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Studies of cyclodextrin effect for glycosylation by galactosyltransferase

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ABSTRACT

Cyclodextrins (CDs) do not disturb HPLC purification and do not form lather, which makes them superior to commonly used surfactants. In this paper, the authors describe the utility of CDs, specifically α-CD, β-CD, and γ-CD, for the synthesis of neoglycolipids from insoluble peptide-linked substrates. γ-CD was found to be well matched to the bulky benzyl group and accelerated the reaction for the substrate having Phe (F), while β-CD was well matched to the methyl group and accelerated the reaction for the substrate having Ala (A). Galactosylation to water-insoluble peptide-linked glucosaminide substrate by galactosyltransferase proceeded 63% at the most, but using either β-CD or γ-CD gave quantitative yield within 10 h.

1. Introduction

It is well known that carbohydrates play key roles in the fields of medicine and biology,[1–3] and carbohydrate-based drug discovery is a subject of current interest and challenge.[4] For example, oseltamivir (Tamiflu from Roche) is a derivative of neuraminic acid and has been developed by computational drug design and synthetic chemistries.[5–7] In addition, a number of biopharmaceuticals are glycoproteins.[8] Carbohydrate moieties of therapeutic glycoproteins are diverse mixtures because they are produced in human or animal cell lines. The degree of diversity is varied, depending on the cell line, the culture conditions and so on. Despite its subtle differences, glycosylation status could influence efficacy, safety, and immunogenicity. Biobetters are improved recombinant protein drugs that are unlike any existing biopharmaceuticals. It is commonly thought that the standardization of carbohydrates on therapeutic glycoproteins could be a key to developing biobetters. Therefore, carbohydrate chemists are expected to contribute to these research fields.

Chemical, enzymatic, and cellular syntheses are the three main methods for obtaining oligosaccharides artificially, and each method has advantages and disadvantages. Enzymatic synthesis can control glycosylation in specific regions as well as controlling stereoselectivities with ease, but substrates must be soluble in water. In addition, some enzymes (e.g. glycosyltransferases) are not stable, which results that their activities are lost during progression of reactions and the reactions are not completed in some cases. We have already reported on approaches to overcome these disadvantages. In particular, applying water-soluble polymer and additive cyclodextrins were found to effectively increase the solubility of the neoglycolipid. This allowed galactosylation by β1,4-galactosyltransferase[9] to proceed, even though the substrate itself was not soluble in water.[10]

Cyclodextrin (CD) is a family of compounds made up of D-glucopyranoside units α1–4 linked in a ring and widely used in food, cosmetic, and pharmaceutical areas. Naturally occurring α-CD, β-CD, and γ-CD are composed of six, seven, and eight D-glucopyranoside units, respectively, and all of them are commercially available. Although these cyclic oligosaccharides are similar forms, their solubilities are different as 145 g/L, 18.5 g/L, and 232 g/L at 25 °C for α-CD, β-CD, and γ-CD, respectively. They are topologically represented as toroids, and size and volume of cavities are reported as 0.57–1.37 nm and 173 A^3, 0.78–1.53 nm and 262 A^3, and 0.95–1.69 nm and 427 A^3 for α-CD, β-CD, and γ-CD, respectively.[11] Exterior of the toroids shows hydrophilicity and interior possesses relatively hydrophobic character, therefore CDs could form host–guest complexes with other hydrophobic molecules and the complexes become to soluble in water. Thermodynamical studies of CD–guest complex were well studied.[12]

Cyclodextrins do not disturb HPLC purification and do not form lather, which makes them superior to commonly used surfactant. In

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previous paper,[10] we have reported relationship between concentrations of γ-CD and compound **9**. Here, we report further details about the effects of cyclodextrins on a variety of peptide-linked neoglycolipids.

2. Results and discussion

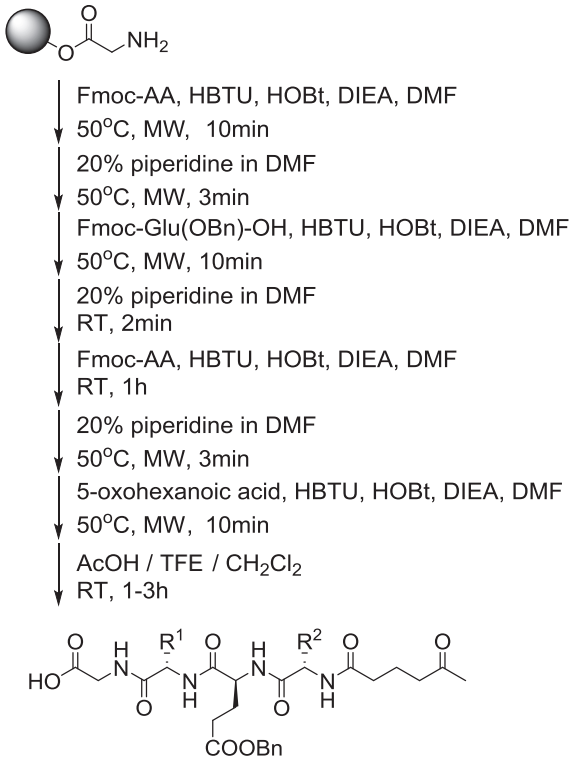
We first considered peptide linkers, which can conjugate with neoglycolipids. To take into account the use of BLase protease,[13] which releases synthesized oligosaccharide from the water-soluble polymer (as shown in the previous report[10]), Glu (E) was essential and hydrophobic amino acids situated next to Glu were preferred. To study the effectiveness of cyclodextrins on reactions for peptide linkers employing β1,4-galactosyltransferase,[9] we designed and synthesized seven kinds of 'oxo-XEYG' peptide linkers (**1**–**7**), where oxo-=$CH_3C(=O)CH_2CH_2CH_2C(=O)$– and X, Y=Gly (G), Ala (A) or Phe (F). These peptide linkers were synthesized by microwave-applied SPPS (solid-phase peptide synthesis) Fmoc strategy (Scheme 1). When the synthesized peptides were released from the supporting resin, the protecting group of side chain of Glu (E) was maintained, in order to account for the coupling with N-terminals of the peptides and 12-azidododecyl glucosaminide (**8**).[14–16] Therefore, we chose a benzyl ester protecting group for the side chain of Glu (E). Deprotection of the Fmoc group on Glu (E) by piperidine and successive amino acid coupling stages were performed then at room temperature for 2 min and 1 h, successively. If those reactions have been performed under microwave irradiation at 50 °C, deprotection of the benzyl ester group and pyroglutamization would have occurred. Finally, **1**[10]–**7** were successfully synthesized in 47–85% overall yields.

Conjugation of 12-azidododecyl glucosaminide (**8**) with the linkers followed by deprotection of benzyl group proceeded in 43–87% yields (Scheme 2). Although cyclodextrins are able to form host–guest complexes, Gly (G) has no side chain, and thus cyclodextrins would not work well in a host–guest system for Gly (G). Therefore, we judged that oxo-GEAG and oxo-AEGG linkers would not need to be tested.

We then studied galactosylation of these substrates by β1,4-galactosyltransferase (Scheme 3). When the reactions of 2 mM of substrate were performed with no additives, **14** (oxo-GEFG-gl) glycosylated in 63% for 10 h, but others proceeded only 36% at the most. Even though the reactions continued for 24 h and 48 h, only **14** (oxo-GEFG-gl) and **10** (oxo-AEAG-gl) were completed, successively, and **9** (oxo-FEFG-gl) was not galactosylated at all.

When 0.1% of Triton X-100 was added to the reactions, reactivity of **10** (oxo-AEAG-gl) and **11** (oxo-GEGG-gl) improved dramatically; the substrates with a single Phe (F) improved only to a small degree. **9** (oxo-FEFG-gl) did not become an enzyme substrate at all (data was not shown), although 1% of Triton X-100 was somewhat helpful.[10] Triton X-100 is a nonionic surfactant, and from these experiments, 0.1% Triton X-100 was found to be effective for increasing the solubility of the substrates with peptide linkers consisting of less hydrophobic amino acids, i.e., Gly (G) and Ala (A), but not for those with more hydrophobic amino acids, i.e., Phe (F).

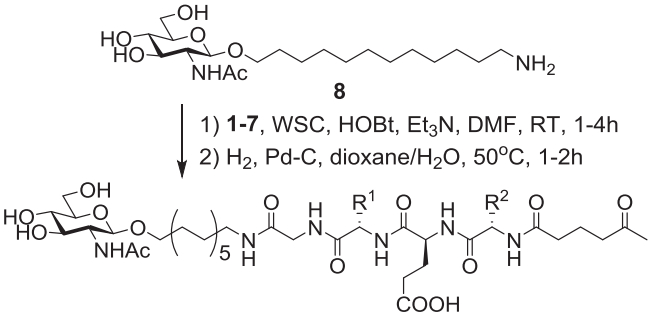
In order to clarify the effects of adding cyclodextrins, we first added 70 mM of α-CD. The subsequent reactions were somewhat improved on those with no additives, but the reactions with 0.1% of Triton X-100 added gave better results. However, when 10 mM of β-CD was added to the reactions, all of the reactions improved. Cyclodextrins are able to form host–guest complexes with hydrophobic molecules. From these experiments, β-CD was found to involve side chains of Ala (A) and Phe (F), namely CH_3– and $C_6H_5CH_2$–, which then increased the solubility of the substrates, resulting in accelerated galactosylation by β1,4-galactosyltransferase. We also observed that the reaction for **11** (oxo-GEGG-gl) was improved, but Gly (G) has no side chain.



1: $R^1 = R^2$ = Bn (58%) **4**: R^1 = Bn, R^2 = Me (63%)
2: $R^1 = R^2$ = Me (78%) **5**: R^1 = Me, R^2 = Bn (57%)
3: $R^1 = R^2$ = H (47%) **6**: R^1 = Bn, R^2 = H (85%)
7: R^1 = H, R^2 = Bn (83%)

AA = Phe, Ala or Gly
MW = microwave irradiation

Scheme 1. Syntheses of peptide linkers. Reagents. Coupling: HBTU, HOBt, DIEA in DMF, deprotection: 20% piperidine in DMF, cleavage: AcOH/CF_3CH_2OH/CH_2Cl_2 (2/2/6, v/v/v).

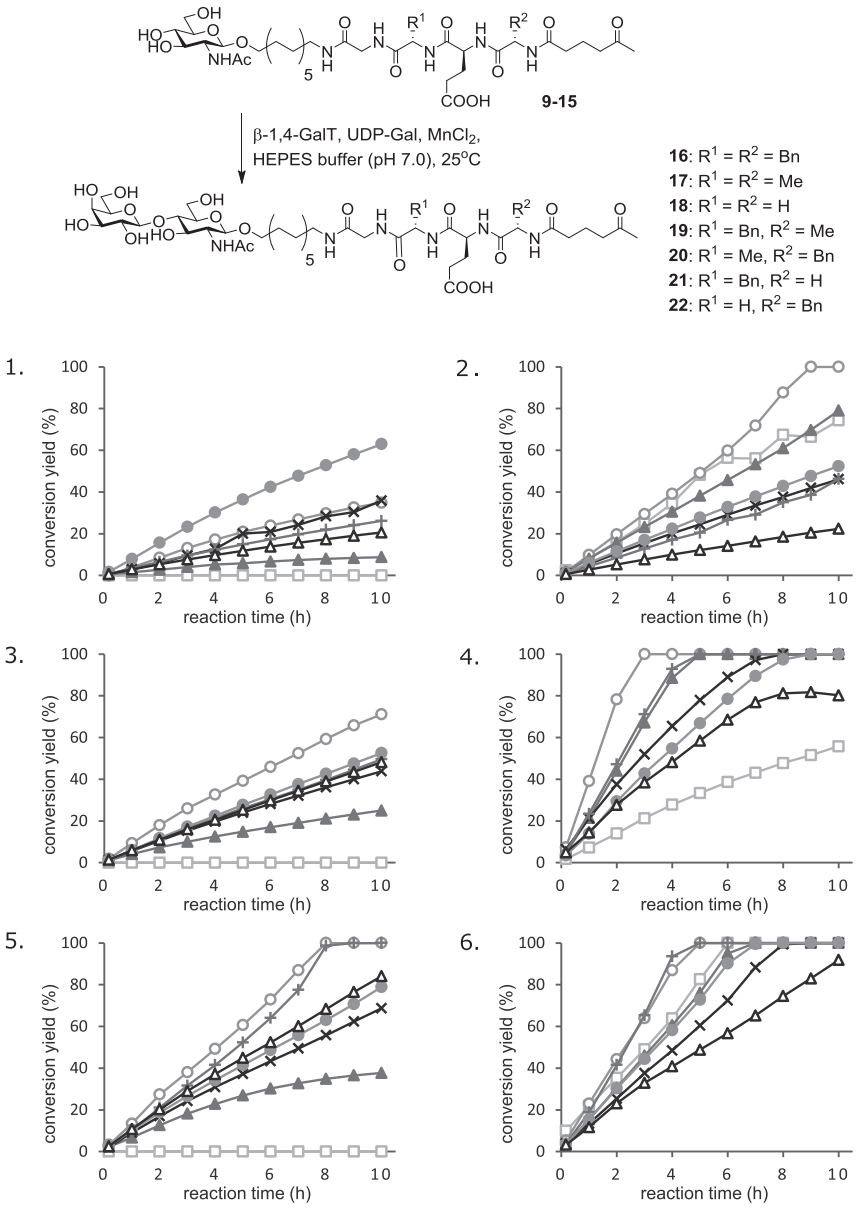


9: $R^1 = R^2$ = Bn (87%) **12**: R^1 = Bn, R^2 = Me (76%)
10: $R^1 = R^2$ = Me (55%) **13**: R^1 = Me, R^2 = Bn (43%)
11: $R^1 = R^2$ = H (51%) **14**: R^1 = Bn, R^2 = H (55%)
15: R^1 = H, R^2 = Bn (56%)

Scheme 2. Conjugation between 12-azidododecyl 2-acetoamido-2-deoxy-β-D-glucopyranoside and the peptide linkers (**1**–**7**).

Therefore, β-CD may have an ability to increase the solubility of substrates like a phase transfer catalyst. Because of the solubility of β-CD itself, we could not test this by adding a higher concentration of β-CD.

Next, we tested the effects of γ-CD. The addition of 70 mM of γ-CD resulted in all seven reactions completing within 12 h. It was especially effective for **9** (oxo-FEFG-gl), but it was less effective for **10** (oxo-AEAG-gl) than for the addition of β-CD. This may be



Scheme 3. Syntheses of lactosamine derivatives by β1,4-galactosyltransferase. Graphs 1: no additives, 2: with 0.1% Triton X-100, 3: with 70 mM α-CD, 4: with 10 mM β-CD, 5: with 10 mM γ-CD, 6: with 70 mM γ-CD. Substrates **9**: square, **10**: white circle, **11**: triangle, **12**: cross, **13**: plus, **14**: gray circle, **15**: white triangle.

because γ-CD was well matched to the bulky side chain of Phe (F), $C_6H_5CH_2$–, but not to the side chain of Ala (A), CH_3–, while it was the inverse for β-CD. All reactions became slower after the addition of 10 mM of γ-CD than after 70 mM γ-CD, and **9** (oxo-FEFG-gl) did not react at all. This indicated that γ-CD worked concentration-dependently. In addition, comparing the addition of 10 mM β-CD to 10 mM γ-CD for **11** (oxo-GEGG-gl) led to the conclusion that β-CD had a stronger ability to solve the substrates than γ-CD.

3. Conclusions

One way to study how cyclodextrins affect substrates at the molecular level is to use NMR. Observation of chemical shift changes of the substrates with or without cyclodextrins could clearly show whether cyclodextrins form host–guest complexes with peptide side chains. These experiments have been difficult, however, due to the poor solubility of the substrates.

Nevertheless this background, we successfully showed that the effectiveness of cyclodextrins is highly dependent upon side chains functional groups. With the results of the previous report,[10] the higher concentration of **9** required the more γ-CD to perform this enzymatic galactosylation, we concluded that cyclodextrins should work mainly in a host–guest system in this enzymatic galactosylation. From the result of 10 mM β-CD (Scheme 3, graph 4), β-CD worked well for methyl group. From comparison of reactions of **12** and **13**, methyl group of sugar side might well coordinate with β-CD. In addition, β-CD could work also to benzyl groups as reactions of **14**, **15**, and **9** have been accelerated. Same as the case of methyl group, benzyl group of sugar side might well recognized that of N-terminal side. Regarding γ-CD, the result that the reactivities for **14** and **15** with 10 mM γ-CD were similar and **14** had been more accelerated than **15** by 70 mM γ-CD indicated that benzyl groups of sugar side might be well involved with γ-CD. The reactions for **9** and **13** were similar in either with 10 mM or with 70 mM γ-CD, thus affinity of γ-CD to Ala (A) and Phe (F) on N-terminal side would be same.

From a synthetic chemistry perspective, discussing the enzymatic reaction by V_0 and Km in this paper is not appropriate,

because we aim to use enzymes as reaction catalyses and to have products in higher yields. We faced many cases in which despite two reaction conditions having the same V_0, one could give a quantitative yield while another had the reaction stop. As seen in Scheme 3, using either β-CD or γ-CD gave quantitative yield within 10 h for these galactosylations, which was desirable from a synthetic chemistry standpoint.

4. Experiments

4.1. General

Polymer supported peptide syntheses with microwave irradiation were performed in the EYELA MWS-1000, and reaction temperatures were monitored by an optional equipped fiber optic probe, which was directly inserted into the reaction medium. All reagents were used as purchased without further purification and were purchased from Wako Pure Chemical Industries, Ltd. (Japan), Nacalai tesque, Inc. (Japan), Dojindo Laboratories (Japan) or Sigma–Aldrich Chemical Co. (USA). Reagents for peptide derivatives were also purchased from Watanabe Chimical Industries, Ltd. (Japan), Peptide Institute, Inc. (Japan) or Novabiochem, Merck group (Germany). UDP-galactose (UDP-Gal) and β1,4-galactosyltransferase (GalT) were purchased from Yamasa Co. (Japan) and Sigma–Aldrich Chemical Co. (USA), respectively. Reactions were monitored by TLC, which was performed with 0.25 mm precoated silica gel $60F_{254}$ on glass from Merck (Darmstadt, Germany). Compounds were detected by dipping the TLC plates in an ethanolic solution of phosphomolybdic acid (5% v/v) or spraying ninhydrin and heating. Silica gel N60 (40–50 nm) from Kanto Chemical (Tokyo, Japan) was used for silica gel chromatography. All NMR data are reported in parts per million downfield shift from tetramethylsilane. ^{1}H NMR spectra and ^{13}C NMR spectra were routinely recorded at 400 MHz and 100 MHz on a BRUKER AVANCE 400 spectrometer at 300 K, respectively, and chemical shifts were expressed relative to methyl proton of tetramethylsilane (δ 0.00 ppm). High-resolution ESI-mass data were recorded by mass spectrometry services at the Center for Instrumental Analysis, Hokkaido University. In lactosamine disaccharide cases, proton positions of glucose and galactose parts were shown by simple positional numbers and numbers with single prime (′), respectively. In peptide linkers, amino acid moieties were numbered from N-terminal.

4.2. Syntheses peptide linkers

All the reactions were carried out in a 20 ml LibraTube®. To a mixture of swelled H-Gly-Trt(2-Cl) resin (0.85 mmol/g, 1.00 g, 0.85 mmol), which was pretreated by DMF for a period of 2 h, Fmoc-Xxx-OH, HBTU (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) (967 mg, 2.55 mmol), and HOBt (1-hydroxybenzotriazole) (345 mg, 2.55 mmol) in DMF (5.5 ml) was added DIEA (*N*,*N*-diisopropylethylamine) (888 μl, 5.10 mmol) at rt. The reaction mixture was shaken for 10 min at 50 °C under microwave irradiation, and the resin was washed with DMF and CH_2Cl_2. The resulting resin in 20% piperidine/DMF (5 ml) and shaken for 3 min at 50 °C under microwave irradiation and washed with DMF and CH_2Cl_2.

To a mixture of the resulting resin, Fmoc-Glu(OBn)-OH (1.18 g, 2.55 mmol), HBTU (967 mg, 2.55 mmol), and HOBt (345 mg, 2.55 mmol) in DMF (5 ml) was added DIEA (888 μl, 5.10 mmol) at rt. The reaction mixture was shaken for 10 min at 50 °C under microwave irradiation, and the resin was washed with DMF and CH_2Cl_2. Then, the resulting resin in 20% piperidine/DMF (5 ml) was shaken for 2 min at rt, and washed with DMF and CH_2Cl_2. To a mixture of the resulting resin, Fmoc-Yyy-OH, HBTU (967 mg, 2.55 mmol), and HOBt (345 mg, 2.55 mmol) in DMF (5 ml) was added DIEA (888 μl, 5.10 mmol) at rt. The reaction mixture was shaken for 1 h at rt, and the resin was washed with DMF and CH_2Cl_2. The resulting resin in 20% piperidine/DMF (5 ml) was shaken for 3 min at 50 °C under microwave irradiation and washed with DMF and CH_2Cl_2. To a mixture of the resulting resin, 5-oxohexanoic acid (304 μl, 2.55 mmol), HBTU (967 mg, 2.55 mmol), and HOBt (345 mg, 2.55 mmol) in DMF (5 ml) was added DIEA (888 μl, 5.10 mmol) at rt. The reaction mixture was shaken for 10 min at 50 °C under microwave irradiation and the resulting resin was washed with DMF and CH_2Cl_2, and dried. A part of the resulting resin was treated with AcOH/CF_3CH_2OH/CH_2Cl_2 (2:2:6 v/v/v, 10 ml) at rt, filtered, and washed with the same cocktail in three times. The combined filtrates were concentrated by streaming of nitrogen gas and the resulting residue was purified by RP-HPLC [column: Inertsil ODS-3 (ϕ20×250 mm); column oven temperature: 40 °C; flow rate: 5 ml/min, detection: 220 nm UV; eluent A: 25 mM ammonium acetate buffer (pH 5.8), eluent B: acetonitrile containing 10%, eluent C: H_2O containing 0.1% TFA and eluent D: acetonitrile containing 0.1% TFA] Elution conditions were shown individually.

*4.2.1. N-(5-Oxohexanoyl)-L-alanyl-L-(γ-benzyl)glutamyl-L-alanyl glycine (**2**).* Fmoc-Xxx-OH and Fmoc-Yyy-OH were Fmoc-Ala-OH (802 mg, 2.58 mmol) and Fmoc-Ala-OH (816 mg, 2.62 mmol), successively. A part (657 mg) of the dried resulting resin (1.415 g) was treated with AcOH/CF_3CH_2OH/CH_2Cl_2 (2:2:6 v/v/v, 10 ml) for 1.5 h at rt. RP-HPLC [eluent B in eluent A, gradient increase from 10% to 40% over 50 min] gave **2** (168 mg, 78%) as an amorphous. ^{1}H NMR (400 MHz, $(CD_3)_2S$=O): δ 8.04–7.99 (3H, m, Ala^1-N*H*, Glu^2-N*H*, and Ala^3-N*H*), 7.73 (1H, t, *J*=4.4 Hz, Gly^4-N*H*), 7.39–7.29 (5H, m, aromatic-*H*), 5.08 (2H, s, PhCH_2O–), 4.30–4.20 (3H, m, Ala^1-Hα, Glu^2-Hα, and Ala^3-Hα), 3.51 (1H, dd, *J*=16.6, 4.4 Hz, Gly^4-Hα), 3.48 (1H, dd, *J*=16.6, 4.4 Hz, Gly^4-Hα), 2.41–2.38 (4H, m, Glu^2-Hγ and –CH_2C(=O)CH_3), 2.08 (2H, t, *J*=7.2 Hz, –NHC(=O)CH_2–), 2.04 (3H, s, –CH_2C(=O)CH_3), 1.97 (1H, m, Glu^2-Hβ), 1.81 (1H, m, Glu^2-Hβ), 1.64 (2H, qui, *J*=7.2 Hz, –C(=O)CH_2C$H_2$$CH_2$C(=O)–), 1.20 (3H, d, *J*=7.2 Hz, Ala^1-Hβ or Ala^3-Hβ), 1.18 (3H, d, *J*=7.2 Hz, Ala^1-Hβ or Ala^3-Hβ). ^{13}C NMR (100 MHz, $(CD_3)_2S$=O): δ 208.17, 172.46, 172.29, 171.69, 171.66, 170.75, 170.37, 136.20, 128.40, 127.92, 127.85, 65.41, 51.52, 48.17, 48.06, 41.92, 34.07, 29.95, 29.69, 27.25, 19.30, 18.24, 17.84. ESI-HRMS: $C_{26}H_{35}N_4O_9$ [M–H]$^-$ calcd (*m*/*z*) 547.24095, found (*m*/*z*) 547.24259.

*4.2.2. N-(5-Oxohexanoyl)-glycinyl-L-(γ-benzyl)glutamyl-glycinyl glycine (**3**).* Fmoc-Xxx-OH and Fmoc-Yyy-OH were Fmoc-Gly-OH (754 mg, 2.54 mmol) and Fmoc-Gly-OH (783 mg, 2.63 mmol), successively. A part (784 mg) of the dried resulting resin (1.304 g) was treated with AcOH/CF_3CH_2OH/CH_2Cl_2 (2:2:6 v/v/v, 10 ml) for 3 h at rt. RP-HPLC [eluent D in eluent C, gradient increase from 10% to 30% over 60 min] gave **3** (125 mg, 47%) as an amorphous. ^{1}H NMR (400 MHz, $(CD_3)_2S$=O): δ 12.56 (1H, br, –C(=O)O*H*), 8.24 (1H, t, *J*=5.6 Hz, Gly^1-N*H*, Gly^3-N*H* or Gly^4-N*H*), 8.11 (1H, t, *J*=5.6 Hz, Gly^1-N*H*, Gly^3-N*H* or Gly^4-N*H*), 8.05–8.02 (2H, m, Glu^2-N*H* and Gly^1-N*H*, Gly^3-N*H* or Gly^4-N*H*), 7.40–7.31 (5H, m, aromatic-*H*), 5.09 (2H, s, PhCH_2O–), 4.32 (1H, dt, *J*=7.6, 6.0 Hz, Glu^2-Hα), 3.82–3.71 (6H, m, Gly^1-Hα, Gly^3-Hα, and Gly^4-Hα), 2.44–2.39 (4H, m, Glu^2-Hγ and –CH_2C(=O)CH_3), 2.11 (2H, t, *J*=7.2 Hz, –NHC(=O)CH_2–), 2.05 (3H, s, –CH_2C(=O)CH_3), 1.99 (1H, m, Glu^2-Hβ), 1.82 (1H, m, Glu^2-Hβ), 1.67 (2H, qui, *J*=7.2 Hz, –C(=O)CH_2C$H_2$$CH_2$C(=O)–). ^{13}C NMR (100 MHz, $(CD_3)_2S$=O): δ 208.16, 172.27, 172.25, 171.15, 171.06, 169.19, 168.94, 136.16, 128.39, 127.94, 127.88, 65.44, 51.73, 42.00, 41.90, 41.69, 40.54, 34.12, 29.88, 29.68, 27.15, 19.29. ESI-HRMS: $C_{24}H_{31}N_4O_9$ [M–H]$^-$ calcd (*m*/*z*) 519.20000, found (*m*/*z*) 519.21090.

*4.2.3. N-(5-Oxohexanoyl)-L-alanyl-L-(γ-benzyl)glutamyl-L-phenylalanyl glycine (**4**).* Fmoc-Xxx-OH and Fmoc-Yyy-OH were Fmoc-

Phe-OH (977 mg, 2.52 mmol) and Fmoc-Ala-OH (799 mg, 2.57 mmol), successively. A part (862 mg) of the dried resulting resin (1.438 g) was treated with AcOH/CF_3CH_2OH/CH_2Cl_2 (2:2:6 v/v/v, 10 ml) for 3 h at rt. RP-HPLC [eluent D in eluent C, gradient increase from 20% to 50% over 60 min] gave **4** (199 mg, 63%) as an amorphous. ^{1}H NMR (400 MHz, $(CD_3)_2S{=}O$): δ 12.57 (1H, br, −C(=O)O*H*), 8.32 (1H, t, *J*=5.8 Hz, Gly^4-N*H*), 7.99 (1H, d, *J*=6.8 Hz, Ala^1-N*H*), 7.89 (1H, d, *J*=8.0 Hz, Glu^2-N*H*), 7.89 (1H, d, *J*=8.0 Hz, Phe^3-N*H*), 7.41–7.11 (10H, m, aromatic-*H*), 5.08 (2H, s, PhC*H*$_2$O−), 4.54 (1H, ddd, *J*=9.6, 8.0, 4.4 Hz, Phe^3-Hα), 4.26–4.17 (2H, m, Glu^2-Hα and Ala^1-Hα), 3.80 (1H, dd, *J*=17.6, 5.8 Hz, Gly^4-Hα), 3.74 (1H, dd, *J*=17.6, 5.8 Hz, Gly^4-Hα), 3.05 (1H, dd, *J*=14.0, 4.4 Hz, Phe^3-Hβ), 2.79 (1H, dd, *J*=14.0, 9.6 Hz, Phe^3-Hβ), 2.39 (2H, t, *J*=7.4 Hz, −C*H*$_2$C(=O)CH$_3$), 2.34–2.19 (2H, m, Glu^2-Hγ), 2.07 (2H, t, *J*=7.4 Hz, −NHC(=O)C*H*$_2$−), 2.03 (3H, s, −CH$_2$C(=O)C*H*$_3$), 1.86 (1H, m, Glu^2-Hβ), 1.74 (1H, m, Glu^2-Hβ), 1.64 (2H, qui, *J*=7.4 Hz, −C(=O)CH$_2$C*H*$_2$CH$_2$C(=O)−), 1.14 (3H, d, *J*=6.8 Hz, Ala^1-Hβ). ^{13}C NMR (100 MHz, $(CD_3)_2S{=}O$): δ 208.13, 172.45, 172.22, 171.71, 171.15, 170.97, 170.54, 137.55, 136.18, 129.14, 128.40, 127.96, 127.86, 126.20, 65.39, 53.46, 51.80, 48.11, 41.91, 40.62, 37.53, 34.06, 29.83, 29.67, 27.23, 19.30, 17.73. ESI-HRMS: $C_{32}H_{39}N_4O_9$ $[M-H]^-$ calcd (*m*/*z*) 623.27225, found (*m*/*z*) 623.27394.

*4.2.4. N-(5-Oxohexanoyl)-L-phenylalanyl-L-(γ-benzyl)glutamyl-L-alanyl glycine (**5**).* Fmoc-Xxx-OH and Fmoc-Yyy-OH were Fmoc-Ala-OH (783 mg, 2.51 mmol) and Fmoc-Phe-OH (994 mg, 2.57 mmol), successively. A part (900 mg) of the dried resulting resin (1.387 g) was treated with AcOH/CF_3CH_2OH/CH_2Cl_2 (2:2:6 v/v/v, 10 ml) for 3 h at rt. RP-HPLC [eluent D in eluent C, gradient increase from 20% to 50% over 60 min] gave **5** (195 mg, 57%) as an amorphous. ^{1}H NMR (400 MHz, $(CD_3)_2S{=}O$): δ 12.54 (1H, br, −C(=O)O*H*), 8.18 (1H, t, *J*=5.8 Hz, Gly^4-N*H*), 8.08 (1H, d, *J*=8.0 Hz, Glu^2-N*H*), 8.04 (1H, d, *J*=8.0 Hz, Phe^1-N*H*), 8.02 (1H, d, *J*=7.2 Hz, Ala^3-N*H*), 7.40–7.15 (10H, m, aromatic-*H*), 5.09 (2H, s, PhC*H*$_2$O−), 4.53 (1H, m, Phe^1-Hα), 4.33–4.26 (2H, m, Glu^2-Hα and Ala^3-Hα), 3.80 (1H, dd, *J*=17.6, 5.8 Hz, Gly^4-Hα), 3.71 (1H, dd, *J*=17.6, 5.8 Hz, Gly^4-Hα), 3.00 (1H, dd, *J*=13.6, 4.0 Hz, Phe^1-Hβ), 2.72 (1H, dd, *J*=13.6, 10.4 Hz, Phe^1-Hβ), 2.40 (2H, t, *J*=8.0 Hz, Glu^2-Hγ), 2.20 (2H, t, *J*=7.2 Hz, −C*H*$_2$C(=O)CH$_3$), 2.02–1.91 (3H, m, −NHC(=O)C*H*$_2$− and Glu^2-Hβ), 1.98 (3H, s, −CH$_2$C(=O)C*H*$_3$), 1.83 (1H, m, Glu^2-Hβ), 1.53 (2H, qui, *J*=7.2 Hz, −C(=O)CH$_2$C*H*$_2$CH$_2$C(=O)−), 1.26 (3H, d, *J*=7.2 Hz, Ala^1-Hβ). ^{13}C NMR (100 MHz, $(CD_3)_2S{=}O$): δ 207.91, 172.25, 172.20, 171.66, 171.36, 170.95, 170.22, 137.93, 136.10, 129.02, 128.32, 127.87, 127.79, 126.07, 65.35, 53.62, 51.50, 47.87, 41.59, 40.46, 37.17, 34.06, 29.86, 29.58, 27.21, 19.14, 18.13. ESI-HRMS: $C_{32}H_{39}N_4O_9$ $[M-H]^-$ calcd (*m*/*z*) 623.27225, found (*m*/*z*) 623.27410.

*4.2.5. N-(5-Oxohexanoyl)-glycinyl-L-(γ-benzyl)glutamyl-L-phenylalanyl glycine (**6**).* **6** was prepared in a half scale of other peptides. Fmoc-Xxx-OH and Fmoc-Yyy-OH were Fmoc-Phe-OH (488 mg, 1.26 mmol) and Fmoc-Gly-OH (372 mg, 1.25 mmol), successively. The resulting dried resulting resin (851 mg) was treated with AcOH/CF_3CH_2OH/CH_2Cl_2 (2:2:6 v/v/v, 10 ml) for 1 h at rt. RP-HPLC [eluent D in eluent C, gradient increase from 20% to 50% over 60 min] gave **6** (208 mg, 85%) as an amorphous. ^{1}H NMR (400 MHz, $(CD_3)_2S{=}O$): δ 12.55 (1H, br, −C(=O)O*H*), 8.23 (1H, m, Gly^1-N*H* or Gly^4-N*H*), 8.06 (1H, m, Gly^1-N*H* or Gly^4-N*H*), 8.02 (1H, d, *J*=8.4 Hz, Phe^3-N*H*), 7.97 (1H, d, *J*=7.6 Hz, Glu^2-N*H*), 7.40–7.14 (10H, m, aromatic-*H*), 5.08 (2H, s, PhC*H*$_2$O−), 4.53 (1H, m, Phe^3-Hα), 4.22 (1H, m, Glu^2-Hα), 3.80 (1H, dd, *J*=17.6, 5.8 Hz, Gly^1-Hα or Gly^4-Hα), 3.75 (1H, dd, *J*=17.6, 5.8 Hz, Gly^1-Hα or Gly^4-Hα), 3.68 (2H, d, *J*=5.2 Hz, Gly^1-Hα or Gly^4-Hα), 3.07 (1H, dd, *J*=13.6, 4.0 Hz, Phe^3-Hβ), 2.81 (1H, dd, *J*=13.6, 10.0 Hz, Phe^3-Hβ), 2.42 (2H, t, *J*=7.2 Hz, −C*H*$_2$C(=O)CH$_3$), 2.27 (2H, t, *J*=7.8 Hz, Glu^2-Hγ), 2.12 (2H, t, *J*=7.2 Hz, −NHC(=O)C*H*$_2$−), 2.05 (3H, s, −CH$_2$C(=O)C*H*$_3$), 1.86 (1H, m, Glu^2-Hβ), 1.72 (1H, m, Glu^2-Hβ), 1.67 (2H, qui, *J*=7.2 Hz, −C(=O)CH$_2$C*H*$_2$CH$_2$C(=O)−). ^{13}C NMR (100 MHz, $(CD_3)_2S{=}O$): δ 208.07, 172.03, 172.12, 171.14, 170.90, 170.55, 169.19, 137.65, 136.10, 129.05, 128.33, 127.94, 127.89, 127.82, 126.14, 65.35, 53.65, 51.77, 41.98, 41.85, 40.58, 37.23, 34.02, 29.73, 29.60, 27.10, 19.20. ESI-HRMS: $C_{31}H_{37}N_4O_9$ $[M-H]^-$ calcd (*m*/*z*) 609.25660, found (*m*/*z*) 609.25813.

*4.2.6. N-(5-Oxohexanoyl)-L-phenylalanyl-L-(γ-benzyl)glutamyl-glycinyl glycine (**7**).* **7** was prepared in a half scale of other peptides (same scale with **6**). Fmoc-Xxx-OH and Fmoc-Yyy-OH were Fmoc-Gly-OH (398 mg, 1.34 mmol) and Fmoc-Phe-OH (523 mg, 1.35 mmol), successively. The resulting dried resulting resin (737 mg) was treated with AcOH/CF_3CH_2OH/CH_2Cl_2 (2:2:6 v/v/v, 10 ml) for 1.5 h at rt. RP-HPLC [eluent D in eluent C, gradient increase from 20% to 40% over 60 min] gave **7** (219 mg, 83%) as an amorphous. ^{1}H NMR (400 MHz, $(CD_3)_2S{=}O$): δ 8.15–8.13 (2H, m, Gly^3-N*H* or Gly^4-N*H*, and Glu^2-N*H*), 8.10–8.05 (2H, m, Gly^3-N*H* or Gly^4-N*H*, and Phe^1-N*H*), 7.40–7.16 (10H, m, aromatic-*H*), 5.10 (2H, s, PhC*H*$_2$O−), 4.55 (1H, ddd, *J*=10.0, 8.0, 4.4 Hz, Phe^1-Hα), 4.32 (1H, dt, *J*=6.0, 7.6 Hz, Glu^2-Hα), 3.79 (2H, d, *J*=5.6 Hz, Gly^3-N*H* or Gly^4-N*H*), 3.75 (2H, d, *J*=5.6 Hz, Gly^3-N*H* or Gly^4-N*H*), 3.03 (1H, dd, *J*=14.0, 4.0 Hz, Phe^1-Hβ), 2.75 (1H, dd, *J*=14.0, 10.6 Hz, Phe^1-Hβ), 2.41 (2H, t, *J*=7.6 Hz, Glu^2-Hγ), 2.21 (2H, t, *J*=7.2 Hz, −C*H*$_2$C(=O)CH$_3$), 2.06–1.95 (3H, m, −NHC(=O)C*H*$_2$− and Glu^2-Hβ), 1.99 (3H, s, −CH$_2$C(=O)C*H*$_3$), 1.85 (1H, m, Glu^2-Hβ), 1.54 (2H, qui, *J*=7.2 Hz, −C(=O)CH$_2$C*H*$_2$CH$_2$C(=O)−). ^{13}C NMR (100 MHz, $(CD_3)_2S{=}O$): δ 208.53, 172.29, 171.87, 171.64, 171.11, 171.07, 168.97, 130.08, 136.21, 129.16, 128.44, 128.01, 127.99, 127.92, 126.20, 65.49, 53.81, 51.87, 41.76, 41.70, 40.60, 37.20, 34.17, 29.90, 29.69, 27.18, 19.27. ESI-HRMS: $C_{31}H_{37}N_4O_9$ $[M-H]^-$ calcd (*m*/*z*) 609.25660, found (*m*/*z*) 609.25836.

4.3. Syntheses of peptide-linked glucosaminide substrates

*4.3.1. 12-[N-(5-Oxohexanoyl)-L-phenylalanyl-L-glutamyl-L-phenylalanyl-glycinyl]-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**9**).*[10] To the solution of **1** (59 mg, 0.13 mmol), HOBt (20 mg, 0.15 mmol), and WSC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) (29 mg, 0.15 mmol) in DMF (3 ml) were added 12-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside acetic acid salt (59 mg, 0.13 mmol) and triethylamine (18 μl, 0.13 mmol) successively. The reaction mixture was stirred for 1 h at rt and directly purified by SiO_2 column chromatography. Eluent of EtOAc/EtOH/H_2O (12:2:1) gave assumed benzyl protected **9**. 10% Pd–C (30 mg) was added to a solution of the resulting material in dioxane/water (1:1 v/v, 10 ml), and the reaction mixture was stirred under hydrogen for 1 h at 50 °C. After removal of Pd–C by Celite filtration, the filtrate was concentrated, and purified by the preparative RP-HPLC [column: Inertsil ODS-3 (ϕ20×250 mm); eluent A: H_2O containing 0.1% TFA, eluent B: acetonitrile containing 0.1% TFA; eluate B gradient increase from 30% to 60% over 50 min; column oven temperature: 40 °C; flow rate: 5 ml/min, detection: 220 nm UV] to give **9**[10] (109 mg, 87%) as an amorphous.

*4.3.2. 12-[N-(5-Oxohexanoyl)-L-alanyl-L-glutamyl-L-alanyl-glycinyl]-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**10**).* To the solution of **2** (83 mg, 0.15 mmol), HOBt (24 mg, 0.17 mmol), and WSC (33 mg, 0.17 mmol) in DMF (3.5 ml) were added 12-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside acetic acid salt (70 mg, 0.15 mmol) and triethylamine (24 μl, 0.17 mmol) successively. The reaction mixture was stirred for 4 h at rt and directly purified by SiO_2 column chromatography. Eluent of EtOAc/EtOH/H_2O (9:2:1) gave benzyl protected **10**. 10% Pd–C (20 mg) was added to a solution of the resulting material in dioxane/water (1:1 v/v, 10 ml), and the reaction mixture was stirred under hydrogen for 2 h at 50 °C. After removal of Pd–C by Celite filtration, the filtrate was concentrated, and purified by the preparative RP-HPLC [column: Inertsil ODS-3 (ϕ20×250 mm); column oven temperature:

40 °C; flow rate: 5 ml/min, detection: 220 nm UV; eluate D in eluent C gradient increase from 10% to 40% over 60 min] to give **10** (70 mg, 55%) as an amorphous. ^{1}H NMR (400 MHz, $(CD_3)_2S{=}O$): δ 12.07 (1H, br, −C(=O)O*H*), 8.12 (1H, t, *J*=6.0 Hz, Gly^4-N*H*), 8.03 (1H, d, *J*=7.2 Hz, Ala^1-N*H* or Ala^3-N*H*), 8.01 (1H, d, *J*=6.8 Hz, Ala^1-N*H* or Ala^3-N*H*), 7.96 (1H, d, *J*=8.0 Hz, Glu^2-N*H*), 7.65–7.61 (2H, m, −N*H*C(=O)CH$_3$ and −CH$_2$CH$_2$N*H*C(=O)−), 4.94 (1H, br, OH-4), 4.86 (1H, br, OH-3), 4.48 (1H, br, OH-6), 4.25 (1H, d, *J*=8.0 Hz, H-1), 4.24–4.17 (3H, m, Ala^1-Hα, Glu^2-Hα, and Ala^3-Hα), 3.73–3.56 (4H, m, −OC*H*$_2$CH$_2$−, H-6b and Gly^4-Hα), 3.46–3.25 (4H, m, H-6a, H-2, −OC*H*$_2$CH$_2$−, and H-3), 3.06–3.01 (4H, m, −CH$_2$C*H*$_2$NH−, H-5 and H-4), 2.42 (2H, t, *J*=7.2 Hz, −C*H*$_2$C(=O)CH$_3$), 2.24 (2H, t, *J*=7.6 Hz, Glu^2-Hγ), 2.10 (2H, t, *J*=7.2 Hz, −NHC(=O)C*H*$_2$−), 2.06 (3H, s, −CH$_2$C(=O)C*H*$_3$), 1.90 (1H, m, Glu^2-Hβ), 1.78 (3H, s, −NHC(=O)C*H*$_3$), 1.75 (1H, m, Glu^2-Hβ), 1.66 (2H, qui, *J*=7.2 Hz, −C(=O)CH$_2$C*H*$_2$CH$_2$C(=O)−), 1.43–1.38 (4H, m, −OCH$_2$C*H*$_2$− and −C*H*$_2$CH$_2$NH−), 1.26–1.21 (16H, m, −OCH$_2$CH$_2$(C*H*$_2$)$_8$−), 1.22 (3H, d, *J*=6.8 Hz, Ala^1-Hβ or Ala^3-Hβ), 1.19 (3H, d, *J*=7.2 Hz, Ala^1-Hβ or Ala^3-Hβ). ^{13}C NMR (100 MHz, $(CD_3)_2S{=}O$): δ 208.05, 173.90, 172.53, 172.20, 171.82, 170.92, 168.77, 168.19, 100.91, 76.86, 74.17, 70.57, 68.13, 60.97, 55.35, 51.66, 48.48, 48.25, 42.00, 41.85, 38.42, 33.97, 29.94, 29.61, 29.03, 28.96, 28.93, 28.74, 28.66, 27.00, 26.27, 25.35, 22.92, 19.19, 17.66, 17.57. ESI-HRMS: $C_{39}H_{67}N_6O_{14}$ $[M-H]^-$ calcd (*m*/*z*) 843.47207, found (*m*/*z*) 843.47382.

*4.3.3. 12-[N-(5-Oxohexanoyl)-glycinyl-L-glutamyl-glycinyl-glycinyl]-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**11**).* To the solution of **3** (100 mg, 0.19 mmol), HOBt (31 mg, 0.23 mmol), and WSC (44 mg, 0.23 mmol) in DMF (4 ml) were added 12-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside acetic acid salt (89 mg, 0.19 mmol) and triethylamine (32 μl, 0.23 mmol) successively. The reaction mixture was stirred for 2.5 h at rt and directly purified by SiO_2 column chromatography. Eluent of EtOAc/EtOH/H_2O (6:2:1) gave assumed benzyl protected **11**. 10% Pd−C (30 mg) was added to a solution of the resulting material in dioxane/water (1:1 v/v, 10 ml), and the reaction mixture was stirred under hydrogen for 1 h at 50 °C. After removal of Pd−C by Celite filtration, the filtrate was concentrated, and purified by the preparative RP-HPLC [column: Inertsil ODS-3 (ϕ20×250 mm); column oven temperature: 40 °C; flow rate: 5 ml/min, detection: 220 nm UV; eluate D in eluent C gradient increase from 10% to 30% over 60 min] to give **11** (80 mg, 51%) as an amorphous. ^{1}H NMR (400 MHz, $(CD_3)_2S{=}O$): δ 12.08 (1H, s, −C(=O)O*H*), 8.22 (1H, t, *J*=5.6 Hz, Gly^1-N*H*, Gly^3-N*H* or Gly^4-N*H*), 8.05–8.01 (3H, m, Glu^2-N*H* and Gly^1-N*H*, Gly^3-N*H* or Gly^4-N*H*), 7.70 (1H, t, *J*=5.6 Hz, −CH$_2$CH$_2$N*H*C(=O)−), 7.64 (1H, d, *J*=8.8 Hz, −N*H*C(=O)CH$_3$), 4.93 (1H, d, *J*=3.2 Hz, OH-4), 4.85 (1H, d, *J*=4.8 Hz, OH-3), 4.48 (1H, t, *J*=5.6 Hz, OH-6), 4.28 (1H, t, *J*=7.6 Hz, Glu^2-Hα), 4.25 (1H, d, *J*=8.0 Hz, H-1), 3.72–3.65 (6H, m, Gly^1-Hα, Gly^3-Hα, Gly^4-Hα, −OC*H*$_2$CH$_2$− and H-6b), 3.44 (1H, m, H-6a), 3.40–3.24 (3H, m, H-2, −OC*H*$_2$CH$_2$− and H-3), 3.05–3.01 (4H, m, −CH$_2$C*H*$_2$NH−, H-5 and H-4), 2.43 (2H, t, *J*=7.2 Hz, −C*H*$_2$C(=O)CH$_3$), 2.25 (2H, t, *J*=7.8 Hz, Glu^2-Hγ), 2.11 (2H, t, *J*=7.2 Hz, −NHC(=O)C*H*$_2$−), 2.06 (3H, s, −CH$_2$C(=O)C*H*$_3$), 1.93 (1H, m, Glu^2-Hβ), 1.78 (3H, s, −NHC(=O)C*H*$_3$), 1.76 (1H, m, Glu^2-Hβ), 1.67 (2H, qui, *J*=7.2 Hz, −C(=O)CH$_2$C*H*$_2$CH$_2$C(=O)−), 1.43–1.38 (4H, m, −OCH$_2$C*H*$_2$− and −C*H*$_2$CH$_2$NH−), 1.26–1.21 (16H, m, −OCH$_2$CH$_2$(C*H*$_2$)$_8$−). ^{13}C NMR (100 MHz, $(CD_3)_2S{=}O$): δ 208.06, 173.84, 172.19, 171.48, 169.17, 168.79, 168.77, 168.22, 100.91, 76.86, 74.17, 70.57, 68.13, 60.97, 55.35, 51.83, 42.07, 41.93, 41.89, 41.84, 38.44, 34.04, 29.86, 29.62, 29.03, 28.97, 28.95, 28.92, 28.73, 28.65, 27.03, 26.28, 25.35, 22.92, 19.21. ESI-HRMS: $C_{37}H_{63}N_6O_{14}$ $[M-H]^-$ calcd (*m*/*z*) 815.44077, found (*m*/*z*) 815.44273.

*4.3.4. 12-[N-(5-Oxohexanoyl)-L-alanyl-L-glutamyl-L-phenylalanyl-glycinyl]-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**12**).* To the solution of **4** (150 mg, 0.24 mmol), HOBt (39 mg, 0.29 mmol), and WSC (55 mg, 0.29 mmol) in DMF (4 ml) were added 12-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside acetic acid salt (112 mg, 0.24 mmol) and triethylamine (40 μl, 0.29 mmol) successively. The reaction mixture was stirred for 1 h at rt and directly purified by SiO_2 column chromatography. Eluent of EtOAc/EtOH/H_2O (9:2:1) gave assumed benzyl protected **12**. 10% Pd−C (40 mg) was added to a solution of the resulting material in dioxane/water (1:1 v/v, 10 ml), and the reaction mixture was stirred under hydrogen for 1 h at 50 °C. After removal of Pd−C by Celite filtration, the filtrate was concentrated, and purified by the preparative RP-HPLC [column: Inertsil ODS-3 (ϕ20×250 mm); column oven temperature: 40 °C; flow rate: 5 ml/min, detection: 220 nm UV; eluate D in eluent C gradient increase from 10% to 50% over 60 min] to give **12** (171 mg, 76%) as an amorphous. ^{1}H NMR (400 MHz, $(CD_3)_2S{=}O$): δ 12.05 (1H, br, −C(=O)O*H*), 8.22 (1H, t, *J*=5.6 Hz, Gly^4-N*H*), 8.02 (1H, d, *J*=7.2 Hz, Ala^1-N*H*), 7.98 (1H, d, *J*=7.6 Hz, Phe^3-N*H*), 7.94 (1H, d, *J*=7.6 Hz, Glu^2-N*H*), 7.64 (1H, d, *J*=8.8 Hz, −N*H*C(=O)CH$_3$), 7.57 (1H, t, *J*=5.6 Hz, −CH$_2$CH$_2$N*H*C(=O)−), 7.26–7.16 (5H, m, aromatic-*H*), 4.94 (1H, br, OH-4), 4.85 (1H, br, OH-3), 4.50–4.42 (2H, m, OH-6 and Phe^3-Hα), 4.25 (1H, d, *J*=8.4 Hz, H-1), 4.23–4.14 (2H, m, Ala^1-Hα and Glu^2-Hα), 3.73–3.66 (3H, m, H-6b, Gly^4-Hα and −OC*H*$_2$CH$_2$−), 3.58 (1H, dd, *J*=16.4, 5.6 Hz, Gly^4-Hα), 3.44 (1H, m, H-6a), 3.40–3.24 (3H, m, H-2, −OC*H*$_2$CH$_2$− and H-3), 3.07–3.01 (5H, m, −CH$_2$C*H*$_2$NH−, Phe^3-Hβ, H-5 and H-4), 2.83 (1H, dd, *J*=13.6, 9.0 Hz, Phe^3-Hβ), 2.41 (2H, t, *J*=7.2 Hz, −C*H*$_2$C(=O)CH$_3$), 2.18–2.12 (2H, m, Glu^2-Hγ), 2.09 (2H, t, *J*=7.2 Hz, −NHC(=O)C*H*$_2$−), 2.05 (3H, s, −CH$_2$C(=O)C*H*$_3$), 1.82 (1H, m, Glu^2-Hβ), 1.78 (3H, s, −NHC(=O)C*H*$_3$), 1.69 (1H, m, Glu^2-Hβ), 1.66 (2H, qui, *J*=7.2 Hz, −C(=O)CH$_2$C*H*$_2$CH$_2$C(=O)−), 1.42–1.38 (4H, m, −OCH$_2$C*H*$_2$− and −C*H*$_2$CH$_2$NH−), 1.27–1.21 (16H, m, −OCH$_2$CH$_2$(C*H*$_2$)$_8$−), 1.15 (3H, d, *J*=7.2 Hz, Ala^1-Hβ). ^{13}C NMR (100 MHz, $(CD_3)_2S{=}O$): δ 208.03, 173.86, 172.53, 171.81, 171.04, 170.94, 168.77, 168.12, 137.50, 129.00, 127.94, 126.17, 100.91, 76.86, 74.17, 70.57, 68.13, 60.97, 55.36, 54.00, 51.89, 48.16, 42.06, 41.85, 38.43, 37.02, 33.99, 29.84, 29.60, 29.03, 28.95, 28.74, 28.68, 27.01, 26.27, 25.35, 22.92, 19.21, 17.62. ESI-HRMS: $C_{45}H_{71}N_6O_{14}$ $[M-H]^-$ calcd (*m*/*z*) 919.50337, found (*m*/*z*) 919.50643.

*4.3.5. 12-[N-(5-Oxohexanoyl)-L-phenylalanyl-L-glutamyl-L-alanyl-glycinyl]-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**13**).* To the solution of **5** (150 mg, 0.24 mmol), HOBt (39 mg, 0.29 mmol), and WSC (55 mg, 0.29 mmol) in DMF (4 ml) were added 12-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside acetic acid salt (111 mg, 0.24 mmol) and triethylamine (40 μl, 0.29 mmol) successively. The reaction mixture was stirred for 1 h at rt and directly purified by SiO_2 column chromatography. Eluent of EtOAc/EtOH/H_2O (9:2:1) containing 1% AcOH gave assumed benzyl protected **13**. 10% Pd−C (40 mg) was added to a solution of the resulting material in dioxane/water (1:1 v/v, 10 ml), and the reaction mixture was stirred under hydrogen for 1 h at 50 °C. After removal of Pd−C by Celite filtration, the filtrate was concentrated, and purified by the preparative RP-HPLC [column: Inertsil ODS-3 (ϕ20×250 mm); column oven temperature: 40 °C; flow rate: 5 ml/min, detection: 220 nm UV; eluate D in eluent C gradient increase from 10% to 50% over 60 min] to give **13** (96 mg, 43%) as an amorphous. ^{1}H NMR (400 MHz, $(CD_3)_2S{=}O$): δ 12.09 (1H, s, −C(=O)O*H*), 8.16 (1H, t, *J*=5.6 Hz, Gly^4-N*H*), 8.10 (1H, d, *J*=6.4 Hz, Ala^3-N*H*), 8.10 (1H, d, *J*=6.4 Hz, Glu^2-N*H*), 8.04 (1H, d, *J*=8.4 Hz, Phe^1-N*H*), 7.65–7.63 (2H, m, −N*H*C(=O)CH$_3$ and −CH$_2$CH$_2$N*H*C(=O)−), 7.26–7.15 (5H, m, aromatic-*H*), 4.93 (1H, br, OH-4), 4.85 (1H, br, OH-3), 4.54 (1H, m, Phe^1-Hα), 4.48 (1H, br, OH-6), 4.28–4.16 (2H, m, Ala^3-Hα and Glu^2-Hα), 4.25 (1H, d, *J*=8.0 Hz, H-1), 3.73–3.66 (3H, m, H-6b, Gly^4-Hα and −OC*H*$_2$CH$_2$−), 3.59 (1H, dd, *J*=16.8, 5.6 Hz, Gly^4-Hα), 3.46–3.43 (1H, m, H-6a), 3.40–3.25 (3H, m, H-2, −OC*H*$_2$CH$_2$− and H-3), 3.06–3.03 (5H, m, −CH$_2$C*H*$_2$NH−, Phe^1-Hβ, H-5 and H-4), 2.71 (1H, dd, *J*=13.2, 11.2 Hz,

Phe[1]-Hβ), 2.27–2.19 (4H, m, $-CH_2C(=O)CH_3$ and Glu[2]-Hγ), 2.01 (2H, m, $-NHC(=O)CH_2-$), 2.00 (3H, s, $-CH_2C(=O)CH_3$), 1.91 (1H, m, Glu[2]-Hβ), 1.79 (1H, m, Glu[2]-Hβ), 1.78 (3H, s, $-NHC(=O)CH_3$), 1.54 (2H, qui, J=7.2 Hz, $-C(=O)CH_2CH_2CH_2C(=O)-$), 1.43–1.38 (4H, m, $-OCH_2CH_2-$ and $-CH_2CH_2NH-$), 1.25–1.19 (19H, m, $-OCH_2CH_2(CH_2)_8-$ and Ala[3]-Hβ). ^{13}C NMR (100 MHz, $(CD_3)_2S=O$): δ 207.91, 173.87, 173.23, 171.71, 171.46, 170.88, 168.77, 168.20, 137.95, 129.02, 127.85, 126.06, 100.91, 76.86, 74.17, 70.58, 68.13, 60.97, 55.36, 53.63, 51.68, 48.54, 42.00, 41.60, 38.44, 37.20, 34.06, 29.88, 29.60, 29.03, 28.94, 28.74, 28.65, 27.12, 26.28, 25.35, 22.92, 19.15, 17.57. ESI-HRMS: $C_{45}H_{71}N_6O_{14}$ $[M-H]^-$ calcd (m/z) 919.50337, found (m/z) 919.50523.

*4.3.6. 12-[N-(5-Oxohexanoyl)-glycinyl-L-glutamyl-L-phenylalanyl-glycinyl]-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**14**).* To the solution of **6** (130 mg, 0.22 mmol), HOBt (35 mg, 0.26 mmol), and WSC (50 mg, 0.26 mmol) in DMF (4 ml) were added 12-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside acetic acid salt (100 mg, 0.22 mmol) and triethylamine (36 μl, 0.26 mmol) successively. The reaction mixture was stirred for 1 h at rt and directly purified by SiO_2 column chromatography. Eluent of EtOAc/EtOH/H_2O (9:2:1) containing 5% AcOH gave assumed benzyl protected **14**.

10% Pd–C (40 mg) was added to a solution of the resulting material in dioxane/water (1:1 v/v, 10 ml), and the reaction mixture was stirred under hydrogen for 2 h at 50 °C. After removal of Pd–C by Celite filtration, the filtrate was concentrated, and purified by the preparative RP-HPLC [column: Inertsil ODS-3 (ϕ20×250 mm); column oven temperature: 40 °C; flow rate: 5 ml/min, detection: 220 nm UV; eluate D in eluent C gradient increase from 10% to 40% over 60 min] to give **14** (108 mg, 55%) as an amorphous. ^{1}H NMR (400 MHz, $(CD_3)_2S=O$): δ 12.05 (1H, br, $-C(=O)OH$), 8.14 (1H, t, J=5.6 Hz, Gly[1]-N*H* or Gly[4]-N*H*), 8.08–8.05 (2H, m, Gly[1]-N*H* or Gly[4]-N*H* and Phe[3]-N*H*), 7.98 (1H, d, J=7.6 Hz, Glu[2]-N*H*), 7.64 (1H, d, J=8.8 Hz, $-NHC(=O)CH_3$), 7.58 (1H, t, J=5.6 Hz, $-CH_2CH_2NHC(=O)-$), 7.27–7.16 (5H, m, aromatic-*H*), 4.92 (2H, br, OH-4 and OH-3), 4.47–4.41 (1H, m, Phe[3]-Hα), 4.25 (1H, d, J=8.4 Hz, H-1), 3.73–3.66 (5H, m, Gly[1]-Hα or Gly[4]-Hα, $-OCH_2CH_2-$ and H-6b), 3.62 (1H, dd, J=16.4, 5.6 Hz, Gly[1]-Hα or Gly[4]-Hα), 3.46–3.25 (4H, m, H-6a, H-2, $-OCH_2CH_2-$, H-3), 3.08–3.01 (5H, m, $-CH_2CH_2NH-$, Phe[3]-Hβ, H-5 and H-4), 2.84 (1H, dd, J=13.6, 9.6 Hz, Phe[3]-Hβ), 2.43 (2H, t, J=7.2 Hz, $-CH_2C(=O)CH_3$), 2.17–2.10 (4H, m, Glu[2]-Hγ and $-NHC(=O)CH_2-$), 2.05 (3H, s, $-CH_2C(=O)CH_3$), 1.81 (1H, m, Glu[2]-Hβ), 1.78 (3H, s, $-NHC(=O)CH_3$), 1.71–1.62 (1H, m, Glu[2]-Hβ), 1.67 (2H, qui, J=7.2 Hz, $-C(=O)CH_2CH_2CH_2C(=O)-$), 1.42–1.38 (4H, m, $-OCH_2CH_2-$ and $-CH_2CH_2NH-$), 1.27–1.20 (16H, m, $-OCH_2CH_2(CH_2)_8-$). ^{13}C NMR (100 MHz, $(CD_3)_2S=O$): δ 208.03, 173.79, 172.27, 171.08, 170.99, 169.20, 168.77, 168.12, 137.62, 129.01, 127.96, 126.18, 100.91, 76.86, 74.17, 70.57, 68.13, 60.97, 55.36, 54.16, 51.85, 42.03, 41.98, 41.85, 38.44, 36.89, 34.02, 29.80, 29.61, 29.03, 28.96, 28.94, 28.74, 28.67, 27.06, 26.27, 25.35, 22.92, 19.19. ESI-HRMS: $C_{44}H_{69}N_6O_{14}$ $[M-H]^-$ calcd (m/z) 905.48772, found (m/z) 905.49089.

*4.3.7. 12-[N-(5-Oxohexanoyl)-L-phenylalanyl-L-glutamyl-glycinyl-glycinyl]-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**15**).* To the solution of **7** (120 mg, 0.20 mmol), HOBt (32 mg, 0.24 mmol), and WSC (46 mg, 0.24 mmol) in DMF (4 ml) were added 12-aminododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside acetic acid salt (93 mg, 0.20 mmol) and triethylamine (34 μl, 0.24 mmol) successively. The reaction mixture was stirred for 1 h at rt and directly purified by SiO_2 column chromatography. Eluent of EtOAc/EtOH/H_2O (9:2:1) containing 5% AcOH gave assumed benzyl protected **15**. 10% Pd–C (40 mg) was added to a solution of the resulting material in dioxane/water (1:1 v/v, 8 ml), and the reaction mixture was stirred under hydrogen for 1 h at 50 °C. After removal of Pd–C by Celite filtration, the filtrate was concentrated, and purified by the preparative RP-HPLC [column: Inertsil ODS-3 (ϕ20×250 mm); column oven temperature: 40 °C; flow rate: 5 ml/min, detection: 220 nm UV; eluate D in eluent C gradient increase from 10% to 40% over 60 min] to give **15** (102 mg, 56%) as an amorphous. ^{1}H NMR (400 MHz, $(CD_3)_2S=O$): δ 12.08 (1H, s, $-C(=O)OH$), 8.15 (1H, d, J=7.6 Hz, Glu[2]-N*H*), 8.11 (1H, t, J=5.6 Hz, Gly[3]-N*H* or Gly[4]-N*H*), 8.09–8.05 (2H, m, Gly[3]-N*H* or Gly[4]-N*H* and Phe[1]-N*H*), 7.69 (1H, t, J=5.6 Hz, $-CH_2CH_2NHC(=O)-$), 7.64 (1H, d, J=8.8 Hz, $-NHC(=O)CH_3$), 7.26–7.15 (5H, m, aromatic-*H*), 4.93 (1H, br, OH-4), 4.85 (1H, bd, J=4.0 Hz, OH-3), 4.55 (1H, m, Phe[1]-Hα), 4.48 (1H, t, J=5.6 Hz, OH-6), 4.27 (1H, m, Glu[2]-Hα), 4.25 (1H, d, J=8.4 Hz, H-1), 3.73–3.66 (2H, m, H-6b and $-OCH_2CH_2-$), 3.72 (2H, d, J=5.6 Hz, Gly[3]-Hα or Gly[4]-Hα), 3.66 (2H, d, J=5.6 Hz, Gly[3]-Hα or Gly[4]-Hα), 3.48–3.41 (1H, m, H-6a), 3.40–3.24 (3H, m, H-2, $-OCH_2CH_2-$ and H-3), 3.08–2.99 (5H, m, $-CH_2CH_2NH-$, Phe[1]-Hβ, H-4 and H-5), 2.72 (1H, dd, J=13.6, 10.8 Hz, Phe[1]-Hβ), 2.27–2.19 (4H, m, $-CH_2C(=O)CH_3$ and Glu[2]-Hγ), 2.01 (2H, m, $-NHC(=O)CH_2-$), 2.00 (3H, s, $-CH_2C(=O)CH_3$), 1.94 (1H, m, Glu[2]-Hβ), 1.80 (1H, m, Glu[2]-Hβ), 1.78 (3H, s, $-NHC(=O)CH_3$), 1.54 (2H, qui, J=7.2 Hz, $-C(=O)CH_2CH_2CH_2C(=O)-$), 1.43–1.37 (4H, m, $-OCH_2CH_2-$ and $-CH_2CH_2NH-$), 1.26–1.19 (16H, m, $-OCH_2CH_2(CH_2)_8-$). ^{13}C NMR (100 MHz, $(CD_3)_2S=O$): δ 207.92, 173.85, 171.72, 171.57, 171.40, 168.77, 168.22, 137.94, 129.02, 127.87, 126.07, 100.91, 76.86, 74.17, 70.58, 68.13, 60.97, 55.36, 53.63, 51.90, 42.11, 41.93, 41.59, 38.45, 37.11, 34.05, 29.83, 29.60, 29.03, 28.97, 28.94, 28.91, 28.74, 28.65, 27.06, 26.28, 25.35, 22.92, 19.15. ESI-HRMS: $C_{44}H_{69}N_6O_{14}$ $[M-H]^-$ calcd (m/z) 905.48772, found (m/z) 905.49107.

4.4. Reactions with galactosyltransferase to yield lactosaminide derivatives

The reactions were performed as a 0.5 ml solution of substrate (**9**–**15**, 2 mM), $MnCl_2$ (10 mM), UDP-Gal (5 mM) β1,4-galactosyltransferase[9] (50 mU/ml), and additives (CDs or Triton-X 100) in 50 mM HEPES buffer (pH 7.0) at 25 °C. The reactions were sampled 15 μl each at 10 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, 12 h, 1 d, and 2 d (not all data were shown in Scheme 3) and monitored by RP-HPLC [column: Inertsil ODS-3 (ϕ4.6×250 mm); column oven temperature: 30 °C; flow rate: 1 ml/min, detection: 220 nm UV].

*4.4.1. 12-[N-(5-Oxohexanoyl)-L-phenylalanyl-L-glutamyl-L-phenylalanyl-glycinyl]-aminododecyl O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**16**).*[10] To obtain physical data of **16**, a 2.5 ml solution of **9** (2 mM) was treated by UDP-Gal (5 mM) and β1,4-galactosyltransferase[9] (50 mU/ml) in 50 mM HEPES buffer (pH 7.0) with $MnCl_2$ (10 mM) and γ-CD (50 mM) at 25 °C for 5 h. The mixture was freeze-dried and the resulting residue was purified by RP-HPLC [eluent D in eluent C gradient increase from 30% to 40% over 30 min] to give **16**[10] (5.8 mg, quant.) as an amorphous. t_R=25 min.

*4.4.2. 12-[N-(5-Oxohexanoyl)-L-alanyl-L-glutamyl-L-alanyl-glycinyl]-aminododecyl O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**17**).* To obtain physical data, six of residual reaction mixtures performed in Scheme 3 were mixed (1.65 ml) and freeze-dried. The resulting residue was purified by RP-HPLC [eluent D in eluent C gradient increase from 20% to 30% over 30 min] to give **17** (2.4 mg, 72%) as an amorphous. t_R=27 min. ^{1}H NMR (400 MHz, $(CD_3)_2S=O$): δ 12.07 (1H, br, $-C(=O)OH$), 8.12 (1H, t, J=5.6 Hz, Gly[4]-N*H*), 8.04 (1H, d, J=7.2 Hz, Ala[1]-N*H* or Ala[3]-N*H*), 8.01 (1H, d, J=6.8 Hz, Ala[1]-N*H* or Ala[3]-N*H*), 7.96 (1H, d, J=7.6 Hz, Glu[2]-N*H*), 7.73 (1H, d, J=8.4 Hz, $-NHC(=O)CH_3$), 7.63 (1H, t, J=5.6 Hz, $-CH_2CH_2NHC(=O)-$), 5.07 (1H, br, OH-2′), 4.78 (1H, br, OH-3′), 4.65 (1H, t, J=4.8 Hz, OH-6′), 4.61 (1H, s, OH-3), 4.58 (1H, t,

J=6.0 Hz, OH-6), 4.50 (1H, d, J=4.8 Hz, OH-4′), 4.29 (1H, d, J=8.0 Hz, H-1), 4.26–4.15 (4H, m, H-1′, Ala^1-Hα, Glu^2-Hα, and Ala^3-Hα), 3.78–3.68 (2H, m, H-6, −OCH_2CH$_2$−), 3.66–3.56 (4H, m, H-6, H-4′ and Gly^4-Hα), 3.54–3.40 (5H, m, H-6′, H-5′, H-4 and H-2), 3.38–3.22 (5H, m, −OCH_2CH$_2$−, H-2′, H-3, H-5, and H-3′), 3.03 (2H, dt, J=5.6, 6.4 Hz, −CH$_2$CH_2NH−), 2.42 (2H, t, J=7.2 Hz, −CH_2C(=O)CH$_3$), 2.24 (2H, t, J=8.0 Hz, Glu^2-Hγ), 2.10 (2H, t, J=7.2 Hz, −NHC(=O)CH_2−), 2.06 (3H, s, −CH$_2$C(=O)CH_3), 1.90 (1H, m, Glu^2-Hβ), 1.77 (3H, s, −NHC(=O)CH_3), 1.75 (1H, m, Glu^2-Hβ), 1.66 (2H, qui, J=7.2 Hz, −C(=O)CH$_2$CH_2CH$_2$C(=O)−), 1.43–1.37 (4H, m, −OCH$_2$CH_2− and, −CH_2CH$_2$NH−), 1.25–1.21 (16H, m, −OCH$_2$CH$_2$(CH_2)$_8$−), 1.22 (3H, d, J=6.8 Hz, Ala^1-Hβ or Ala^3-Hβ), 1.18 (3H, d, J=7.2 Hz, Ala^1-Hβ or Ala^3-Hβ). ^{13}C NMR (100 MHz, (CD$_3$)$_2$S=O): δ 208.06, 173.90, 172.53, 172.21, 171.82, 170.92, 168.51, 168.19, 103.92, 100.79, 81.39, 75.48, 74.87, 73.12, 72.14, 70.50, 68.33, 68.09, 60.40, 54.69, 51.65, 48.48, 48.24, 42.00, 41.85, 38.42. 33.97, 29.92, 29.61, 29.04, 28.93, 28.72, 28.66, 26.99, 26.27, 25.31, 22.86, 19.18, 17.66, 17.57. ESI-HRMS: $C_{45}H_{77}N_6O_{19}$ [M−H]$^-$ calcd (m/z) 1005.52490, found (m/z) 1005.52729.

*4.4.3. 12-[N-(5-Oxohexanoyl)-glycinyl-L-glutamyl-glycinyl-glycinyl]-aminododecyl O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**18**).* To obtain physical data, six of residual reaction mixtures performed in Scheme 3 were mixed (1.65 ml) and freeze-dried. The resulting residue was purified by RP-HPLC [eluent D in eluent C gradient increase from 20% to 30% over 30 min] to give **18** (2.8 mg, 87%) as an amorphous. t_R=24 min. ^{1}H NMR (400 MHz, (CD$_3$)$_2$S=O): δ 12.07 (1H, br, −C(=O)OH), 8.23 (1H, t, J=5.6 Hz, Gly^1-NH, Gly^3-NH or Gly^4-NH), 8.06–8.01 (3H, m, Glu^2-NH and Gly^1-NH, Gly^3-NH or Gly^4-NH), 7.72 (1H, d, J=8.8 Hz, −NHC(=O)CH$_3$), 7.70 (1H, t, J=5.6 Hz, −CH$_2$CH$_2$NHC(=O)−), 5.07 (1H, br, OH-2′), 4.78 (1H, br, OH-3′), 4.65 (1H, dd, J=5.2, 4.0 Hz, OH-6′), 4.61 (1H, s, OH-3), 4.58 (1H, t, J=6.0 Hz, OH-6), 4.50 (1H, d, J=4.4 Hz, OH-4′), 4.29 (1H, d, J=8.0 Hz, H-1), 4.29–4.24 (1H, m, Glu^2-Hα), 4.20 (1H, m, H-1′), 3.79–3.58 (10H, m, H-6b, −OCH_2CH$_2$−, Gly^1-Hα, Gly^3-Hα, Gly^4-Hα, H-6a and H-4′), 3.53–3.41 (5H, m, H-6′, H-5′, H-4, and H-2), 3.40–3.22 (5H, m, −OCH_2CH$_2$−, H-2′, H-3, H-5, and H-3′), 3.03 (2H, dt, J=5.6, 6.4 Hz, −CH$_2$CH_2NH−), 2.43 (2H, t, J=7.2 Hz, −CH_2C(=O)CH$_3$), 2.25 (2H, t, J=7.8 Hz, Glu^2-Hγ), 2.11 (2H, t, J=7.2 Hz, −NHC(=O)CH_2−), 2.06 (3H, s, −CH$_2$C(=O)CH_3), 1.93 (1H, m, Glu^2-Hβ), 1.77 (3H, s, −NHC(=O)CH_3), 1.76 (1H, m, Glu^2-Hβ), 1.67 (2H, qui, J=7.2 Hz, −C(=O)CH$_2$CH_2CH$_2$C(=O)−), 1.44–1.36 (4H, m, −OCH$_2$CH_2− and −CH_2CH$_2$NH−), 1.26–1.22 (16H, m, −OCH$_2$CH$_2$(CH_2)$_8$−). ^{13}C NMR (100 MHz, (CD$_3$)$_2$S=O): δ 208.07, 173.84, 172.19, 171.49, 169.17, 168.80, 168.51, 168.23, 103.92, 100.79, 81.39, 75.48, 74.87, 73.12, 72.14, 70.50, 68.33, 68.09, 60.40, 54.68, 51.83, 42.07, 41.91, 41.89, 41.84, 38.44, 34.04, 29.86, 29.62, 29.03, 28.95, 28.93, 28.72, 28.66, 27.02, 26.28, 25.31, 22.87, 19.21. ESI-HRMS: $C_{43}H_{73}N_6O_{19}$ [M−H]$^-$ calcd (m/z) 977.49360, found (m/z) 977.49586.

*4.4.4. 12-[N-(5-Oxohexanoyl)-L-alanyl-L-glutamyl-L-phenylalanyl-glycinyl]-aminododecyl O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**19**).* To obtain physical data, six of residual reaction mixtures performed in Scheme 3 were mixed (1.65 ml) and freeze-dried. The resulting residue was purified by RP-HPLC [eluent D in eluent C gradient increase from 25% to 40% over 30 min] to give **19** (2.8 mg, 79%) as an amorphous. t_R=22 min. ^{1}H NMR (400 MHz, (CD$_3$)$_2$S=O): δ 12.04 (1H, br, −C(=O)OH), 8.22 (1H, t, J=5.6 Hz, Gly^4-NH), 8.03 (1H, d, J=6.8 Hz, Ala^1-NH), 7.98 (1H, d, J=7.6 Hz, Phe^3-NH), 7.94 (1H, d, J=7.2 Hz, Glu^2-NH), 7.73 (1H, d, J=8.0 Hz, −NHC(=O)CH$_3$), 7.58 (1H, t, J=5.6 Hz, −CH$_2$CH$_2$NHC(=O)−), 7.26–7.16 (5H, m, aromatic-H), 5.07 (1H, br, OH-2′), 4.78 (1H, br, OH-3′), 4.65 (1H, dd, J=5.2, 4.4 Hz, OH-6′), 4.61 (1H, s, OH-3), 4.58 (1H, t, J=6.0 Hz, OH-6), 4.50 (1H, d, J=4.4 Hz, OH-4′), 4.47–4.42 (1H, m, Phe^3-Hα), 4.29 (1H, d, J=8.0 Hz, H-1), 4.25–4.13 (3H, m, Ala^1-Hα, H-1′ and Glu^2-Hα), 3.78–3.55 (6H, m, H-6b, −OCH_2CH$_2$−, Gly^4-Hα, H-6a and H-4′), 3.53–3.41 (5H, m, H-6′, H-5′, H-4, and H-2), 3.40–3.22 (5H, m, −OCH_2CH$_2$−, H-2′, H-3, H-5, and H-3′), 3.07–3.01 (3H, m, −CH$_2$CH_2NH- and Phe^3-Hβ), 2.83 (1H, dd, J=13.6, 9.2 Hz, Phe^3-Hβ), 2.41 (2H, t, J=7.2 Hz, −CH_2C(=O)CH$_3$), 2.18–2.12 (2H, m, Glu^2-Hγ), 2.09 (2H, t, J=7.2 Hz, −NHC(=O)CH_2−), 2.05 (3H, s, −CH$_2$C(=O)CH_3), 1.81 (1H, m, Glu^2-Hβ), 1.77 (3H, s, −NHC(=O)CH_3), 1.71 (1H, m, Glu^2-Hβ), 1.66 (2H, qui, J=7.2 Hz, −C(=O)CH$_2$CH_2CH$_2$C(=O)−), 1.44–1.41 (4H, m, −OCH$_2$CH_2− and −CH_2CH$_2$NH−), 1.25–1.21 (16H, m, −OCH$_2$CH$_2$(CH_2)$_8$−), 1.15 (3H, d, J=6.8 Hz, Ala^1-Hβ). ^{13}C NMR (100 MHz, (CD$_3$)$_2$S=O): δ 208.04, 173.86, 172.53, 171.82, 171.05, 170.95, 168.51, 168.12, 137.50, 129.00, 127.94, 126.17, 103.92, 100.79, 81.39, 75.48, 74.87, 73.13, 72.14, 70.51, 68.33, 68.09, 60.40, 54.69, 54.00, 51.89, 48.15, 42.05, 41.85, 38.43, 37.00, 33.98, 29.84, 29.61, 29.03, 28.95, 28.73, 28.68, 27.01, 26.27, 25.31, 22.86, 19.21, 17.62. ESI-HRMS: $C_{51}H_{81}N_6O_{19}$ [M−H]$^-$ calcd (m/z) 1081.55620, found (m/z) 1081.55823.

*4.4.5. 12-[N-(5-Oxohexanoyl)-L-phenylalanyl-L-glutamyl-L-alanyl-glycinyl]-aminododecyl O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**20**).* To obtain physical data, six of residual reaction mixtures performed in Scheme 3 were mixed (1.65 ml) and freeze-dried. The resulting residue was purified by RP-HPLC [eluent D in eluent C gradient increase from 25% to 40% over 30 min] to give **20** (2.1 mg, 59%) as an amorphous. t_R=21 min. ^{1}H NMR (400 MHz, (CD$_3$)$_2$S=O): δ 12.09 (1H, s, −C(=O)OH), 8.16 (1H, t, J=5.8 Hz, Gly^4-NH), 8.10 (1H, d, J=6.8 Hz, Ala^3-NH), 8.10 (1H, d, J=6.8 Hz, Glu^2-NH), 8.04 (1H, d, J=8.0 Hz, Phe^1-NH), 7.73 (1H, d, J=8.4 Hz, −NHC(=O)CH$_3$), 7.65 (1H, t, J=5.6 Hz, −CH$_2$CH$_2$NHC(=O)−), 7.26–7.15 (5H, m, aromatic-H), 5.07 (1H, br, OH-2′), 4.78 (1H, br, OH-3′), 4.65 (1H, dd, J=5.2, 4.4 Hz, OH-6′), 4.61 (1H, s, OH-3), 4.58 (1H, t, J=6.0 Hz, OH-6), 4.54 (1H, m, Phe^3-Hα), 4.50 (1H, d, J=4.4 Hz, OH-4′), 4.29 (1H, d, J=8.0 Hz, H-1), 4.26–4.17 (3H, m, Ala^3-Hα H-1′ and Glu^2-Hα), 3.78–3.56 (6H, m, H-6b, −OCH_2CH$_2$−, Gly^4-Hα, H-6a and H-4′), 3.53–3.41 (5H, m, H-6′, H-5′, H-4, and H-2), 3.40–3.22 (5H, m, −OCH_2CH$_2$−, H-2′, H-3, H-5, and H-3′), 3.05–3.00 (3H, m, −CH$_2$CH_2NH− and Phe^1-Hβ), 2.71 (1H, dd, J=13.8, 10.6 Hz, Phe^1-Hβ), 2.27–2.19 (4H, m, −CH_2C(=O)CH$_3$ and Glu^2-Hγ), 2.00 (2H, m, −NHC(=O)CH_2−), 2.00 (3H, s, −CH$_2$C(=O)CH_3), 1.91 (1H, m, Glu^2-Hβ), 1.79 (1H, m, Glu^2-Hβ), 1.77 (3H, s, −NHC(=O)CH_3), 1.54 (2H, qui, J=7.2 Hz, −C(=O)CH$_2$CH_2CH$_2$C(=O)−), 1.44–1.36 (4H, m, −OCH$_2$CH_2− and −CH_2CH$_2$NH−), 1.25–1.19 (19H, m, −OCH$_2$CH$_2$(CH_2)$_8$− and Ala^3-Hβ). ^{13}C NMR (100 MHz, (CD$_3$)$_2$S=O): δ 207.92, 173.88, 172.23, 171.71, 171.47, 170.88, 168.51, 168.20, 137.95, 129.02, 127.85, 126.07, 103.92, 100.79, 81.39, 75.48, 74.88, 73.12, 72.14, 70.50, 68.33, 68.09, 60.40, 54.69, 53.63, 51.69, 48.55, 42.00, 41.59, 38.44, 37.20, 34.06, 29.89, 29.60, 29.04, 28.94, 28.73, 28.66, 27.12, 26.28, 25.32, 22.86, 19.15, 17.57. ESI-HRMS: $C_{51}H_{81}N_6O_{19}$ [M−H]$^-$ calcd (m/z) 1081.55620, found (m/z) 1081.55951.

*4.4.6. 12-[N-(5-Oxohexanoyl)-glycinyl-L-glutamyl-L-phenylalanyl-glycinyl]-aminododecyl O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**21**).* To obtain physical data, six of residual reaction mixtures performed in Scheme 3 were mixed (1.65 ml) and freeze-dried. The resulting residue was purified by RP-HPLC [eluent D in eluent C gradient increase from 25% to 40% over 30 min] to give **21** (3.3 mg, 94%) as an amorphous. t_R=21 min. ^{1}H NMR (400 MHz, (CD$_3$)$_2$S=O): δ 12.06 (1H, br, −C(=O)OH), 8.16 (1H, t, J=5.8 Hz, Gly^1-NH or Gly^4-NH), 8.09–8.05 (2H, m, Gly^1-NH or Gly^4-NH and Phe^3-NH), 7.99 (1H, d, J=7.6 Hz, Glu^2-NH), 7.73 (1H, d, J=8.4 Hz, −NHC(=O)CH$_3$), 7.58 (1H, t, J=5.6 Hz, −CH$_2$CH$_2$NHC(=O)−), 7.27–7.16 (5H, m, aromatic-H), 5.07 (1H, br, OH-2′), 4.78 (1H, br, OH-3′), 4.65 (1H, dd, J=5.2, 4.4 Hz, OH-6′), 4.61 (1H, s, OH-3), 4.58 (1H, t, J=6.2 Hz, OH-6), 4.50 (1H, d, J=4.4 Hz, OH-4′), 4.44 (1H, m, Phe^3-Hα), 4.29 (1H, d, J=8.0 Hz, H-1),

4.23–4.16 (2H, m, H-1′ and Glu^2-Hα), 3.78–3.57 (8H, m, H-6b, −OC*H*$_2$CH$_2$−, Gly^1-Hα, Gly^4-Hα, H-6a and H-4′), 3.54–3.41 (5H, m, H-6′, H-5′, H-4, and H-2), 3.40–3.22 (5H, m, −OC*H*$_2$CH$_2$−, H-2′, H-3, H-5, and H-3′), 3.08–3.01 (3H, m, −CH$_2$C*H*$_2$NH− and Phe^3-Hβ), 2.84 (1H, dd, *J*=14.0, 9.6 Hz, Phe^3-Hβ), 2.43 (2H, t, *J*=7.2 Hz, −C*H*$_2$C(=O)CH$_3$), 2.14 (2H, t, *J*=8.4 Hz, Glu^2-Hγ), 2.12 (2H, t, *J*=7.2 Hz, −NHC(=O)C*H*$_2$−), 2.05 (3H, s, −CH$_2$C(=O)C*H*$_3$), 1.83 (1H, m, Glu^2-Hβ), 1.77 (3H, s, −NHC(=O)C*H*$_3$), 1.71–1.62 (1H, m, Glu^2-Hβ), 1.67 (2H, qui, *J*=7.2 Hz, −C(=O)CH$_2$C*H*$_2$CH$_2$C(=O)−), 1.44–1.36 (4H, m, −OCH$_2$C*H*$_2$− and −C*H*$_2$CH$_2$NH−), 1.26–1.21 (16H, m, −OCH$_2$CH$_2$(C*H*$_2$)$_8$−). ^{13}C NMR (100 MHz, (CD$_3$)$_2$S=O): δ 208.04, 173.80, 172.28, 171.08, 170.99, 169.20, 168.51, 168.12, 137.62, 129.01, 127.97, 126.18, 103.92, 100.79, 81.39, 75.48, 74.87, 73.12, 72.14, 70.50, 68.33, 68.09, 60.39, 54.68, 54.16, 51.85, 42.30, 41.96, 41.84, 38.43, 36.89, 34.01, 29.81, 29.61, 29.03, 28.94, 28.72, 28.67, 27.06, 26.27, 25.31, 22.86, 19.19. ESI-HRMS: $C_{50}H_{79}N_6O_{19}$ $[M-H]^-$ calcd (*m*/*z*) 1067.54055, found (*m*/*z*) 1067.54345.

*4.4.7. 12-[N-(5-Oxohexanoyl)-L-phenylalanyl-L-glutamyl-glycinyl-glycinyl]-aminododecyl O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**22**).* To obtain physical data, six of residual reaction mixtures performed in Scheme 3 were mixed (1.65 ml) and freeze-dried. The resulting residue was purified by RP-HPLC [eluent D in eluent C gradient increase from 25% to 40% over 30 min] to give **22** (3.5 mg, quant.) as an amorphous. t_R=22 min. ^{1}H NMR (400 MHz, (CD$_3$)$_2$S=O): δ 12.09 (1H, s, −C(=O)O*H*), 8.15 (1H, d, *J*=7.6 Hz, Glu^2-N*H*), 8.11 (1H, t, *J*=5.6 Hz, Gly^3-N*H* or Gly^4-N*H*), 8.07 (1H, t, *J*=6.0 Hz, Gly^3-N*H* or Gly^4-N*H*), 8.06 (1H, d, *J*=8.4 Hz, Phe^1-N*H*), 7.73 (1H, d, *J*=8.4 Hz, −N*H*C(=O)CH$_3$), 7.69 (1H, t, *J*=5.6 Hz, −CH$_2$CH$_2$N*H*C(=O)−), 7.26–7.15 (5H, m, aromatic-*H*), 5.07 (1H, br, OH-2′), 4.78 (1H, br, OH-3′), 4.65 (1H, dd, *J*=5.2, 4.4 Hz, OH-6′), 4.61 (1H, s, OH-3), 4.58 (1H, t, *J*=6.2 Hz, OH-6), 4.55 (1H, m, Phe^1-Hα), 4.50 (1H, d, *J*=4.4 Hz, OH-4′), 4.29 (1H, d, *J*=7.6 Hz, H-1), 4.29–4.24 (1H, m, Glu^2-Hα), 4.20 (1H, m, H-1′), 3.78–3.58 (8H, m, H-6b, −OC*H*$_2$CH$_2$−, Gly^3-Hα, Gly^4-Hα, H-6a and H-4′), 3.55–3.44 (5H, m, H-6′, H-5′, H-4, and H-2), 3.39–3.22 (5H, m, −OC*H*$_2$CH$_2$−, H-2′, H-3, H-5, and H-3′), 3.05–3.00 (3H, m, −CH$_2$C*H*$_2$NH− and Phe^1-Hβ), 2.72 (1H, dd, *J*=14.0, 10.4 Hz, Phe^1-Hβ), 2.25 (2H, t, *J*=8.4 Hz, Glu^2-Hγ), 2.21 (2H, t, *J*=7.2 Hz, −C*H*$_2$C(=O)CH$_3$), 2.01 (2H, m, −NHC(=O)C*H*$_2$−), 2.00 (3H, s, −CH$_2$C(=O)C*H*$_3$−), 1.94 (1H, m, Glu^2-Hβ), 1.80 (1H, m, Glu^2-Hβ), 1.77 (3H, s, NHC(=O)C*H*$_3$), 1.54 (2H, qui, *J*=7.2 Hz, −C(=O)CH$_2$C*H*$_2$CH$_2$C(=O)−), 1.45–1.35 (4H, m, −OCH$_2$C*H*$_2$− and −C*H*$_2$CH$_2$NH−), 1.25–1.20 (16H, m, −OCH$_2$CH$_2$(C*H*$_2$)$_8$−). ^{13}C NMR (100 MHz, (CD$_3$)$_2$S=O): δ 207.93, 173.85, 171.72, 171.57, 171.40, 168.77, 168.51, 168.22, 137.94, 129.02, 127.87, 126.07, 103.92, 100.79, 81.39, 75.48, 74.87, 73.12, 72.14, 70.50, 68.33, 68.09, 60.40, 54.68, 53.63, 51.90, 42.10, 41.92, 41.59, 38.45, 37.12, 34.05, 29.82, 29.60, 29.03, 28.94, 28.72, 28.65, 27.06, 26.28, 25.31, 22.87, 19.15. ESI-HRMS: $C_{50}H_{79}N_6O_{19}$ $[M-H]^-$ calcd (*m*/*z*) 1067.54055, found (*m*/*z*) 1067.54355.

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