Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 342 (2007) 2173–2181
Carbohydrate RESEARCH

Preparation and characterization of $6^{I},6^{n}$-di-*O*-(L-fucopyranosyl)-β-cyclodextrin (n = II–IV) and investigation of their functions

Yuki Nishi,[a] Naoe Yamane[b] and Toshiko Tanimoto[a,*]

[a]*School of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien, Kyuban-Cho, Nishinomiya 663-8179, Japan*
[b]*JCL Bioassay Corporation, 17-18 Nakahara-Cho, Nishiwaki 677-0032, Japan*

Received 8 November 2006; received in revised form 11 June 2007; accepted 12 June 2007
Available online 23 June 2007

Abstract—Three positional isomers of $6^{I},6^{n}$-di-*O*-(β-L-fucopyranosyl)-cyclomaltoheptaose [$6^{I},6^{n}$-di-*O*-(β-L-Fuc)-β-cyclodextrin, -βCD, n = II–IV] were chemically synthesized using the corresponding authentic compounds, $6^{I},6^{n}$-di-*O*-(*tert*-butyldimethylsilyl)-βCD (n = II–IV), as the fucosyl acceptors, and 2,3,4-tri-*O*-acetyl-L-fucopyranosyl trichloroacetimidate as the fucosyl donor. Their structures were analyzed by HPLC, MS, and NMR spectroscopy. The hemolytic activities of L-Fuc-βCDs were lower than that of βCD, while the solubilities of these branched CDs in water were much higher than that of βCD. The molecular interaction between these compounds and the fucose-binding lectin *Aleuria aurantia* lectin (AAL) was investigated using an optical biosensor based on a surface plasmon resonance (SPR) technique. The order of binding affinity, as a function of the fucose-binding position, was $6^{I},6^{IV}$- > $6^{I},6^{III}$- > $6^{I},6^{II}$-di-*O*-(β-L-Fuc)-βCD > 6-*O*-(β-L-Fuc)-βCD.

Keywords: L-Fucopyranosyl-βCD; Surface plasmon resonance; *Aleuria aurantia* lectin; Hemolytic activity

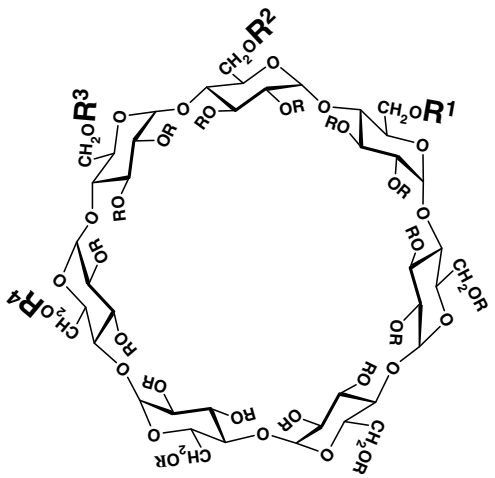
1. Introduction

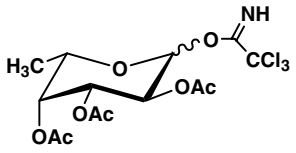
Cyclomaltooligosaccharides (cyclodextrins, CDs) and branched CDs have the ability to form inclusion complexes by incorporating various kinds of compounds into their interior cavities. It is well known that mannose, galactose, and *N*-acetylglucosamine play important roles in the recognition of receptors on the cell surface.[1,2] Branched CDs are expected to be useful as drug carriers in targeted drug delivery systems. In particular, positional isomers of di-branched glycosyl CDs may exhibit differences in their molecular recognition abilities arising from differences in their substitution patterns. We have attempted to synthesize various homogeneous and heterogeneous branched CDs via chemical synthesis[3–7] and enzymatic reactions.[8–12] The water solubilities of branched cyclomaltoheptaose (βCD) derivatives linked with mono- or oligosaccharides containing glucose, galactose, mannose, and maltose are about 50–100 times that of βCD.[13,14] Therefore, the use of branched-βCD inclusion complexes is expected to result in an increase in bioavailability for insoluble and unstable drugs.

L-Fucose (L-Fuc), a deoxyhexose, is a biologically relevant monosaccharide which has been found in some tumor-associated blood-group glycosphingolipids.[15] Fucosyl CDs have not yet been synthesized via enzymatic reactions. In this study, three positional isomers of a di-branched βCD, $6^{I},6^{n}$-di-*O*-(β-L-Fuc)-βCD (n = II–IV) (**10**–**12**), along with a mono-branched βCD, 6-*O*-(β-L-Fuc)-βCD (**13**), were chemically synthesized (Chart 1), and their solubilities in water and hemolytic activities on human erythrocytes were compared with those of βCD. In order to evaluate the molecular recognition abilities of these branched βCDs, their interaction with the fucose-binding lectin *Aleuria aurantia* lectin (AAL)[16–19] was investigated using an optical biosensor based on surface plasmon resonance (SPR).[20]

* Corresponding author. Tel.: +81 798 45 9950; e-mail: tanimoto@mukogawa-u.ac.jp

0008-6215/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2007.06.016





14

	R^1	R^2	R^3	R^4	R
1	Si	Si	H	H	H
2	Si	H	Si	H	H
3	Si	H	H	Si	H
4	H	H	Ac	Ac	Ac
5	H	Ac	H	Ac	Ac
6	H	Ac	Ac	H	Ac
7	Fuc	Fuc	Ac	Ac	Ac
8	Fuc	Ac	Fuc	Ac	Ac
9	Fuc	Ac	Ac	Fuc	Ac
10	β-Fuc	β-Fuc	H	H	H
11	β-Fuc	H	β-Fuc	H	H
12	β-Fuc	H	H	β-Fuc	H
13	β-Fuc	H	H	H	H

Si: *tert*-butyldimethylsilyl.
Fuc: L-fucopyranoside.
β-Fuc: β-L-fucopyranoside.

Chart 1. Structures of compounds **1**–**14**.

2. Results and discussion

2.1. Preparation, separation, and characterization of $6^I,6^n$-di-*O*-(β-L-fucopyranosyl)-cyclomaltoheptaose (*n* = II–IV) (10–12) and 6-*O*-(β-L-fucopyranosyl)-cyclomaltoheptaose (13)

As the key intermediates for the chemical synthesis of positional isomers of di-branched βCDs, we have already synthesized and characterized $6^I,6^n$-di-*O*-(triphenylmethyl)-βCD,[21] $6^I,6^n$-di-*O*-(monomethoxytriphenylmethyl)-βCD,[22] and $6^I,6^n$-di-*O*-(dimethoxytriphenylmethyl)-βCD,[22] as well as three positional isomers of $6^I,6^n$-di-*O*-(*tert*-butyldimethylsilyl)-βCD (*n* = II–IV) (**1**–**3**).[23] The three positional isomers of $6^I,6^n$-di-*O*-(β-L-Fuc)-βCD (*n* = II–IV) (**10**–**12**) were chemically synthesized using the glycosyl acceptors bis(2,3-di-*O*-acetyl)-pentakis(2,3,6-tri-*O*-acetyl)-βCD (**4**–**6**)[24] (which were obtained from the authentic compounds **1**–**3**) and the fucosyl donor 2,3,4-tri-*O*-acetyl-L-fucopyranosyl trichloroacetimidate (**14**).[25] Fucosylation of **4**, **5**, or **6** with **14** in dichloromethane in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as an acid catalyst and molecular sieves for 1 h at −20 °C gave $6^I,6^n$-di-*O*-(L-Fuc)-βCD peracetates (*n* = II–IV) (**7**–**9**) and the mono-fucosylated βCD derivative **X**. The fractions containing **7** and **X**, **8** and **X**, or **9** and **X** were collected from the product by centrifugal chromatography, followed by deacetylation with methanolic sodium methoxide and isolation by HPLC on a TSKgel Amide-80 column (300 × 7.8 mm i.d.) to give $6^I,6^n$-di-*O*-(L-Fuc)-βCD (*n* = II–IV) (**10**–**12**) and 6-*O*-(L-Fuc)-βCD (**13**). At this point (i.e., by HPLC analysis using an aminopropyl-silica column), it was difficult to determine the configurational isomers; that is, whether the two fucoses are linked to βCD by an α-(1→6) linkage, a β-(1→6) linkage, or a mixture of both. Figure 1 shows the elution profiles of compounds **10**–**13** on a LiChroCART NH_2 column, and **10**–**13** and βCD on an ODS column XTerra RP_{18}. The elution sequence using a NH_2-bonded silica and acetonitrile–water system followed the order of molecular size, and the elution time gave qualitative information about the molecular size of the sugar.[26] It became apparent that the mono-branched βCD **13** was distinguishable from the di-branched βCDs **10**–**12**. In contrast, in C_{18}-bonded silica, a reversed phase, the separation mechanism is probably based on hydrophobic chromatography; that is, increased retention with decreasing solubility in water.[27] For instance, because the solubility of βCD is low,[13] it is eluted much later than αCD and γCD, which exhibit high solubility, on the ODS column under the same conditions. Meanwhile, we were aware that the solubilities of branched CDs linked with mono- or oligosaccharides containing glucose, galactose, mannose, and maltose are 50–100 times that of CDs.[14] Therefore, those branched CDs are eluted faster than non-branched CDs on the ODS column. Notwithstanding, although the solubilities of L-Fuc-βCDs are several times higher as compared with βCD (Table 1), they are retained longer than βCD on the ODS column. Because fucose is a 6-deoxyhexose, a change occurs in the hydrophobic interaction between the functional groups and the surface of the stationary phase. On the other hand, configurational isomers can generally be isolated on an ODS column.[3,4,6,7] Analysis of the pure positional isomers **10**–**12** and **13** by HPLC on an ODS column showed a single peak for each (Fig. 1), suggesting that the L-Fuc group is linked to βCD by either an α-(1→6) linkage or a β-(1→6) linkage only.

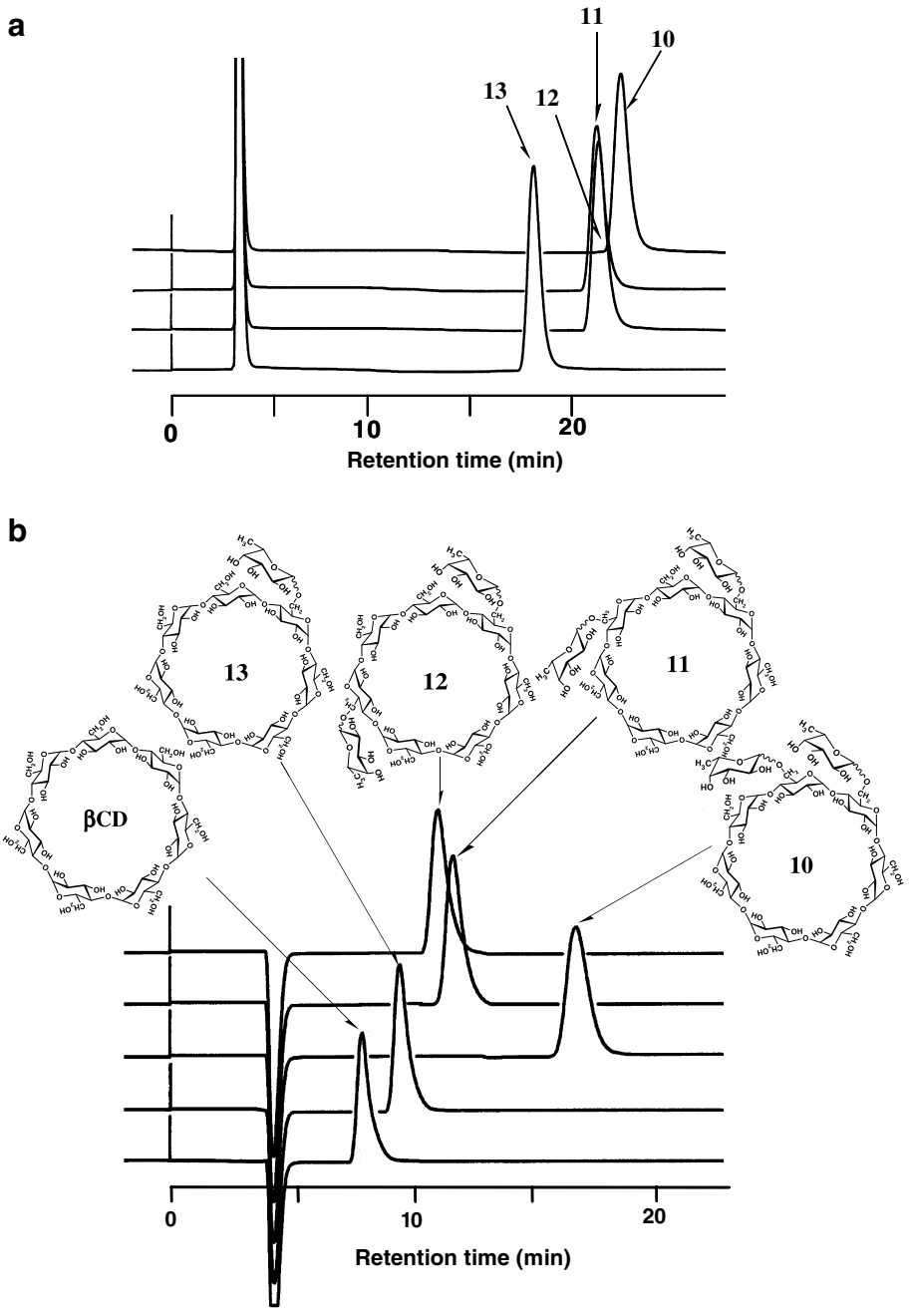


Figure 1. Elution profiles of the three positional isomers of 6^{I},6^{n}-di-*O*-(L-fucopyranosyl)-βCD (n = II–IV) (**10**–**12**) and 6-*O*-(L-fucopyranosyl)-βCD (**13**). Chromatographic conditions: detector, Shodex RI-71; temperature, 30 °C; (a) column, LiChroCART NH_2 (250 × 4.0 mm i.d.); eluent, 17:8 MeCN–water; flow rate, 0.8 mL/min, (b) column, XTerra RP_{18} (150 × 4.6 mm i.d.); eluent, 3:17 MeOH–water; flow rate, 0.5 mL/min.

HRFABMS analysis of **10**–**12** (*m*/*z* 1427.5012, 1427.4922, 1427.4995; M^+) and **13** (*m*/*z* 1281.4359; M^+) supported their molecular formulae (di-branched βCDs and mono-branched βCD, respectively).

NMR analysis was performed using ^1H–^1H COSY and ^1H–^{13}C COSY experiments. All carbon resonances in the spectra of **10**–**13** were assigned (Fig. 2). The assignments of the C-6 signals were confirmed by DEPT (distortionless enhancement by polarization transfer) method.[28] The relative intensities of the signals were also measured. In the spectra of **10**–**13**, the signals due to the fucosylated C-6 (G′-6, δ 68.47–68.72) were downfield shifted by 7–8 ppm compared with the C-6 signals (G-6, δ 61.14–61.47) of the five or six unsubstituted glucoses. The ratio of relative intensities of signals due to G-1, F-1, G-6 and G′-6 were a further structural confirmation of the mono- or di-branched CDs. The C-1 signals (F-1, δ 103.63–103.85) of L-Fuc ($^1C_4$ chair

Table 1. Solubility of 6^{I},6^{n}-di-*O*-(β-L-Fuc)-βCD (n = II–IV) (**10**–**12**), 6-*O*-(β-L-Fuc)-βCD (**13**) and βCD in water

Compounds (100 mg)	H_2O (μL)[a]	
	25 ± 1 °C	50 ± 1 °C
βCD	5000	2000
6^{I},6^{II}-di-*O*-(β-L-Fuc)-βCD (**10**)	68	60
6^{I},6^{III}-di-*O*-(β-L-Fuc)-βCD (**11**)	68	60
6^{I},6^{IV}-di-*O*-(β-L-Fuc)-βCD (**12**)	70	59
6-*O*-(β-L-Fuc)-βCD (**13**)	85	61

[a] The volume (μL) of water required for complete dissolution of compounds (100 mg).

conformation) were downfield shifted compared to the C-1 signals of the βCD ring (G-1, δ 102.15–102.59). In the case of D-glucose[7] or D-galactose[4,6] residues ($^4C_1$ chair conformation) bonded to the CD ring via a β-(1→6) linkage, the C-1 signals were shifted downfield compared to the C-1 signals of the CD ring, but when these residues were bonded to the CD ring via a α-(1→6) linkage, the C-1 signal appeared upfield shifted. In addition, the H-1 signals of L-Fuc were observed at δ 4.39–4.41, $J_{1,2}$, 7.69–7.83 ppm, unequivocally confirming the 6^{I},6^{n}-di-*O*-(β-L-fucopyranosyl)-βCD (n = II–IV) (**10**–**12**) and 6-*O*-(β-L-fucopyranosyl)-βCD (**13**).

2.2. Solubility

The solubilities of L-Fuc-βCDs **10**–**13** and βCD in water at 25 °C and 50 °C are summarized in Table 1. The solubilities of the L-Fuc-βCDs were much higher than those of βCD at both temperatures.

2.3. Hemolytic activity

The hemolytic effects of L-Fuc-βCDs **10**–**13** on human erythrocytes in isotonic soln, compared with βCD are shown in Figure 3. The hemolytic activities became weaker in the order βCD > di-branched βCDs (**10**–**12**) > mono-branched βCD (**13**), and those of the three positional isomers **10**–**12** were almost identical. The hemolytic activities of the native CDs are known to decrease in the order βCD > αCD > γCD;[29] this order is contrary to the order of water-solubility. In the case of L-Fuc-βCDs, although the solubilities of **10**–**13** in water were almost the same (Table 1), the hemolytic activity of **13** was lower than those of **10**–**12**. These results differ remarkably from those of glucosyl-βCDs,[30] which may be attributed to L-Fuc being a 6-deoxyhexose.

2.4. Interactions of L-fucopyranosyl-βCDs with AAL

In order to evaluate the molecular recognition abilities of L-Fuc-βCDs **10**–**13**, the interaction between those compounds and AAL, which is known to be a fucose-specific binding lectin, was investigated using an optical biosensor. The association constants between **10**–**13** and AAL were determined by a SPR method.[31] AAL was immobilized on the surface of a cuvette, and the sugars injected over this surface, so that the lectin on the surface of the biosensor cuvette could be regarded as a model for receptors on the surface of cell membranes. The kinetic and equilibrium constants of the interaction between AAL and L-Fuc-βCDs are listed in Table 2. Although AAL is said to be an α-connected L-fucose-binding lectin,[16–18] the results showed that L-Fuc-βCDs interacted with AAL[19] with binding affinities in the order 6^{I},6^{IV}-di-*O*-(β-L-Fuc)-βCD > 6^{I},6^{III}-di-*O*-(β-L-Fuc)-βCD > 6^{I},6^{II}-di-*O*-(β-L-Fuc)-βCD > 6-*O*-(β-L-Fuc)-βCD when compared in terms of fucose-binding position.

3. Experimental

3.1. General methods

HPLC was performed using a Jasco PU-980 pump, a Rheodyne 7125 injector, and a Shodex RI-71 refractive index detector. HPLC analysis was conducted at constant temperature using a column oven CA-202 (Flom). The columns employed were YMC-Pack SH-343-7 ODS (250 × 20 mm i.d.), TSKgel Amide-80 (300 × 7.8 mm i.d., Tosoh), Hibar LiChroCART NH_2 (250 × 4.0 mm i.d., Kanto Chemical), Waters XTerra RP_{18} (150 × 4.6 mm i.d.) and Hikarisil C18-4D (150 × 4.6 mm i.d.). ^1H and ^{13}C NMR spectroscopic data were recorded for solns in D_2O or $CDCl_3$, using a Jeol GSX-500 or Jeol JNM-ECP 500 spectrometer (^1H: 500 MHz, ^{13}C: 125 MHz). Chemical shifts are expressed in ppm downfield from a Me_4Si signal, with reference to external 1,4-dioxane (67.4 ppm). The conditions for ^1H–^1H COSY and ^1H–^{13}C COSY measurements were the same as those described in a previous paper.[32] HRFABMS was measured in positive-ion mode using a Jeol MS 700 mass spectrometer with xenon atoms, with glycerol as the matrix. MALDITOFMS was carried out on a Vision 2000 instrument (Thermo Bioanalysis), with 2,5-dihydroxybenzoic acid as the matrix. The instrument was operated in positive-ion reflectron mode with an accelerating potential of 7 kV. Optical rotations were determined with a Jasco P-1020 polarimeter at 25 °C. TLC was performed on Silica Gel 60 plates (E. Merck). Melting points were measured using a Yanagimoto micro melting-point apparatus and are uncorrected. A UVIDEC V-530 double beam spectrophotometer (Jasco) was used for determination of absorbances. Centrifugal chromatography was performed with a Harrison Centrifugal Thin-Layer Chromatotron 7924. The optical biosensor IAsys cuvette

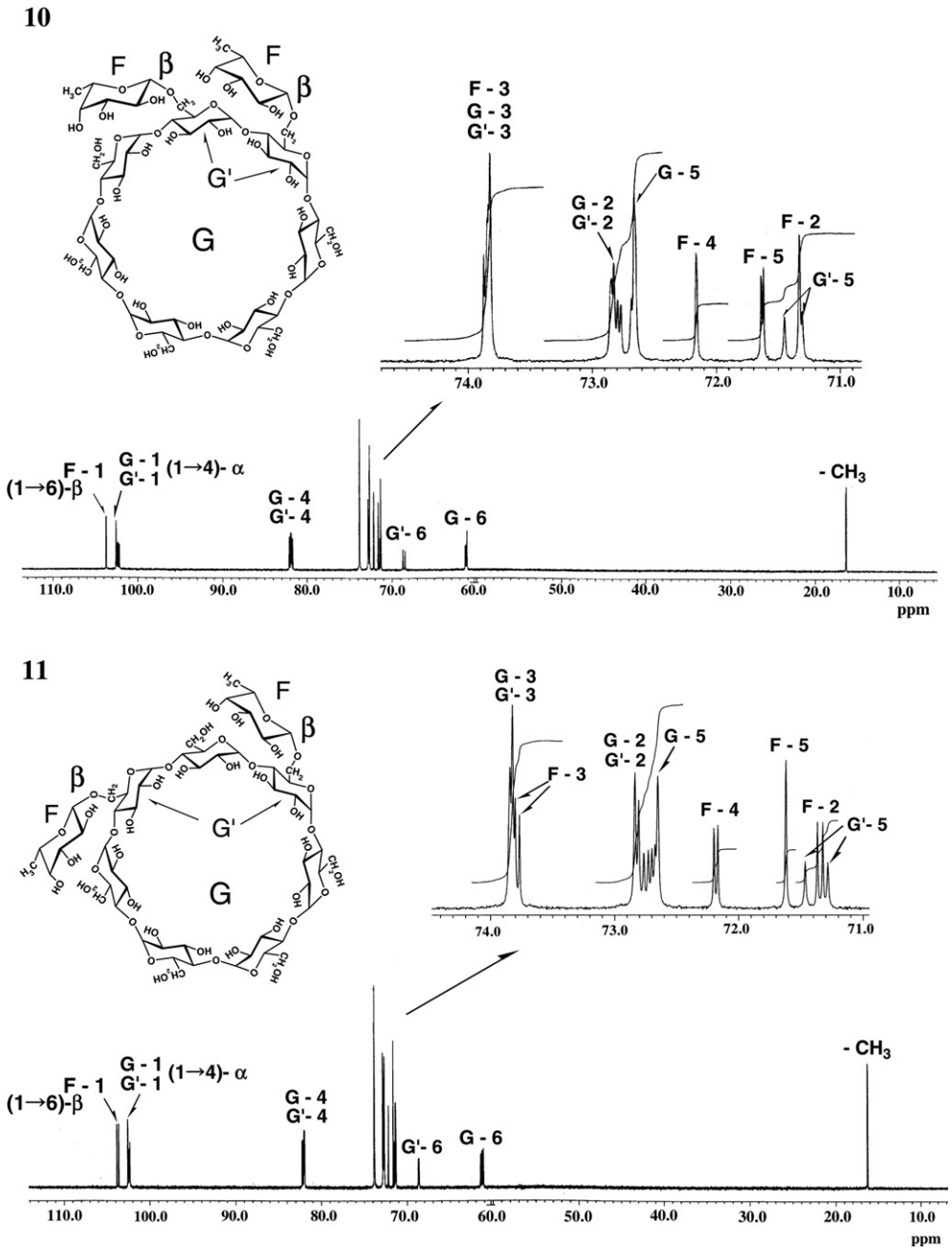


Figure 2. ^{13}C NMR spectra of $6^{I},6^{n}$-di-*O*-(β-L-fucopyranosyl)-βCD (n = II–IV) (**10**–**12**) and 6-*O*-(β-L-fucopyranosyl)-βCD (**13**) measured in D_2O at 125.65 MHz. G-1, -2, -3, -4, -5, and -6 represent the signals of the C-1, -2, -3, -4, -5, and -6 atoms of the βCD ring D-glucopyranose units. G′ is the βCD ring D-glucopyranose unit on which the L-fucopyranose residue is β-(1→6)-linked. F-1, -2, -3, -4, and -5 represent C-1, -2, -3, -4, and -5 of the L-fucopyranose unit on which β-(1→6) is linked directly to βCD.

system, with IAsys software and IAsys cuvettes coated with carboxylate, was from Affinity Sensors.

3.2. Syntheses of substrates

3.2.1. Bis(2,3-di-*O*-acetyl)-pentakis(2,3,6-tri-*O*-acetyl)-cyclomaltoheptaoses (4–6). To a soln of **1** (844 mg, 0.62 mmol), **2** (776 mg, 0.57 mmol), or **3** (1.10 g, 0.80 mmol) in dry pyridine (25–40 mL) was added Ac_2O (8–15 mL), and the mixture was stirred for 5 h at 100 °C and then concentrated. The residue was extracted with $CHCl_3$, and the extract was washed sequentially with aq $NaHCO_3$ and water, then dried with $CaCl_2$ and concentrated to a syrup. To a soln of the residue in dry $CHCl_3$ (40–50 mL) was added $BF_3{\cdot}Et_2O$ (960–1450 μL), in an ice-water bath, with stirring; stirring was continued for 2 h at rt. A soln in $CHCl_3$was washed with water, aq $NaHCO_3$, and water again, and then dried ($CaCl_2$) and concentrated, to give **4** (1.07 g, 95.0%), **5** (0.93 g, 94.6%), and **6** (1.35 g, 97.7%). R_f 0.42 (2:3 hexane–acetone).

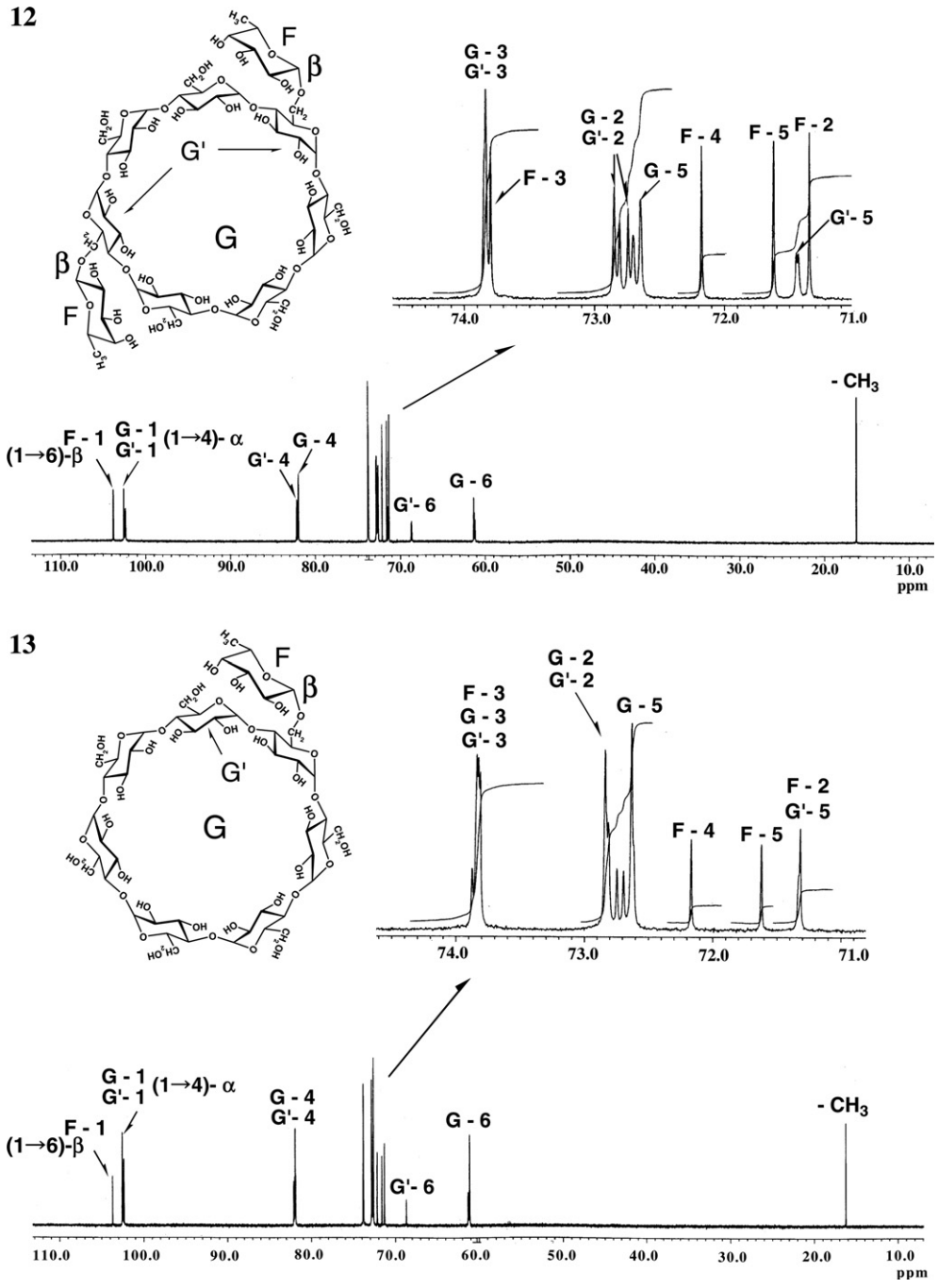


Figure 2 (*continued*)

3.2.2. Synthesis of 2,3,4-tri-*O*-acetyl-L-fucopyranosyl trichloroacetimidate (14) via intermediates tetra-*O*-acetyl-α-L-fucopyranose (15) and 2,3,4-tri-*O*-acetyl-α-L-fucopyranose (16).

3.2.2.1. Tetra-*O*-acetyl-α-L-fucopyranose (15). Acetylation of L-(−)-fucose (5.00 g, 30.5 mmol) was carried out with a mixture of Ac_2O (40 mL) and dry pyridine (50 mL), which was stirred for 2 days at 0 °C. The mixture was concentrated, the residue was extracted with $CHCl_3$, and the extract was washed sequentially with ice-cold aq $NaHCO_3$ and water. The extract was dried ($CaCl_2$) and concentrated to give a colorless syrup (10.6 g) (R_f 0.67 (3:2 hexane–acetone)). Crystallization from EtOH gave **15** as colorless prisms: yield 7.28 g (71.6%); mp 95–96 °C; $[\alpha]_D^{27}$ −120 (*c* 1.0, $CHCl_3$), lit.[33] mp 92–93 °C; $[\alpha]_D^{20}$ −113 (*c* 1.5, $CHCl_3$), lit.[34] mp 90–91 °C; ^{1}H NMR ($CDCl_3$): δ 6.34 (d, $J_{1,2} = 3.2$ Hz, H-1), 5.36–5.30 (m, 3H, H-2, 3, 4), 4.27 (q, H-5), 2.18, 2.14, 2.01, 2.00 (s, 3H, CH_3CO), 1.16 (d, H-6). The ^{1}H NMR results were identical to those in the literature.[34]

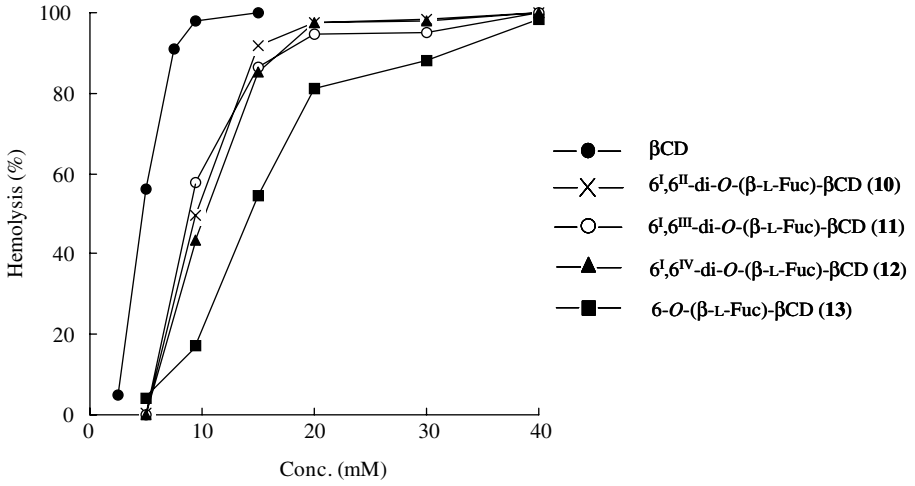


Figure 3. Hemolytic effects of $6^{I},6^{n}$-di-*O*-(β-L-fucopyranosyl)-βCD (n = II–IV) (**10**–**12**), 6-*O*-(β-L-fucopyranosyl)-βCD (**13**), and βCD on human erythrocytes in 60 mM isotonic phosphate buffer saline (pH 7.4).

Table 2. The association equilibrium constant (K_A), association rate constant (k_a), and dissociation rate constant (k_d) of $6^{I},6^{n}$-di-*O*-(β-L-Fuc)-βCD (n = II–IV) (**10**–**12**) and 6-*O*-(β-L-Fuc)-βCD (**13**) with immobilized AAL

Compounds	Kinetic parameter		
	k_a (M^{-1} s^{-1})	k_d (10^{-3} s^{-1})	K_A (M^{-1})
$6^{I},6^{II}$-di-*O*-(β-L-Fuc)-βCD (**10**)	19.79 ± 0.87	4.11 ± 0.20	4820 ± 0.87
$6^{I},6^{III}$-di-*O*-(β-L-Fuc)-βCD (**11**)	4.21 ± 0.06	0.62 ± 0.29	6780 ± 0.06
$6^{I},6^{IV}$-di-*O*-(β-L-Fuc)-βCD (**12**)	4.94 ± 0.15	0.40 ± 0.50	12250 ± 0.15
6-*O*-(β-L-Fuc)-βCD (**13**)	2.43 ± 0.14	1.04 ± 0.45	2330 ± 0.14

^{13}C NMR ($CDCl_3$): δ 90.03 (C-1), 70.67 (C-4), 67.90 (C-3), 67.32 (C-2), 66.57 (C-5), 20.89, 20.65, 20.58, 20.54 ($CH_3C{=}O$), 15.94 (C-6). Anal. Calcd for $C_{14}H_{20}O_9$: C, 50.60; H, 6.07. Found: C, 50.23; H, 6.22.

3.2.2.2. 2,3,4-Tri-*O*-acetyl-α-L-fucopyranose (16). Compound **15** (5.71 g, 17.2 mmol) was stirred with hydrazine acetate (2.38 g, 25.8 mmol) in dry DMF (20 mL) for 5 h at 14 °C. The mixture was then diluted with EtOAc and washed with aq NaCl and ice-water, and the organic layer was dried with $MgSO_4$ and concentrated to give 2,3,4-tri-*O*-acetyl-L-fucopyranose as a syrup (3.0 g, 60%). Crystallization from $(C_2H_5)_2O$ gave pure **16**. Yield 1.2 g (40%); R_f 0.43 (3:2 hexane–acetone); mp 124–125 °C, lit.[35] mp 117 °C, lit.[36] mp 102–103 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.45 (t, H-2), 5.41 (dd, H-4), 5.31 (d, $J_{1,2}$ = 2.3 Hz, H-1), 5.14 (dd, H-3), 4.42 (q, H-5), 3.68 (d, OH), 2.17, 2.09, 1.99 ($CH_3C{=}O$), 1.14 (d, H-6). ^{13}C NMR (125 MHz, $CDCl_3$): δ 90.61 (C-1), 71.40 (C-4), 68.61 (C-3), 67.86 (C-2), 64.39 (C-5), 20.83, 20.68, 20.64 ($CH_3C{=}O$), 15.96 (C-6). The ^{1}H NMR (100 MHz) and ^{13}C NMR (15.08 MHz) spectral data were almost an exact match of the previously reported data.[36] Anal. Calcd for $C_{12}H_{18}O_8$: C, 49.65; H, 6.25. Found: C, 49.43; H; 6.41.

3.2.2.3. 2,3,4-Tri-*O*-acetyl-L-fucopyranosyl trichloroacetimidate (14). To a mixture of **16** (2.90 g, 10.0 mmol) with dry 4 Å molecular sieves (MS) (4 g) in dry CH_2Cl_2 (27 mL) was added 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) (300 μL, 2.0 mmol) and trichloroacetonitrile (10 mL, 0.1 mol), and the mixture was stirred for 1 h at 0 °C. The reaction mixture was diluted with $CHCl_3$, washed with ice-water, and then dried ($CaCl_2$) and concentrated. Centrifugal chromatography of the residue (3:1 → 1:1, hexane–acetone) gave **14** (3.9 g, 89.6%).[25] R_f 0.57 (3:2 hexane–acetone).

3.2.3. $6^{I},6^{n}$-Di-*O*-(β-L-fucopyranosyl)-cyclomaltoheptaose (n = II–IV) (10–12) and 6-*O*-(β-L-fucopyranosyl)-cyclomaltoheptaose (13). A mixture of **4**, **5**, or **6**, with **14**, along with dry powder MS (4 g), in dry CH_2Cl_2 (20–30 mL) was stirred under argon at −20 °C, and a soln of TMSOTf in dry CH_2Cl_2 (2 mL) was added. After stirring for 1 h at −20 °C, triethylamine (1–1.5 mL) was added to the mixture, which was diluted with $CHCl_3$, filtered through Celite, washed sequentially with 1 M H_2SO_4, aq $NaHCO_3$ and water, and then dried and concentrated. The residue was fractionated by centrifugal chromatography with 2:1 → 3:2 → 1:1 hexane–acetone to give fractions containing **7** and mono-fucosylated

Table 3. Fucosylation conditions and products

Fucosyl acceptor		Fucosyl donor **14**	Catalyst (TMSOTf)	Product					
Compound	g (mmol)	g (mmol)	μL (mmol)	Compound	g	Compound	mg	Compound	mg
4	1.7 (0.93)	3.89 (8.96)	168 (0.93)	**7** + **X**	1.97	**10**	496	**13**	119
5	1.4 (0.72)	2.13 (4.90)	135 (0.75)	**8** + **X**	1.78	**11**	340	**13**	152
6	1.4 (0.72)	2.63 (6.05)	135 (0.75)	**9** + **X**	1.50	**12**	416	**13**	90

X = Mono-fucosylated βCD derivative.

βCD derivative **X**, **8** and **X**, or **9** and **X**. The experimental conditions and the yields of the products are summarized in Table 3. Each fraction was individually treated with methanolic 0.5 M CH_3ONa (15.0–19.5 mL) for 1 h at rt, neutralized with Amberlite IR-120B (H^+) resin, filtered, and concentrated. The fractions were a mixture of 6^I,6^n-di-O-(L-Fuc)-βCD (n = II–IV) (**10**–**12**) and 6-*O*-(L-Fuc)-βCD (**13**). Each mixture was isolated by HPLC on a column of TSK-gel Amide-80 with 3:2 MeCN, giving mixtures of **10** (496 mg) and **13** (119 mg), **11** (340 mg) and **13** (152 mg), or **12** (416 mg) and **13** (90 mg). Analysis of the three positional isomers **10**–**12** and **13** by HPLC on a DAISOPAK SP-120-5-ODS-BP column with 9:41 MeOH–water showed a single peak for each. Based on these results and the NMR spectral data, compounds **10**–**13** were identified as 6^I,6^n-di-*O*-(β-L-Fuc)-βCD (n = II–IV) (**10**–**12**) and 6-*O*-(β-L-Fuc)-βCD (**13**), respectively: Compound **10**, $[\alpha]_D^{25}$ +131.14 (*c* 1.23, water); ^{1}H NMR (500 MHz, D_2O): δ 4.4013 and 4.3926 (d, 2H, $J_{1,2}$ = 7.69 and 7.83 Hz, F-1); HRFABMS Calcd for $C_{54}H_{91}O_{43}$ (M+H): 1427.4856. Found: 1427.5012. Compound **11**, $[\alpha]_D^{25}$ +130.11 (*c* 1.06, water); ^{1}H NMR (500 MHz, D_2O): δ 4.4061 and 4.4023 (d, 2H, $J_{1,2}$ 7.83 Hz, F-1); HRFABMS Calcd for $C_{54}H_{91}O_{43}$ (M+H): 1427.4856. Found: 1427.4922. Compound **12**, $[\alpha]_D^{25}$ +134.07 (*c* 1.10, water); ^{1}H NMR (500 MHz, D_2O): δ 4.3976 (d, 2H, $J_{1,2}$ = 7.83 Hz, F-1); HRFABMS Calcd for $C_{54}H_{91}O_{43}$ (M+H): 1427.4856. Found: 1427.4995. Compound **13**, $[\alpha]_D^{25}$ +145.38 (*c* 1.10, water); ^{1}H NMR (500 MHz, D_2O): δ 4.3920 (d, 1H, $J_{1,2}$ = 7.83 Hz, F-1); HRFABMS Calcd for $C_{48}H_{81}O_{39}$ (M+H): 1280.4277. Found: 1281.4359.

3.3. Solubility of L-fucosyl-βCDs 10–13

Water was carefully added in 10–100 μL portions to a glass vessel containing 100 mg of a dried sample of L-Fuc-βCD **10**, **11**, **12** or **13**, and the volumes of solvent required for complete dissolution of the L-Fuc-βCD within 30 min at 25 °C and 50 °C were measured, with vigorous shaking for 30 s periods at 5 min intervals.

3.4. Determination of hemolytic activity

A 0.1% (v/v) human erythrocyte suspension (0.4 mL) in 60 mM isotonic phosphate buffer saline (pH 7.4, PBS) was added to 0.4 mL of PBS containing various concentrations of CDs. The mixture was incubated at 37 °C for 30 min and centrifuged at 1300*g* for 10 min. The percentage hemolysis was expressed in terms of the ratio of the absorbance at 541 nm of hemoglobin released from erythrocytes by CDs to the absorbance after complete hemolysis of erythrocytes in water.

3.5. Immobilization of AAL on optical biosensor cuvette

Immobilization of AAL onto the activated carboxylate surface was performed according to the manufacturer's specifications. Briefly, after equilibration and obtainment of a stable baseline with phosphate buffer saline (PBS, 10 mM KH_2PO_4/Na_2HPO_4, 0.15 M NaCl, pH 7.2), the optical biosensor cuvette coated with carboxylate was activated with a mixture of 400 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and 100 mM *N*-hydroxysuccinimide for 7 min. The activation soln was removed by washing with PBS, and 50 μL of AAL soln (1 mg/mL, in 5 mM maleate buffer, pH 6.0) was added. After 30 min, the remaining active sites were blocked with BSA soln (2 mg/mL in PBS). A baseline was established with PBS.

3.6. Interactions of L-fucosyl-βCDs with immobilized AAL

All binding experiments using IAsys instruments were carried out using 10 mM PBS at 25 °C. Binding of the ligand onto the cuvette was monitored using 50 μL of the sample soln. The association was ended by aspiration of the sample and replacement with the same volume of PBS. Monitoring was continued for a couple of minutes.

Acknowledgments

This study was partly supported by a Grant-in-Aid for Scientific Research (14572103) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and also by a grant from the University-Industry Joint Research Project for Private Universities: matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology) 2004–2009.

The authors thank Dr. Y. Okada for leading the techniques and helpful discussions on solubility and hemolytic assay.

References

1. Morell, A. G.; Irvine, R. A.; Sternlieb, I.; Scheinberg, I. H.; Ashwell, G. *J. Biol. Chem.* **1968**, *243*, 155–159.
2. Ashwell, G.; Morell, A. G. *Adv. Enzymol.* **1974**, *41*, 99–128.
3. Ikuta, A.; Mizuta, N.; Kitahata, S.; Murata, T.; Usui, T.; Koizumi, K.; Tanimoto, T. *Chem. Pharm. Bull.* **2004**, *52*, 51–56.
4. Ikuta, A.; Koizumi, K.; Tanimoto, T. *J. Carbohydr. Chem.* **2003**, *22*, 297–308.
5. Tanimoto, T.; Kishimoto, Y.; Ikuta, A.; Nishi, Y.; Miyake, K. *J. Inclusion Phenom. Macrocyclic Chem.* **2002**, *44*, 297–302.
6. Ikuta, A.; Koizumi, K.; Tanimoto, T. *J. Carbohydr. Chem.* **2000**, *19*, 13–23.
7. Tanimoto, T.; Sakaki, T.; Koizumi, K. *Carbohydr. Res.* **1995**, *267*, 27–37.
8. Tanimoto, T.; Omatsu, M.; Ikuta, A.; Nishi, Y.; Murakami, H.; Nakano, H.; Kitahata, S. *Biosci. Biotechnol. Biochem.* **2005**, *69*, 732–739.
9. Kitahata, S.; Tanimoto, T.; Okada, Y.; Ikuta, A.; Tanaka, K.; Murakami, H.; Nakano, H.; Koizumi, K. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 2406–2411.
10. Kitahata, S.; Tanimoto, T.; Ikuta, A.; Tanaka, K.; Fujita, K.; Hashimoto, H.; Murakami, H.; Nakano, H.; Koizumi, K. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 1223–1229.
11. Hamayasu, K.; Hara, K.; Fujita, K.; Kondo, Y.; Hashimoto, H.; Tanimoto, T.; Koizumi, K.; Nakano, H.; Kitahata, S. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 825–829.
12. Koizumi, K.; Tanimoto, T.; Okada, Y.; Hara, K.; Fujita, K.; Hashimoto, H.; Kitahata, S. *Carbohydr. Res.* **1995**, *278*, 129–142.
13. French, D.; Levine, M. L.; Pazur, J. H.; Norberg, E. *J. Am. Chem. Soc.* **1949**, *71*, 353–356.
14. Okada, Y.; Matsuda, K.; Hara, K.; Hamayasu, K.; Hashimoto, H.; Koizumi, K. *Chem. Pharm. Bull.* **1999**, *47*, 1564–1568.
15. Hakomori, S.-I. *Ann. Rev. Biochem.* **1981**, *50*, 733–764.
16. Yamashita, K.; Kochibe, N.; Ohkura, T.; Ueda, I.; Kobata, A. *J. Biol. Chem.* **1985**, *260*, 4688–4693.
17. Debray, H.; Montreuil, J. *Carbohydr. Res.* **1989**, *185*, 15–26.
18. Fujihashi, M.; Peapus, D. H.; Kamiya, N.; Nagata, Y.; Miki, K. *Biochemistry* **2003**, *42*, 11093–11099.
19. Wimmerova, M.; Mitchell, E.; Sanchez, J.-F.; Gautier, C.; Imberty, A. *J. Biol. Chem.* **2003**, *278*, 27059–27067.
20. Cush, R.; Cronin, J. M.; Stewart, W. J.; Maule, C. H.; Molloy, J.; Goddard, N. J. *Biosens. Bioelectron.* **1993**, *8*, 347–354.
21. Tanimoto, T.; Tanaka, M.; Yuno, T.; Koizumi, K. *Carbohydr. Res.* **1992**, *223*, 1–10.
22. Tanimoto, T.; Ikuta, A.; Koizumi, K. *J. Carbohydr. Chem.* **1997**, *16*, 1445–1455.
23. Nakanishi, N.; Tsujikawa, J.; Hara, N.; Tanimoto, T.; Koizumi, K. *Yakugaku Zasshi* **1990**, *110*, 477–483.
24. Koizumi, K.; Okada, Y.; Nakanishi, N.; Tanimoto, T.; Takagi, Y.; Ishikawa, M.; Ishigami, H.; Hara, K.; Hashimoto, H. *J. Carbohydr. Chem.* **1991**, *10*, 657–670.
25. Schmidt, R. R.; Wegmann, B.; Jung, K.-H. *Liebigs Ann. Chem.* **1991**, 121–124.
26. Verhaar, L. A.; Kuster, B. F. *J. Chromatogr.* **1981**, *220*, 313–328.
27. Horvath, Cs.; Melander, W.; Molnar, I. *J. Chromatogr.* **1976**, *125*, 129–156.
28. Doddrell, D. M.; Pegg, D. T. *J. Am. Chem. Soc.* **1980**, *102*, 6388–6390.
29. Irie, T.; Otagiri, M.; Sunada, M.; Uekama, K.; Ohtani, Y.; Yamada, Y.; Sugiyama, Y. *J. Pharm. Dyn.* **1982**, *5*, 741–744.
30. Okada, Y.; Koizumi, K. *Chem. Pharm. Bull.* **1998**, *46*, 319–323.
31. Fagerstam, L. G.; Frostell-Karlsson, Å.; Karlsson, R.; Persson, B.; Rönnberg, I. *J. Chromatogr.* **1992**, *597*, 397–410.
32. Koizumi, K.; Tanimoto, T.; Fujita, K.; Hara, K.; Kuwahara, N.; Kitahata, S. *Carbohydr. Res.* **1993**, *238*, 75–91.
33. Levvy, G. A.; McAllen, A. *Biochem. J.* **1961**, *80*, 433–435.
34. Nguyen, T. T. M.; Gross, P. H.; Franz, A. H. *ARKIVOC* **2005**, 110–128.
35. Prihar, H. S.; Behrman, E. J. *Biochemistry* **1973**, *12*, 997–1002.
36. Nunez, H. A.; O'Connor, J. V.; Rosevear, P. R.; Barker, R. *Can. J. Chem.* **1981**, *59*, 2086–2095.