Inhibition of *Streptococcus suis* Adhesion by Dendritic Galabiose Compounds at Low Nanomolar Concentration

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A series of mono-, di-, and tetravalent galabiose (Galα1−4Gal) compounds were synthesized in good yields by coupling of a general carboxylic acid-bearing sugar building block to dendritic scaffolds based on the 3,5-di-(2-aminoethoxy)benzoic acid branching unit. Furthermore, a poly(amidoamine)- (PAMAM-) based dendritic galabioside was synthesized containing eight galabiose units. All galabiosides were tested in a hemagglutination assay and a surface plasmon resonance (SPR) competition assay in order to establish their potency in the binding to the bacterial Gram-positive pathogen *Streptococcus suis*. A monovalent galabioside containing a short spacer was used as a reference compound in all the assays. Variations in the scaffold as well as in the spacer arms were introduced to determine their influence on the inhibition. The best inhibitor of hemagglutination was an octavalent galabioside with a minimal inhibitory concentration (MIC) of 0.3 nM, to the best of our knowledge the first example of inhibition of bacterial binding by a soluble carbohydrate at a subnanomolar concentration.

Introduction

Bacterial resistance toward traditional antibiotics is still increasing at an alarming rate.[1] For this reason the development of new and effective preventive methods of bacterial infections is a major priority. The first step toward an infection is the adherence of the bacterial pathogens to the epithelial surfaces of host tissues. Many of these bacterial pathogens adhere to the host via protein−carbohydrate interactions.[2] Inhibitors of the binding of bacterial lectin-like receptors (adhesins) to oligosaccharides present on the cell surface are potential novel antibacterial agents.[3,4] It is likely that, because the bacteria are not killed, bacterial resistance toward these inhibitors will evolve more slowly. Moreover, resistant mutants are likely to be less infective since they have also lost their ability to bind to the tissue cells.

The adhesin−carbohydrate interaction is normally rather weak (*milli*molar concentration),[3] but this is amplified by the fact that both binding partners are present in multivalent arrays.[5] Several groups have shown that, with synthetic multivalent inhibitors, these weak interactions can also be amplified, which has already resulted in high-affinity inhibitors, especially for AB_5 toxins.[6] In the area of bacterial anti-adhesion, relatively few studies using multivalent inhibitor design have been reported to date. Most of them used type I fimbriated *E. coli* as their target.[7] In a recent study, good inhibition was observed. It was found that a lipophilic attachment to mannose ligands already led to a 135-fold enhancement of the inhibitory power over α-D-mannopyranoside. However, multivalency by attaching these spacered mannose moieties to proteins or dendrimers only led to additional enhancements of up to 1 order of magnitude, when compared on a per sugar basis.[8] Autar et al.[9] previously reported studies involving multivalent GalNAcβ1−4Gal-containing compounds targeting the pathogens F1C fimbriated *E. coli* and *Pseudomonas aeruginosa*. As in the case of *E. coli*, spacer effects (3-fold) combined with a modest multivalency effect (6.3-fold) led to a maximum overall enhancement of 19-fold per sugar for a divalent system. Furthermore, with *Streptococcus suis*, Hansen et al.[10] observed a relative increase in inhibitory potency of 50-fold per carbohydrate by using small di-, tri-, and tetravalent galabiose-containing molecules in hemagglutination inhibition experiments. The Gram-positive bacterium *S. suis* can cause meningitis, septicemia, and pneumonia in pigs and also meningitis in humans.[11,12] *S. suis* recognizes the galabiose epitope (Galα1−4Gal) on the terminal and internal positions of various glycolipids, wherein the disaccharide acts as the ligand for pathogen adhesion to cells, as the first step of an infective process. Other pathogenic microbes that bind to the galabiose epitope are the uropathogenic *E. coli* via its PapG adhesin[13] and the bacterium *Pseudomonas aeruginosa* via its PA-IL lectin.[14] Furthermore, glycolipids bearing the galabiose epitope can bind to bacterial enterotoxins, for example, the Shiga-like toxins (SLTs)[15] and verotoxin from *E. coli*.[16] For *S. suis*, extensive specificity studies have been performed which showed that galabiose is the best disaccharide ligand.[17] There are two galabiose-binding subtypes of *S. suis* designated as P_N and P_O, based on the nature of their adhesin specificity.[18] Galabiose was shown to form hydrogen bonds to both adhesins via its HO-2, HO-3, HO-4′, and

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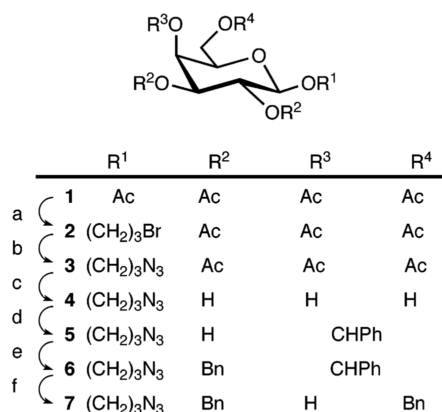
Scheme 1. Synthesis of Galactose Acceptor **7**[a]



	R1	R2	R3	R4
1	Ac	Ac	Ac	Ac
2	(CH2)3Br	Ac	Ac	Ac
3	(CH2)3N3	Ac	Ac	Ac
4	(CH2)3N3	H	H	H
5	(CH2)3N3	H	CHPh	
6	(CH2)3N3	Bn	CHPh	
7	(CH2)3N3	Bn	H	Bn

[a] (a) 3-Bromopropanol, 0 °C, BF_3 Et_2O, 84%; (b) NaN_3, DMF, 100 °C, 87%; (c) NaOMe, MeOH, CH_2Cl_2, 97%; (d) α,α-dimethoxytoluene, *p*-TsOH, DMF, 50 °C, 80%; (e) NaH, TBAI, BnBr, DMF, 75%; (f) TES, TFA, CH_2Cl_2, 0 °C, 76%.

HO-6′, while for the P_O subtype additional weak hydrogen-bonding interactions were detected to HO-6 and HO-3′.[18,19]

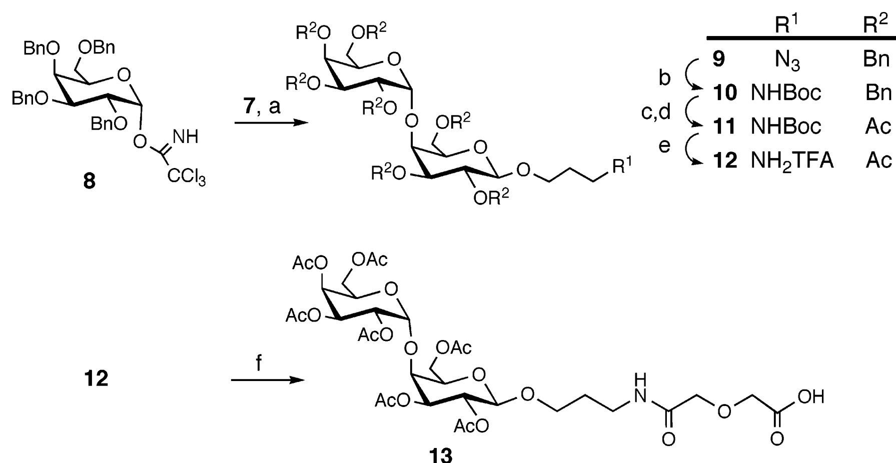
Though the specificities of the carbohydrate binding proteins on bacterial surface are characterized in detail, their relative geometry, in contrast to the structurally well-characterized AB_5 toxins,[20] is mostly unknown. To succeed in inhibitor design, it is likely important to make a structurally diverse set of inhibitors. We here report the synthesis of a diverse set of multivalent galabiose derivatives and their evaluation as inhibitors of *S. suis*. The length and chemical composition of the spacer arms, as well as the nature of the scaffold molecules themselves, were aspects of structural variation. The spacer arms for the compounds described here were considerably longer than those previously reported, since the longer spacers were shown to be more effective.[10] Furthermore studies on AB_5 toxin inhibition reported longer spacers to be generally advantageous.[20] The compounds were evaluated by a hemagglutination assay and a surface plasmon resonance competition assay.

Results and Discussion

Synthesis. All multivalent galabiose derivatives and monovalent reference compounds were synthesized by attachment of the carboxylic acid-containing galabioside **13** (Scheme 2). Compound **13** was synthesized by coupling of 3-azidopropyl 2,3,6-tri-*O*-benzyl-*β*-D-galactopyranoside acceptor **7** (Scheme 1) with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside trichloroacetimidate donor **8** (Scheme 2).[21,22] In our strategy, benzyl protective groups were used to "arm" the O4 position of the galactose acceptor and to favor the α-glycosylated disaccharide. Key building block **13** was coupled to the amine-bearing scaffolds by peptide coupling methods to give the target compounds after sugar deprotection.

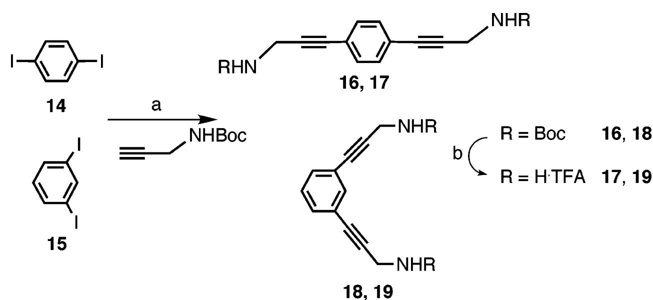
The first target in the synthesis was the benzyl-protected acceptor **7**, which contains a free HO-4 for the glycosidic coupling and an anomeric propyl azide group for further elaboration. As a first step in the synthesis of **7**, 3-bromopropanol was coupled to the anomeric center of 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactose **1**, in the presence of boron trifluoride ethyl etherate ($BF_3{\cdot}Et_2O$) as a catalyst, to give **2** in 84% yield. An azido function was introduced via S_N2 displacement of the bromine, using sodium azide in *N*,*N*-dimethylformamide (DMF). Deacetylation of **3** by use of sodium methoxide in MeOH, followed by benzylidenation with α,α-dimethoxytoluene and *p*-toluenesulfonic acid in DMF, gave **5** in 80% yield. Benzylation of the two remaining free hydroxyl functions of **5** by use of benzylbromide, sodium hydride, and tetrabutylammonium iodide (TBAI) in DMF yielded **6** in 75% yield. Selective opening of the benzylidene ring to the O6 position by use of triethylsilane (TES) and trifluoroacetic acid (TFA)[23] gave the desired acceptor **7** in 76% yield. This reaction was also performed with sodium cyanoborohydride and HCl, which gave slightly lower yields. Coupling of the known "armed" imidate donor **8**[21,22] to acceptor **7** in Et_2O and 15% trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst gave disaccharide **9** in 73% yield (Scheme 2). Due to the solvent effect of Et_2O[21,24] and the low temperature, no *β*-linked disaccharide was isolated. To facilitate deprotection at the final multivalent stage, the benzyl protecting groups were replaced by acetyl groups. Selective reduction of the azido function, by Pd−$CaCO_3$ (Lindlar catalyst) and H_2, followed by the introduction of the *N*-*tert*-butyloxycarbonyl (Boc) protecting group on the free amino group, gave **10** in 92% yield.[25] Debenzylation of **10** with 10% Pd−C and H_2 and subsequent acetyla-

Scheme 2. Synthesis of Galabiose Building block **13**[a]



[a] (a) 15% TMSOTf, Et_2O, 0 °C, 73%; (b) Lindlar catalyst, H_2, Boc_2O, NEt_3, MeOH, EtOAc, 92%; (c) Pd−C, HOAc, H_2, MeOH, EtOAc; (d) pyridine, Ac_2O, 88% over two steps; (e) TFA, H_2O, CH_2Cl_2, 0 °C, quant; (f) diglycolic anhydride, pyridine, dioxane, 90 °C, quant.

Scheme 3. Synthesis of Rigid Divalent Scaffolds[a]



[a] (a) $Pd^{(0)}(PPh_3)_4$, CuI, Et_3N, CH_3CN, 76% (**16**) or 87% (**18**); (b) TFA, CH_2Cl_2, quant (**18**) or quant (**19**).

tion by a 1:1 mixture of pyridine and acetic anhydride gave **11** (88% yield over two steps). Removal of the Boc-protecting group of **11** with trifluoroacetic acid gave compound **12**, which was converted to the key building block **13** by use of diglycolic anhydride in pyridine and dioxane at 90 °C (quantitative over two steps).[9]

To increase the hydrophobicity and rigidity of the spacer arms, in addition to the 3,5-di-(2-aminoethoxy)-benzoic acid-based compounds, two scaffolds **17** and **19** were prepared that included an acetylene moiety (Scheme 3). To fix distinct geometries, 1,3- and 1,4-disubstituted benzene rings were used as scaffold elements. Boc-protected propargylamine was coupled to two commercially available scaffolds, 1,4-diiodobenzene **14** and 1,3-diiodobenzene **15**, in good yields by a Sonogashira coupling with $Pd^0(PPh_3)_4$, CuI, and Et_3N in acetonitrile. During the purification of **16**, the monosubstituted compound was also isolated as a minor side product (16% yield). Removal of the Boc-protecting groups of compounds **16** and **18**, with TFA in CH_2Cl_2, yielded acetylene-containing scaffolds **17** and **19**, both in quantitative yield.

Galabiose derivative **13** was attached to mono-, di-, and tetravalent dendritic scaffolds based on the 3,5-di-(2-aminoethoxy)benzoic acid branching unit.[26,27] These dendrimers have been used as scaffolds for the synthesis of multivalent carbohydrate compounds.[9,28] A general coupling and deprotection route was used to produce mono-, di-, and tetravalent galabiosides **22**, **25**, **32**, and **35** (Scheme 4). Compound **13** was coupled in good yields to the respective (poly)amino compounds by use of BOP [(Benzotriazolo-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate] as a coupling reagent and DIPEA (*N*,*N*-diisopropylethylamine) as a base. Finally, deacetylation with sodium methoxide in MeOH and purification on a C8-cartridge column gave target compounds **22** (80%), **25** (99%), **32** (77%), and **35** (79%). Characterization by 1D and 2D NMR spectroscopy and high-resolution mass spectrometry (HRMS) confirmed the identity of the expected products. By the same protocol, the acetylenic scaffolds **17** and **19** were converted to their corresponding divalent galabiosides **27** and **29**. According to their ^{13}C NMR spectra, the acetylene carbon signals were still present (82 and 86 ppm), indicating that no cyclization to heterocyclic structures took place.[29,30] Poly(amidoamine) (PAMAM) dendrimer scaffolds[31] have extensively been used in the synthesis of dendritic carbohydrate compounds.[32] Galabioside **13** was attached to the commercially available PAMAM dendrimer scaffold **36** containing eight end groups by use of the TBTU coupling reagent [*O*-(benzotriazol-1-yloxy)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate] and DIPEA in dimethyl sulfoxide (DMSO).[33] Compound **37** was purified on an LH20 column with CH_2Cl_2−MeOH (1:1) as the eluent. No free amino functions could be detected by the ninhydrin coloring reagent. Deacetylation by sodium methoxide in MeOH gave target compound **38** (89%) isolated after freeze-drying.

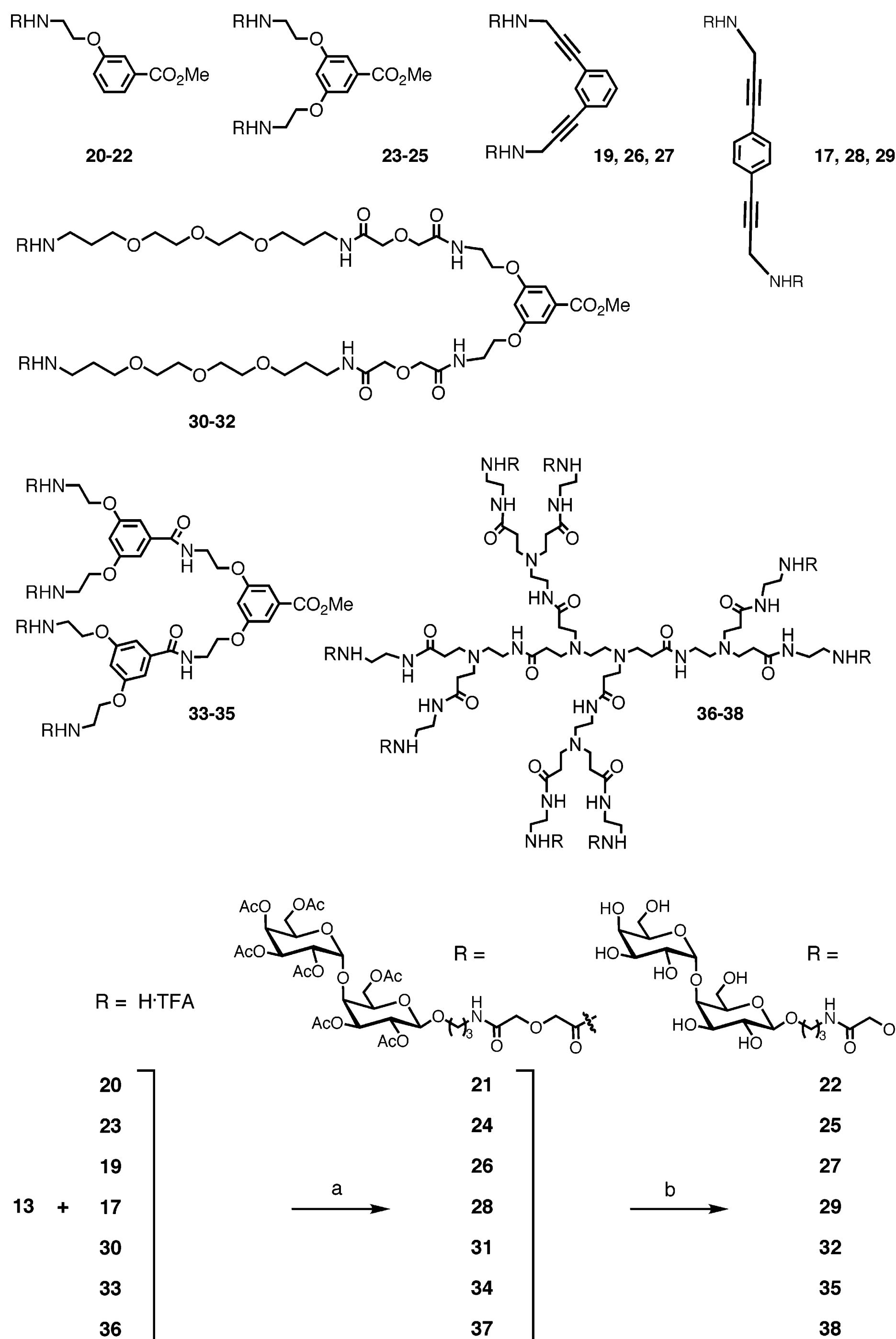
Table 1. Inhibition of the *S. suis* Mediated Hemagglutination of Human Erythrocytes by Galabiose Derivatives

	S. suis 628 (P_N)		*S. suis* 836 (P_O)	
inhibitor	MIC [nM]	rel. pot.[a] (per sugar)[b]	MIC [nM]	rel. pot.[a] (per sugar)[b]
22 monovalent	900	1 (1)	1600	1 (1)
25 divalent	6	150 (75)	70	22 (11)
32 divalent, long arms	9	100 (50)	100	16 (8)
29 divalent rigid 1,4	7	128 (64)	90	18 (9)
27 divalent rigid 1,3	4	225 (112)	50	32 (16)
35 tetravalent	2	450 (112)	40	40 (10)
38 octavalent, PAMAM	0.3	3000 (375)	2	800 (100)

[a] Relative potency = IC_{50}(monovalent **22**)/IC_{50}(multivalent compound). [b] Relative potency per sugar = relative potency/valency.

Inhibition Studies. The synthesized compounds were evaluated in two different assays. The first assay method was a hemagglutination of human erythrocytes, which express glycolipids with the Galα1−4Gal disaccharide on their surface.[18] The *S. suis* bacteria induce agglutination that can be inhibited by galabiosides. The minimal inhibitory concentrations (MIC) required for complete inhibition of hemagglutination were determined for *S. suis* type P_N and P_O and are depicted in Table 1. MIC values in the low nanomolar range were obtained for both bacterial subtypes, indicating the great inhibitory activity of these compounds. For comparison of the inhibition strength of each of the multivalent compounds, the monovalent compound **22** was chosen as a reference compound for determining the multivalency effects. The monovalent compound had MIC values of 900 and 1600 nM for subtypes P_N and P_O, respectively. The MICs for the multivalent compounds were lower than for the monovalent reference compound, also when corrected for valency, indicating a multivalent or cluster effect.[34] Inhibition of the P_O subtype required higher concentrations of inhibitors than for the P_N subtype, and also the response to multivalency effects was smaller for the P_O subtype. Both of these effects may originate from the more strict carbohydrate specificity of its adhesin.[18] The MIC of divalent compound **25** for the P_N subtype was 6 nM, that is, a 150-fold potency increase compared to reference compound **22**. Elongation of the spacer arms (compound **32**) and partial preorganization of the conformation of the divalent compounds by introducing a rigid core structure (compounds **27** and **29**) was hoped to shed light on the favorable geometry of effective divalent binding. The compounds were, however, almost equally as effective as compound **25**. By increasing the valency of the inhibitor molecule from two to four galabiose moieties (compound **35**), a 3-fold increase in relative potency per galabioside was observed. However, the strongest multivalency effects were seen with the octavalent PAMAM compound **38**. For this compound MIC with the P_N subtype of 0.3 nM was determined, which made it 3000-fold more potent (375-fold per sugar) than the reference compound **22**. To the best of our

Scheme 4. Synthesis of Multivalent Galabiosides[a]



[a] (a) BOP, DIPEA, CH_2Cl_2, DMF [however, TBTU, DIPEA, DMSO for **36**]; (b) NaOMe, MeOH.

knowledge, this is the first example of a soluble carbohydrate inhibiting bacterial binding at a subnanomolar concentration.

In the surface plasmon resonance (SPR) assay, pigeon ovomucoid, a glycoprotein rich in galabiose sequences, was immobilized on the sensor chip surface. Whole bacteria (*S. suis* 628, P_N) were added, and the SPR signal (in resonance units, RU) was detected. The whole bacteria showed a clear SPR signal, indicative of their association with the glycoprotein on the chip, and the signal could be inhibited in a dose-dependent manner by the synthesized galabiosides (Figure 1A). An inhibition curve was calculated for each compound and IC_{50} values were determined (Figure 1B; Table 2).

MIC values of the most potent compounds were again approaching the low nanomolar range, confirming their great inhibitory potency. The monovalent compound **22** proved to be a good inhibitor (IC_{50} of 1800 nM), but increasing the valency to two galabiose moieties (**25**) led to an 8-fold more potent inhibitor per galabiose unit (Figure 1, Table 2). Again, increasing the length of spacer arms or partial preorganization of the conformation had no major effects in inhibition compared to compound **25**. By increasing the valency of the inhibitor from two to four galabiose moieties (compound **35**), a strong increase in inhibition potency was observed. An IC_{50} of 7 nM was determined, which made it 256 times more potent than reference compound **22** and 33 times

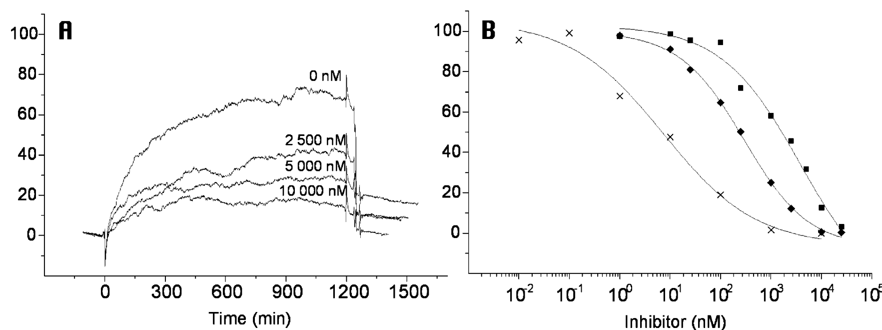


Figure 1. Inhibition of whole bacteria (*S. suis* 628, P_N) binding to pigeon ovomucoid coated sensor chip in a surface plasmon resonance assay. (A) Binding of *S. suis* in the presence of various concentrations of monovalent galabiose compound (RU, resonance units). (B) Inhibition curves for monovalent **22** (■), divalent **25** (◆), and tetravalent **35** (×) galabiose compound (nRU, normalized resonance units).

Table 2. Inhibition of *S. suis* Binding to the Pigeon Ovomucoid Immobilized on a Surface Plasmon Resonance Chip by Galabiose Derivatives

	S. suis 628 (P_N)	
inhibitor	IC_{50} [nM]	rel. pot.[a] (per sugar)[b]
22 monovalent	1800	1 (1)
25 divalent	235	8 (4)
32 divalent, long arms	156	12 (6)
29 divalent rigid 1,4	309	6 (3)
27 divalent rigid 1,3	293	6 (3)
35 tetravalent	7	256 (64)
38 octavalent, PAMAM	7	256 (32)

[a] Relative potency = IC_{50}(monovalent **22**)/IC_{50}(multivalent compound). [b] Relative potency per sugar = relative potency/valency.

more potent than divalent compound **25**, indicating a strong multivalency effect (Figure 1, Table 2). The octavalent PAMAM compound **38** had inhibitory power at similar levels as compound **35**.

To further confirm the results of the SPR experiments, similar inhibition experiments were also performed by use of a previously reported enzyme-linked immunosorbent-type assay (ELISA) with pigeon ovomucoid coated plates (data not shown). The IC_{50} values found in the ELISA assay were higher (IC_{50} compound **22**, 45 μM) than the MIC values of the hemagglutination and also higher than the IC_{50} values of the SPR assay. It was also found that the differences between the compounds were smaller than in the previously mentioned assay. However, in agreement with the SPR assay, the tetravalent **35** was the most potent with a relative potency of 12.

Conclusions

A series of mono- and multivalent galabiosides were synthesized in high yields by coupling of one general galabiose building block to different scaffolds. The synthesized galabiosides were tested for their inhibition of the binding of the bacterial pathogen *S. suis*. Two different assays, hemagglutination and SPR, were used to determine the inhibitory concentrations of the compounds. For comparison of the inhibition strength of each multivalent compound, the monovalent compound **22** was used as a reference compound. In the hemagglutination assay, inhibition of two different subtypes of the adhesin of *S. suis* (P_N and P_O) were tested. All synthesized compounds proved to be excellent inhibitors of the binding of bacteria to galabiosylated surfaces. Strong multivalency effects could be clearly observed, with compounds of higher valency having lower MIC and IC_{50} values. In one case a MIC of 0.3 nM, for octavalent **38**, was determined, representing an increase in potency of 3000-fold, or 375-fold per galabiose unit. This enhancement was much larger than the previously reported most effective compounds, a divalent and a tetravalant galabioside, which both showed a 25-fold enhancement on a per sugar basis for the same *S. suis* subtype and the same assay.[10] Despite good agreement between the data from the two highly different assay methods, some differences were also observed. While the octavalent **38** was the most potent in the hemagglutination assay, the tetravalent **35** showed a higher relative potency per galabiose unit in the SPR assay. When the performance of the four divalent compounds was compared in the two assays, there was no strong correlation to spacer length, rigidity, and orientation (**27** vs **29**). Valency seems to be a stronger determining factor. This result has implications on the orientation and mobility of the different binding sites determining the multivalent enhancement. Our results may bring the anti-adhesion approach against bacterial infections closer to its potential, since strong inhibition was observed as caused by multivalent presentation of the binding galabiose moiety. Further improvements will be pursued by evaluating complementary orientations and valencies. Gratifyingly, we note that multivalent carbohydrates have successfully been used in vivo, as was recently shown in two separate studies focused on the neutralization of the Shiga toxin of *E. coli.*[35]

Experimental Section

General Methods. All chemicals were of reagent grade and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F_{254} (Merck); after examination under UV light, compounds were visualized by heating with 10% (v/v) methanolic H_2SO_4 or with ninhydrin (3 mg mL^{-1}). In the workup procedures of reaction mixtures, organic solutions were washed with appropriate amounts of the indicated aqueous solutions, then dried, and concentrated under reduced pressure at 40 °C. Column chromatography was performed on silica gel 60 (Merck, 0.063–0.200 mm). Final compounds were purified on an Isolute SPE column [IST, 291-1000-F C8(EC)]. ^{1}H NMR spectra were recorded at 300 K with a Varian Gemini-300 (300 MHz) or a Varian Unity INOVA 500 (500 MHz) spectrometer; the δ_H values are given in parts per million (ppm) relative to the signal for internal Me_4Si (δ_H = 0, $CDCl_3$ and CD_3OD) or internal acetone (δ_H = 2.225, D_2O). ^{13}C NMR spectra (APT, 75 MHz) were recorded at 300 K with a Varian Gemini-300 spectrometer; δ_C values are given in ppm relative to the signal of $CDCl_3$ (δ_C = 76.9, $CDCl_3$) or for internal acetone (δ_C = 30.89, D_2O) or internal MeOH (δ_C = 49.50, D_2O). Two-dimensional

$^1H-^1H$ correlation and total correlation spectroscopy (COSY and TOCSY) and $^1H-^{13}C$ correlated heteronuclear single quantum coherence (HSQC) NMR spectra (500 MHz) were recorded at 300 K with a Varian Unity INOVA 500 spectrometer. Exact masses were measured by nanoelectrospray time-of-flight mass spectrometry on a Micromass LCToF mass spectrometer at a resolution of 5000 fwhm. Gold-coated capillaries were loaded with 1 μL of sample (concentration 20 μM) dissolved in a 1:1 (v/v) mixture of CH_3CN-H_2O with 0.1% formic acid. NaI or poly(ethylene glycol) (PEG) was added as internal standard. The capillary voltage was set between 1100 and 1350 V, and the cone voltage was set at 30 V. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) spectra were recorded on a Shimadzu Axima-CFR with α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix and adrenocorticotropin (ACTH) fragment 18−39 for calibration.

3-Azidopropyl 2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranoside (3). A solution of 1,2,3,4,6-penta-*O*-acetyl-*β*-D-galactopyranoside **1** (9.2 g, 23.7 mmol) and 3-bromopropanol (4.9 g, 35.5 mmol) in dry CH_2Cl_2 (100 mL), containing molecular sieve 4 Å (1.5 g), was cooled to 0 °C, and $BF_3{\cdot}Et_2O$ (15 mL, 118.5 mmol) was added in 2 min. The mixture was stirred overnight, neutralized with NEt_3, washed with aqueous saturated $NaHCO_3$, H_2O, and aqueous saturated NaCl, dried (Na_2SO_4), filtered, and concentrated. Reduced-pressure column chromatography (toluene−EtOAc 8:1) of the residue gave **2** (9.36 g, 84%), isolated as a slightly yellow syrup. To a solution of **2** (0.81 g, 1.72 mmol) in dry DMF (5 mL) was added NaN_3 (0.56 g, 8.63 mmol), and the mixture was stirred at 100 °C overnight, then filtered over Hyflo, and coconcentrated with toluene. The residue was dissolved in dichloromethane and washed with aqueous saturated NaCl, dried (Na_2SO_4), filtered, and concentrated to give **3** (0.65 g, 87%), isolated as a slightly yellow syrup. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.84−1.89 (m, 2 H, $OCH_2C\mathit{H}_2CH_2N_3$), 1.98, 2.05, 2.07, and 2.15 (4 s, each 3 H, 4 $COCH_3$), 3.38 (t, 2 H, $OCH_2CH_2C\mathit{H}_2N_3$), 3.58−3.65 (m, 1 H, $OC\mathit{H}HCH_2CH_2N_3$), 3.93−4.00 (m, 2 H, H-5 and $OCH\mathit{H}CH_2CH_2N_3$), 4.10−4.21 (m, 2 H, H-6a and H-6b), 4.50 (d, $J_{1,2}$ = 8.1 Hz, 1 H, H-1), 5.03 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.6 Hz, 1 H, H-3), 5.18 (dd, 1 H, H-2), 5.39 (d, $J_{4,5}$ < 1 Hz, 1 H, H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 23.2, 23.3 (2 C), and 23.4 ($CO\mathit{C}H_3$), 31.6 ($OCH_2\mathit{C}H_2CH_2N_3$), 50.5 ($OCH_2CH_2\mathit{C}H_2N_3$), 63.9 (C-6), 69.1 ($O\mathit{C}H_2CH_2CH_2N_3$), 69.7, 71.5, 73.3, and 73.5 (C-2, C-3, C-4, and C-5), 103.9 (C-1), 172.1, 172.8, 172.9, and 173.1 ($\mathit{C}OCH_3$); HRMS for $C_{17}H_{25}N_3O_{10}$ (M, 431.154) M + NH_4 found 449.189, calcd 449.1883.

3-Azidopropyl 4,6-Di-*O*-benzylidene-*β*-D-galactopyranoside (5). To a solution of **3** (2.6 g, 6.1 mmol) in MeOH (20 mL) and CH_2Cl_2 (6 mL) was added 30% methanolic NaOMe (0.5 mL). The mixture was stirred for 1 h, then neutralized with Dowex 50X8 (H^+), filtered, and concentrated. Reduced-pressure column chromatography (CH_2Cl_2−MeOH 10:1) of the residue gave **4** (1.56 g, 97%), isolated as a colorless syrup. High-resolution MS data for $C_9H_{17}N_3O_6$ (M, 263.1117): M + H found 264.121, calcd 264.1195. To a solution of **4** (4.2 g, 15.9 mmol) in dry DMF (20 mL) were added α,α-dimethoxytoluene (19 mL, 127.6 mmol) and a catalytic amount of *p*-TSOH. The mixture was stirred for 2.5 h at 50 °C and neutralized with NEt_3. After the addition of EtOAc, the mixture was washed with H_2O and aqueous saturated NaCl, dried ($MgSO_4$), filtered, and coconcentrated with toluene. Reduced-pressure column chromatography (toluene−EtOAc, 10:1 → 1:4) of the residue gave **5** (4.5 g, 80%), isolated as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ = 1.91−1.95 (m, 2 H, $OCH_2C\mathit{H}_2CH_2N_3$), 3.43−3.50 (m, 3 H, H-5 and $OCH_2CH_2C\mathit{H}_2N_3$), 3.62−3.79 (m, 3 H, H-2, H-3, and $OC\mathit{H}HCH_2CH_2N_3$), 4.00−4.06 (m, 1 H, $OCH\mathit{H}CH_2CH_2N_3$), 4.09 (dd, $J_{5,6b}$ = 2.1 Hz, $J_{6a,6b}$ = 12.3 Hz, 1 H, H-6b), 4.22 (d, $J_{3,4}$ = 3.3, $J_{4,5}$ < 1 Hz, 1 H, H-4), 4.30 (d, $J_{1,2}$ = 7.5 Hz, 1 H, H-1), 4.34 (dd, $J_{5,6a}$ < 1 Hz, 1 H, H-6a), 5.56 (s, 1 H, $OC\mathit{H}C_6H_5$), 7.36−7.38 and 7.49−7.52 (2 m, 5 H, $OCHC_6\mathit{H}_5$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 30.8 ($OCH_2\mathit{C}H_2CH_2N_3$), 50.1 ($OCH_2CH_2\mathit{C}H_2N_3$), 68.4 and 70.9 (C-6 and $O\mathit{C}H_2CH_2CH_2N_3$), 68.3, 73.1, 74.3, and 77.2 (C-2, C-3, C-4, and C-5), 103.0 and 104.7 (C-1 and $O\mathit{C}HC_6H_5$), 128.3, 130.0, 131.0, and 139.5 ($OCH\mathit{C}_6H_5$); HRMS for $C_{16}H_{21}N_3O_6$ (M, 351.1430) M + H found 352.152, calcd 352.1508.

3-Azidopropyl 2,3-Di-*O*-benzyl-4,6-di-*O*-benzylidene-*β*-D-galactopyranoside (6). To a solution of **5** (2.1 g, 5.97 mmol) in dry DMF (40 mL) was added NaH (0.76 g, 19.1 mmol), and the mixture was stirred for 0.5 h. After the addition of TBAI (0.44 g, 1.19 mmol) and BnBr (1.8 mL, 15.5 mmol), the reaction was stirred for 5 h, and then MeOH was added and the mixture was coconcentrated with toluene. The residue was dissolved in EtOAc, washed with H_2O, aqueous saturated $NaHCO_3$, and H_2O, dried (Na_2SO_4), filtered, and concentrated. Reduced-pressure column chromatography (toluene−EtOAc, 40:1 → 10:1) of the residue gave **6** (2.3 g, 75%), isolated as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ = 1.80−1.95 (m, 2 H, $OCH_2C\mathit{H}_2CH_2N_3$), 3.41 (t, 2 H, $OCH_2CH_2C\mathit{H}_2N_3$), 3.55 (dd, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 3.6 Hz, 1 H, H-3), 3.58−3.64 (m, 2 H, H-5 and $OC\mathit{H}HCH_2CH_2N_3$), 3.82 (dd, $J_{1,2}$ = 8.1 Hz, 1 H, H-2), 3.97−4.02 (m, 2 H, H-6b and $OCH\mathit{H}CH_2CH_2N_3$), 4.10 (d, 1 H, H-4), 4.27 (dd, $J_{5,6a}$ < 1 Hz, $J_{6a,6b}$ = 12.3 Hz, 1 H, H-6a), 4.36 (d, 1 H, H-1), 4.75 and 4.83 (s and dd, each 2 H, 2 $OC\mathit{H}_2C_6H_5$), 5.48 (s, 1 H, $OC\mathit{H}C_6H_5$), 7.28−7.38 and 7.53−7.56 (2 m, 15 H, $OCHC_6\mathit{H}_5$ and 2 $OCH_2C_6\mathit{H}_5$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 31.0 ($OCH_2\mathit{C}H_2CH_2N_3$), 50.1 ($OCH_2CH_2\mathit{C}H_2N_3$), 68.2, 71.0, 73.7, and 77.1 (C-6, $O\mathit{C}H_2CH_2CH_2N_3$, and $O\mathit{C}H_2C_6H_5$), 68.2, 75.5, 80.2, and 81.0 (C-2, C-3, C-4, and C-5), 103.0 and 105.3 (C-1 and $O\mathit{C}HC_6H_5$), 128.3, 129.4−130.7, and 139.7−140.6 ($OCH\mathit{C}_6H_5$); HRMS for $C_{30}H_{33}N_3O_6$ (M, 531.2369) M + Na found 554.2196, calcd 554.2267.

3-Azidopropyl 2,3,6-Tri-*O*-benzyl-*β*-D-galactopyranoside (7). To a solution of **6** (0.61 g, 1.15 mmol) and TES (1.1 mL, 6.9 mmol) in dry CH_2Cl_2 (10 mL) was added, in 1 min at 0 °C, TFA (0.5 mL, 6.9 mmol). The mixture was stirred for 5 h, during which the temperature was allowed to reach room temperature and then neutralized with aqueous saturated $NaHCO_3$. The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated. Reduced pressure column chromatography (toluene−EtOAc 10:1) of the residue yielded **7** (0.47 g, 76%), isolated as a colorless syrup. ^1H NMR (300 MHz, $CDCl_3$) δ = 1.70−1.75 (m, 2 H, $OCH_2C\mathit{H}_2CH_2N_3$), 3.22 (t, 2 H, $OCH_2CH_2C\mathit{H}_2N_3$), 3.75 (dd, $J_{2,3}$ = 9.3 Hz, $J_{3,4}$ = 3.3 Hz, 1 H, H-3), 3.86 (d, $J_{4,5}$ < 1 Hz, 1 H, H-4), 4.23 (d, $J_{1,2}$ = 7.5 Hz, 1 H, H-1), 4.44 and 4.55 (2 s, each 2 H, 2 $OC\mathit{H}_2C_6H_5$), 4.68 (2 d, each 1 H, $OC\mathit{H}_2C_6H_5$), 7.16−7.22 (m, 15 H, 3 $OCH_2C_6\mathit{H}_5$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 28.8 ($OCH_2\mathit{C}H_2CH_2N_3$), 47.8 ($OCH_2CH_2\mathit{C}H_2N_3$), 66.0, 68.8, 71.8, 73.2, and 74.8 (C-6, $O\mathit{C}H_2CH_2CH_2N_3$, and $O\mathit{C}H_2C_6H_5$), 66.3, 72.8, 78.5, and 80.2 (C-2, C-3, C-4, C-5), 103.2 (C-1), 127.2−128.0 and 137.4−138.1 ($OCH\mathit{C}_6H_5$); HRMS for $C_{30}H_{35}N_3O_6$ (M, 533.2526) M + Na found 556.2400, calcd 556.2424.

3-Azidopropyl (2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-*β*-D-galactopyranoside (9). To a solution of **7** (1.10 g, 2.05 mmol) and 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside trichloroacetimidate **8** (2.11 g, 3.08 mmol) in dry Et_2O (50 mL) was added, at 0 °C, TMSOTf (8 μL, 0.46 mmol). The mixture was stirred for 1.5 h, during which the temperature was allowed to reach room temperature, then neutralized with NEt_3, and concentrated. Reduced-pressure column chromatography (toluene−EtOAc 40:1) of the residue gave **9** (1.60 g, 73%), isolated as a slightly yellow syrup. ^1H NMR (300 MHz, $CDCl_3$) δ = 1.81−1.89 (m, 2 H, $OCH_2C\mathit{H}_2CH_2N_3$), 3.38 (t, 2 H, $OCH_2CH_2C\mathit{H}_2N_3$), 3.66 (dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.9 Hz, 1 H, H-2), 4.30 (d, 1 H, H-1), 5.04 (d, $J_{1,2}$ = 2.1 Hz, 1 H, H-1′), 7.14−7.37 (m, 35 H, 7 $OCH_2C_6\mathit{H}_5$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 28.9 ($OCH_2\mathit{C}H_2CH_2N_3$), 48.0 ($OCH_2CH_2\mathit{C}H_2N_3$), 66.2, 67.6, 71.9, 72.0, 72.4, 72.7, 72.9, 73.4, 74.6, and 74.9 (C-6, C-6′, $O\mathit{C}H_2CH_2CH_2N_3$, and $O\mathit{C}H_2C_6H_5$), 69.0, 73.3, 74.3, 74.4, 76.3, 78.6, 78.7, and 80.6 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 100.2 and 103.5 (C-1 and C-1′), 127.0−128.1 and 137.7−138.6 ($OCH\mathit{C}_6H_5$); HRMS for $C_{64}H_{69}N_3O_{11}$ (M, 1055.4932) M + Na found 1078.477, calcd 1078.491.

3-(*N-tert*-Butyloxycarbonyl)aminopropyl (2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-*β*-D-galactopyranoside (10). A suspension of **9** (1.2 g, 1.13 mmol), Lindlar catalyst (0.24 g), Boc_2O (0.27 g, 1.25 mmol), and 3

drops of NEt_3 in 1:1 MeOH−EtOAc (12 mL) was stirred overnight under an H_2 atmosphere. After the addition of EtOAc, the suspension was filtered over Hyflo and concentrated. Reduced-pressure column chromatography (toluene−EtOAc 15:1) of the residue yielded **10** (1.2 g, 92%), isolated as a colorless syrup. ^1H NMR (300 MHz, $CDCl_3$) $\delta = 1.39$ [s, 9 H, $OC(CH_3)_3$], 1.78−1.82 (m, 2 H, $OCH_2CH_2CH_2NH$), 3.38 (dd, $J_{2,3} = 9.9$ Hz, $J_{3,4} = 2.7$ Hz, 1 H, H-3), 3.66 (dd, $J_{1,2} = 7.5$ Hz, 1 H, H-2), 4.00 (d, 1 H, H-4), 4.10 (d, $J_{1,2} = 2.1$ Hz, 1 H, H-2′), 4.30 (d, 1 H, H-1), 5.01 (d, 1 H, H-1′), 7.17−7.37 (m, 35 H, 7 $OCH_2C_6H_5$); ^{13}C NMR (75.5 MHz, $CDCl_3$) $\delta = 28.2$ [$OC(CH_3)_3$], 29.6 ($OCH_2CH_2CH_2NH$), 37.5 ($OCH_2CH_2CH_2NH$), 67.4, 67.7, 71.9, 72.0, 72.7, 72.9, 73.4, 74.6, 74.9, and 78.6 (C-6, C-6′, $OCH_2CH_2CH_2NH$, and $OCH_2C_6H_5$), 69.0, 73.3, 74.3, 74.4, 76.3, 78.5, 78.7, and 80.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 100.2 and 103.6 (C-1 and C-1′), 127.1−128.4 and 137.8−138.7 ($OCHC_6H_5$), 155.7 [$OC(CH_3)_3$]; HRMS for $C_{69}H_{79}NO_{13}$ (M, 1129.5551) M + Na found 1152.542, calcd 1152.553.

3-(*N-tert*-Butyloxycarbonyl)aminopropyl (2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-*β*-D-galactopyranoside (11). A solution of **10** (1.68 g, 1.48 mmol) and 3 drops of HOAc in 1:1 MeOH−EtOAc (12 mL) was stirred overnight under a H_2 atmosphere, then filtered over Hyflo, and concentrated. The residue was dissolved in 1:1 pyridine−acetic anhydride (100 mL) and stirred for 4 h, and then coconcentrated with toluene. Reduced-pressure column chromatography (toluene−EtOAc, 4:1 → 1:1) of the residue gave **11** (1.0 g, 88%), isolated as a white foam. ^1H NMR (300 MHz, $CDCl_3$) $\delta = 1.43$ [s, 9 H, $OC(CH_3)_3$], 1.75−1.81 (m, 2 H, $OCH_2CH_2CH_2NH$), 1.98, 2.03, 2.06, 2.08, 2.10, and 2.13 (6 s, 3, 3, 3, 6, 3, and 3 H, 7 $COCH_3$), 3.26 (t, 2 H, $OCH_2CH_2CH_2NH$), 3.56 (m, 1 H, $OCHHCH_2CH_2NH$), 3.93 (m, 1 H, $OCHHCH_2CH_2NH$), 4.07 (d, $J_{3,4} = 3.0$ Hz, $J_{4,5} < 1$ Hz, 1 H, H-4), 4.57 (d, $J_{1,2} = 8.1$ Hz, 1 H, H-1), 4.80 (dd, $J_{2,3} = 10.8$ Hz, 1 H, H-3), 5.00 (d, $J_{1,2} = 3.6$ Hz, 1 H, H-1′), 5.18 (dd, 1 H, H-2), 5.20 (dd, $J_{2,3} = 11.4$ Hz, 1 H, H-2′), 5.39 (dd, $J_{3,4} = 3.0$ Hz, 1 H, H-3′), 5.57 (d, $J_{4,5} < 1$ Hz, 1 H, H-4′); ^{13}C NMR (75.5 MHz, $CDCl_3$) $\delta = 20.4$−20.7 ($COCH_3$), 28.1 [$OC(CH_3)_3$], 29.4 ($OCH_2CH_2CH_2NH$), 37.4 ($OCH_2CH_2CH_2NH$), 60.2 and 61.7 (C-6 and C-6′), 67.4 ($OCH_2CH_2CH_2NH$), 66.7, 67.1, 67.5, 68.2, 68.3, 71.6, 72.4, and 76.8 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 99.1 and 100.8 (C-1 and C-1′), 155.7 [$OC(CH_3)_3$], 168.9, 169.6, 169.9, 170.1, 170.2, 170.3, and 170.4 ($COCH_3$); HRMS for $C_{34}H_{51}NO_{20}$ (M, 793.3004) M + Na found 816.286, calcd 816.298.

3-Aminopropyl (2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-*β*-D-galactopyranoside Trifluoroacetic Acid Salt (12). To a solution of **11** (222 mg, 0.279 mmol) in CH_2Cl_2 (8 mL) was added, at 0 °C, 90% aqueous TFA (1 mL), and the mixture was stirred for 4 h and then coconcentrated with ethanol to give **12** (225 mg, quantitative), isolated as a white foam. ^{13}C NMR (75.5 MHz, $CDCl_3$) $\delta = 20.4$−20.7 ($COCH_3$), 28.1 [$OC(CH_3)_3$], 29.4 ($OCH_2CH_2CH_2NH$), 37.4 ($OCH_2CH_2CH_2NH$), 60.2 and 61.7 (C-6 and C-6′), 67.4 ($OCH_2CH_2CH_2NH$), 66.7, 67.1, 67.5, 68.2, 68.3, 71.6, 72.4, and 76.8 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 99.1 and 100.8 (C-1 and C-1′), 155.7 [$OC(CH_3)_3$], 168.9, 169.6, 169.9, 170.1, 170.2, 170.3, and 170.4 ($COCH_3$).

Short Spacer (2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-*β*-D-galactopyranoside (13). To a solution of **12** (225 mg, 0.279 mmol) in pyridine (3 mL) and dry dioxane (1 mL) was added a solution of diglycolic anhydride (36 mg, 0.307 mmol) in dry dioxane (1 mL). The mixture was stirred overnight at 90 °C and then coconcentrated with toluene. The residue was dissolved in CH_2Cl_2 and washed with aqueous 1 M $KHSO_4$ and aqueous saturated NaCl, dried (Na_2SO_4), filtered, and concentrated, yielding **13** (226 mg, quantitative), isolated as a white foam. ^1H NMR (300 MHz, $CDCl_3$) $\delta = 1.82$−1.89 (m, 2 H, $OCH_2CH_2CH_2NH$), 2.00, 2.04, 2.06, 2.08, 2.09, 2.10, and 2.14 (7 s, each 3 H, 7 $COCH_3$), 3.81 (t, 2 H, $OCH_2CH_2CH_2NH$), 4.83 (dd, $J_{2,3} = 10.8$ Hz, $J_{3,4} = 2.7$ Hz, 1 H, H-3), 5.00 (d, $J_{1,2} = 3.6$ Hz, 1 H, H-1′), 5.18 (dd, 1 H, H-2), 5.20 (dd, $J_{2,3} = 10.8$ Hz, 1 H, H-2′), 5.40 (dd, $J_{3,4} = 3.3$ Hz, 1 H, H-3′), 5.57 (d, $J_{4,5} < 1$ Hz, 1 H, H-4′); ^{13}C NMR (75.5 MHz, $CDCl_3$) $\delta = 20.2$−20.6 ($COCH_3$), 28.6 ($OCH_2CH_2CH_2NH$), 36.1 ($OCH_2CH_2CH_2NH$), 60.2 and 61.6 (C-6 and C-6′), 67.4 ($OCH_2CH_2CH_2NH$), 68.4 and 70.6 ($COCH_2OCH_2COOH$), 66.6, 67.1, 67.5, 68.1, 68.3, 71.5, 72.3, and 76.8 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 99.0 and 100.7 (C-1 and C-1′), 155.7 [$OC(CH_3)_3$], 169.2, 169.8, 169.9, 170.0, 170.1, 170.2, 170.3, 170.4, and 171.4 ($COCH_3$ and $COCH_2OCH_2COOH$); HRMS for $C_{33}H_{47}NO_{22}$ (M, 809.2590) M + Na found 832.2479, calcd 832.2488.

1,4-Bis[3-(*tert*-butoxycarbonyl)aminoprop-1-ynyl]benzene (16). To a solution of **14** (2.0 g, 6.06 mmol), CuI (0.11 g, 0.6 mmol), and $Pd^{(0)}(PPh_3)_4$ (0.34 g, 0.30 mmol) in dry CH_3CN (10 mL) was added, under N_2, a solution of Boc-protected propargylamine (2.8 g, 18.1 mmol) in dry NEt_3 (1.5 mL) and dry CH_3CN (1 mL). The reaction mixture was stirred under N_2 for 2 days, then diluted with CH_2Cl_2, filtrated over Hyflo, and concentrated. Reduced-pressure column chromatography (hexanes−EtOAc 15:1) of the residue gave **16** (1.77 g, 76%), isolated as a slightly brown solid. ^1H NMR (300 MHz, $CDCl_3$) $\delta = 1.46$ [s, 18 H, 2 $OC(CH_3)_3$], 4.15 (d, $J = 5.7$ Hz, 4 H, 2 CH_2), 4.75 (br s, 2 H, 2 NH), 7.33 (s, 4 H, C_6H_4); ^{13}C NMR (75.5 MHz, $CDCl_3$) $\delta = 28.2$ [$OC(CH_3)_3$], 31.8 (CH_2), 79.9 [$OC(CH_3)_3$], 82.4 (CCH_2), 87.0 (CC_6H_4), 122.5 and 131.4 (C_6H_4), 155.1 (CO); HRMS for $C_{22}H_{28}N_2O_4$ (M, 384.2049) M + Na found 407.2028, calcd 407.1947.

Trifluoroacetic Acid Salt of 1,4-Bis(3-aminoprop-1-ynyl)benzene (17). To a solution of **16** (0.178 g, 0.463 mmol) in CH_2Cl_2 (10 mL) was added, at 0 °C, TFA (0.35 mL). The solution was stirred for 5 h, during which the temperature was allowed to reach room temperature, and then concentrated to give **17** (0.176 g, quantitative), isolated as a slightly brown solid. HRMS for $C_{12}H_{12}N_2$ (M, 184.1000) M + H found 185.1089, calcd 185.1078.

1,3-Bis[3-(*tert*-Butoxycarbonyl)aminoprop-1-ynyl]benzene (18). To a solution of **15** (1.0 g, 3.03 mmol), CuI (6 mg, 0.30 mmol), and $Pd^{(0)}(PPh_3)_4$ (0.17 g, 0.151 mmol) in dry CH_3CN (5 mL) was added, under N_2, a solution of Boc-protected propargylamine (1.41 g, 9.09 mmol) in dry NEt_3 (0.75 mL) and dry CH_3CN (1 mL). The reaction mixture was stirred under N_2 for 20 h, then diluted with CH_2Cl_2, filtrated over Hyflo, and concentrated. Reduced-pressure column chromatography (hexanes−EtOAc 15:1) of the residue gave **18** (1.02 g, 87%), isolated as a slightly yellow solid. ^1H NMR (300 MHz, $CDCl_3$) $\delta = 1.47$ [s, 18 H, 2 $OC(CH_3)_3$], 4.15 (d, $J = 5.7$ Hz, 4 H, 2 CH_2), 4.75 (br s, 2 H, 2 NH); ^{13}C NMR (75.5 MHz, $CDCl_3$) $\delta = 28.2$ [$OC(CH_3)_3$], 30.9 (CH_2), 79.9 [$OC(CH_3)_3$], 82.0 (CCH_2), 85.9 (CC_6H_4), 122.8, 128.2, 131.3, and 134.6 (C_6H_4), 155.1 (CO); HRMS for $C_{22}H_{28}N_2O_4$ (M, 384.2049) M + Na found 407.1951, calcd 407.1947.

Trifluoroacetic Acid Salt of 1,3-Bis(3-aminoprop-1-ynyl)benzene (19). To a solution of **18** (0.148 g, 0.385 mmol) in CH_2Cl_2 (8 mL) was added, at 0 °C, TFA (0.3 mL). The solution was stirred for 4 h, during which the temperature was allowed to reach room temperature, and then concentrated to give **19** (0.146 g, quantitative), isolated as a slightly yellow solid. ^1H NMR (300 MHz, $CDCl_3$) $\delta = 3.96$ (s, 4 H, 2 CH_2), 4.55 (br s, 2 H, 2 NH), 7.32−7.54 (m, 4 H, C_6H_4); ^{13}C NMR (75.5 MHz, $CDCl_3$) $\delta = 29.1$ (CH_2), 80.2 (CCH_2), 85.2 (CC_6H_4), 121.4, 128.2, 131.8 and 134.4 (C_6H_4); HRMS for $C_{12}H_{12}N_2$ (M, 184.1000) M + Na found 185.1019, calcd 185.1078.

General Procedure for Preparation of Acetyl-Protected Galabiose Compounds 21, 24, 26, 28, 31, and 34. To a solution of **17**, **19**, **20**, **23**, **30**, or **33**, **13** (1.1 equiv per NH_2 group), and BOP (1.2 equiv per NH_2 group) in dry CH_2Cl_2 and dry DMF was added dropwise DIPEA (1.1 equiv per NH_2 group). The mixture was stirred and then concentrated. The residue was dissolved in EtOAc, washed with 1 M $KHSO_4$ and aqueous saturated NaCl, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by reduced-pressure column chromatography (CH_2Cl_2−MeOH, 15:1 → 1:1) to give **21**, **24**, **26**, **28**, **31**, or **34**, isolated as white glass.

General Procedure for Preparation of Galabiose Compounds 22, 25, 27, 29, 32, and 35. To a solution of **21**, **24**, **26**, **28**, **31**, or **34** in MeOH (1 mL) was added methanolic

NaOMe (pH 9). The mixture was stirred for 1 h, then neutralized with Dowex 50X8 (H^+), filtered, and concentrated. The residue was applied on a SPE-column, which was eluted with H_2O−AcCN (100:0 → 85:15) to give **22**, **25**, **27**, **29**, **32**, or **35**, isolated as a white solid after freeze-drying.

Galabiose-Specific ^{1}H NMR data (300 MHz, $CDCl_3$) for Compounds 21, 24, 26, 28, 31, 34, and 37. δ = 4.48 (d, H-1), 4.83 (dd, H-3), 5.00 (d, H-1′), 5.17 (dd, 1H, H-2), 5.20 (dd, H-2′), 5.38 (dd, H-3′), 5.56 (d, H-4′)

Monovalent Per-*O*-acetyl-Protected Galabiose Short Spacer (21). Reaction time 1 h; CH_2Cl_2/DMF 14:1; yield 66% (38 mg); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 20.4−20.7 (CO*C*H_3), 29.0 ($OCH_2CH_2CH_2NH$), 36.7 ($OCH_2CH_2CH_2NH$), 38.2 (OCH_2CH_2NH), 52.0 ($C_6H_4COCH_3$), 60.3 and 61.6 (C-6 and C-6′), 66.5 and 68.0 ($OCH_2CH_2CH_2NH$ and OCH_2CH_2NH), 70.9 and 71.0 ($COCH_2OCH_2CO$), 66.8, 67.1, 67.5, 68.3, 68.4, 71.8, 72.3, and 76.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 99.2 and 101.0 (C-1 and C-1′), 114.6, 119.5, 122.2, 129.4, 131.2, and 158.2 ($C_6H_4COCH_3$), 166.6 ($C_6H_4COCH_3$), 168.6−170.5 ($COCH_3$ and CONH); HRMS for $C_{43}H_{58}N_2O_{24}$ (M, 986.3380) M + Na found 1009.3435, calcd 1009.3278.

Monovalent Galabiose Short Spacer (22). Yield 80% (22 mg); ^{1}H NMR (300 MHz, D_2O) δ = 1.76 (m, 2 H, $OCH_2CH_2CH_2NH$), 3.27 (m, 2 H, $OCH_2CH_2CH_2NH$), 3.48 (dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.2 Hz, 1 H, H-2), 3.60−3.68 (m, 7 H, H-3, H-5, H-6a′, H-6b′, $OCHHCH_2CH_2NH$, and OCH_2CH_2NH), 3.73−3.79 (m, 3 H, H-2′, H-6a, and H-6b), 3.83−3.87 (m, 5 H, H-3′, $OCHHCH_2CH_2NH$, and $C_6H_3COCH_3$), 3.97 (s, 4 H, H-4, H-4′, and $COCH_2OCH_2CO$), 4.03 (s, 2 H, $COCH_2OCH_2CO$), 4.17 (m, 2 H, OCH_2CH_2NH), 4.29−4.36 (m, 2 H, H-1 and H-5′), 4.90 (d, $J_{1,2}$ = 3.9 Hz, 1 H, H-1′), 7.17 (dd, 1 H, CH^5), 7.38 (dd, 1 H, CH^4), 7.46 (dd, 1 H, CH^2), 7.56 (dd, 1 H, CH^6), 8.16 and 8.37 (2 t, each 1 H, 2 NH); ^{13}C NMR (75.5 MHz, D_2O) δ = 29.3 ($OCH_2CH_2CH_2NH$), 36.6 ($OCH_2CH_2CH_2NH$), 39.2 (OCH_2CH_2NH), 53.3 ($C_6H_4COCH_3$), 60.7 and 61.1 (C-6 and C-6′), 67.2 (OCH_2CH_2NH), 68.2 ($OCH_2CH_2CH_2NH$), 69.4, 69.5, 69.7, 71.2, 71.4, 73.0, 75.6, 77.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 70.3 (2 C) ($COCH_2OCH_2CO$), 100.6 (C-1′), 103.5 (C-1), 115.6, 121.1, 122.9, and 130.5 ($C_6H_4COCH_3$), 168.7 ($C_6H_4COCH_3$), 172.0 and 172.4 (CONH); HRMS for $C_{29}H_{44}N_2O_{17}$ (M, 692.2640) M + Na found 715.2514, calcd 715.2538.

Divalent Per-*O*-acetyl-Protected Galabiose Short Spacer (24). Reaction time 1 h; CH_2Cl_2/DMF 8:1; yield 88% (135 mg); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 20.3−20.6 ($COCH_3$), 28.9 ($OCH_2CH_2CH_2NH$), 36.5 ($OCH_2CH_2CH_2NH$), 38.1 (OCH_2CH_2NH), 52.0 ($C_6H_3COCH_3$), 60.2 and 61.5 (C-6 and C-6′), 66.4 and 67.9 ($OCH_2CH_2CH_2NH$ and OCH_2CH_2NH), 70.7 and 70.8 ($COCH_2OCH_2CO$), 66.7, 67.0, 67.4, 68.1, 68.3, 71.6, 72.2, and 76.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 99.0 and 100.8 (C-1 and C-1′), 106.3, 107.8, 133.8, and 159.2 ($C_6H_3COCH_3$), 166.1 ($C_6H_3COCH_3$), 168.6−170.3 ($COCH_3$ and CONH); HRMS for$C_{78}H_{109}N_4O_{46}$ (M, 1836.6235) M + Na found 1859.687, calcd 1859.621.

Divalent Galabiose Short Spacer (25). Yield 99% (74 mg); ^{1}H NMR (300 MHz, D_2O) δ = 1.79−1.83 (m, 4 H, 2 $OCH_2CH_2CH_2NH$), 3.30 (m, 4 H, 2 $OCH_2CH_2CH_2NH$), 3.35 (s, 3 H, $C_6H_3COCH_3$), 3.54 (dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.2 Hz, 2 H, 2 H-2), 3.63−3.71 (m, 14 H, 2 each H-3, H-5, H-6a′, H-6b′, $OCHHCH_2CH_2NH$, and OCH_2CH_2NH), 3.78−3.93 (m, 10 H, H-2′, H-3′, H-6a, H-6b, and $OCHHCH_2CH_2NH$), 4.02 (d, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ < 1 Hz, 2 H, 2 H-4), 4.03 (d, $J_{3,4}$ = 3.6 Hz, $J_{4,5}$ < 1 Hz, 2 H, 2 H-4′), 4.04 and 4.10 (2 s, each 4 H, 2 $COCH_2OCH_2CO$), 4.13 (m, 4 H, 2 OCH_2CH_2NH), 4.36 (m, 2 H, 2 H-5′), 4.38 (d, 2 H, 2 H-1), 4.95 (d, $J_{1,2}$ = 3.6 Hz, 2 H, 2 H-1′), 6.70 and 7.05 (2 s, 1 and 2 H, $C_6H_3COCH_3$); ^{13}C NMR (75.5 MHz, D_2O): δ = 29.2 ($OCH_2CH_2CH_2NH$), 36.6 ($OCH_2CH_2CH_2NH$), 39.1 (OCH_2CH_2NH), 53.4 ($C_6H_3COCH_3$), 60.7 and 61.1 (C-6 and C-6′), 67.2 (OCH_2CH_2NH), 68.2 ($OCH_2CH_2CH_2NH$), 69.3, 69.6, 69.8, 71.4, 71.6, 73.0, 75.7, 77.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 70.5 (2 C) ($COCH_2OCH_2CO$), 100.8 (C-1′), 103.5 (C-1), 107.5, 108.9, 132.0, and 159.9 ($C_6H_3COCH_3$), 168.7 ($C_6H_3COCH_3$), 172.0 (2 C) and 172.5 (2 C) (CONH); HRMS for $C_{50}H_{80}N_4O_{32}$ (M, 1248.4756) M + Na found 1271.4650, calcd 1271.4654.

Divalent Per-*O*-acetyl-Protected Short Spacer 1,3-Rigid Galabioside (26). Reaction time 2 h; CH_2Cl_2/DMF 7:1; yield 68% (101 mg); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 20.4−20.7 ($COCH_3$), 28.9 ($OCH_2CH_2CH_2NH$), 29.2 (CCH_2), 36.6 ($OCH_2CH_2CH_2NH$), 60.2 and 61.5 (C-6 and C-6′), 67.9 ($OCH_2CH_2CH_2NH$), 70.8 (2 C, $COCH_2OCH_2CO$), 66.8, 67.1, 67.5, 68.2, 68.4, 71.7, 72.2, and 76.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 81.7 (CCH_2), 85.3 (CC_6H_4), 99.0 and 100.8 (C-1 and C-1′), 122.6, 128.1, 131.3, and 134.5 (CC_6H_4), 168.6−170.4 ($COCH_3$ and CONH); HRMS for $C_{78}H_{102}N_4O_{42}$ (M, 1766.5969) M + Na found 1789.6312, calcd 1789.5867.

Divalent Short Spacer 1,3-Rigid Galabioside (27). Yield 76% (51 mg); ^{1}H NMR (300 MHz, D2O) δ = 1.74 (m, 4 H, 2 $OCH_2CH_2CH_2NH$), 3.25 (m, 4 H, 2 $OCH_2CH_2CH_2NH$), 4.02 and 4.05 (2 s, each 4 H, 2 $COCH_2OCH_2CO$), 4.14 (s, 4 H, 2 CCH_2), 4.82 (d, $J_{1,2}$ = 2.7 Hz, 2 H, 2 H-1′), 7.28, 7.35, and 7.45 (3 m, 4 H, C_6H_4);^{13}C NMR (75.5 MHz, D_2O) δ = 29.2 ($OCH_2CH_2CH_2NH$), 29.6 (CCH_2), 36.6 ($OCH_2CH_2CH_2NH$), 60.5 and 61.1 (C-6 and C-6′), 68.1 ($OCH_2CH_2CH_2NH$), 69.4, 69.6, 69.9, 71.4, 71.6, 73.0, 75.6, 77.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 70.5 (2 C, $COCH_2OCH_2CO$), 82.2 (CCH_2), 86.2 (CC_6H_4), 100.9 (C-1′), 103.5 (C-1), 172.1 (CONH); HRMS for $C_{50}H_{74}N_4O_{28}$ (M, 1178.4490) M + Na found 1201.4894, calcd 1201.4388.

Divalent Per-*O*-acetyl-Protected Short Spacer 1,4-Rigid Galabioside (28). Reaction time 1 h; CH_2Cl_2/DMF 5:1; yield 97% (87 mg); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 20.5−20.8 ($COCH_3$), 28.9 ($OCH_2CH_2CH_2NH$), 29.2 (CCH_2), 36.9 ($OCH_2CH_2CH_2NH$), 60.3 and 61.6 (C-6 and C-6′), 68.1 ($OCH_2CH_2CH_2NH$), 70.9 (2 C, $COCH_2OCH_2CO$), 66.9, 67.2, 67.5, 68.2, 68.5, 71.8, 72.3, and 76.8 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 82.3 (CCH_2), 86.3 (CC_6H_4), 99.1 and 100.9 (C-1 and C-1′), 168.8−170.4 ($COCH_3$ and CONH); HRMS for $C_{78}H_{102}N_4O_{42}$ (M, 1766.5969) M + Na found 1789.6218, calcd 1789.5867.

Divalent Short Spacer 1,4-Rigid Galabioside (29). Yield 93% (50 mg); ^{1}H NMR (300 MHz, D_2O) δ = 1.73 (m, 4 H, 2 $OCH_2CH_2CH_2NH$), 3.24 (m, 4 H, 2 $OCH_2CH_2CH_2NH$), 3.54 (dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.5 Hz, 2 H, 2 H-2), 3.53−3.59 (m, 10 H, 2 each H-3, H-5, H-6a′, H-6b′, and $OCHHCH_2CH_2NH$), 3.66−3.79 (m, 10 H, 2 each H-2′, H-3′, H-6a, H-6b, and $OCHHCH_2CH_2NH$), 3.78 (s, 4 H, 2 H-4 and 2 H-4′), 3.90 and 3.93 (2 s, each 4 H, 2 $COCH_2OCH_2CO$), 4.05 (s, 4 H, 2 CCH_2), 4.12−4.18 (m, 4 H, 2 H-1 and 2 H-5′), 4.82 (d, $J_{1,2}$ = 2.7 Hz, 2 H, 2 H-1′), 7.26 (s, 4 H, C_6H_4); ^{13}C NMR (75.5 MHz, D_2O) δ = 29.2 ($OCH_2CH_2CH_2NH$), 29.6 (CCH_2), 36.6 ($OCH_2CH_2CH_2NH$), 60.5 and 61.1 (C-6 and C-6′), 68.1 ($OCH_2CH_2CH_2NH$), 69.4, 69.6, 69.9, 71.4, 71.6, 73.0, 75.6, 77.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 70.5 (2 C, $COCH_2OCH_2CO$), 82.5 (CCH_2), 87.1 (CC_6H_4), 101.0 (C-1′), 103.6 (C-1), 172.1 (CONH); HRMS for $C_{50}H_{74}N_4O_{28}$ (M, 1178.4490) M + Na found 1201.4495, calcd 1201.4388.

Divalent Per-*O*-acetyl-Protected Galabiose Long Spacer (31). Reaction time 1 h; CH_2Cl_2/DMF 15:1; yield 61% (125 mg); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 20.4−20.6 ($COCH_3$), 28.5 (2 C) and 28.9 ($OCH_2CH_2CH_2NH$), 36.3 and 36.8 (2 C, $OCH_2CH_2CH_2NH$), 38.3 (OCH_2CH_2NH), 52.0 ($C_6H_3COCH_3$), 60.4 and 61.6 (C-6 and C-6′), 66.8, 67.1, 67.5, 68.1, 68.5, 71.7, 72.2, and 76.8 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 99.1 and 100.9 (C-1 and C-1′), 106.0, 107.8, 133.6, and 159.2 ($C_6H_3COCH_3$), 166.3 ($C_6H_3COCH_3$), 169.4−170.3 ($COCH_3$ and CONH); HRMS for $C_{106}H_{160}N_8O_{58}$ (M, 2474.42, average mass) M + Na found 2495.6, calcd 2497.4.

Divalent Galabiose Long Spacer (32). Yield 77% (74 mg); ^{1}H NMR (300 MHz, D_2O) δ = 1.64−1.78 (m, 12 H, 6 $OCH_2CH_2CH_2NH$), 3.18−3.26 (m, 12 H, 6 $OCH_2CH_2CH_2NH$), 4.28 (m, 2 H, 2 H-5′), 4.34 (d, $J_{1,2}$ = 7.5 Hz, 2 H, 2 H-1), 4.95 (d, $J_{1,2}$ = 3.0 Hz, 2 H, 2 H-1′), 6.62 and 6.99 (2 s, 1 and 2 H, $C_6H_3COCH_3$); ^{13}C NMR (75.5 MHz, D_2O) δ = 28.8 (2 C), 29.1 ($OCH_2CH_2CH_2NH$), 36.6, 36.7 (2 C, 2 $OCH_2CH_2CH_2NH$), 39.1 (OCH_2CH_2NH), 53.4 ($C_6H_3COCH_3$), 60.6 and 61.0 (C-6 and C-6′), 67.2 (OCH_2CH_2NH), 68.2, 69.0 (2 C, 2 $OCH_2CH_2CH_2NH$), 69.3, 69.5, 69.7, 71.3, 71.5, 72.9, 75.6, 77.6 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 69.9 and 70.1 (each 2 C, $OCH_2CH_2OCH_2CH_2O$), 70.4 (2 C, $COCH_2OCH_2CO$), 100.8 (C-1′), 103.4 (C-1), 107.3, 108.8, 132.0, and 159.9 ($C_6H_3COCH_3$), 168.7

($C_6H_3COCH_3$), 171.8, 172.0, 172.1, and 172.4 (CONH); HRMS for $C_{78}H_{132}N_8O_{44}$ (M, 1885.91, average mass) M + Na found 1908.9, calcd 1908.9

Tetravalent Per-*O*-acetyl-Protected Galabiose Short Spacer (34). Reaction time 4 h; CH_2Cl_2/DMF 15:1; yield 92% (224 mg); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 19.9−20.2 ($COCH_3$), 28.4 ($OCH_2CH_2CH_2NH$), 35.9 ($OCH_2CH_2CH_2NH$), 37.9 (OCH_2CH_2NH), 53.0 ($C_6H_3COCH_3$), 60.3 and 61.6 (C-6 and C-6′), 65.6, 67.0, and 69.2 ($OCH_2CH_2CH_2NH$, OCH_2CH_2NH, and $COCH_2OCH_2CO$), 66.5, 67.0, 67.4, 67.9, 68.4, 71.5, 72.0, and 76.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 98.8 and 100.6 (C-1 and C-1′), 166.5 ($C_6H_3COCH_3$), 169.6−170.4 ($COCH_3$, CONH); HRMS for $C_{166}H_{226}N_{10}O_{94}$ (M, 3865.58, average mass) M + Na found 3888.6, calcd 3888.6.

Tetravalent Galabiose Short Spacer (35). Yield 79% (121 mg); ^{1}H NMR (300 MHz, D2O) δ = 1.73−1.88 (m, 8 H, 4 $OCH_2CH_2CH_2NH$), 3.23−3.37 (m, 8 H, 4 $OCH_2CH_2CH_2NH$), 4.30−4.38 (m, 8 H, 4 H-1 and 4 H-5′), 4.95 (s, 4 H, 4 H-1′), 6.64 and 6.77 (2 s, 3 and 6 H, 3 $C_6H_3COCH_3$); ^{13}C NMR (75.5 MHz, D_2O) δ = 29.1 ($OCH_2CH_2CH_2NH$), 36.6 ($OCH_2CH_2CH_2NH$), 38.9 (OCH_2CH_2NH), 53.1 ($C_6H_3COCH_3$), 60.6 and 61.1 (C-6 and C-6′), 66.9 (OCH_2CH_2NH), 68.1 ($OCH_2CH_2CH_2NH$), 69.3, 69.5, 69.7, 71.3, 71.5, 73.0, 75.6, 77.6 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, and C-5′), 70.4 ($COCH_2OCH_2CO$), 100.8 (C-1′), 103.5 (C-1), HRMS for $C_{110}H_{170}N_{10}O_{66}$ (M, 2688.55, average mass) M + Na found 2711.9, calcd 2711.6.

Octavalent Per-*O*-acetyl-Protected Short Spacer PAMAM Generation 1 Galabioside (37). PAMAM generation 1 **36** (20% weight solution in MeOH; 7.69 μmol) was evaporated to dryness and dissolved in DMSO (5 mL). Compound **13** (54.8 mg, 67.7 μmol), TBTU (23.7 mg, 73.8 μmol), and DIPEA (62.4 μL, 369 μmol) were added and the mixture was stirred for 16 h and then concentrated. The residue was purified on a LH_{20} column eluted with CH_2Cl_2−MeOH (1:1) to give **37** (34.7 mg, 58%), isolated as a white foam. MALDI-MS for $C_{326}H_{488}N_{34}O_{180}$ (M, 7763.48) M + Na found 7787.0, calcd 7786.5.

Octavalent Short Spacer PAMAM Generation 1 Galabioside (38). To a solution of **37** (40.7 mg, 5.24 μmol) in MeOH (10 mL) was added a 3% solution of NaOMe in MeOH (500 μL) and the mixture was stirred for 2 h, then neutralized with Dowex 50X8 (H^+), filtered, and concentrated. The residue was freeze-dried to give **38** (25.2 mg, 89%), isolated as a white solid. ^{13}C NMR (75.5 MHz, D_2O) δ = 29.2 ($OCH_2CH_2CH_2NH$), 30.0 ($COCH_2CH_2N$), 36.7, 38.8, 39.3 ($OCH_2CH_2CH_2NH$ and NCH_2CH_2NHCO), 49.1, 50.3, and 52.7 [$N(CH_2)_3$], 60.7 and 61.1 (C-6 and C-6′), 68.2, 69.3, 69.6, 69.8, 71.4, 71.6, 72.9, 75.7, 77.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, and $OCH_2CH_2CH_2NH$), 70.5 ($COCH_2OCH_2CO$), 100.8 (C-1′), 103.5 (C-1), 172.1−174.4 (CONH).

Bacterial Strains. The two galabiose-binding strains of *S. suis*, 628 and 836, have slightly different adhesin specificities as has been described previously.[19] The bacteria were stored on THY-agar plates (Todd−Hewitt broth supplemented with 5% yeast extract) and cultivated in a CO_2 incubator. For binding assays they were cultivated in THY broth at 37 °C without shaking.

Hemagglutination. Hemagglutination assays were performed as described previously.[36] Briefly, equal volumes of bacteria and 5% sialidase-treated human erythrocytes were mixed and hemagglutination was visually recorded after 1-h incubation on ice. For inhibition assays, 2-fold dilutions of galabiose compounds (25 μL) were mixed with bacteria (25 μL). After 5 min of incubation at room temperature, 50 μL of the erythrocytes was added. The hemagglutination was recorded as described and the MIC values (the lowest concentration completely inhibiting the hemagglutination) were recorded.

Surface Plasmon Resonance Studies. The SPR studies were performed with whole streptococcal cells.[37] Inhibition of *S. suis* 628 binding to pigeon ovomucoid, a glycoprotein rich in terminal galabiose structures, was measured with a BiaLite instrument (Biacore, Uppsala, Sweden). Pigeon ovomucoid was immobilized on an F1 sensor chip with Biacore amine coupling kit according to manufacturer's instructions. An empty channel, without any immobilized protein, was used as a reference. The bacteria, suspended in HBS-P buffer [10 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.4, 0.15 M NaCl, and 0.005% surfactant P20] to an OD_{600} of 0.3, were injected over the sensor chip at a flow rate of 10 μL/min and the binding to immobilized pigeon ovomucoid was recorded. For inhibition assays, dilutions of the galabiose compounds were made in HBS-P buffer and incubated with the bacteria for 5 min before injection over the sensor chip. Between the injections, the sensor chip surface was regenerated with 10 mM NaOH. For inhibition curve calculations the data from repeated experiments were normalized from 0 to 100 by use of the BIAevaluation program (version 3.0, Biacore). The IC_{50} values, that is, the concentration of the compound that elicited half the maximal response, were defined for each compound.

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Supporting Information Available: NMR and mass spectra of the prepared compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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