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Synthesis of Tris(amino acid)-Substituted Derivatives of α-Cyclodextrin Derivatives

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Abstract : As a part of our efforts to develop artificial molecules with affinity for glucose, we have synthesized amino acid derivatives of α-cyclodextrins(α-CDs). As a first step toward such artificial glucose-binding molecules, synthetic methods for derivatization of α-CDs with amino acids were established. Seven novel tris(amino acid)-substituted α-CDs have been synthesized using a four-step sequence. Azidation of α-cyclodextrin with sodium azide followed by catalytic hydrogenation of the resulting triazidocyclodextrin gave a mixture of triamine regioisomers. Excess N-*t*-BOC-protected amino acids (N-*t*-BOC-L-phenylalanine, N_{α}-*t*-BOC-L-glutamine, N-*t*-BOC-L-glutamic acid α-benzyl ester, N-*t*-BOC-L-asparagine, N-*t*-BOC-L-aspartic acid β-benzyl ester, N-*t*-BOC-L-tryptophan and N-*t*-BOC-O-benzyl-L-tyrosine) were then coupled to this intermediate using dicyclohexylcarbodiimide (DCC) to generate the homotrimeric amido-CDs. Orthogonal deprotection gave the tris (amino acid) CD derivatives in 2.6-3.9% overall yield.

Keywords : bioorganic chemistry, α-cyclodextrin-amino acid conjugates, supramolecular chemistry

Introduction

Development of self-regulated insulin delivery systems is one of the most challenging and important research areas in controlled release. A key element of self-regulated insulin delivery systems is the presence of a reliable and specific glucose sensor. Usually, a glucose sensor is composed of glucose oxidase or concanavalin A that have specific glucose binding properties [1]. Although these glucose-sensitive proteins have been used frequently, they may not be suitable for long-term clinical applications due to stability and/or safety limitations (i.e. they may be easily denatured or elicit immune reactions). For these reasons, it is desirable to develop artificial glucose-binding molecules. Glucose-binding sites of several glucose-binding proteins have been analyzed to find the amino acid residues within the glucose-binding sites that are involved in hydrogen bonding to glucose [2]. Since these proteins show that proper arrangement of amino acids can form glucose-binding sites, we examined the possibility of forming an artificial glucose-binding site by using spatially arranged amino acid moieties. This study was

focused on our attempt to develop synthetic methods for grafting these amino acids onto a cyclodextrin scaffold.

Cyclodextrins (CD) are cyclic oligosaccharides containing six (α-), seven (β-), or eight(γ) α-D-glucopyranosyl units [3,4]. The cylindrical hydrophobic cavity of these molecules imbues them with the capacity to form inclusion complexes with spatially compatible hydrophobic compounds. This property has made CDs widely applicable for separation, solubilization via encapsulation, and as platforms for enzyme models [5-8]. CDs have also been utilized as artificial receptor units due to their favorable biocompatibility and well-developed preparative methods for the selective functionalization of either the 6- or 2-/3- hydroxyl groups on opposing rims of the molecule [9-11]. Total synthesis methods have also been developed [12]. Several attempts have recently been made to derivatize CD with amino acids for molecular recognition and peptidomimetic studies. These reports include the synthesis of CD derivatives that are monosubstituted with either amino acids (cysteine [13], aspartate & glutamate [14], phenylalanine [15], tryptophan [16,17], and tyrosine [18]), or peptide scaffolds [19,20]; heterobifunctional systems [21] and peramino acid derivatives have also been described [22]. We sought a series of related triply-substituted amino acid CDs coupled at the 6-hydroxylmethyl (1^{o}) face of α-CD (Scheme 1) for receptor-ligand binding studies. The amino acids used for this study were phenylalanine, tryptophan, tyrosine, glutamic acid, glutamine, aspartic acid, asparagine.

Materials and Methods

Experimental Methods

All reagents and solvents were used as received, except that DMF was distilled from $CaSO_4$ under reduced pressure at 45°C and α-CD was dried under vacuum for at least 12 h in the presence of P_2O_5 before use. All reactions were conducted under an Ar atmosphere. TLC plates were visualized by UV and sulfuric acid charring. Silica (230-400 mesh) was used for all flash chromatography procedures. HPLC analysis was performed as described by Hanessian *et al.* [23]. ^{1}H NMR chemical shifts are reported relative to the solvents peaks as internal reference. PDMS spectra were acquired using a Bio Ion-20R instrument. The sample (5 mg) was dissolved in 1 ml methanol or water and then subjected to PDMS analysis.

Triazido α-cyclodextrin (1)

Ph_3P (8.90 g, 33.9 mmol), CBr_4 (11.23 g, 33.9 mmol), and NaN_3 (7.35 g, 113 mmol) were added to a dry DMF solution (80 ml) of α-CD (11.0 g, 11.3 mmol) and the reaction stirred for 4 d. The reaction mixture was then concentrated to an oil under vacuum and 800 ml of acetone added. The resulting precipitate was filtered, washed twice with 400 ml acetone, and air dried to give 13.5 g of crude isolate. TLC (4 : 1 CH_3CN : H_2O) indicated that this material was a mixture of four products; tetraazido-α-CD (R_f =

0.63), triazido-α-CD ($R_f = 0.50$), diazido-α-CD ($R_f = 0.3$), and monoazido-α-CD ($R_f = 0.16$). This mixture (6.75 g) was separated via silica gel flash chromatography (5 × 50 cm) using a step gradient of 9 : 1 $CH_3CN : H_2O$ followed by 4 : 1 $CH_3CN : H_2O$. The corresponding tetraazido-, triazido-, diazido-, and monoazido-α-CD fractions were pooled and concentrated by rotary evaporation to give pure tetraazido- (8.3%, 0.503 g), triazido- (17.5%, 1.04 g), diazido- (21.5%, 1.24 g), and monoazido-α-CD (16.2%, 0.91 g). The bold italic symbols in the ^{1}H-NMR spectral assignments below indicates the chemical shifts at the glucose sites bearing the azido group.

Tetraazido-α-cyclodextrin. ^{1}H-NMR (500 MHz, CD_3SOCD_3): 3.24-3.46 (m, H2, H4, **H6**, H_2O), 3.52-3.86 (m, 16H, H3, H5, H6), 4.56-4.70 (m, 2H, OH6), 4.75-4.82 (br, 2H, H1), 4.82-4.89 (m, 4H, ***H1***), 5.38-5.76 (m, 12H, OH2, OH3). Secondary rim : primary rim hydroxyl group proton ratio = OH2 + OH3 : OH6 (Calculated: 6.0, Found: 6.09).

Triazido--cyclodextrin. **(1).** ^{1}H-NMR (500 MHz, CD_3SOCD_3): 3.24-3.46 (m, H2, H4, ***H6***, H_2O), 3.52-3.86 (m, 18H, H3, H5, H6), 4.49-4.70 (m, 3H, OH6), 4.76-4.82 (br, 3H, H1), 4.82-4.88 (m, 3H, ***H1***), 5.38-5.76 (m, 12H, OH2, OH3). Secondary rim:primary rim hydroxyl group proton ratio = OH2 + OH3 : OH6 (Calculated: 4.0, Found: 4.03). PDMS: Calculated $(M+Na)^+$: 1070.9, Found $(M+Na)^+$: 1071.7.

Diazido-α-cyclodextrin. ^{1}H-NMR (200 MHz, CD_3SOCD_3): 3.20-3.51 (br, H2, H4, ***H6***, H_2O), 3.51-3.93 (m, 20H, H3, H5, H6), 4.42-4.71 (br, 4H, OH6), 4.71-4.95 (m, 6H, H1, ***H1***), 5.30-5.77 (br, 12H, OH2, OH3). Secondary rim:primary rim hydroxyl group proton ratio = OH2 + OH3 : OH6 (Calculated: 3.0, Found: 3.0).

Monoazido-α-cyclodextrin. ^{1}H-NMR (200 MHz, CD_3SOCD_3): 3.20-3.51 (br, H2, H4, ***H6***, H2O), 3.51-3.93 (m, 22H, H3, H5, H6), 4.42-4.65 (br, 5H, OH6), 4.72-4.95 (m, 6H, H1, ***H1***), 5.30-5.74 (br, 12H, OH2, OH3). Secondary rim:primary rim hydroxyl group proton ratio = OH2 + OH3 : OH6 (Calculated: 2.25, Found: 2.29).

Triamino α-Cyclodextrin (2)

Triazido-α-CD **1** (200 mg, 0.191 mmol) was dissolved in 1 : 1 $CH_3OH : H_2O$ and 10% Pd/C (20 mg) added to the solution in a Fisher-Porter tube. The bomb was pressurized with H_2 at 40 psi and the reaction conducted at 20°C for 48 h. The reaction was then vented, filtered and the resulting product isolated by rotary evaporation to give 172 mg (93% yield) **2**. TLC (*n*-PrOH : H_2O : NH_4OH, 6 : 2 : 1): $R_f = 0.06$. The bold italics symbols in the following NMR assignments indicate the glucose units containing the amino group. ^{1}H-NMR (300 MHz, D_2O): 2.59-2.83 (m, 3H, ***H6***), 2.83-3.1 (m, 3H, ***H6***), 3.1-3.53 (m, 12H, H2, H4), 3.53-3.97 (m, 18H, H3, H5, H6), 4.78-5.0 (m, 6H, H1, ***H1***). Ratio of H6 + ***H6***:H1 + ***H1*** protons (Calculated: 1, Observed: 1.02). PDMS: Calculated $(M + H)^+$: 971.0, Found $(M + H)^+$: 971.0; Calculated $(M + Na)^+$: 993.0, Found $(M + Na)^+$: 992.8.

General procedure for the synthesis of tris(amino acid) α-cyclodextrin derivatives

N-*t*-BOC-protected amino acids were coupled to the primary amino groups of **2** using DCC. In this study, the amino acid derivatives were used in 10% molar excess with respect to each amine substitute (i.e. 3.3 : 1 ratio relative to **2**).

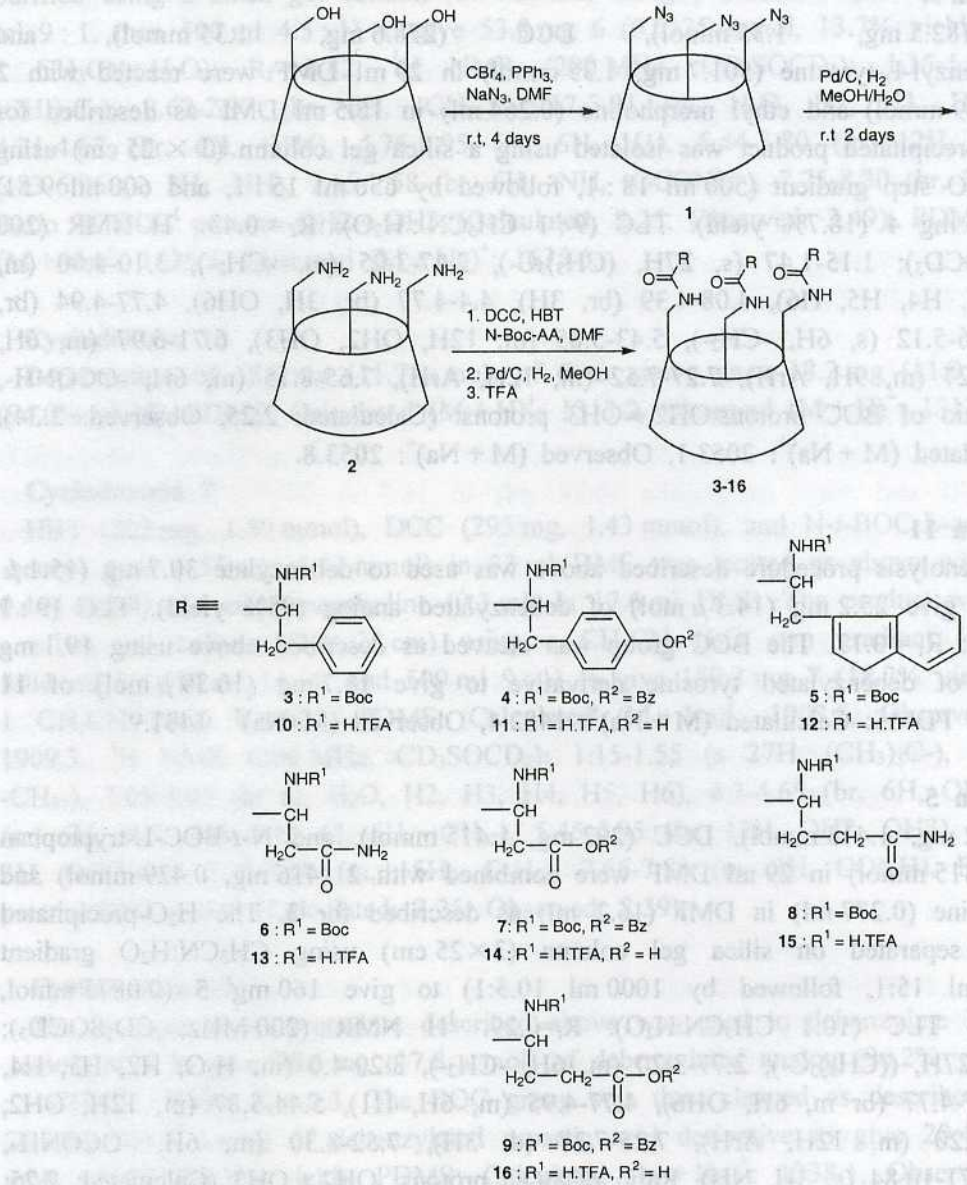
DCC coupling. N-hydroxybenzotriazole (HBT) was added to a DMF solution of the N-*t*-BOC-L-amino acid. This solution was cooled in an ice bath and DCC added. A DMF solution of **2** and ethyl morpholine was then added and the suspension stirred, first for 1 h at 4°C and then for 48 h at 20°C. The reaction mixture was subsequently filtered to remove dicyclohexylurea (DCU), the filtrate concentrated to an oil under vacuum, and the product precipitated by addition of distilled H_2O or Et_2O. The precipitate was filtered, washed with distilled H_2O, and air-dried. The purified products were isolated by silica gel chromatography using a gradient elution scheme (see below) and characterized by ^1H NMR and PDMS. The degree of amino acid substitution was determined by comparing the calculated and observed ratios of BOC protons to α-CD OH2 and OH3 protons. For all derivatives, the observed ratios were slightly higher than the calculated ratios (Calculated: 2.25, Observed: 2.31-2.49, depending on the amino acid substituent), perhaps due to inclusion impurities. Trisubstitution was further verified by PDMS for each derivative.

Deprotection. Debenzylation. Benzyl groups were cleaved by hydrogenation prior to BOC deprotection. The tris(amino acid)-α-CD was dissolved in methanol and stirred for 30 h under 40 psi of H_2 in the presence of 10% Pd/C. After filtration, the reaction mixture was washed with methanol and distilled water. The solution was then evaporated and used as recovered for BOC deprotection.

BOC cleavage. TFA was added to the BOC-protected tris(amino acid)-α-CD's and the mixture stirred for 1 h at 20°C. Distilled water was then added, the mixture evaporated, and the products characterized as the TFA salts.

Cyclodextrin 3

Ethyl morpholine (0.24 ml) and **2** (355 mg, 0.366 mmol) in 13.9 ml DMF were added to a DMF solution (26 ml) of N-*t*-BOC-L-phenylalanine (339.8 mg, 1.28 mmol), HBT (173.1 mg, 1.28 mmol), and DCC (264 mg, 1.28 mmol) and the reaction processed as described above. The H_2O-precipitated product was separated on a silica gel column (3.5 × 20 cm) using a CH_3CN:H_2O step gradient (500 ml 15 : 1, followed by 530 ml 11 : 1, and 650 ml 9 : 1) to give 149.7 mg **3** (23.9% yield). TLC (10 : 1 CH_3CN : H_2O): R_f=0.22. ^1H NMR (200 MHz, CD_3SOCD_3):1.1-1.45 (s, 27H $(CH_3)_3C$-), 2.6-3.08 (br m, 6H, $-CH_2$-) 3.08-3.48 (m, H_2O, H2, H4), 3.48-3.93 (br m, 24H, H3, H5, H6), 4.15-4.38 (br, 3H), 4.45-4.62 (br, 3H, OH6), 4.76-4.91 (s, 6H, H1), 5.42-5.83 (m, 12H, OH2, OH3), 6.76-6.97 (br, 3H, -OCONH-), 7.10-7.38 (m, 15H, $-C_6H_5$), 7.63-8.1 (br, 6H, -CONH-). Ratio of Ph:OH2 + OH3 protons: (Calculated: 1.25, Observed: 1.28). PDMS: Calculated



Scheme 1.

$(M + Na)^+$: 1734.8, Observed $(M + Na)^+$: 1734.9.

Cyclodextrin 10

TFA (100 μl) was added to **3** (18.3 mg, 10.69 μmol) and the reaction processed as described above to give 16.7 mg **10** (89.3% yield). PDMS: Calculated $(M + H)^+$: 1412.5, Observed $(M + H)^+$: 1412.8.

Cyclodextrin 4

HBT (182.5 mg, 1.35 mmol), DCC (278.6 mg, 1.35 mmol), and N-*t*-BOC-*O*-benzyl-L-tyrosine (501.7 mg, 1.35 mmol) in 29 ml DMF were reacted with **2** (397 mg, 0.409 mmol) and ethyl morpholine (0.264 ml) in 15.5 ml DMF as described for **3**. The H_2O-precipitated product was isolated using a silica gel column (3 × 25 cm) using a $CH_3CN : H_2O$ step gradient (500 ml 18 : 1, followed by 650 ml 15 : 1, and 600 ml 9 : 1) to give 155.5 mg **4** (18.7% yield). TLC (9 : 1 $CH_3CN : H_2O$): $R_f = 0.43$. ^1H NMR (200 MHz, CD_3SOCD_3): 1.15-1.47 (s, 27H, $(CH_3)_3C$-), 2.47-3.05 (m, -CH_2-), 3.10-4.00 (m, H_2O, H2, H3, H4, H5, H6), 4.08-4.39 (br, 3H), 4.4-4.77 (br, 3H, OH6), 4.77-4.94 (br, 6H, H1), 4.96-5.12 (s, 6H, -CH_2-), 5.43-5.85 (m, 12H, OH2, OH3), 6.71-6.97 (m, 6H, ArH), 7.07-7.27 (m, 9H, ArH), 7.27-7.52 (m, 12H, ArH), 7.65-8.15 (m, 6H, -OCONH-, -CONH-). Ratio of BOC protons:OH2 + OH3 protons: (Calculated: 2.25, Observed: 2.34). PDMS: Calculated $(M + Na)^+$: 2053.1, Observed $(M + Na)^+$: 2053.8.

Cyclodextrin 11

The hydrogenolysis procedure described above was used to debenzylate 30.7 mg (15.1 μ mol) of **4** to give 25.2 mg (14.3 μ mol) of debenzylated analog (95% yield). TLC (9 : 1 $CH_3CN : H_2O$): $R_f = 0.17$. The BOC group was cleaved as described above using 19.1 mg (10.85 μ mol) of debenzylated tyrosine derivative to give 18.7 mg (10.39 μ mol) of **11** (95.7% yield). PDMS: Calculated $(M + Na)^+$: 1482.4, Observed $(M+Na)^+$: 1481.9.

Cyclodextrin 5

HBT (191.2 mg, 1.415 mmol), DCC (292 mg, 1.415 mmol), and N-*t*-BOC-L-tryptophan (430.7 mg, 1.415 mmol) in 29 ml DMF were combined with **2** (416 mg, 0.429 mmol) and ethyl morpholine (0.277 ml) in DMF (16.2 ml) as described for **3**. The H_2O-precipitated product was separated on silica gel column (3×25 cm) using CH_3CN:H_2O gradient elution (700 ml 15:1, followed by 1000 ml 10.5:1) to give 160 mg **5** (0.0875 mmol, 20.4% yield). TLC (10:1 CH_3CN:H_2O): R_f=0.29. ^1H NMR (200 MHz, CD_3SOCD_3): 0.95-1.53 (s, 27H, $((CH_3)_3C$-), 2.77-3.20 (m, 6H, -CH_2-), 3.20-4.0 (m, H_2O, H2, H3, H4, H5, H6), 4.17-4.77 (br m, 6H, OH6), 4.77-4.95 (m, 6H, H1), 5.44-5.87 (m, 12H, OH2, OH3), 6.63-7.20 (m, 12H, ArH), 7.25-7.37 (d, 3H), 7.52-8.30 (m, 6H, -OCONH-, -CONH-), 10.71-10.84 (s, 3H, NH). Ratio of BOC protons: OH2 + OH3 (Calculated: 2.25, Observed: 2.30). PDMS: Calculated $(M+Na)^+$: 1851.8, Observed $(M+Na)^+$: 1851.6.

Cyclodextrin 12

Deprotection of 18.8 mg (10.28 μ mol) of **5** as above gave 18.2 mg (9.94 μ mol) of **12** (96.7% yield). PDMS: Calculated $(M + H)^+$: 1529.4, Observed $(M + H)^+$: 1528.5.

Cyclodexrin 6

HBT (103.3 mg, 0.76 mmol), DCC (150.8 mg, 0.73 mmol), and N_α-*t*-BOC-L-asparagine (167.1 mg, 0.72 mmol) in 15.8 ml DMF was combined with **2** (230 mg, 0.24 mmol) and ethyl morpholine (0.153 ml) in 9.0 ml DMF as above. The Et_2O-precipitated product was

purified using a silica gel column (3 × 20 cm) using a $CH_3CN : H_2O$ step gradient (400 ml 9 : 1, then 500 ml 4.5 : 1) to give 53.8 mg **6** (0.0325 mmol, 13.7% yield). TLC (4.5 : 1 $CH_3CN : H_2O$): $R_f = 0.42$. ^1H NMR (200 MHz, CD_3SOCD_3): 1.15-1.47 (s, 27H, $(CH_3)_3C$-), 2.62-2.97 (br, 6H, $-CH_2-$), 3.17-3.91 (m, H_2O, H2, H3, H4, H5, H6), 4.21-4.67 (br, 6H, OH6), 4.76-4.95 (m, 6H, H1), 5.44-5.80 (m, 12H, OH2, OH6), 6.83-6.96 (s, 3H, -NH), 7.15-7.58 (br, 6H, -NH, -OCONH-), 7.75-8.30 (br, 3H, -CONH-). Ratio of BOC protons OH2 + OH3 (Calculated: 2.25, Observed: 2.49). PDMS: Calculated $(M + Na)^+$: 1635.5, Observed $(M + Na)^+$: 1635.6.

Cyclodextrin 13

Deprotection of 18.8 mg (11.7 μmol) of **6** as above gave 18.2 mg (11.0 μmol) of **13** (94.0% yield). PDMS: Calculated $(M + H)^+$: 1313.2, Observed $(M + H)^+$: 1313.8.

Cyclodextrin 7

HBT (202 mg, 1.50 mmol), DCC (295 mg, 1.43 mmol), and N-*t*-BOC-L-aspartic acid β-benzyl ester (455 mg, 1.41 mmol) in 33 ml DMF was treated as above with **2** (450 mg, 0.464 mmol) and ethyl morpholine (0.3 ml) in 17.6 ml DMF. The product was isolated on a silica gel column (3 × 25 cm) using a $CH_3CN : H_2O$ step gradient (600 ml 18 : 1, followed by 500 ml 14 : 1, and 500 ml 9 : 1) to give 159.3 mg **7** (18.2% yield). TLC (9 : 1 $CH_3CN : H_2O$) R_f=0.38. PDMS: Calculated $(M + Na)^+$: 1908.8, Observed $(M + Na)^+$: 1909.3. ^1H NMR (200 MHz, CD_3SOCD_3): 1.15-1.55 (s 27H, $(CH_3)_3C$-), 2.47-2.87 (m, $-CH_2-$), 3.05-3.95 (br m, H_2O, H2, H3, H4, H5, H6), 4.3-4.65 (br, 6H, OH6), 4.74-4.95 (m, 6H, H1), 5.01-5.13 (d, 6H, $-CH_2-$), 5.45-5.95 (br, 12H, OH2, OH3), 7.06-7.22 (m, 3H, OCONH), 7.25-7.59 (m, 15H, $-C_6H_5$), 7.66-7.80 (m, 3H, CONH). Ratio of BOC protons:OH2 + OH3 (Calculated: 2.25, Observed: 2.39).

Cyclodextrin 14

The hydrogenolysis procedure described above was used to debenzylate 34.9 mg (18.5 μmol) of **7** to give 28.5 mg (17.8 μmol) of debenzylated analog (96.2% yield). TLC (4 : 1 $CH_3CN : H_2O$): $R_f = 0.3$. The BOC group was then cleaved as described above using 24.9 mg (13.2 μmol) of debenzylated aspartic acid derivative to give 20.4 mg (12.31 μmol) of **14** (93.3% yield). PDMS: Calculated $(M + Na)^+$: 1338.1, Observed $(M + Na)^+$: 1337.6.

Cyclodextrin 8

HBT (183.9 mg, 1.36 mmol), DCC (280.8 mg, 1.36 mmol), and N_a-*t*-BOC-L-glutamine (335.2 mg, 1.36 mmol) in 29 ml DMF were reacted as above with **2** (400 mg, 0.412 mmol) and ethyl morpholine (0.266 ml) in 15.6 ml DMF. The diethyl ether-precipitated product was separated on a silica gel column (3 × 25 cm) using a $CH_3CN : H_2O$ gradient elution (700 ml 9 : 1, followed by 700 ml 4 : 1) to give 145 mg **8** (21.2% yield). TLC (4 : 1 $CH_3CN : H_2O$): $R_f = 0.27$. ^1H NMR (200 MHz, CD_3SOCD_3): 1.17-1.50 (s, 27H, $(CH_3)_3C$-), 1.54-1.95 (br m, 6H, $-CH_2-$), 2.0-2.18 (t, 6H, $-CH_2-$), 3.0-3.85 (br m, H_2O,

H2, H3, H4, H5, H6), 3.85-4.12 (br, 3H), 4.42-4.68 (br, 3H, OH6), 4.68-4.92 (m, 3H, H1), 5.2-5.8 (m, 12H, OH2, OH3), 6.6-6.95 (m, 6H, -NH, -OCONH-), 7.1-7.3 (br, 3H, -NH), 7.35-7.95 (br, 3H, -CONH-). Ratio of BOC protons: OH2 + OH3 protons (Calculated: 2.25, Observed: 2.31). PDMS: Calculated $(M + Na)^+$: 1677.8, Observed $(M + Na)^+$: 1677.7.

Cyclodextrin 15

Deprotection of 17.9 mg (10.8 μmol) of **8** as above gave 17.1 mg (10.1 μmol) of **15** (93.5% yield). PDMS: Calculated $(M + Na)^+$: 1377.5, Observed $(M + Na)^+$ 1377.5.

Cyclodextrin 9

HBT (208.2 mg, 1.54 mmol), DCC (317 mg, 1.54 mmol), and N-*t*-BOC-L-glutamic acid α-benzyl ester (520 mg, 1.54 mmol) in 29.8 ml DMF was treated as above with **2** (427 mg, 0.44 mmol) and ethyl morpholine (0.285 ml) in 16.7 ml DMF. The product was purified on a silica gel column (3 × 25 cm) using a $CH_3CN : H_2O$ step gradient elution (230 ml 19 : 1, followed by 440 ml 15 : 1, 460 ml 10 : 1, and 440 ml 9 : 1) to give 139.1 mg **9** (16.4% yield). TLC (10 : 1 $CH_3CN : H_2O$): $R_f = 0.18$. ^1H NMR (200 MHz, CD_3SOCD_3): 1.13-1.45 (s, 27H, $(CH_3)_3C$-), 1.62-2.06 (br m, 6H, $-CH_2$-), 2.06-2.39 (br, 6H, $-CH_2$-), 3.1-3.44 (m, H_2O, H2, H4), 3.44-3.84 (br m 24H, H3, H5, H6), 3.84-4.06 (m, 3H), 4.45-4.66 (br, 3H, OH6), 4.75-4.95 (m, 6H, H1), 5.02-5.20 (m, 6H, -CH2-), 5.43-5.77 (m, 12H, OH2, OH3), 6.81-7.01 (br, 3H, -OCONH-), 7.20-7.43 (m, 15H, $-C_6H_5$), 7.62-7.92 (br, 3H, -CONH-). Ratio of BOC protons: OH2 + OH3 protons (Calculated: 2.25, Observed: 2.52). PDMS: Calculated $(M + Na)^+$: 1951.1, Observed $(M + Na)^+$: 1951.4.

Cyclodextrin 16

The hydrogenolysis procedure described above was used to debenzylate 30.7 mg (15.9 μmol) of **9** to give 23.6 mg (14.2 μmol) of debenzylated analog (89.3% yield). TLC (4 : 1 $CH_3CN : H_2O$): $R_f = 0.27$. The BOC group was then cleaved as described above using 19.1 mg (11.5 μmol) of debenzylated glutamic acid derivative to give 18.54 mg (10.9 μmol) of **16** (94.8% yield). PDMS: Calculated $(M + Na)^+$: 1380.4, Observed $(M + Na)^+$: 1379.5.

Results and Discussion

α-Cyclodextrin(α-CD) was azidated following the method of Hanessian and co-workers [23]. They reported that treatment of α-CD with triphenylphosphine and carbon tetrabromide (3 equivalents) and lithium azide (10 equivalents) in DMF for 6 h gave a mixture of monoazido, diazido, and triazido α-cyclodextrin in yields of 20%, 26% and 5-10%, respectively. Our pathway (Scheme 1) used similar reaction conditions, except that commercially available sodium azide was used instead of lithium azide, thus

requiring a longer reaction time (4 d) due to the lower solubility of NaN_3. Since we sought the trifunctional α-CD derivatives, we tried to increase the yields of triazido-α-CD (**1**) by increasing the amount of excess NaN_3 in the reaction mixture as well as extending the reaction times. Although higher yields of the triazido α-CD were observed with these modifications, significant amounts of tetraazido α-CD were also produced, making the subsequent separation more tedious. Under these conditions, the respective yields of tetra-, tri-, di-, and monoazido α-CD were 16.2%, 21.5%, 17.5% and 8.3%.

CD **1** was reduced to the corresponding triamino α-CD (**2**) by hydrogenation with Pd/C at 30 psi for 48 h. In the early stages of the hydrogenation reaction, four spots were observed by TLC, corresponding to **1**, amino α-CD (**2**), and the two partially reduced intermediates. After 48 h, this mixture collapsed into a single spot corresponding to **2**, a water soluble material that was characterized by ^{1}H NMR and plasma desorption mass spectrometry (PDMS).

The penultimate target compounds (**3-9**) were prepared by modifying the primary rim of α-CD **2** with orthogonally protected analogs of phenylalanine, tryptophan, tyrosine, aspartic acid, glutamine acid, glutamine and asparagine. This was achieved by coupling 3.3 equivalents of protected amino acid per equivalent of **2**, with yields ranging from 13.7% to 23.9% depending on the amino acid used. The product isolation method employed in our case, however, differed slightly from previously reported methods. Stoddart and co-workers (22) have prepared monofacially substituted CD using DCC in the presence of BOC-protected phenylalanine. After the coupling reaction, the DCU was removed by filtration and the desired BOC-protected per-6-[(phenylalanyl)amino)]-β-CD derivative precipitated from the concentrated reaction mixture by addition of sodium bicarbonate with stirring for 1 h. We were unsuccessful in removing all DCU in this manner, so gradient column chromatography was employed to purify the pure trisubstituted β-CDs **3-9** that were isolated by precipitation from water (BOC-phenylalanine, BOC-glutamic acid, BOC-aspartic acid, BOC-tyrosine, BOC-tryptophan) or diethyl ether solutions (BOC-glutamine, BOC-asparagine). The low yields observed are likely due to incomplete conversion as a result of the sterically hindered regioisomers and the limited stoichiometry of the BOC-amino acids employed. Benzyl and BOC protecting groups were removed in the final step by hydrogenation and TFA treatment, respectively, to give the target receptors **10-16** in >90% yield as their TFA salts.

In conclusion, a direct pathway to the synthesis of regioisomeric α-cyclodextrin derivatives bearing three identical amino acid substituents at the primary rim has been developed. Although the overall efficiency of the route is low, these derivatives may find use as artificial receptors. Glucose affinity studies of compounds **10-16** with a variety of ligands are currently in progress.

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