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A one-step β-selective glycosylation of *N*-acetyl glucosamine and recombinant chitooligosaccharides†

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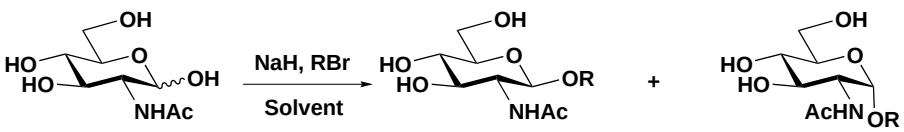
Abstract—*N*-Acetyl glucosamine and chitooligosaccharides are selectively converted into β-glycosides without protection of the other hydroxyl groups by alkylation of the anomeric alkoxides in *N*,*N*-dimethylformamide containing lithium bromide. Addition of the lithium salt notably improves the stereoselectivity of the glycosylation of the monomer and the efficiency of the process with higher oligomers.

Oligosaccharides and glycoconjugates are involved in numerous biological processes,[1] and their chemical synthesis has been enormously improved in the past 20 years making increasingly large structures available for biological studies. This still involves a large number of steps including many protections and deprotections despite some recent promising improvements such as solid supported synthesis. Interesting alternatives to fully chemical syntheses are chemo-enzymatic approaches, where the selectivity of enzymatic reactions is used to produce complex oligosaccharide structures.[2] To transform these molecules into useful biochemical tools, a few selective chemical steps are often necessary. These chemical steps should then be kept as simple as possible.

In this context, Samain et al. recently described a 'recombinant' approach to the chitooligosaccharide synthesis by cultivating, at high density, *Escherichia coli* cells expressing the appropriate enzymes that produce the required oligosaccharides.[3] Our interest was in the chemical modification of these compounds, our goal being the selective introduction of a chemical group that could be used as an anchor for further transformations. We now report that direct anomeric *O*-alkylation[4] of unprotected saccharides[5] gives a simple and straightforward solution to this problem.[6]

In model experiments with the monomer, *N*-acetyl D-glucosamine **1** was suspended in DMF (1 mL per mmol of **1**) and successively treated with sodium hydride (1.3 equiv.) and allyl bromide (3 equiv.) at room temperature for 20 h. After standard work-up and separation from the α-product **2**α[7] (12% yield), the β-allyl glycoside **2**β[7] was isolated in an 82% yield (Scheme 1 and entry 1, Table 1). We noticed that complete dissolving occurred within 1 h, although the reaction was still far from completion. The results are significantly different



1

2β R = allyl
3β R = propargyl
4β R = benzyl
5β R = p-NO_2-benzyl

2α
3α
4α
5α

Scheme 1.

Keywords: carbohydrates; glycosidation; alkylation; salt effects.

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† This paper is dedicated to Professor J. Thiem on the occasion of his 60th birthday.

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Table 1. Alkylation of *N*-acetyl D-glucosamine derivatives

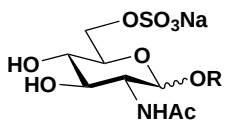
Entry	Substrate	Conditions[a]	Alkylating agent	Reaction time (h)	β-Product[b] (yield, %)	α-Product[b] (yield, %)
1	**1**	DMF	Allyl bromide	20	**2**β (82)	**2**α (12)
2	**1**	DMPU	Allyl bromide		Non-selective reaction	
3	**1**	DMPU[c]	Allyl bromide	1.5	**2**β (30)	**2**α (39)
4	**1**	DMSO	Allyl bromide	20	**2**β (62)	**2**α (13)
5	**1**	DMF, LiBr[d]	Allyl bromide	2.5	**2**β (85)	**2**α (5)
6	**1**	DMF, NaBr[d]	Allyl bromide	1	**2**β (64)	**2**α (4)
7	**1**	DMF	Propargyl bromide	3	**3**β (65)	**3**α (9)
8	**1**	DMF	Benzyl bromide	4	**4**β (75)	**4**α (14)
9	**1**	DMF	*p*-Nitrobenzyl bromide	20	**5**β (58)	**5**α (7)
10	**1**	DMSO	*p*-Nitrobenzyl bromide	20	**5**β (71)	**5**α (11)
11	**6**	DMF[e]	Allyl bromide	20	**7**β (81)	Not isolated

[a] The reaction mixture was stirred at room temperature under Ar with 1 mL of solvent per mmol of substrate, unless otherwise stated.
[b] Yields of isolated products after chromatography on silica gel.
[c] Five mL of solvent per mmol of **1**.
[d] Two equiv. of salt per equiv. of **1**.
[e] 1.2 mL of solvent per mmol of **6**.

from those obtained by Klotz and Schmidt[5a] with *N*-benzoyl D-glucosamine in *N*,*N*′-dimethyl-tetrahydropyrimidin-2-one (DMPU), (53% yield with an α:β ratio of 2.5:1). Replacement of DMF by DMPU in our concentration conditions (1 mL/mmol of sugar) led to a non-selective reaction whereas treatment of *N*-acetyl glucosamine under Klotz and Schmidt conditions provided 69% of **2**α,β with an α:β ratio of 1.3:1 (entries 2 and 3, Table 1). The transformation was also successful with other alkylating reagents such as propargyl bromide and *p*-nitrobenzylbromide (entries 7 and 9, Table 1), both appropriate functional groups for tagging recognition molecular motifs useful in biological studies, and with benzyl bromide (entry 8, Table 1), although with less efficiency. This one-step glycoside synthesis compares well with the standard peracetylation, selective anomeric deprotection, *O*-alkylation at C1 and deacetylation steps,[8] and the classical four-step Koenigs–Knorr synthesis[7] or its 'oxazoline' variant.[9]

Other solvent systems, examined for comparison with the glycosylation of oligomers (see below), like DMSO or DMF containing LiBr[10] (2 equiv. per equiv. of sugar) provided similar results (entries 4 and 5, Table 1) with, however, a shorter reaction time [20 h in DMF (entry 1) versus 2.5 h in DMF-LiBr (entry 5)] and an increase in the stereoselectivity [α:β ratio going from 1:7 (DMF, entry 1) to 1:17 (DMF-LiBr, entry 5)].

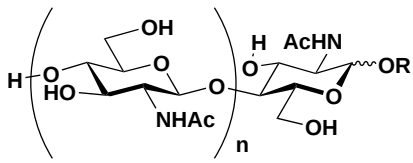
The procedure was also successful in the glycosylation of *N*-acetyl D-glucosamine 6-*O* sulfate **6**, available in one step (SO_3·Pyr, pyridine, 0°C, 54%) from **1**, thus providing β-allyl glycoside **7** in 81% yield (Scheme 2). This experiment serves as a model study for the transformation of *O*-sulfated chitooligomers, the oligosaccharide portions of the bioactive nodulation factors.[11]



6 R = H
7 R = allyl (β only)

Scheme 2.

With DMF, this approach could be extended to chitobiose, providing allyl glycosides **9**[12] in 69% yield (Scheme 3), again showing a significant increase in the β-stereoselectivity in the presence of LiBr (compare entries 1 and 2, Table 2). With the higher chitooligosaccharides (trimer,



R = H
8 n = 1
10 n = 2
12 n = 3
14 n = 4

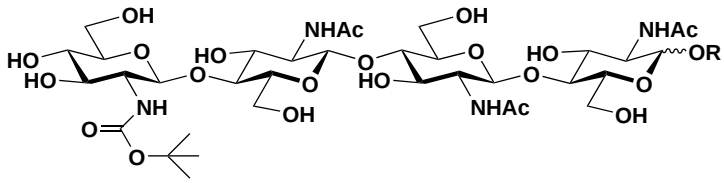
R = allyl
9 n = 1
11 n = 2
13 n = 3
15 n = 4

Scheme 3.

Table 2. Anomeric allylation of unprotected chitooligosaccharides

Entry	Reagent	Conditions[a]	Product (yield, %)[b]	β:α[c]
1	**8**	DMF	**9** (69)	3:1
2	**8**	DMF, LiBr (3 equiv.)	**9** (64)	11:1
3	**10**	DMF	Traces	
4	**10**	DMF, LiBr (4 equiv.)	**11** (68)	6:1
5	**12**	DMF	Traces	
6	**12**	DMSO	**13** (26)	7:1
7	**12**	DMF, LiBr (5 equiv.)[d]	**13** (58)	8:1
8	**14**	DMF	Traces	
9	**14**	DMSO	**15** (19)	10:1
10	**14**	DMF, LiBr (6 equiv.)[d]	**15** (57)	9:1
11	**16**	DMF, LiBr (5 equiv.)[d]	**17** (45)	5:1

[a] The reaction mixture was stirred under Ar at room temperature for 24 h with approx. 0.1 mL of solvent per 20 mg of substrate. The DMF:AllBr ratio was kept to 4:1 (v/v).
[b] Yields of isolated products after chromatography on silica gel.
[c] Anomeric ratio as determined by ^{1}H NMR.
[d] More NaH (0.6 equiv.) was added after 24, 48, and 72 h; total reaction time: 96 h.



16 R = H
17 R = allyl

Scheme 4.

tetramer or pentamer), no dissolving was observed after 24 h of stirring in DMF, and virtually no transformation could be detected by TLC (entries 3, 5 and 8, Table 2). Chitin fragments are notoriously insoluble and solubility has always been recognized as a major problem in their chemical transformation in aprotic or protic solvents.[5a] DMSO as a solvent resulted in a modest improvement leading to selectively allylated chitotetra- and pentaose **13** and **15** with moderate yields (26% and 19% respectively, entries 6 and 9, Table 2). Solubility remained an issue affecting both yield and reproducibility of the reaction. Chitotriose, chitotetraose and chitopentaose were however solubilized in the DMF-LiBr system, and were converted to their allyl glycosides with good yields (57–68%) and selectivities (α:β ratio of 6-9:1) as shown in Table 2 (entries 4, 7 and 10). Finally, the Boc-protected tetramer **16** was converted to allyl glycosides **17** (entry 11, Table 2 and Scheme 4).

The solubilizing effect of salts is well documented both in polysaccharide chemistry, where *N*,*N*-dimethylacetamide with LiCl was originally developed to dissolve chitin[13], cellulose[14] or other polysaccharides, and in peptide chemistry where Seebach has shown that addition of lithium salts greatly increases solubility of peptides in ether solvents.[15] We further observe a salt effect on stereoselectivity with an apparent increase in the reactivity of the β-alkoxide which suggests that discrete complexes may be formed and this important aspect is currently being considered.

We have shown that direct anomeric *O*-alkylation provides a fast, efficient and selective synthesis of glycosides of *N*-acetyl D-glucosamine and derivatives useful as building blocks in carbohydrate chemistry, together with an easy derivatization procedure for chitooligosaccharides.[16] This procedure should also prove useful in the synthesis of glycosides of other bioactive oligosaccharides for biological research.

Acknowledgements

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16. In an attempt to identify some of the challenges chemistry is facing at the beginning of this century, Lippard recently published a 'wishlist'. One of his 22 wishes was the following: "*We wish to create reagents that chemically modify a portion of a molecule without protecting, and later having to deprotect, other reactive sites on the molecule that we do not want to modify*". See: Lippard, S. J. *Chem. Eng. News* **2000**, *78*, *32*, 64.