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Two procedures for the syntheses of labeled sialic acids and their 1,7-lactones

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ABSTRACT

The synthesis of four deuterated sialic acids and their 1,7-lactones has been performed in two ways, one based on sialic acid classical chemistry, and the other involving a direct exchange of the unlabeled acyl group of *N*-acetylneuraminic acid with a labeled one mediated by a perfluorinated amide. The final lactonization is promoted by benzyloxycarbonyl chloride.

1. Introduction

N-Acetylneuraminic acid **1a** (Neu5Ac) and *N*-glycolyl neuraminic acid **2a** (Neu5Gc) are two very important members of sialic acid family (Sias), a group of carbohydrates, sharing the neuraminic acid pyranosidic ring, usually linked, through an α-glycosidic bond to the end of the carbohydrate chains present in glycolipids and glycoproteins of bacteria and humans (Fig. 1).[1] In particular, Sias present in healthy humans derive from Neu5Ac, while those present in bacteria and in human tumors also derive from Neu5Gc. Sias play a central role in numerous biological recognition processes such as lymphocite homing, tumor methastasis and pathogenic bacteria infections.[1–3] As a result of this, much effort has been made to map them in different biological districts and thus many conclusions with regard to their biological roles have already been reached.

Recently, the 1,7-lactone of *N*-acetylneuraminic acid **3a**, first identified in the *Bufo bufo*[4] mucins and as a ligand of human interleukin-4,[5,6] has been described as being present in sialoglycoproteins of the membrane of red blood cells, affected by *Polycythemia vera*, a malignant disorder of haematopoietic stem cells.[7] However, the lactone **3a** has not been isolated but only indirect evidences of its structure have been reported. These are obtained in a GLC–MS screening of Sias present in the glycoconjugated red blood cells affected by *P. vera*, after acidic hydrolysis and derivatization with perfluorinated anhydrides. Due to our interest in Sias chemistry and biochemistry,[8] we decided to search for an analytical protocol suitable to substantiate the reported presence of Sias 1,7-lactones **3a** and **4a** in red blood cells affected by *P. vera*. In our mind the ideal method should permit an analysis of Sias without any derivatization, using HPLC-MS, in order to exclude any possible artefact originating during the commonly used conditions of volatilization.[7] We recently found that the treatment of Sias with perfluorinated anhydrides, under volatilization conditions, can cause the N-transacylation of the *N*-acetyl group,[8b] the dehydration of the anomeric hydroxyl[8c] or other unexpected transformations.[8c,f] Thus, we considered the availability of authentic pure reference standards of the lactones **3a** and **4a** as essential, and of their isotopologues **3b**,**c** and **4b**,**c** necessary as standards in any suitable analytical protocol based on HPLC-MS or for setting-up the appropriate and controlled methods of derivatization.

As a result of our interest, we recently accomplished the first synthesis of lactones Neu5Ac1,7L **3a** and Neu5Gc1,7L **4a**.[8a] Herein, we report the synthesis of their isotopologues **3b**,**c** and **4b**,**c**, labeled with deuterium at the *N*-acyl group and both at the acyl group and at the pyranose ring, prepared in two relatively simple methods by the application of new Sias chemistry. Key intermediates for the synthesis of these compounds were four isotopologues **1b**,**c** and **2b**,**c** of the parent Sias prepared herein.

2. Results and discussion

In our synthetic work, we planned to label the parent sialic acids and then subject them to a lactonization procedure which uses benzyloxycarbonyl chloride (CbzCl) as a promoter of the 1,7-lactonization[8a] (Scheme 1).

Using this method, we could overcome the difficulties connected with the direct labeling of the free 1,7-lactones due to their particular lability. Moreover, since Neu5Gc **2a** is not commercially available and the reported procedures for its preparation from the congener Neu5Ac **1a** were not completely satisfactory,[9] we also decided to search for a more convenient protocol for the transformation of Neu5Ac **1a** into Neu5Gc **2a**. The success of our work would allow us to dispose of four different isotopologues of Sias (i.e., compounds **1b**,**c** and **2b**,**c**), of self consistent utility as standards for a possible analytical protocol for the simultaneous evaluation of the parent Sias and of their 1,7-lactones. Initially we solved the synthetic problem by setting-up a protocol which started from the protected neuraminic acid methyl ester **5** (Scheme 2).[9]

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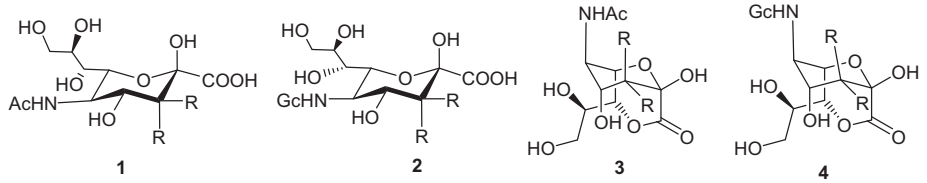
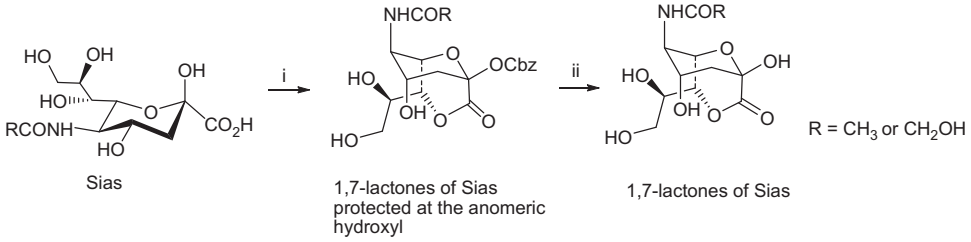


a: Ac = CH_3CO; Gc = $HOCH_2CO$, R = H

b: Ac = CD_3CO; Gc = $HOCD_2CO$, R = H

c: Ac = CD_3CO; Gc = $HOCD_2CO$, R = D

Figure 1.



Scheme 1. Reagents and conditions: (i) Et_3N, THF–DMF (4:3; v/v), 0 °C, then CbzCl, THF, 0–23 °C, 1 h, 70–90%; (ii) H_2, Pd on C 10%, AcOEt, 23 °C, 88–96%.

According to this, the reaction of the methyl ester[9] **5** with trideuterated acetyl chloride in methanol afforded Neu5[$^2H_3$]Ac methyl ester methyl acetal **6** which, after a treatment with aqueous sodium hydroxide and then with acidic sulfonic resin, at 80 °C for 4 h, afforded the trideuterated acid Neu5[$^2H_3$]Ac **1b**. This, upon repeated treatment with NaO[^{2}H] in [$^2H_2$]O, afforded the pentadeuterated isotopologue **1c** deriving from a keto-enolic equilibrium of the starting keto-acid in the labeled basic medium. A parallel procedure allows the conversion of the methyl ester **5** into the Sias **2b** and **2c**. In this case, we introduced a deuterated glycolyl group at the neuraminic acid nitrogen by reaction of the protected methyl ester **5** with dideuterated benzyloxyacetyl chloride and successive hydrogenolysis of the obtained benzylated glycolic acid derivative **7**. Thus, we obtained the deuterated glycolic acid **8** in a two steps, acylation (treatment with labeled benzyloxyacetyl chloride and regeneration of the glycolic hydroxyl). This route permits a quantitative introduction of two deuterium atoms in the methylene group of the acylating agent, by simple basic treatment of the protected glycolic acid in ^2H_2O, a procedure which is not practicable on the free glycolic acid. On the other hand, this two step glycosylation procedure via the protected acyl chloride of glycolic acid is routinely used in our laboratory for the transformation of the unlabeled Neu5Ac **1a** into Neu5Gc **2a** since, in our hands, it is more convenient than the direct acylation of the ester **5** with deuterated glycolic acid and DCC.[9]

From the methyl ester methyl acetal **8**, we prepared the deuterated Neu5Gc acids **2b** and **2c**, adopting the previously set-up procedure. However, while we accomplished the preparation of these deuterated Sias, in our laboratory, we discovered an interesting procedure which allows the N-transacylation of secondary acylamides to different ones via perfluorinated analogues (Fig. 2).[8b]

Thus, we decided to adopt the disclosed N-transacylation reaction to set-up a simplified procedure for the preparation of all the obtained deuterated lactones. In effect, with double successive transacylations, we could obtain the desired deuterated peracylated methyl esters **11** with a simplified procedure, starting from Neu5Ac **1** (Scheme 3). We first prepared the peracylated methyl ester **9**, which then was transacylated to its trideuterated isotopologue **11** which, by simple saponification of its protective groups by treatment with NaOH or with NaOD, afforded the Sias **1b** or **1c**, the first deuterated at the acyl group, the second deuterated both at the acyl group and position 3.

Next, we attempted the N-transacylation of the trifluoroacetamide **10** reacting it with 2,2-dideuterated benzyloxyacetylchloride, under the above reported conditions. Unexpectedly, in this case the reaction afforded very erratic results, even when performed in a large scale and was studied using unlabeled benzyloxyglycolyl chloride. In the best case the desired N-transacylated compound **12b** was obtained in variable but constantly poor yields (8–22%), always being accompanied by a mixture of inseparable compounds apparently derived from a rearrangement and polimerization of Sias.[10] Thus, we decided to discontinue this route considering also that the deuterated glycolic acids useful for the synthesis of the desired lactones **4b** and **4c** were conveniently available from the previously described route (Scheme 2).

In fact, with the four deuterated acids in hand, we performed their lactonization, reacting each of them, dissolved in DMF containing Et_3N, with benzyloxycarbonyl chloride, at 0 °C for 1 h (Scheme 4).[8d]

Under these conditions, the corresponding 2-benzyloxycarbonyl lactones **13–16** are formed, all possessing physicochemical properties, apart from NMR and mass spectra, superimposable to those observed for the unlabeled isotopologues.[8d] Similarly, each protected lactone, by simple hydrogenolysis in the presence of palladium on carbon, afforded the corresponding free 1,7-lactone showing the expected physicochemical properties and ^{1}H and ^{13}C NMR spectra.

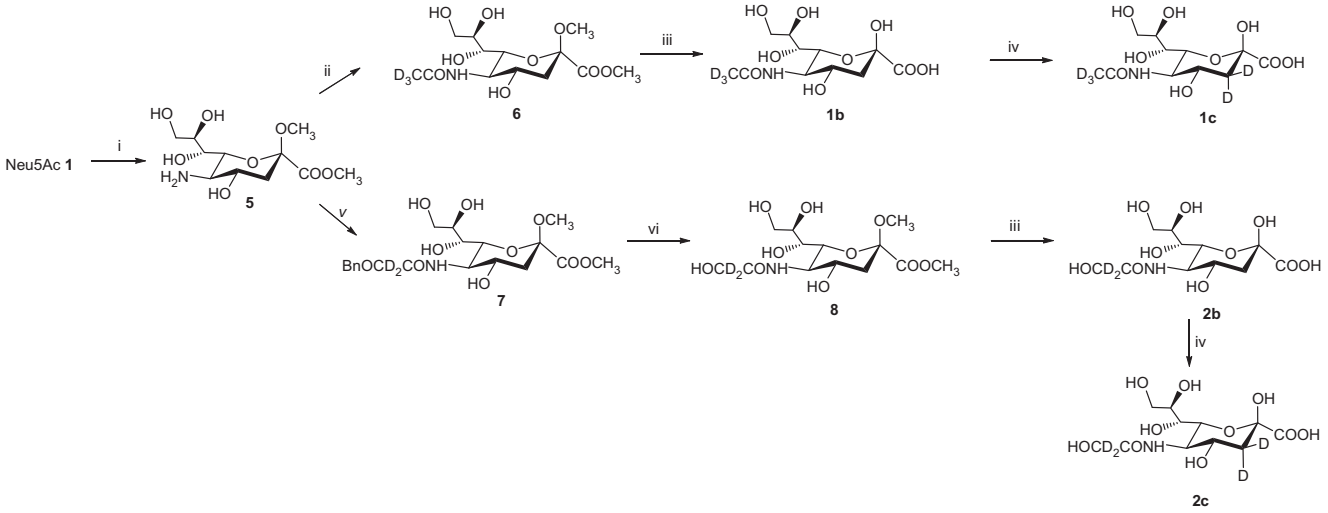
3. Conclusion

In conclusion we have set-up two protocols useful to prepare labeled sialic acids **1b**,**c** **2b**,**c** and their 1,7-lactones **3b**,**c** and **4b**,**c** which are now available for analytical, synthetic and biological studies.

4. Experimental section

4.1. General methods

Melting points are uncorrected. Nuclear magnetic resonance spectra were recorded at 298 K operating at 500.13 MHz for ^{1}H



Scheme 2. Reagents and conditions: (i) HCl in MeOH, 100 °C, 4.5 h; then DOWEX OH^-, 69%; (ii) C[$^2H_3$] COCl, Et_3N, MeOH, 25 °C, 2 h, 84%; (iii) NaOH/H_2O, 40 °C, 0.5 h, then DOWEX H^+, 80 °C, 4 h, 72% (**1b**) or 74% (**2b**); (iv) NaO[^{2}H]/[$^2H_2$]O, 25 °C, 3 h, 84 (**1c**) or 90% (**2c**); (v) BnOC[$^2H_2$]COCl, Et_3N, MeOH 0 °C, 2 h, 83%; (vi) H_2, Pd on C 10%, THF, 23 °C, 92%.

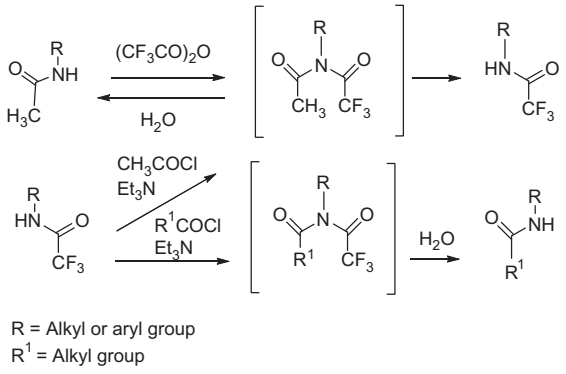
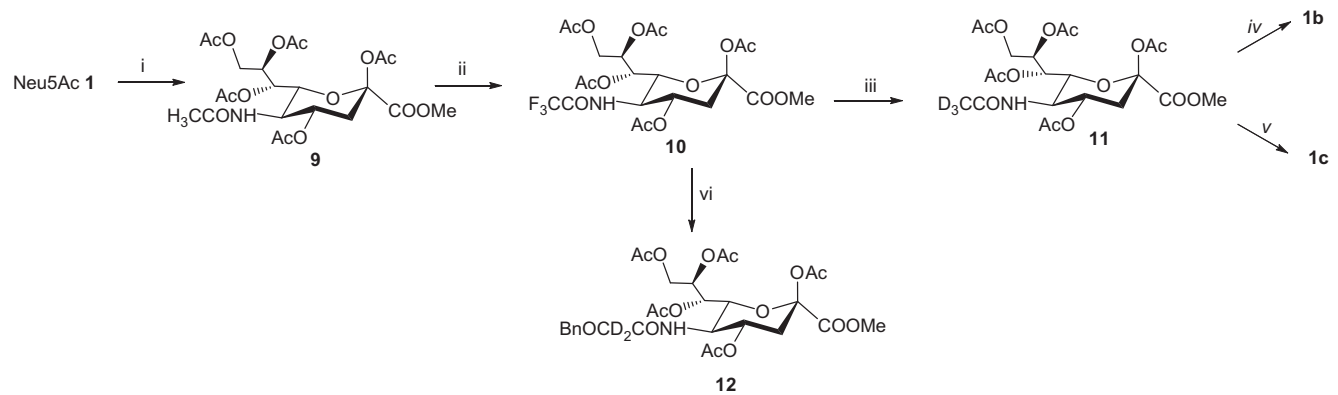
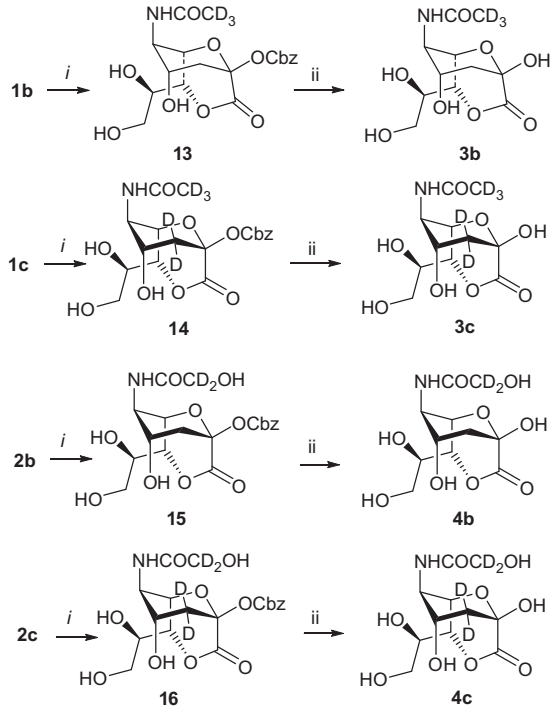


Figure 2.

and 125.76 MHz for ^{13}C. Chemical shifts are reported in parts for million (ppm, δ units) relative to $CDCl_3$ signal fixed at 7.26 ppm for ^1H and at 77.0 ppm for ^{13}C spectra, relative to CD_3OD signal fixed at 3.31 ppm for ^1H and at 49.05 ppm for ^{13}C spectra, relative to DMSO-d_6 signal fixed at 2.50 ppm for ^1H and at 39.43 for ^{13}C spectra and relative D_2O signal refer to $(CH_3)_3COH$, used as a reference standard, at 1.23 ppm for ^1H and at 30.29 for ^{13}C spectra. Proton and carbon assignments were established, if necessary, with ^1H–^1H and ^1H–^{13}C correlated NMR experiments. ^1H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; br s, broad singlet; m, multiplet), coupling constant(s) in Hertz, number of protons 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 concentration is given in g/100 mL. Infrared (IR) spectra were recorded in Nujol. Mass spectrometry was performed using a quadrupole ion-trap mass spectrometer equipped with an 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. 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. Isotopical purity of all target compounds was determined using a JEOL Accuo-ToF instrument in positive ESI mode. All reactions were 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 or 0.2% ninhydrin in ethanol and heat as a developing agent. All flash chromatography was performed with normal phase silica gel, following the general protocol of Still.[11]



Scheme 3. Reagents and conditions: (i) MeOH, DOWEX H^+, 23 °C, 5 h; then Ac_2O, Py, 23 °C, 6 h, 89%; (ii) TFAA, Et_3N, CH_3CN, 135 °C, 5 min, 82%; (iii) C[$^2H_3$] COCl, DIPEA, CH_2Cl_2 0 °C, 20 min, 72%; (iv) NaOH/H_2O, 2.5 h, 82–86%; (v) NaO[^{2}H]/[$^2H_2$]$_2$O, 23 °C, 5 h, 76–80%; (vi) BnOC[$^2H_2$]COCl, DIPEA, CH_2Cl_2, 0 °C, 20 min, 8–22%.



Scheme 4. Reagents and conditions: (i) Et_3N, THF–DMF, 0 °C, then CbzCl, THF, 0–23 °C, 1 h, 70–78%; (ii) H_2, Pd on C 10%, AcOEt, 23 °C, 89–93%.

4.2. *N*-[$^2H_3$]-Acetyl-2-*O*-methyl-β-neuraminic acid methyl ester (6)

[$^2H_3$]-Acetyl chloride (0.84 ml, 11.85 mmol) was added dropwise to a stirred solution of the amine **5** (700 mg, 2.37 mmol) in anhydrous methanol containing Et_3N (3.28 mL, 23.7 mmol), at 0 °C. The mixture was stirred for 2 h at 22 °C and evaporated under reduced pressure. Purification of the crude residue by flash chromatography [eluting with CH_2Cl_2/MeOH (4/1; v/v)] afforded the amide **6** (677 mg, 84%), as a white solid: mp 115–117 (from diethyl ether); $[\alpha]_D^{25} = -7.5$ (*c* 1, CH_3OH); ^1H NMR (CD_3OD) δ 4.03 (m, 1H; H-4), 3.94–3.88 (overlapping, 2H; H-5 and H-6), 3.85 (s, 3H; $COOCH_3$), 3.81–3.75 (overlapping, 2H; H-8 and H-9a), 3.72 (dd, $J_{9b,9a}$ = 11.8, $J_{9b,8}$ = 5.3 Hz, 1H; H-9b), 3.60 (d, $J_{7,8}$ = 8.7 Hz, 1H; H-7), 3.25 (s, 3H; OCH_3), 2.32 (dd, $J_{3a,3b}$ = 13.0, $J_{3a,4}$ = 5.0 Hz, 1H; H-3a), 1.70 (dd, $J_{3b,3a}$ = 13.0 Hz, $J_{3b,4}$ = 12.0 Hz, 1H; H-3b); ^{13}C NMR (CD_3OD) 174.9 (br s, CONH), 172.2 (C-1), 100.7, (C-2), 72.3 (C-6), 71.6 (C-8), 69.4 (C-7), 67.4 (C-4) 64.7 (C-9), 53.8 (C-5), 53.2 ($COOCH_3$), 52.0 (OCH_3), 41.4 (C-3), 23.4 (hept, *J* = 19.2 Hz, *C*D_3CO); MS (ESI positive): *m/z* 363.3 $[M+Na]^+$, 99% isotopically pure. Anal. Calcd for $C_{13}H_{20}D_3NO_9$: C, 45.88; H+D, 7.70; N, 4.12. Found: C, 45.78; H+D, 7.79; N, 4.15.

4.3. *N*-Benzyloxy-[$^2H_2$]-acetyl-2-*O*-metyl-β-neuraminic acid methyl ester 7

Benzyloxy-[$^2H_2$]-acetyl chloride (0.9 ml, 6.33 mmol) was added dropwise to a stirred solution of the amine **5** (700 mg, 2.11 mmol) in anhydrous methanol containing Et_3N (1.7 mL, 12.7 mmol), at 0 °C. The mixture was stirred for 2 h at 22 °C and evaporated under reduced pressure. Purification of the crude residue by flash chromatography [eluting with CH_2Cl_2/MeOH (9/1; v/v)] afforded the amide **7** (785 mg, 83%), as a white solid: mp 136–140 °C (from diethyl ether); $[\alpha]_D^{25} = -35.2$ (*c* 1, H_2O); ^1H NMR (CD_3OD) δ 7.43–7.27 (m, 5H; Ph), 4.61 (AB system, 2H; CH_2Ph), 4.10 (m, 1H; H-4), 3.98–3.9 (overlapping, 2H; H-6 e H-5), 3.84–3.78 (overlapping, 5H; $COOCH_3$, H-8, H-9a), 3.65 (dd, $J_{9b,9a}$ = 12.1 Hz, $J_{9b,8}$ = 6.0 Hz, 1H; H-9a), 3.52 (dd, $J_{7,8}$ = 6.0 Hz, 1H; H-7), 3.28 (s, 3H; OCH_3), 2.35 (dd, $J_{3a,3b}$ = 13.0, $J_{3a,4}$ = 5.00 Hz, 1H; H-3a), 1.65 (dd, $J_{3b,3a}$ = 13.0 Hz, $J_{3b,4}$ = 11.5 Hz, 1H; H-3b); ^{13}C NMR (CD_3OD) δ 173.9 (C-1), 171.0 (br s, CONH), 135.5, 129.5, 129.3, 129.1 (Ph), 100.5, (C-2), 74.4 (CH_2Ph), 72.0 (C-6), 71.5 (C-8), 70.1 (C-7), 67.5 (t, *J* = 21.4 Hz, OCD_2CO), 67.2 (C-4) 65.3. (C-9), 53.6 (C-5), 53.2 ($COOCH_3$), 51.7 (OCH_3) 41.8 (C-3); MS (ESI positive): *m/z* 468.1 $[M+Na]^+$; 99% isotopically pure. Anal. Calcd for $C_{20}H_{27}D_2NO_{10}$: C, 53.93; H+D, 7.01; N, 3.14. Found: C, 55.90; H+D, 6.97; N, 3.12.

4.4. *N*-[$^2H_2$]-Glycolyl-2-*O*-metyl-β-neuraminic acid methyl ester 8

N-Benzyloxy-[$^2H_2$]-acetyl-2-*O*-methyl-β-neuraminic acid methyl ester **7** (650 mg, 1.46 mmol) was dissolved in THF (10 mL) and treated with H_2 in the presence of 10% Pd/C for 3 h at 22 °C. The catalyst was filtered through a pad of Celite and the solvent was evaporated under reduced pressure. The crude residue was then crystallized from a solution of ethanol in water to give the debenzylated compound **8** (477 mg, 92%), as a white solid: mp 138–140; $[\alpha]_D^{25} = +7.1$ (*c* 1, CH_3OH); ^1H NMR (CD_3OD) δ 4.12 (m, 1H; H-4), 3.97–3.93 (overlapping, 2H; H-6 e H-5), 3.83–3.76 (overlapping, 5H; $COOCH_3$, H-8 and H-9a), 3.62 (dd, $J_{9b,9a}$ = 12.0 Hz, $J_{9b,8}$ = 5.9 Hz, 1H; H-9a), 3.52 (dd, $J_{7,8}$ = 6.0 Hz, 1H; H-7), 3.27 (s, 3H; OCH_3), 2.35 (dd, $J_{3a,3b}$ = 13.1, $J_{3a,4}$ = 5.00 Hz, 1H; H-3a), 1.65 (dd, $J_{3b,3a}$ = 13.1 Hz, $J_{3b,4}$ = 11.5 Hz, 1H; H-3b); ^{13}C NMR (CD_3OD) δ 173.7 (C-1), 169.8 (br s, CONH), 100.3, (C-2), 71.9 (C-6), 71.4 (C-8), 70.0 (C-7), 67.5 (t, *J* = 21.2 Hz, OCD_2CO), 67.1 (C-4) 65.3 (C-9), 53.6 (C-5), 53.1 ($COOCH_3$), 51.5 (OCH_3) 41.7 (C-3); MS (ESI positive): *m/z* 378.4 $[M+Na]^+$; 99% isotopically pure. Anal. Calcd for $C_{13}H_{21}D_2NO_{10}$: C, 43.94; H+D, 7.09; N, 3.94. Found: C, 43.90; H+D, 7.02; N, 3.92.

4.5. *N*-[$^2H_3$]-Acetyl-neuraminic acid 1b

A solution of *N*-[$^2H_3$]acetyl-2-*O*-methyl-β-neuraminic acid methyl ester **6** (400 mg, 1.18 mmol) in H_2O (7.0 mL) was treated with an aqueous solution of NaOH (1.8 mL, 1.0 M) at 40 °C for 0.5 h. Then, Dowex® 50WX8 (H^+) resin (500 mg) was added and the solution was warmed at 80 °C for 4 h. After filtration of the resin, lyophilisation of the solution gave a crude residue which, after crystallization, afforded the pure compound **1b** (265 mg, 72%), as a white solid: mp 178–181 (from CH_3CN); $[\alpha]_D^{25} = -34.0$ (*c* 1, H_2O); ^1H NMR (D_2O) δ 4.12–4.04 (overlapping, 2H; H-4, H-6), 3.95 (m, 1H; H-5), 3.86 (d, $J_{9a,9b}$ = 11.8 Hz, 1H; H-9a), 3.77 (m, 1H; H-8), 3.64 (dd, $J_{9b,9a}$ = 11.8, $J_{9b,8}$ = 6.5 Hz, 1H; H-9a), 3.58 (d, $J_{7,8}$ = 9.1 Hz, 1H; H-7), 2.34 (dd, $J_{3a,3b}$ = 13.0, $J_{3a,4}$ = 2.8 Hz, 1H; H-3a), 1.90 (t app., $J_{3b,3a}$ = 13.0 Hz, 1H; H-3b); ^{13}C NMR (D_2O) δ ^{13}C NMR (D_2O) δ 175.5 (br s, *C*OCD_3), 173.7 (C-1), 95.8 (C-2), 71.0 (C-6), 70.8 (C-8), 68.8 (C-7), 67.3 (C-4), 63.8 (C-9), 52.6 (C-5), 39.5 (C-3), 22.7 (hept., *J* = 19.3 Hz, *C*D_3CO); MS (ESI negative): *m/z* 311.1 $[M-H]^-$; 99% isotopically pure. Anal. Calcd for $C_{11}H_{16}D_3NO_9$: C, 42.31; H+D, 7.10; N, 4.49. Found: C, 42.40; H+D, 7.00; N, 4.52.

4.6. *N*-[$^2H_3$]-Acetyl-[3,3-$^2H_2$]-neuraminic acid 1c

N-[$^2H_3$]-acetyl-β-neuraminic acid **1b** (150 mg, 0.48 mmol) was treated with a solution of NaOD in D_2O (0.9 mL, 1.0 M) at 22 °C for 3 h. The solution was neutralized with acidic resin [Dowex® 50WX8 (H^+)], then the resin was filtered and the solution was lyophilized to afford the crude acid **1c** (127 mg, 84%) which, after crystallization shows: mp 178–180 (from CH_3CN); $[\alpha]_D^{25} = -33.2$ (*c* 1, H_2O); ^1H NMR (D_2O) δ 4.08–4.03 (overlapping, 2H; H-4 and H-6), 3.93 (dd, $J_{5,4}$ = 10.2, $J_{5,6}$ = 10.2 Hz 1H; H-5), 3.84 (dd, $J_{9a,9b}$ = 11.9, $J_{9a,8}$ = 2.6 Hz, 1H; H-9a), 3.75 (m, 1H; H-8), 3.62 (dd, $J_{9b,9a}$ = 11.9, $J_{9b,8}$ = 6.3 Hz, 1H; H-9b), 3.56 (dd, $J_{7,8}$ = 9.2, $J_{7,6}$ <1.0 Hz, 1H; H-7); ^{13}C NMR (D_2O) δ 175.6 (br s, *C*OCD_3), 173.8

(C-1), 95.9 (C-2), 71.1 (C-6), 70.8 (C-8), 68.9 (C-7), 67.3 (C-4), 63.8 (C-9), 52.7 (C-5), 39.5 (m, C-3), 22.8 (hept., J = 19.4 Hz, CD_3CO); MS (ESI negative): m/z 313.3 $[M-H]^-$. 99% isotopically pure.

Anal. Calcd for $C_{11}H_{14}D_5NO_9$: C, 42.04; H+D, 7.69; N, 4.46. Found: C, 42.10; H+D, 7.82; N, 4.52.

4.7. *N*-[$^2H_2$]-Glycolyl-neuraminic acid 2b

A solution of *N*-[$^2H_2$]-glycolyl-2-*O*-methyl-β-neuraminic acid methyl ester **8** (400 mg, 1.13 mmol) in H_2O (10 mL) was treated with an aqueous solution of NaOH (0.6 mL, 1.0 M) at 40 °C for 0.5 h. Then, Dowex® 50WX8 (H^+) (360 mg) was added and the solution was warmed at 80 °C for 4 h. Filtration of the resin and crystallization afforded the acid **2b** (272 mg, 74%), as a white solid: mp 182–185 °C (from CH_3CN); $[\alpha]_D^{25} = -36.1$ (c 1, H_2O); ^1H NMR (D_2O) δ 4.15–4.03 (overlapping, 2H, H-4, and H-6), 3.93 (m, 1H; H-5), 3.85 (dd, $J_{9a,9b}$ = 11.8, $J_{9a,8}$ = 2.6 Hz 1H; H-9a), 3.66 (m, 1H; H-8), 3.54 (dd, $J_{9b,9a}$ = 11.8, $J_{9b,8}$ = 6.2 Hz, 1H; H-9b), 3.47 (dd, $J_{7,8}$ = 9.4, $J_{7,6}$ <1.0 Hz, 1H; H-7), 2.26 (dd, $J_{3a,3b}$ = 13.0, $J_{3a,4}$ = 4.9 Hz, 1H; H-3a), 1.82 (dd, $J_{3b,3a}$ = 13.0, $J_{3b,4}$ = 11.7 Hz, 1H; H-3b); ^{13}C NMR (D_2O) δ 176.1 (br s, CD_2CONH), 173.5 (C-1), 95.7, (C-2), 70.7 (C-8), 70.6 (C-6), 68.6 (C-4), 66.9 (C-7), 63.3 (C-9), 61.4 (t, J = 21.2 Hz, $COCD_2OH$), 52.2 (C-5), 39.3 (C-3); MS (ESI negative): m/z 326.3 $[M-H]^-$; 99% isotopically pure. Anal. Calcd for $C_{11}H_{17}D_2NO_{10}$: C, 40.37; H+D, 6.47; N, 4.28. Found: C, 40.48; H+D, 6.51; N, 4.33.

4.8. *N*-[$^2H_2$]-Glycolyl-[3,3-$^2H_2$]-neuraminic acid 2c

N-[$^2H_2$]-Glycolyl-neuraminic acid **2b** (150 mg, 0.46 mmol) was treated with a solution of NaOD in D_2O (0.9 mL, 1.0 M) at 23 °C for 3 h. After neutralization of the solution with acid resin [Dowex® 50WX8 (H^+)], filtration of the resin and lyophilisation of the solution, a crude acid is obtained which, after crystallization, afforded the acid **2c** (136 mg, 90%), as a white solid: mp 175–177 (from CH_3CN); $[\alpha]_D^{25} = -35.0$ (c 1, H_2O); ^1H NMR (D_2O) δ 4.17–4.04 (overlapping, 2H, H-4, and H-6), 3.94 (m, 1H; H-5), 3.85 (dd, $J_{9a,9b}$ = 11.7, $J_{9a,8}$ = 2.6 Hz 1H; H-9a), 3.66 (m, 1H; H-8), 3.53 (dd, $J_{9b,9a}$ = 11.7, $J_{9b,8}$ = 6.1 Hz 1H; H-9b), 3.46 (dd, $J_{7,8}$ = 9.2, $J_{7,6}$ <1.0 Hz, 1H; H-7); ^{13}C NMR (D_2O) δ 175.9 (br s, CD_2CONH), 173.3 (C-1), 95.5, (C-2), 70.6 (C-8), 70.5 (C-6), 68.4 (C-4), 66.6 (C-7), 63.1 (C-9), 61.2 (t, J = 21.4 Hz, $COCD_2OH$), 52.0 (C-5), 39.1 (m, C-3); MS (ESI negative): m/z 328.3 [M−H]; 99% isotopically pure. Anal. Calcd for $C_{11}H_{15}D_4NO_{10}$: C, 40.12; H+D, 7.04; N, 4.25. Found: C, 40.20; H+D, 7.09; N, 4.18.

4.9. 2,4,7,8,9-Penta-*O*-acetyl-5-*N*-[$^2H_3$]-acetyl-β-neuraminic acid methyl ester 11

[$^2H_3$]-Acetyl chloride (0.14 ml, 2.04 mmol) in CH_2Cl_2 (1.8 mL) was added drop wise, under stirring, to a solution of 2,4,7,8,9-penta-*O*-acetyl-5-*N*-(2,2,2-trifluoroacetyl)-β-neuraminic acid methyl ester[8f] **10** (200 mg, 0.34 mmol) containing diisopropylethylamine (1.18 mL, 6.8 mmol) in CH_2Cl_2 (1.8 mL), cooled at 0 °C. After a 20 min the reaction was stopped by addition of an ice cold aqueous solution of HCl (6.0 mL, 1 M). The organic layer was separated and the aqueous solution was extracted twice with dichloromethane. The organic layer was then washed in the sequence with water, with an aqueous solution of sodium hydrogen carbonate and with water, dressed and evaporated under reduced pressure to afford a crude residue which was purified by flash chromatography [eluting with AcOEt/MeOH (95/5; v/v)] to give the ester **11** (131 mg, 72%), as a white solid: mp 133–136 °C; $[\alpha]_D^{20} = -18.3$ (c 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.39–5.32 (overlapping, 2H; H-7, and N–H), 5.24 (m, 1H; H-4), 5.06 (m, 1H; H-8), 4.49 (dd, $J_{9a,9b}$ = 12.3, $J_{9a,8}$ = 1.9 Hz, 1H; H-9a), 4.14–4.08 (overlapping, 3H; H-5, H-6, and H-9b), 3.78 (s, 3H; $COOCH_3$), 2.54 (dd, $J_{3a,3b}$ = 13.5, $J_{3a,4}$ = 4.8 Hz, 1H; H-3a), 2.14 (s, 6H; 2 × CH_3COO), 2.08 (dd, $J_{3b,3a}$ = 13.5, $J_{3b,4}$ = 11.8 Hz, 1H; H-3b), 2.05 (s, 3H; CH_3COO); 2.03 (s, 6H; 2 × CH_3COO); ^{13}C NMR ($CDCl_3$) 170.9, 170.5, 170.2, 170.1, 168.2 (5C, $COCH_3$), 170.3 (br s, CONH), 166.3 (C-1), 97.5 (C-2), 72.8 (C-6), 71.3 (C-8), 68.3 (C-4), 67.9 (C-7), 62.1 (C-9), 53.2 ($COOCH_3$), 49.4 (C-5), 35.9 (C-3), 22.0 (hept, J = 19.4 Hz, CD_3CO), 20.9, 20.8 (2 × $COCH_3$), 20.7 (3 × $COCH_3$); IR 1750, 1721, 1668 cm^{-1}; MS (ESI positive): m/z 559.0 $[M+Na]^+$, 1095.1 $[2\,M+Na]^+$; 99% isotopically pure. Anal. Calcd for $C_{22}H_{28}D_3NO_{14}$: C, 49.25; H+D, 6.39; N, 2.61. Found: C, 49.23; H+D, 6.35; N, 2.68.

4.10. 2,4,7,8,9-Penta-*O*-acetyl-5-*N*-benzyloxy-[$^2H_2$]-acetyl-β-neuraminic acid methyl ester 12

Benzyloxy-[$^2H_2$]-acetyl chloride (0.32 ml, 2.04 mmol) in CH_2Cl_2 (1.8 mL) was added, at 0 °C, to a solution of 2,4,7,8,9-penta-*O*-Acetyl-5-*N*-(2,2,2-trifluoroacetyl)-β-neuraminic acid methyl ester[8f] **10** (200 mg, 0.34 mmol) in CH_2Cl_2 (1.8 mL) containing diisopropylethylamine (1.18 mL, 6.8 mmol). After a 20 min stirring at 0 °C, the reaction was stopped, by adding an ice cold aqueous solution of HCl (6.0 mL, 1 M), and the organic layer was separated. The aqueous phase was extracted twice with dichloromethane and the collected organic layers were washed in the sequence with water, an aqueous solution of $NaHCO_3$ and again with water. Dressing of the solution and evaporation of the organic solvent afforded a crude product which was purified by flash chromatography [eluting with AcOEt/hexane (2/3; v/v)], to afford the compound **12** (17 mg, 8%), as a white solid: mp 114–118 (form CH_3CN); $[\alpha]_D^{20} = -9.3$ (c 1, $CHCl_3$);^1H NMR ($CDCl_3$) δ 7.52–7.40 (m, 5H; Ph), 6.33 (d, $J_{NH,5}$ = 9.6 Hz, 1H; N–H), 5.38 (dd, $J_{7,8}$ = 6.2, $J_{7,6}$ = 2.3 Hz, 1H; H-7), 5.19 (m, 1H; H-4), 5.10 (m, 1H; H-8), 4.44 (dd, $J_{9a,9b}$ = 12.1, $J_{9a,8}$ = 2.3 Hz, 1H; H-9a), 4.15–4.06 (overlapping, 4H; H-5, H-9b, and $PhCH_2$), 4.03 (dd, $J_{6,8}$ = 10.8, $J_{6,7}$ = 2.3 Hz, 1H; H-6), 3.78 (s, 3H; $COOCH_3$), 2.59 (dd, $J_{3a,3b}$ = 13.5, $J_{3a,4}$ = 4.8 Hz, 1H; H-3a), 2.17 (s, 3H, CH_3COO), 2.11 (s, 6H, 2 × CH_3COO), 2.05 (s, 4H; CH_3COO, and H-3b); 1. 97 (s, 3H; CH_3COO); MS (ESI positive): m/z 664.2 $[M+Na]^+$; 99% isotopically pure. Anal. Calcd for $C_{29}H_{35}D_2NO_{15}$: C, 54.29; H+D, 6.13; N, 2.18. Found: C, 54.26; H+D, 6.15; N, 2.14.

4.11. Preparation of the Sias 1,7-lactones from the labeled acids: general procedure

4.11.1. Lactonization

CbzCl (3.2 mmol), dissolved in THF (1.5 mL), was added dropwise to a solution of the appropriate deuterated sialic acid (0.30 mmol), previously dissolved in a mixture of anhydrous THF (1.5 mL) and DMF (3.0 mL) and treated with triethylamine (3.8 mmol) under stirring for 15 min. The solution was then stirred a 0 °C for 15 min, heated to 23 °C and stirred for an additional 60 min. At this time, MeOH (0.3 mL) was added and stirring was continued for 15 min. The solvent was then evaporated under reduced pressure to afford a crude product which was purified by flash chromatography (using the reported solvent system) to afford the desired 1,7-lactone containing a benzyloxycarbonyl group at the anomeric carbon.

4.11.2. Regeneration of the anomeric hydroxy group

The protected 1,7-lactone (0.10 mmol) dissolved in ethyl acetate (10 mL) was hydrogenated in the presence of Pd/C (50 mg, 10%) for 2 h. At this time, the catalyst was filtered and washed with anhydrous THF and the solvent was evaporated under reduced pressure to afford the free 1,7-lactone in a pure form.

4.12. Synthesis of the *N*-[$^2H_3$]-acetyl-β-neuraminic acid 1,7-lactone 3b

(i) Lactonization of the *N*-[$^2H_3$]-acetyl-β-neuraminic acid **1b** (100 mg, 0.32 mmol) according to the general procedure, provided, after purification by flash silica gel chromatography [eluting with AcOEt/MeOH 9:1, v/v], the 2-benzyloxycarbonyl *N*-[$^2H_3$]-acetyl-neuraminic acid 1,7-lactone **13** (103 mg; 75%) as a white solid: mp 123–127 °C (decomp., in sealed tube); $[\alpha]_D^{25} = +16.5$ (*c* 1, CH_3OH); ^1H NMR (CD_3OD) δ 7.36–7.30 (m, 5H; Ph), 5.15 (AB system, 2H; CH_2Ph), 4.64 (s, 1H; H-6), 4.47 (d, $J_{7,8}$ = 8.9 Hz, 1H; H-7), 4.09 (m, 1H; H-4), 4.02 (br s, 1H; H-5), 3.96 (m, 1H; H-8), 3.81 (system ABX, $J_{9a,9b}$ = 11.7, $J_{9a,8}$ = 2.7, 1H; H-9a), 3.77 (system ABX, $J_{9b,9a}$ = 11.7, $J_{9b,8}$ = 4.5 Hz, 1H; H-9b), 2.26 (dd, $J_{3a,3b}$ = 13.6, $J_{3a,4}$ = 3.5, 1H; H-3a), 2.15 (dd, $J_{3b,3a}$ = 13.6, $J_{3b,4}$ = 1.8, 1H; H-3b); ^{13}C NMR (CD_3OD) δ 172.9 (br s, CD_3CONH), 168.0 (C-1), 153.4 (PhCH_2OCO), 136.2, 129.7, 129.6, 129.2 (Ph), 94.9, (C-2), 79.7 (C-7), 73.1 (C-6), 71.9 (C-8), 71.3 (PhCH_2OCO), 67.4 (C-4), 63.3 (C-9), 52.5 (C-5), 36.9 (C-3), 22.2 (hept., *J* = 19.4 Hz, CD_3CO); IR, (Nujol) 3327, 1755 cm^{-1}; MS (ESI negative): *m/z* 427.2 [M−H]; 99% isotopically pure. Anal. Calcd for $C_{19}H_{20}D_3NO_{10}$: C, 53.27; H+D, 6.12; N, 3.27. Found: C, 53.32; H+D, 6.19; N, 3.35.

(ii) Hydrogenation of the 2-benzyloxycarbonyl-*N*-[$^2H_3$]-acetyl-β-neuraminic acid 1,7-lactone **13** (57 mg, 0.13 mmol), according to general procedure, provided the title lactone **3b** (36 mg; 91%) as a white solid: mp 110–113 °C (dec., in sealed tube); $[\alpha]_D^{25} = +25.0$ (*c* 1, THF); (DMSO-d_6) 4.31 (s, 1H; H-6), 4.18 (d, $J_{7,8}$ = 8.5 Hz, 1H; H-7), 3.80 (br s, 1H; H-4), 3.73 (br d, 1H; H-5), 3.59 (m, 1H; H-9a), 3.54 (m, 1H; H-8), 3.45 (dd, $J_{9b,9a}$ = 10.8, $J_{9b,8}$ = 5.0 Hz, 1H; H-9b), 1.93 (br dd, $J_{3a,3b}$ = 13.8, $J_{3a,4}$ = 2.0 Hz, 1H; H-3a), 1.81 (br d, $J_{3b,3a}$ = 13.8 Hz, 1H; H-3b). δ_C (DMSO-d_6) 169.2 (C-1), 168.7 (br s, CD_3CONH), 90.2 (C-2), 77.3 (C-7), 71.1 (C-8), 69.9 (C-6), 65.8 (C-4), 61.7 (C-9), 50.2 (C-5), 36.8 (C-3), 22.1 (hept., *J* = 19.3 Hz, CD_3CO); IR, (nujol) 3328, 1737 cm^{-1}; MS (ESI negative): *m/z* 293.2 $[M-H]^-$; 99% isotopically pure. Anal. Calcd for $C_{11}H_{14}D_3NO_8$: C, 44.90; H+D, 6.85; N, 4.76. Found: C, 44.72; H+D, 6.89; N, 4.69.

4.13. Synthesis of the *N*-[$^2H_3$]-Acetyl-β-[3,3-$^2H_2$]-neuraminic acid 1,7-lactone 3c

(i) The *N*-[$^2H_3$]-acetyl-β-[3,3-$^2H_2$]-neuraminic acid **1c** (100 mg, 0.32 mmol), lactonized according to the general procedure, gave, after purification by flash silica gel chromatography [AcOEt/MeOH 9:1, v/v], the 2-benzyloxycarbonyl-[3,3-$^2H_2$]-*N*-[$^2H_3$]-acetyl-β-neuraminic acid 1,7-lactone **14** (97 mg; 70%) as a white solid: mp 109–111 °C (decomp., in sealed tube; from CH_3CN); $[\alpha]_D^{25} = +17.2$ (*c* 1, CH_3OH); ^1H NMR (CD_3OD) δ 7.37–7.32 (m, 5H; Ph), 5.17 (AB system, 2H; CH_2Ph), 4.63 (s, 1H; H-6), 4.45 (d, $J_{7,8}$ = 9.0 Hz, 1H; H-7), 4.07 (br s, 1H; H-4), 4.02 (m, 1H; H-5), 3.95 (ddd, $J_{8,7}$ = 9.0, $J_{8,9b}$ = 4.5, $J_{8,9a}$ = 2.7 Hz, 1H; H-8), 3.79 (system ABX, $J_{9a,9b}$ = 11.8, $J_{9a,8}$ = 2.7, $J_{9b,8}$ = 4.5 Hz, 2H; H-9a, and H-9b); ^{13}C NMR (CD_3OD) δ 172.8 (br s, CD_3CONH), 167.8 (C-1), 153.2 (PhCH_2OCO), 136.1, 129.6, 129.5, 129.3 (Ph), 94.8 (C-2), 79.7 (C-7), 73.2 (C-6), 71.8 (C-8), 71.2 (PhCH_2OCO), 67.3 (C-4), 63.2 (C-9), 52.5 (C-5), 36.8 (m, C-3), 22.1 (hept., *J* = 19.4 Hz, CD_3CO); IR, (Nujol) 3326, 1753 cm^{-1}; MS (ESI negative): *m/z* 429.1 [M−H]; 99% isotopically pure. Anal. Calcd for $C_{19}H_{18}D_5NO_{10}$: C, 53.02; H+D, 6. 55; N, 3.25. Found: C, 53.10; H+D, 6.59; N, 3.30.

(ii) Hydrogenation of the 2-benzyloxycarbonyl lactone **14** (50 mg, 0.12 mmol), according to the general procedure, gave the title lactone **3c** (32 mg; 93%) as a white solid: mp 110–113 °C (dec., in sealed tube); $[\alpha]_D^{25} = +27.0$ (*c* 1, THF); ^1H NMR (DMSO-d_6) δ 4.30 (s, 1H; H-6), 4.17 (d, $J_{7,8}$ = 8.5 Hz, 1H; H-7), 3.79 (br s, 1H; H-4), 3.72 (br d, 1H; H-5), 3.57 (m, 1H; H-9a), 3.52 (m, 1H; H-8), 3.44 (dd, $J_{9b,9a}$ = 10.8, $J_{9b,8}$ = 5.0 Hz, 1H; H-9b), ^{13}C NMR (DMSO-d_6) δ 169.1 (C-1), 168.6 (br s, CD_3CONH), 90.1 (C-2), 77.2 (C-7), 71.0 (C-8), 69.9 (C-6), 65.7 (C-4), 61.6 (C-9), 50.1 (C-5), 36.7 (m, C-3), 22.1 (hept., *J* = 19.2 Hz, CD_3CO); IR, (nujol) 3326, 1734 cm^{-1}; MS (ESI negative): *m/z* 295.4 [M−H]; 99% isotopically pure. Anal. Calcd for $C_{11}H_{12}D_5NO_8$: C, 44.59; H+D, 7.48; N, 4.73. Found: C, 44.52; H+D, 7.59; N, 4.65.

4.14. Synthesis of *N*-[$^2H_2$]-glycolyl-β-neuraminic acid 1,7-lactone 4b

(i) The *N*-[$^2H_2$]-glycolyl-β-neuraminic acid **2b** (100 mg, 0.30 mmol) lactonized according to the general procedure, gave, after purification by flash chromatography (eluting with AcOEt/MeOH 9:1, v/v), the 2-benzyloxycarbonyl-*N*-[$^2H_2$]-glycolyl-β-neuraminic acid 1,7-lactone **15** (104 mg; 78%), as a white solid: mp 107–109 °C (decomp., in sealed tube; from CH_3CN); $[\alpha]_D^{25} = +9.0$ (*c* 1, CH_3OH); ^1H NMR (CD_3OD) δ 7.37–7.35 (m, 5H; Ph), 5.17 (AB system, 2H; CH_2Ph), 4.65 (br s, 1H; H-6), 4.47 (d, $J_{7,8}$ = 9.0 Hz, 1H; H-7), 4.11 (br m, 1H; H-4), 4.08 (br s, 1H; H-5), 3.95 (ddd, $J_{8,7}$ = 9.0, $J_{8,9b}$ = 4.4, $J_{8,9a}$ = 2.9 Hz, 1H; H-8), 3.78 (ABX system, $J_{9a,9b}$ = 11.6, $J_{9b,8}$ = 4.5, $J_{9a,8}$ = 2.9 Hz, 2H; H-9a, and 9b), 2.22 (dd, $J_{3a,3b}$ = 13.7, $J_{3a,4}$ = 3.5 Hz, 1H; H-3a), 2.16 (dd, $J_{3b,3a}$ = 13.7, $J_{3b,4}$ = 2.2 Hz, 1H; H-3b); ^{13}C NMR (CD_3OD) δ 174.4 (br s, CD_2CONH), 167.7 (C-1), 153.5 (PhCH_2OCO), 136.3, 129.7, 129.7, 129.5 (Ph), 94.8 (C-2), 79.6 (C-7), 73.1 (C-6), 71.9 (C-8), 71.3 (PhCH_2OCO), 67.2 (C-4), 63.4 (C-9), 62.4 (t, *J* = 21.2 Hz, COCD_2OH), 52.0 (C-5), 37.0 (C-3); IR, (Nujol) 3333, 1757 cm^{-1}; MS (ESI positive) *m/z* 466.3 $[M+Na]^+$; 99% isotopically pure. Anal. Calcd for $C_{19}H_{21}D_2NO_{11}$: C, 51.47; H+D, 5.68; N, 3.16. Found: C, 51.51; H+D, 5.71; N, 3.14.

(ii) Hydrogenation of the the 2-benzyloxycarbonyl-*N*-[$^2H_2$]-glycolyl-β-neuraminic acid 1,7-lactone **15** (50 mg, 0.11 mmol), according to the general procedure, gave the title lactone **4b** (31 mg; 89%) as a white solid: mp 111–115 °C (dec., in sealed tube) $[\alpha]_D^{25} = +9.0$ (*c* 1, THF); ^1H NMR (DMSO-d_6) δ 7.56 (d, $J_{NH,5}$ = 8.4 Hz, 1H; NH), 4.32 (br s, 1H; H-6), 4.24 (d, $J_{7,8}$ = 6.2 Hz, 1H; H-7), 3.88–3.83 (m, 1H; H-4), 3.79 (d, $J_{5,NH}$ = 8.4 Hz, 1H; H-5), 3.61–3.50 (overlapping, 2H; H-8, and H-9a), 3.46 (m, 1H; H-9b), 1.87–1.80 (overlapping, 2H; H-3a, and H-3b); ^{13}C NMR (DMSO-d_6) δ 171.0 (br s, CD_2CONH), 169.0 (C-1), 90.3 (C-2), 77.2 (C-7), 71.2 (C-8), 70.0 (C-6), 65.7 (C-4), 61.8 (C-9), 61.1 (t, *J* = 21.0 Hz, COCD_2OH), 49.5 (C-5), 37.0 (C-3); MS (ESI negative): *m/z* 308.2 $[M-H]^-$. 99% isotopically pure. Anal. Calcd for $C_{11}H_{15}D_2NO_9$: C, 42.72; H+D, 6.19; N, 4.53. Found: C, 42.82; H+D, 6.13; N, 4.48.

4.15. Synthesis of *N*-[$^2H_2$]-glycolyl-β-[3,3-$^2H_2$]-neuraminic acid 1,7-lactone 4c

The *N*-[$^2H_2$]-glycolyl-β-[3,3-$^2H_2$]-neuraminic acid **2c** (100 mg; 0.30 mmol), lactonized according to the general procedure gave, after purification by flash chromatography [eluting with AcOEt/MeOH 9:1, v/v)], the 2-benzyloxycarbonyl-*N*-[$^2H_2$]-glycolyl-β-[3,3-$^2H_2$]-neuraminic acid 1,7-lactone **16** (103 mg; 76%), as a white solid: mp 110–113 °C (decomp., in sealed tube; from CH_3CN); $[\alpha]_D^{25} = +8.4$ (*c* 1, CH_3OH); ^1H NMR (CD_3OD) δ 7.38–7.35 (m, 5H; Ph), 5.18 (AB system, 2H; CH_2Ph), 4.65 (br s, 1H; H-6), 4.46 (d, $J_{7,8}$ = 9.1 Hz, 1H; H-7), 4.10 (br s, 1H; H-4), 4.06 (br s, 1H; H-5), 3.94 (ddd, $J_{8,7}$ = 9.0, $J_{8,9b}$ = 4.4, $J_{8,9a}$ = 2.9 Hz, 1H; H-8), 3.77 (ABX system, $J_{9a,9b}$ = 11.6, $J_{9b,8}$ = 4.5, $J_{9a,8}$ = 2.9 Hz, 2H; H-9a, and 9b); ^{13}C NMR (CD_3OD) δ 174.5 (br s, CD_2CONH), 167.8 (C-1), 153.4 (PhCH_2OCO), 136.2, 129.7, 129.6, 129.5 (Ph), 94.7 (C-2), 79.5 (C-7), 73.0 (C-6), 71.8 (C-8), 71.2 (PhCH_2OCO), 67.1 (C-4), 63.3 (C-9), 62.3 (t, *J* = 21.3 Hz, COCD_2OH), 51.9 (C-5), 36.9 (m, C-3); IR, (Nujol) 3330, 1753 cm^{-1}; MS (ESI positive): *m/z* 468.4 $[M+Na]^+$; 99% isotopically pure. Anal. Calcd for $C_{19}H_{19}D_4NO_{11}$: C, 51.23; H+D, 6.11; N, 3.14. Found: C, 51.18; H+D, 5.17; N, 3.14.

(ii) Hydrogenation of the 2-benzyloxycarbonyl-*N*-[$^2H_2$]-glycolyl-β-[3,3-$^2H_2$]-neuraminic acid 1,7-lactone **16** (45 mg, 0.10 mmol), according to the general gave the title lactone **4c** (29 mg; 92%) as a white solid: mp 111–115 °C (dec., in sealed tube); $[\alpha]_D^{25} = +9.3$ (*c* 1, THF); ^{1}H NMR (DMSO-d_6) δ 7.56 (d, $J_{NH,5}$ = 8.3 Hz, 1H; NH), 4.34 (br s, 1H; H-6), 4.25 (d, $J_{7,8}$ = 6.2 Hz, 1H; H-7), 3.87 (d, $J_{4,5}$ = 10.0 Hz, 1H; H-4), 3.79 (d, $J_{5,NH}$ = 8.3 Hz, 1H; H-5), 3.61–3.49 (overlapping, 2H; H-8, and H-9a), 3.47 (m, 1H; H-9b); ^{13}C NMR (DMSO-d_6) δ 171.0 (br s, CD_2CONH), 169.0 (C-1), 90.3 (C-2), 77.2 (C-7), 71.2 (C-8), 70.0 (C-6), 65.7 (C-4), 61.8 (C-9), 61.1 (t, *J* = 21.1 Hz, $COCD_2OH$), 49.5 (C-5), 37.0 (m, C-3); MS (ESI negative): *m/z* 310.4 [M−H]; 99% isotopically pure. Anal. Calcd for $C_{11}H_{13}D_4NO_9$: C, 42.44; H+D, 6.80; N, 4.50. Found: C, 42.38; H+D, 6.83; N, 4.47.

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