Syntheses of Model Compounds Related to an Antigenic Epitope in Pectic Polysaccharides from *Bupleurum falcatum* L. (II)

Yuhua JIN,[a] Noriyasu HADA,[a] Junko OKA,[a] Osamu KANIE,[b] Shusaku DAIKOKU,[b] Yoshimi KANIE,[b] Haruki YAMADA,[c] and Tadahiro TAKEDA*,[a]

[a] *Kyoritsu University of Pharmacy; 1–5–30 Shibakoen, Minato-ku, Tokyo 105–8512, Japan:* [b] *Mitsubishi Kagaku Institute of Life Sciences (MITILS); 11 Minamiooya, Machida, Tokyo 194–8511, Japan: and* [c] *Oriental Medicine Research Center, Kitasato Institute; 5–9–1 Shiroganedai, Minato-ku, Tokyo 108–8642, Japan.*

Received October 12, 2005; accepted December 28, 2005

Stereocontrolled syntheses of model compounds related to a major antigenic epitope against antibupleurum 2IIc/PG-1-IgG from antiulcer pectic polysaccharide are described. A trisaccharide derivative (13) was prepared as a precursor and a novel and simple approach for the rational design of a glycocluster and glycodendrimer was developed, through the syntheses of the fluorescence-labeled glycocluster (2) and glycodendrimer (3).

Key words *Bupleurum falcatum*; glycocluster; glycodendrimer; β-alanine derivative; chemical synthesis

The roots of *Bupleurum falcatum* L. (Japanese name *Saiko*) have been used in Chinese and Japanese herbal medicines for the treatment of chronic hepatitis, nephrosis syndrome, and autoimmune diseases. Yamada *et al.*[1] and Sun *et al.*[2] reported that a potent antiulcer pectic polysaccharide (Bupleuran 2IIc) was isolated from the hot-water extract of the roots. Bupleuran 2IIc consists of a galacturonan region, a "ramified" region (PG-1) composed of a rhamnogalacturonan core with neutral sugar side chains, and a rhamnogalacturonan II-like region[3]; the ramified region has been considered important for the expression of immunopharmacologic activity (Fig. 1). We reported in our previous paper[4] that the synthetic model compound shows specific activity. On the other hand, a polyclonal antibody (antibupleuran 2IIc/PG-1-IgG) against the ramified region of bupleuran 2IIc (antibupleuran 2IIc/PG-1-IgG) was prepared, and the antigenic epitopes were characterized to be 6-linked galactosyl chains with either GlcA or 4-*O*-Me-GlcA as a nonreducing terminal.[5] Bupleuran 2IIc has mitogenic activity in the murine spleen and Peyer's patch cells, and the mitogenic activity was reduced in the presence of the antipolysaccharide antibody (antibupleuran 2IIc/PG-1-IgG). The mitogenic activity of bupleuran 2IIc was reduced with the addition of β-D-GlcA*p*-(1→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p* or β-D-GlcA*p*-(1→6)-β-D-Gal*p*, which are a part of the epitopes of antibupleuran 2IIc/PG-1-IgG.[6] The proposed structure of the antigenic epitopes in PG-1 has been a target for the synthetic studies in our laboratory. Despite the specificity of the binding, it is known that polysaccharide chains generally interact with their protein receptors as a natural cluster. This explains why the binding affinity of a synthetic model compound to the active site is low in various cases.[7] Construction of a glycocluster aimed at their bioactive augmentation is an important problem in glycoscience.[8] For this reason, we synthesized trivalent analogue mono and trivalent analogues of β-D-GlcA4Me-(1→6)-β-D-Gal- and β-D-GlcA4Me-(1→6)-β-D-Gal-(1→6)-β-D-Gal- in the hope of achieving a cluster effect.[9] However, it did not led to a marked augmentation (data not shown). Meanwhile, we developed new peptidic glycoclusters and a glycodendron, which consist of a β-alanine derivative and

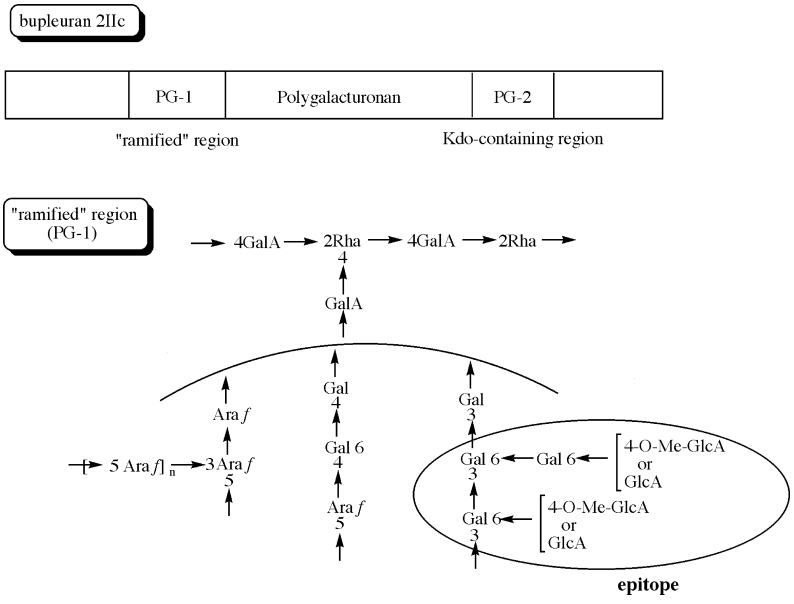


Fig. 1. Structural Model of Bupleuran 2IIc and Its "Ramified" Region (PG-1)

* To whom correspondence should be addressed. e-mail: takeda-td@kyoritsu-ph.ac.jp

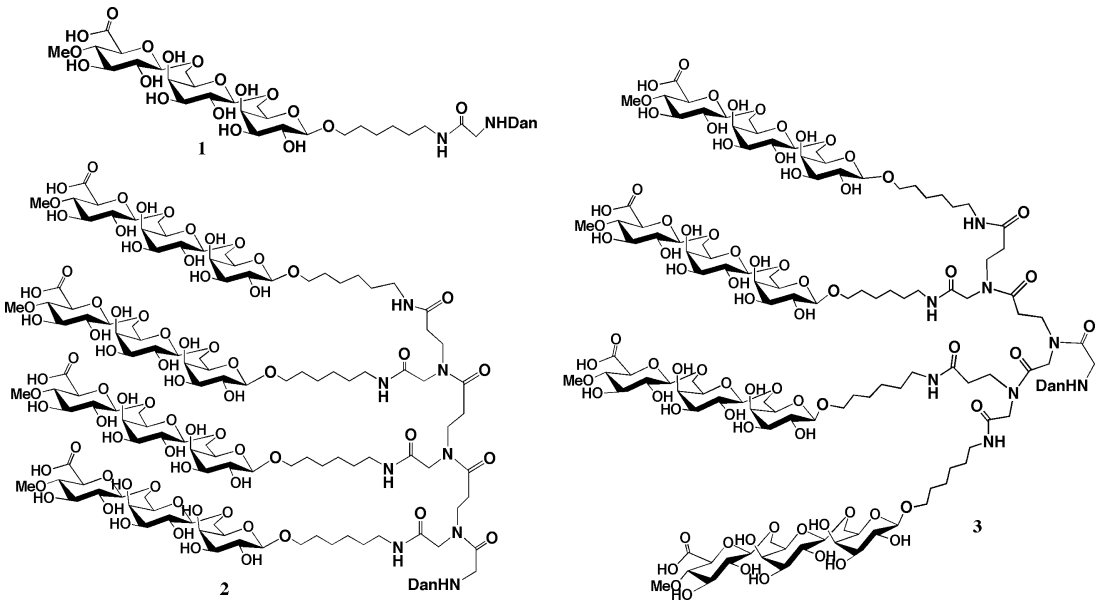
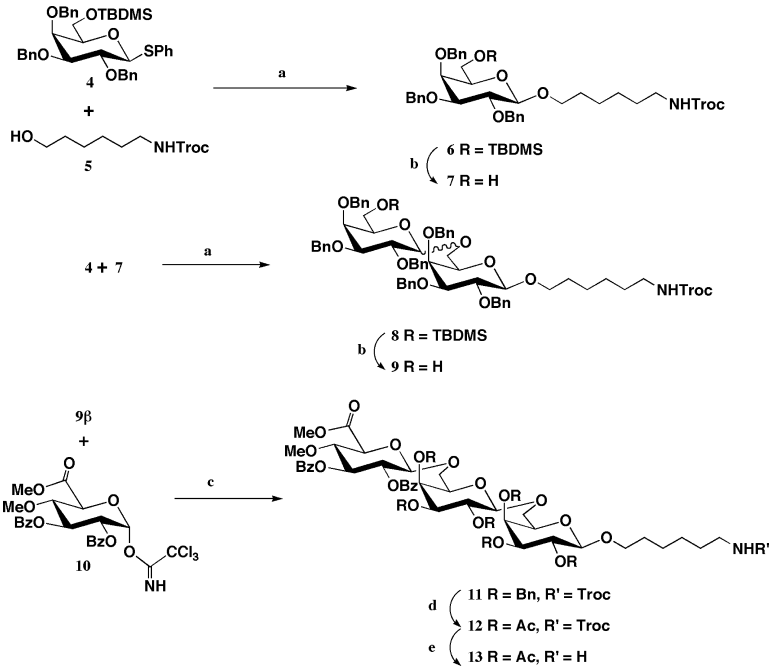


Fig. 2. Structure of Synthetic Glycocluster and Glycodendron



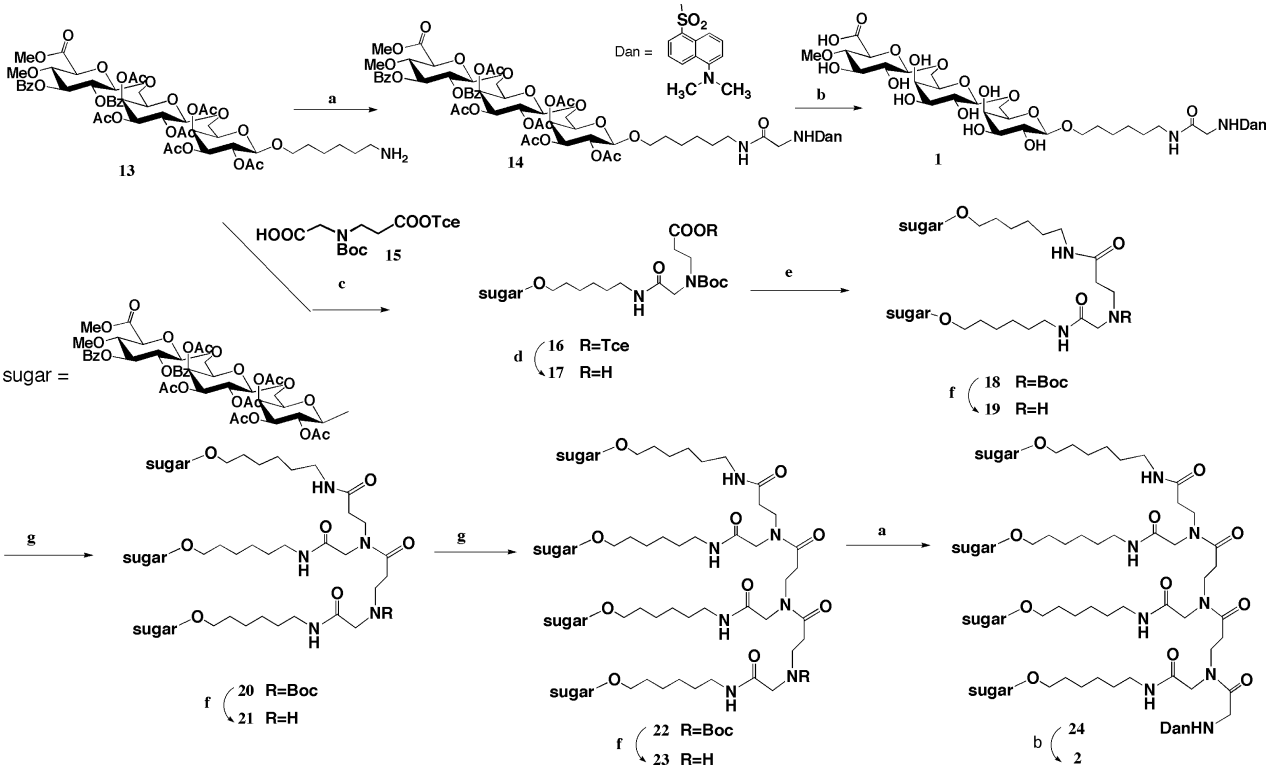
Reagents: (a) NIS, TfOH, EtCN, MSAW-300; (b) TsOH, $CHCl_3$–MeOH (2 : 1); (c) TMSOTf, MS4A, CH_2Cl_2; (d) i) H_2, Pd–C, MeOH–THF (2 : 1), ii) Ac_2O, Pyr.; (e) Zn–AcOH

Chart 1

sugar unit.[10—12] We report here the two types of cluster, **2** and **3**, carrying trisaccharide **1** (Fig. 2) and our attempts to achieve successful augmentation through the cluster effect.

Result and Discussion

Synthesis of Monovalent Trisaccharide Preparation of the designed trisaccharide derivative **1** was straightforward (Chart 1). Monosaccharide derivative **6** was obtained by condensation of phenyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside (**4**), which was prepared by silylation and benzylation of phenyl-1-thio-β-D-galactopyranoside,[13] with the spacer **5** in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) in propionitrile (EtCN).[14,15] Stereochemical control was achieved by the solvent effect of nitrile[16] to give the desired β-glycoside **6** in 81% yield, and the α-glycoside was not detected. The anomeric hydrogen atom of the galactose unit appeared as a signal at δ 4.29 (d, J=7.9 Hz). Removal of the *tert*-butyldimethylsilyl (TBDMS) group was achieved by the treatment of **6** with TsOH, giving the monosaccharide intermediate **7** quantitatively. The coupling reaction of **7** with **4** was carried out as described for the synthesis of **6** and gave compound **8**. Compound **8** was formed as a mixture of anomers (70%) but could not be purified by silica gel column



Reagents: (a) dansyl glycine, DEPC, Et_3N, DMF; (b) NaOMe, MeOH-1,4-dioxane; (c) **15**, DEPC, Et_3N, DMF; (d) Zn–AcOH; (e) **13**, DEPC, Et_3N, DMF; (f) 50% TFA; (g) **17**, DEPC, Et_3N, DMF

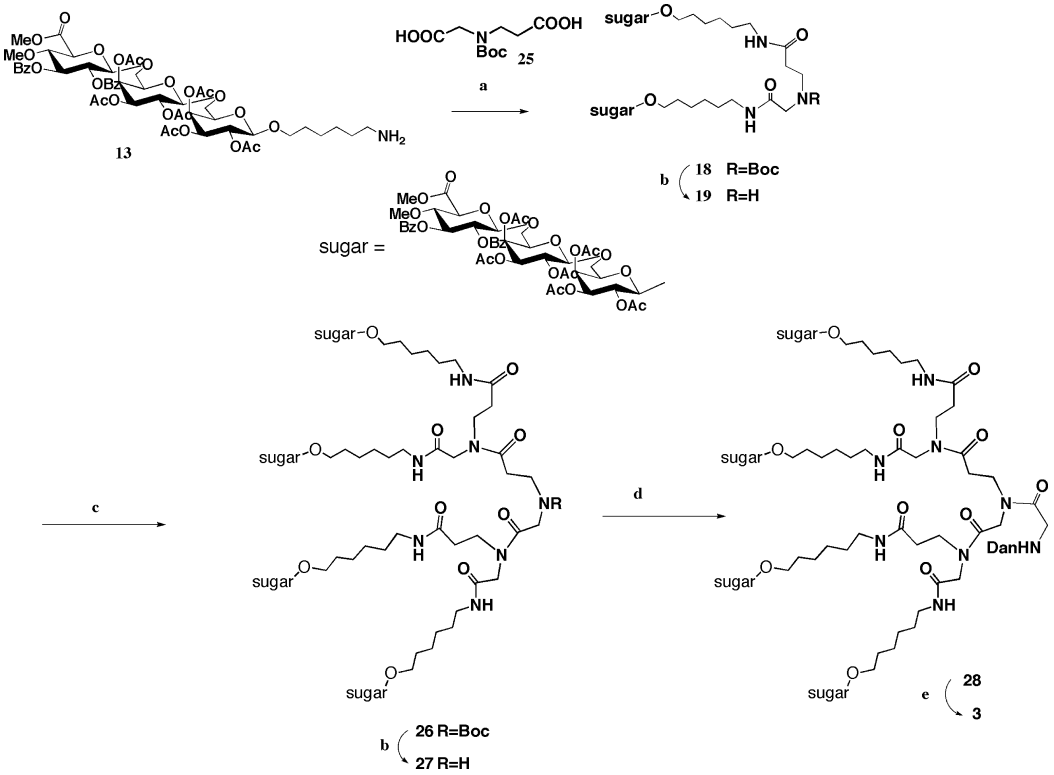
Chart 2

chromatography. The structure of **8** was confirmed after removal of the TBDMS group (**9**, 75%; $\alpha:\beta=1:7$). The glycosylation of the acceptor **9**$\boldsymbol{\beta}$ with the donor **10**[9] was accomplished using trimethylsilyl triflate (TMSOTf) and 4A MS in dichloromethane for 1 h at 0 °C, yielding the desired disaccharide **11** (91%), as evidenced by ^{1}H-NMR spectroscopy (H-1″, 4.72 ppm, $J=7.9$ Hz). Removal of the benzyl groups from **11** by catalytic hydrogenolysis over 10% Pd–C in THF–MeOH and subsequent acetylation gave compound **12** (72%). Selective removal of the Troc group from **12** with Zn–AcOH gave the primary amine **13** (77%) (Chart 1). Compound **13** was condensed with dansyl glycine in the presence of DEPC to give **14** (68%). The removal of all acyl groups and esters with sodium methoxide afforded the monovalent trisaccharide **1** in 83% yield (Chart 2).

Synthesis of a Glycocluster We first synthesized the conventional unit **16** from **15** and **13** to simplify the process. The β-alanine derivative **15** was prepared according to the previously reported method.[10] Elongation of the glycocluster was achieved by the iterative reactions of 1) peptide coupling, 2) deprotection of the *t*-butoxycarbonyl (Boc) group, and 3) deprotection of the trichloroethyl ester (Tce) group. Coupling of unit **15** with the sugar unit **13** in the presence of diethyl phosphorocyanidate (DEPC) in dry DMF gave the glycocluster unit **16** in 92% yield. Subsequent removal of the Tce group with Zn–AcOH afforded **17** (80%). Coupling of **17** with **13** gave the dimer derivative **18** in 89% yield. The Boc group of **18** was removed under acidic conditions with 50% TFA, giving compound **19** (88%), which was subsequently subjected to the next cycle of elongation to give the desired tetramer glycocluster derivative **23** (66%). Finally, dansyl glycine was introduced into tetramer **23** in the presence of DEPC. Complete removal of the *O*-acyl groups and esters provided the target compound **2** in 94% yield (Chart 2).

Synthesis of a Glycodendrimer We chose the convergent approach using the bifunctional linker **25**[11] as the dendron core. In this approach, a coupling reaction of **13** with dendron core **25** was carried out in the presence of DEPC to give **18** in 94% yield, which after removal of the Boc group gave dimer **19** (87%). Coupling of two equivalents of **19** with **25** under the same conditions gave tetramer **26** in 86% yield. The Boc group of **26** was removed to give amine **27** (76%), which was then treated with dansyl glycine in the presence of DEPC to give **28** (73%). Finally, complete deacylation and the hydrolysis of methyl esters afforded the target compound **3** in 80% yield (Chart 3).

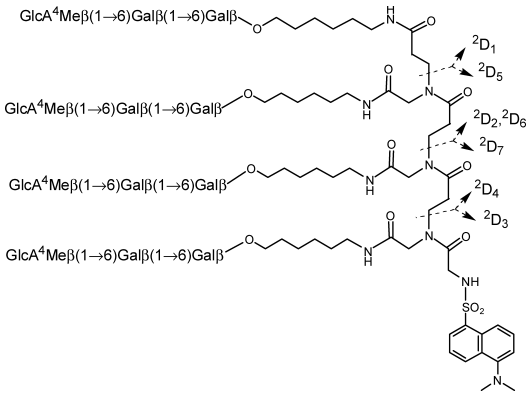
Structural Analysis of 2 and 3 The synthesized compounds **2** (glycocluster) and **3** (glycodendrimer) have the same molecular formula with Mw of 3148.24 but differ in the arrangement of the peptoide scaffolds. Collision-induced dissociation (CID) experiments with **2** and **3** gave distinct spectra showing characteristic daughter ions. The following is a summary of CID MS/MS data obtained in the negative mode. Both parent ions (^{2}P and ^{3}P) were observed as $[M-4H^+ + Na^+]^{3-}$ ($m/z=1055.7$) (Tables 1, 2). One of the daughter ions, $^2D_4$ ($m/z=1094.9$), observed for compound **2** consists of three trisaccharide units, which is not present in compound **3**, and thus it clearly explains the structure of **2**. Also, the ion was found to be a major peak in the spectrum. Other ions listed in the tables support both of the individual structures. Additionally, interesting information was obtained in the experiments. Nitrogen atoms constituting dialkylated amides are present in compounds **2** and **3** where one of the



Reagents: (a) **25**, DEPC, Et_3N, DMF; (b) 50% TFA; (c) **25**, DEPC, Et_3N, DMF; (d) dansyl glycine, DEPC, Et_3N, DMF; (e) NaOMe, MeOH-1,4–dioxane

Chart 3

Table 1. Structural Analysis of Compound **2** by CID MS/MS

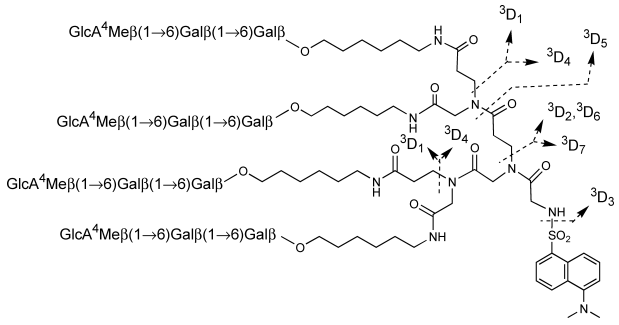


	Ionic structure	Fomula	Exact mass	*m*/*z* (calculated)	*m*/*z* (observed)
^2P	$[M(\mathbf{2})-4H^+ +Na^+]^{3-}$	$[C_{129}H_{205}N_9NaO_{77}S]^{3-}$	3167.2	1055.7	1055.8
$^2D_1$	$[F_1-H^+]^-$	$[C_{28}H_{46}NO_{18}]^-$	684.3	684.3	684.3
$^2D_2$	$[F_2-2H^+]^{2-}$	$[C_{58}H_{95}N_3O_{37}]^{2-}$	1425.6	712.8	712.7
$^2D_3$	$[F_3-H^+]^-$	$[C_{41}H_{61}N_4O_{21}S]^-$	977.4	977.4	977.4
$^2D_4$	$[F_4-3H^+ +Na^+]^{2-}$	$[C_{88}H_{144}N_5NaO_{56}]^{2-}$	2189.8	1094.9	1094.9
$^2D_5$	$[F_5-3H^+ +Na^+]^{2-}$	$[C_{101}H_{157}N_8NaO_{59}S]^{2-}$	2482.4	1241.2	1241.3
$^2D_6$	$[F_2-2H^+ +Na^+]^-$	$[C_{58}H_{95}N_3NaO_{37}]^-$	1448.6	1448.6	1448.6
$^2D_7$	$[F_6-2H^+ +Na^+]^-$	$[C_{71}H_{110}N_6NaO_{40}S]^-$	1741.6	1741.6	1741.8

^2P, parent ion; ^2D_n, daughter ion of a fragment (F_n) from **2**. $^2D_1$ and $^2D_5$ share a fragment structure but differ in the state of acid moieties.

alkyl groups corresponds to a substituted 3-carbonylethyl group and the other corresponds to a substituted 2-carbonylmethyl group. The former linkage tends to cleave under given CID conditions in both **2** and **3**, whereas no cleavage was observed for the latter. Furthermore, when the samples were ionized from solutions containing formic acid, positively charged ion species were obtained. The CID MS/MS of such ions resulted in preferential cleavages of the glycosidic linkages rather than the peptoide linkages (data not shown). These results indicate that the bond energies associated with a given ion depend very heavily on the charge state of the ion. Based on the CID MS/MS results of compounds **2**

Table 2. Structural Analysis of Compound **3** by CID MS/MS



	Ionic structure	Fomula	Exact mass	*m/z* (calculated)	*m/z* (observed)
^{3}P	$[M(\mathbf{3})-4H^+ + Na^+]^{3-}$	$[C_{129}H_{205}N_9NaO_{77}S]^{3-}$	3167.2	1055.7	1055.7
$^3D_1$	$[F_1-H^+]^-$	$[C_{28}H_{46}NO_{18}]^-$	684.3	684.3	684.1
$^3D_2$	$[F_2-2H^+]^{2-}$	$[C_{58}H_{95}N_3O_{37}]^{2-}$	1425.6	712.8	712.7
$^3D_3$	$[F_3-4H^+ + Na^+]^{3-}$	$[C_{117}H_{192}N_8NaO_{75}]^{3-}$	2932.1	977.4	977.6
$^3D_4$	$[F_4-3H^+ + Na^+]^{2-}$	$[C_{101}H_{157}N_8NaO_{59}S]^{2-}$	2482.4	1241.2	1241.2
$^3D_5$	$[F_5-2H^+ + Na^+]^-$	$[C_{55}H_{93}N_3NaO_{36}]^-$	1394.5	1394.5	1394.2
$^3D_6$	$[F_2-2H^+ + Na^+]^-$	$[C_{58}H_{95}N_3NaO_{37}]^-$	1448.6	1448.6	1448.3
$^3D_7$	$[F_6-2H^+ + Na^+]^-$	$[C_{71}H_{110}N_6NaO_{40}S]^-$	1741.6	1741.6	1741.2

^{3}P, parent ion; ^3D_n, daughter ion of a fragment (F_n) from **3**. $^3D_2$ and $^3D_5$ share a fragment structure but differ in the state of acid moieties.

and **3**, these cluster compounds are clearly a linear cluster and a dendrimer.

In conclusion, efficient synthetic strategies in glycoconjugate chemistry were employed to obtain new glycoclusters. The strategies allow changes in the length and pattern of the core portion of the dendrimer. A variety of oligosaccharides can be adopted in the structure. This method should find a wide range of applications.

Experimental

Optical rotations were determined with a Jasco digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded with a JNM A 500 FT NMR spectrometer with Me_4Si as the internal standard for solutions in $CDCl_3$ or CD_3OD. MALDI-TOF-MS was recorded on a Perceptive Voyager RP mass spectrometer. ESI-QIT mass spectra were obtained using a Bruker Esquire 3000 plus. TLC was performed on silica gel 60-F254 (Merck) with detection by quenching of UV fluorescence and by spraying with 5% ninhydrin and 10% H_2SO_4. Column chromatography was carried out on silica gel 60 (Merck).

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside (4) To a solution of phenyl 6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside (4 g, 10.4 mmol) in DMF (10 ml) was added NaH in oil (2.5 g, 62.2 mmol) and BnBr (7.4 ml, 62.2 mmol). The reaction mixture was stirred for 2 h at 0 °C, and then methanol was added to eliminate excess NaH. The reaction mixture was poured into ice water and extracted with ethyl acetate. The extract was washed with water, dried ($MgSO_4$), and concentrated. The product was purified on silica gel column chromatography (hexane : ethyl acetate=20 : 1) to give **4** (4.9 g, 72%). $[\alpha]_D^{23}$ +6.3° (c=5.0, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.58—7.18 (15H, m, Ar-H), 4.99 (1H, d, H-1), 4.79—4.62 (6H, m, benzylmethylene), 3.97—3.93 (2H, m, H-2, H-4), 3.77—3.71 (2H, m, H-6a, H-6b), 3.61 (1H, dd, H-3), 3.45 (1H, t, H-5). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 138.9, 138.4, 138.3, 134.3, 128.7, 128.4, 128.3, 128.1, 127.7, 127.61, 127.58, 127.3, 126.9, 87.7, 84.2, 78.9, 75.6, 74.4, 73.5, 72.8, 61.5, 18.2. MALDI-TOF-MS: Calcd for $C_{39}H_{48}Cl_5NO_8SSiNa$: *m/z* 679.3 $[M+Na]^+$. Found: *m/z* 679.4 $[M+Na]^+$.

***N*-(2,2,2-Trichloroethoxycarbonyl)hexanolamine (5)** To a solution of 2,2,2-trichloroethylchloroformate (4.7 ml, 0.03 mol) in 5 ml of dioxane was added at 0 °C a mixture of hexanolamine (5 g, 0.04 mol) and MgO (3 g) in dioxane (25 ml) and H_2O (25 ml). The reaction mixture was stirred for 16 h at room temperature. Then, ethylacetate was added, the solids were filtered off and washed with 5% HCl, aqueous $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated. The product was purified on silica gel column chromatography (chloroform : methanol=200 : 1) to give **5** (8 g, 64.3%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.03 (1H, s, NH), 4.72 (2H, s, CH_2CCl_3), 3.65 (2H, t, CH_2OH), 3.24 (2H, dd, $NHCH_2$), 1.61—1.36 (8H, m, CH_2×4). MALDI-TOF-MS: Calcd for $C_9H_{16}Cl_3NO_3Na$: *m/z* 314.0 $[M+Na]^+$. Found: *m/z* 314.3 $[M+Na]^+$.

6-*N*-(2,2,2-Trichloroethoxycarbonyl)aminohexyl 2,3,4-tri-*O*-Benzyl-6-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranoside (6) To a solution of **4** (760 mg, 1.16 mmol) and **5** (315 mg, 1.08 mmol) in EtCN (10 ml) was added MSAW-300 (800 mg), and the mixture was stirred for 2 h and then cooled to −60 °C. NIS (391 mg, 1.74 mmol) and TfOH (5.2 μl, 57.9 μmol) were added to the mixture, which was stirred for 1 h at −60 °C and then neutralized with Et_3N. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings were successively washed with saturated $Na_2S_2O_3$ and water, dried ($MgSO_4$), and concentrated. The product was purified on silica gel column chromatography (hexane : ethyl acetate=8 : 1) to give **6** (736 mg, 81.2%). $[\alpha]_D^{23}$ +4.5° (c=0.7, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.92—4.58 (8H, m, benzyl methylene×3, $COOCH_2CCl_3$), 4.29 (1H, d, J=7.9 Hz, H-1), 3.88, 3.48—3.41 (3H, m, H-3, OCH_2), 3.82 (1H, d, H-4), 3.76 (1H, dd, H-2), 3.65 (2H, m, H-6a, 6b), 3.32 (1H, t, H-5), 3.13 (2H, dd, $NHCH_2$). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 104.0, 95.8, 82.2, 79.7, 75.2, 75.1, 74.6, 74.5, 73.7, 73.4, 73.1, 69.7, 61.7. MALDI-TOF-MS: Calcd for $C_{42}H_{58}Cl_3NO_8SiNa$: *m/z* 860.3 $[M+Na]^+$. Found: *m/z* 860.2 $[M+Na]^+$.

6-*N*-(2,2,2-Trichloroethoxycarbonyl)aminohexyl 2,3,4-tri-*O*-Benzyl-β-D-galactopyranoside (7) To a solution of **6** (1.1 g, 1.31 mmol) in 2 : 1 $CHCl_3$–MeOH (12 ml) was added *p*-toluenesulfonic acid (113 mg). The reaction mixture was stirred for 1 h at room temperature. After completion of the reaction and neutralization with Et_3N, the mixture was concentrated and purified on silica gel column chromatography (toluene : acetone= 10 : 1) to give **7** (983 mg, quantitative). $[\alpha]_D^{23}$ +2.7° (c=1.0, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.97—4.65 (8H, m, benzyl methylene×3, $COOCH_2CCl_3$), 4.35 (1H, d, H-1), 3.91—3.48 (7H, m, H-4, 2, 3, 6a, 6b, OCH_2), 3.37 (1H, t, H-5), 3.17 (2H, dd, $NHCH_2$). MALDI-TOF-MS: Calcd for $C_{36}H_{44}Cl_3NO_8Na$: *m/z* 746.2 $[M+Na]^+$. Found: *m/z* 746.4 $[M+Na]^+$.

6-*N*-(2,2,2-Trichloroethoxycarbonyl)aminohexyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-α/β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (8) To a solution of **4** (156 mg, 0.24 mmol) and **7** (143 mg, 0.20 mmol) in EtCN (1.5 ml) was added MSAW-300 (200 mg), and the mixture was stirred for 2 h and then cooled to −60 °C. NIS (80 mg, 0.36 mmol) and TfOH (2.1 μl, 23.4 mmol) were added to the mixture, which was stirred for 1 h, cooled to −60 °C, and then neutralized with Et_3N. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings were successively washed with saturated $Na_2S_2O_3$ and water, then dried ($MgSO_4$) and concentrated. The product was purified on silica gel column chromatography (hexane : ethyl acetate=5 : 1) to give **8** as a mixture of anomers (177 mg, 70.0%). MALDI-TOF-MS: Calcd for $C_{69}H_{86}Cl_3NO_{13}SiNa$: *m/z* 1292.5 $[M+Na]^+$. Found: *m/z* 1292.7

$[M+Na]^+$.

6-*N*-(2,2,2-Trichloroethoxycarbonyl)aminohexyl 2,3,4-tri-*O*-Benzyl-α/β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (9) To a solution of **8** (234 mg, 0.18 mmol) in 2 : 1 $CHCl_3$–MeOH (3 ml) was added *p*-toluenesulfonic acid (80 mg). The reaction mixture was stirred for 1 h at room temperature. After completion of the reaction and neutralization with Et_3N, the mixture was concentrated and purified on silica gel column chromatography (toluene : acetone=10 : 1) to give **9α** (23 mg, 10.8%) and **9β** (159 mg, 74.7%). **9α**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.98—4.57 (15H, m, benzyl methylene×6, $COOCH_2CCl_3$, H-1′), 4.31 (1H, d, $J_{1,2}$=7.5, H-1), 4.06—3.40 (14H, m, H-2, H-2′, H-3, H-3′, H-4, H-4′, H-5, H-5′, H-6a, H-6b, H-6a′, H-6b′, OCH_2), 3.16 (2H, dd, $NHCH_2$). **9β**: $[\alpha]_D^{23}$ +2.7° (*c*=1.0, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.95—4.63 (14H, m, benzyl methylene×6, $COOCH_2CCl_3$), 4.35, 4.28 (2H, d, d, H-1, H-1′), 3.84—3.45 (12H, m, H-2, H-2′, H-3, H-3′, H-4, H-4′, H-6a, H-6b, H-6a′, H-6b′, OCH_2), 3.35 (2H, m, H-5, 5′), 3.12 (2H, dd, $NHCH_2$). MALDI-TOF-MS: Calcd for $C_{63}H_{72}Cl_3NO_{13}Na$: *m/z* 1178.4 $[M+Na]^+$. Found: *m/z* 1178.7 $[M+Na]^+$.

6-*N*-(2,2,2-Trichloroethoxycarbonyl)aminohexyl [methyl(2,3-di-*O*-benzoyl-4-*O*-methyl-β-D-glucopyranosyl)uronate]-(1→6)-2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (11) To a solution of **9β** (159 mg, 0.14 mmol) and **10**[9] (95 mg, 0.17 mmol) in CH_2Cl_2 (2 ml) was added MS4A (500 mg), and the mixture was stirred for 2 h at 0 °C. TMSOTf (3 μl, 16.6 mmol) was added to the mixture, which was stirred for 1 h at 0 °C and then neutralized with Et_3N. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings were successively washed with water, dried ($MgSO_4$), and concentrated. The product was purified on silica gel column chromatography (toluene : acetone=15 : 1) to give **11** (196 mg, 90.9%). $[\alpha]_D^{23}$ +24.5° (*c*=0.5, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.63 (1H, t, H-3″), 5.35 (3H, m, H-2″), 4.72 (1H, d, *J*=7.9 Hz, H-1″), 4.70 (2H, s, $-COOCH_2Cl_3$), 4.29—4.25 (2H, d, H-1, H-1′), 3.76 (3H, s, $COOCH_3$), 3.40 (3H, s, OCH_3). MALDI-TOF-MS: Calcd for $C_{85}H_{92}Cl_3NO_{21}Na$: *m/z* 1590.5 $[M+Na]^+$. Found: *m/z* 1590.8 $[M+Na]^+$.

6-*N*-(2,2,2-Trichloroethoxycarbonyl)aminohexyl [methyl(2,3-di-*O*-benzoyl-4-*O*-methyl-β-D-glucopyranosyl)uronate]-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (12) A solution of **11** (533 mg, 0.34 mmol) in MeOH (8 ml) and THF (4 ml) was hydrogenated over 10% Pd–C (450 mg) for 2 h at room temperature, filtered through Celite, and the residue was washed with MeOH and concentrated. The residue was acetylated with Ac_2O (4 ml) in pyridine (6 ml) for 3 h at room temperature. The reaction mixture was poured into ice water and extracted with $CHCl_3$. The extract was washed sequentially with 5% HCl, aqueous $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated. The product was purified on silica gel column chromatography (toluene : acetone=5 : 1) to give **12** (311 mg, 71.5%). $[\alpha]_D^{23}$ +3.2° (*c*=0.5, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.60 (1H, t, H-3″), 5.36—5.29 (3H, m, H-2″, H-4, H-4′), 5.19—5.10 (2H, dd, dd, H-2, H-2′), 5.01—4.92 (2H, br dd, H-3, H-3′), 4.77 (1H, d, H-1″), 4.72 (2H, s, $-COOCH_2Cl_3$), 4.44 (2H, d, H-1, H-1′), 4.08 (1H, d, H-5″), 3.97—3.40 (15H, m, H-4″, H-5, H-5′, H-6a, H-6b, H-6a′, H-6b′, $COOCH_3$, OCH_2 of sugar unit, OCH_3), 3.20 (2H, s, NCH_2 of sugar unit). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 101.2, 101.0, 100.7, 78.7, 74.2, 72.1, 72.0, 71.8, 71.1, 70.9, 69.9, 69.1, 68.7, 67.5, 67.40, 67.39, 66.8. MALDI-TOF-MS: Calcd for $C_{55}H_{68}Cl_3NO_{27}Na$: *m/z* 1302.3 $[M+Na]^+$. Found: *m/z* 1302.0 $[M+Na]^+$.

6-Aminohexyl [methyl(2,3-di-*O*-benzoyl-4-*O*-methyl-β-D-glucopyranosyl)uronate]-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (13) To a solution of **12** (292 mg, 0.23 mmol) in acetic acid (6 ml) was added zinc powder (500 mg). The reaction mixture was stirred for 16 h at room temperature. After completion of the reaction (TLC monitoring), the mixture was filtered off and washed with $CHCl_3$. The filtrate was concentrated and purified on silica gel column chromatography (chloroform : methanol=10 : 1) to give **13** (194 mg, 76.9%). $[\alpha]_D^{23}$ −2.8° (*c*=0.2, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.61 (1H, t, H-3″), 5.36—5.30 (3H, m, H-2″, H-4, H-4′), 5.17—5.08 (2H, dd, dd, H-2, H-2′), 5.02—4.93 (2H, br dd, H-3, H-3′), 4.78 (1H, d, H-1″), 4.47—4.45 (2H, d, H-1, H-1′), 4.10 (1H, d, H-5″), 3.96—3.40 (15H, m, H-4″, H-5, H-5′, H-6a, H-6b, H-6a′, H-6b′, $COOCH_3$, OCH_2 of sugar unit, OCH_3), 2.95 (2H, s, NCH_2 of sugar unit). MALDI-TOF-MS: Calcd for $C_{52}H_{67}NO_{25}Na$: *m/z* 1128.4 $[M+Na]^+$. Found: *m/z* 1129.1 $[M+Na]^+$.

Compound 14 To a solution of **13** (13.5 mg, 12.7 μmol) and dansyl glycine (5.7 mg, 18.3 μmol) in DMF (1 ml) were added triethylamine (2.6 μl, 18.3 μmol) and DEPC (1.1 μl, 13.4 μmol). The reaction mixture was stirred for 16 h at room temperature. After completion of the reaction, the mixture was extracted with $CHCl_3$, washed with water, dried ($MgSO_4$), and concentrated. The product was purified on silica gel column chromatography ($CHCl_3$: MeOH=50 : 1) to give the dansyl derivative **14** (11.6 mg, 68.1%). $[\alpha]_D^{23}$ +8.2° (*c*=0.3, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.58—7.20 (16H, m, $C_{10}H_6$ of dansyl glycine, Ar-H), 6.40 (1H, t, NH), 5.73 (1H, t, $NHCH_2CO$ of dansyl glycine), δ 5.60 (1H, t, H-3″), 5.36—5.30 (3H, m, H-2″, H-4, H-4′), 5.20—5.09 (2H, dd, dd, H-2, H-2′), 5.03—4.91 (2H, br dd, H-3, H-3′), 4.77 (1H, d, H-1″), 4.49—4.45 (2H, t, H-1, H-1′), 4.09 (1H, d, H-5″), 3.97—3.38 (17H, m, H-4″, H-5, H-5′, H-6a, H-6b, H-6a′, H-6b′, $COOCH_3$, $NHCH_2CO$ of dansyl glycine, OCH_2 of sugar unit, OCH_3), 3.09 (2H, s, NCH_2 of sugar unit), 2.89 (6H, s, $N(CH_3)_2$ of dansyl glycine), 2.10—1.95 (18H, m, $COOCH_3$×6), 1.66 [8H, s, (CH_2)×4]. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.2, 170.1, 170.3, 169.7, 169.4, 168.5, 167.7, 165.5, 165.0, 152.3, 133.4, 133.3, 131.1, 130.2, 130.0, 129.8, 129.5, 129.2, 129.1, 128.9, 128.5, 123.2, 118.2, 115.4, 101.2, 101.0, 100.7, 78.7, 74.2, 74.1, 72.05, 71.97, 71.8, 71.1, 70.9, 69.9, 69.2, 68.8, 67.6, 67.4, 66.9, 60.4, 52.8, 45.9, 45.4, 39.3, 29.7, 29.1, 29.0, 26.4, 25.6, 20.8, 20.74, 20.66, 20.59. MALDI-TOF-MS: Calcd for $C_{66}H_{81}N_3O_{28}SNa$: *m/z* 1418.5 $[M+Na]^+$. Found: *m/z* 1418.6 $[M+Na]^+$.

Compound 1 To a solution of compound **14** (11.0 mg, 11.9 μmol) in 1 : 5 MeOH–H_2O (1.2 ml) was added NaOMe (30 mg), and the mixture was stirred for 14 h at room temperature and then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with MeOH–H_2O. The filtrate and washings were combined and concentrated. Column chromatography (MeOH : H_2O=3 : 1) of the residue on Sephadex LH-20 gave **1** (6 mg, 82.7%). $[\alpha]_D^{23}$ −19.9° (*c*=0.2, H_2O). ^{13}C-NMR (125 MHz, 1 : 1 CD_3OD–D_2O): δ 152.8, 135.1, 131.7, 130.9, 130.6, 130.4, 130.1, 124.9, 120.0, 117.0, 104.8, 104.3, 104.0, 83.5, 77.8, 76.7, 75.0, 74.9, 74.5, 74.2, 74.1, 72.1, 71.4, 70.2, 70.1, 69.91, 69.88, 61.0, 50.0, 46.3, 46.1, 40.2, 30.1, 29.3, 27.1, 26.1. MALDI-TOF-MS: Calcd for $C_{39}H_{59}N_3O_{20}SNa$: *m/z* 944.3 $[M+Na]^+$. Found *m/z* 944.3 $[M+Na]^+$.

Compound 16 To a solution of **13** (120 mg, 0.11 mmol) and β-alanine derivative **15** (49 mg, 0.13 mmol) in DMF (2 ml) were added triethylamine (27 μl, 0.02 mmol) and DEPC (22 μl, 0.02 mmol). The reaction mixture was stirred for 16 h at room temperature. After completion of the reaction, the mixture was extracted with chloroform, washed with water, dried ($MgSO_4$), and concentrated. The product was purified on silica gel column chromatography (toluene : acetone=3 : 1) to give **16** (147 mg, 92.4%). $[\alpha]_D^{23}$ +2.4° (*c*=1.1, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.99—7.38 (10H, m, Ar-H), 6.28 (1H, s, NH), 5.60 (1H, t, H-3″), 5.36—5.29 (3H, m, H-2″, H-4, H-4′), 5.19—5.10 (2H, dd, dd, H-2, H-2′), 5.01—4.92 (2H, br dd, H-3, H-3′), 4.77 (1H, d, H-1″), 4.74 (2H, s, Tce), 4.45—4.43 (2H, br d, br d, H-1, H-1′), 4.08 (1H, d, H-5″), 3.97—3.40 (19H, m, H-4″, H-5, H-5′, H-6a, H-6b, H-6a′, H-6b′, $COOCH_3$, NCH_2CO of β-alanine, OCH_2 of sugar unit, NCH_2 of β-alanine, OCH_3), 3.24 (2H, s, NCH_2 of sugar unit), 2.77 (2H, t, $COCH_2$ of β-alanine), 2.10—1.96 (18H, m, $COOCH_3$×6), 1.77—1.33 [17H, m, *t*-Bu, (CH_2)×4]. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.1, 170.03, 169.92, 169.4, 169.3, 169.2, 168.4, 165.4, 164.9, 137.8, 133.3, 129.7, 129.1, 129.0, 128.4, 128.2, 125.2, 101.2, 100.9, 100.6, 94.7, 81.1, 78.6, 74.1, 72.0, 71.9, 71.7, 71.0, 70.8, 69.9, 69.0, 68.7, 67.4, 67.3, 67.2, 66.7, 60.4, 52.7, 52.5, 44.9, 39.3, 33.2, 33.1, 29.5, 29.4, 29.3, 29.2, 28.4, 28.2, 26.6, 25.5, 21.4, 20.7, 20.7, 20.6, 20.54, 20.51. MALDI-TOF-MS: Calcd for $C_{64}H_{83}Cl_3N_2O_{30}Na$: *m/z* 1487.4 $[M+Na]^+$. Found: *m/z* 1487.8 $[M+Na]^+$.

Compound 17 To a solution of **16** (147 mg, 0.10 mmol) in acetic acid (2 ml) was added zinc powder. The reaction mixture was stirred for 1 h at room temperature. After completion of the reaction (TLC monitoring), the mixture was filtered through Celite. The filtrate was concentrated and purified on silica gel column chromatography (chloroform : methanol=30 : 1) to give **17** (107 mg, 79.8%). $[\alpha]_D^{23}$ +1.7° (*c*=1.9, $CHCl_3$) ^{1}H-NMR (500 MHz, $CDCl_3$): 7.99—7.38 (10H, m, Ar-H), 6.59 (1H, s, NH), 5.61 (1H, t, H-3″), 5.36—5.29 (3H, m, H-2″, H-4, H-4′), 5.19—5.09 (2H, dd, dd, H-2, H-2′), 5.03—4.93 (2H, br d, H-3, H-3′), 4.77 (1H, d, H-1″), 4.46—4.44 (2H, br d, br d, H-1, H-1′), 4.09 (1H, d, H-5″), 3.97—3.40 (19H, m, H-4″, H-5, H-5′, H-6a, H-6b, H-6a′, H-6b′, $COOCH_3$, NCH_2CO of β-alanine, OCH_2 of sugar unit, NCH_2 of β-alanine, OCH_3), 3.30—3.23 (2H, m, NCH_2 of sugar unit), 2.57 (2H, t, $COCH_2$ of β-alanine), 2.10—1.96 (18H, m, OAc×6) 1.77—1.33 [17H, m, *t*-Bu, (CH_2)×4]. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.1, 168.5, 165.5, 165.0, 133.3, 129.8, 129.1, 128.4, 101.2, 100.9, 100.6, 78.6, 74.1, 72.0, 71.8, 70.98, 70.90, 70.0, 69.2, 68.7, 67.5, 67.3, 66.8, 60.4, 52.8, 29.2, 28.1, 26.5, 25.5, 20.8, 20.7, 20.6. MALDI-TOF-MS: Calcd for $C_{62}H_{82}N_2O_{30}Na$: *m/z* 1357.5 $[M+Na]^+$. Found: *m/z* 1358.2 $[M+Na]^+$.

Compound 18 To a solution of **17** (49 mg, 36.7 μmol) and **13** (41 mg, 37.1 μmol) in DMF (2 ml) were added triethylamine (7.7 μl, 40.4 μmol) and DEPC (6.1 μl, 40.4 μmol). The reaction mixture was stirred for 16 h at

room temperature. After completion of the reaction, the mixture was extracted with chloroform, washed with water, dried ($MgSO_4$), and concentrated. The product was purified on silica gel column chromatography (chloroform : methanol=40 : 1) to give **18** (80 mg, 89.3%). $[\alpha]_D^{23}$ +4.5° (c=0.7, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.99—7.38 (20H, m, Ar-H), 5.60 (2H, t, H-3″×2), 5.36—5.29 (6H, m, H-2″×2, H-4×2, H-4″×2), 5.18—5.09 (4H, m, H-2×2, H-2′×2), 5.01—4.92 (4H, br d, H-3×2, H-3′×2), 4.77 (2H, d, H-1″), 4.45—4.43 (4H, br d, br d, H-1×2, H-1′×2), 4.08 (2H, d, H-5″×2), 3.96—3.45 (28H, m, H-4″×2, H-5×2, H-5′×2, H-6a×2, H-6b×2, H-6a′×2, H-6b′×2, $COOCH_3$×2, NCH_2CO, NCH_2 of β-alanine, OCH_2 of sugar unit×2), 3.40 (6H, s, OCH_3×2), 3.23—3.19 (4H, m, NCH_2 of sugar unit×2), 2.40 (2H, m, $COCH_2$ of β-alanine), 2.10—1.95 (36H, m, OAc×6×2), 1.60—1.33 [25H, m, t-Bu, (CH_2)×4×2]. ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.2, 170.0, 169.9, 169.5, 169.3, 168.4, 165.4, 164.9, 133.33, 133.26, 129.8, 129.1, 128.4, 101.2, 100.9, 100.6, 80.7, 78.6, 74.1, 72.0, 71.9, 71.8, 71.0, 70.9, 70.0, 69.1, 68.7, 67.5, 67.3, 67.2, 66.8, 60.4, 52.7, 39.6, 39.4, 29.7, 29.3, 28.2, 26.7, 26.6, 25.6, 20.8, 20.7, 20.61, 20.56. MALDI-TOF-MS: Calcd for $C_{114}H_{147}N_3O_{54}Na$: m/z 2444.9 $[M+Na]^+$. Found: m/z 2445.4 $[M+Na]^+$.

Compound 19 To a solution of **18** (71 mg, 29.3 μmol) in dichloromethane (1 ml) was added trifluoroacetic acid (1 ml). The reaction mixture was stirred for 1 h at room temperature. After completion of the reaction, the mixture was concentrated and purified on silica gel column chromatography (chloroform : methanol=20 : 1) to give **19** (60 mg, 88.1%). $[\alpha]_D^{23}$ +2.7° (c=1.0, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.99—7.38 (20H, m, Ar-H), 6.22 (1H, s, NH), 5.60 (2H, t, H-3″×2), 5.36—5.28 (6H, m, H-2″×2, H-4×2, H-4′×2), 5.18—5.09 (4H, m, H-2×2, H-2′×2), 5.03—4.93 (4H, m H-3×2, H-3′×2), 4.79 (2H, d, H-1″), 4.46 (4H, br d, br d, H-1×2, H-1′×2), 4.09 (2H, d, H-5″×2), 3.97—3.40 (30H, m, H-4″×2, H-5×2, H-5′×2, H-6a×2, H-6b×2, H-6a′×2, H-6b′×2, $COOCH_3$×2, OCH_3×2, OCH_2 of sugar unit×2), 3.26—3.21 (4H, m, NCH_2 of sugar unit×2), 3.01 (2H, t, NCH_2CO of β-alanine), 2.52 (2H, m, NCH_2 of β-alanine), 2.10—1.95 (38H, m, OAc×6×2, $COCH_2$ of β-alanine), 1.56—1.33 [16H, (CH_2)×4×2]. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.0, 169.6, 169.3, 168.4, 165.4, 164.9, 133.33, 133.26, 129.8, 129.13, 129.10, 128.4, 101.2, 100.92, 100.87, 100.7, 100.6, 78.6, 74.12, 74.09, 72.0, 71.93, 71.88, 71.8, 71.01, 70.98, 70.9, 70.0, 69.9, 69.14, 69.09, 68.7, 67.5, 67.4, 67.3, 66.9, 66.8, 60.4, 52.8, 45.4, 39.4, 39.2, 29.32, 29.28, 29.23,29.19, 26.6, 25.6, 25.5, 20.8, 20.7, 20.62, 20.56. MALDI-TOF-MS: Calcd for $C_{109}H_{140}N_3O_{52}Na$: m/z 2345.8 $[M+Na]^+$. Found: m/z 2345.5. $[M+Na]^+$.

Compound 20 To a solution of **19** (34 mg, 14.6 μmol) and **17** (59 mg, 44.2 μmol) in DMF (2 ml) were added triethylamine (7.3 μl, 52.4 μmol) and DEPC (6.2 μl, 40.9 μmol). The reaction mixture was stirred for 16 h at room temperature. After completion of the reaction, the mixture was extracted with chloroform, washed with water, dried (Na_2SO_4), and concentrated. The product was purified on silica gel column chromatography (chloroform : methanol=30 : 1) to give **20** (66 mg, 71.4%). $[\alpha]_D^{23}$ +0.4° (c=0.9, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.99—7.38 (30H, m, Ar-H), 5.60 (3H, t, H-3″×3), 5.36—5.29 (9H, m, H-2″×3, H-4×3, H-4′×3), 5.18—5.09 (6H, m, H-2×3, H-2′×3), 5.01—4.92 (6H, br d, H-3×3, H-3′×3), 4.77 (3H, d, H-1″), 4.45—4.43 (6H, br d, br d, H-1×3, H-1′×3), 4.08 (3H, d, H-5″×3), 3.97—3.40 (53H, m, H-4″×3, H-5×3, H-5′×3, H-6a×3, H-6b×3, H-6a′×3, H-6b′×3, $COOCH_3$×3, NCH_2CO×2, NCH_2×2 of β-alanine, OCH_2 of sugar unit×3, OCH_3×3), 3.22 (6H, m, NCH_2 of sugar unit×3), 2.70—2.40 (4H, m, $COCH_2$×2 of β-alanine), 2.10—1.96 (54H, m, OAc×6×3), 1.55—1.26 [33H, m, t-Bu, (CH_2)×4×3]. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.1, 170.0, 169.9, 169.52, 169.47, 169.3, 168.4, 165.4, 164.9, 133.33, 133.26, 129.8, 129.1, 128.4, 101.2, 100.89, 100.85, 100.6, 78.6, 74.1, 72.0, 71.9, 71.8, 71.0, 70.8, 69.9, 69.1, 68.7, 67.5, 67.3, 67.2, 66.8, 60.4, 52.7, 29.32, 29.29, 26.7, 25.6, 25.5, 20.8, 20.7, 20.61, 20.56. MALDI-TOF-MS: Calcd for $C_{171}H_{219}N_5O_{81}Na$: m/z 3661.3 $C_{114}H_{147}N_3O_{54}Na$. Found: m/z 3661.4 $[M+Na]^+$.

Compound 21 Compound **21** was synthesized from **20** according to the procedure described for the synthesis of **19**. Yield: 49 mg (76.3%). $[\alpha]_D^{23}$ +5.0° (c=0.6, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.99—7.38 (30H, m, Ar-H), 5.60 (3H, t, H-3″×3), 5.36—5.29 (9H, m, H-2″×3, H-4×3, H-4′×3), 5.17—5.09 (6H, m, H-2×3, H-2′×3), 5.03—4.93 (6H, br d, H-3×3, H-3′×3), 4.78 (3H, d, H-1″), 4.47—4.45 (6H, m, H-1×3, H-1′×3), 4.10—4.08 (3H, m, H-5″×3), 3.97—3.40 (49H, m, H-4″×3, H-5×3, H-5′×3, H-6a×3, H-6b×3, H-6a′×3, H-6b′×3, $COOCH_3$×3, NCH_2CO, NCH_2 of β-alanine, OCH_2 of sugar unit×3, OCH_3×3), 3.22 (6H, m, NCH_2 of sugar unit×3), 2.70—2.40 (4H, m, $COCH_2$×2 of β-alanine), 2.10—1.96 (58H, m, OAc×6×3, NCH_2CO, NCH_2 of β-alanine), 1.55—1.26 [24H, m, (CH_2)×4×3]. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.2, 170.05, 169.97, 169.60, 169.57, 169.3, 168.5, 168.4, 165.5, 164.9, 133.33, 133.28, 131.77, 129.8, 129.1, 128.4, 101.7, 101.1, 100.5, 93.3, 78.6, 75.6, 74.1, 73.3, 72.0, 71.9, 71.7, 71.0, 70.9, 70.3, 69.93, 69.89, 69.2, 68.7, 67.5, 67.3, 66.9, 66.8, 66.5, 65.9, 60.0, 59.8, 52.8, 39.5, 29.7, 29.3, 29.2, 26.6, 25.6, 20.8, 20.7, 20.62, 20.56. MALDI-TOF-MS: Calcd for $C_{166}H_{212}N_5O_{79}Na$: m/z 3562.3 $[M+Na]^+$. Found: m/z 3562.4 $[M+Na]^+$.

Compound 22 Compound **22** was synthesized from **21** according to the procedure described for the synthesis of **20**. Yield: 65 mg (96.7%). $[\alpha]_D^{23}$ +2.6° (c=0.8, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.92—7.31 (40H, m, Ar-H), 5.53 (4H, t, H-3″×4), 5.29—5.22 (12H, d, m, H-2″×4, H-4×4, H-4′×4), 5.11—5.02 (8H, m, H-2×4, H-2′×4), 4.94—4.85 (8H, br d, H-3×4, H-3′×4), 4.71 (4H, d, H-1″), 4.37 (8H, dd, H-1×4, H-1′×4), 4.01 (4H, d, H-5″×4), 3.89—3.33 (72H, m, H-4″×4, H-5×4, H-5′×4, H-6a×4, H-6b×4, H-6a′×4, H-6b′×4, $COOCH_3$×4, NCH_2CO of β-alanine×3, NCH_2 of β-alanine×3, OCH_2 of sugar unit×4, OCH_3×4), 3.12 (8H, m, NCH_2 of sugar unit×4), 2.77—2.16 (3H, m, $COCH_2$ of β-alanine×3×1/2), 2.03—1.76 (75H, $COOCH_3$×6×4, $COCH_2$ of β-alanine×3×1/2), 1.48—1.19 [41H, m, t-Bu, (CH_2)×4×4]. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.1, 170.0, 169.9, 169.5, 169.3, 168.4, 165.4, 164.9, 133.33, 133.26, 130.9, 129.8, 129.1, 128.8, 128.4, 101.2, 100.9, 100.7, 78.6, 74.1, 72.0, 71.9, 71.83, 71.75, 71.0, 70.8, 69.9, 69.1, 68.7, 67.5, 67.3, 67.2, 66.8, 66.2, 60.4, 52.7, 39.5, 29.6, 29.3, 29.2, 28.22, 28.19, 26.7, 25.61, 25.6, 20.8, 20.7, 20.61, 20.56. MALDI-TOF-MS: Calcd for $C_{228}H_{291}N_7O_{108}Na$: m/z 4877.7 $[M+Na]^+$. Found: m/z 4877.4 $[M+Na]^+$.

Compound 23 Compound **23** was synthesized from **22** according to the procedure described for the synthesis of **21**. Yield: 42 mg (66.0%). $[\alpha]_D^{23}$ +2.9° (c=0.8, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.91—7.31 (40H, m, Ar-H), 5.53 (4H, t, H-3″×4), 5.29—5.22 (12H, d, m, H-2″×4, H-4×4, H-4′×4), 5.09—5.02 (8H, m, H-2×4, H-2′×4), 4.98—4.86 (8H, br d, H-3×4, H-3′×4), 4.71 (4H, d, H-1″), 4.38 (8H, dd, H-1×4, H-1′×4), 4.02 (4H, d, H-5″×4), 3.92—3.33 (60H, m, H-4″×4, H-5×4, H-5′×4, H-6a×4, H-6b×4, H-6a′×4, H-6b′×4, $COOCH_3$×4, OCH_2 of sugar unit×4, OCH_3×4), 3.10 (8H, m, NCH_2 of sugar unit×4), 2.10—1.89 (78H, OAc×6×4, $COCH_2$ of β-alanine×3), 1.48—1.18 [32H, m, (CH_2)×4×4]. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.2, 170.05, 169.98, 169.6, 169.3, 168.4, 165.5, 164.9, 133.35, 133.28, 129.9, 129.7, 129.1, 128.4, 101.2, 100.9, 100.6, 78.6, 74.12, 74.09, 72.0, 71.9, 71.7, 71.0, 70.9, 69.9, 69.1, 68.7, 67.5, 67.3, 66.8, 60.4, 52.8, 39.5, 29.6, 29.3, 26.7, 25.5, 22.6, 20.8, 20.7, 20.62, 20.56. MALDI-TOF-MS: Calcd for $C_{223}H_{284}N_7O_{106}Na$: m/z 4778.7 $[M+Na]^+$. Found: m/z 4778.3 $[M+Na]^+$.

Compound 24 Compound **24** was synthesized from **23** according to the procedure described for the synthesis of **14**. Yield: 28.4 mg (86.4%). $[\alpha]_D^{23}$ +1.2° (c=1.0, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.47—8.16, 7.11 (6H, m, $C_{10}H_6$ of dansyl glycine), 7.91—7.31 (40H, m, Ar-H), 5.53 (4H, t, H-3″×4), 5.29—5.21 (12H, d, m, H-2″×4, H-4×4, H-4′×4), 5.10—5.02 (8H, m, H-2×4, H-2′×4), 5.00—4.85 (8H, br d, H-3×4, H-3′×4), 4.71 (4H, d, H-1″), 4.36 (8H, dd, H-1×4, H-1′×4), 4.02 (4H, d, H-5″×4), 3.90—3.32 (72H, m, H-4″×4, H-5×4, H-5′×4, H-6a×4, H-6b×4, H-6a′×4, H-6b′×4, $COOCH_3$×4, NCH_2CO of β-alanine×3, NCH_2 of β-alanine×3, OCH_2 of sugar unit×4, OCH_3×4), 3.11 (8H, m, NCH_2 of sugar unit×4), 2.81 (6H, s, NC_2H_6 of dansyl glycine), 2.64—2.24 (3H, m, $COCH_2$ of β-alanine×3×1/2), 2.10—1.89 (75H, m, OAc×6×4, $COCH_2$ of β-alanine×3×1/2), 1.48—1.18 [32H, m, (CH_2)×4×4]. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.1, 170.05, 169.97, 169.6, 169.5, 169.3, 168.4, 165.5, 164.9, 133.35, 133.28, 129.8, 129.6, 129.2, 128.4, 101.2, 100.9, 100.7, 78.6, 74.13, 74.11, 72.0, 71.9, 71.8, 71.0, 70.9, 69.9, 69.1, 68.7, 67.5, 67.3, 66.8, 60.4, 52.8, 45.4, 39.64, 39.56, 29.7, 29.3, 26.7, 26.6, 25.59, 25.56, 20.8, 20.7, 20.62, 20.57. MALDI-TOF-MS: Calcd for $C_{237}H_{297}N_9O_{109}SNa$: m/z 5067.8 $[M+Na]^+$. Found: m/z 5069.5 $[M+Na]^+$.

Compound 2 Compound **2** was synthesized from **24** according to the procedure described for the synthesis of **1**. Yield: 6.8 mg (94.0%): $[\alpha]_D^{23}$ −17.1° (c=0.2, H_2O). ^{13}C-NMR (125 MHz, 1 : 1 CD_3OD–D_2O): δ 104.5, 104.1, 103.8, 83.3, 77.5, 76.5, 74.8, 74.3, 73.94, 73.89, 71.9, 71.4, 69.9, 69.7, 60.9, 49.8, 46.0, 40.4, 31.1, 30.0, 29.6, 27.1, 26.0.

Compound 18 To a solution of **13** (160 mg, 0.14 mmol) and **25** (14.3 mg, 57.9 μmol) in DMF (2 ml) were added triethylamine (30 μl, 0.22 mmol) and DEPC (24 μl, 0.16 mmol). The reaction mixture was stirred for 16 h at room temperature. After completion of the reaction, the mixture was extracted with chloroform, washed with water, dried ($MgSO_4$), and concentrated. The product was purified on silica gel column chromatography (chloroform : methanol=40 : 1) to give **18** (132 mg, 94.1%).

Compound 26 To a solution of **19** (45 mg, 19.4 μmol) and **25** (1.9 mg, 7.7 μmol) in DMF (1 ml) were added triethylamine (4 μl, 29 μmol) and DEPC (3.2 μl, 21.3 μmol). The reaction mixture was stirred for 16 h at

room temperature. After completion of the reaction, the mixture was extracted with chloroform, washed with water, dried ($MgSO_4$), and concentrated. The product was purified on silica gel column chromatography (chloroform : methanol=40 : 1) to give **26** (32 mg, 85.7%). $[\alpha]_D^{23}$ +3.8° (c=0.8, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.99—7.38 (40H, m, Ar-H), 5.60 (4H, t, H-3″×4), 5.36—5.28 (12H, d, m, H-2″×4, H-4×4, H-4′×4), 5.15—5.09 (8H, m, H-2×4, H-2′×4), 5.01—4.92 (8H, br d, H-3×4, H-3′×4), 4.77 (4H, d, H-1″), 4.45—4.42 (8H, br d, br d, H-1×4, H-1′×4), 4.08 (4H, d, H-5″×4), 3.96—3.40 (72H, m, H-4″×4, H-5×4, H-5′×4, H-6a×4, H-6b×4, H-6a′×4, H-6b′×4, $COOCH_3$×4, NCH_2CO×of β-alanine×3, OCH_2 of sugar unit×4, NCH_2 of β-alanine×3, OCH_3×4), 3.16 (8H, m, NCH_2 of sugar unit×4), 2.50 and 2.42 (3H, m, $COCH_2$ of β-alanine), 2.10—1.95 (75H, m, OAc×6×4, $COCH_2$ of β-alanine×3×1/2), 1.54—1.26 [41H, m, t-Bu, (CH_2)×4×4]. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.2, 170.1, 170.0, 169.5, 169.3, 168.4, 165.5, 164.9, 133.35, 133.28, 129.8, 129.2, 128.4, 128.3, 101.2, 100.9, 100.7, 78.6, 74.1, 72.0, 71.93, 71.85, 71.8, 71.0, 70.9, 69.9, 69.1, 68.7, 67.5, 67.3, 66.8, 60.4, 52.8, 39.5, 31.9, 29.7, 29.4, 29.32, 29.26, 28.2, 26.7, 26.64, 26.58, 25.61, 25.57, 22.6, 20.8, 20.7, 20.62, 20.57. MALDI-TOF-MS: Calcd for $C_{228}H_{291}N_7O_{108}$Na: m/z 4877.7 $[M+Na]^+$. Found: m/z 4877.3 $[M+Na]^+$.

Compound 27 Compound **27** was synthesized from **26** according to the procedure described for the synthesis of **19**. Yield: 32 mg (76.0%). $[\alpha]_D^{23}$ +6.4° (c=0.7, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.99—7.38 (40H, m, Ar-H), 5.60 (4H, t, H-3″×4), 5.36—5.28 (12H, d, m, H-2″×4, H-4×4, H-4′×4), 5.17—5.09 (8H, m, H-2×4, H-2′×4), 5.02—4.93 (8H, br d, H-3×4, H-3′×4), 4.78 (4H, d, H-1″), 4.45 (8H, dd, H-1×4, H-1′×4), 4.09 (4H, d, H-5″×4), 3.96—3.40 (71H, m, H-4″×4, H-5×4, H-5′×4, H-6a×4, H-6b××4, H-6a′×4, H-6b′×4, $COOCH_3$×4, NCH_2CO of β-alanine×3, OCH_2 of sugar unit×4, NCH_2 of β-alanine×3, OCH_3×4), 3.16 (8H, m, NCH_2 of sugar unit×4), 2.10—1.95 (75H, m, OAc×6×4, $COCH_2$ of β-alanine×3×1/2). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.1, 170.0, 169.9, 169.3, 168.4, 165.4, 164.9, 133.33, 133.26, 129.7, 129.1, 128.4, 101.2, 100.9, 100.7, 78.6, 74.14, 74.09, 72.0, 71.9, 71.8, 71.0, 70.9, 70.0, 69.1, 68.7, 67.5, 67.3, 67.2, 60.4, 52.7, 29.6, 29.3, 29.2, 26.7, 20.8, 20.7, 20.61, 20.56. MALDI-TOF-MS: Calcd for $C_{223}H_{284}N_7O_{106}$Na: m/z 4778.7 $[M+Na]^+$. Found: m/z 4779.4 $[M+Na]^+$.

Compound 28 To a solution of **27** (22 mg, 4.6 μmol) and dansyl glycine (2.3 mg, 7.5 μmol) in DMF (1 ml) were added triethylamine (1.1 μl, 7.9 μmol) and DEPC (0.8 μl, 5.3 μmol). The reaction mixture was stirred for 16 h at room temperature. After completion of the reaction, the mixture was extracted with chloroform, washed with water, dried ($MgSO_4$), and concentrated. The product was purified on silica gel column chromatography ($CHCl_3$: MeOH=20 : 1) to give the dansyl derivative **28** (17 mg, 73%). $[\alpha]_D^{23}$ −2.5° (c=0.3, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.02—7.39 (40H, m, Ar-H), 5.60 (4H, t, H-3″×4), 5.36—5.28 (12H, d, m, H-2″×4, H-4××4, H-4′×4), 5.12—5.09 (8H, m, H-2×4, H-2′×4), 5.01—4.93 (8H, br d, H-3×4, H-3′×4), 4.77 (4H, d, H-1″), 4.44 (8H, dd, H-1×4, H-1′×4), 4.08 (4H, d, H-5″×4), 3.96—3.40 (72H, m, H-4″×4, H-5×4, H-5′×4, H-6a×4, H-6b×4, H-6a′×4, H-6b′×4, $COOCH_3$×4, NCH_2CO of β-alanine×3, NCH_2 of β-alanine×3, OCH_2 of sugar unit×4, OCH_3×4), 3.16 (8H, m, NCH_2 of sugar unit×4), 2.88 (6H, s, NC_2H_6 of dansyl glycine), 2.10—1.95 (75H, m, OAc×6×4, $COCH_2$ of β-alanine×3×1/2), 1.65—1.25 [32H, m, (CH_2)×4×4]. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.2, 170.1, 170.0, 169.6, 169.4, 168.5, 165.5, 165.0, 133.4, 133.3, 129.8, 129.2, 128.5, 128.4, 101.2, 100.9, 100.7, 78.7, 74.2, 72.1, 71.9, 71.8, 71.1, 70.9, 70.0, 69.2, 68.7, 67.5, 67.4, 67.3, 66.9, 60.5, 52.9, 52.8, 39.6, 29.7, 29.3, 26.8, 25.6, 20.8, 20.74, 20.66, 20.6. MALDI-TOF-MS: Calcd for $C_{237}H_{297}N_9O_{109}$SNa: m/z 5067.8. Found: m/z 5069.5 $[M+Na]^+$.

Compound 3 Compound **3** was synthesized from **28** according to the procedure described for the synthesis of **1**. Yield: 4.5 mg (80%). $[\alpha]_D^{23}$ −8.5° (c=0.2, H_2O). ^{13}C-NMR (125 MHz, 1 : 1 CD_3OD–D_2O): δ 173.8, 171.3, 167.4, 167.4, 167.3, 152.5, 135.5, 131.8, 131.3, 130.50, 130.45, 130.4, 130.2, 124.5, 119.9, 116.6, 103.9, 101.6, 75.5, 75.4, 74.5, 74.0, 73.4, 72.9, 72.1, 71.9, 70.7, 70.6, 70.1, 61.7, 46.4, 45.8, 40.0, 29.8, 29.2, 26.9, 26.0.

CID-MS/MS Experiments of Compounds 2 and 3 All CID experiments were performed on a Bruker Daltonics Esquire 3000 plus (Bruker Daltonics GmbsH, Bremen, German), a quadruapole ion-trap mass spectrometer equipped with an ESI source. The samples were introduced into the ion source *via* infusion (flow rate, 120 μl/h); He pressure; $4.86\times e^{-6}$ mbar; CID time, 40 ms, Other parameters: dry temperature, 250 °C; nebulizer gas (N_2), 10 psi; drying gas (N_2), 6.0 l/min; sample solutions, prepared in a mixed solution of MeOH/water (200 : 1) where concentrations were in the range of pmol/ml; smart frag, off; scan range, 50—1300 m/z; compound stability, 100%; ion charge control target, 5000; and maximum accumulation time, 200 ms. The average of 10 spectra was used as the mass spectra.

Acknowledgments This work was supported under the High-Tech Research Center project of the Ministry of Education, Culture, Sports, Science and Technology of Japan. We gratefully acknowledge financial support in the form of a Sasagawa Scientific Research Grant.

References

1) Yamada H., Sun X.-B., Matsumoto T., Ra K.-S., Hirano M., Kiyohara H., *Planta Med.*, **57**, 555—559 (1991).
2) Sun X.-B., Matsumoto T., Yamada H., *J. Pharm. Pharmocol.*, **43**, 699—704 (1991).
3) Hirano M., Kiyohara H., Matsumoto T., Yamada H., *Carbohydr. Res.*, **251**, 145—162 (1995).
4) Maruyama M., Takeda T., Shimizu N., Hada N., Yamada H., *Carbohydr. Res.*, **325**, 83—92 (2000).
5) Sakurai M., Kiyohara H., Matsumoto T., Tsumuraya Y., Hashimoto Y., Yamada H., *Carbohydr. Res.*, **311**, 219—229 (1998).
6) Sakurai M., Kiyohara H., Yamada H., *Immunology*, **97**, 540—547 (1999).
7) Sauter N. K., Bednarski M. D., Wurzberg B. A., Hanson J. E., Whitesides G. M., Skehel J. J., Wiley D. C. *Biochemistry*, **28**, 8388—8396 (1989).
8) Lee R. T., Lee Y. C., *Glycoconjug. J.*, **17**, 543—551 (2000).
9) Hada N., Ogino T., Yamada H., Takeda T., *Carbohydr. Res.*, **334**, 7—17 (2001).
10) Sato K., Hada N., Takeda T., *Tetrahedron Lett.*, **44**, 9331—9335 (2003).
11) Hada N., Sato K., Jin Y., Takeda T., *Chem. Pharm. Bull.*, **53**, 1131—1135 (2005).
12) Sato K., Hada N., Takeda T., *Carbohydr. Res.*, accepted (2006).
13) Nicolaou K. C., Bockovich N. J., Carcanague D. R., *J. Am. Chem. Soc.*, **115**, 8843—8844 (1993).
14) Veeneman G. H., van Leeuwen S. H., van Boom J. H., *Tetrahedron Lett.*, **31**, 1331—1334 (1990).
15) Konradsso P., Udodong U. E., Fraser-Reid B., *Tetrahedron Lett.*, **31**, 4313—4316 (1990).
16) Schmidt R. R., Behrendt M., Toepfer A., *Synlett*, **11**, 694—696 (1990).