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Synthesis of furan 4′-thio-*C*-nucleosides, their methylsulfonium and sulfoxide derivatives. Evaluation as glycosidase inhibitors

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Abstract—A series of furan *C*-nucleosides having a sulfur atom in the sugar ring were synthesised. The α and β anomers of 3-ethoxycarbonyl-2-methyl-5-(4′-thio-D-erythrofuranosyl)furans **10** and **11** were obtained by acid treatment of (4′-*S*-acetyl-4′-thio-D-*arabino*-tetritol-1-yl)furan **9**. Oxidation of **10** with *m*-chloroperbenzoic acid gave sulfoxide **12** as one epimer at the sulfur atom whereas **11** was transformed into sulfoxide **13** as an epimeric mixture. *S*-Methylation of **10** and **11** with methyl triflate led to sulfonium salts **14** and **15**. The prepared compounds were found to be moderate inhibitors of α-L-fucosidase. © 2003 Elsevier Science Ltd. All rights reserved.

1. Introduction

Glycosidase enzymes acting on cell-surface carbohydrates are involved in processes such as viral infection and tumor metastasis.[1] Synthetic and naturally occurring inhibitors of glycosidases[2–7] have already demonstrated promising therapeutic possibilities in controlling viral infections including HIV,[8] hepatitis,[9] and influenza,[10] tumor cells[11] or carbohydrate-mediated metabolic disorders such as diabetes[12] and Gaucher disease.[13]

Recently, potent α-glucosidase inhibitors salacinol **1**[14] and kotalanol **2**[15] (Fig. 1) have been isolated from *Salacia reticulata* WIGHT[14,15] and *S. oblonga* and *S. chinensis*,[16] traditionally used in the Ayurvedic system of India and Sri Lanka for the treatment of diabetes. The sulfonium structure of these compounds has stimulated different groups to carry out the synthesis of salacinol[17,18] and other carbohydrate-based cyclic sulfonium compounds[19–21](e.g. **3**[20] and **4**[21]) as a new class of glycosidase inhibitors. Heteroanalogues of salacinol having nitrogen[22] or selenium[23] instead of sulfur have also been reported. Previous to salacinol few examples of synthetic sugar-derived cyclic[24] and bicyclic[25] sulfonium compounds were known.

The finding that the new type of imino-*C*-nucleosides **5**[26] and **6**[27] (Fig. 2) are strong and selective glycosidase inhibitors against β-D-galactosidase and α-L-fucosidase, respectively, has promted us to prepare their sulfur analogues in order to study the influence of the heteroatom and the positive charge in their inhibitory properties. Some sugar analogues having divalent sulfur in the ring have shown activity as glycosidase inhibitors, and that activity could be modified after oxidation or alkylation.[21,28,29] In this paper we report the synthesis of **10** and **11** as the sulfur analogues of **5** and **6**, their sulfoxides, and the methyl sulfonium ion derivatives, and evaluate their activity against several glycosidases. Compounds **10** and **11** can be considered as 4′-thio-*C*-nucleosides, many of which exhibit antitumor and antiviral properties.[29–31]

2. Results and discussion

López Aparicio et al.[32] have previously described the synthesis of 4′-thio-D-erythrofuranosyl-furans **10** and **11** as a non-resolved 1:6 α and β mixture by treatment of 5-(4′-*S*-*tert*-butyl-4′-thio-D-*arabino*-tetritol-1-yl)-3-ethoxycarbonyl-2-methylfuran with conc. hydrochloric acid at rt. Instead of this method we prepared the mixture of **10** and **11** by selective tosylation of **7**,[33] followed by nucleophilic displacement with potassium thioacetate and treatment of **9** with 10% trifluoroacetic acid in refluxing 1:1 ethanol–water (Scheme 1). This gave a mixture of **10** and **11** (76%) in a 2:3 α/β ratio, as deduced by ^{1}H NMR. Repeated chromatography on silica gel of this mixture allowed the separation of **10** (29%) and **11** (39%).

The formation of these 4′-thio-*C*-nucleosides **10** and **11** involves the hydrolysis of the thioacetate group and the nucleophilic attack of the thiol group on the stabilised carbocation in C-1′ as described for the synthesis of 4′-thio-D-erythrofuranosyl-imidazoles (Fig. 3).[34]

Keywords: *C*-nucleoside; 4′-thio-nucleoside; sulfoxide; sulfonium salt; glycosidase inhibitor; furan.
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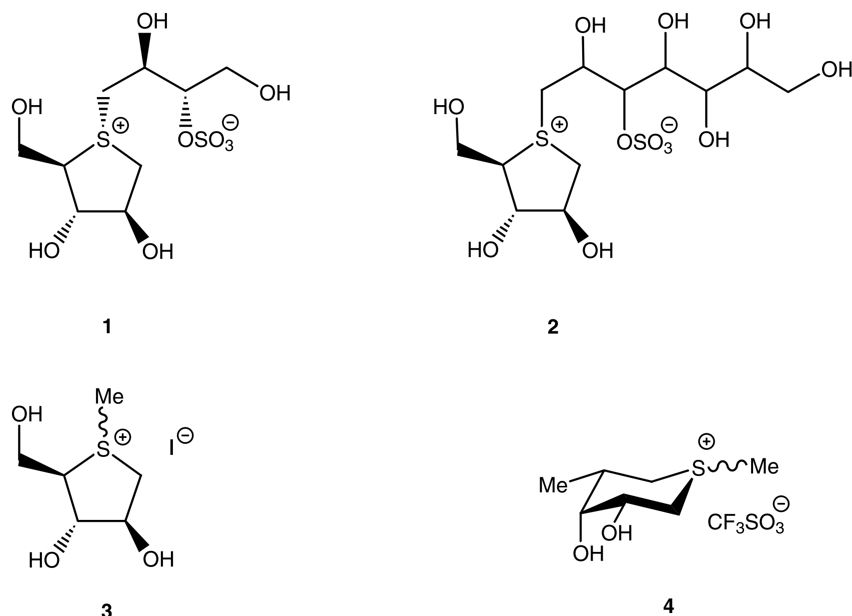


Figure 1.

The anomeric configuration of **10** and **11** was confirmed by the optical rotation values, the α anomer dextrorotatory and the β anomer being strongly levorotatory, in agreement with the reported data for **11**[32] and for the α and β anomers of 4′-thio-D-erythrofuranosyl-imidazoles.[34] The conformational analysis of the thiofuranosyl ring of **10**, based on the relationship of the vicinal coupling constants with the pseudorotational parameters,[35,36] indicates that the α anomer exhibits a strong preference for the *N* conformations E_2, $^3T_2$ and ^3E (not shown), which occupy the northern part of the pseudorotational circle. These conformations are in accordance with the high values (9.8 and 6.9 Hz) for $J_{3',4'}$ (Table 1). On the contrary, the β anomer **11** populates preferentially the *S* conformations ^2E, $^2T_3$ and E_3, according to the high value (7.2 Hz) for $J_{1',2'}$ and the small values (4.8 and 3.8 Hz) for $J_{3',4'}$. Similar conformational behaviour was found for the α and β anomers of 4′-thio-*C*-nucleosides derived from imidazol.[34]

In order to test how a positive charge on the sulfur atom of **10** and **11** would affect their activity as glycosidase inhibitors, we prepared the sulfoxides **12** and **13**. Oxidation of **10** with an equimolecular amount of *m*-chloroperbenzoic acid at −78°C in EtOAc gave sulfoxide **12** in a 93% yield after chromatography (Scheme 2). ^{1}H NMR of **12** showed signals only for one stereoisomer. On the other hand, treatment of the β isomer **11** with 1 equiv. of MCPBA gave sulfoxide **13** in a 95% yield after purification. The ^{1}H NMR spectrum of **13** revealed a 2:3 mixture of stereoisomers, which could not be resolved by chromatography. The vicinal coupling constants for sulfoxides **12** and **13** indicate that the conformation of the thiofuranosyl ring is not modified by *S*-oxidation. The *R* configuration for the only isomer of **12** is deduced from the deshielding effect (0.63 ppm) of the *S*-O group on the H-3′ (Fig. 4). This is similar to the *syn*-axial effect found for thiane oxides that implies a deshielding of the axial β-hydrogens in axial sulfoxides.[21,37] The proposed configuration for **12** implies that the oxygen is added exclusively on the opposite face to the three substituents on the thiolane ring. This is in accordance with the reported steric approach control in oxidations of *cis*-3,4-dihydroxythiolanes with peroxycarboxylic acids.[38]

For sulfoxide **13**, the *R* configuration of the sulfur atom of the minor isomer **13a** could be deduced by the deshielding effect of the oxygen attached to the ring sulfur on H-2′ (0.55 ppm), which is a pseudoaxial orientation in the ^2E conformation. This deshielding effect on H-2′ is absent in the major isomer **13b**, according to the *trans* disposition of the S–O group to H-2′.

Reaction of **10** and **11** with methyl triflate in nitromethane afforded the sulfonium salts **14** and **15**, respectively, in almost quantitative yields (Scheme 2), as mixtures of stereoisomers which showed the same chromatographic mobility. ^{1}H NMR of **14** and **15** in D_2O showed signals for the two possible stereoisomers. The conformational behaviour of the thiofuranosyl ring of these sulfonium salts was found to be almost the same as for the parent thiolanes **10** and **11**, as deduced from the analysis of the vicinal coupling constants. Interestingly, the preferred conformations of the prepared thiolanes, their sulfoxides and sulfonium salts derivatives are strongly dependent on the anomeric configuration; however, they seem to be almost independent of the configuration of the sulfur atom.

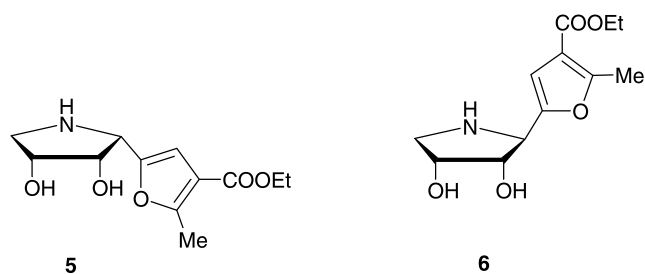
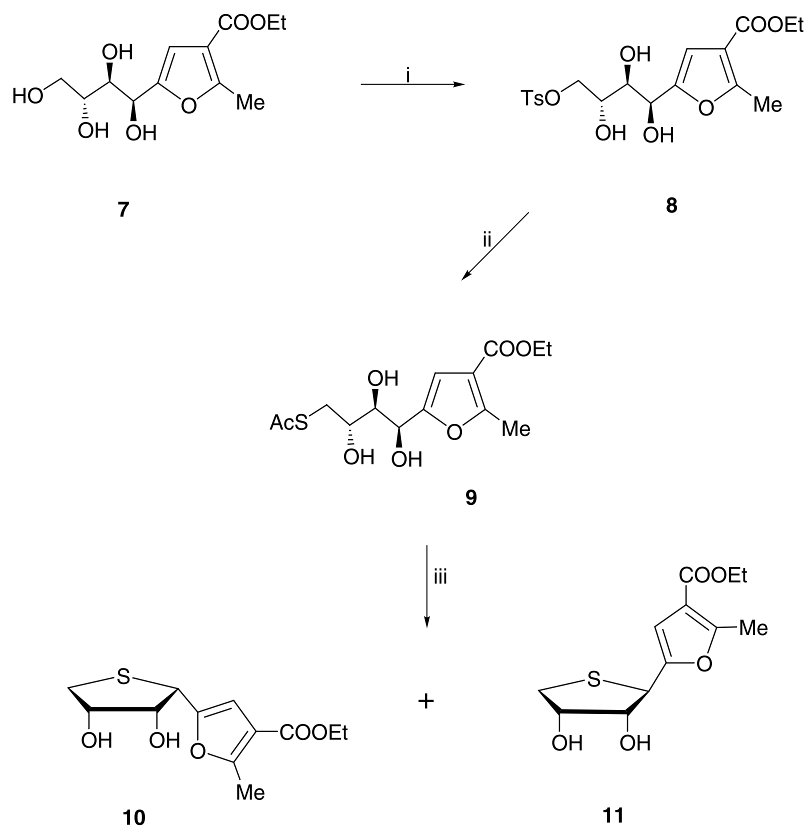


Figure 2.



Scheme 1. *Reagents and conditions*: (i) TsCl, Py, −15°C, 2 h; (ii) KSAc, DMF, rt, 3 h; (iii) 10% TFA, 1:1 EtOH/H_2O, reflux, 1 h.

The stereochemistry of the ring sulfur atom of the methyl sulfonium salts **14** and **15** was determined by NOE correlation experiments involving the SMe groups (Fig. 5). In the case of **14**, the *R* and *S* stereoisomers could be clearly distinguished by the strong NOE effect observed between H-1′ and the SMe group for the *R* isomer (**14a**), which is absent for the *S* isomer. Similarly, for **15** a strong NOE was found between the SMe group and the anomeric proton in the *S* isomer (**15b**).

The ^{1}H NMR of **15** in D_2O just dissolved showed a *R*/*S* mixture in a 2:5 ratio; 3 h after it showed these epimers in an 1:1.2 ratio and signals for the α anomer in a ca. 3%. The higher proportion of the *S* isomer before equilibration could be explained in terms of steric interaction between the methyl group and the bulky substituent on C-1′ during the formation of the sulfonium salt. The equilibration observed would involve the opening of the thiolanium ring to give a stabilised carbocation on C-1′ and a neutral thioether group, whose sulfur is able to attack C-1′ to form the other stereoisomer in the sulfur atom. A slow anomerization took place, as after 6 h at rt only 5% of α anomer was detected. For α anomer **14** just dissolved in D_2O a ca. 1:1 *R*/*S* mixture ratio was observed together with 10% of both stereoisomers of β anomer. After 6 h at rt the signals for the β anomer increased up to 35% in a ca. 1:1 *R*/*S* ratio. The faster anomerization for the α anomers **14** compared with that for the β anomers **15** could be explained in terms of steric hindrance between the furan on C-1′ and the OH on C-2′.

The shielding effect of the SMe group on protons H-4′ that are in a *cis* arrangement is observed by comparison of the

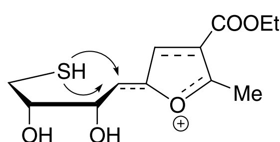
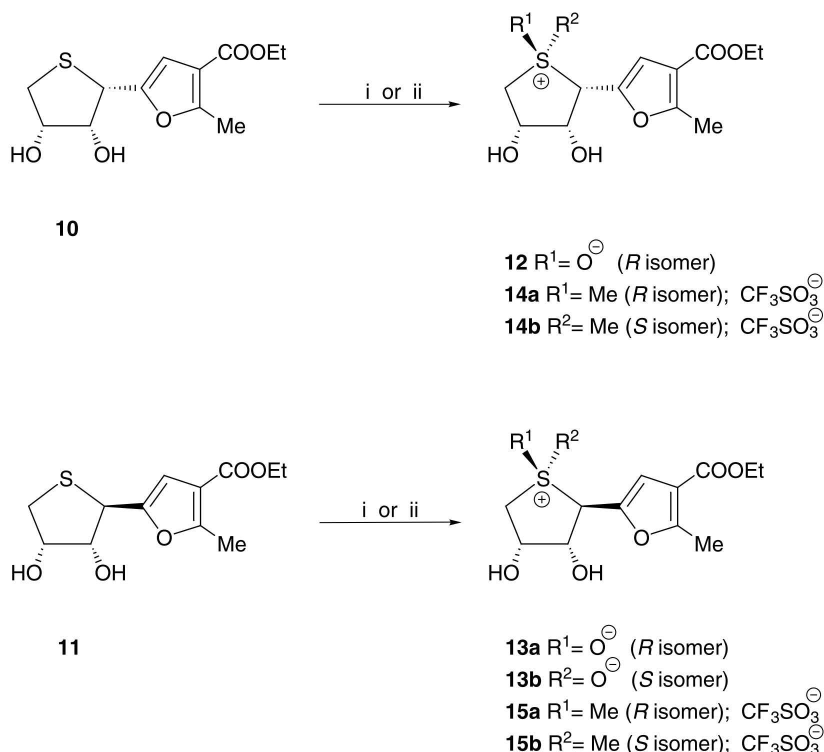


Figure 3.

Table 1. ^{1}H NMR data (δ in ppm, *J* in Hz) for compounds **10**–**15**

Compound	H-1′	H-2′	H-3′	H-4a′	H-4b′	H-4
10[a]	4.54	4.20	4.28	3.02	2.93	6.61
11[a]	4.36	4.23	4.35	3.16	2.81	6.50
12[a]	4.22	4.53	4.91	3.36	3.04	6.81
13a (*R* isomer)[a]	4.44	4.78	4.57	3.75	2.91	6.82
13b (*S* isomer)[a]	4.42	4.30	4.48	3.53	2.89	6.74
14a (*R* isomer)[b]	5.33	4.71	4.92	3.73	3.68	6.96
14b (*S* isomer)[b]	5.40	4.72	4.65	4.04	3.38	7.27
15a (*R* isomer)[b]	5.36	4.97	4.80	3.88	3.68	7.13
15b (*S* isomer)[b]	5.17	4.81	4.77	3.96	3.46	6.95
	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4a'}$	$J_{3',4b'}$	$J_{4a',4b'}$	
10[a]	3.8	3.3	9.8	6.9	9.5	
11[a]	7.2	3.5	4.8	3.8	11.1	
12[a]	3.5	3.0	9.9	7.1	13.7	
13a (*R* isomer)[a]	10.4	3.6	2.1	4.7	14.3	
13b (*S* isomer)[a]	10.0	3.2	4.3	1.9	14.8	
14a (*R* isomer)[b]	3.0	3.0	7.4	9.6	13.2	
14b (*S* isomer)[b]	3.0	3.1	7.3	9.9	12.4	
15a (*R* isomer)[b]	9.8	3.1	2.0	3.8	13.4	
15b (*S* isomer)[b]	9.8	3.0	3.6	1.2	14.4	

[a] CD_3OD, 300 MHz.
[b] D_2O, 500 MHz.



Scheme 2. *Reagents and conditions*: (i) MCPBA (1 equiv.), EtOAc, −78°C, 20 min; (ii) MeOTf, $MeNO_2$, 0°C, 2 h.

chemical shift of H-4′a in **14a** (3.73 ppm) and in **14b** (4.04 ppm). Similarly, H-4′b is shielded in **14b** (3.38 ppm) compared to that proton in **14a** (3.68 ppm). The SMe group also provokes a deshielding effect (ca. 0.3 ppm) on the *cis*-arranged H-3′ as observed by comparison of that proton in **14a** (4.92 ppm) and in **14b** (4.65 ppm). For the sulfonium salts of β configuration, **15a** and **15b**, it was confirmed by NOE experiments that the *cis*-arranged H-4′ to H-3′ is that with the higher $J_{3',4'}$ (3.6 and 3.8 Hz). The shielding effect of the SMe group on the *cis*-arranged H-4′ makes this proton the most shielded proton on C-4′ for **15a** and **15b**.

The SMe group provokes a shielding effect on C-1′ when the methyl group is in a *cis* arrangement to the heterocycle on C-1′ (Table 2). Thus, for the α anomers C-1′ is more shielded (6.3 ppm) for the *S* isomer **14b**, whereas for the β anomers C-1′ is more shielded (5.5 ppm) for the *R* isomer **15a**. It is noteworthy that in sulfoxides **13a** and **13b** the oxygen on the sulfur atom exhibits the same effect as the methyl group, C-1′ being shielded (9.5 ppm) when the oxygen and the heterocycle are *cis*-arranged.

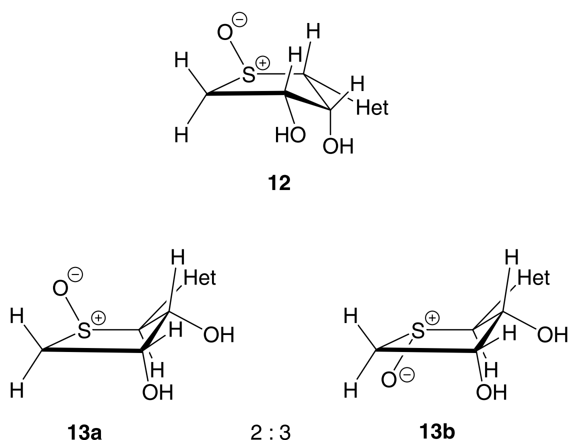


Figure 4. One of the preferred conformations for **12** (E_2) and **13** (^2E).

We have tested compounds **10–15** for their inhibitory activities against several glycosidases and the results are shown in Table 3. The strong inhibitory activity found for the imino-*C*-nucleoside analogue of α configuration **5** against β-galactosidase (*Aspergillus oryzae*, K_i=6.6 μM)[26] contrasts with the absence of activity of the thio analogue **10** and its sulfoxide **12** and sulfonium derivatives **14**. These 4′-thionucleosides in the E_2 conformation have the substituents on the thio sugar ring with an orientation resembling the C-3–C-5 moiety of D-galactose (Fig. 6), as has been suggested for **5**[26] and several polyhydroxylated pyrrolidines.[39] However the lack of inhibitory activity for **10**, **12** and **14** compared to the strong activity of the pyrrolidine isoster **5** may be due to the difference in the geometry of the two rings in terms of puckering and bond lengths,[28a] and the ability of **5** to become protonated at the nitrogen atom in order to mimic oxocarbenium cation **C** and, thus, to interact strongly with a carboxylate group at the enzyme active site.[40,41] In contrast, nitrogen analogues of salacinol **1** have shown less inhibitory activity than the natural sulfonium analogue.[22]

On the other hand, despite the strong activity determined for compound **6** against α-fucosidase (bovine epididymis, K_i=9 μM; human placenta, K_i=15 μM),[27] its counterpart **11** showed only a weak inhibition against α-fucosidase. This activity was found to be improved with positive charge on the sulfur atom, although it was slightly better with the

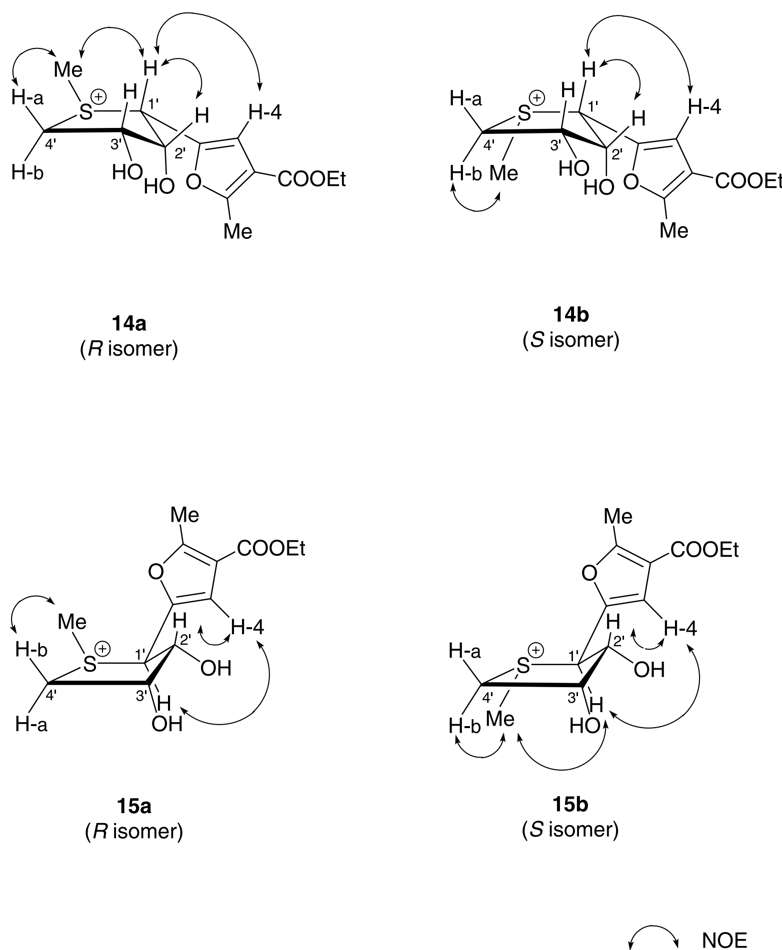


Figure 5. NOE correlations observed for **14** and **15** in one of their preferred conformations.

Table 2. ^{13}C NMR (δ in ppm) for compounds **10–15**

Compound	C-1′	C-2′	C-3′	C-4′	S–Me
10[a]	45.53	75.72	77.68	33.68	–
11[a]	46.18	80.17	75.37	34.22	–
12[a]	72.94	76.50	74.95	55.57	–
13a (*R* isomer)[a]	61.92	76.82	72.80	60.57	–
13b (*S* isomer)[a]	71.44	76.13	74.34	56.61	–
14a (*R* isomer)[b]	61.83	76.15[c]	74.33	43.49	27.20
14b (*S* isomer)[b]	55.52	75.64[c]	74.83	45.49	26.02
15a (*R* isomer)[b]	54.29	76.63	72.73	48.50	23.34
15b (*S* isomer)[b]	59.81	78.29	73.99	45.95	29.89

[a] CD_3OD, 75.5 MHz.
[b] D_2O, 125.7 MHz.
[c] Assignments may have to be reversed.

partial positive charge in sulfoxide **13**, than in sulfonium **15**. These 4′-thionucleosides of β-configuration have the hydroxy groups in the E_3 conformation with the same orientation as those groups on C-3 and C-4 for L-fucose (Fig. 6). The analogues of α configuration were also weak inhibitors, showing that α-fucosidase does not discriminate the anomeric configuration of these nucleoside analogues.

In contrast, the compounds of α configuration **10**, **12**, **14** were not recognised by the α-glucosidase from baker yeast, and an improvement of the inhibitory activity against β-glucosidase from almonds was related to the charge on the sulfur atom, sulfide **10** being non-active, and sulfonium **14** (K_i=250 μM) the most active of them. The compounds of β configuration **11**, **13** and **15** also exhibited a weak

Table 3. Inhibition constants (K_i) in μM

Enzyme	**10**	**11**	**12**	**13**	**14**	**15**
β-Galactosidase (*Aspergillus oryzae*)	NI	NI	NI	NI	NI	NI
α-L-Fucosidase (bovine kidney)	452	853	1220	348	500	663
α-Glucosidase (baker yeast)	NI[a]	1350	NI	496	NI	NI
β-Glucosidase (almonds)	NI	674	5500	771	250	1050

[a] NI: no inhibition.

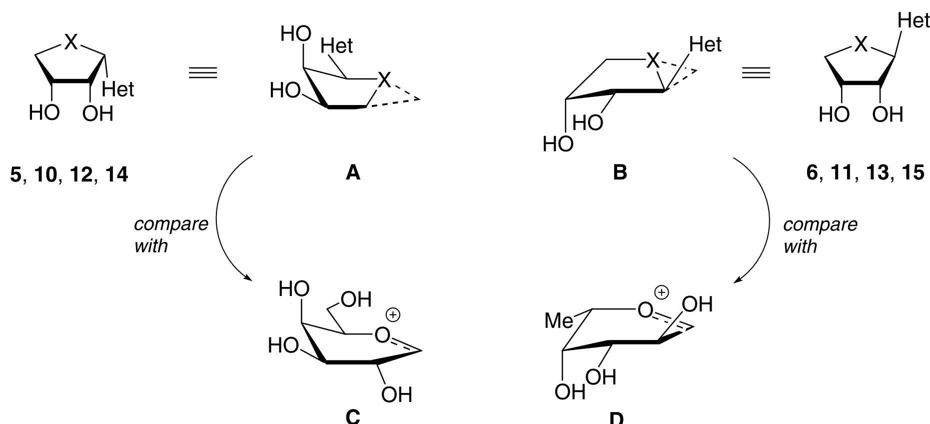


Figure 6. Comparison of the nucleosides analogues **5**, **10**, **12** and **14** in the E_2 conformation, and **6**, **11**, **13** and **15** in the E_3 conformation with the oxocarbenium cations from D-galactose **C** and L-fucose **D**.

activity against the α and β-glucosidases studied, except for the sulfonium **15**, which turned out to be non-active against the α-glucosidase tested.

In conclusion, the results show that 4′-thio-D-erythro-furanosyl-furans of α and β configuration, their sulfoxides and methyl sulfonium salts are weak inhibitors against α-fucosidase from bovine kidney, and not active at all against the β-galactosidase tested. This is in contrast with the activity exhibited by the analogues having a nitrogen in the furanosyl ring that are strong and selective inhibitors of these enzymes. The positive charge on the sulfur atom of these nucleosides analogues does not seem to be relevant for their inhibitory properties.

3. Experimental

3.1. General

Nitromethane was dried over anhydrous $CaCl_2$ and then distilled over 4 Å molecular sieves. Pyridine was refluxed over potassium hydroxide and then distilled. Optical rotations were measured with a Perkin–Elmer 241 polarimeter, and IR spectra (KBr disks) were obtained with an FT-IR Bomem MB-120 spectrophotometer. ^{1}H (300 and 500 MHz) and ^{13}C (75.5 and 125.7 MHz) NMR spectra were recorded on Bruker AMX-300 and AMX-500 spectrometers for solutions in D_2O (internal DOH at 4.75 ppm and internal 1,4-dioxane at 67.4 ppm as reference) and CD_3OD (internal CD_2HOD at 3.30 ppm and internal $^{13}CD_3OD$ at 49.0 ppm as reference). The assignments of ^{1}H and ^{13}C signals were confirmed by homonuclear COSY and heteronuclear 2D correlated spectra, respectively. Mass spectra were recorded on Kratos MS 80 RFA and Micromass AutoSpeQ mass spectrometers. TLC was performed on aluminium pre-coated sheets (E. Merck Silica Gel 60 F_{254}); spots were visualized by UV light, by charring with 10% H_2SO_4 in EtOH and by CEMOL (1% ceric sulfate and 1.5% ammonium molybdate in 10% aqueous H_2SO_4). Column chromatography was performed using E. Merck Silica Gel 60 (40–63 μm).

3.1.1. 3-Ethoxycarbonyl-2-methyl-5-(4′-*O*-*p*-toluenesulfonyl-D-*arabino*-tetritol-1-yl)furan 8. To a solution of 3-ethoxycarbonyl-2-methyl-5-(D-*arabino*-tetritol-1-yl)furan **7**[33] (0.60 g, 2.19 mmol) in pyridine (4 mL) at −15°C was added a solution of *p*-toluenesulfonyl chloride (0.83 g, 4.38 mmol) in dry pyridine (4 mL). After 2 h water was added (three drops) and the solution was concentrated to dryness and co-concentrated several times with toluene/ethanol to afford crude **8**. The residue was purified by column chromatography (30:1 CH_2Cl_2/MeOH) to afford **8** as a syrup (0.72 g, 77%). R_f 0.65 (10:1 EtOAc/Et_2O); $[\alpha]_D^{27}=-3.2°$ (*c* 1.0, $CHCl_3$); IR: ν_{max} 3468, 1704, 1363, 1180, 1093, 822 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ 7.79 (m, 2H, Ar), 7.42 (m, 2H, Ar), 6.54 (s, 1H, H-4), 4.81 (d, 1H, $J_{1',2'}$=2.6 Hz, H-1′), 4.25 (q, 2H, J=7.1 Hz, CH_2CH$_3$), 4.24 (dd, 1H, $J_{3',4a'}$=2.5 Hz, $J_{4a',4b'}$=10.0 Hz, H-4a′), 4.05 (dd, 1H, $J_{3',4b'}$=6.2 Hz, H-4b′), 3.85 (ddd, 1H, $J_{2',3'}$=8.3 Hz, H-3′), 3.68 (dd, 1H, H-2′), 2.51 (s, 3H, CH_3 furan), 2.44 (s, 3H, CH_3 Ar), 1.32 (t, 3H, CH$_2$CH_3); ^{13}C NMR (75.5 MHz, CD_3OD): δ 165.67 (CO), 159.62 (C-2), 155.11 (C-5), 146.41, 134.25, 131.00, 129.10 (Ar), 115.11 (C-3), 108.54 (C-4), 73.64 (C-4′), 73.48 (C-2′), 70.12 (C-3′), 67.44 (C-1′), 61.26 (CH$_2$CH$_3$), 21.56 (CH_3 Ar), 14.64 (CH$_2$CH$_3$), 13.76 (CH_3 furan); FABMS: *m*/*z* 451 [100, $(M+Na)^+$]; HRFABMS calcd for $C_{19}H_{24}NaO_9S$ $(M+Na)^+$ 451.1039, found 451.1029.

3.1.2. 5-(4′-*S*-Acetyl-4′-thio-D-*arabino*-tetritol-1-yl)-3-ethoxycarbonyl-2-methylfuran 9. A solution of **8** (2.34 g, 5.45 mmol) and potassium thioacetate (1.24 g, 10.90 mmol) in *N*,*N*-dimethylformamide (20 mL) was kept at rt for 3 h. Water (80 mL) was added and the compound was extracted with ethyl acetate (4×50 mL). The organic layer was washed with brine (2×50 mL), dried ($MgSO_4$), concentrated and the residue was purified by column chromatography (5:1 Et_2O/hexane) to afford **9** as a syrup (1.38 g, 76%). R_f 0.60 (10:1 EtOAc/Et_2O); $[\alpha]_D^{26}=0.0°$ (*c* 0.8, $CHCl_3$); IR: ν_{max} 3419, 1704, 1424, 1226, 1093, 784 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ 6.57 (s, 1H, H-4), 4.85 (d, 1H, $J_{1',2'}$=2.9 Hz, H-1′), 4.25 (q, 2H, J=7.1 Hz, CH_2CH$_3$), 3.76 (td, 1H, $J_{2',3'}$=7.8 Hz, $J_{3',4a'}$=3.2 Hz, $J_{3',4b'}$=7.8 Hz, H-3′), 3.64 (dd, 1H, H-2′), 3.42 (dd, 1H, $J_{4a',4b'}$=13.8 Hz, H-4a′), 2.97 (dd, 1H, H-4b′), 2.53 (s, 3H, CH_3 furan), 2.32 (s, 3H, SAc), 1.32 (t, 3H, CH$_2$CH_3); ^{13}C NMR (75.5 MHz, CD_3OD): δ 197.67 (S*C*OCH$_3$), 165.60 (CO), 159.61 (C-2), 155.27 (C-5), 115.18 (C-3), 108.55 (C-4), 76.08 (C-2′), 71.21 (C-3′), 67.72 (C-1′), 61.26

(CH_2CH_3), 34.41 (C-4′), 30.45 (SCOCH_3), 14.64 (CH_2CH_3), 13.76 (CH_3 furan); FABMS: *m/z* 355 [100, $(M+Na)^+$]; HRFABMS calcd for $C_{14}H_{20}NaO_7S$ $(M+Na)^+$ 355.0827, found 355.0827.

3.1.3. 3-Ethoxycarbonyl-2-methyl-5-(4′-thio-α-D-erythrofuranosyl)furan 10 and 3-ethoxycarbonyl-2-methyl-5-(4′-thio-β-D-erythrofuranosyl)furan 11. A solution of **9** (0.96 g, 2.88 mmol) in 1:1 ethanol/water (16 mL) containing trifluoroacetic acid (1.6 mL) was boiled under reflux for 1 h. The solution was co-concentrated with ethanol (3×5 mL). The ^{1}H NMR of the residue showed a mixture of the α and β anomers in a 2:3 ratio. Purification by column chromatography (5:1 EtOAc/Et_2O) gave a mixture of **10** and **11** (0.60 g, 76%), which were separated by column chromatography followed by preparative TLC of the non-resolved fractions (40:1 CH_2Cl_2/MeOH). Eluted first was **10** as a white solid (0.23 g, 29%). R_f 0.25 (40:1 CH_2Cl_2/MeOH); $[\alpha]_D^{25}=+32.8°$ (*c* 1.4, $CHCl_3$); IR: ν_{max} 3428, 1712, 1585, 1418, 1236, 1093, 783 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): Table 1 and δ 4.24 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 2.51 (s, 3H, CH_3 furan), 1.32 (t, 3H, CH_2CH_3); ^{13}C NMR (75.5 MHz, CD_3OD): Table 2 and δ 165.67 (CO), 159.85 (C-2), 151.89 (C-5), 115.24 (C-3), 110.60 (C-4), 61.23 (CH_2CH_3), 14.64 (CH_2CH_3), 13.77 (CH_3 furan); CIMS: *m/z* 273 [64, $(M+H)^+$]; HRCIMS calcd for $C_{12}H_{17}O_5S$ $(M+H)^+$ 273.0797, found 273.0784. Eluted second was **11** as a syrup (0.30, 39%). R_f 0.22 (40:1 CH_2Cl_2/MeOH); $[\alpha]_D^{25}=-160.8°$ (*c* 1.0, $CHCl_3$), lit.[32] $[\alpha]_D^{15}=-167°$ (*c* 1.0, $CHCl_3$); IR: ν_{max} 3396, 1720, 1236, 1093, 783 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): Table 1 and δ 4.24 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 2.52 (s, 3H, CH_3 furan), 1.32 (t, 3H, CH_2CH_3); ^{13}C NMR (75.5 MHz, CD_3OD): δ 165.46 (CO), 160.10 (C-2), 153.13 (C-5), 115.23 (C-3), 109.01 (C-4), 61.29 (CH_2CH_3), 14.63 (CH_2CH_3), 13.79 (CH_3 furan); CIMS: *m/z* 273 [100, $(M+H)^+$]; HRCIMS calcd for $C_{12}H_{17}O_5S$ $(M+H)^+$ 273.0797, found 273.0790.

3.1.4. 3-Ethoxycarbonyl-2-methyl-5-(4′-thio-α-D-erythrofuranosyl)furan *S*-oxide 12. To a solution of **10** (30 mg, 0.11 mmol) in EtOAc (2 mL) at −78°C under argon was added a solution of *m*-chloroperbenzoic acid (28 mg, 0.11 mmol) in EtOAc (1 mL). After 20 min the solution was concentrated and the crude product was purified by column chromatography (20:1 CH_2Cl_2/MeOH) to afford **12** (30 mg, 93%). R_f 0.39 (20:1 CH_2Cl_2/MeOH); $[\alpha]_D^{24}=-188.9°$ (*c* 1.0, $CHCl_3$); IR: ν_{max} 3357, 1704, 1521, 1236, 1093, 1013 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): Table 1 and δ 4.27 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 2.56 (s, 3H, CH_3 furan), 1.33 (t, 3H, CH_2CH_3); ^{13}C NMR (75.5 MHz, CD_3OD): Table 2 and δ 165.24 (CO), 160.76 (C-2), 147.11 (C-5), 115.84 (C-3), 111.98 (C-4), 61.42 (CH_2CH_3), 14.62 (CH_2CH_3), 13.74 (CH_3 furan); CIMS: *m/z* 577 [3, (2M+H)], 289 [78, $(M+H)^+$], 271 [100, $(M+H-H_2O)^+$], 253 [87, $(M+H-2H_2O)^+$]; HRCIMS calcd for $C_{12}H_{17}O_6S$ $(M+H)^+$ 289.0746, found 289.0746.

3.1.5. 3-Ethoxycarbonyl-2-methyl-5-(4′-thio-β-D-erythrofuranosyl)furan *S*-oxide 13. To a solution of **11** (24 mg, 0.09 mmol) in EtOAc (2 mL) at −78°C under argon was added a solution of *m*-chloroperbenzoic acid (22 mg, 0.09 mmol) in EtOAc (1 mL). After 20 min the solution was concentrated and the crude product was purified by column chromatography (20:1 CH_2Cl_2/MeOH) to afford **13** (24 mg, 95%). The ^{1}H NMR of **13** showed a mixture of stereoisomers *R* (**13a**) and *S* (**13b**) in a 2:3 ratio. R_f 0.30 (10:1 CH_2Cl_2/MeOH); $[\alpha]_D^{25}=-201.8°$ (*c* 1.1, $CHCl_3$); IR: ν_{max} 3261, 1720, 1418, 1228, 1093, 1013 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) for **13a**: Table 1 and δ 4.27 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 2.57 (s, 3H, CH_3 furan), 1.33 (t, 3H, CH_2CH_3); for **13b**: δ 6.74 (s, 1H, H-4), 4.27 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 2.57 (s, 3H, CH_3 furan), 1.33 (t, 3H, CH_2CH_3); ^{13}C NMR (75.5 MHz, CD_3OD) for **13a**: Table 2 and δ 165.20 (CO), 161.67 (C-2), 145.38 (C-5), 115.91 (C-3), 113.89 (C-4), 61.40 (CH_2CH_3), 14.63 (CH_2CH_3), 13.88 (CH_3 furan); for **13b**: δ 165.08 (CO), 161.35 (C-2), 147.74 (C-5), 115.91 (C-3), 111.56 (C-4), 61.47 (CH_2CH_3), 14.63 (CH_2CH_3), 13.88 (CH_3 furan). CIMS: *m/z* 289 [68, $(M+H)^+$], 253 [100, $(M+H-2H_2O)^+$]; HRCIMS calcd for $C_{12}H_{17}O_6S$ $(M+H)^+$ 289.0746, found 289.0741.

3.1.6. (2*S*,3*R*,4*S*)-2-(3-Ethoxycarbonyl-2-methylfuran-5-yl)-3,4-dihydroxy-1-methylthiolanium triflate 14. To a solution of **10** (42 mg, 0.16 mmol) in dry $MeNO_2$ (1 mL) at 0°C under argon was added methyl triflate (23 μL, 0.21 mmol). After 2 h the solution was concentrated to give pure **14** (66 mg, 98%). The ^{1}H NMR of **14** in D_2O showed a mixture of stereoisomers *R* (**14a**) and *S* (**14b**) in an 1:1 ratio. R_f 0.18 (10:1 CH_2Cl_2/MeOH); $[\alpha]_D^{20}=+67.6°$ (*c* 1.1, H_2O); IR: ν_{max} 3364, 1704, 1434, 1259, 1093, 1037 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) for *R* isomer (**14a**): Table 1 and δ 4.29 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 3.15 (s, 3H, SMe), 2.53 (s, 3H, CH_3 furan), 1.30 (t, 3H, CH_2CH_3); for *S* isomer (**14b**): δ 4.28 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 2.83 (s, 3H, SMe), 2.53 (s, 3H, CH_3 furan), 1.30 (t, 3H, CH_2CH_3); ^{13}C NMR (125.7 MHz, D_2O) for **14a** and **14b**: Table 2 and δ 166.84, 166.77 (CO), 164.58, 163.42 (C-2), 142.42, 140.16 (C-5), 117.72 (C-4 for **14b**), 115.79, 115.59 (C-3), 114.42 (C-4 for **14a**), 62.90 (CH_2CH_3), 14.59, 14.42 (CH_2CH_3, CH_3 furan). FABMS: *m/z* 723 [2, $(2M-TfO)^+$], 459 [3, $(M+Na)^+$], 287 [100, $(M-TfO)^+$]; HRFABMS calcd for $C_{14}H_{19}F_3NaO_8S_2$ $(M+Na)^+$ 459.0371, found 459.0359.

3.1.7. (2*R*,3*R*,4*S*)-2-(3-Ethoxycarbonyl-2-methylfuran-5-yl)-3,4-dihydroxy-1-methylthiolanium triflate 15. To a solution of **11** (36 mg, 0.13 mmol) in dry $MeNO_2$ (1 mL) at 0°C under argon was added methyl triflate (19 μL, 0.17 mmol). After 2 h the solution was concentrated to give pure **15** (57 mg, quantitative). The ^{1}H NMR of **15** in D_2O showed a mixture of stereoisomers *R* (**15a**) and *S* (**15b**) in a 2:5 ratio; after 3 h at rt a 1:1.2 ratio was observed. R_f 0.14 (10:1 CH_2Cl_2/MeOH); $[\alpha]_D^{20}=-79.6°$ (*c* 0.9, H_2O); IR: ν_{max} 3389, 1704, 1434, 1267, 1172, 1093 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) for *R* isomer (**15a**): Table 1 and δ 4.29 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 2.62 (s, 3H, SMe), 2.55 (s, 3H, CH_3 furan), 1.31 (t, 3H, CH_2CH_3); for *S* isomer (**15b**): δ 4.28 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 3.27 (s, 3H, SMe), 2.53 (s, 3H, CH_3 furan), 1.30 (t, 3H, CH_2CH_3); ^{13}C NMR (125.7 MHz, D_2O) for *R* isomer (**15a**): Table 2 and δ 166.57 (CO), 164.63 (C-2), 140.76 (C-5), 117.54 (C-4), 116.05 (C-3), 63.03 (CH_2CH_3), 14.64, 14.49 (CH_2CH_3, CH_3 furan); for *S* isomer (**15b**): δ 166.79 (CO), 163.86 (C-2), 143.07 (C-5), 115.74 (C-3), 114.65 (C-4), 62.96 (CH_2CH_3), 14.64, 14.49 (CH_2CH_3, CH_3 furan). FABMS: *m/z* 723 [2, $(2M-TfO)^+$], 459 [4, $(M+Na)^+$], 287 [100, $(M-TfO)^+$];

HRFABMS calcd for $C_{14}H_{19}F_3NaO_8S_2$ $(M+Na)^+$ 459.0371, found 459.0360.

3.2. Enzyme kinetics

The enzyme assays were carried out as described previously.[42] All assays were performed at pH 6.8 and 32°C. Steady state kinetics was performed and reaction rates were measured after possible slow-onset inhibition was essentially complete. The inhibition constants (K_i) were obtained from the formula $K_i=[I]/(K_{M'}/K_M-1)$, where $K_{M'}$ and K_M are Michaelis–Menten constants with and without inhibitor present (Table 3). $K_{M'}$ and K_M were obtained from a Hanes plot, which was also used to ensure that inhibition was competitive. The following K_M values (without inhibitor) were obtained using 4-nitrophenyl glycosides as substrates, except for β-galactosidase for which 2-nitrophenyl glycoside was used: α-glucosidase (yeast): 0.20 mM, β-glucosidase (almonds): 3.90 mM, α-fucosidase (bovine kidney): 0.34 mM, β-galactosidase (*Aspergillus oryzae*): 1.23 mM. The amount of *p*-nitrophenol was determined colorimetrically at 400 nm and the *o*-nitrophenol at 420 nm.

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