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The synthesis and biological evaluation of lactose-based sialylmimetics as inhibitors of rotaviral infection

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Abstract—Rotaviruses are the most significant cause of gastroenteritis in young children and are responsible for over 600,000 infant deaths annually. The rotaviral haemagglutinin protein (VP8*) of some strains has been implicated in early recognition and binding events of host cell-surface sialoglycoconjugates, and is therefore an attractive target for potential therapeutic intervention. Since *N*-acetylneuraminic acid α(2,3)-linked to galactose is believed to be the minimum binding epitope of rotavirus to host cells, we report here our development of an efficient and flexible synthetic route to a range of lactose-based sialylmimetics of α(2,3)-linked thiosialosides. These compounds were biologically evaluated as inhibitors of rotaviral infection using an in vitro neutralisation assay. The results suggest that these lactose-based sialylmimetics are not inhibitors of the rhesus rotavirus strain; however, they do exhibit modest inhibition of the human (Wa) strain, presumably through inhibition of the rotaviral adhesion process.

1. Introduction

Rotaviral infection is recognised as being responsible for causing severe gastroenteritis in young children,[1] with almost all children, regardless of socioeconomic or environmental conditions, infected by 5 years of age. It has been estimated that over 600,000 infant deaths occur annually, primarily in developing countries, due to diarrhea caused by rotavirus[2] Rotaviruses are also important veterinary pathogens, infecting almost all species of mammals.[3] Although there are several vaccine treatments against rotaviral infection in clinical trials,[4,5] the safety and efficacy of rotaviral vaccines in general has been questioned.[6] The first rotavirus vaccine licensed, Rotashield™ (a tetravalent rhesus-reassortment rotavirus vaccine), was withdrawn in 1999 due to the increased incidence of intussusception.[6–8] More recently, Rota Rix® (GlaxoSmithKline) and RotaTeq® (Merck) have undergone extensive phase III clinical trials, with RotaRix approved for use in Mexico mid-2004. No chemotherapeutic agent has yet been developed to treat rotaviral infection.

Rotaviruses are double-stranded RNA viruses of the family *Reoviridae*.[1] Rotaviruses are highly host-cell specific and sites of mucosal infection are generally limited to mature enterocytes in the mid and upper villous epithelium of the small intestine.[9] Their structures can be simplified as ~1000 Å icosahedral particles consisting of three concentric layers of protein: (1) the outer layer proteins VP4 and VP7; (2) the intermediate layer protein VP6, and (3) the inner core proteins VP1, VP2 and VP3.[1,9] Also known as the inner capsid protein, the core contains the viral genome which consists of 11 segments of AU-rich double-stranded DNA.[1,9] VP4 consists of ~100 Å dimeric spikes that project from the outer core of the virion, and also extend down and interact with VP7 and VP6. The outer layer proteins VP4 and VP7 have been identified as the proteins responsible for receptor binding and cell penetration,[10–14] and independently evoke a response from the immune system as well as being determinants of virulence.[15]

Although the functional properties of VP4 and VP7 have been well defined, the precise cellular recognition site(s) for rotavirus still remains unknown. Elucidation of the

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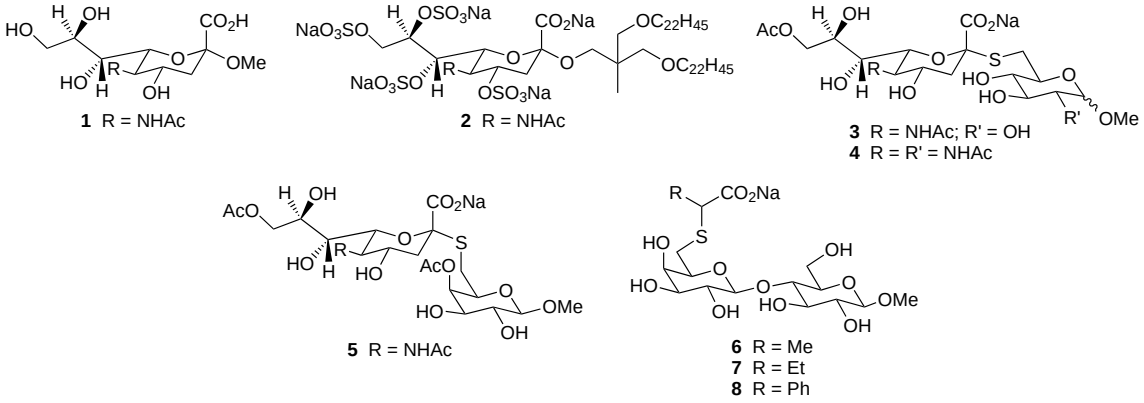
cellular receptor for rotavirus is complicated by the fact that host cell entry is a multi-step process, requiring at least three virus host-cell interactions.[13,14,16–20] The generally accepted model is that rotavirus initially binds to host cell-surface glycoconjugates that contain sialic acid, followed by interactions independent of sialic acid, believed to be part of the lipid domains in which integrins are thought to play an important role.[11,13,16–18,21–24] Details about the precise nature of the initial carbohydrate epitope recognised by rotavirus are complicated by the observations that different rotavirus strains exhibit varying specificities for certain host cell-surface sialoglycoconjugate sequences, although some similarities do exist between human and animal strains.[14,21,23,25] Despite different rotavirus strains recognising different cell-surface sialylglycoconjugates, they can be conveniently classified as being either sialidase-sensitive or sialidase insensitive, which is determined by pre-treatment of cells with a sialidase and then monitoring the change, if any, in the infectivity by rotavirus.[23,26,27] Human strains of rotavirus generally fall into the sialidase-insensitive category, meaning that in vitro treatment of cells with a sialidase has no effect on the ability of human rotaviruses to infect these cells.[23,28,29] It has also been suggested that the initial interaction of rotavirus strains with sialic acids is linked to VP4 P genotype, rather than to species of origin (VP7 G serotype).[30] Several researchers have aimed to investigate the role of sialoglycoconjugates in rotavirus infectivity of host cells.[19,26–28,30–32] In an important investigation, the minimum binding epitope of rotavirus was elucidated to be Neu5Ac-α(2,3)-Gal or Neu5Gc-α(2,3)-Gal after examination of rotavirus binding with 32 different gangliosides.[32] Further studies with chemically modified glycoconjugates determined that the carboxyl group of sialic acid plays an important role in binding to rotavirus.[32] In another study, applied galectins (galactose-recognising proteins) were also found to inhibit rotavirus infection, suggesting that galactose is an important component of the host cell receptor.[21]

Proteolytic treatment of VP4 results in an enhancement of rotavirus infectivity, and leads to the generation of VP8* (the C-terminal fragment responsible for haemagglutination), and VP5* (the N-terminal fragment which contains an internal hydrophobic region and a putative cell fusion region), both of which remain attached to the virion.[33–36] Detailed structural information of VP8* from a rhesus rotavirus strain (RRV) was determined by X-ray crystallography and NMR spectroscopy.[37,38] X-ray crystallography determined the three-dimensional structure of rhesus VP8* in complex with 2-*O*-methyl α-D-*N*-acetylneuraminic acid (α-Me-Neu5Ac, **1**) to 1.4 Å resolution.[37] An interesting observation of the crystal structure reveals a β-sandwich fold that is consistent with the human galectins; however, the VP8* core and the galectins show no significant sequence homology.[37] Between the two β-sheets is a shallow groove that permits Neu5Ac binding to VP8*, and predominant interactions include hydrogen bonding and van der Waals contacts, which are also common to some viral haemagglutinins, such as influenza and Newcastle disease viruses. A subsequent ^{1}H NMR spectroscopic investigation from the same authors further demonstrated that the rhesus VP8* core specifically binds to α-Neu5Ac with a K_d of 1.2 mM.[38] The binding affinities of several α-sialosides were examined by NMR spectroscopy, and it was determined that the haemagglutinin protein requires only α-linked Neu5Ac for binding, irrespective of both the type of carbohydrate the Neu5Ac was linked to and whether the linkage was α(2,3) or α(2,6).[38] The low binding affinities and broad specificity shown by sialic acid binding to the rhesus rotavirus haemagglutinin are consistent with the hypothesis that sialic acid mediates an initial interaction with the host cell that precedes more specific interactions determining rotavirus cell type and host specificity.

The apparent important initial role that sialic acids play in the infection process by rotavirus implies that this may be a good target for therapeutic intervention. In this regard, the highly sulfated sialoside **2** (NMSO3) was found to inhibit four human strains of rotavirus with IC_{50} values in the micromolar range.[39] It was determined that NMSO3 (**2**) was blocking the binding of rotavirus through the VP4 or VP7 proteins, and in an in vitro study, prophylactic oral administration of NMSO3 (**2**) to suckling mice prior to inoculation with the human (MO) strain of rotavirus prevented the onset of diarrhoea.[39] We have previously reported the synthesis and biological evaluation of α(2,6)-linked thiosialosides, for example, **3**–**5**,[40] as well as the lactose-based S-linked sialylmimetics of α(2,6)-thiosialosides **6**–**8**[41] as potential inhibitors of rotaviral infection. The partially acetylated thiosialosides **3**–**5** showed modest rotaviral inhibition, with IC_{50} values in the 10^{-3} M range, against the bovine (NCDV) strain of rotavirus.[40] These results were in concordance with the literature, in which earlier studies by Willoughby and Yolken found that partially acetylated derivatives of Neu5Ac exhibited improved inhibitory activity, in comparison to the non-acetylated versions, against the bovine (NCDV) rotavirus strain.[42] The lactose-based S-linked sialylmimetics of α(2,6)-thiosialosides **6**–**8** were designed to mimic the biologically relevant functionalities of thiosialosides like **3**, in particular the carboxylate functionality of the Neu5Ac residue. The 'methyl' and 'ethyl' lactose-based sialylmimetics **6** and **7**, respectively, showed modest inhibition against both the bovine (NCDV) and human (Wa) strains of rotavirus, whilst the 'phenyl' sialylmimetic **8** was only active against the bovine (NCDV) strain.[41] These results suggested that small alkyl groups are tolerated in the binding pocket of the viral adhesion proteins of the bovine (NCDV) and human (Wa) strains, and that aryl groups may be too sterically demanding (or too hydrophobic) for the human (Wa) strain of rotavirus. Although the level of inhibition is only moderate, these were the first examples of simple carbohydrate derivatives exhibiting inhibition of a human strain of rotavirus.[41]

N-Acetylneuraminic acid α(2,3)-linked to galactose is not only believed to be the minimum binding epitope of rotavirus to host cells,[32] but is a commonly found epitope in several important cell-surface glycoconjugates, and is associated with diseases like cancer (through the



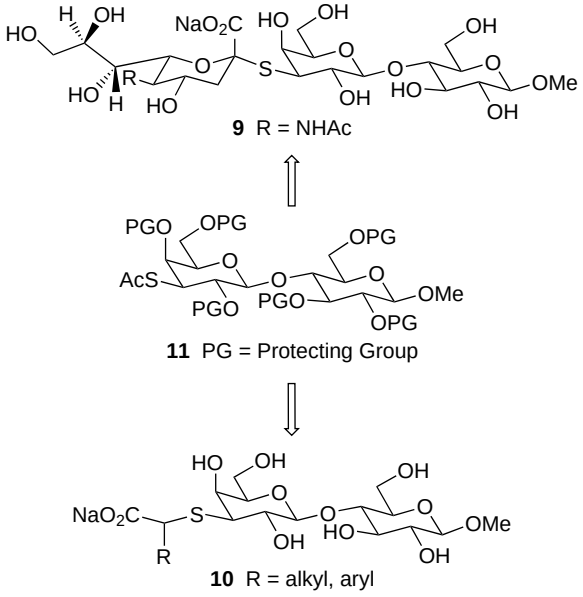
upregulation of α(2,3)-sialyltransferases)[43] and Chagas' disease (and the involvement of *Trypanosoma cruzi trans*-sialidase).[44] In our ongoing endeavour to develop novel sialylmimetics as biological probes for sialic acid-recognising proteins, this paper describes our efforts towards the synthesis of Neu5Ac2-*S*-α(2,3)-Lacβ1Me **9** and lactose-based S-linked sialylmimetics, represented by the general structure **10**, that mimic the key elements of α(2,3)-linked thiosialosides like **9**. Sialylmimetics of the general structure **10** do not need the presence of sulfur to protect them from sialidase-catalysed hydrolysis (as is the case with compound **9**). However, our continued interest in the preparation of sulfur linked sialylmimetics, together with the fact that we can introduce structural variability into our target compounds late in the synthetic sequence (vide infra), prompted us to prepare sialylmimetics like **10**. The biological evaluation of compounds like **9** and **10** as potential inhibitors of rotaviral infection is also described.

2. Results and discussion

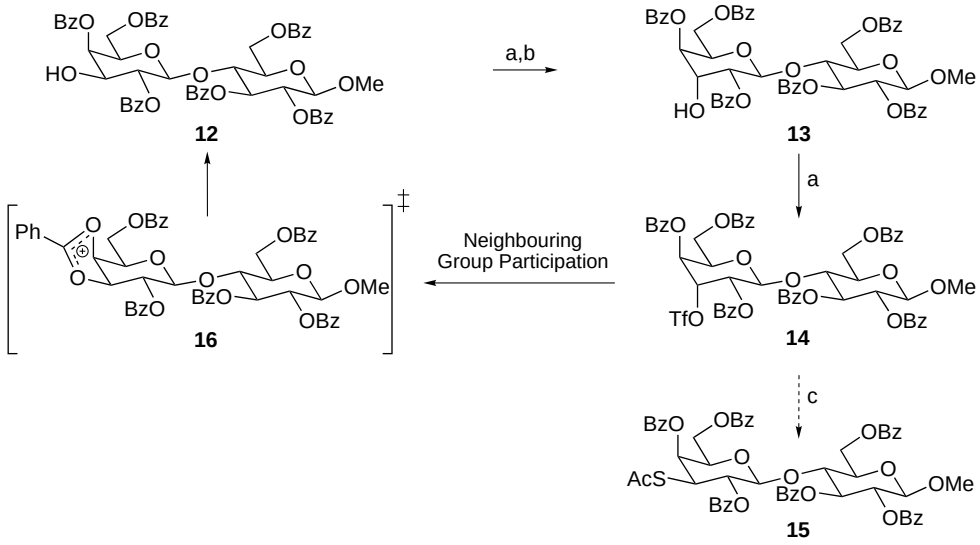
2.1. Synthesis of S-linked α(2,3)-sialyllactoside and sialylmimetics

An important consideration in designing compounds like **9** and **10** as probes for sialic acid-recognising proteins is the metabolic stability of such compounds. In this regard, it has been shown that the thioglycosidic linkage in thiosialosides is resistant to hydrolysis by sialidases.[45] Accordingly, compound **9** was designed with a thioglycosidic linkage to impart a degree of metabolic stability in the final target compound. The lactose-based S-linked sialylmimetics represented by the general structure **10**, wherein the entire Neu5Ac portion has been replaced by a carboxylate group with an appended hydrophobic or hydrophilic moiety, can essentially be considered as mimetics of the α(2,3)-linked thiosialoside **9**. The inclusion of varying functionality in the mimetic portion of **10** was designed to explore the effects of hydrophobic, hydrophilic and sterically demanding groups upon their interaction with a given biomolecule such as rotavirus VP8*. Our approach, as outlined in retrosynthetic terms in Scheme 1, relies on both the thiosialoside **9** and the sialylmimetics of the general structure **10** being accessible by a common C-3′ thiolacetyl lactoside precursor such as **11**.

Typically, transformations involving the introduction of a sulfur functionality in pyranosides can be accomplished by displacement of a leaving group with an appropriate nucleophile,[46] through Mitsunobu chemistry[47] or via the opening of epoxides.[48] We have reported elsewhere a double inversion sequence at the C-3′ position of a lactoside for the successful introduction of a thiolacetate group to give a compound like **11**.[49] From these investigations it was found that a non-participating functionality at C-4′, like a benzylidene acetal, was critical for a double inversion sequence at C-3′ to give the key lactoside **11**.[49] For example, activation of the C-3′ hydroxyl in **12** with a triflate, followed by S_N2 displacement using KNO_2, afforded the desired *gulo*-configured derivative **13** in 84% yield (Scheme 2). However, subsequent formation of the C-3′ triflate **14**, followed by treatment with KSAc, did not yield the desired C-3′ thiolacetylated derivative **15** but the *galacto*-configured 3′-hydroxy compound **12** (Scheme 2).[49] This observation suggested the major reaction pathway to proceed via neighbouring group participation of the C-4′ benzoate in **14**, presumably via the cyclic intermediate **16** shown in Scheme 2. We also observed a similar outcome when other acyl protecting groups at C-4′, like



Scheme 1. Retrosynthetic approach towards the thiosialoside **9** and the sialylmimetics **10**.

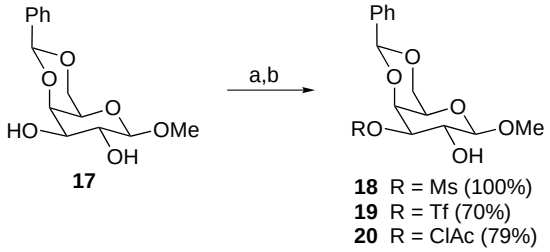


Scheme 2. Reagents and conditions: (a) Tf_2O, pyridine, CH_2Cl_2, −78 to 0 °C, 1 h; (b) KNO_2, DMF, 50 °C, 4 h, 84%; (c) KSAc, DMF, 50 °C, 16 h.

an acetate group, were employed in this sequence of reactions.[49] Subsequent to our findings, there have been other reports of the preparation of C-3′ thiolacetylated lactoside derivatives like **11** that support our findings in relation to the importance of protecting group choice.[50,51]

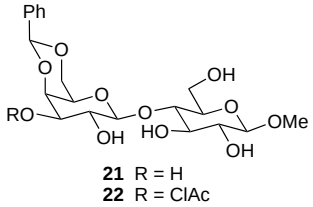
A common transformation for preparing C-3′ functionalised lactoside derivatives generally involves the use of dibutylstannylidene mediated alkylation with *p*-methoxy-benzyl chloride[52] or allylbromide.[53] In most cases, these temporary C-3′ alkyl protecting groups can be selectively cleaved in the presence of most other protecting groups to reveal the hydroxyl group for further elaboration.[54] In our hands, etherifications of this type had proved problematic due to the poor reactivities of the alkyl electrophiles over extended reaction times, resulting in poor yields. We required the synthesis of a key lactoside like **11** with 4′,6′-*O*-bezylidene acetal protection to be efficient in terms of overall chemical yield, to be reproducible on a multi-gram scale, and to provide relatively quick access to an advanced precursor for the preparation of a range of compounds like **10**. To this end, we felt it appropriate to explore regioselective manipulations mediated by dibutyltin oxide.[55,56] using sulfonyl and acyl electrophiles on the 4,6-*O*-benzylidenated galactoside **17** as a model system. Accordingly, **17** was treated with Bu_2SnO in MeOH followed by exposure of the intermediate dibutylstannylidene acetal derivative to either MsCl, Tf_2O and ClAcCl (Scheme 3). All attempts at selectively functionalising the C-3 hydroxyl in **17** were successful. Mesylation was quantitative and afforded exclusively the C-3 functionalised product **18** (Scheme 3). Also, treatment of **17** with Tf_2O via the 2,3-*O*-stannylidene acetal afforded the corresponding C-3 triflate **19** in 70% yield (Scheme 3). Additionally, chloroacetylation of **17** with ClAcCl also yielded the C-3 derivatised product **20** (Scheme 3), although 20% of the 2,3-di-*O*-chloroacetylated compound was also isolated.

With such encouraging results on the galactoside **17**, this chemistry was explored on the known[57] 4′,6′-*O*-benzylidenated lactoside **21**. The initial observation from these

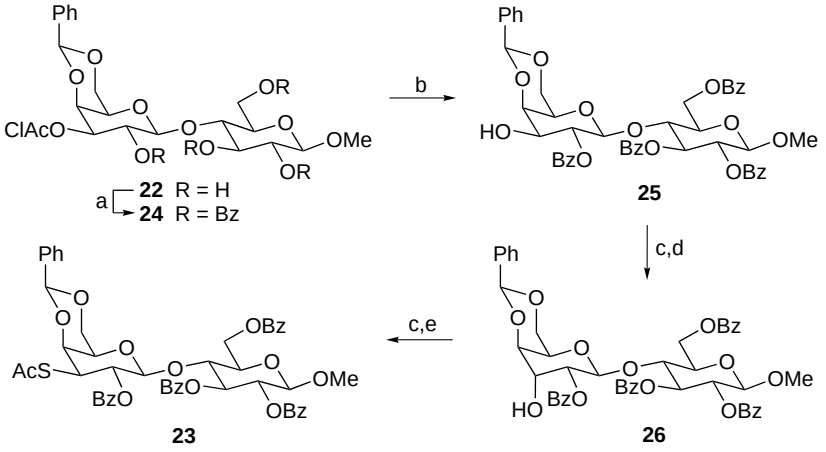


Scheme 3. Reagents and conditions: (a) Bu_2SnO, MeOH, reflux, 2 h; (b) 4 Å sieves, electrophile, toluene, 0 °C, 30 min.

experiments was that they were not as promising as those described for the galactoside model system **17**. The dibutylstannylidene mediated sulfonylations of the 4′,6′-*O*-benzylidenated lactoside **21** afforded complex mixtures of products, in which unreacted starting material was always present. Fortunately, with ClAcCl as the electrophile, the C-3′ chloracetylated protected lactoside **22** was obtained in 70% yield, although a small amount (ca. 19%) of the starting material was also always recovered.



Starting with the C-3′ chloroacetylated lactoside **22**, the successful synthesis of the desired C-3′ thiolacetylated lactoside **23**[49] is shown in Scheme 4. Briefly, complete benzoylation of the hydroxyl groups in the C3′-chloroacetate derivative **22** was achieved by using benzoyl chloride and then BzOTf[58] to give the tetra-*O*-benzoylated lactoside **24**. Dechloroacetylation of **24** with hydrazinium acetate[59] gave the C-3′ hydroxyl derivative **25** in 78% yield. Inversion of configuration at C-3′ via

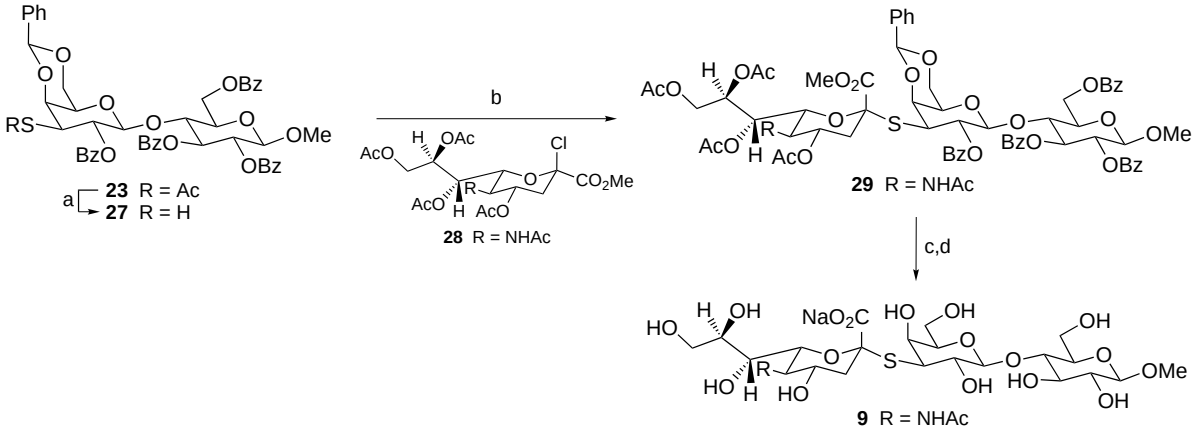


Scheme 4. Reagents and conditions: (a) 4 Å sieves, BzCl, pyridine, CH_2Cl_2, 0 °C to rt, 2 h, and then 4 Å sieves, BzOTf, pyridine, CH_2Cl_2, −78 °C to room temperature, 3 h, 85%; (b) $H_2NNH_2 \cdot HOAc$, DMF, 40 °C, 2 h, 78%; (c) Tf_2O, pyridine, CH_2Cl_2, −78 to 0 °C, 1–2 h; (d) KNO_2, DMF, 50 °C, 16 h, 76%; (e) KSAc, DMF, 50 °C, 16 h, 83%.

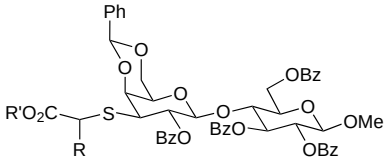
activation with a triflate and subsequent displacement with KNO_2[60] afforded the *gulo*-configured derivative **26** in good yield. Formation of the C-3′ triflate derivative of **26** and treatment with KSAc afforded the desired C-3′ thiolacetylated lactoside derivative **23** in 83% yield over the two steps.

Having prepared the key C-3′ thiolacetylated lactoside derivative **23**, our attention was directed towards the synthesis of Neu5Ac2-*S*-α(2,3)-Lacβ1Me (**9**). Although the first reported synthesis of a lactose-based S-glycosidation reaction to form an α(2,3)-linked thiosialoside was only published recently,[51] the general concept is well established. Our strategy towards the synthesis of **9** employs the successful approach described by Schmidt and coworkers for the synthesis of Neu5Ac2-*S*-α(2,3)-Gal derivatives.[61–63] Preparation of the glycosyl acceptor was achieved by reaction of **23** with hydrazinium acetate[64] to liberate the thiol **27**. Exposure of **27** to the sialosyl chloride **28**[65,66] under base (NaH) promoted S-glycosidation conditions furnished the desired thiosialoside **29** in 57% yield (Scheme 5). Deprotection of the α(2,3)-linked thiosialoside **29** was readily achieved via a two-step process involving initial saponification of the ester protecting groups (NaOH in MeOH) followed by removal of the 4′,6′-*O*-benzylidene acetal (10% aqueous TFA) to afford the target compound **9** in good yield, after formation of the sodium salt and HPLC purification.

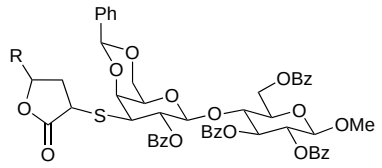
The α(2,3)-linked lactose-based sialylmimetics of the general structure **10** were prepared according to the procedure we have successfully employed previously in the synthesis of a variety of α(2,6)-linked galactose-, glucose- and lactose-based sialylmimetics.[41,67] The general approach is shown in Scheme 6 and involves the selective in situ de-S-acetylation of the C-3′ thiolacetate lactoside **23** with hydrazinium acetate[64] and subsequent reaction with commercially available α-halo-esters in the presence of Et_3N to give the sialylmimetics **30** in excellent yield (Scheme 6). Deprotection of sialylmimetics **30** was readily achieved under the same conditions described for deprotection of the thiosialoside **29**, wherein saponification of the ester protecting groups (NaOH in MeOH) followed by removal of the 4′,6′-*O*-benzylidene acetal (10% aqueous TFA) gave the deprotected lactose-based sialylmimetics **10** in generally high yield after HPLC purification (Scheme 6).



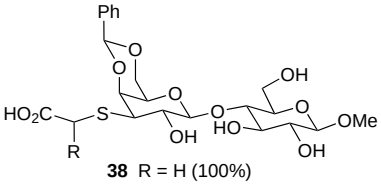
Scheme 5. Reagents and conditions: (a) $H_2NNH_2 \cdot HOAc$, DMF, 3 h, 78%; (b) 4 Å sieves, **28**, NaH, Kryptofix®, THF, 0 °C to rt, ~2 h, 57%; (c) 1 M NaOH, MeOH, rt, 24 h, quantitative; (d) 10% aq. TFA, 0 °C, 8 h, 64%.



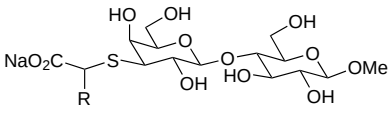
31 R = H; R' = Et (90%)
32 R = Me; R' = Et (84%)
33 R = Et; R' = Me (85%)
34 R = Pr; R' = Et (96%)
35 R = Ph; R' = Me (90%)



36 R = H (85%)
37 R = Me (87%)



38 R = H (100%)
39 R = Me (95%)
40 R = Et (97%)
41 R = Pr (100%)
42 R = Ph (100%)
43 R = CH_2CH_2OH (100%)
44 R = $CH_2CH(OH)CH_3$ (88%)



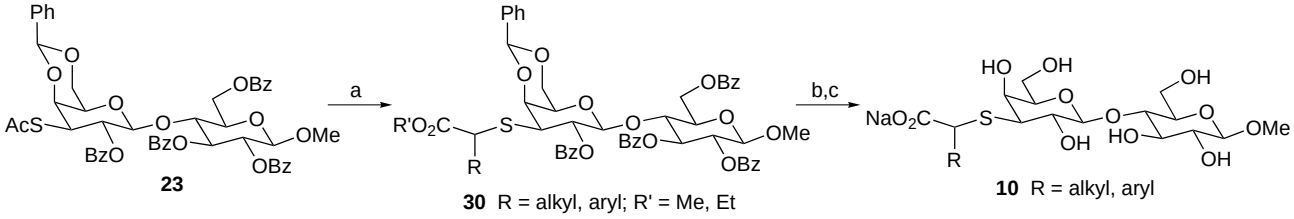
45 R = H (67%)
46 R = Me (66%)
47 R = Et (65%)
48 R = Pr (69%)
49 R = Ph (69%)
50 R = CH_2CH_2OH (70%)
51 R = $CH_2CH(OH)CH_3$ (68%)

The lactose-based sialylmimetics **31**–**37** were prepared in this way.[49] Since the α-halo-esters employed in the coupling with **23** to give the products **32**–**37** were racemic, the products were obtained as mixtures of diastereomers. Interestingly however, it was found that the majority of sialylmimetics **32**–**37** were formed with one diastereomer predominating over the other. Indeed, several of the sialylmimetics were obtained as 2:1 mixtures. Whilst there is no clear explanation for this outcome, the implication from this may be that one face of the α-bromo-ester electrophile is less accessible to the intermediate thiolate nucleophile than the other, presumably due to hydrogen bonding or unfavourable steric interactions with the benzoyl protecting groups. In any event, the high yielding nature of these coupling reactions, together with the fact that racemisation of the undefined chiral centre in the mimetic portion will occur during deprotection, means that this issue was not further pursued. Saponification of compounds **31**–**37** with dilute NaOH in MeOH gave the de-esterified products **38**–**44** in excellent yield, and following debenzylidenation (90% aq. TFA) and HPLC purification, the target compounds **45**–**51** were obtained in consistently high yields.

2.2. Biological evaluation of sialylmimetics as inhibitors of rotaviral infection

Some of the synthesised sialylmimetics were evaluated as potential inhibitors of rotaviral infection using a standard in vitro neutralisation assay[40,41] as described in Section 4. In summary, the assay involves preincubation of the sialylmimetic to be evaluated with either a rhesus (RRV) or human (Wa) strain of rotavirus, prior to incubation onto MA104 cells (an African Green Monkey kidney cell line). After incubation, virus neutralisation was determined using indirect immunofluorescent staining. The results from these biological evaluations are shown in Table 1 and indicate the ability of the sialylmimetic at 10 mM to cause a decrease in rotavirus infectivity of MA104 cells (expressed as a percentage inhibition), unless otherwise indicated.

As the results in Table 1 indicate, none of the sialylmimetics showed any significant inhibition against the rhesus (RRV) strain of rotavirus. The lack of inhibition is perhaps a reflection on the apparent preference for rhesus (RRV) VP8* to recognise α-linked sialic acid only. This is based upon the observation by Dormitzer et al. that the haemagglutinin



23 **30** R = alkyl, aryl; R' = Me, Et **10** R = alkyl, aryl

Scheme 6. Reagents and conditions: (a) 4 Å sieves, H_2NNH_2HOAc, α-halo-ester, Et_3N, DMF, rt, 2 h; (b) 1 M NaOH, MeOH, rt, 24 h; (c) 10% aq. TFA, 0 °C, 8 h.

Table 1. Inhibition of rotavirus by sialylmimetics[a]

Compound	RRV (rhesus)	Wa (human)
9	31	n.t.[b]
45	10	21 (5 mM)
46	15	37
47	<10	30
48	<10	25
49	<10	25
50	<10	0

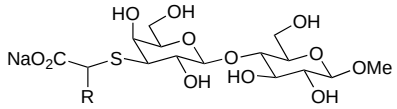
[a] Results are expressed as a percentage inhibition and indicate the decrease in rotavirus infectivity of MA104 cells at 10 mM concentration of compound, unless otherwise indicated.
[b] n.t. indicates the compound was not tested.

(VP8*) of RRV required only α-linked Neu5Ac for binding, and this was irrespective of both the type of carbohydrate the Neu5Ac was linked to, and whether the linkage was α(2,3) or α(2,6).[38] Our previously published results with lactose-based sialylmimetics of α(2,6)-linkages also support this notion with respect to rhesus (RRV) rotavirus.[41] The results obtained from the biological evaluation of the α(2,3)-linked thiosialoside **9** (Table 1) are also consistent with the known specificity of the rhesus (RRV) strain of rotavirus. As expected, the sialoside **9** showed modest inhibition against rhesus rotavirus and is simply an indication that the haemagglutinin protein recognises the Neu5Ac unit of the thiosialoside **9**.

The results shown in Table 1 for inhibition of the human (Wa) strain of rotavirus by the lactose-based sialylmimetics of α(2,3)-sialosides are somewhat more encouraging. Most of these sialylmimetics appear to be modest inhibitors of the human (Wa) strain of rotavirus in comparison to the rhesus (RRV) strain. The observation that the α(2,3)-linked mimetics shown in Table 1 show modest inhibition of Wa (human) rotavirus is consistent with our earlier studies into the inhibition of rotavirus by lactose-based α(2,6)-linked mimetics.[41] These previous studies found that the lactose-based sialylmimetic **7** exhibited 50% inhibition of human rotavirus at 6.25 mM.[41] At this stage it is not appropriate to draw too many conclusions from the data presented here (Table 1), given that no structural information from the human (Wa) haemagglutinin is available. However, it could be speculated that the mimetic portion (alkyl and aryl groups) in these sialylmimetics is tolerated by the human (Wa) strain of rotavirus, either through binding to the haemagglutinin protein or by some other unknown viral adhesion process.

3. Conclusion

In conclusion, we have developed an efficient synthesis of Neu5Ac2-*S*-α(2,3)-Lacβ1Me **9** and have developed a flexible route towards a range of novel lactose-based sialylmimetics of the general structure **10** that mimic the key elements of the thiosialoside **9**. The work described here has contributed towards the development of biological probes and possible inhibitors for sialic acid-recognising proteins that naturally recognise the Neu5Ac-α(2,3)-Gal epitope in general, and has provided a better understanding of the interactions between sialosides or sialylmimetics and rotavirus. The preliminary biological results obtained by evaluation of these compounds against the human (Wa) strain of rotavirus provide some interesting data and clearly show that these compounds interfere in the infectivity of rotavirus in vitro. The albeit modest inhibition shown by these sialylmimetics against the human (Wa) strain of rotavirus provides a valuable starting point for the development of sialylmimetics with improved binding affinities and may ultimately lead to the discovery of a potential chemotherapeutic agent for rotaviral infection.



10 R = alkyl, aryl

4. Experimental section

4.1. General

Infrared spectra were recorded on a Brüker Optik Vector 22 instrument using OPUS (version 3.01) software. IR spectra were obtained in neat $CHCl_3$ solutions between KBr plates. ^1H and ^{13}C NMR spectra were recorded using a Brüker Avance-300 spectrometer unless indicated otherwise. Chemical shifts are expressed in ppm (δ) relative to the solvent used ($CDCl_3$: 7.27, 77.0 for ^1H and ^{13}C, respectively) or relative to external Me_4Si for D_2O spectra. Where due reference is made, chemical shifts indicated with a multiplication (×) descriptor correspond to more than one signal of the same functionality. Assignments indicated with an asterisk (*) correspond to those resonances clearly due to the corresponding diastereomer where such mixtures exist, while (†) indicates overlapping signals. Two dimensional ^1H–^1H COSY and ^1H–^{13}C HSQC NMR spectra were obtained where necessary, in order to assist with spectral assignment. ESI low resolution mass spectra were recorded on a Brüker Daltonics® Esquire 3000 Ion-Trap LC MS, using the positive mode with samples introduced at 180 μL/h. High resolution mass spectrometry was performed at the Department of Chemistry, University of Queensland, Australia, on a Finnigan MAT 900 XL Trap with a Finnigan AP1 III sprayer. Microanalyses were performed at the Department of Chemistry, University of Queensland, Australia, and were recorded on a Carlo Erba Elemental Microanalyser, model 1106. Reactions were monitored by TLC (Merck silica gel plates GF_{254}, Cat. No. 1.05554) and products were generally purified by flash chromatography using Merck silica gel 60 (0.040–0.063 mm, Cat. No. 1.09385) unless otherwise stated. Deprotected products were purified by HPLC using a Phenomenex C_{18} reversed-phase column (semi-preparative). Methyl β-D-galactopyranoside was purchased from Sigma–Aldrich. Methyl β-D-lactoside was prepared by treating hepta-*O*-acetyl-α-lactosylbromide with sodium methoxide.[68] BzOTf was prepared by reaction of BzCl with TfOH and purified

by distillation.[58] All commercial solvents were distilled prior to use. Dried solvents were distilled under N_2 according to Armarego and Perrin.[69]

4.2. Synthesis of 3′-thiolacetyl-lactoside derivatives. Methyl 4,6-*O*-benzylidene-3-*O*-methanesulfonyl-β-D-Galactopyranoside (18)

A suspension of 4,6-*O*-benylidenated galactoside **17**[57] (200 mg, 0.71 mmol) and Bu_2SnO (194 mg, 0.78 mmol) was stirred in refluxing dry MeOH (4 mL) under Ar for 2 h. The mixture was cooled and concentrated (co-evaporation with toluene) under reduced pressure. To the crude stannylidene in dry toluene (4 mL) containing 4 Å sieves was added MsCl (60 μL, 0.78 mmol) under Ar at 0 °C. The reaction mixture was left to stir at 0 °C for 30 min before being filtered through Celite (MeOH as eluant) and concentrated under reduced pressure. Column chromatography (EtOAc/hexane, 2:1 → neat EtOAc, R_f 0.46) afforded **18** (255 mg, quantitative) as an amorphous mass: ^{1}H NMR ($CDCl_3$): δ 3.17 (3H, s, OMs), 3.52 (1H, s, H-5), 3.61 (3H, s, OMe), 4.05–4.13 (2H, m, H-2, H-6), 4.28 (1H, d, $J_{1,2}$ = 7.7 Hz, H-1), 4.38 (1H, dd, $J_{6',6}$ = 12.6, $J_{6',5}$ = 1.3 Hz, H-6′), 4.43 (1H, d, $J_{4,3}$ = 3.6 Hz, H-4), 4.66 (1H, dd, $J_{3,2}$ = 10.0, $J_{3,4}$ = 3.6 Hz, H-3), 5.59 (1H, s, *CH*Ph), 7.35–7.37, 7.51–7.54 (5H, 2× m, CH*Ph*); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 38.6 (OMs), 57.4 (OMe), 66.2 (C-5), 68.7 (C-2), 68.8 (C-6), 74.9 (C-4), 80.3 (C-3), 101.0 (*CH*Ph), 103.9 (C-1), 2× 126.3, 2× 128.1, 129.1 (CH*Ph*), 137.3 (*ipso*Ph); LRMS 383 (M+Na, 100%); HRMS Calcd for $C_{15}H_{20}O_8S$ (M^+) 360.0878. Found 360.0879.

The following were prepared in a similar manner:

4.3. Methyl 4,6-*O*-benzylidene-3-*O*-[(trifluoromethyl)sulfonyl]-β-D-galactopyranoside (19)

In 70% yield (column chromatography EtOAc/hexane, 1:1 → 2:1 → neat EtOAc, R_f 0.62) as a colourless solid: ^{1}H NMR ($CDCl_3$): δ 3.52 (1H, d, $J_{5,4}$ = 1.2 Hz, H-5), 3.60 (3H, s, OMe), 4.07–4.15 (2H, m, H-2, H-6), 4.29 (1H, d, $J_{1,2}$ = 7.7 Hz, H-1), 4.41 (1H, dd, $J_{6',6}$ = 12.6, $J_{6',5}$ = 1.6 Hz, H-6′), 4.45 (1H, dd, $J_{4,3}$ = 3.8, $J_{4,5}$ = 1.2 Hz, H-4), 4.85 (1H, dd, $J_{3,2}$ = 10.0, $J_{3,4}$ = 3.8 Hz, H-3), 5.60 (1H, s, *CH*Ph), 7.35–7.40, 7.49–7.53 (5H, 2× m, CH*Ph*); LRMS 437 (M+Na, 100%); HRMS Calcd for $C_{15}H_{17}O_8F_3S$ (M+Na) 437.0494. Found 437.0493.

4.4. Methyl 4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranoside (20)

In 79% yield (column chromatography EtOAc/hexane, 1:3 → 1:2 → 1:1, R_f 0.12 → neat EtOAc) as a colourless solid: IR 1051, 1759, 3456 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.56 (1H, s, H-5), 3.61 (3H, s, OMe), 4.01–4.12 (2H, m, H-2, H-6), 4.18 (2H, d, OClAc), 4.31 (1H, d, $J_{1,2}$ = 7.7 Hz, H-1), 4.38 (1H, dd, $J_{6',6}$ = 13.7 Hz, H-6′), 4.45 (1H, d, $J_{4,3}$ = 3.5 Hz, H-4), 4.95 (1H, dd, $J_{3,2}$ = 10.2, $J_{3,4}$ = 3.5 Hz, H-3), 5.53 (1H, s, *CH*Ph), 7.36–7.38, 7.49–7.52 (5H, 2× m, CH*Ph*); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 40.9 (OC(O)*CH*$_2$Cl), 57.3 (OMe), 66.3 (C-5), 68.4 (C-2), 69.0 (C-6), 73.1 (C-4), 75.4 (C-3), 101.0 (*CH*Ph), 103.9 (C-1), 2× 126.2, 2× 128.2, 129.1 (CH*Ph*), 137.4 (*ipso*Ph), 167.4 (O*C*(O)CH$_2$Cl); LRMS 381 (M+Na, 100%).

4.5. Methyl 4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (22)

A suspension of 4′,6′-*O*-benylidenated lactoside **21**[57] (200 mg, 0.45 mmol) and Bu_2SnO (123 mg, 0.50 mmol) was stirred in refluxing dry MeOH (4 mL) under Ar for 2 h. The mixture was cooled and concentrated (co-evaporation with toluene) under reduced pressure. To the crude stannylidene in dry DMF (1 mL) and dry toluene (3 mL) containing 4 Å sieves was added ClAcCl (39 μL, 0.50 mmol) under Ar at 0 °C. The reaction mixture was left to stir at 0 °C for 30 min before being filtered through Celite (MeOH as eluant) and concentrated under reduced pressure. Column chromatography (neat EtOAc → EtOAc/MeOH, 30:1 → 10:1 → 4:1, R_f 0.52) afforded **22** [164 mg, 70% (82% based on recovered **21**)] as an amorphous mass: ^{1}H NMR ($CDCl_3$): δ 3.38–3.43 (2H, m, Glc H-2, Glc H-5), 3.54 (3H, s, OMe), 3.66† (1H, d, $J_{5,4}$ = 0.9 Hz, Gal H-5), 3.69† (1H, t, $J_{4,3}$ = $J_{4,5}$ = 9.1 Hz, Glc H-4), 3.79† (1H, t, $J_{3,2}$ = $J_{3,4}$ = 9.1 Hz, Glc H-3), 3.83† (1H, dd, $J_{6,6'}$ = 12.5, $J_{6,5}$ = 3.0 Hz, Glc H-6), 4.01 (1H, dd, $J_{6,6'}$ = 12.5, $J_{6,5}$ = 2.8 Hz, Glc H-6′), 4.05–4.15 (2H, m, Gal H-2, Gal H-6), 4.18 (2H, d, OClAc), 4.22 (1H, d, $J_{1,2}$ = 7.8 Hz, Glc H-1), 4.31 (1H, dd, $J_{6',6}$ = 12.8, $J_{6',5}$ = 1.4 Hz, Gal H-6′), 4.40 (1H, d, $J_{4,3}$ = 3.5, $J_{4,5}$ = 0.9 Hz, Gal H-4), 4.63 (1H, d, $J_{1,2}$ = 8.0 Hz, Gal H-1), 4.99 (1H, dd, $J_{3,2}$ = 10.2, $J_{3,4}$ = 3.5 Hz, Gal H-3), 5.50 (1H, s, *CH*Ph), 7.33–7.42, 7.44–7.54 (5H, 2× m, CH*Ph*); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 41.0 (OC(O)*CH*$_2$Cl), 57.3 (OMe), 61.1 (Glc C-6), 66.5, 74.8 (Glc C-3, Gal C-5), 67.3 (Gal C-2), 68.9 (Gal C-6), 73.2, 74.6 (Glc C-2, Glc C-5), 75.0 (Gal C-3), 77.8 (Glc C-4), 101.0 (*CH*Ph), 103.1 (Gal C-1), 103.6 (Glc C-1), 2× 126.2, 2× 128.3, 129.2 (CH *Ph*), 137.2 (*ipso*Ph), 167.6 (O*C*(O)CH$_2$Cl); LRMS 543 (M+Na, 100%).

4.6. Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (24)

To a stirred solution of **22** (3 g, 5.76 mmol) in dry CH_2Cl_2 (60 mL) containing 4 Å sieves were added dry pyridine (11.18 mL, 0.14 mol), DMAP (cat.) and then BzCl (8.02 mL, 0.07 mmol) under Ar at 0 °C. The mixture was warmed to room temperature and stirred for 2 h before being diluted with CH_2Cl_2 (150 mL), washed with 0.1 M HCl (3× 100 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (EtOAc/hexane, 1:2) gave the desired tetra-*O*-benzoylated derivative **24** [2.70 g, 50% (EtOAc/hexane, 1:1, R_f 0.42)] and the tri-*O*-benzoylated derivative [1.92 g, 40% (EtOAc/hexane, 1:1, R_f 0.40)]. BzOTf (1.02 mL, 4.61 mmol) was added dropwise to a solution of the tri-*O*-benzoylated derivative (1.92 g, 2.30 mmol) in dry CH_2Cl_2 (25 mL) and dry pyridine (559 μL, 6.91 mmol) containing 4 Å sieves under Ar at −78 °C.

The mixture was warmed to 0 °C, left to stir for 1 h and then allowed to warm to room temperature. The mixture was stirred for a further 2 h at room temperature before being diluted with CH_2Cl_2 (100 mL), washed with saturated aqueous $NaHCO_3$ (3× 70 mL) and then 0.1 M HCl (3× 70 mL), dried (Na_2SO_4) and concentrated. Column chromatography (EtOAc/hexane, 2:3 → 1:1) afforded **24** [1.89 g, 88% (overall yield from **22** is 85%)] which was crystallised from EtOAc/hexane to give colourless needles: IR 1071, 1316, 1726 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.92 (1H, s, Gal H-5), 3.51 (3H, s, OMe), 3.56 (1H, appd, $J_{6,6'}$ = 12.4 Hz, Gal H-6), 3.74–3.97 (4H, m, Glc H-5, Gal H-6′, OClAc), 4.18–4.24 (2H, m, Glc H-4, Gal H-4), 4.38 (1H, dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.1 Hz, Glc H-6), 4.61† (1H, d, $J_{1,2}$ = 7.7 Hz, Glc H-1), 4.59–4.63† (1H, m, Glc H-6′), 4.78 (1H, d, $J_{1,2}$ = 7.9 Hz, Gal H-1), 5.03 (1H, dd, $J_{3,2}$ = 10.4, $J_{3,4}$ = 3.6 Hz, Gal H-3), 5.30† (1H, s, *CH*Ph), 5.31† (1H, dd, $J_{2,3}$ = 9.2, $J_{2,1}$ = 7.7 Hz, Glc H-2), 5.62 (1H, dd $J_{2,3}$ = 10.4, $J_{2,1}$ = 7.9 Hz, Gal H-2), 5.83 (1H, t, $J_{3,2} = J_{3,4}$ = 9.2 Hz, Glc H-3), 7.30–7.62, 7.87–8.04 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 40.5 (OC(O) *CH*$_2$Cl), 57.0 (OMe), 62.4 (Glc C-6), 66.2 (Gal C-5), 67.9 (Gal C-6), 69.2 (Gal C-2), 72.3 (C-4), 72.7 (Glc C-2, Glc C-3), 73.5 (Gal C-3), 74.0 (Glc C-3), 76.8 (C-4), 100.8 (*CH*Ph), 101.3 (C-1), 101.5 (C-1), 2× 128.3, 128.4, 128.5, 129.0, 129.6, 129.7, 129.9, 130.2, 133.0, 133.1, 133.4, 133.7, 137.2 (5× Ph), 128.6, 129.4, 129.5, 137.3 (5× *ipso*Ph), 2× 164.9, 165.4, 165.7 (4× O*C*(O)Ph), 167.2 (O*C*(O)CH_2Cl); LRMS 959 (M+Na, 100%); Anal. ($C_{50}H_{45}O_{16}Cl$) C, H.

4.7. Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (25)

To a stirred solution of **24** (2 g, 2.13 mmol) in dry DMF (20 mL) was added hydrazinium acetate (393 mg, 4.27 mmol) under N_2 at room temperature. The mixture was warmed to 40 °C and left to stir for 2 h before being diluted with EtOAc (100 mL), washed with 0.1 M HCl (3× 70 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (EtOAc/hexane, 1:2 → 2:3 → 1:1, R_f 0.25) afforded **25** (1.85 g, 78%) as a colourless solid: ^1H NMR ($CDCl_3$): δ 2.87 (1H, s, Gal H-5), 3.35 (3H, s, OMe), 3.50 (1H, dd, $J_{6,6'}$ = 12.3, $J_{6,5}$ = 1.6 Hz, Gal H-6), 3.61† (1H, appd, $J_{6',6}$ = 12.3 Hz, Gal H-6′), 3.64† (1H, dd, $J_{3,2}$ = 10.0, $J_{3,4}$ = 3.6 Hz, Gal H-3), 3.77 (1H, ddd, $J_{5,4}$ = 9.5, $J_{5,6}$ = 4.4, $J_{5,6'}$ = 2.1 Hz, Glc H-5), 3.92 (1H, d, $J_{4,3}$ = 3.6 Hz, Gal H-4), 4.10 (1H, t, $J_{4,3} = J_{4,5}$ = 9.5 Hz, Glc H-4), 4.40 (1H, dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.4 Hz, Glc H-6), 4.52† (1H, d, $J_{1,2}$ = 7.8 Hz, Glc H-1), 4.55† (1H, dd, $J_{6',6}$ = 12.0, $J_{6',5}$ = 2.1 Hz, Glc H-6′), 4.59 (1H, d, $J_{1,2}$ = 8.0 Hz, Gal H-1), 5.23† (1H, dd, $J_{2,3}$ = 10.0, $J_{2,1}$ = 8.0 Hz, Gal H-2), 5.25† (1H, s, *CH*Ph), 5.28 (1H, dd, $J_{2,3}$ = 9.5, $J_{2,1}$ = 7.8 Hz, Glc H-2), 5.73 (1H, t, $J_{3,2} = J_{3,4}$ = 9.5 Hz, Glc H-3), 7.22–7.55, 7.86–7.97 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 56.9 (OMe), 62.5 (Glc C-6), 66.5 (Gal C-5), 67.9 (Gal C-6), 71.6 (Gal C-3), 72.1 (Glc C-2), 2× 72.9 (Glc C-5, Gal C-2), 73.6 (Glc C-3), 75.0 (Gal C-4), 76.6 (Glc C-4), 2× 101.2 (Gal C-1, *CH*Ph), 101.6 (Glc C-1), 126.5, 128.1, 128.2, 2× 128.3, 2× 129.1, 129.6, 2× 129.7, 129.8, 133.0, 2× 133.1 (5× Ph), 129.2, 129.3, 129.5, 129.7, 137.3 (5× *ipso*Ph), 165.0, 165.4, 165.7, 165.8 (4× O*C*(O)Ph); LRMS 893 (M+Na, 100%).

4.8. Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-gulopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (26)

Tf_2O (218 μL, 1.30 mmol) was added dropwise to a stirred solution of **25** (559 mg, 0.65 mmol) in dry CH_2Cl_2 (11 mL) and dry pyridine (210 μL, 2.60 mmol) under N_2 at −78 °C. The mixture was then warmed to 0 °C and left to stir for 1 h before being diluted with CH_2Cl_2 (40 mL), washed with 0.1 M HCl (20 mL) and H_2O (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. To the crude triflate in dry DMF (11 mL) under N_2 at room temperature was added KNO_2 (276 mg, 3.25 mmol). The mixture was warmed to 50 °C and left to stir for 16 h before being concentrated under reduced pressure. Column chromatography (EtOAc/hexane, 1:2 → 2:3 → 1:1, R_f 0.34) gave **26** (426 mg, 76%) which was crystallised from EtOAc/hexane to give colourless needles: IR 1116, 1272, 1728, 3521 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.25 (1H, s, Gal H-5), 3.39 (3H, s, OMe), 3.49 (1H, dd, $J_{6,6'}$ = 12.4, $J_{6,5}$ = 1.7 Hz, Gal H-6), 3.60 (1H, appd, $J_{6',6}$ = 12.4 Hz, Gal H-6′), 3.75–3.81 (2H, m, Glc H-5, Gal H-4), 4.12 (1H, t, $J_{4,3} = J_{4,5}$ = 9.3 Hz, Glc H-4), 4.18 (1 H, t, $J_{3,2} = J_{3,4}$ = 3.2 Hz, Gal H-3), 4.50-4.60 (3H, m, Glc H-1, Glc H-6, Glc H-6′), 5.04 (1H, d, $J_{1,2}$ = 8.4 Hz, Gal H-1), 5.24† (1H, s, *CH*Ph), 5.25† (1H, dd, $J_{2,1}$ = 8.4, $J_{2,3}$ = 3.2 Hz, Gal H-2), 5.31 (1H, dd, $J_{2,3}$ = 9.3, $J_{2,1}$ = 7.8 Hz, Glc H-2), 5.73 (1H, t, $J_{3,2} = J_{3,4}$ = 9.3 Hz, Glc H-3), 7.18–7.55, 7.88–8.06 (25H, 2× m, 5× Ph). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 57.0 (OMe), 62.6 (Glc C-6), 65.6 (Gal C-6), 68.1 (Gal C-5), 68.8 (Gal C-3), 71.4 (Gal C-2), 72.1 (Glc C-2), 73.0, 75.8 (Glc C-5, Gal C-4), 73.6 (Glc C-3), 76.5 (Glc C-4), 98.6 (Gal C-1), 100.8 (*CH*Ph), 101.6 (Glc C-1), 126.4, 128.0, 128.1, 128.2, 128.3, 128.5, 128.9, 2× 129.6, 129.7, 129.8, 132.8, 133.0, 133.1, 133.3 (5× Ph), 129.1, 129.3, 129.6, 137.5 (5× *ipso*Ph), 164.7, 165.2, 165.4, 166.1 (4× O*C*(O)Ph); LRMS 883 (M+Na, 100%); Anal. ($C_{48}H_{44}O_{15}$) C, H.

4.9. Methyl 3-*S*-acetyl-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (23)

Tf_2O (397 μL, 2.36 mmol) was added dropwise to a stirred solution of **26** (678 mg, 0.79 mmol) in dry CH_2Cl_2 (13 mL) and dry pyridine (382 μL, 4.23 mmol) under N_2 at −78 °C. The mixture was warmed to 0 °C, left to stir for 1 h and then allowed to warm to room temperature. The mixture was stirred for a further 1 h at room temperature before being diluted with CH_2Cl_2 (60 mL), washed with 0.1 M HCl (40 mL) and H_2O (40 mL), dried (Na_2SO_4) and concentrated under reduced pressure. To a stirred solution of the crude triflate in dry DMF (13 mL) was added KSAc (270 mg, 2.36 mmol) under N_2 at room temperature. The mixture was warmed to 50 °C and left to stir for 16 h before being concentrated under reduced pressure. Column chromatography (EtOAc/hexane, 1:2 → 2:3 → 1:1, R_f

0.60) gave **23** (613 mg, 83%) which was crystallised from EtOAc/hexane to give an off-white crystalline solid: ^{1}H NMR ($CDCl_3$): δ 2.11 (3H, s, SAc), 2.98 (1H, s, Gal H-5), 3.40 (3H, s, OMe), 3.53 (1H, dd, $J_{6,6'}$ = 12.4, $J_{6,5}$ = 1.7 Hz, Gal H-6), 3.78–3.86 (3H, m, Glc H-5, Gal H-4, Gal H-6′), 4.08 (1H, dd, $J_{3,2}$ = 11.5, $J_{3,4}$ = 3.3 Hz, Gal H-3), 4.22 (1H, t, $J_{4,3}$ = $J_{4,5}$ = 9.2 Hz, Glc H-4), 4.30 (1H, dd, $J_{6,6'}$ = 12.1, $J_{6,5}$ = 4.2 Hz, Glc H-6), 4.59 (1H, d, $J_{1,2}$ = 7.7 Hz, Glc H-1), 4.65 (1H, dd, $J_{6',6}$ = 12.1, $J_{6',5}$ = 2.0 Hz, Glc H-6′), 4.85 (1H, d, $J_{1,2}$ = 7.7 Hz, Gal H-1), 5.29 (1H, dd, $J_{2,3}$ = 9.2, $J_{2,1}$ = 7.7 Hz, Glc H-2), 5.31 (1H, s, *CH*Ph), 5.40 (1H, dd, $J_{2,3}$ = 11.5, $J_{2,1}$ = 7.7 Hz, Gal H-2), 5.84 (1H, t, $J_{3,2}$ = $J_{3,4}$ = 9.2 Hz, Glc H-3), 7.18–7.61, 7.82–8.01 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 30.4 (SC(O)*CH*$_3$), 46.7 (Gal C-3), 56.9 (OMe), 62.2 (Glc C-6), 67.0 (Glc C-6), 68.4 (Gal C-5), 69.5 (Gal C-2), 72.3, 75.2 (Glc C-5, Gal C-4), 72,7 (Glc C-2), 74.3 (Gal C-5), 76.6 (Glc C-4), 100.9 (*CH*Ph), 101.4 (Glc C-1), 102.6 (Gal C-1), 126.2, 128.0, 128.1, 2× 128.3, 128.4, 128.9, 2× 129.6, 129.7, 129.9, 132.8, 2× 133.1 (5× Ph), 164.7, 165.1, 165.4, 165.5 (4× O*C*(O)Ph), 128.8, 129.3, 129.5, 137.3 (5× *ipso*Ph), 164.7, 165.1, 165.4, 165.5 (4× O*C*(O)Ph), 194.8 (S*C*(O)CH$_3$); LRMS 941 (M+Na, 100%); Anal. ($C_{50}H_{46}O_{15}S$) C, H.

4.10. Synthesis of S-linked α(2,3)-sialyllactoside. Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (27)

To a stirred solution of **23** (300 mg, 0.32 mmol) in dry DMF (3 mL) was added hydrazinium acetate (39 mg, 0.64 mmol) under N_2 at room temperature. The mixture was left to stir for 3 h before being diluted with EtOAc (30 mL), washed with 0.1 M HCl (3× 20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography ($CHCl_3$/hexane, 1:1 → 1:2) afforded **27** [219 mg, 78% (neat $CHCl_3$, R_f 0.25)] as a colourless solid: IR 1114, 1268, 1728 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.12 (1H, d, $J_{SH,3}$ = 11.1 Hz, SH), 2.88 (1H, td, $J_{3,2}$ = $J_{3,SH}$ = 11.1, $J_{3,4}$ = 2.5 Hz, Gal H-3), 2.88 (1H, s, Gal H-5), 3.42 (3H, s, OMe), 3.57 (1H, dd, $J_{6,6'}$ = 12.4, $J_{6,5}$ = 1.8 Hz, Gal H-6), 3.75 (1H, dd, $J_{6',6}$ = 12.4, $J_{6',5}$ = 1.0 Hz, Gal H-6′), 3.83 (1H, ddd, $J_{5,4}$ = 9.5, $J_{5,6}$ = 4.2, $J_{5,6'}$ = 2.0 Hz, Glc H-5), 3.89 (1H, d, $J_{4,3}$ = 2.5 Hz, Gal H-4), 4.18 (1H, t, $J_{4,3}$ = $J_{4,5}$ = 9.5 Hz, Glc H-4), 4.41 (1H, dd, $J_{6,6'}$ = 12.1, $J_{6,5}$ = 4.2 Hz, Glc H-6), 4.59† (1H, d, $J_{1,2}$ = 7.8 Hz, Glc H-1), 4.60† (1H, dd, $J_{6',6}$ = 12.1, $J_{6',5}$ = 2.0 Hz, Glc H-6′), 4.68 (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1), 5.26† (1H, dd, $J_{2,3}$ = 11.1, $J_{2,1}$ = 7.8 Hz, Gal H-2), 5.32† (1H, dd, $J_{2,3}$ = 9.5, $J_{2,1}$ = 7.8 Hz, Glc H-2), 5.35† (1H, s, *CH*Ph), 5.81 (1H, t, $J_{3,2}$ = $J_{3,4}$ = 9.5 Hz, Glc H-3), 7.61–7.62, 7.88–8.04 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 43.5 (Gal C-3), 56.9 (OMe), 62.4 (Glc C-6), 67.9 (Gal C-6), 68.8 (Gal C-5), 72.1 (Glc C-2), 72.6, 72.7 (Glc C-5, Gal C-2), 73.7 (Glc C-3), 75.8 (Gal C-4), 76.3 (Glc C-4), 101.0 (*CH*Ph), 101.4 (Glc C-1), 102.4 (Gal C-1), 126.2, 127.1, 127.9, 3× 128.2, 128.3, 128.8, 129.4, 129.5, 2× 129.6, 129.7, 132.9, 133.0, 133.1 (5× Ph), 129.0, 129.2, 129.4 137.3 (4× *ipso*Ph), 164.8, 165.1, 165.3, 165.6 (4× O*C*(O)Ph); LRMS 899 (M+ Na, 100%).

4.11. Methyl [methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2,3)-*S*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-β-D-galactopyranosyl)-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (29)

To a stirred solution of **27** (205 mg, 0.23 mmol) in dry THF (8 mL) was added NaH (11 mg, 0.27 mmol) under Ar at 0 °C. After 10 min, Kryptofix 21® (10 mg, 0.05 mmol) and then a solution of **28**[65,66] (124 mg, 0.24 mmol) in dry THF (2 mL) were added to the reaction before being warmed to room temperature. After 2 h, the mixture was diluted with EtOAc (40 mL), washed with brine (3× 30 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Column chromatography (EtOAc/hexane, 1:1 → 3:1, R_f 0.13) gave **29** (180 mg, 57%) as an amorphous mass: IR 1113, 1269, 1734, 3018 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.71 (3H, s, NAc), 1.83† , 1.95, 1.99, 2.17 (12H, 4× s, 4× OAc), 1.81–1.87† (1H, m, Neu H-3a), 2.53 (1H, dd, $J_{3e,3a}$ = 12.8, $J_{3e,4}$ = 4.7 Hz, Neu H-3e), 3.33 (1H, s, Gal H-5), 3.38 (3H, s, OMe), 3.45 (1H, d, $J_{4,3}$ = 2.7 Hz, Gal H-4), 3.57 (1H, appd, $J_{6,6'}$ = 11.0 Hz, Gal H-6), 3.68 (1H, dd, $J_{6,5}$ = 10.8, $J_{6,7}$ = 2.4 Hz, Neu H-6), 3.70 (3H, s, CO_2Me), 3.72–3.86 (4H, m, Glc H-5, Gal H-3, Gal H-6′, Neu H-5), 4.22 (1H, dd, $J_{9,9'}$ = 12.7, $J_{9,8}$ = 4.8 Hz, Neu H-9), 4.30-4.38 (2H, m, Glc H-4, Glc H-6), 4.43 (1H, dd, $J_{9',9}$ = 12.7, $J_{9',8}$ = 2.4 Hz, Neu H-9′), 4.55 (1H, d, $J_{1,2}$ = 7.8 Hz, Glc H-1), 4.63 (1H, dd, $J_{6',6}$ = 12.0, $J_{6',5}$ = 2.0 Hz, Glc H-6′), 4.80 (1H, td, $J_{4,3a}$ = $J_{4,5}$ = 11.6, $J_{4,3e}$ = 4.7 Hz, Neu H-4), 5.01 (1H, d, $J_{NH,5}$ = 9.8 Hz, NH), 5.19-5.27 (4H, m, Gal H-1, Gal H-2, Neu H-7, *CH*Ph), 5.30 (1H, dd, $J_{2,3}$ = 9.5, $J_{2,1}$ = 7.8 Hz, Glc H-2), 5.69 (1H, ddd, $J_{8,7}$ = 10.2, $J_{8,9}$ = 4.8, $J_{8,9'}$ = 2.4 Hz, Neu H-8), 5.78 (1H, t, $J_{3,2}$ = $J_{3,4}$ = 9.5 Hz, Glc H-3), 7.20–7.62, 7.91–7.98, 8.13–8.16 (25H, 3× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.6, 20.7, 20.8, 21.3 (4× OC(O) *Me*), 23.1 (NC(O) *Me*), 37.0 (Neu C-3), 45.4, 49.0 (Gal C-3, Neu C-5), 52.9 (CO_2 *Me*), 56.8 (OMe), 62.2 (Neu H-9), 62.8 (Glc C-6), 66.6, 69.0 (Gal C-2, Neu C-7), 67.0 (Gal C-5), 67.8 (Neu C-8), 68.1 (Gal C-6), 69.3 (Neu C-4), 72.3, 73.0, 73.4 (Glc C-2, Glc C-5, Neu C-6), 73.8, (Glc C-3), 75.6 (Gal C-4), 75.9 (Glc C-4), 101.1, 101.4, 101.5 (Glc C-1, Gal C-1, *CH*Ph), 126.3, 127.9, 128.0, 2× 128.2, 128.3, 128.6, 2× 129.6, 129.8, 130.0, 132.6, 132.8, 133.0 (5× Ph), 129.3, 129.6, 130.1, 137.5 (4× *ipso*Ph), 165.2, 165.4, 165.5, 165.6 (4× O*C*(O)Ph), 169.5, 169.9, 170.1, 170.2, 170.7, 170.8 (4× O*C*(O)Me, N*C*(O)Me, *C*O_2Me); LRMS 1372 (M+Na, 100%); HRMS Calcd for $C_{68}H_{71}NO_{26}S$ (M+Na) 1372.3883. Found 1372.390.

4.12. Methyl (5-acetamido-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2,3)-*S*-(4,6-*O*-benzylidene-3-thio-β-D-galactopyranosyl)-(1,4)-*O*-β-D-glucopyranoside

To a stirred solution of **29** (220 mg, 0.16 mmol) in MeOH (5 mL) was added dropwise a 1 M solution of NaOH (~1 mL) until a pH of ~13 was attained. The mixture was left to stir for 24 h before being neutralised with Amberlyte IR-120 (H^+), the resin filtered, washed with aqueous MeOH, and then the solvent was concen-

trated under reduced pressure. The residue was re-dissolved in H_2O (15 mL), the pH adjusted to ~4 with AcOH, washed with EtOAc (3× 30 mL) and the aqueous phase freeze-dried to give the 4′,6′-*O*-benzylidenated thiosialoside (122 mg, quantitative) as a colourless solid: ^{1}H NMR (600 MHz, D_2O): δ 1.76 (1H, dd, $J_{3a,3e}$ = 12.3, $J_{3a,4}$ = 11.8 Hz, Neu H-3$_a$), 1.99 (3H, s, NAc), 2.76 (1H, dd, $J_{3e,3a}$ = 12.3, $J_{3e,4}$ = 4.7 Hz, Neu H-3e), 3.27 (1H, dd, $J_{2,3}$ = 9.1, $J_{2,1}$ = 8.2 Hz, Glc H-2), 3.49 (2H, m, Gal H-2, Gal H-3), 3.54 (3H, s, OMe), 3.55-3.58 (3H, m, Glc H-5, Neu H-6, Neu H-9), 3.61–3.67 (4H, m, Glc H-3, Neu H-4, Neu H-5, Neu H-7), 3.80–3.87 (4H, m, Glc H-4, Glc H-6, Gal H-5, Neu H-9′), 3.91 (1H, ddd, $J_{8,7}$ = 8.8, $J_{8,9}$ = 6.0, $J_{8,9'}$ = 2.3 Hz, Neu H-8), 4.01 (1H, dd, $J_{6',6}$ = 12.0, $J_{6',5}$ = 1.5 Hz, Glc H-6′), 4.18–4.22 (3H, m, Gal H-4, Gal H-6, Gal H-6′), 4.37 (1H, d, $J_{1,2}$ = 8.2 Hz, Glc H-1), 4.59 (1H, d, $J_{1,2}$ = 7.4 Hz, Gal H-1), 5.68 (1H, s, *CH*Ph), 7.41–7.45, 7.48–7.51 (5H, 2× CH*Ph*); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 23.3 (NC(O) *Me*), 40.4 (Neu C-3), 48.2 (Glc C-2), 51.6 (Neu C-5), 57.2 (OMe), 59.8 (Glc C-6), 62.5 (Neu C-9), 67.9 (Gal C-6), 68.1, 68.4, 68.7 (Glc C-5, Gal C-2, Gal C-3), 71.9 (Glc C-2), 2× 72.7 (Neu C-4, Neu C-7), 2× 74.1 (Glc C-3, Neu C-8), 74.8, 75.0 (Glc C-4, Gal C-5), 76.3 (Gal C-4), 78.3 (Neu C-6), 84.1 (Neu C-2), 101.3 (*CH*Ph), 103.0 (Glc C-1), 104.1 (Gal C-1), 126.1, 128.6. 129.7 (CH*Ph*), 136.6 (*ipso*Ph), 174.3 (Neu C-1), 174.9 (N*C*(O)Me); LRMS 797 (M+2Na, 100%).

4.13. Methyl (sodium 5-acetamido-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2,3)-*S*-(3-thio-β-D-galactopyranosyl)-(1,4)-*O*-β-D-glucopyranoside (9)

A solution of the 4′,6′-*O*-benzylidenated thiosialoside (122 mg, 0.16 mmol) in 10% aqueous TFA (7 mL) was stirred at 0 °C for 8 h before being concentrated in vacuo (cold work-up). The residue was dissolved in H_2O (5 mL) and the pH adjusted to ~7.3 with 1 M NaOH before being freeze-dried. Size exclusion chromatography [G10 Sephadex column, H_2O (EtOAc/MeOH/H_2O, 7:2:1, R_f 0.17)] and then HPLC purification (1% CH_3CN in H_2O, 3 mL/min, R_t = 7.74 min) afforded **9** (72 mg, 64%) as a colourless solid: ^{1}H NMR (600 MHz, D_2O): δ 1.79 (1H, dd, $J_{3a,3e}$ = 12.3, $J_{3a,4}$ = 11.7 Hz, Neu H-3a), 1.99 (3H, s, NAc), 2.77 (1H, dd, $J_{3e,3a}$ = 12.3, $J_{3e,4}$ = 4.7 Hz, Neu H-3e), 3.27 (1H, dd, $J_{2,3}$ = 9.1, $J_{2,1}$ = 8.1 Hz, Glc H-2), 3.33 (1H, dd, $J_{3,2}$ = 10.3, $J_{3,4}$ = 2.5 Hz, Gal H-3), 3.36 (1H, dd, $J_{2,3}$ = 10.3, $J_{2,1}$ = 7.0 Hz, Gal H-2), 3.54 (3H, s, OMe), 3.55-3.58 (4H, m, Glc H-3, Glc H-5, Neu H-7, Neu H-9), 3.59–3.66 (3H, m, Neu H-4, Neu H-5, Neu H-6), 3.68–3.69 (2H, m, Gal H-6, Gal H-6′), 3.74 (1H, dd, $J_{5,6}$ = 6.3, $J_{5,6'}$ = 5.8 Hz, Gal H-5), 3.80–3.85 (4H, m, Glc H-4, Glc H-6, Gal H-4, Neu H-9′), 3.89 (1H, ddd, $J_{8,7}$ = 8.8, $J_{8,9}$ = 6.1, $J_{8,9'}$ = 2.4 Hz, Neu H-8), 3.98 (1H, dd, $J_{6',6}$ = 12.3, $J_{6',5}$ = 2.2 Hz, Glc H-6′), 4.37 (1H, d, $J_{1,2}$ = 8.1 Hz, Glc H-1), 4.51 (1H, d, $J_{1,2}$ = 7.0 Hz, Gal H-1); ^{13}C NMR (75.5 MHz, D_2O): δ 22.0 (NC(O) *Me*), 40.5 (Neu C-3), 50.4 (Glc C-2), 51.5 (Neu C-5), 57.2 (OMe), 60.0 (Glc C-6), 61.2 (Gal C-6), 62.5 (Neu C-6), 68.1, 68.3, 68.4, 68.7 (Glc C-4, Gal C-2, Gal C-4, Neu C-7), 71.9 (Neu C-8), 72.8 (Gal C-3), 74.3, 74.8, 74.9 (Glc C-3, Glc C-5, Neu C-4), 77.7 (Gal C-5), 78.0 (Neu C-6), 84.0 (Neu C-2), 103.0 (Glc C-1), 104.1 (Gal C-1), 174.5 (Neu C-1), 174.9 (N*C*(O)Me); LRMS 686 (M+Na, 100%); Anal. ($C_{24}H_{40}NNaO_{18}S4H_2O$) C, H.

4.14. Synthesis of sialylmimetics. Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-3-(ethoxycarbonylmethyl)-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (31)

A solution of **23** (300 mg, 0.32 mmol) in dry DMF (6 mL) was thoroughly degassed (bubbling N_2) for 15 min before the addition of 4 Å sieves and then hydrazinium acetate (59 mg, 0.64 mmol) under N_2 at room temperature. The reaction mixture was left to stir for 1 h before the addition of ethyl bromoacetate (178 μL, 1.6 mmol) and then Et_3N (54 μL, 0.39 mmol). After 2 h, the mixture was diluted with EtOAc (15 mL), washed with 0.1 M HCl (15 mL) and H_2O (15 mL), dried (Na_2SO_4) and concentrated. Column chromatography ($CHCl_3$/hexane, 1:2 → 1:1) yielded **31** [277 mg, 90% (neat $CHCl_3$, R_f 0.2)] which was crystallised from EtOAc/hexane to give a colourless crystalline solid: IR 1071, 1269, 1729 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.13 (3H, t, J_{vic} = 7.1 Hz, CO_2CH_2 *CH_3*), 2.95 (1H, s, Gal H-5), 3.19† (1H, dd, $J_{3,2}$ = 11.2, $J_{3,4}$ = 3.2 Hz, Gal H-3), 3.20† (2H, s, H-2′), 3.41 (3H, s, OMe), 3.58 (1H, appd, $J_{6,6'}$ = 12.2 Hz, Gal H-6), 3.76 (1H, appd, $J_{6',6}$ = 12.2 Hz, Gal H-6′), 3.83 (1H, ddd, $J_{5,4}$ = 9.4, $J_{5,6}$ = 4.3, $J_{5,6'}$ = 2.1 Hz, Glc H-5), 3.99 (2H, q, J_{vic} = 7.1 Hz, CO_2 *4 CH_2*CH_3), 4.91 (1H, d, $J_{4,3}$ = 3.2 Hz, Gal H-4), 4.19 (1H, t, $J_{4,3}$ = $J_{4,5}$ = 9.4 Hz, Glc H-4), 4.37 (1H, dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.3 Hz, Glc H-6), 4.59† (1H, d, $J_{1,2}$ = 7.7 Hz, Glc H-1), 4.61† (1H, dd, $J_{6',6}$ = 12.0, $J_{6',5}$ = 2.1 Hz, Glc H-6′), 4.73 (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1), 5.31† (1H, dd, $J_{2,3}$ = 9.4, $J_{2,1}$ = 7.7 Hz, Glc H-2), 5.33† (1H, s, *CH*Ph), 5.47 (1H, dd, $J_{2,3}$ = 11.2, $J_{2,1}$ = 7.8 Hz, Gal H-2), 5.81 (1H, t, $J_{3,2}$ = $J_{3,4}$ = 9.4 Hz, Glc H-3), 7.25–7.62, 7.88–8.04 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.9 (CO_2CH_2 *CH_3*), 33.4 (C-2′), 48.8 (Gal C-3), 56.9 (OMe), 61.2 (CO_2 *CH_2*CH_3), 62.4 (Glc C-6), 67.9 (Gal C-6), 68.6 (Gal C-5), 71.1 (Gal C-2), 72.2 (Glc C-2), 72.7 (Glc C-5), 73.9 (Glc C-3), 76.3 (Gal C-4), 100.9 (*CH*Ph), 101.4 (Glc C-1), 102.4 (Gal C-1), 126.3, 127.9, 128.2, 128.3, 128.4, 128.7, 2× 129.6, 129.8, 132.9, 133.0, 133.2 (5× Ph), 128.9, 129.3, 129.5, 137.3 (5× *ipso*Ph), 2× 164.8, 165.4, 165.6 (4× O*C*(O)Ph), 170.6 (C-1′); LRMS 985 (M+Na, 100%); Anal. ($C_{52}H_{50}O_{16}S$) C, H.

The following were prepared in a similar manner:

4.15. Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-3-[2′-(ethyl propanoate)]-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (32)

In 84% yield (neat $CHCl_3$, R_f 0.23) as a 2:1 mixture of inseparable diastereomers by coupling between **23** and ethyl (±) 2-bromoproprionate to give a colourless crystalline solid: IR 1071, 1269, 1729 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.03† (1.32*) (2H, d, J_{vic} = 7.2 Hz, H-3′), 1.05† (1.19*) (3H, t, J_{vic} = 6.9 Hz, CO_2CH_2 *CH_3*), 2.90

(2.96*) (1H, s, Gal H-5), 3.25 (3.31*)† (1H, dd, $J_{3,2}$ = 11.4, $J_{3,4}$ = 3.2 Hz, Gal H-3), 3.32–3.38† (1H, m, H-2′), 3.41 (3H, s, OMe), 3.55† (3.57*)† (1H, dd, $J_{6,6'}$ = 12.3, $J_{6,5}$ = 1.7 Hz, Gal H-6), 3.75 (1H, appd, $J_{6',6}$ = 12.3 Hz, Gal H-6′), 3.83 (1H, ddd, $J_{5,4}$ = 9.4, $J_{5,6}$ = 4.2, $J_{5,6'}$ = 1.8 Hz, Glc H-5), 3.90† (4.11*) (2H, q, J_{vic} = 7.2 Hz, CO_2 CH_2CH_3), 3.94† (3.95*) (1H, d, $J_{4,3}$ = 3.2 Hz, Gal H-4), 4.19† (4.20*)† (1H, t, $J_{4,3}$ = $J_{4,5}$ = 9.4 Hz, Glc H-4), 4.38 (1H, dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.2 Hz, Glc H-6), 4.58† (4.59*)† (1H, d, $J_{1,2}$ = 7.5 Hz, Glc H-1), 4.60† (1H, dd, $J_{6',6}$ = 12.0, $J_{6',5}$ = 1.8 Hz, Glc H-6′), 4.73† (4.75*)† (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1), 5.28–5.35† (2H, m, Glc H-2, *CH*Ph), 5.36† (5.51*) (1H, dd, $J_{2,3}$ = 11.4, $J_{2,1}$ = 7.8 Hz, Gal H-2), 5.80† (5.83*)† (1H, t, $J_{3,2}$ = $J_{3,4}$ = 9.4 Hz, Glc H-3), 7.19–7.62, 7.86–7.52 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.8 (16.5*) (CO_2CH_2 CH_3), 14.0 (17.9*) (C-3′), 40.2 (42.5*) (C-2′), 47.2 (49.7*) (Gal C-3), 56.9 (OMe), 61.0 (61.1*) (CO_2 CH_2CH_3), 62.4 (Glc C-6), 67.9 (Gal C-6), 68.5 (68.7*) (Gal C-5), 69.7 (72.2*) (Glc C-2), 72.2 (72.3*) (Gal C-2), 72.7 (72.8*) (Glc C-5), 73.8 (74.0*) (Glc C-3), 75.3 (76.3*) (Gal C-4), 76.4 (Glc C-4), 100.8 (100.9*) (*CH*Ph), 101.4 (Glc C-1), 102.4 (102.5*) (Gal C-1), 126.2, 126.3, 127.9, 2× 128.2, 128.3, 128.4, 128.7, 129.5, 2× 129.6, 129.7, 129.8, 132.9, 133.0, 133.1, 133.2 (5× Ph), 128.8, 129.0, 129.3, 129.5, 132.3 (137.4*) (5× *ipso*Ph), 164.7, 164.8, 164.9, 165.4, 165.6, 165.7 (4× O*C*(O)Ph), 173.0 (173.4*) (C-1′); LRMS 999 (M+Na, 100%); Anal. ($C_{53}H_{52}O_{16}S$) C, H.

4.16. Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-3-[2′-(methyl butanoate)]-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (33)

In 85% yield (neat $CHCl_3$, R_f 0.23) as a 2:1 mixture of inseparable diastereomers by coupling between **23** and methyl (±) 2-bromobutyrate to give a colourless crystalline solid: IR 1070, 1268, 1731 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.47 (0.77*) (3H, t, J_{vic} = 7.3 Hz, H-4′), 1.21–1.35 (1.45–1.65*)† (1H, m, H-3′), 1.45–1.65†, 1.74–1.89 (1H, m, H-3′), 2.94 (2.96*) (1H, s, Gal H-5), 3.09 (3.17*) (1H, dd, J_{vic} = 8.3, 6.6 Hz, H-2′), 3.25 (1H, dd, $J_{3,2}$ = 11.2, $J_{3,4}$ = 3.1 Hz, Gal H-3), 3.41 (3H, s, OMe), 3.55 (3.58*) (1H, dd, $J_{6,6'}$ = 12.2, $J_{6,5}$ = 1.5 Hz, Gal H-6), 3.65 (3H, s, CO_2Me), 3.75 (1H, d, $J_{6',6}$ = 12.2 Hz, Gal H-6′), 3.84 (1H, ddd, $J_{5,4}$ = 9.6, $J_{5,6}$ = 4.2, $J_{5,6'}$ = 1.9 Hz, Glc H-5), 3.88 (3.94*) (1H, d, $J_{4,3}$ = 3.1 Hz, Gal H-4), 4.18† (4.20*)† (1H, t, $J_{4,3}$ = $J_{4,5}$ = 9.6 Hz, Glc H-4), 4.38 (1H, dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.2 Hz, Glc H-6), 4.56-4.63 (2H, m, Glc H-1, Glc H-6′), 4.73† (4.74*)† (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1), 5.28–5.38 (2H, m, Glc H-2, Gal H-2, *CH*Ph) [5.51* (1H, dd, $J_{2,3}$ = 11.2, $J_{2,1}$ = 7.8 Hz, Gal H-2)], 5.80† (5.83*)† (1H, t, $J_{3,2}$ = $J_{3,4}$ = 9.6 Hz, Glc H-3), 7.15–7.56, 7.76–8.00 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 11.5 (11.9*) (C-4′), 24.6 (25.7*) (C-3′), 47.3 (49.4*) (C-2′), 47.7 (49.5*) (Gal C-3), 52.0 (52.3*) (CO_2 *Me*), 57.0 (OMe), 62.5 (Glc C-6), 68.0 (Gal C-6), 68.6 (68.7*) (Gal C-5), 69.9, 72.2, 72.3, 72.4, 2× 72.8 (Glc C-2, Glc C-5, Gal C-2), 73.9 (74.1*) (Glc C-3), 75.4 (76.5*) (Gal C-4), 76.4 (Glc C-4), 100.9 (101.0*) (*CH*Ph), 101.5 (Glc C-1), 102.5 (102.6*) (Gal C-1), 126.3, 126.4, 127.9, 2× 128.3, 2× 128.4, 128.5, 128.8, 3× 129.7, 129.8, 129.9, 133.0, 2× 133.1, 133.3 (5× Ph), 128.9, 129.1, 129.4, 129.6, 137.3 (137.5*) (5× *ipso*Ph), 164.7, 164.9, 165.0, 165.5, 2× 165.7 (4× O*C*(O)Ph), 173.1 (173.6*) (C-1′); LRMS 999 (M+Na, 100%); Anal. ($C_{53}H_{52}O_{16}S$) C, H.

4.17. Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-3-[2′-(ethyl valeroate)]-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (34)

In 96% yield (neat $CHCl_3$, R_f 0.25) as a 2:1 mixture of inseparable diastereomers by coupling between **23** and ethyl (±) 2-bromovalerate to give a colourless crystalline solid: IR 1150, 1342, 1729, 2961 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.47 (0.74*) (3H, t, J_{vic} = 7.3 Hz, H-5′), 0.81–0.98 (1.14–1.31*)† (2H, m, H-4′), 1.07 (1.19*)† (3H, t, J_{vic} = 7.1 Hz, CO_2CH_2 CH_3), 1.14–1.31† (1.40–1.61*) (2H, m, H-3′), 2.90 (2.97*) (1H, s, Gal H-5), 3.11 (3.19–3.23*) (1H, dd, J_{vic} = 9.3, 6.0 Hz, H-2′), 3.23† (3.31*) (1H, dd, $J_{3,2}$ = 11.4, $J_{3,4}$ = 3.1 Hz, Gal H-3), 3.40 (3.41*) (3H, s, OMe), 3.51–3.60 (1H, m, Gal H-6), 3.76 (1H, appd, $J_{6',6}$ = 12.1 Hz, Gal H-6′), 3.82–3.97 (4H, m, Glc H-5, Gal H-4, CO_2 CH_2CH_3) [4.11* (2H, q, J_{vic} = 7.1 Hz, CO_2 CH_2CH_3)], 4.22 (1H, t, $J_{4,3}$ = $J_{4,5}$ = 9.2 Hz, Glc H-4), 4.38 (1H, dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.2 Hz, Glc H-6), 4.58† (4.59*)† (1H, d, $J_{1,2}$ = 7.8 Hz, Glc H-1), 4.66 (1H, dd, $J_{6',6}$ = 12.0, $J_{6',5}$ = 2.0 Hz, Glc H-6′), 4.73 (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1), 5.27–5.35† (2H, m, Glc H-2, Gal H-2) [5.51* (1H, dd, $J_{2,3}$ = 11.4, $J_{2,1}$ = 7.8 Hz, Gal H-2)], 5.32† (1H, s, *CH*Ph), 5.83 (1H, t, $J_{3,2}$ = $J_{3,4}$ = 9.2 Hz, Glc H-3), 7.18–7.61, 7.81–8.05 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.2 (13.4*) (C-5′), 13.8 (14.0*) (CO_2CH_2 CH_3), 20.3 (20.5*) (C-4′), 33.1 (34.3*) (C-3′), 45.0 (47.8*) (C-2′), 47.1 (49.4*) (Gal C-3), 56.8 (OMe), 60.9 (61.0*) (CO_2 CH_2CH_3), 62.3 (Glc C-6), 67.9 (Gal C-6), 68.5 (68.6*) (Gal C-5), 69.4, 69.8, 72.2, 72.6, 72.7, 73.8, 74.0, 74.1, 75.1, 76.3, 76.4, 76.5 (Glc C-2, Glc C-3, Glc C-4, Glc C-5, Gal C-2, Gal C-4), 100.8 (100.9*) (*CH*Ph), 101.4 (Glc C-1), 102.3 (102.5*) (Gal C-1), 2× 126.2, 127.2, 127.8, 127.9, 2× 128.1, 128.2, 2× 128.3, 128.6, 128.8, 129.5, 129.8, 132.8, 132.9, 2× 133.0, 133.1, 137.2 (5× Ph), 128.8, 129.0, 129.2, 129.4, 129.5, 137.3 (137.4*) (5× *ipso*Ph), 2× 164.7, 2× 164.8, 165.1, 165.3, 165.4, 165.5 (4× O*C*(O)Ph), 172.6 (173.0*) (C-1′); LRMS 1027 (M+Na, 100%); Anal. ($C_{55}H_{56}O_{16}S$) C, H.

4.18. Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-3-[2′-(methyl 2-phenylacetate)]-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (35)

In 90% yield (neat $CHCl_3$, R_f 0.23) as a 1:1 mixture of inseparable diastereomers by coupling between **23** and ethyl (±) 2-bromovalerate to give a colourless crystalline solid: IR 1113, 1269, 1732, 3022 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.78† (2.91*) (1H, s, Gal H-5), 2.80† (3.14*) (1H, dd, $J_{3,2}$ = 11.2, $J_{3,4}$ = 3.2 Hz, Gal H-3), 3.28 (3.68*) (3H, s, CO_2 *Me*), 3.41 (3H, s, OMe), 3.49 (3.54*) (1H, dd, $J_{6,6'}$ = 12.4, $J_{6,5}$ = 1.4 Hz, Gal H-6), 3.62–3.76 (2H, m, Gal H-4, Gal H-6′) [3.97* (1H, d, $J_{4,3}$ = 3.2 Hz, Gal H-4)], 3.83 (1H, ddd, $J_{5,4}$ = 9.4,

$J_{5,6}$ = 5.9, $J_{5,6'}$ = 2.0 Hz, Glc H-5), 4.15† (4.20*)† (1H, t, $J_{4,3} = J_{4,5}$ = 9.4 Hz, Glc H-4), 4.38–4.48 (2H, m, Glc H-6, Glc H-6′), 4.52–4.63 (3H, m, Glc H-1, Gal H-1, H-2′) [4.72* (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1)], 5.22–5.34† (1H, m, Glc H-2), 5.31 (1H, s, *CH*Ph), 5.51 (1H, dd, $J_{2,3}$ = 11.2, $J_{2,1}$ = 7.8 Hz, Gal H-2), 5.77† (5.81*† (1H, t, $J_{3,2} = J_{3,4}$ = 9.4 Hz, Glc H-3), 6.79–6.82, 7.01–7.12, 7.28–7.63, 7.87–8.07 (30H, 4× m, 6× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 48.3 (49.6*) (Gal C-3), 52.2 (52.8*(CO_2 *Me*), 52.4 (C-2′), 57.0 (OMe), 62.5 (Glc C-6), 67.9 (Gal C-6), 68.6 (Gal C-5), 71.0 (72.2*) (Gal C-2), 72.1 (72.2*) (Glc C-2), 72.7 (72.8*) (Glc C-5), 73.7 (74.0*) (Glc C-3), 75.9 (76.0*) (Gal C-4), 76.2 (76.4*) (Glc C-4), 100.7 (100.8*) (*CH*Ph), 101.5 (101.6*) (Glc C-1), 102.4 (Gal C-1), 126.3, 126.4, 127.9, 128.0, 128.1, 128.2, 2× 128.3, 128.4, 2× 128.5, 128.7, 128.8, 129.6, 129.7, 129.8, 129.9, 132.9, 133.0, 133.1, 133.2, 133.3 (6× Ph), 128.9, 129.0, 129.3, 129.6, 134.8, 136.4, 137.3 (5× *ipso*Ph), 164.6 (164.8*), 164.9 (165.0*), 165.4, 165.7 (4× O*C*(O)Ph), 170.6 (171.8*) (*C*O_2Me); LRMS 1047 (M+Na, 100%); Anal. ($C_{57}H_{52}O_{16}S$) C, H.

4.19. Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-3-[2′-(γ-butyrolactone)]-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (36)

In 85% yield as a 2:1 mixture of separable diastereomers by coupling between **23** and α-bromo-γ-butyrolactone (chromatography EtOAc/hexane, 2:3 → 1:1, R_f 0.30, 0.19). One diastereomer of **36** as a colourless crystalline solid: IR 1113, 1268, 1731 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.69–1.78, 2.07–2.30 (2H, 2× m, H-3′), 2.89 (1H, s, Gal H-5), 3.32–3.36† (1H, m, H-2′), 3.34† (3H, s, OMe), 3.48 (1H, dd, $J_{6,6'}$ = 11.0, $J_{6,5}$ = 1.3 Hz, Gal H-6), 3.61 (1H, dd, $J_{3,2}$ = 10.3, $J_{3,4}$ = 3.3 Hz, Gal H-3), 3.70 (1H, appd, $J_{6',6}$ = 11.0, Gal H-6′), 3.77 (1H, ddd, $J_{5,4}$ = 9.7, $J_{5,6}$ = 4.0, $J_{5,6'}$ = 1.7 Hz, Glc H-5), 3.96 (1H, d, $J_{4,3}$ = 3.3 Hz, Gal H-4), 4.02–4.23 (3H, m, Glc H-4, H-4′), 4.28 (1H, dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.0 Hz, Glc H-6), 4.53† (1H, d, $J_{1,2}$ = 7.6, Glc H-1), 4.52–4.58† (1H, m, Glc H-6′), 4.68 (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1), 5.24† (1H, dd, $J_{2,3}$ = 9.1, $J_{2,1}$ = 7.6 Hz, Glc H-2), 5.26 (1H, s, *CH*Ph), 5.49 (1H, dd, $J_{2,3}$ = 10.3, $J_{2,1}$ = 7.8 Hz, Gal H-2), 5.77 (1H, t, $J_{3,2} = J_{3,4}$ = 9.1 Hz, Glc H-3), 7.13–7.55, 7.79–7.99 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 29.1 (C-3′), 39.1 (C-2′), 48.0 (Gal C-3), 56.9 (OMe), 62.3 (Glc C-6), 66.7 (C-4′), 67.9 (Gal C-6), 68.4 (Gal C-5), 72.3 (Glc C-5), 72.6 (Glc C-2), 72.7 (Gal C-2), 74.1 (Glc C-3), 76.1 (Gal C-4), 76.5 (Glc C-4), 100.8 (*CH*Ph), 101.4 (Glc C-1), 102.4 (Gal C-1), 126.0, 126.3, 127.9, 2× 128.2, 128.4, 128.6, 128.8, 2× 129.6, 129.9, 132.9, 2× 133.1, 133.3 (5× Ph), 128.8, 129.4, 129.5, 137.4 (5× *ipso*Ph), 164.7, 164.8, 165.4, 165.6 (4× O*C*(O)Ph), 176.6 (C-1′); LRMS 983 (M+Na, 100%); HRMS Calcd for $C_{52}H_{48}O_{16}S$ (M+Na) 983.2561. Found 983.2558. Other diastereomer of **36** as a colourless crystalline solid: IR 1113, 1268, 1731 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.87–1.97, 2.42–2.55 (2H, 2× m, H-3′), 2.95 (1H, s, Gal H-5), 3.40 (3H, s, OMe), 3.43 (1H, dd, $J_{3,2}$ = 11.5, $J_{3,4}$ = 3.1 Hz, Gal H-3), 3.56 (1H, dd, $J_{6,6'}$ = 12.2, $J_{6,5}$ = 1.5 Hz, Gal H-6), 3.66 (1H, dd, J_{vic} = 8.3, 5.3 Hz, H-2′), 3.78† (1H, appd, $J_{6',6}$ = 12.2 Hz, Gal H-6′), 3.82† (1H, ddd, $J_{5,4}$ = 9.5, $J_{5,6}$ = 4.2, $J_{5,6'}$ = 2.2 Hz, Glc H-5), 4.01–4.24 (4H, m, Glc H-4, Gal H-4, H-4′), 4.34 (1H, dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.2 Hz, Glc H-6), 4.59† (1H, d, $J_{1,2}$ = 7.7 Hz, Glc H-1), 4.62† (1H, dd, $J_{6',6}$ = 12.0, $J_{6',5}$ = 2.2 Hz, Glc H-6′), 4.77 (1H, d, $J_{1,2}$ = 7.7 Hz, Gal H-1), 5.30† (1H, dd, $J_{2,3}$ = 9.5, $J_{2,1}$ = 7.7 Hz, Glc H-2), 5.36† (1H, dd, $J_{2,3}$ = 11.5, $J_{2,1}$ = 7.7 Hz, Gal H-2), 5.38† (1H, s, *CH*Ph), 5.82 (1H, t, $J_{3,2} = J_{3,4}$ = 9.5 Hz, Glc H-3), 7.34–7.62, 7.81–8.07 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 30.0 (C-3′), 37.9 (C-2′), 47.5 (Gal C-3), 56.9 (OMe), 62.3 (Glc C-6), 66.7 (C-4′), 68.1 (Gal C-6), 68.7 (Gal C-5), 69.2 (Gal C-2), 72.3, 72.8 (Glc C-2, Glc C-5), 74.1 (Glc C-3), 74.5 (Gal C-4), 76.6 (Glc C-4), 101.1 (*CH*Ph), 101.5 (Glc C-1), 102.6 (Gal C-1), 128.0, 2× 128.3, 2× 128.4, 128.9, 2× 129.7, 129.8, 129.9, 133.0, 2× 133.1, 133.2 (5× Ph), 129.4, 129.5, 137.5 (5× *ipso*Ph), 164.9, 165.3, 165.4, 165.7 (4× O*C*(O)Ph), 175.4 (C-1′); LRMS 983 (M+Na, 100%).

4.20. Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-thio-3-[2′-(γ-valerolactone)]-β-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (37)

In 87% yield as a 2:1:1 mixture of separable diastereomers by coupling between **23** and α-bromo-γ-valerolactone (chromatography EtOAc/hexane, 2:3 → 1:1, R_f 0.44, 0.39, 0.34). One diastereomer of **37** as a colourless crystalline solid: IR 1117, 1267, 1726 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.23 (3H, d, J_{vic} = 6.2 Hz, H-5′), 1.74-1.94 (2H, m, H-3′), 2.93 (1H, s, Gal H-5), 3.40 (3H, s, OMe), 3.45 (1H, dd, J_{vic} = 8.2, 3.0 Hz, H-2′), 3.53 (1H, dd, $J_{6,6'}$ = 12.3, $J_{6,5}$ = 1.5 Hz, Gal H-6), 3.68 (1H, dd, $J_{3,2}$ = 11.3, $J_{3,4}$ = 3.3 Hz, Gal H-3), 3.77 (1H, appd, $J_{6',6}$ = 12.3, Gal H-6′), 3.83 (1H, ddd, $J_{5,4}$ = 9.6, $J_{5,6}$ = 4.0, $J_{5,6'}$ = 1.8 Hz, Glc H-5), 3.98 (1H, d, $J_{3,4}$ = 3.3 Hz, Gal H-4), 4.22 (1H, t, $J_{4,3} = J_{4,5}$ = 9.6 Hz, Glc H-4), 4.32 (1H, dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.0 Hz, Glc H-6), 4.59† (1H, d, $J_{1,2}$ = 7.7 Hz, Glc H-1), 4.60-4.69† (2H, m, Glc H-6′, H-4′), 4.74 (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1), 5.29† (1H, dd, $J_{2,3}$ = 9.6, $J_{2,1}$ = 7.7 Hz, Glc H-1), 5.32† (1H, s, *CH*Ph), 5.55 (1H, dd, $J_{2,3}$ = 11.3, $J_{2,1}$ = 7.8 Hz, Gal H-2), 5.83 (1H, t, $J_{3,2} = J_{3,4}$ = 9.6 Hz, Glc H-3), 7.32–7.62, 7.85–8.02 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 Hz, $CDCl_3$): δ 20.4 (C-5′), 36.8 (C-3′), 40.7 (C-2′), 48.0 (Gal C-3), 56.9 (OMe), 62.3 (Glc C-6), 67.9 (Gal C-6), 68.4 (Gal C-5), 72.4 (Glc C-5), 72.7 (Glc C-2), 72.7 (Gal C-2), 74.2 (Glc C-3), 75.8, 76.1 (Gal C-4, C-4′), 76.6 (Glc C-4), 100.8 (*CH*Ph), 101.4 (Glc C-1), 102.4 (Gal C-1), 128.2, 128.3, 128.5, 128.6, 128.8, 129.6, 2× 129.7, 129.9, 133.0, 133.1, 133.2, 133.4 (5× Ph), 129.4, 129.5, 137.4 (5× *ipso*Ph), 2× 164.8, 165.4, 165.6 (4× O*C*(O)Ph), 176.0 (C-1′); LRMS 997 (M+Na, 100%); HRMS Calcd for $C_{53}H_{50}O_{16}S$ (M+Na) 997.2717. Found 997.2737. Other diastereomer of **37** as a colourless crystalline solid: IR 1117, 1267, 1726 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.29 (3H, d, J_{vic} = 6.3 Hz, H-5′), 1.35–1.45, 2.12–2.22 (2H, 2× m, H-3′), 2.97 (1H, s, Gal H-5), 3.41 (3H, s, OMe), 3.52† (1H, t, J_{vic} = 9.2 Hz, H-2′), 3.56† (1H, dd, $J_{6,6'}$ = 12.4, $J_{6,5}$ = 1.7 Hz, Gal H-6), 3.72† (1H, dd, $J_{3,2}$ = 11.3, $J_{3,4}$ = 3.3 Hz, Gal H-3), 3.76† (1H, dd, $J_{6',6}$ = 12.4, $J_{6',5}$ = 1.0 Hz, Gal H-6′), 3.83 (1H, ddd, $J_{5,4}$ = 9.6, $J_{5,6}$ = 4.0, $J_{5,6'}$ = 2.1 Hz, Glc H-5), 4.15 (1H, d, $J_{4,3}$ = 3.3 Hz, Gal H-4), 4.21 (1H, t, $J_{4,3} = J_{4,5}$ = 9.6 Hz,

Glc H-4), 4.26–4.33 (1H, m, H-4′), 4.63 (1H, dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.0 Hz, Glc H-6), 4.59† (1H, d, $J_{1,2}$ = 7.7 Hz, Glc H-1), 4.58–4.63† (1H, m, Glc H-6′), 4.72 (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1), 5.30 (1H, dd, $J_{2,3}$ = 9.6, $J_{2,1}$ = 7.7 Hz, Glc H-2), 5.35 (1H, s, *CH*Ph), 5.54 (1H, dd, $J_{2,3}$ = 11.3, $J_{2,1}$ = 7.8 Hz, Gal H-2), 5.82 (1H, t, $J_{3,2}$ = $J_{3,4}$ = 9.6 Hz, Glc H-3), 7.32–7.62, 7.86–8.03 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.3 (C-5′), 36.1 (C-3′), 41.2 (C-2′), 48.7 (Gal C-3), 57.0 (OMe), 62.4 (Glc C-6), 67.9 (Gal C-6), 68.5 (Gal C-5), 72.3, 72.8 (Glc C-2, Glc C-5), 74.1 (Gal C-2), 75.4 (Glc C-3), 76.3, 76.5 (Glc C-4, Gal C-4, C-4′), 100.9 (*CH*Ph), 101.5 (Glc C-1), 102.4 (Gal C-1), 126.3, 126.4, 2× 128.0, 128.3, 128.4, 128.6, 128.8, 129.6, 129.7, 129.9, 133.0, 133.1, 133.2, 133.4 (5× Ph), 128.9, 129.4, 137.5 (5× *ipso*Ph), 164.7, 164.9, 165.5, 165.7 (4× O*C*(O)Ph), 176.9 (C-1′); LRMS 997 (M+Na, 100%). Other two diastereomers of **37** as a colourless crystalline solid: IR 1117, 1267, 1726 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.15 (1.23*) (3H, d, J_{vic} = 6.3 Hz, H-5′), 1.50–1.57, 1.94–2.12, 2.49–2.58 (2H, 3× m, H-3′), 2.94 (2.95*(1H, s, Gal H-5), 3.27 (3.50*) (1H, dd, $J_{3,2}$ = 11.5, $J_{3,4}$ = 3.0 Hz, Gal H-3), 3.40 (3.42*) (3H, s, OMe), 3.58 (1H, dd, $J_{6,6'}$ = 11.3, $J_{6,5}$ = 4.5 Hz, Gal H-6), 3.68 (1H, dd, J_{vic} = 7.8, 3.7 Hz, H-2′) [3.80–3.89* (1H, m, H-2′)], 3.75 (1H, appd, $J_{6',6}$ = 11.3 Hz, Gal H-6′), 3.80–3.89 (1H, m, Glc H-5), 4.12–4.51 (4H, m, Glc H-4, Glc H-6, Gal H-4, H-4′), 4.57–4.66 (2H, m, Glc H-1, Glc H-6′), 4.75 (4.78*) (1H, d, $J_{1,2}$ = 7.7 Hz, Gal H-1), 5.26–5.40 (3H, m, Glc H-2, Gal H-2, *CH*Ph), 5.81, 5.82 (1H, t, $J_{3,2}$ = $J_{3,4}$ = 9.1 Hz, Glc H-3), 7.23–7.62, 7.85–8.05 (25H, 2× m, 5× Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.2 (20.8*) (C-5′), 37.1 (37.6*) (C-3′), 38.8 (40.3*) (C-2′), 47.5 (47.6*) (Gal C-3), 56.9 (57.0*) (OMe), 62.3 (62.4*) (Glc C-6), 68.1 (Gal C-6), 68.6 (68.8*) (Gal C-5), 69.2 (69.4*) (Gal C-2), 72.3 (72.4*) (Glc C-5), 72.8 (72.9*) (Glc C-2), 73.9, 74.0, 74.2, 75.2, 75.8, 76.5 (Glc C-3, Glc C-4, Gal C-4, C-4′), 101.0 (*CH*Ph), 101.5 (101.6*) (Glc C-1), 102.6 (Gal C-1), 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 128.8, 128.9, 129.5, 2× 129.7, 2× 129.9, 132.9, 133.1, 133.2, 133.3, 133.4 (5× Ph), 128.9, 129.0, 129.4, 129.5, 137.5 (5× *ispo*Ph), 164.9, 165.0, 165.2, 165.4, 165.6, 165.8 (4× O*C*(O)Ph), 174.8 (175.6*) (C-1′); LRMS 997 (M+Na, 100%).

4.21. Methyl 4,6-*O*-benzylidene-3-thio-3-(carboxymethyl)-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (38)

To a stirred suspension of **31** (330 mg, 0.34 mmol) in MeOH (7 mL) was added dropwise a 1 M solution of NaOH (~1 mL) until a pH of ~13 was attained. The mixture was left to stir for 24 h before being neutralised with Amberlyte IR-120 (H^+), the resin filtered, washed with aqueous MeOH, and then the solvent was concentrated under reduced pressure. The residue was re-dissolved in H_2O (20 mL), the pH adjusted to ~4 with AcOH, washed with EtOAc (3× 30 mL) and the aqueous phase freeze-dried to give 4′,6′-*O*-benzylidenated lactoside (116 mg, quantitative) as a colourless solid: ^{1}H NMR (D_2O): δ 2.97 (1H, dd, $J_{3,2}$ = 11.1, $J_{3,4}$ = 3.0 Hz, Gal H-3), 3.13 (1H, t, $J_{2,1}$ = $J_{2,3}$ = 8.1 Hz, Glc H-2), 3.12 (2H, s, H-2′), 3.40† (3H, s, OMe), 3.40–3.52† (4H, m, Glc H-3, Glc H-4, Glc H-5, Gal H-2), 3.67 (1H, s, Gal H-5), 3.68 (1H, dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.8 Hz, Glc H-6), 3.85 (1H, appd, $J_{6',6}$ = 12.0 Hz, Glc H-6′), 4.08 (2H, apps, Gal H-6, Gal H-6′), 4.65† (1H, d, $J_{4,3}$ = 3.0 Hz, Gal H-4), 4.68† (1H, d, $J_{1,2}$ = 8.1 Hz, Glc H-1), 4.87 (1H, d, $J_{1,2}$ = 7.7 Hz, Gal H-1), 6.00 (1H, s, *CH*Ph), 7.74–7.83, 8.15–8.18 (5H, 2× m, CH*Ph*); ^{13}C NMR (75.5 MHz, D_2O): δ 36.8 (C-2′), 50.3 (OMe), 59.8 (Glc C-6), 68.6 (Gal C-5), 68.9 (Gal C-6), 69.9, 72.7, 74.1, 74.7, 75.8, 78.5 (Glc C-2, Glc C-3, Glc C-4, Glc C-5, Gal C-2, Gal C-4), 101.2 (*CH*Ph), 103.0 (Glc C-1), 103.8 (Gal C-1), 126.1, 128.3, 128.6, 128.7, 131.2 (CH*Ph*), 136.6 (*ipso*Ph), 177.7 (C-1′); LRMS 525 (M+Na, 100%).

The following were prepared in a similar manner:

4.22. Methyl 4,6-*O*-benzylidene-3-thio-3-[2′-(propanoic acid)]-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (39)

In 95% yield as a 2:1 mixture of diastereomers: ^{1}H NMR (D_2O): δ 1.33 (1.37*) (3H, d, J_{vic} = 7.2 Hz, H-3′), 3.12 (3.19*) (1H, dd, $J_{3,2}$ = 11.2, $J_{3,4}$ = 3.2 Hz, Gal H-3), 3.25-3.31 (1H, m, Glc H-2), 3.54† (3H, s, OMe), 3.54-3.66† (4H, m, Glc H-3, Glc H-4, Glc H-5, H-2′), 3.80–3.85 (2H, m, Glc H-6, Gal H-5), 4.00 (1H, appd, $J_{6',6}$ = 12.5 Hz, Glc H-6′), 4.23 (2H, appts, Gal H-6, Gal H-6′), 4.33† (4.35*)† (1H, d, $J_{4,3}$ = 3.2 Hz, Gal H-4), 4.38† (1H, d, $J_{1,2}$ = 8.0 Hz, Glc H-1), 4.59 (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1), 5.72 (5.74*) (1H, s, *CH*Ph), 7.43–7.47, 7.49–7.53 (5H, 2× m, CH*Ph*); ^{13}C NMR (75.5 MHz, D_2O): δ 18.0 (18.5*) (C-3′), 45.3 (46.2*) (C-2′), 49.5 (50.0*) (Gal C-3), 57.2 (OMe), 59.9 (Glc C-6), 68.6 (Gal C-5), 68.8 (68.9*) (Gal C-6), 69.5 (70.2*), 74.2, 74.7, 78.5 (Glc C-3, Glc C-4, Glc C-5, Gal C-2), 72.7 (Glc C-2), 75.1 (76.3*) (Gal C-4), 101.2, 101.4 (*CH*Ph), 103.0 (Glc C-1), 103.9 (Gal C-1), 2× 126.1 (126.2*), 2× 128.5 (128.6*), 129.6 (129.7*) (CH*Ph*), 136.6 (136.7*) (*ipso*Ph), 181.3 (181.4*) (C-1′); LRMS 555 (M+Na, 100%).

4.23. Methyl 4,6-*O*-benzylidene-3-thio-3-[2′-(butanoic acid)]-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (40)

In 97% yield as a 1:1 mixture of diastereomers: ^{1}H NMR (D_2O): δ 0.88 (0.90*) (3H, t, J_{vic} = 7.3 Hz, H-4′), 1.63–1.70 (2H, m, H-3′), 3.08 (3.16*) (1H, dd, $J_{3,2}$ = 11.1, $J_{3,4}$ = 3.1 Hz, Gal H-3), 3.24–3.41 (2H, m, Glc H-2, H-2′), 3.51–3.64† (4H, m, Glc H-3, Glc H-4, Glc H-5, Gal H-2), 3.53† (3H, s, OMe), 3.80† (1H, s, Gal H-5), 3.81† (1H, dd, $J_{6,6'}$ = 12.1, $J_{6,5}$ = 4.6 Hz, Glc H-6), 3.98 (1H, dd, $J_{6',6}$ = 12.1, $J_{6',5}$ = 1.7 Hz, Glc H-6′), 4.21 (2H, appd, Gal H-6, Gal H-6′), 4.31 (4.34*)† (1H, d, $J_{4,3}$ = 3.1 Hz, Gal H-4), 4.37 (1H, d, $J_{1,2}$ = 8.0 Hz, Glc H-1), 4.57 (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1), 5.70 (5.73*) (1H, s, *CH*Ph), 7.41–7.45, 7.48–7.52 (5H, 2× m, CH*Ph*); ^{13}C NMR (75.5 MHz, D_2O): δ 11.3 (11.4*) (C-4′), 25.8 (26.0*) (C-3′), 49.5 (50.0*) (C-2′), 51.9 (53.1*) (Gal C-3), 57.1 (OMe), 59.9 (60.0*(Glc C-6), 68.6 (Gal C-5), 68.8 (68.9*) (Gal C-6), 69.3 (70.1*), 74.2, 74.7, 78.5 (Glc C-3, Glc C-4, Glc C-5, Gal C-2), 74.6 (76.4*) (Gal C-4), 101.2 (101.5*) (*CH*Ph), 103.0 (Glc C-1),

103.9 (Gal C-1), 2× 126.0 (126.1*), 2× 128.5 (128.6*), 129.6 (129.7*) (CH*Ph*), 136.5 (136.6*) (*ipso*Ph), 180.1 (180.7*) (C-1′); LRMS 569 (M + Na, 100%).

4.24. Methyl 4,6-*O*-benzylidene-3-thio-3-[2′-(valeroic acid)]-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (41)

In quantitative yield as a 1:1 mixture of diastereomers: ^{1}H NMR (D_2O): δ 0.83 (0.84*) (3H, t, J_{vic} = 7.3 Hz, H-5′), 1.23–1.38 (2H, m, H-4′), 1.51–1.77 (2H, m, H-3′), 3.08 (3.18*) (1H, dd, $J_{3,2}$ = 11.2, $J_{3,4}$ = 3.2 Hz, Gal H-3), 3.24–3.30 (1H, m, Glc H-2), 3.41 (3.48*) (1H, dd, J_{vic} = 9.2, 7.1, 3.0 Hz, H-2′), 3.53† (3H, s, OMe), 3.53–3.64† (4H, m, Glc H-3, Glc H-4, Glc H-5, Gal H-2), 3.80† (1H, s, Gal H-5), 3.81† (1H, dd, $J_{6,6'}$ = 12.1, $J_{6,5}$ = 4.6 Hz, Glc H-6), 3.98 (1H, dd, $J_{6',6}$ = 12.1, $J_{6',5}$ = 2.1 Hz, Glc H-6′), 4.21 (2H, appd, Gal H-6, Gal H-6′), 4.31 (4.34*) (1H, d, $J_{4,3}$ = 3.2 Hz, Gal H-4), 4.37 (1H, d, $J_{1,2}$ = 8.0 Hz, Glc H-1), 4.57 (4.58*(1H, d, $J_{1,2}$ = 7.7 Hz, Gal H-1), 5.70 (5.74*) (1H, s, *CH*Ph), 7.40–7.46, 7.48–7.53 (5H, 2× m, CH*Ph*); ^{13}C NMR (75.5 MHz, D_2O): δ 13.0 (C-5′), 20.2 (20.3*) (C-4′), 34.6 (34.7*) (C-3′), 49.5 (49.8*) (Gal C-3), 50.0 (51.2*) (C-2′), 57.2 (OMe), 59.8 (59.9*(Glc C-6), 68.6 (Gal C-5), 68.8 (68.9*) (Gal C-6), 69.2 (70.2*), 74.2, 74.7, 78.5 (Glc C-3, Glc C-4, Glc C-5, Gal C-2), 74.4 (76.5*(Gal C-4), 101.2 (101.5*) (*CH*Ph), 103.0 (Glc C-1), 103.9 (Gal C-1), 2× 126.1 (126.2*), 2× 128.5 (128.6*), 129.6 (129.7*) (CH *Ph*), 136.5 (136.6*) (*ipso*Ph), 180.4 (181.0*) (C-1′); LRMS 583 (M+Na, 100%).

4.25. Methyl 4,6-*O*-benzylidene-3-thio-3-[2′-(2′-phenylacetic acid)]-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (42)

In quantitative yield as a 1:1 mixture of diastereomers: ^{1}H NMR (D_2O): δ 2.83 (2.91*) (1H, dd, $J_{3,2}$ = 11.1, $J_{3,4}$ = 3.2 Hz, Gal H-3), 3.17 (1H, t, $J_{2,1} = J_{2,3}$ = 8.0 Hz, Glc H-2), 3.45† (3H, s, OMe), 3.45–3.76† (5H, m, Gal H-2, Gal H-5, Glc H-3, Glc H-4, Glc H-5), 3.86–3.93 (1H, m, Glc H-6), 4.02 (1H, d, $J_{6',6}$ = 12.1 Hz, Glc H-6′), 4.13 (2H, appd, Gal H-6, Gal H-6′), 4.19 (1H, d, $J_{4,3}$ = 3.2 Hz, Gal H-4), 4.28 (1H, d, $J_{1,2}$ = 8.0 Hz, Glc H-1), 4.42 (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1), 4.74 (4.77*) (1H, s, H-2′), 5.24 (5.64*) (1H, s, *CH*Ph), 7.19–7.45 (5H, m, 2× Ph); ^{13}C NMR (75.5 MHz, D_2O): δ 49.7 (49.8*) (Gal C-3), 55.7 (57.2*)† (C-2′), 57.2† (OMe), 59.9 (Glc C-6), 68.5 (68.6*) (Gal C-5), 68.8 (68.9*) (Gal C-6), 69.6 (69.7*), 74.2, 74.8, 78.5 (Gal C-2, Glc C-3, Glc C-4, Glc C-5), 72.7 (Glc C-2), 75.2 (75.6*) (Gal C-4), 101.3 (Gal C-1), 103.0 (Glc C-1), 103.8 (103.9*) (*CH*Ph), 126.2, 127.9, 128.1, 128.3, 2× 128.6, 128.8, 128.9, 129.7 (2× Ph), 136.5 (136.7*), 138.3 (139.6*) (2× *ipso*Ph), 177.1 (177.6*) (C-1′); LRMS 617 (M+Na, 100%).

4.26. Methyl 4,6-*O*-benzylidene-3-thio-3-[2′-(4′-hydroxybutanoic acid)]-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (43)

In quantitative yield as a 1:1 mixture of diastereomers: ^{1}H NMR (D_2O): δ 1.77–2.09 (2H, m, H-3′), 3.13 (3.21*) (1H, dd, $J_{3,2}$ = 11.5, $J_{3,4}$ = 2.9 Hz, Gal H-3), 3.28 (1H, t, $J_{2,1} = J_{2,3}$ = 8.2 Hz, Glc H-2), 3.47–3.70† (5H, m, Gal H-2, Glc H-3, Glc H-4, Glc H-5, H-2′), 3.54† (3H, s, OMe), 3.81† (1H, s, Gal H-5), 3.82† (1H, dd, $J_{6,6'}$ = 12.0, $J_{6,5}$ = 4.6 Hz, Glc H-6), 4.0 (1H, appd, $J_{6',6}$ = 12.0 Hz, Glc H-6′), 4.21–4.39† (3H, m, Gal H-4, Gal H-6, Gal H-6′), 4.38 (1H, d, $J_{1,2}$ = 8.2 Hz, Glc H-1), 4.59 (1H, d, $J_{1,2}$ = 7.8 Hz, Gal H-1), 5.71 (5.74*) (1H, s, *CH*Ph), 7.43–7.45, 7.50–7.54 (5H, 2× m, CH *Ph*); ^{13}C NMR (75.5 MHz, D_2O): δ 34.8 (34.9*) (C-3′), 46.8 (49.8*) (C-2′), 48.1 (50.3*) (Gal C-3), 57.2 (OMe), 59.3 (59.4*) (C-4′), 59.9 (Glc C-6), 68.7 (Gal C-5), 68.9 (69.0*) (Gal C-6), 69.3 (70.2*), 74.2, 74.8, 78.6 (Gal C-2, Glc C-3, Glc C-4, Glc C-5), 72.7 (Glc C-2), 74.8 (76.5*) (Gal C-4), 101.3 (101.5*) (*CH*Ph), 103.1 (Glc C-1), 103.9 (Gal C-1), 2× 126.1 (126.2*), 2× 128.6, 129.7 (129.8*) (CH *Ph*), 136.6 (136.7*) (*ipso*Ph), 182.3 (182.4*) (C-1′); LRMS 585 (M+Na, 100%).

4.27. Methyl 4,6-*O*-benzylidene-3-thio-3-[2′-(4′-hydroxyvaleroic acid)]-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (44)

In 88% yield as a mixture of diastereomers: ^{1}H NMR (D_2O): δ 1.15–1.22 (3H, m, H-5′), 1.69–1.99 (2H, m, H-3′), 3.11–3.25 (1H, m, Gal H-3), 3.32 (1H, t, $J_{2,3} = J_{2,1}$ = 8.5 Hz, Glc H-2), 3.48–3.70† (5H, m, Glc H-3, Glc H-4, Glc H-5, Gal H-2, H-2′), 3.57† (3H, s, OMe), 3.81† (1H, s, Gal H-5), 3.81–3.95† (1H, m, H-4′), 3.86† (1H, dd, $J_{6,6'}$ = 12.3, $J_{6,5}$ = 4.5 Hz, Glc H-6), 4.02 (1H, appd, $J_{6',6}$ = 12.3 Hz, Glc H-6′), 4.23 (2H, appd, Gal H-6, Gal H-6′), 4.34 (1H, apps, Gal H-4), 4.40 (1H, d, $J_{1,2}$ = 8.0 Hz, Glc H-1), 4.61 (1H, d, $J_{1,2}$ = 7.7 Hz, Gal H-1), 5.71 (5.74*) (1H, s, *CH*Ph), 7.46–7.48, 7.53–7.53 (5H, 2× m, CH*Ph*); ^{13}C NMR (75.5 MHz, D_2O): δ 22.4 (22.7*, 22.8*, 22.9*) (C-5′), 41.8 (41.9*) (C-3′), 47.5 (49.1*) (C-2′), 51.8 (Gal C-3), 57.9 (OMe), 60.6 (Glc C-6), 66.0 (66.1*, 66.6*, 66.7*) (C-4′), 69.2 (69.3*) (Gal C-5), 69.4 (69.5*) (Gal C-6), 69.8 (71.0*), 74.9, 75.4, 79.2 (Glc C-3, Glc C-4, Glc C-5, Gal C-2), 75.0 (77.8*) (Gal C-4), 101.9 (102.0*) (*CH*Ph), 103.7 (Glc C-1), 104.5 (Gal C-1), 2× 126.7 (126.8*), 2× 129.2 (129.3*), 130.3 (130.4*) (CH*Ph*), 137.2, (137.3*, 137.4*) (*ipso*Ph), 182.0 (182.0) (C-1′). LRMS 599 (M+Na, 100%).

4.28. Methyl 3-thio-3-(sodium carboxymethyl)-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (45)

A solution of the 4′,6′-*O*-benzylidenated lactoside **38** (170 mg, 0.33 mmol) in 10% aqueous TFA (10 mL) was stirred at 0 °C for 8 h before being concentrated in vacuo (cold work-up). The residue was dissolved in H_2O (5 mL) and the pH adjusted to ~7.3 with 1 M NaOH before being freeze-dried. Size exclusion chromatography [G10 Sephadex column, H_2O (EtOAc/MeOH/H_2O, 7:2:1, R_f 0.17)] and then HPLC purification (5% CH_3CN in H_2O, 3 mL/min, R_t = 4.34 min) afforded **45** (99 mg, 67%) as a colourless solid: ^{1}H NMR (D_2O): δ 3.00 (1H, dd, $J_{3,2}$ = 11.4, $J_{3,4}$ = 2.7 Hz, Gal H-3), 3.31 (1H, t, $J_{2,3} = J_{2,1}$ = 8.1 Hz, Glc H-2), 3.37 (2H, s, H-2′), 3.53 (1H, dd, $J_{2,3}$ = 11.4, $J_{2,1}$ = 7.7 Hz, Gal H-2), 3.57 (3H, s, OMe), 3.59–3.68 (3H, m, Glc H-3, Glc H-4, H-5), 3.71–3.85† (3H, m, H-5, H-6, H-6′), 3.82† (1H, dd, $J_{6,6'}$ = 12.3, $J_{6,5}$ = 4.8 Hz, H-6), 3.98–4.02 (2H, m,

Gal H-4, H-6′), 4.41 (1H, d, $J_{1,2}$ = 8.1 Hz, Glc H-1), 4.52 (1H, d, $J_{1,2}$ = 7.7 Hz, Gal H-1); ^{13}C NMR (75.5 MHz, D_2O): δ 36.1 (C-2′), 52.9 (Gal C-3), 57.2 (OMe), 60.0, 61.2 (Glc C-6, Gal C-6), 67.8, 74.7, 78.2 (Glc C-3, Glc C-4, C-5), 70.0 (Gal C-4), 72.7 (Gal C-2), 74.3 (Glc C-2), 77.5 (C-5), 103.0 (Glc C-1), 103.9 (Gal C-1), 178.1 (C-1′); LRMS 453 (M+Na, 100%); Anal. ($C_{15}H_{25}NaO_{12}S4H_2O$) C, H.

In a similar manner were prepared:

4.29. Methyl 3-thio-3-[2′-(sodium propanoate)]-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (46)

In 66% yield (HPLC, 5% CH_3CN in H_2O, 3 mL/min, R_t = 5.86 min) as a colourless solid: ^{1}H NMR (D_2O): δ 1.33 (1.33*) (3H, d, J_{vic} = 7.0 Hz, H-3′), 2.93 (2.98*) (1H, dd, $J_{3,2}$ = 11.3, $J_{3,4}$ = 2.7 Hz, Gal H-3), 3.25 (1H, t, $J_{2,3} = J_{2,1}$ = 8.3 Hz, Glc H-2), 3.38–3.48 (2H, m, Gal H-2, H-2′), 3.52 (3H, s, OMe), 3.53–3.62 (3H, m, Glc H-3, Glc H-4, H-5), 3.65–3.79† (3H, m, H-5, H-6, H-6′), 3.76† (1H, dd, $J_{6,6'}$ = 12.4, $J_{6,5}$ = 4.8 Hz, H-6), 3.87-3.96 (2H, m, Gal H-4, H-6′), 4.35 (1H, d, $J_{1,2}$ = 8.3 Hz, Glc H-1), 4.46 (1H, d, $J_{1,2}$ = 7.6 Hz, Gal H-1); ^{13}C NMR (75.5 MHz, D_2O): δ 18.1 (18.3*) (C-3′), 44.3 (46.0*) (C-2′), 51.8 (52.7*) (Gal C-3), 57.1 (OMe), 60.0, 61.2 (61.3*) (Glc C-6, Gal C-6), 67.6 (68.3*) (Gal C-4), 69.4 (70.2*) (Gal C-2), 72.8 (Glc C-2), 74.4, 74.7, 78.2 (78.3*) (Glc C-3, Glc C-4, C-5), 77.6 (C-5), 103.1 (Glc C-1), 103.9 (Gal C-1), 181.0 (181.6*) (C-1′); LRMS 467 (M+Na, 100%); Anal. ($C_{16}H_{27}NaO_{12}S2H_2O$) C, H.

4.30. Methyl 3-thio-3-[2′-(sodium butanoate)]-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (47)

In 65% yield (HPLC, 1% CH_3CN in H_2O, 3 mL/min, R_t = 8.29 min) as a colourless solid: ^{1}H NMR (D_2O): δ 0.97† (2.64*)† (3H, t, J_{vic} = 7.3 Hz, H-4′), 1.64-1.87 (2H, m, H-3′), 2.97–3.06 (1H, m, Gal H-3), 3.30 (2H, m, Glc H-2, H-2′), 3.44–3.52 (1H, m, Gal H-2), 3.59 (3H, s, OMe), 3.63–3.69 (3H, m, Glc H-3, Glc H-4, H-5), 3.72–3.86† (3H, m, H-5, H-6, H-6′), 3.83† (1H, dd, $J_{6,6'}$ = 12.3, $J_{6,5}$ = 4.8 Hz, H-6), 3.95 (2H, m, Gal H-4, H-6′), 4.42 (1H, d, $J_{1,2}$ = 8.0 Hz, Glc H-1), 4.53 (4.54*) (1H, d, $J_{1,2}$ = 7.7 Hz, Gal H-1); ^{13}C NMR (75.5 MHz, D_2O): δ 11.4 (C-4′), 26.0 (C-3′), 51.5 (51.9*) (C-2′), 52.9 (53.2*) (Gal C-3), 57.2 (OMe), 60.0 (60.1*), 61.2 (61.3*) (Glc C-6, Gal C-6), 67.5 (68.4*) (Gal C-4), 69.5 (70.3*) (Gal C-2), 72.8 (Glc C-2), 74.4, 74.8, 78.3 (78.4*) (Glc C-3, Glc C-4, C-5), 77.6 (C-5), 103.1 (Glc C-1), 104.0 (Gal C-1), 180.3 (181.0*) (C-1′); LRMS 481 (M+Na, 100%); Anal. ($C_{17}H_{29}NaO_{12}S3H_2O$) C, H.

4.31. Methyl 3-thio-3-[2′-(sodium valeroate)]-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (48)

In 69% yield (HPLC, 3% CH_3CN in H_2O, 3 mL/min, R_t = 6.87 min) as a colourless solid: ^{1}H NMR (D_2O): δ 0.92 (3H, t, J_{vic} = 7.3 Hz, H-5′), 1.33–1.43 (2H, m, H-4′), 1.58–1.84 (2H, m, H-3′), 3.01 (3.02*) (1H, dd, $J_{3,2}$ = 11.4, $J_{3,4}$ = 2.5 Hz, Gal H-3), 3.33 (1H, t, $J_{2,3} = J_{2,1}$ = 8.2 Hz, Glc H-2), 3.41–3.52 (2H, m, Gal H-2, H-2′), 3.59 (3H, s, OMe), 3.61–3.69 (3H, m, Glc H-3, Glc H-4, H-5), 3.74–3.86† (3H, m, H-5, H-6, H-6′), 3.83† (1H, dd, $J_{6,6'}$ = 12.3, $J_{6,5}$ = 4.9 Hz, H-6), 3.94–4.04 (2H, m, Gal H-4, H-6′), 4.42 (1H, d, $J_{1,2}$ = 8.2 Hz, Glc H-1), 4.53 (4.54*) (1H, d, $J_{1,2}$ = 7.7 Hz, Gal H-1); ^{13}C NMR (75.5 MHz, D_2O): δ 13.1 (C-5′), 20.3 (C-4′), 34.7 (34.8*) (C-3′), 49.5 (51.2*) (C-2′), 52.0 (52.9*) (Gal C-3), 57.2 (OMe), 60.0 (60.1*), 61.2 (61.3*) (Glc C-6, Gal C-6), 67.4 (68.6*) (Gal C-4), 69.4 (70.4*) (Gal C-2), 72.8 (Glc C-2), 74.4, 74.8, 78.3 (78.4*(Glc C-3, Glc C-4, C-5), 77.6 (C-5), 103.1 (Glc C-1), 104.0 (Gal C-1), 180.4 (181.2*) (C-1′); LRMS 495 (M+Na, 100%); Anal. ($C_{18}H_{31}NaO_{12}S1.5H_2O$) C, H.

4.32. Methyl 3-thio-3-[2′-(sodium 2′-phenylacetate)]-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (49)

In 69% yield (HPLC, 3% CH_3CN in H_2O, 3 mL/min, R_t = 8.64, 9.20 min) as a mixture of separable diastereomers. One diastereomer of **49** as a colourless solid: ^{1}H NMR (D_2O): δ 2.77 (1H, dd, $J_{3,2}$ = 11.2, $J_{3,4}$ = 2.8 Hz, Gal H-3), 3.31 (1H, t, $J_{2,3} = J_{2,1}$ = 8.0 Hz, Glc H-2), 3.43 (1H, d, $J_{3,4}$ = 2.8 Hz, Gal H-4), 3.54–3.67† (7H, m, Glc H-3, Glc H-4, Glc H-5, Gal H-2, Gal H-5, H-6, H-6′), 3.59† (3H, s, OMe), 3.84 (1H, dd, $J_{6,6'}$ = 12.4, $J_{6,5}$ = 4.4 Hz, H-6), 4.02 (1H, dd, $J_{6',6}$ = 12.4, $J_{6',5}$ = 1.7 Hz, H-6′), 4.42 (1H, d, $J_{1,2}$ = 8.0 Hz, Glc H-1), 4.46 (1H, d, $J_{1,2}$ = 7.7 Hz, Gal H-1), 4.87 (1H, s, H-2′), 7.35– 7.46, 7.51–7.54 (5H, 2× m, Ph); ^{13}C NMR (75.5 MHz, D_2O): δ 51.9 (Gal C-3), 57.1 (C-2′), 57.2 (OMe), 60.1, 61.1 (Glc C-6, Gal C-6), 68.4 (Gal C-4), 70.5, 74.4, 74.7, 77.5, 78.4 (Glc C-3, Glc C-4, Glc C-5, Gal C-2, Gal C-5), 103.1 (Glc C-1), 104.0 (Gal C-1), 127.9, 2× 128.3, 2× 128.9 (Ph), 139.4 (*ipso*Ph), 177.3 (C-1′); LRMS 529 (M+Na, 100%); Anal. ($C_{21}H_{29}NaO_{12}S3H_2O$) C, H. Other diastereomer of **49** as a colourless solid: ^{1}H NMR (D_2O): δ 2.77 (1H, dd, $J_{3,2}$ = 11.3, $J_{3,4}$ = 2.8 Hz, Gal H-3), 3.30 (1H, t, $J_{2,3} = J_{2,1}$ = 8.0 Hz, Glc H-2), 3.52 (1H, dd, $J_{2,3}$ = 11.3, $J_{2,1}$ = 7.7 Hz, Gal H-2), 3.58 (3H, s, OMe), 3.60–3.82† (6H, Glc H-3, Glc H-4, Glc H-5, Gal H-5, H-6, H-6′), 3.79† (1H, dd, $J_{6,6'}$ = 12.5, $J_{6,5}$ = 4.7 Hz, H-6), 3.95–4.00 (2H, m, Gal H-4, H-6′), 4.41† (1H, d, $J_{1,2}$ = 8.0 Hz, Glc H-1), 4.43† (1H, d, $J_{1,2}$ = 7.7 Hz, Gal H-1), 4.86 (1H, s, H-2′), 7.35–7.51 (5H, m, Ph); ^{13}C NMR (75.5 MHz, D_2O): δ 52.2 (Gal C-3), 55.5 (C-2′), 57.2 (OMe), 60.0, 61.2 (Glc C-6, Gal C-6), 67.2 (Gal C-4), 69.7 (Gal C-2), 72.7 (Glc C-2), 74.4, 74.7, 77.5, 78.4 (Glc C-3, Glc C-4, Glc C-5, Gal C-5), 103.0, 103.9 (Glc C-1, Gal C-1), 127.9, 2× 128.2, 2× 128.9 (Ph), 138.5 (*ipso*Ph), 177.6 (C-1′). LRMS 529 (M+Na, 100%).

4.33. Methyl 3-thio-3-[2′-sodium (4′-hydroxy-butanoate)]-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (50)

In 70% yield (HPLC, 1% CH_3CN in H_2O, 3 mL/min, R_t = 4.75 min) as a colourless solid: ^{1}H NMR (D_2O): δ 1.81–2.08 (2H, m, H-3′), 3.03† (3.05*)† (1H, dd, $J_{3,2}$ = 11.6, $J_{3,4}$ = 2.7 Hz, Gal H-3), 3.30 (1H, t, $J_{2,3} = J_{2,1}$ = 8.2 Hz, Glc H-2), 3.42–3.53 (2H, m, Glc H-2, H-2′), 3.57 (3H, s, OMe), 3.59–3.83† (8H, m, Glc

H-3, Glc H-4, Glc H-5, Gal H-5, H-6, H-6′, H-4′), 3.81† (1H, dd, $J_{6,6'}$ = 12.6, $J_{6,5}$ = 4.9 Hz, H-6), 3.93–4.01 (2H, m, Gal H-4, H-6′), 4.40 (1H, d, $J_{1,2}$ = 8.2 Hz, Glc H-1), 4.51† (4.52*)† (1H, d, $J_{1,2}$ = 7.6 Hz, Gal H-1); ^{13}C NMR (75.5 MHz, D_2O): δ 34.7 (34.9*) (C-3′), 46.1 (48.1*) (C-2′), 52.0 (53.0*) (Gal C-3), 57.2 (OMe), 59.3 (C-4′), 60.0 (60.1*), 61.2 (61.3*) (Glc C-6, Gal C-6), 67.4 (68.5*) (Gal C-4), 69.3 (70.4*) (Gal C-2), 72.7 (Glc C-2), 74.4, 74.7, 77.6, 78.2 (78.3*) (Glc C-3, Glc C-4, Glc C-5, Gal C-5), 103.0 (Glc C-1), 103.9 (Gal C-1), 179.7 (180.4*) (C-1′); LRMS 497 (M+Na, 100%); Anal. ($C_{17}H_{29}NaO_{13}S3.\ 5H_2O$) C, H.

4.34. Methyl 3-thio-3-[2′-sodium (4′-hydroxy-valeroate)]-β-D-galactopyranosyl-(1,4)-*O*-β-D-glucopyranoside (51)

In 68% yield (HPLC, 1% CH_3CN in H_2O, 4 mL/min, R_t = 5.49 min) as a colourless solid: ^{1}H NMR (D_2O): δ 1.08–1.11 (3H, m, H-5′), 1.59–1.92 (2H, m, H-3′), 3.20 (1H, t, $J_{2,1} = J_{2,3}$ = 8.3 Hz, Glc H-2), 3.32–3.43 (2H, m, Gal H-2, H-2′), 3.47† (3H, s, OMe), 3.47–3.57† (3H, m, Glc H-3/Gal H-5, Glc H-4, Glc H-5), 3.60–3.74 (4H, m, Glc H-3/Gal H-5, 2× H-6, H-6′), 3.83–3.91 (3H, m, Gal H-4, H-6′, H-4′), 4.30 (1H, d, $J_{1,2}$ = 8.3 Hz, Glc H-1), 4.41 (4.42*) (1H, d, $J_{1,2}$ = 7.7 Hz, Gal H-1); ^{13}C NMR (75.5 MHz, D_2O): δ 21.7 (21.9*, 22.1*, 22.3*) (C-5′), 41.0 (41.3*, 41.4*, 41.5*) (C-3′), 46.0 (46.2*, 48.3*) (C-2′), 52.0 (53.0*, 53.1*) (Gal C-3), 57.2 (OMe), 60.0 (60.1*), 61.2 (61.3*) (Gal C-6, Glc C-6), 65.3 (65.4*, 66.0*, 66.1*), 67.3 (67.4*, 68.5*, 68.6*) (Gal C-4, C-4′), 69.1 (69.2*, 70.4*, 70.5*) (Gal C-2), 72.7 (Glc C-2), 74.4, 74.7, 77.6, 78.2 (78.3*, 78.4*) (Glc C-3, Glc C-4, Glc C-5, Gal C-5), 103.0 (Glc C-1), 103.9 (Gal C-1), 179.7 (179.9*, 180.4*, 180.6*) (C-1′); LRMS 511 (M + Na, 100%); Anal. ($C_{18}H_{31}NaO_{13}S4H_2O$) C, H.

5. Biological evaluation

5.1. Cells

MA104 cells (an African Green Monkey kidney cell line) were used to cultivate rotaviruses of two different serotypes, namely RRV (rhesus) and a strain that affects humans (Wa). MA104 cells were grown at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (FCS), 20 mM Hepes, 26.6 μg/mL gentamicin and 2 μg/mL fungizone.

5.2. Virus

Virus infectivity was activated with 10 μg/mL porcine trypsin (type IX; Sigma) for 30 min at 37 °C before inoculation onto confluent cell monolayers grown in 75 cm^2 plastic cell culture flasks (NUNC, Denmark) at a multiplicity of infection (m.o.i.) of 10 fluorescent cell forming units/cell. After 60 min at 37 °C, the inoculum was removed and infected cells were incubated in DMEM containing 1 μg/mL porcine trypsin until extensive cytopathic effect (c.p.e.) was evident. Cell lysates were frozen and thawed twice, centrifuged (3000*g*, 10 min), and the supernatants were stored at −70 °C. Virus stocks were activated prior to use by treatment of cell lysates with 10 μg/mL porcine trypsin for 30 min at 37 °C.

5.3. Neutralisation assays

For use in neutralisation assays, the virus was titrated to determine a dilution which gave ~200 fluorescent focus forming units/well of the 96-well microtitre tray (NUNC). Sialylmimetics were dissolved in DMEM, brought to pH 7.5 by addition of a small volume of sodium carbonate and incubated for 1 h at 37 °C with trypsin-activated virus. The sialylmimetic-virus mixture was then added to confluent monolayers of MA104 cells grown in 96-well microtitre trays and incubated for 1 h at 37 °C. The inoculum was then removed and replaced with DMEM and the cells were incubated for 16 h at 37 °C in a 5% CO_2 environment. Virus-infected cells were detected by indirect immunofluorescent staining. Rotavirus-infected cell monolayers were fixed and permeabilised in 80% acetone for 10 min. The cells were then washed three times with PBS and covered with 50 μL of hyper-immune rabbit anti-RRV serum diluted 1 in 2000 in PBS and incubated at 37 °C for 30 min. Following this, cells were stained with 30 μL of fluorescein isothiocyanate-conjugated sheep anti-rabbit immunoglobulin (Silenus, Australia) diluted 1 in 100 in PBS. After 30 min at 37 °C, cells were washed three times with PBS and viewed through a fluorescent microscope. Inhibition of virus infection was measured by determining the percentage reduction in the number of rotavirus-infected cells at 10 mM concentration of the sialylmimetic, compared with the number of infected cells in the absence of any sialylmimetic. For those sialylmimetics that showed inhibition at 10 mM, the inhibition studies were repeated with the compound at 5 mM, 2.5 mM, and 1.25 mM concentrations. The percentage inhibition of rotavirus infectivity by the sialylmimetics at 5 mM is also recorded in the results if it were statistically significant. Statistical analysis was determined using the *t* test, with significance set at $p = 0.05$.

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.08.057.

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