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β-Cyclodextrin Conjugates with Glucose Moieties Designed as Drug Carriers: Their Syntheses, Evaluations Using Concanavalin A and Doxorubicin, and Structural Analyses by NMR Spectroscopy

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Abstract: Three kinds of β-cyclodextrin derivatives conjugated with glucose moieties, which were expected as models for a drug carrier targeting the drug delivery systems, were designed and synthesized from β-cyclodextrin and the natural product, 4-hydroxyphenyl-β-D-glucopyranoside called arbutin. Arbutin was used because it had a phenyl group with a hydroxyl function which could be used to link the glucose moiety to β-cyclodextrin. The evaluations of these conjugates as the drug-carrying molecules were done by investigating the molecular interactions with the carbohydrate-binding Concanavalin A (Con A) lectin and the anticancer agent, doxorubicin (DXR), using an SPR optical biosensor. The association constants of the conjugates with immobilized Con A were $2.0 \times 10^3 \sim 8.8 \times 10^3$ M^{-1}. The result showed that the Con A bound to the glucose moieties from arbutin in the conjugates with prospective association constants. The inclusion associations of the conjugates with immobilized DXR reached $2.2 \times 10^5 \sim 1.4 \times 10^8$ M^{-1}. The extremely high inclusion associations for DXR suggested their potential abilities as drug-carrying molecules for carrying DXR. The NMR analyses indicated that the phenyl group of the conjugates greatly served to increase the inclusion associations for DXR. In their DXR inclusion complexes, the formation of the stacking complexes by the π–π interactions between the phenyl groups and the included DXR also enhanced their inclusion abilities for DXR.

Key Words: Cyclodextrin, drug delivery system, drug carrier, stacking effect, arbutin, doxorubicin.

INTRODUCTION

Saccharides are known to be involved in a number of significant biological recognition phenomena at the surfaces of cell membranes. Cyclodextrins (CyDs) have the ability to carry drug molecules within their cavities. Therefore, the CyD derivatives conjugated with one or more saccharide moieties are expected to serve as drug-carrying molecules that are capable of recognizing specific cells, and might be useful as targeting drug delivery systems [1]. However, no targeting drug carrier containing CyDs has yet been reported. To develop effective drug-carrying molecules based on CyD–saccharide conjugates, two technologies are required. The first is a practical method for attaching saccharide moieties to CyD molecules, and the second is an efficient device for increasing the drug inclusion ability of the CyDs so that it is certain that the CyDs will carry the noncovalently included drugs to the targeted cells.

Our previous study successfully demonstrated a preparation of a saccharide-conjugated CyD by the coupling reaction of *N*-α-Fmoc-*N*-ω-(2-acetamide-2-deoxy-β-D-glucopyranosyl)asparagine with *mono*-6-amino-6-deoxy-β-CyD. The conjugate worked as a useful acceptor of the enzymatic glycosidation using the endo-β-*N*-acetylglucosaminidase of *Mucor hiemalis* (Endo-M) to afford several oligosaccharide-branched CyDs [2]. When the inclusion ability of one of the obtained oligosaccharide-branched CyDs with cholic acid was investigated, it had a considerably higher association constant than normal β-CyD [3]. This result suggested that the Fmoc group in the spacer between the oligosaccharide and β-CyD seemed to increase its inclusion behavior for cholic acid. We anticipated that the drug inclusion ability of a saccharide-CyD conjugate would be similarly enhanced by introducing an aromatic group into an appropriate position of the spacer between a saccharide and β-CyD.

We then started the syntheses and evaluations of the structurally simple saccharide-CyD conjugates, which had a phenyl group in the spacers between the saccharide moiety and β-CyD. A commercially available natural product, 4-hydroxyphenyl-β-D-glucopyranoside called arbutin **1** was used as the saccharide source, because it conveniently had a phenyl group with a *p*-hydroxyl function which could be used to link β-CyD. As described in our preliminary letters, three kinds of the glucose-β-CyD conjugates **2-4** were synthesized (Scheme (**1**)) and their inclusion abilities as drug carriers were evaluated using immobilized doxorubicin (DXR, anticancer agent) by an SPR optical biosensor [4]. We found that these conjugates indicated extraordinarily high inclusion associations of $2.2 \times 10^5 \sim 1.4 \times 10^8$ M^{-1} for the immobilized DXR and had potential abilities as drug-carrying molecules. This finding urged us to develop more convenient synthetic approaches to the conjugates **2-4** and to

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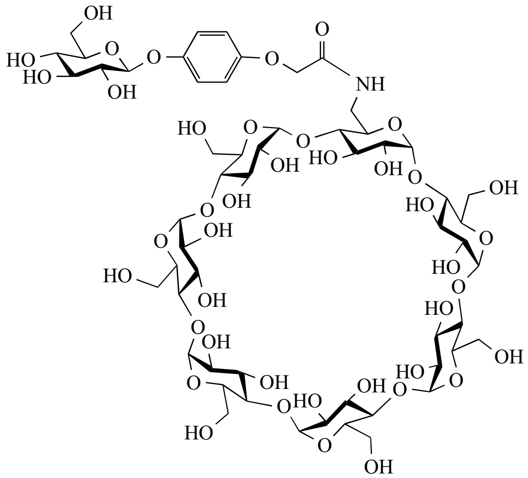

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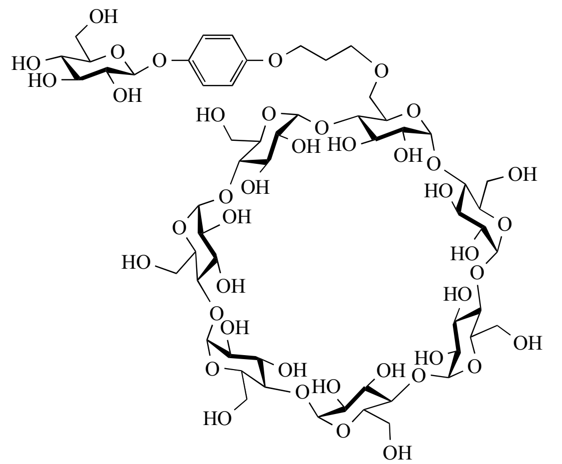

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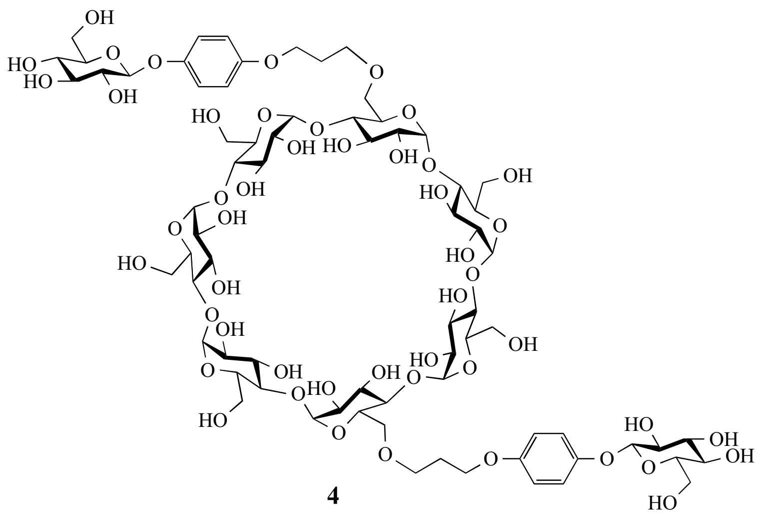

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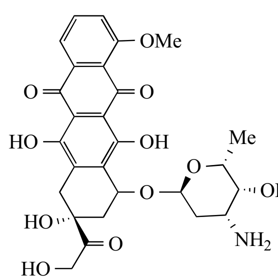
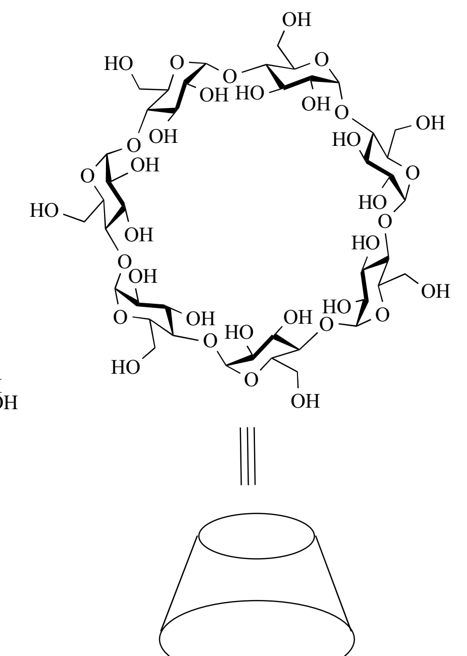
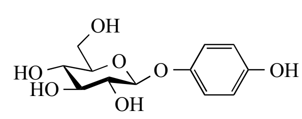


Doxorubicin (DXR)



β-Cyclodextrin (β-CyD)

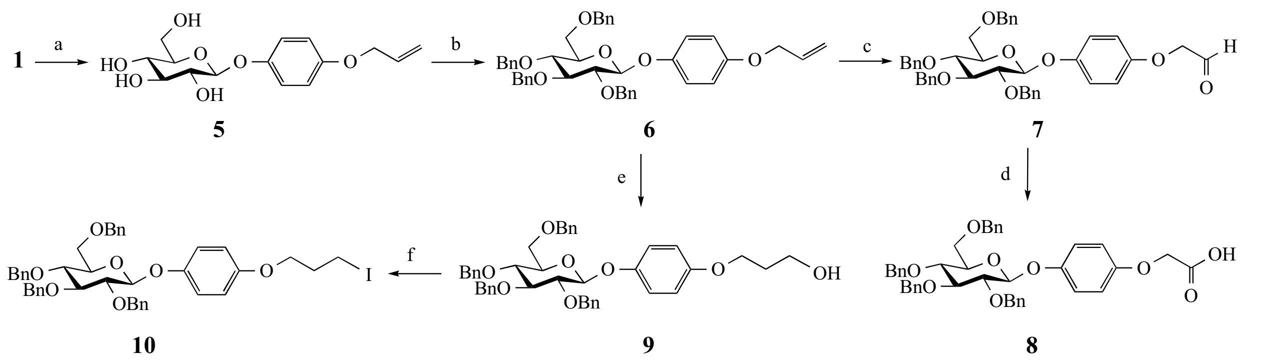


Arbutin (**1**)

Scheme (1). β-CyD conjugates **2**-**4** with glucose moieties.

elucidate the further details of the fundamental properties of **2**-**4** as the drug-carrying molecules.

In this study, we report the syntheses, evaluations and analyses of the β-CyD conjugates **2**-**4**. In particular, 1) the optimization of the synthetic approaches to **2**-**4**, 2) the association constants of the glucose moieties from arbutin in **2**-**4** with immobilized Con A using an SPR optical biosensor, 3) the inclusion associations of **2**-**4** with immobilized DXR by the SPR assay, and 4) the structural analyses of the DXR inclusion complex of **3** by the NMR spectra are described.



Scheme (2). Preparation of arbutin derivatives **8** and **10**.

a) NaOH/H_2O, then dry, AllBr/DMF, 99%; b) NaH, BnBr/DMF, 91%; c) O_3, Ph_3P/CH_2Cl_2, 97%; d) 2-methyl-2-butene, NaH_2PO_4, $NaClO_2$/*t*-BuOH-H_2O, 98%; e) 9-BBN/THF, then NaOHaq., H_2O_2 aq., 95%; f) Ph_3P, I_2/DMF, 83%.

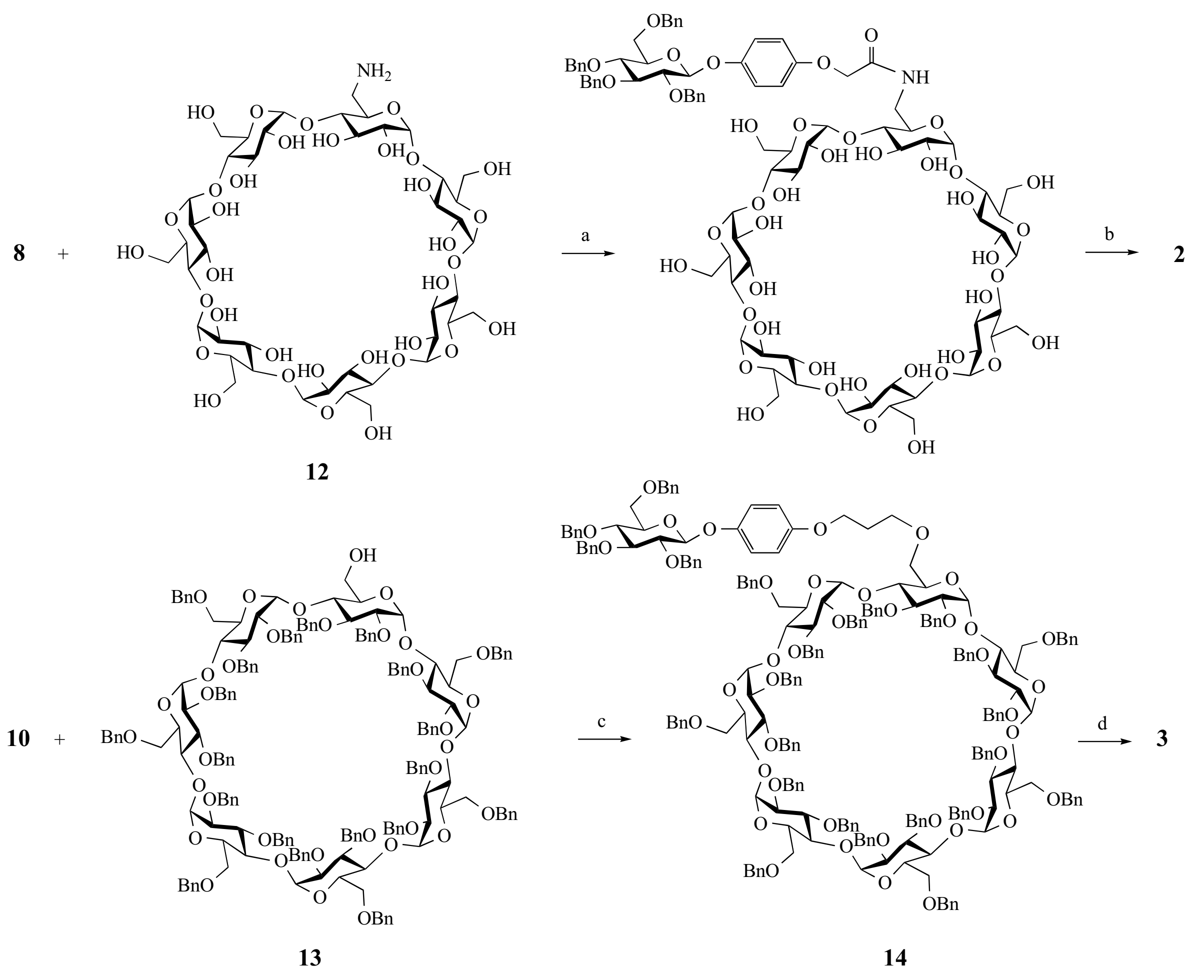
RESULTS AND DISCUSSION

1. Synthesis

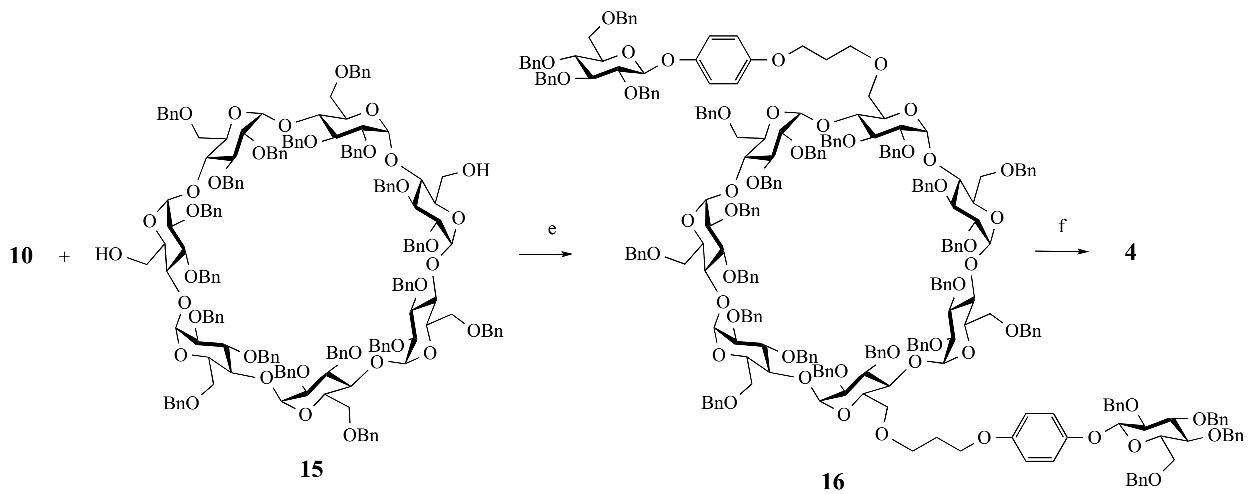
The glucose-branched β-CyD conjugates **2-4** were synthesized by several reaction steps. The two arbutin derivatives (**8** and **10**) with appropriate linkers were prepared from arbutin. The benzyl protected conjugates of **2-4** were obtained by the coupling reactions of **8** with *mono*-6-amino-6-deoxy-β-CyD **12** or of **10** with the partially benzylated β-CyD **13** (or **15**).

1.1. Syntheses of the Arbutin Derivatives 8 and 10

Scheme (**2**) shows the synthetic route of the arbutin derivatives **8** and **10** from arbutin **1**. The allylated compound **5** was obtained by the reaction of allyl bromide and the dry



(Scheme 3. Contd….)



Scheme (3). Synthesis of β-CyD conjugates **2**-**4**.
a) $Me_2P(S)Cl$, DIEA, DMF; b) $H_2/Pd(OH)_2/DMF$, then gel-filtration, 63% from **12**; c) KOH, **10**/DMF, 51%; d) $H_2/Pd(OH)_2$/Ether-MeOH, then gel-filtration, 73%; e) KOH, **10**/DMF, 51%; f) $H_2/Pd(OH)_2$/Ether-MeOH, then gel-filtration, 80%.

sodium salt of **1** in DMF followed by the benzylation of **5** by benzyl bromide-sodium hydride which afforded the synthetic key intermediate **6** in excellent yield.

The ozonization of **6** in the presence of Ph_3P followed by the oxidation of **7** using $NaClO_2$-NaH_2PO_4 afforded the carboxylic compound **8** in good yield.

The hydroboration of **6** with 9-BBN, followed by hydrolysis using aqueous NaOH and by oxidation using aqueous H_2O_2 gave the compound **9** in 95%. The iodonation of **9** using PPh_3-I_2 afforded the arbutin derivative **10** in 92%.

1.2. Syntheses of the β-CyD Conjugates 2-4 with Glucose Moieties

Scheme (**3**) indicates the syntheses of the CyD-arbutin conjugates **2**-**4**. The condensation of **8** with 6-*mono*-amino-deoxy-β-CD **12** using $Me_2P(S)Cl$ was carried out in the presence of DIEA in DMF, followed by the debenzylation using $H_2/Pd(OH)_2$ which afforded the desired conjugate **2** in 63% yield.

The reaction of **10** with the partially benzylated β-CD **13** [5] using KOH in DMF gave the desired benzylated product **14** in 51%. The treatment of **14** with $H_2/Pd(OH)_2$ in ether-methanol provided the desired **3** in 73% yield.

The coupling reaction of **10** with the partially benzylated β-CyD **15** [5] in the presence of KOH in DMF gave a mixture of **4** conjugated with two glucose moieties and the CyD conjugated with a glucose moiety in 51 and 31% yields, respectively. Subsequently, similar debenzylation of **16** using $H_2/Pd(OH)_2$ afforded the desired conjugate **4** in 80% yield.

2. Molecular Interactions with Con A

To evaluate the cell-recognition abilities of **2**-**4**, their molecular interactions with Con A, which was classified as a D-glucose/D-mannose specific lectin, were examined. The bindings of the glucose moieties from arbutin in **2**-**4** to Con A were investigated by measuring the association constants of **2**-**4** with immobilized Con A using an SPR assay.

2.1. An SPR Assay Using Immobilized Con A

The molecular interactions of **2**-**4** with immobilized Con A were examined using an optical biosensor “IAsys” based on SPR (Affinity Sensor, UK). Refer to our reported previously studies about the SPR principle and technique [1r]. The immobilization of Con A on the sensor cuvette of the optical biosensor was carried out under the same conditions as previously reported. Using an acetate buffer of pH 5.3 at 25 °C, Con A was immobilized as a dimer and partly as a tetramer and with the increase in response, R, its immobilization was 3.5 ng/mm^2 based on the sensor sensitivity factor of 640 arc sec/ng.

The association rate constant (k_a) and dissociation rate constant (k_d) were calculated by plotting the slope (k_{on}) between dR/dt and R, changing the concentration of the CyD derivatives **2**-**4**. From the linear plot between k_{on} and the concentration of **2**-**4**, the k_a as the slope and k_d as the intercept were obtained. The interactions of **2**-**4** were measured at the concentration of $10^{-3} \sim 10^{-2}$ M in acetate buffer, pH 5.3, containing 1 mM $CaCl_2$, 1 mM $MnCl_2$ and 100 mM NaCl at 25 °C. Figs. (**1**-**3**) show the kinetic linear plots of **2**-**4**, and Table (**1**) summarizes the k_a, k_d and association constant (K_a) calculated by the relationship, $K_a = k_a/k_d$ of **2**-**4**, respectively.

2.2. Association constants of the conjugates 2-4 with immobilized Con A

The k_a values of **2**-**4** were ca. $5.3 \sim 145$ $M^{-1}s^{-1}$, and the k_d values of those were $1.5 \times 10^{-3} \sim 1.7 \times 10^{-2}$ s^{-1}. Then, their K_a values were calculated as $2.0 \times 10^3 \sim 8.8 \times 10^3$ M^{-1}. The K_a value of the conjugate **4** having two glucose moieties was about $2 \sim 3$ times higher than those of the conjugates **2** and **3** having one glucose moiety. As the reported K_a of methyl α-D-mannopyranoside was 8.2×10^3 M^{-1} [6], these K_a values

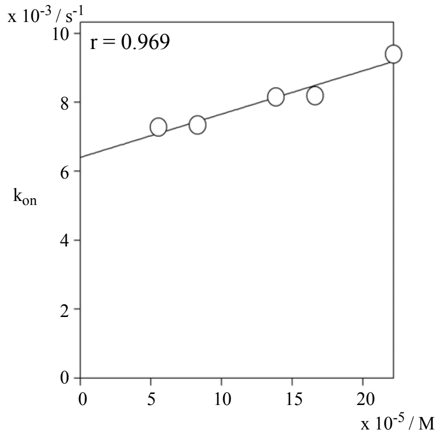


Fig. (1). Kinetic linear plots for the immobilized Con A and the conjugate **2**.

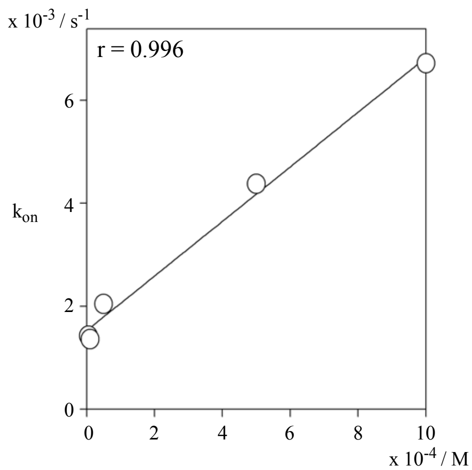


Fig. (2). Kinetic linear plots for the immobilized Con A and the conjugate **3**.

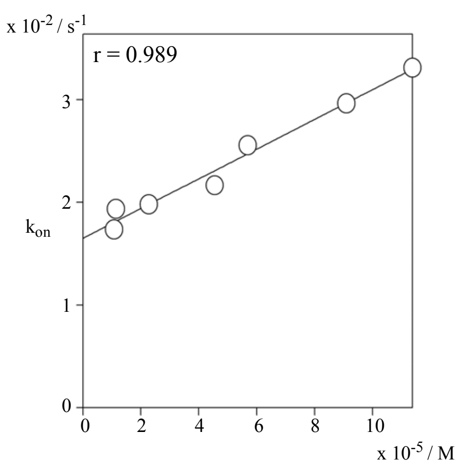


Fig. (3). Kinetic linear plots for the immobilized Con A and the conjugate **4**.

were close to the predicted values. The evaluation study showed that the glucose moieties from arbutin in **2**-**4** maintained their abilities of binding to Con A with the prospective association constants.

Table 1. Kinetic Parameters of Conjugates 2-4 for the Associations with Immobilized Con A

Entry	Conjugate	k_a ($M^{-1}s^{-1}$)	k_d (x 10^{-3} s^{-1})	K_a (x 10^3 M^{-1})
1	**2**	12.5 ± 1.8	6.4 ± 0.3	2.0
2	**3**	5.3 ± 0.3	1.5 ± 0.1	3.5
3	**4**	144.9 ± 9.9	17.0 ± 0.6	8.8

3. Molecular Interactions with DXR

To evaluate the drug inclusion abilities of **2**-**4**, their molecular interactions with DXR, which was an anthracycline-related anticancer agent, were examined. The inclusion associations of **2**-**4** with immobilized DXR were also estimated by the SPR assay.

3.1. An SPR Assay Using Immobilized DXR

The immobilization of DXR on the sensor cuvette of the optical biosensor was carried out under the same conditions as previously reported. The amount of immobilized DXR was 0.67 ng/mm^2. The interactions of **2**-**4** with immobilized ConA were measured at the concentration of 10^{-9} ~ 10^{-4} M in acetate buffer, pH 5.3 at 25 °C. Figs. (**4**-**6**) indicate the kinetic linear plots of **2**-**4**, and Table **2** shows the k_a, k_d, and K_a values of **2**-**4**, respectively.

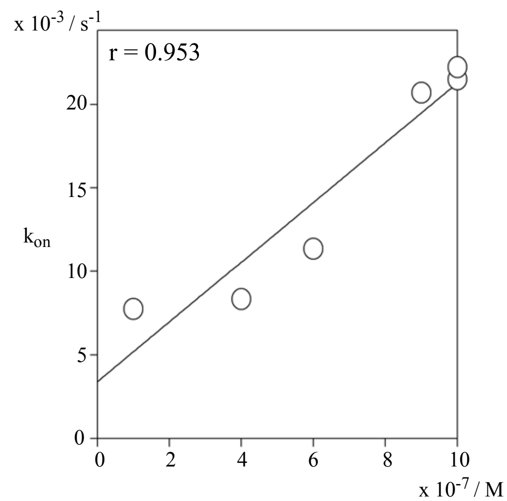


Fig. (4). Kinetic linear plots for the immobilized DXR and the conjugate **2**.

3.2. Inclusion Associations of 2-4 with Immobilized DXR

The k_a values of **2**-**4** were ca. 5 ~ 420 × 10^3 $M^{-1}s^{-1}$ and the k_d values were ca. 3 ~ 21 × 10^{-3} s^{-1}, and their K_a values were calculated to be 1.4 × 10^8 ~ 2.2 × 10^5 M^{-1}, respectively.

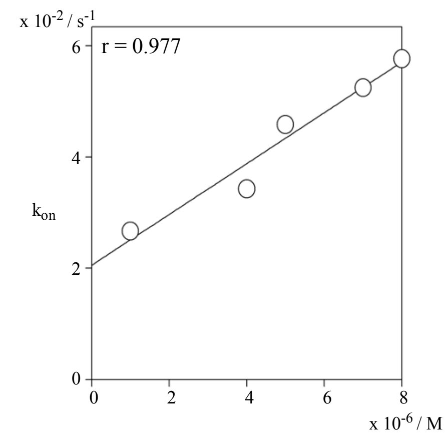


Fig. (5). Kinetic linear plots for the immobilized DXR and the conjugate **3**.

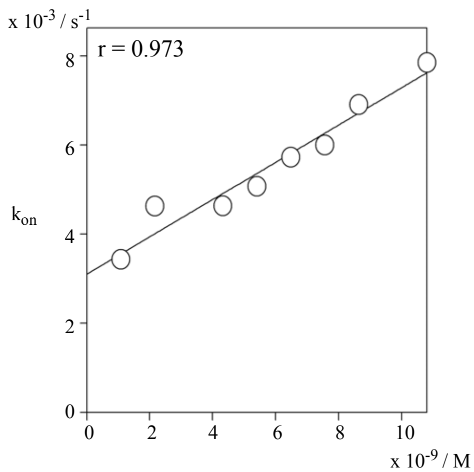
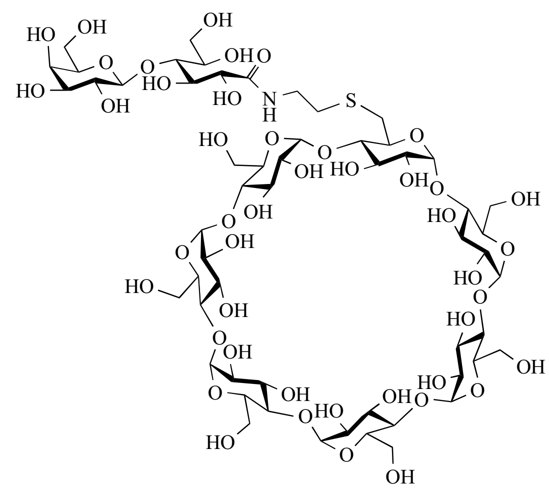


Fig. (6). Kinetic linear plots for the immobilized DXR and the conjugate **4**.

Table **2** contains our reported k_a, k_d, and K_a values of the lactose-branched β-CyD **17** [1k] (Scheme (**4**)) for comparison with those values of **2**-**4**. The K_a values of the conjugates **2** and **3** were about $10^2 \sim 10^3$ times higher than that of **17**. The difference between **2** and **3** in the K_a values would be due to the existence of the amide group in the spacer of **2**, which would generate the hydrogen bonds and make **2** a more rigid "capping structure" than **3**. Surprisingly, the K_a value of the conjugate **4** was about 5×10^4 times higher than that of **17**. In comparison with the K_a value of **17** lacking a phenyl group, these conjugates **2**-**4** had extraordinarily high inclusion associations for DXR. In comparison of the K_a values among the conjugates **2**-**4**, the K_a value of **4** having two phenyl groups in the spacer were about 20 ~ 500 times higher than those of **2** and **3** having one phenyl group. These results suggested that the presence of the phenyl groups in the spacers between the glucose moieties and the β-CyD of **2**-**4** remarkably increased their inclusion abilities for DXR.



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Scheme (4). β-CyD conjugate **17**.

3.3. Speculated Molecular Modelings of the Conjugates 2-4 and their DXR Inclusion Complexes

We speculated that the phenyl groups in the spacer of **2**-**4** would cause the following interactions in the DXR inclusion complexes and enhance the inclusion ability of **2**-**4**. The phenyl group would exhibit a structure like the cap of CyD and this 'pseudo-capping structure' would increase the hydrophobicity of the CyD cavity. The molecular model of **2** including the DXR supported this (Fig. **7**). When DXR was included in the CyD cavity, the stacking complex by the π-π interaction between the phenyl group and DXR would be formed **7**. These phenomena would increase their inclusion associations for DXR as shown in Scheme (**5**). In particular, the CyD conjugate **4** was expected to form the inclusion structure shown in Scheme (**6**); that is, the DXR interposed between the phenyl groups seemed to strengthen the stacking effect.

Table 2. Kinetic Parameters of Conjugates 2-4 for the Associations with Immobilized DXR

Entry	Conjugate	k_a (x 10^3 $M^{-1}s^{-1}$)	k_d (x 10^{-3} s^{-1})	K_a (M^{-1})
1	**2**	17.9 ± 2.8	3.4 ± 2.1	5.3 x 10^6
2	**3**	4.6 ± 0.6	21.0 ± 3.2	2.2 x 10^5
3	**4**	418.1 ± 40.4	3.1 ± 0.3	1.4 x 10^8
4	**17**	0.12 ± 0.05	41 ± 3.0	3.1 x 10^3

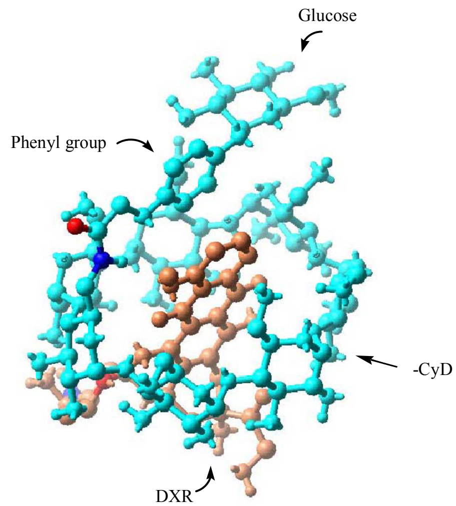
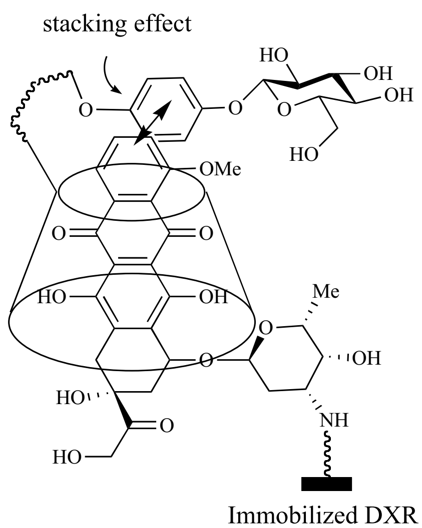
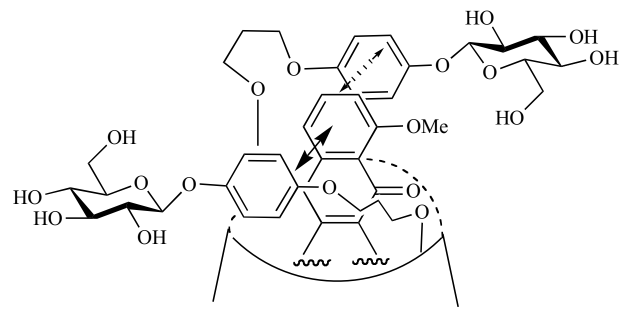


Fig. (7). Molecuar model of the inclusion association between **2** and DXR.



Scheme (5). Expected inclusion and stacking complexes between **2** (or **3**) and DXR.



Scheme (6). Expected inclusion and stacking complexes between **4** and DXR.

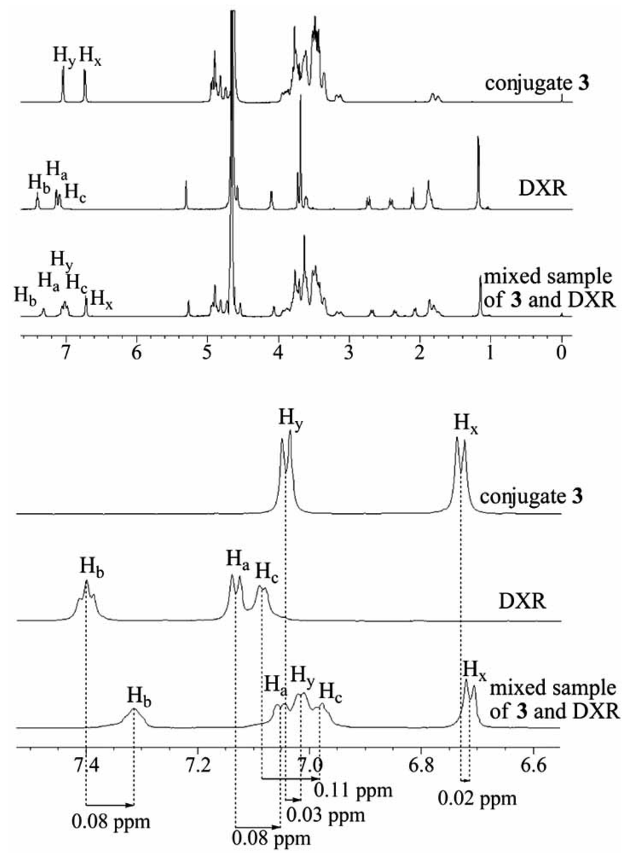


Fig. (8). ^{1}H NMR spectra of **3**, DXR, and the mixed sample of **3** and DXR (1:1).

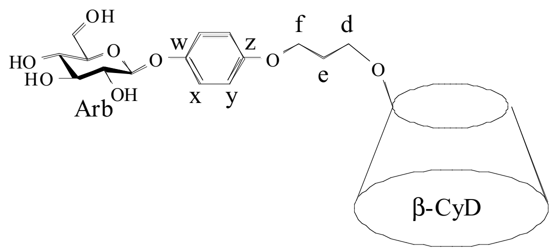
4. Structural Study by NMR Spectroscopy

The study of the structural analyses of the conjugate **3** and of its DXR inclusion complex was done using the NMR spectrometer. In order to ascertain the capping structure of **3** and the formations of the inclusion complex and of the stacking complex between **3** and DXR that we speculated, the ^{1}H NMR, ^{1}H NOESY, and ^{1}H DOSY spectra were measured by a 600 Mz NMR spectrometer.

4.1. ^{1}H NMR Spectra of the Conjugate 3, DXR, and the Mixed Sample of 3 and DXR

Fig. (**8**) indicates the ^{1}H NMR spectra of the conjugate **3**, DXR and the mixed sample of **3** and DXR (1:1) in the D_2O solution at 25 °C. Tables **(3** and **4)** summarizes the ^{1}H and ^{13}C chemical shifts of **3**. In the mixed sample of **3** and DXR, the shift transfers were observed in the partial protons of both **3** and DXR. In particular, the three protons (H_a, H_b and H_c) on the aromatic ring of DXR significantly shifted about 0.15 ~ 0.2 ppm upfield. The protons (H_x and H_y) on the phenyl group of **3** were also observed to transfer upfield slightly. These observations strongly suggested that the inclusion complex was formed in the mixed sample of **3**

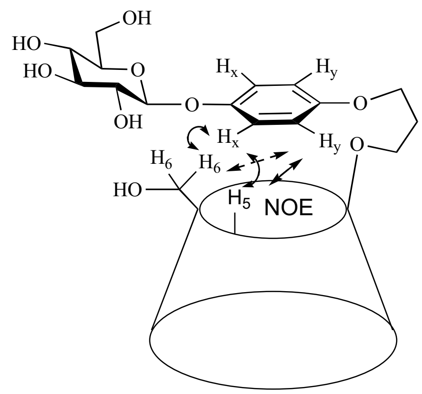
Table 3. **[1]H NMR Assignment of the Conjugate 2**



Chemical shift			ppm				
	H-1	H-2	H-3	H-4	H-5	H-6	
Arb	4.77	3.44	3.44	3.44	3.46	3.54	
CyD	4.48	3.44	3.52	3.12	3.17	3.62, 3.81	
	4.89	3.48	3.85	3.35	3.34	3.81	
	4.92	3.52		3.49	3.44		
	4.95	3.53			3.53		
	4.97				3.67		
Hd							3.92, 3.98
He							1.75, 1.86
Hf							3.37, 3.67
Hx							6.72, 6.74
Hy							7.03, 7.05

Table 4. **[13]C NMR Assignment of the Conjugate 2**

Chemical shift			ppm				
	C-1	C-2	C-3	C-4	C-5	C-6	
Arb	102.9	80.9		69.1		70.8	
CyD	100.9	75.6	73.1	80.1	71.7	59.9	
	101.8	75.9	73.2	80.8	71.9	60.1	
	101.9		73.3	81.1	72.1	60.2	
	102.1		73.4	81.2	72.2	60.3	
	102.2		73.5	81.5	72.3	60.4	
	102.3		74.1	82.4	72.4		
Cd							65.5
Ce							29.9
Cf							67.2
Cw							151.9
Cx							115.6
Cy							118.9
Cz							154.8



Scheme (7). NOE interactions observed in **3**.

and DXR and the three protons on the aromatic ring of DXR were remarkably influenced in the inclusion complex.

4.2. [1]H NOESY Spectra of the Conjugate 3

Fig. (**9**) presents the [1]H NOESY spectra of the conjugate **3**. The long-range NOE interactions were observed between the protons (H_X and H_Y) on the phenyl group and the protons (H-6 and H-5) on the β-CyD (Scheme (**7**)). This observation indicated that the protons on the phenyl group were located close to the protons at the primary side of the β-CyD within 0.5 nm. Therefore, the [1]H NOESY spectra of **3** verified our speculation that the phenyl group capped the primary side of the β-CyD, that is, "the pseudo-capping structure".

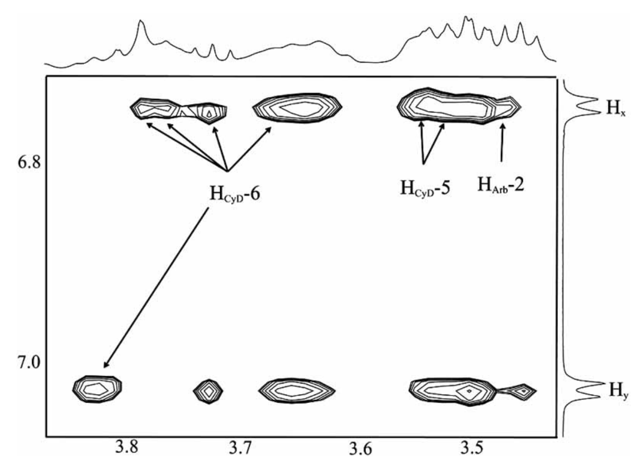
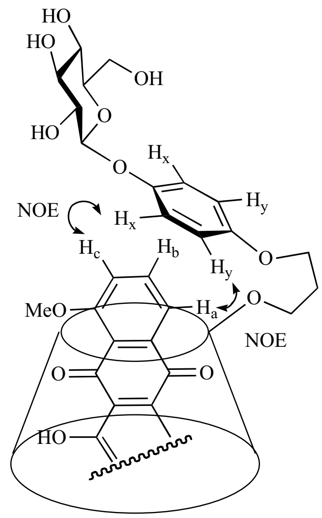


Fig. (9). [1]H NOESY spectra of **3**.

4.3. [1]H DOSY Spectra of the Mixed Sample of the Conjugate 3 and DXR

The [1]H DOSY spectra can conveniently separate the [1]H NMR spectra of each component in a mixed sample by the difference of their diffusion coefficients. Therefore, in the mixed sample including both a host molecule and a guest molecule, the formation of their inclusion complex can be determined by the measurement of the [1]H DOSY spectra.



Scheme (8). NOE interactions observed in inclusion complex of **3** and DXR.

Fig. (**10**) shows the [1]H DOSY spectra of the mixed sample of the conjugate **3** and DXR (1 : 1). The three [1]H NMR spectra were separately observed. Two of them corresponded to the [1]H NMR spectra of **3** and DXR, and the other one was the [1]H NMR spectra indicating that the inclusion complex was composed of **3** and DXR. This observation indicated that the conjugate **3** had the ability to include DXR into its cavity to form the inclusion complex. We could successfully ascertain the formation of the **3**-DXR inclusion complex directly by the [1]H DOSY spectra.

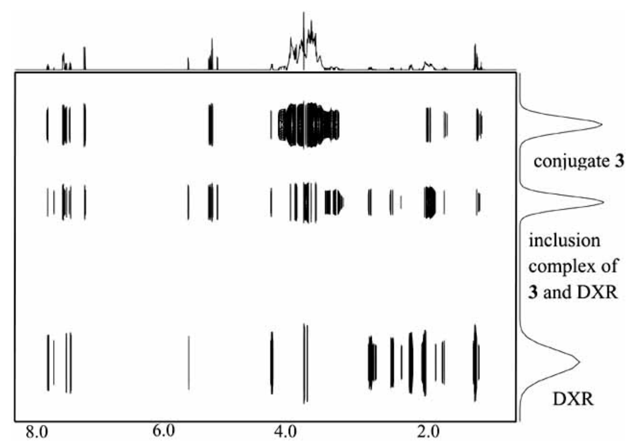


Fig. (10). [1]H DOSY spectra of the mixed sample of **3** and DXR (1:1).

4.4. [1]H NOESY Spectra of the Inclusion Complex Between the Conjugate 3 and DXR

In order to confirm the formation of the stacking complex between the conjugate **3** and DXR, we measured the [1]H NOESY spectra of their inclusion complex. Fig. (**11**) indicates the [1]H NOESY spectra of the mixed sample of the CyD derivative **3**-DXR (1:1). The long-range NOE interactions between the protons (H_X and H_Y) on the phenyl group and the protons (H_X and H_Y) on DXR were observed (Scheme (**8**)).

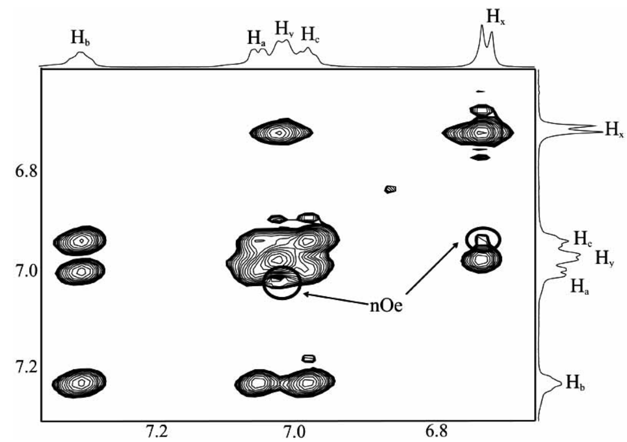


Fig. (11). ^{1}H NOESY spectra of the mixed sample of **3** and DXR (1:1).

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 **3** and the included DXR, as we speculated.

CONCLUSION

The three kinds of β-CyD derivatives **2**-**4** conjugated with glucose moieties were successfully synthesized from arbutin and β-CyD. The evaluations of these conjugates as the drug-carrying molecules were done using the immobilized Con A and DXR by SPR assays. Con A bound to these glucose moieties from arbutin in these conjugates **2**-**4** with prospective association constants of $2.0 \times 10^3 \sim 8.8 \times 10^3$ M^{-1}. These conjugates had extraordinarily high inclusion associations of $2.2 \times 10^5 \sim 1.4 \times 10^8$ M^{-1} for DXR. The NMR analyses of the conjugate **2** and its DXR inclusion complex indicated that the phenyl group in the spacer between the glucose moiety and β-CyD of the conjugate **3** greatly served to increase the inclusion association for DXR in the inclusion complex. The formation of the stacking complex by the π-π interactions between the phenyl group and the included doxorubicin also enhanced the inclusion abilities of **2**-**4** for the DXR. The designed conjugates **2**-**4**, which have high DXR-transportabilities and Con A lectin-recognition abilities, are promising as the models of drug-carrying molecules. The conjugates are expected to have high inclusion associations for anthracycline drugs which have similar structures to the DXR, and the glucose moiety of the conjugates can be changed to other saccharide molecules that are capable of targeting a variety of cells. We believe that this study contributes to the creation of the targeting drug carriers useful for targeting drug delivery systems.

EXPERIMENTAL SECTION

General

The NMR spectra were measured using an ECA-600 (JEOL) spectrometer at 600 MHz (^{1}H), and 150 MHz (^{13}C). The ^{1}H NMR chemical shifts are referenced to the internal standard TMS (δ_H = 0.00). The ^{13}C NMR chemical shifts are referenced to the solvent signal (δ_C = 77.0 for the central line of $CDCl_3$). The ESI-MS spectra were recorded on a Mariner (Applied Biosystems) spectrometer. The MALDI-TOF-MS spectra were recorded on a Voyager DE STR spectrometer. Optical rotations were recorded using a JASCO DIP-360 digital polarimeter. All reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F254 precoated plates (0.25 mm). The associations were measured using an optical biosensor IAsys (Affinity Sensor, UK). Arbutin **1** was purchased from the Tokyo Kasei Kogyo Co., Ltd.

4-(1-Propenyloxy)-phenyl β-D-Glucopyranoside (5)

To a solution of 4-hydroxy-phenyl β-D-glucopyranose (5.02 g, 18.4 mmol) in water (10 mL) was added 0.5 M NaOH aqueous solution (37.0 mL, 1.85 mmol). After the reaction mixture was stirred for 20 min at room temperature, the solvent was evaporated under reduced pressure. The reaction residue was dissolved in DMF (30.0 mL) and allyl bromide (2.0 mL, 23.1 mmol) was added. After the reaction mixture was stirred for 19 h, the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography on a silica-gel (chloroform/methanol = 5/1) to afford **5** (5.72 g, 99% yield) as a white crystal. ^{1}H NMR ($CDCl_3$): δ3.22-3.36 (4H, m, H-2, H-3, H-4, H-5), 3.61 (1H, dd, *J* = 4.9 Hz, *J* = 11.7 Hz, H_a-6), 3.79 (1H, dd, *J* = 1.3 Hz, *J* = 12.3 Hz, H_b-6), 4.39 (2H, td, *J* = 1.3 Hz, *J* = 2.1 Hz, *J* = 4.8 Hz, $OCH_2CH=CH_2$), 4.68 (1H, d, *J* = 6.9 Hz, H-1), 5.14 (1H, dd, *J* = 2.0 Hz, *J* = 10.3 Hz, $OCH_2CH=CH_aH_b$), 5.28 (1H, dd, *J* = 2.1 Hz, *J* = 17.2 Hz, $OCH_2CH=CH_aH_b$), 5.94 (1H, m, $OCH_2CH=CH_2$), 6.76 (2H, d, *J* = 9.7 Hz, OPhO), 6.95 (2H, d, *J* = 8.9 Hz, OPhO); ^{13}C NMR ($CDCl_3$): δ62.5, 70.3, 71.3, 74.9, 77.9, 78.0, 103.3, 116.4, 117.3, 119.1, 135.1, 153.3, 155.5; $[\alpha]^{23}_D = -53.6^o$ (*c* 2.6, CH_3OH); HRMS (ESI): *m/z* calcd for $C_{15}H_{20}O_7 \bullet Na^+$: 335.1101; found 335.1074.

4-(1-Propenyloxy)-phenyl 2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranoside (6)

To a solution of **5** (1.93 g, 6.18 mmol) in DMF (75.0 mL) was added sodium hydride (2.48 g, 103 mmol) at 0 °C. After the reaction mixture was stirred for 20 min, benzyl bromide (8.6 mL, 74.3 mmol) was added. After the reaction mixture was stirred for 12 h at room temperature, the reaction was then quenched by adding methanol (10 mL) and 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 a silica-gel (hexane/ethyl acetate = 4/1) to afford **6** (3.86 g, 93% yield) as a white crystal. ^{1}H NMR ($CDCl_3$): δ3.56-3.58 (1H, m, H-5), 3.64-3.72 (4H, m, H-2, H-3, H-4, H_a-6), 3.78 (1H, dd, *J* = 2.1 Hz, *J* = 11.0 Hz, H_b-6), 4.48-4.49 (2H, m, $OCH_2CH=CH_2$), 4.53-4.60 (3H, m, OCH_2Ph), 4.81-4.85 (3H, m, OCH_2Ph), 4.88 (1H, dd, *J* = 2.1 Hz, *J* = 5.5 Hz, H-1), 4.94 (1H, d, *J* = 11.0 Hz, OCH_2Ph), 5.04 (1H, d, *J* = 11.0 Hz, OCH_2Ph), 5.27 (1H, dd, *J* = 1.4 Hz, *J* = 10.3 Hz, $OCH_2CH=CH_aH_b$), 5.39 (1H, dd, *J* = 1.4 Hz, *J* = 17.2 Hz, $OCH_2CH=CH_aH_b$), 6.04 (1H, m,

OCH_2CH=CH_2), 6.82 (2H, d, *J* = 9.0 Hz, OPhO), 7.01 (2H, d, *J* = 9.0 Hz, OPhO), 7.18-7.36 (20H, m, OCH_2Ph); ^{13}C NMR ($CDCl_3$): δ68.9, 69.3, 73.4, 74.9, 75.0, 75.7, 77.7, 82.0, 84.6, 102.7, 115.5, 117.5, 118.3, 127.6-128.4, 133.4, 138.0-138.5, 151.6, 154.2; $[\alpha]^{23}_D$ = -0.95° (*c* 2.1, $CHCl_3$); HRMS (ESI): *m/z* calcd for $C_{43}H_{44}O_7$•Na^+: 695.2979; found 695.3019.

4-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyloxy)-phenoxy-acetaldehyde (7)

Ozone was bubbled through a stirred solution of **6** (301.4 mg, 0.45 mmol) in CH_2Cl_2 (8.0 mL) at -78 °C for 10 min. After triphenylphosphine (377.5 mg, 1.44 mmol) was added at -78 °C and the reaction temperature was raised to room temperature, the reaction mixture was stirred for 3 h. The solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography on a silica-gel (hexane/ethyl acetate = 1/1) to afford **7** (291.9 mg, 97% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ3.57-3.59 (1H, m, H-5), 3.65-3.69 (3H, m, H-2, H-4, H_a-6), 3.72-3.73 (1H, m, H-3), 3.78 (1H, dd, *J* = 2.0 Hz, *J* = 11.0 Hz, H_b-6), 4.48-4.85 (8H, m, H-2', OCH_2Ph), 4.89 (1H, dd, *J* = 2.0 Hz, *J* = 5.5 Hz, H-1), 4.94 (1H, d, *J* = 11.0 Hz, OCH_2Ph), 5.03 (1H, d, *J* = 11.0 Hz, OCH_2Ph), 9.81 (1H, S, CHO); ^{13}C NMR ($CDCl_3$): δ68.8, 73.2, 73.4, 74.9, 75.0, 75.3, 75.7, 77.7, 82.0, 84.6, 102.5, 115.4, 118.5, 199.4; $[\alpha]^{23}_D$ = -3.48° (*c* 2.9, $CHCl_3$); HRMS (ESI): *m/z* calcd for $C_{42}H_{42}O_8$•Na^+: 697.2777; found 697.2815.

4-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyloxy)-phenoxy-acetic acid (8)

To a solution of **7** (190.3 mg, 0.28 mmol) in *t*-butylalcohol (6.0 mL)-H_2O (3.0 mL) was added $NaClO_2$ (260.5 mg, 2.88 mmol), NaH_2PO_4 (63.8 mg, 0.41 mmol) and 2-metyl-2-butene (130.4 μL, 1.23 mmol). After the reaction mixture was stirred for 4 h, the reaction was quenched by adding 2*N* HCl (1.0 mL) and water (5.0 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 flash column chromatography on a silica-gel (chloroform/methyl alcohol = 5/1) to afford **8** (189.9 mg, 98% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ3.57-3.59 (1H, m, H-5), 3.64-3.69 (3H, m, H-3, H-4, H_a-6), 3.72 (1H, d, *J* = 7.5 Hz, H-2), 3.77 (1H, dd, *J* = 2.0 Hz, *J* = 11.0 Hz, H_b-6), 4.51-4.59 (5H, m, H-2', OCH_2Ph), 4.80-4.84 (3H, m, OCH_2Ph), 4.90 (1H, d, *J* = 10.3 Hz, H-1), 4.94 (1H, d, *J* = 11.0 Hz, OCH_2Ph), 5.02 (1H, d, *J* = 11.0 Hz, OCH_2Ph),; ^{13}C NMR ($CDCl_3$): δ65.5, 68.7, 73.4, 74.9, 75.0, 75.0, 75.7, 77.6, 82.0, 84.6, 102.4, 115.6, 118.4, 173.5; $[\alpha]^{23}_D$ = -5.60° (*c* 2.5, $CHCl_3$); HRMS (ESI): *m/z* calcd for $C_{42}H_{42}O_9$•Na^+: 713.2727; found 713.2878.

4-(3-Hydroxypropyloxy)-phenyl 2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranoside (9)

To a solution of **6** (2.02 g, 3.00 mmol) in THF (7.0 mL) was added in 0.5 M 9-borabicyclo[3.3.1]nonane THF solution (12 mL, 6.00 mmol) at 0 °C. After the reaction mixture was stirred for 24 h at room temperature, 0.5 M NaOH aqueous solution (18.0 mL, 9.0 mmol) and 30% H_2O_2 aqueous solution (3.1 mL, 30.1 mmol) was added. After the reaction mixture were stirred for 16 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 a silica-gel (hexane/ethyl acetate = 2/1) to afford **9** (1.94 g, 95% yield) as a white crystal. ^{1}H NMR ($CDCl_3$): δ2.01-2.04 (2H, m, $OCH_2CH_2CH_2OH$), 3.56 (1H, ddd, *J* = 2.1 Hz, *J* = 5.5 Hz, *J* = 10.3 Hz, H-5), 3.65-3.73 (4H, m, H-2, H-3, H-4, H_a-6), 3.78 (1H, dd, *J* = 1.4 Hz, *J* = 11.0 Hz, H_b-6), 3.84-3.87 (2H, m, $OCH_2CH_2CH_2OH$), 4.08 (2H, t, *J* = 5.5 Hz, $OCH_2CH_2CH_2OH$), 4.52-4.60 (3H, m, OCH_2Ph), 4.81-4.85 (3H, m, OCH_2Ph), 4.88 (1H, dd, *J* = 2.1 Hz, *J* = 5.5 Hz, H-1), 4.94 (1H, d, *J* = 11.0 Hz, OCH_2Ph), 5.04 (1H, d, *J* = 11.0 Hz, OCH_2Ph), 6.81 (2H, d, *J* = 9.0 Hz, OPhO), 7.02 (2H, d, *J* = 9.1 Hz, OPhO), 7.20-7.35 (20H, m, OCH_2Ph); ^{13}C NMR ($CDCl_3$): δ32.0, 60.6, 66.4, 68.9, 73.5, 75.0, 75.0, 75.1, 75.7, 77.7, 82.1, 84.7, 102.7, 115.2, 118.4, 127.6-128.4, 138.0-138.5, 151.6, 154.4; $[\alpha]^{23}_D$ = -1.15° (*c* 2.6, $CHCl_3$); HRMS (ESI): *m/z* calcd for $C_{43}H_{46}O_8$•Na^+: 713.3085; found 713.2863.

4-(3-Iodopropyloxy)-phenyl 2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranoside (10)

To a solution of **9** (461.4 mg, 0.67 mmol) in DMF (7.0 mL) was added triphenylphosphine (889.2 mg, 3.39 mmol) and iodine (873.3 mg, 3.44 mmol) at 70 °C under an argon atmosphere. After the reaction mixture was stirred for 24 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 a preparative silica- gel TLC (hexane/ethyl acetate = 2/1) to afford **10** (441 mg, 83% yield) as a white crystal. ^{1}H NMR ($CDCl_3$): δ2.26 (2H, m, $OCH_2CH_2CH_2I$), 3.37 (2H, t, *J* = 6.9 Hz, $OCH_2CH_2CH_2I$), 3.56-3.58 (1H, m, H-5), 3.64-3.73 (4H, m, H-2, H-3, H-4, H_a-6), 3.78 (1H, dd, *J* = 1.6 Hz, *J* = 11.0 Hz, H_b-6), 3.99 (2H, t, *J* = 6.1 Hz, $OCH_2CH_2CH_2I$), 4.53-4.61 (3H, m, OCH_2Ph), 4.81-4.85 (3H, m, OCH_2Ph), 4.88 (1H, dd, *J* = 2.1 Hz, *J* = 5.5 Hz, H-1), 4.94 (1H, d, *J* = 10.3 Hz, OCH_2Ph), 5.04 (1H, d, *J* = 11.0 Hz, OCH_2Ph), 6.81 (2H, d, *J* = 8.9 Hz, OPhO), 7.03 (2H, d, *J* = 8.9 Hz, OPhO), 7.25-7.36 (20H, m, OCH_2Ph); ^{13}C NMR ($CDCl_3$): δ2.5, 33.0, 67.8, 68.9, 73.5, 75.0, 75.0, 75.1, 75.7, 77.7, 82.1, 84.7, 102.7, 115.4, 118.4, 127.6-128.3, 138.0-138.3, 151.7, 154.3; $[\alpha]^{23}_D$ = -1.52° (*c* 3.9, $CHCl_3$); HRMS (ESI): *m/z* calcd for $C_{43}H_{45}O_7I$•Na^+: 823.2102; found 823.2125.

6A-*O*-(4-β-D-Glucopyranosyloxy-phenoxy)-*N*-(6^A-deoxy-β-cyclodextrin-6^A-yl)-acetamide (2)

To a solution of **8** (27.1 mg, 0.039 mmol) and dimethylphosphinothioyl chloride (5.0 mg, 0.039 mmol) in DMF (6.0 mL) was added diisopropylethylamine (6.8 μL, 0.039 mmol). After the reaction mixture was stirred for 40 min, *mono*-6-amino-6-deoxy-β-cyclodextrin **12** (45.6 mg, 0.040 mmol) 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 (five times) and dissolved in DMF (15 mL). $Pd(OH)_2$ (46.6 mg, 0.30 mmol) was added to the solution and hydrogen was bubbled for 24 h. After the solvent was filtered and evaporated under reduced pressure, the crude product was isolated by adsorbing on HP-20 (DIAION) followed by eluting with methanol to afford **2** (35.5 mg, 63% yield) as a white crystal. MALDI-TOF MS: *m/z* calcd for $C_{56}H_{87}NO_{42}$•Na^+: 1468. 4595; found. 1468.7343.

Heptaxis-(2,3-di-O-benzyl)-6^{B,C,D,E,F,G}-hexa-O-benzyl-6^{A}-O-{3-[4-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyloxy)-phenoxy]-propyl}-β-cyclodextrin (14)

To a solution of **10** (71.2 mg, 0.089 mmol) in DMF (7.0 mL) was added potassium hydrate (132.5 mg, 2.36 mmol) and Heptaxis-(2,3-di-*O*-benzyl)-6^{B,C,D,E,F,G}-hexa-*O*-benzyl-β-cyclodextrin **13** (58.1 mg, 0.020 mmol). After the reaction mixture was stirred for 6h, the reaction was then quenched by adding water (10 mL). The reaction 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 a preparative silica-gel TLC (hexane/ethyl acetate = 2/1) to afford **14** (36.5 mg, 51% yield) as a colorless oil. MALDI-TOF MS: *m/z* calcd for $C_{218}H_{228}O_{42}$ •Na^+: 3540.99; found 3538.27.

6^{A}-*O*-{[3-(4-β-D-Glucopyranosyloxy)-phenoxy]-propyl}-β-cyclodextrin (3)

To a solution of **14** (104.8 mg, 0.029 mmol) in MeOH (3.5 mL) and Et_2O (10.0 mL) was added $Pd(OH)_2$ (42.1 mg, 0.27 mmol). To the solution was bubbled with hydrogen for 3 h. After the solvent was filtered and evaporated under reduced pressure, the crude product was isolated by adsorbing on HP-20 (DIAION) followed by eluting with methanol to afford **3** (35.7 mg, 85% yield) as a white crystal. MALDI-TOF MS: *m/z* calcd for $C_{57}H_{99}O_{42}$ •Na^+: 1469.4799; found 1470.8195.

Heptaxis-(2,3-di-*O*-benzyl)-6^{B,C,E,F,G}-penta-*O*-benzyl-6^{A,D}-bis-O-{3-[4-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl-oxy)-phenoxy]-propyl}-β-cyclodextrin (16)

To a solution of **10** (450.5 mg, 0.56 mmol), heptaxis-(2,3-di-*O*-benzyl)-6^{B,C,E,F,G}-penta-*O*-benzyl-β-cyclodextrin **15** (309.7 mg, 0.11 mmol) in DMF (7.0 mL) were added KOH (740.9 mg, 13.20 mmol) and tetra-*n*-butylammonium iodide (9.0 mg, 0.024 mmol). After the reaction mixture was stirred for 21h, 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 a preparative silica-gel TLC (hexane/ethyl acetate = 3/2) to afford **16** (234.8 mg, 52% yield) as a colorless oil. MALDI-TOF MS: *m/z* calcd for $C_{261}H_{272}O_{49}$ •Na^+: 4212.8685; found 4216.4823.

6^{A,D}-Bis-*O*-{[3-(4-β-D-glucopyranosyloxy)-phenoxy]-propyl}-β-cyclodextrin (4)

To a solution of **16** (50.0 mg, 0.012 mmol) in MeOH (1.0 mL)-Et_2O (2.0 mL) was added $Pd(OH)_2$ (69.1 mg, 0.44 mmol). To the solution was bubbled with hydrogen for 24 h. After the solvent was filtered and evaporated under reduced pressure, the crude product was isolated by adsorbing on HP-20 (DIAION) followed by eluting with methanol to afford **4** (16.7 mg, 80% yield) as a white crystal. MALDI-TOF MS: *m/z* calcd for $C_{72}H_{110}O_{49}$ •Na^+: 1781.6008; found 1781.3431.

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