Preparation of a fluorous protecting group and its application to the chemoenzymatic synthesis of sialidase inhibitor

Kiyoshi Ikeda,* Hitomi Mori and Masayuki Sato*

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2-(Perfluorohexyl)ethoxymethyl chloride was prepared as a novel fluorous protecting reagent. Neu5Ac aldolase-catalyzed chemoenzymatic transformation of *N*-acetyl-D-mannosamine to Neu5Ac derivatives was achieved successfully by using the fluorous reagent not only for hydroxy group protection but also for fluorous tagging. This chemoenzymatic method was applied to the synthesis of 2-deoxy-2,3-didehydrosialic acid 1 known as a potent sialidase inhibitor.

Sialic acids displayed on the surface of mammalian cells are involved in many cell-surface interactions including cell–cell recognition processes, cell adhesion, and viral receptor recognition.[1] Current interests in sialic acid and related molecules stem from their growing importance in biological systems that eventually trigger a variety of biological responses.[2] Among the diverse array of compounds related to sialic acid family, 2,3-unsaturated sialic acid derivatives based on 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (**1**)[3] are promising candidates for designing chemotherapeutics agents against influenza virus.[4]

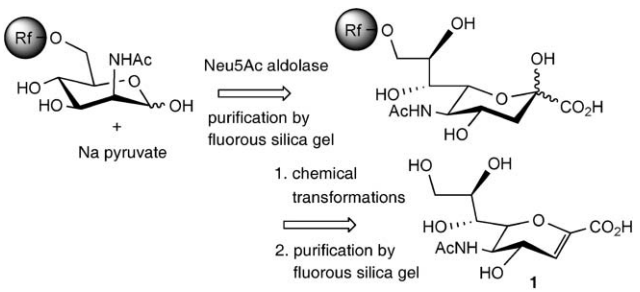
The search for rapid and efficient protocols for the purification of organic compounds is a major concern of modern chemistry. Fluorous techniques for organic synthesis are very attractive for strategic separation of reaction mixtures. Early fluorous synthesis technologies relied on heavy fluorous tags and liquid–liquid extraction to separate tagged-fluorous molecules from untagged organics.[5] In the recently introduced light fluorous synthesis, fewer fluorines are used in the tag and the fluorous solid-phase extraction (FSPE) over fluorous reverse-phase silica gel (FRPS) is proving far superior to binary liquid–liquid extractions for compounds with fewer fluorine atoms.[6] The fluorous tagging method has the advantage of allowing the use of silica gel TLC to monitor the reaction process and allowing us ready purification by a single chromatographic method, greatly reducing time for isolation.

To our knowledge, there is no application of fluorous tagging method to chemoenzymatic synthesis of biologically active carbohydrates. As part of a program aimed at the new sialidase inhibitors, we described the Neu5Ac aldolase-catalyzed chemoenzymatic synthesis of sialic acid derivatives and their inhibitory activities against enzyme.[7] Herein we wish to report the first chemoenzymatic synthesis of sialic acid derivatives from D-ManNAc derivative bearing a fluorous protecting group at the C-6 position of D-ManNAc and its application to synthesis of **1**. Our concept of chemoenzymatic synthesis of **1** using a fluorous tag is shown in Scheme 1.

The first step of the synthesis of **7** began with the per-*O*-acetylation of D-ManNAc, followed by the glycosylation and subsequent deprotection of *O*-acetyl group to give α-glycoside **2** in 68% yield in three steps. Compound **2** was protected by isopropylidene group and then acetylated to give **3** in 84% yield in two steps. Next, the treatment of **3** with 80% AcOH afforded diol **4** in quantitative yield. We synthesized 2-(perfluorohexyl)-ethoxymethyl chloride **5**[8] as a fluorous acetal protecting reagent by the reaction of 2-(perfluorohexyl)ethanol and paraformaldehyde in the presence of dry hydrogen chloride. The 2-(perfluorohexyl)-ethoxymethyl protecting group would have minimal effect on the reactivity of the attached molecules for enzyme reaction, and might be useful for separation by the FRPS method. Selective installation of a fluorous protecting group to the primary hydroxyl function of **4** was successfully carried out with **5** and diisopropylethylamine in DMF to give **6** in 81% yield. Finally, protective acetyl groups and the glycosidic benzyl group of **6** were removed to afford substrate **7** in 94% yield in two steps. Compound **7** was obtained in 52% yield in nine steps from D-ManNAc (Scheme 2).

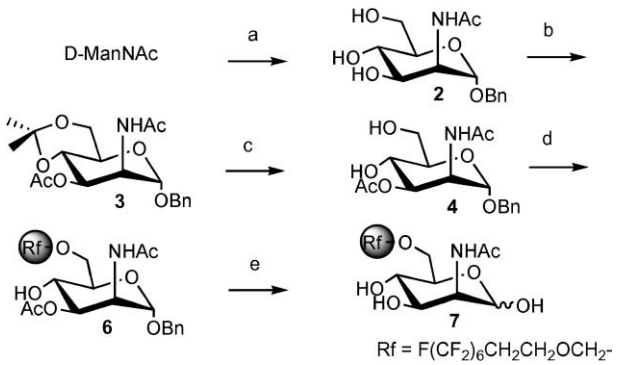
The crucial incubation of Neu5Ac aldolase[9] with **7** and sodium pyruvate in potassium phosphate buffer (KpB) (pH 7.5) in the presence of DTT and $MgCl_2$ at 37 °C for 7 days afforded **8** in 62% yield (Table 1, entry 1), which was desalted by Bio-Gel P-2 gel filtration chromatography using water and purified by FRPS chromatography eluting first with 80% MeOH–H_2O and then with MeOH, without tedious and time consuming ion-exchange chromatography. It should be noted that the addition of HFE-7200[10] for improving the solubility of **7** in KpB resulted in increasing yield of **8** (71%) (Table 1, entry 2).[11]

As depicted in Scheme 3, after esterification of **8** with $TMSCHN_2$, further transformation of **9** into **10**[12] was accomplished with the treatment of AcCl, followed by elimination of HCl with pyridine to give crude 2,3-deoxy sialic acid **10**, which was



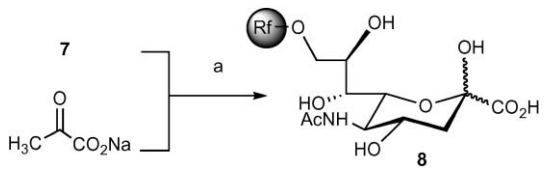
Scheme 1 Rf = fluorous protecting group.

Department of Organic Chemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada Suruga-Ku, Shizuoka, 422-8526, Japan. E-mail: ikeda@ys2.u-shizuoka-ken.ac.jp; Fax: +81-54-264-5108; Tel: +81-54-264-5108

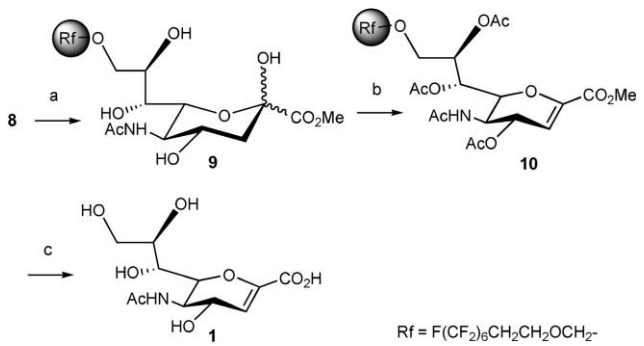


Scheme 2 *Reagents and conditions*: (a) (i) Ac_2O, pyridine, CH_2Cl_2, rt, 24 h, (ii) BnOH, $BF_3{\cdot}OEt_2$, CH_3NO_2, 80 °C, 7 h, (iii) NaOMe, MeOH, 0 °C, 3 h, 68% from D-ManNAc; (b) (i) 2,2-dimethoxypropane, PTSA, DMF, rt, 1 h, (ii) Ac_2O, pyridine, rt, 24 h, 84% from **2**; (c) 80% AcOH, 80 C, quant.; (d) RfCl **5**, $^{i}Pr_2NEt$, DMF, 45 °C, 54 h, 81%; (e) (i) NaOMe, MeOH, 0 °C, 2 h, (ii) 20% $Pd(OH)_2/C$, H_2, MeOH, rt, 24 h, 94% from **6**.

Table 1 Neu5Ac aldolase-catalyzed reaction using fluorous protecting group



Entry	Condition	Yield (%)
1	Neu5Ac aldolase KpB (pH 7.5) $MgCl_2$, DTT, 37 °C, 7 d	62
2	Neu5Ac aldolase HFE-7200-KpB (pH 7.5) (1 : 5) $MgCl_2$, DTT, 37 °C, 7 d	71



Scheme 3 *Reagents and conditions*: (a) $TMSCHN_2$, CH_2Cl_2, rt, 24 h, quant. from **8**; (b) (i) AcCl, rt, 24 h; (ii) pyridine, rt, 1 h, 87% from **9**; (c) (i) NaOMe, MeOH, 0 °C, 2 h; (ii) TMSBr, CH_2Cl_2, 0 °C, 12 h, 67% from **10**.

separated over FRPS chromatography. Finally, after the stepwise removal of acetyl groups and methyl ester group, deprotection of the fluorous acetal group with trimethylsilyl bromide (TMSBr)[13] under mildly acidic conditions gave **1**.[14]

In conclusion, we have demonstrated the first chemoenzymatic synthesis of sialic acid derivatives by using the FSPE technique and its application to the synthesis of sialidase inhibitor **1**. Further application to the synthesis of the bioactive carbohydrates is now in progress.

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Notes and references

1 *Biology of Sialic Acids*, ed. A. Rosenberg, Plenum Press, New York, London, 1995.
2 M. J. Kiefel and M. von Itzstein, *Chem. Rev.*, 2002, **102**, 471.
3 C. T. Holzer, M. von Itzstein, B. Jin, M. S. Pegg, W. P. Stewart and W.-Y. Wu, *Glycoconjugate J.*, 1993, **10**, 40.
4 (*a*) K. Ikeda, M. Sato and Y. Torisawa, *Curr. Med. Chem.*, 2004, **3**, 339; (*b*) J. C. Wilson, R. J. Thomson, J. C. Dyason, P. Florio, K. J. Quelch, S. Abo and M. von Itzstein, *Tetrahedron: Asymmetry*, 2000, **11**, 53.
5 (*a*) I. T. Horvath and J. Rabai, *Science*, 1994, **266**, 72; (*b*) D. P. Cuuran, *Angew. Chem., Int. Ed.*, 1998, **37**, 1174; (*c*) K. Goto, T. Miura, M. Mizuno, H. Takaki, N. Imai, Y. Murakami and T. Inazu, *Synlett*, 2004, 2221; (*d*) D. P. Curran, *Pure Appl. Chem.*, 2000, **72**, 1649.
6 (*a*) D. P. Curran and Z. Y. Luo, *J. Am. Chem. Soc.*, 1999, **121**, 9069; (*b*) D. P. Curran, *Synlett*, 2001, 1488; (*c*) Z. Y. Luo, Q. S. Zhang, Y. Oderaotoshi and D. P. Curran, *Science*, 2001, **291**, 1766.
7 (*a*) M. Murakami, K. Ikeda and K. Achiwa, *Carbohydr. Res.*, 1996, **280**, 101; (*b*) K. Ikeda, F. Kimura, K. Sano, Y. Suzuki and K. Achiwa, *Carbohydr. Res.*, 1998, **312**, 183.
8 *Experimental data for* **5**: Anhydrous hydrogen chloride was passed through a mixture of 2-(perfluorohexyl)ethanol (10.9 g, 30 mmol) and paraformaldehyde powder (0.99 g, 33 mmol) at 20–25 °C for 2 h. The reaction mixture was transferred into a separating funnel and the upper layer was diluted with pentane. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to give 10.9 g (86%) of crude **5** as a colorless oil. Distillation of this oil gave pure **5** (45 mmHg, bp 83–85 °C). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.43–2.49 (m, 2H, $ClCH_2OCH_2C\mathbf{H}_2$), 3.96–4.00 (m, 2H, $ClCH_2OC\mathbf{H}_2CH_2$), 5.50 (m, 2H, $ClC\mathbf{H}_2OCH_2CH_2$). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 29.1 (t, J_{CF} = 22.2 Hz), 60.4, 80.4, 106.7–116.5 (m, C_6F_{13}). ^{19}F NMR ($CDCl_3$, 470 MHz): δ −126.7, −124.2, −123.4, −122.4, −114.0, −81.4. Negative FABMS (TEA) *m/z*: 377 $(M\text{-}Cl)^+$.
9 J. L.-C. Lin, G. -J. Shen, Y. Ichikawa, J. F. Rutant, G. Zapata, W. F. Vann and C. -H. Wong, *J. Am. Chem. Soc.*, 1992, **114**, 3901.
10 $EtOC_4F_9$ is a commercially available fluorocarbon solvent (3 M, Tokyo), which is called Novec™ HFE-7200.
11 *Experimental data of Neu5Ac aldolase catalyzed reaction*: Neu5Ac aldolase [10 unit] was added to a solution of **7** (60 mg, 0.10 mmol) and sodium pyruvate (110 mg, 1.0 mmol) in 0.05 M KpB (pH 7.5) (2 ml) and HFE-7200 (0.4 ml) in the presence of DTT (1.4 mg) and 0.1 M $MgCl_2$ (0.10 ml) at 37 °C. After 2 days, an additional amount of aldolase (10 unit) and sodium pyruvate (110 mg, 1.20 mmol) was added. The mixture was incubated for a further 5 days at 37 °C. The course of the reaction was monitored by TLC. The whole solution was passed through a column of Bio-Gel P-2 gel filtration chromatography using water as eluant. Fractions containing the product were purified on a column of FRPS chromatogaraphy eluted first with 80% MeOH−H_2O and then with MeOH to give **8** (50 mg, 71%), as amorphous after freeze drying. Positive FABMS (NBA) *m/z*: 708 $(M + H)^+$, 730 $(M + Na)^+$.
12 *Selected data for* **10**: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.90 (s, 3H, NHAc), 2.04, 2.06, 2.09 (s, each 3H, OAc), 2.34−2.48 (m, 2H, $OCH_2OCH_2C\mathbf{H}_2$), 3.63 (dd, 1H, $J_{9a,9b}$ = 11.5, $J_{9a,8}$ = 6.9 Hz H-9a), 3.77 (s, 3H, OMe), 3.80–3.84 (m, 2H, $OCH_2OC\mathbf{H}_2CH_2$), 4.07 (dd, 1H, $J_{9b,8}$ = 3.5 Hz H-9b), 4.68, 4.69 (d, each 1H, J_{gem} = 16.6 Hz, $OC\mathbf{H}_2OCH_2CH_2$), 5.98 (d, 1H, $J_{3,4}$ = 3.5 Hz, H-3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.7, 20.8, 20.9, 23.2, 31.4 (t, J_{CF} = 21.6 Hz), 46.5, 52.6, 60.0, 65.8, 67.8, 68.1, 71.9, 95.7, 107.9, 116.1–118.4 (m, C_6F_{13}), 145.0, 161.7, 170.1, 170.3, 170.5, 170.8. ^{19}F NMR ($CDCl_3$, 470 MHz): δ −126.7, −124.2, −123.4, −122.4, −114.1, −81.3. Positive FABMS (NBA) *m/z*: 830 $(M + Na)^+$.
13 S. Hanessian, D. Delorme and Y. Dufresne, *Tetrahedron Lett.*, 1984, **25**, 2515.
14 The spectral data of **1** were consistent with an authentic sample.

