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Carbohydrate Research 340 (2005) 1219–1223
Carbohydrate RESEARCH

Note

9-*O*-Sulfation on α-NeuAc-(2→8)-NeuAc and inter-residue lactonization

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Received 8 November 2004; accepted 28 January 2005

Abstract—Treatment of α-NeuAc-(2→8)-NeuAc (**1**) with SO_3–pyridine (4 equiv) in DMF resulted in selective 9-*O*-sulfation on the nonreducing end residue and the formation of an inter-residual δ-lactone. The lactonization could result from the C-2 carboxylic acid of the nonreducing residue condensing with the hydroxyl group or/and sulfated group at C-9 of the reducing residue to form a six-membered ring between two adjacent sialic acid residues. When α-NeuAc-(2→9)-NeuAc (**5**) was used as a sulfation substrate, only 9-*O*-sulfation on the nonreducing end residue was observed. According to capillary electrophoresis (CE) analysis, 9-*O*-sulfation on the disialic acid is a fast reaction, while sulfation on other hydroxyl groups is insignificant under the conditions used.

Keywords: Sialic acid; Polysialic acid; Regioselective; Sulfation; Lactone; Capillary electrophoresis

Sialic acids are often found in glycoproteins and glycolipids.[1] Thus, α-NeuAc-(2→3)-Gal and α-NeuAc-(2→6)-Gal are presented in tissue antigens, and α-(2→8)-linked oligo- and polysialic acids (PSA) are expressed in certain types of neuron and cancer cells.[2] PSA, like other negatively charged glycoconjugates such as heparin, has been implicated with many biological functions, such as cell development and cancer metastasis.[3] Recent studies indicated that sulfated PSA is able to interact with prion protein,[4] HIV-1 gp120,[5] and fibroblast growth factors,[6] and suppresses neuronal cell death.[4] Hatanaka and coworkers[6] synthesized sulfated PSA by treatment of colominic acid with SO_3–pyridine in DMF at 0 °C, and characterized the product as being sulfated at both the 4-*O* and 9-*O*-positions, but not at the 7-*O*-position because of steric hindrance, and they concluded that no inter-residue lactone was formed.

We have previously applied CE analysis extensively as an effective tool for monitoring the hydrolysis and lactonization of sialic acid oligomers, and revealed that the lactonization is much faster than hydrolysis under acidic conditions.[7] The inter-residue lactonization of sialic acid oligomers decreases the negative charges in the molecules, thus providing the basis for CE analysis. Similarly, the sulfation can also be monitored by CE analysis, because of the introduction of additional negative charges.

Here, we describe the sulfation of α-NeuAc-(2→8)-NeuAc (**1**), a dimeric form of sialic acid, using SO_3–pyridine as a reagent, and observed significant inter-residue lactonization.

Commercially available α-NeuAc-(2→8)-NeuAc (**1**) in DMF was treated with sulfur trioxide–pyridine complex (SO_3–pyridine; 4 equiv) at room temperature (Scheme 1) and reaction aliquots were analyzed by CE at 90 s, 2 h, 4 h, and 5 days, respectively (see Fig. 1). Three triple-charged intermediates, with **2** as a major species, presumably possessing two carboxylates and one sulfate, were formed immediately (see Fig. 1a). As the reaction proceeded, a new doubly charged compound (**3**) was detected after 2 h, and after 4 h two additional minor peaks were also observed in the quadruple-charged

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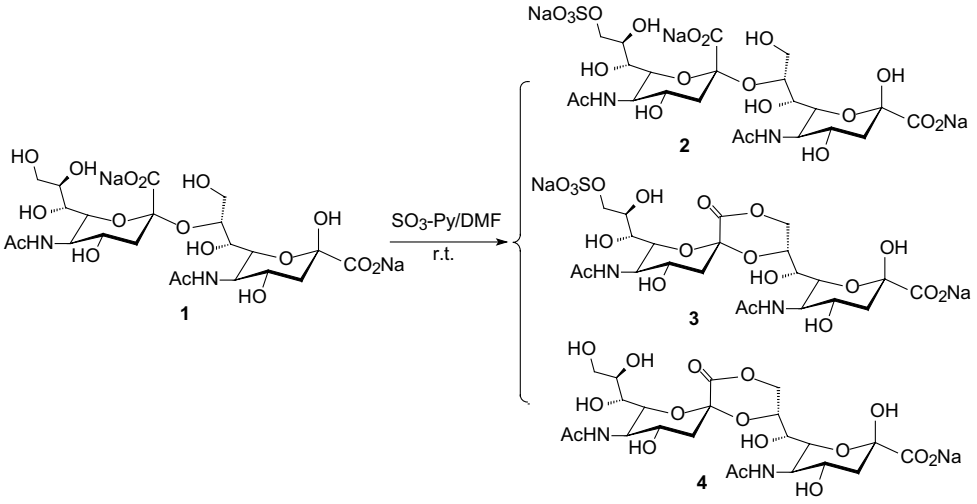
doi:10.1016/j.carres.2005.01.043



Scheme 1.

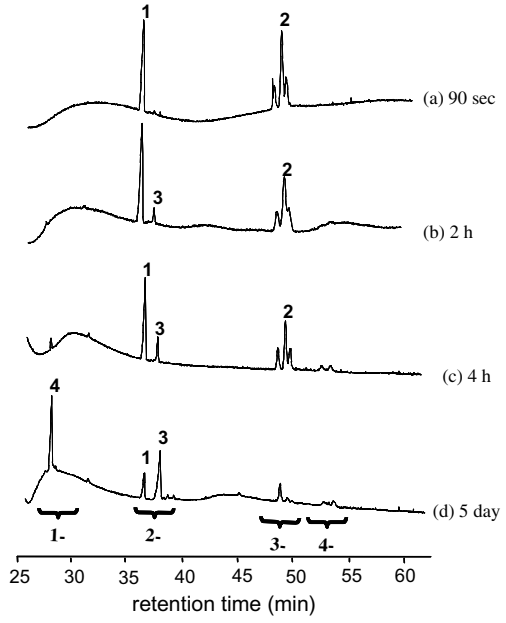


Figure 1. Sulfation of α-NeuAc-(2→8)-NeuAc (**1**).

region, as a result of double sulfation. With prolonged reaction time (5 days), both **1** and **2** were diminished and two major products, **3** and **4**, were found.

When the reaction mixture was quenched after 2 h by the addition of MeOH, we isolated **2** (31%), **3** (11%), and **4** (8%), and **1** (28%) by a mono-Q column with a gradient of NaCl solution as eluent. Both CE and spectroscopic analysis using authentic samples as standards confirmed the structure of **1** and lactone **4**.[7] Monosulfated lactone **3** was characterized by ^{1}H NMR (see Fig. 2a), and its identity further confirmed after transformation into **2** by treatment with 0.5 M $NaHCO_3$. The progressive hydrolysis of **3** was monitored by both CE and ^{1}H NMR analysis (see Fig. 2c and d). The conversion of lactone into carboxylate (**3** to **2**) led to a significant downfield shift of the H-3e′ resonance from 2.64 ppm in **3** to 2.86 ppm in **2** (see Fig. 2), because H-3e′ in **3** is shielded by the carbonyl group in a rigid conformation.

The foregoing results indicated that intramolecular lactonization occurred under the sulfation conditions. However, inter-residue lactonization of PSA in a similar experiment by Hatanak and his co-workers.[6] was not observed, but complete 9-*O*-sulfation was reported. The critical difference between our work and that of Hatanaka's group was that colominic acid with a tributylammonium counter-cation was used in their experiment instead of the sodium salt. Steric hindrance exerted by the bulky cation probably prevented the lactonization by an inter-residue S_N2 reaction by the carboxylate of one residue to the 9-*O*-sulfate of the adjacent residue. In our case, a similar 9-*O*-sulfation in the reducing residue probably occurred, and lactonization occur by the C-2 carboxylic acid of the nonreducing residue condensing with the hydroxyl group and/or sulfated group at C-9 of the reducing residue to form a six-membered ring between two adjacent sialic acid residues. We have no data to exclude one from another.

Our observation shows the sulfation on sialic acid is selective on 9-OH, which is similar to sulfonations in general (tosylation, mesylation).[8] Sulfation on 4-OH and 7-OH was minimal, which in contrast to the previous report[6] that 4-OH was as active as 9-OH in PSA, although different substrates were used. However, it remains unclear whether the lactonization prevented further sulfation of other hydroxy groups. In order to answer this question, α-NeuAc-(2→9)-NeuAc (**5**)[9] was used as a sulfation substrate, because it is known that α-(2→9)-linked polysialic acid forms an inter-residue lactone with difficulty due to steric hindrance.[10]

Sulfation of **5** was performed under same conditions, and aliquots of the reaction mixture were also analyzed

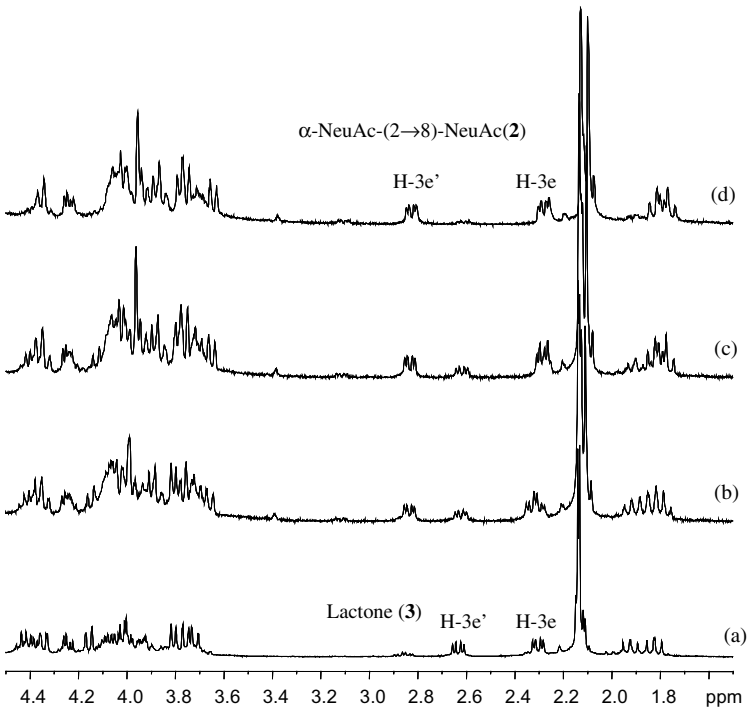


Figure 2. Hydrolysis of lactone (**3**) to 9-*O*-sulfated α-NeuAc-(2→8)-NeuAc (**2**).

by CE (Fig. 3). One triple-charged major product (**6**) was formed immediately and an equilibrium between **5** and **6** was probably reached, because the **5**:**6** ratio did not change with a prolonged reaction times. Compound **6** was isolated and its structure as 9-*O*-sulfated α-NeuAc-(2→9)-NeuAc, was established by NMR and MS (see Scheme 2). Unlike the sulfation of **1**, there was no lactonization from **5**, and neither was a multi-sulfated product detected. Thus, sulfation at the 9-*O*-position of the sialic acid was regioselective.

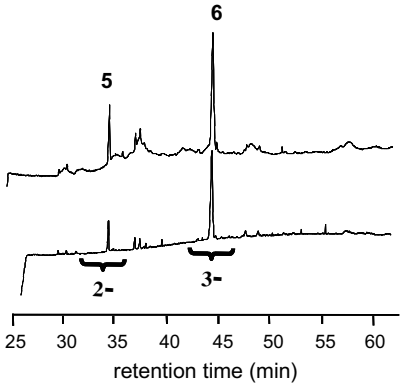
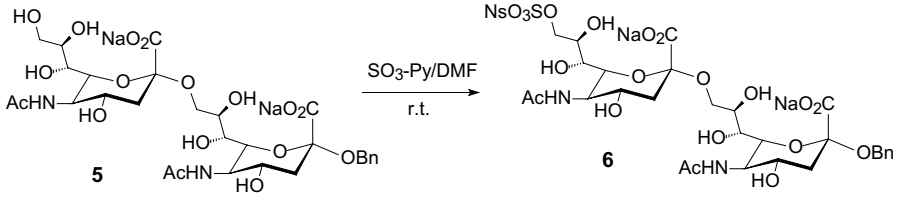


Figure 3. Sulfation of α-NeuAc-(2→9)-NeuAc (**5**).

Sialic acids modified by *O*-acetyl groups are common in glycoconjugates.[11] Modification of sialic acids by *O*-sulfation has also been found on the terminal residue of sea urchin cell-surface polysialic acid,[12] but not on the inner sialic acid residues. However, it is still unknown whether enzymatic sulfation takes place at 9-*O*-position prior to the lactonization; this reaction has been suggested as being significant in biological processes by the modulation of charge density.[13]

In conclusion, we have demonstrated that sulfation of α-NeuAc-(2→8)-NeuAc (**1**) and α-NeuAc-(2→9)-NeuAc (**5**) under SO_3–pyridine conditions occurs primarily at the 9-OH groups. 9-*O*-Sulfation on inner sialic acid residue was not observed, because of the inter-residue lactonization.



Scheme 2.

1. Experimental Section

1.1. General methods

Capillary electrophoresis was performed on a Beckman capillary electrophoresis P/ACE system 2100 (USA) using fused-silica capillaries (117 cm × 75 μm i.d.) and applying 20 kV at 25 °C. Phosphate buffer (50 mM, pH 7.0) was used as the running buffer. The spectra were monitored by UV absorption at 200 nm. Samples were injected into the capillaries by high-pressure nitrogen (20 psi) for 3 s. The capillaries were regenerated by washing with 0.1 N NaOH for 7 min and then doubly distilled water for 5 min.

1.2. Sulfation of 1

To a solution of **1** (37 mg, 0.058 mmol) in DMF (2 mL) was added sulfur trioxide–pyridine complex (SO_3–pyridine; 37 mg, 0.23 mmol), and the mixture was stirred for 2 h at room temperature. After being cooled to 0 °C, the reaction was terminated by the addition of MeOH (2 mL) to decompose the excess of reagent. The mixture was concentrated and the residue purified by a mono-Q column with a gradient of NaCl solution as eluent, with detection by UV absorption, to afford **2** (13.0 mg, 31%), **3** (4.4 mg, 11%), **4** (2.8 mg, 8%) and recovered compound **1** (10.5 mg, 28%). For **2**: ^{1}H NMR (400 MHz, MeOD) δ 4.24 (d, 1H), 4.10–4.14 (m, 1H), 3.52–3.99 (m, 15H), 2.83 (dd, *J* 12.3, 4.8 Hz, 1H), 2.29 (dd, *J* 12.9, 4.8 Hz, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 1.83 (t, *J* 9.7 Hz, 1H), 1.77 (t, *J* 9.6 Hz, 1H); ^{13}C NMR (100 MHz, MeOD) δ 178.18 (C_q), 176.06 (C_q), 175.82 (C_q), 173.67 (C_q), 103.17 (C_q), 97.64 (C_q), 76.27 (CH), 73.68 (CH), 71.30 (CH), 71.09 (CH), 70.12 (CH_2), 69.53 (CH), 68.54 (CH), 67.98 (CH), 61.92 (CH_2), 53.40 (CH), 52.73 (CH), 42.07 (CH_2), 40.31 (CH_2), 23.27 (CH_3), 23.17 (CH_3); ESMS calcd for $C_{22}H_{33}O_{20}N_2SNa_3$ (M) 746.5; found 724.5 (M+H−Na).

1.3. Sulfation of 5

To a solution of **5** (30 mg, 0.04 mmol) in DMF (2 mL) was added SO_3–pyridine (26 mg, 0.16 mmol), and the mixture was stirred for 1 h at room temperature, cooled to 0 °C, and the reaction terminated by the addition of MeOH (2 mL). The mixture was concentrated and the residue purified by a mono-Q column with a gradient of NaCl solution as eluent, with detection by UV absorption to furnish compound **6** (15.0 mg, 45%) as a yellow oil along with recovered compound **5** (6.32 mg, 21%). For **6**: ^{1}H NMR (400 MHz, Me_2SO) δ 8.05 (s, 2H), 7.21–7.39 (m, 5H), 4.85 (b, 2H), 4.66–4.72 (m, 3H), 4.45 (d, 1H, *J* 11.9 Hz), 3.97 (d, 1H, *J* 8.5 Hz), 3.73–3.18 (m, 16H), 2.72 (dd, 1H, *J* 11.6, 4.2 Hz), 2.61 (dd, 1H, *J* 11.7, 4.2 Hz), 1.91 (s, 3H), 1.89 (s, 3H), 1.36 (t, 1H, *J* 11.1 Hz), 1.34 (t, 1H, *J* 11.1 Hz); ^{13}C NMR (100 MHz, Me_2SO) δ 172.34 ($2C_q$), 172.16 (C_q), 170.46 (C_q), 139.06 (C_q), 127.98 (2CH), 127.55 (2CH), 126.92 (CH), 100.12 (C_q), 99.79 (C_q), 72.34 (2CH), 69.83 (CH), 69.47 (CH), 69.39 (CH), 69.16 (CH), 68.34 (CH_2), 67.59 (CH), 67.37 (CH), 66.30 (CH_2), 65.00 (CH_2+CH), 52.93 (CH), 41.82 (CH_2), 41.57 (CH_2), 22.58 ($2CH_3$); FABMS calcd for $C_{29}H_{39}O_{20}N_2SNa_3$: 836.66; found 836.95.

Acknowledgements

The authors thank the National Science Council of Taiwan and Academia Sinica, Taipei, Taiwan, for financial support.

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