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BACTERIAL CELL WALL CONSTITUENTS. II.[1] SYNTHESIS OF O-(N-ACETYL-β-MURAMYL-L-ALANYL-D-ISOGLUTAMINE)-(1→4)-N-ACETYL-D-GLUCOSAMINE

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SUMMARY

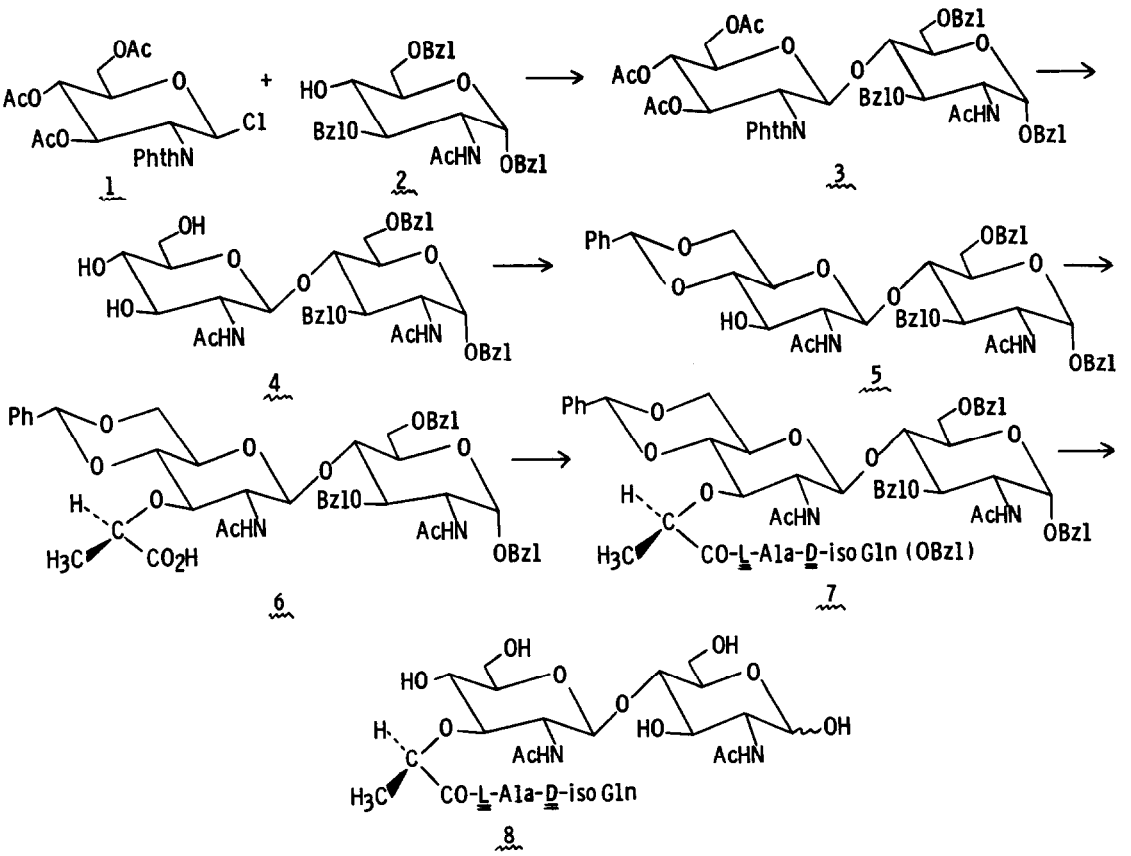
O-(N-Acetyl-β-muramyl-L-alanyl-D-isoglutamine)-(1→4)-N-acetyl-D-glucosamine (8), the repeating disaccharide dipeptide unit obtained by endo-N-acetylglucosaminidase lysis of bacterial cell walls, has been synthesized in a twelve-step sequence from N-acetyl-D-glucosamine.

The glycodipeptide moiety of the rigid polymeric bacterial cell wall peptidoglycan is composed of alternating units of β1→4 linked N-acetyl-D-glucosamine (GlcNAc) and N-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide-MDP), MDP comprising the minimum structure[2] of mycobacteria in Freund's complete adjuvant necessary for immuneadjuvant activity. Therefore, two disaccharide dipeptide structures can be released by enzymic cleavage of the glycosidic linkages in the peptidoglycan, one [MDP-(β1→4)-GlcNAc] having N-acetyl-D-glucosamine and the other [GlcNAc-(β1→4)-MDP] muramyl dipeptide at the reducing end of the β1→4 linked disaccharide. To assist in the elucidation of the minimal structural requirements for the arthritogenicity of peptidoglycans,[3] and, moreover, to provide compounds with potentially enhanced adjuvant properties over MDP, we have synthesized the two possible peptidoglycan isomers. In this communication we report the synthesis of MDP-(β1→4)-GlcNAc (8) in a twelve-step sequence from N-acetyl-D-glucosamine. Structure 8 represents the dipeptide derivative of the disaccharide [MurNAc-(β1→4)-GlcNAc] that is isolated from lysostaphin endo-N-acetylglucosaminidase digests of bacterial cell walls.[4] The synthesis of GlcNAc-(β1→4)-MDP will be described elsewhere.[5]

The strategy employed in the synthesis of 8 required the construction of an appropriately blocked β1→4 linked disaccharide having only the C-3'[6] hydroxyl unprotected and thereby available for conversion to the (R)-lactic acid ether 6. This key intermediate (5) was obtained by condensation of the two D-glucosamine derivatives 1 and 2 and further chemical manipulation. The hydroxyls in the glucosaminyl donor 1 were blocked "temporarily"[7] as acetates, whereas those in the glucosaminyl acceptor 2 (free C-4 hydroxyl) "persistently"[7] as benzyl ethers and benzyl glycoside. The MurNAc-(β1→4)-GlcNAc derivative 6 was then prepared and coupled

with the requisite blocked dipeptide. Final deprotection of the resulting fully blocked disaccharide dipeptide 7 afforded desired 8. Protective groups had been chosen so that they could all be removed in a single hydrogenolytic step.

The synthesis of the blocked chitobiose derivative 3 was accomplished following the method developed by Lemieux and coworkers[8] for the preparation of 2-amino-2-deoxy-β-glycopyranosides using 2-deoxy-2-phthalimidoglycosyl halides. Thus, reaction of 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride (1)[9] with benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (2)[10] in nitromethane in the presence of the silver triflate-collidine complex gave after column chromatography compound 3[11] in 40% yield; $[\alpha]_D^{25}$ + 57° (c 1, $CHCl_3$); n.m.r. (300-MHz, $CDCl_3$): δ 1.74, 1.83, 1.94, and 1.99 (3-proton singlets, 3 OAc and 1 NHAc), 4.86 (d, $J_{1,2}$ 3.8 Hz, H-1), 5.15 (t, $J_{3',4'}$ 9.2 Hz, H-4'), 5.51 (d, $J_{1',2'}$ 8.5 Hz, H-1'), and 5.74 (dd, $J_{2',3'}$ 10.8 Hz, H-3'). De-O-acetylation and de-N-phthalylation of 3



Bzl = benzyl, Phth = phthaloyl, Ph = phenyl

was performed with n-butylamine in refluxing methanol (48h); subsequent N-acetylation with acetic anhydride in methanol afforded compound 4 (69% yield based on 3) having the C-3', C-4', and C-6' hydroxyls free; m.p. 252-256° (dec.) (ethanol), $[\alpha]_D^{25}$ + 72° (c 1.2, MeOH); n.m.r. (300-MHz, CD_3OD): δ1.94 and 1.97 (3-proton singlets, 2 NHAc), 4.82 (d, $J_{1,2}$ 3.9 Hz, H-1), and 5.06 (d, $J_{1',2'}$ 10.8 Hz, H-1'). The presence of a β(1→4) linkage in 4 was confirmed by catalytic hydrogenolysis (Pd in AcOH) of the benzyl ether and benzyl glycoside protective groups to give di-N-acetylchitobiose identical in all respects (m.p., $[\alpha]_D$, 300-MHz n.m.r., t.l.c. mobility) with an authentic sample obtained from chitin.[12] Blocking of the C-4' and C-6' hydroxyls in 4 as a benzylidene acetal (benzaldehyde-$ZnCl_2$) provided the required intermediate 5 with only the C-3' hydroxyl unprotected; yield 90%, m.p. 298-302° (dec.) (methanol), $[\alpha]_D^{25}$ + 58° (c 1.1, DMF); n.m.r. (300-MHz, $CDCl_3$): δ 1.77 and 1.82 (3-proton singlets, 2 NHAc), 4.93 (d, $J_{1,2}$ 3.8 Hz, H-1), and 5.47 (s, benzylic H). The (R)-lactic acid ether 6 was then obtained as an amorphous solid in 91% yield by alkylation[13] of 5 with (S)-2-chloropropionic acid in dioxane in the presence of sodium hydride; $[\alpha]_D^{25}$ + 45° (c 1, MeOH), n.m.r. (300-MHz, DMSO-d_6): δ 1.12 [d, CH_3 (lac)], 1.82 (6-proton singlet, 2 NHAc), and 5.60 (s, benzylic H). The dipeptide was next introduced by the mixed anhydride method (reaction of acid 6 with L-alanyl-D-isoglutamine benzyl ester hydrochloride[14] in DMF in the presence of N-methylmorpholine and isobutyl chloroformate) to afford the fully blocked disaccharide dipeptide 7 in 93% yield; m.p. 295-300° (dec.), $[\alpha]_D^{25}$ + 59° (c 0.54, DMF); n.m.r. (300-MHz, DMSO-d_6): δ 1.19 and 1.23 [2d, CH_3 (ala) and CH_3 (lac)], 1.80 and 1.84 (3-proton singlets, 2 NHAc), 2.34 (t, $-CH_2CO_2Bzl$), 5.07 (s, $-CO_2CH_2Ph$), and 5.66 (s, benzylic H). In the final step of the sequence, the benzyl ester, benzyl ether, benzyl glycoside, and benzylidene acetal protective groups were removed by hydrogenolysis of 7 in glacial acetic acid (Pd, 24 h, atmospheric pressure). Chromatographically homogeneous (silica gel t.l.c., 60:40:10 $CHCl_3$-MeOH-H_2O) O-(N-acetyl-β-muramyl-L-alanyl-D-isoglutamine)-(1→4)-N-acetyl-D-glucosamine (8) was isolated (precipitation by addition of diethyl ether to a methanol-ethanol solution) in 95% yield as an amorphous solid; n.m.r. (300-MHz, D_2O): δ 1.38 and 1.45 [2d, CH_3 (ala) and CH_3 (lac)], 1.99 and 2.04 (3-proton singlets, 2 NHAc), 2.04 (m, $-CH_2CH_2CO_2H$), and 2.29 (t, $-CH_2CO_2H$).

Compound 8 administered as an aqueous solution in mice significantly increased the level of antibodies against bovine serum albumin.[15] Details of its immuneadjuvant properties will be reported elsewhere.

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