Green Chemistry

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: A. Cardona Benages, O. Boutureira, S. Castillon, Y. Diaz and M. I. Matheu, *Green Chem.*, 2017, DOI: 10.1039/C7GC00722A.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/green-chem

View Article Online
DOI: 10.1039/C7GC00722A

Journal Name

ARTICLE

Targeting a Glycosylation: Metal-free and VOC-free Synthesis of *O*-Glycosides in Supercritical CO_2

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Adrià Cardona,[a] Omar Boutureira,[a] Sergio Castillón,[a] Yolanda Díaz,*[a] and M.Isabel Matheu*[a]

Supercritical carbon dioxide ($scCO_2$) is a suitable medium to perform transition metal-free glycosylations in the absence of volatile organic solvents (VOCs) using glycosyl halides as glycosyl donors. The methodology here described can be applied for obtaining *O*-glycosides in a totally green reaction, as well as orthoesters, depending on the reaction conditions. The process is much more sensitive to temperature changes than to pressure modification, glycosyl chlorides requiring higher temperatures to be activated than glycosyl bromides. Pivaloyl groups act as good CO_2-philic units and showed to be the best choice to obtain good stereoselectivities. The relevance of the fluid nature and supercritical conditions was also evidenced.

Introduction

Glycoconjugates have become of special interest in recent years because of the vital role that carbohydrates play in biological recognition processes, such as host-pathogen interaction, cell adhesion, and development, among others.[1,2] The key step in the synthesis of glycoconjugates is the glycosylation reaction, which links a carbohydrate or oligosaccharide with a lipid or a protein.[3]

Since the establishment of its foundations by Michael[4] and Fischer,[5] a large number of glycosylation methods have been described.[6] Nevertheless, the complexity and diversity of glycoconjugates (branched structures, different anomeric configurations, several possible regioisomers, variety of monosaccharides) in relation to the relatively simple sequences of other biopolymers (proteins and nucleic acids) demands the use of efficient synthetic methods and limits automated methodologies.

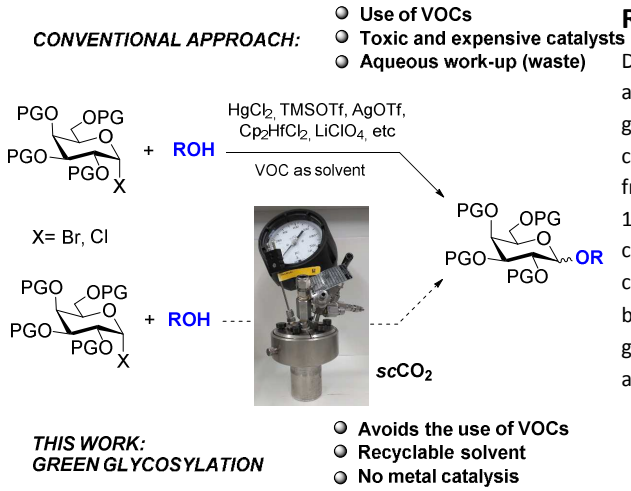
All elements contributing to the glycosylation reaction affect the incidence and selectivity of the process: glycosyl acceptor and donor, protecting and leaving groups, promoter, solvent, temperature, etc. However, the conjunction leaving group/activator-promoter is a key issue for the success of the reaction and hence, there have been continuous efforts to develop novel leaving groups and new promoter/catalyst pairs in order to enhance glycosylation efficiency. In most of the cases, strong Brönsted or Lewis acids, alkylating agents, metallic salts or transition metal complexes are required. This fact implies the elimination of such promoters at the end of the reaction (Scheme 1).

In spite of the increasing interest in green chemical processes, few efforts have been made for obtaining glycoconjugates through a green glycosylation reaction.[7] In contrast, main promoters for this reaction are toxic, expensive and often light- and moisture-sensitive,[8] especially when glycosyl halides are used as donors. Another important aspect in the research towards more sustainable processes in chemical synthesis is the replacement of volatile organic compounds (VOCs) as solvents.[9] However, in the glycosylation reaction, solvents play a critical role in terms of stabilizing the corresponding intermediates, and in the α/β selectivity of the product. Thus, the choice of solvent is a strategic parameter in glycosylation reactions and, in this regard, "green" solvents have been scarcely used, an organic solvent being usually the first and only choice.

[a] *Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, C/ Marcel.lí Domingo 1, 43007, Tarragona, Spain.*

Electronic Supplementary Information (ESI) available. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C7GC00722A



Scheme 1. Conventional approach for the glycosylation from glycosyl halides and our proposal using *sc*CO_2

In this respect, supercritical carbon dioxide (*sc*CO_2) has emerged as an attractive non-toxic, low cost, abundant and easy to recycle green solvent.[10] Furthermore, *sc*CO_2 is easily accessed (*Tc*=31.1°C, *Pc*=72.9 atm, 1071,33 Psi)[11] and can be removed after reaction by simple depressurization. For these reasons, the interest of using this solvent has increased exponentially in recent years.[12]

scCO_2 was considered as a nonpolar solvent mainly because of its low dielectric constant and zero molecular dipole moment. However, it has been claimed that its charge separation and significant bond dipoles producing a quadrupole allows it to act as a weak Lewis acid or Lewis base.[10,13] This microscopic view explains the significant site-specific solute-solvent interactions that it can establish, underscoring the polar nature of this solvent. Indeed, *sc*CO_2 can solubilize nonpolar and polar compounds. On the other hand, many biologically interesting products, such as carbohydrates, are highly polar and tend to exhibit low solubility in *sc*CO_2. However, acetylation of hydroxy groups has been shown to be an efficient strategy to increase solubility in CO_2.[14] This fact points out the possible use of *sc*CO_2 as a promising solvent in glycosylation reactions. In this regard, Hinou and Nishimura described an efficient sulphated-zirconia-promoted glycosylation starting from acetylated sugars in *sc*CO_2.[15]

Furthermore, it has been recently described that *sc*CO_2 promotes the heterolysis of carbon-halogen bonds in aromatic systems with good results in Friedel-Crafts type reactions,[16] which was justified by the cluster effect of *sc*CO_2 in polar solute molecules.[13,17,18] Taking into account that the heterolysis of an activated glycosidic bond is the initial step for the glycosylation reaction and considering that, similarly to Friedel-Craft reactions, a cation is the most common intermediate in this reaction, we hypothesized that the glycosylation reaction starting from glycosyl halides could be performed in *sc*CO_2 and, more interestingly, in the absence of a promoter, additionally avoiding the use of VOCs (Scheme 1).

Results and discussion

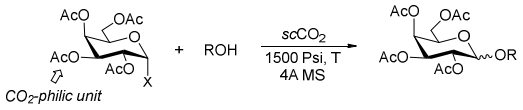
Due to the reported solubility of polyacetylated systems in *sc*CO_2, and the fact that D-galactose is a common carbohydrate found in glycoconjugates, tetra-*O*-acetyl-α-D-galactopyranosyl halides were chosen as glycosyl donors for this study. Preliminary experiments from benzyl alcohol with different galactosyl halides (Cl, Br, I) at 1500 Psi and temperatures ranging between 40 and 90 °C led to the corresponding *O*-glycosides with varying degree of success, the corresponding chlorides and bromides being optimal in terms of balance between reactivity/practical handling. Penta-*O*-acetyl-β-D-galactopyranose was also explored as a glycosyl donor but all attempts working at 1500 Psi led to the recovery of unaltered

View Article Online
DOI: 10.1039/C7GC00722A

starting material, despite using temperatures as high as 100 °C. Thus, the glycosylation conditions were optimised using tetra-*O*-acetyl-α-D-galactopyranosyl bromide **1** and tetra-*O*-acetyl-α-D-galactopyranosyl chloride **2** as glycosyl donors as well as benzyl alcohol (**a**) and cyclohexanol (**b**) as acceptors. Results are summarised in Table 1.

The glycosylation of benzyl alcohol (**a**) with tetra-*O*-acetyl-α-D-galactopyranosyl bromide **1** using a **1**:**a** molar ratio of 1:4 showed that the reaction required minimum pressure and temperature values of 1500 Psi and 60 °C, respectively, to proceed (Table 1, entries 1–2 *vs.*3). Lowering the excess acceptor concentration to 1.4 equiv resulted in almost the same conversion and glycoside yield (Table 1, entry 6), which could be especially important when high value acceptors are concerned. Shorter reaction times were detrimental for the yield and conversion (Table 1, entry 6 *vs.* entries 4, 5). In some cases (results not shown), variable amounts of the lactol or the glycoside unprotected at position C-2 were obtained, which were reduced adding 4Å MS to the reaction mixture.

At first sight, experiments from less reactive donor **2** were anticipated to be more challenging and therefore cyclohexanol, a more demanding acceptor, was used as a model for the reaction condition optimization. Unlike benzyl alcohol, the use of 1.4 equiv of cyclohexanol resulted in incomplete conversion (results not shown), so that, for the sake of exploratory purposes, reactions



1 X = Br **a** R = Bn **3a** R = Bn
2 X = Cl **b** R = Cy **3b** R = Cy

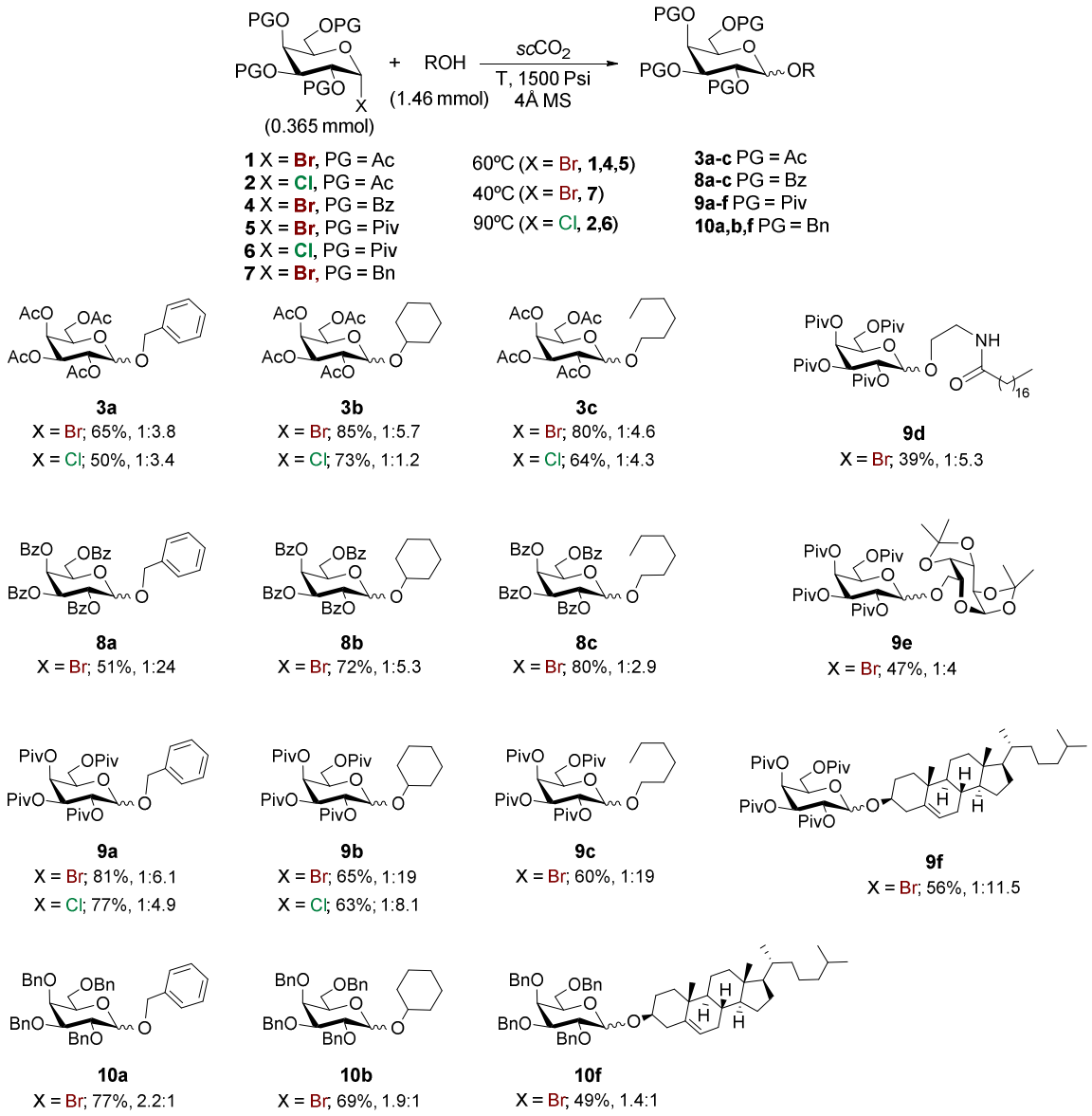
entry[a]	glycosyl donor	alcohol	T (°C)	ROH (equiv.)	t (h)	conv.(%)[b]	yield (%)[c]
1[d]	1	a	40	4	3	n.r.[e]	-
2	1	a	40	4	14	n.r.	-
3	1	a	60	4	14	>98	63
4	1	a	60	1.4	3	77	40
5	1	a	60	1.4	5	89	49
6	1	a	60	1.4	14	95	58
7	2	b	60	4	14	12	n.d.
8[f]	2	b	60	4	14	12	n.d.
9	2	b	60	4	110	30	n.d.
10	2	b	75	4	14	15	n.d.
11	2	b	80	4	14	20	n.d.
12	2	b	100	4	14	>98[g]	n.d.
13	2	b	90	4	14	73	n.d.
14	2	b	90	4	24	>95[h]	73

[a] Galactosyl bromide **1** or **2** (0.365 mmol) and benzyl alcohol (**a**) or cyclohexanol (**b**) were allowed to react in the presence of 4Å MS (ca. 100 mg) in a stainlees steel reactor which was filled with CO_2 until 1500 Psi, unless stated. [b] Conversion was determined by ^{1}H NMR spectroscopy. [c] Isolated yields. [d] The reaction was performed at P = 1200 Psi [e] no reaction. [f] The reaction was performed at P = 3300 Psi. [g] Complex mixture. [h] α/β ratio = 1:1.2.

Table 1. Optimizing conditions for glycosylation in $scCO_2$

further explored with **2** used 4 equiv of the acceptor. Under the optimized conditions set for galactosyl bromide **1** (Table 1, entry 3), reaction of galactosyl chloride **2** led to a poor 12% conversion (Table 1, entry 7),

View Article Online
DOI: 10.1039/C7GC00722A



Scheme 2. Scope of the glycosylation in *sc*CO_2. Conversions of the galactosyl donor are > 98% except for **8a** (88%). Isolated yield and α/β ratio values are shown.

which did not improve by increasing the pressure (Table 1, entry 8). As expected, the reaction from **2** proved slower than that from glycosyl bromide **1** and after 110 h only 30% conversion was achieved (Table 1, entry 9). As already observed with **1**, the reaction is much more sensitive to temperature than to pressure modification.

Thus, galactosyl chloride **2** required higher temperatures to be activated in *sc*CO_2 than its bromide counterpart, the optimal conditions being 90 °C and 1500 Psi (Table 1, entry 14 *vs.* entries 10–13). These results open the way for exploring the possibility of performing orthogonal glycosylations in *sc*CO_2 in the future.

Encouraged by these results, the study was also extended to differently acylated galactosyl bromides **1**, **4**, **5**, and chlorides **2**, **6** and glycosyl acceptors **a–c** (Scheme 2). Taking into account that the progress of the reaction cannot be monitored when working with a pressure reactor and that we were in an exploratory stage, we decided to use 4 equiv of the acceptor, essentially to guarantee full conversion. Tetraacetylated galactosyl bromide **1** afforded moderately good isolated yields of glycosides **3a–c** (65–85 %), with moderate α/β selectivities, which were similar in all acceptors (Scheme 2).

Reactions from benzoyl- and pivaloyl-protected galactosyl bromides **4** and **5** required longer reaction times. For practical reasons the reaction mixtures were allowed to stir for 24 h to

View Article Online
DOI: 10.1039/C7GC00722A

guarantee full conversion. Nevertheless, control experiments from **5** and **b** showed that the reaction can proceed at shorter reaction times (12 h).

Higher temperatures (75–85°C) led to lower yields and stereoselectivities due to decomposition of the galactosyl donor (results not shown). Results from benzoylated galactosyl donor **4** (Scheme 2) to afford glycosides **8b** and **8c** were parallel to those obtained from **1**, except for benzyl alcohol, which rendered **8a** in modest yield but excellent stereoselectivity (1:24 α/β ratio).The better stereoselectivities (from 1:6.1 to 1:19 α/β ratio) were achieved by using the more hindered tetra-*O*-pivaloyl protected galactosyl bromide **5**.

In order to explore the preparation of higher amounts of material, a five-fold more concentrated reaction using **5** as the glycosyl donor with cyclohexanol (**b**) as the acceptor was performed. The reaction proceeded with full conversion leading to glycoside **9b** in similar yields (62%) although with a slight decrease in stereoselectivity (1:9 α/β ratio) compared to the experiment driven in lower concentration (Scheme 2). Stereoselectivity dependence on the concentration has already been observed and could be explained by supramolecular aggregation in the reaction mixture leading to supramers.[19]

Acetyl- and pivaloyl-protected galactosyl chlorides **2** and **6** were treated with benzyl alcohol, cyclohexanol and 1-hexanol, under the optimized glycosylation conditions for glycosyl chlorides to afford the corresponding glycosides **3a–c** and **9a–b**, respectively in moderate to good yields (50–77 %). The stereoselectivities were comparable to those obtained from the galactosyl bromide **1**, with the exception of cyclohexyl tetra-*O*-acetyl-D-galactopyranoside **3b**, which led to almost null stereoselectivity. In the reactions performed from glycosyl chloride **2** the stereoselectivity decreases when the bulkiness or the basicity of the alcohol acceptor increases.

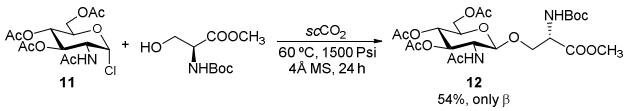
In the light of the results obtained from the differently protected galactosyl donors **1**, **2**, **4**, **5** and **6,** the ester groups tested in this study appear to be good CO_2-philic units.

A selection of interesting acceptors such as the lipidic amidoalcohol **d**, the protected carbohydrate **e** and cholesterol (**f**) were glycosylated with **5** to afford glycolipid **9d**, disaccharide **9e**, and chloresteryl galactoside **9f** in yields ranging from 39% to 56% and α/β selectivities from 1:11.5 (**9f**) to 1:4 (**9e**).

In general, appreciable deviations of isolated yield values with respect of full conversions are observed. The decrease in yield is explained by the formation of the corresponding lactol as a secondary product. The most significant divergences were obtained when less reactive glycosyl acceptors were employed (reactions leading to **9d**-**f**), where 1,3,4,6-tetra-O-pivaloyl-α-D-galactopyranose was also observed as a side product in the crude spectra.[20]

The reaction with the more activated ether-protected glycosyl donor **7** leading to compounds **10a**, **10b**, **10f** proceeded at lower temperature (40 °C) and lower reaction times (3 h) except for **10f**, which required 12 h to be complete, as expected from the lower reactivity of cholesterol. Worthy to note the good yields (ca 70%) obtained from these highly reactive galactosyl donors, except for **10f**, which was obtained in moderate 49% yield due to the competitive formation of the lactol as a secondary product. This result is in line with the lower yields typically reported for cholesterol compared with more simple acceptors. In these cases the α-anomer was the major product obtained, as expected.

Our methodology also proved efficient for the challenging glycosylation of a protected serine derivative with commercially



Scheme 3. GlcNAcylation of a serine derivative in *sc*CO_2.

available 2-acetamidoglucosyl chloride **11**, which afforded *O*-glycoside **12** in 54% yield as the sole anomer together with a glycal side-product. Milder conditions were required in this case due to the higher reactivity of glycosyl donor **11** (Scheme 3).

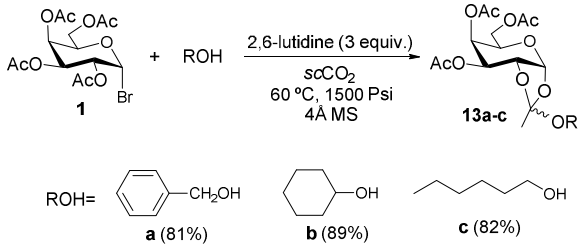
As shown, all experiments undertaken in this study (Scheme 2) led to the formation of variable amounts of the α-glycoside as a minor product as well as the expected β anomer, despite the fact that glycosyl donors with participating groups at position 2 were used. The α anomer could directly arise from the trapping of the oxonium intermediate.[21] Alternatively, its formation could also be explained by the trapping of the acetoxonium ion to give the β anomer followed by a HBr-promoted anomerization process.[22]

To address the origin of the α/β stereoselectivity, the reaction of donor **1** with acceptor **a** was driven in the presence of excess lutidine as an acid scavenger.[23] Under these conditions, no glycoside was observed but instead, ortho-ester **13a** was exclusively obtained in 81% yield. Similar results were obtained from acceptors **b** and **c** (Scheme 4). These experiments open the door to the use of this

View Article Online
DOI: 10.1039/C7GC00722A

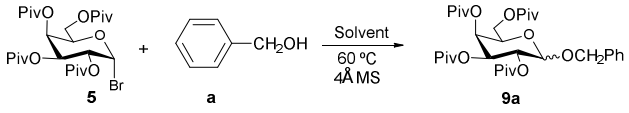
methodology for the synthesis of orthoesters, which are valuable starting materials in oligosaccharide synthesis.[23, 24]

Still, some issues remained incertain. Does the α/β mixture arise from direct trapping of the oxonium/acetoxonium intermediate or from subsequent HBr-promoted anomerization? Conducting experiments at diferent reaction times would indeed shed some light on this issue. A set of experiments with galactosyl donor **1** and cyclohexanol under the optimized conditions at 2 h, 5 h and 14 h led to conversion–(α:β) values of 50%–(1:5.7), 89%–(1:6.1) and >98%–(1:4.3), respectively. Obtaining essentially the same α/β ratio regarless the conversion would suggest that the α anomer results from direct trapping of the onium intermediate, and it is not generated in a subsequent anomerization.



Scheme 4. Orthoester synthesis in *sc*CO_2.

Table 2. Solvent effect in the glycosylation reaction of **a** with **5**.



entry	solvent	time (h)	container	heating source	conv. (%)[a]
1[b]	*sc*CO_2 (1500 Psi)	20	Reactor	Heat	> 95
2[c]	Toluene	20	Schlenk tube	Heat	< 1
3[c]	DCM	20	Schlenk tube	Heat	< 1
4[b]	THF	20	Schlenk tube	Heat	< 1
5[c]	C_6F_6	20	Schlenk tube	Heat	< 1
6[c]	CH_3CN	20	Schlenk tube	Heat	8
7[c]	Toluene	2	Microwave tube	Microwave irradiation	< 1
8[b]	DCM (atm. P)	20	Reactor	Heat	< 1

[a] Conversion was determined by ^{1}H NMR spectroscopy. [b] Glycosylation with galactosyl donor **5** (0.365 mmol, 1 equiv., 15 mM), glycosyl acceptor **a** (1.46 mmol, 4 equiv., 60 mM) and ca. 100 mg of 4Å MS in a 25 mL stainless steel reactor (25 mL Parr reactor). [c] Reaction in 2 mL of solvent with glycosyl donor (0.03 mmol, 1 equiv., 15 mM), glycosyl acceptor (0.12 mmol, 4 equiv., 60 mM) and ca. 10 mg of 4Å MS in a closed schlenk tube.

Furthermore, when cyclohexyl tetra-*O*-acetyl-β-D-galactopyranoside (obtained by purification of the previous α/β 1:5.7 mixture, Scheme 2) was left to stir in *sc*CO_2 under the standard conditions (1500 Psi and 60 °C) for 14 h, intact β-galactoside was recovered after depressurization, ruling out an anomerization process promoted by the supercritical fluid.

Moreover, the presence of molecular sieves has been described as a neutral acid scavenger in some reactions and, in particular, in the glycosylation reaction and, in this respect, could alter the stereoselectivity outcome of the reaction.[25] In our hands, the use of molecular sieves did not produce a change in the stereoselectivity of the glycosylation but, as aforementioned, proved beneficial minimizing the formation of hydrolysis byproducts.

To discard that the activation of galactosyl halides might be exclusively due to a temperature effect, a set of different assays were conducted from galactosyl bromide **5** without any promoter, at the optimal work temperature (60 °C) in the presence of 4Å MS, and different solvents and reaction vessels (Table 2). In the reactions carried out in a Schlenk tube, starting materials were exclusively recovered (Table 2, entries 2–5) regardless the solvent used, except for acetonitrile, which led to 8% conversion (Table 2, entry 6). Note that hexafluorobenzene, a solvent with a quadrupolar moment similar to CO_2,[26] did not give any conversion (Table 2, entry 5). Microwave irradiation did not produce any glycosylated product either (Table 2, entry 7).

The fact that metallic traces coming from the inner wall of the stainless steel reactor might catalyse the glycosylation was also discarded, since no conversion was observed when the reaction was run in CH_2Cl_2 at 60 °C and atmospheric pressure in the reactor (Table 2, entry 8).

Figure 1. (a) Glycosyl donor **5** at atmospheric pressure and r.t.; (b) Glycosyl donor **5** and cyclohexanol at atmospheric pressure and r.t.; (c) Glycosyl donor **5** and cyclohexanol in *sc*CO_2 (1500 Psi, 60 °C) in a reactor with quartz windows.

All these results when compared with those obtained for the same reaction in *sc*CO_2 (Table 2, entry 1) enabled us to underline the unique role of *sc*CO_2, beyond an exclusive thermal effect.

Additional evidence for the activating role of *sc*CO_2 was obtained from a crossover experiment between tetra-*O*-pivaloyl-α-D-galactopyranosyl bromide (**5**) and tetra-*O*-acetyl-α-D-galactopyranosyl chloride (**2**), which were placed together in the reactor at 90 °C and 1500 Psi in the absence of a glycosyl acceptor (see Electronic Supplementary Information (ESI) for experimental details). The crude mixture revealed the presence of three anomeric signals between 6.7 and 6.3 ppm, two of which

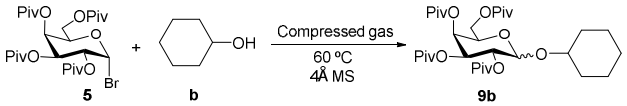
View Article Online
DOI: 10.1039/C7GC00722A

corresponded to starting material **5** and the newly formed tetra-*O*-pivaloyl-α-D-galactopyranosyl chloride (**6**), thus accounting for a halogen exchange process. No traces of tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) were detected in the crude spectrum. Furthermore, the presence of a third anomeric signal might be due to the formation of a partially deacetylated galactosyl donor, although it was not identified.

Solubility of starting materials in the supercritical fluid is a key issue in this process. To shed some light on this regard we performed the reaction by physically separating the galactosyl donor from the acceptor within the reactor vessel. Thus, 2,3,4,6-tetra-*O*-pivaloyl-α-D-galactopyranosyl bromide (**5**) was introduced in a vial placed inside the reactor and the glycosyl acceptor (cyclohexanol) was introduced outside the vial in the same reactor. After 24 h at 60 °C and 1500 Psi (without stirring), NMR analysis of the final vial content showed the presence of some unreacted glycosyl donor with a large amount of product **9b**. A similar situation was found outside the vial where no unreacted glycosyl acceptor but only product **9b**, was present. The experiment was repeated in the absence of the acceptor and introducing only the glycosyl donor **5** into the vial. After 24 h under supercritical conditions, compound **5** was uniformly distributed throughout the reactor.

The use of a reactor equipped with quartz windows led to an indisputable experimental piece of evidence for the solubility of the reactants under *sc*CO_2. Figure 1 shows the change in physical state of the starting material when submitted to *sc*CO_2 conditions. Thus, the mixture of glycosyl donor **5** and cyclohexanol appears as a homogeneous solution in *sc*CO_2 (Figure 1, c) whereas donor **5** alone (Figure 1, a) or the mixture of **5** and cyclohexanol (Figure 1, b) are displayed as heterogeneous at normal conditions.

Table 3. Influence of the fluid nature and supercritical conditions in the glycosylation.



5 **b** **9b**

entry	compressed gas	t (h)	container	P	conv. (%)[a]	α:β ratio[b]
1[c]	*sc*CO_2	24	Reactor	1500 Psi	>95	1:19
2[c]	*sc*Ar	24	Reactor	1500 Psi	25	1.9:1
3[c]	CO_2	24	Reactor	700 Psi	36	1:1.1
4[d]	none	24	Schlenk	atm. P	15	1:10.1
5[c]	none	24	Reactor	atm. P	40	1:1.1

[a] Conversion was determined by ^{1}H NMR spectroscopy. [b] The α:β ratio was determined by ^{1}H NMR spectroscopy. [c] Galactosyl donor (0.365 mmol, 1 equiv.), glycosyl acceptor (1.46 mmol, 4 equiv.) and ca. 100 mg of 4Å MS in a 25 mL stainless steel reactor (25 mL Parr reactor). [d] Galactosyl donor (0.365 mmol, 1 equiv.), glycosyl acceptor (1.46 mmol, 4 equiv.) and ca. 100 mg of 4Å MS in a schlenk tube.

Furthermore, the nature of the supercritical fluid was also evaluated (Table 3). Thus, reaction of 2,3,4,6-tetra-*O*-pivaloyl-α-D-galactopyranosyl bromide **5** and cyclohexanol in Ar under the optimal conditions (1500 Psi, 60 °C) set for *sc*CO_2, proceeded with low conversion and preferential formation of the α anomer (Table 3, entry 2). In fact, the starting mixture showed to be heterogeneous (see images in ESI). Moreover, when the reactants were physically separed inside the reactor vessel and the reaction was conducted in Ar, each starting material remained in its original location after careful depressurization. Although under these conditions, Ar is a supercritical fluid (Tc=150.87 K, Pc=48.26 atm= 710.39 Psi)[27] it does not appear to dissolve the starting materials.

Reaction in *sc*CO_2 (Table 3, entry 1) proved far more superior than that run either in *sc*Ar (Table 3, entry 2) or subcritical CO_2 (Table 3, entry 3), which proceeded with poor conversions and lower stereoselectivities. In addition, reactions in neat conditions led to low conversion (Table 3, entries 4–5), where formation of the product could be accounted for the solvolysis of the galactosyl donor by the own glycosyl acceptor.

During the preparation of this manuscript, a study by Leitner and Reetz was published, concluding that the use of *sc*CO_2 had no activating effect on alkylating reactions through ionization of potentially S_N1-active alkyl halides.[28] Far from questioning the accuracy of this study, we believe that the differences between the systems studied by Leitner/Reetz and also by González-Nuñez (C-C bond formation in Friedel-Crafts and enol ether alkylation) and ours (C-O bond formation in a glycosylation reaction) could tell the difference. The acylated carbohydrates used as substrates in this study are much more complex substrates and present multiple basic sites, potentially able to interact with *sc*CO_2, thus enhancing possible clustering effects with respect to simple hydrocarbon substrates. Be as it may, we would like to present our contribution to the scientific community with the hope that accumulating experimental pieces of evidence will lead us to a more global knowledge about the nature and properties of *sc*CO_2.

Conclusions

The synthesis of *O*-glycosides in *sc*CO_2 has been developed, avoiding the use of VOCs as solvents and in the absence of transition metal as activators.

The best results in terms of glycoside yield and stereoselectivity were obtained using pivaloyl-protected galactosyl bromides working in *sc*CO_2 at 60 °C and 1500 Psi. The scope of the reaction suggests that not only acetyl groups, but also benzoyl and pivaloyl groups can act as CO_2-philic units.

The use of excess lutidine as an acid scavenger biased the reaction outcome towards the formation of the orthoester products in good yields, thus expanding the synthetic methodology for the preparation of this important type of glycosyl donors. Exclusive thermal glycosylation as well as an acid-promoted equilibration of glycoside products have been discarded. The efficiency of *sc*CO_2 over *sc*Ar was also evidenced.

View Article Online
DOI: 10.1039/C7GC00722A

Although this study has focused in beta-glycosylation and the strongly solvent-dependent formation of alpha-glycosides may be anticipated to be more challenging, this reaction protocol is a proof of concept that glycosylation can become a green process.

Acknowledgements

We are indebted to Dr. Anna M. Masdeu-Bultó (URV) for her kind disposition to share her pressure facilities with us. We gratefully acknowledge Prof Walter Leitner at the RWTH Aachen University as well as Prof M. Elena González-Nuñez at the Universitat de València for their helpful discussions. Financial support by the Ministerio de Economia y Competividad, Spain (CTQ2014-58664-R and RYC-2015-17705) and the European Regional Development Fund are gratefully acknowledged. A.C. thanks URV for a predoctoral fellowship. O.B. is a Ramón y Cajal Fellow.

Notes and references

1 L.-X. Wang, B. G. Davis, *Chem. Sci.*, 2013, **4**, 3381; C. R. Bertozzi, L. L. Kiessling, *Science*, 2001, **291**, 2357; R. W. Dwek, *Chem. Rev.*, 1996, **96**, 683.
2 M.C. Galán, D. Benito-Alifonso, G.M. Watt, *Org. Biomol. Chem.* 2011, **9**, 3598.
3 M. A. Wolfert, G.-J. Boons, *Nature Chem. Biol.* 2013, **9**, 776; M. Dalziel, M. Crispin, C. N. Scanlan, N. Zitzmann, R. A. Dwek, *Science* 2014, **343**, 37.
4 A. Michael, *Am. Chem. J.*, 1879, **1**, 305.
5 Fischer, E. *Ber. Dtsch. Chem. Ges.*, 1893, **26**, 2400.
6 B. Yu, J. Sun, X. Yang, *Acc. Chem. Res*. 2012, **45**, 1227; X. Zhu, R.R. Schmidt, *Angew. Chem. Int. Ed.* 2009, **48**, 1900; A.V Demchenko, Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance, Weinheim: Wiley-VCH, 2008
7 K. Sasaki, D. Takahashi, K. Toshima, *Ionic liquids as Green Solvents for Glycosylation Reactions*, Chapter 3 in *Green Solvents II. Properties and applications of Ionic Liquids*, A. Mohammad and Inamuddin, eds, Springer: Dordrecht, 2012; Glycosylation in ionic liquids and acid resin: F.J. Muñoz, S. André, H.-J. Gabius, J.V. Sinisterra, M.J. Hernáiz and R.J. Linhardt, *Green Chem*. 2009, **11**, 373; C. Li, Z. Zhang, Q. Duan and X. Li, *Org. Lett.* 2014, **16**, 3008; I.J. Talisman, V. Kumar, J. Razzaghy, S.V. Malhotra, *Carbohydr. Res*. 2011, **346**, 883; K. Prasad Sethi, K.P. R. Kartha, *Carbohydr. Res*. 2016, **434**, 132; Nagai, K. Sasaki, S. Matsumura and K. Toshima, *Carbohydr. Res.* 2005, **340**, 337; B.K. Gorityala, J. Ma, K. K. Pasunooti, S. Cai and X.-W. Liu, *Green Chem*. 2011, **13**, 573; H. Komatsu and H. Umetani, *Org. Process Res. Dev.* 2002, **6**, 847–850; R.S. Thombal, V.H. Jadhav, *J. Carbohydr. Chem*. 2016, **0**, 1; B. Roy and B. Mukhopadhyay, *Tetrahedron Lett*. 2007, **48**, 3783; H. Hinou, N Saito, T. Maeda, M. Matsuda, Y. Kamiya, S.-I. Nishimura, *J. Carbohydr. Chem.* 2011, **30**, 575.
8 Wang, Z. *Comprehensive Organic Name Reactions and Reagents* ;John Wiley & Sons: Hoboken 2009; pp 1650.
9 D. Adam, *Nature* 2000, **407**, 938.
10 E. J. Beckman, *J. Supercrit. Fluids* 2004, **28**, 121;. W. Leitner. *Nature* 2000, **405**, 129; W. Leitner (ed), P. G. Jessop (ed), *Handbook of Green Chemistry*, *Green Solvents,* Volume 4, *Supercritical Solvents*, Weinheim: Wiley-VCH, 2010
11 E. Ramsey, Q. Sun, Z. Zhang, C. Zhang, W. Gou, *J. Environ. Sci.* 2009, **21**, 720.
12 Examples of reactions in *sc*CO_2: synthesis of tetrasubstituted olefins via palladium catalysts: H.-F. Jiang, Q.-X. Xu, A.-Z. Wang, *J. Supercrit. Fluids*, 2009, **49**, 377; asymmetric Diels-Alder reactions: S. Fukuzawa, K. Metoki, S. Esumi, *Tetrahedron*, 2003, **59**, 10445; olefin metathesis: M. Selva, A. Perosa, M. Fabris, P. Canton, *Green Chem.* 2009, **11**, 229; F. Michalek, D. Mädge, J. Rühe, W. Bannwarth, *Eur. J. Org. Chem.* 2006, 577.; A. Fürstner, L. Ackermann, K. Beck, H. Hori, D. Koch, K. Langemann, M. Liebl, Ch. Six, W. Leitner, *J. Am. Chem. Soc.* 2001, **123**, 9000; oxidation of alcohols: R. Ciriminna, P. Hesemann, J. J. E. Moreau, M. Carraro, S. Campestrini, M. Pagliaro, *Chem. Eur. J*. 2006, **12**, 5220; M. E. González-Núñez, R. Mello, A. Olmos, R. Acerete, G. Asensio, *J. Org. Chem*. 2006, **71**, 1039; formation of esters: M. Rezayat, H. S. Ghaziaskar, *Green Chem.* 2009, **11**, 710; Friedel-Crafts alkylation: I. Komoto, S. Kobayashi, *Org. Lett*. 2002, **4**, 1115; aldol- and Mannich-type reactions: I. Komoto, S. Kobayashi, *J. Org. Chem.* 2004, **69**, 680; the Baylis-Hillman reaction: P. M. Rose, A. A. Clifford, C. M. Rayner, *Chem. Commun.,* 2002, 968; nitroaldol condensations R. Ballini, M. Noè, A. Perosa, M. Selva *J. Org. Chem*. 2008, **73**, 8520; azide-alkyne cycloaddition: B. Grignard, S. Schmeits, R. Riva, C. Detrembleur, P. Lecomte, Christine Jérôme, *Green Chem.*, 2009, **11**, 1525; formation of cyclic carbonates: H.-F. Jiang, A-Z. Wang, H.-L. Liu, C.-R Qi, *Eur. J. Org. Chem.* 2008, 2309; Y. Kayaki, M. Yamamoto, T. Ikariya *J. Org. Chem.* 2007, **72**, 647; J.-Q. Wang, D.-L. Kong, J.-Y. Chen, F. Cai, L.-N. He, *J. Mol. Catal. A: Chem*. 2006, **249**, 143; carbamates and ureas: M. J. Fuchter, C. J. Smith, M. W. S. Tsang, A. Boyer, S. Saubern, J. H. Ryan, A. B. Holmes, *Chem. Commun*. 2008, 2152.
13 P. Raveendran, Y. Ikushima, S. Wallen, *Acc. Res. Chem*. 2005, **38**, 478; E. J. Beckman, J. L. Reynolds, J. A. Gardecki, S. J. V. Frankland, M. L. Horng, M. Maroncelli, *J. Phys. Chem.* 1996, **100**, 10337; J. F. Kauffman, *J. Phys. Chem. A*, 2001, **105**, 3433.
14 P. Raveendran, S. L. Wallen, *J. Am. Chem. Soc.* 2002, **124**, 7274.
15 X.-B. Li, M. Ogawa, T. Monden, T. Maeda, E. Yamashita, M. Naka, M. Matsuda, H. Hinou, S.-I. Nishimura, *Angew. Chem. Int. Ed.* 2006, **45**, 5652; H. Hinou, N. Saito, M. Ogawa, T. Maeda, S.-I. Nishimura, *Int. J. Mol. Sci.* 2009, **10**, 5285.
16 T. Delgado-Abad, J. Martínez-Ferrer, A. Caballero, A. Olmos, R. Mello, M. E. González-Núñez, P. J. Pérez, G. Asensio, *Angew. Chem. Int. Ed.* 2013, **52**, 13298; Delgado-Abad, T.; Martínez-Ferrer, J.; Reig-López, J.; Mello, R.; Acerete, R.; Asensio, G.; González-Núñez, M.E. *RSC Adv.* 2014, **4**, 51016.
17 S-G. Kazarian, M. F. Vincent, F. V. Bright, C. L. Liotta, C. A. Eckert, *J. Am. Chem. Soc.* 1996, **118**, 1729; J. F. Brennecke, J. E. Chateauneuf, *Chem. Rev.* 1999, **99**, 433; M. Saharay, S. Balasubramanian, *J. Phys. Chem. B*, 2007, **111**, 387.
18 D. W. Arnold, S. E. Bradforth, E. H. Kim, D. M. Neumark, *J. Chem. Phys.* 1995, **102**, 3493.
19 L. O. Kononov, N. N. Malysheva, A. V. Orlova, A. I. Zinin, T. V. Laptinskaya, E. G. Kononova, and N. G. Kolotyrkina, *Eur. J. Org. Chem*. 2012, 1926.
20 Additionally, downward bias of the isolated yields due to the setup of the high-pressure equipment leading to small systematic mass loss could not be discarded.
21 L. Bohe, D. Crich, *Carbohydr. Res.* 2015, **403**, 48.
22 W. Pilgrim, P. V. Murphy, *J. Org. Chem*. 2010, **75**, 6747; C. O'Brien, M. Kolápová, N. Pitt, M. Tosin, P. V. Murphy, *Chem. Eur. J.* 2007, **13**, 902. G. Lanz, R. Madsen. *Eur. J. Org. Chem*. 2016, 3119.
23 J. Lu, B. Fraser-Reid, *Org. Lett.* 2004, **6**, 3051; K. V. Radhakrishnan, V. S. Sajisha, J. M. Chacko, *Synlett* 2005, **6**, 997.
24 N.K. Kochetkov, A.J. Khorlin, A.F. Bochkov, *Tetrahedron* 1967, **23**, 693. F. Kong, *Carbohydr. Res.* 2007, **342**, 345.; B. Fraser-Reid, S. Grimme, M. Piacenza, M. Mach. U. Schlueter, *Chem. Eur. J.* 2003, **9**, 4687. N. M. Podvalnyy, S. L. Sedinkin, P. I.

View Article Online
DOI: 10.1039/C7GC00722A

Abronina, A. I. Zinin, K. G. Fedina, V. I. Torgov, L. O. Kononov, *Carbohydr. Res*. 2011, **346**, 7. G. Sureshkumar and S. Hotha, *Tetrahedron Lett*. 2007, **48**, 6564.

25 A. Padwa, J.D. Ginn, S.K. Bur, C.K. Eidell, S. M Lynch, *J. Org. Chem*. 2002, **67**, 3412; H.P. Wessel, N. Ruiz, *J. Carbohydr. Chem*. 1991, **10**, 901.

26 Vrbancich, J.; Ritchie, G.L. *J. Chem. Soc. Faraday Trans 2*, 1980, **76**, 648.

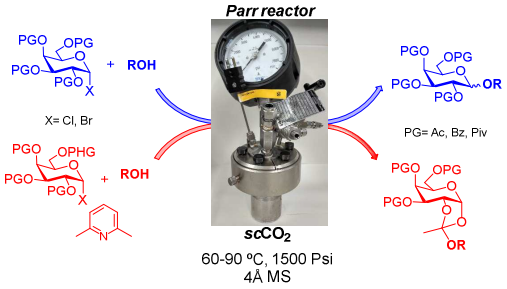
27 Haynes M. W. (2011). *Handbook of Chemistry and Physics* (92nd ed.). CRC Press, Boca Raton, Florida, USA, pp. 4121

28 Y.X Qiao, N. Theyssen,T. Eifert, M.A. Liauw, G. Franciò, K. Schenk, W. Leitner, M.T. Reetz, *Chem. Eur. J*. 2017, **23**, 3898.

View Article Online
DOI: 10.1039/C7GC00722A

Targeting a Glycosylation: Metal-free and VOC-free Synthesis of *O*-Glycosides in Supercritical CO_2

Adrià Cardona,[a] Omar Boutureira,[a] Sergio Castillón,[a] Yolanda Díaz,*[a] and M.Isabel Matheu *[a]



The feasibility of using *sc*CO_2 for obtaining glycoconjugates has been demonstrated, performing metal–free glycosylations without VOCs as solvents.