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Note

A new method for *N*-deacetylation of 2-acetamido-2-deoxy sugars

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N-Deacetylation of 2-acetamido-2-deoxy sugars can be accomplished using strong base in aqueous solution[1] or in methyl sulphoxide[2] under drastic conditions. Hydrazinolysis with anhydrous hydrazine or anhydrous hydrazine together with hydrazine sulphate[3,5] are sometimes useful methods.

We now report a new *N*-deacetylation procedure based upon transamidation with trifluoroacetic anhydride–trifluoroacetic acid followed by cleavage of the resulting *O*- and *N*-trifluoroacetyl functions.

Treatment of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside with 1:1 trifluoroacetic anhydride–trifluoroacetic acid (TFAA/TFA reagent) at room temperature rapidly gave methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-trifluoroacetyl-α-D-glucopyranoside, but at 100° for 48 h, transamidation occurred to give methyl 2-deoxy-2-trifluoroacetamido-3,4,6-tri-*O*-trifluoroacetyl-α-D-glucopyranoside, probably *via* the *N*-tri-

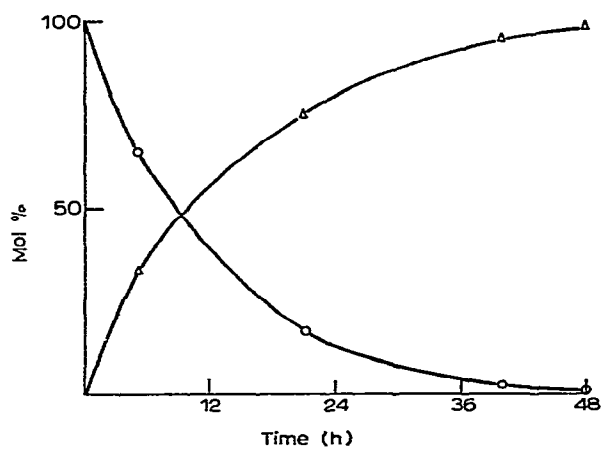


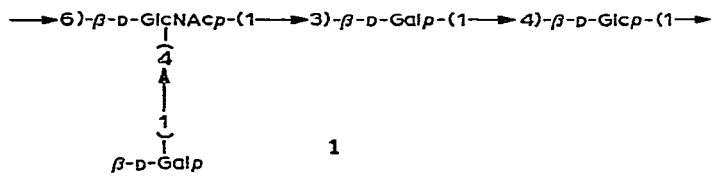
Fig. 1. Treatment of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside with TFAA/TFA at 100°. Analysis of the reaction mixture after *O*-detrifluoroacetylation and reacetylation: methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (○——○) and methyl 2-deoxy-2-trifluoroacetamido-α-D-glucopyranoside (△——△).

fluoroacetyl-*N*-acetyl derivative. The reaction was monitored by g.l.c.–m.s. after *O*-detrifluoroacetylation and acetylation (Fig. 1). After 48 h, no starting material remained and methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranoside was obtained in nearly quantitative yield.

Methyl 2-deoxy-2-trifluoroacetamido-α-D-glucopyranoside could be readily and quantitatively *N*-detrifluoroacetylated with methanolic ammonia or ethanolic sodium borohydride.

N-Deacetylation is a prerequisite for nitrous acid degradation of complex carbohydrates containing 2-acetamido-2-deoxy sugars, and since the glycosidic linkages of hexopyranosides, pentopyranosides, 6-deoxyhexopyranosides, and 6-deoxyhexofuranosides are stable[6] in the TFAA/TFA mixture, the new *N*-deacetylation method was applied to a model polysaccharide.

The capsular polysaccharide[7] from *Diplococcus pneumoniae* Type 14, which has the structure **1**, was treated with the TFAA/TFA reagent and subsequently with aqueous sodium borohydride. Nitrous acid degradation[7] of the *N*-deacetylated polysaccharide gave a product which contained D-glucose and D-galactose in the ratio 1:1.9 and only a trace of 2-acetamido-2-deoxy-D-glucose, demonstrating that *N*-deacetylation was >95%. After reacetylation, sugar analysis and methylation analysis showed that no significant degradation of the polysaccharide had occurred during the *N*-deacetylation. If some degradation of the polysaccharide does occur, a 40:1 TFAA/TFA reagent should be used; this effects transamidation at 100° for 48 h with a minimum of degradation.



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The new method should complement other *N*-deacetylation procedures, and will be especially useful when the carbohydrate structure is sensitive to strong base.

EXPERIMENTAL

Gel chromatography was performed on columns (3 × 140 cm and 2 × 45 cm) of Sephadex G-25 (fine) by elution with water. Optical rotations were measured with a Perkin–Elmer polarimeter Model 241. For g.l.c., a Perkin–Elmer 3920 instrument fitted with a column of 3% of OV-1 on Chromosorb W (80–100 mesh) at 180° was used. G.l.c.–m.s. was performed on a Varian MAT 311-A instrument fitted with a glass-capillary column (25 m × 0.25 mm) wall-coated with SE-30. Mass spectra were recorded at 70 eV with an ionization current of 3 mA and an ion-source temperature of 150°.

Methyl 2-deoxy-2-trifluoroacetamido-α-D-glucopyranoside (**2**). — Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (0.8 g) was treated with TFAA/TFA

reagent (1:1, 10 ml) at 100° for 48 h in a sealed tube. The resulting dark solution was cooled and concentrated to dryness, and a solution of the residue in methanol was basified with aqueous 25% ammonia. After 1 h at room temperature, the solution was concentrated to dryness and the residue was extracted with water (5 ml). The extract was eluted from a column (3 × 140 cm) of Sephadex G-25 (fine) with monitoring by polarimetry. Methyl 2-deoxy-2-trifluoroacetamido-α-D-glucopyranoside (807 mg, 82%), which was eluted in front of three coloured bands, when crystallized from acetone–light petroleum (b.p. 40–60°), had m.p. 136–138°, $[\alpha]_D^{20}$ +99° (*c* 1, water), ν_{max}^{KBr} 1710 cm^{-1} ($NHCOCF_3$) (Found: C, 36.5; H, 5.1; F, 19.1; N, 4.7. $C_9H_{14}F_3NO_6$ calc.: C, 37.4; H, 4.9; F, 19.7; N, 4.8%).

The mass spectrum of the triacetate of **2** contained *inter alia* peaks for $(M - OMe)^+$ at *m/e* 384.0845 (calc. for $C_{14}H_{17}F_3NO_8$, 384.0851) and for $(M - AcOH)^+$ at *m/e* 355.0843 (calc. for $C_{13}H_{16}F_3NO_7$, 355.0847).

N-*Detrifluoroacetylation of* **2**. — (*a*) *With ammonia in aqueous methanol.* A solution of **2** (940 μg) and perseitol (930 μg, internal standard) in 1.5M ammonia in methanol–water (4:1) was stored at room temperature for 48 h. No starting material then remained, and methyl 2-amino-2-deoxy-α-D-glucopyranoside had been formed in quantitative yield as determined by g.l.c.–m.s. of the acetylated reaction product.

(*b*) *With sodium borohydride in aqueous ethanol.* To a solution of **2** (940 μg) and perseitol (930 μg, internal standard) in 90% aqueous ethanol (2 ml) was added sodium borohydride (15 mg). After 10 h at room temperature, only trace amounts of starting material remained, and methyl 2-amino-2-deoxy-α-D-glucopyranoside had been formed in nearly quantitative yield as determined by g.l.c.–m.s. of the acetylated reaction product.

N-*Deacetylation of the capsular polysaccharide S-14*[7]. — The capsular polysaccharide (50 mg) was heated with TFAA/TFA reagent (1:1, 10 ml) at 100° for 48 h in a sealed tube. The resulting dark, clear solution was cooled and concentrated, and a solution of the residue in 1.5M ammonia in methanol–water (4:1, 10 ml) was stored for 1 h and then concentrated to dryness. The product was dissolved in water (10 ml), and sodium borohydride (50 mg) was added. After 10 h at room temperature, the solution was acidified with glacial acetic acid, and boric acid was removed by codistillation with methanol. The *N*-deacetylated polysaccharide (40 mg) was isolated by chromatography on a column (2 × 45 cm) of Sephadex G-25 (fine).

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REFERENCES

1 S. HANESSIAN, *Methods Carbohydr. Chem.*, 6 (1972) 208–214.
2 C. ERBING, K. GRANATH, L. KENNE, AND B. LINDBERG, *Carbohydr. Res.*, 47 (1976) C5–C7.

3 Z. YOSIZAWA, T. SATO, AND K. SCHMID, *Biochim. Biophys. Acta*, 121 (1966) 417–420.
4 B. A. DMITRIEV, Y. A. KNIREL, AND N. K. KOCHETKOV, *Carbohydr. Res.*, 29 (1973) 451–457.
5 B. A. DMITRIEV, Y. A. KNIREL, AND N. K. KOCHETKOV, *Carbohydr. Res.*, 30 (1973) 45–50.
6 B. NILSSON AND S. SVENSSON, unpublished data.
7 B. LINDBERG, J. LÖNNGREN, AND D. A. POWELL, *Carbohydr. Res.*, 58 (1977) 177–186.