Synthesis and Biological Evaluation of Sialylmimetics as Rotavirus Inhibitors

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Rotaviruses cause severe gastroenteritis in infants and are estimated to be responsible for over 600 000 deaths annually, primarily in developing countries. The development of potential inhibitors of this virus is therefore of great interest, particularly since the safety and efficacy of rotaviral vaccines has recently been questioned. This study describes the synthesis of a variety of compounds that can be considered as mimetics of *N*-acetylneuraminic acid thioglycosides and the subsequent in vitro biological evaluation of these sialylmimetics as inhibitors of rotaviral infection. Our results show that readily accessible carbohydrate-based compounds have the potential to act as inhibitors of rotaviral replication in vitro, presumably through inhibition of the rotaviral adhesion process.

Introduction

Rotaviruses are recognized as the single most important cause of severe gastroenteritis in infants[1] and are estimated to be responsible for over 600 000 deaths annually, primarily in developing countries.[2] Rotaviruses are highly host cell specific and infect mature enterocytes in the mid and upper villous epithelium of the small intestine.[1] The rotavirus virion is composed of three concentric layers of proteins surrounding a genome of 11 segments of double-stranded RNA.[1] The outermost layer of the virion is composed of two proteins, VP4 and VP7, which have been implicated in the initial interaction between the virus and the host cell.[1,3–8] VP4 forms dimeric spikes that protrude from the virion surface, with the base of these spikes interacting with VP7.[9,10] While VP4 has been identified as the protein capable of haemagglutination, the precise role of VP7 has not been completely defined although it has been proposed that it modulates the functions of VP4[11,12] and interacts with cell surface molecules after initial attachment.[4] It has also been established that VP4 is associated with the virulence of rotavirus.[13] Both VP4 and VP7 have been shown to independently elicit neutralizing antibodies, confer serotype specificity, and induce protective immunity.[14,15]

The cellular recognition site for rotavirus is not as clearly defined as the roles of VP4 and VP7. Several reports have described efforts directed toward gaining a better understanding of the nature of the site for rotavirus binding.[16–30] A recent study shows that binding and entry of rotavirus is a complex process, with the existence of multiple cell-surface interactions, some of which are common between human and animal strains.[16] Early work implicated glycoproteins as the epitope recognized by rotavirus,[17,18] although more recent findings suggest that glycolipids are the more likely recognition site.[19,20] Investigations into the possible components of these glycoconjugate-based epitopes resulted in the general consensus that animal strains of rotavirus require sialylated glycoconjugates for infection,[5,18,20–24] although human strains appear to be independent of sialic acids.[18,22,25] However, it has also been suggested that many animal strains do not require host cell surface sialic acids for infectivity.[25–27] The assessment of sialic acid dependence or independence is usually determined by pretreatment of cells with a sialidase.[18,25] To further add to the conjecture regarding the requirement (or not) of sialic acids in the rotavirus recognition and binding process, it has recently been shown that human strains of rotavirus bind to ganglioside GM$_1$ on the host cell surface.[20]

Studies into mutations in VP4, specifically at residues 150 (Gly to Glu) and 187 (Lys to Arg), have shown that the infectivity is altered from sialic acid dependent to independent, although the variants still recognize sialic acids.[29] In an attempt to characterize the biochemical nature of the rotavirus cellular recognition site, Guerrero et al. have recently investigated the susceptibility of MA104 cells to infection by rotavirus.[30] The conclusion drawn from this study was that the epitope for rotavirus binding is likely to be a complex of several components including gangliosides, *N*-linked glycoproteins, and other proteins such as integrins.[30] The suggested involvement of integrins in rotavirus infection is further supported by earlier observations which identified integrin binding motifs within VP4 and VP7.[4] The finding that galactose specific lectins appear to show the most inhibitory activity at blocking rotavirus infection of MA104 cells suggests that galactose is involved in the binding of rotavirus to the host cell.[31] This latter work also clearly showed that different strains of rotavirus can have quite different recognition site specificities, even within the group dependent on sialic acids.[31] In

addition, it has been suggested that rotavirus initially interacts with a sialic acid-containing cellular recognition site prior to interacting with a second determinant which is independent of sialic acids.[16,22,31] Recent evidence tends to support the conclusion that sialic acids are highly likely to be involved in the recognition process by most strains of rotavirus.[11,20,32] The consensus at this point appears to be that strains of rotavirus sensitive to sialidase treatment bind to gangliosides containing terminal sialic acid residues, while rotavirus strains insensitive to sialidase treatment recognize internal sialic acids (like that found in GM_1 which are resistant to the action of some sialidases).[20,32]

The involvement of sialic acids in the rotaviral adhesion process has prompted several investigations into the development of potential inhibitors based upon sialic acid glycosides which may mimic elements of the natural cellular recognition site(s).[33-38] In vitro studies have shown that the use of naturally derived substances such as human milk lactadherin,[33] sialyloligosaccharides from egg yolk,[34] and glycophorin A[18,22] resulted in a reduction in rotavirus infectivity. Inhibition of rotavirus infection has also been achieved using exogenous GM_1[20] and the sialylphospholipid **1**.[35] We have

1 R = NHAc, R' = R" = alkyl


2 R = NHAc, R' = H
3 R = NHAc, R' = Ac
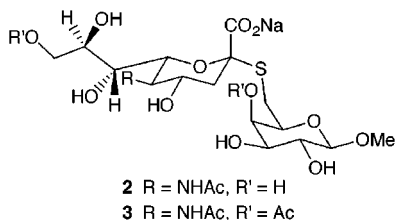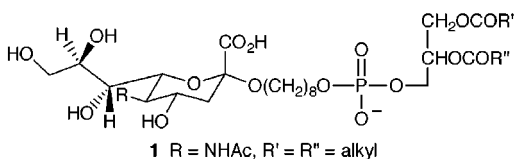
reported the synthesis and rotavirus inhibitory activity of *N*-acetylneuraminic acid thioglycosides of the general structure **2**.[36] We found that the partially O-acetylated thiosialosides (e.g., **3**) showed improved inhibitory activity compared to the corresponding non-O-acetylated thiosialoside toward a bovine rotavirus strain (NCDV).[36] This result is consistent with the earlier findings of Willoughby and Yolken when investigating inhibition of a simian strain of rotavirus (SA11),[37] although their results suggest that multiply O-acetylated *N*-acetylneuraminic acid derivatives may have been responsible for the inhibition.

Despite considerable investigations into the use of *N*-acylneuraminic acid glycosides as potential inhibitors of rotavirus, no chemotherapeutic agent has so far been developed. The development of *N*-acylneuraminic acid glycosides as potential inhibitors is complicated by the complexity of chemistry surrounding *N*-acylneuraminic acid manipulations and glycosidations.[39,40] Several workers have addressed this issue in other areas of sialic acid biology and chemistry by using mimetics of sialic acid. These sialylmimetics retain the structural features essential for interaction with a particular protein but are structurally simpler and consequently more readily

accessible through synthesis.[41-46] Sialylmimetics also have the advantage of being more readily tailored to have improved pharmacological properties, which is an important issue in drug development. Some excellent examples of the success of sialylmimetics include work directed toward the development of mimetics of sialyl Lewis x (**4**) as inhibitors of E-selectin.[41-43] Of the many examples reported, the sialylmimetic **5**, in which the entire sialic acid moiety has been replaced by a carboxylate group with an appended hydrophobic moiety, exemplifies the potential advantages of employing sialylmimetics since it shows higher affinity for E-selectin than sialyl Lewis x itself.[41]

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

5
(0.08mM for E-selectin)
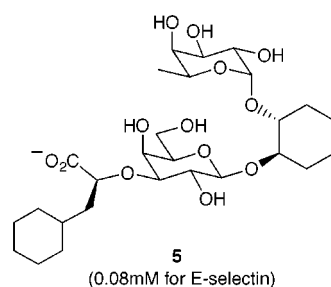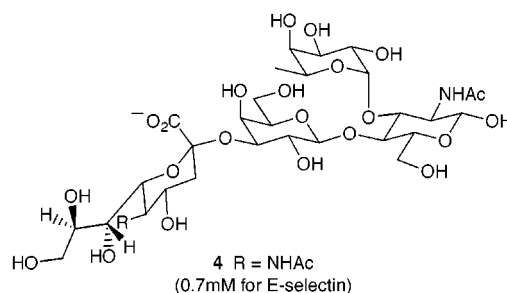
Other examples of sialylmimetics include the influenza virus sialidase inhibitor **6**,[47] which can be considered as a mimetic of Neu5Ac2en (**7**) and is based upon the potent influenza virus sialidase inhibitor Relenza (**8**).[48] We have also been interested in the development of Neu5Ac2en based sialylmimetics[49,50] and have reported the synthesis and sialidase inhibitory activity of **9**.[49]

6

9 R = NHAc or OH

7 R = NHAc, R' = OH
**8** R = NHAc, R' = NHC(NH)NH$_2$
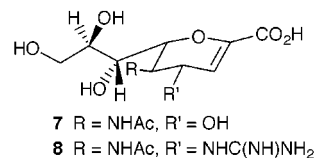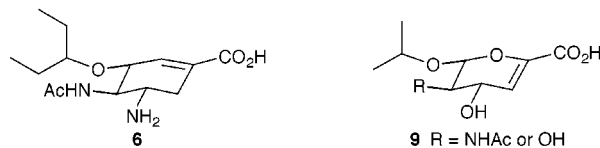
Our interest in the development of novel sialylmimetics as potential inhibitors of sialic acid-recognizing proteins prompted us to investigate mimetics of thiosialosides such as **2** as potential inhibitors of rotavirus. This study describes our efforts toward the synthesis of mimetics of thiosialosides and their evaluation as inhibitors of rotavirus infection.

Scheme 1. Retrosynthetic Approach toward Sialylmimetics

R = alkyl, aryl; X = activating group; PG = protecting group
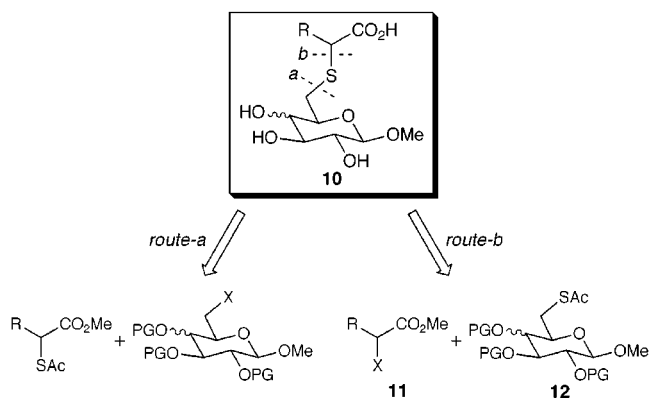
Results and Discussion

(i) Synthesis of Sialylmimetics. Our target compounds for mimetics of α(2,6)-linked thiosialosides such as **2** can be represented by the general structure **10** (Scheme 1). As can be seen, sialylmimetics of the general structure **10** contain a carboxylate group in the same relative position as the sialic acid carboxylate group in thiosialosides such as **2**. In addition, we wished to explore not only the effect of varying the R group α to the carboxylate moiety, in order to investigate the effects of hydrophobicity and steric bulk, but also the nature of the carbohydrate unit. From a retrosynthetic viewpoint it was envisaged that two distinct approaches toward sialylmimetics such as **10** could be employed. We have described elsewhere our preliminary efforts toward the synthesis of compounds of the general structure **10**,[51] and have shown that *route-b* offers the most flexible and efficient access, particularly since many of the proposed acceptors **11** are commercially available.
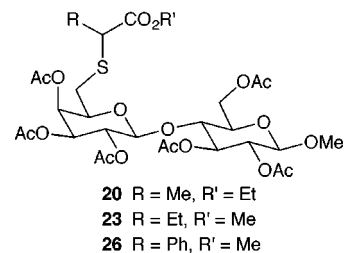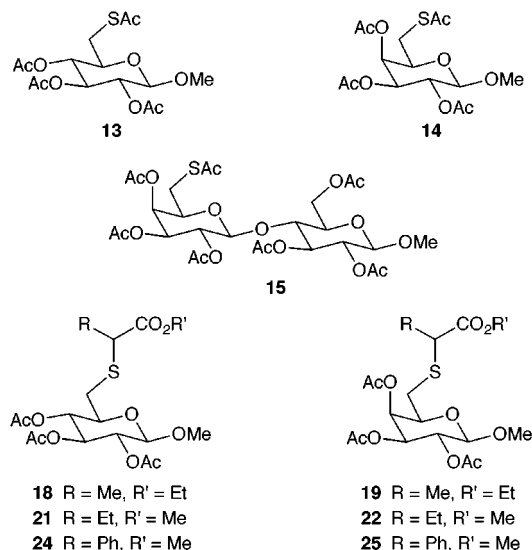
The synthesis of sialylmimetics of the general structure **10** relies upon ready access to the corresponding C-6 thiolacetyl derivatives **12**. Such compounds can readily be obtained from the corresponding 6-*O*-tosyl derivatives via displacement with potassium thioacetate.[51,52] In this way the thiolacetyl derivatives **13** and **14**, corresponding to glucose and galactose, respectively, were obtained in high yield. The requisite C-6′ thiolacetyl lactoside derivative **15** was obtained via the sequence shown in Scheme 2. 4′,6′-*O*-Benzylidenation of methyl β-D-lactoside followed by acetylation gave the 4′,6′-protected lactoside **16**, which was debenzylidenated (90% aqueous TFA) and treated with *p*-toluenesulfonyl chloride to give **17**. Subsequent exposure of **17** to potassium thioacetate (5 equiv) afforded the desired C-6′ thiolacetyl derivative **15**. Interestingly, the C-4′ hydroxyl group in **17** is O-acetylated during the course of the thiolacetylation reaction, as indicated by the presence of six O-acetyl signals in the ^{1}H NMR spectrum of **15**. In addition, the ^{1}H NMR spectrum of **15** contains a one proton doublet at δ 5.37 ($J = 3.0$ Hz) which is characteristic, both in terms of chemical shift and coupling constant, of the H-4 proton of the galactose ring of lactosides when the galactose C-4 hydroxyl is acetylated. At this stage we are yet to determine the source of the acetyl group on the C-4′ hydroxyl, although it is

Scheme 2[a]
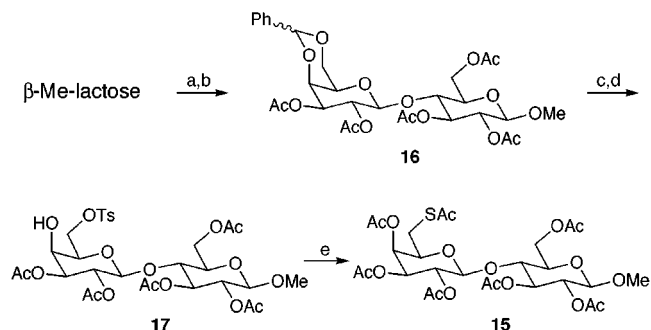
[a] Reagents and conditions: (a) PhCHO, HCO$_2$H, 2 h, rt; (b) Ac$_2$O, pyridine, 16 h, rt, 83%; (c) 90% aq TFA, 2 h, 0 °C, 74%; (d) TsCl, pyridine, 2.5 h, 0 °C, 81%; (e) KSAc (5 equiv), acetone, 56 °C, 48 h, 74%.
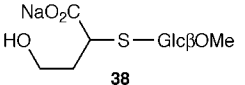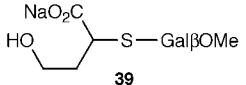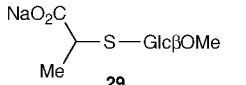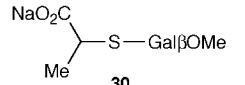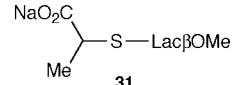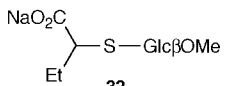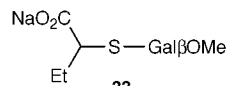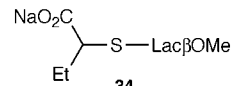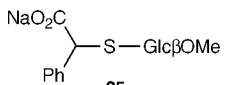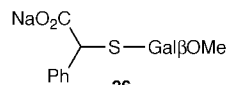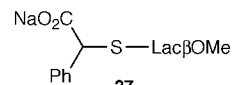
worth remembering that 5 molar equiv of potassium thioacetate is used during the thioacetylation reaction.

Our initial attempts at coupling between the thiolacetyl derivative **14** and methyl 2-bromopropionate utilized Et$_2$NH[53] mediated coupling. Unfortunately, this coupling was very slow with modest yields of ~50% for the desired product after 2 days at 40 °C.[51] However, hydrazinium acetate mediated coupling[54] between the C-6 thiolacetyl derivatives **13**, **14**, and **15** and methyl 2-bromopropionate afforded the sialylmimetics **18**, **19**, and **20**, respectively, in high yield.

18 R = Me, R′ = Et
21 R = Et, R′ = Me
24 R = Ph, R′ = Me

19 R = Me, R′ = Et
22 R = Et, R′ = Me
25 R = Ph, R′ = Me

20 R = Me, R′ = Et
23 R = Et, R′ = Me
26 R = Ph, R′ = Me

In a similar manner, the sialylmimetics **21**−**26** were prepared by coupling between the C-6 thiolacetyl derivatives and the appropriate α-bromo ester. Thus far we had only introduced hydrophobic groups (i.e., R = Me, Et, or Ph) into our sialylmimetics. To investigate the effect of a hydrophilic group, we wanted to introduce a hydroxyl functionality in "R". We chose to use α-bromo-

Table 1. Inhibition of Rotavirus by Sialylmimetics[a]

Compound	NCDV (bovine)	Wa (human)	Compound	NCDV (bovine)	Wa (human)	Compound	NCDV (bovine)	Wa (human)
Neu5Ac—S—GalβOMe **2**	6.25[b]	>25[b]	NaO2C, HO ... S—GlcβOMe **38**	>25	>25	NaO2C, HO ... S—GalβOMe **39**	>25	>25
NaO2C, Me, S—GlcβOMe **29**	>25	>25	NaO2C, Me, S—GalβOMe **30**	>25	>25	NaO2C, Me, S—LacβOMe **31**	12.5	6.25
NaO2C, Et, S—GlcβOMe **32**	>25	>25	NaO2C, Et, S—GalβOMe **33**	25	>25	NaO2C, Et, S—LacβOMe **34**	6.25	6.25
NaO2C, Ph, S—GlcβOMe **35**	>25	>25	NaO2C, Ph, S—GalβOMe **36**	>25	>25	NaO2C, Ph, S—LacβOMe **37**	12.5	25

[a] Results are expressed as the concentration of compound (mM) at which 50% infection of control infected monolayers occurred (IC_{50}).
[b] Results published in ref 36.

γ-butyrolactone for coupling with our C-6 thiolacetyl derivatives, since we knew that hydroxide mediated deprotection of the product from coupling would result in opening of the lactone to give a butyro alcohol. Accordingly, hydrazinium acetate mediated coupling between the C-6 thiolacetyl derivatives **13** and **14** and α-bromo-γ-butyrolactone gave the sialylmimetics **27** (86% yield) and **28** (79% yield), respectively.

29 R = Me
32 R = Et
35 R = Ph
38 R = CH2CH2OH

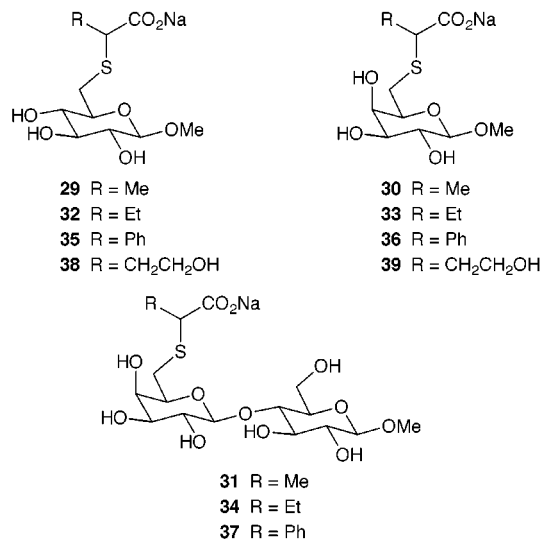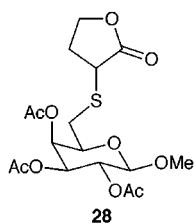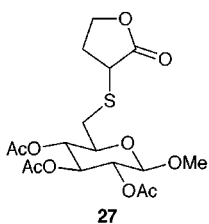30 R = Me
33 R = Et
36 R = Ph
39 R = CH2CH2OH

31 R = Me
34 R = Et
37 R = Ph

27   28

With a series of protected sialylmimetics in hand our attention turned toward deprotection and subsequent biological evaluation. Accordingly, each of the sialylmimetics **18**–**28** was exposed to dilute sodium hydroxide. It has been suggested that highly acidic compounds can produce unreliable results in the rotavirus neutralization assay.[55] In order to eliminate the possibility of spurious results, after acidification during workup (to pH ∼5), each product was dissolved in water and the pH adjusted to 7.3 with sodium hydroxide solution to ensure each compound was present as its sodium salt. In this way, the sialylmimetics **29**–**39** were obtained in good yield after HPLC purification.

(ii) Biological Evaluation of Sialylmimetics as Inhibitors of Rotaviral Infection. The synthesized sialylmimetics were evaluated as potential inhibitors of rotaviral infection using a standard in vitro neutralization assay[36] as detailed in the Experimental Section. Briefly, this method involves the preincubation of the sialylmimetic to be analyzed with a rotavirus strain (either bovine NCDV or human Wa) prior to incubation on MA104 cells. After incubation, virus neutralization was determined using indirect immunofluorescent staining. The results obtained (Table 1) are expressed as the concentration of compound at which 50% infection of

control infected monolayers occurred (IC_{50}). It is clear from the results shown in Table 1 that the glucose- and galactose-based sialylmimetics appear to have no inhibitory activity toward either Wa (human) or NCDV (bovine) strains of rotavirus. While this result was somewhat disappointing, since rotavirus binds to cell surface glycoconjugates it is perhaps not unexpected that monosaccharides would not be recognized by the viral adhesion protein and hence would not inhibit rotaviral adhesion or infection. Consistent with this hypothesis are the results obtained with the lactose-based sialylmimetics. As can be seen (Table 1), each of the three lactose-based sialylmimetics, **31**, **34**, and **37**, show inhibition of rotavirus infection. Significantly, the "methyl" and "ethyl" lactose-based sialylmimetics, **31** and **34**, respectively, show inhibition of the Wa (human) strain of rotavirus. Although the degree of inhibition is only moderate, to the best of our knowledge this represents the first report of relatively simple carbohydrate derivatives exhibiting inhibition of a human strain of rotavirus.

While it is not appropriate to draw too many conclusions from the data presented here (Table 1), it could

be speculated that the "phenyl" lactose-based sialyl-mimetic **37** is too sterically demanding (or too hydrophobic) for the Wa (human) strain of rotavirus. The level of inhibition of **37** against the Wa (human) strain is diminished with respect to the "methyl" and "ethyl" lactose-based sialylmimetics **31** and **34**, respectively. It is also interesting to note that the "ethyl" lactose-based sialylmimetic **34** shows a level of inhibition of the NCDV (bovine) strain comparable to the thiosialoside **2**. On the basis of our present findings, it appears that the minimum determinant for recognition by rotavirus is a disaccharide unit possessing a negative charge.

Experimental Section

General. Infrared spectra were recorded on a Hitachi 270-30 infrared spectrometer as KBr disks. ^{1}H and ^{13}C spectra were recorded using a Brüker DRX-300 spectrometer unless indicated otherwise. Chemical shifts are given in ppm relative to the solvent used (CDCl$_3$: 7.26 for ^{1}H; 77.0 for ^{13}C) or relative to external Me$_4$Si for D$_2$O spectra. Assignments indicated with an asterisk (*) correspond to those resonances clearly due to the other diastereomer where such mixtures exist. Two-dimensional DQF-COSY and HMQC experiments were recorded in order to assist with spectral assignment. Typically, the following parameters were used: DQF-COSY − 16 scans, 512 slices, relaxation delay 2.0s; ^{1}H-^{13}C HMQC − 48 scans, 256 slices, relaxation delay 2.5s. ESI mass spectra were obtained using a Micromass Platform II electrospray spectrometer. 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). Microanalyses were performed at the Department of Chemistry, University of Queensland, Australia. Methyl β-D-glucopyranoside and methyl β-D-galactopyranoside were purchased from Sigma-Aldrich. Methyl β-D-lactoside was prepared by treating hepta-O-acetyl-α-lactosylbromide with sodium methoxide. All solvents were distilled prior to use or were of analytical grade.

1. Synthesis of Thiolacetyl Derivatives 13, 14, and 15. Methyl 2,3,4-Tri-O-acetyl-6-O-p-toluenesulfonyl-β-D-glucopyranoside. To a solution of methyl β-D-glucopyranoside (1.0 g, 5.1 mmol) in pyridine (20 mL) at 0 °C under N$_2$ was added p-TsCl (1.1 g, 5.5 mmol). After the mixture was stirred for 2.5 h, MeOH (2 mL) was added and the solution concentrated in vacuo. The residue was chromatographed through a short column (10 × 2 cm) of silica (EtOAc:MeOH, 8:1, R_f 0.4) to give partially purified methyl 6-O-p-toluenesulfonyl-β-D-glucopyrandoside. Pyridine (10 mL) and Ac$_2$O (5 mL) were added, and the mixture was stirred for 16 h at room temperature before being concentrated under reduced pressure. Column chromatography (EtOAc:hexane, 1:2, R_f 0.3) gave methyl 2,3,4-tri-O-acetyl-6-O-p-toluenesulfonyl-β-D-glucopyranoside (2.1 g, 87%) as an amorphous mass: IR 2800, 1760, 1380, 1250, 1050 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.97, 1.99, 2.02 (3 × 3H, 3 × s, 3 × AcO), 2.45 (3H, s, ArMe); 3.43 (3H, s, OMe); 3.72−3.78 (1H, m, H-5), 4.05 (1H, t, $J_{4,3} = J_{4,5}$ = 9.3 Hz, H-4), 4.06−4.12 (2H, m, H-6/6′), 4.37 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.90 (1H, dd, $J_{2,3}$ 10.2, $J_{2,1}$ 7.8 Hz, H-2), 5.16 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 9.3 Hz, H-3), 7.34 (2H, d, ArH), 7.77 (2H, d, ArH); ESIMS 492 (M + NH$_4$, 100%), 443 (30).

The following was prepared in a similar manner:

Methyl 2,3,4-Tri-O-acetyl-6-O-p-toluenesulfonyl-β-D-galactopyranoside in 82% yield (chromatography EtOAc:hexane, 2:3, R_f 0.3) as an amorphous mass: IR 2800, 1760, 1390, 1250, 1050 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.96, 2.03, 2.04 (3 × 3H, 3 × s, 3 × AcO), 2.45 (3H, s, ArMe), 3.48 (3H, s, OMe); 3.93 (1H, t, $J_{5,6/6'}$ 6.3 Hz, H-5), 4.02 (1H, dd, $J_{6,6'}$ 9.9, $J_{6,5}$ 6.3, H-6), 4.14 (1H, dd, $J_{6',6}$ 9.9, $J_{6',5}$ 6.3, H-6′), 4.36 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 4.98 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 3.3 Hz, H-3), 5.14 (1H, dd, $J_{2,3}$ 10.2, $J_{2,1}$ 8.1 Hz, H-2), 5.35 (1H, d, $J_{4,3}$ 3.3 Hz, H-4), 7.34 (2H, d, ArH), 7.75 (2H, d, ArH); ESIMS 492 (M + NH$_4$, 100%).

Methyl 2,3-Di-O-acetyl-4,6-O-benzylidene-β-D-galacto-pyranosyl-(1,4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (16). Benzaldehyde (30 mL) was added to a solution of methyl β-D-galactopyranosyl-(1,4)-β-D-glucopyranoside (5.0 g, 14 mmol) in HCO$_2$H (30 mL), and the solution was stirred for 2 h at room temperature under N$_2$ before being concentrated under reduced pressure. The residue was chromatographed through a short column (10 × 4 cm) of silica (EtOAc:MeOH, 3:1, R_f 0.4) to give partially purified methyl 4,6-O-benzylidene-β-D-galactopyranosyl-(1,4)-β-D-glucopyranoside. Pyridine (40 mL) and Ac$_2$O (20 mL) were added, and the mixture was stirred for 16 h at room temperature before being concentrated under reduced pressure. Column chromatography (EtOAc:hexane, 2:1, R_f 0.4) gave **16** (7.6 g, 83%) which was crystallized from EtOAc/hexane to give colorless needles: ^{1}H NMR (CDCl$_3$) δ 2.02, 2.05, 2.10 (15H, 3 × s, 5 × AcO), 3.47 (3H, s, OMe), 3.48−3.51 (1H, m, GalH-5), 3.58−3.63 (1H, m, GlcH-5), 3.79 (1H, dd, $J_{4,5} = J_{4,3}$ = 9.4 Hz, GlcH-4), 4.02 (1H, dd, $J_{6,6'}$ 12.4, $J_{6,5}$ 1.4 Hz, GlcH-6), 4.13 (1H, dd, $J_{6,6'}$ 12.0, $J_{6,5}$ 5.0 Hz, GalH-6), 4.29 (1H, dd, $J_{6',6}$ 12.4, $J_{6',5}$ 1.2 Hz, GlcH-6′), 4.32 (1H, d, $J_{4,3}$ 3.7 Hz, GalH-4), 4.39 (1H, d, $J_{1,2}$ 7.9 Hz, GalH-1), 4.47 (1H, d, $J_{1,2}$ 7.9 Hz, GlcH-1), 4.51 (1H, dd, $J_{6',6}$ 12.0, $J_{6',5}$ 1.9 Hz, GalH-6′), 4.87 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 3.7 Hz, GalH-3), 4.90 (1H, dd, $J_{2,3}$ 9.4, $J_{2,1}$ 7.9 Hz, GlcH-2), 5.21 (1H, dd, $J_{3,4} = J_{3,2}$ = 9.4 Hz, GlcH-3), 5.25 (1H, dd, $J_{2,3}$ 10.3, $J_{2,1}$ 7.9 Hz, GalH-2), 5.46 (1H, s, PhCH), 7.33−7.41 (3H, m, Ph), 7.43−7.48 (2H, m, Ph); ^{13}C NMR (75.5 MHz, CDCl$_3$) δ 20.8, 20.9, 21.0 (OC(O)Me), 57.1 (OMe), 62.4 (GlcC-6), 68.8 (GalC-6), 66.8, 69.5, 71.9, 72.4, 72.9, 73.3, 73.5 (GalC-2, GalC-3, GalC-4, GalC-5, GlcC-2, GlcC-3, GlcC-5), 76.3 (GlcC-4), 101.3 (PhCH), 101.6, 101.9 (GalC-1, GlcC-1), 126.8, 128.5, 129.4 (Ph), 137.9 (ipso Ph), 169.1, 169.9, 170.4, 170.6, 170.9 (OC(O)Me).

Methyl 2,3-Di-O-acetyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside. To a solution of **16** (6.0 g, 9.2 mmol) in CH$_2$Cl$_2$ (100 mL) at 0 °C under N$_2$ was added TFA (90% aqueous, 10 mL), and the solution was stirred for 2 h at 0 °C. The mixture was diluted with CH$_2$Cl$_2$ (100 mL), washed with H$_2$O (100 mL), saturated aqueous NaHCO$_3$ (100 mL), and H$_2$O (2 × 100 mL), dried (Na$_2$SO$_4$), and concentrated. Column chromatography (EtOAc:hexane, 10:1, R_f 0.3) gave the title compound (3.8 g, 74%) as an amorphous mass: IR 3495 (br), 1752, 1432, 1370, 1160, 1048 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.03 (6H), 2.06 (6H), 2.10 (3H) (15H, 3 × s, 5 × AcO), 2.67 (2H, brs, OH), 3.46 (3H, s, OMe), 3.54 (1H, t, $J_{5,6/6'}$ 5.2 Hz, GalH-5), 3.60−3.65 (1H, m, GlcH-5), 3.78−3.91 (4H, m, GlcH-4, GlcH-6, GalH-6/6′), 4.10 (1H, d, $J_{4,3}$ 2.9 Hz, GalH-4), 4.38 (1H, d, $J_{1,2}$ 7.8 Hz, GlcH-1), 4.47−4.52 (1H, m, GlcH-6′), 4.48 (1H, d, $J_{1,2}$ 7.8 Hz, GalH-1), 4.86 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 2.9 Hz, GalH-3), 4.88 (1H, dd, $J_{2,3}$ 10.2, $J_{2,1}$ 7.8 Hz, GlcH-2), 5.15−5.24 (2H, m, GlcH-3, GalH-2); ^{13}C NMR (75.5 MHz, CDCl$_3$) δ 20.7, 20.8, 20.9, 21.0 (OC(O)Me), 57.0 (OMe), 61.9, 62.4 (GlcC-6, GalC-6), 67.6, 69.9, 71.8, 72.8, 73.5, 73.8, 74.7, 76.6 (GlcC-2, GlcC-3, GlcC-4, GlcC-5, GalC-2, GalC-3, GalC-4, GalC-5), 101.6, 101.9 (GlcC-1, GalC-1), 169.7, 169.9, 170.5, 170.7, 170.9 (OC(O)Me); ESIMS 589 (M + Na, 100%), 547 (37), 414 (48).

Methyl 2,3-Di-O-acetyl-6-O-p-toluenesulfonyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (17). To a solution of methyl 2,3-di-O-acetyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (3.0 g, 5.3 mmol) in pyridine (40 mL) at 0 °C under N$_2$ was added p-toluenesulfonyl chloride (1.5 g, 7.9 mmol). After the mixture was stirred for 2.5 h at 0 °C, MeOH (2 mL) was added and the solution was concentrated in vacuo. Column chromatography (EtOAc:hexane, 3:2, R_f 0.3) gave **17** (3.1 g, 81%) as an amorphous mass: IR 3500, 2800, 1760, 1390, 1250, 1050 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.93, 2.02, 2.04, 2.06, 2.16 (5 × 3H, 5 × s, 5 × AcO), 2.46 (3H, s, ArMe), 3.47 (3H, s, OMe), 3.57−3.63 (1H, m, GlcH-5), 3.69−3.80 (2H, m, GlcH-4, GalH-5), 4.04 (1H, d, $J_{4,3}$ 3.0 Hz, GalH-4), 4.05−4.16 (3H, m, GlcH-6/6′, GalH-6), 4.26 (1H, dd, $J_{6',6}$ 10.4, $J_{6',5}$ 6.3 Hz, GalH-6′), 4.38 (1H, d, $J_{1,2}$ 7.8 Hz, GlcH-1), 4.45 (1H, d, $J_{1,2}$ 7.8 Hz, GalH-1), 4.86 (1H, dd, $J_{2,3}$ 9.6, $J_{2,1}$ 7.8 Hz, GlcH-2), 4.90 (1H, dd, $J_{3,2}$ 9.9, $J_{3,4}$ 3.0 Hz, GalH-3), 5.10 (1H, dd, $J_{2,3}$ 9.9, $J_{2,1}$ 7.8 Hz, GalH-2), 5.16 (1H, t, $J_{3,4} = J_{3,2}$ = 9.6 Hz, GlcH-3), 7.39 (2H, d, ArH), 7.81

(2H, m, ArH), assignments confirmed by COSY; ^{13}C NMR (75.5 MHz, CDCl$_3$) δ 20.5, 20.6, 20.7 (OC(O)*Me*), 21.5 (Ar*Me*), 56.8 (OMe), 61.9, 66.9 (GlcC-6, GalC-6), 66.3, 69.3, 71.5, 72.1, 72.5, 72.6, 73.0, 75.8 (GlcC-2, GlcC-3, GlcC-4, GlcC-5, GalC-2, GalC-3, GalC-4, GalC-5), 100.6, 101.3 (GlcC-1, GalC-1), 127.6, 130.0 (ArC-2/3), 132.5 (ArC-4), 145.2 (ArC-1), 169.2, 169.5, 169.9, 170.0, 170.4 (O*C*(O)Me); ESIMS 738 (M + NH$_4$, 100%).

Methyl 2,3,4-Tri-*O*-acetyl-6-thiolacetyl-β-D-glucopyranoside (13). To a solution of methyl 2,3,4-tri-*O*-acetyl-6-*O*-p-toluenesulfonyl-β-D-glucopyranoside (2.5 g, 5.3 mmol) in dry acetone (100 mL) at room temperature under N$_2$ was added KSAc (3.0 g, 26.5 mmol). The mixture was heated to reflux for 48 h before allowing to cool to room temperature, filtered through Celite, and concentrated. Column chromatography (EtOAc:hexane, 1;2, R_f 0.3) gave **13** (1.57 g, 79%) as an amorphous mass: IR 1698, 1431, 1374, 1254, 1218, 1158, 1125, 1032 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.95, 2.00, 2.04 (3 × 3H, 3 × s, 3 × AcO), 2.31 (3H, s, AcS), 3.03 (1H, dd, $J_{6,6'}$ 14.2, $J_{6,5}$ 6.9 Hz, H-6), 3.23 (1H, dd, $J_{6',6}$ 14.2, $J_{6',5}$ 2.4 Hz, H-6'), 3.46 (3H, s, OMe), 3.57−3.61 (1H, m, H-5), 4.36 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 4.88−4.96 (2H, m, H-2, H-4) 5.13 (1H, t, $J_{3,2}$ = $J_{3,4}$ = 9.6 Hz, H-3); ^{13}C NMR (CDCl$_3$) δ 20.5, 20.7, 20.8 (OC(O)*Me*), 30.1 (C-6), 30.5 (SC(O)*Me*), 57.0 (OMe), 68.1, 68.9, 71.2, 72.2 (C-2, C-3, C-4, C-5), 102.1 (C-1), 169.5, 170.1, 170.4 (O*C*(O)Me), 194.7 (S*C*(O)Me); ESIMS 396 (M + NH$_4$, 81%), 347 (60), 245 (100).

The following were prepared in a similar manner:

Methyl 2,3,4-Tri-*O*-acetyl-6-thiolacetyl-β-D-galactopyranoside (14) in 77% yield (chromatography EtOAc:hexane, 1:2, R_f 0.3) as an amorphous mass: IR 1760, 1390, 1220, 1135, 1050 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.97, 2.05, 2.14 (3 × 3H, 3 × s, 3 × AcO), 2.34 (3H, s, AcS), 3.06 (1H, dd, $J_{6,6'}$ 13.8, $J_{6,5}$ 6.9 Hz, H-6), 3.12 (1H, dd, $J_{6',6}$ 13.8, $J_{6',5}$ 6.9 Hz, H-6'), 3.53 (3H, s, OMe), 3.69 (1H, t, $J_{5,6/6'}$ 6.9 Hz, H-5), 4.36 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.98 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 3.3 Hz, H-3), 5.17 (1H, dd, $J_{2,3}$ 10.2, $J_{2,1}$ 7.8 Hz, H-2), 5.41 (1H, d, $J_{4,3}$ 3.3 Hz, H-4); ^{13}C NMR (CDCl$_3$) δ 20.4, 20.6, 20.7 (OC(O)*Me*), 28.5 (C-6), 30.3 (SC(O)*Me*), 56.9 (OMe), 68.0, 68.7, 71.0, 72.0 (C-2, C-3, C-4, C-5), 101.9 (C-1), 169.4, 170.0, 170.2 (O*C*(O)Me), 194.5 (S*C*(O)-Me); ESIMS 396 (M + NH$_4$, 27%), 347 (80), 245 (100).

Methyl 2,3,4-Tri-*O*-acetyl-6-thiolacetyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (15) in 74% yield (chromatography EtOAc:hexane, 3:2, R_f 0.4) as an amorphous mass: IR 1760, 1390, 1220, 1130, 1050 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.95, 2.03, 2.05, 2.08, 2.12, 2.16 (6 × 3H, 6 × s, 6 × AcO), 2.35 (3H, s, AcS), 3.04 (2H, d, $J_{6/6',5}$ 7.2 Hz, GalH-6/6'), 3.48 (3H, s, OMe), 3.58−3.66 (2H, m, GlcH-5, GalH-5), 3.83 (1H, t, $J_{4,3}$ = $J_{4,5}$ = 9.6 Hz, GlcH-4), 4.10 (1H, dd, $J_{6,6'}$ 12.0, $J_{6,5}$ 4.8 Hz, GlcH-6), 4.39 (1H, d, $J_{1,2}$ 7.8 Hz, GlcH-1), 4.45 (1H, d, $J_{1,2}$ 7.8 Hz, GalH-1), 4.50 (1H, dd, $J_{6',6}$ 12.0, $J_{6',5}$ 1.8 Hz, GlcH-6'), 4.90 (1H, dd, $J_{2,3}$ 9.6, $J_{2,1}$ 7.8 Hz, GlcH-2), 4.92 (1H, dd, $J_{3,2}$ 9.6, $J_{3,4}$ 3.0 Hz, GalH-3), 5.07 (1H, dd, $J_{2,3}$ 9.6, $J_{2,1}$ 7.8 Hz, GalH-2), 5.21 (1H, t, $J_{3,4}$ = $J_{3,2}$ = 9.6 Hz, GlcH-3), 5.37 (1H, d, $J_{4,3}$ 3.0 Hz, GalH-4); [13]C (75.5 MHz, CDCl$_3$) δ 20.1, 20.2, 20.4, 20.5, 20.6, 20.8 (OC(O)*Me*), 28.1 (GalC-6), 30.3 (SC(O)*Me*), 56.8 (OMe), 62.1 (GlcC-6), 69.1, 71.1, 71.5, 72.1, 72.3, 72.6, 72.7, 74.8 (GlcC-2, GlcC-3, GlcC-4, GlcC-5, GalC-2, GalC-3, GalC-4, GalC-5), 100.6, 101.3 (GlcC-1, GalC-1), 168.9, 169.7, 169.9, 170.2, 173.1, 173.4 (O*C*(O)Me), 194.2 (S*C*(O)Me); ESIMS 684 (M + NH$_4$, 64%), 670 (40), 347 (100); Found C, 49.0; H, 5.8; S, 4.8%. C$_{27}$H$_{38}$O$_{17}$S requires C, 48.65; H, 5.75; S, 4.8%.

2. Synthesis of Sialylmimetics. Methyl 2,3,4-Tri-*O*-acetyl-6-thio-6-[2′-(ethyl propanoate)]-β-D-glucopyranoside (18). A solution of the 6-thiolacetyl derivative **13** (0.5 g, 1.32 mmol) in dry *N,N*-DMF (10 mL) was degassed[56] for 20 min. H$_2$NNH$_2$·AcOH (140 mg, 1.52 mmol) was added and the solution stirred for 30 min before the addition of ethyl 2-bromopropionate (260 mg, 1.45 mmol) and Et$_3$N (212 μL, 1.52 mmol). After 4 h at room temperature under N$_2$, the mixture was diluted with EtOAc (30 mL) and washed with dilute HCl (1 M, 30 mL) and H$_2$O (2 × 30 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. Chromatography (Et$_2$O: hexane, 2:3, R_f 0.3) gave **18** (530 mg, 92%) as colorless crystals (Et$_2$O/hexane): mp 98−100 °C; IR 1755, 1372, 1216, 1162,

1065, 1036 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.28 (1.29*) (3H, t, J 7.2 Hz, CO$_2$CH$_2$C*H*$_3$), 1.41 (3H, d, $J_{3',2'}$ 7.2 Hz, H-3′), 1.99 (3H, s, AcO), 2.04 (6H, s, 2 × AcO), 2.72 (1H, dd, $J_{6,6'}$ 14.4, $J_{6,5}$ 7.5 Hz, H-6), 2.81−2.94 (2H, m, H-6′, H-2′), 3.50 (3H, s, OMe), 3.62−3.68 (1H, m, H-5), 4.16 (4.19*) (2H, q, J 7.2 Hz, CO$_2$C*H*$_2$-CH$_3$), 4.40 (4.42*) (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.95 (1H, dd, $J_{2,3}$ 9.3, $J_{2,1}$ 7.8 Hz, H-2), 4.98 (1H, dd, $J_{3,4}$ 9.6, $J_{3,2}$ 9.3 Hz, H-3), 5.13− 5.21 (1H, m, H-4); ^{13}C NMR (75.5 MHz, CDCl$_3$) δ 14.0 (14.1*) (CO$_2$CH$_2$*C*H$_3$), 17.0 (C-3′), 20.5, 20.6, 20.7 (3 × OC(O)*Me*), 32.0 (32.7*) (C-6), 41.5 (41.9*) (C-2′), 56.8 (OMe), 61.0 (61.1*) (CO$_2$*C*H$_2$CH$_3$), 71.3 (71.6*), 72.7 (72.8*), 73.8, 74.6 (C-2, C-3, C-4, C-5), 101.4 (101.5*) (C-1), 169.3, 169.5, 170.2, 172.6 (C-1′, 3 × O*C*(O)Me); ESIMS 459 (M + Na, 35%), 454 (M + NH$_4$, 100); HRESIMS Found 454.17453. C$_{18}$H$_{28}$O$_{10}$S·NH$_4$ requires 454.17469.

The following were prepared in a similar manner:

Methyl 2,3,4-Tri-*O*-acetyl-6-thio-6-[2′-(ethyl propanoate)]-β-D-galactopyranoside (19) in 87% yield by coupling between the 6-thiolacetyl galactoside derivative **14** and ethyl 2-bromopropionate (chromatography EtOAc:hexane, 1:2, R_f 0.3) as an amorphous mass: IR 1756, 1370, 1222, 1166, 1054 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.27−1.31 (3H, m, CO$_2$CH$_2$C*H*$_3$), 1.43 (1.44*) (3H, d, $J_{3',2'}$ 7.2 Hz, H-3′), 1.97, 2.05, 2.15 (3 × 3H, 3 × s, 3 × AcO), 2.65 (2.76*) (1H, dd, $J_{6,6'}$ 13.8, $J_{6,5}$ 6.9 Hz, H-6), 2.89 (2.98*) (1H, dd, $J_{6',6}$ 13.8, $J_{6',5}$ 7.2 Hz, H-6′), 3.38 (3.45*) (1H, q, $J_{2',3'}$ 7.2 Hz, H-2′), 3.52 (3H, s, OMe), 3.76 (3.79*) (1H, dd, $J_{5,6'}$ 7.2, $J_{5,6}$ 6.9 Hz, H-5), 4.17 (4.20)* (2H, q, J 7.2 Hz, CO$_2$C*H*$_2$CH$_3$), 4.37 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 5.01 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 3.3, H-3), 5.17 (1H, dd, $J_{2,3}$ 10.2, $J_{2,1}$ 7.8 Hz, H-2), 5.40 (1H, d, $J_{4,3}$ 3.3 Hz, H-4); ^{13}C NMR (75.5 MHz, CDCl$_3$) δ 14.4 (CO$_2$CH$_2$*C*H$_3$), 17.1 (17.4)* (C-3′), 20.8, 20.9, 21.0 (3 × OC(O)*Me*) 30.9 (31.0*) (C-6), 41.3 (41.5*) (C-2′), 56.8 (OMe), 61.1 (61.2*) (CO$_2$*C*H$_2$CH$_3$), 69.2, 71.4, 73.5, 73.8, (C-2, C-3, C-4, C-5), 101.9 (C-1), 169.3, 169.9 (170.0*), 170.1 (170.2*) (3 × O*C*(O)Me), 172.6 (172.7*) (C-1′); ESIMS 454 (M + NH$_4$, 40%), 109 (100); HRESIMS Found 454.17453. C$_{18}$H$_{28}$O$_{10}$S·NH$_4$ requires 454.17469.

Methyl 2,3,4-Tri-*O*-acetyl-6-thio-6-[2′-(ethyl propanoate)]-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (20) in 89% yield by coupling between the 6-thiolacetyl lactoside derivative **15** and ethyl 2-bromopropionate (chromatography EtOAc:hexane, 1:1, R_f 0.4) as an amorphous mass: IR 1760, 1370, 1252, 1165, 1060 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.25−1.31 (3H, m, CO$_2$CH$_2$C*H*$_3$), 1.43 (1.44*) (3H, d, $J_{3',2'}$ 7.2 Hz, H-3′), 1.96, 2.03, 2.04, 2.06, 2.13, 2.16 (6 × 3H, 6 × s, 6 × AcO), 2.72 (1H, dd, $J_{6,6'}$ 13.8, $J_{6,5}$ 6.9 Hz, GalH-6), 2.82 (1H, dd, $J_{6',6}$ 13.8, $J_{6',5}$ 6.9 Hz, GalH-6′), 3.48 (3H, s, OMe), 3.60− 3.67 (1H, m, GlcH-5), 3.72 (1H, t, $J_{5,6}$ = $J_{5,6'}$ = 6.9 Hz, GalH-5), 3.84 (3.86*) (1H, t, $J_{4,3}$ = $J_{4,5}$ = 9.3 Hz, GlcH-4), 4.07 (4.10*) (2H, q, J 7.2 Hz, CO$_2$C*H*$_2$CH$_3$), 4.13−4.21 (2H, m, GlcH-6/6′), 4.40 (1H, d, $J_{1,2}$ 8.1 Hz, GlcH-1), 4.47 (1H, d, $J_{1,2}$ 7.8 Hz, GalH-1), 4.87−4.92 (2H, m, GlcH-2, GalH-3), 5.07 (1H, dd, $J_{2,3}$ 10.2, $J_{2,1}$ 7.8 Hz, GalH-2), 5.21 (1H, t, $J_{3,4}$ = $J_{3,2}$ = 9.3 Hz, GlcH-3), 5.39 (1H, d, $J_{4,3}$ 3.3 Hz, GalH-4); ^{13}C NMR (CDCl$_3$) δ 14.4 (CO$_2$-CH$_2$*C*H$_3$), 17.3 (C-3′), 20.4, 20.5, 20.6, 20.7, 20.8 (3 × OC(O)-*Me*), 30.2 (30.5*) (GalC-6), 48.3 (48.7*) (C-2′), 56.8 (OMe), 61.1 (CO$_2$*C*H$_2$CH$_3$), 61.9 (62.0*) (GlcC-6), 69.7, 69.9, 70.4, 71.3, 71.8, 72.3, 72.7 (GlcC-2, GlcC-3, GlcC-4, GlcC-5, GalC-2, GalC-3, GalC-4, GalC-5), 100.3, 101.4 (GlcC-1, GalC-1), 168.9, 169.5, 169.8, 170.1, 170.3, 170.8 (6 × O*C*(O)Me), 172.2 (C-1′); ESIMS 742 (M + NH$_4$, 100%), 405 (25); HRESIMS Found 742.26029. C$_{30}$H$_{44}$O$_{18}$S·NH$_4$ requires 742.25921.

Methyl 2,3,4-Tri-*O*-acetyl-6-thio-6-[2′-(methyl butanoate)]-β-D-glucopyranoside (21) in 90% yield by coupling between the 6-thiolacetyl glucoside derivative **13** and methyl 2-bromobutyrate (chromatography EtOAc:hexane, 1:2, R_f 0.3) as an amorphous mass: IR 1754, 1434, 1376, 1220, 1158, 1032 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 0.99 (3H, t, $J_{4',3'}$ 7.5 Hz, H-4′), 1.71− 1.83 (2H, m, H-3′), 1.99 (s, 3H, AcO), 2.05 (6H, s, 2 × AcO), 2.70 (1H, dd, $J_{6,6'}$ 14.1, $J_{6,5}$ 7.5 Hz, H-6), 2.88 (1H, dd, $J_{6',6}$ 14.1, $J_{6',5}$ 3.0 Hz, H-6′), 3.29 (1H, t, $J_{2',3'}$ 7.8 Hz, H-2′), 3.51 (s, 3H, OMe), 3.62 (1H, ddd, $J_{5,4}$ 9.3, $J_{5,6}$ 7.5, $J_{5,6'}$ 3.0 Hz, H-5), 3.73 (3.74*) (3H, s, CO$_2$*Me*), 4.41 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.94 (1H, dd, $J_{2,3}$ 9.3, $J_{2,1}$ 7.8 Hz, H-2), 4.99 (1H, t, $J_{4,3}$ = $J_{4,5}$ = 9.3 Hz,

H-4), 5.17 (1H, t, $J_{3,4} = J_{3,2}$ = 9.3 Hz, H-3); ^{13}C NMR (CDCl$_3$) δ 11.8 (C-4'), 20.5, 20.6 (3 × OC(O)*Me*), 24.7 (C-3'), 32.5 (C-6), 48.9 (C-2'), 52.0 (OMe), 56.8 (CO$_2$*Me*), 71.4, 72.7, 73.7, 74.4 (C-2, C-3, C-4, C-5), 101.4 (C-1), 169.3, 169.5, 170.1, 172.4 (3 × O*C*(O)Me, C-1'); ESIMS 454 (M + NH$_4$, 70%), 405 (100%).

Methyl 2,3,4-Tri-*O*-acetyl-6-thio-6-[2′-(methyl butanoate)]-β-D-galactopyranoside (22) in 88% yield by coupling between the 6-thioacetyl galactoside derivative **14** and methyl 2-bromobutyrate (chromatography EtOAc:hexane, 1:2, R_f 0.3) as an amorphous mass: IR 1750, 1420, 1380, 1370, 1200, 1034 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 0.99 (3H, t, $J_{4',3'}$ 7.3 Hz, H-4'), 1.65–1.92 (2H, m, H-3'), 1.97, 2.05, 2.14 (3 × 3H, 3 × s, 3 × AcO), 2.64 (2.73*) (1H, dd, $J_{6,6'}$ 13.8, $J_{6,5}$ 6.3 Hz, H-6), 2.86 (2.91*) (1H, dd, $J_{6',6}$ 13.8, $J_{6',5}$ 7.3 Hz, H-6'), 3.15 (3.23*) (1H, t, $J_{2',3'}$ 7.2 Hz, H-2'), 3.52 (3H, s, OMe), 3.73 (3H, s, CO$_2$Me), 3.73–3.79 (1H, m, H5), 4.37 (4.38*) (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 5.00 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 3.3 Hz, H-3), 5.17 (1H, dd, $J_{2,3}$ 10.2, $J_{2,1}$ 7.8 Hz, H-2), 5.40 (5.42*) (1H, d, $J_{4,3}$ 3.3 Hz, H-4), assignments confirmed by COSY; ^{13}C NMR (CDCl$_3$) δ 11.7 (11.8*) (C-4'), 20.3, 20.4, 20.5 (3 × OC(O)*Me*), 24.6 (24.7*) (C-3'), 30.8 (30.9*) (C-6), 48.3 (48.4*) (C-2'), 52.1 (OMe), 56.8 (56.9*) (CO$_2$*Me*), 68.2, 68.7, 71.0, 72.6 (C-2, C-3, C-4, C-5), 102.0 (C-1), 169.3, 169.8, 170.1 (3 × O*C*(O)Me), 172.7 (172.8*) (C-1'); ESIMS 454 (M + NH$_4$, 70%); HRESIMS Found 454.17471. C$_{18}$H$_{28}$O$_{10}$S·NH$_4$ requires 454.17469.

Methyl 2,3,4-Tri-*O*-acetyl-6-thio-6-[2′-(methyl butanoate)]-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (23) in 89% yield by coupling between the 6-thioacetyl lactoside derivative **15** and methyl 2-bromobutyrate (chromatography EtOAc:hexane, 1:1, R_f 0.2) as an amorphous mass: IR 1755, 1570, 1390, 1225, 1055 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 0.99 (1.00*) (3H, t, $J_{4',3'}$ 7.2 Hz, H-4'), 1.64–1.91 (2H, m, H-3'), 1.95, 2.02, 2.04, 2.06, 2.12, 2.15 (6 × 3H, 6 × s, 6 × AcO), 2.60 (2.69*) (1H, dd, $J_{6,6'}$ 13.8, $J_{6,5}$ 6.3 Hz, GalH-6), 2.80 (2.86*) (1H, dd, $J_{6',6}$ 13.8, $J_{6',5}$ 7.3 Hz, GalH-6'), 3.17 (3.19*) (1H, t, $J_{2',3'}$ 7.2 Hz, H-2'), 3.48 (3H, s, OMe), 3.62–3.73 (2H, m, GlcH-5, GalH-5), 3.74 (3.75*) (3H, s, CO$_2$Me), 3.86 (1H, t, $J_{4,3} = J_{4,5}$ = 9.3 Hz, GlcH-4), 4.08–4.15 (1H, m, GlcH-6), 4.39 (1H, d, $J_{1,2}$ 7.8 Hz, GlcH-1), 4.46 (1H, d, $J_{1,2}$ 8.1 Hz, GalH-1), 4.48–4.53 (1H, m, GlcH-6'), 4.87 (1H, dd, $J_{2,3}$ 9.3, $J_{2,1}$ 7.8 Hz, GlcH-2), 4.94 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 3.3 Hz, GalH-3), 5.06 (1H, dd, $J_{2,3}$ 10.2, $J_{2,1}$ 8.1 Hz, GalH-2), 5.21 (1H, t, $J_{3,4} = J_{3,2}$ = 9.3 Hz, GlcH-3), 5.41 (1H, d, $J_{4,3}$ 3.3 Hz, GalH-4); ^{13}C NMR (75.5 MHz, CDCl$_3$) δ 11.8 (C-4'), 20.3, 20.5, 20.6, 20.7 21.5 (6 × OC(O)*Me*), 24.5 (24.9*) (C-3'), 30.4 (30.6*) (GalC-6), 48.3 (48.7*) (C-2'), 52.3 (OMe), 57.0 (CO$_2$*Me*), 62.0 (GlcC-6), 67.8, 67.9, 69.1, 71.2, 71.3, 72.5, 74.7 (GlcC-2, GlcC-3, GlcC-4, GlcC-5, GalC-2, GalC-3, GalC-4, GalC-5), 100.3, 101.3 (GlcC-1, GalC-1), 168.9, 169.8, 170.1, 170.3, 170.8, 172.8 (C-1', 6 × O*C*(O)Me); ESIMS 742 (M + NH$_4$, 40%), 327 (100%); HRESIMS Found 742.25802. C$_{30}$H$_{44}$O$_{18}$S·NH$_4$ requires 742.25921.

Methyl 2,3,4-Tri-*O*-acetyl-6-thio-6-[2′-(methyl 2-phenylacetate)]-β-D-glucopyranoside (24) in 90% yield by coupling between the 6-thioacetyl glucoside derivative **13** and methyl 2-bromo-2-phenylacetate (chromatography EtOAc:hexane, 1:2, R_f 0.2) as an amorphous mass: mp 138–140 °C; IR 1753, 1372, 1213, 1142, 1064, 1038 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.97, 1.99, 2.04 (3 × 3H, 3 × s, 3 × AcO), 2.53 (1H, dd, $J_{6,6'}$ 14.7, $J_{6,5}$ 8.1 Hz, H-6), 2.64 (1H, dd, $J_{6',6}$ 14.7, $J_{6',5}$ 2.7 Hz, H-6'), 3.55 (3H, s, OMe), 3.59–3.65 (1H, m, H-5), 3.72 (3H, s, CO$_2$Me), 4.38 (4.40*) (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.76 (4.85*) (1H, s, H-2'), 4.87 (1H, dd, $J_{4,3} = J_{4,5}$ = 9.3 Hz, H-4), 4.96 (1H, dd, $J_{2,3}$ 9.3, $J_{2,1}$ 7.8 Hz, H-2), 5.14 (5.15)* (1H, dd, $J_{3,4} = J_{3,2}$ = 9.3 Hz, H-3), 7.30–7.46 (5H, m, Ph), assignments confirmed by COSY; ^{13}C NMR (CDCl$_3$) δ 20.5, 20.6 (3 × OC(O)*Me*), 32.0 (32.7*) (C-6), 52.5 (52.6*) (C-2'), 52.7 (OMe), 56.9 (57.0*) (CO$_2$*Me*), 71.2, 72.6, 72.7*, 73.9, 74.8 (C-2, C-3, C-4, C-5), 101.5 (C-1), 128.2, 128.4, 128.6 (Ph), 135.7 (ipso-Ph), 169.4, 170.1, 170.9 (C-1', 3 × O*C*(O)Me); ESIMS 502 (M + NH$_4$, 93%), 453 (100%). Found C, 54.5; H, 5.8; S, 6.3%. C$_{22}$H$_{28}$O$_{10}$S requires C, 54.5; H, 5.8; S, 6.6%.

Methyl 2,3,4-Tri-*O*-acetyl-6-thio-6-[2′-(methyl 2-phenylacetate)]-β-D-galactopyranoside (25) in 90% yield by coupling between the 6-thioacetyl galactoside derivative **14** and

methyl 2-bromo-2-phenylacetate (chromatography EtOAc:hexane, 1:2, R_f 0.25) as an amorphous mass: IR 3136, 2964, 2856, 1744, 1400, 1136, 1020 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.96, 2.04, 2.11 (3 × 3H, 3 × s, 3 × AcO), 2.48 (2.53*) (1H, dd, $J_{6,6'}$ 13.8, $J_{6,5}$ 6.8 Hz, H-6), 2.69 (2.82*) (1H, dd, $J_{6',6}$ 13.8, $J_{6',5}$ 7.5 Hz, H-6'), 3.50 (3H, s, OMe), 3.51–3.63 (1H, m, H-5), 3.73 (3.74*) (3H, s, CO$_2$Me), 4.27 (4.29*) (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.64 (4.81*) (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 3.3 Hz, H-3), 4.73 (4.75*) (1H, s, H-2'), 5.13 (1H, dd, $J_{2,3}$ 10.2, $J_{2,1}$ 7.8 Hz, H-2), 5.31 (5.32*) (1H, d, $J_{4,3}$ 3.3 Hz, H-4), 7.29–7.46 (5H, m, Ph), assignments confirmed by COSY; ^{13}C NMR (CDCl$_3$) δ 20.5, 20.7 (3 × OC(O)*Me*), 30.9 (31.6*) (C-6), 52.4 (52.7*) (C-2'), 52.5 (OMe), 56.9 (57.0*) (CO$_2$*Me*), 68.5, 68.7*, 71.0, 72.9, 73.5, 74.8* (C-2, C-3, C-4, C-5), 102.0 (102.1*) (C-1), 128.3, 128.6, 128.7, 129.3 (Ph), 139.7 (ipso-Ph), 169.4, 170.0, 170.8, 170.9 (C-1', 3 × O*C*(O)Me); ESIMS 502 (M + NH$_4$, 55%), 453 (100%); HRESIMS Found 502.17473. C$_{22}$H$_{28}$O$_{10}$S·NH$_4$ requires 502.17469.

Methyl 2,3,4-Tri-*O*-acetyl-6-thio-6-[2′-(methyl 2-phenylacetate)]-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (26) in 87% yield by coupling between the 6-thioacetyl lactoside derivative **15** and methyl 2-bromo-2-phenylacetate (chromatography EtOAc:hexane, 2:1, R_f 0.4) as an amorphous mass: IR 2825, 1750, 1550, 1370, 1250, 1050 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.92, 1.95, 2.02, 2.04, 2.06, 2.12 (6 × 3H, 6 × s, 6 × AcO), 2.57 (1H, dd, $J_{6,6'}$ 13.8, $J_{6,5}$ 6.9 Hz, GalH-6), 2.70 (1H, dd, $J_{6',6}$ 13.8, $J_{6',5}$ 6.3 Hz, GalH-6'), 3.47 (3H, s, OMe), 3.52–3.68 (2H, m, GlcH-5, GalH-5), 3.75 (3H, s, CO$_2$Me), 3.84 (1H, t, $J_{4,3} = J_{4,5}$ = 9.3 Hz, GlcH-4), 4.09–4.15 (1H, m, GlcH-6), 4.36 (1H, d, $J_{1,2}$ 7.5 Hz, GlcH-1), 4.40 (1H, d, $J_{1,2}$ 8.1 Hz, GalH-1), 4.49 (1H, dd, $J_{6'6}$ 11.7, $J_{6',5}$ 2.7 Hz, GlcH-6'), 4.62 (4.64*) (1H, s, H-2'), 4.88 (1H, dd, $J_{3,2}$ 10.5, $J_{3,4}$ 3.3 Hz, GalH-3), 4.92–5.04 (2H, m, GlcH-2, GalH-2), 5.19 (1H, t, $J_{3,4} = J_{3,2}$ = 9.3 Hz, GlcH-3), 5.32 (5.35*) (1H, d, $J_{4,3}$ 3.3 Hz, GalH-4), 7.32–7.48 (5H, m, Ph); ^{13}C NMR (75.5 MHz, CDCl$_3$) δ 20.3, 20.4, 20.5, 20.6, 20.7 (6 × OC(O)*Me*), 30.8 (31.0*) (GalC-6), 51.9 (52.2*) (C-2'), 52.7 (OMe), 56.8 (CO$_2$*Me*), 61.9 (GlcC-6), 67.9, 69.1, 71.1, 71.6, 72.5, 72.6, 75.3, (75.5*) (GlcC-2, GlcC-3, GlcC-4, GlcC-5, GalC-2, GalC-3, GalC-4, GalC-5), 100.2 (100.3*), 101.3 (101.4*) (GlcC-1, GalC-1), 127.8, 128.1, 128.4, 128.7 (Ph), 135.3 (*ipso*-Ph), 169.0, 169.5, 169.7, 169.8, 170.0, 170.3, 170.8 (C-1', 6 × O*C*(O)Me); ESIMS 790 (M + NH$_4$, 100%), 748 (35%); HRESIMS Found 790.25943. C$_{34}$H$_{44}$O$_{18}$S·NH$_4$ requires 790.25921.

Methyl 2,3,4-Tri-*O*-acetyl-6-thio-6-[2′-(γ-butyrolactone)]-β-D-glucopyranoside (27) in 86% yield by coupling between the 6-thioacetyl glucoside derivative **13** and α-bromo-γ-butyrolactone (chromatography EtOAc:hexane, 2:1, R_f 0.3) as an amorphous mass: IR 1765, 1380, 1255, 1220, 1170, 1040 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.94, 2.03, 2.05 (3 × 3H, 3 × s, 3 × AcO), 2.05–2.13 (1H, m, H-3a'), 2.58–2.62 (1H, m, H-3b'), 2.68 (1H, dd, $J_{6,6'}$ 14.7, $J_{6,5}$ 6.0 Hz, H-6), 3.30 (1H, dd, $J_{6',6}$ 14.7, $J_{6',5}$ 2.7 Hz, H-6'), [3.02* (2H, d, $J_{6/6',5}$ 5.3 Hz, H-6/6')], 3.51 (3H, s, OMe), 3.57 (3.71*) (1H, dd, $J_{2',3a'}$ 8.5, $J_{2',3b'}$ 4.7 Hz, H-2'), 3.73–3.81 (1H, m, H-5), 4.27–4.40 (2H, m, H-4'), 4.42 (4.43*) (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 4.96 (1H, dd, $J_{2,3}$ 9.3, $J_{2,1}$ 7.9 Hz, H-2), 5.00 (1H, t, $J_{4,3} = J_{4,5}$ = 9.3 Hz, H-4), 5.15 (5.17)* (1H, t, $J_{3,4} = J_{3,2}$ = 9.3 Hz, H-3), assignments confirmed by COSY; ^{13}C NMR (CDCl$_3$) δ 20.6, 20.7, 20.8 (3 × OC(O)*Me*), 29.8 (30.0*) (C-3'), 31.3 (32.5*) (C-6), 39.2 (39.6*) (C-2'), 57.1 (OMe), 66.6 (66.7*) (C-4'), 70.6, 71.3 (71.4*), 72.8 (72.9*), 73.8 (C-2, C-3, C-4, C-5), 101.5 (101.6*) (C-1), 169.4 (169.5*), 169.6, 170.2 (170.3*) (3 × O*C*(O)Me), 175.4 (175.6*) (C-1'); ESIMS 438 (M + NH$_4$, 100%), 389 (90%); HRESIMS Found 438.14349. C$_{17}$H$_{24}$O$_{10}$S·NH$_4$ requires 438.14339.

Methyl 2,3,4-Tri-*O*-acetyl-6-thio-6-[2′-(γ-butyrolactone)]-β-D-galactopyranoside (28) in 79% yield by coupling between the 6-thioacetyl glucoside derivative **14** and α-bromo-γ-butyrolactone (chromatography EtOAc:hexane, 2:1, R_f 0.2) as an amorphous mass: IR 1775, 1380, 1260, 1225, 1165, 1065 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.95, 2.03, 2.13 (3 × 3H, 3 × s, 3 × AcO), 2.05–2.11 (1H, m, H-3a'), 2.57–2.66 (1H, m, H-3b'), 2.74 (1H, dd, $J_{6,6'}$ 14.0, $J_{6,5}$ 5.8 Hz, H-6), 3.16 (1H, dd, $J_{6',6}$ 14.0, $J_{6',5}$ 8.0 Hz, H-6'), [2.92* (2H, ABq, H-6/6')], 3.49 (3H, s, OMe), 3.55 (1H, dd, $J_{2',3a'}$ 8.5, $J_{2',3b'}$ 4.9 Hz, H-2'), 3.83–3.91 (1H, m,

H-5), 4.25−4.41 (2H, m, H-4′), 4.38 (4.39*) (1H, d, $J_{1,2}$ 8.0 Hz, H-1), 5.00 (1H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 3.3 Hz, H-3), 5.14 (1H, dd, $J_{2,3}$ 10.2, $J_{2,1}$ 8.0 Hz, H-2), 5.40 (5.41*) (1H, d, $J_{4,3}$ 3.3 Hz, H-4), assignments confirmed by COSY; ^{13}C NMR (CDCl₃) δ 20.5, 20.6, 20.7 (3 × OC(O)*Me*), 29.6 (29.8*) (C-3′), 30.9 (31.0*) (C-6), 39.3 (39.8*) (C-2′), 56.9 (OMe), 66.6 (C-4′), 68.3 (68.5*), 68.8, 71.1, 72.8 (73.1*) (C-2, C-3, C-4, C-5), 102.0 (C-1), 169.5, 170.0 (170.1*), 170.3 (170.5*), 175.2 (175.4*) (C-1′); ESIMS 438 (M + NH₄, 65%), 389 (100%). Found C, 48.2; H, 5.6; S, 7.75%. C₁₇H₂₄O₁₀S requires C, 48.6; H, 5.75; S, 7.6%.

3. Deprotection of Sialylmimetics. Methyl 6-Thio-6-[2′-(sodium propanoate)]-β-D-glucopyranoside (29). To a solution of **18** (200 mg, 0.46 mmol) in dry MeOH (10 mL) at room temperature under N₂ was added NaOH (1 M, 1.5 mL). After being stirred for 15 h, the mixture was neutralized [IR-120 (H⁺) resin, to pH ∼ 5] and the resin removed by filtration, washed with aqueous MeOH, and concentrated under reduced pressure. The residue was dissolved in H₂O (10 mL) and the pH adjusted to 7.3 with NaOH (0.5 M and then 0.05 M) before being lyophilized. The residue was purified using HPLC (reverse phase: H₂O) to give **29** (97 mg, 70%) as an amorphous mass: IR 3450 (br), 2932, 1615, 1460, 1395, 1070 cm⁻¹; ^{1}H NMR (D₂O) δ 1.36 (3H, d, $J_{3',2'}$ 7.2 Hz, H-3′), 2.75 (1H, dd, $J_{6,6'}$ 14.1, $J_{6,5}$ 8.4 Hz, H-6), 3.06 (1H, dd, 1H, $J_{6',6}$ 14.1, $J_{6',5}$ 2.7 Hz, H-6′), 3.22−3.27 (1H, m, H-5), 3.31−3.36 (1H, m, H-4), 3.41−3.49 (3H, m, H-2, H-3, H-2′), 3.55 (3H, s, OMe), 4.34 (1H, d, $J_{1,2}$ 7.8 Hz, H-1); ^{13}C NMR (D₂O) 18.1 (C-3′), 32.2 (32.4*) (C-6), 45.9 (46.1*) (C-2′), 57.2 (OMe), 72.5, 72.6, 73.2, 75.5 (C-2, C-3, C-4, C-5), 103.2 (103.5*) (C-1), 181.0 (181.1*) (C-1′); ESIMS 305 (M + 1, 100%), 272 (60%), 251(65%); HRESIMS Found 300.11110. C₁₀H₁₈O₇S·NH₄ requires 300.11170.

The following were prepared in a similar manner:

Methyl 6-Thio-6-[2′-(sodium propanoate)]-β-D-galactopyranoside (30) in 71% yield (reverse phase HPLC, H₂O) as an amorphous mass: IR 3450 (br), 1610, 1465, 1405, 1365, 1210, 1080 cm⁻¹; ^{1}H NMR (D₂O) δ 1.34 (3H, d, $J_{3',2'}$ 7.2 Hz, H-3′), 2.76−2.83 (2H, m, H-6/6′), 3.40−3.46 (2H, m, H-5, H-2′), 3.52 (3H, s, OMe), 3.64 (1H, dd, $J_{2,3}$ 9.3, $J_{2,1}$ 7.8 Hz, H-2), 3.70−3.74 (1H, m, H-3), 3.90−3.95 (1H, m, H-4), 4.27 (1H, d, $J_{1,2}$ 7.8 Hz, H-1); ^{13}C NMR (D₂O) δ 18.6 (C-3′), 31.6 (31.8*) (C-6), 46.5 (C-2′), 57.8 (OMe), 70.0, 71.3, 73.4, 74.4 (74.7*) (C-2, C-3, C-4, C-5), 104.4 (C-1), 181.7 (C-1′); ESIMS 327 (M + Na, 70%), 305 (40%), 272 (50%), 251(52%); HRESIMS Found 300.11112. C₁₀H₁₈O₇S·NH₄ requires 300.11170.

Methyl 6-Thio-6-[2′-(sodium propanoate)]-β-D-galactopyranosyl-(1,4)-β-D-glucopyranoside (31) in 63% yield (reverse phase HPLC, H₂O) as an amorphous mass: IR 3500 (br), 1610, 1480, 1415, 1065 cm⁻¹; ^{1}H NMR (500 MHz, D₂O) δ 1.30 (3H, d, $J_{3',2'}$ 7.0 Hz, H-3′), 2.72 (1H, dd, $J_{6,6'}$ 13.9, $J_{6,5}$ 5.6 Hz, GalH-6), 2.78−2.85 (1H, m, GalH-6′), 3.26 (1H, dd, $J_{2,3}$ 9.1, $J_{2,1}$ 8.0 Hz, GlcH-2), 3.36 (3.37*) (1H, q, $J_{2',3'}$ 7.0 Hz, H-2′), 3.45 (1H, dd, $J_{2,3}$ 9.1, $J_{2,1}$ 7.8 Hz, GalH-2), 3.51 (3H, s, OMe), 3.55−3.61 (4H, m, GlcH-3, GlcH-4, GlcH-5, GalH-3), 3.69−3.75 (2H, m, GlcH-6, GalH-5), 3.89 (3.90*) (1H, d, $J_{4,3}$ 3.3 Hz, GalH-4), 3.92 (1H, d, $J_{6',6}$ 12.8 Hz, GlcH-6′), 4.34 (1H, d, $J_{1,2}$ 8.0 Hz, GlcH-1), 4.38 (4.40*) (1H, d, $J_{1,2}$ 7.8 Hz, GalH-1), assignments confirmed by COSY; ^{13}C NMR (75.5 MHz, D₂O) δ 18.4 (18.6*) (C-3′), 31.8 (GalC-6), 46.0 (46.5*) (C-2′), 57.8 (OMe), 60.9 (GlcC-6), 70.0 (70.1*), 71.4, 73.1, 73.4, 74.1 (74.6*), 75.1 (75.4*), 79.6 (79.8*) (GlcC-2, GlcC-3, GlcC-4, GlcC-5, GalC-2, GalC-3, GalC-4, GalC-5), 103.6, 103.7 (GlcC-1, GalC-1), 181.6 (C-1′); ESIMS 489 (M+Na, 60%), 467 (M+1, 35%), 184 (75%); HRESIMS Found 462.16437. C₁₆H₂₈O₁₂S·NH₄ requires 462.16452.

Methyl 6-Thio-6-[2′-(sodium butanoate)]-β-D-glucopyranoside (32) in 73% yield (reverse phase HPLC, H₂O) as an amorphous mass: IR 3425 (br), 2940, 1600, 1465, 1395, 1080 cm⁻¹; ^{1}H NMR (D₂O) δ 0.88 (3H, t, $J_{4',3'}$ 7.2 Hz, H-4′), 1.68−1.77 (2H, m, H-3′), 2.70 (1H, dd, 1H, $J_{6,6'}$ 14.1, $J_{6,5}$ 8.4 Hz, H-6), 3.05 (1H, dd, 1H, $J_{6',6}$ 14.1, $J_{6',5}$ 2.7 Hz, H-6′), 3.18−3.25 (2H, m, H-4, H-5), 3.35−3.43 (3H, m, H-2, H-3, H-2′), 3.48 (3H, s, OMe), 4.26 (1H, d, $J_{1,2}$ 7.8 Hz, H-1); ^{13}C NMR (D₂O) 11.4 (C-4′), 25.7 (C-3′), 32.4 (32.6*) (C-6), 52.9 (53.4*) (C-2′), 57.2 (OMe), 72.5 (72.7*), 73.2, 74.5, 75.5 (C-2, C-3, C-4, C-5), 103.2

(C-1), 180.3 (C-1′); ESIMS 341 (M + Na, 30%), 319 (M + 1, 90%), 265 (100%).

Methyl 6-Thio-6-[2′-(sodium butanoate)]-β-D-galactopyranoside (33) in 77% yield (reverse phase HPLC, H₂O) as an amorphous mass: IR 3450 (br), 1605, 1455, 1395, 1075 cm⁻¹; ^{1}H NMR (D₂O) δ 0.89 (3H, t, $J_{4',3'}$ 7.2 Hz, H-4′), 1.54−1.78 (2H, m, H-3′), 2.76−2.82 (2H, m, H-6/6′), 3.23−3.28 (1H, m, H-5), 3.39 (1H, dd, $J_{2,3}$ 9.6, $J_{2,1}$ 7.8 Hz, H-2), 3.48 (3H, s, OMe), 3.56 (1H, dd, $J_{3,2}$ 9.6, $J_{3,4}$ 3.3 Hz, H-3), 3.66 (1H, t, $J_{2',3'}$ 6.6 Hz, H-2′), 3.87 (1H, d, $J_{4,3}$ 3.3 Hz, H-4), 4.21 (1H, d, $J_{1,2}$ 7.8 Hz, H-1); ^{13}C NMR (D₂O) 12.0 (12.1*) (C-4′), 26.3 (C-3′), 31.7 (31.8*) (C-6), 53.4 (53.7*) (C-2′), 57.8 (OMe), 70.0 (70.1*), 71.3, 73.5, 74.2 (74.7*) (C-2, C-3, C-4, C-5), 104.4 (C-1), 181.1 (C-1′); ESIMS 341 (M + Na, 100%), 319 (M + 1, 30%). Found C, 39.0; H, 6.55; S, 9.3%. C₁₁H₁₉O₇SNa·H₂O requires C, 39.3; H, 6.3; S, 9.5%.

Methyl 6-Thio-6-[2′-(sodium butanoate)]-β-D-galactopyranosyl-(1,4)-β-D-glucopyranoside (34) in 78% yield (reverse phase HPLC, H₂O) as an amorphous mass: IR 3460 (br), 1605, 1460, 1400, 1050 cm⁻¹; ^{1}H NMR (500 MHz, D₂O) δ 0.87 (0.88*) (3H, t, $J_{4',3'}$ 7.2 Hz, H-4′), 1.59−1.68 (2H, m, H-3′), 2.71 (1H, dd, $J_{6,6'}$ 13.9, $J_{6,5}$ 5.5 Hz, GalH-6), 2.76−2.83 (1H, m, GalH-6′), 3.17 (1H, t, $J_{2',3'}$ 7.2 Hz, H-2′), 3.26 (1H, dd, $J_{2,3}$ 8.7, $J_{2,1}$ 8.0 Hz, GlcH-2), 3.44 (1H, dd, $J_{2,3}$ 9.8, $J_{2,1}$ 7.8 Hz, GalH-2), 3.50 (3H, s, OMe), 3.55−3.61 (4H, m, GlcH-3, GlcH-4, GlcH-5, GalH-3), 3.68−3.72 (1H, m, GalH-5), 3.74 (1H, dd, $J_{6,6'}$ 12.2, $J_{6,5}$ 4.6 Hz, GlcH-6), 3.88 (1H, d, $J_{4,3}$ 3.1 Hz, GalH-4), 3.91 (1H, d, $J_{6',6}$ 12.2 Hz, GlcH-6′), 4.34 (1H, d, $J_{1,2}$ 8.0 Hz, GlcH-1), 4.38 (4.40*) (1H, d, $J_{1,2}$ 7.8 Hz, GalH-1), assignments confirmed by COSY; ^{13}C NMR (D₂O) δ 12.1 (C-4′), 26.2 (26.3*) (C-3′), 31.9 (GalC-6), 53.2 (53.7*) (C-2′), 57.6 (OMe), 60.8 (GlcC-6), 69.9, 71.4, 73.1, 73.7, 74.0, 74.6, 75.4, 79.6 (GlcC-2, GlcC-3, GlcC-4, GlcC-5, GalC-2, GalC-3, GalC-4, GalC-5), 103.5, 103.6 (GlcC-1, GalC-1), 181.1 (C-1′); ESIMS 503 (M + Na, 35%), 481 (M+1, 50%), 265 (65%); HRESIMS Found 476.17991. C₁₇H₃₀O₁₂S·NH₄ requires 476.18017.

Methyl 6-Thio-6-[2′-(sodium 2′-phenylacetate)]-β-D-glucopyranoside (35) in 88% yield (reverse phase HPLC, H₂O) as an amorphous mass: IR 3460 (br), 1605, 1455, 1390, 1070 cm⁻¹; ^{1}H NMR (D₂O) δ 2.48 (1H, dd, $J_{6,6'}$ 14.1, $J_{6,5}$ 8.1 Hz, H-6), 2.85 (1H, d, $J_{6',6}$ 14.1 Hz, H-6′), 3.10−3.26 (3H, m, H-2, H-3, H-4), 3.33−3.44 (1H, m, H-5), 3.48 (3H, s, OMe), 4.09 (4.27*) (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.63 (4.67*) (1H, s, H-2′), 7.27−7.38 (5H, m, Ph); ^{13}C NMR (D₂O) δ 33.0 (33.4*) (C-6), 57.4 (57.8*) (C-2′), 57.9 (OMe), 73.2, 73.8, 75.6, 76.2 (C-2, C-3, C-4, C-5), 103.9 (C-1), 128.4, 128.9, 129.5 (Ph), 139.5 (ipso Ph), 178.0 (C-1′); ESIMS 389 (M + Na, 67%), 367 (M + 1, 70%), 313 (100%). Found C, 49.3; H, 5.4; S, 8.8%. C₁₅H₁₉O₇SNa requires C, 49.2; H, 5.2; S, 8.75%.

Methyl 6-Thio-6-[2′-(sodium 2′-phenylacetate)]-β-D-galactopyranoside (36) in 80% yield (reverse phase HPLC, H₂O) as an amorphous mass: IR 3480 (br), 1605, 1465, 1395, 1080 cm⁻¹; ^{1}H NMR (D₂O) δ 2.66−2.74 (2H, m, H-6/6′), 3.26−3.41 (3H, m, H-2, H-3, H-5), 3.44 (3H, s, OMe), 3.75 (1H, d, $J_{4,3}$ 3.3 Hz, H-4), 4.03 (4.04*) (1H, d, $J_{1,2}$ 7.5 Hz, H-1), 4.71 (4.74*) (1H, s, H-2′), 7.32−7.38 (5H, m, Ph); ^{13}C NMR (D₂O) δ 31.7 (31.9*) (C-6), 57.5 (57.7*) (C-2′), 57.9 (OMe), 69.6, 71.2, 73.8, 74.3 (C-2, C-3, C-4, C-5), 104.4 (C-1), 128.5, 128.8, 129.5 (Ph), 139.9 (ipso Ph), 178.0 (C-1′); ESIMS 367 (M + 1, 60%), 313 (100%); HRESIMS Found 362.12677. C₁₅H₂₀O₇S·NH₄ requires 362.12735.

Methyl 6-Thio-6-[2′-(sodium 2′-phenylacetate)]-β-D-galactopyranosyl-(1,4)-β-D-glucopyranoside (37) in 81% yield (reverse phase HPLC, H₂O) as an amorphous mass: IR 3450 (br), 1605, 1390, 1095, 1060 cm⁻¹; ^{1}H NMR (500 MHz, D₂O) δ 2.60 (1H, dd, $J_{6,6'}$ 14.0, $J_{6,5}$ 6.7 Hz, GalH-6), 2.64−2.70 (1H, m, GalH-6′), 3.22−3.31 (2H, m, GlcH-2, GalH-5), 3.37−3.41 (2H, m, GalH-3, H-2′), 3.43 (1H, dd, $J_{3,2}$ 9.6, $J_{3,4}$ 3.0 Hz, GalH-3), 3.50 (3.51*) (3H, s, OMe), 3.51−3.57 (3H, m, GlcH-3, GlcH-4, GlcH-5), 3.69 (1H, m, GlcH-6), 3.77 (3.79*) (1H, d, $J_{4,3}$ 3.0 Hz, GalH-4), 3.89 (3.90*) (1H, dd, $J_{6',6}$ 12.1, $J_{6',5}$ 2.0 Hz, GlcH-6′), 4.09 (4.18*) (1H, d, $J_{1,2}$ 7.6 Hz, GalH-1), 4.32 (4.33*) (1H, d, $J_{1,2}$ 8.0 Hz, GlcH-1), 7.29−7.43 (5H, m, Ph), assignments confirmed by COSY; ^{13}C NMR (D₂O) δ 30.9 (31.7*)

(GalC-6), 56.9 (57.1*) (C-2′), 57.2 (OMe), 60.1 (GlcC-6), 68.8, 70.7, 72.5, 72.8, 72.9, 74.3, 74.7, 78.9 (GlcC-2, GlcC-3, GlcC-4, GlcC-5, GalC-2, GalC-3, GalC-4, GalC-5), 102.9, 103.0 (GlcC-1, GalC-1), 127.9, 128.2, 128.9 (Ph), 139.2 (ipso Ph), 177.2 (C-1′); ESIMS 551 (M + Na, 25%), 529 (M + 1, 30%), 265 (100%); HRESIMS Found 524.18018. $C_{21}H_{30}O_{12}S \cdot NH_4$ requires 524.18017.

Methyl 6-Thio-6-[2′-(sodium 4′-hydroxy-butanoate)]-β-D-glucopyranoside (38) in 79% yield (reverse phase HPLC, H_2O) as an amorphous mass: IR 3480 (br), 1660, 1580, 1420, 1060 cm^{-1}; ^{1}H NMR (D_2O) δ 1.78−1.89 (1H, m, H-3′), 2.03−2.10 (1H, m, H-3′), 2.69−2.77 (1H, m, H-2′), 2.83 (2.99*) (1H, dd, $J_{6,6'}$ 14.1, $J_{6,5}$ 6.9 Hz, H-6), 3.13−3.24 (2H, m, H-6′, H-2), 3.28−3.37 (2H, m, H-4, H-5), 3.48−3.52 (1H, m, H-3), 3.51 (3.52*) (3H, s, OMe), 4.14 (4.17*) (1H, d, $J_{1,2}$ 7.6 Hz, H-1), 4.28−4.42 (2H, m, H-4′); ^{13}C NMR (D_2O) δ 32.4 (32.5*) (C-6), 34.6 (34.7*) (C-3′), 47.7 (48.1*) (C-2′), 57.2 (OMe), 59.4 (C-4′), 72.5, 73.2, 74.5, 75.5 (C-2, C-3, C-4, C-5), 103.2 (C-1), 172.7 (C-1′); ESIMS 357 (M + Na, 40%), 335 (M + 1, 70%), 281 (80%).

Methyl 6-Thio-6-[2′-(sodium 4′-hydroxy-butanoate)]-β-D-galactopyranoside (39) in 87% yield (reverse phase HPLC, H2O) as an amorphous mass: IR 3480 (br), 1660, 1610, 1210, 1065 cm^{-1}; ^{1}H NMR (D_2O) δ 1.80−1.93 (1.97−2.08*) (1H, m, H-3′), 2.20−2.33 (1H, m, H-3′), 2.73−2.95 (2H, m, H-6, H-2′), 3.00 (3.14*) (1H, dd, $J_{6',6}$ 12.9, $J_{6',5}$ 7.2 Hz, H-6′), 3.48 (1H, dd, $J_{2,3}$ 9.3, $J_{2,1}$ 8.1 Hz, H-2), 3.57 (3H, s, OMe), 3.65 (1H, dd, $J_{3,2}$ 9.3, $J_{3,4}$ 3.0 Hz, H-3), 3.72−3.84 (2H, m, H-4′), 3.93−3.98 (1H, m, H-4), 4.31 (1H, d, $J_{1,2}$ 8.1 Hz, H-1); ^{13}C NMR (D_2O) δ 30.0 (30.3*) (C-6), 31.7 (31.8*) (C-3′), 41.6 (41.8*) (C-2′), 57.8 (OMe), 60.1 (C-4′), 70.2, 71.2, 73.4, 74.5 (C-2, C-3, C-4, C-5), 104.5 (C-1), 180.2 (C-1′); ESIMS 335 (M + 1, 40%), 317 (100%).

4. Biological Evaluation. Cells. MA104 cells, a monkey kidney cell line, were grown at 37 °C in RPMI supplemented with 10% foetal calf serum, 20 mM HEPES, 26.6 μg/mL gentamicin, and 2 μg/mL fungizone.

Virus. All viruses were cultivated in MA104 cells. Virus was preactivated with 10 μg/mL porcine trypsin (type IX; Sigma) for 30 min at 37 °C before inocculating onto confluent cell monolayers grown in 200 mL flat surface glass bottles at a multiplicity of infection (m.o.i.) of 10 fluorescent cell forming units/cell. After 60 min at 37 °C, the infected cells were incubated in Eagles minimal medium containing 1 μg/mL porcine trypsin until extensive cytopathic effect (c.p.e.) was evident. Cell lysates were frozen and thawed twice, centrifuged (3000g, 10 min), and the supernatants were stored at −70 °C. Virus stocks were activated prior to use by incubation of cell lysates with 10 μg/mL porcine trypsin for 30 min at 37 °C. The reaction was stopped by the addition of foetal calf serum (final concentrated of 2%). For use in neutralization assays, the virus was titrated to determine a dilution which gave ∼200 fluorescent focus forming units/well of the 96-well microtiter tray.

Neutralization Assays. Sialylmimetics (starting concentration, 25 mM) were serially diluted (2-fold) in virus diluent (Hanks balanced salt solution containing 0.002% gelatin + 0.01 M HEPES, pH 7.5) and incubated with equal volumes of rotavirus for 1 h at 37 °C. The sialylmimetic−virus mixture was then added to confluent monolayers of MA104 cells grown in 96-well microtiter trays and left for 1 h at 37 °C. The inoculum was then removed and replaced with maintenance medium (Eagles MEM with 10 mM HEPES) and left for 16 h at 37 °C in a 5% CO_2 environment. Neutralization was determined by indirect immunofluorescent staining.

Indirect Immunofluorescence. Rotavirus infected cell monolayers were fixed and permeabilized 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-CRW8 serum diluted 1 in 2000 and incubated at 37 °C for 30 min. Following this, cells were stained with 30 μL of fluorescein isothiocyanate-conjugated anti-rabbit immunoglobulin diluted 1 in 200. After 30 min at 37 °C cells were washed three times with PBS and viewed through a fluorescent microscope. Results were expressed as the concentration of the compound at which 50% infection of control infected monolayers occurred.

Conclusion

We have clearly demonstrated an efficient synthesis of a variety of novel sialylmimetics and have evaluated these compounds as inhibitors of two strains of rotavirus. Our results show that glucose- and galactose-based sialylmimetics are not inhibitors of rotaviral infection. However, on the basis of this study it appears that the minimum epitope required for recognition by rotavirus is a disaccharide unit with a negative charge. We are presently investigating lactose-based sialylmimetics with altered steric demand and hydrophobicity in order to further explore these interesting results.

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