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Chiral 5-Methyl-trihydroxypyrrolidines—Preparation from 1,2-Oxazines and Glycosidase Inhibitory Properties

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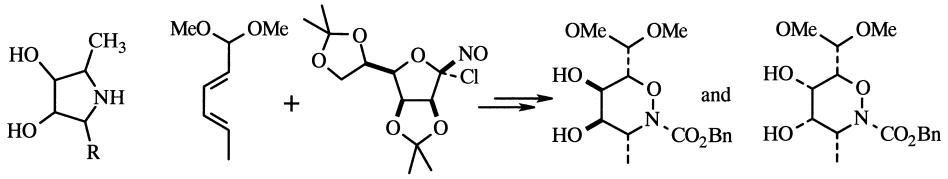
Abstract—Chemical transformations of chiral 1,2-oxazines **4**, **5** gave the 2,5,6-trideoxy-2,5-imino D-alditols **12b**, **13b** in the D-altritol and D-talitol series, respectively. Basic aldehyde epimerisation led to the D-allitol isomer **15b**. Compound **12b** is a potent α-L-fucosidase and α-D-galactosidase inhibitor. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Polyhydroxypiperidine and polyhydroxypyrrolidine amino-sugars are monosaccharide analogues which mimic these sugars in their pyranose or furanose forms so that they are generally potent inhibitors of the corresponding glycosidases.[1] Among these amino-sugars, type **1a**–**c** 5-methyl-polyhydroxypyrrolidines, which are also called ω-deoxy-aza-sugars, have been recently synthesised and studied as glycosidase inhibitors. L-Arabinose derivatives of type **1b**,**c** are potent α-L-rhamnosidase inhibitors;[2] the (±)-ribose analogues were synthesised, but not tested.[3] Some 5-methyl-pyrrolidine-triols **1a** were obtained by chemio-enzymatic synthesis by Wong et al.[4–6] and proved to be α-L-fucosidase inhibitors,[4,5] and also rather weak α-[1,3]-fucosyl-transferase inhibitors.[7] The all-*trans* isomer is a natural product isolated from the seeds of a tree of the *Leguminoseae* family (*Sophoreae* tribu) and is a weak β-D-mannosidase inhibitor.[8]

We have already described the de novo chiral synthesis of 6-deoxy-nojirimycin and of some of its isomers from sorbaldehyde dimethylacetal **2** via asymmetric hetero-Diels–Alder cycloaddition with the α-chloronitroso-D-mannose derivative **3** and with excellent asymmetric induction (ee>99%); chemical modifications of the primary Diels–Alder adduct gave the intermediate oxazane-diols **4** and **5** (Scheme 1).[9] Starting from these two diols, we describe herein the synthesis of three 5-methyl-trihydroxypyrrolidines of type **1a**, as well as their glycosidase inhibitory properties. The principle of this synthesis is a ring reduction of 1,2-oxazane in pyrrolidine and a base-catalysed epimerisation of the aldehyde group. A preliminary communication relating to this work has already been published.[10]



1a R = CH_2OH
b R = OH
c R = H

2 **3** **4** **5**

Scheme 1.

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Synthesis of Pyrrolidine Ring

Modifications of the oxazane ring required protection of the diol moieties in **4**, **5** as acetonides **6**, **7**, respectively, which was achieved according to Ref. 11 in dimethoxy-propane with Amberlyst-15 (H^+) as catalyst. Hydrogenolysis of the N–O bond over Pd/C as described earlier for the diols **4**, **5**[9] led to the linear protected 5-amino-5,6-dideoxy-D-hexoses **8a**, **9a**, in the D-allose and D-fucose series, respectively, which were *N*-reprotected at once with benzyl chloroformiate in aqueous 1N NaOH to give **8b**, **9b** in 67–80% overall yields from the diols **4**, **5** after chromatographic purification. Esterification of the remaining alcohol function with methane sulfonyl chloride and NEt_3 gave quantitatively the mesylates **8c**, **9c** which were easily cyclised to the protected 2,5-*trans* pyrrolidines **10a**, **11a**, respectively, with aq. 2.5 N NaOH in EtOH at 80°C in 70–75% yield after chromatography (Scheme 2).

Selective acidic deprotection of the acetal moiety was carried out in dry acetone with Amberlyst-15 (H^+), according to Coppola's method[12] at 40°C and led easily to aldehyde **10b** from **10a**, but its isomer **11b** from **11a** was more difficult to obtain.

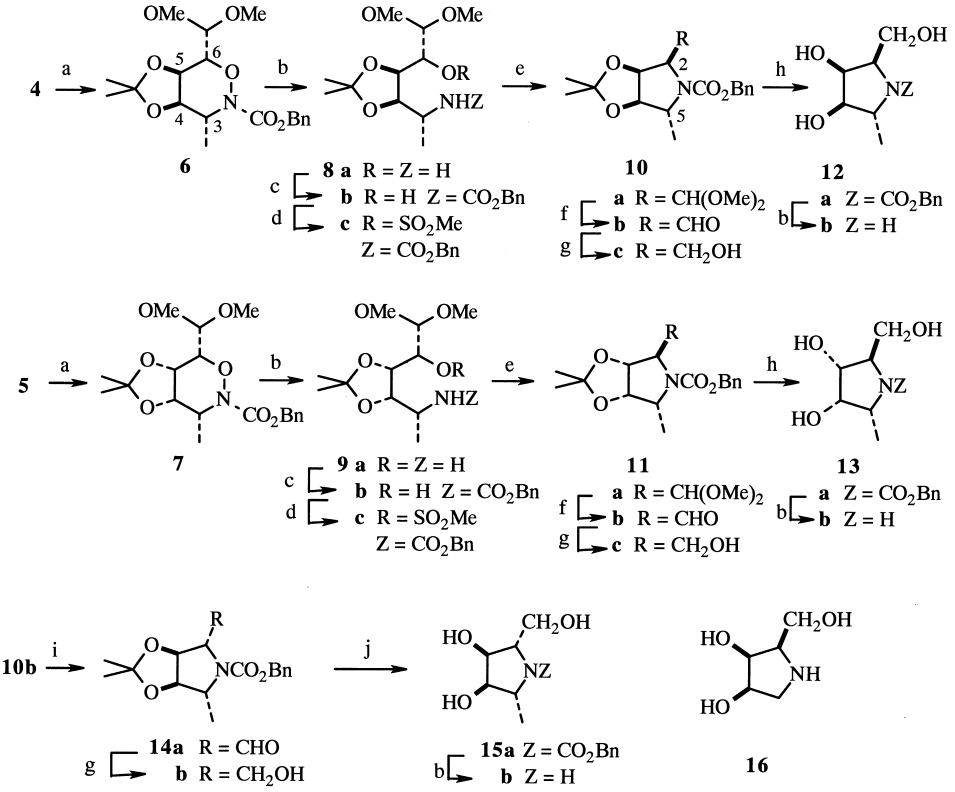
Crude aldehydes **10b**, **11b** were reduced by $NaBH_4$ in MeOH into the corresponding alcohols **10c**, **11c** in 67 and 78% yield from acetals **10a**, **11a**, respectively. Deprotection of these alcohols by Amberlyst-15 (H^+) in MeOH into **12a**, **13a**, then by hydrogenolysis over Pd/C, led to the final trihydroxypyrrolidines, i.e. to the crystalline **12b** (2,5,6-trideoxy-2,5-imino-D-altritol) and the oily **13b** (2,5,6-trideoxy-2,5-imino-D-talitol) in 80–85% yields from the corresponding alcohols **10c**, **11c**. In these syntheses, intermediary compounds are generally not purified and only characterised by ^1H NMR.

The enantiomer of the talitol derivative **13b** was assumed by Wong to have been obtained by chemio-enzymatic synthesis,[4] nevertheless its physical properties are quite different from those of **13b** as obtained by us and correspond presumably to another isomer[13] (see Structural Analysis).

Aldehyde Epimerisation

Isomeric aldehydes **10b**, **11b** appear to be stable compounds under normal conditions, nevertheless they cannot be chromatographed without some degradations. They present in their ^1H NMR spectra two sets of distinct resonances for two rotamers at 300 K; the origin of this unusual highly hindered internal rotation of the carbamate N–CO_2Bn moiety[14] is probably a steric bulkiness between this group and the two neighbouring substituents, the CHO(1) and the Me(6) groups. This bulkiness is particulary severe for **10b** because of the proximity of the acetonide ring, so that, even at 330 K, the ^1H NMR spectrum was not resolved.

The supposed instability of some similar pyrrolidine-2-carbaldehydes, which is reported in the literature,[15] was usually explained as a spontaneous epimerisation and is probably attributed to such a rotamery.



Scheme 2. (a) Dimethoxypropane/Amberlyst 15 (H^+). (b) H_2/Pd–C. (c) $ClCO_2Bn$/NaOH. (d)$ClSO_2Me/NEt_3$. (e) 1 N NaOH/MeOH. (f) Amberlyst 15 (H^+)/acetone. (g) $NaBH_4$/MeOH. (h) Amberlyst 15 (H^+)/EtOH. (i)CO_3Na_2/MeOH. (j) H^+/MeOH.

Table 1. Glycosidases inhibition values as IC_{50} or (K_i) for pyrrolidines **12b**, **13b**, **15b** [α-D-glucosidase from yeast, β-D-glucosidases from almond, α-D-mannosidase from Jack bean, α-L-fucosidase from bovine kidney, α-D-galactosidase from green coffee beans; n.i.: no or weak (IC_{50}>1 mM) inhibition]

	α-D-Glucosidase	β-D-Glucosidase	α-D-Mannosidase	α-L-Fucosidase	α-D-Galactosidase
12b	n.i.	1.2 mM	(53 μM)	(9 μM)	(5 μM)
13b	n.i.	(100 μM)	n.i.[a]	1 mM	2 mM
15b	n.i.	n.i.	n.i.	(120 μM)	(70 μM)
16[18]	n.i.	350 μM	14 μM	n.i.	0.2 μM

[a] Activation (+78% at 1 mM).

Epimerisation of the carbaldehyde group of **10b** was possible indeed, but it required basic conditions; in this case Na_2CO_3 in MeOH was a strong enough base to trigger complete epimerisation into the thermodynamically more stable 2,5-*cis* isomer **14a** after 1 h at rt.[16] Attemps to epimerise its isomer **11b** failed using the same experimental conditions; more drastic conditions led to degradation. In this latter case, the formation of the all-*cis* isomer is obviously not favoured.

Immediate reduction of **14a** by addition of $NaBH_4$ to the isomerisation solution gave directly the corresponding alcohol **14b** in 54% yield from **10b**. Deprotection in **15a** with Amberlyst 15 (H^+) followed by hydrogenolysis over Pd/C, led to trihydroxypyrrolidine (2,5,6-trideoxy-2,5-imino-D-allitol) **15b** in 87% yield.

Structural Analysis of the Pyrrolidines

^{1}H NMR data of the synthesised pyrrolidines **10a**–**c**, **11a**–**c**, **14a**,**b**, **12a**,**b**, **13a**,**b**, **15a**,**b** were carefully determined because of strong rotation hindrances for the *N*-acyl derivatives and are presented in Table 2 (see Experimental). The structures of these products could be ascertained unambiguously by the chemical transformations which were carried out starting from the oxazane-diols **4**, **5**. Nevertheless, in the *N*-acylated pyrrolidines, steric interaction between the *N*-acyl group and the substituents in 2- and 5-positions forces the pyrrolidine ring to adopt a conformation in which the dihedral angles of both *trans* vicinal H-C(2), H-C(3) and/or H-C(4), H-C(5) protons are close to 90°, so that the corresponding couplings are small or null (0–2.5 Hz). This is clearly observed for the *trans* vicinal couplings *J*(2,3) in **11a**–**c**, **13a**; for *J*(2,3) and *J*(4,5) in **14a**,**b** and for *J*(4,5) in **10a**–**c**, **12a**.

Similar results have been observed for methyl furanosides in which the anomeric effect favours a quasi-axial position for the anomeric substituent so that *trans J*(1,2) values are small (0–1.5 Hz).[17]

As a consequence, the suppression of this steric bulkiness by *N*-deprotection led to a conformational change for the final pyrrolidines **12b**, **13b**, **15b** and the above *trans*-couplings are larger (5–9 Hz, see Table 2).

If we consider, in particular, the case of **13b**, its stereostructure results from one of its *N*-acylated derivatives **11c**, **13a** and the relative position of the CH_2OH(1) group on the same side as H-C(3), H-C(4) and H-C(5) was ascertained with the derivative **13a** by nOe experiments: irradiation of CH_2(1) signals gave an enhancement of 8 and 6% of the ones of HC(3) and H-C(4), respectively; irradiation of H-C(5) signal gave an enhancement of 6% of the one of H-C(4).

Inhibitions of Glycosidases

The inhibition properties of pyrrolidine-triols **12b**, **13b**, **15b** on various glycosidases are reported in Table 1. Inhibition constants (K_i) determined for the most potent derivatives indicate a competitive inhibition. It is noteworthy that α-L-fucosidase and α-D-galactosidase show similar inhibition profiles with the three evaluated compounds. It underlines the importance of the *cis*-2,3-diol group in these pyrrolidines similar to the structure of the parent sugars, α-L-fucose and α-D-galactose. In addition, the presence of the CH_2OH functionality improves the affinity when the same orientation as the *cis*-diol group is considered, D-altritol **12b** being ca. 10 times more potent on both enzymes than D-allitol **15b**. The activity of the other studied glucosidases is not significantly inhibited by these compounds, except **13b** on β-D-glucosidase (K_i=100 μM) and **12b** on α-D-mannosidase (K_i=53 μM).

Comparison of our results for **12b** with literature data related to compound **16**[18] (Table 1) shows that the presence of the methyl-(6) substituent is essential for the recognition by α-L-fucosidase. An orientation of this methyl group identical to L-fucose should increase the affinity to the enzyme.

Conclusion

We have presented a simple synthesis as well as the glycosidase inhibitor properties of three pyrrolidine-triols, starting from the known oxazane-diols **4** and **5**, with overall yields of 31% for **12b** and 27% for **15b** from diol **4**, and of 33% for **13b** from diol **5**.

Experimental

General

Flash chromatography (FC): silica gel (Merck 60, 230–400 mesh). TLC: Al-roll silica gel (Merck 60, F_{254}). Mp: Kofler hot bench or Büchi-SMP20 apparatus, corrected. IR spectra (ν in cm^{-1}): Perkin–Elmer 157 G, $[\alpha]_D$: Schmidt-Haensch Polartronic Universal polarimeter. HPLC measurements:

liquid chromatograph Hewlett-Packard 190. ^{1}H and ^{13}C NMR (62.9 MHz) spectra: Bruker AC-F250; tetramethylsilane (TMS) in $CDCl_3$, CD_3OD ($\delta(CD_3OD)$=3.30), (D_6)-DMSO (δ((D_6)-DMSO)=2.50) or natrium (D_4)-trimethylsilyl-propionate (D_4-TSP) in D_2O (^{1}H-NMR) and $CDCl_3$, CD_3OD, or (in D_2O) CH_3OH or dioxane [$\delta(CDCl_3)$=77.0, $\delta(CD_3OD)$=49.0, in D_2O $\delta(CH_3OH)$=50.0, δ(dioxane)= 67.4 with respect to TMS] (^{13}C-NMR) as internal references; δ in ppm and J in Hz. ^{13}C attributions were ascertained by ^{1}H–^{13}C correlation. NOe experiments were carried out in argon saturated solutions. High resolution (HR)-MS were measured on a MAT-311 spectrometer at the University of Rennes. Microanalyses were carried out by the Service Central de Microanalyses du CNRS, F-69390 Vernaison, or Service de Microanalyse de l'ICSN-CNRS, F-91168 Gif sur Yvette.

Reagents and solvents: 5% Pd/C catalyst, benzyl chloroformate, 2,2-dimethoxypropane, $MeSO_2Cl$ were purchased from Fluka, Amberlyst-15 from Rohm & Haas, triethylamine was distilled. Usual solvents were freshly distilled, dry EtOH and MeOH distilled over Mg/MgI_2, CH_2Cl_2 was kept over Na_2CO_3.

1,2-Oxazane derivatives

Benzyl (3*R*)-*t*-4,*t*-5-isopropylidenedioxy-*c*-6-dimethoxymethyl-*r*-3-methyl-1,2-oxazane-2-carboxylate (6). To a solution of diol **4**[9] (2.21 g, 6.48 mmol) in 2,2-dimethoxypropane (8 ml) was added Amberlyst-15 (H^+) (0.13 g) and the solution was stirred at 40°C for 2 h. The catalyst was discarded by filtration, washed with acetone and the solvents were evaporated to give **6** as a brownish oil (2.6 g, quant.). For analytical purposes, it was purified by FC (AcOEt/cyclohexane, 1:1).

6: yellowish oil, $[\alpha]_D^{20}=-99$ (c=1.0, $CHCl_3$). IR ($CHCl_3$): 2985, 2925, 1705, 1450, 1405, 1385, 1355, 1330, 1305, 1290, 1230, 1130, 1070, 900, 865, 695. ^{1}H NMR ($CDCl_3$, 298 K): 7.36 (m, 5H arom.); 5.15, 5.27 (2d, J=12.3 Hz, CH_2Ph); 4.64 (dq, H-C(3)); 4.43 (d, H-C(1′)); 4.35 (dd, H-C(5)); 4.05 (dd, H-C(4)); 3.97 (dd, H-C(6)); 3.42, 3.44 (2s, 2 OMe); 1.36 (d, Me-C(3)); 1.33, 1.36 (2s, 2 Me). J(3,4)=1.2 Hz, J(3,Me-3)=7.2 Hz, J(4,5)=5.2 Hz, J(5,6)= 8.8 Hz, J(6,1′)=3.4 Hz. ^{13}C NMR ($CDCl_3$, 300 K): 155.5 (NCO_2); 136.0, 128.4, 128.2, 128.2 (Ph); 109.7 (CMe_2)); 102.3 (C(1′)); 79.3 (C(6)); 74.7 (C(4)); 68.9 (C(5)); 67.7 (CH_2Ph); 54.8, 54.5 (2 OMe); 51.5 (C(3)); 28.0, 26.3 (2 Me); 16.0 (Me-C(3)). R_f=0.36 (AcOEt/cyclohexane 1:1). Anal. calcd for $C_{19}H_{27}NO_7$ (381.42): C 59.83, H 7.14, N 3.67; found: C 59.5, H 7.1, N 3.7.

Benzyl (3*R*)-*c*-4,*c*-5-isopropylidenedioxy-*c*-6-dimethoxymethyl-*r*-3-methyl-1,2-oxazane-2-carboxylate (7). Same procedure as for **6** from diol **5**[9] (2.65 g, 7.75 mmol) in 2,2-dimethoxypropane (10 ml) with Amberlyst-15 (H^+) (0.16 g) at 45°C for 5 h to give pure **7** after washing with cold i-Pr_2O (1.975 g, 67%).

7: beige crystals. Mp=107–108°C (i-Pr_2O/cyclohexane). $[\alpha]_D^{21}=-40$ (c=1.0, $CHCl_3$). IR (KBr): 2980, 2930, 1695, 1410, 1375, 1350, 1300, 1245, 1210, 1135, 1100, 1070, 1045, 1025, 1010, 965, 875, 750, 700. ^{1}H NMR ($CDCl_3$, 300 K): 7.36 (m, 5 H arom.); 5.24, 5.13 (2d, J=12.2 Hz, CH_2Ph); 4.69 (d, J=8.0 Hz, H-C(1′)); 4.38 (m, H-C(3), H-C(4)); 4.24 (m, H-C(5)); 4.11 (dd, J=3.0 Hz, 8.0 Hz, H-C(6)); 3.44, 3.34 (2s, 2 OMe); 1.53,1.35 (2s, 2 Me); 1.30 (m, Me HzC(3)). ^{1}H NMR (C_6D_6, 300 K): 7.27 (m, 2 H arom.); 7.08 (m, 3 H arom.); 5.13, 5.05 (2d, J=12.4 Hz, CH_2Ph); 4.87 (d, H-C(1′)); 4.42 (quint., H-C(3)); 3.93 (dd, H-C(4)); 4.01 (dd, H-C(5)); 4.11 (dd, H-C(6)); 3.22, 3.40 (2s, 2 OMe); 1.39 (d, Me-C(3)); 1.39, 1.15 (2s, 2 Me). J(1′,6)=8.0 Hz, J(3,4)=7.3 Hz, J(3,Me-3)=6.9 Hz, J(4,5)= 6.1 Hz, J(5,6)=2.8 Hz. ^{13}C NMR ($CDCl_3$, 300 K): 154.8 (NCO_2); 135.7, 128.5, 128.3, 128.2 (Ph); 109.6 (CMe_2)); 99.9 (C(1′)); 78.3 (C(6)); 71.2 (C(5)); 70.7 (CH_2Ph); 67.8 (C(4)); 56.0 (OMe); 51.5 (C(3)); 50.9 (OMe); 25.6, 25.5 (2 Me); 14.6 (Me HzC(3)). R_f=0.48 (AcOEt/cyclohexane 1:1). Anal. calcd for $C_{19}H_{27}NO_7$ (381.42): C 59.83, H 7.14, N 3.67; found: C 59.9, H 7.1, N 3.7.

Pyrrolidine derivatives

5-Amino-3,4-*O*-isopropylidene-5,6-dideoxy-D-allose dimethylacetal (8a) and 5-(benzyloxy-carbonylamino)-3,4-*O*-isopropylidene-5,6-dideoxy-D-allose dimethylacetal (8b). A solution of crude **6** (2.07 g corresponding to 5.06 mmol) in EtOH (8 ml) was hydrogenolysed over 5% Pd/C (0.1 g) at 50°C for 1 day (after 8 h, another 0.1 g Pd/C was added). The catalyst was discarded by centrifugation, washed with EtOH and the solvents were evaporated to give crude **8a** (1.4 g, quant.).

To a stirred solution of crude **8a** (1.4 g, 5.06 mmol) in distilled water (10 ml) was added aq. 2.5 N NaOH (6 ml, 15 mmol) and $ClCO_2Bn$ (1.1 ml, 7.6 mmol, 1.5 equiv.). After 3 h at rt, the solution was extracted with CH_2Cl_2 (5×5 ml), the organic phases were dried ($MgSO_4$) and evaporated. The resulting yellow oil (2.3 g) was purified by FC (AcOEt/cyclohexane, 1:1 on 100 g silica gel) and gave pure **8b** (1.55 g, 80% yield from **4**).

8a: yellowish oil characterised by ^{1}H NMR ($CDCl_3$, 300 K): 4.51 (d, H-C(1)); 4.25 (dd, H-C(3)); 3.86 (dd, H-C(2)); 3.80 (dd, H-C(4)); 3.55, 3.50 (2 s, 2 OMe); 3.15 (dq, H-C(5)); 2.62 (broad s, OH, NH_2); 1.40, 1.34 (2 s, 2 Me); 1.26 (d, Me(6)). J(1,2)=1.8 Hz, J(2,3)=9.6 Hz, J(3,4)=5.4 Hz, J(4,5)=9.4 Hz, J(5,Me(6))=6.4 Hz.

8b: yellow oil. $[\alpha]_D^{20}=-4$ (c=1.0, $CHCl_3$). IR ($CHCl_3$): 3560, 3435, 2990, 2935, 2840, 1710, 1510, 1450, 1380, 1370, 1230, 1070, 910, 870, 695. ^{1}H NMR ($CDCl_3$, 298 K): 7.32 (m, 5 H arom.); 5.08, 5.10 (2d, J=12.4 Hz, CH_2Ph); 5.39 (d, NH-C(5)); 4.47 (d, H-C(1)); 3.80 (ddd, H-C(2)); 4.08 (dd, H-C(3)); 4.16 (dd, H-C(4)); 4.05 (ddq, H-C(5)); 3.54, 3.44 (2s, 2 OMe); 2.54 (d, OH-C(2)); 1.42, 1.34 (2s, 2 Me); 1.30 (d, Me(6)). J(1,2)=1.8 Hz, J(2,OH-2)=4.8 Hz, J(2,3)=9.6 Hz, J(3,4)=5.4 Hz, J(4,5)= 6.0 Hz, J(5,Me(6))=6.6 Hz, J(5,NH-5)=8.0 Hz. R_f=0.28 (AcOEt/cyclohexane 1:1). Anal. calcd for $C_{19}H_{29}NO_7$ (383.44): C 59.51, H 7.62, N 3.65; found: C 59.3, H 7.7, N 3.6.

5-(Benzyloxycarbonylamino)-3,4-*O*-isopropylidene-2-*O*-methanesulfonyl-5,6-dideoxy-D-allose dimethylacetal (8c) and 2,5-(benzyloxycarbonylimino)-3,4-*O*-isopropyl-

Table 2. ^{1}H NMR data ($CDCl_3$) of pyrrolidines **10a**–**c**, **12a**,**b**, **11a**–**c**, **13a**,**b**, **14a**,**b**, **15a**,**b** (250 MHz, 300 K, δ in ppm, J in Hz)

	Ha-C(1)	Hb-C(1)	H-C(2)	H-C(3)	H-C(4)	H-C(5)	Me(6)	CH_2[a]		Me[b]	
10a[c]		5.09	3.82	4.75	4.26	4.14	1.23	5.11	5.12	1.30	1.46
10b[d] maj		9.32	4.14	5.03	4.47	4.36	1.23	5.10	5.12	1.28	1.43
min		9.43	4.15	4.99	4.47	4.28	1.14	5.12	5.23	1.28	1.46
10c[e]	3.92	3.96	3.78	4.75	4.30	4.19	1.12	5.13	5.18	1.28	1.42
12a	4.26	3.99	3.75	4.51	3.80	3.93	1.16	5.07	5.19		
12b[f]	3.77	3.60	3.39	4.20	3.71	3.06	1.20				
11a[g,h]		4.43	4.11	4.79	4.57	3.91	1.38	5.08	5.25	1.33	1.47
11b[i]		9.59	4.54	4.67	4.59	3.95	1.43	5.11		1.33	1.50
11c[h,j]	3.70	3.92	3.97	4.54	4.62	4.04	1.34	5.11	5.16	1.33	1.48
13a[h,k]	3.73	3.73	3.86	4.08	4.23	4.01	1.32	5.12	5.16		
13a[l]	3.53	3.44	3.64	4.02	4.16	3.81	1.20	5.05	5.10		
13b[m]	3.65	3.58	3.10	3.95	3.82	3.15	1.14				
13b[f]	3.70	3.63	3.18	4.04	3.96	3.24	1.14				
14a[h]		9.66	4.57	4.92	4.34	4.33	1.15	5.19		1.32	1.45
14b[i,n]	3.75	3.70	4.13	4.61	4.34	4.17	1.25	5.15		1.30	1.44
15a[i,o]	3.87	3.78	3.81	4.12	3.86	3.93	1.22	5.15			
15b[f]	3.71	3.67	3.09	3.92	3.60	3.05	1.22				

	J(1a,1b)	J(1a,2)	J(1b,2)	J(2,3)	J(3,4)	J(4,5)	J(5,6)
10a		6.4		5.4	6.2	1.9	6.8
10b[d] maj.		3.3		7.2	5.8	0	6.9
min.		3.4		6.6	5.8	0	6.9
10c	12.7	4.8	4.8	5.5	6.2	0	7.0
12a	11.4	3.4	nd	8.7	4.8	0	6.7
12b[f]	11.0	6.8	6.8	4.4	4.4	8.8	6.4
11a		1.5		0	6.3	6.5	6.5
11b		1.4		2.0	6.2	5.8	6.5
11c[j]	11.2	4.0	4.0	2.0	6.3	6.2	6.6
13a		4.6	4.6	2.6	4.9	7.2	6.6
13a[l]	10.8	3.3	6.5	1.1	4.6	7.8	6.6
13b[m]	11.2	4.2	5.8	7.7	4.4	3.3	6.7
13b[f]	11.5	4.6	6.3	7.7	4.4	3.4	6.7
14a		0		1.9	5.6	0	7.0
14b[n]	11.1	5.4	5.4	1.9	5.7	1.4	6.9
15a	10.2	2.9	4.9	5.6	4.0	2.4	6.7
15b[f]	11.6	5.1	5.6	5.2	6.1	7.3	6.5

[a] Benzyl protons, J=12.3 Hz, Ph: 7.30–7.38.
[b] Acetonide methyls.
[c] Acetal OMe: 3.47, 3.48.
[d] Two rotamers.
[e] 330 K, OH–C(1): 4.6 (br. s).
[f] In D_2O.
[g] Acetal OMe: 3.30, 3.38.
[h] 336 K.
[i] 333 K.
[j] OH–C(1): 2.3 (br. s), J(1a,OH-1)=J(1b,OH-1)=4.8 Hz.
[k] 3 OH: ca. 2.8, 2.83, 2.65 (3 br. s); J(1a,OH-1)=J(1b,OH-1)=6.2 Hz.
[l] In (D_6)-DMSO, 350 K, 3 OH: ca. 4.45 (br. s).
[m] In CD_3OD.
[n] OH–C(1): 2.2 (br. s), J(1a,OH-1)=4.8 Hz, J(1b,OH-1)=6.2 Hz.
[o] 400 MHz, 3 OH: 2.32 (d, J=3.6 Hz), 2.37 (d, J=5.7 Hz), 2.84 (s).

idene-2,5,6-trideoxy-D-altrose dimethylacetal (benzyl (2*S*)-*c*-3,*c*-4-isopropylidenedioxy-*r*-2-dimethoxymethyl-*t*-5-methyl-pyrrolidine-1-carboxylate, 10a). To a stirred solution of **8b** (1.56 g, 4.08 mmol) and NEt_3 (0.7 ml, 5.1 mmol, 1.25 equiv.) in CH_2Cl_2 (9 ml), was added at −10°C $MeSO_2Cl$ (0.35 ml, 4.5 mmol, 1.1 equiv.). After 1 h at −10°C, Et_2O (20 ml) was added, the organic phase was washed with water (3×10 ml), the aqueous phases were extracted with Et_2O (3×10 ml), the organic phases dried ($MgSO_4$) and evaporated to give **8c** (1.88 g, quant.).

A solution of **8c** (1.82 g, 3.95 mmol) in EtOH (2.5 ml) and aq. 2.5N NaOH (4.75 ml, 11.8 mmol, 3 equiv.) was stirred at 80°C for 1.5 day. H_2O (15 ml) was added and the solution was extracted with AcOEt (3×10 ml), the organic phases were dried ($MgSO_4$) and evaporated. Crude **10a** (1.2 g) was purified by FC (CH_2Cl_2/Et_2O 9:1 on 60 g silica gel) to give pure **10a** (1.03 g, 72% yield from **8b**).

8c: yellow oil, characterised by ^{1}H NMR ($CDCl_3$, 298 K): 7.33 (m, 5 H arom.); 5.34 (d, NH-C(5)); 5.07 (s, C*H*$_2$Ph); 4.92 (dd, H-C(2)); 4.58 (d, H-C(1)); 4.41 (dd, H-C(3)); 4.35 (dd, H-C(4)); 3.81 (sext., H-C(5)); 3.52, 3.47 (2 s, 2 OMe); 3.20 (s, SO_2*Me*); 1.45, 1.36 (2s, 2 Me); 1.34 (d, Me(6)). J(1,2)=2.6 Hz, J(2,3)=6.8 Hz, J(3,4)=5.7 Hz, J(4,5)=7.0 Hz, J(5,NH-5)=7.8 Hz, J(5,Me(6))=6.8 Hz. R_f=0.39 (AcOEt/cyclohexane 1:1).

10a: yellow oil, $[\alpha]_D^{20}$=−46 (c=1.0, $CHCl_3$). IR ($CHCl_3$): 2980, 2930, 1700, 1450, 1410, 1380, 1350, 1290, 1235, 1185, 1120 1065, 965, 865, 690. ^{1}H NMR: see Table 2. ^{13}C NMR ($CDCl_3$, 300 K): 155.2 (N*C*O$_2$); 136.6, 128.4, 128.0, 127.9 (Ph); 112.2 (*C*Me$_2$)); 103.9 (C(1)); 84.3 (C(4)); 79.0 (C(3)); 66.8 (*C*H$_2$Ph); 62.2 (C(2)); 59.8 (C(5)); 57.1, 56.2 (2 OMe); 26.1, 24.7 (2 Me); 17.1 (Me(6)). R_f=0.37 (CH_2Cl_2/Et_2O 9:1). Anal. calcd for $C_{19}H_{27}NO_6$ (365.42): C 62.45, H 7.45, N 3.83; found: C 61.9, H 7.5, N 3.8.

5-Amino-3,4-*O*-isopropylidene-5,6-dideoxy-D-fucose dimethylacetal (9a), 5-(benzyloxy-carbonylamino)-3,4-*O*-isopropylidene-5,6-dideoxy-D-fucose dimethylacetal (9b), 5-(benzyloxycarbonylamino)-3,4-*O*-isopropylidene-2-*O*-methanesulfonyl-5,6-dideoxy-D-fucose dimethylacetal (9c) and 2,5-(benzyloxycarbonylimino)-3,4-*O*-isopropylidene-2,5,6-trideoxy-D-talose dimethylacetal (benzyl (2*S*)-*t*-3,*t*-4-isopropylidenedioxy-*r*-2-dimethoxymethyl-*t*-5-methyl-pyrrolidine-1-carboxylate, (11a)). Same procedure as for **8a** from **7** (1.675 g, 4.39 mmol) in EtOH (17 ml) over 5% Pd/C (88 mg) at 50°C for 14 h to give crude **9a** (1.3 g, quant.) which was *N*-protected as for **8b** in H_2O (8 ml), 2.5 N NaOH (5.3 ml), with $ClCO_2Bn$ (0.93 ml, 6.59 mmol, 1.5 equiv.) for 4.5 h at rt. Purification by FC (AcOEt/cyclohexane 1:1) gave pure **9b** (1.68 g, quant.).

Same procedure as for **8c** with **9b** (1.68 g, 4.39 mmol) in CH_2Cl_2 (10 ml), NEt_3 (0.92 ml, 6.6 ml, 1.5 equiv.) with $ClSO_2Me$ (0.375 ml, 4.8 mmol, 1.1 equiv.) to give **9c** (2.1 g, quant.). **9c** was cyclised with the same procedure as for **10a** in EtOH (9 ml) with 2.5 N NaOH (5.3 ml) at 80°C for 14 h to give **11a** (1.17 g, 73% from **7**) after FC (AcOEt/cyclohexane 3:7 on 60 g silica gel).

9a: yellowish oil characterised by ^{1}H NMR ($CDCl_3$, 300 K): 4.42 (d, H-C(1)); 3.70 (dd, H-C(2)); 4.38 (dd, H-C(3)); 4.11 (dd, H-C(4)); 3.29 (dq, H-C(5)); 4.04 (br. s, OH, NH_2); 3.49, 3.46 (2s, 2 OMe); 1.55, 1.37 (2s, 2 Me); 1.35 (d, Me(6)). J(1,2)=7.5 Hz, J(2,3)=0.8 Hz, J(3,4)=7.9 Hz, J(4,5)=1.9 Hz, J(5,Me(6))=6.7 Hz.

9b: yellowish oil characterised by ^{1}H NMR ($CDCl_3$, 300 K): 7.32 (m, 5 H arom.); 5.32 (br. s, NH-C(5)); 5.09, 5.10 (2d, C*H*$_2$Ph, J=12.4 Hz); 4.34 (d, H-C(1)); 3.68 (ddd, H-C(2)); 4.24 (dd, H-C(3)); 4.08 (dd, H-C(4)); 4.03 (m, H-C(5)); 3.44, 3.39 (2s, 2 OMe); 2.36 (d, OH-C(2)); 1.52, 1.37 (2s, 2 Me); 1.28 (d, Me(6)). J(1,2)=6.7 Hz, J(2,OH-2)=4.6 Hz, J(2,3)= 3.1 Hz, J(3,4)=6.6 Hz, J(4,5)=4.0 Hz, J(5,NH-5)=7.1 Hz, J(5,Me(6))=6.4 Hz. R_f=0.21 (AcOEt/cyclohexane 1:1).

9c: yellow oil, characterised by ^{1}H NMR ($CDCl_3$, 333 K): 7.34 (m, 5 H arom.); 5.13 (s, C*H*$_2$Ph); 4.88 (br d, NH-C(5)); 4.51 (d, H-C(1)); 4.99 (dd, H-C(2)); 4.43 (dd, H-C(3)); 4.03 (dd, H-C(4)); 4.14 (ddq, H-C(5)); 3.51, 3.46 (2s, 2 OMe); 2.96 (s, SO_2*Me*); 1.52, 1.38 (2s, 2 Me); 1.21 (d, Me(6)). J(1,2)=4.6 Hz, J(2,3)=8.8 Hz, J(3,4)=6.6 Hz, J(4,5)=2.4 Hz, J(5,Me(6))=6.6 Hz, J(5,NH-5)=9.3 Hz. R_f=0.33 (AcOEt/cyclohexane 1:1).

11a: yellow oil. $[\alpha]_D^{20}=-93$ (c=1.0, $CHCl_3$). IR ($CHCl_3$): 2990, 2930, 2830, 1685, 1450, 1410, 1385, 1375, 1350, 1320, 1305, 1240, 1190, 1165, 1140, 1110, 1070, 1025, 870, 695. ^{1}H NMR: see Table 2. R_f=0.49 (AcOEt/cyclohexane 1:1). Anal. calcd for $C_{19}H_{27}NO_6$ (365.42): C 62.45, H 7.45, N 3.83; found: C 62.4, H 7.4, N 3.9.

2,5-(Benzyloxycarbonylimino)-3,4-*O*-isopropylidene-2,5,6-trideoxy-D-altrose (benzyl (2*S*)-*r*-2-formyl-*c*-3,*c*-4-isopropylidenedioxy-*t*-5-methyl-pyrrolidine-1-carboxylate, (10b)). A solution of **10a** (1.03 g, 2.83 mmol) in dry acetone (12 ml) was stirred at 40°C for 1 h with Amberlyst-15 (H^+) (0.28 g, 0.1 g pro mmol acetal). The catalyst was discarded by filtration and washed with acetone, the solvents were evaporated to give crude **10b**.

10b: characterised by ^{1}H NMR (two rotamers ca. 55:45): see Table 2.

2,5-(Benzyloxycarbonylimino)-3,4-*O*-isopropylidene-2,5,6-trideoxy-D-altritol (benzyl (2*R*)-*r*-2-hydroxymethyl-*c*-3,*c*-4-isopropylidenedioxy-*t*-5-methyl-pyrrolidine-1-carboxylate, (10c)). To a stirred solution of crude **10b** (0.274 g, 0.79 mmol) in dry MeOH (1.5 ml) under Ar was added $NaBH_4$ (60 mg, 1.58 mmol, 2 equiv.). After 30 min at rt, acetone (2 ml), aq. 1N HCl (0.8 ml, 0.8 mmol, 1 equiv.) and water (10 ml) were successively added and the solution extracted with Et_2O (4×5 ml). Organic solutions were dried ($MgSO_4$) and evaporated. Crude **10c** (0.3 g) was purified by FC (CH_2Cl_2/Et_2O 9:1 on 50 g silica gel) to give **10c** (170 mg, 67% yield from **10a**).

10c: yellow oil. $[\alpha]_D^{20}=-31$ (c=1.0, $CHCl_3$). IR ($CHCl_3$): 3400, 2980, 2930, 1675, 1445, 1415, 1375, 1355, 1305, 1265, 1230, 1130, 1100, 1090, 1055, 1000, 855. ^{1}H NMR: see Table 2. Anal. calcd for $C_{17}H_{23}NO_5$ (321.37): C 63.53, H 7.21, N 4.36; found: C 63.6, H 7.3, N 4.3.

2,5-(Benzyloxycarbonylimino)-3,4-*O*-isopropylidene-2,5,6-trideoxy-D-talose (benzyl (2*S*)-*r*-2-formyl-*t*-3,*t*-4-isopropylidenedioxy-*t*-5-methyl-pyrrolidine-1-carboxylate, (11b)) and 2,5-(benzyloxycarbonylimino)-3,4-*O*-isopropylidene-2,5,6-trideoxy-D-talitol (benzyl (2*S*)-*r*-2-hydroxymethyl-*t*-3,*t*-4-isopropylidenedioxy-*t*-5-methyl-pyrrolidine-1-carboxylate, (11c)). To a stirred solution of **11a** (0.85 g, 2.32 mmol) in acetone (9.5 ml) under Ar at 40°C was added Amberlyst-15 (H^+) (1.22 g) in six times (four additions of 0.23 g at 0, 3, 4.5, 7 h and two additions of 0.15 g at 11 and 15 h). After 17.5 h the conversion was complete and the same treatment as for **10b** gave crude **11b** (0.96 g, quant.) which was reduced with the same procedure as for **10b** in dry MeOH (3.7 ml) with $NaBH_4$ (90 mg, 2.3 mmol, 1 equiv.) to give after FC (CH_2Cl_2/Et_2O 9:1) **11c** (0.58 g, 78% from **11a**).

11b: yellow-brownish oil characterised by ^{1}H NMR: see Table 2. R_f=0.25 (CH_2Cl_2/Et_2O 9:1).

11c: yellow oil. $[\alpha]_D^{21}=-62$ (c=1.0, $CHCl_3$). IR ($CHCl_3$): 2980, 2940, 1690, 1455, 1415, 1395, 1355, 1310, 1240, 1165, 1140, 1100, 1070, 1030, 870. ^{1}H NMR: see Table 2. ^{13}C NMR ($CDCl_3$, 333 K): 168.3 (N*C*O$_2$); 136.7, 128.6, 128.2, 128.1 (Ph); 112.0 (*C*Me$_2$)); 81.6 (C(3)); 80.1 (C(4)); 67.1 (*C*H$_2$Ph); 64.8 (C(2)); 63.0 (C(1)); 58.1 (C(5)); 26.2, 25.2 (2 Me); 15.4 (Me(6)). R_f=0.08 (CH_2Cl_2/Et_2O 9:1), R_f=0.35 (AcOEt/cyclohexane 7:3). No satisfactory elemental analysis.

2,5-(Benzyloxycarbonylimino)-2,5,6-trideoxy-D-altritol (benzyl (2*R*)-*c*-3,*c*-4-dihydroxy-*r*-2-hydroxymethyl-*t*-5-methyl-pyrrolidine-1-carboxylate, (12a)) and 2,5-imino-2,5,6-trideoxy-D-altritol ((2*R*,3*S*,4*R*,5*R*)-2-hydroxymethyl-5-methyl-pyrrolidine-3,4-diol, (12b)). A solution of **10c** (0.327 g, 1.02 mmol) in EtOH (2 ml) was stirred with Amberlyst-15 (H^+) (40 mg, 40 mg pro mmol acetonide) at

80°C for 7 h. The catalyst was discarded and washed with MeOH, the solvents were evaporated to give **12a** (0.29 g, quant.).

A solution of **12a** (0.29 g, 1.02 mmol) in EtOH (2 ml) was hydrogenolysed over 5% Pd/C (21 mg) at 40°C for 1 h. The catalyst was discarded by centrifugation and washed with EtOH, the solvents were evaporated to give **12b** (0.12 g, 80% from **10c** after recrystallisation in MeOH/Et_2O).

12a: yellow oil, characterised by ^{1}H NMR ($CDCl_3$, 298 K, two rotamers in 70:30 proportions): see Table 2 for the major rotamer, 7.35 (m, 5 H arom.); 5.05–5.21 (m, CH_2Ph); 4.51 (dd, J=4.8, 8.7 Hz, H-C(3)); 4.26 (dd, J=3.4, 11.4 Hz, Ha-C(1) maj.); 3.95, 3.79 (2m, Ha-C(1) min., Hb-C(1), H-C(2), H-C(4), H-C(5); 1.24, 1.16 (2d, J=6.7 Hz, Me(6) min. and maj.). R_f=0.43 (AcOEt/EtOH 9:1).

12b: colourless crystals, mp=118–120°C (MeOH/Et_2O). $[\alpha]_D^{20}$=+36 (c=0.83, MeOH). IR (KBr): 3390, 3260, 2910, 1440, 1370, 1320, 1105, 1080, 1040, 1020, 950, 880, 810, 765, 685. ^{1}H NMR: see Table 2. ^{13}C NMR (D_2O, 300 K): 65.3 (C(1)); 63.5 (C(2)); 76.6 (C(3)); 83.3 (C(4)); 59.6 (C(5)); 21.8 (Me(6)). Anal. calcd for: $C_6H_{13}NO_3$ (147.17): C 48.96, H 8.90, N 9.52; found: C 48.6, H 8.9, N 9.3.

2,5-(Benzyloxycarbonylimino)-2,5,6-trideoxy-D-talitol (benzyl (2*R*)-*t*-3,*t*-4-dihydroxy-*r*-2-hydroxymethyl-*t*-5-methyl-pyrrolidine-1-carboxylate, (13a)) and 2,5-imino-2,5,6-trideoxy-D-talitol ((2*R*,3*R*,4*S*,5*R*)-2-hydroxymethyl-5-methyl-pyrrolidine-3,4-diol, (13b)). Same procedure as for **12a** from **11c** (0.317 g, 0.99 mmol) in EtOH (2 ml) with Amberlyst-15 (H^+) (40 mg) for 4 h at 80°C to give **13a** (0.257 g, 92%) after FC (AcOEt on 30 g silica gel). Hydrogenolysis of **13a** as for **12a** in EtOH (2 ml) over 5% Pd/C (18 mg) for 1 h at 40°C gave **13b** (0.125 g, 93%) after evaporation of the solvent.

13a: colourless oil, characterised by ^{1}H NMR: see Table 2. R_f=0.05 (AcOEt/cyclohexane 7:3), R_f=0.34 (AcOEt/EtOH 9:1).

13b: colourless oil. $[\alpha]_D^{19}$=+46 (c=1.0, CH_3OH). IR (KBr): 3380, 2900, 1400, 1370, 1335, 1230, 1195, 1155, 1110, 1075, 1060, 1025, 1000, 940, 875, 800. ^{1}H NMR: see Table 2. ^{13}C NMR (CD_3OD, 300 K): 63.8 (C(1)); 64.3 (C(2)); 75.9 (C(3)); 75.4 (C(4)); 56.4 (C(5)); 14.8 (Me(6)). Anal. calcd for: $C_6H_{13}NO_3$ (147.17): C 48.96, H 8.90, N 9.52; found: C 48.9, H 8.8, N 9.3.

2,5-(Benzyloxycarbonylimino)-3,4-*O*-isopropylidene-2,5,6-trideoxy-D-allose (benzyl (2*R*)-*r*-2-formyl-*t*-3,*t*-4-isopropylidenedioxy-*c*-5-methyl-pyrrolidine-1-carboxylate, (14a)), 2,5-(benzyloxycarbonylimino)-3,4-*O*-isopropylidene-2,5,6-trideoxy-D-allitol (benzyl (2*S*)-*r*-2-hydroxymethyl-*t*-3,*t*-4-isopropylidenedioxy-*c*-5-methyl-pyrrolidine-1-carboxylate, (14b)). A solution of crude **10b** (0.876 g, 2.75 mmol) in MeOH (10 ml) was stirred with Na_2CO_3 (0.29 g, 2.74 mmol, 1 equiv.) at rt under Ar for 1 h to give a solution of **14a** which was reduced at once by adding $NaBH_4$ (0.207 g, 5.49 mmol, 2 equiv.) and stirring for 15 min. Same treatment as for **10c** (addition of acetone (5 ml), aq. 1N HCl (2.7 ml), water (30 ml) and extraction with Et_2O (4×10 ml)), gave after FC (AcOEt/cyclohexane 7:3) pure **14b** (0.49 g, 54% from **10a**).

14a can be isolated by evaporation of the isomerisation solution as a yellowish oil, characterised by ^{1}H NMR ($CDCl_3$, 336 K): see Table 2. ^{1}H NMR ($CDCl_3$, 300 K, two rotamers 50:50): 9.70, 9.63 (2s, CHO(1), two rotamers); 7.35 (m, 5 H arom.); 5.20, 5.15 (2s, CH_2Ph, two rotamers); 4.93 (s, H-C(3)); 4.64, 4.51 (2s, H-C(2), two rotamers); 4.36 (d, J=5.7 Hz, H-C(4)); 4.32 (m, H-C(5)); 1.43, 1.31 (2 Me); 1.16, 1.12 (2d, J=7.0 Hz, Me(6), two rotamers). R_f=0.29 (CH_2Cl_2/Et_2O 9:1).

14b: yellow oil. $[\alpha]_D^{20}$=+13 (c=1.0, $CHCl_3$). IR ($CHCl_3$): 3430, 2985, 2940, 1685, 1450, 1410, 1375, 1355, 1325, 1265, 1235, 1155, 1130, 1100, 1060 1020, 865, 690. ^{1}H NMR: see Table 2. ^{13}C NMR ($CDCl_3$, 333 K): 155.6 (NCO_2); 136.7, 128.5, 128.1, 127.8, (Ph); 112.0 (CMe_2); 85.6 (C(4)); 81.9 (C(3)); 67.3 (CH_2Ph); 66.9 (C(2)); 64.2 (C(()); 60.5 (C(5)); 27.4, 25.4 (2 Me); 19.7 (Me(6)). R_f=0.37 (AcOEt/cyclohexane 7:3). Anal. calcd for $C_{17}H_{23}NO_5$ (321.37): C 63.53, H 7.21, N 4.36; found: C 63.8, H 7.3, N 4.3.

2,5-(Benzyloxycarbonylimino)-2,5,6-trideoxy-D-allitol (benzyl (2*S*)-*t*-3,*t*-4-dihydroxy-*r*-2-hydroxymethyl-*c*-5-methyl-pyrrolidine-1-carboxylate, (15a)). Same procedure as for **12a**, starting from **14b** (0.40 g, 1.25 mmol) in EtOH (4 ml) with Amberlyst 15 (H^+) (50 mg) at 80°C for 18 h. Purification by FC (AcOEt/cyclohexane 7:3 on 30 g silica gel) gave pure **15a** (0.313 g, 90%).

15a: yellow oil. $[\alpha]_D^{20}$=+9 (c=1.0, $CHCl_3$). IR ($CHCl_3$): 3385, 2940, 1670, 1450, 1415, 1355, 1145, 1085, 690. ^{1}H NMR: see Table 2. ^{13}C NMR ($CDCl_3$, 336 K): 156.7 (NCO_2); 136.4, 128.5, 128.1, 127.8 (Ph); 76.2 (C(4)); 72.7 (C(3)); 67.4 (CH_2Ph); 65.2 (C(2)); 63.7 (C(1)); 60.1 (C(5)); 18.8 (Me(6)). R_f=0.07 (AcOEt/cyclohexane 7:3). Anal. calcd for $C_{14}H_{19}NO_5$ (281.31): C 59.77, H 6.81, N 4.98; found: C 59.8, H 6.5, N 4.8.

2,5-Imino-2,5,6-trideoxy-D-allitol ((2*S*,3*S*,4*R*,5*R*)-2-hydroxymethyl-5-methyl-pyrrolidine-3,4-diol, (15b)). Hydrogenolysis of **15a** (0.20 g, 0.71 mmol) as for **12b** in EtOH (2 ml) over 5% Pd/C (14 mg) for 3 h at 40°C gave **15b** (0.102 g, 97%).

15b: colourless oil. $[\alpha]_D^{20}$=−2 (c=1.0, MeOH). ^{1}H NMR: see Table 2. ^{13}C NMR (D_2O, 300 K): 65.2 (C(1)); 62.1 (C(2)); 72.5 (C(3)); 77.3 (C(4)); 58.2 (C(5)); 17.3 (Me(6)). Anal. calcd for $C_6H_{13}NO_3$ (147.17): C 48.96, H 8.90, N 9.52; found: C 48.7, H 8.9, N 9.4.

Enzyme inhibitions

Glycosidase activities were determined at 25°C at the optimal pH of each enzyme[19] with the corresponding *p*-nitrophenyl glycopyranoside as substrate against α-D-glucosidase (EC 3.2.1.20) from baker's yeast (9 units per mg of protein, K_m=0.3 mM, pH=6.8), β-D-glucosidase (EC 3.2.1.21) from almonds (20–40 units per mg of protein, K_m=2 mM, pH=5.0), α-D-mannosidase (EC 3.2.1.24) from Jack beans (ca. 20 units per mg of proteins,

K_m=2 mM, pH=4.5), α-L-fucosidase (EC 3.2.1.51) from bovine kidney (5–15 units per mg, K_m=0.15 mM, pH=6.0) and α-D-galactosidase (EC 3.2.1.22) from green coffee beans (10 units per mg, K_m=0.3 mM, pH=6.0). Glycosidases and corresponding *p*-nitrophenyl glycopyranosides were obtained from Sigma Chemical Co. The release of *p*-nitrophenol was measured continuously at 400 nm to determine initial velocities.[20] All kinetics were started by enzyme addition in a 1 ml assay medium using substrate concentrations around the K_m value of each enzyme. K_i values were determined for the most potent inhibitors using the Dixon graphical method,[21] IC_{50} values were determined for weak inhibitions (at a substrate concentration equal to K_m value) and correspond to the inhibitor concentration required for 50% inhibition of the enzyme in our experimental conditions.

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