The first synthesis of *N*-acetylneuraminic acid 1,7-lactone†

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***N*-Acetylneuraminic acid is transformed into its until now unavailable and rather unwieldy 1,7-lactone, *via* the manageable 2-benzyloxycarbonyl *N*-acetylneuraminic acid 1,7-lactone which generates the free lactone in quantitative yield by hydrogenolysis.**

Sialic acids, a negatively charged nine-carbon monosaccarides, play pivotal roles in many physiologically and pathologically important processes such as cellular recognition and communication, bacterial and viral infection, and tumor metastasis (Fig. 1).[1–3] The more typical members of the family are *N*-acetylneuraminic acid (Neu5Ac) **1**, *N*-glycolylneuraminic acic (Neu5Gc) **2** and 2-keto-3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid (KDN) **3**.

Of particular interest, among newly discovered sialic acids is the *N*-acetylneuraminic acid 1,7-lactone (Neu5Ac1,7L) **4a** since it has been proposed to be present in glycoproteins where it may play a role but has never been isolated.[3–7] Its synthesis and characterization are therefore of some importance taking into account that there is just indirect evidence of its involvement in several biological events.[4–8]

Moreover, the availability of the lactone **4a** is important also for the assessment of suitable analytical methods for its qualitative and quantitative evaluation in glycoconjugated compounds after acid hydrolysis. Thus, we programmed and satisfactorily accomplished its synthesis by a procedure herein reported. In our work, we did not consider the direct lactonization of Neu5Ac **1** by means of *N*,*N*-dicyclohexylcarbodiimide (DCC) in pyridine since it was reported that, in these conditions, a partial 1,4-intramolecular lactonization of Neu5Ac **1** occurs to afford a mixture of the bicyclic 1,4-lactone[9,10] **5a** and of the starting Neu5Ac **1**. We excluded also the lactonization mediated by usual acylic chlorides or anhydrides on the basis of the extensive work of Ogura and coworkers[11–13] and of Gervay *et al.*[8,14,15] who reported the formation of a variety of peracylated and partially acylated 1,7-lactones **4b** and **4c**, and of peracylated 1,4-lactones **5b**. Also some preliminary attempts to perform a direct chemoselective 1,7-lactonization of Neu5Ac **1**, under catalysis of protic and aprotic acids, performed in our laboratory, were unsuccessful. Thus, we decided to search for a two-step lactonization protocol which, at the end, was the successful solution of the problem (Scheme 1).

The helpful idea was to use a bulky and easily removable acylating agent, less reactive than usual acid chlorides, to promote the lactonization. However, the successful result was not immediate, due to the very particular experimental conditions (solvent mixture and sequence of reagent mixing) required by the successful reaction. In fact, we found them and transformed the Neu5Ac **1** in to the lactone **6**, in good yields (76%), only after various attempts (see ESI†). The Neu5Ac **1** was treated, at 0 °C with a large excess of benzyloxycarbonyl chloride (CbzCl), in a mixture of DMF–THF, containing a comparable excess of triethylamine.‡ Under these conditions, the lactone **6** forms, acylated exclusively at the anomeric hydroxyl, probably as a consequence of the proximity of the anomeric hydroxy group to an initially formed mixed anhydride of Neu5Ac **1**.[11] We choose CbzCl for the activation of the carboxyl group of the Neu5Ac **1**, considering that it is bulky and relatively scarcely reactive with alcohols. In fact, according with the literature,[16] in the absence of added 4-dimethylaminopyridine or of 1,4-diazabicyclo[2.2.2]octane, CbzCl should not react with the secondary hydroxyls. In the meantime we accurately searched for the more appropriate reaction solvent, stoichiometry and time, to avoid the acylation of the primary hydroxyl at C-9. In addition, we were also confident that the initial entrance of a bulky protective group to the anomeric position could facilitate the assembling and the purification of the successively formed acetal-lactone **6**. Moreover, the bulky group could favour the lactonization, by flipping the ring of Neu5Ac **1** from the $^2C_5$ to the $^5C_2$ conformation more convenient for the final 1,7-ring closure. In effect, the acetalic lactone **6**, is a stable compound which is

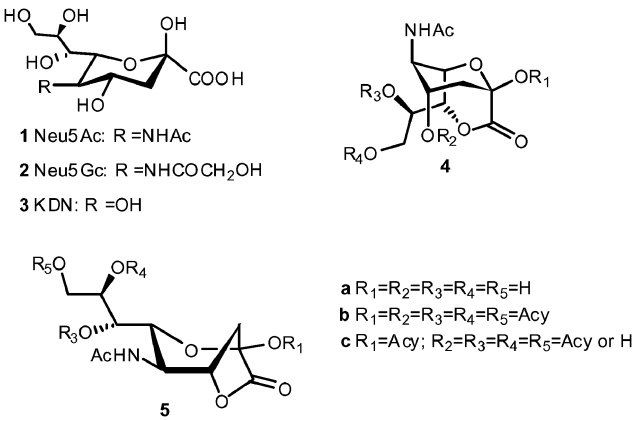

Fig. 1 Sialic acids and lactones.

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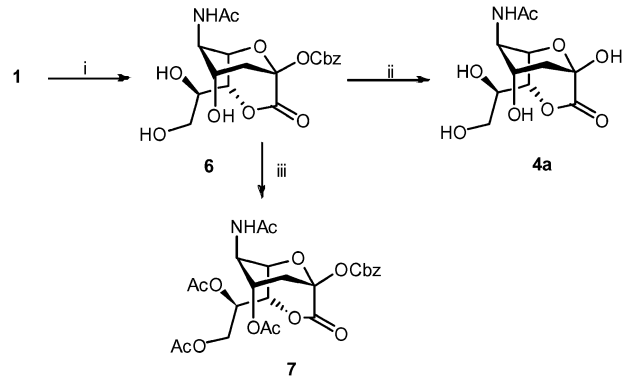
† Electronic supplementary information (ESI) available: Experimental details for the preparation of compounds **4a**, **7** and **9** and $^1H/^{13}C$ NMR spectra; COSY, HSQC, HMBC for compounds **4a**, **6**, **7** and **9**. See DOI: 10.1039/b810447f



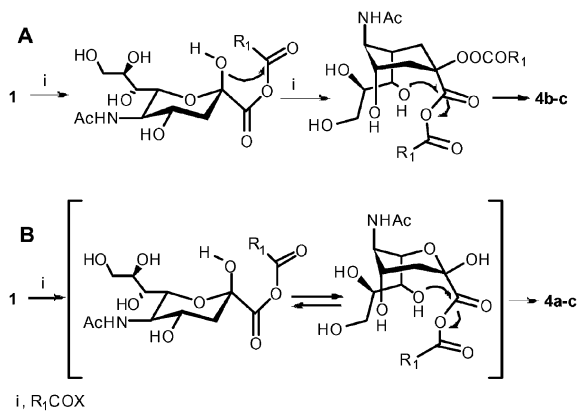
Scheme 1 *Reagents and conditions*: (i), CbzCl, Et_3N, THF–DMF (4 : 3; v/v), 0 → 23 °C, 24 h, 76%; (ii), H_2, Pd on C 10%, AcOEt, 25 °C, 96%; (iii), Ac_2O, py, 12 h, 23 °C, 96%.

easily isolated by chromatography on silica and purified to a crystalline compound showing the correct mass spectrum§ and the expected IR absorption with the carbonyl stretching band at 1759 cm^{-1}, characteristic for a 1,7-lactone.[11] The lactone **6** is stable in a number of protic and aprotic solvents at room temperature and shows ^1H and ^{13}C NMR spectra in agreement with its assigned structure. Complete proton and carbon resonance assignments were achieved using the combination of 1D and 2D experiments.† In particular, the ^1H NMR spectrum showed small coupling constant values ($^3J_{HH}$ = 1–4 Hz) for all the pyranose ring protons and evident $^4J_{HH}$ between protons at C-4 and C-6 and between those at C-3 and C-5. This indicates that the pyranose ring of **6** is in a $^5C_2$ conformation. The position of the carbobenzyloxy group at the 2-carbon was first derived from the resonances of the methylene protons at C-9, which resonate at high field (3.83–3.78 ppm) in agreement with their α-position to the 9-hydroxyl group.§ On the other hand, protons at C-4 and C-8 with the expected resonances[11] and an evident coupling between the H-7 proton and the C-1 carbon, evidenced in HMBC experiment, were diagnostic for the presence of a 1,7-lactone. In agreement with this conclusion, acetylation of the lactone **6**, using acetic anhydride in pyridine at 23 °C, afforded the 4,8,9-tri-O-acetylated bicyclic 1,7-lactone **7**, the ^1H NMR spectrum of which assured the correct structure, showing, in addition to a coupling constant ($^3J_{CH}$), between the acetate carbonyl carbon and the 9, 8 and 4 protons, the expected shifts of these proton signals at lower field in respect to those of the parent compound **6**. In some previous experiments, we had observed that operating in DMF containing triethylamine, in various conditions of temperature, no lactone forms, but, after some days at room temperature, the known[12] benzyl ester of Neu5Ac **1** is isolated, probably deriving from the reaction of benzyl alcohol formed in a slow decomposition of the reagent. Similarly, using THF alone or other solvents (py, CH_3CN–DMF, *etc.*) the reaction appeared to follow a more complex and different course. Thus, our favorable result of the reaction, due to the use of DMF–THF mixture, is quite surprisingly but, at the moment, in absence of a detailed study, any attempted rationalization appears higly speculative.

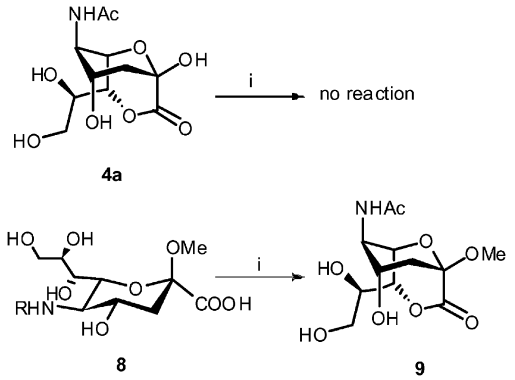
The preparation of the Neu5Ac1,7L **4a** was then accomplished by hydrogenolysis of the lactone **6** in anhydrous ethyl acetate, under Pd-catalysis (Scheme 1). The lactone **4a**¶ was quantitatively obtained by simple filtration of the catalyst and evaporation of the solvent under reduced pressure. It shows the expected mass spectrum and molecular formula together with appropriate IR and NMR spectra. The lactone **4a** is stable for almost six months in crystalline form at 4 °C but, in neutral or acidic aqueous solutions, at 25 °C, slowly decomposes, in a maximum of 12 h, to afford a complex mixture of products. On the other hand, in our first attempts to regenerate the anomeric hydroxyl of the lactone **6** by hydrogenolysis performed in aqueous methanol (94%), we obtained only the known[17] methyl ester of Neu5Ac **1**, which clearly derives from the opening of the lactone **4a** by action of the alcoholic solvent.

Our results are in line with the results obtained by others[8,11] for the formation of peracylated 1,7-lactones and appear to support the brilliant mechanism postulated by Ogura and co-workers to explain the formation of their mixture of acylated 1,7-lactones.[11–13] According to these authors, the formation in sequence of two mixed anhydrides, between Neu5Ac **1** and the acylating agent occurs during the reaction. The first formed anhydride acylates the anomeric hydroxyl, while the successive anhydride promotes the lactonization with the hydroxyl at carbon-7 (Scheme 2, A). Moreover, in our opinion, an alternative mechanism, in which the benzyloxycarbonylation of the anomeric hydroxyl of the lactone **4a** is a final reaction and follows the lactonization should be excluded (Scheme 2, B). In fact, in the lactone **4a** the normal reactivity of the hydroxy groups known for the Neu5Ac **1** could be reversed.

With the Neu5Ac1,7L **4a** in hand, we were able to strongly support the mechanism postulated by Ogura and co-workers[11] which could be operative also in our case. In effect, treating the free lactone **4a** with an excess of CbzCl in a mixture of DMF–THF, we did not observe any reaction, thus confirming that the benzyloxycarbonylation of the anomeric hydroxyl of **4a** occurs before and not after the lactonization. On the other hand, we supported the idea that the initial presence of a protected anomeric hydroxyl of Neu5Ac **1** could facilitate the lactonization of the hydroxy acid, with a second experiment in which we subjected the methyl acetal[18] **7** of the Neu5Ac **1** to our lactonization reaction. In effect, the reaction occurs in good yields (73%) affording the lactone **9** which is stable to work-up and is easily isolated in pure form (Scheme 3), thus



Scheme 2 Lactonization mechanism in the acylation.



Scheme 3 *Reagents and conditions*: (i), CbzCl, Et_3N, THF–DMF (4 : 3; v/v), 0 → 23 °C, 24 h, 73%.

also confirming that the presence of a protected hydroxyl group at the anomeric carbon stabilizes the sialic lactones. This reaction has a course different from that observed by Gervay *et al*. in the benzoylation of an isotopologue of the 2-methoxylactone **7** in pyridine which affords a perbenzoylated 1,4-lactone **5** in preference to the 1,7-lactone **4b**. Preliminary experiments performed on the Neu5Gc **2** and on the KDN **3**, show for the first, a parallel behaviour, and for the second, the necessity to perform an in depth study, which is ongoing in our laboratory.

In conclusion, the observed instability of the free Neu5Ac1,7L **4a** to aqueous solvents suggests that attention should be given to disclose the possible presence of this lactone in biological media in order to avoid its total or partial destruction during its acidic cleavage from glycolipids or sialoglycoproteins. On the other hand, our results evidence the opposite risk to synthesize acylated Neu5Ac1,7L **4a** during derivatization of various sialic acids in protocols using acylation to protect them before analysis, for example by GLC.[19] Work to ascertain these risks and to set-up analytical methods which circumvent these problems are in progress in our laboratory.

Notes and references

‡ The best rigorous conditions found were: CbzCl (4.0 mL, 28 mmol) dissolved in THF (15.0 mL) was added dropwise to a solution of anhydrous THF (25.0 mL) containing triethylamine (5.0 mL; 36 mmol) under stirring, at 0 °C. At this point solid sialic acid (900 mg; 2.91 mmol) was added followed by DMF (30.0 mL). The mixture was then stirred at 23 °C for 24 h. At this time, MeOH (40 mL) was added and stirring continued for 2 h. After evaporation of the MeOH–THF mixture, the residual DMF was removed under high vacuum to afford a crude residue which was chromatographed on silica (eluting with 10% MeOH in AcOEt), to give the pure lactone **6** (940.0 mg; 76% yield); mp 122–124 °C (decomp., in sealed tube); $[\alpha]_D$ = +21, (CH_3OH, c = 1); IR (Nujol) 3331, 1759 cm^{-1}; δ_H (500.13 MHz; CD_3OD, T = 298 K) 7.39–7.34 (5H, m, Ph), 5.18 (2H, AB system, CH_2Ph), 4.63 (1H, br s, H-6), 4.47 (1H, d, $J_{7,8}$ 9.1 Hz, H-7), 4.10 (1H, br m, H-4), 4.04 (1H, br s, H-5), 3.98 (1 H, ddd, $J_{8,7}$ 9.1 Hz, $J_{8,9b}$ 4.5 Hz, $J_{8,9a}$ 2.9 Hz, H-8), 3.79 (2H, ABX system, $J_{9a,9b}$ 11.7 Hz, $J_{9a,8}$ 2.9 Hz, $J_{9b,8}$ 4.5 Hz, H_2-9), 2.29 (1H, dd, $J_{3a,3b}$ 13.8 Hz, $J_{3a,4}$ 3.5 Hz, H-3a), 2.16 (1H, dd, $J_{3b,3a}$ 13.8 Hz, $J_{3b,4}$ 1.8 Hz, H-3b), 2.01 (3H, s, CH_3CONH); δ_C (125.76 MHz; CD_3OD, T = 298 K) 172.9 (CH_3CONH), 168.0 (C-1), 153.6 ($PhCH_2OCO$), 136.4, 129.8, 129.7, 129.6 (Ph), 94.9, (C-2), 79.8 (C-7), 73.2 (C-6), 72.0 (C-8), 71.3 ($PhCH_2OCO$), 67.5 (C-4), 63.4 (C-9), 52.6 (C-5), 37.0 (C-3), 22.4 (CH_3CONH); MS (ESI negative): m/z 424.1 (M − H), 848.9 (2M − H).

§ All new compounds gave satisfactory microanalytical and mass spectroscopic data.

¶ *Selected data* for **4a**: mp 110–113 °C (decomp., in sealed tube); $[\alpha]_D$ = +23 (THF, c = 1); IR (Nujol) 3330, 1740 cm^{-1}; δ_H (500.13 MHz; DMSO-d_6, T = 298 K) 4.32 (1H, s, H-6), 4.20 (1H, d, $J_{7,8}$ 8.5 Hz, H-7), 3.81 (1H, br s, H-4), 3.74 (1H, d, $J_{5,NH}$ 7.5 Hz, H-5), 3.60 (1H, m, H-9a), 3.53 (1H, m, H-8), 3.46 (1H, dd, $J_{9b,9a}$ 10.8 Hz, $J_{9b,8}$ 5.2 Hz, H-9b), 1.95 (1H, br dd, $J_{3a,3b}$ 13.6 Hz, $J_{3a,4}$ 2.0 Hz, H-3a), 1.87 (3H, s, CH_3CONH), 1.81 (1H, br d, $J_{3b,3a}$ 13.6 Hz, H-3b); δ_C (125.76 MHz; DMSO-d_6, T = 298 K) 169.3 (C-1), 168.8 (CH_3CONH), 90.3 (C-2), 77.4 (C-7), 71.2 (C-8), 70.0 (C-6), 65.9 (C-4), 61.9 (C-9), 50.3 (C-5), 36.9 (C-3), 22.3 (CH_3CONH). MS (ESI negative): m/z 290.0 (M − H), 581.3 (2M − H).

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