Syntheses and Doxorubicin-Inclusion Abilities of β-Cyclodextrin Derivatives with a Hydroquinone α-Glycoside Residue Attached at the Primary Side

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This paper describes syntheses and doxorubicin-inclusion abilities of β-cyclodextrin (CyD) derivatives with a hydroquinone α-glycoside residue attached at the primary side. The hydroquinone glycoside having an α-D-glucosidic or 2-acetamido-2-deoxy-α-D-glucosidic linkage became a useful component for providing an α-D-glucose- or 2-acetamido-2-deoxy-α-D-glucose–β-CyD conjugate. The surface plasmon resonance analyses of these β-CyD derivatives for the anticancer agent, doxorubicin, indicated that they had excellent inclusion associations on the order of 10^5 M^{-1} for the immobilized doxorubicin.

Key words synthesis; β-cyclodextrin; hydroquinone; surface plasmon resonance; saccharide-attached cyclodextrin; doxorubicin

Our recent study showed that the β-glucose–β-cyclodextrin (CyD) conjugates **1** and **2** as shown in Fig. 1 were successfully prepared from β-CyD and arbutin (4-hydroxyphenyl β-glucopyranoside, **3**).[1—3] Arbutin **3** is a naturally occurring hydroquinone glycoside containing a β-D-glucosidic linkage. An appropriate linker necessary to connect **3** with a β-CyD derivative was conveniently introduced into the phenolic alcohol of **3**. The inclusion association constants (K_a) of **1** and **2** for the immobilized doxorubicin (DXR, anticancer agent) measured using a surface plasmon resonance (SPR) optical biosensor were 10^5—10^6 M^{-1}. These K_a values are remarkably high when compared with the inclusion association of the lactose–β-CyD conjugate **4**[4] or normal β-CyD which was on the order of 10^3 M^{-1}. The β-CyD derivatives **1** and **2** were characterized by the existence of the phenyl group in the spacer between the glucose and β-CyD. The π–π stacking interaction effect between the phenyl group and DXR would increase their inclusion associations for DXR.[3] Thus, our former study showed that the hydroquinone glycoside could be a synthetically useful component for providing a saccharide–β-CyD conjugate which is expected to have an excellent DXR-inclusion ability.[5]

The CyD derivatives attached to saccharide moieties are expected to carry drug molecules to specific cells, because they have both the drug-inclusion ability of CyDs and the cell-recognition of saccharides.[4,6—22] Then, our next objective was the synthesis of a novel β-CyD conjugate having another hydroquinone glycoside. This paper describes the synthesis of two novel β-CyD derivatives attached to a hydroquinone glycoside containing an α-D-glucopyranosidic or 2-acetamido-2-deoxy-α-D-glucopyranosidic linkage and the evaluation of their DXR-inclusion abilities.

Results and Discussion

We designed two novel β-CyD derivatives **5** and **6** attached to a hydroquinone glycoside containing an α-D-glucosidic or 2-acetamido-2-deoxy-α-D-glucosidic linkage as shown in Fig. 1.

Chart 1 shows the synthetic procedure of **5** using 4-hydroxyphenyl α-glucopyranoside (α-arbutin, **7**). The reaction of allyl bromide (1.2 eq) with the dry sodium salt of **7** in *N*,*N*-dimethylformamide (DMF) for 70 h gave the allylated compound **8** in 84% yield. The benzylation of **8** using benzyl bromide (4.8 eq) and NaH (16 eq) in DMF for 21 h afforded compound **9** in 99% yield. The hydroboration of **9** with 9-borabicyclo[3.3.1]nonane (9-BBN) (2 eq) in tetrahydrofuran (THF) at 0 °C for 5 h, followed by oxidation using aq. H_2O_2 (10 eq) and by hydrolysis with aq. NaOH (3 eq) for 48 h gave compound **10** in 94% yield. The iodination of **10** with iodine (4 eq) in the presence of Ph_3P (4 eq) in DMF at 35 °C for 2 h quantitatively gave compound **11** in 89% yield. The condensation of **11** (3.8 eq) with the benzylated β-CyD **12**[23] using KOH (*ca.* 120 eq) and tetra-*n*-butylammonium iodide (*n*-Bu_4NI) (0.5 eq) in DMF for 64 h gave **13** in 69% yield. The treatment of **13** with H_2–$Pd(OH)_2$ in DMF for 6 h provided the desired compound **5**, which was purified by gel-filtration using LH 20 (MeOH) in 98% yield.

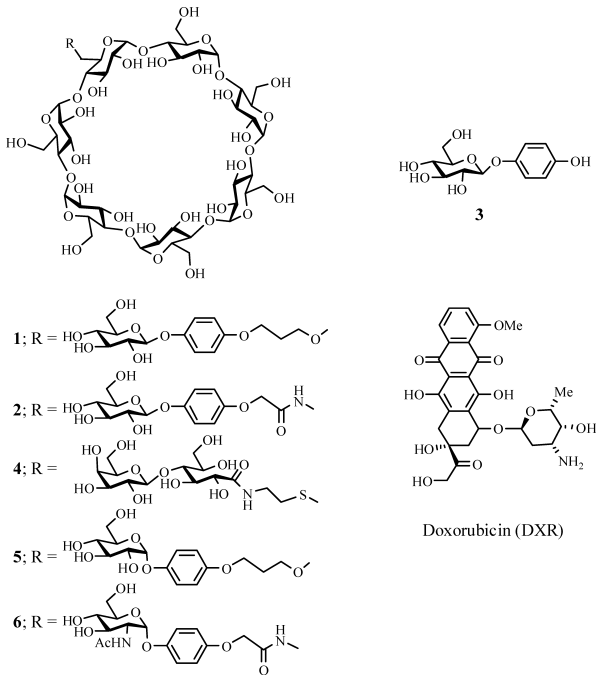

Fig. 1. Saccharide–β-CyD Conjugates (**1**, **2**, **4**, **5**, **6**), Arbutin (**3**) and DXR

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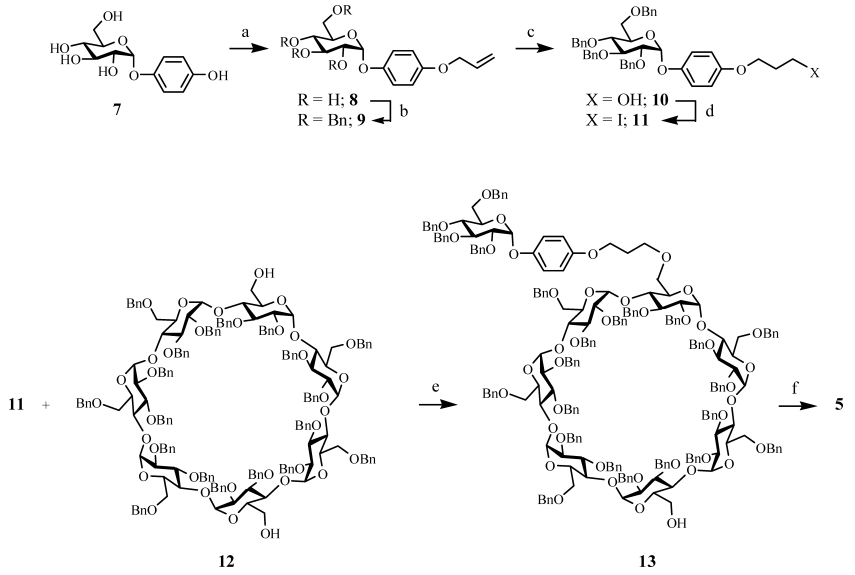
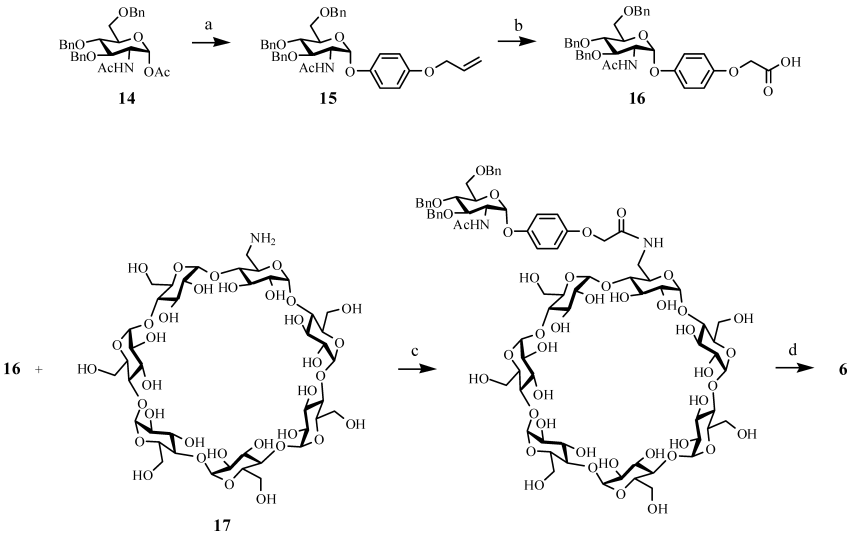


a) 1) 0.5 M NaOH aq., H_2O, 2) AllBr, DMF, 84%, b) BnBr, NaH, DMF, 99%, c) 1) 9-BBN, THF, 2) H_2O_2 aq., 0.5 M NaOH aq., 94%, d) Ph_3P, I_2, DMF, 35 °C, 89%, e) KOH, *n*-Bu_4NI, DMF, 69%, f) $Pd(OH)_2$, H_2 gas, DMF, 98%.

Chart 1. Synthetic Approach to the D-Glucose–β-CyD Conjugate (**5**)



a) $Yb(OTf)_3$, $BF_3 \cdot OEt_2$, 4-allyloxyphenol, CH_2Cl_2, 61%, b) 1) O_3 gas, Ph_3P, 2) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, H_2O, 70%, c) $Me_2P(S)Cl$, DIEA, DMF, d) $Pd(OH)_2$, H_2 gas, DMF, 56%.

Chart 2. Synthetic Approach to the 2-Acetamido-2-deoxy-α-D-glucose–β-CyD Conjugate (**6**)

Chart 2 shows the synthetic procedure of **6**. The glycosylation of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucosyl acetate (**14**)[24] to 4-allyloxyphenol (0.84 eq) was carried out using $Yb(OTf)_3$ (0.84 eq) and $BF_3 \cdot OEt_2$ (0.025 eq) in CH_2Cl_2 at room temperature for 24 h that gave the hydroquinone glycoside derivative **15** in 61% yield.[25] The ozonization of **15** in the presence of Ph_3P (10 eq), followed by oxidation using $NaClO_2$ (10 eq)–NaH_2PO_4 (1.8 eq) in the presence of 2-methyl-2-butene in *t*-BuOH–H_2O afforded compound **16** in 70% yield. The condensation of **16** with 6-monoamino-6-monodeoxy-β-CyD (**17**) using $Me_2P(S)Cl$ (1.6 eq) in the presence of *N*,*N*-diisopropylethylamine (DIEA) (2 eq) in DMF for 24 h, followed by the debenzylation using H_2–$Pd(OH)_2$ produced the desired product **6**, which was purified by gel-filtration using DIAION HP 20 (MeOH) in 56% yield.

Next, the inclusion associations of **5** and **6** with immobilized DXR were estimated by using an SPR optical biosensor according to the reported method.[22] Figure 2 indicates the kinetic linear plots of **5** and **6**. The association rate constant (k_a) and dissociation rate constant (k_d) of **5** (or **6**) were $1.4\times10^4\ M^{-1}\ s^{-1}$ ($1.3\times10^3\ M^{-1}\ s^{-1}$) and $4.1\times10^{-2}\ s^{-1}$ ($2.7\times10^{-3}\ s^{-1}$), and K_a of **5** (or **6**), calculated by the relationship of $K_a = k_a/k_d$, was $3.5\times10^5\ M^{-1}$ ($4.6\times10^5\ M^{-1}$). The conjugates **5** and **6** indicated high inclusion associations for immobilized DXR. The K_a values of **5** and **6** were about 100 times higher than that of the β-CyD conjugate **4** having no phenyl group and almost corresponded to those of **1** and **2** of 10^5—$10^6\ M^{-1}$ levels. These results suggested the formation of the π–π stacking inclusion complex between the conjugate **5** (or **6**)

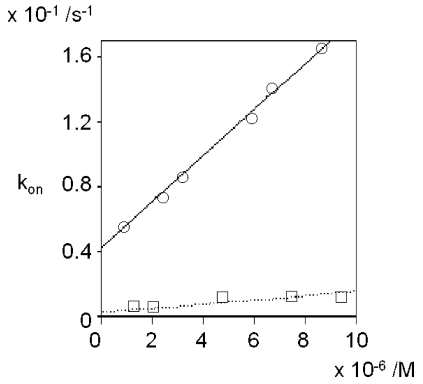


Fig. 2. Kinetic Linear Plots for the Immobilized DXR and the Conjugates **5** (○; r=0.998) and **6** (□; r=0.988).

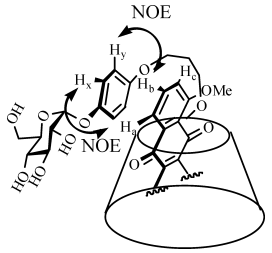


Fig. 3. NOE Interactions Observed in the Inclusion Complex of **5** and DXR

and DXR. In the measurement of the ^{1}H-NOESY spectra of the mixed sample of **5** and DXR (1 : 1) in D_2O, the NOE interactions between the protons (H_x; d; 6.72 ppm and H_y; d; 7.01 ppm) on the phenyl group and the protons (H_a and H_c; br; 7.20 ppm and H_b; br; 7.46 ppm) on DXR were observed as shown in Fig. 3.[3] The observation indicated that these protons were located close to each other and there was a strong possibility of the formation of the stacking complex between the phenyl group of **5** and the included DXR, as we speculated. The phenyl group in the conjugates **5** and **6** would also contribute to the increase in the inclusion associations for the immobilized DXR.

Conclusions

We demonstrated the synthesis of two novel β-CyD derivatives **5** and **6** attached to a hydroquinone glycoside. The hydroquinone glycosides **7** and **15** containing an α-D-glucosidic or 2-acetamido-2-deoxy-α-D-glucosidic linkage were synthetically useful components for providing these CyDs. The SPR analyses indicated that the conjugates **5** and **6**, which had the phenyl group derived from a hydroquinone glycoside in the spacers between the saccharide and β-CyD, indicated the excellent inclusion associations of 3.5—4.6×10^5 M^{-1} for the immobilized DXR. The newly synthesized β-CyD derivatives **5** and **6**, which have high DXR-transportabilities, are promising models of drug-carrying molecules.

Experimental

General The ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectra were recorded by a JEOL ECA-600 spectrometer. The NOESY experiment was done at 25 °C using a mixing time of 2 ms. Melting points (mp) were measured by a B-545 (BÜCHI Labortechnik AG) and are uncorrected. Optical rotations were recorded by a JASCO DIP-360 digital polarimeter. The HR-MS were obtained using a Mariner spectrometer (PerSeptive Biosystems Inc.). The MALDI-TOF-MS spectra were recorded by a Voyager DE STR spectrometer. Preparative TLC was performed on Merck silica gel 60GF254. The column chromatography was conducted using silica gel 60N (40—50 μm, Kanto Chemical Co., INC.) All anhydrous solvents were purified according to the standard methods. The immobilization of DXR on the sensor cuvette of the SPR optical biosensor was carried out under the same conditions as previously reported.[1—3] The amount of immobilized DXR was 1.1 ng/mm^2. The interactions of **5** and **6** with the immobilized DXR were measured at the concentration of 10^{-6}—10^{-5} M in acetate buffer, pH 5.3 at 25 °C.

4-Allyloxyphenyl α-D-Glucopyranoside (8) A 0.5 M NaOH aqueous solution (8 ml, 4 mmol) was added to **7** (1000 mg, 3.7 mmol). After the reaction mixture was stirred for 3 h at room temperature, the solvent was evaporated under reduced pressure and the reaction residue was crystallized. The reaction residue was dissolved in DMF (80 ml) and allyl bromide (413 μl, 4.8 mmol) was added. After the reaction mixture was stirred for 70 h, the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica-gel (chloroform/methanol=9/1) to afford **8** (963 mg, 84%) as white crystals. mp: 159.0—160.2 °C; $[\alpha]_D^{23}$ +154° (c=1.1, CH_3OH); ^{1}H-NMR (CD_3OD) δ: 3.40 (1H, t, J=11.0 Hz, H-4), 3.53 (1H, m, dd, J=3.4 Hz, J=9.7 Hz, H-2), 3.68—3.72 (2H, m, H-5, H_a-6), 3.76 (1H, dd, J=4.1 Hz, J=9.6 Hz, H_b-6), 3.82 (1H, t, J=8.9 Hz, H-3), 4.48 (2H, dt, J=1.3 Hz, J=5.5 Hz, C$\underline{H}_2$CH=CH$_2$), 5.21 (1H, dd, J=1.4 Hz, J=10.3 Hz, CH=C$\underline{H}_a\underline{H}_b$), 5.32 (1H, d, J=4.1 Hz, H-1), 5.36 (1H, dd, J=1.4 Hz, J=18.5 Hz, CH=C$\underline{H}_a\underline{H}_b$), 6.03 (1H, m, C$\underline{H}$=CH$_2$), 6.84 (2H, dd, J=2.7 Hz, J=9.6 Hz, Ph), 7.08 (2H, dd, J=2.1 Hz, J=13.1 Hz, Ph); ^{13}C-NMR (CD_3OD) δ: 62.4 (C-6), 70.3 (C$\underline{H}_2$CH=CH$_2$), 71.6 (C-4), 73.4 (C-2), 74.3 (C-5), 75.0 (C-3), 100.3 (C-1), 116.6 (Ph), 119.6 (Ph), 117.3 (CH=C$\underline{H}_2$), 135.1 (C$\underline{H}$=CH$_2$), 152.8 (Ph), 155.6 (Ph); HR-MS (ESI) m/z: 335.1124 (Calcd for $C_{15}H_{20}O_7$: M$^+$+Na, 335.1101).

4-Allyloxyphenyl 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranoside (9) To a solution of **8** (458 mg, 1.5 mmol) in DMF (30 ml) was added sodium hydride (585 mg, 24.4 mmol) at 0 °C. After the reaction mixture was stirred for 45 min, benzyl bromide (9 ml, 7 mmol) was added. After the reaction mixture was stirred for 21 h at room temperature, the reaction was then quenched by adding methanol (50 ml) and water (50 ml). The mixture was extracted with EtOAc (three times) and the combined organic solvent was dried over anhydrous Na_2SO_4. The organic solvent was filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica-gel (hexane/ethyl acetate=8/1) to afford **9** (973 mg, 99%) as white crystals. mp: 57.6—58.2 °C; $[\alpha]_D^{23}$ +73° (c=1.3, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 3.59 (1H, dd, J=2.0 Hz, J=10.3 Hz, H_a-6), 3.69—3.73 (2H, m, H-2, H_b-6), 3.75 (1H, t, J=9.0 Hz, H-4), 3.91—3.93 (1H, m, H-5), 4.18 (1H, t, J=8.9 Hz, H-3), 4.40 (1H, d, J=12.4 Hz, C$\underline{H}_2$Ph), 4.47—4.50 (3H, m, C$\underline{H}_2$CH=CH$_2$, C$\underline{H}_2$Ph), 4.58 (1H, d, J=11.7 Hz, C$\underline{H}_2$Ph), 4.68 (1H, d, J=12.3 Hz, C$\underline{H}_2$Ph), 4.79 (1H, d, J=11.7 Hz, C$\underline{H}_2$Ph), 4.85 (1H, d, J=11.0 Hz, C$\underline{H}_2$Ph), 4.87 (1H, d, J=11.7 Hz, C$\underline{H}_2$Ph), 5.04 (1H, d, J=11.0 Hz, C$\underline{H}_2$Ph), 5.27 (1H, dd, J=1.3 Hz, J=10.3 Hz, CH=C$\underline{H}_a\underline{H}_b$), 5.36 (1H, d, J=3.4 Hz, H-1), 5.40 (1H, dd, J=1.4 Hz, J=17.2 Hz, CH=C$\underline{H}_a\underline{H}_b$), 6.04 (1H, m, C$\underline{H}$=CH$_2$), 6.82 (2H, dd, J=2.8 Hz, J=6.9 Hz, Ph), 7.00 (2H, dd, J=2.1 Hz, J=6.9 Hz, Ph), 7.24—7.39 (20H, m, Ph); ^{13}C-NMR ($CDCl_3$) δ: 68.3 (C-6), 69.3 (C$\underline{H}_2$CH=CH$_2$), 70.7 (C-5), 73.2 (C$\underline{H}_2$), 73.4 (C$\underline{H}_2$), 75.1 (C$\underline{H}_2$), 75.7 (C$\underline{H}_2$), 77.4 (C-4), 79.7 (C-2), 81.9 (C-3), 96.3 (C-1), 115.4 (Ph), 117.5 (CH=C$\underline{H}_2$), 118.1 (Ph), 127.6—128.4 (Ph), 133.4 (C$\underline{H}$=CH$_2$), 137.8 (Ph), 138.0 (Ph), 138.2 (Ph), 138.8 (Ph), 150.8 (Ph), 154.0 (Ph); HR-MS (ESI) m/z: 695.2985 (Calcd for $C_{43}H_{44}O_7$: M$^+$+Na, 695.2979).

4-*O*-(3-Hydroxypropyl)phenyl 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranoside (10) To a solution of **9** (303 mg, 0.45 mmol) in THF (7 ml) was added in 0.5 M 9-BBN THF solution (2 ml, 1 mmol) at 0 °C. After the reaction mixture was stirred for 5 h at room temperature, a 0.5 M NaOH aqueous solution (2.7 ml, 1.4 mmol) and a 30% H_2O_2 aqueous solution (0.46 ml, 4.5 mmol) were added. After the reaction mixture was stirred for 48 h, the reaction was then quenched by adding water (10 ml). The mixture was extracted with EtOAc (three times) and the combined organic solvent was dried over anhydrous Na_2SO_4. The organic solvent was filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica-gel (hexane/ethyl acetate=3/1) to afford **10** (291 mg, 94%) as a colorless oil. $[\alpha]_D^{23}$ +11° (c=1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 1.74 (1H, s, OH), 1.99 (2H, m, C$\underline{H}_2$CH$_2$OH), 3.53 (1H, dd, J=1.3 Hz, J=10.3 Hz, H_a-6), 3.64—3.69 (2H, m, H-2, H_b-6), 3.71 (1H, t, J=8.9 Hz, H-4), 3.82 (2H, m, C$\underline{H}_2$CH$_2$CH$_2$OH), 3.85—3.88 (1H, m, H-5), 4.03 (2H, t, J=5.5 Hz, C$\underline{H}_2$OH), 4.13 (1H, t, J=9.6 Hz, H-3), 4.36 (1H, d, J=11.7 Hz, C$\underline{H}_2$Ph), 4.44 (1H, d, J=10.3 Hz, C$\underline{H}_2$Ph), 4.53 (1H, d, J=12.3 Hz, C$\underline{H}_2$Ph), 4.63 (1H, d, J=11.7 Hz, C$\underline{H}_2$Ph), 4.75 (1H, d,

J=11.7 Hz, C$\underline{H}_2$Ph), 4.81 (1H, d, J=11.0 Hz, C$\underline{H}_2$Ph), 4.83 (1H, d, J=11.0 Hz, C$\underline{H}_2$Ph), 5.00 (1H, d, J=11.0 Hz, C$\underline{H}_2$Ph), 5.31 (1H, d, J=3.4 Hz, H-1), 6.76 (2H, d, J=8.9 Hz, Ph), 6.96 (2H, d, J=8.9 Hz, Ph), 7.20—7.34 (20H, m, Ph); ^{13}C-NMR ($CDCl_3$) δ: 32.0 ($\underline{C}H_2CH_2OH$), 60.7 ($\underline{C}H_2CH_2CH_2OH$), 66.4 ($\underline{C}H_2OH$), 68.3 (C-6), 70.7 (C-5), 73.3 ($\underline{C}H_2$), 73.4 ($\underline{C}H_2$), 75.1 ($\underline{C}H_2$), 75.8 ($\underline{C}H_2$), 77.5 (C-2), 79.7 (C-4), 82.0 (C-3), 96.3 (C-1), 115.2 (Ph), 118.1 (Ph), 127.6—128.45 (Ph), 137.8—138.7 (Ph), 150.9 (Ph), 154.1 (Ph); HR-MS (ESI) m/z: 713.3099 (Calcd for $C_{43}H_{46}O_8$: M^++Na, 713.3085).

4-*O*-(3-Iodoxypropyl)phenyl 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranoside (11) To a solution of **10** (100 mg, 0.14 mmol) in DMF (3 ml) were added triphenylphosphine (156 mg, 0.6 mmol) and iodine (148 mg, 0.58 mmol) at 35 °C under argon. After the reaction mixture was stirred for 2 h, the reaction was then quenched by adding water (10 ml). The mixture was extracted with EtOAc (three times) and the combined organic solvent was dried over anhydrous Na_2SO_4. The organic solvent was filtered and evaporated under reduced pressure. The crude product was purified by preparative silica-gel TLC (hexane/ethyl acetate=6/1) to afford **11** (103 mg, 89%) as a colorless oil. $[\alpha]_D^{23}$ +102° (c=1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 2.25 (2H, m, C$\underline{H}_2$CH$_2$I), 3.37 (2H, t, J=6.9 Hz, C$\underline{H}_2$I), 3.59 (1H, dd, J=2.0 Hz, J=10.3 Hz, H$_a$-6), 3.69—3.74 (2H, m, H-2, H$_b$-6), 3.75 (1H, t, J=10.3 Hz, H-4), 3.91 (1H, m, H-5), 3.98 (2H, t, J=5.5 Hz, C$\underline{H}_2$CH$_2$CH$_2$I), 4.18 (1H, t, J=8.9 Hz, H-3), 4.41 (1H, d, J=12.4 Hz, C$\underline{H}_2$Ph), 4.49 (1H, d, J=11.0 Hz, C$\underline{H}_2$Ph), 4.58 (1H, d, J=11.7 Hz, C$\underline{H}_2$Ph), 4.68 (1H, d, J=11.7 Hz, C$\underline{H}_2$Ph), 4.79 (1H, d, J=12.4 Hz, C$\underline{H}_2$Ph), 4.85 (1H, d, J=10.4 Hz, C$\underline{H}_2$Ph), 4.87 (1H, d, J=11.0 Hz, C$\underline{H}_2$Ph), 5.04 (1H, d, J=10.3 Hz, C$\underline{H}_2$Ph), 5.36 (1H, d, J=3.4 Hz, H-1), 6.80 (2H, d, J=8.9 Hz, Ph), 7.00 (2H, d, J=8.9 Hz, Ph), 7.13—7.39 (20H, m, Ph); ^{13}C-NMR ($CDCl_3$) δ: 2.6 ($\underline{C}H_2$I), 33.0 ($\underline{C}H_2CH_2$I), 67.7 ($\underline{C}H_2CH_2CH_2$I), 68.3 (C-6), 70.7 (C-5), 73.3 ($\underline{C}H_2$), 73.4 ($\underline{C}H_2$), 75.1 ($\underline{C}H_2$), 75.8 ($\underline{C}H_2$), 77.4 (C-4), 79.7 (C-2), 82.0 (C-3), 96.3 (C-1), 115.3 (Ph), 118.1 (Ph), 127.6—128.5 (Ph), 137.8—138.8 (Ph), 150.9 (Ph), 154.0 (Ph); HR-MS (ESI) m/z: 823.2098 (Calcd for $C_{43}H_{45}O_7I$: M^++Na, 823.2102).

Heptaxis-(2,3-di-*O*-benzyl)-6^{B,C,E,F,G}-penta-*O*-benzyl-6^A-*O*-(3-*O*-[4-*O*-{2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside-1-yl}phenyl]propane-1-yl)β-cyclodextrin or Heptaxis-(2,3-di-*O*-benzyl)-6^{B,C,E,F,G}-penta-*O*-benzyl-6^D-*O*-(3-*O*-[4-*O*-{2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside-1-yl}phenyl]propane-1-yl)β-cyclodextrin (13) To a solution of **11** (84 mg, 0.1 mmol), **12** (75 mg, 0.026 mmol) in DMF (4 ml) were added KOH (179 mg, 3.19 mmol) and n-Bu_4NI (4.9 mg, 0.013 mmol). After the reaction mixture was stirred for 64 h, the reaction was then quenched by adding water (10 ml). The mixture was extracted with EtOAc (three times) and the combined organic solvent was dried over anhydrous Na_2SO_4. The organic solvent was filtered and evaporated under reduced pressure. The crude product was purified by preparative silica-gel TLC (hexane/ethyl acetate=3/1) to afford **13** (64 mg, 69%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.87 (2H, m, C$\underline{H}_2$CH$_2$OPh), 3.36—5.39 (106H, m, CyD, H-1, H-2, H-3, H-4, H-5, H-6, C$\underline{H}_2$CH$_2$C$\underline{H}_2$OPh, C$\underline{H}_2$Ph), 6.73 (2H, dd, J=3.4 Hz, J=8.9 Hz, Ph), 6.96 (2H, dd, J=1.4 Hz, J=8.9 Hz, Ph), 7.05—7.38 (115H, m, Ph); ^{13}C-NMR ($CDCl_3$) δ: 14.2, 21.0, 29.5, 60.3—82.0, 96.3, 98.3—98.7, 115.1, 118.1, 126.9—128.4, 137.8—139.3, 150.7, 154.2; MALDI-TOF-MS m/z: 3540.6 (Calcd for $C_{218}H_{228}O_{42}$: M^++Na, 3540.6).

6^A-*O*-(3-*O*-[4-*O*-{α-D-Glucopyranoside-1-yl}phenyl]propane-1-yl)β-cyclodextrin (5) To a solution of **13** (56 mg, 0.015 mmol) in DMF (5 ml) was added palladium hydroxide (76 mg, 0.49 mmol). Hydrogen was bubbled through the solution for 6 h. After the solvent was filtered and evaporated under reduced pressure, the crude product was isolated by adsorption on LH 20 followed by eluting with methanol to afford **5** (22 mg, 98%) as white crystals. mp: 284.1—286.0 °C; $[\alpha]_D^{23}$ +97° (c=1.0, CH_3OH); ^{1}H-NMR (D_2O) δ: 1.88 (2H, m, C$\underline{H}_2$CH$_2$OPh), 3.43—4.08 (52H, m, CyD-2, CyD-3, CyD-4, CyD-5, CyD-6, H-2, H-3, H-4, H-5, H-6, C$\underline{H}_2$CH$_2$C$\underline{H}_2$OPh), 4.90—5.06 (7H, m, CyD-1), 5.56 (1H, d, J=2.1 Hz, H-1), 6.82 (2H, d, J=8.2 Hz, Ph), 7.12 (2H, d, J=8.9 Hz, Ph); ^{13}C-NMR (D_2O) δ: 29.4, 60.2—74.5, 81.2—82.9, 98.2, 101.4—102.7, 115.9—118.3, 151.3, 154.7; MALDI-TOF-MS m/z: 1469.2 (Calcd for $C_{57}H_{90}O_{42}$: M^++Na, 1469.5).

4-Allyloxyphenyl 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranoside (15) $Yb(OTf)_3$ (635 mg, 1 mmol) was added to a solution of **14** (653 mg, 1.2 mmol), 4-allyloxyphenol (153 mg, 1 mmol) and $BF_3\cdot OEt_2$ (3.9 μl, 0.03 mmol) in CH_2Cl_2 (8 ml) at 0 °C. The resulting mixture was stirred for 24 h at room temperature. The reaction was then quenched by the addition of a sat. $NaHCO_3$ solution (5 ml). The reaction mixture was extracted with CH_2Cl_2, and the organic layer was washed with water and a sat. NaCl solution. After the organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure. The crude product was purified by preparative silica-gel TLC (ethyl acetate/hexane=2/1) to give **15** (389 mg, 61%) as white crystals. mp: 195.0—197.0 °C; $[\alpha]_D^{23}$ +159° (c=1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 1.58 (3H, s, CH_3), 3.63 (1H, dd, J=2.0 Hz, J=11.0 Hz, H$_a$-6), 3.76 (1H, dd, J=3.4 Hz, J=11.0 Hz, H$_b$-6), 3.34—3.90 (2H, m, H-3, H-4), 3.92—3.94 (1H, m, H-5), 4.38 (1H, dt, J=2.7 Hz, J=8.9 Hz, H-2), 4.45—4.47 (3H, m, C$\underline{H}_2$CH=CH$_2$, C$\underline{H}_a$H$_b$Ph), 4.57 (1H, d, J=10.3 Hz, C$\underline{H}_2$Ph), 4.61 (1H, d, J=11.7 Hz, C$\underline{H}_2$Ph), 4.70 (1H, d, J=12.3 Hz, C$\underline{H}_2$Ph), 4.83 (1H, d, J=10.4 Hz, C$\underline{H}_2$Ph), 4.90 (1H, d, J=11.7 Hz, C$\underline{H}_2$Ph), 5.27—5.31 (2H, m, CH=C$\underline{H}_a$H$_b$, NH), 5.34 (1H, dd, J=2.1 Hz, J=17.9 Hz, CH=CH$_a$H$_b$), 5.41 (1H, d, J=3.4 Hz, H-1), 6.03 (1H, m, C$\underline{H}$=CH$_2$), 6.81 (2H, d, J=9.0 Hz, Ph), 6.90 (2H, d, J=8.9 Hz, Ph), 7.20—7.37 (15H, m, Ph); ^{13}C-NMR ($CDCl_3$) δ: 23.4 (CH_3), 52.5 (C-2), 68.4 (C-6), 69.3 ($\underline{C}H_2$CH=CH$_2$), 71.6 (C-5), 73.4 ($\underline{C}H_2$Ph), 74.8 ($\underline{C}H_2$Ph), 75.1 ($\underline{C}H_2$Ph), 78.3 (C-4), 79.9 (C-3), 92.2 (C-1), 115.6 (Ph), 117.6 (CH=$\underline{C}H_2$), 117.9 (Ph), 127.6—128.7 (Ph), 133.3 ($\underline{C}$H=CH$_2$), 134.1—166.8 (Ph), 169.8 (C=O); HR-MS (ESI) m/z: 646.2787 (Calcd for $C_{38}H_{41}NO_7$: M^++Na, 646.2775).

4-*O*-(Carboxymethyl)phenyl 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranoside (16) Ozone was bubbled through a stirred solution of **15** (389 mg, 0.62 mmol) in CH_2Cl_2 (15 ml) at −78 °C for 2 h. After triphenylphosphine (497 mg, 1.9 mmol) was added at −78 °C and the reaction temperature was raised to room temperature, the reaction mixture was stirred for 2 h. The solvent was then evaporated under reduced pressure. To a solution of the crude product in t-butylalcohol (10 ml)–H_2O (2 ml) was added $NaClO_2$ (561 mg, 6.2 mmol), NaH_2PO_4 (116 mg, 0.74 mmol) and 2-methyl-2-butene (305 μl, 2.9 mmol). After the reaction mixture was stirred for 15 h, the reaction was quenched by adding 2 M HCl (1 ml) and water (5 ml). After the reaction mixture was extracted with CH_2Cl_2 (three times), the combined organic solvent was dried over anhydrous Na_2SO_4. The organic solvent was filtered and evaporated under reduced pressure. The crude product was purified by preparative silica-gel TLC (chloroform/methanol=5/1) to afford **16** (281 mg, 70%) as white crystals. mp: 165.0—165.9 °C; $[\alpha]_D^{23}$ +79° (c=1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 1.90 (3H, s, CH_3), 3.63 (1H, d, J=10.9 Hz, H$_a$-6), 3.77 (1H, dd, J=4.2 Hz, J=11.0 Hz, H$_b$-6), 3.83 (3H, m, H-4, C$\underline{H}_2$COOH), 3.94—3.98 (2H, m, H-3, H-5), 4.37 (1H, s, NH), 4.38 (1H, dt, J=3.4 Hz, J=11.0 Hz, H-2), 4.46 (1H, d, J=12.4 Hz, C$\underline{H}_2$Ph), 4.54 (1H, d, J=10.3 Hz, C$\underline{H}_2$Ph), 4.61 (1H, d, J=12.4 Hz, C$\underline{H}_2$Ph), 4.76 (1H, d, J=11.0 Hz, C$\underline{H}_2$Ph), 4.81 (1H, d, J=11.0 Hz, C$\underline{H}_2$Ph), 4.89 (1H, d, J=11.0 Hz, C$\underline{H}_2$Ph), 5.36 (1H, d, J=4.2 Hz, H-1), 6.96 (2H, d, J=9.0 Hz, Ph), 7.18 (2H, d, J=7.6 Hz, Ph), 7.29—7.39 (15H, m, Ph); ^{13}C-NMR ($CDCl_3$) δ: 22.5 (CH_3), 52.6 (C-2), 67.4 (C-6), 71.3 (C-5), 73.2 ($\underline{C}H_2$Ph), 74.8 ($\underline{C}H_2$Ph), 74.9 ($\underline{C}H_2$Ph), 78.0 ($\underline{C}H_2$COOH), 78.1 (C-4), 79.9 (C-3), 97.2 (C-1), 115.9 (Ph), 117.9 (Ph), 127.5—128.3 (Ph), 133.0—138.2 (Ph), 150.9 (Ph), 180.0 (C=O); HR-MS (ESI) m/z: 664.2508 (Calcd for $C_{37}H_{39}NO_9$: M^++Na, 664.2517).

4-*O*-(2-Acetamido-2-deoxy-α-D-glucopyranoside-1-yl)phenyl-*N*-(6^A-deoxy-β-cyclodextrin-6^A-yl)acetamide (6) To a solution of **16** (41 mg, 0.062 mmol) in DMF (1.5 ml) were added dimethylphosphinothioyl chloride (13 mg, 0.097 mmol) and DIEA (21 μl, 0.12 mmol). After the reaction mixture was stirred for 40 min, **17** (84 mg, 0.074 mmol) in DMF (1.5 ml) was added. After the reaction mixture was stirred for 24 h, the solvent was evaporated under reduced pressure. The resulting reaction mixture was washed with diethyl ether (eight times) and dissolved in DMF (3 ml). Palladium hydroxide (29 mg, 0.18 mmol) was added to the solution and hydrogen was bubbled through it for 24 h. After the solvent was filtered and evaporated under reduced pressure, the crude product was isolated by adsorption on HP 20 (DIAION) followed by eluting with methanol to afford **6** (52 mg, 56%) as white crystals. mp: 281.0—283.0 °C; ^{1}H-NMR (D_2O) δ: 1.92 (3H, s, CH_3), 3.21—3.93 (51H, m, CyD, H-2, H-3, H-4, H-5, H-6, C$\underline{H}_2$C(O)NH), 4.65—4.96 (7H, m, CyD), 5.59 (1H, d, J=3.4 Hz, H-1), 6.63 (2H, d, J=8.9 Hz, Ph), 7.01 (2H, d, J=9.0 Hz, Ph); MALDI-TOF-MS m/z: 1510.1 (Calcd for $C_{58}H_{90}N_2O_{42}$: M^++Na, 1509.5).

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References and Notes

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