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Low Molecular Weight Antagonists of the Myelin-Associated Glycoprotein: Synthesis, Docking, and Biological Evaluation

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The injured adult mammalian central nervous system is an inhibitory environment for axon regeneration due to specific inhibitors, among them the myelin-associated glycoprotein (MAG), a member of the Siglec family (sialic-acid binding immunoglobulin-like lectin). In earlier studies, we identified the lead structure **5**, which shows a 250-fold improved in vitro affinity for MAG compared to the tetrasaccharide binding epitope of GQ1bα (**1**), the best physiological MAG ligand described so far. By modifying the 2- and 5-position, the affinity of **5** could be further improved to the nanomolar range (→**19a**). Docking studies to a homology model of MAG allowed the rationalization of the experimental binding properties. Finally, pharmacokinetic parameters (stability in the cerebrospinal fluid, logD and permeation through the BBB) indicate the drug-like properties of the high-affinity antagonist **19a**.

Introduction

The injured adult mammalian central nervous system (CNS) lacks the ability for axon regeneration,[1,2] predominantly due to specific inhibitors expressed on residual myelin and on astrocytes recruited to the site of injury.[3−7] Several inhibitor proteins have been identified, one of them being the myelin-associated glycoprotein (MAG).[8] MAG is a transmembrane glycoprotein[9] belonging to the family of the sialic acid-binding immunoglobulin like lectins, the so-called Siglecs.[10,11] On the surface of neurons, MAG interacts with two classes of targets: proteins of the family of Nogo receptors (NgR[a])[12,13] and the gangliosides GD1a and GT1b.[11,14−16] Although the relative roles of gangliosides and NgRs as MAG ligands have yet to be resolved,[8,17] in some systems, MAG inhibition is completely reversed by sialidase treatment, suggesting that MAG uses sialylated glycans as its major axonal ligands.[18] Therefore, blocking MAG with potent antagonists may be a valuable therapeutic approach to enhance axon regeneration.

So far, the native carbohydrate ligand with the highest affinity to MAG is the ganglioside GQ1bα (Figure 1).[19] Recently, MAG affinity of a partial structure of GQ1bα, the tetrasaccharide **1**, could clearly be correlated with its ability to reverse MAG-mediated inhibition of axonal outgrowth.[20] To reduce the structural complexity of tetrasaccharide **1** and, at the same time, improve its pharmacodynamic and pharmacokinetic properties, numerous MAG antagonists have been prepared. Because the affinity of a series of gangliosides indicated that not only the terminal Neu5Acα(2−3)Gal structure is essential for MAG binding but also the internal sialic acids, the corresponding sialylated and sulfated analogues were synthesized.[21,22] Furthermore, structural information obtained by trNOE NMR[23] and STD NMR[24] suggested that the Galβ(1−3)Gal*N*Ac core contributes to binding mainly by hydrophobic contacts. This was confirmed by the successful replacement of this disaccharide substructure by noncarbohydrate linkers.[25] In addition, the α(2−6)-linked sialic acid could also be replaced by lipophilic substituents.[22] Finally, a pivotal simplification was reported by Kelm and Brossmer when they found that sialic acid derivatives modified in the 2- (e.g., **2**),[26,27] 5- (e.g., **3**),[26,27] or 9-position[26,28] (e.g., **4**) exhibited enhanced antagonistic activity.[26] Combining the best modifications found for the 2[29]- and 9-position[30] in one molecule led to antagonists, e.g., **5**, with affinities in the low micromolar range.[30]

In this communication, we report a small library of MAG antagonists based on lead compound **5**. The binding properties, evaluated by a hapten binding assay, surface plasmon resonance (SPR), and competitive NMR experiments were rationalized by docking studies to a homology model of MAG. Finally, according to pharmacokinetic parameters, e.g., stability in the cerebrospinal fluid, the drug-likeness of the identified high-affinity antagonists could be demonstrated.

Results and Discussion

The sialic acid derivatives reported by Kelm and Brossmer[26] and our group[29,30] exhibit MAG affinities in the low μM range. An example is the neuraminic acid (Neu5Ac) derivative

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[a] BBB, blood−brain barrier; CHO, Chinese hamster ovary; ClAc, 2-chloroacetyl; DCE, 1,2-dichloroethane; DCM, dichloromethane; DMAP, 4-dimethylamino-pyridine; DMF, *N*,*N*-dimethylformamide; FAc, 2-fluoroacetyl; Gal, galactose; Gal*N*Ac, *N*-acetylgalactosamine; IgG, immunoglobulin G; K_D, dissociation constant; MS, molecular sieve; Neu5Ac, *N*-acetylneuraminic acid; NgR, Nogo receptor; NIS, *N*-iodosuccinimide; NMR, nuclear magnetic resonance; nosyl, 2-nitrobenzylsulfonyl; PAMPA, parallel artificial membrane permeation assay; PDB, Protein Data Bank; *i*PrOH, 2-propanol; RP, reversed phase; STD NMR, saturation transfer difference NMR; TfOH, trifluoroacetic acid; THF, tetrahydrofurane; TMS, trimethylsilyl; trNOE, transfer nuclear Overhauser enhancement; *p*-Ts, *p*-tolylsulfonyl.

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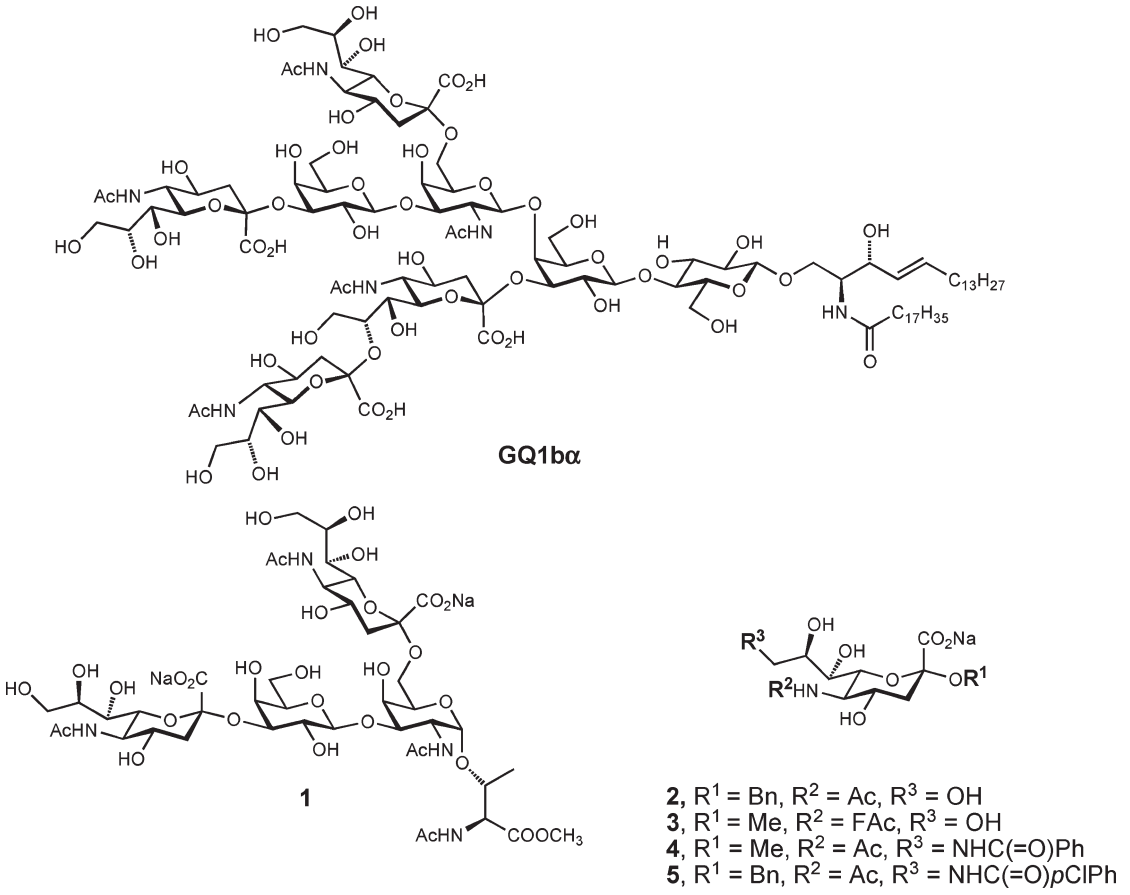


Figure 1. MAG antagonists; GQ1bα, the partial structure **1**,[19,20] and sialic acid derivatives **2**−**5**.[26,30]

5[30] with a K_D of 17 μM. Because a broad optimization effort for the 9-position had led to the identification of *p*-chlorobenzamide as the best substitutent,[30] this position was not further investigated. The changeover from a methyl substituent in the 2-position to a benzyl group was rewarded by a 10-fold gain in affinity.[26] This effect can be rationalized by the results of STD-NMR investigations,[23] indicating a hydrophobic interaction of the α-face of D-galactose (see **1** in Figure 1) with MAG. When the hydrophobic contact was further extended by replacing the benzyl group with phenoxybenzyl or biphenyl substituents, only marginally improved affinities were detected.[29] Because the sheer enlargement of the hydrophobic group did not exhibit improved affinities, the electron density of the aglycone was altered in a next step. Finally, with the substituents in the 2- and 9-position set, a further optimization of the acyl group in the 5-position was conducted.[26,27]

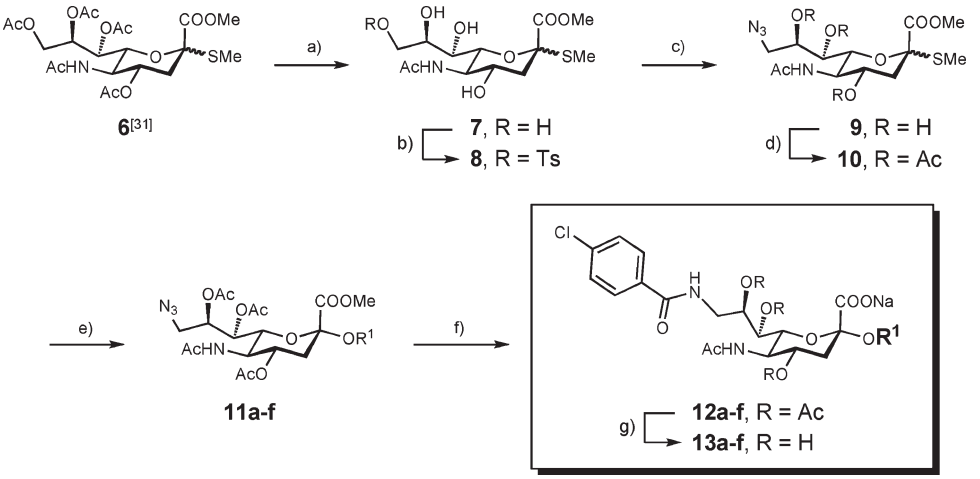
Synthesis of Sialosides 13a−f. Starting from Neu5Ac, thioglycoside **6** was synthesized according to a reported procedure.[31] After deprotection by Zemplén conditions (→**7**), the hydroxy group in the 9-position was selectively tosylated (→**8**).[32] Substitution of the tosylate using sodium azide and 15-crown-5 in DMF (→**9**)[33] followed by acetylation yielded the sialyl donor **10**. For the introduction of the aglycone, **10** was then reacted with various benzyl alcohols (see Table 1, entries 2−9) in the presence of the promotors NIS/TfOH.[34] The sialosides **11a−f** were obtained as separable anomeric mixtures (α/β = 7/1 to 9/1). Amidation with *p*-chlorobenzoylchloride under modified Staudinger conditions[35] (→**12a−f**) and final deprotection gave the amides **13a−f** in good yields (Scheme 1, Table 1).

Synthesis of Sialosides 19a−g. As reported earlier, halogenation of the *N*-acetyl group at the 5-position increases the binding affinity toward MAG by a factor of 10−20.[26] Therefore, both the *N*-fluoroacetyl and *N*-chloroacetyl derivatives **19a** and **19b** were prepared. As sulfonamides adapt a different geometry[36] compared to the corresponding amides, **19c** and **19d** allow a further exploration of the binding site. Finally, the cyclopropyl and cyclobutyl derivatives **19f** and **19g** were synthesized in order to explore the possibility for extended hydrophobic contacts.

For the cleavage of the *N*-acetate group, **10** was treated with hydrazine in the presence of $(Boc)_2O$[37,38] and subsequently reacetylated (→**14**). Deprotection with TMSCl and PhOH[39] (→**15**), followed by acylation with carboxylic or sulfonic acid derivatives, yielded **16a−d**. Glycosylation using 2,3-difluorobenzyl alcohol (→**17a−d**), amidation using modified Staudinger conditions[35] (→**18a−d**) and final deprotection gave the test compounds **19a−d**. When methanolic NaOH was used in the final deprotection step of **18b**, a nucleophilic substitution occurred, leading to a 1:1 mixture of the desired **19b** and the methoxyacetamide derivative **19e** (Scheme 2).

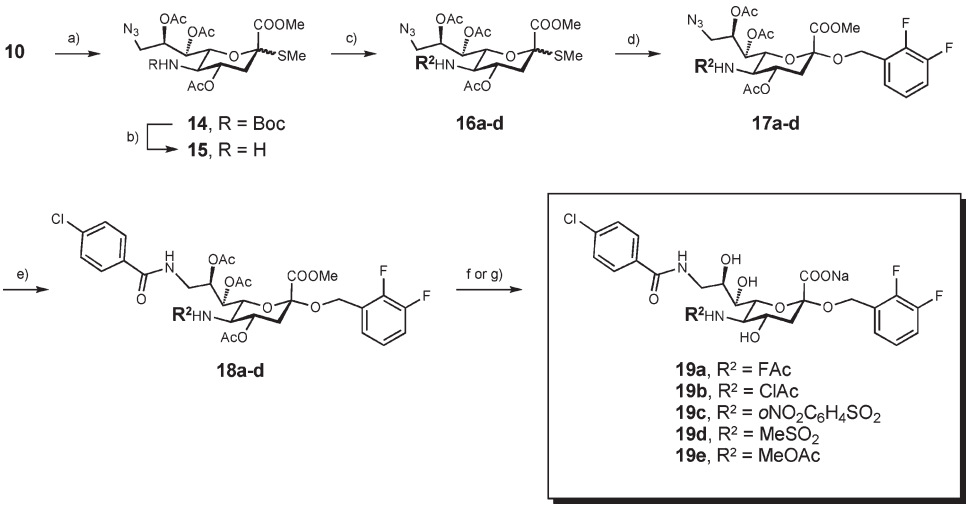
For the synthesis of the two remaining test compounds **19f** and **19g**, an analogue approach starting from the thiosialoside **10** was accomplished. However, in contrast to the synthesis of **19a−e**, a different sequence of the modifications conducted at the 2-, 5-, and 9-position was performed (Scheme 3). Cleavage of the *N*-acetate (→**14**) and amidation under modified Staudinger conditions yielded compound **20**. Next, *N*-deprotection followed by *N*-acylation (→**22f** and **22g**) and benzylation (→**23f** and **23g**) yielded, after final

Scheme 1[a]



[a] (a) NaOMe, MeOH (61%); (b) *p*-TsCl, pyridine (66%); (c) NaN_3, 15-crown-5, DMF (78%); (d) Ac_2O, DMAP, pyridine (73%); (e) R^1OH, NIS, TfOH, MeCN (**11a**−**f**, α-isomers: 54−76%; *β*-isomers: 8−11%); (f) PPh_3, *p*-chlorobenzoylchloride, DCE, rt (**12a**−**f**, 45−60%); (g) 10% aq NaOH; Dowex 50 × 8, Na^+ form (**13a**−**f**, 39−70%).

Scheme 2[a]



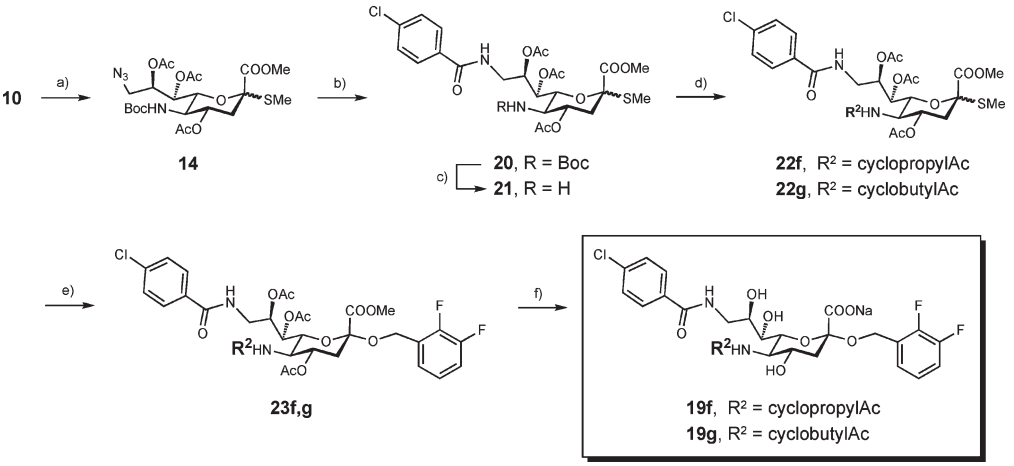
[a] (a) (i) Boc_2O, DMAP, THF, 60 °C, 4 h, (ii) $N_2H_4 \cdot H_2O$, MeOH, rt, 24 h, (iii) Ac_2O, pyridine (76%); (b) 4 M PhOH, 4 M TMSCl in abs DCM (70%);[39] (c) acylation agent, NEt_3, DMAP, abs DCM, rt, 4 h or $[ClCH_2C(=O)]_2O$, NEt_3, dioxane/H_2O, rt (**16a**−**d**, 66−85%); (d) 2,3-difluorobenzyl alcohol, NIS, TfOH, MeCN (**17a**−**d**, α-isomers: 56−68%; *β*-isomers: 8−11%); (e) PPh_3, *p*-chlorobenzoylchloride, DCE, rt (**18a**−**d**, 48−58%); (f) 10% aq LiOH, THF/H_2O; Dowex 50 × 8, Na^+ form (**19a**−**d**, 30−60%); (g) **18b**, 10% aq NaOH; Dowex 50 × 8, Na^+ form (**19b**, 21%, **19e**, 19%).

deprotection, the desired cycloalkylacetic acid derivatives **19f** and **19g** in excellent overall yields.

Biological Evaluation. For the evaluation of the binding properties of the sialosides **13a**−**f** and **19a**−**g**, two previously reported assay formats were applied: a fluorescent hapten binding assay[40] and a SPR based biosensor (Biacore) experiment[22] (Table 1). For the hapten inhibition assay, a recombinant protein consisting of the three *N*-terminal domains of MAG and the Fc part of human IgG (MAG_{d1-3}-Fc) was produced by expression in CHO cells and affinity purification on protein A-agarose.[40] The relative inhibitory concentrations (rIC_{50}) of the test compounds as competitive ligands were determined in microtiter plates coated with fetuin as the binding target for MAG_{d1-3}-Fc. By complexing the Fc-part with alkaline phosphatase-labeled anti-Fc antibodies and measuring the initial velocity of fluorescein release from fluorescein diphosphate, the amount of bound MAG_{d1-3}-Fc could be determined. At least three independent titrations were performed for each compound tested with seven or eight concentrations in triplicates. The affinities were measured relative to the reference compound **5** (rIC_{50} of 1, Table 1, entry 2). For the K_D determination in the Biacore assay, MAG_{d1-3}-Fc was immobilized on a dextran chip containing a surface of covalently bound protein A. A reference cell providing only protein A was used to compensate unspecific binding to the matrix.

By analyzing the affinities of **13a**−**f**, a substantial increase in affinity was achieved when the aromatic aglycone was halogenated in *ortho*- and *meta*-positions (entries 8 and 9). Less effective were halogens in the *para*-position (entries 4 and 5). In addition, with fluorine instead of chlorine, consistently slightly higher affinities (entries 4 and 8 vs entries 5 and 9) were obtained. With the symmetric pentafluoro benzyl group in **13c** (entry 6), an increase in affinity was expected, caused by an improved preorganization of the aglycone in the bioactive conformation. The observed

Scheme 3[a]



[a] (a) (i) Boc_2O, DMAP, THF, 60 °C, 4 h, (ii) $N_2H_4 \cdot H_2O$, MeOH, rt, 24 h, (iii) Ac_2O, pyridine (76%); (b) PPh_3, *p*-chlorobenzoylchloride, DCE, rt (48%); (c) 4 M PhOH, 4 M TMSCl in abs DCM (63%);[39] (d) R^2COCl, NEt_3, DMAP, abs DCM, rt, 4 h (**22f**, 75%, **22g**, 39%); (e) 2,3-difluorobenzyl alcohol, NIS, TfOH, MeCN (**23f**, α: 73%, *β*: 8%; **23g**, α: 72%, *β*: 8%); (f) 10% aq LiOH, THF/H_2O; Dowex 50 × 8, Na^+ form (**19f**, 44%; **19g**, 50%).

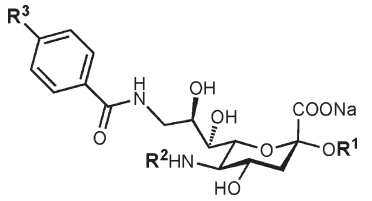
diminished affinity, i.e., a loss of a factor 3 compared to **13f**, may be the result of steric hindrance. Finally, when a 2-naphthylmethyl substituent was introduced (**13d**), a slight improvement of affinity compared to the benzyl substituent as present in **5** was obtained, presumably due to a favorable $\pi-\pi$ interaction of the extended aromatic system. In a next step, substituent R^2 was optimized based on the so far most active antagonist **13f**. Kelm and Brossmer have demonstrated that halogenated acetamides in the 5-position of sialic acid derivatives strongly improve binding of MAG antagonists.[26] When this observation was applied to our series, the nanomolar fluoroacetamide **19a** (entry 10) was obtained. For chloroacetamide derivative **19b** (entry 11), the effect was less pronounced. Interestingly, an equally potent antagonist was achieved with the phenylsulfone substituent (**19c**, entry 12), while sulfone **19d** (entry 13) suffers from a drastic loss in activity. This is quite unexpected because an increase in the size of the acyl substituent was reported to lead to a reduction in affinity,[27] an observation that was confirmed by compounds **19e**−**g** (entries 14−16).

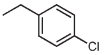
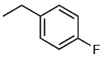
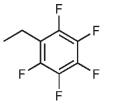
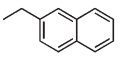
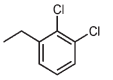
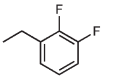
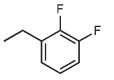
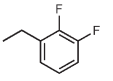
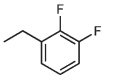
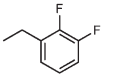
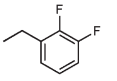
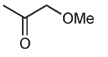
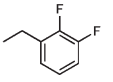
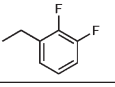
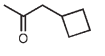
Stability in Cerebrospinal Fluid. For nerve regeneration, MAG antagonists will most likely be applied to the CNS by a local infusion. We therefore tested the stability of the fluoroacetate **19a** and the corresponding acetate **13f** in artificial cerebrospinal fluid (aCSF)[41] and, as a control, in buffer solution for 19 h at 37 °C. According to LC-MS analysis, more than 95% of the initial concentrations of both antagonists were recovered from both media, predicting a high stability in the CNS, the target compartment of an in vivo application. Furthermore, $\log D_{\text{octanol/water}}$ values from −0.27 to 0.87 (see Table 1) might be beneficial for an intrathecal application because these distribution coefficients suggest a loss from the CNS compartment by a passive transport mechanism to be unlikely. This hypothesis is further supported by the results of the BBB-PAMPA assay showing log P_e values for **13f** and **19a** in the range of −10. For values below −5.7,[42] no passive permeation through the BBB is expected.

Surface Plasmon Resonance (SPR). The interaction between MAG and MAG antagonists was analyzed by SPR experiments.[43−45] For this purpose, MAG_{d1-3}-Fc was immobilized on protein A, which on its part was covalently linked to a carboxymethyl dextrane surface of the chip. Whereas earlier Biacore investigations with MAG antagonists produced traditional sensorgrams,[22] consistently negative sensorgrams, i.e., a net decrease in resonance units, were obtained with the compound series reported in Table 1. As an example, the sensorgram of **13f** is shown in Figure 2a. When fitted to a binding isotherm, these negative sensorgrams appear to clearly result from specific receptor−ligand interactions. To elucidate the origin of this unusual result, numerous factors such as the buffer capacity, the ion strength of the buffer, type, and matrix of the sensor chip as well as the applied type of immobilization of MAG_{d1-3}-Fc were analyzed.

As a result of ionizable functional groups of the analytes, pH variabilities could occur, a phenomenon previously reported by Mannen et al.[46] To avoid this effect, a sufficient buffer capacity, 50 mM instead of 10 mM HEPES, was applied. Furthermore, to exclude ionic repulsion effects, measurements were carried out at increased salt concentrations (150−500 mM NaCl). In addition, the effect of potential nonspecific binding to the dextran matrix or to protein A was analyzed by adding either dextran (1 mg/mL) to the buffer system or by conducting the experiment on a regenerated protein A surface. Because all these modifications of assay parameters did not provide an indication for the cause of the negative sensorgrams, different dextran biosensor chips, varying in carboxylate density (CM5 vs CM4), were analyzed as well. Although the reduction in signal intensity correlated well with the degree of functionalization of the chip surface, no influence on the sign of the sensorgrams could be detected (see Table S1 in Supporting Information). A further explanation for the negative sensorgrams could be a ligand-induced conformational change of the immobilized receptor leading to a decrease of its hydrodynamic radius and, as a consequence, yielding a negative refraction index.[47,48] Because the negative refraction index correlates with the analyte concentration, we mirrored the negative sensorgrams, for an example see the sensorgram of **13f** in Figure 2b, to obtain SPR-determined equilibrium dissociation constants (K_Ds, see Table 1).

Table 1. Relative Inhibitory Concentrations (rIC_{50}) Relative to Reference Compound **5**, K_D Values, and $logD_{7.3}$ of Sialosides **13a−f** and **19a−g**



Entry	Compound	R^1	R^2	R^3	rIC_{50}[a]	K_D [μM]	logD (pH 7.3)
1	**4**[26-28]	$-CH_3$	Ac	H	18.00	137[b]	n.d.[c]
2	**5**[30]		Ac	Cl	1.00	17	n.d.[c]
3	**24**[30]		Ac	H	1.50	26	n.d.[c]
4	**13a**		Ac	Cl	1.30	15	0.36
5	**13b**		Ac	Cl	1.20	13	-0.11
6	**13c**		Ac	Cl	0.26	6.1	-0.11
7	**13d**		Ac	Cl	0.74	11.6	0.58
8	**13e**		Ac	Cl	0.50	4.3	0.53
9	**13f**		Ac	Cl	0.30	2.4	-0.27
10	**19a**			Cl	0.02	0.5	-0.26
11	**19b**			Cl	0.07	2.1	0.35
12	**19c**			Cl	0.05	1.4	0.87
13	**19d**			Cl	0.60	17	-0.17
14	**19e**			Cl	0.14	2.3	0.06
15	**19f**			Cl	0.10	4.1	0.31
16	**19g**			Cl	0.14	5.8	0.75

[a] rIC_{50} is the concentration when 50% of the protein are inhibited, measured relative to reference compound **5**. [b] The affinity of compound **4** was measured using different protein batches, resulting in K_Ds of 137 μM and 105 μM (Table 2). All affinity data given in Table 1 were obtained with the protein batch showing a K_D of 137 μM for compound **4**; [c] n.d. not determined

To justify this procedure, we analyzed whether K_Ds obtained by mirroring the negative sensorgrams can be correlated with rIC_{50} values determined by the fluorescent hapten binding assay[40] (Figure 3). K_Ds from previously reported compounds[25,30] (see Table S2, Supporting Information), which exhibit much higher molecular weights and therefore

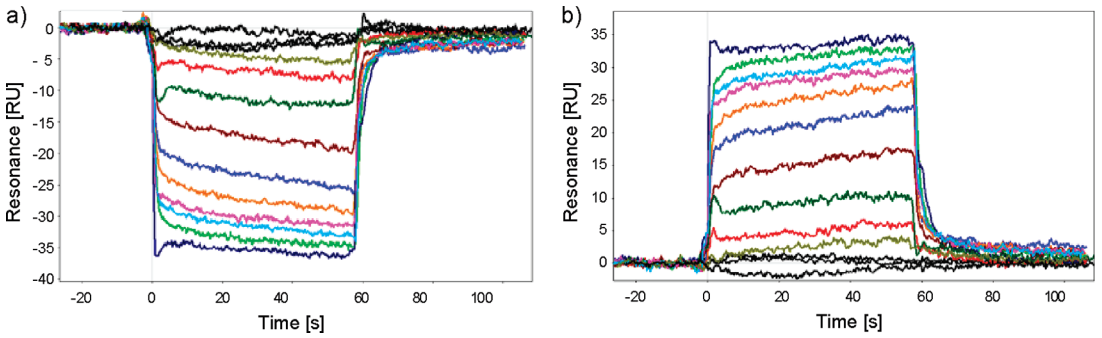


Figure 2. (a) Biacore sensorgrams for **13f** after subtraction of the reference; (b) mirrored sensorgrams of **13f**.

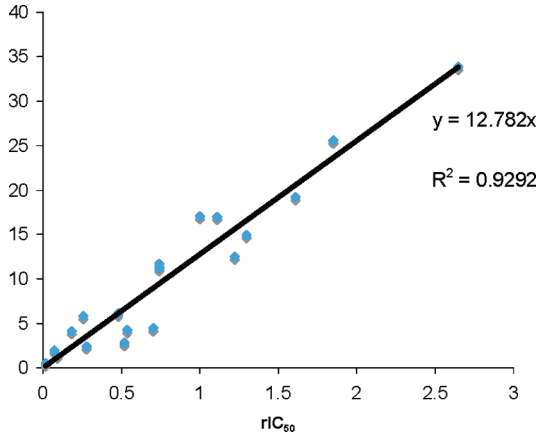


Figure 3. Correlation of K_D values obtained by SPR measurements with rIC_{50} values determined in the competitive inhibition assay;[40] Compounds **5**, **13a**−**f**, **19a**−**g**, and the compounds **25**−**29** from Table S2 (Supporting Information) were included.

generate positive sensorgrams, were also included in this correlation. The obtained correlation factor R^2 of 0.93 clearly suggests that the mirroring procedure does not falsify the binding information.

Determination of Relative Affinities by NMR. A further argument for the acceptance of the above-described mirroring of the sensorgrams was accomplished by competitive NMR experiments.[49] The approach is based on the molecular weight dependence of selective inversion recovery experiments (sT1). In the absence of binding, a selectively inverted NMR signal of a ligand requires a relatively long time to recover back to equilibrium. By contrast, if the ligand binds to a receptor, the time required to recover back to equilibrium is reduced. As a result, the binding of a ligand to a receptor is detectable through sT1 experiments. Furthermore, these sT1 experiments can be used for the ranking of the affinities of ligands relative to a reference compound.

For our purpose, the binding of antagonist **4** to MAG_{d1-3}-Fc was used as reference (K_D determined by Biacore is 137 μM), whereas compounds **13f** and **25**[22] (for the structure, see Supporting Information) were chosen as competitors because they fulfill two criteria. First, SPR experiments with both **13f** and **25** gave comparable K_D values, 2.4 and 2.8 μM, respectively, and therefore require comparable concentrations for the observation of competitive binding. Second, the observed sensorgrams of compounds **13f** and **25** are of opposite sign, negative and positive, respectively.

In a first NMR experiment, it was demonstrated that **4** binds to MAG_{d1-3}-Fc according to the large differences between selective inversion recovery of the *para*-hydrogen of the benzamide substituent in the presence or absence of MAG_{d1-3}-Fc (Figure 4a). For a quantitative evaluation of the relative affinities of **13f** and **25**, a titration curve describing the concentration dependence of the selective inversion recovery time of **4** was required (Figure 4b). The selective inversion time constants (sT1) were fit to a one-site binding model.

On the basis of the titration curve shown in Figure 4b, the determination of the relative affinities of **13f** and **25** became possible. With a NMR sample consisting of MAG_{d1-3}-Fc and compound **4** (500 μM), sT1 for the initial point of the titration curve was remeasured. The obtained sT1 of 1.03 ± 0.08 s compared to 1.05 ± 0.06 s for the first sample indicated a high degree of reproducibility. In a second step, 25 μM of either compound **13f** or **25** were added. Then, the sT1s were measured and the apparent concentration of compound **4** determined. When 25 μM of compound **13f** were added, the sT1 increased to 1.84 ± 0.06 s, indicating an apparent concentration of compound **4** of 1.36 mM. Subtracting the actual concentration of compound **4** from its apparent concentration and dividing the result by the concentration of the inhibitor yields a relative affinity of 34.4 ± 2.1 for compound **13f** with respect to the reference compound **4** with the relative affinity of 1. With the identical procedure, a relative affinity of 17.6 ± 1.0 for compound **25** with respect to compound **4** was found (Figure 5).

The competitive NMR assay demonstrates that compounds **13f** and **25** both bind to MAG_{d1-3}-Fc with an affinity more than 1 order of magnitude greater than reference compound **4**. The relative affinities displayed in Figure 5 are in good agreement with the corresponding values determined by Biacore and the hapten inhibition assay.[40] Allowing a factor of 2 in the estimation of a compound's affinity with a single technology implies that the comparison of affinities between two assay formats may differ by as much as a factor of 4. Because for any of the three assays employed the affinities of **13f** and **25** to MAG_{d1-3}-Fc are within this range, the mirroring of the negative sensorgrams for the determination of K_D values is further justified.

Determination of Enthalpic and Entropic Contributions to Binding. For the elucidation of the thermodynamic parameters of the MAG/antagonist interaction, K_Ds were measured in the Biacore assay at different temperatures. The analysis of a library of ligands containing structural modifications at the 2-, 5-, and 9-position of the neuraminic acid scaffold should allow the assignment of the enthalpic and entropic contributions to the various structural elements. The K_D values were determined at six different temperatures, starting at 5 °C and elevating the temperature by 5 °C

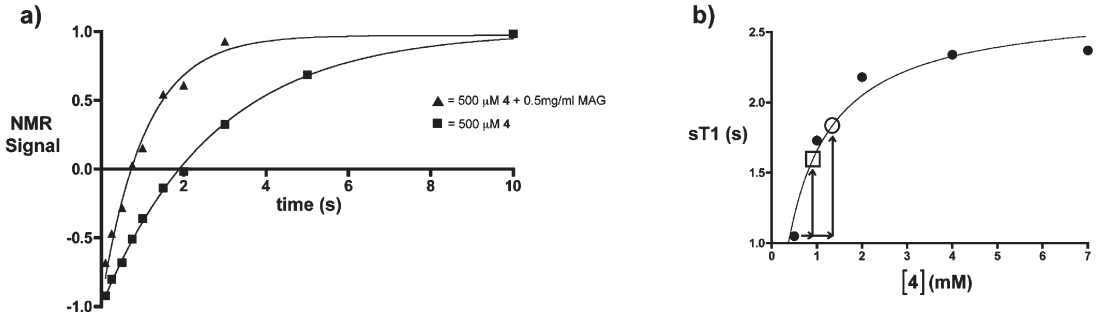


Figure 4. (a) Selective inversion recovery (sT1) of the *para*-hydrogen of the benzamide in compound **4**, either in the presence of MAG_{d1-3}-Fc (filled triangles) or in the absence of MAG_{d1-3}-Fc (filled squares). The normalized NMR signal represents the intensity of the *para*-hydrogen at a particular time divided by its intensity after 60 s of relaxation. (b) Titration of MAG_{d1-3}-Fc with compound **4**, and the observed sT1 (filled circles), observed sT1 when 500 μM of **4** were mixed with 25 μM **13f** (hollow circle), and observed sT1 when 500 μM of **4** were mixed with 25 μM **25** (hollow square). Vertical and horizontal arrows indicate the extent of attenuated relaxation of **4** when mixed with 25 μM or competitor **13f** or **25** and the apparent concentration of **4**, respectively.

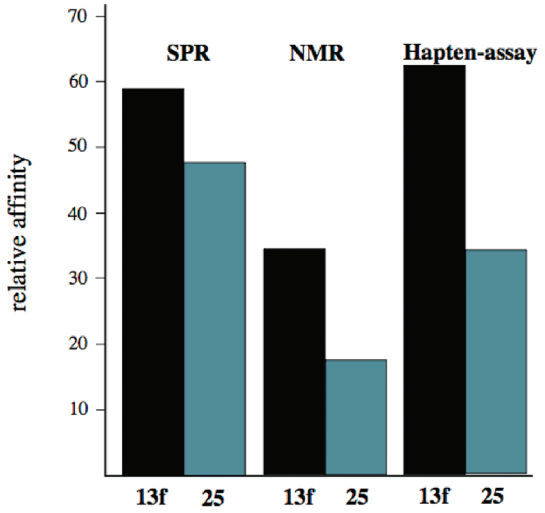


Figure 5. Affinity of compounds **13f** (black) and **25** (gray), relative to the affinity of compound **4** (= relative affinity of 1). The relative affinities are determined by NMR, Biacore, and the fluorescent hapten assay.[40]

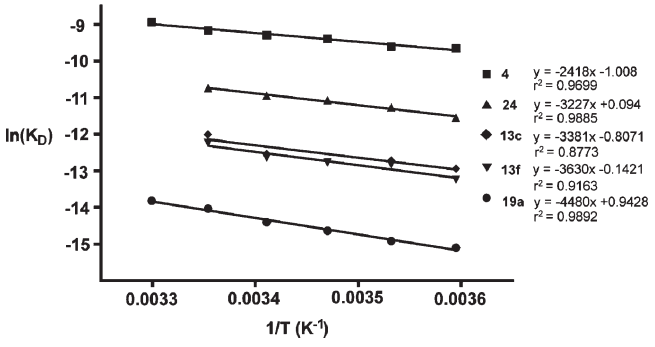


Figure 6. Van't Hoff plot. Measured data (dots) and corresponding linear fits according to eq 1.

$$\ln K_D = \frac{\Delta H}{RT} - \frac{\Delta S}{R} \quad (1)$$

steps up to 30 °C. The values were fitted according to eq 1 (Figure 6) leading to ΔH and ΔS[50] (Table 2).

The analysis (Table 2) revealed that the improvement of the binding energies ΔG resulted mainly from enhanced binding enthalpies ΔH. The substitution of the methoxy group at position 2 in **4** by a benzyloxy group (**24**, entry 18)[30] increased binding enthalpy by more than 6 kJ/mol and at the same time caused substantial entropy costs upon binding. With the pentafluorobenzyloxy substituent (**13c**, entry 19), both ΔH and ΔS were improved. Apparently, the interaction of the reducing end substituent can be optimized with an electron-poor aromatic ring. When the 2,3-difluorinated benzyloxy substituent (**13f**, entry 20) was introduced, ΔG could be further improved, mainly by a favorable enthalpy change. On the other hand, entropy costs increased as a result of the asymmetric substitution. The binding energy of the most active compound **19a** (entry 21, *N*-acetate replaced by *N*-fluoroacetate) is mainly based on a further enthalpic improvement. Unfortunately, in this case entropy costs of more than 2 kJ/mol have to be compensated, probably due to a specific conformational orientation requested for an optimal interaction of the FAc group.

Table 2. Weighting of ΔH and ΔS with Respect to ΔG

entry	compd	ΔH [kJ/mol]	$-T^a\Delta S^*$ [kJ/mol]	ΔG [kJ/mol]	K_D [μM]
17	**4**[30]	−20.09	−2.52	−22.61	106[b]
18	**24**[30]	−26.85	0.25	−26.60	22
19	**13c**	−28.13	−1.98	−30.1	6.1
20	**13f**	−30.31	−0.20	−30.52	2.4
21	**19a**	−37.33	2.42	−34.91	0.5

[a] T = 298.13 K. [b] The affinity of compound **4** was measured using different protein batches, resulting in K_Ds of 137 μM and 106 μM. For the data given in Table 2, the protein batch showing a K_D of 106 μM for compound **4** was used.

Structure−Affinity Relationships. To interpret the binding affinities at the molecular level, we performed molecular docking studies. In the absence of a crystal structure, we used a homology model of the ligand-binding domain of MAG, which was recently applied successfully to a series of MAG antagonists (Figure 7).[22] The compounds were manually docked using the salt bridge between the carboxylate of the sialosides with Arg118 and the hydrogen bond of C(9)-NH to the backbone nitrogen of Thr128 as anchor points. Finally, the protein−ligand complexes were fully minimized in aqueous solution.

For validation purposes, 12 compounds were docked and their binding strength quantified. Because these compounds bind at the protein surface, the contribution of solvation and entropy are difficult to estimate from thermodynamic docking studies. We therefore performed a series of molecular-dynamical simulations (4.0×10^{-9} s at 300 K) to elucidate the

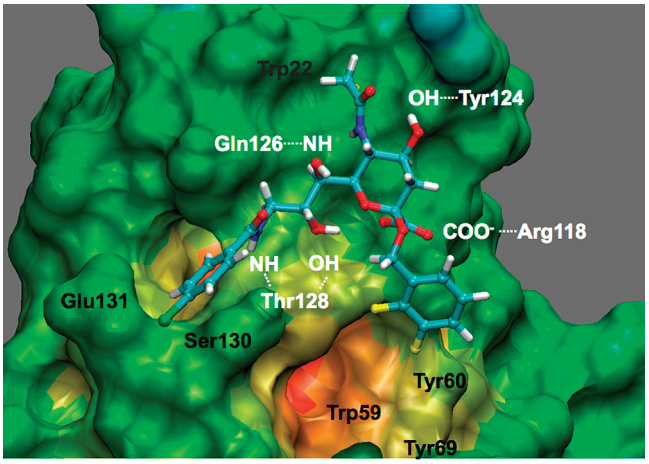


Figure 7. Homology model of MAG[22] complexed with **19a** (most active compound of the series). Amino acids in white are forming hydrogen bonds and amino acids in black contribute to hydrophobic pockets. The salt bridge formed by the carboxylate group of **19a** with Arg118 and a hydrogen bond by C(9)-NH and the backbone nitrogen of Thr128 were used as anchor points for the docking. Hydrophobic interactions are established by the fluoroacetamido group and the side chains of Trp22 and Tyr124, the *p*-chlorobenzamide, and the side chains of Ser130 and Glu131, and the reducing end substituent, the 2,3-difluorobenzyl and the side chains of Trp59, Tyr60, and Tyr69, lining the main hydrophobic pocket. The image has been generated using VMD.[51]

kinetic aspects of binding and to quantify the contribution of hydrogen bonding over time. Details are given in the Supporting Information.

Upon analyzing the docking studies, the binding affinity could be associated to hydrophilic as well as hydrophobic interactions. The most important contribution is the salt bridge between the carboxylic acid and Arg118.[26,52,53] Additionally, hydrogen-bond formations between 5-NH and the backbone carbonyl of Gln126, the carboxylate and the OH of Thr128, 8-OH and the backbone NH of Thr128 and 9-NH and the backbone carbonyl of Thr128 are observed. This latter finding is in good agreement with previous studies, where the abolishment of a hydrogen bond donor at position 9 resulted in a reduced binding affinity.[26] A considerable contribution to the binding affinity results from hydrophobic interactions. Thus, the *p*-chlorobenzamide is shown to point into a hydrophobic pocket, built by Ser130 and Glu131. A second hydrophobic pocket, which hosts the aglycone substituents, is defined by the side chains of Trp59, Tyr60, and Tyr69. With respect to different substitution patterns, the dichloro compound **13e** (Table 1, entry 8) shows a 4-fold and the difluoro compound **13f** (see Table 1, entry 9), a 7-fold enhancement in affinity compared to reference compound **5**, indicating a charge transfer complex with the electron rich aromatic ring of Tyr60. The only moderate improvement in binding affinity for the halogenated compounds **13a** and **13b** could be due to a steric clash of the *p*-substitutent with the protein. Compound **13c** was synthesized as a symmetric analogon of compound **13f** in order to compensate entropy loss due to the orientation of the 2,3-difluorobenzyl ring. Again, that there is no improvement of the binding affinity in comparison to compound **13f** may be the consequence of a steric clash based on the *p*-substitutent. Finally, the improved binding of **13d** might result from favorable $\pi-\pi$ interactions of the naphthalene with Tyr69.

Some of the compounds modified at the 5-position seem to undergo a favorable $\sigma-\pi$ interaction with Trp22. In case of **19a** and **19b**, we assume that the positively polarized hydrogens of the FAc or ClAc, respectively, stick favorably into the aromatic ring.[54] As fluorine is more electronegative, the polarization of the hydrogens is stronger and therefore the interaction is more favorable. For compounds **19f** and **19g**, additional hydrophobic interactions are possible, but the binding site seems to be spatially limited.[27] The reduced affinity of **19d** could be a consequence of the different bond angle for the sulfonamide substituent compared to an acetate in the same position (**13f**, entry 9), leading to different spatial requirements. Whereas methylsulfonamide **19d** shows a decrease in binding affinity, the nosyl substituent (**19c**) shows an opposite behavior. This might be due to the formation of a charge transfer complex with Trp22. To summarize, modifications at the reducing end improved binding affinity by a factor of 7 (**5**→**13f**). Combined with the best modification at the 5-position, the high affinity ligand **19a** was obtained.

Conclusion

In conclusion, the nanomolar affinity of the sialic acid derivative **19a** containing a difluorobenzyl substituent at the 2-, a fluoroacetate at the 5-, and *p*-chlorobenzamide at the 9-position clearly indicates the additivity of the beneficial effect of the various modifications. In addition, the thermodynamic analysis reveals that the improved affinity of **19a** predominantly results from an increased binding enthalpy and not from an entropy gain. The beneficial pharmacokinetic properties, e.g., a high stability in the cerebrospinal fluid, also support the drug-like properties of the newly identified MAG antagonist. However, due to the shallow binding site generally responsible for short half-lifes of carbohydrate−protein complexes,[22,55−58] it remains to be shown whether the complex formed by **19a** and MAG exhibits sufficient kinetic stability for in vivo applications.

Experimental Section

Chemistry. NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^{1}H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC, TOCSY). Chemical shifts are expressed in ppm using residual $CHCl_3$, CHD_2OD, and HDO as references. Optical rotations were measured using Perkin-Elmer polarimeters 241 and 341. MS analyses were carried out using a Waters Micromass ZQ Detector system. The spectra were recorded in positive or negative ESI mode. The HPLC/HRMS analyses were carried out using a Agilent 1100 equipped with a photodiode array detector and a Micromass QTOF I equipped with a 4 GHz digital-time converter. All target compounds exhibit a purity of ≥95%. Reactions were monitored by TLC using glass plates coated with silica gel 60 F_{254} (Merck) and visualized by using UV light and/or by charring with a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). Column chromatography was performed on silica gel (Uetikon, 40−60 mesh). Methanol was dried by refluxing with sodium methoxide and distilled immediately before use. Pyridine was freshly distilled under argon over CaH_2. Dichloromethane (DCM), dichloroethane (DCE), acetonitrile (MeCN), toluene, and benzene were dried by filtration over Al_2O_3 (Fluka, type 5016 A basic). Molecular sieves (3 Å) were activated under vacuum at 500 °C for 2 h immediately before use. Compound **6** was prepared according to a published procedure.[31] HPLC chromatograms and ^{1}H NMR spectra of the target compounds can be found in the Supporting Information.

Methyl (Methyl 5-Acetamido-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (7). Compound **6** (217 mg, 42.0 mmol) was dissolved in dry MeOH (8.0 mL) and treated with NaOMe (1 M, 1.0 mL) for 2 h. The reaction mixture was neutralized with Amberlyst 15, filtered over a pad of celite, and the celite washed thoroughly with MeOH. The solvent was evaporated under reduced pressure, and the crude product was purified by chromatography on silica gel (1% gradient MeOH in DCM) to yield **7** as a white foam (90.0 mg, 61%). ^{1}H NMR (500 MHz, CD_3OD) δ 1.97 (dd, J = 11.5, 13.9 Hz, 1H, H-3a), 2.03 (s, 3H, SMe), 2.09 (s, 3H, NHAc), 2.47 (dd, J = 4.9, 13.9 Hz, 1H, H-3b), 3.54 (d, J = 9.4 Hz, 1H, H-7), 3.67 (dd, J = 5.6, 11.6 Hz, 1H, H-9a), 3.81−3.86 (m, 6H, H-5, H-8, H-9b, OMe). 4.11 (m, 2H, H-4, H-6). ^{13}C NMR (CD_3OD) δ 11.2 (SMe), 22.7 (NHAc), 41.2 (C-3), 53.1 (OMe), 54.1 (C-5), 65.2 (C-9), 68.3 (C-4), 70.2 (C-7), 71.2 (C-8), 72.6 (C-6), 84.6 (C-2), 170.8, 175.0 (2 CO). ESI-MS calcd for $C_{13}H_{23}NO_8S$ $[M + Na]^+$ 376.10; found m/z 376.10.

Methyl (Methyl 5-Acetamido-3,5-dideoxy-2-thio-9-tosyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (8). To a solution of **7** (3.20 g, 9.07 mmol) in freshly distilled pyridine (80 mL) *p*-toluenesulfonyl chloride (1.90 g, 10.0 mmol) was added at 0 °C and the mixture was stirred for 2 h at 0 °C. Afterward tosyl chloride (0.70 g, 3.68 mmol) was added and the solution stirred continuously for 16 h at 5 °C. The reaction mixture was warmed to rt, methanol (20 mL) was added and stirring continued for 30 min. After evaporation of the solvents, the crude product was purified by chromatography on silica gel (DCM:MeOH, 19:1) to give **8** as a foam (3.00 g, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.79 (dd, J = 11.2, 13.4 Hz, 1H, H-3a), 1.96 (s, 3H, SMe), 1.99 (s, 3H, NHAc), 2.37 (s, 3H, CH_3), 2.74 (dd, J = 3.7, 13.2 Hz, 1H, H-3b), 3.25 (d, J = 9.5 Hz, 1H, H-6), 3.41 (d, J = 9.7 Hz, 1H, H-7), 3.66 (s, 3H, OMe), 3.98 (m, 1H, H-8), 4.10 (m, 1H, H-9a), 4.24 (m, 1H, H-9b), 6.94 (d, J = 7.2 Hz, 1H, N*H*Ac), 7.28, 7.72 (AA′, BB′ of AA′BB′, J = 8.2 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 11.9 (SMe), 21.7 (CH_3), 23.0 (NHAc), 40.4 (C-3), 52.7 (C-5), 53.7 (OMe), 67.8, 68.6, 69.1 (C-4, C-7, C-8), 72.0 (C-6), 82.4 (C-2), 128.0, 130.0, 132.4, 145.1 (6 C−Ar), 170.4, 174.2 (2 CO). ESI-MS calcd for $C_{20}H_{29}NO_{10}S_2$ $[M + Na]^+$ 530.11; found m/z 530.19.

Methyl (Methyl 5-Acetamido-9-azido-3,5,9-trideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (9). Compound **8** (160 mg, 0.32 mmol) was dissolved in dry DMF (5 mL). NaN_3 (103 mg, 1.58 mmol) and 15-crown-5 (28.6 mg, 0.13 mmol) were added successively and the reaction mixture was stirred at 60 °C for 24 h. After filtration through a pad of celite, the solvent was evaporated and the residue was purified by chromatography on silica gel (gradient 1% MeOH in DCM) to yield **9** (96.0 mg, 78%). ^{1}H NMR (500 MHz, CD_3OD) δ 1.79 (m, 1H, H-3a), 2.03 (s, 3H, SMe), 2.17 (s, 3H, NHAc), 2.77 (dd, J = 4.5, 12.8 Hz, 1H, H-3b), 3.39 (dd, J = 6.2, 12.7 Hz, 1H, H-9a), 3.48 (m, 2H, H-6, H-7), 3.57 (dd, J = 2.3, 12.7 Hz, 1H, H-9b), 3.69 (m, 1H, H-4), 3.79 (m, 1H, H-5), 3.86 (s, 3H, OMe), 3.93 (ddd, J = 2.6, 5.7, 8.5 Hz, 1H, H-8), 4.01 (m, 1H, H-6). ^{13}C NMR (CD_3OD) δ 12.0 (SMe), 22.7 (NHAc), 41.8 (C-3), 53.6 (C-5), 53.8 (OMe), 55.2 (C-9), 69.0 (C-4), 71.0 (C-7), 71.7 (C-8), 76.8 (C-6), 171.7, 175.2 (2 CO). ESI-MS calcd for $C_{13}H_{22}N_4O_7S$ $[M + Na]^+$ 401.11; found m/z 401.15.

Methyl (Methyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (10). Compound **9** (87.0 mg, 0.23 mmol) was dissolved in dry pyridine (1.0 mL) and cooled to 0 °C. After 15 min, DMAP (4.47 mg, 0.04 mmol) and Ac_2O (0.5 mL) were added successively. The reaction mixture was warmed to rt and stirred for 14 h. The solvent was evaporated and the residue purified by chromatography on silica gel (toluene/ethylacetate, 1:3) to afford **10** (85.0 mg, 73%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.81 (s, 3H, NHAc), 1.91 (m, 1H, H-3a), 1.97, 2.05, 2.10 (3 s, 9H, 3 OAc), 2.13 (s, 3H, SMe), 2.67 (dd, J = 4.4, 12.5 Hz, 1H, H-3b), 3.19 (dd, J = 5.7, 13.4 Hz, 1H, H-9a), 3.59 (m, 1H, H-9b), 3.76 (m, 4H, H-6, OMe), 4.01 (m, 1H, H-5), 4.81 (m, 1H, H-4), 4.91 (d, J = 7.7 Hz, 1H, N*H*Ac), 5.24 (m, 2H, H-7, H-8). ^{13}C NMR ($CDCl_3$) δ 12.1 (SMe), 20.9, 21.1 (3C, 3 OAc), 23.2 (NHAc), 37.8 (C-3), 49.4 (C-5), 50.6 (C-9), 53.0 (OMe), 68.1 (C-4), 69.7 (C-7), 72.3 (C-6), 74.6 (C-8), 88.4 (C-2), 170.2, 170.3, 171.0 (5C, CO). ESI-MS calcd for $C_{19}H_{28}N_4O_{10}S$ $[M + Na]^+$ 527.40; found m/z 527.21.

General Procedure for the Synthesis of Compounds 11a−f, 17a−d, 18f,g. Compound **10** (0.12 mmol) was dissolved in dry acetonitrile (2.0 mL) under argon. The alcohol (0.26 mmol) and powdered MS 3 Å (80.0 mg) were added. The mixture was stirred at rt for 1.5 h. Then the suspension was cooled to −40 °C and subsequently treated with *N*-iodosuccinimide (0.60 mmol) and triflic acid (0.06 mmol in 0.2 mL MeCN). After 30 min, the reaction mixture was warmed to −30 °C and stirring continued for 16 h. The mixture was then warmed to rt, stirred for another 2 h, and filtered through a pad of celite. The celite was washed with DCM (10 mL), and the filtrate was subsequently washed with 20% aqueous $Na_2S_2O_3$ (1 mL) and saturated aqueous $NaHCO_3$ (3 × 5 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel.

General Procedure for the Synthesis of Compounds 12a−f, 20, 18a−d. Compounds **11a−f** (**14** or **17a−d**) (0.09 mmol) and *p*-chlorobenzoyl chloride (0.36 mmol) were dissolved in dry DCE (3.0 mL) under argon. Triphenylphosphine (0.18 mmol) in dry DCE (1.5 mL) was added after 5 min, and the solution was stirred at rt for 24 h. The reaction mixture was diluted with DCM (10 mL) and washed with saturated aqueous $NaHCO_3$ (3 × 10 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel.

General Procedure for the Deprotection of 12a−f, 18b. Compound **12a−f** (**18b**) (0.06 mmol) was dissolved in MeOH (1.5 mL) and treated with 10% aqueous NaOH (0.3 mL). The reaction mixture was stirred at rt for 3 h. Then the reaction mixture was neutralized with 7% aqueous HCl (0.2 mL). The solvent was evaporated, and the crude product was purified by chromatography on RP-18.

General Procedure of the Deprotection of 18a−d,f−g. Compound **18a−d,f−g** (0.03 mmol) was dissolved in THF/H_2O (2 mL/0.5 mL) and was reacted with LiOH (0.28 mmol). The crude product was purified on RP-18 (10% gradient MeOH in water) followed by ion exchange chromatography (Dowex-50) and P2 size exclusion chromatography to yield **19**.

Methyl (4-Chlorobenzyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (11a). Compound **10** (61.0 mg, 0.12 mmol) was reacted with 4-chlorobenzyl alcohol (38.0 mg, 0.26 mmol), *N*-iodosuccinimide (134 mg, 0.601 mmol), and triflic acid (6.00 μL, 9.00 mg, 0.06 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient *i*PrOH in petrol ether/DCM 8:4) to yield **11a** (55.0 mg, 76%) as a colorless oil. $[\alpha]^{20}_D$ −0.02 (*c* 0.28, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.90 (s, 3H, NHAc), 2.03 (m, 4H, OAc, H-3a), 2.17, 2.20 (2 s, 6H, 2 OAc), 2.65 (dd, J = 4.6, 12.9 Hz, 1H, H-3b), 3.26 (dd, J = 5.7, 13.4 Hz, 1H, H-9a), 3.55 (dd, J = 2.8, 13.4 Hz, 1H, H-9b), 3.71 (s, 3H, OMe), 4.12 (m, 2H, H-5, H-6), 4.41, 4.76 (A, B of AB, J = 12.3 Hz, 2H, C*H*$_2$Ar), 4.87 (m, 1H, H-4), 5.15 (d, J = 9.8 Hz, 1H, NH), 5.35 (m, 2H, H-7, H-8) 7.30 (m, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 20.8, 20.9, 21.1 (3 OAc), 23.3 (NHAc), 38.0 (C-3), 49.4 (C-5), 51.0 (C-9), 52.8 (OMe), 66.1 (*C*H$_2$Ar), 67.9 (C-7), 68.9 (C-4), 69.4 (C-8), 72.8 (C-6), 98.6 (C-2), 128.4, 129.1, 133.5, 135.7 (6 C−Ar), 168.3, 170.3, 170.3, 171.0 (5C, 5 CO). ESI-MS calcd for $C_{25}H_{31}ClN_4O_{11}$ $[M + Na]^+$ 621.17; found m/z 621.24.

Methyl (4-Fluorobenzyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)-onate (11b). Compound **10** (62.0 mg, 0.12 mmol) was reacted with 4-fluorobenzyl alcohol (38.0 mg, 0.26 mmol), *N*-iodosuccinimide (134 mg, 0.601 mmol), and triflic acid (6.00 μL, 9.00 mg, 0.06 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient *i*PrOH in petrol ether/DCM 8:4) to yield **11b** (53.0 mg, 76%) as a colorless oil. $[\alpha]^{20}_D$

−0.01, (*c* 2.6, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.89 (s, 3H, NHAc), 2.02 (m, 1H, H-3a), 2.03, 2.17, 2.20 (3 s, 9H, 3 OAc), 2.65 (dd, J = 4.5, 12.8 Hz, 1H, H-3b), 3.29 (dd, J = 5.4, 13.3 Hz, 1H, H-9a), 3.58 (dd, J = 2.0, 13.1 Hz, 1H, H-9b), 3.71 (s, 3H, OMe), 4.13 (m, 2H, H-5, H-6), 4.41, 4.76 (A, B of AB, J = 11.9 Hz, 2H, CH_2Ar), 4.85 (m, 1H, H-4), 5.35 (m, 2H, H-8, H-7), 7.02 (t, J = 8.6 Hz, 2H, CH_{ar}), 7.31 (dd, J = 5.6, 8.2 Hz, 2H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 20.8, 20.9, 21.1 (3 OAc), 23.2 (NHAc), 49.3 (C-3), 51.0 (C-5), 52.8 (C-9), 53.5 (OMe), 66.1 (CH_2Ar), 68.0 (C-7), 68.9 (C-4), 69.7 (C-8), 72.8 (C-6), 98.5 (C-2), 115.2 (J = 21.7 Hz), 129.6 (J = 8.4 Hz), 132.9 (J = 2.9 Hz), 162.4 (J = 246.0 Hz) (6 C−Ar), 168.4, 170.2, 170.3, 170.4, 171.0 (6C, 6 CO). ESI-MS calcd for $C_{25}H_{31}FN_4O_{11}$ $[M + Na]^+$ 605.20; found m/z 605.22.

Methyl (Pentafluorobenzyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (11c). Compound **10** (60.0 mg, 0.12 mmol) was reacted with pentafluorobenzyl alcohol (60.0 mg, 0.30 mmol), *N*-iodosuccinimide (32.0 mg, 0.14 mmol), and triflic acid (4.00 μL, 7.00 mg, 0.04 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient *i*PrOH in petrol ether/DCM 8:4) to yield **11c** (47.0 mg, 66%) as a colorless oil. $[\alpha]^{20}_D$ −0.03 (*c* 0.5, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.90 (s, 3H, NHAc), 1.94 (dd, J = 10.4, 12.6 Hz, 1H, H-3a), 2.03, 2.19, 2.21 (3 s, 9H, 3 OAc), 2.59 (dd, J = 4.6, 12.8 Hz, 1H, H-3b), 3.30 (dd, J = 5.6, 13.5 Hz, 1H, H-9a), 3.59 (dd, J = 2.9, 13.5 Hz, 1H, H-9b), 3.86 (s, 3H, OMe), 4.12 (m, 2H, H-5, H-6), 4.43 (A of AB, J = 11.0 Hz, 1H, CH_2Ar), 4.87 (m, 1H, H-4), 4.90 (B of AB, J = 10.7 Hz, 1H, CH_2Ar), 5.19 (d, J = 9.3 Hz, 1H, NH), 5.39 (m, 2H, H-7, H-8). ^{13}C NMR ($CDCl_3$) δ 20.8, 20.9, 21.1 (3 OAc), 23.2 (NHAc), 37.7 (C-3), 49.4 (C-5), 51.0 (C-9), 53.0 (OMe), 54.0 (CH_2Ar), 67.8, 68.8, 69.3 (C-4, C-7, C-8), 72.9 (C-6), 98.7 (C-2), 167.8, 170.3 (5C, 5 CO). ESI-MS calcd for $C_{25}H_{27}F_5N_4O_{11}$ $[M + Na]^+$ 677.16; found m/z 677.32.

Methyl (2-Naphthyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (11d). Compound **10** (50.0 mg, 0.10 mmol) was reacted with 2-naphthalenemethanol (24.0 mg, 0.15 mmol), *N*-iodosuccinimide (27.0 mg, 0.12 mmol), and triflic acid (4.00 μL, 7.00 mg, 0.04 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient *i*PrOH in petrol ether/DCM 8:4) to yield **11d** (37.0 mg, 61%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.85 (s, 3H, NHAc), 2.09 (m, 1H, H-3a), 2.03, 2.15, 2.21 (3 s, 9H, 3 OAc), 2.26 (dd, J = 6.0, 13.5 Hz, 1H, H-9a), 3.59 (dd, J = 2.9, 13.5 Hz, 1H, H-9b), 3.67 (s, 3H, OMe), 4.14 (m, 2H, H-5, H-6), 4.63 (A of AB, J = 12.2 Hz, 1H, CH_2Ar), 4.89 (m, 1H, H-4), 4.96 (B of AB, J = 12.2 Hz, 1H, CH_2Ar), 5.34 (m, 2H, H-7, NH), 5.38 (m, 1H, H-8), 7.45−7.48 (m, 3H, CH_{ar}), 7.79−7.83 (m, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 21.3, 21.5 (3C, 3 OAc), 23.6 (NHAc), 38.5 (C-3), 49.9 (C-5), 51.3 (C-9), 53.1 (OMe), 67.4 (CH_2Ar), 68.4 (C-7), 69.4 (C-4), 70.2 (C-8), 73.3 (C-6), 126.1, 126.4, 126.5, 126.9, 128.1, 128.3, 128.4 (10 C−Ar), 170.7 (6C, 6 CO). ESI-MS calcd for $C_{29}H_{34}N_4O_{11}$ $[M + Na]^+$ 637.33; found m/z 637.20.

Methyl (2,3-Dichlorobenzyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (11e). Compound **10** (45.0 mg, 0.09 mmol) was reacted with 2,3-dichlorobenzyl alcohol (24.0 mg, 0.13 mmol), *N*-iodosuccinimide (24.0 mg, 0.12 mmol), and triflic acid (3.00 μL, 6.00 mg, 0.04 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient *i*PrOH in petrol ether/DCM 8:4) to yield **11e** (30.0 mg, 54%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.83 (s, 3H, NHAc), 2.01 (t, J = 12.9 Hz, 1H, H-3a), 1.97, 2.08, 2.11 (3 s, 9H, 3 OAc), 2.63 (dd, J = 4.7, 12.9 Hz, 1H, H-3b), 3.19 (dd, J = 6.0, 13.6 Hz, 1H, H-9a), 3.50 (dd, J = 3.0, 13.6 Hz, 1H, H-9b), 3.72 (s, 3H, OMe), 4.05 (m, 2H, H-5, H-6), 4.50, 4.82 (A, B of AB, J = 13.0 Hz, 2H, CH_2Ar), 4.85 (m, 1H, H-4), 5.13 (d, J = 8.0 Hz, 1H, N*H*Ac), 5.24 (m, 2H, H-7, H-8), 7.14 (t, J = 8.0 Hz, 1H, CH_{ar}), 7.34 (d, J = 8.1 Hz, 2H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 21.3, 21.5 (3C, 3 OAc), 23.6 (NHAc), 38.2 (C-3), 49.9 (C-5), 51.2 (C-9), 53.4 (OMe), 64.7 (CH_2Ar), 68.4 (C-8), 69.3 (C-4), 70.5 (C-7), 73.5 (C-6), 109.6 (C-2), 127.6, 127.7, 130.0 (6C, 6 C−Ar), 168.5, 170.5, 170.6, 170.7, 171.4 (5 CO). ESI-MS calcd for $C_{25}H_{30}Cl_2N_4O_{11}$ $[M + Na]^+$ 655.13; found m/z 655.07.

Methyl (2,3-Difluorobenzyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (11f). Compound **10** (86.0 mg, 0.17 mmol) was reacted with 2,3-difluorobenzyl alcohol (29.0 μL, 37.0 mg, 0.26 mmol), *N*-iodosuccinimide (46.0 mg, 0.21 mmol), and triflic acid (6.00 μL, 10.0 mg, 0.07 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient *i*PrOH in petrol ether/DCM 8:4) to yield **11f** (67.0 mg, 66%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.82 (s, 3H, NHAc), 1.95 (m, 1H, H-3a), 1.96, 2.10, 2.13 (3 s, 9H, 3 OAc), 2.58 (m, 1H, H-3b), 3.20 (m, 1H, H-9a), 3.50 (m, 1H, H-9b), 3.72 (s, 3H, OMe), 4.06 (m, 2H, H-5, H-6), 4.46, 4.75 (A, B of AB, J = 12.0 Hz, 2H, CH_2Ar), 4.82 (m, 1H, H-4), 5.29 (m, 2H, H-8, H-7), 7.05 (m, 3H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 21.3, 21.6 (3C, 3 OAc), 23.6 (NHAc), 38.2 (C-3), 49.8 (C-5), 51.3 (C-9), 53.3 (OMe), 60.8 (CH_2Ar), 68.4 (C-7), 69.3 (C-8), 70.3 (C-4), 73.4 (C-6), 99.1 (C-2), 117.4, 119.1 (J = 17.0 Hz), 124.3, 125.5 (6 C−Ar), 168.5, 170.5, 170.6, 170.8, 171.4 (5 CO). ESI-MS calcd for $C_{25}H_{30}F_2N_4O_{11}$ $[M + 2Na]^+$ 645.99; found m/z 645.26.

Methyl (4-Chlorobenzyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12a). Compound **11a** (55.0 mg, 0.09 mmol) was reacted with *p*-chlorobenzoyl chloride (46.0 μL, 63.0 mg, 0.36 mmol) and triphenylphosphine (47.0 mg, 0.18 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient of MeOH in DCM) to yield **12a** (35.0 mg, 59%) as a yellow solid. $[\alpha]^{20}_D$ −0.01 (*c* 2.9, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.89 (s, 3H, NHAc), 2.05 (m, 4H, H-3a, OAc), 2.14, 2.27 (2 s, 6H, 2 OAc), 2.67 (dd, J = 4.6, 12.8 Hz, 1H, H-3b), 2.92 (dt, J = 3.3, 14.9 Hz, 1H, H-9a), 3.65 (s, 3H, OMe), 4.05 (dd, J = 2.0, 10.7 Hz, 1H, H-6), 4.21 (q, J = 10.4 Hz, 1H, H-5), 4.36 (ddd, J = 3.0, 8.7, 15.1 Hz, 1H, H-9b), 4.41, 4.78 (A, B of AB, J = 12.3 Hz, 2H, CH_2Ar), 4.84 (m, 1H, H-4), 5.15 (dd, J = 2.0, 10.0 Hz, 1H, H-7), 5.31 (m, 2H, N*H*Ac, H-8), 7.10 (dd, J = 4.0, 8.5 Hz, 1H, NH), 7.29 (m, 4H, CH_{ar}), 7.41, 7.77 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 20.9, 21.2, 21.3 (3 OAc), 23.2 (NHAc), 38.1 (C-3), 38.4 (C-9), 49.5 (C-5), 52.7 (OMe), 66.1 (CH_2Ar), 67.9 (C-7), 68.2 (C-8), 69.0 (C-4), 72.2 (C-6), 98.4 (C-2), 128.4, 128.5, 128.8, 129.2, 132.0, 132.6, 133.5, 135.7, 137.7 (12 C−Ar), 168.1, 170.4, 171.2, 172.6 (6C, 6 CO). ESI-MS calcd for $C_{32}H_{36}Cl_2N_2O_{12}$ $[M + Na]^+$ 733.16; found m/z 733.25.

Methyl (4-Fluorobenzyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12b). Compound **11b** (52.0 mg, 0.09 mmol) was reacted with *p*-chlorobenzoyl chloride (46.0 μL, 63.0 mg, 0.36 mmol) and triphenylphosphine (52.0 mg, 0.19 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient of MeOH in DCM) to yield **12b** (37.0 mg, 60%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.85 (s, 3H, NHAc), 2.00 (m, 1H, H-3a), 2.03, 2.11, 2.26 (3 s, 9H, 3 OAc), 2.68 (dd, J = 4.5, 12.7 Hz, 1H, H-3b), 2.96 (dt, J = 3.5, 15.0 Hz, 1H, H-9a), 3.61 (s, 3H, OMe), 4.12 (d, J = 10.7 Hz, 1H, H-6), 4.23 (q, J = 10.2 Hz, 1H, H-5), 4.36 (ddd, J = 3.0, 8.7, 15.3 Hz, 1H, H-9b), 4.40, 4.78 (A, B of AB, J = 11.9 Hz, 2H, CH_2Ar), 4.87 (m, 1H, H-4), 5.20 (dd, J = 1.1, 9.9 Hz, 1H, H-7), 5.35 (dt, J = 2.9, 9.9 Hz, 1H, H-8), 5.99 (m, 1H, N*H*Ac), 7.00 (t, J = 8.7 Hz, 2H, CH_{ar}), 7.17 (dd, J = 3.8 Hz, 8.1 Hz, 1H, NH), 7.49 (dd, J = 5.4 Hz, 8.5 Hz, 1H, CH_{ar}), 7.38, 7.76 (AA′, BB′ of AA′BB′, J = 8.4 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 21.2, 21.3 (3 OAc), 23.1 (NHAc), 38.3 (C-3), 38.5 (C-9), 49.4 (C-5), 52.6 (OMe), 66.1 (CH_2Ar), 68.0 (C-7), 68.2 (C-8), 69.2 (C-4), 72.2 (C-6), 98.4 (C-2), 115.0, 115.2 (J = 21.3 Hz), 128.6, 129.6, 129.7 (J = 7.5 Hz), 132.0, 132.8, 132.9, 137.7, 161.3, 163.5 (J = 275.1 Hz), 168.1, 166.3 (J = 237.5 Hz) (12 C−Ar), 170.39, 170.4, 171.0, 172.5 (6C, 6 CO). ESI-MS calcd for $C_{32}H_{36}ClFN_2O_{12}$ $[M + Na]^+$ 717.19; found m/z 717.34.

Methyl (Pentafluorobenzyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12c). Compound **11c** (47.0 mg, 0.07 mmol) was reacted with *p*-chlorobenzoyl chloride (36.0 μL, 49.0 mg, 0.28 mmol) and triphenylphosphine (41.0 mg, 0.16 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient of MeOH in DCM) to yield **12c** (25.0 mg, 45%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.86 (s, 3H, NHAc), 1.94 (m, 1H, H-3a), 2.02, 2.16, 2.19 (3 s, 9H, 3 OAc), 2.60 (dd, J = 4.5, 12.8 Hz, 1H, H-3b), 3.55 (dd, J = 6.3, 12.3 Hz, 1H, H-9a), 3.81 (m, 4H, OMe, H-9b), 4.07 (d, J = 10.3 Hz, 1H, H-6), 4.20 (dd, J = 2.0, 10.8 Hz, 1H, H-5), 4.45, 4.93 (A, B of AB, J = 11.3 Hz, 2H, CH_2Ar), 5.37 (dd, J = 2.1, 8.1 Hz, 1H, H-7), 5.46 (m, 1H, H-8), 5.68 (d, J = 9.6 Hz, 1H, NHAc), 7.38, 8.00 (AA′, BB′ of AA′BB′, J = 8.4 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 21.0, 21.2, 21.3 (3 OAc), 23.3 (NHAc), 37.8 (C-3), 43.7 (C-9), 49.5 (C-5), 53.1 (OMe), 54.1 (CH_2Ar), 68.3, 69.0 (C-4, C-7), 70.0 (C-8), 72.9 (C-6), 98.8 (C-2), 128.8, 128.9, 129.1, 131.5, 132.9, 133.2, 133.3 (12 C−Ar), 167.6, 167.9, 168.7, 170.3, 170.4, 170.6 (6 CO). ESI-MS calcd for $C_{32}H_{32}ClF_5N_2O_{12}$ $[M + Cl]^-$ 801.45; found m/z 801.36.

Methyl (2-Naphthyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12d). Compound **11d** (50.0 mg, 0.08 mmol) was reacted with *p*-chlorobenzoyl chloride (42.0 μL, 57.0 mg, 0.32 mmol) and triphenylphosphine (47.0 mg, 0.18 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient of MeOH in DCM) to yield **12d** (25.0 mg, 47%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.87 (s, 3H, NHAc), 2.04 (s, 3H, OAc), 2.10 (t, J = 12.4 Hz, 1H, H-3a), 2.14, 2.24 (2 s, 6H, 2 OAc), 2.72 (dd, J = 4.5, 12.8 Hz, 1H, H-3b), 2.92 (m, 1H, H-9a), 3.59 (s, 3H, OMe), 4.11 (m, 1H, H-6), 4.23 (q, J = 10.3 Hz, 1H, H-5), 4.34 (ddd, J = 3.0, 8.5, 15.2 Hz, 1H, H-9b), 4.63 (A of AB, J = 12.3 Hz, 1H, CH_2Ar), 4.87 (dt, J = 4.6, 12.3 Hz, 1H, H-4), 4.98 (B of AB, J = 12.3 Hz, 1H, CH_2Ar), 5.17 (d, J = 9.8 Hz, 1H, H-7), 5.34 (d, J = 9.9 Hz, 1H, H-8), 5.50 (m, 1H, NHAc), 7.11 (m, 1H, NH), 7.39 (AA′ of AA′BB′, J = 8.5 Hz, 2H, CH_{ar}), 7.44−7.47 (m, 3H, CH_{ar}), 7.76 (BB′ of AA′BB′, J = 8.5 Hz, 2H, CH_{ar}), 7.75−7.83 (m, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 21.3, 21.5, 21.6 (3 OAc), 23.5 (NHAc), 38.6 (C-3), 38.9 (C-9), 49.9 (C-5), 53.0 (OMe), 67.4 (CH_2Ar), 68.4 (C-7), 68.7 (C-8), 69.5 (C-4), 72.6 (C-6), 98.9 (C-2), 126.2, 126.3, 126.5, 127.0, 128.0, 128.3, 128.9, 129.0, 129.2, 132.4, 133.1, 133.3, 133.5, 135.1 (14 C−Ar), 166.6, 168.7, 170.8, 171.5, 172.9 (6C, 6 CO). ESI-MS calcd for $C_{36}H_{39}ClN_2O_{12}$ $[M + Na]^+$ 749.22; found m/z 749.25.

Methyl (2,3-Dichlorobenzyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12e). Compound **11e** (47.0 mg, 0.07 mmol) was reacted with *p*-chlorobenzoyl chloride (38.0 μL, 52.0 mg, 0.30 mmol) and triphenylphosphine (43.0 mg, 0.16 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient of MeOH in DCM) to yield **12e** (25.0 mg, 47%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.78 (t, J = 12.4 Hz, 1H, H-3a), 1.80 (s, 3H, NHAc), 1.97, 2.04, 2.17 (3 s, 9H, 3 OAc), 2.63 (dd, J = 4.8, 12.8 Hz, 1H, H-3b), 2.90 (ddd, J = 3.3, 3.7, 15.1 Hz, 1H, H-9a), 3.64 (s, 3H, OMe), 4.01 (d, J = 10.8 Hz, 1H, H-6), 4.15 (q, J = 10.4 Hz, 1H, H-5), 4.23 (m, 1H, H-9b), 4.49 (A of AB, J = 13.0 Hz, 1H, CH_2Ar), 4.30 (m, 2H, H-4, CH_2Ar), 5.11 (d, J = 1.6 Hz, 1H, H-7), 5.20 (m, 1H, H-8), 5.55 (d, J = 8.8 Hz, 1H, NHAc), 7.03 (dd, J = 3.8 Hz, 1H, NH), 7.13 (t, J = 7.8 Hz, 1H, CH_{ar}), 7.39 (m, 2H, CH_{ar}), 7.68, 7.93 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 21.3, 21.5, 21.6 (3 OAc), 23.5 (NHAc), 38.2 (C-3), 39.0 (C-9), 49.9 (C-5), 53.2 (OMe), 64.8 (CH_2Ar), 68.4 (C-8), 68.8 (C-7), 69.5 (C-4), 72.7 (C-6), 110.0 (C-2), 127.6, 128.1, 130.0, 131.8, 133.0, 136.0, 138.1 (12 C−Ar), 160.0, 168.0, 170.6, 170.9, 171.5, 172.8 (6 CO). ESI-MS calcd for $C_{32}H_{35}Cl_3N_2O_{12}$ $[M + Na]^+$ 767.13; found m/z: 767.12.

Methyl (2,3-Difluorobenzyl 5-Acetamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12f). Compound **11f** (67.0 mg, 0.11 mmol) was reacted with *p*-chlorobenzoyl chloride (57.0 μL, 78.0 mg, 0.45 mmol) and triphenylphosphine (65.0 mg, 0.25 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient of MeOH in DCM) to yield **12f** (44.0 mg, 55%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.80 (s, 3H, NHAc), 2.04 (m, 4H, H-3a, OAc), 2.14, 2.27 (2 s, 6H, 2 OAc), 2.65 (dd, J = 4.6, 12.8 Hz, 1H, H-3b), 2.97 (dt, J = 3.5, 15.0 Hz, 1H, H-9a), 3.74 (s, 3H, OMe), 4.08 (m, 1H, H-6), 4.23 (q, J = 10.4 Hz, 1H, H-5), 4.33 (m, 1H, H-9b), 4.52, 4.84 (A, B of AB, J = 12.0 Hz, 2H, CH_2Ar), 5.17 (dd, J = 1.9, 9.8 Hz, 1H, H-7), 5.31 (m, 1H, H-8), 5.43 (m, 1H, NHAc), 7.75 (m, 4H, NH, CH_{ar}), 7.39, 7.77 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 21.3, 21.5, 21.6 (3 OAc), 23.5 (NHAc), 38.3 (C-3), 38.9 (C-9), 49.9 (C-5), 53.2 (OMe), 60.8 (CH_2Ar), 68.3 (C-8), 68.7 (C-7), 69.4 (C-4), 72.7 (C-6), 98.0 (C-2), 117.3, 117.4 (J = 17.0 Hz), 125.7, 128.8, 129.2, 132.4, 132.5 (J = 9.0 Hz), 133.0, 138.1 (12 C−Ar), 166.7, 168.4, 170.7, 170.9, 171.6, 172.9 (6 CO). ESI-MS calcd for $C_{32}H_{35}ClF_2N_2O_{12}$ $[M + Na]^+$ 735.18; found m/z 735.15.

Sodium (4-Chlorobenzyl 5-Acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (13a). Compound **12a** (25.0 mg, 0.03 mmol) was treated with 10% aqueous NaOH (0.2 mL) in MeOH (1.0 mL). The crude product was purified by chromatography on RP-18 (5% gradient of MeOH in H_2O) to yield **13a** as a white solid (15.0 mg, 50%). $[\alpha]^{20}_D$ −0.25 (*c* 0.41, H_2O). ^{1}H NMR (500 MHz, CD_3OD) δ 1.55 (dd, J = 3.3, 11.9 Hz, 1H, H-3a), 1.90 (s, 3H, NHAc), 2.81 (dd, J = 2.0, 12.2 Hz, 1H, H-3b), 3.34 (dd, J = 1.8, 9.0 Hz, 1H, H-7), 3.41 (dd, J = 7.8, 13.6 Hz, 1H, H-9a), 3.55−3.63 (m, 3H, H-4, H-5, H-6), 3.67 (dd, J = 3.1 Hz, 13.6 Hz, 1H, H-9b), 3.94 (m, 1H, H-8), 4.40, 4.70 (A, B of AB, J = 11.6 Hz, 2H, CH_2Ar), 7.18, 7.25 (AA′, BB′ of AA′BB′, J = 8.4 Hz, 4H, CH_{ar}), 7.36, 7.72 (AA′, BB′ of AA′BB′, J = 8.6 Hz, 4H, CH_{ar}). ^{13}C NMR (CD_3OD) δ 22.6 (NHAc), 42.7 (C-3), 44.6 (C-9), 54.2 (C-5), 66.5 (CH_2Ar), 69.6 (C-4), 71.2 (C-8), 72.5 (C-7), 74.4 (C-6), 102.1 (C-2), 129.2, 129.7, 130.1, 130.6, 134.0, 134.5, 138.6, 138.8 (12 C−Ar), 169.2, 174.3, 175.5 (3 CO). HRMS calcd for $C_{25}H_{28}Cl_2N_2O_9$ $[M - H]^-$ 615.0891; found 615.0888.

Sodium (4-Fluorobenzyl 5-Acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (13b). Compound **12b** (37.0 mg, 0.05 mmol) was treated with 10% aqueous NaOH (0.3 mL) in MeOH (1.2 mL). The crude product was purified by chromatography on RP-18 (5% gradient of MeOH in H_2O) to yield **13b** as a white solid (21.1 mg, 70%). $[\alpha]^{20}_D$ −0.22 (*c* 0.33, H_2O). ^{1}H NMR (500 MHz, CD_3OD) δ 1.54 (m, 1H, H-3a), 1.90 (s, 3H, NHAc), 2.80 (m, 1H, H-3b), 3.35 (m, 1H, H-7), 3.42 (m, 1H, H-9a), 3.58−3.69 (m, 4H, H-4, H-5, H-6, H-9b), 3.96 (m, 1H, H-8), 4.39, 4.69 (A, B of AB, J = 11.2 Hz, 2H, CH_2Ar), 6.90 (t, J = 8.8 Hz, 2H, CH_{ar}), 7.27 (m, 2H, CH_{ar}), 7.35 (m, 2H, CH_{ar}), 7.72 (BB′ of AA′BB′, J = 8.5 Hz, 2H, CH_{ar}). ^{13}C NMR (CD_3OD) δ 22.7 (NHAc), 42.7 (C-3), 44.5 (C-9), 54.2 (C-5), 66.7 (CH_2Ar), 69.6 (C-4), 71.4 (C-8), 72.5 (C-7), 74.4 (C-6), 102.1 (C-2), 115.8 (J = 12.5 Hz), 130.1, 129.7, 131.1 (J = 21.3 Hz), 138.6, 162.0 (12 C−Ar), 164.6, 169.3, 174.4 (3 CO). HRMS calcd for $C_{25}H_{28}ClFN_2O_9$ $[M - H]^-$ 577.1367; found 577.1369.

Sodium (Pentafluorobenzyl 5-Acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (13c). Compound **12c** (25.0 mg, 0.03 mmol) was treated with 10% aqueous NaOH (0.2 mL) in MeOH (1.0 mL). The crude product was purified by chromatography on RP-18 (5% gradient of MeOH in H_2O) to yield **13c** as a white solid (8.00 mg, 39%). $[\alpha]^{20}_D$ −0.02 (*c* 0.32, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 1.63 (t, J = 12.1 Hz, 1H, H-3a), 1.97 (s, 3H, NHAc), 2.71 (dd, J = 4.7, 12.4 Hz, 1H, H-3b), 3.50 (m, 2H, H-7, H-9a), 3.65 (ddd, J = 4.7, 9.5, 11.9 Hz, 1H, H-4), 3.74−3.79 (m, 3H, H-5, H-6, H-9b), 3.88 (ddd, J = 2.9, 7.8, 8.8 Hz, 1H, H-8), 4.66, 4.86 (A, B of AB, J = 11.7 Hz, 2H, CH_2Ar), 7.50, 7.73 (AA′, BB′ of AA′BB′, J = 8.6 Hz, 4H, CH_{ar}). ^{13}C NMR (D_2O) δ 21.9 (NHAc), 40.3 (C-3), 42.6 (C-9), 51.8 (C-5), 54.1 (CH_2Ar), 68.1 (C-4), 69.8 (C-7), 70.3 (C-8), 72.8 (C-6), 101.0 (C-2), 128.7, 128.7, 132.2, 136.2, 137.5, 139.1

(12 C−Ar), 170.1, 172.8, 175.0 (3 CO). HRMS calcd for $C_{25}H_{23}ClF_5N_2NaO_9$ $[M - H]^-$ 625.1018; found 625.1015.

Sodium (2-Naphthyl 5-Acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (13d). Compound **12d** (25.0 mg, 0.03 mmol) was treated with 10% aqueous NaOH (0.2 mL) in MeOH (1.0 mL). The crude product was purified by chromatography on RP-18 (5% gradient of MeOH in H_2O) to yield **13d** as a white solid (14.0 mg, 70%). $[\alpha]^{20}_D$ −0.37 (*c* 0.46, H_2O). ^{1}H NMR (500 MHz, CD_3OD) δ 1.60 (m, 1H, H-3a), 1.90 (s, 3H, NHAc), 2.84 (d, J = 11.8 Hz, H-3b), 3.35 (d, J = 8.9 Hz, H-7), 3.42 (dd, J = 7.7, 13.7 Hz, H-9a), 3.60−3.68 (m, 4H, H-4, H-5, H-6, H-9b), 4.60 (A of AB, J = 11.5 Hz, 1H, CH_2Ar), 3.96 (m, 1H, H-8), 4.89 (B of AB, J = 11.5 Hz, 1H, CH_2Ar), 7.31−7.36 (m, 4H, CH_{ar}), 7.40 (dd, J = 1.5, 8.4 Hz, 1H, CH_{ar}), 7.67−7.72 (m, 6H, CH_{ar}). ^{13}C NMR (CD_3OD) δ 21.7 (NHAc), 43.3 (C-3), 43.4 (C-9), 53.1 (C-5), 68.6 (CH_2Ar), 71.3 (C-7), 72.0 (C-4), 73.3 (C-6), 110.0 (C-2), 125.7, 125.9, 126.3, 126.4, 127.6, 127.7, 127.9, 128.6, 129.1 (16 C−Ar), 174.6, 175.3 (3C, CO). HRMS calcd for $C_{29}H_{30}ClN_2NaO_9$ $[M + Na]^+$ 631.1438; found 631.1435.

Sodium (2,3-Dichlorobenzyl 5-Acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (13e). Compound **12e** (25.0 mg, 0.03 mmol) was treated with 10% aqueous NaOH (0.2 mL) in MeOH (1.0 mL). The crude product was purified by chromatography on RP-18 (5% gradient of MeOH in H_2O) to yield **13e** as a white solid (10.0 mg, 50%). $[\alpha]^{20}_D$ −0.19 (*c* 0.53, H_2O). ^{1}H NMR (500 MHz, CD_3OD) δ 1.69 (t, J = 11.8 Hz, 1H, H-3a), 1.93 (s, 3H, NHAc), 2.01 (dd, J = 9.1, 11.8 Hz, 1H, H-3b), 3.36 (dd, J = 1.7, 9.0 Hz, 1H, H-7), 3.45 (dd, J = 7.6, 13.6 Hz, 1H, H-9a), 3.59 (m, 1H, H-6), 3.65−3.70 (m, 3H, H-4, H-5, H-9b), 3.92 (m, 1H, H-8), 4.65, 4.89 (A, B of AB, J = 13.6 Hz, 2H, CH_2Ar), 7.18 (t, J = 7.9 Hz, 1H, CH_{ar}), 7.32 (dd, J = 1.5, 8.0 Hz, 1H, CH_{ar}), 7.39 (AA′ of AA′BB′, J = 8.5 Hz, 2H, CH_{ar}), 7.49 (dd, J = 1.1, 7.7 Hz, 1H, CH_{ar}), 7.74 (BB′ of AA′BB′, J = 8.5 Hz, 2H, CH_{ar}). ^{13}C NMR (CD_3OD) δ 21.6 (NHAc), 41.5 (C-3), 43.4 (C-9), 53.1 (C-5), 63.5 (CH_2Ar), 68.6 (C-4), 70.2 (C-8), 71.5 (C-7), 73.4 (C-6), 102.5 (C-2), 113.3, 117.0, 118.4, 127.4, 127.5, 128.6, 128.9, 129.0, 139.6 (12 C−Ar), 163.8, 174.5 (3C, 3 CO). HRMS calcd for $C_{25}H_{27}Cl_3N_2O_9$ $[M + Na]^+$ 627.0682; found 627.0683.

Sodium (2,3-Difluorobenzyl 5-Acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (13f). Compound **12f** (44.0 mg, 0.06 mmol) was treated with 10% aqueous NaOH (0.3 mL) in MeOH (1.5 mL). The crude product was purified by chromatography on RP-18 (5% gradient of MeOH in H_2O) to yield **13f** as a white solid (23.0 mg, 64%). $[\alpha]^{20}_D$ −0.18 (*c* 1.13, H_2O). ^{1}H NMR (500 MHz, CD_3OD) δ 1.56 (m, 1H, H-3a), 1.90 (s, 3H, NHAc), 2.84 (dd, J = 3.0, 12.2 Hz, 1H, H-3b), 3.37 (d, J = 9.0 Hz, 1H, H-7), 3.46 (dd, J = 7.7, 13.6 Hz, 1H, H-9a), 3.59 (m, 1H, H-6), 3.63−3.70 (m, 3H, H-4, H-5, H-9b), 3.96 (m, 1H, H-8), 4.60, 4.85 (A, B of AB, J = 12.2 Hz, 2H, CH_2Ar), 7.04 (m, 2H, CH_{ar}), 7.24 (t, J = 6.5 Hz, 1H, CH_{ar}), 7.38, 7.75 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH_{ar}). ^{13}C NMR (CD_3OD) δ 21.6 (NHAc), 41.5 (C-3), 43.5 (C-9), 53.1 (C-5), 59.2 (CH_2Ar), 68.6 (C-4), 70.3 (C-8), 71.4 (C-7), 73.4 (C-6), 101.0 (C-2), 115.9, 116.1 (J = 17.3 Hz), 124.2, 125.3, 128.6, 129.1, 133.5, 137.6 (12 C−Ar), 168.3, 173.0, 174.5 (3 CO). HRMS calcd for $C_{25}H_{27}ClF_2N_2O_9$ $[M + Na]^+$ 595.1273; found 595.1272.

Methyl (Methyl 5-*tert*-Butyloxycarbonylamino-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (14). Compound **10** (85.0 mg, 0.17 mmol) was dissolved in dry THF (0.7 mL) under argon. Boc_2O (74.0 mg, 0.34 mmol) was added to the reaction mixture, followed by DMAP (4.50 mg, 0.02 mmol). The reaction mixture was heated up to 60 °C for 5 h. After cooling to rt, MeOH (0.7 mL) and $N_2H_4 \cdot H_2O$ (52 μL, 1.1 mmol) were added and stirring was continued for 16 h. The reaction mixture was washed successively with 0.1 M HCl (1 × 5 mL), 0.5 M $CuSO_4$ (1 × 5 mL), saturated aqueous $NaHCO_3$ (2 × 5 mL), and H_2O (1 × 5 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a yellow oil. The crude product was reacted with acetic anhydride (0.9 mL) in dry pyridine (1.7 mL). A catalytic amount of DMAP was added, and stirring was continued at rt. After 18 h, the reaction mixture was washed with $CuSO_4$ (0.5 M, 4 × 5 mL), saturated aqueous $NaHCO_3$ (1 × 5 mL), and H_2O (1 × 5 mL). The organic layer was dried over Na_2SO_4, filtered, and the solvent was evaporated. The crude product was purified by chromatography on silica gel (EA:PE, gradient 1:1 to 2:1) to yield **14** (72 mg, 76%) as a white foam. $[\alpha]^{20}_D$ 0.31 (*c* 1.68, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37 (s, 9H, *t*-Butyl), 1.95 (m, 1H, H-3a), 2.02 (s, 3H, SMe), 2.10, 2.14, 2.17 (3 s, 9H, 3 OAc), 2.73 (dd, J = 4.6, 12.7 Hz, 1H, H-3b), 3.25 (dd, J = 6.0, 13.5 Hz, 1H, H-9a), 3.61 (dd, J = 3.2, 13.5 Hz, 1H, H-9b), 3.73 (m, 1H, H-6), 3.80 (m, 4H, OMe, H-5), 4.25 (d, J = 10.4 Hz, 1H, NH), 4.78 (m, 1H, H-4), 5.29 (m, 1H, H-8), 5.41 (m, 1H, H-7). ^{13}C NMR ($CDCl_3$) δ 12.1 (SMe), 20.8, 21.0, 21.3 (3 OAc), 27.9 (3C, C-(CH_3)$_3$), 38.0 (C-3), 50.4 (C-9), 50.6 (C-5), 52.9 (OMe), 67.6 (C-7), 68.1 (C-8), 70.0 (C-4), 73.7 (C-6), 80.2 (C-2), 83.0 (C(CH$_3$)$_3$), 155.2 (CONH), 168.2, 170.0, 170.6, 174.0 (4 CO). ESI-MS calcd for $C_{22}H_{34}N_4O_{11}S$ $[M + Na]^+$ 585.19; found m/z 585.15.

Methyl (Methyl 5-Amino-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (15). Compound **14** (120 mg, 0.21 mmol) was dissolved in 4 M PhOH (in abs DCM; 7.5 mL) and 4 M TMSCl (in abs DCM; 1.5 mL). The reaction mixture was stirred at rt for 2 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$ (2 × 5 mL) and H_2O (1 × 5 mL). The organic layer was dried over Na_2SO_4, filtered, and the solvent was evaporated. The crude product was purified by chromatography on silica gel (EA:PE, gradient 1:1 to 8:1) to yield **15** as a white foam (74 mg, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.01 (s, 3H, SMe), 2.04 (m, 1H, H-3a), 2.10, 2.11, 2.19 (3 s, 9H, 3 OAc), 2.71 (dd, J = 4.7, 12.8 Hz, 1H, H-3b), 3.38 (d, J = 10.5 Hz, 1H, H-6), 3.66 (dd, J = 3.3, 13.3 Hz, 1H, H-9a), 3.70 (dd, J = 2.8, 13.3 Hz, 1H, H-9b), 3.76 (dd, J = 1.3, 9.4 Hz, 1H, H-7), 3.78 (s, 3H, OMe), 3.98 (dt, J = 8.0, 10.5 Hz, 1H, H-5), 4.93 (dd, J = 6.9, 10.7 Hz, 1H, H-4), 5.26 (t, J = 3.9, 9.4 Hz, 1H, H-8), 5.94 (d, J = 7.8 Hz, 2H, NH_2). ^{13}C NMR ($CDCl_3$) δ 12.0 (SMe), 21.1, 21.2, 23.2 (3 OAc), 37.7 (C-3), 51.2, 52.1, 52.9 (3C, C-5 C-9, OMe), 66.8, 69.3, 70.0 (3C, C-4, C-7, C-8), 75.3 (C-6), 82.2 (C-2), 167.9, 170.2, 172.4, 172.9 (4 CO). ESI-MS calcd for $C_{17}H_{26}N_4O_9S$ $[M + Na]^+$ 485.14; found m/z 485.13.

Methyl (Methyl 5-Fluoroacetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (16a). Compound **15** (74.0 mg, 0.16 mmol) was dissolved in dry DCM (1.8 mL) and cooled to 0 °C. Then, monofluoroacetic chloride was added dropwise (30.0 mg, 31.0 μL, 0.32 mmol), followed by the addition of NEt_3 (324 mg, 0.45 mL, 3.2 mmol) and DMAP (9.8 mg, 0.08 mmol). Stirring was continued overnight, and the reaction mixture was allowed to come to rt. The brown solution was washed with saturated aqueous $NaHCO_3$ (3 × 5 mL), saturated aqueous NaCl (1 × 5 mL), and H_2O (1 × 5 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (EA:PE, gradient 1:1 to 2:1) to yield **16a** (71 mg, 85%). $[\alpha]^{20}_D$ 0.34 (*c* 1.2, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.98 (m, 1H, H-3a), 2.03 (s, 3H, SMe), 2.12, 2.15, 2.21 (3 s, 9H, 3 OAc), 2.79 (dd, J = 4.7, 12.8 Hz, 1H, H-3b), 3.24 (dd, J = 4.8, 13.6 Hz, 1H, H-9a), 3.64 (dd, J = 2.8, 13.6 Hz, 1H, H-9b), 3.84 (s, 3H, OMe), 3.90 (dd, J = 1.9, 10.7 Hz, 1H, H-6), 4.14 (m, 1H, H-5), 4.71 (m, 2H, CH_2F), 4.92 (td, J = 4.7, 11.6 Hz, 1H, H-4), 5.32 (m, 2H, H-7, H-8), 6.09 (dd, J = 3.3, 10.3 Hz, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 12.2 (SMe), 20.9 (3C, OAc), 37.8 (C-3), 48.6 (C-5), 50.6 (C-9), 53.1 (OMe), 67.8 (C-7), 69.4, 69.6 (2C, C-4, C-8), 74.1 (C-6), 80.8 (J = 186.1 Hz, CH_2F), 83.1 (C-2), 168.2, 170.2, 170.6 (5C, CO). ESI-MS calcd for $C_{19}H_{27}FN_4O_{10}S$ $[M + Na]^+$ 545.14; found m/z 545.15.

Methyl (Methyl 5-Chloroacetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (16b). Compound **15** (55.0 mg, 0.12 mmol) was dissolved in dioxane/water (0.5 mL/0.1 mL), treated with triethylamine

(48.6 mg, 34 μL, 0.48 mmol), and cooled to 0 °C. Then, chloroacetic anhydride (41.0 mg, 0.48 mmol) was added, and stirring was continued at rt for 3 h. The reaction mixture was diluted with $CHCl_3$ (10.0 mL) and washed successively with saturated aqueous $NaHCO_3$ (3 × 5 mL) and H_2O (1 × 5 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (gradient, PE:EA; 1:1 to 1:2) to yield **16b** as a white foam (42 mg, 66%). $[\alpha]^{20}_D$ 0.33 (*c* 0.95, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.91 (t, *J* = 12.2 Hz, 1H, H-3a), 1.96 (s, 3H, SMe), 2.06, 2.08, 2.13 (3 s, 9H, 3 OAc), 2.72 (dd, *J* = 4.7 Hz, 12.8 Hz, 1H, H-3b), 3.18 (m, 1H, H-9a), 3.59 (m, 1H, H-9b), 3.78 (s, 3H, OMe), 3.85 (A of AB, *J* = 15.0 Hz, 1H, CH_2Cl), 3.86 (m, 1H, H-6), 3.93 (B of AB, *J* = 15.0 Hz, 1H, CH_2Cl), 4.03 (m, 1H, H-5), 4.90 (dt, *J* = 4.7 Hz, 11.5 Hz, 1H, H-4), 5.24 (m, 2H, H-7, H-8), 6.28 (d, *J* = 10.1 Hz, 1H, NH).^{13}C NMR ($CDCl_3$) δ 12.1 (SMe), 20.8, 20.9, 21.1 (3 OAc), 37.9 (C-3), 42.5 (*C*H_2Cl), 49.7 (C-9), 50.6 (C-5), 53.1 (OMe), 67.1, 67.9, 69.2 (3C, C-4, C-7, C-8), 74.2 (C-6), 83.1 (C-2), 166.6, 167.8, 170.3, 170.6 (5C, CO). ESI-MS calcd for $C_{19}H_{27}ClN_4O_{10}S$ $[M + Na]^+$ 561.10; found *m*/*z* 561.37.

Methyl (Methyl 5-*o*-Nitrotoluenesulfonamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (16c). Compound **15** (73.0 mg, 0.16 mmol) was dissolved in dry DCM (3.0 mL), and it was cooled to 0 °C. Nosylchloride (105 mg, 0.47 mmol), NEt_3 (34.0 μL, 48.0 mg, 0.47 mmol), and DMAP (10.0 mg, 0.08 mmol) were added successively. The reaction mixture was stirred at rt overnight. Then it was washed with saturated aqueous $NaHCO_3$ (2 × 5 mL) and H_2O (1 × 5 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (EA:PE, gradient 1:1 to 2:1) to yield **16c** (81 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.85 (m, 1H, H-3a), 2.02 (s, 3H, SMe), 2.10, 2.13, 2.21 (3 s, 9H, 3 OAc), 2.80 (m, 1H, H-3b), 3.32 (dd, *J* = 6.2, 13.4 Hz, 1H, H-9a), 3.57 (dd, *J* = 3.2, 13.4 Hz, 1H, H-9b), 3.80 (m, 1H, H-5), 3.82 (s, 3H, OMe), 3.91 (d, *J* = 10.5 Hz, 1H, H-6), 4.97 (td, *J* = 4.7, 11.4 Hz, 1H, H-4), 5.30 (m, 2H, H-7, H-8), 5.75 (d, *J* = 9.4 Hz, 1H, NH), 7.70 (m, 2H, CH_{ar}), 7.90 (d, *J* = 7.9, 1H, CH_{ar}), 8.10 (d, *J* = 6.5, 1H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 12.1 (SMe), 20.5, 21.1, 21.1 (3 OAc), 38.1 (C-3), 50.8 (C-9), 53.2, 53.5 (2C, C-5, OMe), 68.8 (C-7), 69.7 (C-4), 70.4 (C-8), 74.7 (C-6), 82.8 (C-2), 125.5, 130.4, 133.5, 135.5, 147.5 (6C, C−Ar), 167.8, 170.4 (4C, CO). ESI-MS calcd for $C_{23}H_{29}N_5O_{13}S_2$ $[M - H]^-$ 646.12; found *m*/*z* 646.56.

Methyl (Methyl 5-Methylsulfonamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (16d). Compound **15** (50.0 mg, 0.11 mmol) was dissolved in dry DCM (2.0 mL) under argon atmosphere and subsequently cooled to 0 °C. Methanesulfonylchloride (25.0 μL, 37.0 mg, 0.32 mmol), NEt_3 (45.0 μL, 33.0 mg, 0.32 mmol), and a catalytic amount of DMAP were added successively. The reaction mixture was stirred at 0 °C overnight. Then it was washed with saturated aqueous $NaHCO_3$ (2 × 5 mL) and H_2O (1 × 5 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (EA:PE, gradient 1:1 to 2:1) to yield **16d** (30 mg, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.98 (t, *J* = 12.3 Hz, 1H, H-3a), 2.03 (s, 3H, SMe), 2.13, 2.17 (3 s, 9H, 3 OAc), 2.68 (dd, *J* = 4.7, 12.7 Hz, 1H, H-3b), 3.14 (s, 3H, CH_3), 3.29 (dd, *J* = 6.1, 13.4, 1H, H-9a), 3.66 (m, 2H, H-5, H-9b), 3.77 (s, 3H, OMe), 3.88 (dd, *J* = 1.6, 10.6 Hz, 1H, H-6), 4.55 (d, *J* = 9.8 Hz, 1H, NH), 5.16 (td, *J* = 4.7, 11.5 Hz, 1H, H-4), 5.26 (m, 1H, H-8), 5.48 (dd, *J* = 1.6, 7.2 Hz, 1H, H-7). ^{13}C NMR ($CDCl_3$) δ 14.3 (SMe), 20.9, 21.1, 21.4 (3 OAc), 31.7 (*C*H_3), 38.2 (C-3), 50.7 (C-9), 52.5 (C-5), 53.0 (OMe), 68.3, 69.6 (2C, C-7, C-8), 70.4 (C-4), 74.5 (C-6), 82.8 (C-2), 167.8, 170.3, 171.7, 172.2 (4 CO). ESI-MS calcd for $C_{18}H_{28}N_4O_{11}S_2$ $[M + Na]^+$ 563.12; found *m*/*z* 563.18.

Methyl ((2,3-Difluorobenzyl) 5-Fluoroacetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (17a). Compound **16a** (55.0 mg, 0.16 mmol) was dissolved in dry acetonitrile (2 mL). Powdered MS 3 Å (50 mg) and 2,3-difluorobenzyl alcohol (35.0 μL, 42.0 mg, 0.29 mmol) were added. The reaction mixture was stirred at rt for 1.5 h. Then the suspension was cooled to −40 °C and was subsequently treated with *N*-iodosuccinimide (35.0 mg, 0.16 mmol) and triflic acid (8.00 μL, 13.0 mg, 0.09 mmol). After 30 min, the reaction mixture was warmed to −30 °C and stirring was continued for 20 h. After warming to rt, the mixture was filtered through a pad of celite and washed with 20% $Na_2S_2O_3$ (1 × 2 mL), saturated aqueous $NaHCO_3$ (3 × 5 mL), and H_2O (1 × 5 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (1% gradient *i*PrOH in petrol ether/DCM 2:1) to yield **16a** (43 mg, 66%) as a colorless oil. $[\alpha]^{20}_D$ −0.04 (*c* 0.73, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.03 (s, 3H, OAc), 2.04 (m, 1H, H-3a), 2.16, 2.21 (2 s, 6H, 2 OAc), 2.69 (dd, *J* = 4.7, 12.9 Hz, 1H, H-3b), 3.26 (dd, *J* = 5.3, 13.5 Hz, 1H, H-9a), 3.58 (dd, *J* = 2.8, 13.5 Hz, 1H, H-9b), 3.79 (s, 3H, OMe), 4.21 (m, 2H, H-5, H-6), 4.53 (A of AB, *J* = 12.0 Hz, 1H, CH_2Ar), 4.63 (m, 2H, CH_2F), 4.83 (B of AB, *J* = 12.3 Hz, 1H, CH_2Ar), 4.95 (ddd, *J* = 4.7, 10.0, 12.1 Hz, 1H, H-4), 5.35 (m, 2H, H-7, H-8), 6.18 (dd, *J* = 3.3, 9.0 Hz, 1H, N*H*FAc), 7.11 (m, 3H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 20.9 (3C, OAc), 37.9 (C-3), 48.6 (C-5), 50.9 (C-9), 53.0 (OMe), 60.5 (*C*H_2Ar), 67.8 (C-7), 68.7 (C-8), 69.3 (C-4), 72.6 (C-6), 80.1 (*J* = 186.1 Hz, *C*H_2F), 98.7 (C-2), 116.9, 117.1, 125.1, 126.6 (6C, C−Ar), 168.0, 170.2, 170.7 (5C, CO). ESI-MS calcd for $C_{25}H_{29}F_3N_4O_{11}$ $[M + Na]^+$ 641.17; found *m*/*z* 641.14.

Methyl ((2,3-Difluorobenzyl) 5-Chloroacetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (17b). Compound **16b** (63.0 mg, 0.12 mmol) was reacted with 2,3-difluorobenzyl alcohol (41.0 μL, 0.36 mmol), *N*-iodosuccinimide (42.0 mg, 0.19 mmol), and triflic acid (8.0 μL, 14.4 mg, 0.1 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient *i*PrOH in PE:DCM, 8:4) to yield **17b** (50 mg, 68%) as a colorless oil. $[\alpha]^{20}_D$ −0.01, (*c* 1.05, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.02 (m, 1H, H-3a), 2.03, 2.16, 2.21 (3 s, 9H, 3 OAc), 2.70 (dd, *J* = 4.6, 12.9 Hz, 1H, H-3b), 3.28 (dd, *J* = 5.8, 13.5 Hz, 1H, H-9a), 3.59 (dd, *J* = 3.0, 13.5 Hz, 1H, H-9b), 3.81 (s, 3H, OMe), 3.93, 4.01 (A, B of AB, *J* = 15.0 Hz, 2H, CH_2Cl), 4.11 (m, 1H, H-5), 4.24 (dd, *J* = 2.1, 10.7 Hz, 1H, H-6), 4.54, 4.83 (A, B of AB, *J* = 12.0 Hz, 2H, CH_2Ar), 4.99 (m, 1H, H-4), 5.32 (dd, *J* = 2.1, 7.8 Hz, 1H, H-7), 5.36 (m, 1H, H-8), 6.41 (d, *J* = 10.1 Hz, 1H, NH), 7.06−7.17 (m, 3H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 20.8, 20.9, 21.1 (3 OAc), 37.9 (C-3) 42.4 (*C*H_2Cl), 49.7 (C-5), 50.9 (C-9), 60.5 (*C*H_2Ar), 67.9, 68.1, 68.4 (3C, C-4, C-7, C-8), 72.6 (C-6), 98.7 (C-2), 117.0, 123.9, 125.1, 126.5, 149.5, 151.4 (6 C−Ar), 166.7, 168.0, 170.2, 170.3, 170.7 (5 CO). ESI-MS calcd for $C_{25}H_{29}Cl_2FN_4O_{11}$ $[M + Na]^+$ 657.14; found *m*/*z* 657.29.

Methyl ((2,3-Difluorobenzyl) 5-(*o*-Nitrotoluenesulfonamido)-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (17c). Compound **16c** (62.0 mg, 0.10 mmol) was reacted with 2,3-difluorobenzyl alcohol (30.0 μL, 38.0 mg, 0.29 mmol), *N*-iodosuccinimide (32.0 mg, 0.14 mmol), and triflic acid (7.00 μL, 12.0 mg, 0.08 mmol). The crude product was purified by chromatography on silica gel (toluene/EA, gradient 1:1 to 2:1) to yield **17c** (44 mg, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.84 (t, *J* = 12.4 Hz, 1H, H-3a), 2.08, 2.17, 2.28 (3 s, 9H, 3 OAc), 2.64 (dd, *J* = 4.6, 12.8 Hz, 1H, H-3b), 3.29 (m, 1H, H-9a), 3.45 (m, 1H, H-9b), 3.73 (s, 3H, OMe), 3.79 (m, 2H, H-5, H-7), 4.12 (d, *J* = 8.8 Hz, 1H, H-6), 4.46, 4.77 (A, B of AB, *J* = 12.0 Hz, 2H, CH_2Ar), 4.91 (td, *J* = 4.6, 11.5 Hz, 1H, H-4), 5.25 (d, *J* = 7.8 Hz, 1H, H-8), 5.60 (d, *J* = 9.4 Hz, 1H, NH), 7.09 (m, 3H, CH_{ar}), 7.69 (m, 2H, CH_{ar}), 7.85 (d, *J* = 7.9 Hz, 1H, CH_{ar}), 8.07 (d, *J* = 7.8 Hz, 1H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 21.1 (3C, OAc), 38.1 (C-3), 51.1 (C-5), 53.1 (C-9), 53.6 (OMe), 60.5 (*C*H_2Ar), 68.7, 68.8 (2C, C-4, C-7), 69.8 (C-8), 73.2 (C-6), 125.5, 130.4, 133.5, 147.6 (12C, C−Ar), 168.0, 170.1, 170.3, 170.5 (4 CO). ESI-MS calcd for $C_{29}H_{31}F_2N_5O_{14}S$ $[M + Na]^+$ 766.16; found *m*/*z* 766.14.

Methyl ((2,3-Difluorobenzyl) 5-Methylsulfonamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (17d). Compound **16d** (56.0 mg, 0.10 mmol) was reacted with 2,3-difluorobenzyl alcohol (33.0 μL, 42.0 mg, 0.29 mmol), *N*-iodosuccinimide (35.0 mg, 0.16 mmol), and triflic acid (8.00 μL, 13.0 mg, 0.09 mmol). The crude product was purified by chromatography on silica gel (1% gradient *i*PrOH in petrol ether/DCM 2:1) to yield **17d** (37 mg, 56%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.95 (t, J = 12.5, 1H, H-3a), 2.13, 2.18 (2 s, 9H, 3 OAc), 2.61 (dd, J = 4.6, 12.7 Hz, 1H, H-3b), 3.02 (s, 3H, CH_3), 3.31 (dd, J = 6.3, 13.5 Hz, 1H, H-9a), 3.60 (dd, J = 3.3, 13.5 Hz, 1H, H-9b), 3.71 (m, 4H, H-5, OMe), 4.12 (dd, J = 1.7, 10.7 Hz, 1H, H-6), 4.54, 4.81 (A, B of AB, J = 11.8 Hz, 2H, CH_2Ar), 5.09 (m, 1H, H-4), 5.31 (m, 1H, H-8), 5.49 (dd, J = 1.7, 7.5 Hz, 1H, H-7), 7.11 (m, 3H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 20.9, 21.1, 21.4 (3 OAc), 38.1 (C-3), 42.3 (CH_3), 50.8 (C-9), 52.6, 53.0 (2C, OMe, C-5), 60.4 (CH_2Ar), 68.1 (C-4), 68.8 (C-7), 70.0 (C-8), 72.8 (C-6), 98.5 (C-2), 116.9, 117.0, 124.0, 125.1 (6C, C−Ar), 167.7, 170.1, 170.7, 172.1 (4 CO). ESI-MS calcd for $C_{24}H_{30}F_2N_4O_{12}S$ [M + Na]$^+$ 659.15; found m/z 659.24.

Methyl ((2,3-Difluorobenzyl) 5-Fluoroacetamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (18a). Compound **17a** (55.0 mg, 0.09 mmol) was dissolved in dry DCM (2 mL). *p*-Chlorobenzoyl chloride (45.0 μL, 62.0 mg, 0.36 mmol) and triphenylphosphine (47.0 mg, 0.18 mmol) were added. The reaction mixture was stirred at rt overnight. Afterward, the reaction mixture was washed with saturated aqueous $NaHCO_3$ (3 × 5 mL) and H_2O (1 × 5 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (EA:PE, gradient 1:1 to 2:1) to yield **18a** (31 mg, 48%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.97 (m, 1H, H-3a), 1.97, 2.09, 2.18 (3 s, 9H, 3 OAc), 2.64 (dd, J = 4.6, 12.8 Hz, 1H, H-3b), 2.94 (dd, J = 3.9, 15.0 Hz, 1H, H-9a), 3.69 (s, 3H, OMe), 4.09 (m, 1H, H-6), 4.22 (m, 2H, H-5, H-9b), 4.46 (A of AB, J = 12.0 Hz, 1H, CH_2Ar), 4.64 (m, 2H, CH_2F), 4.78 (B of AB, J = 12.0 Hz, 1H, CH_2Ar), 4.86 (m, 1H, H-4), 5.11 (dd, J = 2.0, 9.8 Hz, 1H, H-7), 5.27 (m, 1H, H-8), 6.12 (dd, J = 3.3, 10.0 Hz, 1H, NHFAc), 6.89 (dd, J = 4.3, 7.9 Hz, 1H, NH), 7.03 (m, 3H, CH_{ar}), 7.34, 7.67 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 20.8, 21.1 (3C, OAc), 37.9 (C-3), 38.7 (C-9), 48.7 (C-5), 52.9 (OMe), 60.5 (CH_2Ar), 67.8 (C-7), 68.2 (C-8), 68.7 (C-4), 72.0 (C-6), 80.0 (J = 186.0 Hz, CH_2F), 98.6 (C-2), 117.1, 124.0, 125.3, 126.6, 128.4, 128.8, 131.5, 132.6, 137.8 (12C, C−Ar), 166.4, 167.8, 168.3, 170.5, 170.7, 172.3 (6 CO). ESI-MS calcd for $C_{32}H_{34}ClF_3N_2O_{12}$ [M + Na]$^+$ 753.18; found m/z 753.19.

Methyl ((2,3-Difluorobenzyl) 5-Chloroacetamido-4,7,8-tri-*O*-acetyl-9-(4-chloro-benzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (18b). Compound **17b** (52.2 mg, 0.08 mmol) was reacted with *p*-chlorobenzoyl chloride (23.0 μL, 31.0 mg, 0.18 mmol) and triphenylphosphine (47.0 mg, 0.18 mmol). The crude product was purified by chromatography on silica gel (0.5% gradient of MeOH in DCM) to yield in **18b** (25.0 mg, 48%). $[\alpha]^{20}_D$ 0.04 (*c* 1.06, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.00 (m, 1H, H-3a), 2.03, 2.16, 2.25 (3 s, 9H, 3 OAc), 2.71 (m, 1H, H-3b), 3.01 (m, 1H, H-9a), 3.77 (s, 3H, OMe), 3.97 (m, 2H, CH_2Cl), 4.11 (m, 1H, H-6), 4.20 (m, 1H, H-5), 4.29 (m, 1H, H-9b), 4.54, 4.85 (A, B of AB, J = 11.9 Hz, 2H, CH_2Ar), 4.95 (m, 1H, H-4), 5.18 (m, 1H, H-7), 5.31 (m, 1H, H-8), 6.39 (m, 1H, NHAc), 6.97 (m, 1H, NH), 7.06−7.15 (m, 3H, CH_{ar}), 7.40 (m, 2H, CH_{ar}), 7.74 (m, 2H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 20.8, 21.2 (3C, OAc), 38.0 (C-3), 38.6 (C-9), 42.4 (CH_2Cl), 49.8 (C-5), 52.9 (OMe), 60.4 (CH_2Ar), 67.8, 68.3, 68.5 (3C, C-4, C-7, C-8), 72.1 (C-6), 98.6 (C-2), 117.0 (J = 17.5 Hz), 124.0, 125.3, 126.5, 128.4, 128.9, 132.7, 137.8 (12 C−Ar), 166.4, 166.7, 167.9, 170.5, 170.7, 172.3 (6 CO). ESI-MS calcd for $C_{32}H_{34}Cl_2F_2N_2O_{12}$ [M + Na]$^+$ 769.14; found m/z 769.34.

Methyl ((2,3-Difluorobenzyl) 5-(*o*-Nitrotoluenesulfonamido)-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (18c). Compound **17c** (44.0 mg, 0.06 mmol) was dissolved in dry DCE (2 mL). *p*-Chlorobenzoyl chloride (30.0 μL, 40.0 mg, 0.23 mmol) and triphenylphosphine (31.0 mg, 0.12 mmol) were added. The reaction mixture was stirred at rt overnight. The crude product was purified by chromatography on silica gel (EA:PE, gradient 1:1 to 2:1) to yield **18c** (29 mg, 58%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.90 (m, 1H, H-3a), 2.04, 2.14, 2.32 (3 s, 9H, 3 OAc), 2.69 (dd, J = 4.5, 12.7 Hz, 1H, H-3b), 2.92 (d, J = 15.2 Hz, 1H, H-9a), 3.75 (s, 3H, OMe), 3.91 (m, 1H, H-5), 4.12 (m, 1H, H-6), 4.38 (m, 1H, H-9b), 4.52 (A of AB, J = 11.9 Hz, 1H, CH_2Ar), 4.84 (m, 2H, H-4, CH_2Ar), 5.31 (m, 2H, H-7, H-8), 5.64 (d, J = 9.4 Hz, 1H, NH), 7.13 (m, 4H, CH_{ar}), 7.21 (m, 1H, NH), 7.40 (m, 3H, CH_{ar}), 7.72 (m, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 20.3, 21.2, 21.3 (3 OAc), 38.2, 38.3 (2C, C-3, C-9), 53.0 (OMe), 53.7 (C-5), 60.6 (CH_2Ar), 68.2, 68.4 (2C, C-7, C-8), 69.0 (C-4), 72.6 (C-6), 98.2 (C-2), 117.0, 123.9, 125.6, 128.8, 130.3, 131.5, 132.1, 132.8, 133.3 (18C, C−Ar), 166.6, 167.7, 169.9, 170.4, 172.4 (5 CO). ESI-MS calcd for $C_{36}H_{36}ClF_2N_3O_{15}S$ [M + Na]$^+$ 878.15; found m/z 878.28.

Methyl ((2,3-Difluorobenzyl) 5-Methylsulfonamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (18d). Compound **17d** (35.0 mg, 0.06 mmol) was dissolved in dry DCM (2 mL). *p*-Chlorobenzoyl chloride (28.0 μL, 38.0 mg, 0.22 mmol) and triphenylphosphine (29.0 mg, 0.11 mmol) were added. The reaction mixture was stirred at rt overnight. The crude product was purified by chromatography on silica gel (EA:PE, gradient 1:1 to 2:1) to yield **18d** (21 mg, 52%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.98 (t, J = 13.6 Hz, 1H, H-3a), 2.05, 2.10, 2.13 (3 s, 9H, 3 OAc), 2.67 (dd, J = 4.6, 12.7 Hz, 1H, H-3b), 2.99 (s, 3H, CH_3), 3.05 (m, 1H, H-9a), 3.70 (s, 3H, OMe), 3.77 (m, 1H, H-5), 4.09 (d, J = 1.5 Hz, 1H, H-6), 4.30 (ddd, J = 3.0, 8.2, 15.1 Hz, 1H, H-9b), 4.54 (A of AB, J = 12.1 Hz, 1H, CH_2Ar), 4.59 (d, J = 9.6 Hz, 1H, NH), 4.83 (B of AB, J = 11.9 Hz, 1H, CH_2Ar), 4.99 (ddd, J = 4.6, 10.4, 12.2 Hz, 1H, H-4), 5.30 (m, 1H, H-8), 5.37 (dd, J = 1.7, 9.6 Hz, 1H, H-7), 7.13 (m, 4H, CH_{ar}, NH), 7.40, 7.75 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 21.2, 21.2, 21.3 (3 OAc), 29.7 (C-3), 38.2, 38.8 (2C, C-9, CH_3), 42.3 (C-5), 52.8 (OMe), 60.5 (CH_2Ar), 68.1, 68.5, 69.1 (3C, C-4, C-7, C-8), 72.4 (C-6), 98.4 (C-2), 116.9, 117.1, 125.3, 128.4, 128.9, 132.1, 132.7, 137.8 (12C, C−Ar), 166.4, 167.7, 170.5, 171.6, 172.6 (5 CO). ESI-MS calcd for $C_{31}H_{35}ClF_2N_2O_{13}S$ [M + Na]$^+$ 771.15; found m/z 771.29.

Sodium ((2,3-Difluorobenzyl) 5-Fluoroacetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (19a). Compound **18a** (28.0 mg, 0.04 mmol) was dissolved in THF/water (2.0 mL/0.5 mL) and was reacted with LiOH (9.00 mg, 0.38 mmol). The reaction mixture was stirred at rt for 4 h. 7% HCl (aq) was added to adjust the pH to 7. The crude product was purified by reversed-phase chromatography (RP-18, 10% gradient MeOH in H_2O) followed by ion exchange chromatography (Dowex-50) and P2 size exclusion chromatography to yield **19a** (7.0 mg, 30%) as a white foam. $[\alpha]^{20}_D$ −0.73 (*c* 0.08, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 1.59 (t, J = 12.2, 1H, H-3a), 2.65 (dd, J = 4.7, 12.4, 1H, H-3b), 3.31 (dd, J = 7.7, 14.0 Hz, 1H, H-9a), 3.41 (dd, J = 1.8, 8.8 Hz, 1H, H-5), 3.64 (m, 3H, H-4, H-8, H-9b), 3.77 (dd, J = 1.9, 10.5 Hz, 1H, H-6), 3.84 (m, 1H, H-7), 4.52 (A of AB, J = 11.7 Hz, 1H, CH_2Ar), 4.70 (m, 2H, CH_2Ar, CH_2F), 4.81 (d, J = 5.1 Hz, 1H, CH_2F), 7.02 (m, 3H, CH_{ar}), 7.39, 7.62 (AA′, BB′ of AA′BB′, J = 8.7 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 40.4, 42.6 (2C, C-3, C-9), 57.2 (C-5), 60.6 (CH_2Ar), 68.0 (C-4), 69.7 (C-7), 70.3 (C-8), 72.3 (C-6), 87.0 (CH_2F), 101.3 (C-2), 117.2, 125.8, 128.6, 128.8, 132.1, 137.5, 141.7 (12C, C−Ar), 173.1, 175.3, 189.7 (3 CO). HRMS calcd for $C_{25}H_{26}ClF_3N_2O_9$ [M − H]$^-$ 589.1206; found 589.1191.

Sodium ((2,3-Difluorobenzyl) 5-Chloroacetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (19b). Compound **18b** (21.0 mg, 0.03 mmol) was treated with LiOH (6.7 mg, 0.3 mmol) in THF/water (2.0 mL/0.5 mL). The crude product was purified by chromatography on silica gel

(0.1% gradient of H_2O in DCM/MeOH; 2:1) followed by ion exchange chromatography (Dowex 50) and P2 size exclusion chromatography to yield **19b** as a white solid (10 mg, 60%). ^{1}H NMR (500 MHz, MeOD) δ 1.58 (dd, J = 10.7, 12.1 Hz, 1H, H-3a), 2.82 (m, 1H, H-3b), 3.35 (m, 1H, H-7), 3.46 (m, 1H, H-9a), 3.68 (m, 4H, H-4, H-5, H-6, H-9b), 3.93 (m, 2H, CH_2Cl), 4.57, 4.84 (A, B of AB, J = 12.1 Hz, 2H, CH_2Ar), 7.01 (m, 3H, CH$_{ar}$), 7.35, 7.72 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH$_{ar}$). ^{13}C NMR (MeOD) δ 43.0 (C-3), 44.8 (C-9), 45.0 (*C*H$_2$Cl), 54.0 (C-5), 63.1 (*C*H$_2$Ar), 70.5, 72.2, 72.9 (3C, C-4, C-7, C-8), 74.9 (C-6), 103.8 (C-2), 119.7, 126.9, 128.4, 128.9, 131.2, 131.3, 140.1 (10 C−Ar), 170.0, 170.5, 173.11 (3 CO). HRMS calcd for $C_{25}H_{25}Cl_2F_2N_2NaO_9$ [M + Na]$^+$ 651.0701; found m/z 651.0700.

Sodium ((2,3-Difluorobenzyl) 5-(*o*-Nitrotoluenesulfonamido)-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (19c). Compound **18c** (29.0 mg, 0.03 mmol) was dissolved in THF/H_2O (2 mL/0.5 mL) and was reacted with LiOH (8.00 mg, 0.33 mmol). The crude product was purified on RP-18 (10% gradient MeOH in water) followed by ion exchange chromatography (Dowex-50) and P2 size exclusion chromatography to yield **19c** (10 mg, 40%). $[\alpha]^{20}_D$ −0.35 (c 0.10, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 1.45 (t, J = 12.2 Hz, 1H, H-3a), 2.52 (dd, J = 4.5, 12.3 Hz, 1H, H-3b), 3.16 (t, J = 9.8 Hz, 1H, H-5), 3.43 (m, 2H, H-4, H-9a), 3.60 (dd, J = 2.9, 14.2 Hz, 1H, H-9b), 3.67 (m, 2H, H-6, H-8), 3.74 (dd, J = 1.1, 8.9 Hz, 1H, H-7), 4.50 (A of AB, J = 11.7 Hz, 1H, CH_2Ar), 4.65 (m, 1H, CH_2Ar), 7.04 (m, 3H, CH$_{ar}$), 7.42 (AA′ of AA′BB′, J = 8.5 Hz, 2H, CH$_{ar}$), 7.60 (m, 4H, CH$_{ar}$), 7.98 (BB′ of AA′BB′, J = 7.6 Hz, 2H, CH$_{ar}$). ^{13}C NMR (D_2O) δ 40.4 (C-3), 42.4 (C-9), 56.5 (C-5), 60.5 (*C*H$_2$Ar), 69.4 (C-7), 69.5 (C-4), 70.3 (C-8), 73.8 (C-6), 101.2 (C-2), 117.1, 124.2, 125.8, 128.7, 130.2, 132.1, 132.5, 137.5, 146.8 (18C, C−Ar), 170.0, 173.3 (2 CO). HRMS calcd for $C_{29}H_{27}ClF_2N_3NaO_{12}S$ [M + Na]$^+$ 760.0767; found m/z 760.0775.

Sodium ((2,3-Difluorobenzyl) 5-Methylsulfonamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (19d). Compound **18d** (21.0 mg, 0.03 mmol) was dissolved in THF/H_2O (2 mL/0.5 mL) and was reacted with LiOH (7.00 mg, 0.28 mmol). The crude product was purified on RP-18 (10% gradient MeOH in water) followed by ion exchange chromatography (Dowex-50) and P2 size exclusion chromatography to yield **19d** (10 mg, 59%). $[\alpha]^{20}_D$ −0.44 (c 0.07, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 1.56 (t, J = 12.2 Hz, 1H, H-3a), 2.62 (dd, J = 4.6, 12.4 Hz, 1H, H-3b), 3.02 (s, 3H, CH_3), 3.23 (t, J = 10.1 Hz, 1H, H-5), 3.40 (dd, J = 7.8, 14.7 Hz, 1H, H-9a), 3.48 (td, J = 4.6, 11.9 Hz, 1H, H-4), 3.66 (m, 3H, H-6, H-8, H-9b), 3.75 (d, J = 8.7 Hz, 1H, H-7), 4.51 (A of AB, J = 11.8 Hz, 1H, CH_2Ar), 4.66 (m, 1H, CH_2Ar), 7.02 (m, 3H, CH$_{ar}$), 7.40, 7.63 (AA′, BB′ of AA′BB′, J = 8.6 Hz, 4H, CH$_{ar}$). ^{13}C NMR (D_2O) δ 40.9, 41.3 (2C, C-3, C-9), 42.5 (*C*H$_3$), 55.5 (C-5), 60.6 (*C*H$_2$Ar), 68.6 (C-7), 69.3 (C-4), 70.3 (C-8), 73.2 (C-6), 101.2 (C-2), 112.4, 117.2, 124.4, 125.8, 128.7, 128.8, 137.5 (12C, C−Ar), 173.1, 187.7 (2 CO). HRMS calcd for $C_{24}H_{26}ClF_2N_2NaO_{10}S$ [M + Na]$^+$ 653.0760; found m/z 653.0759.

Sodium ((2,3-Difluorobenzyl) 5-Methoxyacetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (19e). Compound **18b** (10 mg, 0.01 mmol) was treated with 10% aqueous NaOH (0.3 mL) in MeOH (1.5 mL) at rt for 4 h. The crude product was purified by chromatography on RP-18 (5% gradient of MeOH in H_2O) to yield **19e** (2.3 mg, 19%) and **19b** (1.7 mg, 21%) as white solids. $[\alpha]^{20}_D$ −19.1 (c 0.46, MeOH). ^{1}H NMR (500 MHz, D_2O) δ 1.67 (t, J = 12.2 Hz, 1H, H-3a), 2.73 (dd, J = 4.6, 12.4 Hz, 1H, H-3b), 3.28 (s, 3H, OMe), 3.41−3.56 (m, 2H, H-7, H-9a), 3.62−3.79 (m, 3H, H-4, H-8, H-9b), 3.82−3.89 (m, 2H, H-5, H-6), 3.95, 3.98 (A, B of AB, J = 15.6 Hz, 2H, CH_2OMe), 4.62, 4.78 (A, B of AB, J = 11.7 Hz, 2H, CH$_2$Ar), 7.00−7.22 (m, 3H, CH$_{ar}$), 7.49, 7.71 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH$_{ar}$). ^{13}C NMR (D_2O) δ 40.4 (C-3), 42.4 (C-9), 51.5 (C-5), 58.9 (OMe), 60.6 (CH$_2$Ar), 67.9 (C-4), 69.6 (C-7), 70.2 (C-8), 70.8 (*C*H$_2$OMe), 72.4 (C-6), 101.2 (C-2), 110.0, 117.1, 117.2, 124.4, 125.9, 128.7, 128.8, 132.1 (12C, C−Ar), 173.0, 173.5 (3C, CO). HRMS calcd for $C_{26}H_{28}ClF_2N_2O_{10}Na$ [M + Na]$^+$ 647.1195; found m/z 647.5573.

Sodium ((2,3-Difluorobenzyl) 5-Cyclopropylamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (19f). Compound **23f** (31.0 mg, 0.04 mmol) was dissolved in THF/water (2.0 mL/0.5 mL) and was reacted with LiOH (10.0 mg, 0.42 mmol). The crude product was purified on RP-18 (10% gradient MeOH in H_2O) followed by ion exchange chromatography (Dowex-50) and P2 size exclusion chromatography to yield **19f** (11 mg, 44%). $[\alpha]^{20}_D$ −0.1 (c 0.26, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 0.59 (m, 2H, CH_2), 0.70 (m, 2H, CH_2), 1.44 (m, 1H, CH), 1.56 (t, J = 11.8 Hz, 1H, H-3a), 2.64 (dd, J = 3.5, 11.8 Hz, 1H, H-3b), 3.37 (d, J = 8.2 Hz, 1H, H-7), 3.47 (m, 1H, H-9a), 3.63 (m, 4H, H-5, H-6, H-8, H-9b), 4.52, 4.69 (A, B of AB, J = 11.6 Hz, 2H, CH_2Ar), 7.04 (m, 3H, CH$_{ar}$), 7.40, 7.63 (AA′, BB′ of AA′BB′, J = 8.1 Hz, 4H, CH$_{ar}$). ^{13}C NMR ($CDCl_3$) δ 6.8, 7.0 (2C, *C*H$_2$), 14.1 (*C*H), 40.5, 42.3 (2C, C-3, C-9), 51.9 (C-5), 60.5 (*C*H$_2$Ar), 68.0 (C-4), 69.4 (C-7), 70.1 (C-8), 72.8 (C-6), 101.2 (C-2), 117.1, 124.4, 125.9, 128.7, 132.1, 137.6 (12C, C−Ar), 170.0, 173.1, 178.3 (3 CO). HRMS calcd for $C_{27}H_{28}ClF_2N_2O_9$ [M − H]$^-$ 597.1457; found 597.1454.

Sodium ((2,3-Difluorobenzyl) 5-Cyclobutylamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (19g). Compound **23g** (44.0 mg, 0.06 mmol) was dissolved in THF/water (2.0 mL/0.5 mL) and was reacted with LiOH (14.0 mg, 0.58 mmol). The crude product was purified on RP-18 (10% gradient MeOH in H_2O) followed by ion exchange chromatography (Dowex-50) and P2 size exclusion chromatography to yield **19g** (17 mg, 49%). $[\alpha]^{20}_D$ −0.2 (c 0.11, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 1.55 (m, 1H, H-3a), 1.85 (m, 6H, CH_2), 2.63 (dd, J = 3.9, 12.2 Hz, 1H, H-3b), 2.99 (quint, J = 8.8 Hz, 1H, CH), 3.32 (d, J = 8.6 Hz, 1H, H-9a), 3.61 (m, 6H, H-4, H-5, H-6, H-7, H-8, H-9b), 4.52, 4.68 (A, B of AB, J = 11.4 Hz, 2H, CH_2Ar), 7.05 (m, 3H, CH$_{ar}$), 7.40, 7.63 (AA′, BB′ of AA′BB′, J = 7.5 Hz, 4H, CH$_{ar}$). ^{13}C NMR (D_2O) δ 17.4, 24.5 (3C, *C*H$_2$), 39.2 (*C*H), 40.5 (C-3), 42.1 (C-9), 51.6 (C-5), 60.5 (*C*H$_2$Ar), 67.8 (C-4), 69.3 (C-7), 70.1 (C-8), 72.7 (C-6), 110.0 (C-2), 115.6, 123.3, 125.9, 128.8, 150.9 (12C, C−Ar), 170.0, 175.8. 179.7 (3 CO). ESI-MS calcd for $C_{28}H_{30}ClF_2N_2NaO_9$ [M + Na]$^+$ 657.1403; found m/z 657.1401.

Methyl (Methyl 5-*tert*-Butyloxycarbonylamino-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-tride-oxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (20). Compound **14** (167 mg, 0.30 mmol) was dissolved in dry DCE (5 mL). *p*-Chlorobenzoyl chloride (150 μL, 210 mg, 1.19 mmol) and triphenylphosphine (156 mg, 0.59 mmol) were added. The reaction mixture was stirred at rt overnight. Afterward, the reaction mixture was washed with saturated aqueous $NaHCO_3$ (3 × 5 mL) and H_2O (1 × 5 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (EA:PE, gradient 1:1 to 2:1) to yield **20** (95 mg, 48%) as a white foam. ^{1}H NMR (500 MHz, CD_2Cl_2) δ 1.39 (s, 9H, C(CH$_3$)$_3$), 1.92 (s, 3H, SMe), 2.01 (s, 6H, 2 OAc), 2.12 (m, 1H, H-3a), 2.23 (s, 3H, OAc), 2.56 (dd, J = 4.7, 13.8 Hz, 1H, H-3b), 3.00 (td, J = 3.2, 15.2 Hz, 1H, H-9a), 3.79 (m, 4H, OMe, H-5), 4.23 (d, J = 10.6 Hz, 1H, H-6), 4.41 (ddd, J = 3.1, 8.8, 14.4 Hz, 1H, H-9b), 4.52 (dd, J = 2.9, 10.0 Hz, 1H, N*H*Ac), 5.09 (m, 1H, H-8), 5.16 (td, J = 4.6, 11.0 Hz, 1H, H-4), 5.33 (m, 1H, H-7), 7.23 (d, J = 3.9 Hz, 1H, NH), 7.54, 8.08 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH$_{ar}$). ^{13}C NMR (CD_2Cl_2) δ 11.7 (SMe), 21.2, 21.3, 21.4 (3 OAc), 28.5 (C(*C*H$_3$)$_3$), 37.8 (C-3), 38.4 (C-9), 51.5 (C-5), 53.3 (OMe), 68.9 (C-7), 70.0 (C-4), 70.6 (C-8), 71.5 (C-6), 80.5 (C-2), 85.3 (*C*(CH$_3$)$_3$), 129.3, 132.4, 138.0, 141.8 (6C, C−Ar), 155.7 (CONH), 166.7, 168.6, 170.5, 170.7, 172.4 (5 CO). ESI-MS calcd for $C_{29}H_{39}ClN_2O_{12}S$ [M + Na]$^+$ 697.18; found m/z 697.25.

Methyl (Methyl 5-Amino-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (21). Compound **20** (95.0 mg, 0.14 mmol) was dissolved in 4 M PhOH (in DCM; 4 mL) and 4 M TMSCl

(in DCM; 2 mL). The reaction mixture was stirred at rt for 2 h. The crude product was purified by chromatography on silica gel (EA:PE, gradient 1:1 to 8:1) to yield **21** as a white foam (51 mg, 63%). ^{1}H NMR (500 MHz, CD_2Cl_2) δ 1.95 (s, 3H, SMe), 1.99 (dd, J = 5.7, 14.0 Hz, 1H, H-3a), 2.04, 2.06, 2.19 (3 s, 9H, 3 OAc), 2.57 (dd, J = 4.7, 13.7 Hz, 1H, H-3b), 2.62 (t, J = 10.0 Hz, 1H, H-5), 3.34−3.42 (m, 1H, H-9a), 3.76 (s, 3H, OMe), 4.06 (dd, J = 1.4, 10.0 Hz, 1H, H-6), 4.17 (ddd, J = 3.3, 7.0, 15.0 Hz, 1H, H-9b), 4.98 (td, J = 4.7, 11.4 Hz, 1H, H-4), 5.18 (dt, J = 3.7, 7.5 Hz, 1H, H-8), 5.61 (dd, J = 1.5, 7.5 Hz, 1H, H-7), 6.90 (m, 2H, NH_2), 7.43, 7.74 (AA′, BB′ of AA′BB′, J = 8.6 Hz, 4H, CH_{ar}). ^{13}C NMR (CD_2Cl_2) δ 11.8 (SMe), 21.3, 21.4, 21.4 (3 OAc), 37.0 (C-3), 39.4 (C-9), 52.2 (C-5), 53.3 (OMe), 69.5 (C-7), 71.3 (C-8), 73.0 (C-4), 73.8 (C-6), 84.7 (C-2), 129.1, 129.3, 130.1, 133.5 (6C, C−Ar), 166.3, 168.2, 170.3, 170.4, 171.4 (5 CO). ESI-MS calcd for $C_{24}H_{31}ClN_2O_{10}S$ $[M + Na]^+$ 597.13; found m/z 597.14.

Methyl (Methyl 5-Cyclopropylamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (22f). Compound **21** (74.0 mg, 0.16 mmol) was dissolved in dry DCM (2.0 mL) under argon atmosphere. Cyclopropanoyl chloride (24.0 μL, 28.0 mg, 0.27 mmol), NEt_3 (37.0 μL, 27.0 mg, 0.27 mmol), and a catalytic amount of DMAP were added successively. The reaction mixture was stirred at rt for 3.5 h. Then it was washed with saturated aqueous $NaHCO_3$ (3 × 5 mL) and H_2O (1 × 5 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (EA:PE, gradient 1:1 to 2:1) to yield **22f** (43 mg, 75%). $[\alpha]^{20}_D$ 0.48 (c 2.16, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.70 (m, 2H, CH_2), 0.91 (m, 2H, CH_2), 1.22 (m, 1H, CH), 2.01 (m, 4H, H-3a, SMe), 2.10, 2.12, 2.20 (3 s, 9H, 3 OAc), 2.71 (dd, J = 4.6, 12.7 Hz, 1H, H-3b), 2.86 (m, 1H, H-9a), 3.73 (dd, J = 2.0, 10.7 Hz, 1H, H-6), 3.76 (s, 3H, OMe), 4.24 (q, J = 10.5 Hz, 1H, H-5), 4.36 (ddd, J = 2.9, 8.7, 11.6 Hz, 1H, H-9b), 4.84 (td, J = 4.6, 11.6 Hz, 1H, H-4), 5.12 (dd, J = 2.0, 10.1 Hz, 1H, H-7), 5.25 (dt, J = 2.7, 10.1 Hz, 1H, H-8), 5.42 (d, J = 10.3 Hz, 1H, 5-NH), 7.18 (dd, J = 4.0, 8.7 Hz, 1H, NH), 7.39, 7.75 (AA′, BB′ of AA′BB′, J = 8.6 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 7.4 (2C, CH_2), 12.1 (CH), 14.6 (SMe), 21.1 (3C, 3 OAc), 37.8, 38.2 (2C, C-3, C-9), 49.4 (C-5), 52.9 (OMe), 67.9, 68.2 (2C, C-7, C-8), 69.7 (C-4), 73.9 (C-6), 82.8 (C-2), 128.8, 129.4, 132.7, 137.7 (6C, C−Ar), 166.1, 167.7, 170.3, 171.2, 172.4, 173.7 (6 CO). ESI-MS calcd for $C_{28}H_{35}ClN_2O_{11}S$ $[M + Na]^+$ 665.17; found m/z 665.06.

Methyl (Methyl 5-Cyclobutylamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (22g). Compound **21** (60 mg, 0.1 mmol) was dissolved in dry DCM (2.4 mL). Cyclobutanecarbonyl chloride (36 μL, 37 mg, 0.3 mmol), NEt_3 (44 μL, 32 mg, 0.3 mmol), and a catalytic amount of DMAP were added successively. The reaction mixture was stirred at rt overnight. Then it was washed with saturated aqueous $NaHCO_3$ (3 × 5 mL) and H_2O (1 × 5 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (EA:PE, gradient 1:1 to 2:1) to yield **22g** (27 mg, 39%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.00 (m, 4H, SMe, H-3a), 2.03 (s, 3H, OAc), 2.08 (m, 2H, CH_2), 2.13 (s, 3H, OAc), 2.18 (m, 4H, CH_2), 2.27 (s, 3H, OAc), 2.71 (dd, J = 4.6, 12.7 Hz, 1H, H-3b), 2.86 (m, 2H, CH, H-9a), 3.71 (dd, J = 2.1, 10.8 Hz, 1H, NH), 3.77 (s, 3H, OMe), 4.20 (t, J = 10.4 Hz, 1H, H-5), 4.37 (ddd, J = 2.9, 8.8, 15.2 Hz, 1H, H-9b), 4.82 (td, J = 4.6, 11.6 Hz, 1H, H-4), 5.08 (d, J = 10.3 Hz, 1H, H-6), 5.27 (m, 2H, H-7, H-8), 7.13 (dd, J = 4.0, 8.6 Hz, 1H, 5-NH), 7.38, 7.74 (AA′, BB′ of AA′BB′, J = 8.6 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 12.1 (SMe), 18.2 (CH_2), 20.9 (3C, OAc), 24.9 (2C, CH_2), 37.8, 38.2 (2C, C-3, C-9), 39.8 (CH), 49.2 (C-5), 53.0 (OMe), 68.0, 68.1, 69.6 (3C, C-4, C-7, C-8), 73.8 (C-4), 82.8 (C-2), 128.4, 132.7, 137.7 (6C, C−Ar), 166.1, 167.7, 170.3, 172.6, 175.0 (6C, CO). ESI-MS calcd for $C_{29}H_{37}ClN_2O_{11}S$ $[M + Na]^+$ 679.18; found m/z 679.11.

Methyl ((2,3-Difluorobenzyl) 5-Cyclopropylamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (23f). Compound **22f** (37.0 mg, 0.06 mmol) was reacted with 2,3-difluorobenzyl alcohol (18.0 μL, 23.0 mg, 0.16 mmol), *N*-iodosuccinimide (20.0 mg, 0.09 mmol), and triflic acid (4.00 μL, 7.00 mg, 0.05 mmol). The crude product was purified by chromatography on silica gel (1% gradient *i*PrOH in petrol ether/DCM 2:1) to yield **23f** (31 mg, 72%). $[\alpha]^{20}_D$ 0.23 (c 1.73, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.79 (m, 2H, CH_2), 1.00 (m, 2H, CH_2), 1.29 (m, 1H, CH), 2.08 (m, 1H, H-3a), 2.09, 2.21, 2.29 (3 s, 9H, 3 OAc), 2.72 (dd, J = 4.5, 12.8 Hz, 1H, H-3b), 2.98 (m, 1H, H-9a), 3.82 (s, 3H, OMe), 4.11 (m, 1H, H-6), 4.34 (m, 1H, H-5), 4.41 (m, 1H, H-9b), 4.59, 4.89 (A, B of AB, J = 12.0 Hz, 2H, CH_2Ar), 4.95 (m, 1H, H-4), 5.21 (dd, J = 1.8, 10.0 Hz, 1H, H-7), 5.36 (m, 1H, H-8), 5.49 (d, J = 10.2 Hz, 1H, 5-NH), 7.19 (m, 4H, NH, CH_{ar}), 7.47, 7.84 (AA′, BB′, J = 8.5 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 7.1, 7.4 (2C, 2 CH_2), 14.6 (SMe), 20.8, 21.2, 21.3 (3 OAc), 25.4 (CH), 38.0, 38.4 (2C, C-3, C-9), 49.5 (C-5), 52.8 (OMe), 60.5 (CH_2Ar), 67.9, 68.4, 68.9 (3C, C-4, C-7, C-8), 72.5 (C-6), 98.6 (C-2), 115.8, 117.1, 123.9, 125.4, 126.7, 128.5, 132.7, 137.7 (12C, C−Ar), 166.2, 168.0, 170.3, 171.2, 172.4, 173.8 (6 CO). ESI-MS calcd for $C_{34}H_{37}ClF_2N_2O_{12}$[M + Na]$^+$ 761.20; found m/z 761.16.

Methyl ((2,3-Difluorobenzyl) 5-Cyclobutylamido-4,7,8-tri-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (23g). Compound **22g** (53.0 mg, 0.08 mmol) was reacted with 2,3-difluorobenzyl alcohol (25.0 μL, 33.0 mg, 0.23 mmol), *N*-iodosuccinimide (27.0 mg, 0.12 mmol), and triflic acid (6.00 μL, 10.0 mg, 0.07 mmol). The crude product was purified by chromatography on silica gel (1% gradient *i*PrOH in petrol ether/DCM 2:1) to yield **23g** (44 mg, 72%). $[\alpha]^{20}_D$ 0.23 (c 1.7, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.85 (m, 1H, H-3a), 1.99 (s, 3H, OAc), 2.04 (m, 4H, 2 CH_2), 2.13 (s, 3H, OAc), 2.18 (m, 2H, CH_2), 2.27 (s, 3H, OAc), 2.63 (dd, J = 4.5, 12.7 Hz, 1H, H-3b), 2.84 (quint, J = 10.0 Hz, 1H, CH), 2.91 (dt, J = 3.5, 15.0 Hz, 1H, H-9a), 3.73 (s, 3H, OMe), 4.03 (d, J = 12.5 Hz, 1H, H-6), 4.22 (q, J = 10.4 Hz, 1H, H-5), 4.33 (ddd, J = 2.8, 8.6, 15.1 Hz, 1H, H-9b), 4.51 (A of AB, J = 11.8 Hz, 1H, CH_2Ar), 4.82 (m, 2H, H-4, CH_2Ar), 5.10 (dd, J = 1.7, 9.9 Hz, 1H, H-7), 5.17 (d, J = 10.4 Hz, 1H, 5-NH), 5.26 (m, 1H, H-8), 5.36 (d, J = 10.2 Hz, 1H, 5-NH), 7.09 (m, 3H, CH_{ar}), 7.19 (dd, J = 4.9, 8.3 Hz, 1H, NH), 7.37, 7.73 (AA′, BB′ of AA′BB′, J = 8.4 Hz, 4H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 18.1 (2C, CH_2), 21.2 (3C, OAc), 25.3 (CH_2), 37.9 (C-3), 38.3 (C-9), 39.7 (CH), 49.1 (C-5), 52.8 (OMe), 60.3 (CH−Ar), 67.8, 68.2, 68.8 (3C, C-4, C-7, C-8), 72.3 (C-6), 98.5 (C-2), 116.4, 117.1, 123.7, 125.3, 128.7, 132.5, 137.6 (12C, C−Ar), 167.9, 171.1, 172.4, 175.0 (6C, CO). ESI-MS calcd for $C_{35}H_{39}ClF_2N_2O_{12}$ $[M + Na]^+$ 775.22; found m/z 775.25.

Hapten Inhibition Assays with MAG_{d1-3}-Fc. Murine MAG_{d1-3}-Fc was affinity purified from CHO-Lec 3.2.8.1 cell culture supernatant as described before,[40] dialyzed against 10 mM phosphate buffer pH 7.4, sterile filtered, and stored at 4 °C. The purified protein is stable for several months. The protein was analyzed by an ELISA and binding assay with immobilized fetuin. Inhibition assays for MAG were performed as described previously.[26,30,40] In brief, fetuin was immobilized in microtiter plates and binding of MAG-Fc was determined in the presence of seven to eight different concentrations for each inhibitor using alkaline phosphatase-labeled anti-Fc antibodies. The half-maximal inhibitory concentrations were determined from corresponding binding curves and used to calculate relative inhibitory concentrations (rIC_{50}).

SPR Analysis. The SPR measurements were performed on a Biacore 3000 surface plasmon resonance based optical biosensor (Biacore AB, Sweden). Sensor chips (CM5 and CM4), immobilization kits, maintenance supply and HBS-EP (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% v/v surfactant P20) were purchased from Biacore AB (HBS-EP ready-to-use; degassed and filtered). CM5 (CM4, respectively) chips were preconditioned prior to usage by injecting a series of

conditioning solutions. A flow rate of 50 μL/min was used and 2 × 20 μL of 50 mM NaOH, 10 mM HCl, 0.1% SDS, and 100 mM H_3PO_4 were injected. The carboxy groups on the CM5 (CM4) chip were activated for 10 min with a 1:1 mixture of 0.1 M *N*-hydroxysuccinimide (NHS) and 0.1 M 3-(*N*,*N*-dimethylamino)propyl-*N*-ethylcarbodiimide (EDC) at a flow rate of 10 μL/min. Protein A (P6031) was purchased from Sigma. A sample and a reference surface were prepared sequentially or in parallel. For immobilizing protein A, a stock solution (1 mg/mL in 50 mM phosphate buffer, pH 7.0) was diluted in 10 mM sodium acetate, pH 5.0 to obtain a concentration of 30 μg/mL. This solution was then injected over the activated surface for 10 min at a flow rate of 10 μL/min. Protein A densities around 4000 RU and 5000 RU were achieved. Flow cells were blocked with a 10 min injection of 1 M ethanolamine, pH 8.0. For capturing, MAG_{d1-3}-Fc solution (expressed and purified as described[40]) was diluted to a 30−40 μg/mL concentration using HBS-EP. Afterward, MAG_{d1-3}-Fc was injected at a flow rate of 1 μL/min for 10 min. The surface was equilibrated overnight at a flow rate of 5 μL/min, achieving densities around 2000 RU. 10-fold dilution series were freshly prepared in eluent buffer immediately before use. All binding experiments were conducted at 25 °C (except thermodynamic measurements) at a flow rate of 20 μL/min. The samples were injected over 1 min, followed by 1 min dissociation. Each sample was measured with a duplicate of one concentration, using a randomized concentration order. Several buffer samples were injected before the first concentration, and one blank between each concentration, which were used for the double blank referencing during data processing. Double referencing was applied to correct for bulk effects and other systematic artifacts. Data processing and equilibrium binding constant determinations were accomplished with Scrubber (BioLogic Software, Version 1.1 g or 2.0a). Kinetic data were simultaneously fit using the nonlinear regression program Clamp or Scrubber 2.0a.

Stability Test. To dermine the stability of a compound in the central nervous system, an artificial cerebrospinal fluid (aCSF) was prepared based on published data.[41] The following concentrations (all in mM/L) were used: sodium 140, chlorine 125, hydrogen carbonate 22.5, potassium 2.9, calcium 1.15, magnesium 1, urea 4.16, and glucose 3.2. Because the composition of proteins in the CSF is comparable to the serum but at a lower concentration (the ratio of liquor protein to serum protein is 4×10^{-3}),[59] 0.4% v/v of human plasma (Sigma-Aldrich) was added and the pH was adjusted to 7.3. Then 100 μM solutions of the compound were prepared and shaken at 37 °C and 300 rpm on an Eppendorf-Thermomixer Comfort. Samples were withdrawn after 0, 30, 60, 120, 180 min and 20 h, respectively. The value assigned to every time point was the average of a triplicate measurement. The quantification of the samples was performed on a Agilent 1100 series HPLC instrument with a UV-DAD spectrometer using the ChemStation software.

$logD_{7.3}$ Determination. Two similar ratios of octanol to buffer were chosen according to the expected logD value, whereas every ratio was measured as a triplicate. Phosphate buffer at pH 7.3 was prepared and shaken overnight together with octanol in order to mutually saturate the two phases. Upon separation of the two layers, the buffer phase was withdrawn and mixed with an analyte stock solution in DMSO to yield a final concentration of 10^{-4} M. Both phases were transferred to a PCR plate, which was covered with aluminum foil (Axygen PCR-AS-200) and shaken for 1 h at 1200 rpm and 25 °C on a PHMP-4 instrument (Grant-bio). After 2.5 h at room temperature, the aqueous phases were transferred to microvials, centrifuged for 30 s, and analyzed by HPLC (Agilent 1100 series). The values were accepted if the mean values of the two ratios did not differ by more than 0.1 unit.

BBB-PAMPA.[42] Consumables (system solution, P/N 110151; brain sink buffer, P/N 110674; BBB-1 lipid solution, P/N 110672; preloaded PAMPA sandwich with stirring devices, P/N 110 212) were purchased from pION. Each donor compartment of the preloaded PAMPA plate was filled with 200 μL of pION's system solution at pH 7.4, containing the analytes at a concentration of 50 μM. Then 150 μL of the same solution were transferred to an UV-plate (UV-Star, Greiner Bio-one) and UV spectra were recorded as reference on a SpectraMax instrument (Molecular Devices). The filter membranes of the acceptor compartments were impregnated with 5 μL of BBB-1 lipid solution and each compartment was filled with 200 μL of brain sink buffer. The system was assembled and individual stirring of the wells was induced by pION's GutBox to yield an unstirred water layer thickness of 40 μm. After 30 min, the UV data of the acceptor and the donor plate were acquired on a SpectraMax instrument (Molecular Devices) and analyzed by the PAMPA Evolution command software (version 3.4, pION).

NMR. Shigemi NMR tubes were used to reduce the sample volume needed for measurement to 250 μL. MAG_{d1-3}-Fc protein was diluted from a stock solution of 1 mg/mL by a factor of 2 using 99.8% D_2O (Armar Chemicals). Following dilution, the 0.5 mg/mL MAG_{d1-3}-Fc was in a solvent of 50% D_2O and 50% H_2O, with 0.01% NaN_3 with a buffer of 5 mM PBS. Stock solutions of **4** were prepared in D_2O at 100, 50, and 20 mM and added to the NMR samples containing MAG_{d1-3}-Fc for both the titration curve and competition experiments. Stock solutions of **13f** and **25** were prepared in D_2O at 5 mM to add to the NMR samples containing MAG_{d1-3}-Fc for the competition experiments. All NMR experiments were carried out at 300 K on a Bruker DRX500 spectrometer, equipped with *Z*-gradient SEI probe. The pulse sequence used for the selective inversion recovery experiments began with a selective 25 ms I-Burp-1[60] 180° pulse applied to the *para*-hydrogen of the benzamide group of compound **4**. This proton does not overlap with any other resonances of **13f** and **25**. A further benefit of the *para*-hydrogen of the benzamide group of compound **4** was that its resonance frequency was sufficiently different from the water resonance, thus avoiding complications due to radiation damping.[61] Following the selective inversion pulse, a 1 ms gradient pulse was applied to dephase any residual transverse magnetization. The gradient pulse was followed by a variable delay to allow for the recovery of longitudinal magnetization. The delay was followed by a DPFGSE water suppression sequence to suppress the magnetization from the 50% H_2O.[61]

For each selective inversion recovery time measurement (sT1), 10 experiments were performed. These experiments consisted of increasing delays following the selective inversion pulse and gradient of 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 5, and 10 s. Then 32 scans, preceded by eight dummy scans, were measured for the concentrations of compound **4** of 500 μM and 1 mM. Sixteen scans, preceded by eight dummy scans, were measured for the concentrations of compound **4** of 2, 4, and 7 mM. For the competitive experiments with 25 μM of either **13f** or **25** added to 500 μM of **5**, 32 scans, preceded by eight dummy scans, were measured. A delay of 20 s following the measurement of each transient was inserted to allow the magnetization to return to equilibrium. Prior to the measurement upon addition of either **13f** or **25**, a 1 h incubation time for equilibration was allowed. The NMR data were analyzed using XWINNMR version 3.5 operating on a PC running under Linux OS. The spectra were apodized with an exponential decay function with 2 Hz line broadening. The inversion recovery data, as well as the one-site binding model, were fit using Prism 4 (GraphPad Software Inc., San Diego, CA).

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Supporting Information Available: Surface plasmon resonance assay, structure of compounds implemented additionally

in the Biacore−Hapten assay correlation, HRMS data, HPLC traces, and ^{1}H spectra of target compounds **13a**−**f**, **19a**−**g**. This material is available free of charge via the Internet at http://pubs.acs.org.

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