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Note

A facile two-step synthesis of *N*-acetylmuramic acid by selective functionalization of HO-3 of 2-acetamido-2-deoxy-D-glucose*

HANS MERTEN AND REINHARD BROSSMER†
Institut für Biochemie II, Universität Heidelberg, Im Neuenheimer Feld 328, D-6900 Heidelberg (F.R.G.)

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N-Acetylmuramic acid (MurNAc) has been used as a starting material for the preparation of *N*-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP) and derivatives thereof. The syntheses of MurNAc[1–3] and of protected compounds[4,5] for the preparation of MDP analogues involve laborious multi-stage procedures. 2-Methyl-(1,2-dideoxy-5,6-*O*-isopropylidene-α-D-glucofurano-[2,1-*d*])-2-oxazoline[6] (**1**), which can be used for a convenient synthesis of *N*-acetylmuramic acid, is accessible from 2-acetamido-2-deoxy-D-glucose (GlcNAc) in one step and the oxazoline moiety contains the NAc group of MurNAc in a masked form. Moreover, the lactate side-chain can be introduced selectively at HO-3 without further protection. After treatment of **1** with sodium hydride, a nucleophilic substitution reaction was effected with (*S*)-2-chloropropionic acid[7]. The major portion of the products could be isolated after acidification and *N*-acetylmuramic acid (**2**) was then obtained by mild hydrolysis in aqueous acetic acid. The ^{1}H-n.m.r. spectrum [$(CD_3)_2SO$] of the crystalline α-anomer showed a broad signal (δ 12–14) for COOH. The c.d. spectrum of MurNAc confirmed previous results[8] and indicated the (*R*) configuration of the carboxyethyl moiety.

After the completion of this work, the racemization of (*S*)-2-chloropropionic acid in the presence of NaH was reported[5,9], yielding the (*S*)-1-carboxyethyl diastereomer of **2** (isomuramic acid). We did not observe any racemization, and, in another communication, formation of only a small amount of racemization product (5%) was reported[10]. The use of ethyl (2*S*)-2-(trifluoromethylsulfonyloxy)-propionate[9] might be advantageous in preventing racemization.

Following the reaction of **1** with (*S*)-2-chloropropionic acid, control of the work-up conditions and subsequent treatment with diazomethane gave a 6:1

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†Author for correspondence.

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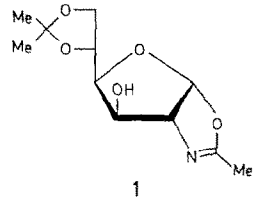

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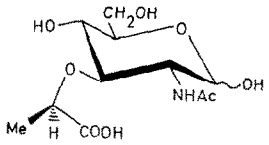

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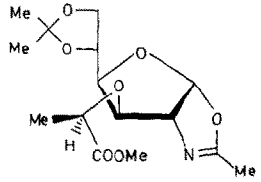

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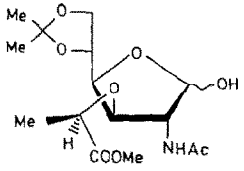

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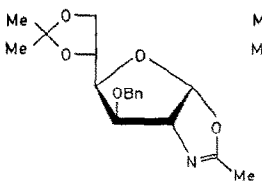
mixture of the oxazoline **3** and the furanose derivative **4** which could be resolved by column chromatography. Mild acid hydrolysis opened the oxazoline ring of **3** to afford $\alpha\beta$-**4**. The analytical data for **3** and **4** confirmed the introduction of a lactate residue, the presence of the oxazoline structure in **3**, and the structure of **4**. Thus, the use of **1** reduces the synthesis of MurNAc to a two-step process.

The usefulness of **1** for the functionalization of HO-3 of GlcNAc was demonstrated by benzylation. Treatment of **1** with benzyl bromide in the presence of NaH gave the 3-*O*-benzyl derivative **5**. Mild acid hydrolysis converted **5** into the $\alpha\beta$-furanose derivative **6**. More drastic hydrolysis of **5** yielded 2-acetamido-3-*O*-benzyl-2-deoxy-α,β-D, glucopyranose[11,12] (**7**), obtained hitherto only by a laborious process.



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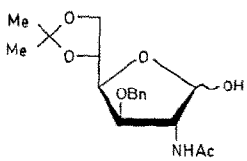

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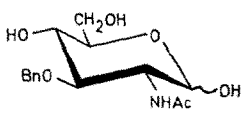

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EXPERIMENTAL

General methods. — Melting points were measured with a Bock-Monoscop and are corrected. T.l.c. was performed on Silica Gel 60 F_{254} (Merck) and column chromatography on Silica Gel 60 (Merck, 63–200 μm). Optical rotations were

determined with a Perkin–Elmer 241 polarimeter. A Perkin–Elmer 297 Spectrophotometer was used for measurement of i.r. spectra. ^{1}H-N.m.r. spectra were recorded with a Bruker WM-300 (300 MHz), WM 250 (250 MHz), or Varian EM 390 (90 MHz) spectrometer. The pH values were determined with a Metrohm Herisau Präzisions-pH-Meter E 510. Hexane, 1,4-dioxane, *N,N*-dimethylformamide, and ether were dried before use.

2-Acetamido-3-O-[(R)-1-carboxyethyl]-2-deoxy-α-D-glucopyranose (MurNAc) (**2**). — A 50% dispersion of sodium hydride in mineral oil (4.8 g, 100 mmol) was washed with hexane, the NaH was dispersed in 1,4-dioxane (30 mL), and a solution of **1** (1.29 g, 5.30 mmol) in 1,4-dioxane (100 mL) was added quickly. The mixture was stirred at 80–90° for 30 min, then at 65°. (*S*)-2-Chloropropionic acid (2 mL, 2.3 g; 21.2 mmol), $[\alpha]_D^{21}$ −16° (liquid, 10 cm) {lit.[7] $[\alpha]_D^{25}$ −16.7° (liquid, 10 cm)}, was added, followed by 1,4-dioxane (50 mL) to suppress initially the briskly reacting solution. After ~5 h at 65°, the mixture was cooled to room temperature and stirred overnight. The reaction was monitored by t.l.c. (6:1:1 1-propanol–aqueous 25% ammonia–water) and, when complete, the mixture was cooled until the 1,4-dioxane began to crystallise. The pH was adjusted to 8 by the addition of 2M HCl (~40 mL). Subsequently, most of the solvent was evaporated, and chloroform (250 mL) and water (100 mL) were added. The resulting solution was cooled to 0°, the pH of the aqueous layer was adjusted to 3 by the addition of 2M HCl (~7 mL), the chloroform layer was separated as rapidly as possible, and the aqueous layer was extracted with $CHCl_3$ (5 × 100 mL). The combined organic layers were washed quickly with water (100 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated. A solution of the syrupy residue in aqueous 80% acetic acid (100 mL) was stirred overnight, then concentrated, and water and toluene were distilled thrice from the residue, an aqueous solution of which was lyophilized to afford *αβ*-**2** (1.13 g, 73%). Crystallization from methanol–ethyl acetate gave *α*-**2** as the methanolate[3], m.p. 124–127°, $[\alpha]_D^{20}$ +57° (10 min)→+40° (final value; *c* 1.6, water); ν_{max}^{KBr} 3300 (OH, broad), 1710 (C=O), 1640 (Amide I), 1540 cm^{-1} (Amide II); lit.[3] m.p. 126–128°, $[\alpha]_D$ +48.3° (4 h) (*c* 0.3, water). ^{1}H-N.m.r. data (300 MHz, D_2O) for *αβ*-**2**: *δ* 5.29 (d, 0.8 H, $J_{1,2}$ 3.5 Hz, H-1*α*), 4.7 (H-1*β*, partly covered by HDO), 4.45 (q, 0.8 H, J_{CH,CH_3} 7.1 Hz, $CH_3CH\alpha$), 4.36 (q, 0.2 H, J_{CH,CH_3} 7.1 Hz, $CH_3CH\beta$), 3.87–3.51 (m, 6 H, H-2,3,4,5,6,6′*αβ*), 2.02 (s, 2.4 H, NAc*α*), 2.00 (s, 0.6 H, NAc*β*), 1.41 (d, 2.4 H, $CH_3CH\alpha$), 1.40 (d, 0.6 H, $CH_3CH\beta$); *αβ*-ratio, 4:1; {250 MHz, $[(CD_3)_2SO]$} *α*-**2**: *δ* 14–12 (bs, 1 H, COOH), 5.2 (bs, 1 H, H-1).

Anal. Calc. for $C_{11}H_{19}NO_8 \cdot CH_3OH$ (325.31): C, 44.30; H, 7.13; N, 4.31. Found: C, 4.18; H, 7.31; N, 4.22.

2-Methyl-(1,2-dideoxy-5,6-O-isopropylidene-3-O-[(R)-1-(methoxycarbonyl)-ethyl]-α-D-glucofurano)-[2,1-d]-2-oxazoline (**3**). — As described for the preparation of **2**, a solution of **1** (3.27 g, 13.4 mmol) in 1,4-dioxane (100 mL) was added to a suspension of NaH (12.4 g, 258.3 mmol) in 1,4-dioxane (100 mL). After 30 min at 80–90°, the temperature was lowered to 65° and (*S*)-2-chloropropionic acid (3.8 mL, 4.4 g; 40.3 mmol) was added dropwise, followed quickly by 1,4-dioxane (50

mL). The chloroform layer was dried (Na_2SO_4) and concentrated, and a solution of the syrupy residue in ether (100 mL) was treated with ethereal diazomethane until the mixture remained yellow. After evaporation of the solvent, column chromatography (ethyl acetate) of the residue, using silica gel which had been treated with a few drops of triethylamine, gave **3** (2.21 g, 50%), isolated as a syrup, $[\alpha]_D^{20}$ +17° (*c* 1, chloroform); ν_{max}^{film} 2930 (CH), 1730 (C=O), 1650 cm^{-1} (N=C). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ 6.13 (d, 1 H, $J_{1,2}$ 5.3 Hz, H-1), 4.76 (dq, 1 H, $J_{2,N=CCH_3}$ 1.8 Hz, H-2), 4.31 (q, 1 H, J_{CH,CH_3} 7.1 Hz, C*H*CH_3), 4.25 (ddd, 1 H, $J_{4,5}$ 8.4, $J_{5,6}$ 6.2, $J_{5,6'}$ 5.3 Hz, H-5), 4.12 (dd, 1 H, $J_{6,6'}$ 8.8 Hz, H-6), 4.01 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-3), 3.99 (dd, 1 H, H-6'), 3.78 (s, 3 H, COOMe), 3.71 (dd, 1 H, H-4), 2.02 (d, 3 H, $N=CCH_3$), 1.43 (d, 3 H, C*H*$_3$CH), 1.39 (s, 3 H, CH_3), 1.35 (s, 3 H, CH_3).

Anal. Calc. for $C_{15}H_{23}NO_7$ (329.34): C, 54.70; H, 7.04; N, 4.25. Found: C, 53.98; H, 7.30; N, 4.21.

2-Acetamido-2-deoxy-5,6-O-isopropylidene-3-O-[(R)-1-(methoxycarbonyl)-ethyl]-D-glucofuranose (**4**). — (*a*) Obtained (360 mg, 8%) as a side product of the preceding synthesis of **3**.

(*b*) A solution of **3** in methanol (20 mL), water (10 mL), and glacial acetic acid (0.1 mL) was stirred for 24 h at room temperature and then concentrated, and toluene was distilled four times from the residue. Column chromatography (ethyl acetate) then afforded $\alpha\beta$-**4** (970 mg, 82%), m.p. 85–87°, $[\alpha]_D^{20}$ +41° (15 min)→+39° (final value; *c* 0.5, methanol); ν_{max}^{KBr} 3260 (OH, NH), 2950 (CH), 1730 (C=O), 1610 (amide I), 1560 cm^{-1} (amide II). ^{1}H-N.m.r. data {300 MHz, [$(CD_3)_2SO$]}: δ 7.97 (d, 0.15 H, $J_{NH,2}$ 7.1 Hz, NHβ), 7.63 (d, 0.85 H, $J_{NH,2}$ 8.0 Hz, NHα), 6.54 (d, 0.85 H, $J_{HO,1}$ 4.9 Hz, HO-1α), 6.08 (d, 0.15 H, $J_{HO,1}$ 6.6 Hz, HO-1β), 5.26 (dd, 0.85 H, $J_{1,2}$ 4.9 Hz, H-1α), 4.98 (dd, 0.15 H, $J_{1,2}$ 2.6 Hz, H-1β), 4.27 (q, 0.85 H, J_{CH,CH_3} 7 Hz, C*H*$CH_3\alpha$), 4.24–3.75 (m, 6.15 H, H-2,3,4,5,6,6'$\alpha\beta$, C*H*$CH_3\beta$), 3.66 (s, 0.45 H, COOMeβ), 3.65 (s, 2.55 H, COOMeα), 1.85 (s, 2.55 H, NAcα), 1.82 (s, 0.45 H, NAcβ), 1.33–1.26 (m, 9 H, 2 $CH_3\alpha\beta$ + C*H*$_3$CH$\alpha\beta$); $\alpha\beta$-ratio, 5.5:1.

Anal. Calc. for $C_{15}H_{25}NO_8$ (347.36): C, 51.86; H, 7.25; N, 4.03. Found: C, 52.03; H, 7.10; N, 3.92.

2-Methyl-(3-O-benzyl-1,2-dideoxy-5,6-O-isopropylidene-α-D-glucofurano)-[2,1-d]-2-oxazoline (**5**). — A 50% dispersion of sodium hydride in mineral oil (3.5 g, 36 mmol) was washed with hexane. Under nitrogen, the NaH was dispersed in *N,N*-dimethylformamide (50 mL), and a solution of **1** (3.5 g, 14.4 mmol) in *N,N*-dimethylformamide (30 mL) was added quickly. After ~45 min, the evolution of hydrogen ceased. A solution of freshly distilled benzyl bromide (4.3 mL, 6.16 g, 36 mmol) in *N,N*-dimethylformamide (5 mL) was added dropwise during 15 min, and stirring was continued under nitrogen for 3 h. Methanol (15 mL) was added, most of the solvent was evaporated, and a solution of the residue in ethyl acetate (140 mL) was washed with water (40 mL), dried (Na_2SO_4), and concentrated. Column chromatography (ethyl acetate) of the residue on silica gel that had been treated with 5 drops of triethylamine afforded **5** (3.0 g, 63%), isolated as a syrup, $[\alpha]_D^{19}$

$-36°$ (*c* 1.2, chloroform); ν_{max}^{film} 2970 (CH), 2930 (CH), 2870 (CH), 1660 cm^{-1} (N=C). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ 7.36–7.29 (m, 5 H, Ph), 6.12 (d, 1 H, $J_{1,2}$ 5.3 Hz, H-1), 4.72 (ABq, 1 H, $J_{A,B}$ 11.7 Hz, CH_AH_B), 4.66 (ABq, 1 H, CH_AH_B), 4.52 (dq, 1 H, $J_{1,2}$ 5.3, $J_{2,N=CCH_3}$ 1.8 Hz, H-2), 4.38 (ddd, 1 H, $J_{4,5}$ 7.1, $J_{5,6}$ 6.4, $J_{5,6'}$ 5.7 Hz, H-5), 4.10 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-3), 4.10 (dd, 1 H, $J_{6,6'}$ 8.8 Hz, H-6), 4.02 (dd, 1 H, H-6'), 3.85 (dd, 1 H, H-4), 2.02 (d, 3 H, $N=CCH_3$), 1.41 (s, 3 H, CH_3), 1.37 (s, 3 H, CH_3).

Anal. Calc. for $C_{18}H_{23}NO_5$ (333.37): C, 64.85; H, 6.95; N, 4.20. Found: C, 64.74; H, 6.89; N, 4.00.

*2-Acetamido-3-*O*-benzyl-2-deoxy-5,6-*O*-isopropylidene-*D*-glucofuranose* (**6**). — A solution of **5** (1.04 g, 3.12 mmol) in methanol (6.6 mL), water (3.3 mL), and glacial acetic acid (50 μL) was stirred at room temperature for 16 h, then concentrated, and toluene was evaporated several times from the residue. Column chromatography (1:1 ethyl acetate–acetone) then afforded $\alpha\beta$-**6** (860 mg, 79%), m.p. 127–128°, $[\alpha]_D^{20}$ $-11°$ (15 min)$\rightarrow -13°$ (final value; *c* 0.6, methanol); ν_{max}^{KBr} 3450 (OH), 3400 (NH), 2980 (CH), 1670 (Amide I), 1560 cm^{-1} (Amide II). ^{1}H-N.m.r. data {300 MHz, $[(CD_3)_2SO]$}: δ 8.04 (d, 0.45 H, $J_{NH,2}$ 7.5 Hz, NHβ), 7.74 (d, 0.55 H, $J_{NH,2}$ 8.2 Hz, NHα), 7.38–7.25 (m, 5 H, Ph), 6.61 (d, 0.55 H, $J_{HO,1}$ 4.4 Hz, HO-1α), 6.33 (d, 0.45 H, $J_{HO,1}$ 5.8 Hz, HO-1β), 5.32 (dd, 0.55 H, $J_{1,2}$ 4.9 Hz, H-1α), 5.06 (dd, 0.45 H, $J_{1,2}$ 2.2 Hz, H-1β), 4.68 (ABq, 0.45 H, $J_{A,B}$ 11.5 Hz, $CH_AH_B\beta$), 4.63 (ABq, 0.55 H, $J_{A,B}$ 11.7 Hz, $CH_AH_B\alpha$), 4.53 (ABq, 0.45 H, $CH_AH_B\beta$), 4.50 (ABq, 0.55 H, $CH_AH_B\alpha$), 4.36–3.77 (m, 6 H, H-2,3,4,5,6,6'$\alpha\beta$), 1.87 (s, 1.65 H, NAcα), 1.84 (s, 1.35 H, NAcβ), 1.32 (s, 3 H, $CH_3\alpha\beta$), 1.28 (s, 1.35 H, $CH_3\beta$), 1.27 (s, 1.45 H, $CH_3\alpha$); $\alpha\beta$-ratio, 1.25:1.

Anal. Calc. for $C_{18}H_{25}NO_6$ (351.40): C, 61.52; H, 7.17; N, 3.98. Found: C, 61.76; H, 7.40; N, 3.92.

*2-Acetamido-3-*O*-benzyl-2-deoxy-*D*-glucopyranose* (**7**). — A solution of **5** (300 mg, 0.90 mmol) in aqeous 80% acetic acid (10 mL) was stirred at room temperature for 16 h, then diluted with water (5 mL), and concentrated, and water and toluene were distilled thrice from the residue. A solution of the residue in water was freeze-dried. Recrystallization of the product from ethyl acetate afforded **7** (263 mg, 94%), m.p. 195–198° (lit.[11] 199–201°), $[\alpha]_D^{18}$ $+34°$ (15 min$\rightarrow +20°$ (final value; *c* 1, water); ν_{max}^{KBr} 3480 (OH), 3320 (NH), 2950 (CH), 1650 (Amide I), 1560 cm^{-1} (Amide II). ^{1}H-N.m.r. data (90 MHz, CD_3OD): δ 7.5–7.3 (m, 5 H, Ph), 5.05 (d, 1 H, $J_{1,2}$ 3 Hz, H-1), 1.95 (s, 3 H, NAc).

Anal. Calc. for $C_{15}H_{21}NO_6$ (311.33): C, 57.86; H, 6.80; N, 4.50. Found: C, 57.87; H, 6.90; N, 4.44.

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