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

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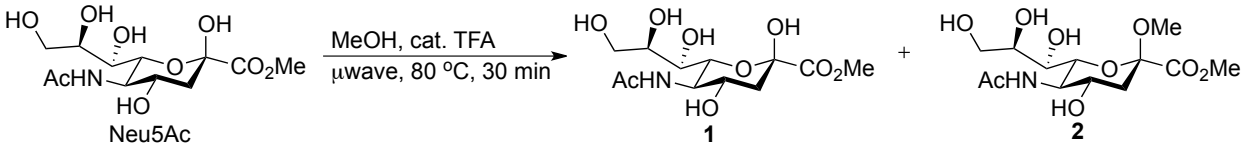
Supporting Information

General Procedures

Nuclear magnetic resonance (NMR) spectra were recorded on Varian Inova 500 MHz and Varian Vx 300 MHz spectrometers. Chemical shifts were referenced to residual $CHCl_3$ (δ_H = 7.26), CHD_2OD (δ_H = 4.87), $(CHD_2)_2CO$ (δ_H = 2.05), and HDO (δ_H = 4.79). Electrospray ionization mass spectra were recorded using Thermo Ion Trap ESI mass spectrometer. Microwave-assisted synthesis was performed using the Biotage Initiator+ SP Wave microwave synthesizer. *N*-acetylneuraminic acid (Neu5Ac; sialic acid) was purchased from Carbosynth and used as received. All reagents were obtained from commercial suppliers and used as received.

Synthesis of Neu5Ac1Me (1) and Neu5Acβ1,2Me$_2$ (2)



In general Neu5Ac was added to a thick walled microwave tube, suspended in anhydrous MeOH, followed by drop-wise addition of TFA to the stirring reaction mixture. The reaction tube was then sealed and subjected to microwave irradiation. After irradiation, an aliquot of the reaction mixture was analyzed by TLC, ESI-MS, and ^{1}H NMR spectroscopy.

The first trials were done using (0.200 g, 0.647 mmol) Neu5Ac at 80 ˚C using 0.2 and 0.4 equiv of TFA. The starting material was consumed at 50 and 30 minutes respectively, but gave compound **2** as minor product (Table S1, Fig S1 and S2). Trials performed at higher temperatures using 0.2 g Neu5Ac and 0.4 equiv TFA were carried out as well favoring **2** but eventually led to other decomposition products (Table S2, Fig. S3 and S4).

When applying the optimized methods on a 1 gram scale, it became necessary to reduce the concentration of Neu5Ac to minimize the amount of **2**, finding 0.16 M Neu5Ac (1 g in 20 mL MeOH) to be ideal, giving **1** in quantitative yields (Figure S5).

Table S1. Microwave-assisted esterification of Neu5Ac (0.20 g, 0.647 mmol) at 80 ˚C with variable time using 0.2 and 0.4 equiv of TFA.

Entry	Time (min)	TFA (0.2 Equiv.) Ratio (**1**:**2**)	TFA (0.4 Equiv.) Ratio (**1:2**)
1	20	n.d.	No reaction
2	30	n.d.	1.0:0.1
3	40	No reaction	1.0:0.1
4	50	1.0:0.1	1.0:0.1
5	60	1.0:0.1	1.0:0.1
6	90	1.0:0.1	n.d.

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). n.d. = not determined

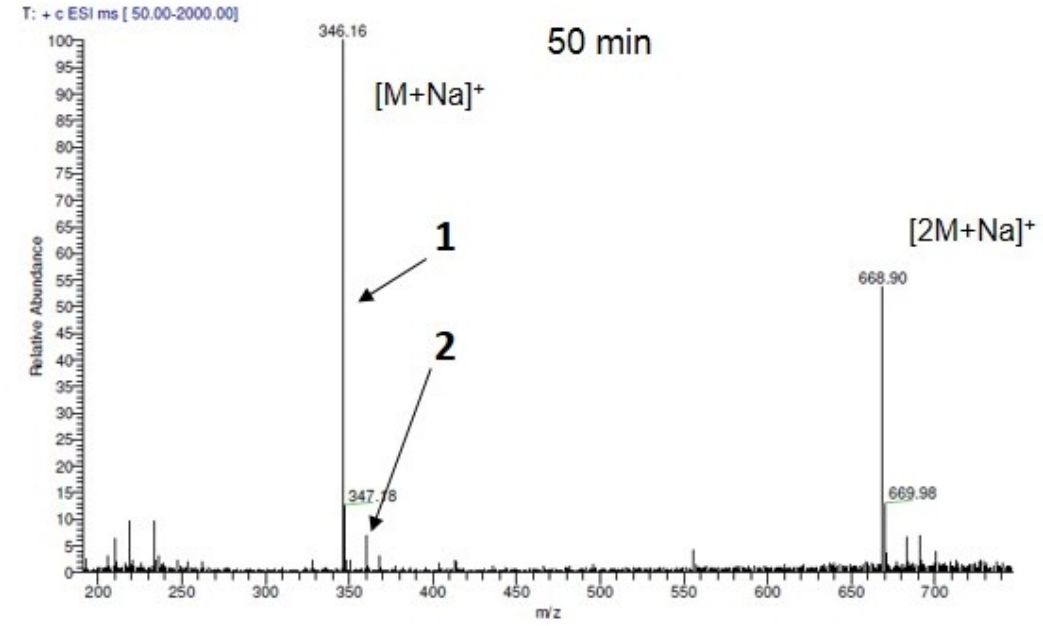


Figure S1. ESI-MS after esterification of Neu5Ac (0.20 g, 0.647 mmol), 80 °C, 50 minutes, and 0.2 equiv TFA.

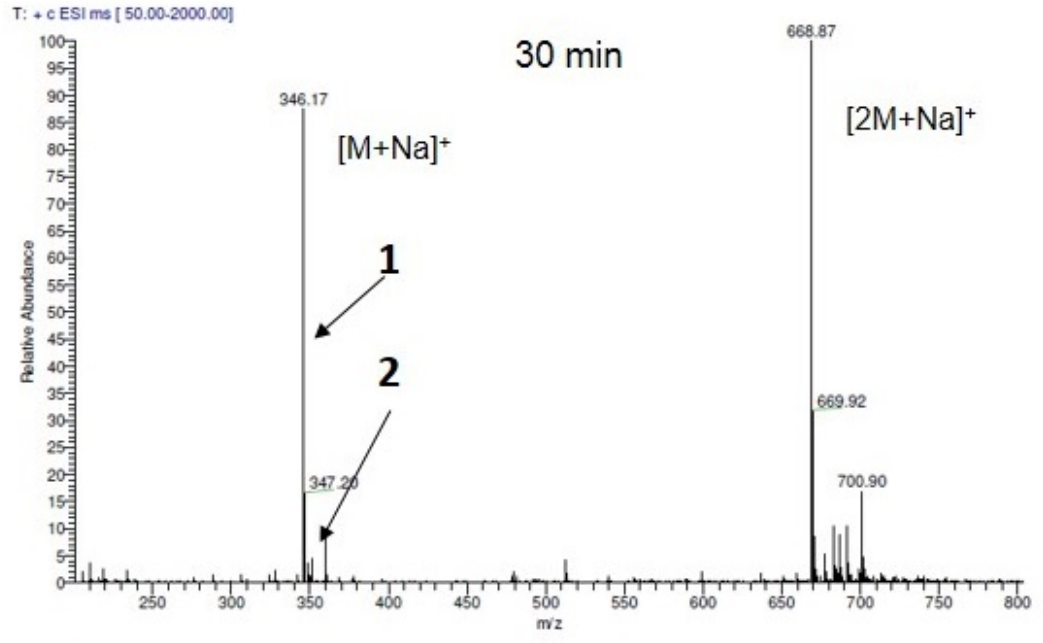


Figure S2. ESI-MS from esterification of Neu5Ac (0.20 g, 0.647 mmol), 80 °C, 30 minutes, and 0.4 equiv TFA.

Table S2. Microwave-assisted esterification of Neu5Ac (0.20 g) using 0.4 equiv of TFA at high temperatures for 5 minutes.

Entry	Temperature (°C)	Ratio (**1:2**)
1	130	1.0:1.6
2	140	1.0:2.0
3	150	1.0:1.9
4	160	1.0:1.0

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).

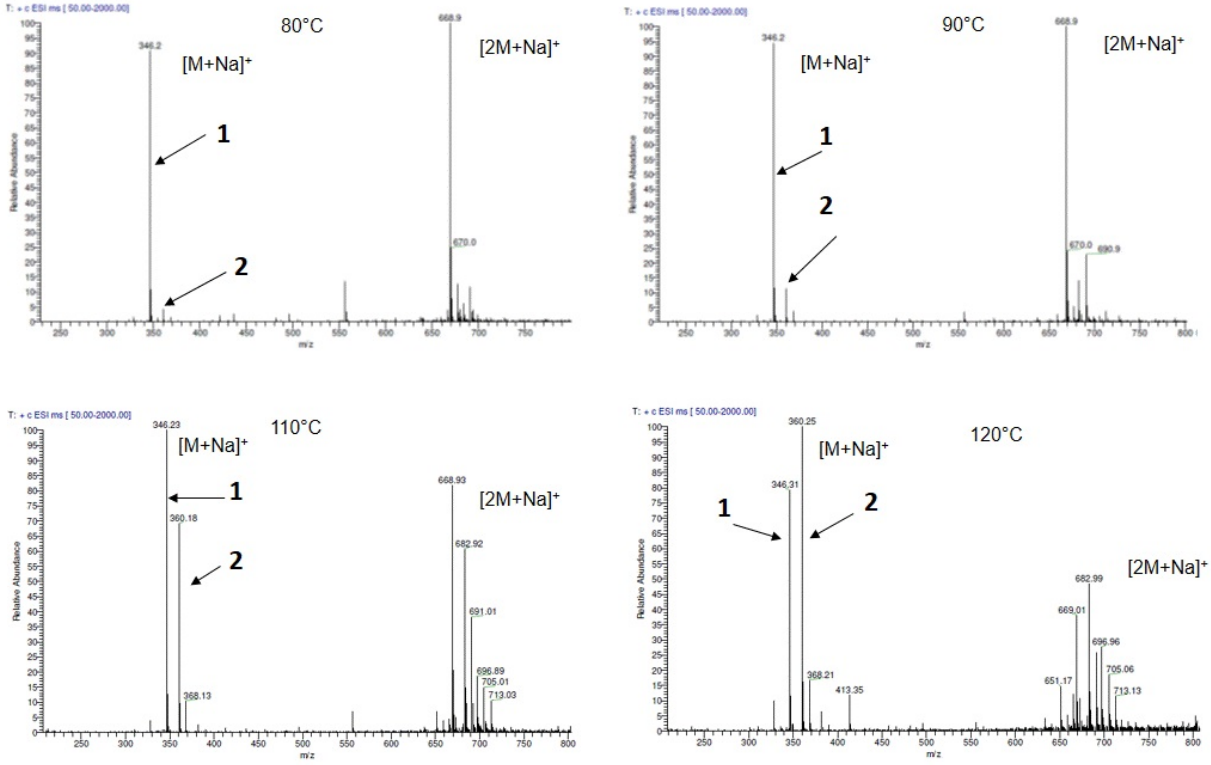


Figure S3. ESI-MS after esterification of Neu5Ac (0.20 g) at different temperatures using 0.4 equiv of TFA and 30 min irradiation.

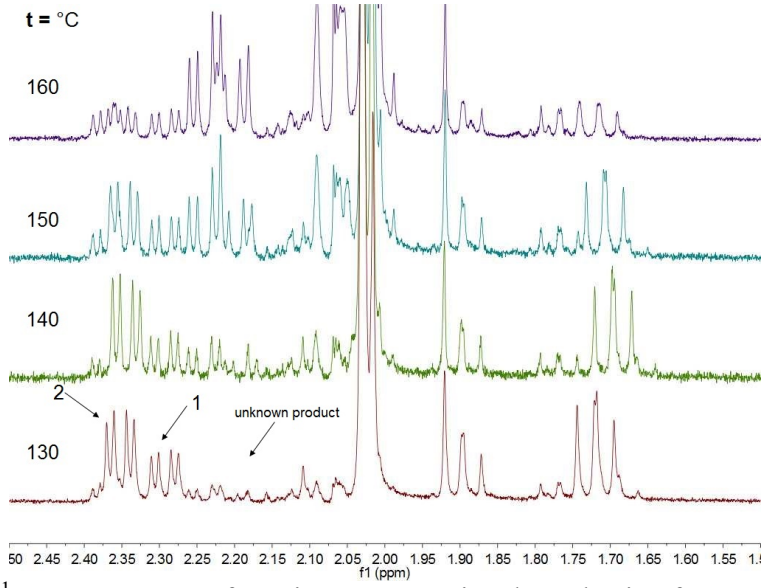


Figure S4. Stacked ^{1}H NMR spectra for microwave-assisted synthesis of Neu5Ac1Me (**1**) carried out with 0.4 equiv of TFA at higher temperature for 5 minutes. The doublet of doublets at δ 2.35 is due to the β-methyl glycoside Neu5Acβ1,2Me$_2$ **(2)**. At 150 and 160 °C the compounds **1** and **2** are the minor components, whereas the rest are uncharacterized side products.

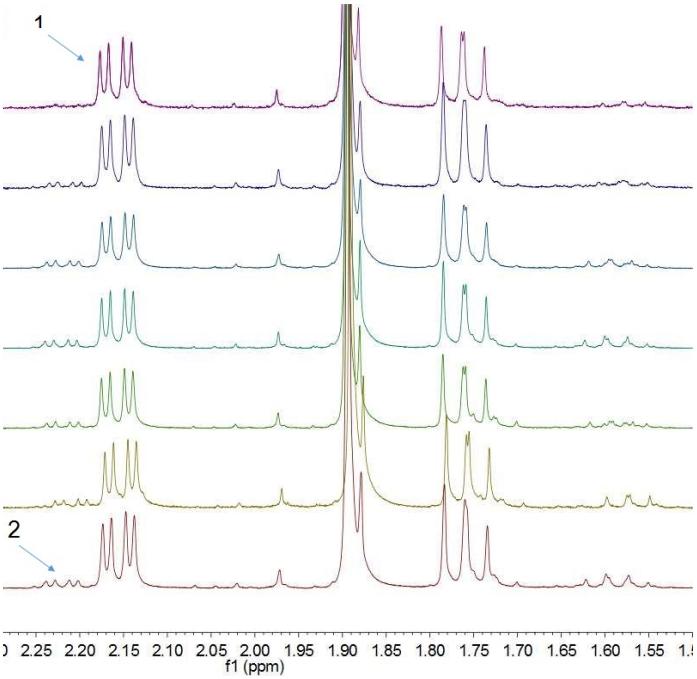


Figure S5. Stacked ^{1}H NMR spectra (500 MHz, D_2O, 298 K) for gram scale microwave-assisted synthesis of Neu5Ac1Me (**1**) using 0.4 equiv of TFA at 80 °C for 30 minutes at different Neu5Ac concentrations carried out with 0.4 equiv. of TFA at variable dilution (0.38, 0.31, 0.28, 0.25, 0.23, 0.19, and 0.14 M from bottom to top) for 30 minutes. The doublet of doublets at δ 2.35 is due to the β-methyl glycoside Neu5Acβ1,2Me$_2$ (**2**).

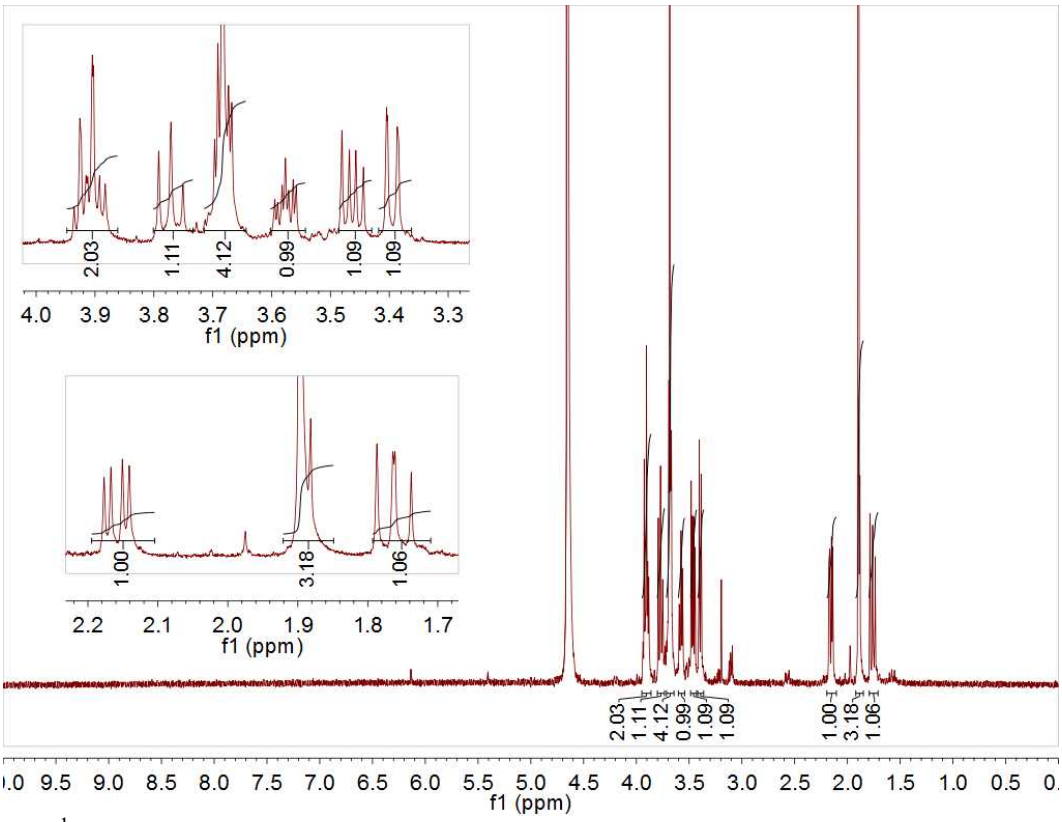


Figure S6. ^{1}H NMR Spectrum of purified compound **1** from large-scale synthesis (D_2O, 298 K, 500 MHz).

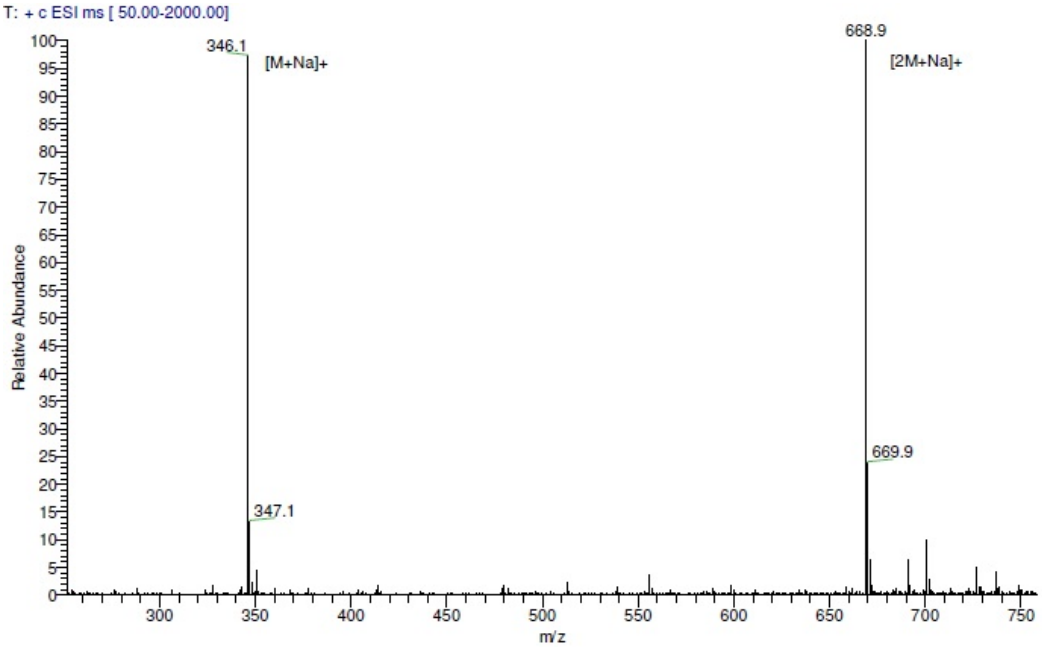
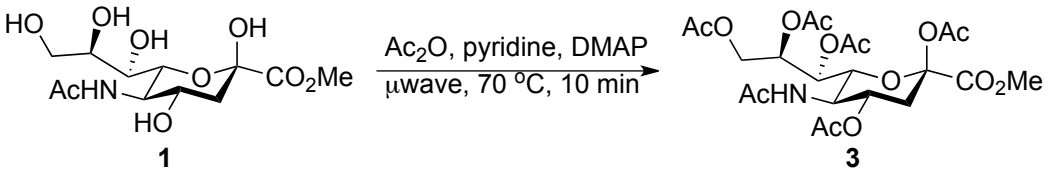


Figure S7. ESI-MS of **1** of purified compound **1** from scale up synthesis.

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



1 **3**

Compound **1** (0.2 g, 0.647 mmol 1.0 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 irradiated for 10 min at 90 °C. After the reaction, the solvent was evaporated to yield an oily material that was azeotroped with toluene (15 mL x 3) to yield the crude product. Although the desired product was obtained, material decomposition was also observed. Further optimization was done as specified in Table S3; we found 70 °C to be optimal.

For the scale up reaction, compound **1** (1.0 g, 3.23 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. After the reaction, the solvent was evaporated to yield an oily material that was azeotroped with toluene (15 mL x 3) followed by by silica column chromatography using CH_2Cl_2/MeOH (49:1) as eluent to yield **3** (93%).

Table S3. Microwave-assisted synthesis of Neu5Ac1Me *O*-peracetate **3** at variable temperature for 10 minutes.*

Entry	Temperature (°C)	Major Product (O-acetylation)
1	50	Penta
2	60	Tetra
3	70	>90% Penta
4	80	Penta

*The reactions were carried out at 0.2 g (0.647 mmol) scale. The crude products were analyzed by ^{1}H NMR spectroscopy and ESI-MS.

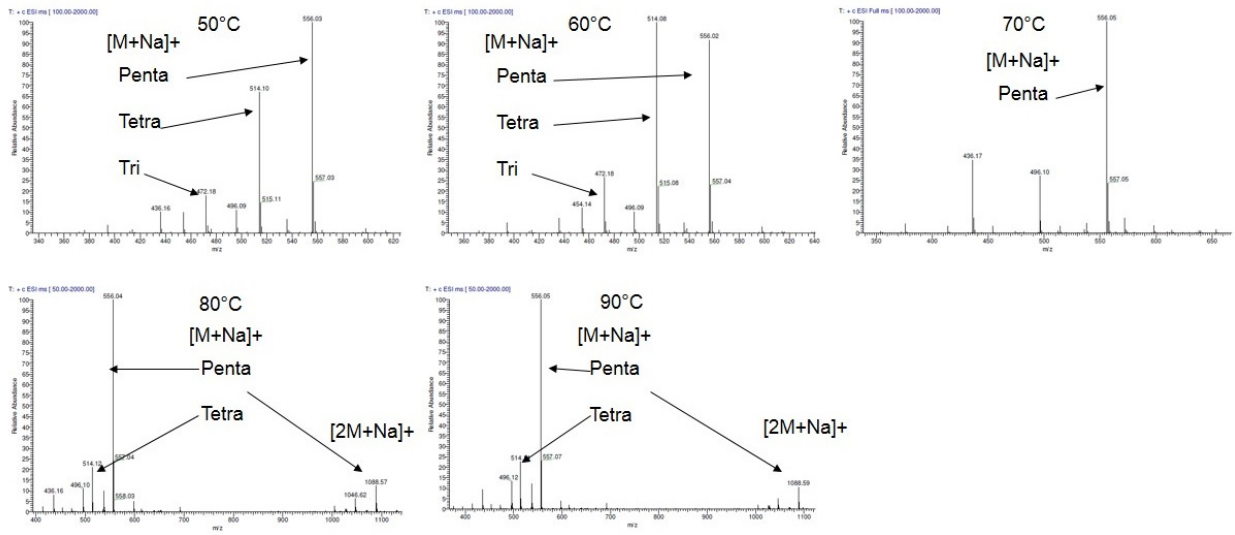


Figure S8. ESI-MS of partially peracetylated products after 10 minutes of irradiation at varying temperatures.

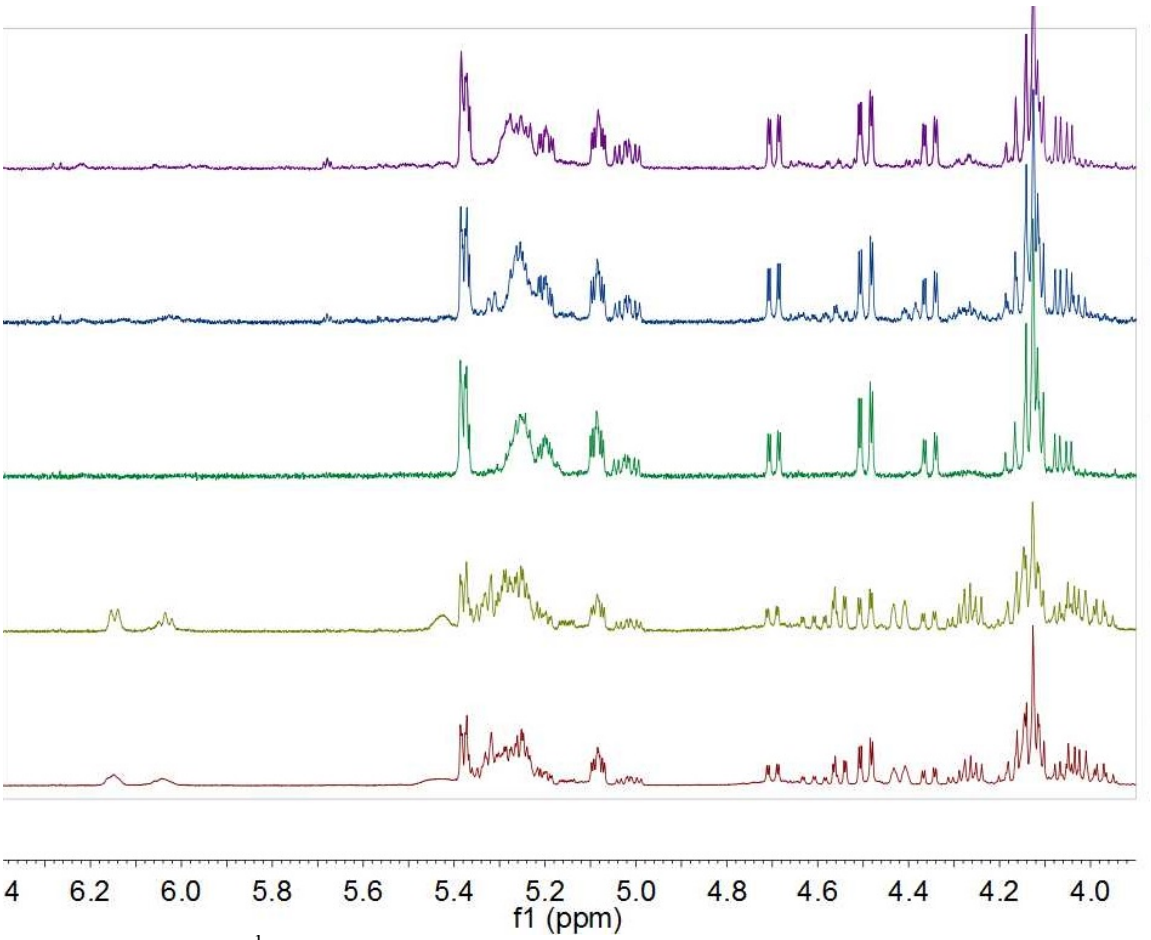


Figure S9. Stacked ^{1}H NMR spectra after acetylation at 50, 60, 70, 80, and 90 °C (from top to bottom), 10 minute irradiation time.

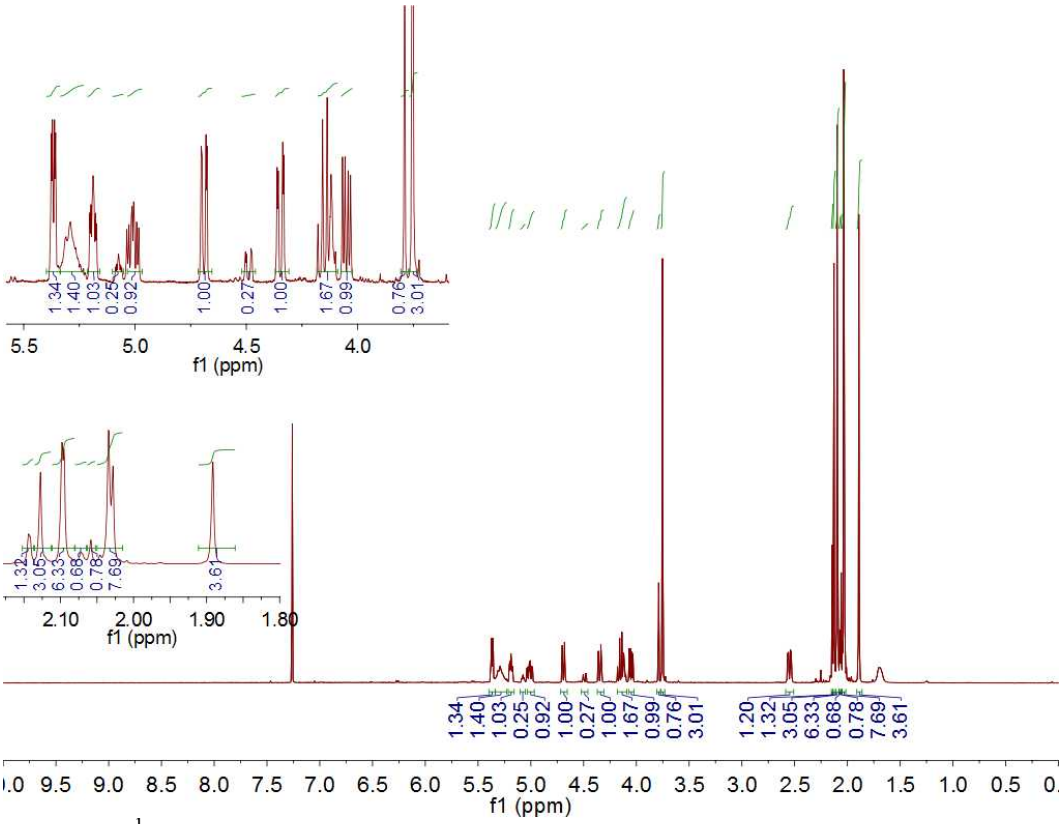


Figure S10. ^{1}H NMR spectrum of **3** from Route A scale up ($CDCl_3$, 500 MHz, 298 K).

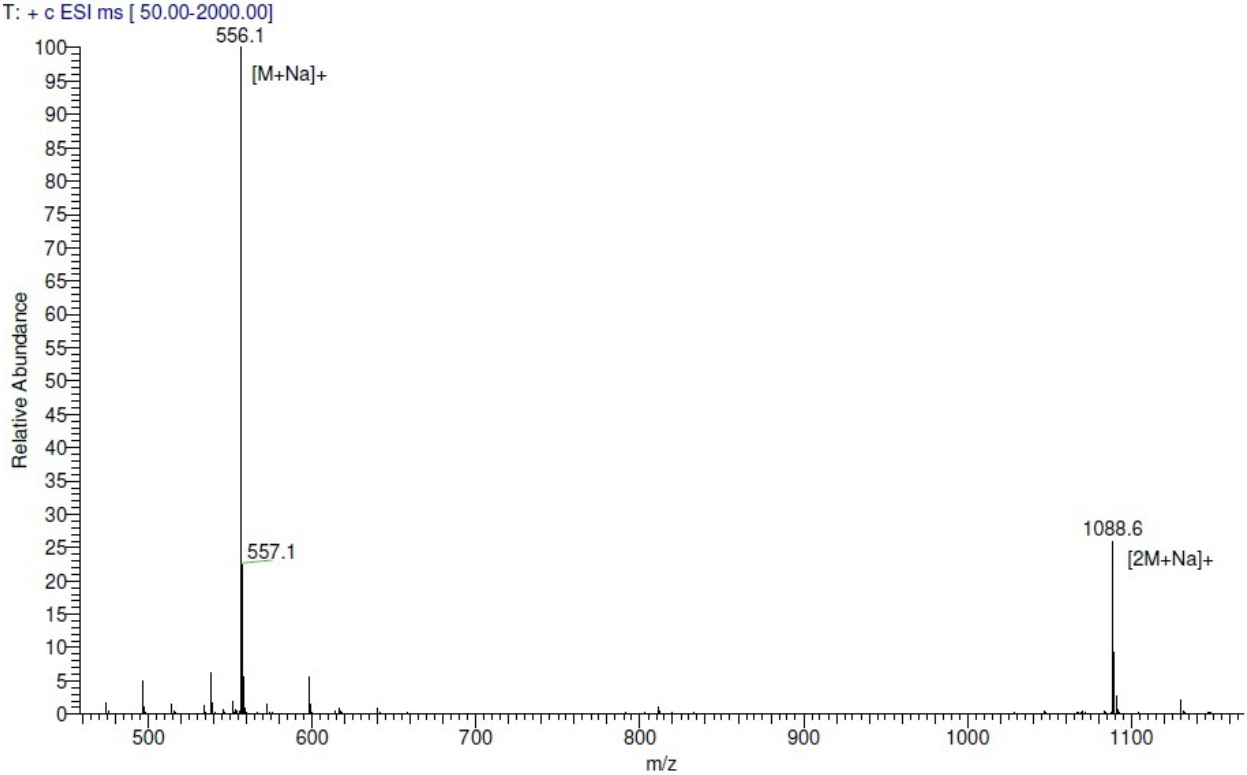
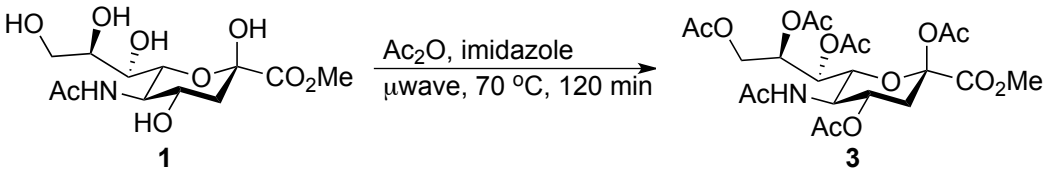


Figure S11. ESI-MS of purified **3** from Route A scale up synthesis.

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



1 3

Compound **1** (1.0 g, 3.09 mmol, 1 equiv) was transferred to a microwave tube and dissolved in a minimal amount of DMF at ~1.2mL/mmol. Imidazole was dissolved in the stirring solution of **1** followed by the addition of Ac_2O. The vessel was sealed and heated by microwave irradiation and the reaction progress monitored by TLC and ESI-MS. The reaction mixture was evaporated to remove Ac_2O followed by lyophilization. The mixture was resuspended in H_2O and extracted with DCM. The organic layer was then washed three times with saturated Na_2CO_3 solution, dried over Na_2SO_4, and evaporated to dryness yielding purified **3** (1.64 g, 98 %) as a white fluffy solid. ^{1}H NMR data is consistent with literature[2] (Figure S8). ESI-MS: $[M+Na]^+$ calculated for $C_{20}H_{29}NNaO_{13}^+$, 556.2; found, 556.1, (Figure S9).

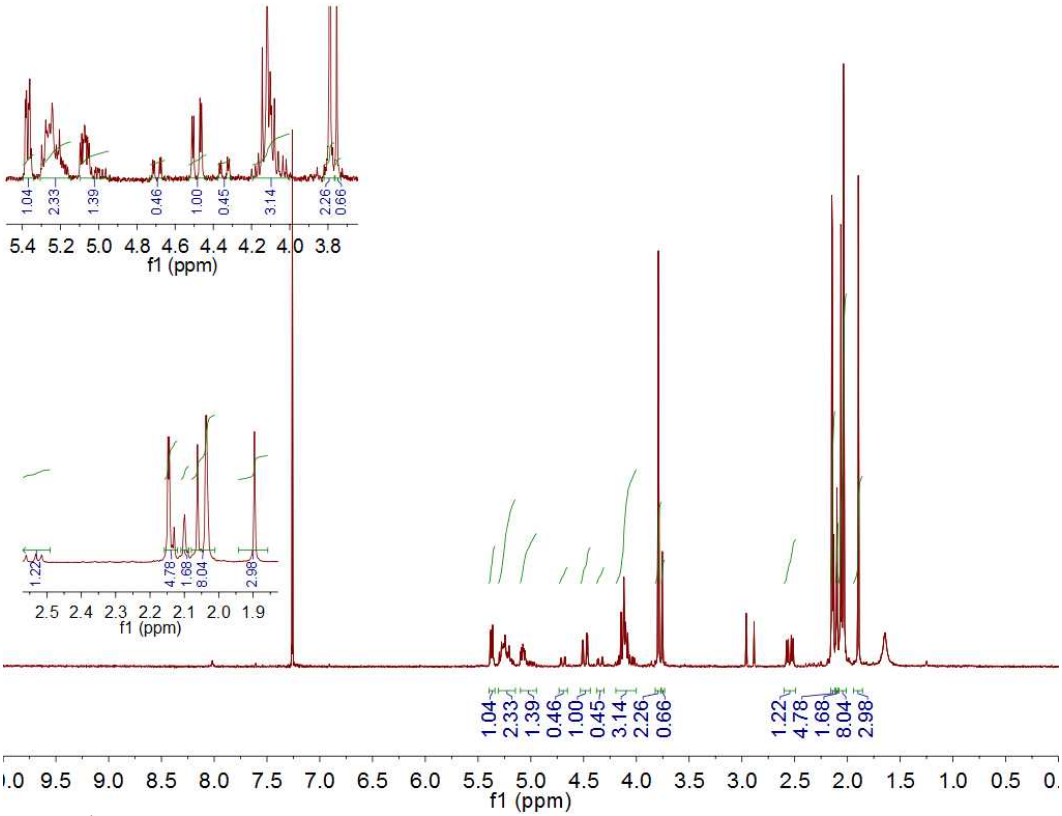


Figure S12: ^{1}H NMR spectrum of purified **3** by Route B scale up synthesis ($CDCl_3$, 500 MHz, 298 K).

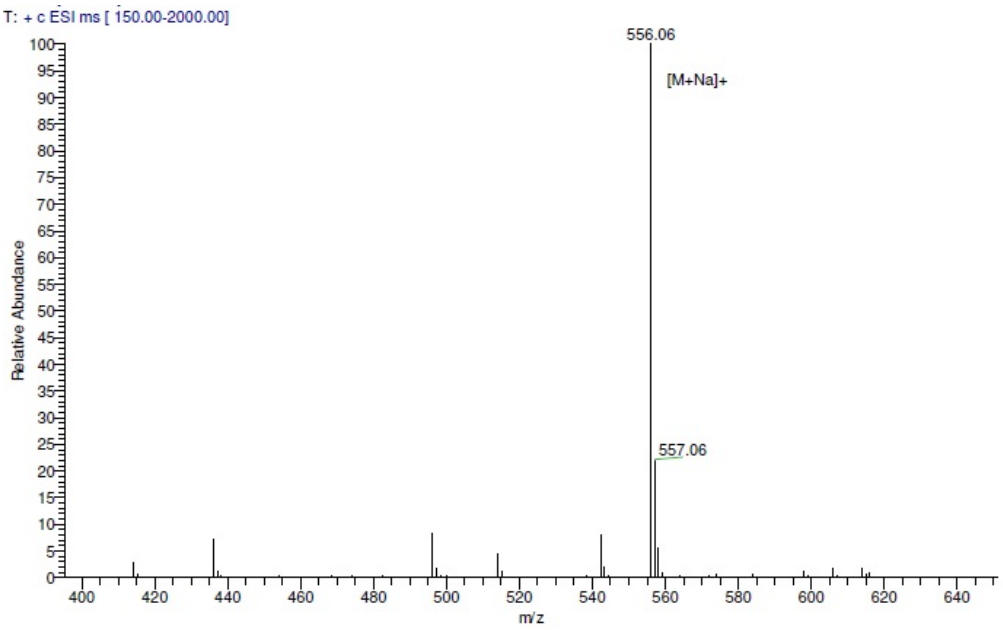
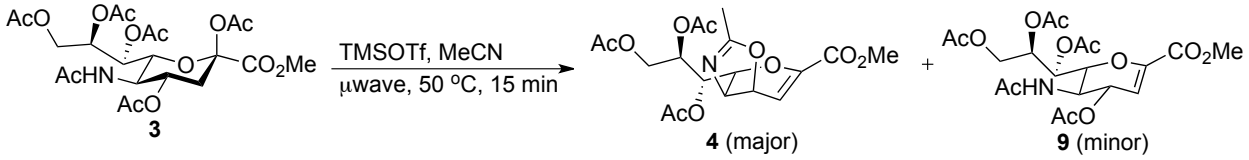


Figure S13: ESI-MS of purified **3** by Route B scale up synthesis.

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



To a glass microwave reactor tube was added compound **3** (1.0 g, 1.88 mmol). The tube was capped, evacuated, filled with Ar and this process was repeated three times, followed by the addition of anhydrous MeCN (15 mL). TMSOTf (0.7 mL, 3.87 mmol, 2.1 equiv.) was then added and mixture irradiated at 50 °C for 15 min.

At the end of the reaction, the mixture was cooled in an ice bath for five minutes at 0 °C. A saturated solution of $NaHCO_3$ solution (15.0 mL) was added and the mixture stirred for five minutes while in the ice bath. The pH of the reaction mixture was maintained at 9. The mixture was extracted with EtOAc (3 x 25 mL). The combined organic layer was washed with water (10 mL) and then dried over $MgSO_4$. The solvent was evaporated to obtain the crude material (0.79 g). The compound was dissolved in EtOAc (5 mL) and purified through a silica column and eluted with EtOAc. Compound **4** was isolated as a white solid (0.47 g, 60 %). ^{1}H NMR spectrum (Figure S10) is consistent with the literature.[3] ESI-MS: $[M+Na]^+$ calculated for $C_{18}H_{23}NNaO_{10}^+$, 436.1; found, 436.2, (Figure S11). Compound **9** was formed as a minor product and was isolated at 30 % yield (0.27 g). Its ^{1}H NMR spectrum (Figure S12) is consisted with the literature.[4] ESI-MS: $[M+Na]^+$ calculated for $C_{20}H_{27}NNaO_{12}^+$, 496.1; found, 436.1, (Figure S13)

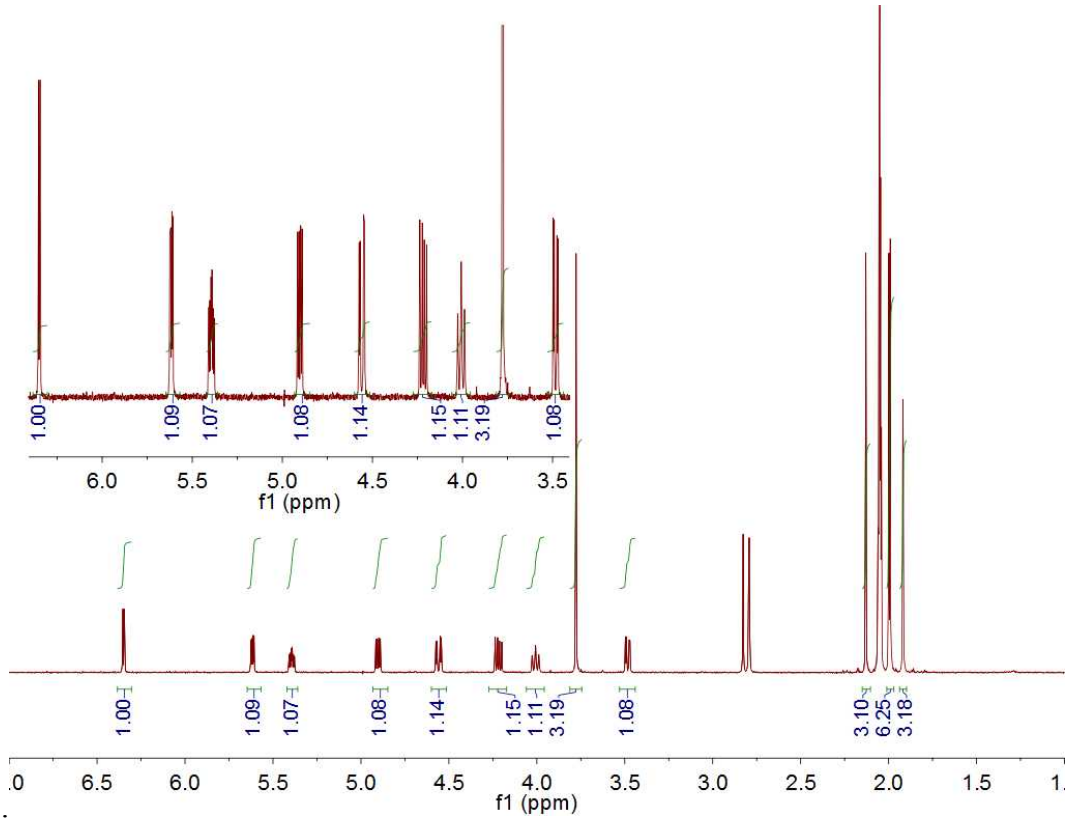


Figure S14. ^{1}H NMR spectrum of **4** ($(CD_3)_2CO$, 500 MHz, 298 K).

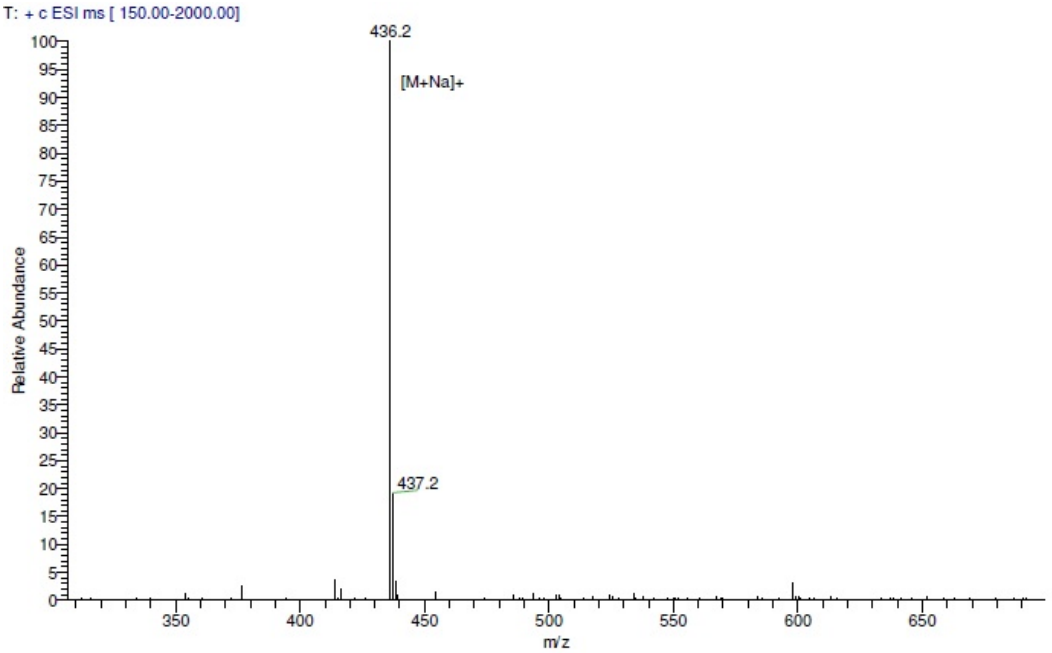


Figure S15. ESI-MS of **4**.

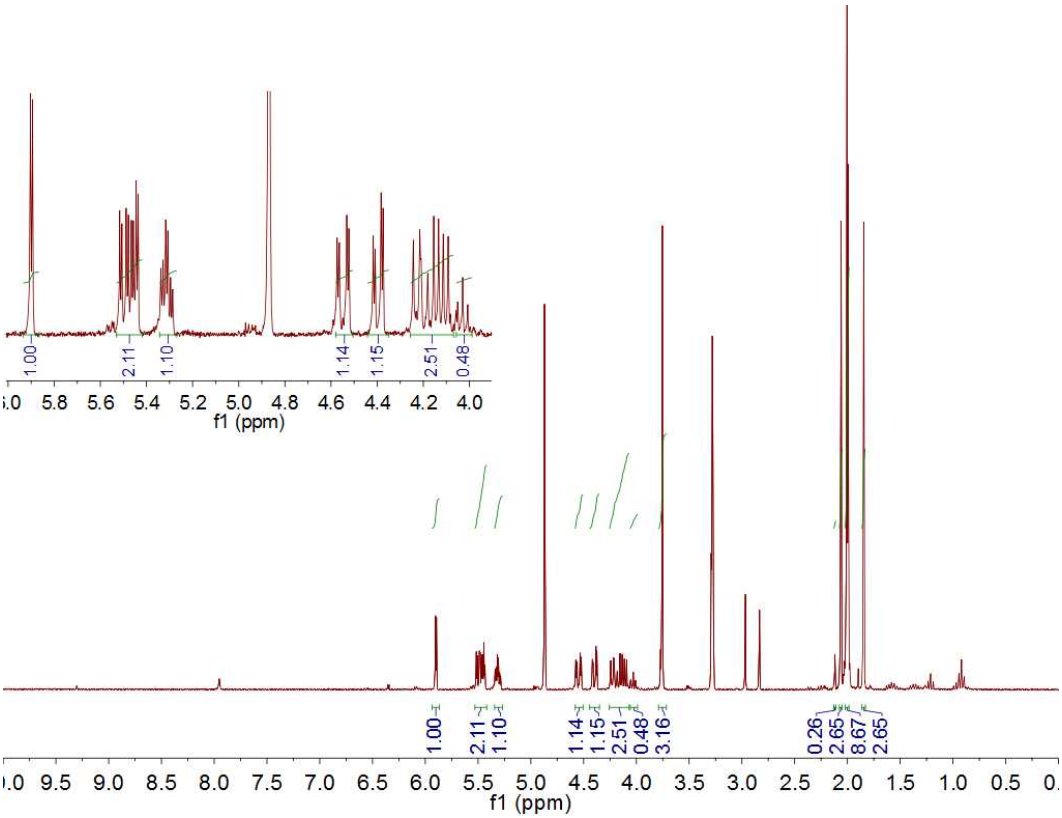


Figure S16. ^{1}H NMR spectrum of **9** (CD_3OD, 300 MHz, 298 K).

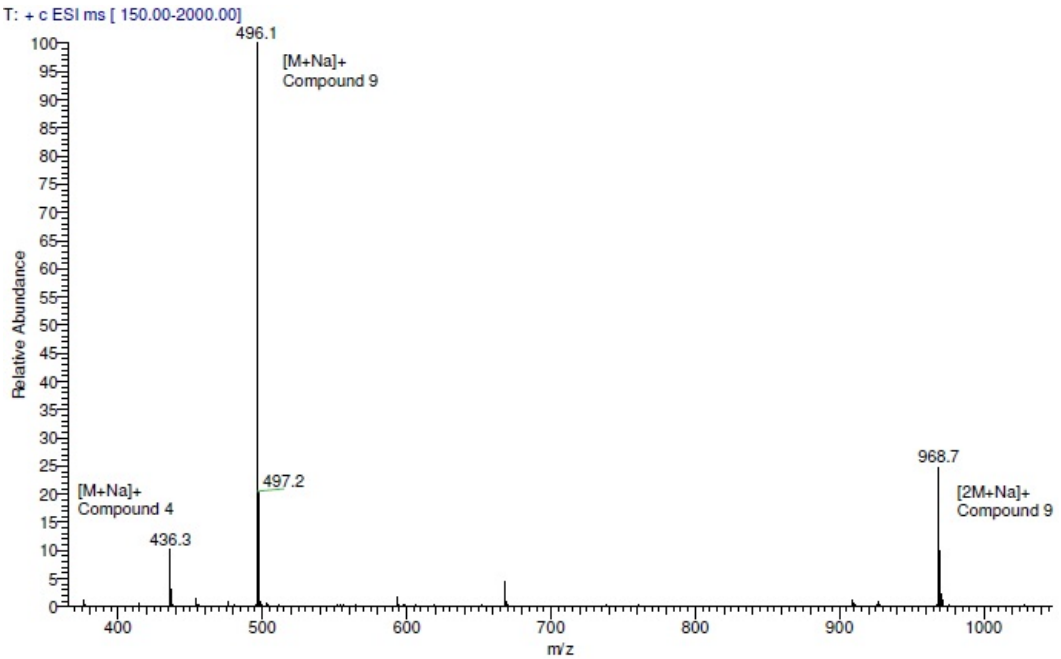


Figure S17. ESI-MS of **9**.

References:

1. P. Chopra, R. J. Thomson, I. D. Grice and M. von Itzstein, *Tetrahedron Lett*., **2012**, *53*, 6254.
2. A. Marra, and P. Sinay, *Carbohydr. Res.,* **1989**, *190*, 317.
3. E. Schreiner, E. Zbiral, R. G. Kleineidam, and R. Schauer, *Liebigs Ann.Chem., **1991,*** 129.
4. F. Baumberger, A. Vasella and R. Schauer, *Helv. Chim. Acta*, 1986, **69**, 1927.