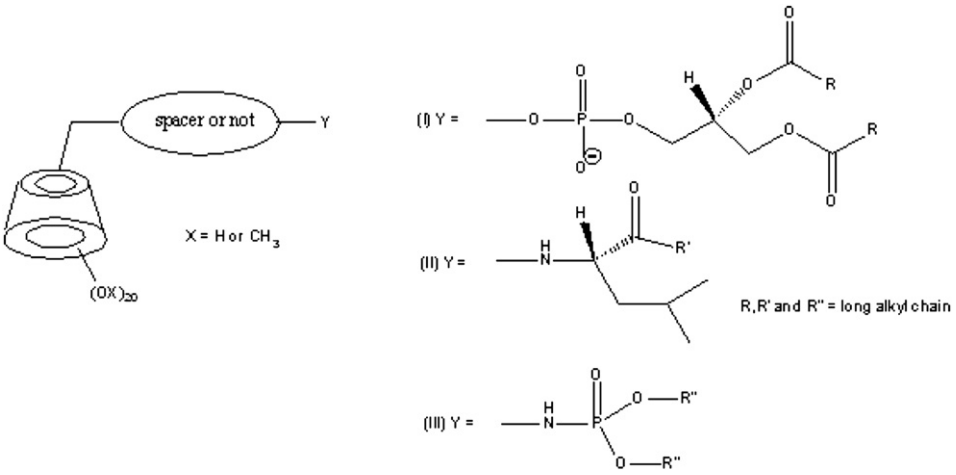


Scheme 1. Families of amphiphilic cyclodextrin derivatives designed in the laboratory.

expected to combine self-assembly properties of (I) and efficient access of (II). These new derivatives of β-cyclodextrin were obtained from 6-amino-6-deoxy-β-CD, permethylated or not, bearing a spacer arm or not, and two oleyl chains. The introduction of these two lipid chains was achieved in one step by making use of an Atherton–Todd coupling. This reaction enables the formation of a phosphorus–nitrogen bond by the reaction of an amine with dioleylphosphite. Of note this reaction has been widely employed for the synthesis of cationic [14,15] and neutral [16,17] amphiphilic compounds that have been used as gene carrier for both in vitro [18,19] an in vivo [20] transfection assays.

The physico-chemical properties of this new family of compounds were studied in terms of self-assembling properties in aqueous media. Tensioactive properties of synthesized compounds have been measured by interfacial tension in pure water and several buffers. Dynamic Light Scattering (DLS) measurements have been recorded to measure the mean size diameter and the polydispersity index. Moreover, the zeta potential of the supramolecular objects was determined. The effect of different parameters, including the nature of the aqueous medium, the temperature and the time, on the stability of the nanoparticles has been also evaluated. The yield of encapsulation inside the nanoparticles was determined by the quantification of carboxyfluorescein after purification of nanoparticles by exclusion chromatography. Finally, the potential utility of such nanoparticles for drug delivery was assessed through the investigation of the encapsulation of scopolamine derivatives in several physiological aqueous solutions.

2. Materials and methods

2.1. Chemicals

Native β-cyclodextrin was obtained from Wacker Chemicals (Germany). Dioleylphosphite was synthesized as previously reported [13]. Other chemicals were purchased from Sigma, Acros (Belgium) and Fluka Chemie GmbH (Germany). All the solvents employed for the reactions were distilled once before use. Deuterated solvents were purchased from Eurisotop (France).

2.2. Product characterization method

Stepwise control of the reactions has been readily achieved using ESI-MS in the positive ion mode using a ZQ 4000 quadrupole mass spectrometer (Waters-Micromass, Manchester, U.K.). High-resolution electrospray mass spectra (ESI-HRMS) in the positive ion mode were obtained on a Q-TOF *Ultima Global* instrument (Waters-Micromass, Manchester, U.K.). Data acquisition and processing were performed with MassLynx 4.0 software.

Structure elucidation of the final products has been readily achieved using standard ^{1}H, ^{13}C, COSY, and HSQC NMR experiments (300 ms of spin-lock, 22 dB of attenuation) performed at 500.13 MHz or 300.13 MHz using Bruker DRX 500 or AMX 300 spectrometers. Me_4Si was used as an internal standard. Measurements were performed at 298 K. ^{1}H NMR data spectra were collected using 16 K data points. Samples were dissolved in $CDCl_3$. UV spectra were recorded on a Cary 50 Bio UV–Vis (Varian).

Analytical TLC was performed using Silica Gel 60 F_{254} plates (Merck, Germany) (eluent: CH_2Cl_2/MeOH 9:1) followed by charring with vanillin-H_2SO_4.

2.3. Nanoparticles characterization

The mean diameter and the size distribution of the nanoparticles were measured using a quasi-elastic light scattering method with a Zetasizer Nano ZS (Malvern instrument) or a DynaPro Nanostar (Wyatt). The experimental temperature was 25 ± 0.3 °C. The polydispersity index (PI) is a dimensionless measure of the broadness of the size distribution. Zeta potential (mV) of the nanoparticles was measured by Malvern Zetasizer Nano ZS in order to evaluate their surface charge and their potential stability in the different eluents. All measurements were made in triplicate and expressed as mean ± SD.

A Gel Permeation Chromatography system associated with a Multi Angle Laser Light Scattering (GPC–MALLS experiments) and a Photon Correlation Detector were used to determinate the nanoparticle size and shape. Briefly, 20 μL of 2.5 mg/mL nanoparticle samples in water were injected into the GPC columns (Shodex OHpak SB-807 HQ, 8 × 300 mm, exclusion range > 500,000,000 g mol^{-1}, particle size) with water and sodium azide as a mobile phase. A DAWN® 8+ laser photometer (Wyatt Corporation, Santa Barbara, CA) and a refractive index detector (RID-10A, Shimadzu) were used to obtain the radii of gyration (Rg). The Rg were calculated from the scattering data processed with the ASTRA software (ASTRA™ 5.3.4, Wyatt Corp.) according to the Zimm formalism employing seven scattering angles between 20° and 130°. The hydrodynamic radii (Rh) were measured with a DYNAPRO Nanostar photon correlation detector photometer (Wyatt Corp., Santa Barbara, CA) at angle of 90°.

^{31}P NMR Hahn Echo experiments with high power proton decoupling were performed at magnetic fields of 7.0 T or 11.7 T. The static NMR experiments (^{31}P resonance frequency of 121.49 MHz) were done on a Bruker ASX-300 spectrometer (Bruker, Wissembourg, France) using a 7 mm CP-MAS probe and the MAS NMR experiments were realized on a Bruker DRX 500 (Bruker, Wissembourg, France) at a frequency of 202.47 MHz with a 4 mm CP-MAS probe. A stable spinning rate was obtained at 4.7 kHz. The 90° pulse length was set to 7 μs at 121.49 MHz and 7.5 μs at 202.47 MHz. A 40 μs delay was used between the 90° pulse and the 180° pulse. Respectively 38,900 and 2600 scans were acquired for static and MAS experiments. The recycling time was set to 5 s. Line broadenings of 100 Hz and 10 Hz were applied respectively for static and MAS experiments. All measurements were performed at 25 °C. The chemical shifts were referenced relative to external H_3PO_4.

2.4. Surface tension measurement

Surface tension (γ) was measured at 20 °C for each solution, obtained by dilution of a mother solution S_0 (83.2 μM for example with phosphate buffer solution) 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 h) using the Willielmy method (Tensimat N3 Prolabo tensiometer). The critical micellar concentration value (CMC) was performed using graph $\gamma = f(\log C)$, in which C indicates the molar concentration of the solution.

2.5. Calibration curve of carboxyfluorescein fluorescence intensity in water

From a mother solution of 0.1 mg/mL of carboxyfluorescein in water a dilution was done to obtain a 10^{-6} mg/mL solution. Then, from this preparation, ten dilutions were prepared from 10^{-7} to 10^{-6} mg/mL. Measurements of fluorescence are obtained from these different dilutions using a fluorimeter (VersaFluor™ Fluorometer BIO-RAD) to trace the calibration curve of carboxyfluorescein fluorescence intensity in water ($R^2 = 0.994$). The excitation filter used was 485–495 nm and the emission filter was 505–515 nm.

2.6. Calibration curve absorbance of scopolamine derivatives

Different solutions of scopolamine and N-methyl-scopolamine were prepared from 10^{-5} mol/L to 10^{-4} mol/L. Measurements of absorbance are obtained from these solutions using a UV–Visible spectrophotometer (Cary 50 Bio, Varian) to trace the calibration curve of scopolamine derivatives absorbance in different aqueous media: NaCl 0.9% ($R^2 = 0.994$ for scopolamine and N-methyl-scopolamine) Glucose 5% ($R^2 = 0.990$ for scopolamine and $R^2 = 0.997$ N-methyl-scopolamine), phosphate buffer ($R^2 = 0.995$ for scopolamine and $R^2 = 0.981$ N-methyl-scopolamine). For measurements on entrapment of scopolamine derivatives in nanoparticles, a blank has been done with nanoparticles alone in the aqueous media. The wavelengths used were 197 nm for scopolamine and 202 nm for *N*-methyl-scopolamine.

2.7. Synthesis and product characterization

2.7.1. 6-Di-oleylphosphoramidyl-6^I-deoxy-2^I,3^I-di-O-methyl-hexakis(2^{II-VII},3^{II-VII},6^{II-VII}-tri-O-methyl)-cyclomaltoheptaose 4

Lyophilized 6^I-Amino-6^I-deoxy-2^I,3^I-di-*O*-methyl-hexakis(2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl)-cyclomaltoheptaose (500 mg, 0.353 mmol, 1 eq) and dioleylphosphite (206 mg, 0.353 mmol, 1 eq) were dissolved in anhydrous dichloromethane (1 mL) under argon atmosphere. At 0 °C, a spatula of molecular sieves 3 Å, bromotrichloromethan (35 μL, 0.353 mmol, 1 eq) and diisopropylethylamine (60 μL, 0.353 mmol, 1 eq) were added. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 1 h. The solvent was removed by evaporation under reduced pressure. Then, diethyl ether was added to precipitate diisopropylethylamine which was eliminated by filtration. The solvent was removed by evaporation under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 98:2) with 35% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 5.37–5.31 (*m*, 4H, H_{diene}); 5.14–5.09 (*m*, 7H, H_1, H_I); 4.00–3.75 (*m*, 13H, H_5, H_V, H_{6a}); 3.95 (*t*, 4H, $H_{1'}$, $J_{1',2'} = 7$ Hz); 3.66–3.44 (*m*, 20H, H_3, H_{III}, H_4, H_{IV}, H_{6b}); 3.63 (*s*, 18H, $O_{(6)}CH_3$); 3.50 (*s*, 21H, $O_{(3)}CH_3$); 3.38 (*s*, 21H, $O_{(2)}CH_3$); 3.30 (*m*, 2H, H_{VIa}, H_{VIb}); 3.18 (*m*, 6H, H_2, H_{II}); 3.11 (dd, 1H, H_{II}, $J_{I,II} = 9.5$ Hz, $J_{II,III} = 3$ Hz); 2.00 (*m*, 8H, $H_{8'}$, $H_{11'}$); 1.64 (*m*, 8H, $H_{2'}$, $H_{3'}$); 1.28 (*m*, 40H, CH_2); 0.88 (*t*, 6H, $H_{18'}$, $J_{17',18'} = 7$ Hz).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 130,4, 130,1 (4C, C=C); 99.7–99.1 (7C, C_1, C_I); 82.8–81.8 (14C, C_2, C_{II}, C_3, C_{III}); 80.9–80.5 (7C, C_4, C_{IV}); 71.4–71.0 (13C, C_5, C_V, C_6); 66.7 (2C, C_7, C_I); 61.9–61.6 (6C, $O_{(6)}CH_3$); 59.6–59.3 (7C, $O_{(3)}CH_3$); 59.0–58.7 (7C, $O_{(2)}CH_3$); 42.5 (1C, C_{VI}); 31.0, 30.9 (4C, $C_{2'}$, $C_{3'}$); 33.0–23.1 (20C, CH_2); 27.6 (4C, $C_{8'}$, $C_{11'}$); 14.5 (2C, $C_{18'}$) Elemental analysis $C_{98}H_{180}NO_{37}P$, H_2O found C, 58.35; H, 8.84, N, 0.67 requires C, 58.46; H, 9.11, N, 0.70.

HRMS (ESI): *m*/*z*: 2017.1956 ($[M + Na]^+$, ($C_{98}H_{180}NO_{37}NaP$) requires 2017.1870).

2.7.2. 6^I-Amido-β-alanyl-(6-Di-oleylphosphoramidyl)-6^I-deoxy-2^I,3^I-di-O-methyl-hexakis(2^{II-VII},3^{II-VII},6^{II-VII}-tri-O-methyl)-cyclomaltoheptaose 5

Lyophilized 6^I-Amido-β-alanyl-6^I-deoxy-2^I,3^I-di-*O*-methyl-hexakis(2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl)-cyclomaltoheptaose (100 mg, 0.067 mmol, 1 eq) and dioleylphosphite (39 mg, 0,067 mmol, 1 eq) were dissolved in anhydrous dichloromethane (350 μL) under argon atmosphere. At 0 °C, a spatula of molecular sieves 3 Å, bromotrichloromethane (66 μL, 0.674 mmol, 10 eq) and diisopropylethylamine (117 μL, 0.674 mmol, 10 eq) were added. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 1 h. The solvent was removed by evaporation under reduced pressure. Then, diethyl ether was added to precipitate diisopropylethylamine which was eliminated by filtration. The solvent was removed by evaporation under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 98:2) to give 5 with the yield of 66%.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.19 (*s*, 1H, NHCO); 5.34 (*m*, 4H, H_{diene}); 5.16–5.07 (*m*, 7H, H_1, H_I); 3.95 (*t*, 4H, $H_{1'}$, $J_{1',2'} = 9.5$ Hz); 3.97–3.75 (*m*, 13H, H_5, H_V, H_{6a}); 3.69–3.45 (*m*, 20H, H_3, H_{III}, H_4, H_{IV}, H_{6b}); 3.63 (*s*, 18H, $O_{(6)}CH_3$); 3.51–3.49 (*s*, 21H, $O_{(3)}CH_3$); 3.38 (*s*, 21H, $O_{(2)}CH_3$); 3.21–3.16 (*m*, 2H, $H_{19'}$); 3.19 (dd, 7H, H_2, H_{II}, $J_{1,2} = J_{I,II} = 15.5$ Hz, $J_{2,3} = J_{II,III} = 5.5$ Hz); 2.37 (*t*, 2H, $H_{20'}$, $J_{19'-20'} = 11$ Hz); 2.01–1.97 (*m*, 8H, $H_{8'}$, $H_{11'}$); 1.67–1.62 (*m*, 4H, $H_{2'}$, $H_{3'}$); 1.25 (*m*, 40H, CH_2); 0.87 (*t*, 6H, $H_{18'}$,, $J_{17'-18'} = 10.5$ Hz).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 171.6 (1C, NHCO); 130.4, 130.2 (4C, C=C); 99.5–99.1 (7C, C_1, C_I); 82.3–80.7 (21C, C_2, C_{II}, C_3, C_{III}, C_4, C_{IV}); 71.8–70.2 (14C, C_5, C_V, C_6, C_{VI}); 66.9 (2C, $C_{1'}$); 62.0 (6C, O $C_{(6)}CH_3$); 59.5–58.7 (14C, $O_{(3)}CH_3$, $O_{(2)}CH_3$); 38.2 (2C, $C_{19'}$, $C_{20'}$); 32.3–23.1 (20C, CH_2); 14.5 (2C, $C_{18'}$).

Elemental analysis $C_{101}H_{185}N_2O_{38}P$, H_2O found C, 57.93; H, 8.89, N, 1.35 requires C, 58.19; H, 9.04, N, 1.34.

HRMS (ESI): *m*/*z*: 2088.2278 ($[M + Na]^+$, ($C_{101}H_{185}N_2O_{38}NaP$) requires 2088.2241).

2.7.3. 6-Di-stearylphosphoramidyl-6^I-deoxy-2^I,3^I-di-O-methyl-hexakis(2^{II-VII},3^{II-VII},6^{II-VII}-tri-O-methyl)-cyclomaltoheptaose 6

Lyophilized 6^I-Amino-6^I-deoxy-2^I,3^I-di-*O*-methyl-hexakis(2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl)-cyclomaltoheptaose (100 mg, 0.071 mmol, 1 eq) and distearylphosphite (83 mg, 0.141 mmol, 2 eq) were

dissolved in anhydrous chloroform (350 μL) under argon atmosphere. At 0 °C, bromotrichloromethane (139 μL, 1.413 mmol, 20 eq) and diisopropylethylamine (2 M) (707 μL, 1.43 mmol, 20 eq) were added. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 1 h 30 min. The solvent was removed by evaporation under reduced pressure. Then, diethyl ether was added to precipitate diisopropylethylamine which was eliminated by filtration. The solvent was removed by evaporation under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 100:0 to 97:3) to give 5 with the yield of 14%.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 5.14–5.08 (*m*, 7H, H_1, H_I); 4.25–3.90 (*m*, 4H, $H_{1'}$); 3.90–3.70 (*m*, 13H, H_5, H_V, H_{6a}); 3.70–3.45 (*m*, 20H, H_3, H_{III}, H_4, H_{IV}, H_{6b}); 3.63 (*s*, 18H, $O_{(6)}CH_3$); 3.52 (*s*, 21H, $O_{(3)}CH_3$); 3.37 (*s*, 21H, $O_{(2)}CH_3$); 3,33–2.25 (*m*, 2H, H_{VI}); 3.23–3.08 (*m*, 7H, H_2, H_{II}); 1.66 (*m*, 8H, $H_{2'}$, $H_{3'}$); 1.25 (*m*, 56H, CH_2); 0.87 (*m*, 6H, $H_{18'}$).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 99.2–99.1 (7C, C_1, C_I); 82.2-81-9 (14C, C_2, C_{II}, C_3, C_{III}); 80.7–80.3 (7C, C_4, C_{IV}); 71.6–71.2 (14C, C_5, C_V, C_6); 66.5 (2C, $C_{1'}$); 61.7–61.5 (6C, $O_{(6)}CH_3$); 59.2 (7C, $O_{(3)}CH_3$); 58.6–58.5 (7C, $O_{(2)}CH_3$); 42,2 (1C, C_{VI}); 30.7–30.6 (4C, $C_{2'}$, $C_{3'}$); 32.1–22.8 (28C, CH_2); 14.3 (2C, $C_{18'}$).

HRMS (ESI): *m*/*z*: 2021.2156 ($[M + Na]^+$, ($C_{98}H_{184}NO_{37}NaP$ requires 2021.2183).

2.7.4. 6-Di-oleylphosphoramidyl-6^I-deoxy-cyclomaltoheptaose 8

Lyophilized 6^I-Amino-6^I-deoxy-cyclomaltoheptaose (100 mg, 0.088 mmol, 1 eq) and dioleylphosphite (500 mg, 0.882 mmol, 10 eq) were dissolved in anhydrous *N*,*N*-dimethylformamide (4 mL) under argon atmosphere. At 0 °C, a spatula of molecular sieves 3 Å, bromotrichloromethane (87 μL, 0.882 mmol, 10 eq) and diisopropylethylamine (2 M) (2.21 mL, 4.409 mmol, 50 eq) were added. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 10 days. The solvent was removed by evaporation under reduced pressure. Then, diethyl ether was added to precipitate diisopropylethylamine which was eliminated by filtration. The solvent was removed by evaporation under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 98:2) to give with the yield of 4%.

^{1}H NMR (600 MHz, DMSO) δ (ppm): 5.9–5.6 (*m*, 14H, OH_2, OH_{II}, OH_3, OH_{III}); 5.45–5.25 (*m*, 4H, H_{diene}); 5–4.75 (*m*, 7H, H_1, H_I); 4.47 (*s*, 6H, OH_6); 3.90–3.75 (*m*, 4H, $H_{1'}$); 3.75–3.00 (*m*, 42H, H_2, H_{II}, H_3, H_{III}, H_4, H_{IV}, H_5, H_V, H_6, H_{VI}); 2.1–1.8 (*m*, 8H, $H_{8'}$, $H_{11'}$); 1.7–1.4 (*m*, 4H, $H_{2'}$); 1.23 (*m*, 40H, CH_2); 0.85–0.83 (*m*, 6H, $H_{18'}$).

^{13}C NMR (150 MHz, DMSO) δ (ppm): 129.8 (4C, $C_{9'}$, $C_{10'}$); 102.0 (7C, C_1, C_I); 81.7 (7C, C_2, C_{II}); 73.1–72.2 (21C, C_3, C_{III}, C_4, C_{IV} C_5, C_V); 65.4 (2C, $C_{1'}$); 60.0 (7C, C_6, C_{VI}); 31.4–22.2 (22C, $C_{3'}$–$C_{7'}$, $C_{12'}$–$C_{17'}$); 29.2 (2C, $C_{2'}$); 26.8 (4C, $C_{8'}$, $C_{11'}$); 14.1 (2C, $C_{18'}$).

HRMS (ESI): *m*/*z*: 1714.8962([M + H]$^+$, ($C_{78}H_{141}NO_{37}P$ requires1714.8920).

2.7.5. 6^I -Amido-β-alanyl -6^I-deoxy-cyclomaltoheptaose 9

Fmoc-β-alanine (206 mg, 0.661 mmol, 1.5 eq) was dissolved in anhydrous *N*,*N*-dimethylformamide (25 mL) under Argon atmosphere. Then, 1-Hydroxybenzotriazole solution (595 mg, 4.409 mmol, 10 eq) and *N*,*N*-Diisopropylcarbodiimide (0.69 mL, 4.409 mmol, 10 eq) were added. After stirring for 2 h, a 6^I-Amido-6^I-deoxy-cyclomaltoheptaose solution (500 mg dissolved in 15 mL of *N*,*N*-dimethylformamide with few drops of triethylamine) was added. The reaction mixture was stirred at room temperature for 1 night. Diethyl ether was added on the solution to precipitate the cyclodextrins. The residue was filtered on silica gel. The product and piperidine (328 μL, 3.329 mmol, 19 eq) were dissolved in anhydrous *N*,*N*-dimethylformamide. The reaction mixture was stirred at 90 °C for few hours. Diethyl ether was added on the solution to precipitate the cyclodextrins. The residue was purified on ion exchange column (AGMP50) to give the yield of 23%.

^{1}H NMR (600 MHz, DMSO) δ (ppm): 5.25–4.6 (*m*, 7H, H_1, H_I); 4.5–3.5 (*m*, 28H, H_3, H_{III}, H_5, H_V, H_6, H_{VI}); 3.5–3.1(*m*, 18, H_2, H_{II} H_4, H_{IV}, $H_{19'}$); 3.1–2.75 (*m*, 2H, $H_{20'}$).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 167 (1C, NHCO); 102.2 (7C, C_1, C_I); 81.9 (7C, C_2, C_{II}); 73.2–72.3 (21C, C_3, C_{III}, C_4, C_{IV}, C_5, C_V); 60.2 (7C, C_6); 40.4–38.7 (2C, $C_{19'}$, $C_{20'}$).

HRMS (ESI): *m*/*z*: 1205.4353 ($[M + H]^+$, ($C_{45}H_{77}N_2O_{35}$ requires 1205.4307).

2.8. Nanoparticle preparation

6^I-Amido-β-alanyl-(6-Di-oleylphosphoramidyl)-6^I-deoxy-2^I,3^I-di-*O*-methyl-hexakis(2$^{II–VII}$,3$^{II–VII}$,6$^{II–VII}$-tri-*O*-methyl)-cyclomalto heptaose (5 mg, 2.42 μmol) **5** was dissolved with or not host compound (0.8 mg, 2.13 μmol of carboxyfluorescein; 0.7 mg, 2.05 μmol of scopolamine; 0.8 mg, 2.01 μmol of *N*-methyl-scopolamine) in 0.5 mL of chloroform which was slowly evaporated under N_2 flow to form a thin film. The preparation was dried under vacuum overnight. The following day, the film was hydrated (2 mL of aqueous solution). The solution has been shaken by vortex for 15 min, put for few hours in ultrasound bath and sonicated for 2 min 30 s (power: 20% of 100 W, pulse: 1 s). The excess of host compound which was not encapsulated in the nanoparticles was eliminated by size exclusion chromatography (Sephadex G50, lengh: 17 cm, diameter: 1 cm, calibrated with blue dextran (V_0 = 7 mL) and vitamin B12, (V_t = 25 mL). The loaded nanoparticles were eluted first at a V_e of 7 mL and the small free molecule, not entrapped, were then eluted at a V_e of 23 mL. Then, the solutions have been preserved at 4 °C overnight and investigated by DLS using Malvern instrumentation the next day. The solutions were analyzed for carboxyfluorescein by fluorescence spectrophotometry and for scopolamine derivatives by UV–vis spectrophotometer (Cary 50 Bio, Varian) at wavelength of 256 nm. Loading capacity expressed as the associated drug percentage was calculated according to the following equation:

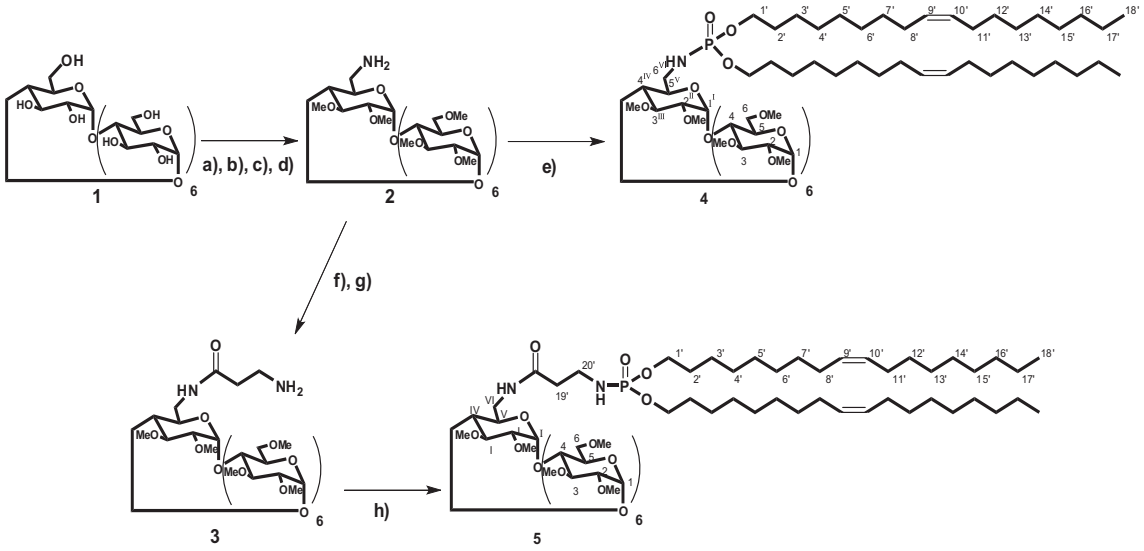
Associated drug % = 100*[determinate guest quantity (μmol)/initial guest quantity (μmol)]

3. Results and discussion

3.1. Synthesis

We have previously reported that the functionalization of CD's deeply impacted their self-assembling in water. Among the structural alterations initially studied, we have shown that the structure of the hydrophobic anchor, the cyclodextrin methylation degree and the molecular structure of the spacer arm were some important factors [21]. The synthesis of phospholipidyl-CDs has been described and these compounds have been able to self-organize in pure water and also in buffers [5]. Their ability to cross the Blood Brain Barrier (BBB) using in vitro model was demonstrated [22]. However the cost, the tedious synthesis and their rather poor chemical stability have led us to investigate the synthesis of some CD derivatives with two long chains branched on an amino-acid moiety [13]. Unfortunately these modifies CD's were poorly soluble in aqueous media.

Based on these preliminary results, our interest was focused on the design of amphiphilic cyclodextrin that would be easily accessible and that would form stable supramolecular assemblies in aqueous media. Hence, we have selected the Atherton–Todd reaction which has been first reported in 40's and that produces



a) TsCl (8 eq), $CuSO_4$, H_20, 4h b) LiN_3 (17 eq), H_20, 4h at 90°C, 15h at RT c) MeI(180 eq), NaH(58 eq), DMF, 24h d) PPh_3 (5 eq), NH_4OH, DMF, 24h, 16% e) Dioleylphosphite (1 eq), $CBrCl_3$ (1 eq), DIPEA (1 eq), CH_2Cl_2, 35% f) β-alanineFmoc (1,5 eq), HOBt (10 eq), DIC (10 eq), DMF, 77% g) piperidine (20% in $CHCl_3$), 62% h) Dioleylphosphite (1 eq), $CBrCl_3$ (10 eq), DIPEA (10 eq), CH_2Cl_2, 66%

Scheme 2. Synthesis of dioleylphosphoramidyl-permethylated-β-CD **4** and **5.**

a phosphoramide functional group by the reaction of an amine with dialkylphosphite in presence of carbon tetrachloride [23]. The use of dialkylphosphite possessing two lipid chains (e.g. oleyl, linoleyl) as substrate in the Atherton–Todd reaction have been more recently proposed for the design of cationic lipophosphoramides that have been employed as vector for gene delivery [24].

The 6-amino-6-deoxy-permethylated-β-cyclodextrin **2** was obtained in four steps from native β-CD **1** using classical way [25–29]: monotosylation, azidation, methylation of free hydroxyls and Staudinger reaction to reduce azido group in amine group (Scheme 2). To study the effect of the distance between the polar head (β-CD) and the lipid moiety, we synthesized 6-amido-β-alanyl-6-deoxy-permethylated-β-CD **3** in two steps from **2** [30]. The peptidic coupling was performed with DIC in 77% yield and the deprotection of fmoc group by piperidine led to **3** in 62% yield.

With the both amino cyclodextrin derivatives in hands, we tried to transpose the Atherton–Todd reaction to the cyclodextrin's chemistry. The amine **2** was then able to react with dioleylphosphite according to classical Atherton–Todd reaction conditions to offer phosphoramidyl-β-CD **4** in 35% yield. The optimization of the yield for this reaction was not attempted since we have later shown that no self-assembling properties were obtained with **4** in water.

The amine **3** could then react with dioleylphosphite in presence of $CBrCl_3$ and DIPEA to synthesize **5.** The conditions were optimized (Table 1) and the yield was improved to 66%. This better yield is likely explained by the easier accessibility of the amine in compound **3**. These two new compounds were caracterized by NMR and Mass spectrometry.

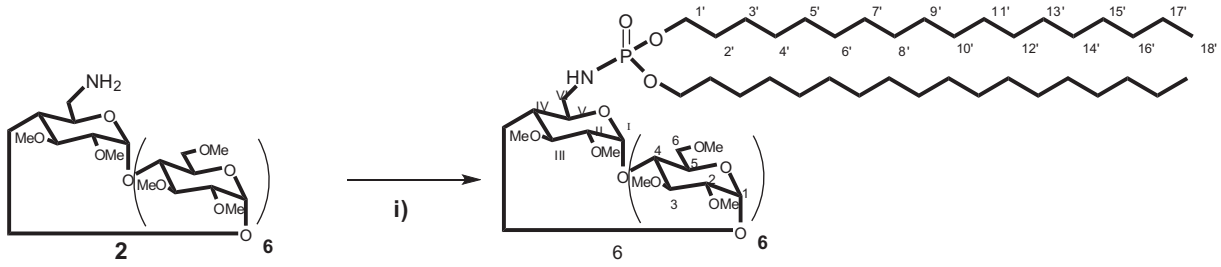
Table 1
Number of equivalents of reagents and yields in Todd–Atherton reaction on **3**.

Phosphite (eq)	$CBrCl_3$ (eq)	DIPEA (eq)	Yield (%) of 5
1	1	1	14
1	10	10	66

To evaluate the consequence of the presence of a double bound in the oleyl lipid chains on the supramolecular properties, the synthesis of the analogs of **4** and **5** without double bond (stearyl chains) was carried out (Scheme 3). The reaction of the amine **2** with the distearylphosphite was achieved following the same conditions as those used for dioleylphosphite but, unfortunately, the yield was dramatically lower. Even with 2 equivalents of distearylphoshite, compound **6** was produced in low yield (14%). In the case of amine **3** that possess a spacer arm, the reaction was tested with few conditions of temperature, solvents, and reagent quantities but without further yield improvement. The produced compound appeared to have a low stability and seemed to degrade before purification so it was impossible to isolate enough amounts of compound for its characterization and for the evaluation of its supramolecular properties.

In general, permethylated-β-CD is usually preferred to free β-CD since its solubility in both organic solvent and water is better. Nevertheless, the permethylation step induced structural changes that likely impacts the supramolecular properties of these CD's when they are placed in water. With the aim to dispose of both permethylated and free CD's functionalized with a lipophosphoramidyl moiety, we have also engaged free amino-CD in the Atherton–Todd reaction (Scheme 4).

The 6-amino-6-deoxy-β-CD **7** was obtained in three steps from β-CD **1** by monotosylation, azidation and Staudinger reaction to reduce azide group in amine [26,27]. Unfortunately, **7** was not soluble in usual organic solvents. Among different solvents and combination of solvents tested (MeOH, CH_2Cl_2/DMF 5:5 and 1:2 v:v, and DMF), DMF was found as the best solvent. Unfortunately, the product **8** was obtained in only 4% yield. This low yield was probably due to the numerous purifications of this very polar compound. **8** was obtained in so low amount that only partial characterization was carried out and the study of its supramolecular organization in water was not attempted. Finally, the same reaction has been carried out on **9** (obtained in two steps from **7**) which is a precursor possessing a spacer arm between the CD and



i) Distearylphosphite (2 eq), $CBrCl_3$ (10 eq), DIPEA (10 eq), CH_2Cl_2, 14%

Scheme 3. Synthesis of distearylphosphoramidyl-permethylated-β-CD **6.**

the amino group. Unfortunately only traces of the expected compound **10** have been obtained.

To summarize this part, the Atherton–Todd reaction can be carried out on methylated amino cyclodextrin derivatives using optimized conditions. The presence of a spacer arm is in favor of better yields. The use of free hydroxyl amino-CD derivatives as starting material precludes the Atherton–Todd reaction probably due to their too poor solubility in solvents. Nevertheless, self-assembling properties of compounds **4** and **5** have been evaluated.

3.2. Tensioactive properties of nanoparticles

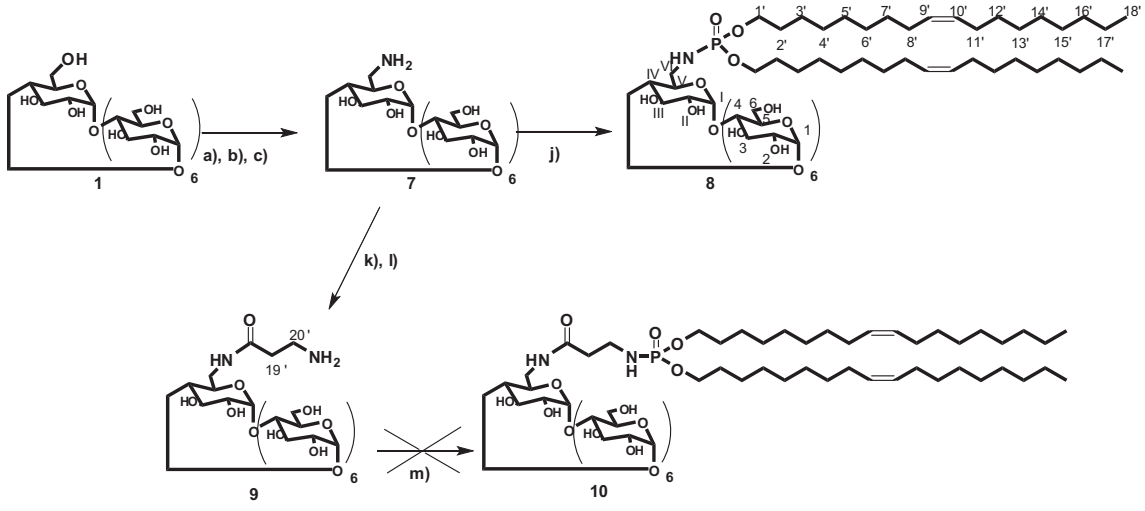
The behavior of compounds **4** and **5** in water was first studied. It is worthy to note that these two compounds are composed by an extremely hydrophobic part and a large hydrophilic head-group. Consequently, a strong cooperation in self-aggregation in water and in buffers is expected. Firstly, surface tension measurements were used to evidence the self-assembly properties in water. Surface tension was measured for a series of solutions of **4** and **5** of increasing concentrations in pure water and in two different buffered solutions at 20 °C (Fig. 1).

From these experiments, the solubility were estimated and displayed in Table 2. Compound **4** is insoluble in water and in buffered solutions and does not produce supramolecular aggregates. Compound **5** exhibits a rather low solubility in water. The same measurements performed in buffered solution (phosphate buffer 0.1 M pH = 7.5 or tris buffer 0.05 M pH = 8.6) showed a weak increase of the solubility values.

It can be observed that these two compounds differ from only one point: compound **4** corresponds to the direct bonding of the phosphoramide on the position 6 of the cyclodextrin, conversely, in **5**, an alanyl spacer is added between the CD and the dioleylphosphoramidyl moiety. These results are in agreement with those already reported [31].

3.3. Nanoparticle size studies

The method used to form nanoparticles was similar to that of liposomes preparation and not the conventionally one used with amphiphilic cyclodextrins, namely nanoprecipitation. The recovery yield of formulation is around 84% in water. The results described in the Table 3 were obtained after the preparation of the nanoparticles and before the storage at 4 °C.



a) TsCl (8 eq), $CuSO_4$, H_2O, 4h b) LiN_3 (17 eq), H_2O, 4h at 90°C, 15h at RT c) PPh_3 (5 eq), NH_4OH, DMF, 24h, 90% j) Dioleylphosphite (10 eq), $CBrCl_3$ (2 eq), DIPEA (50 eq), DMF 4% k) β-alanineFmoc (1,5 eq), HOBt (10 eq), DIC (10 eq), DMF g) piperidine (20% in DMF), 22% m) Dioleylphosphite (1 eq), $CBrCl_3$ (10 eq), DIPEA (10 eq), DMF

Scheme 4. Synthesis of the dioleylphosphoramidyl-β-CD **8** and **10.**

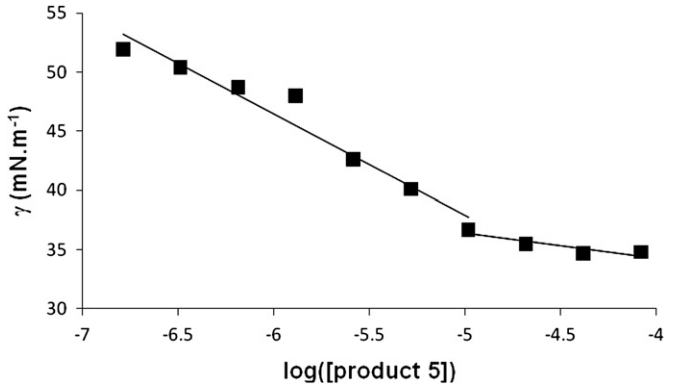


Fig. 1. Surface tension of 5 in phosphate buffer at 20 °C as function of concentration.

Hydrodymanic diameter, polydispersity index (PI) and zeta potential of nanoparticles obtained in pure water, glucose 5%, NaCl 0.9% at 25 °C, are reported in Table 3. The mean size of nanoparticles as measured by DLS ranged from 180 to 280 nm with PI values of 0.1–0.2. It should be pointed out that PI value below 0.25 generally signed the presence of monodisperse particles suspensions. The zeta potentials of nanoparticles were measured in order to estimate the potential stability of the system due to a possible mechanism of dissociation or ion adsorption in water. It is indeed frequently claimed that a value lower than −30 mV or higher than +30 mV is in favor of stable nanoparticles. In first approximation, stability of nanoparticles is higher in water than in glucose 5% solution (−46 mV *vs* −36 mV).

As temperature of storage seemed to be a key-point for the stability, we realized 3 successive cycles of storage: 25 °C for 24H and then 4 °C for 24H (Fig. 2). The size of the nanoparticles in pure water and in glucose 5% solution was then measured by DLS each day. In the both cases, the size measured at 25 °C is about 200–280 nm while at 4 °C the size measured at 25 °C is around 130 nm. These results suggest that the phenomenon is not perfectly stable as a function of storage time.

In order to better characterize our particles, the hydrodynamic radius (R_h) and the radius of gyration (R_g) by dynamic and static light scattering were determined. It is well known that the ratio R_g/R_h is related to the spatial density distribution and the degree of draining of a scattering object in solution or dispersion. In the case of "true" liposomes, the theoretical values will be around 1.0, revealing a thin-wall hollow sphere. The determined value of 0.694, close to the theoretical value of 0.774, means a quite uniform and nondraining hard sphere [32]. Furthermore, ^{31}P NMR spectrum of **5** in water, compared to its powder spectrum, showed a broad and symmetric peak centered on the isotropic resonance (9.7 ppm) revealing that auto-assembly occurred (Fig. 3).

3.4. Evaluation of the encapsulation properties of nanoparticles

To validate the encapsulation step of our nanoparticles, a hydrophilic fluorescent molecule, the carboxyfluorescein was chosen as host molecule (Fig. 4). This fluorescent dye carboxyfluorescein was usually used as guest model [33]. Cyclodextrin nanoparticles were prepared with carboxyfluorescein at self-quenching concentration (see material and method section). Encapsulated carboxyfluorescein was separated from free dye by size exclusion chromatography (Sephadex G50). The amount carboxyfluorescein within nanoparticles (6.7 g/L *ie* 178 μM) was measured by fluorescence using the calibration curve.

Recovery yield of encapsulation is around 42%. The entrapment efficiency is in the order of 0.89 μmol carboxyfluorescein per 2 μmol of cyclodextrin. It should be noted that for liposomes, the entrapment is usually about 1:100 [33] but loading efficiency could reached 40% in the case of nanoparticles made from amphiphilic cyclodextrin [34]. In the same experimental conditions, the measured size of nanoparticles of **5** in pure water was found as 114 nm in absence of carboxyfluorescein and 103 nm in presence of dye. The high entrapment efficiency observed with

Table 2
Determination of the solubility values of compounds **4** and **5** in several media.

compound	4	5
Water	Insoluble	5.4×10^{-5} M
Tris Buffer	Insoluble	2.8×10^{-4} M
Phosphate Buffer	Insoluble	1.7×10^{-5} M

Table 3
Properties of **5** nanoparticles in several aqueous media at 25 °C. PI: Polydispersity index.

Solvent	Diameter (nm)	PI	Zeta potential (mV)
Pure water pH = 5.6	191 ± 19	0.192	−46
Glucose 5% pH = 5.6	282 ± 25	0.119	−36
NaCl 0.9%, pH = 5.6	175 ± 20	0.202	>−30

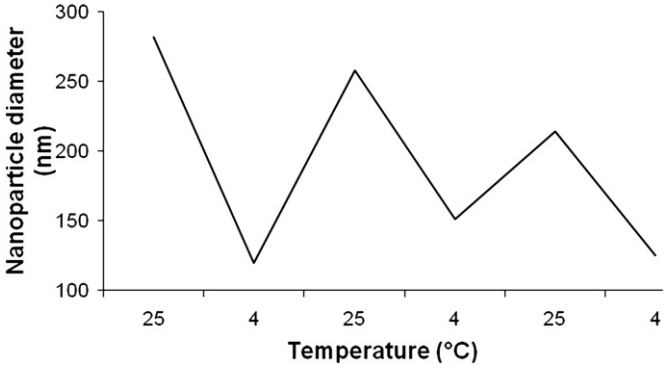


Fig. 2. Effect of storage temperature cycle on the nanoparticle size in glucose 5% solution.

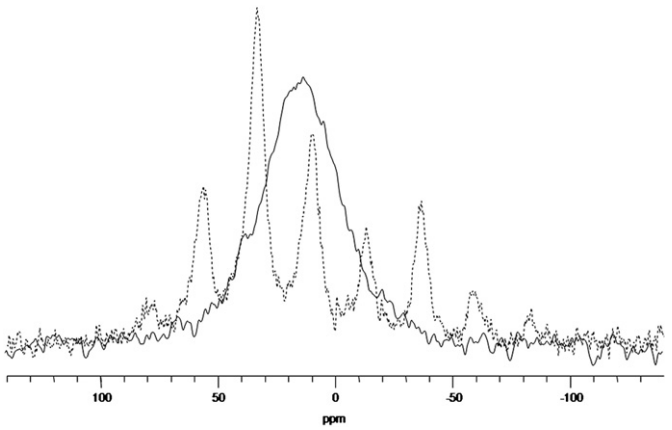


Fig. 3. Static ^{31}P NMR spectrum of the nanoparticles formed in water from the compound 5 and MAS ^{31}P NMR spectrum at 4.7 kHz spinning rate of the corresponding powder sample (dotted line).

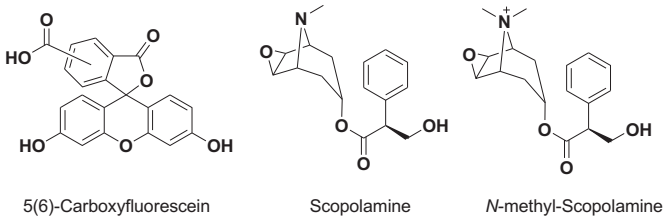


Fig. 4. Chemical structure of guest molecules.

carboxyfluorescein was not correlated to an increase of the size of the nanoparticles. Moreover it could not be explained by interaction of carboxyfluorescein with cavity of the cyclodextrin since an NMR study performed on permethylated-β-CD used as headgroup model and carboxyfluorescein has demonstrated that no inclusion complex has been observed. However, adsorption of carboxyfluorescein on the nanoparticle surface can not be excluded.

Then, to verify the efficiency of our nanoparticles to pass through the blood brain barrier, two molecules, scopolamine and *N*-methyl-scopolamine, were chosen (Fig. 4). Indeed, these two drugs have the same amnesic action but scopolamine can cross the blood brain barrier contrary to the *N*-methyl-scopolamine. NMR studies, in water, have demonstrated that no inclusion complex occurred in presence of permethylated β-cyclodextrin and scopolamine or *N*-methyl-scopolamine. Cyclodextrin nanoparticles were prepared in presence of these drugs in glucose 5%, NaCl 0.9% and phosphate buffered solutions. The size of obtained nanoparticles in presence or in absence of drug has been measured by DLS and the values are collected in Table 4. The nanoparticles sizes were ranging from 100 to 200 nm. In presence of drug, the nanoparticle size seemed to be in the same order of magnitude than those formed without scopolamine derivatives.

The nature of the medium had a significant influence on the loading efficiency (percentage of associated drug): in phosphate buffer, no encapsulation with scopolamine is observed while 22% loading inside nanoparticles could be reached with *N*-methyl-scopolamine. On the other hand, encapsulation performed in NaCl 0.9% gave 8% and 14% of associated scopolamine and *N*-methyl-scopolamine respectively. The most stable nanoparticles have been obtained in glucose 5% solution with PI values lower than 0.20 (0.176 and 0.107 for scopolamine and N-methyl-scopolamine respectively) and zeta potential values ranging from −33 to −40 mV. In these conditions nanoparticles loaded with scopolamine and *N*-methyl-scopolamine remained stable more than one week without increase of size or PI value. The percentage of associated drug were slightly weaker (13% and 6%) but remained correct for applications.

Table 4
Size of nanoparticles in presence or in absence of drug as measured by DLS.

Average nanoparticle diameter (nm)		Associated drug (%)
Phosphate Buffer (pH = 7.5)		
Cyclodextrin **5** alone	112 ± 20	
5 with scopolamine	118 ± 20	nd
5 with *N*-methyl-scopolamine	101 ± 20	59 μM (yield = 22%)
NaCl 0.9%		
Cyclodextrin **5** alone	175 ± 20	
5 with scopolamine	135 ± 50	30 μM (yield = 8%)
5 with *N*-methyl-scopolamine	140 ± 75	37 μM (yield = 14%)
Glucose 5%		
Cyclodextrin **5** alone	140 ± 25	
5 with scopolamine	132 ± 50	20 μM (yield = 13%)
5 with *N*-methyl-scopolamine	130 ± 20	8 μM (yield = 6%)

In conclusion, our study demonstrates the potentiality of these new lipophosphoramidyl-β-CD obtained by an Atherton–Todd reaction optimized for β-CD to form nanoparticles. Their self-assemblies are stable both with time and temperature in physiological conditions such as glucose 5% solution. Furthermore, preliminary experiments of drugs of interest have shown promising entrapment properties in the nanoparticles. In vivo assays are occurring on mice's in our laboratories. Indeed, measurements of behavioral effects (such as memorization and learning test) of these drugs carried or not by nanoparticles will be performed.

Acknowledgments

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.biochi.2011.09.005.

References

[1] H. Dodziuk, Cyclodextrins and their complexes, in: Chemistry, Analytical Methods, Applications. Wiley-VCH, New York, 2006.
[2] F. Sallas, R. Darcy, Advances in synthesis and supramolecular chemistry, Eur. J. Org. Chem. (2008) 957–969.
[3] E. Bilensoy, A.A. Hincal, Recent advances and future directions in amphiphilic cyclodextrin nanoparticles, Expert Opin. Drug Deliv. 6 (2009) 1161–1173.
[4] R. Auzely-Velty, B. Perly, O. Tache, T. Zemb, P. Jehan, P. Guenot, J.P. Dalbiez, F. Djedaini-Pilard, Cholesteryl-cyclodextrins: synthesis and insertion into phospholipid membranes, Carbohydr. Res. 318 (1999) 82–90.
[5] S. Moutard, B. Perly, P. Godé, G. Demailly, F. Djedaïni-Pilard, Novel glycolipids based on cyclodextrins, J. Incl. Phenom. Macrocylic. Chem. 44 (2002) 317–322.
[6] D. Gallois-Montbrun, N. Thiebault, V. Moreau, G. Lebas, J.-C. Archambault, S. Lesieur, F. Djedaıni-Pilard, Direct synthesis of novel amphiphilic cyclodextrins, Incl. Phenom. Macrocylic. Chem. 57 (2007) 131–135.
[7] L. Choisnard, A. Gèze, J.L. Putaux, Y.S. Wong, D. Wouessidjewe, Nanoparticles of β-cyclodextrin esters obtained by self-assembling of biotransesterified β-cyclodextrins, Biomacromolecules 7 (2006) 515–520.
[8] S. McNicholas, A. Rencurosi, L. Lay, A. Mazzaglia, L. Sturiale, M. Perez, R. Darcy, Amphiphilic *N*-glycosyl-thiocarbamoyl cyclodextrins: synthesis, self-assembly, and fluorimetry of recognition by *lens culinaris* lectin, Biomacromolecules 8 (2007) 1851–1857.
[9] W. Abdelwahed, G. Degobert, A. Dubes, H. Parrot-Lopez, H. Fessi, Sulfated and non-sulfated amphiphilic β-cyclodextrins: impact of their structural properties on the physicochemical properties of nanoparticles, Int. J. Pharm. 351 (2008) 289–295.
[10] M. Lahiani-Skiba, F. Bounoure, S. Shawky-Tous, P. Arnaud, M. Skiba, Optimization of entrapment of metronidazole in amphiphilic β-cylodextrin nanospheres, J. Pharm. Biomed. Anal. 41 (2006) 1017–1735.
[11] A. Noomen, S. Hbaieb, H. Parrot-Lopez, R. Kalfat, H. Fessi, N. Amdouni, Y. Chevalier, Emulsions of β-cyclodextrins grafted to silicone for the transport of antifungal drugs, Mat. Sci. Eng. C 28 (2008) 705–715.
[12] A. Gèze, L. Choisnard, J.L. Putaux, D. Wouessidjewe, Colloidal systems made of biotransfered α, β and γ cyclodextrins grafted with C10 alkyls chains, Mat. Sci. Eng. C 29 (2009) 458–462.
[13] A. Angelova, C. Fajolles, C. Hocquelet, F. Djedaïni-Pilard, S. Lesieur, V. Bonnet, B. Perly, G. Lebas, L. Mauclaire, Physico-chemical investigation of asymmetrical peptidolipidyl-cyclodextrins, J. Colloid Interface Sci. 322 (2008) 304–314.
[14] F. Lamarche, M. Mével, T. Montier, L. Burel-Deschamps, P. Giamarchi, R. Tripier, T. Le Gall, D. Cartier, P. Lehn, J.C. Clément, P.A. Jaffrès, Lipophosphoramidates as lipidic part of lipospermines for gene delivery, Bioconjug. Chem. 18 (2007) 1575–1582.
[15] M. Mével, T. Montier, F. Lamarche, P. Delépine, T. Le Gall, J.J. Yaouanc, P.A. Jaffrès, D. Cartier, P. Lehn, J.C. Clément, Dicationic lipophosphoramidates as DNA carriers, Bioconjug. Chem. 18 (2007) 1604–1611.
[16] P. Midoux, C. Pichon, J.J. Yaouanc, P.A. Jaffrès, Chemical vectors for gene delivery: a current review on polymers, peptides and lipids containing histidine or imidazole as nucleic acids carriers, Br. J. Pharmacol. 157 (2009) 166–178.

[17] M. Mével, C. Neveu, C. Gonçalves, J.J. Yaouanc, C. Pichon, P.A. Jaffrès, P. Midoux, Novel neutral imidazole-lipophosphoramides for transfection assays, Chem. Commun. (2008) 3124–3126.
[18] M. Mével, G. Breuzard, J.J. Yaouanc, J.C. Clément, P. Lehn, C. Pichon, P.A. Jaffrès, P. Midoux, Synthesis and transfection activity of new cationic phosphoramidate lipids: high efficiency of an imidazolium derivative, Chem. Biol. Chem. 9 (2008) 1462–1471.
[19] V. Laurent, A. Fraix, T. Montier, S. Cammas-Marion, C. Ribault, T. Benvegnu, P.A. Jaffrès, P. Loyer, Highly efficient gene transfer into hepatocyte-like HepaRG cells: new means for drug metabolism and toxicity studies, Biotechnol. J. 5 (2010) 314–320.
[20] T. Le Gall, D. Loizeau, E. Picquet, N. Carmoy, J.J. Yaouanc, L. Deschamps, P. Delépine, P. Giamarchi, P.A. Jaffrès, P. Lehn, T. Montier, A novel cationic lipophosphoramidate with di-unsaturated lipid chains: synthesis, physicochemical and transfection activities, J. Med. Chem. 53 (2010) 1496–1508.
[21] R. Auzély-Velty, F. Djedaïni-Pilard, S. Désert, B. Perly, T. Zemb, Micellization of hydrophobically modified cyclodextrins: I-Micellar structure, Langmuir 16 (2000) 3727–3734.
[22] B. Perly, S. Moutard, F. Djedaïni-Pilard, Amphiphilic cyclodextrins from a general concept to properties and applications, Pharma Chem. 1-2 (2005) 4–9.
[23] F.R. Atherton, H.T. Openshaw, A.R. Todd, Studies on phosphorylation. Part II. The reaction of dialkyl phosphites with polyhalogen compounds in presence of bases. A new method for the phosphorylation of amines, J. Chem. Soc. (1945) 660–663.
[24] T. Montier, T. Benvegnu, P.A. Jaffrès, J.J. Yaouanc, P. Lehn, Progress in cationic lipid-mediated gene transfection: a series of bio-inspired lipids as an example, Curr. Gene Ther. 8 (2008) 296–312.
[25] J. Defaye, S. Crouzy, N. Evrard, H. Law, Procédé de Preparation Régiosélectif de Derives Monosulfonylés en C6 des CDs (1999) French Patent WO 9961483.
[26] F. Djedaïni-Pilard, N. Azaroual Bellanger, M. Gosnat, D. Vernet, B. Perly, Potential formation of intramolecular inclusion complexes in peptide-cyclodextrins as evidenced by NMR spectroscopy, J. Chem. Soc. Perkin Trans. 2 (1995) 723–730.
[27] F. Djedaïni-Pilard, J. Désalos, B. Perly, Synthesis of a new molecular carrier: N-(Leu-enkephalin)yl 6-amido-6-deoxy-cyclomaltoheptaose, Tetrahedron Lett. 34 (1993) 2457–2460.
[28] S. Hanessian, A. Benalil, C. Laferrière, The synthesis of functionalised cyclodextrins as scaffolds and templates for molecular diversity, catalysis, and inclusion phenomena, J. Org. Chem. 60 (1995) 4786–4797.
[29] L.D. Melton, K.N. Slessor, Synthesis of monosubstituted cyclohexaamyloses, Carbohydr. Res. 18 (1971) 29–37.
[30] A. Favrelle, V. Bonnet, C. Sarazin, F. Djedaïni-Pilard, Synthesis of polyenyl derivatives of permethylated β-cyclodextrin, Tetrahedron Asymmetry 19 (2008) 2240–2245.
[31] D. Shen, H. Yu, Y. Huang, Densely grafting copolymers of ethyl cellulose through atom transfer radical polymerization, J. Polym. Sci. A1 43 (2005) 4099–4108.
[32] F. Djedaïni-Pilard, B. Perly, M. Roux, Self-assemblies of amphiphilic cyclodextrins, Eur. Biophys. J. 36 (2007) 861–867.
[33] P. Falvey, C.W. Lim, R. Darcy, T. Revermann, U. Karst, M. Giesbers, A.T.M. Marcelis, A. Lazar, A.W. Coleman, D.N. Reinhoudt, B.J. Ravoo, Bilayer vesicles of amphiphilic cyclodextrins: host membranes that recognize guest molecules, Chem. Eur. J. 11 (2005) 1171–1180 and references herein.
[34] B.B. Ghera, F. Perret, Y. Chevalier, H. Parrot-Lopez, Novel nanoparticles made from amphiphilic perfluoroalkyl α-cyclodextrin derivatives: preparation, characterization and application to the transport of acyclovir, Int. J. Pharm. 375 (2009) 155–162.