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Stereocontrolled synthesis of enantiomeric imidazolopiperidinoses and imidazoloazepanoses using Wittig/dihydroxylation reactions

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Abstract—A new route for the synthesis of imidazolosugars—6-deoxyimidazolopiperidinoses **1** and **2** and 7-deoxyimidazoloazepanoses **3** and **4** in two enantiomerical forms—is reported. The new synthetic approach is based on two key reactions: (i) stereocontrolled Wittig *Z*-olefination of an imidazolecarbaldehyde with phosphoranes containing one chiral centre, obtained from (*S*)- and (*R*)-1,2,4-butantriol; (ii) diastereoselective cis-dihydroxylation of previously obtained olefins. All synthesised imidazolosugars were evaluated as potential inhibitors of glycosidases.

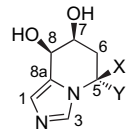
1. Introduction

Among the natural and synthetic inhibitors of glycosidases there are structural analogues of monosaccharides in which the endocyclic oxygen atom is replaced by a nitrogen atom. Thus, naturally occurring polyhydroxylated pyrrolidines (pyrrolidinoses), such as DMDP (2,5-bis-hydroxymethyl-3,4-dihydroxy-pyrrolidine),[1] and polyhydroxylated piperidines (piperidinoses), such as DNJ (1-deoxynojirimycin),[2] exhibit specific glycosidase inhibitory activity. In recent years some efforts have been directed to the synthesis of the seven-membered ring analogues, the polyhydroxyazepanes (azepanoses).[3–9] Some of them exhibit even higher inhibition potency than their five- and six-membered counterparts.[10]

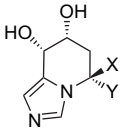
The enzymatic mechanism of catalytic polysaccharide hydrolysis, using glycosidases, has been well studied. The putative oxocarbenium ion-like transition state (TS), which is presumably produced during both glycosylation and deglycosylation steps of glycosides, appears to be in a half-chair conformation. This TS can be mimicked by some putative inhibitors, for example, with azasugars fused to a tetrazole, a triazole or an imidazole.[11,12] In 1991 we reported the first synthesis of the imidazolosugars.[13] In 1992 it was showed that the natural imidazolosugar nagstatine is a very potent inhibitor of *N*-acetyl-glucosaminidases.[14]

Imidazolosugars, as potential glycosidase inhibitors, have been the subject of interest of several research groups.[15–18] Our previous research in this area concerned the syntheses of imidazolopyrrolidinoses[19] and imidazolopiperidinoses.[20] In addition, one imidazoloazepanose has been reported by us so far.[21] All our products have been obtained in multistep synthetic pathways from the respective monosaccharides.

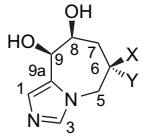
We now report the syntheses of novel imidazolopiperidinoses **1** and **2** and imidazoloazepanoses **3** and **4**, each in both enantiomeric forms.



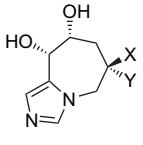
1 X = H, Y = CH_2OH
ent-2 X = CH_2OH, Y = H



ent-1 X = CH_2OH, Y = H
2 X = H, Y = CH_2OH



3 X = H, Y = OH
ent-4 X = OH, Y = H



ent-3 X = OH, Y = H
4 X = H, Y = OH

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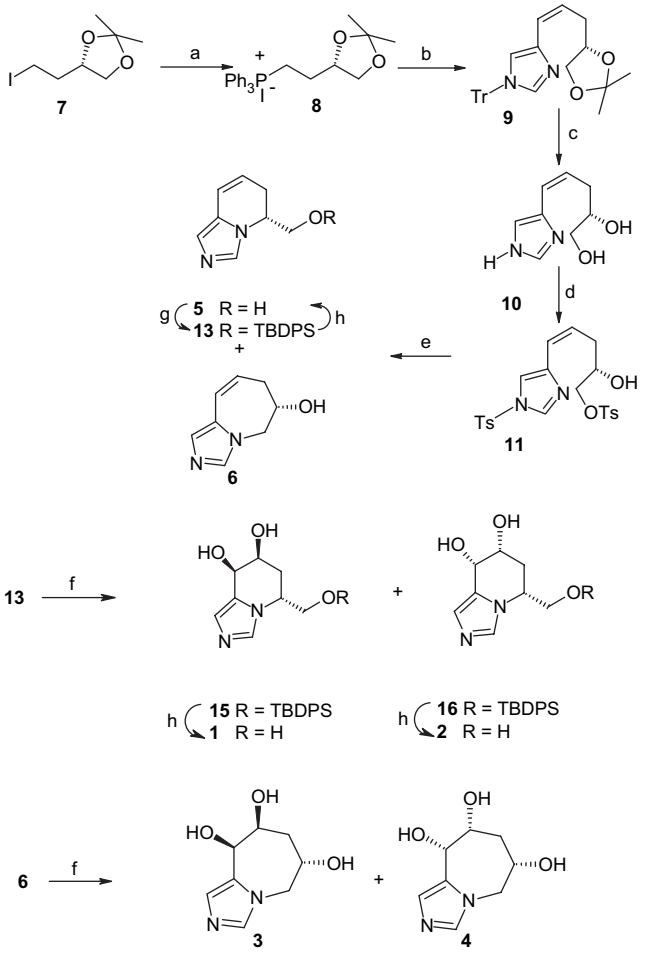
2. Results and discussion

2.1. Synthetic strategy

Our approach is based on two key reactions:

(i) stereocontrolled Wittig olefination of chiral phosphoranes, obtained from (*S*)- and (*R*)-1,2,4-butantriol, with imidazolecarbaldehyde, to yield, after subsequent cyclisation, the mixture of olefins **5** and **6** and **ent-5** and **ent-6**, respectively.

(ii) Diastereoselective cis-dihydroxylation of olefin **5** to imidazolopiperidinoses **1** and **2**, and olefin **6** to imidazoloazepanoses **3** and **4** (Scheme 1). In the same conditions from olefin **ent-5** imidazolopiperidinoses **ent-1** and **ent-2**, and from olefin **ent-6** imidazoloazepanoses **ent-3** and **ent-4** were obtained.

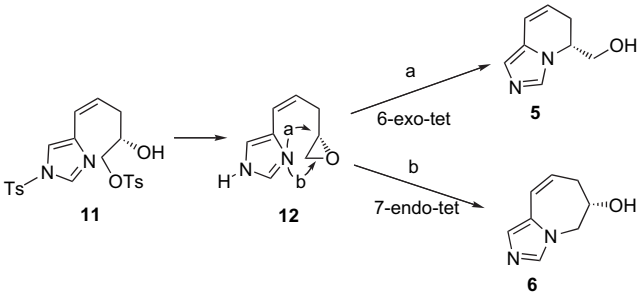


Scheme 1. Reagents and conditions: (a) Ph_3P, toluene, reflux, 30 h; (b) $NaNH_2$/^{t}BuOK 9:1, ether, rt, 4 h, then 4-formyl-1-tritylimidazole in THF at −75 °C; (c) 2 M HCl_{aq}/THF, 6 h, 40 °C; (d) TsCl, pyridine, −10 °C, 12 h; (e) 1 M $NaOH_{aq}$, acetone, rt, 12 h; (f) $K_2OsO_4\cdot 2H_2O$, $K_3Fe(CN)_6$, K_2CO_3 and $MeSO_2NH_2$, ^{t}BuOH/H_2O (1:1), 48 h, 45 °C; (g) TBDPSCl, DMAP, pyridine, rt, 12 h; (h) TBAF, THF, rt, 12 h.

2.2. *Z*-Wittig olefination and cyclisation

(*S*)-1,2,4-Butantriol, readily accessible from *S*-malic acid,[22] was transformed by the known procedure[23,24] to iodide **7**. Triphenylphosphonium iodide **8**, obtained from **7** in 92% yield, and 4-formyl-1-tritylimidazole[25] were used as components in the Wittig olefination. High *Z*-stereoselectivity (*Z*/*E*=93:7) was achieved owing to a suitable selection of bases ($NaNH_2$/^{t}BuOK=9:1).[26] The *Z*-olefin **9** was isolated in 69% yield from the *Z*/*E* mixture by flash chromatography.

Following the acid removal of the protecting groups (HCl/THF) leading to **10**, subsequent tosylation in pyridine at −10 °C gave the ditosylate **11**. Crude **11** without isolation, treated with sodium hydroxide in water and acetone (2:1) gave the mixture of (*S*)-imidazoazepinol **6** and (*R*)-hydroxymethyl-imidazopyridine **5** in a 3:1 ratio (Scheme 1). The formation of this mixture suggests the reaction pathway via the epoxide **12**, followed by the opening of epoxy function leading to imidazoazepinol **6** (7-*endo*-tet-cyclisation) and hydroxymethylpyridine **5** with inversion in configuration at C-5 (6-*exo*-tet-cyclisation)[27] (Scheme 2).



Scheme 2. Proposed pathways of cyclisation.

Separation of **5** and **6** appeared very difficult by means of flash chromatography. However, the imidazoazepinol **6** crystallises partially from the mixture when dissolved in ethyl acetate/methanol (10:1). The complete separation of **5** and **6** was achieved after selective protection of the primary alcohol function of **5**.

Thus the mother liquor was treated with *tert*-butyldiphenylsilyl chloride (TBDPSCl) in pyridine to give the *O*-silylated derivative **13**, easily isolated by flash chromatography. After removal of the silyl protecting group from **13** by means of tetrabutylammonium fluoride (TBAF) in THF, pure **5** was obtained in 71% yield.

The same reaction conditions as used in the preparation of olefins **5** and **6** were applied for the synthesis of olefins **ent-5** and **ent-6**, starting from (*R*)-1,2-*O*-isopropylidene-1,2,4-butantriol, accessible from L-ascorbic acid by the known procedure.[28–30]

2.3. Diastereoselective cis-dihydroxylations

The cis-dihydroxylations of olefins **5** and **6** were performed in catalytic conditions using $K_2OsO_4\cdot 2H_2O$ (0.06 equiv) as a nonvolatile Os source in combination with $K_3Fe(CN)_6$ (3 equiv) as co-oxidant in the presence of K_2CO_3 (3 equiv) and $MeSO_2NH_2$ (2 equiv) in the two-phase ^{t}BuOH/H_2O (1:1) system.[31] We utilised only the internal asymmetric induction caused by the existing chiral centre without resorting

to external sources of chirality. The cis-dihydroxylations of dihydroimidazoazepinol **6** or **ent-6** proceeded preferentially from the opposite side to the hydroxy group on the existing chiral centre, to give the mixture of **3** and **4** or **ent-3** and **ent-4** diastereomers, respectively, in a 3:1 ratio. The diastereomers **3** and **4** were separated by flash chromatography.

The cis-dihydroxylation of both enantiomers of dihydrohydroxymethyl-imidazopyridine **5** resulted in the formation of a chromatographically inseparable mixture of diastereomers **1** and **2**. Therefore, the olefin **5** was silylated under standard conditions to give TBDPS derivative **13**. The dihydroxylation of compound **13** led to mixture of diastereomers **15** and **16**. Isomers **15** and **16** were chromatographically separated and isolated in a 2:1 ratio (63% overall yield).

The preference of attack of the oxidative agent from the opposite side to the existing $TBDPSOCH_2$ group is a consequence of steric hindrance caused by the bulky silyl group. The silyl group of **15** was removed by treatment with Bu_4NF to give pure **1** in 60% yield. The same reaction starting from **16** led to the formation of **2** in 60% yield.

The enantiomers **ent-1**, **ent-2**, **ent-3** and **ent-4** were obtained in the same reaction conditions as used above starting from olefins **ent-5** and **ent-6**.

2.4. Structural analysis

Structures of (6*S*)-6,7-dihydro-5*H*-imidazo[1,5-*a*]azepine-6-ol **6**, (6*S*,8*S*,9*R*)-6,7,8,9-tetrahydro-5*H*-imidazo[1,5-*a*]-azepin-6,8,9-triol **3** and (6*R*,8*R*,9*S*)-6,7,8,9-tetrahydro-5*H*-imidazo[1,5-*a*]azepin-6,8,9-triol **ent-3** were clearly demonstrated by the X-ray diffraction analysis as shown in Figure 1. The molecules adopt an extended conformation with the planar imidazole moiety fused with the seven-membered azepine ring along the aromatic N2–C7 bond. In **6** the latter is constrained by the endocyclic C5═C6 double bond and exists in the distorted envelope conformation with C2, N2, C7, C6, C5 and C4 almost coplanar and C3 situated at the flap. The **3** and **ent-3** are enantiomers with virtually the same conformation. In particular, the azepine ring adopts a chair conformation. The C2, C3, C5 and C6 atoms form the central plane of the chair while C4 and both N2 and C7 atoms are situated at the opposite flaps. Although imidazole and azepine fragments exist in a number of compounds investigated by X-ray analysis the combination in which they are fused along the aromatic 1,5 bond is unique among the crystal structures reported to date (Cambridge Structural Database,[32] version 5.27, January 2006 update). In all investigated compounds the hydroxyl hydrogen atoms bonded to O1 are involved in strong intermolecular hydrogen bonds with the imidazole N1 atoms. In **3** and **ent-3** those interactions are supplemented by intermolecular hydrogen bonds in which the hydroxyl O2 and O3 atoms are also involved.

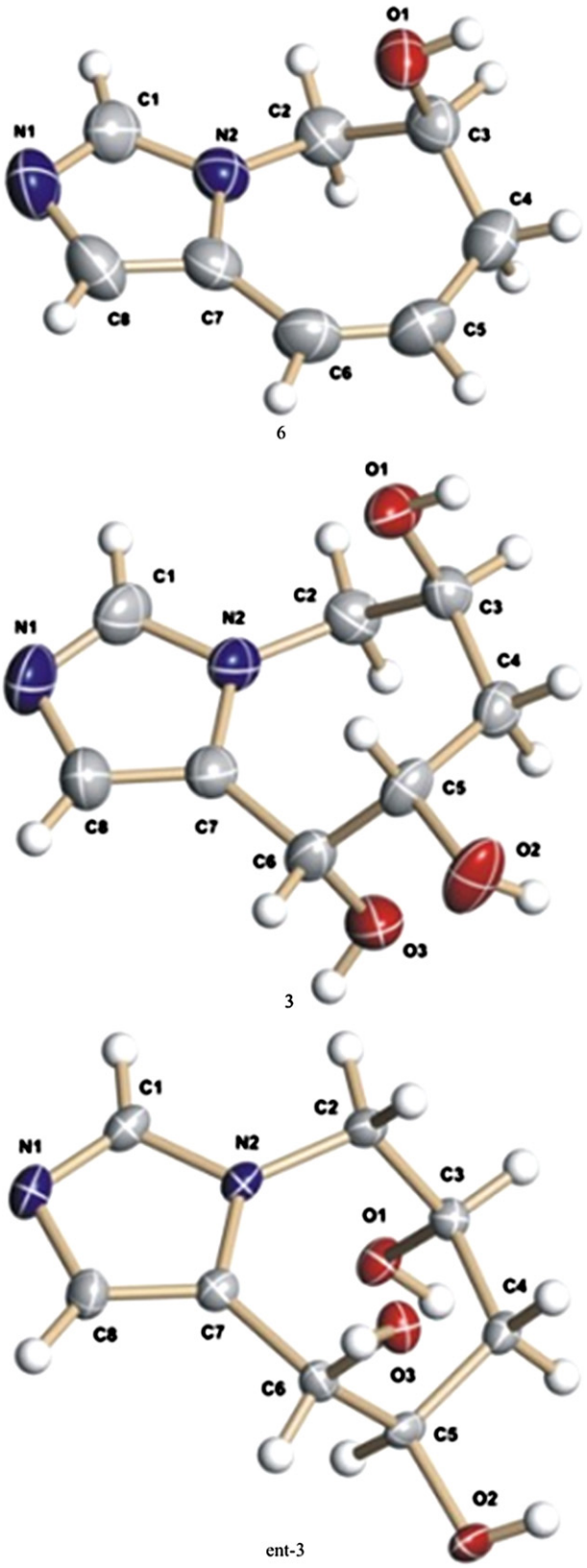


Figure 1. Molecular structures of **6**, **3** and **ent-3**. Displacement ellipsoids are drawn at the 50% probability level.

Structures of both imidazolopiperidinoses **1** and **2** were deduced from ^1H and ^{13}C NMR spectra by application of the nuclear Overhauser effect (NOE). For example, in compound **ent-1** cross signal H6–H8 was observed in two dimensional ROESY spectrum, cross signal H5–H7 was found in compound **ent-2** (see numbering given in Section 1).

2.5. Enzymatic assays

Compounds **1**, **2**, **3** and **4**, in both enantiomeric forms, have been evaluated as potential inhibitors of six commercially available glycosidases. Compound **1** as the (5*S*,7*R*,8*S*) enantiomer inhibited the α-D-galactosidase (*Aspergillus niger*) with K_i value of 120 μM (IC_{50}=240 μM +/−18 μM), while the (5*R*,7*S*,8*R*) enantiomer was inactive towards this enzyme. The remaining compounds were inactive towards the pool of evaluated D-glycosidases.

3. Experimental

3.1. General

Flash chromatography: silica gel (Merck 60, 230–400 mesh). TLC: silica gel on plastic sheets (Merck 60 HF_{254}); the spots were viewed under UV or by heating with a heatgun after spraying with a solution of $KMnO_4$ (20 g) and Na_2CO_3 (40 g) in H_2O (1 l). Mp: Büchi-SMP-30 apparatus; corrected values. Optical rotations were measured at 20 °C: Perkin–Elmer 241 polarimeter. ^1H and ^{13}C NMR spectra: Bruker 250 AC 250 spectrometer at 300 K. Internal references for ^1H NMR: $SiMe_4$ (δ=0.00), $CDCl_3$ (δ=7.26), CD_3OD (δ=3.30), [D_4]TSP for spectra in D_2O (δ=0.00); for ^{13}C NMR: $CDCl_3$ (δ=77.03), CD_3OD (δ=49.02); δ in parts per million and *J* in hertz. HRMS: Finnigan MAT 95 (Finnigan MAT GmbH, Germany).

3.1.1. (*S*)-3,4-*O*-Isopropylidene-3,4-dihydroxybutyltriphenylphosphonium iodide (8) and (*R*)-3,4-*O*-isopropylidene-3,4-dihydroxybutyltriphenylphosphonium iodide (ent-8). To a stirred solution of iodide **7**[24] (8.31 g, 33 mmol) in anhydrous toluene (30 ml) was added dropwise a solution of triphenylphosphine (12.80 g, 49 mmol) in anhydrous toluene (60 ml) at room temperature. The mixture was refluxed for 30 h until complete disappearance of **7** (TLC). The resulting suspension was filtered and the solid was washed with toluene and Et_2O to obtain **8** (15.55 g, 92%) as colourless crystals. Mp 219–221 °C; $[\alpha]_D$ +2.62 (*c* 1.00, $CHCl_3$); ν_{max} (KBr): 3112, 2864, 1584, 1560, 1480, 1432, 1372, 1252, 1108, 696, 664, 620 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.85–7.72 (15H, m, *H* arom.), 4.56–4.51 (1H, m, C(3)*H*), 4.47–4.28 (1H, m, C(1)H_aH_b), 4.16 (1H, dd, *J* 8.6, 6.4 Hz, C(4)H_aH_b), 3.62 (1H, dd, *J* 8.6, 5.6 Hz, C(4)H_aH_b), 3.58–3.39 (1H, m, C(1)H_aH_b), 2.21–2.06 (1H, m, C(2)H_aH_b), 1.82–1.60 (1H, m, C(2)H_aH_b), 1.32 (3H, s, CH_3), 1.29 (3H, s, CH_3); δ_C (60 MHz, $CDCl_3$) 134.78 and 134.73 (d, $^4J_{C-P}$ 3 Hz, *C p*-arom.), 133.43 and 133.27 (d, $^2J_{C-P}$ 10 Hz, *C o*-arom.), 130.42 and 130.22 (d, $^3J_{C-P}$ 12 Hz, *C m*-arom.), 118.2 and 116.8 (d, $^1J_{C-P}$ 678 Hz, *C s*-arom.), 108.8 (*C*$(CH_3)_2$), 72.7 (*C*-3), 68.4 (*C*-4), 30.6 (*C*-2), 26.5 (*C*H_3), 24.8 (*C*H_3), 19.8 and 19.0 (d, $^1J_{C-P}$ 48 Hz, *C*-1). HRMS (CI, NH_3) MH^+, found: 519.0948; $C_{25}H_{28}PO_2I$ requires: 519.0950.

The same procedure as for the preparation of **8** was used starting from **ent-7** (9.23 g, 36.6 mmol) to give **ent-8** as a colourless crystals (17.3 g, 93%).

ent-8: $[\alpha]_D$ −2.7 (*c* 1.00, $CHCl_3$).

3.1.2. (*S*)(*Z*)-1-[1′-(Triphenylmethyl)-1′*H*-imidazol-4′-yl]-4,5-*O*-isopropylidene-pent-1-en-4,5-diol (9) and (*R*)(*Z*)-1-[1′-(triphenylmethyl)-1′*H*-imidazol-4′-yl]-4,5-*O*-isopropylidene-pent-1-en-4,5-diol (ent-9). To the phosphonium iodide **8** (3.12 g, 6 mmol) were added $NaNH_2$ (398 mg, 10.2 mmol), ^{t}BuOK (45 mg) and anhydrous Et_2O (15 ml) under argon at room temperature. The mixture was stirred at room temperature for 4 h. To the stirred dark yellow suspension was added dropwise a solution of 4-formyl-1-tritylimidazole (2.04 g, 6 mmol) in anhydrous THF at −78 °C. The stirring was continued at −78 °C overnight. Then water (60 ml) was added and the reaction mixture was extracted with CH_2Cl_2 (3×100 ml). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2/acetone 2:1) to give **9** as pale yellow foam (1.89 g, 69%). R_f (CH_2Cl_2/acetone 2:1) 0.64; $[\alpha]_D$ +17.1 (*c* 1.00, $CHCl_3$); ν_{max} (film): 3192, 3024, 3008, 2992, 1552, 1512, 1492, 1464, 1288, 1176, 1100, 764, 720 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.42 (1H, d, *J* 1.0 Hz, C(2′)*H*), 7.39–7.38 (9H, m, *H* arom.), 7.19–7.12 (6H, m, *H* arom.), 6.77 (1H, d, *J* 1.0 Hz, C(4′)*H*), 6.33 (1H, d, *J* 11.6 Hz, C(1)*H*), 5.56 (1H, dt, *J* 11.6, 7.0 Hz, C(2)*H*), 4.27–4.17 (1H, m, C(4)*H*), 4.01 (1H, dd, *J* 8.0, 6.0 Hz, C(5)H_aH_b), 3.58 (1H, dd, *J* 8.0, 7.6 Hz, C(5)H_aH_b), 2.82 (2H, dt, *J* 7.3, 1.5 Hz, C(3)H_aH_b, C(3)H_aH_b), 1.37 (3H, s, CH_3), 1.34 (3H, s, CH_3); δ_C (60 MHz, $CDCl_3$) 142.3 (*C s*-arom.), 138.9 (*C*-5′), 138.5 (*C*-2′), 129.7 (*C o*-arom.), 128.0 (*C m*-arom. and *C p*-arom.), 124.7 (*C*-1), 123.1 (*C*-4′), 120.8 (*C*-2), 108.7 (*C*$(CH_3)_2$), 75.6 (*C*Ph_3), 75.2 (*C*-4), 68.9 (*C*-5), 32.8 (*C*-3), 26.8 (*C*H_3), 25.6 (*C*H_3). HRMS (CI, NH_3) MH^+, found: 451.2386; $C_{30}H_{31}N_2O_2$ requires: 451.2387.

The same procedure as for the preparation of **9** was used starting from **ent-8** to give **ent-9** as pale yellow foam with the same yield.

ent-9: $[\alpha]_D$ −16.9 (*c* 1.00, $CHCl_3$).

3.1.3. (*S*)(*Z*)-1-(Imidazol-4′(5′)-yl)pent-1-en-4,5-diol (10) and (*R*)(*Z*)-1-(imidazol-4′(5′)-yl)pent-1-en-4,5-diol (ent-10). To a stirred solution of **9** (92.67 g, 5.93 mmol) in THF (20 ml) was added 2 M HCl (36 ml). The stirring was continued at 40 °C for 6 h. The reaction mixture was neutralised with saturated aq K_2CO_3 solution, concentrated in vacuo and coevaporated several times with dry ethanol in order to remove water. The residue was extracted a few times with dry ethanol and after evaporation was purified by flash chromatography ($CHCl_3$/EtOH 2:1) to give **10** (895 mg, 90%) as a colourless foam. R_f ($CHCl_3$/EtOH 2:1) 0.30; $[\alpha]_D$ +20.1 (*c* 1.00, MeOH); ν_{max} (film): 3500, 3440, 3232, 1640, 1592, 1464, 1360, 1264, 1216, 1088, 956, 832 cm^{-1}; δ_H (250 MHz, acetone-d_6) 7.68 (1H, s, C(2′)*H*), 7.11 (1H, s, C(4′)*H*), 6.27 (1H, d, *J* 11.6 Hz, C(1)*H*), 5.61 (1H, dt, *J* 11.6, 8.3 Hz, C(2)*H*), 3.72 (1H, dddd, *J* 6.0, 5.7, 5.2, 4.6 Hz, C(4)*H*), 3.51 (1H, dd, *J* 10.9, 4.6 Hz, C(5)H_aH_b), 3.47 (1H, dd, *J* 10.9, 5.7 Hz, C(5)H_aH_b), 2.87–2.75 (2H, m, C(3)H_aH_b, C(3)H_aH_b); δ_C (60 MHz, CD_3OH) 136.6 (*C*-2′), 135.3 (*C*-5′), 127.4 (*C*-2), 122.9 (*C*-1), 121.8 (*C*-4′), 73.2 (*C*-4), 66.6 (*C*-5), 34.3 (*C*-3). HRMS (CI, NH_3) MH^+, found: 169.0977; $C_8H_{13}N_2O_2$ requires: 169.0979.

The same procedure as for the preparation of **10** was used starting from **ent-9** to give **ent-10** as a colourless foam.

ent-10: $[\alpha]_D$ −20.0 (*c* 1.00, MeOH).

3.1.4. (5*R*)-5-*tert*-Butyldiphenylsilyloxymethyl-5,6-dihydroimidazo[1,5-*a*]pyridine (13), (6*S*)-6,7-dihydro-5*H*-imidazo[1,5-*a*]azepine-6-ol (6) and (5*S*)-5-*tert*-butyldiphenylsilyloxymethyl-5,6-dihydroimidazo[1,5-*a*]pyridine (ent-13), (6*R*)-6,7-dihydro-5*H*-imidazo[1,5-*a*]-azepine-6-ol (ent-6). To a stirred solution of compound **10** (720 mg, 4.28 mmol) in anhydrous pyridine (32 ml) was added TsCl (2.28 g, 11.98 mmol) at −10 °C. The stirring was continued overnight at −10 °C until the disappearance of the starting material (TLC). Water (10 ml) was added to the reaction mixture at −5 °C and stirring was continued at this temperature for 1 h. After extraction with CH_2Cl_2 (5×30 ml) the organic layer was concentrated in vacuo and aq NaOH solution (1 M, 108 ml) and acetone (50 ml) were added to the residue. The reaction mixture was stirred overnight at room temperature. After concentration in vacuo the residue was purified by flash chromatography ($CHCl_3$/EtOH 3:1) to give the mixture of **6** and **5** in a 3:1 ratio (NMR). The azepinol **6** (219 mg) partially crystallised from the mixture **6** and **5** dissolved in AcOEt/MeOH (10:1). The mother liquor was evaporated in vacuo to dryness.

To a stirred solution of the resulted mixture of isomers **5** and **6** (231 mg) in anhydrous pyridine (20 ml) was added 4-dimethylaminopyridine (DMAP) (60 mg), and then *tert*-butyldiphenylsilyl chloride (^{t}BuPh$_2$SiCl) (275 mg, 1 mmol) was added dropwise. The stirring was continued at room temperature overnight. After evaporation in vacuo the residue was purified by flash chromatography ($CHCl_3$/EtOH 20:1) to give **13** (270 mg) as a yellow thick oil and **6** (99 mg) ($CHCl_3$/EtOH 2:1) as yellowish crystals, which were recrystallised (AcOEt/MeOH) mp 154–156 °C (dec).

Compound **13**: R_f ($CHCl_3$/EtOH 20:1) 0.30; $[\alpha]_D$ +0.9 (*c* 1.00, $CHCl_3$); ν_{max} (film): 3072, 2992, 2936, 2904, 2856, 1588, 1544, 1472, 1424, 1392, 1200, 1104, 976, 876, 840, 824, 676, 620 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.87–7.29 (11H, m, C(3)*H*, *H* arom.), 7.18 (1H, d, *J* 1.0 Hz, C(1)*H*), 6.40 (1H, d, *J* 11.6 Hz, C(8)*H*), 5.59 (1H, dt, *J* 11.6, 8.0 Hz, C(7)*H*), 4.30 (1H, m, C(5)*H*), 3.76–3.60 (2H, m, C(9)H_aH$_b$, C(9)H$_a$$H_b$), 2.65–2.32 (2H, m, C(6)H_aH$_b$, C(6)H$_a$$H_b$), 1.07 (9H, s, SiC(C$H_3$)$_3$); δ_C (60 MHz, $CDCl_3$) 136.7 (*C*-3), 135.4 (*C o*-arom.), 132.8 and 132.5 (*C s*-arom.), 129.8 (*C p*-arom.), 127.7 (*C m*-arom.), 126.8 (*C*-8a), 125.2 (*C*-1), 119.5 (*C*-7), 116.7 (*C*-8), 65.0 (*C*-9), 54.04 (*C*-5), 26.7 (SiC(*C*H$_3$)$_3$), 25.5 (*C*-6), 19.0 (Si*C*(CH$_3$)$_3$). HRMS (CI, NH_3) MH$^+$, found: 389.2049; $C_{24}H_{29}N_2OSi$ requires: 389.2050.

Compound **6**: R_f ($CHCl_3$/EtOH 3:1) 0.20; $[\alpha]_D$ +62.9 (*c* 0.50, MeOH); ν_{max} (film): 3480, 2144, 3064, 3008, 2992, 2872, 1536, 1504, 1496, 1432, 1256, 1224, 992, 944, 692, 620 cm^{-1}; δ_H (250 MHz, D_2O) 7.58 (1H, s, C(3)*H*), 6.95 (1H, s, C(1)*H*), 6.40 (1H, d, *J* 12.6 Hz, C(9)*H*), 5.61 (1H, dt, *J* 12.6, 7.8 Hz, C(8)*H*), 4.32–4.07 (3H, m, C(6)*H*, C(5)H_aH$_b$, C(5)H$_a$$H_b$), 2.77–2.48 (2H, m, C(7)H_aH$_b$, C(7)H$_a$$H_b$); δ_C (60 MHz, CH_3OD) 139.7 (*C*-3), 132.2 (*C*-9a), 129.8 (*C*-1), 123.8 (*C*-8), 117.2 (*C*-9), 67.6 (*C*-6), 54.1 (*C*-5), 40.3 (*C*-7). HRMS (CI, NH_3) MH$^+$, found: 151.0871; $C_8H_{11}N_2O$ requires: 151.0873.

The same procedure as for the preparation of **13** and **6** was used starting from **ent-10** to give **ent-13** and **ent-6**.

ent-6: $[\alpha]_D$ −63.0 (*c* 1.00, MeOH).

ent-13: $[\alpha]_D$ −0.9 (*c* 1.00, $CHCl_3$).

3.1.5. (5*R*)-5,6-Dihydro-5-hydroxymethyl-imidazo[1,5-*a*]-pyridine (5) and (5*S*)-5,6-dihydro-5-hydroxymethyl-imidazo[1,5-*a*]pyridine (ent-5). To a stirred solution of **13** (422 mg, 1.17 mmol) in anhydrous THF (3 ml) the solution of TBAF in THF (1.1 M, 2.2 ml, 2.34 mmol) was added. The mixture was stirred at room temperature overnight until the disappearance of the starting material (TLC). After evaporation in vacuo the residue was purified by flash chromatography to give **5** (16 mg, 71%) as a colourless film. R_f ($CHCl_3$/EtOH/25% aq NH_3 20:10:1) 0.60; $[\alpha]_D$ −54.0 (*c* 1.00, MeOH); ν_{max} (film): 3416, 3032, 2968, 2904, 2872, 1656, 1520, 1464, 1400, 1288, 1248, 1056, 884, 664, 620 cm^{-1}; δ_H (250 MHz, CH_3OD) 7.68 (1H, s, C(3)*H*), 6.83 (1H, s, C(1)*H*), 6.47 (1H, d, *J* 10.0 Hz, C(8)*H*), 5.77 (1H, dt, *J* 10.0, 5.0 Hz, C(7)*H*), 4.29 (1H, m, C(5)*H*), 3.65 (2H, m, C(9)H_aH$_b$, C(9)H$_a$$H_b$), 2.54 (2H, m, C(6)$H_aH_b$, C(6)H$_a$$H_b$); δ_C (60 MHz, CH_3OD) 137.8 (*C*-3), 128.6 (C-8a), 124.8 (*C*-1), 122.3 (*C*-7), 117.8 (*C*-8), 64.0 (*C*-9), 55.7 (*C*-5), 26.5 (*C*-6); FABMS: 151.0 ([M+H]$^+$). HRMS (CI, NH_3) MH$^+$, found: 151.0873; $C_8H_{11}N_2O$ requires: 151.0873.

The same procedure as for the preparation of **5** was used starting from **ent-13** to give **ent-5** as a colourless film.

ent-5: $[\alpha]_D$ +53.9 (*c* 1.00, MeOH).

3.1.6. (6*S*,8*S*,9*R*)-6,7,8,9-Tetrahydro-5*H*-imidazo[1,5-*a*]-azepin-6,8,9-triol (3), (6*S*,8*R*,9*S*)-6,7,8,9-tetrahydro-5*H*-imidazo[1,5-*a*]azepin-6,8,9-triol (4) and (6*R*,8*R*,9*S*)-6,7,8,9-tetrahydro-5*H*-imidazo[1,5-*a*]azepin-6,8,9-triol (ent-3), (6*R*,8*S*,9*R*)-6,7,8,9-tetrahydro-5*H*-imidazo-[1,5-*a*]azepin-6,8,9-triol (ent-4). To a stirred solution of $MeSO_2NH_2$ (307 mg, 3.23 mmol), $K_3Fe(CN)_6$ (1.27 g, 3.87 mmol) and K_2CO_3 (535 mg, 3.87 mmol) in ^{t}BuOH (3 ml) and H_2O (3 ml) was added $K_2OsO_4\cdot 2H_2O$ (24 mg, 0.065 mmol) at room temperature. Then a solution of **6** (194 mg, 1.29 mmol) in ^{t}BuOH (4 ml) and H_2O (4 ml) was added dropwise at room temperature. The stirring was continued at 45 °C for 2 days until the disappearance of the starting material (TLC). The reaction was quenched at 0 °C by the addition of Na_2SO_3 (2.03 g, 16.13 mmol), then warmed to room temperature and stirred for 2 h. After evaporation in vacuo the solid residue was extracted with EtOH. The solvent was removed in vacuo and the crude product was chromatographed ($CHCl_3$/EtOH/25% aq NH_3 10:10:1) to give **3** (132 mg) and **4** (49 mg)) in 76% overall yield.

Compound **3**: R_f ($CHCl_3$/EtOH/25% aq NH_3 10:10:1) 0.27; mp 212–216 °C (dec) (recrystallised from AcOEt/MeOH); $[\alpha]_D$ −39.6 (*c* 0.50, MeOH); ν_{max} (film): 3912, 3720, 3344, 3136, 1640, 1532, 1452, 1432, 1384, 1308, 1008, 956, 660, 620 cm^{-1}; δ_H (250 MHz, CD_3OD) 7.53 (1H, s, C(3)*H*), 6.89 (1H, s, C(1)*H*), approx. 4.90 (1H, s, C(9)*H*—overlapped with H_2O signal from methanol), 4.18 (1H, dm, *J* 12.8 Hz, C(5)H_aH$_b$), 4.18 (1H, dd, *J* 12.8, 9.8 Hz, C(5)H$_a$$H_b$), 3.71 (1H, dddd, *J* 10.7, 3.7, 1.8, 1.6 Hz, C(6)*H*), 3.67 (1H, m, C(8)*H*), 2.39 (1H, dt, *J* 12.9, 10.7 Hz, C(7)H_aH$_b$), 2.08 (1H, dm, *J* 12.9 Hz, C(7)H$_a$$H_b$); δ_C (60 MHz, CD_3OD) 139.9 (*C*-3), 132.6 (*C*-9a), 128.8

(C-1), 72.1 (*C*-6), 69.5 (*C*-8), 69.2 (*C*-9), 53.5 (*C*-5), 40.8 (*C*-7). HRMS (CI, NH_3) MH^+, found: 185.0927; $C_8H_{13}N_2O_3$ requires: 185.0928.

Compound **4**: R_f ($CHCl_3$/EtOH/25% aq NH_3 10:10:1) 0.34; mp 196–198 °C (dec) (recrystallised from AcOEt/MeOH); $[\alpha]_D$ +54.6 (*c* 0.50, MeOH); ν_{max} (film): 3912, 3720, 3344, 3136, 1640, 1532, 1452, 1432, 1384, 1308, 1008, 956, 660, 620 cm^{-1}; δ_H (250 MHz, CD_3OD) 7.59 (1H, s, C(3)*H*), 6.95 (1H, s, C(1)*H*), approx. 4.90 (1H, s, C(9)*H*—overlapped with H_2O signal from methanol), 4.22–4.13 (3H, m C(8)*H*, C(5)H_aH_b, C(5)H_a*H*$_b$), 3.97 (1H, dddd, *J* 10.9, 4.8, 2.1, 1.5 Hz, C(6)*H*), 2.25 (1H, ddd, *J* 13.7, 10.9, 2.1 Hz, C(7)H_aH_b), 2.02 (1H, dm, *J* 13.7 Hz, C(7)H_a*H*$_b$); δ_C (60 MHz, CD_3OD) 140.5 (*C*-3), 129.9 (*C*-9a), 128.1 (*C*-1), 69.9 (*C*-9) and (*C*-6), 66.7 (*C*-8), 52.7 (*C*-5), 39.2 (*C*-7). HRMS (CI, NH_3) MH^+, found: 185.0927; $C_8H_{13}N_2O_3$ requires: 185.0928.

The same procedure as for the preparation of **3** and **4** was used starting from **ent-6** to give **ent-3** and **ent-4**.

ent-3: $[\alpha]_D$ +39.7 (*c* 1.00, MeOH).

ent-4: $[\alpha]_D$ −54.5 (*c* 0.50, MeOH).

3.1.7. (5*R*,7*S*,8*R*)-5-*tert*-Butyldiphenylsilyloxy-methyl-5,6,7,8-tetrahydro-imidazo[1,5-*a*]pyridine-7,8-diol (15), (5*R*,7*R*,8*S*)-5-*tert*-butyldiphenylsilyloxy-methyl-5,6,7,8-tetrahydro-imidazo[1,5-*a*]pyridine-7,8-diol (16) and (5*S*,7*R*,8*S*)-5-*tert*-butyldiphenylsilyloxy-methyl-5,6,7,8-tetrahydro-imidazo[1,5-*a*]pyridine-7,8-diol (ent-15), (5*S*,7*S*,8*R*)-5-*tert*-butyldiphenylsilyloxy-methyl-5,6,7,8-tetrahydro-imidazo[1,5-*a*]pyridine-7,8-diol (ent-16). To a stirred solution of $MeSO_2NH_2$ (96 mg, 1.01 mmol), $K_3Fe(CN)_6$ (398 mg, 1.21 mmol) and K_2CO_3 (167 mg, 1.21 mmol) in ^{t}BuOH (2.5 ml) and H_2O (2.5 ml) was added $K_2OsO_4\cdot 2H_2O$ (7.4 mg, 0.02 mmol) at room temperature. Then a solution of **13** (145 mg, 0.40 mmol) in ^{t}BuOH (3.5 ml) was added dropwise at room temperature. The stirring was continued at 45 °C for two days until the disappearance of the starting material (TLC). Na_2SO_3 (633 mg, 5.03 mmol) was added at 0 °C and the reaction mixture was evaporated to dryness. The residue was chromatographed ($CHCl_3$/EtOH 3:1) to give **15** (66 mg) and **16** (33 mg) in 63% overall yield.

Compound **15**: R_f ($CHCl_3$/EtOH 3:1) 0.31; $[\alpha]_D$ +6.6 (*c* 1.00, $CHCl_3$); ν_{max} (film): 3360, 3104, 3072, 2984, 2928, 2872, 2856, 1728, 1520, 1468, 1456, 1424, 1112, 704, 664, 620 cm^{-1}; δ_H (250 MHz, CD_3OD) 7.54 (1H, s, C(3)*H*), 7.52–7.30 (10H, m, *H* arom.), 7.01 (1H, s, C(1)*H*), 4.73 (1H, d, *J* 3.5 Hz, C(8)*H*), 4.45 (1H, m, C(5)*H*), 4.18 (1H, dt, *J* 8.2, 2.6 Hz, C(7)*H*), 3.94 (1H, dd, *J* 10.9, 5.5 Hz, C(9)H_aH_b), 3.81 (1H, dd, *J* 10.9, 3.8 Hz, C(9)H_a*H*$_b$), 2.30 (1H, ddd, *J* 14.0, 8.2, 6.1 Hz, C(6)H_aH_b), 1.96 (1H, ddd, *J* 14.0, 6.9, 2.6 Hz, C(6)H_a*H*$_b$), 1.01 (9H, s, (SiC(CH_3)$_3$)); δ_C (60 MHz, CD_3OD) 136.7 (*C*-3), 136.6 (*C m*-arom.), 134.0 and 133.7 (*C s*-arom.), 131.2 (*C*-8a), 129.3 (*C p*-arom.), 129.0 (*C o*-arom.), 126.5 (*C*-1), 68.2 (*C*-9), 67.3 (*C*-7), 65.7 (*C*-8), 53.7 (*C*-5), 30.2 (*C*-6), 27.3 (SiC(*C*H_3)$_3$), 18.4 (Si*C*(CH$_3$)$_3$). HRMS (CI, NH_3) MH^+, found: 423.2105; $C_{23}H_{31}N_2O_3Si$ requires: 423.2106.

Compound **16**: R_f ($CHCl_3$/EtOH 3:1) 0.36; $[\alpha]_D$ +18.9 (*c* 1.00, $CHCl_3$); ν_{max} (film): 3360, 3104, 3072, 2984, 2928, 2872, 2856, 1728, 1520, 1468, 1456, 1424, 1112, 704, 664, 620 cm^{-1}; δ_H (250 MHz, CD_3OD) 7.87 (1H, s, C(3)*H*), 7.70–7.39 (10H, m, *H* arom.), 6.98 (1H, s, C(1)*H*), 4.79 (1H, d, *J* 3.4 Hz, C(8)*H*), 4.32 (1H, dddd, *J* 6.4, 6.3, 3.9, 0.9 Hz, C(5)*H*), 4.02–3.88 (3H, m, C(7)*H*, C(9)H_aH_b, C(9)H$_a$*H*$_b$), 2.16 (1H, ddd, *J* 12.7, 6.3, 5.2 Hz, C(6)H_aH_b), 1.93 (1H, ddd, *J* 12.7, 5.9, 0.9 Hz, C(6)H$_a$*H*$_b$), 1.06 (9H, s, (SiC(CH_3)$_3$); δ_C (60 MHz, CD_3OD) 136.9 (*C*-3), 136.7 (*C m*-arom.), 133.9 and 133.8 (*C s*-arom.), 131.2 (*C*-8a), 129.3 (*C o*-arom.), 129.0 (*C p*-arom.), 126.4 (*C*-1), 68.6 (*C*-9), 68.4 (*C*-7), 64.0 (*C*-8), 56.4 (*C*-5), 28.5 (*C*-6), 27.3 (SiC(*C*H_3)$_3$), 19.9 (Si*C*(CH$_3$)$_3$); FABMS: 423.2 ([M+H]$^+$). HRMS (CI, NH_3) MH^+, found: 423.2104; $C_{23}H_{31}N_2O_3Si$ requires: 423.2106.

The same procedure as for the preparation of **15** and **16** was used starting from **ent-13** to give **ent-15** and **ent-16**.

ent-15: $[\alpha]_D$ −6.8 (*c* 1.40, $CHCl_3$).

ent-16: $[\alpha]_D$ −19.1 (*c* 1.70, $CHCl_3$).

3.1.8. (5*R*,7*S*,8*R*)-5,6,7,8-Tetrahydro-5-hydroxymethyl-imidazo[1,5-*a*]pyridine-7,8-diol (1) and (5*S*,7*R*,8*S*)-5,6,7,8-tetrahydro-5-hydroxymethylimidazo[1,5-*a*]pyridine-7,8-diol (ent-1). To a stirred solution of compound **15** (66 mg, 0.156 mmol) in anhydrous THF (1 ml) was added the solution of TBAF in THF (1.1 M, 75 µl, 0.312 mmol). The mixture was stirred at room temperature overnight until the disappearance of the starting material (TLC). After evaporation in vacuo the residue was chromatographed ($CHCl_3$/EtOH/25% aq NH_3 20:10:1). After evaporation of the solvents the residue was dissolved in H_2O (2 ml). This aq solution was successively passed over *Amberlite CG 400* (OH^-) and *Amberlite CG 120* (H^+) columns. Elution of **1** was performed with 2 M aq NH_3 to give **1** (17 mg, 59%) as a yellow film after lyophilisation. R_f ($CHCl_3$/EtOH/25% aq NH_3 20:10:1) 0.14; $[\alpha]_D$ +22.9 (*c* 1.00, H_2O); ν_{max} (film): 3368, 3128, 2936, 2872, 2856, 1552, 1520, 1472, 1404, 1152, 1112, 704, 680, 664, 620 cm^{-1}; δ_H (250 MHz, CD_3OD) 7.94 (1H, s, C(3)*H*), 7.08 (1H, s, C(1)*H*), 4.84 (1H, d, *J* 3.0 Hz, C(8)*H*), 4.22 (1H, m, C(5)*H*), 3.99 (1H, dt, *J* 11.6, 3.7 Hz, C(7)*H*), 3.95 (1H, dd, *J* 12.2, 3.7 Hz, C(9)H_aH_b), 3.75 (1H, dd, *J* 12.2, 5.5 Hz, C(9)H$_a$*H*$_b$), 2.09 (1H, dt, *J* 13.1, 11.6 Hz, C(6)H_aH_b), 1.93 (1H, dt, *J* 13.1, 4.3 Hz, C(6)H$_a$*H*$_b$); δ_C (60 MHz, CD_3OD) 135.4 (*C*-8a), 129.9 (*C*-3), 125.2 (*C*-1), 66.4 (*C*-7), 62.9 (*C*-8), 61.6 (*C*-9), 54.1 (*C*-5), 26.1 (*C*-6). HRMS (CI, NH_3) MH^+, found: 185.0917; $C_8H_{13}N_2O_3$ requires: 185.0928.

The same procedure as for the preparation of **1** was used starting from **ent-15** to give **ent-1**.

ent-1: $[\alpha]_D$ −22.7 (*c* 0.95, H_2O).

3.1.9. (5*R*,7*R*,8*S*)-5,6,7,8-Tetrahydro-5-hydroxymethyl-imidazo[1,5-*a*]pyridine-7,8-diol (2) and (5*S*,7*S*,8*R*)-5,6,7,8-tetrahydro-5-hydroxymethylimidazo[1,5-*a*]pyridine-7,8-diol (ent-2). The same procedure as described for **1** was used starting from **16** (75 mg, 0.177 mmol) to give after workup compound **2** (19 mg, 59%). R_f ($CHCl_3$/EtOH/25%

aq NH_3 20:10:1) 0.14; $[\alpha]_D$ +14.9 (*c* 0.50, H_2O); ν_{max} (film): 3368, 3128, 2936, 2872, 2856, 1552, 1520, 1472, 1404, 1152, 1112, 704, 680, 664, 620 cm^{-1}; δ_H (250 MHz, CD_3OD) 7.90 (1H, s, C(3)*H*), 7.04 (1H, s, C(1)*H*), 4.73 (1H, d, *J* 1.8 Hz, C(8)*H* partially overlapped with H_2O signal from methanol), 4.28 (1H, m, C(5)*H*), 4.17 (1H, m, C(7)*H*), 3.89 (1H, dd, *J* 12.2, 4.3 Hz, C(9)H_aH_b), 3.63 (1H, dd, *J* 12.2, 4.9 Hz, C(9)H_aH_b), 2.20 (1H, ddd, *J* 9.8, 6.1, 1.8 Hz, C(6)H_aH_b), 1.89 (1H, ddd, *J* 9.8, 7.9, 1.8 Hz, C(6)H_aH_b); δ_C (60 MHz, CD_3OD) 135.3 (*C*-8a), 129.8 (*C*-3), 124.3 (*C*-1), 65.1 (*C*-7), 63.8 (*C*-8), 63.1 (*C*-9), 51.4 (*C*-5), 27.7 (*C*-6). FABMS: 184.9 ([M+H]$^+$). HRMS (CI, NH_3) MH^+, found: 185.0928; $C_8H_{13}N_2O_3$ requires: 185.0928.

ent-2: $[\alpha]_D$ −15.0 (*c* 1.00, H_2O).

3.2. X-ray diffraction analysis

Single crystals were obtained by recrystallisation from ethyl acetate/*n*-hexane (1:1) mixture at room temperature. X-ray diffraction data were collected on a Bruker Smart Apex CCD area detector diffractometer, graphite-monochromated radiation λ (Cu Kα=1.54178 Å). All structures were solved with direct methods and refined on F^2 by full matrix least-squares method. Hydrogen atoms were localised on difference Fourier maps and further refined with individual temperature factors. Computer programs used are: data collection SMART APEX,[33] data reduction SAINT-Plus,[34] semiempirical absorption correction based on multiple scanned equivalent reflections SADABS,[35] structure solution, refinement and molecular graphics SHELXTL.[36] Crystallographic data (excluding structure factors) for structures reported herein have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge on application to: The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk]. Any request should be accompanied by a full literature citation.

3.2.1. (6*S*)-6,7-Dihydro-5*H*-imidazo[1,5-*a*]azepine-6-ol (6). Formula $C_8H_{10}N_2O$, M_w=150.18, colourless crystal 0.40×0.20×0.10 mm, *a*=4.6209(1), *b*=7.2461(1), *c*=22.6157(2) Å, *V*=757.25(1) Å^3, ρ=1.317 g cm^{-3}, μ=7.27 cm^{-1}, *Z*=4, crystal system: orthorhombic, space group $P2_12_12_1$, *T*=293 K, ω scans ($\Delta\omega$=0.3°), 8742 reflections collected ($5\geq h\geq -5$; $8\geq k\geq -8$; $27\geq l\geq -27$), $2\theta_{max}\geq 142°$, 1455 unique reflections (R_{int}=0.016) and 1452 observed reflections [$I\geq 2\sigma(I)$], 142 refined parameters, R_{all}=0.028, $wR(F^2)$=0.079, *S*=1.08, max (min) residual electron density $\Delta\rho_{max}$=0.14, $\Delta\rho_{min}$=−0.13 eÅ^{-3}, Flack parameter[37] with 556 Friedel pairs η=0.05(24). Crystallographic data deposited as supplementary publication CCDC 602460.

3.2.2. (6*S*,8*S*,9*R*)-6,7,8,9-Tetrahydro-5*H*-imidazo[1,5-*a*]-azepin-6,8,9-triol (3). Formula $C_8H_{12}N_2O_3$, M_w=184.20, colourless crystal 0.40×0.20×0.20 mm, *a*=4.8638(1), *b*=13.2335(2), *c*=13.2205(2) Å, *V*=850.94(3) Å^3, ρ=1.438 g cm^{-3}, μ=9.30 cm^{-1}, *Z*=4, crystal system: orthorhombic, space group $P2_12_12_1$, *T*=293 K, ω scans ($\Delta\omega$=0.3°), 9662 reflections collected ($5\geq h\geq -5$; $16\geq k\geq -15$; $16\geq l\geq -16$), $2\theta_{max}\geq 142°$, 1610 unique reflections (R_{int}=0.023) and 1606 observed reflections [$I\geq 2\sigma(I)$], 168 refined parameters, R_{all}=0.030, $wR(F^2)$=0.076, *S*=1.06, max (min) residual electron density $\Delta\rho_{max}$=0.15, $\Delta\rho_{min}$=−0.19 eÅ^{-3}, Flack parameter with 640 Friedel pairs η=0.00(19). Crystallographic data deposited as supplementary publication CCDC 602462.

3.2.3. (6*R*,8*R*,9*S*)-6,7,8,9-Tetrahydro-5*H*-imidazo[1,5-*a*]-azepin-6,8,9-triol (ent-3). Formula $C_8H_{12}N_2O_3$, M_w=184.20, colourless crystal 0.40×0.15×0.10 mm, *a*=4.8168(1), *b*=13.1830(1), *c*=13.2008(1) Å, *V*=838.25(1) Å^3, ρ=1.460 g cm^{-3}, μ=9.30 cm^{-1}, *Z*=4, crystal system: orthorhombic, space group $P2_12_12_1$, *T*=135 K, ω scans ($\Delta\omega$=0.3°), 9518 reflections collected ($5\geq h\geq -4$; $16\geq k\geq -16$; $16\geq l\geq -16$), $2\theta_{max}\geq 142°$, 1591 unique reflections (R_{int}=0.014) and 1591 observed reflections [$I\geq 2\sigma(I)$], 168 refined parameters, R_{all}=0.024, $wR(F^2)$=0.060, *S*=1.06, max (min) residual electron density $\Delta\rho_{max}$=0.16, $\Delta\rho_{min}$=−0.20 eÅ^{-3}, Flack parameter with 630 Friedel pairs η=0.01(16). Crystallographic data deposited as supplementary publication CCDC 602461.

3.3. Enzymatic assays

Glycosidases [α-mannosidase (EC 3.2.1.24) from Jack beans (Sigma M 7257), β-mannosidase (EC 3.2.1.25) from snail acetone powder (Sigma M 9400), α-glucosidase (EC 3.2.1.20) from baker's yeast (Sigma G-5003), β-glucosidase (EC 3.2.1.21) from almonds (Sigma G-4511), α-galactosidase (EC 3.2.1.22) from green coffee beans (Sigma G-8507), β-galactosidase (EC 3.2.1.23) from *Escherichia coli* (Sigma G-4155)] and their corresponding substrates were purchased from Sigma Co. Spectrophotometric assays were performed at the optimum pH for each enzyme, with *p*-nitrophenyl-α-D-mannopyranoside as a substrate for α-mannosidase (K_m=5 mM, pH=4.5), *p*-nitrophenyl-β-D-mannopyranoside for β-mannosidase (K_m=0.9 mM, pH=4.5), *p*-nitrophenyl-α-D-glucopyranoside for α-glucosidase (K_m=0.2 mM, pH=7), *p*-nitrophenyl-β-D-glucopyranoside for β-glucosidase (K_m=5 mM, pH=5.0), *p*-nitrophenyl-α-D-galactopyranoside for α-D-galactosidase (K_m=0.8 mM, pH=6.5) and *p*-nitrophenyl-β-D-galactopyranoside for β-D-galactosidase (K_m=0.2 mM, pH=7). The release of *p*-nitrophenol was measured continuously at 405 nm with a spectrophotometer HP-8453 to determine initial velocities. 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 or phosphate buffer 20 mM according to the desired pH value) using substrate concentrations around the K_m value of each enzyme. The substrates were dissolved in water/ethanol 50:50 (v/v). The K_i value was determined for the most potent inhibitor, by the Dixon graphical procedure.[38,39]

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.01.016.

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