Synthesis of Tn, Sialyl Tn and HIV-1-Derived Peptide Antigen Conjugates Having a Lipid A Analog as an Immunoadjuvant for Synthetic Vaccines

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Conjugates (3—5) of Tn, sialyl Tn and HIV-1-derived peptide antigen with a *N*-tetradecanoyl L-serine-β-alanine-containing D-glucosamine derivative, structurally related to lipid A, as an immunoadjuvant for the development of totally synthetic vaccines against cancers or HIV were synthesized. The mitogenic activity of compounds 3, 4 and 5 was stronger than that of lipid A analogs (1, 2).

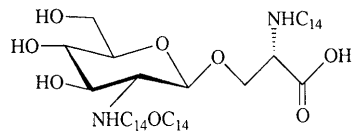
Key words Tn; sialyl Tn; lipid A analog; HIV-1-derived peptide; synthetic vaccine; mitogenic activity

Lipid A is well known to be responsible for expression of many of the biological activities, such as endotoxicity, adjuvanticity, and antitumor activity, of the lipopolysaccharide (LPS) of Gram-negative bacteria.[1] Many compounds related to lipid A partial structures have been synthesized with the aim of enhancing biological activities.[2] We have already reported the synthesis and biological activities of *N*-acylated L-serine, L-threonine and L-homoserine-containing D-glucosamine derivatives structurally similar to the lipid A disaccharide backbone.[3] As a result, it was found that *N*-tetradecanoyl L-serine-linked lipid A analog (**1**) exhibited potent mitogenic activity. In addition, we have recently reported that compound **2**, with β-alanine introduced into compound **1**, possessed the same activity as **1**.[4]

In recent years, development of totally synthetic vaccines against cancers or human immunodeficiency virus (HIV) using a synthetic immunoadjuvant, such as *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) and lipopeptide analogs, has been attempted.[5] We planned to develop completely synthetic vaccines which consist of lipid A analog (**2**) as a synthetic immunoadjuvant, covalently coupled to a low-molecular-weight antigen. We selected Tn [α-D-GalNAc-(1→*O*)-Ser], sialyl Tn [α-D-Neu5Ac-(2→6)-α-D-GalNAc-(1→O)-Ser] and HIV-1 envelope glycoprotein gp120-derived peptide (RIQRGPGRAFVTI: I13)[6] epitopes as antigens. Tn and sialyl Tn epitopes have been identified as tumour-associated carbohydrate antigens present in glycoproteins on the surface of cancer cells.[7] Further, the Tn and sialyl Tn epitopes have been discovered on the envelope glycoprotein gp120 of HIV.[8] On the other hand, I13 has been identified as an antigenic peptide that could stimulate a cytotoxic T lymphocyte (CTL) response and induce T helper (Th) cell activity.[6] In this paper, we describe synthetic details for the preparation of conjugates (**3**—**5**) of the Tn, sialyl Tn and HIV-1 derived peptide (I13) antigens with *N*-teradecanoyl L-serine-β-alanine-containing D-glucosamine derivative (**2**), structurally related to lipid A as an immunoadjuvant, and their biological effects.

Several syntheses of the Tn and sialyl Tn antigens have been reported.[9] We achieved the synthesis of Tn and sialyl Tn antigen derivatives (**14**, **25**), with an ethylene diamine introduced as a spacer, suitable for coupling to lipid A analog (**2**), by the routes shown in Charts 3, 4.

First, synthesis of Tn antigen derivative (**14**) was carried out as follows. Treatment of **6**[10] with thiophenol (PhSH) in the presence of boron trifluoride etherate (BF_3OEt_2) in CH_2Cl_2 gave thiophenylglycoside (**7**) in 91% yield. Coupling of **7** and **8** with *N*-bromosuccinimide (NBS), iodine, tetrabutylammonium trifluoromethanesulfonate (TBAOTf) as a promoter, and 4 Å molecular sieves in CH_2Cl_2 gave the α-glycoside (**9**) in 65% yield.[11] The α-configuration of **9** was determined from the coupling constant (3.6 Hz) of the signal due to the anomeric proton in the proton magnetic resonance (^{1}H-NMR) spectrum. Reduction of the azido group in **9** with thioacetic acid in pyridine–CH_2Cl_2 gave **10** in 68% yield. After the benzyl group of **10** was removed by hydrogenolysis



1

C_{14}: $CH_3(CH_2)_{12}C(O)$-

$C_{14}OC_{14}$: $CH_3(CH_2)_{10}\overset{(R)}{\underset{|}{C}}HCH_2C(O)$-
$CH_3(CH_2)_{12}C(O)O$

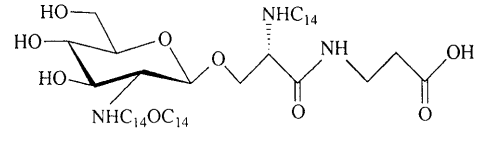


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Chart 1

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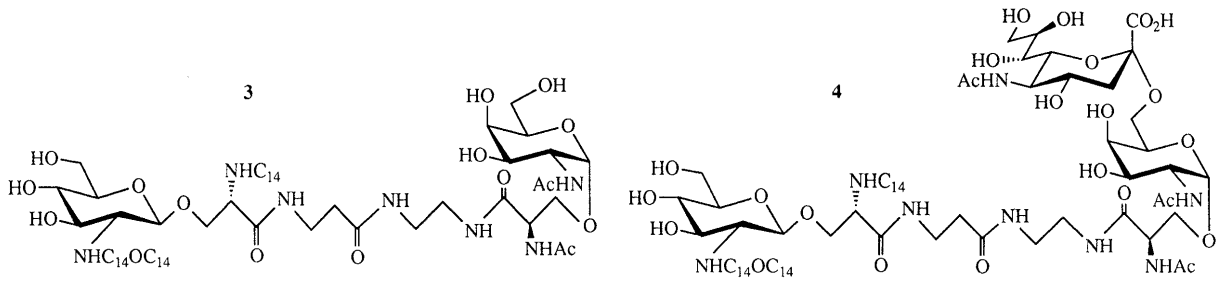
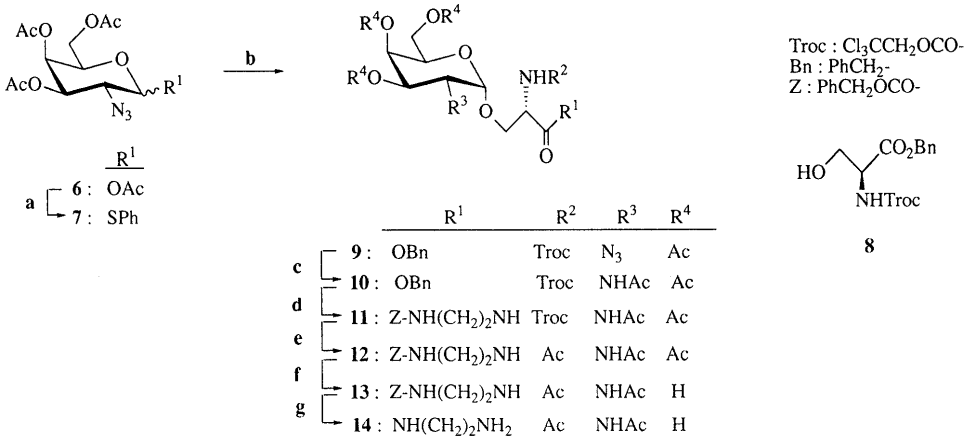


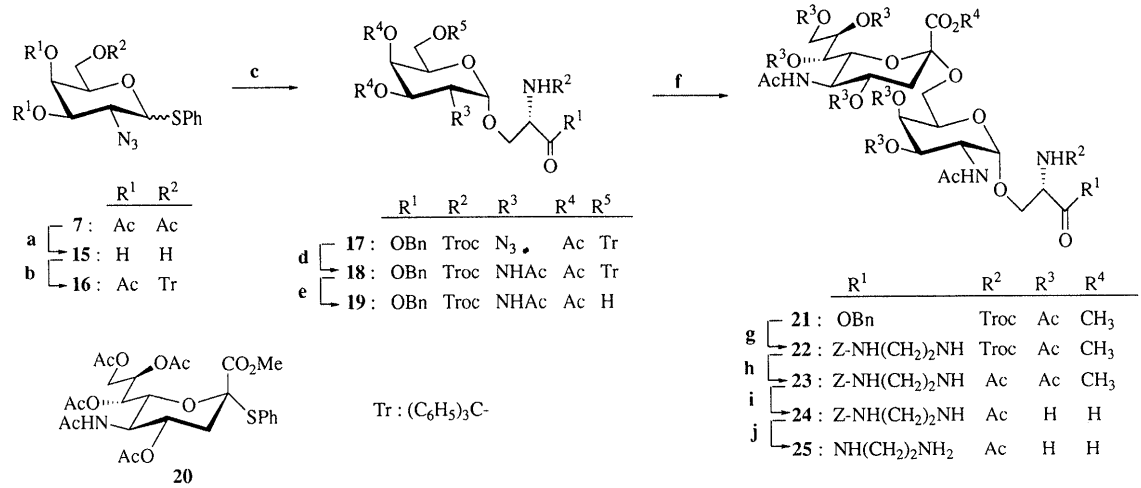
Chart 2



		R^1	R^2	R^3	R^4
a	**6**	OAc			
a	**7**	SPh			
c	**9**	OBn	Troc	N_3	Ac
c	**10**	OBn	Troc	NHAc	Ac
d	**11**	Z-NH$(CH_2)_2$NH	Troc	NHAc	Ac
e	**12**	Z-NH$(CH_2)_2$NH	Ac	NHAc	Ac
f	**13**	Z-NH$(CH_2)_2$NH	Ac	NHAc	H
g	**14**	NH$(CH_2)_2NH_2$	Ac	NHAc	H

reagents : a) PhSH, BF_3•Et_2O in CH_2Cl_2 ; b) **8**, NBS, I_2, TBAOTf, MS 4Å in Et_2O ; c) AcSH, in pyridine-CH_2Cl_2 ; d) 1) Pd-black, H_2 in THF ; 2) Z-NH$(CH_2)_2NH_2$•HCl, WSC•HCl, HOBt, Et_3N in DMF ; e) 1) Zn in AcOH ; 2) Ac_2O, DMAP in pyridine-CH_2Cl_2 ; f) 0.1NKOH in MeOH ; g) $Pd(OH)_2$, H_2 in MeOH.

Chart 3



		R^1	R^2	R^3	R^4	R^5
a	**7**	Ac	Ac			
a	**15**	H	H			
b	**16**	Ac	Tr			
d	**17**	OBn	Troc	N_3	Ac	Tr
d	**18**	OBn	Troc	NHAc	Ac	Tr
e	**19**	OBn	Troc	NHAc	Ac	H

		R^1	R^2	R^3	R^4
g	**21**	OBn	Troc	Ac	CH_3
g	**22**	Z-NH$(CH_2)_2$NH	Troc	Ac	CH_3
h	**23**	Z-NH$(CH_2)_2$NH	Ac	Ac	CH_3
i	**24**	Z-NH$(CH_2)_2$NH	Ac	H	H
j	**25**	NH$(CH_2)_2NH_2$	Ac	H	H

reagents : a) NaOMe in MeOH ; b) 1)TrCl, DMAP in pyridine-DMF ; 2) Ac_2O ; c) **8**, NBS, I_2, TBAOTf, MS 4Å in Et_2O ; d) AcSH in pyridine-CH_2Cl_2 ; e) 80%AcOH ; f) **20**, NBS, I_2, TBAOTf, MS 3Å in CH_3CN ; g) 1) Pd-black, H_2 in MeOH-THF ; 2) Z-NH$(CH_2)_2NH_2$•HCl, WSC•HCl, HOBt, Et_3N in DMF ; h) 1) Zn in AcOH ; 2) Ac_2O, DMAP in pyridine-CH_2Cl_2 ; i) 0.1NKOH in MeOH ; j) $Pd(OH)_2$, H_2 in MeOH.

Chart 4

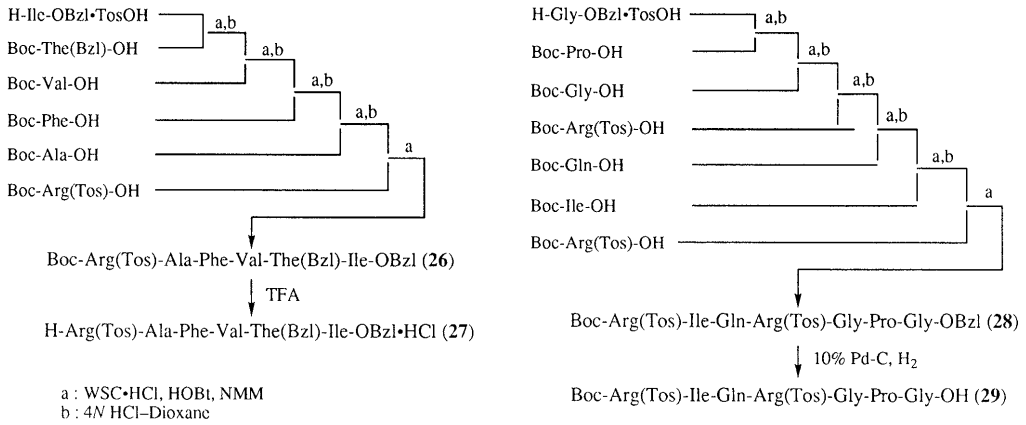
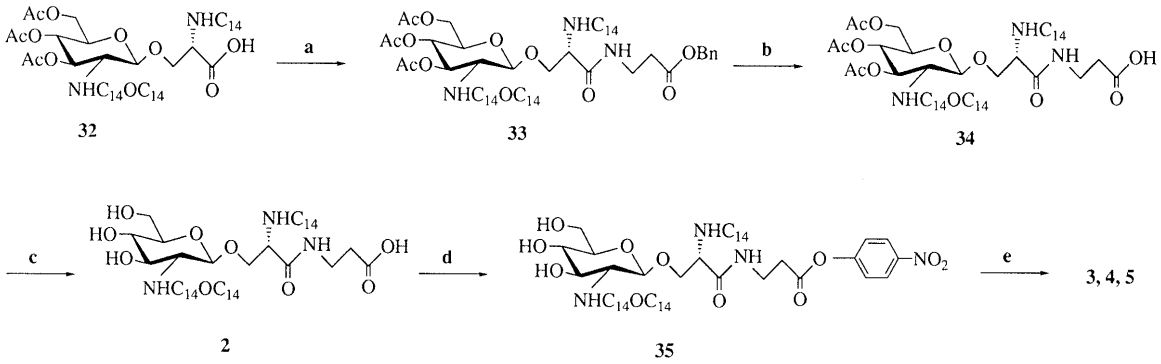


Chart 5



reagents : a) Z-NH(CH$_2$)$_2$NH$_2$•HCl, WSC•HCl, HOBt, Et$_3$N in DMF ; b) Pd-black, H$_2$ in THF ;
c) conc. NH$_4$OH in MeOH-THF ; d) *p*-nitrophenol, WSC•HCl in DMF ; e) **14**, **25** or **31**, NMM in DMF.

Chart 6

over palladium-black in tetrahydrofuran (THF), a *N*-benzyloxycarbonylaminoethylamino (Z-EDA) group was introduced to the carboxyl group by the water-soluble carbodiimide (WSC)–1-hydroxy-1*H*-benzotriazole (HOBt) coupling method, to give **11** in 80% yield over two steps. Cleavage of the 2,2,2-trichloroethoxycarbonyl (Troc) group of **11** with activated zinc powder in acetic acid (AcOH), followed by acetylation using Ac_2O in the presence of 4-dimethylaminopyridine (DMAP) in pyridine–CH_2Cl_2, gave **12** in 56% yield over two steps. *O*-Deacylation of the acetyl groups of **12** with 0.1 N aquoeus KOH in MeOH afforded **13** in 92% yield. Finally, hydrogenolytic removal of the benzyloxycarbonyl group using palladium hydroxide in MeOH gave the desired product **14** in almost quantitative yield.

Next, sialyl Tn antigen derivative (**25**) was synthesized as follows. Methanolysis of **7** in the presence of sodium methoxide gave the triol **15** in 95% yield. The 6-hydroxyl group of **15** was selectively protected with triphenylmethyl (Tr) chloride in the presence of DMAP in pyridine–*N*,*N*-dimethylformamide (DMF), and the remaining hydroxyl groups were subsequently acylated with Ac_2O to give **16** in 81% yield. As described for **9**, glycosylation of **8** and **16** gave the α-glycoside (**17**) in 70% yield. Reduction of the azido group in **17** with thioacetic acid in pyridine–CH_2Cl_2 gave **18** in 66% yield. Removal of the Tr group with 80% aqueous AcOH at 60 °C gave the sialosyl acceptor (**19**) in 71% yield. Sialylation of **19** and sialosyl donor (**20**) with NBS and I_2 and TBAOTf in CH_3CN at −40 °C gave the α-glycoside (**21**) in 55% yield. The α-configuration of **21** was indicated by ^{1}H-NMR spectrum which showed signals for H-3eq. and H-4 of Neu5Ac at δ 2.57 and 4.84, respectively.[12] As described for the preparation of **14** from **10**, after removal of the benzyl group of **21**, a Z-EDA group was introduced to the carboxyl group by the WSC–HOBt coupling method to give **22** in 64% yield over two steps. Cleavage of the Troc group, followed by acetylation gave **23** in 73% yield over two steps. *O*-Deacylation of **23** afforded **24** in 98% yield. Finally, hydrogenolytic removal of the benzyloxycarbonyl group gave the desired product **25** in almost quantitative yield.

HIV-1-derived peptide (**31**) was synthesized according to the reaction sequence shown in Chart 5. **26** and **28** were pre-

pared by a stepwise peptide coupling strategy using the WSC–HOBt method. Deprotection of the butoxycarbonyl (Boc) group of **26** with trifluoroacetic acid (TFA) afforded **27** in 92% yield. The benzyl group of **28** was removed by hydrogenolysis over 10% palladium carbon to afford **29** in 90% yield. Coupling of **27** and **29** by the WSC–HOBt method gave **30** in 73% yield. All protecting groups in **30** were removed with anhydrous HF in anisole to give the peptide antigen (**31**) in 90% yield.

The Tn antigen derivative (**14**), sialyl Tn antigen derivative (**25**) and HIV-1-derived peptide (**31**)–lipid A analog (**2**) conjugates were constructed as shown in Chart 6. A Z-EDA group was introduced to the carboxyl group of **32**[3a] by the WSC–HOBt coupling method to give **33** in 75% yield. Catalytic hydrogenolysis of the benzyl group using palladium-black gave **34** in 98% yield. Removal of the acetyl groups with concentrated NH_4OH in MeOH–THF then gave lipid A analog (**2**) in 74% yield. Compound **2** was coupled with *p*-nitrophenol in the presence of WSC in DMF to give *p*-nitrophenyl ester (**35**) in 55% yield. Finally, this activated ester (**35**) was condensed with **14**, **25** and **31** in the presence of *N*-methylmorpholine (NMM) in DMF at room temperature for 4 d to give conjugates **3**, **4** and **5** in 47%, 43% and 58% yields, respectively, after purification by chromatography on a silica gel column and Sephadex LH-20, followed by lyophilization from a H_2O suspension.

The structures of all compounds were characterized by ^{1}H-NMR spectroscopy, infrared (IR) spectroscopy, and fast-atom bombardment (FAB) mass spectroscopy.

In a preliminary examination of mitogenic activity towards the splenocytes of C3H/He mice,[13] conjugates **3**, **4** and **5** exhibited potent activities in comparision with lipid A analogs (**1**, **2**). These results suggest that lipid A analog conjugates can be expected to induce antigen-specific immune responses. Further, studies on the biological activites of conjugates **3**, **4** and **5** are in progress.

Experimental

All melting points are uncorrected. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. IR spectra were recorded on a JASCO A-202 infrared spectrophotometer. FAB-MS were recorded on a JEOL JMS-SX 102 spectrometer. ^{1}H-NMR spectra were taken on a JEOL JNM-GX 270 (270 MHz) spectrometer. ^{13}C-NMR spectra were recorded with a JEOL JNM-GX 270 (67.5 MHz) spectrometer. ^{1}H and ^{13}C chemical shifts (δ) are given in ppm relative to Me_4Si (δ=0) in $CDCl_3$ or CD_3OD as an internal standard. The abbreviations of signal patterns are as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Column chromatography was carried out on Silica gel 60 (70—230 mesh, Merck). Thin-layer chromatography (TLC) on Silica gel 60-F_{254} (Merck) was used to monitor reactions and to ascertain the purity of reaction products. The spots were visualized by spraying the plates with 5% aqueous sulfuric acid and heating.

Phenyl-3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-D-galactopyranoside (7) Boron trifluoride etherate (430 mg, 3.0 mmol) was added dropwise to a solution of **6** (450 mg, 1.2 mmol) and thiophenol (150 mg, 1.3 mmol) in CH_2Cl_2 (10 ml) at 0 °C. The mixture was stirred at room temperature for 4 h, diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using hexane–AcOEt (2 : 1) to give **7** (470 mg, 91%, α : β=6 : 5) as an amorphous powder, $[\alpha]_D$ +82.2° (c=0.31, $CHCl_3$). IR (ν neat): 2110, 1735, 695 cm^{-1}. ^{1}H-NMR ($CDCl_3$): (α-compound) δ: 1.99, 2.07, 2.16 (each 3H, s, $OCOCH_3$), 3.89 (1H, t, J=6.2 Hz, H-6), 4.15 (1H, dd, J=9.2, 7.0 Hz, H-6), 4.32 (1H, dd, J=11.1, 5.4 Hz, H-2), 4.76 (1H, m, H-5), 5.18 (1H, dd, J=11.1, 3.0 Hz, H-3), 5.49 (1H, br d, H-4), 5.70 (1H, d, J=5.4 Hz, H-1), 7.31—7.38 (3H, m, Ph), 7.48—7.54 (2H, m, Ph). Positive FAB-MS m/z: 423 $(M+H)^+$. (β-compound) δ: 2.03, 2.05, 2.09 (each 3H, s, $OCOCH_3$), 3.65 (1H, t, J=6.2 Hz, H-6), 4.15 (1H, dd, J=9.2, 7.0 Hz, H-6), 4.53 (1H, d, J=10.1 Hz, H-1), 4.65 (1H, m, H-5), 4.87 (1H, dd, J=10.3, 3.2 Hz, H-3), 5.36 (1H, m, H-4), 7.31—7.38 (3H, m, Ph), 7.60—7.64 (2H, m, Ph).

***N*-Trichloroethoxycarbonyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-L-serine Benzyl Ester (9)** A solution of **7** (40 mg, 0.095 mmol) and *N*-trichloroethoxycarbonyl-L-serine benzyl ester **8** (63 mg, 0.17 mmol) in anhydrous Et_2O (3 ml) was stirred for 1 h at room temperature under argon in the presence of 4 Å powdered molecular sieves (800 mg). The mixture was cooled to −20 °C, then NBS (67 mg, 0.38 mmol), iodine (96 mg, 0.38 mmol), and TBAOTf (18 mg, 0.047 mmol) were added. The mixture was stirred for 1 h at the same temperature and for 2 h at room temperature. After removal of the insoluble materials by filtration, the filtrate was washed successively with 10% aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using hexane–AcOEt (4 : 1) to give **9** (42 mg, 65%) as an amorphous powder, $[\alpha]_D$ +79.0° (c=0.68, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 2.04, 2.05, 2.13 (each 3H, s, $OCOCH_3$), 3.60 (1H, dd, J=11.2, 3.6 Hz, H-6), 3.87—4.12 (4H, m, H-5, 6, OCH_2CHNH), 4.42—4.49 (1H, m, OCH_2CHNH), 4.61—4.79 (2H, m, CH_2CCl_3), 4.90 (1H, d, J=3.6 Hz, H-1), 5.24 (2H, br s, CH_2Ph), 5.25—5.40 (2H, m, H-3,4), 6.16 (1H, d, J=8.2 Hz, NH), 7.25—7.42 (5H, m, Ph). ^{13}C-NMR ($CDCl_3$) δ: 20.9, 21.0 (q, $OCOCH_3$), 55.0 (d, OCH_2CHNH), 57.6 (d, C-2), 61.9 (t, C-6), 67.5 (d, C-4), 67.6 (t, CH_2Ph), 67.8 (d, C-3), 68.2 (d, C-5), 70.1 (t, OCH_2CHNH), 75.0 (t, CH_2CCl_3), 95.5 (s, CH_2CCl_3), 99.7 (d, C-1), 128.6, 128.9, 129.0 (d, Ph), 135.1 (s, Ph), 154.5, 169.2, 170.0, 170.2, 170.7 (s, C=O). Positive FAB-MS m/z: 683 $(M+H)^+$.

***N*-Trichloroethoxycarbonyl-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine Benzyl Ester (10)** **9** (42 mg, 0.062 mmol) was dissolved in CH_2Cl_2–pyridine (4 : 1, 5 ml), and thioacetic acid (1 ml, 12 mmol) was added to the solution with ice cooling under argon. The reaction mixture was stirred at room temperature for 8 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH (40 : 1) to give **10** (29 mg, 68%) as an amorphous powder, $[\alpha]_D$ +64.7° (c=1.16, $CHCl_3$). IR (Nujol): 1740, 1656 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.90 (3H, s, $NHCOCH_3$), 2.00, 2.08, 2.15 (each 3H, s, $OCOCH_3$), 3.87—4.11 (5H, m, H-5, 6, OCH_2CHNH) 4.46—4.67 (2H, m, H-2, OCH_2CHNH), 4.75 (2H, s, CH_2CCl_3), 4.84 (1H, d, J=3.6 Hz, H-1), 5.03—5.38 (4H, m, H-3, 4, CH_2Ph), 5.99 (1H, d, J=9.2 Hz,NH), 6.61 (1H, d, J=8.6 Hz, NH), 7.28—7.44 (5H, m, Ph). ^{13}C-NMR ($CDCl_3$) δ: 20.6, 20.7 (q, $OCOCH_3$), 23.0 (q, $NHCOCH_3$), 47.6 (d, C-2), 54.7 (d, OCH_2CHNH), 61.8 (t, C-6), 67.1 (d, C-3), 67.3 (d, C-5), 67.8 (t, CH_2Ph), 68.1 (d, C-4), 69.2 (t, OCH_2CHNH), 74.7 (t, CH_2CCl_3), 95.2 (s, CH_2CCl_3), 98.7 (d, C-1), 128.2, 128.5, 128.8 (d, Ph), 134.6 (s, Ph), 154.2, 169.7, 170.30, 170.33, 170.4, 174.9 (s, C=O). Positive FAB-MS m/z: 699 $(M+H)^+$.

***N*-Trichloroethoxycarbonyl-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-(*N'*-benzyloxycarbonylaminoethyl)amide (11)** Palladium–black (40 mg) was added to a solution of **10** (82 mg, 0.12 mmol) in THF (5 ml), and the mixture was stirred under a hydrogen atmosphere for 10 h at room temperature. The catalyst was filtered off and the filtrate concentrated under reduced pressure. The residue and Z-EDA·HCl (46 mg, 0.20 mmol) were dissolved in DMF (6 ml), and WSC·HCl (38 mg, 0.20 mmol) and TEA (40 mg, 0.40 mmol) were added to the solution with ice cooling under argon. The mixture was stirred for 1 h at 0 °C and 20 h at room temperature. The reaction mixture was diluted with AcOEt and washed with ice-H_2O, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH (40 : 1) to give **11** (74 mg, 80%) as an amorphous powder, $[\alpha]_D$ +66.8° (c=1.20, $CHCl_3$). IR (Nujol): 1740, 1656 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.89 (3H, s, $NHCOCH_3$), 2.01, 2.09, 2.15 (each 3H, s, $OCOCH_3$), 3.34—3.42 (4H, m, $NHCH_2CH_2NH$), 3.76—4.19 (5H, m, H-5, 6, OCH_2CHNH) 4.39—4.42 (1H, m, H-2), 4.55—4.66 (1H, m, OCH_2CHNH), 4.74 (2H, s, CH_2CCl_3), 4.93 (1H, d, J=3.6 Hz, H-1), 5.01—5.20 (3H, m, H-3, CH_2Ph), 5.37 (1H, d, J=3.2 Hz, H-4), 5.55 (1H, br s, NH), 6.33 (1H, d, J=7.6 Hz, NH), 6.54 (1H, d, J=8.9 Hz, NH), 7.20—7.41 (6H, m, Ph, NH). ^{13}C-NMR ($CDCl_3$) δ: 20.6, 20.7 (q, $OCOCH_3$) 23.0 (q, $NHCOCH_3$), 40.3, 41.4 (t, CH_2), 47.4 (d, C-2), 54.5 (d, OCH_2CHNH), 61.8 (t, C-6), 66.9 (d, C-4),67.1 (d, C-3), 67.6 (t, CH_2Ph), 68.3 (d, C-5), 69.1 (t, OCH_2CHNH), 74.7 (t, CH_2CCl_3), 95.2 (s, CH_2CCl_3), 98.6 (d, C-1), 128.3, 128.5, 128.6 (d, Ph), 135.9 (s, Ph), 154.3, 158.0, 169.2, 170.3, 170.5, 170.6, 170.9 (s, C=O). Positive FAB-MS m/z: 785 $(M+H)^+$.

***N*-Acetyl-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine-(*N'*-benzyloxycarbonylaminoethyl)amide (12)** Activated zinc powder (180 mg) was added to a solution of **11** (60 mg, 0.077 mmol) in AcOH (5 ml), and the mixture was stirred at 38—40°C for

18 h. After removal of the insoluble materials by filtration, the solvent was evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2, and this solution was washed with saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 (3 ml), and Ac_2O (78 mg, 0.77 mmol), pyridine (0.5 ml) and DMAP (10 mg, 0.077 mmol) were added to the solution with ice cooling under argon. After stirring at 0 °C for 1 h and for 17 h at room temperature, the reaction mixture was diluted with CH_2Cl_2, and washed successively with 2 N HCl, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH (30 : 1) to give **12** (28 mg, 56%), $[\alpha]_D$ +77.4° (c=0.56, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.97, 1.98, 2.04, 2.15, 2.19 (each 3H, s, $OCOCH_3$), 3.25—3.46 (4H, m, $NHCH_2CH_2NH$), 3.71—3.78 (1H, m, H-5), 3.98—4.15 (4H, m, H-6, OCH_2CHNH) 4.50—4.72 (2H, m, H-2, OCH_2CHNH), 4.90 (1H, d, J= 3.6 Hz, H-1), 5.01—5.20 (3H, m, H-3, CH_2Ph), 5.37 (1H, d, J=3.0 Hz, H-4), 5.52 (1H, br s, NH), 6.57 (1H, d, J=9.6 Hz, NH), 6.72 (1H, d, J=7.6 Hz, NH), 7.21—7.43 (6H, m, Ph, NH). ^{13}C-NMR ($CDCl_3$) δ: 21.0 (q, $OCOCH_3$), 23.4 (q, $NHCOCH_3$), 40.7, 41.7 (t, $NHCH_2CH_2NH$), 47.7 (d, C-2), 52.8 (d, OCH_2CHNH), 62.2 (t, C-6), 67.35 (d, C-4),67.36 (t, CH_2Ph), 67.4 (t, OCH_2CHNH), 68.7 (d, C-3, 5), 99.0 (d, C-1), 128.2, 128.7, 128.9 (d, Ph), 136.2 (s, Ph), 158.3, 170.1, 170.7, 170.8, 170.9, 171.1, 171.3 (s, C=O). Positive FAB-MS m/z: 653 $(M+H)^+$.

***N*-Acetyl-3-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine-(*N'*-benzyloxycarbonylaminoethyl)amide (13)** **12** (21 mg, 0.032 mmol) was dissolved in MeOH (3 ml), and 0.1 N KOH (1.7 ml, 0.17 mmol) was added to the solution at 0 °C under argon. The mixture was stirred for 2 h at 0 °C and then for 1 h at room temperature. The solution was adjusted to pH 3 with ion exchange resin (Amberlite IR-120) and the resin removed by filtration. The filtrate was evaporated to dryness and the residue was purified by gel filtration (LH-20) using CH_2Cl_2–MeOH–H_2O (12 : 8 : 1) to give **13** (16 mg, 92%) as an amorphous powder. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 1.94, 1.97 (each 3H, s, $NHCOCH_3$), 5.00 (2H, br s, CH_2Ph), 7.27 (5H, s, Ph). ^{13}C-NMR ($CDCl_3$–CD_3OD) δ: 21.8, 21.9 (q, $NHCOCH_3$), 39.4, 39.8 (t, $NHCH_2CH_2NH$), 49.7 (d, C-2), 53.0 (d, OCH_2CHNH), 61.5 (t, C-6), 66.2, 68.2, 68.7, 68.9, 70.9 (C-3, 4, 5, CH_2Ph, OCH_2CHNH), 98.4 (d, C-1), 127.3, 127.6, 128.0 (d, Ph), 135.8 (S, Ph), 154.5, 170.4, 171.5, 172.4 (s, C=O). Positive FAB-MS m/z: 527 $(M+H)^+$.

***N*-Acetyl-3-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine-aminoethylamide (14)** Palladium hydroxide (30 mg) was added to a solution of **13** (24 mg, 0.046 mmol) in MeOH (5 ml), and the mixture was stirred under a hydrogen atmosphere for 3 h at room temperature. The catalyst was filtered off and the filtrate was concentrated under reduced pressure to give **14** (18 mg, quant.). Compound **14** was used for the subsequent acylation without further purification. Positive FAB-MS m/z: 393 $(M+H)^+$.

Phenyl-2-azido-2-deoxy-1-thio-D-galactopyranoside (15) **7** (370 mg, 0.87 mmol) was dissolved in MeOH (9 ml), and sodium methoxide (47 mg, 0.87 mmol) was added to the solution at 0 °C. The mixture was stirred for 1 h at 0 °C and then for 8 h at room temperature. It was adjusted to pH 3 with ion exchange resin (Amberlite IR-120) and the resin removed by filtration. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH–H_2O (10 : 10 : 1) to give **15** (250 mg, 95%) as a white powder, mp 133—135 °C, $[\alpha]_D$ +129.4° (c=1.00, MeOH). IR (ν neat): 3388, 2102 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.46 (1H, m, H-5), 3.58 (1H, dd, J=9.5 Hz, H-2), 3.84 (4H, m, H-3, 4, 6), 4.15 (1H, m, H-5), 4.47 (1H, d, J=9.2 Hz, H-1β), 7.23—7.37 (3H, m, Ph), 7.50—7.54 (2H, m, Ph). ^{13}C-NMR ($CDCl_3$) δ: 62.4 (t, C-6), 62.36 (d, C-3), 69.0 (d, C-2), 73.9 (d, C-4), 77.7 (d, C-5), 87.1(d, C-1β), 128.2, 129.1, 132.2 (d, Ph), 132.4 (s, Ph), 169.5, 170.0, 170.2 (s, C=O), 87.8 (d, C-1α). Positive FAB-MS m/z: 297 $(M+H)^+$.

Phenyl-3,4-di-*O*-acetyl-2-azido-6-*O*-triphenylmethyl-2-deoxy-1-thio-β-D-galactopyranoside (16) Triphenylmethyl chloride (440 mg, 1.6 mmol) was added to a solution of **15** (190 mg, 0.64 mmol) and DMAP (78 mg, 0.63 mmol) in pyridine–DMF (9 : 1, 6 ml) at room temperature. After the mixture was stirred at 70 °C for 10 h, acetic anhydride (647 mg, 6.3 mmol) was added with ice cooling. After stirring at room temperature for 4 h, the reaction mixture was diluted with CH_2Cl_2, washed with water, saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using hexane–AcOEt (2 : 1) to give **16** (320 mg, 81%) as an amorphous powder, $[\alpha]_D$ +104.2° (c=1.00, $CHCl_3$). IR (ν neat): 2110, 1742, 1215, 746 cm^{-1}. ^{1}H-NMR ($CDCl_3$): (α-compound) δ: 1.91, 2.08 (each 3H, s, $OCOCH_3$), 3.02 (1H, dd, J=9.5, 6.8 Hz, H-6), 3.23 (1H, dd, J=9.5, 5.9 Hz, H-6), 4.22 (1H, dd, J=11.1, 5.4 Hz, H-2), 4.69 (1H, t, J=6.5 Hz, H-5), 5.20 (1H, dd, J= 11.1, 3.2 Hz, H-3), 5.56 (1H, m, H-4), 5.61 (1H, d, J=5.4 Hz, H-1α), 7.23—7.38 (18H, m, Ph), 7.49—7.60 (2H, m, Ph). Positive FAB-MS m/z: 624 $(M+H)^+$. (β-compound) δ: 1.88, 2.03 (each 3H, s, $OCOCH_3$), 3.07 (1H, dd, J=9.5, 7.3 Hz, H-6), 3.41 (1H, dd, J=9.5, 5.9 Hz, H-6), 3.60 (1H, t, J= 10.4 Hz, H-2), 3.70 (1H, m, H-5), 4.50 (1H, d, J=10.2 Hz, H-1β), 4.86 (1H, dd, J=10.4, 3.1 Hz, H-3), 5.46 (1H, br d, H-4), 7.23—7.40 (18H, m, Ph), 7.49—7.60 (2H, m, Ph).

***N*-Trichloroethoxycarbonyl-3-*O*-(3,4-di-*O*-acetyl-2-azido-2-deoxy-6-*O*-triphenylmethyl-α-D-galactopyranosyl)-L-serine Benzyl Ester (17)** The same procedure as described for the preparation of **9** provided a crude product from **16** (410 mg, 0.66 mmol), **8** (490 mg, 1.3 mmol), NBS (470 mg, 2.6 mmol), iodine (670 mg, 2.6 mmol), TBAOTf (51 mg, 0.13 mmol), and 4 Å powdered molecular sieves in anhydrous Et_2O (20 ml), and this was purified by silica gel column chromatography using hexane–AcOEt (4 : 1) to give **17** (410 mg, 70%) as an amorphous powder, $[\alpha]_D$ +33.5° (c=0.37, $CHCl_3$). IR (Nujol): 2110, 1745, 740 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.79, 1.98 (each 3H, s, OCOCH3), 2.88 (1H, m, H-6), 3.25 (1H, dd, J=9.0, 3.1 Hz, H-6), 3.44 (1H, dd, J=11.1, 3.5 Hz, H-2), 3.89—4.13 (3H, m, H-5, OCH_2CHNH), 4.50—4.59 (3H, m, CH_2CCl_3, OCH_2CHNH), 4.77 (1H, d, J= 3.7 Hz, H-1), 5.13—5.24 (3H, m, H-3, CH_2Ph), 5.43—5.49 (1H, m, H-4), 6.02 (1H, d, J=8.4 Hz, NH), 7.13—7.32 (20H, m, Ph). ^{13}C-NMR ($CDCl_3$) δ: 20.4, 20.7 (q, $OCOCH_3$), 54.7 (d, OCH_2CHNH), 57.6 (d, C-2), 60.9 (t, C-6), 67.6 (d, C-4), 67.9 (t, CH_2Ph), 68.0 (d, C-3), 68.6 (d, C-5), 69.9 (t, OCH_2CHNH), 74.6 (t, CH_2CCl_3), 86.9 (s, CPh_3), 95.3 (s, CH_2CCl_3), 99.6 (d, C-1), 127.1, 127.9, 128.5, 128.6, 128.7, 128.8 (d, Ph), 143.3 (s, Ph), 154.4, 169.1, 169.5, 169.7 (s, C=O). Positive FAB-MS m/z: 883 $(M+H)^+$.

***N*-Trichloroethoxycarbonyl-3-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-triphenylmethyl-α-D-galactopyranosyl)-L-serine Benzyl Ester (18)** The same procedure as described for the preparation of **10** provided a crude product from **17** (1.31 g, 1.5 mmol), pyridine (8 ml) and thioacetic acid (8.6 ml, 120 mmol), and this was purified by silica gel column chromatography using CH_2Cl_2–MeOH (40 : 1) to give **18** (880 mg, 66%) as an amorphous powder, $[\alpha]_D$ +29.3° (c=0.20, $CHCl_3$). IR (Nujol): 1744, 1554cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.91 (3H, s, $NHOCOCH_3$), 1.92, 2.00 (each 3H, s, $OCOCH_3$), 3.01 (1H, m, H-6), 3.32 (1H, dd, J=9.2, 3.1 Hz, H-6), 3.90—4.02 (3H, m, H-5, OCH_2CHNH), 4.45 (1H, ddd, J=12.3, 8.6, 3.7 Hz, H-2), 4.56—4.59 (1H, m, OCH_2CHNH), 4.71 (2H, s, CH_2CCl_3), 4.74 (1H, d, J=3.7 Hz, H-1), 5.07 (1H, dd, J=11.6, 3.6 Hz, H-3), 5.17, 5.20 (each 1H, d, J=12.2, CH_2Ph), 5.44 (1H, br d, H-4), 5.65 (1H, d, J=9.5 Hz, NH), 6.08 (1H, d, J=6.5 Hz, NH), 7.20—7.40 (20H, m, Ph). ^{13}C-NMR ($CDCl_3$) δ: 20.6, 20.8, 23.17 (q, $OCOCH_3$), 47.9 (d, C-2), 54.7 (d, OCH_2CHNH), 61.2 (t, C-6), 67.3 (d, C-4), 67.5 (t, CH_2Ph), 68.4 (d, C-3), 68.6 (d, C-5), 69.4 (t, OCH_2CHNH), 74.7 (t, CH_2CCl_3), 86.9 (s, CPh_3), 95.1 (s, CH_2CCl_3), 99.2 (d, C-1), 127.1, 127.9, 128.5 (d, Ph), 143.6 (s, Ph), 154.2, 169.6, 169.8, 170.1, 171.0 (s, C=O). Positive FAB-MS m/z: 899 $(M+H)^+$.

***N*-Trichloroethoxycarbonyl-3-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine Benzyl Ester (19)** A mixture of **18** (890 mg, 0.99 mmol) and 80% aqueous AcOH (25 ml) was stirred at 60 °C for 6 h. The mixture was diluted with CH_2Cl_2, washed with water and brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH (30 : 1) to give **19** (230 mg, 71%) as an amorphous powder, $[\alpha]_D$ +58.0° (c=1.00, $CHCl_3$). IR (Nujol): 3332, 1743, 1659, 1537 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.85 (3H, s, $NHOCOCH_3$), 1.95, 2.10 (each 3H, s, $OCOCH_3$), 3.40 (1H, m, H-6), 3.56 (1H, m, H-6), 3.90—4.01 (3H, m, H-5, OCH_2CHNH), 4.43—4.56 (2H, m, H-2, OCH_2CHNH), 4.69 (2H, s, CH_2CCl_3), 4.74 (1H, d, J=3.8 Hz, H-1), 5.01 (1H, dd, J=11.3, 3.2 Hz, H-3), 5.13, 5.16 (each 1H, d, J=14.9, CH_2Ph), 5.22 (1H, br d, H-4), 5.76 (1H, d, J=9.7 Hz, NH), 6.46 (1H, d, J=6.5 Hz, NH), 7.25—7.35 (5H, m, Ph). ^{13}C-NMR ($CDCl_3$) δ: 20.6, 20.8, 23.17 (q, $OCOCH_3$), 47.9 (d, C-2), 54.8 (d, OCH_2CHNH), 60.9 (d, C-4), 67.8 (t, CH_2Ph), 68.2 (d, C-3), 69.6 (t, OCH_2CHNH), 69.8 (d, C-5), 74.7 (t, CH_2CCl_3), 95.2 (s, CH_2CCl_3), 99.1 (d, C-1), 127.6, 127.9, 128.8 (d, Ph), 134.6 (s, Ph), 146.8, 154.3, 169.7, 170.3, 171.7 (s, C=O). Positive FAB-MS m/z: 657 $(M+H)^+$.

***O*-[Methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate]-(2→6)-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-*N*-(trichloroethoxycarbonyl)-L-serine Benzyl Ester (21)** The same procedure as described for the preparation of **9** provided a crude product from **19** (200 mg, 0.30 mmol), sialosyl donor **20** (210 mg, 0.36 mmol), NBS (260 mg, 1.5 mmol), iodine (380 mg, 1.5 mmol), TBAOTf (71 mg, 0.18 mmol), 3 Å powdered molecular sieves (600 mg) in CH_3CN (6 ml) at −40 °C for 4 h, and this was purified by silica gel column chromatography using CH_2Cl_2–MeOH (30 : 1) to give **21** (190 mg, 55%) as an amorphous powder, $[\alpha]_D$ +18.9° (c=0.2, $CHCl_3$). IR (Nujol): 3312, 1745, 1663, 1540 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.88, 1.93,

1.99, 2.02, 2.04, 2.12, 2.15, 2.16 (each 3H, s, $OCOCH_3$), 2.54 (1H, dd, J=13.0, 4.6 Hz, H-3b), 3.32 (1H, dd, J=10.3, 4.6 Hz, H-6a), 3.76—3.84 (4H, m, H-6a, $COOCH_3$), 3.90—4.14 (4H, m, H-6b, H-9b, OCH_2CHNH), 4.30 (1H, dd, J=12.4, 2.4 Hz, H-9b), 4.59—4.65 (2H, m, H-2a, OCH_2CHNH), 4.76—4.78 (3H, m, H-1a, CH_2CCl_3), 4.83—4.85 (1H, m, H-4b), 5.08 (1H, dd, J=11.3, 3.5 Hz, H-3a), 5.18, 5.20 (each 1H, s, CH_2Ph), 5.24—5.38 (4H, m, H-4a, 7b, 8b, NH), 5.86 (1H, d, J=9.7 Hz, NH), 6.51 (1H, d, J=8.1 Hz, NH), 7.32—7.42 (5H, m, Ph). ^{13}C-NMR ($CDCl_3$) δ: 19.7, 20.7, 21.0 (q, $OCOCH_3$), 23.1, 24.2 (q, $NHCOCH_3$), 37.7 (t, C-3b), 47.5 (d, C-2a), 49.3 (d, C-5b), 52.9 (q, $COOCH_3$), 54.7 (d, OCH_2CHNH), 62.4 (t, C-6a), 63.2 (t, C-9b), 67.2 (d, C-4a), 67.3 (d, C-4b), 67.7 (t, CH_2Ph), 68.1 (t, OCH_2CHNH), 68.3 (d, C-3a), 68.5 (d, C-5a), 68.8 (d, C-8b), 70.8 (d, C-7b), 72.5 (d, C-6b), 74.7 (t, CH_2CCl_3), 95.2 (s, CH_2CCl_3), 98.8 (s, C-2b), 99.0 (d, C-1a), 128.4, 128.6, 128.8 (d, Ph), 134.7 (s, Ph), 154.4, 167.7, 169.8, 170.0, 170.2, 170.4, 170.5, 170.7, 170.9 (s, C=O). Positive FAB-MS m/z: 1130 $(M+H)^+$.

***O*-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate]-(2→6)-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-*N*-(trichloroethoxycarbonyl)-L-serine-(*N'*-benzyloxycarbonylaminoethyl)amide (22)** The same procedure as described for the preparation of **11** provided a crude product from **21** (120 mg, 0.11 mmol), palladium-black (200 mg) and MeOH–THF (1 : 3) (8 ml), followed by treatment with Z-EDA · HCl (44 mg, 0.19 mmol), WSC · HCl (36 mg, 0.19 mmol), HOBt (29 mg, 0.19 mmol), triethylamine (TEA) (38 mg, 0.38 mmol) and DMF (6 ml). This product was purified by silica gel column chromatography using CH_2Cl_2–MeOH (20 : 1) to give **22** (82 mg, 64%) as an amorphous powder, $[\alpha]_D$ +11.8° (c=0.4, $CHCl_3$). IR (Nujol): 3318, 1652, 1540, 740 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.88, 1.93, 1.99, 2.02, 2.04, 2.12, 2.15, 2.16 (each 3H, s, $COCH_3$), 2.53 (1H, dd, J=13.2, 4.9 Hz, H-3b), 3.28—3.36 (3H, m, H-6a, $NHCH_2CH_2NH$), 3.43 (2H, m, $NHCH_2CH_2NH$), 3.71—3.82 (4H, m, H-6a, $COOCH_3$), 3.90—3.97 (1H, m, H-5b), 4.01—4.13 (5H, m, H-5a, 6b, 9b, OCH_2CHNH) 4.34 (1H, dd, J=12.4, 2.4 Hz, H-9b), 4.42—4.46 (1H, m, OCH_2CHNH) 4.55—4.62 (1H, m, H-2a), 4.74—4.75 (3H, m, H-4b, CH_2CCl_3), 4.90 (1H, d, J=3.5 Hz, H-1a), 5.10 (2H, s, CH_2Ph), 5.13—5.20 (2H, m, H-3a, 8b), 5.32 (2H, m, H-7b, NH×2), 5.42 (1H, br d, H-4a), 6.37—6.40 (2H, m, NH), 7.33—7.43 (7H, m, Ph, NH×2). ^{13}C-NMR ($CDCl_3$) δ: 20.65, 20.7, 20.8, 20.9, 21.1 (q, $OCOCH_3$) 23.1, 23.2 (q, $NHCOCH_3$), 37.5 (t, C-3b), 40.4, 41.2 (t, $NHCH_2CH_2NH$), 47.5 (d, C-2a), 49.2 (d, C-5b), 52.9 (q, $COOCH_3$), 54.8 (d, OCH_2CHNH), 62.3 (t, C-6a), 63.0 (t, C-9b), 66.9 (d, C-4a), 67.2 (d, C-4b), 67.3 (t, CH_2Ph), 67.4 (t, OCH_2CHNH), 68.1 (d, C-3a), 68.4 (d, C-5a), 68.5 (d, C-8b), 68.8 (d, C-7b), 72.6 (d, C-6b), 74.7 (t, CH_2CCl_3), 95.2 (s, CH_2CCl_3), 98.8 (d, C-1a, C-2b), 127.9, 128.3, 128.6 (d, Ph), 136.0 (s, Ph), 154.5, 157.7, 167.7, 169.4, 169.7, 170.2, 170.4, 170.8, 170.9, 171.0, 172.0 (s, C=O). Positive FAB-MS m/z: 1216 $(M+H)^+$.

***O*-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate]-(2→6)-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-*N*-(acetyl)-L-serine-(*N'*-benzyloxycarbonylaminoethyl)amide (23)** The same procedure as described for the preparation of **12** provided a crude product from **22** (34 mg, 0.028 mmol), activated zinc powder (110 mg) and AcOH (5 ml), followed by treatment with Ac_2O (80 mg, 0.78 mmol), DMAP (10 mg, 0.077 mmol) and pyridine (0.5 ml). This product was purified by silica gel column chromatography using CH_2Cl_2–MeOH (30 : 1) to give **23** (22 mg, 73%) as an amorphous powder, $[\alpha]_D$ +17.5° (c=0.44, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.88, 1.96, 1.99, 2.01, 2.02, 2.07 (each 3H, s, $COCH_3$), 2.54 (1H, dd, J=12.9, 4.6 Hz, H-3b), 3.32—3.55 (5H, m, H-6a, $NHCH_2CH_2NH$), 3.78 (3H, s, $COOCH_3$), 3.70—3.96 (2H, m, H-5b, 6a), 4.04—4.14 (5H, m, H-5a, 6b, 9b, OCH_2CHNH), 4.31—4.36 (1H, m, H-9b), 4.52—4.76 (2H, m, H-2a, OCH_2CHNH) 4.86—4.95 (1H, m, H-4b), 4.88 (1H, d, J=3.6 Hz, H-1a), 5.10 (2H, s, CH_2Ph), 5.16—5.40 (5H, m, H-3a, 4a, 7b, 8b, NH), 6.52 (1H, d, J=9.6 Hz, NH), 6.82 (1H, d, J=7.3 Hz, NH), 7.18—7.43 (7H, m, Ph, NH×2). ^{13}C-NMR ($CDCl_3$) δ: 20.7, 20.8, 21.1 (q, $OCOCH_3$), 23.1, 23.2 (q, $NHCOCH_3$), 37.6 (t, C-3b), 40.4, 41.2 (t, $NHCH_2CH_2NH$), 47.4 (d, C-2a), 49.2 (d, C-5b), 52.8 (q, $COOCH_3$), 52.9 (d, OCH_2CHNH), 62.3 (t, C-6a), 63.0 (t, C-9b), 66.1 (d, C-4a), 67.1 (d, C-4b), 67.3 (t, CH_2Ph), 67.5 (t, OCH_2CHNH), 68.1 (d, C-3a), 68.5 (d, C-5a), 68.8 (d, C-8b), 69.0 (d, C-7b), 72.6 (d, C-6b), 98.7 (s, C-2b), 99.0 (d, C-1a), 127.9, 128.3, 128.6 (d, Ph), 136.0 (s, Ph), 157.7, 167.7, 169.9, 170.1, 170.3, 170.5, 170.6, 170.91, 170.94 (s, C=O). Positive FAB-MS m/z: 1084 $(M+H)^+$.

***O*-[5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl]-(1→3)-L-serine-(*N'*-benzyloxycarbonylaminoethyl)amide (24)** As described for **13**, **23** (13 mg, 0.012 mmol) was treated with 0.1 N KOH (1.2 ml, 0.12 mmol) in MeOH (3 ml) to give **24** (7.4 mg, 98%) as an amorphous powder, $[\alpha]_D$ +2.4° (c=0.20, MeOH). IR (Nujol): 3650, 1718, 1542 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 1.72—1.79 (1H, m, H-3b), 1.94, 1.98, 2.03 (each 3H, s, $NHCOCH_3$), 2.77—2.85 (1H, m, H-3b), 5.33 (1H, br d, NH), 7.34 (5H, s, Ph), 7.55—7.68 (2H, m, NH×2). ^{13}C-NMR ($CDCl_3$–CD_3OD) δ: 21.5, 21.7, 21.8 (q, $NHCOCH_3$), 39.1 (t, C-3b), 39.6, 40.5 (t, $NHCH_2CH_2NH$), 49.6 (d, C-2a), 52.3 (d, C-5b), 53.1 (d, OCH_2CHNH), 62.4 (t, C-6a), 63.3 (t, C-9b), 66.0, 67.5, 67.6, 68.1, 68.8, 68.9, 71.3, 72.8 (C-3a, 4a, 4b, 5a, 6b, 7b, 8b, CH^2Ph, OCH_2CHNH), 97.9 (s, C-2b), 100.4 (d, C-1a), 127.2, 127.4, 127.9 (d, Ph), 134.2 (s, Ph), 154.1, 169.8, 170.4, 171.7, 172.4, 173.8 (s, C=O). Positive FAB-MS m/z: 818 $(M+H)^+$, 840 $(M+Na)^+$.

***O*-[5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl]-(1→3)-L-serine-aminoethylamide (25)** As described for **14**, **24** (14 mg, 0.017 mmol) was hydrogenated in the presence of palladium hydroxide (30 mg) in MeOH (4 ml) to give **25** (11 mg, quant.). It was used for the following reaction without further purification. Positive FAB-MS m/z: 684 $(M+H)^+$.

***N*^{w}-*p*-Toluenesulfonyl-L-arginyl-L-alanyl-L-phenylalanyl-L-valyl-*O*-benzyl-L-threonyl-L-isoleucine Benzyl Ester Hydrochloride (27)** TFA (10 ml) was added to **26** (1.5 g, 1.42 mmol) with ice cooling. After being stirred for 40 min at room temperature, the reaction mixture was evaporated *in vacuo*. The residue was dissolved in 4 N HCl–Dioxane (10 ml), and the mixture was evaporated *in vacuo* to afford **27** (1.4 g, 92%) as a white powder. It was used for the following reaction without further purification.

***N*^{a}-*tert*-Butoxycarbonyl-*N*$^\omega$-*p*-toluenesulfonyl-L-arginyl-L-isoleucyl-L-glutaminyl-*N*$^\omega$-*p*-toluenesulfonyl-L-arginyl-glycyl-L-prolyl-glycine (29)** As described for **14**, **28** (324 mg, 0.25 mmol) was hydrogenated in the presence of 10% Pd–C (120 mg) in MeOH (20 ml) to give **29** (270 mg, 90%). It was used for the following reaction without further purification.

***N*^{a}-*tert*-Butoxycarbonyl-*N*$^\omega$-*p*-toluenesulfonyl-L-arginyl-L-isoleucyl-L-glutaminyl-*N*$^\omega$-*p*-toluenesulfonyl-L-arginyl-glycyl-L-prolyl-glycyl-*N*$^\omega$-*p*-toluenesulfonyl-L-arginyl-L-alanyl-L-phenylalanyl-L-valyl-*O*-benzyl-L-threonyl-L-isoleucine Benzyl Ester (30)** HOBt (39 mg, 0.25 mmol), WSC · HCl (48 mg, 0.25 mmol) and *N*-methylmorpholine (25 mg, 0.25 mmol) were added with stirring to a solution of **27** (269 mg, 0.25 mmol), **29** (270 mg, 0.23 mmol) and *N*-methylmorpholine (25 mg, 0.25 mmol) in DMF (20 ml) at 0 °C. The mixture was stirred for 1 h at the same temperature and then for 16 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 and the solution was washed with 10% citric acid, saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH (10 : 1) to give **30** (371 mg, 73%) as a white solid, $[\alpha]_D$ −21.4° (c=0.55, $CHCl_3$: MeOH=3 : 2). Positive FAB-MS m/z: 2214 $(M+H)^+$.

L-Arginyl-L-isoleucyl-L-glutaminyl-L-arginyl-glycyl-L-prolyl-glycyl-L-arginyl-L-alanyl-L-phenylalanyl-L-valyl-L-threonyl-L-isoleucine (31) Freshly distilled HF (2 ml) was added to a solution of **30** (208 mg, 0.094 mmol) in anisole (0.5 ml) at −78 °C. After being stirred for 1 h at 0 °C, the reaction mixture was evaporated *in vacuo*. Et_2O was added to the residue and the whole was filtered to give a crude product. This product was purified by gel filtration (LH-20) using 1% aqueous AcOH to give **31** (124 mg, 90%) as a white amorphous powder, $[\alpha]_D$ −20.5° (c=0.46, MeOH). Positive FAB-MS m/z: 1471$(M+H)^+$.

***N*-Tetradecanoyl-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosyl]-L-seryl-β-alanine Benzyl Ester (33)** **32** (165 mg, 0.16 mmol) and β-alanine benzyl ester *p*-TsOH (84 mg, 0.24 mmol) were dissolved in DMF (6 ml), and WSC · HCl (46 mg, 0.24 mmol), HOBt (37 mg, 0.24 mmol) and TEA (24 mg, 0.24 mmol) were added to the solution with ice cooling under argon. The mixture was stirred for 1 h at 0 °C and then for 20 h at room temperature. The reaction mixture was diluted with AcOEt and the solution was washed with ice-H_2O, saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using hexane–AcOEt (1 : 1) to give **33** (144 mg, 75%) as an amorphous powder, $[\alpha]_D$ −2.1° (c=0.86, $CHCl_3$). IR (Nujol): 1745, 1648 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=6.9 Hz, $-CH_3$), 1.26 (58H, br s, $-CH_2-$), 1.40—1.63 (6H, m, $-CH_2-$), 2.00, 2.03, 2.07 (each 3H, s, $OCOCH_3$), 2.08—2.36 (5H, m, $-CH_2-$), 2.46 (1H, dd, J=6.3, 14.5 Hz, $NHCOCH_2CH(OCO)$), 2.61 (2H, t, J=6.6 Hz, $NHCH_2CH_2$), 3.49—3.78 (4H, m, H-2, 5, $NHCH_2CH_2$), 3.86—3.91 (1H, m, OCH_2CHNH), 3.97 (1H, dd, J=10.9, 5.0 Hz, OCH_2CHNH), 4.14—4.18 (1H, m, H-6), 4.30 (1H, dd, J=12.2, 5.3 Hz, H-6), 4.62—4.69 (1H, m, OCH_2CHNH), 4.73 (1H, d, J=8.3 Hz, H-1), 5.00—5.27 (5H, m, H-3, 4, CH_2Ph, $NHCOCH_2CH(OCO)$), 6.15 (1H, d, J=8.6 Hz, NH), 6.53 (1H, d, J=6.9 Hz, NH), 7.06 (1H, t, J=6.3 Hz, $NHCH_2$), 7.26—

7.38 (5H, m, Ph). Positive FAB-MS *m/z*: 1201 $(M+H)^+$.

***N*-Tetradecanoyl-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosyl]-L-seryl-β-alanine (34)** Palladium-black (70 mg) was added to a solution of **33** (110 mg, 0.092 mmol) in THF (7 ml), and the mixture was stirred under a hydrogen atmosphere for 20 h at room temperature. The catalyst was filtered off and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH (10 : 1) to give **34** (100 mg, 98%) as an amorphous powder, $[\alpha]_D$ −3.7° (*c*=0.92, $CHCl_3$: MeOH=3 : 2). ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (9H, t, *J*=6.9 Hz, $-CH_3$), 1.25 (58H, br s, $-CH_2-$), 1.42—1.60 (6H, m, $-CH_2-$), 2.02, 2.04, 2.10 (each 3H, s, $OCOCH_3$), 2.17—2.52 (8H, m, $-CH_2-$, $NHCH_2CH_2$), 3.36—4.18 (7H, m, H-2, 5, 6, $NHCH_2CH_2$, OCH_2CHNH), 4.29 (1H, dd, *J*= 12.2, 5.0 Hz, H-6), 4.51—4.56 (1H, m, OCH_2CHNH), 4.65 (1H, d, *J*= 8.3 Hz, H-1), 5.00—5.17 (2H, m, H-4, $NHCOCH_2CH(OCO)$), 5.20 (1H, t, *J*=9.2 Hz, H-3). Positive FAB-MS *m/z*: 1111 $(M+H)^+$.

***N*-Tetradecanoyl-*O*-[2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosyl]-L-seryl-β-alanine (2) 34** (70 mg, 0.063 mmol) was dissolved in a solution of concentrated NH_4OH (2 ml) in MeOH–THF (1 : 1, 20 ml). The mixture was stirred for 20 h at room temperature, and the solvent was removed by evaporation. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH–H_2O (12 : 8 : 1) to give **2** (46 mg, 74%) as an amorphous powder, $[\alpha]_D$ −5.2° (*c*=0.34, $CHCl_3$: MeOH=3 : 2). ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (9H, t, *J*=6.9 Hz, $-CH_3$), 1.25 (58H, br s, $-CH_2-$), 1.52—1.60 (6H, m, $-CH_2-$), 2.07—2.57 (8H, m, $-CH_2-$, $NHCH_2CH_2$), 3.18—4.63 (12H, m, H-1, 2, 3, 4, 5, 6, $NHCH_2CH_2$, OCH_2CHNH, OCH_2CHNH), 5.23 (1H, m, $NHCOCH_2CH(OCO)$). Positive FAB-MS *m/z*: 985 $(M+H)^+$, 1007 $(M+Na)^+$.

***N*-Tetradecanoyl-*O*-[2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosyl]-L-seryl-β-alanine *p*-Nitrophenyl Ester (35)** To a solution of **2** (31 mg, 0.031 mmol) and *p*-nitrophenol (31 mg, 0.22 mmol) in DMF (4 ml) was added WSC·HCl (31 mg, 0.16 mmol) with ice cooling. After being stirred for 16 h at room temperature, the mixture was poured into H_2O (3 ml). The insoluble materials were collected by filtration and dried. This product was purified by silica gel column chromatography using CH_2Cl_2–MeOH (20 : 1) to give **35** (18 mg, 53%) as a yellow solid, $[\alpha]_D$ −18.4° (*c*=0.22, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, *J*=6.9 Hz, $-CH_3$), 1.25 (58H, br s, $-CH_2-$), 1.52—1.60 (6H, m, $-CH_2-$), 2.07—2.57 (6H, m, $-CH_2-$), 2.88 (2H, t, *J*=6.3 Hz, $NHCH_2CH_2$), 3.19—3.99 (10H, m, H-2, 3, 4, 5, 6, $NHCH_2CH_2$, OCH_2CHNH), 4.55 (1H, d, *J*=7.6 Hz, H-1), 4.57—4.70 (1H, m, OCH_2CHNH), 5.17—5.23 (1H, m, $NHCOCH_2CH(OCO)$), 6.46 (1H, br d, NH), 6.61 (1H, d, *J*=6.3 Hz, NH), 7.33 (2H, d, *J*=9.2 Hz, Ph), 8.28 (2H, d, *J*=8.9 Hz, Ph). Positive FAB-MS *m/z*: 1106 $(M+H)^+$, 1128 $(M+Na)^+$.

***N*-Tetradecanoyl-*O*-[2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosyl]-L-seryl-β-alanyl[*N*-acetyl-3-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine]-aminoethylamide (3)** NMM (20 mg, 0.2 mmol) was added to a solution of **14** (12 mg, 0.031 mmol) and **35** (40 mg, 0.036 mmol) in DMF (4 ml) at 0 °C. The mixture was stirred at room temperature for 4 d. After evaporation of the solvent, the residue was purified by silica gel column chromatography (elution with CH_2Cl_2 : MeOH : H_2O=12 : 8 : 1) and gel filtration (LH-20) (elution with CH_2Cl_2 MeOH : H_2O=12 : 8 : 1) to give **3** (20 mg, 47%), after lyophilization from a H_2O suspension as a white amorphous powder, $[\alpha]_D$ +3.3° (*c*=0.28, $CHCl_3$: MeOH=2 : 3). ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (9H, t, *J*=6.9 Hz, $-CH_3$), 1.25 (58H, br s, $-CH_2-$), 1.52—1.75 (6H, m, $-CH_2-$), 1.87, 1.99 (each 3H, s, $NHCOCH_3$), 2.21—2.45 (8H, m, $NHCH_2CH_2NHCONH$, $COCH_2CH(OCO)$, $-CH_2-$), 4.72 (1H, d, *J*=3.6 Hz, H-1b), 5.08—5.12 (1H, m, $COCH_2CH(OCO)$). ^{13}C-NMR ($CDCl_3$) δ: 13.5 (q, CH_3), 21.9, 22.0 (q, $NHCOCH_3$), 22.3, 24.7, 25.0, 25.2, 25.3, 28.8, 28.9, 29.0, 29.1, 29.2, 29.3, 31.5, 34.2, 35.6, 37.1, 38.4, 39.1, 41.36, 41.38 (t, CH_2), 49.6 (d, C-2b), 53.1, 53.2 (d, OCH_2CH), 55.5 (d, C-2a), 60.8, 61.3 (t, C-6a, b), 68.4, 68.5, 68.6, 68.7, 70.0, 71.0, 71.2, 73.9, 75.8 (C-3a, 3b, 4a, 4b, 5a, 5b, OCH_2×2, $COCH_2CH(OCO)$), 98.2 (d, C-1b), 100.9 (d, C-1a), 170.5, 170.6, 171.9, 172.6, 172.8, 173.9, 174.5, 176.2 (s, C=O). Positive FAB-MS *m/z*: 1381 $(M+Na)^+$.

***N*-Tetradecanoyl-*O*-[2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosyl]-L-seryl-β-alanyl[*O*-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid]-(2→6)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-*N*-(acetyl)-L-serine]-aminoethylamide (4)** As described for **3**, NMM (10 mg, 0.1 mmol) was added to a solution of **25** (12 mg, 0.0176 mmol) and **35** (22 mg, 0.02 mmol) in DMF (4 ml) at 0 °C. The mixture was stirred at room temperature for 4 d. After evaporation of the solvent, the residue was purified by silica gel column chromatography (elution with CH_2Cl_2 : MeOH : H_2O=12 : 8 : 1) and gel filtration (LH-20) (elution with CH_2Cl_2 : MeOH : H_2O=12 : 8 : 1) to give **4** (12.5 mg, 43%), after lyophilization from a H_2O suspension as a white powder, $[\alpha]_D$ −7.2° (*c*=0.10, $CHCl_3$: MeOH=2 : 3). ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (9H, t, *J*=6.9 Hz, $-CH_3$), 1.25 (58H, br s, $-CH_2-$), 1.51—1.63 (6H, m, $-CH_2-$), 2.03, 2.04, 2.07 (each 3H, s, $NHCOCH_3$), 2.17—2.59 (8H, m, $-CH_2-$), 5.27—5.36 (1H, m, $NHCOCH_2CH(OCO)$). ^{13}C-NMR ($CDCl_3$–CD_3OD) δ: 13.6 (q, CH_3), 22.1, 22.2, 22.6 (q, $NHCOCH_3$), 23.0, 24.3, 25.1, 25.3, 25.4, 28.9, 29.1, 29.2, 29.3, 29.4, 29.7, 31.6, 35.1, 35.2, 35.5, 35.6, 35.8 (t, $-CH_2-$), 37.2 (t, C-3c), 39.1, 43.7 (t, $NHCH_2CH_2NH$), 40.5 (t, $-CH_2-$), 47.9 (d, C-2b), 49.3 (d, C-5c), 52.9 (d, OCH_2CHNH), 54.4 (d, OCH_2CHNH), 55.5 (d, C-2a), 60.7, 63.0 (t, C-6a, 6b), 63.6 (t, C-9c), 67.7 (d, C-4b),67.8 (d, C-4c), 69.1 (d, C-4a), 69.3, 69.9 (t, OCH_2CHNH),70.4 (d, C-3b), 70.6 (d, C-5b), 71.1 (d, C-8c),71.7 (d, C-7c), 72.3 (d, C-6c), 72.8 (d, $COCH_2CH(OCO)$), 75.1 (d, C-3a), 76.0 (d, C-5a), 98.0 (s, C-2c), 98.1 (d, C-1b), 101.2 (d, C-1a), 162.5, 167.9, 170.4, 171.8, 170.2, 172.3, 172.8 (s, C=O). Positive FAB-MS *m/z*: 1694 $[(M+Na)^+$ for $C_{80}H_{144}NaN_8O_{27}]$.

***N*-Tetradecanoyl-*O*-[2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosyl]-L-seryl-β-alanyl-L-arginyl-L-isoleucyl-L-glutaminyl-L-arginyl-glycyl-L-prolyl-glycyl-L-arginyl-L-alanyl-L-phenylalanyl-L-valyl-L-threonyl-L-isoleucine (5)** As described for **3**, NMM (10 mg, 0.1 mmol) was added to a solution of **31** (16 mg, 0.011 mmol) and **35** (12 mg, 0.011 mmol) in DMF (3 ml) at 0 °C. The mixture was stirred at room temperature for 4 d. After evaporation of the solvent, the residue was purified by gel filtration (LH-20) (elution with CH_2Cl_2 : MeOH : H_2O=12 : 8 : 1) to give **5** (16 mg, 58%), after lyophilization from a H_2O suspension as a white amorphous powder, $[\alpha]_D$ −15.1° (*c*=0.17, $CHCl_3$: MeOH=2 : 3). Positive FAB-MS *m/z*: 2438 $(M+H)^+$.

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