The Synthesis of Imidazol Sugars Which Mimic Cyclic Carboxonium Ions Formed During the Glycosidase-Catalysed Hydrolysis of Oligo- and Polysaccharides

Jacques Streith,*[a] Hélène Rudyk,[a] Théophile Tschamber,[a] Céline Tarnus,[a] Christiane Strehler,[a] Dariusz Deredas,[b] and Andrzej Frankowski[b]

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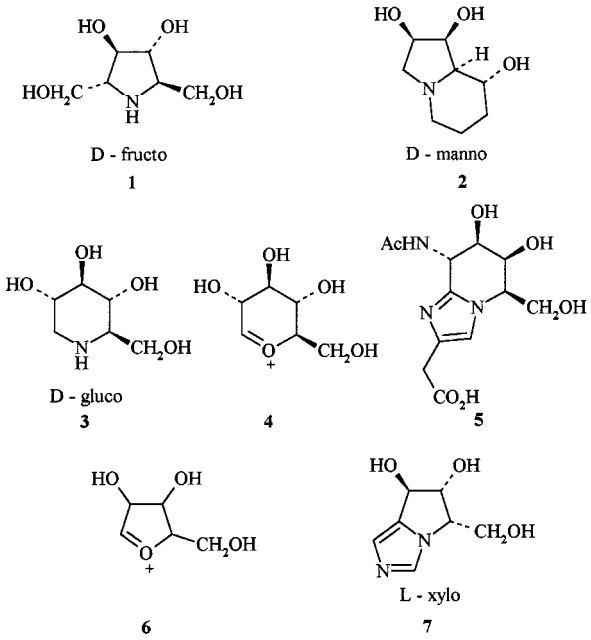
Some naturally occurring carbohydrates, of which several hydroxy groups had been selectively protected, were condensed with formamidine to give the expected imidazole derivatives in the D-arabino (**9**), D-lyxo (**12**), L-xylo (**17**), D-threo (**21**), and in the L- and D-erythro (**24**) series. Introduction of a strong leaving group at the remaining free alcohol function of these products led at once to intramolecular S_N2 cyclisation to the corresponding bicyclic aza sugar derivatives. This was followed by total deprotection to give the target aza sugars in the L-xylo (**7**), L-ribo (**14**), D-arabino (**19**), as well as in the D-threo (**22**) and the L- and D-erythro (**26**) series. Inhibitory assays with four glycosidases showed that the D-arabino aza sugar **19** is the only potent inhibitor (for an α-mannosidase of jack bean).

Introduction

Naturally occurring amino deoxysugars which have a pyrrolidine skeleton such as 3,4-dihydroxy-2,5-bis(hydroxymethyl)pyrrolidine (DMDP) (**1**),[1][2] a derivative of D-fructose, or swainsonine (**2**),[3][4] a derivative of D-mannose, are potent glycosidase inhibitors. Both of these compounds are devoid of the anomeric hydroxy group, a feature which is common to most amino sugars found in nature. The six-membered aza sugars, i.e. piperidinoses like deoxynojirimycine (DNJ) (**3**),[4][5] are more widespread in nature and have been studied in greater depth than the five-membered pyrrolidinoses, particularly when it comes to their interaction with the corresponding glycosidases whose action they inhibit. Thanks to the elaborate X-ray investigations by Phillips with a lysozyme-inhibitor complex,[6] and also to the more recent findings by Withers who used the two anomeric 1,5-difluoroglucopyranose derivatives as irreversible glycosidase inhibitors,[7] cyclic half-chair carboxonium ions like **4** are the widely accepted intermediates during glycosidase-induced hydrolysis of pyranose-type oligo- and polysaccharides. To the best of our knowledge nagstatine (**5**),[8] a potent inhibitor of *N*-acetylaminoglucosaminidase, is the only stable imidazole sugar found in nature (so far) which corresponds to the postulated half-chair cyclic carboxonium ion, or, stated differently, the transition state (TS) on its way to it.

As to pyrrolidinoses they appear as very flexible envelope conformations which interconvert at a fast rate. They do not appear to be highly specific inhibitors. Quite often though pyrrolidinoses (both natural and artificial) are good to excellent glycosidase inhibitors.[1]



Scheme 1. Some glycosidase inhibitors and postulated carboxonium ion intermediates

Since pyrrolidines **1** and **2** are known to be good glycosidase inhibitors, and since there is an obvious analogy between the postulated carboxonium ion intermediates **4** and **6**,[9] it seemed reasonable to synthesize flattened pyrrolidine sugar derivatives which correspond to carboxonium ion **6**. Imidazole L-xylo aza sugar **7**, the synthesis of which was published in 1991,[10] seemed to be such a potential mimic (i.e. after *N*-protonation in the enzymatic active site). It was surmised that **7**, as well as some stereoisomers of **7**, would show inhibitory properties against glycosidases. In fact it

[a] Ecole Nationale Supérieure de Chimie, Université de Haute-Alsace,
3 rue Alfred Werner, F-68093 Mulhouse, France
Fax: (internat.) + 33-3/89336815
E-mail: J.Streith@univ-mulhouse.fr
[b] Institute of Organic Chemistry, Technical University,
ul. Zwirki 36, PL-90924 Lodz, Poland

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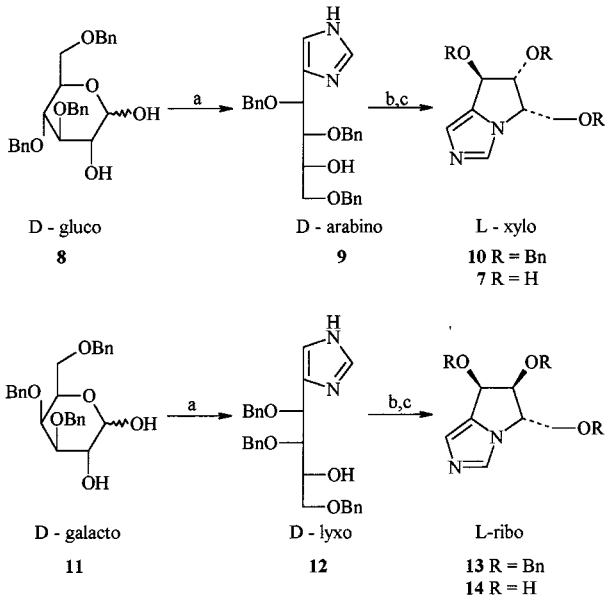
took several years before it was possible to determine some inhibitory properties of the target molecules, i.e. aza sugar derivatives in general. Herein is described the synthesis of several imidazole sugars which are pyrrolidinose derivatives, the starting material being carbohydrates taken out of the chiral pool. Furthermore some inhibitory data, obtained with these aza sugars as determined with but a few glycosidases, are described.

L-Xylo, L-Ribo, and D-Arabino Imidazole Sugars

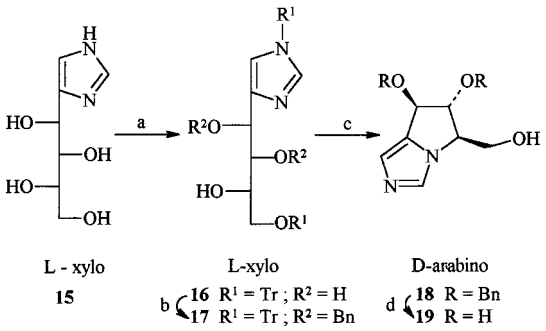
The synthesis of the L-xylo derivative **7** had already been described in a previous paper and the stereostructure ascertained by an X-ray diffraction analysis.[10] In Scheme 2 a second, more straightforward, synthesis of **7** is described starting from the known tri-*O*-benzyl D-glucopyranose derivative **8**.[11] Compound **8** was condensed with formamidine in the presence of ammonia under pressure at 85−90°C, according to a method developed previously.[12] This led to the D-arabino derivative **9** in poor yield (15%). Addition of triflic anhydride to a pyridine solution of **9** in the cold led to intramolecular cyclisation (S_N2) and gave the bicyclic product **10** which was submitted to catalytic hydrogenolysis whereby the crystalline compound **7** (m.p. 227−228°C)[10] was formed.



Scheme 2. Reagents and conditions: (a) NH_3, formamidine acetate, 90°C; (b) CH_2Cl_2, Tf_2O, pyridine, −40 to −20°C; (c) H_2, $Pd(OH)_2$/C, acetic acid

The L-ribo stereoisomer **14** was obtained according to a similar reaction sequence starting from the known tri-*O*-benzyl D-galactopyranose derivative **11**.[11] This latter compound was condensed with formamidine in the presence of ammonia in a pressure vessel (as above) whereby the D-lyxo derivative **12** was obtained. The final reaction steps, i.e. intramolecular cyclisation of the corresponding triflic ester followed by hydrogenolytic debenzylation proceeded as above and gave the crystalline (m.p. 191−192°C) target molecule **14** (see Scheme 2).

The D-arabino stereoisomer **19** was obtained from the imidazolo L-xylo compound **15** prepared previously[12] (see Scheme 3): Tritylation with TrCl in pyridine gave specifically the bis(trityl) derivative **16** in good yield (85%). It was followed by two-fold *O*-benzylation of **16** with benzyl bromide in THF in the presence of sodium hydride and of the solid catalyst Bu_4NI at 0°C to give **17** (62%). The third hydroxy group proved to be inert due to steric crowding, as expected from some previous findings.[10] The last two steps were performed as above for **7** and **14** and led to the target molecule **19** (m.p. 220−221°C, in 56% overall yield from **17**).

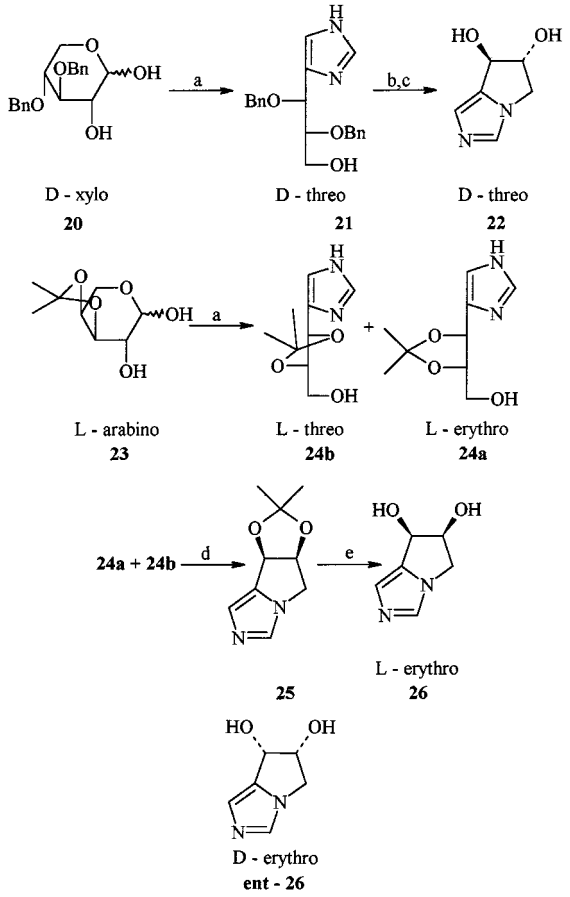


Scheme 3. Reagents and conditions: (a) TrCl, pyridine, DMAP, 75°C; (b) THF, NaH, Bu_4NI, BnBr, 0°C; (c) CH_2Cl_2, pyridine, Tf_2O, −40 to −20°C; (d) H_2, $Pd(OH)_2$/C, acetic acid

D-Threo-, D- and L-Erythro Imidazole Sugars

The D-threo imidazole sugar **22** was synthesised starting from the known di-*O*-benzyl derivative **20** of D-xylose.[11] Condensation with formamidine in ammonia under pressure at 95°C gave imidazole derivative **21** (45%). Sequential reaction of **21** with triflic anhydride in pyridine and dichloromethane (which led to intramolecular cyclisation) and debenzylation by catalytic hydrogenolysis, led to the target D-threo product **22** as a crystalline compound (m.p. 171−172°C, 40% overall yield from **21**).

The L-erythro imidazole sugar **26** was prepared in three steps starting from the known L-arabinose derivative **23**:[13] Condensation with formamidine in ammonia under pressure, as above, led to a 7:3 mixture of the imidazole L-erythro derivative **24a** and the rather unexpected L-threo isomer **24b** in an overall yield of 70%. Reaction of the preceding mixture with triflic anhydride in pyridine/dichloromethane or with α-tosyl chloride, followed by addition of acetic anhydride and heating,[14] led to the L-erythro compound **25** only. The protection group of **25** was removed with trifluoroacetic acid and water; L-erythro isomer **26** was isolated by column chromatography as colourless crystals (m.p. 171−172°C) in an overall yield of 57% from **24** (see Experimental Section). The D enantiomer **ent-26** was prepared in the same manner starting from D-arabinose, i.e. from **ent-23**.



Scheme 4. Reagents and conditions: (a) NH_3, formamidine acetate; (b) CH_2Cl_2, pyridine, Tf_2O; (c) H_2, $Pd(OH)_2$, acetic acid; (d) $PhCH_2SO_2Cl$, pyridine then Ac_2O, 85°C; (e) CF_3COOH/H_2O

Enzymatic Assays

The imidazole sugars were evaluated as inhibitors of glycosidases at the optimum pH (see Experimental Section and Table 1). The dihydroxy compounds **22, 26**, and **ent-26** showed no significant inhibition of the four glycosidases evaluated (at up to 1 mM concentration), **22** being the most potent with a slight inhibition of α-mannosidase of jack bean (IC_{50} = 500 μM).

Amongst the trihydroxy compounds derivative **14** was inactive on the four glycosidases tested. The hydroxymethylene group in **7** and **19** had no effect on α- and β-glucosidase and β-mannosidase activities up to 1 mM concentration in our assays, whatever the configuration of the imidazole sugar.

Compound **19** (IC_{50} = 10 μM) proved to be the most potent and selective competitive inhibitor of α-mannosidase activity (50 times more potent than the parent compound **22** which lacks the CH_2OH group). In addition comparison with **7** (IC_{50} = 150 μM, 3 times more potent than **22,** IC_{50} = 500 μM), clearly indicates that the D configuration and the presence of the hydroxymethylene group of the imidazole sugar are essential for increased affinity for α-mannosidase.

Table 1. Inhibitory activities (IC_{50} expressed in μM) of four selected compounds

Glycosidases	**14**	**22**	**7**	**19**
α-mannosidase of jack beans	> 1000	500	150	10 (K_i = 5 μM)
β-mannosidase of snails	> 1000	> 1000	> 1000	> 1000
α-glucosidase of baker's yeast	> 1000	> 1000[a]	> 1000	> 1000
β-glucosidase of almonds	> 1000	> 1000	> 1000	> 1000

[a] 30% inhibition with [I] = 1mM.

Experimental Section

General: Flash chromatography (FC): Silica gel (Merck 60, 230−400 mesh). − TLC: Silica gel HF_{254} (Merck). − Optical rotation: Schmitt-Haensch Polartronic Universal and Perkin−Elmer−241 polarimeters. − NMR (300 K): Bruker AC-250 spectrometer using double irradiation techniques; tetrametylsilane (TMS; ^1H NMR) and $CDCl_3$ [δ($CDCl_3$) = 77.00 with respect to TMS; ^{13}C NMR] as internal references. − Mass spectra (MS and HRMS): ZabSpec TOF Micromass spectrometer at 8 kV (source temp. 40°C) in a *m*-nitrobenzylic alcohol matrix in the "LSIMS with Cs^+, Positive" ionisation mode, or with a Varian MAT 311 spectrometer with electronic impact, performed at the Centre Régional de Mesures Physiques de l'Ouest at the University of Rennes. The H^+ exchange resin Amberlite CG-6000 is from Rohm & Haas. $Pd(OH)_2$/C (20% Pd) is moistened with H_2O (ca. 50%).

(1*R*,2*R*,3*R*)-1-(4′-Imidazolyl)-1,2,4-tri-*O*-benzylbutane-1,2,3,4-tetrol (9): Ammonia (ca. 20 mL) was condensed in a 200-mL stainless steel pressure vessel cooled in a dry-ice/acetone bath, followed by the addition of a mixture of 3,4,6-tri-*O*-benzylglucose[11] (1.16 g, 2.57 mmol) and formamidine acetate (513 mg, 1.9 equiv.). The pressure vessel was sealed and heated to 90°C (the pressure reached 40−45 atm) with stirring for 30 h. The pressure vessel was then cooled in a dry-ice/acetone bath, opened, taken out and the ammonia allowed to evaporate. The resulting brownish oil was dissolved in MeOH (10 mL) and CH_2Cl_2 (15 mL), and the solution concentrated to dryness to eliminate the remaining ammonia. The resulting oil was taken up in MeOH and passed through a column of Amberlite CG-6000. The elution of **9** was performed with MeOH containing 5% NH_4OH. After evaporation of the solvent, the residue was purified by FC (Et_2O/MeOH/NH_4OH, 95:5:3) and **9** was obtained as a yellowish resin (176 mg, 15%). − ^1H NMR ($CDCl_3$): δ = 7.40 (s, 2′-H), 7.22−7.15 (H arom.), 6.83 (5′-H), 4.68 (d, 1-H), 4.48−4.16 (m, 3 × CH_2Ph), 3.85 (m, 3-H), 3.70 (dd, 2-H), 3.51−3.49 (m, 2 × 4-H, AB system); $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 6.8 Hz. − MS; *m/z* (%): 459 (100) $[M + H]^+$, 351 (21) $[(M + H) - PhCH_2OH]^+$. − HRMS: calcd. for $[M + H]^+$ ($C_{28}H_{31}N_2O_4$) 459.2284; found 459.2290.

(5*S*,6*R*,7*R*)-6,7-Dihydro-5-hydroxymethyl-5*H*-pyrrolo[1,2-*c*]-imidazole-6,7-diol (7): Tf_2O (0.8 mL, 3 equiv.) was added to a stirred solution of **9** (732 mg, 1.60 mmol) and pyridine (0.8 mL, 6.0 equiv.) in CH_2Cl_2 (7.0 mL) under Ar at −40°C. After 45 min at −20°C, the reaction was neutralised by adding solid $NaHCO_3$, H_2O (2.0 mL) and CH_2Cl_2 (10 mL). After extraction, the organic phase was dried with $MgSO_4$, filtered and concentrated to yield **10** as a resin. This was dissolved in acetic acid (10 mL) and hydrogenated with H_2 (1 atm) at $Pd(OH)_2$ with stirring. After 4 d, the solu-

tion was filtered and concentrated. The residue was dissolved in H_2O, passed through Amberlite CG-6000 (H^+), desorbed with 5% NH_4OH, concentrated and chromatographed (FC; Et_2O/MeOH/conc. NH_4OH, 7:3:0.2) to obtain **7** after recrystallisation (EtOH + 2% Et_2O) (71 mg; 26%), m.p. 227−228°C (ref.[11] 227−228°C). − ^{1}H- and ^{13}C-NMR data are identical to those reported in the literature.[10]

(1*R*,2*S*,3*R*)-1-(4′-Imidazolyl)-1,2,4-tri-*O*-benzylbutane-1,2,3,4-tetrol (12): Same procedure as for **9** from **11** (2.43 g, 5.39 mmol) and formamidine acetate (1.12 g, 2.0 equiv.). **12** was obtained after FC (Et_2O/MeOH/NH_4OH, 98:2:2) (728 mg, 30%) as a yellowish resin. − $[\alpha]_D = -32$ (c = 1, MeOH). − ^{1}H NMR ($CDCl_3$, 330 K): δ = 7.49 (d, 2′-H), 7.36−7.17 (m, H arom.), 6.93 (d, 5′-H), 4.72 (d, 1-H), 4.56−4.37 (m, 3 × CH_2Ph, 1 s, 2 AB systems, J = 11.0 Hz and 11.7), 4.05−3.95 (m, 2-H and 3-H), 3.53 (m, 2 × 4-H); $J_{1,2}$ = 4.8 Hz, $J_{2',5'}$ = 1.0 Hz. − ^{13}C NMR ($CDCl_3$): δ = 137.9 137.8 (C arom.), 135.6 (C-2′), 134.1 (C-4′), 128.9−127.5 (C arom.), 120.6 (C-5′), 80.6 (C-2), 75.3 (C-1), 74.4 73.4 71.1 (3 × CH_2Ph), 70.7 (C-4), 70.2 (C-3). − MS; *m*/*z* (%): 459 (100) $[M + H]^+$, 351 (28) $[(M + H) - PhCH_2OH]^+$. − HRMS: calcd. for $[M + H]^+$ ($C_{28}H_{31}N_2O_4$) 459.2284; found 459.2290.

(5*S*,6*S*,7*R*)-6,7-Dihydro-5-hydroxymethyl-5*H*-pyrrolo[1,2-*c*]-imidazole-6,7-diol (14): Same procedure as for **7**. From **12** (1.69 g, 3.68 mmol), Tf_2O (1.85 mL, 3 equiv.), pyridine (1.85 mL) and CH_2Cl_2 (16 mL), **13** was obtained. After hydrogenolysis with H_2 (1 atm) on $Pd(OH)_2$ in acetic acid (20 mL, 24 h) and FC (Et_2O/MeOH/conc. NH_4OH, 8:2:0.2), the latter gave compound **14** (154 mg, 25%) as a colourless powder (MeOH + 2% Et_2O), m.p. 191−192°C. − $[\alpha]_D = -58$ (c = 1, MeOH). − ^{1}H NMR (D_2O): δ = 7.69 (3-H), 6.96 (1-H), 4.98 (d, 7-H), 4.47 (dd, 6-H), 4.21 (5-H), 4.16 (CH_aHOH), 3.85 (CH_bHOH); $J_{6,7}$ = 5.2 Hz, $J_{5,6}$ = 6.6 Hz, $J_{5,Ha}$ = 3.1 Hz, $J_{5,Hb}$ = 5.0 Hz, $J_{Ha,Hb}$ = 12.4 Hz (the J values of the second-order signals were calculated with the Panic Program from Bruker). − ^{13}C NMR (CD_3OD): δ = 137.5 (C-8), 132.3 (C-3), 122.5 (C-1), 77.9 (C-6), 66.0 (C-7), 64.6 (C-5), 62.2 (CH_2OH). − MS(Varian); *m*/*z* (%): 170 (11) $[M]^+$, 152 (4) $[M - H_2O]^+$, 97 (100). − HRMS: calcd. for $C_7H_{10}N_2O_3$ (170.06914); found 170.0687.

(1*R*,2*S*,3*S*)-4-*O*-Triphenylmethyl-1-[1′-(triphenylmethyl)-1′*H*-imidazol-4′-yl]butane-1,2,3,4-tetrol (16): TrCl (32.98 g, 2.2 equiv.) and DMAP (600 mg) were added to a solution of **15** (10.12 g, 53.78 mmol) in pyridine (210 mL). The suspension was divided into two batches and each one was heated at 70−75°C for ca. 4 h until the TrCl was entirely consumed (TLC). Pyridine was distilled under vacuum and H_2O and CH_2Cl_2 were added to the residue. The organic phase was extracted, washed with brine, dried ($MgSO_4$) and filtered. The two reaction batches were concentrated together and the residue purified (FC; AcOEt then AcOEt/MeOH, 9:1). **16** was eluted after the trityl derivatives. The contaminated fractions were chromatographed again (FC). Total amount of **16**: 30.62 g (85%). − $[\alpha]_D^{20} = -3$ (c = 2.0, $CHCl_3$). − IR (KBr): $\tilde{\nu}$ = 3410 cm^{-1}, 3055, 3020, 2910, 1590, 1475, 1210, 1120, 740, 700. − ^{1}H NMR ($CDCl_3$): δ = 7.44 (2′-H), 7.44−7.20 and 7.13−7.10 (H arom.), 6.86 (5′-H), 4.69 (1-H), 3.93 (3-H), 3.88 (2-H), 3.30 (m, 2 × 4-H); $J_{2',5'}$ = 1.4 Hz, $J_{1,2}$ = 5.0 Hz, $J_{2,3}$ = 2.6 Hz, $J_{3,4a}$ = 5.7 Hz. − ^{13}C NMR ($CDCl_3$): δ = C arom. not mentioned, 140.5 (C-4′), 138.7 (C-2′), 74.0 (C-2), 70.7 (C-3), 69.6 (C-1), 65.2 (C-4). − $C_{45}H_{40}N_2O_4$ (672.8): calcd. C 80.33, H 5.99, N 4.16; found C 80.2, H 5.9, N 4.2.

(1*R*,2*R*,3*S*)-1,2-*O*-Dibenzyl-4-triphenylmethyl-1-[1′-triphenylmethyl-1′*H*-imidazol-4′-yl]butane-1,2,3,4-tetrol (17): NaH in oil (50%) (0.2 g, ca 4.15 mmol) at −5°C was added to a stirred solution of **16** (0.82 g; 1.22 mmol) in anhyd. THF (15 mL). The reaction mixture was kept at −5°C until the evolution of H_2 had ceased. To this solution were added Bu_4NI (5 mg) and BnBr (340 µL, 2.4 equiv.). Stirring was continued at 0°C for 24 h until complete disappearance of the starting material (TLC). If the reaction stopped at the monobenzyl derivative, further NaH and $PhCH_2Br$ were added. The mixture was treated with MeOH (0.5 mL) and stirred at room temp. for another 0.5 h. After evaporation of the solvents, the residue was taken up in H_2O and extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated. The residue was purified by FC (AcOEt/C_6H_{12}, 3:7) to yield **17** as a colourless foam (644 mg, 62%). − $[\alpha]_D^{20} = -20$ (c = 0.75, $CHCl_3$). − IR (KBr): $\tilde{\nu}$ = 3450 cm^{-1}, 3050, 3000, 2918, 2850, 1590, 1490, 1450, 1210, 1120, 1080, 1050. − ^{1}H NMR ($CDCl_3$): δ = 7.50 (d, 2′-H), 7.4−6.95 (H arom.), 6.85 (d, 5′-H), 4.72−4.33 (2 × AB system, 2 × CH_2Ph), 4.67 (d, 1-H), 4.00 (m, 2-H), 3.89 (m, 3-H), 3.36 (dd, 4-H$_a$), 3.11 (dd, 4-H$_b$); $J_{2',5'}$ = 1.4 Hz, $J_{1,2}$ = 7.6 Hz, $J_{2,3}$ = 1.5 Hz, $J_{3,4a}$ = 5.9 Hz, $J_{3,4b}$ = 7.7 Hz, $J_{4a,4b}$ = 8.9 Hz. − ^{13}C NMR ($CDCl_3$): δ = C arom. not mentioned, 139.0 (C-2′), 138.2 (C-4′), 120.8 (C-5′), 81.3 (C-2), 78.3 (C-1), 69.8 (C-3), 64.3 (C-4).

(5*R*,6*R*,7*R*)-6,7-Di-*O*-benzyl-6,7-dihydro-5-hydroxymethyl-5*H*-pyrrolo[1,2-*c*]imidazole-6,7-diol (18): Anhyd. pyridine (1.5 mL) and freshly distilled Tf_2O (1.5 mL) were added dropwise to a stirred solution of **17** (2.55 g, 2.99 mmol) in anhyd. CH_2Cl_2 (25 mL) at −55°C under Ar. Stirring was continued from −55°C to −20°C for 2 h. MeOH was added to the resulting yellow solution. This solution was concentrated at room temp. The residue was taken up in THF (25 mL) to which was added aq. HCl 6N (50 drops). The resulting mixture was heated to reflux for 1.5 h (TLC), and neutralised with $NaHCO_3$. The mixture was concentrated and the residue purified by FC ($CHCl_3$/MeOH, 9.25:0.75 to 9:1) to yield **18** (696 mg, 66%) as a colourless resin. − $[\alpha]_D^{20} = -32$ (c = 0.27, $CHCl_3$). − IR (film): $\tilde{\nu}$ = 3150 cm^{-1}, 3040, 2940, 2860, 1475, 1455, 1240, 1100, 1080, 1015, 750, 700. − ^{1}H NMR ($CDCl_3$): δ = 7.57 (s, 3-H), 7.38−7.25 (H arom.), 6.93 (s, 1-H), 4.81 (d, 7-H), 4.41 (dd, 6-H), 4.32 (m, 5-H), 3.93 (dd, CH_aHOH), 3.79 (dd, CH_bHOH); $J_{5,6}$ = 2.5 Hz, $J_{5,Ha}$ = 4.4 Hz, $J_{5,Hb}$ = 7.6 Hz, $J_{6,7}$ = 1.8 Hz, $J_{Ha,Hb}$ = 11.4 Hz. − ^{13}C NMR ($CDCl_3$): δ = C arom. not mentioned, 133.7 (C-8), 131.7 (C-3), 123.0 (C-1), 90.0 (C-6), 76.1 (C-7), 64.1 (C-5), 63.0 (C-9). − $C_{21}H_{22}N_2O_3$ (350.4): calcd. C 71.98, H 6.33, N 8.00; found C 71.9, H 6.3, N 8.0.

(5*R*,6*R*,7*R*)-6,7-Dihydro-5-hydroxymethyl-5*H*-pyrrolo[1,2-*c*]-imidazole-6,7-diol (19): $Pd(OH)_2$/C (80 mg) was added to a solution of **18** (350 mg, 1.00 mmol) in AcOH (15 mL), and the resulting suspension stirred at room temp. under H_2 (1 atm) for 24 h until complete disappearence of **18** (TLC). The catalyst was removed by centrifugation and successively washed with AcOH and H_2O. The combined filtrates were concentrated and the resulting residue was dissolved in H_2O. This aq. solution was passed through an Amberlite CG-6000 (H^+) column. Desorbtion of **19** was performed with a 5% NH_4OH solution and the solution concentrated to dryness. The residue was purified (FC; Et_2O/MeOH/conc. NH_4OH, 7:3:0.5). **19**: 194 mg (57%). M.p. (MeOH): 220−221°C. − $[\alpha]_D^{20}$ = +9 (c = 1, H_2O). − IR (KBr): $\tilde{\nu}$ = 3350 cm^{-1}, 3160, 3100, 2940, 2490, 1670, 1505, 1485, 860, 790. − ^{1}H NMR (CD_3OD): δ = 7.66(3-H), 6.87 (1-H), 4.83 (7-H), 4.32 (6-H), 4.10 (5-H), 4.02 (CH_aHOH), 3.75 (CH_bHOH); $J_{5,6}$ = 3.4 Hz, $J_{5,Ha}$ = 3.8 Hz, $J_{5,Hb}$ = 7.2 Hz, $J_{6,7}$ = 3.4 Hz, $J_{Ha,Hb}$ = 11.4 Hz. − ^{13}C NMR (CD_3OD): δ = 137.9 (C-8), 132.2 (C-3), 121.6 (C-1), 85.4 (C-6), 73.5 (C-7), 67.0 (C-5), 63.1 (CH_2OH). − $C_7H_{10}N_2O_3$ (170.2): calcd C 49.41, H 5.92, N 16.46; found C 49.5, H 6.0, N 16.3.

(1*R*,2*R*)-1,2-Di-*O*-benzyl-1-(4′-imidazolyl)propane-1,2,3-triol (21): Same procedure as for **9**. **21** was obtained after FC (Et_2O/MeOH/conc. NH_4OH, 95:5:2) (1.38 g, 45%) as a yellowish resin from **20** (3.00 g, 9.08 mmol) and formamidine acetate (1.89 g, 18.16 mmol), which were heated for 40 h at 95°C. − $[\alpha]_D = -45$. − ^{1}H NMR ($CDCl_3$): δ = 7.30 (2′-H), 7.14−7.09 (H arom.), 6.74 (5′-H), 4.58 and 4.30 (2 × CH_2Ph), 4.57 (d, 1-H), 3.71 (ddd, 2-H), 3.52 (dd, 3-H_a), 3.42 (dd, 3-H_b); $J_{1,2}$ = 6.6 Hz, $J_{2,3a}$ = 3.6 Hz; $J_{2,3b}$ = 4.6 Hz, $J_{3a,3b}$ = 12.2 Hz. − ^{13}C NMR ($CDCl_3$): δ = 139.1, 138.2, 128.1−127.3 (C arom.), 138.2 (C-4′), 135.2 (C-2′), 117.8 (C-5′), 81.7 (C-2), 75.9 (C-1), 73.1 and 71.1 (2 × *C*H_2Ph), 61.3 (C-3). − MS; *m*/*z* (%): 339 (100) $[M + H]^+$, 231 (64) $[(M + H) - PhCH_2OH]^+$. − HRMS; calcd. for $[M + H]^+$ ($C_{20}H_{23}N_2O_3$) 339.1709; found 339.1708.

(6*R*,7*R*)-6,7-Dihydro-5*H*-pyrrolo[1,2-*c*]imidazole (22): Triflic anhydride (1.9 mL) was added (under Ar at −40°C) to a stirred solution of **21** (1.275 g, 3.78 mmol) in CH_2Cl_2 (13 mL) and pyridine (1.9 mL), then allowed to slowly warm up to 0°C. After 1 h, CH_2Cl_2 (10 mL) and some solid $NaHCO_3$ were added. After addition of water, the organic phase was extracted, dried ($MgSO_4$), filtered and concentrated to dryness. AcOH (10 mL) and $Pd(OH)_2$/C were added to the residue and the resulting suspension was stirred under H_2 (1 atm) for 72 h until complete disappearence of the benzyl derivative (TLC). The catalyst was removed by filtration and the solution concentrated. The residue was dissolved in H_2O and the solution passed through an Amberlite CG-6000 column. Desorbtion of **22** was performed with NH_4OH (2 N). After concentration, the residue was chromatographed (Et_2O/MeOH/conc. NH_4OH, 8:2:0.2) to give **22** (210 mg, 40%) as a colourless powder (EtOH + 2% Et_2O), m.p. 171−172°C. − $[\alpha]_D^{20} = -36$ (*c* = 1, MeOH). − ^{1}H NMR (CD_3OD): δ = 7.57 (s, 3-H), 6.89 (s, 1-H), 4.80 (d, 7-H), 4.63 (ddd, 6-H), 4.38 (5-H_a), 3.86 (5-H_b); $J_{5a,5b}$ = 11.8 Hz, $J_{5a,6}$ = 5.2 Hz, $J_{5b,6}$ = 2.6 Hz, $J_{6,7}$ = 2.2 Hz. − ^{13}C NMR (CD_3OD): δ = 138.6 (C-8), 132.6 (C-3), 122.3 (C-1), 83.6 (C-6), 73.0 (C-7), 52.1 (C-5). − MS (Varian); *m*/*z* (%): 140 (50) $[M]^+$, 122 (30) $[M - H_2O]^+$, 97 (100). − HRMS; calcd. for $[M]^+$ ($C_6H_8N_2O_2$) 140.05857; found 140.0592.

(1*R*,2*S*)-1-(4′-Imidazolyl)-1,2-*O*-isopropylidenepropane-1,2,3-triol (24a) and (1*S*,2*S*)-1-(4′-Imidazolyl)-1,2-*O*-isopropylidenepropane-1,2,3-triol (24b): Same procedure as for **9**. **23**[13] (27.00 g, 141.8 mmol) and formamidine acetate (1.2 equiv., 17.8 g) were heated for 16 h at 80°C. After evaporation of NH_3, the residue was passed through an Amberlite CG-6000 column (desorbtion with a 5% NH_4OH solution). After concentration, the residue was chromatographed (FC; Et_2O/MeOH, 7:3) which led to a mixture of **24a** and **24b** (9.8 g, 35%) (**24a**/**24b** = 70:30, ^{1}H NMR). This mixture was used as such for further reactions.

24a: ^{1}H NMR (CD_3OD): δ = 7.69 (d, 2′-H), 7.04 (dd, 5′-H), 5.29 (1-H), 4.39 (2-H), 3.29 (m, 3-H_a and 3-H_b), 1.55 and 1.42 (2 s, 2 × CH_3); $J_{2',5'}$ = 1.3 Hz, $J_{1,5'}$ = 0.7 Hz, $J_{1,2}$ = 7.0 Hz, $J_{2,3a}$ = 5.4 Hz, $J_{2,3b}$ = 6.2 Hz.

24b: ^{1}H NMR (CD_3OD): δ = 7.70 (d, 2′-H), 7.14 (d, 5′-H), 4.86 (d, 1-H), 4.17 (ddd, 2-H), 3.74 (3-H_a), 3.58 (3-H_b), 1.47 and 1.44 (2 × CH_3); $J_{2',5'}$ = 1.2 Hz, $J_{1,2}$ = 8.8 Hz, $J_{2,3a}$ = 3.1 Hz, $J_{2,3b}$ = 5.1 Hz, $J_{3a,3b}$ = 12.2 Hz.

(6*S*,7*R*)-6,7-Dihydro-6,7-*O*-isopropylidene-5*H*-pyrrolo[1,2-*c*]-imidazole-6,7-diol (25): Pyridine (2.7 mL, 4.0 equiv.) and triflic anhydride (2.7 mL, 2.0 equiv.) were added at −20°C to a stirred solution of the mixture of **24a** and **24b** (1.62 g; 8.17 mmol, **24a**/**24b** = 70:30) in CH_2Cl_2 (10mL). After stirring for 5 h, the reaction was allowed to warm up to 0°C; then it was neutralised with aq. $NaHCO_3$ and extracted with CH_2Cl_2. The organic phase was dried ($MgSO_4$), filtered and concentrated to dryness. After (FC; CH_2Cl_2/CH_3OH 95:5), only **25** was isolated (215 mg, 22% from the mixture **24a**/**24b**, yield not optimised) as a yellowish powder. − $[\alpha]_D^{20}$ = +106 (*c* = 1, MeOH). − ^{1}H NMR (CD_3OD): δ = 7.55 (3-H), 6.89 (1-H), 5.53 (d, 7-H), 5.37 (ddd, 6-H), 4.26 (dd, 5-H_a), 4.16 (dd, 5-H_b), 1.38 and 1.24 (2 × CH_3); $J_{6,7}$ = 5.8 Hz, $J_{5a,6}$ = 5.3 Hz, $J_{5b,6}$ = 1.8 Hz, $J_{5a,5b}$ = 12.8 Hz. − ^{13}C NMR (CD_3OD): δ = 144.0 (C-8), 132.5 (C-3), 122.3 (C-1), 114.0 $C(CH_3)_2$, 85.7 (C-6), 74.9 (C-7), 51.3 (C-5), 27.5 and 26.1 (2 × CH_3). − MS (Varian); *m*/*z* (%): 180 (40) $[M]^+$, 165 (75) $[M - CH_3]^+$, 123 (44), 43 (100). − HRMS; calcd. for $[M]^+$ ($C_9H_{12}N_2O_2$) 180.08987; found 180.0902.

(6*S*,7*R*)-6,7-Dihydro-5*H*-pyrrolo[1,2-*c*]imidazole-6,7-diol (26): α-Tosyl chloride (3.95 g, 3.0 equiv.) was added in small portions to a stirred solution of the mixture **24a**/**24b** (1.37 g, 6.91 mmol, 70:30) in pyridine (7 mL) at 0°C.[15] The reaction mixture was allowed to warm up to room temp. After 30 min, Ac_2O (3.0 equiv.) was added and the solution heated for 1 h at 85°C. The solvents were evaporated under reduced pressure. The residue (containing **25**) was treated with CF_3COOH/H_2O (1:3) and stirred overnight. After addition of MeOH (5 mL) and evaporation of the solvents under reduced pressure, the residue was passed through an Amberlite CG-6000 column and desorbed with a 5% NH_4OH solution After evaporation of the solvents, FC of the residue gave **22** (389 mg, 57%) as colourless crystals, m.p. 171−172°C. − $[\alpha]_D^{20} = -15$ (*c* = 1, MeOH). − ^{1}H NMR (CD_3OD): 7.56 (2-H), 6.91 (1-H), 4.90 (7-H), 4.65 (6-H), 4.27 (5-H_a), 3.86 (5-H_b); $J_{6,7}$ = 5.2 Hz, $J_{5a,6}$ = 6.6 Hz, $J_{5b,6}$ = 6.6 Hz, $J_{5a,5b}$ = 11.1 Hz. − ^{13}C NMR: δ = 138.1 (C-8), 132.7 (C-3), 122.5 (C-1), 76.7 (C-6), 66.5 (C-7), 50.2 (C-5). − $C_6H_8N_2O_2$ (140.1): calcd.C 51.42, H 5.75, N 19.99; found C 51.3, H 5.8, N 19.8.

(6*S*,7*R*)-6,7-Dihydro-5*H*-pyrrolo[1,2-*c*]imidazole (ent-26): Same procedure as for **26**, starting from the D-arabinose derivative **ent-23**: m.p. 171−172°C. − $[\alpha]_D^{20} = +15$ (*c* = 1, MeOH).

Enzymatic Assays: Glycosidases [α-mannosidase (EC 3.2.1.24) from jack beans (approx. 20 units per mg of protein), β-mannosidase (EC 3.2.1.25) from snails (5−30 units per mL), α-glucosidase (EC 3.2.1.20) from baker's yeast (9 units per mg of protein), β-glucosidase (EC 3.2.1.21) from almonds (20−40 units per mg of solid)] and their corresponding substrates were purchased from Sigma Co. Spectrophotometric assays were performed at the optimum pH of each enzyme[15] with *p*-nitrophenyl α-L-mannopyranoside as a substrate for α-mannosidase (K_m = 2 mM, pH = 5.0), *p*-nitrophenyl β-L-mannopyranoside as a substrate for α-mannosidase (K_m = 1.3 mM, pH = 5.0), *p*-nitrophenyl α-L-glucopyranoside as a substrate for α-glucosidase (K_m = 0.3 mM, pH = 6.8) and *p*-nitrophenyl β-L-glucopyranoside as a substrate for β-glucosidase (K_m = 2 mM, pH = 5.0). The release of *p*-nitrophenol was measured continuously at 400 nm to determine initial velocities.[16] All kinetics were performed at 25°C and the reaction was started by the addition of enzyme in a 1-mL assay medium [acetate buffer (50 mM, pH = 5.0) or phosphate buffer (20 mM pH = 6.8)] using substrate concentrations around the K_m value of each enzyme. The K_i value was determined for the most potent inhibitors, by using the Dixon graphical procedure.[17] IC_{50} values (determined for weak inhibition) correspond to the inhibitor concentration required for 50% inhibition of the enzyme[17] in our experimental conditions.

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