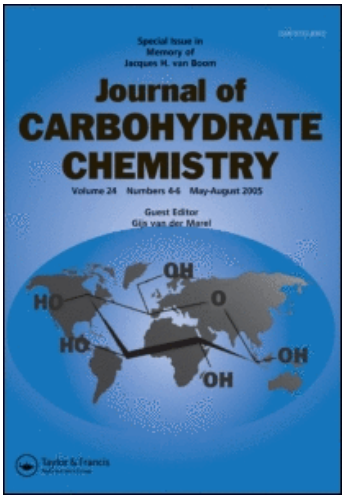

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Synthesis and Biological Activities of*N*-Acetyl-6-*O*-acyl-1-thiomuramoyl-l-alanyl-d-isoglutamine Derivatives

Akira Hasegawa[a]; Yuichi Hioki[a]; Etzuko Yamamoto[a]; Makoto Kiso[a]; Ichiro Azuma[b]
[a] Department of Agricultural Chemistry, Gifu University, Gifu, Japan [b] Institute of Immunological Science, Hokkaido University, Sapporo, Japan

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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF N-ACETYL-6-O-ACYL-1-S-ACYL-1-THIOMURAMOYL-L-ALANYL-D-ISOGLUTAMINE DERIVATIVES*

Akira Hasegawa, Yuichi Hioki, Etzuko Yamamoto, Makoto Kiso

Department of Agricultural Chemistry

Gifu University, Gifu 501-11, Japan

Ichiro Azuma

Institute of Immunological Science, Hokkaido University

Sapporo 060, Japan

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ABSTRACT

A variety of N-[2-O-(2-acetamido-6-O-acyl-1-S-acyl-2,3-dideoxy-1-thio-β-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl esters were synthesized. Their immunoadjuvant activities were examined in guinea-pigs.

INTRODUCTION

Recently, it has been shown that lipophilic derivatives[1-5] of N-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP) and its carbohydrate analogs carrying strong, adjuvant activity causes potent antitumor and

*Studies on Immunoadjuvant Active Compounds, Part 32. For Part 31, see ref. 1.

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antiinfection activities, based on the immune reaction, that are not found for MDP itself, and abolishes the pyrogenicity, which is one of the side effects of MDP. However, the position of introduction of lipophilicity into the molecule is critical for the activity, as the lipophilic analogs[6] at both C-2 and C-6 in 6-amino-6-deoxymuramoyl dipeptide (having strong, adjuvant activity) completely lack activity. In view of these facts, it seems important to elucidate the relationship between the position of introduction of lipophilicity into the sugar moiety of MDP analogs, and the biological activities.

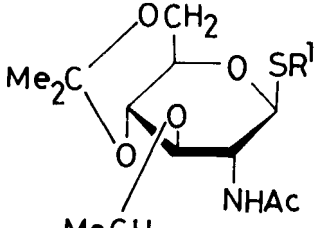
We now describe the synthesis of lipophilic derivatives at both C-1 and C-6 in 1-thiomuramoyl-L-alanyl-D-isoglutamine methyl ester[4], and their biological activities.

RESULTS AND DISCUSSION

Treatment of N-[2-O-(2-acetamido-2,3-dideoxy-4,6-O-isopropylidene-1-thio-β-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester[4] (1) with tetradecanoyl chloride in pyridine-dichloromethane at -30°C gave the 1-S-tetradecanoyl derivatives (2) as crystals in 75% yield; this was converted, by hydrolytic removal of the isopropylidene group under mild, acidic conditions, into N-[2-O-(2-acetamido-2,3-dideoxy-1-S-tetradecanoyl-1-thio-β-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (4) in quantitative yield. On the other hand, in order to obtain the lipophilic derivatives of compound 6[4], bearing the lipid moiety at C-6 of the sugar skeleton, when treated with hexanoic anhydride in pyridine-dichloromethane at -30°C, compound 6 selectively afforded the 6-O-hexanoyl derivative 7 in 74% yield. Condensation of 6 with decanoyl, tetradecanoyl, or octadecanoyl chloride at -30°C, gave the corresponding 6-O-(fatty acyl)-derivatives (8-10) in good yields.

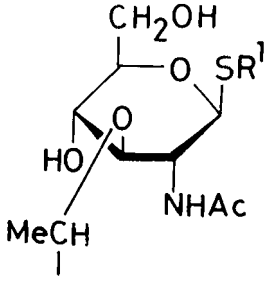
For the synthesis of 1-S-acyl-6-O-acyl derivatives of 1-thio-N-acetylmuramoyl-L-alanyl-D-isoglutamine methyl ester, condensation of compounds 3-5 with hexanoic anhydride or decanoyl, tetradecanoyl, or octadecanoyl chloride, according to the same way described for compounds 2 and 7, yielded a variety of the desired, lipophilic derivatives (11-22) in good yields.

The immunoadjuvant activities of the compounds thus obtained on the induction of the delayed type of hypersensitivity to N-acetyl-L-



1 R^1 = H

2 R^1 = $CO(CH_2)_{12}Me$

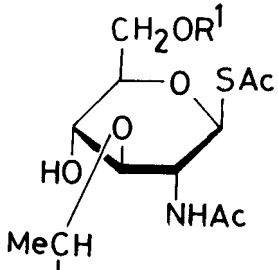


R^1=$CO(CH_2)_nMe$

3 R^1: n=8

4 n=12

5 n=16



6 R^1 = H

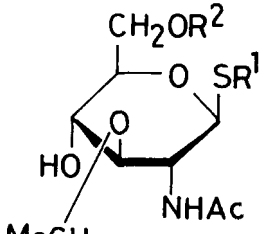
R^1=$CO(CH_2)_nMe$

7 R^1: n=4

8 n=8

9 n=12

10 n=16



	R^1=$CO(CH_2)_mMe$,	R^2=$CO(CH_2)_nMe$
11	R^1: m=8	R^2: n=4
12	m=8	n=8
13	m=8	n=12
14	m=8	n=16
15	m=12	n=4
16	m=12	n=8
17	m=12	n=12
18	m=12	n=16
19	m=16	n=4
20	m=16	n=8
21	m=16	n=12
22	m=16	n=16

TABLE 1

Adjuvant Activities of the Lipophilic Analogs of N-Acetyl-l-thio-muramoyl-L-alanyl-D-isoglutamine on Induction of Delayed-type of Hypersensitivity to ABA-N-Acetyltyrosine in Guinea-pigs.

Compounds[a]	Skin Reaction with ABA-BSA[b] (50 µg) (diam. in mm ± SE)[c] at	
	24 h	48 h
7	16.4 ± 0.7	13.0 ± 0.7
8	14.4 ± 2.0	12.0 ± 1.4
9	17.0 ± 0.9	17.0 ± 0.5
10	18.8 ± 0.7	17.0 ± 0.9
11	17.9 ± 0.5	13.8 ± 0.6
12	16.0 ± 1.0	13.0 ± 1.0
13	14.0 ± 0.6	12.0 ± 0.8
14	14.3 ± 2.2	11.0 ± 2.5
15	16.0 ± 0.3	15.1 ± 0.5
16	17.3 ± 0.8	13.3 ± 0.2
17	16.5 ± 1.2	14.8 ± 0.6
18	17.0 ± 1.2	16.5 ± 0.6
19	17.4 ± 0.8	17.5 ± 0.9
20	14.3 ± 1.1	13.8 ± 0.7
21	16.5 ± 1.0	14.9 ± 0.7
22	18.5 ± 0.6	16.7 ± 0.4
MDP	17.0 ± 0.9	14.0 ± 1.0
Control[d]	0	0

[a]Dose: 10 µg. [b]Azobenzenearsonate-N-acetyl-L-tyrosine-bovine serum albumin. [c]The data indicate the average diameter ± the standard error (SE) of the skin reaction (induration) of four guinea-pigs. [d]ABA-N-acetyl-L-tyrosine in Freund's incomplete adjuvant.

tyrosine-3-azobenzene-4'-arsonate (ABA-N-acetyltyrosine) in guinea-pigs were examined[6b] (see Table I). All of the compounds were equally as active as MDP as adjuvant. The results clearly indicate that introduction of fatty acyl group at C-6, or at both C-1 and C-6 of the sugar moiety in N-acetyl-1-thiomuramoyl-L-alanyl-D-isoglutamine methyl ester is favorable for the activity. In the previous papers[4,7], we knew that 1-O-acyl-MDP derivatives were less active than the 1-S-acyl-MDP analogs. The protective activity in mice infected with E. coli (E-77156) was examined.[8] All of the compounds provided efficient protection, especially compounds 13, 17, 19, and 20 protected completely.

EXPERIMENTAL

General methods. Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were determined with a Union MP-201 polarimeter at 25°C, and IR spectra were recorded with a Jasco A-100 spectrophotometer. NMR data were recorded at 90 MHz with a Hitachi R-22 spectrometer. Preparative chromatography was performed on silica gel (Waco Co.; 200 mesh) with the solvent systems specified. Evaporations were conducted in vacuo.

N-[2-O-(2-Acetamido-1-S-tetradecanoyl-2,3-dideoxy-4,6-O-isopropylidene-1-thio-β-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (2). To a solution of 1[4] (150 mg) in dry pyridine (2 mL) and dichloromethane (5 mL) was added dropwise, with stirring, a solution of tetradecanoyl chloride (100 mg) in dichloromethane (2 mL) at -30°C and the mixture was stirred for 60 min at -20°C; the residue extracted with chloroform. The extract was washed with water, dried (sodium sulfate), and concentrated to a syrup which was chromatographed on a column of silica gel (20 g) with (a) 150:1, (b) 70:1, and (c) 30:1 chloroform-methanol. Eluant (c) gave amorphous 2 (155 mg; 75%); mp 182°, $[\alpha]_D$ + 6.5° (c 0.46, 1:1 chloroform-methanol); IR (KBr): 3270 (NH), 2940 and 2850 (Me, methylene), 1740 and 1260 (ester), 1700 (S-acetyl), 1660, 1540, 1535, and 1530 (amide), and 860 cm^{-1} (Me_2C); NMR ($CDCl_3$): δ 0.88 (near t, 3H, J_{Me,CH_2} 6.0 Hz, $MeCH_2$), 1.25 (s, 22H, $11CH_2$), 1.26-1.40 (m, 12H, Me_2C, 2MeCH), 1.98 (s, 3H, AcN), 3.67 (s, 3H, MeO), 5.22 (d, 1H, $J_{1,2}$ 10.5 Hz, H-1), and 6.37, 7.18, 7.40, and 7.74 (5H, 3NH, NH_2).

Anal. Calcd for $C_{37}H_{64}N_4O_{11}S$: C, 57.49; H, 8.35; N, 7.25. Found: C, 57.42; H, 8.43; N, 7.19.

N-[2-O-(2-Acetamido-2,3-dideoxy-1-S-tetradecanoyl-1-thio-β-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (4). A solution of 2 (80 mg) in 80% aqueous acetic acid (2 mL) was heated for 2 h at 45°C, and concentrated to a crystalline mass. Recrystallization from ether gave 4 in quantitative yield; mp 174-175°, $[\alpha]_D$ + 37.5° (c 0.4, methanol); IR (KBr): 3350-3230 (OH, NH), 2930 and 2850 (Me, methylene), 1740 and 1250 (ester), 1700 (S-acyl), and 1640 and 1530 cm^{-1} (amide).

Anal. Calcd for $C_{34}H_{66}N_4O_{11}S$: C, 55.71; H, 8.25; N, 7.65. Found: C, 55.64; H, 8.09; N, 7.60.

N-[2-O-(2-Acetamido-1-S-acetyl-2,3-dideoxy-6-O-hexanoyl-1-thio-β-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (7). To a solution of 6^4 (150 mg) in dry pyridine (3 mL) and dichloro methane (1 mL) was added, with stirring, a solution of hexanoic anhydride (62.5 mg) in dichloromethane (1 mL) at -30°C, the mixture was stirred for 2 h at -30°C; methanol (0.5 mL) was added to the mixture, which was concentrated. The residue was chromatographed on a slica gel plate (Kieselgel 60F-254) with 5:1 chloroform-methanol, to afford 7 (130 mg; 74%) as crystals; mp 161-163°, $[\alpha]_D$ + 25.7° (c 0.48, 1:1 chloroform-methanol); IR (KBr): 3380 (OH), 3280 (NH), 2950, 2930, and 2850 (Me, methylene), 1730 and 1240 (ester), 1690 (S-acetyl), and 1640 and 1550 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.90 (near t, 3H, J_{Me,CH_2} 6.0 Hz, $MeCH_2$), 1.25-1.44 (m, 12H, 2MeCH, $3CH_2$), 1.95 (s, 3H, AcN), 2.37 (s, 3H, AcS), 3.71 (s, 3H, MeO), and 5.13 (d, 1H, $J_{1,2}$ 11.0 Hz, H-1).

Anal. Calcd for $C_{28}H_{46}N_4O_{12}S$: C, 50.74; H, 7.00; N, 8.45. Found: C, 50.66; H, 7.15; N, 8.43.

N-[2-O-(2-Acetamido-1-S-acetyl-6-O-decanoyl-2,3-dideoxy-1-thio-β-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (8). To a solution of 6 (150 mg) in dry pyridine (3 mL) and dichloromethane (1 mL) was added, with stirring, a solution of decanoyl chloride (56 mg) in dichloromethane (1 mL) at -30°C, and the mixture was stirred for 2 h at -30°C; methanol (0.5 mL) was added to the mixture, which was treated with Amberlite IR-45 (OH^-) resin. The resin was filtered off, and washed with methanol, and the filtrate and wash-

ings were combined, and concentrated. The residue was chromatographed on a silica gel plate (Kieselgel 60F-254) with 5:1 chloroform-methanol, to give 8 (160 mg; 84%) as crystals; mp 189-191°, $[\alpha]_D$ + 18.5° (c 0.4, 1:1 chloroform-methanol); IR (KBr): 3400 (OH), 3280 (NH), 2930 and 2850 (Me, methylene), 1730 and 1240 (ester), 1690 (S-acetyl), and 1660, 1640, and 1545 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88 (near t, 3H, J_{Me,CH_2} 5.2 Hz, $MeCH_2$), 1.28 (s, 14H, $7CH_2$), 1.30-1.44 (2d, 6H, 2MeCH), 1.93 (s, 3H, AcN), 2.37 (s, 3H, AcS), 3.70 (s, 3H, MeO), and 5.12 (d, 1H, $J_{1,2}$ 11.0 Hz, H-1).

Anal. Calcd for $C_{32}H_{54}N_4O_{12}S$: C, 53.46; H, 7.57; N, 7.79. Found: C, 53.45; H, 7.61; N, 7.83.

N-[2-O-(2-Acetamido-1-S-acetyl-2,3-dideoxy-6-O-tetradecanoyl-1-thio-β-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (9). Compound 9 was obtained as crystals in 75% yield, according to the procedure described for 8; mp 192-193°, $[\alpha]_D$ + 25.5° (c 0.6, 1:1 chloroform-methanol); IR (KBr): 3380 (OH), 3280 (NH), 2930 and 2850 (Me, methylene), 1740, 1730, and 1230 (ester), 1690 (S-acetyl), and 1670, 1660, 1640, 1545, and 1530 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88 (near t, 3H, J_{Me,CH_2} 5.2 Hz, $MeCH_2$), 1.27 (s, 22H, $11CH_2$), 1.36, 1.42 (2d, 6H, $J_{Me,CH}$ 6.5 Hz, 2MeCH), 1.93 (s, 3H, AcN), 2.37 (s, 3H, AcS), 3.69 (s, 3H, MeO), and 1.52 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1).

Anal. Calcd for $C_{36}H_{62}N_4O_{12}S$: C, 55.79; H, 8.06; N, 7.23. Found: C, 55.68; H, 8.03; N, 7.15.

N-[2-O-(2-Acetamido-1-S-acetyl-2,3-dideoxy-6-O-octadecanoyl-β-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (10). Compound 10 was obtained as crystals in 87% yield, according to the procedure described for 8; mp 193-196°, $[\alpha]_D$ + 13° (c 0.5, 1:1 chloroform-methanol); IR (KBr): 3400-3250 (OH, NH), 2940 and 2850 (Me, methylene), 1740 and 1250 (ester), 1700 (S-acetyl), and 1670, 1640, and 1540 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88 (near t, 3H, J_{Me,CH_2} 5.0 Hz, $MeCH_2$), 1.27 (s, 30H, $15CH_2$), 1.28-1.44 (2d, 6H, 2MeCH), 1.90 (s, 3H, AcN), 2.35 (s, 3H, AcS), 3.69 (s, 3H, MeO), and 5.11 (d, 1H, $J_{1,2}$ 10.5 Hz, H-1).

Anal. Calcd for $C_{40}H_{70}N_4O_{12}S$: C, 57.81; H, 8.49; N, 6.74. Found: C, 57.65; H, 8.33; N, 6.59.

N-[2-O-(2-Acetamido-1-S-acyl-2,3-dideoxy-6-O-hexanoyl-1-thio-β-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl esters

(11, 15, and 19) were prepared according to the method described for 7, and other 6-O-acyl-1-S-acyl-N-acetyl-1-thiomuramoyl-L-alanyl-D-iso-glutamine methyl esters (12-14, 16-18, and 20-22) were synthesized as described for 8, from compounds 3^4, 4, and 5^4 by 6-O-acylation.

Compound 11 was obtained as crystals in 87% yield; mp 182-183°, $[\alpha]_D$ + 22.5° (c 0.9, 1:1 chloroform-methanol); IR (KBr): 3400 (OH), 3280 (NH), 2950, 2930, and 2850 (Me, methylene), 1740, 1730, and 1245 (ester), 1690 (S-acyl), and 1660, 1640, 1550, and 1540 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.87 (near 2t, 6H, J_{Me,CH_2} 5.4 Hz, $2MeCH_2$), 1.36, 1.42 (2d, 6H, $J_{Me,CH}$ 6.4 Hz, 2MeCH), 1.90 (s, 3H, AcN), 3.69 (s, 3H, MeO), and 5.10 (d, 1H, $J_{1,2}$ 10.8 Hz, H-1).

Anal. Calcd for $C_{36}H_{62}N_4O_{12}S$: C, 55.79; H, 8.06; N, 7.23. Found: C, 55.84; H, 8.16; N, 7.22.

Compound 12 was obtained as crystals (69% yield); mp 192-193°, $[\alpha]_D$ + 26.2° (c 0.8, 1:1 chloroform-methanol); IR (KBr): 3420 (OH), 3280 (NH), 2950 and 2860 (Me, methylene), 1730, 1260, and 1230 (ester), 1700 (S-acyl), and 1680, 1660, 1550, and 1540 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88 (near t, 6H, J_{Me,CH_2} 5.8 Hz, $2MeCH_2$), 1.27 (s, 28H, $14CH_2$), 1.37, 1.42 (2d, 6H, $J_{Me,CH}$ 6.6 Hz, 2MeCH), 1.90 (s, 3H, AcN), 3.69 (s, 3H, MeO), and 5.11 (d, 1H, $J_{1,2}$ 10.4 Hz, H-1).

Anal. Calcd for $C_{40}H_{70}N_4O_{12}S$: C, 57.81; H, 8.49; N, 6.74. Found: C, 57.66; H, 8.49; N, 6.58.

Compound 13 was obtained as crystals (70% yield); mp 192-193°, $[\alpha]_D$ + 24.0° (c 0.9, 1:1 chloroform-methanol); IR (KBr): 3420 (OH), 3280 (NH), 2940 and 2850 (Me, methylene), 1730 and 1240 (ester), 1700 (S-acyl), and 1680, 1660, 1640, 1550, and 1540 cm^{-1} (amide): NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88 (near t, 6H, J_{Me,CH_2} 6.0 Hz, $2MeCH_2$), 1.27 (s, 36H, $18CH_2$), 1.37, 1.41 (2d, 6H, $J_{Me,CH}$ 6.8 Hz, 2MeCH), 1.90 (s, 3H, AcN), 3.69 (s, 3H, MeO), and 5.11 (d, 1H, $J_{1,2}$ 11.0 Hz, H-1).

Anal. Calcd for $C_{44}H_{78}N_4O_{12}S$: C, 59.57; H, 8.86; N, 6.32. Found: C, 59.55; H, 8.74; N, 6.31.

Compound 14 was obtained as crystals (74% yield); mp 191-192°, $[\alpha]_D$ + 22.5° (c 0.6, 1:1 chloroform-methanol); IR (KBr): 3420 (OH), 3280 (NH), 2930 and 2850 (Me, methylene), 1730 and 1230 (ester), 1690 (S-acyl), and 1680, 1660, 1640, 1550, and 1540 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88 (near t, 6H, J_{Me,CH_2} 5.0 Hz, $2MeCH_2$), 1.27 (s, 44H, $22CH_2$), 1.36 (d, 3H, $J_{Me,CH}$ 6.0 Hz, MeCH), 1.39 (d, 3H, $J_{Me,CH}$

7.0 Hz, MeCH), 1.90 (s, 3H, AcN), 3.69 (s, 3H, MeO), and 5.10 (s, 1H, $J_{1,2}$ 11.0 Hz, H-1).

Anal. Calcd for $C_{48}H_{86}N_4O_{12}S$: C, 61.12; H, 9.12; N, 5.94. Found: C, 61.29; H, 9.23; N, 5.94.

Compound 15 was obtained as crystals (65% yield); mp 162-164°, $[\alpha]_D$ 32.0° (c 0.3, 1:1 chloroform-methanol); IR (KBr): 3400 (OH), 3280 (NH), 2940 and 2850 (Me, methylene), 1740, 1730, and 1245 (ester), 1700 (S-acyl), 1680, 1660, 1640, 1550, and 1530 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88, 0.90 (2t, 6H, J_{Me,CH_2} 5.0 Hz, $2MeCH_2$), 1.27 (s, 28H, $14CH_2$), 1.37 (d, 3H, $J_{Me,CH}$ 6.2 Hz, MeCH), 1.41 (d, 3H, $J_{Me,CH}$ 6.8 Hz, MeCH), 1.93 (s, 3H, AcN), 3.71 (s, 3H, MeO), and 5.12 (d, 1H, $J_{1,2}$ 10.4 Hz, H-1).

Anal. Calcd for $C_{40}H_{70}N_4O_{12}S$: C, 57.81; H, 8.49; N, 6.74. Found: C, 57.69; H, 8.45; N, 6.73.

Compound 16 was obtained as crystals (89% yield); mp 196-197°, $[\alpha]_D$ + 26.0° (c 0.4, 1:1 chloroform-methanol); IR (KBr): 3400 (OH), 3280 (NH), 2930 and 2850 (Me, methylene), 1740, 1725, and 1240 (ester), 1690 (S-acyl), 1660, 1640, 1550, and 1540 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88 (near t, 6H, J_{Me,CH_2} 5.2 Hz, $2MeCH_2$), 1.27 (s, 36H, $18CH_2$), 1.37 (d, 3H, $J_{Me,CH}$ 6.4 Hz, MeCH), 1.41 (d, 3H, $J_{Me,CH}$ 6.8 Hz, MeCH), 1.95 (s, 3H, AcN), 3.69 (s, 3H, MeO), and 5.12 (s, 1H, $J_{1,2}$ 10.0 Hz, H-1).

Anal. Calcd for $C_{44}H_{78}N_4O_{12}S$: C, 59.57; H, 8.86; N, 6.32. Found: C, 59.55; H, 8.98; N, 6.25.

Compound 17 was obtained as crystals (79% yield); mp 192-193°, $[\alpha]_D$ + 25.0° (c 0.5, 1:1 chloroform-methanol); IR (KBr): 3400 (OH), 3280 (NH), 2930 and 2850 (Me. methylene), 1740, 1730, and 1230 (ester), 1700 (S-acyl), and 1680, 1660, 1640, 1550, and 1540 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88 (near t, 6H, J_{Me,CH_2} 5.6 Hz, $2MeCH_2$), 1.27 (s, 44H, $22CH_2$), 1.37, 1.41 (2d, 6H, $J_{Me,CH}$ 6.8 Hz, 2MeCH), 1.92 (s, 3H, AcN), 3.70 (s, 3H, MeO), and 5.11 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1).

Anal. Calcd for $C_{48}H_{86}N_4O_{12}S$: C, 61.12; H, 9.12; N, 5.94. Found: C, 60.96; H, 9.28; N, 5.88.

Compound 18 was obtained as crystals (74% yield); mp 169-170°, $[\alpha]_D$ + 22.5° (c 0.5, chloroform-methanol); IR (KBr): 3400-3300 (OH, NH), 2930 and 2850 (Me, methylene), 1740, 1720, and 1250 (ester), 1690 (S-acyl), and 1660, 1550, and 1535 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD):

δ 0.88 (near t, 6H, J_{Me,CH_2} 5.4 Hz, $2MeCH_2$), 1.27 (s, 52H, $26CH_2$), 1.38, 1.41 (2d, 6H, $J_{Me,CH}$ 6.6 Hz, 2MeCH), 1.93 (s, 3H, AcN), 3.69 (s, 3H, MeO), and 5.11 (d, 1H, $J_{1,2}$ 10.6 Hz, H-1).

Anal. Calcd for $C_{52}H_{94}N_4O_{12}S$: C, 62.49; H, 9.48; N, 5.61. Found: C, 62.45; H, 9.33; N, 5.49.

Compound 19 was obtained as crystals (62% yield); mp 175-176°, $[\alpha]_D$ + 15.8° (c 0.5, 1:1 chloroform-methanol); IR (KBr): 3430 (OH), 3280 (NH), 2930 and 2850 (Me, methylene), 1730 and 1240 (ester), 1690 (S-acyl), and 1680, 1660, 1640, and 1550 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88, 0.90 (2t, 6H, J_{Me,CH_2} 5.4 Hz, $2MeCH_2$), 1.27 (s, 36H, $18CH_2$), 1.38, 1.41 (2d, 6H, $J_{Me,CH}$ 6.6 Hz, 2MeCH), 1.90 (s, 3H, AcN), 3.70 (s, 3H, MeO), and 5.11 (d, 1H, $J_{1,2}$ 10.5 Hz, H-1).

Anal. Calcd for $C_{44}H_{78}N_4O_{12}S$: C, 59.57; H, 8.36; N, 6.32. Found: C, 59.33; H, 8.41; N, 6.25.

Compound 20 was obtained as crystals (71% yield); mp 175-176°, $[\alpha]_D$ + 18.0° (c 0.9, 1:1 chloroform-methanol); IR (KBr): 3430 (OH), 3280 (NH), 2930 and 2850 (Me, methylene), 1730, 1260, and 1230 (ester), 1700 (S-acyl), and 1660, 1640, and 1545 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88 (neat t, 6H, J_{Me,CH_2} 5.8 Hz, $2MeCH_2$), 1.27 (s, 44H, $22CH_2$), 1.38, 1.41 (2d, 6H, $J_{Me,CH}$ 6.6 Hz, 2MeCH), 1.90 (s, 3H, AcN), 3.70 (s, 3H, MeO), and 5.11 (d, 1H, $J_{1,2}$ 10.4 Hz, H-1).

Anal. Calcd for $C_{48}H_{86}N_4O_{12}S$: C, 61.12; H, 9.19; N, 5.94. Found: C, 61.06; H, 9.30; N, 5.75.

Compound 21 was obtained as crystals (70% yield); mp 195-198°, $[\alpha]_D$ + 21.5° (c 0.9, 1:1 chloroform-methanol); IR (KBr): 3400 (OH), 3280 (NH), 2930 and 2850 (Me, methylene), 1740 and 1230 (ester), 1700 (S-acyl), and 1660, 1640, and 1550 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88 (near t, 6H, J_{Me,CH_2} 5.4 Hz, $2MeCH_2$), 1.27 (s, 52H, $26CH_2$), 1.38, 1.42 (2d, 6H, $J_{Me,CH}$ 6.6 Hz, 2MeCH), 1.91 (s, 3H, AcN), 3.69 (s, 3H, MeO), and 5.12 (d, 1H, $J_{1,2}$ 11.0 Hz, H-1).

Anal. Calcd for $C_{52}H_{94}N_4O_{12}S$: C, 62.49; H, 9.48; N, 5.61. Found: C, 62.38; H, 9.58; N, 5.53.

Compound 22 was obtained as crystals (85% yield); mp 193-195°, $[\alpha]_D$ + 8.7° (c 0.63, 2:1 chloroform-methanol); IR (KBr): 3400 (OH), 3280 (NH), 2930 and 2850 (Me, methylene), 1740 and 1230 (ester), 1690 (S-acyl), and 1660, 1640, and 1550 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.88 (near t, 6H, J_{Me,CH_2} 5.6 Hz, $2MeCH_2$), 1.27 (s, 60H, $30CH_2$),

1.37, 1.41 (2d, 6H, $J_{Me,CH}$ 6.6 Hz, 2MeCH), 1.91 (s, 3H, AcN), 3.69 (s, 3H, MeO), and 5.10 (d, 1H, $J_{1,2}$ 10.6 Hz, H-1).

Anal. Calcd for $C_{56}H_{102}N_4O_{12}S$: C, 63.72; H, 9.74; N, 5.31. Found: C, 63.63; H, 9.98; N, 5.25.

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REFERENCES

1. A. Hasegawa, E. Seki, M. Kiso, and I. Azuma, Agric. Biol. Chem., in press.
2. S. Kusumoto, M. Inage, T. Shiba, I. Azuma, and Y. Yamamura, Tetrahedron Lett., 4899 (1978).
3. S. Kobayashi, T. Fukuta, I. Imada, M. Fujino, I. Azuma, and Y. Yamamura, Chem. Pharm. Bull., 27, 3193 (1979).
4. A. Hasegawa, Y. Hioki, M. Kiso, H. Okumura, and I. Azuma, Carbohydr. Res., 123, 183 (1983).
5. K. Matsumoto, T. Otani, T. Une, Y. Osada, H. Ogawa, and I. Azuma, Infect. Immun., 39, 1029 (1983).
6. (a) A. Hasegawa, E. Tanahashi, and M. Kiso, Carbohydr. Res., 103, 251 (1982); (b) I. Azuma, H. Okumura, I. Saiki, Y. Tanio, M. Kiso, A. Hasegawa, and Y. Yamamura, Infect. Immun., 32, 1305 (1981).
7. A. Hasegawa, Y. Hioki, M. Kiso, and I. Azuma, Carbohydr. Res., 123, 63 (1983).
8. I. Azuma et al., unpublished results.