Design and Synthesis of Peptide Mimetics of GDP-Fucose: Targeting Inhibitors of Fucosyltransferases

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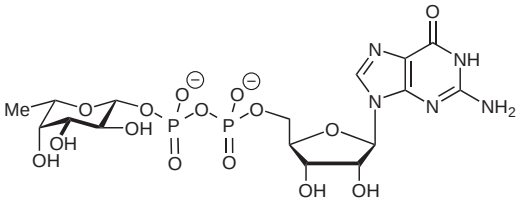
Abstract: Novel peptide mimetics of GDP-fucose were designed and synthesized targeting inhibitors of the fucosyltransferases that transfer L-fucose from GDP-fucose to oligosaccharides, on the basis of the background that nikkomycin Z, a peptide mimetic of UDP-*N*-acetylglucosamine, shows potent inhibitory activity toward an *N*-acetylglucosamine transfer enzyme. The synthetic routes of the GDP-fucose mimetics take advantage of an enzymatic aldol reaction catalyzed by L-threonine aldolase to prepare the guanine carrying β-hydroxy-α-L-amino acid, a key synthetic intermediate.

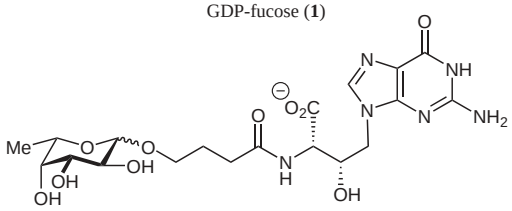
Key words: aldol reactions, amino acids, enzymes, inhibitors, nucleotides

A group of fucosyltransferases that transfer an L-fucose unit from guanosine 5′-diphospho-β-L-fucose (GDP-fucose, **1**) to D-galactose or *N*-acetyl-D-glucosamine residues of glycoconjugates forms a family of glycosyltransfer enzymes, which utilize sugar nucleotides as the substrates of glycosyl donors. The biological functions of the fucosyltransferases, which afford L-fucosylated oligosaccharides, e.g., Le^y and Le^b, have been revealed in the last decade.[1] The activity of an α-1,2-fucosyltransferase, for instance, affects the immune responses to bacterial infection through the mucous membrane since a glycoprotein related to the immunity is activated to secrete into the mucous liquid by the fucosylation.[2] Moreover, the activities of the fucosyltransferases including the above enzyme are, in general, hereditary. Therefore, the selective inhibitors and/or inducers of the fucosyltransferases are promising candidates targeting custom-made medicine that is usable for specific patients or their families having the same gene. However, only a few tediously prepared inhibitors have been reported so far.[3]

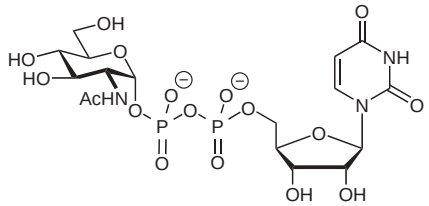
Targeting useful and easily available inhibitors of the fucosyltransferases, we designed novel peptide mimetics of GDP-fucose (**2a**, **2b**) on the basis of the fact that an *N*-acetylglucosamine transfer enzyme, which utilizes uridine 5′-diphospho-*N*-acetyl-α-D-glucosamine (UDP-GlcNAc, **4**) as the substrate, was potently inhibited by nikkomycin Z (**3**),[4] a mimetic of **4** (Figure 1).[5]



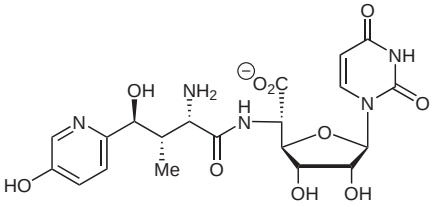
GDP-fucose (**1**)



Peptide mimetic of GDP-fucose
(**2a**: α-isomer, **2b**: β-isomer)



UDP-*N*-acetyl-D-glucosamine (**4**)



nikkomycin Z (**3**)

Figure 1 Structure of sugar nucleotids and their mimetics

Upon designing the GDP-fucose analogs (**2a**, **2b**), we focused on the fact that the C-terminal's peptide bond that also exists in the structure of **3** can be a bioisoster of the diphosphate part of **1** as well as that of **4** in terms of having the negative charge and high polarity. In addition, the hydroxyl groups at the β-position of the guanine-carrying amino acid parts in **2a** and **2b** were expected to behave as the 2′- and/or 3′-hydroxyl group of **1**.

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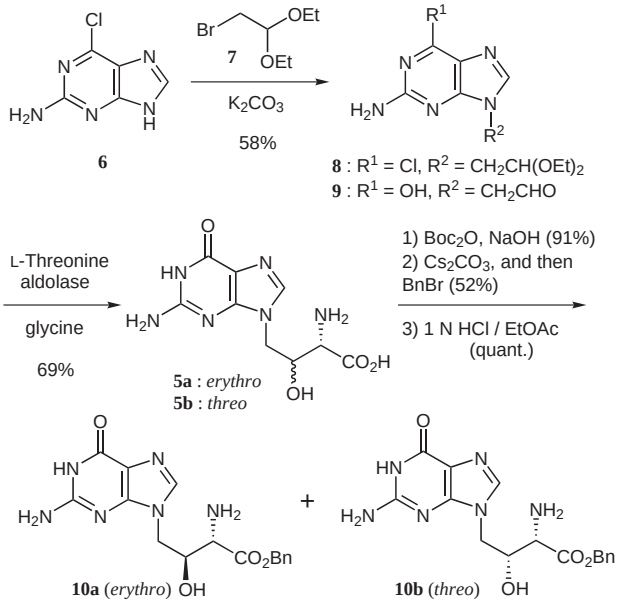
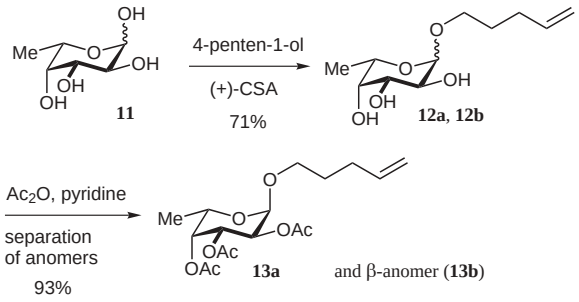


Scheme 1 Preparation of guanine carrying amino acids **5a**,**b** and their benzyl esters **10a**,**b**

In the present paper, we report the facile synthesis of **2a** and **2b**, in which the L-threonine aldolase-catalyzed reaction, an enzymatic aldol reaction,[6] is advantageous in preparing the guanine carrying β-hydroxy-α-L-amino acid with the L-*erythro* configuration (**5a**)[7], a key synthetic intermediate.

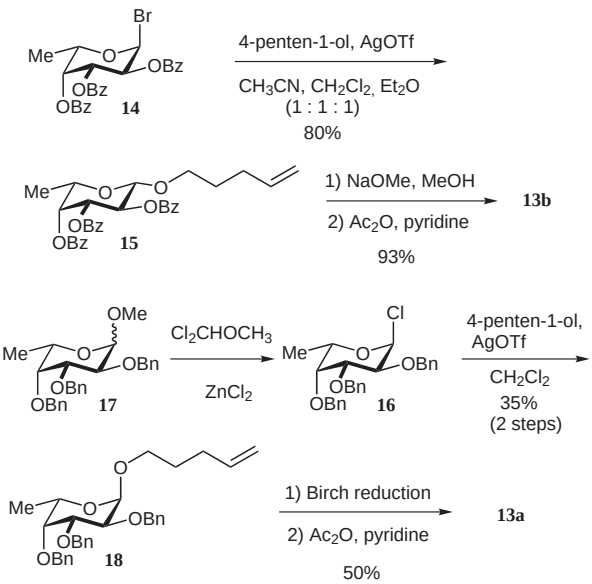
2-Amino-6-chloropurine (**6**) and 2-bromo-1,1-diethoxyethane (**7**) were heated in the presence of potassium carbonate at 100 °C in DMF to give α-(2-amino-6-chloroprin-9-yl)acetaldehyde diethylacetal (**8**, 58%). The diethyl acetal **8** was hydrolyzed with 1 M hydrochloric acid to aldehyde **9** and the reaction mixture was diluted with Tris-buffer (pH 7.5, 200 mM). After adding glycine (50 equiv to **9**) and L-threonine aldolase solution (450 units/200 mL) from *Candida humicola* (AKU 4586), the reaction mixture was incubated at 30 °C for 24 hours. Heating the reaction medium at 100 °C for 30 minutes to terminate the reaction, followed by celite filtration, evaporation in vacuo, and purification by column chromatography on ODS eluted with hot water (50 °C) afforded guanine carrying β-hydroxy-α-L-amino acid (69%, 2 steps), albeit a mixture of *erythro* (**5a**) and *threo* (**5b**) isomers.[8] Separation of these stereoisomers was attained by converting to benzyl esters **10a**,**b** in 3 steps [protection of the amino group of **5a** and **5b** with *t*-butoxycarbonyl (Boc) group, making cesium salt of the carboxylic acid, reaction with benzyl bromide, and treatment with 1 M HCl/EtOAc], and the following purification by silica gel column chromatography ($CHCl_3$:MeOH:H_2O = 8:1.7:0.1).

On the other hand, L-fucose (**11**) was derived to 1-*O*-(4-pentenyl)-α-L-fucose (**12a**) and/or 1-*O*-(4-pentenyl)-β-L-fucose (**12b**), since the terminal olefins of these L-fucosides can be derived with oxidative agents to carboxylic acid to form an amide bond with the α-amino group of **10a**. L-Fucose (**11**) was first treated with 4-pentenol in the presence of camphor sulfonic acid to afford a mixture of α-L-fucoside (**12a**) and β-L-fucoside (**12b**, 71%), and L-fucofuranoside (18%) albeit obtained the yield of L-fucopyranoside (**12a**,**b**) was no higher than that of the literature.[9] Acetylation of the L-fucopyranoside mixture made the separation of the two isomers possible by silica gel chromatography (*n*-hexane:EtOAc = 7:1) to afford 1-*O*-pentenyl 2,3,4-tri-*O*-acetyl-α-L-fucoside (**13a**)[10] and 1-*O*-pentenyl 2,3,4-tri-*O*-acetyl-β-L-fucoside (**13b**)[11] (93%) in the ratio of 65:35.

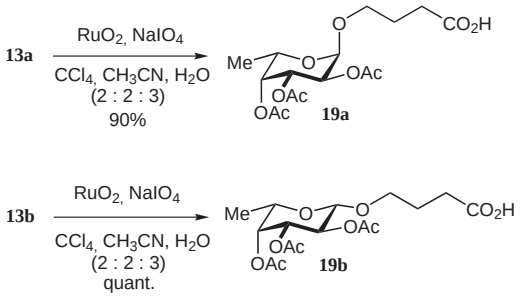


Scheme 2 Preparation of 1-*O*-pentenyl fucosides (**13a**,**b**)

Neighboring group participation effect of the benzoyl group at the C-2 position was used to obtain 1-*O*-pentenyl β-L-fucoside derivative as a predominant product. 1α,2,3,4-Tetra-*O*-benzoyl-L-fucose was, namely, treated with HBr/HOAc in acetic anhydride and dichloromethane to afford 1-bromo-2,3,4-tri-*O*-benzoyl-L-fucose (**14**).[12] 4-Penten-1-ol was L-fucosylated with **14** in the presence of silver triflate in a relatively polar mixed solvent composed of acetonitrile, dichloromethane, and diethyl ether (1:1:1) to give 1-*O*-(4-pentenyl) 2,3,4-tri-*O*-benzoyl-β-L-fucoside (**15**, 80%, 2 steps). Debenzoylation with sodium methoxide followed by acetylation gave **13b** (93%).



Scheme 3 Respective preparation of α- and β-L-fucosides (**13a**,**b**)



Scheme 4 Preparation of fucose carboxylates (**19a**,**b**)

On the other hand, 1-*O*-pentenyl α-L-fucoside derivative can be solely prepared by using an anomeric effect in a non-polar solvent. 4-Penten-1-ol was L-fucosylated with 1-chloro-2,3,4-tri-*O*-benzyl-α-L-fucose (**16**), prepared from 1-*O*-methyl 2,3,4-tri-*O*-benzyl-L-fucoside (**17**) with dichloromethoxy-methane/$ZnCl_2$ in $CHCl_3$, to afford 1-*O*-(4-pentenyl) 2,3,4-tri-*O*-benzyl-α-L-fucoside (**18**, 2 steps, 50%).[13] Debenzylation of **18** under Birch reduction conditions followed by conventional acetylation gave **13a** (50%).

Both α- and β-L-fucosides (**13a**,**b**) were respectively treated with ruthenium tetroxide[14] to afford 1-*O*-(1-carboxylbutyl-4-oxyl) 2,3,4-tri-*O*-acetyl-α-L-fucoside (**19a**) and 1-*O*-(1-carboxylbutyl-4-oxyl) 2,3,4-tri-*O*-acetyl-β-L-fucoside (**19b**) in excellent yields.

Finally, both of the *erythro* isomer of guanine carrying amino acid (**10a**) corresponding to the nucleotide part, and the sugar units (**19a** and **19b**) were coupled with DCC/HOBt/NMM to give fully protected GDP-fucose analogs (**20a**,**b**)[15,16] (43% and 83%, respectively). Cleavage of the acetyl groups in **20a** and **20b** with sodium methoxide in methanol smoothly proceeded and was also accompanied with ester exchange from benzyl to methyl ester, which was quantitatively hydrolyzed in aqueous sodium hydroxide to afford the target molecules **2a**[17] and **2b**[18].

Neither **2a** nor **2b** unfortunately showed inhibitory activity toward fucosyltransferases (FUT-3, FUT-6),[19,20] however, this result and structure comparison of **2a**,**b** with **3** provided useful information in that both the hydroxyl and amino groups on the γ-hydroxybutyric chain were found to be indispensable for the incorporation into the glycosyltransferases by forming a chelate with Mn^{2+} or Mg^{2+}, the essential metal ion for glycosyltransferase-catalyzed reactions. Synthesis of GDP-fucose analogs carrying the hydroxyl and amino groups on the γ-hydroxybutyric linker is now in progress.

Acknowledgment

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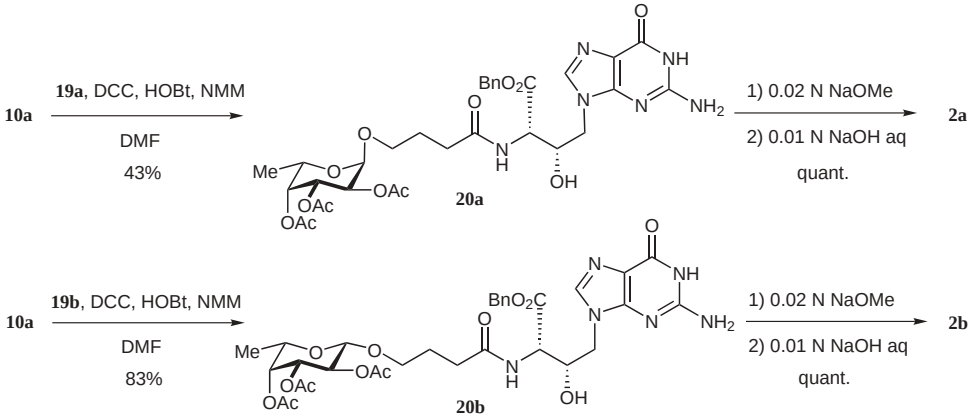
References

(1) For example, see:Taniguchi, N.; Honke, K.; Fukuda, M. *Handbook of Glycosyltransferases and Related Genes*; Springer-Verlag, **2002**, 205.
(2) Stapleton, A.; Stround, M. R.; Hakomori, S.; Stamm, W. E. *Infect. Immun.* **1998**, *66*, 3856.
(3) Compain, P.; Martin, O. R. *Bioorg. Med. Chem.* **2001**, *9*, 3077.
(4) (a) König, W. A.; Hahn, H.; Rathman, R.; Hass, W.; Keckeisen, A.; Hagenmaier, H.; Bormann, C.; Dehler, W.; Kurth, R.; Zähner, H. *Liebigs Ann. Chem.* **1986**, 407. (b) Saksena, A. K.; Lovey, R. G.; Girijavallabhan, V. M.; Guzik, H.; Ganguly, A. K. *Tetrahedron Lett.* **1993**, *34*, 3267.
(5) Dahn, U.; Hagenmeier, H.; Höhne, H.; König, W. A.; Wolf, G.; Zähner, H. *Arch. Microbiol.* **1976**, *107*, 143.
(6) (a) Vassilev, V. P.; Uchiyama, T.; Kajimoto, T.; Wong, C. H. *Tetrahedron Lett.* **1995**, *36*, 4081. (b) Vassilev, V. P.; Uchiyama, T.; Kajimoto, T.; Wong, C. H. *Tetrahedron Lett.* **1995**, *36*, 5063. (c) Shibata, K.; Shingu, K.; Vassilev, V. P.; Nishide, K.; Fujita, T.; Node, M.; Kajimoto, T.; Wong, C. H. *Tetrahedron Lett.* **1996**, *37*, 2791. (d) Fujii, M.; Miura, T.; Kajimoto, T.; Ida, Y. *Synlett* **2000**, 1046. (e) Nishide, K.; Shibata, K.; Fujita, T.; Kajimoto, T.; Wong, C. H. *Heterocycles* **2000**, *52*, 1191. (f) Miura, T.; Kajimoto, T. *Chirality* **2001**, 577.



Scheme 5 Synthetic route of peptidic mimetics of GDP-fucose (**2a**,**b**)

(7) Miura, T.; Fujii, M.; Shingu, K.; Koshimizu, I.; Naganoma, J.; Kajimoto, T.; Ida, Y. *Tetrahedron Lett.* **1998**, *39*, 7313.
(8) (a) The absolute stereochemistry of the α-carbon was determined to be L-configuration by the observation that **5a** and **5b** were not substrates of the D-amino acid oxidase but those of the L-amino acid oxidase. The *erythro*- and *threo*-configuration was determined by converting to the corresponding oxazolidones treating with ethyl chlorocarbonate in 1 M aq NaOH. See also:Saeed, A.; Yong, D. W. *Tetrahedron* **1992**, *48*, 2507. (b) Kaneko, T.; Inui, T. *Bull. Chem. Soc. Jpn.* **1961**, *82*, 1075.
(9) Udodong, U. E.; Rao, C. S.; Fraser-Reid, B. *Tetrahedron Lett.* **1992**, *48*, 4713.
(10) Compound **13a**: ^{1}H NMR ($CDCl_3$): δ = 1.14 (d, 3 H, *J* = 6.5 Hz, Me-6), 1.69 (m, 2 H), 2.00, 2.08, 2.17 (s, each 3 H, 3 × Ac), 2.13 (m, 2 H), 3.41 (dt, 1 H, *J* = 10.0, 6.5 Hz, A part of AB type), 3.69 (dt, 1 H, *J* = 10.0 Hz, B part of AB type), 4.16 (br q, 1 H, *J* = 6.5 Hz, H-5), 4.95–5.02 (m, 2 H), 5.05 (d, 1 H, *J* = 4.0 Hz, H-1), 5.11 (dd, 1 H, *J* = 4.0, 10.5 Hz, H-2), 5.30 (dd, 1 H, *J* = 1.0, 3.5 Hz, H-4), 5.35 (dd, 1 H, *J* = 3.5, 10.5 Hz, H-3), 5.81 (ddt, 1 H, *J* = 10.5, 17.0, 7.5 Hz). ^{13}C NMR ($CDCl_3$): δ = 15.8, 20.6 × 2, 20.7, 28.4, 30.0, 64.2, 67.5, 68.0, 68.2, 71.1, 96.0, 115.0, 137.7, 170.0, 170.4, 170.6.
(11) Compound **13b**: ^{1}H NMR ($CDCl_3$): δ = 1.23 (d, 3 H, *J* = 6.5 Hz, Me-6), 1.70 (m, 2 H), 2.00, 2.06, 2.18 (s, each 3 H, 3 × Ac), 2.11 (m, 2 H), 3.49 (m, 1 H), 3.81 (br q, 1 H, *J* = 6.5 Hz, H-5), 3.92 (dt, 1 H, *J* = 9.5, 6.0 Hz), 4.43 (d, 1 H, *J* = 7.5, H-1), 4.95–5.05 (m, 2 H), 5.02 (dd, 1 H, *J* = 3.0, 10.5 Hz, H-3), 5.20 (dd, 1 H, *J* = 7.0, 10.5 Hz, H-2), 5.24 (br d, 1 H, *J* = 3.0 Hz, H-4), 5.80 (ddt, 1 H, *J* = 10.0, 17.0, 6.5 Hz).
(12) Ichikawa, Y.; Sim, M. M.; Wong, C. H. *J. Org. Chem.* **1992**, *57*, 2943.
(13) Iversen, T.; Bundle, D. R. *J. Org. Chem.* **1981**, *46*, 5389.
(14) Carlsen, P. H. J.; Katzuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.
(15) Compound **20a**: ^{1}H NMR (CD_3OD): δ = 0.99 (d, 3 H, *J* = 6.5 Hz, Me-6), 1.80 (m, 2 H), 1.85, 1.92, 2.04 (s, each 3 H, 3 × Ac), 2.30 (t, 2 H, *J* = 7.5 Hz), 3.33 (dt, 1 H, *J* = 10.0, 6.0 Hz), 3.60 (dt, 1 H, *J* = 10.0, 6.0 Hz), 4.01 (dd, 1 H, *J* = 9.0, 15.0 Hz, H-γ), 4.07 (br q, 1 H, *J* = 6.5 Hz, H-5), 4.14 (dd, 1 H, *J* = 3.0, 15.0 Hz, H-γ′), 4.16 (m, 1 H, H-β), 4.53 (d, 1 H, *J* = 5.5 Hz, H-α), 4.88 (d, 1 H, *J* = 3.5 Hz, H-1), 4.94 (dd, 1 H, *J* = 3.5, 11.0 Hz, H-2 or H-3), 5.05, 5.08 (d, each 1 H, AB type, *J* = 13.0 Hz, C*H*$_2$Ph), 5.15 (dd, 1 H, *J* = 1.0, 3.5 Hz, H-4), 5.20 (dd, 1 H, *J* = 3.5, 11.0 Hz, H-2 or H-3), 7.25 (m, 5 H, Ph), 7.53 (s, 1 H, guanine H-8). FAB MS: Calcd for $C_{32}H_{40}N_6O_{13}$: 716.3. Found: 717.4.
(16) Compound **20b**: ^{1}H NMR (CD_3OD): δ = 1.06 (d, 3 H, *J* = 6.5 Hz, Me-6), 1.76 (m, 2 H), 1.84, 1.94, 2.04 (s, each 3 H, 3 × Ac), 2.25 (t, 2 H, *J* = 8.0 Hz), 3.45 (dt, 1 H, *J* = 10.0, 6.0 Hz), 3.74 (dt, 1 H, *J* = 10.0, 6.0 Hz), 3.81 (br q, 1 H, *J* = 6.5 Hz, H-5), 4.03 (dd, 1 H, *J* = 8.5, 14.0 Hz, H-γ), 4.16 (dd, 1 H, *J* = 4.0, 14.0 Hz, H-γ′), 4.19 (m, 1 H, H-β), 4.44 (d, 1 H, *J* = 7.5 Hz, H-1), 4.51 (d, 1 H, *J* = 5.5 Hz, H-α), 4.94 (dd, 1 H, *J* = 7.5, 10.5 Hz, H-2), 4.98 (dd, 1 H, *J* = 3.5, 10.5 Hz, H-3), 5.06, 5.09 (d, each 1 H, AB type, *J* = 12.5 Hz, C*H*$_2$Ph), 5.11 (dd, 1 H, *J* = 1.0, 3.5 Hz, H-4), 7.30 (m, 5 H, Ph), 7.56 (s, 1 H, guanine H-8). FAB MS: Calcd for $C_{32}H_{40}N_6O_{13}$: 716.3. Found: 717.4.
(17) Compound **2a**: ^{1}H NMR (D_2O): δ = 1.01 (d, 3 H, *J* = 6.5 Hz, Me-6), 1.73 (m, 2 H), 2.24 (m, 2 H), 3.34 (m, 1 H), 3.54 (m, 1 H), 3.59 (dd, 1 H, *J* = 4.0, 10.0 Hz, H-2), 3.62 (br d, 1 H, *J* = 3.5 Hz, H-4), 3.70 (dd, 1 H, *J* = 3.5, 10.0 Hz, H-3), 3.88 (br q, 1 H, *J* = 6.5 Hz, H-5), 3.91 (dd, 1 H, *J* = 8.0, 14.5 Hz, H-γ), 4.04 (dd, 1 H, *J* = 6.0, 14.5 Hz, H-γ′), 4.18 (d, 1 H, *J* = 3.0 Hz, H-α), 4.37 (m, 1 H, H-β), 4.71 (d, 1 H, *J* = 4.0 Hz, H-1), 7.63 (s, 1 H, guanine H-8). MALDI-TOF MS: Calcd for $C_{19}H_{28}N_6O_{10}$ + Na^+: 523.2. Found: 523.1.
(18) Compound **2b**: ^{1}H NMR (D_2O): δ = 1.01 (d, 1 H, *J* = 6.5 Hz, Me-6), 1.72 (m, 2 H), 2.23 (m, 2 H), 3.24 (dd, 1 H, *J* = 7.5, 10.0 Hz, H-2), 3.38 (dd, 1 H, *J* = 3.5, 10.0 Hz, H-3), 3.46 (dt, 1 H, *J* = 10.5, 6.5 Hz, A part of AB type), 3.48–3.55 (m, 2 H, H-4 and H-5), 3.72 (dt, 1 H, *J* = 10.5, 6.5 Hz, B part of AB type), 3.95–4.12 (m, 3 H, H-β and H_2-γ), 4.13 (d, 1 H, *J* = 7.5 Hz, H-1), 4.19 (d, 1 H, *J* = 5.5 Hz, H-α), 7.62 (s, 1 H, guanine H-8). FAB MS: Calcd for $C_{19}H_{28}N_6O_{10}$ + Na^+: 523.2. Found: 523.2.
(19) Nishihara, S.; Nakazato, M.; Kudo, T.; Kimura, H.; Ando, T.; Narimatsu, H. *Biochem. Biophys. Res. Commun.* **1993**, *190*, 42.
(20) The assay was performed in 50 mM cacodylate buffer (pH 6.8) containing 5 mM ATP, 10 mM L-Fuc, 25 mM $MnCl_2$, 15 mM acceptor substrate, Galβ1-3GlcNAcβ1-3Galβ1-4Glc-2-aminobenzamide (for FUT 3) or Galβ1-4GlcNAcβ1-3Galβ1-4Glc-2-aminobenzamide (for FUT 6), 75 μM donor substrate GDP-Fuc, and **2a** or **2b** (0 mM for the positive control; 0.75 mM, and 7.5 mM respectively for the inhibitory assay). After incubation at 37 °C for 2 h in the presence of the fucosyltransferases (FUT 3 or FUT 6), the enzyme reaction was terminated by heating at 97 °C for 5 min followed by adding H_2O. After centrifugation of the reaction mixture, in order to detect the fucosylated products and estimate their amounts, each supernatant was filtered and subjected to reverse-phase HPLC analysis on TSK-gel ODS-80Ts QA column (4.6 × 250 mm; Tosoh, Tokyo, Japan) and eluted with 20 mM ammonium acetate buffer (pH 4.0) containing 7% MeOH at flow rate of 1.0 mL/min at 50 °C, with monitoring by a fluorescence spectrophotometer (JASCO FP-920; Nihon Bunkoh, Tokyo, Japan).