CHEMISTRY LETTERS, pp. 1051-1054, 1986.

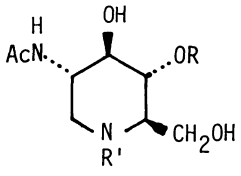
Synthesis of 2-Acetamido-1,5-imino-1,2,5-trideoxy-D-mannitol and of 2-Acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol, a Potent and Specific Inhibitor of a Number of β-N-Acetylglucosaminidases

George W. J. FLEET,* Paul W. SMITH, Robert J. NASH,+ Linda E. FELLOWS,+
Raj B. PAREKH,++ and Thomas W. RADEMACHER++
Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY, UK
+Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK
++Department of Biochemistry, Oxford University, Oxford OX1 3QY, UK

The stereochemical outcome of the azide displacement of triflates derived from a piperidin-3-ol depends on the protecting group on the ring nitrogen and allows the synthesis of 2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol (a potent and specific inhibitor of a number of β-N-acetylglucosaminidases) and of 2-acetamido-1,5-imino-1,2,5-trideoxy-D-mannitol.

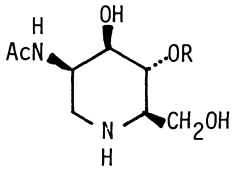
A number of glycosidase enzymes have been found to be elevated in malignancy and associated with tumor cell invasion and degradation of basement membrane components. Elevations of serum β-N-acetylglucosaminidase and β-glucuronidase most commonly have been found to occur, and these enzymes have been shown to be secreted into the extracellular medium by many different tumor cell types in vitro. It is therefore of considerable interest to develop inhibitors against these enzymes.[1,2] Several specific inhibitors of glycosidase activity, which structurally resemble azapyranoses, have been isolated from plants[3] and microorganisms. Deoxynojirimycin 3 inhibits the enzyme catalysed hydrolysis of glucopyranosides by a number of glucosidases;[4,5] 3 is related structurally to glucopyranosides by removal of the anomeric substituent and replacement of the pyranose ring oxygen by an amino group. Deoxymannojirimycin 4, similarly structurally related to mannopyranosides, is a specific inhibitor of some mannosidases.[6] The synthetic compound 1,5-dideoxy-1,5-imino-L-fucitol has been demonstrated to be a highly potent inhibitor of the enzymic activity of a number of α-L-fucosidases.[7] This paper describes the synthesis of 1, the corresponding analogue of N-acetylglucosamine (2-acetamido-2-deoxy-D-glucose) and of the epimer 2, the analogue of N-acetylmannosamine; a preliminary evaluation of these compounds as glycosidase inhibitors is reported.

The benzyloxycarbonyl protected amine 6,[8] with only the C-5 hydroxyl group of the original sugar unprotected, was esterified with trifluoromethanesulphonic anhydride in dichloromethane in the presence of pyridine at -30 °C and the resulting triflate was treated with sodium azide in dimethyl formamide at 60 °C for 12 h to give as the major isolated product the azide 8[9] in which the



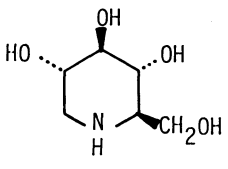
1 R=R'=H

14 R=Bzl; R'=Z

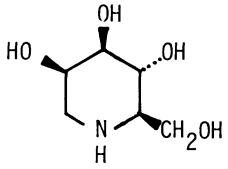


2 R=H

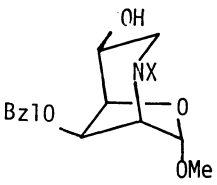
15 R=Bzl



3



4

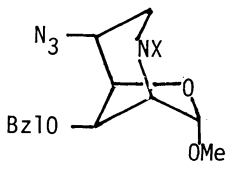


5 X=H

6 X=Z

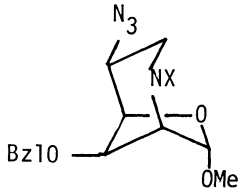
7 X=BOC

[Bzl=$PhCH_2$; Ac=MeCO; Z=$PhCH_2OCO$; BOC=Bu^tOCO]



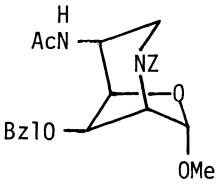
8 X=Z

9 X=BOC

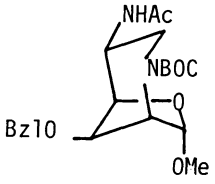


10 X=Z

11 X=BOC



12



13

configuration at C-5 of the sugar had been inverted in the displacement reaction [yield 40% from 6], together with the epimeric azide 10 formed in 20% yield. Reduction of 8 with sodium hydrogen telluride,[10] followed by acetylation with acetic anhydride in pyridine gave the amide 12 as a syrup, $[\alpha]_D^{20}$ +88° (c, 0.41 in $CHCl_3$) in 92% yield. Hydrolysis of the furanoside 12 with aqueous trifluoroacetic acid, followed by sodium borohydride reduction of the resulting lactol gave 14 in 81% yield; removal of the benzyl and benzyloxycarbonyl protecting groups in 14 by hydrogenolysis in the presence of palladium black in acetic acid gave 1, mp 227-228 °C, $[\alpha]_D^{20}$ +35° (c, 0.3 in H_2O) in 92% yield [26% overall yield from 6]. When the minor azide 10 was subjected to the same sequence of reactions, 2 was formed.

The t-butyloxycarbonyl protected amine 7, mp 103 °C, $[\alpha]_D^{20}$ +17.7° (c, 0.38 in $CHCl_3$), may be prepared by protection of the amino function in 5[8] with di-t-butyl dicarbonate. Conversion of 7 to the corresponding triflate followed by treatment with sodium azide in dimethyl formamide at 70 °C for 48 h gave the azide 11, $[\alpha]_D^{20}$ +44.7° (c, 1.33 in $CHCl_3$) as the major product [45% yield] with only a small amount of the epimer 9. Hydrogenation of the azide 7 [palladium black in ethanol] followed by acetic anhydride in pyridine formed the crystalline amide 13, mp 109-110 °C, in 83% yield. Hydrolysis of 13 caused simultaneous removal of the BOC protecting group and hydrolysis of the furanoside to the corresponding lactol; reduction of the lactol with sodium borohydride gave 15 which on hydrogenation in acetic acid in the presence of palladium black gave 2, mp 203-207 °C, $[\alpha]_D^{20}$ -53.3° (c, 0.12 in H_2O) [52% yield from 13], identical to the sample of 2 made from 10. When the epimer 9 was subjected to the same series of transformations as the azide 11, the N-acetyl glucosamine analogue 1 was formed, identical in all respects to the sample prepared from 8.

There is a marked contrast in the behaviour of the triflates derived from the alcohols 6 and 7, which only differ in the carbamate protecting group. Thus, the reaction of azide ion with the triflate derived from the BOC protected alcohol

7 leads to displacement mainly with retention of configuration at C-5 of the original sugar and occurs much more slowly than the displacement of the triflate derived from the benzyloxycarbonyl protected analogue 6, which results in predominant inversion of configuration at C-5. Neighbouring group participation by BOC and benzyloxycarbonyl protected amines in nucleophilic displacements is well known;[11] this work indicates that the BOC group is a more effective neighbouring group than benzyloxycarbonyl. The difference in neighbouring group effects of the two groups may arise from the greater inductive effect of the t-butyl group in comparison to the benzyl group.

The appearance of the three protons on C-1 and C-5 in the ^{1}H NMR spectra of the N-acetylglucosamine analogue 1[12] is identical to that of the corresponding protons in deoxynojirimycin 3; a different characteristic pattern for the corresponding protons in the N-acetylmannosamine analogue 2[13] and in deoxymannojirimycin 4 is also observed.

The inhibitory action of 1 and 2 on the hydrolysis of the corresponding nitrophenyl glycopyranosides catalysed by α-glucosidase (yeast), β-glucosidase (almonds), α-galactosidase (green coffee beans), β-galactosidase (Aspergillus niger), α-L-fucosidase (bovine epididymis), β-xylosidase (Aspergillus niger) and a number of β-N-acetylglucosaminidases was determined.[14] The N-acetylglucosamine analogue 1 was a potent competitive inhibitor of β-N-acetylglucosaminidases from Jack Bean (50% inhibition 3.4×10^{-7}M, K_I 2.3×10^{-7}M), from human placenta (50% inhibition 6.0×10^{-6}M, K_I 9.0×10^{-7}M) and from bovine kidney (50% inhibition 7.5×10^{-6}M, K_I 6.0×10^{-7}M); weaker inhibition of the bond and aglycon specific N-acetylglucosaminidase from Streptococcus pneumoniae[15] was observed (50% inhibition 3.2×10^{-4}M) while no inhibition of Aspergillus niger N-acetylglucosaminidase was found. Also, 1 did not inhibit the catalytic action of any of the other glycosidases at 3×10^{-4} M. In marked contrast, the N-acetylmannosamine analogue 2 showed no significant inhibition of the glycosidase activity of any of the enzymes.

In summary, 2-acetamido-1.5-imino-1,2,5-trideoxy-D-glucitol 1 is a potent and specific inhibitor of a number of β-N-acetylglucosaminidases, although the lack of inhibition of the glucosaminidase from Aspergillus niger is noteworthy; the inhibitory activity of this compound 1 requires the correct stereochemistry at the acetamidocarbon, since the epimer 2 causes no significant inhibition of glucosaminidase activity. These preliminary results indicate that 1 may have interesting properties in several areas of biochemistry.[16]

References

1) R.J.Bernacki, M.J.Niedbala, and W. Korytnyk, Cancer Metastasis Rev., 4, 81 (1985).

2) R.Csuk and B.I.Glanzer, J. Chem. Soc., Chem. Commun., 1986, 343.

3) S.V.Evans, L.E.Fellows, T.K.M.Shing, and G.W.J.Fleet, Phytochemistry, 24, 1953 (1985); A.M.Scofield, L.E.Fellows, R.J.Nash, and G.W.J.Fleet, Life Science, submitted for publication.

4) M.P.Dale, H.E.Ensley, K.Kern, K.A.R.Sastry, and L.D.Byers, Biochemistry, 24, 3530 (1985).
5) U.Fuhrmann, E.Bause, and H. Ploegh, Biochim. Biophys. Acta, 825, 95 (1985).
6) A.D.Elbein, G.Legler, A.Tlutsy, W.McDowell, and R.Schwarz, Arch. Biochem. Biophys., 235, 579 (1984).
7) G.W.J.Fleet, A.N.Shaw, S.V.Evans, and L.E.Fellows, J. Chem. Soc., Chem. Commun., 1985, 841; W.Watkins, and G. Fleet, unpublished results.
8) G.W.J.Fleet and P.W.Smith, Tetrahedron Lett., 26, 1469 (1985).
9) Satisfactory spectral and/or analytical data were obtained for all new compounds.
10) H. Suzuki, and K. Takaoka, Chem. Lett., 1984, 1733.
11) J.K.Thottathil and J.L.Moniot, Tetrahedron Lett., 27, 151 (1986).
12) N-Acetylglucosamine analogue 1: ^{1}H NMR (300 MHz) in D_2O δ 1.82 (s, Me), 2.25 (dd, H1a), 2.37 (m, H5), 2.88 (dd, H1e), 3.12, 3.21 (2m, H3,H4), 3.47 (dd, H6), 3.55 (m, H2), 3.64 (dd, H6'); J(1a,1e) 12.6, J(1a,2) 11.5, J(1e,2) 4.9, J(5,6) 6.0, J(5,6') 3.0, J(6,6') 11.6 Hz. ^{13}C NMR (125 MHz) in D_2O δ 22.77 (q, Me), 47.65 (t, CH_2NH), 52.88 (d, CHNH), 61.16 (d, CHOH), 61.96 (t, CH_2OH), 72.69 (d, CHOH), 76.55 (d, CHNAc), 175.07 (s, C=O).
13) N-Acetylmannosamine analogue 2: ^{1}H NMR (300 MHz) in D_2O δ1.87 (s, Me), 2.36 (ddd, H5), 2.63 (dd, H1), 2.84 (dd, H1'), 3.28 (t, H4), 3.54 (dd, H6), 3.55 (dd, H3), 3.64 (dd, H6'), 4.05 (dt, H2); J(1,1') 13.7, J(1,2) 2.4, J(1',2) 2.4, J(2,3) 4.7, J(3,4) 9.8, J(4,5) 9.6, J(5,6) 5.5, J(5,6') 3.0, J(6,6') 11.7 Hz. ^{13}C NMR 125 MHz) in D_2O δ 22.08 (q, Me), 46.52 (t, CH_2NH), 50.54 (d, CHNH), 60.74 (d, CHOH), 68.70 (d,CHOH), 61.01 (t, CH_2OH), 73.03 (d, CHNAc), 174.65 (s, C=O).
14) The nitrophenyl glycopyranoside substrates and the enzymes were obtained from Sigma, except for Streptococcus pneumoniae, isolated by a modification of the method of L.R.Glasgow, J.C.Paulson, and R.L.Hill, J. Biol. Chem., 252, 8615 (1977). The enzyme assay for Jack Bean β-N-acetylglucosaminidase was carried out as follows: 200 μl enzyme (6.4 g/ml), 400 μl 50 mM trisodium citrate pH 5.0, 400 μl 4 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide incubated for various times, then add 800 μl 0.25 M NaOH and read absorbance at 408 nm. Identical assays were used for placental and bovine kidney β-N-acetylglucosaminidases (except that the buffer pH was 4.25) and for Aspergillus niger β-N-acetylglucosaminidase (buffer pH 4). 50% Inhibition of enzymic activity by S. pneumoniae β-N-acetylglucosaminidase was measured using 3 mM p-nitrophenyl N-acetyl-β-D-glucosaminide in 0.1 M citrate at pH 6.0. To 100 μl of this solution, containing varying concentrations of 1 ,5 μl of the enzyme solution was added and the mixture incubated at 37 ^{o}C; at various times (0-20 min), 15 μl of solution were removed into 1.0 ml of 0.5 M sodium carbonate solution and the initial rate of enzymic activity at each concentration of 1 determined. Assays for the other enzymes are given in Ref. 1.
15) K.Yamashita, T.Ohkura, H. Yoshima, and A. Kobata, Biochem. Biophys. Res. Commum., 100, 226 (1981).
16) An SERC post graduate award (to PWS) is acknowledged. RBP and TWR are members of the Oxford Oligosaccharide group supported by the Monsanto Company. TWR is a member of the Oxford Enzyme Group.

(Received March 22, 1986)