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CHEMICAL COMBINATION OF 6-DEOXY-6-MYCOLOYLAMINO-α,α-TREHALOSE AND *N*-ACETYL-6-*O*-(AMINOACYL)MURAMOYL DIPEPTIDE*

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ABSTRACT

6-Deoxy-6-mycoloylamino-α,α-trehalose, a biologically active derivative of 6,6′-di-*O*-mycoloyl-α,α-trehalose (TDM), and *N*-acetyl-6-*O*-(aminoacyl)-muramoyl dipeptide (MDP) were joined chemically by a succinic acid unit. The compounds synthesized showed activities that are characteristic of both TDM and MDP.

INTRODUCTION

6,6′-Di-*O*-mycoloyl-α,α-trehalose (trehalose 6,6′-dimycolate; TDM), which is a glycolipid purified from mycobacteria, has been shown to have various biological activities, such as immunoadjuvant, antitumor, and antiinfection activity. Previously, it was reported[2] that TDM shows a stronger antitumor effect when employed in combination with *N*-acetylmuramoyl dipeptide (MDP) than when used alone. It was also shown that 6,6′-dideoxy-6,6′-bis(mycoloylamino)-α,α-trehalose, which was established as[3] being a more beneficial immunomodulator than TDM, exhibits a very potent synergistic effect on tumor-regressive activity when combined[4] with 6-*O*-stearoyl-MDP. We have reported[5] that the new synthetic immunomodulators combining a biologically active derivative of *N*-acetylmuramoyl dipeptide and lipid A exhibit efficient antitumor activity, as well as strong immunoadjuvant activity. We now describe the chemical joining of 6-deoxy-6-mycoloylamino-α,α-trehalose and *N*-acetyl-6-*O*-(aminoacyl)muramoyl dipeptide by succinic acid, and the biological activities of the resulting conjugates.

RESULTS AND DISCUSSION

Treatment of 5-aminopentanoic acid (**1a**) and 8-aminooctanoic acid (**1b**) with 2-(*tert*-butoxycarbonylthio)-4,6-dimethylpyrimidine[6] in the presence of tri-

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*Studies on Immunoadjuvant-active Compounds, Part 40. For Part 39, see ref. 1.

$H_2N(CH_2)_nCO_2H$

1 a: $n = 4$

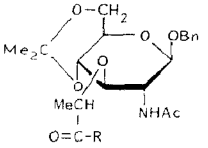
2 b: $n = 7$

$\overset{H}{\text{Boc-N}}(CH_2)_nCO_2H$

2 a

2 b

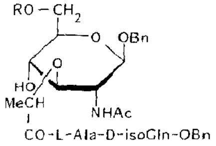
Boc = Me_3COCO



3 R = OH

4 R = -L-Ala-D-isoGln-OBn

Bn = $PhCH_2$



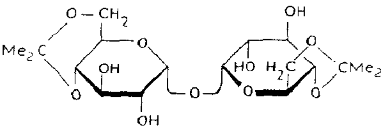
5 R = H

6 a,b R = $-\overset{O}{\overset{\|}{C}}(CH_2)_nNHBoc$

7 a,b R = $-\overset{O}{\overset{\|}{C}}(CH_2)_nNH_3^+\cdot CF_3CO_2^-$

Bn = benzyl

Boc = tert-butoxycarbonyl



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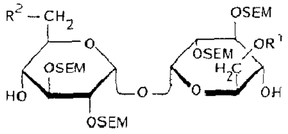


9 R^1 = H, R^2 = OH

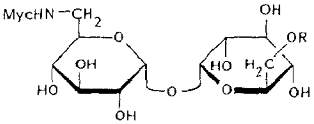
10 R^1 = H, R^2 = OTs

11 R^1 = H, R^2 = N_3

12 R^1 = $-\overset{O}{\overset{\|}{C}}(CH_2)_2\overset{O}{\overset{\|}{C}}O-SE$, R^2 = N_3

13 R^1 = $-\overset{O}{\overset{\|}{C}}(CH_2)_2\overset{O}{\overset{\|}{C}}O-SE$, R^2 = NH_2

14 R^1 = $-\overset{O}{\overset{\|}{C}}(CH_2)_2\overset{O}{\overset{\|}{C}}O-SE$, R^2 = NHMyc



15 R = $-\overset{O}{\overset{\|}{C}}(CH_2)_2\overset{O}{\overset{\|}{C}}OH$

Ts = $O_2SC_6H_4Me$-*p*

SE = $Me_3SiC_2H_4$

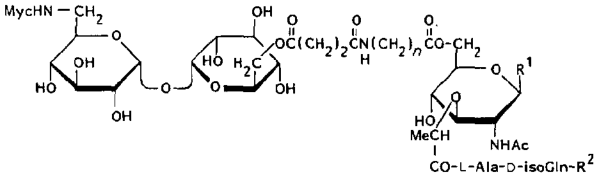
SEM = $Me_3SiC_2H_4OCH_2$

Myc = mycoloyl

ethylamine gave respectively 5-(*tert*-butoxycarbonylamino)pentanoic acid (**2a**) and 8-(*tert*-butoxycarbonylamino)octanoic acid (**2b**) in almost quantitative yields. These acids were coupled with *N*-{2-*O*-[benzyl 2-acetamido-2,3-dideoxy-β-D-glucopyranosid-3-yl]-D-lactoyl}-L-alanyl-D-isoglutamine benzyl ester (**5**), using dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) as the activating agents, to afford the 6-*O*-(*tert*-butoxycarbonylamino)acyl-MDP derivatives (**6a** and **6b**) in 44–46% yields. Hydrolytic removal of the *tert*-butoxycarbonyl group in compounds **6a** and **6b** with trifluoroacetic acid gave the corresponding trifluoroacetates of the 6-*O*-aminoacyl derivatives (**7a** and **7b**), which were key intermediates for the synthesis of the desired conjugates (**16a** and **16b**).

Treatment of 4,6:4′,6′-di-*O*-isopropylidene-α,α-trehalose (**8**), which was easily prepared from α,α-trehalose, with chloro-(2-trimethylsilylethoxy)methane[7] in dichloromethane in the presence of diisopropylethylamine gave the fully protected α,α-trehalose, and selective hydrolysis of the isopropylidene groups under mildly acidic conditions afforded 2,3,2′,3′-tetrakis-*O*-[(2-trimethylsilylethoxy)methyl]-α,α-trehalose (**9**) as a suitable starting material. The (2-trimethylsilylethoxy)methyl group is a very useful protecting group, because it can be easily removed by a Lewis acid in dichloromethane or other of the usual organic solvents. Treatment of compound **9** with 1.2 equiv. of *p*-toluenesulfonyl chloride gave the 6-*O*-tosyl derivative (**10**) in 44% yield, and **10** was converted into the 6-azido-6-deoxy-2,3,2′,3′-tetrakis-*O*-[(2-trimethylsilylethoxy)methyl]-α,α-trehalose (**11**) in good yield. Before introduction of a mycoloyl group onto O-6 of the trehalose derivative, the hydroxyl group on C-6′ was substituted with a 3-[(2-trimethylsilyl)-ethoxy)carbonyl]propanoyl group by a procedure described below. Treatment of succinic anhydride with 2-trimethylsilylethanol (1 equiv.) in the presence of DMAP afforded 3-[(2-trimethylsilylethoxy)carbonyl]propanoic acid. This acid was introduced onto the hydroxyl group at C-6′ of compound **11** by the DCC–DMAP method as described for compounds **6a** and **6b**. Reduction of the azide group in compound **12** with hydrogen in the presence of 10% palladium-on-carbon in methanol gave the 6-amino derivative of α,α-trehalose (**13**). The introduction of a mycoloyl group onto the amino group of compound **13** was performed in 76% yield by using *N*-(mycoloyloxy)succinimide[8]. The average molecular weight of mycolic acid was calculated to be $C_{80}H_{158}O_{3.5}$. Removal of the 2-trimethylsilylethyl and (2-trimethylsilylethoxy)methyl groups in compound **14** with boron trifluoride etherate gave the desired 6-*O*-(3-carboxypropanoyl)-6′-deoxy-6′-(mycoloylamino)-α,α-trehalose (**15**) in quantitative yield.

Coupling of **15** with **7a** and **7b** was conducted, with DCC and *N*-hydroxysuccinimide (HOSu) as the activating agents in the presence of triethylamine, to afford *N*-{2-*O*-[benzyl 2-acetamido-6-*O*-{5-[3-(6-deoxy-6-mycoloylamino-α,α-trehalos-6′-yloxycarbonyl)propanamido]pentanoyl}-2,3-dideoxy-β-D-glucopyranosid-3-yl]-D-lactoyl}-L-alanyl-D-isoglutamine benzyl ester (**16a**) and the octanoyl analog (**16b**) in 74 and 60% yield, respectively. Hydrogenolytic removal of the benzyl groups in compounds **16a** and **16b** with hydrogen and palladium-black catalyst in tetrahydrofuran–acetic acid gave the desired products (**17a** and **17b**, respectively) in almost quantitative yields.



16 a,b $R^1 = R^2 = OBn$
17 a,b $R^1 = \sim OH$, $R^2 = OH$

TABLE I

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

Compounds[a]	*Dose (μg)*	*Skin reaction with ABA-BSA*[a] *(50 μg) (diam. in mm ±s.e.)*[b] *at*	
		24 h	*48 h*
17a	10	21.2 ±0.9	15.0 ±0.6
	100	20.1 ±0.2	17.5 ±0.5
17b	10	19.3 ±1.0	16.7 ±0.7
	100	18.6 ±0.7	15.3 ±0.3
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 (ABA)–*N*-acetyl-L-tyrosine–bovine serum albumin (BSA). [b]The data indicate the average diameter ± the standard error, (s.e.) 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 INTRAPERITONEAL ADMINISTRATION OF MDP–TDM CONJUGATES

Compounds[a]	*Dose (μg)*[b]	*Radioactivity in viable target cells (cpm ±s.d.)*[c]
17a	50	435 ±73 (89%)
17b	50	311 ±13 (92%)
TDM	50	476 ±68 (88%)
Tumor cells alone	—	3898 ±110

[a]Mice were injected i.p. with MDP–TDM conjugates as indicated, 5 days before harvest of macrophages. [b]All samples were suspended in PBS. [c]Target cells (B16-BL6) labeled with ^{125}I-UdR were plated into culture wells to obtain an 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.

The compounds thus obtained showed potent activity not only on the induction of delayed-type hypersensitivity in guinea pigs as an MDP analog, but also on the activation of mouse peritoneal macrophages as a TDM analog (see Tables I and II)[9,10].

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) was accomplished with the solvent systems (v/v) specified.

*5-(*tert*-Butoxycarbonylamino)pentanoic acid* (**2a**). — To a stirred solution of 5-aminopentanoic acid (**1a**; 1.6 g, 13.7 mmol) in a mixture of water (10 mL) and triethylamine (2.9 mL) was added a solution of 2-(*tert*-butoxycarbonylthio)-4,6-dimethylpyrimidine (5.0 g, 20.8 mmol) in 1,4-dioxane (3 mL), and the mixture was stirred for 12 h at room temperature. After removal of the solvent by lyophilization, the residue was washed with hexane (4 × 10 mL). Pure compound **2a** (2.0 g, 96.6%) was obtained as an amorphous mass; m.p. 44.5°; ν 3400–2400 (broad, carboxylic acid), 3320 (NH), 3000 and 2840 (methyl, methylene), 1700 (C=O), and 1680 and 1560 cm^{-1} (amide).

Anal. Calc. for $C_{10}H_{19}NO_4$ (217.27): C, 55.28; H, 8.82; N, 6.45. Found: C, 55.25; H, 8.95; N, 6.39.

*8-(*tert*-Butoxycarbonylamino)octanoic acid* (**2b**). — Compound **2b** was obtained in quantitative yield from 8-aminooctanoic acid as described for **2a**; m.p. 51.0°.

Anal. Calc. for $C_{13}H_{25}NO_4$ (259.35): C, 60.21; H, 9.72; N, 5.40. Found: C, 60.30; H, 9.76; N, 5.14.

N-*[2-*O*-(Benzyl 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-β-D-glucopyranosid-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine benzyl ester* (**4**). — To a solution of **3** (1.22 g, 2.88 mmol) in dry 1,4-dioxane (15 mL) were added, with stirring, *N*-hydroxysuccinimide (HOSu; 0.40 g, 3.46 mmol) and dicyclohexylcarbodiimide (DCC; 3.0 g, 4.33 mmol), and the mixture was stirred for 30 min at room temperature. L-Alanyl-D-isoglutamine benzyl ester trifluoroacetate (1.26 g, 3.46 mmol) and triethylamine (0.56 mL, 4.33 mmol) were added, and the mixture was stirred for 1 h at room temperature; the precipitates were filtered off, and washed with 1,4-dioxane. The filtrate and washings were combined and evaporated to a syrup which was extracted with dichloromethane. The extract was washed with water, dried (sodium sulfate), and evaporated to a syrup, which was chromatographed on a column of silica gel with 30:1 dichloromethane–methanol, affording compound **4** (1.67 g, 83.0%); m.p. 92.0–92.8°, $[\alpha]_D$ −18.2° (*c* 0.8, 1:1 dichloromethane–methanol); ν 3300 (NH), 1730 and 1200 (ester), 1650 and 1530 (amide), 860 (CMe_2), and 695 cm^{-1} (phenyl).

Anal. Calc. for $C_{36}H_{48}N_4O_{11}$ (712.79): C, 60.66; H, 6.79; N, 7.86. Found: C, 60.76; H, 6.51; N, 8.12.

N-*[2-*O*-(Benzyl 2-acetamido-2,3-dideoxy-β-D-glucopyranosid-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine benzyl ester* (**5**). — Compound **4** (1.5 g) was treated with 80% aqueous acetic acid (20 mL) for 1 h at 45°, and the solution cooled and evaporated, to yield compound **5** quantitatively; m.p. 170.2–171.8°, $[\alpha]_D$ −6.9° (*c* 0.8, 1:2 dichloromethane–methanol); ν 3200 (OH and NH), 1730 (ester), 1650 and 1530 (amide), and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{33}H_{44}N_4O_{11}$ (672.73): C, 58.92; H, 6.59; N, 8.33. Found: C, 58.99; H, 6.53; N, 8.11.

N-[*2-*O-{*Benzyl 2-acetamido-6-*O-[*5-(*tert-*butoxycarbonylamino)pentanoyl*]-*2,3-dideoxy-β-*D-*glucopyranosid-3-yl*}-D-*lactoyl*]-L-*alanyl-*D-*isoglutamine benzyl ester* (**6a**). — To a solution of **5** (150 mg, 0.23 mmol) in *N,N*-dimethylformamide (DMF; 5 mL) were added the acid **2a** (59.3 mg, 0.27 mmol), DCC (93.8 mg, 0.45 mmol), and 4-(dimethylamino)pyridine (DMAP; 13.5 mg, catalytic amount), and the mixture was stirred for 3 h at room temperature, and evaporated. The residue was chromatographed on a column of silica gel with 25:1 dichloromethane–methanol, to afford **6a** (89.0 mg, 46.2%); $[\alpha]_D$ −4.7° (*c* 0.9, dichloromethane); ν 3400 (OH and NH), 1720 (ester), and 1650 and 1530 cm^{-1} (amide); ^1H-n.m.r. data (CD_3OD): δ 1.35, 1.37 (2 d, 6 H, 2 $CHCH_3$), 1.41 (s, 9 H, CMe_3), 1.50, 1.52 (2 m, 4 H, $CH_2CH_2CH_2CH_2$), 1.89 (s, 3 H, CH_3CON), 1.90, 2.25 (2 m, 2 H, CH_2CH_2CO of isoGln), 2.45 (m, 4 H, 2 CH_2CO), 3.0–3.6 (m, 6 H, H-2,3,4,5,6a,6b), 4.1–4.4 (3 m, 3 H, 2 $CHCH_3$ and $CHCONH_2$), 4.43 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.55, 4.81 (2 d, 2 H, J_{gem} 12.1 Hz, OCH_2Ph), 5.11 [s, 2 H, $C(O)OCH_2Ph$], and 7.3–7.4 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{43}H_{61}N_5O_{14}$ (871.98): C, 59.23; H, 7.05; N, 8.03. Found: C, 59.14; H, 6.94; N, 7.82.

N-[*2-*O-{*Benzyl 2-acetamido-6-*O-[*8-(*tert-*butoxycarbonylamino)octanoyl*]-*2,3-dideoxy-β-*D-*glucopyranosid-3-yl*}-D-*lactoyl*]-L-*alanyl-*D-*isoglutamine benzyl ester* (**6b**). — Compound **6b** was obtained by the same procedure as for **6a**, in 44.0% yield; $[\alpha]_D$ −4.5° (*c* 0.9, dichloromethane).

Anal. Calc. for $C_{46}H_{67}N_5O_{14}$ (914.06): C, 60.45; H, 7.39; N, 7.66. Found: C, 60.38; H, 7.55; N, 7.61.

N-{*2-*O-[*Benzyl 2-acetamido-6-*O-*(5-aminopentanoyl)-2,3-dideoxy-β-*D-*gluco-pyranosid-3-yl*]-D-*lactoyl*}-L-*alanyl-*D-*isoglutamine benzyl ester trifluoroacetate* (**7a**). — Compound **6a** (85 mg) was treated with trifluoroacetic acid (2 mL) for 1 h at 0°, and the solution evaporated. The residue was lyophilized from 1,4-dioxane, and subjected to the next reaction without purification.

N-{*2-*O-[*Benzyl 2-acetamido-6-*O-*(8-aminooctanoyl)-2,3-dideoxy-β-*D-*gluco-pyranosid-3-yl*]-D-*lactoyl*}-L-*alanyl-*D-*isoglutamine benzyl ester trifluoroacetate* (**7b**). — Compound **6b** was converted into **7b** by the procedure described for **7a**.

*4,6:4′,6′-Di-*O-*isopropylidene-α,α-trehalose* (**8**). — To a suspension of α,α-trehalose dihydrate (10 g) in DMF (100 mL) were added 2,2-dimethoxypropane (20 mL) and *p*-toluenesulfonic acid (0.4 g), and the mixture was stirred for 7.5 h at room temperature, made neutral with triethylamine, and evaporated. The solid residue was purified by chromatography on a column of silica gel. Elution with 50:1 dichloromethane–methanol, followed by 10:1 dichloromethane–methanol, gave **8** as a syrup (9.7 g, 79%); $[\alpha]_D$ +130° (*c* 0.8, chloroform).

Anal. Calc. for $C_{18}H_{30}O_{11}$: C, 51.18; H, 7.16. Found: C, 51.37; H, 7.34.

*2,3,2′,3′-Tetrakis-*O-[*(2-trimethylsilylethoxy)methyl*]-*α,α-trehalose* (**9**). — To a solution of **8** (11 g, 26.0 mmol) in dichloromethane (200 mL) were added chloro-

(2-trimethylsilylethoxy)methane (25 g, 156.3 mmol) and diisopropylethylamine (23 g, 182.3 mmol). The mixture was stirred for 12 h at 30°, and evaporated. The residue was extracted with dichloromethane, the extract was successively washed with 2M hydrochloric acid, 10% sodium carbonate, and water, dried (sodium sulfate), and evaporated to a syrup. The residue was chromatographed on a column of silica gel with 300:1 dichloromethane–methanol, to afford the (2-trimethylsilylethoxy)methylated compound (22 g, 91.7%); this (19.1 g) was treated with 80% aqueous acetic acid (100 mL) for 30 min at 45°, and evaporated. The residue was chromatographed on a column of silica gel with 100:1 dichloromethane–methanol, to give the desired product **9** (15.0 g, 88.9%); m.p. 67.0–69.0°, $[\alpha]_D$ +74.3° (*c* 0.4, dichloromethane); ν 3400 (OH), and 860 and 830 cm^{-1} (Si–C); ^{1}H-n.m.r. data ($CDCl_3$): δ 0–0.1 [m, 36 H, 4 Si(CH_3)$_3$], 0.95 (m, 8 H, 4 CH_2SiMe_3), 3.4–4.0 (m, 20 H, H-2,3,4,5,6a,6b,2′,3′,4′,5′6a′,6b′, 4 $CH_2CH_2SiMe_3$), 4.55–4.80 (m, 12 H, 4 OCH_2O, 4 O*H*), and 5.12 (d, 2 H, $J_{1,2}$ = $J_{1',2'}$ =2.45 Hz, H-1,1′).

Anal. Calc. for $C_{36}H_{78}O_{15}Si_4$ (863.35): C, 50.08; H, 9.11. Found: C, 50.17; H, 9.23.

6-O-Tosyl-2,3,2′,3′-tetrakis-O-[(2-trimethylsilylethoxy)methyl]-α,α-trehalose (**10**). — To a solution of **9** (13 g, 15.6 mmol) in dry pyridine (65 mL) was added, with stirring, *p*-toluenesulfonyl chloride (3.4 g, 17.8 mmol) at 0°. The mixture was stirred for 2 h at room temperature, then methanol (excess) was added to the mixture, which was evaporated. The residue was extracted with dichloromethane, the extract was successively washed with 2M hydrochloric acid, 10% sodium carbonate, and water, dried (sodium sulfate), and evaporated to a syrup. The residue was chromatographed on a column of silica gel with 100:1 dichloromethane–methanol, to give **10** (6.8 g, 44.4%) as a syrup; $[\alpha]_D$ +30.3° (*c* 1.6, dichloromethane); ν 3400 (OH), 1380 and 1180 (tosyl), and 860 and 830 cm^{-1} (Si–C); ^{1}H-n.m.r. data ($CDCl_3$): δ 0–0.1 [m, 36 H, 4 Si(CH_3)$_3$], 0.90 (m, 8 H, 4 CH_2SiMe_3), 2.41 (s, 3 H, CH_3Ph), 3.4–4.4 (m, 20 H, H-2,3,4,5,6a,6b,2′,3′,4′,5′,6a′,6b′, 4 $CH_2CH_2SiMe_3$), 4.55–4.80 (m, 11 H, 4 OCH_2O, 3 O*H*), 4.99, 5.01 (2 d, 2 H, H-1,1′), 7.29, and 7.76 (2 d, 4 H, 4 Ph-*H*).

Anal. Calc. for $C_{43}H_{84}O_{17}SSi_4$ (1017.54): C, 50.76; H, 8.32. Found: C, 50.98; H, 8.31.

6-Azido-6-deoxy-2,3,2′,3′-tetrakis-O-[(2-trimethylsilylethoxy)methyl]-α,α-trehalose (**11**). — A solution of **10** (6.5 g, 6.4 mmol) in DMF (100 mL) was treated with sodium azide (2 g, 31.9 mmol), and the mixture was stirred for 48 h at 100°, and evaporated. The residue was extracted with dichloromethane, the extract successively washed with 2M hydrochloric acid, 10% sodium carbonate and water, dried (sodium sulfate), and evaporated to a syrup. The residue was chromatographed on a column of silica gel with 250:1 dichloromethane–methanol, to afford **11** (4.6 g, 80.0%); $[\alpha]_D$ +29.6° (*c* 0.5, dichloromethane); ν 3400 (OH), 2100 (azide), and 860 and 830 cm^{-1} (Si–C).

Anal. Calc. for $C_{36}H_{77}N_3O_{14}Si_4$ (888.36): C, 48.67; H, 8.74; N, 4.73. Found: C, 48.83; H, 8.91; N, 4.88.

6-Azido-6-deoxy-6′-O-{3-[2-(trimethylsilylethoxy)carbonyl]propanoyl}-2,3,2′,3′-tetrakis-O-[(2-trimethylsilylethoxy)methyl]-α,α-trehalose (**12**). — To a stirred solution of succinic anhydride (270 mg, 2.7 mmol) in 1,2-dichloroethane (10 mL) were added 2-trimethylsilylethanol (0.39 mL, 2.7 mmol) and DMAP (50 mg, catalytic amount), and the mixture was stirred for 4 h at 50°. Compound **11** (1.6 g, 1.8 mmol) in 1,2-dichloroethane (10 mL) and DCC (743 mg, 3.6 mmol) were added to the mixture, and it was stirred for 8 h at 50°. After addition of methanol (excess) to quench the reaction, the mixture was concentrated. Compound **12** was roughly purified by a column of silica gel, and applied to the next reaction without further purification.

6-Amino-6-deoxy-6′-O-{3-[(2-trimethylsilylethoxy)carbonyl]propanoyl}-2,3,2′,3′-tetrakis-O-[(2-trimethylsilylethoxy)methyl]-α,α-trehalose (**13**). — A solution of crude **12** in methanol (100 mL) was hydrogenolyzed in the presence of 10% palladium-on-carbon (300 mg) for 2 h at room temperature. The catalyst was filtered off, and the filtrate was evaporated to a syrup, which was chromatographed on a column of silica gel with 50:1 dichloromethane–methanol to give compound **12** (1.34 g, 70% yield, based on **11**); $[\alpha]_D$ +25.5° (*c* 0.6, dichloromethane); ν 3400 (OH and NH_2), 1740 (ester), and 860 and 840 cm^{-1} (Si–C); ^{1}H-n.m.r. data ($CDCl_3$): δ 0–0.1 [m, 45 H, 5 $Si(CH_3)_3$], 0.9–1.1 (m, 10 H, 5 CH_2SiMe_3), 2.5–2.7 (m, 4 H, succinoyl), 3.1–4.9 (m, 34 H, ring protons, 5 $CH_2CH_2SiMe_3$, 4 OCH_2O, and O*H*), 4.99, 5.01 (2 d, 2 H, $J_{1,2} = J_{1',2'}$ = 3.30 Hz, H-1,1′).

Anal. Calc. for $C_{45}H_{95}NO_{17}Si_5$ (1062.67): C, 50.86; H, 9.01; N, 1.32. Found: C, 51.05; H, 9.30; N, 1.03.

6-Deoxy-6-(mycoloylamino)-2,3,2′,3′-tetrakis-O-[(2-trimethylsilylethoxy)-methyl]-6′-O-{3-[(2-trimethylsilylethoxy)carbonyl]propanoyl}-α,α-trehalose (**14**). — To a solution of **13** (278.5 mg, 0.27 mmol) in dichloromethane (10 mL) were added *N*-(mycoloyloxy)succinimide[6] (400 mg, 0.31 mmol) and triethylamine (1 drop). The mixture was stirred for 8 h at room temperature, and evaporated to a syrup which was chromatographed on a column of silica gel with 250:1 dichloromethane–methanol, to give **14** (530 mg, 75.9%). Compound **14** solidified from ether–methanol; m.p. 28.0–29.0°, $[\alpha]_D$ +7.5° (*c* 0.4, dichloromethane); ν3400 (OH and NH_2), 2900, 2840, 1470, and 720 (mycoloyl), 1740 (ester), 1650 and 1540 (amide), and 860 and 840 cm^{-1} (Si–C).

Anal. Calc. for $C_{125}H_{251}NO_{19.5}Si_5$ (2220.8): C, 67.61; H, 11.39; N, 0.63. Found: C, 67.32; H, 11.13; N, 0.58.

6-O-(3-Carboxypropanoyl)-6′-deoxy-6′-(mycoloylamino)-α,α-trehalose (**15**). — To a solution of compound **14** (100 mg, 45 μmol) in dichloromethane (5 mL) was added boron trifluoride etherate (0.5 mL), and the mixture was stirred for 30 min at room temperature, poured into methanol, and compound **14**, an amorphous mass, collected by filtration; m.p. 93.0–95.0°, $[\alpha]_D$ +33.0° (*c* 0.4, dichloromethane); ν 3400 (OH), 3000–2400 (broad, carboxylic acid), 2930, 2850, 1470, and 720 (mycoloyl), 1740 (ester), and 1640 and 1550 cm^{-1} (amide).

Anal. Calc. for $C_{96}H_{183}NO_{15.5}$ (1549.51): C, 72.09; H, 11.53; N, 0.88. Found: C, 71.98; H, 11.24; N, 1.13.

N-{*2-O-[Benzyl 2-acetamido-6-O-{5-[3-(6-deoxy-6-mycoloylamino-α,α-trehalos-6′-yloxycarbonyl)propanamido]pentanoyl}-2,3-dideoxy-β-*D*-glucopyranosid-3-yl]-*D*-lactoyl}-*L*-alanyl-*D*-isoglutamine benzyl ester* (**16a**). — To a solution of **15** (100 mg, 63 μmol) in dichloromethane (10 mL) were added, with stirring, DCC (26 mg, 0.13 mmol) and HOSu (11 mg, 95 μmol), and the mixture was stirred for 30 min at room temperature. A solution of compound **7a** (63.5 mg, 76 μmol) in DMF (5 mL) and triethylamine (1 drop) were added to the mixture, which was stirred for 10 h at room temperature and evaporated; the residue was chromatographed on a column of silica gel with 5:1 dichloromethane–methanol, to give compound **16a** (105 mg, 73.8%), $[\alpha]_D$ +12.8° (*c* 1.0, dichloromethane); ν 3400 (OH and NH), 2950, 2870, 1470, and 720 (mycoloyl), 1740 (ester), and 1660 and 1550 cm^{-1} (amide).

Anal. Calc. for $C_{134}H_{234}N_6O_{26.5}$ (2353.35): C, 68.39; H, 10.02; N, 3.57. Found: C, 68.59; H, 9.80; N, 3.74.

N-{*2-O-[Benzyl 2-acetamido-6-O-{8-[3-(6-deoxy-6-mycoloylamino-α,α-trehalos-6′-yloxycarbonyl)propanamido]octanoyl}-2,3-dideoxy-β-*D*-glucopyranosid-3-yl]-*D*-lactoyl}-*L*-alanyl-*D*-isoglutamine benzyl ester* (**16b**). — Compound **16b** was obtained in 60.8% yield by the procedure described for **15a**; $[\alpha]_D$ +16.6° (*c* 0.9, dichloromethane).

Anal. Calc. for $C_{137}H_{240}N_6O_{26.5}$ (2395.43): C, 68.69; H, 10.10; N, 3.51. Found: C, 68.72; H, 10.30; N, 3.39.

N-{*2-O-[2-Acetamido-6-O-{5-[3-(6-deoxy-6-mycoloylamino-α,α-trehalos-6′-yloxycarbonyl)propanamido]pentanoyl}-2,3-dideoxy-*D*-glucopyranos-3-yl]-*D*-lactoyl}-*L*-alanyl-*D*-isoglutamine* (**17a**). — A solution of **16a** (103 mg) in tetrahydrofuran (5 mL) and acetic acid (5 mL) was hydrogenolyzed in the presence of palladium black (50 mg) for 2 days at room temperature, the catalyst filtered off, and the filtrate evaporated to a syrup. Compound **17a** (85 mg, 88.1%) was obtained as an amorphous mass by solidification from methanol; $[\alpha]_D$ +26.1° (*c* 0.8, dichloromethane); ν 3400 (OH and NH), 2930, 2860, 1450, and 720 (mycoloyl), 1730 (ester), and 1650 and 1550 cm^{-1} (amide).

Anal. Calc. for $C_{120}H_{222}N_6O_{26.5}$ (2173.10): C, 66.33; H, 10.30; N, 3.87. Found: C, 66.52; H, 10.05; N, 3.98.

N-{*2-O-[2-Acetamido-6-O-{8-[3-(6-mycoloylamino-α,α-trehalos-6′-yloxycarbonyl)propanamido]octanoyl}-2,3-dideoxy-*D*-glucopyranos-3-yl]-*D*-lactoyl}-*L*-alanyl-*D*-isoglutamine* (**17b**). — Compound **17b** was obtained, by the procedure described for **16a**, in 93.7% yield; $[\alpha]_D$ +25.8° (*c* 0.8, dichloromethane).

Anal. Calc. for $C_{123}H_{228}N_6O_{26.5}$ (2215.18): C, 66.69; H, 10.37; N, 3.79. Found: C, 66.50; H, 10.18; N, 3.88.

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