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REGIOSELECTIVE ESTER HYDROLYSIS OF SIALIC ACID DERIVATIVES CATALYZED BY MOLECULARLY IMPRINTED POLYMERS

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Abstract – Regioselective ester hydrolysis of sialic acid derivatives catalyzed by molecularly imprinted polymers was studied. A sialic acid derivative having 8-*O*-methylphosphonate was used as the template for molecular imprinting polymer (MIP). By mimicking the tetrahedral intermediate for ester hydrolysis, this MIP catalyzed the regioselective hydrolysis of the 8-*O*-acetyl group of per-*O*-acetylated sialic acid derivative at an increased rate.

N-Acetylneuraminic acid (sialic acid, Neu5Ac) and its various analogs play essential roles in a variety of biochemical and biological processes.[1] Gangliosides containing the Neu5Acα(2-8)Neu5Ac unit in their molecules are of interest in connection with the important biological roles of glycolipids.[2] Although effort has been made to develop regio- and stereoselective syntheses of α(2-8)-linked polysialogangliosides, the problem of differential protection of potentially competing functional groups is still formidable.[3] For instance, conventional means for selecting just one of the four hydroxyl groups of per-*O*-acetylated sialic acid derivative **5** to function as a glycosyl acceptor typically requires several steps of protection and deprotection, followed by tedious chromatographic purification; therefore, effective methods for the synthesis of oligosialosides are still required.

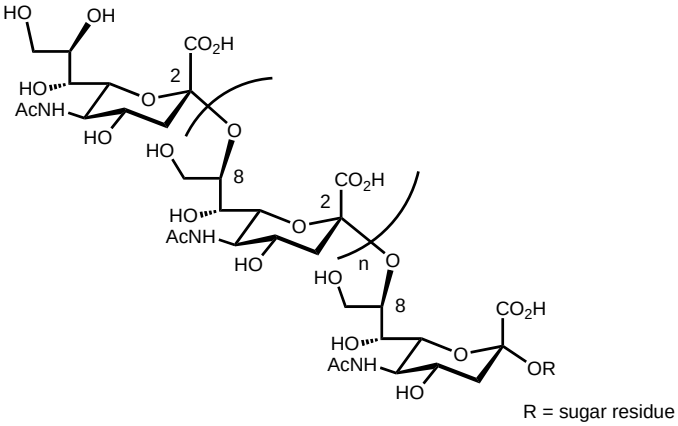
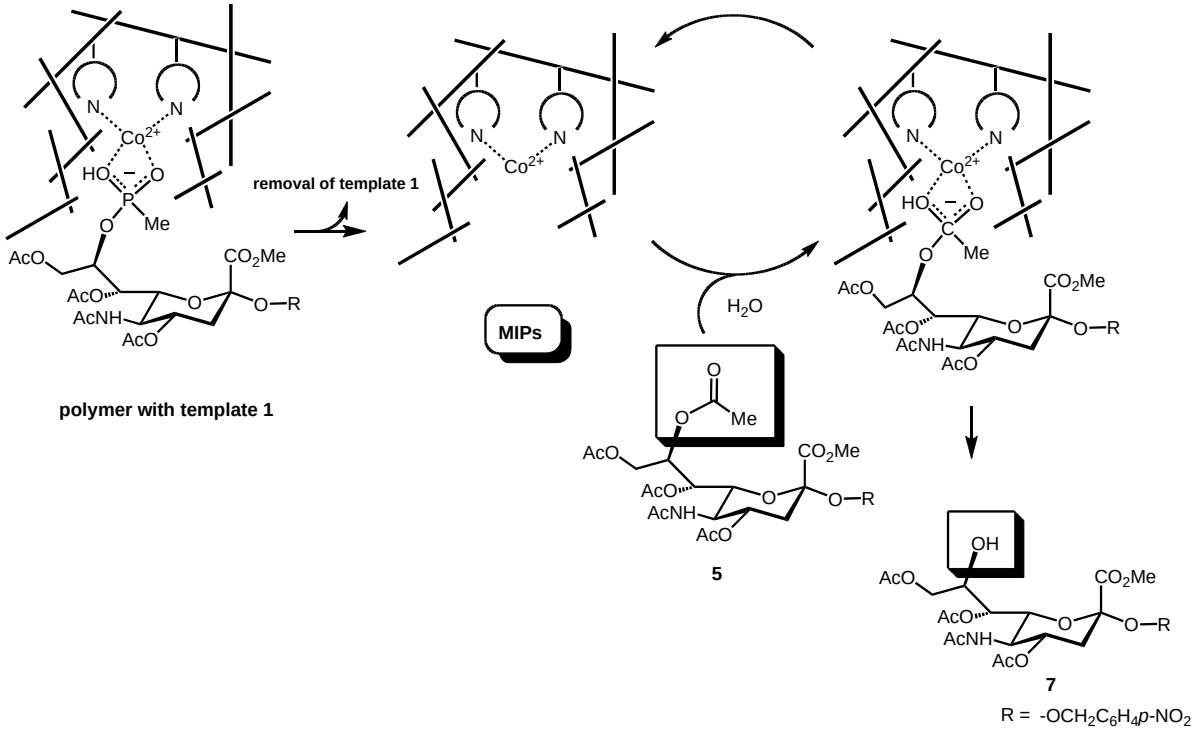


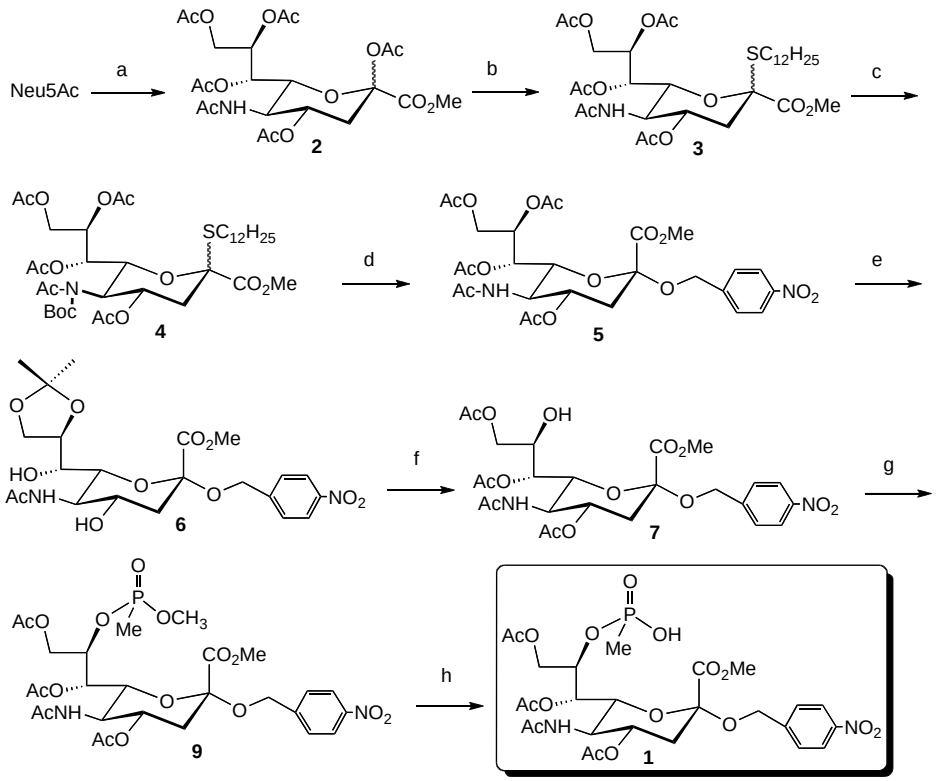
Figure 1. Structure of Neu5Acα(2-8)Neu5Ac sequence

Molecular imprinting (MI)[4] is a known synthetic technique for developing polymeric artificial receptors to a given target molecule. The main merit of imprinted catalysts is their similarity to a natural enzyme that catalyzes a specific reaction. Furthermore, imprinted catalysts are easier to prepare and handle than enzymes. Whereas enzymes and catalytic antibodies[5] degrade under harsh conditions, such as high temperature, chemically aggressive media, and high and low pH, imprinted polymers show better catalytic activity and stability in most cases. Thus, molecular imprinting catalysts are promising for a wide range of applications, including chromatographic purification,[6] solid-phase extraction,[7] and reaction catalysis.[4c] For the construction of α(2-8)-linked polysialogangliosides, we report our preliminary results on the synthesis of molecular imprinting polymers (MIPs) that are capable of discriminating chemically identical functional groups in the same molecule, catalyzing regioselective 8-*O*-acetyl deprotection of **5** (Scheme 1).



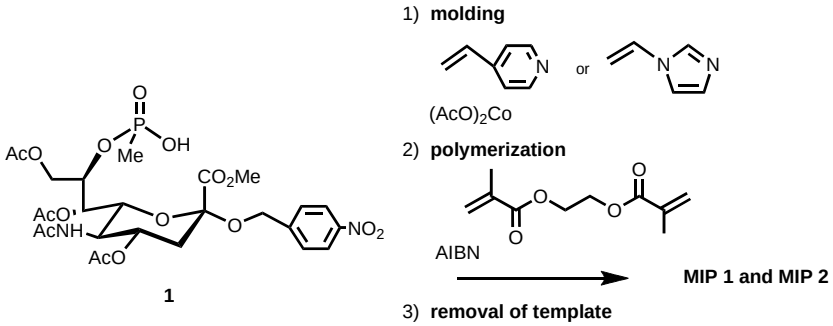
Scheme 1. Regioselective hydrolysis of **5** by MIPs

Template **1**, which possesses a phosphonate group[8] mimicking the tetrahedral intermediate for regioselective ester hydrolysis of **5**, was prepared from *N*-acetyneuraminic acid, as outlined in scheme 2. Initially, the acetate of Neu5Ac **2**, derived from Neu5Ac, was converted to the β-2-thioglycoside **3** in 90% yield in three steps. Our *N,N*-diacylated donor **4**[9] was prepared by treating **3** with Boc_2O and DMAP in THF in 99% yield. The glycosylation of **4** with *p*-nitrobenzyl alcohol[10] by NIS and TfOH in CPME[11] at -40 °C gave α-glycoside in 97% yield with α-anomer as the major product (α/β = 7:1), which was treated with TFA to give compound **5** in 91% yield. Zemplen *O*-deacetylation of **5** with 0.1 M sodium methoxide in MeOH followed by subsequent protection by 2,2-dimethoxypropane and Amberlite 120 (H^+) in DMF afforded the 8,9-*O*-isopropylidene compound **6** in 85% yield in two steps. Compound **6** was treated with Ac_2O and pyridine, followed by deprotection of the isopropylidene group with 80% AcOH to afford the 8,9-diol compound in 82% yield in two steps, whose selective 9-*O*-acetylation with acetyl chloride and pyridine at -40 °C gave 9-*O*-acetyl compound **7** in 87% yield. The phosphorylation of **7** with methyl methylphosphonochloridate **8**[12] and pyridine gave 8-*O*-methylphoshorylated compound **9** in 94% yield, as chromatographically inseparable ca. 1:1 mixtures of diastereomer. Finally, the demethylation of methylphosphoryl group of **9** with trimethylsilyl bromide, and subsequent hydrolysis of TMS group gave template **1**,[13] in quantitative yield, as a single isomer.



Scheme 2. *Reagents and conditions*: (a) 1) MeOH, IR120(H^+), 2) Ac_2O, pyridine, quant. in two steps; (b) 1-dodecanethiol, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 90%; (c) Boc_2O, DMAP,THF, 99%; (d) 1) *p*-$NO_2C_6H_4CH_2OH$, NIS, TfOH, CPME, -40 °C, 97% (α/β=7:1); 2) TFA, CH_2Cl_2, 91%; (e) 1) NaOMe, MeOH, 2) 2,2-dimethoxypropane, PTSA, DMF, 85%, in two steps; (f) 1) Ac_2O, pyridine; 2) 80% AcOH, 82% in two steps; 3) AcCl, pyridine, CH_2Cl_2, -40 °C, 87%; (g) ClP(=O)MeOMe **8**, NEt_3, DMAP, CH_2Cl_2, 94%; (h) 1) TMSBr,CH_2Cl_2, 2) THF-H_2O (9:1), quant. in two steps.

For the preparation of MIPs (Scheme 3), azobis(isobutyronitrile) (AIBN) (12 mg, 0.070 mmol) as a radical initiator was added to a stirred solution of template **1** (2.5 mg, 0.0075 mmol), 4-vinylpyridine (38 mg, 0.36 mmol) or 1-vinylimidazole (34 mg, 0.36 mmol), ethylene glycol dimethacrylate (350 mg, 1.8 mmol) and cobalt(II) acetate tetrahydrate[8] (45 mg, 0.18 mmol) in deaerated methanol (20 mL). Polymerization was performed in refluxing methanol for 5 h. After crushing, the resultant polymer was thoroughly washed with soxhlet extraction in phosphate buffer (100 mM, pH 7) and methanol overnight to remove the printing molecule **1** as a template and afford blue cross-linked polymer MIP 1 (284 mg) and MIP 2 (320 mg), respectively. As a control, nonimprinted polymers MIP 1-B and 2-B were prepared in the presence of cobalt but without **1**, respectively (Table 1, entries 2 and 4).



Scheme 3. Preparation of MIPs derived from **1**

Table 1. Preparation of MIPs derived from **1**

entry	MIPs	functional monomers	template **1**	Co^{2+}
1	MIP 1	4-vinylpyridine	+	+
2	MIP 1-B	4-vinylpyridine	-	+
3	MIP 2	1-vinylimidazole	+	+
4	MIP 2-B	1-vinylimidazole	-	+
5	MIP 3	1-vinylimidazole	+	-

The catalytic activities of MIP 1, 2, 3 and of their controls MIP 1-B, 2-B were investigated in the regioselective hydrolysis of substrates **5** to **7** in 1 mL vials containing polymer (10 mg) and solvent (0.30 mL acetonitrile / phosphate 0.05M, pH 6.0, 7.6 and 9.1: 1/1) by adding substrate **5** (5 mg). The vials were then shaken mechanically at 20 °C for 2 days. The polymer was removed by filtration, and the filtrate was concentrated to dryness. To evaluate hydrolytic activity, HPLC (C-18 reversed phase, mobile phase: chloroform/methanol 50:1) was performed by injecting the supernatant and UV monitoring (254 nm). Table 2 gives control data of the hydrolysis of **5** with added nonimprinted polymers MIP1-B, 2-B and Co^{2+}. MIP1-B and 2-B did not catalyze the regioselective hydrolysis of 8-*O*-Ac of **5**.

Table 2. Hydrolysis of **5** by MIP 1-B or MIP 2-B

product	MIPs	pH 6.0	pH 7.6	pH 9.1
recovered product **5**	MIP1-B	99%	99%	67%
	MIP2-B	99%	99%	66%
hydrolyzed product **7**	MIP1-B	0%	0%	2%
	MIP2-B	0%	0%	1%
non-selectively hydrolyzed product	MIP1-B	1%	1%	31%
	MIP2-B	1%	1%	33%

Conditions; Column: Wakopack Wakosil 10SIL, Eluent: $CHCl_3$: MeOH = 50 : 1, Flow: 1ml/min Detected at 254nm.

Table 3 shows the results obtained by regioselective hydrolysis of **5** by MIP 1 or 2. Interestingly, MIP 2 exhibited moderate rate enhancements of **7** at pH 7.6 without non-selectively hydrolyzed product. This reaction was strongly dependent on the pH value.

Table 3. Regioselective hydrolysis of **5** by MIP 1 or MIP 2

product	MIPs	pH 6.0	pH 7.6	pH 9.1
recovered product **5**	MIP1	99%	99%	93%
	MIP2	97%	94%	92%
hydrolyzed product **7**	MIP1	1%	1%	4%
	MIP2	3%	6%	7%
non-selectively hydrolyzed product	MIP1	0%	0%	3%
	MIP2	0%	0%	1%

Conditions; Column: Wakopack Wakosil 10SIL, Eluent: $CHCl_3$: MeOH = 50 : 1, Flow: 1ml/min Detected at 254nm.

Table 4 shows the results obtained by hydrolysis of **5** with MIP 3. It was found that Co^{2+} has an important role in the regioselective hydrolysis of 8-*O*-Ac of **5**.

Table 4. Hydrolysis of **5** by MIP 3

product	pH 6.0	pH 7.6	pH 9.1
recovered product **5**	99%	99%	94%
hydrolyzed product **7**	0%	0%	3%
non-selectively hydrolyzed product	1%	1%	3%

Conditions; Column: Wakopack Wakosil 10SIL, Eluent: $CHCl_3$: MeOH = 50 : 1, Flow: 1ml/min Detected at 254nm.

In summary, molecular imprinting of a functionally active polymer (MIP 2) using a transition state analogue **1** as a template led to enhanced rates of ester hydrolysis of **5** to **7**. Although modest, these rate enhancements should be considered as a first step in the development of imprinted polymers for the regioselective hydrolysis of per-*O*-acetylated sialic acid derivative.

ACKNOWLEDGEMENTS

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13. *Selected data for* **1**: IR ($CHCl_3$) cm^{-1}: 3371, 1749, 1666 and 1593. ^{1}H-NMR ($CDCl_3$) δ: 1.55 (3H, d, $^2J_{H\text{-}P}$ = 18 Hz, CH_3PO-), 1.92 (3H, s, CH_3CONH-), 2.01, 2.05 (each 3H, s, CH_3CO-), 2.09-2.13 (4H, m, CH_3CO-, H-3ax), 2.72 (1H, dd, $J_{3e,3a}$ = 13 Hz, $J_{3e,4}$ = 4.6 Hz, H-3eq), 3.77 (3H, s, CH_3COO-), 4.06-4.22 (3H, m, H-5, 9, 9'), 4.59-4.62 (2H, m, -OCH_2PhNO_2), 4.80-5.03 (3H, m, H-4, 6, 8), 5.43 (1H, dd, $J_{7,6}$ = 2.0 Hz, $J_{7,8}$ = 4.6 Hz, H-7), 7.49-7.60 (2H, m, aromatic-H), 8.12-8.19 (2H, m, aromatic-H). ^{31}P-NMR (81 MHz, $CDCl_3$) ™: 33.2. FAB-MS (NBA) *m/z*: 663 $(M+H)^+$. FAB HRMS calcd for $C_{26}H_{36}N_2O_{16}P$ $(M+H)^+$ 663.1801, Found 663.1796.