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Synthesis of Lactose-Based *S*-Linked Sialylmimetics of α(2,3)-Sialosides

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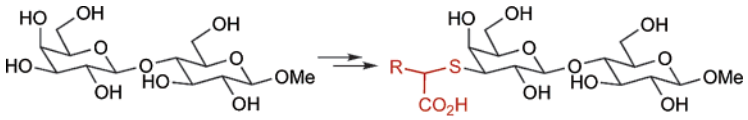
ABSTRACT



3 R = alkyl, aryl

A new approach toward the synthesis of lactose-based *S*-linked sialylmimetics of α(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 acids[5] 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 cell-surface glycoconjugates. This epitope is believed to be the minimum sequence required for the binding of rotavirus to host cells[14] and is overexpressed on the cell surface of some metastatic cancers as a result of the upregulation of α(2,3)-sialyltransferases.[15] *N*-Acetylneuraminic acid α(2,3)-linked to galactose (**1**) is also identified as a key recognition sequence of the trypanosomal α(2,3)-*trans*-sialidase.[16] This same se-

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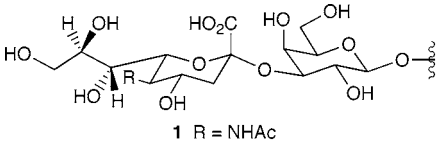
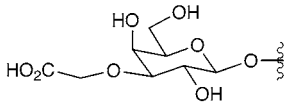
quence is also found within the biologically significant sialyl Lewis x structure.[6,7] In light of the importance of *N*-acetylneuraminic acid α(2,3)-linked to galactose, several groups have investigated the synthesis of sialylmimetics of this epitope, especially in relation to their interaction with 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.



1 R = NHAc



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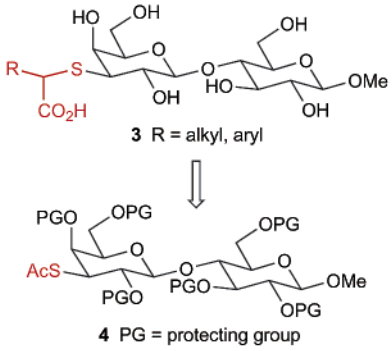
As part of our continued interest in the synthesis of sialylmimetics as probes for sialic acid-recognizing proteins,[17,18] we had reason to prepare lactose-based *S*-linked sialylmimetics of the general structure **3**. From a retrosynthetic point of view (Scheme 1) the key feature toward sialymimetics such as **3** is the ability to introduce a thiolacetyl group at the C-3 position of the galactose ring, to give compound **4**.

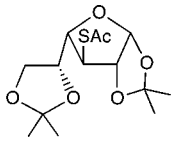
Scheme 1. Retrosynthetic Analysis



3 R = alkyl, aryl

4 PG = protecting group

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 obtained 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**.



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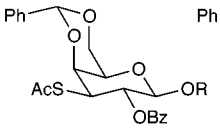
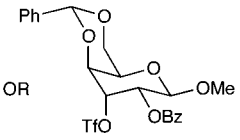


6 R = TDS
9 R = Me



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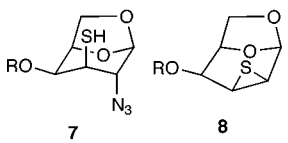

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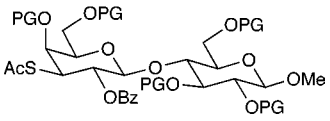

11 PG = protecting group

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 opening[23] furnished the C-3′-hydroxy lactoside derivative **13** (Scheme 2). Introduction of a triflate at C-3′ in **13** followed by inversion with KNO_2[24] 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 chemistry[25] 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 ^{1}H 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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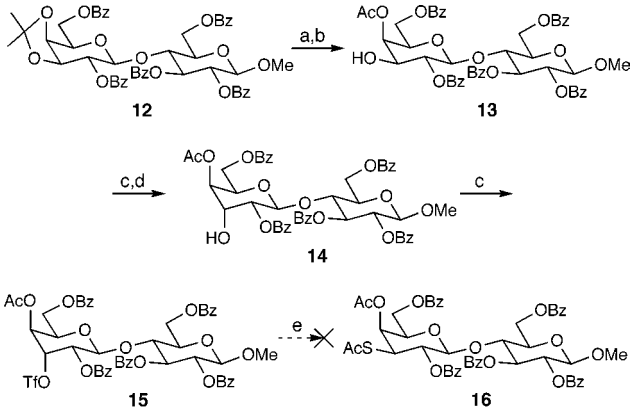
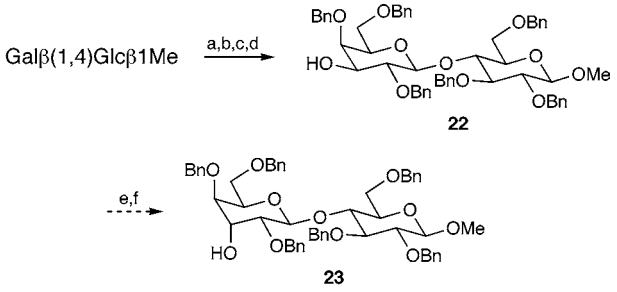
(25) See, for example: von Itzstein, M.; Jenkins, M. J.; Mocerino, M. *Carbohydr. Res.* **1990**, *208*, 287−288.

Scheme 2. Initial Approach toward the Synthesis of C-3′-Thiolacetylated Lactosides[a]



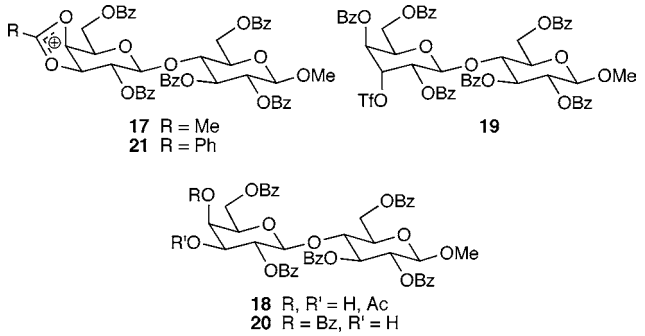
[a] Conditions: (a) 90% aq TFA, rt, 0.5h, 90%; (b) $(MeO)_3CCH_3$, *p*-TsOH·H_2O, DMF, rt, 2 h, 93%; (c) Tf_2O, py, CH_2Cl_2, −78 to 0 °C, 1 h; (d) KNO_2, DMF, 50 °C, 16 h; (e) KSAc, DMF.

Scheme 3. Alternative Approach toward the Synthesis of C-3′-Thiolacetylated Lactosides[a]



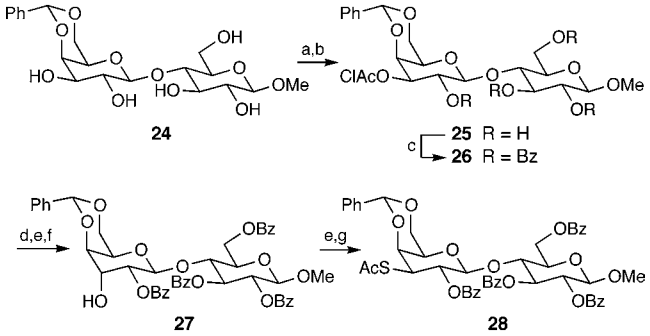
[a] Conditions: (a) Bu_2SnO, toluene, 110 °C, 16 h; (b) *p*-MeOBnCl, toluene, TBAI, 50 °C, 72h; (c) BnBr, NaH, DMF, 0 °C to rt, 15 h; (d) CAN, CH_3CN, H_2O, 0 °C to rt, 1 h; (e) Tf_2O, py, CH_2Cl_2, −78 to 0 °C, 1 h; (f) KNO_2, DMF, 50 °C, 50 h.

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-mediated[27] 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 conditions[28] 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 oxide-mediated acylation[29] with chloroacetyl chloride (Scheme 4).

Scheme 4. Successful Synthesis of C-3′-Thiolacetylated Lactosides[a]



[a] Conditions: (a) Bu_2SnO, MeOH, 65 °C, 2 h; (b) ClAcCl, toluene, DMF, 0 °C, 0.5 h; (c) (i) BzCl, py, DMAP, CH_2Cl_2, 2h, (ii) BzOTf, py, CH_2Cl_2, 1 h, 85%; (d) H_2NNH_2·HOAc, DMF, 40 °C, 2 h, 85%; (e) Tf_2O, py, CH_2Cl_2, −78 to 0 °C; (f) KNO_2, 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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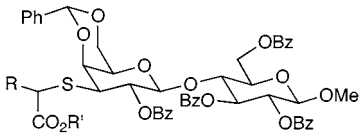
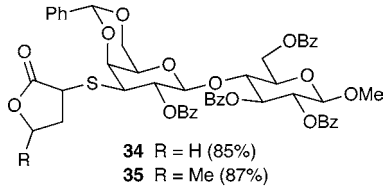
mixture of tetra-*O*-benzoylated and tri-*O*-benzoylated products, with the latter believed to have the C-2 hydroxyl on the galactose ring unprotected. Exposure of **25** to benzoyl chloride for extended reaction times (>4 h) resulted in the formation of complex reaction mixtures, possibly due to the 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 acetate[31] 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.

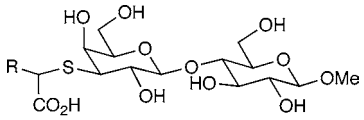


29 R = H; R' = Et (90%)
30 R = Me; R' = Et (84%)
31 R = Et; R' = Me (85%)
32 R = Pr; R' = Et (96%)
33 R = Ph; R' = Me (83%)



34 R = H (85%)
35 R = Me (87%)

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



36 R = H
37 R = Me
38 R = Et
39 R = Pr
40 R = Ph
41 R = CH_2CH_2OH
42 R = $CH_2CH(OH)CH_3$

In conclusion, we have developed a novel approach toward the synthesis of lactose-based *S*-linked sialylmimetics of α(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 acid-recognizing proteins and will report these results in due course.

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

OL035733D

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