Joined use of oxazolidinone and desymmetric amino protection: a new strategy for protection of glucosamine

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ABSTRACT

Joined use of *N*-benzyl oxazolidinone and *N*-benzyl-*N*-benzyloxycarbonyl (*N*-BnCbz) desymmetric amino-protecting function is reported. The new synthetic approach enables the facile preparation of type 1 and type 2 LacNAc disaccharides in satisfactory yields. One-pot deprotection of *N*-BnCbz and *O*-benzyl ether is achieved by hydrogenolysis under mild conditions.

A number of naturally occurring glycoconjugates contain *N*-acetyl glucosamines that glycosylate at C-3 and C-4 positions.[1] Typical examples are the Lewis blood group antigens, which contain either Gal-β(1→3)-GlcNAc (type 1 LacNAc) or Gal-β(1→4)-GlcNAc (type 2 LacNAc) backbone.[2] Some of these blood group antigens such as Lewis Y antigen have been proven to be specific tumor markers for cancer diseases; thus, they are attractive targets for various biomedical investigations.[3] To sustain these research activities, the supply of pure oligosaccharide samples and their conjugates is crucial. One of the important factors in oligosaccharide synthesis is the effective formation of glycosidic bonds. However, due to steric hindrance and hydrogen-bonding interaction, the C-3 and C-4 hydroxyl functions in *N*-acetyl glucosamine are weakly nucleophilic, and therefore glycosylations of these hydroxyl functions are often problematic.[4,5] To solve these problems, different amino-protecting groups have been designed, which include *N*-phthaloyl (*N*-Phth),[6] *N*-tetrachlorophthaloyl (*N*-TCPhth),[7] *N*-dithiasuccinoyl (*N*-Dts),[8] *N*-trichloroethoxycarbonyl (*N*-Troc),[9] *N*-trichloroacetyl (*N*-TCA),[10] *N*-trifluoroacetyl (*N*-TFA),[11] *N,N*-diacetyl (*N*-Ac$_2$),[12] *N*-*p*-nitrobenzyloxy-carbonyl (*N*-PNZ),[13] *N*-dimethyl-phosphoryl (*N*-DMP),[14] and others.[15] In routine practice, the amino function of glucosamine is often masked with a protecting function in the early stage of synthesis. After a series of protecting group manipulations and glycosylations, this amino-protecting group has to be removed in the final stage. This standard strategy demands the use of a robust protecting function to survive different conditions, but such a function has to be taken off in the end. Therefore, it is not easy to design a single protecting function embracing both features. A point in case is the use of *N*-Phth protection, which is stable to different reaction conditions,[5] but its removal is non-trivial.[15,16]

In 2001, Kerns and co-workers reported using N-unprotected oxazolidinone for the protection of C-3 hydroxyl and C-2 amino functions in glucosamine.[17] This function was later elaborated to *N*-acetyl[18–22] and *N*-benzyl oxazolidinone derivatives.[23–25] The primary goal of using oxazolidinone function is to search for a good α-directing glucosamine donor.[17] Subsequent studies reveal some degree of inconsistency in the stereochemical preference of glycosylations.[22,25,26] We speculated that other than stereochemical preference, the unique feature of *N*-benzyl oxazolidinone may impart additional utilities (Fig. 1).

Our rationale is grounded on the following facts. Firstly, the 'tied-up' C-3 hydroxyl and C-2 amino functions reduce the steric hindrance at C-4 position and therefore should facilitate its glycosylation.[21] Secondly, the oxazolidinone protection has been shown to decrease the reactivity of the anomeric-leaving function,[22,27] which paves the way for the reactivity-based glycosylation.[28] Thirdly, the hydrolytic opening of oxazolidinone and reprotection of amine function lead to the formation of desymmetric amino-protected glucosamine, which to the best of our knowledge has rarely been studied in the literature.[9b] In the light of the discussion above, this study reports a useful strategy for the protection of glucosamine capitalizing the *N*-benzyl oxazolidinone and its derived desymmetric *N*-benzyl-*N*-benzyloxycarbonyl (*N*-BnCbz) functions.
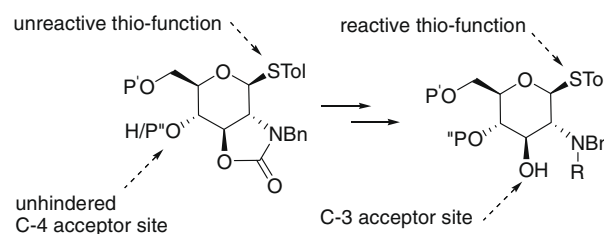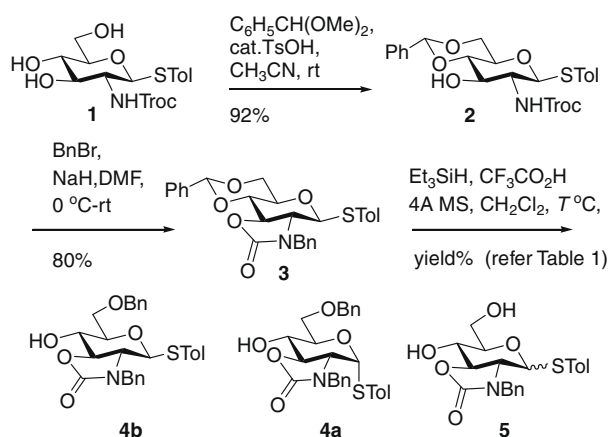

Figure 1. *N*-Benzyl oxazolidinone-protected glucosamine and its derived disubstituted-desymmetric amino-protected glucosamine.

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In the beginning, 2-Troc-2-deoxy thioglucopyranoside **1** prepared from glucosamine[28] was converted to 4,6-O-benzylidene-2N-benzyl-2,3-N,O-carbonyl-2-deoxy thioglucopyranoside **3** via benzylidene acetal intermediate **2** (Scheme 1).[25] However, the reductive ring opening of benzylidene acetal **3** required considerable experimentation (Table 1). Previous efforts using either sodium cyanoborohydride–hydrogen chloride (NaBH$_3$CN/HCl)[29] or triethylsilane–boron trifluoride etherate (Et$_3$SiH/BF$_3$·Et$_2$O)[30] led to β→α anomerization. This undesirable reaction is attributable to the coordination of BF$_3$ to ring oxygen atom that promotes the endocyclic cleavage of C1–O5 linkage.[24,31] After some investigations, using triethylsilane–trifluoroacetic acid (Et$_3$SiH/TFA) at low reaction temperature was found to be effective for the reduction of β→α anomerization.[32] To our delight, N-benzyl-2,3-N,O-carbonyl-protected β-thioglucopyranoside **4b** was formed exclusively in high 80% yield at −20 °C (Table 1, entry 3). However, anomerization of **4b** to α-anomer **4a** and trace amount of complete deacetalation product **5** were observed at higher reaction temperatures (Table 1, entries 1 and 2). Noted that the use of the literature procedure resulted in a 1:6 α/β-anomeric mixture (Table 1, entry 4).[24] The β-anomeric configuration of **4b** was supported by the ^{13}C chemical shift at 86.7 ppm and $^1J_{CH}$ coupling constant of 161 Hz.[33]

After the preparation of glucosamine acceptor **4b**, this study proceeded to synthesize a desymmetric amino-protected glucosamine acceptor (Scheme 2). In this regard, N-benzyl oxazolidinone-protected glucosamine thioglycoside **6**[25] was treated with t-BuOK to produce benzylamine derivative **7**,[25] which was chemoselectively converted to desymmetric N-benzyl-N-benzyloxycarbonyl (N-BnCbz)-protected glucosamine thioglycoside **8**.[34] Subsequent glycosylation of aglycon acceptor **9** with thioglycoside **8** using N-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) as promoters furnished glucosamine glycoside **10**.[35] Noted that the assignment of ^{1}H NMR spectra of **8** and **10** was difficult due to the peak broadening of the resonance signals.[36] Nonetheless, their preliminary identifications were evidenced by HRMS.
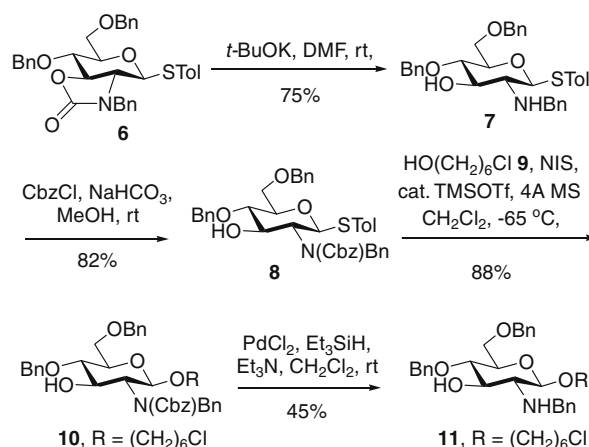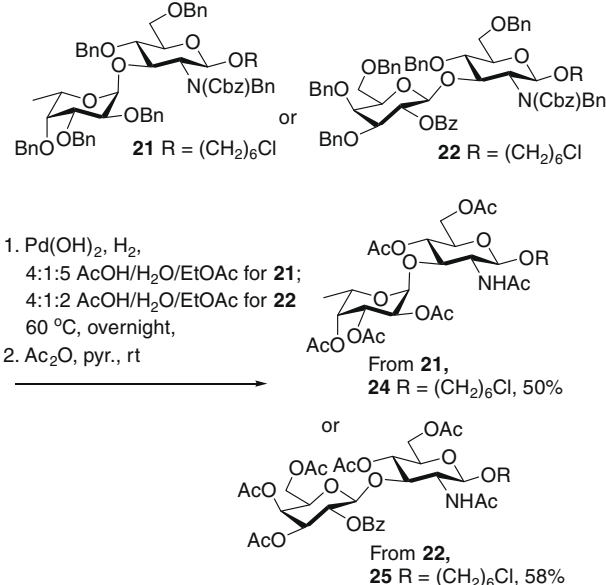

Scheme 2. Synthesis of desymmetric (N-BnCbz)-protected glucosamine acceptor **10**.

Further support of their structures could be obtained by high temperature NMR spectroscopy, as demonstrated for glycoside **10** (ca VT-NMR from rt to 100 °C in deuterated DMSO solvent).[37] The broadening of resonance signal is due to the presence of the Cbz carbamate function because such a broadening phenomenon had gone for glucosamine glycoside **11**, in which the Cbz function was removed.

With glucosamine acceptors **4b** and **10** in hand, the stage was ready to study their glycosylations with known thioglycosides **12**–**16** (Table 2).[38] Glycosylations of **4b** with thiogalactopyranoside **12** and thiofucopyranoside **13** produced Gal-α(1→4)-GlcNAc disaccharide **17** and Fuc-α(1→4)-GlcNAc disaccharide **18** as the single anomers (Table 2, entries 1 and 2). Intriguingly, the thiotolyl function in thioglycoside **18** underwent β→α anomerization forming an inseparable 1:3.5 α/β-anomeric mixture. Though this anomerization can be explained by C1–O5 endocyclic bond cleavage as described before,[31] it is unclear why the same anomerization did not occur in the glycosylation of **12**. Due to the deactivation of oxazolidinone function, self-condensation of **4b** did not occur under the present reaction conditions.[22,27] Glycosylations of **4b** with thioglycosides **14** and **15** furnished type 2 LacNAc disaccharides **19** and **20** in high yields (Table 2, entries 3 and 4). For glycosylations of

Scheme 1. Synthesis of glucosamine acceptor **4b**.

Table 1
Reaction conditions and results of reductive benzylidene ring opening of thioglycoside **3**

Entry	Acid (equiv)	Et$_3$SiH (equiv)	T (°C)	Yield (%) of **4**[a]	α:β
1	TFA (6)	5	25	35	1:1
2	TFA (6)	5	0	57	1:10
3	TFA (6)	5	−20	80	β only
4	BF$_3$ (2)	12	−20	65	1:6[b]

[a] Total yield of **4a** and **4b** after chromatography purification.
[b] The method was referred to Ref. 23.

Scheme 3. Deprotection of disaccharides **21** and **22**.

Table 2
Glycosylation studies of glucosamine acceptors **4b** and **10**

thioglycoside donor   +   glucosamine acceptor

12, **13**, **14**, **15** or **16**;   **4b** or **10**

NIS, cat. TMSOTf,
4A MS, CH$_2$Cl$_2$, T °C
$\longrightarrow$ disaccharide

17, **18**, **19**, **20**,
21, **22**, or **23**

Entry	Thioglycoside donor	Glucosamine acceptor	T (°C)	Disaccharide product	Yield (%)
1	**12**	**4b**	−70	**17**	70
2	**13**	**4b**	−60	**18** (α:β = 1:3.5)	85
3	**14**	**4b**	−65	**19**	65
4	**15**	**4b**	−70	**20**	80
5	**13**	**10**	−70	**21** R = (CH$_2$)$_6$Cl	93
6	**15**	**10**	−65	**22** R = (CH$_2$)$_6$Cl	80
7	**16**	**10**	−65	**23** R = (CH$_2$)$_6$Cl	73

glucosamine acceptor **10**, thioglycoside donors **13**, **15**, and **16** were employed. All the glycosylations furnished the expected disaccharide products **21**–**23** in high (73–93%) yields (Table 2, entries 5–7). For NMR spectroscopy of disaccharides **21**–**23**, the phenomenon of resonance peak broadening was also observed.

After studying the glycosylation properties of glucosamine acceptors **4b** and **10**, we next explored appropriate deprotection methods for selected disaccharide products. As the deprotection methods for oxazolidinone have already been developed,[23] this study focused on the deprotection of desymmetric amino protection of Fuc-α(1→3)-GlcNAc glycoside **21** and type 1 LacNAc glycoside **22** (Scheme 3). An advantage of using N-Cbz protection in glucosamine is that it can be removed along with the benzyl ether and benzylamine functions during Pd-catalyzed hydrogenolysis.[39]

In our hands, the optimization of reaction conditions was required. Ultimately, Pd(OH)$_2$ was found to be the most effective catalyst for the deprotection of *N*-BnCbz and *O*-Bn in **21** and **22** (Scheme 3).[23,40,41] Both hydrogenolysis reactions were performed in AcOH/H$_2$O/EtOAc solvent mixtures under 1 atm H$_2$ at 60 °C. For NMR characterization, the resulting debenzylated products were further acetylated to produce the peracetyl Fuc-α(1→3)-Glc-NAc glycoside **24** and type 1 LacNAc glycoside **25**.

In summary, this study reports a versatile amino protection strategy for glucosamine by the joined use of *N*-benzyl oxazolidinone and desymmetric *N*-BnCbz function. The scope of investigation includes the installation, deprotection, and application of these protecting functions. As glucosamine constitutes the key component in different oligosaccharide structures, the results of this study should be found useful for their preparation.

Acknowledgments

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.02.021.

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