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Note

(3*R*,4*R*,5*S*)-5-Acetamido-3,4-piperidinediol: a selective hexosaminidase inhibitor*

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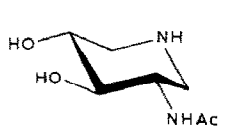
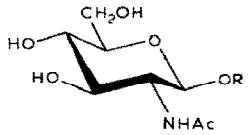
Recent interest in trimming and elongation processes of oligosaccharides during the late stages of glycoprotein biosynthesis[1] has led to the synthesis of several highly specific, naturally occurring D-glucosidase and D-mannosidase inhibitors[2–4]. However, many other important glycosidases that are involved in the catabolism of macromolecular polysaccharides, glycosaminoglycans, and related glycoconjugates are insensitive to these inhibitors. In bacteria, for instance, exo and endo-*N*-acetylhexosaminidases are required in order to expand the rigid, highly crosslinked, peptidoglycan cell-wall during growth and division[5]. Inhibitors of these enzymes, if selective, might complement the antibiotic action of β-lactam drugs, and prove to be of considerable therapeutic value. Herein, we report a simple synthesis of the title compound (**1**), which specifically inhibits the enzymic hydrolysis of *N*-acetylglucosamine linkages (*cf.*, formula **2**).



1



2

Methyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-D-glucopyranoside (**3**) prepared as a mixture of anomers according to a known procedure[6], was converted into its 6-bromo derivative **4** (mixture of anomers) in two steps ($MsCl$–Et_3N, and then LiBr–2-butanone). When heated with activated zinc, benzylamine, and $NaBH_3CN$ in 9:1 1-propanol–water at reflux, reductive ring-opening of **4** followed by *in situ*, reductive amination[7] furnished the acetamidoalkene **5** (53%), along with the iso-

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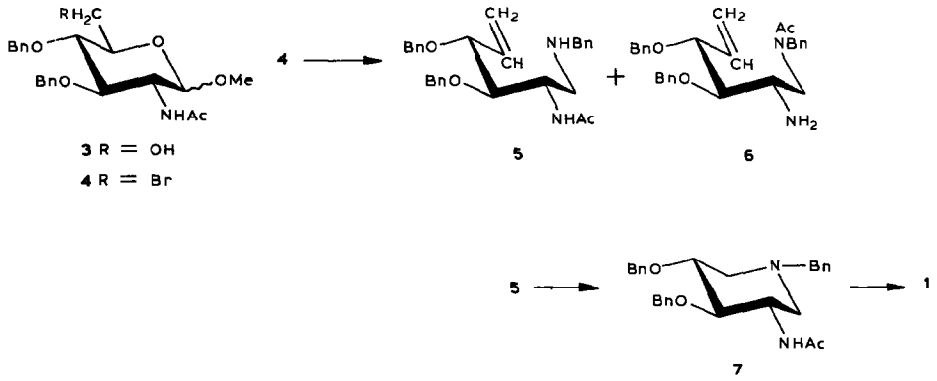
meric *N*-benzylacetamide **6** (30%) resulting from intramolecular acetyl migration. These two products were readily separated by flash chromatography, and **6** could be converted back into **5** simply by heating. Ozonolysis of **5** (TFA salt) in CH_2Cl_2 at $-78°$, with Me_2S workup, followed by *in situ*, reductive amination ($NaBH_3CN$–CH_3OH) gave the protected piperidine **7** (30%). Debenzylation (Pd–C, EtOH, HCl) of **7** afforded compound **1** (90%) as a hygroscopic solid. Bovine β-hexosaminidase was 50% inhibited by **1** at 0.10mM, whereas almond β-D-glucosidase, bovine β-D-galactosidase, endoglycosidase F, and endoglycosidase H were unaffected at 1.0mM.



EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover Unimelt apparatus and are uncorrected. ^{1}H-N.m.r. spectra were recorded at 300 MHz with a Bruker WM-300 spectrometer. For solutions of samples in $CDCl_3$, tetramethylsilane was used as the internal standard, and for those in D_2O, the HOD peak was used as an internal reference. Chemical-ionization mass spectrometry (c.i.m.s.) was performed by using isobutane as the reagent gas.

*Methyl 2-acetamido-3,4-di-*O*-benzyl-6-bromo-2,6-dideoxy-*D*-glucopyranoside* (**4**). — To a solution of methyl 2-acetamido-3,4,-di-*O*-benzyl-2-deoxy-D-glucopyranoside (11.5 g, 27 mmol), as a 2.5:1 mixture of the α and β anomers[6] in CH_2Cl_2 (250 mL) cooled to 0° were added triethylamine (6.0 mL) and then mesyl chloride (2.5 mL, 32 mmol). After stirring for 1.5 h, saturated $NaHCO_3$ (50 mL) and water (200 mL) were added, and the mixture was extracted with CH_2Cl_2 (3 × 200 mL). The extracts were combined, dried, and evaporated to a solid which was heated at reflux for 2 h in 2-butanone (175 mL) containing LiBr (60 g, 0.69 mol). The mixture was cooled, diluted with water (500 mL), and extracted with CH_2Cl_2 (3 × 150 mL). The extracts were combined, dried, and evaporated, to afford 13 g (45% overall) of **4** as a 2.5:1 mixture of the α and β anomers: α *anomer*, R_F 0.23 (1:1 EtOAc–hexane); ^{1}H-n.m.r ($CDCl_3$): δ 7.34–7.24 (10 H, benzyl), 5.30 (d, NHAc, *J* 10 Hz), 4.92–4.60

(m, anomeric H, O-CH_2-Ph), 4.25 (dt, H-5, *J* 4, 10 Hz), 3.82–3.38 (m, H-2,3,4,6, 6′), 3.34 (s, OCH_3), and 1.83 (s, $COCH_3$): *β anomer*, R_F 0.30 (1:1 EtOAc–hexane); ^{1}H-n.m.r. ($CDCl_3$): δ 7.34–7.24 (10 H, benzyl), 5.67 (d, *J* 8 Hz, N*H*Ac), 4.92–4.60 (m, anomeric H, O-CH_2-Ph), 4.11 (t, H-5, *J* 7.5 Hz), 3.82–3.38 (m, H-2,3,4,6,6′), 3.48 (s, OCH_3), and 1.85 (s, $COCH_3$); μ_{max}^{KBr} 3300, 3090, 3070, 3040, 2920, 1650, 1555, 1455, 1380, 1360, 1070, and 1050 cm^{-1}; c.i.m.s. *m/z* 481 (25%), 480 (100%, M + 1), 479 (27%), and 478 (99%, M + 1).

Anal. Calc. for $C_{23}H_{35}BrNO_5$: C, 57.86; H, 7.33, N, 2.90. Found: C. 57.98; H, 7.61; N, 2.99.

(2S,3R,4R)-2-Acetamido-1-(benzylamino)-3,4-bis(benzyloxy)-hex-5-ene (**5**) *and (2S,3R,4R)-1-(N-acetylbenzylamino)-2-amino-3,4-bis(benzyloxy)-hex-5-ene* (**6**). — To a suspension of acid-washed zinc powder (1.75 g, 26.7 mmol) in 9:1 1-propanol–water (13 mL) was added a mixture of bromide **4** (0.217 g, 0.45 mmol) and benzylamine (1.1 mL, 10 mmol). Sodium cyanoborohydride (0.073 g, 1.16 mmol) was then added, and the vigorously stirred mixture was heated at reflux for 3.25 h, cooled, filtered through Celite, and the filtrate treated with 5% aqueous HCl (30 mL) for 20 min and then basified to pH 13 with 15% aqueous NaOH. The resulting mixture was extracted with CH_2Cl_2 (3 × 150 mL), and the extracts were combined, and concentrated *in vacuo* to remove solvent and most of the benzylamine. Flash chromatography with 1:1:0.1 EtOAc–hexane–Et_3N, then EtOAc, and then 3:17 CH_3OH–EtOAc afforded **5** (0.111 g, 53%) and **6** (0.061 g, 30%) as oils.

For **5**: R_F 0.64 (99:1 EtOAc–Et_3N); $[\alpha]_D^{20}$ −19.2° (*c* 1.2, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.19 (15 H, *N*- and *O*-benzyl), 5.85 (d, *J* 9.2 Hz; N*H*Ac), 5.70 (ddd, H-5, C*H*=, $J_{4,5}$ 7.4, J_{trans} 17, J_{cis} 10.7 Hz), 5.31, 5.39 (2 dd, H-6,6′, J_{gem} 1.1 Hz), 4.91, 4.50 (ABq, *J* 11 Hz, OCH_2Ph), 4.63, 4.38 (ABq, *J* 11.7 Hz, OCH_2Ph), 4.17 (broad q, H-2, $J_{2,3}$ 1.2 Hz), 3.94 (dd, H-4, $J_{3,4}$ 7.7, $J_{4,5}$ 7.4 Hz), 3.81 (dd, H-3, $J_{2,3}$ 1.2 Hz), 3.75, 3.62 (ABq, 2 H, *J* 13.2 Hz; PhCH_2N), 2.68, 2.57 (2 dd, H-1,1′, $J_{1,1}$ 11.7, $J_{1,2}$ 8.2, $J_{1',2}$ 6.0 Hz), and 1.89 (s, $COCH_3$); μ_{max}^{KBr} 3430, 3300, 3090, 3065, 3035, 2880, 1660, 1495, 1455, 1370, and 1130–1050 cm^{-1}; c.i.m.s. *m/z* 460 (13%), 459 (29%, M + 1), and 441 (100%).

Anal. Calc. for $C_{29}H_{34}N_2O_3$: C, 75.95; H, 7.47; N, 6.11. Found: C. 75.89; H, 7.58; N, 6.25.

For **6** R_F 0.08 (99:1 EtOAc–Et_3N); $[\alpha]_D^{20}$ −7.5° (*c* 0.5, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.10 (m, *N*- and *O*-benzyl), 5.94 (ddd, H-5, C*H*=, $J_{4,5}$ 7.2, J_{cis} 10.4, J_{trans} 17.4 Hz), 5.44, 5.31 (2 dd, H-6,6′, J_{gem} 1.2 Hz), 4.92, 4.68 (Abq, *J* 11.9 Hz, OCH_2Ph), 4.60, 4.38 (ABq, *J* 11.5 Hz, OCH_2Ph), 4.35 (t, H-4), 4.30, 4.00 (ABq, *J* 13 Hz, PhCH_2N), 4.13 (br, t, H-2), 3.41 (dd, H-3, $J_{3,4}$ 6.6, $J_{2,3}$ 3.8 Hz), 3.12 (m, H-1,1′), 2.01 (s, $COCH_3$), and 2.05–1.85 (br. m, NH_2); μ_{max}^{film} 3600–3000, 3095, 3065, 3035, 2940, 2880, and 1620 cm^{-1} high-resolution m.s. (20 eV). Calc. for $C_{29}H_{34}N_2O_3$: 458.6059; Found: 458.6075.

(3R,4R,4S)-5-Acetamido-N-benzyl-3,4-bis(benzyloxy)piperidine (**7**). — To a solution of aminoalkene **5** (0.210 g, 0.46 mmol) in CH_2Cl_2 (10 mL) was added trifluoroacetic acid (0.6 mL), and the mixture was evaporated *in vacuo*. The resul-

ting, viscous oil was dissolved in CH_2Cl_2 (13 mL) and treated with a saturated solution of ozone in CH_2Cl_2 (13 mL; 0.52 mmol of O_3) at −78°. After 5 min, a solution of dimethyl sulfide (0.050 mL, 0.70 mmol) and $NaBH_3CN$ (0.083 g, 1.32 mmol) in CH_3OH (10 mL) was added, and the mixture was allowed to warm to room temperature. After 20 h, saturated aqueous $NaHCO_3$ solution was added, and the mixture extracted with CH_2Cl_2 (3 × 20 mL). The extracts were combined, dried, and evaporated *in vacuo*, and the residue chromatographed with 1:1 EtOAc–hexane, to afford 0.059 g (30%) of **7** as an oil; R_F 0.25 (1:1 EtAOc–hexane); $[\alpha]_D^{20}$ −12° (*c* 0.3, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 7.39–7.29 (15 H, *N*-, *O*-benzyl), 6.62 (bs, N*H*Ac), 4.67, 4.57 (ABq, OCH_2Ph, *J* 12 Hz), 4.47, 4.37 (ABq, OCH_2Ph, *J* 11.5 Hz), 4.25 (m, H-5), 3.59, 3.49 (2 m, H-3,4), 3.58, 3.48 (ABq, NCH_2Ph, *J* 13 Hz), 2.73, 2.51 (2 br, m, each 2 H; H-2,2′,6,6′), and 1.81 (s, $COCH_3$); μ_{max}^{film} 3410, 3350–3250, 3090, 3065, 3035, 2950–2800, 1650, 1550, 1495, 1455, 1100, 1065, 1025, 905, 730, and 695 cm^{-1}; high-resolution m.s. (20 eV). Calc. for $C_{28}H_{32}N_2O_3$: 444, 5788: Found: 444.5816.

(3R,4R,5S)-5-Acetamido-3,4-piperidinediol (**1**). — To a solution of piperidine **7** (56.4 mg, 0.12 mmol) in EtOH (2 mL) was added 4M methanolic HCl (0.5 mL). The solution was evaporated *in vacuo*, the residue dissolved in EtOH (5 mL), and 10% Pd–C (0.045 g) was added. Hydrogen was bubbled through the suspension for 16 h; then, the mixture was filtered through Celite, and the filtrate evaporated *in vacuo*, to give 24 mg (90%) of **1** as a very hygroscopic glass; $[\alpha]_D^{20}$ +21° (*c* 0.4, MeOH); ^{1}H-n.m.r. (D_2O): δ 4.09 (dt, H-5; $J_{5,6} = J_{5,6'} = 4.8$, $J_{4,5}$ 10.3 Hz), 3.90 (ddd, H-3, $J_{2,3}$ 4.8, $J_{2',3}$ 8.8, $J_{3,4}$ 10.3 Hz), 3.65 (t, H-4, *J* 10.3 Hz), 3.60–3.49 (m, H-6eq,2eq), 3.00 (t, H-6ax,2ax, *J* 11.8 Hz), and 2.07 (s, $COCH_3$); high-resolution m.s. (20 eV). Calc. for $C_7H_{14}N_2O_3$: 174.2011, Found: 174.2030.

Bioassays. — Compound **1** was tested against almond β-D-glucosidase, bovine liver β-D-glucosiduronase, bovine β-D-galactosidase, and bovine β-*N*-acetylhexosaminidase, using the corresponding monosaccharide *p*-nitrophenyl β-D-glycosides as substrates at pH 5.00 (50mM HOAc–NaOAc buffer) and a substrate concentration of 5mM. Assays were conducted in disposable, rimless culture-tubes (10 × 75 mm; Kimble Company) which had been rinsed with 1% aqueous bovine serum albumin solution and then baked to dryness. [This procedure prevented enzyme from sticking to the test-tube walls.] Enzyme–buffer–inhibitor mixtures (200 μL total volume; triplicate runs) were pre-incubated for 5 min at 37°; substrate was then added and, after incubation (15 min), each run was quenched with pH 10.4 glycine buffer, and absorbances were read at 400 nm using a glycine buffer blank. In control runs, distilled, de-ionized water was substituted for the inhibitor.

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REFERENCES

1 R. T. SCHWARTZ AND R. DATEMA, *Trends Biochem. Sci.*, 9 (1984) 32–34.
2 Pyrrolidines: (a) G. W. J. FLEET, S. J. NICHOLAS, P. W. SMITH, S. V. EVANS, L. E. FELLOWS, AND R. J. NASH, *Tetrahedron Lett.*, (1985) 3127–3130; (b) C. J. RULE, B. A. WURZBURG, AND B. GANEM, *ibid.*, (1985) 5379–5380; (c) M. J. EIS, C. J. RULE, B. A. WURZBURG, AND B. GANEM, *ibid.*, (1985) 5397–5398.
3 (a) Deoxynojirimycin: S. INOUYE, T. TSURUOKA, T. ITO, AND T. NIIDA, *Tetrahedron*, 23 (1968) 2125–2144; (b) Deoxymannonojirimycin: L. E. FELLOWS, E. A. BELL, D. G. LYNN, F. PILKIEWICZ, I. MIURA, AND K. NAKANISHI, *J. Chem. Soc., Chem. Commun.*, (1979) 977–978.
4 (a) Castanospermine: R. SAUL, J. P. CHAMBERS, R. J. MOLYNEUX, AND A. D. ELBEIN, *Arch. Biochem. Biophys.*, 221 (1983) 593–597; (b) swainsonine: P. R. DORLING, C. R. HUXTABLE, AND S. M. COLEGATE, *Biochem. J.*, 191 (1980) 649–651.
5 H. J. ROGERS, H. R. PERKINS, AND J. B. WARD, *Microbial Cell Walls and Membranes*, Chapman and Hall, New York, 1980.
6 (a) R. W. JEANLOZ, *J. Chem. Soc.*, 74 (1952) 4597–4599; (b) *ibid.* 76 (1954) 558–560; (b) A. S. SHASHKOV, A. YU. EVSTIGNEEV, AND V. A. DEREVITSKAYA, *Bioorg. Khim.*, 4 (1978) 1495–1499; *Chem. Abstr.*, 90 (9179) 168–904*e*.
7 R. C. BERNOTAS AND B. GANEM, *Tetrahedron Lett.*, (1985) 1123–1126.