Carbohydrate Research, 247 (1993) 341–345
Elsevier Science Publishers B.V., Amsterdam

Note

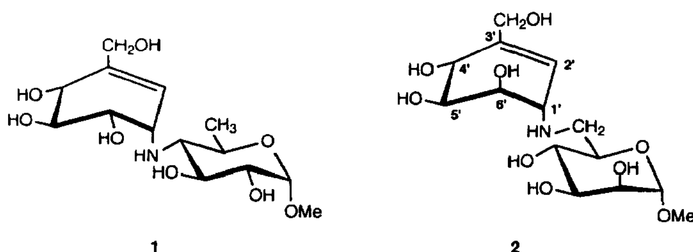
An imino-linked carba-disaccharide α-D-mannosidase inhibitor

Sylvain Cottaz [a], John S. Brimacombe [a,*] and Michael A.J. Ferguson [b]

[a] *Departments of Chemistry and* [b] *Biochemistry, The University of Dundee, Dundee DD1 4HN (United Kingdom)*

(Received March 15th, 1993; accepted March 29th, 1993)

The finding that such imino-linked carba-disaccharides as methyl acarviosin[1] (**1**) are potent glycosidase inhibitors has sparked off interest in the synthesis of analogous carba-disaccharides for biological testing. In connection with a project concerned with inhibition of the biosynthesis of glycosyl phosphatidylinositol (GPI) anchors, we had occasion to synthesise the unsaturated (1 → 6)-imino-linked carba-disaccharide **2** having the '*α-manno*' configuration, which was also tested as a potential inhibitor of α-D-mannosidase and other glycosidases. The results of these tests and a synthesis of **2** are reported herein.

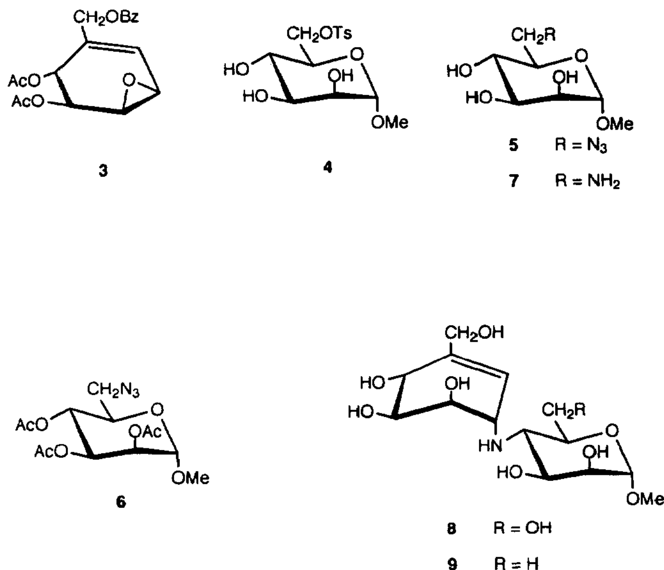


The strategy used for the synthesis of **2** involved a coupling reaction between (1*R*)-(1,2,3/4)-3,4-di-*O*-acetyl-1,2-anhydro-5-benzoyloxymethylcyclohex-5-ene-1,2,3,4-tetraol[2] (**3**) and methyl 6-amino-6-deoxy-α-D-mannopyranoside (**7**) in the expectation[3] of favoured diaxial opening of the epoxide ring at the allylic position. A straightforward synthesis of **7** was based on one outlined in a Japanese patent[4], in which the first step entailed regioselective toluene-*p*-sulphonylation of methyl α-D-mannopyranoside to give the primary tosylate **4**. Displacement of the tosyloxy group of **4** with sodium azide in DMF then furnished **5**, which was conveniently

* Corresponding author.

0008-6215/93/$06.00 © 1993 – Elsevier Science Publishers B.V. All rights reserved

isolated and characterised as the triacetate **6**[4]. Zemplén deacetylation of **6** (→ **5**) and subsequent catalytic reduction of the azido group of **5** gave **7**.



Coupling of an excess of **7** with the enantio-pure epoxide **3**[2] gave, after deacylation and chromatography, the carba-disaccharide **2** in 78% yield. The structure of **2** was confirmed by the ^{1}H NMR spectrum (500 MHz, D_2O) which revealed H-1′, 6′ at δ4.00 (dd, $J_{1',2'}$, ~ 1, $J_{1',6'}$, 3.9 Hz) and 3.82 (dd, $J_{5,6}$, 2.5 Hz), respectively, exhibiting the appropriate spin–spin couplings.

The carba-disaccharide **2** exhibited good inhibitory activity against Jack bean α-D-mannosidase at pH 4.5 under standard conditions[5,6] but was inactive against yeast α-D-glucosidase, almond β-D-glucosidase, coffee bean α-D-galactosidase, Jack bean β-D-galactosidase, and snail β-D-mannosidase. The high degree of selectivity shown by **2** against Jack bean α-D-mannosidase is noteworthy. Lineweaver–Burk plots * (*p*-nitrophenyl α-D-mannopyranoside as the substrate, K_M 2.5 mM, ref 6) demonstrated the competitive nature of the inhibition and yielded a K_i value of 30 μM. Interestingly, the positional isomer **8** of **2** was inactive against Jack bean α-D-mannosidase, although it exhibited moderate inhibitory activity against yeast α-D-glucosidase; the corresponding 6-deoxy analogue **9** had mild inhibitory activity against both of these glycosidases[5].

The inhibitory activity against Jack bean α-D-mannosidase was removed completely on *N*-acetylation of **2**, pointing to the importance of the basic NH group in the inhibitory process, although the intervention of a steric effect as a result of

* These plots were submitted as supplementary material and are available (from J.S.B.) on request.

N-acetylation cannot be ruled out. Since the carba-disaccharide **2**, pK_a 8.75 (determined by titration), would be fully protonated under the test conditions (pH 4.5), it is not unreasonable to infer that, as in other cases[7], the protonated form **2**-H^+ is responsible for inhibition.

EXPERIMENTAL

General methods.—Melting points were measured on a Reichart hot-plate apparatus and are uncorrected. Optical rotations were obtained using a Perkin–Elmer 141 polarimeter at ambient temperature. ^{1}H NMR spectra (500 MHz) were recorded for solutions in D_2O at 20°C with a Bruker AC-500 spectrometer with Me_2CO (δ2.07) as an internal standard. FABMS were measured in the positive ionisation mode with a VG 250/70 SE instrument; thioglycerol–glycerol was used as the liquid matrix. Flash-column chromatography was performed on columns of Silica Gel 60 (230–400 mesh, Merck); TLC was performed on Silica Gel 60F_{254} (Merck) with detection by UV light or charring with dil H_2SO_4. Light petroleum refers to the fraction having the boiling range 60–80°C.

Methyl 2,3,4-tri-O-acetyl-6-azido-6-deoxy-α-D-mannopyranoside (**6**).—A cooled (0°C) solution of methyl α-D-mannopyranoside (5 g, 25.8 mmol) and toluene-*p*-sulphonyl chloride (5.9 g, 31 mmol) in pyridine (50 mL) was stirred for 2 h and then concentrated under reduced pressure. Flash-column chromatography (1:1 CH_2Cl_2–Me_2CO) of the residue gave the tosylate **4** (10 g) slightly contaminated with ditosylated derivatives.

A stirred solution of **4** (10 g) in DMF (100 mL) containing sodium azide (10 g, 154 mmol) was heated at 100°C for 1.5 h and then concentrated under reduced pressure. A solution of the residue in pyridine (50 mL) was treated with Ac_2O (50 mL) and 4-dimethylaminopyridine (30 mg) at room temperature for 1 h and then diluted with CH_2Cl_2. The solution was washed in turn with dil HCl, aq $NaHCO_3$, and aq NaCl, dried ($MgSO_4$), and concentrated under reduced pressure. Flash-column chromatography (5:1 light petroleum–EtOAc) gave **6** (5.98 g, 67%); mp 99–100°C (from Et_2O), lit.[4] mp 99–100°C; $[\alpha]_D$ + 63° (*c* 1, $CHCl_3$), ν_{max} 2100 cm^{-1} (N_3). Anal. Calcd for $C_{13}H_{19}N_3O_8$: C, 45.2; H, 5.5; N, 12.2. Found: C, 45.5; H, 5.5; N, 12.0.

Methyl 6-amino-6-deoxy-α-D-mannopyranoside (**7**).—A solution of **6** (3.45 g, 10 mmol) in MeOH (100 mL) was treated with 1 M methanolic NaOMe (1 mL) at room temperature for 45 min and then neutralised with Amberlite IR-120 (H^+) resin. After filtration, the solution was concentrated under reduced pressure to give the azide **5** (2.2 g, ~ 100%) as a syrup; ν_{max} 3400 (OH) and 2100 cm^{-1} (N_3).

A solution of **5** (1.15 g, 5.25 mmol) in MeOH (20 mL) containing platinum oxide (0.25 g) was shaken under a slight overpressure of H_2 at room temperature for 24 h and then filtered through Celite. Evaporation of the solvent under reduced pressure gave **7** (1 g, 99%) as a hygroscopic white foam; $[\alpha]_D$ +78° (*c* 1.2, MeOH); ν_{max} 3400 (OH and NH_2) and 1600 cm^{-1} (NH). ^{1}H NMR data (D_2O): δ 2.65 (dd,

1 H, $J_{5,6}$ 7, $J_{6,6'}$ 13.5 Hz, H-6), 2.80 (dd, 1 H, $J_{5,6'}$ 2.8 Hz, H-6′), 3.21 (s, 3 H, OMe), 3.30 (ddd, 1 H, H-5), 3.39 (t, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 3.50 (dd, 1 H, $J_{2,3}$ 3.6 Hz, H-3), 3.71 (dd, 1 H, H-2), and 4.50 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1).

Methyl 6-deoxy-6-[(1S)-(1,4/5,6)-4,5,6-trihydroxy-3-hydroxymethylcyclohex-2-en-1-ylamino]-α-D-mannopyranoside (**2**).—A solution of **7** (31 mg, 160 μmol) and (1*R*)-(1,2,3/4)-3,4-di-*O*-acetyl-1,2-anhydro-5-benzoyloxymethylcyclohex-5-ene-1,2,3,4-tetraol[2] (**3**; 20 mg, 58 μmol) in propan-2-ol (1 mL) was heated in a sealed tube for 48 h at 60°C, cooled, and concentrated under reduced pressure. The residue in MeOH (2 mL) was treated with 1 M methanolic NaOMe (0.2 mL) at room temperature for 12 h, neutralised with Amberlyst-15 (H^+) resin, and concentrated under reduced pressure. Flash-column chromatography (1:1 $CHCl_3$–MeOH) gave **2** (16 mg, 78%); $[\alpha]_D$ +40° (*c* 1.2, H_2O). ^{1}HNMR data (D_2O): δ2.75 (dd, 1 H, $J_{5,6a}$ 8.4, $J_{6a,6b}$ 12.4 Hz, H-6a), 2.87 (dd, 1 H, $J_{5,6b}$ 2.8 Hz, H-6b), 3.27 (s, 3 H, OMe), 3.30 (bd, 1 H, $J_{4',5'}$ 7.3 Hz, H-4′), 3.41 (t, 1 H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 3.53 (ddd, 1 H, H-5), 3.59 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-3), 3.77 (m, 2 H, H-2,5′), 3.82 (dd, 1 H, $J_{5',6'}$, 2.5 Hz, H-6′), 4.00 (dd, 1 H, $J_{1',2'} \sim 1$, $J_{1',6'}$ 3.9 Hz, H-1′), 4.01 (s, 2 H, CH_2OD), 4.60 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), and 5.68 (m, 1 H, H-2′). Anal. Calcd for $C_{14}H_{25}NO_9 \cdot 4H_2O$: C, 39.7; H, 7.85; N, 3.35. Found: C, 40.2; H, 7.3; N, 3.0.

Inhibition studies.—The activities of all the glycosidases were determined in the presence of the carba-disaccharide **2** at a concentration of 1 mM by estimation of the *p*-nitrophenol liberated on hydrolysis of the corresponding *p*-nitrophenyl glycopyranoside at a concentration of 3 mM.

Incubations were conducted at 25°C using 15 mU/mL of the enzyme for 7 min before 0.5 M Na_2CO_3 (2 vol) was added to terminate the reaction. Absorbance measurements at 400 nm were carried out immediately thereafter. Assay conditions were as follows: Jack bean α-D-mannosidase in 50 mM sodium citrate–0.2 mM zinc acetate buffer at pH 4.5; yeast α-D-glucosidase in 100 mM sodium phosphate buffer at pH 7.0; coffee bean α-D-galactosidase in 100 mM sodium citrate–sodium phosphate buffer at pH 6.0; almond β-D-glucosidase in 50 mM sodium citrate buffer at pH 4.5; Jack bean β-D-galactosidase in 50 mM sodium citrate buffer at pH 3.5; snail β-D-mannosidase in 100 mM sodium acetate buffer at pH 4.0. In all experiments, bovine serum albumin (1 mg/mL) was added.

Enzymes were used as obtained commercially.

ACKNOWLEDGEMENTS

We thank the SERC for financial support, Dr. S.W. Homans for recording the ^{1}H NMR spectra, and Dr. C.A. Redman for the FABMS.

REFERENCES

1 B. Junge, F.-R. Heiker, J. Kurz, L. Müller, D.D. Schmidt, and C. Wunsche, *Carbohydr. Res.*, 128 (1984) 235–268.

2 S. Ogawa and T. Takagaki, *J. Org. Chem.*, 50 (1985) 2356–2359.
3 S. Ogawa, K. Yasuda, T. Takagaki, Y. Iwasawa, and T. Suami, *Carbohydr. Res.*, 141 (1985) 329–334.
4 Y. Ito and T. Okuda, Japanese Patent 8003, 1967; *Chem. Abstr.*, 67 (1967) 117 217a.
5 S. Ogawa and Y. Nakamura, *Carbohydr. Res.*, 226 (1992) 79–89.
6 Y.T. Li and S.C. Li, *Methods Enzymol.*, 28 (1972) 702–713.
7 M.K. Tong, G. Papandreou, and B. Ganem, *J. Am. Chem. Soc.*, 112 (1990) 6137–6139; M.P. Dale, H.E. Ensley, K. Kern, K.A.R. Sastry, and L.D. Byers, *Biochemistry*, 24 (1985) 3530–3539.