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5-*epi*-Deoxyrhamnojirimycin is a potent inhibitor of an α-L-rhamnosidase: 5-*epi*-deoxymannojirimycin is not a potent inhibitor of an α-D-mannosidase

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Abstract

Whereas deoxyrhamnojirimycin (LRJ) **1** shows no significant inhibition of naringinase (an α-L-rhamnosidase), its C-5 epimer **2** is a potent and specific inhibitor of the enzyme and demonstrates the value of unambiguous chemical synthesis of such materials in the evaluation of their biological properties. In contrast, moderately weak inhibition towards an α-D-mannosidase is shown by both deoxymannojirimycin (DMJ) **5** and its C-5 epimer **6**. Mimics of L-rhamnose which are recognised by enzymes that synthesise or process L-rhamnose may inhibit either the biosynthesis of the sugar or its incorporation into mycobacterial cell walls, providing new strategies for the treatment of diseases such as tuberculosis and leprosy. Molecular modelling studies provide a rationale for the surprisingly potent activity of the C-5 epimer **2** compared with LRJ **1** and support a general hypothesis that potent piperidine glycosidase inhibitors mimic the $^4H_3$ conformation of the relevant glycopyranosyl cation intermediate.

1. Introduction

There exists a very wide range of naturally occurring[1] and synthetic[2] polyhydroxylated piperidines and pyrrolidines that mimic individual sugar moieties. Piperidine analogues of carbohydrates in which the ring oxygen of a sugar is replaced by nitrogen and the anomeric hydroxyl group is replaced by hydrogen are usually effective inhibitors of the corresponding glycosidases,[3] but a change of configuration of one

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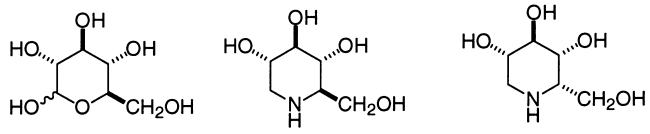
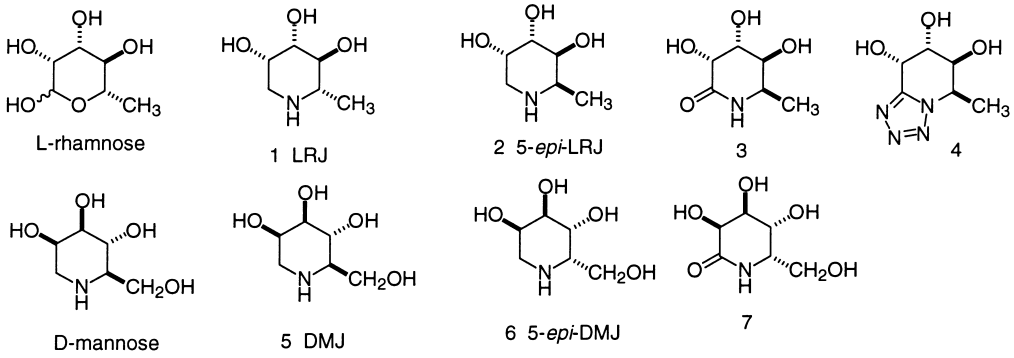
of the ring carbon atoms usually causes a dramatic loss of glycosidase inhibition. Thus deoxynojirimycin (DNJ) is a very good inhibitor of many α-glucosidases whereas 5-*epi*-DNJ is only a very weak inhibitor.[4]



Accordingly, L-deoxyrhamnojirimycin (LRJ) **1**, the corresponding analogue of L-rhamnose, might be predicted to inhibit naringinase [an α-L-rhamnosidase (EC 3.2.1.40)]. However, a paper reporting the synthesis of LRJ **1** from D-gulonolactone stated that the compound had no significant inhibition of naringinase.[5] In contrast, it was claimed that LRJ prepared by an alternative procedure was a potent inhibitor of naringinase with a K_i of 34 μM towards naringinase.[6] The apparent discrepancy in these results was investigated by Wong. He repeated both syntheses and found that, whilst the materials had essentially identical physical data, the sample prepared from D-gulonolactone showed no significant inhibition of naringinase (K_i 490 μM) whereas that prepared by a sequence involving an aldolase-catalysed step[7] was a good inhibitor of the enzyme (K_i 62 μM).[8] Wong suggested that the difference between the two materials might be due to traces of an impurity which was a strong inhibitor of naringinase. On the basis of the possible ambiguities in the aldolase-mediated syntheses, he proposed that the inhibition of the enzyme was due to the presence of a small, undetectable[9] amount of 5-*epi*-LRJ **2**,[10] the C-5 epimer of **1**.



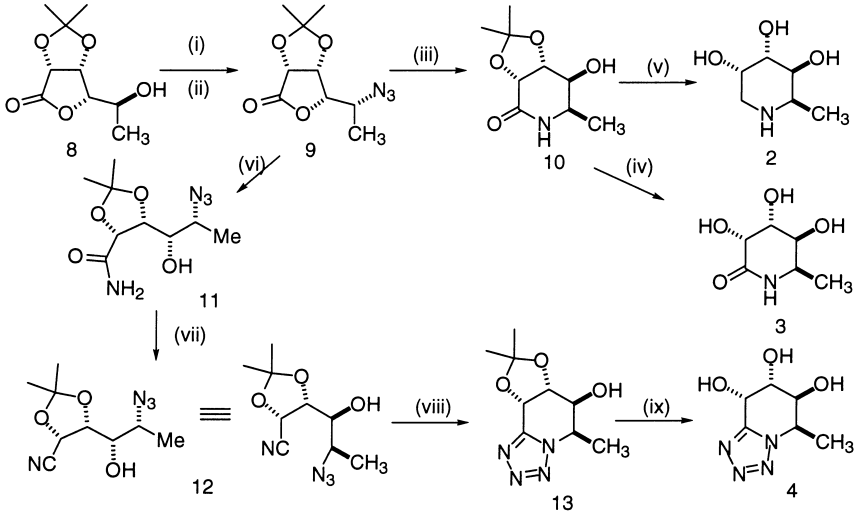
Mimics of L-rhamnose, as well as — or in preference to — their ability to inhibit L-rhamnosidase activity, may be recognised by enzymes which are involved in the biosynthesis of rhamnose or associated with its incorporation into cell walls of mycobacteria. Such materials may provide novel chemotherapeutic strategies for the treatment of diseases such as tuberculosis or leprosy.[10a,11] L-Rhamnose is the enantiomer of 6-deoxy-D-mannose; on the basis of known potent inhibitors of α-D-mannosidases, some powerful inhibitors of L-rhamnosidase have been described.[11d] Deoxymannojirimycin (DMJ) **5**, first isolated from *Lonchocarpus sericeus*,[12] is a potent inhibitor of mannosidase I of glycoprotein processing but otherwise is a rather weak inhibitor of most other α-mannosidases;[13] indeed, DMJ is generally a more potent inhibitor of α-L-fucosidases than of mannosidases.[14] By analogy with the relative qualities of **1** and **2** as inhibitors of naringinase, it was possible that 5-*epi*-DMJ **6**[15] may be a stronger inhibitor in general of mannosidases than is DMJ **5**. This paper reports the unambiguous synthesis of 5-*epi*-LRJ **2** and confirms Wong's suggestion that **2** is a very good inhibitor of naringinase. The corresponding lactam **3** and tetrazole **4** were also prepared as potential L-rhamnose mimics; other lactams and tetrazoles have been shown to be effective glycosidase inhibitors.[10a,11a,d,16] The preparation of *epi*-DMJ **6** from mannose is also described; there is little difference between the ability of DMJ **5** and 5-*epi*-DMJ **6** to

inhibit mannosidases, but the latter is a significantly weaker inhibitor of α-L-fucosidases; some of this work has appeared as a preliminary publication.[10a]

Molecular modelling studies are reported which provide a good rationale for the much stronger inhibition of naringinase by the 5-*epi* compound **2** than by **1** itself. Further modelling studies may give rise to predictions of novel structures that should be good rhamnosidase inhibitors and thus provide guides for structures that interact with enzymes that handle L-rhamnose, and might give clues to new approaches to the study of mycobacterial cell wall biosynthesis.

2. Synthesis

The synthesis of 5-*epi*-LRJ **2**, the lactam **3**, and the tetrazole **4** requires introduction of nitrogen with inversion of configuration at C-5 of L-rhamnose (Scheme 1). Thus, L-rhamnose was first converted, as previously described,[17] to the lactone acetonide **8**[17a] in which only the side chain C-5 hydroxyl group is unprotected. Esterification of **8** with triflic anhydride in the presence of excess pyridine gave the corresponding triflate which, with sodium azide in DMF, afforded the fully protected azide **9** in 67% yield. The azide **9** is the key divergent intermediate in the synthesis of all three 5-*epi*-L-rhamno targets **2**, **3** and **4**.



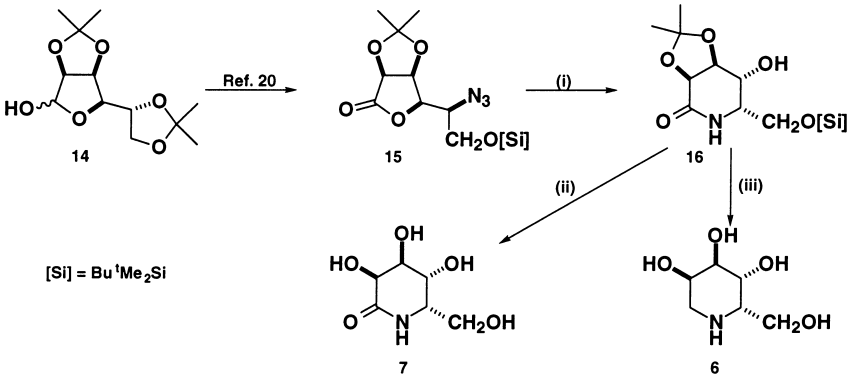
Scheme 1. (i) Tf_2O/pyridine/CH_2Cl_2/−40°C; (ii) NaN_3/DMF; (iii) H_2/10%Pd–C/MeOH; (iv) CF_3COOH:H_2O (3:2); (v) $BH_3 \cdot Me_2S$/THF then conc. HCl/EtOH; (vi) NH_3/MeOH; (vii) $(CF_3CO)_2O$/pyridine/−30°C; (viii) Δ/toluene; (ix) CF_3COOH:H_2O (1:1)

Hydrogenation of a solution of **9** in methanol in the presence of palladium on carbon caused reduction to the corresponding amine which underwent intramolecular acylation to give the lactam **10** (74% yield). Subsequent deprotection with aqueous trifluoroacetic acid gave the easily crystallised unprotected 1,5-lactam **3** in 89% yield. The protected lactam **10** was reduced with borane:dimethylsulphide complex in THF. Treatment of the crude product mixture with methanolic hydrogen chloride caused both the decomposition of the borane adduct formed as well as removal of the acetonide protecting group to produce, after purification by ion-exchange chromatography, 5-*epi*-LRJ **2** in 71% yield.

The bicyclic tetrazole **4** was synthesised in a manner essentially analogous to methods described previously,[10a,11a,d,16i] and required the synthesis of an open chain 5-azidonitrile derivative **12** as a substrate for a key thermally induced intramolecular [1,3]-dipolar cycloaddition. Reaction of azido-

lactone **9** with a methanolic solution of ammonia gave the ring opened primary amide **11** in 99% yield. Dehydration of **11** was achieved by treatment with excess trifluoroacetic anhydride in pyridine, followed by methanolic work-up, and gave the azidonitrile **12** (84% yield). Cyclisation of nitrile **12** to give the protected tetrazole **13** (71% yield) was induced by heating in anhydrous toluene; the formation of a small amount of azidolactone **9** (16% yield) was also observed. Hydroxynitriles are particularly prone to hydrolysis; intramolecular closure of the hydroxyl group onto the electrophilic nitrile group leads to the formation of a cyclic imidate ester which is readily converted to the corresponding lactone.[18] Treatment of protected tetrazole **13** with aqueous trifluoroacetic acid afforded target 5-*epi*-tetrazole **4** in 79% yield.

For the synthesis of 5-*epi*-DMJ **6** and the corresponding δ-lactam **7**,[19] diacetone mannose **14** was converted to the azidolactone **15** as previously described (Scheme 2).[20] Hydrogenation of the azide **15** afforded the protected lactam **16** via the corresponding 4-amino lactone in 80% yield. Deprotection of **16** to L-gulono-1,5-lactam **7** was achieved using aqueous trifluoroacetic acid in 90% yield. Reduction of the protected lactam **16** using borane as its dimethylsulphide complex in THF followed by treatment with aqueous acid in ethanol afforded 5-*epi*-DMJ **6** in 92% yield.



Scheme 2. (i) H_2/10% Pd–C/EtOH; (ii) CF_3COOH:H_2O (2:1); (iii) $BH_3 \cdot Me_2S$/THF then conc. HCl/EtOH

2.1. *Effect on glycosidases*

The inhibitory effects of LRJ **1**, 5-*epi*-LRJ **2**, DMJ **5**, and 5-*epi*-DMJ **6**, the corresponding lactam **3**, and the tetrazole derivative of **3** towards a panel of glycosidases have been studied. Only 5-*epi*-LRJ **2** showed significant inhibition against an α-L-rhamnosidase from *Penicillium decumbens* with a K_i of 1 μM (naringinase from Sigma). None of **1**, **3**, **4**, **5** and **6** were inhibitory at 770 μM using *p*-nitrophenyl α-L-rhamnopyranoside as substrate. **2** was weakly inhibitory to almond β-glucosidase at 970 μM (60% inhibition). 5-*epi*-DMJ **6** was a poor inhibitor of jack bean α-mannosidase (52%) and bovine epididymis α-L-fucosidase (40%) at 770 μM; DMJ **5** has a K_i of 5 μM against the bovine fucosidase used here and is reported as an inhibitor of mannosidases (K_i 110 μM against jack bean mannosidase)[14] but there was no inhibition by 775 μM concentrations of the compounds **1**–**6** of the following enzymes: yeast α-glucosidase, green coffee bean α-galactosidase, *E. coli* β-galactosidase and jack bean β-*N*-acetylglucosaminidase. The substrates were all 5 mM *p*-nitrophenylglycosides and enzyme concentrations were 1.4 μg/ml in the assay mixture.[14] All inhibition of enzymes shown by these compounds is competitive.

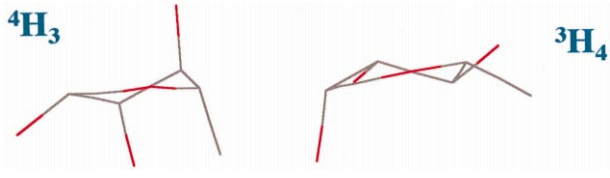
The low level of inhibition of naringinase shown by LRJ **1** confirmed the results of previous assays of chemical syntheses. Wong's suggestion that the inhibition by the product of the aldolase-catalysed routes was due to 5-*epi*-LRJ's **2** potency is correct and shows that the inhibitory activity shown by enzymatically

synthesised samples of LRJ **1** is consistent with the presence of **2** as an impurity. However the potent (K_i 1.0 μM) action of **2** towards naringinase is in stark contrast to the complete lack of inhibition shown by the corresponding 5-*epi*-lactam **3** and 5-*epi*-tetrazole **4**.

5-*epi*-DMJ **6** inhibits the action of jack bean α-mannosidase but is weaker than DMJ itself and **6** is ineffective as an inhibitor of the bovine α-L-fucosidase compared with DMJ **5**. Therefore, 5-*epi*-LRJ **2** is a more potent and specific inhibitor than 5-*epi*-DMJ **6** as evidenced by its strong activity against naringinase and its weak inhibition of the almond β-glucosidase. The unambiguous synthesis of **6** explains the inhibition of α-mannosidase noted by Legler and Jülich[13c] which was thought to be due to contamination by DMJ **5**.

2.2. Molecular orbital calculations

Molecular modelling techniques were used to rationalise the surprising biological activity of 5-*epi*-LRJ **2** in comparison to LRJ **1**. It has been suggested that inhibitors of glycosidases are effective because they have structures which resemble those of the respective glycopyranosyl cations that are the putative intermediates of enzyme-catalysed hydrolysis.[21,22] A recent molecular graphics structure–activity study[23,24] of inhibitors of α-D-mannosidases (EC 3.2.1.24) has suggested that they do indeed mimic the mannopyranosyl cation reaction intermediate. DFT molecular orbital methods were used to determine the geometries of the two possible half-chair forms ($^4H_3$ and $^3H_4$) of the rhamnopyranosyl cation.



2.3. Molecular modelling

The simulated annealing calculations with subsequent minimisation located the lowest energy conformations of LRJ **1** and its C-5 epimer **2**. For LRJ **1**, the lowest energy structure was the $^1C_4$ chair conformation in which the substituents are all equatorial except 2-OH. The lowest energy conformer of the C-5 epimer **2** was the $^4C_1$ (opposite) chair conformation in which the 2-OH and 5-Me substituents are equatorial and the remainder axial. This conformation is also supported by a $^3J_{1,2}$ proton NMR coupling constant value of 10.3 Hz for **2** which is consistent with H-1 and H-2 in a diaxial arrangement. As in previous studies,[23,24] each ring conformation was used as a potential model to rationalise inhibitor structure–activity relationships. The lowest energy conformation of the biologically active C-5 epimer of LRJ **2** was found to be an excellent match with the $^4H_3$ half-chair structure of the rhamnopyranosyl cation. The $^3H_4$ rhamnopyranosyl cation geometry was a very poor fit to the lowest energy C-5 epimer geometry. The lowest energy conformation of the biologically inactive LRJ **1** was a very poor match with the $^4H_3$ rhamnopyranosyl structure. Interestingly, a $^4H_3$ half-chair conformation for the glucosyl cation was also proposed by Sinnott[25] as being consistent with studies of the mechanism of β-glycosidases; it was also found that the $^4H_3$ conformation provided the most useful SAR model for mannosidases. Fig. 1 shows the superimposition of the lowest energy conformations of LRJ **1** and its C-5 epimer **2** on the $^4H_3$ structure of the rhamnopyranosyl cation.

For the C-5 epimer of LRJ **2** the ring hydroxyl oxygen atoms and ring heteroatoms superimpose well, in spite of the conformation of the piperidine ring not being identical to the half-chair conformation of the glucosyl cation pyran ring. The ring carbon atoms also superimpose quite well.

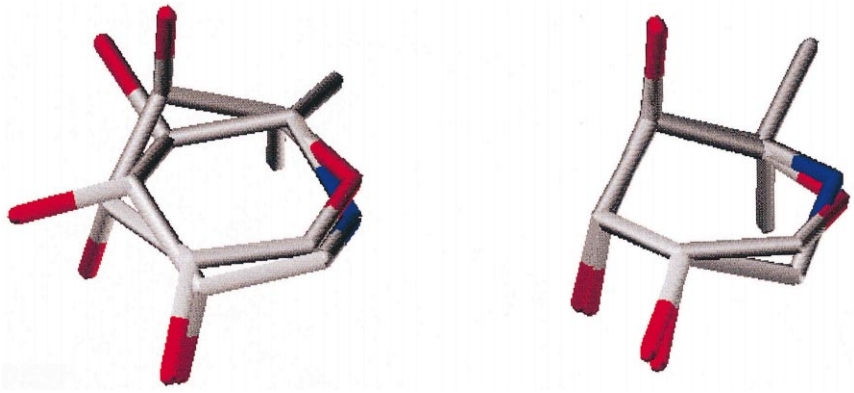


Fig. 1. Superimposition of lowest energy conformers of LRJ **1** (left) and C-5-*epi*-LRJ **2** (right) on the $^4H_3$ conformation of the rhamnopyranosyl cation. Hydrogens have been omitted for clarity

The dominant factors which modulate rhamnosidase inhibition appear to be a ring conformation which matches the $^4H_3$ ring conformation of the rhamnopyranosyl cation, and hydroxyl substituents which are topographical analogues of those in the rhamnopyranosyl cation. The 5-methyl group of LRJ **1** and the epimer **2** appear to modulate inhibition by controlling the conformation of the piperidine ring via steric interactions with the hydroxyl groups. In the case of the C-5 epimer **2**, the piperidine ring adopts a conformation which causes the hydroxyl groups to closely match the orientation in the rhamnopyranosyl cation. In LRJ **1**, the ring conformation is such that the hydroxyls adopt orientations clearly different from those of the cation. Presumably this interferes with the recognition processes in the active site, as previous work has shown that the stereochemistry and positions of the hydroxyl groups of substrates and inhibitors of glycosidase inhibitors are crucial for activity. The apparent tolerance of the active site at certain positions in inhibitors (e.g. the 5-methyl group in LRJ **1** and its epimer **2**) has been observed in other glycosidases. For example, the topographical analogue of the 4-OH in mannosidase inhibitors has only a small influence on inhibitory activity,[23] a finding in agreement with those of kinetic studies of β-glucosidase inhibitors by Dale et al.[26]

The model based on the geometry of the rhamnopyranosyl cation provides a useful rationale to explain the surprising biological activity of LRJ and its epimer, and extends the paradigm for glycosidase inhibition, providing a third example where the $^4H_3$ form of the glycopyranosyl cation is the favoured form for templating of inhibitors. We are currently extending this model for other rhamnosidase inhibitors and are assessing its value in designing other inhibitors of this enzyme.

Simulated annealing experiments were also carried out on DMJ **5** and its C-5 epimer **6**. In both cases the lowest energy conformations were $^4C_1$, in which the C-3 and C-4 hydroxyl groups are equatorial. Such conformations do not superimpose well (Fig. 2) on the mannopyranosyl cation model published previously.[23,24] For DMJ **5** this conformation is further stabilised by the formation of an intramolecular hydrogen bond in low polarity environments, as has been shown for several glycosidase inhibitors by NMR experiments.[27] Superimposition of the heteroatoms of DMJ **5** onto the topographically equivalent heteroatoms in the mannopyranosyl cation model gave an RMS error of 0.9 Å. This relatively poor alignment is consistent with the low activity of compounds **5** and **6** against jack bean α-mannosidase.

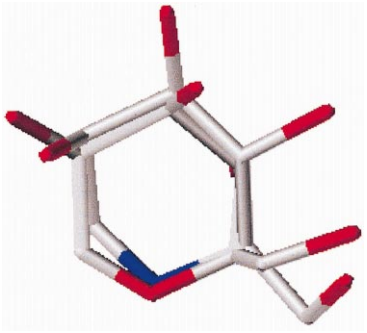


Fig. 2. Superimposition of lowest energy conformer of deoxymannojirimycin **5** on the $^4H_3$ conformation of the mannopyranosyl cation model. Hydrogens have been omitted for clarity

3. Experimental

Melting points were recorded on a Kofler hot block and are uncorrected. Proton nuclear magnetic resonance (δ_H) spectra were recorded on a Varian Gemini 200 (200 MHz), Bruker AC 200 (200 MHz) or Bruker AM 500 (500 MHz) spectrometer. Carbon-13 nuclear magnetic resonance (δ_C) spectra were recorded on a Varian Gemini 200 (50.3 MHz), Bruker AC 200 (50.3 MHz) or Bruker AM 500 (125 MHz) 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 carbon-13 nuclear magnetic resonance spectra run in D_2O, 1,4-dioxan (δ_C 67.3 ppm) or methanol (δ_C 49.6 ppm) were used. The following abbreviations were used to explain multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; qu, quintet; m, multiplet; br, broad; p, pseudo. Infra-red spectra were recorded on a Perkin–Elmer 1750 FT-IR or Perkin–Elmer Paragon 1000 spectrophotometer. Mass spectra (*m/z*) were recorded on a VG Micromass 20-250, ZAB1F, VG Platform, or VG Autospec spectrometer using desorption chemical ionisation (NH_3, DCI), chemical ionisation (NH_3, CI), electrospray (ES), or atmospheric pressure chemical ionisation (APCI) as stated. Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1 dm; concentrations are given in g/100 ml. 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 (TLC) 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 2 M sulphuric acid. Flash chromatography was carried out using Sorbsil C60 40/60 silica. Aqueous orthophosphate solution buffering to pH ~7 (pH 7 buffer) was prepared through the dissolution of 85 g KH_2PO_4 and 14.5 g NaOH in 950 ml 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*.

*3.1. 5-Azido-5,6-deoxy-2,3-*O*-isopropylidene-*D*-gulono-1,4-lactone **9***

Triflic anhydride (0.54 ml, 1.7 equiv.) was added dropwise to a solution of pyridine (0.58 ml, 3.6 equiv.) and 2,3-*O*-isopropylidene-L-rhamnono-1,4-lactone **8**[17] (400 mg, 1.98 mmol) in freshly distilled dichloromethane (20 ml) under nitrogen at −40°C. After 2 h, TLC (ethyl acetate:hexane, 1:1) showed the presence of a small amount of starting material (R_f 0.2) and a major product (R_f 0.7). Further pyridine (0.12 ml, 0.75 equiv.) and triflic anhydride (0.1 ml, 0.3 equiv.) were added to the reaction solution.

After 4 h, TLC showed no starting material and a major product. The reaction solution was diluted with dichloromethane (50 ml) and then washed with dilute hydrochloric acid (30 ml, 2 M), distilled water (30 ml), buffer (30 ml) and then brine (30 ml), dried (magnesium sulphate), filtered and the solvent removed. The residue was dissolved in *N*,*N*-dimethylformamide (10 ml) under nitrogen, and sodium azide (390 mg, 3.0 equiv.) was added to the resulting solution. After 26 h, TLC (ethyl acetate:hexane, 1:1) showed the consumption of triflate and the formation of a major product (R_f 0.55). The solvent was removed and the residue dissolved in ethyl acetate (60 ml). The resulting solution was washed with brine (3×20 ml) and the aqueous layers were further extracted with ethyl acetate (60 ml). The organic fractions were combined, dried (magnesium sulphate), filtered and the solvent removed. The residue was purified by flash chromatography (ethyl acetate:hexane, 1:3) to give the azidolactone **9** (303 mg, 67%) as a white solid, m.p. 78–81°C (ether/hexane). $[\alpha]_D^{21}$ −118.8 (*c*, 1.33 in $CHCl_3$). ν_{max} (KBr) 3300 cm^{-1} (OH), 2125 cm^{-1} (N_3), 1779 cm^{-1} (C=O). *m/z* (APCI+): 232 ($M+H^+-N_2$+MeOH, 8), 200 ($M+H^+-N_2$, 100%). δ_H ($CDCl_3$, 500 MHz) 1.35 (d, 3H, H-6, $J_{5,6}$ 6.7 Hz), 1.41, 1.50 (s×2, 3H×2, $C(CH_3)_2$), 3.91 (dq, 1H, H-5, $J_{5,6}$ 6.7 Hz, $J_{4,5}$ 9.0 Hz), 4.26 (dd, 1H, H-4, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ 9.2 Hz), 4.75 (dd, 1H, H-3, $J_{2,3}$ 5.1 Hz, $J_{3,4}$ 3.6 Hz), 4.85 (d, 1H, H-2, $J_{2,3}$ 5.2 Hz). δ_C ($CDCl_3$, 50.3 MHz) 15.3 (q, C-6), 25.9, 26.7 (q×2, $C(CH_3)_2$), 57.6, 75.7, 76.2, 82.0 (d×4, C-2, C-3, C-4, C-5), 114.7 (s, $C(CH_3)_2$), 173.3 (s, C-1). Anal. found: C, 47.79; H, 5.69; N, 18.47%; $C_9H_{13}N_3O_4$ requires C, 47.57; H, 5.77; N, 18.49%.

*3.2. 6-Deoxy-2,3-*O*-isopropylidene-*D*-gulono-1,5-lactam **10***

Palladium on carbon (10%, 30 mg) was added to a solution of azidolactone **9** (190 mg, 0.837 mmol) in anhydrous methanol (6 ml). The solution was thoroughly degassed and stirred under hydrogen. After 7 h, TLC showed the consumption of starting material [R_f 0.55 in ethyl acetate:hexane (1:1); R_f 0.9 in methanol:ethyl acetate (1:9)] and the formation of a major product [R_f 0.2 in methanol:ethyl acetate (1:9)]. The reaction solution was filtered through a pad of Celite and the solvent removed. The residue was purified by flash chromatography (ethanol:ethyl acetate, 1:9) to give the lactam **10** (125 mg, 74%) as a white crystalline solid, m.p. 151–152°C (ethyl acetate). $[\alpha]_D^{21}$ +39.0 (*c*, 0.52 in $CHCl_3$). ν_{max} (KBr) 3300 cm^{-1} (OH), 1667 cm^{-1} (C=O). *m/z* (APCI+): 202 ($M+H^+$, 100), 144 (22%). δ_H ($CDCl_3$, 500 MHz) 1.35 (d, 3H, H-6, $J_{5,6}$ 6.8 Hz), 1.39, 1.48 (s×2, 3H×2, $C(CH_3)_2$), 2.63 (br s, 1H, OH), 3.81 (qt, 1H, H-5, *J* 1.6 Hz, $J_{5,6}$ 6.9 Hz), 3.85 (br s, 1H, H-4), 4.48 (dd, 1H, H-3, $J_{2,3}$ 6.7 Hz, $J_{3,4}$ 3.3 Hz), 4.53 (dd, 1H, H-2, *J* 0.7 Hz, $J_{2,3}$ 6.7 Hz), 5.86 (br s, 1H, NH). δ_C ($CDCl_3$, 50.3 MHz) 16.2 (q, C-6), 26.4, 26.4 (q×2, $C(CH_3)_2$), 46.9, 69.7, 72.8, 76.4 (d×4, C-2, C-3, C-4, C-5), 110.1 (s, $C(CH_3)_2$), 170.0 (s, C-1). Anal. found: C, 53.62; H, 7.67; N, 6.69%; $C_9H_{15}NO_4$ requires C, 53.72; H, 7.51; N, 6.96%.

*3.3. 6-Deoxy-*D*-gulono-1,5-lactam **3***

The protected lactam **10** (55 mg, 0.274 mmol) was dissolved in aqueous trifluoroacetic acid (60% v/v, 2 ml). After 5 h, TLC (methanol:ethyl acetate, 1:9) showed the consumption of starting material (R_f 0.6) and the formation of a major product (R_f 0.1). The solvent was removed and the residue crystallised from ethanol/ethyl acetate to give 6-deoxy-D-gulono-1,5-lactam **3** (39 mg, 89%) as a white crystalline solid, m.p. 192–193°C (ethanol/ethyl acetate). $[\alpha]_D^{25}$ +66.9 (*c*, 0.86 in H_2O). ν_{max} (KBr) 3400 cm^{-1} (OH), 1670 cm^{-1} (C=O). *m/z* (APCI+): 162 ($M+H^+$, 100), 126 (87%). δ_H (D_2O, 500 MHz) 1.12 (d, 3H, H-6, $J_{5,6}$ 6.9 Hz), 3.80 (qd, 1H, H-5, $J_{4,5}$ 3.0 Hz, $J_{5,6}$ 6.9 Hz), 3.90 (dd, 1H, H-4, $J_{3,4}$ 4.8 Hz, $J_{4,5}$ 3.0 Hz), 4.17 (dd, 1H, H-3, $J_{2,3}$ 3.8 Hz, $J_{3,4}$ 4.6 Hz), 4.25 (d, 1H, H-2, $J_{2,3}$ 3.6 Hz). δ_C (D_2O, 50.3 MHz) 15.0 (q, C-6), 47.9, 66.0, 69.4, 70.1 (d×4, C-2, C-3, C-4, C-5), 173.9 (s, C-1). Anal. found: C, 44.73; H, 6.80; N, 8.49%; $C_6H_{11}NO_4$ requires C, 44.72; H, 6.88; N, 8.69%.

3.4. 5-epi-*LRJ (1,5,6-trideoxy-1,5-imino-*D*-gulitol)* **2**

A solution of borane:dimethylsulphide complex in THF (0.4 ml, 2 M) was added dropwise to a solution of the protected lactam **10** (106 mg, 0.527 mmol) in freshly distilled THF (2 ml) under nitrogen. After 25 h, anhydrous methanol was added dropwise until no further effervescence was observed. The reaction solvent was removed and the residue repeatedly dissolved in methanol (3×5 ml) and evaporated under reduced pressure. The residue was then dissolved in ethanol (1 ml), a small amount of concentrated hydrochloric acid added (3 drops) and the resulting solution cooled to −15°C. After 147 h, the solvent was removed, the residue purified by ion-exchange chromatography [Amberlite IR-120(plus), eluent aqueous ammonia solution 1 M] and freeze-dried to give 5-*epi*-LRJ **2** (55 mg, 71%) as an amorphous solid. $[\alpha]_D^{24}$ +6.3 (*c*, 0.96 in H_2O) {lit.,[10b] $[\alpha]_D^{20}$ −3 (*c*, 1 in H_2O)}. ν_{max} (KBr) 3400 cm^{-1} (OH, NH). *m/z* (APCI+): 148 ($M+H^+$, 100), 130 ($M+H^+-H_2O$, 5%). δ_H (D_2O, 500 MHz) 0.94 (d, 3H, H-6, $J_{5,6}$ 6.9 Hz), 2.59 (dd, 1H, H-1, $J_{1,1'}$ 12.8 Hz, $J_{1,2}$ 10.3 Hz), 2.71 (dd, 1H, H-1′, $J_{1,1'}$ 12.9 Hz, $J_{1',2}$ 4.7 Hz), 2.91 (m, 1H, H-5), 3.61 (dd, 1H, H-4, *J* 2.0 Hz, *J* 4.4 Hz), 3.77 (ddd, 1H, H-2, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 10.0 Hz, $J_{1',2}$ 4.4 Hz), 3.81 (m, 1H, H-3), consistent with literature.[10b] δ_C (D_2O, 50.3 MHz) 15.7 (q, C-6), 44.8 (t, C-1), 49.3 (d, C-5), 66.6, 71.3, 72.9 (d×3, C-2, C-3, C-4), consistent with literature.[10b] HRMS *m/z* (CI, NH_3): found 148.096890 ($M+H^+$); $C_6H_{14}NO_3$ requires 148.097368.

3.5. 5-Azido-5,6-dideoxy-2,3-O-*isopropylidene-*D*-gulononamide* **11**

Ammonia was bubbled through a solution of 5-azido-5,6-dideoxy-2,3-*O*-isopropylidene-D-gulono-1,4-lactone **9** (160 mg, 0.705 mmol) in a freshly prepared saturated solution of ammonia in anhydrous methanol (7 ml). After 1 h, TLC (ethyl acetate) showed the complete conversion of starting material (R_f 0.8) to a single product (R_f 0.4). The solvent was removed to give the open chain amide **11** (170 mg, 99%) as a hygroscopic white solid, m.p. 78–81°C (ethyl acetate). $[\alpha]_D^{23}$ +13.1 (*c*, 1.13 in acetone). ν_{max} (KBr) 3400 cm^{-1} (OH, NH), 2111 cm^{-1} (N_3), 1684 cm^{-1} (amide I), 1586 cm^{-1} (amide II). *m/z* (APCI+): 245 ($M+H^+$, 46), 217 ($M+H^+-N_2$, 100%). δ_H (CD_3CN, 500 MHz) 1.23 (d, 3H, H-6, $J_{5,6}$ 6.7 Hz), 1.37, 1.56 (s×2, 3H×2, $C(CH_3)_2$), 3.60 (pqu, 1H, H-5, *J* 6.4 Hz), 3.68 (dd, 1H, H-4, $J_{3,4}$ 3.1 Hz, $J_{4,5}$ 5.9 Hz), 4.46 (dd, 1H, H-3, $J_{2,3}$ 7.9 Hz, $J_{3,4}$ 3.1 Hz), 4.50 (d, 1H, H-2, $J_{2,3}$ 7.9 Hz), 6.06, 6.63 (br s×2, 1H×2, NH_2). δ_C (CD_3CN, 125 MHz) 16.3 (q, C-6), 24.7, 26.6 (q×2, $C(CH_3)_2$), 60.9, 73.3, 76.6, 77.9 (d×4, C-2, C-3, C-4, C-5), 110.5 (s, $C(CH_3)_2$), 173.3 (s, C-1). Anal. found: C, 44.25; H, 6.56; N, 22.51%; $C_9H_{16}O_4N_4$ requires C, 44.26; H, 6.60; N, 22.94%.

3.6. 5-Azido-5,6-dideoxy-2,3-O-*isopropylidene-*D*-gulononitrile* **12**

Trifluoroacetic anhydride (0.43 ml, 5 equiv.) was added dropwise to a solution of 5-azido-5,6-dideoxy-2,3-*O*-isopropylidene-D-gulononamide **11** (150 mg, 0.615 mmol) in dry pyridine (5 ml) under nitrogen at −35°C. After 2 h, TLC (ethyl acetate) showed the consumption of starting material (R_f 0.4) and the formation of a major product (R_f 0.9). Methanol (1 ml) was added, the reaction solution warmed to room temperature and the solvent removed. The residue was dissolved in ethyl acetate (15 ml), washed with brine (10 ml), dried (magnesium sulphate), filtered and the solvent removed. The residue was purified by flash chromatography (ethyl acetate:hexane, 2:3) to give the azidonitrile **12** (117 mg, 84%) as an amorphous solid. $[\alpha]_D^{24}$ −82.1 (*c*, 0.78 in CH_3CN). ν_{max} (KBr) 3450 cm^{-1} (OH), 2111 cm^{-1} (N_3). *m/z* (APCI+): 227 ($M+H^+$, 100), 201 (8%). δ_H (CD_3CN, 500 MHz) 1.36 (d, 3H, H-6, $J_{5,6}$ 6.7 Hz), 1.38, 1.54 (s×2, 3H×2, $C(CH_3)_2$), 3.50 (qd, 1H, H-5, $J_{4,5}$ 3.4 Hz, $J_{5,6}$ 6.4 Hz), 3.58 (d, 1H, OH, $J_{4,OH}$ 6.5 Hz), 3.79 (ptd, 1H, H-4, $J_{4,5}$ 3.4 Hz, *J* 6.6 Hz), 4.32 (dd, 1H, H-3, $J_{2,3}$ 5.5 Hz, $J_{3,4}$ 6.7 Hz), 4.95 (d, 1H, H-2, $J_{2,3}$

5.5 Hz). δ_C (CD_3CN, 125 MHz) 15.4 (q, C-6), 25.7, 27.1 (q×2, C(CH_3)$_2$), 59.0, 66.4, 73.8, 78.8 (d×4, C-2, C-3, C-4, C-5), 112.7 (s, C(CH_3)$_2$), 117.9 (s, C-1). Anal. found: C, 48.05; H, 6.03; N, 28.01%; $C_9H_{14}N_4O_3$ requires C, 47.78; H, 6.24; N, 24.76%.

*3.7. (5*R,*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* **13**

The amide **12** (110 mg, 0.486 mmol) was dissolved in deuteriated toluene (1.65 ml) and the resulting solution heated to 102°C under nitrogen. After 33 h, proton NMR and TLC (ethyl acetate:hexane, 1:1) showed no starting material (R_f 0.45) and the formation of a major product (R_f 0.2) and a minor product (R_f 0.7). The solvent was removed and the residue purified by flash chromatography (ethyl acetate:hexane, 1:1) to give the lactone **9** (18 mg, 16%) as a white solid and the protected tetrazole **13** (78 mg, 71%) as a white foam. $[\alpha]_D^{24}$ −23.4 (*c*, 0.84 in $CHCl_3$). ν_{max} (KBr) 3400 cm^{-1} (OH). *m/z* (APCI+): 227 ($M+H^+$, 100%). δ_H ($CDCl_3$, 500 MHz) 1.24, 1.47 (3H×2, s×2, C(CH_3)$_2$), 1.87 (d, 1H, CH_3, $J_{5,Me}$ 6.8 Hz), 2.65 (br s, 1H, OH), 4.42 (m, 1H, H-6), 4.67 (qd, 1H, H-5, $J_{5,Me}$ 6.8 Hz, $J_{5,6}$ 2.1 Hz), 4.72 (dd, 1H, H-7, $J_{6,7}$ 3.8 Hz, $J_{7,8}$ 6.0 Hz), 5.59 (d, 1H, H-8, $J_{7,8}$ 6.0 Hz). δ_C ($CDCl_3$, 125 MHz) 13.9 (q, CH_3), 25.1, 26.9 (q×2, C(CH_3)$_2$), 52.7, 66.6, 70.4, 75.0 (d×4, C-5, C-6, C-7, C-8), 111.6 (s, C(CH_3)$_2$), 150.2 (s, C-8a). Anal. found: C, 47.76; H, 6.16; N, 24.95%; $C_9H_{14}N_4O_3$ requires C, 47.78; H, 6.24; N, 24.76%.

*3.8. (5*R,*6*S,*7*R,*8*S*)-5,6,7,8-Tetrahydro-5-methyl-tetrazolo[1,5*-a*]pyridine-6,7,8-triol* **4**

The protected tetrazole **13** (68 mg, 0.301 mmol) was dissolved in aqueous trifluoroacetic acid (50% v/v, 3 ml). After 5 h, TLC (ethyl acetate) showed no starting material (R_f 0.6) and the formation of a major product (R_f 0.1). The solvent was removed and the residue purified by flash chromatography (methanol:ethyl acetate, 1:19) to give the tetrazole **4** (44 mg, 79%) as a colourless oil. $[\alpha]_D^{23}$ +24.3 (*c*, 0.6 in acetone). ν_{max} (film) 3400 cm^{-1} (OH). *m/z* (APCI−): 221 ($M+Cl^-$, 13), 185 ($M-H^+$, 100), 149 (18), 125 (34), 113 (57%). δ_H (CD_3OD, 500 MHz) 1.71 (d, 3H, CH_3, $J_{5,Me}$ 6.8 Hz), 4.19 (dd, 1H, H-6, $J_{5,6}$ 2.8 Hz, $J_{6,7}$ 5.1 Hz), 4.28 (dd, 1H, H-7, $J_{6,7}$ 5.0 Hz, $J_{7,8}$ 3.7 Hz), 4.68 (qd, 1H, H-5, $J_{5,Me}$ 6.8 Hz, $J_{5,6}$ 2.7 Hz), 5.11 (d, 1H, H-8, $J_{7,8}$ 3.6 Hz). δ_C (CD_3OD, 125 MHz) 14.7 (q, CH_3), 54.7, 63.9, 71.2, 72.0 (d×4, C-5, C-6, C-7, C-8), 155.2 (s, C-8a). HRMS *m/z* (CI, NH_3): found 187.083165 ($M+H^+$); $C_6H_{11}N_4O_3$ requires 187.083115.

3.9. 6-O-tert-*Butyldimethylsilyl-2,3*-O-*Isopropylidene*-L-*gulono-1,5-lactam* **16**

The lactone **15**[20] (307 mg, 0.86 mmol) was dissolved in ethanol (8 ml) and palladium on charcoal (25 mg) was added. The solution was degassed and stirred under hydrogen. After 23 h, TLC (ethyl acetate:hexane, 1:1) showed one major product (R_f 0.2) and no starting material (R_f 0.8). The reaction mixture was passed through a Celite plug and the solvents removed. Purification by flash chromatography gave the gulono-1,5-lactam **16** (227 mg, 80%) as a colourless oil. $[\alpha]_D^{22}$ −10.6 (*c*, 1.16 in $CHCl_3$). ν_{max} ($CHCl_3$) 3300 cm^{-1} (OH), 1673 cm^{-1} (C=O). *m/z* (APCI+): 332 ($M+H^+$, 100%). δ_H ($CDCl_3$, 500 MHz) 0.12, 0.12 (s×2, 3H×2, Si(CH_3)$_2$), 0.90 (s, 9H, SiC(CH_3)$_3$), 1.39, 1.47 (s×2, 3H×2, C(CH_3)$_2$), 3.66–3.68 (m, 2H, OH, H-4/H-5), 3.91 (dd, 1H, H-6, $J_{5,6}$ 3.4 Hz, $J_{6,6'}$ 10.9 Hz), 3.98 (dd, 1H, H-6′, $J_{5,6'}$ 4.5 Hz, $J_{6,6'}$ 10.9 Hz), 4.13–4.15 (m, 1H, H-4/H-5), 4.43 (dd, 1H, H-3, $J_{2,3}$ 6.7 Hz, $J_{3,4}$ 3.8 Hz), 4.54 (d, 1H, H-2, $J_{2,3}$ 6.7 Hz), 6.42 (br s, 1H, NH). δ_C ($CDCl_3$, 50.3 MHz) −5.64, −5.56 (q×2, Si(CH_3)$_2$), 18.1 (s, SiC(CH_3)$_2$), 24.5, 26.7 (q×2, C(CH_3)$_2$), 25.8 (q, SiC(CH_3)$_2$), 52.2, 68.5, 73.4, 76.1 (d×4, C-2, C-3,

C-4, C-5), 63.5 (t, C-6), 110.2 (s, $C(CH_3)_2$), 170.1 (s, C-1). Anal. found C, 54.31; H, 9.10; N, 4.21%; $C_{15}H_{29}NO_5Si$ requires C, 54.35; H, 8.82; N, 4.21%.

3.10. L-*Gulono-1,5-lactam* **7**

The protected lactam **16** (73 mg, 0.22 mmol) was dissolved in trifluoroacetic acid (4 ml) and water (2 ml) and stirred for 4 h, after which time TLC (ethyl acetate) showed one product (R_f 0.1) and no starting material (R_f 0.4). The solvents were removed and the residue coevaporated with toluene (2×5 ml). The residue was dissolved in the minimum amount of hot ethanol and a few drops of ethyl acetate were added. After 3 days, the resulting crystals were washed with ethyl acetate giving L-gulono-1,5-lactam **7** (35 mg, 90%), m.p. 171–173.5°C {lit.,[19] could not be crystallised}. $[\alpha]_D^{22}$ −20.6 (*c*, 0.65 in H_2O) {lit.,[19] $[\alpha]_D$ −27.0 (*c*, 0.5 in H_2O)}. δ_H (D_2O, 500 MHz) 3.61–3.67 (m, 1H, H-5), 3.72–3.78 (m, 2H, H-6, H-6′), 4.16 (dd, 1H, H-4, $J_{4,5}$ 2.8 Hz, $J_{3,4}$ 4.8 Hz), 4.19 (dd, 1H, H-3, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 4.8 Hz), 4.35 (d, 1H, H-2, $J_{2,3}$ 3.5 Hz), consistent with literature.[19]

3.11. 5-epi-*DMJ (1,5-dideoxy-1,5-imino*-L-*gulitol)* **6**

The protected lactam **16** (127 mg, 0.38 mmol) was dissolved in dry tetrahydrofuran (2.5 ml) and the solution stirred under nitrogen. Borane:dimethyl sulphide (2 M in THF, 0.75 ml) was added. After 16 h, methanol was added to the reaction mixture until effervescence ceased. The solvents were removed and further methanol (4×5 ml) distilled off the residue, which was then dissolved in ethanol (3 ml). A few drops of concentrated hydrochloric acid were added and the mixture stirred for 3 h. The solvent was removed and the residue dissolved in water (1 ml) and purified by ion-exchange chromatography to give 5-*epi*-DMJ **6** (57 mg, 92%) as a colourless oil. $[\alpha]_D^{21}$ +8.8 (*c*, 0.25 in H_2O) {lit.,[15c,d] $[\alpha]_D$ +9 (*c*, 0.58 in H_2O), lit.,[15b] $[\alpha]_{578}^{20}$ −21 (*c*, H_2O)}. δ_H (D_2O, 500 MHz) 2.86–2.91 (m, 1H, H-1), 3.06 (dd, 1H, H-1′, $J_{1,1'}$ 12.3 Hz, $J_{1',2}$ 4.9 Hz), 3.20–3.23 (m, 1H, H-5), 3.67 (dd, 1H, H-6, $J_{5,6}$ 8.0 Hz, $J_{6,6'}$ 11.8 Hz), 3.73 (dd, 1H, H-6′, $J_{5,6'}$ 5.6 Hz, $J_{6,6'}$ 11.8 Hz), 3.95–3.98 (m, 2H, H-3, H-4), 4.06 (ddd, 1H, H-2, $J_{1,2}$ 11.2 Hz, $J_{1',2}$ 4.9 Hz, $J_{2,3}$ 2.9 Hz), consistent with literature.[15c,d]

3.12. *Enzyme assays*[14]

α-Glucosidase (brewer's yeast), α-fucosidase (bovine epididimus), β-glucosidase (almond emulsin), α-galactosidase (green coffee bean), β-galactosidase (*E. coli*), α-mannosidase (jack bean), β-*N*-acetylglucosaminidase (bovine and jack bean) and naringinase (*Penicillium decumbens*) (20 μl of 10 μg/ml commercially available solution) were purchased from Sigma and assayed using the appropriate *p*-nitrophenylglycopyranoside (5 mM in 100 μl) as a substrate, at the optimum pH of the enzyme at 30°C in the absence and presence of each of the compounds to be tested and quenched after a period of 10 min by the addition of glycine solution (pH 10.4).

3.13. *Molecular orbital calculations*

Molecular orbital calculations were performed on a Cray Y-MP4 computer using the DGausss DFT molecular orbital package in the UniChem suite of programs.[28] Full geometry optimisation was carried out on each half-chair form of the rhamnopyranosyl cation. The calculations used the DZVP basis functions with BLYP non-local functionals. The starting geometries for the two possible half-chair forms of the rhamnopyranosyl cation were derived from the corresponding mannopyranosyl cation

geometries obtained from a molecular orbital study.[23] The calculations used hydroxyl torsion angles which maximised the number of intramolecular hydrogen bonds, as found by Csonka et al. using DFT calculations.[29]

3.14. Molecular modelling

The molecular modelling was carried out on a Silicon Graphics Indigo 2xl workstation (R4400) using Tripos Sybyl[30] software (version 6.3). Crystal structures were available for L-rhamnose.[31] The geometries of the other inhibitors were obtained by modification of these crystal structures, and by the use of the molecular modification and building capabilities of Sybyl. The derived geometries were optimised by means of a molecular mechanics calculation using the Tripos force field.[32] Simulated annealing calculations were used to sample the conformational space accessible to the piperidine rings of LRJ and its epimer, and to derive the lowest energy conformations. The calculations were run for 10 annealing cycles with an initial equilibration period of 1000 fs at a temperature of 1000 K. The time increment in the dynamics calculations was 0.5 fs and the coupling time for temperature regulations was 2.0 fs. The annealing phase used an exponential annealing function cooling from 1000 K to 200 K over 2000 fs. Final geometries were optimised using the Tripos force field prior to superimposition. The superimpositions were accomplished by a least squares fit after choosing pairs of topographically equivalent atoms on the two molecules being studied. The choice of topographically equivalent atoms for the superimpositions was usually clear, with hydroxyl groups of inhibitors and cation being superimposed, as were the pyranose ring oxygen atom and the heterocyclic nitrogen atoms of the inhibitors.

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