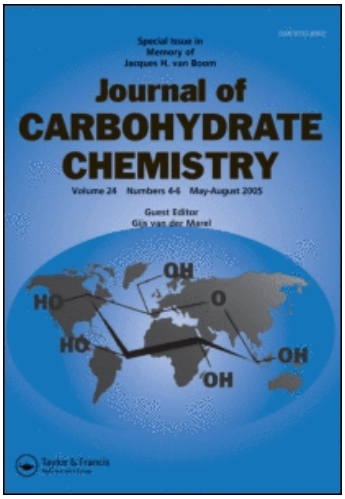

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SYNTHESIS AND DRUG COMPLEXATION STUDIES WITH β-CYCLODEXTRINS FLUORINATED ON THE PRIMARY FACE

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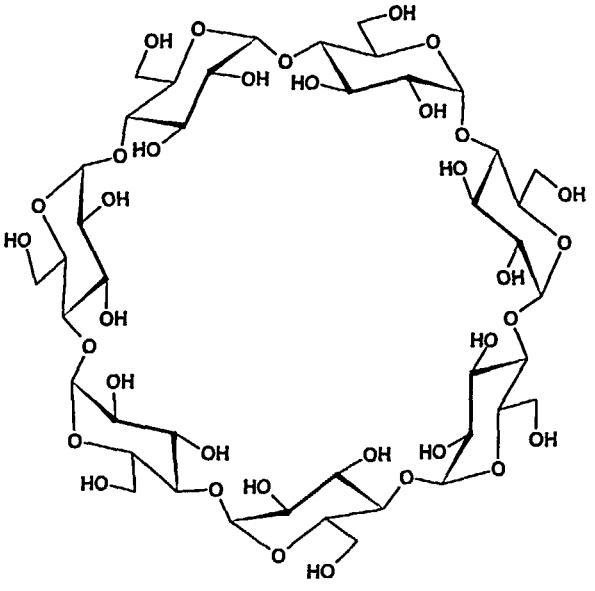
ABSTRACT

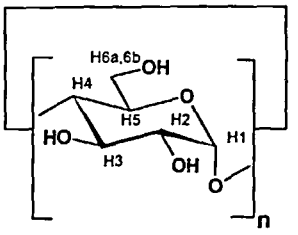
Three fluorinated β-cyclodextrin derivatives, namely **5**, **9** and **12**, were prepared with the hope that the fluorine reporter group may assist in direct evaluation of the complexation properties of these potential drug carriers. Two of the synthesized derivatives, the previously reported monofluoro-β-cyclodextrin **9** and the novel trifluoroethylthio-β-cyclodextrin **12**, displayed reasonable aqueous solubility and thus were suitable for drug-cyclodextrin complexation studies. Preliminary NMR results (^{1}H and ^{19}F) on the host-guest complex formation of both of these cyclodextrin derivatives with amantidine, a therapeutic agent employed in the treatment of Influenza A infections, are also presented.

INTRODUCTION

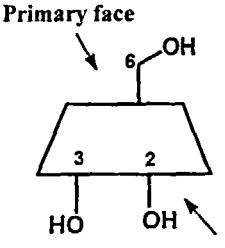
Cyclodextrins (CyDs) are a homologous family of cyclic oligosaccharides that are constructed of α-(1→4)-linked glucopyranose units.[1] The three major cyclodextrins consist of six (α-CyD), seven (β-CyD) and eight (γ-CyD) monosaccharide subunits, and in terms of molecular architecture, can be viewed as toroidal baskets.[2] This intrinsic



β-Cyclodextrin



α-Cyclodextrin (n = 6)
β-Cyclodextrin (n = 7)
γ–Cyclodextrin (n=8)



Scheme 1: Cyclodextrins

cyclic nature has enabled CyDs to function as hosts in the formation of host-guest inclusion complexes, and these macrocycles have found widespread medicinal application as carriers of pharmaceuticals and biochemicals.[3] The basket-like architecture segregates the primary hydroxyl moieties on one face (primary face) and the secondary hydroxyl groups on the other (secondary face) and thus, the scaffold is amenable to regioselective exploitation (Scheme 1).

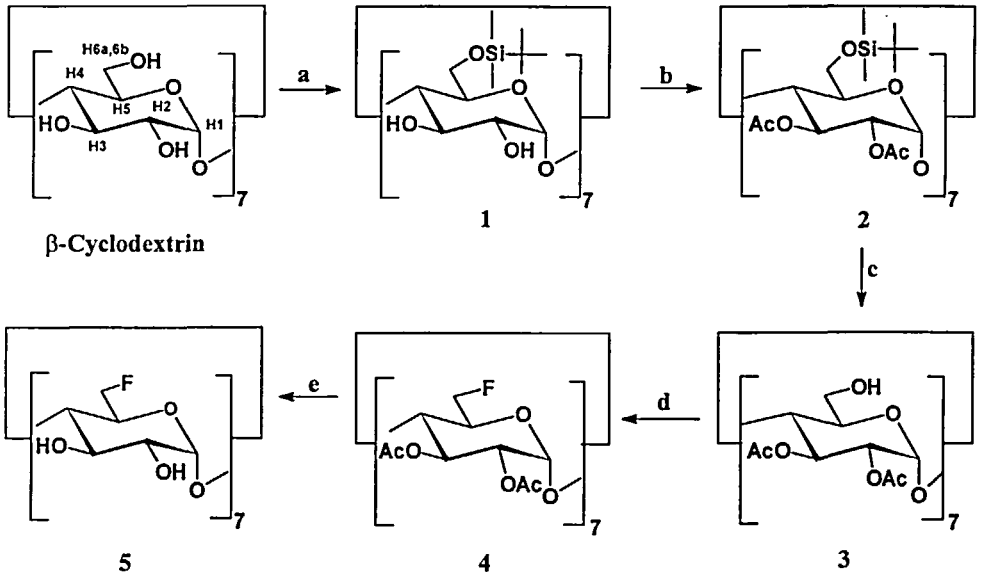
Confirmation of the formation of a host-guest complex has traditionally been accomplished by laborious X-ray crystallographic studies.[4] More recently, detailed proton NMR studies have revealed that upon complexation in aqueous solution, the magnitude of the change in chemical shift of the CyD H3 and H5 protons can be correlated to both complex stability and depth of penetration of the guest molecule into the cavity.[5] In the case of fluorobenzoate guests, ^{19}F NMR has been employed to monitor complexation events. Encapsulation of the ^{19}F-labelled guest has revealed small but measurable (< 2.5 ppm) chemical shift variations.[6] It seemed reasonable to us that the reverse situation,

namely a fluorine-labelled CyD may serve as a more general probe for investigating complexation events by ^{19}F NMR, particularly in the case of aromatic guests.

Very few fluorinated cyclodextrin derivatives have been reported to date,[7] thus, our first task was to establish methodology for the preparation of fluorinated-CyD (F-CyD) derivatives for ^{19}F NMR analysis of complex formation. Fluorination of the cyclodextrin scaffold is more readily accomplished at the primary face and is the focus of this report. We also describe herein our preliminary results on the preparation of F-CyD complexes.

RESULTS AND DISCUSSION

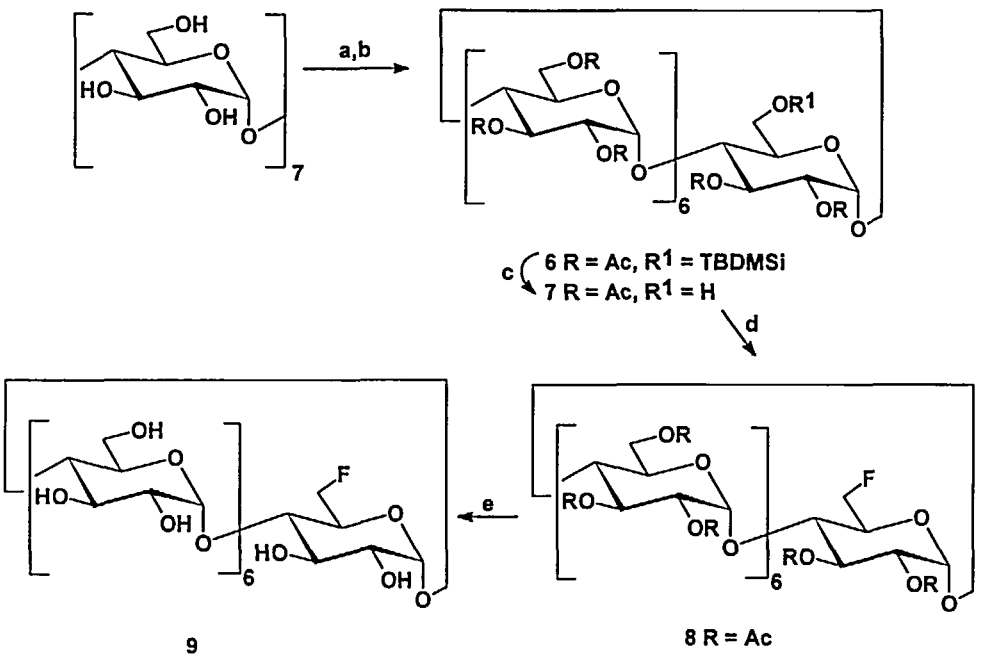
As part of our ongoing interest in the preparation of drug delivery agents with labels suitable for magnetic resonance imaging (MRI), CyDs bearing fluorine atoms on the primary face (compounds **5**, **9**, and **12**) were synthesized. The *tert*-butyldimethylsilyl (TBDMS) group[8] has proven its value as a protecting group in innumerable instances, and its utility has been extended to include selective protection of the primary face of cyclodextrins.[9] Furthermore, treatment of CyDs with >14 equivalents of TBDMS-Cl has resulted in regioselective discrimination between the two types of secondary hydroxyl groups[10] and subjection of the 2,6-di-*O*-TBDMSi-CyD to migration/alkylation conditions opens the door for expanding the repertoire of novel chemically modified CyDs.[11,12] The synthesis of the symmetrical heptakis-(6-deoxy-6-fluoro)-β-CyD **5** was initiated by reaction of β-CyD with 7.7 equivalents of *tert*-butyldimethylsilyl chloride in dry pyridine to provide **1** (Scheme 2) as per literature protocol.[9] The ^1H NMR spectral data for this precursor (in $CDCl_3$ solution) was in good agreement with proton NMR data reported in a recent detailed study.[13] Acetylation of **1** in 2:1 pyridine-acetic anhydride was sluggish at room temperature as has been previously noted,[14] thus, heating (100 °C, 4.5 h) was required in order to obtain the fully protected CyD derivative **2** in 84% yield after chromatography over silica gel. The ^{13}C NMR spectrum of **2** indicated a highly symmetrical structure consistent with earlier reported data.[10] Desilylation was accomplished with boron trifluoride etherate in chloroform,[15] and the reaction proceeded smoothly to provide the heptakis-(2,3-di-*O*-acetyl) derivative **3** (78%). Conversion of **3**



Scheme 2: a) TBDMSiCl/Py, b) 2:1 Py/Ac_2O, c) BF_3·Et_2O, d) DAST, e) NaOMe/MeOH.

(wherein only the primary hydroxyl moieties are exposed) to the heptafluoro-CyD **4** was performed with excess diethylaminosulfur trifluoride (DAST) in chloroform. The yield for this transformation was low (35%). *O*-Deacylation in methanolic sodium methoxide solution yielded the heptakis-(6-fluoro-6-deoxy)-β-CyD **5**. Diagnostic signals in the ^{13}C NMR spectrum of highly symmetrical CyD derivative **5** (DMSO-d_6) were found at 82.34 ppm ($J_{C,F}$ = 168 Hz, C6) and 70.15 ppm ($J_{C,F}$ = 18 Hz, C5). The ^{19}F NMR spectrum of **5** displayed the anticipated triplet of doublets and heteronuclear decoupling experiments involving irradiation of the H5 (3.86 ppm) and H6 (4.62 ppm) protons collapsed the ^{19}F NMR signal to the corresponding triplet and doublet, respectively.

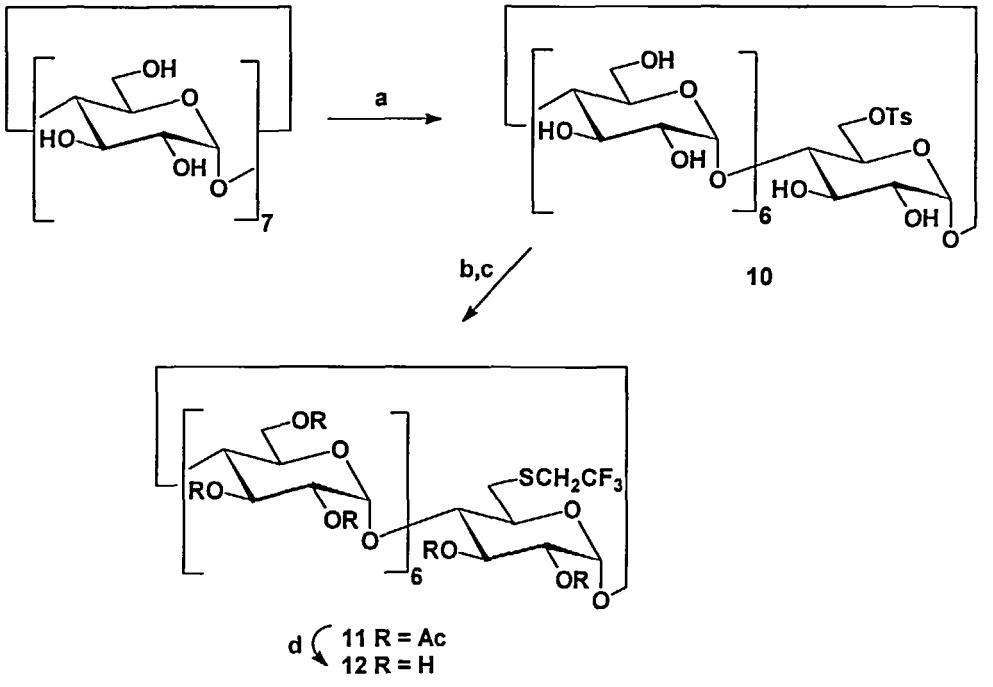
Due to the poor aqueous solubility of **5**, the previously reported mono-6-deoxy-6-fluoro-β-CyD **9**[7] was also prepared by minor modification of the literature protocol (Scheme 3). Silylation of β-cyclodextrin was carried out with 1.25 equivalents of *tert*-butyldimethylsilyl chloride in dry pyridine. After 4 h, a large excess of acetic anhydride was introduced and the acetylation reaction was continued overnight at 60 °C. This reaction sequence provided **6** in 40% yield after purification over silica gel. Again, de-*O*-



Scheme 3: a) 1.25 equiv TBDMSiCl/Py, b) Py/Ac_2O, c) $BF_3{\cdot}Et_2O$, d) DAST, e) NaOMe/MEOH.

silylation was accomplished with boron trifluoride etherate and monohydroxyl compound **7** was produced in 91% yield. Subsequently, compound **7** was converted into the corresponding monofluoro-CyD **8** by treatment with DAST and was then purified by column chromatography over silica gel. The yield of the mono-fluorination reaction was 89% as could be predicted from the previous fluorination reaction leading to **4**. De-*O*-acetylation of **8** in methanolic sodium methoxide furnished mono-6-deoxy-6-fluoro-β-CyD **9**. The ^{19}F NMR spectrum and elemental analysis for this compound have previously been reported,[7] and we have expanded the characterization to also include mass, ^{1}H and ^{13}C NMR spectral data. The proton NMR spectrum revealed heteronuclear couplings of 47 Hz and 30 Hz for geminal and vicinal couplings respectively for the fluorinated glucose moiety. Again, although this fluorinated derivative displayed unfavorable aqueous solubility properties, its solubility was superior to that of heptafluoro-CyD **5**.

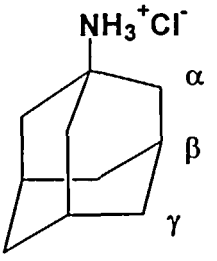
In an attempt to improve aqueous solubility and ^{19}F signal intensity, the synthesis of trifluoroethylthio-β-CyD was initiated. The known mono-6-*O*-toluenesulfonyl-β-CyD



Scheme 4: a) TsCl/Py, b) $CF_3CH_2S^-Na^+$, c) Py/Ac_2O, d)NaOMe/MeOH.

10[16] (Scheme 4) was reacted with 2,2,2-trifluoroethanethiol (excess) at 70 °C in 0.7 M aqueous sodium hydrogen carbonate and the crude material obtained upon work-up was then directly acetylated to provide **11** (62%) after purification over silica gel. Finally, de-*O*-acetylation of **11** in methanolic sodium methoxide afforded the 6-deoxy-6-trifluoroethylthio-β-CyD **12**. Although **12** can be recrystallized from water, this cyclodextrin derivative displays improved aqueous solubility over that of both **5** and **9**.

We have utilized NMR to investigate complex formation between β-cyclodextrin and amantidine (adamantanamine hydrochloride) **13** which is currently employed in the prophylactic and therapeutic treatment of Influenza A virus infections. During the course of our investigation with native β-CD, we have found that complex formation between equimolar amounts of β-cyclodextrin and 1-adamantanamine hydrochloride is rapid, and upon host-guest complexation, the host's H3 and H5 protons are shielded by 0.1 and 0.08 ppm, respectively. The notable H5 shielding implies reasonable penetration of the guest



13 (amantidine)

into the cavity. Concomitantly, the 1-adamantanamine β (methine) protons at 2.16 ppm are deshielded to 2.35 ppm, while the γ proton signal at 1.70 ppm has moved to 1.80 ppm. Irradiation of the guest's β-protons results in NOE enhancements of 3% and 1% for the H3 and H5 CyD protons, respectively. The observed enhancements are consistent with those reported by Jamie *et al.*[17] for the β-CyD/1-bromoadamantane host-guest complex. Irradiation of the guest's α- and γ-protons resulted in lower NOE enhancements (1.1% and <0.3% for the H3 and H5 protons, respectively). As additional substantiation, the correct mass (calcd *m/z* 1286.5, found *m/z* 1286.4) for the complex was observed by electrospray MS in the positive ion mode.

Complexation of amantidine with monofluoro-CyD **9** led to both improved aqueous solubility of the host as well as detectable perturbations in the proton spectrum. The correct mass (calcd *m/z* 1289.12, found *m/z* 1289.7) for the complex was observed by electrospray MS in the positive ion mode. Similarly, complex formation with an equivalent of 1-adamantanamine hydrochloride dramatically improved the aqueous solubility of host **12**, but exerted virtually no effect on the solubility of host **5**. Significant changes were observed in the proton NMR spectrum of **12** upon inclusion of **13** (Figure 1). Notably, the six anomeric proton resonance at 4.92 ppm is partially resolved upon complex formation and the single proton triplet at 3.92 ppm is shifted upfield. A single proton doublet of doublets (J_d = 4.5 Hz and 12.5) and a single proton doublet (J_d = 11.0 Hz) have apparently moved downfield to 4.05 and 3.9 ppm, respectively, from the complex multiplet housing a majority of the protons in the ring region. Presumably, these

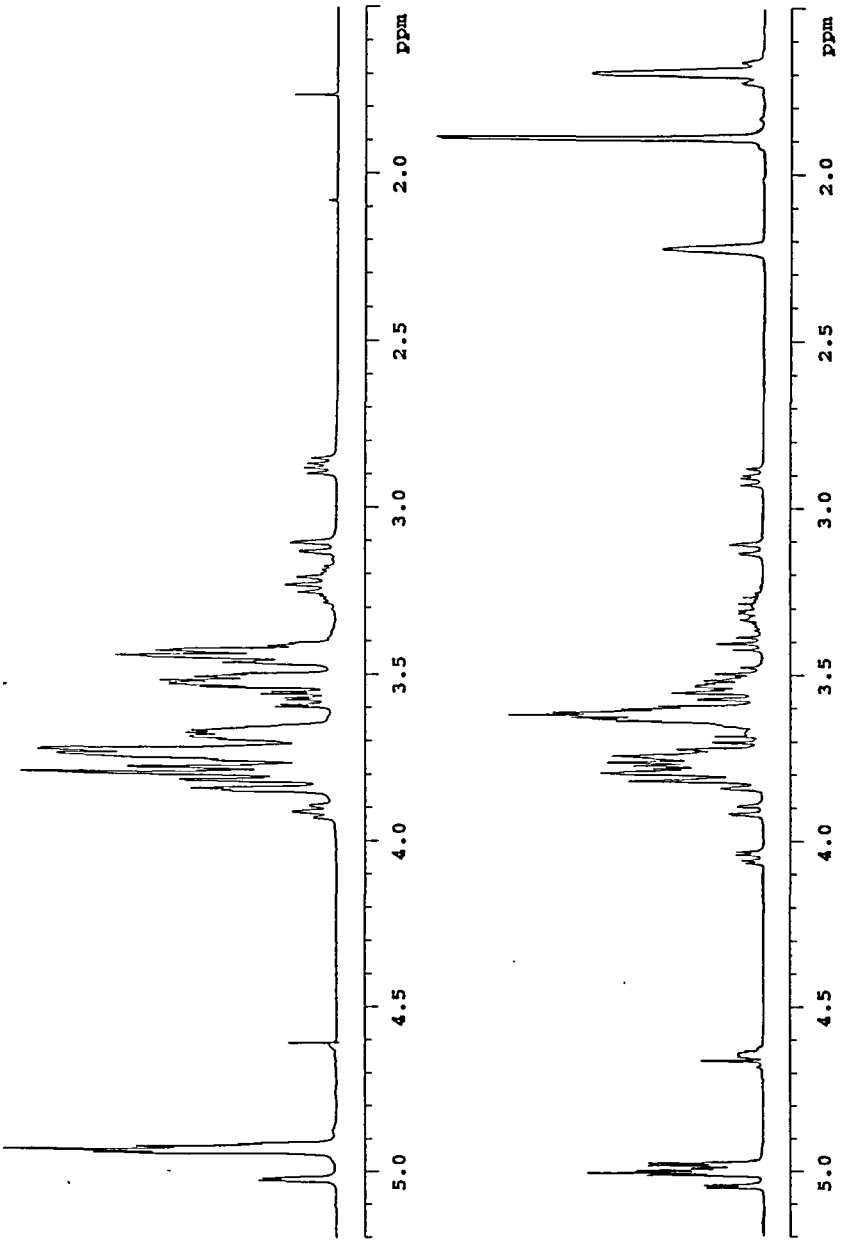


Figure 1. 500 MHz ^{1}H NMR spectrum of **12** (top) and the **12/13** inclusion complex (bottom).

are H6 protons of a glucose moiety and can serve as a handle for complete assignment. Also, a triplet at 3.24 ppm is seen to move downfield to 3.4 ppm. The ^{1}H NMR spectrum of the host-guest complex is complicated and a more detailed analysis is currently underway. No significant changes, however, were noted in the shift of the ^{19}F triplet. Again, the correct mass (calcd *m/z* 1384.5, found *m/z* 1384.6) for the complex was observed by electrospray MS in the positive ion mode.

CONCLUSION

Three cyclodextrin derivatives fluorinated on the primary face have been prepared and one of these, namely **12**, has been found to possess suitable aqueous solubility for drug delivery of hydrophobic drug molecules. Preliminary studies on the complexation properties of this host have been initiated and a more detailed NMR investigation (2D homonuclear HOHAHA and 2D NOE under spin-locked conditions[18]) is currently underway. Hopefully, refined analysis of guest complexes with **12** may shed light on the potential geometric preferences inherent in complexes involving unsymmetrically derivatized CyD hosts.[19] The fluorine substitution on the CyD skeleton may serve as a label for monitoring drug complex localization *in vivo* by ^{19}F MRI. As a further complexation probe, fluorination of the secondary face is underway.

EXPERIMENTAL

General Methods. NMR spectra were recorded on a Bruker AM 300 spectrometer (^{1}H: 300 MHz; ^{19}F: 282 MHz; ^{13}C: 75 MHz) in $CDCl_3$ or D_2O solution unless otherwise stated. Chemical shifts in $CDCl_3$ solutions are reported in parts per million downfield from TMS, or in the case of D_2O solutions, using HOD set at δ 4.82 (25 °C) unless otherwise specified. Homonuclear NOE experiments of the complexes were performed on a Varian Unity 500 spectrometer. ^{13}C NMR spectral assignments were aided by the J-MOD technique.[20] ^{19}F NMR spectra are reported in parts per million using external C_6F_6 (set at 0.0 ppm) as a reference for $CDCl_3$ solutions, and external $CF_3CO_2^-Na^+$ (set at 0.0 ppm) for D_2O solutions. Fast atom bombardment (FAB) mass spectra

were obtained with a Kratos AE1 MS9 mass spectrometer while Electrospray (ES) mass spectra were obtained with a Micromass VG ZabSpec TOF instrument in the positive ion mode. Reactions were monitored by thin-layer chromatography (TLC) on Kieselgel 60 F_{254} (Merck) and visualization was accomplished by charring with 5% methanolic sulfuric acid. Column chromatography was performed using Merck 9385 silica gel (40 - 63μ). Cyclodextrin was dried *in vacuo* over P_2O_5 at 60 °C prior to use. Chloroform was distilled from P_2O_5 and pyridine was distilled from CaH_2 and stored over 3 Å molecular sieves.

Heptakis(6-*O*-*tert*-butyldimethylsilyl)cyclomaltoheptaose (1). Compound **1** was synthesized according to the procedure published by Fügedi:[10] yield 80%; ^{1}H NMR ($CDCl_3$) δ 0.04, 0.06 (2s, 6H, $SiCH_3$), 0.88 (s, 9H, s, 3H, CCH_3), 3.47 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.66 (dd, 1H, $J_{1,2}$ = 3.5 Hz, H-2), 3.72 (d, 1H, $J_{6a,6b}$ = 11.5 Hz, H-6a), 3.92 (dd, 1H, $J_{5,6b}$ = 2.5 Hz, H-6b), 4.06 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.0 Hz, H-4), 4.90 (d, 1H, H-1), 5.28 (s, 1H, OH), 6.74 (s, 1H, OH); ^{13}C NMR δ -5.2 ($SiCH_3$), -5.1 ($SiCH_3$), 18.3 ($\underline{C}CH_3$), 25.9 ($C\underline{C}H_3$), 61.7 (C-6), 72.6, 73.4, 73.6 (C-2,3,5), 81.8 (C-4), 102.0 (C-1).

Heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)cyclomaltoheptaose (2). The fully protected derivative **2** was synthesized according to the procedure published by Takeo *et al*:[14] yield 84%; R_f 0.25 (10:10:1 hexane-ethyl acetate-ethanol); ^{1}H NMR ($CDCl_3$) δ 0.06, 0.07 (2s, 6H, $SiCH_3$), 0.91 (s, 9H, $C(CH_3)_3$), 2.07, 2.08 (2s, 6H, $COCH_3$), 3.75 (d, 1H, $J_{6a,6b}$ = 11.5 Hz, H-6a), 3.90 (m, 2H, H-4, H-5), 4.07 (d, 1H, H-6b), 4.73 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 5.17 (d, 1H, H-1), 5.36 (t, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3); ^{13}C NMR δ -5.7 ($SiCH_3$), -5.4 ($SiCH_3$), 17.8 ($\underline{C}CH_3$), 20.3, 20.5 (2s, $CO\underline{C}H_3$) 25.4 ($C\underline{C}H_3$), 61.4 (C-6), 70.8, 71.1, 71.5 (C-2,3,5), 74.9 (C-4), 96.1 (C-1), 169.0 (C=O), 170.3 (C=O).

Heptakis(2,3-di-*O*-acetyl)cyclomaltoheptaose (3). Compound **3** was synthesized according to the procedure published by Takeo *et al*:[14] yield 79%; R_f 0.5 (3:1 chloroform-methanol); ^{1}H NMR (9:1 $CDCl_3$-CD_3OD) δ 1.97, 2.01 (2s, 6H, $COCH_3$), 3.71 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.0 Hz, H-4); 3.78-3.90 (m, 3H, H-5, H-6a, H-6b) 4.67 (s, 1H, OH), 4.70 (dd, 1H, $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 5.03 (d, 1H, H-1), 5.24 (dd, 1H, H-3).

Heptakis(2,3-di-*O*-acetyl-6-deoxy-6-fluoro)cyclomaltoheptaose (4). To a solution of **3** (290 mg, 0.17 mmol) in dry chloroform cooled to 5 °C under argon, was

added dropwise, a solution of diethylaminosulfur trifluoride (1.1 mL, 8.25 mmol) in chloroform (1.9 mL), and the reaction mixture was then stirred at room temperature for 3 h. The reaction mixture was diluted with chloroform, cooled to 5 °C, and the excess fluorinating agent was quenched by the addition of ice-water. The layers were separated and the organic solution was successively washed with saturated aqueous sodium hydrogen carbonate, water, brine, and then dried (Na_2SO_4), filtered and concentrated. Column chromatography over silica gel using 70:1 then 60:1 chloroform-methanol furnished the desired heptafluoro compound **4** (102 mg, 35%) as a foam: R_f 0.14 (19:1 chloroform-methanol); ^{1}H NMR ($CDCl_3$) δ 2.08, 2.10 (2s, 6H, $COCH_3$), 3.82 (t, 1H, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4), 4.05 (ddd, 1H, $J_{6a,5} < 1.0$ Hz, $J_{5,6b} = 2.5$ Hz, $J_{5,F} = 27.0$ Hz, H-5), 4.62 (ddd, 1H, $J_{6a,6b} = 11.0$ Hz, $J_{6a,F} = 48.0$ Hz, H-6a), 4.78 (ddd, 1H, $J_{6b,F} = 48.0$ Hz, H-6b), 4.82 (dd, 1H, $J_{1,2} = 3.5$ Hz, $J_{2,3} = 9.0$ Hz, H-2), 5.15 (d, 1H, H-1), 5.36 (t, 1H, H-3); ^{19}F NMR (external C_6F_6) δ -71.8 (td, $J_{F,5} = 27.0$ Hz, $J_{F,6} = 48.0$ Hz, CH_2F); ^{13}C NMR δ 20.7, 20.8 (2s, $CO\underline{C}H_3$), 70.3, 70.5, 70.7 (C-2,3,5), 75.3 (obscured by $CDCl_3$), 75.4, 82.0 (d, $J_{C6,F} = 174.0$ Hz, C-6), 96.6(C-1), 169.5 (C=O), 170.5 (C=O).

Anal. Calcd for $C_{70}H_{91}O_{42}F_7$: C, 48.39; H, 5.28. Found: C, 48.74; H, 5.24.

Heptakis(6-deoxy-6-fluoro)cyclomaltoheptaose (5). A solution of heptafluoro-CyD **4** (86 mg, 49 μM) in 1:1 methanol-water containing 1 mmol of sodium methoxide was stirred at room temperature overnight. The cloudy solution was neutralized with acetic acid and the solvents were removed. The resulting material, which displayed poor solubility characteristics, was purified by column chromatography over silica gel using 65:35:2 then 65:35:6 chloroform-methanol-water to give 47 mg (82%) of **5** as a white solid: R_f 0.3 (65:35:6 chloroform-methanol); ^{1}H NMR (DMSO-d_6) δ 3.28-3.48 (m, 2H, H-2, H-4), 3.65 (t, 1H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 3.86 (dd, 1H, $J_{4,5} = 9.0$ Hz, $J_{5,F} = 26.0$ Hz, H-5), 4.62 (d, 2H, $J_{6,F} = 47.5$ Hz, H-6a, H-6b), 4.88 (d, 1H, $J_{1,2} = 3.0$ Hz, H-1), 6.1 (br s, 2H, OH); ^{19}F NMR (DMSO-d_6, external $CF_3CO_2^-Na^+$) δ -65.5 (td, CH_2F), heteronuclear decoupling of the H-6 proton (δ 4.62) collapsed the ^{19}F signal to a doublet while irradiation of the H-5 proton (δ 3.86) yielded a triplet; ^{13}C NMR (DMSO-d_6) δ 70.2 (d, $J_{C,F} = 18$ Hz, C-5), 71.9 and 72.9 (C-2, C-3), 81.1 (C-4), 82.3 (d, $J_{C,F} = 168$ Hz, C-6), 102.1 (C-1). FAB-MS Calcd for $C_{42}H_{63}O_{28}F_7Na$: 1171.4. Found: 1171.9.

(2,3-Di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)hexakis(2,3,6-tri-*O*-acetyl)cyclomaltoheptaose (6). Compound **6** was synthesized according to the procedure published

by Fügedi *et al.*[9] yield 40%; R_f 0.35 (1:1 hexane-acetone); ^{1}H NMR ($CDCl_3$) δ 0.0 (2s, 6H, $SiCH_3$), 1.82 (s, 9H, $C(CH_3)_3$), 1.98-2.18 (20s, 60H, $COCH_3$), 3.60-3.79 (m, 6H, H-4s), 3.84 (t, 1H, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4), 4.0-4.58 (m, 21H, H-5s, H-6s), 4.68-4.85 (m, 7H, H-2s), 4.98- 5.14 (m, 7H, H-1s), 5.15-5.39 (m, 7H, H-3s).

(2,3-Di-*O*-acetyl)hexakis(2,3,6-tri-*O*-acetyl)cyclomaltoheptaose (7). To a solution of **6** (188 mg, 0.09 mmol) in dry ethanol free chloroform (6 mL), was added dropwise, boron trifluoroetherate (150 μL, 1.17 mmol) and the reaction was stirred at room temperature for 45 min. Analysis by TLC (1:1 hexane-acetone) revealed that the reaction was complete and the reaction mixture was cooled to 0-5 °C, then quenched with saturated aqueous sodium hydrogen carbonate. The layers were separated and the organic extract was washed with saturated aqueous sodium hydrogen carbonate, dried (Na_2SO_4), filtered and concentrated. Column chromatography over silica gel using 1:1 hexane-acetone gave 161 mg (91%) of **7**: R_f 0.14 (1:1 hexane-acetone); ^{1}H NMR ($CDCl_3$) δ 2.04-2.15 (20s, 60H, $COCH_3$), 3.66-3.77 (m, 6H, H-4s), 3.78 (t, 1H, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4), 3.87-4.65 (m, 22H, H-5s, H-6s, OH), 4.75-4.89 (m, 7H, H-2s), 5.05-5.16 (m, 7H, H-1s), 5.22-5.38 (m, 7H, H-3s).

Anal. Calcd for $C_{82}H_{110}O_{55}$: C, 49.85, H; 5.61. Found: C, 49.96, H; 5.37.

(2,3-Di-*O*-acetyl-6-deoxy-6-fluoro)hexakis(2,3,6-tri-*O*-acetyl)cyclomaltoheptaose (8). To a solution of **7** (38 mg, 0.02 mmol) in dry chloroform (2 mL) was added a solution of diethylaminosulfur trifluoride (50 μL, 0.38 mmol) in chloroform (250 μL) and the reaction was stirred at room temperature for 1 h. The reaction was then diluted with chloroform and quenched with ice water. The organic layer was washed with saturated aqueous sodium hydrogen carbonate, brine, dried (Na_2SO_4), filtered and concentrated. Column chromatography over silica gel using 1:1 hexane-acetone afforded 34 mg (89%) of **8**: R_f 0.17 (1:1 hexane-acetone); ^{1}H NMR ($CDCl_3$) δ 2.04-2.18, (20s, 60H, $COCH_3$), 3.65-3.79 (m, 6H, H-4s), 3.84 (t, 1H, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4), 4.05-4.36 (m, 14H, H-5s, H-6s), 4.52-4.65 (m, 7H, H-6s), 4.75-4.90 (m, 7H, H-2s), 5.08-5.19 (m, 7H, H-1s), 5.25-5.39 (m, 7H, H-3s); ^{19}F NMR ($CDCl_3$) δ -72.6 (m).

Anal. Calcd for $C_{82}H_{109}O_{54}F$: C, 49.80; H, 5.56. Found: C, 49.77; H, 5.39.

(6-Deoxy-6-fluoro)hexakiscyclomaltoheptaose (9). A solution of **8** (31 mg, 16 μmol) in 2:1 methanol-water (3 mL) containing sodium methoxide (0.5 mmol) was

stirred at room temperature overnight. The reaction mixture was then deionized with IR 120(H^+) resin, filtered and concentrated. Column chromatography over silica gel using 7:7:2 2-propanol-water-ammonia provided 15 mg (83 %) of **9**: mp 234 °C (decomp.); lit.[7] mp < 200 °C; R_f 0.09 (5:4:1 chloroform-methanol-water); ^{1}H NMR (D_2O) δ 3.43-3.60 (m, 14H, H-2s, H-4s), 3.65-3.80 (m, 18H, H-5s, H-6s), 3.81-3.94 (m, 7H, H-3s), 3.95 (m, 1H), 4.85-4.55 (m, partially obscured by HOD, possibly 2H), 4.95-5.0 (m, 7H, H-1s); ^{19}F NMR δ -158.5 (td, $J_{F,6}$ = 47.0 Hz, $J_{F,5}$ = 30.0 Hz). FAB-MS, Calcd for $C_{42}H_{69}O_{34}F$·Na: 1159.98. Found: 1160.2.

(6-*O*-*p*-Toluenesulfonyl)hexakiscyclomaltoheptaose (10). Compound **10** was synthesized according to the procedure published by Melton and Slessor:[16] yield 32%; mp 177 °C (decomp.); lit.[21] mp 179 °C (decomp.); R*f* 0.5 (10:10:7 ethyl acetate-2-propanol-water); ^{1}H NMR (D_2O) δ 2.43 (s, 3H, CH_3), 3.15-3.77 (m), 4.1-4.4 (m), 4.77 (br s), 5.48-5.60 (m), 7.45 (d, 2H, J_d = 8.0 Hz), 7.77 (d, 2H, J_d = 8.0 Hz).

[2,3-Di-*O*-acetyl-6-deoxy-6-(2',2',2'-trifluoroethylthio)]hexakis(2,3,6-tri-*O*-acetyl)cyclomaltoheptaose (11). A mixture of **10** (650 mg, 0.5 mmol), 2,2,2-trifluoroethanethiol (900 μL, 10 mmol) and sodium hydrogen carbonate (1.17 g, 14 mmol) in water (20 mL) was strirred at 70 °C under argon for 72 h. The reaction mixture was treated with excess acetic acid and was concentrated in a well vented fume hood. The resulting solid was triturated with ether, filtered and then washed several times with ether. The crude mixture was then acetylated by treatment with 2:1 pyridine-acetic anhydride (21 mL) and stirring was continued for 72 h. The reaction mixture was concentrated to dryness and subsequent column chromatography over silica gel using 8:8:1 hexane-ethyl acetate-ethanol furnished 644 mg (62%) of **11**: R_f 0.15 (7:7:1 hexane-ethyl acetate-ethanol); ^{1}H NMR ($CDCl_3$) δ 2.04-2.16 (20s, 60H, $COCH_3$), 3.05 (dd, 1H, $J_{6,6}$ = 14.0 Hz, $J_{6,5}$ = 6.0 Hz, H-6), 3.16-3.43 (m, 3H, H-6, CH_2CF_3), 3.62-3.76 (m, 6H, H-4s), 3.81 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, H-4), 4.05-4.66 (m, 19H, H-5s, H-6s), 4.71-4.89 (m, 7H, H-2s), 5.03-5.16 (m, 7H, H-1s), 5.21-5.39 (m, 7H, H-3s); ^{19}F NMR ($CDCl_3$, external C_6F_6) δ 94.6 (t, 1H, J_t = 10.0 Hz).

Anal. Calcd for $C_{84}H_{111}O_{54}SF_3$: C, 48.65; H, 5.39. Found: C, 48.44; H, 5.35.

[6-Deoxy-6-(2',2',2'-trifluoroethylthio)]hexakiscyclomaltoheptaose (12). Trifluoroethylthio derivative **11** (634 mg, 0.31 mmol) was dissolved in 1:1 methanol-

water (10 mL) containing potassium hydroxide (84 mg, 1.5 mmol) and the reaction mixture was stirred at room temperature for 48 h. The cloudy solution was neutralized by the addition of excess acetic acid and the solvents were evaporated. Column chromatography over silica gel using 2:1:1 ethyl acetate-methanol-water followed by recrystallization from water gave 330 mg (88%) of **12** as a crystalline compound: mp 287 °C (decomp.); ^{1}H NMR (D_2O) δ 3.08 (dd, $J_{5,6}$ = 8.0 Hz, $J_{6,6}$ = 14.0 Hz), 3.62-4.05 (m, 41H), 5.13 (d, 6H, $J_{1,2}$ = 3.5 Hz, H-1s), 5.23 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1); ^{19}F NMR (external $CF_3CO_2^-Na^+$) δ 9.04 (t, J_t = 10.0 Hz). FAB-MS Calcd for $C_{44}H_{71}O_{34}F_3SNa$: 1256.07. Found: 1255.3. FAB-MS Calcd for $C_{44}H_{71}O_{34}F_3S$: 1233.07. Found: 1232.35.

Preparation of Inclusion Complexes for ES-MS Studies. For mass spectrometric analysis of inclusion complexes of 1-adamantanamine hydrochloride with fluorocyclodextrins **9** and **12**, equimolar amounts of guest and host were prepared at a concentration of 1mg/mL in water. Aliquots of 5-10 μL of this solution were analyzed by electrospray mass spectrometry in the positive ion mode.

Preparation of Inclusion Complexes for NMR Studies. Equimolar amounts of 1-adamantanamine hydrochloride with fluorocyclodextrins **9** and **12** were prepared at a concentration of approximately 18 mg/mL in D_2O.

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