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Note

A convenient synthesis of 6-*N*-methylpurpurosamine C

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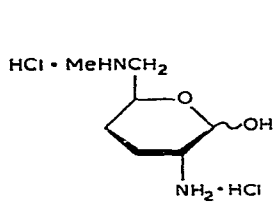
Purpurosamine C, 2,6-diamino-2,3,4,6-tetradeoxy-α-D-*erythro*-hexopyranose[1,2], was first found as a constituent of a gentamicin group antibiotic, gentamicin C_{1a}, and several syntheses of this diaminotetradeoxyhexose have been reported[2–6]. More recently, the presence of the 6-*N*-methylpurpurosamine C (**1**) moiety in the molecule of sagamicin[7] (identical[8] with gentamicin C_{2b}), another antibiotic of the gentamicin group, was described. Also, **1** was comprised in a semisynthetic kanamycin derivative[9], 3′,4′-dideoxy-6′-*N*-methylkanamycin B, as its building unit. The 3,4-dideoxy and 6-methylamino structure in the aminodeoxyhexose part of the antibiotics just described was shown to protect against inactivation caused by aminocyclitol antibiotic modifying enzymes that phosphorylate the 3′-hydroxyl group and *N*-acylate the 6′-amino group and are present in R factor-containing, Gram-negative bacteria[8–10]. In this Note we describe a convenient synthetic method of **1**, starting from a readily available D-glucosamine derivative. Since no chromatographic purification is needed in the following reaction-sequence, except the final one, this method provides a suitable procedure for a large-scale preparation of **1**.

Treatment of benzyl 2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside[11] (**2**) with excess methanesulfonyl chloride in dry pyridine overnight at 5° gave crystalline benzyl 2-(benzyloxycarbonyl)amino-2-deoxy-3,4,6-tri-*O*-methylsulfonyl-α-D-glucopyranoside (**3**) in 98% yield. When the trimesylate **3** was heated under reflux in 30% methylamine in methanol solution for 18 h, benzyl 2-(benzyloxycarbonyl)-amino-2,6-dideoxy-6-methylamino-3,4-di-*O*-methylsulfonyl-α-D-glucopyranoside (**4**) was obtained as pure crystals, in 74% yield, only by recrystallization of the reaction product. Under the reaction conditions just described, the selective substitution of the primary mesyloxy with a methylamino group proceeded smoothly without any significant reaction of the secondary mesylate group or any other side-reactions[12,13], and a relatively good yield (74%) of **4** was obtained. After protection of the free methylamino group with a benzyloxycarbonyl group, 3,4-unsaturation was introduced by the following modification of the method of Tipson and Cohen[14]: Heating of the dimesylate **5** with sodium iodide and zinc dust in dry *N*,*N*-dimethylformamide for 6 h at 93–95° gave benzyl 2-(benzyloxycarbonyl)amino-6-(benzyloxycarbonyl)methyl-amino-2,6-dideoxy-α-D-*erythro*-hex-3-enopyranoside (**6**) in 67% yield.



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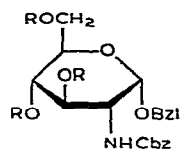

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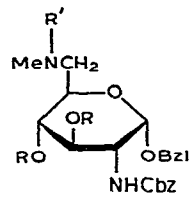
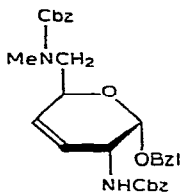
3 R = Ms



4 R = Ms, R' = H
5 R = Ms, R' = Cbz



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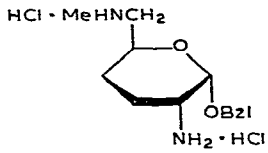

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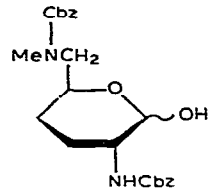

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Treatment of **6** with hydrogen in the presence of 10% palladium-on-charcoal as a catalyst at 2.7 atm at room temperature gave in 86% yield benzyl 2-amino-2,3,4,6-tetradeoxy-6-methylamino-α-D-*erythro*-hexopyranoside (**7**). Acid hydrolysis with hydrochloric acid gave 6-*N*-methylpurpurosamine C (**1**), obtained in 65% yield as the amorphous hydrochloride, further characterized as the crystalline *N,N'*-dibenzyloxycarbonyl derivative **8**. Synthetic **1** was compared on t.l.c. with an acid hydrolyzate of sagamicin, which contains natural 5-*N*-methylpurpurosamine C. The spot corresponding to natural 6-*N*-methylpurpurosamine C was identical with that of synthetic **1** in two solvent systems.

EXPERIMENTAL

General methods. — Solutions were concentrated at 50° under reduced pressure. Melting points were determined on a Yamato Model MP-21 apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-180 automatic polarimeter. ^{1}H-N.m.r. spectra were recorded on JEOL JNM-MH-60 and JEOL PS-100 spectrometers, respectively; chemical shifts are given in p.p.m. from the DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) or Me_4Si (tetramethylsilane) peaks, both compounds being used as internal standards. Mass spectra were measured on a Hitachi RMU-6M spectrometer by direct insertion of samples. I.r. spectra were recorded with a Hitachi 215 spectrometer. T.l.c. analyses were performed with Silica gel G plates (E. Merck) and cellulose plates (E. Merck). Spots were detected on silica gel plates by spraying with 50% sulfuric acid and heating, and on cellulose plates by spraying with an aniline phthalate or ninhydrin reagent.

Benzyl 2-(benzyloxycarbonyl)amino-2-deoxy-3,4,6-tri-O-methylsulfonyl-α-D-glucopyranoside (**3**). — To a solution of benzyl 2-(benzyloxycarbonyl)amino-2-deoxy-

α-D-glucopyranoside (**2**, 25 g, 60 mmol) in dry pyridine (250 ml), was added methanesulfonyl chloride (42.5 g, 0.37 mol) at 0° with stirring. The mixture was stored overnight at 5° and then the solvent was evaporated. The residue was dissolved in chloroform, and washed with aqueous sodium hydrogencarbonate and water. The chloroform layer was dried (sodium sulfate) and evaporated to yield a colorless crystalline mass that was recrystallized from chloroform–ethanol to give **3** (38 g, 98%), m.p. 148–149°, $[\alpha]_D^{25.5}$ +96.5° (*c* 1.0, chloroform); ν_{max}^{Nujol} 1170 cm^{-1} (SO_2); n.m.r. (60 MHz, $CDCl_3$): δ 2.84, 3.06 and 3.19 (each 3 H, 3s, S-CH_3), 7.38 (10 H, aromatic H).

Anal. Calc. for $C_{24}H_{31}NO_{13}S_3$: C, 45.20; H, 4.90; N, 2.20; S, 15.08. Found: C, 45.28; H, 4.86; N, 2.10; S, 15.06.

Benzyl 2-(benzyloxycarbonyl)amino-2,6-dideoxy-6-methylamino-3,4-di-O-methylsulfonyl-α-D-glucopyranoside (**4**). — A stirred solution of **3** (33 g, 52 mmol) in 30% methylamine in a methanol solution (260 ml) was heated for 18 h under reflux. T.l.c. (silica gel) in 20:1 (v/v) chloroform–methanol showed almost disappearance of **3** (R_F 0.81) and appearance of a new major product (R_F 0.48). Evaporation of the solvent yielded a white crystalline mass, which was recrystallized from ethanol to give the free base of **4** as white needles (22 g, 74.2%), m.p. 155–157°, $[\alpha]_D^{25.5}$ +104.7° (*c* 1.0, chloroform). A methanolic solution of the free base (1 g) was adjusted to pH 4 with dil. hydrochloric acid and the solution was concentrated to give crystals, which were recrystallized from 3:1 (v/v) methanol–ethanol to give the hydrochloride of **4** as white needles (524 mg), m.p. 216.5–217.5° (dec.), $[\alpha]_D^{26}$ +87.5° (*c* 1.0, *N,N*-dimethylformamide); n.m.r. (60 MHz, dimethyl sulfoxide-d_6): δ 2.64 (3 H, s, N-CH_3), 3.16, 3.38 (each 3 H, 2s, S-CH_3), and 7.33 (10 H, aromatic H).

Anal. Calc. for $C_{24}H_{32}N_2O_{10}S_2 \cdot HCl$: C, 47.36; H, 5.46; Cl, 5.82; N, 4.60; S, 10.52. Found: C, 47.10; H, 5.48; Cl, 5.63; N, 4.55; S, 10.41.

Benzyl 2-(benzyloxycarbonyl)amino-6-(benzyloxycarbonyl)methylamino-2,6-dideoxy-3,4-di-O-methylsulfonyl-α-D-glucopyranoside (**5**). — To a stirred mixture of **4** (22 g, 38 mmol) in *p*-dioxane (300 ml) and sodium hydroxide (4 g) in water (20 ml) was added dropwise benzyloxycarbonyl chloride (8 g, 47 mmol) over a period of 1 h at 0–5°, and stirring was continued for 5 h at room temperature. The insoluble material was filtered off and the filtrate was evaporated to yield a residue that crystallized on trituration with hexane. The product was recrystallized from ethanol to give **5** (26 g, 96%), m.p. 121–122°, $[\alpha]_D^{25.5}$ +90.0° (*c* 1.0, chloroform); n.m.r. (100 MHz, $CDCl_3$): δ 2.93, 3.08 (each 3 H, 2s, S-CH_3), 3.03, 3.20 (N-CH_3)*, and 7.36 (15 H, aromatic H).

Anal. Calc. for $C_{32}H_{38}N_2O_{12}S_2$: C, 54.38; H, 5.42; N, 3.96; S, 9.07. Found: C, 54.80; H, 5.52; N, 3.90; S, 8.77.

Benzyl 2-(benzyloxycarbonyl)amino-6-(benzyloxycarbonyl)methylamino-2,6-dideoxy-α-D-erythro-hex-3-enopyranoside (**6**). — To a solution of **5** (25 g, 35 mmol)

*The *N*-methyl group gave two sharp singlets of almost equal intensity (total 3 H) at room temperature. At 40°, however, these peaks were simplified to a sharp singlet at δ 3.12. Similar multiplicity was reported for the *N*-methyl groups of *N,N'*-diacetyl-2-deoxy-*N,N'*-5-*O*-trimethylstreptamine[15].

in dry *N,N*-dimethylformamide (200 ml) were added sodium iodide (126 g) and zinc dust (60.4 g) and the mixture was stirred for 6 h at 93–95° (oil-bath temperature). T.l.c. (5:1, v/v, benzene–ethyl acetate) of the resulting reaction mixture showed the formation of a single product (R_F 0.56) and no starting material (R_F 0.30) was detected. After filtration and addition of water (200 ml), the reaction mixture was extracted with ethyl ether. The residue obtained on concentration of the extract was crystallized from ethanol to give 12.7 g of **6** (67%), m.p. 67.5–68.5°, $[\alpha]_D^{25.5}$ +13.0° (*c* 1.0, chloroform); n.m.r. (60 MHz, $CDCl_3$): δ 2.98 (3 H, s, N-CH_3), 5.62 (2 H, broad, olefinic H), 7.25, and 7.30 (15 H, aromatic H).

Anal. Calc. for $C_{30}H_{32}N_2O_6$: C, 69.75; H, 6.24; N, 5.42. Found: C, 69.52; H, 6.27; N, 5.39.

*Benzyl 2-amino-2,3,4,6-tetradeoxy-6-methylamino-α-*D-erythro-*hexopyranoside dihydrochloride* (**7**). — To a solution of **6** (12 g, 35 mmol) in 5:1 (v/v) *p*-dioxane–water (60 ml), was added 10% palladium-on-charcoal (2.4 g), and the solution was hydrogenated for 10 h under 2.7 atm at room temperature. The solid precipitate formed was redissolved by addition of dilute hydrochloric acid (pH 4.0). The residue obtained on concentration of the filtrate was crystallized from ethanol to give 6.5 g of **7** (86.4%), m.p. 253.5–255° (dec.), $[\alpha]_D^{26.5}$ +88.7° (*c* 1.0, water); n.m.r. (60 MHz, D_2O): δ 1.5–2.2 (4 H, m, 3,4-methylene H), 2.74 (3 H, s, N-CH_3), 3.06–3.20 (2 H, m, 6-methylene H), 3.30–3.80 (1 H, m, 5-methine H), 3.90–4.50 (1 H, m, 2-methine H), 5.27 (1 H, d, *J* 3 Hz, aromatic H), and 7.58 (5 H, aromatic H): m.s.: 251 (MH^+), 250 (M^+), 220 ($MH^+-CH_3NH_2$), 206, 159, 142, and 114.

Anal. Calc. for $C_{14}H_{22}N_2O_2 \cdot 2HCl$: C, 52.02; H, 7.48; N, 8.67; Cl, 21.94. Found: C, 52.18; H, 7.57; N, 8.76; Cl, 21.47.

6-N-Methylpurpurosamine C dihydrochloride (**1**). — A solution of 7 (6.0 g) in 1:1 (v/v) conc. hydrochloric acid–water (30 ml) was heated for 6 h to 80–83°. On t.l.c. (cellulose; 15:10:3:12, v/v, 1-butanol–pyridine–acetic acid–water), the solution showed the presence of only one spot, positive with ninhydrin and the aniline phthalate reagent (R_F 0.38). The pH of the solution was adjusted to 5.0–5.5 with Amberlite IR-45 (OH^-) anion-exchange resin. After evaporation, the residue was chromatographed on a column of Sephadex LH-20 with methanol to give **1** (2.8 g, 64.8%) m.p. 178–180° (dec.) $[\alpha]_D^{21}$ +33.7° (*c* 1.08 water); n.m.r. (100 MHz D_2O): δ 1.3–2.5 (4 H, m, 3,4-methylene H), 2.9–4.5 (4 H, m, 6-methylene H, 2,5-methine H), 2.76 (s, α-N-CH_3), 2.78 (s, β-N-CH_3) [$\alpha:\beta = 1:2$], 4.86 (d, *J* 10 Hz, β-anomeric H), 5.38 (d, *J* 3 Hz, α-anomeric H) [$\alpha:\beta = 1:2$].

Anal. Calc. for $C_7H_{16}N_2O_2 \cdot 2HCl$: C, 36.06; H, 7.78; N, 12.02. Found: C, 35.85; H, 8.20; N, 11.05.

*2-(Benzyloxycarbonyl)amino-6-(benzyloxycarbonyl)methylamino-2,6-dideoxy-*D-erythro-*hexopyranose* (**8**). — To a stirred mixture of **1** (1 g, 4 mmol) in methanol (15 ml) and sodium carbonate (7 g, 66 mmol) in water (15 ml) was added dropwise a solution of *N*-benzyloxycarbonyloxysuccinimide (2.5 g, 10 mmol) in methanol (20 ml) within a period of 1 h at room temperature. After 2 h, a solid began to precipitate; it was dissolved by addition of chloroform. The chloroform solution

was washed with water and evaporated. The residue was treated with ethyl ether to yield a crystalline mass, which was recrystallized from chloroform–ethyl ether to give **8** (1.29 g, 68.8%), m.p. 129–131°, $[\alpha]_D^{26}$ +31.7° (*c* 1.0, chloroform); ν_{max}^{Nujol} 1680, 1690 (COO^-) 750 and 690 cm^{-1} (monosubstituted benzene); n.m.r. (60 MH_3, $CDCl_3$): δ 1.20–2.30 (m, 4 H, 3,4-methylene H), 2.92 and 2.97 (each s, 3 H, N-CH_3), 5.06 (broad signals, 5 H, anomeric H (α,β anomer and benzylmethylene H), 7.28 (10 H, aromatic H); m.s.: *m/e* 429 (MH^+), 411 (MH^+–H_2O), 320, 212, and 91.

Anal. Calc. for $C_{23}H_{28}N_2O_6$: C, 64.47; H, 6.59; N, 6.54. Found: C, 64.33; H, 6.62; N, 6.59.

Chromatographic comparison of **1** *with natural* 6-N-*methylpurpurosamine C.* — A solution of sagamicin[7] (1.0 mg) in 6M hydrochloric acid (0.1 ml) was sealed in a small glass tube and heated for 6 h at 110°. The solution was transferred to a round-bottom flask with water and evaporated several times from water to remove excess hydrochloric acid. The residue was finally dissolved in water (0.1 ml) to give a solution that was spotted on cellulose plates and co-chromatographed with synthetic **1**. The natural 6-*N*-methylpurpurosamine C in the acid hydrolyzate gave spots detected with ninhydrin and the aniline phthalate reagent and having the following R_F values: 0.52 (lit.[7] 0.550) in 15:10:3:12 v/v, 1-propanol–pyridine–acetic acid–water, and 0.18 (lit.[7] 0.167) in 5:6:1:3 v/v, pyridine–ethyl acetate–acetic acid–water. The spot corresponding to the natural product could not be differentiated from the spot of **1** in these solvent systems.

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