# Di-, Tri-, and Tetravalent Dendritic Galabiosides That Inhibit Hemagglutination by *Streptococcus suis* at Nanomolar Concentration

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**Abstract:** Galabiose (Galα1-4Gal) derivatives were coupled to carrier molecules having mono-, di-, tri-, and tetrameric functionalities, thus creating mono- to tetravalent dendritic galabiosides (**1**−**9**). The di- to tetravalent galabiosides were several hundred times more efficient than the monomeric galabiosides in inhibiting hemagglutination by the Gram-positive bacterium *Streptococcus suis*, resulting in complete inhibition at low nanomolar concentrations. This is unprecedented in the field of inhibition of bacterial adhesion by soluble compounds.

## Introduction

Bacterial resistance against traditional antibiotics is a growing medical problem.[1] New principles for treatment of infections are therefore highly desirable. One possibility would be to inhibit the attachment of the infecting bacteria to the host's cell surfaces; such attachment is often a prerequisite for the later stages of infection, colonization, and invasion of tissues.[2] Since the chemical structure of the adhesion inhibitors most likely would be quite similar to the natural attachment ligands used by the bacteria, it is unlikely that mutations (resistance) would give them the capability to overcome the inhibitory effect of the antiadhesive drug whithout impairing their ability to adhere to the host cells. In the virus field, lack of formation of resistant mutants was observed in connection with the successful development of a potent sialidase-based inhibitor of influenza virus replication.[3]

Many bacterial species carry surface-bound lectins that recognize saccharide moieties on the surface of the host cells and, thus, use these as anchoring points.[4] However, each saccharide−lectin interaction is normally rather weak, which is compensated by multivalent binding;[5,6] an *Escherichia coli* cell carries multiple lectin molecules for this purpose.[2]

We are currently interested in the development of inhibitors of different pathogens that recognize the galabiose (Galα1-4Gal) moiety of glycolipids. The work is based on earlier determinations of the galabiose epitopes used for adhesion by uropathogenic *E. coli*[7] (a Gram-negative species) and the piglet pathogen *Streptococcus suis*[8] (a Gram-positive species).

The effective inhibitory concentrations ($IC_{50}$ or MIC values) were found in the micro- to millimolar region for most of the galabiose derivatives we have tested as inhibitors of *E. coli* and *S. suis* hemagglutination. Such inhibitor concentrations are typical and probably too high for practical treatment of infected tissues. However, strong inhibitors have been prepared and various aromatic α-mannosides inhibit the adherence of type 1 fimbriated *E. coli* to yeast and guinea pig intestinal epithelial cells at low micromolar concentrations.[9]

Inhibition of influenza virus sialidase, as well as inhibition of influenza virus replication, was accomplished at the low nanomolar level by a designed guanidino derivative of *N*-acetylneuraminic acid.[3] Other approaches toward efficient inhibitors of sugar-binding proteins use saccharides coupled in a multivalent fashion to carriers, producing, for example, polymers, dendrimers, and solid materials capped with various sugars.[10] However, it is reasonable to believe that polymers of high molecular weight will be reluctant to cross biological membranes and, consequently, their absorbance into animal tissues and their systemic *in vivo* antiadhesive function would be impaired. Absorption of the inhibitors by cells is of course not relevant when the inhibition is expected to occur by topical administration or in the gut, but would be crucial for treatment of, for example, *S. suis* infections, which cause meningitis, septicemia, and pneumonia in pigs and also meningitis in humans.[11]

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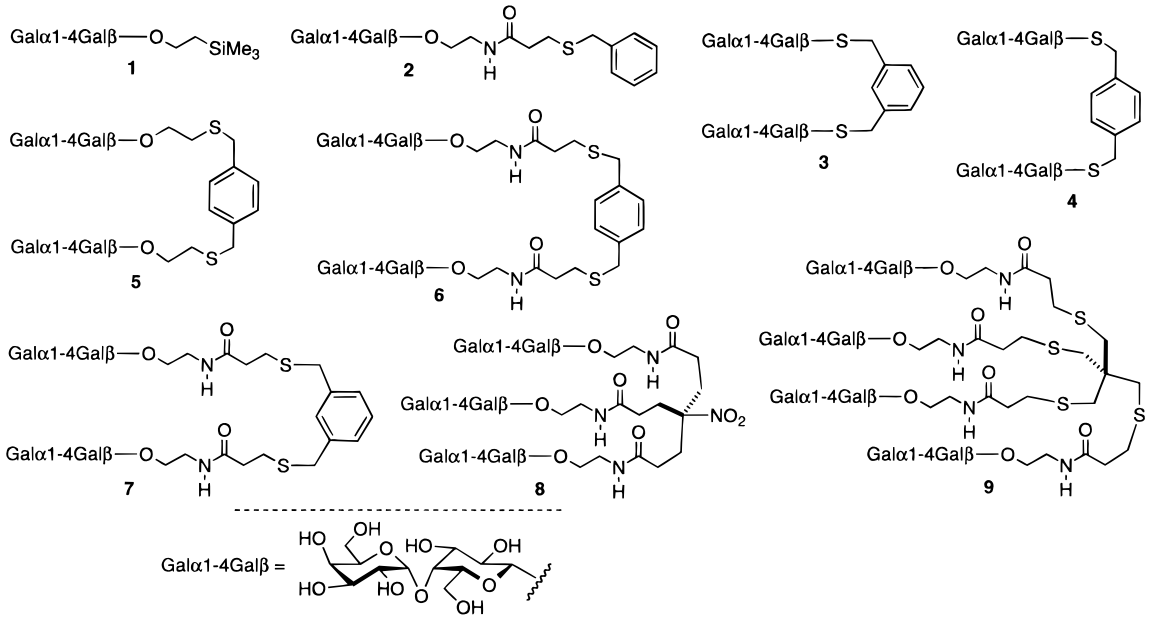
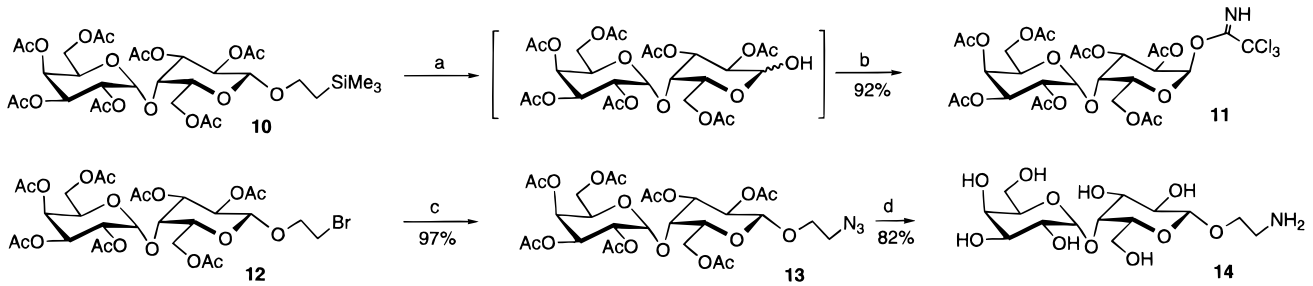



**Figure 1.** Dendritic galabiosides used as inhibitors of hemagglutination by *S. suis* bacteria.

**Scheme 1**[a]




[a] Key: (a) $CF_3COOH$, $CH_2Cl_2$, 22 °C, 30 min; (b) $Cl_3CCN$, DBU, 0 °C, 50 min; (c) $NaN_3$, 15-crown-5, 75 °C, 24 h; (d) MeONa, MeOH, then $H_2$, Pd/C.

We now report the synthesis and biological evaluation of the novel mono- to tetravalent galabiosides **2**−**9** (Figure 1). Some of these compounds were found to inhibit the agglutination of human erythrocytes by *S. suis* bacteria, at inhibitor concentrations as low as 2 nM. This result is unprecedented in the field of bacterial antiadhesion by soluble inhibitors. The high inhibitory power of **6**−**9**, combined with the moderate molecular weight (∼1−2 kD), makes these saccharides potentially useful as starting points for the practical development of useful antiadhesive drugs.

## Results and Discussion

**I. Synthesis of Di- to Tetravalent Galabiosides.** The galabioside moieties **11**, **12**, and **14** were synthesized as described above (Scheme 1) and coupled to the various scaffold molecules shown in Schemes 2 and 3, thus producing the mono- to tetradentate galabiosides **2**−**9**, useful as inhibitors of hemagglutination by the *S. suis* bacteria. The 2-(trimethylsilyl)-ethyl (TMSEt) galabioside **10**[12] was treated with trifluoroacetic acid, which removed the TMSEt group.[12] The crude hemiacetal was then transformed into the α-trichloroacetimidate **11** by treatment with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU),[13] leaving **11** in an overall yield of 92%.

The 2-bromoethyl galabioside **12**[14] was treated with sodium azide to give **13** (97%). Removal of the *O*-acetyl groups of **13**, followed by hydrogenolysis of the azido group, gave the amine **14** (82%).

Commercially available 1,3- and 1,4-benzenedimethanethiol were glycosylated with the imidate **11** (2.5 equiv), using boron trifluoride etherate as promoter,[13] which gave **15** and **16** (Scheme 2) in 65 and 63% yield, respectively. Removal of the *O*-acetyl groups of **15** and **16** gave the dimeric galabiosides **3** (94%) and **4** (92%). Treatment of 1,4-benzenedimethanethiol with the 2-bromoethyl galabioside **12** (2.5 equiv) and cesium carbonate[15] gave **17** in 55% yield. De-*O*-acetylation of **17** gave the dimeric galabioside **5** (93%).

Alkylation of benzylthiol, 1,4-benzenedimethanethiol, and 1,3-benzenedimethanethiol with 3-bromopropionic acid (Scheme 3) gave the scaffold mono- and diacids **18** (65%), **19** (77%), and **20** (66%), respectively. The commercially available tetra-(bromomethyl)methane (Scheme 3) was treated with methyl 3-mercaptopropionate and cesium carbonate,[15] which gave the tetraester **22** (54%). Hydrolysis of the ester groups gave the tetraacid **23** (96%).

The 2-aminoethyl galabioside **14** was coupled via amide bonds to the mono- to tetraacids **18**−**21** and **23**, which furnished the mono- to tetravalent galabiosides **2** and **6**−**9**, respectively.

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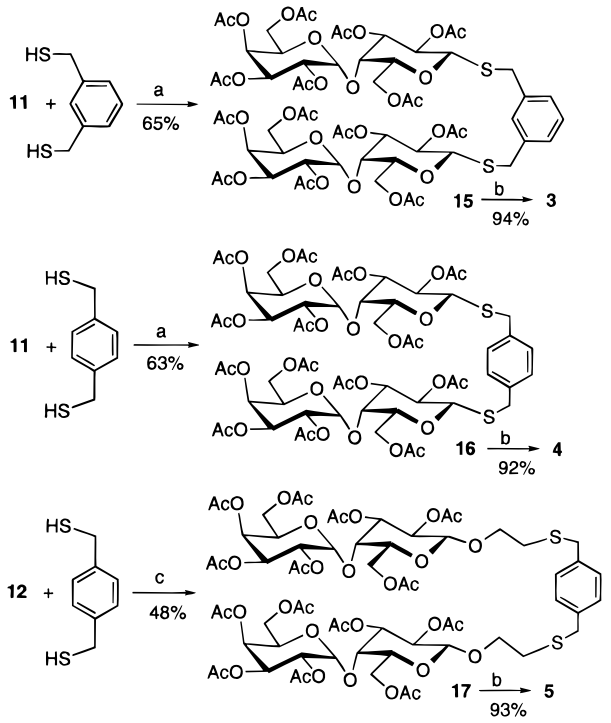
**Scheme 2**[a]




[a] Key: (a) $BF_3{\cdot}OEt_2$, $CH_2Cl_2$, 22 °C, 30−120 min; (b) MeONa, MeOH, 22 °C, 6.5−15 h; (c) $Cs_2CO_3$, DMF, 22 °C, 15 h.

This flexible methodology permits easy variation of the carbohydrate moieties, which would provide ligands for other applications. The amide linkages were obtained by first activating the carboxyl groups of the acids and isolating the resulting active esters. Without the crucial isolation step, a complex mixture was obtained, resulting in a low yield of the desired amide. The acids **18**−**21** were activated by *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC)-mediated esterification with *N*-hydroxysuccinimide (NHS),[16] and the active esters were isolated and coupled with the galabiosylamine **14** (1.1 equiv per ester group), which gave the galabiosyl amides **2** (98%), **6** (97%), **7** (97%), and **8** (98%). Attempted reaction of the tetraacid **23** under the same conditions as above and coupling with **14** left a mixture of several compounds, and the yield of **9** was estimated by NMR to be ∼40%. Several alternative procedures were investigated, but none of them gave **9** in high yield. The best result was obtained by transforming **23** into the corresponding pentafluorophenyl ester (isolated yield 32%), using pentafluorophenol and diisopropylcarbodiimide (DIC).[17] Treatment of **14** with the pentafluorophenyl ester gave **9** (55%) after purification by preparative TLC. The symmetry of compounds **3**−**9** simplified the interpretation of NMR spectra; all signals from structurally equivalent protons and carbons had the same chemical shifts, agreeing with those of other galabiosides.[18]

**II. Inhibition of Hemagglutination of *S. suis* Bacteria.** Glycolipids of the globoseries are present on the surface of human red blood cells (erythrocytes).[19] Galabiose (Galα1-4Gal$\beta$) is a common structural unit in these glycolipids.

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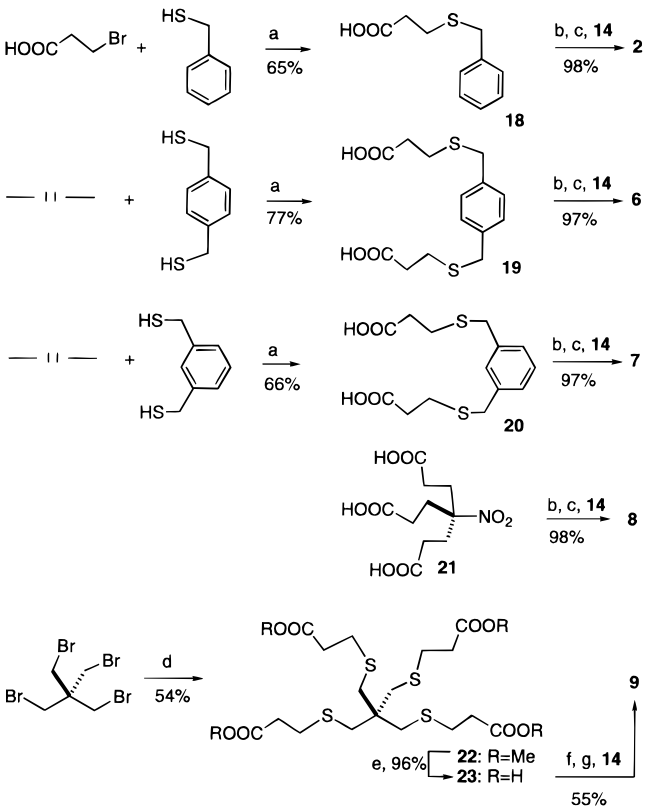
**Scheme 3**[a]




[a] Key: (a) NaH, DMF, 22 °C, 15 h; (b) NHS, EDC, DMF, 22 °C, 15−18 h; (c) pyridine, 60 °C, 8 h; (d) $Cs_2CO_3$, DMF, 22 °C, 15 h; (e) LiOH, MeOH, $H_2O$, 22 °C, 15 h; (f) $F_5C_6OH$, DIC, DMF, 0 → 22 °C, 16 h; (g) HOBt, $Et_3N$, DMF, 22 °C, 16 h.

Bacteria having galabiose-specific lectins on the surface will agglutinate erythrocytes, due to a multivalent recognition of the cell-surface sugar residues by the bacterial protein molecules. Most cells in the body carry these glycolipids. Therefore, the erythrocyte−bacteria agglutination system is useful for testing the adhesion of bacteria to cell surfaces, and inhibition *in vitro* by water-soluble saccharides can be used to probe the inhibitory efficacy and map the binding epitopes of synthetic saccharide analogs. It is important that the same batches of erythrocytes and bacteria are used in such investigations; experiments performed with different batches can give rather different absolute MIC (or $IC_{50}$) values but the relative values for a series of inhibitors are fairly constant.[20] The procedure is described in the Experimental Section, and the concentrations of the different saccharide inhibitors needed for complete inhibition of hemagglutination are given in Table 1.

The results show a clear connection between inhibitory efficacy and the number of galabiose units present on the inhibitor. We have earlier shown that the glycolipid globotriosyl ceramide is the most efficient receptor for *S. suis* on the erythrocyte surface, whereas globotetraosyl ceramide shows much weaker inhibitory acticity. The hydrogen-bonding pattern of the recognition of galabiosides by *S. suis* bacteria was determined with the help of synthetic monodeoxygalabiosides.[8] However, the protein has not yet been obtained in a form suitable for detailed complexation studies with galabiosides and it is at present not known in what type of structure the protein occurs on the bacterial surface.

The monomeric TMSEt galabioside **1**[12] has been used as a standard inhibitor in earlier investigations of bacterial adhesion

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**Table 1.** Inhibition of Agglutination of Human Erythrocytes by *S. suis* Bacteria

| inhibitor | inhibitory concentration[a] (nM) of *S. suis* (strain)[b] | |
|---|---|---|
| | type $P_N$ (628) | type $P_O$ (836) |
| **1** | 1800 | 1300 |
| **2** | 300 | 300 |
| **3** | 130 | 76 |
| **4** | 90 | 51 |
| **5** | 100 | 64 |
| **6** | 6 | 3 |
| **7** | 10 | *c* |
| **8** | 25 | 16 |
| **9** | 3 | 2 |

[a] Complete inhibition of hemagglutination. [b] Described in ref 8. [c] Not determined.

(*S. suis*,[8] *E. coli*,[7] and the *E. coli* pilus protein PapG).[7,21] Changing to an aromatic amide aglycon made the galabioside **2** more efficient than **1** by a factor of 6. The dimeric galabiosides **3**−**5** are 14−25 times more efficient than **1**. A large increase in inhibitory power was displayed by the di- and tetravalent galabiosides **6**, **7**, and **9**, being several hundred times more efficient than **1**. The trimer **8** was somewhat less efficient than **6**, **7**, and **9**. The high efficacy of **6**−**9** might be due to the rather long and flexible aromatic linkers, as compared with the shorter linkers of **3**−**5** and **8**, allowing effective adhesion to the bacterial surface proteins. However, the fine molecular details of the recognition remain to be determined.

In conclusion, we have demonstrated that very low concentrations of synthetic oligomeric saccharides of moderate molecular weight can inhibit the agglutination of human erythrocytes by *S. suis* bacteria. This is the first example of inhibition of bacterial adhesion by low nanomolar concentrations of a soluble saccharide inhibitor.

## Experimental Section

NMR spectra were recorded on 300 or 400 MHz instuments. $^1$H NMR spectral assignments were made by the double-resonance technique COSY. Concentrations were made using rotary evaporation with bath temperatures at or below 40 °C. Anhydrous $Na_2SO_4$ was used as drying agent for the organic extracts in the workup procedures. TLC was performed on Kiselgel 60 $F_{254}$ plates (Merck). Column chromatography was performed using $SiO_2$ (Matrex LC-gel 60 Å, 35−70 MY, Grace). Compounds **1**,[7] **10**,[12] and **12**[14] were synthesized as described. Compound **21** is commercially available.

**2-(5-Phenyl-4-thiapentanoylamido)ethyl 4-*O*-α-D-Galactopyranosyl-*β*-D-galactopyranoside (2).** Compound **18** (30 mg, 0.15 mmol) was dissolved in dry *N*,*N*-dimethylformamide (2 mL), and *N*-hydroxysuccinimide (NHS, 26.5 mg, 0.229 mmol) and *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC, 44 mg, 0.229 mmol) were added. The mixture was stirred at 22 °C for 18 h, and water and dichloromethane were added. The organic layer was dried and concentrated. The residue was chromatographed (EtOAc/heptane 2:1) to give the *N*-hydroxysuccinimide ester of **18** (43 mg, 98%). The ester (13 mg, 0.044 mmol) was added to a solution of **14** (20 mg, 0.052 mmol) in dry pyridine (4 mL). The mixture was stirred at 60 °C for 8 h and concentrated. The residue was chromatographed (Varian Mega Bond Elut Column C18; $H_2O$/MeOH 9:1 → 8:2 → 7:3 → 6:4 → 5:5, 5 mL each) to give **2** (24.6 mg, 98%): $[\alpha]^{22}_D$ +66 (*c* 0.7, $H_2O$); $^1$H NMR ($D_2O$) $\delta$ 7.18−7.32 (m, 5H, Ar), 4.82 (d, 1 H, $J$ = 3.7 Hz, H-1′), 4.33 (d, 1 H, $J$ = 7.7 Hz, H-1), 4.19 (t, 1 H, $J$ = 6.7 Hz, H-5′), 3.88 (d, 1 H, $J$ = 3.0 Hz, H-4), 3.85 (bd, 1 H, $J$ = 2.8 Hz, H-4′), 3.80−3.90 (m, 1 H, OC*H*$_2$CH$_2$NH), 3.53−3.80 (m, 11 H, H-3,5,6,2′,3′,6′, C*H*$_2$-CH$_2$NH-, PhCH$_2$S), 3.43 (dd, 1 H, $J$ = 7.7, 10.2 Hz, H-2), 3.20−3.39 (m, 2 H, CH$_2$N), 2.62 (t, 2 H, $J$ = 7.0 Hz, SC*H*$_2$CH$_2$CO), 2.40 (t, 2 H, $J$ = 6.8 Hz, SCH$_2$C*H*$_2$CO); $^{13}$C NMR ($D_2O$) $\delta$ 175.0, 138.8, 129.3, 129.2, 127.7, 103.4, 100.7, 77.7, 75.4, 72.7, 71.3, 71.2, 69.5, 69.3, 69.0, 68.9, 60.9, 60.5, 39.8, 35.7, 35.6, 27.1; HRMS calcd for $C_{24}H_{38}O_{12}NS$ (M + H) 564.2115, found 564.2096.

**Bisgalabioside 3.** Compound **15** (47 mg, 0.0334 mmol) was dissolved in methanol (4 mL), and the mixture was treated with a catalytic amount of sodium methoxide for 6.5 h, neutralized with Duolite C436 ($H^+$), and concentrated. The residue was chromatographed ($CH_2Cl_2$/MeOH/$H_2O$ 5:5:1) to give **3** (25.7 mg, 94%): $[\alpha]^{24}_D$ +23 (*c* 0.6, $H_2O$); $^1$H NMR ($D_2O$) $\delta$ 7.17−7.31 (m, 4 H, Ar), 4.81 (d, 2 H, $J$ = 3.9 Hz, H-1′), 4.22 (d, 2 H, $J$ = 9.1 Hz, H-1), 4.21 (bt, 2 H, $J$ = 6.8 Hz, H-5′), 4.05 (d, 2 H, $J$ = 13.5 Hz, PhC*H*$_2$S), 4.00 (d, 2 H, $J$ = 13.5 Hz, PhC*H*$_2$S), 3.92 (bd, 2 H, $J$ = 2.6 Hz, H-4), 3.89 (bd, 2 H, $J$ = 3.1 Hz, H-4′), 3.77 (dd, 2 H, $J$ = 3.2, 10.5 Hz, H-3′), 3.69 (dd, 2 H, $J$ = 3.8, 10.5 Hz, H-2′), 3.60−3.69 (m, 4 H, H-6), 3.49−3.60 (m, 6 H, H-5,6′), 3.49 (dd, 2 H, $J$ = 2.7, 9.7 Hz, H-3), 3.44 (t, 2 H, $J$ = 9.6 Hz, H-2); $^{13}$C NMR ($D_2O$) $\delta$ 136.4, 131.3, 128.2, 100.7, 85.1, 79.1, 77.8, 74.1, 71.1, 70.0, 69.5, 69.3, 69.1, 60.8, 60.3, 31.5; HRMS calcd for $C_{32}H_{50}O_{20}S_2Na$ (M + Na) 841.2235, found 841.2252.

**Bisgalabioside 4.** Compound **16** (35 mg, 0.0249 mmol) was treated as in the preparation of **3** to give **4** (20.4 mg, 92%): $[\alpha]^{26}_D$ −1 (*c* 0.5, $H_2O$); $^1$H NMR ($D_2O$) $\delta$ 7.26 (s, 4 H, Ar), 4.81 (d, 2 H, $J$ = 3.9 Hz, H-1′), 4.20 (bt, 2 H, $J$ = 6.6 Hz, H-5′), 4.16 (d, 2 H, $J$ = 9.3 Hz, H-1), 3.91 (bd, 2 H, $J$ = 2.4 Hz, H-4), 3.90 (d, 2 H, $J$ = 13.4 Hz, PhC*H*$_2$S), 3.89 (m, 2 H, H-4′), 3.79 (d, 2 H, $J$ = 13.5 Hz, PhC*H*$_2$S), 3.77 (dd, 2 H, $J$ = 3.2, 10.5 Hz, H-3′), 3.69 (dd, 2 H, $J$ = 3.9, 10.8 Hz, H-2′), 3.45−3.70 (m, 12 H, H-3,5,6,6′), 3.45 (t, 2 H, $J$ = 9.7 Hz, H-2); $^{13}$C NMR ($D_2O$) $\delta$ 137.4, 129.7, 100.7, 84.9, 79.2, 77.9, 74.1, 71.1, 70.0, 69.5, 69.3, 69.1, 60.8, 60.4, 33.8; HRMS calcd for $C_{32}H_{50}O_{20}S_2Na$ (M + Na) 841.2235, found 841.2214.

**Bisgalabioside 5.** Compound **17** (50 mg, 0.0334 mmol) was dissolved in dry methanol (4 mL), and the mixture was treated with a catalytic amount of sodium methoxide overnight, neutralized with Duolite C436 ($H^+$), and concentrated. The residue was chromatographed ($CH_2Cl_2$/MeOH/$H_2O$ 10:4:1) to give **5** (28.2 mg, 93%): $[\alpha]^{26}_D$ +145 (*c* 0.5, $H_2O$); $^1$H NMR ($D_2O$) $\delta$ 7.25 (s, 4 H, Ar), 4.84 (d, 2 H, $J$ = 3.9 Hz, H-1′), 4.26 (d, 2 H, $J$ = 7.7 Hz, H-1), 4.23 (bt, 2 H, $J$ = 6.8 Hz, H-5′), 3.90 (bd, 2 H, $J$ = 3.2 Hz, H-4), 3.89 (bd, 2 H, $J$ = 3.3 Hz, H-4′), 3.53−3.86 (m, 24 H, H-3,5,6,2′,3′,6′, OC*H*$_2$CH$_2$S, PhC*H*$_2$S), 3.41 (dd, 2 H, $J$ = 7.7, 10.2 Hz, H-2), 2.61 (dt, 2 H, $J$ = 1.3, 6.5 Hz, OCH$_2$C*H*$_2$S); $^{13}$C NMR ($D_2O$) $\delta$ 137.9, 129.7, 103.4, 100.6, 77.4, 75.4, 72.7, 71.2, 71.1, 69.5, 69.3, 69.2, 69.1, 60.9, 60.3, 35.3, 30.5; HRMS calcd for $C_{36}H_{58}O_{22}S_2Na$ (M + Na) 929.2759, found 929.2763.

**Bisgalabioside 6.** Compound **19** (10 mg, 0.0318 mmol) was dissolved in dry *N*,*N*-dimethylformamide (2 mL), and *N*-hydroxysuccinimide (11 mg, 0.096 mmol) and *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC, 18.5 mg, 0.097 mmol) were added. The mixture was stirred at 22 °C overnight, and water (10 mL) and dichloromethane (30 mL) were added. The organic layer was dried and concentrated. The residue was chromatographed (EtOAc/heptane 2:1) to give the *N*-hydroxysuccinimide ester of **19** (14.6 mg, 91%). The diester (14.6 mg, 0.0287 mmol) was added to a solution of **14** (24.3 mg, 0.0632 mmol) in dry pyridine (7 mL). The mixture was stirred at 60 °C for 8 h and concentrated. The residue was chromatographed (Varian Mega Bond Elut Column C18; $H_2O$/MeOH 9:1 → 8:2 → 7:3 → 6:4 → 5:5, 5 mL each) to give **6** (29.1 mg, 97%): $[\alpha]^{20}_D$ +67 (*c* 0.2, $H_2O$); $^1$H NMR ($D_2O$) $\delta$ 7.22 (s, 4 H, Ar), 4.82 (d, 2 H, $J$ = 3.7 Hz, H-1′), 4.33 (d, 2 H, $J$ = 7.7 Hz, H-1), 4.19 (t, 2 H, $J$ = 6.5 Hz, H-5′), 3.89 (bd, 2 H, $J$ = 3.0 Hz, H-4), 3.85 (bd, 2 H, $J$ = 3.2 Hz, H-4′), 3.82 (m, 2 H, OC*H*$_2$CH$_2$N), 3.53−3.79 (m, 22 H, H-3,5,6,2′,3′,6′, OC*H*$_2$CH$_2$N, PhC*H*$_2$S), 3.42 (dd, 2 H, $J$ = 7.7, 10.2 Hz, H-2), 3.30 (m, 4 H, OCH$_2$C*H*$_2$N), 2.60 (t, 4 H, $J$ = 6.9 Hz, SC*H*$_2$CH$_2$CO), 2.39 (t, 4 H, $J$ = 6.8 Hz, SCH$_2$C*H*$_2$CO); $^{13}$C NMR ($D_2O$) $\delta$ 174.9, 137.7, 129.6, 103.4, 100.7, 77.7, 75.4, 72.7, 71.3, 71.2, 69.5, 69.3, 69.0, 68.9, 60.9, 60.5, 39.8, 35.7, 35.2, 27.1; HRMS calcd for $C_{42}H_{69}O_{24}N_2S_2$ (M + H) 1049.3682, found 1049.3684.

**Bisgalabioside 7.** Compound **20** (15 mg, 0.048 mmol) was dissolved in dry dichloromethane (2 mL), and *N*-hydroxysuccinimide (17 mg, 0.147 mmol) and *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC, 27 mg, 0.143 mmol) were added. The mixture was stirred at 22 °C overnight under $N_2$. Water (10 mL) and dichloromethane (30 mL) were added. The organic layer was dried and concentrated. The residue was chromatographed (EtOAc/heptane 2:1) to give the *N*-hydroxysuccinimide ester of **20** (19 mg, 79%). The

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diester (8.4 mg, 0.017 mmol) was added to a solution of **14** (14 mg, 0.036 mmol) in dry pyridine (5 mL). The mixture was stirred at 60 °C for 8 h and concentrated. The residue was chromatographed (Varian Mega Bond Elut Column C18; $H_2O$/MeOH 9:1 → 8:2 → 7:3 → 6:4 → 5:5, 5 mL each) to give **7** (16.8 mg, 97%): $[\alpha]^{20}_D$ +47 (*c* 0.9, $H_2O$); $^1$H NMR ($D_2O$) $\delta$ 7.15−7.29 (m, 4 H, Ar), 4.83 (d, 2 H, *J* = 3.8 Hz, H-1′), 4.34 (d, 2 H, *J* = 7.7 Hz, H-1), 4.20 (t, 2 H, *J* = 6.4 Hz, H-5′), 3.89 (bd, 2 H, *J* = 3.0 Hz, H-4), 3.86 (bd, 2 H, *J* = 3.4 Hz, H-4′), 3.83 (dd, 2 H, *J* = 4.4, 6.5 Hz, O*CH*$_2$CH$_2$NH), 3.53−3.80 (m, 22 H, including H-2′,3′,3), 3.43 (dd, 2 H, *J* = 7.7, 10.2 Hz, H-2), 3.23−3.40 (m, 4 H, OCH$_2$*CH*$_2$NH), 2.61 (t, 4 H, *J* = 7.1 Hz, S*CH*$_2$CH$_2$CO), 2.41 (t, 4 H, *J* = 6.8 Hz, SCH$_2$*CH*$_2$CO); $^{13}$C NMR ($H_2O$) $\delta$ 174.9, 139.1, 129.6, 129.4, 128.0, 103.4, 100.6, 77.6, 75.4, 72.6, 71.3, 71.1, 69.4, 69.2, 69.0, 68.8, 60.8, 60.4, 39.7, 35.7, 35.3, 26.9; HRMS calcd for $C_{42}H_{68}O_{24}N_2S_2Na$ (M + Na) 1071.3501, found 1071.3506.

**Trisgalabioside 8.** Nitromethanetrispropionic acid (**21**) was converted to the *N*-hydroxysuccinimide triester as described.[16] The triester (10.5 mg, 0.0185 mmol) was added to a solution of **14** (22.5 mg, 0.058 mmol) in dry pyridine (6 mL). The mixture was stirred at 60 °C for 8 h and concentrated. The residue was chromatographed (Varian Mega Bond Elut Column C18; $H_2O$/MeOH 9:1 → 8:2 → 7:3 → 6:4 → 5:5, 5 mL each) to give **8** (25.0 mg, 98%): $[\alpha]^{22}_D$ +101 (*c* 0.3, $H_2O$); $^1$H NMR ($D_2O$) $\delta$ 4.83 (d, 3 H, *J* = 3.9 Hz, H-1′), 4.34 (d, 3 H, *J* = 7.7 Hz, H-1), 4.24 (t, 3 H, *J* = 6.5 Hz, H-5′), 3.91 (d, 6 H, *J* = 3.1 Hz, H-4,4′), 3.85 (m, 3 H, -OC*H*$_2$CH$_2$N), 3.78 (dd, 3 H, *J* = 3.0, 10.4 Hz, H-3′), 3.53−3.74 (m, 24 H, H-3,5,6,2′,6′, OC*H*$_2$CH$_2$N), 3.43 (dd, 3 H, *J* = 7.7, 10.2 Hz, H-2), 3.31 (m, 6 H, OCH$_2$C*H*$_2$N), 2.17 (s, 12 H, CH$_2$CH$_2$CO); $^{13}$C NMR ($D_2O$) $\delta$ 174.9, 103.4, 100.6, 93.7, 77.5, 75.5, 72.7, 71.3, 71.2, 69.5, 69.3, 69.1, 68.8, 60.9, 60.5, 39.8, 30.8, 30.4; HRMS calcd for $C_{52}H_{91}O_{38}N_4$ (M + H) 1379.5311, found 1379.5250.

**Tetrakisgalabioside 9.** Compound **23** (50 mg, 0.102 mmol) and pentafluorophenol (226 mg, 1.23 mmol) were suspended in dry *N*,*N*-dimethylformamide (10 mL), the mixture was cooled to 0 °C, and diisopropylcarbodiimide (DIC, 0.095 mL, 0.614 mmol) was added. The mixture was stirred for 1 h at 0 °C and at 22 °C overnight. Water (3 mL) was added, and the mixture was extracted with ether (100 mL). The combined organic extracts were dried and concentrated. The residue was chromatographed (EtOAc/heptane 1:8) to give the pentafluorophenyl ester of **23** (37.4 mg, 32%). The ester (12.1 mg, 0.0105 mmol) was added to a solution of **14** (43.4 mg, 0.113 mmol) in *N*,*N*-dimethylformamide (5 mL) and triethylamine (3 mL), and 1-hydroxybenzotriazole (HOBt, 15 mg, 0.111 mmol) was added. The mixture was stirred for 16 h at 22 °C and concentrated. The residue was purified by preparative TLC (Merck silica gel 60 $F_{254}$, $CH_2Cl_2$/MeOH/$H_2O$ 4:12:3) to give **9** (11.2 mg, 55%): $^1$H NMR ($D_2O$) $\delta$ 4.84 (d, 4 H, *J* = 4.0 Hz, H-1′), 4.35 (d, 4 H, *J* = 7.7 Hz, H-1), 4.24 (t, 4 H, *J* = 6.5 Hz, H-5′), 3.82−3.95 (m, 4 H, CH$_2$C*H*$_2$O), 3.91 (d, 8 H, *J* = 3.1 Hz, H-4,4′), 3.55−3.82 (m, 36 H, H-3,2′,3′,6′, CH$_2$C*H*$_2$O), 3.44 (dd, 4 H, *J* = 7.7, 10.1 Hz, H-2), 3.25−3.42 (m, 8 H, CH$_2$N), 2.76 (t, 8 H, *J* = 6.7 Hz, S*CH*$_2$CH$_2$CO), 2.63 (s, 8 H, CCH$_2$S), 2.48 (t, 8 H, *J* = 6.6 Hz, SCH$_2$C*H*$_2$CO); $^{13}$C NMR ($D_2O$) $\delta$ 174.9, 103.5, 100.6, 77.6, 75.5, 72.7, 71.3, 71.2, 69.5, 69.4, 69.1, 68.9, 60.9, 60.5, 43.8, 39.8, 38.1, 36.3, 29.2; HRMS calcd for $C_{73}H_{128}O_{48}N_4S_4Na$ (M + Na) 1979.6479, found 1979.6489.

**2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranosyl Trichloroacetimidate (11).** 2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-*β*-D-galactopyranoside **10**[12] (1.06 g, 1.44 mmol) was dissolved in dry dichloromethane (7.5 mL) at 22 °C under Ar. Trifluoroacetic acid (15 mL) was added,[12] and the mixture was stirred at 22 °C. After 30 min, *n*-propyl acetate and toluene were added and the mixture was concentrated and co-concentrated with toluene to give the corresponding hemiacetal (920 mg). The crude hemiacetal was dissolved in dry dichloromethane (22 mL), and trichloroacetonitrile (5.5 mL, 54.6 mmol) was added. The mixture was cooled to 0 °C under Ar. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 0.33 mL, 2.22 mmol) was added. After 50 min of stirring at 0 °C, the mixture was washed with ice cold saturated aqueous $NaHCO_3$ (20 mL), dried, and concentrated. The residue was chromatographed (heptane/EtOAc 1:1) to give **11** (1030 mg, 92%): $[\alpha]^{24}_D$ +144 (*c* 1.0, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 8.68 (s, 1 H, NH), 6.60 (d, 1 H, *J* = 3.7 Hz, H-1), 5.57 (dd, 1 H, *J* = 1.3, 3.2 Hz, H-4′), 5.42 (dd, 1 H, *J* = 3.6, 12.5 Hz, H-3′), 5.38 (dd, 1 H, *J* = 3.6, 12.5 Hz, H-2′), 5.30 (dd, 1 H, *J* = 2.7, 11.2 Hz, H-3), 5.24 (dd, 1 H, *J* = 3.6, 11.1 Hz, H-2), 5.02 (d, 1 H, *J* = 3.6 Hz, H-1′), 4.53 (bt, 1 H, *J* = 6.6 Hz, H-5 or 5′), 4.27−4.38 (m, 3 H), 4.06−4.18 (m, 3 H), 2.14, 2.12, 2.04, 2.03, 2.00 (5 s, 21 H, Ac); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.5, 170.3, 170.1, 169.9, 169.7, 160.7, 99.0, 93.5, 90.8, 70.6, 69.4, 68.1, 67.7, 67.2, 67.1, 66.7, 61.8, 60.7, 21.0, 20.7, 20.6, 20.5; HRMS calcd for $C_{28}H_{36}O_{18}NCl_3Na$ (M + Na) 802.0896, found 802.0895.

**2-Azidoethyl 2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-*β*-D-galactopyranoside (13).** To a mixture of **12**[14] (495 mg, 0.666 mmol), dry *N*,*N*-dimethylformamide (10 mL), and 15-crown-5 (0.132 mL, 0.667 mmol) was added sodium azide (130 mg, 2.00 mmol). The mixture was stirred at 75 °C for 24 h. Water (20 mL) and toluene (60 mL) were added, and the organic layer was dried and concentrated. The residue was chromatographed (EtOAc/heptane 3:1) to give **13** (454 mg, 97%): $[\alpha]^{23}_D$ +68 (*c* 0.6, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 5.58 (dd, 1 H, *J* = 1.2, 3.3 Hz, H-4′), 5.41 (dd, 1 H, *J* = 3.3, 11.0 Hz, H-3′), 5.24 (dd, 1 H, *J* = 7.7, 10.8 Hz, H-2), 5.21 (dd 1 H, *J* = 3.6, 11.0 Hz, Hz, H-2′), 5.01 (d, 1 H, *J* = 3.6 Hz, H-1′), 4.82 (dd, 1 H, *J* = 2.8, 10.8 Hz, H-3), 4.58 (d, 1 H, *J* = 7.8 Hz, H-1), 4.54 (ddd, 1 H, *J* = 1.5, 5.7, 8.4 Hz, H-5′), 4.46 (dd, 1 H, *J* = 6.7, 11.2 Hz, H-6), 4.04−4.22 (m, 5 H, OC*H*$_2$CH$_2$N$_3$, H-4,6,6′), 3.82 (bt, 1 H, *J* = 6.6 Hz, H-5), 3.71 (ddd, 1 H, *J* = 3.3, 8.7, 10.7 Hz, OC*H*$_2$CH$_2$N$_3$), 3.55 (ddd, 1 H, *J* = 3.4, 8.6, 13.3 Hz, OCH$_2$C*H*$_2$N$_3$), 3.29 (ddd, 1 H, *J* = 3.3, 4.4, 13.4 Hz, OCH$_2$C*H*$_2$N$_3$), 2.14, 2.11, 2.09, 2.08, 2.06, 2.05, 2.00 (7 s, 3 H each, Ac); $^{13}$C NMR ($CDCl_3$) $\delta$ 171.1, 171.0, 170.88, 170.86, 170.5, 170.2, 169.6, 101.3, 99.9, 77.5, 73.1, 72.4, 69.0, 68.8, 68.6, 68.3, 67.8, 67.5, 62.4, 60.9, 50.9, 21.4, 21.2, 21.1, 21.05; HRMS calcd for $C_{28}H_{39}O_{18}N_3Na$ (M + Na) 728.2126, found 728.2128.

**2-Aminoethyl 4-*O*-(α-D-galactopyranosyl)-*β*-D-galactopyranoside (14).** Compound **13** (86 mg, 0.122 mmol) was treated with methanol (1 mL) and a catalytic amount of sodium methoxide, and the mixture was neutralized with Duolite C436 ($H^+$) and concentrated. The residue was dissolved in a mixture of ethanol (3 mL) and aqueous hydrochloric acid (1.22 mL, 0.1 M) and hydrogenated ($H_2$, 10% Pd/C, 1 atm) for 2 h. The mixture was filtered through Celite and concentrated. The residue was dissolved in water and passsed through a column of Duolite A147 ($OH^-$) and concentrated. The residue was chromatographed (Varian Mega Bond Elut Column C18; $H_2O$/MeOH 9:1 → 8:2 → 7:3 → 6:4 → 5:5, 5 mL each) to give **14** (38.6 mg, 82%): $[\alpha]^{22}_D$ + 104 (*c* 0.5, $H_2O$); $^1$H NMR ($D_2O$) $\delta$ 4.79 (d, 1 H, *J* = 3.8 Hz, H-1′), 4.26 (d, 1 H, *J* = 7.7 Hz, H-1), 4.16 (bt, *J* = 6.3 Hz, H-5′), 3.86 (bs, 2 H, H-4,4′), 3.61−3.90 (m, 5 H, H-5,6,2′,3′, OC*H*$_2$CH$_2$), 3.48−3.60 (m, H-3,6,6′, OC*H*$_2$CH$_2$), 3.38 (dd, 1 H, *J* = 7.7, 10.2 Hz, H-2), 2.65 (m, 2 H, CH$_2$C*H*$_2$N), 1.75 (s, partly exchanged, NH$_2$); $^{13}$C NMR ($D_2O$) $\delta$ 103.9, 101.2, 78.0, 75.6, 73.5, 72.1, 71.7, 71.6, 70.1, 69.8, 69.4, 61.2, 60.4, 40.7; HRMS calcd for $C_{14}H_{28}O_{11}N$ (M + H) 386.1662, found 386.1670.

A sample of **14** was acetylated: $^1$H NMR ($CDCl_3$) $\delta$ 6.03 (bs, 1 H, NH), 5.58 (dd, 1 H, *J* = 1.3, 3.3 Hz, H-4′), 5.41 (dd, 1 H, *J* = 3.3, 11.1 Hz, H-3′), 5.22 (dd, 1 H, *J* = 3.6, 11.0 Hz, H-2′), 5.20 (dd, 1 H, *J* = 7.7, 10.8 Hz, H-2), 5.01 (d, 1 H, *J* = 3.7 Hz, H-1′), 4.82 (dd, 1 H, *J* = 2.7, 10.8 Hz, H-3), 4.53 (bt, 1 H, *J* = 7.6 Hz, H-5′), 4.49 (d, 1 H, *J* = 7.8 Hz, H-1), 4.45 (dd, 1 H, *J* = 7.0, 11.2 Hz, H-6), 4.10−4.21 (m, 3 H, H-6,6′), 4.09 (bd, 1 H, *J* = 2.9 Hz, H-4), 3.84−3.91 (m, 1 H, OC*H*$_2$CH$_2$), 3.81 (bt, 1 H, *J* = 6.7 Hz, H-5), 3.66−3.75 (m, 1 H, OC*H*$_2$-CH$_2$), 3.49 (m, 2 H, OCH$_2$C*H*$_2$N), 2.15, 2.12, 2.10, 2.09, 2.08, 2.04, 2.02, 2.01 (8 s, 3 H each, Ac); HRMS calcd for $C_{30}H_{43}O_{19}NNa$ (M + Na) 744.2327, found 744.2321.

**Acetylated Bisgalabioside 15.** To a solution of **11** (130 mg, 0.167 mmol) and 1,3-benzenedimethanethiol (11.3 mg, 0.067 mmol) in dichloromethane (2 mL) was added boron trifluoride etherate (0.017 mL, 0.135 mmol) under Ar. After 2 h at 22 °C, saturated aqueous sodium hydrogen carbonate (10 mL) and dichloromethane (20 mL) were added. The organic layer was dried and concentrated. The residue was chromatographed (EtOAc/heptane 2:1) to give **15** (60.9 mg, 65%): $[\alpha]^{24}_D$ +29 (*c* 0.5, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.2−7.3 (m, 4 H, Ar), 5.56 (bd, 2 H, *J* = 3.4 Hz, H-4′), 5.36 (dd, 2 H, *J* = 3.4, 11.1 Hz, H-3′), 5.22 (t, 2 H, *J* = 10.0 Hz, H-2), 5.19 (dd, 2 H, *J* = 3.9, 11.0 Hz, H-2′), 5.00 (d, 2 H, *J* = 3.6 Hz, H-1′), 4.78 (dd, 2 H, *J* = 2.7, 10.3 Hz, H-3), 4.40−4.51 (m, 4 H), 4.28 (d, 2 H, *J* = 9.8 Hz, H-1), 4.00−4.19 (m, 12 H), 3.72 (bt, 2 H, *J* = 6.5 Hz, H-5), 2.13, 2.12, 2.11, 2.05,

2.02, 1.98, 1.978 (7 s, 6 H each, Ac); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.7, 170.6, 170.5, 170.4, 170.1, 169.8, 169.2, 135.4, 131.1, 127.7, 99.3, 82.5, 77.2, 75.8, 73.8, 68.5, 67.8, 67.3, 67.1, 62.4, 60.6, 31.2, 20.92, 20.86, 20.8, 20.7; HRMS calcd for $C_{60}H_{78}O_{34}S_2Na$ (M + Na) 1429.3714, found 1429.3711.

**Acetylated Bisgalabioside 16.** To a solution of **11** (258 mg, 0.33 mmol) and 1,4-benzenedimethanethiol (20.1 mg, 0.118 mmol) in dichloromethane (2 mL) was added boron trifluoride etherate (0.031 mL, 0.247 mmol) under Ar. After 30 min at 22 °C, saturated aqueous sodium hydrogen carbonate (10 mL) and dichloromethane (20 mL) were added. The organic layer was dried and concentrated. The residue was chromatographed (EtOAc/heptane 2:1) to give **16** (104.2 mg, 63%): $[\alpha]^{24}_D$ +38 (*c* 0.9, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.23 (s, 4 H, Ar), 5.53 (dd, 2 H, $J$ = 0.9, 3.2 Hz, H-4′), 5.35 (dd, 2 H, $J$ = 3.2, 11.0 Hz, H-3′), 5.25 (t, 2 H, $J$ = 10.2 Hz, H-2), 5.17 (dd, 2 H, $J$ = 3.6, 11.1 Hz, H-2′), 4.99 (d, 2 H, $J$ = 3.5 Hz, H-1′), 4.79 (dd, 2 H, $J$ = 2.7, 10.3 Hz, H-3), 4.38−4.50 (m, 4 H), 4.28 (d, 2 H, $J$ = 10.0 Hz, H-1), 4.01−4.17 (m, 10 H), 3.94 (d, 2 H, $J$ = 12.7 Hz, PhC$H_2$S), 3.83 (d, 2 H, $J$ = 12.7 Hz, PhC$H_2$S), 3.72 (t, 2 H, $J$ = 6.4 Hz, H-5), 2.10, 2.08, 2.07, 2.03, 2.00, 1.99, 1.95 (7 s, 6 H each, Ac); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.6, 170.5, 170.4, 170.1, 169.8, 169.2, 136.0, 129.3, 99.1, 82.3, 77.1, 75.9, 73.7, 68.5, 67.8, 67.3, 67.1, 67.06, 62.3, 60.6, 33.1, 20.9, 20.8, 20.7, 20.6; HRMS calcd for $C_{60}H_{78}O_{34}S_2Na$ (M + Na) 1429.3714, found 1429.3726.

**Acetylated Bisgalabioside 17.** To a solution of 1,4-benzenedimethanethiol (10 mg, 0.058 mmol) and **12**[14] (105 mg, 0.141 mmol) in dry *N*,*N*-dimethylformamide (3 mL) was added cesium carbonate (50 mg, 0.153 mmol), and the resulting mixture was stirred at 22 °C overnight. Water (10 mL) and dichloromethane (30 mL) were added. The organic layer was dried and concentrated. The residue was chromatographed (EtOAc/heptane 2:1) to give **17** (49 mg, 55%): $[\alpha]^{23}_D$ +65 (*c* 0.6, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.28 (s, 4 H, Ar), 5.57 (dd, 2 H, $J$ = 1.1, 3.3 Hz, H-4′), 5.39 (dd, 2 H, $J$ = 3.3, 11.0 Hz, H-3′), 5.21 (dd, 2 H, $J$ = 3.6, 11.2 Hz, H-2′), 5.19 (dd, 2 H, $J$ = 7.6, 11.0 Hz, H-2), 5.01 (d, 2 H, $J$ = 3.7 Hz, H-1′), 4.82 (dd, 2 H, $J$ = 2.8, 10.8 Hz, H-3), 4.53 (bt, 2 H, $J$ = 6.7 Hz, H-5′), 4.48 (d, 2 H, $J$ = 7.7 Hz, H-1), 4.45 (dd, 2 H, $J$ = 6.7, 11.1 Hz, H-6), 4.08−4.21 (m, 6 H, H-6,6′), 4.07 (bd, 2 H, $J$ = 2.0 Hz, H-4), 3.96−4.05 (m, 2 H, OC$H_2$CH$_2$S), 3.79 (t, 2 H, $J$ = 6.5 Hz, H-5), 3.75 (s, 4 H, PhC$H_2$S), 3.59−3.69 (m, 2 H, OC$H_2$CH$_2$S), 2.60−2.73 (m, 4 H, OCH$_2$C$H_2$S), 2.14, 2.11, 2.09, 2.08, 2.05, 2.04, 1.99 (7 s, 6 H each, Ac); $^{13}$C NMR ($CDCl_3$) $\delta$ 171.2, 171.0, 170.91, 170.87, 170.6, 170.2, 169.6, 137.6, 129.5, 101.6, 99.8, 77.7, 73.1, 72.4, 69.7, 69.0, 68.3, 67.8, 67.5, 62.4, 60.9, 36.7, 31.2, 21.4, 21.22, 21.21, 21.16, 21.11, 21.08; HRMS calcd for $C_{64}H_{86}O_{36}S_2$-Na (M + Na) 1517.4238, found 1517.4249.

**(4-Carboxy-2-thiabutyl)benzene (18).** Benzenemethanethiol (0.050 mL, 0.42 mmol) was dissolved in dry *N*,*N*-dimethylformamide (4 mL), and sodium hydride (89 mg, 80% in oil, 2.97 mmol) and 3-bromopropionic acid (195 mg, 1.27 mmol) were added. The mixture was stirred at 22 °C under $N_2$ overnight. Water, aqueous ammonium chloride (10%), acetic acid, and toluene were added. The organic layer was dried and concentrated. The residue was chromatographed (EtOAc/heptane/AcOH 3:12:1) to give **18** (54 mg, 65%): $^1$H NMR ($CDCl_3$/$CD_3OD$ 1:1) $\delta$ 5.85−5.99 (m, 5 H, Ar), 2.39 (s, 2 H, PhC$H_2$S), 1.31 (bt, 2 H, SC$H_2$CH$_2$CO), 1.18 (bt, 2 H, SCH$_2$C$H_2$CO); $^{13}$C NMR ($CDCl_3$/$CD_3OD$ 1:1) $\delta$ 173.5, 137.2, 127.8, 127.4, 126.0, 35.0, 33.3, 25.1; HRMS calcd for $C_{10}H_{11}O_2SNa_2$ (M − H + 2Na) 241.0275, found 241.0263.

**Bis(4-carboxy-2-thiabutyl)-1,4-benzene (19).** To a solution of 1,4-benzenedimethanethiol (50 mg, 0.294 mmol) and 3-bromopropionic acid (269.5 mg, 1.76 mmol) in dry *N*,*N*-dimethylformamide (4 mL) was added sodium hydride (110 mg, 80% in oil, 3.66 mmol). The mixture was stirred overnight at 22 °C under Ar. Methanol (1 mL), acetic acid (5 mL), and toluene (60 mL) were added. The organic layer was dried and concentrated. The residue was chromatographed (EtOAc/heptane/AcOH 2:6:1) to give **19** (70.7 mg, 77%): $^1$H NMR ($CDCl_3$/$CD_3OD$ 1:1) $\delta$ 7.25 (s, 4 H, Ar), 3.70 (s, 4 H, ArCH$_2$S), 2.64 (bt, 4 H, SC$H_2$CH$_2$CO), 2.50 (bt, 4 H, SCH$_2$C$H_2$CO); $^{13}$C NMR ($CDCl_3$/$CD_3OD$ 1:1) $\delta$ 174.9, 137.4, 129.3, 36.0, 34.6, 26.5; HRMS calcd for $C_{14}H_{18}O_4S_2Na$ (M + Na) 337.0544, found 337.0545.

**Bis(4-carboxy-2-thiabutyl)-1,3-benzene (20).** To a solution of 1,3-benzenedimethanethiol (50 mg, 0.294 mmol) in dry *N*,*N*-dimethylformamide (4 mL) was added sodium hydride (106 mg, 80% in oil, 3.53 mmol), followed by 3-bromopropionic acid (270 mg, 1.76 mmol). The mixture was stirred overnight at 22 °C under $N_2$. Aqueous ammonium chloride (10%, 4 mL), acetic acid (3 mL), and toluene (50 mL) were added. The organic layer was dried and concentrated. The residue was chromatographed (EtOAc/heptane/AcOH 2:6:1) to give **20** (60.5 mg, 66%): $^1$H NMR ($CDCl_3$) $\delta$ 7.18−7.32 (m, 4 H, Ar), 3.73 (s, 4 H, ArCH$_2$S), 2.67 (bt, 4 H, $J$ = 6.6 Hz, SC$H_2$CH$_2$CO), 2.58 (bt, 4 H, $J$ = 6.6 Hz, SCH$_2$C$H_2$CO); $^{13}$C NMR ($CDCl_3$) $\delta$ 178.6, 138.8, 129.8, 129.4, 128.1, 36.6, 34.8, 26.2; HRMS calcd for $C_{14}H_{18}O_4S_2Na$ (M + Na) 337.0544, found 337.0536.

**Tetra(4-methoxycarbonyl-2-thiabutyl)methane (22).** Tetra(bromomethyl)methane (100 mg, 0.258 mmol) and methyl 3-mercaptopropionate (0.335 mL, 3.09 mmol) were dissolved in dry *N*,*N*-dimethylformamide (4 mL), and cesium carbonate (840 mg, 2.58 mmol) was added. The mixture was stirred at 22 °C overnight. Water (5 mL) and dichloromethane (60 mL) were added, and the organic layer was dried and concentrated. The residue was chromatographed (EtOAc/heptane 2:1) to give **22** (76.5 mg, 54%): $^1$H NMR ($CDCl_3$) $\delta$ 3.69 (s, 12 H, OMe), 2.83 (t, 8 H, $J$ = 7.3 Hz, SC$H_2$CH$_2$), 2.71 (s, 8 H, CCH$_2$S), 2.63 (t, 8 H, $J$ = 7.3 Hz, CH$_2$C$H_2$CO); $^{13}$C NMR ($CDCl_3$): $\delta$ 172.6, 52.2, 44.4, 38.7, 35.2, 29.0; HRMS calcd for $C_{21}H_{37}O_8S_4$ (M + H) 545.1371, found 545.1368.

**Tetra(4-carboxy-2-thiabutyl)methane (23).** Compound **22** (49 mg, 0.09 mmol) was dissolved in methanol/water (4 mL, 6:1), and lithium hydroxide (25.5 mg, 1.06 mmol) was added. The mixture was stirred at 22 °C overnight, filtered, and concentrated. The residue was chromatographed (EtOAc/heptane/AcOH 1:3:1) to give **23** (42.2 mg, 96%): $^1$H NMR ($CD_3OD$/$D_2O$ 6:1) $\delta$ 2.83 (t, 8 H, $J$ = 7.0 Hz, SC$H_2$-CH$_2$CO), 2.74 (s, 8 H, CCH$_2$S), 2.61 (t, 8 H, $J$ = 7.0 Hz, CH$_2$C$H_2$-CO); $^{13}$C NMR ($CD_3OD$/$D_2O$ 6:1) $\delta$ 177.7, 46.8, 40.7, 37.6, 31.2; HRMS calcd for $C_{17}H_{28}O_8S_4Na$ (M + Na) 511.0565, found 511.0574.

**Inhibition of Hemagglutination.** The experiments were performed as described earlier.[8] In short, 50 $\mu$L of sialidase-treated human erythrocytes (5% hematocrit) were mixed with 25 $\mu$L of a suspension of *S. suis* bacteria ($\sim 10^8$ cfu/mL), preincubated with 25 $\mu$L of the serially diluted saccharide derivatives **1**−**9**, and incubated on ice for 2 h. The lowest concentrations giving complete inhibition of the hemagglutination were recorded (Table 1).

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