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Synthesis of new enantiopure thiepane derivatives and their evaluation as glycosidase inhibitors

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Abstract—Starting from inexpensive alcohol sugars we have synthesised a series of oxygen- and nitrogen-containing enantiopure thiepanes to investigate the structural and stereochemical requirements for inhibitory activity against a variety of glycosidases. An interesting trend was observed on analysis of the biological screening results for these derivatives.

1. Introduction

Glycosidases belong to a very important class of carbohydrate and glycoconjugate modifying enzymes and are involved in a wide range of important biological pathways. The possibility of blocking or modifying these processes using glycosidase inhibitors has attracted the interest of researchers in designing and synthesising new molecules with potential therapeutic activity in many diseases, such as viral and other infections,[1,2] diabetes,[3] cancer,[4] and HIV (human immunodeficiency virus).[5] Among the various types of glycosidase inhibitors, iminosugars or azasugars–monosaccaride analogues having a nitrogen atom instead of oxygen in the ring-have been widely investigated[6] whereas the corresponding thio-analogues have received only minor attention: the synthesis and biological activity as glycosidase inhibitors has been reported for only a limited number of seven-membered sulfurated cyclic compounds so far.[7] The inhibitory action of seven-membered rings such as tetrahydroxy thiepanes[7] and tetrahydroxy azepanes[8,5] has been tentatively ascribed to the flexibility of the seven-membered ring, which mimics the hypothetical transition state of enzymatic glycosidic cleavage.[8,9] Even more noteworthy is the potent HIV-1 protease inhibition reported for some orally bioavailable thiepane derivatives.[10]

The interesting biological activity and the reported low cytotoxicity[10] of these compounds prompted us to investigate extensively a wider range of thiepanes (Fig. 1) to determine the structural and stereochemical features able to confer upon these molecules the highest activity as glycosidase inhibitors.

The biological activity of compounds **3**–**5**[11] carrying an isopropylidene protecting group for the C(4) and C(5) OH groups, which generates a new bicyclic *trans* fused system, has been evaluated with respect to that already known for tetrahydroxy derivatives **1** and **2**.[7]

Furthermore, to investigate the effect of varying the substituents and the stereochemistry on the inhibitory activity we synthesised a series of new nitrogen-contain-

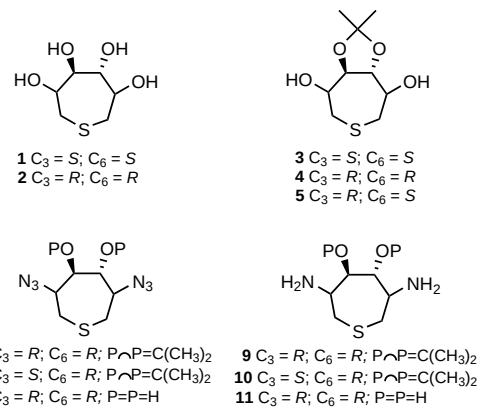

Figure 1.

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ing enantiopure diastereoisomeric thiepanes such as the 3,6-diazido (**6**, **7** and **8**) and the 3,6-diamino (**9**, **10**, **11**) derivatives starting from inexpensive alcohol sugars such as D-mannitol and D-sorbitol,.

2. Synthesis

Thiepanes **3** and **5** were prepared, respectively from D-mannitol,[12] and D-sorbitol.[12] From these substrates, new enantiopure 3,6-diazido and 3,6-diamino-4,5-dihydroxythiepanes were synthesised with free or *O*-isopropylidene protected OH groups using a straightforward and high yielding procedure.

On treatment with MsCl in pyridine, compound **3**[11] gave the dimesyl derivative **12** (Scheme 1). Nucleophilic substitution of **12** with NaN_3 afforded the diazido derivative **6**, which, after catalytic reduction, led in very good yield to the related diamine **9**. By acidic treatment of **6** we also obtained compound **8** with two free hydroxy groups. This compound could be reduced, in analogy to **6**, to the corresponding diamino derivative **11**.

All products were obtained in enantiomerically pure form since the original stereochemistry of the asymmetric carbons remained untouched or, as in the case of the nucleophilic substitution products, underwent complete inversion.

Some difficulties were encountered when the reaction sequence reported in Scheme 1 was applied to **13**, the dimesyl thiepane derivative obtained from compound **5** having D-sorbitol stereochemistry (Scheme 2). In fact, by treatment of **13** with NaN_3 in DMSO a mixture of three products **7**, **6** and **15** was obtained in a 6/3/1 ratio.

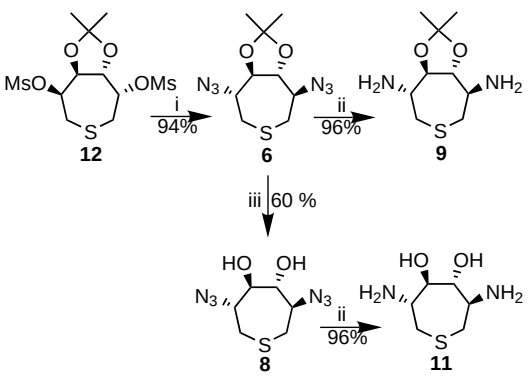
In this case we assume that both compounds **6** and **15** might derive from the bicyclic intermediate **14**, which undergoes nucleophilic attack by N_3^- on two different carbons leading, respectively to **6**, epimer of **7**, and to the ring contracted product **15**. This behaviour can be easily justified considering the ability of the medium-size rings to undergo transannular reactions. A ring contraction reaction leading to a five-membered sulfurated ring starting from 3,6-dimethoxy-4,5-dimesyl thiepane derivative with D-sorbitol stereochemistry[13] was previously observed.

In the present case the intermolecular nucleophilic substitution on the two mesyl groups competes with the nucleophilic transannular substitution leading to the intermediate **14**. An effort to extend further this synthetic protocol to 3,6-diazido thiepane derivatives having the stereochemistry of the L-iditol was unsuccessful. Serious difficulties were encountered in the nucleophilic substitution of the dimesyl derivative with NaN_3 and the expected diazido derivative was obtained in very poor yield with large amounts of ring-contraction products.

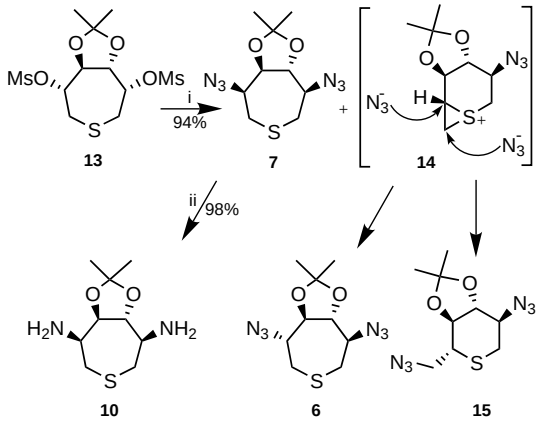
2.1. Enzyme inhibition studies

All of these compounds were subjected to kinetic investigations to correlate their activity as potential inhibitors with the structural modifications on the thiepane moiety.

As regards tetrahydroxy derivatives, simple introduction of the isopropylidene protection on the hydroxy groups at C(4) and C(5) resulted, in general, in a beneficial effect on the inhibitory activity. As evidenced by values in Table 1, a moderate activity towards α-glucosidase ($K_i = 6300$ μM) is displayed by compound **3** in contrast with the lack of activity reported[7] for the tetrahydroxy thiepane **1**. Even more remarkable is the increase in activity towards the α-glucosidase on moving from compound **2**[7] ($K_i = 3900$ μM) to **4** ($K_i = 240$ μM) by introduction of the isopropylidene group. In substrates **1** and **2** the free hydroxyl groups in the 4 and 5 positions of the ring might give rise to lower selectivity in the interaction with the active site of the enzyme, as compared to that of the more rigid structure of corresponding bicyclic derivatives **3** and **4**. In addition, these latter compounds are competitive inhibitors of α-glucosidase, but are almost completely ineffective towards β-glucosidase.

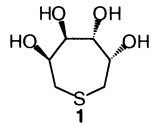
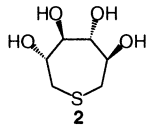
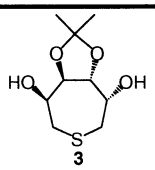
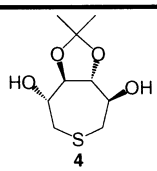
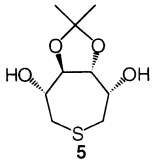


Scheme 1. (i) NaN_3, DMSO; (ii) H_2, Pd/C 10%, MeOH; (iii) aq. H_2SO_4 0.1N.



Scheme 2. (i) NaN_3, DMSO; (ii) H_2, Pd/C (10%), MeOH.

Table 1. Ki (M) of thiepane derivatives towards α- and β-glucosidases[a]

Inhibitor	 1	 2	 3	 4	 5
Conc (mol/L) [b]			(2.00×10^{-3})	(1.38×10^{-4})	(6.86×10^{-4})
α-Glucosidase	N.I.[c,d]	3900 [d]	6300	240	26 % [e]
β-Glucosidase	10 %[d]	28 % [d]	10 % [e]	N.I.[c]	N.I.[c]

[a] All enzymatic reactions were performed in 0.1 M HEPES buffer (pH = 6.86) at 37°C. Standard errors are in the range of 5-10%.
[b] In parentheses the inhibitor concentration is reported.
[c] N.I.= no inhibition.
[d] Reference 7.
[e] For weak inhibitors the percentage of initial velocity at $[S]/K_m = 1$ is reported.

These results suggest that substrates **3** and **4** bearing an isopropylidene group are preferred by α-glucosidase over derivatives **1**, **2** containing free hydroxyl groups.

The thiepane derivative **5**, which contains the isopropylidene group and differs from **3** and **4** in the configuration of only one carbon (C(3) and C(6), respectively), exhibits a weak inhibitory effect and exclusively towards α-glucosidase. In this case the results are in agreement with the circumstance that inhibitors possessing a C_2-symmetric cyclic structure display more marked biological properties.[5]

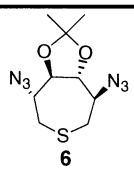
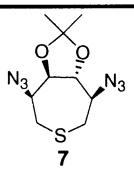
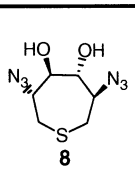
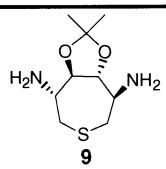
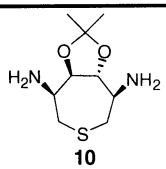
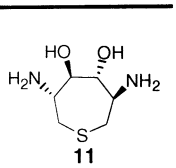
To evaluate the effect of varying the substituents in the thiepane ring on the biological activity a series of diazido and diamino derivatives has been tested.

An interesting observation which emerges from the inhibition activity data of these compounds (Table 2), is that the diazido derivatives **6** and **7** are specific for α-glucosidase and are better inhibitors than the related diamines **9** and **10**. Among these latter derivatives, compound **9** displays the wider range of inhibition being active towards all the glycosidases tested and is the best competitive inhibitor of β-glucosidase ($K_i = 190$ μM). Moreover it is the only substrate effective toward α-galactosidase.

On the other hand compound **10** (which differs from **9** in the configuration at C(3)-the carbon carrying an NH_2 group) is more effective towards α-glucosidase ($K_i = 280$ μM), but is a weaker non-competitive inhibitor of β-glucosidase ($K_i = 800$ μM) in comparison with **9**, which shows $K_i = 410$ and 190 μM, respectively.

We believe that the stereochemical discrimination of α- and β-glycosidases is counterbalanced by the strong electrostatic interactions between the positively charged inhibitor[14] and the enzyme, which allows substrate **10** to bind to different positions from the active site.

Table 2. Ki (M) of thiepane derivatives toward various glycosidases[a]

Inhibitor	 6	 7	 8	 9	 10	 11
Conc (mol/L)[b]	(2.5×10^{-5})	(5.0×10^{-5})	(5.0×10^{-5})	(6.5×10^{-4})	(5.0×10^{-4})	(5.0×10^{-5})
α-Glucosidase	35	170	10 % [d]	410	280	760
β-Glucosidase	N.I. [c]	N.I. [c]	N.I. [c]	190	800	N.I. [c]
α-Galactosidase	N.I. [c]	N.I. [c]	N.I. [c]	2000	10% [d]	10% [d]
α-Mannosidase	N.I. [c]	N.I. [c]	N.I. [c]	17 % [d]	N.I. [c]	11% [d]

[a] All enzymatic reactions were performed in 0.1 M HEPES buffer (pH = 6.86) at 37°C. Standard errors are in the range of 5-10%.
[b] In parentheses the inhibitor concentration is reported.
[c] N.I.= no inhibition.
[d] For weak inhibitors the percentage of initial velocity at $[S]/K_m = 1$ is reported.

The K_i values, reported in Table 2 outline the importance, within the same class of compounds, of the stereochemistry on the inhibitory effect as evidenced by comparison of compounds **6** and **7**, **9** and **10** which are couples of epimers of diazido and diamino thiepanes, respectively.

Even in the series of diazido and diamino derivatives the comparison of the inhibition constants of α-glucosidase obtained for **6** and **9** with the values obtained from **8** and **11** suggests that the loss of the isopropylidene group results in a dramatic lowering or total loss of inhibitory activity in line with the findings for the tetrahydroxy derivatives reported in Table 1.

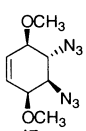
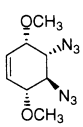
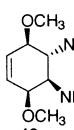
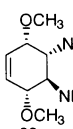
Finally bicyclic diamino compound **9** and diazido derivative **6** with the same stereochemical pattern, clearly show that replacement of the NH_2 groups in the 3 and 6 positions of the seven-membered ring with N_3 groups, leads to a strong improvement in the binding, modifying the K_i values from 410 to 35 μM and to a change from competitive to non competitive inhibition. At this stage it is possible to hypothesise that the N_3 group interacts more favourably than NH_3^+ with the hydrophilic region and binds to different regions from the active site of the enzyme.

2.2. Appendix

The inhibitory activity observed in the case of diazido thiepanes prompted us to extend this investigation to other cyclic diazides. To this aim conduritol derivatives **17** and **18**, precursors of the previously investigated[15] diamino derivatives **19** and **20**, were taken into consideration.

From the results reported in Table 3 it clearly appears that all the above compounds behave as specific α-glucosidase inhibitors and the diazido derivatives show the better results.

Table 3. Ki (μM) of conduritol derivatives towards α- and β-glucosidases (glcase)

Inhibitor	 17	 18	 19	 20
Conc. (mol/L)[a]	(7.0×10^{-5})	(8.9×10^{-5})	(3.8×10^{-3})	(1.1×10^{-4})
α-glcase	320	40 [b]	5300 [c]	700 [c]
β-glcase	N.I.	N.I.	N.I.	N.I.

[a] In parentheses the inhibitor concentration is reported.

[b] IC_{50} value at $[S]/K_m = 1$.

[c] Reference 16.

3. Conclusions

From the readily available thiepanes **3** and **5** we have developed an easy and efficient synthesis of enantiomerically pure 3,6-diazido and 3,6-diamino-4,5-dihydroxythiepanes.

Samples of these new compounds were submitted to biological screening to assess any activity as glycosidase inhibitors. Our results highlight some interesting trends, such as the favourable inhibitory effect displayed by presence of the isopropylidene group, the weak beneficial effect due to the presence of the amino group and finally the large enhancement of the inhibitory activity coupled with a high selectivity towards α-glucosidase which is seen upon introduction of azide groups.

4. Experimental

4.1. General

All moisture sensitive reactions were performed in flame-dried glassware equipped with rubber septa under positive pressure of dry nitrogen. Organic extracts were dried over $CaSO_4$. Melting points are uncorrected. Preparative flash chromatographic experiments were performed using ICN silica gel 230–400 mesh. For TLC precoated glass plates were used (Stratochrom SIF_{254}, 0.25 mm thick) and the spots were developed at 110°C with an aqueous solution of $(NH_4)_6Mo_7O_{24}$ (2.5%) and $(NH_4)_4Ce(SO_4)_4$ (1%) in 10% H_2SO_4 or $KMnO_4$ 0.1 M/H_2SO_4 1 M 1/1. Yields are for isolated compounds. Unless specified otherwise ^1H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, in $CDCl_3$ as solvent. Chemical shifts are in ppm downfield of TMS and signal multiplicities were established by DEPT experiments. Signal assignments, if necessary, were elucidated by decoupling ^1H NMR and by 2D ^1H–^1H and ^1H–^{13}C NMR experiments. Optical rotations were measured at 589 nm. Infrared spectra were recorded on a FT IR spectrophotometer. Mass spectra were recorded using electron impact (70 eV). Solvents and reagents were dried as follows: DMSO was distilled under vacuum from CaH_2 and CH_2Cl_2 was refluxed over and distilled from CaH_2. Light petroleum had bp 35–60°C.

4.2. (+)-(3*R*,4*R*,5*R*,6*R*)-3,6-Diazido-4,5-*O*-isopropylidenethiepane, 6

To a solution of (−)-(3*S*,4*R*,5*R*,6*S*)-3,6-dimesyl-4,5-*O*-isopropylidenethiepane **12** (0.12 g (0.32 mmol) of[11b] in dry DMSO (1.5 mL), was added NaN_3 (0.10 g, 1.50 mmol). After stirring for 19 h at 120°C, the reaction mixture was diluted with AcOEt (50 mL). The organic layer was washed with H_2O then with brine and dried by evaporation of the solvent to afford **6** (0.08 g, 0.30 mmol, 94%), which was used without further purification. Nevertheless, a sample was purified by flash chromatography (SiO_2; light petroleum/Et_2O, 5/1) for characterisation, to afford the title compound as a white solid, mp 78–79°C. ^1H NMR δ: 4.12–4.00 (m,

2H, CHO), 3.80–3.62 (m, 2H, CHN), 2.80 (dd, 2H, C*H*HS; $J=15.39$, 5.13 Hz), 2.65 (dd, 2H, CH*H*S; $J=15.38$, 6.27 Hz), 1.43 (s, 6H, CH_3). ^{13}C NMR δ: 110.3 (C), 81.0 (2CHO), 64.3 (2CHN), 36.7 ($2CH_2$), 27.0 ($2CH_3$). $[\alpha]_D^{25}=71.4$ ($c=1.22$, $CHCl_3$). m/z (EI): 270 (<1), 255 (17), 227 (100), 184 (40), 126 (23). IR (Nujol) $\nu_{max}/cm^{-1}=2970$, 2115, 1360, 1170, 890.

4.3. (+)-(3*R*,4*R*,5*R*,6*R*)-3,6-Diamino-4,5-*O*-isopropylidenethiepane 9

To a solution of the diazido thiepane **6** (0.14 g, 0.53 mmol) in MeOH (7.4 mL) was added a suspension of Pd/C (10% w/w, 0.06 g) in MeOH (2.2 mL). After stirring for 24 h at room temperature under 0.5 atm of hydrogen pressure, the reaction mixture was filtered through Celite washing repeatedly with MeOH. By evaporation of the solvent pure **9** was obtained as a pale yellow oil (0.11 g, 0.51 mmol, 96%) of. ^1H NMR δ: 3.73–3.58 (m, 2H, CHO), 3.08–2.89 (m, 2H, CHN), 2.66 (dd, 2H, C*H*HS; $J=14.92$, 5.00 Hz), 2.42 (dd, 2H, CH*H*S; $J=14.90$, 6.25 Hz), 1.75 (bs, 4H, NH_2), 1.25 (s, 6H, CH_3). ^{13}C NMR δ: 108.5 (C), 83.2 (2CHO), 55.6 (2CHN), 39.4 ($2CH_2S$), 26.9 ($2CH_3$). $[\alpha]_D^{25}=56.4$ ($c=0.82$, $CHCl_3$). m/z (EI): 218 (21), 201 (31), 157 (37), 126 (55), 43 (100). IR (neat) $\nu_{max}/cm^{-1}=3480$, 2990, 2910, 1600, 1380, 1370, 1250, 1080, 880, 755.

4.4. (+)-(3*R*,4*R*,5*R*,6*R*)-3,6-Diazido-4,5-dihydroxythiepane 8

Compound **6** (0.34 g, 1.28 mmol) was treated with aqueous H_2SO_4 (1N, 2.7 mL) at 95°C for 12 h. The reaction mixture was neutralised with 10% aqueous Na_2CO_3 solution. The solid residue, obtained after evaporation of the solvent was extracted with CH_2Cl_2. After drying and evaporating the organic layer 0.30 g of a crude product was obtained, which was purified by flash chromatography (SiO_2; CH_2Cl_2/Et_2O, 10/1) to afford pure **8** as a white solid (0.18 g, 0.78 mmol, 60%), mp 53–55°C. ^1H NMR δ: 3.95–3.52 (m, 6H, 2CHO, 2CHN and 2OH), 2.88–2.60 (m, 4H, $2CH_2S$). ^{13}C NMR δ: 74.0 (2CHO), 66.8 (2CHN), 35.9 ($2CH_2S$). $[\alpha]_D^{25}=84.2$ ($c=0.99$, $CHCl_3$). m/z (EI): 230 (1), 187 (45), 144 (31), 86 (31), 43 (100). IR (CCl_4) $\nu_{max}/cm^{-1}=$ 3274, 2916, 2108, 1070.

4.5. (+)-(3*R*,4*R*,5*R*,6*R*)-3,6-Diamino-4,5-dihydroxythiepane 11

The reduction of **8** was carried out applying the procedure adopted for the synthesis of **9** using **8** (0.11 g, 0.47 mmol), a 1/1, $MeOH/H_2O$ solution (6.8 mL) and Pd/C (10% w/w 0.05 g) in MeOH (1 mL). The pure title compound **11** was obtained as a white solid (80 mg, 0.45 mmol, 96%); mp 104–106°C. ^1H NMR (CD_3OD) δ: 3.48 (dd, 2H, CHO, $J=5.77$, 1.92 Hz), 3.05–2.82 (m, 4H, 2CHN and 2CH*H*S), 2.68 (dd, 2H, 2C*H*HS, $J=$ 14.60, 6.60 Hz). ^{13}C NMR (CD_3OD) δ: 76.9 (2CHO), 58.6 (2CHN), 38.5 ($2CH_2S$). $[\alpha]_D^{25}=76.2$ ($c=0.79$, MeOH). m/z (EI): 178 (2), 161 (6), 117 (20), 72 (40), 43 (100). IR (Nujol) $\nu_{max}/cm^{-1}=3400$, 2960, 1609, 730.

4.6. (+)-(3*S*,4*R*,5*R*,6*R*)-3,6-Diazido-4,5-*O*-isopropylidenethiepane 7

The title compound was obtained analogously to **6** using **13** (0.12 g, 0.32 mmol),[16] dry DMSO (1.5 mL), NaN_3 (0.10 g, 1.50 mmol) and heating for 19 h at 85°C. The crude reaction mixture obtained after workup was a mixture of **7**, **6** and **15** in a 6:3:1 ratio. These products were separated by flash chromatography (SiO_2; light petroleum/Et_2O, 12/1) obtaining **7** as a white solid (0.05 g, 0.19 mmol, 60%); mp 63–64°C and **6** (0.02 g, 0.076 mmol, 20%) and **15** (0.013 g, 0.05 mmol, 15%) as colourless oils. Spectroscopic data for **7**: ^1H NMR δ: 4.42–4.32 (m, 1H, CHO), 4.30–4.23 (m, 2H, CHO and CHN), 3.74–3.63 (m, 1H, CHN, irradiating at 2.68 ppm was obtained a doublet with $J=8.10$ Hz), 2.93 (dd, 1H, C*H*HS, $J=15.43$, 5.02 Hz), 2.76 (dd, 1H, C*H*HS, $J=$ 15.41, 5.19 Hz), 2.65 (dd, 1H, CH*H*S, $J=15.39$, 6.04 Hz), 2.51 (dd, 1H, CH*H*S, $J=15.57$, 5.70 Hz), 1.50 (s, 3H, CH_3), 1.43 (s, 3H, CH_3). ^{13}C NMR δ: 110.5 (C), 79.6 (CHO), 78.6 (CHO), 64.5 (CHN), 59.6 (CHN), 36.7 (SCH_2), 36.6 (SCH_2), 27.3 (CH_3), 26.3 (CH_3). $[\alpha]_D^{25}=52.5$ ($c=0.87$, $CHCl_3$). m/z (EI): 270 (<1), 255 (27), 227 (85), 184 (14), 126 (9), 85 (24), 43 (100). IR (CCl_4) $\nu_{max}/cm^{-1}=2990$, 2107, 1374, 1077, 1005. Spectroscopic data for **15**: ^1H NMR δ: 3.80–3.40 (m, 4H), 3.23–3.08 (m, 2H), 2.79 (dd, 1H, C*H*HS, $J=13.68$, 4.44 Hz), 2.65 (dd, 1H, CH*H*S, $J=13.70$, 4.41 Hz), 1.83 (s, 3H, CH_3), 1.80 (s, 3H, CH_3). ^{13}C NMR δ: 110.1 (C), 81.6 (CHO), 78.4 (CHO), 62.0 (CHN), 51.7 (CH_2N), 45.7 (CHS), 31.6 (CH_2S), 27.0 (CH_3), 26.9 (CH_3). m/z (EI): 270 (<1), 255 (30), 227 (75), 169 (42), 43 (100). IR (film) $\nu_{max}/cm^{-1}=2980$, 2110, 1350, 1010.

4.7. (−)-(3*S*,4*R*,5*R*,6*S*)-3,6-Diamino-4,5-*O*-isopropylidenethiepane 10

The title compound was obtained analogously to **9**, using 0.11 g (0.41 mmol) of **7**, 5.7 mL of MeOH and Pd/C (10% w/w 0.05 g) in 1.7 mL MeOH, were obtained 0.09 g (0.40 mmol, 98%) of the pure diamino derivative **10** as yellow oil. ^1H NMR (CD_3OD) δ: 4.28 (dd, 1H, CHO, $J=9.19$, 3.85 Hz), 4.10–4.00 (m, 1H, CHO), 3.61–3.53 (m, 1H, CHN), 3.25–3.15 (m, 1H, CHN), 3.12 (dd, 1H, C*H*HS, $J=15.11$, 5.22 Hz), 2.95 (dd, 1H, CH*H*S, $J=15.20$, 5.16 Hz), 2.71–2.55 (m, 2H, CH_2S), 1.48 (s, 3H, CH_3), 1.42 (s, 3H, CH_3). ^{13}C NMR (CD_3OD) δ: 110.3 (C), 80.8 (CHO), 79.9 (CHO), 57.2 (CHN), 50.5 (CHN), 38.9 ($2CH_2S$), 27.8 (CH_3), 27.6 (CH_3). $[\alpha]_D^{25}=-22.4$ ($c=0.91$, CH_3OH). m/z (EI): 218 (11), 201 (14), 157 (37), 126 (56), 58 (28), 43 (100). IR (film) $\nu_{max}/cm^{-1}=3358$, 2983, 2909, 1593, 1370, 1247, 1069, 882.

5. Inhibition studies

5.1. Materials

α-Glucosidase (EC 3.2.1.20) was obtained from baker's yeast, β-glucosidase (EC 3.2.1.21) was obtained from almonds, α-galactosidase (EC 3.2.1.22) was obtained from green coffee beans, α-mannosidase (EC 3.2.1.24)

was obtained from jack beans; *p*-nitrophenyl glucosides were purchased from Sigma. Other products (Merck) were of analytical degree.

5.2. Kinetics of glycosidase inhibition

Inhibition studies were carried out at a single substrate concentration. Aqueous solutions of inhibitors were used except for **5** and **6** which were dissolved in water/EtOH 50% mixture and for **17** and **18** in absolute EtOH.

Kinetics of hydrolysis of glycosides were performed at pH 6.85 in presence of 0.1 M of *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethane sulfonic acid/K salt buffer (HEPES) at 37±0.1°C. The amount of enzyme used was varied between 0.05–0.20 units. Aliquots of stock solution of inhibitor were added by a Hamilton syringe to a thermostatted buffer solution containing the enzyme poured into a 10 mm cell in the thermostatically controlled cell holder of a Perkin–Elmer Lambda 6 spectrophotometer and the solution was incubated for 15 min. An appropriate quantity of stock *p*-nitrophenylglucoside solution thermostatically controlled at 37°C was then added and the initial rates were followed at $\lambda = 400$ nm monitoring the formation of *p*-nitrophenol. Parallel kinetic runs were performed in presence of EtOH which modifies significatively only the value of V_m.

Time-dependent inhibition was evidenced with the thiepane **6** assaying α-glucosidase in absence and in presence of inhibitor after 2, 30, 60 min of incubation at 37°C. In this case all the kinetics were followed after 30 min and the results were compared against control kinetics performed under the same conditions.

Competitive inhibition constants K_i were calculated from eq. $K_i = K_m \times [I_o]/(K'_m - K_m)$[17] (where K_m and K'_m are the apparent dissociation constants in the absence or presence of inhibitor (I_o), respectively. Non-competitive inhibition constants K_i were calculated from the equation $K_i = V'_{max} \times [I_o]/(V_{max} - V'_{max})$[17] where V_{max} and V'_{max} are the apparent maximum rates measured in absence and in presence of inhibitor (I_o), respectively.
The values of kinetic parameters were calculated by the non-linear least squares programme.[18]

5.3. pK_a Determinations

The apparent pK_a values of the conjugate acid of diamino compounds **9** and **10** were determined potentiometrically by titrating a 0.005 M solution with 0.1 M HCl under a nitrogen atmosphere at 25°C, using a microburette syringe type SB2 titration equipment and a Knick Ph meter mod. 643 fitted with an Ingold HA-405 combined standardised glass electrode.[19] Owing to overlapping of the two ionisation processes, the pK_a values were calculated by the Martin method.[20] The values obtained are p$K_{a1} = 5.1$; p$K_{a2} = 8.1$ for **9** and p$K_{a1} = 6.7$; p$K_{a2} = 8.0$ for **10**.

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