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Preliminary communication

Synthesis and immunoadjuvant activities of novel *N*-acylmuramoyl dipeptides related to the lipid A constituent of the bacterial lipopolysaccharide*

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Bacterial cell-wall components have a variety of interesting immunomodulating activities. In recent years, much progress[2] has been made regarding the synthetic peptidoglycan derivatives as strong immunostimulants.

In our continuing effort[3] to elucidate the relationships between the structure of the carbohydrate moiety and the biological activity of muramoyl dipeptide (MDP)**, which is the minimal, adjuvant-active structure of bacterial, cell-wall peptidoglycan[4], we have demonstrated that not only is the sugar moiety essential for adjuvant activity, but also that the combining site of the lactoyl-dipeptide moiety and its configuration are critically important[3e]. However, the sugar skeleton (2-amino-2-deoxy-D-glucose) was exchangeable with 2-amino-2-deoxy-D-mannose[3a] or -D-galactose[3e], while retaining the activity. In addition to these findings, it has been shown that the 6-hydroxyl group can be replaced by an amino or acylamino group[3c,d], but that the activity is decreased dramatically by deoxygenation[1,3c,d], deamination[3b], or halogenation[1]. Moreover, the 2-acetamido group could be replaced by a free amino[3b], a methylamino[5], or a hydroxyl[3b] group without decreasing the activity.

We have recently developed[6] a new synthetic route to analogs of the lipid A component of the bacterial lipopolysaccharide (LPS), in order to elucidate the relationships between chemical structure and biological activity. The preliminary investigation[7] revealed that 2-deoxy-2-(D-3-hydroxytetradecanoylamino)-D-glucose and one of its β-(1→6)-linked disaccharide derivatives had antitumor, as well as *Limulus*-lysate gelation, activities. In view of the fact that the synthetic, lipophilic MDP derivatives showed potent antitumor and anti-infection activities[2] that were not found for MDP itself, our

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**A summary of this study, involving the biological investigation of the synthetic, repeating disaccharide–dipeptide units[3f], was presented at the Xth International Symposium on Carbohydrate Chemistry, Sydney, Australia, in July 1980.

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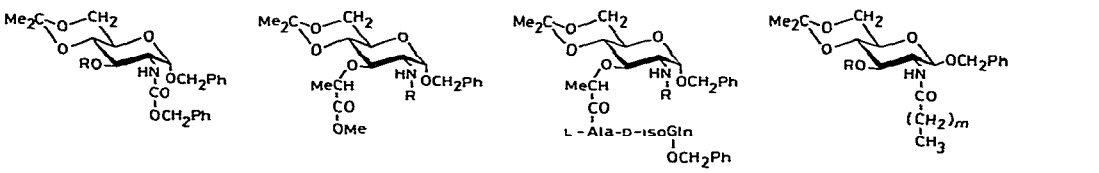
interest has been directed to the synthesis of new, lipophilic, MDP analogs related to the lipid A constituent of LPS. We now describe the synthesis of novel *N*-acylmuramoyl dipeptides, and their immunoadjuvant activities on the induction of delayed-type hypersensitivity in guinea-pigs.

Benzyl 2-(benzyloxycarbonylamino)-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (**1**), a syrup, $[\alpha]_D$ +99.8° (*c* 0.5, chloroform), was prepared by (benzyloxycarbonyl)ation of benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside, m.p. 144–148° (dec.), $[\alpha]_D$ +123.2° (*c* 1.11, chloroform), which can be obtained almost quantitatively by treatment of the corresponding 2-acetamido derivative[3a] with hydrazine hydrate under reflux. Compound **1** can also be synthesized by direct acetalation of benzyl 2-(benzyloxycarbonylamino)-2-deoxy-α-D-glucopyranoside[8] with 2,2-dimethoxypropane in dry 1,4-dioxane containing a trace of *p*-toluenesulfonic acid[9].

For the synthesis of the *N*-hexadecanoyl or *N*-octadecanoyl derivative, we employed the corresponding benzyl 2-(acylamino)-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside as the starting material: **15** (*m* = 14), m.p. 114–117°, $[\alpha]_D$ −67.4° (*c* 0.5, chloroform); and **16** (*m* = 16), m.p. 118°, $[\alpha]_D$ −65.5° (*c* 1, chloroform), which were prepared stepwise by *O*-deacetylation of the benzyl 3,4,6-tri-*O*-acetyl-2-(acylamino)-2-deoxy-β-D-glucopyranosides[6a], and subsequent 4,6-*O*-isopropylidenation as just described.



1 R = H
2 R = CHCO$_2$Me (Me)

3 R = H
4 R = C(CH$_2$)$_{12}$CH$_3$ (O)
5–7 R = CCH$_2$C(CH$_2$)$_n$CH$_3$ (O, O)
5 *n* = 10
6 *n* = 12
7 *n* = 14
8–10 R = CCH$_2$CH(CH$_2$)$_n$CH$_3$ (O, OH)
8 *n* = 10
9 *n* = 12
10 *n* = 14

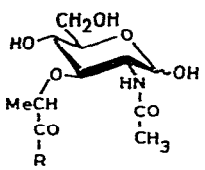
11 R = C(CH$_2$)$_{12}$CH$_3$ (O)
12–14 R = CCH$_2$CH(CH$_2$)$_n$CH$_3$ (O, OH)
12 *n* = 10
13 *n* = 12
14 *n* = 14

15 R = H, *m* = 14
16 R = H, *m* = 16
17–18 R = CHCO$_2$Me (Me)
17 *m* = 14
18 *m* = 16
19–20 R = CHCO-L-Ala-D-isoGln (Me; OCH$_2$Ph)
19 *m* = 14
20 *m* = 16

The isopropylidene derivatives **1**, **15**, and **16** were condensed with L-2-chloropropionic acid in the presence of sodium hydride, to give 3-*O*-(D-1-carboxyethyl) derivatives, which were converted into the methyl esters by treatment with diazomethane in ether solution: **2**, m.p. 136–137°, $[\alpha]_D$ +83.2° (*c* 0.202, chloroform); **17**, m.p. 108–110°, $[\alpha]_D$ −28.8° (*c* 1.5, chloroform); and **18**, m.p. 96–97°, $[\alpha]_D$ −31.2° (*c* 0.5, chloroform). The benzyloxycarbonyl group of **2** was removed by brief hydrogenation

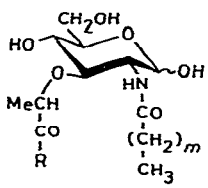
(30–40 min) in methanol in the presence of 10% palladium–carbon catalyst, to afford **3**, which was converted, by treatment with *N*-(tetradecanoyloxy)succinimide or *N*-(3-oxo-tetra-, -hexa-, or -octa-decanoyloxy)succinimide, into the corresponding *N*-fatty acyl derivatives: **4** (80%), m.p. 45–47°, $[\alpha]_D$ +76.4° (*c* 0.2, chloroform); and **5**–**7** (55–70%) as amorphous materials. Compounds **5**–**7** were dissolved in methanol, and treated with sodium borohydride at 0°, to give 3-hydroxy-fatty acyl derivatives **8**–**10** in quantitative yields. After de-esterification of **4** and **8**–**10** with 0.1M aqueous potassium hydroxide in 1,4-dioxane, the acids formed were coupled with the benzyl ester of L-alanyl-D-isoglutamine, using dicyclohexylcarbodiimide and *N*-hydroxysuccinimide as activating agents, to yield the protected *N*-acylmuramoyl dipeptides as amorphous materials: **11**, $[\alpha]_D$ +60.9° (*c* 0.33, chloroform); **12** (*n* = 10), $[\alpha]_D$ +50.6° (*c* 1.1, chloroform); **13** (*n* = 12), $[\alpha]_D$ +54.5° (*c* 0.59, chloroform); and **14** (*n* = 14), $[\alpha]_D$ +45.0° (*c* 0.69, chloroform). Similar treatment of **17** and **18** gave **19** (*m* = 14), $[\alpha]_D$ −7.62° (*c* 1.94, chloroform) and **20** (*m* = 16), $[\alpha]_D$ −8.3° (*c* 0.7, chloroform), respectively.

Hydrolytic removal of the 4,6-*O*-isopropylidene group from **11**, **12**–**14**, **19**, and **20** with 70% acetic acid, and subsequent hydrogenation in the presence of 10% palladium–carbon catalyst, gave the desired (*N*-acylmuramoyl)-L-alanyl-D-isoglutamines as amorphous materials after lyophilization: **21** (*m* = 12), $[\alpha]_D$ +12.2° (*c* 0.34, methanol); **22** (*m* = 14), $[\alpha]_D$ +13.0° (*c* 0.2, water); **23** (*m* = 16), $[\alpha]_D$ +15.3° (*c* 0.4, water); **24** (*n* = 10), $[\alpha]_D$ +19.7° (*c* 0.46, methanol); **25** (*n* = 12), $[\alpha]_D$ +23.7° (*c* 0.95, methanol); and **26** (*n* = 14), $[\alpha]_D$ +6.8° (*c* 0.205, methanol). In order to clarify the effect of the



MDP

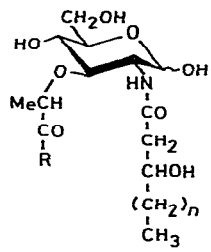
R = L-Ala-D-isoGln



21 *m* = 12

22 *m* = 14

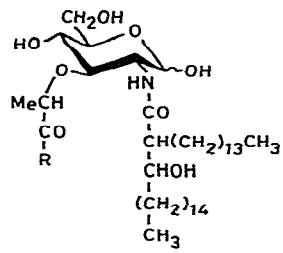
23 *m* = 16



24 *n* = 10

25 *n* = 12

26 *n* = 14



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alkyl-chain length of the *N*-acyl moiety, we also prepared 2-deoxy-2-(3-hydroxy-2-tetradecyl-octadecanoylamino)-3-*O*-(D-2-propionyl-L-alanyl-D-isoglutamine)-D-glucopyranose (**27**), amorphous, $[\alpha]_D$ +11.3° (*c* 0.2, methanol, equil.), starting from the 3-hydroxy-2-tetradecyl-octadecanoyl[10] derivative of compound **3**.

The immunoadjuvant activities of **21**–**27** on the induction of delayed-type hypersensitivity to azobenzenearsonate-*N*-acetyl-L-tyrosine (ABA-*N*-acetyl-L-tyrosine)[11] in guinea-pigs were examined. As shown in Table I, all of the synthetic MDP analogs exhibited potent activity. Compound **21** showed not only induration, but also a clear necrotic reaction.

TABLE I

ADJUVANT ACTIVITY OF NOVEL *N*-ACYLMURAMOYL DIPEPTIDES (21–27) ON THE INDUCTION OF DELAYED-TYPE HYPERSENSITIVITY TO ABA-*N*-ACETYL-L-TYROSINE IN GUINEA-PIGS

Compound[a]	*Skin reaction*[b] *with ABA-BSA (50 μg) (diam. in mm ±SE)*[c] *at*	
	24 h	*48 h*
21	21.0 ±1.2	19.4 ±0.8
22	20.8 ±0.4	18.3 ±0.4
23	21.0 ±0.8	16.5 ±0.6
24	21.1 ±1.1	21.1 ±0.8
25	20.5 ±0.4	17.8 ±0.6
26	20.1 ±1.0	19.5 ±1.1
27	21.5 ±1.0	18.0 ±0.7
N-Acetylmuramoyl-L-alanyl-D-isoglutamine (MDP)	20.3 ±0.8	19.8 ±0.9
Control[d]	0	0

[a] At a dose of 100 μg. [b] Four animals. [c] The data indicate the average diameter ± standard-error (SE) of the skin reaction (induration); ABA-BSA, azobenzenearsonate–*N*-acetyl-L-tyrosine–bovine serum albumin. [d] ABA-*N*-acetyl-L-tyrosine in Freund's incomplete adjuvant (FIA), or FIA alone.

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