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Microwave-assisted synthesis of *N*-glycolylneuraminic acid derivatives

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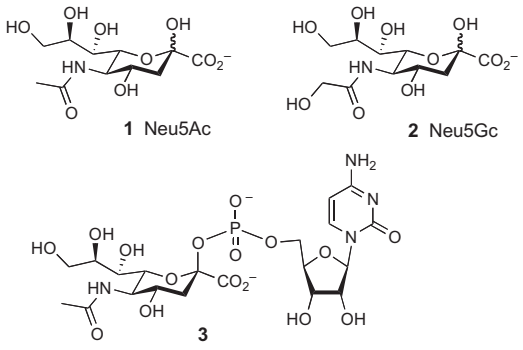
ABSTRACT

A rapid, efficient and scalable synthesis of biologically-relevant *N*-glycolylneuraminic acid derivatives from the natural *N*-acetyl (Neu5Ac) precursors has been developed. Microwave irradiation provides accelerated de-N-acetylation compared to more traditional methods, with optimised NaOH-promoted de-N-acetylation in only 15 min. The prepared amines were readily re-N-acylated to afford the corresponding *N*-glycolyl (Neu5Gc) analogues.

The sialic acids are a family of nine-carbon acidic monosaccharides, with approximately 50 different forms currently known arising from substitutions on the 3-deoxy-non-2-ulosonic acid template.[1] They are typically found α-linked to the non-reducing end of glycans associated with glycoproteins and glycolipids, and also in polymeric form.[1,2] Sialic acids participate in a range of biologically-important processes, including aspects of cell–cell interactions, cell differentiation, tumour metastasis and host–pathogen recognition phenomena.[2]

The most abundant mammalian-relevant members of the sialic acid family are *N*-acetylneuraminic acid (Neu5Ac, **1**) and *N*-glycolylneuraminic acid (Neu5Gc, **2**).[1] In contrast to other mammalian systems, Neu5Gc is not synthesised naturally in humans as a consequence of the inactivation of the human CMP-Neu5Ac hydroxylase (CMAH) gene[3] that encodes for the enzyme responsible for conversion of the activated nucleotide sugar CMP-Neu5Ac (**3**) into CMP-Neu5Gc.[3] Interestingly, despite the lack of the 'natural' biosynthetic pathway to Neu5Gc, **2** has been found in glycoconjugates as a cell surface component of human tumours,[4–6] and also in small amounts in normal tissues.[7]

The presence of the 'foreign' Neu5Gc epitope in human cancers has generated substantial interest in the use of Neu5Gc-containing glycans for immunotherapy and vaccine development.[6] The broader introduction of 'non-natural' N-acyl groups on neuraminic acid derivatives has also been investigated for potential use in cancer immunotherapy,[8] as well as being widely used in studies of both human and microbial sialic acid recognising proteins.[9,10] In addition, neuraminic acids carrying functionalised N-acyl groups have been exploited in metabolic engineering of cell-surface glycans.[10] From a synthetic chemistry standpoint, alteration of the acetamido group at C-5 has been used to enhance the outcome of sialic acid glycosidations.[11]



As part of our continuing studies of sialic acid recognising proteins, we required a rapid and efficient method for the synthesis of *N*-glycolylneuraminic acid derivatives. Alternatively N-acylated neuraminic acids have been accessed by chemical or enzymatic synthesis from the corresponding N-acylated mannosamine precursors.[10] However, we required a method that would allow us to alter the N-acyl group in already functionalised and glycosidi-

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cally-linked Neu5Ac derivatives. Towards this end, various methods to directly de-N-acetylate Neu5Ac-based compounds, under strongly basic[12–17] or acidic[18] conditions have been reported. These methods, however, generally require reaction at high temperature, over long reaction times.

For numerous thermally-driven organic reactions, microwave irradiation has been used as an alternative source of energy to accelerate chemical transformations.[19] Reduced reaction time, increased purity and higher yields are some of the major advantages often obtained when using microwave-assisted synthesis.[20] The scope of microwave irradiation in chemical manipulations of carbohydrates,[21] and in particular sialic acids,[22–24] however, has been relatively poorly explored.

Herein, we report the development of optimised microwave irradiation based reaction conditions for the de-N-acetylation of selected Neu5Ac compounds, and the further elaboration of the resultant amines to the corresponding N-glycolylated derivatives. Traditional thermal de-N-acetylation methods under acidic conditions would readily hydrolyse an O-glycosidic linkage. We therefore selected basic reaction conditions to investigate the microwave irradiation of the selected Neu5Ac substrates.

To begin this study, we selected the protected α-methyl glycoside of Neu5Ac, **4**,[25] as a representative α-linked sialoside to gauge the stability of the glycosidic linkage, and of the sialoside substrate in general, to basic conditions under microwave irradiation[26] (Table 1). In an initial screening, different concentrations of aq NaOH (0.2–4.0 M) were employed. We began with microwave conditions that we have previously shown[24] to be optimal for the synthesis of the β-methyl glycoside of Neu5Ac: a reaction temperature of 120 °C (at a microwave power of 100 W) for a period of 15 min. The reaction progress was monitored by TLC using ninhydrin as an amine-active stain. De-N-acetylation of the C-5 acetamido group was confirmed by ^{1}H NMR spectroscopy of the reaction mixture (in NaOH/D_2O) where a characteristic upfield shift of H-5 from δ 3.80 ppm[27] for the acetamido derivative Neu5Acα2Me, to 2.51 ppm in **5**[12] (Neuα2Me, as the sodium salt), and the lack of the acetamido methyl protons (δ 2.03 in Neu5Acα2Me[27]) were observed.

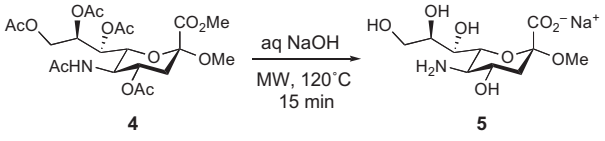
The concentration of alkali significantly influenced the rate of reaction, with lower concentrations (0.2–0.6 M) resulting only in saponification (Table 1, entries 1–3) while the highest alkali concentration (Table 1, entry 7) resulted in some decomposition of the substrate and/or product (based on TLC analysis). However, a significant yield of the desired de-N-acetylated product, Neuα2Me **5**, was obtained with 2.0 M NaOH (Table 1, entry 6) without any observed decomposition.

Based on the above results (Table 1), 2.0 M NaOH was selected as the optimum alkali concentration. We then proceeded to determine the optimum reaction time and temperature (Table 2). A progressive increase in the conversion into **5** was obtained with increasing exposure to microwave irradiation at a fixed temperature of 120 °C (100 W max. power) for 5 (57% conversion into **5**), 10 (72% conversion) or 15 (91% conversion) minutes. Irradiating for more than 15 min, however, while maintaining excellent conversion into **5** also resulted in some degradation of the carbohydrate substrate and/or product (Table 2, entries 5 and 6). In evaluating the effect of the reaction temperature, while maintaining the reaction time at 15 min, we observed that a temperature of 100 °C resulted in only saponification with little de-N-acetylation (36% conversion into **5**). Furthermore, employing a reaction temperature above 120 °C (140 °C or 160 °C) led to increasing levels of carbohydrate degradation. An optimal yield of **5** was therefore obtained at a reaction temperature of 120 °C after exposure to microwave irradiation for 15 min.

To investigate the influence of reaction scale on the microwave-based synthesis of **5**, we increased the mass of substrate **4** by 20-fold to 1.0 g. Reaction under the optimised microwave irradiation conditions (15 min at 120 °C, at a maximum power of 100 W) afforded the desired 5-amino derivative **5** in 80% yield (Table 3, entry 1). The general application of this method was demonstrated by reaction of the alternative β-methyl glycoside, Neu5Ac1,β2Me$_2$ (**6**;[24], Table 3, entry 2), the peracetylated unsaturated derivative Neu5Ac2en1Me (**8**;[28], Table 3, entry 3) and the α-2,8-linked Neu5Ac homopolymer (~100 residues, **10**; Table 2, entry 4) under the optimised reaction conditions.[29] To our delight, we found that for each substrate we achieved excellent isolated yields of the desired target 5-amino derivatives, in significantly shorter times than reported[12–14] for reactions using conventional heating (15 min as compared to, e.g., 16 h for reaction of **4**[12] or **8**[13] with Ba(OH)$_2$), and avoiding the extensive work-up typically required with reagents such as Ba(OH)$_2$.[12,13]

Finally, the target N-glycolyated derivatives were readily prepared from the free amines by reaction with acetoxyacetyl chlo-

Table 1
Microwave-assisted de-N-acetylation of **4** at 120 °C: optimisation of alkali concentration[a]



4 5

Entry	(NaOH) (M)	Conversion into **5**[b] (%)
1	0.2	0[c]
2	0.4	0[c]
3	0.6	0[c]
4	0.8	10
5	1.0	16
6	2.0	91
7	4.0	90[d]

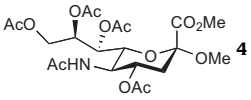
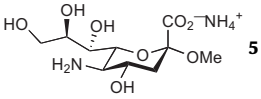
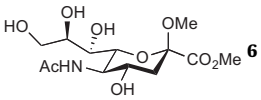
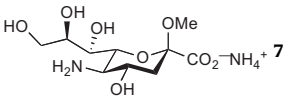
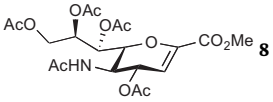
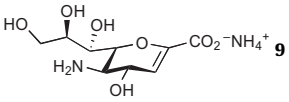
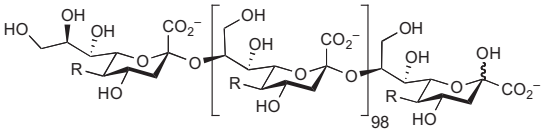
[a] Reaction was carried out on **4** (50 mg, 0.10 mmol) in NaOH solution (0.2–4.0 M in D_2O, 1 mL) in a sealed reaction vessel, under MW irradiation (max. 100 W) at 120 °C for 15 min.
[b] Conversion into **5** was determined from the ^{1}H NMR (300 MHz, NaOH/D_2O) spectrum of the reaction mixture, based on comparison of the integration of H-5 of **5** at δ 2.51, with that of the combined H-3 equiv for **4** and **5** at δ 1.31–1.39 ppm. See Supplementary data.
[c] Partial to complete saponification was observed.
[d] Decomposition of the carbohydrate was observed.

Table 2
Microwave-assisted de-N-acetylation of **4** in 2.0 M NaOH: optimisation of time and temperature[a]

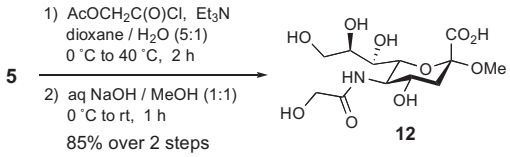
Entry	Reaction conditions	Conversion into **5** (%)
1	5.0 min, 120 °C	57[b]
2	10.0 min, 120 °C	72[b]
3	12.5 min, 120 °C	90
4	15.0 min, 120 °C	91
5	17.5 min, 120 °C	95[c]
6	20.0 min, 120 °C	94[c]
7	15.0 min, 100 °C	36[b]
8	15.0 min, 140 °C	99[c]
9	15.0 min, 160 °C	100[c]

[a] Reaction was carried out on **4** (50 mg, 0.10 mmol) in NaOH solution (2.0 M in D_2O, 1 mL) in a sealed reaction vessel, under MW irradiation (max. 100 W) and the specified conditions.
[b] Partial to complete saponification was observed.
[c] Decomposition of the carbohydrate was observed.

Table 3
Microwave-assisted de-N-acetylation of *N*-acetylneuraminic acid (Neu5Ac) derivatives in 2.0 M NaOH at 120 °C for 15 min[a]

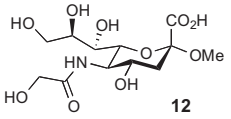
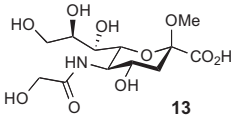
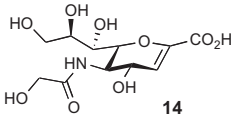
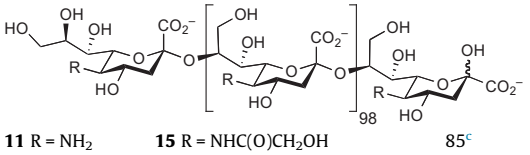
Entry	Substrate	Product	Isolated yield (%)
1	 **4**	 **5**	80
2	 **6**	 **7**	82
3	 **8**	 **9**	85
4	 **10** R = NHAc	**11** R = NH_2	82[b]

[a] Reaction was carried out on the Neu5Ac substrate (1.0 g of **4**, **6** or **8**; 0.1 g of **10**) in 2.0 M aq NaOH (15 mL, or 5 mL for **10**) in a sealed reaction vessel, under microwave irradiation at 120 °C for 15 min. Products **5**, **7** and **9** were isolated by elution from Dowex® 50W × 8 (H^+) resin with 1.5 M aq NH_4OH. Product **11** was purified by dialysis.
[b] Isolated as the sodium salt.



Scheme 1. N-glycolylation of Neuα2Me **5**.

Table 4
Synthesis of *N*-glycolylneuraminic acid (Neu5Gc) derivatives from the corresponding 5-amino (Neu) compounds[a]

Entry	Substrate	*N*-Glycolyl-neu product	Yield[b] (%)
1	**5**	 **12**	85
2	**7**	 **13**	91
3	**9**	 **14**	80
4	**11** R = NH_2	 **15** R = $NHC(O)CH_2OH$	85[c]

[a] Reaction conditions: (i) acetoxyacetyl chloride (2.0 mol equiv), Et_3N, 1,4-dioxane/water (5:1), 0–40 °C, 2 h; (ii) MeOH/0.1 M aq NaOH (1:1), 0 °C to rt, 1 h. Products **12**, **13** and **14** were purified by elution from Dowex® 1 × 2 (Cl^-) ion exchange resin with 1.0 M formic acid, followed by RP-HPLC. Product **15** was purified by dialysis.
[b] Isolated yield over two steps.
[c] Isolated as the triethylammonium salt.

ride, followed by de-O-acetylation, which afforded the corresponding *N*-glycolylneuraminic acid derivatives **12**–**15** in high yields (Scheme 1, Table 4).

In conclusion, we have elaborated a rapid and convenient approach that employs microwave irradiation for the facile de-N-acetylation of *N*-acetylneuraminic acid derivatives in good yield. The ready accessibility of 5-amino neuraminic acid derivatives using this method should facilitate the synthesis of *N*-glycolyl, and alternative N-functionalised neuraminic acid derivatives, for studies of sialic acid recognising proteins.

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Supplementary data

Supplementary data (full experimental details and scanned spectra for all synthesized compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.07.107.

References and notes

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29. *General procedure for the de-N-acetylation of Neu5Ac derivatives under microwave irradiation conditions—exemplified for the synthesis of* ***5***: In a Teflon septum sealed 30 mL pressure tube, a mixture of Neu4,5,7,8,9Ac$_5$1,α2Me$_2$ (**4**) (1.0 g, 1.98 mmol) and 2 M NaOH (15 mL) was irradiated for 15 min at 120 °C. After completion of the holding time, the reaction mixture was cooled and concentrated to give a yellow residue. The residue was taken up in H_2O and added to a column of Dowex® 50W × 8 (H^+) resin (20 g). The column was washed with H_2O (200 mL) and with 1.5 M NH_4OH (100 mL). Fractions containing the product were combined, concentrated and lyophilised to afford Neuα2Me (**5**) as a yellowish fluffy solid in 80% yield.