Published in final edited form as:
Angew Chem Int Ed Engl. 2015 January 19; 54(4): 1275–1278. doi:10.1002/anie.201409797.

The Isothiocyanato Moiety. An Ideal Protecting Group for Stereoselective Sialic Acid Glycoside Synthesis and Subsequent Diversification**

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Abstract

The preparation of a crystalline, peracetyl adamantanyl thiosialoside donor protected by an isothiocyanate group is described. On activation at -78 C in the presence of typical carbohydrate acceptors this donor gives high yields of the corresponding sialosides with exquisite α-selectivity. The high selectivity extends to the 4-O-benzyl-protected 3-OH acceptors that are typically less reactive and selective than galactose 3,4-diols. Treatment of the α-sialosides with tris(trimethylsilyl)silane or allyltris(trimethylsilyl)silane sialosides replaces the C5-N5 bond by a C-H or a C-C bond. Reaction of the isothiocyanate-protected sialosides with thioacids achieves conversion into amides. Reaction of the isothiocyanate with an amine gives a thiourea, which can be converted to a guanidine. The very high α-selectivities observed with the new donor and the rich chemistry of the isothiocyante function considerably extend the scope for optimization at the sialoside 5-position.

Keywords

glycosylation; isothiocyanate; guanidine; thiourea; deamination

In recent years major steps have been taken toward the establishment of high-yielding and highly α-selective chemical sialidation reactions.[1] For the most part advances have centered around modification of the N-5 protecting group,[2] culminating in the discovery of the 4-*O*-,5-*N*-oxazolidine systems[3] and their *N*-acetyl variants,[3c, 4] which afford excellent yields and selectivities. Nevertheless, the potential applications of sialic acid glycosides and their oligomers in medicine,[5] and the consequent need for larger scale synthesis, drive the continued search for improved methods.

**Support of this work by the NIH (GM62160) is gratefully acknowledged. The 600 MHz NMR spectrometer in the Lumigen Instrument Center at Wayne State University was acquired with NSF support (MRI-084043). DC thanks Dr Peter Fügedi, Debrecen, for a helpful discussion.

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201xxxxxx.

The N-5 position also plays a prominent role in the development of sialic acid glycosides with improved properties for application as therapeutic agents and/or vaccines.[6] Such N-5 modified systems are either produced chemoenzymatically,[6b, 7] or chemically by removal of the N-5 protecting group post-glycosylation followed by derivatization.[6a, 6c, 8] We now reveal that protection of N-5 in the form of an isothiocyanate provides a sialyl donor that is not only exquisitely α-selective in its coupling reactions but which also, by taking advantage of the versatile chemistry[9] of the isothiocyanate group, affords facile access to an unprecedented range of functionality in the so-formed glycosides. The isothiocyanate **2**, previously obtained in low yield as a by-product in the synthesis of an *N*-acetyl-4-*O*,5-*N*-oxazolidinthione-proected sialyl donor,[10] was procured in 59% yield by treatment of the β-*S*-adamantanyl thiosialoside **1**[4b, 10] with HCl in ether, followed by phenyl chlorothionoformate and aqueous sodium bicarbonate, and finally acetic anhydride in pyridine (Scheme 1). Donor **2** is a stable, readily handled white crystalline solid.

Activation of donor **2** at -78 °C in 2/1 dichloromethane and acetonitrile in the presence of 1.2 equivs of various acceptors (Scheme 2) afforded the corresponding glycosides **3**-**7** (Table 1). The anomeric configuration of the products was assigned on the basis of the heteronuclear $^3J_{C1,H3ax}$ coupling constant, which followed the typical pattern.[11] An authentic sample of the β-anomer of **5** was obtained in 20% yield by coupling **2** with methyl 2,4,6-tri-*O*-benzyl-α-D-galactopyranoside **10** in pure dichloromethane at -30 °C (Figure 1), and enabled confirmation of its absence in the couplings conducted at -78 °C in the dichloromethane/acetonitriloe mixture. A by-product of this latter reaction conducted at -30 °C, obtained in 27% yield, was methyl 3-*O*-(1-adamantanyl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside **13** (Figure 1) arising from formation of the 1-adamantanyl cation in the reaction mixture at the higher temperature.

The formation of a single anomer of **5** at -78 °C (Table 1, entry 3) is especially noteworthy. Typically, 4-*O*-protected galactopyranosyl 3-OH acceptors give only poor selectivity in sialidation reactions, even with the oxazolidinone-proected donors,[4] hence the common use of the more reactive and selective 3,4-galactosyl diols. The excellent selectivities obtained with the isothiocyanate-protected donor are all the more remarkable when contrasted with the coupling reactions of related azide-protected sialyl donors **14** (Figure 1),[12] which are reported to be competent donors for coupling to primary alcohols,[13] but to be much less selective with secondary alcohols.[2, 13c]

Two possibilities were envisaged for the greater selectivity of the isothiocyanate **2** over the structurally-related azides **14**. First, as the isothiocyanate group is considerably more polar than the azido and isocyanate groups (dipole moments of $C_6H_5N_3$, $C_6H_5N{=}C{=}O$, and PhN=C=S in Debye units, respectively: 1.82, 2.43, 2.69[14]), it is possible that the isothiocyanate simply serves as a strongly electron-withdrawing group and promotes S_N2 glycosylation as has been proposed[15] for the oxazolidinone system. Alternatively, consistent with current models for the through-space stabilization of glycosyl oxocarbenium ions,[16] it is possible that a transient intermediate sialyl oxocarbenium ion preferentially adopts the $^5H_4$ conformation **15** benefitting from stabilization by the pseudoaxial 4-*O*-acetate and the isothiocyanate groups, with the latter providing significant steric shielding to the β-face (Figure 1). In a competition experiment designed to begin to probe this question a

1:1:1 mixture of the isothiocyanate **2**, the *N*-acetyloxazolidinone **16** (Figure 2), and acceptor **10** was activated at -78 °C by the addition of 0.2 equiv of triflic acid. After standard work up the disaccharides **5** and **17** (Figure 2) were isolated in 3 and 51% yield, respectively, the latter as a 4:1 α:β-mixture. Consistent with this result, donors **2** and **16** were recovered from this experiment in 73 and 17% yield, respectively. While this experiment does not exclude the involvement of oxocarbenium ions such as **15**, it establishes the isothiocyanate-protected donor **2** is less reactive than the *N*-acetyloxazolidinone-protected donor **16** under the usual conditions consistent with the highly electron-withdrawing nature of the isothiocyanate moiety.

Turning to the post-glycosylation derivatization of the isothiocyanate group, in a modification of the Saegusa-Barton[17] radical deamination protocol heating of disaccharide **5** with tris(trimethylsilyl)silane[18] and AIBN in toluene at reflux afforded the 5-deamino-α-sialoside **18** (Scheme 3). Acetylation of the residual alcohol in **4** followed by AIBN-initiated reaction with allyltris(trimethylsilyl)silane[19] in toluene at reflux gave the 5-deamino-5-allyl-α-sialoside **19** as a single equatorial diastereoisomer consistent with earlier reports[20] on radical C-C-bond formation at the 4-position of glucopyranosides (Scheme 3). Reaction of *N*-Boc-L-methionine thioacid and benzyloxy thioacetic acid, both derived by deprotection of the corresponding 9-fluorenylmethyl thioesters,[21] with disaccharide **5** gave the modified sialosides **20** and **21** in good yield (Scheme 3). The greater ease of reaction of thioacids with isothiocyanates[22] than with unactivated azides[23] is noteworthy. In a further example of the isothiocyanate to amide transformation, the residual alcohol in disacccharide **7** was acetylated and the product 7-Ac allowed to react with benzyloxy thioacetic acid in the presence of piperidine in DMF at 40 °C to give the disialoside **22** containing a protected glycolyl amide and an acetamide (Scheme 3).

In a further demonstration of the possibilities afforded by the isothiocyanate group, disaccharide **5** was treated first with 2-phenylethylamine to give the thiourea **23** in 90% yield. Subsequent reaction with methyl iodide gave an isothiourea **24**, which on heating with ammonia in DMF gave the guanidine **25** (Scheme 4).

Finally, selected disaccharides were subjected to a two-step deprotection protocol involving the saponification of all esters followed by hydrogenolysis over palladium-charcoal in aqueous buffer (Scheme 5 and Table 2). In this manner novel sialosides either completely lacking subsitution at the 5-position (Table 2, entry 1) or in which the amido function has been replaced by an alkyl chain (Table 2, entry 2) become available for the first time. A variety of N5 amides can readily produced, as exemplified by the important N-glycoyl chain (Table 2, entry 3), and even guanidines (Table 2, entry 4) may be easily accessed in this manner.

Overall, the crystalline sialyl donor **2** affords very highly stereoselective access to a range of sialyl saccharides. Because of the richness of isothiocyanate chemistry, such isothiocyanate-protected saccharides offer direct introduction of a range of standard and novel functionality at the 5-position post-glycosylation, frequently in a single reaction step. In combination with the stereospecific oxidative deamination methods recently developed in our laboratory,[24] this chemistry opens up the 5-position of the sialic acid glycosides, beyond the modified

amides accessible by current methods, as a promising locus for the optimization of their diverse biological properties.

Experimental Section

General coupling protocol

A solution of donor **2** (0.15 mmol), acceptor (0.18 mmol), and activated 4 Å acid-washed powdered molecular sieves (300 mg, 2.0 g/mmol) in anhydrous CH_2Cl_2:MeCN (2:1, 2 mL) was stirred for 5 h under Ar, and then cooled to -78 °C, followed by addition of NIS (42 mg, 0.18 mmol) and TfOH (2 μL, 0.02 mmol). The reaction mixture was stirred at -78 °C for 5 h and then quenched with DIPEA (7 μL). The mixture was diluted with CH_2Cl_2, filtered through Celite, washed with 20% aqueous $Na_2S_2O_3$ solution, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with EtOAc:hexane mixtures to afford the desired coupled product.

General Protocol for Amide Formation from Isothiocyanates

To the required 9-fluorenylmethyl (thioester (0.03 mmol) at room temperature was added piperidine (0.21 mmol) in DMF (500 μL). The reaction mixture was stirred for 15 min, then diluted with $CHCl_3$ (3 mL). The resulting solution was washed with 1*N* HCl aq. (3 mL) and brine (3 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was dried under high vacuum, and dissolved in dry CH_2Cl_2 (0.5 mL) before addition of the isothiocyanate (0.02 mmol). The reaction mixture was stirred for 36 h at room temperature before the volatiles were removed *in vacuo*. The residue was purified by column chromatography on silica gel eluting with EtOAc:hexane mixtures to afford the corresponding amide.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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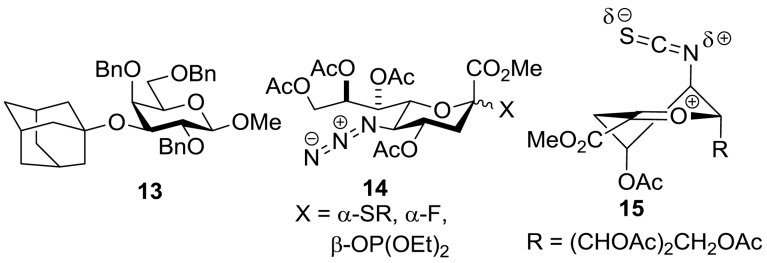


Figure 1. Structures of the Adamantanyl Ether 13, of Azido-Protected Sialyl Donors 14, and of the $^{5}H_{4}$ Conformer the Hypothetical Oxocarbenium Ion 15

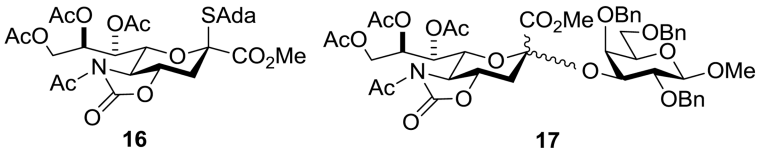
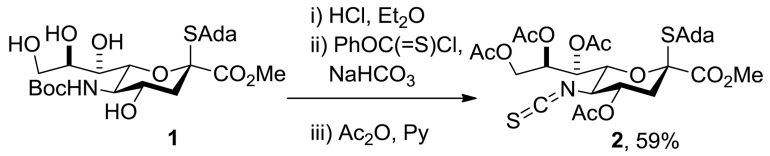
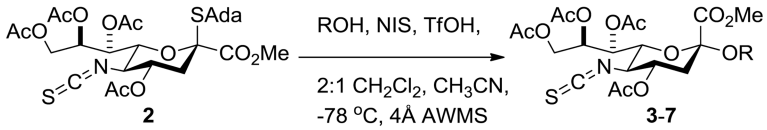


Figure 2. Structures of the *N*-acetyloxazolidionone-protected donor 16 and glycoside 17 from the competition experiment



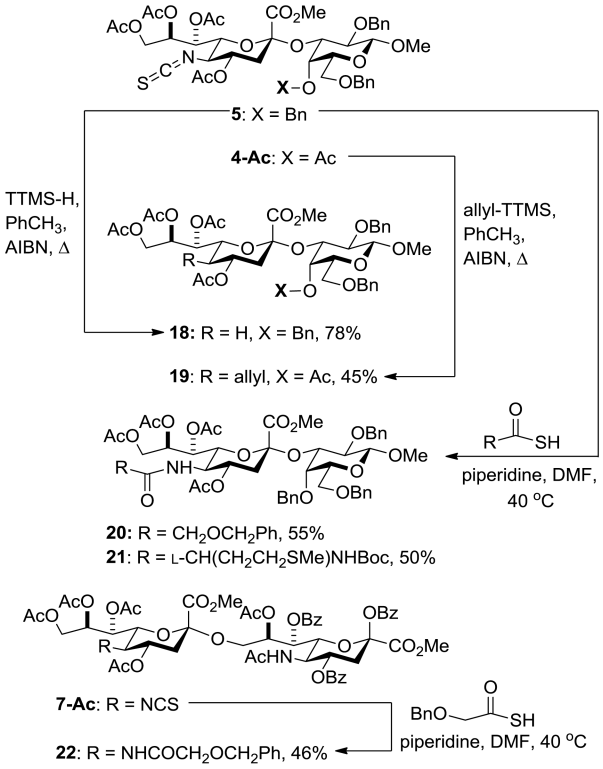
Scheme 1.

Synthesis of the Isothiocyanate **2**. Abbreviations: Ada = 1-adamantanyl, Boc = *tert*-butyloxycarbonyl, Py = pyridine



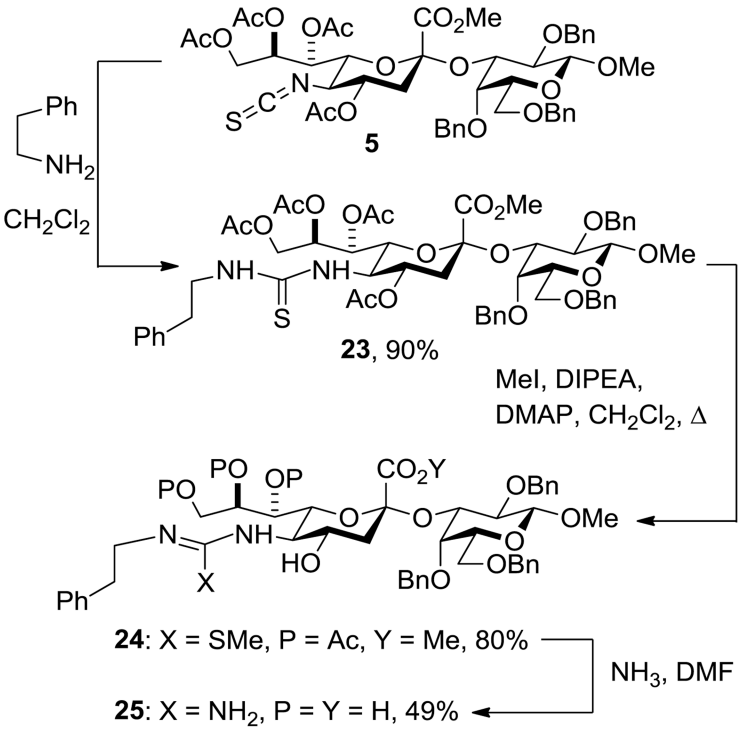
Scheme 2.

Glycosylation with Isothiocyanate **2**. Abbreviations: NIS = *N*-iodosuccinimide, TfOH = trifluoromethanesulfonic acid, AWMS = acid-washed molecular sieves.



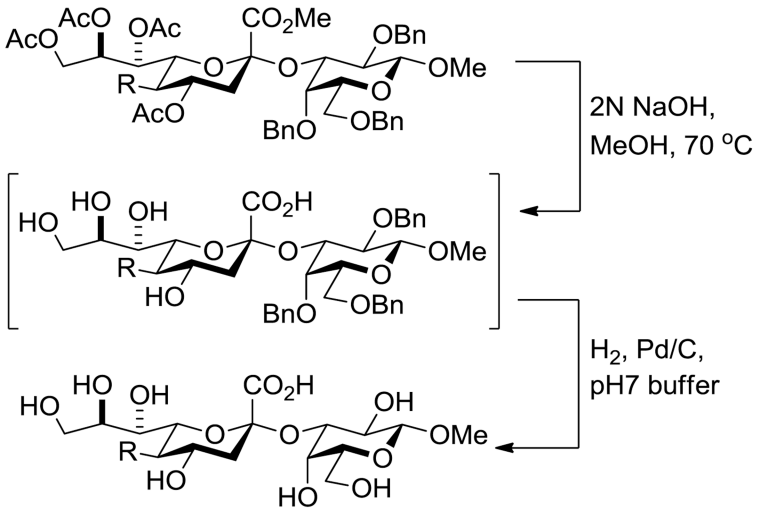
Scheme 3.

Formation of Desamino and Amido Derivatives from Isothiocyanate **5**. Abbreviation: TTMS = tris(trimethylsilyl)silyl



Scheme 4.

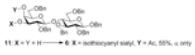
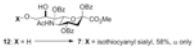
Synthesis of Thiourea and Guanidine Derivatives. Abbreviations: DIPEA = diisopropylethylamine, DMAP = 4-dimethylaminopyridine, DMF = dimethylformamide



Scheme 5. Deprotection of Selected Disaccharides

Table 1

Glycosylation reactions with isothiocyanate 2[a][b]

Entry	Acceptor	Product, Yield, selectivity
1		
2		
3		
4[c]		
5		

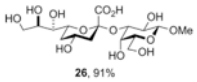
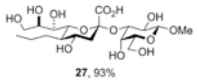
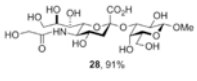
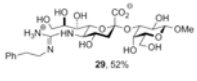
[a] Bn = benzyl, Bz = benzoyl, isothiocyanyl sialyl = [methyl (4,7,8,9-tetra-O-acetyl-3-deoxy-5-isothiocyanyl D-glycero-D-galacto-α,β-nonulopyranosid)onate].

[b] unless otherwise stated all reactions were conducted at -78 °C with 1.2 equiv of acceptor in 2:1 dichloromethane:acetonitrile.

[c] After glycosylation the crude reaction mixture was acetylated to facilitate purification.

Table 2

Deprotection Reactions

Entry	Substrate	Product, Yield, selectivity
1	**18**	 **26**, 91%
2[a]	**19**	 **27**, 93%
3	**20**	 **28**, 91%
4	**25**	 **29**, 52%

[a]Concomitant hydrogenation of the allyl group took place in the course of the hydrogenolytic debenzylation.