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Synthesis and biological activity of the (–)-(2*R*,3*S*,4*S*)-3-azido-4-methoxy-2-(1′*S*-methoxy-2′-azido)ethyl-thiolane

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Abstract

A stereospecific ring contraction reaction, promoted by NaN_3, was detected starting from a thiepane derivative obtained from D-sorbitol, an inexpensive alcohol sugar. The major polyfunctionalized thiolane derivative obtained was investigated as a potential glycosidase inhibitor.

1. Introduction

The polyhydroxythiolanes are the thioanalogues of the polyhydroxypyrrolidines, a class of compounds that have been recognized as important glycosidase inhibitors. Nevertheless, few examples are reported for the synthesis of cyclic polyhydroxylated five-membered sulfides[1] or sulfonium salts.[2] On the other hand these small-ring sulfur heterocycles have recently received considerable attention due to their use as sugar mimics in the synthesis of thionucleosides.[3] It is worth noting that a potent α-glucosidase inhibitor, isolated from *Salacia reticulata*, features the structure of a polyhydroxylated thiolanium salt.[4] Due to their significance we have studied the formation of a five-membered ring thiosugar derivative.

2. Results and discussion

In order to develop new syntheses for enantiomerically pure thiolane derivatives we have examined a ring contraction reaction, which occurs on a seven-membered sulfur heterocycle, by nucleophilic transannular substitution. The (3*S*,4*S*,5*S*,6*S*)-3,4,5,6-tetrahydroxy thiepane can be

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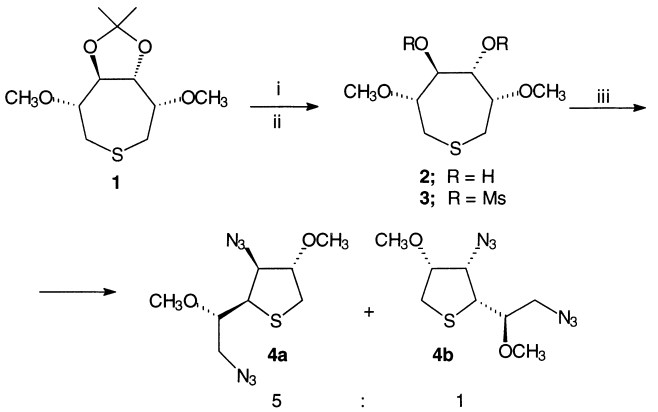
easily synthesized starting from D-mannitol, by means of a simple procedure.[5,6] We have applied this method as such, but starting from D-sorbitol, for the synthesis of the corresponding thiosugar, which can be quantitatively protected to the dimethoxy derivative **1** (Scheme 1). This latter compound was deprotected under acidic conditions to give the hydroxy derivative **2** which was transformed into the dimesyl derivative **3**. Treatment of **3** with NaN_3 in DMSO at 120°C gave a crude reaction mixture consisting of two diastereoisomers **4a** and **4b** in a 5:1 ratio (75% yield).



Scheme 1. (i) 0.1N H_2SO_4 (96%); (ii) MsCl, pyridine (95%); (iii) NaN_3, DMSO (75%)

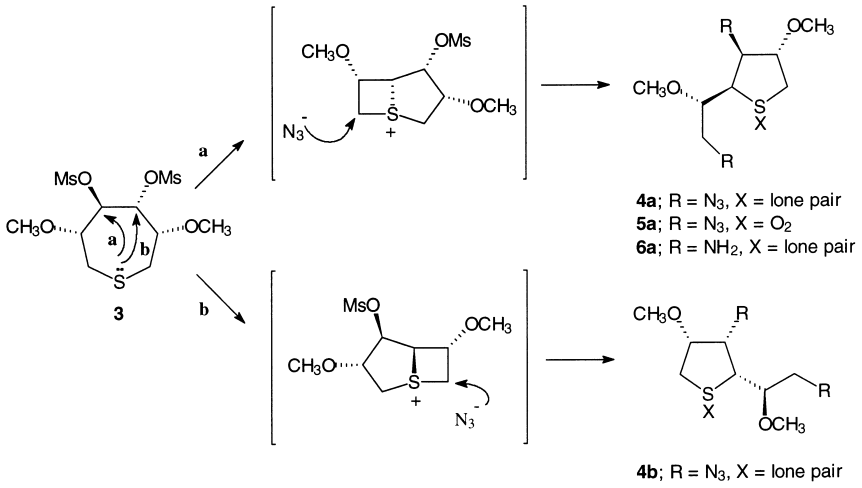
Examples of ring contractions on polyhydroxylated thiepanes can be found in the literature.[6] In particular we have reported[7] a stereospecific ring contraction reaction, which occurs on treating polyhydroxylated thiepanes with Me_3SiI. Under those conditions six-membered sulfur heterocyclic compounds were exclusively obtained. From the data reported in Scheme 1 it appears that the ring contraction reaction, deriving from nucleophilic transannular substitution, competes favourably with the nucleophilic substitution on the two mesyl groups, thus affording **4a** and **4b**. A favourable stereochemistry and the displacement of a very good leaving group promotes the formation of a [3.2.0] bicyclic intermediate (Scheme 2). Due to the absence of a C_2-symmetry axis two different [3.2.0] bicyclic sulfonium salt intermediates can be generated by nucleophilic transannular attack of sulfur on the carbons carrying the mesyl groups. The subsequent attack of N_3^- α to the sulfur of the strained four-membered rings affords the two thiolane derivatives **4a**,**b**.

It is worth noting that the analogous dimesylthiepane derivative, synthesized from D-mannitol, treated with NaN_3 does not suffer the same ring contraction reaction[8] and gives exclusively an S_N2 reaction. On the other hand the thiepane **7** (Scheme 3), having the stereochemistry of L-iditol, shows the formation of the thiolane derivative **8** only in a small amount (22%).

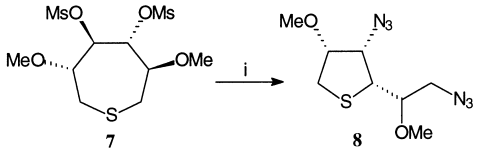
These findings are relevant to the related stereochemistry of the four stereogenic centres.

In particular from the L-iditol derivative **7**, due to a C_2-symmetry axis, **8** was the only thiolane isolated from the complex crude mixture. Other byproducts presumably derive from isomerization of the stereogenic centres and elimination of methansulfonic acid, as reported in the literature.[9]

By means of two consecutive flash chromatographic purifications **4a** (Scheme 2) was obtained pure, in 45% yield; **4b** was, in contrast, obtained >90% pure (8% yield). The yield of 45% related to **4a** can be considered quite acceptable especially in regard to the smooth synthesis of the starting thiepane derivative, obtained from the inexpensive alcohol sugar D-sorbitol. In light of the potential biological activity of these compounds we have tried to elucidate completely their



Scheme 2.



Scheme 3.

structure. The structures of **4a** and **4b**, reported in Scheme 1, were established on the basis of analytical and spectroscopic data (2D ^{1}H–^{1}H-homo and ^{13}C–^{1}H-heteronuclear shift-correlation spectra); furthermore, we confirmed the structural assignment of the major diastereoisomer by X-ray crystallography. For this purpose we have oxidized compound **4a** to the related sulfone **5a** (Scheme 2), obtaining a pure white crystalline product. The overall molecular geometry and the crystallographic numbering of **5a** are shown in Fig. 1.

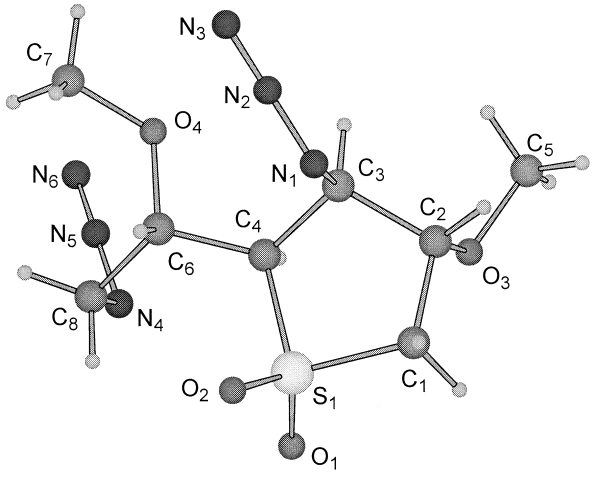


Figure 1.

The absolute configurations at the C(2), C(3), C(4) and C(6) stereogenic centres have been unambiguously assigned through X-ray crystallography as *S*, *S*, *R* and *S*, respectively, on the basis of the Flack parameter[10] (–0.0159 for correct absolute structure). The thiolane penta-atomic ring shows an envelope conformation at C(3) (dev. 0.29 Å) with the azido substituent, as well as the methoxy one, occupying an axial position. As usually observed in azido complexes, the N–N–N system is not symmetric: N(1)–N(2) and N(4)–N(5) bond lengths, 1.216(4) and 1.234(5) Å, are significantly longer than N(2)–N(3) and N(5)–N(6), respectively. Actually, the latter are 1.116(4) and 1.129(5) Å long, with the short N–N bond always between the N atom in the middle and the N terminal atom. Bond lengths and angles for **5a** are reported in Table 1S (see supplementary material). A network of intermolecular hydrogen bonding of the kind C–H···O and C–H···N is responsible for its crystal packing. A short intramolecular H-bond occurs at C(4)–H(4)···N(4), with donor···acceptor distance of 2.951(4) Å and a donor-H···acceptor angle of 104.8°.

The X-ray structure of **5a** corroborates the reaction mechanism proposed in Scheme 2. In view of the importance of the amino functionalities[11] we submitted to reduction with Pd/C a pure sample of the major thiolane derivative **4a**, obtaining nearly quantitatively the diamino compound **6a** (Scheme 2). The behaviour of this compound towards the main glycosidases has been examined.

2.1. Glycosidase inhibition studies

The hydrolysis of the *p*-nitro α-D-glucopyranoside by the α-glucosidase was weakly inhibited by the diamino compound **6a** ($K_i = 3300$ μM) and the inhibition was apparently competitive. The hydrolysis of the *p*-nitro α-D-glucopyranoside by the α-fucosidase was reduced by 17%. The hydrolysis of the *p*-nitro β-D-glucopyranoside by the β-glucosidase and of *p*-nitrophenyl α-D-galactopyranoside by α-galactosidase was only reduced by 13%.

3. Conclusion

Using a simple procedure we have synthesized, in acceptable yield, a new sulfur sugar derivative, starting from a natural inexpensive alcohol sugar. The preliminary results related to the biological activity seem to be promising. Due to the importance of the polyfunctionalized thiolanes, we intend to extend our investigations to completely unprotected compounds and verify the possibility of improving the ring contraction reaction starting from other polyfunctionalized thiepanes. We also intend to enlarge the screening on the biological activity studying the inhibitory effect of these substrates on different glycosidases.

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 were uncorrected. Preparative flash chromatographic experiments were performed using

ICN silica gel 230–400 mesh. For TLC, pre-coated 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 FT-IR 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.

4.2. (–)-(2R,3S,4S)-3-Azido-4-methoxy-2-(1′S-methoxy-2′-azido)ethyl-thiolane **4a** and (2R,3S,4R)-3-azido-4-methoxy-2-(1′S-methoxy-2′-azido)ethyl-thiolane **4b**

To 0.36 g (1 mmol) of the thiepane derivative **3**,[9] dissolved in 5 mL of DMSO, 0.20 g (3 mmol) of NaN_3 were added. After 19 h at 120°C the reaction mixture was diluted with 150 mL of AcOEt, washed with H_2O then with brine. A crude reaction mixture [0.19 g (75%)], constituted by the two diastereoisomers **4a** and **4b** in a 5:1 ratio, were obtained. These compounds were separated by two consecutive flash chromatographic purifications (SiO_2; petroleum ether:Et_2O dry:cyclohexane 50:30:20). Compound **4a** was obtained pure in 45% yield as colourless oil; **4b** instead was obtained in 8% yield (>90% pure). For **4a**: ^1H NMR δ 4.31 (dd, 1H, CHN_3, J 4.0, 1.9 Hz), 4.04–4.02 (m, 1H, HC_4), 3.81 (dd, 1H, CHS, J 9.9, 4.0 Hz), 3.75 (dd, 1H, C*H*HN_3, J 13.6, 2.8 Hz), 3.64–3.54 (m, 1H, CHO), 3.49 (s, 3H, CH_3), 3.38 (s, 3H, CH_3), 3.17–3.06 (m, 2H, CH*H*N_3 and C*H*HS), 2.93 (d, 1H, CH*H*S, J 11.7 Hz). ^{13}C NMR δ 86.0 (CHO), 80.2 (CHO), 68.2 (CHN_3), 57.4 (CH_3), 57.1 (CH_3), 52.1 (CH_2N_3), 50.0 (CHS), 34.4 (CH_2S). m/z (EI): 230 (<1), 202 (17), 118 (35), 89 (63), 58 (52), 45 (100). $[\alpha]_D^{20} = -42.6$ ($c = 1.41$, $CHCl_3$). IR ($CHCl_3$) ν_{max}/cm^{-1} = 3006, 2934, 2107, 1260, 1112. Anal. calcd for $C_8H_{14}N_6O_2S$: C, 37.20; H, 5.46. Found: C, 37.28; H, 5.50. For **4b**: ^1H NMR δ 4.05–3.60 (m, 2H), 3.50–3.15 (m, 4H), 3.43 (s, 3H, CH_3 superimposed), 3.35 (s, 3H, CH_3 superimposed), 3.05–2.50 (m, 2H). ^{13}C NMR δ 86.2 (CHO), 80.2 (CHO), 67.0 (CHN_3), 59.4 (CH_3), 58.2 (CH_3), 52.1 (CH_2N_3), 47.8 (CHS), 30.3 (CH_2S).

4.3. (–)-(2R,3S,4S)-3-Azido-4-methoxy-2-(1′S-methoxy-2′-azido)ethyl-thiolane-S,S-oxide **5a**

To 0.51 g (2.0 mmol) of **4a**, dissolved in 7 mL of CH_2Cl_2 and cooled at 0°C, 0.79 g (4.58 mmol) of 50% *m*-chloroperbenzoic acid were added. After 3 h at room temperature, $Na_2S_2O_5$ was added to destroy the unreacted peracid. The reaction mixture was diluted with CH_2Cl_2 and washed with a saturated solution of $NaHCO_3$. The organic layer, washed with brine and dried, gave, after evaporation of the solvent, 0.57 g (98%) of a crude product which was purified by flash chromatography (SiO_2; CH_2Cl_2:Et_2O 95:5) to give a white crystalline solid which was subsequently recrystallized by addition of petroleum ether to a solution of the product in CH_2Cl_2; mp 103–104°C. ^1H NMR δ 4.55–4.50 (m, 1H, $CHSO_2$), 4.09–4.05 (m, 1H, CHO), 3.95 (dd, 1H, C*H*HN, J 13.9, 2.7 Hz), 3.92–3.86 (m, 1H, CHO), 3.76 (dd, 1H, CHN, J 10.2, 4.9 Hz), 3.46 (s, 3H, CH_3), 3.39 (s, 3H, CH_3), 3.35 (dd, 1H, CH*H*N, J 13.9, 2.5 Hz), 3.29 (m, 2H, CH_2SO_2). ^{13}C NMR δ 78.3 (CHO), 75.1 (CHO), 63.9 (CH), 61.4 (CH), 57.4 (CH_3), 57.1 (CH_3), 55.2 (CH_2), 49.4 (CH_2). m/z (EI): 290 (<1), 234 (17), 179 (12), 121 (100), 112 (18), 84 (18), 58 (46). $[\alpha]_D^{20} = -28.3$ ($c = 1.05$,

$CHCl_3$). IR (Nujol) $\nu_{max}/cm^{-1} = 2990$, 2115, 1305, 1110. Anal. calcd for $C_8H_{14}N_6O_4S$: C, 33.10; H, 4.86. Found: C, 33.15; H, 4.81.

4.4. (–)-(2R,3S,4S)-3-Amino-4-methoxy-2-(1′S-methoxy-2′-amino)ethyl-thiolane **6a**

To 0.26 g (1.00 mmol) of **4a**, dissolved in 16 mL of CH_3OH, were added 0.12 g of Pd/C suspended in 4.2 mL of CH_3OH. Treating for 24 h this stirred suspension with H_2 at 1 atm, 0.20 g (97%) of **6a** were recovered by filtration on Celite and subsequent evaporation of the solvent, as a pale orange oil. m/z (EI): 174 (< 1), 148 (16), 128 (48), 118 (52), 86 (100). $[\alpha]_D^{20} = -51.2$ ($c = 1.13$, $CHCl_3$). IR ($CHCl_3$) $\nu_{max}/cm^{-1} = 3380$, 2937, 1600, 1461, 1088, 856. ^{1}H NMR δ 3.80–3.65 (m, 1H, CHN), 3.60–3.22 (m, 3H), 3.35 (s, 3H, CH_3, superimposed), 3.25 (s, 3H, CH_3, superimposed), 3.10–2.55 (m, 8H). ^{13}C NMR δ 88.5 (CHO), 79.7 (CHO), 57.8 ($CHNH_2$), 56.9 (CH_3), 56.8 (CH_3), 50.8 (CH_2NH_2), 42.4 (CHS), 33.5 (CH_2S). Anal. calcd for $C_8H_{18}N_2O_2S$: C, 46.58; H, 8.79. Found: C, 46.60; H, 8.71.

4.5. (–)-(2R,3S,4R)-3-Azido-4-methoxy-2-(1′S-methoxy-2′-azido)ethyl-thiolane **8**

The same procedure applied for the synthesis of **4a** and **4b** was followed using 320 mg of (+)-1,6-anhydrous-4,5-di-*O*-methansulfonyl-3,6-dimethoxy-1,6-thia-L-iditol **7**,[12] 286 mg of NaN_3 and 4.3 mL of DMSO. The crude product was purified by flash chromatography (SiO_2; light petroleum:Et_2O 5:1), giving 50 mg of the title compound as a pale yellow oil. $[\alpha]_D^{20} = -14.4$ ($c = 0.82$, $CHCl_3$). ^{1}H NMR δ 4.03 (t, 1H, CHN, J 5.0 Hz), 3.84 (q, 1H, CHO, J 5.4 Hz), 3.65–3.25 (m, 4H), 3.47 (s, 3H, CH_3, superimposed), 3.38 (s, 3H, CH_3, superimposed), 3.02 (dd, 1H, C*H*HS, J 11.3, 5.4 Hz), 2.77 (dd, 1H, CH*H*S, J 11.3, 5.8 Hz). ^{13}C NMR δ 87.1 (CHO), 82.6 (CHO), 68.4 (CHN_3), 58.5 (OCH_3), 57.8 (OCH_3), 51.2 (CH), 49.5 (CH_2N), 32.7 (CH_2S). m/z (EI): 202 (17), 128 (18), 118 (51), 89 (71), 45 (100). IR ($CHCl_3$) $\nu_{max}/cm^{-1} = 2995$, 2100, 1263, 1107. Anal. calcd for $C_8H_{14}N_6O_2S$: C, 37.20; H, 5.46. Found: C, 37.25; H, 5.53.

4.6. X-Ray crystallography

Crystals of **5a** were grown from CH_2Cl_2 and petroleum ether and used for data collection on an Enraf–Nonius CAD4 diffractometer. Structure was solved by direct methods and refined anisotropically for all non-hydrogen atoms, using full-matrix least-squares on F.[10] Hydrogen atoms were geometrically positioned and refined using the 'riding' model with the isotropic equivalent thermal parameters equal to 1.2 (1.5 for methyl groups) of that of their parent atoms. The final Fourier maps revealed non-significant residual density. All the calculations were performed using the SHELXS-86[10] and SHELXL-93[13] programs. Schakal 97[14] and Platon 97[15] have been used for graphics and geometrical calculations, respectively.

Atomic coordinates and anisotropic temperature factors have been deposited at the Cambridge Crystallographic Data Centre, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ as SUPP (CCDC 140423).

4.7. Inhibition analysis of glycosidase

The enzymatic activity was carried out on a single substrate concentration (1.8×10^{-3} M) in 0.1 M *N*-(2-hydroxyethyl)piperazine-*N*′-ethansulfonic (HEPES) buffer at pH 6.86 or in 0.05 M

sodium acetate buffer at pH 6.0 with α-fucosidase, in the presence of appropriate *p*-nitrophenylglucoside at 37°C. The amount of enzyme used changes from 0.2 to 0.02 units. The rates of hydrolysis of *p*-nitrophenylglucosides were followed spectrophotometrically measuring at $\lambda = 400$ nm the *p*-nitrophenol released on a Perkin–Elmer Lambda 6 instrument. The initial velocity was followed for less than 5% of the reaction. The enzymes used were: α-glucosidase (EC 3.2.1.20) from baker's yeast, β-glucosidase (EC 3.2.1.21) from sweet almonds, α-galactosidase (EC 3.2.1.22) from green coffee beans, α-fucosidase (EC 3.2.1.51) from bovine kidney and were obtained by the Sigma Chemical Company. The value of the inhibition constant K_i was calculated by a non-linear least-squares analysis using FigP (Biosoft) programme.

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