Pergamon

Tetrahedron Letters 39 (1998) 8101–8104

TETRAHEDRON LETTERS

A Convenient Synthesis of α- and β-D-Glucosamine-1-phosphate and Derivatives

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Received 23 July 1998; accepted 30 August 1998

Abstract: Derivatives of α- and β-D-glucosamine-1-phosphate and of α-D-galactosamine-1-phosphate, as well as free β-D-glucosamine-1-phosphate, were prepared stereoselectively and in high yield by way of the opening of the 1,2-oxazoline derived from the corresponding 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-hexopyranosyl bromide with dibenzyl phosphate.

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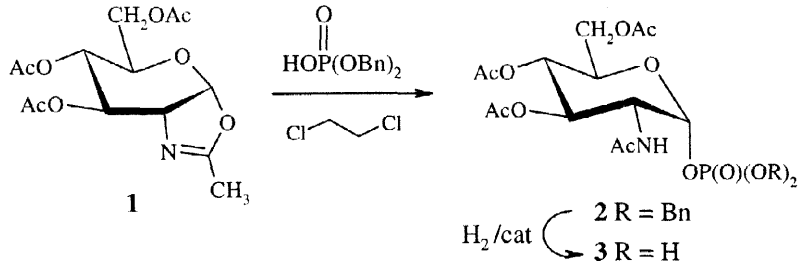
To prepare analogs of UDP-GalNAc and UDP-GlcNAc as probes of HexNAc-transferase-mediated processes,[1] we became interested in the synthesis of derivatives of hexosamine-1-phosphates carrying a N-trifluoroacetyl group. This group replaces advantageously the acetamido group in amino sugars[2] and related compounds: it provides a convenient reporter group for binding studies[3,4a] and its presence can, in certain cases, increase the affinity of the substrate analog for carbohydrate-binding proteins and enzymes.[4] In addition, the N-$COCF_3$ analog of UDP-GlcNAc was shown recently to be a substrate for the 'core' GlcNAc-transferase involved in the biosynthesis of O-linked glycoproteins.[5]

Among the numerous methods available for the preparation of glycosyl phosphates,[6] the most reliable one for hexosamine-1-phosphates is the opening of an oxazoline with dibenzyl phosphate.[7] This method provides directly and exclusively the thermodynamic α-anomer of the phosphotriester; extensive investigations



by Jeanloz and cowokers[7c] failed to reveal the formation of the labile kinetic β-epimer. The free β-phosphate (β-epimer of **3**) has been prepared in low yield by the reaction of **1** with anhydrous phosphoric acid[8] and by other, tedious methods.[9]

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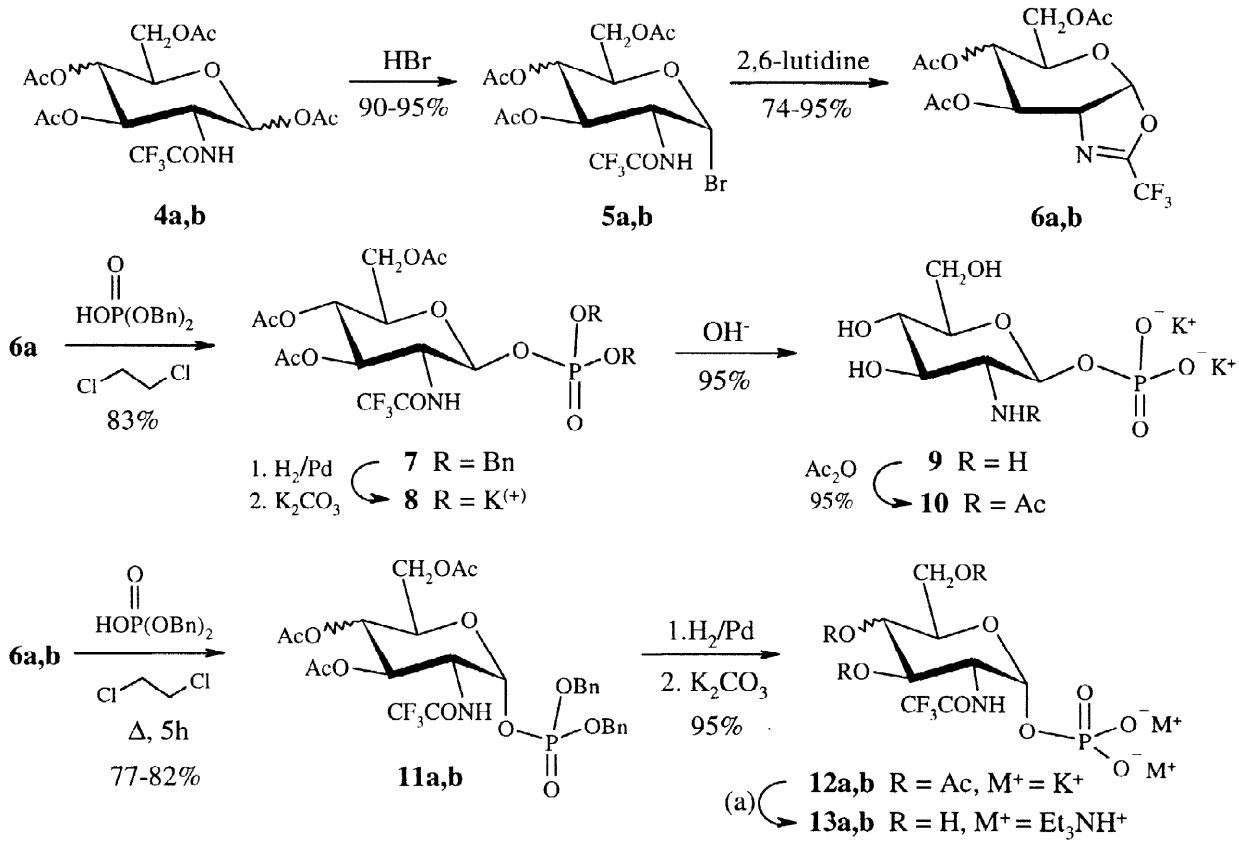
PII: S0040-4039(98)01838-3

We report herein the synthesis of N-trifluoroacetyl α-D-hexosamine-1-phosphates by way of the oxazoline procedure as well as a convenient method for the preparation of β-D-glucosamine-1-phosphate and derivatives.

The peracetates of 2-deoxy-2-trifluoroacetamido-D-gluco- and D-galactopyranose **4a** and **4b**[10] were converted into the bromides **5a** and **5b** in high yield using HBr in acetic acid.[10a] The labile bromides were immediately treated with 2,6-lutidine (1.1 equiv.) in acetonitrile,[11] thus affording in 1 h the corresponding trifluoromethyl oxazolines **6a** and **6b**.[12] These reactive oxazolines could be isolated in excellent yield after rapid purification by flash chromatography (petroleum ether/EtOAc 2:1 containing 0.1 % Et_3N) and stored at -20°C for several days with minimal degradation. The *gluco* oxazoline **6a** had been obtained previously from the bromide in the course of a glycosylation[13] in the presence of a silver salt and was recently identified again in a reaction mixture by Tanner and coworkers.[5] The potential of these oxazolines as precursors of hexosaminyl phosphates was then investigated.



(a) i. guanidine, MeOH, 30 min, R.T.; ii, Dowex-H^+; iii, Et_3N
a = *gluco* series, **b** = *galacto* series

The reaction of the D-*gluco* oxazoline **6a** with dibenzyl phosphate gave a single product; the NMR data[14] of this product were entirely consistent with the structure of the β-phosphotriester **7**. Remarkably, this compound was sufficiently stable to be purified by flash chromatography and isolated in 83% yield. Compound **7** was found to be a convenient precursor of derivatives of β-D-glucosamine-1-phosphate and of the parent compound. The benzyl phosphates were hydrogenolyzed and the resulting monoester neutralized with K_2CO_3 to afford the β-phosphate **8** in 96% yield. All of the acyl groups were then cleaved by saponification with aqueous

hydroxide, thus providing the free β-D-glucosamine-1-phosphate **9** in essentially quantitative yield (spectrum in Fig. 1). This compound had been prepared previously by a lengthy procedure and its configuration ascertained only on the basis of optical rotation data.[9a] Finally, reacetylation of **9** using acetic anhydride in the presence of K_2CO_3 gave N-acetyl-β-D-glucosamine-1-phosphate **10** in high yield. That the stereochemical integrity of the β-phosphate was conserved throughout the sequence was demonstrated by the identity of the ^{13}C-NMR spectrum of **10** with the reported data.[15] This compound is an essential component of the glycosyl donor (β-GlcNAc-P-polyprenol) involved in the biosynthesis of lipoteichoic acids.[16]

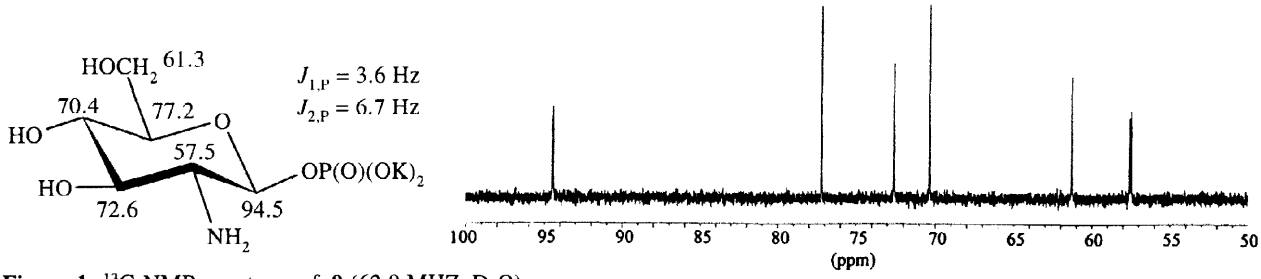


Figure 1: ^{13}C-NMR spectrum of **9** (62.9 MHZ, D_2O)

When the reaction of the oxazolines **6a** and **6b** was performed at higher temperature (5h at reflux in 1,2-dichloroethane), the initially formed β-phosphotriesters isomerized completely to the more stable α-phosphates **11a**[17] and **11b**. Both of these compounds could be purified by flash chromatography before debenzylation and were isolated in 82% and 77% yield, respectively. Debenzylation of **11a** and **11b** was performed as described for **7** and afforded the α-D-hexosaminyl-1-phosphates **12a** and **12b** in high yield. After extensive experimentation, the acetates could be cleaved selectively without affecting the trifluoroacetamido group by treatment with guanidine[18] (2 equiv.) in MeOH (30 min); the mixture was neutralized by passage through an acidic ion exchanger (H^+ form) and the products were then converted to their triethylammonium salts **13a**[19] and **13b**. These compounds can be further modified or used as precursors of modified sugar nucleotides. Further work in this direction is in progress.

The remarkable difference of reactivity that characterizes the glycosyl phosphates derived from hexosamines carrying an acetyl group or its trifluoro equivalent at N-2 is probably the consequence of the strong electronic inductive effect of the trifluoroacetyl group: this effect opposes the formation of a positive charge at the anomeric center and therefore stabilizes the glycosyl phosphate function, thus preventing its spontaneous isomerisation under acidic conditions.

Acknowledgement: The award of a fellowship to P.B. by the "Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche" is gratefully acknowledged.

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12. Data for **6b**: ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 2.06 (s, 6H), 2.13 (s, 3H) (3 $COCH_3$), 4.10 - 4.35 (m, 4H, H-2,5,6A,6B), 4.92 (dd, 1H, J = 3.5, 7.7 Hz, H-3), 5.47 (narrow dd, 1H, J = 1.9, 3.5 Hz, H-4), 6.32 (d, 1H, J = 7.2 Hz, H-1); ^{13}C-NMR ($CDCl_3$, 62.9 MHz): δ 20.1, 20.2, 20.3, 61.2, 62.3, 64.2, 69.9, 70.7, 104.9, 115.7 (q, $J_{C,F}$ = 275 Hz, CF_3), 155.9 (q, $J_{C,F}$ = 41 Hz, CCF_3), 169.5, 169.5, 170.1.
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14. Data for **7**: $[\alpha]_D^{20}$ - 6.7 (*c* 4.7, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 1.97, 2.01, 2.05 (3s, 3 x 3H, 3 $COCH_3$), 3.69 (ddd, 1H, J = 2.2, 5.1, 10.5 Hz, H-5), 4.08 (dd, 1H, J = 2.2, 12.6 Hz, H-6A), 4.21 (dd, 1H, J = 5.2, 12.5 Hz, H-6B), 4.41 (br. q, 1H, H-2), 5.02 (A of ABX, 2H, $J_{H,P}$ = 7.0, $J_{H,H}$ = 11.6 Hz) and 5.11 (B of ABX, 2H, $J_{H,P}$ = 6.9, $J_{H,H}$ = 11.9 Hz) (2 $OCH_2C_6H_5$), 5.14 (t, 1H, J = 10 Hz, H-4), 5.42 (dd, 1H, J = 9.4, 9.7 Hz, H-3), 5.61 (t, 1H, $J_{H,H} \approx J_{H,P} \approx$ 8.2 Hz, H-1), 7.20 - 7.35 (m, 10H, 2 $OCH_2C_6H_5$), 8.70 (d, 1H, J = 9.4 Hz, NH); ^{13}C-NMR ($CDCl_3$, 62.9 MHz): δ 20.2, 20.3, 20.3, 54.0 (d, $J_{C,P}$ = 9.1 Hz, C-2), 61.4, 67.8, 69.9 (d, $J_{C,P}$ = 4.6 Hz) and 70.0 (d, $J_{C,P}$ = 4.8 Hz) [$(C_6H_5CH_2O)_2P$], 71.8, 72.5, 96.1 (d, $J_{C,P}$ = 4.2 Hz, C-1), 115.7 (q, $J_{C,F}$ = 288 Hz, CF_3), 127.6 - 128.5 (Ar-CH's), 134.7 (d, $J_{C,P}$ = 7.9 Hz) and 135.1 (d, $J_{C,P}$ = 7.9 Hz) (2 Ar-C's), 157.5 (q, $J_{C,F}$ = 37.6 Hz, CCF_3), 169.1, 170.3, 170.8.
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17. Data for **11a**: $[\alpha]_D^{20}$ - 53.3 (*c* 0.73, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 2.00, 2.02, 2.04 (3s, 3 x 3H, 3 $COCH_3$), 3.97 (dd, 1H, J = 1.8, 12.3 Hz, H-6A), 4.12 (ddd, 1H, J = 1.8, 4, 10 Hz, H-5), 4.20 (dd, 1H, J = 3.8, 12.3 Hz, H-6B), 4.39 (ddt, 1H, $J_{H,H}$ = 3, 8.6, 10.8 Hz, $J_{H,P}$ = 3 Hz, H-2), 5.02 (d, 2H, $J_{H,P}$ = 9.3 Hz) and 5.06 (d, 2H, $J_{H,P}$ = 8.8 Hz) (2 $OCH_2C_6H_5$), 5.16 (t, 1H, J = 9.8 Hz, H-4), 5.30 (dd, 1H, J = 9.5, 10.8 Hz, H-3), 5.70 (dd, 1H, $J_{H,H}$ = 3.2 Hz, $J_{H,P}$ = 6.4 Hz, H-1), 7.2 - 7.4 (m, 10H, 2 C_6H_5), 7.62 (d, 1H, J = 8.8 Hz, NH); ^{13}C-NMR ($CDCl_3$, 62.9 MHz): δ 20.3, 20.5, 20.6, 52.4 (d, $J_{C,P}$ = 7.9 Hz, C-2), 61.0, 67.2, 69.6, 69.7, 70.1 [2C, $J_{C,P}$ = 5.5 Hz, $(C_6H_5CH_2O)_2P$], 95.0 (d, $J_{C,P}$ = 6.7 Hz, C-1), 115.5 (q, CF_3), 128.0 - 128.9 (Ar-CH's), 134.9 (d, $J_{C,P}$ = 6.1 Hz) and 135.1 (d, $J_{C,P}$ = 6.7 Hz) (2 Ar-C's), 157.6 (q, $J_{C,F}$ = 38.1 Hz, CCF_3), 169.1, 170.5, 170.9.
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19. Data for **13a**: ^{13}C-NMR ($CDCl_3$, 62.9 MHz): δ 8.9, 47.3, 55.4 (d, $J_{C,P}$ = 7.3 Hz, C-2), 61.1, 70.5, 71.1, 73.3, 93.3 (d, $J_{C,P}$ = 6.1 Hz, C-1), 116.5 (q, $J_{C,F}$ = 286 Hz, CF_3), 160.1 (q, $J_{C,F}$ = 38.1 Hz, CCF_3).