Efficient Two Step β-Glycoside Synthesis from *N*-Acetyl D-Glucosamine: Scope and Limitations of Copper(II) Triflate-Catalyzed Glycosylation

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Abstract

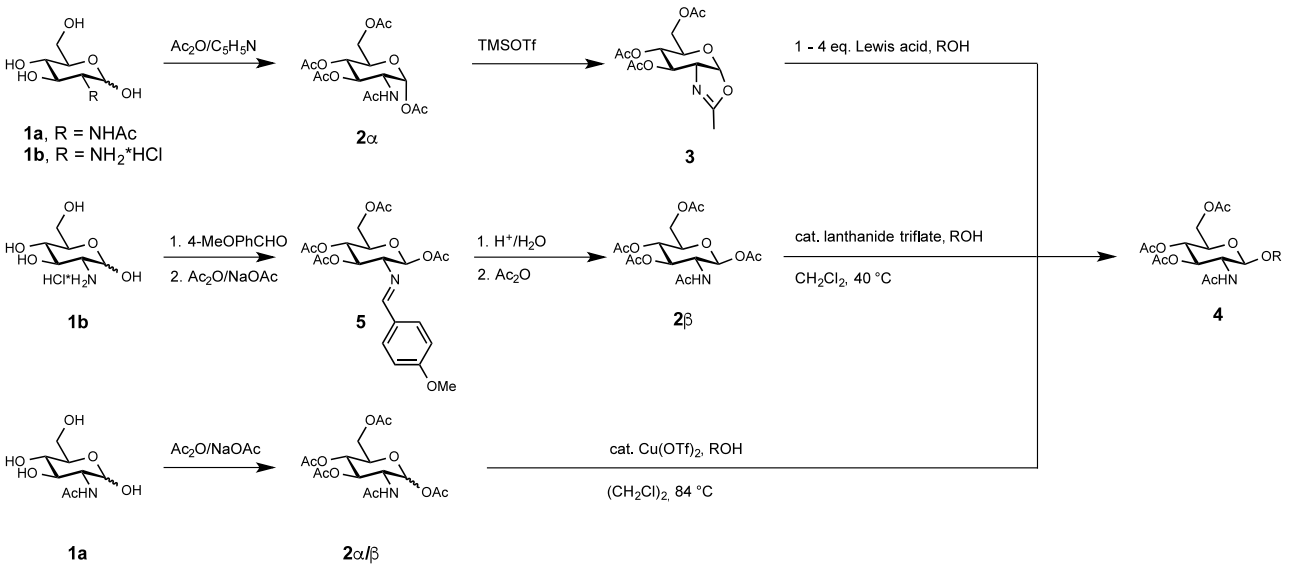
β-Linked glycosides of *N*-acetyl glucosamine are widespread in nature. Their direct synthesis is hampered by the low reactivity of GlcNAc as a glycosyl donor. We report a selective and rapid copper(II) triflate-catalyzed two-step synthesis of β-glycosides of GlcNAc from cheap GlcNAc as starting material without purification of intermediates. α-Specific glycosylation can be achieved by increasing the amount of catalyst and extending reaction times.

Introduction

β-Glycosides of *N*-acetyl D-glucosamine (GlcNAc) are widespread in nature and found, among others, in protein *N*- and *O*-glycosylation, in polysaccharides of bacterial and fungal cell walls, as well as in natural product glycosylation. The synthesis of β-linked glycoconjugates of *N*-acetyl glucosamine is challenging compared to hexoses with an acetylated 2-hydroxy group trans to the newly formed glycosidic bond.[1] The latter ones can often be easily accessed by glycosylation with the corresponding hexose pentaacetates.[2] For 2-deoxy 2-acetamido hexoses such as GlcNAc (**1a**) this is generally not the case. Therefore, a variety of activating groups and protecting groups for *N*-acetyl glucosamine donors have been developed to yield smooth glycosylation reactions, with the disadvantage of cumbersome steps for introducing and removing these functional groups.[1b] Kelly and Jensen, however, reported one case of a direct glycosylation of o-nitrobenzyl alcohol with both anomers of *N*-acetyl glucosamine tetraacetate (**2α**, **2β**) at high temperature or equimolar Lewis acid treatment,[3] and Aguilera *et al.* reported the β-selective glycosylation of benzyl alcohol with the readily accessible α-acetate **2α** using stoichiometric amounts of $SnCl_4$.[4] In general, the direct chemical synthesis of β-glycosides (**4**) of *N*-acetyl glucosamine is usually carried out *via* the more reactive oxazoline donor **3** and Lewis acid activation, *e.g.*, trimethylsilyl triflate[5] or transition metal salts[6] (Scheme 1). Oxazoline **3** is synthesized from *N*-acetyl D-glucosamine (**1a**) or more expensive D-glucosamine hydrochloride (**1b**) in 2 steps *via* tetraacetate **2α**. Jensen and Davis reported on a direct glycosylation with the β-acetate **2β** under mild conditions catalyzed by metal triflates.[7] In contrast to β-acetates of hexoses, which are easily obtained by irreversibly trapping the kinetic β-product with acetic anhydride at high temperatures, the β-selective synthesis of 2-deoxy 2-acetamido analogs (*e.g.*, **2β**) requires several chemical steps. **2β** is selectively obtained *via* the intermediate imine **5** after four

chemical steps from glucosamine **1b**.[7b,8] The more readily accessible α-anomer **2α** is unreactive under the mild conditions reported by Jensen and co-workers.[7b] However, using ytterbium triflate and harsh conditions (170 °C with microwave irradiation), benzyl alcohol could be glycosylated with an α/β-mixture (ratio 10/1) of **2**.[9] Recently, Beau and co-workers reported on the use of $Fe(OTf)_3$ as Lewis acid catalyst for glycosylation reactions with **2β**.[10] Attemps using **2α** as donor did not yield any product, even under harsh conditions (80 °C with microwave irradiation).

In the course of our research on cyanoethyl β-glycosides, we identified copper(II) triflate as suitable catalyst for a β-selective glycosylation with the readily accessible anomeric mixture of *N*-acetyl glucosamine tetraacetate (**2α/β**) as glycosyl donor.



Scheme 1: The synthesis of β-glycosides of *N*-acetyl glucosamine (**4**) is widely performed *via* the stable oxazoline **3** using excess amounts of Lewis acid. A soft method was developed[7b] using catalytic amounts of lanthanide triflates for glycosylation with **2β** which can be obtained in 4 chemical steps. In this study, readily accessible **2α/β** was used as glycosyl donor in a direct $Cu(OTf)_2$-catalyzed glycosylation reaction.

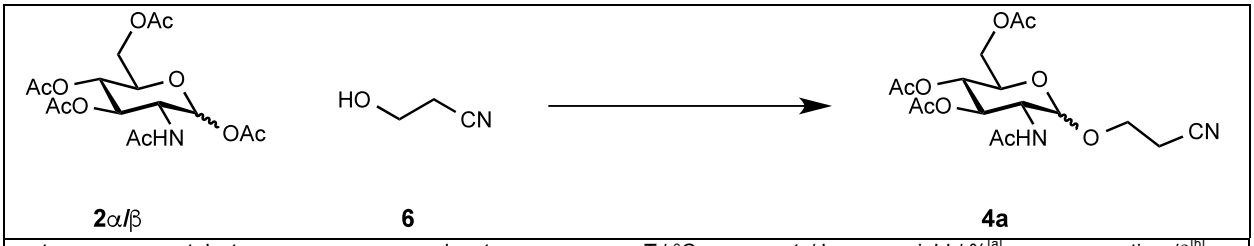
Results & Discussion

In general, glycosylation reactions proceed with moderate to good yields. In order to increase these yields, mid to large excess of valuable glycosyl donor is used. In the search for economic glycosylation conditions for β-glycosides of GlcNAc, we intended to use an anomeric mixture of *N*-acetyl glucosamine tetraacetate (**2α/β**) as glycosyl donor and excess of low cost acceptor alcohols. The donor **2α/β** was readily synthesized by acetylation of GlcNAc with acetic anhydride and sodium acetate as base at elevated temperatures (Scheme 1). After aqueous quenching of the reaction, **2α/β** (α/β 5:1) was obtained in 70% yield as precipitate without the requirement for additional chromatographic purification.

2α/β was then used in the screening for donor-efficient glycosylation conditions, *i.e.*, by using excess of acceptor alcohols. Neither classical boron trifluoride-catalyzed glycosylation conditions in absence (entry 1, Table 1) or in presence of molecular sieves (entry 2) nor lanthanum triflate-catalyzed conditions (entry 3) reported by Jensen and co-workers[7b] for the β-acetate resulted in any reaction. Exposure to harsher conditions using microwave irradiation at 80 °C was also unsuccessful (entry 4). Subsequently, we increased the reaction temperature by changing the solvent from dichloromethane to dichloroethane (DCE). However, attempts using lanthanum triflate in DCE resulted in gel formation of the reaction mixture without observable product formation (entry 5). In analogy to Christensen *et al.* for **2β**, we employed the transition metal catalyst copper(II) triflate in refluxing DCE for glycosylation of excess acceptor with **2α/β** and a smooth reaction to the β-glycoside in high yield and selectivity was observed (entry 6). Changing the solvent to

toluene or acetonitrile generally led to reduced β-selectivity at increased reaction rates (entries 7, 8).

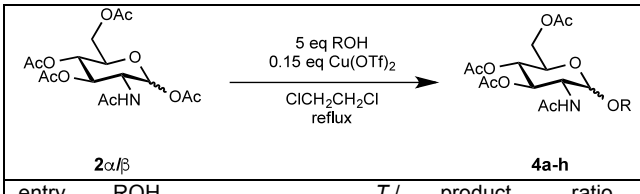
Table 1: Screening for reaction conditions for direct glycosylation of **2α/β** with acceptor **6**. [a]isolated yields of glycosides, [b]determined by ^{1}H-NMR spectroscopy.



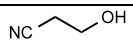
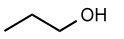
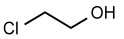
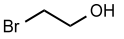
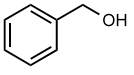
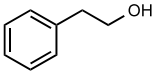
2α/β **6** **4a**

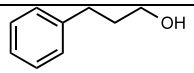
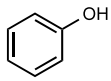
entry	catalyst	solvent	*T* / °C	*t* / h	yield / %[a]	ratio α/β[b]
1	BF_3*OEt_2, 4 eq.	CH_2Cl_2	0 - r.t.	18	-	
2	MS 3Å, BF_3*OEt_2, 5 eq.	CH_2Cl_2	0 - r.t	19	-	
3	$La(OTf)_3$, 0.15 eq.	CH_2Cl_2	40	48	-	
4	$La(OTf)_3$, 0.15 eq.	CH_2Cl_2	80 (μW)	0.5	-	
5	$La(OTf)_3$, 0.15 eq.	$(CH_2Cl)_2$	84	6	- (gel formed)	
6	$Cu(OTf)_2$, 0.15 eq.	$(CH_2Cl)_2$	84	49	71	1:8.1
7	$Cu(OTf)_2$, 0.15 eq.	MeCN	82	18	79	1:3.8
8	$Cu(OTf)_2$, 0.15 eq.	PhMe	111	18	60	1:2.7

Table 2: Copper(II) triflate-catalyzed direct glycosylation of **2α/β** with various acceptor alcohols. [a]isolated yields of glycosides, [b]determined from isolated yields of α- and β-glycoside, respectively.



2α/β **4a-h**

entry	ROH	*T* / h	product, yield / %[a]	ratio α/β[b]
1		48	**4a**, 69	1:14
2		22	**4b**, 54	1:1.4
3		20	**4c**, 57	1:2.8
4		26	**4d**, 45	1:3.5
5		7	**4e**, 42	1:8.6
6		26	**4f**, 62	1:2.1

7		6	**4g**, 38	1:8.2
8		9	**4h**, 27	1:1.7
9		6	**4i**, 27	1:1.5

The scope of the glycosylation conditions in DCE with copper(II) catalysis was analyzed by using a series of acceptor alcohols (Table 2). Electron-rich and electron-poor primary alcohols, as well as benzylic or secondary alcohols and phenol could be glycosylated in a β-selective manner. The degree of β-selectivity varied from 1.4- to 14-fold with the highest selectivity obtained for **4a**, *i.e.*, the acceptor with the strongest electron withdrawing properties. For some products we observed rapid anomerization by TLC and/or LCMS. The anomerization reaction of the rather stable β-glycoside **4a** to the thermodynamically more stable α-isomer was monitored by NMR spectroscopy over time (Figure 1). This anomerization is likely to proceed *via* acid catalysis, as reported for acetylated glucosides by Lindberg.[11] Addition of a sterically hindered pyrazine base (TTBP) for the glycosylation of phenol did not improve selectivity (data not shown).

Interestingly, a related α-selective glycosylation with **2α** or **2β** using $FeCl_3$ was reported by Wei *et al.*.[12] Except for the Lewis acid employed, the authors used similar reaction conditions to the ones reported here. Therefore, the observed anomeric α-selectivity is likely to result from differences in anomerization rates as a consequence of the more Lewis acidic iron(III) chloride. In addition, Wei *et al.* analyzed the effect of $FeCl_3$ on isolated β-glycosides and observed a rapid anomerization *via* an intermediate detected by TLC, which the authors assigned to oxazoline **3**.

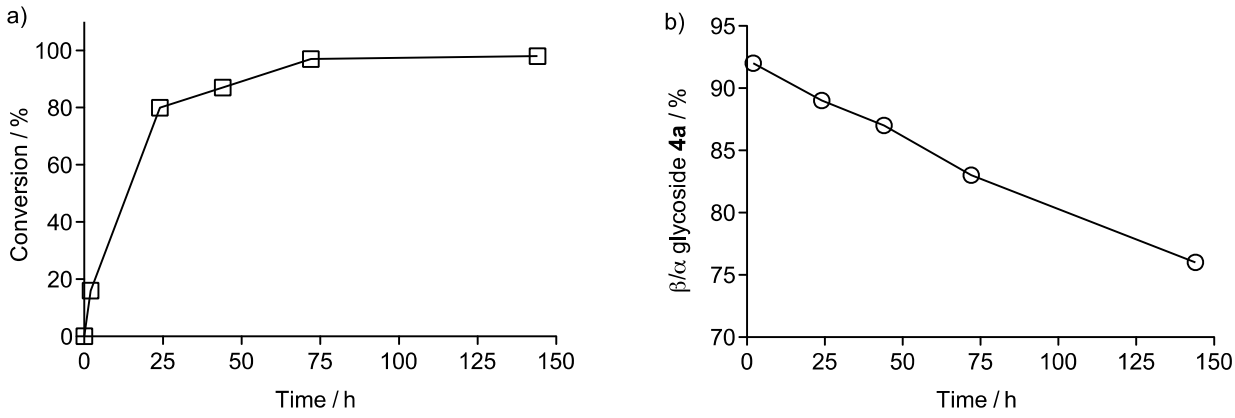


Figure 1: Reaction monitoring of glycosylation of **6** with **2α/β** to glycoside **4a** by ^{1}H-NMR spectroscopy over time. a) conversion of starting material to **4a**, b) ratio of β-glycoside **4a** in the product formed decreases due to anomerization.

In order to localize the limiting factor of the reaction we analyzed the anomerization of pure β-**4f** under different conditions in analogy to previous reports.[7b,13] β-Glycoside **4f** was exposed to glycosylation conditions in presence or in absence of $Cu(OTf)_2$ and/or acetic acid, which is one reaction product of this glycosylation reaction (Figure 2a). The pure β-anomer of glycoside **4f** was stable in DCE at 84 °C. Addition of 1 equivalent acetic acid was not sufficient to induce anomerization even under prolonged reaction times. In presence of the catalyst $Cu(OTf)_2$ the β-glycoside anomerized time dependent and formation of corresponding α-anomer was detected under prolonged reaction times. This anomerization promoting effect of $Cu(OTf)_2$ was further potentiated in the presence of acetic acid yielding 70% α-**4f** after 48 h. Thus, under the reaction conditions described, anomerization of a given glycoside can be expected after extended reaction times and the reaction must therefore be followed to optimize yields and selectivity. A similar trend for the anomerization was observed for three further β-glycosides (**4b**, **4e**, **4i**) when tested under

reaction conditions (Figure 2b). The pure β-acetate of **2** was included and also showed anomerization to the corresponding α-epimer (Figure 2b).

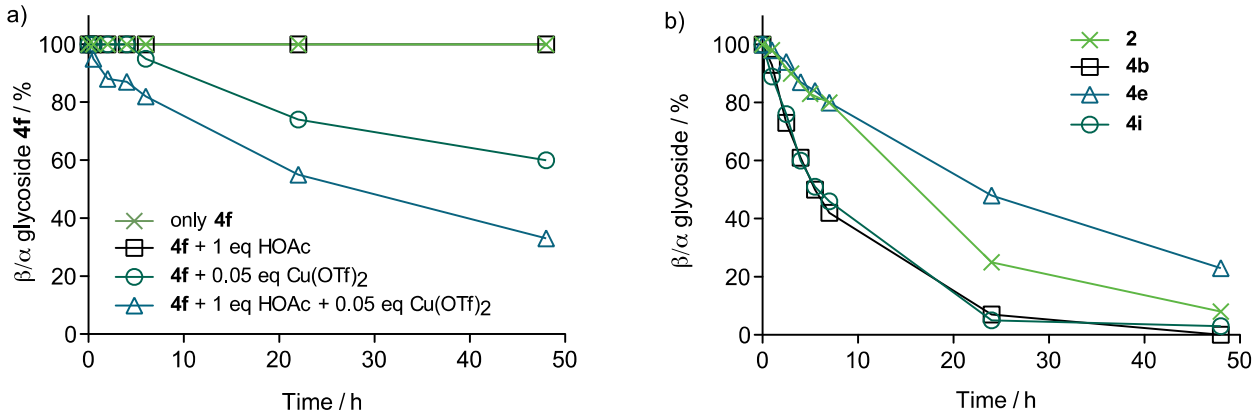


Figure 2: a) Anomerization of pure β glycoside **4f** under different conditions. b) Anomerization of pure β-glycosides **2,** and **4b, e, i** in presence of 0.05 eq $Cu(OTf)_2$ and 1 eq HOAc. All reactions were carried out in $ClCH_2CH_2Cl$ at 84 °C and the β/α-ratio was quantified by LCMS.

In order to further investigate the mechanism of this copper triflate-catalyzed glycosylation reaction, the reaction kinetics using different donors with 2-phenylethanol as acceptor were studied in a first step. Glycosylations using either pure **2β**, **2α**, or the mixture **2α/β** (5:1) as donors were performed (Figure 3a). While **2β** quantitatively reacted within 30 minutes to the phenethyl glycoside **4f**, the reaction times with pure **2α**, or the mixture **2α/β** were significantly prolonged (Figure 3b).

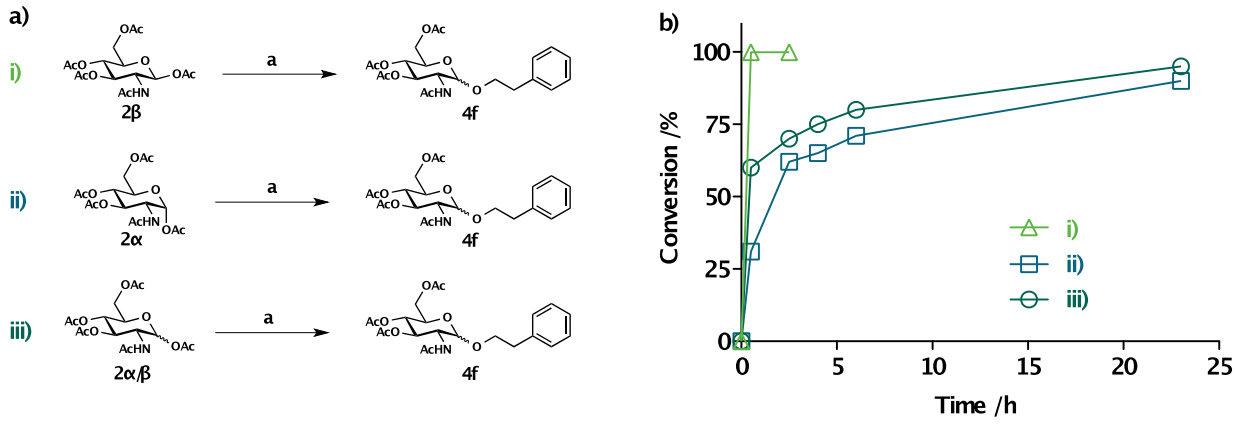


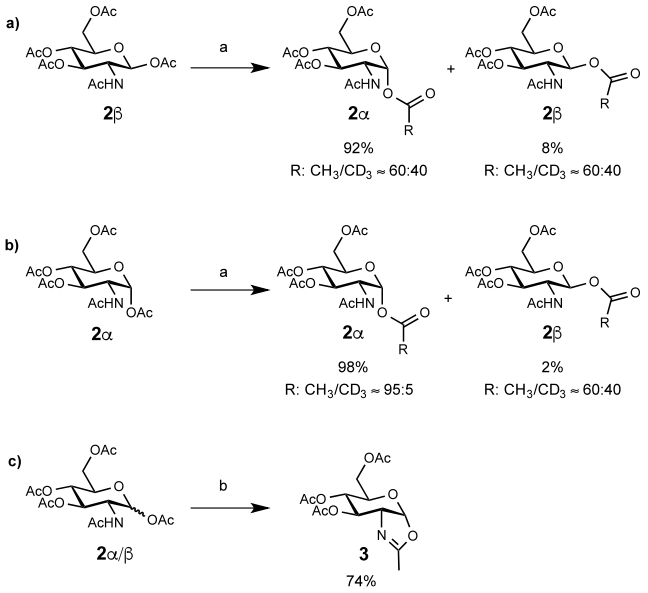
Figure 3: a) Glycosylation reaction using donors with varying anomeric configuration and 2-phenylethanol**.** i) pure **2β**; ii) pure **2α** and iii) **2α/β** (5:1) mixture. Reagents and conditions: (a) 0.05 eq $Cu(OTf)_2$, $ClCH_2CH_2Cl$, 5 eq $Ph(CH_2)_2OH$, 84 °C. b) Kinetics of the glycosylation reaction i), ii), and iii).

Acetic acid is formed as a reaction product in the glycosylation reactions, and was shown to contribute to the anomerization of the glycosides formed (Figure 2a). In order to analyze a possible first anomerization of the acetates before a subsequent glycosylation reaction, both anomers of **2** were studied under reaction conditions in absence of acceptor alcohols but in presence of equimolar amounts of isotopically labelled acetic acid (Scheme 2a, 2b). Using **2β** as donor, 92% anomerization to the α-epimer was observed and deuterated acetic acid was readily incorporated in ratios that corresponded to its isotopic availability. In contrast, **2α** was more stable and with only 2% epimerization and deuterated acetic acid was incorporated into **2α** only at basal level. These results further underpin the low reactivity of **2α** compared to **2β** and, at the same time, demonstrate that the glycosylation reaction takes place for **2α** without prior anomerization to the more reactive **2β**.

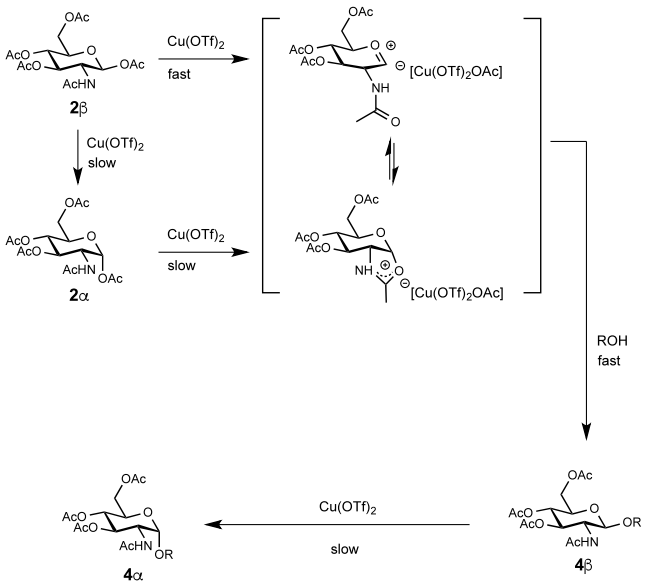
To get insight into the mechanism of this glycosylation reaction, we also performed high temperature ^{1}H- and ^{13}C-NMR in deuterated dichloroethane. The line width of the signals was broadened due to the presence of paramagnetic copper(II), resulting in a poorly resolved ^{1}H-NMR spectrum. Continuous monitoring of the reaction process did not reveal characteristic oxazoline signals, neither in ^{1}H-NMR spectra nor in ^{13}C-NMR spectra of superior resolution (data not shown). Attempts to trap reaction intermediates using hydride reagents, *e.g.* triethylsilane or sodium cyanoborohydride, were unsuccessful in our hands. However, exposure of **2α/β** to equimolar amounts of copper(II) triflate followed by basic work-up resulted in the isolation of oxazoline **3** in 74% yield (Scheme 2c). This was

indicative for the generation of an oxazolinium intermediate during the glycosylation reaction and further explains its β-selectivity (Scheme 3).

Interestingly, Wittmann and Lennartz previously reported that $Cu(OTf)_2$ does not catalyze glycosylation with oxazoline **3.**[6] Our results suggest that $Cu(OTf)_2$ activates donor **2** via an oxazolium intermediate, which then either reacts *in situ* with the acceptor alcohols to the corresponding glycosides, or can be trapped with base in absence of alcohols to give oxazoline **3**.



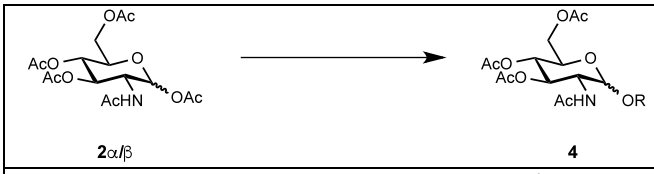
Scheme 2: Intermediates of the copper(II) triflate mediated glycosylation. Anomerization of *N*-acetyl glucosamine tetraacetate (**2**) under reaction conditions for a) **2α** and b) **2β** and incorporation of the deuterated acetic acid indicate an exocyclic anomerization mechanism and a direct glycosylation of **2α** due to absence of **2β** formation. c) Oxazoline donor **3** was isolated after basic treatment of the reaction mixture. Reagents and conditions: (a) 1 eq $DOOCCD_3$, 0.05 eq $Cu(OTf)_2$, $ClCH_2CH_2Cl$, 84 °C, 48 h; (b) 1.2 eq $Cu(OTf)_2$, $ClCH_2CH_2Cl$, 84 °C, 7 h; 2.8 eq Et_3N, r.t., 15 min.



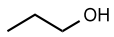
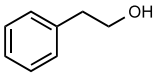
Scheme 3: Plausible mechanism of the copper(II) triflate mediated glycosylation.

Jensen and co-workers demonstrated the temperature dependence of the product anomerization during the glycosylation reaction.[13] These results and the mechanistic studies performed here indicate differences in reaction rates for donor activation, glycosylation and product anomerization as depicted in Scheme 3. As the limiting step was the formation of the oxazolinium intermediate from the poorly reactive α-acetate, our idea was thus to enhance the β-selectivity by accelerating donor activation with equimolar catalyst amounts and at high temperature followed by a shift to 40 °C prior to addition of the acceptor in order to prevent product anomerization. Reactions of **2α/β** with three different acceptors were then performed under two different reaction conditions (Table 3): A, both donor activation and glycosylation at high temperatures yielded high α-selectivity due to anomerization; whereas under condition B, the initial formation of the glycosylation intermediate at high temperatures followed by a shift to 40 °C reliably yielded the glycosides with β-selectivity of 98 to 99%.

Table 3: Tuning anomeric selectivity of the copper(II)-catalyzed glycosylation reaction. Condition A: 1 eq. $Cu(OTf)_2$, 5 eq ROH, $ClCH_2CH_2Cl$, 84 °C, 6 h. Condition B: 1 eq. $Cu(OTf)_2$, $ClCH_2CH_2Cl$, 84 °C, 4 h; 5 eq ROH 40 °C, 2-4 h. [a]Isolated yields of pure α-glycoside (entry 1, 3, 5) or pure β-glycoside (entry 2, 4, 6). [b]α/β-Ratio was determined by LCMS analysis of the crude reaction mixture.



2α/β **4**

entry	ROH	condition	product, yield / %[a]	ratio α/β[b]
1		A	**4b**, 56	77:23
2		B	**4b**, 56	1:99
3		A	**4h**, 35	88:12
4		B	**4h**, 62	2:98
5		A	**4i**, 76	94:6
6		B	**4i**, 52	1:99

In summary, we report a simple and direct synthesis of β-glycosides of *N*-acetyl D-glucosamine. GlcNAc was acetylated to give the anomeric mixture of tetraacetate **2α/β** as pure solid after aqueous work-up. This material was subsequently employed as donor in a copper(II) triflate-catalyzed glycosylation of a variety of acceptor alcohols. Good yields with β-selectivities up to 14:1 were obtained. Partial anomerization to the thermodynamically more stable α-glycoside was observed upon prolonged reaction times or with more reactive glycosides. Careful monitoring of the glycosylation reaction by TLC or HPLC is crucial for optimizing β-selectivity. This is in contrast to the soft method developed by Jensen and co-workers[7b] which, however, requires the labor and cost intensive donor **2β**. Furthermore, we provide a method that allows the precise tuning to either α- or β-glycoside formation, however, at the cost of equimolar catalyst amounts. The method reported here provides a rapid and economic access to β-linked glycosides of N-acetyl glucosamine in

two steps from cheap *N*-acetyl glucosamine as starting material without the need for laborious purification of intermediates. Consequently, we anticipate widespread use in industrial or large-scale syntheses of different β-glycosides of GlcNAc.

Supporting Information Summary

Supporting information includes experimental section, ^{1}H and ^{13}C-NMR spectra.

Acknowledgments

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Keywords

α-glycosides, β-glycosides, copper(II) triflate, glycosylation, *N*-acetyl glucosamine

References

[1] a) J. Banoub, P. Boullanger, D. Lafont, *Chem. Rev.* **1992**, *92*(6), 1167–1195; b) A. F. G. Bongat, A. V. Demchenko, *Carbohydr. Res.* **2007**, *342*(3-4), 374–406.

[2] *Carbohydrates Essential Molecules of Life*. (Eds.: R. V. Stick, S. J. Williams), Elsevier B. V., Oxford, **2009**, pp. 133–202.

[3] N. M. Kelly, K. J. Jensen, *J. Carb. Chem.* **2001**, *20*(7 & 8), 537–548.

[4] B. Aguilera, A. Fernández-Mayoralas, C. Jaramillo, *Tetrahedron.* **1997**, *53*(16), 5863–5876.

[5] T. Ogawa, K. Beppu, S. Nakabayashi, *Carbohydr. Res.* **1981**, *93*(1), C6–C9.

[6] V. Wittmann, D. Lennartz, *Eur. J. Org. Chem.* **2002**, 1363–1367.

[7] a) S. I. van Kasteren, H. B. Kramer, H. H. Jensen, S. J. Campbell, J. Kirkpatrick, N. J. Oldham, D. C. Anthony, B. G. Davis, *Nature.* **2007**, *446*(7139), 1105–1109; b) H. Christensen, M. S. Christiansen, J. Petersen, H. H. Jensen, *Org. Biomol. Chem.* **2008**, *6*(18), 3276–3283.

[8] H. Myszka, D. Bednarczyk, M. Najder, W. Kaca, *Carbohydr. Res.* **2003**, *338*(2), 133–141.

[9] H. Myszka, D. Bednarczyk, M. Najder, W. Kaca, *Carbohydr. Res.* **2003**, *338*(2), 133–141.

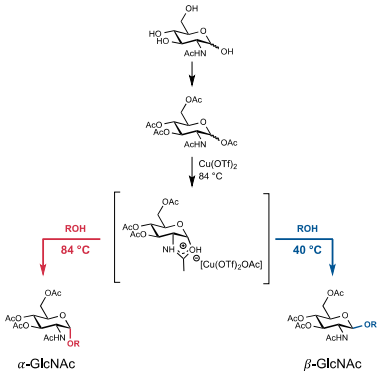
[10] a) A. Stevenin, F.-D. Boyer, J.-M. Beau, *Eur. J. Org. Chem.* **2012**, 1699–1702; b) A. Xolin, S. Norsikian, F.-D. Boyer, J.-M. Beau, *Eur. J. Org. Chem.* **2016**, *2016*(20), 3408–3418.

[11] B. Lindberg, *Acta Chem. Scand.* **1948**, *2,* 426–429.

[12] G. Wei, X. Lv, Y. Du, *Carbohydr. Res.* **2008**, *343*(18), 3096–3099.

[13] M. R. Rasmussen, M. H. S. Marqvorsen, S. K. Kristensen, H. H. Jensen, *J. Org. Chem.* **2014**, *79*(22), 11011–11019.

Graphical Table of Contents entry



β-Linked glycosides of *N*-acetyl glucosamine are widespread in nature. Their direct synthesis is hampered by the low reactivity of GlcNAc as a glycosyl donor. We report a selective and rapid copper(II) triflate-catalyzed two-step synthesis of β-glycosides of GlcNAc from cheap GlcNAc as starting material without purification of intermediates. α-Specific glycosylation can be achieved by increasing the amount of catalyst and extending reaction times.