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Chemoselective Synthesis of Sialic Acid 1,7-Lactones

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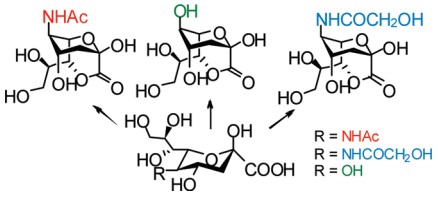
Received April 15, 2010

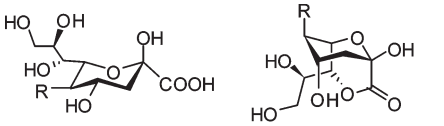


The chemoselective synthesis of the 1,7-lactones of *N*-acetylneuraminic acid, *N*-glycolylneuraminic acid, and 3-deoxy-D-glycero-D-galacto-nononic acid is accomplished in two steps: a simple treatment of the corresponding free sialic acid with benzyloxycarbonyl chloride and a successive hydrogenolysis of the formed 2-benzyloxycarbonyl 1,7-lactone. The instability of the 1,7-lactones to protic solvents has been also evidenced together with the rationalization of the mechanism of their formation under acylation conditions. The results permit to dispose of authentic 1,7-sialolactones to be used as reference standards and of a procedure useful for the preparation of their isotopologues to be used as inner standards in improved analytical procedures for the gas liquid chromatography−mass spectrometry (GLC−MS) analysis of 1,7-sialolactones in biological media.

Introduction

N-Acetylneuraminic acid **1** (Neu5Ac), *N*-glycolylneuraminic acid **2** (Neu5Gc), and 3-deoxy-D-glycero-D-galacto-nononic acid **3** (KDN; Figure 1) are the three more representative members of the sialic acids (Sias) family, a group of biological important nine carbon monosaccharides,[1] that at the moment form a family with more than 60 members, mainly differing for the substitution at the alcoholic hydroxyls.[1a,c,2] Sias are usually linked as α-acetals to the nonreducing end of the carbohydrate chains of glycolipids and glycoproteins, where they are involved in many important biological recognition processes as lymphocyte homing, tumor metastasis, and pathogenic bacteria infections.[1]

Among the members of the Sias family, the *N*-acetylneuraminic acid 1,7-lactone **4** (Neu5Ac 1,7 L) and the *N*-glycolyneuraminic acid 1,7-lactone **5** (Neu5Gc 1,7 L), indirectly identified in various glycoproteins and in the membranes of



1 Neu5Ac: R =NHAc
2 Neu5Gc: R =NHCOCH$_2$OH
3 KDN: R =OH
4 R = NHAc
5 R = NHCOCH$_2$OH
6 R = OH

FIGURE 1. Representatives sialic acids and their 1,7-lactones.

diseased erythrocytes,[3] appear of particular interest for their unusual structure and the unconventional β geometry of their glycosidic bond in glycoconjugated matrixes.[4] The lactones have not been isolated but indirectly identified in glycoconjugates, by gas liquid chromatography−mass spectrometry (GLC−MS) analysis,[3] after acidic hydrolysis of their acetalic bond and volatilization by treatment with perfluorinated anhydrides. As part of a larger program, directed to assess suitable analytical protocols for the identification

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Published on Web 07/22/2010 DOI: 10.1021/jo100732j

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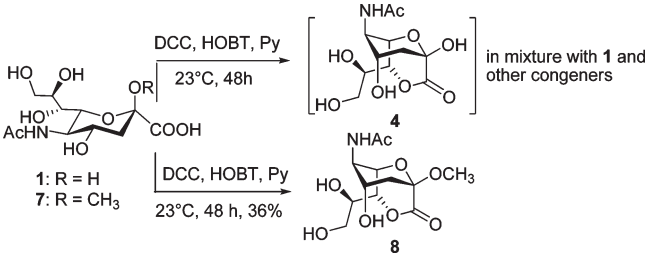
of Sias,[5] we recently reported[6] the first synthesis of the 1,7-lactone derivative **4**. Herein, we noticeably expand our initial seeding results, describing in detail our synthetic studies about the preparation of 1,7-lactone derivatives (**4**, **5**, and **6**) from Neu5Ac, Neu5Gc, and KDN, improving the procedure previously reported for the preparation of the 1,7-lactone **4** and reporting some information on the stability of these lactones.

Results and Discussion

In our initial unsatisfactory experiments, we confirmed the literature report according to which the direct lactonization of free Neu5Ac **1**, by reaction with dicyclohexylcarbodiimide (DCC), results into an inseparable mixture of the starting acid and lactones.[7] This was observed even when the reaction was performed in a pyridine solution containing 1-hydroxybenzotriazole hydrate (HOBT), a compound able to improve yields and speed of many condensation reactions (Scheme 1).[8,9] In this case, after a 48 h reaction, the presence of a major compound, with the polarity and the mass spectrum of the successively prepared lactone **4**, could be monitored by TLC. However, the compound partially decomposed on TLC and resisted all isolation attempts (Scheme 1).

SCHEME 1



Considering that the instability of the lactone **4** to the common purification procedures could be due to the presence of the free anomeric hydroxyl, which allows the reversible opening of its pyranose ring, in successive experiments, we performed the reaction on the Neu5Ac methyl acetal **7** (Scheme 1).[10] In this case, we isolated in low yield (36%), after column chromatography, a lactone to which the structure **8** could be assigned on the base of its analytical data (Scheme 1). However, since the regeneration of its anomeric hydroxyl, in the presence of the lactone ring was a difficult problem, we discontinued this route. In the same time, we realized that any successful preparation of the free lactone **4** from Neu5Ac **1**, required the selective protection of the anomeric hydroxyl of the acid with a group easily cleavable under neutral, not hydrolytic conditions, with a reaction, ideally quantitative, in order to avoid any purification of the final 1,7-lactone. Thus, we devised to attempt the lactonization of Neu5Ac **1**, activating its carboxylic group by benzyloxycarbonyl chloride (CbzCl), a bulky, scarcely reactive, acyl chloride.[11] In fact, the extensive work of Ogura[12] and of Gervay,[13] on the formation of peracylated or partially acylated 1,7- and 1,4-lactones of Sias, under acylation conditions, showed, as a common feature, the formation of lactones with an acylated anomeric hydroxyl group, even in compounds containing some free alcoholic hydroxyls. This suggested that any lactone, eventually obtained in the reaction promoted by CbzCl, should be a stable and easily isolable compound, since it was protected at the anomeric hydroxyl. On the other hand, CbzCl, being less reactive than the common acyl chlorides, presented the advantage to not react with the secondary and primary hydroxy groups of the sialic acid.[11] Moreover, the eventually formed esters could be cleaved under hydrogenolysis conditions in a suitable solvent. Unfortunately, in our first attempts, with the reaction of Neu5Ac **1** with CbzCl, in DMF, no lactonization occurred, but after a long reaction time, the benzyl ester **9** was obtained as the only product,[12b,14] probably formed by the benzyl alcohol deriving from the decomposition of CbzCl during the long reaction time (Scheme 2).

However, performing the reaction of Neu5Ac **1** with a large excess of CbzCl and triethylamine (10 mol equiv) in a mixture of DMF−THF, we obtained the 2-benzyloxycarbonylated lactone **10**, in satisfactory yields (76%), after a reaction time of 24 h at 0 °C (Scheme 2, procedure a). Successively, taking in consideration the mechanism of the lactonization reaction, described later, we reacted the triethylammonium salt of Neu5Ac **1** with CbzCl and obtained a much more rapid reaction (1 h in place of 24 h; Scheme 2, procedure b).

The lactone **10**, isolated in pure crystalline form by column chromatography, showed the expected physicochemical properties, with a carbonyl stretching band at 1770 cm^{-1}, diagnostic for the lactone ring.[12a] Moreover, by reaction with acetic anhydride in pyridine, it afforded, as expected, the triacetylated lactone **11**, possessing the appropriate physicochemical properties.

Interesting, no trace of the possible isomeric 1,4-lactone was found in the crude reaction mixture (thin-layer chromatography (TLC), NMR). This could appear in contrast with the Ogura et al.[12] and Gervay et al.[13] reports on sialic acid lactonization, under acylation conditions, where 1,4-lactones accompany the 1,7-isomers. However, our results can be easily rationalized considering that all 1,4-lactones obtained by Ogura et al. and Gervay et al. have a constantly 7-acylated hydroxyl. This appears to suggest 1,4-lactones of the sialic acid form, in concurrence with 1,7-isomers, when the 7-hydroxy groups have been already esterified by the acylating agent. In our case, the CbzCl is unable to acylate any alcoholic hydroxyl, then only the more stable 1,7-lactone forms. However, in absence of any additional experimental evidence, any additional rationalization appears highly speculative.

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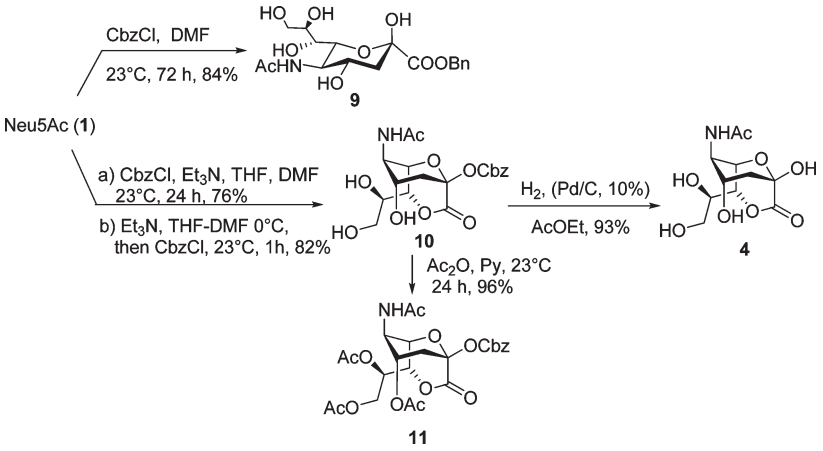
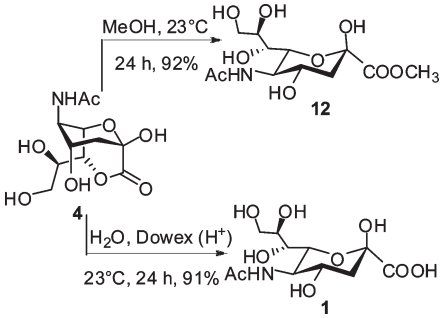
SCHEME 2



The lactone **10**, stable in a number of protic and aprotic solvents, by simple catalytic hydrogenolysis in anhydrous ethyl acetate afforded the parent lactone **4**, in quantitative yields (Scheme 2). The lactone **4** is unstable in protic solvents such as methanol or water. In fact, by simple treatment with methanol at room temperature, it afforded the corresponding Neu5Ac methyl ester **12**[15] (Scheme 3).

SCHEME 3



Moreover, when the lactone **4** was dissolved in D_2O, it could be characterized by a complete NMR analysis, but in some hours, it was opened to afford Neu5Ac **1**, identified by NMR, together with a minor amount of inseparable forms which are known to accompany it to an extent depending on the pH.[16] Similarly, when the lactone **4** was dissolved in water, in the presence of ion exchange resins (Dowex H^+), it was quantitatively transformed into pure Neu5Ac **1**, isolated by lyophilization (Scheme 3). These findings suggest the improbable survival of this lactone to the acidic hydrolysis of the glycoconjugated, performed to cleave its acetalic bond, before its identification by GLC−MS.[2,3]

With the 1,7-lactone in hand, we did some experiments directed to ascertain the mechanism of lactonization under acylation conditions, intuitively proposed by Ogura et al.[12a] for the formation of perbenzoylated Sias lactones (Figure 2, path A). According to this mechanism, the initial formation of a mixed anhydride occurs which permits the esterification of the otherwise scarcely reactive anomeric hydroxyl. This should facilitate the 1,7-lactonization, eliminating any possible hydrogen bond of this hydroxyl from the β-side of the molecule and favoring the flipping of the Neu5Ac **1** ring from the $^2C_5$ to the $^5C_2$ conformation. Then, a second mixed anhydride forms and accomplishes the lactonization.

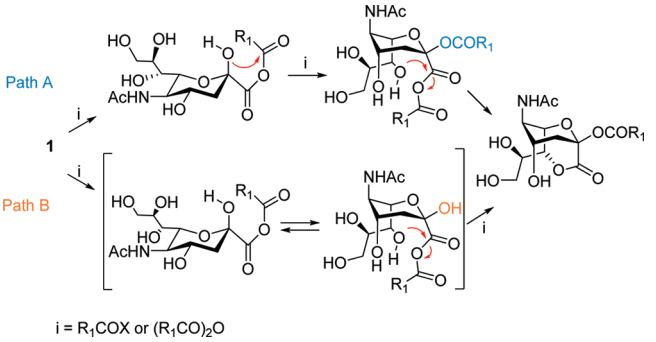
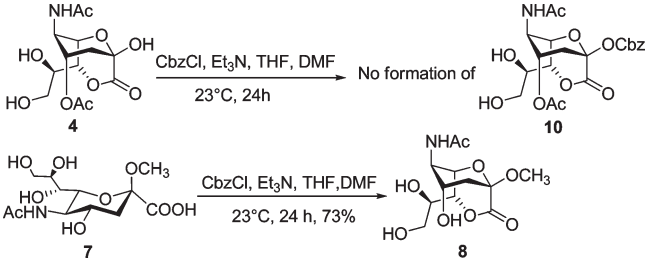


FIGURE 2. Lactonization mechanism under acylation conditions.

Moreover, we considered that an alternative mechanism (Figure 2, path B), in which the benzyloxycarbonylation of the anomeric hydroxyl of the lactone **4** follows the lactonization, could not be excluded. In order to support or reject this possibility, we performed two separate experiments. First we treated the free lactone **4** with CbzCl, under the lactonization conditions, and observed that no trace of the benzyloxycarbonylated lactone **10** forms in the reaction (Scheme 4).

SCHEME 4



This confirmed the Ogura suggestion[12] and excluded that the benzyloxycarbonylation of the anomeric hydroxyl of **4** can follow the formation of the lactone ring. Additional

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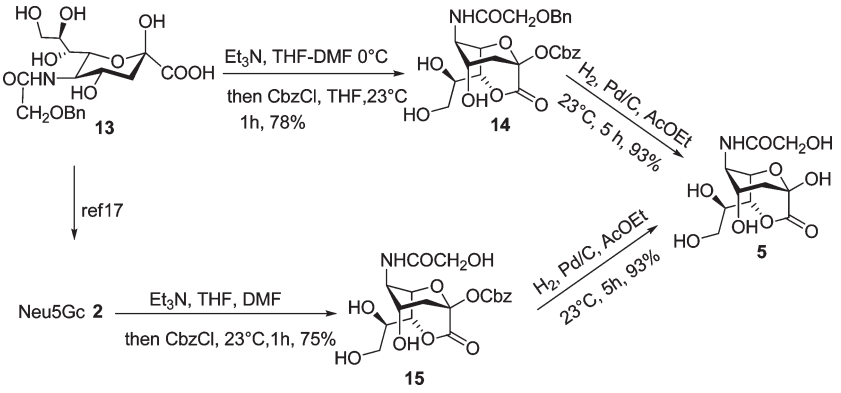
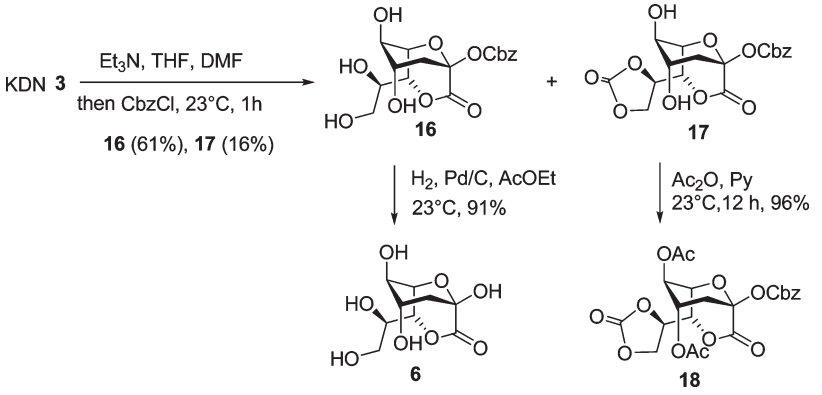
SCHEME 5



SCHEME 6



support to this rationalization was obtained subjecting the acetal **7** to the lactonization reaction (Scheme 4). In this case we obtained the corresponding lactone **8**, in more satisfactory yields (73%), in agreement with the view that the protected anomeric hydroxy group of Neu5Ac **1** facilitates the lactonization.

This possible mechanism prompted us to react the triethylammonium salt of Neu5Ac **1** with CbzCl in order to facilitate the initial formation of the mixed anhydride and to accelerate the lactonization reaction of Neu5Ac, which really, in this case, was completed in a short time (1 h; Scheme 2).

The synthesis of the 1,7-lactone **5**, deriving from the Neu5Gc **2**, could be complicated by the presence of an additional primary hydroxy group in the starting acid which could interfere forming an hydrogen bond with the hydroxyl group at C-7. Thus, we decided to perform the first lactonization reaction on the benzyl ether **13** of Neu5Gc (Scheme 5). We considered also that, being that the benzyl ether **13** is a key intermediate in the protocol proposed by Ogura et al.[17] for the preparation of Neu5Gc from the commercial Neu5Ac, we would not have extended the synthetic route to obtain the desired lactone **5**.

In fact, after the lactonization of the protected Neu5Gc **13**, the benzyl group could be cleaved in the final hydrogenolysis regenerating the anomeric hydroxyl of the lactone **5**. If operative, this route could be even shorter than that involving the direct lactonization of Neu5Gc prepared by a preventive debenzylation of the hydroxyacyl group of compound **13** (Scheme 5).

In effect, reacting the benzylether **13** with CbzCl, according to our more favorable procedure, we obtained, in good yields (78%), the 1,7-lactone **14**, as a stable compound, isolated by chromatography on silica (Scheme 5). The lactone **14** showed the expected analytical data and, after hydrogenolysis, afforded the free lactone **5** with appropriate physicochemical properties.

At this point, being more familiar with the management of the lactone **5**, we attempted its direct preparation, i.e., by treatment of the triethylammonium salt of Neu5Gc **2** with CbzCl. In this way, we obtained the 1,7-lactone **15**, in good yields (75%) and in a short time (1 h). This lactone, after regeneration of the anomeric hydroxyl group by hydrogenolisis, afforded the 1,7-lactone **5**, identical in all respects to that obtained previously.

Finally, we attempted the lactonization of KDN **3**, which in spite of the possible formation of a 1,5-β-lactone, requiring a total equilibration of the carboxylic group of KDN **3** from the α- to the β-position, afforded the 2-benzyloxycarbonyl 1,7-lactone **16** as a major compound in satisfactory yields (61%; Scheme 6). This was accompanied by a less polar derivative, obtained in low yields (16%), to which the structure of the 8,9-carbonate 1,7-lactone **17** could be assigned, on the basis of its physicochemical properties and those of its triacetate **18**, obtained from its treatment with acetic anhydride.

The lactone **16** showed analytical data in agreement with the proposed structure. Moreover by hydrogenolysis in the

(17) Ogura, H.; Furuhata, K.; Itoh, M. Shitori, Y. JP Patent 226539/85, 1985; EU Patent 0222172B1, 1991.

presence of palladium on carbon, it afforded the free lactone **6**, in pure form and in quantitative yields. The formation of the cyclic carbonate **17**, even if unexpected to the light of the previous reactions herein described, is not surprising, on the basis of the chemical literature where the formation of cyclic carbonates in carbohydrate chemistry by action of benzyl-chloroformiate in the presence of bases has been reported.[18] The carbonate group could derive from an inner transesterification of a 9-benzyloxycarbonyl sialic acid with the hydroxy group at C-8.

In conclusion, herein we report a complete picture of the synthetic accessibility of 1,7-lactones **4**, **5**, and **6** deriving from the more representative Sias.

The availability of these lactones, and the possibility to prepare their isotopologues, labeled at the pyranose ring, following the procedure herein reported, should facilitate both analytical studies directed to map their presence in different biological tissues, by means of GLC−MS methodologies, and studies directed to ascertain their possible formation in vitro, by ^{13}C NMR experiments.[13] Furthermore, the availability of the lactones **4**−**6** is of interest for a possible synthesis of glycoconjugated bonding with an unusual β-geometry at the acetalic bond.

Experimental Section

General Methods. Melting points are uncorrected. Nuclear magnetic resonance spectra were recorded at 298 K operating at 500.13 MHz for ^1H 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. 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 are given in grams/100 mL. Infrared (IR) spectra were recorded in Nujol. 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. 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 (60 F_{254}) using UV light, 50% sulfuric acid or 0.2% ninhydrin in ethanol, and heat as developing agents. All flash chromatography was performed with normal phase silica gel, following the general protocol of Still.[19]

***N*-Acetyl-β-neuraminic acid 1,7-lactone (4).** The 2-benzyloxycarbonyl-*N*-acetyl-β-neuraminic acid 1,7-lactone **10** (425 mg, 1.00 mmol), dissolved in ethyl acetate (350 mL), was hydrogenated in the presence of Pd on carbon (200 mg, 10%) for 2 h. At this time, the catalyst was filtered and washed with anhydrous tetrahydrofuran (THF). The solvent was then evaporated under reduced pressure to afford the title compound **4** (271 mg; 93%) as a white solid: mp 109−113 °C (decomposition, in sealed tube); $[\alpha]_D^{25} = +23$ (concn 1, THF). Anal. Calcd for $C_{11}H_{17}NO_8$: C, 45.36; H, 5.88; N, 4.81. Found: C, 45.23; H, 5.92; N, 4.78. The compound was identical in all respect with that reported.[6]

***N*-Glycolyl-β-neuraminic acid 1,7 Lactone (5). 1. Starting from the Benzyloxyderivative 14.** The 2-benzyloxycarbonyl-*N*-benzyloxyacetyl-β-neuraminic acid 1,7-lactone **14** (50 mg, 0.09 mmol), dissolved in ethyl acetate (38 mL), was hydrogenated in the presence of Pd on carbon (25 mg, 10%) for 5 h. At this time, the catalyst was filtered and washed with anhydrous THF. The solvent was then removed under reduced pressure to afford the title compound **5** (27 mg; 93%) as a white solid: mp 112−116 °C (decomposition, in sealed tube); $[\alpha]_D^{25} = +7.6$, (concn 1, THF). ^1H NMR (DMSO-d_6) δ 7.58 (d, $J_{NH,5} = 8.4$ Hz, 1H, NH); 7.33 (s, 1H, OH at C-2); 5.56 (brd, $J = 2.3$ Hz, 1H, OH at C-4); 5.52 (t, $J = 5.5$; OH at C-5); 5.21 (d, $J = 6.2$ Hz, 1H, OH at C-8); 4.64 (br t, $J = 5.4$ Hz, 1H, OH at C-9); 4.34 (br s, 1H, H-6); 4.25 (d, $J_{7,8} = 6.2$ Hz, 1H, H-7); 3.89−3.83 (overlapping, 3H, H-4 and $CO\underline{CH_2}OH$); 3.80 (d, $J_{5,NH} = 8.4$ Hz, 1H, H-5); 3.62−3.51 (overlapping, 2H, H-8 and H-9a); 3.47 (m, 1H, H-9b); 1.89−1.81 (overlapping, 2H, H-3a and H-3b). ^{13}C NMR (DMSO-d_6) δ 171.0 ($\underline{C}H_2CONH$), 169.0 (C-1), 90.2, (C-2), 77.1 (C-7), 71.2 (C-8), 70.0 (C-6), 65.7 (C-4), 61.8 (C-9), 61.1 ($CO\underline{C}H_2OH$), 49.6 (C-5), 37.1 (C-3). MS (ESI negative) m/z 306.0 $[M - H]^-$, 612.8 $[2M - H]^-$. Anal. Calcd for $C_{11}H_{17}NO_9$: C, 43.00; H, 5.58; N, 4.56. Found: C, 43.10; H, 5.43; N, 4.48.

2. Starting from Compound 15. The 2-benzyloxycarbonyl *N*-glycolyl-β-neuraminic acid 1,7-lactone **15** (50 mg, 0.11 mmol), dissolved in ethyl acetate (38 mL), was hydrogenated in the presence of palladium on carbon (25 mg, 10%) for 2 h. At this time, the catalyst was filtered and washed with anhydrous THF. The solvent was then evaporated under reduced pressure to afford the title compound **5** (32 mg; 93%) as a white solid: mp 112−116 °C (decomposition, in sealed tube); $[\alpha]_D^{25} = +7.6$, (concn 1, THF). MS (ESI negative) m/z 306.0 $[M - H]^-$, 612.8 $[2M - H]^-$. Anal. Calcd for $C_{11}H_{17}NO_9$: C, 43.00; H, 5.58; N, 4.56. Found: C, 43.18; H, 5.47; N, 4.49. The compound was identical in all respects to that described above.

3-Deoxy-D-glycero-D-galacto-nononic Acid 1,7 Lactone (6). The 2-benzyloxycarbonyl 3-deoxy-D-glycero-D-galacto-nononic 1,7-lactone **16** (50 mg, 0.13 mmol), dissolved in ethyl acetate (38 mL), was hydrogenated in the presence of palladium on carbon (10 mg, 10%) for 2 h. At this time, the catalyst was filtered, washed with anhydrous THF, and the solvent was evaporated under reduced pressure to afford the title compound **6** (30 mg; 91%) as a white solid: mp 172−178 °C (decomposition, in sealed tube); $[\alpha]_D^{25} = +9.7$, (concn 1, THF). ^1H NMR (DMSO-d_6) δ 7.06 (s, 1H, OH at C-2); 5.22−5.17 (overlapping, 3H, OH at C-4, C-5 and C-8); 4.35 (br s, 1H, H-6); 4.09 (d, $J_{7,8} = 8.8$ Hz, 1H, H-7); 3.84 (br s, 1H, H-4); 3.63−3.51 (overlapping, 2H, H-8 and H-9a); 3.45 (m, 1H, H-9b); 3.36 (br s, 1H, H-5); 1.92 (dd, $J_{3a,3b} = 13.5$, $J_{3a,4} = 3.1$, 1H, H-3a); 1.73 (br d, $J_{3b,3a} = 13.5$, 1H, H-3b). ^{13}C NMR (DMSO-d_6) δ 169.4 (C-1), 90.3, (C-2), 76.5 (C-7), 71.4 (C-8), 71.1 (C-6), 68.9 (C-5), 67.5 (C-4), 61.9 (C-9), 36.7 (C-3). MS (ESI negative) m/z 248.7 $[M - H]^-$, 498.8 $[2M - H]^-$. Anal. Calcd for $C_9H_{14}O_8$: C, 43.20; H, 5.64. Found: C, 43.12; H, 5.49.

2-Methyl *N*-acetyl-β-neuraminic Acid 1,7 Lactone (8). 1. By Reaction of 2-Methyl *N*-acetyl-β-neuraminic Acid 7 with DCC. The 2-methyl *N*-acetyl-β-neuraminic acid **7** (94 mg, 0.31 mmol) was dissolved in anhydrous pyridine (1 mL) and treated with DCC (192 mg, 0.93 mmol) containing HOBT (3 mg). The mixture was then stirred at 23 °C for 48 h. At this time, MeOH (1 mL) was added and stirring was continued for 2 h. Then, the solvent was removed under vacuum and the crude residue obtained was purified by chromatography on silica gel (eluting with AcOEt/MeOH, 9:1, v/v) to afford the pure lactone **8** (32 mg; 36%): a glass; $[\alpha]_D^{25} = +15$ (concn 1, CH_3OH). MS (ESI negative) m/z 304.4 $[M - H]^-$, 609.1 $[2M - H]^-$. Anal. Calcd for

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$C_{12}H_{19}NO_8$: C, 47.21; H, 6.27; N, 4.59. Found: C, 47.09; H, 6.33; N, 4.50. Other physicochemical properties were identical to those previously reported.[6]

2. Reaction of 2-Methyl *N*-acetyl-*β*-neuraminic Acid 7 with CbzCl. CbzCl (0.4 mL, 2.8 mmol), dissolved in THF (1.5 mL) was added dropwise to a stirred solution of anhydrous THF (2.5 mL) containing trietylamine (0.50 mL, 36 mmol), at 0 °C. Then, Neu5Ac **1** (94 mg, 0.31 mmol) dissolved in DMF (3.0 mL) was added and the mixture was stirred at 23 °C, for 24 h. At this time, MeOH (1.0 mL) was added and stirring was continued for 1 h. After evaporation of the solvent under high vacuum (0.1 mmHg), a crude residue was obtained which, after purification by flash chromatography (eluting with AcOEt/MeOH, 9:1, v/v), afforded the pure lactone **8** (67 mg; 73%): a glass; $[\alpha]_D^{25}$ = +15 (concn 1, CH_3OH). MS (ESI negative) *m*/*z* 304.4 $[M - H]^-$, 609.3 $[2M - H]^-$. Anal. Calcd for $C_{12}H_{19}NO_8$: C, 47.21; H, 6.27; N, 4.59. Found: C, 47.12; H, 6.29; N, 4.55. Other physicochemical properties were identical to those previously reported.[6]

***N*-Acetyl-*β*-neuraminic Acid Benzyl Ester (9).** To a well stirred solution of CbzCl (0.44 mL, 3.1 mmol) in DMF (1.6 mL), solid *N*-acetyl-*β*-neuraminic acid (100 mg, 0.32 mmol) was added at 0 °C. Then the mixture was stirred at 23 °C for 72 h. At this time, MeOH (1.0 mL) was added and the stirring was continued for 15 min. After evaporation of the solvent under high vacuum (0.1 mmHg), the crude residue obtained was chromatographed (eluting with CH_2Cl_2/MeOH, 8:2, v/v) to afford the pure compound **9** (107 mg; 84%) as a white solid: mp 184−185 °C; $[\alpha]_D^{25}$ = −41, (concn 1, H_2O). MS (ESI positive) *m*/*z* 422.4 $[M + Na]^+$, 821.8 $[2M + Na]^+$. Anal. Calcd for $C_{18}H_{25}NO_9$: C, 54.13; H, 6.31; N, 3.51%. Found: C, 54.00; H, 6.10; N, 3.42. Other physicochemical properties were identical to those previously reported[12b] and of an authentic sample of our laboratory.

2-Benzyloxycarbonyl-*N*-acetyl-*β*-neuraminic Acid 1,7-Lactone (10). 1. Procedure a. CbzCl (4.0 mL, 28.0 mmol) dissolved in THF (15 mL) was added to a stirred solution of anhydrous THF (25 mL) containing triethylamine (5.0 mL, 36.0 mmol), at 0 °C. Then, Neu5Ac **1** (900 mg, 2.91 mmol) dissolved in DMF (30 mL) was added and the mixture was stirred at 23 °C, for 24 h. At this time, MeOH (1.5 mL) was added and the stirring was continued for 15 min. Then, the solvent was removed under high vacuum (0.1 mmHg) to afford, after chromatography on silica (eluting with AcOEt/MeOH, 9:1, v/v), the pure lactone **10** (940 mg; 76%) as a white solid: mp 122−124 °C (decomposition, in sealed tube); $[\alpha]_D^{25}$ = +22.0, (concn 1, CH_3OH). ^{1}H NMR (CD_3OD) *δ* 7.39−7.34 (m, 5H; Ph); 5.18 (AB system, 2H, CH_2Ph); 4.63 (br s, 1H, H-6); 4.45 (d, $J_{7,8}$ = 9.1 Hz, 1H, H-7); 4.10 (br m, 1H, H-4); 4.01 (br s, 1H, H-5); 3.95 (ddd, $J_{8,7}$ = 9.1, $J_{8,9b}$ = 4.5, $J_{8,9a}$ = 2.9 Hz, 1H, H-8); 3.79 (system ABX, $J_{9a,9b}$ = 11.7, $J_{9a,8}$ 2.9, $J_{9b,8}$ 4.5 Hz, 2H, H-9a and H-9b); 2.27 (dd, $J_{3a,3b}$ = 13.8, $J_{3a,4}$ = 3.5, 1H, H-3a); 2.14 (dd, $J_{3b,3a}$ = 13.8, $J_{3b,4}$ = 1.8, 1H, H-3b); 2.01 (s, 3H, CH_3CONH). ^{13}C NMR (CD_3OD) *δ* 172.9 (CH_3CONH), 168.0 (C-1), 153.6 ($PhCH_2OCO$), 136.4, 129.8, 129.7, 129.3 (5C-Ph), 94.9, (C-2), 79.8 (C-7), 73.2 (C-6), 72.0 (C-8), 71.3 ($PhCH_2$-OCO), 67.5 (C-4), 63.4 (C-9), 52.6 (C-5), 37.0 (C-3), 22.4 (CH_3CONH). IR, (nujol) 3331, 1770 cm^{-1}. MS (ESI negative) *m*/*z* 424.1 $[M - H]^-$, 848.9 $[2M - H]^-$. Anal. Calcd for $C_{19}H_{23}$-NO_{10}: C, 53.65; H, 5.45; N, 3.29. Found: C, 53.54; H, 5.39; N, 3.34.

2. Procedure b. Triethylamine (5.0 mL, 36.0 mmol) was added to a stirred solution of Neu5Ac **1** (900 mg, 2.91 mmol) in THF and DMF (20 and 25 mL) and the stirring was continued at 0 °C, for 5 min. Then, CbzCl (4.0 mL, 28.0 mmol), dissolved in THF (15 mL), was added dropwise and the mixture was stirred at 23 °C, for 1 h. Then, MeOH (1.5 mL) was added and the stirring was continued for 15 min. After evaporation of the solvent under high vacuum (0.1 mmHg), a crude residue was obtained which, after purification by flash chromatography (eluting with AcOEt/MeOH, 9:1, v/v), afforded the pure lactone **10** (1.15 g; 82%) as a white solid: mp 122−124 °C (decomposition, in sealed tube); $[\alpha]_D^{25}$ = +21.4, (concn 1, CH_3OH). IR, (nujol) 3331, 1770 cm^{-1}. MS (ESI negative) *m*/*z* 424.1 $[M - H]^-$, 848.9 $[2M - H]^-$. Anal. Calcd for $C_{19}H_{23}NO_{10}$: C, 53.65; H, 5.45; N, 3.29. Found: C, 53.60; H, 5.35; N, 3.30. Other physicochemical properties were identical to those reported above.

4,8,9-Tri-*O*-Acetyl-2-benzyloxycarbonyl-*N*-acetyl-*β*-neuraminic Acid 1,7-Lactone (11). The 2-benzyloxycarbonyl-*N*-acetyl-*β*-neuraminic acid 1,7-lactone **10** (213.0 mg, 0.50 mmol), dissolved in pyridine (1.5 mL), was treated with acetic anhydride (0.70 mL, 7.4 mmol) containing a trace of 4-dimethylamino pyridine, and the solution was stirred at 23 °C, for 24 h. At this time the reaction was worked-up and the organic layers were evaporated under reduced pressure to afford a crude compound which, after flash chromatography (eluting with AcOEt/MeOH, 99:1, v/v), afforded the pure lactone **11** (265 mg; 96%): a glass; $[\alpha]_D^{25}$ +41 (concn 1, $CHCl_3$). MS (ESI positive) *m*/*z* 574.2 $[M + Na]^+$, 606.1 $[M + Na + MeOH]^+$, 1124.9 $[2M + Na]^+$. Anal. Calcd for $C_{25}H_{29}NO_{13}$: C, 54.45; H, 5.30; N, 2.54. Found: C, 54.50; H, 5.15; N, 2.35. Other physicochemical properties were identical to those previously reported.[6]

Reaction of the *N*-acetyl-*β*-neuraminic Acid 1,7-Lactone with Methanol. The *N*-acetyl-*β*-neuraminic acid 1,7-lactone **4** (50.0 mg, 0.17 mmol) was dissolved in MeOH and the solution was kept at 23 °C for 24 h. Then, the solvent was removed, under reduced pressure, to afford the pure *N*-acetyl-*β*-neuraminic acid methyl ester **12** (51 mg; 92%) as a white solid: mp 182−184 °C; $[\alpha]_D^{25}$ = −32.2, (concn 1, H_2O). MS (ESI positive) *m*/*z* 346.1 $[M + Na]^+$. Anal. Calcd for $C_{12}H_{21}NO_9$: C, 44.58; H, 6.55; N, 4.33. Found: C, 44.67; H, 6.45; N, 4.39. The ester was identical in all respects with an authentic sample of our laboratory.[15]

Reaction of the *N*-Acetyl-*β*-neuraminic Acid 1,7-Lactone with Water and Dowex 50WX8 (H^+). The *N*-acetyl-*β*-neuraminic acid 1,7-lactone **4** (50 mg, 0.17 mmol), dissolved in H_2O (3 mL), was treated with acidic resin (Dowex 50WX8, H^+) and the mixture was stirred for 24 h, at 23 °C. Then, the resin was filtered and washed with water. The solution was then lyophilized to afford pure Neu5Ac **1** (48 mg; 91%) as a white solid: mp 185−187 °C; $[\alpha]_D^{25}$ = −32.2, (concn 1, H_2O). MS (ESI positive) *m*/*z* 332.2 $[M + Na]^+$. The compound was identical in all respects to an authentic sample of commercial Neu5Ac.

2-Benzyloxycarbonyl-*N*-benzyloxyacetylneuraminic Acid 1,7 Lactone (14). Triethylamine (0.19 mL, 1.34 mmol) was added to a well stirred solution of the known[17] *N*-benzyloxyacetyl-*β*-neuraminic acid **13** (45 mg; 0.11 mmol) in THF and DMF (1.0 mL in 1.5 mL) and the reaction mixture was stirred for 5 min, at 0 °C. Then, CbzCl (0.15 mL, 1.07 mmol) dissolved in THF (1.5 mL) was added dropwise and the solution was stirred at 23 °C, for 1 h. At this time, MeOH (1 mL) was added and the stirring was continued for 15 min. The solvent was then evaporated under vacuum (0.1 mmHg) to afford a crude residue which, after chromatography (eluting with AcOEt/MeOH, 9:1, v/v), afforded the lactone **14** (45 mg; 78%): a glass; $[\alpha]_D$ = +9.5, (concn 1, CH_3OH). ^{1}H NMR (CD_3OD) *δ* 7.40−7.27 (overlapping, 10H, Ph); 5.18 (AB system, 2H, CH_2Ph); 4.65 (br s, 1H, H-6); 4.60 (s, 2H, OCH_2Ph); 4.48 (d, $J_{7,8}$ = 9.1 Hz, 1H, H-7); 4.09 (br m, 1H, H-4); 4.06 (br s, 1H, H-5); 4.01 (s, 2H, $COCH_2$-OPh); 3.96 (ddd, $J_{8,7}$ = 9.1, $J_{8,9b}$ = 4.3 Hz, $J_{8,9a}$ = 2.7 Hz, 1H, H-8); 3.79 (ABX system, $J_{9a,9b}$ = 11.7, $J_{9b,8}$ = 4.3, $J_{9a,8}$ = 2.7 Hz, 2H, H-9a and H-9b); 2.16 (ABX system, $J_{3a,3b}$ = 14.1, $J_{3a,4}$ = 3.3, $J_{3b,4}$ = 2.4 Hz, 2H, H-3a e H-3b). ^{13}C NMR (CD_3OD) *δ* 171.9 (CH_2CONH), 167.7 (C-1), 153.5 ($PhCH_2OCO$), 138.6, 136.3, 129.8, 129.7, 129.6, 129.4, 129.3, 129.2 (10C-Ph), 94.8, (C-2), 79.6 (C-7), 74.6 ($COCH_2OCH_2Ph$), 73.1 (C-6), 71.9 (C-8), 71.4 ($PhCH_2OCO$), 70.0 ($COCH_2OCH_2Ph$), 67.2 (C-4), 63.3 (C-9), 52.1 (C-5), 37.0 (C-3). MS (ESI positive) *m*/*z* 554.2 $[M + Na]^+$. Anal. Calcd for $C_{26}H_{29}NO_{11}$: C, 58.75; H, 5.50; N, 2.64. Found: C, 58.60; H, 5.25; N, 2.70.

2-Benzyloxycarbonyl-*N*-glycolyl-*β*-neuraminic Acid 1,7-Lactone (15). Triethylamine (3.5 mL; 1.34 mmol) was added to a well stirred mixture of *N*-glycolyl-*β*-neuraminic acid **2** (94 mg; 0.29 mmol) in THF and DMF (3.0 mL in 4.0 mL) and the reaction mixture was stirred at 0 °C for 5 min. Then, CbzCl (0.41 mL, 2.80 mmol) dissolved in THF (2.5 mL) was added dropwise to the reaction mixture which was stirred for 1 h, at 23 °C. At this time, MeOH (1 mL) was added and the stirring was continued for 15 min. After evaporation of the solvent, under high vacuum (0.1 mHg), a crude residue was obtained that, after flash chromatography (eluting with AcOEt/MeOH, 9:1, v/v) afforded the lactone **15** (97.0 mg; 75%): a glass; $[\alpha]_D^{25} = +7.6$, (concn 1, CH_3OH). ^{1}H NMR (CD_3OD) 7.38−7.33 (5H, m, Ph); 5.18 (AB system, 2H, CH_2Ph); 4.67 (br s, 1H, H-6); 4.48 (d, $J_{7,8} = 9.1$ Hz, 1H, H-7); 4.12 (br m, 1H, H-4); 4.08 (br s, 1H, H-5); 4.03 (s, 2H, $COCH_2OH$); 3.96 (ddd, $J_{8,7} = 9.1$, $J_{8,9b} = 4.4$, $J_{8,9a} = 2.9$ Hz, 1H, H-8); 3.79 (ABX system, $J_{9a,9b} = 11.7$, $J_{9b,8} = 4.5$, $J_{9a,8} = 2.9$ Hz, 2H, H-9a and 9b); 2.23 (dd, $J_{3a,3b} = 13.8$, $J_{3a,4} = 3.5$ Hz, 1H, H-3a); 2.16 (dd, $J_{3b,3a} = 13.8$, $J_{3b,4} = 2.2$ Hz, 1H, H-3b). ^{13}C NMR (CD_3OD) *δ* 174.5 (CH_2CONH), 167.8 (C-1), 153.5 ($PhCH_2OCO$), 136.4, 129.8, 129.7, 129.3 (5C-Ph), 94.9 (C-2), 79.7 (C-7), 73.2 (C-6), 72.0 (C-8), 71.4 ($PhCH_2OCO$), 67.3 (C-4), 63.4 (C-9), 62.6 ($COCH_2OH$), 52.1 (C-5), 37.1 (C-3). IR, (nujol) 3331, 1759 cm^{-1}. MS (ESI positive) *m*/*z* 464.1 $[M + Na]^+$. Anal. Calcd for $C_{19}H_{23}NO_{11}$: C, 51.70; H, 5.25; N, 3.17. Found: C, 51.50; H, 5.45; N, 3.37.

2-Benzyloxycarbonyl-3-deoxy-D-glycero-D-galacto-2-nononic Acid 1,7-Lactone (16) and 2-Benzyloxycarbonyl-8,9-*O*-carbonate-3-deoxy-D-glycero-D-galacto-2-nononic Acid 1,7-Lactone (17). Triethylamine (5.1 mL, 37.0 mmol) was added to a solution of 2-keto-3-deoxy-D-glycero-D-galacto-2-nononic acid **3** (800 mg; 2.98 mmol) in DMF and THF (20 mL in 35 mL) at 0 °C. After a 5 min stirring, CbzCl (4.1 mL, 29.0 mmol) dissolved in THF (12.0 mL) was added dropwise and the mixture was stirred at 23 °C, for 1 h. At this time, MeOH (1 mL) was added and the stirring was continued for 15 min. After evaporation of the MeOH−THF solution, the residual DMF was eliminated under high vacuum (0.1 mmHg) to afford a crude residue which was purified by flash chromatography to afford first (eluting with AcOEt/hexane 8:2, v/v) the lactone **17** (195 mg; 16%): a glass; $[\alpha]_D^{23}$ +38,4 (concn 1, $CHCl_3$). ^{1}H NMR (CD_3OD) *δ* 7.44−7.33 (5H, Ph); 5.23−5.18 (AB system, 2H, CH_2Ph); 5.11 (m, 1H, H-8); 4.91 (t, $J_{9a,9b} = 8.7$, $J_{9b,8} = 5.6$ Hz, 1H, H-9a); 4.77 (d, $J_{7,8} = 6.3$ Hz, 1H, H-7); 4.66 (dd, $J_{9a,9b} = J_{9a,8} = 8.7$ Hz, 1H, H-9b); 4.37 (s, 1H, H-6); 4.12 (m, 1H, H-4); 3.76 (br s, 1H, H-5); 2.34 (dd, $J_{3a,3b} = 13.7$, $J_{3,4} = 3.3$ Hz, 1H, H-3a); 2.16 (dd, $J_{3a,3b} = 13.7$, $J_{3,4} = 2.0$ Hz, 1H, H-3b). ^{13}C NMR (CD_3OD) *δ* 167.3 (C-1), 165.5 (CO at C-8 and C-9), 153.6 ($PhCH_2OCO$), 136.6, 129.8, 129.7, 129.3 (5C-Ph), 95.2, (C-2), 78.2 (C-7), 76.8 (C-8), 74.9 (C-6), 71.5 ($OCOCH_2$), 70.0 (C-5), 68.7 (C-4), 67.4 (C-9), 36.4 (C-3). MS (ESI negative) *m*/*z* 409.7 $[M - H]^-$, 819.7 $[2M - H]^-$. Anal. Calcd for $C_{18}H_{18}O_{11}$: C, 52.69; H, 4.42. Found: C, 52.90; H, 4.19. Additional elution (using AcOEt/MeOH 9:1, v/v) afforded the pure lactone **16** (698 mg; 61%): mp 135−136; $[\alpha]_D^{25} = +2.0$ (concn 1, CH_3OH). ^{1}H NMR (CD_3OD) *δ* 7.40−7.33 (overlapping, 5H, Ph); 5.17 (AB system, 2H, CH_2Ph); 4.66 (br s, 1H, H-6); 4.36 (d, $J_{7,8} = 9.2$ Hz, 1H, H-7); 4.10 (br m, 1H, H-4); 3.99 (ddd, $J_{8,7} = 9.2$, $J_{8,9b} = 4.5$ Hz, $J_{8,9a} = 2.5$ Hz, 1H, H-8); 3.79 (ABX system, $J_{9a,9b} = 11.7$, $J_{9a,8} = 2.5$, $J_{9b,8} = 4.5$ Hz, 2H, H-9a and H-9b); 3.67 (br s, 1H, H-5); 2.29 (dd, $J_{3a,3b} = 13.6$, $J_{3a,4} = 3.3$ Hz, 1H, H-3a); 2.08 (br d, $J_{3b,3a} = 13.6$ Hz, 1H, H-3b). ^{13}C NMR (CD_3OD) *δ* 168.4 (C-1), 153.6 ($PhCH_2OCO$), 136.3, 129.7, 129.3 (5C- Ph), 95.1, (C-2), 78.9 (C-7), 74.9 (C-6), 71.8 (C-8), 71.3 ($PhCH_2OCO$), 70.8 (C-5), 69.0 (C-4) 63.5 (C-9), 36.6 (C-3). MS (ESI negative) *m*/*z* 382.7 $[M - H]^-$, 766.7 $[2M - H]^-$. Anal. Calcd for $C_{17}H_{20}O_{10}$: C, 53.13; H, 5.25. Found: C, 53.00; H, 5.36.

4,5-Di-*O*-Acetylated-2-benzyloxycarbonyl-8,9-*O*-carbonate-3-deoxy-D-glycero-D-galacto-nononic Acid 1,7-Lactone (18). The 2-benzyloxycarbony-8,9-*O*-carbonate-3-deoxy-D-glycero-D-galacto-nononic acid 1,7-lactone **17** (213.0 mg, 0.52 mmol) was dissolved in pyridine (1.5 mL) and treated with acetic anhydride (0.70 mL, 0.52 mmol), containing a trace of 4-dimethylaminopyridine, at 23 °C, for 12 h. Then MeOH (0.5 mL) was added and the solution was concentrated under reduce pressure to afford a crude residue which was recovered with ethyl acetate and washed in the sequence with an aqueous solution of HCl, with water, with an aqueous $NaHCO_3$ solution, and again with water. Elimination of the solvent, under reduced pressure, afforded a crude residue which, after flash chromatography (eluting with hexane/AcOEt, 6:4, v/v), afforded the triacetate **18** (246 mg; 96%): mp 116−117 °C; $[\alpha]_D^{23}$ +41 (concn 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) *δ* 7.41−7.32 (5H, Ph); 5.24 (m, 1H, H-8); 5.18 (AB system, 2H, CH_2Ph); 5.16 (br m, 1H, H-4); 4.93 (br s, 1H, H-5); 4.71 (t, $J_{9a,9b} = J_{9a,8} = 9.4$ Hz, 2H, H-9a); 4.66 (dd, $J_{9b,9a} = 9.4$, $J_{9b,8} = 5.6$ Hz, 1H, H-9b); 4.56 (br s, 1H, H-6), 4.53 (d, $J_{7,8} = 9.3$ Hz, 1H, H-7); 2.39−2.32 (m, 2H, H-3a and H-3b); 2.16 (s, 3H, $COCH_3$ at C-5); 2.07 (s, 3H, $COCH_3$ at C-4). *δ* ^{13}C NMR ($CDCl_3$) 169.1 ($COCH_3$ at C-5), 168.6 ($COCH_3$ at C-4), 163.4 (C-1), 153.5 (CO at C-8 and C-9), 152.1 ($PhCH_2OCO$), 133.8, 129.0, 128.7, 128.3 (5C- Ph), 93.3, (C-2), 77.0 (C-7), 72.7, (C-8), 71.7 (C-6), 71.0 ($OCOCH_2$), 67.4 (C-5), 66.9 (C-9), 66.7 (C-4), 33.4 (C-3), 20.7 (2 × $COCH_3$). MS (ESI positive) *m*/*z* 516.9 $[M + Na]^+$. Anal. Calcd for $C_{22}H_{22}O_{13}$: C, 53.45; H, 4.49. Found: C, 53.26; H, 4.39.

Acknowledgment. This work was supported financially by the Italian MiUR (Ministero dell'Università e della Ricerca).

Supporting Information Available: Spectroscopic data of compounds **4**, **5**, **6**, **8**, **9**, **10**, **11**, **12**, **14**, **15**, **16**, **17**, and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.