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A facile microwave-assisted protocol for rapid synthesis of *N*-acetylneuraminic acid congeners†

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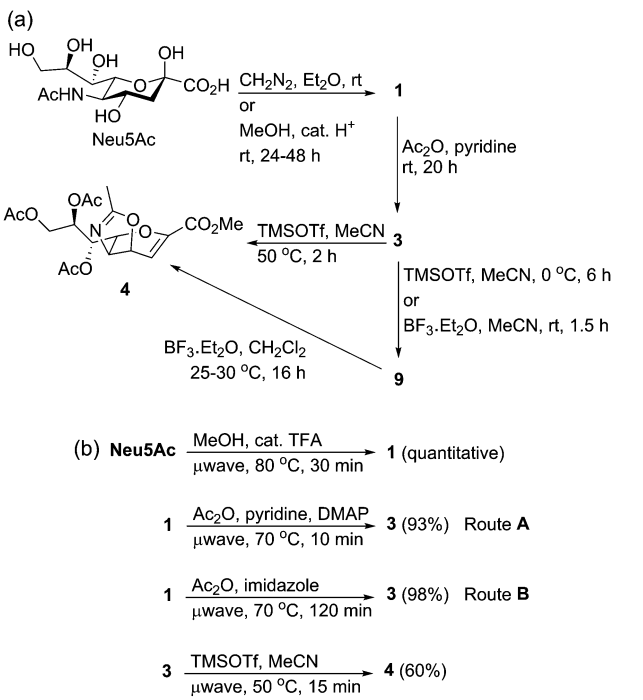
We developed a simple, rapid and efficient microwave irradiation-assisted protocol that is 1- to 2-orders of magnitude faster than conventional techniques, providing an expedient access to the sialic acid congeners Neu5Ac1Me (1), Neu5Acβ1,2Me$_2$ (2), Neu5Ac1Me *O*-peracetate (3) and 4,5-oxazoline of Neu5Ac2en1Me *O*-peracetate (4).

N-Acetylneuraminic acid (sialic acid; Neu5Ac) is a sugar amino acid found as the terminal monosaccharide of many glycoconjugates on mammalian cell surfaces. It plays a crucial role in vital biological processes as a mediator of intercellular communication and adhesion, viral receptor recognition, and various (patho)physiological processes.[1,2] Thus, many efforts have been devoted to the design and synthesis of Neu5Ac congeners as chemical probes[3] and antiviral agents.[2,4] For example, von Itzstein developed the neuraminidase inhibitor zanamivir[4] (4-guanidino-Neu5Ac2en, **5**) from Neu5Ac that is being stockpiled around the world to combat potential influenza pandemic. However, neuraminidase inhibitor drug resistance is emerging, which has sparked renewed interest in sialic acid analogues. Recently, Withers and co-workers reported that 2,3-difluoro-4-guanidino-Neu5Ac (**6**) is active against zanamivir-resistant influenza virus strains,[5] demonstrating the importance of the chemistry of functionalized sialic acid.

The key intermediate in the preparation of **5**, **6** and other related C-4 substituted congeners of Neu5Ac2en (**7**) is the peracetylated 4-azido-2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid methyl ester (**8**). Compound **8** can be conveniently prepared from the 4,5-oxazoline derivative of *O*-peracetylated Neu5Ac2en (**4**) *via* S_N2 type azidation and oxazoline ring opening upon treatment with $TMSN_3$ or LiN_3.[6] The oxazoline is easily synthesized through Lewis acid-promoted intramolecular β-attack of the 5-acetamido group at the adjacent allylic C-4 position of the glycal. The two common methods to prepare **4** are: (a) treatment of **3** with trimethylsilyl trifluoromethanesulfonate (TMSOTf)[7,8] and (b) treatment of Neu5Ac2en1Me *O*-peracetate (**9**) with $SnCl_4$ or $BF_3{\cdot}Et_2O$.[9] Although these reactions are efficient and are complete within 2–16 h (Scheme 1a), synthesis of their precursors **3** and **9** is traditionally more cumbersome. The synthesis of **3** requires esterification and peracetylation, which can then undergo elimination to give the glycal **9** using TMSOTf or $BF_3{\cdot}Et_2O$ (Scheme 1a). Standard C-1 esterification can be performed using the highly reactive but



Scheme 1 (a) Conventional synthesis and (b) optimized microwave irradiation-assisted synthesis of Neu5Ac congeners.

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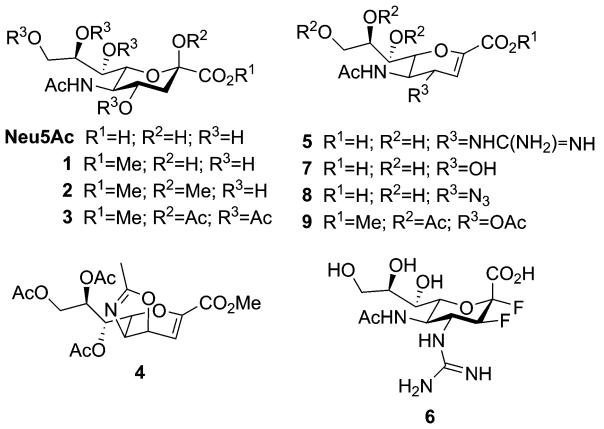

Fig. 1 Structure of Neu5Ac and some of its congeners.

toxic and explosive CH_2N_2 or by Fischer esterification using MeOH and catalytic trifluoroacetic acid (TFA) or dry Dowex 50x8 (H^+) resin, requiring 24–48 h to yield Neu5Ac1Me (**1**). Neu5Ac peracetylation is typically accomplished using excess Ac_2O and pyridine under ambient conditions for ~20 h to generate Neu5Ac1Me *O*-peracetate (**3**).[8,10–13] Although these have very slow reaction kinetics, they are still the current methods of choice (Fig. 1).

We successfully demonstrated the utility of Neu5Ac derivatives as useful building blocks for solid phase synthesis of amide-linked oligomers with stable secondary structures and remarkable proteolytic stability.[12,14] As part of our continuing studies on unnatural Neu5Ac peptides, we employed the standard methods described above to prepare Neu5Ac congeners, but found these to be too sluggish. Recently, there has been increasing interest in microwave energy-assisted heating to drive chemical reactions. Among other advantages, microwave heating dramatically reduces reaction times from days or hours to minutes, allowing rapid access to target molecules.[15] Despite these benefits, microwave-assisted synthesis remains underutilized for the chemical manipulation of sialic acid.[16–18] In order to expedite the synthesis of Neu5Ac congeners, we examined the effect of microwave irradiation to accelerate these chemical transformations. Herein we report a microwave-assisted protocol for the gram-scale preparation of **1**, **2**, **3** and **4**, at reaction rates that are 1- to 2-orders of magnitude faster than conventional techniques.

Since Neu5Ac1Me **1** is the most common synthetic derivative of Neu5Ac, we started the optimization of its synthesis by modifying the method described by Schauer.[13] Initial screening was carried out by treating Neu5Ac (0.20 g, 0.65 mmol) with catalytic TFA in anhydrous MeOH. We used the Biotage Initiator SP Wave synthesizer and set the microwave-assisted heating at 80 °C with variable power for various times (Scheme 1b). The reaction progress was monitored by thin layer chromatography, which indicated that Neu5Ac was completely consumed after 30 and 50 min at 80 °C by using 0.4 and 0.2 equiv. of TFA, respectively (see ESI†). Investigation by electrospray ionization mass spectrometry (ESI-MS) indicated that the major product (90–95%) formed in both treatments was **1** (*m*/*z* 346.1, $[M + Na]^+$).

^{1}H NMR analysis showed a broad singlet at δ 3.82 corresponding to the OMe peak of the C-1 methyl ester and a doublet of doublets at δ 2.29 (H-3$_{eq}$, J = 13.0, 4.9 Hz). We noticed a minor product with *m*/*z* 360.2 ($[M + Na]^+$; $\Delta m/z$ from **1** = 14.1) indicating methylation of C-2 OH to form the methyl sialoside Neu5Acβ1,2Me$_2$ **2** (see ESI†). This can be expected since **2** has been previously prepared using MeOH/H^+ under ambient[11] and microwave conditions.[16]

To investigate if irradiation at higher temperatures would favor the formation of **1** within 30 min, we treated Neu5Ac with 0.4 equiv. TFA at varying temperatures (80–120 °C at 10 degree increments, Table 1). It was interesting that the reaction mixture yielded higher amounts of **2** at higher temperatures. This observation also correlated well with the increase in ^{1}H NMR resonance intensity of the H-3$_{eq}$ of **2** (δ 2.35, dd, J = 13.5, 4.9 Hz) that confirms its increased formation with temperature, reaching a ratio of 1 : 1 at 120 °C based on resonance peak area integration (Fig. 2). The above finding seemed to offer a handle for directing the formation of **2**. Further irradiation to higher temperatures resulted in the

Table 1 Optimization of microwave-assisted synthesis of **1** at 30 min using variable temperatures

Entry	Temperature (°C)	Ratio (**1** : **2**)
1	80	1.00 : 0.05
2	90	1.00 : 0.23
3	100	1.00 : 0.27
4	110	1.00 : 0.55
5	120	1.00 : 1.00

Reactions were carried out using 200 mg of Neu5Ac (0.65 mmol) and 0.4 equiv. of TFA in anhydrous MeOH (3 mL). Ratios were measured by integrating the H-3$_{eq}$ resonance peak of products in ^{1}H-NMR (500 MHz, D_2O, 298 K). Neu5Ac1Me (**1**): δ 2.30 (dd, $J_{3eq,3ax}$ 13.1, $J_{3eq,4}$ 4.9 Hz); Neu5Acβ1,2Me$_2$ (**2**): 2.35 (dd, 1H, $J_{3eq,3ax}$ 13.1, $J_{3eq,4}$ 5.0 Hz).

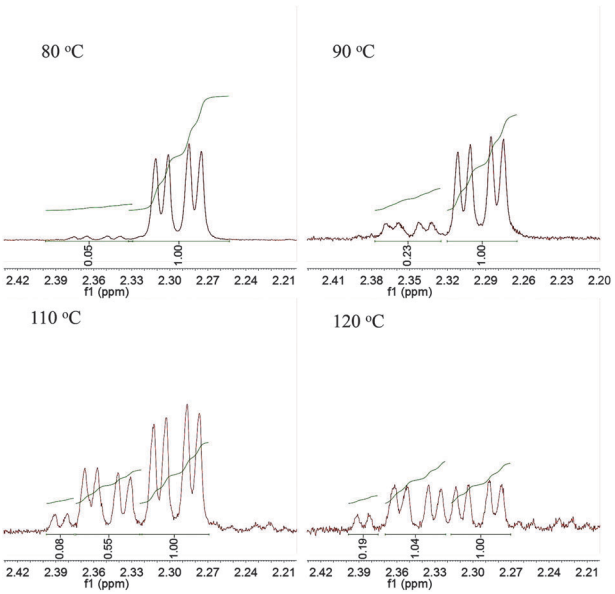

Fig. 2 ^{1}H NMR spectra of crude esterification reaction mixtures showing increasing amounts of **2** at higher temperatures (500 MHz, D_2O).

Table 2 Optimization of the scale-up synthesis of **1**

Entry	MeOH (mL)	Ratio (**1** : **2**)
1	8	1.0 : 0.1
2	9	1.0 : 0.2
3	10	1.0 : 0.1
4	11	1.0 : 0.2
5	12	1.0 : 0.2
6	15	1.0 : 0.1
7	20	1.0 : 0.0

Reactions were carried out using 1.0 g of Neu5Ac (3.23 mmol) and 0.4 equiv. of TFA in anhydrous MeOH. The mixture was microwave irradiated to 80 °C for 30 min. Ratios were measured by integrating the H-3_{eq} resonance peak of products in ^{1}H-NMR (500 MHz, D_2O, 298 K). Neu5Ac1Me (**1**): δ 2.30 (dd, $J_{3eq,3ax}$ 13.1 Hz, $J_{3eq,4}$ 4.9 Hz); Neu5Acβ1,2Me_2 (**2**): 2.35 (dd, 1H, $J_{3eq,3ax}$ 13.1 Hz, $J_{3eq,4}$ 5.0 Hz).

formation of compound **2**, and concurrently, other uncharacterized products (Fig. 2 and ESI†). Since our interest is to prepare the methyl ester **1** in large amounts, we proceeded with the optimization, maintaining the conditions at 80 °C for 30 min using 1 g of Neu5Ac. At this point we found it necessary to dilute the reaction mixture to 20 mL (0.16 M Neu5Ac in MeOH) to produce **1** as the sole product (Table 2 and ESI†) in quantitative isolated yield. A previous report on an efficient microwave-assisted synthesis of **1** used Dowex 50x8 (H^+) as catalyst.[16] However, it was noted that resin age and quality influence the outcomes of the reaction, requiring tedious resin conditioning followed by storage under inert conditions. The use of TFA as described in our present work offers an economical and simple alternative towards consistent and reproducible preparation of Neu5Ac1Me.

The standard method for peracetylating carbohydrates uses excess Ac_2O in pyridine at room temperature. We examined the effect of microwave irradiation on the synthesis of Neu5Ac1Me *O*-peracetate **3** by treating **1** with Ac_2O (25 equiv.), pyridine (30 equiv.), and *N*,*N*-dimethylaminopyridine (DMAP, 5 mol%) and irradiating the mixture to 40 °C which went to completion at 45 min. Noting the mild temperature compared to the esterification conditions used in microwave-assisted reactions, we dramatically increased the temperature to 90 °C with the expectation that the kinetics of this thermally driven reaction would benefit from further heating. We found that **1** consumed within 10 min but this also occurred with concomitant partial decomposition of the starting material. We investigated irradiation conditions at 50, 60, 70, 80 °C at 10 min and found 70 °C to be optimal, leading to clean formation of **3** (ESI†). These conditions were translated to a larger scale (1.0 g) giving 93% isolated yield of **3**.

Despite the efficiency of the optimized microwave-assisted peracetylation as described above, it is still desirable to avoid the use of hazardous and noxious reagents like pyridine when possible as well as avoid solvent intensive and laborious column chromatographic purification which restrict our method's general utility. To overcome these limitations, we investigated a more benign peracetylation method[19] using catalytic imidazole (0.6 equiv.), 10 equiv. of Ac_2O, and MeCN as solvent followed by irradiation to 70 °C for 10 min. To our dismay, compound **1** was minimally soluble in MeCN and we only obtained a mixture of partially peracetylated products in very low yields that did not go to completion even after prolonged irradiation (240 min). We optimized and translated the conditions to gram scale synthesis by using DMF as solvent, 5 equiv. of imidazole, 25 equiv. of Ac_2O, and irradiating for 120 min to form the desired compound **3** as the sole product (Table 3). The mixture was concentrated, re-suspended in CH_2Cl_2, washed with aq. Na_2CO_3, and dried under reduced pressure to yield purified **3** (1.64 g, 98% isolated yield). The simpler purification and avoidance of pyridine and related base catalysts more than compensates for the longer irradiation time, making this method amenable and very attractive for peracetylation of carbohydrates in multigram quantities.

Table 3 Optimization of peracetylation using imidazole at 70 °C

Entry	Imidazole (equiv.)	Reaction time (min)	Yield (% of **3**)
1	0.6	240	5[a]
2	1.0	240	20[a]
3	2.0	240	40[a]
4	5.0	120	100[b]

Reactions were carried out using 25 equiv. Ac_2O, 70 °C, in DMF. [a] Yields estimated using TLC and ESI-MS, major products were partially acetylated compounds. [b] Confirmed by TLC, ESI-MS, and ^{1}H NMR.

We proceeded with the synthesis of **4** by treating the solution of **3** in anhydrous MeCN with TMSOTf and irradiating the mixture to 50 °C at various reaction times. The reaction was quenched by titrating the mixture with Na_2CO_3 to pH = 9.0 followed by extraction with EtOAc. We obtained the oxazoline **4** in 15 min under these conditions. The preparation was scaled up using 1.0 g of **3** and gave the desired product in 60% isolated yield that is comparable with previous synthesis of **4** using conventional conductive heating.[8,20]

There is a dearth of literature on the chemical manipulation of sialic acid using microwave irradiation,[16–18] which calls for improved synthetic methodologies that take advantage of this technology. Recently, von Itzstein and co-workers reported on a fast and convenient one-step microwave-assisted de-*N*-acetylation of Neu5Ac congeners using 2.0 M aqueous NaOH.[18] This significantly improved method could be utilized as an alternative for the tedious, multi-step deprotection of **9** and other Neu5Ac congeners.[14,21] These findings are complementary to our current efforts in expediting access to sialic acid derivatives by microwave-assisted reactions.

In conclusion, we have developed simple, rapid, and efficient microwave-assisted protocols for the synthesis of Neu5Ac1Me (**1**), Neu5Acβ1,2Me_2 (**2**), Neu5Ac1Me *O*-peracetate (**3**), and the 4,5-oxazoline derivative of Neu5Ac2en1Me *O*-peracetate (**4**). These microwave-assisted reactions expedited the syntheses of compounds **1**, **2**, **3** and **4** at rates that are 1- to 2-orders of magnitude faster than standard procedures. These methods use benign and readily available reagents as well as streamlined the purification of **3** through simple solvent extraction. These protocols are amenable to gram scale preparation of Neu5Ac congeners and offer convenient access to important intermediates in sialic acid chemistry and in our studies of unnatural Neu5Ac peptides.

Experimental section

Synthesis of Neu5Ac1Me (1)

Neu5Ac (1.0 g, 3.2 mmol, 1 equiv.) was transferred to a thick walled microwave tube and suspended in 20 mL anhydrous MeOH (Neu5Ac conc. = 0.16 M). TFA (1.29 mmol, 0.4 equiv.) was added dropwise to the stirring reaction mixture, the tube sealed, and the mixture subjected to microwave irradiation using the Biotage Initiator SP Wave microwave reactor for 30 min at 80 °C. The mixture was concentrated under reduced pressure to give **1** in quantitative yield.

Synthesis of Neu5Ac1Me *O*-peracetate (3): route A

Compound **1** (1.0 g, 3.1 mmol, 1 equiv.) was transferred to a thick walled microwave tube followed by the addition of DMAP (5.0 mol%). Pyridine (30 equiv.) and Ac_2O (25 equiv.) were added and the tube was sealed. The reaction mixture was microwave irradiated at 70 °C for 10 min. The mixture was concentrated under reduced pressure and azeotroped with 15 mL toluene to yield an oily material that was purified by silica column chromatography using CH_2Cl_2–MeOH (49 : 1) as an eluent to give **3** in 93% yield.

Synthesis of Neu5Ac1Me *O*-peracetate (3): route B

Compound **1** (1.0 g, 3.1 mmol, 1 equiv.) was transferred to a microwave reactor tube and dissolved in 3.9 mL DMF. Imidazole (5 equiv.) was added to the stirring solution of **1** followed by the addition of Ac_2O (25 equiv.). The vessel was sealed and heated by microwave irradiation to 70 °C for 120 min. The reaction mixture was evaporated to dryness, resuspended in H_2O, and extracted with DCM. The organic layer was washed three times with saturated Na_2CO_3 solution, dried over Na_2SO_4, and evaporated to dryness yielding purified **3** in 98% yield.

Synthesis of the 4,5-oxazoline derivative of Neu5Ac2en1Me *O*-peracetate (4)

To a microwave reactor tube compound **3** (1.0 g, 1.9 mmol, 1 equiv.) was added. The vessel was capped, evacuated, and filled with Ar. The air purging process was repeated three times followed by the addition of anhydrous MeCN (15 mL) and TMSOTf (0.7 mL, 2.1 equiv.). The mixture was irradiated at 50 °C for 15 min, cooled to 0 °C in an ice bath for 5 min, and treated with saturated solution of Na_2CO_3 to pH = 9. The mixture was extracted with EtOAc (3 × 25 mL), the combined organic layer washed with water, dried over $MgSO_4$, evaporated to dryness, and purified by silica column chromatography using EtOAc as an eluent to give compound **4** in 60% yield.

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