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SYNTHESIS OF CMP-SIALIC ACID MIMICS THAT HAVE 5-FLUOROURACIL FOR CYTOSINE AND THE C-TERMINAL'S PEPTIDE BOND FOR THE PHOSPHATE GROUP: TARGETING INHIBITORS OF SIALYLTRANSFERASES

Shinji Nakahara,[1] Toru Tanaka,[1] Kazuharu Noguchi,[2] Kenji Nozaki,[2] Shuichi Tsuji,[3] Tsuyoshi Miura,[4] and Tetsuya Kajimoto[1]*

[1]Department of Biotechnology, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei-shi, Tokyo 184-8588, Japan; [2]Han-no Research Center, Taiho Pharmaceutical Co., Ltd., 1-27 Misugidai, Han-no-shi, Saitama 357-8527, Japan; [3]The Glycoscience Institute, Ochanomizu University, 2-1-1 Otsuka, Bunkyo-ku, Tokyo 112-8555, Japan; [4]The Noguchi Institute, 1-8-1 Kaga, Itabashi-ku, Tokyo 173-0003, Japan

Abstract – Novel mimics of cytidine 5'-monophosphate-sialic acid (CMP-sialic acid) were designed and synthesized for targeting inhibitors of sialyltransferases on the basis of the phenomenon that the tautomerization of 5-fluorouracil from the lactam to the lactim form produces a structure similar to that of cytosine, and the *C*-terminal's peptide bond can be a bioisoster of a phosphate group. Since γ-N^1-(5-fluorouracilyl)-β-hydroxy-α-L-amino acid, a key synthetic intermediate, was easily prepared using the enzyme-catalyzed aldol reaction, the synthesis of the mimics of the CMP-sialic acid was attained in a short step.

Sialyl oligosaccharides, in which sialic acids link to the non-reducing ends of glycoconjugates on cell surfaces, play important roles in cell differentiation, immune responses, bacterial and viral infections, and other cell-cell recognition events.[1] Therefore, the inhibitors toward sialyltransferases that transfer sialic acid from cytidine 5'-phospho-β-D-ribose-sialic acid (CMP-sialic acid: **1**) to galactose or other sialic acid residues of oligosaccharides would be excellent candidates as anti-inflammatory agents, anti-metastastic agents, and agents for auto-immune diseases.[2] Despite a number of CMP-sialic acid analogs being synthesized as the inhibitors of sialyltransferases and a few of them exhibited potent inhibitory activities, the synthetic routes of the inhibitors required very tedious procedures to provide a sufficient amount for

clinical tests or a wide-range of laboratory use.[3] We have also reported the design and synthesis of two CMP-sialic acid analogs (**2a**, **2b**), in which the *C*-terminal's peptide bond was chosen as a bioisoster of the phosphate group of **1** in terms of having a negative charge and high polarity (Figure 1).[4] These analogs were capable of being easily prepared on a large scale using the L-threonine aldolase(L-TA)-catalyzed reaction as a key step, however, it had only slight inhibitory activities toward sialyltransferases.[5]

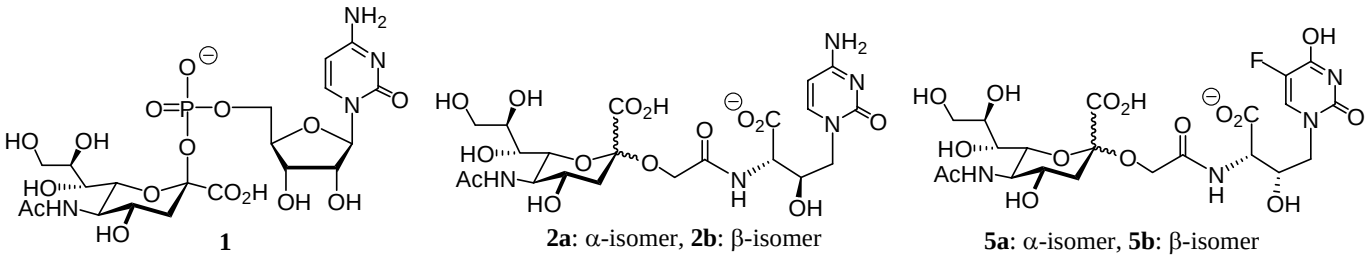
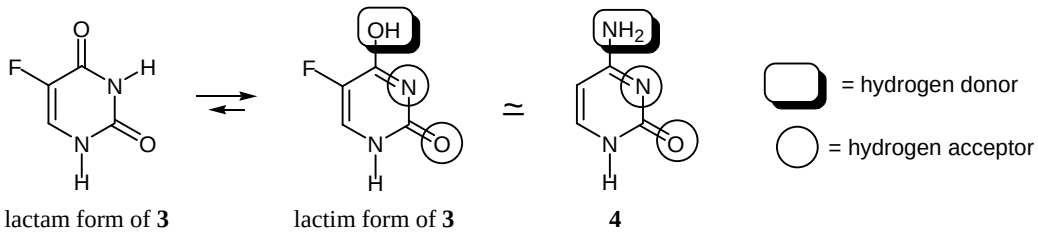


Figure 1 Structures of CMP-sialic acid (**1**) and its mimetics (**2a**, **2b**, **5a**, **5b**)

After a precise comparison of the predictable stable conformations of **2a** and **2b** with that of **1**, we became aware of the possibility to raise the activity by changing the stereochemistry of the β-hydroxy-α-amino unit in **2a** and **2b** from the *threo*- to *erythro*-configuration. However, the preparation of the β-hydroxy-α-amino unit using the L-TA-catalyzed reaction[6] predominantly afforded the isomer with the *threo*-configuration (*threo* : *erythro* = 3.5 : 1).

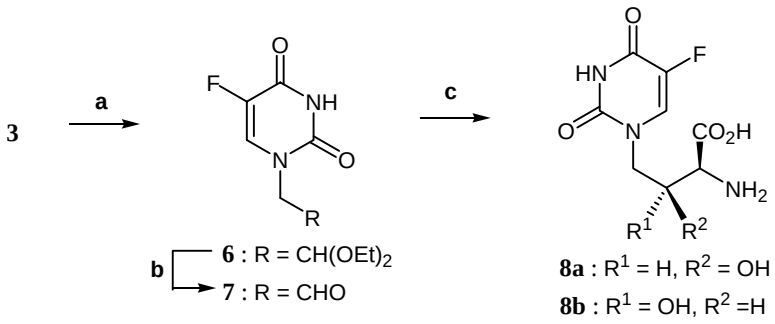
On the other hand, tautomerization of 5-fluorouracil (**3**) from the lactam to the lactim form produces a structure similar to cytosine (**4**) in terms of orientation of the hydrogen donor and the hydrogen acceptor, which would tightly interact with the active site of sialyltransferases by forming hydrogen bonds (Scheme 1).[7] And the tautomerism equilibrium declines to the lactim form by the strong electronegativity of fluorine.



Scheme 1 Tautomerization of 5-fluorouracile (**3**) and its structural similarity of cytosine (**4**)

Since replacing the cytosine part of **2a** and **2b** with 5-fluorouracil (**3**) showed the possibility to positively affect the stereochemistry of the β-hydroxy-α-amino unit upon being formed by the L-TA catalyzed reaction (*vide infra*), we now report the synthesis of novel mimics of CMP-sialic acid (**5a**, **5b**).

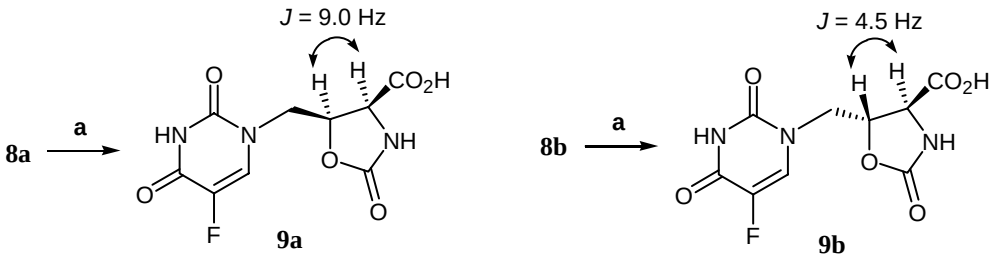
5-Fluorouracil (**3**) was treated with 1-bromo-2,2-diethoxyethane in the presence of sodium carbonate in DMF at 100 °C to afford the N^1-alkylated 5-fluorouracil (**6**)[9] (51%). A 2-fold volume of Tris-buffer solution (500 mM, pH 6.5) was added to the aqueous solution of 1 M HCl, in which the diethyl acetal (**6**) was converted to the aldehyde (**7**) (100 °C, 30 min), and then glycine (30 eq. to **6**) and L-threonine aldolase from *Candida humicola* AKU 4586 (300 units) were successively added (Scheme 2). The reaction mixture was incubated with moderate shaking for 15 h, and heated at 100 °C for 30 min in order to deactivate the aldolase, then filtered through celite. The filtrate was passed through a column chromatography over activated charcoal, washed with distilled water, and elution with methanol afforded a mixture of the *erythro* and *threo* isomers of the γ-(5-fluorouracilyl)-β-hydroxy-α-L-amino acid (**8a**, **8b**) (79%, 2 steps), which was easily separated by C_{30} reverse-phase chromatography (Nomura Chemical Co., Ltd., Japan) with medium pressure (mobile phase: 0.01 M HCl).[8]



a) $BrCH_2CH(OEt)_2$, Na_2CO_3 (51%), b) aq. HCl, c) L-threonine aldolase, glycine (79%, 2 steps)

Scheme 2 Preparation of β-hydroxy-α-L-amino acids having 5-fluorouracil residue at γ-position (**8a**, **8b**)

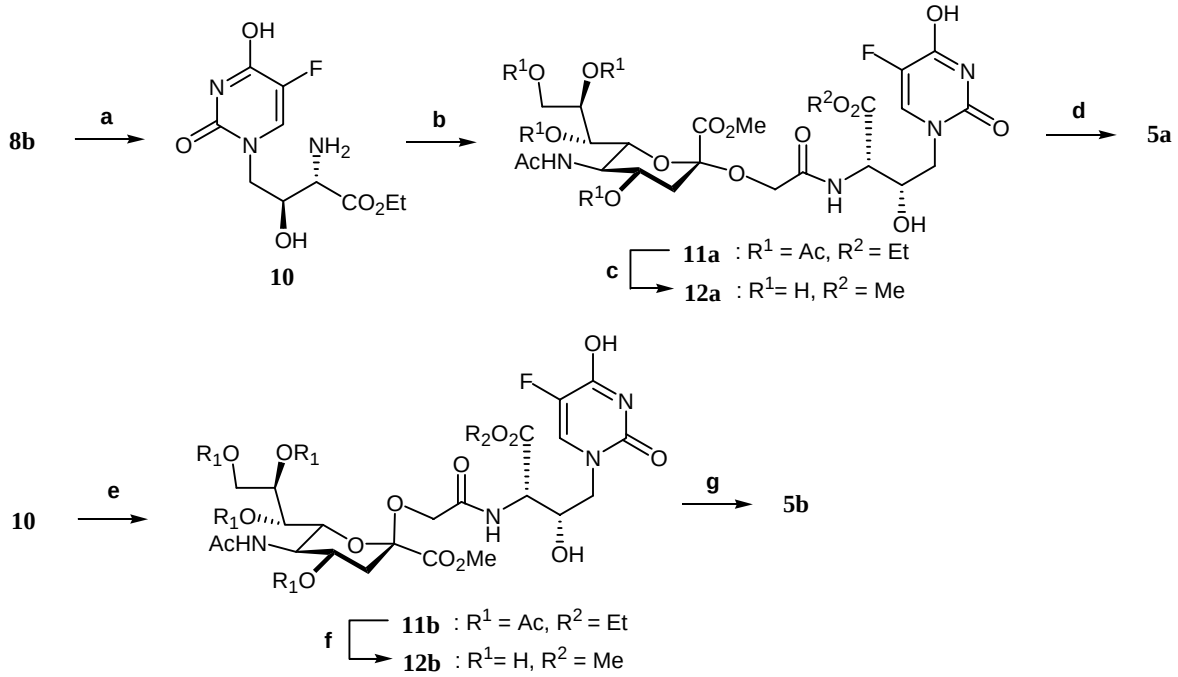
The ratio of the isomers eluted faster and later was 1.5 : 1, and the absolute configuration of both isomers was determined to be the L-configuration by consumption analysis using L- and D-amino acid oxidases. On the other hand, the relative configurations of **8a** and **8b** were determined from the coupling constants between the α- and β-protons observed in the ^{1}H NMR spectra, after converting each isomer to the oxazolin-2-one (**9a**, **9b**) by treatment with chloroethyl carbonate in 1M sodium hydroxide. The major isomer with the coupling constant of 4.5 Hz has the *threo* configuration, while a minor isomer with the constant of 9.0 Hz has the *erythro* one (Scheme 3).[10]



a) $ClCO_2Et$, 1M aq. NaOH

Scheme 3 Preparation of oxazolin-2-ones (**9a, 9b**) from **8a** and **8b**

γ-(5-Fuorouracilyl)-β-hydroxy-α-L-amino acid with the *erythro*-configuration (**8b**) was treated with ethanol in the presence of *p*-toluenesulfonic acid to give the ethyl ester (**10**) (61%), which was linked with methyl 2-*O*-α-4,7,8,9-tetra-*O*-acetyl-*N*-acetylneuraminyl acetic acid and methyl 2-*O*-β-4,7,8,9-tetra-*O*-acetyl-*N*-acetylneuraminyl acetic acid[4] by a conventional method (DCC/HOBt/NMM),[11] to afford the fully protected CMP-sialic acid analogs having 5-fluorouracil (**11a**, **11b**)[12,13] (83% and 53%, repectively). Deacetylation of **11a** and **11b** was done with sodium methoxide in methanol to afford the dimethyl esters (**12a**, **12b**) (90% and 39 %, respectively), which were further hydrolyzed with 0.01 M NaOH to give the target compounds (**5a**, **5b**)[14, 15] (96% and 21%, respectively) (Scheme 4).



a) EtOH, *p*-TsOH (61%), b) 2-O-α-4,7,8,9-tetra-*O*-acetyl-*N*-acetylneuraminyl acetic acid, DCC, HOBt, NMM (83%), c) MeONa (90%), d) 0.01M aq. NaOH (96%), e) 2-*O*-β-4,7,8,9-tetra- *O*-acetyl-*N*-acetylneuraminyl acetic acid, DCC, HOBt, NMM(53%), f) MeONa (39%), g) 0.01M aq. NaOH (21 %).

Scheme 4 Synthetic routes of CMP-sialic acid mimics (**5a**, **5b**)

The inhibitory activities of **5a** and **5b** toward α2,6-sialyltransferase from rat liver (purchased from Sigma Co. Ltd., S-2769) were preliminary assayed, and found to be *ca.* 500 μM in the value of IC_{50}.

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We thank Professors T. Nohara and T. Ikeda (Kumamaoto University) for their kind mass spectra measurements of **11a**, **11b**, and Professor T. Hamamoto (Jichi Medical School) for his inhibitory assay of **5a**, **5b** toward the sialyltransferase.

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2. For example, In *Handbook of Glycosyltransferases and Related Genes*, ed. by N. Taniguchi, K. Honke, and M. Fukuda, Springer-Verlag, 2002.
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4. For example, nikkomycin Z, an analog of UDP-*N*-acetyl-D-glucosamine, also has a *C*-terminal peptide bond for the phosphate group and exhibits potent inhibitory activity toward a *N*-acetyl-D-glucosaminyltransferase. See also (a) W. A. Konig, H. Hahn, R. Rathman, W. Hass, A. Keckeisen, H. Hagenmaier, C. Bormann, W. Dehler, R. Kurth, and H. Zahner, *Liebig Ann. Chem.*, 1986, 407. (b) A. K. Saksena, R. G. Lovey, V. M. Girijavallabhan, H. Guzik, and A. K. Ganguly, *Tetrahedron Lett.*, 1993, **34**, 3267.
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7. I. Kijima-Suda, Y. Miyamoto, S. Toyoshima, M. Itoh, and T. Osawa, *Cancer Res.*, 1986, **46**, 858.
8. C_{30} reverse phase column was purchased from Nomura Chemicals Co., Ltd., under the trade name “Develosil 30”.
9. **6** : ^{1}H-NMR ($CDCl_3$) δ: 1.20 (6H, t, *J* = 7.0 Hz), 3.53, 3.76 (each 2H, q, *J* = 7.0 Hz), 3.76 (2H, d, *J* = 5.0 Hz), 4.60 (1H, t, *J* = 5.0 Hz), 7.38 (1H, d, *J* = 6.0 Hz)。
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11. W. Konig and R. Geiger, In *Peptides*; ed. by E. Scoffone, North-Holland Pub. Co., Amsterdam, 1969, 17.
12. **11a** : ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, *J* = 7.5 Hz, $CO_2CH_2CH_3$), 1.97 (1H, br t, *J* = 13.0 Hz, H-3*ax*), 1.78, 1.94, 2.02, 2.06 (each 3H, s, 5xAc), 2.65 (1H, dd, *J* = 4.5, 12.0 Hz, H-3*eq*), 3.57 (1H, dd, A part of AB type *J* = 8.5, 14.0 Hz, H-γ’), 3.79 (3H, s, CO_2CH_3), 3.97 (1H, t, *J* = 10.5 Hz, H-5), 3.98 (1H, dd, *J* = 15.5 Hz, -OCH_2CO-) 4.00 (1H, dd, *J* = 5.5, 11.0 Hz, H-9), 4.05 (1H, dd, B part of AB

type, *J* = 4.0, 14.0 Hz, H-γ), 4.11 (1H, dd, *J* = 2.5, 11.0 Hz, H-9'), 4.18 (4H, m, H-β, H-6, and -OC*H*$_2$CH$_3$), 4.24 (1H, d, *J* = 15.5 Hz, -OC*H*$_2$CO-), 4.54 (1H, d, *J* = 5.0 Hz, H-α), 4.82 (1H, ddd, *J* = 4.5, 10.5, 13.0 Hz, H-4), 5.25 (1H, dd, *J* = 2.5, 9.0 Hz, H-7), 5.43 (1H, ddd, *J* = 2.5, 5.5, 11.0 Hz, H-8), 7.71 (1H, d, *J* = 6.0 Hz, 5-FU H-6). FAB MS: found 807.3, Calcd for $C_{32}H_{43}N_4O_{19}F$, 806.7.

13. **11b** : ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, t, *J* = 7.5 Hz, -OC*H*$_2$CH$_3$), 1.83 (1H, t, *J* = 13.0 Hz, H-3*ax*), 1.77, 1.90, 1.92, 1.95, 2.03 (each 3H, s, 5xAc), 2.50 (1H, dd, *J* = 5.0, 13.0 Hz, H-3*eq*), 3.55 (1H, dd, *J* = 9.0, 14.5 Hz, H-γ), 3.84 (1H, br t, *J* = 10.0 Hz, H-5), 3.93 (1H, dd, *J* = 7.0 Hz, 12.0 Hz, H-9), 3.90-4.20 (9H, m, H-β, H-γ', H-6, -OC*H*$_2$CH$_3$ x2, and -OC*H*$_2$CO-), 4.56 (1H, d, *J* = 5.5 Hz, H-α), 4.60 (1H, dd, *J* = 2.5, 12.0 Hz, H-9'), 5.15 (1H, m, *J* = 5.0 Hz, H-8), 5.26 (1H, ddd, *J* = 4.5, 10.0, 12.5 Hz, H-4), 5.31 (1H, dd, *J* = 1.5, 5.0 Hz, H-7), 7.72 (1H, d, *J* = 6.0, 5-FU H-6). FAB MS: found 807.3, Calcd for $C_{32}H_{43}N_4O_{19}F$, 806.7.
14. **5a** : ^{1}H-NMR (D_2O) δ: 1.64 (1H, t, *J* = 12.5 Hz, H-3ax), 1.83 (3H, s, Ac), 2.60 (1H, dd, *J* = 5.0, 12.5 Hz, H-3eq), 4.10-4.22 (2H, m, H-α and γ), 3.30-4.10 (10H, m), 4.25 (1H, dt, *J* = 9.0, 3.0 Hz, H-β), 7.72 (1H, d, *J* = 6.0 Hz, 5FU H-6).
15. **5b** : ^{1}H-NMR (D_2O) δ: 1.53 (1H, dd, J = 11.0, 13.0 Hz, H-3*ax*), 1.87 (3H, s, Ac), 2.29 (1H, dd, *J* = 5.0, 13.0 Hz, H-3*eq*), 3.30-3.95 (9H, m), 4.05 (2H, m), 4.18 (1H, dd, *J* = 4.5, 14.0 Hz, H-γ), 4.22 (1H, dd, *J* = 3.0, 9.0 Hz, H-β), 7.46 (1H, d, *J* = 6.0 Hz, 5-FU H-6).