Tetrahedron Letters 53 (2012) 6254–6256

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Rapid and clean microwave-assisted synthesis of *N*-acetylneuraminic acid methyl ester and its β-methyl glycoside

Pradeep Chopra, Robin J. Thomson, I. Darren Grice, Mark von Itzstein *

Institute for Glycomics, Griffith University Gold Coast Campus, Queensland 4222, Australia

ARTICLE INFO

Article history:
Received 18 June 2012
Revised 30 August 2012
Accepted 6 September 2012
Available online 14 September 2012

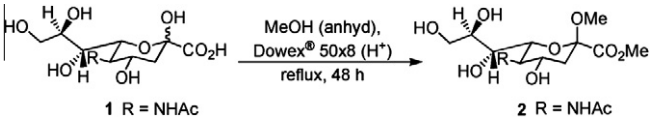
Keywords:
Sialic acid
Microwave
N-Acetylneuraminic acid methyl ester
β-Methyl glycoside

ABSTRACT

The effect of microwave irradiation on the synthesis of *N*-acetylneuraminic acid methyl ester and its β-methyl glycoside is investigated. On a 1 g scale, a high yield (94%) of the methyl ester was obtained after 15 min at 80 °C. Acceleration of the glycosidation reaction was achieved, with the β-methyl glycoside isolated in good yield following optimisation of reaction conditions to 15 min at 120 °C.

Sialic acids comprise a family of over 50 nine-carbon acidic monosaccharides, with the most prevalent member being *N*-acetylneuraminic acid (Neu5Ac, **1**).[1] They are widely distributed in nature, typically glycosidically linked to the non-reducing end of a range of glycoprotein- and glycolipid-associated glycans that are present on cell surfaces.[2–4] These carbohydrates are intimately involved in a wide range of biological processes, ranging from cell–cell interactions, cell differentiation and tumour metastasis, to host–pathogen recognition phenomena.[5–7] In order to investigate the requirements of sialic acid recognizing proteins, in particular the enzymes involved in sialic acid metabolism such as CMP-sialate synthase (CMAS), sialidases and Neu5Ac aldolase, extensive manipulations have been carried out on Neu5Ac, resulting in modification at each carbon.[8] In this context, we recently reported the synthesis of C-9 oxidised derivatives of α- and β- (**2**) methyl glycosides of *N*-acetylneuraminic acid methyl ester.[9]

The functionalisation of *N*-acetylneuraminic acid (**1**) typically commences with the methyl esterification of the anomeric carboxy group to provide Neu5Ac1Me (**3**) and is followed by, in specific instances, glycosidation of the anomeric hydroxy group. Both the β-methyl glycoside of *N*-acetylneuraminic acid methyl ester [Neu5Acβ1,2Me$_2$ (**2**)] and methyl ester **3** itself, are useful intermediates in sialic acid chemistry. Kuhn et al. first reported[10] the synthesis of **2** in 1966, which involved refluxing **1** with dry Dowex® 50x8 (H^+) resin in anhydrous methanol for 24 h (Scheme 1). This method is still favoured today despite the long reaction time.



Scheme 1.

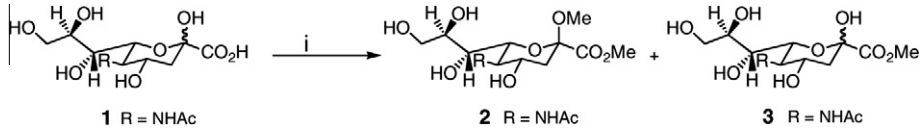
Microwave irradiation has been used in some thermally driven organic reactions as an alternative source of heat energy to accelerate chemical transformations.[11,12] Some of the major advantages of using microwave-assisted synthesis include reduced reaction times, increased purity and higher yields.[13] The use of microwave irradiation in chemical manipulations of carbohydrates is still relatively in its infancy,[14] although some well known reactions such as Fischer glycosylation have been reported.[15] To date, microwave-assisted synthesis has been poorly explored in the chemistry of sialic acids.[16] Herein we report on the optimisation of the rapid and facile synthesis of both **2** and **3** using microwave irradiation.

To examine the effect of microwave irradiation on the synthesis of the methyl ester of **1** and its β-methyl glycoside using Dowex® 50x8 (H^+) resin in anhydrous methanol, initial screening was carried out to optimise microwave[17] irradiation time (Scheme 2). We employed a reaction temperature of 100 °C at a maximum microwave power of 100 W for a period of 5, 10, 15 and 20 min, respectively (Table 1).[18]

The progress of the reaction was monitored by thin-layer chromatography (TLC) and ^{1}H NMR spectroscopy.[18] TLC analysis showed that after 5 min complete consumption of starting material **1** had occurred. Investigation by ^{1}H NMR spectroscopy (in D_2O) indicated that the major product formed was Neu5Ac1Me

* Corresponding author. Tel.: +61 7 5552 7025; fax: +61 7 5552 9040.
E-mail address: m.vonitzstein@griffith.edu.au (M. von Itzstein).

0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2012.09.017



Scheme 2. Reagents and conditions: (i) MeOH (anhyd), Dowex® 50Wx8 (H^+) resin, 100 °C, maximum power 100 W.

Table 1
Microwave-assisted synthesis of **2** and **3** at 100 °C[a]

Entry	Time (min)	Ratio (**2**:**3**)[b]
1	5	0.06:1.0
2	10	0.83:1.0
3	15	1.0:0.70
4	20	1.0:1.0

[a] Reaction was carried out on 50 mg (0.16 mmol) of **1** at 100 °C under microwave irradiation.[18]

[b] Ratio of reaction products (**2**:**3**) in the reaction mixture as monitored by ^{1}H NMR (300 MHz, D_2O, ppm): δ 2.42 (dd, 1H, $J_{3eq,3ax}$ 12.9 Hz, $J_{3eq,4}$ 5.1 Hz, H-3$_{eq}$) Neu5Acβ1,2Me$_2$ (**2**), δ 2.40 (dd, 1H, $J_{3eq,3ax}$ 12.9 Hz, $J_{3eq,4}$ 5.1 Hz, H-3$_{eq}$) Neu5Ac1Me (**3**).

(**3**). This was established by the presence of a broad singlet at δ 3.75 corresponding to COOMe. Also formed as a very minor component was Neu5Acβ1,2Me$_2$ (**2**), as indicated by the presence of a singlet at δ 3.30, corresponding to the anomeric OMe of **2**. ^{1}H NMR analysis of the 10 min reaction, revealed the clear presence of a doublet of doublets at δ 2.42 and two singlets at δ 3.30 and δ 3.86, corresponding to H-3$_{eq}$, OMe and COOMe, respectively, of **2**, confirming the formation of **2**, although **3** was still the predominant reaction product in a ratio of 0.83:1.0 (**2**:**3**). Interestingly, irradiation of the reaction mixture for 15 min resulted in the dominant formation of **2** in the ratio of 1.0:0.70, as indicated by the integration of the respective H-3$_{eq}$ signals in the ^{1}H NMR spectrum of the reaction mixture (see Table 1).

A progressive increase in the quantity of the β-methyl glycoside **2** was obtained with increasing microwave irradiation from 5 to 10 to 15 min. However, irradiating for 20 min resulted in decomposition of the carbohydrate. Based on this observation, 15 min of microwave exposure was selected as the optimum reaction time. We then proceeded to optimise the reaction temperature, employing temperatures ranging from 80 to 180 °C (Table 2). Temperatures below 100 °C favoured the formation of Neu5Ac1Me (**3**), while above 120 °C increasing levels of decomposition were observed. It is evident from Table 2 that the optimum reaction temperature for the synthesis of **3** is 80 °C. The optimum reaction temperature for the synthesis of **2** was 120 °C. While maintaining the temperature at 120 °C, further optimisation for the synthesis

Table 2
Optimisation of the reaction conditions: temperature[a]

Entry	Temp (°C)	Ratio (**2**:**3**)[b]
1	80	0.10:1.0
2	90	0.15:1.0
3	100	0.46:1.0
4	110	1.0:0.50
5	120	1.0:0.20
6	130	1.0:0.58
7	140	1.0:0.47
8	160	0.24:1.0
9	180	0.20:1.0

[a] Reaction was carried out on 50 mg (0.16 mmol) of **1** for 15 min under microwave irradiation.[18]

[b] Composition of the reaction products (**2**:**3**) in the reaction mixture as monitored by ^{1}H NMR (300 MHz, D_2O, ppm): δ 2.42 (dd, 1H, $J_{3eq,3ax}$ 12.9 Hz, $J_{3eq,4}$ 5.1 Hz, H-3$_{eq}$) Neu5Acβ1,2Me$_2$ (**2**), δ 2.40 (dd, 1H, $J_{3eq,3ax}$ 12.9 Hz, $J_{3eq,4}$ 5.1 Hz, H-3$_{eq}$) Neu5Ac1Me (**3**).

Table 3
Optimisation of the reaction conditions: time at 120 °C[a]

Entry	Reaction time (min)	Ratio (**2**:**3**)[b]
1	10	1.0:0.25
2	12	1.0:0.25
3	15	1.0:0.20
4	18	1.0:0.22
5	20	1.0:0.20

[a] Reactions carried out on 50 mg (0.16 mmol) of **1** at 120 °C under microwave irradiation.[18]

[b] Composition of the reaction products (**2**:**3**) in the reaction mixture as monitored by ^{1}H NMR (300 MHz, D_2O, ppm): δ 2.42 (dd, 1H, $J_{3eq,3ax}$ 12.9 Hz, $J_{3eq,4}$ 5.1 Hz, H-3$_{eq}$) Neu5Acβ1,2Me$_2$ (**2**), δ 2.40 (dd, 1H, $J_{3eq,3ax}$ 12.9 Hz, $J_{3eq,4}$ 5.1 Hz ,H-3$_{eq}$) Neu5Ac1Me (**3**).

of **2** was then carried out by exploring five reaction times between 10 and 20 min (Table 3). The optimum reaction time for the synthesis of **2** at this temperature was found to be 15 min.

It was of interest to determine if methyl ester **3** could be formed as the sole reaction product under the microwave irradiation conditions. It was found that by decreasing the amount of Dowex® 50x8 (H^+) resin catalyst (from 0.05[18] to 0.01 g of resin per 0.05 g of **1**) and decreasing the reaction concentration, using the conditions of 80 °C and 15 min reaction time, only ester **3** was formed, as indicated by TLC and ^{1}H NMR spectroscopy.[19]

To investigate the effect of microwave irradiation on the scale of synthesis of **2** and **3** we increased the mass of starting material **1** by 20-fold to 1.0 g.[20] The optimised microwave irradiation conditions for the preparation of **2** (15 min at 120 °C at maximum power of 100 W) afforded the desired compound **2** in 63% yield, with **3** also isolated in 15% yield [(**2**:**3** ratio, 1.0:0.23)].[20] The determined optimised reaction conditions for the preparation of ester **3** provided the target compound in 94% isolated yield.[21]

In conclusion, we have developed and optimised a rapid and convenient method employing microwave irradiation for a facile access to *N*-acetylneuraminic acid methyl ester (**3**) in excellent yield and its β-methyl glycoside (**2**). Although the yield of **2** was comparable to the conventional method of synthesis, [10,21] the major advantage of this approach was that the reaction time was greatly reduced from 24 h to 15 min with microwave assistance. Additionally, there is a significant reduction in the amount of solvent and acidic resin required under our conditions. Scale-up synthesis for both **2** and **3** was demonstrated to be feasible and with advancement in microwave reactors the often required large-scale production of grams to kilograms of product would appear to be an available option, with little or no further procedural modifications required.

Acknowledgments

P.C. thanks Griffith University (Research Scholarship) and the Institute for Glycomics for financial support. M.v.I. is grateful to the Australian Research Council and the National Health and Medical Research Council for financial support.

References and notes

1. (a) Schauer, R. *Angew. Chem., Int. Ed.* **1973**, *12*, 127–138; (b) Angata, T.; Varki, A. *Chem. Rev.* **2002**, *102*, 439–469.

2. Varki, A. *Glycobiology* **1992**, *2*, 25–40.
3. Schauer, R. *Glycoconjugate J.* **2000**, *17*, 485–499.
4. Chen, X.; Varki, A. *ACS Chem. Biol.* **2010**, *5*, 163–176.
5. Varki, A. *Trends Mol. Med.* **2008**, *14*, 351–360.
6. Hedlund, M.; Padler-Karavani, V.; Varki, N. M.; Varki, A. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 18936–18941.
7. Inoue, S.; Sato, C.; Kitajima, K. *Glycobiology* **2010**, *20*, 752–762.
8. (a) Zbiral, E. In *Carbohydrates—Synthetic Methods and Applications in Medicinal Chemistry*; Ogura, H., Hasegawa, A., Suami, T., Eds.; VCH: Weinheim, 1992; pp 304–339; (b) von Itzstein, M.; Thomson, R. J. *Curr. Med. Chem.* **1997**, *4*, 185–210.
9. Kiefel, M. J.; Chopra, P.; Madge, P. D.; Szyczew, A.; Thomson, R. J.; Grice, I. D.; von Itzstein, M. *Tetrahedron Lett.* **2011**, *52*, 98–100.
10. Kuhn, R.; Lutz, P.; MacDonald, D. L. *Chem. Ber.* **1966**, *99*, 611–617.
11. Gedye, R.; Smith, F.; Westaway, K.; Ali, H.; Baldisera, L.; Laberge, L.; Rousell, J. *Tetrahedron Lett.* **1986**, *27*, 279–282.
12. Giguere, R. J.; Bray, T. L.; Duncan, S. M.; Majetich, G. *Tetrahedron Lett.* **1986**, *27*, 4945–4948.
13. (a) Kappe, C. O. *Angew. Chem., Int. Ed.* **2004**, *43*, 6250–6284; (b) Caddick, S.; Fitzmaurice, R. *Tetrahedron* **2009**, *65*, 3325–3355.
14. (a) Corsaro, A.; Chiacchio, U.; Pistarà, V.; Romeo, G. *Curr. Org. Chem.* **2004**, *8*, 511–538; (b) Corsaro, A.; Chiacchio, U.; Pistarà, V.; Romeo, G., 2nd ed. In *Microwaves in Organic Synthesis*; Loupy, A., Ed.; Wiley-VCH, 2006; Vol. 1, pp 579–614; (c) Richel, A.; Laurent, P.; Wathelet, B.; Wathelet, J.-P.; Paquot, M. C. R. *Chimie* **2011**, *14*, 224–234.
15. (a) Nüchter, M.; Ondruschka, B.; Lautenschläger, W. *Synth. Commun.* **2001**, *31*, 1277–1283; (b) Bornaghi, L. F.; Poulsen, S.-A. *Tetrahedron Lett.* **2005**, *46*, 3485–3488; (c) Roy, D. K.; Bordoloi, M. *J. Carbohydr. Chem.* **2008**, *27*, 300–307.
16. (a) Šardzík, R.; Noble, G. T.; Weissenborn, M. J.; Martin, A.; Webb, S. J.; Flitsch, S. L. *Beilstein J. Org. Chem.* **2010**, *6*, 699–703; (b) Patane, J.; Trapani, V.; Villavert, J.; McReynolds, K. D. *Carbohydr. Res.* **2009**, *344*, 820–824.
17. A CEM Discover® SP Explorer Hybrid-12 microwave system with single mode cavity was used in this study. The microwave can perform reactions with volumes of 0.2–75 mL and run open vessel or pressurised vessel reactions. The system embodies volume-independent infrared temperature or fibre optic temperature measurement, automated power control, air-cooling for simultaneous cooling (PowerMax) and reaction quenching and ActiVent self-venting technology for pressure relief during or after reaction.
18. *General procedure for the synthesis of Neu5Acβ1,2Me$_2$ (**2**) and Neu5Ac1Me (**3**) under microwave irradiation conditions*: In a Teflon (septum)-sealed 10 mL pressure tube, a mixture of *N*-acetylneuraminic acid (**1**) (0.05 g, 0.16 mmol) and dry Dowex® 50x8 (H^+) resin (0.05 g) in anhydrous MeOH (2 mL) was irradiated for a specific time at a specific temperature. After completion of the holding time, an aliquot of the reaction mixture was analysed by TLC and ^{1}H NMR spectroscopy. ^{1}H NMR spectra were recorded in D_2O to allow observation of the anomeric OMe which is overlapped by residual MeOH when the spectra are run in CD_3OD.
It should be noted, we observed that the age and quality of the resin used influenced reaction outcomes. In the reported experiments the resin was prepared according to the following procedure: A slurry of Dowex® 50x8 (H^+) resin (50 g) in H_2O (400 mL) was gently agitated, allowed to settle, and the H_2O was decanted off. The resin was then similarly washed with MeOH (2 × 200 mL) and finally with anhydrous MeOH (200 mL). The washed resin was dried on a rotary evaporator, then under high vacuum for 16 h, and was finally stored under N_2 or Ar.
19. Optimised conditions for the synthesis of Neu5Ac1Me (**3**) from *N*-acetylneuraminic acid (**1**) (0.05 g, 0.16 mmol), that consistently gave high yields (>92%), used less of the freshly dried resin (0.01 g) and an increased solvent volume (3 mL), with reaction at 80 °C for 15 min.
20. To explore larger scale syntheses of **2** and **3** a mixture of *N*-acetylneuraminic acid (**1**) (1.0 g, 3.24 mmol) and dry Dowex® 50x8 (H^+) resin (1.0 g for the synthesis of **2** or 0.2 g for the synthesis of **3**) in anhydrous MeOH (25 mL) was irradiated for 15 min at either 120 °C (for the synthesis of **2**) or 80 °C (for the synthesis of **3**). After completion of the holding time, the reaction mixture was cooled to room temperature, the resin was removed by filtration over Celite, and the filtrate was concentrated under reduced pressure to give a syrup.
*Synthesis of Neu5Acβ1,2Me$_2$ (**2**)*: The crude product mixture was dissolved in a small volume of EtOAc–MeOH, adsorbed onto silica, and purified using a Reveleris® flash chromatography system [40 g column; flow rate 30 mL/min; eluent: 100% EtOAc to 4:1 EtOAc–MeOH] to provide Neu5Acβ1,2Me$_2$ (**2**) (0.68 g, 63%) and Neu5Ac1Me (**3**) (0.15 g, 15%).
Compound **2**: R_f = 0.55 (EtOAc–MeOH–H_2O 7:2:1); ^{1}H NMR (CD_3OD): δ 1.62 (1H, dd, $J_{3ax,\ 3eq}$ 12.9 Hz, $J_{3ax,\ 4}$ 11.4 Hz, H-3$_{ax}$), 1.99 (3H, s, NAc), 2.33 (1H, dd, $J_{3eq,\ 3ax}$ 12.9 Hz, $J_{3eq,\ 4}$ 5.1 Hz, H-3$_{eq}$), 3.25 (3H, s, OMe), 3.49 (1H, d, $J_{6,5}$ 9.0 Hz, H-6), 3.64 (1H, dd, $J_{9A,9B}$ 12.0 Hz, $J_{9A,8}$ 6.3 Hz, H-9A), 3.77–3.84 (7H, m, H-5, H-7, H-8, H-9B, CO_2Me), 3.94–4.01 (1H, m, H-4); ^{13}C NMR (CD_3OD): δ 21.9 [NC(O)*Me*], 39.0 (C-3), 50.87 (OMe), 51.5 (C-5), 53.4 (CO_2*Me*), 63.2 (C-9), 66.3 (C-4), 67.8, 69.6 and 70.4 (C-6, C-7, C-8), 99.0 (C-2), 170.3 (C-1), 174.6 [NC(O)Me]; LRMS: $C_{13}H_{23}NO_9$ *m*/*z* 360.3 ([M+Na]$^+$, 100%).
Compound **3**: R_f = 0.50 (EtOAc–MeOH–H_2O 7:2:1); ^{1}H NMR (CD_3OD): δ 1.84 (1H, dd, $J_{3ax,\ 3eq}$ 12.9 Hz, $J_{3ax,\ 4}$ 11.4 Hz, H-3$_{ax}$), 2.00 (3H, s, NAc), 2.17 (1H, dd, $J_{3eq,\ 3ax}$ 12.9 Hz, $J_{3eq,\ 4}$ 5.1 Hz, H-3$_{eq}$), 3.45 (1H, dd, $J_{7,6}$ 1.5 Hz, $J_{7,8}$ 9.3 Hz, H-7), 3.57 (1H, dd, $J_{9A,9B}$ 11.1 Hz, $J_{9A,8}$ 5.7 Hz, H-9A), 3.66 (1H, m, H-8), 3.76 (3H, s, CO_2Me), 3.79–3.83 (2H, m, H-5, H-9B), 3.96 (1H, dd, $J_{6,7}$ 1.5 Hz, $J_{6,5}$ 10.5 Hz, H-6), 4.02 (1H, m, H-4); ^{13}C NMR (CD_3OD): δ 22.6 [NC(O)*Me*], 40.7 (C-3), 53.1 (CO_2*Me*), 54.3 (C-5), 64.8 (C-9), 67.8 (C-4), 70.1, 71.6 and 72.6 (C-6, C-7, C-8), 96.6 (C-2), 171.7 (C-1), 175.1 [NC(O)Me]; LRMS: $C_{12}H_{21}NO_9$ *m*/*z* 345.8 ([M+Na]$^+$, 100%).
*Synthesis of Neu5Ac1Me (**3**)*: The crude syrupy product was dried under high vacuum to provide an amorphous solid. The amorphous solid was washed with EtOAc (3 × 10 mL) and dried under high vacuum to provide **2** (0.99 g, 94%). Characterisation is as given above.
21. *General procedure for the conventional synthesis of Neu5Acβ1,2Me$_2$ (**2**)*: A mixture of *N*-acetylneuraminic acid (**1**) (5.0 g, 16.18 mmol) and dry Dowex® 50x8 (H^+, 12.5 g) in anhydrous MeOH (250 mL) was refluxed for 48 h, then cooled to room temperature and the resin removed by filtration. The filtrate was concentrated under reduced pressure to give a yellow syrup. The syrup was then dissolved in a small volume of EtOAc–MeOH (3:1, v/v). On standing at ~4 °C, crystals were deposited which were further washed with ethyl acetate to obtain crystalline **2** (3.4 g, 62%). R_f = 0.2 (EtOAc–MeOH, 5:1).