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ESTERASE MEDIATED REGIOSELECTIVE DEACETYLATION OF ULOSONIC ACID

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ABSTRACT

The regioselective deacetylation of peracetylated derivatives of *N*-acetylneuraminic acid and KDN was achieved by hydrolysis with esterase isolated from *Rhodosporidium toruloides*.

One of the major problems in synthetic carbohydrate chemistry is the preparation of selectively protected saccharide building blocks. Many strategies for the synthesis of partially protected mono-, di- and oligosaccharides have been developed that rely on conventional chemical methods. These strategies often require multiple protection/deprotection steps to procure suitably protected saccharides. The partial chemical hydrolysis of peracylated sugars represents an alternative approach to selective protection that is largely unattractive, because of the poor regioselectivity and the harsh conditions required often cause carbohydrate precursors to

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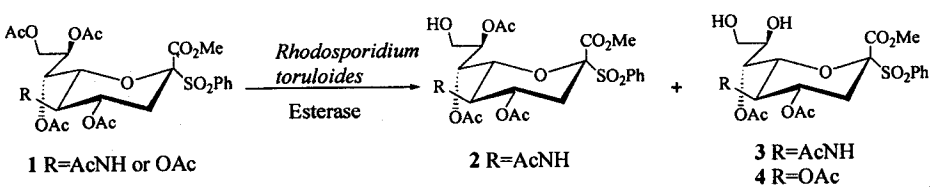
undergo decomposition. In contrast, enzymatic approaches usually rely on milder conditions and often show high selectivity.

Enzymes have been employed successfully in the regioselective hydrolysis of peracylated sugars affording useful building blocks for oligosaccharide synthesis.[1–6]

Cell surface bound glycoconjugates have been shown to play pivotal roles in various biological process, both in normal and pathological states.[7] Ulosonic acids normally occupy the terminal position of carbohydrates in glycoconjugates and participate directly in these processes.[8] Our laboratory has been interested in synthesis of ulosonic acid containing glycoconjugates to elucidate their biological role and to establish structure-activity relationships.[9,10] Toward this purpose, an enzymatic approach was explored as a simplified general method to regioselectively prepare deacetylated ulosonic acid derivatives having free OH at C-9. In this paper, we report the use of the crude esterase from *Rhodosporidium toruloides* to prepare the monodeacetylated and dideacetylated *N*-acetyl neuraminic acid (NANA) and dideacetylated 3-deoxy-D-glycero-D galactonulosonic acid (KDN) derivatives. *R. toruloides* esterase was selected for this study, since Crout and co-workers had previously reported the regioselective deacetylation of various peracetylated hexoses to afford derivatives having free OH at their C6 position.[11,12]

Ulosonic acid derivatives **1**[13] were subjected to the esterase-catalyzed hydrolysis as shown in Scheme 1. The hydrolysis was conducted by suspending the substrate and the esterase in sodium citrate, sodium phosphate buffer (pH 5.0). The insolubility of fully acetylated ulosonic acid derivatives in the sodium citrate phosphate buffer was overcome by dissolving the substrate in tetrahydrofuranacetone (1 : 1, v/v) before adding it to the buffer containing the enzyme. The mixture was stirred at 30°C and the reaction was monitored by thin-layer chromatography (TLC). Once the starting material was consumed, typically after 24 h, the enzyme was removed by filtration. The filtrate was then extracted with methylene chloride, followed by column chromatography to obtain the deacetylated products. Peracetylated



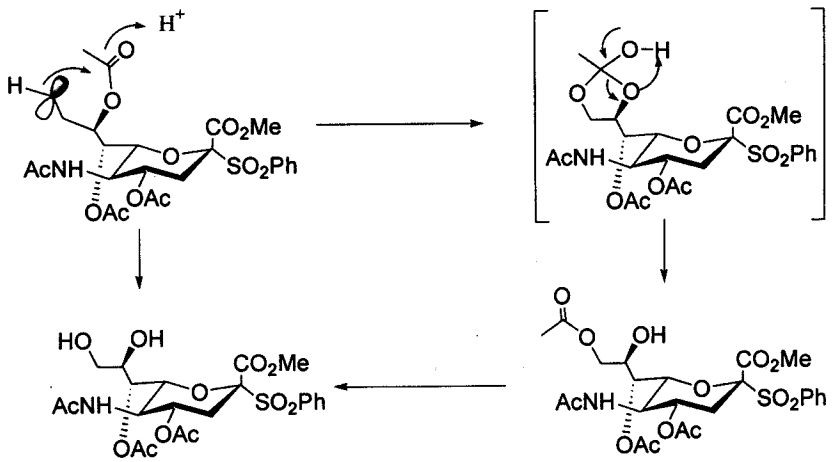
1 R=AcNH or OAc

2 R=AcNH

3 R=AcNH
4 R=OAc

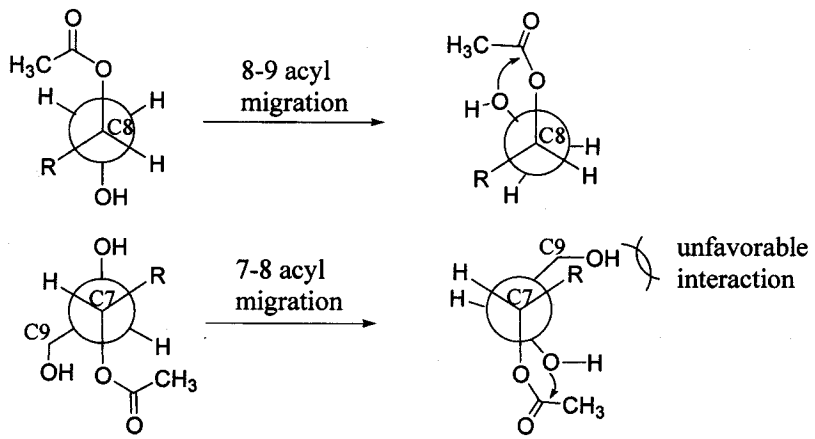
Scheme 1. Esterase mediated deprotection of peracetylated ulosonic acid derivatives.

NANA was converted into two products, 9-hydroxy and 8,9-dihydroxy derivatives, **2** and **3**. The site of the deacetylation was established by comparing the ^{1}H NMR spectra of the deacetylated products with those of the starting materials. In the C-9 deprotected product **2**, the C-9 protons in the ^{1}H NMR spectra are shifted upfield owing to the loss of the deshielding effect of the acetate groups. Complete conversion of peracetylated NANA also afforded an equal amount of the dihydroxy product **3**, having free OH group at C-9 and C-8. A mechanism by which the dihydroxy derivative can be formed through an 8–9 acyl migration, followed by further esterase-catalyzed hydrolysis is shown in Scheme 2. This mechanism is supported by the initial formation (at <25% conversion) of exclusively the 9-hydroxy product **2**. Furthermore, treatment of the purified **2** with additional esterase cleanly gives rise to compound **3**.



Scheme 2. Proposed mechanism for 8–9 acetyl-migration.

The 8–9 acyl migration did not give rise to a subsequent 7–8 acyl migration. No evidence of 7,8,9-tri-deacetylated species was found on treatment of **1** with esterase. Furthermore, no tri-deacetylated product is observed even following extensive esterase treatment of either **2** or **3**. The presumed oxonium ion intermediate in 8–9 acyl migration proceeds through a stable a five-membered ring intermediate (Scheme 3). In contrast, the corresponding intermediate for 7–8 acyl migration would proceed through a more highly strained five-membered ring. Thus, since 7–8 acyl migration should be considerably slower than 8–9 acyl migration, the fully acetylated



Scheme 3. Proposed difference between 8–9 and 7–8 acetyl migrations.

derivative of KDN (14) was hydrolyzed quantitatively by *R. toruloides* esterase affording only the 8,9-dihydroxylated derivative **4**.

The work described in this paper provides a useful method for the regiospecific deacetylation peracetylated NANA and KDN at C-9 and C-8. C-9 deprotected monosaccharide can be isolated from peracetylated NANA in one step, in contrast to the traditional chemical approach that requires the use of complex protection/deprotection strategies. Subsequent oxidation of primary hydroxyl group to an aldehyde should permit samarium iodide catalyzed *C*-glycosylation[13,14] to afford *C*-glycoside analogues of polysialic (colominic acid).

General Procedure of Esterase-Catalysed Hydrolyses of Acetylated Monosaccharides

Peracetylated ulosonic acid (10 mg in 1 ml of 1 : 1 THF : acetone) was suspended in 10 ml of sodium citrate–sodium phosphate buffer (pH 5.0, 50 mM/100 mM). After addition of the *R. toruloides* esterase (25 mg in 2 ml reaction media), the reaction mixture was stirred at 30°C, and was followed by TLC. When peracetylated starting sugars had completely disappeared (after 24 h), the enzyme was removed by filtration and the filtrate was extracted with dichloromethane. The organic fraction was combined and dried (anhydrous $NaSO_4$), and concentrated by rotary-evaporation. The residue was then purified by flash chromatography.

Selected Data for Deacetylated Compounds

Mono-deacetylated compound 2: ^{1}H NMR (500 MHz, $CDCl_3$), 7.84–7.92 (m, 2H, aromatic), 7.74 (t, 1H, aromatic), 7.56–7.64 (m, 2H, aromatic), 5.20 (d, 1H, $J = 10$ Hz, NH), 5.06 (d, 1H, $J = 10$ Hz, H-7), 4.87 (ddd, 1H, $J = 3.5$ Hz, H-4), 4.58 (d, 1H, $J = 10$ Hz, H-8), 4.03 (q, 1H, $J = 10$ Hz, H-5), 3.87 (dd, 1H, $J = 1.5$ Hz, 10 Hz, H-6), 3.85 (s, 3H, OCH_3), 3.79 (t, 1H, H-9a), 3.40 (d, 1H, $J = 10$ Hz H-9b), 3.01 (dd, 1H, $J = 4.5$ Hz, 13 Hz, H-3eq), 2.84 (m, 1H, OH), 2.31 (t, 1H, $J = 13$ Hz, H-3ax), 2.12, 2.07, 2.05 1.87 (4s, 12H, 4Ac). MS, 596, $(M + Na)^+$.

Di-deacetylated compound 3: ^{1}H NMR (500 MHz, $CDCl_3$), 7.876 (m, 2H, aromatic), 7.76 (t, 1H, aromatic), 7.60 (m, 2H, aromatic), 5.382 (d, 1H, $J = 10$ Hz, NH), 4.92–4.84 (m, 2H, H-7, H-4), 4.047 (q, 1H, $J = 10$ Hz, H-5), 3.807 (dd, 1H, $J = 2$, 11 Hz, H-6), 3.56 (dt, 1H, H-9a), 3.99 (bm, 1H, H-8), 3.4 (bd, 1H, H-9b), 3.23 (d, 1H, $J = 5$ Hz, OH), 3.03 (dd, $J = 5$, 13 Hz, H-3eq), 2.31 (t, 1H, $J = 12.5$ Hz, H-3ax), 2.07, 2.05, 1.84 (3s, 9H, 3Ac). MS, 554, $(M + Na)^+$.

Di-deacetylated compound 4: 7.85–7.92 (m, 2H, aromatic), 7.77 (t, 1H, aromatic), 7.56–7.65 (m, 2H, aromatic), 4.88–4.95 (m, 3H, H-4, H-5, H-7), 3.97 (s, 3H, OCH_3), 4.87 (dd, 1H, $J = 1.5$, 9.6 Hz, H-6), 3.50–3.56 (m, 2H, H-8, H-9a), 3.41–3.43 (m, 1H, H-9b), 3.21 (d, $J = 5$ Hz, 1H, OH), 3.13 (dd, 1H, $J = 4$, 12 Hz, H-3eq), 2.34 (t, 1H, $J = 12$ Hz), 2.06, 2.04, 1.96 (3s, 9H, 3Ac).

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REFERENCES

1. Hennen, W.J.; Sweers, H.M.; Wang, Y.F.; Wong, C.H. J. Org. Chem. **1988**, *53*(21), 4939.
2. Chang, K.Y.; Wu, S.H.; Wang, K.T. Carbohydr. Res. **1991**, *222*, 121.
3. Chang, K.Y.; Wu, S.H.; Wang, K.T. J. Carbohydr. Chem. **1991**, *10*(2), 251.
4. Bruno, F.F.; Dordick, J.S.; Kaplan, D.L.; Akkara, J.A. Regioselective Enzymatic Transesterification of Polysaccharides in Organic Solvents. **1998**, *684*, 167–174.

5. Chen, X.M.; Johnson, A.; Dordick, J.S.; Rethwisch, D.G. Macromol. Chem. Phys. **1994**, *195*, 3567.
6. Gardossi, L.; Khan, R.; Konowicz, P.A.; Gropen, L.; Paulsen, B.S. J. Mol. Catal. B-Enzym. **1999**, *6*, 89.
7. Varki, A. Glycobiology **1993**, *3*, 97.
8. Varki, A. Glycobiology **1992**, *2*, 25.
9. Kuberan, B.; Linhardt, R.J. Curr. Org. Chem. **2000**, *4*, 653.
10. Linhardt, R.J.; Kuberan, B.; Polat, T.; Koketsu, M.; Wang, Q. Abstr. Pap. Am. Chem. Soc. **2000**, *219*, 51-CARB.
11. Horrobin, T.; Tran, C.H.; Crout, D. J. Chem. Soc.-Perkin Trans. 1 **1998**, 1069.
12. Chaplin, D.; Crout, D.H.G.; Bornemann, S.; Hutchinson, D.W.; Khan, R. J. Chem. Soc.-Perkin Trans. 1 **1992**, 235.
13. Vlahov, I.R.; Vlahova, P.I.; Linhardt, R.J. J. Am. Chem. Soc. **1997**, *119*, 1480.
14. Polat, T.; Du, Y.; Linhardt, R.J. Synlett **1998**, 1195.

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