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Synthesis of Novel Sialylmimetics as Biological Probes

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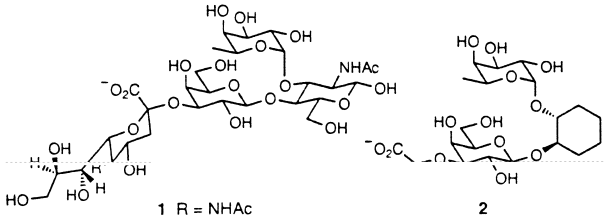
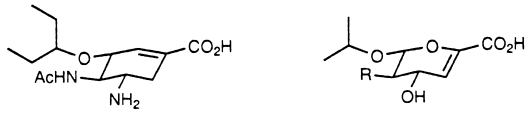
Abstract—Glycomimetics are increasingly being recognised as powerful tools in the search for novel compounds that possess useful biological properties. This paper describes our preliminary efforts towards the development of novel mimetics of sialic acid thioglycosides. These sialylmimetics are readily prepared and have been shown, in some instances, to have biological properties similar to sialic acid thioglycosides. © 2001 Elsevier Science Ltd. All rights reserved.

Sialic acids are involved in a number of important biological processes including cell–cell communication and cell–microbe interactions.[1–4] The importance of sialic acids in these processes, especially with respect to human disease states, has led to interest in the synthesis of natural and modified sialic acids[5–7] both as probes of sialic acid-recognising proteins, and as potential glycopharmaceuticals. The complexity of sialic acid chemistry, particularly in the areas of functional group manipulations and glycosidation reactions,[5,7] together with the inherently poor pharmacological properties of carbohydrate-based compounds has resulted in recent interest in the development of mimetics of sialic acids.[8–12] These sialylmimetics are designed to retain the structural features essential for interaction with a particular protein, but are structurally simpler compounds with potentially improved pharmacological profiles. Some excellent examples of the success of sialylmimetics as potential pharmaceuticals include work directed towards the development of mimetics of sialyl Lewis x (**1**), particularly those designed to inhibit E-selectin. This has resulted in several compounds (e.g., **2**), that exhibit comparable affinity for E-selectin when compared to sialyl Lewis x.[8–10,13] It is pertinent to note that, in addition to significant backbone alterations, the sialylmimetic **2** contains a simple carboxylate group in place of the entire sialic acid moiety present in sialyl Lewis x. Considerable effort has also been directed towards the development of sialylmimetics as inhibitors of sialidases.[14–17] This work has focused on mimetics of 2,3-didehydrosialic acids and includes the carbocyclic derivative **3**,[14] a potent inhibitor of influenza virus sialidase, and mimetics based on uronic acids,[15–17] such as compounds of the general structure **4**.



1 R = NHAc
(0.7mM for E-selectin)

2
(0.5mM for E-selectin)



3

4 R = NHAc or OH

As part of our continued interest in sialic acid chemistry and biochemistry, we have been interested in further developing methods that provide ready access into a range of related sialylmimetics that would serve as probes for a number of sialic acid-recognising proteins. We have also had a long term interest in α-(2,6)-linked thiosialosides typified by **5**, both as metabolically stable biological probes for sialic acid-recognising proteins,[18–20] and as potential inhibitors of rotaviral infection.[21] Our interest in the development of novel sialylmimetics prompted us to investigate the synthesis of sialylmimetics of the general structure **6**, which can be considered as mimetics of thiosialosides like **5**.

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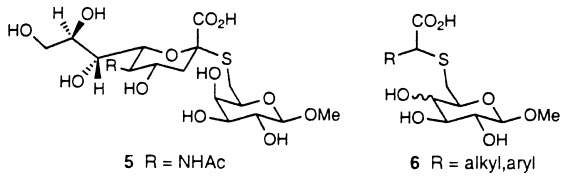
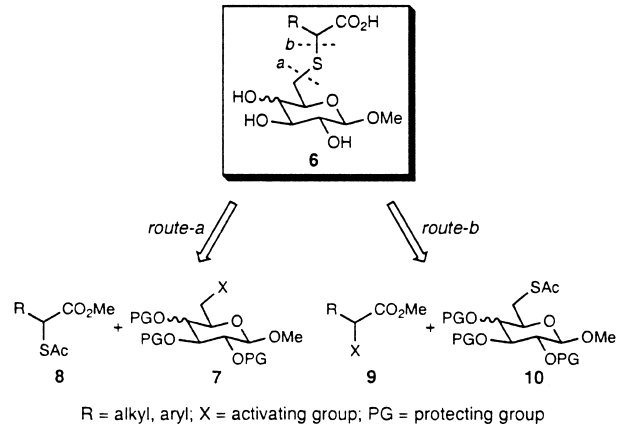


5 R = NHAc 6 R = alkyl,aryl

As can be seen from the proposed sialylmimetic **6**, the entire sialic acid portion has been replaced by a carboxylate functionality and a variable group 'R'. Our intention in opting to prepare sialylmimetics of the general structure **6** was to investigate the effects of replacing the entire sialic acid moiety with a group that maintains the charge present in thiosialosides. In addition, the variable 'R' group in **6** would allow us the opportunity to explore the effects of hydrophobicity, hydrophilicity or steric bulk, on a range of sialic acid-recognising proteins. Importantly, the carboxylate functionality of **6** is in the same relative position as the sialic acid carboxylate group in thiosialosides like **5**. Others have shown that replacement of an entire sialic acid moiety with a carboxylate group can lead to compounds that have improved (or in some cases comparable) affinity for particular proteins.[8]

From a retrosynthetic viewpoint, there are two distinct pathways towards sialylmimetics of the general structure **6** (Scheme 1). The first approach ('*route-a*') involves coupling between a C-6 activated carbohydrate like **7** and the α-thiolacetyl carboxylate derivative **8**. The advantage of this route is that it is analogous to the approach that we have previously used to prepare thiosialosides like **5**,[18,21] and as such we already have access to the requisite C-6 activated carbohydrates **7**. However, a potential drawback of '*route-a*' is that every time we wish to alter the variable 'R' group in sialylmimetics like **6**, we would have to prepare the requisite α-thiolacetyl ester derivative **8**.

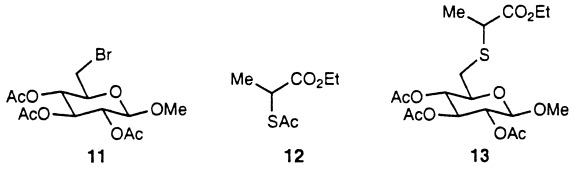
The alternative approach ('*route-b*') involves introduction of the thiolacetyl group onto the carbohydrate portion of the mimetic before coupling with an α-halo-ester derivative (e.g., **9**). Requisite C-6 thiolacetylated carbohydrates of the general structure **10** would be directly available from C-6 activated carbohydrates like **7**. The potential advantage of '*route-b*' is that several α-halo-esters are commercially available, which facilitates the ease of introduction of the variable 'R' group. Comparison of the two routes towards sialylmimetics like **6** depends on the relative ease of preparation of the respective thiolacetyl derivatives **8** and **10**, and the efficiency of the subsequent coupling reactions. To determine the most effective and flexible approach towards sialylmimetics of the general structure **6**, we have examined both routes towards such compounds.



R = alkyl, aryl; X = activating group; PG = protecting group

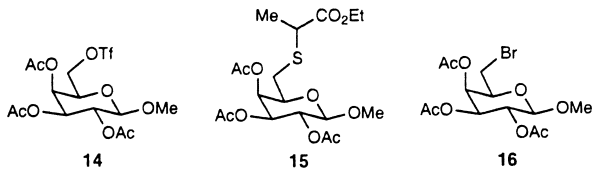
Scheme 1. Retrosynthetic approach towards sialylmimetics.

As depicted in Scheme 1 the synthesis of sialylmimetics via '*route-a*' involves coupling between a C-6 activated carbohydrate derivative (i.e., **7**) and the α-thiolacetyl ester derivative **8**. We have previously described the synthesis of the 6-bromo-6-deoxy glucoside derivative **11** from methyl β-D-glucopyranoside.[18,21] The appropriate coupling partner was prepared by treatment of commercially available ethyl 2-bromopropionate with potassium thioacetate (2.5 equiv) in acetone at room temperature for 18 h, to give the desired α-thiolacetyl derivative **12** in 82% yield after distillation (Kügelrohr bp 70–75 °C @ 5 mmHg). Following our earlier studies towards the synthesis of thiosialosides,[18,21] a mixture of the 6-bromo-6-deoxy glucoside derivative **11** and the thiolacetyl ester derivative **12** was treated with Et_2NH in *N,N*-dimethylformamide at room temperature under N_2. Coupling was found to be extremely slow, with the desired sialylmimetic **13** obtained in a moderate 62% yield after 2 days.



11 12 13

Identification of the product from the coupling reaction between **11** and **12** was evident from examination of the ^{1}H NMR spectrum that showed characteristic signals for the H-6 protons at δ 2.72 and δ 2.89 indicating they are adjacent to sulfur. The remainder of the resonances in the ^{1}H NMR spectrum of **13** are consistent with the structure as shown. Attempts at improving the efficiency of the coupling between **11** and **12** by raising the reaction temperature to 45 °C resulted in extensive decomposition of starting materials. Furthermore, attempted coupling between the α-thiolacetyl ester derivative **12** and the 6-*O*-trifluoromethanesulfonyl galactoside derivative **14**[21] resulted in a complex reaction product containing none of the desired galactose-based sialylmimetic **15**. Interestingly, and contrary to our previous studies towards the synthesis of thiosialosides,[21] exposure of the 6-bromo-6-deoxy-galactoside derivative **16** to **12** in *N,N*-DMF containing Et_2NH at 25 °C smoothly furnished **15** in 71% yield.

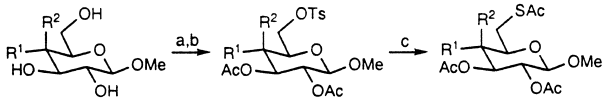


14 15 16

For the alternative approach ('*route-b*' in Scheme 1) to be a viable strategy towards sialylmimetics of the general

structure **6**, we required an efficient synthesis of C-6 thiolacetyl derivatives like **10**. Accordingly, treatment of the previously described[18,21] 6-bromo-6-deoxy derivatives **11** and **16** with potassium thioacetate furnished the corresponding 6-thiolacetyl derivatives **17** and **18**, respectively, in >90% yield.

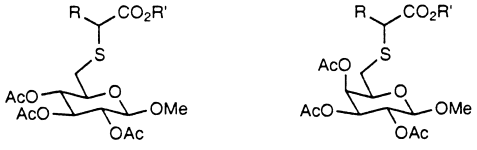
Although this approach had provided access to the desired compounds, the 6-bromo-6-deoxy derivatives **11** and **16** had themselves been derived from the corresponding 6-*O*-*p*-toluenesulfonyl derivatives **19** and **20**.[18,21] We therefore decided a more efficient route to the C-6 thiolacetyl derivatives **17** and **18** would be directly from the 6-*O*-*p*-toluenesulfonyl derivatives **19** and **20**. Towards this end, exposure of the 6-*O*-*p*-toluenesulfonyl derivatives **19** and **20** to potassium thioacetate in acetone at reflux[22] afforded the C-6 thiolacetyl derivatives **17** and **18**, respectively, in ~80% yield after chromatography (Scheme 2). The yield for the introduction of the thiolacetyl group is slightly lower for tosylate displacement versus bromide displacement. However, the overall sequence from commercially available material is more efficient, since one step (bromide displacement of the 6-*O*-*p*-toluenesulfonyl group to give the 6-bromo-6-deoxy derivative) has been eliminated and the overall chemical yield is comparable.



R^1 = OH, R^2 = H; R^1 = H, R^2 = OH — **19** R^1 = OAc, R^2 = H; **20** R^1 = H, R^2 = OAc — **17** R^1 = OAc, R^2 = H; **18** R^1 = H, R^2 = OAc

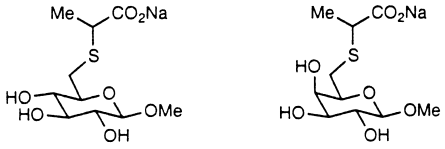
Scheme 2. Synthesis of C-6 thiolacetyl derivatives. Reagents and conditions: (a) TsCl (1.1 equiv), pyridine, 0 °C, 2.5 h; (b) Ac_2O, pyridine, 15 h; (c) KSAc (2.5 equiv), acetone, 55 °C, 48 h.

With the requisite C-6 thiolacetyl derivatives **17** and **18** readily available, our attention turned to their coupling to commercially available α-bromo-ester derivatives. Attempts at coupling the C-6 thiolacetyl glucoside derivative **17** and ethyl 2-bromopropionate, utilising our Et_2NH mediated coupling procedure,[18] resulted in only a modest yield (~50%) of the desired sialylmimetic **13** even after extended reaction times (2 days) at elevated temperature (45 °C). Fortunately, hydrazine acetate mediated coupling[23,24] between the C-6 thiolacetate derivatives **17** and **18** and ethyl 2-bromopropionate afforded the sialylmimetics **13** and **15**, respectively, in high yield. In the same way, the sialylmimetics **21**–**24** were prepared by coupling the C-6 thiolacetyl derivatives **17** and **18** with the corresponding commercially available α-bromo-esters. In all cases the purified products were obtained in yields above 80% after chromatography.



13 R = Me, R' = Et; **21** R = Et, R' = Me; **23** R = Ph, R' = Me — **15** R = Me, R' = Et; **22** R = Et, R' = Me; **24** R = Ph, R' = Me

Although the sialylmimetics **13**, **15**, and **21**–**24** are mixtures of diastereomers, we felt they would serve as useful biological probes for proteins involved in sialic acid recognition processes. As part of our continued interest in the development of α-(2,6)-linked thiosialosides as potential inhibitors of rotavirus,[21] we undertook a preliminary investigation of the rotavirus inhibitory activity of some of the sialylmimetics described above. Although there is some conjecture as to if cell-surface sialic acid residues are essential for recognition by rotavirus, the current consensus is that most strains of rotavirus require sialic acid for the recognition process.[25] Despite reports suggesting that partial *O*-acetylation in the glycerol side chain of sialic acid glycosides results in compounds with improved inhibition of some animal strains of rotavirus,[21,26] it remains to be proven if sialylmimetics like those described above can be recognised by rotavirus. As part of our preliminary investigations towards this end, treatment of the sialylmimetics **13** and **15** with dilute NaOH (1.0 M) gave the deprotected sialylmimetics **25** and **26**, in ~70% yield after HPLC purification.



25 **26**

Analysis of the sialylmimetics **25** and **26** as inhibitors of rotaviral infection involved the use of a standard in vitro neutralisation assay as we have described previously.[21] Neither sialylmimetics **25** nor **26** showed any inhibition against the NCDV (bovine) or the Wa (human) strains of rotavirus (IC_{50} >25 mM for **25** and **26** against both strains).[27] Previously we have reported that the thiosialoside **5** shows some inhibition (IC_{50} 6.25 mM[21]) against the NCDV (bovine) strain of rotavirus. It remains to be determined why the sialylmimetics **25** and **26** do not inhibit rotavirus. It may be that, unlike the interaction of sialyl Lewis x mimetics such as **2** with selectins, the rotavirus recognition/adhesion process requires interaction with sialic acid functional groups in addition to the carboxylate moiety. The incorporation of hydrophilic groups at 'R' in sialylmimetics like **6** may prove interesting in this regard. Alternatively, the sialylmimetics **25** and **26** are only monosaccharides and therefore may not carry sufficient non-sialic acid carbohydrate information for recognition by the rotavirus adhesion protein. It is worth noting that the E-selectin inhibitor, sialylmimetic **2**, does incorporate additional carbohydrate information by virtue of the fucose moiety.

In summary, we have described a simple and efficient synthesis of mimetics of α-(2,6)-linked thiosialosides. The method described provides maximum flexibility in terms of the introduction of alternative functionality in the unit that replaces the sialic acid moiety, and utilises readily accessible starting materials. Although we are yet to demonstrate the usefulness of such sialylmimetics as probes for sialic acid-recognising proteins, we are currently investigating sialylmimetics with different

functionality at the variable 'R' group, as well as alternative mono- and disaccharide templates. Results from the analysis of further sialylmimetics as inhibitors of rotaviral infection, and as probes for sialic acid-recognising proteins, will be published in due course.

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24. General procedure for hydrazine acetate mediated couplings: A solution of the C-6 thiolacetyl derivative (1.0 mmol) in dry DMF (10 mL) was degassed for 20 min by bubbling N_2 into the solution. $H_2NNH_2 \cdot AcOH$ (1.15 mmol) was added and the solution stirred for 30 min before the addition of the α-bromo-ester (1.1 mmol) and Et_3N (1.15 mmol). After 4 h under N_2 the mixture was diluted with EtOAc (20 mL) and washed with dil HCl (1 M, 20 mL), H_2O (20 mL), dried (Na_2SO_4), and concentrated.
25. See for example: Guo, C.-T.; Nakagomi, O.; Mochizuki, M.; Ishida, H.; Kiso, M.; Ohta, Y.; Suzuki, T.; Miyamoto, D.; Hidari, K. I.-P. J.; Suzuki, Y. *J. Biochem.* **1999**, *126*, 683. Delorme, C.; Brüssow, H.; Sidoti, J.; Roche, N.; Karlsson, K.-A.; Neeser, J.-R.; Teneberg, S. *J. Virol.* **2001**, *75*, 2276.
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27. Each sialylmimetic was incubated with a rotavirus strain prior to incubation on MA104 cells. After incubation, virus neutralisation was determined using indirect immunofluorescent staining. Results are expressed as the concentration of sialylmimetic at which 50% infection of control infected monolayers occurred (IC_{50}).