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SYNTHESIS AND IMMUNOADJUVANT ACTIVITY OF 2,2′-*O*-[2,2′-DIACETAMIDO-2,3,2′,3′-TETRADEOXY-6,6′-DI-*O*-(2-TETRADECYLHEXADECANOYL)-α,α′-TREHALOSE-3,3′-DIYL]BIS(*N*-D-LACTOYL-L-ALANYL-D-ISOGLUTAMINE)*

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ABSTRACT

The disaccharide of 6-*O*-(2-tetradecylhexadecanoyl)muramoyl dipeptide coupled through an α-(1↔1)-α linkage, named in the title, and an analog bearing a single peptide moiety, have been synthesized from 2,2′-diazido-2,2′-dideoxy-α,α′-trehalose. The immunoadjuvant activities of the products were examined.

INTRODUCTION

Trehalose 6,6′-dimycolate (TDM), a mycobacterial glycolipid, was found to have a variety of biological activities. In the course of chemical and immunopharmacological investigation of TDM[2,3] it has been reported that TDM shows potent antitumor activity in combination with 6-*O*-stearoyl-*N*-acetylmuramoyl dipeptides[3]. In this paper, we describe the synthesis and immunoadjuvant activity of 2,2′-*O*-[2,2′-diacetamido-2,3,2′,3′-tetradeoxy-6,6′-di-*O*-(2-tetradecylhexadecanoyl)-α,α′-trehalose-3,3′-diyl]bis(*N*-D-lactoyl-L-alanyl-D-isoglutamine), which has structural features related to both TDM and *N*-acetylmuramoyl dipeptide (MDP).

RESULTS AND DISCUSSION

The synthesis of nitrogenous disaccharides structurally related to α,α-trehalose has been performed by two types of procedures. One involves the introduction of nitrogen into α,α-trehalose[4–6], and the other the coupling of nitrogenous glucose derivatives[7]. In the present work, we synthesized the desired nitrogenous α,α-trehalose, which is the important intermediate for the preparation of the title compound, by the latter method. Condensation of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranosyl trichloroacetimidate[8] (**1**) with 3,4,6-tri-*O*-acetyl-2-azido-

*Studies on Immunoadjuvant-Active Compounds, Part 41. For Part 40, see ref. 1.

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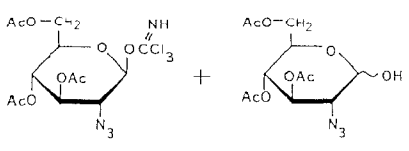
2-deoxy-D-glucopyranose (**2**), obtained from the α-isomer of **1**, in the presence of trimethylsilyl trifluoromethanesulfonate afforded 3,4,6,3′,4′,6′-hexa-*O*-acetyl-2,2′-diazido-2,2′-dideoxy-α,α'-trehalose (**3**; 12% yield) and its α,β isomer (50%). Deacetylation of **3** and subsequent isopropylidenation gave 2,2′-diazido-2,2′-dideoxy-4,6:4′,6′-di-*O*-isopropylidene-α,α'-trehalose (**5**) in high yield. Compound **5** was condensed with L-2-chloropropionic acid (5 equiv.) in dry *N*,*N*-dimethylformamide (DMF) to give the corresponding 3,3′-di-*O*- and 3-*O*-(D-1-carboxyethyl) derivatives (**6** and **11**). The coupling of **6** and **11** with L-alanyl-D-isoglutamine benzyl ester was conducted with dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (HOSu) as the activating agents to give 2,2′-*O*-(2,2′-diazido-2,3,2′,3′-tetradeoxy-4,6:4′,6′-di-*O*-isopropylidene-α,α'-trehalose-3,3′-diyl)bis(*N*-D-lactoyl-L-alanyl-D-isoglutamine benzyl ester) (**7**) and *N*-[2-*O*-(2-azido-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranosyl 2-azido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine benzyl ester (**12**) in 14% and 26% yields, respectively, based on the compound **5**. Hydrolysis of the isopropylidene groups from **7** and **12** under mild acidic conditions gave the corresponding 4,6-diol derivatives **8** and **13** quantitatively. The ^{1}H-n.m.r. data indicated that products **8** and **13** were pure even with respect to the stereochemistry of the lactoyl groups.

The introduction of the 2-tetradecylhexadecanoyl group into the primary positions of **8** and **13** was performed by using the free carboxylic acid in the presence of 4-dimethylaminopyridine (DMAP) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (water soluble carbodiimide, WSC), instead of by the displacement reaction of the potassium salt of the carboxylic acid on the



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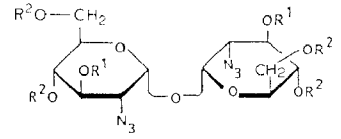
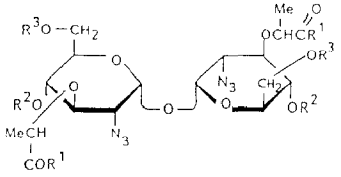


3 $R^1 = R^2 = Ac$

4 $R^1 = R^2 = H$

5 $R^1 = H$, $R^2, R^2 = Ipd$

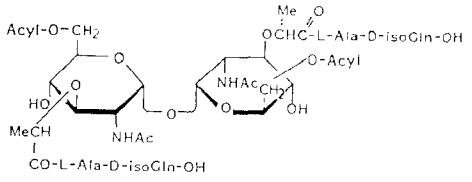


6 $R^1 = OH$, R^2, $R^3 = Ipd$

7 $R^1 = -L\text{-}Ala\text{-}D\text{-}isoGln\text{-}OBn$, R^2, $R^3 = Ipd$

8 $R^1 = -L\text{-}Ala\text{-}D\text{-}isoGln\text{-}OBn$, $R^2 = R^3 = H$

9 $R^1 = -L\text{-}Ala\text{-}D\text{-}isoGln\text{-}OBn$, $R^2 = H$, $R^3 = Acyl$



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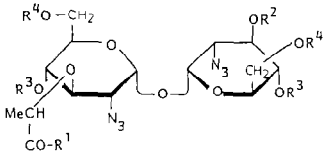
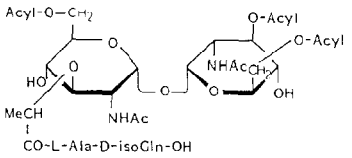
Ipd: isopropylidene Bn: benzyl Acyl: 2-tetradecylhexadecanoyl

tosylates of the primary alcohol functions[9]. Treatment of **8** with 2-tetradecylhexadecanoic acid (2.5 equiv.) in the presence of DMAP and WSC in dry DMF for **3** h at 40° gave the 6,6′-di-*O*-acyl derivative (**9**) in 62% yield. On the other hand, compound **13** was converted into the 6,3′,6′-tri-*O*-acyl derivative **14** under the same conditions. The final products (**10** and **15**) were obtained by the hydrogenation of compounds **9** and **14** in the presence of palladium black in a mixture of tetrahydrofuran and acetic anhydride. The i.r. spectra showed the disappearance of the azido and benzyl groups.





15

11 R^1 = OH, R^2 = H, R^3 , R^4 = Ipd

12 R^1 = -L-Ala-D-isoGln-OBn, R^2 = H, R^3 , R^4 = Ipd

13 R^1 = -L-Ala-D-isoGln-OBn, R^2 = R^3 = R^4 = H

14 R^1 = -L-Ala-D-isoGln-OBn, R^2 = R^4 = acyl, R^3 = H

Ipd : isopropylidene Bn: benzyl Acyl: 2-tetradecylhexadecanoyl

TABLE I

ADJUVANT ACTIVITY OF MDP ANALOGS ON DELAYED-TYPE HYPERSENSITIVITY TO ABA-*N*-ACETYLTYROSINE IN GUINEA PIGS

Compound	*Dose (μg)*	*Skin reaction to 50 μg of ABA-BSA[a] (diameter in mm ± s.e.)[b] at*	
		24 h	*48 h*
10	10	8.6 ±2.9	0
	100	21.2 ±0.6	17.7 ±1.9
15	10	3.6 ±0.2	0
	100	9.2 ±5.1	5.0 ±5.0
MDP	10	19.8 ±1.0	15.7 ±0.9
	100	20.1 ±0.2	17.3 ±0.3
Control[c]		3.9 ±0.6	0

[a]Azobenzenearsonate-*N*-acetyl-L-tyrosine–bovine serum albumin. [b]The data indicate the average diameter ± the standard error (s.c.) of the skin reaction (induration) of four guinea pigs. [c]ABA-*N*-acetyltyrosine in Freund's incomplete adjuvant.

TABLE II

In situ ACTIVATION OF MOUSE PERITONEAL MACROPHAGES BY ADMINISTRATION OF MDP ANALOGS

Compounds[a]	*Dose*[b] *(μg)*	*Radioactivity in viable target cells (c.p.m. ±s.d.)*[c]
10	50	314 ±55 (92%)[d]
15	50	460 ±89 (88%)
TDM	50	476 ±68 (88%)
Tumor cells alone		3898 ±110

[a]Mice were injected i.p. with the compounds indicated 5 days before harvest of the macrophages. [b]All samples were suspended in phosphate-buffered saline (PBS). [c]Target cells (B16-BL6) labeled with [^{125}I]deoxyuridine were plated into culture wells to obtain in initial target:effector-cell ratio of 1:10. After 72 h the culture wells were washed twice with PBS and the adherent viable cells were lysed with 0.1 mL of 0.5M NaOH. The radioactivity of the lysate was measured in a gamma counter. [d]Proportion (in %) of tumor cells destroyed by macrophages.

The immunoadjuvant activities of the compounds thus prepared were examined using induction of delayed-type hypersensitivity (DTH) to *N*-acetyl-L-tyrosine-3-azobenzene-4′-arsonate (azobenzenearsonate-*N*-acetyltyrosine, ABA-*N*-acetyltyrosine) in guinea-pigs[10] as the characteristic activity of MDP, and the *in situ* activation of mouse peritoneal macrophages[11] as that of TDM. Compound **10** showed weak but distinct activity on DTH, while compound **15** was inactive (Table I). Both **10** and **15** exhibited macrophage-activating activity as strong as that of TDM (Table II).

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were determined with a Union PM-201 polarimeter, and i.r. spectra were recorded with a Jasco A-100 spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 270 MHz with a JEOL JNM-GX-270 spectrometer, for solutions in chloroform-*d* unless otherwise noted. T.l.c. was performed on Silica Gel 60 (Merck, aluminum sheets), and column chromatography on silica gel (Wako Co., 200 or 300 mesh) with the solvent systems (v/v) specified.

3,4,6-Tri-O-acetyl-2-azido-2-deoxy-D-glucopyranose (**2**). — A solution of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl trichloroacetimidate[8] (100 mg) in 80% aqueous acetone (5 mL) was treated with Amberlite IR-120 (H^+) ion-exchange resin for 3 h at room temperature. After the resin was filtered off, the filtrate was concentrated. The residue obtained was chromatographed on a column of silica gel with 50:1 dichloromethane–methanol to give **2** (73 mg, quant.) as an anomeric mixture (α:β 7:3); i.r. (neat): ν_{max} 3400 (broad, OH), 2100 (azide), and 1750 cm^{-1} (ester); ^{1}H-n.m.r. data ($CDCl_3$): δ 2.0–2.2 (m, 9 H, 3 CH_3CO), 3.41 (dd, 0.7 H, $J_{1,2}$ 3.3, $J_{2,3}$ 10.2 Hz, H-2α), 3.50 (dd, 0.3 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.2 Hz, H-2β),

4.1–4.7 (m, 3 H, H-5,6a,6b), 4.75 (d, 0.3 H, H-1β), 5.0–5.2 (m, 1.3 H, H-2β,4), 5.40 (d, 0.7 H, H-1α), and 5.54 (dd, 0.7 H, $J_{3,4}$ 9.2 Hz, H-3α).

Anal. Calc. for $C_{12}H_{17}N_3O$ (331.28): C, 43.51; H, 5.17; N, 12.68. Found: C, 43.33; H, 5.00; N, 12.51.

3,4,6,3',4',6'-Hexa-O-acetyl-2,2'-diazido-2,2'-dideoxy-α,α'-trehalose (**3**) *and its α,β-isomer.* — Compound **2** (500 mg, 1.51 mmol) and 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranosyl trichloroacetimidate (**1**; 750 mg, 1.58 mmol) were dissolved in dichloromethane (30 mL), and the mixture was stirred for 24 h at room temperature in the presence of Drierite (1 g). The mixture was cooled to 0°, then trimethylsilyl trifluoromethanesulfonate (0.04 mL in 0.3 mL of dichloromethane, 0.02 mmol) was added. After 1 h, the reaction mixture was treated with excess solid sodium hydrogencarbonate and aqueous sodium hydrogencarbonate solution. Filtration from the insoluble materials gave a mixture to which dichloromethane was added, then the organic layer was washed with water, dried with sodium sulfate, and concentrated. The oily residue was chromatographed on a column of silica gel with 3:1 hexane–ethyl acetate to afford **3** (117 mg, 12%) and its α,β-isomer (488 mg, 50%). Compound **3** had $[\alpha]_D$ +163.2° (*c* 0.4, dichloromethane); i.r. (neat): ν_{max} 2100 (azide) and 1750 cm^{-1} (ester); ^{1}H-n.m.r. data ($CDCl_3$): δ 2.06, 2.09, 2.10 (3 s, 9 H, 3 CH_3CO), 3.74 (dd, 1 H, $J_{1,2}$ 3.66, $J_{2,3}$ 10.26 Hz, H-2), 4.11 (dd, 1 H, $J_{5,6}$ 3.3, J_{gem} 13.55 Hz, H-6a), 4.24–4.32 (m, 2 H, H-5,6b), 5.08 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.52 Hz, H-4), 5.19 (d, 1 H, H-1), and 5.44 (dd, 1 H, H-3).

Anal. Calc. for $C_{24}H_{32}N_6O_{15}$ (644.55): C, 44.72; H, 5.00; N, 13.04. Found: C, 44.63; H, 5.07; N, 13.07.

The α,β isomer had m.p. 146.0–148.0°, $[\alpha]_D$ +63.9° (*c* 0.6, dichloromethane).

2,2'-D-Azido-2,2'-dideoxy-4,6:4',6'-di-O-isopropylidene-α,α'-trehalose (**5**). — To a solution of **3** (100 mg) in methanol (5 mL) was added 28% sodium methoxide in methanol (1 drop), and after 1 h the solution was treated with Amberlite IR-120 (H^+) resin to remove the base. The resin was filtered off, and the filtrate and washings were concentrated to give **4**. This was taken up in *N,N*-dimethylformamide (DMF; 5 mL), 2,2-dimethoxypropane (2 mL) and *p*-toluenesulfonic acid monohydrate (25 mg) were added, the mixture was stirred for 12 h at room temperature, and then neutralized with triethylamine. After concentration the residue was chromatographed on a column of silica gel with 100:1 dichloromethane–methanol to afford compound **5** (60 mg, 82% based on **3**); $[\alpha]_D$ +129.6° (*c* 1.0, dichloromethane); i.r.: ν_{max} 3400 (broad, OH), 2100 (azide), and 850 cm^{-1} (Me_2C); ^{1}H-n.m.r. data ($CDCl_3$): δ 1.46, 1.53 (2 s, 12 H, 4 CCH_3), 3.39 (dd, 2 H, $J_{1,2}$ 3.70, $J_{2,3}$ 9.90 Hz, H-2,2'), 3.59 (t, 2 H, $J_{3,4} = J_{4,5}$ = 9.20 Hz, H-4,4'), 3.76–3.92 (m, 6 H, H-5,6a,6b,5',6'a,6'b), 4.13 (t, 2 H, H-3,3'), and 5.12 (d, 2 H, H-1,1').

Anal. Calc. for $C_{18}H_{28}N_6O_9$ (472.26): C, 45.76; H, 5.97; N, 17.79. Found: C, 45.59; H, 6.18; N, 17.76.

2,2'-O-(2,2'-Diazido-2,3,2',3'-tetradeoxy-4,6:4',6'-di-O-isopropylidene-α,α'-trehalose-3,3'-diyl)bis(N-D-lactoyl-L-alanyl-D-isoglutamine benzyl ester) (**3**) *and*

N-[2-O-*(2-azido-2-deoxy-4,6-O-isopropylidene-α-*D*-glucopyranosyl 2-azido-2,3-dideoxy-4,6-O-isopropylidene-α-*D*-glucopyranosid-3-yl)-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine benzyl ester* (**12**). — To a stirred solution of **5** (200 mg, 0.42 mmol) in DMF (10 mL) was added 60% suspension of sodium hydride in oil (66 mg of NaH, 2.75 mmol) at room temperature. The mixture was kept for 1 h, then L-2-chloropropionic acid (0.2 mL, 2.2 mmol) was added with stirring. The mixture was stirred for 2 h at room temperature, and concentrated. The residue was extracted with dichloromethane, and the extract was successively washed with 2M hydrochloric acid and water, dried (sodium sulfate), and concentrated to a syrup. The syrup was chromatographed on a column of silica gel with 25:1 dichloromethane–methanol to give a mixture of **6** and **11** (150 mg).

To a solution of the foregoing mixture of **6** and **11** in dry 1,4-dioxane (6 mL) were added, with stirring, *N*-hydroxysuccinimide (HOSu; 120 mg, 1.04 mmol) and dicyclohexylcarbodiimide (DCC; 240 mg, 1.17 mmol), and the mixture was stirred for 30 min at room temperature. L-Alanyl-D-isoglutamine benzyl ester trifluoroacetate (300 mg, 0.66 mmol) and triethylamine (1 drop) were added to the mixture, which was further stirred for 1 h at room temperature, then the precipitate was filtered off and washed with 1,4-dioxane. The filtrate and washings were combined and concentrated to a syrup, which was chromatographed on a column of silica gel with (*a*) 50:1 and (*b*) 25:1 dichloromethane–methanol. Eluants *a* and *b* afforded compound **7** (76 mg, 14%) and **12** (100 mg, 26%), respectively. Compound **7** had $[\alpha]_D$ +51.7° (*c* 1.5, dichloromethane); i.r.: ν_{max} 3350 (NH), 2120 (azide), 1740 (ester), 1680, 1540 (amide), and 860 cm^{-1} (Me_2C).

Anal. Calc. for $C_{60}H_{76}N_{12}O_{21}$ (1195.25): C, 54.26; H, 6.24; N, 14.06. Found: C, 54.33; H, 6.34; N, 13.98.

Compound **12** had $[\alpha]_D$ +73.7° (*c* 2.0, dichloromethane); i.r.: ν_{max} 3400 (OH and NH), 2130 (azide), 1740 (ester), 1660, 1540 (amide), and 860 cm^{-1} (Me_2C).

Anal. Calc. for $C_{36}H_{51}N_9O_{15}$ (833.85): C, 51.86; H, 6.17; N, 15.12. Found: C, 51.75; H, 6.09; N, 15.36.

*2,2'-O-(2,2'-Diazido-2,3,2',3'-tetradeoxy-α,α'-trehalose-3,3'-diyl)bis(*N-*D-lactoyl-*L*-alanyl-*D*-isoglutamine benzyl ester)* (**8**). — A solution of **7** (100 mg) in 80% aqueous acetic acid (2 mL) was heated for 2 h at 45°, and then concentrated to give **8** in quantitative yield; $[\alpha]_D$ +81.1° (*c* 0.4, 1:2 dichloromethane–methanol); i.r. ν_{max} 3350 (OH and NH), 2120 (azide), 1730 (ester), 1660, and 1540 cm^{-1} (amide); ^{1}H-n.m.r. data (1:2 $CDCl_3$–CD_3OD): δ 1.40, 1.42 (2 d, 12 H, 4 CH_3CH), 1.93, 2.25 (2 m, 4 H, 2 CH_2CH_2CO of isoGln), 2.47 (t, 4 H, 2 CH_2CO of isoGln), 3.30–3.85 (m, 12 H, H-2,3,4,5,6a,6b,2',3',4',5',6a', and 6b'), 4.31–4.57 (m, 6 H, 2 NHC*H* of isoGln and 4 CH_3CH), 5.11 (s, 4 H, 2 $PhCH_2$), 5.34 (d, 2 H, $J_{1,2} = J_{1',2'}$ = 3.67 Hz, H-1,1'), and 7.33 (s, 10 H, Ph-H).

Anal. Calc. for $C_{48}H_{66}N_{12}O_{19}$ (1115.12): C, 51.70; H, 5.97; N, 15.07. Found: C, 51.53; H, 5.68; N, 14.94.

N-[2-O-*(2-azido-2-deoxy-α-*D*-glucopyranosyl 2-azido-2,3-dideoxy-α-*D*-glucopyranosid-3-yl)-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine benzyl ester* (**13**). — Compound

13 was synthesized from **12** in quantitative yield by the procedure described for **8**; $[\alpha]_D$ +113.7° (*c* 0.6, 1:2 dichloromethane–methanol); i.r.: ν_{max} 3400 (OH and NH), 2100 (azide), 1740 (ester), 1660, and 1540 cm^{-1} (amide); ^{1}H-n.m.r. data (1:2 $CDCl_3$–CD_3OD): δ 1.41, 1.42 (2 d, 6 H, 2 CH_3CH), 1.92, 2.23 (2 m, 2 H, CH_2CH_2CO of isoGln), 2.47 (t, 2 H, CH_2CO of isoGln), 3.29–3.91 (m, 12 H, H-2,3,4,5,6a,6b, 2′,3′,4′,5′,6a′, and 6b′), 4.34–4.41 (m, 3 H, NHC*H* of isoGln and CH_3CH), 5.12 (s, 2 H, $PhCH_2$), 5.22, 5.33 (2 d, J = 3.67, J = 3.30 Hz, H-1,1′), and 7.34 (s, 5 H, Ph-H).

Anal. Calc. $C_{30}H_{43}N_9O_{15}$ (846.8): C, 47.81; H, 5.71; N, 16.73. Found: C, 48.07; H, 5.58; N, 16.63.

*2,2′-*O*-[2,2′-Diazido-2,3,2′,3′-tetradeoxy-6,6′-di-*O*-(2-tetradecylhexadecanoyl)-α,α′-trehalose-3,3′-diyl]bis(*N*-D-lactoyl-L-alanyl-D-isoglutamine benzyl ester)* (**9**). — To a solution of **8** (38.0 mg, 0.031 mmol) in dry DMF (4 mL) were added 2-tetradecylhexadecanoic acid (35.0 mg, 0.078 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (water soluble carbodiimide, WSC; 23.9 mg, 0.12 mmol), and 4-dimethylaminopyridine (3.8 mg, 0.031 mmol), and the mixture was stirred for 3 h at 40°. After concentration of the mixture, the residue was chromatographed on a column of silica gel with 100:1 dichloromethane–methanol to afford compound **9** (40.0 mg, 62%); $[\alpha]_D$ +18.5° (*c* 0.4, dichloromethane); i.r.: ν_{max} 3300 (OH and NH), 2940, 2860 (methyl and methylene), 2100 (azide), 1740 (ester), 1650, and 1540 cm^{-1} (amide); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 12 H, 4 CH_3CH_2), 5.10 (s, 4 H, 2 $PhCH_2$), 5.17 (d, 2 H, $J_{1,2} = J_{1',2'}$ = 2.93 Hz, H-1,1′), and 7.33 (s, 10 H, Ph-H).

Anal. Calc. for $C_{108}H_{182}N_{12}O_{21}$ (2090.74): C, 65.35; H, 9.24; N, 8.47. Found: C, 65.15; H, 9.35; N, 8.18.

*N-{2-*O*-[2-azido-2-deoxy-3,6-di-*O*-(2-tetradecylhexadecanoyl)-α-D-glucopyranosyl 2-azido-2,3-dideoxy-6-*O*-(2-tetradecylhexadecanoyl)-α-D-glucopyranosid-3-yl]-D-lactoyl}-L-alanyl-D-isoglutamine benzyl ester* (**14**). — Compound **14** was obtained from **13** in 63% yield by the procedure described for **9**; $[\alpha]_D$ +41.8° (*c* 0.8, dichloromethane); i.r.: ν_{max} 3400 (OH and NH), 2950, 2890 (methyl and methylene), 2140 (azide), 1750 (ester), 1670, and 1540 cm^{-1} (amide); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 18 H, 6 CH_3CH_2), 5.12 (s, 2 H, $PhCH_2$), 5.20, 5.22 (2 d, 2 H, H-1,1′), 5.33 (t, 1 H, H-3), and 7.34 (s, 5 H, Ph-H).

Anal. Calc. for $C_{120}H_{217}N_9O_{17}$ (2058.14): C, 70.03; H, 10.63; N, 6.13. Found: C, 70.01; H, 10.33; N, 6.24.

*2,2′-*O*-[2,2′-Diacetamido-2,3,2′,3′-tetradeoxy-6,6′-di-*O*-(2-tetradecylhexadecanoyl)-α,α′-trehalose-3,3′-diyl]bis(*N*-D-lactoyl-L-alanyl-D-isoglutamine)* (**10**). — To a solution of **9** (40.0 mg) in tetrahydrofuran (10 mL) and acetic anhydride (1 mL) was added palladium black prepared from palladium chloride (50 mg), and the mixture was stirred for 12 h in a hydrogen atmosphere. The catalyst was filtered off and washed with tetrahydrofuran. The filtrate and washings were combined, and concentrated. The residue was chromatographed on a column of silica gel with 10:1 dichloromethane–methanol to give compound **10** (23.0 mg, 62%); $[\alpha]_D$ +19.1°

(*c* 0.2, dichloromethane); i.r.: ν_{max} 3300 (OH and NH), 2930, 2850 (methyl and methylene), 1740 (ester), 1660, and 1540 cm^{-1} (amide).

Anal. Calc. for $C_{98}H_{178}N_8O_{23}$ (1836.54): C, 64.09; H, 9.77; N, 6.10. Found: C, 63.85; H, 9.51; N, 6.30.

N-{*2-O-[2-acetamido-2-deoxy-3,6-di-O-(2-tetradecylhexadecanoyl)-α-*D-*glucopyranosyl 2-acetamido-2,3-dideoxy-6-O-(2-tetradecylhexadecanoyl)-α-*D-*glucopyranosid-3-yl]-*D-*lactoyl*}-L-*alanyl*-D-*isoglutamine* (**15**). — Compound **15** was obtained from **14** by the procedure described for **10** in 74% yield; $[\alpha]_D$ +24.0° (*c* 0.6, dichloromethane); i.r.: ν_{max} 3300 (OH and NH), 2930, 2850 (methyl and methylene), 1740 (ester), 1660, and 1540 cm^{-1} (amide).

Anal. Calc. for $C_{117}H_{219}N_5O_{19}$ (2000.05): C, 70.26; H, 11.04; N, 3.50. Found: C, 70.10; H, 11.33; N, 3.71.

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