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

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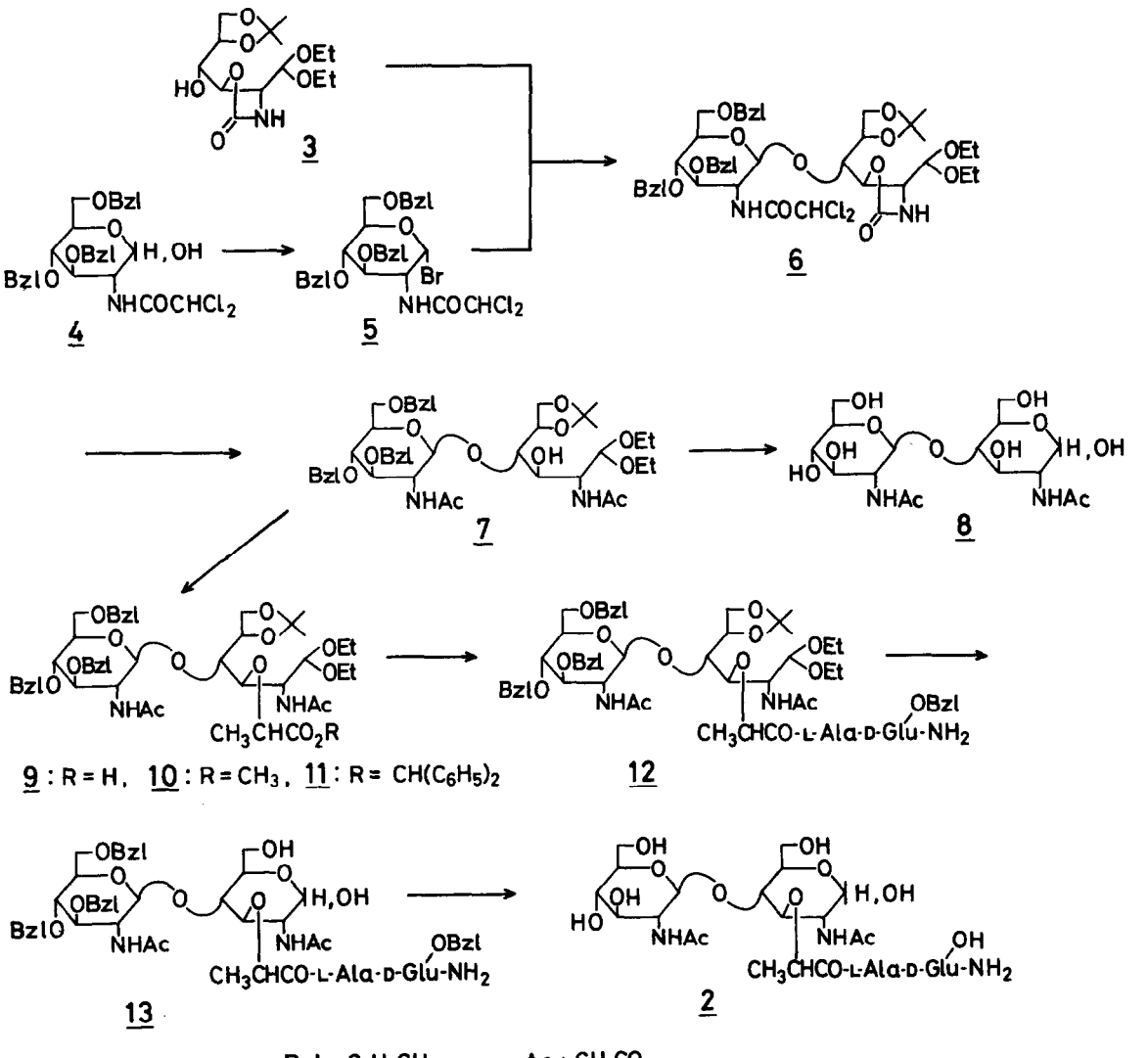
Our synthetic studies on muramyl peptides have significantly elucidated the relationship between the immunoadjuvant activity and the chemical structure.[2)] In these investigations, *N*-acetylmuramyl-L-alanyl-D-isoglutamine (1) as common structure of peptidoglycans in many bacterial cell walls had been revealed to be the most important and minimum moiety responsible for the adjuvant activity from the results of immunological tests of many structural analogs mainly in the peptide parts.[2,3)] On the other hand, little has been known about the role of glycan chain of the cell wall on the adjuvant activity so far. In this connection, we aimed to synthesize *N*-acetyl-β-D-glucosaminyl-(1-4)-*N*-acetyl-muramyl-L-alanyl-D-isoglutamine (2), which has never been synthesized before though it corresponds to a common but longer building unit than 1 in many bacterial peptidoglycans.

Mercer and Sinaÿ had already reported the synthesis of a derivative of the disaccharide portion of 2, *i.e.*, *N*,*O*-heptaacetyl-β-D-glucosaminyl-(1-4)-muramic acid methyl ester.[4)] Since their synthetic method seemed to be not applicable to our purpose for several reasons,[5)] a new route had to be exploited for the preparation of the disaccharide dipeptide (2). In our method, the molecule of 2 was constructed by the following steps. 1) Synthesis of a β(1-4) linked glucosamine disaccharide. 2) Transformation of the glucosamine residue on its reducing terminal into muramic acid. 3) Coupling of the muramic acid moiety of the disaccharide with the dipeptide and 4) the final deprotection.

A formation of the β(1-4) linkage between two glucosamine residues was performed by means of Koenigs-Knorr reaction of *N*-acylglucosaminyl bromide with an open chain form of protected glucosamine, *i.e.*, 2-*N*,3-*O*-carbonyl-5,6-*O*-isopropylidene-D-glucosamine diethyl acetal (3). Thus, 3,4,6-tri-*O*-benzyl-D-glucosamine[6)] was treated with pentachlorophenyl dichloroacetate[7)] to afford 3,4,6-tri-*O*-benzyl-*N*-dichloroacetyl-D-glucosamine (4) (82%, mp 217-219°C dec),[8,9)] which was then converted into the corresponding glucosaminyl bromide (5) *via* 1-*O*-*p*-nitrobenzoate (mp 173-174°C).[10)] The bromide (5) was dried *in vacuo* over KOH and P_2O_5 and used without purification for the coupling reaction

(stirring in dry benzene at room temperature for 24 hr in the presence of $Hg(CN)_2$) with 3 which was prepared according to Heins *et al.*[11] A disaccharide derivative (6) was obtained after purification by preparative TLC on silica gel ($CHCl_3$ - methanol, 40 : 1, twice developing) (33%, mp 52-57°C, $[\alpha]_D^{17}$ -11.0° (*c* 1, $CHCl_3$)).[8] Removal of the dichloroacetyl group and opening of the oxazolidone ring in 6 by alkaline hydrolysis (refluxing in 50% aqueous dioxane in the presence of $Ba(OH)_2$ for 16 hr) followed by acetylation of the newly formed amino groups (treating with acetic anhydride in pyridine and then with sodium methoxide in methanol) yielded *N*-acetyl-4-*O*-(*N*-acetyl-3,4,6-tri-*O*-benzyl-



β-D-glucosaminyl)-5,6-*O*-isopropylidene-D-glucosamine diethyl acetal (7) (89%, syrup). This compound possessing a free hydroxyl group only at C-3 position in the open chain moiety is the appropriate key intermediate for the conversion into a glucosaminyl muramic acid disaccharide.[12]

For the purpose of an unequivocal confirmation of β(1-4) linkage in 6 and 7, the all protecting groups except *N*-acetyl in 7 were removed (heating in 60% aqueous acetic acid followed by catalytic hydrogenolysis) to give *N*,*N*'-diacetylchitobiose (8) (mp 242-243°C dec, $[\alpha]_D^{22}$ +16.3° (*c* 0.4, H_2O)) which was identified with an authentic specimen of natural origin (mp 240-241°C dec, $[\alpha]_D^{22}$ +17.5° (*c* 0.4, H_2O) by means of NMR and TLC.

D-Lactic acid ether moiety was introduced to the 3-hydroxyl group on 7 by reaction with L-2-chloropropionic acid (3 equivalents) in dry dioxane in the presence of NaH (7 equivalents) at 60°C overnight.[13] The resultant β-D-glucosaminyl-(1-4)-muramic acid derivative (9) was treated with diazomethane or better with diphenyldiazomethane to be isolated as pure methyl ester (10) (25%, syrup)[14] or diphenylmethyl ester (11) (33%, mp 87-92°C, $[\alpha]_D^{18}$ +21.0° (*c* 0.4, $CHCl_3$)).[8] After alkaline hydrolysis of 10 (1N KOH in methanol), the regenerated glucosaminyl-(1-4)-muramic acid (9) was coupled with the dipeptide moiety, *i.e.*, L-alanyl-D-isoglutamine benzyl ester, by means of the conventional Eintopf procedure using *N*-hydroxysuccinimide and dicyclohexylcarbodiimide in THF. The protected disaccharide dipeptide (12), *i.e.*, *N*-acetyl-4-*O*-(*N*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucosaminyl)-5,6-*O*-isopropylidenemuramyl-L-alanyl-D-isoglutamine benzyl ester diethyl acetal, was obtained as colorless solid (59%, mp 55-65°C, $[\alpha]_D^{18}$ +9.5° (*c* 0.2, $CHCl_3$))[8] after silica gel column chromatography and precipitation from benzene - hexane. The NMR spectrum of 12 in $CDCl_3$ showed signals corresponding to the desired structure for both sugar and peptide moieties. The final deprotection was performed in two steps. On heating 12 in 60% aqueous acetic acid (at 60°C for 70 min), the ketal and the acetal groups were removed simultaneously to give 13 (57%, mp 187-194°C dec, $[\alpha]_D^{18}$ +8.5° (*c* 0.1, pyridine))[8] after silica gel column chromatography. Catalytic hydrogenolysis of 13 with palladium black in methanol afforded the final product (2) in a quantitative yield. Although it showed a single spot on silica gel TLC (butanol - acetic acid - water, 4:1:2) in this stage, it was further purified by reprecipitation from methanol - acetone (hygroscopic powder; mp 170-178°C dec; $[\alpha]_D^{20}$ +4.0° (*c* 1, H_2O, after 2 min), $[\alpha]_D^{24}$ +0.6° and $[\alpha]_{365}^{24}$ -21.7° (after equilibration)). The correct structure of this product was confirmed by NMR as well as elemental analysis. The disaccharide dipeptide (2) thus prepared exhibited rather high adjuvant activity compared to the muramyl dipeptide (1). Details on the biological activity of this compound will be reported elswhere.

References and Footnotes

1) This work was presented at the 26th International Congress of pure and

Applied Chemistry, Tokyo, September, 1977.
2) a) S. Kotani, Y. Watanabe, F. Kinoshita, T. Shimono, I. Morisaki, T. Shiba S. Kusumoto, Y. Tarumi, and K. Ikenaka, *Biken J.*, 18, 105 (1975). b) S. Kusumoto, Y. Tarumi, K. Ikenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 49, 553 (1976). c) S. Kotani, F. Kinoshita, Y. Watanabe, I. Morisaki, T. Shimono, K. Kato, T. Shiba, S. Kusumoto, K. Ikenaka, and Y. Tarumi, *Biken J.*, 20, 125 (1977) and references cited therein.
3) C. Merser, P. Sinaÿ, and A. Adam, *Biochem. Biophys. Res Commun.*, 66, 1316 (1975).
4) C. Mercer and P. Sinaÿ, *Tetrahedron Lett.*, 1973, 1029.
5) First of all, removal of *O*-acetyl groups inevitably leads to decomposition of the disaccharide, because alkaline conditions will cause β-elimination of lactic acid moiety in muramic acid part. On the other hand, a procedure of acetolysis must be employed for 1,6-anhydride-ring opening. Therefore, this synthetic approach *via* acetyl derivative suffers from the serious defect by this reason.
6) T. D. Inch and H. G. Fletcher Jr., *J. Org. Chem.*, 31, 1810 (1965).
7) M. Fujino and H. Hatanaka, *Chem. Pharm. Bull.*, 16, 929 (1968).
8) Satisfactory elemental analysis was obtained.
9) The dichloroacetyl group as *N*-protection was shown to be promissing in Koenigs-Knorr reaction: D. Shapiro, A. J. Acher, and E. S. Rachaman, *J. Org. Chem.*, 32, 3767 (1967). On the other hand, the corresponding *N*-acetyl derivative did not give a good result in the following bromination and glycosidation reaction in our preliminary experiment.
10) This indirect preparation of glucosyl bromide was described by M. Dejter-Juszysky and H. M. Flowers, *Carbohyd. Res.*, 18, 219 (1971). The procedure gave a good result even in the presence of benzyl groups.
11) K. Heins, K. Propp, R. Harrison, and H. Paulsen, *Chem. Ber.*, 100, 2655 (1967).
12) The glucosamine derivative (3) used above has not only the open chain structure with free 4-hydroxyl group favorable for (1-4)-glycoside formation,[4] but also very suitable protecting groups of the oxazolidone ring for our purpose of selective regeneration of the 3-hydroxyl group after glycoside formation.
13) Y. Matsushima and J. T. Park, *J. Org. Chem.*, 27, 3581 (1962).
14) This methyl ester (10) was deprotected by heating in hot aqueous acetic acid followed by hydrogenolysis. The product was treated with acetic anhydride in pyridine and then with $ZnCl_2$ in acetic anhydride to afford a peracetyl methyl ester (mp 231-233°C dec, $[\alpha]_D^{18.5}$ +43°). Comparison of the physical data of this product with those of peracetyl-β-D-glucosaminyl-(1-4)-muramic acid methyl ester reported in the literature[4] (natural: mp 235-236°C dec, $[\alpha]_D^{22}$ +40°; synthetic: mp 237-238°C dec, $[\alpha]_D^{20}$ +38°) clearly assured the desired structure of 10.

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