Carbohydrate Research, 77 (1979) C1–C4

Preliminary communication

Synthesis of *O*-(2-acetamido-2-deoxy-β-D-glucosyl)-(1→4)-*N*-acetylmuramoyl-L-alanyl-D-isoglutamine, the repeating disaccharide-dipeptide unit of the bacterial cell-wall peptidoglycan*

PHILIPPE L. DURETTE†, ERIC P. MEITZNER, and T. Y. SHEN

Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065 (U.S.A.)

(Received August 2nd, 1979; accepted for publication, August 27th, 1979)

As the glycodipeptide moiety of the rigid, polymeric, bacterial cell-wall peptidoglycan is composed of alternating units of β-(1→4)-linked 2-acetamido-2-deoxy-D-glucose (GlcNAc) and *N*-acetylmuramoyl-L-alanyl-D-isoglutamine ("muramoyl dipeptide", MDP**), two disaccharide dipeptide structures can be released by enzymic cleavage of the glycosidic linkages in the peptidoglycan, one [namely, β-MDP-(1→4)-GlcNAc] having a 2-acetamido-2-deoxy-D-glucose residue, and the other [β-GlcNAc-(1→4)-MDP], a muramoyl dipeptide residue at the reducing end of the β-(1→4)-linked disaccharide. To assist in the identification of the minimal, structural components required for the arthritogenic property of the peptidoglycan molecule***, and, moreover, to provide compounds having adjuvant activity potentially enhanced over that of MDP, we have synthesized both possible peptidoglycan isomers. We had previously described[1] the preparation of β-MDP-(1→4)-GlcNAc. The present work reports the synthesis of β-GlcNAc-(1→4)-MDP (**10**) in a fourteen-step sequence from 2-acetamido-2-deoxy-D-glucose. Structure **10** represents the dipeptide derivative of the disaccharide [β-GlcNAc-(1→4)-MurNAc] that is isolated from lysozyme (an *N*-acetylmuramidase) digests of bacterial cell-walls[4].

The strategy devised for the synthesis of **10** involved the construction of an appropriately protected, β-(1→4)-linked disaccharide having only the 3-hydroxyl group†† at the reducing end unprotected and available for conversion into the (*R*)-lactic acid ether **8**. This intermediate (**7**) was prepared *via* condensation of the 2-amino-2-deoxy-D-glucosyl donor **1** (hydroxyl groups protected "temporarily"[5] as their acetates) with the 2-amino-2-deoxy-D-glucosyl acceptor **2** (having a free 4-hydroxyl group) in which the 1- and 6-

*Bacterial Cell-Wall Constituents, Part III. For Part II, see ref. 1.

†To whom enquiries should be addressed.

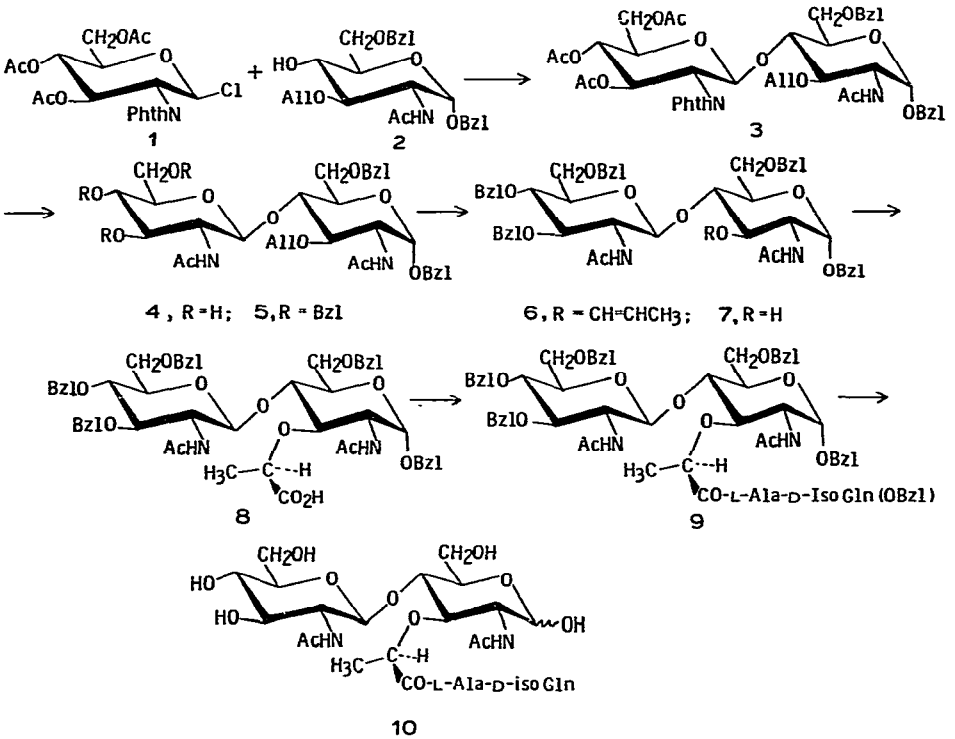
**Muramoyl dipeptide (MDP) has been identified[2] as the minimum structure of mycobacteria, in Freund's complete adjuvant, necessary for immuneadjuvant activity.

***Although adjuvanticity and arthritogenicity are inherent in the peptidoglycan molecule, these two properties appear to depend on different, minimal, structural requirements; for a discussion, see ref. 3, and references cited therein.

††The ring positions of the pyranosyl group (at the nonreducing end) of the disaccharide are designated with primed numbers.

hydroxyl groups were protected "persistently"[5] as a benzyl glycoside and benzyl ether, respectively, and the 3-hydroxyl group temporarily as its allyl ether. The β-(1→4)-linked disaccharide derivative **3** obtained was modified to give compound **5**, in which the 3′-, 4′-, and 6′-hydroxyl groups were protected persistently as benzyl ethers. The allyl group in **5** was then removed, to afford intermediate **7**, which was converted into the β-GlcNAc-(1→4)-MurNAc derivative **8**. Coupling of **8** with the requisite, protected dipeptide, and subsequent deprotection of the resulting, completely protected disaccharide-dipeptide **9** afforded the desired **10***. Protecting groups had been closen so that they could all be removed in a single, hydrogenolytic step.

The synthesis of the protected chitobiose derivative **3** was achieved by following the method of Lemieux and co-workers[7] for the preparation of 2-amino-2-deoxy-β-aldopyranosides using 2-deoxy-2-phthalimidoglycosyl halides. Thus, reaction of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride[8] (**1**) with benzyl 2-acetamido-3-



All = allyl, Bzl = benzyl, Ph = phenyl, and Phth = phthaloyl.

*During the course of this work, an alternative synthesis (16 steps) of **10**, in which the β-(1→4)-linkage between the two 2-amino-2-deoxy-D-glucose residues was formed by means of a Koenigs–Knorr reaction of a 2-deoxy-2-(dichloroacetyl)amino-D-glucosyl bromide with an acyclic form of a protected 2-amino-2-deoxy-D-glucose derivative, was reported[6].

O-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside* (2) in nitromethane in the presence of the silver triflate-*s*-collidine complex gave, after h.p.l.c.†, compound 3** in 38% yiled, $[\alpha]_D^{25}$ +50° (*c* 1.2, $CHCl_3$); n.m.r. (300-MHz, $CDCl_3$): δ 1.84, 1.93, 2.02, and 2.06 (3-proton singlets, 3 OAc and 1 NHAc), 4.85 (d, $J_{1,2}$ 3.8 Hz, H-1), 5.12 [d, =CH(*c*), $J_{2,3\,(allyl)}$ 10.5 Hz], 5.16 (t, $J_{3',4'} = J_{4',5'}$ = 9.4 Hz, H-4'), 5.27 [d, =CH(*t*), $J_{2,3\,(allyl)}$ 17 Hz], 5.48 (d, N*H*Ac), 5.53 (d, $J_{1',2'}$ 8.8 Hz, H-1'), 5.74 (t, H-3'), and 5.83 (m, $OCH_2CH{=}CH_2$). *O*-Deacetylation and *N*-dephthaloylation of **3** was performed with butylamine in refluxing methanol during 48 h, and subsequent *N*-acetylation with acetic anhydride in methanol afforded compound **4** (68% yield, based on **3**) having the 3'-, 4'-, and 6'-hydroxyl groups free; m.p. 268–270° (dec.) (from methanol–diethyl ether), $[\alpha]_D^{25}$ +73° (*c* 1.2, MeOH); n.m.r. (300-MHz, CD_3OD): δ 1.94 and 1.95 (3-proton singlets, 2 NHAc), 4.80 (d, H-1), 5.14 [d, =CH(*c*)], 5.27 [d, =CH(*t*)], and 5.94 (m, $OCH_2CH{=}CH_2$). Benzylation of **4** (with α-bromotoluene, BaO, and $Ba(OH)_2 \cdot 8H_2O$ in $HCONMe_2$ at r.t.) gave the 3',4',6'-tribenzyl ether **5** in 69% yield, m.p. 214–218° (dec.) (darkening at 200–214°) (from methanol), $[\alpha]_D^{25}$ +58° (*c* 1.1, $CHCl_3$); n.m.r. (300-MHz, $CDCl_3$): δ 1.68 and 1.94 (3-proton singlets, 2 NHAc) and 5.02 (d, $J_{1,2}$ 3.7 Hz, H-1). The 3-*O*-allyl group in **5** was selectively cleaved, to afford the alcohol **7**, by catalytic isomerization[10] [$(PPh_3)_3RhCl$, DABCO, 7:3:1 ethanol–toluene–water under reflux] to the 1-propenyl ether **6**, and subsequent hydrolysis ($HgCl_2$ in[11] oxolane–H_2O); yield 64% (based on **5**), m.p. 220.5–221.5° (from methanol), $[\alpha]_D^{25}$ +74° (*c* 1.5, $CHCl_3$). The presence of a β-(1→4)-linkage in **7** was confirmed by catalytic hydrogenolysis (Pd in AcOH) of the benzyl ether and benzyl glycoside protecting groups, to give di-*N*-acetyl-chitobiose, identical in all respects (m.p., $[\alpha]_D$, 300-MHz n.m.r. spectrum, and t.l.c. mobility) with an authentic sample obtained from chitin[12]. Alkylation of **7** with (*S*)-2-chloropropionic acid in 1,4-dioxane in the presence of sodium hydride provided the (*R*)-lactic acid ether **8** in 86% yield, m.p. 125–129°, $[\alpha]_D^{25}$ +43° (*c* 1.2, $CHCl_3$); n.m.r. (300-MHz, Me_2SO-d_6): δ 1.22 [d, CH_3 (lac)], 1.81 and 1.83 (3-proton singlets, 2 NHAc), and 5.20 (d, H-1). Introduction of the dipeptide was performed by the mixed anhydride method (reaction of acid **8** with L-alanyl-D-isoglutamine benzyl ester hydrochloride[13] in $HCONMe_2$ in the presence of 4-methylmorpholine and isobutyl chloroformate); the fully protected disaccharide-dipeptide **9** was obtained in 84% yield, m.p. 214–216° (dec.), $[\alpha]_D^{25}$ +35° (*c* 1, $CHCl_3$); n.m.r. (300-MHz, Me_2SO-d_6): δ 1.15 and 1.26 [2 d, CH_3 (ala) and CH_3 (lac)], 1.80 (6-proton singlet, 2 NHAc), 1.80 and 2.00 (2 m, $-CH_2CH_2CO_2Bzl$), 2.35 (t, $-CH_2CO_2Bzl$), 4.94 (d, H-1), and 5.08 (s, $-CO_2CH_2Ph$). The benzyl ester, benzyl ether, and benzyl glycoside protecting groups in **9** were removed by hydrogenolysis in glacial acetic acid (Pd, 48 h, atmospheric pressure). Chromatographically homogeneous (t.l.c. on silica gel, 6:4:1 $CHCl_3$–MeOH–H_2O) *O*-(2-acetamido-2-deoxy-β-D-glucosyl)-(1→4)-*N*-acetyl-

*This compound was prepared by modification of the procedure[9] of Jacquinet and Sinaÿ. Separation of **2** from the 4,6-dibenzylated coproduct was readily achieved by preparative h.p.l.c. on silica gel, using 5:1 chloroform–ethyl acetate as eluant.

†H.p.l.c. was performed on dual Prep-PAK™ 500 silica columns using a Waters Associates Prep LC/System 500 with 9:1 dichloromethane–diethyl ether as eluant.

**All compounds gave microanalyses, and exhibited n.m.r.- and mass-spectral characteristics, in agreement with their structures.

muramoyl-L-alanyl-D-isoglutamine (**10**) was isolated (precipitation by addition of diethyl ether to a methanolic solution) in 96% yield as an amorphous solid; n.m.r. (300-MHz, D_2O): δ 1.40 and 1.45 [2 d, CH_3 (ala) and CH_3 (lac)], 1.97 and 2.05 (3-proton singlets, 2 NHAc), and 2.34 (t, -CH_2CO_2H).

Compound **10** administered to mice as an aqueous solution enhanced antibody titers against bovine serum albumin[14].

ACKNOWLEDGMENTS

The authors thank Mr. Herman Flynn for n.m.r.-spectral measurements, and Mr. Jack Gilbert and his associates for microanalyses.

REFERENCES

1 P. L. Durette, E. P. Meitzner, and T. Y. Shen, *Tetrahedron Lett.*, (1979) 4013–4016.
2 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; S. Kotani, Y. Watanabe, F. Kinoshita, T. Shimono, I. Morisaki, T. Shiba, S. Kusumoto, Y. Tarumi, and K. Ikenaka, *Biken J.*, 18 (1975) 105–111.
3 T. Koga, K. Maeda, K. Onoue, K. Kato, and S. Kotani, *Mol. Immunol.*, 16 (1979) 153–162.
4 N. Sharon, *Complex Carbohydrates, Their Chemistry, Biosynthesis, and Function*, Addison–Wesley, London, 1975, pp. 393–417.
5 P.J. Pfäffli, S. H. Hixson, and L. Anderson, *Carbohydr. Res.*, 23 (1972) 195–206.
6 S. Kusumoto, K. Yamamoto, and T. Shiba, *Tetrahedron Lett.*, (1978) 4407–4410.
7 R. U. Lemieux, T. Takeda, and B. Y. Chung, *Am. Chem. Soc. Symp. Ser.*, 39 (1976) 90–115.
8 S. Akiya and T. Osawa, *Chem. Pharm. Bull.*, 8 (1960) 583–587.
9 J.-C. Jacquinet and P. Sinaÿ, *J. Org. Chem.*, 42 (1977) 720–724.
10 E. J. Corey and J. W. Suggs, *J. Org. Chem.*, 38 (1973) 3224; P. A. Gent and R. Gigg, *Chem. Commun.*, (1974) 277–278.
11 R. Gigg and C. D. Warren, *J. Chem. Soc., C*, (1968) 1903–1911.
12 M. Shaban and R. W. Jeanloz, *Carbohydr. Res.*, 19 (1971) 311–318.
13 S. Kusumoto, Y. Tarumi, K. Ikenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 49 (1976) 533–539.
14 A. Friedman, to be published.