Synthesis of New Peptidic Glycoclusters Derived from β-Alanine. Part 2: Optionally Modulated Distance between Side-Chain Branched Points

Noriyasu HADA, Koji SATO, Yuhua JIN, and Tadahiro TAKEDA*

Kyoritsu University of Pharmacy; 1–5–30 Shibakoen, Minato-ku, Tokyo 105–8512, Japan.
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The synthesis of an asymmetric glycocluster 1 has been achieved using two glycocluster units 12 and 13, prepared by coupling the cluster chain unit 4 with each ω-amino acid (β-alanine and 6-aminocapronic acid) trichloroethyl ester, and peptidic C-terminal block glycocluster 16, prepared by coupling the bifunctional linker 14 with sugar unit 9. This method facilitated the synthesis of the cluster optionally modulated the distance between the side-chain branched points by using various ω-amino acids. We also synthesized glycodendron 2 using the same intermediate.

Key words glycocluster; ω-amino acid; D-galactose; glycodendron; unit synthesis

It is worthwhile noting that multivalency is a powerful design approach to increase the binding strength of synthetic carbohydrate ligands with protein receptors.[1—3] Some carbohydrate chemists have developed methods to make glycoclusters for that reason,[4—8] however, these clusters are symmetrical structures and there are few asymmetrical ones. Asymmetrical clusters are expected not only to enhance binding affinity but also may resolve steric conformation of protein receptors. Therefore, we have devised three types of new glycocluster (Fig. 1). In our previous paper,[9] we synthesized the glycocluster using diverse 'glycocluster units' that make it possible to modulate optionally the length of the side chain by insertion of ω-amino acid as spacer between the main chain and the carbohydrate moiety (Fig. 1B). The glycocluster units are composed of β-alanine derivative as a cluster-chain unit, sugar unit and ω-amino acids as a spacer. They provide diversity using different ω-amino acids between the cluster-chain unit and sugar unit. This method is advantageous for the synthesis of diverse glycoclusters to combine several diverse glycocluster units.

It is also important to consider not only side chain length but also the distance between the side chain branched points to give diversity to the glycoclusters. The steric distribution of carbohydrate residues on glycoconjugates will affect their interaction with lectins. Therefore, two kinds of ω-amino acids (β-alanine and 6-aminocapronic acid) as spacers of different length were attached to the cluster-chain unit, and diverse glycocluster units were synthesized. The glycoclusters were elongated by coupling these units (Fig. 1C).

In this paper we report the synthesis of glycocluster **1** which makes it possible to modulate optionally the distance between the side chain branch points (Fig. 2). Furthermore, we undertook to build a glycodendron derivative **2** using cluster-chain unit **16** as a dendron core.

Synthesis of Glycocluster First, glycocluster units **12** and **13** were prepared as follows. Removal of the trichloroethyl group from cluster-chain unit **3**, which was prepared according to the previous paper,[9] by Zn-AcOH provided acid-free cluster-chain unit **4** (93%). Coupling of compound **4** with each ω-amino acid (β-alanine and 6-aminocapronic acid) trichloroethyl ester as a spacer in the presence of diethyl phosphorocyanidate (DEPC) in dry DMF

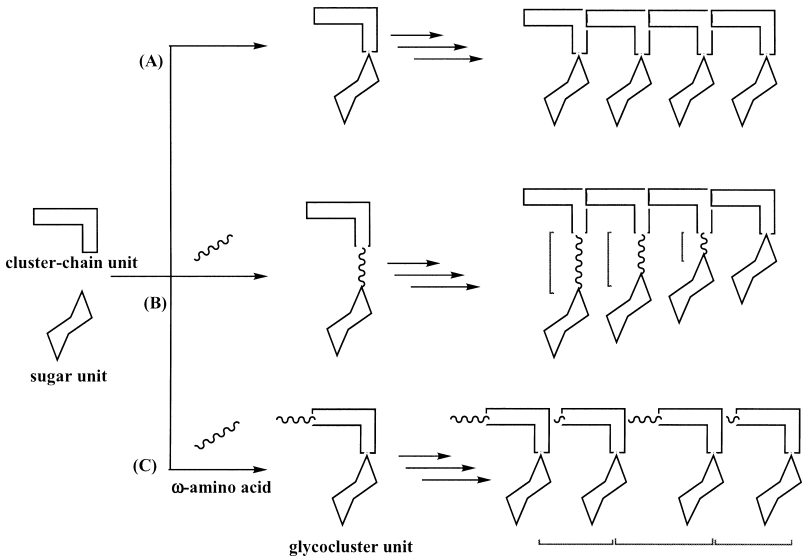


Fig. 1. Elongation of Glycoclusters: (A) Using a Conventional Glycocluster Unit for Elongation, (B) ω-Amino Acid Insertion between Cluster-Chain Units and Sugar Units for Modulation Side-Chain Spacer, (C) ω-Amino Acid Attachment with Cluster-Chain Units for Modulation of Side-Chain Branched Points

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

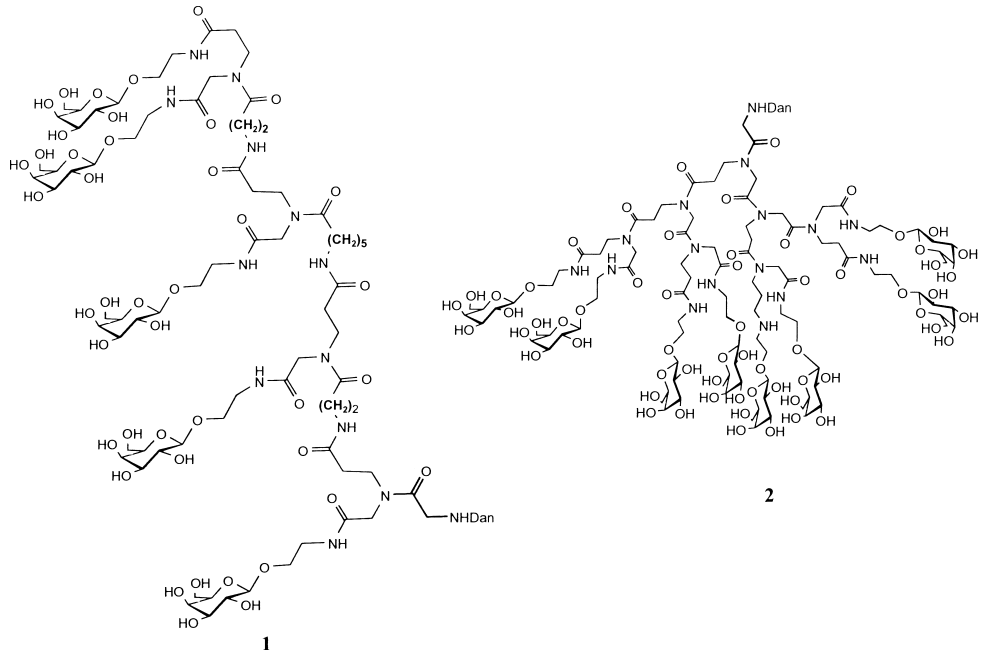


Fig. 2. Structures of Synthetic Glycocluster and Glycodendron

for 16 h at room temperature gave compounds **5** (80%) and **6** (72%), respectively. Subsequent deprotection of the benzyl group in **5** and **6** under neutral conditions by hydrogenation over 10% Pd-C afforded compounds **7** (87%) and **8** (94%), respectively. Furthermore, coupling of **7** and **8** with sugar unit **9**, which is a simple D-galactose derivative[9] in the presence of DEPC as described for **5** and **6** gave glycocluster units **10** (77%) and **11** (79%) of varied lengths corresponding to the main chain of the cluster. To elongate this peptidic cluster from the C-terminal to the N-terminal, removal of the trichloroethyl group from **10** and **11** by Zn-AcOH gave the desired acid-free glycocluster units **12** (82%) and **13** (83%).

Next, for the synthesis of the C-terminal block compound **16** with respect to the peptidic main chain, saponification of compound **3** by 1 M NaOH solution in 1,4-dioxane gave compound **14** in 85% yield. Coupling of **14** with sugar unit **9** in the presence of DEPC in dry DMF gave **15** in 67% yield. Compound **15** was transformed into the target C-terminal block compound **16** by treatment with 50% TFA in dichloromethane (81%). Coupling of **16** with **12** in the presence of DEPC as described for **5** gave the trimer derivative **17** in 73% yield. The Boc group of **17** was removed under acidic conditions with 50% TFA giving a secondary amine-free compound **18**, which was subsequently subjected to repeated couplings, and deprotection of the Boc group for elongation gave the desired pentamer glycocluster derivative **22** in 87% yield. Finally, pentamer **22** was subsequently treated with dansyl glycine in the presence of DEPC, and complete removal of the *O*-benzoyl groups provided the target compound **1** in 87% yield (two steps) with free hydroxyl groups on the asymmetric glycocluster **1** (Chart 1).

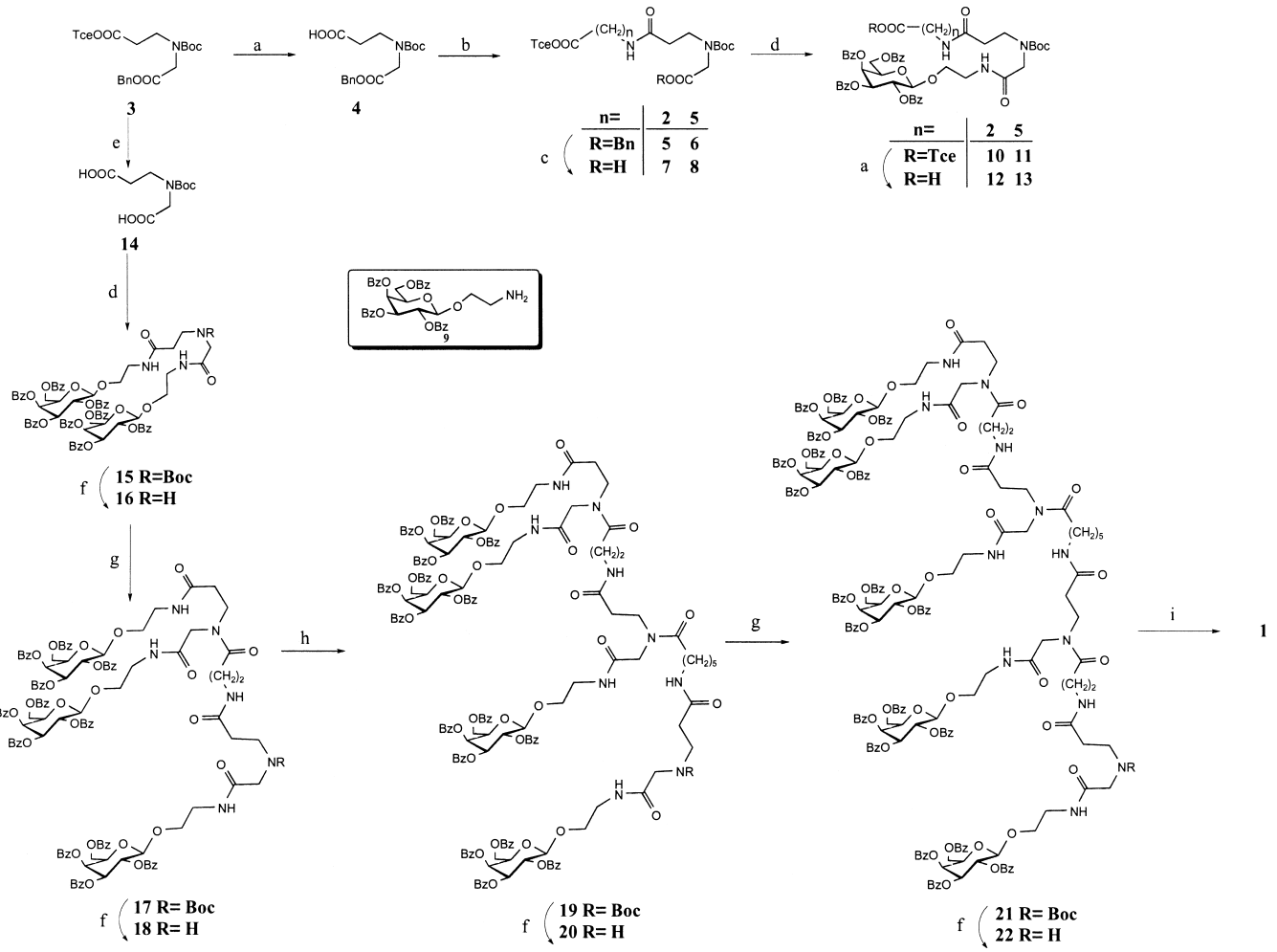
Synthesis of Glycodendron Glycodendrimers are part of the emerging class of synthetic macromolecules which first appeared in 1993.[10] They were originally designed to adjust the binding affinities of carbohydrate ligands to protein receptors. There are two different main strategies to build glycodendrimers. Two well-known approaches that are well recognized for dendrimer syntheses are divergent[11,12] and convergent[13—15] growth. It is difficult in the divergent approach to ascertain the completeness of the conjugation and partial defects are only detectable by spectroscopic method with great difficulty because the carbohydrate portions are all added at once and at the end of the dendrimer synthesis. Therefore, we chose the convergent approach using bifunctional linker **14** as the dendron core, which was used in glycocluster synthesis.

For this approach, coupling of two equivalent dimer **16** with dendron core **14** in the presence of DEPC gave **23** in 90% yield, and removal of the Boc group using 50% TFA gave the tetramer **24** (88%). Coupling of two equivalent **24** with **14** under the same conditions gave the octamer **25** in 91% yield. The Boc group of **25** was removed under acidic conditions with 50% TFA to give the free secondary amine **26** (82%), which was subsequently treated with dansyl glycine in the presence of DEPC. Finally, complete de-benzoylation afforded the target compound **2** in 80% yield (two steps). There were no by-products generated in the peptide condensation (Chart 2).

In conclusion, efficient and widely applicable synthetic strategies in glycoconjugate chemistry have been achieved to obtain new glycoclusters. They are capable of modulating optionally not only the length of the side-chain but also the distance between the side-chain branched points. We have succeeded in the synthesis of various kinds of glycoclusters from limited and economical materials. Providing diversity to the glycocluster, a specific glycocluster library will become possible.

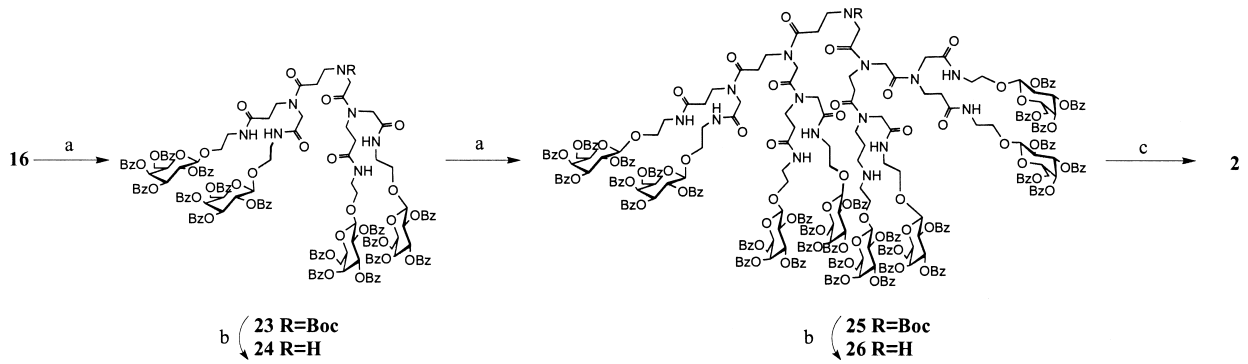
Experimental

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 $CDCl_3$, CD_3OD. MALDI-TOF-MS was recorded on a Perceptive Voyager RP mass spectrometer. TLC was performed on silica gel 60-F254 (E. Merck) with detection by quenching of UV fluorescence and by spraying with 5% ninhydrin and 10%



Reagents: (a) Zn-AcOH; (b) ω-amino acid 2,2,2-trichloroethyl ester, DEPC, Et_3N, DMF; (c) Pd-C, H_2, THF; (d) **9**, DEPC, Et_3N, DMF; (e) 1 M NaOH, 1,4-dioxane; (f) 50% TFA; (g) **12**, DEPC, Et_3N, DMF; (h) **13**, DEPC, Et_3N, DMF; (i) (1) Dansyl glycine, DEPC, Et_3N, DMF. (2) NaOMe, MeOH-1,4-dioxane.

Chart 1



Reagents: (a) **14**, DEPC, Et_3N, DMF; (b) 50% TFA; (c) (1) Dansyl glycine, DEPC, Et_3N, DMF. (2) NaOMe, MeOH-1,4-dioxane.

Chart 2

H_2SO_4. Column chromatography was carried out on silica gel 60 (E. Merck). Several oligomers gave mixtures of rotamers as seen from ^{1}H- and ^{13}C-NMR spectra.[5)]

Compound 4 To a solution of **3**[9)] (504 mg, 1.07 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 by silica gel column chromatography (chloroform : methanol : acetic acid=30 : 1 : 0.1) as elute to give **4** (337 mg, 93.0%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.37—7.31 (5H, m, Ar-H), 5.15 (2H, s, benzyl methylene), 4.08, 4.00 (2H, s, NCH_2CO), 3.56, 3.53 (2H, dd, J=6.1 Hz, J=6.7 Hz, NCH_2 of β-alanine), 2.70, 2.64 (2H, dd, J=6.1 Hz, J=6.7 Hz, CH_2CO of β-alanine), 1.46, 1.34 (9H, 2s, *t*-Bu). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 177.6, 177.2, 170.23, 170.16, 155.4, 155.1, 135.40, 135.37, 128.6, 128.5, 128.4, 128.3, 128.3, 80.9, 80.8, 66.9, 66.8, 51.1, 50.2, 44.8, 44.7, 33.9, 33.5, 28.3, 28.1. MALDI-TOF-MS: Calcd for $C_{17}H_{23}NO_6$: *m*/*z* 337. Found: *m*/*z* 360 $[M+Na]^+$.

Compounds **12** and **13** were prepared according to the same procedures as described for the preparation of **4**.

Compound 5 To a solution of **4** (224 mg, 0.66 mmol) and β-alanine

2,2,2-trichloroethyl ester (341 mg, 0.87 mmol) in DMF (2 ml) were added triethylamine (133 μl, 0.95 mmol) and DEPC (133 μl, 0.88 mmol). The reaction mixture was stirred for 16 h at room temperature. After completion of the reaction, the mixture was extracted with ethyl acetate, washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography (benzene : acetone = 5 : 1) as elute to give **5** (286 mg, 79.8%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.35 (5H, m, Ar-H), 6.52 and 6.44 (s, 1H, NH), 5.16 (2H, s, benzyl methylene), 4.77 (2H, s, CH_2CCl_3), 4.05 and 3.97 (2H, s, NCH_2CO), 3.58—3.53 (4H, m, NCH_2 of β-alanine ×2), 2.69 (2H, br t, $COCH_2$ of β-alanine), 2.39 and 1.93 (2H, br t, $COCH_2$ of β-alanine) 1.47 and 1.34 (9H, 2s, *t*-Bu). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.3, 170.5, 170.3, 155.3, 135.4, 128.6, 128.4, 128.3, 128.2, 94.7, 80.6, 74.0, 66.8, 50.6, 50.3, 45.7, 45.3, 36.3, 35.7, 34.8, 33.9, 28.3, 28.1. MALDI-TOF-MS: Calcd for $C_{22}H_{29}Cl_3N_2O_7$: *m/z* 538. Found: *m/z* 561 $[M+Na]^+$.

The following compounds **6**, **10**, **11** and **15** were prepared according to the same procedures as described for the preparation of **5**.

Compound 6 Yield 72.3%; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.37—7.34 (5H, m, Ar-H), 6.31 and 6.22 (1H, s, NH), 5.16 (2H, s, benzyl methylene), 4.74 (2H, s, CH_2CCl_3), 4.05 and 3.97 (2H, s, NCH_2CO), 3.59 (2H, t, CH_2), 3.21 (2H, t, CH_2), 2.47 (4H, t, CH_2×2), 1.71 (2H, m, CH_2), 1.52 (2H, m, CH_2), 1.40 (2H, m, CH_2), 1.46 and 1.35 (9H, 2s, *t*-Bu). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.8, 171.0, 170.2, 155.4, 135.4, 128.6, 128.43, 128.36, 128.2, 95.0, 80.6, 73.8, 66.8, 50.4, 46.0, 45.2, 39.3, 36.4, 35.8, 33.7, 29.0, 28.3, 28.1, 26.2, 24.3. MALDI-TOF-MS: Calcd for $C_{25}H_{35}Cl_3N_2O_7$: *m/z* 580. Found: *m/z* 603 $[M+Na]^+$.

Compound 7 A solution of **5** (286 mg, 0.53 mmol) in THF (2 ml) was hydrogenated over 10% Pd-C (102 mg) for 2 h at room temperature, then filtered through Celite. The filtrate was concentrated and purified by silica gel column chromatography (benzene : acetone = 1 : 3) as elute to give **7** (206 mg, 86.5%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.00 and 6.79 (1H, s, NH), 4.77 (2H, s, CH_2CCl_3), 4.02 and 3.97 (2H, s, NCH_2CO), 3.60—3.55 (4H, m, NCH_2×2), 2.73 and 2.65 (2H, t, $COCH_2$), 2.51 and 2.44 (2H, br t, $COCH_2$), 1.46 and 1.43 (9H, 2s, *t*-Bu). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 172.7, 172.5, 170.6, 155.5, 94.7, 81.2, 74.0, 50.4, 50.0, 45.8, 45.3, 35.3, 35.1, 33.7, 33.5, 28.3, 28.1. MALDI-TOF-MS: Calcd for $C_{15}H_{23}Cl_3N_2O_7$: *m/z* 448. Found: *m/z* 471 $[M+Na]^+$.

Compound 8 A solution of **6** (131 mg, 0.22 mmol) in THF (2 ml) was hydrogenated over 10% Pd-C (106 mg) for 2 h at room temperature, then filtered through Celite. The filtrate was concentrated and purified by silica gel column chromatography (benzene : acetone = 1 : 3) as elute to give **8** (103 mg, 93.7%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.75 (1H, s, NH), 4.75 (2H, s, CH_2CCl_3), 3.95 (2H, s, NCH_2CO), 3.59 (2H, t, NCH_2 of β-alanine), 3.23 (2H, dd, NCH_2 of 6-aminocapronic acid), 2.50—2.46 (4H, m, $COCH_2$ of β-alanine, $COCH_2$ of 6-aminocapronic acid, 1.71 (2H, m, CH_2), 1.54 (2H, m, CH_2), 1.45 (9H, s, *t*-Bu), 1.40 (2H, m, CH_2). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 172.3, 172.0, 155.7, 95.0, 81.1, 73.8, 50.3, 45.2, 39.6, 35.3, 33.7, 28.7, 28.1, 26.2, 24.2. MALDI-TOF-MS: Calcd for $C_{18}H_{29}Cl_3N_2O_7$: *m/z* 490. Found: *m/z* 513 $[M+Na]^+$.

Compound 10 Yield 76.8%; $[\alpha]_D^{23}$ +57.8° (*c* = 1.1, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.10—7.23 (20H, m, Ar-H), 6.95 (1H, s, NH), 6.36 (s, NH), 6.00 (1H, d, H-4), 5.77 (1H, dd, H-2), 5.64 (1H, dd, H-3), 4.90 (1H, br d, H-1), 4.74 (2H, s, CH_2CCl_3), 4.68 (1H, m, H-6a), 4.45—4.34 (2H, m, H-5, H-6b), 3.99 and 3.77 (2H, m, OCH_2 of sugar unit), 3.53—3.46 (8H, m, NCH_2CO, NCH_2 of sugar unit, NCH_2 of β-alanine×2), 2.70 (2H, t, $COCH_2$), 2.32 (2H, m, $COCH_2$), 1.40 (9H, s, *t*-Bu). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.5, 169.9, 166.0, 165.5, 165.4, 133.7, 133.5, 13.4, 133.3, 130.0, 129.7, 129.2, 129.1, 129.0, 128.7, 128.6, 128.5, 128.3, 101.7, 94.8, 80.7, 73.9, 71.5, 71.4, 69.9, 69.8, 68.1, 62.2, 62.0, 52.1, 41.2, 35.0, 28.2. MALDI-TOF-MS: Calcd for $C_{51}H_{54}Cl_3N_3O_{16}$: *m/z* 1069.3. Found: *m/z* 1092.6 $[M+Na]^+$.

Compound 11 Yield 79.0%; $[\alpha]_D^{23}$ +60.4° (*c* = 1.1, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.10—7.23 (20H, m, Ar-H), 6.54 (1H, s, NH), 6.37 (1H, s, NH), 6.00 (1H, d, H-4), 5.76 (1H, dd, H-2), 5.65 (1H, dd, H-3), 4.89 (1H, d, H-1), 4.73 (2H, s, CH_2CCl_3), 4.68 (1H, dd, H-6a), 4.45—4.38 (2H, m, H-5, H-6b), 4.00 and 3.77 (2H, m, OCH_2 of sugar unit), 3.58—3.48 (6H, m, NCH_2 of sugar unit, NCH_2 of β-alanine, NCH_2CO), 3.19 (2H, m, NCH_2 of 6-aminocapronic acid), 2.45—2.36 (4H, m, CH_2×2), 1.68 (2H, m, CH_2), 1.55—1.39 (13H, m, *t*-Bu, CH_2×2). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.9, 166.0, 165.5, 133.7, 133.4, 130.0, 129.7, 129.0, 128.7, 128.6, 128.5, 128.3, 101.6, 95.0, 80.7, 73.8, 71.6, 71.4, 69.9, 68.1, 51.9, 39.6, 33.7, 28.9, 28.2, 26.3, 24.3. MALDI-TOF-MS: Calcd for $C_{54}H_{60}Cl_3N_3O_{16}$: *m/z* 1111.3. Found: *m/z* 1134.5 $[M+Na]^+$.

Compound 12 Yield 82.4%; $[\alpha]_D^{23}$ +54.2° (*c* = 1.2, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.09—7.14 (20H, m, Ar-H), 6.92 (1H, s, NH), 6.56 (1H, s, NH), 6.00 (1H, d, H-4), 5.76 (1H, br dd, H-2), 5.65 (1H, br dd, H-3), 4.90 (1H, br d, H-1), 4.68 (1H, m, H-6a), 4.44—4.38 (2H, m, H-5, H-6b), 3.99 and 3.78 (2H, m, OCH_2 of sugar unit), 3.58—3.46 (8H, m, NCH_2 of sugar unit, NCH_2CO, NCH_2 of δ-alanine×2), 2.55 (2H, m, CCH_2 of β-alanine), 2.35 (2H, m, CCH_2 of β-alanine), 1.38 (9H, s, *t*-Bu). MALDI-TOF-MS: Calcd for $C_{49}H_{53}N_3O_{16}$: *m/z* 939.3. Found: *m/z* 962.7 $[M+Na]^+$.

Compound 13 Yield 83.3%; $[\alpha]_D^{23}$ +58.5° (*c* = 1.1, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.10—7.22 (20H, m, Ar-H), 6.74 (1H, s, NH), 6.63 (1H, s, NH), 6.00 (1H, d, H-4), 5.76 (1H, dd, H-2), 5.65 (1H, dd, H-3), 4.89 (1H, br d, H-1), 4.67 (1H, dd, H-6a), 4.46—4.38 (2H, m, H-5, H-6b), 3.99 and 3.77 (2H, m, OCH_2 of sugar unit), 3.61—3.47 (6H, NCH_2 of sugar unit, NCH_2CO, NCH_2 of β-alanine), 3.19 (2H, m, NCH_2 of 6-aminocapronic acid), 2.35—2.29 (4H, m, CH_2×2), 1.61 (2H, m, CH_2), 1.50—1.33 (13H, m, *t*-Bu, CH_2×2). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 176.8, 171.3, 166.1, 165.5, 165.4, 133.7, 133.6, 133.4, 133.3, 130.0, 129.7, 129.2, 129.1, 128.9, 128.7, 128.6, 128.5, 128.3, 101.7, 80.8, 71.5, 71.4, 69.9, 68.7, 68.1, 62.0, 51.8, 45.8, 39.3, 33.7, 28.7, 28.2, 26.1, 24.2. MALDI-TOF-MS: Calcd for $C_{52}H_{59}N_3O_{16}$: *m/z* 981.4. Found: *m/z* 1004.8 $[M+Na]^+$.

Compound 14 To a solution of **3** (746 mg, 1.59 mmol) in 1,4-dioxane (5 ml) was added 1 M NaOH solution (2 ml). The reaction mixture was stirred for 6 h at room temperature. After completion of the reaction, the mixture was washed with ethyl acetate. Then the water layer was acidified with 5% citric acid solution and extracted with ethyl acetate, washed with water, dried (Mg_2SO_4), and concentrated to give **14** (335 mg, 85.1%). ^{1}H-NMR (500 MHz, CD_3OD): δ 4.00 and 3.97 (2H, s, NCH_2CO), 3.53 (2H, t, NCH_2 of β-alanine), 2.59 (2H, t, *J* = 6.7 Hz, $COCH_2$ of β-alanine), 1.48 and 1.42 (9H, 2s, *t*-Bu). ^{13}C-NMR (125 MHz, CD_3OD): δ 175.8, 175.7, 157.4, 157.1, 81.9, 81.8, 51.3, 50.4, 34.7, 34.1, 28.6, 28.5. MALDI-TOF-MS: Calcd for $C_{10}H_{17}NO_6$: *m/z* 247.1. Found: *m/z* 270.5 $[M+Na]^+$.

Compound 15 Yield 67.2%; $[\alpha]_D^{23}$ +80.7° (*c* = 1.1, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.09—7.22 (40H, m, Ar-H), 7.06 (1H, s, NH), 6.49 (1H, s, NH), 6.00 (2H, br d, H-4×2), 5.77 (2H, m, H-2×2), 5.63 (2H, br dd, H-3×2), 4.88 (2H, br d, H-1×2), 4.66 (2H, m, H-6a×2), 4.44 (2H, m, H-6b×2), 4.35 (2H, m, H-5×2), 3.98 and 3.75 (4H, m, OCH_2 of sugar unit×2), 3.60—3.35 (8H, m, NCH_2CO, NCH_2 of β-alanine, NCH_2 of sugar unit×2), 2.10 (2H, m, $COCH_2$ of β-alanine), 1.38 (9H, s, *t*-Bu). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 169.8, 166.0, 165.5, 165.3, 133.6, 133.4, 133.3, 130.0, 129.7, 129.3, 129.1, 129.0, 128.7, 128.5, 128.3, 101.7, 80.6, 71.5, 71.4, 69.8, 68.9, 68.0, 61.9, 52.3, 45.8, 39.3, 35.1, 28.2. MALDI-TOF-MS: Calcd for $C_{82}H_{79}N_3O_{24}$: *m/z* 1489.5. Found: *m/z* 1512.8 $[M+Na]^+$.

Compound 16 To a solution of **15** (191 mg, 0.13 mmol) 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 by silica gel column chromatography (chloroform : methanol = 60 : 1) as elute to give **16** (144 mg, 81.1%). $[\alpha]_D^{23}$ +92.6° (*c* = 1.0, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.01—7.22 (40H, m, Ar-H), 6.15 (1H, t, NH), 6.00 (2H, m, H-4), 5.81—5.76 (2H, m, H-2), 5.66—5.61 (2H, m, H-3), 4.89—4.84 (2H, m, H-1), 4.69—4.65 (2H, m, H-6a), 4.45—4.41 (2H, m, H-6b), 4.37—4.34 (2H, m, H-5), 4.02—3.98 and 3.56—3.51 (4H, m, OCH_2 of sugar unit), 3.79—3.70 and 3.46—3.37 (4H, m, NCH_2 of sugar unit), 2.96 (2H, s, NCH_2CO), 2.52 (2H, t, NCH_2 of β-alanine), 2.04—1.89 (3H, m, $COCH_2$ of β-alanine, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.5, 166.0, 165.5, 133.6, 133.5, 133.3, 130.0, 129.7, 129.3, 129.2, 128.9, 128.6, 128.5, 128.3, 101.6, 101.4, 71.6, 71.4, 71.3, 69.9, 69.8, 69.0, 68.7, 68.1, 62.0, 51.6, 45.2, 38.9, 38.8, 35.0. MALDI-TOF-MS: Calcd for $C_{77}H_{71}N_3O_{22}$: *m/z* 1389.5. Found: *m/z* 1413.0 $[M+Na]^+$.

The following compounds **18**, **20**, **22**, **24** and **26** were prepared according to the same procedures as described for the preparation of **16**.

Compound 17 To a solution of carboxyl acid **12** (43 mg, 46.0 μmol) and amine **16** (52 mg, 37.0 μmol) in DMF (2 ml) were added triethylamine (8 μl, 57.7 μmol) and DEPC (6 μl, 40.7 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 by silica gel column chromatography ($CHCl_3$: MeOH = 30 : 1) as elute to give **17** (62 mg, 72.9%). $[\alpha]_D^{23}$ +72.4° (*c* = 1.6, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.08—7.21 (60H, m, Ar-H), 6.00 (3H, br d, H-4), 5.77 (3H, br dd, H-2), 5.64 (3H, br dd, H-3), 4.91 (3H, br d, H-1), 4.67 (3H, m, H-6a), 4.43 (3H, m, H-6b), 4.37 (3H, m, H-5), 3.95 and 3.77 (6H, m, OCH_2 of sugar unit×3), 3.58—3.43 (16H, m, NCH_2 of sugar unit×3, NCH_2CO×2, NCH_2 of β-alanine×3), 2.59—2.00

(6H, m, CH_2CO of β-alanine×3), 1.36 (9H, s, *t*-Bu). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.0, 165.5, 133.6, 133.3, 130.0, 129.7, 129.3, 128.9, 128.6, 128.5, 128.3, 101.6, 101.6, 101.4, 80.5, 71.6, 71.4, 69.9, 69.8, 68.7, 68.1, 62.0, 46.0, 39.2, 39.1, 39.0, 35.3, 28.2. MALDI-TOF-MS: Calcd for $C_{126}H_{122}N_6O_{37}$: *m/z* 2310.8. Found: *m/z* 2334.1 $[M+Na]^+$.

The following compounds **19**, **21**, **23** and **25** were prepared according to the same procedures as described for the preparation of **17**.

Compound 18 Yield 93.5%; $[\alpha]_D^{23}$ +82.5° (*c*=1.2, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.08—7.22 (60H, m, Ar-H), 6.00 (3H, br d, H-4), 5.77 (3H, br dd, H-2), 5.65 (3H, br dd, H-3), 4.89 (3H, br d, H-1), 4.68 (3H, m, H-6a), 4.44—4.36 (6H, m, H-5, H-6b), 3.96 and 3.77 (6H, m, OCH_2 of sugar unit×3), 3.48—3.38 (10H, m, NCH_2 of sugar unit×3, NCH_2CO×2), 3.03, 2.67 and 2.51 (6H, NCH_2 of β-alanine×3), 2.30—2.01 (7H, m, CH_2CO of β-alanine×3, NH). MALDI-TOF-MS: Calcd for $C_{121}H_{114}N_6O_{35}$: *m/z* 2210.7. Found: *m/z* 2233.6 $[M+Na]^+$.

Compound 19 Yield 87.8%; $[\alpha]_D^{23}$ +39.2° (*c*=1.3, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.09—7.21 (80H, m, Ar-H), 5.99 (4H, br d, H-4), 5.76 (4H, dd, H-2), 5.65 (4H, br dd, H-3), 4.89 (4H, br d, H-1), 4.67 (4H, m, H-6a), 4.44—4.37 (8H, m, H-5, H-6b), 3.96 and 3.74 (8H, m, OCH_2 of sugar unit), 3.58—3.42 (22H, m, NCH_2 of sugar unit, NCH_2CO, NCH_2×4), 3.15 (2H, m, NCH_2), 2.48—2.09 (10H, m, CH_2CO), 1.53—1.35 (15H, m, *t*-Bu, CH_2×3). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.0, 165.5, 133.6, 133.5, 133.3, 130.0, 129.7, 129.3, 129.2, 129.0, 128.7, 128.5, 128.3, 101.6, 71.5, 71.4, 69.9, 68.1, 62.0, 45.9, 39.2, 29.7, 28.2, 24.2. MALDI-TOF-MS: Calcd for $C_{173}H_{171}N_9O_{50}$: *m/z* 3174.1. Found: *m/z* 3197.7 $[M+Na]^+$.

Compound 20 Yield 47.2%; $[\alpha]_D^{23}$ +82.5° (*c*=0.6, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.07—7.21 (80H, m, Ar-H), 6.00 (4H, br d, H-4), 5.76 (4H, br dd, H-2), 5.66 (4H, br dd, H-3), 4.91 (4H, br d, H-1), 4.68 (4H, m, H-6a), 4.44—4.37 (8H, m, H-5, H-6b), 3.96—3.16 (32H, m, OCH_2 of sugar unit, NCH_2 of sugar unit, NCH_2CO, NCH_2, NCH_2), 2.48—2.09 (10H, m, CH_2CO), 1.75—1.43 (6H, m, CH_2). MALDI-TOF-MS: Calcd for $C_{168}H_{163}N_9O_{48}$: *m/z* 3074.1. Found: *m/z* 3097.3 $[M+Na]^+$.

Compound 21 Yield 91.0%; $[\alpha]_D^{23}$ +65.0° (*c*=0.4, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.07—7.21 (100H, m, Ar-H), 6.00 (5H, br d, H-4), 5.76 (5H, br dd, H-2), 5.65 (5H, br dd, H-3), 4.90 (5H, br d, H-1), 4.67 (5H, m, H-6a), 4.42—4.38 (10H, m, H-5, H-6b), 3.95—3.13 (42H, m, OCH_2, NCH_2), 2.61—2.10 (14H, m, CCH_2), 1.52—1.34 (15H, m, CH_2, *t*-Bu). MALDI-TOF-MS: Calcd for $C_{217}H_{214}N_{12}O_{63}$: *m/z* 3995.4. Found: *m/z* 4018.5 $[M+Na]^+$.

Compound 22 Yield 87.1%; $[\alpha]_D^{23}$ +70.2° (*c*=0.4, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.06—7.21 (100H, m, Ar-H), 6.00 (5H, br d, H-4), 5.75 (5H, br dd, H-2), 5.66 (5H, br dd, H-3), 4.90 (5H, br d, H-1), 4.67 (5H, m, H-6a), 4.38 (10H, m, H-5, H-6b), 3.96—3.19 (42H, m, OCH_2, NCH_2), 2.75—2.10 (14H, m, CCH_2), 1.41 (6H, m, CH_2). MALDI-TOF-MS: Calcd for $C_{212}H_{206}N_{12}O_{61}$: *m/z* 3895.3. Found: *m/z* 3918.5 $[M+Na]^+$.

Compound 1 To a solution of **22** (13 mg, 3.3 μmol) and dansyl glycine (3 mg, 9.7 μmol) in DMF (1 ml) were added triethylamine (3.0 μl, 21.4 μmol) and DEPC (1.6 μl, 10.7 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 by silica gel column chromatography ($CHCl_3$: MeOH=20 : 1) as elute to give dansyl derivatives (12 mg, 86.8%). To a solution of this compound (12 mg, 2.8 μmol) in 1,4-dioxane–MeOH (1 : 1) (2 ml) was added NaOMe (40 mg), and the mixture was stirred for 4 h at room temperature, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with MeOH–H_2O and water. The filtrate and washings were combined and concentrated. Column chromatography (MeOH : H_2O=1 : 1) of the residue on Sephadex LH-20 gave **1** (5.8 mg, quant.). $[\alpha]_D^{23}$ +6.8° (*c*=0.4, H_2O). MALDI-TOF-MS: Calcd for $C_{82}H_{79}N_3O_{24}$: *m/z* 2104.9. Found *m/z* 2128.2 $[M+Na]^+$.

The following compound **2** was prepared according to the same procedures as described for the preparation of **1**.

Compound 23 Yield 89.6%; $[\alpha]_D^{23}$ +71.7° (*c*=0.9, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.07—7.21 (80H, m, Ar-H), 6.00 (4H, d, H-4), 5.76 (4H, br dd, H-2), 5.65 (4H, br dd, H-3), 4.87 (4H, br d, H-1), 4.65 (4H, m, H-6a), 4.42—4.36 (8H, m, H-5, H-6b), 3.95 and 3.70 (8H, m, OCH_2 of sugar unit×4), 3.47—3.35 (20H, m, NCH_2 of sugar unit×4, NCH_2CO×3, NCH_2 of β-alanine×3), 2.06 (6H, m, CH_2CO of β-alanine), 1.43, 1.40, 1.36, 1.35, 1.33 and 1.30 (9H, s, *t*-Bu). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.0, 165.5, 133.6, 133.3, 129.9, 129.7, 129.3, 129.0, 128.6, 128.5, 128.2, 101.6, 71.5, 71.4, 69.84, 68.79, 68.1, 61.8, 51.5, 39.1, 28.3. MALDI-TOF-MS: Calcd for $C_{164}H_{155}N_7O_{48}$: *m/z* 2990.0. Found: *m/z* 3013.2 $[M+Na]^+$.

Compound 24 Yield 88.0%; $[\alpha]_D^{23}$ +78.0° (*c*=1.0, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.08—7.21 (80H, m, Ar-H), 6.00 (4H, d, H-4), 5.76 (4H, br dd, H-2), 5.65 (4H, br dd, H-3), 4.90 (4H, br d, H-1), 4.66 (4H, m, H-6a), 4.43—4.36 (8H, m, H-5, H-6b), 3.96—3.03 (28H, m, OCH_2, NCH_2), 2.06 (6H, m, CCH_2). MALDI-TOF-MS: Calcd for $C_{159}H_{147}N_7O_{46}$: *m/z* 2889.9. Found: *m/z* 2913.3 $[M+Na]^+$.

Compound 25 Yield 90.6%; $[\alpha]_D^{23}$ +65.6° (*c*=0.9, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.08—7.20 (160H, m, Ar-H), 6.00 (8H, br d, H-4), 5.76 (8H, br dd, H-2), 5.65 (8H, br dd, H-3), 4.90 (8H, br d, H-1), 4.66 (8H, m, H-6a), 4.37 (16H, m, H-5, H-6b), 3.95—3.38 (52H, m, OCH_2, NCH_2), 2.57—2.12 (14H, m, CCH_2), 1.36 and 1.31 (9H, 2s, *t*-Bu). MALDI-TOF-MS: Calcd for $C_{328}H_{307}N_{15}O_{96}$: *m/z* 5991.0. Found: *m/z* 5914.1 $[M-Boc+Na]^+$.

Compound 26 Yield 82.1%; $[\alpha]_D^{23}$ +65.0° (*c*=0.4, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.03—7.20 (160H, m, Ar-H), 5.99 (8H, br d, H-4), 5.75 (8H, br dd, H-2), 5.65 (8H, br dd, H-3), 4.90 (8H, br d, H-1), 4.66 (8H, m, H-6a), 4.38 (16H, m, H-5, H-6b), 3.94—2.07 (66H, m, CH_2). MALDI-TOF-MS: Calcd for $C_{323}H_{299}N_{15}O_{94}$: *m/z* 5890.9. Found: *m/z* 5914.6 $[M+Na]^+$.

Compound 2 Yield 80.2%; $[\alpha]_D^{23}$ +8.4° (*c*=0.4, H_2O). MALDI-TOF-MS: Calcd for $C_{113}H_{185}N_{17}O_{65}S$: *m/z* 2852.1. Found: *m/z* 2875.5 $[M+Na]^+$.

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