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# SYNTHESIS OF 4-*O*-(*N*-ACETYL-β-D-GLUCOSAMINYL)-6-*O*-ACYL-*N*-ACETYLMURAMYL-L-ALANYL-D-ISOGLUTAMINE[1)]

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Summary: Long-chain acyl derivatives of the disaccharide dipeptide, which is a common building unit of bacterial cell walls, were synthesized in order to test their immunostimulating activity.

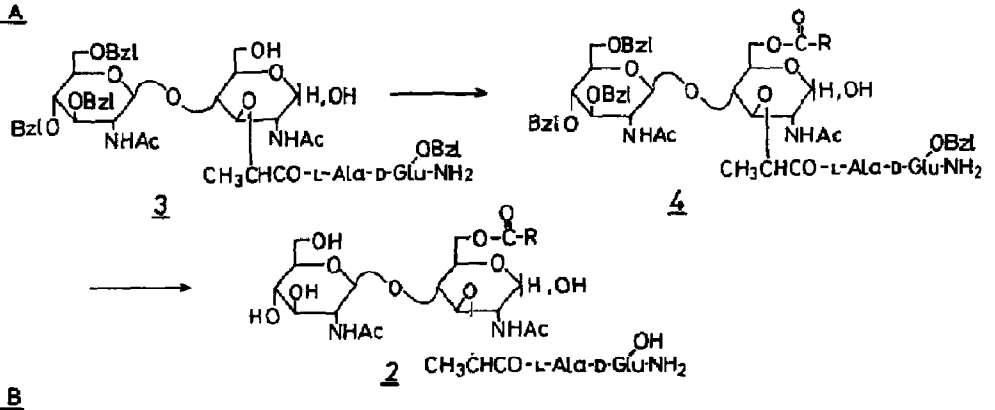
It was demonstrated that the unique immunopotentiating activities including the antitumor effect[2,3,4)] could be produced by addition of lipophilic character to the molecule of *N*-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP), which is the minimum effective structure required for the immunoadjuvant activity of bacterial cell walls.[5)] Thus, some of 6-*O*-acyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamines (1) suppressed tumor growth in mice though to a lesser extent than natural BCG cell wall.[2,3)] Meanwhile, we also showed that synthetic 4-*O*-(*N*-acetyl-β-D-glucosaminyl)-*N*-acetylmuramyl-L-alanyl-D-isoglutamine,[6)] which corresponds to a common but longer building unit in cell walls, has more potent adjuvant activity than MDP.[7)] It could be therefore expected that *O*-acyl derivatives of the disaccharide dipeptide would have stronger antitumor activity than 1. In this communication, synthesis of a series of 4-*O*-(*N*-acetyl-β-D-glucosaminyl)-6-*O*-acyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine (2) *via* two alternative pathways is described.

Acylation of a disaccharide dipeptide derivative (route A) was first examined. An intermediate in our previous synthesis,[6)] *i.e.*, 4-*O*-(*N*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucosaminyl)-*N*-acetylmuramyl-L-alanyl-D-isoglutamine

benzyl ester (3), could serve as a starting material for this purpose. Though 3 gave a rather complex mixture in the reaction with stearoyl chloride and pyridine, the selective acylation of its primary hydroxyl group could be accomplished in the following manner. Reaction of 3 with stearic acid and dicyclohexylcarbodiimide (DCC) (each 1.2 equivalent) in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP)[8] and 1-hydroxybenzotriazole (HOBt) in tetrahydrofuran (THF) afforded 4-*O*-(*N*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucosaminyl)-6-*O*-stearoyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine benzyl ester (4a) (58%, mp 203-205°C, $[\alpha]_{365}^{20}$ -28.7°)[9] after silica gel column chromatography. Hydrogenolytic deprotection of 4a in the presence of palladium black in THF-methanol gave the desired stearoyl disaccharide dipeptide (2a) (98%, mp 183-185°C dec, $[\alpha]_{365}^{20}$ -8.91°).[9,10] The corresponding 2-tetradecylhexadecanoyl and 3-hydroxy-2-docosylhexacosanoyl derivatives (2b and c) were also prepared in the same manner. However, the yields of the acylation products (4b and c, see Table 1) were very low. Rather long reaction period (96 hr at room temperature) with DCC might have caused the product to be dehydrated at isoglutamine residue in some extent.

Thus, another possibility of route B was next examined, where the disaccharide was first acylated and then coupled with the dipeptide moiety. Compound 5 [6] was converted with phenyldiazomethane into benzyl ester (6) which was then heated in 60% aqueous acetic acid (at 60°C for 70 min) to give 4-*O*-(*N*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucosaminyl)-*N*-acetylmuramic acid benzyl ester (7) (53%, mp 185-189°C, $[\alpha]_{365}^{20}$ +8.3°).[9] Acylation of 7 with 2-tetradecylhexadecanoic or 3-hydroxy-2-docosylhexacosanoic acid by the same method (DCC-DMAP-HOBt) mentioned above gave the corresponding 4-*O*-(*N*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucosaminyl)-6-*O*-acyl-*N*-acetylmuramic acid benzyl ester (8b or c).[9] After removal of the all benzyl groups in 8b,c by hydrogenolysis, the products (9b,c) without further purification were condensed respectively with L-alanyl-D-isoglutamine benzyl ester by means of the Eintopf procedure using *N*-hydroxysuccinimide and DCC in THF. The acyl disaccharide dipeptide benzyl esters (10b,c) were obtained as syrupy materials after silica gel column chromatography. These were hydrogenolyzed in THF-methanol to afford the free

Route A



Route B

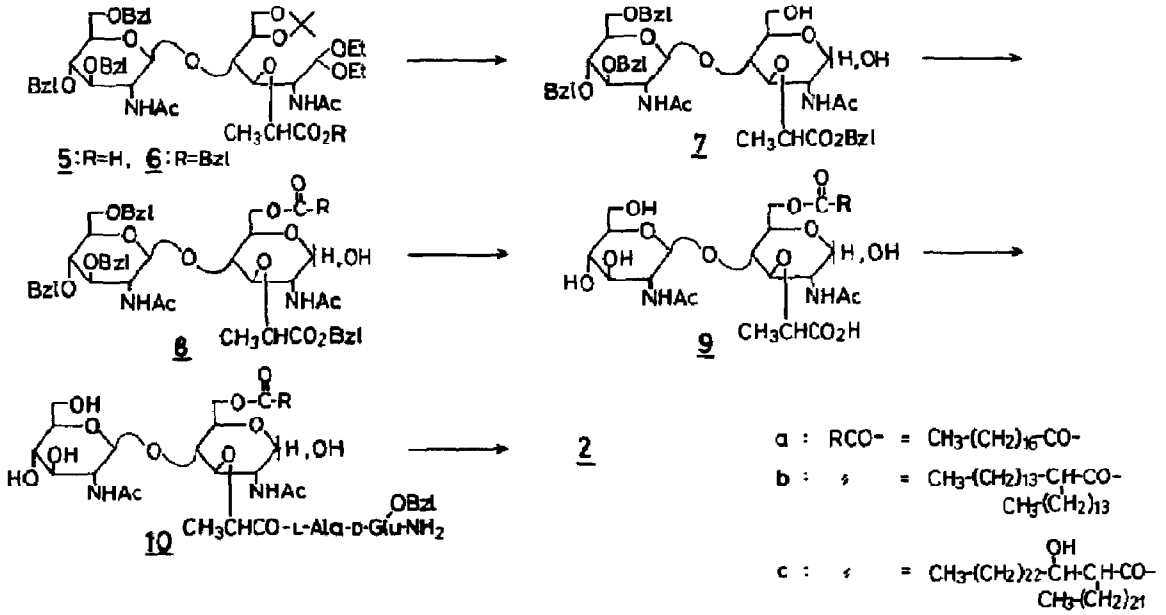


Table 1 Yields and physical constants of 4, 8 and 2

| | Yield (%) | $[\alpha]_{365}$ [a] | Mp (°C) | | Yield (%) | $[\alpha]_{365}$ [a] | Mp (°C) |
|---|---|---|---|---|---|---|---|
| 4b | 21 | -24.9° | 195 - 198 | 4c | 28 | -22.7° | 185 - 191 |
| 8b | 70 | +32.0° | 110 - 112 | 8c | 53 | +26.2° | 125 - 129 |
| 2b | 96 | - 7.6° | 190 - 194 [b] | 2c | 60 | - 4.0° | 155 - 165 [b] |

a) $c$ 0.5 in $CHCl_3$ - methanol (9:1).

b) see Ref. 10 in the text.

acyl disaccharide dipeptide (2b and c), which were identical with the corresponding compounds obtained above *via* route A. The yields and physical properties of the products as well as their intermediates are summarized in Table 1. In view of the yields and easiness of the purification of intermediates, route B seemed to be preferable than A, especially for the preparation of 2b and c with large acyl groups.

The results of the biological tests will be soon reported elsewhere.

## References and Footnotes


1) This work was presented at the 41th National Meeting of the Chemical Society of Japan, Osaka, April, 1980; cf. Abstract p.1112.

2) a) S. Kusumoto, S. Okada, T. Shiba, I. Azuma, and Y. Yamamura, *Tetrahedron Lett.*, 1976, 4287. b) T. Shiba, S. Okada, S. Kusumoto, I. Azuma, and Y. Yamamura, *Bull. Chem. Soc. Jpn.*, 51, 3307 (1978).

3) S. Kusumoto, M. Inage, T. Shiba, I. Azuma, and Y. Yamamura, *Tetrahedron Lett.*, 1978, 4899.

4) K. Tobe, H. Ogawa, T. Kusama, O. Nagase, N. Ozawa, M. Inage, S. Kusumoto, T. Shiba, I. Azuma, presented at the 100th Annual Meeting of the Pharmaceutical Society of Japan, April, 1980. *Infect. Immun.*, to be submitted.

5) a) F. Ellouz, A. Adam, R. Ciorbaru, and E. L. Lederer, *Biochem. Biophys. Res. Commun.*, 59, 1317 (1974). b) S. Kotani, Y. Watanabe, F. Kinoshita, T. Shimono, I. Morisaki, T. Shiba, S. Kusumoto, Y. Tarumi, and K. Ikenaka, *Biken J.*, 18, 105 (1975).

6) S. Kusumoto, K. Yamamoto, T. Shiba, *Tetrahedron Lett.*, 1978, 4407.

7) M. Tsujimoto, F. Kinoshita, T. Okunaga, S. Kotani, S. Kusumoto, K. Yamamoto, T. Shiba, *Microbiol. Immunol.*, 23, 933 (1979).

8) W. Steglich, G. Höfle, *Angew. Chem.*, 81, 1001 (1969).

9) Satisfactory elemental analysis was obtained.

10) This compound was obtained as colorless solid by lyophilization showing no definite melting point.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