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Synthesis and glycosidase inhibition of new enantiopure 2,3-diamino conduritols

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Abstract—A new 2,3-diamino conduritol, isoster of conduritol F, was obtained starting from D-sorbitol. In the synthetic sequence an unprecedented transannular cyclization led, as a side product, to a bicyclic compound bearing a cyclopropyl ring. The synthesis of the completely deprotected 2,3-diamino conduritol, isoster of conduritol B, was devised via the intermediate *O*-benzylation of the OH groups. The *O*-methylated and deprotected 2,3-diamino conduritols were evaluated as inhibitors of α- and β-glucosidase. © 2001 Elsevier Science Ltd. All rights reserved.

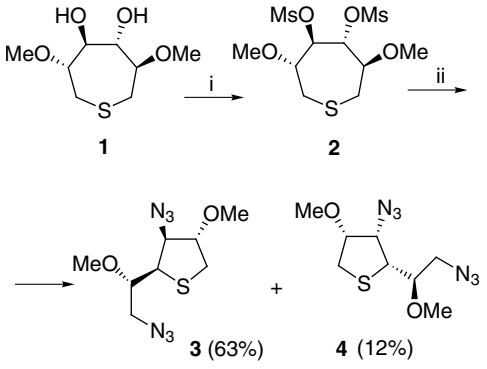
1. Introduction

We have previously reported a smooth and general synthetic procedure, mediated by sulfur, to produce enantiopure conduritols from inexpensive alcohol sugars.[1,2] On the other hand, the potential biological activity of 1,4- and 1,3-diamino conduritols has been widely reported as well as their applications to the synthesis of new antibiotics,[3–7] and of cytostatic platinum complexes.[8,9] This prompted us to apply our sulfur-mediated strategy to the synthesis of the previously unknown 2,3-diamino conduritols. The first example of the synthesis of a 2,3-diamino conduritol using D-mannitol as the starting alcohol sugar was thus reported[10] by introduction of two azido groups on the dimesylated thiepane precursor. Extension of this protocol to the synthesis of 2,3-diamino-1,4-dimethoxy conduritol F, starting from D-sorbitol, differing from D-mannitol only in the stereochemistry of a single chiral centre, did not lead however to the expected nucleophilic substitution since only the ring contraction products **3** and **4** were obtained (Scheme 1).[11]

This behaviour could be easily justified because a distinctive feature of medium-size rings is their ability to undergo transannular reactions. In fact we previously reported that eight- and nine-membered thiacycloalkenes give reactions of transannular cyclisation, promoted by the sulfur atom, under acidic catalysis[12,13] which lead to thioniabicyclic sulfonium salts. Analogously in the hydroxylated seven-membered sulfurated cyclic thioethers a transannular cyclisation was obtained by means of Me_3SiI, resulting in a stereospecific ring contraction reaction.[14] Herein we describe our further work directed towards the synthesis of amino conduritols focused on the preparation of 2,3-diamino-1,4-dimethoxy conduritol F. For the sake of comparison of the biological activity of 2,3-diamino-1,4-dimethoxy conduritols with that of the corresponding *O*-deprotected counterparts, a mild and straightforward procedure for the synthesis of 2,3-diamino conduritol E with two unprotected OH groups has been devised as well.

2. Results and discussion

To circumvent the problem caused by the interesting but undesired reaction leading to compounds **3** and **4** via stereospecific transannular cyclisation following an S_N2 mechanism, the thiepane derivative **2** was oxidised to the sulfone **5**



Scheme 1. (i) MsCl, pyridine; (ii) NaN_3, DMSO.

Keywords: cyclitols; thiosugars; amino alcohols; transannular reactions; biologically active compounds.

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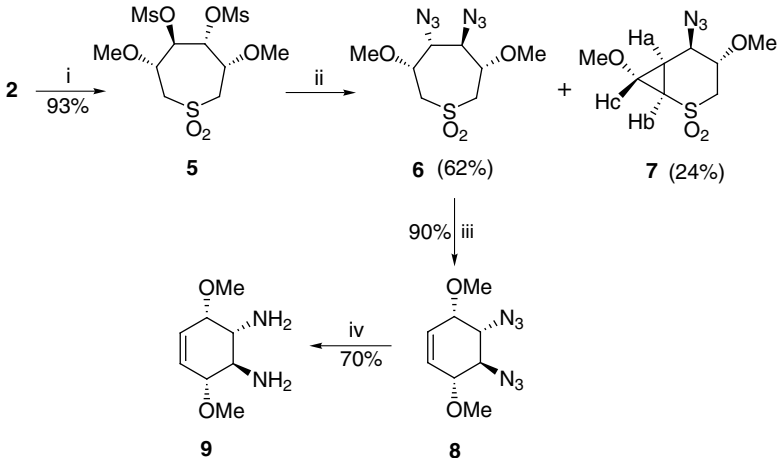
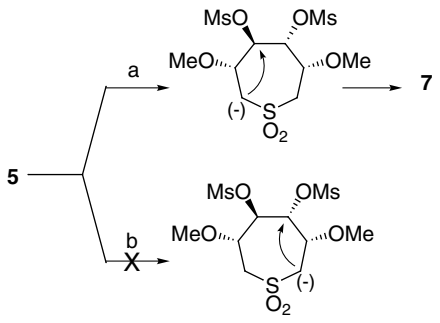


Scheme 2. (i) *m*-CPBA, CH_2Cl_2; (ii) NaN_3, DMSO; (iii) KOH, CCl_4, *t*-BuOH, H_2O; (iv) Et_3N, $HS(CH_2)_3SH$, MeOH.

before treatment with NaN_3 (Scheme 2). The expected derivative **6** was obtained in 62% yield together with 24% of the bicyclic compound **7**. The presence and the stereochemistry of the cyclopropyl ring were inferred from the values of the chemical shifts and of the CH coupling constants in the ^1H and ^{13}C NMR spectra (see Section 4).

The formation of **7** emerges in this reaction sequence as an interesting feature and its presence clearly indicates an unpredictable transannular cyclisation. We hypothesised that the basicity of NaN_3 may generate two different α-SO_2 carbanions and the contemporaneous presence of two good leaving groups at C_4 and C_5 would favour a nucleophilic intramolecular substitution (Scheme 3). Since only one diastereoisomer was obtained, one transannular attack (route a) is favoured over the other (route b), probably due to the stereochemistry of the substrate.

To the best of our knowledge this behaviour has never been reported. In order to verify the ability of the NaN_3 to generate a carbanion, we treated $PhSO_2CH_3$ with 1 equiv. of NaN_3 in DMSO under the usual reaction conditions. The recovery, upon quenching of the reaction mixture with D_2O, of $PhSO_2CH_2D$ confirmed our hypothesis. With the aim of eliminating **7** by decreasing the acidity of the protons α to the sulfur moiety, we have also oxidised **2** to the related sulfoxide. Treatment of this substrate with NaN_3 resulted, however, only in a complex mixture of unidentified compounds.



Scheme 3.

Compounds **6** and **7** were easily separated by flash chromatography and the diazido derivative **6** afforded (Scheme 2), through a Ramberg–Bäcklund reaction, the enantiopure diazido conduritol **8**. Some difficulties were finally encountered in the reduction of **8** to the related diamino derivative **9**, presumably due, even in this case, to the stereochemistry of the substrate. In fact, treatment of this derivative with $LiAlH_4$ (successful in the case of the 2,3-diamino-1,4-dimethoxy conduritol B)[10] led mainly to isomerisation of the starting material. Hydrogenation over Lindlar catalyst, or treatment with Ac_2O and Me_3SiCl gave the same result. The reduction performed with Bu_3SnH and AIBN was more promising but the numerous chromatographic purifications, needed to eliminate the stannyl derivatives, lowered the yield to 23%. Finally by using[15] 1,3-propanedithiol and Et_3N the conversion of **8** was achieved successfully leading to **9** in 70% yield.

The inhibitory power towards α- and β-glycosidase of the 2,3-diamino conduritols was investigated on the *O*-methyl protected diamino derivatives **9** and **10**[10] derived from D-sorbitol and D-mannitol, respectively (Fig. 1). Moreover, in order to evaluate the effect exploited on the biological activity by the protection at the OH groups, we devised a route to the synthesis of **11**, the deprotected counterpart of **10**.

However, since the deprotection conditions normally used to remove the methyl groups can give rise to problems in the presence of two amino groups, **11** was obtained from the more suitable *O*-benzyl analogue. This modification applied to the thiepane derivative synthesised from D-mannitol is shown in Scheme 4. The 3,6-dihydroxy-4,5-di-*O*-isopropylidenethiepane **12**[16,17] was protected as dibenzyl ether at the

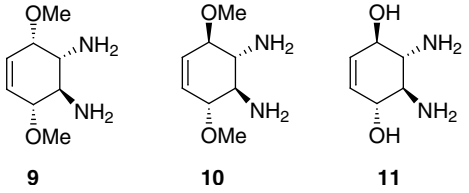
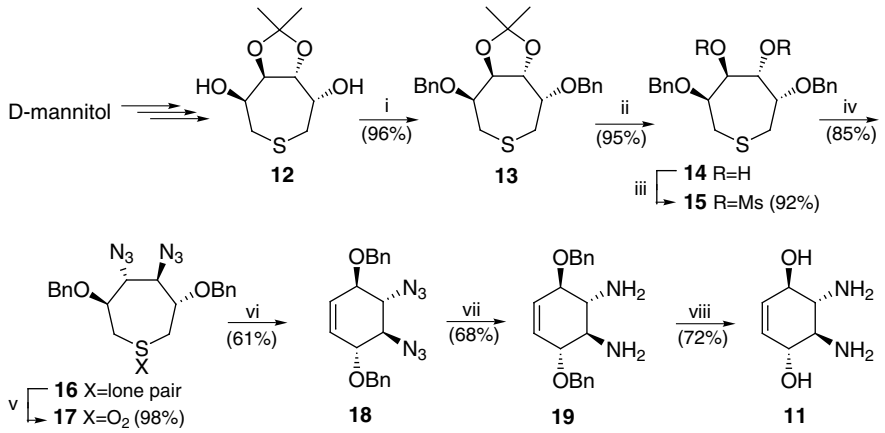


Figure 1.



Scheme 4. (i) NaH, BzBr, THF; (ii) CF_3COOH/H_2O 1:10, CH_3CN; (iii) MsCl, pyridine; (iv) NaN_3, DMSO; (v) *m*-CPBA, CH_2Cl_2; (vi) KOH, CCl_4, *t*-BuOH, H_2O; (vii) Et_3N, $HS(CH_2)_3SH$, MeOH; (viii) BCl_3, CH_2Cl_2.

C_3 and C_6 hydroxyl groups giving **13**. Then the propylidene group was easily removed under acidic conditions to obtain **14** and the hydroxyl groups were dimesylated to give **15**. Subsequent treatment with NaN_3 led to the diazido derivative **16** which, upon oxidation to the sulfone **17** with *m*-CPBA and Ramberg–Bäcklund reaction, afforded **18**. After reduction of the two diazido groups the related diamino compound **19** was obtained. Finally, the 2,3-diamino conduritol **11** with all deprotected groups was obtained in good yield by treatment of **19** with BCl_3.[18]

The diamino derivatives **9** and **10** derived from D-sorbitol and D-mannitol and **11**, the unprotected counterpart of **10**, were evaluated as inhibitors of α- and β-glucosidase.

The hydrolysis of *p*-nitro α-D-glucopyranoside by the α-glucosidase was weakly inhibited by the diamino compound **9** (K_i=700 μM) and the inhibition was apparently competitive. Regarding the diamino derivative **10** the inhibition was non-competitive but less effective (K_i=5300 μM). Both the diamino derivatives proved to be specific for the α-glucosidase while they were completely ineffective towards the hydrolysis of the *p*-nitro β-D-glucopyranoside by the β-glucosidase.

Analogously **11**, with the same stereochemistry as **10**, was submitted to biological evaluation. This compound is a good α-glucosidase competitive inhibitor (K_i=190 μM), while it is almost inactive toward β-glucosidase.

The better inhibition activity of 2,3-diamino conduritol **11**, in comparison with the dimethoxy compound **10**, can be ascribed to the presence of hydroxy groups in 1,4 position on the cyclohexene ring, which interact favourably with the hydrophilic region in the active site of α-glucosidase. The presence of a six-membered aminocyclohexene ring could be an important factor for the inhibition, as shown by acarbose, a naturally occurring potent inhibitor of glucoamylase. The polyhydroxylated cyclohexene may mimic the hypothetical oxonium ion transition state that is generated during the hydrolysis catalysed by glycosidases.[19]

Further investigations in this field are in progress.

3. Conclusions

The synthesis of the new enantiopure (+)-2,3-diamino-1,4-dimethoxy conduritol F (**9**) was achieved in good yield. The reaction sequence was nevertheless modified to avoid the reactions of transannular cyclisation encountered when the starting alcohol sugar was D-sorbitol. 2,3-Diamino conduritol, an isoster of conduritol F, was also obtained using benzyl as a removable protecting group.

4. Experimental

4.1. General

All moisture sensitive reactions were performed in flame-dried glassware equipped with rubber septa under a 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 0.1 M $KMnO_4$/1 M H_2SO_4 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 an FTIR spectrophotometer. Mass spectra were recorded using electron impact (70 eV). Solvents and reagents were obtained dry as follows: DMSO was distilled under vacuum from CaH_2 and CH_2Cl_2 was refluxed over and distilled from CaH_2. Light petroleum used had bp 35–60°C.

4.1.1. (−)-1,6-Dideoxy-3,4-methanesulfonyl-2,5-di-*O*-methyl-1,6-thia-D-sorbitol-*S*,*S*-oxide (5). 0.56 g (1.5 mmol) of (−)-1,6-dideoxy-3,4-methansulfonyl-2,5-di-*O*-methyl-1,6-thia-D-sorbitol (**2**)[16,17] were dissolved in CH_2Cl_2 (7.5 mL) and treated with 1.03 g of 50% *m*-CPBA

for 4 h at room temperature. The crude reaction mixture, diluted with CH_2Cl_2 (15 mL), was treated with a saturated solution of $Na_2S_2O_5$ to destroy the excess of *m*-CPBA, then washed with a saturated solution of $NaHCO_3$. By evaporation of the organic layer, 0.55 g (1.4 mmol, 93%) of **5** was obtained. The title compound, recovered as a white crystalline product, mp 214–216°C, was used for the next reaction, without any further purification. In fact, the purification of this compound by flash chromatography on SiO_2 carried to decomposition of the product. ^{1}H NMR: δ 4.99 (m, 2H, 2CHOMs), 4.18 (m, 1H, C*H*OCH$_3$), 3.90 (m, 1H, C*H*OCH$_3$), 3.73 (m, 2H, CH_2SO_2), 3.50 (s, 3H, OCH_3), 3.45 (s, 3H, OCH_3), 3.45 (m, 2H, CH_2SO_2), 3.22 (s, 3H, CH_3SO_2), 3.20 (s, 3H, CH_3SO_2). ^{13}C NMR: δ 81.0 (CHO), 79.6 (CHO), 76.3 (CHO), 74.8 (CHO), 57.9 (CH_3O), 57.2 (CH_3O), 54.6 (CH_2S), 52.5 (CH_2S), 38.7 (CH_3SO_2), 38.2 (CH_3SO_2). $[\alpha]_D^{27}=-0.2$ (c=1.60, CH_3CN). *m/z*: 317 (8), 285 (18), 221 (19), 178 (100), 165 (49), 121 (76), 99 (32), 79 (78). IR (KBr) ν_{max} (cm^{-1}): 2920, 1470, 1310, 1110. Anal. Calcd for $C_{10}H_{20}O_{10}S_3$: C, 30.30; H, 5.08. Found: C, 30.32; H, 5.11.

4.1.2. (−)-(3*R*,4*R*,5*R*,6*S*)-4,5-Diazido-3,6-dimethoxythiepane-*S*,*S*-oxide (6) and *cis*-(−)-(1*R*,4*S*,5*R*,6*R*,7*R*)-5-azido-4,7-dimethoxy-thiabicyclo[4.1.0]heptane-*S*,*S*-oxide (7). 200 mg (0.50 mmol) of the dimesyl derivative **5** were dissolved in DMSO (2.5 mL) then 160 mg (24.6 mmol) of NaN_3 were added. After 19 h at 120°C the reaction mixture, diluted with 70 mL of EtOAc, was washed twice with H_2O (2×15 mL) then with brine (20 mL). The organic layer, dried and evaporated, gave 130 mg of a mixture of **6** and **7** in a 2:1 ratio, respectively. These products were separated by flash chromatography (SiO_2; CH_2Cl_2, EtOAc 0.5%) obtaining 90 mg (0.31 mmol, 62%) of **6** as a yellow oil and 30 mg (0.12 mmol, 24%) of **7** as a white crystalline product, mp 72–74°C.

Spectroscopic data for **6**. ^{1}H NMR: δ 3.72 (m, 6H), 3.60 (s, 3H, OCH_3), 3.42 (s, 3H, OCH_3), 3.17 (m, 2H). ^{13}C NMR: δ 78.9 (CHO), 75.1 (CHO), 66.8 (CHN), 65.2 (CHN), 58.2 (CH_3O), 57.7 (CH_3O), 55.3 (CH_2SO_2), 53.5 (CH_2SO_2). $[\alpha]_D^{25}=-6.3$ (c=0.71, $CHCl_3$). *m/z*: 176 (7), 121 (5), 91 (24), 85 (18), 58 (100). IR (neat) ν_{max} (cm^{-1}): 2950, 2120, 1460, 1315, 1140. Anal. Calcd for $C_8H_{14}N_6O_4S$: C, 33.10; H, 4.86, N; 28.95. Found: C, 33.12; H, 4.90; N, 28.95.

The chemical shifts of **7** were assigned by means of 2D-NMR experiments: COSY, HETCOR and HET2DJ. ^{1}H NMR: δ 3.82 (dd, 1H, C_5H, J=9.82, 7.11 Hz,), 3.62 (dd, 1H, C_7H, J=4.70, 3.05 Hz), 3.41 (s, 3H, CH_3), 3.37 (s, 3H, CH_3), 3.24 (m, 2H, C_4H and C_3H*H*), 2.77 (2dd, 2H, C_1H and C_3*H*H superimposed; irradiating at δ=2.15 ppm the J=10.79, 3.05 Hz were assigned to C_1H and the J=13.39, 12.03 Hz were assigned to C_3*H*H), 2.15 (ddd, 1H, C_6H, J=10.80, 7.11, 4.74 Hz). ^{13}C NMR, δ: 77.2 (C_4), 61.8 (C_7), 59.6 (C_5), 58.6 (CH_3), 57.8 (CH_3), 53.8 (C_3), 42.9 (C_1), 27.8 (C_6). HET2DJ: J_{C_1}H=177.42 Hz, JC_7H=187.05 Hz, JC_6H=168.33 Hz. $[\alpha]_D^{25}=-33.8$ (c=0.94, $CHCl_3$). *m/z*: 220 (<1), 173 (4), 140(3), 97 (53), 82 (60), 70 (75), 58 (94). IR (KBr) ν_{max} (cm^{-1}): 2948, 2118, 1313, 1141. Anal. Calcd for $C_8H_{13}N_3O_4S$: C, 38.86; H, 5.30; N, 16.99. Found: C, 38.89; H, 5.33; N, 17.01.

4.1.3. (+)-(1*S*,2*R*,3*R*,4*R*)-2,3-Diazido-1,4-dimethoxycyclohex-5-ene (8). To 0.20 g (0.70 mmol) of **6**, CCl_4 (2.9 mL), *t*-BuOH (1.9 mL) and H_2O (0.3 mL) were added under N_2. After complete dissolution of **6**, 1.94 g of finely powdered KOH were added and a vigorous stirring was continued for 2 h. Finally, H_2O was added and the reaction mixture was extracted with CH_2Cl_2 giving 0.14 g (0.60 mmol, 90%) of **8** as yellow oil, which was used without any further purification. Nevertheless the title compound can be purified by flash chromatography (SiO_2; CH_2Cl_2/light petroleum 3:1). ^{1}H NMR: δ 6.05–5.93 (ddd, 1H, CH═, J=10.15, 4.64, 1.61 Hz), 5.92–5.84 (dd, 1H, CH═, J=10.12, 1.90 Hz), 3.97–3.80 (m, 2H, 2CH), 3.73 (m, 1H, CH), 3.45 (s, 6H, 2CH_3O), 3.15 (dd, 1H, CHN_3, J=11.35, 3.70 Hz). ^{13}C NMR δ: 130.4 (CH═), 126.1 (CH═), 80.9 (CHO), 74.6 (CHO), 61.9 (CHN_3), 61.8 (CHN_3), 58.2 (CH_3O), 57.0 (CH_3O). $[\alpha]_D^{25}=+33.2$ (c=1.30, $CHCl_3$). *m/z*: 224 (<1), 154 (70), 114 (100), 99 (48), 71 (52). IR (neat) ν_{max} (cm^{-1}): 2950, 2120, 1470, 1280, 1100. Anal. Calcd for $C_8H_{12}N_6O_2$: C, 42.85; H, 5.39; N, 37.48. Found: C, 42.82; H, 5.41; N, 37.52.

4.1.4. (+)-(1*S*,2*R*,3*R*,4*R*)-2,3-Diamino-1,4-dimethoxycyclohex-5-ene (9). To 60 mg (0.27 mmol) of the diazido derivative **8**, dissolved in MeOH (2.4 mL), were added 0.27 mL (2.73 mmol) of 1,3-propanedithiol and 0.38 mL (2.73 mmol) of Et_3N. After 48 h the solid formed was removed by filtration and the reaction mixture was evaporated. The residue, treated with 5 mL of methanol and 5 mL of Et_2O, was extracted with 2 M HCl. The aqueous layer was neutralised with Na_2CO_3 then extracted with CH_2Cl_2. 33 mg (0.19 mmol, 70%) of the title compound were obtained as viscous oil. ^{1}H NMR: δ 6.05–5.82 (m, 2H, 2CH═), 3.67 (m, 1H, CHO), 3.55 (d, 1H, CHO, J=8.12 Hz), 3.32 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 2.95 (bs, 4H, 2NH_2), 2.85 (m, 1H, CHN), 2.72 (dd, 1H, CHN, J=11.16, 3.86 Hz). ^{13}C NMR δ: 130.5 (CH═), 126.6 (CH═), 82.4 (CHOH), 75.5 (CHOH), 57.5 (CH_3O), 56.0 (CH_3O), 53.9 (CHN), 52.5 (CHN). $[\alpha]_D^{25}=+87.4$ (c=1.31, $CHCl_3$). *m/z*: 182 (62), 151 (18), 114 (100) 99 (55), 71 (22). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3420, 2910, 1720, 1640. Anal. Calcd for $C_8H_{16}N_2O_2$: C, 55.77; H, 9.37; N, 16.27. Found: C, 55.80; H, 9.41; N, 16.31.

4.1.5. (−)-1,6-Dideoxy-2,5-di-*O*-benzyl-3,4-isopropylidene-1,6-thia-D-mannitol (13). To 150 mg (3.12 mmol) of 50% NaH, washed with light petroleum, was added under nitrogen 7 mL of THF and 568 mg (2.58 mmol) of the thiepane derivative **12**.[16,17] The reaction mixture was stirred for 30 min and treated with a solution of 0.3 mL of benzyl bromide and 2 mg (0.012 mmol) of KI in 1.8 mL of THF. After 7 h, 150 mg (3.12 mmol) of 50% NaH and 0.3 mL (2.71 mmol) of benzyl bromide were added. Stirring was continued for 12 h, then H_2O was added and the mixture extracted with Et_2O (3×30 mL). The organic layer was dried and evaporated to give 0.99 g (2.39 mmol, 96%) of **13** as a yellow solid which was purified by flash chromatography (SiO_2; CH_2Cl_2/light petroleum 3:1), giving a solid with mp 55–57°C. ^{1}H NMR: δ 7.40 (m, 10H, ArH), 4.90 (d, 2H, 2CH*H*Ph, J=12.09 Hz), 4.70 (m, 2H, 2CHO, irradiating at 4.18 ppm a singlet was obtained), 4.69 (d, 2H, 2C*H*HPh, J=12.09 Hz), 4.18 (m, 2H, 2CHOBn), 2.90 (dd, 2H, 2CH*H*S, irradiating at 4.18 ppm a doublet was obtained; J=15.01 Hz), 2.63 (dd, 2H, 2C*H*HS, irradiating at

4.18 ppm a doublet was obtained; J=15.26 Hz), 1.50 (s, 6H, $2CH_3$). ^{13}C NMR: δ 138.8 (2CAr), 128.4 (4CHAr), 127.7 (4CHAr), 127.6 (2CHAr), 109.4 ($C(CH_3)_2$), 77.8 (2CHO), 74.2 (2CHO), 73.5 ($2CH_2O$), 36.9 ($2CH_2S$), 27.0 ($2CH_3$). $[\alpha]_D^{25}=-47.2$ (c=1.19, $CHCl_2$). m/z: 400 (1), 385 (3), 342 (2), 251 (4), 234 (5), 91 (100). IR (CCl_4) ν_{max} (cm^{-1}): 2940, 1250, 1080, 700. Anal. Calcd for $C_{21}H_{24}O_4S$: C, 67.72; H, 6.49. Found: C, 67.76; H, 6.52.

4.1.6. (−)-1,6-Dideoxy-2,5-di-*O*-benzyl-3,4-dihydroxy-1,6-thia-D-mannitol (14). 415 mg (1.04 mmol) of the (−)-1,6-dideoxy-2,5-di-*O*-benzyl-3,4-*O*-isopropylidene-1,6-thia-D-mannitol (**13**) was dissolved in 1.7 mL of CH_3CN. The mixture was treated with 0.34 mL of a CF_3COOH/H_2O 1:10 solution. After 24 h at 95°C, the reaction mixture was neutralised with a 10% $NaHCO_3$ solution. By evaporation of the reaction mixture a solid residue was obtained which was extracted with CH_2Cl_2. The organic layer was dried and evaporated to give 345 mg (0.96 mmol, 95%) of **14**, as a yellow oil, which could be used without any further purification for the next reaction. Nevertheless the title compound could be purified by flash chromatography (SiO_2; Et_2O/light petroleum 1:1). ^1H NMR: δ 7.60 (m, 10H, ArH), 4.95 (d, 2H, 2C*H*HPh, J=11.75 Hz), 4.87 (d, 2H, 2CH*H*Ph, J=11.75 Hz), 4.43 (d, 2H, 2CHO, J= 0.82 Hz), 4.17 (m, 2H, 2CHOBn), 3.16 (dd, 2H, 2C*H*HS, J=14.68, 4.03 Hz), 3.10 (bs, 2H, 2OH), 3.01 (dd, 2H, 2CH*H*S, J=14.68, 7.02 Hz). ^{13}C NMR: δ 138.1 (2CAr), 128.6 (4CHAr), 128.0 (4CHAr), 127.9 (2CHAr), 78.1 (2CHO), 72.2 ($2CH_2Ph$), 71.3 (2CHO), 32.8 ($2CH_2S$). $[\alpha]_D^{25}=-87.0$ (c=0.84, $CHCl_3$). m/z: 360 (<1), 342 (1), 91 (100). IR (neat) ν_{max} (cm^{-1}): 3450, 2970, 1080, 730. Anal. Calcd for $C_{20}H_{24}O_4S$: C, 66.64; H, 6.71. Found: C, 66.60 H, 6.75.

4.1.7. (−)-1,6-Dideoxy-2,5-di-*O*-benzyl-3,4-di-*O*-mesyl-1,6-thia-D-mannitol (15). To 166 mg (0.46 mmol) of the (−)-1,6-dideoxy-2,5-di-*O*-benzyl-3,4-dihydroxy-1,6-thia-D-mannitol (**14**) dissolved in 0.5 mL of pyridine, 0.11 mL (1.42 mmol) of methanesulfonyl chloride was added. The mixture was stirred for 5 h at 15°C. The reaction mixture, neutralised at 0°C with 2 M HCl, was extracted with CH_2Cl_2 (3×30 mL). The organic layer was washed with brine (20 mL) and dried. By evaporation of the solvent 220 mg (0.43 mmol, 92%) of **15** were obtained, as a yellow oil, which could be used without any further purification for the next reaction. Nevertheless the title compound could be purified by flash chromatography (SiO_2; Et_2O/light petroleum 1:1). ^1H NMR: δ 7.35 (m, 10H, ArH), 5.23 (bs, 2H, 2CHOMs), 4.71 (d, 2H, 2C*H*HPh, J=11.46 Hz), 4.62 (d, 2H, 2CH*H*Ph, J=11.46 Hz), 4.32 (m, 2H, 2CHOBn), 2.98 (s, 6H, $2CH_3SO_2$), 2.92 (dd, 2H, 2C*H*HS, J=14.94, 3.86 Hz), 2.77 (dd, 2H, 2CH*H*S, J=14.84, 7.48 Hz). ^{13}C NMR: δ 137.2 (2CAr), 128.6 (4CHAr), 128.5 (4CHAr), 128.4 (2CHAr), 79.6 (2CHO), 77.1 (2CHO), 72.7 ($2CH_2Ph$), 38.4 ($2CH_3$), 31.3 ($2CH_2S$). $[\alpha]_D^{25}=-51.7$ (c=1.37, $CHCl_3$). IR (CCl_4) ν_{max} (cm^{-1}): 2920, 1380, 1180, 700. Anal. Calcd for $C_{22}H_{28}O_8S_3$: C, 51.15; H, 5.46. Found: C, 51.18 H, 5.42.

4.1.8. (−)-(3*S*,4*R*,5*R*,6*S*)-4,5-Diazido-3,6-di-*O*-benzylthiepane (16). The reaction was carried out using the same procedure adopted for the synthesis of **6** using 113 mg (0.22 mmol) of (−)-1,6-dideoxy-2,5-di-*O*-benzyl-3,4-di-*O*-mesyl-1,6-thia-D-mannitol (**15**), 1.1 mL of DMSO and 72 mg (1.10 mmol) of NaN_3. After 2 h at 120°C the reaction mixture was diluted with 47 mL of EtOAc. By usual workup 77 mg (0.19 mmol, 85%) of **16** were obtained, as a dark oil, which could be used without any further purification for the next reaction. Nevertheless the title compound could be obtained 95% pure by flash chromatography (SiO_2; light petroleum/Et_2O 10:1). A sample was again purified by thin line preparative chromatography obtaining a pure yellow oil. ^1H NMR: δ 7.38 (m, 10H, ArH), 4.66 (d, 2H, 2CH*H*Ph, J=11.66 Hz), 4.58 (d, 2H, 2C*H*HPh, J=11.58 Hz), 4.14 (m, 2H, 2CHO), 3.91 (m, 2H, 2CHN), 2.85 (m, 4H, $2CH_2S$). ^{13}C NMR: δ 137.5 (2CAr), 128.3 (4CHAr), 128.2 (4CHAr), 128.1 (2CHAr), 78.2 (2CHO), 71.9 ($2CH_2O$), 64.5 (2CHN), 32.4 ($2CH_2S$). $[\alpha]_D^{25}=-44.8$ (c=1.08, $CHCl_3$). m/z: 382 (<1), 354 (1), 284 (82), 91 (100). IR (CCl_4) ν_{max} (cm^{-1}): 3000, 2100, 1450, 1280, 1100, 700. Anal. Calcd for $C_{20}H_{22}N_6O_2S$: C, 58.52; H, 5.40; N, 20.47. Found: C, 58.55; H, 5.42; N, 20.50.

4.1.9. (−)-(3*S*,4*R*,5*R*,6*S*)-4,5-Diazido-3,6-di-*O*-benzylthiepane-*S*,*S*-oxide (17). The title compound was obtained using the same procedure used to synthesised **5** starting from 53 mg (0.13 mmol) of (−)-(3*S*,4*R*,5*R*,6*S*)-4,5-diazido-3,6-di-*O*-benzylthiepane (**16**), 1.1 mL of CH_2Cl_2 and 91 mg (0.26 mmol) of 50% *m*-CPBA. Sulfone **17** was obtained as a yellow oil (56 mg, 98%), which can be used without any further purification. This compound was purified by flash chromatography (SiO_2; CH_2Cl_2/EtOAc 99.5:0.5) obtaining a pure viscous oil. ^1H NMR: δ 7.32 (m, 10H, ArH), 4.53 (d, 2H, 2CH*H*Ph, J=11.69 Hz), 4.51 (d, 2H, 2C*H*HPh, J=11.71 Hz), 4.13 (m, 2H, 2CHO), 3.79 (bs, 2H, 2CHN), 3.68 (m, 2H, 2CH*H*S), 3.33 (m, 2H, 2C*H*HS). ^{13}C NMR: δ 137.5 (2CAr), 128.7 (4CHAr), 128.6 (4CHAr), 128.5 (2CHAr), 73.0 (2CHO), 71.8 ($2CH_2Ph$), 64.7 (2CHN), 54.8 ($2CH_2S$). $[\alpha]_D^{25}=-32.3$ (c=1.10, $CHCl_3$). m/z: 413 (<1), 386 (<1), 132 (10), 91 (100). IR (CCl_4) ν_{max} (cm^{-1}): 3040, 2900, 2100, 1380, 1100. Anal. Calcd for $C_{20}H_{22}N_6O_4S$: C, 54.29; H, 5.01; N, 18.99. Found: C, 54.32; H, 5.04; N, 19.02.

4.1.10. (−)-(1*R*,2*R*,3*R*,4*R*)-2,3-Diazido-1,4-di-*O*-benzylcyclohex-5-ene (18). This was obtained analogously to **8** using 0.22 g (0.49 mmol) of the (−)-(3*S*,4*R*,5*R*,6*S*)-4,5-diazido-3,6-di-*O*-benzylthiepane-*S*,*S*-oxide (**17**), 1.8 mL of CCl_4, 1.2 mL of *t*-BuOH, 0.2 mL of H_2O and 1.18 g of KOH. The reaction mixture was vigorously stirred for 7 h and after workup was obtained 0.11 g of **18** (0.30 mmol, 61%) as a yellow oil. A sample was purified by flash chromatography (SiO_2; light petroleum/Et_2O 1:1) obtaining a white crystalline product, mp 50–52°C. ^1H NMR: δ 7.32 (m, 10H, ArH), 5.82 (m, 2H, 2CH=), 4.71 (d, 2H, 2C*H*HPh, J=11.76 Hz), 4.62 (d, 2H, 2CH*H*Ph, J=11.76 Hz), 4.22 (m, 2H, 2CHN), 3.94 (m, 2H, 2CHOBn). ^{13}C NMR: δ 137.8 (2CAr), 128.7 (CH), 128.3 (CH), 128.1 (CH), 128.0 (CH), 73.4 (2CHO), 72.5 ($2CH_2Ph$), 59.8 (2CHN). $[\alpha]_D^{25}=-226.3$ (c=0.68, $CHCl_3$). m/z: 347 (<1), 306 (4), 257 (2), 106 (12), 91 (100). IR (CCl_4) ν_{max} (cm^{-1}): 3000, 2100, 1250, 700. Anal. Calcd for $C_{20}H_{20}N_6O_2$: C, 63.82; H, 5.36; N, 22.33. Found: C, 63.85; H, 5.32; N, 22.30.

4.1.11. (−)-(1*R*,2*R*,3*R*,4*R*)-2,3-Diamino-1,4-di-*O*-benzylcyclohex-5-ene (19). To 71 mg (0.19 mmol) of the diazido

derivative **18** dissolved in 4.1 mL of MeOH were added 0.190 mL (1.89 mmol) of 1,3-propanedithiol and 0.270 mL (1.94 mmol) of Et_3N. After 48 h at room temperature, the solid formed was removed by filtration and the filtrate evaporated. The residue, treated with 5 mL of CH_3OH and 5 mL of Et_2O, was extracted with 2 M HCl (5 mL). The aqueous layer was neutralised with Na_2CO_3 then extracted with CH_2Cl_2 (3×20 mL). The title compound (58 mg, 68%) was obtained as a viscous oil. ^{1}H NMR: δ 7.48–7.13 (m, 10H, ArH), 5.95 (bs, 4H, $2NH_2$), 5.85 (s, 2H, 2CH═), 4.58 (d, 2H, 2C*H*HPh, J=10.97 Hz), 4.47 (2H, 2CH*H*Ph, J=11.10 Hz), 4.02 (bs, 2H, 2CHO), 3.44 (bs, 2H, 2CHN). ^{13}C NMR: δ 138.1 (2CAr), 129.0 (CH), 128.6 (CH), 128.4 (CH), 128.2 (CH), 128.0 (CH), 72.8 (2CHO), 72.4 ($2CH_2$), 49.4 (2CHN). $[\alpha]_D^{25}$=−190.7 (c= 1.31, $CHCl_3$). m/z: 324 (<1), 266 (12), 91 (100), 58 (42). IR (neat) ν_{max} (cm^{-1}): 3372, 3300, 3000, 1650, 770. Anal. Calcd for $C_{20}H_{24}N_2O_2$: C, 74.05; H, 7.46; N, 8.63. Found: C, 74.07; H, 7.48; N, 8.65.

4.1.12. (1*R*,2*R*,3*R*,4*R*)-2,3-Diamino-1,4-dihydroxycyclohex-5-ene (11). To 42 mg (0.13 mmol) of the (−)-(1*R*,2*R*,3*R*,4*R*)-2,3-diamino-1,4-di-*O*-benzylcyclohex-5-ene (**19**), dissolved in 8 mL of CH_2Cl_2 and cooled to −78°C, was added 0.64 mL (0.64 mmol) of BCl_3 (1 M in hexane). After 2 h the reaction mixture was warmed to 0°C and stirred overnight, then cooled to −78°C and quenched with MeOH (2 mL). The reaction mixture was evaporated under reduced pressure (0.1 mmHg) for 2 h obtaining 13.5 mg (0.094 mmol, 72%) of the title compound as a dark oil. ^{1}H NMR (CD_3OD): δ 6.00 (dd, 2H, 2CH═, J=2.56, 1.10 Hz), 4.44 (m, 2H, 2CHO), 3.88 (dd, 2H, 2CHN, J=2.52, 1.30 Hz). ^{13}C NMR (CD_3OD): δ 130.3 (2CH═), 63.9 (2CHO), 51.3 (2CHN). The dark solution was unsuitable for $[\alpha]_D^{25}$ determination. m/z: 143 (<1), 83 (6), 69 (18), 57 (39), 55 (51). IR (neat) ν_{max} (cm^{-1}): 3400, 2950, 1074, 1056. Anal. Calcd for $C_6H_{12}N_2O_2$: C, 49.99; H, 8.39; N, 19.43. Found: C, 49.97; H, 8.41; N, 19.40.

4.2. Kinetics of glycosidase inhibition

α-Glucosidase (EC 3.2.1.20) from baker's yeast, β-glucosidase (EC 3.2.1.21) from sweet almonds, and *p*-nitroglucosides were purchased from Sigma Chemical.

The enzymatic activity was measured by incubating the enzyme and inhibitor in 0.1 M *N*-(2-hydroxyethyl)piperazine-*N*′-ethansulfonic acid/K (HEPES) salt buffer solution at pH=6.86 at 37°C for 15 min. Appropriate quantity of the *p*-nitrophenylglucoside stock solution (0.01–0.05 M), thermostated at 37°C, was added by a Hamilton syringe and the initial rate was followed at λ=400 nm on a Perkin–Elmer Lambda 6 spectrophotometer. The amount of enzyme used was 0.02 units. Inhibition kinetics were carried out at 1.14×10^{-4} M for **9**, 3.8×10^{-3} M for **10** and 2.3×10^{-4} M for **11**. The competitive inhibition constant K_i were calculated from the equation: $K_i=K_m\times[I_0/K'_m-K_m]$,[20] where K_m and K'_m are the apparent dissociation constant in the absence and presence of inhibitor (I_0), respectively. The parameters were calculated by a non-linear least squares method.[21]

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