Synthesis of tri- and tetramines containing two 2,3-dihydroxypyrrolidine moieties and their inhibitory activity toward α-mannosidases

Sandrine Gerber-Lemaire,*[a] Florence Popowycz,[a] Eliazar Rodriguez-García,[a] Catherine Schütz,[a] Ana T. Carmona Asenjo,[b] Inmaculada Robina,[b] and Pierre Vogel[a]

[a] Institut de chimie moléculaire et biologique, Ecole Polytechnique Fédérale de Lausanne, BCH, CH-1015 Lausanne, Switzerland and [b] Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, E-41071 Sevilla, Spain
E-mail: Sandrine.Gerber@epfl.ch

Dedicated to Professor Josef Muchowski on the occasion of his 65th birthday

(received 20 Feb 03; accepted 08 Apr 03; published on the web 16 Apr 03)

Abstract

Through the reductive amination of *N*-[(*tert*-butoxy)carbonyl]-2,5-dideoxy-2,5-imino-3,4-*O*-isopropylidene-L-ribose with tetramethylenediamine, hexamethylenediamine, 2,7-diaminofluorene, 4,4'-diaminodiphenylmethane and 1,4-(diaminomethyl)benzene, five tetramines containing two (2*R*,3*R*,4*S*)-2-aminomethylpyrrolidine-3,4-diol moieties have been prepared and assayed for their inhibitory activities toward 24 glycosidases. Tetramines containing the tetramethylene or benzene-1,4-dimethylene linkers are more potent α-mannosidase inhibitors than simple (2*R*,3*R*,4*S*)-2-aminomethylpyrrolidine-3,4-diols. Triamines such as (2*S*,3*R*,4*S*)-bis(3,4-dihydroxy-pyrrolidin-2-ethyl)amine were also prepared and shown to be better α-mannosidase inhibitors than (2*S*,3*R*,4*S*)-2-(2-aminoethyl)pyrrolidin-3,4-diol.

Keywords: α-Mannosidase inhibitors, polyamines containing hydroxylated pyrrolidines, reductive amination

Introduction

Cell sociology involves a language based on molecular recognition between cell-surface carbohydrates and proteins.[1] The biosynthesis of the surface oligosaccharides uses glycosyltransferases and glycosidases as catalysts. Inhibitors of these enzymes[2] are important molecular tools for glycobiology, and can be used to modulate cellular functions. They are also potential drugs in new therapeutic strategies.[3] Among the most potent glycosidase inhibitors are polyhydroxypiperidines (1,5-dideoxy-1,5-iminoalditols) that are mimics of the glycosyl cation

intermediates liberated during enzyme-catalyzed hydrolytic processes.[4,5] Derivatives of 3,4-dihydroxypyrrolidines (1,4-dideoxy-1,4-iminoalditols) also emerge as an important class of glycosidase[4a,5,6] and glycosyltransferase[7] inhibitors. Simple *meso*-3,4-dihydroxypyrrolidine **1** is a non-selective, weak inhibitor of several glycosidases (Figure 1).[8] We have found that derivatives **2b** with (2*R*)-aminomethyl side chains can be highly selective and competitive inhibitors of α-mannosidases, especially for Ar = phenyl, thiophenyl.[8]

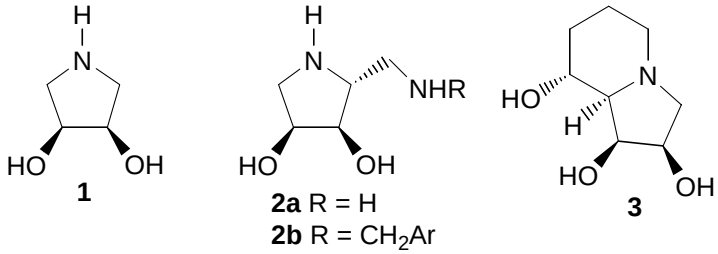


Figure 1. Inhibitors of glycosidases and glycosyltransferases.

Clinical trials have shown that swainsonine **3**, a natural α-mannosidase inhibitor that contains a 4-amino-4-deoxy-mannofuranoside moiety,[9,10] reduces solid tumors and hematological malignancies.[11] Analogues of **3** have also shown interesting properties.[12] Mannosidase inhibitors mediate increased secretion of mutant α1-antitrypsin Z. They are thus leads in the development of drugs for the chemoprophylaxis of liver injury and emphysema in patients with α1-antitrypsin Z deficiency.[13] Mannostatin A and B isolated from the soil microorganism *Streptoverticillum verticillus*[14] and a synthetic analogue[15] are probably the most potent inhibitors of α-mannosidases reported so far.[16] Often α-mannosidase inhibitors that are monosaccharide mimics[4a,17] also inhibit other types of glycosidases,[18] in particular α-L-fucosidases.[4a,19] To become a drug, a good inhibitor must satisfy a number of conditions apart from its low toxicity and enzyme specificity.[20] We have envisioned that polyamines containing two (2*R*,3*R*,4*S*)-2-(aminomethyl)-3,4-dihydroxypyrrolidine fragments could be alternative α-mannosidase inhibitors with improved pharmacological properties. We report here the synthesis of five tetramines **4** (Figure 2). We have also prepared triamine **5** that contains two (2*S*,3*R*,4*S*)-2-(1-aminoeth-2-yl)-3,4-dihydroxypyrrolidine moieties, as well as its enantiomer *ent*-**5**. These new compounds have been assayed for their inhibitory activity toward 24 commercially available glycosidases, and in particular toward α-mannosidase from *jack bean*, an enzyme known to be a useful model for mammalian α-mannosidases such as Golgi α-mannosidase II.[21] Whereas triamine *ent*-**5** does not inhibit any of the enzyme tested (except for a poor 38% inhibition of β-glucosidase from almond at 1 mM concentration), its enantiomer **5** is a moderate inhibitor of α-mannosidase from jack bean (K_i = 74 μM) and from almond (K_i = 92 μM). Among the five tetramines **4**, best inhibitory activities toward these enzymes were found with **4a** and **4e**. But contrary to inhibitors of type **2b**, these polyamines are less enzyme selective.

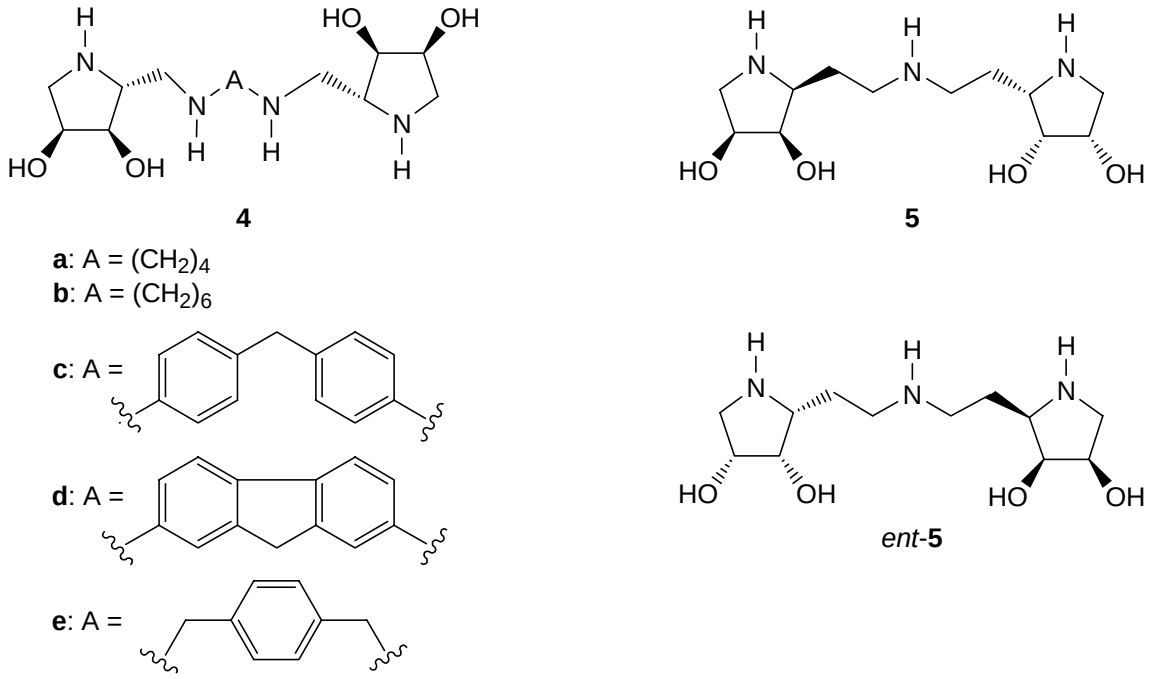
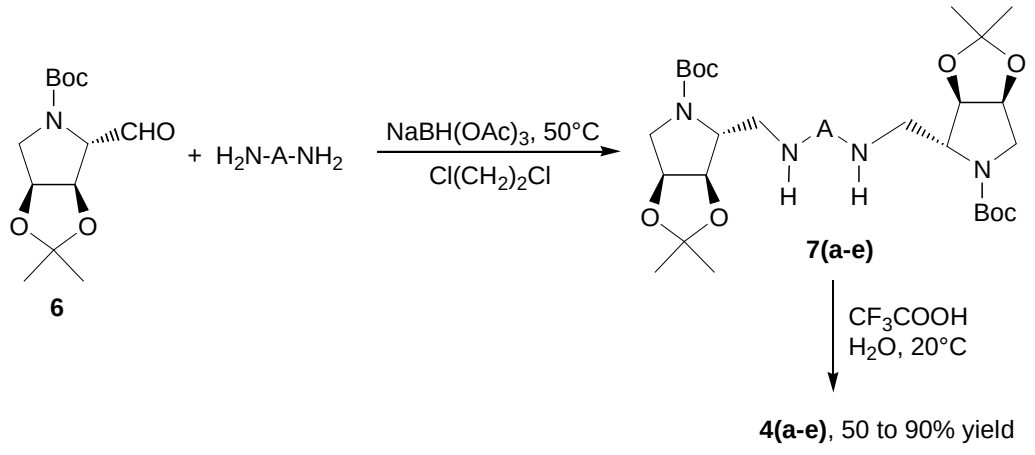


Figure 2. Tri- and tetramines containing two 2,3-dihydroxypyrrolidine moieties.

Results and Discussion

Synthesis of the polyamines

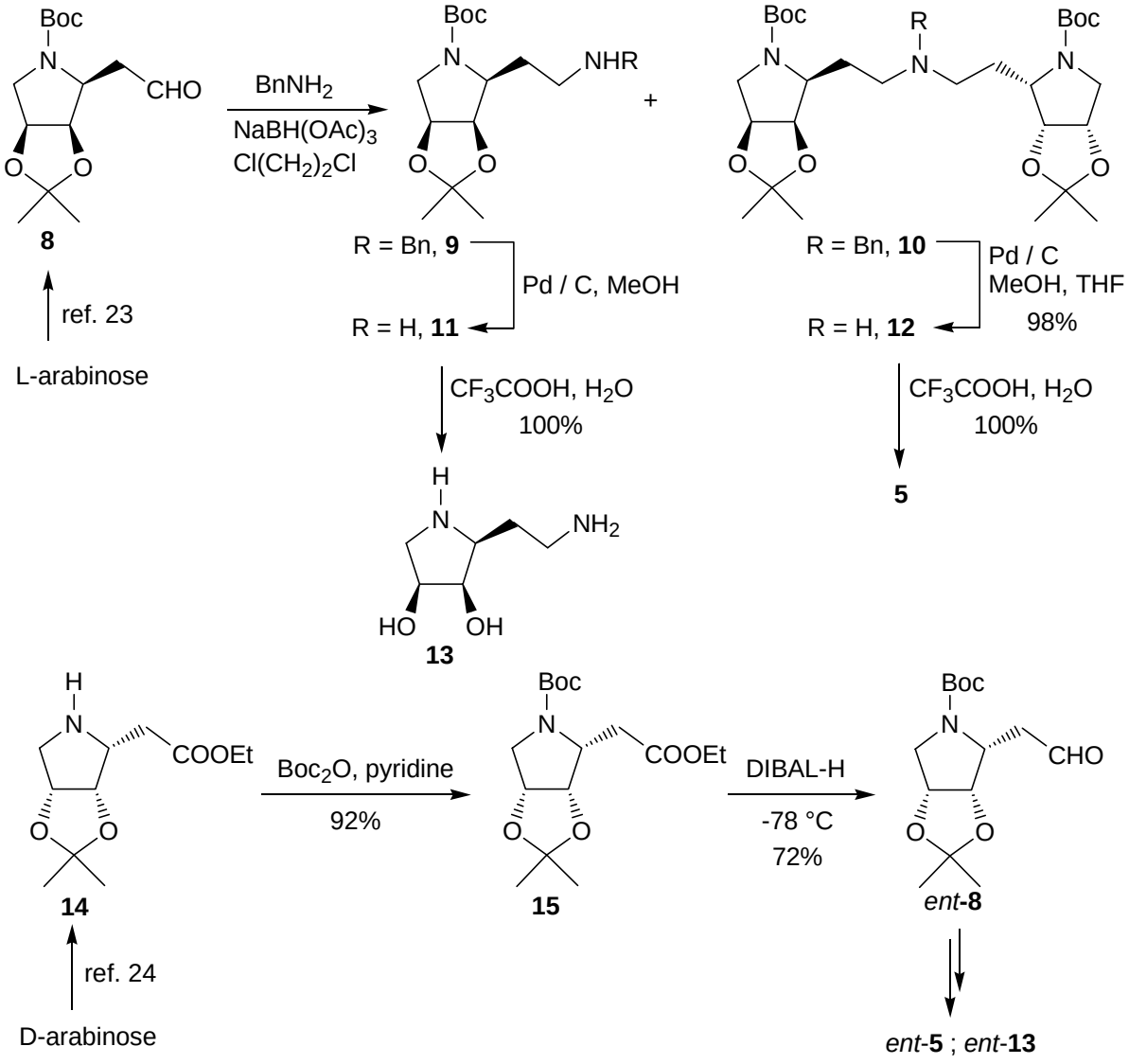
Tetramines **4** were all prepared from aldehyde **6**[8] by reaction with the corresponding diamine H_2N-A-NH_2 (1.8 equivalent) in the presence of $NaBH(OAc)_3$[22] for *in situ* reduction of the resulting diimine intermediate (Scheme 1).



Scheme 1. Synthesis of tetramines **4**.

The so-formed semi-protected tetramines were treated with aqueous CF_3COOH, at room temperature, to cleave the Boc and acetonide moieties. Overall yields based on **6** ranged from 50 to 90%.

Triamines **5** and *ent*-**5** were derived from aldehydes **8** and *ent*-**8**, themselves derived from L- and D-arabinose, respectively[23,24] (Scheme 2). Treatment of a 1:1.1 mixture of **8** and benzylamine with $NaBH(OAc)_3$ in 1,2-dichloroethane resulted in the formation of **9** and **10** with 46% and 18% yield, respectively. Using a half equivalent of benzylamine, **10** was obtained in 55% yield. Hydrogenolysis of the benzyl group (10% Pd / charcoal, THF/MeOH) gave **11** in 98% yield. Deprotection under acidic conditions provided **5** in almost quantitative yield. The same reactions were applied to *ent*-**8** providing *ent*-(**9**–**13**). Compound *ent*-**8** was obtained from known **14**[24] after Boc-protection and reduction with DIBAL-H.



Scheme 2. Preparation of triamines **5** and *ent*-**5**.

Glycosidase inhibitory activities

Appropriate p-nitrophenyl pyranosides were used as substrates and commercially available glycosidases (see below and Table) were used as catalysts of the buffered hydrolysis under optimal pH.[25] At 1 mM concentration and under optimal pH conditions tetramines **4** and triamines **5** and *ent*-**5** did not inhibit the following enzymes: α-L-fucosidase from bovine epididymis, α-D-galactosidases from coffee bean, *Aspergillus niger* and *E. coli*, β-galactosidase from *orizae*, β-D-mannosidase from *Helix pomatia*, β-*N*-acetylgalactosamidase from jack bean, bovine epididymis A and B. The inhibitory activities toward other glycosidases are reported in Table 1.

We have found that (2*R*,3*R*,4*S*)-2-aminomethylpyrrolidine-3,4-diol **2a** is a weak inhibitor of α-mannosidase from jack bean and from almond. This diamine also moderately inhibits β-galactosidases, α-glucosidases and β-glucosidases. Derivatives **2b** are much better and more selective α-mannosidase inhibitors.[8] Thus, we expected that compounds **4** and **5** would also show improved inhibitory activities toward α-mannosidases. This is indeed the case for **4a** with the tetramethylene linker, and for **4e** with the *p*-benzenedimethylene spacer. Both are competitive inhibitors. The bad surprise is that these tetramines also inhibit other glycosidases, moderately though, except for **4a** which is a good, non-competitive inhibitor of β-glucosidase from almond. This result suggests that **4a** "sticks" to this enzyme and inhibits it for allosteric reasons, a mechanism different from that making **4a** a competitive inhibitor of α-mannosidases. Tetramine **4b** with the hexamethylene linker and analogues **4c** and **4d** with diphenylmethane linkers are poor inhibitors in terms of both potency and selectivity. They are even worse than simple diamine **2a**. As (2*S*,3*R*,4*S*)-2-(2-aminoethyl)pyrrolidine-3,4-diol **13** is a weak inhibitor of α-mannosidase, although the side chain is in a β-configuration rather than α, we envisioned that triamine **5** might have improved inhibitory activity. Interestingly, we find **5** to be a more potent α-mannosidase inhibitor than **13**. Unfortunately, it is not a more selective inhibitor than **13** because it inhibits moderately a few α-glucosidases, β-glucosidases and α-N-acetyl-galactosamidase from chicken liver (Table 1). As expected, triamine *ent*-**5**, which does not share the configuration of any of the hexoses liberated during the hydrolytical process catalyzed by the enzymes used in this study, ignores all these glycosidases.

Conclusions

The conjugation of two (2*R*,3*R*,4*S*)-2-(2-aminomethyl)pyrrolidine-3,4-diols by their primary amines to alkane or arene linkers can generate potent α-mannosidase inhibitors. This work opens a new road in the search for new glycosidase inhibitors. Analogues of tetramines **4a** and **4e** that will be more enzyme selective remain to be made.

Table 1. Inhibitory activities of diamines **2a**, **2b**, triamines **5** and *ent*-**5** and tetramines **4a-4e**. Percentage of inhibition at 1mM concentration, IC_{50} (in parenthesis) and Ki in μM, optimal pH, 35°C[25,26]

Enzyme / inhibitor	2a	2b	4a	4b	4c	4d	4e	5	*ent*-5
β-galactosidase from									
E-coli	92%	24%	95%	43%	47%	ni	37%	ni	ni
bovine liver	ni	26%	ni	24%	95%	82%	41%	ni	ni
Aspergillus niger	24%	ni	ni	ni	ni	ni	40%	22%	ni
jack bean	76%	ni	45%	23%	ni	ni	39%	31%	ni
α-glucosidase from									
yeast (maltase)	24%	ni	88%	37%	ni	ni	55%	ni	ni
rice (maltase)	53%	ni	ni	ni	26%	ni	ni	ni	ni
baker yeast (isomaltase)	98%	ni	ni	69%	ni	ni	86%	50%	ni
Aspergillus niger	ni	ni	ni	ni	ni	ni	28%	26%	ni
(amyloglucosidase)									
Rhyzopus mold	ni	ni	ni	ni	26%	ni	39%	ni	ni
(amyloglucosidase)									
β-glucosidase from									
almonds	97%	68%	97%(160)	87%(110)	35%	52%	85%(99)	37%	38%
Ki =			8(NC)	110 (C)			65(C)		
caldocellum sacch.	93%	ni	90%	76%	36%	29%	67%	26%	ni
α-mannosidase from									
jack bean	81%	92%	76%(330)	72%	ni	47%	95%(50)	71%(300)	ni
Ki =	53(C)	7.4(C)	21 (C)				12 (C)	74 (C)	
almonds	51%	69%	85%(92)	70%	39%	ni	81%(145)	65%(280)	ni
Ki =		7(C)	10 (C)				48 (C)	92 (C)	
β-xylosidase from									
Aspergillus niger	ni	ni	ni	ni	ni	ni	26%	ni	ni
α–*N*–acetylgalactosamidase									
chicken liver	ni	ni	ni	92%(100)	ni	ni	91%(53)	ni	ni
Ki =				43 (C)			31 (C)		

ni = no inhibition, C = competitive, NC = non-competitive

Experimental Section

General Procedures. All commercially available reagents (Fluka, Aldrich) were used without further purification. Solvents were dried by standard methods. Light petroleum ether used refers to the fraction boiling at 40–60 °C. Solutions after reactions and extractions were evaporated in a

rotatory evaporator under reduced pressure. Liquid/solid flash chromatography (FC): columns of silica gel (Merck No.9385 silica gel 60, 240–400 mesh). TLC for reaction monitoring: Merck silica gel $60F_{254}$ plates; detection by UV light, Pancaldi reagent [$(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O] or $KMnO_4$. IR spectra: Perkin-Elmer-1420 spectrometer. Optical rotations were determined at room temperature on a Jasco DIP-370 polarimeter. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. ^1H NMR spectra: Bruker-ARX-400 spectrometer (400 MHz), Bruker AMX-300 spectrometer (300 MHz); δ(H) in ppm relative to the solvent's residual ^1H signal [$CHCl_3$, δ(H) 7.27; CH_3OD, δ(H) 3.31; D_2O, δ(H) 4.79; DMSO-d_6, δ(H) 2.54] as internal reference; all ^1H assigments were confirmed by 2D-COSY-45 and 2D-NOESY spectra. ^{13}C NMR spectra: same instrument as above (100.6 MHz and 75.4 MHz); δ(C) in ppm relative to the solvent's C-signal [$CDCl_3$, δ(C) 77.0; CD_3OD, δ(C) 49.8; DMSO-d_6 δ(C) 39.7] as internal reference; all ^{13}C assigments were confirmed by 2D-HMQC; coupling constants *J* in Hz. MS: Nermag R 10-10C, chemical ionization (NH_3) mode *m*/*z* (amu) [% relative to base peak (100%)]. High resolution mass spectrometry: Micromass AutoSpecQ, resolution of 10000 (5% valley definition). Elemental analyses: Ilse Beetz, D-96301 Kronach, Germany.

Glycosidase inhibitions. A known protocol was applied.[25,26] We verified that the delay of inhibitor/enzyme incubation did not affect the inhibition measurements. Under standard conditions, optimal inhibitory activities were measured after five minutes of incubation.

Reductive amination. General procedure A. To a solution of *N*-[(*t*-butoxy)carbonyl]-2,5-dideoxy-2,5-imino-3,4-*O*-isopropylidene-L-ribose (200 mg, 0.737 mmol) in anhydrous 1,2-dichloroethane (7 mL) were added the diamine (0.6 eq, 0.442 mmol) and $NaBH(OAc)_3$ (1.8 eq, 281 mg, 1.327 mmol). The solution was stirred at 50 °C for 12 h and then poured into a sat. aq solution of $NaHCO_3$ (20 mL). The mixture was extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was directly used in the deprotection step.

Reductive amination. General procedure B. To a solution of *N*-[(*t*-butoxy)carbonyl]-2,3,6-trideoxy-3,6-imino-4,5-*O*-isopropylidene-L- (or D-) *arabino*-hexose (1 mmol) in anhydrous 1,2-dihloroethane (3 mL) were added benzylamine (118 mg, 1.1 mmol) and $NaBH(OAc)_3$ (276 mg, 1.3 mmol). The solution was stirred at r.t. for 3 h and then poured into a sat. aq solution of $NaHCO_3$ (20 mL). The mixture was extracted with EtOAc (3 x 20 mL) and the combined organic extracts were dried ($MgSO_4$). After solvent evaporation under reduced pressure the residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 60:1 to 5:1).

Deprotection. General procedure C. A solution of bis (pyrrolidine) derivatives in CF_3COOH / H_2O (4:1; 5–10%) was stirred at 20 °C for 2 h. After solvent evaporation *in vacuo*, the residue was purified by flash chromatography on silica gel (MeCN/aq NH_3).

Deprotection. General procedure D. A solution of the protected pyrrolidine derivative (0.1 mmol) in CF_3COOH H_2O (4:1; 3 mL) was stirred at 20 °C for 2 h. The mixture was passed through a Dowex 50WX8 (100–200 mesh) column and eluted, successively with MeOH (30 mL), H_2O (30 mL) and NH_4OH (10%, 50 mL). The fractions containing the unprotected product were concentrated to yield the corresponding pyrrolidine derivative.

(2*R*,3*R*,4*S*)-2-[[4-[[[(2*R*,3*R*,4*S*)-3,4-Dihydroxypyrrolidin-2-yl]methyl]amino]butyl]amino-methyl]pyrrolidine-3,4-diol (4a). Procedure A was applied to 1,4-diaminobutane (45 μL, 0.442 mmol) to afford crude **7a** (180 mg). Deprotection according to procedure C gave **4a** (127 mg, 90%, 2 steps) as a pale orange oil. R_f = 0.15 (MeCN / NH_4OH 1:1). $[\alpha]_{589}^{25}$ = –106, $[\alpha]_{577}^{25}$ = –262, $[\alpha]_{546}^{25}$ = –409, $[\alpha]_{435}^{25}$ = –690, $[\alpha]_{405}^{25}$ = –1114 (*c* = 0.25, H_2O). IR (film): $\tilde{\nu}$ 3500–2900, 1440, 1200, 1140, 840, 800, 710, 695 cm^{-1}. UV (MeCN): λ_{max} (ε) 195 (1360). ^1H NMR (D_2O): δ 4.11 (m, 2H, H-4, H-4^{IV}), 3.94 (dd, 2H, ^3J = 10.7, ^3J = 3.9 Hz, H-3, H-3^{IV}), 3.75 (ddd, 2H, ^3J = 10.7, ^3J = 5.1, ^3J = 3.3 Hz, H-2, H-2^{IV}), 3.23 (dd, 2H, ^2J = 9.3, ^3J = 2.1, H-5, H-5^{IV}), 3.13 (dd, 2H, ^2J = 12.6, ^3J = 5.1, H-1', H-1'"), 3.09–3.01 (m, 2H, H-5, H-5^{IV}), 2.81 (dd, 2H, ^2J = 12.6, ^3J = 3.3, H-1', H-1'"), 2.72–2.64 (m, 4H, H-1", H-4"), 1.71–1.56 (m, 4H, H-2", H-3"). ^{13}C NMR (D_2O): δ 76.9 (d, C-3, C-3^{IV}), 73.5 (d, C-4, C-4^{IV}), 60.7 (d, C-2, C-2^{IV}), 53.9 (t, C-5, C-5^{IV}), 52.6 (t, C-1, C-1'"), 50.4 (t, C-1", C-4"), 26.3 (t, C-2", C-3"). CI-MS: *m/z* 319 (100, M + H^+), 293 (74), 204 (33), 133 (35), 102 (36), 84 (55). Anal. calcd for $C_{14}H_{30}N_4O_4$ (318.42): C, 52.81; H, 9.50. Found: C, 52.79; H, 9.32.

(2*R*,3*R*,4*S*)-2-[[6-[[[(2*R*,3*R*,4*S*)-3,4-Dihydroxypyrrolidin-2-yl]methyl]aminohexyl]amino-methyl]pyrrolidine-3,4-diol (4b). Procedure A was applied to 1,6-diaminohexane (51 mg, 0.442 mmol) to afford crude **7b** (155 mg). Deprotection according to procedure C gave **4b** (86 mg, 56% yield, 2 steps) as a colorless oil. R_f = 0.1 (MeCN, NH_4OH 1/1). $[\alpha]_{589}^{25}$ = –54 (c = 0.5, H_2O). IR (film): $\tilde{\nu}$ 3500–2900, 1450, 1195, 1150, 840, 800, 705, 700 cm^{-1}. UV (MeCN): λ_{max} (ε) 197 (1450). ^1H NMR (D_2O): δ 4.18 (m, 2H, H-4, H-4^{IV}), 3.95 (dd, 2H, ^3J = 5.4, ^3J = 2.7 Hz, H-3, H-3^{IV}), 3.75 (m, 2H, H-2, H-2^{IV}), 3.29 (m, 2H, H-5, H-5^{IV}), 3.15 2H, (2H, dd, ^2J = 13.2, ^3J = 4.8, H-1', H-1'"), 3.09 (m, 2H, H-5, H-5^{IV}), 2.94 (dd, 2H, ^2J = 13.2, ^3J = 3.1, H-1', H-1'"), 2.73 (m, 4H, H-1", H-6"), 1.65-1.54 (m, 4H, H-2", H-5"), 1.38 (m, 4H, H-3", H-4"). ^{13}C NMR (D_2O): δ 77.0 (d, C-3, C-3^{IV}), 71.7 (d, C-4, C-4^{IV}), 60.7 (d, C-2, C-2^{IV}), 54.2 (t, C-5, C-5^{IV}), 52.6 (t, C-1, C-1'"), 50.9 (t, C-1", C-6"), 29.7 (t, C-2", C-5"), 26.3 (t, C-3", C-4"). CI-MS: *m/z* 347 (28, M + H^+), 274 (9), 232 (12), 117 (100), 98 (85), 86 (63). Anal. calcd for $C_{16}H_{34}N_4O_4$ (346.47): C, 55.47; H, 9.89; N, 16.17. Found: C, 55.18; H, 9.70; N, 16.01.

(2*R*,3*R*,4*S*)-2-[4-[4-[[[(2*R*,3*R*,4*S*)-3,4-Dihydroxypyrrolidin-2-yl]methyl]amino]benzyl]phenyl-aminomethyl]pyrrolidine-3,4-diol (4c). Procedure A was applied to 4,4'-diaminodiphenyl-methane (88 mg, 0.442 mmol) to afford crude **7c** (150 mg). Deprotection according to procedure C gave **4c** (113 mg, 60% yield, 2 steps) as a pale yellow oil. R_f = 0.10 (MeCN/NH_4OH 4:1). $[\alpha]_{589}^{25}$ = +27, $[\alpha]_{577}^{25}$ = +34, $[\alpha]_{546}^{25}$ = +41 (c = 0.9, MeOH). IR (film): $\tilde{\nu}$ 3400–3200, 2950, 1675, 1515, 1450, 1205, 1140, 1025, 725 cm^{-1}. UV (MeCN): λ_{max} (ε) 260 (7250), 207 (13980). ^1H NMR (MeOD): δ 6.94, 6.68 (2d, 8H, ^3J = 8.5 Hz, H-2", H-6", H-3^{VI}, H-5^{VI}), 4.29 (m, 2H, H-4, H-4^{VI}), 4.08 (dd, 2H, ^3J = 8.6, ^3J = 4.0 Hz, H-3, H-3^{VI}), 3.80 (bs, 2H, 2H-1'"), 3.76 (ddd, 2H, ^3J =

9.1, ^3J = 8.6, ^3J = 3.7 Hz, H-2, H-2^{VI}), 3.60 (dd, 2H, ^2J = 14.4, ^3J = 3.7 Hz, H-1', H-1^{V}), 3.48 (dd, 2H, ^2J = 14.4, ^3J = 4.0 Hz, H-5, H-5^{VI}), 3.45 (dd, 2H, ^2J = 14.4, ^3J = 3.7 Hz, H-1', H-1^{V}), 3.27 (dd, 2H, ^2J = 14.4, ^3J = 1.9 Hz, H-5, H-5^{VI}). ^{13}C NMR (MeOD): δ 133.3 (s, C-1", C-4^{IV}), 133.2 (d, C-2", C-6", C-3^{IV}, C-5^{IV}), 122.3 (s, C-4", C-1^{IV}), 116.9 (d, C-3", C-5", C-2^{IV}, C-6^{IV}), 77.3 (d, C-3, C-3^{VI}), 73.5 (d, C-4, C-4^{VI}), 64.0 (d, C-2, C-2^{VI}), 53.3 (t, C-5, C-5^{VI}), 47.1 (t, C-1', C-1^{V}), 43.8 (t, C-1'"). Anal. calcd for $C_{23}H_{30}N_4O_4$ (426.51): C, 64.77; H, 7.09; N, 13.14. Found: C, 64.34; H, 7.28; N, 12.99.

(2*R*,3*R*,4*S*)-2-[[7-[[[(2*R*,3*R*,4*S*)-3,4-Dihydroxypyrrolidin-2-yl]methyl]amino]-9*H*-fluoren-2-yl]aminomethyl]pyrrolidine-3,4-diol (4d). Procedure A was applied with 2,7-diaminofluorene (87 mg, 0.442 mmol) to afford crude **7d** (153 mg). Deprotection according to procedure C gave **4d** (94 mg, 50% yield, 2 steps) as a pale yellow oil. R_f = 0.09 (MeCN/NH_4OH 2/1). $[\alpha]_{589}^{25}$ = –62, $[\alpha]_{577}^{25}$ = –74, $[\alpha]_{546}^{25}$ = –103, $[\alpha]_{435}^{25}$ = –107, $[\alpha]_{405}^{25}$ = –130 (*c* = 1, MeOH). IR (film): $\tilde{\nu}$ 3400–3200, 2960, 1675, 1520, 1455, 1210, 1135, 125, 880, 765 cm^{-1}. UV (MeCN): λ_{max} (ε) 308 (5820), 215 (5680), 203 (6200). ^{1}H NMR (MeOD): δ 7.43 (d, 2H, ^3J = 8.1 Hz, H-3", H-6"), 6.92 (bs, 2H, H-1", H-8"), 6.71 (d, 2H, ^3J = 8.1 Hz, H-4", H-5"), 4.32 (m, 2H, H-4, H-4^{IV}), 4.13 (dd, 2H, ^3J = 8.5, ^3J = 4.0 Hz, H-3, H-3^{IV}), 3.82 (ddd,2H, ^3J = 8.5, ^3J = 8.4, ^3J = 3.8 Hz, H-2, H-2^{IV}), 3.73 (bs, 2H, H-9"), 3.67 (dm, 2H, ^2J = 13.3 Hz, H-5, H-5^{IV}), 3.52 (m, 2H, H-5, H-5^{IV}), 3.51 (dd, 2H, ^2J = 12.6, ^3J = 4.0 Hz, H-1', H-1'"), 3.30 (dd, 2H, ^2J = 12.6, ^3J = 1.8 Hz, H-1', H-1'"). ^{13}C NMR (MeOD): δ 148.2, 146.2 (2s, C-4a", C-4b", C-8a", C-9a"), 121.0 (s, C-2", C-7"), 120.5 (d, C-3", C-6"), 114.2 (d, C-4", C-5"), 111.9 (d, C-1", C-8"), 75.7 (d, C-3, C-3^{IV}), 71.9 (d, C-4, C-4^{IV}), 62.5 (d, C-2, C-2^{IV}), 51.7 (t, C-1', C-1'"), 45.7 (d, C-5, C-5^{IV}), 42.1 (t, C-9"). CI-MS: *m/z* 427 (21, M + H$^+$), 370 (14), 311 (50), 197 (28), 98 (100), 80 (57). Anal. calcd for $C_{23}H_{30}N_4O_4$ (426.51): C, 64.77; H, 7.09. Found: C, 64.88; H, 7.23.

(2*R*,3*R*,4*S*)-2-[[4-[[[(2*R*,3*R*,4*S*)-3,4-Dihydroxypyrrolidin-2-yl]methyl]aminomethyl]benzyl]-aminomethyl]pyrrolidine-3,4-diol (4e). Procedure A was applied with 1,4-(diamino-methyl)benzene (60 mg, 0.442 mmmol) to afford crude **7e** (157 mg). Deprotection according to procedure C gave **4e** (89 mg, 55% yield, 2 steps) as a colorless oil. R_f = 0.14 (MeCN/NH_4OH 1:1). $[\alpha]_{589}^{25}$ = + 57, $[\alpha]_{577}^{25}$ = + 77, $[\alpha]_{546}^{25}$ = + 83, $[\alpha]_{435}^{25}$ = + 93, $[\alpha]_{405}^{25}$ = + 110 (*c* = 0.65, H_2O). IR (film): $\tilde{\nu}$ 3500–3000, 1675, 1425, 1200, 1130, 835, 800, 740, 700 cm^{-1}. UV (MeCN): λ_{max} (ε) 197 (5600). ^{1}H NMR (D_2O): δ 7.50 (bs, 4H, H_{arom}), 4.26 (m, 2H, H-4, H-4^{VI}), 3.94 (s, 4H, 2H-1", 2H-1^{IV}), 3.87 (dd, 2H, ^3J = 7.6, 5.0 Hz, H-3, H-3^{VI}), 3.30–3.25 (m, 4H, H-2, H-2^{VI}, H-5, H-5^{VI}), 2.99-2.94 (m, 4H, H-1', H-1^{V}, H-5, H-5^{VI}), 2.79 (dd, 2H, ^2J = 12.5, ^3J = 8.6 Hz, H-1', H-1V). ^{13}C NMR (D_2O): δ 137.4 (s, C-1'", C-4'"), 129.2 (d, C-2'", C-3'", C-5'", C-6'"), 75.2 (d, C-3, C-3^{VI}), 70.9 (d, C-4, C-4^{VI}), 59.9 (t, C-1", C-1^{IV}), 52.1 (t, C-5, C-5^{VI}), 50.6 (d, C-2, C-2^{VI}), 50.0 (t, C-1', C-1^{V}). CI-MS : *m/z* 368 (24, M$^+$), 252 (7), 133 (100), 117 (59). Anal. calcd for $C_{18}H_{32}N_4O_4$ (368.48): C 58.67; H 8.75; N 15.21. Found: C 58.42, H 8.60, N 15.12.

***N*-(*tert*-Butoxycarbonyl)-(2*S*,3*R*,4*S*)-2-[2-(benzylamino)ethyl]-3,4-*O*-isopropylidenepyrrolidine-3,4-diol (9) and *N*,*N*-bis[*N*-(*tert*-butoxycarbonyl)-[(2*S*,3*R*,4*S*)-3,4-*O*-isopropylidenoxypyrrolidinyl]ethyl]benzylamine (10).** Procedure B was applied to carbaldehyde **8**[23] (298 mg, 1.05 mmol) affording **9** (178.8 mg, 46%) as oil and **10** (123.4 mg, 18%) as white solid.

9. $[\alpha]_{589}^{25}$ = +46 (c = 0.94, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ 3335, 1705, 1470, 1405, 1085, 735, 695 cm^{-1}. ^1H NMR (DMSO-d_6, 90 ºC): δ 7.36–7.27 (m, 5H, H_{arom}), 4.71–4.64 (m, 2H, H-3, H-4), 3.83 (m, 1H, H-2), 3.75 (d, 1H, ^2J = 13.6, CH_2Ph), 3.70 (d, 1H, ^2J = 13.6, CH_2Ph), 3.67 (dd, 1H, ^3J = 7.0, ^2J = 12.2, H-5), 3.13 (dd, 1H, ^3J = 3.3, H-5), 3.00 (bs, 1H, NH), 2.65 (ddd, 1H, ^2J = 11.6, H-2'), 2.59 (ddd, 1H, H-2'), 1.93 (dq, 1H, ^3J = 6.5, ^2J = 13.3, H-1'), 1.81 (dq, 1H, ^3J = 6.1, H-1'), 1.39 (s, 9H, $C(CH_3)_3$), 1.42 and 1.26 (2s, 6H, $C(CH_3)_2$). ^{13}C NMR (DMSO-d_6 90ºC): δ 153.4 (s, CO), 139.7 (s, Carom), 127.5, 127.4, 125.9 (3d, C_{arom}), 111.2 (s, $C(CH_3)_2$), 79.3, 76.7 (2d, C-3, C-4), 78.4 (s, CMe_3), 57.3 (d, C-2), 52.4 (t, CH_2Ph), 50.0 (t, C-5), 45.3 (t, C-2'), 28.8 (t, C-1'), 27.6 (q, $C(CH_3)_3$), 26.0, 24.6 (2q, $C(CH_3)_2$). CI-MS: *m/z* 377 (100, $[M+H]^+$). CI-HRMS: *m/z* 377.2439 (calcd for $C_{21}H_{32}N_2O_4$+H: 223.1446).

10. $[\alpha]_{589}^{25}$ = +81 (c = 0.98, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ 1700, 1400, 1165, 1100, 870, 735 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.36–7.16 (m, 5H, H_{arom}), 4.49 (m, 2H, H-4), 4.53 (dd, 2H, ^3J = 6.1, ^3J = 6.1, H-3), 3.83 (ddd, 2H, ^3J = 9.3, ^3J = 6.1, ^3J = 5.4, H-2), 3.80 (d, 1H, ^2J = 12.9, CH_2Ph), 3.77 (dd, ^2J = 12.1, ^3J = 7.1, H-5), 3.60 (d, 1H, ^2J = 12.9, CH_2Ph), 3.24 (dd, 2H, ^2J = 12.1, ^3J = 4.7, H-5), 2.63 (dt, ^2J = 12.8, ^3J = 7.8, H-2'), 2.50 (ddd, 2H, ^2J = 12.8, ^3J = 8.4, ^3J = 4.4, H-2'), 1.42 (s, 18H, ^{t}Bu), 1.47, 1.27 (2s, 6H, $C(CH_3)_2$). ^{13}C NMR ($CDCl_3$): δ 154.2 (s, CO), 140.2 (s, Carom), 129.0, 127.8, 126.3 (3d, Carom), 112.2 (s, $C(CH_3)_2$), 79.3 (d, C-3), 77.3 (s, CMe_3), 77.3 (d, C-4), 58.2 (d, C-2), 58.0 (t, CH_2Ph), 50.7, 50.6 (2t, C-5, C-2'), 28.3 (q, $C(CH_3)_3$), 26.5 (t, C-1'), 26.7, 25.0 (2q, $C(CH_3)_2$). CI-MS: *m/z* 646 (100, M + H^+). CI-HRMS: *m/z* 646.4069 (calcd for $C_{35}H_{55}N_3O_8$+H: 646.4067).

***N*-(*tert*-Butoxycarbonyl)-(2*S*,3*R*,4*S*)-2-aminoethyl-3,4-*O*-isopropylidenepyrrolidine-3,4-diol (11).** A solution of **9** (131.6 mg, 0.35 mmol) in abs. EtOH (7 mL) was hydrogenated with catalyst Pd/C (10%, 55 mg) at 1 atm for 2 h. The mixture was filtered through Celite, and the filtrate was evaporated to give **11** (101 mg, 100%) as a syrup. $[\alpha]_{589}^{25}$ = +48 (c = 0.6, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ 1695, 1400, 1090, 800, 735 cm^{-1}. ^1H NMR (DMSO-d_6, 90 ºC): δ 4.77–4.70 (m, 2H, H-3, H-4), 3.84 (m, 1H, H-2), 3.68 (dd, 1H, ^2J = 12.0, ^3J= 7.2, H-5), 3.14 (dd, 1H, ^3J = 3.4, H-5), 2.66-2.62 (m, 3H, H-2', H-2', NH), 1.87-1.71 (m, 3H, H-1', H-1', NH), 1.43 and 1.29 (2s, 6H $C(CH_3)_2$), 1.41 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (DMSO-d_6, 90 ºC): δ 153.4 (s, CO), 111.2 (s, $C(CH_3)_2$), 79.2, 76.8 (2d, C-4, C-3), 78.3 (s, $C(CH_3)_3$), 56.9 (d, C-2), 49.9 (t, C-5), 38.2 (t, C-2'), 32.4 (t, C-1'), 27.6 (q, $C(CH_3)_3$), 25.9 and 24.6 (2q, $C(CH_3)_2$). CI-MS: *m/z* 287 (85, $[M+H]^+$). CI-HRMS: *m/z* 287.1971 (calcd for $C_{21}H_{33}N_2O_4$+H: 287.1980).

***N*,*N*-Bis-[*N*-(*tert*-butoxycarbonyl)-[(2*S*,3*R*,4*S*)-3,4-*O*-isopropylidenoxy-pyrrolidinyl]ethyl]-amine (12).** A solution of **10** (115 mg, 0.18 mmol) in THF/MeOH (1:1, 4 mL) was hydrogenated with Pd/C (10%, 28 mg) at 1 atm for 2.5 h. The mixture was filtered through a pad of Celite and evaporated in vacuo to afford **11** as a white solid (97 mg, 98% yield). $[\alpha]_{589}^{25}$ = +57 (c = 0.77, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ 3335, 1705, 1470, 1405, 1085, 865, 735 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.77–4.70 (m, 4H, H-3, H-4), 3.84 (m, 2H, H-2), 3.68 (dd, 2H, ^2J = 12.0, ^3J = 7.2, H-5), 3.14 (dd, 2H, ^2J = 12.0, ^3J = 3.4, H-5), 2.66-2.62 (m, 4H, H-2'), 1.87-1.71 (m, 5H, H-1', NH), 1.43, 1.29 (2s, 6H, $C(CH_3)_2$), 1.41 (s, 18H, CMe_3). ^{13}C NMR (DMSO-d_6): δ 153.4 (s, CO), 111.2 (s, $C(CH_3)_2$), 79.2 (d, C-3), 78.3 (s, CMe_3), 76.8 (d, C-4), 56.9 (d, C-2), 49.9 (t, C-5), 38.2 (t, C-2'),

32.4 (t, C-1'), 27.6 (q, $C(CH_3)_3$), 25.9, 24.6 (2q, $C(CH_3)_2$). CI-MS: *m/z* 556 (100, M + H^+). CI-HRMS: *m/z* 556.3593 (calcd for $C_{28}H_{49}N_3O_8$+H: 556.3598).

(2*S*, 3*R*, 4*S*)-2-Aminoethylpyrrolidine-3,4-diol (13). Deprotection of **11** (94.3 mg, 0.33 mmol) according to procedure D gave **13** (47 mg, 98%) as viscous oil. $[\alpha]_{589}^{25}$ = +16 (c = 1.1, MeOH). ^{1}H NMR (MeOD): δ 4.20 (m, 1H, H-4), 3.92 (t, 1H, ^3J = 4.3, H-3), 3.00–2.92 (m, 2H, H-2, H-5), 2.84-2.77 (m, 3H, H-5b, H-2', H-2'), 1.84 (dq, 1H, ^2J = 14.0, ^3J = 7.1, H-1'), 1.71 (dq, 1H, ^3J = 7.0, H-1'). ^{13}C NMR (MeOD): δ 73.9, 73.4 (2d, C-4, C-3), 60.6 (d, C-2), 51.5 (d, C-5), 39.8 (d, C-2'), 32.2 (d, C-1'). CI-MS: *m/z* 147 (100, $[M+H]^+$). CI-HRMS: *m/z* 147.1134 (calcd for $C_6H_{14}N_2O_2$+H: 147.1135).

***N,N*-Bis-[[(2*S*, 3*R*, 4*S*)-3,4-dihydroxy-pyrrolidinyl]ethyl]amine (5).** Deprotection of **12** (100 mg, 0.18 mmol) according to procedure D gave triamine **5** (49.5 mg, 100%) as viscous oil. $[\alpha]_{589}^{25}$ = +8 (c = 0.5, MeOH). IR (KBr): $\tilde{\nu}$ 3295, 1690, 1460, 1410, 1095, 805 cm^{-1}. ^{1}H NMR (MeOD): δ 4.20 (m, 2H, H-4), 3.92 (dd, 2H, ^3J = 4.3, ^3J = 4.2, H-3), 3.00–2.92 (m, 4H, H-2, H-5), 2.84-2.77 (m, 6H, H-5, H-2'), 1.84 (dq, 2H, ^2J = 14.0, ^3J = 7.1, H-1'), 1.71 (dq, 2H, ^2J = 14.0, ^3J = 7.0, H-1'). ^{13}C NMR (MeOD): δ 73.9 (d, C-3), 73.4 (d, C-4), 60.6 (d, C-2), 51.5 (t, C-5), 39.8 (t, C-2'), 32.2 (t, C-1'). CI-MS: *m/z* 276 (80, M + H^+). CI-HRMS: *m/z* 276.1922 (calcd for $C_{28}H_{49}N_3O_8$+H: 276.1923).

Ethyl *N*-(*tert*-butoxycarbonyl)-2,3,6-trideoxy-3,6-imino-4,5-*O*-isopropylidene-D-arabino-2-hexanoate (15). To a solution of ethyl 2,3,6-trideoxy-3,6-imino-4,5-*O*-isopropylidene-D-arabino-2-hexanoate (**14**)[24] (2.87 g, 12.5 mmol) in dry pyridine (35 mL) was added a solution of $(Boc)_2O$ (3.06 g, 13.8 mmol) in pyridine (20 mL). The reaction was left at r.t. for 2 h and then evaporated. The crude product was dissolved in AcOEt (100 mL) and washed twice with brine. The organic layer was dried (Na_2SO_4), filtered and concentrated. Column chromatography of the residue (ether/petroleum ether, 1:5 to 1:2), gave **15** (3.78 g, 92%) as an oil. $[\alpha]_{589}^{25}$ = –68 (c = 1.2, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ 2980, 2940, 1720, 1700, 1380, 1090 cm^{-1}. ^{1}H NMR (DMSO-d_6, 90 ºC): δ 4.76 (m, 1H, H-4), 4.73 (m, 1H, H-5), 4.13 (m, 1H, H-3), 4.08 (q, 2H, ^2J = 7.1, CH_2CH_3), 3.60 (dd, 1H, ^3J = 6.5, ^2J = 12.7, H-6), 3.26 (dd, 1H, ^3J = 2.4, H-6'), 2.85 (dd, 1H, ^3J = 4.7, ^2J = 16.0, H-2), 2.50 (dd, 1H, ^2J = 9.6, H-2'), 1.40 (s, 9H, $C(CH_3)_3$), 1.41,1.27 (2s, 6H, $C(CH_3)_2$), 1.19 (t, 3H, CH_2CH_3). ^{13}C NMR (DMSO-d_6 90ºC): δ 170.0 (s, CO), 153.3 (s, CO of Boc), 111.1 (s, $C(CH_3)_2$), 78.9 (d, C-4), 78.7 (s, CMe_3), 76.9 (d, C-5), 59.0 (t, CH_2CH_3), 56.0 (d, C-3), 50.0 (t, C-6), 33.8 (t, C-2), 27.6 (q, $C(CH_3)_3$), 25.4, 24.5 (2q, $C(CH_3)_2$), 13.4 (q, CH_2CH_3). CI-MS: *m/z* 330 (60, $[M+H]^+$). Anal. calcd for $C_{16}H_{27}NO_6$ (329.39): C, 58.34; H, 8.26; N, 4.25. Found: C, 58.49; H, 8.16; N, 4.32.

***N*-(*tert*-Butoxycarbonyl)-2,3,6-trideoxy-3,6-imino-4,5-*O*-isopropyliden-D-arabinohexose (*ent*-8).** To a solution of ethyl *N*-(*tert*-butoxycarbonyl)-*2*,3,6-trideoxy-3,6-imino-4,5-*O*-isopropyliden-D-*arabino*-2-hexanoate (**15**) (0.76 g, 2.32 mmol) in dry dichloromethane (10 mL), was added dropwise a solution of DIBAL-H in dichloromethane (1 M, 4.6 mL, 4.6 mmol) at –78 ºC under Ar. After 2 h at –78ºC MeOH (4 mL) was slowly added, and the reaction mixture was left to warm up to r.t. Then the mixture was cooled to 0 ºC, HCl (1M, 10 mL) was added, and the mixture was extracted with dichloromethane (4x50 mL). The organic layer was washed

with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), filtered and evaporated. Column chromatography of the residue (ether/petroleum ether 1:4 to 1:2) gave *ent*-**8** (0.48 g, 72%) as viscous oil. $[\alpha]_{589}^{25}$ = 80 (c = 0.68, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ 2935, 1725, 1400, 1090 cm^{-1}. 1H NMR (DMSO-d_6, 90 ºC): δ 9.69 (t, 1H, ^3J = 1.7, CHO), 4.79–4.71 (m, 2H, H-4, H-5), 4.21 (c, 1H, ^3J = 6.6, H-3), 3.60 (dd, 1H, ^3J = 6.4, ^2J = 12.3, H-6), 3.29 (dd, 1H, ^3J= 2.3, H-6'), 2.74 (dd, 2H, H-2, H-2'), 1.40 (s, 9H, $C(CH_3)_3$), 1.41, 1.27 (2s, 6H, $C(CH_3)_2$). ^{13}C NMR (DMSO-d_6, 90 ºC): δ 200.0 (s, CHO), 153.4 (s, CO), 111.1 (s, $C(CH_3)_2$), 79.0, 76.8 (2d, C-4, C-5), 78.9 (s, CMe_3), 55.2 (d, C-3), 50.3 (t, C-6), 42.9 (t, C-2), 27.6 (q, $C(CH_3)_3$), 25.6 and 24.5 (2q, $C(CH_3)_2$). FAB-MS: m/z 286 (20, $[M+H]^+$). Anal. calcd for $C_{14}H_{23}NO_5$ (285.34): C, 58.93; H, 8.12; N, 4.91. Found: C, 58.69; H, 8.39; N, 5.16.

***N*-(*tert*-Butoxycarbonyl)-(2*R*,3*S*,4*R*)-2-[2-(benzylamino)ethyl]-3,4-*O*-isopropylidenepyrrolidine-3,4-diol (*ent*-9) and *N*,*N*-bis[*N*-(*tert*-butoxycarbonyl)-[(2*R*,3S,4*R*)-3,4-*O*-isopropylidenoxypyrrolidinyl]ethyl]benzylamine (*ent*-10).** Procedure B was applied to carbaldehyde *ent*-**8** (327 mg, 1.15 mmol) to afford *ent*-**9** (155.3 mg, 36%) as an oil and *ent*-**10** (95.5 mg, 13%) as a white solid.

***ent*-9.** $[\alpha]_{589}^{25}$ = –47 (c = 0.7, CH_2Cl_2). CI-HRMS: *m/z* 377.2439 (calcd for $C_{21}H_{32}N_2O_4$+H: 377.2446).

***ent*-10.** $[\alpha]_{589}^{25}$ = –88 (c = 0.54, CH_2Cl_2). CI-MS: *m/z* 668 (40, M + NH_4^+), 646 (60, M + H^+). CI-HRMS: *m/z* 646.4057 (calcd for $C_{35}H_{55}N_3O_8$+H: 646.4067). NMR and IR spectra were identical to those of its enantiomer **10**.

***N*-(*tert*-Butoxycarbonyl)-(2*R*,3*S*,4*R*)-2-aminoethyl-3,4-*O*-isopropylidene-pyrrolidine-3,4-diol (*ent*-11).** A solution of *ent*-**9** (146.3 mg, 0.39 mmol) in abs. EtOH (8 mL) was hydrogenated with catalyst Pd/C (10%) (62 mg) at 1 atm for 2 h. The mixture was filtered through Celite and the filtrate was evaporated to give *ent*-**11** (111 mg, 100%) as syrup. $[\alpha]_{589}^{25}$ = –55 (c = 0.8, CH_2Cl_2). CIMS: *m*/*z* 287 [50%, $(M+H)^{+}$]. CI-NSHR: *m*/*z* 287.1963 (cald for $C_{14}H_{26}N_2O_4$+H: 287.1971). This product showed NMR and IR spectra identical to those of its enantiomer **11**.

***N*,*N*-Bis[*N*-(*tert*-butoxycarbonyl)-[(2*R*,3S,4*R*)-3,4-*O*-isopropylidenoxy-pyrrolidinyl]ethyl] amine (*ent*-12).** A solution of *ent*-**10** (90 mg, 0.14 mmol) in THF-MeOH (1.5 mL / 1.5 mL) was hydrogenated for 1.5 h under 1 atm with Pd/C (10% on charcoal, 22 mg). The mixture was filtered through a pad of Celite and concentrated in vacuo to afford *ent*-**11** (78 mg, 100%) as white solid. $[\alpha]_{589}^{25}$ = –62 (c = 0.45, CH_2Cl_2). CI-MS: *m/z* 556 (100, M + H^+). CI-HRMS: *m/z* 556.3589 (calcd for $C_{28}H_{49}N_3O_8$+H: 556.3598). NMR and IR spectra were identical to those of its enantiomer **12**.

(2*R*,3*S*,4*R*)-2-Aminoethylpyrrolidine-3,4-diol (*ent*-13). Deprotection of *ent*-**11** (102 mg, 0.36 mmol) according to procedure D gave *ent*-**13** (52 mg, 91%) as thick oil. $[\alpha]_{589}^{25}$ = –12 (c = 0.1, MeOH). CI-HRMS *m*/*z* 147.1136 (calcd for $C_6H_{14}N_2O_2$+H: 147.1134). This product showed NMR spectra identical to those of its enantiomer **13**.

***N*,*N*-bis-[[(2*R*,3*S*,4*R*)-3,4-Dihydroxypyrrolidinyl]ethyl]amine (*ent*-5).** Deprotection of *ent*-12 (77 mg, 0.14 mmol) according to procedure D gave *ent*-5 (37 mg, 97%) as oil. $[\alpha]_{589}^{25}$ = –10 (c =

0.78, MeOH). CI-MS: *m/z* 276 (80, M + H^+). CI-HRMS *m/z* 276.1919 (calcd for $C_{28}H_{49}N_3O_8$+H: 276.1923). NMR and IR spectra were identical to those of its enantiomer **5**.

Acknowledgments

The Swiss National Science Foundation (grants n° 20-63667.00 and 2100-063567.00/1), the European COST (COST D13/0001/99) program, the "Office Fédéral de l'Education et de la Science" (Bern), the "Dirreción General de Investigación Científica y Técnica" of Spain (grant n° BQU-2001-3779) and SOCRATES (EPFL/Sevilla) programs are gratefully acknowledged for financial support. We also thank Dr. Vladimir Kren of the Academy of Sciences of the Czech Republic for suggesting to us this investigation.

References

1. (a) Morenem, K. W.; Trimble, R. B.; Herscovics, A. *Glycobiology* **1994**, *4*, 113. (b) Varki, A. *Glycobiology* **1993**, *3*, 97. (c) Crocker, P. R.; Feizi, T. *Curr. Opin. Struct. Biol*. **1996**, *6*, 679. (d) Dwek, R. A. *Chem. Rev*. **1996**, *96*, 683.
2. (a) Kirby, A. J. *Acc. Chem. Res*. **1984**, *17*, 305. (b) Gorenstein, D. G. *Chem. Rev*. **1987**, *87*, 1047. (c) Sinnott, M. L. *Chem. Rev*. **1990**, *90*, 1171. (d) Jeong, J. H.; Murray, B. W.; Takayama, S.; Wong, C. H. *J. Am. Chem. Soc*. **1996**, *118*, 4227. (e) Ganem, B. *Acc. Chem. Res*. **1996**, *29*, 340. (f) Bols, M. *Acc. Chem. Res*. **1998**, *31*, 1. (g) Heightman, T. D.; Vasella, A. T. *Angew. Chem., Int. Ed.* **1999**, *38*, 750. (h) Ichikawa, Y.; Lin, Y. C.; Dumas, D. P.; Shen, G. J.; Garcia-Jonceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C. H. *J. Am. Chem. Soc*. **1992**, *114*, 9283. (i) Qiao, L. Murray, B. W.; Shimazakin, M.; Schultz, J.; Wong, C. H. *J. Am. Chem. Soc*. **1996**, *118*, 7653. (j) Jefferies, I.; Bowen, B. R. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1171. (k) Palcic, M. M.; Heerze, L. D.; Srivastava, O. P.; Hindsgaul, O. J. *Biol. Chem*. **1989**, *264*, 17174.
3. (a) Fernandes, B.; Sagman, U.; Auger, M.; Demetrio, M.; Dennis, J. W. *Cancer Res*. **1991**, *51*, 718. (b) Robinson, K. M.; Begovic, M. E.; Rhinerhardt, M. E.; Heineke, E. W.; Ducep, J. B.; Kastner, P. R.; Marshall, F. N.; Danzin, C. *Diabetes* **1991**, *40*, 825. (c) Platt, F. M.; Reinkensmeier, G.; Dwek, R. A.; Butters, T. D. *J. Biol. Chem*. **1997**, *272*, 19365. (d) Lapierre, F.; Holme, K.; Lam, L.; Tressler, R. J.; Storm, N.; Wee, J.; Stack, R. J.; Castellot, J.; Tyrrell, D. J. *Glycobiology* **1996**, *6*, 355. (e) Mehta, A.; Zizmann, N.; Rudd, P. M.; Block, T. M.; Dwek, R. A. *FEBS Lett*. **1998**, *430*, 17. (f) Kolter, T. *Angew. Chem., Int. Ed.* **1997**, *36*, 1955. (g) Fan, Q. J.; Ishii, S.; Asano, N.; Suzuki, Y. *Nat. Med*. **1999**, *5*, 112. (h) Cox, T.; Lachman, R.; Hollack, C.; Aerts, J.; van Weely, S.; Hrebicek, M.; Platt, F.; Butters, T.; Dwek, R.; Moyses, C.; Gow, I.; Elstein, D.; Zimran, A. *Lancet* **2000**, *355*, 1481.

4. (a) Stütz, A. E. *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*; Wiley-VCH: Weinheim, 1999. (b) Asano, N.; Nash, R. J.; Molineux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1645. (c) Asano, N.; Nishida, M.; Kato, A.; Kizu, H.; Matsui, K.; Shimida, Y.; Itoh, T.; Baba, M.; Watson, A. A.; Nash, R. J.; de Q Lilley, X.; Watkin, D. J.; Fleet, G. W. J. *J. Med. Chem*. **1998**, *41*, 2565. (d) Martin, O. R.; Saavedra, O. M.; Xie, F.; Liu, L.; Picasso, S.; Vogel, P.; Kizu, H.; Asano, N. *Bioorg. Med. Chem. Lett*. **2001**, *9*, 1269.
5. (a) Jespersen, T. M.; Dong, W.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, M. *Angew. Chem., Int. Ed.* **1994**, *33*, 1778. (b) Ichikawa, Y.; Igarashi, Y. *Tetrahedron Lett*. **1995**, *36*, 4585. (c) Igarashi, Y.; Ichikawa, M.; Ichikawa, Y. *Bioorg. Med. Chem. Lett*. **1996**, *6*, 553. (d) Ichikawa, Y.; Igarashi, Y.; Ichikawa, M.; Suhura, Y. *J. Am. Chem. Soc*. **1998**, *120*, 5854. (e) Williams, S. J.; Hos, R.; Whiters, S. G. *J. Am. Chem. Soc*. **2000**, *122*, 2223. (f) Nishimura, Y.; Shitara, E.; Adachi, H.; Toyoshima, M.; Nakajima, M.; Okami, Y.; Takeuchi, T. *J. Org. Chem*. **2000**, *65*, 2. (j) Liu, H.; Liang, X.; Sϕhoel, H.; Bülow, A.; Bols, M. *J. Am. Chem. Soc*. **2001**, *123*, 5116, (h) Jensen, H.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 905.
6. (a) Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash. R. J. *Tetrahedron Lett*. **1985**, *26*, 3127. (b) Asano, N.; Oseki, K.; Kizu, H.; Matsui, K. *J. Med. Chem*. **1994**, *37*, 3701. (c) Asano, N.; Nishida, M.; Miyauchi, M.; Ikeda, K.; Yamamoto, M.; Kizu, H.; Kameda, Y.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J. *Phytochemistry* **2000**, *53*, 379.
7. (a) Saotome, C.; Kanie, Y.; Kanie, O.; Wong, C. H. *Bioorg. Med. Chem*. **2000**, *8*, 2249 references cited therein. (b) Saotome, C.; Wong, C. H.; Kanie, O. *Chem. Biol*. **2001**, *8*, 1061.
8. (a) Popowycz, F.; Gerber-Lemaire, S.; Demange, R.; Rodriguez-García, E.; Carmona-Asenjo, A. T.; Robina, I.; Vogel, P. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 2489. (b) Popowycz, F.; Gerber-Lemaire, S.; Rodriguez-García, E.; Schütz, C.; Vogel, P. *Helv. Chim. Acta*, in press.
9. (a) White, S. L.; Nagai, T.; Akiyama, S. K.; Reeves, E. J.; Grzegorzewski, K.; Olden, K. *Cancer Commun*. **1991**, *3*, 83. (b) Olden, K.; Breton, P.; Grzegorzewski, K.; Yasuda, Y.; Gause, B. L.; Oredipe, O. A.; Newton, S. A.; White, S. L. *Pharmacol.Ther*. **1991**, *50*, 285. (c) Asano, N. *J. Enzyme Inhibition* **2000** *15*, 215. (d) Carver, J.; Dennis, J. W.; Shah, P. US Patent 5773239A, 30 Jun. 1998; *Chem. Abstr*. **1998**, *129*, 95683.
10. Colegate, S. M.; Dorling, P. R.; Huxtable, C. R. *Austr. J. Chem.* **1979**, *32*, 2257.
11. Elbein, A. D.; Molyneux, R. D. *Iminosugars as Glycosidase Inhibitors; Nojirimycin and Beyond*, A. E. Stütz, Ed; Wiley-VCH: Weinheim, 1999, Chapt.11, pp 216–251.
12. (a) Goss, P. E.; Baptiste, J.; Fernades, B.; Baker, M.; Dennis, J. W. *Cancer Res*. **1994**, *54*, 1450. (b) Goss, P. E.; Reid, C. L.; Bailey, D.; Dennis, J. W. *Clin. Cancer Res*. **1997**, *3*, 1077. (c) P. D. Rye, N. V. Bovin, E. V. Vlasova, R. A. Walker, *Glycobiology* **1995**, *5*, 385.
13. (a) Pearson, W. H.; Guo, L. *Tetrahedron Lett*. **2001**, *42*, 8267. (b) Pearson, W. H.; Perlmutter, D. H. *Tetrahedron Lett*. **2001**, *42*, 8273.

14. (a) Aoyagi, T.; Yamamoto, T.; Kojiri, K.; Morishima, H.; Nagai, M.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot*. **1989**, *42*, 883. (b) Morishima, H.; Kojiri, K.; Yamamoto, T.; Aoyagi, T.; Nakamura, H.; Iitaka, Y. *J. Antibiot*. **1989**, *42*, 100. (c) Tropea, J. E.; Kaushal, G. P.; Pastuszak, I.; Mitchell, M.; Aoyagi, T.; Molyneux, R. J.; Elbein, A. D. *Biochem*. **1990**, *29*, 10062. (d) Berecibar, A.; Grandjean, C.; Siriwardena, A. *Chem. Rev*. **1999**, *99*, 779.
15. Ogawa, S.; Morikawa, T. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 1047.
16. (a) Ogawa, S.; Morikawa, T. *Eur. J. Org. Chem*. **2000**, 1759. (b) Ogawa, S.; Morikawa, T. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 1499. (c) Wong, C. H.; Provencher, L.; Porco, J. A.; Jung, S. H.; Wang, Y. F.; Chen, L. R.; Wang, R.; Steensma, D. H. *J. Org. Chem*. **1995**, *60*, 1492.
17. Winkler, D. A. *J. Med. Chem*. **1996**, *39*, 4332.
18. See e.g. Davis, B. G.; Brandstetter, T. W.; Hackett, L.; Winchester, B. G.; Nash, R. J.; Watson, A. A.; Griffiths, R. C.; Smith, C.; Fleet, G. W .J. *Tetrahedron* **1999**, *55*, 4489.
19. (a) Andersen, S. M.; Ekhart, C.; Lundt, I.; Stütz, A. E. *Carbohydr. Res*. **2000**, *326*, 22. (b) Kim, Y. J.; Takatsuki, A.; Kogoshi, N.; Kitahara, T. *Tetrahedron* **1999**, *55*, 8353. (c) Joubert, M.; Defoin, A.; Tarnus, C.; Streith, J. *Synlett* **2000**, 1366.
20. See e.g. (a) Lipper, R. A. *Modern Drug Discovery* **1999**, 55. (b) Lipinsky, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev*. **1997**, *23*, 3.
21. Howard, S.; He, S.; Withers, S. G. *J. Biol. Chem*. **1998**, *273*, 2067.
22. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem*. **1996**, *61*, 3849.
23. (a) Thompson, D. K.; Hubert, C. N.; Wightman, R. H. *Tetrahedron* **1993**, *49*, 3827. (b) Cardona, F.; Robina, I.; Vogel, P. *J. Carbohydr. Chem*. **2000**, *19*, 555.
24. Robina, I.; Gearing, R. P.; Buchanan, J. G.; Wightman, R. H. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2622;
25. (a) Brandi, A.; Cicchi, S.; Cordero, F. M.; Frigneoli, B.; Goti, A.; Picasso, S.; Vogel, P. *J. Org. Chem*. **1995**, *60*, 6806. (b) Picasso, S.; Chen. Y.; Vogel, P. *Carbohydr. Lett*. **1994**, *1*, 1.
26. Saul, R.; Chambers, J. P.; Molyneux, R. J.; Elbein, A. D. *Arch. Biochem. Biophys*. **1983**, *221*, 593.