Reaction of *N*-acetylneuraminic acid derivatives with perfluorinated anhydrides: a short access to *N*-perfluoracylated glycals with antiviral properties†

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Received 7th April 2010, Accepted 26th May 2010
First published as an Advance Article on the web 17th June 2010
DOI: 10.1039/c004938g

An efficient short protocol for the preparation of *N*-perfluoroacylated glycals of neuraminic acid, by simple short treatment of differently protected *N*-acetylneuraminic acid with perfluorinated anhydrides in acetonitrile at 135 °C, is reported, together with a rationalitazion of the reaction that allows the alternative formation of *N*-perfluoroacylated 1,7-lactones to be previewed under the same reaction conditions.

Introduction

N-acetylneuraminic acid **1** (Neu5Ac, Fig. 1), a well known member of the sialic acid family, fulfils several important cellular recognition processes[1,2] and, *inter alia*, represents the ligand recognized on the host cell membrane by the influenza A virus.[3] Moreover, terminal Neu5Ac **1**, linked to the influenza virus receptors, may be cleaved by virus sialidase (neuraminidase; NA), thus causing the release of the virus from the infected cells.[3]

Therefore, much effort has been made to design and synthesize novel NA inhibitors to be used as anti-influenza drugs.[4] Two of them are the clinically used Zanamivir **2** (4-deoxy-4-guanidino-Neu5Ac2en; Relenza™),[3] obtained from Neu5Ac **1** *via* the key intermediate glycal **3** (Fig. 1),[5,6] and the *N*-trifluoroacetamido glycal **4**,[7] a congener which has shown the higher *in vitro* inhibitory action on the NA of influenza A and B viruses.[4] However, while much effort has been made[4,6] to synthesize the peracetylated glycal **3**, little attention has been devoted to the preparation of the fluorinated glycal **5**, and of its homologues **6** and **7**, which could be potentially useful intermediates for the preparation of the perfluorinated Zanamivir analogue **4** and of other active congeners.[8]

As a result of our interest in the chemistry of sialic acid, we herein disclose an efficient and short synthesis of the *N*-perfluoroacylated glycals **5–7**, by simple treatment of the easily available peracetylated Neu5Ac methyl ester **8** (Fig. 1), with the appropriate perfluorurated anhydride[9,10] (trifluoracetic, pentafluoropropionic and heptafluorobutyric anhydride: TFAA, PFPAA and HFBAA). Moreover, we have identified the structural characteristic of the sialic acids derivatives which, by treatment with perfluorinated anhydrides, can afford transacylated glycals. Furthermore we have discriminated them from others that, under the same reaction conditions, afford saturated *N*-transacylated 1,7-lactones.

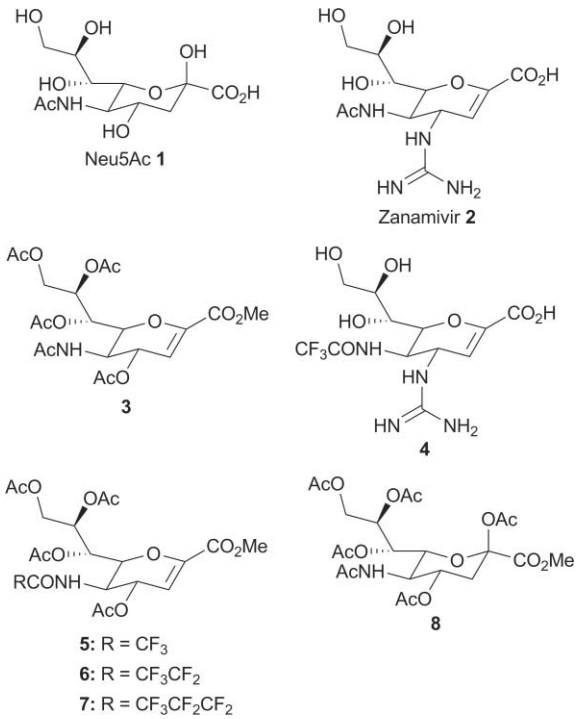

Fig. 1 Structures of some sialic acids and their glycals.

Result and discussion

When we started our work, no preparation of the glycals **5–7**, was reported in the literature, where just the glycal **5** was described, as a by-product of a glycosidation reaction.[11] Moreover, at least in principle, the glycal **4** could be obtained by protection of the known, but not easily available,[12–14] parent hydroxyl acid.

Our initial work allowed access to the glycals **5–7** in four steps (Scheme 1, Path A), performing their preparation, by direct *N*-transacylation of the acetylglycal **3** with the appropriate perfluorinated anhydride (at 135 °C for 10 min in CH_3CN), according to a very recent method set-up in our laboratory.[10] Successively, on searching for a possible additional shortening of the glycals **5–7** preparation, we considered the possibility to bypass the formation of the intermediate glycal **3** and to obtain

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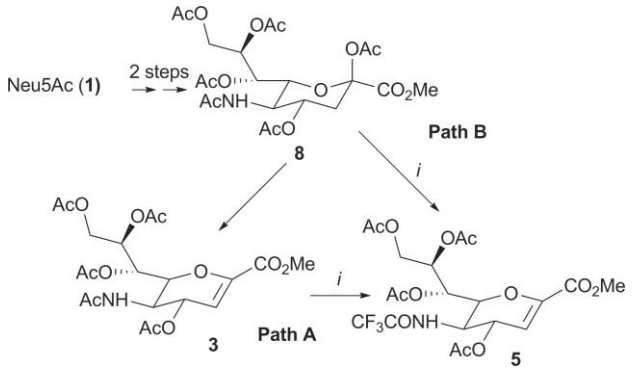
† Electronic supplementary information (ESI) available: Experimental details and characterisation data. See DOI: 10.1039/c004938g

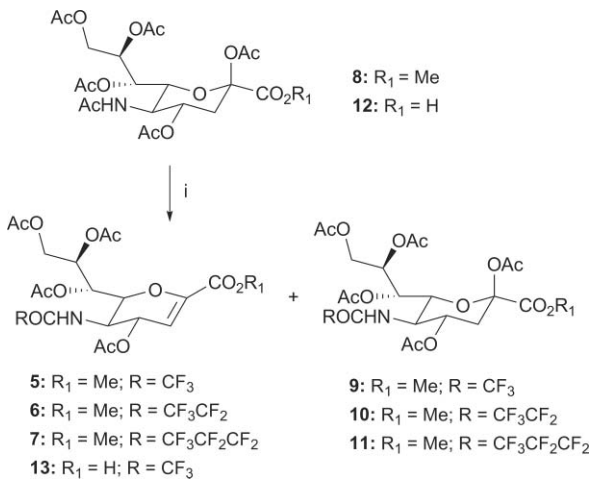


Scheme 1 Synthesis of the glycal **5**: by direct *N*-transacylation of glycal **3** (path A) or by direct reaction of the peracetylated ester **8** (path B). *Reagents and conditions*: i, TFAA, MeCN, 135 °C, 10–15 min, in a sealed tube.

them by direct reaction of the peracetylated ester **8** with the appropriate perfluorinated anhydride (Scheme 1, Path B). We were confident that subjecting the saturated sialic ester **8** to the *N*-transacylation,[10] the acidic medium of the reaction could promote the formation of the glycal **5**, by elimination of elements of acetic acid (Scheme 1).

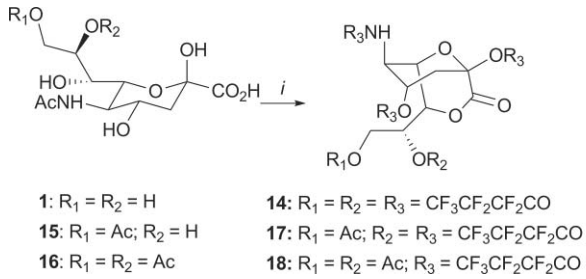
In effect, on reacting the ester **8** with the appropriate anhydride, at different temperatures and times (between 80–135 °C for 5–15 min), we observed that, according with our prediction, the glycals **5–7** accompanied the *N*-transacylated esters **9–11**. These compounds were obtained in the best yields (40–55%) after 5 min reactions at 135 °C (see experimental section).

Moreover, when the heating was prolonged to 15 min, at 135 °C, the glycals **5–7** were the only reaction products, thus obtained in three steps from the commercial Neu5Ac **1** (Scheme 2). Furthermore, on reacting the peracetylated acid **12**, obtained in one step from Neu5Ac **1**, with TFAA in CH_3CN at 135 °C, for 15 min, we obtained the glycal **13** in good yields (68%; Scheme 2). This glycal could permit the preparation of possible NA inhibitors by selective elaboration of the carboxylic group in the presence of the acetates esters.



Scheme 2 *Reagents and conditions*: i, TFAA or PFPAA or HFBAA, MeCN, 80–135 °C, 5–15 min, in a sealed tube.

Finally, we subjected free Neu5Ac **1** to a direct treatment with HFBAA, to verify or exclude if, under the reaction conditions, the formation of the corresponding unprotected glycal could occur in a single step. In fact we had already shown that the perfluoroacylation of the free hydroxyls could be reversed by simple treatment of the final reaction mixture with aqueous methanol.[10] Moreover, performing the reaction with HFBAA in CD_3CN, under various reaction conditions (times and temperatures), we did not observe the formation of any perfluorinated glycal (NMR), but obtained variable amounts of a 1,7-lactone of assigned structure **14** (Scheme 3).



Scheme 3 *Reagents and conditions*: i, HFBAA, MeCN, 135 °C, 15 min, in a sealed tube.

This lactone, quantitatively formed at 135 °C, in agreement with the observed instability of 1,7-sialolactones having an anomeric hydroxyl free or protected with a labile group,[15] was not isolable in pure form by chromatography on silica. However, it could be subjected to a GLC-MS inspection and to a complete NMR analysis[15] (see experimental section).

The formation of the lactone **14** was not expected on the basis of the reported literature, which excludes the formation of any artefact sialic lactone in the treatment of sialic acid with perfluorinated anhydrides, under the same conditions herein used.[9] However the observed lactonization of sialic acid and the transacylation are in keeping with some pioneering results of Ogura *et al.* and with those we have very recently disclosed[10,15] suggesting that acyl anhydride can promote 1,7-lactonization.[16]

In agreement with this rationalization also the sialic acids **15** and **16**, acetylated at the C-9 or at the C-8 and the C-9 hydroxyls, in the same reaction, afford the corresponding lactones **17** and **18**, in this case, accompanied by minor amounts of by products.

Conclusions

The protocol for the preparation of sialic acid perfluorinated glycals herein reported is of interest since it allows us to obtain key intermediates for the synthesis of perfluorinated congeners of the antiviral agent Zanamivir, useful as inhibitors of the NA of influenza A and B viruses. Moreover, the experiments performed to understand the mechanism of the glycal formation has permitted evidence of the formation of *N*-transacylated 1,7-lactones by simple reaction of free sialic acid with perfluorinated anhydrides. Thus, it allows some knowledge to be acquired that is useful not only in organic synthesis but also in analytical chemistry where they can permit a better rationalization of the results obtained in sialic acids analysis in biological tissues where the presence of 1,7-lactones has been suggested after volatilization of sialic acids by treatment with perfluorinated anhydrides.[9]

Experimental

General information

Solvents were dried using standard methods and distilled before use. The reactions are thermostated by block heater -BBA- Grant Boekel apparatus. The progress of all reactions was monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60 F_{254}) using UV light, 50% sulfuric acid, anisaldehyde/H_2SO_4/EtOH solution or 0.2% ninhydrin in ethanol and heat as developing agent. Flash chromatography was performed with normal phase silica gel (E. Merck 230–400 mesh silica gel), following the general protocol of Still *et al.*[17] GLC was performed by Hewlett 5890 PACKARD Series II using HP-5 30 m x 0.32 mm, 0.25 μm film-thickness column. Melting points were measured on a SMP3 mp apparatus (Stuart Scientific, USA) and are not corrected. NMR spectra were recorded at 25 °C on a Bruker AM-500 spectrometer operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C. The chemical shifts are reported in ppm and coupling constants are given in Hz, relative to CD_3OD signal fixed at 3.31 ppm for ^{1}H spectra and to CD_3OD signal fixed at 49.05 ppm for ^{13}C spectra, relative to $CDCl_3$ signal fixed at 7.26 ppm for ^{1}H spectra and to $CDCl_3$ signal fixed at 77.00, relative to CD_3CN signal fixed at 1.94 ppm for ^{1}H spectra and to CD_3CN signal fixed at 1.24 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. Data for ^{1}H NMR are recovered as follows: chemical shift (ppm), number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), coupling constant(s) in Hz, assignment of proton(s). In some cases, reported in the text, the ^{1}H NMR inspection was performed on the total reaction mixture using CD_3CN as a solvent. Optical rotations were taken on a Perkin-Elmer 241 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 per 100 mL. Infrared (IR) spectra were recorded in CH_2Cl_2 solution using a Perkin-Elmer 1420 instrument. Mass spectrometry was performed using Finnigan LCQ_{Deca} quadrupole ion-trap mass spectrometer equipped with an ESI ion source (Finnigan ThermoQuest, San Jose, CA, USA). 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.

Work-up refers to successive washing of the organic layer with an ice cold aqueous $NaHCO_3$ saturated solution and water, to drying over Na_2SO_4, and evaporation of the solvent under reduced pressure.

Preparation of *N*-perfluoroacylneuraminic acid glycals

The acylamide (0.2 mmol) dissolved in CH_3CN (0.60 mL) was reacted with the appropriate perfluorinated anhydride (0.6–1.4 mmol, 3–7 molar equivalents) at 135 °C for 5–15 min in a sealed tube. Then the reaction mixture was cooled, followed by addition of methanol (0.20 mL) and evaporation under reduced pressure to afford a crude residue which, after usual work-up and rapid chromatography, using the designed solvent system, afforded the appropriate glycal. This procedure was used both for the exclusive *N*-transacylation and for the *N*-transacylation coupled with elimination reaction.

4,7,8,9-Tetra-*O*-acetyl-2,3-dehydro-2-deoxy-5-*N*-(2,2,2-trifluoroacetyl)-β-neuraminic acid methyl ester 5. Method A. Reacting the glycal **3**[18] (95 mg; 0.20 mmol) with TFAA (0.11 mL, 0.8 mmol) the title glycal **5** was obtained, after a 10 min reaction. Usual work-up and flash chromatography (eluting with hexane–AcOEt; 6 : 4, v/v) afforded the glycal **5** (83 mg, 79%): as a solid: m.p. 115–116 °C; $[\alpha]_D^{20}$ + 50.8 (*c* 1 in $CHCl_3$); (Found: C, 45.65; H, 4.49; N, 2.53 Calc. for $C_{20}H_{24}F_3NO_{12}$: C, 45.55; H, 4.59; N, 2.66%); ν_{max}/cm^{-1} 1750,1724, 1660; ^{1}H NMR ($CDCl_3$) δ 7.21 (1H, d, $J_{NH,2}$ = 9.0 Hz, N–H), 5.97 (1H, d, $J_{3,4}$ = 2.6 Hz, 3-H), 5.65 (1H, dd, $J_{4,5}$ = 8.0, $J_{4,3}$ = 2.6 Hz, 4-H), 5.47 (1H, dd, $J_{7,8}$ = 4.3, $J_{7,6}$ = 3.0 Hz, 7-H), 5.29 (1H, m, 8-H), 4.71 (1, dd, $J_{9a,9b}$ = 12.4, $J_{9a,8}$ = 2.6 Hz, 9a-H), 4.49 (1H, dd, $J_{6,5}$ = 9.7, $J_{6,7}$ = 3.0 Hz, 6-H), 4.34 (1H, m, 5-H), 4.18 (1H, dd, $J_{9b,9a}$ = 12.4, $J_{9b,8}$ = 7.3 Hz, 9b-H), 3.81 (3H, s, $COOCH_3$); 2.11 (3H, s, CH_3COO at C-7), 2.06 (6H, overlapping, 2XCH_3COO), 2.04 (s, 3H; CH_3COO); ^{13}C NMR ($CDCl_3$) δ 170.7 (3C, CH_3COO at C-4, CH_3COO at C-8 and CH_3COO at C-9), 170.0 (CH_3COO at C-7), 161.3 (C-1), 157.4 (q, J_{C-F} = 38 Hz; $COCF_3$), 145.3 (C-2), 120.0-110.0 (1C, CF_3), 107.7 (C-3), 75.9 (C-6), 71.3 (C-8), 67.4 (C-4 and C-7), 61.8 (C-9), 52.7 ($COOCH_3$), 47.8 (C-5), 20.8 (CH_3COO), 20.6 (2C, CH_3COO), 20.5 (CH_3COO); MS (ESI positive) m/z 550.2 [M+Na]$^+$, 1077.8 [2M+Na]$^+$. Method B. Reacting 4,7,8,9-tetra-*O*-acetyl-5-*N*-acetyl-β-neuraminic acid methyl ester **8**[19] (107 mg, 0.2 mmol) with TFAA (0.11 mL, 0.8 mmol) for 15 min, the glycal **5** (77 mg, 73%) was obtained after flash chromatography (eluting with hexane–AcOEt; 6 : 4, v/v). It showed: m.p. 114–116 °C; $[\alpha]_D^{20}$ + 50.8 (*c* 1 in $CHCl_3$); (Found: C, 45.65; H, 4.49; N, 2.53 Calc. for $C_{20}H_{24}F_3NO_{12}$: C, 45.55; H, 4.59; N, 2.66%). All physico-chemical properties were superimposable to those of the compound described above.

4,7,8,9-Tetra-*O*-acetyl-2,3-dehydro-2-deoxy-5-*N*-(2,2,3,3,3-pentafluoropropyonil)-β-neuraminic acid methyl ester 6. Method A. Reacting the glycal **3**[18] (95 mg; 0.20 mmol) with PFPAA (0.16 mL, 0.8 mmol) the title glycal **6** was obtained, after a 10 min reaction. Usual work-up and flash chromatography (eluting with hexane–AcOEt; 6 : 4, v/v) afforded the glycal **6** (90 mg, 78%): as a solid: m.p. 120–122 °C; $[\alpha]_D^{20}$ + 55.3 (*c* 1 in $CHCl_3$); (Found: C, 43.50; H, 4.35; N, 2.50 Calc. for $C_{21}H_{24}F_5NO_{12}$: C, 43.68; H, 4.19; N, 2.43%); ν_{max}/cm^{-1} 1747,1728, 1661; ^{1}H NMR ($CDCl_3$) δ 7.10 (1H, d, $J_{NH,2}$ = 9.3 Hz, N–H), 5.98 (1H, d, $J_{3,4}$ = 2.8 Hz, 3-H), 5.70 (1H, dd, $J_{4,5}$ = 8.0, $J_{4,3}$ = 2.8 Hz, 4-H), 5.43 (1H, dd, $J_{7,8}$ = 4.5, $J_{7,6}$ = 3.0 Hz, 7-H), 5.31 (1H, m, 8-H), 4.67 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9a,8}$ = 2.9 Hz, 9a-H), 4.51 (1H, dd, $J_{6,5}$ = 9.7, $J_{6,7}$ = 3.0 Hz, 6-H), 4.37 (1H, m, 5-H), 4.19 (1H, dd, $J_{9b,9a}$ = 12.4, $J_{9b,8}$ = 6.8 Hz, 9b-H), 3.84 (3H, s, $COOCH_3$); 2.13 (3H, s, CH_3COO), 2.08 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO); ^{13}C NMR ($CDCl_3$) δ 170.7-170.5 (3C, CH_3COO at C-4, CH_3COO at C-8 and CH_3COO at C-9), 170.0 (CH_3COO at C-7), 161.3 (C-1), 158.1 (t, J_{C-F} = 26 Hz; $COCF_2CF_3$), 145.2 (C-2) 120.0- 112.0 (2C, CF_2CF_3), 107.7 (C-3), 75.6 (C-6), 70.9 (C-8), 67.3 (C-4 and C-7), 61.8 (C-9), 52.2 ($COOCH_3$), 47.7 (C-5), 20.8 (CH_3COO), 20.6 (CH_3COO), 20.5 (2C,CH_3COO); MS (ESI positive) m/z 600.1 [M+Na]$^+$, 1176.4 [2M+Na]$^+$. Method B. Reacting 4,7,8,9-tetra-*O*-acetyl-5-*N*-acetyl-β-neuraminic acid methyl ester **8**[19] (107 mg, 0.2 mmol) with PFPAA (0.16 mL, 0.8 mmol) for 15 min, the

glycal **6** (87 mg, 75%) was obtained after flash chromatography (eluting with hexane–AcOEt; 6 : 4, v/v). It showed: m.p. 120–122 °C; $[\alpha]_D^{20}$ + 55.3 (*c* 1 in $CHCl_3$); (Found: C, 43.50; H, 4.35; N, 2.50 Calc. for $C_{21}H_{24}F_5NO_{12}$: C, 43.68; H, 4.19; N, 2.43%); with all physico-chemical properties superimposable to those of the compound described above.

4,7,8,9-Tetra-*O*-acetyl-2,3-dehydro-2-deoxy-5-*N*-(2,2,3,3,4,4,4 heptafluorobutanoyl)-β-neuraminic acid methyl ester 7. Method A. Reacting the glycal **3**[18] (95 mg; 0.20 mmol) with HFBAA (0.19 mL, 0.8 mmol) the title glycal **7** was obtained, after a 10 min reaction. Usual work-up and flash chromatography (eluting with hexane–AcOEt; 6 : 4, v/v) afforded the glycal **7** (102 mg, 81%): as a solid: m.p. 115–116 °C; $[\alpha]_D^{20}$ + 49.1 (*c* 1 in $CHCl_3$); (Found: C, 42.20; H, 3.70; N, 2.40 Calc. for $C_{22}H_{24}F_7NO_{12}$: C, 42.11; H, 3.86; N, 2.23%); ν_{max}/cm^{-1} 1746, 1729, 1668; ^1H NMR ($CDCl_3$) δ 7.21 (1H, d, $J_{NH,2}$ = 9.0 Hz, N–H), 5.97 (1H, d, $J_{3,4}$ = 2.6 Hz, 3-H), 5.70 (1H, dd, $J_{4,5}$ = 9.0, $J_{4,3}$ = 2.6 Hz, 4-H), 5.43 (1H, dd, $J_{7,8}$ = 4.4, $J_{7,6}$ = 2.8 Hz, 7-H), 5.30 (1H, m, 8-H), 4.66 (1H, dd, $J_{9a,9b}$ = 12.4, $J_{9a,8}$ = 2.6 Hz, 9a-H), 4.53 (1H, dd, $J_{6,5}$ = 9.8, $J_{6,7}$ = 2.8 Hz, 6-H), 4.35 (1H, q app., $J_{5,4} = J_{5,6} = J_{5,NH}$ = 9.0 Hz, 5-H), 4.19 (1H, dd, $J_{9b,9a}$ = 12.4, $J_{9b,8}$ = 6.8 Hz, 9b-H), 3.81 (3H, s, $COOCH_3$); 2.14 (3H, s, CH_3COO at C-7), 2.07 (3H, s, CH_3COO), 2.06 (3H, s, CH_3COO), 2.00 (3H, s, CH_3COO); ^{13}C NMR ($CDCl_3$) δ 170.6 (3C, CH_3COO at C-4, CH_3COO at C-8 and CH_3COO at C-9), 170.1 (CH_3COO at C-7), 161.2 (C-1), 157.8 (t, J_{C-F} = 27 Hz; $COCF_2CF_2CF_3$), 145.1 (C-2), 122.0- 109.0 (3C, $CF_2CF_2CF_3$), 107.3 (C-3), 75.6 (C-6), 70.9 (C-8), 67.4 (C-4 and C-7), 61.8 (C-9), 52.7 ($COOCH_3$), 47.8 (C-5), 20.8 (CH_3COO), 20.6 (2C, CH_3COO), 20.5 (CH_3COO); MS (ESI positive) *m*/*z* 650.0 $[M+Na]^+$, 1276.2 $[2M+Na]^+$. Method B. Reacting 4,7,8,9-tetra-*O*-acetyl-5-*N*-acetyl-β-neuraminic acid methyl ester **8**[19] (107 mg, 0.2 mmol) with HFBAA (0.19 mL, 0.8 mmol) for 15 min, the glycal **7** (87 mg, 75%) was obtained after flash chromatography (eluting with hexane–AcOEt; 6 : 4, v/v). It showed: m.p. 114–117 °C; $[\alpha]_D^{20}$ + 50.3 (*c* 1 in $CHCl_3$); (Found: C, 42.20; H, 3.70; N, 2.40 Calc. for $C_{22}H_{24}F_7NO_{12}$: C, 42.11; H, 3.86; N, 2.23%); with all physico-chemical properties superimposable to those of the compound described above.

4,7,8,9-Tetra-*O*-acetyl-2,3-dehydro-2-deoxy-5-*N*-(2,2,2-trifluoroacetyl)-β-neuraminic acid 13. Reacting the 4,7,8,9-tetra-*O*-acetyl-5-*N*-acetyl-β-neuraminic acid **12**[20] (107 mg, 0.2 mmol) with TFAA (0.11 mL, 0.8 mmol) the title glycal **13** was obtained, after a 15 min reaction. Usual work-up and flash chromatography (eluting with AcOEt–MeOH; 85 : 15, v/v) afforded the glycal **13** (73 mg, 71%): as a solid: m.p. 115–116 °C; $[\alpha]_D^{20}$ + 50.8 (*c* 1 in CH_3OH); (Found: C, 45.44; H, 4.60; N, 2.50 Calc. for $C_{20}H_{24}F_3NO_{12}$: C, 45.55; H, 4.59; N, 2.66%); ν_{max}/cm^{-1} 1741,1730, 1662; ^1H NMR (CD_3OD) δ 5.75 (1H, d, $J_{3,4}$ = 2.5 Hz, 3-H), 5.62 (1H, dd, $J_{4,5}$ = 8.6, $J_{4,3}$ = 2.5 Hz, 4-H), 5.49 (1H, m, 8-H), 5.43 (1H, dd, $J_{7,8}$ = 7.0, $J_{7,6}$ = 2.1 Hz, 7-H), 4.55 (1H, dd, $J_{9a,9b}$ = 12.5, $J_{9a,8}$ = 2.7 Hz, 9a-H), 4.42 (1H, dd, $J_{6,5}$ = 10.6, $J_{6,7}$ = 2.1 Hz, 6-H), 4.24-4.19 (2H, overlapping, 5-H and 9b-H), 2.08 (3H,s, CH_3COO), 2.04 (3H, s, CH_3COO), 2.01 (6H, overlapping, CH_3COO); ^{13}C NMR (CD_3OD) δ 172.6 (CH_3COO at C-9), 172.2 (CH_3COO at C-4), 171.7 (CH_3COO at C-8), 171.4 (CH_3COO at C-7), 169.2 (C-1), 159.2 (q, J_{C-F} = 38 Hz; $COCF_3$), 151.6 (C-2), 120.0-116.0 (1C, CF_3), 105.3 (C-3), 76.4 (C-6), 71.4 (C-8), 71.1 (C-4), 68.7 (C-7), 63.1 (C-9), 48.5 (C-5), 20.8 (CH_3COO), 20.7 (3C, CH_3COO); MS (ESI negative) *m*/*z* 511.8 $[M - H]^-$.

Saturated *N*-perfluoroacylneuraminic acid. The acylamide (0.2 mmol) dissolved in CH_3CN (0.60 mL) was reacted with the appropriate perfluorinated anhydride (0.6–1.4 mmol, 3–7 molar equivalents) at 135 °C for 5 min in a sealed tube. Then the reaction mixture was cooled, followed by addition of methanol (0.20 mL) and evaporation under reduced pressure to afford a crude residue which, after usual work-up, and rapid chromatography, using the designed solvent system, afforded a mixture of the appropriate glycal and of the relative saturated derivative.

2,4,7,8,9-Penta-*O*-acetyl-5-*N*-(2,2,2-trifluoroacetyl)-β-neuraminic acid methyl ester 9. Starting with 4,7,8,9-tetra-*O*-acetyl-5-*N*-acetyl-β-neuraminic acid methyl ester **8**[19] (107 mg, 0.2 mmol), after a 5 min reaction, with TFAA (0.11 mL, 0.8 mmol) and flash chromatography (eluting with hexane–AcOEt; 7 : 3, v/v to 6 : 4, v/v) a mixture of saturated derivative **9** (58 mg, 49%) and of glycal **5** (51 mg, 48%) was obtained. Compound **9**, as a glass, showed: $[\alpha]_D^{20}$ −22.3 (*c* 1 in $CHCl_3$); (Found: C, 45.94; H, 4.78; N, 2.35. Calc. for $C_{22}H_{28}F_3NO_{14}$: C, 45.98; H, 4.80; N, 2.38%);ν_{max}/cm^{-1} 1752,1725, 1667; ^1H NMR ($CDCl_3$) δ 6.60 (d, $J_{NH,2}$ = 9.2 Hz, 1H; N–H), 5.43 (1H, m, 4-H), 5.37 (1H, m, 7-H), 5.12 (1H, m, 8-H), 4.51 (1H, d, $J_{9a,9b}$ = 12.5, 9a-H), 4.31 (1H, br d, $J_{6,5}$ = 10.5, 6-H), 4.17 (1H, dd, $J_{9b,9a}$ = 12.5, $J_{9b,8}$ = 5.8 Hz, 9b-H), 4.08 (1H, m, 5-H), 3.83 (3H, s, $COOCH_3$); 2.62 (1H, br d, $J_{3a,3b}$ = 13.4 Hz, H-3a) 2.18 (6H, overlapping, $2XCH_3COO$), 2.16-2.02 (4H, overlapping, CH_3COO and H-3b), 2.06 (6H, overlapping, $2XCH_3COO$); ^{13}C NMR ($CDCl_3$) δ 171.1, 170.8, 170.6, 170.1, 168.2, 166.1, 157.4 (q, J_{C-F} = 38 Hz; $COCF_3$), 116.6 (1C, J_{C-F} = 287 Hz; CF_3), 97.3, 71.8, 71.7, 67.7, 67.5, 62.0, 53.3, 49.9, 35.8, 20.9, 20.7, 20.6, 20.5; MS (ESI positive) *m*/*z* 610.0 $[M+Na]^+$, 1197.1 $[2M+Na]^+$. Glycal **5** showed: m.p. 115–116 °C; : $[\alpha]_D^{20}$ + 50.3 (*c* 1 in $CHCl_3$); (Found: C, 45.58; H, 4.45; N, 2.62 Calc. for $C_{20}H_{24}F_3NO_{12}$: C, 45.55; H, 4.59; N, 2.66%). All physico-chemical properties were superimposable to those of the compound described above.

2,4,7,8,9-Penta-*O*-acetyl-5-*N*-(2,2,3,3,3-pentafluoropropyonil)-β-neuraminic acid methyl ester 10. Starting from 4,7,8,9-tetra-*O*-acetyl-5-*N*-acetyl-β-neuraminic acid methyl ester **8**[19] (0.107 g, 0.2 mmol) and PFPAA (0.16 mL, 0.8 mmol) after a reaction for 5 min at 135 °C a mixture of saturated derivative **10** (61 mg, 41%) and of the glycal **6** (47 mg, 41%) was obtained after column chromatography (eluting with hexane–AcOEt; 7 : 3, v/v to 6 : 4, v/v). Compound **10**, as a glass, showed: $[\alpha]_D^{20}$ −18.5 (*c* 1 in $CHCl_3$); (Found: C, 43.80; H, 4.10; N, 2.38 Calc. for $C_{21}H_{24}F_5NO_{12}$: C, 43.68; H, 4.19; N, 2.43%); ν_{max}/cm^{-1} 1747,1718, 1669; ^1H NMR ($CDCl_3$) δ 7.00 (1H, d, $J_{NH,2}$ = 9.2 Hz, N–H), 5.42 (1H, ddd, $J_{4,3b} = J_{4,5}$ = 10.8, $J_{4,3a}$ = 4.8 Hz, 4-H), 5.32 (1H, m, 7-H), 5.04 (1H, br s, 8-H), 4.51 (1H, d, $J_{9a,9b}$ = 12.5, 9a-H), 4.30 (1H, br d, $J_{6,5}$ = 10.6, 6-H), 4.16 (1H, dd, $J_{9b,9a}$ = 12.5, $J_{9b,8}$ = 6.5 Hz, 9b-H), 4.08 (1H, m, 5-H), 3.85 (3H, s, $COOCH_3$); 2.58 (1H, dd, $J_{3a,3b}$ = 13.4, $J_{3a,4}$ = 4.8 Hz, 3a-H) 2.15 (6H, overlapping, $2XCH_3COO$), 2.09-2.05 (4H, overlapping, CH_3COO and 3b-H), 2.03 (3H, s, CH_3COO), 2.02 (3H, s, CH_3COO); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.6, 170.5, 170.1, 168.2, 166.1, 158.1 ($COCF_2CF_3$), 116.6-110.0 (2C, CF_2CF_3), 97.1, 71.6, 71.4, 67.6, 67.3, 61.9, 53.3, 50.1, 35.9, 20.9, 20.7, 20.6, 20.5; MS (ESI positive) *m*/*z* 610.0 [M+Na]+, 1298.3 [2M+Na]+. Glycal **6** showed: m.p. 120–122 °C; $[\alpha]_D^{20}$ + 55.3 (*c* 1 in $CHCl_3$); (Found: C,

43.55; H, 4.31; N, 2.52 Calc. for $C_{21}H_{24}F_5NO_{12}$: C, 43.68; H, 4.19; N, 2.43%); with all physico-chemical properties superimposable to those of the compound described above.

2,4,7,8,9-Penta-*O*-acetyl-5-*N*-(2,2,3,3,4,4,4-heptafluorobutanoyl)-β-neuraminic acid methyl ester 11. Starting from 4,7,8,9-tetra-*O*-acetyl-5-*N*-acetyl-β-neuraminic acid methyl ester **8**[19] (0.107 g, 0.2 mmol) and HFBAA (0.19 mL, 0.8 mmol) after a reaction for 5 min at 135 °C a mixture of saturated derivative **11** (73 mg, 53%) and of the glycal **7** (53 mg, 42%) was obtained after column chromatography (eluting with hexane–AcOEt; 7 : 3, v/v to 6 : 4, v/v). Compound **11** showed: $[\alpha]_D^{20}$ −22.5 (*c* 1 in $CHCl_3$); (Found: C, 42.08; H, 3.90; N, 2.35 Calc. for $C_{22}H_{24}F_7NO_{12}$: C, 42.11; H, 3.86; N, 2.23%); ν_{max}/cm^{-1} 1752,1715, 1669; ^{1}H NMR ($CDCl_3$) δ 6.83 (1H, d, $J_{NH,2} = 9.1$ Hz, N–H), 5.48 (1H, ddd, $J_{4,3b} = J_{4,5} = 11.0$, $J_{4,3a} = 5.0$ Hz, 4-H), 5.28 (1H, dd, $J_{7,8} = 5.7$, $J_{7,6} = 1.7$ Hz, 7-H), 5.09 (1H, ddd, $J_{8,9b} = J_{8,7} = 5.7$, $J_{8,9a} = 2.4$ Hz 8-H), 4.47 (1H, dd, $J_{9a,9b} = 12.5$, $J_{9b,8} = 5.7$ Hz Hz, 9a-H), 4.36 (1H, dd, $J_{6,5} = 10.5$, $J_{6,7} = 1.7$ Hz, 6-H), 4.14 (1H, dd, $J_{9b,9a} = 12.5$, $J_{9b,8} = 5.7$ Hz, 9b-H), 3.99 (1H, m, 5-H), 3.80 (3H, s, $COOCH_3$); 2.62 (1H, dd, $J_{3a,3b} = 13.5$, $J_{3a,4} = 5.0$ Hz, 3a-H) 2.16 (6H, overlapping, 2XCH_3COO), 2.06 (3H, s, CH_3COO), 2.05-2.00 (7H, overlapping, 2XCH_3COO and 3b-H); ^{13}C NMR ($CDCl_3$) δ 170.5, 170.4, 170.3, 170.12, 168.2, 166.1, 157.9 (1C, *C*O*C*$F_2C F_2CF_3$), 120.0-108.0 (3C, CO*C*$F_2C F_2CF_3$), 97.1, 71.3, 70.9, 67.6, 67.1, 61.8, 53.3, 50.6, 35.9, 20.7, 20.6 (3C) 20.5; MS (ESI positive) *m*/*z* 710.6 $[M+Na]^+$. Anal. Calcd for $C_{24}H_{28}F_7NO_{14}$: C, 41.93; H, 4.11; N, 2.04. Found: C, 41.89; H, 4.08; N, 2.07. Glycal **7** showed: m.p. 114–117 °C; $[\alpha]_D^{20}$ +50.3 (*c* 1 in $CHCl_3$); with all physico-chemical properties superimposable to those of the compound described above.

1,7-Lactonization and *N*-transacylation of sialic acids by action of HFBAA

Treatment of Neu5Ac 1 with HFBAA. The reaction was performed treating Neu5Ac **1** (30 mg, 0.1 mmol), dissolved in CD_3CN (0.300 mL), with HFBAA (0.034 mL, 1.4 mmol) at 135 °C for 15 min, and the reaction mixture was subjected to NMR analyses. The ^{1}H NMR spectrum showed the absence of any olefinic signal between 5.6–6.5 ppm, attributable to the proton at C-3 of sialic glycals. On the contrary it showed diagnostic signals for the presence of a 1,7-lactone. ^{13}C analyses of the reaction mixture, performed after evaporation of CD_3CN and dilution of the reaction mixture with $CDCl_3$, confirmed the presence of the lactone ring and of the perfluorinated amide group in place of the starting acetyl group. Compound **14** showed: ^{1}H NMR (CD_3CN) δ 8.23 (1H, d, $J_{NH,5} = 7.6$ Hz, N–H), 5.80 (1H, dt, $J_{8,7} = J_{8,9b} = 5.7$, $J_{8,9a} = 2.6$ Hz, 8-H), 5.61 (1H, br s, 4-H), 5.04 (1H, d, $J_{8,7} = 5.7$ Hz, 7-H), 4.96 (1H, dd, $J_{9a,9b} = 13.0$, $J_{9a,8} = 2.6$ Hz, 9a-H), 4.75 (1H, dd, $J_{9b,9a} = 13.0$, $J_{9b,8} = 5.7$ Hz, 9b-H), 4.66 (1H, br s, 6-H), 4.54 (1H, br d, $J_{5,NH} = 7.6$ Hz, 5-H), 2.77 (1H, dd, $J_{3a,3b} = 15.4$, $J_{3a,4} = 4.1$ Hz, 3a-H), 2.51 (1H, br d, $J_{3b,3a} = 15.4$ Hz, 3b-H); ^{1}H NMR ($CDCl_3$) δ 7.34 (1H, d, $J_{NH,5} = 7.8$ Hz, N–H), 5.69 (1H, br d, $J_{8,7} = 9.0$ Hz, 8-H), 5.53 (1H, br s, 4-H), 5.04 (1H, br d, $J_{9a,9b} = 13.0$, 9a-H), 4.85 (1H, d, $J_{8,7} = 9.0$ Hz, 7-H), 4.77 (1H, dd, $J_{9b,9a} = 13.0$, $J_{9b,8} = 2.8$ Hz, 9b-H), 4.50 (1H, br d, $J_{5,NH} = 7.8$ Hz, 5-H), 4.32 (1H, br s, 6-H), 2.62-2.59 (2H, m, 3a-H and 3b-H); ^{13}C NMR ($CDCl_3$) 160.1 (C-1), 158-100 (overlapping *C*O*C*$F_2C F_2CF_3$), 93.63 (C-2), 74.9 (C-7), 72.8 (C-8), 71.6 (C-6), 69.9 (C-4), 63.3 (C-9), 48.6 (C-5), 32.7 (C-3). All attempt to isolate the formed lactone were unsuccessful.

Treatment of Neu5,9Ac$_2$ (15) with HFBAA. The reaction was performed treating Neu5,9Ac$_2$ **15** (35 mg, 0.1 mmol), dissolved in CD_3CN (0.300 mL), with HFBAA (0.034 mL, 1.4 mmol) at 135 °C for 15 min, and the reaction mixture was subjected to NMR analyses. The ^{1}H NMR spectrum showed the absence of any olefinic signal between 5.6–6.5 ppm, attributable to the proton at C-3 of sialic glycals. On the contrary it showed diagnostic signals for the presence of a 1,7-lactone. Compound **17** showed: ^{1}H NMR ($CDCl_3$) δ 7.11 (1H, d, $J_{NH,5} = 8.4$ Hz, N–H), 5.58 (1H, br m, 8-H), 5.51 (1H, br s, 8-H), 4.86-4.80 (2H, overlapping, 7-H and 9a-H), 4.45-4.35 (2H, overlapping, 5-H and 6-H), 4.30 (1H, dd, $J_{9b,9a} = 12.8$, $J_{9b,8} = 3.5$ Hz, 9b-H), 2.62-2.59 (2H, m, 3a-H and 3b-H), 2.12 (s, 3H, $COCH_3$ at C-9).

Treatment of Neu5,8,9Ac$_3$ (16) with HFBAA. The reaction was performed treating Neu5,8,9Ac$_3$ **16** (40 mg, 0.1 mmol), dissolved in CD_3CN (0.300 mL), with HFBAA (0.034 mL, 1.4 mmol) at 135 °C for 15 min, and the reaction mixture was subjected to NMR analyses. The ^{1}H-NMR spectrum showed the absence of any olefinic signal between 5.6–6.5 ppm, attributable to the proton at C-3 of sialic glycals. On the contrary it showed diagnostic signals for the presence of a 1,7-lactone. Compound **18** showed: ^{1}H NMR ($CDCl_3$) δ 7.15 (1H, d, $J_{NH,5} = 7.7$ Hz, N–H), 5.52 (1H, br d, 4-H), 5.44 (1H, m, 8-H), 4.76-4.71 (2H, overlapping, $J_{8,7} = 8.3$ Hz, 7-H and 9a-H), 4.47 (1H, br s, 6-H), 4.39 (1H, br d, $J_{5,NH} = 7.7$ Hz, 5-H), 4.32 (1H, dd, $J_{9b,9a} = 12.8$, $J_{9b,8} = 3.5$ Hz, 9b-H), 2.62 (1H, br d, $J_{3a,3b} = 15.4$, 3a-H), 2.51 (1H, dd, $J_{3a,3b} = 15.4$, $J_{3b,4} = 4.2$ Hz, 3b-H), 2.14 (3H, s, $COCH_3$), 2.11(3H, s, $COCH_3$).

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