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Note

2-Acetamido-2-deoxy-5-thio-D-glucopyranose (5-thio-*N*-acetyl-D-glucosamine)

REZSŐ BOGNAR, PÁL HERCZEGH,
Research Group for Antibiotics, Hungarian Academy of Sciences, H-4010 Debrecen (Hungary)
ROY L. WHISTLER, AND EMEKA B. MADUMELU
Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907 (U.S.A.)
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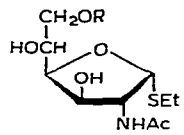
N-Acetylneuraminic acid (NANA) is an important constituent of the cell wall of higher animals[1], usually as a non-reducing end of the hetero-oligosaccharide portion of cell-surface glycoproteins and of glycolipids. 2-Acetamido-2-deoxy-D-glucose is the precursor in the biosynthesis of NANA, and disturbance of this process can cause a decreased NANA level in the cell surface.

Removal of NANA by sialidase ("unmasking") may give rise to specific tumour-immunity and offers a possibility for cancer immunotherapy[2]. An inhibitor of NANA biosynthesis could act similarly to sialidase in preventing the accumulation of NANA on the surface of tumour cells and thereby cause them to become immunogenic. From this point of view, a close analogue of 2-acetamido-2-deoxy-D-glucose, namely the 5-thio derivative, is potentially useful, because similar analogues of biologically important sugars also possess inhibitory activity for the transport of the parent compounds[3].

We now report a synthesis of 2-acetamido-2-deoxy-5-thio-D-glucopyranose. Since the preliminary account of part of this work was presented during the IXth International Symposium on Carbohydrate Chemistry (London, April 10th, 1978), another synthesis of the title compound has been described[4].

Treatment of ethyl 2-acetamido-2-deoxy-1-thio-α-D-glucofuranoside[5] (**1**) with 1 mol. equiv. of benzoyl chloride gave the 6-benzoate **2**, which was converted into the 5-tosylate **3**. Treatment of **3** with sodium methoxide afforded ethyl 2-acetamido-5,6-anhydro-1-thio-β-L-idofuranoside (**4**), which was converted into the 5,6-epithio-1-thio-D-*gluco* derivative **5** by reaction with thiourea. Opening of the thi-irane ring in **5** by potassium acetate in acetic acid–acetic anhydride and acetolysis of the product **6** gave, among other products, the tetra-acetate (**7**) of 2-acetamido-2-deoxy-5-thio-α-D-glucose **8**, which was isolated by column chromatography. The chemical shift of the H-5 multiplet in the n.m.r. spectrum of **7** occurred at unusually high field (δ 3.5) due to the sulfur in the ring. Zemplén deacetylation[6] of **7** gave the crystalline title compound **8**.



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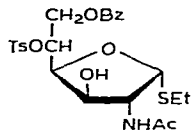
2 R = Bz



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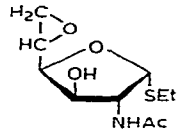

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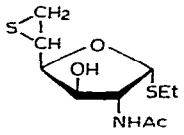

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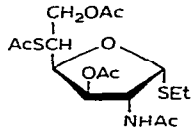

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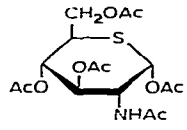

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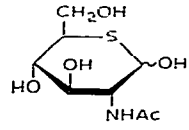

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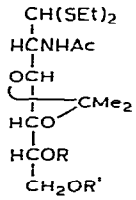
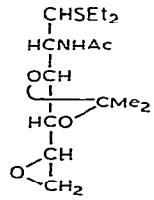
In another preparation of **8**, the 3,4-*O*-isopropylidene derivative[7] **9** was subjected to transformations at C-5,6 similar to those described for **1**. Compound **9** was monobenzoylated to give **10** and subsequently mesylated to yield **11**. A better route to **11** involved treatment of the dimesylate **12** with sodium benzoate. The product **11** was converted into the 5,6-anhydro-L-*ido* derivative **13** which, with thiourea, gave the thi-irane **14**. Treatment of **14** with potassium acetate and acetolysis of the product **15** gave **7**.



9 R = R' = H
10 R = H, R' = Bz
11 R = Ms, R' = Bz
12 R = R' = Ms



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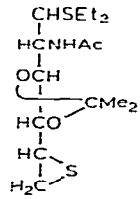

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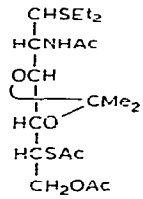

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This second synthesis was the more convenient, and involved high yields and easily crystallisable intermediates.

EXPERIMENTAL

General methods. — Melting points were measured on a Kofler block and were uncorrected. U.v. spectra were recorded with a Unicam SP 800 spectrophotometer. I.r. spectra were obtained for KBr pellets, using a Unicam SP 200 photometer. N.m.r. spectra were recorded with JEOL JMH-100 (^{1}H) and Varian XL-100 instruments (^{13}C). Optical rotations were measured with a Perkin–Elmer 241 polarimeter.

*Ethyl 2-acetamido-6-O-benzoyl-2-deoxy-1-thio-α-*D*-glucofuranoside* (**2**). — To a solution of ethyl 2-acetamido-2-deoxy-1-thio-α-D-glucofuranoside[5] (6 g, 22.6 mmol) in pyridine (70 ml) was added dropwise at −15° a solution of benzoyl

chloride (4.25 ml, 33 mmol) in dry chloroform (10 ml). The mixture was kept at 0° for 4 h and then at 25° for 15 h, poured into ice and water, and extracted with chloroform (4 × 100 ml). The extract was washed successively with 0.5M sulfuric acid and saturated, aqueous sodium hydrogencarbonate, dried ($MgSO_4$), and concentrated. Crystallisation of the product (8.9 g) from chloroform–ether gave **2** (4.72 g, 56.7%), m.p. 127–128°, $[\alpha]_D^{23}$ +96° (*c* 0.5, chloroform), ν_{max} 1738 cm^{-1} (ester).

Anal. Calc. for $C_{17}H_{23}NO_6S$: C, 55.26; H, 6.27; N, 3.79; S, 8.68. Found: C, 55.11; H, 6.10; N, 3.83; S, 8.73.

*Ethyl 2-acetamido-6-O-benzoyl-2-deoxy-1-thio-5-O-toluene-*p*-sulfonyl-α-*D*-glucofuranoside* (**3**). — To a solution of **2** (7.8 g, 21 mmol) in pyridine (30 ml) was added toluene-*p*-sulfonyl chloride (3.81 g, 20 mmol) portionwise at 0° during 20 min. After 20 h, the solvent was evaporated and a solution of the residue in chloroform (200 ml) was washed sequentially with aqueous sodium hydrogencarbonate, 0.5M sulfuric acid, and aqueous sodium hydrogencarbonate, dried, and concentrated. Elution of the residue from a column of silica gel with light petroleum–acetone (8:2) gave **3** (3.0 g, 27.2%), m.p. 96°, $[\alpha]_D^{23}$ +82° (*c* 0.6, chloroform).

Anal. Calc. for $C_{24}H_{29}NO_8S_2$: C, 55.05; H, 5.58; N, 2.68; S, 12.24. Found: C, 55.22; H, 5.62; N, 2.65; S, 12.01.

*Ethyl 2-acetamido-5,6-anhydro-2-deoxy-1-thio-β-*L*-idofuranoside* (**4**). — To a solution of **3** (2.9 g) in chloroform (5 ml) was added a solution of sodium methoxide in methanol (from 307 mg of Na and 12 ml of methanol) at −15°. The mixture was stored at −15° for 0.5 h and then for 2 h at 0°, washed with saturated, aqueous sodium chloride, dried, and concentrated. The residue was crystallised from methanol–ether, to give **4** (0.96 g, 70%), m.p. 143–144°, $[\alpha]_D^{23}$ +147° (*c* 0.5, chloroform).

Anal. Calc. for $C_{10}H_{17}NO_4S$: C, 48.56; H, 6.93; N, 5.66; S, 12.96. Found: C, 48.80; H, 6.78; N, 5.55; S, 13.07.

*Ethyl 2-acetamido-2,5,6-trideoxy-5,6-epithio-1-thio-α-*D*-glucofuranoside* (**5**). — Compound **4** (408 mg) was treated with a solution of thiourea (130 mg) in methanol (15 ml) for 24 h at 25°. Ice and water were added (20 ml), and the mixture was concentrated under diminished pressure. The residue was extracted with chloroform (3 × 50 ml), the combined extracts were dried and concentrated, and the residue was crystallised from methanol–ether, to give **5** (327 mg, 8.2%), m.p. 162–165°, $[\alpha]_D^{23}$ +39.5° (*c* 0.5, chloroform).

Anal. Calc. for $C_{10}H_{17}NO_3S_2$: C, 45.59; H, 6.51; N, 5.32; S, 24.34. Found: C, 45.74; H, 6.62; N, 5.21; S, 23.88.

*Ethyl 2-acetamido-3,6-di-O-acetyl-5-S-acetyl-2-deoxy-1,5-dithio-α-*D*-glucofuranoside* (**6**). — A solution of **5** (450 mg) in a mixture of acetic acid (1 ml) and acetic anhydride (3 ml) containing potassium acetate (300 mg) was boiled under reflux for 22 h and then cooled, poured into ice and water, and, after 2 h, extracted with chloroform (3 × 50 ml). The extract was washed with aqueous sodium hydrogencarbonate, dried, treated with fuller's earth, and concentrated to give **6** (400 mg, 59%), m.p. 133–135°, $[\alpha]_D^{23}$ +142° (*c* 0.5, chloroform), λ_{max} 227 nm (thioacetyl), ν_{max} 1692 cm^{-1} (thioacetyl).

Anal. Calc. for $C_{16}H_{28}NO_7S_2$: C, 46.81; H, 6.87; N, 3.40; S, 15.62. Found: C, 47.10; H, 6.99; N, 3.52; S, 15.54.

*2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-5-thio-α-*D*-glucopyranose* (**7**). — A solution of **6** (140 mg) in acetic anhydride (1.6 ml), acetic acid (0.33 ml), and sulfuric acid (0.03 ml) was kept for 5 days at 25°. Anhydrous sodium acetate was added and then toluene (20 ml), and the solvents were evaporated under reduced pressure. Elution of the residue from a column of silica gel with light petroleum–acetone (7:3) gave **7** (50 mg, 35%), m.p. 174–175°, $[\alpha]_D^{23}$ +195.5° (*c* 0.4, methanol). N.m.r. data ($CDCl_3$): ^1H, δ 3.5 (o, 1 H, H-5), 4.65 (o, 1 H, H-2), 5.2 (q, 1 H, H-3), 5.4 (q, 1 H, H-4), and 5.95 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1); ^{13}C, 72.79 (C-1), 55.26 (C-2), 71.33 and 71.69 (C-3,4), 39.84 (C-5), and 61.20 p.p.m. (C-6).

Anal. Calc. for $C_{16}H_{23}NO_9S$: C, 47.39; H, 5.72; N, 3.46; S, 7.91. Found: C, 47.55; H, 5.82; N, 3.40; S, 7.77.

*2-Acetamido-6-*O*-benzoyl-2-deoxy-3,4-*O*-isopropylidene-5-*O*-methanesulfonyl-*D*-glucose diethyl dithioacetal* (**11**). — (*a*) To a solution of 2-acetamido-2-deoxy-3,4-*O*-isopropylidene-D-glucose diethyl dithioacetal[5] (3.67 g, 10 mmol) in pyridine (31 ml) was added dropwise at −15° a solution of benzoyl chloride (2.05 g, 14.6 mmol) in chloroform (6 ml). The mixture was kept at 0° for 4 h and at 25° for 15 h, and then poured onto cracked ice and extracted with chloroform. The extract was washed with 0.5M sulfuric acid and saturated, aqueous sodium hydrogencarbonate, dried, and concentrated, to give 2-acetamido-6-*O*-benzoyl-2-deoxy-3,4-*O*-isopropylidene-D-glucose diethyl dithioacetal[5] as a syrup (4.4 g). ^1H-N.m.r. data ($CDCl_3$): δ 7–8 (m, 5 H, Ph).

Compound **10** (9 g) was treated with methanesulfonyl chloride (5 ml) in pyridine (100 ml) for 4 h at 25°. The mixture was poured into ice and water, and extracted with chloroform; the extract was washed with 0.5M sulfuric acid and aqueous sodium hydrogencarbonate, and then dried to give **11** (5.3 g, 50.5%). Recrystallisation from ether–light petroleum gave material having m.p. 73–74°, $[\alpha]_D^{23}$ +36° (*c* 3, chloroform).

Anal. Calc. for $C_{23}H_{38}NO_8S_3$: C, 49.97; H, 6.93; N, 2.53; S, 17.40. Found: C, 50.10; H, 6.85; N, 2.52; S, 17.35.

(*b*) Compound **9** (15.6 g) was treated with pyridine (150 ml) containing mesyl chloride (10 ml) for 20 h at 0°. The mixture was worked-up in the usual manner, to give 2-acetamido-2-deoxy-3,4-*O*-isopropylidene-5,6-di-*O*-methanesulfonyl-D-glucose diethyl dithioacetal (**12**, 15.5 g). Recrystallisation from benzene–light petroleum gave material having m.p. 110–111°, $[\alpha]_D^{23}$ +21° (*c* 2.4, chloroform).

Anal. Calc. for $C_{17}H_{33}NO_9S_4$: C, 38.99; H, 6.35; N, 2.67; S, 24.49. Found: C, 39.20; H, 6.34; N, 2.67; S, 24.30.

Compound **12** (2.6 g) was heated with sodium benzoate (1.5 g) in *N*,*N*-dimethylformamide (50 ml) for 5 h at 100°. The mixture was poured into water, extracted with chloroform, dried, and concentrated. The residue was recrystallised from ether–light petroleum, to give **11** (1.92 g, 70.6%).

2-Acetamido-5,6-anhydro-2-deoxy-3,4-O-isopropylidene-L-idose diethyl dithioacetal (**13**). — To a solution of **11** (4.5 g) in chloroform (80 ml) was added methanolic

sodium methoxide (prepared from 10 ml of methanol and 425 mg of sodium) at −15°. The mixture was kept at 0° for 2 h, and then extracted with saturated, aqueous sodium chloride, dried, and concentrated. Recrystallisation of the residue from ether–light petroleum gave **13** (2.1 g, 73%), m.p. 109–110°, $[\alpha]_D^{23}$ −2° (*c* 1.2, chloroform).

Anal. Calc. for $C_{15}H_{27}NO_4S_2$: C, 51.54; H, 7.79; H, 4.01; S, 18.35; Found: C, 51.52; H, 7.75; N, 3.89; S, 18.57.

2-Acetamido-2,5,6-trideoxy-5,6-epithio-3,4-O-isopropylidene-D-glucose diethyl dithioacetal (**14**). — To a solution of **13** (1.68 g) in methanol (70 ml) was added thiourea (390 mg), and the mixture was kept at 25° for 3 days. Water (100 ml) was then added and the methanol was evaporated under diminished pressure. The aqueous solution was extracted with chloroform, and the extract was dried and concentrated. Recrystallisation of the residue from ether–light petroleum gave **14** (1.2 g, 68.6%), m.p. 114°, $[\alpha]_D^{23}$ −12.5° (*c* 1.2, chloroform).

Anal. Calc. for $C_{15}H_{27}NO_3S_3$: C, 49.28; H, 7.45; N, 3.83; S, 26.31. Found: C, 49.11; H, 7.77; N, 3.82; S, 26.41.

2-Acetamido-6-O-acetyl-5-S-acetyl-2-deoxy-3,4-O-isopropylidene-5-thio-D-glucose diethyl dithioacetal (**15**). — A mixture of **14** (1.09 g), acetic acid (1 ml), acetic anhydride (5 ml), and potassium acetate (0.60 g) was boiled under reflux for 6.5 h and then poured onto cracked ice. The syrupy residue solidified during 2 h. The crystals were collected and dried, to give **12** (1.1 g, 75%), m.p. 138°, $[\alpha]_D^{23}$ +14.5° (*c* 2.76, chloroform), ν_{max} 1702 cm^{-1} (SAc).

A mixture of **15** (2.5 g), acetic anhydride (25 ml), acetic acid (5 ml), and sulfuric acid (0.5 ml) was stored at 0° for 3 days, and then poured into aqueous sodium hydrogencarbonate and extracted with chloroform. The dried extract was concentrated and the residue was eluted from silica gel with light petroleum–acetone (7:3), to give **7** (420 mg), m.p. 174–175°, $[\alpha]_D^{23}$ +195.5° (chloroform).

2-Acetamido-2-deoxy-5-thio-D-glucose (**8**). — Zemplén deacetylation[6] of **7** (0.5 g) and elution of the product from silica gel with chloroform–methanol (7:3) gave **8** (120 mg), m.p. 115–117°, $[\alpha]_D^{23}$ +88° (*c* 1, methanol; equil.).

Anal. Calc. for $C_8H_{15}NO_5S$: C, 40.49; H, 6.37; N, 5.90; S, 13.50. Found: C, 40.71; H, 6.42; N, 5.98; S, 13.25.

ACKNOWLEDGMENTS

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