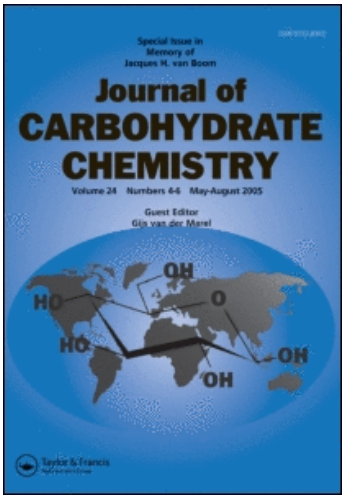

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Synthesis of 1-Thio-*N*-acetylmuramoyl-L-alanyl-D-isoglutamine Derivatives, and Their Biological Activities. XX

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Communication

SYNTHESIS OF 1-THIO-N-ACETYLMURAMOYL-L-ALANYL-D-ISOGLUTAMINE DERIVATIVES, AND THEIR BIOLOGICAL ACTIVITIES, XX*.

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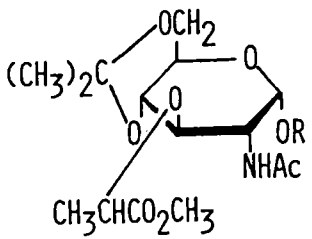
In the course of an investigation[2] on the relationship between the immunoadjuvant activity and the structure of the carbohydrate moiety in N-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP), which is the minimal, immunoadjuvant-active component of bacterial cell-wall peptidoglycan, we demonstrated that not only is restricted configuration of the sugar moiety important for the activity[2] but also that chemical modifications[3-5] of the functional groups in the carbohydrate moiety produce various, important effects on the manifestation of activity. It has been shown that lipophilic deriva-

* Part XIX, see ref. 1.

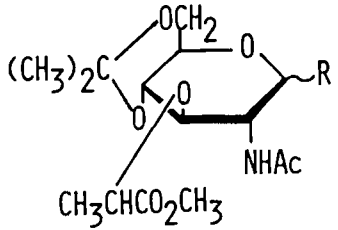
tives[6-8] of MDP bearing the lipid moiety at C-6 of the sugar skeleton, or at the end of the peptide chain, have strong antitumor and anti-infection activities that are not for MDP itself. In addition, we have also observed that introduction[3b,9,10] of lipophilic character at C-2 in muramoyl-L-alanyl-D-isoglutamine, or at C-6 in N-acetyl-6-amino-6-deoxy-muramoyl-L-alanyl-D-isoglutamine, causes potent antitumor activity based on the immune reaction, as well as strong, immunoadjuvant activities.

In view of these facts, we now describe the synthesis of 1-thio-N-acetylmuramoyl-L-alanyl-D-isoglutamine, and its lipophilic derivatives (1-S-hexadecanoyl, and 1-S-hexadecanyl derivatives), and their immunoadjuvant and anti-infection activities.

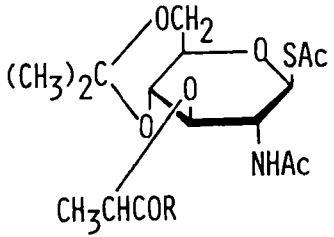
Treatment of benzyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-3-O-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (1) with chromium trioxide-pyridine complex[11] in the presence of acetic anhydride in dichloromethane at 45° gave crystalline 2 in 92% yield; mp 135-137°, $[\alpha]_D$ +144° (c 1.5, methanol). Hydrolysis of the 1-O-benzoyl group in 2 with sodium methoxide in methanol gave 3 in 93% yield; mp 180-184°, $[\alpha]_D$ +44.3° (c 0.47, chloroform), which on displacement of the hydroxyl group by treatment[12] with hexamethylphosphorous triamide and carbon tetrachloride in dichloromethane afforded the expected 2-acetamido-2-deoxy-4,6-O-isopropylidene-3-O-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranosyl chloride (4) in high yield. Treatment of 4 with potassium thioacetate in dry acetone gave the β-thioacetate 5 in good yield; mp 171-173°, $[\alpha]_D$ +8.1° (c 0.4, chloroform), which was converted, via hydrolysis of the S-acetyl and methyl ester groups, and subsequent S-acetylation, into 2-acetamido-3-O-(D-1-carboxyethyl)-2-deoxy-4,6-O-isopropylidene-1-thio-β-D-glucopyranose (6) in 78% yield; mp 193-200° (dec.), $[\alpha]_D$ +10.5° (c 0.3, chloroform). Coupling of 6 with L-alanyl-D-isoglutamine methyl ester using dicyclohexylcarbodiimide and N-hydroxysuccinimide in dry 1,4-dioxane gave 7 in 95% yield; mp 148-151°, $[\alpha]_D$ +11° (c 0.2, chloroform). Hydrolytic removal of the isopropylidene group in 7 under mild, acidic conditions afforded 13 in quantitative yield; mp 158-162°, $[\alpha]_D$ +62° (c 0.2, 1:1 chloroform-



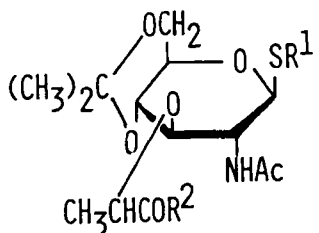
1 R = Bn
2 R = Bz
Bn = $PhCH_2$
Bz = PhCO



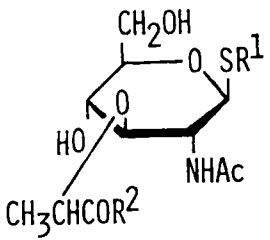
3 R = OH
4 R = Cl



5 R = OCH_3
6 R = OH
7 R = a
a = L-Ala-D-isoGln-OCH_3



8 R^1 = H, R^2 = a
9 R^1 = H, R^2 = b
10 R^1 = $-CO(CH_2)_{14}CH_3$, R^2 = a
11 R^1 = $-CO(CH_2)_{14}CH_3$, R^2 = b
12 R^1 = $-(CH_2)_{15}CH_3$, R^2 = a



13 R^1 = Ac, R^2 = a
14 R^1 = H, R^2 = b
15 R^1 = H, R^2 = b
16 R^1 = $-CO(CH_2)_{14}CH_3$, R^2 = a
17 R^1 = $-CO(CH_2)_{14}CH_3$, R^2 = b
18 R^1 = $-(CH_2)_{15}CH_3$, R^2 = a
19 R^1 = $-(CH_2)_{15}CH_3$, R^2 = b
b = L-Ala-D-isoGln

methanol), which was treated with sodium methoxide in methanol to give 2-acetamido-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine methyl ester)-1-thio-β-D-glucopyranose (14) in 95% yield; mp 117-125° (dec.), $[\alpha]_D$ +24° (c 0.2, methanol). Saponification of 14 with 0.2M aqueous potassium hydroxide in methanol gave the desired 1-thio-MDP (15); mp 157-166° (dec.), $[\alpha]_D$ +20° (c 0.2, methanol).

2-Acetamido-1-S-acetyl-2-deoxy-4,6-O-isopropylidene-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine methyl ester)-1-thio-β-D-glucopyranose (7) served as a starting material for the synthesis of all of the lipophilic, 1-thio-MDP derivatives at C-1 of the sugar moiety.

Condensation of 8, formed by selective hydrolysis of the S-acetyl group in 7, with hexadecanoyl chloride in pyridine-dichloromethane gave 10, which was converted, by hydrolytic removal of the isopropylidene group under mild acidic conditions, into 2-acetamido-2-deoxy-1-S-hexadecanoyl-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine methyl ester)-1-thio-β-D-glucopyranose (16) in good yield; mp 181-183°, $[\alpha]_D$ +63° (c 0.2, 1:1 chloroform-methanol). Treatment of 9, derived from 7 by hydrolysis, with hexadecanoyl chloride according to the procedure just described gave the 1-S-hexadecanoyl derivative 11 in good yield; mp 103-106°, $[\alpha]_D$ +42° (c 0.5, 1:1 chloroform-methanol), compound 11 hydrolyzed with 80% aqueous acetic acid by heating at 45° for 1 h, to yield 2-acetamido-2-deoxy-1-S-hexadecanoyl-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine)-1-thio-β-D-glucopyranose (17) in almost quantitative yield; mp 125-129°, $[\alpha]_D$ +62.5° (c 0.2, 1:1 chloroform-methanol).

On the other hand, treatment of sodium salt of 8, derived from 7 by addition of sodium methoxide in methanol, with hexadecanyl bromide afforded the 1-S-hexadecanyl derivative 12 in 93% yield after column chromatography; mp 93-96°, $[\alpha]_D$ +35° (c 0.3, chloroform). Hydrolytic removal of the isopropylidene group in 12 under mildly acidic conditions gave 18; mp 179-182°, $[\alpha]_D$ +44° (c 0.2, 1:1 chloroform-methanol), which was saponified with 0.2M potassium hydroxide in 1,4-dioxane-methanol to afford 1-S-hexadecanyl 2-acetamido-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine)-1-thio-β-D-gluco-

TABLE 1

Adjuvant Activity of 1-Thio Derivatives of N-Acetylmuramoyl-L-alanyl-D-isoglutamine (MDP) on the Induction of Delayed-type Hypersensitivity to ABA-N-acetyltyrosine in Guinea-pigs.

Compound	Dose (μg)	Skin Reaction with ABA-BSA[a] (50 μg) (diam. in mm)[b] at	
		24 h	48 h
8	100	15.5	12.3
11	100	(11.1)	0
13	100	20.4	19.1
	10	19.8	19.8
14	100	20.6	19.1
	10	18.5	17.9
15	100	19.6	17.4
	10	20.6	18.5
16	100	19.6	17.8
17	100	19.4	19.9
18	100	(11.6)	(9.5)
19	100	(14.9)	(12.4)
MDP	100	20.2	18.2
	10	20.0	19.1
Control[c]		0	0

[a]ABA-N-acetyl-L-tyrosine-bovine serum albumin. [b]The data indicate the average diameter of skin reaction (induration) of four guinea-pigs; the values in parentheses indicate the size of erythema. [c]ABA-N-acetyl-L-tyrosine in Freund's incomplete adjuvant.

pyranoside (19) in quantitative yield; mp 110–115° (dec.), $[\alpha]_D$ +24° (c 0.2, methanol).

The immunoadjuvant activities of compounds 8, 11, and 13–19 thus obtained on the induction of the delayed-type of hypersensitivity to N-acetyltyrosine-3-azobenzene-4'-arsonic acid (ABA-N-acetyltyrosine) were examined[13] in guinea-pigs (see Table 1).

Compounds 13–17 showed strong activities, comparable to that of MDP, whereas other compounds exhibited weak, or no adjuvant activity. The results indicate that, for activity, the substituent on C-1 is not restricted to the hydroxyl group, and can be replaced by the thiol or S-acyl group.

The protective activities of compounds 8, 13, 14, 16, 17, and and 19 in mice infected with E. coli (E 77156) were examined.[14] Compounds 14, 16, and 17 provided efficient protection, but 8, 13, and 19 were inactive.

New compounds gave elemental analysis and IR and NMR data in agreement with the structures assigned.

REFERENCES

1. A. Hasegawa, E. Tanahashi, Y. Goh, and M. Kiso, Carbohydr. Res., 103, 273 (1982).

2. A. Hasegawa, Y. Kaneda, Y. Goh, K. Nishihori, M. Kiso, and I. Azuma, Carbohydr. Res., 94, 143 (1981), and references cited therein.

3. (a) A. Hasegawa, H. Okumura, M. Kiso, I. Azuma, and Y. Yamamura, Agric. Biol. Chem., 44, 1301 (1980); (b) 44, 1309 (1980).

4. A. Hasegawa, E. Tanahashi, Y. Goh, M. Kiso, and I. Azuma, Carbohydr. Res., 92, 81 (1981).

5. A. Hasegawa, H. Okumura, K. Nishihori, Y. Kaneda, M. Kiso, and I. Azuma, Carbohydr. Res., 97, 337 (1981).

6. (a) S. Kusumoto, M. Inage, T. Shiba, I. Azuma, and Y. Yamamura, Tetrahedron Lett., 4899 (1978); (b) S. Kobayashi, T. Fukuda, I. Imada, M. Fujino, I. Azuma, and Y. Yamamura, Chem. Pharm. Bull., 27, 3193 (1979); (c) K. Matsumoto, H. Ogawa, T. Kusama, O. Nagase, N. Sawai, M. Inage, S. Kusumoto, T. Shiba, and I. Azuma, Infect. Immun., 32, 748 (1981).

7. M. Parant, F. Audibert, L. Chedid, M. Level, P. Lefrancier, and E. Lederer, Infect. Immun., 27, 826 (1980).

8. E. Yarkoni, E. Lederer, and H. J. Rapp, Infect. Immun., 32, 273 (1981).

9. M. Kiso, Y. Goh, E. Tanahashi, A. Hasegawa, H. Okumura, and I. Azuma, Carbohydr. Res., 90, c8 (1981).

10. H. Okumura, K. Kamisango. I. Saili, Y. Tanio, I. Azuma, M. Kiso, A. Hasegawa, and Y. Yamamura, Agric. Biol. Chem., 46, 507 (1982).

11. P. J. Garegg and B. Samuelsson, Carbohydr. Res., 67, 267 (1978).

12. F. Chretien, Y. Chapleur, B. Castro, and B. Gross, J. Chem. Soc. Perkin 1, 381 (1980).

13. I. Azuma, K. Kamisango, I. Saiki, Y. Tanio, S. Kobayashi, and Y. Yamamura, Infect. Immun., 29, 1193 (1980).

14. Y. Osada, M. Mitsuyama, T. Une, K. Matsumoto, T. Otani, M. Satoh, H. Ogawa, and K. Nomoto, Infect. Immun., 37, 292 (1982).