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Tetrazoles of Manno- and Rhamno-Pyranoses: Contrasting Inhibition of Mannosidases by [4.3.0] but of Rhamnosidase by [3.3.0] Bicyclic Tetrazoles

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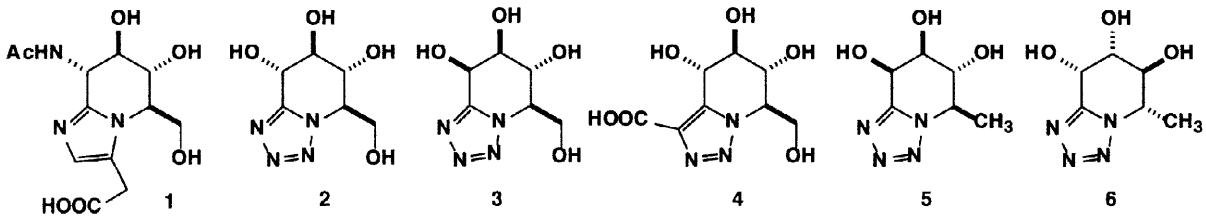
Abstract: The synthesis of tetrazoles derived from D-manno and D- rhamnopyranose from L-gulonolactone and of L-rhamnopyranose from D-gulonolactone is described. These and other materials are assessed as inhibitors of glycosidases. The [4.3.0] tetrazoles of D-manno- and D-rhamnopyranose are inhibitors of human liver α-mannosidase. In contrast the D-furanose analogues show no inhibitory activity whilst the [3.3.0] L-rhamno furanotetrazole is a potent rhamnosidase inhibitor, a potential inhibitor of mycobacterial cell wall biosynthesis and as such may provide a strategy for the treatment of tuberculosis. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

There are a large number of naturally occurring polyhydroxylated nitrogen heterocycles which may be considered to be mimics of sugars.[1] Their synthesis,[2] biosynthesis[3] and biological activity[4] have been the subject of a number of reviews. In particular the inhibitory activity of these and similar mimics towards glycosidases and other sugar processing enzymes has prompted discussion of their mechanism of action[5,6] and of their use in potential therapeutic strategies for the treatment of viral infections,[7] cancer,[8] diabetes[9] and tuberculosis.[10] Of these only nagstatin (**1**) contains an aromatic ring linking the pseudoanomeric centre to the ring nitrogen. Nagstatin is a powerful inhibitor of hexosaminidases,[11] several analogues have been studied[12] and its total synthesis has been reported.[13] The often higher potency displayed by such and similar bicyclic mimics has been attributed to their greater rigidity; the polyhydroxylated heterocyclic moiety being effectively locked in a conformation favoring inhibition.[5]



1 2 3 4 5 6

The enhanced potency of bicyclic aromatic compounds is therefore a valuable strategy in the design of inhibitors and has driven the synthesis of a number of tetrazoles,[14-20] triazoles[21-24] and imidazoles[11,12,21] which are fused to pyranoses and furanoses. Vasella designed the glucotetrazole **2**[17] as a bicyclic neutral transition state analogue inhibitor of β-glucosidases and glycogen phosphorylase.[18] The

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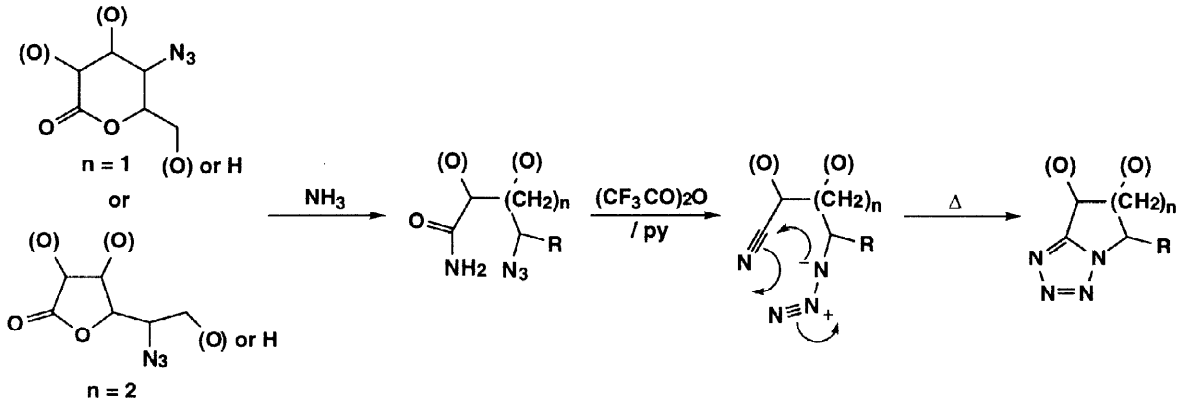
mannose analogue **3**[19] is an inhibitor of mannosidases and the syntheses of galacto- and N-acetylglucosamino-analogues have recently been described.[20] The syntheses of the glucotriazole **4**, a competitive inhibitor of glycogen phosphorylase, and of the corresponding galacto- isomer[24] have been reported.

Previous syntheses of pyranose tetrazoles, such as **2** or **3**, have relied on the configurational inversion of open-chain alcohols and the subsequent displacement of tosylate by azide and *in situ* cyclization.[20] Alternatively, they may be prepared from the corresponding glyconolactams.[25] This paper describes efficient syntheses of the D-pyranose tetrazoles **3** and **5** from L-gulonolactone and of the L-rhamnopyranotetrazole **6** from D-gulonolactone. The following paper details the synthesis of the corresponding furanose tetrazoles.[26] Moreover, the inhibitory properties of these compounds further confirms that although the mirror-image relationship between D-mannosidases and L-rhamnosidases may be used to design highly potent rhamnosidase inhibitors,[15] the substrate specificity of these two enzymes is subtly different.[27] Preliminary details of these syntheses have been reported previously.[14,15]

Synthesis

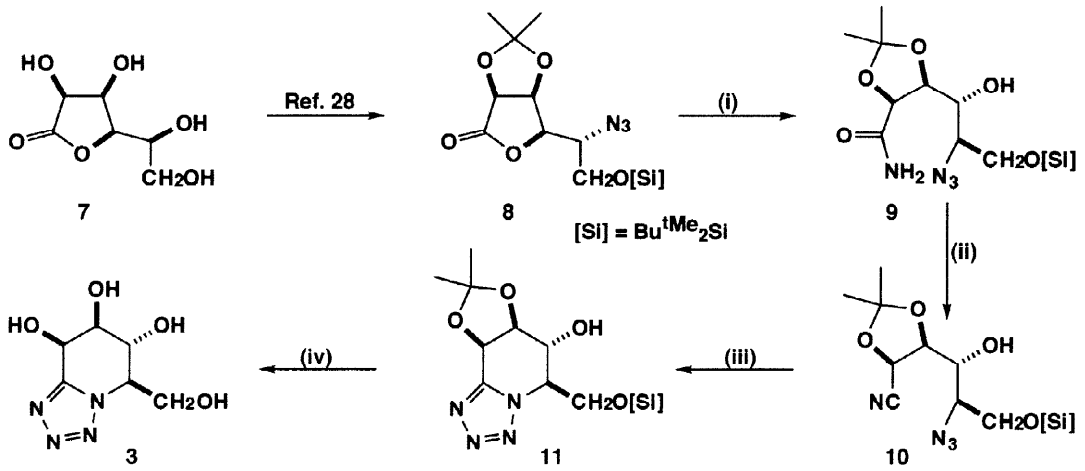
The following syntheses exploit the intramolecular [1,3] dipolar cycloaddition of azide and nitrile groups [Scheme 1] as a powerful method for the formation of bicyclic systems from acyclic precursors. The substrates to these key cyclizations may be formed through the elaboration of the corresponding azido lactones; in addition they require protection of the hydroxyl group α to the nitrile group to prevent collapse. The *cis* stereochemistry of the C-2 and C-3 hydroxyl groups of all of the compounds described allows this to be achieved conveniently through the formation of isopropylidene ketals.



Scheme 1

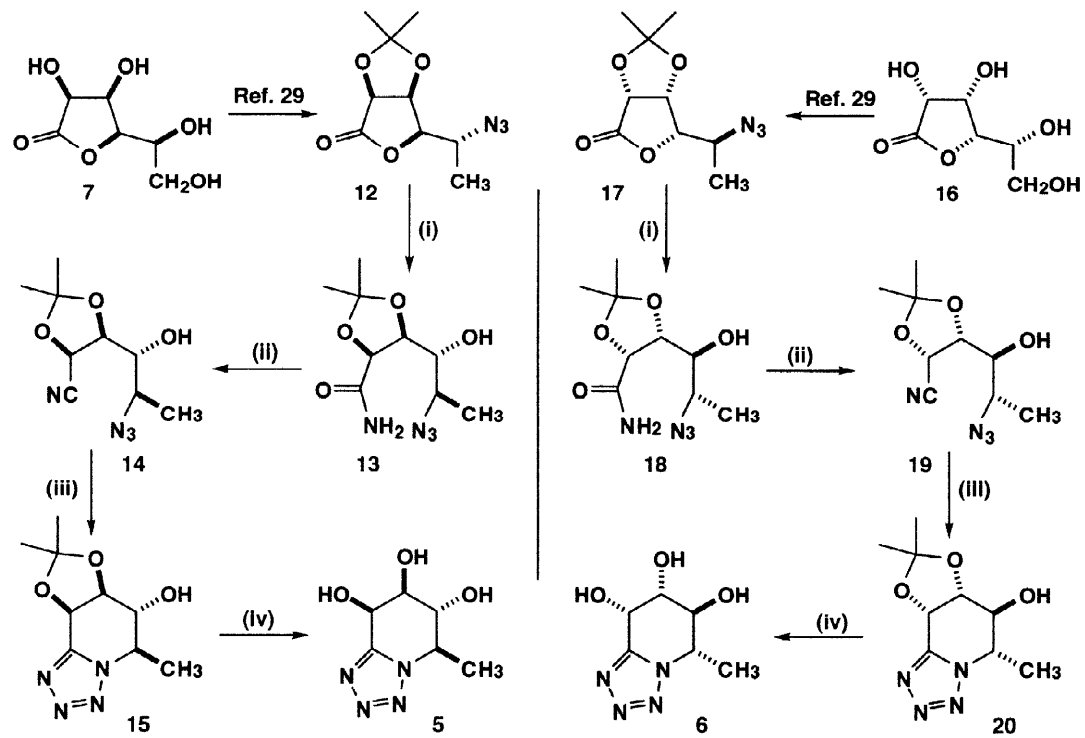
For the synthesis of the D-mannotetrazole **3**, L-gulonolactone **7** was converted to the azide **8** as previously described.[28] Treatment of **8** with a saturated solution of ammonia in methanol resulted in clean and efficient ring opening and the quantitative formation of the open chain primary amide **9** [Scheme 2]. Overall dehydration of **9** to the nitrile **10** was achieved in 75% yield using excess trifluoroacetic anhydride in pyridine, followed by methanolic work-up. A solution of the azidonitrile **10** in anhydrous toluene was heated at 100-105°C for 3 days and effected an intramolecular [1,3] dipolar cycloaddition which resulted in the formation of the tetrazole **11** in 91% yield. Hydrolysis of **11** by aqueous trifluoroacetic acid allowed the

removal of both the silyl ether and acetonide protecting groups and afforded the mannopyranotetrazole **3**[19] in 81% yield [55% overall from **8**].



Scheme 2: (i) NH_3/MeOH, 100% (ii) $(CF_3CO)_2O$/pyridine/-30°C, 75% (iii) Δ/toluene, 91% (iv) CF_3COOH:H_2O, (1:1), 81%

A similar synthetic approach was adopted for the D-rhamnotetrazole **5** and its enantiomer the L-rhamnotetrazole **6**, involving the initial conversion of the appropriate enantiomer of gulonolactone to the corresponding 6-deoxyazide. Thus, L-gulonolactone **7** was converted to the D-azide **12** according to published methods [Scheme 3].[29] Ammonia in methanol with **12** gave amide **13** [100% yield], which upon dehydration gave nitrile **14** [82% yield]. Thermally induced cyclization of **14** resulted, after 3 days, in the formation of tetrazole **15** [67% yield]. Treatment of **15** with aqueous acid afforded the target D-rhamnotetrazole **5** in 85% yield [47% yield from **12**].



Scheme 3: (i) NH_3/MeOH, 100% for **13**, 99% for **18** (ii) $(CF_3CO)_2O$/pyridine/-30°C, 82% for **14**, 85% for **19** (iii) Δ/toluene, 67% for **15**, 75% for **20** (iv) CF_3COOH:H_2O (1:1), 85% for **5**, 79% for **6**

The L-rhamnotetrazole **6** was prepared by an identical sequence from D-gulonolactone **16**. Following conversion of **16** to the L-deoxyazide **17**,[29] treatment of **17** with ammonia in methanol afforded amide **18** [99% yield]. Dehydration of **18** to nitrile **19** [85% yield] and subsequent cyclization resulted in the formation of tetrazole **20** [75% yield]. Deprotection of **20** using aqueous trifluoroacetic acid afforded L-rhamnotetrazole **6** in 79% yield [50% yield from **17**, Scheme 3].

Inhibitory Data

All six tetrazoles described in this and the following paper have been evaluated as inhibitors of glycosidases and details of their biological activities published.[14,15] In addition, an assessment of the relative activities of the D-tetrazoles as compared with those of other inhibitors with regard to structural feature has previously been delineated. A detailed re-evaluation is therefore not required, however there are certain valuable structural generalizations that be made [Table 1], particularly in light of the recently determined and highly unusual inhibitory profile of the α-L-rhamnosidase from *P. decumbens*, naringinase[27]:

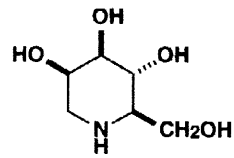
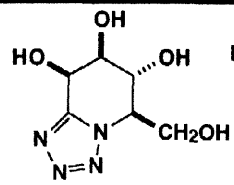
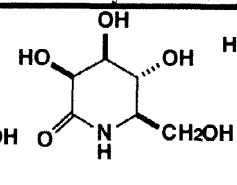
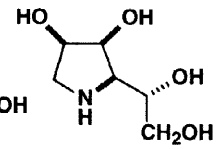
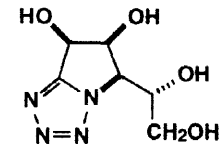
(i) *The activities of the [3.3.0] tetrazoles relative to those of the [4.3.0] tetrazoles show opposing profiles.* The potent inhibition of naringinase (α-L-rhamnosidase) shown by the L-rhamnofuranose tetrazole **32** [K_i 56 μM] is in contrast to the weak activity shown by the L-rhamnopyranose tetrazole **6** [25% inhibition]. The D-pyranose tetrazoles **3** and **5** are potent and specific inhibitors of human liver α-mannosidase [56% and 92% inhibition, respectively] whereas the D-furanotetrazoles **24** and **28** show a complete lack of activity. These apparently contradictory results may indicate a marked difference in the degree of protonation required for D- and L- sugar inhibitors.[30]

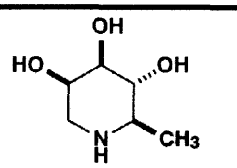
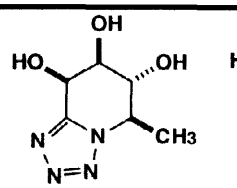
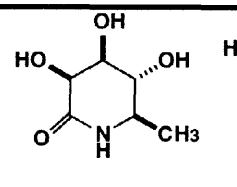
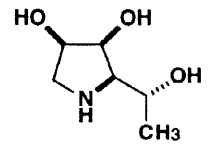
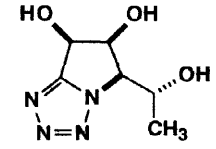
(ii) *Azarhamnofuranose analogues are the most potent inhibitors of α-rhamnosidase.* Polyhydroxylated pyrrolidines bearing L-rhamnose stereochemistry, such as L-(+)-swainsonine, DRAM **31**, and furanotetrazole **32** are the most potent inhibitors of α-L-rhamnosidase naringinase.[14] Indeed, the inhibitory potency of polyhydroxylated piperidines **6**,**29**,**30** bearing L-rhamnose stereochemistry is so uniformly low that L-rhamnopyranose tetrazole **6** is the only piperidine analogue to demonstrate any inhibitory activity.

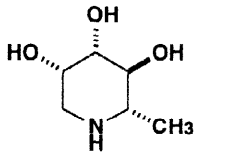
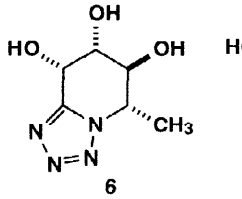
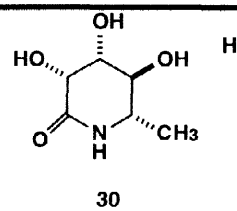
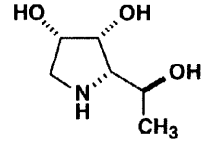
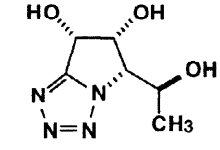
(iii) *Although in general azamannofuranose analogues are more potent inhibitors of α-mannosidase than are azamannopyranoses, this may not be true for analogues containing an sp^2 carbon at the anomeric position.* Pyrrolidines, such as swainsonine, DIM **23** and 6-deoxyDIM **27**, are the most powerful inhibitors of lysosomal α-mannosidase, usually several orders of magnitude more potent than piperidine analogues such as DMJ **21** and DRJ **25**.[31] However, the non-basic tetrazoles **24** and **28** show no inhibitory effects. In contrast, non-basic piperidine analogues of mannose, such as lactams **22** and **26**, and tetrazoles **3** and **5**, are at least as powerful inhibitors of α-mannosidases as are basic piperidines such as **21** and **25**.

(iv) The C-6 hydroxyl group in both furanose and pyranose mimics of mannose does not contribute significantly to the binding of inhibitors to α-mannosidase. It has been suggested that C-6 OH group assists the binding of inhibitors to the active site[32] and claimed that the position of the C-6 OH group of the mannosyl cation is a critical determinant of activity.[33] It is clear that for basic piperidines (**21** *versus* **25**), for pyranotetrazoles (**3** *versus* **5**) and δ-lactams (**22** *versus* **26**), the C-6 OH contributes little to the binding of the inhibitor to the enzyme. In fact, in the case of pyrrolidines, DIM **23**[34] is a weaker inhibitor than 6-deoxyDIM **27**.[35]

Table 1: Percentage Inhibition at ~1mM of compounds of Glycosidases

Inhibitor → / Enzyme ↓	 21	 3	 22	 23	 24
α-mannosidase (Human Liver)	58 (K_i 7.5 x 10^{-4}M)	56	94 (K_i 1.3x10^{-5} M)	89 (K_i 1.3 x 10^{-5}M)	0
β-mannosidase (Human Liver)	4	28	98 (I_{50} 1 x 10^{-5}M)	10	4

Inhibitor → / Enzyme ↓	 25	 5	 26	 27	 28
α-mannosidase (Human Liver)	55 (I_{50} 1 x 10^{-3}M)	92	86 (I_{50} 1 x 10^{-4}M)	100 (K_i 1.3 x 10^{-6}M)	0
β-mannosidase (Human Liver)	+8	2	40	+6	4

Inhibitor → / Enzyme ↓	 29	 6	 30	 31	 32
α-mannosidase (Jack Bean)	0	0	0	0	0
α-rhamnosidase (*P. decumbens*)	0	25	0	100 (K_i 1.0 x10^{-6} M)	100 (K_i 5.6 x10^{-5} M)

In summary, this and the following paper report efficient syntheses of tetrazole analogues of both pyranoses and furanoses. Whilst the D-pyranotetrazoles are potent mannosidase inhibitors, the D-furanotetrazoles are not. In contrast, the tetrazole of L-rhamnofuranose is a much more potent inhibitor of rhamnosidase than the pyranose equivalent. This comparison of the inhibitory properties of tetrazoles **3**,**5**,**6**,**24**,**28**,**32** with other mannose and rhamnose mimics represents another chapter in the emerging story of a mirror-image relationship between mannosidases and rhamnosidases that is punctuated by fascinating subtleties. It is the greater understanding of these key differences that will allow the specificities of glycoprocessing enzyme inhibitors to be finely honed.[36]

Experimental

Melting points were recorded on a Kofler hot block and are uncorrected. Proton nuclear magnetic resonance (δ_H) spectra were recorded, unless otherwise stated on a Varian Gemini 200 (200MHz), Bruker AC 200 (200MHz) or Bruker AM 500 (500MHz) spectrometer. Carbon-13 nuclear magnetic resonance (δ_C) spectra were recorded, unless otherwise stated on a Varian Gemini 200 (50.3MHz), Bruker AC 200 (50.3MHz) or Bruker AM 500 (125MHz) spectrometer and multiplicities were assigned using DEPT sequence. All chemical shifts are quoted on the δ-scale using residual solvent as an internal standard; for samples carbon-13 nuclear magnetic resonance spectra run in D_2O, 1,4-dioxan (δ_C 67.3ppm) or methanol (δ_C 49.6ppm) were used. The following abbreviations were used to explain multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; p, pseudo. Infra-red spectra were recorded on a Perkin Elmer 1750 IR Fourier Transform or Perkin Elmer Paragon 1000 spectrophotometer. Mass spectra (*m/z*) were recorded on a VG Micromass 20-250, ZAB1F, VG Platform, or VG Autospec spectrometers using desorption chemical ionization (NH_3, DCI), chemical ionization (NH_3, CI), electrospray (ES) as stated. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm; concentrations are given in g/100ml. Hydrogenations were executed at atmospheric pressure under an atmosphere of hydrogen gas maintained by an inflated balloon. The removal of water, aqueous acetic acid or aqueous trifluoroacetic acid as solvents was aided by co-evaporation with toluene. Microanalyses were performed by the microanalysis service of the Dyson-Perrins Laboratory. Thin layer chromatography (t.l.c.) was carried out on aluminium or plastic sheets coated with $60F_{254}$ silica. Plates were developed using a spray of 0.2% w/v cerium (IV) sulphate and 5% ammonium molybdate in 2M sulphuric acid. Flash chromatography was carried out using Sorbsil C60 40/60 silica. Aqueous orthophophate solution buffering to pH ~7 (pH 7 buffer) was prepared through the dissolution of 85g KH_2PO_4 and 14.5g NaOH in 950ml distilled water. Solvents and commercially available reagents were dried and purified before use according to standard procedures; hexane was distilled at 68°C before use to remove involatile fractions. All solvents were removed *in vacuo*.

5-Azido-6-O-tert-butyldimethylsilyl-5-deoxy-2,3-O-isopropylidene-D-mannonoamide ***9***: 5-Azido-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-2,3-*O*-isopropylidene-D-mannono-1,4-lactone **8** (5.06 g, 14.3 mmol) prepared as described in ref. 28 was dissolved in dry methanol (50 ml) and ammonia was bubbled through the solution for 30 min when t.l.c. (ethyl acetate/hexane 1:1) showed no starting material (R_f 0.7) and one major product (R_f 0.2). The solvent was removed to give the title compound **9** (5.35 g, 100%) as a colourless oil, which was pure enough for further reactions. For analytical purposes a small sample was purified by flash chromatography (ethyl acetate/hexane 1:4 to 3:1). (Found: C, 48.32; H, 8.26; N, 14.72%. $C_{15}H_{30}N_4O_5Si$ requires C, 48.11; H, 8.07; N, 14.96%). $[\alpha]_D^{25}$ -67.1 (*c*, 1.0 in $CHCl_3$). ν_{max} (film) 3480, 3300 cm^{-1} (br, NH_2, OH), 2100 cm^{-1} (N_3), 1690 cm^{-1} (C=O). *m/z* (CI, NH_3): 375 ($M+H^+$, 10), 174 (100%). δ_H ($CDCl_3$) 0.07, 0.08 (s x 2, 3H x 2, $Si(CH_3)_2$), 0.90 (s, 9H, $SiC(CH_3)_3$), 1.40, 1.56 (s x 2, 3H x 2, $C(CH_3)_2$), 3.13 (s br, 1H, OH), 3.49 (ddd, 1H, H-5, $J_{4,5}$ 9.1Hz, $J_{5,6}$ 7.5Hz, $J_{5,6'}$ 3.0Hz), 3.78 (dd, 1H, H-6, $J_{5,6}$ 7.4Hz, $J_{6,6'}$ 10.7Hz), 3.87 (d, 1H, H-4, $J_{4,5}$ 9.1Hz), 4.08 (dd, 1H, H-6', $J_{5,6'}$ 3.0Hz, $J_{6,6'}$ 10.7Hz), 4.51 (d, 1H, H-3, $J_{2,3}$ 8.4Hz), 4.65 (d, 1H, H-2, 8.4Hz), 6.50, 6.60 (s br x 2, 1H x 2, NH_2). δ_C ($CDCl_3$) -5.6 (q, $Si(CH_3)_2$), 18.1 (s, $SiC(CH_3)_3$), 24.1, 25.7, 26.2 (q x 3, $SiC(CH_3)_3$, $C(CH_3)_2$), 64.5, (t, C-6), 64.4, 67.3, 75.1, 76.5 (d x 4, C-2, C-3, C-4, C-5), 109.6 (s, $C(CH_3)_2$), 173.4 (s, C-1).

5-Azido-6-O-tert-butyldimethylsilyl-5-deoxy-2,3-O-isopropylidene-D-mannononitrile ***10***: 5-Azido-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-2,3-*O*-isopropylidene-D-mannonoamide **9** (5.35 g, 14.3 mmol) was dissolved in dry pyridine (40 ml) and cooled under dry nitrogen to -30°C. Trifluoroacetic anhydride (9.90 ml, 71.2 mmol) was added and the reaction stirred for 1 h when t.l.c. (ethyl acetate/hexane 1:1) showed no starting material (R_f 0.2) and one product (R_f 0.7). Methanol (10 ml) was added and the reaction mixture was allowed to warm

up to room temperature. Solvents were removed and the residue and dissolved in ethyl acetate (100 ml). The solution was washed with brine (75 ml), dried (magnesium sulphate), filtered and the solvent removed. Purification by flash chromatography (ethyl acetate/hexane 1:4) and recrystallisation from hexane afforded the title compound **10** (3.8 g, 75%) as a white solid, m.p. 77-80°C. (Found: C, 50.39; H, 8.18; N, 15.94%. $C_{15}H_{28}N_4O_4Si$ requires C, 50.54; H, 7.92; N, 15.72%). $[\alpha]_D^{25}$ +5.4 (*c*, 1.0 in $CHCl_3$). ν_{max} (KBr) 3460 cm^{-1} (br, OH), 2120 cm^{-1} (N_3). *m/z* (CI, NH_3): 374 ($M+NH_4^+$, 20), 357 ($M+H^+$, 100), 331 (85), 174 (70%). δ_H ($CDCl_3$) 0.13 (s, 6H, $Si(CH_3)_2$), 0.93 (s, 9H, $SiC(CH_3)_3$), 1.42, 1.60 (s x 2, 3H x 2, $C(CH_3)_2$), 2.75 (d, 1H, OH, *J* 4.2 Hz), 3.47 (ddd, 1H, H-5, *J* 6.4Hz, *J* 8.6Hz, $J_{5,6'}$ 3.6Hz), 3.87 - 3.98 (m, 2H, H-4, H-6), 4.15 (dd, 1H, H-6', $J_{5,6'}$ 3.6Hz, $J_{6,6'}$ 10.7Hz), 4.25 (dd, 1H, H-3, $J_{2,3}$ 5.6Hz, $J_{3,4}$ 6.5Hz), 4.94 (d, 1H, H-2, $J_{2,3}$ 5.6Hz). δ_C (d_8-toluene) -5.4 (q, $Si(CH_3)_2$), 18.6 (s, $SiC(CH_3)_3$), 25.8, 26.2, 27.0 (q x 2, $SiC(CH_3)_3$, $C(CH_3)_2$), 64.1, (t, C-6), 64.4, 66.7, 69.9, 79.0 (d x 4, C-2, C-3, C-4, C-5), 111.6 (s, $C(CH_3)_2$), 117.0 (s, C-1).

(5R, 6R, 7S, 8R)-5,6,7,8-Tetrahydro-5-(tert-butyldimethylsilyloxy)methyl-7,8-O-isopropylidene-tetrazolo[1,5-a]pyridine-6,7,8-triol ***11****:* 5-Azido-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-2,3-*O*-isopropylidene-D-mannononitrile **10** (1070 mg, 3.00 mmol) was dissolved in dry toluene (20 ml) and heated to 100 - 105°C for 3 d when t.l.c. (ethyl acetate/hexane 1:1) showed no starting material (R_f 0.7) and one product (R_f 0.3). The solvent was removed and the residue was purified by flash chromatography (ethyl acetate/hexane 1:1) to afford the title compound **11** (969 mg, 91%) as a white solid, m.p. 117°C. (Found: C, 50.33; H, 7.91; N, 15.67%. $C_{15}H_{28}N_4O_4Si$ requires C, 50.54; H, 7.92; N, 15.72%). $[\alpha]_D^{25}$ +5.5 (*c*, 1.0 in $CHCl_3$). ν_{max} (film) 3340 cm^{-1} (br, OH). *m/z* (CI, NH_3): 357 ($M+H^+$, 100%). δ_H ($CDCl_3$): 0.05, 0.08 (s x 2, 3H x 2, $Si(CH_3)_2$), 0.86 (s, 9H, $SiC(CH_3)_3$), 1.43, 1.50 (s x 2, 3H x 2, $C(CH_3)_2$), 2.80 (d, 1H, OH, *J* 3.5 Hz), 4.24 (dd, 1H, CHH'OSi, $J_{5,H}$ 4.5Hz, $J_{H,H'}$ 10.4Hz), 4.33 (dd, 1H, CHH'OSi, $J_{5,H'}$ 6.2Hz, $J_{H,H'}$ 10.4 Hz), 4.51 - 4.64 (m, 3H, H-5, H-6, H-7), 5.52 (d, 1H, H-8, $J_{7,8}$ 6.0Hz). δ_C ($CDCl_3$): -5.6, -5.5 (q x 2, $Si(CH_3)_2$), 18.1 (s, $SiC(CH_3)_3$), 24.8, 25.7, 26.9 (q x 3, $SiC(CH_3)_3$, $C(CH_3)_2$), 61.5, (t, CHH'OSi), 63.3, 66.1, 66.3, 76.7 (d x 4, C-5, C-6, C-7, C-8), 111.8 (s, $C(CH_3)_2$), 149.9 (s, C-8a).

(5R, 6R, 7S, 8R)-5,6,7,8-Tetrahydro-5-hydroxymethyl-tetrazolo[1,5-a]pyridine-6,7,8-triol ***3****:*

(5*R*, 6*R*, 7*S*, 8*R*)-5,6,7,8-Tetrahydro-5-(*tert*-butyldimethylsilyloxy)methyl-7,8-*O*-isopropylidene-tetrazolo[1,5-a]pyridine-6,7,8-triol **11** (677 mg, 1.90 mmol) was dissolved in trifluoroacetic acid/water 1:1 (14 ml) and stirred for 18 h when t.l.c. (ethyl acetate) showed no starting material (R_f 0.7) and one product (R_f 0.0). The solvent was removed and the residue was crystallised from ethanol to give the title compound **3** (312 mg, 81%) as a white solid, m.p. 172°C [lit.,[15] m.p. 159-161°C]. (Found: C, 35.34; H, 4.85; N, 27.39%. $C_6H_{10}N_4O_4$ requires C, 35.65; H, 4.99; N, 27.71%). $[\alpha]_D^{25}$ -63.8 (*c*, 1.0 in MeOH) {lit.,[15] $[\alpha]_D^{25}$ -64.0 (*c*, 0.995 in MeOH)}. ν_{max} (KBr) 3340 cm^{-1} (br, OH). *m/z* (DCI, NH_3): 203 ($M+H^+$, 100%). δ_H (CD_3OD) 4.05 (dd, 1H, H-7, $J_{7,8}$ 3.9Hz, $J_{6,7}$ 7.5Hz), 4.12 (dd, 1H, CHH'OH, $J_{5,H}$ 3.2Hz, $J_{H,H'}$ 11.5Hz), 4.33 (dd, 1H, CHH'OH, $J_{5,H'}$ 4.8Hz, $J_{H,H'}$ 11.5Hz), 4.35 - 4.38 (m, 1H, H-5), 4.47 (dd, 1H, H-6, $J_{5,6}$ 5.0Hz, $J_{6,7}$ 7.5Hz), 5.13 (d, 1H, H-8, $J_{7,8}$ 3.9Hz). δ_C (CD_3OD) 61.1 (t, CHH'OH), 63.0, 65.6, 67.6, 72.6 (d x 4, C-5, C-6, C-7, C-8), 155.2 (s, C-8a).

5-Azido-5,6-dideoxy-2,3-O-isopropylidene-D-mannonoamide ***13****:* 5-Azido-5,6-dideoxy-2,3-*O*-isopropylidene-D-mannono-1,4-lactone **12** (767 mg, 3.38 mmol) prepared as described in ref. 29 was dissolved in dry methanol (20 ml) and ammonia was bubbled through the solution for 20 min when t.l.c. (ethyl acetate) showed no starting material (R_f 0.9) and one product (R_f 0.3). The solvent was removed to give the title compound **13** (825 mg, 100%) as a white solid, m.p. 100-101°C. (Found: C, 44.52; H, 6.42; N, 23.12%. $C_9H_{16}N_4O_4$ requires C, 44.26; H, 6.60; N, 22.94%). $[\alpha]_D^{25}$ -110.1 (*c*, 1.0 in acetone). ν_{max} (KBr) 3540, 3480, 3280 cm^{-1} (br, NH_2, OH), 2130, 2100 cm^{-1} (N_3), 1710, 1670 cm^{-1} (C=O). *m/z* (CI

NH_3): 245 ($M+H^+$, 100%). δ_H (CD_3CN) 1.31 (d, 3H, H-6, $J_{5,6}$ 6.6 Hz), 1.37, 1.55 (s x 2, 3H x 2, $C(CH_3)_2$), 2.93 (s br, 1H, OH), 3.51 (dd, 1H, H-4, $J_{3,4}$ 1.7 Hz, $J_{4,5}$ 1.3Hz), 3.51 (m, 1H, H-5), 4.51 (d, 1H, H-2, $J_{2,3}$ 8.2 Hz), 4.55 (dd, 1H, H-3, $J_{2,3}$ 8.2Hz, $J_{3,4}$ 1.7Hz), 5.97, 6.60 (s br x 2, 1H x 2, NH_2). δ_C (CD_3CN) 15.9 (q, C-6), 24.7, 26.5 (q x 2, $C(\underline{C}H_3)_2$), 59.8, 71.8, 76.3, 77.3 (d x 4, C-2, C-3, C-4, C-5), 110.4 (s, $\underline{C}(CH_3)_2$), 174.2 (s, C-1).

5-Azido-5,6-dideoxy-2,3-O-isopropylidene-D-mannononitrile ***14****:* 5-Azido-5,6-dideoxy-2,3-*O*-isopropylidene-D-mannonoamide **13** (825 mg, 3.38 mmol) was dissolved in dry pyridine (10 ml) and cooled under dry nitrogen to -30°C. Trifluoroacetic anhydride (2.35 ml, 16.9 mmol) was added and the reaction stirred for 1.5 h when t.l.c. (ethyl acetate/hexane 1:3) showed no starting material (R_f 0.0) and one product (R_f 0.5). Methanol (5 ml) was added and the reaction mixture was allowed to warm to room temperature. Solvents were removed and the residue dissolved in ethyl acetate (50 ml). The solution was washed with brine (25 ml), dried (magnesium sulphate), filtered and the solvent removed. Purification by flash chromatography (ethyl acetate/hexane 1:3) afforded the title compound **14** (624 mg, 82%) as colourless oil. (Found: C, 47.78; H, 6.51; N, 24.77%. $C_9H_{14}N_4O_3$ requires C, 47.78; H, 6.24; N, 24.76%). $[\alpha]_D^{25}$ -8.3 (*c*, 1.0 in acetone). ν_{max} (film) 3490 cm^{-1} (br, OH), 2100 cm-1 (N_3). m/z (CI, NH_3): 227 ($M+H^+$, 100%). δ_H (CD_3CN) 1.37 (d, 3H, H-6, $J_{5,6}$ 6.4Hz), 1.37, 1.54 (s x 2, 3H x 2, $C(CH_3)_2$), 3.42 - 3.48 (m, 1H, H-5), 3.53 (d, *J* 6.6Hz, 1H, OH), 3.69 (q, 1H, H-4,*J* 6.6Hz), 4.23 (t, 1H, H-3, *J* 6.0Hz), 5.01 (d, 1H, H-2, $J_{2,3}$ 5.6Hz). δ_C (d_8-toluene) 15.3 (q, C-6), 25.8, 27.1 (q x 2, $C(\underline{C}H_3)_2$), 58.5, 66.6, 73.7, 78.9 (d x 4, C-2, C-3, C-4, C-5), 111.8 (s, $\underline{C}(CH_3)_2$), 117.4 (s, C-1).

(5R, 6R, 7S, 8R)-5,6,7,8-Tetrahydro-7,8-O-isopropylidene-5-methyl-tetrazolo[1,5-a]pyridine-6,7,8-triol ***15****:* 5-Azido-5,6-dideoxy-2,3-*O*-isopropylidene-D-mannononitrile **14** (445 mg, 1.97 mmol) was dissolved in dry toluene (20 ml) and heated to 100 - 105°C under nitrogen for 3 d when t.l.c. (ethyl acetate) showed no starting material (R_f 0.7) and one product (R_f 0.5). The solvent was removed and the residue was purified by flash chromatography (ethyl acetate : hexane, 1:1) to afford the title compound **15** (298 mg, 67%) as a white solid, m.p. 133 - 135°C. (Found: C, 47.88; H, 5.99; N, 24.80%. $C_9H_{14}N_4O_3$ requires C, 47.78; H, 6.24; N, 24.76%). $[\alpha]_D^{25}$ +29.9 (*c*, 1.0 in $CHCl_3$). ν_{max} (KBr) 3300, 3200 cm^{-1} (br, OH). *m/z* (CI, NH_3): 227 ($M+H^+$, 100%). δ_H ($CDCl_3$) 1.37, 1.47 (s x 2, 3H x 2, $C(CH_3)_2$), 1.77 (d, 3H, CH_3, $J_{5,Me}$ 7.0Hz), 3.59 (d, 1H, OH, *J* 3.7 Hz), 4.32 - 4.34 (m, 1 H, H-5), 4.67 - 4.72 (m, 2H, H-6, H-7), 5.53 (d, 1H, H-8, $J_{7,8}$ 6.2Hz). δ_C (d_6-DMSO) 17.5 (q, CH_3), 24.7, 26.6 (q x 2, $C(\underline{C}H_3)_2$), 57.4, 65,7, 69.7, 76.6 (d x 4, C-5, C-6, C-7, C-8), 110.6 (s, $\underline{C}(CH_3)_2$), 149.6 (s, C-8a).

(5R, 6R, 7S, 8R)-5,6,7,8-Tetrahydro-5-methyl-tetrazolo[1,5-a]pyridine-6,7,8-triol ***5****:* (5*R*, 6*R*, 7*S*, 8*R*)-5,6,7,8-Tetrahydro-7,8-*O*-isopropylidene-5-methyl-tetrazolo[1,5-a]pyridine-6,7,8-triol **15** (286 mg, 1.26 mmol) was dissolved in trifluoroacetic acid/water 1:1 (10 ml) and stirred for 18 h when t.l.c. (ethyl acetate) showed no starting material (R_f 0.5) and one product (R_f 0.1). The solvent was removed and the residue was purified by flash chromatography (ethyl acetate/methanol 20:1) to afford the title compound **5** (200 mg, 85%) as a colourless oil. (Found: C, 38.54; H, 5.50; N, 30.32%. $C_6H_{10}N_4O_3$ requires C, 38.71; H, 5.41; N, 30.09%). $[\alpha]_D^{25}$ -66.6 (*c*, 1.0 in acetone). ν_{max} (KBr) 3400 cm^{-1} (br, OH). *m/z* (DCI, NH_3): 187 ($M+H^+$, 100%). δ_H (CD_3OD): 1.78 (d, 3H, CH_3, $J_{5,Me}$ 6.8Hz), 4.03 (dd, 1 H, H-7, *J* 3.6, 7.7 Hz), 4.07 (dd, 1 H, H-6, *J* 5.2, 7.7 Hz), 4.55 - 4.39 (m, 1 H, H-5), 5.13 (d, 1H, H-8, $J_{7,8}$ 3.6Hz). δ_C (CD_3OD): 18.8 (q, CH_3), 60.2, 63.0, 72.3, 72.4 (d x 4, C-5, C-6, C-7, C-8), 154.4 (s, C-8a).

5-Azido-5,6-dideoxy-2,3-O-isopropylidene-L-mannonoamide ***18****:* Ammonia was bubbled through a solution of 5-azido-5,6-dideoxy-2,3-*O*-isopropylidene-L-mannono-1,4-lactone **17** (190mg, 0.705mmol) prepared as described in ref. 29 in a freshly prepared saturated solution of ammonia in anhydrous methanol (7 ml). After 30 minutes, t.l.c (ethyl acetate) showed the complete conversion of starting material (R_f 0.9) to a single

product (R_f 0.3). The solvent was removed to give 5-azido-5,6-dideoxy-2,3-*O*-isopropylidene-L-mannonoamide **18** (202 mg, 99%) as a white solid, m.p. 103-105°C (ether). $[\alpha]_D^{23}$ +120.5 (*c*, 1.3 in acetone), identical in all other respects to the enantiomer **13** above.

5-Azido-5,6-dideoxy-2,3-O-isopropylidene-L-mannononitrile ***19****:* Trifluoroacetic anhydride (0.55 ml, 5.35equiv.) was added dropwise to a solution of 5-azido-5,6-dideoxy-2,3-*O*-isopropylidene-L-mannonoamide **18** (180 mg, 0.738 mmol) in dry pyridine (6 ml) under nitrogen at -30°C. After 2h, t.l.c (ethyl acetate : hexane, 1:3) showed the consumption of starting material (R_f 0.0) and the formation of a major product (R_f 0.4). Methanol (1 ml) was added, the reaction solution warmed to room temperature and the solvent removed. The residue was dissolved in ethyl acetate (20 ml) and washed with brine (10 ml). The organic fraction was dried (magnesium sulphate), filtered and the solvent removed. The residue was purified by flash chromatography (ethyl acetate : hexane, 1:3) to give 5-azido-5,6-dideoxy-2,3-*O*-isopropylidene-L-mannononitrile **19** (142 mg, 85%) as a yellow oil; $[\alpha]_D^{23}$ +6.4 (*c*, 1.12 in acetone), identical in all other respects to the enantiomer **14** above.

(5S,6S,7R,8S)-5,6,7,8-tetrahydro-7,8-O-isopropylidene-5-methyl-tetrazolo[1,5-a]pyridine-6,7,8-triol ***20****:* 5-Azido-5,6-dideoxy-2,3-*O*-isopropylidene-L-mannononitrile **19** (126 mg, 0.558mmol) was dissolved in anhydrous toluene (5.5 ml) and the resulting solution heated to 105°C under nitrogen. After 73h, t.l.c. (ethyl acetate : hexane, 3:1) showed consumption of starting material (R_f 0.8) and the formation of a major product (R_f 0.4). The solvent was removed and the residue purified by flash chromatography (ethyl acetate : hexane, 1:1) to give (5*S*,6*S*,7*R*,8*S*)-5,6,7,8-tetrahydro-7,8-*O*-isopropylidene-5-methyl-tetrazolo[1,5-a]pyridine-6,7,8-triol **20** (94 mg, 75%) as a white solid; m.p. 134-135°C (ethyl acetate/hexane). $[\alpha]_D^{23}$ -33.4 (*c*, 0.96 in $CHCl_3$), identical in all other respects to the enantiomer **15** above.

(5S,6S,7R,8S)-5,6,7,8-Tetrahydro-5-methyl-tetrazolo[1,5-a]pyridine-6,7,8-triol ***6****:* (5*S*,6*S*,7*R*,8*S*)-5,6,7,8-Tetrahydro-7,8-*O*-isopropylidene-5-methyl-tetrazolo[1,5-a]pyridine-6,7,8-triol **20** (75 mg, 0.301 mmol) was dissolved in aqueous trifluoroacetic acid (50% v/v, 2.8 ml). After 5h, t.l.c. (ethyl acetate) showed no starting material (R_f 0.7) and the formation of a major product (R_f 0.2). The solvent was removed and the residue purified by flash chromatography (methanol : ethyl acetate, 1:19) to give (5*R*,6*S*,7*R*,8*S*)-5,6,7,8-tetrahydro-5-methyl-tetrazolo[1,5-a]pyridine-6,7,8-triol **6** (44 mg, 79%) as a colourless oil; $[\alpha]_D^{22}$ +60.6 (*c*, 2.22 in acetone), identical in all other respects to the enantiomer **5** above.

Enzyme Assays: Human liver glycosidases were assayed in the absence and presence [1 mM] of each of the compounds using the appropriate buffered 4-umbelliferyl glycosides as substrates as described previously.[23] α-Mannosidase (Jack Bean) and naringinase (*Penicillium decumbens*) (20 μl of 0.25μg/ml commercially available solution) were assayed using the appropriate *p*-nitrophenyl glycopyranoside (5 mM in 100μl) as a substrate, at the optimum pH of the enzyme at 30°C in the absence and presence [1 mM] of each of the compounds to be tested and quenched after a period of 10 minutes by the addition of glycine solution (pH 10.4).

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