Cumulative Coupling Hot Paper

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# The Isothiocyanato Moiety: An Ideal Protecting Group for the Stereoselective Synthesis of Sialic Acid Glycosides and Subsequent Diversification**

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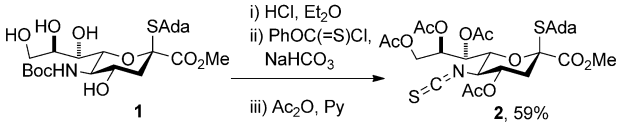
***Abstract:*** *The preparation of a crystalline, peracetyl adamantanyl thiosialoside donor protected by an isothiocyanate group is described. On activation at −78 °C in the presence of typical carbohydrate acceptors, this donor gives high yields of the corresponding sialosides with exquisite α-selectivity. The high selectivity extends to the 4-O-benzyl-protected 3-OH acceptors, which are typically less reactive and selective than galactose 3,4-diols. Treatment of the α-sialosides with tris(trimethylsilyl)silane or allyltris(trimethylsilyl)silane results in replacement of the C5−N5 bond by a C−H or a C−C bond. The reaction of the isothiocyanate-protected sialosides with thioacids generates amides, while reaction with an amine gives a thiourea, which can be converted into 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.*

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 N5 protecting group,[2] and have culminated 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 syntheses, drive the continued search for improved methods.

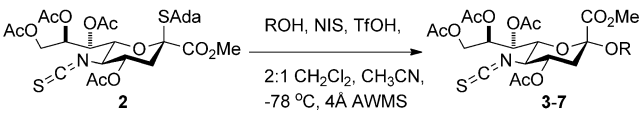
The N5-position also plays a prominent role in the development of sialic acid glycosides with improved properties for application as therapeutic agents and/or vaccines.[6] Such N5-modified systems are produced either chemoenzymatically[6b,7] or chemically by removal of the N5 protecting group post-glycosylation followed by derivatization.[6a,c,8] We now reveal that protection of N5 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-protected sialyl donor,[10] was procured in 59% yield by treatment of the β-*S*-adamantanyl thiosialoside **1**[4b,10] with HCl in diethyl 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.




***Scheme 1.*** Synthesis of the isothiocyanate **2**. Ada = 1-adamantanyl, Boc = *tert*-butyloxycarbonyl, Py = pyridine.

Activation of donor **2** at −78 °C in 2:1 dichloromethane/acetonitrile in the presence of 1.2 equiv of various acceptors (Scheme 2) afforded the corresponding glycosides **3**–**7** as single α-anomers (Table 1). The anomeric configuration of the products was assigned on the basis of the heteronuclear $^{3}J_{C1,H3ax}$ 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-β-D-galactopyranoside **10** in pure dichloromethane at −30 °C, and enabled confirmation of its absence in the coupling reactions conducted at −78 °C in the dichloromethane/acetonitrile 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**; Scheme 3), which arises from the 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




***Scheme 2.*** Glycosylation with isothiocyanate **2**. NIS = *N*-iodosuccinimide, TfOH = trifluoromethanesulfonic acid, AWMS = acid-washed molecular sieves.

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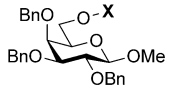
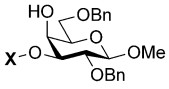
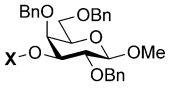
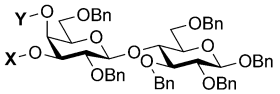
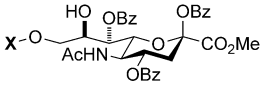
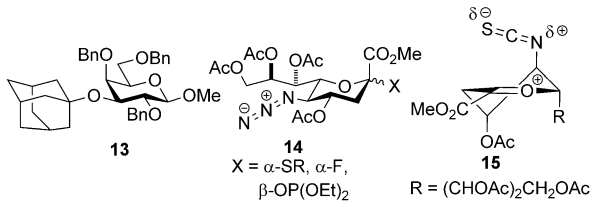
***Table 1:*** Glycosylation reactions with isothiocyanate **2**.[a,b]

| Entry | Acceptor | Product, yield, selectivity |
|---|---|---|
| 1 |  | **8**: **X** = H → **3**: **X** = isothiocyanyl sialyl, 80%, α only |
| 2 |  | **9**: **X** = H → **4**: **X** = isothiocyanyl sialyl, 79%, α only |
| 3 |  | **10**: **X** = H → **5**: **X** = isothiocyanyl sialyl, 87%, α only |
| 4[c] |  | **11**: **X** = **Y** = H → **6**: **X** = isothiocyanyl sialyl, **Y** = Ac, 55%, α only |
| 5 |  | **12**: **X** = H → **7**: **X** = isothiocyanyl sialyl, 58%, α only |

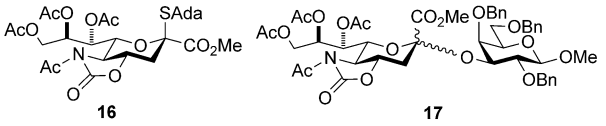
[a] Bn = benzyl, Bz = benzoyl, isothiocyanyl sialyl = methyl (4,7,8,9-tetra-*O*-acetyl-3-deoxy-5-isothiocyanyl D-glycero-D-galacto-α,β-nonulopyranosid)onate. [b] Unless otherwise stated all reactions were conducted at −78 °C with 1.2 equiv of acceptor in 2:1 dichloromethane/acetonitrile. [c] After glycosylation, the crude reaction mixture was acetylated to facilitate purification.




***Scheme 3.*** Structures of the adamantanyl ether **13**, azido-protected sialyl donors **14**, and the $^5H_4$ conformer of the hypothetical oxocarbenium ion **15**.

sialidation reactions, even with the oxazolidinone-protected 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** (Scheme 3),[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 $C_6H_5N{=}C{=}S$: 1.82, 2.43, 2.69 D, respectively),[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**, which benefits from stabilization by the pseudoaxial 4-*O*-acetate and the isothiocyanate groups, with the latter providing significant steric shielding to the β-face (Scheme 3). In a competition experiment designed to begin to probe this question, a 1:1:1 mixture of the isothiocyanate **2**, the *N*-acetyloxazolidinone **16** (Scheme 4), and acceptor **10** was



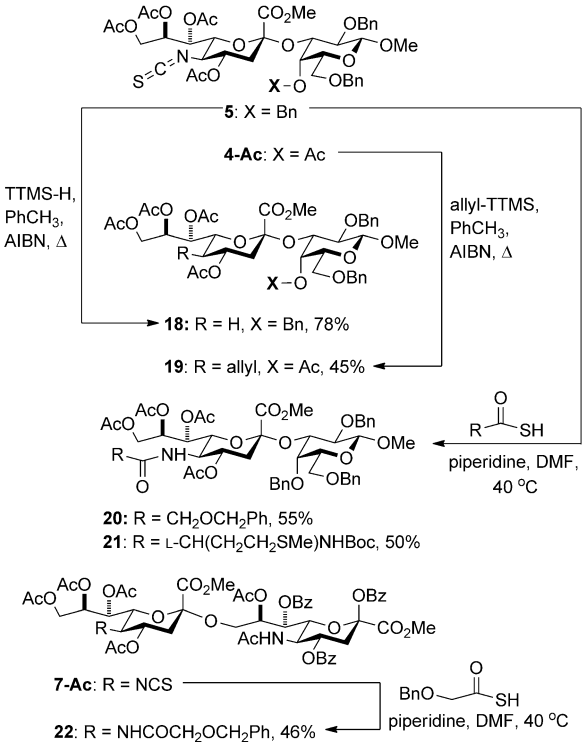

***Scheme 4.*** Structures of the *N*-acetyloxazolidionone-protected donor **16** and glycoside **17** from the competition experiment.

activated at −78 °C by the addition of 0.2 equiv triflic acid. After standard work up, the disaccharides **5** and **17** (Scheme 4) 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 that 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 procedure, heating disaccharide **5** with tris(trimethylsilyl)silane[18] and AIBN in toluene at reflux afforded the 5-deamino-α-sialoside **18** (Scheme 5). Acetylation of the residual alcohol in **4** followed by an 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 5). The reaction of *N*-Boc-L-methionine thioacid and benzyloxythioacetic 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 5). 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** treated with benzyloxythioacetic 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 5).

In a further demonstration of the possibilities afforded by the isothiocyanate group, disaccharide **5** was treated first with

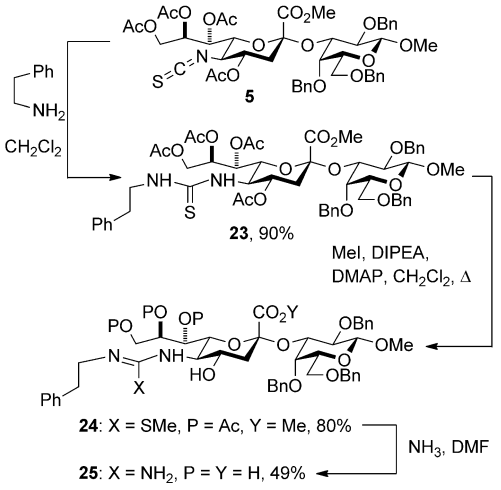
  



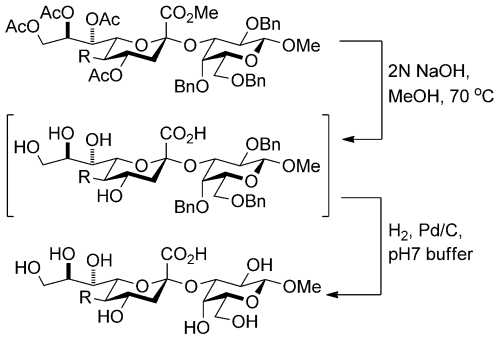

***Scheme 5.*** Formation of desamino and amido derivatives from isothiocyanate **5**. AIBN = azobisisobutyronitrile, TTMS = tris(trimethylsilyl)-silyl.

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 6).

Finally, selected disaccharides were subjected to a two-step deprotection procedure involving the saponification of all esters followed by hydrogenolysis over palladium/charcoal in aqueous buffer (Scheme 7 and Table 2). In this manner,




***Scheme 6.*** Synthesis of thiourea and guanidine derivatives. DIPEA = diisopropylethylamine, DMAP = 4-dimethylaminopyridine, DMF = dimethylformamide.

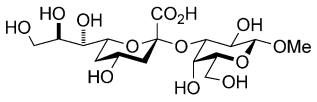
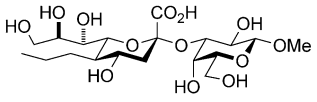
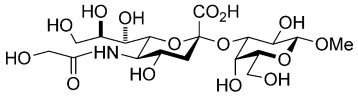
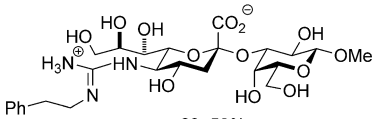



***Scheme 7.*** Deprotection of selected disaccharides.

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 be 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. As a consequence of the richness of isothiocyanate chemistry, such isothiocyanate-protected saccharides offer the 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.

***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.

## Experimental Section

General coupling procedure: 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$^{-1}$) 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 the 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 procedure for amide formation from isothiocyanates: Piperidine (0.21 mmol) in DMF (500 μL) was added to the required 9-fluorenylmethyl thioester (0.03 mmol) at room temperature. The reaction mixture was stirred for 15 min, then diluted with $CHCl_3$ (3 mL). The resulting solution was washed with aq 1 N HCl (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.

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**Keywords:** deamination · glycosylation · guanidine · isothiocyanate · thiourea

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