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Direct access to new β-D-galactofuranoconjugates: application to the synthesis of galactofuranosyl-L-cysteine and L-serine

Dancho Danalev [†], Laurent Legentil [*], Richard Daniellou, Caroline Nugier-Chauvin, Vincent Ferrières [*]

Ecole Nationale Supérieure de Chimie de Rennes, CNRS, UMR 6226, Avenue du Général Leclerc, CS 50837, 35708 Rennes Cedex 7, France
Université européenne de Bretagne, France

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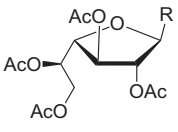
ABSTRACT

Galactofuranose post-translational modifications, although quite rare, were detected in some biomolecules produced by parasites. While hexopyranosides were already linked to various peptides and proteins, few hexofuranosides have been artificially conjugated to amino acids. We thus report herein a robust glycosylation methodology to obtain *S*-alkyl, *O*-serine and *S*-cysteine-β-D-galactofuranosides starting from readily available galactofuranose donors. *O*-Acetyl, thioimidoyl and acetimidoyl donors were compared in terms of yields and selectivity when reacted with mercaptans, L-cysteine and L-serine. Acetimidates turned out to be the best notably for amino acids glycosylation.

1. Introduction

β-D-Galactofuranosyl moiety forms an original motif in nature. Completely unknown in mammalian systems,[1] it has been found in various bacteria, fungi and parasites as part of complex glycoconjugates.[2,3] These latter were involved as cell-wall constituents or were incorporated into proteins by post-translational modifications. Post-translational glycosylations add particular properties to biomolecules, favour stability against proteolysis, and in certain cases increase the biological activities of the protein itself.[4,5] For example, β-galactofuranosyl residues are integrated to the O-linked glycan of *Aspergillus niger* glucoamylase,[6] the invertase produced by *Aspergillus nidulans*[7] or the N-linked glycans of α-galactosidase A from *A. niger*.[8] This latter microorganism is an important fungi involved in the industrial production of many substances for food production and preservation notably. In particular, α-galactosidase A catalytically trims α-linked galactose from oligosaccharides. It was suggested that the presence of terminal β-galactofuranosyl residues on the N-linked glycans stopped further elongation of the oligomer. However, the biological significance of this monosaccharide has yet to be fully elucidated and its associated antigenicity has to be fully understood. Recently our group showed that oligofuranoses possessed some adjuvant properties.[9] Moreover, expression of glycoproteins leads in general to a heterogeneous mixture of glycoforms that differ in the nature and site of glycosylation. This phenomenon added difficulties to identify the exact effect of β-galactofuranosyl motif on the activity. Consequently, definition of a straightforward strategy for regioselective glycosylation on the native proteins is of primordial importance.

Proper timing glycosylation and assembly strategy are vital issue for production of glycopeptides and glycoproteins. They have been extensively reviewed in the previous years.[10–14] These techniques implied the use of pre-established glycosylated amino acids as building-blocks. As far as we are aware, only stereoselective syntheses of pentofuranosyl amino acids were reported and involved arabinosyl or ribosyl glycosylation to serine or asparagine.[15–17] In this general context, we thus identified and synthesized a family of β-D-galactofuranosyl (β-D-Gal*f*) conjugates that can be used as simple sugar probes for further protein ligation (Fig. 1). Our targets were designed around β-D-Gal*f* connected to either a functionalized thioalkyl arm (**1a** to **1d**) or the side chain of L-cysteine (**1e**) or serine (**1f**). Interestingly, thioglycosidic bonds in **1a** to **1e** are



	R
1a	SBn
1b	SCH_2CO_2Et
1c	$S(CH_2)_2CO_2Me$
1d	$S(CH_2)_3Cl$
1e	$SCH_2CH(NHAc)CO_2Et$
1f	$OCH_2CH(NHCbz)CO_2Me$

Figure 1. Targeted β-D-galactofuranosides.

* Corresponding author. Tel.: +33 223 238 140; fax: +33 223 238 046 (L.L.).
E-mail address: laurent.legentil@ensc-rennes.fr (L. Legentil).
† Present address: University of Chemical Technology and Metallurgy, Department of Organic Chemistry, Sofia 1756, Bulgaria, 8 Blvd. Kliment Ohridski, Bulgaria. Tel.: +359 2 81 63 418, fax: +359 2 868 54 88.

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known to be more stable against acidic and enzymatic hydrolysis compared to their O-counterpart.[18]

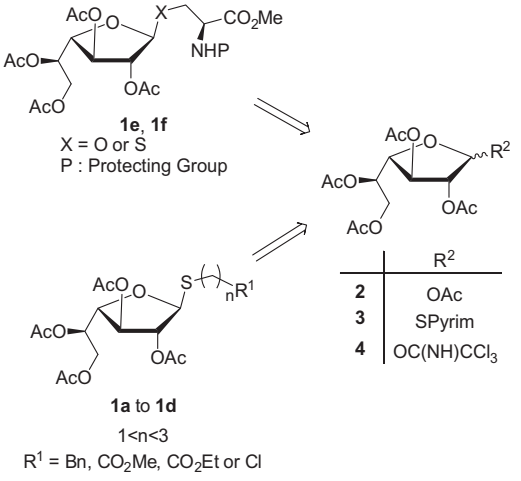
Grafting of serine onto different glycosides was widely described in particular in the hexopyranose series.[19,20] However, direct O-glycosylation of the side chain of the protected serine usually necessitates activation of the carbohydrate donor such as glycosyl bromide or thiophenyl glycoside.[17] As for cysteine, it is one of the least abundant amino acids in proteins and was popularized as a good linker for glycopeptide synthesis by different groups.[21] Its glycosylation on pyranoses followed two main pathways; anomeric nucleophilic thiolate anions generated from pyranosyl isothiouronium could substitute iodo-alanine[22] in good to excellent yields while Koenigs–Knorr reaction allowed moderate access to thioglycosidic bond.[23] Considering the literature, there is, therefore, a need to develop a new methodology for amino acids glycosylation in particular in the furanose series. Indeed, no example describes direct glycosylation of cysteine or serine on galactofuranoses. Consequently we thought to evaluate the potential of acetate, thioimidate and trichloroacetimidate as furanosyl donors for direct glycosylation with alkyl mercaptans and amino acid acceptors (Scheme 1).

2. Results and discussion

First a model study for direct glycosylation between the available per-*O*-acetylated-D-galactofuranose **2**[24] and benzyl mercaptan was implemented (Table 1). Best yields occurred in a non-protic and non-participating solvent such as dichloromethane. The nature of the Lewis acid seemed to influence greatly the outcome of the reaction. Weak one as copper(II) triflate failed to initiate the reaction while the strongest trimethylsilyltriflate resulted in the formation of one side product namely 2,3,5,6-tetra-*O*-acetyl galactose. However, boron trifluoride diethyl etherate presents the right acidity balance to afford the aimed benzyl 1-thio-galactofuranoside **1a** as a pure 1,2-*trans* anomer in 81% yield (Table 1, entry 1). To ensure best yields and diastereoselectivity, the reaction proceeded at 0 °C. Indeed, when performed at room temperature, the resulting reaction mixture was polluted with some 1,2-*cis* anomer. β-Configuration was obtained thanks to the presence of the participating acetate group at position 2 and was confirmed by a small NMR coupling constant between anomeric proton H-1 and H-2 ($J_{1,2}$ = 2 Hz).[25]

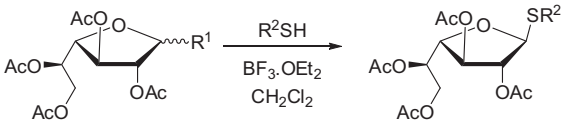
To extend the study, another well documented donor **3**[26] was also used (Table 1). We chose a pyrimidazoyl aglycon as it led to the best yields for glycosylation with various alcohols when acetate protected. It had also the advantage to react readily at room temperature with complete diastereoselectivity. However, glycosylation of benzyl mercaptan under previous conditions provided **1a** in a much lower yield of 53% (entry 2). Similar conclusions were reached when our protocol was extended to various thiol nucleophiles; 2-mercaptoacetic acid ethyl ester (Mpe-OEt, entries 3 and 4), 3-mercaptopropanoic acid methyl ester (Mpa-OMe, entries 5 and 6) and 3-chloropropane mercaptan (entries 7 and 8). These functionalized thioalkyl derivatives could serve as arm linkers for further ligation to peptides or proteins.[27] Furthermore, replacement of the natural amino acid cysteine with Mpa in peptide or proteins often leads to significant increase of their biological activities.[28] Once again, in general, per-O-acetylated donor **2** turned out to be better in terms of yields and low temperature was required to access pure 1,2-*trans* diastereoisomers **1b** to **1d**.

Direct ligation either on N-terminal or side chain of serine, cysteine or glycine provided the simplest access to glycopeptides. Consequently, we have adapted our methodology for direct glycosylation of serine and cysteine derivatives (Table 2). Surprisingly, our protocol could not be reproduced for the synthesis of *S*-galactofuranosyl cysteine **1e**. Indeed while *N*-acetyl-L-cysteine methyl ester was not intrinsically chemically different from the other thiol acceptors, ligation to per-O-acetylated donor **2** appeared to be unsuccessful (entry 1). Good yields were however obtained with thioimidate **3** but the reaction turned out to be sluggish (entry 3). To overcome these difficulties, a third donor, namely galactofuranosyl trichloroacetimidate **4** was used.[29] It was obtained according to a two-step procedure starting from **2** (Scheme 2). Firstly, the anomeric position was carefully deacetylated with hydrazine acetate[30] in dimethylformamide. It has never been described be-



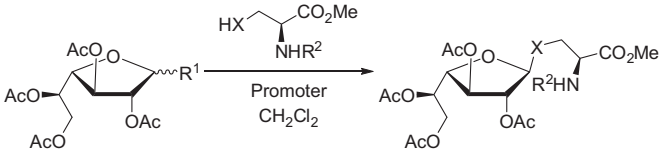
Scheme 1. Proposed retrosynthesis for β-D-galactofuranosides **1a–f**.

Table 1
Access to alkyl 1-thio-β-D-galactofuranosides **1a–d**

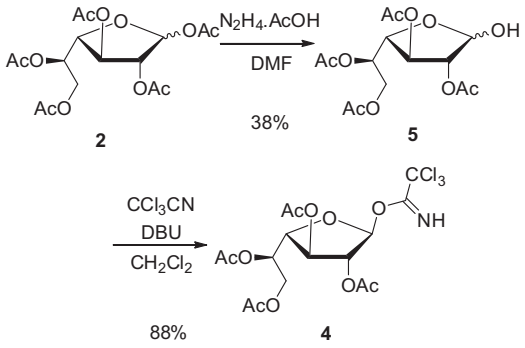


Entry	Donor (R^1)	T (°C)	Product	R^2	Yield (%)
1	**2** (α,β-OAc)	0	**1a**	SBn	81
2	**3** (β-SPyrim)	20	**1a**	SBn	53
3	**2** (α,β-OAc)	0	**1b**	SCH_2CO_2Et	69
4	**3** (β-SPyrim)	20	**1b**	SCH_2CO_2Et	63
5	**2** (α,β-OAc)	0	**1c**	$S(CH_2)_2CO_2Me$	63
6	**3** (β-SPyrim)	20	**1c**	$S(CH_2)_2CO_2Me$	74
7	**2** (α,β-OAc)	0	**1d**	$S(CH_2)_3Cl$	83
8	**3** (β-SPyrim)	20	**1d**	$S(CH_2)_3Cl$	52

Table 2
Access to *S*-galactofuranosyl cysteine and *O*-galactofuranosyl serine **1e–f**



Entry	Donor (R^1)	Conditions (T, °C)	Product (X)	R^2	Yield (%)
1	**2** (α,β-OAc)	$BF_3{\cdot}OEt_2$ (0)	**1e** (*S*)	Ac	—
2	**2** (α,β-OAc)	$BF_3{\cdot}OEt_2$ (0)	**1f** (*O*)	Cbz	65
3	**3** (β-SPyrim)	$BF_3{\cdot}OEt_2$ (20)	**1e** (*S*)	Ac	79
4	**3** (β-SPyrim)	$BF_3{\cdot}OEt_2$ (20)	**1f** (*O*)	Cbz	—
5	**3** (β-SPyrim)	$Cu(OTf)_2$ (20)	**1f** (*O*)	Cbz	48
6	**4** [β-$OC(NH)CCl_3$]	TMSOTf (0)	**1e** (*S*)	Ac	80
7	**4** [β-$OC(NH)CCl_3$]	TMSOTf (0)	**1f** (*O*)	Cbz	75



Scheme 2. Synthesis of β-D-galactofuranosyl trichloroacetimidate **4**.

fore on furanosides as previous groups have preferentially used thiophenyl furanosides as intermediates.

On the assumption that furanoses are more reactive than pyranoses, regioselectivity was ensured by limiting reaction time to 1 h. Under these conditions, the corresponding hemiacetal was isolated in 38% yield at best. Beyond this time, mixture was observed. Further conversion into the desired trichloroacetimidate **4** was achieved by reaction with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in 88% yield.

As expected, trichloroacetimidate **4** proved to be the best donor and allowed us to readily glycosylate cysteine under common TMSOTf activation in 80% yield (Table 2, entry 6).[31]

As for the introduction of serine, *t*-butyloxycarbonyl protection turned out to be not compatible with the acidic conditions employed. Gratifyingly, *N*-benzylcarbamate serine methyl ester was a good acceptor and reacted with the various donors **2** (Table 2, entry 2), **3** (entries 4 and 5) and **4** (entry 7). Interestingly, a weaker Lewis acid, copper(II) triflate was required with thioimidate **3**. These results illustrated the importance of the Lewis acid nature for glycosylation reactions.[21] Optimized yields were obtained once again with trichloroacetimidate **4** to give *O*-galactofuranosyl serine in 75% yield.

3. Conclusion

To summarize, new galactofuranosyl amino acids building blocks were synthesized starting from readily available per-O-acetylated and pyrimidoyl donors. It included galactofuranosyl serine and a small library of alkyl 1-thio-galactofuranosides. The incorporation of cysteine proved to be more challenging and forced us to use the more reactive trichloroacetimidate donor. Further studies to conjugate hexofuranosyl amino acids and analogues onto more complex biomolecules are presently under investigation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.01.001.

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31. *Typical procedure for S-furanosylation of cysteine*: A mixture of trichloroacetimidate donor **4** (40 mg, 81 μmol), *N*-acetyl-L-cysteine methyl ester (11 mg, 62 μmol) and 4 Å molecular sieves in CH_2Cl_2 (2 mL) were stirred at RT for 10 min. Then after cooling to 0 °C, trimethylsilyltriflate (4 μL, 20 μmol) was added and the reaction was monitored by TLC (cyclohexane/AcOEt 1:1). After 1 h stirring, no starting material remained. So the mixture was neutralized with Et_3N (0.5 mL), filtered through a small pad of Celite and evaporated under vacuum. The crude oil obtained was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 99:1) to give **1e** (25 mg, 79%) as colourless oil. $[\alpha]_D^{20} = -9$ (*c* 1, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.44 (d, 1H, *J* = 7.4 Hz, NH), 5.36 (td, 1H, *J* = 7.2, 4.3 Hz, H-5), 5.30 (d, 1H, *J* = 2.0 Hz, H-1), 5.05–5.01 (m, 2H, H-2, H-3), 4.88 (td, 1H, *J* = 7.4, 5.0 Hz, C*H*CH_2S), 4.30 (dd, 1H, *J* = 12.0, 4.3 Hz, H-6), 4.35–4.32 (m, 1H, H-4), 4.18 (dd, 1H, *J* = 12.0, 7.2 Hz, H-6′), 3.77 (s, 3H, CO_2CH_3), 3.18 (dd, 1H, *J* = 14.1, 5.0 Hz, CH_2S), 3.04 (dd, 1H, *J* = 14.1, 5.0 Hz, CH_2S), 2.14, 2.11, 2.08, 2.06, 2.04 (5s, 15H, COCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.6, 170.03, 169.99, 169.85, 169.8 (CO), 88.6 (C-1), 81.8 (C-2), 79.8 (C-4), 76.4 (C-3), 69.1 (C-5), 62.5 (C-6), 52.8 (CO_2CH_3), 51.9 (*C*HCH_2S), 33.2 (CH*C*H_2S), 23.0 (NHCO*C*H_3), 20.8, 20.7, 20.6 (CO*C*H_3). HRMS (ESI): calcd for $C_{20}H_{29}O_{12}NSNa$ [M+Na]$^+$ 530.1308, found 530.1401.