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Note

An inexpensive route to 2-azido-2-deoxy-D-mannose and its conversion into an azido analog of *N*-acetylneuraminic acid*

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Azidonitration is one possible route to 2-azido-2-deoxy-D-mannose derivatives. Under the improved conditions described by Paulsen *et al.*[1], the reaction lasts 6 days at −40°. The year before, Kiso *et al.*[2] reported a synthesis of compound **6** by nucleophilic substitution of the triflate of the D-glucose derivative[3] **1**. We report herein a protocol which minimizes the cost by use of the inexpensive 1-imidazolesulfonate-leaving group introduced by Hanessian and Vatèle[4].

The reaction of 1-imidazolesulfonate **2**, prepared in quantitative yield from alcohol **1**, with sodium azide in *N,N*-dimethylformamide at 70° was complete within 3 h and gave two products, one of which was the known[2] *manno* azide **6**. The 64% yield was somewhat better than the yield observed[2] with the triflate. The contaminant is probably a product of β-elimination, as a compound having the same R_F on t.l.c. was observed as the product of the reaction of **2** with tetrabutylammonium benzoate, and as the unique product of reaction with sodium cyanide. This behavior would be expected if H-3 is in an "anti" relationship to the leaving group in **2**, and indeed, the observed $J_{2,3}$ coupling constant, 1.5 Hz, is compatible with this conformation (Fig. 1). Thus a chromatographic separation cannot be avoided as the last stage.

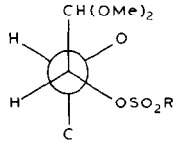

Fig. 1. Suggested Newmann projection of **2** along the C-2–C-3 bond (R = 1-imidazolyl).

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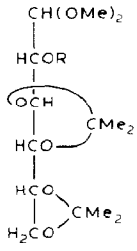

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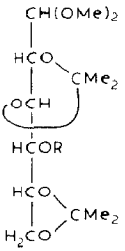
2 R = Im



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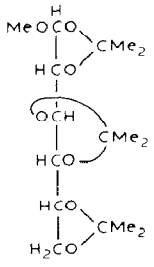
4 R = Im



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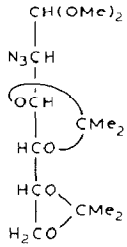

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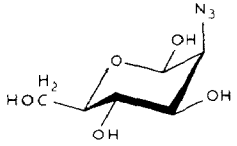

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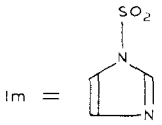


Stevens[3] reported that the treatment of D-glucose with 2,2-dimethoxypropane, methanol, and sulfuric acid gave, in 1 h at room temperature, a mixture of **1** (23%), **3, 5,** and 1,2:4,5-di-*O*-isopropylidene-α-D-glucofuranose. Unfortunately, the dimethyl acetal could only be conveniently isolated by fractional crystallization of the mixed benzoate, when it accumulated in the mother liquors[3]. Since the reagent, *N,N'*-sulfuryldiimidazole is inexpensive, it was also used in derivatizing the crude mixture, and a chromatographic separation of the product on ten times its weight of silica gel afforded a mixture of 1-imidazolesulfonates **2** and **4**. Nucleophilic substitution with sodium azide gave azide **6**, separated by conventional chromatography from two more polar compounds, one of which being probably the 4-azido-4-deoxy-D-*galacto* derivative[2] but this was not further investigated. The

TABLE I

^{1}H-N.M.R. DATA[a] FOR 2-AZIDO-2-DEOXY-D-MANNOPYRANOSE (**7**)

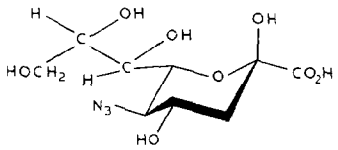
Atom	*This work*	*Litt.*[c]
H-1α	5.26 (d, 0.45, H, $J_{1,2}$ 1.5 Hz)	5.05 (d, 0.64 H, J 1.7 Hz)
H-1β	5.04 (d, 0.55 H, $J_{1,2}$ 1 Hz)	4.81 (d, 0.36 H, $J_{1,2}$ 1.5 Hz)
H-2α	3.99 (dd, 0.45 H, $J_{2,3}$ 4 Hz)	3.84 (dd, 0.64 H)
H-2β	4.04 (dd, $J_{2,3}$ 4 Hz)	3.88 (dd, 0.36 H, $J_{2,3}$ 3.7 Hz)
H-3	4.06 (dd, $J_{3,4}$ 10 Hz)	3.94 (dd, 0.64 H, $J_{2,3}$ 3.9, $J_{3,4}$ 9.3 Hz)
H-4α	3.61 (dd, $J_{4,5}$ 10 Hz)	3.55 (dd, 0.64 H)
H-4β	3.49 (dd, 0.55 H, $J_{4,5} = J_{3,4} = 10$ Hz)	3.40 (dd, 0.36 H)
H-5β	3.35 (ddd, 0.55 H, $J_{4,5}$ 10, $J_{5,6a}$ 2, $J_{5,6b}$ 6 Hz)	3.16 (ddd, 0.36 H, $J_{4,5}$ 9.6, $J_{5,6a}$ 2.9, $J_{5,6b}$ 5.7 Hz)

[a]δ Values. [b]At 250 MHz for a solution in D_2O. [c]At 400 MHz, for a solution in CD_3CN (ref. 1).

yield of azide **6** from D-glucose was 10%; however, it seems more reasonable, in the present preparative context, to consider the yield in terms of the only expensive chemical involved, that is (in France) 2,2-dimethoxypropane (9 mL/mmol of **6**).

Acid hydrolysis of the dimethyl acetal **6** gave the free sugar **7** which appeared on t.l.c. as two contiguous spots, with near equal intensities. The ^{1}H-n.m.r. for a solution in D_2O indicated a slight excess of the β-D anomer. Otherwise, the chemical shifts and J values were very similar to those already reported[1] for a solution in CD_3CN (Table I).

The incubation during 24 h of the azido sugar **7** with sodium pyruvate in the presence of sialate aldolase (*N*-acetylneuraminate pyruvate-lyase; EC 4.1.3.3) gave 5-azido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulosonic acid (**8**) in 78% yield. The composition of the ammonium salt corresponded to a hemihydrate after prolonged drying at 60° in high vacuum. The 200-MHz ^{1}H-n.m.r. spectrum of the free acid was a typical sialic acid spectrum. In the sialate aldolase cleavage reaction, the azido analog **8** showed more affinity for the enzyme (K_m 2.2mM) and a smaller V_{max} (0.1) than *N*-acetylneuraminic acid under the same conditions (K_m 5mM, V_{max} 0.4).



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EXPERIMENTAL

General methods. — See previous publication[5].

2-Azido-2-deoxy-3,4;5,6-di-O-*isopropylidene*-aldehydo-D-*mannose dimethyl acetal* (**6**). — Repetition of the procedure of Stevens[3] starting from D-glucose (20 g), methanol (150 mL), 2,2-dimethoxypropane (100 mL), and H_2SO_4 (3 mL) gave a mixture of **1, 3, 5**, and 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose (23.4 g).

Part of this (19.14 g) was dissolved in anhydrous *N,N*-dimethylformamide (250 mL) and stirred for 15 min at room temperature in the presence of NaH (60% suspension in oil; 3 g). Then it was cooled to −40°, and after the addition of *N,N'*-sulfuryldiimidazole (14.85 g), stirred again for 1 h at −30°. The solution was cooled to −40°, treated with methanol, and allowed to warm up to room temperature. The usual partition between ether and water gave a gum which was adsorbed onto a silica gel column (200 g). Elution with 4:1 hexane–ethyl acetate gave (*S*)-1,2:3,4:5,6-tri-*O*-isopropylidene-1-methoxy-D-glucitol (**5**) (5.25 g). Then 1:1 hexane–ethyl acetate eluted a mixture of 1-imidazolesulfonates **2** and **4** (15.6 g).

This was dissolved in anhydrous *N,N*-dimethylformamide, NaN_3 (5.7 g) was added, and the solution was heated for 3 h at 70°. After the usual partition between ether and water, silica gel chromatography (9:1 hexane–ethyl acetate) gave the azido compound **6** (2.93 g), syrup, $[\alpha]_D^{20}$ +9° (*c* 1.4, dichloromethane); ν_{max}^{film} 2120 (N_3), 850, and 875 cm^{-1} (Me_2C); ^{1}H-n.m.r. (250 MHz, $CDCl_3$, Me_4Si): δ 1.37 (2 s, with 1 Hz separation, 6 H, 2 Me_2), 1.44 (s, 6 H, 2 Me_2), 3.42 (s, 3 H, MeO), 3.49 (s, 3 H, MeO), 3.77 (dd, 1 H, $J_{1,2}$ 7, $J_{2,3}$ 4 Hz, H-2), ~4.60 (m, H-3), and 4.52 (d, 1 H, H-1); lit.[2] $[\alpha]_D$ +8.4° (*c* 0.5, chloroform); ν_{max}^{film} 2120, 880, and 855 cm^{-1}; ^{1}H-n.m.r. (90 MHz, $CDCl_3$): δ 1.35 and 1.42 (2 s, 12 H), 3.40 and 3.47 (2 s, 6 H), 3.73 (dd, 1 H, *J* 6.8 and 2.6 Hz), and 4.50 (d, 1 H, *J* 6.8 Hz).

2-Azido-2-deoxy-D-*mannose* (**7**). — A solution of **6** (0.5 g) in 0.25M H_2SO_4 (10 mL) was kept for 1 h at 100°, and then cooled to room temperature and deionized on a Dowex 2 (HCO_2^-) column. The effluent was evaporated to dryness to give the free sugar **7** as a syrup (293 mg; 95%) which appeared practically pure by t.l.c. (3:3:1, 2-propanol–ethyl acetate–water, R_F 0.82 and 0.87). Traces of less polar contaminants were removed by silica gel column chromatography in 4:1 dichloromethane–methanol $[\alpha]_D^{20}$ +14.4° (*c* 5, methanol); ^{1}H-n.m.r., see Table I; lit.[1] $[\alpha]_D^{20}$ −36.4° (*c* 1.1, methanol); ^{1}H-n.m.r., see Table I.

2-O-(1-Imidazolesulfonyl)-3,4;5,6-di-O-isopropylidene-aldehydo-D-*mannose dimethyl acetal* (**2**). — ^{1}H-N.m.r. (250 MHz, $CDCl_3$, Me_4Si): δ 1.38, 1.40, 1.50 (12 H, 2 CMe_2), 3.20, 3.45 (2 s, 2 × 3 H, 2 OMe), 3.87 (dd, 1 H, $J_{5,6b}$ 6, $J_{6a,6b}$ 6 Hz, H-6b), 3.93 (dd, 1 H, $J_{5,6}$ 8 Hz, H-6a), 4.07 (ddd, 1 H, $J_{4,5}$ 6 Hz, H-5), 4.21 (dd, 1 H, $J_{3,4}$ 8 Hz, H-4), 4.26 (dd, 1 H, $J_{2,3}$ 1.5 Hz, H-3), 4.45 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), and 4.88 (d, 1 H, H-2).

5-Azido-3,5-dideoxy-D-glycero-D-galacto-*nonulosonic acid* (**8**). — The progress of the reaction and the chromatographic separation were monitored by t.l.c. on silica gel plates (7:3 propanol–water). The suspension of immobilized[5] *N*-acetylneuraminate pyruvate-lyase (EC 4.1.33; 12 units) was gently stirred with 2-azido-2-deoxy-D-mannose (1 mmol), sodium pyruvate (10 mmol), dithiothreitol (10^{-2}M), and NaN_3 (0.01%) in 0.05M phosphate buffer (pH 7.0; 10 mL), at 37° under N_2 for 24 h. The gel was removed by filtration. Compound **8** in the filtrate was purified by anion-exchange chromatography on a column (33 × 2.5 cm) of Dowex 1 (HCO_2^-) using a 0–2M gradient of formic acid as eluent, and finally separated from the solvent by freeze-drying (220 mg; 78%); ^{1}H-n.m.r. (200 MHz, D_2O, Me_4Si as external reference): δ 1.86 (dd, $J_{3a,4}$ 11.5, ^2J 13 Hz, H-3*a*), 2.26 (dd, $J_{3b,4}$ 5 Hz, H-3*e*), 3.51 (dd, $J_{4,5} = J_{5,6}$ = 10 Hz, H-5), 3.62 (dd, $J_{8,9a}$ 6, ^2J 11.2 Hz, H-9*a*), 3.73 (m, H-8), 3.80 (dd, $J_{6,7}$ 1, $J_{7,8}$ 8 Hz, H-7), 3.84 (dd, $J_{8,9b}$ 2.5 Hz, H-9b), 3.92 (dd, H-6), and 4.07 (m, H-4). Michaelis constant for enzymic cleavage with aldolase, estimated following a described protocol[6]: K_m 2.2mM.

The ammonium salt was obtained by neutralisation to pH 7 with dilute ammonia, freeze-drying, and heating at 60°/6Pa for 16 h.

Anal. Calc. for $C_9H_{18}N_4O_8 \cdot 0.5\ H_2O$: C, 33.85; H, 6.00; N, 17.55; O, 42.60. Found: C, 33.97; H, 6.01; N, 16.87; O, 42.94.

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