Synthesis of Lactose-Based S-Linked Sialylmimetics of $\alpha(2,3)$ -Sialosides

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A new approach toward the synthesis of lactose-based S-linked sialylmimetics of $\alpha(2,3)$ -linked sialosides is described. These compounds, represented by the general structure 3, were prepared from methyl β -D-lactoside in 11 steps. It was found that the choice of protecting group was crucial to allow the efficient introduction of sulfur at the 3-position of the galactose ring.

Sialic acids are a family of carbohydrates that, as α -ketosidically linked components of cell surface glycoconjugates, play significant roles in a number of important biological processes.^{1–4} Typically found at the terminus of these glycoconjugates, sialic acids are ideally located to participate in carbohydrate-protein interactions that mediate a range of biological processes. These processes include cell-cell adhesion and communication, microbial pathogenesis, and cellular response to changes in environment.¹⁻⁴ Specific examples of these interactions include the binding of influenza virus haemagglutinin to cell-surface sialic acids⁵ and the interaction of selectins with sialyl Lewis x during the recruitment of leukocytes in the inflammation process.^{6,7}

The growing realization of the importance of α -ketosidically linked sialic acids in biological processes has generated intensive research into sialic acid chemistry and biochemistry.8-11 The complexity associated with the synthesis of structurally modified sialosides as potential inhibitors or probes of sialic acid-recognizing proteins^{8,9,12} has led to great interest in the development of compounds that mimic the key components of sialosides involved in important interactions with biomolecules. These sialylmimetics are structurally simpler than the sialoside upon which they are based, making them more amenable to synthesis as well as having potentially improved pharmacological profiles.^{6,8,13}

N-Acetylneuraminic acid α -(2,3)-linked to galactose (1) is a commonly found epitope in a number of important cellsurface glycoconjugates. This epitope is believed to be the minimum sequence required for the binding of rotavirus to host cells¹⁴ and is overexpressed on the cell surface of some metastatic cancers as a result of the upregulation of $\alpha(2,3)$ -sialyltransferases.¹⁵ N-Acetylneuraminic acid $\alpha(2,3)$ -linked to galactose (1) is also identified as a key recognition sequence of the trypanosomal $\alpha(2,3)$ -trans-sialidase.¹⁶ This same se-

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quence is also found within the biologically significant sialyl Lewis x structure.^{6,7} In light of the importance of *N*-acetyl-neuraminic acid $\alpha(2,3)$ -linked to galactose, several groups have investigated the synthesis of sialylmimetics of this epitope, especially in relation to their interaction with the selectins.^{6,7} Of the multitude of such sialylmimetics that have been reported,^{6–8} the majority are represented by the general structure **2** wherein the entire *N*-acetylneuraminic acid residue has been replaced by a simple carboxylate group that is attached to C-3 of the galactose ring via an ether linkage.



As part of our continued interest in the synthesis of sialylmimetics as probes for sialic acid-recognizing proteins,^{17,18} we had reason to prepare lactose-based *S*-linked sialylmimetics of the general structure **3**. From a retrosynthetic point of view (Scheme 1) the key feature toward sialymimetics



such as **3** is the ability to introduce a thiolacetyl group at the C-3 position of the galactose ring, to give compound **4**.

A study of the literature revealed surprisingly few examples of C-3 thio-substituted galactose derivatives. The C-3 thiolacetyl Gal_f derivative **5** was prepared from the corresponding 3-OTf Gul_f derivative, which itself was obtained in 5 steps from diacetone glucose.¹⁹ The 3-thiolacetyl galactose derivative **6** was prepared in 7 steps from galactose, with the key step involving displacement of the corresponding 3-OTf gulose derivative with KSAc.²⁰ In an alternative approach, the 1,6-anhydro-3-thio-Gal derivative **7** was ob-

tained from the 2,3-episulfide 8.²¹ It was however noted that nucleophilic azide opening of the 2,3-episulfide 8 was "somewhat difficult", and also resulted in the formation of some of the undesired 2-thio-3-azido analogue.²¹ Our own preliminary efforts toward the synthesis of C-3 thio substituted galactosides (e.g., 9) involved displacement of the corresponding 3-OTf gulose derivative 10.²² Interestingly, our studies had shown that the presence of a benzoate group at C-2 in 10 was crucial to the success of the reaction. The use of a benzyl ether at C-2 led to poor yields of the desired C-3 thiolacetylated galactosides, due in part to the generation of eliminated byproducts.²² Bearing these previous reports and our own observations in mind, our initial pivotal target compound was the 3'-thiolacetyl-2'-O-benzoate-lactoside derivative 11.



Of the multitude of possible routes toward 11, our initial strategy involved temporary protection of the 3-position of the galactose ring and then benzoylation of the remaining hydroxyl groups. Accordingly, treatment of methyl β -D-lactoside with 2,2-dimethoxypropane and subsequent benzoylation gave the fully protected lactoside 12. De-O-isopropylidenation of 12 and subsequent ortho ester formation and opening²³ furnished the C-3'-hydroxy lactoside derivative 13 (Scheme 2). Introduction of a triflate at C-3' in 13 followed by inversion with KNO2²⁴ provided the desired C-3' inverted compound 14 in 86% yield. Although the triflate 15 could be readily formed from 14, all attempts at displacement of this triflate with KSAc to give the desired C-3' thiolacetylated product 16 failed. The attempted direct conversion of 14 to 16 using Mitsunobu chemistry²⁵ resulted in the recovery of unreacted 14. Additionally, treatment of 14 with thiourea (in an attempt to form the pseudo thiourea hydrobromide analogue of 16)²⁶ also failed, providing the galacto-configured 3-hydroxy compound 13.

Careful examination of the ¹H NMR data of the products obtained by reaction of **15** with KSAc showed that the acetate group at C-4' had scrambled, presumably through the cyclic

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^{*a*} Conditions: (a) 90% aq TFA, rt, 0.5h, 90%; (b) (MeO)₃CCH₃, *p*-TsOH·H₂O, DMF, rt, 2 h, 93%; (c) Tf₂O, py, CH₂Cl₂, -78 to 0 °C, 1 h; (d) KNO₂, DMF, 50 °C, 16 h; (e) KSAc, DMF.



^{*a*} Conditions: (a) Bu₂SnO, toluene, 110 °C, 16 h; (b) *p*-MeOBnCl, toluene, TBAI, 50 °C, 72h; (c) BnBr, NaH, DMF, 0 °C to rt, 15 h; (d) CAN, CH₃CN, H₂O, 0 °C to rt, 1 h; (e) Tf₂O, py, CH₂Cl₂, -78 to 0 °C, 1 h; (f) KNO₂, DMF, 50 °C, 50 h.

intermediate 17, to give products 18. In an attempt to overcome this problem of neighboring group participation by the C-4' acetate in 15, the strategy shown in Scheme 2 was repeated except that a benzoate was introduced at C-4' using trimethyl orthobenzoate (step b, Scheme 2). Unfortunately, exposure of the triflate 19 to KSAc also failed to furnish any C-3' thiolacetyl lactoside, but rather gave the C-3' hydroxyl derivative 20, also presumably via the cyclic intermediate 21. Clearly, the presence of a participating ester functionality at C-4' in compounds such as 15 and 19 was causing difficulties in our strategy. To prevent this neighboring group participation, we opted to use a benzyl protecting group at C-4'. Accordingly, the selectively benzylated lactoside 22 was prepared from methyl β -D-lactoside (29% over 4 steps), with the key initial step being the dibutylstannylene-mediated²⁷ selective *p*-methoxy-benzyl protection of the C-3' hydroxyl (Scheme 3). Attempted inversion of configuration at C-3' in 22, via the triflate as described above, unfortunately gave a complex reaction mixture from which the desired C-3' inverted derivative 23 could only be obtained in very poor yield.



The complexity of the chemistry associated with the use of benzyl protecting groups, especially during ¹H NMR analysis of reaction products, together with the need to not have a participating group at C-4', prompted us to approach the synthesis of C-3' thiolacetylated lactosides such as **4** using a 4',6'-O-benzylidene protecting group strategy. Accordingly, under standard conditions²⁸ the 4',6'-O-benzylidenated lactoside **24** was obtained from methyl β -D-lactoside. Our need now was to find an appropriate protecting group for the C-3' hydroxyl in **24** that could be easily introduced and also selectively removed in the presence of benzoate protecting groups. After some experimentation, it was found that this could be best achieved using dibutylstannylene oxidemediated acylation²⁹ with chloroacetyl chloride (Scheme 4).



^{*a*} Conditions: (a) Bu₂SnO, MeOH, 65 °C, 2 h; (b) ClAcCl, toluene, DMF, 0 °C, 0.5 h; (c) (i) BzCl, py, DMAP, CH₂Cl₂, 2h, (ii) BzOTf, py, CH₂Cl₂, 1 h, 85%; (d) H₂NNH₂·HOAc, DMF, 40 °C, 2 h, 85%; (e) Tf₂O, py, CH₂Cl₂, -78 to 0 °C; (f) KNO₂, DMF, 50 °C, 16 h, 76%; (g) KSAc, DMF, 50 °C, 16 h, 83%.

In this way, the C-3' chloroacetyl protected lactoside **25** was obtained in 89% yield (based on recovered starting material, **24** ca. 15%).

Complete benzoylation of the hydroxyl groups in **25** to give **26** required the use of benzoyl chloride followed by benzoyl triflate. Using only benzoyl chloride resulted in a

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mixture of tetra-*O*-benzoylated and tri-*O*-benzoylated products, with the latter believed to have the C-2 hydroxyl on the galactose ring unprotected. Exposure of **25** to benzoyl chloride for extended reaction times (>4 h) resulted in the formation of complex reaction mixtures, possibly due to the in situ loss of the chloroacetyl group as well as incomplete benzoylation. Dechloroacetylation of **26** and inversion of stereochemistry at C-3', again via the triflate, gave **27**. To our delight, formation of the C-3' triflate derivative of **27** and subsequent treatment with KSAc afforded the desired C-3' thiolacetylated lactoside derivative **28** in 83% yield over the two steps.

With the desired C-3' thiolacetylated lactoside **28** now readily accessible from methyl β -D-lactoside in nine steps (overall yield 34%), we turned our attention to the preparation of sialylmimetics of the general structure **3**. The approach we utilized was based upon our previously described synthesis of C-6' thio-linked lactose-based sialylmimetics.^{18,30} Accordingly, exposure of the C-3' thiolacetyl lactoside derivative **28** to a series of commercially available α -halo esters in the presence of hydrazine acetate³¹ gave the corresponding lactose-based sialylmimetics **29–35** in consistently excellent yield (indicated in parentheses).

Deprotection of the sialylmimetics 29-35 was readily achieved via a two-step process. First, the ester protecting groups were removed by saponification (NaOH in MeOH), and then the 4',6'-O-benzylidene was cleaved using 10% aq TFA.

Ph
$$O$$
 OBz
R S OBz OBz OBz
29 R = H; R' = Et (90%)
30 R = Me; R' = Et (84%)
31 R = Et; R' = Me (85%)
32 R = Pr; R' = Et (96%)
33 R = Ph: R' = Me (83%)



In this way, the lactose-based sialylmimetics 36-42 were each obtained in >70% yield over the two steps after purification.



In conclusion, we have developed a novel approach toward the synthesis of lactose-based S-linked sialylmimetics of $\alpha(2,3)$ -sialosides. The synthesis of the key C-3' thiolacetylated lactoside **28** from methyl β -D-lactoside is efficient and can be routinely carried out on multigram scale, providing relatively quick access to an advanced precursor. We are currently evaluating some of the sialylmimetics described for their interaction with a variety of sialic acidrecognizing proteins and will report these results in due course.

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Supporting Information Available: ¹H NMR data and spectrum for compound **28**, together with experimental procedures and ¹H NMR data and spectra for selected sialylmimetics. This material is available free of charge via the Internet at http://pubs.acs.org.

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