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Synthesis and conformational analysis of 1,2-cis fused bicyclic α-D-galactofuranosyl thiocarbamate from per-*O*-*tert*-butyldimethylsilyl-β-D-galactofuranosyl isothiocyanate

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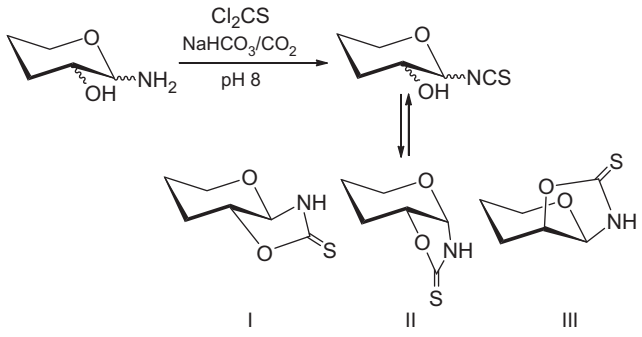
ABSTRACT

Per-*O*-*tert*-butyldimethylsilyl-α,β-D-galactofuranosyl isothiocyanate (**4**) was synthesized by the reaction of per-*O*-TBS-β-D-galactofuranose (**1**) with KSCN, promoted by TMSI. Upon O-desilylation (1,2-dideoxy-α-D-galactofuranoso)[1,2*d*]-1,3-oxazolidine-2-thione (**6**), the cis-fused bicyclic thiocarbamate was obtained as the only product. Conformational analysis, aided by molecular modelling, showed two stable twist forms ($^{3}T_{4}$ and $^{4}T_{O}$) for the five-membered sugar ring in **6**. In aqueous solution, the equilibrium favours the first conformation (3:1 ratio). On the other hand, this ratio decreases for less polar solvents.

1. Introduction

Glycosyl isothiocyanates are useful building blocks in carbohydrate synthesis due to the versatility of the heterocumulene group, whose strong electrophilicity enables them to take part readily in cyclo- and nucleophilic addition reactions.[1,2] They are precursors of thiohydantoins, glycosyl thioureas, heterocyclic derivatives, such as nucleoside analogues, and other N-linked glycoconjugates of biological interest.[3] The versatility of glycosyl isothiocyanates as synthons has prompted the development of various synthetic methods for O-protected glycopyranosyl isothiocyanates, being the most widely used in the treatment of the O-protected glycosyl halide with thiocyanate salts. Depending on the thiocyanate counterion and the solvent, a glycosyl thiocyanate or isothiocyanate is obtained, and conditions for the isomerization of thiocyanate to isothiocyanate have also been described.[1,2] Other methods have been developed, including one involving oxazoline intermediates.[4]

On the other hand, fully unprotected glycopyranosyl isothiocyanates in equilibrium with the 1,2-cyclic thiocarbamates, which are produced as the result of the intramolecular reaction between HO-2 and the isothiocyanate group, have also received attention. The resulting bicyclic structures are important motifs in organic chemistry (Scheme 1).[2,5] The procedure usually employed for the synthesis of free glycopyranosyl isothiocyanates, involves treatment of the glycopyranosylamines with thiophosgene in a buffered medium (Scheme 1).[6] Depending on the relative configurations of C-1 and C-2 (1,2-cis or 1,2-trans), a different equilibrium with the cis- or trans-1,2-cyclic thiocarbamate is observed. Thus, β-D-galacto- and β-D-glucopyranosyl isothiocyanates are in a solvent-dependent equilibrium with the trans-fused bicyclic thiocarbamates (type I, Scheme 1),[7,8] while β-mannosylamine or α-glucosylamine afford the cis-fused thiocarbamate (types II or III, Scheme 1), as the only product.[9] The differential reactivity of these 1,2-bicyclic thiocarbamates towards amines has been



Scheme 1. Synthesis of free glycopyranosyl isothiocyanates and possible 1,2-cyclic thiocarbamates in equilibrium, according to their 1,2-relative configurations.

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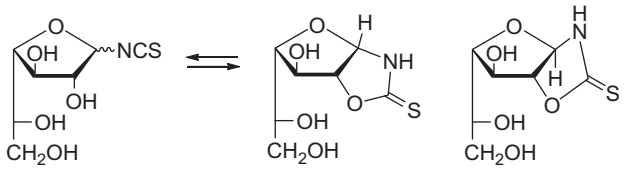


Scheme 2. Free galactofuranosyl isothiocyanate and possible 1,2-cyclic thiocarbamates in equilibrium.

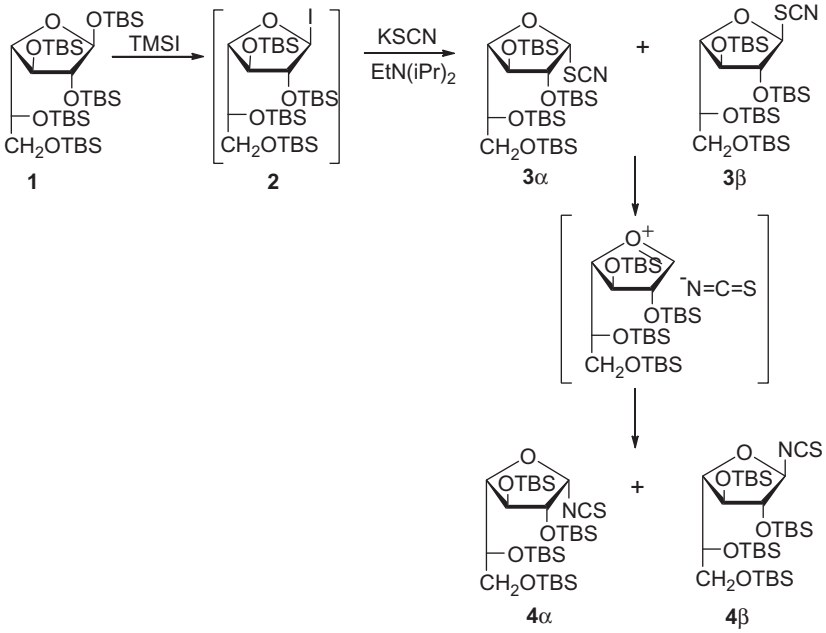
considered as indicative of the relative stability of the cyclic thiocarbamates, which results from the strain in the ring fusion. The trans-fused bicyclic thiocarbamates (type I, Scheme 1) smoothly react with amines leading to the thioureide derivatives and a wide range of glycosyl thioureas have thus been prepared.[8] The cis-bicyclic thiocarbamate derived from mannose, instead, did not react at all with amines as a latent isothiocyanate.[9]

As part of an ongoing project for the synthesis of useful compounds for the characterization of enzymes related to galactofuranose glycobiology and the development of inhibitors of the Gal*f*-processing enzymes,[10] we have focused our attention on the synthesis of free galactofuranosyl isothiocyanate and its possible equilibrium with the cyclic thiocarbamate forms (Scheme 2). We have previously developed the synthesis of per-O-acylated-1,2-*trans*-galactofuranosyl and pentofuranosyl isothiocyanates prepared from per-O-acylated precursors via the glycosyl chlorides or bromides. By nucleophilic addition of alcohols, amines and aminoacids, a variety of *N*-glycosyl derivatives of D-Gal*f* were prepared, and the usefulness of per-*O*-benzoyl-β-D-galactofuranosyl isothiocyanate as a chiral resolving agent of amines and aminoacids was demonstrated.[11,12] Moreover, *N*-β-D-galactofuranosyl-4-imidazoline- and 4-methoxyimidazolidine-2-thione derivatives were obtained from the galactofuranosyl isothiocyanate derivative and evaluated as β-D-galactofuranosidase inhibitors, showing a weak activity.[13]

Despite the simplicity of the procedure previously developed,[11,12] synthesis of unprotected galactofuranosyl isothiocyanate would require other precursors, due to the reactivity of the isothiocyanate group with the nucleophiles required for the de-O-benzoylation. The isothiocyanation of the 1-aminoglycopyranosyl derivative required the use of thiophosgene,[6,9] a toxic and dangerous agent. In addition, the amino derivative of D-Gal*f* is not available. We studied in the present work the isothiocyanation of per-*O*-TBS-β-D-galactofuranose (**1**) and the synthesis of the galactofuranosyl 1,2-cis-fused bicyclic thiocarbamate **6** obtained as the only product upon deprotection. The stability of this compound and its reactivity with amines were evaluated, and its conformational features were determined with the aid of molecular modelling. As the knowledge of the three-dimensional structures of carbohydrates is essential for understanding their biological and physical functions, the conformational features of **6** were determined with the aid of molecular modelling.

2. Results and discussion

We have recently reported the synthesis of per-*O*-TBS-β-D-galactofuranose (**1**), obtained as a crystalline product in just one high yield step from D-galactose.[14] This precursor could be convenient for the synthesis of free galactofuranosyl isothiocyanate, as the O-deprotection could be performed by treatment with non nucleophilic reagents. We have also investigated the glycosylation of this donor by in situ activation as the galactofuranosyl iodide **2**, and we demonstrated the effectiveness of this mild glycosylation method for several D-Gal*f* containing molecules.[14] Following the reported protocol, compound **1** was treated with 1.2 equiv of TMSI in anhydrous CH_2Cl_2 at 0 °C during 30 min, and then $EtN(iPr)_2$ and a solution of KSCN (3.0 equiv) in anhydrous acetonitrile were added. After 2 h, TLC examination showed the formation of a main product of $R_f = 0.57$ (10:1 hexane–EtOAc) and a faster migrating compound ($R_f = 0.80$). Total conversion to the product of $R_f = 0.80$ occurred, either after 48 h under the reaction conditions, or after the work up on storage at −20 °C (Scheme 3). The lower migrating component was identified as thiocyanate **3αβ** on the basis of the δ_{SCN} ^{13}C resonances at 112.8 and 112.6 ppm. For the faster migrating compound the δ_{NCS} 139.0 and 138.0 observed supported the isothiocyanate structure present as an anomeric mixture. The anomeric region of the ^1H NMR spectrum of a 2 h reaction mixture is shown in Figure 1 and the product distribution at different reaction



Scheme 3. Synthesis of 2,3,5,6-tetra-*O*-*tert*-butyldimethylsilyl-α,β-D-galactofuranosyl isothiocyanate (**4αβ**).

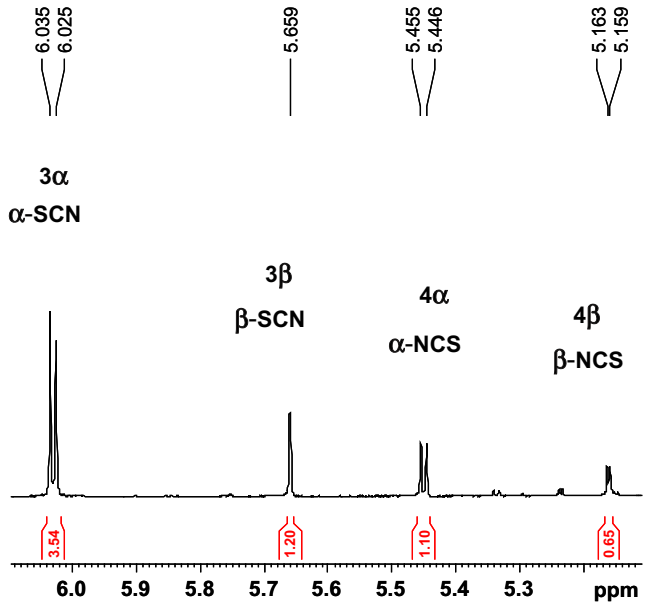


Figure 1. Anomeric region of the ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of crude reaction of **1** with KSCN promoted by TMSI (2 h of reaction).

Table 1
Product distribution for the reaction of per-*O*-TBS-β-D-galactofuranose (**1**) with KSCN, promoted by TMSI

Entry	Time (h)	Product distribution			
		3α	**3β**	**4α**	**4β**
1[a]	2	57	19	15	9
2[a]	24	4	12	34	50
3[a]	48	—	—	38	62
4[b]	48	—	—	36	54
5[a]	96	—	—	25	75

[a] Determined by ^{1}H NMR spectroscopy.
[b] Pure products isolated after column chromatography.

times is shown in Table 1. After 2 h of reaction the main product was thiocyanate **3α** ($J_{1,2}$ = 4.6 Hz). Lesser amounts of **3β** ($J_{1,2}$ <0.5 Hz) and **4α** $J_{1,2}$ = 4.5 Hz) were present, and **4β** ($J_{1,2}$ = 2.0 Hz) was the minor component (Table 1, entry 1). After 48 h of reaction the reaction mixture was composed of isothiocyanates **4β** and **4α** in a 3:2 ratio (Table 1, entries 3 and 4), and under longer times the mixture was even further enriched in **4β** (Table 1, entry 5).

The final composition indicates that the thermal isomerization of thiocyanate (kinetic product) to isothiocyanate[1,2] is accompanied by anomerization. Initially, formation of α-thiocyanate **3α** is favored, as a result of a S_N2 like attack of thiocyanate ion on β-iodide.[14] During the rearrangement to the thermodynamically more stable isothiocyanate (via the oxonium ion), the S_N1 attack of isothiocyanate occurs mainly from the β-face as a result of the steric effect of the bulky substituent at C-2. This moderate diastereoselectivity is in agreement with that previously observed for the glycosidation of **1** with less sterically demanding nucleophiles.[14] In the case of per-O-benzoylated furanosyl isothiocyanates the rearrangement of the thiocyanate derivatives to the corresponding isothiocyanates was faster, and no intermediate product was detected.[11,12] The stabilization of the oxacarbenium intermediate due to the anchimeric participation of the benzoyloxy groups, which increases the rate of this reaction, should be responsible for this effect.

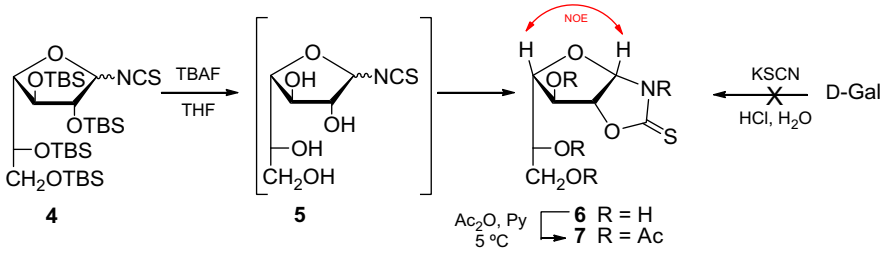
The isothiocyanates **4β** and **4α** were separated by column chromatography. The ^{1}H NMR spectrum of **4β** showed the H-1signal as a doublet at δ 5.16 with a small $J_{1,2}$ (2.0 Hz), indicating a trans relationship for H-1 and H-2 and hence a β configuration.[15] The presence of the NCS group was confirmed by the IR absorption band at 2025 cm^{-1} and ^{13}C NMR resonances at δ 139.0 (NCS) and 91.3 (C-1), similar to those observed for per-O-benzoylated galactofuranosyl isothiocyanate.[11,12] Signals at 88.2 and 85.1 ppm for C-4 and C-2, respectively, were also characteristic of β-anomers of galactofuranosyl derivatives.[16] For **4α** the resonance of H-1 was observed at 5.45 ppm ($J_{1,2}$ = 4.5 Hz), and signals corresponding to C-1 and NCS function were observed at 87.2 and 138.0 ppm, respectively. The other signals were in complete agreement with the α-D-galactofuranosyl pattern.[17]

Deprotection of **4** was accomplished with *n*-Bu_4NF (TBAF) in THF.[18] Several procedures for removing the excess of the desilylating agent and tetrabutylammonium salts involving ionic resins were described,[19,20] but in our hands, the best results were obtained by column chromatography purification of the crude desilylated product using EtOAc–MeOH with Et_3N as solvent.

Either starting from the anomeric mixture **4α,β** or from pure **4α**, the only product isolated was compound **6** (20% and 76%, respectively), which was stable under storage at −20 °C. The formation of this compound must take place through the free isothiocyanate **5** (not detected), followed by nucleophilic attack of the HO-2 on the isothiocyanate group of the α-anomer. The cis-fused bicyclic structure of **6** was clearly supported by the NMR spectra, which showed a signal at 190.8 ppm, corresponding to the thiocarbonyl group (C=S) of five-membered cyclic thiocarbamates,[21] and a significant deshielding of the C-2 signal (93.5 ppm) with respect to C-2 signal in **4β** and **4α** (δ 85.4 and 79.0). The α-anomeric configuration was confirmed by the large $J_{1,2}$ value (5.9 Hz) and the NOE effect observed between H-1 and H-4 (Scheme 4). Internal $^3J_{H,H}$ of **6** were consistent with those observed for glycofurano[2,1-*d*]imidazolidin-2-ones, also having the cis-fused difuranosic system.[22] In the IR spectrum the absorption band at 1512 cm^{-1} indicated the NHC=S group. No equilibrium between **6** and free isothiocyanate **5** in D_2O or acetone-d_6 was detected. Thus, the only product formed was the cis-fused bicyclic compound **6**. As expected, considering the enormous strain of a bicyclic trans-fused system with two five-membered rings, the *trans*-thiocarbamate was not formed, contrasting with the behaviour of the free β-D-*gluco*- and -galactopyranosyl isothiocyanates which can afford both trans- and cis-fused bicyclic thiocarbamates.[7,8]

A procedure for the preparation of carbohydrate-derived oxazolidine-2-ones and 2-thiones consists of treatment of O-unprotected reducing sugar with KNCO[23] or KNCS,[24] respectively, in acidic medium. This method is extremely time-consuming, even though it has been used for a long time. Pentofuranosyl oxazolidin-2-ones thus obtained were efficiently used to prepare quinazolinedione nucleosides.[25] The procedure was applied for D-galactose but a type I or type II structure (Scheme 1) was assigned to the product.[26] Later, Jochims et al. assigned the cis-fused bicyclic structure of compound **6,** obtained after several weeks of reaction, on the basis of the ^{1}H NMR spectrum (Table 2).[27] In our hands that reaction was extremely slow, and after 48 h, although the furanosic carbamate **6** was the only product, the yield was extremely low (ca. 7%). During the work-up reversion of the reaction was observed.

In order to further confirm the structure of compound **6**, the *N*-acetyl-tri-O-acetylated derivative **7** was obtained by treatment of **6** with acetic anhydride in pyridine at 5 °C during 24 h. Under this condition, only O-acylation was observed for analogue glycofurano[2,1-*d*]imidazolin-2-ones, as acylation of the ring nitrogen required the presence of zinc chloride.[22] ^{1}H NMR spectrum showed the signal for the NAc group at 2.83 ppm and three singlets between 2.23 and 2.04 ppm, corresponding to the OAc groups. These substituents were also evidenced in the ^{13}C NMR spectrum, which



Scheme 4. Synthesis of 1,2-**cis** fused bicyclic α-D-galactofuranosyl thiocarbamate and NOESY correlation to confirm the relative configurations of C-1 and C-4.

Table 2
Comparison of three-bond coupling constants (Hz) calculated for **6a**, and experimental for **6**, with the expected Boltzmann-averaged ratios

Method	$J_{H1,H2}$	$J_{H2,H3}$	$J_{H3,H4}$	Ratio $^3T_4/^4T_O$
B3LYP 6-311+G**, gas phase	5.94	1.50	3.96	58:42
B3LYP 6-311+G**+ PCM[a]	5.93	1.22	3.23	69:31
Experimental in water (this work)	5.9	1.1	2.8	75:25[b]
Experimental in DMSO (from[27])	5.9	1.6	4.4	50:50[b]

[a] Using the polarizable continuum method in water.
[b] Approximate experimental ratios, calculated from the coupling constants.

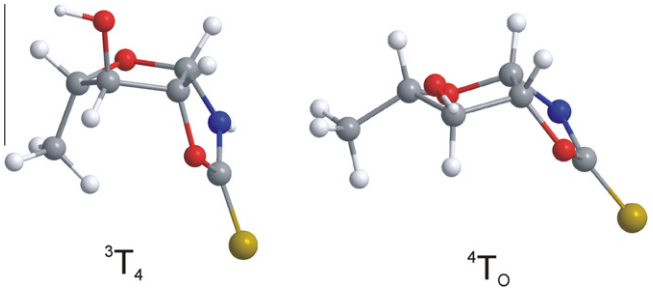


Figure 2. Depiction of the most stable conformers of **6a** in each twist conformation, as calculated by B3LYP/6-311+G(d,p).

showed a signal at 26.0 ppm for the NAc group, and three signals at 20.8–20.5 ppm, whereas the remaining displacements agreed with the acetylation effects. The presence of 3-*O*-acetyl group induces a downfield shift (0.7 ppm) in the H-3 signal and an upfield shift (0.3 ppm) for H-2.

The behaviour of bicyclic sugar 1,2-thiocarbamates towards amines is considered an indication of the stability.[7,9] Therefore, we treated **6** with benzylamine in THF at room temperature. Under this condition, persilylated isothiocyanate **4** led to the corresponding glycosylthioureide after 2 h, whereas compound **6** was stable (Scheme 4). This result showed that cis-fused thiocarbamate 6 is stable towards nucleophiles, as it does not act as a latent isothiocyanate in reactions with amines. This result is in agreement with those of other cis-fused thiocarbamates, given their low ring strain.[9]

The conformational features of the five-membered rings in **6** were investigated, given their conformational flexibility either as free entities or as constituents of more complex biomolecules such as DNA or RNA.[28] The conformational features of five-membered rings are not as simple as those of their six-membered ring counterparts: cyclopentane is not flat, but puckered, and it undergoes a rapid interconversion of conformers with similar energies (pseudorotation). When five-membered rings are substituted, different degrees of puckering and altered rotational barriers occur, and thus some puckered conformations become more stable than others.[29] Usually computer-aided calculations (molecular modelling) show the presence of a few conformational minima in those substituted rings but with a low-energy barrier between them. The combination of accurate molecular modelling with experimental determinations, mainly three-bond NMR coupling constants, has shown great success not only to assess conformational features, but also to determine configurations.[30,31] It has to be pointed out that sometimes many combinations of Boltzmann-averaged rapidly interconverting conformers or a single one can match experimental coupling constants.[31,32] To model the conformational behaviour of **6** in aqueous solution, an analogue where the dihydroxyethyl side chain in C-4 was replaced by a methyl group (**6a**, 1,2,5-trideoxy-β-l-arabinoso)[1,2*d*]-1,3-oxazolidine-2-thione) was constructed. This analogue, besides reducing the system complexity, avoids modelling the intramolecular hydrogen bonds for the side chain of **6**, which are certainly not important in aqueous solution. Compound **6a** was analysed by DFT calculations at the B3LYP/6-311+G(d,p) level. Only two twist conformers (^4T_O and $^3T_4$) of the sugar ring were found to be stable, both leaving the flattest part of the ring closer to the ring-fusion bond. However, each of the twist conformers finds three stable conformers according to the arrangement of the HO-3 group. The geometries of each twist conformation are shown in Figure 2, whereas the calculated Boltzmann-averaged vicinal coupling constants (using the Karplus equation as parameterized by Haasnoot et al.[33]) are shown in Table 2.

The DFT calculations show similar energies for both twist conformations. The Boltzmann-averaged populations indicated a 58:42 ratio of $^3T_4$ to ^4T_O conformers (Table 2). When a solvent model is added (PCM[34] in water), the relative stability of the $^3T_4$ conformers increases, to indicate a 69:31 ratio. The experimental coupling constants observed in water agree quite well with those calculated with the solvent model (Table 2), suggesting a 3:1 ratio for the twist conformers in aqueous solution. On the other hand, the experimental coupling constants obtained in DMSO[27] are compatible with those calculated in gas phase (Table 2), suggesting a 1:1 ratio of both twist conformations. Thus, the experimental data can only be explained in terms of a 'virtual' conformation, in which two conformations are populated enough so as to give a set of coupling constants with no meaning for a single conformation, but only explainable in terms of an average of two ring conformations. In all the calculations the thiocarbamate ring showed an almost planar shape, slightly puckered towards $^NT_1$. The experimental coupling constants obtained previously for the 2-methylthio-(D-galacto)oxazoline,[35] structurally related with **6a**, are also compatible with mixtures of both ring conformations, although higher proportions of ^4T_O conformations appear: in water the ratio is about 1:1, whereas in DMSO the $^4T_O/^3T_4$ ratio is about 3:1. The predominance of the $^3T_4$ conformation for **6**/**6a** can be explained in terms of the preference of hydroxyl groups for a quasi-axial arrangement already observed for five-membered rings[30,32,36–38] in spite of the quasi-axial arrangement, which this conformation poses to the bulkier substituent. It should be noted that we have also carried out the same calculations for the C-4 epimer of 6a, (1,2-dideoxy-α-D-xylofuranoso)[1,2*d*]-1,3-oxazolidine-2-thione, the analogue of the glucofuranose derivative). These calculations indicate that the stability of the $E_4/^3T_4$ conformers is quite larger

than that of the $^4E/^4T_O$ conformers, suggesting that the coupling constants can be explained just in terms of the former conformers. The experimental data obtained for the glucofuranose derivatives of thiocarbamates and methylthiooxazolines agree with these calculations.[35] This data can also be explained in terms of a quasi-axial O-3, present in the major conformation. However, in this case, the weight of this conformer is larger because this conformer has also a quasi-equatorial bulky substituent.

In the framework of our project in galactofuranose glycobiology,[10] we tested compound **6** against the *exo* β-D-galactofuranosidase from *Penicillium fellutanum*. As expected on the basis of the α-configuration, it did not show inhibitory activity.

3. Conclusion

Per-*O*-*tert*-butyldimethylsilyl-α,β-D-galactofuranosyl isothiocyanate (**4**) was synthesized by reaction of per-O-silylated derivative **1** with KSCN, promoted by TMSI. The method previously developed for glycofuranosyl isothiocyanates, involved the per-O-acylated precursors and the glycosyl bromides or chlorides as intermediates.[11,12] In comparison, the method now studied is globally faster because the glycosyl iodide is readily formed in 0.5 h, instead of 24 h, but less diastereoselective, affording α/β mixtures. Nevertheless, it is slower in the thiocyanate nucleophilic attack step, allowing detecting the isomerization to the isothiocyanate and anomerization. On the other hand, compound **4** could be O-deprotected by treatment with non nucleophilic reagents, without affecting the isothiocyanate function. The only product thus obtained was the cis-fused thiocarbamate **6**. The coupling constant data, integrated with the aid of molecular modelling allowed us to determine that the sugar five-membered ring shape in **6** can be represented as a fast equilibrium between a $^3T_4$ and a ^4T_O conformation in a 3:1 ratio in aqueous solution.

4. Experimental

4.1. General synthetic methods

Analytical thin layer chromatography (TLC) was performed on Silica Gel 60 F254 (Merck) aluminum supported plates (layer thickness 0.2 mm) with solvent systems given in the text. Visualization of the spots was effected by exposure to UV light and charring with a solution of 10% (v/v) sulfuric acid in EtOH, containing 0.5% *p*-anisaldehyde. Column chromatography was carried out with Silica Gel 60 (230–400 mesh, Merck). Optical rotations were measured with a Perkin–Elmer 343 digital polarimeter. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AMX 500 spectrometer. Assignments of ^1H and ^{13}C were assisted by 2D ^1H-COSY and HSQC experiments. High resolution mass spectra (HRMS ESI$^+$) were recorded in a Bruker micrOTOF-Q II spectrometer.

4.2. 2,3,5,6-Tetra-*O*-*tert*-butyldimethylsilyl-α,β-D-galactofuranosyl isothiocyanate (4αβ)

A solution of **1** (0.140 g, 0.19 mmol) in anhydrous CH_2Cl_2 (10 mL) containing dry 4 Å powdered molecular sieves was cooled to 0 °C and stirred during 10 min under Ar. Then, iodotrimethylsilane (1.2 equiv, 0.030 mL, 0.23 mmol) was added and the solution was stirred at 0 °C during 0.5 h. TLC monitoring then showed complete transformation of **1** in two products (R_f = 0.70 and 0.54, 10:1 hexane–EtOAc), previously attributed to the iodide intermediate **2** and its product of hydrolysis 2,3,4,5-tetra-*O*-TBS-β-D-galactofuranose, formed on the silica gel plate. Then, $EtN(iPr)_2$ (0.037 mL, 0.23 mmol) and a solution of KSCN (0.054 g, 0.57 mmol) in acetonitrile (10 mL) were added by syringe and the stirring was continued until consumption of the components of R_f = 0.70 and 0.54. The reaction was allowed to reach room temperature and the stirring was continued for 48 h. The solution was diluted with CH_2Cl_2, washed with $NaHCO_3$ (ss) and water, dried (Na_2SO_4) and concentrated. The crude mixture (β/α 3:2 ratio) was purified by column chromatography (99:1 hexane–EtOAc) affording syrupy compound **4** (76 mg, 90%) as a partially resolved anomeric mixture. For the **4β**: R_f = 0.60 (20:1 hexane–EtOAc), $[\alpha]_D$ −51 (*c* 0.9, $CHCl_3$), IR (KCl): 2025 cm^{-1} (NCS); ^1H NMR (500 MHz, $CDCl_3$): δ 5.16 (d, *J* = 2.0 Hz, 1H, H-1), 4.18 (dd, *J* = 2.0, 4.0 Hz, 1H, H-3), 4.13–4.11 (m, 2H, H-2,4), 3.76 (m, 1H, H-5), 3.66 (dd, *J* = 6.5, 10.5 Hz, 1H, H-6), 3.58 (dd, *J* = 5.2, 10.5 Hz, 1H, H-6′), 0.90–0.89 (SiC(CH_3)$_3$), 0.11–0.06 (Si(CH_3)$_2$). ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 139.0 (NCS), 91.3 (C-1), 88.4 (C-4), 85.4 (C-2), 78.9 (C-3), 73.5 (C-5), 65.0 (C-6), 26.0–25.7 (SiC(CH_3)$_3$), 18.4–17.8 (SiC(CH_3)$_3$), −4.0 to (−4.5) (Si(CH_3)$_2$).

For **4α**: R_f = 0.47 (20:1 hexane–EtOAc), $[\alpha]_D$ +32 (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 5.45 (d, *J* = 4.5 Hz, 1H, H-1), 4.26 (apparent t, *J* = 5.2 Hz, 1H, H-3), 4.07 (dd, *J* = 4.5, 5.0 Hz, 1H, H-2), 3.89 (dd, *J* = 4.2, 5.2 Hz, 1H, H-4), 3.78 (ddd, *J* = 4.2, 5.5, 7.0 Hz, 1H, H-5), 3.63 (dd, *J* = 10.0, 7.0 Hz, 1H, H-6), 3.59 (dd, *J* = 5.5, 10.0 Hz, 1H, H-6′), 0.95–0.87 (SiC(CH_3)$_3$), 0.16–0.04 (Si(CH_3)$_2$). ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 138.0 (NCS), 87.2 (C-1), 85.2 (C-4), 79.0 (C-2), 75.6 (C-3), 72.2 (C-5), 64.5 (C-6), 25.9–25.7 (SiC(CH_3)$_3$), 18.4–17.8 (SiC(CH_3)$_3$), −4.0 to (−4.2) (Si(CH_3)$_2$). HRMS (ESI): calcd for $C_{31}H_{71}N_2O_5SSi_4$ $[M+NH_4]^+$: 695.4155, found 695.4162.

4.3. (1,2-Dideoxy-α-D-galactofuranoso)[1,2*d*]-1,3-oxazolidine-2-thione (6)

To a solution of **4α** (68 mg, 0.1 mmol) in freshly distilled THF (5 mL), cooled at 0 °C, (nBu)$_4$NF (209 mg, 0.8 mmol) was added.[18] The solution was stirred for 30 min until TLC monitoring showed consumption of the starting material. The solvent was evaporated and the syrup was purified by two consecutive column chromatographies (90:10:0.1 and 99:1:0.5→90:10:0.5 EtOAc–MeOH–Et_3N) to afford 16.8 mg (76%) of compound **6**, R_f = 0.55 (7:1:2 nPrOH–NH_3–H_2O); $[\alpha]_D$ +25 (*c* 1.4, acetone); ^1H NMR (500 MHz, D_2O): δ 6.01 (d, *J* = 5.9 Hz, 1H, H-1), 5.30 (dd, *J* = 5.9, 1.1 Hz, 1H, H-2), 4.57 (d, *J* = 2.8 Hz, 1H, H-3), 4.09 (dd, *J* = 2.8, 5.6 Hz, 1H, H-4), 3.69 (dd, *J* = 6.9, 14.2 Hz, 1H, H-6), 3.62 (m, 2H, H-5,6′). ^{13}C NMR (125.8 MHz, D_2O): δ 190.8 (C=S), 93.6 (C-2), 90.9 (C-1), 87.9 (C-4), 76.6 (C-3), 71.8 (C-5), 64.0 (C-6). IR (KCl): 1512 cm^{-1} (C=S). HRMS (ESI) calcd for $C_7H_{12}NO_5S$ $[M+H]^+$: 222.0431, found 222.0454.

4.4. *N*-Acetyl-(3,5,6-tri-*O*-acetyl-1,2-dideoxy-α-D-galactofuranoso)[1,2*d*]-1,3-oxazolidine-2-thione (7)

To a stirred solution of **6** (7.5 mg, 0.039 mmol) in dry pyridine (1 mL) at 0 °C, acetic anhydride (0.050 mL) was added. After 24 h at 5 °C the reaction mixture was coevaporated under vacuum with methanol and toluene, affording syrupy compound **7** (11.1 mg, 84%), R_f = 0.37 (1:1 hexane–EtOAc); $[\alpha]_D$ +36 (*c* 0.9, $CHCl_3$); ^1H NMR (500 MHz; $CDCl_3$): δ 6.51 (d, *J* = 5.7 Hz, 1H, H-1), 5.28–5.24 (m, 2H, H-3,5), 5.07 (d, *J* = 5.7 Hz, 1H, H-2), 4.34 (dd, *J* = 2.0, 5.0 Hz, 1H, H-4), 4.25 (dd, *J* = 2.0, 5.0 Hz, 1H, H-6), 4.17 (dd, *J* = 12.0, 6.4 Hz, 1H, H-6′), 2.83 (s, 3H, NCOCH_3), 2.13, 2.12, 2.04 (3 s, 9H, OCOCH_3). ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 183.5 (C=S), 170.6, 170.4, 170.2, 169.4 (C=O), 90.9 (C-1), 85.2 (C-2), 84.9 (C-4), 77.0 (C-3), 69.5 (C-5), 62.5 (C-6), 26.0 (NCOCH_3), 20.8, 20.6, 20.5 (OCOCH_3). HRMS (ESI) calcd for $C_{15}H_{20}NO_9S$ $[M+H]^+$: 390.0853, found 390.0851.

4.5. Computational methods

Quantum mechanical calculations were performed using GAUSSIAN 03W with standard basis sets (6-311+G(d,p)) and default

minimization methods and termination conditions.[39] Different starting envelopes and twists, stable for similar compounds were used for the **6a** structure. However, all of them converged to either the $^{4}T_{O}$ or the $^{3}T_{4}$ twist forms. By rotation of the C-3–O-3 bond, three different conformers of each twist shape were found. The six conformers were characterized as minima by showing no imaginary frequency. A similar study was made with the C-4 epimer (glucofuranose analogue) although in this case, one of the three $^{4}T_{O}$ rotamers converges to other geometry by rotating its HO-3. Thus, only 5 conformers are present for this compound. All of the five-membered ring shapes were characterized according to their Cremer-Pople puckering parameters,[40] and named as depicted elsewhere.[41] The free energy of solvation was estimated by the polarizable continuum method (PCM, with water as solvent) of Barone and co-workers,[34] on the gas-phase geometries obtained by the DFT procedure, that is, with no further optimization.

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Supplementary data

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