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Zhu Guan
Université Pierre et Marie Curie, Paris

Yali Wang
University of Nebraska-Lincoln, yali.wang@huskers.unl.edu

Yong Chen
Université Pierre et Marie Curie, Paris

Yongmin Zhang
Université Pierre et Marie Curie, Paris, yongmin.zhang@upmc.fr

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Novel approach for synthesis of 2:1 permethylated β-cyclodextrin–C_{60} conjugate

Zhu Guan,[1, 2] Yali Wang,[1] Yong Chen,[1] Lihe Zhang,[2] and Yongmin Zhang,[1, 3]

1. Laboratoire de Chimie Organique, UMR CNRS 7611, Tour 44/45, Université Pierre et Marie Curie, 4 Place Jussieu, 75005 Paris, France

2. Peking University Health Science Center, State Key Laboratory of Natural and Biomimetic Drugs, 38 Xueyuan Rd, Haidian District, Beijing 100083, China

3. ZJU-ENS Joint Laboratory of Medicinal Chemistry, Zhejiang University, Hangzhou 310058, China

Corresponding author — Y. Zhang, tel 33 1 44276153; fax 33 1 44275504; email yongmin.zhang@upmc.fr

Present address for Y. Chen — State Key Laboratory of Elemento-Organic Chemistry, Department of Chemistry, Nankai University, Tianjin 300071, China.

Abstract

Amphiphilic cyclodextrin–fullerene conjugates have potential biological activity, due to their water solubility. In order to study the influence of the linker of these conjugates on solubility and aggregation, a permethylated β-cyclodextrin–C_{60} conjugate with a short linker –$(CH_2)_2$NHCO–, which is attached to the secondary face of β-cyclodextrin was synthesized. Its solubility in water and its UV spectrum in CH_2Cl_2 and water were investigated.

Keywords: synthesis, β-cyclodextrin, fullerene, C_{60} , amphiphilic, conjugate

1. Introduction

C_{60}, the most representative among fullerenes, is currently considered as a powerful building block in material science and biological chemistry due to its unique photo-, electrophysical, and chemical properties. However, lack of solubility in polar solvents and formation of aggregates in aqueous solution limit its application in biological field.[1] There have been several attempts to overcome the natural repulsion of fullerenes for water. Generally the water-soluble fullerenes could be obtained either by covalent addition of hydrophilic appendages or by complex formation with host molecules.[2]

Cyclodextrins (CDs) are naturally occurring oligomers of α-1,4-linked d-glucose units, with a unique hollow-truncated-cone geometry (Fig. 1).[3] They are known to function as host molecules making inclusion complexes with hydrophobic guest molecules in aqueous solution.[4] By formation of complex with CD, water-soluble C_{60} was first obtained in 1992 by Andersson and co-workers.[5] In 1994 Yoshida and co-workers reported[6] first preparation of the stable, water-soluble γ-CD–C_{60} (2:1) complex ('bicapped buckminster-fullerene'), they used the complex in a spectral investigation for elucidation of the molecular recognition of C_{60} by γ-CD.

However, in the case of 2:1 γ-CD–C_{60} complexation, different equilibria, such as (1) and (2) may take place in solution

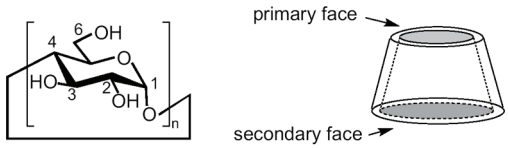


Figure 1. Structures of the cyclodextrin (CD) cavities. α-CD: *n* = 6; β-CD: *n* = 7; γ-CD: *n* = 8.

(Scheme 1), so that if some other substrates with sufficient affinity for γ-CD were present, C_{60} could be displaced and possibly precipitate. A covalent binding between C_{60} and γ-CD would probably impede this displacement.[2]

So far, few works have been reported on the CD–C_{60} conjugates. The first water-soluble CD–C_{60} conjugate was described by Samal and Geckeler in 2000.[7] Liu and co-workers reported in 2005 that the CD–C_{60} conjugates with DNA-cleaving properties exhibited a moderate water solubility (2.5 mg/mL).[8]

We recently prepared a new type of 2:1 permethylated β-cyclodextrin–C_{60} conjugate (Fig. 2) **1**, which showed a high

$$C_{60} + CD \rightleftharpoons (C_{60},CD) \quad (1)$$

$$(C_{60},CD) + CD \rightleftharpoons (CD,C_{60},CD) \quad (2)$$

Scheme 1. Equilibria between γ-CD and C_{60} in water.

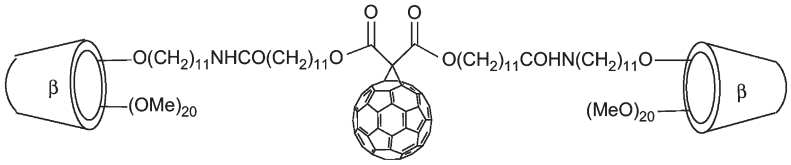


Figure 2. Highly water-soluble CD–C_{60} conjugate **1**.

water solubility up to 320 mg/mL.[9] However, UV and NMR spectra showed the presence of aggregates. Although this high water solubility is convenient for application to biological systems, micellar aggregation may induce chemical,[10] electrochemical,[11] or photophysical[12] properties differing from those of the isolated fullerene molecule.

It was postulated that this kind of conjugate could be present in water equilibria between conformers such as **A**, **B**, and **C** (Fig. 3). Conformers **A** and **B** could form micelle-like aggregates, while **C** could exist as a non-associated species by forming an internal complexation.[13]

2. Results and discussion

Methylated β-cyclodextrin was chosen to be appendage of C_{60} because methylated CD derivatives are thought to be able to enhance the solubility in an aqueous medium.[14] Moreover, inclusion complexes of methylated CDs are usually more stable than the correspondent complexes of unmodified CDs.[15] The secondary face of CD was chosen to be a linkage connecting point due to its larger diameter than that of primary face, which is important for C_{60} inclusion.

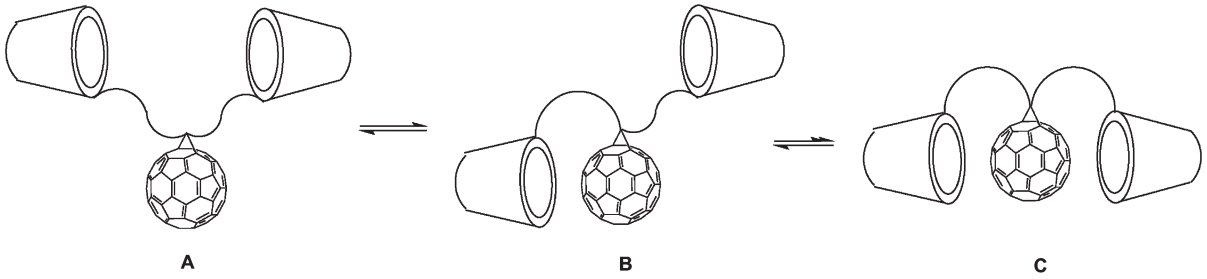


Figure 3. Postulated equilibria of the CD–C_{60} conjugate in water.

In molecule **1**, the longer linkers between two CDs and C_{60} gave a higher freedom for C_{60} molecule, which favors the formation of the conformers **A** and **B**, we supposed that a shorter linker should force the CDs and C_{60} to form an inclusion complex (conformer **B**). In order to study the influence of the linker on solubility and aggregation, we have prepared a new CD–C_{60} conjugate **2** in which the two linkers are much shorter than their analogues (Fig. 4). Herein, we describe the synthesis of this conjugate.

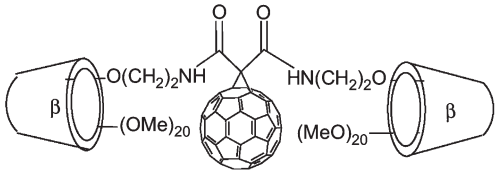
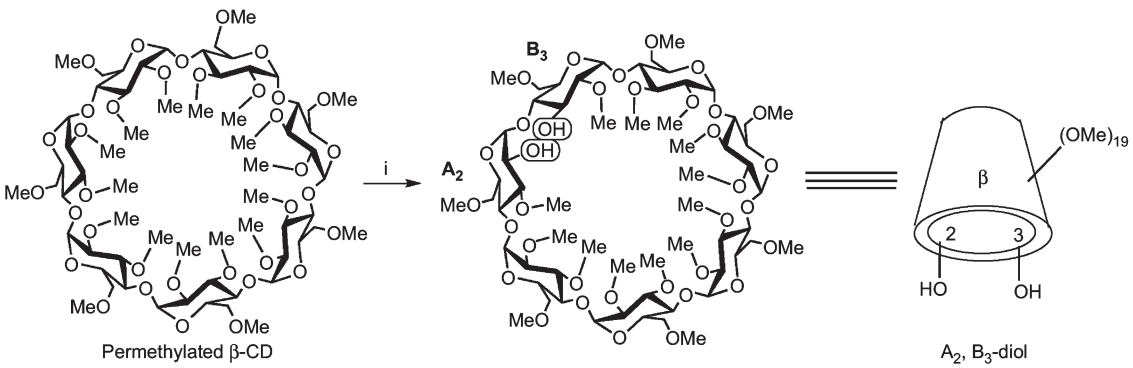


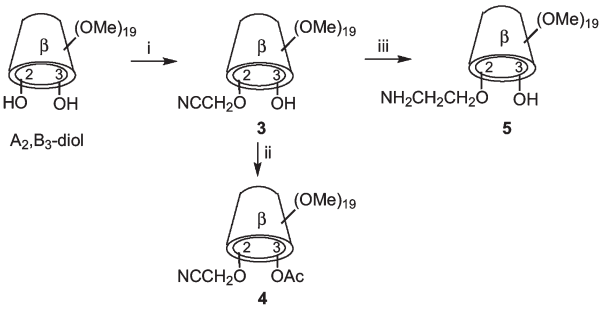
Figure 4. CD–C_{60} conjugate **2**.

The key intermediate of this synthesis is a permethylated β-CD A_2,B_3-diol, which was regioselectively prepared according to our previously reported procedure[9] from the commercially available permethylated β-CD in 56% yield (Scheme 2).

Instead of preparing an azidoalkyltosylate for conjugate **1**, we used commercially available bromoacetonitrile to react with the A_2,B_3-diol, giving monoalkylated derivative **3** in 91% yield via a selective alkylation. Here KOH was selected instead of NaOH as basic reagent since the reaction gave a better yield with KOH. In this step, the hydroxyl group at 2-position is more acidic than that at 3-position, it could be first deprotonated in the presence of strong base[16] to react with bromoacetonitrile, giving regioselectively the 2-alkyl CD. The regioselectivity of this reaction was demonstrated by analysing the structure of compound **3** based on NMR and MS data. This structure was further confirmed from the ^{1}H NMR spectrum of derivative **4**, obtained from **3** by acetylation (90% yield); which displayed a deshielded signal for H_3 of the glucose unit



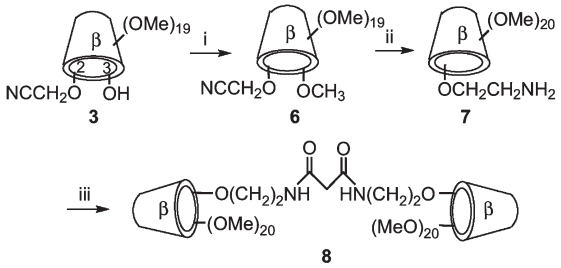
Scheme 2. Reagents and conditions: (i) DIBAL-H, (9 equiv), 0 °C, 18 h (56%).



Scheme 3. Reagents and conditions: (i) KOH, THF, $BrCH_2CN$; 1 h (91%); (ii) Ac_2O, pyridine, 40 °C, 24 h (90%); (iii) H_2, Pd–C, 1 N HCl, CH_3OH, 4 h (64%).

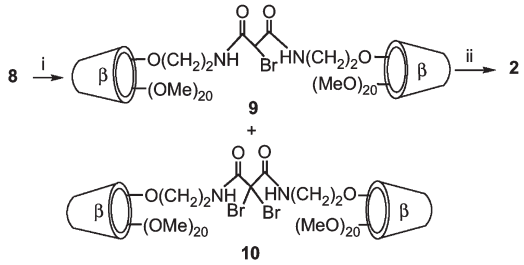
B at 5.41 ppm, indicating that alkylation of the A_2,B_3-diol took place at position 2 to afford compound **3**. In order to reduce the cyano group of **3**, $LiAlH_4$ and BH_3–THF were first used, but neither of them gave desired amino product. We then found that hydrogen in the presence of Pd–C could reduce cyano group of **3** to give the amino derivative **5** in a satisfactory yield (Scheme 3).

The free hydroxyl group of compound **3** was then methylated to provide cyanoalkyl permethylated β-CD **6** in 87% yield. Reduction of the cyano group of **6**, as described for **3**, gave aminoalkyl permethylated β-CD **7** in good yield, which was condensed with malonyl dichloride to afford CD dimer **8** (Scheme 4).



Scheme 4. Reagents and conditions: (i) CH_3I, NaH, DMF, rt, 2 h (87%); (ii) H_2, Pd–C, 1 N HCl, CH_3OH, 4 h (80%); (iii) malonyl dichloride, Et_3N, CH_2Cl_2, rt, 10 h (34%).

With **8** in hand, we logically used Hirsch–Bingle reaction to perform the condensation with C_{60}, the reaction we used for preparation of conjugate **1**.[9] Surprisingly, the reaction failed to produce the methanofullerene derivative. A two-step reaction was then tested and proved to be successful. The first step consisted in a mono bromination of dimer **8** to provide **9**. In this step, dibromide **10** was also formed. After optimization, we found that when 1.2 equiv of bromine was used and the reaction was carefully monitored by TLC and timely quenched, monobromide **9** could be isolated in 40% yield and dibromide **10** in 20% yield. The starting material was recovered in about 40% yield, which could be reused for the same bromination. Finally monobromo β-CD **9** was treated with C_{60} in the presence DBU to afford target compound **2**, as shown in Scheme 5.



Scheme 5. Reagents and conditions: (i) Br_2, Et_3N, CH_2Cl_2 (60%, **9**/**10** = 2:1); (ii) C_{60}, DBU, toluene, rt, 1 h (33%).

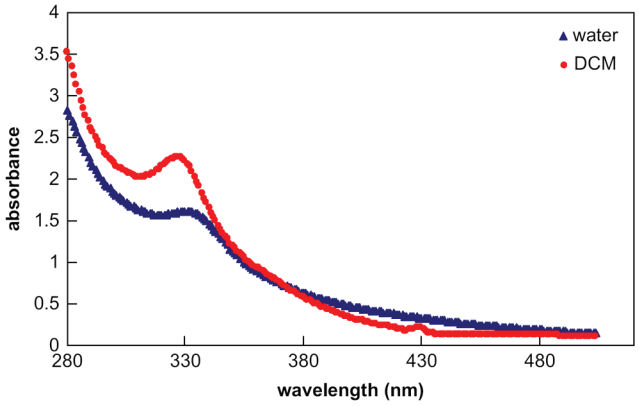


Figure 5. UV–vis spectra of conjugate **2** in DCM (2.0×10^{-4} mmol) and water (3.0×10^{-4} mmol).

The structure of conjugate **2** was fully characterized by ^1H NMR, ^{13}C NMR, MS, and UV–vis techniques. The UV–vis absorption spectra in dichloromethane (DCM) and water of **2** are reported in Figure 5. In DCM, the typical absorption features of fullerene[60] mono-adduct could be observed: an absorption at ~330 nm, a weak peak at ~430 nm, and a broad weak band at ~480 nm. The weak absorption at ~430 nm indicates conjugate **2** can exist as a non-associated species in DCM without aggregate formation. But in water, no absorption could be observed around 430 nm, indicating the existence of aggregates in water.

Conjugate **2** cannot be rapidly dissolved in water. A mixture of **2** (3 mg) in water (100 μL) stored at 4 °C for more than 24 h gave a clear brown solution.

3. Conclusion

We have described the synthesis of a novel CD–C_{60} conjugate (Fig. 4) in which two shorter linkers [-$(CH_2)_2$NHCO-] were used to connect CD and C_{60}, with two amide groups being linked directly to the carbon atom of the methanofullerene instead of two ester groups as in our former conjugates. For the preparation of alkyl CD, a selective alkylation was realized in a more convenient way than preparing conjugate **1** and its analogues, and a two-step reaction was used for achieving the synthesis of methanofullerene.

Compared with other conjugates we prepared previously,[9], [17] and [18] conjugate **2** displays a lower solubility (30 mg/mL), and the shorter linker (-CH_2CH_2-) does not reduce the formation of aggregates. Since it is possible that the affinity of β-CD for C_{60} is not strong enough, the distance between C_{60} and CD could play an important role in formation of internal complexation, a conjugate with medium size of linker is being synthesized. Due to their interesting water-soluble property, these amphiphilic fullerene derivatives, which can be transported in biological systems, are particularly desirable for biological testing.

4. Experimental

4.1. General

Optical rotations were measured at room temperature with a Perkin Elmer Model 241 digital polarimeter, using a 10 cm, 1 mL cell. Microanalyses were performed by Service de Microanalyse de l'Université Pierre et Marie Curie, 4 Place Jussieu, 75005 Paris, France. Fast Atom Bombardment Mass Spectra FABMS were obtained with a JMS-700 spectrometer, and ESI-MS were obtained with Bruker DALTONTICS DataAnaly-

sis 3.3. NMR spectra were recorded on a Bruker DRX 400 spectrometer at ambient temperature. ^{1}H NMR chemical shifts are referenced to residual protic solvent ($CDCl_3$, δ_H = 7.30). ^{13}C NMR chemical shifts are referenced to the solvent signal (δ_C = 77.0 for the central line of $CDCl_3$). Reactions were monitored by thin-layer chromatography (TLC) on a pre-coated silica gel 60 F_{245} plate (layer thickness 0.2 mm; E. Merck, Darmstadt, Germany) and detection by charring with sulfuric acid. Flash column chromatography was performed on silica gel 60 (230–400 mesh, E. Merck).

4.2. Synthesis of 2-O-cyanomethyl-3-hydroxyl-permethylated β-cyclodextrin (3)

To a solution of A_2,B_3-diol (420 mg, 0.3 mmol) in anhydrous THF (40 mL) was added KOH (50 mg, 0.9 mmol) under argon, the reaction mixture was stirred at room temperature for 24 h. Diluted $BrCH_2CN$ (31 μL, 0.45 mmol) by THF (1 mL) was dropped into the solution and stirred for 30 min. Then the reaction was stopped with acetic acid. After removing the solvent by evaporation under reduced pressure, the residue was dissolved in CH_2Cl_2, washed with saturated brine, and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by flash chromatography, eluting with 25:1 CH_2Cl_2/CH_3OH to give compound **3** as a white amorphous solid (395 mg, 91%). R_f = 0.42 (CH_2Cl_2/CH_3OH 10:1); $[\alpha]_D$ +141 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.18 (d, 2H, *J* = 3.6 Hz, 2×H_1), 5.10 (d, 1H, *J* = 3.5 Hz, H_1), 5.15–5.12 (m, 4H, 4×H_1), 4.60 (2d, 2H, *J* = 16.2 Hz, CH_2–CN); ^{13}C NMR (100 MHz, $CDCl_3$): δ 115.47 (1C, CN), 100.41, 99.69, 99.46, 99.43, 98.90, 98.87, 98.61 (7C, 7×C_1), 83.43, 82.38, 82.16, 82.13, 82.04, 81.99, 81.88, 81.74, 81.65, 81.58, 81.39, 81.36, 81.31, 81.09, 80.95, 80.80, 80.30, 80.10, 79.82 (21C, 7×C_2, C_3, C_4), 71.67, 71.02, 70.93, 70.89, 70.86, 70.72, 69.83 (7C, 7×C_5), 71.29, 71.23, 71.17, 70.82, 70.65 (7C, 7×C_6), 62.07, 61.44, 61.40, 61.19, 58.98, 58.91, 58.89, 58.87, 58.85, 58.76, 58.48, 58.40, 58.35, 58.33, 58.30 (19C, 19×OCH_3), 57.26 (1C CH_2–CN); FABMS: *m*/*z* 1462.7 (M+Na^+). Anal. Calcd for $C_{63}H_{109}NO_{35}$ 3H_2O: C, 50.63; H, 7.76; N, 0.94. Found: C, 50.61; H, 7.79; N, 1.14.

4.3. Synthesis of 2-O-cyanomethyl-3-O-acetyl-permethylated β-cyclodextrin (4)

A mixture of **3** (460 mg, 0.31 mmol), Ac_2O (5.5 mL) in anhydrous pyridine (11 mL) was stirred at room temperature for 24 h under argon. After removing the solvent by evaporation under reduced pressure, the residue was dissolved in CH_2Cl_2, washed with brine, and dried over $MgSO_4$. The solvent was evaporated, and the residue was isolated by flash chromatography, eluting with ethyl acetate/isopropanol/H_2O 10:1:0.3 to give compound **4** as a white amorphous solid (417 mg, 90%). R_f = 0.5 (CH_3CN/CH_3OH 16:1); $[\alpha]_D$ +113 (*c* 0.75, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.41 (m, 1H, H_3), 5.33 (d, 1H, *J* = 3.6 Hz, H_1), 5.16–5.12 (m, 4H, 4×H_1), 5.08 (d, 1H, *J* = 3.5 Hz, H_1), 5.02 (d, 1H, *J* = 3.4 Hz, H_1), 4.50 (2d, 2H, *J* = 16.1 Hz, CH_2–CN), 3.31 (2d, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10.3 Hz, H_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.24 (1C, C═O), 116.27 (1C, CN), 99.60, 99.59, 99.45, 98.88, 98.78, 98.76, 98.68 (7C, 7×C_1), 82.42, 82.24, 82.20, 82.10, 81.86, 81.72, 81.63, 81.61, 81.59, 81.57, 81.50, 81.47, 81.39, 80.73, 80.68, 80.65, 80.58 (21C, 7×C_2, C_3, C_4), 71.77, 71.50, 71.08, 70.98, 70.94, 70.77, 70.43 (7C, 7×C_5), 71.95, 71.54, 71.30, 71.12, 70.89, 70.47 (7C, 7×C_6), 61.98, 61.76, 61.67, 61.41, 60.85, 59.36, 59.09, 59.07, 58.93, 58.88, 58.69, 58.33, 58.26, 58.00 (19C, 19×OCH_3), 57.19 (1C, CH_2CN), 21.48 (1C, O═CCH_3); FABMS: *m*/*z* 1504.7 (M+Na^+). Anal. Calcd for $C_{65}H_{111}O_{36}N$: C, 52.66; H, 7.55; N, 0.94. Found: C, 52.44; H, 7.60; N, 1.12.

4.4. Synthesis of 2-O-aminoethyl-3-hydroxyl-permethylated β-cyclodextrin (5)

To a mixture of **3** (390 mg, 0.28 mmol), Pd/C (10%, 160 mg) in CH_3OH (10 mL) was added 1 N HCl aqueous solution (0.4 mL, 0.4 mmol). The reaction mixture was stirred at room temperature for 4 h under hydrogen. After filtration, the filtrate was concentrated by evaporation under reduced pressure, the residue was purified by flash chromatography, eluting with $CH_2Cl_2/CH_3OH/NH_4OH$ 10:1:0.05 to provide compound **5** as a white amorphous solid (248 mg, 64%). R_f = 0.25 (CH_2Cl_2/CH_3OH 6:1); $[\alpha]_D$ +150 (*c* 1.05, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.16–5.09 (m, 6H, 4×H_1, NH_2), 5.08 (d, 1H, *J* = 3.4 Hz, H_1), 5.03 (d, 1H, *J* = 3.6 Hz, H_1), 2.93 (m, 2H, CH_2N); ^{13}C NMR (100 MHz, $CDCl_3$): δ 100.56, 99.72, 99.30, 99.09, 98.72, 98.62, 98.48 (7C, 7×C_1), 82.83, 82.55, 82.08, 81.97.81.84, 81.77, 81.50, 81.43, 81.42, 81.26, 81.02, 80.97, 80.53, 79.99, 79.88, 79.79, 79.27 (21C, 7×C_2, C_3, C_4), 74.57 (1C, OCH_2), 71.23, 71.18, 71.05, 70.81 (7C, 7×C_6), 71.43, 70.72, 70.67, 70.58 (7C, 7×C_5), 61.65, 61.26, 61.21, 61.17, 61.11, 61.07, 58.76. 58.70, 58.66, 58.38, 58.24, 58.23, 58.19, 58.17, 58.13 (19C, 19×OCH_3), 41.48 (1C, CH_2NH_2); FABMS: *m*/*z* 1466.8 (M+Na^+). Anal. Calcd for $C_{63}H_{113}O_{35}N$ 3H_2O: C, 50.49; H, 8.00; N, 0.93. Found: C, 50.56; H, 8.00; N, 0.93.

4.5. Synthesis of 2-O-cyanomethyl-permethylated β-cyclodextrin (6)

To a mixture of **3** (380 mg, 0.26 mmol) and NaH (60%, 54 mg, 1.34 mmol) in anhydrous DMF (4 mL) was added CH_3I (80 μL, 1.34 mmol), the reaction mixture was stirred at room temperature for 10 h. CH_3OH (1 mL) was added to quench the reaction. After removal of solvent by evaporation, the residue was purified by flash chromatography, eluted with cyclohexane/acetone 2:1 to give compound **6** (330 mg, 87%) as a white foam. R_f = 0.32 (cyclohexane/acetone 1:1); $[\alpha]_D$ +135.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.15–5.10 (m, 6H, 6×H_1), 5.06 (d, 1H, $J_{1,2}$ = 3.6 Hz, H_1), 4.50 (2d, 2H, *J* = 15.9 Hz, CH_2CN); ^{13}C (100 MHz, $CDCl_3$): δ 116.15 (1C, CN), 98.97, 98.91, 98.88, 98.86, 98.85 (7C, 7×C_1), 81.97, 81.95, 81.93, 81.90, 81.89, 81.80, 81.75, 81.70, 81.69, 81.63, 81.58, 80.54, 80.34, 80.27, 80.24, 80.18, 79.97 (21C, 7×C_2, C_3, C_4), 71.01, 70.91, 70.87, 70.82, 70.74, 70.61 (7C, 7×C_5), 71.38, 71.32, 71.29, 71.26 (7C, 7×C_6), 61.71, 61.42, 61.39, 61.37, 61.32, 61.27, 61.21, 58.92, 58.89, 58.87, 58.85, 58.74, 58.62, 58.56, 58.53, 58.49, 58.47, 58.39, 58.35, 58.32 (20C, 20×OCH_3), 56.35 (1C, CHCN); HRMS (FAB): calcd for: 1476.6834 (M+Na^+), found: *m*/*z* 1476.6898.

4.6. Synthesis of 2-O-aminoethyl-permethylated-cyclodextrin (7)

To a mixture of **6** (300 mg, 0.23 mmol), Pd–C (10%, 125 mg) in CH_3OH (10 mL) was added 1 N HCl aqueous solution (0.3 mL, 0.3 mmol). The mixture was stirred at room temperature for 2 h under hydrogen. After filtration and removal of the solvent, the residue was purified by flash chromatography, eluted with $CH_2Cl_2/CH_3OH/NH_3 \cdot H_2O$ 10:1:0.05 to give **7** (269 mg, 80%) as a white foam. R_f = 0.14 (CH_2Cl_2/CH_3OH 8:1); $[\alpha]_D$ +113.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.17–5.09 (m, 6H, 6×H_1), 5.05 (d, 1H, $J_{1,2}$ = 3.2 Hz, H_1), 3.27–3.23 (m, 2H, CH_2NH_2); ^{13}C (100 MHz, $CDCl_3$): δ 99.38, 99.24, 99.09, 99.01, 98.98, 98.76, 98.63 (7C, 7×C_1), 82.45, 82.22, 82.15, 82.03, 81.94, 81.80, 81.77, 81.68, 81.63, 81.59, 81.50, 81.26, 81.21, 81.04, 80.60, 80.36, 80.22, 80.16, 79.99, 79.87, 79.79 (21C, 7×C_2, C_3, C_4), 71.61, 71.52, 71.46, 71.20, 71.11 (7C, 7×C_6), 71.39, 71.27, 70.92, 70.83, 70.76, 70.68 (7C, 7×C_5), 67.73 (1C, OCH_2), 61.78,

61.75, 61.60, 61.51, 61.44, 61.28, 61.25, 61.19, 61.11, 59.14, 58.90, 58.87, 58.84, 58.77, 58.51, 58.39, 58.37, 58.26, 58.23, 58.18 (20C, 20×OCH_3), 40.83 (1C, CH_2NH_2); HRMS (FAB): calcd for: 1458.7328 ($M+H^+$), found: *m/z* 1458.7313.

4.7. Synthesis of permethylated β-CD dimer (8)

A solution of **7** (770 mg, 0.52 mmol) in anhydrous CH_2Cl_2 (65 mL) in ice-bath was added Et_3N (183 µL, 1.32 mmol) and malonyl dichloride (26 µL, 0.27 mmol) under argon. The mixture was stirred at room temperature for 10 h. After removal of solvent, the residue was purified by flash chromatography, eluted with EtOAc/CH_3OH 8:1 to provide **8** (270 mg, 34%) as colorless syrup. R_f = 0.29 (EtOAc/CH_3OH 6:1); $[\alpha]_D$ +133.5 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.94 (t, 2H, 2×NH), 5.19 (d, 4H, $J_{1,2}$ = 3.6 Hz, 4×H_1), 5.17–5.13 (m, 8H, 8×H_1), 5.03 (d, 2H, $J_{1,2}$ = 3.6 Hz, 2×H_1); ^{13}C (100 MHz, $CDCl_3$): δ 127.24 (2C, 2×C═O), 99.26, 99.13, 99.05, 99.04, 98.84, 98.83, 98.80 (14C, 14×C_1), 82.15, 82.03, 81.90, 81.85, 81.80, 81.74, 81.68, 81.66, 81.62, 81.56, 81.45, 80.60, 80.58, 80.49, 80.45, 80.41, 80.39, 82.23, 79.99, 79.89, 71.03, 70.95, 70.83, 70.68 (56C, 14×C_2, C_3, C_4, C_5), 71.58, 71.52, 71.49, 71.38, 71.18, 70.79 (14C, 14×C_6), 69.91 (2C, 2×OCH_2), 61.86, 61.68, 61.59, 61.56, 61.34, 61.25, 61.21, 58.99, 58.96, 58.93, 58.91, 58.81, 58.47, 58.42, 58.24 (40C, 40×OCH_3), 42.56, 39.74 (3C, $COCH_2CO$, 2×CH_2NHCO); HRMS (ESI): calcd for: 2984.4471 ($M+H^+$), found: *m/z* 2984.4449.

4.8. Synthesis of bromo permethylated β-CD dimer (9) and dibromo permethylated β-CD dimer (10)

A solution of **8** (50 mg, 0.016 mmol) in anhydrous CH_2Cl_2 (1 mL) in ice-bath under argon was added Et_3N (12 µL, 0.086 mmol) and Br_2 (1 µL, 0.018 mmol). The reaction solution was stirred at room temperature and monitored by TLC. The reaction should be stopped when the dibromide product appear. After removal of solvent, the residue was purified by flash chromatography, eluted with EtOAc/CH_3OH 15:1 to give **9** (20 mg, 40%) and **10** (10 mg, 20%) both as white foam. Compound **9**: R_f = 0.29 (EtOAc/CH_3OH 5:1); $[\alpha]_D$ +48.2 (*c* 0.4, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.93 (t, 2H, *J* = 5.5 Hz, 2×NH), 5.17–5.11 (m, 12H, 12×H_1), 5.06 (d, $J_{1,2}$ = 3.5 Hz, 2H, 2×H_1); ^{13}C NMR (125 NMR, $CDCl_3$): δ 166.17 (2×CONH), 99.26, 99.25, 99.23 (14C, 14×C_1), 82.64, 82.50, 82.38, 82.22, 82.12, 82.08, 82.05, 80.91, 77.75, 77.43, 77.19, 77.18, 77.15, 77.13, 77.10, 77.06, 77.03, 77.02, 77.01, 71.99, 71.97, 71.32, 71.31, 71.19 (70C, 14×C_2, C_3, C_4, C_5, C_6, OCH_2), 61.71, 59.41, 51.36, 58.98, 58.94, 8.72 (40C, 40×Me); HRMS (ESI): calcd for: 3062.3576 ($M+H^+$), found: *m/z* 3062.3196.

Compound **10**: R_f = 0.33 (EtOAc/CH_3OH 5:1); $[\alpha]_D$ +185.8 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.97 (t, 2H, *J* = 5.6 Hz, 2×NH), 5.21–5.12 (m, 12H, 12×H_1), 5.04 (d, 2H, *J* = 3.5 Hz, 2×H_1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.17 (2C, 2×CONH), 99.55, 99.26, 99.15, 99.02, 99.00, 98.81, 98.76 (14C, 14×C_1), 82.26, 82.14, 82.05, 81.97, 81.91, 81.82, 81.75, 81.69, 81.21, 80.87, 80.70, 80.42, 80.24, 80.17, 80.11, 80.07, 80.03, 79.91, 71.16, 71.10, 70.96, 70.85, 70.76, 70.74 (56C, 14×C_2, C_3, C_4, C_5), 71.52, 71.48, 71.37, 71.29, 71.23, 69.91 (16C, 14×C_6, 2×OCH_2), 61.80, 61.57, 61.51, 61.36, 61.30, 61.28, 58.97, 58.93, 58.81, 58.75, 58.51, 58.31 (40C, 40×Me), 57.00, 41.85 (CH_2NH, CBr_2); HRMS (ESI): calcd for: 3157.2946 ($M+NH_4^+$), found: *m/z* 3157.2945.

4.9. Synthesis of 2:1 β-cyclodextrin/fullerene[60] conjugate (2)

To a solution of **9** (10 mg, 3.27×10^{-3} mmol) and C_{60} (4 mg, 5.5×10^{-3} mmol) in anhydrous toluene, DBU (2 µL, 0.013 mmol) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was directly chromatographed, eluting first with cyclohexane to remove excess C_{60}, then cyclohexane/acetone 3:2 to provide compound **2** (4 mg, 33%) as brown solid. R_f = 0.29 (cyclohexane/acetone 1:1); $[\alpha]_D$ +16.5 (*c* 0.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.95 (t, 2H, *J* = 5.5 Hz, 2×NH), 5.15–5.11 (m, 12H, 12×H_1), 5.02 (d, 2H, $J_{1,2}$ = 3.5 Hz, 2×H_1); ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.48 (2×CONH), 146.69, 145.92, 145.71, 145.38, 145.16, 144.67, 144.50, 144.28, 143.74, 142.99, 142.32, 142.14, 140.87, 140.75, 137.95, 137.83 (C_{60}-sp^2C), 99.44, 98.93, 98.80 (14C, 14×C_1), 81.93, 81.78, 81.17, 80.35, 80.09, 79.85, 77.26, 77.00, 76.75, 71.46, 71.35, 71.09, 70.97, 70.84, 69.99 (70C, 14×C_2, C_3, C_4, C_5, C_6, 2×OCH_2, C_{60}-sp^3C), 62.06, 61.90, 61.51, 61.32, 58.96, 58.72, 58.61, 58.46 (40C, 40×Me), 41.09, 29.68 (3C, 2×CH_2NH, bridgehead C); HRMS (ESI): calcd for: 3724.4134 ($M+Na^+$), found: *m/z* 3724.4115.

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