Amino Acid Derivatives of β-Cyclodextrin

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The syntheses of the heptaamino acid-substituted β-cyclodextrins per-6-[(phenylalanyl)amino]-β-cyclodextrin (**6**), per-6-cysteinyl-β-cyclodextrin (**7**), as well as the per-2,3-dimethyl-per-6-cysteinyl-β-cyclodextrin (**12**) are described. The amino acids were coupled to the primary face of the β-cyclodextrin torus using the backbone carboxylic acid functionality of phenylalanine and the side chain thiol group of cysteine. In the case of the heptacysteinyl derivatives, polyzwitterionic compounds were obtained and shown to be highly water soluble.

Introduction

Composed of six, seven, or eight α-1,4-linked D-(+)-glucopyranose units, the cyclodextrins (α, β, and γ, respectively) are cyclic oligosaccharides[1] which have an overall shape reminiscent of a lampshade or truncated cone. The most significant characteristic of the cyclodextrins is their ability to form[2] inclusion complexes in aqueous solutions with a wide variety of substrates. Since compounds that are complexed by an appropriate cyclodextrin often display much improved water solubilities, the cyclodextrins may act as enhancers of the aqueous solubilities of small organic molecules.[3−5] In this context, cyclodextrins have found applications[6] in the solubilization of organic compounds when organic solvents cannot be employed because of their toxicity, flammability, or expense.

As a consequence of its good binding ability toward aromatic units, as well as its ready availability, β-cyclodextrin has emerged at present as the naturally-occurring cyclodextrin with the highest commercial potential. It is, therefore, unfortunate that its aqueous solubility is so low.[7] As a result, the extensive investigations into the chemical modifications of cyclodextrins[8] have been concerned primarily with influencing their solubilities as well as with modifying their binding behaviors.

The synthesis of cyclodextrins that are singly substituted with amino acids has been reported previously.[9−15] However, only one report of cyclodextrins that are symmetrically substituted with amino acids has appeared recently in the literature.[16] Despite their relative ease of characterization, highly-substituted, symmetrical cyclodextrins present a synthetic challenge. Nonetheless, the challenge is worthwhile since a higher degree of substitution should confer upon cyclodextrin derivatives characteristics which are expected to deviate more strongly from those of the native cyclodextrin. As a consequence of these considerations, amino acids have been identified as residues to attach to all the primary hydroxyl groups on β-cyclodextrin. Here, we report the efficient syntheses (Schemes 1−3) of β-cyclodextrin derivatives which are fully substituted on their primary faces with the amino acids L-phenylalanine and L-cysteine.

Results and Discussion

The opportunity for the symmetric derivatization of β-cyclodextrin was found in an important reaction reported recently by Defaye and Gadelle.[17] This reaction allows the selective replacement of all the primary hydroxyl groups of a cyclodextrin by iodine atoms. Thus, treatment of β-cyclodextrin (**1**) with I_2 and Ph_3P in DMF at 70 °C afforded (Scheme 1) the heptaiodide **2**. The reaction was performed as described in the literature.[17] However, the workup procedure, which was recom-

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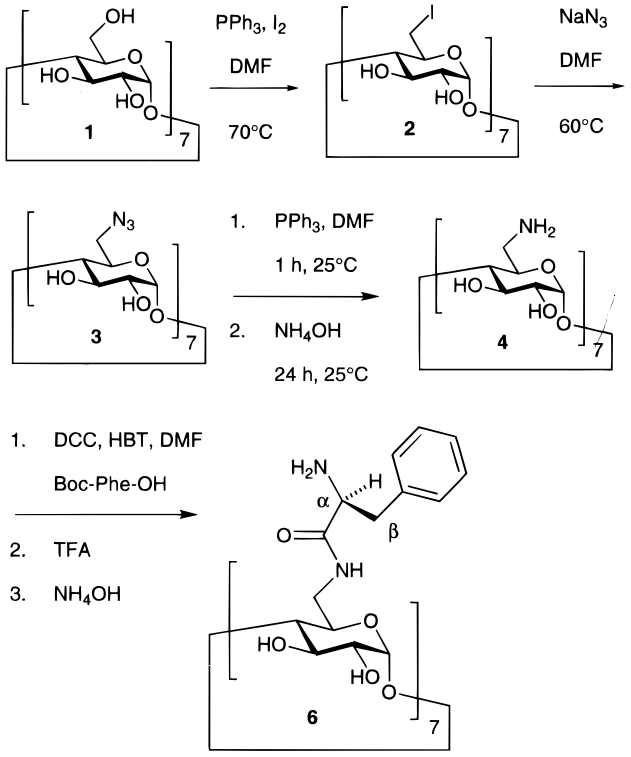
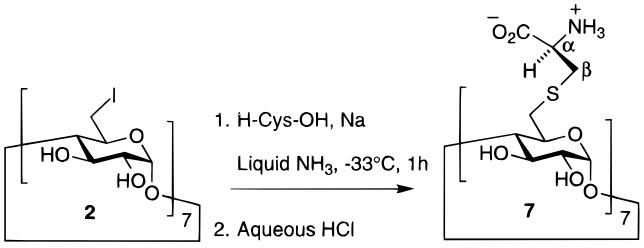
Scheme 1



mended by Defaye and Gadelle,[17] proved to be unsatisfactory in our hands. And so, a simple and efficient method for purifying the desired product by selective precipitation, followed by Soxhlet extraction, was developed. We believe that this method is superior even to a revised workup procedure which was reported subsequently.[18] Next, the heptaiodide **2** was treated with NaN_3 in DMF to give in 96% yield the heptaazide **3**[19] which was found to be a stable, easy-to-handle compound. Treating the heptaazide **3** with a Pd/C catalyst under 1 atm of H_2 did not prove to be successful as a means of reducing the azide functional groups to the corresponding amino functions.[20] However, a different reduction procedure, employing Ph_3P, was successful. This reduction of **3** proceeded very efficiently, and subsequent treatment of the intermediate with aqueous NH_3 solution allowed the isolation of the heptaamine **4**, which surprisingly, proved to be extremely insoluble in H_2O as well as in organic solvents. However, as the pH of an aqueous suspension of **4** was lowered to neutrality or slightly acidic conditions, the compound dissolved rapidly. Indeed, for NMR spectroscopic analysis, **4** had to be converted into its HCl salt, which is highly soluble in D_2O.

In the first instance, phenylalanine was chosen as the amino acid residue because of its inert side chain. Moreover, we argued that a method that would allow the exhaustive coupling of phenylalanine to the β-cyclodextrin torus would be applicable to any other amino acid which bears a less sterically demanding, inert side chain. The peptide coupling procedure employed in the case of phenylalanine was analogous to that introduced by

Scheme 2



Geiger and König.[21] *N*-Boc-phenylalanine (Boc = *tert*-butyloxycarbonyl) was treated with dicyclohexylcarbodiimide (DCC) in DMF in the presence of *N*-hydroxybenzotriazole (HBT) and *N*-methylmorpholine in order to couple it exhaustively with per-6-amino-β-cyclodextrin (**4**). The Boc-protected per-6-[(phenylalanyl)amino]-β-cyclodextrin derivative **5** was characterized only by fast atom bombardment mass spectrometry (FABMS) since hindered rotation associated with the Boc carbamate bonds gives rise to substantial line broadening in the ^1H NMR spectrum at 300 MHz. Removal of the Boc groups from **5** was achieved in neat trifluoroacetic acid (TFA), yielding the per-6-[(phenylalanyl)amino]-β-cyclodextrin (**6**) after a basic workup. Both the FABMS and the ^1H NMR spectra confirmed the fully substituted nature of the compound which is soluble in H_2O under neutral or acidic conditions. However, in alkaline aqueous media, as the free amine, per-6-[(phenylalanyl)amino]-β-cyclodextrin (**6**), is insoluble.

In order to address this problem, an amino acid derivative of a cyclodextrin was targeted that would retain the zwitterionic character of the pendant amino acids. This goal could only be reached if the amino acids were attached by their side chain functionalities[22] to the cyclodextrin torus. A synthetic approach, using cysteine and the per-6-iodo-β-cyclodextrin (**2**), proved to be successful (Scheme 2). The thiol group in the cysteine side chain can be converted easily under basic conditions into the thiolate anion, which can be used to displace[23] all the iodine atoms as anions from **2**. The best experimental conditions for generating the thiolate anion and inducing it to react with the heptaiodide **2** were found to involve the dissolution of cysteine or cystine in liquid NH_3 at −33 °C followed by the addition of 2 equiv of Na metal. Any

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(23) Further studies showed that nucleophilic displacement of iodide anions from per-6-iodo-β-cyclodextrin, employing poorer nucleophiles or elevated temperatures, favors the intramolecular substitution reaction, resulting in the formation of 3,6-anhydro-D-glucopyranose residues within the structure of per-6-iodo-β-cyclodextrin. See: Fujita, K.; Tahara, T.; Egashira, Y.; Yamamura, H.; Imoto, T.; Koga, T.; Fujioka, T.; Mihashi, K. *Chem. Lett.* **1988**, 705−708. Fujita, K.; Yamamura, H.; Imoto, T.; Fujioka, T.; Mihashi, K. *J. Org. Chem.* **1988**, *53*, 1943−1947. Fujita, K.; Egashira, Y.; Tahara, T.; Imoto, T.; Koga, T. *Tetrahedron Lett.* **1989**, *30*, 1285−1288. Fujita, K.; Tahara, T.; Koga, T. *Chem. Lett.* **1989**, 821−824. Fujita, K.; Tahara, T.; Yamamura, H.; Imoto, T.; Koga, T.; Fujioka, T.; Mihashi, K. *J. Org. Chem.* **1990**, *55*, 877−880. Yamamura, H.; Fujita, K. *Chem. Pharm. Bull.* **1991**, *39*, 2505−2508. Gadelle, A.; Defaye, J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 78−80. Ashton, P. R.; Ellwood, P.; Staton, I.; Stoddart, J. F. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 80−81. Ashton, P. R.; Ellwood, P.; Staton, I.; Stoddart, J. F. *J. Org. Chem.* **1991**, *56*, 7274−7280. Yamamura, H.; Ezaka, T.; Kawase, Y.; Kawai, M.; Butsugan, Y.; Fujita, K. *J. Chem. Soc., Chem. Commun.* **1993**, 636−637.

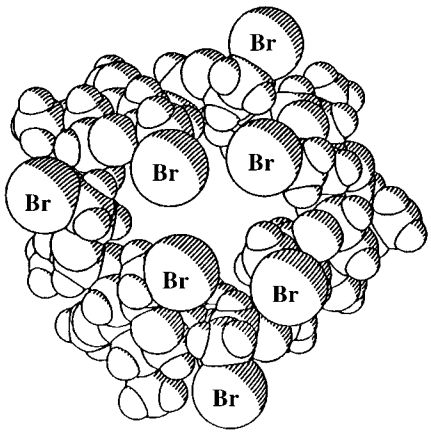
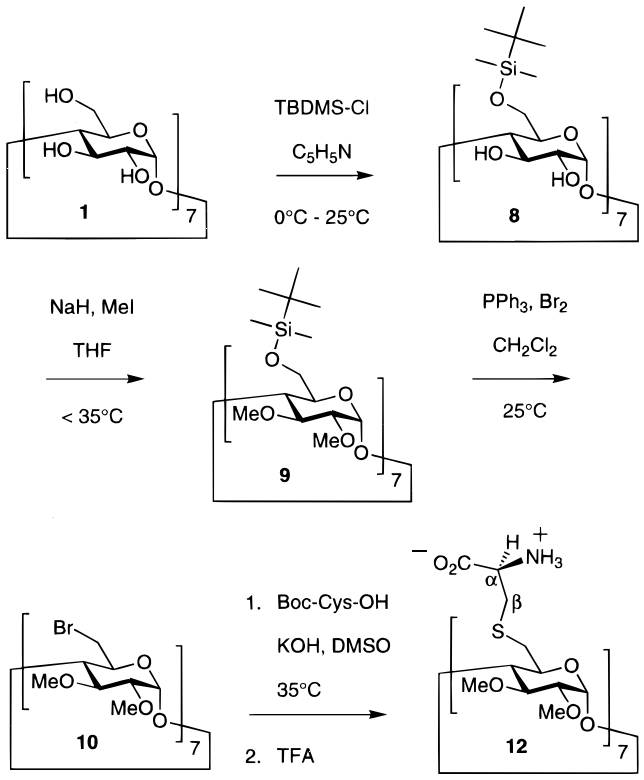


Figure 1. Space-filling representation of the solid state structure of per-6-bromo-per-2,3-*O*-dimethyl-β-cyclodextrin (**10**) viewed from the primary face of the molecule.

excess of Na had to be destroyed by the addition of NH_4Cl before the per-6-iodo-β-cyclodextrin (**2**) was added to the reaction mixture, which was maintained at the temperature of refluxing NH_3 for 1 h before the NH_3 was allowed to evaporate. After the NH_3 had evaporated completely, the residue was dissolved in a small amount of H_2O and neutralized to pH 7 by the addition of 2 N aqueous HCl. The heptazwitterionic compound **7** was then precipitated using an excess of EtOH to give a white hygroscopic powder. The solubility of **7** in H_2O was found to be similar to that of amino acids: at high and low pH, **7** displays very high solubility, whereas at neutral pH, concentrated solutions are turbid. Similarly, in the ^{1}H-NMR spectrum of **7**, the chemical shifts of all the signals are dependent on the pH, with the largest change in chemical shift being exhibited by the α protons of the cysteine residues. The hepta-Na salt of **7** is highly deliquescent and H_2O soluble, so much so that precipitation cannot be achieved at any concentration. A viscous mass and finally a glass forms as an aqueous solution is concentrated.

Protection of a more permanent kind for the secondary hydroxyl groups on β-cyclodextrin (**1**) was investigated next as a means of improving the organic solubility of per-6-cysteinyl-β-cyclodextrin (**7**), which is only soluble in H_2O. Methylation emerged as the method of choice for the modification of the secondary hydroxyl groups of β-cyclodextrin **1**. It was hoped that the analogue of **7** in which the secondary hydroxyl groups were methylated would exhibit better solubility characteristics in organic solvents. Direct methylation of **7** or the precursor heptaiodide **2** was not contemplated seriously because of the anticipated large number of side reactions. Consequently, a completely new synthesis of the methylated derivative of **7** was proposed. The selectivity with which all the primary hydroxyl groups of a cyclodextrin can be converted into TBDMS ether groups[24−26] and the possibility of direct replacement[27] of these TBDMS ether functions with bromine atoms suggested to us an efficient route (Scheme 3) to the desired compound **12** starting from β-cyclodextrin **1**. Per-6-(*tert*-butyldimethylsilyl)-β-cyclodextrin (**8**) was prepared from **1** using a literature

Scheme 3



procedure.[24,25] The per-2,3-O-dimethyl derivative **9** was accessible by exhaustive methylation of **8**, using a large excess of MeI and oil-free NaH in THF at room temperature. Desilylation and bromination were accomplished in one step by adding **9** to a suspension of Ph_3PBr_2 in CH_2Cl_2. As the reaction proceeded, the suspension cleared slowly. The product was recrystallized from Me_2CO and characterized as per-6-bromo-per-2,3-*O*-dimethyl-β-cyclodextrin (**10**). Analytically pure **10** could be obtained by flash column chromatography on silica gel using 70% *t*-BuOMe in hexane as the eluant. Even at this stage, the solubility of **10** in low boiling organic solvents was noted to be much enhanced compared with that of the unmethylated analog, per-6-bromo-β-cyclodextrin,[17] which could be prepared by a procedure analogous to that employed in the synthesis of per-6-iodo-β-cyclodextrin (**2**). Single crystals of the per-6-bromo-per-2,3-*O*-dimethyl-β-cyclodextrin (**10**), which proved to be of suitable quality for X-ray crystallographic analysis,[28] were grown by slow evaporation from an Me_2O solution. The X-ray crystal structure reveals (Figure 1) that the conformation of the molecule departs significantly from C_7 symmetry. The α-1,4-linked D-glucopyranose rings adopt different orientations with respect to each other, resulting in four of the bromomethyl groups being directed outward and three inward, partially blocking the free passage through the core of the molecule. The loss of symmetry within **10** is undoubtedly a consequence of the loss of the chain of circumferal intramolecular hydrogen bonds between the secondary hydroxyl groups of adjacent D-glucopyranose units in β-cyclodextrin.[29] The increased flexibility of **10** becomes apparent when its crystal structure is compared to that

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of its unmethylated analog.[30] Attempts were made to react the heptabromide **10** with an *N*-protected cysteine derivative. For example, per-6-bromo-per-2,3-*O*-dimethyl-β-cyclodextrin (**10**) was reacted with *N*-Boc-cysteine[31] under basic conditions. A variety of different conditions[32,33] were tried from which one employing powdered KOH in DMSO[34] emerged as the most effective method. These reaction conditions resulted in a very short reaction time of only a few minutes at room temperature to give a product which was found to move as one component by TLC. The ^{1}H NMR spectrum of **11** shows only extremely broad signals. Also, it could not be characterized by a variety of mass spectrometric techniques. As in the case of the Boc-protected per-6-[(phenylalanyl)amino]-β-cyclodextrin (**5**), this compound exhibits slow interconversions between the *Z* and *E* conformations for the carbamoyl functions associated with each of the seven cysteine residues on the per-(2,3-*O*-dimethyl)-β-cyclodextrin torus. Deprotection of **11** in neat TFA gave compound **12**, which displayed sharp signals in its ^{1}H NMR spectrum. As expected, compound **12** showed improved solubilities in organic solvents. For example, it dissolves readily in DMF, DMSO, and C_5H_5N, and as its hepta-TFA salt, it dissolves in Me_2CO and THF.

Conclusion

The synthetic methodology which is described in this paper affords gram quantities of the β-cyclodextrin derivatives **6**, **7**, and **12**, which are symmetrically substituted with either phenylalanine or cysteine. It is hoped that these chemically modified cyclodextrins will show higher and more selective complexation and solubilization of guests than the naturally occurring cyclodextrins. Furthermore, these amino acid derivatives of β-cyclodextrin may be used to assemble peptides on β-cyclodextrins.[16,35]

Experimental Section

General. Unless otherwise stated, chemicals were used as received. When dry solvents are referred to, they were dried by the following procedures: (i) DMF was dried over CaH_2 and filtered, (ii) pyridine (C_5H_5N) was dried over CaH_2 and distilled, and (iii) THF was distilled under N_2 from a solution containing the Na/Ph_2CO ketal. Thin layer chromatography (TLC) was performed on aluminum sheets coated with silica gel. Where appropriate, the TLC plates were scrutinized by ultraviolet light, before being developed with 5% H_2SO_4 in EtOH, followed by heating in an oven or, preferably, by using a heat gun. Column chromatography was performed using silica gel (particle size 0.040−0.063 mm). Fast atom bombardment mass spectra (FABMS) were obtained with a spectrometer on which the FAB unit was operated at 8 keV by use of a krypton primary atom beam with a 3-nitrobenzyl alcohol (NOBA) matrix. Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry was carried out using a gentisic acid (2,5-dihydroxybenzoic acid) matrix. Liquid secondary ion mass spectrometry (LSIMS) was performed using a cesium ion beam. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded at either 270, 300, or 400 MHz using the solvent peaks as internal references. ^{13}C NMR spectra were recorded at either 75 or 100 MHz.

Per-6-iodo-β-cyclodextrin (2). The reaction conditions for the preparation of this compound are analogous to those described by Defaye and Gadelle;[14] *i.e.*, Ph_3P (40.1 g, 153 mmol) was dissolved with stirring in dry DMF (160 mL). To this solution was carefully added I_2 (40.5 g, 160 mmol) over 10 min with the evolution of heat: the solution reaches approximately 50 °C. Dry β-cyclodextrin (**1**) (11.6 g, 10.2 mmol) was then added to this dark brown solution, and the temperature was raised to 70 °C. At this temperature, the solution was stirred under an atmosphere of N_2 for 18 h. Heating was then discontinued and the solution concentrated under reduced pressure by the removal of DMF (approximately 100 mL). NaOMe in MeOH (3 M, 60 mL) was then prepared by adding Na (4.2 g) to MeOH (60 mL) under an inert atmosphere with efficient cooling. This NaOMe solution was then added to the reaction vessel with cooling, and the reaction mixture was stirred for 30 min. The following reaction workup deviates from the literature procedure. Instead of precipitating the product with ice−water, the reaction mixture was poured into MeOH (800 mL) to form a precipitate, which was washed with MeOH, superficially dried, and Soxhlet extracted with MeOH for 20 h or until no more discoloration of the solvent could be detected. The product was removed from the Soxhlet extractor and allowed to air dry before being dried under high vacuum. Compound **2** (17.9 g, 92%) was recovered as a white powder: FABMS *m*/*z* 1927 for $[M + Na]^+$, calcd for $C_{42}H_{63}O_{28}I_7$ M 1904; δ_H (300 MHz, CD_3SOCD_3) 3.28 (t, J = 9 Hz, 7H), 3.34−3.48 (m, 14H), 3.54−3.68 (m, 14H), 3.80 (bd, J = 9 Hz, 7H), 4.99 (d, J = 3 Hz, 7H), 5.94 (d, J = 2 Hz, 7H), 6.05 (d, J = 6.5 Hz, 7H); δ_C (75 MHz, CD_3SOCD_3) 9.5, 71.0, 72.0, 72.3, 86.0, 102.2.

Per-6-azido-β-cyclodextrin (3). Per-6-iodo-β-cyclodextrin (**2**) (2.99 g, 1.57 mmol) was dissolved in DMF (50 mL), and NaN_3 (1.00 g, 15.4 mmol) was added. The resulting suspension was stirred at 60 °C under an atmosphere of N_2 for 20 h. The suspension was then concentrated under reduced pressure to a few milliliters before a large excess of H_2O was added. A fine white precipitate was formed and was filtered off carefully. The precipitate was washed with H_2O and dried under high vacuum to yield a stable white powder. The product never showed any sign of decomposition, even when the dry powder was heated to 80 °C. Compound **3** (2.01 g, 98%) was recovered: IR (Nujol suspension on NaCl plates) 3355 cm^{-1} (OH), 2106 cm^{-1} (N_3); LSIMS *m*/*z* 1332 for $[M - H + Na]^+$, 1354 for $[M - 2H + 2Na]^+$, 1376 for $[M - 3H + 3Na]^+$; calcd for $C_{42}H_{63}N_{21}O_{28}$ M 1310; δ_H (300 MHz, CD_3SOCD_3) 3.30−3.42 (m, 14H), 3.53−3.65 (m, 14H), 3.68−3.82 (m, 14H), 4.91 (d, J = 3 Hz, 7H), 5.77 (d, J = 2 Hz, 7H), 5.92 (d, J = 7 Hz, 7H); δ_C (75MHz, CD_3SOCD_3) 51.4 , 70.4, 72.1, 72.7, 83.3, 102.1. Anal. Calcd for $C_{42}H_{63}N_{21}O_{28}$: C, 38.5; H, 4.81; N, 22.4. Found: C, 38.5; H, 5.05; N, 21.9.

Per-6-amino-β-cyclodextrin (4). The heptaazide **3** (2.01 g, 1.53 mmol) was dissolved in DMF (40 mL), and Ph_3P (6.36 g, 24.2 mmol) was added. The evolution of N_2 can be observed by the formation of bubbles in the reaction vessel. After 1 h, during which time the evolution of N_2 ceased, concentrated aqueous NH_3 (6 mL, approximately 35%) was added dropwise

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to the solution. Shortly after the addition of the NH_3 solution was complete, the reaction mixture turned into an off-white suspension. It was stirred at rt for 18 h before the resulting suspension was concentrated under reduced pressure to approximately 10 mL. The product was then precipitated by the addition of EtOH (100 mL). The precipitate was washed with EtOH and dried under high vacuum to yield a white solid. Compound **4** (1.69 g, 98%) was recovered. To allow characterization by NMR spectroscopy, the HCl salt of **4** was formed by suspending compound **4** in a small volume of H_2O followed by the addition of a dilute solution of HCl until the pH had reached 6. At this pH, a clear solution formed which gave a yellow glass when evaporated under reduced pressure: $[\alpha]_D$ +112° (c = 1 in H_2O at 25 °C); FABMS m/z 1128 for $[M]^+$, calcd for $C_{42}H_{77}O_{28}N_7$ M 1128; δ_H (300 MHz, D_2O) 3.26 (dd, J = 7, 13 Hz, 7H), 3.44 (dd, J = 3, 13 Hz, 7H), 3.57 (t, J = 9 Hz, 7H), 3.66 (dd, J = 3.5, 9.5 Hz, 7H), 3.98 (dd, J = 9, 9.5 Hz, 7H), 4.15−4.25 (ddd, J = 3, 7, 9 Hz, 7H), 5.15 (d, J = 3.5 Hz, 7H); δ_C (75MHz, D_2O) 42.9, 70.5, 74.3, 74.8, 84.8, 104.1.

Per-6-[(*N*-Boc-phenylalanyl)amino]-β-cyclodextrin (5). *N*-Boc-phenylalanine[21] (0.850 g, 3.20 mmol) was dissolved in dry DMF (40 mL). HBT (0.460 g, 3.40 mmol) was added, and the solution was cooled to 0 °C in an ice bath. DCC (0.670 g, 3.25 mmol) was then added, and the temperature was maintained at 0 °C for a further 60 min. The reaction mixture was then allowed to warm to rt, during which time dicyclohexylurea precipitated out. It was stirred for a further 60 min at ambient temperature, a suspension of per-6-amino-β-cyclodextrin (**4**) (0.510 g, 0.452 mmol) and ethylmorpholine (0.34 mL) in DMF (20 mL) was added to the reaction vessel, and the reaction mixture was stirred at rt for 20 h. The precipitated dicyclohexylurea was filtered off, and the filtrate was concentrated under reduced pressure at 50 °C to obtain an oil. Then, saturated aqueous $NaHCO_3$ (200 mL) was added to this oil to give a suspension which was stirred for 1 h and then filtered. The precipitate was washed with H_2O and dried under high vacuum. Compound **5** (1.13 g, 89%) was recovered. As the ^1H NMR spectrum showed very broad signals, this compound was only identified by FABMS: m/z 2880 for $[M + Na]^+$, calcd for $C_{140}H_{196}N_{14}O_{49}$ M 2857.

Per-6-[(phenylalanyl)amino]-β-cyclodextrin (6). Per-6-[(*N*-Boc-phenylalanyl)amino]-β-cyclodextrin (**5**) (1.13 g, 0.395 mmol) was added to neat TFA (2 mL). The solution was agitated for 1 h before the TFA was evaporated off under reduced pressure at rt. The resulting oil was added to Et_2O (50 mL) and sonicated to give a fine suspension which was carefully filtered. The precipitate was washed with Et_2O and dried under high vacuum to give a yellow/orange hepta-TFA acid salt. This solid was dissolved in a small amount of H_2O and precipitated by the addition of concentrated aqueous NH_3 solution. The precipitate was filtered off and dried under high vacuum. Compound **6** (0.295 g, 35%) was recovered: $[\alpha]_D$ +78° (c = 1 in DMF at 25 °C); FABMS m/z 2158 for $[M]^+$, calcd for $C_{105}H_{140}N_{14}O_{35}$ M = 2158; δ_H (300 MHz, CD_3SOCD_3) 1.71 (bs, 14H), 2.56 (dd, J = 9, 14 Hz, 7H), 2.90 (dd, J = 4, 14 Hz, 7H), 3.15−3.65 (m, 42H), 3.85 (bd, J = 9 Hz, 7H), 4.77 (bd, J = 3 Hz, 7H), 5.78 (bs, 7H), 5.87 (d, J = 6.5 Hz, 7H), 7.13-7.25 (m, 35H), 8.08 (bs, 7H); δ_C (75MHz, CD_3SOCD_3) = 39.2, 41.2, 55.7, 70.2, 72.3, 72.7, 83.2, 102.0, 126.1, 128.0, 129.4, 138.9, 175.1. Anal. Calcd for $C_{119}H_{147}F_{21}N_{14}O_{49}$ (the hepta-TFA salt): C, 48.9; H, 5.04; N, 6.72. Found: C, 48.8; H, 5.52; N, 7.06.

Per-6-cysteine-β-cyclodextrin (7). Cysteine (0.993 g, 8.19 mmol) was weighed into a three-necked round-bottom flask. The flask was cooled in an *i*-PrOH/dry ice bath, and NH_3 (100 mL) was condensed into the vessel using a dry ice condenser. Na (0.38 g, 16.5 mmol) was then added to the solution, and the reaction mixture was allowed to warm to NH_3 reflux temperature (−33 °C). After the dark blue color had persisted for 20 min, the excess of Na was destroyed by the addition of NH_4Cl. Per-6-iodo-β-cyclodextrin (**2**) (2.208 g, 1.15 mmol) was then added to the solution, and the reaction mixture was stirred for 30 min. The NH_3 was allowed to evaporate over 2 h. The solid residue was then taken up in a small volume of H_2O to give a white suspension, which was filtered through a pad of Celite. The hepta-Na salt could then be precipitated from this filtrate by the addition of an excess of EtOH. The precipitate was washed with EtOH and dried under high vacuum at 80 °C to give a deliquescent white solid (2.19, 94%). To obtain the zwitterionic compound **7**, the aqueous solution was neutralized with dilute HCl before precipitation of the product was effected with EtOH, $[\alpha]_D$ +73° (c = 1 in 1 M $NaOH_{aq}$ at 25 °C). This compound did not give a FABMS under a variety of different conditions: MALDI-TOFMS m/z 1859 for $[M + H]^+$, 1881 for $[M + Na]^+$, 1900 for $[M - H + 2Na]^+$, 1921 for $[M - 2H + 3Na]^+$, calcd for $C_{63}H_{105}N_7O_{42}S_7$ M 1857; δ_H (300 MHz, 0.1 M NaOD in D_2O) 2.82 (dd, J = 8, 14 Hz, 7H), 2.93 (dd, J = 7.5, 14 Hz, 7H), 3.05 (dd, J = 5, 14 Hz, 7H), 3.13 (bd, J = 14 Hz, 7H), 3.47 (dd, J = 5, 8 Hz, 7H), 3.56 (t, J = 9 Hz, 7H), 3.58 (dd, J = 4, 9 Hz, 7H), 3.87 (t, J = 9 Hz, 7H), 3.95 (bdd, J = 7.5, 9 Hz, 7H), 5.08 (d, J = 4 Hz, 7H); δ_C (75 MHz, 0.1 M NaOD in D_2O) 36.2, 41.1, 58.1, 74.5, 74.7, 75.5, 86.1, 104.0, 182.2. Elemental analysis was performed on the hepta-Na salt. Anal. Calcd for $C_{63}H_{98}O_{42}N_7S_7Na_7 \cdot 7H_2O$: C, 35.4; H, 5.24; N, 4.58. Found: C, 35.3; H, 5.33; N, 4.47.

Per-6-(*tert*-butyldimethylsilyl)-β-cyclodextrin (8). Dry β-cyclodextrin (**1**) (9.04 g, 7.96 mmol) was dissolved under vigorous stirring in dry C_5H_5N (100 mL). The solution was cooled on an ice bath, producing a thick gel. A solution of TBDMSCl (14.5 g, 96.2 mmol) in dry C_5H_5N (150 mL) was then added dropwise to the cooled reaction vessel over 3.5 h. During this time, the gel liquified. Cooling was continued for a further 3 h before the solution was allowed to warm to rt. After a further 18 h at rt, the solvent was removed under reduced pressure to give a white solid, which was taken up in CH_2Cl_2 (300 mL). The CH_2Cl_2 layer was washed with $KHSO_4$ (200 mL, 1 M) to remove any residual C_5H_5N, followed by saturated aqueous NaCl solution. The CH_2Cl_2 layer was recovered and evaporated to dryness. Compound **8** (14.64, 95%) was recovered. In order to remove any last traces of TBDMSCl and to obtain high purity samples for analytical purposes, the product can be subjected to column chromatography on silica gel using EtOAc/hexane as eluant: LSIMS m/z 1958 for $[M + Na]^+$, calcd for $C_{84}H_{168}O_{35}Si_7$ M 1935; δ_H (300 MHz, $CDCl_3$) 0.03 (s, 21H), 0.04 (s, 21H) 0.86 (s, 63H), 3.55 (dd, J = 9.5, 9.5 Hz, 7H), 3.59 (bs, 7H), 3.65 (dd, J = 3, 9.5 Hz, 7H), 3.70 (bd, J = 11 Hz, 7H), 3.89 (dd, J = 2, 11 Hz, 7H), 4.03 (dd, J = 9.5, 9.5 Hz, 7H), 4.88 (d, J = 3 Hz, 7H), 5.26 (s, 7H), 6.72 (s, 7H); δ_C (75 MHz, $CDCl_3$) −5.2, −5.1, 18.3, 25.9, 61.7, 72.6, 73.4, 73.6, 81.8, 102.0.

Per-6-(*tert*-butyldimethylsilyl)per-2,3-methyl-β-cyclodextrin (9). NaH 60% (11.6 g, 289 mmol) was weighed into the reaction vessel. Hexane (100 mL) was added, and the mixture was stirred for 1 min before the suspension was allowed to settle and the hexane was decanted off. This hexane washing procedure was repeated two more times to obtain oil-free NaH. Per-6-(*tert*-butyldimethylsilyl)-β-cyclodextrin (**8**) (16.80 g, 8.68 mmol) was dissolved in dry THF (100 mL) and added very carefully to the NaH with cooling. Once the evolution of H_2 had subsided, MeI (20 mL, 321 mmol) was added and stirring was commenced. The reaction mixture was protected from light and placed under an atmosphere of N_2. Cooling was maintained for the first hour of the reaction, after which time the suspension was allowed to warm to rt. After 16 h, the reaction mixture was cooled on an ice bath and MeOH was added dropwise to destroy the excess of NaH and MeI. Once all the NaH had been destroyed, the THF was removed under reduced pressure and the residue was suspended in CH_2Cl_2. The CH_2Cl_2 layer was washed with H_2O, followed by saturated aqueous NaCl solution. The CH_2Cl_2 layer was recovered and evaporated to dryness to yield an off-white solid. Compound **9** (18.30 g, 99%) was recovered: $[\alpha]_D$ +95° (c = 1 in $CHCl_3$ at 25 °C); FABMS m/z 2153 for $[M + Na]^+$, calcd for $C_{98}H_{196}O_{35}Si_7$ M 2130; δ_H (300 MHz, $CDCl_3$) 0.02 (s, 21H), 0.03 (s, 21H), 0.86 (s, 63H), 3.04 (dd, J = 3.5, 10 Hz, 7H), 3.47−3.75 (m, 28H), 3.49 (s, 21H), 3.64 (s, 21H), 4.10 (dd, J = 2, 12 Hz, 7H), 5.18 (d, J = 3.5 Hz, 7H); δ_C (75 MHz, $CDCl_3$) −5.2, −4.8, 18.3, 25.9, 58.6 and 61.5, 62.3, 72.2, 78.7, 82.0, 82.2, 98.1.

Per-6-bromo-per-2,3-methyl-β-cyclodextrin (10). Ph_3PBr_2 was freshly prepared by careful addition of Br_2 (1.1 mL, 21 mmol) to Ph_3P (5.59 g, 21.3 mmol) dissolved in CH_2Cl_2 (100 mL) maintained on an ice bath If the solution is slightly red

in color, more Ph_3P may be added to dispel the coloration. When the reaction mixture was warmed to rt, a white suspension was formed. Per-6-(*tert*-butyldimethylsilyl)per-2,3-methyl-β-cyclodextrin (**9**) (5.99 g, 2.81 mmol) was then added to this suspension, and the reaction mixture was stirred for 24 h at rt, during which time the suspension cleared. The CH_2Cl_2 solution was then washed with saturated aqueous $NaHCO_3$ solution, followed by saturated aqueous NaCl solution. The CH_2Cl_2 layer was recovered and concentrated under reduced pressure to yield a thick oil. The oil was triturated with EtOH to yield a white precipitate after sonication. The precipitate was allowed to settle for 10 min before filtering it off. Compound **10** (3.93 g, 79%) was recovered. Further purification was often not necessary. However, analytically pure samples may be prepared by submitting the product to column chromatography on silica gel using a *t*-BuOMe/hexane eluant. Crystals of compound **10** were grown by slow evaporation from a concd Me_2CO solution in only a few hours: $[\alpha]_D$ +109° (c = 1 in $CHCl_3$ at 25 °C); FABMS m/z 1794 for $[M + Na]^+$, calcd for $C_{56}H_{91}Br_7O_{28}$ M 1771; δ_H (300 MHz, $CDCl_3$) 3.22 (dd, J = 3.5, 9.5 Hz, 7H), 3.47−3.65 (m, 14H), 3.53 (s, 21H), 3.65 (s, 21H), 3.76−3.95 (m, 21H), 5.24 (d, J = 3.5 Hz, 7H); δ_C (75 MHz, $CDCl_3$) 34.3, 58.9 and 61.5, 70.8, 81.5, 81.6, 81.9, 98.3 (C-1). Anal. Calcd for $C_{56}H_{91}Br_7O_{28}$: C, 38.0; H, 5.18. Found: C, 37.9; H, 5.50.

Per-6-[*N*-(*tert*-butyloxycarbonyl)cysteinyl]-per-2,3-methyl-β-cyclodextrin (11). Per-6-bromo-per-2,3-methyl-β-cyclodextrin (**10**) (2.03 g, 1.15 mmol) was dissolved in DMSO (35 mL). *N*-Boc-cysteine[31] (2.43 g, 10.2 mmol) was then added to the solution, followed by powdered KOH (3.258 g, 58 mmol). The suspension was placed under an atmosphere of N_2 and stirred at rt for 1 h. The solution was then partitioned between aqueous $KHSO_4$ (150 mL, 1 M) and EtOAc (100 mL). The EtOAC layer was washed with saturated aqueous NaCl solution (3 × 75 mL) and then dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was then applied to a column and eluted using 10% MeOH in CH_2Cl_2 to give a white solid. Compound **11** (2.00 g, 63%) was recovered, $[\alpha]_D$ +88° (c = 1 in $CHCl_3$ at 25 °C). It was not possible to obtain a mass spectrum of this compound despite applying a range of different mass spectrometric techniques to this sample: δ_H (300 MHz, $CDCl_3$) 1.46 (bs, 63H), 2.80−3.90 (2 bs, 81H), 4.80 (bs, 7H). Anal. Calcd for $C_{112}H_{189}N_7O_{56}S_7 + 7.CH_2Cl_2$: C, 42.5; H, 6.06; N, 2.93. Found: C, 42.5; H, 6.09; N, 3.05.

Per-6-cysteinyl-per-2,3-methyl-β-cyclodextrin (12). Per-6-[*N*-(*tert*-butyloxycarbonyl)cysteinyl]-per-2,3-methyl-β-cyclodextrin (**11**) was dissolved in neat TFA, and the reaction mixture was stirred at rt for 1 h. The TFA was then removed under reduced pressure, ensuring that the temperature did not exceed rt. The residual glass was dissolved in a minimum of H_2O and neutralized using *i*-Pr_2EtN followed by precipitation with an excess of Me_2CO. The flask was allowed to stand for 2 h before the precipitate was filtered off in near-quantitative yield, $[\alpha]_D$ +78° (c = 1 in DMF at 25 °C). The specific optical rotation for this compound was also determined on the hepta-TFA salt: $[\alpha]_D$ +49° (c = 1 in H_2O at 25 °C); MALDI-TOFMS m/z 2079 for $[M + Na]^+$, calcd for $C_{77}H_{133}O_{42}N_7S_7$ M 2053; the hepta-TFA salt of **12** was analyzed by ^{1}H-NMR δ_H (300 MHz, D_2O) 3.02 (bd, J = 13 Hz, 7H), 3.10−3.20 (m, C-6, 14H), 3.25 (dd, J = 4, 13 Hz, 7H), 3.30 (dd, J = 3, 9 Hz, 7H), 3.43 (s, 21H), 3.54 (s, 21H), 3.57 (m, 7H), 3.70 (t, J = 9 Hz, 7H), 3.91−3.98 (m, 7H), 4.21 (dd, J = 4, 8 Hz, 7H), 5.19 (d, J = 3 Hz, 7H); δ_C (75 MHz, D_2O) = 36.7, 37.5, 56.8, 60.8, 62.9, 74.5, 82.5, 83.4, 100.5, 176.0.

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