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PAPER

A simple synthesis of *N*-perfluoroacylated and *N*-acylated glycals of neuraminic acid with a cyclic aminic substituent at the 4α position as possible inhibitors of sialidases†

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A simple protocol for the synthesis of *N*-perfluoroacylated and *N*-acylated glycals of neuraminic acid, with a secondary cyclic amine (morpholine or piperidine) at the 4α position, has been set-up, starting from peracetylated *N*-acetylneuraminic acid methyl ester that undergoes, sequentially to its direct *N*-transacylation followed by a C-4 amination, a β-elimination, and a selective hydrolysis of the ester functions, without affecting the sensitive perfluorinated amide.

Introduction

The viral enzyme neuraminidase (NA) is a glycoprotein identified as a valid target for the development of new therapies against the influenza virus.[1] In fact, NA, which is expressed at the surface of the virus, is responsible for the viral release from the infected cell, where the virus is bound to the terminal sialic acid (Neu5Ac **1**, Fig. 1) of host cell sialoglycoproteins.[2–6] Therefore inhibitors of NA enzymatic activity have been shown to reduce the diffusion of the viral infection.[1] Along this line, synthetic efforts for the development of new NA inhibitors,[7–10] led to the clinically used Zanamivir **2** (4-deoxy-4-guanidino-Neu5Ac2en; Relenza™),[11–14] and its isostere Oseltamivir **3** (Tamiflu™),[15] which differs by a cyclohexene in place of the pyranosic ring. Notably, both inhibitors possess a basic substituent at position 4α, a feature that, together with the glycal structure, appears to improve the activity against NA. Actually, the structures of these drugs were inspired by that of the 2,3-unsaturated *N*-acetylneuraminic acid **4** (Neu5Ac2en, DANA), the first sialic acid glycal showing an inhibitory activity against NA (Fig. 1).[16]

Similarly, glycal **5** (FANA), which differs from DANA for a trifluoroacetyl in place of an acetylamido group, has shown an improved activity *in vitro* against *Vibrio cholerae*[17] and influence NA.[18] However, while many efforts have been made to synthesize 4α-modified analogues of DANA, little attention has been directed to the preparation of FANA congeners, which appears unjustified as they could be equally active against NA. Truthfully, this is possibly due to the long-standing lack of general methods for the FANA preparation[19] and to the struggle in preparing its 4α-substituted derivatives.

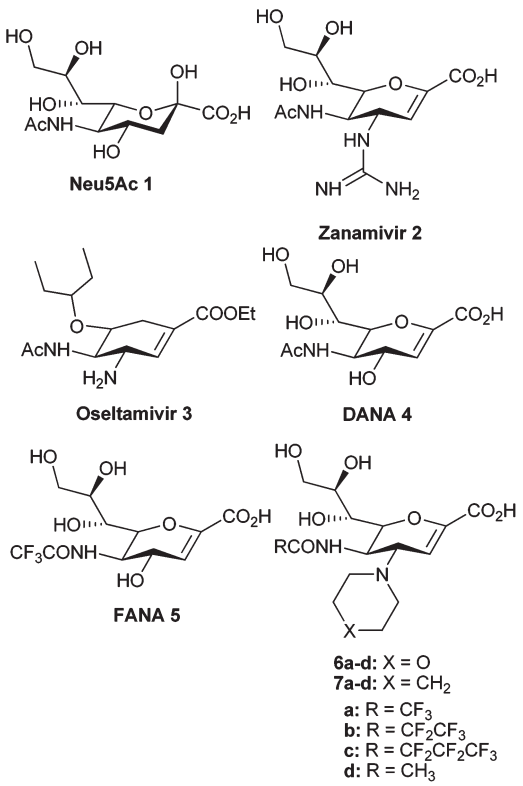

Fig. 1 Structures of Neu5Ac acid and of its glycals.

These difficulties were only recently overcome in our laboratory, where the FANA and some homologues were suitably

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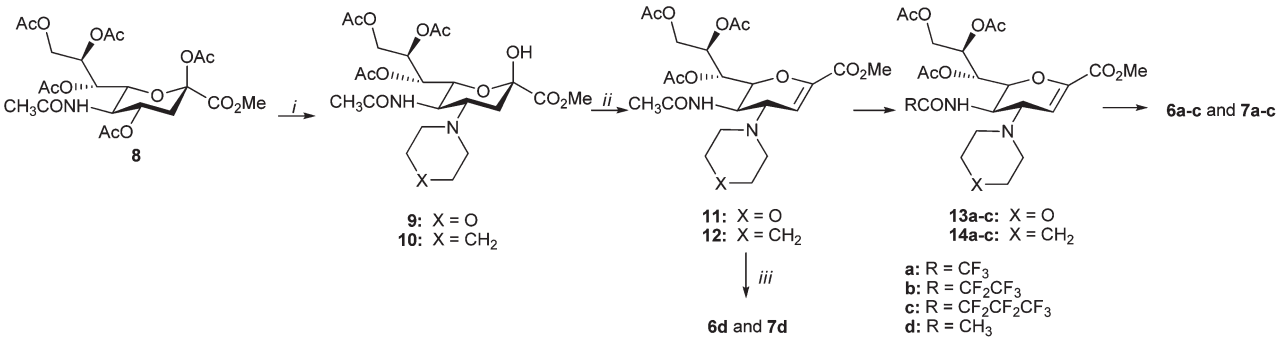


Scheme 1 Reagents and conditions: i, cyclic amine, MeCN, 60 °C, 5–7 h, 73–80%; ii, TFAA, MeCN, 23 °C, 10 min, then TFA, MeCN, 135 °C, 5 min, 78–80%; iii, K_2CO_3, MeOH–H_2O (10 : 1, v/v), 23 °C, 12 h, 85–91%.

synthesized.[20–22] Moreover, the synthesis of 4α-substituted FANA derivatives, starting from commercial Neu5Ac **1**, still represents an attractive goal since it requires some synthetic difficulties, such as the introduction of the glycal function and the insertion of a substituent at the C-4α position of a *N*-perfluoroacylated Neu5Ac, to be overcome. Furthermore, the presence of a perfluorinated acyl amido group, which is more prone to hydrolysis than the corresponding Neu5Ac amide,[23] in their structure poses the problem of its survival of the overall synthetic protocol, including an expected final hydrolytic regeneration of the esterified functions, leading to the target free glycals. Herein we report a synthetic strategy, which addresses and overcomes these problems and allows the successful synthesis of glycals **6a–d** and **7a–d**, analogues of FANA and DANA, with a basic substituent (piperidine or morpholine ring) at 4α position.

Results and discussion

The first step of our initial synthetic approach was the conversion of peracetylated Neu5Ac methyl ester **8**,[24] prepared from Neu5Ac **1**, into the 4α-substituted hydroxyesters **9** and **10**, adopting the Liu *et al.* procedure for the amination of the peracetylated sialic acid esters[25] (Scheme 1).

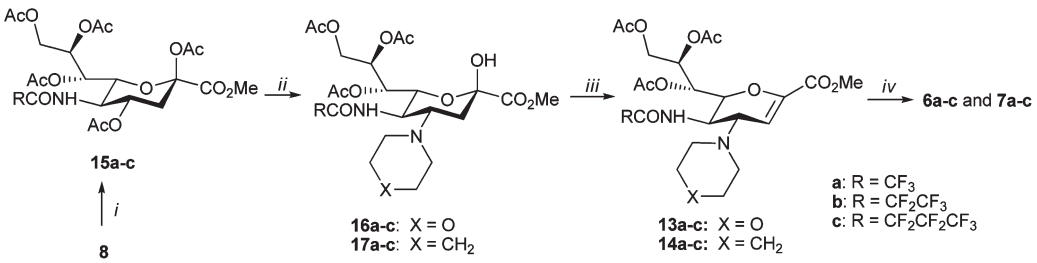
We were confident that they would generate the protected glycals **11** and **12**, precursors of the desired perfluorinated glycals **13a–c** and **14a–c** *via* *N*-transacylation of their amido group. Selective hydrolysis of their ester functions could then afford FANA derivatives **6a–c** and **7a–c**. Moreover, we considered that the glycals **11** and **12** could afford the unreported 4α-substituted DANA derivatives **6d** and **7d**, by simple selective hydrolysis.

In fact, we could prepare the hydroxy esters **9** and **10** by treating the methyl ester **8** with morpholine or piperidine at 25 °C for 24 h,[25] (Scheme 1). Both reactions afforded the desired α-substituted esters in high yields, moreover, by exchanging the reaction solvent (CH_3CN in place of pyridine), and increasing the reaction temperature to 60 °C we noticeably shortened the reaction time to 5 and 7 h, respectively, without affecting the yields. As expected from the sound mechanism proposed by the authors,[25] insertion of the base at the 4α-position of compound **8** occurs with regeneration of the 2-hemiacetalyc hydroxyl in the final compounds **9** and **10**. This, at first, appeared as an inconvenience for the following glycal formation that, in all previously reported procedures, requires the activation of the 2-hydroxyl as acetate ester, as glycosyl chloride or as thioglycoside.[26,27] In fact, initial attempts to perform the direct dehydration of 2-hydroxylated compounds **9** and **10**, afforded unsatisfactory results under several different conditions, even those that afforded glycal **11** as a undesired product.[28]

We eventually activated the anomeric hydroxyls of compounds **9** and **10** by perfluoroacetylation with TFAA, adopting a procedure we previously set up in our work on C-glycoside synthesis promoting the elimination of anomeric hydroxyls.[29–32] Thus, we performed the dehydration of compounds **9** and **10**, by acylation of their 2-hydroxy groups with TFAA, at 23 °C for 10 min, followed by treatment of the formed 2-trifluoroacetates with trifluoroacetic acid (TFA) in CH_3CN at 135 °C for 5 min. Under these conditions compounds **11** and **12** could be systematically obtained in very good repetitive yields (80% and 78% respectively).

The structure of both glycals was assigned on the basis of their elemental analyses and physicochemical and spectroscopic properties. In particular the coupling constant values between the olefinic proton at C-3 and the β-proton at C-4 signals, for both compounds ($J_{3,4}$ = 2.1 and 2.4 Hz) were diagnostic for the presence of an olefinic bond and of an allylic α-orientated amino group at C-4. This assumption was also supported by the coupling constant values between the β-proton at C-4 signals and the adjacent α-proton at C-5, for both glycals ($J_{4,5}$ = 8.0 and 8.1 Hz).[33–35] In fact, in all known sialic acid glycals with an epimeric substituent at the C-4 β-position, in agreement with the molecular geometry, the first coupling constant has a noticeably higher value while the second one has a lower value ($J_{3,4}$ = 5.1–5.7 and $J_{4,5}$ = 5.1–5.7 Hz).[35]

The successful formation of the glycals **11** and **12** from the saturated compounds **9** and **10**, prompted us to attempt the direct transformation of the normal hydroxy amides **9** and **10** into the transacylated FANA congeners **13a–c** and **14a–c**, adapting our protocol for the contemporaneous exchange of *N*-acyl groups and glycal formation of sialic acid.[20–22] Thus, we treated the hydroxy amides **9** and **10** first with the appropriate perfluorinated anhydride for 10 min at room temperature, then we heated the formed 2-perfluoroacylated esters in CH_3CN at 135 °C.[21] However, all attempts to perform this "*one pot*" transformation were quite unsuccessful as the desired *N*-transacylated



Scheme 2 Reagents and conditions: i, TFAA, Et_3N, MeCN, 135 °C, 5 min, 81–89%; ii, cyclic amine, MeCN, 60 °C, 5–7 h, 71–82%; iii, TFAA, MeCN, 23 °C, 10 min, then TFA, MeCN, 135 °C, 5 min, 77–82%; iv, Et_3N, MeOH–H_2O (2 : 1, v/v), 23 °C, 12 h, 84–94%.

compounds **13a–c** and **14a–c** could be obtained in very low yields (10–15%), only with forced reaction conditions, *i.e.* heating for 30 min with a strong excess of perfluorinated anhydrides (20 : 1). The perfluorinated glycals were always accompanied by major amounts of the normal glycals **11** and **12**. Moreover, in agreement with these negative results, our attempts to *N*-transacylate the glycals **11** and **12**, by treatment with TFAA in CH_3CN, at 135 °C, were unsuccessful and afforded the *N*-transacylated compounds **13a–c** and **14a–c** in poor yields, always accompanied by the starting glycals and by a series of unidentified compounds, also lacking of the substituent at C-4, as showed by mass spectrometry and NMR evidences. Thus we decided to discontinue this route and to utilize the protected glycals **11** and **12** to prepare the free glycals **6d** and **7d** by simple basic hydrolysis. Then, hypothesizing that the unsuccessful *N*-transacylation could be due to the chemical or steric interference of the basic substituent at C-4, we decided to modify our protocol by inverting the reaction order, first *N*-transacylating the peracetylated methyl ester **8** (Scheme 2) and then subjecting the *N*-transacylated methyl esters **15a–c** to the C-4 α-amination. We were confident that this would allow to successfully obtain the *N*-perfluoroacylated esters **16a–c** and **17a–c**, as their direct C-4 α-amination should proceed well, similarly to that of the acetate **8**. In fact, we could satisfactory prepare the saturated *N*-transacylated esters **15a–c**, using perfluorinated anhydrides, in the presence of Et_3N,[22] which avoids losing the 2-acetoxy group, essential for the subsequent 4α amination.

We eventually performed the reaction treating esters **15a–c** with morpholine or piperidine, in pyridine (at 25 °C for 24 h), or in CH_3CN (at 60 °C for 5–7 h). The reaction works well in all cases and affords the desired hydroxy compounds **16a–c** and **17a–c** in high yields (ranging from 71% to 82%). The hydroxy compounds were then dehydrated, using the TFAA activation and the acid treatment, as described before, affording the corresponding glycals **13a–c** and **14a–c** in very satisfactory yields (ranging from 77% to 82%), with the correct physico-chemical properties. In particular, ^1H NMR spectra were nearly superimposable to those of the acetylated compounds, and, as expected, they differed by the lack of the acyl group proton and the NH proton signals that, in all perfluorinated compounds, resonate at lower fields (6.64–7.09 ppm in place of 5.35–5.72 ppm). Also in these compounds, the coupling constants values of β-proton at C-4 were diagnostic for an α-stereochemistry of the amino group. Finally, in the ^{13}C NMR spectra, the carbon atom of the amidic carbonyl group of all perfluorinated congeners resonates at higher fields (157.5–158.5 ppm) than those of the acetylated congeners (170.0–171.0 ppm).

Table 1 Inhibition of the sialidase from *Vibrio cholerae* by DANA, FANA and the analogues **6a–d** and **7a–d**

Compound	K_i[a] (M)
DANA	$2.1 \pm 0.027 \times 10^{-5}$
FANA	$1.6 \pm 0.052 \times 10^{-6}$
6d	$1.2 \pm 0.82 \times 10^{-3}$
7d	$1.4 \pm 0.96 \times 10^{-3}$
6a	$1.4 \pm 0.10 \times 10^{-4}$
6b	$1.5 \pm 0.10 \times 10^{-4}$
6c	$1.4 \pm 0.12 \times 10^{-4}$
7a	$4.2 \pm 0.35 \times 10^{-4}$
7b	$4.6 \pm 0.15 \times 10^{-4}$
7c	$4.4 \pm 0.10 \times 10^{-4}$

[a] Each value represents the mean ± standard deviation of two or three independent experiments carried out in triplicate.

At this point, to obtain the free glycals **6a–c** and **7a–c**, we had to solve the problem of the selective regeneration of the carboxylic and hydroxylic functions of the protected glycals **13a–c** and **14a–c**, retaining their fluorinated amido groups. Some basic hydrolyic conditions were inefficient ($NaHCO_3$, $Zn(CH_3COO)_2$, or Na_3PO_4, in aqueous methanol) or not selective (LiOH, Li_2CO_3, tetramethylguanidine or Cs_2CO_3, in aqueous methanol), while the use of K_2CO_3, in aqueous methanol, afforded the correct final perfluorinated glycals **6b–c** and **7b–c**, in very good yields (80–90%), but caused an unselective hydrolysis of the glycal **13a**, affording a complex mixture of compounds. Hydrolysis with aqueous NH_3 in methanol caused the selective regeneration of the alcoholic functions, but transformed the sialic acid esters into the corresponding amides. Finally, we obtained the free acidic glycals **6a–c** and **7a–c**, performing the hydrolysis with Et_3N, in aqueous methanol, at 23 °C for 12 h. The reaction is slow, but allows all the perfluoroacylated glycals **6a–c** and **7a–c** to be prepared in good yields and with the expected physicochemical properties. In particular the survival of the perfluorinated acyl groups in their molecule was evident from the chemical shift position of their carbonyl carbon in the ^{13}C-NMR spectra and from the inspection of their coupling constants with the fluorine atoms at the respective α-carbons that are easily identifiable in the spectra.

The obtained glycals **6a–d** and **7a–d** were then inspected for their possible inhibitory action on *Vibrio cholerae* sialidase (Table 1). Assays were conducted using methods previously reported from our department.[36,37]

The data for inhibition, obtained for all the 4α-amino substituted derivatives, compared with those obtained for the parent

DANA and FANA congeners, that are in good agreement with those reported,[17] show that none of the new compounds are as effective as the parent glycal. In fact, in all cases a significant decrease of the anti sialidase activity is observed.

Moreover, we also performed an *in vitro* antiviral screening assay using influenza A viruses H1N1 (ATCC: VR1520) and H3N2 (ATCC: VR544) adopting a well proven method[38,39] to screen drug candidates for their potential antiviral activities.

In all cases, no positive activity was observed, and the viral replication was found to be unchanged in the presence of all tested compounds. These results suggest that the introduction of a 4α-cyclic amino substituent significantly reduces the anti-NA and the antiviral activities of 5-acylated neuraminic acid glycals, evidence that must be considered in programming the synthesis of new possible NA inhibitors.

Conclusions

In our work we demonstrated that the challenging synthesis of normal and perfluorinated glycals of Neu5Ac, bonding a cyclic secondary amine at the 4α position, can be efficiently performed using a simple procedure which appears general in scope. At the same time, we have set-up a simple procedure, of general utility, for the hydrolysis of acetates and methyl esters of Neu5Ac **1** in the presence of a labile trifluoroacylated amido groups. Unfortunately, a biological screening of the anti NA and anti influenza A viruses activity of the synthesized glycals, showed that no one of them is a possible candidate for additional studies. This negative result is however useful to programme future synthesis of NA inhibitors.[25]

Experimental

General information

Melting points are uncorrected. Nuclear magnetic resonance spectra were recorded at 298 K operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C. Chemical shifts are reported in parts per million (ppm, δ units), and in $CDCl_3$ are relative to $CDCl_3$ signal (fixed at 7.26 ppm for ^{1}H and at 77.0 ppm for ^{13}C spectra). In D_2O spectra, chemical shifts are relative to D_2O, referenced to *t*-BuOH as internal standards (signal fixed at 1.24 ppm for ^{1}H and at 30.29 ppm for ^{13}C spectra). Proton and carbon assignments were established, if necessary, with ^{1}H-^{1}H and ^{1}H-^{13}C correlated NMR experiments. ^{1}H NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet, t app, apparent triplet, br s, broad singlet; m, multiplet), coupling constant(s) in hertz, assignment of proton(s). Optical rotations were taken on a polarimeter equipped with a 1 dm tube; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1} and the concentrations are given in g/100 mL. Mass spectrometry was performed using a quadrupole ion trap mass spectrometer equipped with an electrospray (ESI) ion source. The spectra were collected in continuous flow mode by connecting the infusion pump directly to the ESI source. Solutions of compounds were infused at a flow rate of 5 μL min^{-1}. The spray voltage was set at 5.0 kV in the positive and at 4.5 kV in the negative ion mode with a capillary temperature of 220 °C. Full-scan mass spectra were recorded by scanning a *m*/*z* range of 100–2000. All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F 254) using UV light, 50% sulfuric acid or 0.2% ninhydrin in ethanol, and heat as developing agents. Work-up refers to successive washing of the organic layer with an ice cold aqueous $NaHCO_3$ saturated solution and water, drying over Na_2SO_4 and evaporation of the solvent under reduced pressure. Rapid chromatography was performed with normal phase silica gel.[40] *Vibrio cholerae* neuraminidsase was from Sigma Aldrich (N7885, 1–5 units per mg protein). Influenza A viruses H1N1 (ATCC: VR1520) and H3N2 (ATCC: VR544) were kindly provided by Prof. Fabrizio Pregliasco, from Milan University.

Methyl 5-acetamido-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (9). The ester **8** (213 mg, 0.40 mmol) and morpholine (0.35 mL, 4.0 mmol) were reacted in pyridine, according to the procedure reported by Liu *et al.*,[25] to afford the title compound **9**, as a white solid (168 mg, 81%): m.p. 153–155 °C; $[\alpha]_D^{20}$ + 26.6 (*c* 1 in $CHCl_3$); (Found: C, 50.88; H, 6.58; N, 5.60; Calc. for: $C_{22}H_{34}N_2O_{12}$ C, 50.96; H, 6.61; N, 5.40%); ^{1}H NMR ($CDCl_3$) δ 5.35 (1H, dd, $J_{7,8}$ = 6.1, $J_{7,6}$ = 2.1 Hz, 7-H), 5.34–5.26 (1H, br s, N–H), 5.23–5.17 (1H, m, 8-H), 4.66 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 2.0 Hz, 9a-H), 5.29 (1H, br s, OH), 4.13–4.06 (2H, overlapping, 5-H and 6-H), 3.99 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.5 Hz, 9b-H), 3.85 (3H, s, $COOCH_3$), 3.66–3.52 (4H, overlapping, $N(CH_2CH_2)_2O$), 2.92 (1H, br s, 4-H), 2.68–2.63 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.44–2.25 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.11 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.04–1.98 (5H, overlapping, CH_3COO, 3a-H and 3b-H), 1.94 (3H, s, CH_3COO); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.5, 170.4, 170.2 (4C, $3XCH_3COO$ and $NHCOCH_3$), 169.8 (C-1), 95.1 (C-2), 72.3 (C-6), 71.3 (C-8), 68.6 (C-7), 67.5 (2C, $N(CH_2CH_2)_2O$), 62.8 (C-9), 61.6 (C-4), 53.4 ($COOCH_3$), 48.6 (2C, $N(CH_2CH_2)_2O$), 46.3 (C-5), 29.5 (C-3), 23.2 ($NHCOCH_3$), 20.9, 20.8, 20.7 (3C, $3XCH_3COO$); MS (ESI positive) *m*/*z* 518.9 $[M + H]^+$, 541.1, $[M + Na]^+$, 1058.8 $[2M + Na]^+$.

An α-aminated compound **9**, with correct elemental analyses and physico-chemical properties superimposable to that above described was also obtained in 80% yields when the reaction was performed in CH_3CN (4 mL), heating at 60 °C for 5 h.

Methyl 5-acetamido-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (10). The ester **8** (362 mg, 0.68 mmol) and piperidine (0.68 mL, 6.8 mmol) were reacted in pyridine, according to the procedure reported by Liu *et al.*,[25] to afford the title compound **10**, as a white solid (270 mg, 76%): m.p. 132–133 °C; $[\alpha]_D^{20}$ + 20.3 (*c* 1 in $CHCl_3$); (Found: C, 53.14; H, 6.93; N, 5.32; Calc. for: $C_{23}H_{36}N_2O_{11}$ C, 53.48; H, 7.02; N, 5.42%); ^{1}H NMR ($CDCl_3$) δ 5.38 (1H, d, $J_{NH,5}$ = 9.9 Hz, N–H), 5.32 (1H, br d, $J_{7,8}$ = 6.3 Hz, 7-H), 5.24–5.20 (1H, m, 8-H), 4.43 (1H, br d, $J_{9a,9b}$ = 12.2 Hz, 9a-H), 4.14–4.08 (2H, overlapping, OH and 5-H), 4.03 (1H, br d, $J_{6,5}$ = 10.0 Hz, 6-H), 4.00 (1H, dd, $J_{9b,9a}$ = 12.2, $J_{9b,8}$ = 7.3 Hz, 9b-H), 3.85 (3H, s, $COOCH_3$), 3.02–2.97 (1H, m, 4-H), 2.67–2.59 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.34–2.26 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.10 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO), 2.02–1.95 (5H, overlapping, CH_3COO, 3a-H and 3b-H), 1.95 (3H, s, CH_3CONH), 1.54–1.31

(6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 170.7, 170.6, 170.5, 170.4 (4C, $3XCH_3COO$ and $NHCOCH_3$), 170.0 (C-1), 95.3 (C-2), 72.7 (C-6), 71.4 (C-8), 68.7 (C-7), 62.9 (C-9), 61.8 (C-4), 53.2 ($COOCH_3$), 49.5 (2C, $N(CH_2CH_2)_2CH_2$), 46.8 (C-5), 29.5 (C-3), 26.5 (2C, $N(CH_2CH_2)_2CH_2$), 24.6 (1C, $N(CH_2CH_2)_2CH_2$), 23.2 ($NHCOCH_3$), 21.0, 20.9, 20.8 (3C, $3XCH_3COO$); MS (ESI positive) *m/z* 517.1 $[M + H]^+$, 539.1, $[M + Na]^+$, 1054.8 $[2M + Na]^+$. An α-aminated compound **10**, with correct elemental analyses and physico-chemical properties superimposable to that above described was also obtained in 73% yields when the reaction was performed in CH_3CN (4 mL) heating at 60 °C for 7 h.

Methyl 5-acetamido-2,6-anhydro-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (11). The 2-hydroxy ester **9** (104 mg, 0.20 mmol) dissolved in CH_3CN (0.60 mL), was reacted with the TFAA (84 μL, 0.6 mmol) at 23 °C for 10 min. Then, the solvent and the excess TFAA were evaporated under a nitrogen flow to afford a crude residue that was recovered in CH_3CN (0.60 mL) and treated with TFA (46 μL, 0.6 mmol) at 135 °C for 5 min, in a sealed tube. Then, the reaction mixture was cooled, diluted with methanol (0.20 mL) and evaporated under reduced pressure. The obtained crude product (88 mg) that was then purified by rapid chromatography, to afford the appropriate glycal **11**, as a white solid, (80 mg, 80%): m.p. 110–112 °C; $[\alpha]_D^{20}$ + 65.0 (*c* 1 in $CHCl_3$); (Found: C, 52.76; H, 6.15; N, 5.79; Calc. for: $C_{22}H_{32}N_2O_{11}$ C, 52.79; H, 6.44; N, 5.60%); ^1H NMR ($CDCl_3$) δ 6.10 (1H, d, $J_{3,4}$ = 3.1 Hz, 3-H), 5.57 (1H, dd, $J_{7,8}$ = $J_{7,6}$ = 4.0 Hz, 7-H), 5.55–5.38 (1H, br s, N–H), 5.35 (1H, ddd, $J_{8,9b}$ = 7.2, $J_{8,7}$ = 4.0, $J_{8,9a}$ = 3.3 Hz, 8-H), 4.62 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 3.3 Hz, 9a-H), 4.38–4.32 (1H, m, 5-H), 4.29 (1H, dd, $J_{6,5}$ = 8.0, $J_{6,7}$ = 4.0 Hz, 6-H), 4.17 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.2 Hz, 9b-H), 3.80 (3H, s, $COOCH_3$), 3.73–3.59 (4H, overlapping, $N(CH_2CH_2)_2O$), 3.32 (1H, br s, 4-H), 2.83–2.70 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.67–2.55 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.11 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO), 1.96 (3H, s, CH_3CONH); ^{13}C NMR ($CDCl_3$) δ 170.5, 170.2, 170.1, 170.0 (4C, $3XCH_3COO$ and $NHCOCH_3$), 161.8 (C-1), 144.6 (C-2), 108.7 (C-3), 77.2 (C-6), 71.0 (C-8), 68.2 (C-7), 67.1 (2C, $N(CH_2CH_2)_2O$), 62.6 (C-4), 62.1 (C-9), 52.3 ($COOCH_3$), 49.7 (2C, $N(CH_2CH_2)_2O$), 43.8 (C-5), 23.2 ($NHCOCH_3$), 20.8 (1C, CH_3COO), 20.7 (2C, $2XCH_3COO$); MS (ESI positive) *m/z* 523.1 $[M + Na]^+$, 1022.5 $[2M + Na]^+$.

Methyl 5-acetamido-2,6-anhydro-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (12). The 2-hydroxy ester **10** (103 mg, 0.20 mmol), dissolved in CH_3CN (0.60 mL), was reacted with the TFAA (84 μL, 0.6 mmol) at 23 °C for 10 min. Then, the solvent and the TFAA excess were evaporated under a nitrogen flow to afford a crude residue which was recovered in CH_3CN (0.60 mL) and treated with TFA (46 μL, 0.6 mmol) at 135 °C for 5 min, in a sealed tube. Then, the reaction mixture was cooled, diluted with methanol (0.20 mL) and evaporated under reduced pressure to afford a crude product (90 mg) that was purified, by rapid chromatography, to afford the glycal **12** (81 mg, 78%), as a white solid: m.p. 137–138 °C; $[\alpha]_D^{20}$ + 57.0 (*c* 1 in $CHCl_3$); (Found: C, 55.40; H, 6.51; N, 5.59; Calc. for: $C_{23}H_{34}N_2O_{10}$ C, 55.41; H, 6.87; N, 5.62%); ^1H NMR ($CDCl_3$) δ 6.06 (1H, d, $J_{3,4}$ = 3.0 Hz, 3-H), 5.61 (1H, d, $J_{NH,5}$ = 9.4 Hz, N–H), 5.50 (1H, dd, $J_{7,8}$ = $J_{7,6}$ = 3.7 Hz, 7-H), 5.30 (1H, ddd, $J_{8,9b}$ = 7.4, $J_{8,7}$ = 3.7, $J_{8,9a}$ = 3.3 Hz, 8-H), 4.64 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 3.3 Hz, 9a-H), 4.31–4.25 (1H, m, 5-H), 4.20 (1H, dd, $J_{6,5}$ = 8.6, $J_{6,7}$ = 3.7 Hz, 6-H), 4.14 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.4 Hz, 9b-H), 3.75 (3H, s, $COOCH_3$), 3.23 (1H, dd, $J_{4,5}$ = 8.1, $J_{4,3}$ = 3.0 Hz, 4-H), 2.65–2.57 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.47–2.40 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.09 (3H, s, CH_3COO), 2.03–2.00 (6H, overlapping, $2XCH_3COO$), 1.89 (3H, s, CH_3CONH), 1.54–1.32 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 170.5, 170.2, 170.1, 170.0 (4C, $3XCH_3COO$ and $NHCOCH_3$), 162.0 (C-1), 144.2 (C-2), 110.7 (C-3), 77.4 (C-6), 71.3 (C-8), 68.2 (C-7), 63.1 (C-4), 62.1 (C-9), 52.2 ($COOCH_3$), 50.5 (2C, $N(CH_2CH_2)_2CH_2$), 43.9 (C-5), 26.4 (2C, $N(CH_2CH_2)_2CH_2$), 24.4 (1C, $N(CH_2CH_2)_2CH_2$), 23.3 ($NHCOCH_3$), 20.8 (2C, $2XCH_3COO$), 20.7 (1C, CH_3COO); MS (ESI positive) *m/z* 499.1 $[M + H]^+$, 521.1 $[M + Na]^+$, 1018.7 $[2M + Na]^+$.

5-Acetamido-2,6-anhydro-4-(morpholin-4-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (6d). The protected glycal **11** (50 mg, 0.10 mmol), dissolved in aqueous methanol (1.0 mL, 1 : 2 v/v) saturated with K_2CO_4, was stirred for 12 h at 23 °C. Then the solution was treated with a acidic resin [DOWEX 50WX8 (H^+)] and stirred for 15 min. The solution was filtered and the solvent was removed under reduced pressure to afford the free glycal **6d** (31 mg, 85%), as a white solid: m. p. 182–184 °C; $[\alpha]_D^{20}$ + 28.6 (*c* 1 in CH_3OH): (Found: C, 49.85; H, 6.76; N, 7.71; Calc. for: $C_{15}H_{24}N_2O_8$, C, 49.99; H, 6.71; N, 7.77%); ^1H NMR (D_2O) δ 5.77 (1H, d, $J_{3,4}$ = 2.8 Hz, 3-H), 4.36 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 9.4 Hz, 5-H), 4.17 (1H, d app, $J_{6,5}$ = 9.4 Hz, 6-H), 3.9 (1H, ddd, $J_{8,7}$ = 9.2, $J_{8,9b}$ = 6.2, $J_{8,9a}$ = 2.7 Hz, 8-H), 3.80 (1H, dd, $J_{9a,9b}$ = 11.9, $J_{9a,8}$ = 2.7 Hz, 9a-H), 3.79–3.80 (5H, overlapping, $N(CH_2CH_2)_2O$ and 4-H), 3.64 (1H, dd, $J_{9b,9a}$ = 11.9, $J_{9b,8}$ = 6.2 Hz, 9b-H), 3.60 (1H, dd, $J_{7,8}$ = 9.2, $J_{7,6}$ = 1.0Hz, 7-H), 2.81–2.74 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.70–2.64 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.04 (CH_3CONH); ^{13}C NMR (D_2O) δ 174.4 ($NHCOCH_3$), 168.9 (C-1), 151.2 (C-2), 101.2 (C-3), 75.8 (C-6), 69.9 (C-8), 68.5 (C-7), 66.3 (2C, $N(CH_2CH_2)_2O$), 63.1 (C-9), 62.7 (C-4), 48.6 (2C, $N(CH_2CH_2)_2O$), 42.8 (C-5), 22.3 ($NHCOCH_3$); MS (ESI negative) *m/z* 359.3 $[M - H]^-$.

5-Acetamido-2,6-anhydro-4-(piperidin-1-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (7d). The protected glycal **12** (50 mg, 0.10 mmol), dissolved in aqueous methanol (1.0 mL, 1 : 2 v/v) saturated with K_2CO_4, was stirred for 12 h at 23 °C. Then the solution was treated with a acidic resin [DOWEX 50WX8 (H^+)] and stirred for 15 min. The solution was filtered and the solvent was removed under reduced pressure to afford the free glycal **7d** (32 mg, 91%) as a white solid: m. p. 166–168 °C; $[\alpha]_D^{20}$ + 24.3 (*c* 1 in CH_3OH); (Found: Calc. for: C, 53.63; H, 7.28; N, 7.80; $C_{16}H_{26}N_2O_7$ C, 53.62; H, 7.31; N, 7.82%); ^1H NMR (D_2O) δ 5.67 (1H, br s, 3-H), 4.51 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 10.2 Hz, 5-H), 4.23 (1H, d, $J_{6,5}$ = 10.2 Hz, 6-H), 3.89–3.82 (1H, m, 8-H), 3.78 (1H, dd, $J_{9a,9b}$ = 12.0, $J_{9a,8}$ = 1.8 Hz, 9a-H), 3.61–3.45 (3H, overlapping, 4-H, 9b-H and 7-H),

3.43–3.20 (2H, overlapping, N(CH_2CH_2)$_2CH_2$), 3.10–2.74 (2H, overlapping, N(CH_2CH_2)$_2CH_2$), 1.85 (3H, s, CH_3CONH), 1.84–1.27 (6H, overlapping, N(CH_2CH_2)$_2CH_2$); ^{13}C NMR (D_2O) δ 175.7 ($NHCOCH_3$), 168.6 (C-1), 153.9 (C-2), 97.4 (C-3), 76.2 (C-6), 70.3 (C-8), 68.5 (C-7), 65.7 (C-9), 63.7 (C-4), 50.5 (2C, N(CH_2CH_2)$_2CH_2$), 43.0 (C-5), 23.9 (2C, N(CH_2CH_2)$_2CH_2$), 22.8 (1C, N(CH_2CH_2)$_2CH_2$), 21.9 (CH_3CONH); MS (ESI negative) *m/z* 357.1 [M − H]$^-$.

Amination at the C-4 α-position of the *N*-perfluoroacylated neuraminic acid methyl esters 15a–c. General procedure. The appropriate *N*-perfluoracylated derivative[20] (0.40 mmol), dissolved in CH_3CN (4 mL) was reacted with the appropriate cyclic amine (4.0 mmol) under stirring at 60 °C, for the reported time. Then, the solvent was evaporated under reduced pressure, and the residue was purified by rapid chromatography.

Methyl 5-(2,2,2-trifluoroacetamido)-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (16a). Starting with the amido ester **15a**[20] (235 mg, 0.40 mmol) and morpholine (0.35 mL, 4.0 mmol) the amino derivative **16a** was obtained, after 5 h, as a white solid (181 mg, 79%): m.p. 149–151 °C; $[\alpha]_D^{20}$ + 23.6 (*c* 1 in $CHCl_3$); (Found: C, 46.26; H, 5.48; N, 4.96; Calc. for: $C_{22}H_{31}F_3N_2O_{12}$ C, 46.16; H, 5.46; N, 4.89%); ^{1}H NMR ($CDCl_3$) δ 6.78 (1H, d, $J_{NH,5}$ = 10.2 Hz, N–H), 5.34 (1H, dd, $J_{7,8}$ = 4.7, $J_{7,6}$ = 2.4 Hz, 7-H), 5.28 (1H, ddd, $J_{8,9b}$ = 7.3, $J_{8,7}$ = 4.7, $J_{8,9a}$ = 2.4 Hz, 8-H), 4.74 (1H, br s, OH), 4.55 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9a,8}$ = 2.4 Hz, 9a-H), 4.30 (1H, dd, $J_{6,5}$ = 10.3, $J_{6,7}$ = 2.4 Hz, 6-H), 4.06–3.98 (2H, overlapping, 5-H and 9b-H), 3.87 (3H, s, $COOCH_3$), 3.65–3.50 (4H, overlapping, N(CH_2CH_2)$_2$O), 3.00–2.96 (1H, m, 4-H), 2.71–2.65 (2H, overlapping, N(CH_2CH_2)$_2$O), 2.33–2.27 (2H, overlapping, N(CH_2CH_2)$_2$O), 2.17 (3H, s, CH_3COO), 2.09–1.98 (8H, overlapping, 2XCH_3COO, 3a-H and 3b-H); ^{13}C NMR ($CDCl_3$) δ 171.2, 170.8, 169.8 (3C, 3XCH_3COO), 169.4 (C-1), 157.8 (q, $J_{C,F}$ = 37 Hz, $COCF_3$), 122.0–110.0 (1C, CF_3), 95.1 (C-2), 71.4 (C-6 and C-8), 68.7 (C-7), 67.1 (2C, N(CH_2CH_2)$_2$O), 62.6 (C-9), 60.9 (C-4), 53.4 ($COOCH_3$), 48.7 (2C, N(CH_2CH_2)$_2$O), 46.8 (C-5), 29.4 (C-3), 20.8, 20.7, 20.5 (3C, 3XCH_3COO); MS (ESI positive) *m/z* 573.2 [M + H]$^+$, 595.1 [M + Na]$^+$, 1166 [2M + Na]$^+$.

Methyl 5-(2,2,2-trifluoroacetamido)-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (17a). Starting with the amido ester **15a**[20] (235 mg, 0.40 mmol) and piperidine (0.40 mL, 4.0 mmol) the amino derivative **17a** was obtained, after 7 h, as a white solid (171 mg, 75%): m.p. 160–162 °C; $[\alpha]_D^{20}$ + 36.3 (*c* 1 in $CHCl_3$); (Found: C, 48.37; H, 5.89; N, 4.79; Calc. for: $C_{23}H_{33}F_3N_2O_{11}$ C, 48.42; H, 5.83; N, 4.91%); ^{1}H NMR ($CDCl_3$) δ 6.65 (1H, br s, N–H), 5.33 (1H, dd, $J_{7,8}$ = 4.6, $J_{7,6}$ = 2.1 Hz, 7-H), 5.32–5.28 (1H, m, 8-H), 4.66 (1H, br s, OH), 4.55 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9,8}$ = 2.2 Hz, 9a-H), 4.27 (1H, dd, $J_{6,5}$ = 10.2, $J_{6,7}$ = 2.1 Hz, 6-H), 4.09–3.98 (2H, overlapping, 5-H and 9b-H), 3.86 (3H, s, $COOCH_3$), 2.96–2.92 (1H, m, 4-H), 2.63–2.57 (2H, overlapping, N(CH_2CH_2)$_2CH_2$), 2.26–2.19 (2H, overlapping, N(CH_2CH_2)$_2$-CH_2), 2.14 (3H, s, CH_3COO), 2.09 (3H, s, CH_3COO), 2.06–1.95 (5H, overlapping, CH_3COO, 3a-H and 3b-H), 1.55–1.32 (6H, overlapping, N(CH_2CH_2)$_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 171.3, 170.9, 170.0 (3C, 3XCH_3COO), 169.8 (C-1), 157.8 (q, $J_{C,F}$ = 37 Hz, $COCF_3$), 120.0–110.0 (1C, CF_3), 95.4 (C-2), 72.0 (C-6), 71.7 (C-8), 69.0 (C-7), 62.7 (C-9), 61.4 (C-4), 53.4 ($COOCH_3$), 49.8 (2C, N(CH_2CH_2)$_2CH_2$), 47.3 (C-5), 29.6 (C-3), 26.5 (2C, N(CH_2CH_2)$_2CH_2$), 24.7 (1C, N(CH_2CH_2)$_2CH_2$), 20.9, 20.8, 20.7 (3C, 3XCH_3COO); MS (ESI positive) *m/z* 571.2 [M + H]$^+$, 593.1 [M + Na]$^+$, 1162.6 [2M + Na]$^+$.

Methyl 5-(2,2,3,3,3-pentafluoropropionamido)-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (16b). Starting with the amido ester **15b**[20] (255 mg, 0.40 mmol) and morpholine (0.35 mL, 4.0 mmol) the amino derivative **16b** was obtained, after 5 h, as a white solid (204 mg, 82%): m.p. 154–156 °C; $[\alpha]_D^{20}$ + 23.6 (*c* 1 in $CHCl_3$); (Found: C, 44.58; H, 4.82; N, 4.38; Calc. for: $C_{23}H_{31}F_5N_2O_{12}$ C, 44.38; H, 5.02; N, 4.50%); ^{1}H NMR ($CDCl_3$) δ 6.63 (1H, d, $J_{NH,5}$ = 10.0 Hz, N–H), 5.33 (1H, dd, $J_{7,8}$ = 5.5, $J_{7,6}$ = 2.2 Hz, 7-H), 5.25 (1H, ddd, $J_{8,9b}$ = 7.6, $J_{8,7}$ = 5.5, $J_{8,9a}$ = 2.3 Hz, 8-H), 4.67 (1H, br s, OH), 4.50 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 2.3 Hz, 9a-H), 4.27 (1H, dd, $J_{6,5}$ = 10.3, $J_{6,7}$ = 2.2 Hz, 6-H), 4.06–3.97 (2H, overlapping, 5-H and 9b-H), 3.87 (3H, s, $COOCH_3$), 3.64–3.52 (4H, overlapping, N(CH_2CH_2)$_2$O), 3.01 (1H, ddd, $J_{4,3a}$ = $J_{4,5}$ = 11.2, $J_{4,3b}$ = 4.7 Hz, 4-H), 2.71–2.64 (2H, overlapping, N(CH_2CH_2)$_2$O), 2.36–2.30 (2H, overlapping, N(CH_2CH_2)$_2$O), 2.12 (3H, s, CH_3COO), 2.09–1.99 (8H, overlapping, 2XCH_3COO, 3a-H and 3b-H); ^{13}C NMR ($CDCl_3$) δ 171.4, 171.2, 170.0 (3C, 3XCH_3COO), 169.3 (C-1), 158.3 (t, $J_{C,F}$ = 26 Hz; $COCF_2CF_3$), 120.0–108.0 (2C, CF_2CF_3), 95.4 (C-2), 71.6 (2C, C-6 and C-8), 69.1 (C-7), 67.2 (2C, N(CH_2CH_2)$_2$O), 62.8 (C-9), 60.9 (C-4), 53.7 ($COOCH_3$), 49.0 (2C, N(CH_2CH_2)$_2$O), 47.4 (C-5), 29.4 (C-3), 20.9, 20.8, 20.7 (3C, 3XCH_3COO); MS (ESI positive) *m/z* 623.6 [M + H]$^+$, 646.7 [M + Na]$^+$, 1268.1 [2M + Na]$^+$.

Methyl 5-(2,2,3,3,3-pentafluoropropionamido)-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-non-2-ulopyranosidonate (17b). Starting with the amido ester **15b**[20] (255 mg, 0.40 mmol) and piperidine (0.40 mL, 4.0 mmol) the amino derivative **17b** was obtained, after 7 h, as a white solid (176 mg, 71%): m.p. 151–153 °C; $[\alpha]_D^{20}$ + 23.0 (*c* 1 in $CHCl_3$); (Found: C, 46.51; H, 5.26; N, 4.42; Calc. for: $C_{24}H_{33}F_5N_2O_{11}$ C, 46.45; H, 5.36; N, 4.51%); ^{1}H NMR ($CDCl_3$) δ 6.92 (1H, d, $J_{NH,5}$ = 8.2 Hz, N–H), 5.31 (1H, dd, $J_{7,8}$ = 4.3, $J_{7,6}$ = 2.1 Hz, 7-H), 5.29–5.26 (1H, m, 8-H), 4.76 (1H, br s, OH), 4.58 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 2.2 Hz, 9a-H), 4.30 (1H, dd, $J_{6,5}$ = 10.3, $J_{6,7}$ = 2.1 Hz, 6-H), 4.14–4.05 (1H, m, 5-H), 4.00 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.7 Hz, 9b-H), 3.85 (3H, s, $COOCH_3$), 2.94 (1H, ddd, $J_{4,3a}$ = $J_{4,5}$ = 11.0 Hz, $J_{4,3b}$ = 5.0 Hz, 4-H), 2.63–2.53 (2H, overlapping, N(CH_2CH_2)$_2CH_2$), 2.23–2.16 (2H, overlapping, N(CH_2CH_2)$_2CH_2$), 2.13 (3H, s, CH_3COO), 2.07 (3H, s, CH_3COO), 2.03–1.86 (5H, overlapping, CH_3COO, 3a-H and 3b-H), 1.52–1.31 (6H, overlapping, N(CH_2CH_2)$_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 171.7, 170.9, 169.9 (3C, 3XCH_3COO), 169.8 (C-1), 158.3 (t, $J_{C,F}$ = 27 Hz; $COCF_2CF_3$), 120.0–110.0 (2C, CF_2CF_3), 95.4 (C-2), 72.3 (C-6), 72.0 (C-8), 69.2 (C-7), 62.7 (C-9), 61.1 (C-4), 53.3 ($COOCH_3$), 49.8 (2C, N(CH_2CH_2)$_2CH_2$), 47.0 (C-5), 29.3 (C-3), 26.2 (2C, N(CH_2CH_2)$_2CH_2$), 24.7 (1C, N(CH_2CH_2)$_2CH_2$), 20.8, 20.7, 20.6 (3C, 3XCH_3COO); MS (ESI

positive) *m/z* 621.2 $[M + H]^+$, 643.7 $[M + Na]^+$, 1264.1 $[2M + Na]^+$.

Methyl 5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-β-D-*glycero*-D-*galacto*-non-2-ulopyranosidonate (16c). Starting with the amido ester **15c**[20] (275 mg, 0.40 mmol) and morpholine (0.35 mL, 4.0 mmol) the amino derivative **16c** was obtained, after 5 h, as a white solid (215 mg, 80%): m.p. 148–150 °C; $[\alpha]_D^{20}$ + 36.5 (*c* 1 in $CHCl_3$); (Found: C, 42.38; H, 4.76; N, 4.28; Calc. for: $C_{24}H_{31}F_7N_2O_{12}$ C, 42.86; H, 4.65; N, 4.17%); ^{1}H NMR ($CDCl_3$) δ 6.87 (1H, d, $J_{NH,5}$ = 9.8 Hz, N–H), 5.31 (1H, dd, $J_{7,8}$ = 4.8, $J_{7,6}$ = 2.3 Hz, 7-H), 5.26 (1H, ddd, $J_{8,9b}$ = 7.3, $J_{8,7}$ = 4.8, $J_{8,9a}$ = 2.3 Hz, 8-H), 4.80 (1H, br s, OH), 4.57 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9a,8}$ = 2.3 Hz, 9a-H), 4.31 (1H, dd, $J_{6,5}$ = 10.3, $J_{6,7}$ = 2.3 Hz, 6-H), 4.06–4.02 (1H, m, 5-H), 3.99 (1H, dd, $J_{9b,9a}$ = 12.4, $J_{9b,8}$ = 7.3 Hz, 9b-H), 3.87 (3H, s, $COOCH_3$), 3.63–3.51 (4H, overlapping, N(CH_2CH_2)$_2$O), 3.00 (1H, ddd, $J_{4,3a} = J_{4-5}$ = 11.0, $J_{4,3b}$ = 5.3 Hz, 4-H), 2.71–2.65 (2H, overlapping, N(CH_2CH_2)$_2$O), 2.32–2.27 (2H, overlapping, N(CH_2CH_2)$_2$O), 2.13 (3H, s, CH_3COO), 2.09–1.99 (8H, overlapping, 2XCH_3COO, 3a-H and 3b-H); ^{13}C NMR ($CDCl_3$) δ 171.2, 171.0, 169.8 (3C, 3XCH_3COO), 169.7 (C-1), 158.2 (t, $J_{C,F}$ = 26 Hz; $COCF_2CF_2CF_3$), 121.0–108.0 (3C, $CF_2CF_2CF_3$), 95.3 (C-2), 71.4 (2C, C-6 and C-8), 69.0 (C-7), 67.0 (2C, N(CH_2CH_2)$_2$O), 62.6 (C-9), 60.7 (C-4), 53.5 ($COOCH_3$), 48.8 (2C, N(CH_2CH_2)$_2$O), 47.2 (C-5), 29.2 (C-3), 20.7, 20.6, 20.5 (3C, 3XCH_3COO); MS (ESI positive) *m/z* 673.1 $[M + H]^+$, 695.0 $[M + Na]^+$.

Methyl 5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-β-D-*glycero*-D-*galacto*-non-2-ulopyranosidonate. (17c). Starting with the amido ester **15c**[20] (255 mg, 0.40 mmol) and piperidine (0.40 mL, 4.0 mmol) the amino ester **17c** was obtained, after 7 h, as a white solid (190 mg, 71%): m.p. 140–142 °C; $[\alpha]_D^{20}$ + 31.2 (*c* 1 in $CHCl_3$); (Found: C, 44.34; H, 4.81; N, 4.24; Calc. for: $C_{25}H_{33}F_7N_2O_{11}$ C, 44.78; H, 4.96; N 4.18%); ^{1}H NMR ($CDCl_3$) δ 7.12 (1H, br s, N–H), 5.32–5.26 (2H, overlapping, 7-H and 8-H), 4.95 (1H, br s, OH), 4.63 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9,8}$ = 2.4 Hz, 9a-H), 4.32 (1H, dd, $J_{6,5}$ = 10.3, $J_{6,7}$ = 2.2 Hz, 6-H), 4.18–4.04 (1H, m, 5-H), 3.99 (1H, dd, $J_{9b,9a}$ = 12.4, $J_{9b,8}$ = 7.4 Hz, 9b-H), 3.84 (3H, s, $COOCH_3$), 2.97–2.90 (1H, m, 4-H), 2.62–2.54 (2H, overlapping, N(CH_2CH_2)$_2CH_2$), 2.22–2.15 (2H, overlapping, N(CH_2CH_2)$_2CH_2$), 2.13 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.03–1.96 (5H, overlapping, CH_3COO, 3a-H and 3b-H), 1.50–1.30 (6H, overlapping, N(CH_2CH_2)$_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 171.7, 170.0, 169.9 (3C, 3XCH_3COO), 169.8 (C-1), 158.3 (t, $J_{C,F}$ = 27 Hz; $COCF_2CF_2CF_3$), 120.0–105.0 (3C, $CF_2CF_2CF_3$), 95.4 (C-2), 72.3 (C-6), 72.0 (C-8), 69.3 (C-7), 62.6 (C-9), 60.9 (C-4), 53.3 ($COOCH_3$), 49.7 (2C, N(CH_2CH_2)$_2CH_2$), 47.2 (C-5), 29.3 (C-3), 26.2 (2C, N(CH_2CH_2)$_2$-CH_2), 24.7 (1C, N(CH_2CH_2)$_2CH_2$), 20.7 (CH_3COO), 20.6 (2C, 2XCH_3COO); MS (ESI positive) *m/z* 671.2 $[M + H]^+$, 693.1 $[M + Na]^+$, 1362.6 $[2M + Na]^+$.

Dehydration of 4α-aminated 2-hydroxy peracetylated sialic acid esters 16a–c and 17a–c. General procedure. The 2-hydroxy peracetylated sialic acid amino ester (0.20 mmol), dissolved in CH_3CN (0.60 mL), was reacted with the TFAA (84 μL, 0.6 mmol) at 23 °C for 10 min. Then, the solvent and the excess TFAA were evaporated under a nitrogen flow and the crude was dissolved in CH_3CN (0.60 mL) and treated with TFA (46 μL, 0.6 mmol) at 135 °C for 5 min, in a sealed tube. The reaction mixture was cooled, diluted with methanol (0.20 mL) and evaporated under reduced pressure. The obtained crude residue was purified by rapid chromatography, to afford the appropriate glycal.

Methyl 2,6-anhydro-5-(2,2,2-trifluoroacetamido)-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate. (13a). Starting from the 2-hydroxy compound **16a** (114 mg, 0.20 mmol) the glycal **13a** (88 mg, 79%) was obtained, as a white solid: m.p. 138–140 °C; $[\alpha]_D^{20}$ + 84.9 (*c* 1 in $CHCl_3$); (Found: C, 47.99; H, 5.46; N, 5.11; Calc. for: $C_{22}H_{29}F_3N_2O_{11}$ C, 47.66; H, 5.27; N, 5.05%); ^{1}H NMR ($CDCl_3$) δ 7.09 (1H, d, $J_{NH,5}$ = 9.6 Hz, N–H), 6.10 (1H, d, $J_{3,4}$ = 2.6 Hz, 3-H), 5.50 (1H, dd, $J_{7,8}$ = 4.3, $J_{7,6}$ = 2.7 Hz, 7-H), 5.30–5.25 (1H, m, 8-H), 4.77 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9a,8}$ = 3.0 Hz, 9a-H), 4.34 (1H, dd, $J_{6,5}$ = 9.0, $J_{6,7}$ = 2.7 Hz, 6-H), 4.30–4.24 (1H, m, 5-H), 4.11 (1H, dd, $J_{9b,9a}$ = 12.4, $J_{9b,8}$ = 7.6 Hz, 9b-H), 3.78 (3H, s, $COOCH_3$), 3.65–3.53 (4H, overlapping, N(CH_2CH_2)$_2$O), 3.36 (1H, dd, $J_{4,5}$ = 9.1, $J_{4,3}$ = 2.6 Hz, 4-H), 2.74–2.67 (2H, overlapping, N(CH_2CH_2)$_2$O), 2.45–2.43 (2H, overlapping, N(CH_2CH_2)$_2$O), 2.09 (3H, s, CH_3COO), 2.02 (6H, overlapping, 2XCH_3COO); ^{13}C NMR ($CDCl_3$) δ 171.0, 170.5, 169.9 (3C, 3XCH_3COO), 161.5 (C-1), 157.6 (q, $J_{C,F}$ = 37 Hz, $COCF_3$), 145.0 (C-2), 122.0–110.0 (1C, CF_3), 108.4 (C-3), 76.6 (C-6), 71.3 (C-8), 68.1 (C-7), 67.1 (2C, N(CH_2CH_2)$_2$O), 62.6 (C-4), 62.0 (C-9), 52.4 ($COOCH_3$), 49.3 (2C, N(CH_2CH_2)$_2$O), 44.2 (C-5), 22.8 ($NHCOCH_3$), 20.7, 20.6, 20.5 (3C, 3XCH_3COO); MS (ESI positive) *m/z* 555.8 $[M + H]^+$, 577.1 $[M + Na]^+$, 1131.5 $[2M + Na]^+$.

Methyl 2,6-anhydro-5-(2,2,2-trifluoroacetamido)-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (14a). Starting from the 2-hydroxy compound **17a** (114 mg, 0.20 mmol) the glycal **14a** (84 mg, 77%) was obtained, as a white solid: m.p. 123–125 °C; $[\alpha]_D^{20}$ + 89.5 (*c* 1 in $CHCl_3$); (Found: C, 50.26; H, 5.67; N, 4.98; Calc. for: $C_{23}H_{31}F_3N_2O_{10}$ C, 50.00; H, 5.66; N, 5.07%); ^{1}H NMR ($CDCl_3$) δ 6.96 (1H, br d, $J_{NH,5}$ = 8.7 Hz, N–H), 6.10 (1H, d, $J_{3,4}$ = 2.6 Hz, 3-H), 5.48 (1H, dd, $J_{7,8}$ = 4.1, $J_{7,6}$ = 2.6 Hz, 7-H), 5.28 (1H, ddd, $J_{8,9b}$ = 7.3, $J_{8,7}$ = 4.1, $J_{8,9a}$ = 3.1 Hz, 8-H), 4.74 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 3.1 Hz, 9a-H), 4.34–4.24 (2H, overlapping, 6-H and 5-H), 4.13 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.3 Hz, 9b-H), 3.78 (3H, s, $COOCH_3$), 3.36 (1H, dd, $J_{4,5}$ = 8.6, $J_{4,3}$ = 2.2 Hz, 4-H), 2.66–2.58 (2H, overlapping, N(CH_2CH_2)$_2CH_2$), 2.41–2.34 (2H, overlapping, N(CH_2CH_2)$_2CH_2$), 2.10 (3H, s, CH_3COO), 2.03 (6H, overlapping, 2XCH_3COO), 1.54–1.34 (6H, overlapping, N(CH_2CH_2)$_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 170.9, 170.5, 170.0 (3C, 3XCH_3COO), 161.7 (C-1), 157.7 (q, $J_{C,F}$ = 37 Hz, $COCF_3$), 144.5 (C-2), 120.0–110.0 (1C, CF_3), 110.1 (C-3), 76.7 (C-6), 71.4 (C-8), 68.1 (C-7), 63.1 (C-4), 62.1 (C-9), 52.3 ($COOCH_3$), 50.3 (2C, N(CH_2CH_2)$_2CH_2$), 44.6 (C-5), 26.3 (2C, N(CH_2CH_2)$_2CH_2$), 22.8 (1C, N(CH_2CH_2)$_2CH_2$), 20.7 (2C, 2XCH_3COO), 20.5 (CH_3COO); MS (ESI positive) *m/z* 553.3 $[M + H]^+$, 575.1 $[M + Na]^+$, 1129.4 $[2M + Na]^+$.

Methyl 2,6-anhydro-5-(2,2,3,3,3-pentafluoropropionamido)-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (13b). Starting from the 2-hydroxy

compound **16b** (124 mg, 0.20 mmol) the glycal **13b** (97 mg, 80%) was obtained, as a white solid: m.p. 128–130 °C; $[\alpha]_D^{20}$ + 78.2 (*c* 1 in $CHCl_3$); (Found: C, 45.98; H, 4.68; N, 4.69; Calc. for: $C_{23}H_{29}F_5N_2O_{11}$ C, 45.70; H, 4.84; N, 4.63%); ^{1}H NMR ($CDCl_3$) δ 6.95 (1H, d, $J_{NH,5}$ = 9.7 Hz, N–H), 6.13 (1H, d, $J_{3,4}$ = 2.5 Hz, 3-H), 5.48 (1H, dd, $J_{7,8}$ = 4.2, $J_{7,6}$ = 2.7 Hz, 7-H), 5.29 (1H, ddd, $J_{8,9b}$ = 7.3, $J_{8,7}$ = 4.2, $J_{8,9a}$ = 3.0 Hz, 8-H), 4.75 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 3.0 Hz, 9a-H), 4.37 (1H, dd, $J_{6,5}$ = 9.6, $J_{6,7}$ = 2.7 Hz, 6-H), 4.32–4.25 (1H, m, 5-H), 4.14 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.3 Hz, 9b-H), 3.80 (3H, s, $COOCH_3$), 3.67–3.54 (4H, overlapping, $N(CH_2CH_2)_2O$), 3.37 (1H, dd, $J_{4,5}$ = 9.3, $J_{4,3}$ = 2.5 Hz, 4-H), 2.75–2.68 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.47–2.41 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.10 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO); ^{13}C NMR ($CDCl_3$) δ 170.9, 170.5, 169.9 (3C, $3XCH_3COO$), 161.5 (C-1), 158.3 (t, J_{C-F} = 26 Hz, $COCF_2CF_3$), 145.0 (C-2), 120.0–108.0 (2C, CF_2CF_3), 108.2 (C-3), 76.4 (C-6), 71.2 (C-8), 68.1 (C-7), 67.0 (2C, $N(CH_2CH_2)_2O$), 62.8 (C-4), 62.0 (C-9), 52.5 ($COOCH_3$), 49.2 (2C, $N(CH_2CH_2)_2O$), 44.4 (C-5), 20.8, 20.6, 20.5 (3C, $3XCH_3COO$); MS (ESI positive) *m*/*z* 605.2 $[M + H]^+$, 627.1 $[M + Na]^+$.

Methyl 2,6-anhydro-5-(2,2,3,3,3-pentafluoropropionamido)-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (14b). Starting from the 2-hydroxy compound **17b** (124 mg, 0.20 mmol) the glycal **14b** (98 mg, 81%) was obtained, as a white solid: m.p. 140–143 °C; $[\alpha]_D^{20}$ + 59.7 (*c* 1 in $CHCl_3$); (Found: C, 47.92; H, 5.12; N, 4.56; Calc. for: $C_{24}H_{31}F_5N_2O_{10}$ C, 47.84; H, 5.19; N, 4.65%); ^{1}H NMR ($CDCl_3$) δ 6.88 (1H, br s, N–H), 6.12 (1H, d, $J_{3,4}$ = 2.5 Hz, 3-H), 5.46 (1H, dd, $J_{7,8}$ = 4.4, $J_{7,6}$ = 2.5 Hz, 7-H), 5.29 (1H, ddd, $J_{8,9b}$ = 7.2, $J_{8,7}$ = 4.4, $J_{8,9a}$ = 2.8 Hz, 8-H), 4.73 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 2.8 Hz, 9a-H), 4.35 (1H, dd, $J_{6,5}$ = 9.6, $J_{6,7}$ = 2.5 Hz, 6-H), 4.32–4.26 (1H, m, 5-H), 4.14 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.2 Hz, 9b-H), 3.79 (3H, s, $COOCH_3$), 3.37 (1H, dd, $J_{4,5}$ = 8.6, $J_{4,3}$ = 2.5 Hz, 4-H), 2.67–2.59 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.40–2.32 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.11 (3H, s, CH_3COO), 2.04 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO), 1.55–1.35 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.4, 170.0 (3C, $3XCH_3COO$), 161.7 (C-1), 158.2, (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_3$), 144.6 (C-2), 120.0–110.0 (2C, CF_2CF_3), 109.7 (C-3), 76.6 (C-6), 71.2 (C-8), 68.1 (C-7), 63.2 (C-4), 62.0 (C-9), 52.4 ($COOCH_3$), 50.3 (2C, $N(CH_2CH_2)_2CH_2$), 44.9 (C-5), 26.2 (2C, $N(CH_2CH_2)_2CH_2$), 24.4 (1C, $N(CH_2CH_2)_2CH_2$), 20.8, 20.6, 20.5 (3C, $3XCH_3COO$); MS (ESI positive) *m*/*z* 603.2 $[M + H]^+$, 625.3 $[M + Na]^+$.

Methyl 2,6-anhydro-5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4-(morpholin-4-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (13c). Starting from the 2-hydroxy compound **16c** (135 mg, 0.20 mmol) the glycal **13c** (105 mg, 81%) was obtained, as a white solid: m.p. 127–129 °C; $[\alpha]_D^{20}$ + 77.7 (*c* 1 in $CHCl_3$); (Found: C, 44.15; H, 4.53; N, 4.20; Calc. for: $C_{24}H_{29}F_7N_2O_{11}$ C, 44.04; H, 4.47; N, 4.28%); ^{1}H NMR ($CDCl_3$) δ 6.97 (1H, d, $J_{NH,5}$ = 9.5 Hz, N–H), 6.13 (1H, d, $J_{3,4}$ = 2.4 Hz, 3-H), 5.48 (1H, dd, $J_{7,8}$ = 4.3, $J_{7,6}$ = 2.8 Hz, 7-H), 5.32–5.27 (1H, m, 8-H), 4.73 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 3.0 Hz, 9a-H), 4.39 (1H, dd, $J_{6,5}$ = 9.5, $J_{6,7}$ = 2.8 Hz, 6-H), 4.31–4.22 (1H, m, 5-H), 4.15 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.2 Hz, 9b-H), 3.80 (3H, s, $COOCH_3$), 3.67–3.52 (4H, overlapping, $N(CH_2CH_2)_2O$), 3.40 (1H, dd, $J_{4,5}$ = 9.2, $J_{4,3}$ = 2.4 Hz, 4-H), 2.75–2.67 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.48–2.40 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.11 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.4, 170.0 (3C, $3XCH_3COO$), 161.5 (C-1), 158.1 (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_2CF_3$), 145.0 (C-2), 126.0–108.0 (3C, $CF_2CF_2CF_3$), 108.2 (C-3), 76.3 (C-6), 71.1 (C-8), 68.0 (C-7), 66.9 (2C, $N(CH_2CH_2)_2O$), 62.6 (C-4), 61.6 (C-9), 52.5 ($COOCH_3$), 49.2 (2C, $N(CH_2CH_2)_2O$), 44.6 (C-5), 20.8, 20.6, 20.5 (3C, $3XCH_3COO$); MS (ESI positive) *m*/*z* 654.9 $[M + H]^+$, 677.1 $[M + Na]^+$, 1330.5 $[2M + Na]^+$.

Methyl 2,6-anhydro-5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4-(piperidin-1-yl)-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (14c). Starting from the 2-hydroxy compound **17c** (134 mg, 0.20 mmol) the glycal **14c** (103 mg, 79%) was obtained, as a white solid: m.p. 137–139 °C; $[\alpha]_D^{20}$ + 93.0 (*c* 1 in $CHCl_3$); (Found: C, 45.94; H, 4.68; N, 4.33; Calc. for: $C_{25}H_{31}F_7N_2O_{10}$ C, 46.02; H, 4.79; N, 4.29%); ^{1}H NMR ($CDCl_3$) δ 6.90 (1H, br s, N–H), 6.12 (1H, d, $J_{3,4}$ = 2.6 Hz, 3-H), 5.45 (1H, dd, $J_{7,8}$ = 4.3, $J_{7,6}$ = 2.6 Hz, 7-H), 5.30–5.26 (1H, m, 8-H), 4.73 (1H, dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 2.9 Hz, 9a-H), 4.37 (1H, dd, $J_{6,5}$ = 9.0, $J_{6,7}$ = 2.6 Hz, 6-H), 4.28–4.21 (1H, m, 5-H), 4.14 (1H, dd, $J_{9b,9a}$ = 12.3, $J_{9b,8}$ = 7.2 Hz, 9b-H), 3.77 (3H, s, $COOCH_3$), 3.39 (1H, dd, $J_{4,5}$ = 9.1, $J_{4,3}$ = 2.6 Hz, 4-H), 2.68–2.57 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.39–2.31 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.10 (3H, s, CH_3COO), 2.03 (3H, s, CH_3COO), 2.02 (3H, s, CH_3COO), 1.53–1.34 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.2, 169.8 (3C, $3XCH_3COO$), 161.5 (C-1), 158.0, (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_2CF_3$), 144.4 (C-2), 120.0–110.0 (2C, $CF_2CF_2CF_3$), 109.5 (C-3), 76.4 (C-6), 71.1 (C-8), 67.9 (C-7), 63.0 (C-4), 61.8 (C-9), 52.1 ($COOCH_3$), 50.2 (2C, $N(CH_2CH_2)_2CH_2$), 44.7 (C-5), 26.0 (2C, $N(CH_2CH_2)_2CH_2$), 24.2 (1C, $N(CH_2CH_2)_2CH_2$), 20.6, 20.4, 20.3 (3C, $3XCH_3COO$); MS (ESI positive) *m*/*z* 653.1 $[M + H]^+$, 675.2 $[M + Na]^+$, 1326.6 $[2M + Na]^+$.

Selective hydrolysis of 4α-aminated *N*-perfluoracyl glycals 13a–c and 14a–c. General procedure. The appropriate protected glycal (0.1 mmol) dissolved in methanol–water (1.5 mL, 2 : 1 v/v) was treated with Et_3N (0.90 mL) under stirring for 12 h at 23 °C. Then the solvent was removed under reduced pressure and the residue was recovered with water and lyophilized many times until complete elimination of Et_3N.

2,6-Anhydro-5-(2,2,2-trifluoroacetamido)-4-(morpholin-4-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (6a). Starting from the protected glycal **13a** (55 mg, 0.10 mmol) the free glycal **6a** (39 mg, 93%) was obtained, as a white solid: m.p. 187–189 °C; $[\alpha]_D^{20}$ + 28.6 (*c* 1 in CH_3OH); (Found: C, 43.54; H, 5.06; N, 6.68; Calc. for: $C_{15}H_{21}F_3N_2O_8$ C, 43.48; H, 5.11; N, 6.76%); ^{1}H NMR (D_2O) δ 5.84 (1H, d, $J_{3,4}$ = 2.2 Hz, 3-H), 4.50 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 10.0 Hz, 5-H), 4.33 (1H, d, $J_{6,5}$ = 10.0 Hz, 6-H), 3.96 (1H, ddd, $J_{8,7}$ = 9.3, $J_{8,9b}$ = 6.6, $J_{8,9a}$ = 2.6 Hz, 8-H), 3.89 (1H, dd, $J_{9a,9b}$ = 12.0, $J_{9a,8}$ = 2.6 Hz, 9a-H), 3.77–3.59 (6H, overlapping, $N(CH_2CH_2)_2$, 4-H and 9b-H), 3.56 (1H, d app, $J_{7,8}$ = 9.3 Hz, 7-H), 2.83–2.75 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.63–2.56 (2H, overlapping, $N(CH_2CH_2)_2O$); ^{13}C

NMR (D_2O) δ 169.8 (C-1), 159.3 (q, $J_{C,F}$ = 37 Hz, $COCF_2$), 150.5 (C-2), 121.0–110.0 (1C, CF_3), 104.3 (C-3), 75.9 (C-6), 70.6 (C-8), 69.3 (C-7), 67.2 (2C, $N(CH_2CH_2)_2O$), 63.8 (C-9), 62.1 (C-4), 49.0 (2C, $N(CH_2CH_2)_2O$), 44.7 (C-5); MS (ESI negative) *m/z* 413.3 $[M - H]^-$.

2,6-anhydro-5-(2,2,2-trifluoroacetamido)-4-(piperidin-1-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (7a). Starting from compound **14a** (55 mg, 0.10 mmol) the free glycal **7a** was obtained (35 mg, 85%), as slightly yellow solid: m. p. 155–157 °C; $[\alpha]_D^{20}$ + 31.2 (*c* 1 in CH_3OH); (Found: C, 46.56; H, 5.58; N, 6.79; Calc. for: $C_{16}H_{23}F_3N_2O_7$ C, 46.60; H, 5.62; N, 6.79%); ^1H NMR (D_2O) δ 5.99 (1H, d, $J_{3,4}$ = 2.7 Hz, 3-H), 4.60 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 9.3 Hz, 5-H), 4.41 (1H, d app., $J_{6,5}$ = 9.3 Hz, 6-H), 3.91 (1H, ddd, $J_{8,7}$ = 9.0, $J_{8,9b}$ = 6.1, $J_{8,9a}$ = 2.2 Hz, 8-H), 3.87 (1H, dd, $J_{9a,9b}$ = 12.0, $J_{9a,8}$ = 2.2 Hz, 9a-H), 3.78 (1H, br s, 4-H), 3.66 (1H, dd, $J_{9b,9a}$ = 12.0, $J_{9b,8}$ = 6.1 Hz, 9b-H), 3.62 (1H, d, $J_{7,8}$ = 9.0 Hz, 7-H), 2.82–2.73 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 2.69–2.59 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 1.64–1.45 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR (D_2O) δ 169.3 (C-1), 160.2 (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_3$), 152.2 (C-2), 119.5–107.4 (2C, CF_2CF_3), 107.3 (C-3), 76.0 (C-6), 70.5 (C-8), 69.0 (C-7), 63.7 (C-9), 59.7 (C-4), 50.6 (2C, $N(CH_2CH_2)_2CH_2$), 44.0 (C-5), 24.7 (2C, $N(CH_2CH_2)_2CH_2$), 23.1 (1C, $N(CH_2CH_2)_2CH_2$); MS (ESI negative) *m/z* 411.3 $[M - H]^-$.

2,6-anhydro-5-(2,2,3,3,3-pentafluoropropionamido)-4-(morpholin-4-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (6b). Starting from the protected glycal **13b** (60 mg, 0.10 mmol) the free glycal **6b** (42 mg, 91%) was obtained, as a white solid: m. p. 144–146 °C; $[\alpha]_D^{20}$ + 26.4 (*c* 1 in CH_3OH); (Found: C, 41.25; H, 4.50; N, 6.00; Calc. for: $C_{16}H_{21}F_5N_2O_8$ C, 41.39; H, 4.56; N, 6.03%); ^1H NMR (D_2O) δ 5.84 (1H, d, $J_{3,4}$ = 2.2 Hz, 3-H), 4.75 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 9.6 Hz, 5-H), 4.44 (1H, d, $J_{6,5}$ = 9.6 Hz, 6-H), 4.40 (1H, dd, $J_{4,5}$ = 9.6, $J_{4,3}$ = 2.4 Hz, 4-H), 4.04–3.96 (2H, overlapping, $N(CH_2CH_2)_2O$), 3.96–3.88 (3H, overlapping, $N(CH_2CH_2)_2O$ and 8-H), 3.84 (1H, dd, $J_{9a,9b}$ = 11.9, $J_{9a,8}$ = 2.5 Hz, 9a-H), 3.58 (1H, dd, $J_{9b,9a}$ = 11.9, $J_{9b,8}$ = 6.3 Hz 9b-H), 3.56–3.48 (3H, overlapping, $N(CH_2CH_2)_2O$ and 7-H), 3.29–3.21 (2H, overlapping, $N(CH_2CH_2)_2O$); ^{13}C NMR (D_2O) δ 167.5 (C-1), 160.0 (t, $J_{C,F}$ = 26 Hz, $COCF_2$), 153.3 (C-2), 119.5–107.4 (2C, CF_2CF_3), 96.6 (C-3), 75.4 (C-6), 69.7 (C-8), 68.2 (C-7), 63.9 (3C, $N(CH_2CH_2)_2O$ and C-9), 63.0 (C-4), 48.7 (2C, $N(CH_2CH_2)_2O$), 43.2 (C-5); MS (ESI negative) *m/z* 463.2 $[M - H]^-$.

2,6-anhydro-5-(2,2,3,3,3-pentafluoropropionamido)-4-(piperidin-1-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (7b). Starting from the protected glycal **14b** (60 mg, 0.10 mmol) the free glycal **7b** (39 mg, 84%) was obtained, as slightly yellow solid: m.p. 155–157 °C; $[\alpha]_D^{20}$ + 22.3 (*c* 1 in CH_3OH); (Found: C, 44.09; H, 4.96; N, 6.03; Calc. for: $C_{17}H_{23}F_5N_2O_7$ C, 44.16; H, 5.01; N, 6.06%); ^1H NMR (D_2O) δ 5.80 (1H, d, $J_{3,4}$ = 2.5 Hz, 3-H), 4.73 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 10.0 Hz, 5-H), 4.40 (1H, d app, $J_{6,5}$ = 10.0 Hz, 6-H), 4.22 (1H, br s, 4-H), 3.96 (1H, ddd, $J_{8,7}$ = 9.3, J_{8-9b} = 6.3, $J_{8,9a}$ = 2.4 Hz, 8-H), 3.89 (1H, dd, $J_{9a,9b}$ = 11.9, $J_{9a,8}$ = 2.4 Hz, 9a-H), 3.62 (1H, dd, $J_{9b,9a}$ = 11.9, $J_{9b,8}$ = 6.3 Hz, 9b-H), 3.54 (1H, d app., $J_{7,8}$ = 9.3 Hz, 7-H), 3.33–3.19 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 3.09–2.96 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 1.81–1.55 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR (D_2O) δ 168.3 (C-1), 159.8 (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_3$), 152.4 (C-2), 119.5–107.4 (2C, CF_2CF_3), 99.3 (C-3), 75.4 (C-6), 69.8 (C-8), 68.3 (C-7), 63.3 (C-9), 63.1 (C-4), 50.2 (2C, $N(CH_2CH_2)_2CH_2$), 43.3 (C-5), 23.7 (2C, $N(CH_2CH_2)_2CH_2$), 22.0 (1C, $N(CH_2CH_2)_2CH_2$); MS (ESI negative) *m/z* 461.1 $[M - H]^-$.

2,6-anhydro-5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4-(morpholin-4-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (6c). Starting from the protected glycal **13c** (65 mg, 0.10 mmol) the free glycal **6c** (48 mg, 94%) was obtained, as a white solid: m. p. 148–151 °C; $[\alpha]_D^{20}$ + 25.7 (*c* 1 in CH_3OH); (Found: C, 39.65; H, 4.09; N, 5.50; Calc. for: $C_{17}H_{21}F_7N_2O_8$ C, 39.70; H, 4.12; N, 5.45%); ^1H NMR (D_2O) δ 5.84 (1H, d, $J_{3,4}$ = 2.4 Hz, 3-H), 4.52 (1H, t app., $J_{5,4}$ = $J_{5,6}$ = 9.9 Hz, 5-H), 4.34 (1H, d, $J_{6,5}$ = 9.9 Hz, 6-H), 3.95 (1H, ddd, $J_{8,7}$ = 9.4, $J_{8,9b}$ = 6.6, $J_{8,9a}$ = 2.5 Hz, 8-H), 3.89 (1H, dd, $J_{9a,9b}$ = 11.9, $J_{9a,8}$ = 2.5 Hz, 9a-H), 3.79–3.68 (5H, overlapping, $N(CH_2CH_2)_2O$ and 4-H), 3.61 (1H, dd, $J_{9b,9a}$ = 11.9, $J_{9b,8}$ = 6.6 Hz, 9b-H), 3.56 (1H, d, $J_{7,8}$ = 9.4 Hz, 7-H), 2.88–2.81 (2H, overlapping, $N(CH_2CH_2)_2O$), 2.69–2.62 (2H, overlapping, $N(CH_2CH_2)_2O$); ^{13}C NMR (D_2O) δ 169.8 (C-1), 159.9 (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_2CF_2$), 150.6 (C-2), 120.0–111.5 (3C, $CF_2CF_2CF_3$), 104.0 (C-3), 75.8 (C-6), 70.6 (C-8), 69.3 (C-7), 67.2 (2C, $N(CH_2CH_2)_2O$), 63.8 (C-9), 62.4 (C-4), 49.0 (2C, $N(CH_2CH_2)_2O$), 45.0 (C-5); MS (ESI negative) *m/z* 513.2 $[M - H]^-$.

2,6-anhydro-5-(2,2,3,3,4,4,4-heptafluorobutanamido)-4-(piperidin-1-yl)-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (7c). Starting from the protected glycal **14c** (65 mg, 0.10 mmol) the free glycal **7c** (44 mg, 85%) was obtained, as a white solid: m.p. 188–190 °C; $[\alpha]_D^{20}$ + 24.9 (*c* 1 in CH_3OH); (Found: C, 42.10; H, 4.50; N, 5.43; Calc. for: $C_{18}H_{23}F_7N_2O_7$ C, 42.19; H, 4.52; N, 5.47%); ^1H NMR (D_2O) δ 5.80 (1H, d, $J_{3,4}$ = 1.6 Hz, 3-H), 4.74 (1H, under water signal, 5-H), 4.40 (1H, d app, $J_{6,5}$ = 10.0 Hz, 6-H), 4.22 (1H, br s, 4-H), 3.96 (1H, m, 8-H), 3.88 (1H, dd, $J_{9a,9b}$ = 11.9, $J_{9a,8}$ = 1.8 Hz, 9a-H), 3.62 (1H, dd, $J_{9b,9a}$ = 11.9, $J_{9b,8}$ = 6.4 Hz, 9b-H), 3.55 (1H, d app., $J_{7,8}$ = 9.2 Hz, 7-H), 3.30–3.23 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 3.23–3.15 (2H, overlapping, $N(CH_2CH_2)_2CH_2$), 1.93–1.47 (6H, overlapping, $N(CH_2CH_2)_2CH_2$); ^{13}C NMR (D_2O) δ 168.3 (C-1), 159.8 (t, $J_{C,F}$ = 26 Hz, $COCF_2CF_2CF_3$), 152.4 (C-2), 119.5–107.4 (3C, $CF_2CF_2CF_3$), 99.3 (C-3), 75.4 (C-6), 69.8 (C-8), 68.3 (C-7), 63.3 (C-9), 63.1 (C-4), 50.2 (2C, $N(CH_2CH_2)_2CH_2$), 43.3 (C-5), 23.7 (2C, $N(CH_2CH_2)_2CH_2$), 22.0 (1C, $N(CH_2CH_2)_2CH_2$); MS (ESI negative) *m/z* 511.2 $[M - H]^-$.

Biological assays

Sialidase enzyme assay. *Vibrio cholerae* sialidase inhibition activity was performed essentially according to Venerando *et al.*,[36,37] but using the fluorescent artificial substrate 4-methylumbelliferyl *N*-acetylneuraminic acid (4-MU-Neu5Ac).

Briefly, the incubation mixture (final volume of 100 µL) contained 0.1–0.5 mU of *Vibrio cholerae* sialidase, various amounts of 4-MU-Neu5Ac, 80 µg of bovine serum albumin (BSA), 2.5 µmol of Tris/HCl buffer (pH 6.9). After incubation at 37 °C for 5–10 min, the reactions were stopped by the addition of

1.5 mL of 0.2 M glycine buffered with NaOH at pH 10.2, and sialidase activity was determined by spectrofluorometric measurement of the 4-methylumbelliferone released (λ excitation 365 nm, λ emission 445 nm). One unit of sialidase was defined as the amount releasing 1 μmol of *N*-acetylneuraminic acid min^{-1} at 37 °C. K_m and apparent V_{max} values were determined by the method of Lineweaver and Burk using five different 4-MU-Neu5Ac concentrations. To measure the inhibitory constants K_i, typical Vo/[S] experiments were carried out in the presence of three different inhibitor concentrations. To obtain a value for K_i, the kinetic data were fitted to the standard equation for competitive inhibition.

The results are the mean of at least two experiments carried out in triplicate.

***In vitro* screening of glycals 6a–d and 7a–d for their potential antiviral activities.** The test was that routinely used in the Department of one of the authors (R. M.). Briefly, 50 μL of serial 2-fold dilutions of each glycal were incubated overnight with 100 μL of MDCK cells giving a final cell count of 30 000 cells per well in a 96-well microtitre plate (Nunc A/S Roskilde, Denmark), for the drug to equilibrate with the cells. 50 μL of virus at a concentration of 100 50% tissue culture infectivity dose (TCID50) was added to each well and the plate incubated at 37 °C in 5% CO_2 for 2 days. Viral replication was assessed by two methods: real-time RT-PCR using the described protocol[38,39] was carried out on nucleic acid extracted from the supernatant of the culture, using the Roche Lightcycler system (Roche Diagnostics, Mannheim, Germany); FITC-labeled anti-nucleoprotein monoclonal antibody (Chemicon International, Temecula, CA) was used to stain the remaining cell monolayer and viewed under an ultraviolet light microscope. Controls consisting of virus and cell only, cell only, virus only and serial 2-fold dilution of amantadine in place of substance in study were included in each plate. Cytotoxicity of the drugs was also assessed at all concentrations used in the antiviral assay.

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Notes and references

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