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

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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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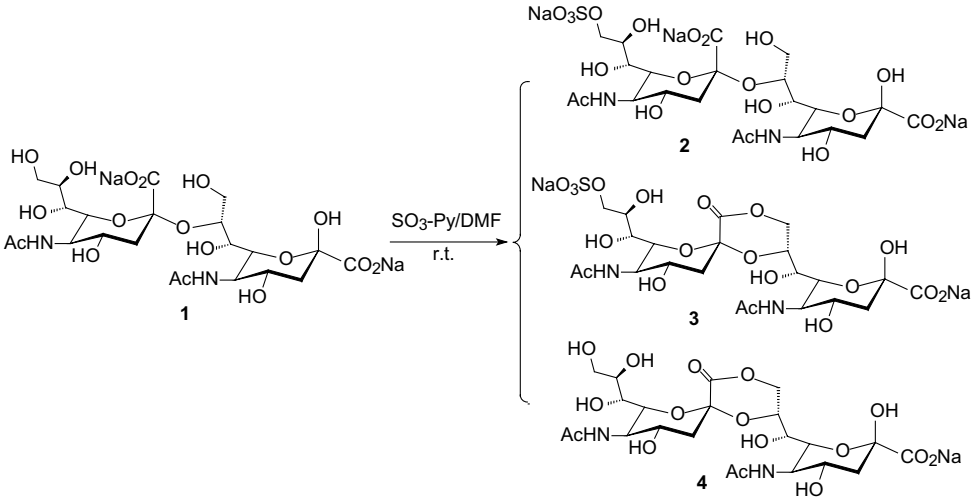

Scheme 1.

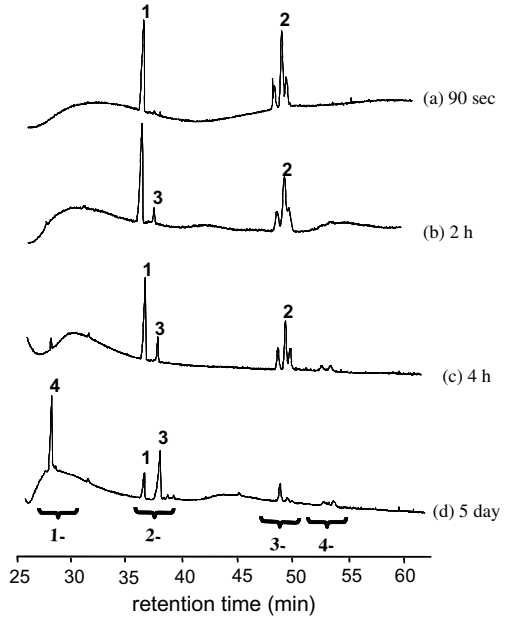

Figure 1. Sulfation of α-NeuAc-(2→8)-NeuAc (**1**).

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