Poly-6-cationic amphiphilic cyclodextrins designed for gene delivery

Colin Byrne, Florence Sallas, Dilip K. Rai, Julien Ogier and Raphael Darcy*

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A new series of amphiphilic cyclodextrins containing cationic groups at the 6-positions and alkyl or biolabile ester groups at the 2-positions has been synthesised. Selective 2-*O*-allylation followed by photochemical addition of lipophilic thiols made it possible to control lipophilicity in these mesomolecules and allow solubility and self-assembly in water. The cationic groups are cysteamine-derived, while the alkyl and ester groups are C_1–C_{16} and benzyl ester groups. This is a new general synthetic route to a potentially wide range of polycationic cyclodextrins capable of acting as gene delivery vectors by condensing DNA and forming liquid crystalline complexes with oligonucleotides.

Introduction

Cyclodextrins are cyclic oligosaccharides which are well known for their ability to form molecular complexes in water.[1] They are also unique as readily available monodisperse oligosaccharides which have structures with multiple equivalent sites for modification. This has led to their conversion to mesomolecular amphiphiles in which multiple polar groups on one side of the macrocycle are balanced by lipophilic groups on the other side. The amphiphilic designs enable formation of diverse supramolecular assemblies with molecular recognition properties.[2]

We have assessed polycationic cyclodextrins (CDs) as condensers and delivery vectors for DNA in cell transfection, and have shown that similar but amphiphilic polycationic cyclodextrins are superior to the non-amphiphilic CDs as vectors.[3,4] Since then, other examples have been synthesised as vectors including both cationic[5,6] and cationic amphiphilic CDs.[7] Also, CDs have been conjugated to the polymeric vector polyethylene imine[8] and incorporated into other cationic polymers,[9] dendrimers[10] and polyrotaxanes[11] for gene delivery. Incorporation of cyclodextrins has been shown to improve targeting through their inclusion of adamantyl, where adamantyl is conjugated with oligonucleotide[12] or polyethylenglycol (PEG),[13] and to reduce toxicity in certain cases. These cyclic oligosaccharides are therefore now well established as versatile core molecules in the efforts by chemists to create successful non-viral gene vectors.

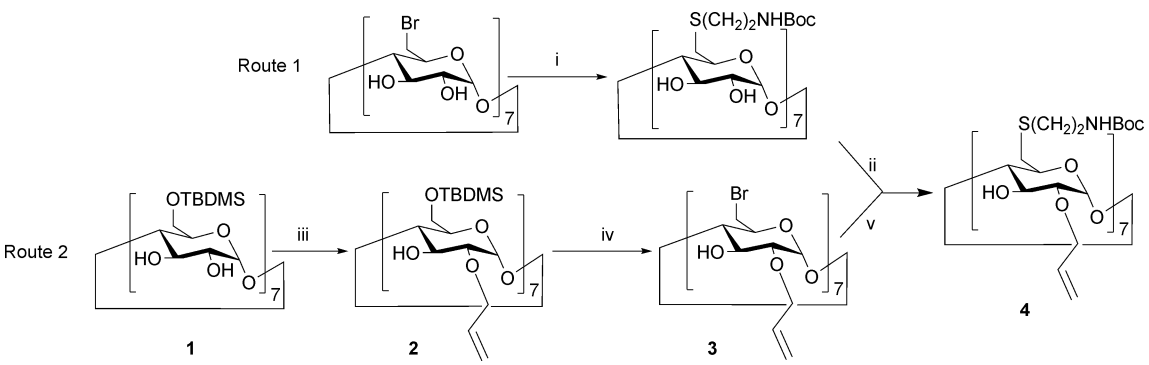
Here we describe the synthesis of a new series of cationic amphiphilic cyclodextrins containing biolabile ester groups, as well as their non-ester analogues, that have been designed for gene delivery purposes. In this series, the potentially cationic amino groups are attached at the 6-positions of the glucosyl units, while the lipophilic groups, including those containing the biolabile ester links, are at the 2-positions. The relative placing of multiple groups, namely polar groups on the primary-OH side and lipophilic chains on the secondary-OH side, is therefore inverted compared with previous examples of cyclodextrin vectors designed by us, and provides new possibilities for design of more efficient vectors.

Results and discussion

Synthesis of the polycationic amphiphiles involved a choice, as already described for amphiphilic cyclodextrins in general,[2] of which groups to introduce first – the polar or the lipophilic. Here (Scheme 1, Route 1) we could either use the 6-deoxy-6-brominated intermediate for introduction first of the polar groups on the primary hydroxyl side, or protect these positions (Route 2) and introduce first the allyl groups (which will be extended to form the lipophilic groups) on the secondary side.

Starting from the 6-perbromo-β-cyclodextrin, bromide was replaced with thiolate anion of Boc-protected cysteamine. Allylation

Centre for Synthesis and Chemical Biology, School of Chemistry and Chemical Biology, Ollscoil na hEireann, University College Dublin, Belfield, Dublin 4, Ireland. E-mail: raphael.darcy@ucd.ie



Scheme 1 *Reagents and conditions*: Route 1: (i) *t*BuOK, $BocNH(CH_2)_2SH$, DMF, 80 °C, 3 d; (ii) NaH, allyl bromide, r.t., 24 h. Route 2: (iii) NaH, allyl bromide, r.t., 24 h; (iv) PPh_3, Br_2, CH_2Cl_2, r.t., 24 h; (v) NaH, $BocNH(CH_2)_2SH$, DMF, r.t., 16 h.

of the 2-OH groups[14] then gave **4** as an intermediate to which the lipophilic groups could be attached photochemically (see below). However, this route gave a low overall yield for compound **4**.

The second route was therefore preferred, starting from the 6-OH-protected heptakis (6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (**1**).[15,16] Its 2-*O*-allylation[14] formed **2**, in which the unsaturated chains made possible photochemical addition of thiols. Before that addition, the TBDMS groups were replaced by bromines[17,18] and these by Boc-protected cysteamine groups. The method adopted for the synthesis of heptakis [2-*O*-allyl-6-(*N*-Boc-2-aminoethylthio)-6-deoxy]-β-cyclodextrin (**4**) was adapted from one described by Leon-Ruaud and Plusquellec[19] for the synthesis of 6-alkylthio-6-deoxy glycosides. A considerable excess of thiol anion (up to five equivalents) is often used here, but in our case, with the use of two equivalents and a reaction time of 16 hours, the reaction was high-yielding.

Persubstitution was confirmed by ^{1}H NMR (Fig. 1). The protons corresponding to the methylenes of the cysteamine moiety were visible as two broad singlets at 2.73 and 3.30 ppm respectively. Also visible was the singlet of the *tert*-butyl group at 1.43 ppm. This integrated for 63 protons compared with the *H-3*,*H-5* signal of the CD, indicating that full substitution had occurred. In the ^{13}C NMR (Fig. 2) the Boc carbonyl carbon *C-12*, along with the methyl carbons *C-14* showed at 156.0 and 28.7 ppm respectively. The quaternary *tert*-butyl carbon *C-13* was not distinctly visible since it overlapped with *C-2* of the CD, while *C-6* of the CD and *C-10* had the same shift of 33.7 ppm.

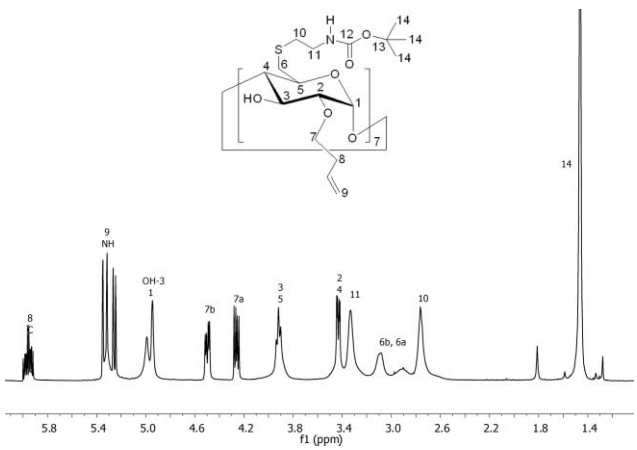


Fig. 1 ^{1}H NMR of cyclodextrin **4** in $CDCl_3$.

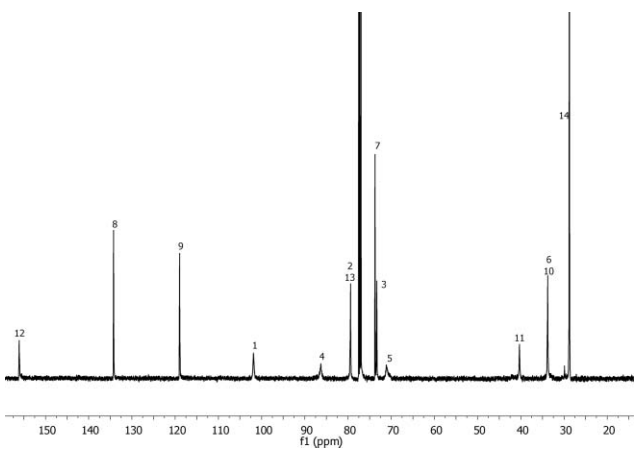


Fig. 2 ^{13}C NMR of cyclodextrin **4** in $CDCl_3$.

Ester groups or disulfide groups in synthetic gene vectors may be expected to facilitate release of DNA within the acidic environment of the endosome and the reductive environment of the cytoplasm after cell-uptake. Disulfide links have been incorporated into gene vectors[20] and into vector·DNA nanoparticles.[21] Amphiphilic CDs having disulfide linkages have been synthesised,[22] though these were charge-neutral and not capable of condensing DNA. Small cationic lipids for gene delivery such as DOTAP,[23] which contains ester groups, their analogues based on carnitine,[24] as well as orthoester lipids,[25] potentially exploit the acidic environment of endosomes with its esterases. Polymeric ester vectors have also been applied and shown to have reduced cell toxicity.[26] A series of DOTAP analogues have been synthesised that incorporate additional benzyl ester groups within the lipophilic tails.[27]

Previously synthesised amphiphilic CD gene vectors were based on alkyl chains of varying length[4] that lacked labile groups. We were interested here to see if the incorporation of biolabile esters in this new series of gene vectors would improve the ability to transfect plasmid DNA to the cell.

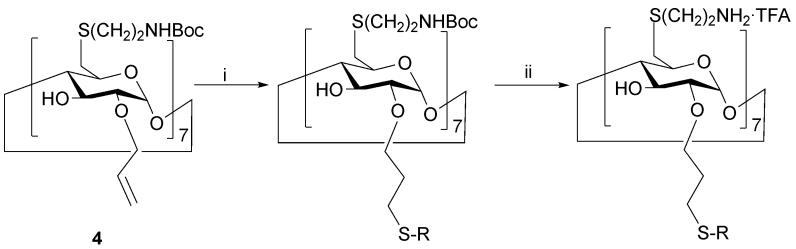
For ester group incorporation, we chose the thiol-to-allyl photoaddition reaction described by Fulton and Stoddart.[14] β-Mercaptopropionic acid esters (compounds **5–9** as described in the Experimental section) were added to CD **4** photochemically (Scheme 2). To reduce disulfide formation, the optimum concentration of 5 mM CD in methanol was used, with additions of toluene in some cases to maintain solubilisation throughout the reaction.

^{1}H NMR was the most effective method for determining whether the reaction had reached completion. Typically, an aliquot (1 mL) of the reaction solution was concentrated and used for ^{1}H NMR analysis in $CDCl_3$. Complete disappearance of the multiplet corresponding to allylic protons of CD **4** (5.97–5.89 ppm) together with the appearance of a multiplet at 1.89–1.83 ppm corresponding to the formation of a new CH_2 group confirmed that the reaction had reached completion; in the purified product the ^{13}C NMR spectrum showed the shift of *C-8* and *C-9* of the allyl ether in CD **4** from 134.2 and 118.9, to 29.7 and 28.5 ppm.

In order to assess the effectiveness of the bio-labile ester groups incorporated into CDs **10–15** in gene delivery, cyclodextrin vectors **25–27** lacking the ester moiety were prepared for comparison. Similar conditions to those described for synthesis of the CD ester derivatives (**10–15**) were used for their preparation (Scheme 2).

When introduction of the 2-*O*-lipophilic groups was finally completed by the above photochemical addition to allyl, the 6-cysteamine groups were deprotected. Removal of the Boc protecting groups from compounds **10–18** left the trifluoroacetate salts **19–27**. The Boc carbonyl (150.0 ppm), quaternary carbon (79.3 ppm) and *tert*-butyl (28.6 ppm) carbons were now absent, and for the TFA salts two sets of signals were visible as quartets at 163.1 ppm (F_3CC=O) and 118.2 ppm (CF_3) (Fig. 3, 4).

ESI-MS used to characterise these compounds showed predominantly doubly charged species of the analytes. An example of an exact mass measurement mass spectrum is shown in



Scheme 2 *Reagents and conditions*: (i) R–SH (propionate esters **5–9**), AIBN, MeOH; (ii) CH_2Cl_2, TFA, r.t. * Converted to hepta-hydrochloride.

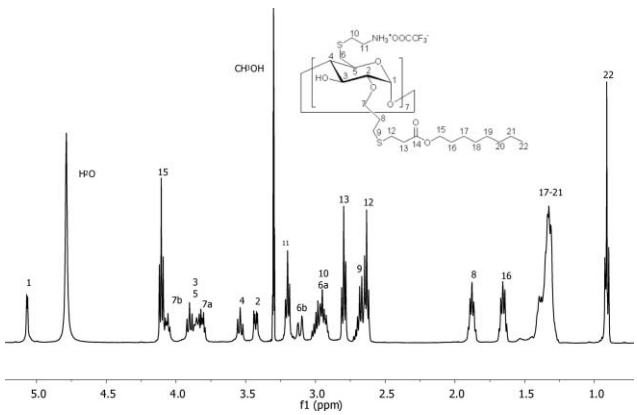


Fig. 3 ^{1}H NMR of **21** in CD_3OD.

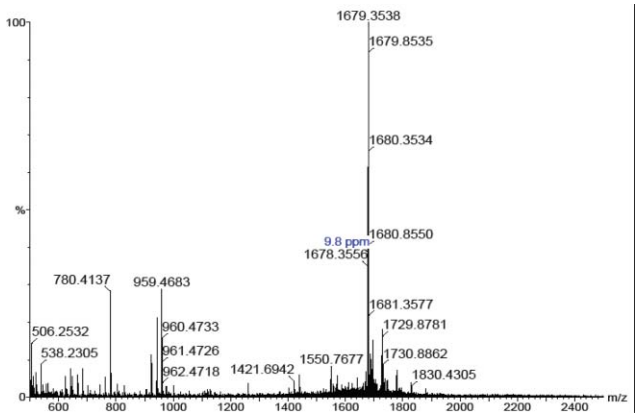


Fig. 5 ESI mass spectrum of **21**.

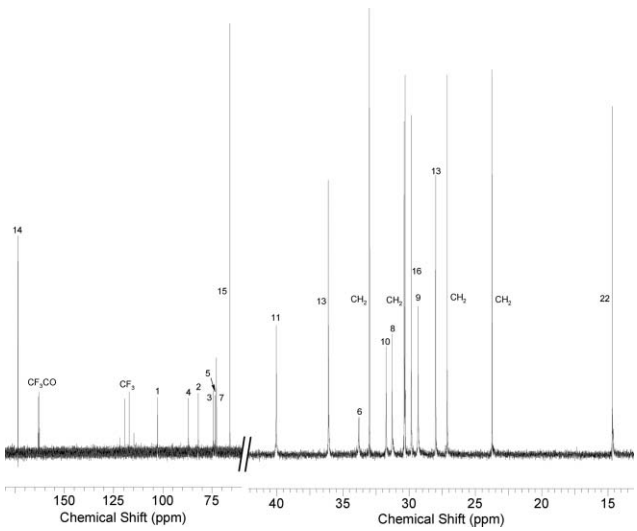


Fig. 4 ^{13}C NMR of **21** in CD_3OD.

Fig. 5. Mass measurement accuracies of between 5–10 ppm were obtained for the doubly charged species using the lock mass (Table 1).

Conclusions

This series of cyclodextrin vectors has been synthesised by a procedure involving attachment and photochemical extension of the lipophilic chains selectively at the 2-positions of the glucose rings. This selectivity for the 2-position limits the number of lipophilic groups on the secondary-OH side to seven, rather than the fourteen for 2,3-derivatisation, and preserves solubility in water to a degree allowing self-assembly. Of previous cyclodextrin amphiphiles having this configuration, that is, a lipophilic secondary-OH side, only 6-sulfated[28] and 6-glycosylated[29] cyclodextrin amphiphiles had solubility and self-assembly properties.

The route also makes it possible to have multiple and potentially variable cationic groups for oligonucleotide condensation at the 6-positions, opening the way to many new possibilities for the improvement of cyclodextrin gene vectors.

Using these methods, vectors have been synthesised in which the lipophilic chains incorporate potentially biolabile ester groups, for assessment as a means of controlled drug release from vesicles or nanoparticles. The assembly properties of these cyclodextrin amphiphiles, alone and with DNA, as well as their assessment as non-viral gene delivery vectors, are now being investigated and will be reported later.

Table 1 ESI high resolution mass measurements and their accuracies

Compound	Chemical formula	Calculated m/z $[M + 2H]^{2+}$	Observed m/z $[M + 2H]^{2+}$	Mass measurement accuracy (ppm)
CH_3 ester (**19**)	$C_{105}H_{189}O_{42}N_7S_{14}$	1334.9558	1334.9646	6.6
C_6 ester (**20**)	$C_{140}H_{259}O_{42}N_7S_{14}$	1580.2296	1580.2438	8.98
C_8 ester (**21**)	$C_{154}H_{287}O_{42}N_7S_{14}$	1678.3392	1678.3556	9.8
C_{12} ester (**22**)	$C_{182}H_{343}O_{42}N_7S_{14}$	1874.5583	1874.5818	12.5
C_{16} ester (**23**)	$C_{210}H_{399}O_{42}N_7S_{14}$	2070.7774	2070.7981	9.99
C_8 alkyl (**25**)	$C_{133}H_{259}O_{28}N_7S_{14}$	1426.2652	1426.2769	8.2
C_{12} alkyl (**26**)	$C_{161}H_{315}O_{28}N_7S_{14}$	1622.4843	1622.4994	9.3
C_{16} alkyl (**27**)	$C_{189}H_{371}O_{28}N_7S_{14}$	1818.7034	1818.7234	10.1

Experimental

Reactions were carried out in dry solvents under a nitrogen atmosphere. All solvents and reagents (Aldrich) were used without further purification. DMF was purchased in Sureseal™ bottles over molecular sieves and stored under nitrogen. Triphenylphosphine was recrystallised from ethanol and dried *in vacuo* for 6 h at 50 °C. β-Cyclodextrin (Aldrich) was dried for 12 h at 100 °C *in vacuo*.

Reactions were monitored by TLC on precoated aluminium plates of silica gel (Merck $60F_{254}$ 0.25 mm). Carbohydrates were made visible by dipping in ethanol containing 5% sulfuric acid by volume, and charring. For esters, a stain of 21 g $(NH_4)_6Mo_7O_{24}$, 1 g $Ce(SO_4)_2$, 1 L water and 31 mL conc. H_2SO_4 was used, followed by charring. For all other compounds a UV lamp (254 nm) was used.

Column chromatography was performed on silica gel (Merck Kieselgel 60, 0.04–0.063 μm). Gel filtration chromatography was performed on Sephadex G-25 or LH-20 (Pharmacia Biotech.).

^{1}H NMR and ^{13}C NMR spectra were recorded at 25 °C with Varian spectrometers at 300 and 75, 400 and 100, or 500 and 125 MHz.

Electrospray ionisation mass spectrometry (ESI-MS) was performed in the positive ion mode on a liquid chromatography time-of-flight mass spectrometer (LCT, Waters Corporation, Manchester, UK). The samples were introduced into the ion source by an LC system (Waters Alliance 2795, Waters Corporation, USA) in acetonitrile:water (60:40 v/v) at 200 μL/min. The capillary voltage of the mass spectrometer was 3 kV, with sample cone voltage of 120 V for the high mass scale (m/z 500–4500) and 100 V for low mass scale scan (m/z 500–2500). For exact mass determination, the instrument was externally calibrated for the mass range m/z 500 to m/z 2500. A lock (reference) mass (m/z 1347.7359) was used. The analyte to lock mass ion abundance ratio was maintained at 1:1.

Compounds **1**[15,16] and **2**[14] were prepared by literature methods.

Heptakis(2-*O*-allyl-6-bromo-6-deoxy)-β-cyclodextrin (3)

To a stirred solution of triphenylphosphine (1.74 g, 6.6 mmol), in freshly distilled CH_2Cl_2 (50 mL), cooled to 0 °C, was added bromine (0.34 mL, 6.5 mmol) dropwise. Any remaining red coloration was dispelled by addition of more triphenylphosphine. The solution was allowed to warm to room temperature, while a colourless precipitate formed. Heptakis(2-*O*-allyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (**2**) (1.94 g, 0.88 mmol), in CH_2Cl_2 (10 mL), was added dropwise. The reaction mixture was stirred for 24 h at room temperature. Evaporation under reduced pressure gave an oily residue, which was triturated with EtOH (60 mL) and sonicated for 1 h to precipitate the product. The precipitate was sonicated twice more with EtOH (2 × 60 mL), then dried *in vacuo* at 40 °C for 12 h to obtain **3** (1.2 g, 74%) as a colourless solid. Found: C 40.58, H 4.73, Br 30.42%; $C_{63}H_{91}O_{28}Br_7$ requires C 40.78, H 4.94, Br, 30.14%; δ_H (500 MHz, $CDCl_3$) 5.97-5.89 (m, 7H, C*H*=CH_2), 5.32 (dd, J_{gem} = 1.3 Hz, J_{vic} = 17.1 Hz, 7H, CH=CH_2trans), 5.25 (d, J = 10.4 Hz, 7H, CH=CH_2cis), 4.97 (d, J = 3.6 Hz, 7H, H-1), 4.94 (s, 7H, OH-3), 4.48 (dd, J = 5.2 Hz, J = 12.4 Hz, 7H, OCH_b), 4.24 (dd, J = 7 Hz, J = 12.4 Hz, 7H, OCH_a), 3.96 (t, J = 9.2 Hz, 7H, H-3), 3.86-3.81 (m, 14H, H-5, H-6_b), 3.70 (dd, J = 6.1 Hz, J = 11.5 Hz, 7H, H-6_a), 3.45 (dd, J = 3.6 Hz, J = 9.6 Hz, 7H, H-2), 3.33 (t, J = 9.2 Hz, 7H, H-4); δ_C (125 MHz, $CDCl_3$) 133.9 (*C*H=CH_2), 119.2 (CH_2=CH), 101.9 (C-1), 86.2 (C-4), 78.9 (C-2), 73.7 (OCH_2), 73.0 (C-3), 70.7 (C-5), 33.3 (C-6).

Heptakis [2-*O*-allyl-6-(*N*-Boc-2-aminoethylthio)-6-deoxy]-β-cyclodextrin (4)

Sodium hydride (95%, 0.19 g, 7.5 mmol) was added in portions to a stirred solution of *N*-Boc-2-aminoethyl thiol (1.43 g, 8.1 mmol) dissolved in anhydrous DMF (10 mL). The solution was stirred for 10 min at room temperature then for 20 min at 80 °C before cooling to room temperature. Heptakis(2-*O*-allyl-6-bromo-6-deoxy)-β-cyclodextrin (**3**) (1.0 g, 0.5 mmol), dissolved in anhydrous DMF (5 mL), was added dropwise and the resulting solution was stirred at room temperature for 18 h under nitrogen. The solution was concentrated under reduced pressure to yield an oily residue; this was dissolved in ethyl acetate (30 mL) which was washed with water (40 mL) and brine (40 ml), dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (SiO_2, cyclohexane-ethyl acetate 50:50) to afford **4** (1.0 g, 74%). Found C 52.98, H 7.46, N 3.74, S 9.17. $C_{112}H_{189}O_{42}N_7S_7$ requires C, 53.17; H, 7.53; N, 3.88; S, 8.87%; δ_H(500 MHz, $CDCl_3$) 5.97-5.89 (m, 7H, C*H*=CH_2), 5.32-5.21 (m, 21H, CH_2=CH, NH), 4.96-4.92 (m, 14H, OH-3, H-1), 4.47 (dd, J = 5.3 Hz, J = 12.5 Hz, 7H, OCH_b), 4.23 (dd, J = 6.9 Hz, J = 12.4 Hz, 7H, OCH_a), 3.91-3.87 (m, 14H, H-3, H-5), 3.41-3.39 (m, 14H, H-4, H-2), 3.3 (br s, 7H, NCH_2), 3.05-2.87 (m, 14H, H-6_b, H-6_a), 2.73 (br s, 14H, SCH_2), 1.43 (s, 63H, $C(CH_3)_3$); δ_C (125 MHz, $CDCl_3$) 156.0 (C=O), 134.2 (*C*H=CH_2), 118.9 (CH_2=CH), 101.8 (C-1), 86.2 (C-4), 79.3 (C-2, *C*$(CH_3)_3$), 73.6 (OCH_2), 73.3 (C-3), 71.0 (C-5), 40.2 (CH_2N), 33.7 (SCH_2, C-6), 28.7 (C(*C*$H_3)_3$).

Synthesis of 3-mercaptopropionic acid esters

General procedure

To mercaptopropionic acid (20 mmol) in solution in the appropriate alcohol (60 mmol) was added two drops of concentrated sulfuric acid. The reaction solution was stirred overnight at room temperature, then diluted with CH_2Cl_2 and washed with distilled water. The organic layer was dried ($MgSO_4$) and evaporated, and the residue was purified by column chromatography on silica gel.

Hexyl 3-mercaptopropionate (5). Column chromatography (cyclohexane-ethyl acetate 90:10) gave 5.6 g, 63% (Found: C, 56.88; H, 9.44; S, 16.76. $C_9H_{18}O_2S$ requires C, 56.80; H, 9.53; S, 16.85%). δ_H(300 MHz, $CDCl_3$) 4.10 (t, J = 6.7 Hz, CO_2CH_2), 2.82-2.74 (m, 2H, CH_2SH), 2.67-2.62 (m, 2H, CH_2CO_2), 1.66-1.58 (m, 3H, $CO_2CH_2CH_2$, SH), 1.37-1.27 (m, 6H, $CH_2 \times 3$), 0.89 (t, J = 6.8 Hz, 3H, CH_3); δ_C (75 MHz, $CDCl_3$) 171.8 (C=O), 65.1 (CO_2CH_2), 31.6, 28.7, 25.7, 22.7 ($CH_2 \times 4$), 20.0 (CH_2SH), 14.1 (CH_3).

Octyl 3-mercaptopropionate (6). Column chromatography (CH_2Cl_2) gave 2.50 g, 61% (Found C, 60.32; H, 10.03; S, 14.44. $C_{11}H_{22}O_2S$ requires C, 60.50; H, 10.15; S, 14.68%). δ_H(300 MHz, $CDCl_3$) 4.08 (t, J = 6.7 Hz, 2H, CO_2CH_2), 2.80-2.72 (m, 2H, CH_2SH), 2.65-2.6 (m, 2H, CH_2CO_2), 1.64-1.57 (m, 3H, SH, $CO_2CH_2CH_2$), 1.35-1.26 (m, 10H, $CH_2 \times 5$), 0.86 (t, J = 6.7 Hz, 3H, CH_3); δ_C (75 MHz, $CDCl_3$) 171.8 (C=O); 65.0 (CO_2CH_2); 38.7 (CH_2CO_2); 31.9, 29.30, 29.28, 28.7, 26.0, 22.7 ($CH_2 \times 6$); 19.9 (CH_2SH); 14.2 (CH_3).

Dodecyl 3-mercaptopropionate (7). The dodecyl ester (3.74 g, 73%) was obtained as colourless oil (Found C, 60.32; H, 10.03; S 14.44. $C_{15}H_{30}O_2S$ requires C, 60.51; H, 10.16; S, 14.68%). δ_H(300 MHz, $CDCl_3$) 4.05 (t, J = 6.7 Hz, 2H, CO_2CH_2), 2.76-2.69 (m, 2H, CH_2SH), 2.62-2.57 (m, 2H, CH_2CO_2), 1.62-1.56 (m, 3H, SH, $CO_2CH_2CH_2$), 1.26-1.22 (m, 18H, $CH_2 \times 9$), 0.84 (t, J = 6.7 Hz, 3H, CH_3); δ_C (75 MHz, $CDCl_3$) 171.6 (C=O), 64.9 (CO_2CH_2), 38.6 (CH_2CO_2), 32.0-22.7 (alkyl chain), 19.8 (CH_2SH), 14.1 (CH_3).

Hexadecyl 3-mercaptopropionate (8). This reaction was performed at 50 °C. Column chromatography (CH_2Cl_2) gave 4.62 g, 74% (Found C, 69.33; H, 11.32; S, 10.08. $C_{19}H_{38}O_2S$ requires C, 69.03; H, 11.59; S, 9.70%). δ_H(400 MHz, $CDCl_3$) 4.09 (t, J = 6.75 Hz, CO_2CH_2), 2.79-2.73 (m, 2H, CH_2SH), 2.65-2.61 (m, 2H, CH_2CO_2), 1.65-1.58 (m, 3H, SH, $CO_2CH_2CH_2$), 1.35-1.24 (m, 26H, $CH_2 \times 13$), 0.87 (t, J = 6.7 Hz, 3H, CH_3); δ_C (100 MHz, $CDCl_3$) 171.8 (C=O), 65.0 (CO_2CH_2), 38.7 (CH_2CO_2), 32.1-22.8 (alkyl chain), 19.9 (CH_2SH), 14.2 (CH_3).

Benzyl 3-mercaptopropionate (9). Column chromatography (cyclohexane-ethyl acetate 90:10) gave 0.75 g, 54% (Found C, 61.25; H, 6.14; S, 15.93. $C_{10}H_{12}O_2S$ requires C, 61.20; H, 6.16; S, 16.34%). δ_H(400 MHz, $CDCl_3$) 7.38-7.33 (m, 5H, C_6H_5), 5.16 (s, 2H, C$H_2C_6H_5$), 2.83-2.77 (m, 2H, CH_2SH), 2.72-2.69 (m, 2H, CH_2CO_2), 1.64 (t, J = 8.2 Hz, 1H, SH); δ_C (100 MHz, $CDCl_3$) 171.5 (C=O), 135.8 (OCH_2CPh), 128.7, 128.5, 128.4 (Ph), 66.6 (CH_2Ph), 38.6 (CH_2CO_2), 19.9 (CH_2SH).

Heptakis[6-(*N*-Boc-2-amino-ethylthio)-6-deoxy-2-*O*-methoxycarbonylethylsulfanylpropyl]-β-cyclodextrin (10). Heptakis [2-*O*-allyl-6-(*N*-Boc-2-aminoethylthio)-6-deoxy]-β-cyclodextrin (**4**) (0.37 g, 0.15 mmol) was dissolved in MeOH (30 mL) using gentle heat in a quartz test-tube. Methyl-3-mercaptopropionate (0.33 mL, 3.1 mmol) and AIBN (0.012 g, 0.1 mmol) were added and the reaction solution was degassed for 30 min with nitrogen. The reaction solution was irradiated (λ = 254 nm) for 6 h, then concentrated and the residue purified by column chromatography (SiO_2, ethyl acetate-cyclohexane 60:40) to afford **10** (0.4 g, 82%) as a colourless resin (Found C, 49.74; H, 7.22; N, 3.10; S, 13.04. $C_{140}H_{245}O_{56}N_7S_{14}$ requires C, 49.88; H, 7.33; N, 2.91; S, 13.32%). δ_H(500 MHz, $CDCl_3$) 5.30 (br s, 7H, NH), 4.92 (br s, 14H, H-1, OH-3), 4.06-4.01 (m, 7H, OCH_b), 3.86-3.82 (m, 14H, H-3, H-5), 3.77-3.73 (m, 7H, OCH_a), 3.69 (s, 21H, CO_2CH_3), 3.39-3.30 (m, 28H, H-4, H-2, CH_2N), 3.06-2.89 (m, 14H, H-6_b, H-6_a), 2.79-2.73 (m, 28H, CH_2CO_2, SCH_2), 2.63-2.59 (m, 28H, S$CH_2CH_2CO_2$, $OCH_2CH_2CH_2$), 1.89-1.84 (m, 14H, OCH_2CH_2), 1.43 (s, 63H, $C(CH_3)_3$); δ_C (125 MHz, $CDCl_3$) 172.4 (CH_2C=O), 155.9 (NC=O), 101.6 (C-1), 86.0 (C-4), 80.8 (C-2), 79.3 ($C(CH_3)_3$), 73.0 (C-3); 71.7 (OCH_2), 71.1 (C-5), 51.8 (CO_2CH_3), 40.1 (CH_2N), 34.7 ($SCH_2CH_2CO_2$), 33.5 (SCH_2CH_2N, C-6), 29.7 (OCH_2CH_2), 28.6 ($C(CH_3)_3$), 28.5 ($OCH_2CH_2CH_2$), 26.9 (CH_2CO_2).

Heptakis[6-(*N*-Boc-2-amino-ethylthio)-6-deoxy-2-*O*-hexyloxycarbonylethylsulfanylpropyl]-β-cyclodextrin (11). This compound was prepared as described for **10** starting from **4** (0.4 g, 0.15 mmol) and hexyl-3-mercaptopropionate (**5**) (0.63 g, 3.3 mmol). Irradiation for 24 h, then column chromatography (SiO_2, cyclohexane-ethyl acetate 60:40) afforded **11** (0.45 g, 74%) as a colourless resin (Found C, 52.23; H, 7.75; N, 2.49; S, 11.52. $C_{175}H_{315}O_{56}N_7S_{14}.8H_2O$ requires C, 52.46; H, 8.33; N, 2.45; S, 11.18%). δ_H(500 MHz, $CDCl_3$) 5.30 (br s, 7H, NH), 4.91 (br s, 14H, OH-3, H-1), 4.08-4.01 (m, 21H, CO_2CH_2, OCH_b), 3.85-3.81 (m, 14H, H-3, H-5), 3.76-3.72 (m, 7H, OCH_a), 3.38 - 3.30 (m, 28H, H-4, H-2, CH_2N), 3.04-2.86 (m, 14H, H-6_b, H-6_a), 2.77-2.71 (m, 28H, CH_2CO_2, SCH_2CH_2N), 2.62-2.06 (m, 28H, $OCH_2CH_2CH_2$, $SCH_2CH_2CO_2$), 1.89-1.83 (m, 14H, OCH_2CH_2), 1.63-1.58 (m, 14H, $CO_2CH_2CH_2$), 1.42 (s, 63H, $C(CH_3)_3$), 1.40-1.25 (m, 42H, $CH_2 \times 3$), 0.87 (t, J = 6.8 Hz, 21H, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 172.0 (CH_2C=O), 155.9 (NC=O), 101.6 (C-1), 86.1 (C-4), 80.9 (C-2), 79.3 ($C(CH_3)_3$), 73.0 (C-3), 71.7 (OCH_2), 71.1 (C-5); 64.9 (CO_2CH_2), 40.1 (CH_2N); 35.0 ($SCH_2CH_2CO_2$), 33.6 (SCH_2CH_2N, C-6), 31.5, 25.6, 22.6 ($CH_2 \times 3$), 29.7 (OCH_2CH_2), 28.64, 28.59, 28.54 ($C(CH_3)_3$, $CO_2CH_2CH_2$, $OCH_2CH_2CH_2$), 27.0 (CH_2CO_2), 14.1(CH_2CH_3).

Heptakis[6-(*N*-Boc-2-amino-ethylthio)-6-deoxy-2-*O*-octyloxycarbonylethylsulfanylpropyl]-β-cyclodextrin (12). This compound was prepared as described for **10** starting from **4** (0.3 g, 0.1 mmol) and octyl-3-mercatopropionate (**6**) (0.58 g, 2.7 mmol). Irradiation for 19 h, then column chromatography (SiO_2, cyclohexane-ethyl acetate 65:35 to 50:50) afforded **12** (0.45 g, 86%) as a colourless resin (Found C, 55.72; H, 8.44; N, 2.21; S, 10.79. $C_{189}H_{343}O_{56}N_7S_{14}$ requires C, 55.93; H, 8.52; N, 2.42; S, 11.06%). δ_H(500 MHz, $CDCl_3$) 5.31 (br s, 7H, NH), 4.92 (br s, 14H, OH-3, H-1), 4.08-4.02 (m, 21H, CO_2CH_2, OCH_b), 3.85-3.82 (m, 14H, H-3, H-5), 3.77-3.73 (m, 7H, OCH_a), 3.38-3.31 (m, 28H, H-4, H-2, CH_2N), 3.05-2.87 (m, 14H, H-6_b, H-6_a), 2.78-2.73 (m, 28H, CH_2CO_2, SCH_2CH_2N), 2.63-2.57 (m, 28H, $OCH_2CH_2CH_2$, $SCH_2CH_2CO_2$,), 1.90-1.84 (m, 14H, OCH_2CH_2), 1.64-1.58 (m, $CO_2CH_2CH_2$), 1.43 (s, 63H, $C(CH_3)_3$), 1.34-1.24 (m, 70H, $CH_2 \times 5$), 0.87 (t, J = 6.9 Hz, 21H, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 172.1 (CH_2C=O), 156.0 (NC=O), 101.7 (C-1), 86.1 (C-4), 80.9 (C-2), 79.3 ($C(CH_3)_3$), 73.1 (C-3), 71.8 (OCH_2), 71.1

(C-5), 65.0 (CO_2CH_2), 40.2 (CH_2N), 35.0 ($SCH_2CH_2CO_2$), 33.6 (C-6, SCH_2CH_2N), 31.9, 29.32, 29.29, 26.0, 22.8 ($CH_2 \times 5$), 29.8 (OCH_2CH_2), 28.74, 26.64, 28.59 ($C(CH_3)_3$, $OCH_2CH_2CH_2$, $CO_2CH_2CH_2$), 27.1 (CH_2CO_2), 14.2 (CH_2CH_3).

Heptakis[6-(*N*-Boc-2-amino-ethylthio)-6-deoxy-2-*O*-dodecyloxycarbonylethylsulfanylpropyl]-β-cyclodextrin (13). This compound was prepared as described for **10** starting from **4** (0.15 g, 0.05 mmol) and dodecyl-3-mercaptopropionate **7** (0.34 g, 1.2 mmol). The reaction solution was irradiated for 24 h after which an oily resin had separated. Column chromatography (SiO_2, cyclohexane-ethyl acetate 60:40) afforded **13** (0.17 g, 65%) as a colourless resin (Found C, 58.26; H, 8.95; N, 1.92. $C_{217}H_{399}O_{56}N_7S_{14}$ requires C, 58.55; H, 9.03; N, 2.20%). δ_H(400 MHz, $CDCl_3$) 5.31 (br s, 7H, NH), 4.92 (br s, 14H, H-1, OH-3), 4.09-4.02 (m, 21H, CO_2CH_2, OCH_b), 3.86-3.84 (m, 14H, H-3, H-5), 3.76-3.74 (m, 7H, OCH_a), 3.38-3.31 (m, 28H, H-4, H-2, CH_2N), 3.04-2.87 (m, 14H, H-6_b, H-6_a), 2.79-2.75 (m, 28H, CH_2CO_2, SCH_2CH_2N), 2.64-2.57 (m, 28H, $CH_2SCH_2CH_2CO_2$), 1.43 (s, 63H, $C(CH_3)_3$), 1.32-1.25 (m, 126H, $CH_2 \times 9$), 0.87 (t, J = 6.8 Hz, 21H, CH_2CH_3); δ_C (100 MHz, $CDCl_3$): 172.1 ($CH_2C{=}O$), 156.0 (NC=O), 101.7 (C-1), 86.2 (C-4), 80.9 (C-2), 73.4 ($C(CH_3)_3$), 73.1 (C-3), 71.8 (OCH_2), 71.1 (C-5), 65.0 (CO_2CH_2), 40.2 (CH_2N), 35.0 ($SCH_2CH_2CO_2$), 33.6 (C-6, SCH_2CH_2N), 32.0-22.8 (CH_2 alkyl, OCH_2CH_2, CH_2CO_2), 28.8, 28.7, 28.6 ($C(CH_3)_3$, $OCH_2CH_2CH_2$, $CO_2CH_2CH_2$), 14.3 (CH_2CH_3).

Heptakis[6-(*N*-Boc-2-amino-ethylthio)-6-deoxy-2-*O*-hexadecyloxycarbonylethylsulfanylpropyl]-β-cyclodextrin (14). This compound was prepared as described for **10** starting from **4** (0.4 g, 0.15 mmol) and hexadecyl-3-mercaptopropionate (**8**) (1.1 g, 3.3 mmol) and AIBN (0.012 g, 0.1 mmol) dissolved in toluene (3 mL) were added to the solution, which was then degassed for 30 min with nitrogen. Irradiation for 24 h and column chromatography (SiO_2, cyclohexane-ethyl acetate 60:40) afforded **14** (0.46 g, 60%) as a colourless solid (Found C, 60.63; H, 9.24; N, 2.48; S, 9.03. $C_{245}H_{455}O_{56}N_7S_{14}$ requires C, 60.75; H, 9.47; N, 2.02; S, 9.27%). δ_H(500 MHz, $CDCl_3$) 5.29 (br s, 7H, NH), 4.92 (br s, 14H, H-1, OH-3), 4.09-4.02 (m, 21H, CO_2CH_2, OCH_b), 3.86-3.82 (m, 14H, H-3, H-5), 3.77-3.73 (m, 7H, OCH_a), 3.38-3.31 (m, 28H, H-4, H-2, CH_2N), 3.05-2.89 (m, 14H, H-6_b, H-6_a), 2.78-2.74 (m, 28H, CH_2CO_2, SCH_2CH_2N), 2.64-2.57 (m, 28H, $CH_2SCH_2CH_2CO_2$), 1.90-1.85 (m, 14H, OCH_2CH_2), 1.64-1.58 (m, 14H, $CO_2CH_2CH_2$), 1.43 (s, 63H, $C(CH_3)_3$), 1.32-1.25 (m, 182H, $CH_2 \times 13$), 0.87 (t, J = 6.9 Hz, 21H, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 172.1 ($CH_2C{=}O$), 156.0 (NC=O), 101.7 (C-1), 86.2 (C-4), 80.9 (C-2), 79.3 ($C(CH_3)_3$), 73.1 (C-3), 71.8 (OCH_2), 71.0 (C-5), 65.0 (CO_2CH_2), 40.2 (CH_2N), 35.0 ($SCH_2CH_2CO_2$), 33.6 (C-6, SCH_2CH_2N), 32.0-22.8 (CH_2 alkyl chain, OCH_2CH_2, CH_2CO_2), 14.4 (CH_2CH_3).

Heptakis[6-(*N*-Boc-2-amino-ethylthio)-6-deoxy-2-*O*-benzyloxycarbonylethylsulfanylpropyl]-β-cyclodextrin (15). This compound was prepared as described for **10** starting from **4** (0.3 g, 0.1 mmol) and benzyl-3-mercaptopropionate (**9**) (0.47 g, 2.4 mmol). The reaction mixture was irradiated for 5h. Column chromatography (SiO_2, cyclohexane-ethyl acetate 50:50) gave **15** (0.23 g, 52%) as a colourless resin (Found C, 55.88; H, 7.29; N, 1.94; S, 10.66. $C_{182}H_{273}O_{56}N_7S_{14}$ requires C, 55.99; H, 7.05; N, 2.51; S, 11.50%). δ_H(500 MHz, $CDCl_3$) 7.36-7.29 (m, 35H, C_6H_5), 5.31 (br s, 7H, NH), 5.12 (s, 14H, CO_2CH_2), 4.92 (br s, 14H, H-1, OH-3), 4.04-4.00 (m, 7H, OCH_b), 3.86-3.83 (m, 14H, H-3, H-5), 3.75-3.71 (m, 7H, OCH_a), 3.39-3.30 (m, 28H, H-4, H-2, NCH_2), 3.05-2.87 (m, 14H, H-6_b, H-6_a), 2.79-2.74 (m, 28H, CH_2CO_2, SCH_2), 2.65-2.58 (m, 28H, $SCH_2CH_2CO_2$, $OCH_2CH_2CH_2$), 1.87-1.82 (m, 14H, OCH_2CH_2), 1.43 (s, 63H, $C(CH_3)_3$); δ_C (125 MHz, $CDCl_3$) 171.8 (CH_2CO_2), 156.0 (NCO_2), 136.0 (OCH_2CPH), 128.7, 128.38, 128.35 (Ph), 101.7 (C-1), 86.2 (C-4), 80.9 (C-2), 79.4 ($C(CH_3)_3$), 73.1 (C-3), 71.8 (OCH_2CH_2), 71.0 (C-5), 66.6 (CH_2Ph), 40.2 (NCH_2), 35.0 ($SCH_2CH_2CO_2$), 33.6 (SCH_2CH_2N, C-6), 29.8 (OCH_2CH_2), 28.64 ($C(CH_3)_3$), 28.58 ($OCH_2CH_2CH_2$), 27.0 (CH_2CO_2).

Heptakis[6-(*N*-Boc-2-amino-ethylthio)-6-deoxy-2-*O*-octylsulfanylpropyl]-β-cyclodextrin (16). This compound was prepared as described for **10** starting from **4** (0.14 g, 0.05 mmol) and 1-octyl thiol (0.21 mL, 1.1 mmol). The reaction mixture was irradiated for 1 h. Column chromatography (SiO_2, cyclohexane/ethyl acetate: 65:35) gave **18** (0.1 g, 51%) as a cloudy wax (Found C, 56.90; H, 8.77; N, 3.06. $C_{168}H_{315}O_{42}N_7S_{14}$ requires C, 56.77; H, 8.93; N, 2.76%). δ_H(500 MHz, $CDCl_3$) 5.31 (br s, 7H, NH), 4.93 (br s, 14H, H-1, OH-3), 4.08-4.03 (m, 7H, OCH_b), 3.87-3.83 (m, 14H, H-3, H-5), 3.79-3.74 (m, 7H, OCH_a), 3.38-3.32 (m, 28H, H-4, H-2, CH_2N), 3.06-2.87 (m, 14H, H-6_b, H-6_a), 2.74 (br s, 14H, SCH_2CH_2N), 2.59 (t, J = 7.3 Hz, 14H, $OCH_2CH_2CH_2$), 2.51 (t, J = 7.4 Hz, 14H, $SCH_2CH_2CH_2$), 1.90-1.85 (m, 14H, OCH_2CH_2), 1.60-1.54 (m, 14H, $SCH_2CH_2CH_2$), 1.44 (s, 63H, $C(CH_3)_3$), 1.39-1.28 (m, 70H, $(CH_2 \times 5)$, 0.88 (t, J = 6.8 Hz, 21H, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 156.0 (C=O), 101.7 (C-1), 86.2 (C-4), 80.9 (C-2), 79.4 ($C(CH_3)_3$),73.1 (C-3), 72.0 (OCH_2), 71.1 (C-5); 40.2 (CH_2N), 33.7 (C-6, SCH_2CH_2N), 32.3 ($SCH_2(CH_2)n$), 32.0, 29.42, 29.37, 22.8 ($CH_2 \times 4$), 30.0 (OCH_2CH_2) 29.8 ($SCH_2CH_2CH_2$), 29.1 ($SCH_2CH_2CH_2$), 28.7 ($OCH_2CH_2CH_2$, $C(CH_3)_3$), 14.2 (CH_2CH_3).

Heptakis[6-(*N*-Boc-2-amino-ethylthio)-6-deoxy-2-*O*-dodecylsulfanylpropyl]-β-cyclodextrin (17). This compound was prepared as described for **10** starting from **4** (0.4 g, 0.15 mmol) and 1-dodecyl thiol (0.79 mL, 3.3 mmol). The reaction mixture was irradiated for 8 h (after 1 h the minimum amount of toluene was added to clear the solution). Column chromatography (SiO_2, cyclohexane/ethyl acetate: 65:35) gave **19** (0.34 g, 54%) as a colourless solid (Found C, 59.51; H, 9.31; N, 2.37; S, 11.07. $C_{196}H_{371}O_{42}N_7S_{14}$ requires C, 59.64; H, 9.47; N, 2.48; S, 11.37%). δ_H(500 MHz, $CDCl_3$) 5.30 (br s, 7H, NH), 4.93 (br s, 14H, H-1, OH-3), 4.07-4.03 (m, 7H, OCH_b), 3.86-3.83 (m, 14H, H-3, H-5), 3.78-3.73 (m, 7H, OCH_a), 3.38-3.31 (m, 28H, H-4, H-2, CH_2N), 3.05-2.87 (m, 14H, H-6_b, H-6_a), 2.74 (br s, 14H, SCH_2CH_2N), 2.58 (t, J = 7.2 Hz, 14H, $OCH_2CH_2CH_2$), 1.59-1.54 (m, 14H, $SCH_2CH_2(CH_2)_n$), 1.44 (s, 63H, $C(CH_3)_3$), 1.38-1.34 (m, 14H, $SCH_2CH_2CH_2$), 1.31-1.26 (m, 112H, $CH_2 \times 8$), 0.88 (t, J = 6.9 Hz, 21H, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 156.1 (C=O), 101.8 (C-1), 86.3 (C-4), 81.0 (C-2), 80.0 ($C(CH_3)_3$), 73.3 (C-3), 72.2 (OCH_2), 71.2 (C-5), 40.4 (CH_2N), 33.8 (C-6, SCH_2CH_2N), 32.4 ($SCH_2CH_2CH_2$), 32.2, 29.96, 29.93, 29.90, 29.89, 29.86, 29.6, 29.3, 22.9 ($CH_2 \times 8$), 30.1 (OCH_2CH_2), 28.8 ($OCH_2CH_2CH_2$, $C(CH_3)_3$), 14.3 (CH_2CH_3).

Heptakis[6-(*N*-Boc-2-amino-ethylthio)-6-deoxy-2-*O*-hexadecylsulfanylpropyl]-β-cyclodextrin (18). This compound was prepared as described for **10** starting from **4** (0.30 g, 0.1 mmol) and

1-hexadecyl thiol (0.5 g, 2 mmol). After irradiation for 2 h the minimum amount of toluene was added to clear the solution before irradiation for a further 22 h. Column chromatography (SiO_2, cyclohexane/ethyl acetate: 65:35) afforded **18** (0.3 g, 58%) as a colourless solid (Found C, 62.03; H, 9.76; N, 2.56; S, 10.10. $C_{224}H_{427}O_{42}N_7S_{14}$ requires C, 61.99; H, 9.92; N, 2.26; S, 10.34%). δ_H(500 MHz, $CDCl_3$) 5.30 (br s, 7H, NH), 4.93 (br s, 14H, H-1, OH-3), 4.06-4.03 (m, 7H, OCH_b), 3.86-3.83 (m, 14H, H-3, H-5), 3.78-3.73 (m, 7H, OCH_a), 3.37-3.31 (m, 28H, H-4, H-2, CH_2N), 2.58 (t, J = 7.0 Hz, 14H, $OCH_2CH_2CH_2$), 2.50 (t, J = 7.3 Hz, 14H, $SCH_2(CH_2)_n$), 1.90-1.84 (m, 14H, OCH_2CH_2), 1.59-1.53 (m, 14H, $SCH_2CH_2(CH_2)_n$), 1.44 (s, 63H, $C(CH_3)_3$), 1.38-1.25 (m, 182H, $CH_2 \times 13$), 0.87 (t, J = 6.9 Hz, 21H, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 156.1 (C=O), 101.8 (C-1), 86.2 (C-4), 81.0 ($C(CH_3)_3$), 79.5 (C-2), 73.2 (C-3), 72.2 (OCH_2), 71.1 (C-5), 40.3 (CH_2N), 33.8 (C-6, SCH_2CH_2N), 32.4 ($SCH_2(CH_2)_n$), 32.2, 30.0-29.3, 22.9 (CH_2 alkyl chain), 30.1 (OCH_2CH_2), 28.8 ($C(CH_3)_3$, $OCH_2CH_2CH_2$), 14.3 (CH_2CH_3).

Heptakis[6-(2-amino-ethylthio)-6-deoxy-2-*O*-methoxycarbonylethylsulfanylpropyl]-β-cyclodextrin hepta-*N*-trifluoroacetate (19). To a stirred solution of cyclodextrin **10** (0.2 g, 0.05 mmol) in CH_2Cl_2 (2 mL) was added TFA (0.23 mL, 2.95 mmol). The reaction mixture was stirred for 16 h at room temperature then concentrated under reduced pressure below 30 °C. Size-exclusion chromatography on Sephadex LH-20–100 (MeOH) gave **19** (0.14 g, 77%) as a colourless solid. δ_H (500 MHz, CD_3OD) 5.07 (d, J = 3.5 Hz, 7H, H-1), 4.07-4.03 (m, 7H, OCH_b), 3.94-3.90 (m, 14H, H-5, H-3), 3.84-3.80 (m, 7H, OCH_a), 3.70 (s, 21H, OCH_3), 3.57 (t, J = 9.2 Hz, 7H, H-4), 3.45-3.42 (m, 7H, H-2), 3.21 (t, J = 7.0 Hz, 14H, SCH_2CH_2N), 3.28 (d, J = 13 Hz, 7H, H-6_b), 3.03-2.91 (m, 21H, CH_2N, H-6_a), 2.82-2.79 (m, 14H, CH_2CO_2), 2.78-2.64 (m, 28H, $CH_2SCH_2CH_2CO_2$), 1.91-1.86 (m, 14H, OCH_2CH_2); δ_C (125 MHz, CD_3OD) 174.2 (CH_2C=O), 163.4 (F_3CC=O), 118.5 (CF_3), 102.6 (C-1), 86.7 (C-4), 82.0 (C-2), 74.2 (C-3), 73.0 (C-5), 72.7 (OCH_2), 52.4 (OCH_3), 40.0 (CH_2N), 35.7 ($SCH_2CH_2CO_2$), 33.8 (C-6), 31.8 ($SCH2CH_2N$), 31.1 (OCH_2CH_2), 29.3 ($OCH_2CH_2CH_2$), 27.9 (CH_2CO_2).

Heptakis[6-(2-amino-ethylthio)-6-deoxy-2-*O*-hexyloxycarbonylethylsulfanylpropyl]-β-cyclodextrin hepta-*N*-trifluoroacetate (20). This compound was prepared as described for **19** starting from **11** (0.135 g, 0.04 mmol). This yielded the TFA salt as a colourless solid which was then dissolved in methanol. The pH was adjusted to 2 with 0.1M HCl before the solvent was evaporated. Size-exclusion chromatography on Sephadex LH-20–100 (MeOH) gave **20** as a colourless solid (74 mg, 58%). δ_H (500 MHz, CD_3OD) 5.12 (d, J = 3.5 Hz, 7H, H-1), 4.13-4.06 (m, 21H, CO_2CH_2, OCH_b), 3.94-3.81 (m, 21H, H-3, H-5, OCH_a), 3.56 (t, J = 9.2 Hz, 7H, H-4), 3.45 (dd, J = 3.5 Hz, J = 9.6 Hz, 7H, H-2), 3.25-3.19 (m, 21H, CH_2N, H-6′), 3.09-2.98 (m, 21H, SCH_2CH_2N, H-6), 2.81 (t, J = 7.1 Hz, 14H, CH_2CO_2), 2.74-2.63 (m, 28H, $CH_2SCH_2CH_2CO_2$), 1.90-1.86 (m, 14H, OCH_2CH_2), 1.69-1.64 (m, 14H, $CO_2CH_2CH_2$), 1.44-1.34 (m, 42H, $CH_2 \times 3$), 0.94 (t, J = 7.0 Hz, 21H, CH_3); δ_C (125 MHz, CD_3OD) 173.8 (C=O), 102.6 (C-1), 87.0 (C-4), 82.0 (C-2), 74.4 (C-3), 72.7 (C-5), 65.9 (OCH_2), 40.2 (CH_2N), 36.1 ($SCH_2CH_2CO_2$), 34.1 (C-6); 32.7, 26.7, 23.7 ($CH_2 \times 3$), 31.7 (SCH_2CH_2N), 31.3 (OCH_2CH_2), 29.8 ($CO_2CH_2CH_2$), 29.3 ($OCH_2CH_2CH_2$), 28.0 (CH_2CO_2), 14.6 (CH_3).

Heptakis[6-(2-amino-ethylthio)-6-deoxy-2-*O*-octyloxycarbonylethylsulfanylpropyl]-β-cyclodextrin hepta-*N*-trifluoroacetate (21). This compound was prepared as described for **19** starting from **12** (0.22 g, 0.05 mmol) with a reaction time of 24 h. Size-exclusion chromatography gave **21** (0.18 g, 89%) as a colourless solid. δ_H (500 MHz, CD_3OD) 5.08 (d, J = 3.5 Hz, 7H, H-1), 4.13-4.05 (m, 21H, CO_2CH_2, OCH_b), 3.93-3.80 (m, 21H, H-3, H-5, OCH_b), 3.55 (t, J = 9.1 Hz, 7H, H-4), 3.44 (dd, J = 3.5 Hz, J = 9.6 Hz, 7H, H-2), 3.21 (t, J = 7.1 Hz, 14H, CH_2N), 3.12 (d, J = 12.9 Hz, 7H, H-6_b), 3.03-2.92 (m, 21H, SCH_2CH_2N, H-6_a), 2.81 (t, J = 14.3 Hz, CH_2CO_2), 2.74-2.63 (m, 28H, $CH_2SCH_2CH_2CO_2$), 1.92-1.86 (m, 14H, OCH_2CH_2), 1.69-1.64 (m, 14H, $CO_2CH_2CH_2$), 1.41-1.32 (m, 56H, $CH_2 \times 5$), 0.92 (t, J = 7.0 Hz, 21H, CH_3); δ_C (125 MHz, CD_3OD) 173.7 (CH_2C=O),163.1 (F_3CC=O), 118.2 (CF_3), 102.7 (C-1), 87.0 (C-4), 82.0 (C-2), 74.3 (C-3), 73.5 (C-5), 72.7 (OCH_2), 65.9 (CO_2CH_2), 40.0 (CH_2N), 36.1 ($SCH_2CH_2CO_2$), 33.8 (C-6), 33.0, 30.4, 30.3, 27.1, 23.8 ($CH_2 \times 5$), 31.7 (SCH_2CH_2N), 31.3 (OCH_2CH_2), 29.8 ($CO_2CH_2CH_2$), 29.3 ($OCH_2CH_2CH_2$), 28.0 (CH_2CO_2), 14.7 (CH_3).

Heptakis[6-(2-amino-ethylthio)-6-deoxy-2-*O*-dodecyloxycarbonylethylsulfanylpropyl]-β-cyclodextrin hepta-*N*-trifluoroacetate (22). This compound was prepared as described for **19** starting from **13** (0.1 g, 0.02 mmol) with a reaction time of 24 h. Evaporation and drying *in vacuo* for 8 h gave **22** (84 mg, quant.) as a cream solid. δ_H (500 MHz, $CDCl_3$) 8.00 (br s, NH_2, 14H), 4.91 (br s, 14H, H-1, OH-3), 4.12-4.06 (m, 21H, CO_2CH_2, OCH_b), 3.84-3.76 (m, 21H, H-3, H-5, OCH_a), 3.49-2.76 (br m, 70H H-4, H-2, CH_2N, H-6_b, H-6_a, SCH_2CH_2N, CH_2CO_2), 2.64-2.58 (m, 28H, $CH_2SCH_2CH_2CO_2$), 1.87 (br s, 14H, OCH_2CH_2), 1.64-1.59 (m, 14H, $CO_2CH_2CH_2$), 1.33-1.26 (m, 126H, $CH_2 \times 9$), 0.88 (t, J = 7 Hz, 21H, CH_3); δ_C (125 MHz, $CDCl_3$) 172.2 (C=O), 161.9 (CF_3C=O), 116.6 (CF_3), 101.7 (C-1), 73.3 (C-3), 71.8 (OCH_2), 71.5 (C-5); 65.0 (CO_2CH_2), 38.6 (CH_2N), 35.1, 32.1, 30.5, 29.8-29.4, 28.8, 28.6, 27.1, 26.1, 22.8 (C-6, SCH_2CH_2N, OCH_2CH_2, $SCH_2CH_2CO_2$, $CH_2 \times 10$ alkyl chain), 14.2 (CH_3).

Heptakis[6-(2-amino-ethylthio)-6-deoxy-2-*O*-hexadecyloxycarbonylethylsulfanylpropyl]-β-cyclodextrin hepta-*N*-trifluoroacetate (23). This compound was prepared as described for **19** starting from **14** (0.15 g, 0.031 mmol) with a reaction time of 24 h. Size-exclusion chromatography gave **23** (128 mg, quant.) as a colourless solid. δ_H (500 MHz, $CDCl_3$) 8.00 (br s, 14H, NH2), 4.92 (br s, 14H, H-1, OH-3), 4.09-4.02 (m, 14H, CO_2CH_2, OCH_b), 3.85-3.75 (m, 21H, H-3, H-5, OCH_a), 3.44-2.76 (br m, 70H, H-4, H-2, SCH_2CH_2N, H-6_b, H-6_a, CH_2CO_2), 2.63-2.58 (m, 28H, $CH_2SCH_2CH_2CO_2$), 1.88-1.86 (m, 14H, OCH_2CH_2), 1.65-1.59 (m, 14H, $CO_2CH_2CH_2$), 1.43-1.26 (m, 182H, $CH_2 \times 13$), 0.88 (t, J = 6.9 Hz, 21H, CH_3); δ_C (125 MHz, $CDCl_3$) 172.5 (C=O), 161.3 (CF_3C=O), 115.9 (CF_3), 101.6 (C-1), 85.5 (C-4), 80.6 (C-2), 73.2 (C-3), 71.9 (OCH_2), 71.7 (C-5), 65.2 (CO_2CH_2), 38.6 (CH_2N), 35.0, 32.1, 31.6, 30.5, 29.9-29.4, 28.8, 28.6, 27.7, 26.1, 25.3, 22.8 (C-6, SCH_2CH_2N, $OCH_2CH_2CH_2$, $SCH_2CH_2CO_2$, $CH_2 \times 14$ alkyl chain), 14.2 (CH_3).

Heptakis[6-(2-amino-ethylthio)-6-deoxy-2-*O*-benzyloxycarbonylethylsulfanylpropyl]-β-cyclodextrin hepta-*N*-trifluoroacetate (24). This compound was prepared as described for **19** starting from **15** (83 mg, 0.02 mmol) with a reaction time of 18 h.

Size-exclusion chromatography on Sephadex LH-20–100 (MeOH) gave **24** (38 mg, 45%) as a colourless resin. δ_H (500 MHz, CD_3OD) 7.32-7.28 (m, 42H, C_6H_5), 5.09 (s, 14H, OCH_2Ph), 5.04 (s, 7H, H-1), 4.05-4.01 (m, 7H, OCH_b), 3.95 (t, J = 9.0 Hz, 7H, H-3), 3.77-3.74 (m, 21H, H-5, OCH_a), 3.52 (t, J = 9.0 Hz, 7H H-4), 3.40 (dd, J = 3.2 Hz, J = 9.6 Hz, 7H, H-2), 3.21-t, J = 7.3 Hz, 14H, CH_2N), 3.08 (d, J = 13.3 Hz, 7H, H-6_b), 3.03-2.91 (m, 21H, SCH_2CH_2N, H-6_a), 2.75 (t, J = 7.4 Hz, 14H, CH_2CO_2), 2.64-2.57 (m, 28H, $CH_2SCH_2CH_2CO_2$), 1.81-1.80 (m, 14H, OCH_2CH_2); δ_C (125 MHz, CD_3OD) 173.4 (CH_2C=O), 163.1 (CF_3C=O), 137.6 (CH_2CPh), 129.6-129.2 (Ph), 116.8 (CF_3), 102.6 (C-1), 86.8 (C-4), 81.9 (C-2), 74.4 (C-3), 73.2 (C-5), 72.7 (OCH_2), 67.4 (CH_2Ph), 39.9 (CH_2N), 36.0 ($SCH_2CH_2CO_2$), 33.6 (C-6), 31.7 (SCH_2CH_2N), 31.3 (OCH_2CH_2), 29.2 ($OCH_2CH_2CH_2$), 27.8 (CH_2CO_2).

Heptakis[6-(2-amino-ethylthio)-6-deoxy-2-*O*-octylsulfanylpropyl]-β-cyclodextrin hepta-*N*-trifluoroacetate (25). This compound was prepared as described for **19** starting from **16** (50 mg, 0.01 mmol) with a reaction time of 48 h. Size-exclusion chromatography on Sephadex LH-20–100 (MeOH) gave **25** (34 mg, 67%) as a glass-like solid. δ_H (500 MHz, CD_3OD) 5.07 (d, J = 3.5 Hz, 7H, H-1), 4.12-4.08 (m, 7H, OCH_b), 3.94-3.80 (m, 21H, H-3, H-5, OCH_a), 3.54 (t, J = 9.2 Hz, 7H, H-4), 3.44 (dd, J = 3.6 Hz, J = 9.6 Hz, 7H, H-2), 3.21-3.11 (m, 21H, CH_2N, H-6_b), 3.02-2.90 (m, 21H, SCH_2CH_2N, H-6_a), 2.70-2.61 (m, 14H, $OCH_2CH_2CH_2$), 2.56 (t, J = 7.3 Hz, 14H, $SCH_2(CH_2)_n$), 1.92-1.86 (m, 14H, OCH_2CH_2), 1.65-1.59 (m, 14H, $SCH_2CH_2(CH_2)_n$), 1.44-1.34 (m, 70H, $CH_2 \times 5$), 0.95-0.91 (m, 21H, CH_3); δ_C (125 MHz, CD_3OD) 163.1 (CF_3C=O), 117.9 (CF_3), 102.6 (C-1), 86.9 (C-4), 81.9 (C-2), 74.5 (C-3), 73.2 (C-5), 72.8 (OCH_2), 40.1 (CH_2N), 33.8 (C-6), 33.1, 30.5, 30.4, 30.1, 23.8 ($CH_2 \times 5$), 33.0 ($SCH_2(CH_2)_n$), 31.9 (SCH_2CH_2N), 31.5 (OCH_2CH_2), 31.0 ($SCH_2CH_2(CH_2)_n$), 29.4 ($OCH_2CH_2CH_2$), 14.7 (CH_3).

Heptakis[6-(2-amino-ethylthio)-6-deoxy-2-*O*-dodecylsulfanylpropyl]-β-cyclodextrin hepta-*N*-trifluoroacetate (26). This compound was prepared as described for **19** starting from **17** (0.2 g, 0.05 mmol) with a reaction time of 24 h. Evaporation, and drying under high vacuum for 8 h, gave **26** (164 mg, quant.) as a glass-like solid. δ_H (500 MHz, $CDCl_3$) 8.00 (br s, 14H, NH_2), 4.91 (br s, 14H, H-1, OH-3), 4.04 (br s, 7H, OCH_b), 3.84-3.75 (br m, 21H, H-3, H-5, OCH_a), 3.41-2.75 (br m, 56H H-4, H-2, H-6_b, H-6_a, SCH_2CH_2N), 2.59 (t, J = 7.2 Hz, 14H, $OCH_2CH_2CH_2$), 2.51 (t, J = 7.3 Hz, 14H, $SCH_2(CH_2)_n$), 1.89-1.86 (m, 14H, OCH_2), 1.59-1.54 (m, 14H, $SCH_2CH_2CH_2$), 1.37-1.27 (m, 126H, $CH_2 \times 9$), 0.88 (t, 14H, J = 6.9 Hz, CH_3); δ_C (125 MHz, $CDCl_3$) 162.8 (CF_3C=O), 116.7 (CF_3), 101.7 (C-1), 85.7 (C-4), 80.6 (C-2), 73.3 (C-3), 72.1 (OCH_2), 71.6 (C-5), 38.7 (CH_2N), 32.3, 32.1, 30.5, 30.0, 29.8-29.2, 28.7, 22.9 (C-6, SCH_2CH_2N, $OCH_2CH_2CH_2$, $CH_2 \times 11$), 14.3 (CH_3).

Heptakis[6-(2-amino-ethylthio)-6-deoxy-2-*O*-hexadecylsulfanylpropyl]-β-cyclodextrin hepta-*N*-trifluoroacetate (27). This compound was prepared as described for **19** starting from **18** (0.15 g, 0.04 mmol) with a reaction time of 16 h. The product was dried without heating to obtain **27** (126 mg, quant.) as a cream solid. δ_H (500 MHz, $CDCl_3$) 7.81 (br s, 14H, NH_2), 4.91 (br s, 14H, H-1, OH-3), 4.02 (br s, 7H, OCH_b), 3.84-3.76 (br m, 21H, H-3, H-5, OCH_a), 3.43-2.71 (br m,56H, H-4, H-2, H-6_b, H-6_a, SCH_2CH_2N), 2.58 (t, J = 7 Hz, 14H, $OCH_2CH_2CH_2$), 2.50 (t, J = 7.3 Hz, $SCH_2CH_2CH_2$), 1.87 (m, 14H, OCH_2CH_2), 1.60–1.54 (m, 14H, $SCH_2CH_2CH_2$), 1.37–1.26 (m, 182H, $CH_2 \times 13$), 0.88 (t, J = 6.9 Hz, 21H, CH_3); δ_C (125 MHz, $CDCl_3$) 161.5 (CF_3C=O), 116.0 (CF_3), 101.6 (C-1), 85.6 (C-4), 80.6 (C-2), 73.2 (C-3), 72.1 (OCH_2), 71.7 (C-5), 38.6 (CH_2N), 32.3, 32.1, 31.6, 30.5, 29.9-29.2, 28.6, 22.8 (C-6, SCH_2CH_2N, $OCH_2CH_2CH_2$, $CH_2 \times 15$), 14.2 (CH_3).

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