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Derivatization Procedures for Reducing Oligosaccharides, Part 2: Chemical Transformation of 1-Deoxy-1-(4-Trifluoroacetamidophenyl)aminoalditols

ELISABETH KALLIN[1], HANS LÖNN[2] and THOMAS NORBERG[2]

[1]*Department of Carbohydrate Chemistry, University of Lund, S-223 70 Lund, Sweden*
[2]*Organic Synthesis Department, BioCarb AB, S-223 70 Lund, Sweden*

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Three chemical transformations of oligosaccharide 1-deoxy-1-(4-trifluoroacetamidophenyl)aminoalditols are described. 1) Oxidation with hydrogen peroxide to give the corresponding reducing oligosaccharides. 2) Oxidation with cerium ammonium nitrate to give the corresponding 1-amino-1-deoxyalditols. 3) Treatment with acetic anhydride to give the corresponding *N*-acetylated derivatives, which are more stable towards oxidation.

In a previous paper [1], we showed that reductive amination of reducing oligosaccharides with 4-trifluoroacetamidoaniline (TFAN)/sodium cyanoborohydride gave 1-deoxy-1-(4-trifluoroacetamidophenyl)aminoalditols (TFAN derivatives), which were useful in the preparation of glycoconjugates or in chromatographic separations. We have now further studied the chemical reactions of oligosaccharide TFAN derivatives, and we here report two different oxidative degradation reactions. Hydrogen peroxide converts an oligosaccharide TFAN derivative back into the parent sugar, whereas cerium ammonium nitrate converts it into the corresponding 1-amino-1-deoxyalditol (Fig. 1). Furthermore, we have found that *N*-acetylation of an oligosaccharide TFAN derivative renders it more stable towards oxidative reagents, including air.

Results

Oxidation with Hydrogen Peroxide

Treatment of the TFAN derivative of lactose (**1**) with aqueous hydrogen peroxide containing acetic acid at room temperature for 20 h gave, after purification, lactose in 76% yield. Treatment of the TFAN derivative of lacto-*N*-tetraose in the same way gave 63% of the parent sugar. Omitting the acetic acid resulted in slower reactions, while replacing it with trifluoroacetic acid gave similar results. Other oxidants tested (oxygen, iron(III) chloride, silver nitrate, sodium periodate) gave inferior results.

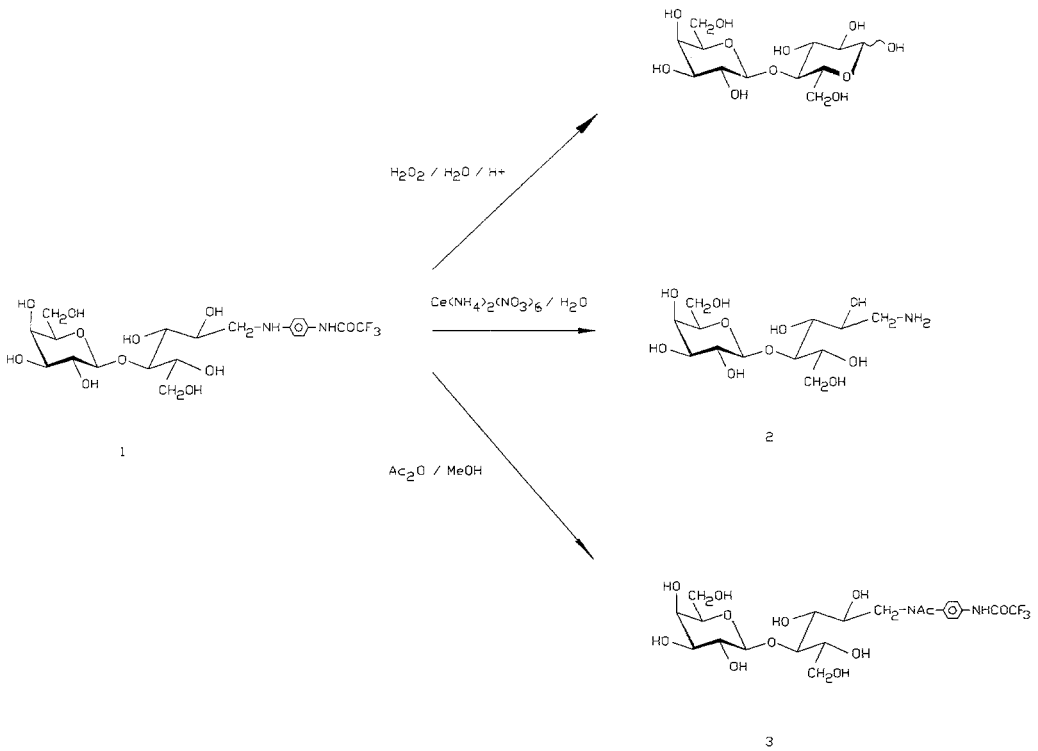

Figure 1. Reactions of the TFAN derivative of lactose.

Oxidation with Cerium Ammonium Nitrate

Treatment of an aqueous solution of (**1**) with cerium ammonium nitrate at 0°C for 2 h gave the corresponding aminoalditol (**2**) in 94% yield. The compound was indistinguishable from a sample prepared [2] by reductive amination with ammonium acetate/sodium cyanoborohydride. Reaction of other oligosaccharide TFAN derivatives gave similar results (Table 1).

N-Acetylation

Treatment of the TFAN derivative of lactose (**1**) with acetic anhydride/methanol, 1/9 by vol, at room temperature resulted in rapid and quantitative *N*-acetylation of the secondary amine function. The derivative (**3**) was more stable towards oxidation than the parent amine. For example, an aqueous solution of (**1**) gradually became miscoloured during a week in contact with air, while no degradation or miscolouring was seen with (**3**) under these conditions.

Table 1. Oxidation of oligosaccharide TFAN derivatives with cerium ammonium nitrate. 20 mg starting material was used in each experiment.

TFAN-derivative	Yield aminoalditol (%)
Lacto-*N*-tetraose	66
Lacto-*N*-fucopentaose I	83
Lacto-*N*-fucopentaose II	75
Lacto-*N*-fucopentaose III	79
Lacto-*N*-difucohexaose I	83

Discussion

Oligosaccharide TFAN derivatives have already been shown [1] to be useful for conjugations or chromatographic separations. Since they are *p*-phenylenediamine derivatives, they are relatively sensitive towards oxidising reagents. The stability can be increased by *N*-acetylation, and we now recommend this simple extra transformation if later conjugation to other macromolecules (such as proteins or matrixes) are intended. On the other hand, the sensitivity of the TFAN derivatives towards oxidation makes possible the reverse reaction of reductive amination, i.e. oxidative deamination, with resulting formation of the reducing sugars. Of the oxidants tested, hydrogen peroxide in acidic solutions was most effective for this kind of transformation. The yields in the two examples presented here (76 and 63%) can probably be further improved. Thus, it is now possible to derivatize an oligosaccharide mixture with TFAN, separate it into its components by virtue of the good chromatographic properties of the TFAN derivatives, and recover the reducing oligosaccharides in fair yields by hydrogen peroxide treatment of the TFAN derivatives.

The mechanism of the oxidation reaction probably involves attack of OH^+ or its equivalent on the free electron pair of the secondary amine nitrogen (Fig. 2), followed by elimination of water from the hydroxylamine formed to give the imine, which then hydrolyzes to give the sugar aldehyde function. Similar reactions are known [3-5]. Preliminary experiments with the analogous oligosaccharide aniline derivatives showed that these can also be oxidized in the same manner, although in this case the reaction required a longer time.

In contrast to the reaction with hydrogen peroxide, reaction of TFAN derivatives with cerium ammonium nitrate gave the 1-amino-1-deoxyalditols. The initial step in this mechanism is probably electron abstraction (Fig. 3). Further reaction of the formed quinonediimide could lead to the observed product as outlined. Similar reactions are known to occur [3, 6], e.g. during the process of photographic development [7] with *p*-phenylenediamine developers. In contrast to the oxidation with hydrogen peroxide, the phenylenediamine structure is necessary here for good yields, since the analogous oligosaccharide aniline derivatives gave complex reaction mixtures with cerium ammonium nitrate (preliminary experiments).

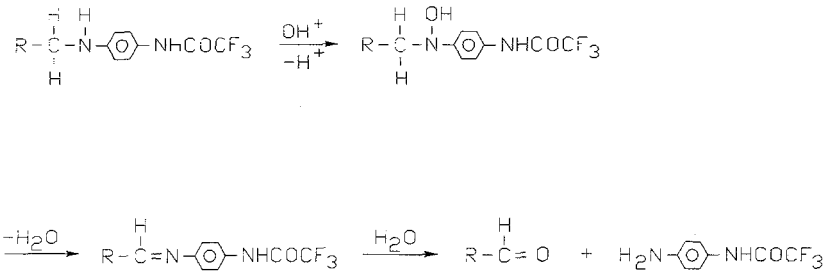

Figure 2. Proposed reaction path for oxidation of sugar TFAN derivative with hydrogen peroxide.

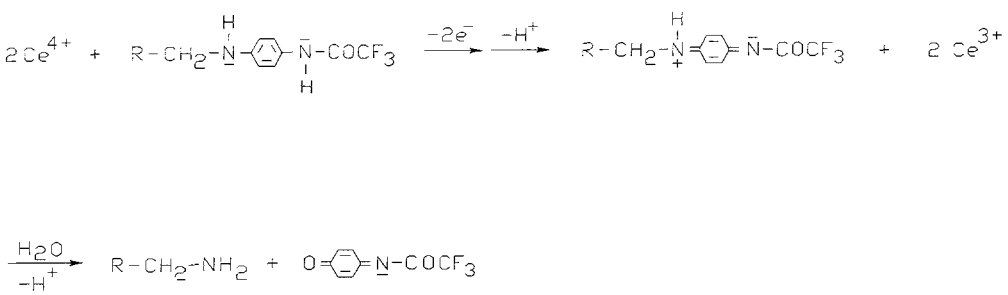

Figure 3. Proposed reaction path for oxidation of sugar TFAN derivative with cerium ammonium nitrate.

Experimental

General Methods

Degassed distilled water was used. All reactions were performed under nitrogen. Concentrations were performed at < 40°C bath temperature. Optical rotations were recorded at 23°C with a Perkin-Elmer 241 polarimeter. NMR spectra were recorded at 300 K in ^2H_2O, using a Bruker AM 500 instrument. The following reference signals were used: acetone δ 2.225 (^1H), dioxane δ 67.4 (^{13}C). Only selected NMR data are reported. Thin layer chromatography was performed on silica gel 60 F-254 (Merck, Darmstadt, W. Germany) with EtOAc/HOAc/MeOH/H_2O, 6/3/3/2 by vol, as eluant. The spots were visualized with UV light or by charring with sulfuric acid. The oligosaccharide TFAN derivatives used were obtained as described before [1]. Bond Elut Si and C-18 cartridges were from Analytichem International (Harbor City, CA, USA). Bio-Gel P-2 columns (Bio-Rad, Richmond, CA, USA) were packed and eluted with water containing 0.05% 2,2,2-trichlorobutanol and 0.02% acetic acid.

*Oxidation of Lactose-TFAN (**1**) with H_2O_2. Regeneration of Lactose*

A solution of 100 mg lactose-TFAN in 1.0 ml water containing 100 μl 30% H_2O_2 and 100 μl acetic acid was left at room temperature. After 48 h TLC showed that most of the starting material had disappeared. The reaction mixture was freed from by-products by passing the brown aqueous solution through, successively, a column of silica gel, and octadecyl silane gel (Bond Elut, Si and C-18 respectively, 1000 mg columns). The water eluate was concentrated to 2 ml and applied on a Bio-Gel P-2 column. Lyophilization of the appropriate fractions yielded lactose (49 mg, 76%). The ^1H-NMR spectrum of the product was identical to that of lactose.

Oxidation of 48 mg lacto-*N*-tetraose-TFAN by the above method yielded lacto-*N*-tetraose (24 mg, 63%), as verified by ^1H-NMR spectroscopy.

*4-O-β-D-Galactopyranosyl-1-amino-1-deoxy-D-glucitol (**2**)*

*a) from (**1**) by oxidative cleavage with cerium ammonium nitrate:* To a solution of 100 mg lactose-TFAN in 3.0 ml water was added a solution of 310 mg $Ce(NH_4)_2(NO_3)_6$ in 3.0 ml water at 0°C. After 2 h TLC showed that all starting material had disappeared. The brownish solution was passed through a column of octadecyl silane gel (Bond Elut, C-18, 1000 mg column) in order to remove miscolouring by-products. The column was washed thoroughly with water and the combined water eluates were evaporated and applied to a Bio-Gel P-2 column. Lyophilization of the appropriate fractions gave (**2**) (61 mg, 94%). $[\alpha]_D$ +10.2° (H_2O, c = 1.0), $[\alpha]_D^{25}$[2] +10.6° (H_2O, c = 0.1). ^{13}C-NMR data: δ 42.5 (C-1); 61.9, 62.7 (C-6, C-6'); 69.3, 69.5, 71.4, 71.6, 71.7, 73.2, 75.9, 79.0 (C-2 - C-5, C-2' - C-5'); 103.6 (C-1'). FAB-MS of the product showed an M+1 ion at m/z 344.

Other milk oligosaccharide TFAN derivatives were oxidized by the above method to give the yields shown in Table 1.

b) from lactose by reductive amination [2]: A solution of 1.0 g lactose, 90 g ammonium acetate and 1.8 g sodium cyanoborohydride in 100 ml water was stirred at 50°C for 16 h.

TLC then indicated that all lactose was reduced. Most of the ammonium acetate was removed by evaporation at 1.3 kPa at 50°C bath temperature with addition of 3 × 50 ml water. In order to prevent extensive formation of bubbles during the gel filtration excess sodium cyanoborohydride was removed. This was done by dissolving the residue in water and stirring it overnight at room temperature with 50 ml anion exchange resin (Dowex 1-X2, Cl^--form).

Gel filtration on Bio-Gel P-2 gave (**2**) (0.94 g, 94%). The ^{13}C-NMR spectrum was indistinguishable from that of (**2**) prepared by method a).

*N-Acetylated Lactose-TFAN Derivative (**3**)*

A solution of 100 mg lactose-TFAN in 10 ml MeOH/acetic anhydride, 9/1 by vol, was left at room temperature. The reaction was monitored on TLC, where the acetylated compound corresponded to the slower moving spot. After 2 h the solvents were evaporated and the residue co-distilled twice with water. Finally the product was taken up in water and lyophilized to give **3** (104 mg, 96%). ^{13}C-NMR data: δ 22.8 (NAc); 52.2 (C-1); 61.5, 62.8 (C-6, C-6'); 69.3, 70.2, 70.7, 71.9, 72.2, 73.3, 75.9, 81.3 (C-2 - C-5, C-2' - C-5'); 104.0 (C-1'); 116.7 (q, J 286 Hz, CF_3); 124.2-141.0 (C-Ar); 157.9 (q, J 38.2 Hz, **C**OCF_3); 175.4 (**C**OCH_3). FAB-MS of the product showed an M+1 ion at m/z 572.

After several weeks in aqueous solution at room temperature (**3**) showed no signs of degradation, whereas a similar solution of (**1**) gradually became miscoloured.

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