Carbohydrate Research 345 (2010) 1348–1359

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Kinetic and thermodynamic properties of MAG antagonists

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ARTICLE INFO

Article history:
Received 21 January 2010
Received in revised form 4 March 2010
Accepted 9 March 2010
Available online 16 March 2010

Keywords:
Carbohydrate mimetics
Siglecs
Myelin-associated glycoprotein (MAG)
Thermodynamics of carbohydrate–protein interactions
Kinetics of carbohydrate–lectin interactions

ABSTRACT

Paraplegia is caused by injuries of the central nervous system (CNS) and especially young people suffer from these severe consequences as, for example, the loss of motor functions. The lack of repair of the injured nerve strands originates from the inhibitory environment for axon regeneration in the CNS. Specific inhibitory proteins block the regrowth of nerve roots. One of these neurite outgrowth inhibitors is the myelin-associated glycoprotein (MAG), which is a member of the Siglec family (sialic acid-binding immunoglobulin-like lectin). In previous studies, we identified potent small molecule MAG antagonists. In this communication, we report new neuraminic acid derivatives modified in the 4- and 5-position, and the influence of various structural modifications on their kinetic and thermodynamic binding properties.

1. Introduction

Paraplegia is caused by injuries of the central nervous system (CNS). A therapy for full regeneration of injured nerve strands is not yet available. The lack of regeneration originates from the inhibitory environment in the CNS,[1,2] that is, specific inhibitors on residual myelin and on astrocytes, which are recruited to the site of injury.[3–5] In the last decade, several inhibitor proteins have been identified, one of them being the myelin-associated glycoprotein (MAG).[6] MAG is a transmembrane glycoprotein, belonging to the Siglec family (sialic acid-binding immunoglobulin-like lectin).[7,8] On the surface of neurons, MAG interacts with two classes of targets: proteins of the Nogo receptor family[9,10] and gangliosides, primarily the gangliosides GD1a and GT1b.[11–14] Although the relative role of Nogo receptors and gangliosides as MAG ligands has yet to be resolved, in some systems, neurite outgrowth can be initiated by sialidase treatment, suggesting that the sialic acid-mediated interactions of MAG predominantly contribute to the inhibitory process.[15] Therefore, blocking MAG with potent glycomimetic antagonists may be a valuable therapeutic approach to enhance axon regeneration. Based on the best known natural ligand of MAG identified to date, the ganglioside GQ1bα (Fig. 1), different series of antagonists have been developed.[16–21]

With neuraminic acid derivatives such as **1**,[16] Kelm et al. reported a remarkable simplification of the relevant tetrasaccharide binding epitope of GQ1bα. Further reported modifications are related to lipophilic interactions. Thus, antagonists with a lipophilic core, for example, the biphenyl derivatives **2**[18] or a lipophilic replacement of the α-(2→6)-linked Neu5Ac, for example, **3**[19] were synthesized (Fig. 1).

The concept of drug discovery is based upon selectively addressing particular biological targets preferably by low molecular weight compounds. In vitro determined drug–target interactions are classically rated in terms of binding parameters such as IC_{50}'s and K_D's. An alternative perspective on drug optimization is the residence time of the drug–target binary complex,[22] as quantified by the dissociation half-life ($t_{1/2}$). Potential advantages of a long residence time are extended duration of the pharmacological effect and target selectivity.[22,23] Especially in the field of carbohydrate–lectin interactions, this is a crucial point to address. As a result of the shallow and water accessible binding sites of lectins, carbohydrates bind with only low affinity and show very fast dissociation off-rates, leading to $t_{1/2}$ in the range of seconds. Examples

Abbreviations: AIBN, α,α′-azodiisobutyronitrile; aq, aqueous; BnBr, benzyl bromide; DCM, dichloromethane; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; FAc, fluoro-acetyl; HBS-E, HEPES/NaCl/EDTA buffer; HBS-EP, HEPES–NaCl–EDTA–P20 buffer; ITC, isothermal titration calorimetry; K_D, dissociation constant; MS, mass spectrometry; Neu5Ac, *N*-acetylneuraminic acid; NgR, Nogo receptor; NMR, nuclear magnetic resonance; PDC, pyridinium dichromate; PPTS, pyridinium *p*-toluenesulfonate; RP, reversed phase; SPR, surface plasmon resonance; STD-NMR, saturation transfer difference nuclear magnetic resonance spectroscopy; THF, tetrahydrofuran.

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doi:10.1016/j.carres.2010.03.010

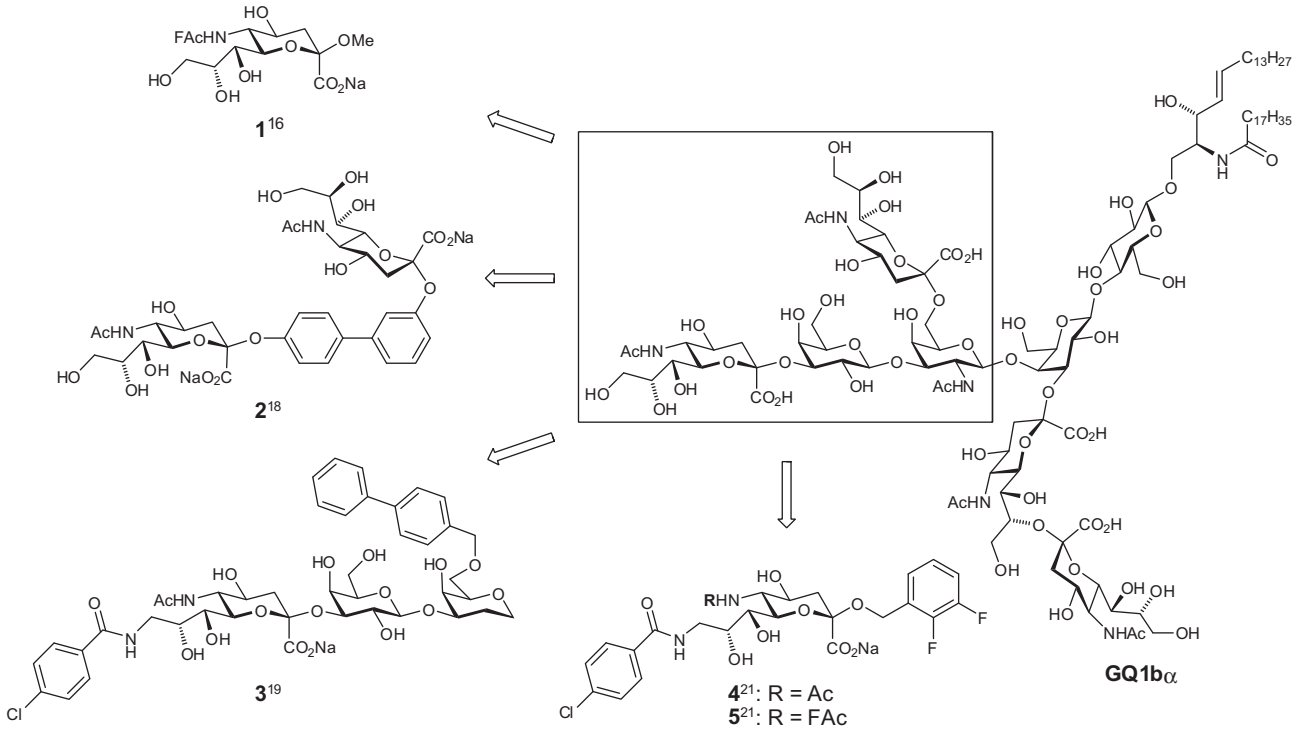


Figure 1. MAG antagonists **1**[16], **2**[18], **3**[19] and **4**, **5**[21]derived from the tetrasaccharide core structure (highlighted in box) of GQ1bα.

Table 1
Carbohydrate–protein interactions: thermodynamic and kinetic binding parameters

Protein	Ligand	K_D (μM)	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	$t_{1/2}$ (s)
P-Selectin[24]	PSGL-1	0.3	$4{\cdot}10^6$	1.4	0.5
E-Selectin[25]	ESL-1	62	$4{\cdot}10^4$	3.0	0.2
GSLA-2 mAb[26]	Sialyl Lewis[a]	4.3	$1.1{\cdot}10^5$	0.48	1.5
MAG[19]	Neu5Ac derivative **3**[19]	2.8	$3.5{\cdot}10^5$	0.8	0.9

of thermodynamic and kinetic parameters for carbohydrate–protein interactions are summarized in Table 1.

For medical applications, an improved $t_{1/2}$ of the drug–protein complex is beneficial, because the therapeutic effect can be reached with a lower dose. Zanamivir is one of the prominent examples, where a carbohydrate-based lead was optimized to yield a drug with a dramatically improved kinetic behavior, showing a half-life of 33 min of its complex with the B/Memphis/3/89 (H3N2) influenza virus.[27] In this communication, we present various MAG antagonists modified at the 4- and 5-position with the aim to modulate their kinetic properties. In general, lead optimization is often achieved by additional lipophilic contacts and thereby improving the binding entropy. As a result of the increased lipophilicity, the dissociation half-life ($t_{1/2}$) of the drug–target complex is extended.[28,29] The starting point for our investigation was MAG antagonist **5**,[21] a result of an extended optimization program focusing exclusively on the improvement of its thermodynamic binding properties.[17–19,21]

2. Results and discussion

Recently, we reported the synthesis and biological evaluation of a series of MAG antagonists with affinities in the low micromolar range.[21] Furthermore, pharmacokinetic parameters such as stability and membrane penetration indicated that the antagonists **4** and **5** (Fig. 1) fulfill the basic requirements for lead compounds. As halogenated acetates at the 5-position led to a drastic improvement of the binding affinity,[16,21] we investigated the impact of this position on the thermodynamic properties and also examined its influence on the dissociation half-life time. Molecular modeling studies with a homology model of MAG[30] suggested that the hydroxy group in the 4-position is not directly involved in the binding process[19] and therefore provides a possibility for derivatization. Because additional hydrophobic contacts based on the 4-position and an inverted configuration at C-4 are expected to alter the thermodynamic and kinetic behavior, we synthesized a small library of antagonists and analyzed their binding properties by surface plasmon resonance.

2.1. A MAG antagonist modified in the 5-position of the Neu5Ac scaffold

With isothermal titration calorimetry, we determined the thermodynamic parameters ΔH, ΔS, and ΔG[31] of antagonist **5** interacting with a recombinant protein consisting of the three N-terminal domains of MAG and the Fc part of human IgG ($MAG_{d1\text{-}3}$-Fc).[32] For the ITC experiment, a solution of **5** (500 μM, HBS-E buffer) was injected into a solution of $MAG_{d1\text{-}3}$-Fc (48.35 μM, HBS-E buffer) at 25 °C (Fig. 2).

The experimental data were fitted to a theoretical titration curve (one site binding model) using *Origin version 7* software (MicroCal) and the thermodynamic parameters calculated according to the equation shown in Table 2. The ITC experiment confirmed the high potency of **5**, having a K_D in the nanomolar

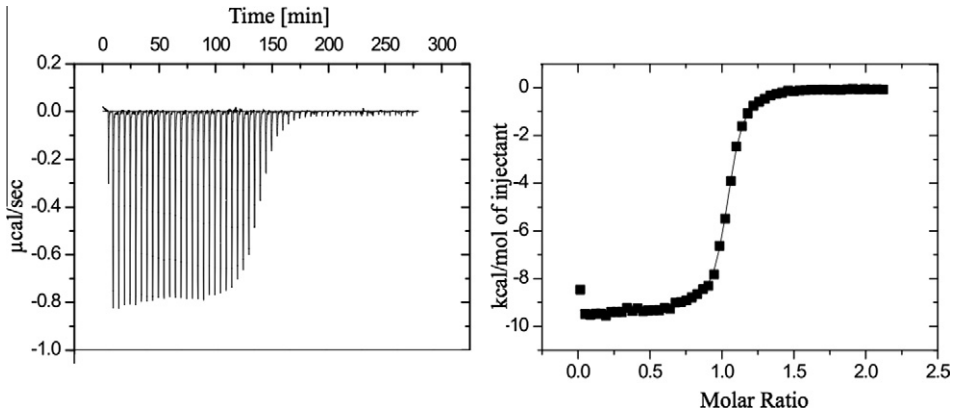


Figure 2. Enthalpogram (left) and corresponding fit (right) of the titration of $MAG_{d1\text{-}3}$-Fc with antagonist **5**. For the fit, the first injection was not taken into account.

Table 2
Thermodynamic parameters of antagonist **5**

Ligand	*N*	K_D (nM)	ΔG (kJ/mol)*	ΔH (kJ/mol)*	$T\Delta S$ (kJ/mol)*
5	1.03	142	−39.1	−39.2	−0.14

ΔH, ΔS, and ΔG were calculated according to the equation $\Delta G = \Delta H - T\Delta S = RT\ln K_A = -RT\ln K_D$; *N* represents the stoichiometry, * values' accuracy ±5%.

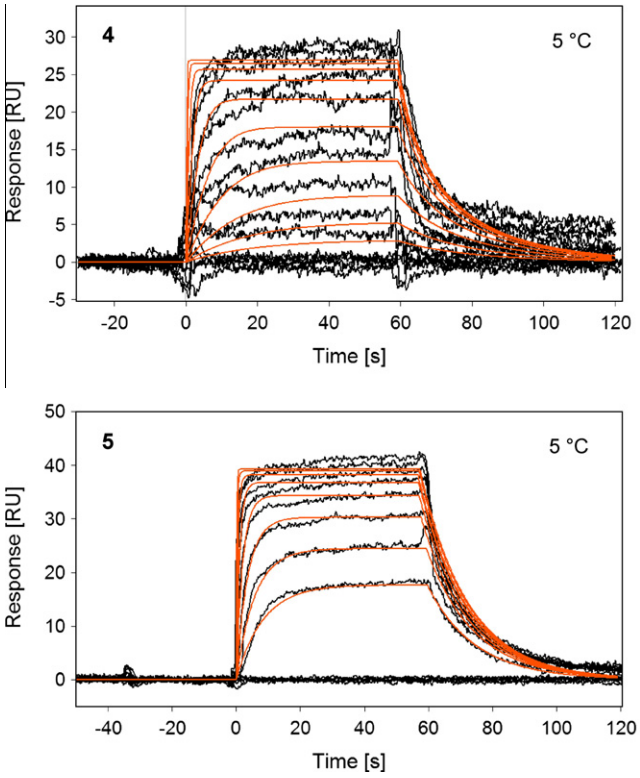
range.[21] Interestingly, the interaction is exclusively enthalpy driven.[33,34]

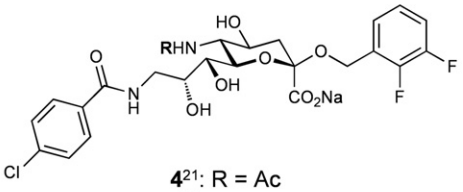
In a next step, the kinetic binding properties of **5** were determined by surface plasmon resonance (SPR) (Fig. 3). Because the off-rate at 25 °C turned out to be very fast, the Biacore experiment was repeated at lower temperature. At 5 °C, a clear slowdown of k_{off} for **5** (R: FAc) compared to that for **4** (R: Ac) was observed.

2.2. MAG antagonists modified in the 4-position of the Neu5Ac scaffold

As an increased lipophilicity often leads to prolonged residence times,[35] hydrophobic substituents were introduced in the 4-position. Furthermore, we planned to investigate the influence of the configuration at this position on the kinetic binding behavior.

Starting from the Neu5Ac donor **6**,[36] compound **8** was obtained according to published procedures (Scheme 1).[21] After deacetylation under Zemplén conditions (→**9**), the acetonide[37] **10** was formed with the two hydroxy groups in the 7- and 8-position, permitting selective modifications of the 4-position. To obtain the 4-deoxy compound **14**, the 4-hydroxy group in **10** was transformed into the corresponding thiocarbonate **12**, a precursor for a Barton deoxygenation using tributyltin hydride.[38] Cleavage of the aceto-

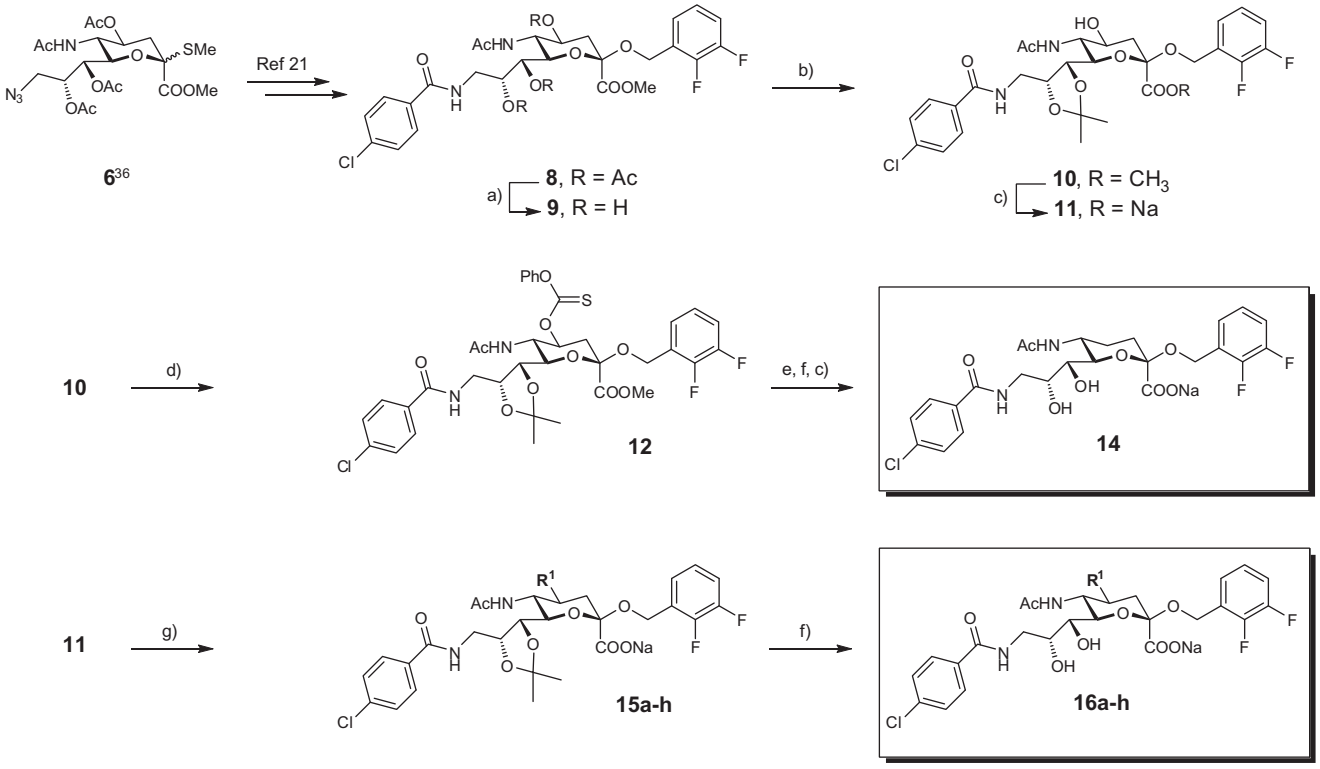




4[21]: R = Ac
5[21]: R = FAc

Compound	k_{on} [$M^{-1}s^{-1}$]	k_{off} [s^{-1}]	$t_{1/2}$ [s]
4 5 °C	$9.4 \cdot 10^4$	0.153	4.5
5 5 °C	$2.75 \cdot 10^5$	0.07	9.9
5 25 °C	$2.8 \cdot 10^5$	0.154	4.5

Figure 3. Kinetic fits of compounds **4** and **5** at 5 °C. In the case of **5**, an increase of the dissociation half-life ($t_{1/2}$) by a factor of 2 was observed. For the fitting of the sensorgrams Scrubber 2.0c was applied.



Scheme 1. Reagents and conditions: (a) NaOMe, MeOH (61%); (b) $MeO_2C(CH_3)_2$, PPTS, MeCN (60%); (c) 10% NaOH (aq), MeOH (42%); (d) $C_7H_5ClO_2S$, DCM, pyridine (83%); (e) *n*-Bu_3SnH, AIBN, toluene, 100 °C (→**13**, 20%); (f) 80% AcOH (aq), 60 °C (→**16a**–**h**, 10–80%); (g) (i) $(R^1O)_2C{=}O$, DMAP, pyridine (→**15a**, 80%, **15b**, 61%); or (ii) BnBr, KOH (aq, 50%), 18-crown-6, DCM, 60 °C (→**15c**, 60%); or (iii) R^1NCO, DMAP, pyridine (→**15d**–**h**, 20–37%).

nide under acidic conditions followed by hydrolysis of the methyl ester yielded test compound **14** in excellent overall yield.

Starting from **11**, the hydroxy group in the 4-position was either acylated with the corresponding anhydrides (→**15a**,**b**), the corresponding isocyanides (→**15d**–**h**), or reacted with benzyl bromide under phase-transfer catalysis conditions (→**15c**). Finally, the acetonide was cleaved under acidic conditions to yield the test compounds **16a**–**h** (Scheme 1, Table 3).

The 4-disubstituted antagonist **19** was obtained in a two-step procedure (Scheme 2). Oxidation of **10** with pyridinium dichromate under acidic conditions[39] yielded **17**; however, due to the instability of the acetonide only in moderate 30% yield. Various other conditions, for example, using molecular sieves instead of acetic anhydride[40] did not lead to notably improved yields. Then, **17** was reacted with the tetramethylzirconium complex[41] followed by the cleavage of the acetonide to yield **18** with an acceptable stereoselectivity (11% of the *S*-stereoisomer was formed). The zirconium complex was chosen in order to avoid undesired side reactions (e.g., enolization) as reported earlier by Hartmann et al.[42] Final deprotection gave compound **19**.

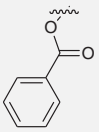
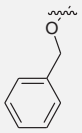
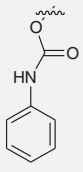
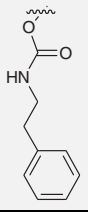
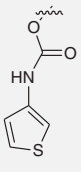
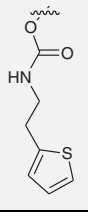
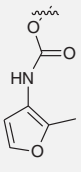
Test compound **22** was obtained by reduction of **17** with $BH_3{\cdot}NH_3$, yielding the 4-hydroxy compound **20** [(*R*)-stereoisomer] with the inverted configuration at C-4 compared to Neu5Ac.[43] Finally, removal of the acetonide and hydrolysis of the methyl ester gave compound **22**.

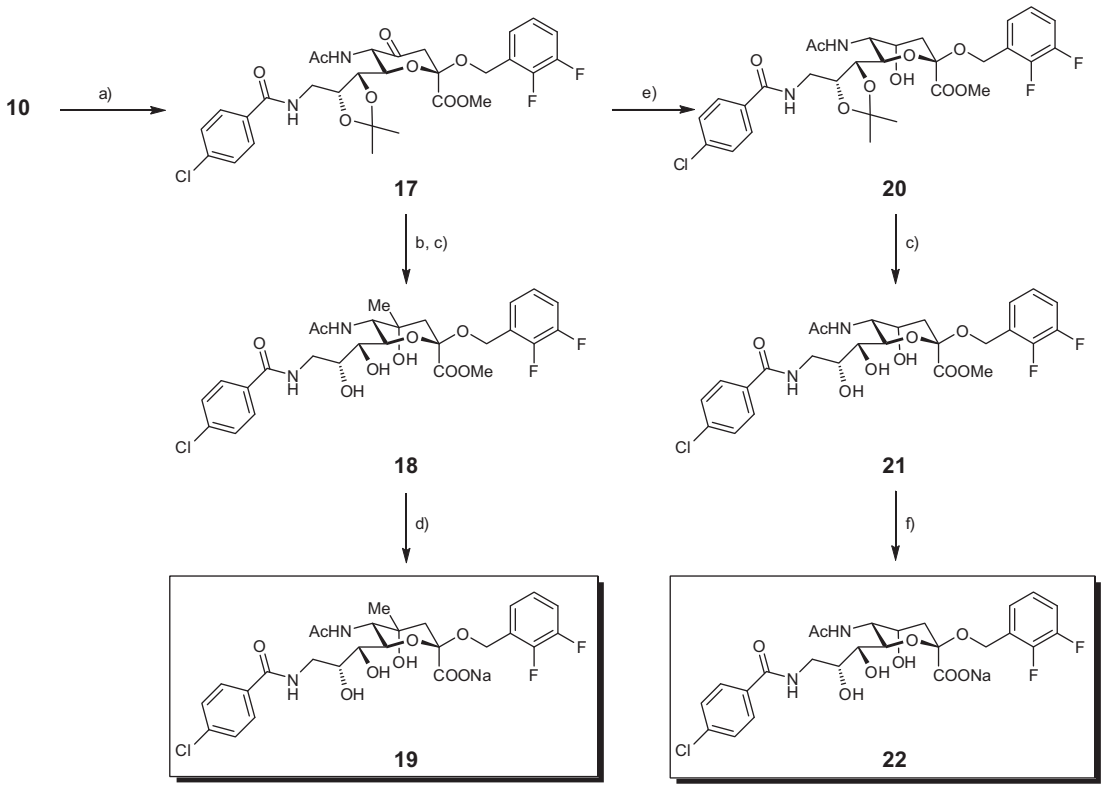
2.3. Biological evaluation and kinetic studies

First, the affinity of the test compounds **14**, **16a**–**h**, **19**, and **22** toward MAG was determined by a surface plasmon resonance based biosensor (Biacore) experiment.[21] Fc-$MAG_{d1\text{-}3}$-Fc[32] 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.

Dilution series of the compounds were prepared either in pure HEPES-buffer or in buffer containing 3% DMSO and passed over the flow cells. As reported earlier,[21] negative sensorgrams were obtained (after subtraction of the reference cell). After their mirroring, they could be fitted to a one-to-one binding model using Scrubber 2.0c (Table 4). The kinetic parameters k_{on} and k_{off} were obtained by applying a global fit (Scrubber 2.0c).

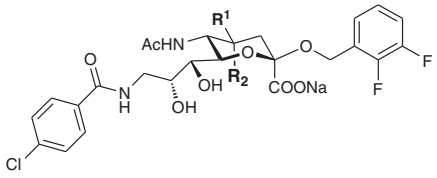
Table 3
Substituents R^1 in antagonist **16**

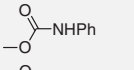
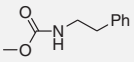
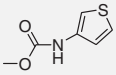
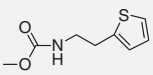
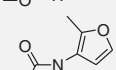
16a	16b	16c	16d	16e	16f	16g	16h
							



Scheme 2. Reagents and conditions: (a) PDC, Ac_2O, DCM, rt (30%); (b) Me_4Zr, THF, −78 °C, 50% NH_4Cl (aq); (c) 80% AcOH (aq), 60 °C (→**18**, 32 + 11% (*S*)-stereoisomer, two steps; →**21**, 90%); (d) LiOH (aq), THF (34%); (e) $BH_3{\cdot}NH_3$, MeOH, 0 °C (36%); (f) 10% NaOH (aq) (34%).

Table 4
Affinities and kinetic parameters of Neu5Ac derivatives modified in the 4-position



Compd	R^1	R^2	K_D (μM)	k_{on} ($M^{-1}\,s^{-1}$)	k_{off} (s^{-1})	$t_{1/2}$ (s)
4[21]	OH	H	2.0	1.5×10^5	0.54	1.2
14	H	H	11.4	2.8×10^4	0.28	2.5
16a	OAc	H	11.5	4.6×10^4	0.52	1.3
16b	OBz	H	26.0	1.6×10^4	0.41	1.7
16c	OBn	H	2.1	1.6×10^4	0.33	2.1
16d		H	257	2.7×10^4	7.00	0.1
16e		H	114	5.3×10^6	>100	<0.01
16f		H	15.6	n.d.	n.d.	n.d.
16g		H	n.b.	n.d.	n.d.	n.d.
16h		H	49.4	2.2×10^4	1.10	0.6
19	Me	OH	30.0	2.4×10^{-4}	0.53	1.3
22	H	OH	9.0	3.6×10^{-4}	0.33	2.1

n.d., not determined; n.b., not binding.

The deoxy compound **14** showed a decrease in affinity by a factor of six. The inversion of the configuration at the 4-position (→**22**) or the introduction of a methyl group in equatorial position (→**19**) led also to a drop in binding affinity (factor of 4 and 15, respectively). Originally, we assumed based on docking studies to a homology model of MAG[30] that the hydroxy group in the 4-position is not directed toward the protein and can therefore be modified. Our results, however, suggest that the equatorial 4-hydroxy contributes to binding, maybe by hydrogen bonding, as the change of the configuration at C-4 also leads to a decreased affinity. Furthermore, a steric clash of the methyl group in **19** could be responsible for a further reduction of affinity.

In case of antagonists **16a**,**b** and **16d**–**h**, a pronounced drop in the binding affinity or even a complete abolishment of binding was observed. In contrast, **16c** showed the same binding affinity as reference compound **4**. The loss in potency for **16a**,**b** and **16d**–**h** is probably the consequence of the rigid geometry of ester and carbamate substituents, leading to a steric clash with the protein, whereas the benzyl ether in **16c** seems to adapt a favorable spatial arrangement compensating the impact of the 4-hydroxy to binding affinity.

Beside the decreased binding affinity, no substantial improvement of the kinetic properties could be achieved. All compounds showed fast dissociation rate constants, leading to residence times in the range of seconds (Table 4). Despite the increased lipophilicity, even **16c** does not show an extended dissociation half-life.

3. Conclusion

A small library of MAG antagonists modified in the 4-position of the Neu5Ac scaffold was synthesized with the goal to improve the half-life of the antagonist-MAG complex. Although all modifica-

tions in the 4-position were not successful, the investigation of modifications in the 5-position led to a reduction of the dissociation rate constant k_{off}, although only by a factor of 2. In conclusion, the prolongation of the residence time remains a challenge and will be a critical issue in further studies on the kinetic properties of glycomimetics.

4. Experimental

4.1. General methods

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, ROESY, and NOESY). Chemical shifts are expressed in ppm using residual $CHCl_3$, CHD_2OD, CHD_2CN, 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. 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 aq 10% H_2SO_4). Column chromatography was performed on silica gel (Fluka, 40–60 mesh). MeOH was dried by heating at reflux 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 or 4 Å) were activated in vacuo at 500 °C for 2 h immediately before use. Compounds **6**–**8** were prepared according to a published procedure.[21,36]

4.2. Synthesis and characterization of compounds 9–22

4.2.1. Methyl [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (9)

Compound **8**[21] (217 mg, 42.0 mmol) was dissolved in dry MeOH (8.0 mL) and treated with methanolic 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 was washed thoroughly with MeOH. The solvent was evaporated under reduced pressure and the crude product was purified by chromatography on silica gel (1% gradient of MeOH in DCM) to yield **9** (90.0 mg, 61%) as a white foam. ^{1}H NMR (500 MHz, CD_3OD) δ 1.80 (t, J = 12.3 Hz, 1H, H-3a), 1.97 (s, 3H, NHAc), 2.72 (dd, J = 4.5, 12.8 Hz, 1H, H-3b), 3.45 (d, J = 8.8 Hz, 1H, H-7), 3.55 (dd, J = 7.3, 13.8 Hz, 1H, H-9a), 3.61–3.74 (m, 2H, H-4, H-6), 3.75–3.90 (m, 5H, OMe, H-5, H-9), 4.04 (td, J = 3.2, 8.8 Hz, 1H, H-8), 4.68, 4.86 (A, B of AB, J = 12.1 Hz, 2H, CH_2Ar), 7.06–7.24 (m, 3H, CH_{ar}), 7.46, 7.82 (AA′, BB′ of AA′BB′, J = 8.4 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 22.7 (NHAc), 41.6 (C-3), 45.1 (C-9), 53.4 (OMe), 53.8 (C-5), 68.5 (C-4), 70.8 (C-8), 72.1 (C-7), 74.9 (C-6), 117.8, 125.4, 126.4, 128.0, 129.7, 130.1, 131.3, 133.0, 134.4, 138 (12C, C-Ar), 169.6, 170.6, 175.0 (3CO). ESI-MS calcd for $C_{26}H_{29}ClF_2N_2O_9$ $[M+Na]^+$: 609.14; found m/z 609.19.

4.2.2. Methyl [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (10)

Compound **9** (20.0 mg, 30 μmol) was dissolved in dry MeCN (250 μL) and 2,2-dimethoxypropane (17 μL, 0.12 mmol) was added. After cooling to 0 °C PPTS (3.0 mg, 10 μmol) was added. The reaction mixture was stirred at rt overnight. After completion of the reaction, IRA-93 was added and stirring was continued for 30 min. After filtration, the solvent was evaporated and the pure product **10** (13 mg, 60%) was obtained by chromatography on silica gel (1% gradient of MeOH in DCM). ^{1}H NMR (500 MHz, CD_3OD): δ 1.33, 1.51 (2s, 6H, 2 C(CH_3)$_2$), 1.75 (t, J = 12.4 Hz, 1H, H-3a), 1.99 (s, 3H, NHAc), 2.67 (dd, J = 4.3, 12.6 Hz, 1H, H-3b), 3.56–3.65 (m, 1H, H-4), 3.83 (s, 3H, OMe), 3.86–4.00 (m, 3H, H-5, H-6, H-9a), 4.03 (dd, J = 3.1, 14.1 Hz, 1H, H-9b), 4.26 (d, J = 6.8 Hz, 1H, H-7), 4.48 (ddd, J = 3.3, 7.1, 8.7 Hz, 1H, H-8), 4.56–4.69 (m, 1H, CH_2Ar), 4.96 (B of AB, J = 11.6 Hz, 1H, CH_2Ar), 7.09–7.27 (m, 4H, NH, CH_{ar}), 7.46, 7.83 (AA′, BB′ of AA′BB′, J = 8.6 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 23.0 (NHAc), 25.8, 27.1 (C(CH_3)$_2$), 41.6, 41.8 (2C, C-3, C-9), 53.2 (OMe), 54.3 (C-5), 61.0 (CH_2Ar), 68.4 (C-4), 74.3 (C-6), 75.8 (C-7), 77.7 (C-8), 100.5 (C-2), 110.2 (C(CH$_3$)$_2$), 117.9, 118.0, 125.4, 126.7, 129.7, 130.1, 130.2, 134.3, 138.7 (12C, C-Ar), 169.3, 170.4, 173.9 (3CO). ESI-MS calcd for $C_{29}H_{33}ClF_2N_2O_9$ $[M+Na]^+$: 649.18; found m/z 649.15.

4.2.3. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (11)

Compound **10** (20.0 mg, 30 μmol) was dissolved in MeOH (2 mL) and 10% aq NaOH (0.1 mL) was added. After 2 h, the solution was neutralized with 7% aq HCl. Then the solvent was evaporated and the pure product **11** (13 mg, 42%) was obtained by chromatography on RP-8 (10% of MeOH in water). $[\alpha]_D^{20}$ −33.4 (c 0.37, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.27, 1.41 (2s, 6H, 2 (CH_3)$_2$), 1.65 (t, J = 12.0 Hz, 1H, H-3a), 1.92 (s, 3H, NHAc), 2.75 (dd, J = 4.3, 12.0 Hz, 1H, H-3b), 3.57 (ddd, J = 4.3, 10.3, 12.0 Hz, 1H, H-4), 3.82–3.99 (m, 3H, H-5, H-9a, H-9b), 4.11 (d, J = 10.3 Hz, 1H, H-6), 4.18 (d, J = 6.8 Hz, 1H, H-7), 4.35 (dd, J = 7.1, 12.5 Hz, 1H, H-8), 4.61, 4.89 (A, B of AB, J = 10.6 Hz, 2H, CH_2Ar), 7.00–7.16 (m, 2H, CH_{ar}), 7.30 (t, J = 6.8 Hz, 1H, CH_{ar}), 7.40, 7.91 (AA′, BB′ of AA′BB′, J = 8.6 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 22.9 (NHAc), 25.4, 27.4 (C(CH_3)$_2$), 41.9, 42.1 (2C, C-3, C-9), 54.7 (C-5), 60.3 (CH_2Ar), 69.75 (C-4), 73.1 (C-6), 75.8 (C-7), 76.5 (C-8), 102.8 (C-2), 109.3 (C(CH$_3$)$_2$), 117.0, 117.1, 125.2, 126.5, 129.6, 130.6, 134.1, 138.6 (12C, C-Ar), 169.5, 173.9, 174.4 (3CO). ESI-MS calcd for $C_{28}H_{30}ClF_2N_2NaO_9$ $[M+H]^+$: 634.15; found m/z 635.16.

4.2.4. Methyl [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-4-*O*-(phenoxycarbonothioyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (12)

Compound **10** (96 mg, 0.15 mmol) was dissolved in dry pyridine/DCM (2/1; 1.5 mL). After cooling to 0 °C phenyl chlorothioformate (162 μL, 1.20 mmol) was added dropwise. Stirring was continued for 2 h at rt and then MeOH (2 mL) was added. After evaporation of the solvents, the residue was dissolved in DCM (5 mL) and washed with 0.5 M aq $CuSO_4$ (1 mL) and water (1 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by chromatography on silica gel (1% gradient of MeOH in DCM) to yield **12** (14 mg, 83%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.37, 1.54 (2s, 6H, C(CH_3)$_2$), 1.97–2.00 (m, 4H, NHAc, H-3a), 3.01 (dd, J = 4.6, 12.3 Hz, 1H, H-3b), 3.87 (s, 3H, OMe), 3.96–4.02 (m, 1H, H-9a), 4.05 (dd, J = 3.2, 14.2 Hz, 1H, H-9b), 4.15 (d, J = 10.3 Hz, 1H, H-6), 4.27–4.37 (m, 2H, H-5, H-7), 4.53 (td, J = 3.4, 8.6 Hz, 1H, H-8), 4.66, 5.02 (A, B of AB, J = 11.5 Hz, 2H, CH_2Ar), 5.59 (td, J = 4.6, 12.0 Hz, 1H, H-4), 7.07 (d, J = 8.3 Hz, 2H, CH_{ar}), 7.13–7.18 (m, 1H, CH_{ar}), 7.21–7.27 (m, 2H, CH_{ar}), 7.30 (t, J = 7.4 Hz, 1H, CH_{ar}), 7.43 (t, J = 7.9 Hz, 2H, CH_{ar}), 7.48, 7.84 (AA′, BB′ of AA′BB′, J = 8.6 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 23.0 (NHAc), 25.8, 27.0 (C(CH_3)$_2$), 37.9 (C-3), 41.7 (C-9), 51.3 (C-5), 53.4 (OMe), 61.3 (CH_2Ar), 73.9 (C-6), 75.5 (C-7), 77.7 (C-8), 80.1 (C-4), 100.1 (C-2), 110.4 (C(CH$_3$)$_2$), 118.0, 122.1, 122.9, 125.5, 126.8, 126.9, 127.3, 127.7, 129.7,

130.1, 130.6, 134.2, 138.8, 152.6, 154.6, 154.9 (18C, C-Ar), 169.3, 169.8, 173.5 (3CO), 196.0 (CS). ESI-MS calcd for $C_{36}H_{37}ClF_2N_2O_{10}S$ $[M+Na]^+$: 785.17; found *m*/*z* 785.27.

4.2.5. Methyl [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,4,5,9-tetradeoxy-7,8-*O*-isopropylidene-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (13)

Compound **12** (96 mg, 0.13 mmol) was dissolved in dry toluene (4 mL). After the sequential addition of freshly distilled *n*-Bu_3SnH (340 μL, 1.3 mmol) and AIBN (29 mg, 0.18 mmol), the reaction mixture was stirred at 100 °C for 1 h. After evaporation of the solvent, the crude product was purified by chromatography on silica gel (1% gradient MeOH in DCM) to yield **13** (15.3 mg, 20%) as a white foam. ^{1}H NMR (500 MHz, CD_3OD): δ 1.33 (s, 3H, CH_3), 1.35–1.42 (m, 1H, H-4a), 1.51 (s, 3H, CH_3), 1.81 (td, *J* = 4.0, 13.8 Hz, 1H, H-3a), 1.94 (s, 3H, NHAc), 2.00–2.07 (m, 1H, H-4b), 2.37 (dt, *J* = 3.4, 13.1 Hz, 1H, H-3b), 3.81 (s, 3H, OMe), 3.92–4.02 (m, 2H, H-9a, H-9b), 4.02–4.08 (m, 2H, H-5, H-6), 4.26 (d, *J* = 7.6 Hz, 1H, H-7), 4.48 (ddd, *J* = 3.3, 7.0, 8.6 Hz, 1H, H-8), 4.56, 4.96 (A, B of AB, *J* = 11.6 Hz, 2H, C*H*$_2$Ar), 7.10–7.20 (m, 3H, CH_{ar}), 7.46, 7.83 (AA′, BB′ of AA′BB′, *J* = 8.7 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 22.8 (NHAc), 25.8, 27.1 (C(*C*H$_3$)$_2$), 27.5 (C-4), 32.8 (C-3), 41.7 (C-9), 46.2 (C-5), 53.0 (OMe), 61.0 (*C*H$_2$Ar), 75.9, 76.4, 77.7 (C-6, C-7, C-8), 100.9 (C-2), 110.2 (*C*(CH$_3$)$_2$), 117.8, 125.4, 126.7, 129.7, 130.1, 134.3, 138.7 (12C, C-Ar), 169.3, 170.8, 172.9 (3CO). ESI-MS calcd for $C_{29}H_{33}ClF_2N_2O_8$ $[M+Na]^+$: 633.18; found *m*/*z* 633.12.

4.2.6. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,4,5,9-tetradeoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (14)

Compound **13** (15.0 mg, 20 μmol) was dissolved in 80% aq AcOH (2.5 mL) and heated for 3 h at 60 °C. After TLC showed completion of the reaction, the reaction mixture was cooled to rt. The pH was set to 10 by addition of 10% aq NaOH. Stirring was continued for 4 h and then the reaction mixture was neutralized with 7% aq HCl. After evaporation of the solvents, the crude product was purified by chromatography on RP-8 (5% gradient of MeOH in water) to yield **14** (4.5 mg, 34%) as a white solid. $[\alpha]_D^{20}$ −29.7 (*c* 0.13, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.47 (q, *J* = 11.2 Hz, 1H, H-4a), 1.68 (td, *J* = 3.7, 13.3 Hz, 1H, H-3a), 1.94 (s, 3H, NHAc), 2.00–2.09 (m, 1H, H-4b), 2.35–2.47 (m, 1H, H-3b), 3.40–3.58 (m, 2H, H-7, H-9a), 3.70–3.81 (m, 3H, H-6, H-8, H-9b), 3.87 (td, *J* = 4.0, 11.1 Hz, 1H, H-5), 4.61, 4.79 (A, B of AB, *J* = 11.8 Hz, 2H, C*H*$_2$Ar), 7.00–7.27 (m, 3H, CH_{ar}), 7.53, 7.75 (AA′, BB′ of AA′BB′, *J* = 8.5 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, D_2O): δ 21.8 (C-4), 26.9 (NHAc), 31.7 (C-3), 44.5 (C-9), 51.7 (C-5), 60.5 (*C*H$_2$Ar), 68.2, 70.1 (C-7, C-8), 75.8 (C-6), 99.3 (C-2), 117.0, 124.3, 125.8, 126.5, 128.7, 128.8, 132.1, 137.5 (12C, C-Ar), 174.2, 186.3 (3C, 3CO). HRMS calcd for $C_{25}H_{26}ClF_2N_2NaO_{11}$ $[M]^+$: 601.1141; found *m*/*z* 601.1154.

4.2.7. Sodium [(2,3-difluorobenzyl) 5-acetamido-4-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (15a)

Compound **11** (13.2 mg, 20 μmol) was dissolved in dry pyridine (0.5 mL). After cooling to 0 °C, acetic anhydride (0.25 mL, 2.64 mmol) and DMAP (1.0 mg, 8.0 μmol) were added. The reaction mixture was allowed to reach rt and stirring was continued for 24 h. After completion of the reaction, the solvent was removed by co-evaporation with toluene. Then DCM (5 mL) was added and the organic layer was washed with 0.5 M aq $CuSO_4$ (2 mL), satd aq $NaHCO_3$ (3 × 2 mL), and H_2O (2 mL). Finally, the crude product was purified by chromatography on silica gel (10% gradient MeOH in DCM) to yield **15a** (11.2 mg, 80%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.29, 1.43 (2s, 6H, C(*CH*$_3$)$_2$), 1.70 (t, *J* = 11.5 Hz, 1H, H-3a), 1.86 (s, 3H, NHAc), 1.93 (s, 3H, OAc), 2.75 (dd, *J* = 4.5, 11.5 Hz, 1H, H-3b), 3.86–4.07 (m, 2H, H-5, H-9a), 4.15 (dd, *J* = 4.0, 14.5 Hz, 1H, H-9b), 4.20–4.28 (m, 2H, H-6, H-7), 4.39–4.46 (m, 1H, H-8), 4.63, 4.93 (A, B of AB, *J* = 11.9 Hz, 2H, C*H*$_2$Ar), 5.05–5.17 (m, 1H, H-4), 7.00–7.14 (m, 2H, CH_{ar}), 7.16–7.27 (m, 1H, CH_{ar}), 7.37, 7.84 (AA′, BB′ of AA′BB′, *J* = 7.9 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 20.9 (OAc), 22.9 (NHAc), 25.8, 27.2 (C(*CH*$_3$)$_2$), 39.2, 40.2 (C-3, C-9), 52.4 (C-5), 60.4 (*CH*$_2$Ar), 71.5, 72.7, 75.8, 76.9 (C-4, C-6, C-7, C-8), 101.3 (C-2), 110.1 (*C*(CH$_3$)$_2$), 117.2, 117.3, 125.3, 126.4, 129.6, 130.5, 138.9, 140.1 (12C, C-Ar), 170.6, 172.3, 173.5, 177.9 (4CO). ESI-MS calcd for $C_{30}H_{32}ClF_2N_2NaO_{10}$ $[M+H]^+$: 677.16; found *m*/*z* 677.21.

4.2.8. Sodium [(2,3-difluorobenzyl) 5-acetamido-4-*O*-benzoyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (15b)

Compound **11** (10.0 mg, 20 μmol) was dissolved in dry pyridine (0.5 mL) at rt. After cooling to 0 °C, benzoic anhydride (100 mg, 0.44 mmol) and DMAP (1.0 mg, 8.0 μmol) were added. The reaction mixture was allowed to reach rt and stirring was continued for 24 h. After completion of the reaction, the solvent was removed by co-evaporation with toluene. Then DCM (5 mL) was added and the organic layer was washed with 0.5 M aq $CuSO_4$ (2 mL), satd aq $NaHCO_3$ (3 × 2 mL), and H_2O (2 mL). Finally, the crude product was purified by chromatography on silica gel (10% gradient of MeOH in DCM) to yield **15b** (7.1 mg, 61%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.31, 1.47 (2s, 6H, C(*CH*$_3$)$_2$), 1.76 (s, 3H, NHAc), 1.82–1.96 (m, 1H, H-3a), 2.93 (dd, *J* = 4.2, 11.8 Hz, 1H, H-3b), 3.97 (dd, *J* = 6.0, 13.4 Hz, 1H, H-9a), 4.10–4.20 (m, 1H, H-9b), 4.25 (d, *J* = 6.5 Hz, 1H, H-7), 4.27–4.37 (m, 2H, H-5, H-6), 4.46 (dd, *J* = 6.1, 12.4 Hz, 1H, H-8), 4.67, 4.98 (A, B of AB, *J* = 11.7 Hz, 2H, C*H*$_2$Ar), 5.23–5.40 (m, 1H, H-4), 6.98–7.15 (m, 2H, CH_{ar}), 7.27 (t, *J* = 6.5 Hz, 1H, CH_{ar}), 7.42 (d, *J* = 7.9 Hz, 2H, CH_{ar}), 7.48 (t, *J* = 7.4 Hz, 2H, CH_{ar}), 7.55 (t, *J* = 7.5 Hz, 1H, CH_{ar}), 7.88 (d, *J* = 8.3 Hz, 2H, CH_{ar}), 7.92 (d, *J* = 7.5 Hz, 2H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 22.8 (NHAc), 25.7, 27.3 (C(*CH*$_3$)$_2$), 30.8 (C-3), 39.3 (C-9), 42.3 (C-5), 52.0 (CH_2Ar), 72.6, 72.9, 75.9, 76.8 (C-4, C-6, C-7, C-8), 110.0 (C-2), 111.4 (*C*(CH$_3$)$_2$), 117.3, 129.1, 129.5, 129.6, 130.5, 130.6, 130.7, 131.2, 133.0, 134.3, 135.1 (18C, C-Ar), 172.0 (4C, 4CO). ESI-MS calcd for $C_{35}H_{34}ClF_2N_2NaO_{10}$ $[M+Na]^+$: 739.18; found *m*/*z* 739.27.

4.2.9. Sodium [(2,3-difluorobenzyl) 5-acetamido-4-*O*-benzyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (15c)

Compound **11** (10 mg, 20 μmol) was dissolved in DCM/50% aq KOH (1/3, 0.5/1.5 mL). After adding 18-crown-6 (1.0 mg) and benzyl bromide (60 μL, 0.5 mmol), the reaction mixture was heated to 60 °C and stirring was continued for 18 h. The solvents were removed under reduced pressure and the crude product was purified by chromatography on silica gel (10% gradient of MeOH in DCM) to yield **15c** (6.8 mg, 60%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.32, 1.47 (2s, 6H, C(*CH*$_3$)$_2$), 1.64–1.78 (m, 1H, H-3a), 1.94 (s, 3H, NHAc), 3.02 (dd, *J* = 4.0, 12.0 Hz, 1H, H-3b), 3.56–3.64 (m, 1H, H-4), 3.97 (dd, *J* = 6.1, 13.3 Hz, 1H, H-9a), 4.00–4.11 (m, 2H, H-5, H-9b), 4.22 (d, *J* = 10.5 Hz, 1H, H-6), 4.27 (d, *J* = 6.7 Hz, 1H, H-7), 4.44 (dd, *J* = 6.4, 12.8 Hz, 1H, H-8), 4.49 (A of AB, *J* = 12.0 Hz, 1H, C*H*$_2$Ar), 4.69 (A′, B′ of A′B′, *J* = 11.9 Hz, 2H, C*H*$_2$Ar), 4.99 (B of AB, *J* = 11.8 Hz, 1H, C*H*$_2$Ar), 7.08–7.19 (m, 3H, CH_{ar}), 7.22–7.28 (m, 5H, CH_{ar}), 7.46, 7.92 (AA′, BB′ of AA′BB′, *J* = 8.4 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 21.5 (NHAc), 24.1, 25.8 (C(*CH*$_3$)$_2$), 37.8 (C-3), 40.5 (C-9), 51.7 (C-5), 58.8 (CH_2Ar), 70.2 (C-6), 71.7 (*CH*$_2$Ar), 74.4 (C-4), 75.3, 75.4 (C-7, C-8), 100.7 (C-2), 108.1 (*C*(CH$_3$)$_2$), 115.6, 115.7, 123.8, 124.9, 127.0, 127.1, 127.8, 128.1, 128.9, 129.0, 132.6, 137.1, 138.6 (18C, C-Ar), 167.8, 171.9, 173.4 (3CO). ESI-MS calcd for $C_{35}H_{36}ClF_2N_2NaO_9$ $[M-H]^-$: 701.21; found *m*/*z* 701.52.

4.2.10. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-4-*O*-phenylcarbamoyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosid] onate (15d)

Compound **11** (30.0 mg, 50 μmol) was dissolved in dry pyridine (1.0 mL). Phenyl isocyanate (24 μL, 0.24 mmol) and DMAP (1.0 mg, 8.0 μmol) were added. The reaction mixture was stirred for 24 h and then water (0.1 mL) was added. After removal of the solvents under reduced pressure, the crude product was purified by chromatography on silica gel (10% gradient of MeOH in DCM) to yield **15d** (10 mg, 29%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.25, 1.38 (2s, 6H, C(CH_3)$_2$), 1.85–1.92 (m, 4H, NHAc, H-3a), 2.76 (dd, J = 4.8, 13.0 Hz, 1H, H-3b), 3.50–3.64 (m, 1H, H-9a), 3.79–3.94 (m, 1H, H-9b), 4.10 (d, J = 10.4 Hz, 1H, H-7), 4.28–4.35 (m, 3H, H-5, H-6, H-8), 4.58, 4.70 (A, B of AB, J = 11.9 Hz, 2H, CH_2Ar), 5.21 (td, J = 4.7, 11.0 Hz, 1H, H-4), 6.96 (t, J = 7.3 Hz, 1H, CH_{ar}), 7.00–7.13 (m, 2H, CH_{ar}), 7.21 (dd, J = 7.7, 8.3 Hz, 2H, CH_{ar}), 7.23–7.28 (m, 1H, CH_{ar}), 7.31 (AA′ of AA′BB′, J = 8.6 Hz, 2H, CH_{ar}), 7.35 (d, J = 8.0 Hz, 2H, CH_{ar}), 7.84 (BB′ of AA′BB′, J = 8.6 Hz, 2H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 22.9 (NHAc), 25.5, 27.6 (C(CH_3)$_2$), 38.3 (C-3), 42.5 (C-9), 52.1 (C-5), 60.7 (CH_2Ar), 70.9, 71.6 (C-4, C-7), 76.1, 76.8 (C-6, C-8), 101.8 (C-2), 109.3 (C(CH_3)$_2$), 117.5, 119.7, 124.0, 125.6, 126.0, 128.6, 128.7, 129.4, 129.5, 129.8, 130.6, 134.0, 138.6, 140.1, 150.4, 150.5, 152.4 (18C, C-Ar), 155.1 (NC(O)O), 169.4, 173.7, 173.9 (3CO). ESI-MS calcd for $C_{35}H_{35}ClF_2N_3O_{10}$ [M−H]$^-$: 730.20; found m/z 730.33.

4.2.11. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-4-*O*-phenylethylcarbamoyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (15e)

Compound **11** (30.0 mg, 50 μmol) was dissolved in dry pyridine (1.5 mL). Phenylethyl isocyanate (50 μL, 0.42 mmol), DMAP (3.0 mg, 24.0 μmol), and NEt_3 (20 μL) were added. The reaction mixture was stirred at 60 °C for 24 h and then water (0.2 mL) was added. After removal of the solvents under reduced pressure, the crude product was purified by chromatography on silica gel (10% gradient of MeOH in DCM) to yield **15e** (7.3 mg, 20%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.30, 1.35 (2s, 6H, (CH_3)$_2$), 1.79 (t, J = 12.3 Hz, 1H, H-3a), 1.88 (s, 3H, NHAc), 2.61–2.78 (m, 3H, H-3b, CH_2), 3.18–3.26 (m, 2H, CH_2), 3.53–3.70 (m, 1H, H-9a), 3.87 (d, J = 12.1 Hz, 1H, H-9b), 4.07 (d, J = 10.6 Hz, 1H, H-7), 4.18–4.26 (m, 2H, H-5, H-6), 4.27–4.36 (m, 1H, H-8), 4.56, 4.69 (A, B of AB, J = 11.6 Hz, 2H, CH_2Ar), 5.08 (td, J = 4.8, 11.0 Hz, 1H, H-4), 7.01–7.09 (m, 2H, CH_{ar}), 7.14 (d, J = 7.5 Hz, 4H, CH_{ar}), 7.19–7.27 (m, 2H, CH_{ar}), 7.31, 7.83 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH_{ar}); ^{13}C NMR (CD_3OD) δ 23.1 (NHAc), 25.8, 27.7 (C(CH_3)$_2$), 36.8 (CH_2), 38.4 (C-3), 42.6 (C-9), 43.4 (CH_2), 52.1 (C-5), 60.7 (CH_2Ar), 70.9 (C-7), 71.5 (C-4), 75.7 (C-6), 76.8 (C-8), 101.7 (C-2), 109.2 (C(CH_3)$_2$), 117.4, 125.5, 125.2, 126.0, 127.4, 128.7, 129.5, 129.9, 130.0, 130.4, 130.6, 134.1, 140.5, 150.5 (18C, C-Ar), 158.2 (COONH), 169.4, 173.5, 173.6 (3CO). ESI-MS calcd for $C_{37}H_{39}ClF_2N_3O_{10}$ [M−H]$^-$: 758.23; found m/z 758.37.

4.2.12. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-4-*O*-(3-thienylcarbamoyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (15f)

Compound **11** (20.0 mg, 30 μmol) was dissolved in dry pyridine (0.5 mL). 3-Thienyl isocyanate (12 μL, 0.1 mmol) and DMAP (1.0 mg, 8.0 μmol) were added. The reaction mixture was stirred for 24 h and then water (0.1 mL) was added. After removal of the solvents under reduced pressure, the crude product was purified by chromatography on silica gel (10% gradient of MeOH in DCM) to yield **15f** (8.0 mg, 35%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.25, 1.38 (2s, 6H, C(CH_3)$_2$), 1.87–1.90 (m, 4H, H-3a, NHAc), 2.76 (dd, J = 4.3, 12.8 Hz, 1H, H-3b), 3.48–3.63 (m, 1H, H-9a), 3.81–3.94 (m, 1H, H-9b), 4.10 (d, J = 10.4 Hz, 1H, H-7), 4.27–4.36 (m, 3H, H-5, H-6, H-8), 4.58, 4.70 (A, B of AB, J = 11.6 Hz, 2H, CH_2Ar), 5.15–5.29 (m, 1H, H-4), 6.93 (d, J = 5.4 Hz, 1H, CH_{ar}), 7.02–7.08 (m, 1H, CH_{ar}), 7.09–7.19 (m, 2H, CH_{ar}), 7.20–7.25 (m, 2H, CH_{ar}), 7.31 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 22.8 (NHAc), 25.5, 27.7 (C(CH_3)$_2$), 38.3 (C-3), 42.5 (C-9), 52.1 (C-5), 60.7 (CH_2Ar), 70.9, 71.8 (C-4, C-7), 76.1, 76.8 (C-6, C-8), 101.8 (C-2), 108.1 (C(CH_3)$_2$), 109.3, 117.5, 117.6, 121.8, 125.3, 125.4, 125.6, 126.0, 129.5, 130.6, 134.1, 138.0, 138.6, 150.5, 152.5 (16C, C-Ar), 155.2 (NC(O)O), 169.4, 173.6, 173.9 (3CO). ESI-MS calcd for $C_{33}H_{33}ClF_2N_3O_{10}S$ [M−H]$^-$: 736.15; found m/z 736.26.

4.2.13. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-4-*O*-(2-(2-thienyl)ethylcarbamoyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (15g)

Compound **11** (28.0 mg, 40 μmol) was dissolved in dry pyridine (0.5 mL). 2-(2-Thienyl)ethyl isocyanate (27.5 mg, 0.18 mmol) and DMAP (1.0 mg, 8.0 μmol) were added. The reaction mixture was stirred for 24 h at 45 °C, then cooled to rt, and treated with water (0.1 mL). After removal of the solvents under reduced pressure, the crude product was purified by chromatography on silica gel (10% gradient of MeOH in DCM) to yield **15g** (16 mg, 37%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.20, 1.27 (2s, 6H, C(CH_3)$_2$), 1.71 (t, J = 12.3 Hz, 1H, H-3a), 1.81 (s, 3H, NHAc), 2.62 (d, J = 4.8, 12.3 Hz, 1H, H-3b), 2.81–2.91 (m, 2H, CH_2), 3.15–3.22 (m, 2H, CH_2), 3.95–4.04 (m, 3H, H-7, H-9a, H-9b), 4.16–4.25 (m, 2H, H-5, H-8), 4.46–4.55 (m, 1H, H-6), 4.64, 4.88 (A, B of AB, J = 12.8 Hz, 2H, CH_2Ar), 4.98–5.08 (m, 1H, H-4), 6.64–6.74 (m, 1H, CH_{ar}), 6.75–6.81 (m, 1H, CH_{ar}), 6.84–6.93 (m, 1H, CH_{ar}), 6.94–7.00 (m, 1H, CH_{ar}), 7.07 (d, J = 4.9 Hz, 1H, CH_{ar}), 7.10–7.20 (m, 1H, CH_{ar}), 7.23, 7.74 (AA′, BB′ of AA′BB′, J = 8.3 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 23.0 (NHAc), 25.9, 27.7 (C(CH_3)$_2$), 31.0 (CH_2), 38.6 (C-3), 43.5 (C-9), 49.9 (CH_2), 52.1 (C-5), 60.8 (CH_2Ar), 71.0 (C-7), 75.8 (C-4), 76.1 (C-8), 76.9 (C-6), 101.6 (C-2), 109.4 (C(CH_3)$_2$), 117.7, 124.7, 126.3, 127.9, 129.5, 130.4, 130.6, 134.0, 138.7, 139.0, 140.0, 142.4, 142.5, 148.6, 150.5, 152.4 (16 C-Ar), 158.4 (NC(O)O), 169.4, 173.5, 173.6 (3CO). ESI-MS calcd for $C_{35}H_{38}ClF_2N_3O_{10}S$ [M+Na]$^+$: 788.19; found m/z 788.42.

4.2.14. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-4-*O*-((2-methylfuran-3-yl)carbamoyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (15h)

Compound **11** (37.0 mg, 50 μmol) was dissolved in dry pyridine (0.5 mL). 2-Methylfuran-3-yl isocyanate (22.0 mg, 0.18 mmol) and DMAP (1.0 mg, 8.0 μmol) were added. The reaction mixture was stirred for 24 h and then water was added. After removal of the solvents under reduced pressure, the crude product was purified by chromatography on silica gel (10% gradient of MeOH in DCM) to yield **15h** (11.0 mg, 28%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.20, 1.33 (2s, 6H, C(CH_3)$_2$), 1.72–1.82 (m, 1H, H-3a), 1.85 (s, 3H, NHAc), 2.08 (s, 3H, CH_3), 2.68 (dd, J = 4.4, 12.8 Hz, 1H, H-3b), 3.40–3.53 (m, 1H, H-9a), 3.84 (d, J = 12.8 Hz, 1H, H-9b), 4.04 (d, J = 10.2 Hz, 1H, H-7), 4.20–4.29 (m, 3H, H-5, H-6, H-8), 4.51, 4.63 (A, B of AB, J = 11.7 Hz, 2H, CH_2Ar), 5.11 (td, J = 4.2, 10.7, 11.1 Hz, 1H, H-4), 6.36 (s, 1H, CH_{ar}), 6.97–7.20 (m, 4H, CH_{ar}), 7.27, 7.78 (AA′, BB′ of AA′BB′, J = 8.4 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 11.2 (CH_3), 22.9 (NHAc), 25.6, 27.6 (C(CH_3)$_2$), 38.3 (C-3), 42.5 (C-9), 52.2 (C-5), 60.7 (CH_2Ar), 70.9 (C-7), 71.9 (C-4), 76.1 (C-8), 76.9 (C-6), 101.8 (C-2), 109.3 (C(CH_3)$_2$), 111.4, 117.5, 117.6, 120.4, 125.5, 126.0, 126.4, 129.5, 130.6, 134.2, 138.6, 138.9, 140.8, 150.5 (16C, C-Ar), 156.4 (NC(O)O), 169.4, 173.6 (3C, 3CO). ESI-MS calcd for $C_{34}H_{36}ClF_2N_3O_{11}$ [M+Na]$^+$: 758.18; found m/z 758.42.

4.2.15. Sodium [(2,3-difluorobenzyl) 5-acetamido-4-*O*-acetyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (16a)

Compound **15a** (11.0 mg, 20 μmol) was dissolved in 80% aq AcOH (1.5 mL) and stirred for 3 h at 60 °C. Then the reaction mixture was cooled to rt and neutralized with 10% aq NaOH. After removal of the solvents under reduced pressure the crude product was purified on RP-18 (10% gradient of MeOH in water) to yield **16a** (8.5 mg, 80%). $[\alpha]_D^{20}$ −32.8 (*c* 0.24, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.79 (t, *J* = 12.1 Hz, 1H, H-3a), 1.91 (s, 3H, NHAc), 2.03 (s, 3H, OAc), 2.75 (dd, *J* = 4.8, 12.4 Hz, 1H, H-3b), 3.42 (dd, *J* = 7.9, 14.2 Hz, 1H, H-9a), 3.52 (d, *J* = 10.0 Hz, 1H, H-7), 3.69–3.80 (m, 2H, H-8, H-9b), 3.91 (d, *J* = 11.7 Hz, 1H, H-6), 4.04 (t, *J* = 10.3 Hz, 1H, H-5), 4.62, 4.87 (A, B of AB, *J* = 11.8 Hz, 2H, C*H*$_2$Ar), 4.91 (td, *J* = 4.9, 11.5 Hz, 1H, H-4), 7.08 (dt, *J* = 8.4, 13.1 Hz, 2H, CH_{ar}), 7.17 (t, *J* = 6.7 Hz, 1H, CH_{ar}), 7.49, 7.72 (AA′, BB′ of AA′BB′, *J* = 8.5 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, D_2O): δ 20.2 (OAc), 21.8 (NHAc), 37.3 (C-3), 42.6 (C-9), 49.3 (C-5), 60.6 (*C*H$_2$Ar), 69.6, 70.2, 70.8, 72.1 (C-4, C-6, C-7, C-8), 101.1 (C-2), 117.0, 124.3, 125.8, 126.5, 128.6, 128.8, 132.1, 137.5, 138.0, 147.5, 149.0, 149.5 (12 C-Ar), 167.0, 172.8, 173.1, 174.5 (4CO). HRMS calcd for $C_{27}H_{29}ClF_2N_2O_9$ [M+Na]$^+$: 637.1379; found *m*/*z* 637.1362.

4.2.16. Sodium [(2,3-difluorobenzyl) 5-acetamido-4-*O*-benzoyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (16b)

Prepared from **15b** (7.1 mg, 10 μmol) according to the procedure described for **16a**. Purification on RP-18 (10% gradient of MeOH in water) yielded **16b** (4.1 mg, 60%). $[\alpha]_D^{20}$ −7.4 (*c* 0.28, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.80 (s, 3H, NHAc), 1.96 (t, *J* = 12.0 Hz, 1H, H-3a), 2.90 (dd, *J* = 4.9, 12.4 Hz, 1H, H-3b), 3.43 (dd, *J* = 8.0, 14.3 Hz, 1H, H-9a), 3.56 (d, *J* = 8.7 Hz, 1H, H-7), 3.73–3.83 (m, 2H, H-8, H-9b), 4.00 (dd, *J* = 1.8, 10.6 Hz, 1H, H-6), 4.24 (t, *J* = 10.3 Hz, 1H, H-5), 4.66, 4.81 (A, B of AB, *J* = 11.9 Hz, 2H, C*H*$_2$Ar), 5.13 (ddd, *J* = 4.8, 10.2, 11.6 Hz, 1H, H-4), 7.01–7.17 (m, 2H, CH_{ar}), 7.20 (t, *J* = 6.9 Hz, 1H, CH_{ar}), 7.46–7.56 (m, 4H, CH_{ar}), 7.65 (t, *J* = 7.5 Hz, 1H, CH_{ar}), 7.74, 7.96 (AA′, BB′ of AA′BB′, *J* = 8.6 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, D_2O): δ 21.7 (NHAc), 37.4 (C-3), 42.6 (C-9), 49.4 (C-5), 60.7 (*C*H$_2$Ar), 69.6 (C-7), 70.2 (C-8), 71.7 (C-4), 72.2 (C-6), 100.0 (C-2), 117.0, 117.2, 125.8, 128.7, 128.8, 128.9, 129.4, 132.1, 133.9, 137.5 (18C, C-Ar), 172.9, 174.3 (4C, 4CO). HRMS calcd for $C_{32}H_{30}ClF_2N_2NaO_{10}$ [M+Na]$^+$: 721.1345; found *m*/*z* 721.1353.

4.2.17. Sodium [(2,3-difluorobenzyl) 5-acetamido-4-*O*-benzyl-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (16c)

Prepared from **15c** (6.8 mg, 10 μmol) according to the procedure described for **16a**. Purification on RP-18 (10% gradient of MeOH in water) yielded **16c** (2.0 mg, 36%). $[\alpha]_D^{20}$ −8.4 (*c* 0.16, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.55 (t, *J* = 12.0 Hz, 1H, H-3a), 1.77 (s, 3H, NHAc), 2.86 (dd, *J* = 4.6, 12.4 Hz, 1H, H-3b), 3.27–3.39 (m, 2H, H-7, H-9a), 3.42–3.51 (m, 1H, H-4), 3.60–3.69 (m, 3H, H-6, H-8, H-9b), 3.79 (t, *J* = 10.2 Hz, 1H, H-5), 4.39, 4.55 (A, B of AB, *J* = 11.8 Hz, 2H, C*H*$_2$Ar), 4.61, 4.70 (A′, B′ of A′B′, *J* = 11.6 Hz, 2H, C*H*$_2$Ar), 7.00–7.12 (m, 3H, CH_{ar}), 7.20–7.35 (m, 5H, CH_{ar}), 7.41, 7.62 (AA′, BB′ of AA′BB′, *J* = 8.6 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, D_2O): δ 21.9 (NHAc), 37.8 (C-3), 49.5 (C-9), 50.0 (C-5), 69.7, 70.2, 71.2 (C-7, C-8, *C*H$_2$Ar), 72.6 (C-6), 75.6 (C-4), 101.4 (C-2), 126.0, 128.3, 128.6, 128.7, 128.8, 132.1, 136.7 (18C, C-Ar), 170.0, 172.9, 174.5 (3CO). HRMS calcd for $C_{32}H_{32}ClF_2N_2NaO_9$ [M+H]$^+$: 685.1741; found *m*/*z* 685.1745.

4.2.18. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-4-*O*-phenylcarbamoyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (16d)

Prepared from **15d** (10 mg, 10 μmol) according to the procedure described for **16a**. Purification on RP-18 (10% gradient of MeOH in water) yielded **16d** (1.5 mg, 15%). $[\alpha]_D^{20}$ −1.0 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.78 (s, 3H, NHAc), 1.88–1.99 (m, 1H, H-3a), 2.68 (d, *J* = 9.1 Hz, 1H, H-3b), 3.33–3.49 (m, 1H, H-9a), 3.60 (d, *J* = 10.7 Hz, 1H, H-9b), 3.74 (d, *J* = 5.4 Hz, 1H, H-7), 4.14 (d, *J* = 2.6 Hz, 1H, H-8), 4.19–4.46 (m, 2H, H-5, H-6), 4.57, 4.79 (A, B of AB, *J* = 11.2 Hz, 2H, C*H*$_2$Ar), 5.26 (td, *J* = 4.9, 10.7 Hz, 1H, H-4), 6.95 (t, *J* = 7.2 Hz, 1H, CH_{ar}), 7.13 (d, *J* = 6.0 Hz, 2H, CH_{ar}), 7.19 (t, *J* = 7.7 Hz, 3H, CH_{ar}), 7.28–7.35 (m, 2H, CH_{ar}), 7.41, 7.82 (AA′, BB′ of AA′BB′, *J* = 8.3 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 22.8 (NHAc), 38.9 (C-3), 44.2 (C-9), 51.2 (C-5), 60.4 (*C*H$_2$Ar), 69.4 (C-7), 70.8 (C-4), 72.5 (C-8), 73.3 (C-6), 103.0 (C-2), 117.6, 117.7, 119.8, 124.2, 125.6, 126.3, 128.1, 129.4, 129.8, 130.3, 133.4, 139.1, 139.9, 150.6 (18C, C-Ar), 154.9 (NC(O)O), 169.9, 174.3, 177.6 (3CO). ESI-MS calcd for $C_{32}H_{32}ClF_2N_3O_{10}$ [M+Na]$^+$: 714.16; found *m*/*z* 714.30.

4.2.19. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-4-*O*-phenylethylcarbamoyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (16e)

Prepared from **15e** (7.3 mg, 10 μmol) according to the procedure described for **16a**. Purification on RP-18 (10% gradient of MeOH in water) yielded **16e** (1.5 mg, 20%). $[\alpha]_D^{20}$ −5.6 (*c* 0.11, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.71–1.83 (m, 1H, H-3a), 1.87 (s, 3H, NHAc), 2.38–2.51 (m, 1H, H-3b), 2.53–2.68 (m, 2H, CH_2), 3.05–3.18 (m, 2H, CH_2), 3.31–3.47 (m, 2H, H-8, H-9a), 3.48–3.65 (m, 2H, H-7, H-9b), 4.05–4.29 (m, 2H, H-5, H-6), 4.46 (A, B of AB, *J* = 11.4 Hz, 2H, C*H*$_2$Ar), 5.04 (dt, *J* = 5.3, 10.5, 11.0 Hz, 1H, H-4), 6.93–7.19 (m, 8H, CH_{ar}), 7.32, 7.72 (AA′, BB′ of AA′BB′, *J* = 8.2 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 22.8 (NHAc), 37.0 (C-3), 39.0, 43.4 (2 CH_2), 44.6 (C-9), 51.2 (C-5), 60.4 (*C*H$_2$Ar), 70.0 (C-4), 70.9, 72.1 (C-7, C-8), 72.3 (C-6), 102.8 (C-2), 117.6, 125.3, 126.3, 126.6, 127.2, 127.3, 129.5, 129.8, 130.2, 130.3, 133.7, 134.2, 138.7, 139.0, 140.4, 152.5 (18C, C-Ar), 158.0 (NC(O)O), 169.9, 174.0, 175.9 (3CO). HRMS calcd for $C_{34}H_{36}ClF_2N_3O_{10}$ [M+Na]$^+$: 764.1774; found *m*/*z* 764.1791.

4.2.20. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-4-*O*-(3-thienylcarbamoyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (16f)

Prepared from **15f** (8.0 mg, 10 μmol) according to the procedure described for **16a**. Purification on RP-18 (10% gradient of MeOH in water) yielded **16f** (2.1 mg, 31%). $[\alpha]_D^{20}$ −17.8 (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.76 (t, *J* = 11.9 Hz, 1H, H-3a), 1.88 (s, 3H, NHAc), 3.02 (dd, *J* = 5.0, 12.1 Hz, 1H, H-3b), 3.44 (dd, *J* = 1.7, 9.0 Hz, 1H, H-7), 3.48 (dd, *J* = 7.7, 13.7 Hz, 1H, H-9a), 3.76 (dd, *J* = 3.1, 13.7 Hz, 1H, H-9b), 3.84 (dd, *J* = 1.3, 10.5 Hz, 1H, H-6), 3.97–4.08 (m, 2H, H-5, H-8), 4.66, 4.92 (A, B of AB, *J* = 12.2 Hz, 2H, C*H*$_2$Ar), 4.98 (td, *J* = 5.0, 11.0 Hz, 1H, H-4), 6.94 (d, *J* = 4.9 Hz, 1H, CH_{ar}), 7.04–7.11 (m, 2H, CH_{ar}), 7.17 (s, 1H, CH_{ar}), 7.23 (dd, *J* = 3.2, 5.1 Hz, 1H, CH_{ar}), 7.25–7.32 (m, 1H, CH_{ar}), 7.43, 7.80 (AA′, BB′ of AA′BB′, *J* = 8.6 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 22.6 (NHAc), 39.7 (C-3), 44.4 (C-9), 51.7 (C-5), 60.3 (*C*H$_2$Ar), 71.4 (C-8), 72.2 (C-7), 72.5 (C-4), 74.0 (C-6), 108.0 (C-2), 108.1, 117.0, 121.8, 122.4, 125.2, 125.4, 126.3, 129.7, 130.1, 134.4, 138.6 (16C, C-Ar), 152.4 (NC(O)O), 174.9 (3C, 3CO). HRMS calcd for $C_{30}H_{30}ClF_2N_3O_{10}S$ [M−H]$^-$: 720.1206; found *m*/*z* 720.1209.

4.2.21. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-4-*O*-(2-(2-thienyl)ethylcarbamoyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (16g)

Prepared from **15g** (16.0 mg, 20 μmol) according to the procedure described for **16a**. Purification on RP-18 (10% gradient of MeOH in water) yielded **16g** (1.5 mg, 10%). $[\alpha]_D^{20}$ −5.9 (*c* 0.18, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.73–1.83 (m, 4H, H-3a, NHAc), 2.54 (dd, *J* = 5.0, 13.0 Hz, 1H, H-3b), 2.85 (t, *J* = 7.0 Hz, 2H, CH_2), 3.16–3.22 (m, 2H, CH_2), 3.28 (dd, *J* = 6.4, 14.0 Hz, 1H, H-9a),

3.45 (dd, J = 5.0, 14.0 Hz, 1H, H-9b), 3.66 (d, J = 5.6 Hz, 1H, H-7), 4.00–4.13 (m, 2H, H-5, H-8), 4.20 (d, J = 10.6 Hz, 1H, H-6), 4.50, 4.69 (A, B of AB, J = 11.7 Hz, 2H, CH_2Ar), 5.07 (td, J = 4.9, 10.9 Hz, 1H, H-4), 6.71 (d, J = 3.0 Hz, 1H, CH_{ar}), 6.79–6.85 (m, 1H, CH_{ar}), 7.05–7.19 (m, 4H, CH_{ar}), 7.36, 7.78 (AA′, BB′ of AA′BB′, J = 8.6 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 22.9 (NHAc), 30.9 (CH_2), 43.5 (C-3), 44.0 (C-9), 46.7 (CH_2), 51.2 (C-5), 60.5 (CH_2Ar), 69.2, 70.6, 72.3 (C-4, C-7, C-8), 73.9 (C-6), 103.0 (C-2), 117.6, 117.7, 124.7, 125.7, 126.1, 126.3, 127.9, 129.8, 130.4, 133.3, 139.2, 142.4 (16C, C-Ar), 157.9 (NC(O)O), 169.8, 174.2, 174.6 (3CO). ESI-MS calcd for $C_{32}H_{34}ClF_2N_3O_{10}S$ [M+Na]$^+$: 748.14; found m/z 748.29.

4.2.22. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-4-*O*-((2-methylfuran-3-yl)carbamoyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (16h)

Prepared from **15h** (11.0 mg, 10 μmol) according to the procedure described for **16a**. Purification on RP-18 (10% gradient of MeOH in water) yielded **16h** (2.4 mg, 25%). $[\alpha]_D^{20}$ −2.4 (c 0.37, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.71 (dd, J = 7.9, 11.6 Hz, 1H, H-3a), 1.82 (s, 3H, NHAc), 2.07 (s, 3H, CH_3), 2.50 (dd, J = 5.1, 12.3 Hz, 1H, H-3b), 3.31 (d, J = 9.4 Hz, 1H, H-7), 3.44 (dd, J = 6.5, 13.8 Hz, 1H, H-9a), 3.71 (dd, J = 2.6, 13.6 Hz, 1H, H-9b), 3.84–3.97 (m, 1H, H-8), 4.06 (t, J = 11.5 Hz, 1H, H-6), 4.18 (t, J = 10.6 Hz, 1H, H-5), 4.45, 4.69 (A, B of AB, J = 11.2 Hz, 2H, CH_2Ar), 5.11–5.21 (m, 1H, H-4), 6.35 (s, 1H, CH_{ar}), 7.00–7.06 (m, 2H, CH_{ar}), 7.11 (s, 1H, CH_{ar}), 7.18 (s, 1H, CH_{ar}), 7.37, 7.74 (AA′, BB′ of AA′BB′, J = 8.4 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 11.3 (CH_3), 22.9 (NHAc), 39.3 (C-3), 44.1 (C-9), 60.4 (CH_2Ar), 67.5, 70.2, 71.0 (C-4, C-7, C-8), 72.0 (C-6), 101.9 (C-2), 111.4, 112.2, 117.2, 125.4, 126.7, 129.7, 130.2, 130.3, 134.1, 138.6, 138.8, 140.8, 143.5 (16C, C-Ar), 156.4 (NC(O)O), 169.8, 176.7, 180.5 (3CO). HRMS calcd for $C_{31}H_{32}ClF_2N_3O_{11}$ [M+Na]$^+$: 718.1592; found m/z 718.1584.

4.2.23. Methyl [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-α-D-*manno*-2,4-nonudiulopyranosid]onate (17)

Compound **10** (63.0 mg, 10 μmol) was dissolved in dry DCM (2.5 mL). Pyridinium dichromate (26.0 mg, 70 μmol) was added followed by the addition of acetic anhydride (28 μL, 0.3 mmol). The reaction mixture was stirred at rt for 4 h. After addition of 2-propanol (1 mL) the mixture was co-evaporated three times with toluene. Purification by chromatography on silica gel (EtOAc) yielded **17** (20 mg, 30%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.37, 1.52 (2s, 6H, C(CH_3)$_2$), 2.17 (s, 3H, NHAc), 2.94 (d, J = 15.2 Hz, 1H, H-3a), 3.29 (d, J = 15.0 Hz, 1H, H-3b), 3.82 (s, 3H, OMe), 3.85–3.96 (m, 1H, H-9a), 4.02 (dt, J = 6.4, 13.0 Hz, 1H, H-9b), 4.27 (d, J = 6.4 Hz, 1H, H-7), 4.38–4.52 (m, 2H, H-5, H-6), 4.53–4.59 (m, 1H, H-8), 4.64, 5.07 (A, B of AB, J = 11.5 Hz, 2H, CH_2Ar), 6.06 (d, J = 6.9 Hz, 1H, NH), 6.97–7.21 (m, 4H, NH, CH_{ar}), 7.40, 7.76 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, $CDCl_3$): δ 23.1 (NHAc), 25.4, 26.9 (C(CH_3)$_2$), 44.3 (C-3), 47.6 (C-9), 53.7 (C-5), 56.3 (OMe), 60.4 (CH_2Ar), 73.2 (C-7), 74.6 (C-8), 75.3 (C-6), 99.8 (C-2), 109.1 (C(CH$_3$)$_2$), 117.2, 124.1, 124.2, 125.2, 125.9, 126.0, 128.5, 128.6, 128.8, 132.7, 137.8 (12C, C-Ar), 167.2, 170.7, 173.0, 200.1 (4CO). ESI-MS calcd for $C_{29}H_{31}ClF_2N_2O_9$ [M+Na]$^+$: 647.16; found m/z 647.13.

4.2.24. Methyl [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-4-methyl-D-*glycero*-α-D-*talo*-nonulopyranosid]onate (18)

$ZrCl_4$ (50.0 mg, 0.13 mmol, 4.0 equiv) was dried for 30 min at 30 °C under high vacuum. After addition of dry THF (1.7 mL), the suspension was heated up to 50 °C for 20 min. Afterwards the colorless solution was cooled to −54 °C. MeLi (1 M in hexane, 0.5 mL) was added dropwise and the pale yellow solution was warmed up to 0 °C and stirring was continued for 30 min. After cooling to −78 °C, a solution of **17** (20.0 mg, 30 μmol) in THF (0.5 mL) was added. The reaction mixture was stirred for 3 h and then allowed to warm to 0 °C. After addition of semi-satd aq NH_4Cl (0.5 mL), the reaction mixture was extracted with DCM (5 mL). The combined organic layers were dried over Na_2SO_4, filtered, and the solvents were evaporated. Afterwards the crude mixture was dissolved in 80% aq AcOH (1 mL) and stirred for 3 h at 60 °C. After removal of the solvents under reduced pressure, the pure product **18** was obtained by chromatography on silica gel (1% gradient of MeOH in DCM) (6.0 mg, 32% + 2.0 mg, 11% (*S*)-stereoisomer). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24 (s, 3H, CH_3), 2.07 (s, 3H, NHAc), 2.96 (d, J = 15.0 Hz, 1H, H-3a), 3.35 (d, J = 15.0 Hz, 1H, H-3b), 3.48 (d, J = 8.9 Hz, 1H, H-7), 3.73 (dd, J = 6.9, 13.1 Hz, 1H, H-9a), 3.79 (s, 3H, OMe), 3.82–3.90 (m, 2H, H-6, H-9b), 4.09–4.18 (m, 1H, H-8), 4.68–4.80 (m, 2H, H-5, CH_2Ar), 4.95 (B of AB, J = 11.7 Hz, 1H, CH_2Ar), 6.55 (d, J = 6.6 Hz, 1H, NHAc), 7.00–7.18 (m, 4H, NH, CH_{ar}), 7.39, 7.74 (AA′, BB′ of AA′BB′, J = 8.5 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, $CDCl_3$): δ 23.0 (NHAc), 29.0 (CH_3), 45.2 (C-9), 47.5 (C-3), 52.8 (C-5), 53.6 (OMe), 69.7 (C-8), 70.5 (C-7), 71.5 (C-6), 98.1 (C-2), 122.8, 128.5, 128.6, 129.0, 129.2 (12C, C-Ar), 178.1 (3C, 3CO). ESI-MS calcd for $C_{30}H_{35}ClF_2N_2O_9$ [M+Na]$^+$: 663.19; found m/z 663.05.

4.2.25. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-4-methyl-D-*glycero*-α-D-*talo*-nonulopyranosid]onate (19)

Compound **18** (6.0 mg, 10 μmol) was dissolved in THF (1.0 mL) and LiOH (9.0 mg, 0.4 mmol) in water (1.0 mL) was added. The mixture was stirred at rt for 4 h and neutralized with 7% aq HCl. After removal of the solvents under reduced pressure, the pure product **19** (2.0 mg, 34%) was obtained by chromatography on RP-8 (5% gradient of MeOH in water) followed by Dowex 50X8 ion-exchange and P2 size exclusion chromatography. $[\alpha]_D^{20}$ −6.3 (c 0.26, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.19 (s, 3H, CH_3), 1.79 (d, J = 14.1 Hz, 1H, H-3a), 2.02 (s, 3H, NHAc), 2.60 (d, J = 14.1 Hz, 1H, H-3b), 3.45 (dd, J = 7.3, 13.6 Hz, 1H, H-9a), 3.50 (dd, J = 1.9, 8.6 Hz, 1H, H-7), 3.65–3.83 (m, 2H, H-8, H-9b), 3.88 (d, J = 10.6 Hz, 1H, H-5), 4.31 (dd, J = 2.0, 10.6 Hz, 1H, H-6), 4.56, 4.76 (A, B of AB, J = 12.0 Hz, 2H, CH_2Ar), 7.03–7.23 (m, 3H, CH_{ar}), 7.53, 7.76 (AA′, BB′ of AA′BB′, J = 8.6 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, D_2O): δ 21.8 (NHAc), 26.0 (CH_3), 42.6 (C-9), 44.8 (C-3), 52.1 (C-5), 60.1 (CH_2Ar), 70.3, 70.5, 70.6, 71.4 (C-4, C-6, C-7, C-8), 100.4 (C-2), 117.0, 124.3, 125.9, 126.7, 128.7, 128.8, 132.2, 137.5 (12C, C-Ar), 170.0, 174.4, 174.6 (3CO). HRMS calcd for $C_{26}H_{30}ClF_2N_2O_9$ [M+Na]$^+$: 585.1427; found m/z 585.1439.

4.2.26. Methyl [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-7,8-*O*-isopropylidene-D-*glycero*-α-D-*talo*-nonulopyranosid]onate (20)

$BH_3{\cdot}NH_3$ (7.0 mg, 0.22 mmol) was dissolved in MeOH (0.5 mL) at 0 °C followed by the addition of **17** (30.0 mg, 50 μmol) in MeOH (0.5 mL). After stirring for 2 h at 0 °C, the solvent was evaporated under reduced pressure and the crude product was purified by chromatography on silica gel (2% gradient of 2-PrOH in DCM/MeOH 10:1) to yield **20** (11.0 mg, 36%) as a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 1.30, 1.48 (2s, 6H, C(CH$_3$)$_2$), 1.88 (dd, J = 2.0, 13.7 Hz, 1H, H-3a), 1.95 (s, 3H, NHAc), 2.62 (dd, J = 3.9, 13.7 Hz, 1H, H-3b), 4.02 (d, J = 3.8 Hz, 1H, H-4), 4.07 (t, J = 6.2 Hz, 2H, H-9a, H-9b), 4.13 (dd, J = 2.6, 10.7 Hz, 1H, H-5), 4.20 (d, J = 6.9 Hz, 1H, H-7), 4.37 (d, J = 10.7 Hz, 1H, H-6), 4.43 (A of AB, J = 11.4 Hz, 1H, CH_2Ar), 4.47 (tt, J = 4.5, 8.8 Hz, 1H, H-8), 4.90 (B of AB, J = 11.4 Hz, 1H, CH_2Ar), 7.04–7.13 (m, 1H, CH_{ar}), 7.13–7.22 (m, 2H, CH_{ar}), 7.44, 7.82 (AA′, BB′ of AA′BB′, J = 8.6 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 22.7 (NHAc), 25.8, 27.1 (C(CH_3)$_2$), 40.8

(C-3), 41.9 (C-9), 50.1 (C-5), 52.7 (OMe), 60.5 (CH_2Ar), 66.3 (C-4), 71.5 (C-7), 76.3 (C-6), 77.7 (C-8), 98.9 (C-2), 110.1 ($C(CH_3)_2$), 117.8, 125.4, 126.8, 128.3, 129.7, 130.1, 130.2, 134.3, 138.7 (12C, C-Ar), 169.3, 171.4, 173.1 (3CO). ESI-MS calcd for $C_{29}H_{33}Cl_2F_2N_2O_9$ $[M+Na]^+$: 649.18; found *m*/*z* 649.22.

4.2.27. Methyl [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*talo*-nonulopyranosid]onate (21)

Compound **20** (20.0 mg, 30 μmol) was dissolved in 80% aq AcOH (1.5 mL) and stirred for 3 h at 60 °C. Then, the reaction mixture was cooled to rt and neutralized with 10% aq NaOH. After evaporation of the solvents, the crude product was purified by chromatography on silica gel (10% gradient of MeOH in DCM) to yield **21** (17 mg, 90%) as a colorless oil. ^{1}H NMR (500 MHz, CD_3OD): δ 1.89–1.97 (m, 4H, H-3a, NHAc), 2.62 (dd, *J* = 3.4, 13.9 Hz, 1H, H-3b), 3.42 (dd, *J* = 5.1, 13.8 Hz, 1H, H-6), 3.52 (dd, *J* = 7.6, 13.8 Hz, 1H, H-9a), 3.70 (s, 3H, OMe), 3.78 (dd, *J* = 3.3, 13.9 Hz, 1H, H-9b), 4.01–4.11 (m, 3H, H-4, H-5, H-8), 4.35 (d, *J* = 11.4 Hz, 1H, H-7), 4.45, 4.76 (A, B of AB, *J* = 11.8 Hz, 2H, CH_2Ar), 7.00–7.21 (m, 3H, CH_{ar}), 7.41, 7.78 (AA′, BB′ of AA′BB′, *J* = 8.6 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD): δ 22.7 (NHAc), 41.1 (C-3), 44.9 (C-9), 49.8 (C-5), 53.2 (OMe), 60.3 (CH_2Ar), 67.0 (C-4), 71.3 (C-8), 71.8 (C-7), 72.5 (C-6), 99.0 (C-2), 117.9, 118.0, 125.5, 125.6, 126.7, 128.5, 128.6, 129.8, 130.2, 134.6, 138.8 (12C, C-Ar), 169.6, 172.0, 174.3 (3CO). ESI-MS calcd for $C_{26}H_{29}ClF_2N_2O_9$ $[M+Na]^+$: 587.25; found *m*/*z* 587.25.

4.2.28. Sodium [(2,3-difluorobenzyl) 5-acetamido-9-(4-chlorobenzamido)-3,5,9-trideoxy-D-*glycero*-α-D-*talo*-nonulopyranosid]onate (22)

Compound **21** (17.0 mg, 30 μmol) was dissolved in MeOH (3 mL) and 10% aq NaOH (0.1 mL) was added. After 2 h the reaction was neutralized with 7% aq HCl. The solvents were evaporated and the residue was purified by chromatography on RP-8 (10% gradient of MeOH in water) to yield **22** (2.0 mg, 34%). $[\alpha]_D^{20}$ −41.6 (*c* 0.37, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.85 (dd, *J* = 2.9, 14.2 Hz, 1H, H-3a), 1.96 (s, 3H, NHAc), 2.62 (dd, *J* = 3.4, 14.2 Hz, 1H, H-3b), 3.44 (dd, *J* = 7.9, 14.2 Hz, 1H, H-9a), 3.49 (dd, *J* = 1.8, 8.9 Hz, 1H, H-7), 3.67–3.79 (m, 2H, H-8, H-9b), 4.00 (dd, *J* = 2.8, 10.7 Hz, 1H, H-5), 4.13 (q, *J* = 2.9 Hz, 1H, H-4), 4.37 (dd, *J* = 1.8, 10.7 Hz, 1H, H-6), 4.53, 4.73 (A, B of AB, *J* = 11.7 Hz, 2H, CH_2Ar), 6.97–7.25 (m, 3H, CH_{ar}), 7.49, 7.73 (AA′, BB′ of AA′BB′, *J* = 8.5 Hz, 4H, CH_{ar}); ^{13}C NMR (126 MHz, CD_3OD) δ 21.8 (NHAc), 39.2 (C-3), 42.6 (C-9), 48.2 (C-5), 60.1 (CH_2Ar), 65.8 (C-4), 69.4 (C-6), 70.2, 70.3 (C-7, C-8), 100.0 (C-2), 117.0, 125.9, 128.7, 128.8, 132.1, 137.5 (12C, C-Ar), 170.0, 174.5 (3C, 3CO). HRMS calcd for $C_{25}H_{27}ClF_2N_2O_9$ $[M+Na]^+$: 595.1273; found *m*/*z* 595.1276.

4.3. Surface plasmon resonance (SPR) analysis

The SPR measurements were performed on a Biacore 3000 surface plasmon resonance-based optical biosensor (Biacore AB, Sweden). Sensor chips (CM5), 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 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 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 4'000 to 5'000 RU were achieved. Flow cells were blocked with a 10-min injection of 1 M ethanolamine, pH 8.0. For capturing, $MAG_{d1\text{-}3}$-Fc solution (expressed and purified as described[32]) was diluted to a 30–40 μg/mL concentration using HBS-EP. Afterwards, $MAG_{d1\text{-}3}$-Fc was injected at a flow rate of 1 μL/min for 10 min. Using HBS-EP, the surface was equilibrated overnight at a flow rate of 5 μL/min, achieving densities around 2000 RU. Ten-fold dilution series were freshly prepared in eluent buffer immediately before use (→**14**, **16a**–**c**, **19**, and **22**). All binding experiments were conducted at 25 °C 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 was 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 2.0c). Kinetic data were simultaneously fit using Scrubber 2.0c. For the DMSO assay, DMSO (for molecular biology, >99.9%) was purchased from Fluka. The stock solution of the test compounds (→**16d**–**h**) was prepared in DMSO and was kept in glass vials to eliminate contaminations by, for example, softeners. The running buffer was 3% DMSO in HBS-EP. The surface was equilibrated at a flow of 5 μL/min until the baseline was stable. In order to eliminate the influence of DMSO on the signals, a calibration curve was done. Therefore, two solutions were prepared (A = 1 mL running buffer + 50 μL HBS-EP; B = 1 mL running buffer + 1 μL DMSO). Solutions A and B were mixed as indicated in Table 5 and used for calibration. DMSO calibration solutions were injected after five blank injections and before the sample solutions. The test compounds were diluted before measuring with HBS-EP to achieve a content of 3% DMSO. The DMSO calibration was accomplished directly in Scrubber© (version 2.0c).

4.4. Isothermal titration calorimetry

ITC experiments were performed using a VP-ITC instrument from MicroCal, Inc. (Northampton, MA). The measurements were performed at 25 °C. Injections of 5 μL ligand solutions were added from a computer controlled 300 μL microsyringe at an interval of 5 min into the sample cell solution of $MAG_{d1\text{-}3}$-Fc (cell volume 1.4512 mL) with stirring at 307 rpm. A control experiment was performed, where the identical ligand solutions were injected into buffer without protein. The enthalpogram showed negligible heat development, resulting from dilution effects. The assay buffer was HBS-E (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, pH 7.4). The concentration of $MAG_{d1\text{-}3}$-Fc was 48.4 μM, and 500 μM antagonist was injected. The experimental data were fitted to a theoretical titration curve (one site binding model) using *Origin version 7* software (MicroCal), with ΔH (enthalpy change in kcal/mol), K_A (association constant in M^{-1}), and *N* (number of binding sites) as adjustable parameters. The quantity $c = K_A \cdot Mt(0)$, where Mt(0) is the initial macromolecule concentration, is of importance in titra-

Table 5
Calibration solutions

Calibration	1	2	3	4	5
A (μL)	400	300	200	100	0
B (μL)	0	100	200	300	400

tion microcalorimetry.[31] The experiment was performed with a *c* value of 340. Thermodynamic parameters were calculated from Eq. (1),

$$\Delta G = \Delta H - T\Delta S = RT \ln K_A = -RT \ln K_D \quad (1)$$

where ΔG, ΔH, and ΔS are the changes in free energy, enthalpy, and entropy of binding, respectively, T is the absolute temperature, and R = 1.98 cal/mol/K. For reasons of consistency the values were converted to kJ (1 cal = 4.1868 J).

4.5. HPLC

The concentration of $MAG_{d1\text{-}3}$-Fc was determined via HPLC against a standard curve of BSA at 210 nm using a Beckmann Gold system, with UV detection (210 nm). The column used was Poros R1/10 10 μm (100 × 2 mm, Dr. Maisch HPLC Markensäulen, po10.r1.s1002, Morvay Analytik GmbH). The running buffers were A: H_2O + 0.1% TFA and B: 90% MeCN + 0.09% TFA. All measurements were performed at 75 °C, applying a gradient of 20–90% running buffer B within 20 min at a flow rate of 0.2 mL/min.[44]

Acknowledgments

We are greatly indebted to Dr. Francis Bitsch and Peggy Brunet Lefeuvre, Novartis, for providing a HPLC method for protein concentration determination and for providing the VP-ITC instrument as well as for advices regarding the experimental setup and analyzing the data. In addition, we would like to thank the Swiss National Science Foundation (project 200020-120628), the German Ministry for Education and Research ((BMBF, project 031632A), and the Tönjes-Vogt Foundation (project XXI) for their support of this work.

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