

Synthesis of galactofuranose-containing disaccharides using thioimidoyl-type donors

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Abstract—Four galactofuranose-containing disaccharides have been prepared utilising various thioimidates [Gal_f-SC(=NR)XR'] and suitably protected acceptors as key precursors. We observed that the efficiency of the coupling reactions was particularly dependent on the aglycon present on the furanosyl donor when copper(II) ions were used as the promoter, and that activation could be correlated with the nature of the third heteroatom, X.

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1. Introduction

In mammalian biomolecules, hexoses are exclusively found in the pyranose form. Nevertheless, numerous efforts in the structural elucidation of compounds isolated from various microorganisms have clearly established the presence of hexofuranosides in some oligo- and polysaccharides.^{1–4} Consequently, it is legitimate to wonder about the gain for such organisms to biosynthesize hexofuranose-containing glycoconjugates considering that furanosides are generally less stable than their pyranosidic counterparts.⁵ Moreover, many of these microorganisms are pathogenic; however, the role of hexofuranosides in the development of diseases and/or the way in which they interact with receptors are not yet clearly elucidated.⁶ Because mammalian cells are devoid of the ability to biosynthesize and/or to metabolize glycofuranosides, such molecules are of great interest to increase knowledge relating to their biological and physicochemical roles, and the biological mechanisms connected with them. This observation favourably argues

for the increasing attention that has been paid to new chemical syntheses of oligofuranosides of varying complexities.^{6–15}

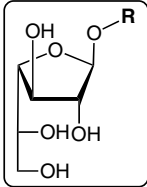
Among hexoses present in a furanose form, three natural conjugates have been found containing D-glucose (D-Glc)^{16–18} and one with D-mannose (D-Man).¹⁹ However, D-fucose (D-Fuc),^{20,21} (not L as in mammalian cells) and D-galactose (D-Gal) are the most widely distributed. The D-Gal_f residue (Table 1) can be connected either to pyranosyl units, that is, D-Man_p,^{22,23} D-Glc_p,^{24,25} D-Gal_p,²¹ N-acetyl-D-galactosamine (D-Gal_p-NAc)^{26–28} or glucosamine (D-Glc_p-NAc),²⁹ L-rhamnose (L-Rhap),^{30–32} D-fructose (D-Frup)³³ or to galactofuranosyl entities.^{29,34,35,23} Among these natural products, most of the Gal_f units are connected with their aglycon through β-D-(1→6) or β-D-(1→5) linkages (Table 1).

In this context, and considering our interest in the preparation of hexofuranose-containing derivatives,^{9–11} disaccharides **1–4** were selected as targets of biological interest (Fig. 1). The sequence β-D-Gal_f-(1→6)-α-D-Glc_p as in **1** is a subunit of the O-antigenic glycoside produced by the K-12 strain of *Escherichia coli*,²⁴ a bacteria that has been commonly used for genetic studies. The second saccharidic structure β-D-Gal_f-(1→6)-α-D-Man_p, which alternatively presents furanosyl and pyranosyl entities (**2**) was designed as a building block for further

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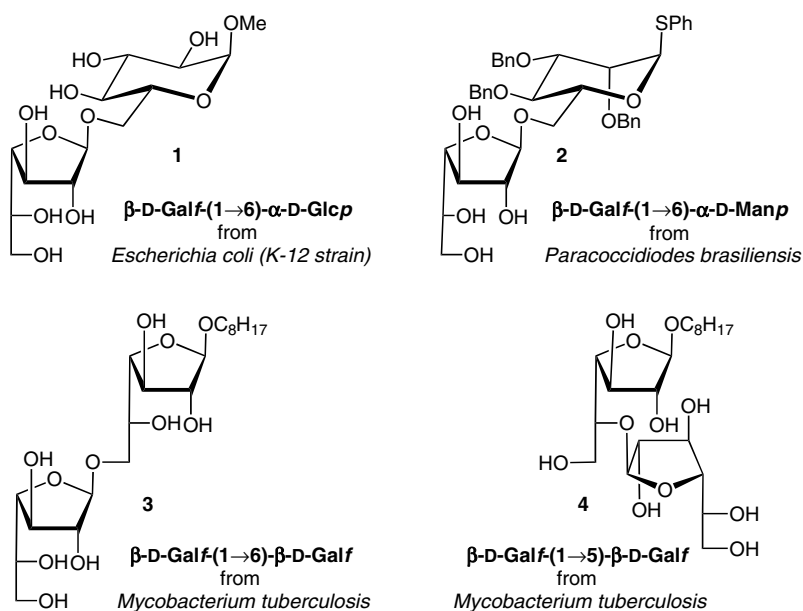
Table 1. Selection of microorganisms biosynthesizing oligofuranosides



β -D-Galf(1→R)

6)-D-Galf		5)-D-Galf	
<i>Arachniotus</i>	<i>Aspergillus</i> ^a	<i>Actinobacillus</i> ^a	<i>Arachniotus</i>
<i>Bionectria</i>	<i>Cryphonectria</i> ^a	<i>Aspergillus</i> ^a	<i>Bionectria</i>
<i>Hypocrea</i>	<i>Calonectria</i>	<i>Byssochlamys</i>	<i>Chaetosartorya</i>
<i>Discula</i>	<i>Fusarium</i>	<i>Cryphonectria</i> ^a	<i>Discula</i>
<i>Gibberella</i>	<i>Mycobacterium</i> ^a	<i>Eupenicillium</i>	<i>Gymanascella</i>
<i>Myrothecium</i>	<i>Renibacterium</i> ^a	<i>Hypocrea</i>	<i>Mycobacterium</i> ^a
<i>Penicillium</i>	<i>Renibacterium</i> ^a	<i>Nectria</i>	<i>Penicillium</i>
<i>Talaromyces</i>	<i>Trichoderma</i>	<i>Talaromyces</i>	<i>Trichoderma</i>
6)-D-Glcp		6)-D-Manp	
<i>Escherichia coli K12</i>		<i>Aspergillus</i> ^a	<i>Epidermophyton</i>
		<i>Microsporum</i>	<i>Paraccidioides</i> ^a
		<i>Rocella decipens</i>	<i>Trichophyton</i>

^a These microorganisms are potentially pathogenic.

**Figure 1.** Structure of targeted galactofuranose-containing disaccharides 1–4.

elongation to yield oligosaccharides found in *Paracoccidioides brasiliensis*.³⁶ This pathogenic fungus is known to affect the lungs, lymphoid and mucocutaneous tissues of rural farm workers in South and Central America. The glycosidic parts of digalactofuranosides **3** (β -D-Galf(1→6)- α -D-Galf) and **4** (β -D-Galf(1→5)- α -D-Galf) are found in the cell wall that ensures the membrane stability of *Mycobacterium tuberculosis*,³⁰ the lethal bacteria responsible for the death of more than 2 million people each year.

These four disaccharides were also chosen as model compounds to probe the structural effects in glycosyl-

ation reactions based on the use of galactofuranosyl thioimidates as furanosyl donors. This family of donors belongs to the thioglycoside-type donors but they potentially differ from more standard thioglycosides because they can be activated by ‘remote activation’ (Fig. 2).^{37,38}

The first outcome of this mechanistic feature is that some differences in the reactivity of the donors can be connected with the structure of the aglycon and/or the nature of the promoter. This is the reason why we have selected thioimidoyl furanosides **5–8** as donors (Fig. 3). These compounds differ from each other in (i) the nature of the X group (X = NH or S, the latter having ‘softer’

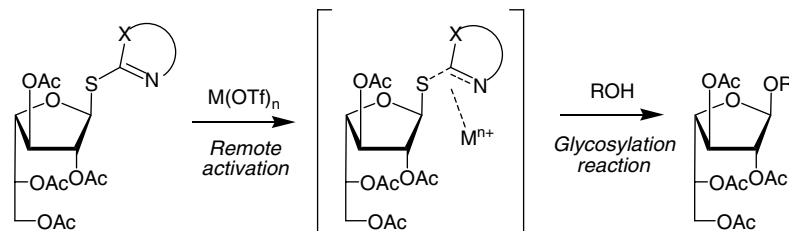


Figure 2. Expected activation of thioimidates.

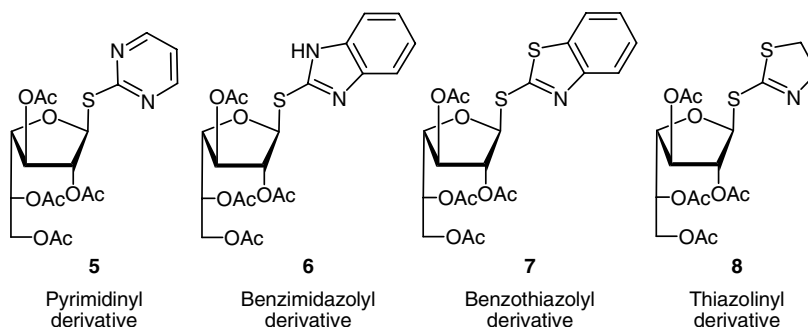


Figure 3. Structure of donors 5–8.

basic properties compared to the amino group, as based upon the HSAB principle), (ii) the basicity of the heterocycle and (iii) the size of the aglycon.

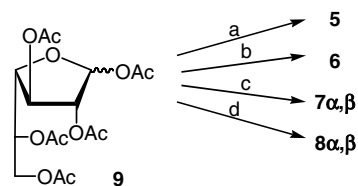
Moreover, because thioimidates are likely to be activated not directly at the anomeric sulfur atom, orthogonality with thioethyl or thiophenyl glycosides can be expected.^{39,40} We describe herein the synthesis of naturally occurring galactofuranose-containing disaccharides and our evaluation of four galactofuranosyl thioimidates in reactions with acceptors of different steric accessibilities, for example, primary versus secondary hydroxyl groups or furanosyl versus pyranosyl entities.

2. Results and discussion

To prepare 1,2-*trans* furanosides 1–4, the β -directing effect was achieved through the assistance of a 2-*O*-acetyl protecting group on the galactosyl donor. Consequently, the required galactofuranosides were obtained from peracetylated galactofuranose (9)⁴¹ according to a Ferrier reaction⁴² catalyzed by the boron trifluoride-etherate complex. Excess catalyst was used to shift the thione/thiol equilibrium towards the thiol form and to limit the formation of the N-glycoside as a by-product.⁴³ Donors 5–7⁴⁴ were thus obtained in good to excellent yields (Scheme 1). This synthesis was also extended to the new derivative 8,⁴³ because the thiazolanyl group was used as the versatile leaving group in 1,2-*cis* glycosidation reactions of pyranosyl donors.^{39,45,46} In the preparation of compounds 5–8, we observed that the nature of the third heteroatom X of the thioimidoyl residue influenced the

diastereocontrol of the Ferrier reaction. Better ratios favouring the expected β -anomer were obtained with a nitrogen atom ($\alpha/\beta = 0:1$) instead of a sulfur ($1:4.2 < \alpha/\beta < 1:6.7$). Better selectivities were nevertheless observed by reducing the reaction time but this modification also resulted in lower yields. However, owing to the presence of the 2-*O*-participating group, this parameter was not detrimental for further glycosylation reactions.

On the other hand, acceptors 10–13 (Fig. 4) are known compounds and were prepared as previously described (10,^{5,47} 11,⁴⁸ 12,⁴⁹ 13⁵⁰). Nevertheless, an interesting optimization for the synthesis of 12 was performed. The preparation of the latter began (Scheme 2) with the regioselective tritylation of an anomeric mixture of the well-known octyl galactofuranoside 14 α,β ^{51,52} by trityl chloride (TrCl) in the presence of *N,N*-dimethyl-4-aminopyridine (DMAP). Unfortunately, a disappointing reaction was observed under these conditions (yields lower than 5%). To overcome this limitation, the desired etherification was performed



Scheme 1. Synthesis of thioimidates 5–8. Reagents and conditions: (a) (99%, $\alpha/\beta = 0:1$);⁴⁴ (b) (91%, $\alpha/\beta = 0:1$);⁴⁴ (c) (83%, $\alpha/\beta = 1:6.7$);⁴⁴ (d) 2-mercaptothiazoline, $\text{BF}_3 \cdot \text{OEt}_2$ (93%, $\alpha/\beta = 1:4.2$).

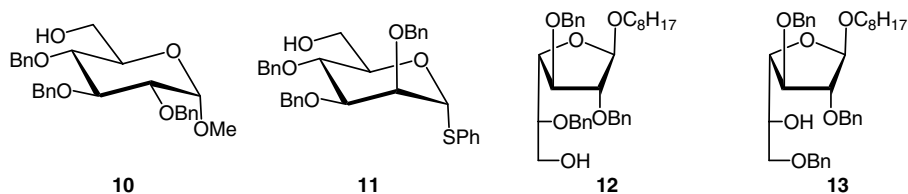
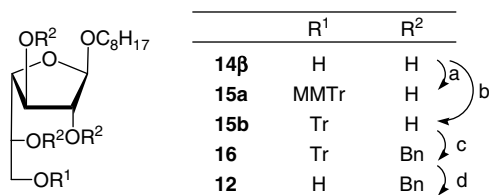


Figure 4. Structures of required acceptors 10–13.



Scheme 2. Optimized synthesis of acceptor 12. Reagents: (a) MMTrCl, DMAP (64%); (b) TrCl–DMAP salt (80%); (c) BnBr, NaH (99%); (d) AcOH, H₂O (78%).

from the pure 1,2-*trans*-furanoside **14β**, which could be selectively crystallized from **14α,β** in diethyl ether.⁵²

To explain the apparent inhibition of the Williamson etherification by the 1,2-*cis*-anomer, we assumed that the standard tritylation reagent was not reactive enough, and more importantly, that the nucleophilic base DMAP could engage in competitive reactions, that is, (i) nucleophilic activation of TrCl and (ii) possible deprotonation of the more acidic and more hindered 2-hydroxyl function in the 1,2-*cis* galactoside **14α**. Such a property, which proved to be of particular interest in selective 2-O-protection,⁵³ was suspected to be a factor that disfavoured the protection of the primary hydroxyl. On this basis, the introduction of the trityl group was first attempted starting from the pure **14β** and the more reactive *p*-methoxyphenyldiphenyl chloride (MMTrCl). The target product **15a** was then isolated in an increased 64% yield after only 4 h at room temperature and chromatographic purification. Secondly, the use of the electrophilic salt previously prepared from trityl chloride and DMAP⁵⁴ also gave increased performance because after 24 h at reflux in dichloromethane, the triol **15b** was obtained in a good and reproducible 80% yield on a multi-gram scale (20 g). Subsequent benzylation of **15b** under standard conditions followed by detritylation in aqueous acetic acid afforded the desired compound **12** in 77% overall yield. This approach is highly competitive with those proposed by Plusquellec et al.⁴⁹ or Reynolds and co-workers.⁵⁰

For subsequent glycosidation of donors **5–8**, we were first interested in synthesizing the β-D-Galp-(1→6)-α-D-Glcp disaccharide residue found in *E. coli* (K-12 strain) and particularly focused our attention on the nature of the promoter (Table 2). To ensure selective activation of the thioimidoyl donor, no halonium ion was used as promoter. After experimentation, we observed the

disappearance of the donor, thus showing its activation, with the Lewis acids trimethylsilyl trifluoromethanesulfonate (TMSOTf) and copper(II) trifluoromethanesulfonate. These results also showed the significant influence of the nature of the leaving group because the thiazol-2-yl-type donors **7** and **8** (entries 8–11, X = S) demonstrated very poor activity in the glycosylation of **10** while the desired disaccharide **17** was isolated in good 58% and 67% yield starting from the pyrimidinyl and benzimidazolyl derivatives **6** (X = NH) and **5** (X = N), respectively (entries 2, 4, 5 and 7). Moreover, the most efficient coupling was obtained with the more thiophilic copper(II) salt (entries 1/2, 6/7 and 10/11); adding only one molar equivalent of this promoter resulted in a significantly decreased yield (entries 2/3). It is also interesting to note that the modification of the initial equimolar donor/acceptor ratio, whatever the sense, had a positive influence on the yield of the reaction as the desired coupling product **17** was isolated, after deacetylation under Zemplen transesterification, in ~75% yield (entries 4 and 5). Further removal of the benzyl groups by hydrogenolysis afforded the target disaccharide **1** in 97% yield.

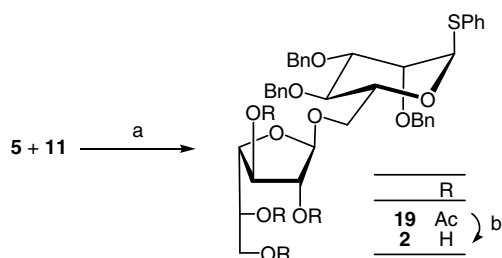
This preliminary study allowed us to select the 2-thiopyrimidinyl aglycon as the most appropriate leaving group in O-glycosylation reactions activated by copper(II) triflate. The use of the thiopyrimidinyl donor was further successfully extended to the preparation of the β-D-Galp-(1→6)-α-D-Manp disaccharide **2**, using thiomannopyranoside **11**, which bears a primary hydroxyl, as the acceptor (Scheme 3). The donor **5** thus smoothly reacted with **11** and afforded, after deacetylation and chromatographic purification, the desired disaccharide **2** in 60% yield.

Subsequently, the preparation of the disaccharides **3** and **4**, fragments of the glycosidic skeleton of the galactan produced by *M. tuberculosis*, required the use of glycosyl acceptors in a furanose form. These compounds are well known to be very sensitive to conformational changes not only because the five-membered ring is more flexible than the pyranosyl counterpart⁶ but also because the side arm is less constrained, thus increasing the number of possible rotamers. Consequently, this molecular parameter may influence the progress of the glycosylation reaction. Indeed, modest yields were obtained for the synthesis of protected disaccharides **20** (30%) and **21** (14%) (Scheme 4) and the yields could

Table 2. Model glycosidic coupling for selection of the most appropriate donor

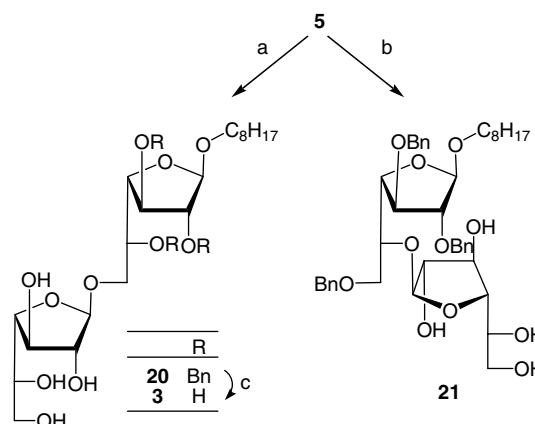
Entry	Donor	Aglycon	Donor/acceptor	Promoter (equiv)	17 (overall yield, %)
1	5		1:1	TMSOTf (2)	18
2	5		1:1	Cu(OTf) ₂ (2)	67
3	5		1:1	Cu(OTf) ₂ (1)	23
4	5		1:2	Cu(OTf) ₂ (2)	74
5	5		2:1	Cu(OTf) ₂ (2)	77
6	6		1:1	TMSOTf (2)	nd
7	6		1:1	Cu(OTf) ₂ (2)	58
8	7		1:1	TMSOTf (2)	nd
9	7		1:1	Cu(OTf) ₂ (2)	nd
10	8		1:1	TMSOTf (2)	18
11	8		1:1	Cu(OTf) ₂ (2)	24

nd: not determined.

**Scheme 3.** Orthogonal glycosylation. Reagents: (a) Cu(OTf)₂; (b) NaOMe, MeOH (60% for two steps).

not be improved even with a more standard promoter such as NIS/TMSOTf. A final debenzoylation performed in a hydrogen atmosphere catalyzed by palladium(II) acetate afforded **3** in good yield.

As expected, the results presented here suggested that the reactions were dependant on the nature of the electrophilic and nucleophilic partners and also on the promoter. We first observed a better reactivity for primary hydroxyl groups and showed that the higher yields of coupling were obtained with pyranosyl acceptors versus furanosyl ones. Secondly, the glycosylation could be achieved due to activation of the donor, that is, interac-

**Scheme 4.** Synthesis of digalactofuranosides **3** and **21**. Reagents: (a) (i) **12**, Cu(OTf)₂; (ii) NaOMe, MeOH (30% for two steps); (b) **13**, Cu(OTf)₂; (ii) NaOMe, MeOH (14% for two steps); (c) H₂, Pd(OAc)₂ (**3**: 67% for two steps).

tion between the aglycon and the reactive catalytic species, Cu²⁺. On the basis of the pK_a of the free aglycons (Table 3), the activation process could not be simply ascribed to this parameter because donors **5** and **8β** have two heterocycles with similar acidity but a significantly

Table 3. Selected physicochemical properties of donors **5–8**

Entry	Donor	Heterocycle	pK _a ^a	δ _{H-1} (ppm)	δ _{C-1} (ppm)
1	5		1.65 ^b	6.47	87.5
2	6		0.31 ^b	5.94	89.2
3	7β		−3.17 ^b	6.27	89.3
4	8β		1.28 ^b	5.39	88.4

^a The pK_a values correspond to the unglycosylated heterocycles.

^b The pK_a values were obtained from 'calculated properties' for each compound in SciFinder Scholar v.2006.

different reactivity (Table 2, entries 2 and 11). On the other hand, Hanessian et al. could correlate the high glycosylating ability of MOP hexopyranosides to upfield and downfield chemical shifts for ¹H and ¹³C, respectively.³⁷ Unfortunately, such a correlation could not explain the results observed with thioimidates **5–8** (Table 3).

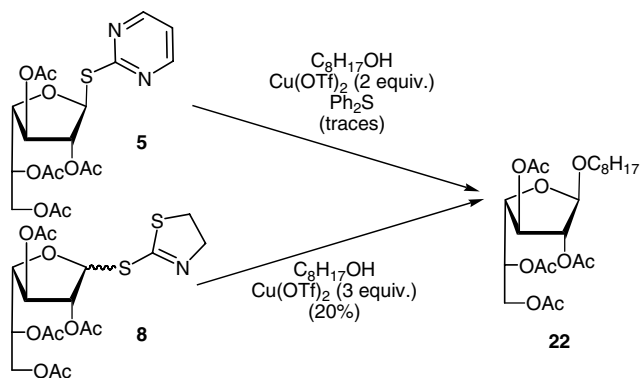
To rationalize our results, two main sets of experiments, already published, have also to be considered. First, by comparison with the phosphorylation of thioimidates of the same series⁴⁴ for which we had concluded better suitability between phosphoric acid and the benzimidazolyl derivative, it would have been difficult to predict the higher efficiency of the 2-pyrimidinyl

thiofuranoside **5** in O-glycosylation reactions with saccharide acceptors. Second, it was also very surprising to notice the low reactivity of the disarmed donor **8** belonging to the thiazolyl family, although such donors were presented as an interesting alternative to standard glycosylation using either armed or disarmed thioglycopyranosides as an activator.^{40,55} As a consequence, the nature of the interactions between the activating cation and the thioimidoyl function was suspected to be responsible for these data. Indeed, in the present study, the borderline Lewis acidic cupric cations, according to the hard and soft acid/base concept, could interact with three different centres: the exocyclic sulfur atom, the nitrogen atom and the X group (X = S or NH) of the heterocycle. A computational approach of the relative charges on atoms possibly involved in the glycosylation process gave the values summarized in Table 4. The resulting data showed that similar values were obtained for all donors **5–8** for S-1, N-1 and C-1. On the assumption that thiophenylglycosides are not activated by cupric ions, it was expected that the exocyclic sulfur S-1 was not involved in the activation process, thus corroborating a remote activation mechanism. The main difference was however obtained for the X atom, because the charge on S-2 in donors **7** and **8** was significantly lower than that calculated for N-2 in **5** and **6**. We then hypothesized that a stronger interaction between S-2 and Cu²⁺ was not able to really activate the furanosyl donor but may be also responsible for the observed lack of reactivity using **7** and **8**.

To probe this hypothesis, two supplementary reactions were further performed (Scheme 5). Thus, the Cu²⁺-activated glycosylation of *n*-octanol was carried out using either thiazolyl derivative **8** in the presence of a large excess of copper(II) triflate or donor **5** in the presence of diphenylsulfide as a scavenger of cupric cat-

Table 4. Selective data for galactofuranosyl donors **5–8**

Entry	Donor	Heterocycle	C-1 anomeric	S-1	N-1	X
1	5		0.365	−0.462	−0.218	−0.230 (N-2)
2	6		0.348	−0.430	−0.274	−0.173 (N-2)
3	7β		0.368	−0.405	−0.203	−0.394 (S-2)
4	8β		0.368	−0.396	−0.210	−0.385 (S-2)



Scheme 5. Influence of additive diphenylsulfide or excess $\text{Cu}(\text{OTf})_2$ on glycosidation of **5** and **8**, respectively.

ions. The resulting peracetylated octyl galactofuranoside **22** was thus isolated in moderate 20% yield, close to that obtained for **17** under similar conditions. However, the use of diphenylsulfide really inhibited the desired coupling since only traces of **22** were observed by monitoring the reaction by thin layer chromatography. This result therefore support the notion that a sulfur atom present on the heterocycle could modulate the activation of thioimidates by Cu^{2+} .

In conclusion, the synthesis of relevant galactofuranose-containing disaccharides was achieved through the remote activation of four furanosyl thioimidates using copper(II) triflate as an activator. The large heterogeneousness of the results was first connected with the size of the glycosyl acceptors (pyranosyl vs furanosyl) and was secondly ascribed to the presence on the heterocyclic aglycon of an endocyclic sulfur atom or amino group. Better coordination of the cupric cations by this sulfur than by a nitrogen atom manifested in a lack of reactivity of the thiazole-like donors. Further studies are currently underway to investigate the potential orthogonality of these families of thioimidates in the presence of various promoters.

3. Experimental

3.1. General methods

All reactions were performed at rt under N_2 atmosphere. Pyrimidin-2-yl 2,3,5,6-tetra-*O*-acetyl-1-thio- β -D-galactofuranoside (**5**),⁴⁴ benzimidazol-2-yl 2,3,5,6-tetra-*O*-acetyl-1-thio- β -D-galactofuranoside (**6**),⁴⁴ benzothiazol-2-yl 2,3,5,6-tetra-*O*-acetyl-1-thio- β -D-galactofuranoside (**7**)⁴⁴ and octyl 2,3,5-tri-*O*-benzyl- β -D-galactofuranoside (**12**)⁴⁹ were synthesized as previously described. Octyl 2,3,6-tri-*O*-benzyl- β -D-galactofuranoside (**13**) was obtained as described by Pathak et al.⁵⁰ Phenyl 2,3,4-tri-*O*-benzyl-1-thio- α -D-mannopyranoside (**11**) was synthesized as reported by Martín-Lomas et al.⁴⁸ Methyl

2,3,4-tri-*O*-benzyl- α -D-glucopyranoside (**10**) was obtained as described by Reitz et al.⁵⁶ Reactions were monitored by TLC (E. Merck 60 F₂₅₄ Silica Gel non activated plates) and compounds were visualized by UV-absorption and/or charring with a 5% solution of H_2SO_4 in EtOH. Preparative chromatography was conducted on Geduran Si 60 (40–63 μm) Silica Gel. Melting points were determined on a Reichert microscope and are uncorrected. Optical rotations were measured on a Perkin–Elmer 341 polarimeter. ^1H , ^{13}C , HMBC, HMQC and COSY NMR spectra were recorded at the ENSCR on a Bruker ARX 400 spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C analyses. 1D and 2D spectra of unprotected disaccharides were recorded on a Bruker Avance 500 spectrometer at 500 MHz for ^1H and 125 MHz for ^{13}C , by the ‘Service de Spectrométrie de RMN Haut Champ du Centre Régional de Mesures Physiques de l’Ouest (CRMPO, Rennes, France)’. Chemical shifts are given in δ units measured from the solvent signal. The HRMS were performed at the CRMPO with a MS/MS ZabSpec TOF Micromass by ESI in positive mode. Microanalyses were performed by the ‘Service de Microanalyse de l’ICSN (Gif sur Yvette, France)’ and by the CRMPO.

3.2. Thiazolin-2-yl 2,3,5,6-tetra-*O*-acetyl-1-thio-D-galactofuranoside (**8**)

To a solution of 1,2,3,5,6-penta-*O*-acetyl-D-galactofuranose (**9**)⁴¹ (2.00 g, 5.12 mmol) in CH_2Cl_2 (82 mL) were added 2-mercaptothiazoline (1.83 g, 15.37 mmol) and $\text{BF}_3 \cdot \text{OEt}_2$ (5.8 mL, 46.08 mmol). The solution was stirred at rt for 24 h and was diluted with CH_2Cl_2 (80 mL). The reaction mixture was washed with a satd aq NaHCO_3 (3×50 mL) and with H_2O (3×100 mL). The aq layers thus obtained were extracted with CH_2Cl_2 (3×50 mL) and the combined organic layers were dried (MgSO_4) and finally concentrated. Purification of the residue by column chromatography (9:1, CH_2Cl_2 –EtOAc) as eluent gave **8** in anomeric mixture ($\alpha/\beta = 1:4.2$) as a colourless oil (2.15 g, 93%): TLC (9:1, CH_2Cl_2 –EtOAc): $R_f = 0.3$; **8 α** : ^1H NMR (CDCl_3): δ 6.40 (d, 1H, $J_{1,2} = 5.1$ Hz, H-1), 5.51 (dd, 1H, $J_{2,3} = 3.3$ Hz, H-2), 5.33 (dt, 1H, $J_{4,5} = 7.6$ Hz, $J_{5,6a} = J_{5,6b} = 4.3$ Hz, H-5), 5.23 (dd, 1H, $J_{3,4} = 3.8$ Hz, H-3), 4.38–4.11 (m, 5H, H-4, H-5a', H-5b', H-6a, H-6b), 3.40 (t, 2H, $^3J = 8.0$ Hz, H-4a', H-4b'), 2.15, 2.13, 2.09, 2.04 (4s, 12H, CH_3CO); ^{13}C NMR (CDCl_3): δ 170.6, 170.1, 169.6, 169.3 (C=O), 163.3 (C-2'), 85.9 (C-1), 81.7 (C-4), 76.4 (C-2), 76.2 (C-3), 69.5 (C-5), 64.3 (C-5'), 62.6 (C-6), 35.6 (C-4'), 21.0, 20.8, 20.7, 20.6 (CH_3CO); **8 β** : ^1H NMR (CDCl_3): δ 6.21–6.20 (m, 1H, H-1), 5.39 (dt, 1H, $J_{4,5} = 7.4$ Hz, $J_{5,6a} = J_{5-6b} = 4.1$ Hz, H-5), 5.28 (t, 1H, $J_{1,2} = J_{2,3} = 1.8$ Hz, H-2), 5.10 (ddd, 1H, $J_{3,4} = 4.8$ Hz, H-3), 4.38–4.35 (m, 1H, H-4), 4.36 (dd, 1H, $J_{6a,6b} = 12.0$ Hz, H-6a), 4.28, 4.19 (2dt, 2H,

$^2J = 14.8$ Hz, $^3J = 7.9$ Hz, H-5a', H-5b'), 4.18 (dd, 1H, H-6b), 3.40 (t, 2H, H-4a', H-4b'), 2.12, 2.12, 2.11, 2.05 (4s, 12H, CH₃CO); ^{13}C NMR (CDCl₃): δ 170.6, 170.1, 169.7, 169.4 (C=O), 162.8 (C-2'), 88.4 (C-1), 81.7 (C-4), 80.9 (C-2), 76.4 (C-3), 69.2 (C-5), 64.3 (C-5'), 62.6 (C-6), 35.4 (C-4'), 20.9, 20.8, 20.8, 20.7 (CH₃CO); HRMS Calcd for C₁₇H₂₃NaNO₉S₂ [M+Na]⁺: 472.0712. Found: 472.0712; Anal. Calcd for C₁₇H₂₃NO₉S₂: C, 45.43; H, 5.16; N, 3.12. Found: C, 45.09; H, 5.15; N, 3.16.

3.3. General procedure A: glycosylation reactions

To a solution of thioimidoyl galactofuranoside (0.23 mmol) in CH₂Cl₂ (10 mL/g) were successively added the appropriate acceptor (0.12 mmol or 0.46 mmol; depending on the acceptor) and Cu(OTf)₂ (163 mg, 0.45 mmol). The mixture was stirred at rt for a given period and the reaction was quenched by addition of a few drops of Et₃N. Insoluble materials were removed by filtration and the filtrate was concentrated to dryness. The residue thus obtained was then purified by chromatography on silica gel. The fractions containing the target protected disaccharide were pooled, concentrated under reduced pressure and diluted in dry MeOH (20 mL). A 0.1 M solution of NaOMe in MeOH (2.3 mL, 0.23 mmol) was then added and the mixture was stirred at rt overnight. The reaction was quenched by adding a few drops of AcOH and the solution was concentrated under reduced pressure. The partially deprotected disaccharide was finally recovered by chromatographic purification.

3.4. Methyl β -D-galactofuranosyl-(1 \rightarrow 6)-2,3,4-tri-O-benzyl- α -D-glucopyranoside (17)

This compound was produced according to the general procedure A from the pyrimidin-2-yl galactofuranoside **5** (226 mg, 0.51 mmol) and from the glucopyranoside acceptor **10** (119 mg, 0.26 mmol). After stirring for 2 h and work-up, the resulting disaccharide was purified by chromatography using 3:2, light petroleum–EtOAc as eluant [TLC (3:2, light petroleum–EtOAc): $R_f = 0.4$]. The mixture thus obtained was deacetylated as described. The partially deprotected disaccharide **17** was purified by chromatography (19:1, CH₂Cl₂–MeOH) and was then isolated as a white solid (124 mg, 77%): mp 98 °C (CH₂Cl₂–MeOH); $[\alpha]_D^{20} -1.0$ (*c* 1.1, MeOH); TLC (19:1, CH₂Cl₂–MeOH): $R_f = 0.1$; ^1H NMR (CD₃OD): δ 7.38–7.24 (m, 15H, C₆H₅), 4.92–4.62 (m, 6H, OCH₂Ph), 4.87 (d, 1H, $J_{1',2'} = 1.8$ Hz, H-1'), 4.71 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.00 (dd, 1H, $J_{3',4'} = 6.4$ Hz, $J_{2',3'} = 0.8$ Hz, H-3'), 3.97 (dd, 1H, H-2'), 3.94 (dd, 1H, $J_{4',5'} = 3.1$ Hz, H-4'), 3.92 (dd, 1H, $J_{6a,6b} = 11.2$ Hz, $J_{5,6a} = 1.8$ Hz, H-6a), 3.87 (dd, 1H, $J_{2,3} = 9.4$ Hz, $J_{3,4} = 9.2$ Hz, H-3), 3.74–3.69 (m, 2H, H-5, H-5'),

3.62–3.57 (m, 3H, H-6b, H-6a', H-6b'), 3.54 (dd, 1H, H-2), 3.51 (dd, 1H, $J_{4,5} = 9.9$ Hz, H-4), 3.38 (s, 3H, OCH₃); ^{13}C NMR (CD₃OD): δ 140.1, 139.7, 139.6 (C_{ipso}), 129.5, 129.4, 129.3, 129.2, 129.0, 128.9, 128.7, 128.6 (C₆H₅), 109.8 (C-1'), 99.1 (C-1), 84.3 (C-4'), 83.2 (C-2'), 83.0 (C-3), 81.4 (C-2), 79.0 (C-4), 78.6 (C-3'), 76.5, 76.0, 74.0 (OCH₂Ph), 72.3 (C-5'), 71.5 (C-5), 67.6 (C-6), 64.6 (C-6'), 55.6 (OCH₃); HRMS Calcd for C₃₄H₄₂KO₁₁ [M+K]⁺: 665.2364. Found: 665.2365.

3.5. Phenyl β -D-galactofuranosyl-(1 \rightarrow 6)-2,3,4-tri-O-benzyl-1-thio- α -D-mannopyranoside (2)

This disaccharide was obtained according to the general procedure A from the thio galactofuranoside **5** (68 mg, 0.15 mmol) and the mannopyranosidic acceptor **11** (168 mg, 0.31 mmol). The acetylated intermediate **19** was purified by chromatography (4:1 \rightarrow 3:2, light petroleum–EtOAc). The fractions containing this product [TLC (4:1, light petroleum–EtOAc): $R_f = 0.1$] were pooled, concentrated and subjected to Zemplén deacetylation. The disaccharide **2** thus generated was purified by flash chromatography (19:1, CH₂Cl₂–MeOH). This compound was then isolated as a colourless oil (65 mg, 60%): $[\alpha]_D^{20} +30.2$ (*c* 1.0, MeOH); TLC (19:1, CH₂Cl₂–MeOH): $R_f = 0.2$; ^1H NMR (CD₃OD): δ 7.48–7.45, 7.39–7.26 (2 m, 20H, C₆H₅), 5.47 (d, 1H, $J_{1,2} = 1.5$ Hz, H-1), 4.91–4.86, 4.70–4.54 (2 m, 6H, OCH₂Ph), 4.87–4.86 (m, 1H, H-1'), 4.20 (ddd, 1H, $J_{5,6b} = 5.6$ Hz, $J_{5,6a} = 1.8$ Hz, H-5), 4.05 (dd, 1H, $J_{2,3} = 3.0$ Hz, H-2), 4.00–3.94 (m, 2H, H-2', H-3'), 3.96–3.92 (m, 2H, H-4', H-6a), 3.92 (dd, 1H, $J_{4,5} = 9.6$ Hz, H-4), 3.82 (dd, 1H, $J_{3,4} = 9.2$ Hz, H-3), 3.70 (ddd, 1H, $J_{5',6a'} = J_{5',6b'} = 6.1$ Hz, $J_{4',5'} = 3.0$ Hz, H-5'), 3.64 (dd, 1H, $J_{6a,6b} = 11.2$ Hz, H-6b), 3.60–3.57 (m, 2H, H-6a', H-6b'); ^{13}C NMR (CD₃OD): δ 139.8, 139.4, 139.3 (C_{ipso} OCH₂Ph), 135.4 (C_{ipso} SPh), 133.3, 130.2, 129.5, 129.4, 129.2, 129.2, 129.0, 128.9, 128.8, 128.8 (C₆H₅), 109.6 (C-1'), 87.3 (C-1), 84.7 (C-4'), 83.0 (C-2'), 81.0 (C-3), 79.0 (C-3'), 77.4 (C-2), 76.1, 73.2, 72.9 (OCH₂Ph), 75.9 (C-4), 73.7 (C-5), 72.4 (C-5'), 67.5 (C-6), 64.7 (C-6'); HRMS Calcd for C₃₉H₄₄NaO₁₀S [M+Na]⁺: 727.2553. Found: 723.2550.

3.6. Octyl β -D-galactofuranosyl-(1 \rightarrow 6)-2,3,5-tri-O-benzyl- β -D-galactofuranoside (20)

The disaccharide **20** was synthesized according the general procedure A from thioimidoyl galactofuranoside **5** (181 mg, 0.41 mmol) and the galactofuranosidic acceptor **12** (459 mg, 0.82 mmol). The acetylated intermediate was then purified by flash chromatography (4:1 \rightarrow 3:2, light petroleum–EtOAc) [TLC (4:1, light petroleum–EtOAc): $R_f = 0.4$]. The resulting crude oil was further deacetylated as described. The partially benzylated disaccharide **20** was purified by chromatography on sil-

ica gel eluting with 19:1, CH₂Cl₂–MeOH, and was then isolated as a colourless oil (88 mg, 30%): $[\alpha]_{\text{D}}^{20}$ –81.3 (*c* 1.4, MeOH); TLC (19:1, CH₂Cl₂–MeOH): R_{f} = 0.2; ¹H and ¹³C NMR spectra were identical to the reported data;⁵⁰ HRMS Calcd for C₄₁H₅₆NaO₁₁ [M+Na]⁺: 747.3720. Found: 747.3721.

3.7. Octyl β-D-galactofuranosyl-(1→5)-2,3,5-tri-O-benzyl-β-D-galactofuranoside (21)

This compound was obtained as described in procedure A from the galactofuranosidic donor **5** (220 mg, 0.50 mmol) and the acceptor **13** (137 mg, 0.24 mmol). The partially acetylated disaccharide was then isolated by chromatographic purification (4:1, light petroleum–EtOAc) [TLC (7:3, light petroleum–EtOAc): R_{f} = 0.3] and the fractions containing this product were combined, concentrated and subjected to Zemplen transesterification. The disaccharide **21** was purified by chromatography on silica gel (19:1, CH₂Cl₂–MeOH) and could be finally isolated as a colourless oil (24 mg, 14%): $[\alpha]_{\text{D}}^{20}$ –60.9 (*c* 1.0, MeOH); TLC (19:1, CH₂Cl₂–MeOH): R_{f} = 0.2; ¹H and ¹³C NMR spectra were identical to the reported data;⁵⁰ HRMS Calcd for C₄₁H₅₆NaO₁₁ [M+Na]⁺: 747.3720. Found: 747.3726.

3.8. General procedure B: debenzilation reactions

AcOH (2.5 mL) and Pd(OAc)₂ (5 mg) were added to a solution of disaccharide (30 mg) in EtOAc (2.5 mL). After stirring under hydrogen (1 atm) at rt for 72 h, the mixture was filtered, concentrated under reduced pressure and traces of AcOH were coevaporated with toluene (5 × 5 mL). The totally unprotected disaccharide was then isolated by flash chromatography on silica gel.

3.9. Methyl β-D-galactofuranosyl-(1→6)-α-D-glucopyranoside (1)

This compound was synthesized according to the general procedure B from the benzylated disaccharide **17** (31 mg, 0.05 mmol). The target product **1** was then purified by chromatography on silica gel (3:3:1, EtOAc–*i*-PrOH–H₂O), and was isolated as a colourless hygroscopic foam (17 mg, 97%): $[\alpha]_{\text{D}}^{20}$ +13.7 (*c* 0.3, H₂O); TLC (3:3:1, EtOAc–*i*-PrOH–H₂O): R_{f} = 0.4; ¹H NMR (D₂O): δ 4.95 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1'), 4.72 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.03 (dd, 1H, $J_{2',3'}$ = 3.9 Hz, H-2'), 3.99 (dd, 1H, $J_{3',4'}$ = 6.6 Hz, H-3'), 3.93 (dd, 1H, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6a}$ = 1.4 Hz, H-6a), 3.91 (dd, 1H, $J_{4',5'}$ = 4.1 Hz, H-4'), 3.75 (ddd, 1H, $J_{5',6b'}$ = 7.3 Hz, $J_{5',6a'}$ = 4.3 Hz, H-5'), 3.70 (ddd, 1H, $J_{4,5}$ = 9.3 Hz, $J_{5,6b}$ = 5.4 Hz, H-5), 3.66 (dd, 1H, H-6b), 3.64 (dd, 1H, $J_{6a',6b'}$ = 11.7 Hz, H-6a'), 3.59 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 3.58 (dd, 1H, H-6b'), 3.49 (dd, 1H, H-2), 3.37 (t, 1H, H-4), 3.34

(s, 1H, OCH₃); ¹³C NMR (D₂O): δ 107.9 (C-1'), 99.3 (C-1), 82.6 (C-4'), 81.0 (C-2'), 76.6 (C-3'), 73.0 (C-3), 71.1 (C-2), 70.7 (C-5'), 70.6 (C-5), 69.5 (C-4), 66.7 (C-6), 62.7 (C-6'), 55.2 (OCH₃); HRMS Calcd for C₁₃H₂₄NaO₁₁ [M+Na]⁺: 379.1216. Found: 379.1219.

3.10. Octyl β-D-galactofuranosyl-(1→6)-β-D-galactofuranoside (3)

The unprotected disaccharide **3** could be obtained according the general procedure B from the partially benzylated disaccharide **21** (67 mg, 0.09 mmol). The target glycoconjugate **3** could then be isolated after chromatographic purification using 19:1, CH₂Cl₂–MeOH as eluent, as a colourless oil (28 mg, 67%): $[\alpha]_{\text{D}}^{20}$ +12.6 (*c* 1.5, H₂O); TLC (19:1, CH₂Cl₂–MeOH): R_{f} = 0.3; ¹H and ¹³C NMR spectra were identical to the reported data;^{49,50} HRMS Calcd for C₂₀H₃₈NaO₁₁ [M+Na]⁺: 477.2312. Found: 477.2292.

3.11. Calculations of the charges

Hyperchem Molecular Visualization and Simulation software (Hypercube, Waterloo, Ontario, Canada, software HyperChem 7.5) was used to calculate the charges on the interesting atoms. The initial geometries of the compounds **5–8** were built by means of standard parameters, applying an Amber molecular mechanics force field and then optimized by TNDO geometrical optimization. The resulting charge values were not considered individually but were rather used to make comparisons between similar species. Selected data are summarized in Table 4.

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