

Synthesis of a Useful Anomeric Thioacetate of an *N*-Acetyllactosamine Derivative and its Application[†]

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Abstract

A novel anomeric β -thioacetate of an *N*-acetyllactosamine derivative was efficiently synthesized in high yield from the known 2-azido glycosyl chloride using thioacetic acid as a convenient reagent. The synthesis involved not only an S_N2 replacement of the chloride by a carbothiolate anion but also a reductive acetamidation of the azide group. Applications of the thioacetate for glycosidation were demonstrated to provide both *O*- and *S*-glycosides in high yields. Furthermore, both intermediates gave a new class of glycoclusters that included thioglycosidic linkages.

Keywords: N-acetyllactosamine; glycosides; thioacetic acid; thioacetates; glycoclusters

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N-Acetyllactosamine (LacNAc; Gal β 1 \rightarrow 4GlcNAc) is known as an extremely valuable core structure of glycoconjugates such as glycoproteins and glycolipids.¹ In order to use oligosaccharides for elucidating their functional roles in biological systems, synthetic construction of oligosaccharides, including LacNAc, has been reported by several groups.² Unfortunately, LacNAc or its precursor to be used as the starting material for such a purpose has not been readily obtained. Lemieux *et al.* introduced an azidonitration reaction followed by treatment of the products using Et₄NCl for peracetylated D-lactal to provide crystalline *O*-(2,3,4,6-Tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-3,6-di-*O*-acetyl-2-azido-2-deoxy- α -D-glucopyranosyl chloride **1** as the precursor of *N*-acetyllactosamine.³ The chloride, however, has not been widely used for glycosciences. In this study, we therefore investigate further manipulation of this unused chloride to obtain useful derivatives of *N*-acetyllactosamine. In this communication, we describe a stereospecific synthesis of the novel peracetylated *N*-acetyllactosaminyl 1- β -thioacetate **2** by modified Lemieux's protocol and the applicability of **2** as a glycosyl donor.

Since Rosen *et al.* initially reported a convenient and highly chemoselective reduction of azides to provide the corresponding acetamide in good yield,⁴ we examined the use of a slight modification of this procedure⁵ for preparation of *N*-acetyllactosaminyl chloride. Freshly prepared 2-azido-1- α -chloride **1** was treated with a large amount of thioacetic acid in the presence of pyridine at rt. The reaction proceeded smoothly on TLC to give a major product after purification by silica gel chromatography. In order to elucidate the structure of the product, the IR spectrum of the compound was first measured and showed absorptions at 1753, 1703, 1674, and 1541 cm⁻¹ and the disappearance of absorption at around 2100 cm⁻¹ due to the azide functional group ($\nu_{N=N=N}$). The absorptions at 1753, 1674, and 1541 cm⁻¹ were assigned to an ester ($\nu_{C=O}$), an amide I ($\nu_{C=O}$), and an amide II (δ_{N-H}), respectively. There was still an unknown absorption at 1703 cm⁻¹. A flame reaction of a compound on the copper metal reminded us of Beilstein test to detect a halide atom in the compound. Consequently, Beilstein test was performed on the product, and the results showed a negative color, indicating vanishment of the chloride atom in the product. Our attention was then focused on replacement of the chloride atom at the anomeric center. A ¹H NMR spectrum of the product revealed a signal assignable to H-1 at δ 5.09 as a doublet with $J_{1,2}$ 10.9 Hz and SAc at δ 2.35 ppm as a singlet. Accordingly, the structure was confirmed to be a

β -thioacetate **2** of *N*-acetylactosamine, and the yield was estimated to be 71.6%,[†] $[\alpha]_{\text{D}}^{24} +7.3^{\circ}$ (*c* 0.15, methanol), IR (KBr) 1703 cm^{-1} [$\nu_{\text{C=O}}$ (SAc)], ^1H NMR (CDCl_3) δ 4.49 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1') and 5.76 (d, 1 H, $J_{2,\text{NH}}$ 9.8 Hz, NH). We propose that the simultaneous reactions of thioacetic acid with 2-azido-1- α -chloride **1** include a usual reduction followed by acetylation at the azido functional group and a nucleophilic replacement by the carbothiolate anion from the β face at the anomeric carbon to provide **2** as the sole product.

Scheme 1

Since the preparation and the structural assignment of **2** had been accomplished, we turned our attention to the potency of **2** as a glycosyl donor. To the best of our knowledge, no direct activation of thioacetate as a donor for glycoside synthesis has been reported.⁶ Thus, thioacetate **2** was treated with NIS—TfOH as a typical glycosidation protocol using thioglycoside as a donor. Interestingly, the reaction proceeded smoothly to give the oxazoline derivative **3**⁷, which is known as a useful precursor for an *O*-glycoside, in 70.0% yield after purification by silica gel chromatography, ^1H NMR (CDCl_3) δ 3.46 (d, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 5.64 (d, 1 H, $J_{2,3}$ 9.4 Hz, H-3), and 5.91 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1). The oxazoline **3** was then converted into its *O*-glycoside by the method previously reported⁸ to yield the corresponding β -glycoside **4**.

Scheme 2

Given the success of the conversion of thioacetate **2** into an *O*-glycoside, we examined the further transformation of thioacetate **2** to a thioglycoside that can be used as a potential inhibitor of glycosidases. The removal of *S*-acetyl of **2** in the presence of sodium methoxide in methanol⁹ gave the thiolate anion **5**, which was successively allowed to react with 5-bromo-1-pentene to afford the corresponding β -thioglycoside **6** in 78.9% yield after reacetylation, $[\alpha]_{\text{D}}^{22} -32.3^{\circ}$ (*c* 0.48, chloroform), ^1H NMR (CDCl_3) δ 2.13 (m, 2 H, SCH_2), 4.42 (d, 1 H, $J_{1,2}$ 10.3 Hz, H-1), 4.50 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1') and 5.58 (d, 1 H, $J_{2,\text{NH}}$ 9.7 Hz, NH).

In our ongoing synthetic study of artificial glycoconjugates, synthetic assembly of carbohydrate moieties using carbosilane dendrimers has been achieved using β -cyclodextrin,¹⁰ globotriaose,¹¹ functional monosaccharides¹², and sialyllactose.¹³ Since such carbohydrate moieties were found in our previous investigation to be coupled to carbosilane dendrimers through a spacer arm including a sulfide linkage, the

[†]All new compounds with the specific rotation data gave satisfactory results of elemental analyses.

formation of thioglycoside demonstrated here, which is referred to as a model compound without an aglycon, was then examined for construction of carbosilane dendrimers uniformly functionalized with *N*-acetyllactosamine residues through thioglycoside linkages. The incorporation of *N*-acetyllactosamine residues into a carbosilane **7**¹¹⁾ under conditions similar to those used for the preparation of **6** proceeded smoothly and gave the corresponding glycocluster **8** in 68.7% yield, $[\alpha]_{\text{D}}^{23} -39.9^{\circ}$ (*c* 0.093, CHCl₃), integral ratio of the H atoms by ¹H NMR: SiCH₂:SCH₂:Ph:H-1' = 6:6:5:3, FABMS calcd for [M+H⁺]: 2183.69; Found *m/z*: 2183.62. An analogue of the thioglycoside-type glycocluster was also prepared in a manner similar to that used for the preparation of **4** using triol **9**¹⁴⁾ as a cluster-type alcohol to provide glycocluster **10** in 33.0 yield, $[\alpha]_{\text{D}}^{28} -14.2^{\circ}$ (*c* 0.427, MeOH), integral ratio of the H atoms by ¹H NMR: SiCH₂:Ph:H-1' = 6:5:3, FABMS calcd for [M+H⁺]: 2135.76; Found *m/z*: 2135.50, which was an attractive compound in comparison with thioglycoside-type glycocluster for activity against glycosidase and glycosyltransferase, respectively. Finally, the protected glycoclusters **8** and **10** were quantitatively deprotected by a combination of typical Zemplén's manner and saponification to afford corresponding water soluble **11**, IR 1652 (Amide I; $\nu_{\text{C=O}}$) and 1558 (Amide II; $\delta_{\text{N-H}}$) cm⁻¹, FABMS calcd for [M+H⁺]: 1426.50; Found *m/z*: 1426.41, and **12**, IR 1649 ($\nu_{\text{C=O}}$) and 1556 ($\delta_{\text{N-H}}$) cm⁻¹, FABMS calcd for [M+H⁺]: 1378.57; Found *m/z*: 1378.40, respectively.

Scheme 3

In conclusion, we have successfully demonstrated the preparation of a novel thioacetate **2** of *N*-acetyllactosamine and the efficient activation of **2** for glycoside synthesis yielding a couple of glycosides through an *O*- or *S*-glycosidic linkage, respectively. Further transformations of **2**, including synthetic assembly of **2** using other carbosilane dendrimers as core scaffolds and enzymatic elongation of sialic acid for the cluster-type compounds, are now in progress, and the results will be reported elsewhere.

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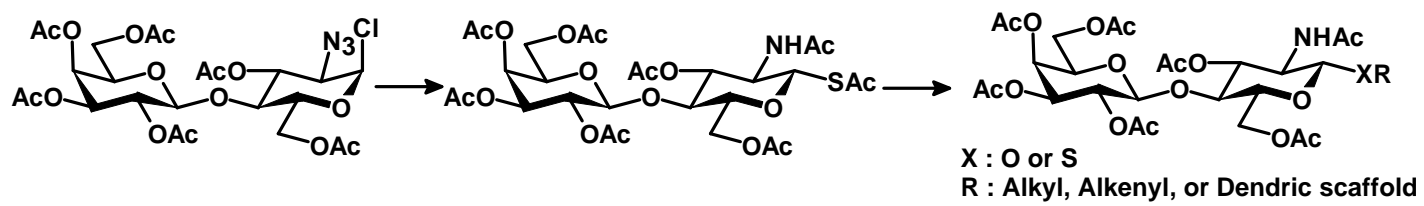
Legends to Schemes

Scheme 1. *Reagents and conditions:* i) AcSH (large excess), Pyr, rt, 4 h.

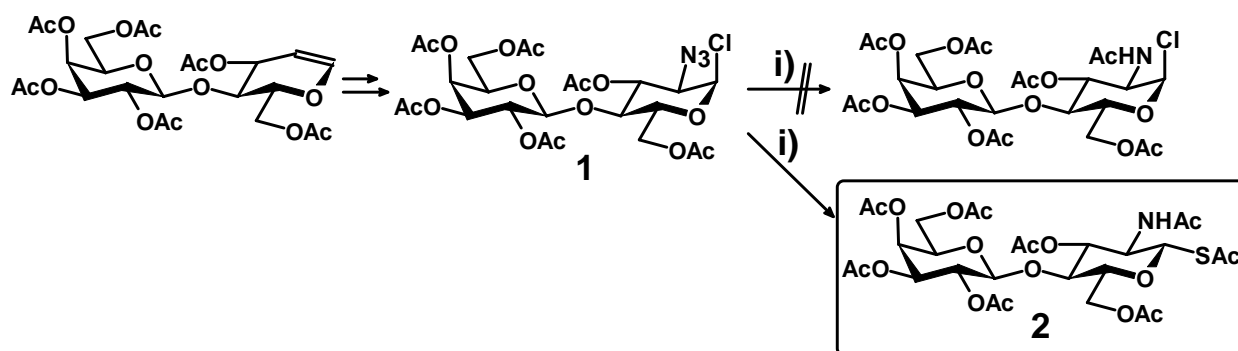
Scheme 2. *Reagents and conditions:* i) NIS (4 molar excess), TfOH (4 molar excess), CH₂ClCH₂Cl, 0 °C→rt, 4 h, ii) 4-penten-1-ol, CSA, CH₂ClCH₂Cl, 90 °C, iii) NaOMe, MeOH, -40 °C, iv) 5-bromo-1-pentene (3 molar excess), 2 h, -40 °C, then Ac₂O—Pyr, rt.

Scheme 3. *Reagents and conditions:* i) NaOMe, MeOH, -40 °C, then, **7** (ref. 11), -40 °C, overnight, then, Ac₂O—Pyr, rt., ii) **9** (ref. 14), CSA, CH₂ClCH₂Cl, 90 °C.

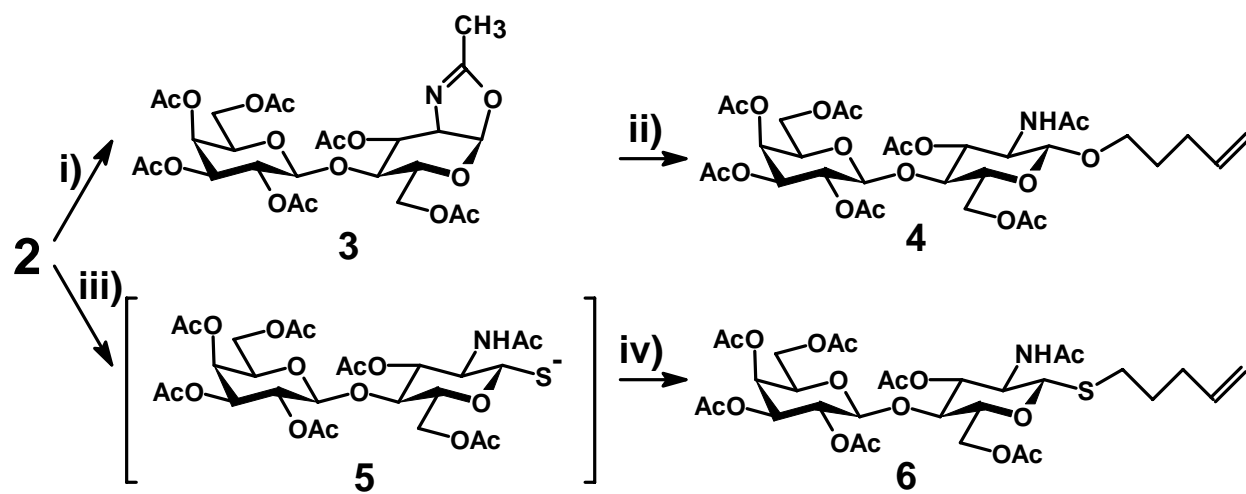
Abstract



Scheme 1



Scheme 2



Scheme 3

