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Preliminary communication

Synthesis of *N*-acetyl-6-amino- and -6-(acylamino)-6-deoxymuramoyl dipeptides, and their immunoadjuvant activities*

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In the course of studies[1–4] on the relationship between the structure of the carbohydrate moiety and activity in *N*-acetylmuramoyl-L-alanyl-D-isoglutamine, which is the minimal structure[5,6] required for the immunoadjuvant activity of bacterial, cell-wall peptidoglycans, we have demonstrated that the carbohydrate moiety is not restricted to 2-acetamido-2-deoxy-D-glucose. In view of this fact, we have synthesized a variety of carbohydrate analogs[7] of the muramoyl dipeptides in order to clarify the structural requirement in the carbohydrate moiety for the activity.

In this communication, we describe a synthesis of 6-amino- and 6-(acylamino)-6-deoxy-*N*-acetylmuramyl dipeptides, and their immunoadjuvant and antitumor activities. Treatment of benzyl 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside[1,2] (**1**) with methanol in the presence of Amberlite IR-120 (H^+) ion-exchange resin at 70° (bath temp.) gave crystalline **2** in high yield; m.p. 136°, $[\alpha]_D^{20}$ +151° (*c* 1.0, chloroform). Selective *O*-mesylation of **2** gave the 6-mesylate **3** (85%), $[\alpha]_D^{20}$ +117° (*c* 0.3, chloroform), which, on (tetrahydropyran-2-yl)ation afforded **4** quantitatively; $[\alpha]_D^{20}$ +100° (*c* 0.3, chloroform). Displacement of the mesyloxy group in **4** by treatment with sodium azide in *N*,*N*-dimethylformamide for 6 h at 70° gave the expected benzyl 2-acetamido-6-azido-2,6-dideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-4-*O*-(tetrahydropyran-2-yl)-α-D-glucopyranoside (**5**) in 85% yield; $[\alpha]_D^{20}$ +128° (*c* 0.3, methanol). De-esterification of **5** with 0.1M aqueous potassium hydroxide in 1,4-dioxane for 5 min at 0° afforded **6** (86%); m.p. 146°, $[\alpha]_D^{20}$ +134° (*c* 1.1, methanol). Coupling of **6** with the benzyl esters of L-alanyl-D-isoglutamine[8], L-valyl-D-isoglutamine[9], and *O*-benzyl-L-seryl-D-isoglutamine[10], using dicyclohexylcarbodiimide–*N*-hydroxysuccinimide in 1,4-dioxane, respectively, af-

*Studies on Immunoadjuvant Active Compounds, Part V. For Part IV, see ref. 4.
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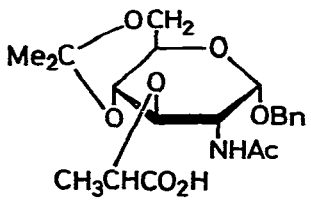

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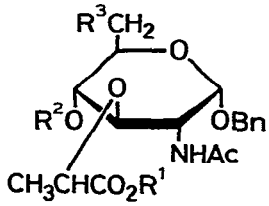
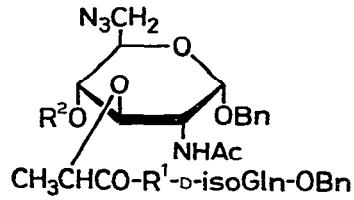


2 R^2=H, R^3=OH
3 R^2=H, R^3=OMs
4 R^2=THP, R^3=OMs
5 R^2=THP, R^3=N_3
6 R^1=H, R^2=THP, R^3=N_3
(2-5, R^1=Me)



7a,b R^1=L-Ala
8a,b R^1=L-Val
9a,b R^1=L-Ser-OBn
(a, R^2=THP; b, R^2=H)

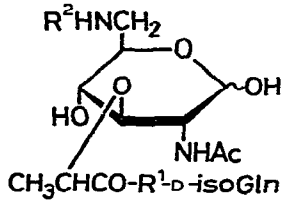
Bn = $PhCH_2$, THP = tetrahydropyran-2-yl

forded the corresponding *N*-acetyl-6-azido-6-deoxymuramoyl dipeptide derivatives in almost quantitative yields: **7a**, m.p. 204° (dec.), $[\alpha]_D^{20}$ +90.5° (*c* 0.3, 1 : 1 chloroform–methanol); **8a**, m.p. 205° (dec.), $[\alpha]_D^{20}$ +82° (*c* 0.3, 1 : 1 chloroform–methanol); and **9a**, m.p. 188° (dec.), $[\alpha]_D^{20}$ +74.5° (*c* 0.3, 1 : 1 chloroform–methanol).

Hydrolytic removal of the tetrahydropyran-2-yl group in **7a**, **8a**, and **9a** under mild, acidic conditions gave **7b** (95%), m.p. 219–220° (dec.), $[\alpha]_D^{20}$ +99° (*c* 0.7, acetic acid) {lit.[11] m.p. 221–225° (dec.), $[\alpha]_D^{25}$ +98°}; **8b** (90%), m.p. 219–221° (dec.), $[\alpha]_D^{20}$ +83° (*c* 0.5, acetic acid); and **9b** (88%), m.p. 214–215° (dec.), $[\alpha]_D^{20}$ +87° (*c* 0.5, acetic acid), respectively.

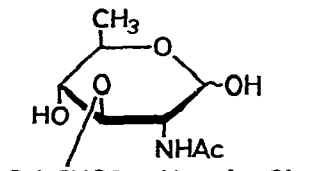
Hydrogenolytic removal of the protecting groups and reduction of the azide group in compounds **7b**, **8b**, and **9b**, in 30 : 10 : 3 (v/v) methanol–water–acetic acid, with hydrogen in the presence of 10% Pd–C catalyst at 30° gave the desired *N*-acetyl-6-amino-6-deoxymuramoyl-L-alanyl-D-isoglutamine (**10**, 95%), m.p. 133–134° (dec.), $[\alpha]_D^{20}$ +34° (*c* 1.0, water; equil.); the corresponding L-valyl-D-isoglutamine (**11**, 92%), m.p. 144–145° (dec.), $[\alpha]_D^{20}$ +30° (*c* 0.4, water; equil.); and the L-seryl-D-isoglutamine (**12**, 94%), m.p. 135–136° (dec.), $[\alpha]_D^{20}$ +26° (*c* 0.5, water; equil.), respectively. 6-*N*-Acetylation of **10** gave compound **13**; m.p. 155–156° (dec.), $[\alpha]_D^{20}$ +25° (*c* 0.4, methanol; equil.). Compound **10** was treated in *N*,*N*-dimethylformamide with two molar equiv. of *N*-(stearoyloxy)succinimide, affording *N*-acetyl-6-deoxy-6-(stearoylamino)muramoyl-L-alanyl-D-isoglutamine (**14**) in 48% yield (after purification by chromatography on a column of silica gel); m.p. 162–164° (dec.), $[\alpha]_D^{20}$ +15.5° (*c* 0.2, 1 : 1 chloroform–methanol; equil.). When treated with *N*-(mycoloyloxy)succinimide according to the procedure just described, compound **10** yielded **15**; m.p. 157–158° (dec.), $[\alpha]_D^{20}$ +14° (*c* 0.7, 1 : 1 chloroform–methanol; equil.).

The adjuvant activities of the *N*-acetyl-6-amino-6-deoxymuramoyl dipeptides **10**–**12** and the *N*-acetyl-6-(acylamino)-6-deoxymuramoyl-L-alanyl-D-isoglutamines (**13**–**15**) thus obtained, and of the carbohydrate analogs[7] (**16** and **17**), on the induction of delayed-type hypersensitivity on ABA-*N*-acetyltyrosine in guinea pigs were examined[12].



10 R^1=L-Ala, R^2=H
11 R^1=L-Val, R^2=H
12 R^1=L-Ser, R^2=H
13 R^1=L-Ala, R^2=Ac
14 R^1=L-Ala, R^2=stearoyl
15 R^1=L-Ala, R^2=mycoloyl

N-Acetyl-6-amino-6-deoxymuramoyl-L-seryl-D-isoglutamine (**12**) showed adjuvant activity clearly stronger than that of the original *N*-acetylmuramoyl-L-alanyl-D-isoglutamine, whereas compounds **10**, **11**, **13**, and **14** exhibited activities comparable to that of the muramoyl dipeptide. However, compounds **15**, **16**, and **17** had no adjuvant activity.



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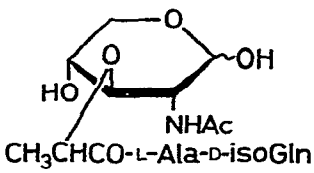

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The results show that the substituent on C-6 in the carbohydrate moiety of the minimal adjuvant-active structure, *N*-acetylmuramoyl-L-alanyl-D-isoglutamine, can be replaced by an amino or an acylamino group, and that the substituents seem to be very critical for manifestation of the immunoadjuvancy of the *N*-acetylmuramoyl dipeptide, as replacement of the hydroxyl group at C-6 by a hydrogen atom, giving the D-*xylo* analog (**17**), almost abolished the adjuvant activity.

On the other hand, compound **15** and the long-chain acylamino analogs[13] showed distinct, antitumor activity in animal tests[14].

New compounds gave elemental analyses and i.r.- and n.m.r.-spectral data compatible with the structures assigned.

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REFERENCES

1 A. Hasegawa, K. Bito, and M. Kiso, *Gifu Daigaku Nogakubu Kenkyu Hokoku*, 40 (1977) 95–100.
2 A. Hasegawa, Y. Kaneda, M. Amano, M. Kiso, and I. Azuma, *Agric. Biol. Chem.*, 42 (1978) 2187–2189.
3 A. Hasegawa, H. Okumura, and M. Kiso, *Gifu Daigaku Nogakubu Kenkyu Hokoku*, 42 (1979) 171–177.
4 M. Kiso, Y. Kaneda, H. Okumura, A. Hasegawa, I. Azuma, and Y. Yamamura, *Carbohydr. Res.*, 79 (1980) C17–C19.
5 F. Ellouz, A. Adam, R. Ciorbaru, and E. Lederer, *Biochem. Biophys. Res. Commun.*, 59 (1974) 1317–1325; C. Merser, P. Sinaÿ, and A. Adam, *ibid.*, 66 (1975) 1316–1322.
6 S. Kotani, I. Morisaki, Y. Watanabe, F. Kinoshita, T. Shimono, T. Shiba, S. Kusumoto, Y. Tarumi, and K. Ikenaka, *Biken J.*, 18 (1975) 105–111.
7 A. Hasegawa, Y. Kaneda, H. Okumura, Y. Goh, and M. Kiso, manuscript in preparation.
8 S. Kusumoto, Y. Tarumi, K. Ikenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 49 (1976) 533–539.
9 S. Kobayashi and M. Fuzino, unpublished results.
10 S. Kotani, Y. Watanabe, F. Kinoshita, I. Morisaki, K. Kato, T. Shiba, S. Kusumoto, Y. Tarumi, and K. Ikenaka, *Biken J.*, 20 (1977) 39–45.
11 P. L. Durette, A. Rosegay, M. A. R. Walsh, and T. Y. Shen, *Tetrahedron Lett.*, (1979) 291–294.
12 I. Azuma, K. Sugimura, T. Taniyama, M. Yamawaki, Y. Yamamura, S. Kusumoto, S. Okada, and T. Shiba, *Infect. Immun.*, 14 (1976) 18–27.
13 A. Hasegawa, H. Okumura, M. Kiso, I. Azuma, and Y. Yamamura, manuscript in preparation.
14 Y. Yamamura, I. Azuma, K. Sugimura, M. Yamawaki, M. Uemiya, S. Kusumoto, S. Okada, and T. Shiba, *Proc. Jpn. Acad.*, 53 (1977) 63–66.