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Synthesis and characterisation of sulfated amphiphilic α-, β- and γ-cyclodextrins: application to the complexation of acyclovir☆

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Abstract

The synthesis of sulfated amphiphilic α-, β- and γ-cyclodextrins was achieved according to the standard protection–deprotection procedure. The formation of inclusion complexes between the amphiphilic α-, β- and γ-cyclodextrins and an antiviral molecule, acyclovir (ACV) was investigated by UV–visible spectroscopy (UV–Vis) and electrospray ionisation mass spectrometry (ESIMS). UV–Vis spectroscopy allowed determination of the stoichiometry and stability constants of complexes, whereas ESIMS, a soft ionisation technique, allowed the detection of the inclusion complexes. The results showed that the non-sulfated amphiphilic cyclodextrins exhibit a 1:2 stoichiometry with acyclovir, while sulfated amphiphilic cyclodextrins, except γ-cyclodextrin, exhibit a 1:1 stoichiometry indicating the loss of one interaction site. Non-covalent interactions between acyclovir and non-sulfated amphiphilic cyclodextrins appear to take place both in the cavity of the cyclodextrin and inside the hydrophobic zone generated by alkanoyl chains. In contrast, in the case of sulfated amphiphilic cyclodextrins, the interactions appear to involve only the hydrophobic region of the alkanoyl chains.

Keywords: Amphiphilic cyclodextrins; Sulfated cyclodextrins; Acyclovir; Inclusion complexes; UV characterisation; Electrospray ionisation mass spectrometry

1. Introduction

Cyclomaltohexaose, cyclomaltoheptaose, cyclomaltooctaose (α-, β- and γ-cyclodextrins) are cyclic oligosaccharides composed of six, seven and eight D-glucopyranose residues, respectively, linked into a macrocycle by α-(1→4) glycosidic bonds. The cyclodextrins (CDs) are well known for their ability to interact with lipophilic molecules to form non-covalent inclusion (or host–guest) complexes.[2–4] CD complexes are currently used in the pharmaceutical industry to improve aqueous solubility, stability, and bioavailability of drugs. However, the low solubility and the hemolytic activity of β-cyclodextrin prevent its use by a parenteral route.[5,6]

Cyclodextrins can be chemically modified to prevent such drawbacks and to improve their properties. The introduction of sulfate groups onto the hydroxyl groups of cyclodextrins confers higher aqueous solubility and a wide range of biological activities[4] such as anti-inflammatory, anti-lipemic, anti-angiogenic,[7] and antiviral[8–10] properties. Furthermore, this particular class of cyclodextrins does not exhibit hemolytic activity[11] and even protects erythrocytes against hemolysis.[12] Introduction of lipophilic groups at the upper or the lower rims of cyclodextrins generates amphiphilic cyclodextrins.[13–17] Amphiphilic cyclodextrins are of considerable interest for pharmaceutical application in view of their capacity for self-organising in water at physiological pH. Micellar aggregates,[18] vesicles,[19,20] nanospheres,[21] nanocapsules [22] and solid lipid nanoparticles[23] can be prepared from amphiphilic cyclodextrins with the aim of provid-

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ing versatile carrier and delivery systems for drug molecules.

9-[(2-Hydroxyethoxy)-methyl]-guanine (Acyclovir, Fig. 1) is an acyclic synthetic analogue of purine nucleosides with antiviral activity. It is active against *Herpes simplex* virus (HSV1 and HSV2), *Varicella zoster* virus (VZV), *Epstein–Barr* virus (EBV) and *cytomegalovirus* (CMV).[24] Acyclovir inhibits the viral DNA polymerase after phosphorylation by viral thymidine kinase. Acyclovir formulations do not allow suitable drug levels at target sites following oral, local or parenteral administration, due to the low water solubility and low lipid bilayer solubility of the drug.[25]

We aim to combine the inclusion properties of cyclodextrins with the antiviral activity of sulfated cyclodextrins and with the self-organisation ability of amphiphilic cyclodextrins to enhance the biodisponibility of drug molecules. The present paper reports the synthesis and characterisation of sulfated amphiphilic α-, β- and γ-cyclodextrins and the preparation and characterisation of the non-sulfated and sulfated amphiphilic α-, β- and γ-cyclodextrins inclusion complexes with acyclovir. Sulfated amphiphilic cyclodextrin/acyclovir complexes have been investigated in solution by UV–visible spectroscopy (UV–Vis) and by electrospray ionisation mass spectrometry (ESIMS).

2. Results and discussion

2.1. Synthesis of sulfated amphiphilic α-, β- and γ-cyclodextrins

The synthesis of sulfated amphiphilic α-, β- and γ-cyclodextrins was carried out by a sequential approach (Fig. 2) involving total protection of the primary hydroxyl groups with *tert*-butyldimethylsilyl chloride. Acylation at O-2 and O-3 positions was realised with hexanoyl anhydride in the presence of 4-dimethylaminopyridine (DMAP). 6-*O*-*tert*-Butyldimethylsilyl-α-, β- and γ-cyclodextrins were found to be acylated with respectively 12, 14 and 16 hexanoyl chains at the O-2 and O-3 positions, yielding respectively **4**, **5** and **6**. As pointed out by Hölfe and coworkers,[26] and confirmed by Lesieur and coworkers[27] in the cyclodextrin series, acylation performed with hexanoyl chloride in the presence of DMAP may result in over-acylation. Indeed,

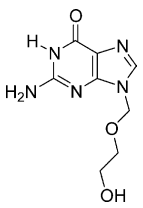

Fig. 1. Molecular structure of acyclovir.

the greater reactivity of hexanoyl chloride compared to hexanoyl anhydride may lead to auto-condensation of hexanoyl chains, leading to β-hexanoylhexanoate double chains. In our case of 6-*O*-persilylated CD derivatives, either hexanoyl substituents or hexanoylhexanoate chains may be introduced at o-2 and o-3 positions resulting in compounds with an average number of 21 hexanoyl substituents as determined by ESIMS for compound **14**.

Deprotection of the *tert*-butyldimethylsilyl groups with boron trifluoride etherate led to **7**, **8**, **9 and 15**.

Sulfation with an excess of sulfur trioxide pyridine complex provided persulfated α-, β- and γ-cyclodextrins on the primary hydroxyl rim **10**, **11**, **12** and **17**. In the β-CD series, use of only 8 equivalents of the sulfation reagent allowed preparation of cyclodextrin derivatives **13** and **16** with an average degree of sulfation of **4**.

2.2. Determination of the stoichiometry of complexes

The continuous variation method was used to determine the stoichiometry of the sulfated and non-sulfated amphiphilic cyclodextrin/acyclovir complexes. The difference of UV–Vis absorption between acyclovir complexed with amphiphilic cyclodextrins and the uncomplexed molecule (ΔA) was measured at 256 nm in water for the sulfated amphiphilic cyclodextrins **10**–**13** and in ethanol for the non-sulfated amphiphilic cyclodextrins **7**–**9**, **15** due to their total insolubility in water. The results are summarised in Table 1. For sulfated amphiphilic CDs, experiments were carried out at different CD concentrations below the critical micellar concentration ($CMC = 10^{-4}$ mol L^{-1}). For the non-sulfated amphiphilic cyclodextrin/acyclovir complexes, the Job plots shows a maximum at a molar ratio of 0.66 indicating 1:2 stoichiometry of the complexes. The Job plots of sulfated amphiphilic cyclodextrin/acyclovir complexes show a maximum at a molar ratio of 0.5 indicating a 1:1 stoichiometry of complexes, with the exception of the complex **12** with acyclovir where the maximum occurs at $r = 0.66$ indicating a 1:2 complex stoichiometry. Sulfation of amphiphilic cyclodextrins, except for the γ-cyclodextrin series, results in the loss of one complexation site. It can be suggested that in case of non-sulfated amphiphilic cyclodextrins, one molecule of acyclovir interacts with the cavity of the cyclodextrin while another molecule of acyclovir interacts with the hydrophobic site generated by the hexanoyl chains. This is consistent with the results of Von Plessing Rossel and coworkers[28] who demonstrated that β-cyclodextrin forms a 1:1 complex with acyclovir and those of Ling and Darcy[29] where 2:1 complexation between sodium anthraquinone-2-sulfonate and 6-*S*-hydroxyethylated 6-thio-γ-cyclodextrins was determined. This indicates that expansion of the hydrophobic cavity of β-cyclodextrin by substitution at the secondary face has led to

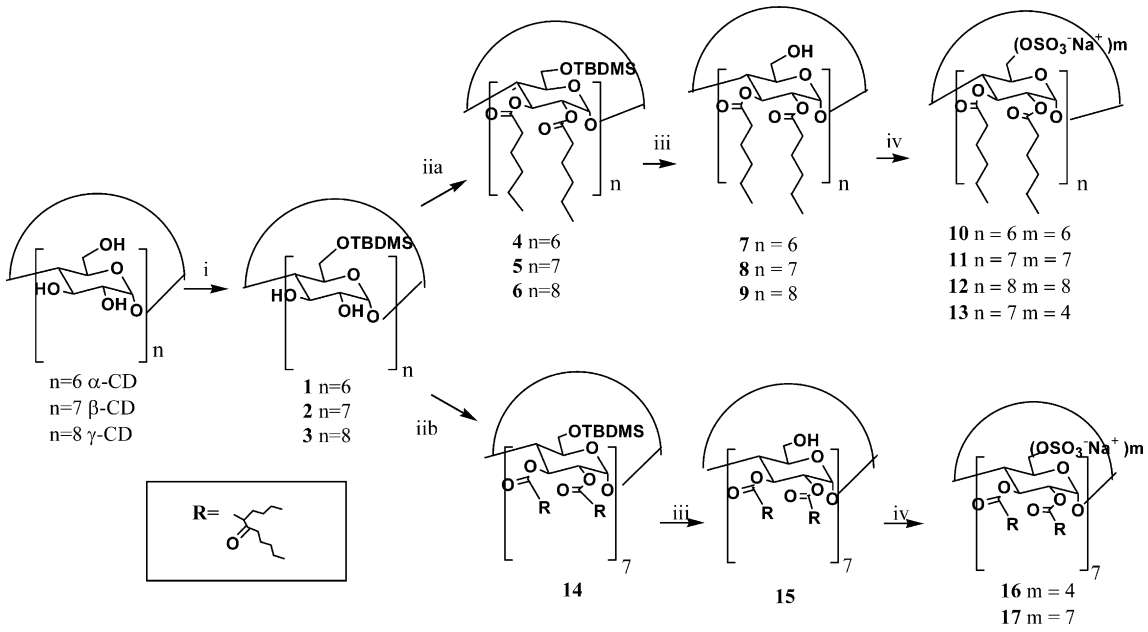

Fig. 2. Synthesis of sulfated and non-sulfated amphiphilic cyclodextrins: (i) TBDMSCl–pyridine; (iia) hexanoyl anhydride–DMAP–pyridine; (iib) hexanoyl chloride–DMAP–pyridine; (iii) $BF_3 \cdot Et_2O$–$CHCl_3$; (iv) $SO_3 \cdot Pyr$–pyridine.

Table 1
Stoichiometry and stability constants of complexes

Complex	Stoichiometry	K_{11} (mol L^{-1})	Solvent
β-CD/ACV[28]	1:1	22	Water
7/ACV	1:2		EtOH
10/ACV	1:1	895	Water
8/ACV	1:2		EtOH
13/ACV	1:1	1647	Water
11/ACV	1:1	1036	Water
15/ACV	1:2		EtOH
16/ACV	1:1		EtOH
17/ACV	1:1		EtOH
9/ACV	1:2		EtOH
12/ACV	1:2		Water

modification of the complexation site of included molecules. The presence of sulfate groups (from 4 to 7) at the primary hydroxyl face of the cyclodextrin may hinder the interaction of acyclovir with the cavity of the cyclodextrins preventing the complexation of a second molecule of acyclovir. In the case of **12**, the larger cavity could increase the distance between sulfate groups at the primary face allowing interactions between the cavity of γ-cyclodextrin derivatives and a second molecule of acyclovir.

2.3. Stability constants of complexes

Absorption intensity of acyclovir at constant concentration was measured in water at room temperature at 256 nm as a function of added **10**, **11** and **13**. Absorption intensity of acyclovir increased with the concentration of the sulfated amphiphilic cyclodextrins before reaching a plateau. To estimate the simple 1:1 equilibrium constant, the double-reciprocal (Benesi–Hildebrand) plot was used (Fig. 3). The Benesi–Hildebrand plots were linear (correlation coefficient = 0.998 for **10**, 0.997 for **11** and 0.994 for **13**). The stability constant is given by the Benesi–Hildebrand equation:

$$\frac{l}{\Delta A} = \frac{1}{[\mathrm{ACV}]_t K_{11} \Delta\varepsilon_{11} [\mathrm{CD}]} + \frac{1}{[\mathrm{ACV}]_t \Delta\varepsilon_{11}}$$

where l is the path length, ΔA the absorbency change, $[\mathrm{ACV}]_t$ the total acyclovir concentration, K_{11} the stability constant,

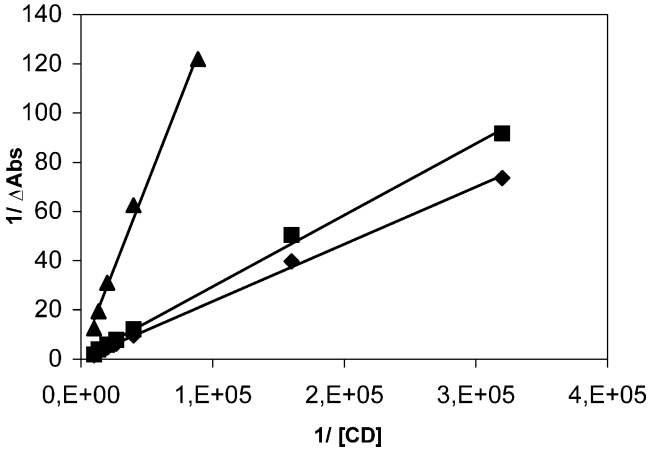

Fig. 3. Double-reciprocal plot for **10** (◆), **13** (▲) and **11** (■). ACV at constant concentration (5×10^{-7} mol L^{-1}) in the absence and in the presence of increasing concentrations of sulfated amphiphilic cyclodextrins (from 3.13×10^{-6} to 10^{-4} mol L^{-1}).

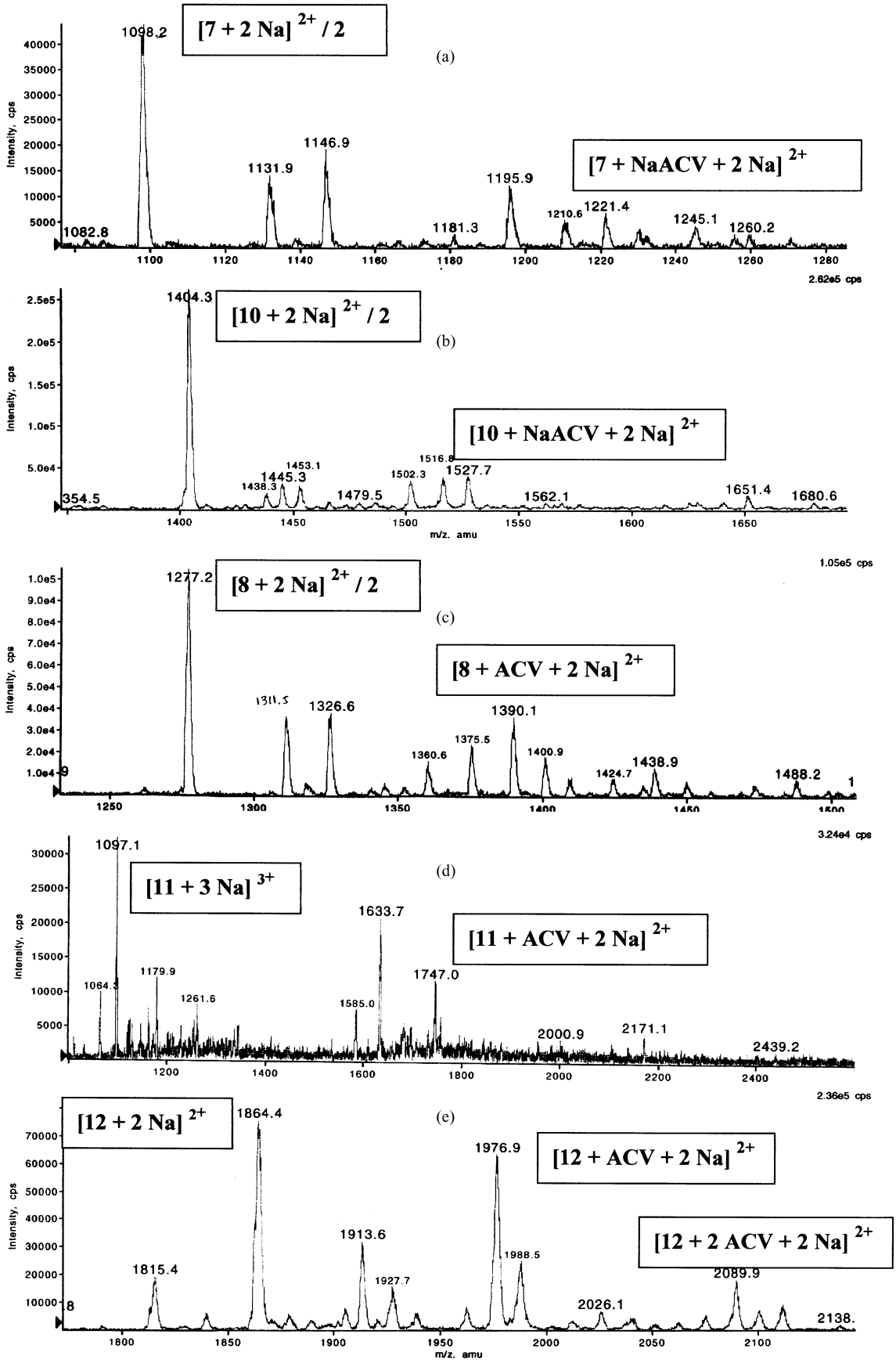

Fig. 4. Electrospray mass spectra for: (a) **7**/ACV complexes; (b) **10**/ACV complexes; (c) **8**/ACV complexes; (d) **11**/ACV complexes; and (e) **12**/ACV complexes.

$\Delta\varepsilon_{11}$ the molar absorptivity change and [CD] the cyclodextrin concentration. The K_{11} values calculated are shown in Table 1 and are 895 mol L^{-1} for **10/acyclovir**, 1036 mol L^{-1} for **11/acyclovir** and 1647 mol L^{-1} for **13/acyclovir**. These values are considerably higher than the value calculated for the native β-cyclodextrin (22 mol L^{-1}).[28] This also indicates a difference in the site of complexation of acyclovir with sulfated amphiphilic cyclodextrins compared to native β-cyclodextrin. The stability constant values are significantly higher for the sulfated amphiphilic β-cyclodextrins series as compared to the sulfated amphiphilic α-cyclodextrin series. Furthermore, the stability constant is higher if the sulfated amphiphilic β-cyclodextrins possess fewer sulfate groups.

2.4. Electrospray ionisation mass spectrometry (ESIMS)

Electrospray ionisation mass spectrometry provides confirmation of the interaction of acyclovir with the hydrophobic cavity of cyclodextrin. With this soft ionisation technique, ions existing in solution can be transferred into gas phase without breaking non-covalent interaction, which are predominant forces in the host–guest interaction.

Electrospray mass spectra are given in Fig. 4. For complexes of non-sulfated amphiphilic cyclodextrins, **7** (Fig. 4(a)) and **8** (Fig. 4(c)), only complexes with acyclovir of 1:1 stoichiometry are observed at *m/z* 1210.6 and 1390.1 corresponding to the $[M+ACV+2\ Na]^{2+}$ cations. For **7**, a peak at *m/z* 1221.4 corresponding to $[M+NaACV+2\ Na]^{2+}$ indicates the complexation of the sodium salt of acyclovir. For complexes of ACV with the sulfated amphiphilic cyclodextrins, **10** (Fig. 4(b)) and **11** (Fig. 4(d)), peaks at *m/z* 1516.8 and 1747.0 corresponding to the $[M+ACV+2\ Na]^{2+}$ cations were observed. A peak at *m/z* 1527.7 indicates the complexation of the sodium salt of acyclovir by the sulfated amphiphilic α-cyclodextrin. Lastly, for the complexes of **12** (Fig. 4(e)), peaks at *m/z* 1976.9 and 2089.9 corresponding to $[M+ACV+2\ Na]^{2+}$ and $[M+2\ ACV+2\ Na]^{2+}$ indicate the presence of complexes of 1:1 and 1:2 stoichiometry in the nebulated phase. The fact that complexes of 1:2 stoichiometry for **7** and **8** are not observed in the nebulated phase suggests that the complexation of the second molecule of acyclovir is weak. Comparing these results with those of the sulfated derivatives and with the stability constant of native β-CD–acyclovir complex, it can reasonably be assumed that the second molecule is complexed weakly into the cavity of the non-sulfated amphiphilic α- and β-cyclodextrins. Furthermore, the presence of the complex of 1:2 stoichiometry with **12** should indicate a stronger interaction of the second molecule of acyclovir with the larger cavity of the γ-CD.

In conclusion, we have prepared sulfated amphiphilic cyclodextrins in the α-, β-, and γ-series. These modified cyclodextrins retain their inclusion properties towards acyclovir, an antiviral drug. These amphiphilic cyclodextrins, nevertheless, have different complexation behaviors compared to the native cyclodextrins. In the case of sulfated amphiphilic α- and β-cyclodextrins, acyclovir appear to interact only with the lipophilic environment generated by the acyl chains grafted onto the lower rim of the cyclodextrin. In the case of non-sulfated cyclodextrins and for sulfated derivatives in the γ-CDs series, two molecules of acyclovir interact with one molecule of modified cyclodextrin which probably indicates inclusion of one molecule of acyclovir within the CD cavity and interaction of the second one with the lipophilic acyl chains. These CDs can self-organise in water as micellar aggregates or nanospheres, which may provide additional opportunity for the drug transport. Experiments are currently undertaken to study the loading capacity of sulfated amphiphilic cyclodextrins nanospheres towards acyclovir.

3. Experimental

3.1. Materials and methods

All chemicals were purchased from Aldrich and were used without further purification. Acyclovir (9-[(2-hydroxyethoxy)-methyl]-guanine] was extracted from Zovirax© (GlaxoWellcome, Evreux, France) by precipitation in water and recrystallisation from MeOH. Purity was checked by ^1H NMR and UV–Vis spectroscopy. α-, β- and γ-Cyclodextrins were generously provided by Wacker (Lyon, France) and were dried under diminished pressure at 120 °C for 48 h before use. Elemental analysis were performed at the Service Central de Microanalyses of CNRS (Lyon, France). ^1H and ^{13}C NMR experiments were performed at 300 and 75 MHz, respectively, using a Bruker DRX300 spectrometer.

3.2. Hexakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-hexanoyl)cyclomaltohexaose (4)

Hexanoic anhydride (34 mL, 0.14 mol, 48 equiv) and DMAP (13.28 g, 0.11 mol, 36 equiv) were added to a stirred solution of **1**[30] (5 g, 3.02 mmol) in dry Py (100 mL). The mixture was heated under N_2 at 70 °C for 2 days then cooled to room temperature (rt) and poured into cold water (300 mL). The organic layer was removed, poured into CH_2Cl_2 (100 mL) and washed with dilute HCl (10%), water and $NaHCO_3$. After drying over Na_2SO_4, the organic layer was concentrated and MeOH was added to the residue. The white precipitate was isolated by filtration and washed with

MeOH. The crude product was purified by recrystallisation in 95:5 MeOH–$CHCl_3$ (2.16 g, 25%); $[\alpha]_D$ −14.5° (*c* 2.20, $CHCl_3$); δ_H ($CDCl_3$, 300 MHz) 5.34 (t, 6 H, *J* 10.1 Hz, H-3), 5.10 (d, 6 H, *J* 3.20 Hz, H-1), 4.68 (dd, 6 H, H-2), 4.14 (d, 6 H, *J* 10.73 Hz, H-6a), 4.03 (t, 6 H, *J* 8.85 Hz, H-4), 3.87 (d, 6 H, H-5), 3.70 (d, 6 H, H-6b), 2.32 (m, 24 H, H-2′), 1.60 (m, 24 H, H-3′), 1.31 (m, 48 H, H-4′, H-5′), 0.90 (s, 90 H, H-6′), 0 (s, 36 H, C*H*$_3$–Si); δ_C ($CDCl_3$, 75 MHz) 173.9 (C-1′), 171.9 (C-1′), 96.5 (C-1), 75.1 (C-4), 72.4 (C-5), 71.6–71.5 (C-2, C-3), 62.4 (C-6), 34.3 (C-2′), 31.8 (C-4′), 26.3 ((CH$_3$)$_3$–*C*), 24.8 (C-3′), 22.8 (*C*-5′), 18.6 ((*C*H$_3$)$_3$–C), 14.3 (C-6′), −4.6 (*C*H$_3$–Si). ESIMS: *m/z* 2858.2 $[M+Na]^+$.

3.3. Heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-hexanoyl)cyclomaltoheptaose (5)

Hexanoic anhydride (122.3 mL, 0.53 mol, 56 equiv) and DMAP (48.4 g, 0.4 mol, 42 equiv) were added to a stirred solution of **2**[30] (18.2 g, 9.4 mmol) in dry Py (250 mL). The mixture was heated under N_2 at 70 °C for 2 days then cooled to rt and poured into cold water (600 mL). The organic layer was removed, poured into CH_2Cl_2 (300 mL) and washed with dilute HCl (10%), water and $NaHCO_3$. After drying over Na_2SO_4, the organic layer was concentrated and MeOH was added to the residue. The white precipitate was isolated by filtration and washed with MeOH. The crude product was purified by recrystallisation in 95:5 MeOH–$CHCl_3$ (19.8 g, 63%); $[\alpha]_D$ +62.5° (*c* 5.12, CH_2Cl_2); δ_H ($CDCl_3$, 300 MHz) 5.34 (dd, 7 H, J_{3-4} 8.3, J_{3-2} 10.1 Hz, H-3), 5.08 (d, 7 H, *J* 3.58 Hz, H-1), 4.64 (dd, 7 H, H-2), 4.03 (m, 7 H, J_{6a-6b} 11.5 Hz, H-6a), 3.81 (m, 14 H, H-4, H-5), 3.65 (m, 7 H, H-6b), 2.35 (m, 28 H, H-2′), 1.52 (m, 28 H, H-3′), 1.26 (m, 56 H, H-4′, H-5′), 0.83 (m, 105 H, H-6′, C*H*$_3$–C), 0.00 (s, 42 H, C*H*$_3$–Si); δ_C ($CDCl_3$, 75 MHz) 173.9 (C-1′), 172.0 (C-1′), 96.8 (C-1), 75.5 (C-4), 72.2 (C-5), 71.5–71.2 (C-2, C-3), 62.3 (C-6), 34.5 (C-2′), 31.8 (C-4′), 26.3 ((CH$_3$)$_3$–*C*), 24.8 (C-3′), 22.8 (C-5′), 18.6 ((*C*H$_3$)$_3$–C), 14.3 (C-6′), −4.5 (*C*H$_3$–Si). ESIMS: *m/z* 1676.6 $[M+2\ Na]^{2+}$.

3.4. Octakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-hexanoyl)cyclomaltooctaose (6)

Hexanoic anhydride (20 mL, 87 mmol, 64 equiv) and DMAP (7.98 g, 65 mmol, 48 equiv) were added to a stirred solution of **3**[31] (3 g, 1.36 mmol) in dry Py (100 mL). The mixture was heated, under N_2 at 70 °C for 2 days then cooled to rt and poured into cold water (300 mL). The organic layer was removed, poured into CH_2Cl_2 (100 mL) and washed with dilute HCl (10%), water and $NaHCO_3$. After drying over Na_2SO_4, the organic layer was concentrated to a residue and MeOH was added to this residue. The crude product was isolated by filtration and washed with MeOH. The white precipitate was purified by recrystallisation in 95:5 MeOH–$CHCl_3$ (1.35 g, 26%); $[\alpha]_D$ −50.8° (*c* 3.4, $CHCl_3$); δ_H ($CDCl_3$, 300 MHz) 5.42 (t, 8 H *J* 9.79 Hz, H-3), 5.24 (d, 8 H, *J* 3.39 Hz, H-1), 4.68 (dd, 8 H, H-2), 4.10 (m, 8 H, H-6), 3.87 (m, 16 H, H-4, H-5), 3.77 (m, 8 H, H-6a), 2.52 (m, 32 H, H-2′), 1.62 (m, 32 H, H-3′), 1.33 (m, 64 H, H-4′, H-5′), 0.90 (s, 120 H, H-6′, C*H*$_3$–C), 0.06 (s, 48 H, CH$_3$–Si); δ_C ($CDCl_3$, 75 MHz) 173.8 (C-1′), 172.1 (C-1′), 96.03 (C-1), 72.33 (C-5), 62.20 (C-6), 34.5 (C-2′), 31.9 (C-4′), 26.3 ((CH$_3$)$_3$–*C*), 24.8 (C-3′), 22.82 (C-5′), 18.69 ((*C*H$_3$)$_3$–C), 14.36 (C-6′), −4.60 (*C*H$_3$–Si). ESIMS: *m/z* 1913.6 $[M+2\ Na]^{2+}$.

3.5. Hexakis(2,3-di-*O*-hexanoyl)cyclomaltohexaose (7)

To a stirred solution of **4** (1.82 g, 0.66 mmol) in $CHCl_3$ (20 mL) was added $BF_3{\cdot}Et_2O$ (1 mL, 7.97 mmol, 12 equiv). The mixture was stirred under N_2 at rt for 12 h, then poured into cold water (100 mL). The organic layer was removed and washed with water, $NaHCO_3$, and water then dried with Na_2SO_4. The organic layer was concentrated to dryness to yield **7** (1.35 g, 96%); $[\alpha]_D$ −15.2° (*c* 2.1, $CHCl_3$); δ_H ($CDCl_3$, 300 MHz) 5.36 (t, 6 H, H-3), 5.08 (d, 6 H, *J* 3.96 Hz, H-1), 5.16 (dd, 6 H, H-2), 4.44 (d, 6 H, H-6a), 4.20 (t, 6 H, H-4), 3.80 (m, 12 H, H-5, H-6b), 2.20 (m, 24 H, H-2′), 1.57 (m, 24 H, H-3′), 1.50 (m, 48 H, H-4′, H-5′), 0.91 (m, 36 H, C*H*$_3$); δ_C ($CDCl_3$, 75 MHz) 173.7 (C-1′), 172.2 (C-1′), 96.5 (C-1), 77.6 (C-4), 72.7 (C-5), 71.0 (C-2, C-3), 61.6 (C-6), 34.3 (C-2′), 31.7 (C-4′), 24.7 (C-3′), 22.7 (C-5′), 14.3 (C-6′). ESIMS: *m/z* 1097.8 $[M+2\ Na]^{2+}$. Anal. Calcd for $C_{108}H_{180}O_{42}{\cdot}H_2O$: C, 59.82; H, 8.46. Found: C, 59.89; H, 8.78.

3.6. Heptakis(2,3-di-*O*-hexanoyl)cyclomaltoheptaose (8)

To a stirred solution of **5** (19.8 g, 6 mmol) in $CHCl_3$ (200 mL) was added $BF_3{\cdot}Et_2O$ (11 mL, 87 mmol, 14 equiv). The mixture was stirred under N_2 at rt for 12 h, then poured into cold water (600 mL). The organic layer was removed and washed with water, $NaHCO_3$, and water then dried with Na_2SO_4. The organic layer was concentrated to dryness to yield **8** (15 g, 99%); $[\alpha]_D$ +90.5° (*c* 4.8, CH_2Cl_2); δ_H ($CDCl_3$, 300 MHz) 5.36 (t, 7 H, *J* 9.1 Hz, H-3), 5.08 (d, 7 H, *J* 3.8 Hz, H-1), 4.78 (dd, 7 H, H-2), 4.03 (m, 14 H, H-6a, H-5), 3.89 (dd, 7 H, $J_{6'-5}$ 5, $J_{6'-6}$ 12 Hz, H-6′), 3.75 (t, 7 H, *J* 9 Hz, H-4), 2.35 (m, 28 H, H-2′), 1.59 (m, 28 H, H-3′), 1.32 (m, 56 H, H-4′, H-5′), 0.91 (m, 45 H, H-6′); δ_C ($CDCl_3$, 75 MHz) 173.7 (C-1′), 172.2 (C-1′), 96.5 (C-1), 77.6 (C-4), 72.7 (C-5), 71.0 (C-2, C-3), 61.6 (C-6), 34.3 (C-2′), 31.7 (C-4′) 24.7 (C-3′), 22.7 (C-5′), 14.3 (C-6′). ESIMS: *m/z* 1277.07 $[M+2\ Na]^{2+}$. Anal. Calcd for $C_{126}H_{210}O_{49}$: C, 60.33; H, 8.38; O, 31.29. Found: C, 60.63; H, 8.50; O, 30.72.

3.7. Octakis(2,3-di-*O*-hexanoyl)cyclomaltooctaose (9)

To a stirred solution of **6** (1.35 g, 0.36 mmol) in $CHCl_3$ (15 mL) was added $BF_3{\cdot}Et_2O$ (0.54 mL, 8.2 mmol, 16 equiv). The mixture was stirred under N_2 at rt for 12 h, then poured into cold water (100 mL). The organic layer was removed and washed with water, $NaHCO_3$ and water then dried with Na_2SO_4. The organic layer was then concentrated to dryness to yield **9** (926 mg, 91%); $[\alpha]_D$ −36.3° (*c* 3.45, $CHCl_3$); δ_H ($CDCl_3$, 300 MHz) 5.40 (t, 8 H, *J* 9.24 Hz, H-3), 5.15 (d, 8 H, $J_{1,2}$ 3.8 Hz, H-1), 4.77 (dd, 8 H, H-2), 3.93 (m, 16 H, H-6a, H-5), 3.76 (dd, 8 H, $J_{6'-5}$ 5, $J_{6'-6}$ 12 Hz, H-6b), 2.43 (t, 8 H, H-4), 2.35 (m, 32 H, H-2′), 1.61 (m, 32 H, H-3′), 1.32 (m, 64 H, H-4′, H-5′), 0.92 (m, H-6′); δ_C ($CDCl_3$, 75 MHz) 173.7 (C-1′), 172.2 (C-1′), 96.5 (C-1), 77.6 (C-4), 72.7 (C-5), 71.0 (C-2, C-3), 61.6 (C-6), 34.3 (C-2′), 31.7 (C-4′), 24.7 (C-3′), 22.7 (C-5′), 14.3 (C-6′). ESIMS: *m/z* 1456.6 $[M+2\ Na]^{2+}$. Anal. Calcd for $C_{144}H_{240}O_{56}$: C, 60.32; H, 8.44. Found: C, 60.73; H, 8.47.

3.8. Hexakis(6-*O*-sulfo-2,3-di-*O*-hexanoyl)cyclomaltohexaose (10)

To a stirred solution of **7** (1.09 g, 0.5 mmol) in dry Py (50 mL) was added the sulfur trioxide pyridine complex (2.42 g, 0.015 mmol, 30 equiv). The mixture was heated at 60 °C for 3 days under N_2 then cooled at rt and concentrated to dryness. The amorphous powder was dissolved in $CHCl_3$ and salts were removed by washing the organic layer with dilute HCl (10%), water and $NaHCO_3$. The organic layer was dried with Na_2SO_4 and concentrated to dryness to yield **10**, 1.28 g (92%); $[\alpha]_D$ −16.6 (*c* 2.1, $CHCl_3$); δ_H (Me_2SO-d_6, 300 MHz) 5.36 (t, 7 H, *J* 9.1 Hz, H-3), 5.08 (d, 7 H, *J* 3.8 Hz, H-1), 4.8 (dd, 7 H, H-2), 4.03 (m, 14 H, H-6a, H-5), 3.8 (dd, 7 H, $J_{6',5}$ 5, $J_{6',6}$ 12 Hz, H-6b), 3.7 (t, 7 H, *J* 9 Hz, H-4), 2.35 (m, 28 H, H-2′), 1.6 (m, 28 H, H-3′), 1.3 (m, 56 H, H-4′, H-5′), 0.9 (m, 45 H, H-6′); δ_C (Me_2SO-d_6, 75 MHz) 173.3 (C-1′), 172.2 (C-1′), 96.6 (C-1), 80.0 (C-4), 75.5 (C-5), 71.3 (C-2, C-3), 65.3 (C-6), 34.2 (C-2′), 31.7 (C-4′), 24.7 (C-3′), 22.7 (C-5′), 14.5 (C-6′). ESIMS: *m/z* 1404.3 $[M+2\ Na]^{2+}$. Anal.Calcd for $C_{108}H_{174}Na_6O_{60}S_6{\cdot}5\ H_2O$: C, 45.47; H, 6.50; Na, 4.84; S, 6.74. Found: C, 45.48; H, 6.60; Na, 4.67; S, 6.5.

3.9. Heptakis(6-*O*-sulfo-2,3-di-*O*-hexanoyl)cyclomaltoheptaose (11)

To a stirred solution of **8** (5 g, 2 mmol) in dry Py (250 mL) was added the sulfur trioxide pyridine complex (11.11 g, 70 mmol, 35 equiv). The mixture was heated at 60 °C for 3 days under N_2 then cooled at rt and concentrated to dryness. The amorphous powder was dissolved in $CHCl_3$ and salts were removed by washing the organic layer with dilute HCl (10%), water and $NaHCO_3$. The organic layer was dried with Na_2SO_4 and concentrated to dryness, (5 g, 90%); $[\alpha]_D$ +112.8° (*c* 3.28, CH_2Cl_2); δ_H (Me_2SO-d_6, 500 MHz) 5.32 (t, 7 H, *J* 9.46 Hz, H-3), 5.12 (d, 7 H, *J* 2.6 Hz, H-1), 4.64 (dd, 7 H, H-2), 4.21 (dd, 7 H, H-6a), 4.08 (dd, 7 H, *J* 9.46 Hz, H-5), 4.00 (d, 7 H, H-6b), 3.85 (d, 7 H, *J* 8.83 Hz, H-4), 2.37 (m, 14 H, H-2′), 2.17 (m, 14 H, H-2′), 1.51 (m, 28 H, H-3′), 1.26 (m, 56 H, H-4′, H-5′), 0.86 (m, 45 H, H-6′); δ_C (Me_2SO-d_6, 125 MHz) 173.3 (C-1′), 172.2 (C-1′), 96.6 (C-1), 80.1 (C-4), 75.5 (C-5), 71.3 (C-2, C-3), 65.4 (C-6), 34.2 (C-2′), 31.7 (C-4′), 24.7 (C-3′), 22.7 (C-5′), 14.5 (C-6′). ESIMS: *m/z* 1634.07 $[M+2\ Na]^{2+}$, 1097.22 $[M+3\ Na]^{3+}$. Anal. Calcd for $C_{126}H_{203}Na_7O_{70}S_7{\cdot}10\ H_2O$: C, 44.47; H, 6.56; Na, 4.73; S, 6.58. Found: C, 44.87; H, 6.70; Na, 4.15; S, 6.3.

3.10. Octakis(6-*O*-sulfo-2,3-di-*O*-hexanoyl)cyclomaltooctaose (12)

To a stirred solution of **9** (736 mg, 0.3 mmol) in dry Py (50 mL) was added the sulfur trioxide pyridine complex (1.62 g, 10 mmol, 40 equiv). The mixture was heated at 60 °C for 3 days under N_2 then cooled at rt and concentrated to dryness. The amorphous powder was dissolved in $CHCl_3$ and salts were removed by washing the organic layer with dilute HCl (10%), water and $NaHCO_3$. The organic layer was dried with Na_2SO_4 and concentrated to dryness to yield **12**, 841 mg (93%) as an amorphous powder; $[\alpha]_D$ −24.18° (*c* 3.35, $CHCl_3$); δ_H (Me_2SO-d_6, 300 MHz) 5.29 (t, 8 H, H-3), 5.14 (d, 8 H, H-1), 4.67 (dd, 8 H, H-2), 4.10 (m, 16 H, H-6a, H-5), 3.96 (dd, 8 H, H-6b), 3.81 (t, 8 H, H-4), 2.35 (m, 32 H, H-2′), 1.51 (m, 32 H, H-3′), 1.25 (m, 64 H, (H-4′, H-5′), 0.85 (m, H-6′); δ_C (Me_2SO-d_6, 75 MHz) 173.0 (C-1′), 172.4 (C-1′), 95.9 (C-1), 79.8 (C-4), 73.2 (C-5), 71.4 (C-2, C-3), 65.3 (C-6), 34.1 (C-2′), 31.7 (C-4′), 24.7 (C-3′), 22.7 (C-5′), 14.4 (C-6′). ESIMS: *m/z* 1864.4 $[M+2\ Na]^{2+}$, 1250.5 $[M+3\ Na]^{3+}$. Anal. Calcd for $C_{144}H_{232}Na_8O_{80}S_8{\cdot}15\ H_2O$: C, 43.77; H, 6.68; Na, 4.65; S, 6.49. Found: C, 43.93; H, 6.55; Na, 4.82; S, 6.30.

3.11. Heptakis(2,3-di-O-hexanoyl)-tetrakis(6-O-sulfo)cyclomaltoheptaose (13)

To a stirred solution of **8** (5 g, 2 mmol) in dry Py (250 mL) was added the sulfur trioxide pyridine complex (2.55 g, 16 mmol, 8 equiv). The mixture was heated at 60 °C for 3 days under N_2 then cooled at rt and concentrated to dryness. The procedure can be carried out in a similar manner to that previously described for **11**, 92% yield (5.3 g); $[\alpha]_D$ +152.9° (*c* 1.7, CH_2Cl_2); δ_H (Me_2SO-d_6, 500 MHz) 5.29 (m, 7 H, H-3), 5.10 (d, 7 H, H-1), 4.80–4.57 (m, 7 H, H-2), 4.27–3.94 (m, 14 H, H-6a, H-5), 3.84 (dd, 7 H, H-4), 2.34 (m, 14 H, H-2′), 2.18 (m, 14 H, H-2′), 1.51 (m, 28 H, H-3′), 1.27 (m, 56 H, H-4′, H-5′), 0.86 (m, 45 H, H-6′); δ_C (Me_2SO-d_6, 125 MHz)

173.3 (C-1′), 172.2 (C-1′), 96.6 (C-1), 84.4 (C-4), 75.4 (C-5, C-2, C-3), 65.3 (C-6), 34.2 (C-2′), 31.6 (C-4′), 24.7 (C-3′), 22.8 (C-5′), 14.5 (C-6′). ESIMS: *m/z* 1481.23 [M+2 Na]$^{2+}$. Anal. Calcd for $C_{126}H_{206}Na_4O_{61}S_4 \cdot 5\ H_2O$: C, 50.33; H, 6.85; Na, 3.06; S, 4.26. Found: C, 49.98; H, 6.95; Na, 3.82; S, 4.52.

3.12. Synthesis of over-acylated β-cyclodextrin derivatives

3.12.1. Per 6-*O*-silylated compound 14. Conditions used for the preparation of **5** were followed but with hexanoyl chloride (16.2 mL, 0.11 mol, 28 equiv), DMAP (21.3 g, 0.17 mol, 42 equiv) and **2**[29] (8.1 g, 4.1 mmol) in dry Py (200 mL) at 60 °C for 3 days. The crude product was purified by recrystallisation in 95:5 MeOH–$CHCl_3$ (9 g, 65%); $[\alpha]_D$ +62.5° (*c* 5.12, CH_2Cl_2); δ_H ($CDCl_3$, 300 MHz) 5.32 (m, 7 H, H-3), 5.08 (m, 7 H, H-1), 4.51 (m, 7 H, H-2), 3.67 (m, 28 H, H-5, H-6a, H-6b, H-4), 3.51 (m, –CH–), 2.49–2.37 (m, H-2′), 1.77 (m, –H-4′), 1.65 (m, H-3′), 1.28 (m, H-5′), 0.84 (m, –H-6′), (s, $CH_3)_3$–C), 0.00 (s, CH_3–Si–); δ_C ($CDCl_3$, 75 MHz) 206.8 (C-1′), 178.0 (C-1′), 173.7 (C-1′), 168.0 (C-1′), 96.5 (C-1), 75.5 (C-4), 72.2 (C-5), 71.5–71.2 (C-2, C-3), 64.1 (C-6), 33.9 (C-2′), 31.6 (C-4′), 29.9 (C-2′), 28.4 (C-2′), 26.3 ($(CH_3)_3$–C), 24.8 (C-3′), 24.2 (–C-5′), 23.1 (C-5′), 18.6 ($(CH_3)_3$–C), 14.3 (C-6′), −4.6 (CH_3–Si). ESIMS: *m/z* 1972.0 [M with 20 chains+2 Na]$^{2+}$, 2020.5 [M with 21 chains+2 Na]$^{2+}$, 2069.5 [M with 22 chains+2 Na]$^{2+}$.

3.12.2. Per-6-*O*-unprotected compound 15. To a stirred solution of **14** (9 g, 2.7 mmol) in $CHCl_3$ (200 mL) was added $BF_3 \cdot Et_2O$ (4.9 mL, 38 mmol, 14 equiv). The mixture was stirred under N_2 at rt for 12 h, then poured into cold water (600 mL). The organic layer was removed and washed with water, $NaHCO_3$, and water then dried with Na_2SO_4. The organic layer was concentrated to dryness (7.3 g, 85% yield); $[\alpha]_D$ +80.5° (*c* 5.34, CH_2Cl_2); δ_H ($CDCl_3$, 300 MHz) 5.13 (m, 7 H, H-3), 4.89 (m, 7 H, H-1), 4.55 (m, 7 H, H-2), 3.78 (m, 21 H, H-5, H-6a, H-6b), 3.49 (H-4), 2.22 (m, H-2′), 1.64 (m, H-4′), 1.43 (m, H-3′), 1.13 (m, H-5′), 0.70 (H-6′). ESIMS: *m/z* 1568.54 81 [M with 20 chains+2 Na]$^{2+}$, 1617.67 [M with 21 chains+2 Na]$^{2+}$, 1666.41 [M with 22 chains+2 Na]$^{2+}$. Anal. Calcd for $C_{168}H_{280}O_{56} \cdot 5\ H_2O$: C, 61.42; H, 8.53. Found: C, 61.52; H, 8.68.

3.12.3. Tetra-6-*O*-sulfated compound 16. Conditions used for the preparation of **11** were followed but with **15** (5 g, 2 mmol), and the sulfur trioxide pyridine complex (2.55 g, 16 mmol, 8 equiv) in dry Py (250 mL) at 60 °C for 3 days (2.6 g, 76% yield); $[\alpha]_D$ +108.8° (*c* 4.96, CH_2Cl_2); δ_H (Me_2SO-d_6, 300 MHz) 5.33 (m, 7 H, H-3), 5.10 (m, 7 H, H-1), 4.64 (m, 7 H, H-2), 3.85 (m, 21 H, H-5, H6a, H6b), 3.58 (H-4), 2.50 (m, H-2′), 1.73 (m, H-4′), 1.54 (m, H-3′), 1.26 (m, H-5′), 0.85 (m, H-6′); δ_C (Me_2SO-d_6, 75 MHz) 206.3 (C-1′), 173.0 (C-1′), 172.2 (C-1′), 168.2 (C-1′), 96.2 (C-1), 75.6 (C-4), 72.7 (C-5), 70.9–69.9 (C-2, C-3), 65.4 (C-6), 34.1 (C-2′), 31.5 (C-4′), 29.9 (C-2′), 28.2 (C-2′), 24.8 (C-3′), 24.2 (C-5′), 22.7 (C-5′), 14.5 (C-6′). ESIMS (−): *m/z* 1778.35 [M (4 sulfates)-2 Na]$^{2-}$. Anal. Calcd for $C_{168}H_{276}Na_4O_{68}S_4 \cdot 3\ H_2O$: C, 55.17; H, 7.55; Na, 2.52; S, 3.50. Found: C, 55.33; H, 7.76; Na, 2.59; S, 3.40.

3.12.4. Per-6-*O*-sulfated compound 17. Similar experiments as for **11** carried out with **15** (5 g, 2 mmol), and the sulfur trioxide pyridine complex (11.11 g, 70 mmol, 35 equiv) in dry Py (250 mL) at 60 °C for 3 days afforded **17** (3.3 g, 94%); $[\alpha]_D$ +91.5° (*c* 4.48, CH_2Cl_2); δ_H (Me_2SO-d_6, 300 MHz) 5.33 (m, 7 H, H-3), 5.07 (m, 7 H, H-1), 4.59 (m, 7 H, H-2), 4.05 (m, 21 H, H-5, H-6a, H-6b), 3.57 (H-4), 2.50 (m, H-2′), 1.73 (m, H-4′), 1.43 (m, H-3′), 1.24 (m, H-5′), 0.85 (m, H-6′); δ_C (Me_2SO-d_6, 75 MHz) 206.3 (C-1′), 173.0 (C-1′), 172.2 (C-1′), 168.2 (C-1′), 95.9 (C-1), 74.7 (C-4), 73.8.7 (C-5), 71.0–69.8 (C-2, C-3), 65.5 (C-6), 35.2 (C-2′), 31.5 (C-4′), 29.5 (C-2′), 28.3 (C-2′), 27.0 (C-3′), 24.5 (C-5′), 22.9 (C-5′), 14.5 (C-6′). ESIMS (−): *m/z* 1280.17 [M−3 Na]$^{3-}$. Anal. Calcd for $C_{168}H_{273}Na_7O_{77}S_7 \cdot 10\ H_2O$: C, 49.33; H, 6.68; Na, 3.94; S, 5.48. Found: C, 49.68; H, 6.94; Na, 3.92; S, 5.21.

3.13. Determination of the stoichiometry of acyclovir/sulfated amphiphilic cyclodextrin complexes

The continuous variation method was adopted to determine the stoichiometry of the complexes. The total concentration of the two species, acyclovir and amphiphilic cyclodextrin, was kept constant (7.5×10^{-5} mol L^{-1}), and the mole ratio (*r*) was varied from 0 to 1. The differences of absorption intensity of acyclovir in absence and in presence of amphiphilic cyclodextrins were measured for a given mole ratio by a UV–Vis spectrophotometer (Shimadzu UV-2401 PC spectrophotometer).

3.14. Determination of the stability constant of complexes

The absorption of acyclovir was measured at 256 nm at constant concentration (5.0×10^{-7} M) in absence and in presence of increasing concentrations of amphiphilic cyclodextrins (from 3.13×10^{-6} to 10^{-4} M) in water, at rt. Alternatively, complex formation was investigated by mass spectrometry. For these purpose, ESIMS was carried out in the positive mode on a Applied biosystems API 165 spectrometer. The complexes were prepared in 1:1:0.1% MeOH–water–HCOOH by dissolving acyclovir and the corresponding amphiphilic cyclodextrin at a molar ratio of 5:1. The mass spectrometer was operated at 5 kV. The samples were introduced into the ion

source by an infusion pump operating with a flow rate of 5 μL min^{-1}.

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References

1. Dubes, A.; Bouchu, D.; Lamartine, R.; Parrot-Lopez, H. *Tetrahedron Lett.* **2001**, *42*, 9147–9151.
2. Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 803–822.
3. Szejtli, J. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L.; Davies, J. E. D.; MacNicol, D. D.; Vögtle, F.; Lehn, J.-M., Eds.; Vol. 3; Pergamon: Oxford, 1996.
4. Uekama, K.; Hirayama, F.; Irie, T. *Chem. Rev.* **1998**, *98*, 2045–2076.
5. Bar, R.; Olitzer, S. *Appl. Microbiol. Biotechnol.* **1994**, *41*, 574–577.
6. Irie, T.; Uekama, K. *J. Pharm. Sci.* **1997**, *86*, 147–162.
7. Folkman, J.; Weisz, P. B.; Joullie, M. M.; Li, W. W.; Ewing, W. R. *Science* **1989**, *243*, 1490–1493.
8. Leydet, A.; Moullet, C.; Roque, J. P.; Witvrouw, M.; Pannecouque, C.; Andrei, G.; Snoeck, R.; Neyts, J.; Schols, D.; De Clercq, E. *J. Med. Chem.* **1998**, *41*, 4927–4932.
9. Schols, D.; De Clercq, E.; Witvrouw, H.; Nakashima, H.; Snoeck, R.; Pauwels, R.; Van Schepdael, A.; Claes, P. *Antiviral Chem. Chemother.* **1991**, *2*, 45–53.
10. Witvrouw, M.; Desmyter, J.; De Clercq, E. *Antiviral Chem. Chemother.* **1994**, *5*, 345–359.
11. Shiotani, K.; Uehata, K.; Irie, T.; Uekama, K.; Thompson, D. O.; Stella, V. J. *Pharm. Res.* **1995**, *12*, 78–84.
12. Weisz, P. B.; Kumor, K.; Macarak, E. J. *Biochem. Pharmacol.* **1993**, *45*, 1011–1016.
13. Lin, J.; Creminon, C.; Perly, B.; Djedaini-Pilard, F. *J. Chem. Soc. Perkin Trans. 2* **1998**, 2639–2646.
14. Gèze, A.; Aous, S.; Baussanne, I.; Putaux, J.-L.; Defaye, J.; Wouessidjewe, D. *Int. J. Pharm.* **2002**, 301–305.
15. Zhang, P.; Ling, C-C; Coleman, A. W.; Parrot-Lopez, H.; Galons, H. *Tetrahedron Lett.* **1991**, *32*, 2769–2770.
16. Zhang, P.; Parrot-Lopez, H.; Tchoreloff, P.; Baszkin, A.; Ling, C. C.; De Rango, C.; Coleman, A. W. *J. Phys. Org. Chem.* **1992**, *5*, 518–528.
17. Mazzaglia, A.; Donohue, R.; Ravoo, B. J.; Darcy, R. *Eur. J. Org. Chem.* **2001**, 1715–1721.
18. Auzely-Velty, R.; Djedaïni-Pilard, F.; Desert, S.; Perly, B.; Zemb, T. *Langmuir* **2000**, *16*, 3727–3734.
19. Ravoo, B. J.; Darcy, R. *Angew. Chem., Int. Ed.* **2000**, *39*, 4324–4326.
20. Sukegawa, T.; Furuike, T.; Niikura, K.; Yamagishi, A.; Monde, K.; Nishimura, S.-I. *Chem. Commun.* **2002**, 430–431.
21. Perrier, E.; Terry, N.; Rival, D.; Coleman, A. W. *Jpn. Kokai Tokkyo Koho* **2001**, Jp 2001323002; *Chem. Abstr.,* **2001**, *135*, 376708u.
22. Skiba, M.; Wouessidjewe, D.; Fessi, H.; Devissaguet, J.-P.; Duchêne, D.; Puisieux, F. *PCT Applic. Fr*. 93/00593; **1993**; *Chem. Abstr.,* **1994**, *120*, 144164b.
23. Dubes, A.; Parrot-Lopez, H.; Shahgaldian, P.; Coleman, A. W. *J. Colloid Interface Sci.* **2003**, *259*, 103–111.
24. Laskin, O. L.; Langsteth, J. A.; Sral, R.; Demiranda, P.; Keeney, R.; Lietman, P. S. *Antimicrob. Agents Chemother.* **1982**, *21*, 393–398.
25. Fresta, M.; Panico, A. M.; Bucolo, C.; Giannavola, C.; Puglisi, G. *J. Pharm. Pharmacol.* **1999**, *51*, 565–576.
26. Hölfe, G.; Steglich, W.; Vordrüggen, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 569–583.
27. Lesieur, S.; Charon, D.; Lesieur, P.; Ringard-Lefebvre, C.; Muguet, V.; Duchêne, D.; Wouessidjewe, D. L. *Chem. Phys. Lipids* **2000**, *106*, 127–144.
28. Von Plessing Rossel, C.; Sepulveda Carreno, J.; Rodriguez-Baeza, M.; Alderete, J. B. *Quimica Nova* **2000**, *23*, 749–752; *Chem. Abstr.,* **2001**, 170610x.
29. Ling, C.-C.; Darcy, R. *J. Chem. Soc. Chem. Commun.* **1993**, 203–205.
30. Takeo, K.; Uemura, K.; Mitoh, H. *J. Carbohydr. Chem.* **1988**, *7*, 293–308.
31. Fugedi, P. *Carbohydr. Res.* **1989**, *192*, 366–369.