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COMMUNICATION

Chemoselectivity in Self-Promoted Glycosylation: *N*- versus *O*-Glycosylation.

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Abstract: Self-promoted glycosylation using trichloroacetimidates and sulfonamides have recently been developed. In this communication, we study the parameters controlling the chemoselectivity between a nucleophilic sulfonamide nitrogen and an alcohol, both contained in the same molecule. The influence of solvent polarity and concentrations have been studied, and it has been revealed how the chemoselectivity, and to some extend the stereoselectivity, can be controlled. The experimental results furthermore give insight into the reaction mechanism of this self-promoted glycosylation and the mechanism of glycosylation reactions in general.

The glycosylation reaction is the most studied reaction in carbohydrate chemistry.[1-4] It involves a glycosyl donor, which must be activated and acts as electrophile, and a glycosyl acceptor which plays the role as the nucleophile.[5] Based on the kind of acceptor involved, different types of glycosyl bonds can be formed.[6-11] Among them, the C-O bond and the C-N bond are of particular interest, as they are ubiquitous in Nature and hence have received much attention.[12-14] *O*-glycosides are omnipresent with different functions and relevance from a biological point of view.[5] *N*-glycosylation is crucial in post-translational modifications, where monosaccharides are connected to proteins via a glycosyl amide bond linkage.[13,15] Other biologically active *N*-glycosides, like *N*-glycosyl sulfonamides, have recently gained attention. These can be obtained via a palette of reactions e.g. synthetized from addition of sulfamides to acetyl-protected glycals (via Ferrier rearrangement) or from monosaccharides, both in the presence of boron trifluoride etherate.[16,17] Some of these compounds have been found to be carbonic anhydrase (CA) inhibitors.[17-19] The same biological activity has been shown by *O*-glycosides containing aromatic sulfonamide residues.[20-22] A different synthetic pathway was developed for this type of compounds where a 1,3-dipolar cycloaddition reaction was used in order to generate 1,4-disubstituted 1,2,3-triazole glycoconjugate sulfonamides from alkyne-substituted sugars and azido aromatic sulfonamides.[20,21] Due to the general importance of *N*-glycosides, the interest in studying the "Glycome" as well as drug properties of small molecules, glycosyl sulfonamides and the development of new simple, selective and mild methods are highly important.

Recently, we reported that sulfonamides react with trichloroacetimidates (TCAs) in a stereospecific and self-promoted manner forming *N*-glycosides (Figure 1). [19] The acidity

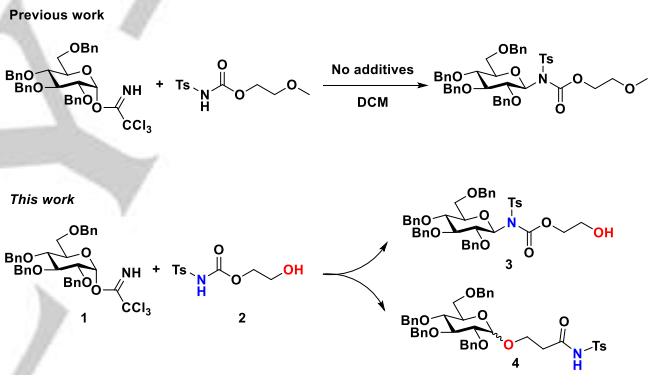
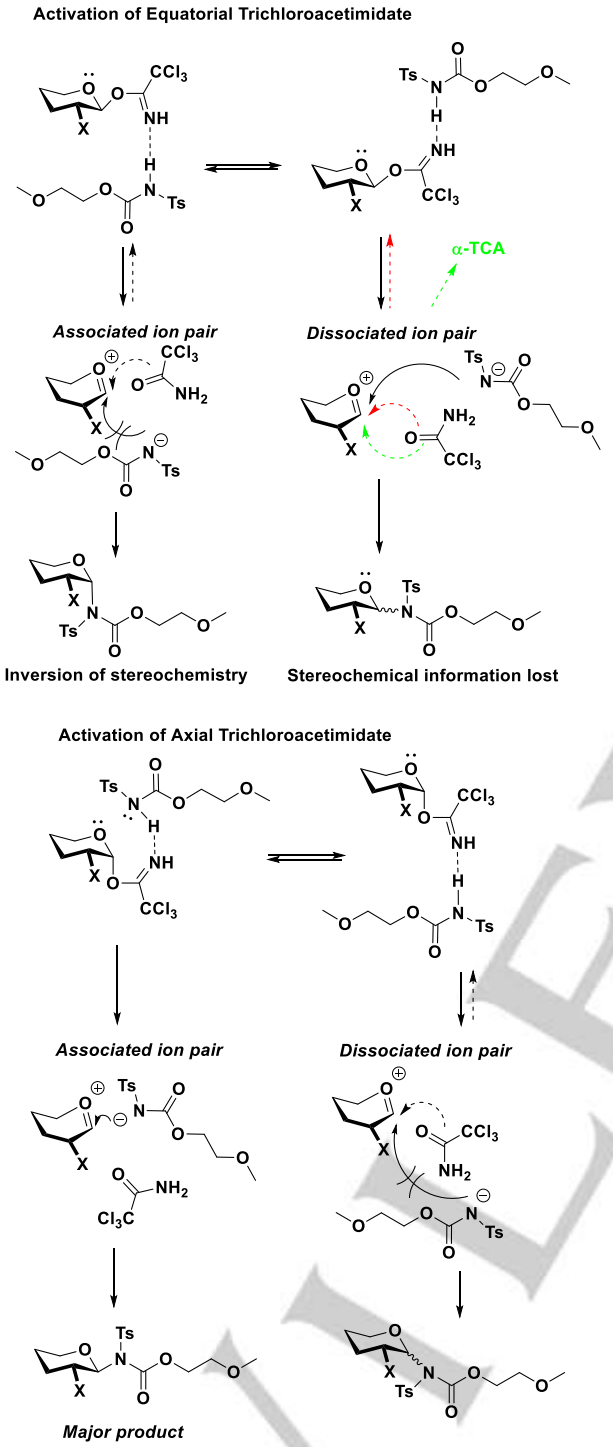


Figure 1 Self-promoted glycosylations. Previous work was focused on self-promoted *N*-glycosylation.[19] This work studies the chemoselectivity when having two nucleophiles in the same molecule – high-lighted in red and blue.

of the sulfonamide functionality makes them able to activate TCA donors with a consecutive nucleophilic attack to the glycosyl cation (Scheme 1) i.e. they act as both catalysts and nucleophiles (glycosyl acceptors). The reactions have been found to be highly stereospecific, which is very useful as TCAs often can be synthesized in a stereoselective manner.[23] One can thereby determine the *N*-glycoside stereochemistry already when synthesizing the glycosyl donor. In our preceding work, it was also realized that the degree of stereospecificity was dependent on the anomeric configuration. Axial TCAs often resulted in high equatorial selectivity, whereas the opposite was not always the case. This difference in selectivity, when using the preferred solvent for glycosylations (DCM), was explained by two mechanistic pathways (Scheme 1). The activation of the glycosyl donor can either lead to the formation of an associated or dissociated ion pair. When the TCA is axially oriented, the leaving group departure and nucleophilic attack are more aligned and follows a more associated IP (Ion Pair) mechanism resulting in shielding one site and hence nucleophilic attack from the opposite site of the glycosyl cation. When β-TCAs are activated and

substituted a more dissociative reaction path is followed and the nucleophilic attack is sterically hindered by the leaving group, as well as the substituents of the pyranose ring (Scheme 1). Consequently, this can lead to *in situ* anomerisation (Scheme 1 in green and red), resulting in formation of the α-TCA, with a consecutive loss of stereochemical information from the glycosyl donor and hence a mixture of products.[19] Contact and solvent-separated ion pairs are on the borderline between a S_N1 and a S_N2 mechanism. Small changes in the reaction condition can therefore shift the balance to either site. Important parameters in this is e.g. the polarity of the solvent and its ability to promote a charge separation.[3,24,25] The dissociative reaction path allows two competing reaction pathways resulting in low anomeric selectivity. In this work, we study the reaction mechanism in self-promoted glycosylations and how it is influenced by the reaction conditions.



Scheme 1 Proposed mechanism for self-promoted N-glycosylation reactions. Axial TCAs results in a more associative mechanism and hence inversion of stereochemistry. Equatorial TCAs on the other hand follows a more dissociative resulting in less stereospecificity and anomerisation of the TCA.

Trichloroacetimidates are one of the most used glycosyl donor types. They are prepared under basic conditions, starting from the hemiacetal. Depending on the conditions, mainly the α- or β-anomer can be obtained.[19,23] This study primary involves the thermodynamically more stable axial TCA (α) as this will give raise to the equatorial product in the glycosylation and has a greater stability.

As a model glycosyl donor the perbenzylated glucopyranosyl trichloroacetimidate **1** was synthesized and as a model glycosyl acceptor 2-hydroxyethyl tosylcarbamate **2**.[19] This acceptor contains both a primary alcohol and a sulfonamide, but only the later can activate the TCA. Our hypothesis is that a mechanism proceeding through an associative mechanism leads primary to the *N*-glycoside with high stereospecificity, whereas a more dissociative pathway gives more of the *O*-glycoside and this with lower anomeric selectivity. As the reaction is self-promoted only the two reactants and solvent are present, hence ruling out effects of metals ions, counter ions and consequently reduce the complexity significantly.

Initially, the reactions were performed in DCM as this is the most commonly used solvent for glycosylations. This was done on a preparative scale in order to separate the closely eluting reaction products, which were to be used as references for the determination of product ratios from crude-NMR. The isolated yields (34% for *N*-glycoside and 40% for *O*-glycoside) were found to be in accordance with the ratio obtained from crude NMR.

Glycosylating **2** gave, as expected, a mixture of *O*- and *N*-glycosides. The *N*-glycosylation was very selective towards the β-product **3** in line with earlier work (associative mechanism).[19] The *O*-glycosides **4** on the other hand were found to be a 1:1 mixture suggesting a reaction under diffusion control and presumably through a highly reactive glycosyl cation (dissociative mechanism). With DCM as a reference point, other solvents were then used to study the effects of polarity and solvent properties. Interestingly, a change in the chemoselectivity was observed going from the least polar solvent in the study, toluene (entry 1, Table 1) to the most polar $MeNO_2$ (entry 4, Table 1). The difference in ratio is going from very *N*-selective to *O*-selective, but without a change in the anomeric selectivity of the *O*-glycosides, which also seems unaffected when using a participating solvent like THF (See SI for details). Using MeCN (not shown) results in the formation of a *N*-glycosyl acetimidate product, confirming the participation of acetonitrile and in line with earlier work.[19]

Table 1. Effect of the increasing polarity of the solvent.

Entry	**1** (M)	**2** (M)	Solv.	*N*- vs *O*-glycosides
1[a]	0.073	0.110	PhMe	87:13
2	0.073	0.110	DCM	46:54
3	0.073	0.110	THF	56:44
4	0.073	0.110	$MeNO_2$	0:100

The donor and acceptor were dissolved in the solvent at rt. and the reaction progress was followed by TLC [a] Performed at 40˚C. See SI for more detail and anomeric ratios of the *O*-glycosides.

When increasing the charge separation by using more polar solvents, the hydroxyl group competes with the amide being the negatively charged counter-ion. In more polar solvents, the reaction is consequently shifted towards the *O*-glycosylation (dissociative mechanism). In less polar solvent like DCM there is less charge separation and the reaction results in both *N*- and *O*-glycosides.

With the reaction on the borderline between mechanistic pathways it was expected that the concentration would also influence the reaction outcome. Therefore, the change of concentration of the sulfonamide was studied in both toluene and DCM (Table 2). Increasing the concentration of the acceptor without changing the concentration of **1** resulted in a decrease in *N*-selectivity in DCM (table 2). This change in reaction outcome can be explained by a second molecule of **2** attacking the dissociated IP resulting in more *O*-glycoside. This also explains why only a small excess of **2** is sufficient to get a change in the chemoselectivity (entry 7 and 8, Table 2).

Table 2. Effect of changing the concentration of sulphonamide **2**.

Entry	**1** (M)	**2** (M)	Solv.	N- vs O-glycosides
1[a]	0.073	0.0146	PhMe	100:0
2[a]	0.073	0.049	PhMe	85:15
3[a]	0.073	0.110	PhMe	87:13
4[a]	0.073	0.367	PhMe	47:53
5	0.073	0.0146	DCM	70:30
6	0.073	0.049	DCM	70:30
7	0.073	0.091	DCM	60:40
8	0.073	0.110	DCM	46:54
9	0.073	0.367	DCM	14:86

The donor and acceptor were dissolved in the solvent at rt. and the reaction progress was followed by TLC [a] Performed at 40˚C. See SI for more detail and anomeric ratios of the *O*-glycosides.

A different trend was observed in toluene (entry 3, Table 2). The low polarity of the solvent resulted in a decrease of the rate of reaction and therefore the reaction temperature was increased to 40 °C in order to be complete within 48 hours. The increase of temperature has no effect on the ratio between *N*- and *O*-glycosylation (entry 1 and 2, Table 3). Because of the lower solvent polarity the ion pairs are tighter and the reaction with the alcohol is less competitive. The slow conversion of the sulfonamide to its dissociated form combined with the activation mode, based on a pre-complexation, ensures the presence of **2** upon activation, but also an intermolecular competition from the free alcohol giving the *O*-glycosylation (Scheme 1), even if it is not predominant (entry 2 and 3, Table 2). Sub-stoichiometric amounts of the acceptor is therefore required in order to get a more selective *N*-glycosylation as a bimolecular (H-bond complex) reaction is favored and a termolecular reaction unlikely (entry 1, Table 2).

A rearrangement of α-TCA donor can compete in the reaction (Scheme 1), producing the glucosyl trichloroacetamide as side product, both with high and low concentration of **2** (see SI).

The participation of a second molecule **2** was also supported by the results obtained when increasing the concentration of the reaction both in toluene and DCM (Table 3), where more of the *O*-glycoside formed.

Table 3. Effect of changing the concentration of the reaction keeping the ratios.

Entry	**1** (M)	**2** (M)	Solv.	*N*- vs *O*-glycosides
1[a]	0.146	0.220	PhMe	83:17
2[a]	0.073	0.110	PhMe	87:13
3[a]	0.0146	0.022	PhMe	75:25
4	0.146	0.220	DCM	27:73
5	0.073	0.110	DCM	46:54
6	0.0146	0.022	DCM	56:44

The donor and acceptor were dissolved in the solvent at rt. and the reaction progress was followed by TLC [a] Performed at 40˚C. See SI for more detail and anomeric ratios of the *O*-glycosides.

To study the effect of other counter ions, in the self-promoted glycosylation, lithium salts were added. It has earlier been demonstrated that counter-ions influences the outcome of glycosylations and also that the Lewis acidity of Li^+ is enough to activate a TCA. [26,27] Using **1**, 20 mol% of TfOLi or Tf_2NLi and **2** in DCM completely changed the chemoselectivity and only the *O*-glycoside was observed (Table 4). Thus, the salt can change the reaction course of the self-promoted chemoselective glycosylation resembling a common acid catalyzed *O*-glycosylation. [26] The *O*-glycoside **4** was obtained as a 1:1 mixture as the major product suggesting a dissociated mechanism under diffusion control.

When performing the same reaction, without sulfonamide present, but with EtOH as the glycosyl acceptor, a similar stereoselectivity was obtained, suggesting that the sulfonamide is not participating in the activation when lithium salts are present (Table 5).

As described above, β-TCA donors often gives rise to a mixture of anomers. When mixing **5** and **2** in DCM, a 40:60 mixture of α- and β-*N*-glycosides was obtained confirming that the reaction proceeds through a less associated reaction pathway and therefore gives lower selectivity (Scheme 1). Furthermore, it was noticed that the α-*N*-glycosides decomposed on the silica column biasing the product ratio and yield.

Table 4 Effect of adding litium salts.

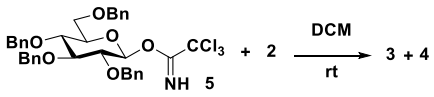
Entry	Cat.	Acceptor	N- vs O-glycosides
1	TfOLi	2	0:100
2	Tf_2NLi	2	0:100

The donor **1** (0.073 M) and acceptor (0.110 M) were dissolved in the solvent at rt. and the reaction progress was followed by TLC. See SI for more detail and anomeric ratios of the *O*-glycosides.

Table 5 Effect of adding litium salts.

Entry	Cat.	Acceptor	α : β product
3	TfOLi	EtOH	56:44
4	Tf_2NLi	EtOH	25:75
5	/	EtOH	/

The donor **1** (0.073 M) and acceptor (0.110 M) were dissolved in the solvent at rt. and the reaction progress was followed by TLC.



Scheme 3 Self-promoted glycosylation using the β-TCA **5** and **2**. A mixture of anomers of *N*-glycosides was obtained and the α-*N*-glycosides was found to be unstable upon purification.

In conclusion, we have demonstrated that the self-promoted *N*-glycosylation of sulfonamides takes place in a concerted manner and presumably through an activated H-bond complex. When increasing the ratio of the sulfonamide a competing reaction pathway involving another molecule of the sulfonamide results in the formation of *O*-glycosides. The same effect can be obtained by adding lithium salts, which acts as both acid and nucleophilic catalysts, forming an intermediate glycosyl triflate. Consequently, all stereochemical information is lost. As the reaction is on the borderline between mechanisms, solvents influence the outcome dramatically. Apolar solvents favors tight ion pair leading to an associative mechanism, i.e. S_Ni-type, whereas polar solvents leads to a dissociative mechanism closer to the S_N1 mechanism. By tuning the parameters one can control the chemoselectivity and to some extend the stereoselectivity.

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Keywords: Glycosylation • Self-promoted • Chemoselectivity • Stereoselectivity • N-Glycosides

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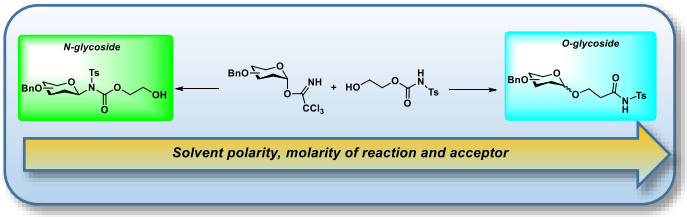

The chemo- and stereoselectivity of a self-promoted glycosylation is studied by changing solvents, molarity and ratio between the glycosyl donor and acceptor. The outcome of the reaction is found to be strongly dependent on these parameters and can to a large extend be controlled. S_Ni and S_N1 are suggested as to be the competing mechanistic pathways.

Key topic: Glycosylation • Self-promoted • Chemoselectivity • Stereoselectivity • N-Glycosides