# Synthesis and Evaluation as Glycosidase Inhibitors of Carbasugar-Derived Spirodiaziridines, Spirodiazirines, and Spiroaziridines

by **Peter Kapferer**, **Véronique Birault**[1]), **Jean-François Poisson**[2]), and **Andrea Vasella***

Laboratorium für Organische Chemie, ETH-Hönggerberg, HCI, CH-8093 Zürich

Dedicated to Professor *Wolfgang Steglich* on the occasion of his 70th birthday

The spirodiaziridines **6** and **9**, potential inhibitors of $\alpha$- and $\beta$-glucosidases, were prepared from the validoxylamine A-derived cyclohexanone **5**. The trimethylsilyl protecting groups of **5** are crucial for the formation of **6** in good yields. Oxidation of **6** gave **7**. The diaziridine **6** ($pK_{HA} = 2.6$) and the diazirine **7** did not inhibit the $\beta$-glucosidases from almonds, the $\beta$-glucosidase from *Caldocellum saccharolyticum*, and the $\alpha$-glucosidase from yeast. The *N*-benzyl diaziridine **9** is a very weak inhibitor of the $\alpha$-glucosidase, but did not inhibit the $\beta$-glucosidases. To see whether the weak inhibition is due to the low basicity of the diaziridines or to geometric factors, we prepared the spiro-aziridines **21** and **25** and 1-epivalidamine (**32**). The known cyclohexanone **10** was methylenated and epoxidised to **16** and **17**. Azide opening of **16** and **17**, mesylation, $LiAlH_4$ reduction, and deprotection gave the aziridines **21** and **25** respectively. 1-Epivalidamine (**32**) was prepared from the known carba-glucose **29**. The aziridine **25** ($pK_{HA} = 6.8$) is a weak irreversible inhibitor of the $\beta$-glucosidase from *Caldocellum saccharolyticum* and a weak reversible inhibitor of the $\alpha$-glucosidase from yeast, but did not inhibit the $\beta$-glucosidases from almonds. The poorly stable aziridine **21** weakly inhibited the three enzymes. Similarly, 1-epivalidamine ($pK_{HA} = 8.4$) proved only a weak inhibitor. The known cyclopentylamine **34** ($pK_{HA} = 7.9$), however, is a micromolar inhibitor of these enzymes. The much stronger inhibition by **34** is related to the pseudoaxial orientation of its amino group.

**Introduction.** – Alkoxydiaziridines of type **1** (*Scheme 1*) possess *trans*-oriented N-atom lone-pairs located above and below the average plane of the pyranose ring and oriented more or less parallel to the ring plane. These diaziridines may, thus, inhibit both $\alpha$- and $\beta$-glucosidases[3]). The inhibition of $\beta$-glycosidases by diaziridines of this type may provide evidence for the correlation between the change of the pyranose-ring conformation, orienting the scissile bond in a pseudoaxial orientation, and the proton transfer to the glycosidic heteroatom characterising the reaction coordinate linking the ground and transition states of the rate-determining step. The diaziridines **1** may be accessible by deprotection of the known benzyl ethers **2** [2]. We were, however, concerned about the low basicity and the fragility of alkoxydiaziridines in aqueous solution. Exploratory experiments indeed showed that the diaziridines **1** are not sufficiently stable [3], so that we aimed at the synthesis of the carba analogues **6**. To differentiate between the effects of shape and charge on the inhibition, we also required the related spiro-aziridines **21** and **25**[4]) (*Scheme 2*), and 1-epivalidamine

[1]) Postdoctoral fellow from January 1996 to October 1997.

[2]) Postdoctoral fellow from November 2000 to October 2001.

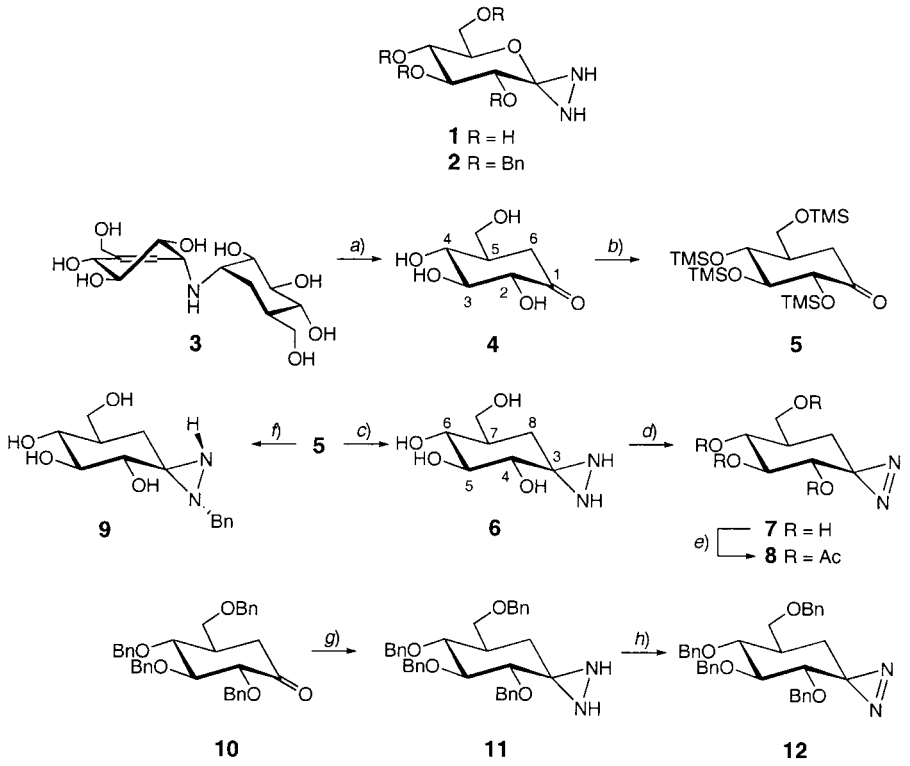
[3]) No diaziridines are known to inhibit glycosidases. Carbohydrate-derived diaziridines with the hydrazi group spiro-linked to other centers have been synthesized by *Kurz et al.* as intermediates in the synthesis of diazirines [1].

[4]) A range of carbohydrate-derived fused aziridines are known, and many of them inhibit glycosidases [4–12]. Carbohydrate derived spiro-aziridines have been prepared as intermediates in the syntheses of C(3)-branched 3-amino sugars, as reviewed in [13].

(**32**[5]), *Scheme 3*) [14]; their basicities are expected to increase in the order **6** < **21**, **25** < **32**.

**Results and Discussion.** – *Synthesis of the Diaziridines and Diazirines.* We planned to synthesise the diaziridines **6** by treating the trimethylsilyl ether **5** of validone (**4**) [15] with $NH_3$ and hydroxylamine-*O*-sulfonic acid in MeOH [16–19], although *Kurz et al.* [1] and *Thieme* [20] reported low yields for a related synthesis of a 4-hydrazipyranoside. Oxidation of **6** should provide the diazirine **7**.

*Scheme 1*




*a*) *N*-Bromosuccinimide (NBS), MeCN, $H_2O$ ([15]); 50%. *b*) $Me_3SiCl$ (TMSCl), 1,1,1,3,3,3-hexamethyldisilazane (HMDS), pyridine; 27% (chrom.). *c*) $NH_3$, MeOH, then $NH_2OSO_3H$; 50%. *d*) $I_2$, $Et_3N$, MeOH; 89%. *e*) $Ac_2O$, pyridine; 84% from **6**. *f*) $BnNH_2$, MeOH, then $NH_2OSO_3H$; 27%. *g*) $NH_3$, MeOH, then $NH_2OSO_3OH$; 35%. *h*) $I_2$, $Et_3N$, MeOH, $CH_2Cl_2$; 85%.

Validone (**4**) was prepared by *N*-bromosuccinimide (NBS) cleavage of validoxylamine A[6]) (**3**) [15] and isolated in a yield of 50% (*Scheme 1*). Trimethylsilylation of **4**

[5]) 1-Epivalidamine (**32**) is a competitive inhibitor of pig kidney trehalase ($K_i = 1.2$ μM) [14]. To the best of our knowledge, the inhibition by this compound against the enzymes studied here has not been reported.

[6]) We thank Dr. *A. G. O'Sullivan*, *Syngenta*, Basel, for a generous gift of validoxylamine A.

[21] gave crude **5** (94%) that was isolated by chromatography (27%)[7]). Treatment of a solution of **5** in MeOH and $NH_3$ with hydroxylamine-*O*-sulfonic acid gave crude **6** (69%), which oxidised iodide in acidic solution [22–24]. Flash chromatography gave a solution of **6**, pure by TLC, but complete evaporation of the solvent (AcOEt, i-PrOH, $H_2O$), even below 10°, resulted in partial decomposition. Pure **6** was obtained in 50% yield by ion-exchange column chromatography of a concentrated solution.

The diaziridine **6** was also obtained by treating validone (**4**) with $NH_3$ and hydroxylamine-*O*-sulfonic acid in MeOH. However – in agreement with previously reported results [1][20] – the yield of **6** was lower (28% of crude **6**), and the reaction less clean, so that **6** could not be obtained pure. The $Me_3Si$ groups are cleaved off during the diaziridine syntheses and appear to play an active role in the diaziridine formation. This might be due to the *in situ* formation of 1,1,1,3,3,3-hexamethyldisilazane (HMDS), or to sequestration of $H_2O$ formed in the condensation of $NH_3$ with the ketone, rather than to protection of the $\beta$-hydroxy ketone **4**. To address this issue, we tested the transformation of validone (**4**) in the presence of HMDS, and we also examined the transformation of the benzylated validone **10** [15] into the benzyl-protected diaziridine **11** (*Table 1*). Treatment of **4** with HMDS and then with hydroxylamine-*O*-sulfonic acid led to a lower yield of the diaziridine (20% of crude **6**) than the reaction with $NH_3$/hydroxylamine-*O*-sulfonic acid (28%). Using HMDS and $NH_3$ improved the yield somewhat (38%), but it was still significantly lower than the one realised from **5** (69%). Treating the benzylated validone **10** with $NH_3$ and hydroxylamine-*O*-sulfonic acid yielded 35% of **11**, whereas the reaction with HMDS and $NH_3$/hydroxylamine-*O*-sulfonic acid provided **11** in slightly lower yield (29%). Treating **10** with excess $Me_3SiCl$ and $NH_3$/hydroxylamine-*O*-sulfonic acid resulted in a significantly higher yield (50%), suggesting that the trimethylsilyl ether **5** acts as a $H_2O$ scavenger.

Table 1. *Synthesis of* **6** *and* **11** *in the Presence of Silylating Agents*[a])

| *Entry* | Starting material | Reagents | Product (yield) |
|---|---|---|---|
| *1* | **4** | $NH_3$, then $NH_2OSO_3H$ | **6** (28%)[b]) |
| *2* | **4** | $HN(SiMe_3)_2$ (4 equiv.), then $NH_2OSO_3H$ | **6** (20%)[b]) |
| *3* | **4** | $HN(SiMe_3)_2$ (2 equiv.), $NH_3$, then $NH_2OSO_3H$ | **6** (38%)[b]) |
| *4* | **5** | $NH_3$, then $NH_2OSO_3H$ | **6** (69%)[b]) |
| *5* | **10** | $NH_3$, then $NH_2OSO_3H$ | **11** (35%) |
| *6* | **10** | $HN(SiMe_3)_2$ (2 equiv.), $NH_3$, then $NH_2OSO_3H$ | **11** (29%) |
| *7* | **10** | $Me_3SiCl$ (4 equiv.), $NH_3$, then $NH_2OSO_3H$ | **11** (52%) |

[a]) Conditions: a solution of the starting material in MeOH was treated with $HN(SiMe_3)_2$ at r.t. (*Entries 2, 3,* and *6*) or with $Me_3SiCl$ at −20° (*Entry 7*), saturated with $NH_3$ at −20°, treated dropwise with $NH_2OSO_3H$ (1 equiv.), stirred at −20° for 3 h, and allowed to warm to r.t. overnight. [b]) Crude after FC.

Oxidation of the diaziridine **6** with $I_2$ in MeOH in the presence of $Et_3N$ gave a mixture of the diazirine **7** and $Et_3N$ (2.5:1), which could not be separated even by repeated flash chromatography (FC); replacing $Et_3N$ with the more volatile $Me_3N$ led

[7]) An attempt to purify **5** by bulb-to-bulb distillation (130°, 0.5 bar) resulted in partial decomposition. Only chromatographically purified samples of **5** gave good results in the subsequent step.

to a mixture of **7** and several unidentified by-products. Acetylation gave the tetra-acetate **8** (84% from **6**). Pure diazirine **7** (89%) was obtained from **7** · $Et_3N$ by weak acid ion-exchange chromatography. Treatment of the silylated validone **5** with $BnNH_2$ and hydroxylamine-*O*-sulfonic acid in MeOH gave, after FC, a *ca.* 1:4 mixture of the *N*-benzyl diaziridine **9** and $BnNH_2$, from which pure **9** (27%) was obtained by chromatography on a weak acid ion exchanger. The *N*-benzyl diaziridine **9** results from equatorial attack of hydroxylamine-*O*-sulfonic acid on the *N*-benzyl imine formed *in situ* from **5** and $BnNH_2$[8]).

The diaziridines **6**, **11**, and **9** oxidise iodide to iodine in acidic medium [22–24], and **11** shows a characteristic IR N–H band at 3259 $cm^{-1}$ ($CHCl_3$) [27][28]. According to the $^1$H-NMR spectrum in ($D_6$)DMSO, **6** is a *ca.* 3:2 mixture of two diastereoisomers. NH Groups of the major isomer appear as two *d* at 2.30 and 2.13 ppm ($J$ = 8.1 Hz) and NH of the minor isomer as two *d* at 2.24 and 2.07 ppm, respectively ($J$ = 8.1 Hz). Upon addition of $CF_3COOH$, the two diastereoisomers equilibrate rapidly, and the spectrum of a single compound is observed. C(3) of **6** appears as a *s* at 60.02 ppm (*cf.* [29]). The $^1$H-NMR spectrum ($CDCl_3$) shows that **11** is a *ca.* 4:1 mixture of diastereoisomers. NH Groups exchanged with $D_2O$; the signals of the major diastereoisomer resonate as two *d* at 2.64 and 1.57 ppm, respectively ($J$ = 7.9 Hz), while only one *d* resonating at 1.44 ppm ($J$ = 8 Hz) could be observed for the other isomer (the other signal is hidden between 2.26–2.06 ppm). C(3) resonates as a *s* at 57.06 ppm. According to the $^1$H-NMR spectrum ($D_2O$), the *N*-benzyl diaziridine **9** is a single stereoisomer. Its configuration was determined by NOE difference spectra. Upon irradiation at 2.04 ppm ($H_{eq}$–C(8)), a NOE of 4 and 6%, respectively, was observed for the benzylic H-atoms. Upon irradiation of the benzylic H-atoms at 3.92 and 3.81 ppm, a NOE of 6% for $H_{eq}$–C(8) and one of 8% for H–C(7) were observed. Irradiation at 3.62 ppm (H–C(4)) did not lead to a NOE of the benzylic H-atoms. C(3) appears as a *s* at 62.90 ppm and Ph*C*$H_2$ as a *t* at 57.27 ppm (*cf.* [30]). The UV spectrum of **7** ($H_2O$) shows a typical maximum at 340 nm ($\varepsilon$ = 68) [27][31]. A large chemical-shift difference is observed for the two H–C(8) ($\Delta\delta \approx 1.2$ ppm). The signal for the equatorial H-atom is shifted upfield, an observation characteristic for spiro-diazirines [18][27][32]. The C(3) *s* (32.34 ppm) is shifted upfield by 27.7 ppm as compared to that of **6** (for similar upfield shifts of 1-azipyranoses, see [2]). The UV spectrum of the diazirine **12** (obtained in 85% yield by oxidation ($I_2$, $Et_3N$) of **11**) in $CH_2Cl_2$ shows a maximum at 340 nm ($\varepsilon$ = 92). The IR N=N band ($CHCl_3$) is observed at 1590 $cm^{-1}$ [31][27]. The chemical-shift difference for the two H–C(8) ($\Delta\delta$ = 1.38 ppm) is again large. The C(3) *s* of **12**, (28.82 ppm) is shifted upfield by 28.2 ppm as compared to **11**. The vicinal coupling constants for the ring H of **6**, **7**, **8**, **11**, **12**, and **9** evidence a $^6C_3$ conformation.

*Synthesis of the Spiro-aziridines. Wittig* methylenation of the cyclohexanone **10** gave the alkene **13** (77%), but, of our attempts to transform **13** into an aziridine[9]), only the reaction with chloramine T and phenyl(trimethyl)ammonium tribromide in MeCN [44] gave the *N*-tosyl aziridine **14** (18%), along with 2% of the ring-opened tosyl amide **15** (*Scheme 2*). The low yield of the aziridination prompted us to investigate the synthesis of the aziridines *via* the epoxides **16** and **17** (*cf.* [45][46]). These epoxides were obtained in 25 and 61% yield, respectively, by treating the alkene **13** with *m*-chloroperbenzoic acid (*m*CPBA)/$NaHCO_3$. On a scale of 700 mg, their separation required HPLC, and the yields were somewhat lower (19 and 48%, resp.). Treatment of

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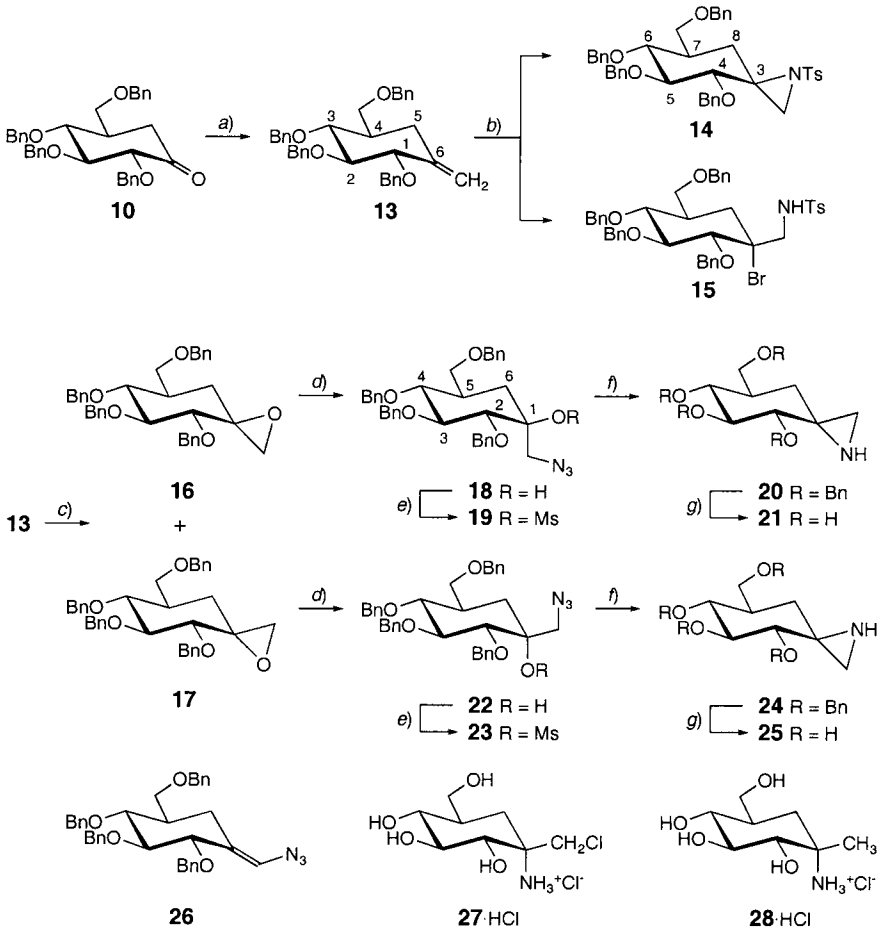
[8]) Equatorial attack of hydroxylamine-*O*-sulfonic acid on an intermediary imine has also been observed by *Shustov et al.* in the synthesis of 5-methyl-3,3-pentamethylenediaziridine [25]. For a discussion of the diastereoselective addition of $NH_3$ or $H_2NMe$ to glyconolactone oxime mesylates in the formation of alkoxydiaziridines, see [26].

[9]) The alkene **13** did not react with $TsN_3$, toluene, 110° [33][34]; $TfN_3$, $CH_2Cl_2$ [35]; diphenylphosphoryl azide, toluene, 70°; NBS, $NaN_3$, DME/$H_2O$ (or DMF) [36]; I-NCO, $Et_2O$ then KOH, MeOH [37–39]. I-NCO, MeCN, then KOH, MeOH gave **16** (36%) and unidentified by-products. ICl/$NaN_3$ ([40–43]) gave 34% of a mixture of $IN_3$ addition products, which, on treatment with $LiAlH_4$, gave **13** (43%) (*cf.* [40]; see *Exper. Part*).

*Scheme 2*




*a*) $Ph_3P=CH_2$, THF; 77%. *b*) Chloramine T, $PhMe_3NBr_3$, MeCN; 18% of **14**, 2% of **15**. *c*) 3-Chloroperbenzoic acid (*m*CPBA), $NaHCO_3$, $CH_2Cl_2$; 25% of **16**, 61% of **17**. *d*) $NaN_3$, DMF; 91% of **18**; 88% of **22**. *e*) MsCl, DMAP, 2,5-lutidine; 95% of **19**, 4% of **26**; 99% of **23**. *f*) $LiAlH_4$, THF; 83% of **20**; 74% of **24**. g) Na, $NH_3$, THF; 45% of **25**; (yield of **21**: see text).

**16** and **17** with $NaN_3$ in DMF gave the azido alcohols **18** (91%) and **22** (88%). Mesylation (MsCl, 4-(dimethylamino)pyridine (DMAP), 2,5-lutidine) [47] of **18** yielded 95% of the methanesulfonate **19** besides 4% of the elimination product **26**, while mesylation of **22** gave the methanesulfonate **23** (99%) exclusively. The azido methanesulfonates **19** and **23** were transformed into aziridines **20** (83%) and **24** (74%), respectively, by treatment with $LiAlH_4$ in THF (*cf.* [48]). Debenzylation of the aziridine **24** under *Birch* conditions gave the aziridine **25**, which was was isolated in a yield of 45% by chromatography first on an ion exchanger and then on a CN phase. *Birch* debenzylation of the aziridine **20**, followed by chromatography, led to the crude aziridine **21**, which decomposed on the CN phase column. Even just evaporating a

methanolic solution of some batches of **21** led to decomposition[10]). Crude **21** (containing small amounts of an unidentified by-product and some salts) was obtained by chromatography of the reaction product over a *Sephadex G 10* column, and used for the enzyme assay. Hydrogenation of the benzylated aziridine **20** over Pd/C in MeOH/HCl gave a mixture of the ring-opened **27** and **28** as their hydrochlorides (1.6:1). Elution of crude **21** from a *Sephadex C 25* column with aqueous HCl gave **27**·HCl[11]).

The $^1$H-NMR spectrum of the *N*-tosyl aziridine **14** shows two br. *s* at 2.63 and 2.59 ppm ($CH_2$(2)). Their small coupling constants are typical for aziridines [53]. C(3) appears as a *s* at 51.73 ppm and C(2) as a *t* at 28.77 ppm. The configuration of **14** was determined by NOE difference spectra. Upon irradiation at 2.63 ppm (H–C(2)), a NOE of 2% was observed for H–C(5), and irradiation at 2.59 ppm (H′–C(2)) led to a NOE of 1% for H–C(7) and for the $CH_2$(8) *multiplet*. Irradiation at 3.78 ppm (H–C(4)) did not lead to an observable NOE for $CH_2$(2). Irradiation at 3.39 ppm (H–C(5)) caused an NOE of 1.5% for H–C(2). The vicinal coupling constants evidence a $^6C_3$ conformation. The mass spectrum of the ring-opened tosyl derivative **15** shows two $[M+Na]^+$ peaks at *m/z* 808 and 806 of about equal intensity. $CH_2$–C(1) resonate as two *dd* at 3.18 ppm ($J$ = 13.4, 6.5 Hz) and 3.04 ppm ($J$ = 13.4, 8.1 Hz), and NH as a *dd* at 3.62 ppm ($J$ = 7.8, 6.2 Hz), evidencing that the NHTs moiety is bound to $CH_2$–C(1) and not to C(1). H–C(2) appears as a *d* at 3.13 ppm ($J$ = 9.0 Hz), indicating that C(1) is fully substituted. The configuration of C(1) was deduced from the large downfield shifts for H–C(5) (2.29–2.15 ppm) and H–C(3) (4.03 ppm), which indicate that Br–C(1) is axial.

The configuration of the epoxides was determined by NOE difference spectra. Irradiation of H–C(2) of **16** at 3.18 ppm caused a NOE of 1% for H–C(5). Upon irradiation at 2.56 ppm (H′–C(2)), a NOE of 7% was observed for $H_{eq}$–C(8). For **17**, irradiation at 2.98 ppm (H–C(2)) led to a NOE of 1% for H–C(4). Irradiation at 2.59 ppm (H′–C(2)) led to a NOE of 4% for $H_{eq}$–C(8). The pseudo-axial O–C(3) of **17** leads to a downfield shift for H–C(5) ($\Delta\delta$ = 0.29 ppm) and H–C(7) ($\Delta\delta \approx$ 0.2 ppm), as compared to **16**. This shift confirms the assignment of the configuration of **16** and **17**. The azido alcohols **18** and **22** show strong IR bands for the $N_3$ and OH groups. C$H_2$–$N_3$ resonate as two *d* at 3.74 and 3.34 ppm ($J$ = 12.8 Hz) for **18**, at 3.29 and 3.22 ppm ($J$ = 11.8 Hz) for **22**, at 4.10 and 3.45 ppm ($J$ = 14.0 Hz) for the methanesulfonate **19**, and at 4.17 and 3.90 ppm ($J$ = 12.1 Hz) for the methanesulfonate **23**. H–C(3) and H–C(5) of **22** and **23** (axial O–C(1)) are shifted downfield as compared to **18** ($\Delta\delta \approx$ 0.3 ppm for H–C(3) and $\Delta\delta \approx$ 0.4 ppm for H–C(5)) and **19** ($\Delta\delta$ = 0.37 ppm for H–C(3) and $\Delta\delta \approx$ 0.4 ppm for H–C(5)). H–C(2) ($\Delta\delta \approx$ 0.8 ppm) and $H_{ax}$–C(6) ($\Delta\delta$ = 0.94 ppm) of **19** are shifted downfield as compared to **18**. A downfield shift of *ca.* 20 ppm is observed for the C(1) *s* of **19** (95.31 ppm) and **23** (94.00 ppm) as compared to **18** and **22**, confirming that the O-atom is bound to C(1). The coupling constants for the ring H-atoms evidence a $^4C_1$ conformation for **18**, **22**, **19**, and **23**. The geminal coupling constants for the axial $CH_2$–$N_3$ substituent of **18** and **19** are larger than those of the equatorial $CH_2$–$N_3$ of **22** and **23** ($\Delta J$ = 1.0 to 1.9 Hz). The tertiary alcohol **22** and the methanesulfonate **23** can adopt a conformation in which the RO group is *gauche* to both vicinal $CH_2$ H-atoms, an orientation of an electron-withdrawing substituent that leads to a decreased (absolute) geminal coupling constant [54][55]. The structure of the elimination product **26** is evidenced by a strong IR band at 2109 cm$^{-1}$ for the $N_3$ group, by a br. *t* at 6.36 ppm ($J$ = 1.2 Hz) for C$H$–$N_3$, and by the mass spectrum (*m/z* 570, corresponding to $[M+Na-N_2]^+$. The $^{13}$C-NMR spectrum shows alkene signals at 126.55 and 120.32 ppm. The coupling constants for the ring H-atoms evidence a flattened $^3C_6$ conformation. The (*E*)-configuration of the olefinic C=C bond was not proven. $CH_2$(2) of the aziridines **20** and **24** appear as two br. *s* at 1.87 and 1.40 ppm for **20**, and at 1.98 and 1.29 ppm for **24**. NH of **20** and **24** appear as a br. signal at 1.17–0.87 and 1.18–0.85 ppm, respectively. C(3) resonates as a *s* at 37.42 ppm for **20** and at 37.86 ppm for **24**, and C(2) as a *t* at 27.17 ppm for **20** and at 26.97 ppm for **24**. The coupling constants for the ring H-atoms of **20** evidence a $^6C_3$ conformation. Signal overlap for the ring H-atoms of **24** prevented a conformational analysis. $CH_2$(2) of the aziridine **25** resonate as two br. *s* at 2.02 and 1.34 ppm, respectively. C(3) appears as a *s* at 39.90 ppm and C(2) as a *t* at 27.36 ppm. The coupling constants for the ring H-atoms evidence a $^6C_3$ conformation. The p$K_{HA}$ value of **25** is 6.8, and equilibration of the N-configurational isomers should be fast at pH 7. $CH_2$(2) of the crude aziridine **21** resonate as two br. *s* at 1.96 and 1.53 ppm, respectively. The coupling

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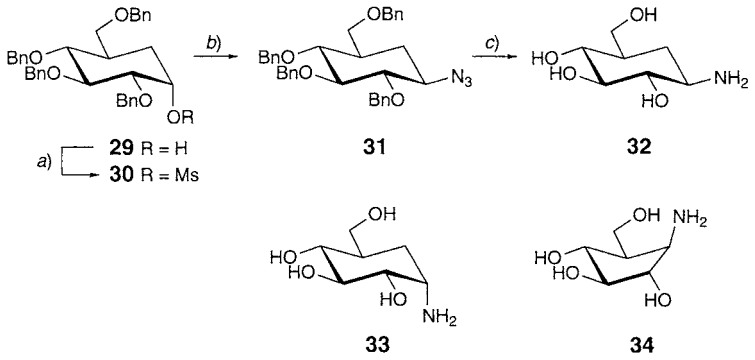
[10]) For other examples of related, unstable aziridines see *Martin* and *Saavedra* [9], and *Paulsen et al.* [11]. *Hassner* et *al.* reported that some aziridines decompose upon concentration of their solution in organic solvents, unless scrupulously dried [40].

[11]) For similar acid-catalysed regioselective ring openings of aziridines, *cf.* [49–52].

constants evidence a $^6C_3$ conformation. In the HR-MS, the $[M+1]^+$ peak appears at 190.1076 (calc. 190.1079). The structure of the ring-opened **27**·HCl and **28**·HCl was assigned on the basis of MS and $^1$H-NMR data. The ESI$^+$-MS spectrum of the mixture of **27**·HCl and **28**·HCl shows peaks at 224 ($[M+\text{MeOH}+1]^+$) and 192 ($[M+1]^+$) for **28**, and pairs of peaks with an intensity ratio of 1:2 (typical for chloro compounds) at 250 and 248 ($[M+\text{Na}]^+$), and 228 and 226 ($[M+1]^+$), and peaks at 222 ($[M-\text{Cl}+\text{MeOH}]^+$) and 190 ($[M-\text{Cl}]^+$, *i.e.*, $[M(\mathbf{21})+1]^+$) for **27**. In the $^1$H-NMR spectrum of the mixture **27**·HCl/**28**·HCl, Me of **28**·HCl resonates as a *s* at 1.43 ppm. $CH_2$–C(1) of **27**·HCl resonates as two *d* ($J=12.3$ Hz) at 4.11 and 3.85 ppm. The large geminal coupling constant excludes the structure of an aziridinium ion. The large downfield shift indicates that Cl and not $NH_3^+$ is bound to $CH_2$–C(1).

*1-Epivalidamine. Asano et al.* prepared 1-epivalidamine (**32**) by reductive amination of validone (**4**), thus obtaining **32** (22%) and **33** (36%) after separation by ion-exchange chromatography [14] (*Scheme 3*). To avoid this separation, we prepared **32** from the known axial alcohol **29** [56] that was transformed into the methanesulfonate **30** (93%). Substitution of the methanesulfonate with $NaN_3$ in DMF gave the azide **31** (94%) that was hydrogenated over Pd/C in MeOH/HCl to yield 1-epivalidamine (**32**) in an almost quantitative yield.

*Scheme 3*




*a*) MsCl, pyridine; 93%. *b*) $NaN_3$, DMF; 94%. *c*) $H_2$ (6 bar), Pd/C, MeOH, HCl; 100%.

*Enzyme-Inhibition Studies.* The diaziridines **6** and **9**, and the aziridines **21** and **25** are weak inhibitors of the $\beta$-glucosidase from *Caldocellum saccharolyticum*, the $\beta$-glucosidases from almonds, and the $\alpha$-glucosidase from brewer's yeast (*Table 2*). At pH 6.8, **6** did not inhibit the $\beta$-glucosidases, while the $\alpha$-glucosidase was inhibited only very weakly at a concentration of 8.8 mM. At pH 4.2, **6** inhibited the $\beta$-glucosidase from *Caldocellum saccharolyticum* very weakly and proved inactive against the sweet almond $\beta$-glucosidases. At pH 6.8, the *N*-benzyl diaziridine **9** was a weak inhibitor of the $\alpha$-glucosidase ($IC_{50}=1.7$ mM); as expected, the hydrophobic aglycon mimic leads to a (slightly) stronger inhibition [57]. The diazirine **7** did not significantly inhibit any of the enzymes tested. Also, the aziridine **25** did not inhibit the almond $\beta$-glucosidases (pH 6.8). It proved a weak irreversible inhibitor of the $\beta$-glucosidase from *Caldocellum saccharolyticum*, and a weak reversible inhibitor of the $\alpha$-glucosidase from brewer's yeast; the inhibition of the $\alpha$-glucosidase was abolished at pH 5.0. The crude aziridine **21** appears to be a weak reversible inhibitor of the $\beta$-glucosidases, and a weak

irreversible inhibitor of the $\alpha$-glucosidase[12]). Irreversible inhibition by the aziridines most likely results from an acyloxylating aziridine ring opening. Also 1-epivalidamine (**32**) proved only a millimolar inhibitor of all three enzymes, approximately as potent as its epimer **33** [58].

Table 2. *Inhibition of the β-Glucosidases from Sweet Almonds, the β-Glucosidase from* Caldocellum saccharolyticum *and the α-Glucosidase from Brewer's Yeast* ([$IC_{50}$] in mM at pH 6.8) *by the Diaziridines* **6** *and* **9**, *the Diazirine* **7**, *the Aziridines* **21** *and* **25**, *Epivalidamine* (**32**), *Validamine* (**33**) [58], *and the Cyclopentylamine* **34** [61]

| Inhibitor ($pK_{HA}$) | β-Glucosidase (sweet almonds) | β-Glucosidase (*C. saccharolyticum*) | α-Glucosidase (brewer's yeast) |
|---|---|---|---|
| **6** (2.6) | no inh. at 8.8 mM | no inh. at 2.2 mM[a]) | no inh. at 8.8 mM |
| **9** | no inh. at 3.7 mM | [b]) | *ca.* 1.7 |
| **7** | no inh. at 5.8 mM | [b]) | no inh. at 11.6 mM |
| **21**[c]) | *ca.* 30% inh. | *ca.* 10% inh. | *ca.* 50% inh.[d]) |
| **25** (6.8) | no inh. at 1.3 mM | 3[e]) (irreversible) | *ca.* 1[f]) |
| **32** (8.4) | 1.7 | 1.0 | 0.7 |
| **33**[g]) | 1.5 | – | 0.58 |
| **34**[h]) (7.9) | 0.0034[e]) | 0.00018[e]) | 0.013[e]) |

[a]) At pH 4.2, *ca.* 20% inhibition at 6.6 mM. [b]) Not determined. [c]) *Ca.* 100 μM solution. [d]) After 20 min, irreversible. [e]) $K_i$ Value. [f]) At pH 5.0: no inhibition at 1.3 mM. g) Data from [58]. h) Inhibition data from [61a].

The very low inhibition by the diaziridines, aziridines, and epivalidamine suggests that structural factors rather than basicity[13]) are at the root of the weak interaction of the glycosidases tested. This is highlighted by the striking differences in the inhibitory action of the cyclohexane derivative **32** (($IC_{50}$ *ca.* 1 mM; $pK_{HA} = 8.4$) and the cyclopentane derivative **34** (*Scheme 3*) [60] ($K_i$ between 0.2 and 13 μM [61]; $pK_{HA} =$ 7.9 ). The small difference in the $pK_{HA}$ of the two compounds cannot account for this difference in the affinity to the enzymes. Both inhibitors will be mostly in the protonated form at pH 6.8. To further study the inhibition of the β-glucosidases from almonds by **34**, we examined the pH dependence of the inhibition between pH 5.0 and 7.8 (*Figure*). Between pH 6.2 and 7.8, the inhibition was nearly constant, but it was significantly reduced at pH 5.0. This behaviour evidences that the protonated form of **34** binds to the enzyme; it is similar to the one of β-glucosyl pyridinium ion [62].

The stronger inhibition by the cyclopentane derivative may be traced back to the ease with which it adapts a conformation with a pseudoaxial amino group, whereas the cyclohexane derivatives are fixed in a chair conformation. On the basis of stereoelectronic effects, it was proposed that β-glucosidases cleave their substrate from a conformation where a lone pair of the ring O-atom is antiperiplanar to the scissile bond [63]. Crystal-structure analyses have shown such substrate distortion in enzyme–substrate complexes for hen eggwhite lysozyme [64], the endoglucanase I from *Fusarium oxysporum* (family 7) [65], the chitobiase from *Serratia marcescens* (family 20) [66],

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[12]) The concentration of **21** in the enzyme tests was assumed 100 μM. This is an upper limit, as this concentration implies a quantitative yield of the *Birch* reduction of **20**.

[13]) Basicity does have some effect, and the lower basicity of **6** as compared to the one of **25** may contribute to the particularly low inhibition by **6**. *Legler* has reported that only glycosylamines with a $pK_{HA}$ higher than 5 are strong glycosidase inhibitors [59].

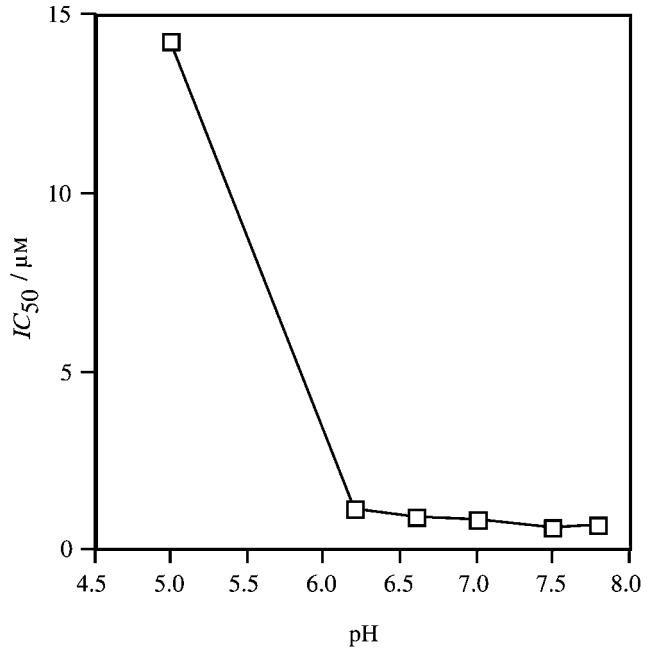


Figure. *pH Dependence of the inhibition of almond β-glucosidases by* **34**.

and the β-glycosidase Cel5A from *Bacillus agaradhaerens* (family 5) [67]. The transition state for the glycoside cleavage by these enzymes lies on the reaction coordinate between the distorted substrate and an oxycarbenium cation (with $\Phi(C(5)-O-C(1)-C(2) \approx 0^\circ)$, with the exact location of the transition state probably depending on the glycosidase family. Substrate distortion has not been demonstrated for family 1 glycosidases, but the aminocyclopentitol **34** is suggested to be a stronger inhibitor of the β-glucosidases on account of the pseudoaxial $NH_2$ group. The cyclopentylamine thereby mimics the distorted substrate and is, thus, closer to the transition state.

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## Experimental Part

*General*. Solvents were freshly distilled from $CaH_2$ ($CH_2Cl_2$, MeOH, DMF), Na/benzophenone (THF, toluene), Na ($BnNH_2$), or BaO (2,6-lutidine). Reactions were carried out under Ar, unless stated otherwise. Anal. TLC: *Merck* precoated silica-gel *60 F-254* plates; detection by treatment with a soln. of 5% $(NH_4)_6Mo_7O_{26} \cdot 4\,H_2O$, 0.1% $Ce(SO_4)_2 \cdot H_2O$ in 10% $H_2SO_4$ soln. Flash chromatography (FC): silica gel *60* (40–63 μm). M.p.: uncorrected. Optical rotations: 1-dm cell, 589 nm. FT-IR Spectra: absorption in $cm^{-1}$. NMR Spectra: chemical shifts in ppm relative to TMS; coupling constants in Hz. FAB-MS in 3-nitrobenzyl alcohol (NOBA) matrix. HR-MALDI-MS: in 2,5-dihydroxybenzoic acid (DHB) matrix. The $pK_{HA}$ values of **25**, **32**, and **34** were determined by potentiometric titration in $H_2O$. The $pK_{HA}$ value of **6** was estimated as the pH, at which $c(\mathbf{6}) = c(\mathbf{6} \cdot HCl)$ as described in [62]. The β-glucosidases from almonds (*Fluka*), the β-glucosidase from

*Caldocellum saccharolyticum* (*Sigma*), and the $\alpha$-glucosidase from brewer's yeast (*Sigma*) were used without further purification.

*(2*R*,3*S*,4*R*,5*R*)-2,3,4-Trihydroxy-5-(hydroxymethyl)cyclohexanone* (**4**). A suspension of **3** (2.0 g, 5.96 mmol) in MeCN (30 ml) and $H_2O$ (6 ml) was treated with NBS (1.6 g, 8.98 mmol), stirred for 15 h, and evaporated. FC (90 g of silica gel; AcOEt/i-PrOH/$H_2O$ 8:2:1) gave **4** (520 mg, 50%) as a brown amorphous solid. A pure sample (14 mg) was obtained by reversed-phase *C8* HPLC ($H_2O$) and lyophilisation. $R_f$ (PrOH/AcOH/$H_2O$ 4:1:1) 0.53. $^1$H-NMR (200 MHz, $D_2O$): 4.25 (*d*, $J = 10$, H–C(2)); 3.84–3.60 (*m*, H–C(4), CH–C(5), CH′–C(5)); 3.50–3.30 (*m*, H–C(3)); 2.63–2.41 (*m*, 2 H–C(6)); 2.00–1.44 (*m*, H–C(5)). $^{13}$C-NMR (50 MHz, $D_2O$): 212.9 (*s*, C(1)); 81.1, 80.8, 74.4 (3*d*, C(2), C(3), C(4)); 64.3 (*t*, $CH_2$–C(5)); 43.6 (*d*, C(5)); 41.7 (*t*, C(6)). CI-MS ($NH_3$): 194 (77, $[M + NH_4]^+$), 176 (7, $M^+$), 141 (26, $[M + 1 - 2\ H_2O]^+$), 123 (100, $[M + 1 - 3\ H_2O]^+$), 110 (75), 98 (16), 86 (17), 73 (22), 55 (19).

*(2*R*,3*S*,4*R*,5*R*)-2,3,4-Tris(trimethylsilyloxy)-5-[(trimethylsilyloxy)methyl]cyclohexanone* (**5**). At 25°, a soln. of **4** (630 mg, 3.59 mmol) was treated with HMDS (10 ml, 48.0 mmol) and $Me_3SiCl$ (5 ml, 39.5 mmol), stirred for 6.5 h, evaporated, and dried in h.v. The residue was digested with $CH_2Cl_2$ (3 × 50 ml) and filtered. The org. phase was washed with ice-cold $H_2O$ (75 ml), dried ($MgSO_4$), and evaporated. FC (30 g of silica gel; hexane/AcOEt/$Et_3N$ 400:10:0.4) of the residue (1.28 g, brown oil) gave **5** (448 mg, 27%). Colourless oil. $R_f$ (hexane/AcOEt 9:1) 0.63. $^1$H-NMR (300 MHz, $CDCl_3$): 4.03 (*dd*, $J = 9.5, 1.1$, H–C(2)); 3.76 (*t*, $J = 9.8$, H–C(4)); 3.76 (*dd*, $J = 10.0, 3.1$, CH–C(5)); 3.47 (*dd*, $J = 10.0, 2.5$, CH–C(5)); 3.46 (*t*, $J = 9.0$, H–C(3)); 2.40 (*td*, $J = 14.0, 1.1$, $H_{ax}$–C(6)); 2.30 (*dd*, $J = 14.0$, 4.7, $H_{eq}$–C(6)); 1.70–1.56 (*m*, H–C(5)); 0.19, 0.15, 0.14, 0.09 (4*s*, 4 $Me_3Si$). $^{13}$C-NMR (75 MHz, $CDCl_3$): 207.24 (*s*, C=O); 80.20, 79.76, 73.50 (3*d*, C(2), C(3), C(4)); 61.76 (*t*, $CH_2$–C(5)); 41.57 (*t*, C(6)); 39.45 (*d*, C(5)). FAB-MS (NOBA): 465 (14, $[M + 1]^+$), 464 (31, $M^+$), 447 (42, $[M - OH]^+$), 359 (68, $[M - TMS - O_2]^+$), 331 (32), 305 (13).

*(4*S*,5*S*,6*R*,7*R*)-7-(Hydroxymethyl)-1,2-diazaspiro[2.5]octane-4,5,6-triol* (**6**). At −20°, a soln. of **5** (260 mg, 0.56 mmol) in MeOH (15 ml) was saturated with $NH_3$, treated dropwise with a soln. of hydroxylamine-*O*-sulfonic acid (63 mg, 0.56 mmol) in MeOH (5 ml), stirred for 3 h, allowed to warm to r.t., and stirred overnight. Filtration, evaporation, and FC (60 g of silica gel; AcOEt/i-PrOH/$H_2O$ 4:2:1) gave a colourless soln. of **6**. After removal of most of the AcOEt *in vacuo* below 30°, this soln. was applied to an ion-exchange column (5 ml of *Dowex 50-WX-8*, $H^+$ form, washed with MeOH (50 ml) and $H_2O$ to pH 7). After washing with $H_2O$ (200 ml), elution with 2% aq. $NH_3$, and lyophilisation gave colourless, amorphous **6** (53 mg, 50%). $R_f$ (AcOEt/i-PrOH/$H_2O$ 4:2:1) 0.13. $R_f$ (acetone/$H_2O$ 2:1) 0.72. $R_f$ (i-PrOH/$H_2O$ 4:1) 0.43. M.p. 68–76°. $[\alpha]_D^{25} = 22.2$ ($c = 1.50$, $H_2O$). p$K_{HA} = 2.6$. $^1$H-NMR (300 MHz, ($D_6$)DMSO; 3:2 mixture of diastereoisomers): 4.87–4.68 (*m*, 1.4 OH); 4.61–4.52 (*m*, 1.2 OH); 4.41–4.27 (*m*, 1.4 OH); 3.61–2.85 (*m*, H–C(4), H–C(5), H–C(6), $CH_2$–C(7)); 2.30 (br. *d*, $J = 8.1$, 0.6 H), 2.24 (br. *d*, $J = 8.1$, 0.4 H), 2.13 (br. *d*, $J = 8.1$, 0.6 H), 2.07 (br. *d*, $J = 8.1$, 0.4 H) (2 NH); 1.80 (*t*, $J = 13.4$, 0.4 $H_{ax}$–C(8)); 1.60 (*t*, $J = 13.0$, 0.6 $H_{ax}$–C(8)); 1.57–1.37 (*m*, H–C(7)); 1.27–1.15 (*m*, $H_{eq}$–C(8)). $^1$H-NMR (300 MHz, ($D_6$)DMSO/$CF_3COOH$): 3.70 (*d*, $J = 9.0$, H–C(4)); 3.56 (*dd*, $J = 10.6$, 3.1, CH–C(7)); 3.38 (*dd*, $J = 10.6$, 6.2, CH′–C(7)); 3.16 (*t*, $J = 9.0$, H–C(6)); 3.06 (*t*, $J = 9.0$, H–C(5)); 1.95 (br. *t*, $J = 14.9$, $H_{ax}$–C(8)); 1.52–1.47 (*m*, H–C(7), $H_{eq}$–C(8)). $^1$H-NMR (500 MHz, $CD_3OD$; 4:3 mixture of diastereoisomers): 3.74–3.63 (*m*, 2.57 H); 3.48 (*d*, $J = 9.1$, 0.43 H–C(4)); 3.38–3.34 (*m*, 1.43 H); 3.19 (*t*, $J = 9.3$, 0.57 H); 2.04 (*t*, $J = 13.6$, 0.43 H–C(8)); 1.89 (*t*, $J = 13.3$, 0.57 H–C(8)); 1.80–1.71 (*m*, 0.57 H–C(7)); 1.71–1.63 (*m*, 0.43 H–C(7)); 1.37 (*td*, $J = 14.5$, 3.9, H′–C(8)). $^{13}$C-NMR (75 MHz, $D_2O$; *ca.* 1:1 mixture of diastereoisomers): 80.37, 79.87 (2*d*); 75.50, 75.21 (2*d*); 72.19 (*d*); 64.70 (*t*, $CH_2$–C(7)); 60.02 (*s*, C(3)); 43.78, 43.50 (2*d*, C(7)); 34.91 (*t*, C(8)). ESI-MS: 445 (4, $[2M + 1 + 2\ MeOH]^+$), 419 (8, $[2M + K]^+$), 403 (100, $[2M + Na]^+$), 381 (12, $[2M + 1]^+$), 360 (6), 338 (4), 245 (16, $[M + Na + MeOH]^+$), 229 (10, $[M + K]^+$), 213 (26, $[M + Na]^+$), 191 (23, $[M + 1]^+$). HR-FAB-MS (NOBA): 213.0858 ($C_7H_{14}N_2NaO_4^+$, $[M + Na]^+$; calc. 213.0851), 191.1026 ($C_7H_{15}N_2O_4^+$, $[M + H]^+$; calc. 191.1027).

*(4*S*,5*S*,6*R*,7*R*)-7-(Hydroxymethyl)-1,2-diazaspiro[2.5]oct-1-ene-4,5,6-triol* (**7**). A soln. of $I_2$ (*ca.* 30 mg) in MeOH (1 ml) was added dropwise at 25° to a soln. of **6** (55 mg, 0.29 mmol) in MeOH (8 ml) and $Et_3N$ (0.1 ml, 0.72 mmol) until the brown colour persisted. Evaporation and FC (20 g of silica gel; $CH_2Cl_2$/MeOH 9:1) gave a mixture of **7** and $Et_3N$ (2.5:1, 60 mg). This mixture was applied to an ion-exchange column (7 ml of *Dowex-CCR-2*, $H^+$ form, washed with MeOH (100 ml) and $H_2O$ to pH 7). Elution with $H_2O$ and lyophilisation gave **7** (48.8 mg, 89%). Colourless syrup. $R_f$ (AcOEt/i-PrOH/$H_2O$ 4:2:1) 0.75. $[\alpha]_D^{25} = 37.2$ ($c = 1.03$, $H_2O$). UV ($H_2O$): 340 (68). $^1$H-NMR (300 MHz, $D_2O$): 3.86 (*d*, $J = 9.3$, H–C(4)); 3.77 (*dd*, $J = 11.2, 3.1$, CH–C(7)); 3.69–3.66 (*m*, CH′–C(7)); 3.54 (*t*, $J = 9.3$, H–C(5)); 3.48 (*t*, $J = 9.3$, H–C(6)); 2.00–1.85 (*m*, H–C(7), $H_{ax}$–C(8)); 0.72 (*dd*, $J = 13.1$, 2.8, $H_{eq}$–C(8)). $^{13}$C-NMR (75 MHz, $D_2O$): 80.60, 75.38, 71.93 (3*d*, C(4), C(5), C(6)); 64.68 (*t*, $CH_2$–C(7)); 43.46 (*d*, C(7)); 32.34 (*s*, C(3)); 31.76 (*t*, C(8)). HR-ESI-MS (MeOH): 211.069 ($C_7H_{12}NaN_2O_4^+$, $[M + Na]^+$; calc. 211.0695).

*(4S,5S,6R,7R)-4,5,6-Tris(acetoxy)-7-[(acetoxy)methyl]-1,2-diazaspiro[2.5]oct-1-ene* (**8**). At 0°, a soln. of a mixture of **7** and $Et_3N$ (84 mg, prepared as described above from **6** (79 mg, 0.42 mmol)) in pyridine (10 ml) was treated with $Ac_2O$ (1 ml), allowed to warm to r.t. overnight, and poured into ice-water (50 ml). The aq. phase was extracted with $CH_2Cl_2$ (4 × 50 ml). Drying ($Na_2SO_4$), evaporation, and FC (30 g of silica gel; hexane/AcOEt 3:1) gave **8** (124 mg, 84%). Colourless oil. $R_f$ (hexane/AcOEt 3:1) 0.15. $[\alpha]_D^{25} = 47.4$ ($c = 1.54$, $CHCl_3$). UV ($CH_2Cl_2$): 229 (130), 334 (83). FT-IR (1.5%, $CHCl_3$): 3038*w*, 2954*w*, 1751*s*, 1594*w*, 1443*w*, 1377*m*, 1248*s*, 1131*w*, 1064*m*, 1033*m*, 978*w*, 932*w*, 896*w*, 846*w*. $^1$H-NMR (300 MHz, $CDCl_3$): 5.33 (*t*, $J = 9.7$, H–C(5)); 5.15 (*d*, $J = 9.7$, H–C(4)); 5.13 (*dd*, $J = 10.6$, 9.3, H–C(6)); 4.07 (*dd*, $J = 11.5$, 5.6, CH–C(7)); 3.90 (*dd*, $J = 11.5$, 3.1, CH′–C(7)); 2.40–2.29 (*m*, H–C(7)); 2.05, 2.03, 1.98, 1.87 (4*s*, 4 AcO); 2.02 (*dd*, $J = 15.1$, 9.0, $H_{ax}$–C(8)); 0.79 (*dd*, $J = 15.1$, 4.4, $H_{eq}$–C(8)). $^{13}$C-NMR (75 MHz, $CDCl_3$): 170.86, 170.11, 170.00, 169.03 (4*s*, 4 C=O); 73.69, 71.07, 68.26 (3*d*, C(4), C(5), C(6)); 62.66 (*t*, $CH_2$–C(7)); 37.28 (*d*, C(7)); 29.67 (*t*, C(8)); 26.75 (*s*, C(3)); 20.68, 20.58, 20.55, 20.11 (4*q*, 4 Me). FAB-MS (NOBA): 379 (11, $[M+Na]^+$), 357 (24, $[M+1]^+$), 297 (9, $[M-AcO]^+$), 269 (100, $[M-AcO-N_2]^+$), 227 (21), 208 (11), 167 (93). Anal. calc. for $C_{15}H_{20}N_2O_8$ (356.33): C 50.56, H 5.66, N 7.86; found: C 50.80, H 5.58, N 7.64.

*(1R,2R,3S,4S,5S,6R,7R)-1-Benzyl-7-(hydroxymethyl)-1,2-diazaspiro[2.5]octane-4,5,6-triol* (**9**). A soln. of **5** (466 mg, 1.0 mmol) in MeOH (30 ml) was treated at 25° with $BnNH_2$ (4.5 ml, 41.2 mmol), stirred for 1 h, cooled to 0°, treated dropwise with a soln. of hydroxylamine-*O*-sulfonic acid (116 mg, 1.00 mmol) in MeOH (5 ml), allowed to warm to r.t. and stirred overnight. Filtration, evaporation, and repeated FC (50 g of silica gel; AcOEt/MeOH 1:0 → 4:1) gave a mixture of **9** and $BnNH_2$ (1:4, 487 mg), which was dissolved in $H_2O$, applied on an ion-exchange column (15 g of *Dowex-CCR-2*, $H^+$ form, washed with MeOH (100 ml) and $H_2O$ to pH 7), and eluted with $H_2O$. Lyophilisation gave colourless, amorphous **9** (76 mg, 27%). $R_f$ (AcOEt/i-PrOH/$H_2O$ 4:2:1) 0.51. M.p. 52–58°. $[\alpha]_D^{25} = 59.7$ ($c = 1.53$, $H_2O$). $^1$H-NMR (300 MHz, $D_2O$): 7.49–7.38 (*m*, 5 arom. H); 3.92 (*d*, $J = 14.0$, irrad. at 2.04 → NOE of 4%, PhC*H*); 3.81 (*d*, $J = 14.0$, irrad. at 2.04 → NOE of 6%, PhC*H*); 3.75 (*dd*, $J = 11.5$, 3.4, CH–C(7)); 3.64, (*dd*, $J = 11.5$, 5.9, CH′–C(7)); 3.62 (*d*, $J = 9.3$, H–C(4)); 3.43 (*t*, $J = 9.0$, irrad. at 3.62 → NOE of 9%, H–C(6)); 3.36 (*t*, $J = 9.0$, H–C(5)); 2.04 (*dd*, $J = 14.6$, 4.1, irrad. at 3.92 and 3.81 → NOE of 6%, $H_{eq}$–C(8)); 1.93 (br. *t*, $J = 14.0$, irrad. at 3.62 → NOE of 4%, $H_{ax}$–C(8)); 1.53–1.40 (*m*, irrad. at 3.81 and 3.75 → NOE of 8%, H–C(7)). $^{13}$C-NMR (75 MHz, $CD_3OD$): 140.07 (*s*); 129.83 (*d*, 2 C); 129.65 (*d*, 2 C); 128.52 (*d*); 79.66, 74.45, 73.10 (3*d*, C(4), C(5), C(6)); 63.96 (*t*, $CH_2$–C(7)); 62.87 (*s*, C(3)); 57.19 (*t*, Ph$CH_2$); 43.02 (*d*, C(7)); 27.44 (*t*, C(8)). HR-MALDI-MS (DHB): 303.1310 ($C_{14}H_{20}NaN_2O_4^+$, $[M+Na]^+$; calc. 303.1316), 281.1492 ($C_{14}H_{21}N_2O_4^+$, $[M+H]^+$; calc. 281.1496). Anal. calc. for $C_{14}H_{20}N_2O_4 \cdot 0.25H_2O$: C 59.04, H 7.26, N 9.84; found: C 58.79, H 7.31, N 10.00.

*(4S,5S,6R,7R)-4,5,6-Tris(benzyloxy)-7-[(benzyloxy)methyl]-1,2-diazaspiro[2.5]octane* (**11**). At −20°, a suspension of **10** [15] (115 mg, 0.21 mmol) in MeOH (5 ml) was saturated with $NH_3$, treated dropwise with a soln. of hydroxylamine-*O*-sulfonic acid (24.3 mg, 0.21 mmol) in MeOH (2.5 ml), stirred for 3 h, allowed to warm to r.t., and stirred overnight. Filtration, evaporation, and FC (17 g of silica gel; hexane/AcOEt 3:1) gave **11** (41 mg, 35%). Colourless, amorphous. $R_f$ (hexane/AcOEt 1:1) 0.49. M.p. 76–78°. $[\alpha]_D^{25} = 29.4$ ($c = 0.98$, $CHCl_3$). FT-IR (1%, $CHCl_3$): 3259*w*, 3089*w*, 3066*w*, 3008*s*, 2908*m*, 2862*m*, 1951*w*, 1876*w*, 1811*w*, 1730*w*, 1603*w*, 1497*m*, 1454*s*, 1404*w*, 1358*s*, 1274*w*, 1175*m*, 1149*s*, 1097*s*, 1028*s*, 1016*w*, 913*w*, 872*w*, 855*w*. $^1$H-NMR (200 MHz, $CDCl_3$, 4:1 mixture of diastereoisomers) 7.35–7.22 (*m*, 20 arom. H); 5.00 (*d*, $J = 10.0$, PhC*H*); 4.91 (*d*, $J = 10.8$, PhC*H*); 4.89 (*d*, $J = 11.6$, PhC*H*); 4.82 (*d*, $J = 10.8$, PhC*H*); 4.70 (*d*, $J = 10.4$, PhC*H*); 4.59 (*d*, $J = 11.2$, PhC*H*); 4.47 (*s*, Ph$CH_2$); 3.90 (*d*, $J = 9.6$, H–C(4)); 3.77 (*dd*, $J = 9.1$, 3.7, CH–C(7)); 3.69 (*t*, $J = 10.0$, H–C(6)); 3.56 (*t*, $J = 9.6$, H–C(5)); 3.48 (*dd*, $J = 9.1$, 2.5, CH′–C(7)); 2.64 (br. *d*, $J = 7.9$, 0.8 H, exchange with $D_2O$, NH); 2.26–2.06 (0.2 H, exchange with $D_2O$, NH); 2.26 (br. *t*, $J = 12.9$, $H_{ax}$–C(8)); 2.19–2.06 (*m*, H–C(7)); 1.57 (*d*, $J = 7.9$, 0.8 H, exchange with $D_2O$, NH); 1.44 (*d*, $J = 8$, 0.2 H, exchange with $D_2O$, NH); 1.35 (*dd*, $J = 12.9$, 2.9, $H_{eq}$–C(8)). $^{13}$C-NMR (75 MHz, $CDCl_3$, one set of signals): 139.00, 138.88, 138.62, 138.50 (4*s*); 128.62–127.79 (several *d*); 88.20, 80.33, 79.49 (3*d*, C(4), C(5), C(6)); 76.38, 76.01, 75.51, 73.21 (4*t*, 4 Ph$CH_2$); 69.42 (*t*, $CH_2$–C(7)); 57.06 (*s*, C(3)); 39.96 (*d*, C(7)); 34.80 (*t*, C(8)). FAB-MS (NOBA): 551 (100, $[M+1]^+$), 534 (14, $[M-NH_2]^+$), 531 (10), 461 (18), 459 (22, $[M-Bn]^+$), 443 (96, $[M-BnO]^+$), 428 (27). Anal. calc. for $C_{35}H_{38}N_2O_4$ (550.70): C 76.34, H 6.95, N 5.09; found: C 76.04, H 6.66, N 4.98.

*(4S,5S,6R,7R)-4,5,6-Tris(benzyloxy)-7-[(benzyloxy)methyl]-1,2-diazaspiro[2.5]oct-1-ene* (**12**). At 0°, a soln. of **11** (61 mg, 0.11 mmol) in MeOH (10 ml) and $CH_2Cl_2$ (10 ml) was treated with $Et_3N$ (0.1 ml, 0.72 mmol) and, dropwise, with a soln. of $I_2$ (*ca.* 30 mg) in MeOH (1 ml), until the brown colour persisted. Evaporation and FC (10 g of silica gel; hexane/AcOEt 10:1) gave **12** (52 mg, 85%). Colourless, amorphous. $R_f$ (hexane/AcOEt 3:1) 0.73. M.p. 59–61°. $[\alpha]_D^{25} = 49.7$ ($c = 1.03$, $CHCl_3$). UV ($CH_2Cl_2$): 232 (846), 252 (846), 258 (906), 340 (92). FT-IR (1%, $CHCl_3$): 3089*w*, 3066*w*, 3007*m*, 2907*m*, 2864*m*, 2791*w*, 1950*w*, 1869*w*, 1810*w*, 1750*w*, 1590*m* , 1558*w*, 1497*m*, 1454*s*, 1400*w*, 1357*m*, 1329*w*, 1309*w*, 1150*m*, 1131*s*, 1096*s*, 1043*s*, 1028*s*, 1005*m*, 912*w*, 868*w*. $^1$H-NMR

(300 MHz, $CDCl_3$): 7.37–7.19 (*m*, 20 arom. H); 4.93 (*d*, *J* = 10.9, PhC*H*); 4.88 (*d*, *J* = 10.9, PhC*H*); 4.82 (*d*, *J* = 10.9, PhC*H*); 4.57 (*d*, *J* = 10.9, PhC*H*); 4.42 (*s*, PhC$H_2$); 4.26 (*d*, *J* = 11.2, PhC*H*); 4.21 (*d*, *J* = 11.2, PhC*H*); 3.82 (*t*, *J* = 9.2, H–C(5)); 3.70 (*d*, *J* = 9.2, H–C(4)); 3.65–3.57 (hidden by other signals, H–C(6)); 3.63 (*dd*, *J* = 9.0, 4.4, CH–C(7)); 3.42 (*dd*, *J* = 9.0, 3.4, CH′–C(7)); 2.13–2.05 (*m*, H–C(7)); 2.05 (br. *t*, *J* = 12.8, $H_{ax}$–C(8); 0.67 (br. *d*, *J* = 10.6, $H_{eq}$–C(8)). $^{13}$C-NMR (75 MHz, $CDCl_3$): 138.94, 138.79, 138.49, 137.39 (4*s*); 128.67–127.75 (several *d*); 86.39, 80.55, 78.10 (3*d*, C(4), C(5), C(6)); 75.86, 75.57, 73.23, 73.19 (4*t*, 4 PhC$H_2$); 69.46 (*t*, C$H_2$–C(7)); 39.66 (*d*, C(7)); 30.21 (*t*, C(8)); 28.82 (*s*, C(3)). FAB-MS (NOBA): 549 (60, $[M+1]^+$), 457 (15, $[M-Bn]^+$), 441 (18, $[M-BnO]^+$), 413 (22, $[M-BnO-N_2]^+$), 307 (33). Anal. calc. for $C_{35}H_{36}N_2O_4$ (548.68): C 76.62, H 6.61, N 5.11; found: C 76.75, H 6.73, N 4.88.

*(1*S*,2*S*,3*R*,4*R*)-1,2,3-Tris(benzyloxy)-4-[(benzyloxy)methyl]-6-methylidenecyclohexane* (**13**). A soln. of **10** (1.17 g, 2.18 mmol) in THF (20 ml) was treated dropwise at −20° with a soln. of $Ph_3P{=}CH_2$ (prepared at 0° from $Ph_3PMeBr$ (1.17 g, 3.27 mmol) and BuLi (2.05 ml, 1.6M in hexane) in THF (15 ml)), allowed to warm slowly for 3 h, treated with acetone (5 ml), and poured into sat. aq. $NH_4Cl$ soln. (150 ml). The aq. phase was extracted with $CH_2Cl_2$ (3 × 100 ml), and the extract was dried ($Na_2SO_4$), and evaporated. FC (150 g of silica gel; cyclohexane/AcOEt 20:1) gave **13** (0.90 g, 71%). Colourless oil. $R_f$ (cyclohexane/AcOEt 3:1) 0.75. $[\alpha]_D^{25} = 56.7$ (*c* = 1.53, $CHCl_3$). FT-IR (1.5%, $CHCl_3$): 3089*w*, 3066*m*, 3008*m*, 2908*m*, 2862*m*, 2789*w*, 1951*w*, 1876*w*, 1811*w*, 1657*w*, 1605*w*, 1496*m*, 1454*s*, 1400*w*, 1356*m*, 1309*w*, 1149*m*, 1100*s*, 1028*s*, 912*m*, 865*w*. $^1$H-NMR (300 MHz, $CDCl_3$): 7.45–7.24 (20 arom. H); 5.22 (br. *d*, *J* = 0.9, CH=C(6)); 5.05 (*d*, *J* = 10.9, PhC*H*); 4.99 (*d*, *J* = 1.6, CH′=C(6)); 4.96 (*d*, *J* = 10.9, PhC*H*); 4.86 (*d*, *J* = 10.9, PhC*H*); 4.81 (*d*, *J* = 11.5, PhC*H*); 4.73 (*d*, *J* = 11.2, PhC*H*); 4.58 (*d*, *J* = 10.9, PhC*H*); 4.50 (*s*, PhC$H_2$); 3.96 (br. *d*, *J* = 9.3, H–C(1)); 3.66 (*dd*, *J* = 9.0, 1.9, CH–C(4)); 3.64 (*dd*, *J* = 9.0, 7.2, H–C(3)); 3.56 (*dd*, *J* = 9.0, 2.8, CH′–C(4)); 3.53 (*t*, *J* = 9.0, H–C(2)); 2.48 (*dd*, *J* = 13.4, 4.4, $H_{eq}$–C(5)); 2.16 (br. *t*, *J* = 13.4, $H_{ax}$–C(5)); 1.84–1.75 (*m*, H–C(4)). $^{13}$C-NMR (75 MHz, $CDCl_3$): 144.38 (*s*, C(6)); 139.34 (*s*); 139.09 (*s*); 138.83 (*s*, 2 C); 128.65–127.72 (several *d*); 108.28 (*t*, C$H_2$=C(6)); 88.37, 83.82, 81.23 (3*d*, C(1), C(2), C(3)); 75.81, 75.44, 73.68, 73.26 (4*t*, 4 PhC$H_2$); 70.31 (*t*); 43.36 (*d*, C(4)); 33.95 (*t*, C(5)). FAB-MS (NOBA): 535 (3, $[M+1]^+$), 335 (9), 280 (15), 279 (17), 263 (13), 262 (10), 221 (13), 207 (22). Anal. calc. for $C_{36}H_{38}O_4$ (534.69): C 80.87, H 7.16; found: C 80.60, H 7.17.

*(3*S*,4*S*,5*S*,6*R*,7*R*)-4,5,6-Tris(benzyloxy)-7-[(benzyloxy)methyl]-1-[(4-methylphenyl)sulfonyl]-1-azaspiro[2.5]octane* (**14**) and *4-Methyl*-N*-{[(1*S*,2*R*,3*S*,4*R*,5*R*)-2,3,4-tris(benzyloxy)-5-[(benzyloxy)methyl]-1-bromocyclohexyl]methyl}benzenesulfonamide* (**15**). A soln. of **13** (500 mg, 0.94 mmol) in MeCN (50 ml) was treated with chloramine T (2.13 g, 9.35 mmol) and $PhMe_3N \cdot Br_3$ (123 mg, 0.33 mmol). The mixture was stirred for 4.5 h, diluted with AcOEt (250 ml), washed with $H_2O$ (150 ml) and brine (150 ml), dried ($Na_2SO_4$), and evaporated. FC (80 g of silica gel; cyclohexane/AcOEt 9:1) of the residue (1.2 g) gave a mixture of **14** and **15** (283 mg), which was separated by prep. HPLC (silica gel; toluene/AcOEt 40:1) to yield **14** (120 mg, 18%) and **15** (15 mg, 2%).

*Data of* **14**: Colourless oil. $R_f$ (toluene/AcOEt 10:1) 0.47. Prep. HPLC (toluene/AcOEt 40:1): $t_R$ 22.8 min. $[\alpha]_D^{25} = -9.2$ (*c* = 1.56, $CHCl_3$). FT-IR (1.5%, $CHCl_3$): 3064*w*, 3008*m*, 2866*m*, 1952*w*, 1810*w*, 1599*w*, 1496*w*, 1454*m*, 1357*m*, 1321*s*, 1158*s*, 1099*s*, 995*s*, 910*w*, 859*m*. $^1$H-NMR (300 MHz, $CDCl_3$): 7.84 (*d*, *J* = 8.4, 2 arom. H); 7.40–7.08 (22 arom. H); 4.87 (*d*, *J* = 10.6, PhC*H*); 4.84 (*d*, *J* = 10.0, PhC*H*); 4.74 (*d*, *J* = 10.6, PhC*H*); 4.73 (*d*, *J* = 10.3, PhC*H*); 4.59 (*d*, *J* = 10.3, PhC*H*); 4.53 (*d*, *J* = 10.9, PhC*H*); 4.51 (*d*, *J* = 11.8, PhC*H*); 4.44 (*d*, *J* = 12.1, PhC*H*); 3.78 (*d*, *J* = 9.3, H–C(4)); 3.70 (*dd*, *J* = 9.0, 4.4, CH–C(7)); 3.63 (*dd*, *J* = 9.3, 10.6, irrad. at 3.78 → NOE of 3%, H–C(6)); 3.51 (*dd*, *J* = 9.0, 2.5, CH′–C(7)); 3.39 (*t*, *J* = 9.2, irrad. at 2.63 → NOE of 2%, H–C(5)); 2.63 (br. *s*, irrad. at 3.39 → NOE of 1.5%, H–C(2)); 2.59 (br. *s*, irrad. at 2.63 → NOE of 19%, H′–C(2)); 2.45 (*s*, Me); 2.42–2.35 (*m*, irrad. at 3.78 → NOE of 1%, irrad. at 2.59 → NOE of 1%, 2 H–C(8)); 1.88–1.78 (*m*, irrad. at 3.39 → NOE of 9%, irrad. at 2.59 → NOE of 1%, H–C(7)). $^{13}$C-NMR (75 MHz, $CDCl_3$): 144.25 (*s*); 138.83, 138.55, 138.16 (3*s*, 1*s* hidden by noise or other signals); 129.82–127.58 (several *d*); 86.49, 80.96, 80.18 (3*d*, C(4), C(5), C(6)); 76.11, 75.93, 75.54, 73.35 (4*t*, 4 PhC$H_2$); 69.50 (*t*, C$H_2$–C(7)); 51.73 (*s*, C(3)); 41.58 (*d*, C(7)); 35.82 (*t*, C(8)); 28.77 (*t*, C(2)); 21.65 (*q*, Me). MALDI-MS (DHB): 742 (67, $[M+K]^+$), 726 (100, $[M+Na]^+$). Anal. calc. for $C_{43}H_{45}NO_6S$ (703.90): C 73.37, H 6.44, N 1.99; found: C 73.36, H 6.44, N 1.96.

*Data of* **15**: Colourless oil. $R_f$ (toluene/AcOEt 10:1) 0.45. Prep. HPLC (toluene/AcOEt 40:1): $t_R$ 27.2 min. $^1$H-NMR (300 MHz, $CDCl_3$): 7.56 (*d*, *J* = 8.4, 2 arom. H); 7.40–7.18 (22 arom. H); 4.94 (*d*, *J* = 10.9, PhC*H*); 4.92 (*d*, *J* = 11.8, PhC*H*); 4.89 (*d*, *J* = 11.2, PhC*H*); 4.87 (*d*, *J* = 10.9, PhC*H*); 4.73 (*d*, *J* = 12.1, PhC*H*); 4.55 (*d*, *J* = 10.9, PhC*H*); 4.44 (*s*, PhC$H_2$); 4.03 (*t*, *J* = 9.2, H–C(3)); 3.70 (*dd*, *J* = 9.0, 4.1, CH–C(5)); 3.62 (*dd*, *J* = 7.8, 6.2, irrad.. at 3.04 → *d*, NH); 3.57 (*dd*, *J* = 10.9, 9.3, H–C(4)); 3.40 (*dd*, *J* = 9.0, 2.2, CH′–C(5)); 3.18 (*dd*, *J* = 13.4, 5.6, CH–C(1)); 3.13 (*d*, *J* = 9.0, irrad. at 4.03 → *s*, H–C(2)); 3.04 (*dd*, *J* = 13.4, 8.1, CH′–C(1)); 2.34 (*s*, Me); 2.29–2.15 (*m*, H–C(5)); 2.09–1.91 (*m*, 2 H–C(6)). FAB-MS (NOBA): 808 (9, $[M(^{81}Br)+Na]^+$), 806 (8,

$[M(^{79}Br)+Na]^+$), 786 (59, $[M(^{81}Br)+H]^+$), 784 (100, $[M(^{81}Br)-H]^+$, $[M(^{79}Br)+H]^+$), 782 (51, $[M(^{79}Br)-H]^+$), 695 (14), 694 (33), 693 (12), 692 (29), 614 (11), 613 (23), 461 (15), 460 (54).

*Iodoazidation and Reduction of* **13**. A soln. of $NaN_3$ (61 mg, 94 μmol) in MeCN (1 ml) at 0° was treated with ICl (7.3 mg, 45 μmol), stirred for 10 min, treated with **13** (20 mg, 37 μmol), allowed to warm to r.t., stirred for 20 h, and poured into $H_2O$ (20 ml). The aq. phase was extracted with AcOEt (2 × 20 ml), and the combined org. phases were washed with 5% $Na_2S_2O_3$ (20 ml) and $H_2O$ (2 × 20 ml), and dried ($Na_2SO_4$). Evaporation and FC (20 g of silica gel; cyclohexane/AcOEt 20:1) gave a mixture ($^1$H-NMR) of iodo azides (9 mg, 34%). Colourless oil. $R_f$ (toluene/AcOEt 10:1) 0.69. FT-IR (1%, $CHCl_3$): 2106*s* ($N_3$). HR-MS (MALDI): 726.1802 ($C_{36}H_{38}IN_3NaO_4^+$ $[M+Na]^+$; calc. 726.1805).

A soln. of this mixture (9 mg, 13 μmol) in THF (2 ml) was treated at 0° with $LiAlH_4$ (2.5 g, 64 μmol), stirred for 70 min, and treated with MeOH (1 ml), and 1M NaOH (0.5 ml). Filtration through *Celite*, evaporation, and FC (20 g of silica gel; cyclohexane/AcOEt 20:1) gave **13** (3 mg, 43%).

*(3*S*,4*R*,5*S*,6*R*,7*R*)- and (3*R*,4*R*,5*S*,6*R*,7*R*)-4,5,6-Tris(benzyloxy)-7-[(benzyloxy)methyl]-1-oxaspiro-[2.5]octane* (**16** and **17**, resp.). A soln. of **13** (20 mg, 37.4 mmol) in $CH_2Cl_2$ (1 ml) was treated with $NaHCO_3$ (7 mg, 84.2 mmol) and *m*CPBA (23 mg, 93.5 mmol), and stirred for 220 min. The mixture was diluted with $CH_2Cl_2$ (25 ml), washed with sat. aq. $NaHCO_3$ soln. (3 × 20 ml) and brine (20 ml), dried ($Na_2SO_4$), and evaporated. FC (20 g of silica gel; cyclohexane/AcOEt 9:1) gave **16** (5.2 mg, 25%) and **17** (61%).

*Data of* **16**: Colourless oil. $R_f$ (cyclohexane/AcOEt 3:1) 0.66. $[\alpha]_D^{25} = 36.0$ ($c = 1.32$, $CHCl_3$). FT-IR (1.3%, $CHCl_3$): 3065*w*, 3008*m*, 2922*m*, 2863*m*, 1953*w*, 1877*w*, 1811*w*, 1726*w*, 1603*w*, 1496*w*, 1454*m*, 1358*m*, 1099*s*, 841*w*. $^1$H-NMR (300 MHz, $CDCl_3$): 7.37–7.19 (20 arom. H); 4.91 (*d*, *J* = 10.9, 2 PhC*H*); 4.78 (*d*, *J* = 10.9, PhC*H*); 4.77 (*d*, *J* = 10.9, PhC*H*); 4.62 (*d*, *J* = 10.9, PhC*H*); 4.54 (*d*, *J* = 10.9, PhC*H*); 4.45 (*s*, PhC$H_2$); 3.73 (br. *d*, *J* = 9.3, H–C(4)); 3.61–3.54 (*m*, H–C(5), H–C(6), CH–C(7), irrad. at 3.18 → NOE of 1% for *t* at 3.54 of H–C(5)); 3.50 (*dd*, *J* = 8.7, 2.8, CH′–C(7)); 3.18 (*dd*, *J* = 5.3, 1.3, H–C(2)); 2.56 (*d*, *J* = 5.3, H′–C(2)); 2.09 (*td*, *J* = 13.1, 2.0, $H_{ax}$–C(8)); 1.93–1.81 (*m*, H–C(7)); 1.46 (*dd*, *J* = 13.4, 3.4, irrad. at 2.56 → NOE of 7%, $H_{eq}$–C(8)). $^{13}$C-NMR (75 MHz, $CDCl_3$): 138.81 (br. *s*); 128.62–127.79 (several *d*); 86.83, 80.75, 80.26 (3*d*, C(4), C(5), C(6)); 75.93, 75.56, 75.51, 73.23 (4*t*, 4 PhC$H_2$), 69.89 (*t*, C$H_2$–C(7)); 59.77 (*s*, C(3)); 49.61 (*t*, C(2)); 40.06 (*d*, C(7)); 32.64 (*t*, C(8)). FAB-MS (NOBA): 1213 (21), 663 (35), 647 (19), 572 (16, $[M+Na-1]^+$), 550 (44, $M^+$), 549 (20, $[M-1]^+$), 548 (84, $[M-2]^+$), 480 (20), 459 (13), 458 (30), 444 (15), 442 (36), 391 (12), 338 (14), 306 (19), 288 (15), 271 (13), 260 (11), 259 (40), 245 (16), 242 (12), 219 (47), 217 (12), 203 (13), 197 (12), 181 (100).

*Data of* **17**: Colourless, amorphous. $R_f$ (cyclohexane/AcOEt 3:1) 0.55. $[\alpha]_D^{25} = 16.5$ ($c = 1.47$, $CHCl_3$). FT-IR (1.5%, $CHCl_3$): identical to that of **16**. $^1$H-NMR (300 MHz, $CDCl_3$): 7.36–7.22 (20 arom. H); 4.94 (*d*, *J* = 10.9, PhC*H*); 4.92 (*d*, *J* = 10.9, PhC*H*); 4.88 (*d*, *J* = 10.9, PhC*H*); 4.86 (*d*, *J* = 11.5, PhC*H*); 4.61 (*d*, *J* = 11.5, PhC*H*); 4.58 (*d*, *J* = 10.9, PhC*H*); 4.43 (*s*, PhC$H_2$); 3.83 (*t*, *J* = 9.3, H–C(5)); 3.75 (*dd*, *J* = 9.0, 3.4, CH–C(7)); 3.69 (*d*, *J* = 9.6, irrad. at 2.98 → NOE of 1%, H–C(4)); 3.66 (br. *t*, *J* = 9.6, H–C(6)); 3.41 (*dd*, *J* = 9.0, 1.9, CH′–C(7)); 2.98 (*d*, *J* = 5.0, H–C(2)); 2.59 (*d*, *J* = 5.0, H′–C(2)); 2.09–2.00 (*m*, H–C(7), $H_{ax}$–C(8)); 1.36 (br. *d*, *J* = 10.0, irrad. at 2.59 → NOE of 4%, $H_{eq}$–C(8)). $^{13}$C-NMR (75 MHz, $CDCl_3$): 138.94, 138.70, 138.07 (3*s*, 1*s* hidden by other signal or noise); 128.68–127.79 (several *d*); 86.75, 80.81, 78.55 (3*d*, C(4), C(5), C(6)); 75.93, 75.47, 75.38, 73.18 (4*t*, 4 PhC$H_2$); 69.54 (*t*, C$H_2$–C(7)); 58.76 (*s*, C(3)); 49.70 (*t*, C(2)); 39.80 (*d*, C(7)); 32.17 (*t*, C(8)). FAB-MS (NOBA): 662 (16), 646 (12), 572 (22, $[M+Na-1]^+$), 550 (32, $M^+$), 549 (26, $[M-1]^+$), 548 (77, $[M-2]^+$), 459 (10), 458 (24), 442 (28), 335 (9), 288 (8), 271 (17), 260 (8), 259 (32), 245 (14), 241 (8), 219 (32), 217 (8), 203 (9), 197 (11), 181 (100). Anal. calc. for $C_{36}H_{38}O_5$ (550.69): C 78.52, H 6.95; found: C 78.67, H 6.99.

*(1*S*,2*R*,3*S*,4*R*,5*R*)-1-(Azidomethyl)-2,3,4-tris(benzyloxy)-5-[(benzyloxy)methyl]cyclohexan-1-ol* (**18**). A soln. of **16** (174 mg, 0.32 mmol) in DMF (25 ml) was treated with $NaN_3$ (255 mg, 3.93 mmol), stirred at 100° for 20 h, cooled, diluted with AcOEt (200 ml), washed with $H_2O$ (100 ml) and brine (100 ml), dried ($Na_2SO_4$), and evaporated. FC (40 g of silica gel; cyclohexane/AcOEt 9:1) gave colourless, amorphous **18** (170 mg, 91%). $R_f$ (cyclohexane/AcOEt 3:1) 0.52. M.p. 79–80°. $[\alpha]_D^{25} = 16.2$ ($c = 1.50$, $CHCl_3$). FT-IR (1.5%, $CHCl_3$): 3555*w*, 3066*w*, 3008*m*, 2867*m*, 2107*s*, 1954*w*, 1812*w*, 1589*w*, 1496*w*, 1453*m*, 1359*m*, 1282*m*, 1065*s*, 912*w*, 871*w*. $^1$H-NMR (300 MHz, $CDCl_3$): 7.38–7.19 (20 arom. H); 4.91 (*d*, *J* = 11.5, PhC*H*); 4.87 (*d*, *J* = 10.9, PhC*H*); 4.85 (*s*, PhC$H_2$); 4.79 (*d*, *J* = 10.6, PhC*H*); 4.54 (*d*, *J* = 10.6, PhC*H*); 4.46 (*s*, PhC$H_2$); 3.74 (br. *d*, *J* = 12.8, 1 H, $CH_2N_3$); 3.66 (*dd*, *J* = 9.0, 4.1, CH–C(5)); 3.62–3.49 (*m*, H–C(2), H–C(3), H–C(4)); 3.45 (*dd*, *J* = 9.0, 2.5, CH′–C(5)); 3.34 (*d*, *J* = 12.8, 1 H, $CH_2N_3$); 2.13 (*s*, OH); 2.06 (*dd*, *J* = 12.8, 2.5, $H_{eq}$–C(6)); 1.75–1.62 (*m*, H–C(5)); 1.58 (br. *t*, *J* = 13.1, $H_{ax}$–C(6)). $^{13}$C-NMR (75 MHz, $CDCl_3$): 138.76, 138.60 (2 br. *s*); 128.88–127.86 (several *d*); 86.81, 85.41, 81.06 (3*d*, C(2), C(3), C(4)); 76.06, 75.78 (2*t*, 2 PhC$H_2$); 75.68 (*s*, C(1)); 75.54, 73.26 (2*t*, 2 PhC$H_2$); 69.62 (*t*, C$H_2$–C(5)); 54.49 (*t*, C$H_2N_3$); 38.64 (*d*, C(5)); 33.80 (*t*, C(6)). MALDI-MS (DHB): 616 (68,

$[M+Na]^+$), 590 (34), 588 (45, $[M+Na-N_2]^+$), 573 (19), 566 (20, $[M+1-N_2]^+$), 559 (100), 480 (20, $[M+Na-N_2-BnOH]^+$), 460 (19), 458 (60, $[M+1-N_2-BnOH]^+$). Anal. calc. for $C_{36}H_{39}N_3O_5$ (593.72): C 72.83, H 6.62, N 7.08; found: C 72.65, H 6.62, N 6.91.

*(1*R*,2*R*,3*S*,4*R*,5*R*)-1-(Azidomethyl)-2,3,4-tris(benzyloxy)-5-[(benzyloxy)methyl]cyclohexan-1-ol* (**22**). As described above for **18**, **22** (186 mg, 88%) was obtained from **17** (195 mg, 0.35 mmol) and $NaN_3$ (280 mg, 4.31 mmol). Colourless oil. $R_f$ (cyclohexane/AcOEt 3:1) 0.45. $[\alpha]_D^{25}=9.1$ ($c=1.50$, $CHCl_3$). FT-IR (1.5%, $CHCl_3$): apart from a band at 3559*m* identical to the spectrum of **18**. $^1$H-NMR (300 MHz, $CDCl_3$): 7.61–7.20 (20 arom. H); 4.98 (*d*, $J=10.9$, PhC*H*); 4.95 (*d*, $J=11.2$, PhC*H*); 4.85 (*d*, $J=11.1$, 2 PhC*H*); 4.62 (*d*, $J=11.2$, PhC*H*); 4.57 (*d*, $J=10.9$, PhC*H*); 4.48 (*d*, $J=12.1$, PhC*H*); 4.44 (*d*, $J=12.1$, PhC*H*); 3.87 (*t*, $J=9.3$, H–C(3)); 3.76 (*dd*, $J=9.0$, 4.1, CH–C(5)); 3.54 (*dd*, $J=10.9$, 9.3, H–C(4)); 3.46 (*d*, $J=9.3$, H–C(2)); 3.43 (*dd*, $J=9.0$, 2.5, CH′–C(5)); 3.29 (*d*, $J=11.8$, 1 H, $CH_2N_3$); 3.22 (*d*, $J=11.8$, 1 H, $CH_2N_3$); 2.41 (*d*, $J=2.2$, OH); 2.21–2.09 (*m*, H–C(5)); 1.80 (*dd*, $J=14.3$, 4.1, $H_{eq}$–C(6)); 1.67 (*td*, $J=14.3$, 2.2, $H_{ax}$–C(6)). $^{13}$C-NMR (75 MHz, $CDCl_3$): 139.02, 138.73, 138.13 (3*s*, 1*s* hidden by noise or other signals); 128.76–127.81 (several *d*); 85.67, 81.54, 81.01 (3*d*, C(2), C(3), C(4)); 75.78 (2 *t*), 75.31 (*t*, 3 Ph*C*H$_2$); 74.67 (*s*, C(1)); 73.27 (*t*, Ph*C*H$_2$); 69.71 (*t*, *C*H$_2$–C(5)); 57.89 (*t*, $CH_2N_3$); 37.39 (*d*, C(5)); 33.02 (*t*, C(6)). MALDI-MS (DHB): 616 (47, $[M+Na]^+$), 590 (37), 588 (72, $[M+Na-N_2]^+$), 573 (24), 566 (12, $[M+1-N_2]^+$), 559 (100), 480 (18, $[M+Na-N_2-BnOH]^+$), 460 (8), 458 (14, $[M+1-N_2-BnOH]^+$). Anal. calc. for $C_{36}H_{39}N_3O_5$ (593.72): C 72.83, H 6.62, N 7.08; found: C 72.81, H 6.66, N 6.99.

*(1*S*,2*R*,3*S*,4*R*,5*R*)-1-(Azidomethyl)-2,3,4-tris(benzyloxy)-5-[(benzyloxy)methyl]cyclohexane-1-methanesulfonate* (**19**) and *(1*S*,2*S*,3*R*,4*R*)-6-(Azidomethylidene)-1,2,3-tris(benzyloxy)-4-[(benzyloxy)methyl]cyclohexane* (**26**). A soln. of **18** (165 mg, 0.28 mmol) in 2,6-lutidine (2.2 ml) was treated at 0° with MsCl (0.1 ml, 1.25 mmol) and DMAP (2 mg), stirred for 7.5 h, diluted with $Et_2O$ (120 ml), washed with cold brine (2 × 60 ml), dried ($Na_2SO_4$), and evaporated. FC (25 g of silica gel; cyclohexane/AcOEt 9:1) gave **26** (6 mg, 4%) and **19** (177 mg, 95%).

*Data of* **19**: Colourless oil. $R_f$ (toluene/AcOEt 10:1) 0.49. $^1$H-NMR (300 MHz, $CDCl_3$): 7.41–7.17 (20 arom. H); 4.98 (*d*, $J=10.9$, PhC*H*); 4.92 (*d*, $J=10.9$, PhC*H*); 4.88 (*d*, $J=10.3$, PhC*H*); 4.87 (*d*, $J=10.9$, PhC*H*); 4.84 (*d*, $J=11.2$, PhC*H*); 4.55 (*d*, $J=10.9$, PhC*H*); 4.54 (*d*, $J=12.5$, PhC*H*); 4.49 (*d*, $J=12.1$, PhC*H*); 4.38 (*d*, $J=9.3$, H–C(2)); 4.10 (br. *d*, $J=13.4$, 1 H, $CH_2N_3$); 3.67 (*dd*, $J=9.0$, 4.7, CH–C(5)); 3.66 (*t*, $J=9.2$, H–C(4)); 3.53 (*t*, $J=9.3$, H–C(3)); 3.51 (*dd*, $J=9.0$, 2.5, CH′–C(5)); 3.45 (*d*, $J=14.0$, 1 H, $CH_2N_3$); 3.02 (*s*, MsO); 2.66 (*dd*, $J=13.4$, 3.7, $H_{eq}$–C(6)); 2.52 (br. *t*, $J=13.1$, $H_{ax}$–C(6)); 1.79–1.66 (*m*, H–C(5)). $^{13}$C-NMR (75 MHz. $CDCl_3$): 138.62, 138.47 (2 br. *s*); 128.70–127.79 (several *d*); 95.31 (*s*, C(1)); 85.91, 84.70, 80.28 (3*d*, C(2), C(3), C(4)); 76.22, 75.91, 75.59, 73.26 (4*t*, 4 Ph*C*H$_2$); 69.18 (*t*, *C*H$_2$–C(5)); 52.94 (*t*, *C*H$_2$N$_3$); 41.14 (*q*, MsO); 38.64 (*d*, C(5)); 30.94 (*t*, C(6)). MALDI-MS (DHB): 570 (100, $[M+Na-HOMs-N_2]^+$).

*Data of* **26**: Colourless oil. $R_f$ (toluene/AcOEt 10:1) 0.67. FT-IR (1.5%, $CHCl_3$): 3089*w*, 3067*w*, 3008*m*, 2914*w*, 2862*m*, 2109*s*, 1950*w*, 1732*w*, 1668*w*, 1604*w*, 1496*w*, 1454*m*, 1358*m*, 1327*w*, 1275*w*, 1151*m*, 1130*m*, 1095*m*, 1028*m*, 909*w*, 843*w*. $^1$H-NMR (300 MHz, $CDCl_3$): 7.36–7.18 (20 arom. H); 6.36 (br. *t*, $J=1.2$, $CHN_3$); 4.93 (*d*, $J=10.9$, PhC*H*); 4.87 (*d*, $J=10.9$, PhC*H*); 4.81 (*d*, $J=10.9$, PhC*H*); 4.75 (*d*, $J=11.5$, PhC*H*); 4.69 (*d*, $J=11.5$, PhC*H*); 4.53 (*d*, $J=10.9$, PhC*H*); 4.46 (*s*, PhC*H*$_2$); 3.92 (br. *d*, $J=7.5$, H–C(1)); 3.65 (*dd*, $J=9.0$, 4.7, CH–C(4)); 3.58 (*t*, $J=9.5$, H–C(3)); 3.52–3.47 (*dd*, $J=9.9$, 2.5, partly hidden by other signals, CH′–C(4)); 3.45 (*t*, $J\approx8.7$, H–C(2)); 2.73 (*dd*, $J=13.7$, 3.7, $H_{eq}$–C(5)); 1.80 (br. *t*, $J=13.4$, $H_{ax}$–C(5)); 1.72–1.60 (*m*, H–C(4)). $^{13}$C-NMR (75 MHz, $CDCl_3$): 139.07; 138.73; 138.39; 128.73–127.81; 126.55; 120.32; 88.59; 82.58; 80.80; 75.67; 75.36; 74.02; 73.24; 69.93; 42.24; 30.39. MALDI-MS (DHB): 588 (5), 586 (1, $[M+K-N_2]^+$), 570 (100, $[M+Na-N_2]^+$), 566 (4), 548 (17, $[M+1-N_2]^+$).

*(1*R*,2*R*,3*S*,4*R*,5*R*)-1-(Azidomethyl)-2,3,4-tris(benzyloxy)-5-[(benzyloxy)methyl]cyclohexane-1-methanesulfonate* (**23**). As described above, treatment of **22** (103 mg, 0.17 mmol) with MsCl (0.06 ml, 0.77 mmol) and DMAP (1 mg) in 2,6-lutidine (3 ml) at 0° gave **23** (116 mg, 99%). Colourless oil. $R_f$ (toluene/AcOEt 10:1) 0.38. $^1$H-NMR (300 MHz, $CDCl_3$): 7.42–7.19 (20 arom. H); 4.96 (*d*, $J=11.2$, PhC*H*); 4.91 (*d*, $J=10.9$, PhC*H*); 4.89 (*d*, $J=10.6$, PhC*H*); 4.85 (*d*, $J=10.9$, PhC*H*); 4.69 (*d*, $J=11.2$, PhC*H*); 4.56 (*d*, $J=10.9$, PhC*H*); 4.48 (*d*, $J=11.8$, PhC*H*); 4.43 (*d*, $J=12.1$, PhC*H*); 4.17 (*d*, $J=11.8$, 1 H, $CH_2N_3$); 3.90 (*t*, $J=9.3$, H–C(3)); 3.90 (*d*, $J=12.1$, 1 H, $CH_2N_3$); 3.79 (*dd*, $J=9.3$, 3.7, CH–C(5)); 3.62 (*dd*, $J=10.9$, 9.3, H–C(4)); 3.54 (*d*, $J=9.7$, H–C(2)); 3.43 (*dd*, $J=9.3$, 2.5, CH′–C(5)); 3.07 (*s*, MsO); 2.40 (*dd*, $J=14.9$, 3.4, $H_{eq}$–C(6)); 2.25–2.13 (*m*, H–C(5)); 1.89 (*dd*, $J=14.6$, 13.4, $H_{ax}$–C(6)). $^{13}$C-NMR (75 MHz, $CDCl_3$): 138.83, 138.76, 138.55, 138.29 (4*s*); 128.70–127.87 (several *d*); 94.00 (*s*, C(1)); 84.66, 81.06, 80.54 (3*d*, C(2), C(3), C(4)); 76.04, 75.77, 75.62, 73.34 (4*t*, 4 Ph*C*H$_2$); 69.00 (*t*, *C*H$_2$–C(5)); 53.46 (*t*, *C*H$_2$N$_3$); 40.71 (*q*, MsO); 37.83 (*d*, C(5)); 32.67 (*t*, C(6)). MALDI-MS (DHB): 570 (100, $[M+Na-HOMs-N_2]^+$).

*(3*R*,4*S*,5*S*,6*R*,7*R*)-4,5,6-Tris(benzyloxy)-7-[(benzyloxy)methyl]-1-azaspiro[2.5]octane* (**20**). A soln. of **19** (165 mg, 0.25 mmol) in THF (15 ml) was treated at 0° with $LiAlH_4$ (49 mg, 1.2 mmol), stirred for 3.5 h, treated with MeOH (14 ml) and 1M NaOH (3 ml), filtered through *Celite*, and evaporated. FC (20 g of silica gel; cyclohexane/AcOEt 1:1) gave **20** (112 mg, 83%). Colourless oil. $R_f$ (cyclohexane/AcOEt 1:1) 0.14. $[\alpha]_D^{25} = 32.1$ ($c = 1.50$, $CHCl_3$). FT-IR (1.5%, $CHCl_3$): 3296*w*, 3066*m*, 3007*s*, 2911*m*, 2863*m*, 1952*w*, 1877*w*, 1811*w*, 1725*w*, 1585*w*, 1497*m*, 1453*m*, 1359*m*, 1152*s*, 1095*s*, 909*m*, 869*w*. $^1$H-NMR (300 MHz, $CDCl_3$): 7.38–7.24 (20 arom. H); 4.94 (*d*, $J = 10.9$, PhC*H*); 4.92 (br. *d*, $J = 12.1$, 2 PhC*H*); 4.86 (*d*, $J = 10.9$, PhC*H*); 4.62 (*d*, $J = 10.9$, PhC*H*); 4.57 (*d*, $J = 11.2$, PhC*H*); 4.48 (*d*, $J = 12.1$, PhC*H*); 4.44 (*d*, $J = 12.1$, PhC*H*); 3.79 (*dd*, $J = 9.0$, 3.7, CH–C(7)); 3.78 (*d*, $J = 9.0$, H–C(4)); 3.68 (*t*, $J = 9.5$, H–C(6)); 3.60 (*t*, $J = 9.2$, H–C(5)); 3.45 (*dd*, $J = 9.0$, 2.2, CH′–C(7)); 2.15–2.06 (*m*, H–C(7)); 2.01 (*t*, $J = 12.9$, $H_{ax}$–C(8)); 1.87 (br. *s*, H–C(2)); 1.40 (br. *s*, H′–C(2)); 1.29–1.21 (*m*, $H_{eq}$–C(8)); 1.17–0.87 (br. *s*, NH). $^{13}$C-NMR (75 MHz, $CDCl_3$): 139.05, 138.86, 138.21 (3*s*, 1*s* hidden by noise or other signals); 128.72–127.75 (several *d*); 87.79, 81.25, 79.20 (3*d*, C(4), C(5), C(6)); 75.96, 75.88, 75.44, 73.19 (4*t*, 4 PhC$H_2$); 69.84 (*t*, C$H_2$–C(7)); 40.29 (*d*, C(7)); 37.42 (*s*, C(3)); 33.62 (*t*, C(8)); 27.17 (*t*, C(2)). MALDI-MS (DHB): 588 (2, $[M+K]^+$), 572 (100, $[M+Na]^+$), 550 (4, $[M+1]^+$). Anal. calc. for $C_{36}H_{39}NO_4$ (549.71): C 78.66, H 7.15, N 2.55; found: C 78.57, H 7.12, N 2.48.

*(3*S*,4*S*,5*S*,6*R*,7*R*)-4,5,6-Tris(benzyloxy)-7-[(benzyloxy)methyl]-1-azaspiro[2.5]octane* (**24**). As described above, a soln. of **23** (116 mg, 0.17 mmol) in THF (11 ml), was treated with $LiAlH_4$ (35 mg, 0.86 mmol) at 0° for 9 h to yield **24** (70 mg, 74%). Colourless oil. $R_f$ (cyclohexane/AcOEt 1:1) 0.22. $[\alpha]_D^{25} = 8.8$ ($c = 1.42$, $CHCl_3$). FT-IR (1.4%, $CHCl_3$): identical to that of **20**. $^1$H-NMR (300 MHz, $CDCl_3$): 7.38–7.23 (20 arom. H); 4.93 (*d*, $J =$ 10.9, PhC*H*); 4.92 (*d*, $J = 11.8$, PhC*H*); 4.91 (*d*, $J = 10.9$, PhC*H*); 4.86 (*d*, $J = 10.9$, PhC*H*); 4.59 (*d*, $J = 10.9$, PhC*H*); 4.58 (*d*, $J = 11.5$, PhC*H*); 4.46 (*s*, PhC$H_2$); 3.65–3.57 (*m*, H–C(4), H–C(5), H–C(6), CH–C(7)); 3.49 (*dd*, $J = 9.0$, 2.8, CH′–C(7)); 2.00 (br. *t*, $J \approx 12.5$, $H_{ax}$–C(8)); 1.98 (br. *s*, H–C(2)); 1.93–1.81 (*m*, H–C(7)); 1.31–1.25 (*m*, $H_{eq}$–C(8)); 1.29 (br. *s*, H′–C(2)); 1.18–0.85 (br. *s*, NH). $^{13}$C-NMR (75 MHz, $CDCl_3$): 138.89, 138.71, 138.45 (3*s*, 1*s* hidden); 128.85–127.78 (several *d*); 88.61, 81.54, 79.60 (3*d*, C(4), C(5), C(6)); 75.75, 75.64, 75.51, 73.21 (4*t*, 4 PhC$H_2$); 70.02 (*t*, C$H_2$–C(7)); 40.92 (*d*, C(7)); 37.86 (*s*, C(3)); 34.32 (*t*, C(8)); 26.97 (*t*, C(2)). MALDI-MS (DHB): identical to that of **20**. Anal. calc. for $C_{36}H_{39}NO_4$ (549.71): C 78.66, H 7.15, N 2.55; found: C 78.86, H 7.32, N 2.60.

*(3*R*,4*S*,5*S*,6*R*,7*R*)-7-(Hydroxymethyl)-1-azaspiro[2.5]octane-4,5,6-triol* (**21**). A soln. of **20** (10 mg, 18 mmol) in $NH_3$ (5 ml) and THF (2 ml) at −78° was treated with Na in small pieces (*ca.* 20 mg), until the blue colour persisted for 1 h, treated with $NH_4Cl$ (*ca.* 40 mg), until the blue colour disappeared, allowed to warm to r.t. overnight, and evaporated. The residue was dried in h.v., suspended in abs. MeOH, and filtered. After evaporation, the suspension of the residue in abs. EtOH was filtered. Evaporation and chromatography on *Sephadex G-10* (20 × 1 cm, eluant $H_2O$), and lyophilisation gave a mixture of **21**, an unidentified by-product, and inorganic material (13 mg, colourless powder). $R_f$ (PrOH/AcOH/$H_2O$ 4:1:1) 0.31. $^1$H-NMR (300 MHz, $D_2O$): 3.80 (*d*, $J = 9.3$, H–C(4)); 3.78 (*dd*, $J = 11.5$, 3.1, CH–C(7)); 3.70 (*dd*, $J = 11.2$, 5.0, CH′–C(7)); 3.45 (*t*, $J = 9.5$, H–C(6)); 3.36 (*t*, $J = 9.2$, H–C(5)); 1.96 (br. s, H–C(2)); 1.94 (*s*, impurity); 1.87–1.78 (*m*, H–C(7), $H_{ax}$–C(8)); 1.53 (br. *s*, H′–C(2)); 1.35 (*d*, $J = 6.9$, impurity); 1.26 (br. *d*, $J = 10.6$, $H_{eq}$–C(8)). HR-MALDI-MS (DHB): 190.1076 ($C_8H_{16}NO_4^+$, $[M+H]^+$; calc. 190.1079).

*(1*S*,2*S*,3*S*,4*R*,5*R*)-1-Amino-1-(chloromethyl)-5-(hydroxymethyl)cyclohexane-2,3,4-triol Hydrochloride* (**27**·HCl) and *(1*S*,2*S*,3*S*,4*R*,5*R*)-1-Amino-5-(hydroxymethyl)-1-methylcyclohexane-2,3,4-triol Hydrochloride* (**28**·HCl). A soln. of **20** (12 mg, 21.8 mmol) in MeOH (2 ml) was acidified (pH 4) by dropwise addition of a soln. of conc. HCl (1 drop) in MeOH (1 ml) (20 drops), treated with 10% Pd on C (10 mg), and stirred under a $H_2$ atmosphere for 110 min. Filtration through a pad of *Celite* (washed with 20 ml of 0.1N HCl in MeOH and with 50 ml of MeOH) with MeOH, and evaporation gave a mixture of **27**·HCl and **28**·HCl (7.6 mg, 3:2). $R_f$ (PrOH/AcOH/$H_2O$ 4:1:1) 0.35. $^1$H-NMR (300 MHz, $D_2O$; **27**·HCl/**28**·HCl 3:2): 4.06, 3.79 (2*d*, $J = 11.8$, 1.2 H); 3.83–3.33 (*m*, 5 H); 2.32 (br. *d*, $J = 12.1$, 0.6 H, H–C(6)); 2.04 (*dd*, $J = 14.8$, 3.3, 0.4 H, H–C(6)); 1.81–1.57 (*m*, 2 H, H–C(5), H′–C(6)); 1.43 (*s*, 1.2 H, Me). ESI$^+$-MS: **27**: 250 (6, $[M(^{37}Cl)+Na]^+$), 248 (11, $[M(^{35}Cl)+Na]^+$), 228 (14, $[M(^{37}Cl)+1]^+$), 226 (34, $[M(^{35}Cl)+1]^+$), 222 (34, $[M-Cl+MeOH]^+$), 190 (100, $[M-Cl]^+$); **28**: 224 (8, $[M+MeOH+1]^+$), 192 (94, $[M+1]^+$).

*Compound* **27**·HCl. At −60°, Na (70 mg, 2 mmol) was dissolved in $NH_3$ (3 ml) and THF (5 ml), stirred for 15 min, treated dropwise with a soln. of **20** (31 mg, 0.05 mmol) in THF (1 ml), and stirred for 30 min. at −50°. The blue mixture was treated with $NH_4Cl$ in small portions, until it became colourless, and $NH_3$ was allowed to evaporate. The remaining soln. was diluted with MeOH, filtered through *Celite*, and evaporated. The residue was purified by chromatography on *Sephadex C-25* (elution with HCl 0.01M–1M). Lyophilisation of the eluate gave a mixture of **27**·HCl and $NH_4Cl$ (64 mg). $^1$H-NMR (300 MHz, $D_2O$): 4.11 (*d*, $J = 12.3$, CH–C(1)); 3.85

(*d*, *J* = 12.3, CH′–C(1)); 3.81 (*dd*, *J* = 11.4, 3.3, CH–C(5)); 3.75 (*dd*, *J* = 11.1, 5.4, CH′–C(5)); 3.71–3.61 (*m*, 2 H); 3.44 (*ddd*, *J* = 9.6, 7.8, 1.5, 1 H); 2.37 (*dd*, *J* = 14.1, 2.4, H–C(6)); 1.87–1.65 (*m*, H–C(5), H′–C(6)).

*(3S,4S,5S,6R,7R)-7-(Hydroxymethyl)-1-azaspiro[2.5]octane-4,5,6-triol* (**25**). A soln. of **24** (33 mg, 60 mmol) in $NH_3$ (10 ml) and THF (2.5 ml) was treated at −78° with Na in small pieces (*ca.* 30 mg), until the blue colour persisted for 1 h, treated with $NH_4Cl$ (*ca.* 50 mg), until the blue colour disappeared, and allowed to warm to r.t. overnight. The solvent was evaporated, the residue dried in h.v., suspended in abs. MeOH, and filtered. After evaporation, the suspension of the resulting residue in abs. EtOH was filtered, and the filtrate evaporated. The residue (138 mg) was adsorbed on neutral *Dowex 50-WX-8* (3 ml, washed with $H_2O$). After washing with $H_2O$ (50 ml), elution with 2% aq. $NH_3$ gave crude **25** (6 mg). Chromatography (25 g of *Nucleoprep 20 CN*, MeOH) gave colourless, amorphous **25** (5.2 mg, 45%). $R_f$ (PrOH/AcOH/$H_2O$ 4:1:1) 0.26. $[\alpha]_D^{25} = 6.0$ ($c = 0.31$, $H_2O$). $pK_{HA} = 6.78$. $^1$H-NMR (300 MHz, $CD_3OD$): 3.75 (*dd*, *J* = 10.9, 3.7, CH–C(7)); 3.58 (*dd*, *J* = 10.9, 5.9, CH′–C(7)); 3.48 (*d*, *J* = 9.0, H–C(4)); 3.30 (*t*, *J* = 9.2, H–C(6)); 3.22 (*t*, *J* = 9.0, H–C(5)); 2.02 (br. *s*, H–C(2)); 1.73 (br. *t*, *J* = 12.5, $H_{ax}$–C(8)); 1.68–1.57 (*m*, H–C(7)); 1.34 (br. *s*, H′–C(2)); 1.29 (*dd*, *J* = 12.5, 2.8, $H_{eq}$–C(8)). $^{13}$C-NMR (75 MHz, $CD_3OD$): 80.44, 75.30, 72.11 (3*d*, C(4), C(5), C(6)); 64.25 (*t*, $CH_2$–C(7)); 43.77 (*d*, C(7)); 39.90 (*s*, C(3)); 34.27 (*t*, C(8)); 27.36 (*t*, C(2)). HR-MALDI-MS (DHB): 401 (69, $[2M + Na]^+$), 379 (71, $[2M + 1]^+$), 212.0891 (19) ($C_8H_{15}NNaO_4^+$, $[M + Na]^+$; calc. 212.0899), 207.1337 (23) ($C_8H_{19}N_2O_4^+$, $[M + NH_4]^+$; calc. 207.1345), 190.1074 (100) ($C_8H_{16}NO_4^+$, $[M + H]^+$); calc. 190.1079).

*(1S,2S,3S,4R,5R)-2,3,4-Tris(benzyloxy)-5-[(benzyloxy)methyl]cyclohexane-1-methanesulfonate* (**30**). A soln. of **29** [56] (200 mg, 0.37 mmol) in pyridine (6 ml) at 0° was treated with MsCl (0.12 ml, 1.49 mmol), stirred for 45 min at 0° and for 90 min at r.t., diluted with AcOEt (100 ml), washed with $H_2O$ (100 ml) and brine (100 ml), dried ($Na_2SO_4$), and evaporated. FC (20 g of silica gel; cyclohexane/AcOEt 9:1) gave colourless, amorphous **30** (212 mg, 93%). $R_f$ (cyclohexane/AcOEt 3:1) 0.40. M.p. 137–138°. $^1$H-NMR (300 MHz, $CDCl_3$): 7.38–7.21 (20 arom. H); 5.22–5.18 (*m*, H–C(1)); 4.94 (*d*, *J* = 10.9, PhC*H*); 4.90 (*d*, *J* = 9.7, PhC*H*); 4.84 (*d*, *J* = 10.6, PhC*H*); 4.78 (*d*, *J* = 11.2, PhC*H*); 4.69 (*d*, *J* = 10.9, PhC*H*); 4.55 (*d*, *J* = 10.9, PhC*H*); 4.43 (*s*, PhC$H_2$); 3.84 (*t*, *J* = 9.3, H–C(3)); 3.77 (*dd*, *J* = 9.0, 3.4, CH–C(5)); 3.59 (*dd*, *J* = 10.9, 9.3, H–C(4)); 3.49 (*dd*, *J* = 9.7, 2.8, H–C(2)); 3.40 (*dd*, *J* = 9.3, 2.2, CH′–C(5)); 3.01 (*s*, MsO); 2.18–2.05 (*m*, H–C(5), $H_{eq}$–C(6)); 1.77 (br. *t*, *J* = 13.7, $H_{ax}$–C(6)). $^{13}$C-NMR (75 MHz, $CDCl_3$): 138.9, 138.55, 137.78 (3*s*, 1*s* hidden by other signals); 128.76–127.84 (several *d*); 83.39, 81.20, 80.34, 79.07 (4*d*, C(1), C(2), C(3), C(4)); 75.89, 75.54, 73.40, 73.21 (4*t*, 4 PhC$H_2$); 69.08 (*t*, $CH_2$–C(5)); 39.03 (*d*, C(5)); 37.42 (*q*, MsO); 30.32 (*t*, C(6)). FAB-MS (NOBA): 639 (25, $[M + Na]^+$), 615 (100, $[M - 1]^+$), 525 (48), 435 (6), 391 (7). Anal. calc. for $C_{36}H_{40}O_7S$ (616.77): C 70.11, H 6.54; found: C 69.88, H 6.47.

*(1R,2S,3S,4R,5R)-1-Azido-2,3,4-tris(benzyloxy)-5-[(benzyloxy)methyl]cyclohexane* (**31**). A soln. of **30** (210 mg, 0.34 mmol) in DMF (15 ml) was treated with $NaN_3$ (221 mg, 3.4 mmol), stirred at 120° for 3 h, cooled to r.t., diluted with AcOEt (60 ml), washed with $H_2O$ (50 ml) and brine (50 ml), dried ($Na_2SO_4$), and evaporated. FC (30 g of silica gel; cyclohexane/AcOEt 20:1) gave colourless, amorphous **31** (181 mg, 94%). $R_f$ (cyclohexane/AcOEt 9:1) 0.36. M.p. 69–70°. $[\alpha]_D^{25} = 49.5$ ($c = 1.49$, $CHCl_3$). FT-IR (1.5%, $CHCl_3$): 3066*w*, 3006*w*, 2865*w*, 2103*s*, 1497*w*, 1454*m*, 1359*m*, 1260*w*, 1089*m*, 1028*m*, 910*w*. $^1$H-NMR (300 MHz, $CDCl_3$): 7.38–7.18 (20 arom. H); 4.93–4.83 (*m*, 5 PhC*H*); 4.53 (*d*, *J* = 10.9, PhC*H*); 4.46 (*s*, PhC$H_2$); 3.60 (*dd*, *J* = 9.0, 5.0, CH–C(5)); 3.59–3.35 (*m*, 5 H); 2.04 (*dt*, *J* = 13.4, 3.7, $H_{eq}$–C(6)); 1.82–1.69 (*m*, H–C(5)); 1.48 (*q*, *J* = 13.0, $H_{ax}$–C(6)). $^{13}$C-NMR (75 MHz, $CDCl_3$): 138.84, 138.63, 138.50, 138.24 (4*s*); 128.67–127.86 (several *d*); 86.91, 85.05, 80.62 (3*d*, C(2), C(3), C(4)); 75.91 (*t*, 2 PhC$H_2$); 75.52, 73.34 (2*t*, 2 PhC$H_2$); 69.71 (*t*, $CH_2$–C(5)); 63.21 (*d*, C(1)); 40.01 (*d*, C(5)); 30.68 (*t*, C(6)). FAB-MS (NOBA): 614 (29), 565 (38, $[M + 2]^+$), 554 (13), 540 (11), 537 (20), 510 (18), 496 (12), 461 (100), 452 (13), 444 (20), 429 (19), 422 (17), 408 (14), 392 (10), 339 (29), 329 (12). Anal. calc. for $C_{35}H_{37}N_3O_4$ (563.70): C 74.58, H 6.62, N 7.45; found: C 74.61, H 6.71, N 7.23.

*(1R,2S,3S,4R,6R)-4-Amino-6-(hydroxymethyl)cyclohexane-1,2,3-triol* (**32**). A soln. of **31** (50 mg, 88 mmol) in MeOH (12 ml) was treated with conc. aq. HCl (2 drops) and Pd on C (10%, 40 mg), stirred in a $H_2$ atmosphere (6 bar) for 15 h, filtered through *Celite*, and evaporated. The residue was adsorbed on neutral *Dowex 50 WX8* (3 ml, washed with $H_2O$). After washing with $H_2O$ (70 ml), elution with 2% aq. $NH_3$ gave **32** (16 mg, 100%). $R_f$ (PrOH/AcOH/$H_2O$ 4:1:1) 0.31. $[\alpha]_D^{25} = 17$ ($c = 0.42$, $H_2O$; [14]: 17.2 ($c = 0.57$, $H_2O$)). $^1$H-NMR (300 MHz, $D_2O$): 3.77 (*dd*, *J* = 11.2, 3.4, CH–C(6)); 3.62 (*dd*, *J* = 11.2, 6.2, CH′–C(6)); 3.31–3.26 (*m*, 2 H); 3.14–3.08 (*m*, 1 H); 2.74 (*ddd*, *J* = 13.1, 9.65, 4.0, H–C(4)); 1.92 (*dt*, *J* = 13.1, 4.0, $H_{eq}$–C(5)); 1.75–1.60 (*m*, H–C(6)); 1.15 (br. *q*, *J* = 12.6, $H_{ax}$–C(5)). $^{13}$C-NMR (75 MHz, $D_2O$): 80.26, 80.10, 75.68 (3*d*, C(1), C(2), C(3)); 65.09 (*t*, $CH_2$–C(6)); 54.81 (*d*, C(4)); 44.05 (*d*, C(6)); 33.77 (*t*, C(5)).

*Inhibition Studies.* The $IC_{50}$ values were determined based on a range of inhibitor concentrations (typically 4–8 concentrations) that bracket the $IC_{50}$ value.

a) *Inhibition of the β-Glucosidase from Almonds in the pH Range of 6.2–7.8.* $IC_{50}$ values were determined at 37° in 0.08M $KH_2PO_4/K_2HPO_4$ or $NaH_2PO_4/Na_2HPO_4$ buffer, with 4-nitrophenyl β-D-glucopyranoside as the substrate ($[S] \approx K_M$). The enzymatic reaction was started after incubation of the enzyme for 10–60 min in the presence of the inhibitor by the addition of substrate. The increase of absorption per min at 400 nm was taken as the relative rate for the hydrolysis of the substrate. The increase was linear during all measurements (1 min). $IC_{50}$ Values were determined by plotting the relative rate of substrate hydrolysis *vs.* the inhibitor concentration. Determination of the inhibitor concentration corresponding to half the relative rate measured in the absence of the inhibitor gave the appropriate $IC_{50}$ value. To check for slow inhibition, the dependence of the inhibition on the incubation time was determined.

b) *Inhibition of the β-Glucosidase from Almonds at pH 4.2.* See *a*. $IC_{50}$ Values were determined at 37° in 0.08M citric acid/$Na_2HPO_4$ buffer (pH 4.2). The enzymatic reaction was started after incubation of the enzyme for 15 min in the presence of the inhibitor by the addition of substrate. The mixture was incubated at 37° for 5 min, and the reaction was quenched by the addition of 0.2M borate buffer (pH 9.0). The absorption at 400 nm was measured immediately and taken as the relative rate for the hydrolysis of the substrate.

c) *Inhibition of the β-Glucosidase from* Caldocellum saccharolyticum. As described in *a* or *b*, respectively. All measurements were performed at 55°.

d) *Inhibition of the α-Glucosidase from Brewer's Yeast at pH 6.8.* As described in *a*. All measurements were performed at 37°, with 4-nitrophenyl α-D-glucopyranoside as substrate.

e) *Inhibition of the α-Glucosidase from Brewer's Yeast and the β-Glucosidase from Almonds at pH 5.0.* As described in *a*, *b*, and *d*, with 0.08M AcOH/AcONa buffer (pH 5.0).

f) *Determination of* $K_i$ *and* $k_i$ *Values for the Irreversible Inhibition of the β-Glucosidase from* Caldocellum saccharolyticum *by* **25**. *Cf.* [6][68][69]. The enzyme was incubated with various concentrations of inhibitor (5.0–0.5 mM) at 55° in 0.08M $KH_2PO_4/K_2HPO_4$ buffer (pH 6.8). Aliquots (50 µl) were taken at appropriate time intervals, diluted into 900 µl of buffer, and the residual enzyme activity was measured by the increase of the absorbance at 400 nm, which was observed after the addition of substrate. For each inhibitor concentration, ln of the residual activity was plotted *vs.* time, fitted to a straight line, and the first-order rate constant for the inactivation determined from the slope of the line. Replotting the reciprocal of these rate constants *vs.* the reciprocal of the inhibitor concentrations gave a straight line, from which $K_i$ and $k_i$ values were determined.

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