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Preliminary communication

A general, convenient synthesis of the repeating, disaccharide–dipeptide unit of the bacterial, cell-wall peptidoglycan by the oxazoline method*

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The basal, bacterial cell-wall peptidoglycans are usually made up of alternating β-(1,4)-linked pyranosides of 2-acetamido-2-deoxy-D-glucose (GlcNAc) and *N*-acetylmuramic acid joined to peptide. In 1974–1975, Adam *et al.*[2,3] and Kotani *et al.*[4] reported that the partial, monomeric structure, namely, *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP) is the minimum structure required for the immunoadjuvant activities. However, the recent study of Tsujimoto *et al.*[5] has shown that 2-acetamido-2-deoxy-β-D-glucosyl-(1→4)-*N*-acetylmuramoyl-L-alanyl-D-isoglutamine, one of the repeating disaccharide–dipeptide units, has immunoadjuvant activities higher than those of MDP. In view of this fact, as well as in continuation of our interest in elucidating the relationship between the structure of the carbohydrate moiety in and the activity of MDP analogs[6], we have sought a facile preparation of the repeating disaccharide–dipeptide units.

The use of oxazoline derivatives is a well established method for introducing β-linked GlcNAc units into synthetic oligosaccharides[7], and Warren and Jeanloz[8] recently reported that 1,2-dichloroethane is a better solvent than the conventional toluene–nitromethane for glycosylation by this method. Also very recently, it has been found that, when the couplings of allyl 2-acetamido-3,6-di-*O*-benzyl[9]- and -3,6-di-*O*-(2-butenyl)[10]-2-deoxy-β-D-glucopyranoside with 2-methyl-(4-*O*-acetyl-3,6-di-*O*-benzyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline[11] were conducted in 1,2-dichloroethane containing a 10–20 mmolar proportion of *p*-toluenesulfonic acid, the desired β-(1,4)-linked disaccharides could be isolated crystalline in 30–40% yield. In this communication, we describe a synthesis of the repeating disaccharide–dipeptide units of the bacterial cell-wall peptidoglycan, one being [β-MDP-(1→4)-GlcNAc] and the other [β-GlcNAc-(1→4)-MDP], by using the improved oxazoline procedure.

The most familiar oxazoline, namely, **1**, which was used in the present disaccharide synthesis as the glycosyl donor, was prepared by treatment of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose with anhydrous ferric chloride in dichloromethane[12]. As acceptors, we employed benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[13] (**2**) and benzyl 2-acetamido-6-*O*-benzyl-3-*O*-(2-butenyl)-α-D-glucopyranoside (**3**), m.p. 144–146°, $[\alpha]_D$ +100.3° (*c* 1, $CHCl_3$), which was readily prepared, stepwise, by crotylation of benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside[1], hydrolytic

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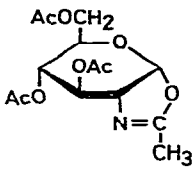
removal of the isopropylidene group, and selective benzylation of the primary hydroxyl group at C-6.



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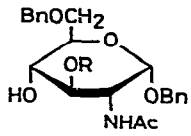

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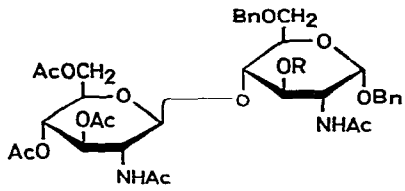
3 R=Cro



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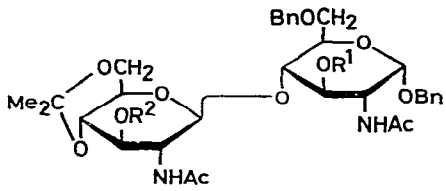
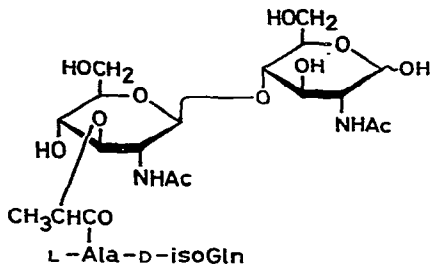


6 R^1=Bn, R^2=H
7 R^1=Cro, R^2=H
8 R^1=Bn, R^2=a
9 R^1=H, R^2=Bn
10 R^1=a, R^2=Bn
11 R^1=Bn, R^2=b
12 R^1=b, R^2=Bn

Bn = $PhCH_2$
Cro = crotyl = 2-butenyl
a = $CH(CH_3)CO_2H$
b = $CH(CH_3)CO$-L-Ala-D-isoGln-OBn

The glycosylation reaction was conducted by adding **1** (3 molar equiv.) in 1,2-dichloroethane solution to a mixture of acceptor **2** or **3** (1 molar equiv.), *p*-toluenesulfonic acid (~0.15 molar equiv.), and 1,2-dichloroethane; the final concentration of the acid was adjusted to 10–20 mM by adding 1,2-dichloroethane, and the mixture was stirred for 10–20 h at the reflux temperature, the reaction being monitored periodically by t.l.c. When the oxazoline had almost completely disappeared, the mixture was extracted with chloroform, and the extract was successively washed with 10% sodium carbonate and water, dried, and evaporated, to give a residue containing disaccharide, unreacted acceptor, and decomposition products derived from **1**. The disaccharide was isolated from the residue by chromatography on a column of silica gel using chloroform and 200:1 to 100:1 chloroform–methanol as eluants. The disaccharide fraction thus obtained crystallized from hot ethanol to give **4** (30.4%, based on **2**), m.p. 249–250°, $[\alpha]_D$ +63.5° (*c* 1, $CHCl_3$); or **5** (36.1%, based on **3**), m.p. 248–250°, $[\alpha]_D$ +41° (*c* 0.5, $CHCl_3$), as fine needles. Most of the unreacted acceptor could be recovered by rechromatography. Such yields may, therefore, be considered satisfactory (compared with those obtained by the Koenigs–Knorr reaction[14] and the Lemieux method[15]). The structures of **4** and **5** were confirmed by converting each of them into di-*N*-acetylchitobiose.

O-Deacetylation of **4** and **5**, and subsequent 4′,6′-*O*-isopropylidenation[16] gave **6**, m.p. 251–252°, $[\alpha]_D$ +60.4° (*c* 0.5, $CHCl_3$); and **7**, m.p. 244–245°, $[\alpha]_D$ +40.5° (*c* 0.4, $CHCl_3$), in high yields. Compound **6** was condensed with L-2-chloropropionic acid in the



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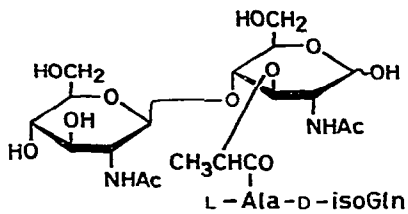

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presence of sodium hydride, to give **8** (91%), m.p. 232–233°, $[\alpha]_D$ +75° (*c* 0.4, $CHCl_3$). On the other hand, benzylation of **7** and removal of the 2-butenyl group with potassium *tert*-butoxide in dimethyl sulfoxide[17] afforded **9**, m.p. 204–206°, $[\alpha]_D$ +84.3° (*c* 0.4, $CHCl_3$), as needles. The yield from the decrotylation was ~80%, although the reaction was almost quantitative according to t.l.c. Compound **9** was condensed with L-2-chloropropionic acid as just described, to give **10** (60%), m.p. 136–138°, $[\alpha]_D$ +59.1° (*c* 0.5, $CHCl_3$). Couplings of **8** and **10** with L-alanyl-D-isoglutamine benzyl ester were conducted with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide as the activating agents, to afford the corresponding lactoyl-dipeptide derivatives: **11** (88%), m.p. 256–258° (dec.), $[\alpha]_D$ +49.4° (*c* 0.5, $CHCl_3$); and **12** (62%), m.p. 206–208° (dec.), $[\alpha]_D$ +40.5° (*c* 0.38, $CHCl_3$), respectively. Hydrolytic removal of the 4,6-*O*-isopropylidene group from **11** and **12** with 60% acetic acid, and subsequent hydrogenation in the presence of 10% palladium–carbon catalyst gave the desired disaccharide–dipeptide units: **13**, $[\alpha]_D$ +10.7° (*c* 1, H_2O; equil.); and **14**, $[\alpha]_D$ −2° (*c* 1, H_2O; equil.) {lit.[14] $[\alpha]_D^{24}$ +0.6° and $[\alpha]_{365}^{24}$ −21.7° (*c* 1, H_2O; equil.)}, as amorphous solids. All new compounds were characterized by i.r. and n.m.r. spectra, and had elemental compositions in satisfactory accord with theory.

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