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# Synthesis of C-9 oxidised *N*-acetylneuraminic acid derivatives as biological probes

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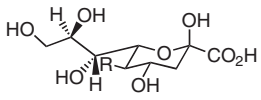
ABSTRACT

Sialic acids are 9-carbon acidic sugars involved in a number of important biological processes and human diseases. As part of our ongoing interest in the development of novel sialic acids as biological probes, we have developed an efficient and simple synthesis of C-9 oxidised sialic acid derivatives. The key oxidative step involves the use of TEMPO under carefully controlled aqueous pH conditions.

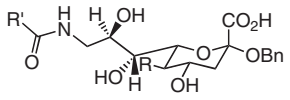


Sialic acids are typically found in nature at the non-reducing terminus of a range of glycoproteins and glycolipids present on the surface of cells.[1–4] These 9-carbon acidic sugars are intimately involved in a wide range of biological processes, ranging from cell–cell interactions, cell differentiation, tumour metastasis and pathogen-host recognition phenomena.[1,4–7] The most common member of the sialic acid family in humans is *N*-acetylneuraminic acid (Neu5Ac, **1**), which is usually found α(2,3)- or α(2,6)-linked to either galactose or galactosamine residues,[1,8] or α(2,8)-linked to itself.[9] The importance of sialic acid in pathogen-host recognition events is of particular interest, since gaining a better understanding of the way a particular pathogen utilizes sialic acid to either infect a host or to evade detection by the host is necessary in order to develop new drugs to combat such events.

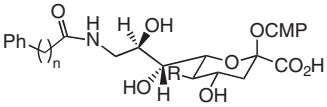
We have had ongoing interest in the development of novel sialic acid derivatives as probes for a number of important human pathogens, including the influenza virus,[10,11] rotavirus,[12,13] *Vibrio cholerae*[14] and *Plasmodium falciparum*.[15] As part of our investigations into the carbohydrate binding protein VP8* from rotavirus,[12,16] as well as the link between sialic acid and the metastatic potential of cancer cells, we required an efficient synthesis of sialic acids modified at the C-9 position. There have been numerous reports of the synthesis of modified sialic acid derivatives, and the reader is directed to comprehensive reviews.[4,17,18] For the synthesis of C-9 modified sialic acids, recent examples include the preparation of hydrophobic chain-extended compounds such as **2** as probes for myelin-associated glycoprotein.[19] Several different types of sialic acid derivatives have been used as probes for sialyltransferases, including cytidine monophosphate (CMP)-activated phenyl substituted alkyl amide derivatives (e.g., **3**)[20] or glycerol side-chain modified compounds.[21] Our own previous efforts towards C-9 modified sialic acids were related to simple C-9 ether, ester and chain-extended compounds (e.g., **4**) as probes for Neu5Ac aldolase.[22] For the majority of these C-9 modified sialic acids, the synthetic strategy employed relies on the fact that the C-9 hydroxy group is primary, and hence is more reactive and less sterically hindered than the other hydroxy groups in the molecule.



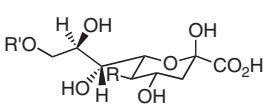
**1** R = NHAc



**2** R = NHAc, R' = alkyl or aryl



**3** R = NHAc



**4** R = NHAc, R' = alkyl, acyl

In this current work, we were particularly interested in sialic acid analogues wherein the C-9 hydroxy group was oxidized to a

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carboxylic acid. Such a modification would then allow subsequent elaboration of the carboxyl group with amines to provide rapid access to a series of amide-based compounds that would serve as valuable probes for a series of sialic acid recognizing proteins. Although 9-carboxy-Neu5Ac and 9-carboxy-Neu5Acα2Me have been reported as probes for haemagglutination studies,[23] to the best of our knowledge the synthesis of these compounds has never been described in detail, although Brossmer et al.[24] reported that they used 'catalytic oxidation'. We therefore describe our synthesis of both the α- and β-methyl glycosides of 9-carboxy-Neu5Ac.

Our approach towards C-9 oxidised sialic acids is summarized in retrosynthetic terms in Scheme 1. Following directly from some of our previous work,[25] the desired 9-carboxy-derivative **5** should be available by oxidation of the 9-hydroxy-Neu5Ac derivative **6**, which itself is obtained from the methyl sialoside **7** via a series of simple and high yielding protecting group manipulations.

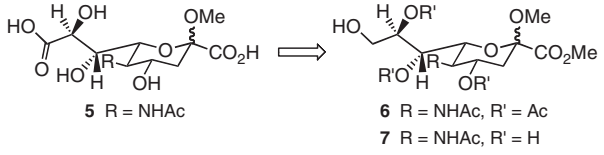
Our initial studies towards the preparation of 9-carboxy-Neu5Ac centred on the α-methyl glycoside series. Accordingly, selective silylation of the C-9 hydroxy group in **8**[26] followed by acetylation and desilylation gave the pivotal 9-hydroxy derivative **9** in high yield (Scheme 2). Whilst there are numerous methods in the literature for oxidation of a primary hydroxy to a carboxy group,[27,28] we opted to use a 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) mediated approach, since this reagent is known to be quite mild and the reaction can be conducted under relatively neutral conditions.[29] We reasoned that such mild conditions would be needed with our substrate, especially because of the acid labile nature of the α-methyl sialoside group. Our initial attempts at TEMPO oxidation of the C-9 hydroxy group in **9** produced mixed results, with low (~20%) and variable yields of the desired product **10**.

Under TEMPO conditions, it is necessary to use aqueous base in order for the reaction to proceed efficiently. We felt that the acetate groups present in the sialoside **9** may be labile under such reaction conditions and, in conjunction with the newly introduced carboxy group at C-9, would result in a compound that would be partially water soluble. Given that the work-up for a TEMPO oxidation normally involves an aqueous wash, it is reasonable to conclude that our low and variable yields for **10** were due to loss of the compound during the aqueous work-up. To test this hypothesis, we repeated the TEMPO oxidation on compound **9**, but used a modified work-up procedure wherein the aqueous wash was removed, and instead the crude reaction mixture was carefully acidified (dil HCl), concentrated to dryness using a rotary evaporator, and then subjected to acetylation conditions (pyridine/acetic anhydride) overnight.[30] In this way, the C-9 oxidised sialic acid derivative **10** was obtained in 66% yield after chromatographic purification. Deprotection of the ester groups in **10** was achieved efficiently and smoothly using aqueous lithium hydroxide solution, to give the bis-carboxylic acid α-sialoside **11** in good yield after HPLC purification.

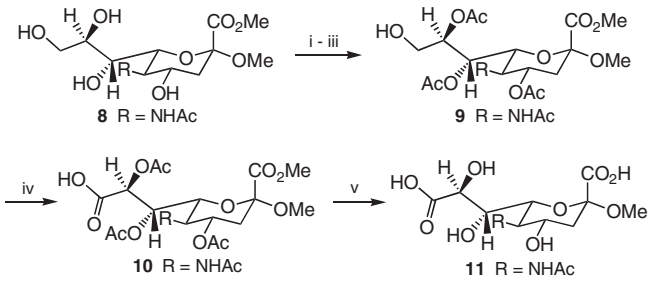
Our synthesis of the β-methyl sialoside analogue of **11** followed a similar route to that depicted in Scheme 2. Briefly, treatment of Neu5Ac with acidic methanol according to the method of Kuhn et al.[31] gave the methyl ester β-methyl glycoside **12**. Selective C-9 silylation, followed by acetylation, gave the 9-*O*-silyl ether **13**, as previously described.[25] Careful acid-promoted removal of the silyl ether furnished the 9-hydroxy derivative **14** in modest yield (Scheme 3). TEMPO-mediated oxidation of **14** gave the 9-carboxy derivative **15**. Given our initial water solubility problems in the α-series, we reasoned that an alternative option was to esterify the newly formed acid functionality. Accordingly, the crude TEMPO oxidation product **15** was exposed to methyl iodide in DMF, to give **16** in 41% yield from **14** after acetylation. De-esterification of **16** gave the bis-carboxylic acid β-sialoside **17** in good yield after HPLC purification.

During the aqueous acetic acid promoted removal of the 9-*O*-silyl ether from **13**, we found it extremely difficult to obtain pure **14**. As we have described in detail previously,[25] this is due to acetate group migration from the 8-position to the 9-position, resulting in the 9-hydroxy product **14** being contaminated with the corresponding 8-hydroxy-9-acetoxy analogue. In our hands, no amount of manipulation of experimental conditions could overcome the presence of small amounts of the 8-hydroxy-9-acetoxy analogue in our purified **14**. In an attempt to overcome problems associated with minor contaminants, the TEMPO oxidation was repeated directly on the methyl ester β-methyl glycoside **12**. To our delight, after acetylation of the crude reaction product, the 9-carboxy β-sialoside **15** was obtained directly from **12** in a very acceptable 56% yield (Scheme 4). Notably, a direct TEMPO oxidation of **8** afforded the corresponding 9-carboxy α-methyl glycoside in a 40% isolated yield.

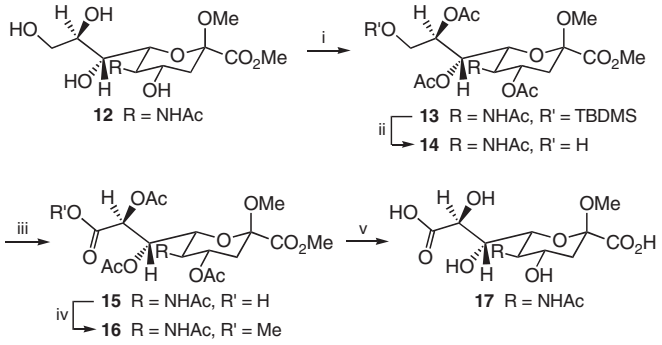
In summary, our synthesis of C-9 oxidised sialosides is efficient (compound **10** in 43% overall yield from **8**; compound **15** in 46% overall yield from Neu5Ac), and provides ready access to compounds (e.g., **10** and **14**) that could serve as useful intermediates



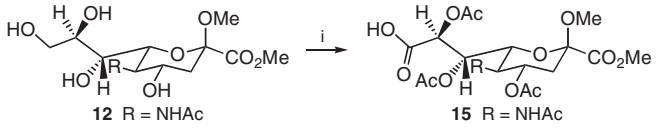

**Scheme 1.** Proposed approach towards C-9 oxidised sialic acids.




**Scheme 2.** Reagents and conditions: (i) TBDMSCl, pyridine, 3 h; (ii) $Ac_2O$, pyridine, 16 h, 82% over two steps; (iii) 80% aq AcOH, 50 °C, 30 min, 79% based on recovered starting material; (iv) TEMPO, $CH_2Cl_2$, satd aq $NaHCO_3$ containing KBr and $Bu_4NBr$, aq NaOCl, 1 h, then aq HCl to pH ~2, then $Ac_2O$, pyridine, 16 h, 66%; (v) MeOH, LiOH (2 M), 12 h, 62%.




**Scheme 3.** Reagents and conditions: (i) Ref. 25; (ii) 80% aq AcOH, 50 °C, 20 min, 69% based on recovered starting material; (iii) TEMPO, $CH_2Cl_2$, satd aq $NaHCO_3$ containing KBr and $Bu_4NBr$, aq NaOCl, 1 h, then aq HCl to pH ~2; (iv) DMF, MeI, 16 h, then $Ac_2O$, pyridine, 16 h, 41% from **14**; (v) NaOMe, MeOH, 3 h, then NaOH (1 M), 4 h, 76%.




**Scheme 4.** Reagents and conditions: (i) TEMPO, $CH_2Cl_2$, satd aq $NaHCO_3$ containing KBr and $Bu_4NBr$, aq NaOCl, 1 h, then aq HCl to pH ~2, then $Ac_2O$, pyridine, 16 h, 56%.

for other glycerol side-chain modified sialosides. The use of the products described herein as probes for sialic acid recognizing proteins will be described in detail elsewhere.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.10.156.

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30. Typical procedure for modified TEMPO oxidation is as follows: To a solution of the alcohol **9** (1.10 g, 2.37 mmol) and TEMPO (5 mg, 0.03 mmol) in $CH_2Cl_2$ (15 mL) at 0 °C was added a solution of satd aq $NaHCO_3$ (4.5 mL) containing KBr (26 mg, 0.22 mmol) and $Bu_4NBr$ (38 mg, 0.12 mmol). The biphasic mixture was stirred vigorously whilst a solution of NaOCl (10–15%, 5.5 mL) containing satd aq $NaHCO_3$ (2.6 mL) and satd aq NaCl (4.4 mL) was added over a 15 min period. The mixture was allowed to warm to room temperature and stirred for 45 min before an additional portion of NaOCl (10–15%, 4.5 mL) was added. After a further 15 min the pH was adjusted to ~2 using dil. HCl (4 M) and the entire mixture concentrated in vacuo. The residue was dissolved in pyridine (15 mL) and $Ac_2O$ (8 mL), and stirred for 14 h at room temperature. The mixture was concentrated to dryness, the residue dissolved in EtOAc (50 mL) and washed with dil HCl (1 M, 30 mL), $H_2O$ (30 mL), dried ($Na_2SO_4$) and concentrated. Flash chromatographic purification on silica gel (EtOAc/MeOH, 7:1) gave the desired 9-carboxy derivative **10** in 66% yield.
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