ARTICLE

OBC
www.rsc.org/obc

Chemo-enzymatic synthesis of fluorinated 2-*N*-acetamidosugar nucleotides using UDP-GlcNAc pyrophosphorylase

Fei Feng,[a] Kiyoshi Okuyama,[b] Kenichi Niikura,[a] Takashi Ohta,[a] Reiko Sadamoto,[d] Kenji Monde,[a] Toshitada Noguchi[b] and Shin-Ichiro Nishimura *[a,c]

[a] *Laboratory for Bio-Macromolecular Chemistry, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 001-0021, Japan*

[b] *Bioproducts Laboratory, Biochemicals Division, YAMASA Corporation, Chiba 288-0056, Japan*

[c] *National Institute of Advanced Industrial Science and Technology, Sapporo 062-8517, Japan*

[d] *Shionogi Laboratory of Biomolecular Chemistry, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 001-0021, Japan. E-mail: shin@glyco.sci.hokudai.ac.jp*

Received 24th February 2004, Accepted 16th April 2004
First published as an Advance Article on the web 11th May 2004

Two non-natural fluorinated 2-*N*-acetamidosugar nucleotides, uridine 5′-diphosphate (UDP) 2-acetamido-2,4-dideoxy-4-fluoro-α-D-glucopyranose (UDP-4-FGlcNAc) **1** and its galacto isomer (UDP-4-FGalNAc) **2**, were enzymatically constructed by treating chemically synthesized fluorinated 2-*N*-acetamidosugar 1-phosphates as the donor with UDP 2-acetamido-2-deoxy-α-D-glucopyranose pyrophosphorylase in the presence of uridine 5′-triphosphate (UTP).

Introduction

N-Acetylglucosamine (GlcNAc) and *N*-acetylgalactosamine (GalNAc) are common *N*-acetyl amino sugars found ubiquitously in nature and in the oligosaccharide chains of glycoconjugates, and they play important roles in biological recognition processes. For example, it was suggested that β1–6 branched GlcNAc residues of the glycans of cell-surface proteins induce malignant alterations and cancer metastasis.[1] These *N*-acetyl amino sugars are derived and transferred from the corresponding UDP-GlcNAc or UDP-GalNAc into the non-reducing terminus of the oligosaccharide chains by glycosylation reactions with glycosyltransferases. Therefore, glycosyltransferase inhibitors based on UDP-GlcNAc or UDP-GalNAc structures would be a candidate for novel anticancer drugs.

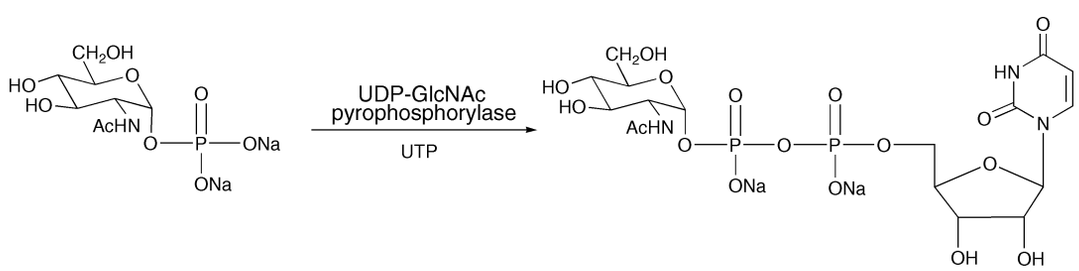
Because of the similarities in bond length and polarization between the C–OH and C–F groups, some of the fluorinated sugars act as glycosylation inhibitors; for example, 4-deoxy-4-fluoro-mannopyranose inhibited the synthesis of lipid-linked oligosaccharides[2] and 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose inhibited the metabolism of amino sugars.[3]

We focus our attention on the non-natural amino sugar nucleotides containing fluorine atoms at the C-4 position as inhibitors of glycosyltransferases. So far, fluoro-sugar nucleotides have been mainly chemically synthesized.[4] However, the substitution of an enzymatic step into the synthetic route offers advantages over conventional chemical synthesis in that there is less decomposition of the diphosphate, resulting in higher yields. A few reports have shown that pyrophosphorylase can simplify the synthetic route to non-natural sugar nucleotides. Pyrophosphorylase catalyzes the formation of the diphosphate bond of sugar nucleotides, and is responsible for the biosynthetic pathway of natural sugar nucleotides. C.-H. Wong *et al.* showed that UDP-glucose pyrophosphorylase catalyzes the transformation from 2-deoxy-2-fluoro-α-D-galactopyranosyl phosphate (2FGal-1-P) to UDP-(2-deoxy-2-fluoro)-galactose (UDP-2FGal).[5] Further, UDP-GlcNAc pyrophosphorylase catalyzes the synthesis of UDP-GlcNAc from GlcNAc-1-phosphate and UTP as shown in Scheme 1. Hartman and Coward reported only preliminary results indicating that this enzyme can transfer a non-natural substrate such as 5-FGlcNAc-1-phosphate,[6] however, the usefulness of the enzyme to create non-natural sugar nucleotides was not fully evaluated.

In the present study, we report a convenient and versatile approach to construct the fluorine-substituted 2-*N*-acetamidosugar nucleotides UDP-4-FGlcNAc **1** and UDP-4-FGalNAc **2**, by employing a combined chemical synthesis and UDP *N*-acetylglucosamine pyrophosphorylase.[7]

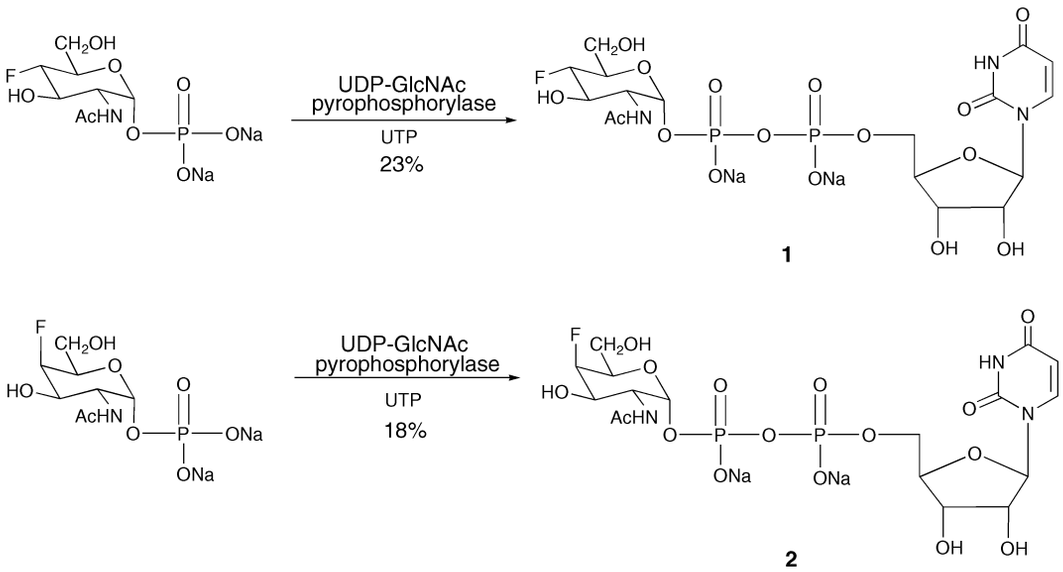
Results and discussion

As illustrated in Scheme 2, we considered that enzymatic reactions of 2-acetamido-2,4-dideoxy-4-fluoro-α-D-glucopyranose 1-phosphate (4-FGlcNAc-1-P) or 2-acetamido-2,4-dideoxy-4-fluoro-α-D-galactopyranose 1-phosphate (4-FGalNAc-1-P) and

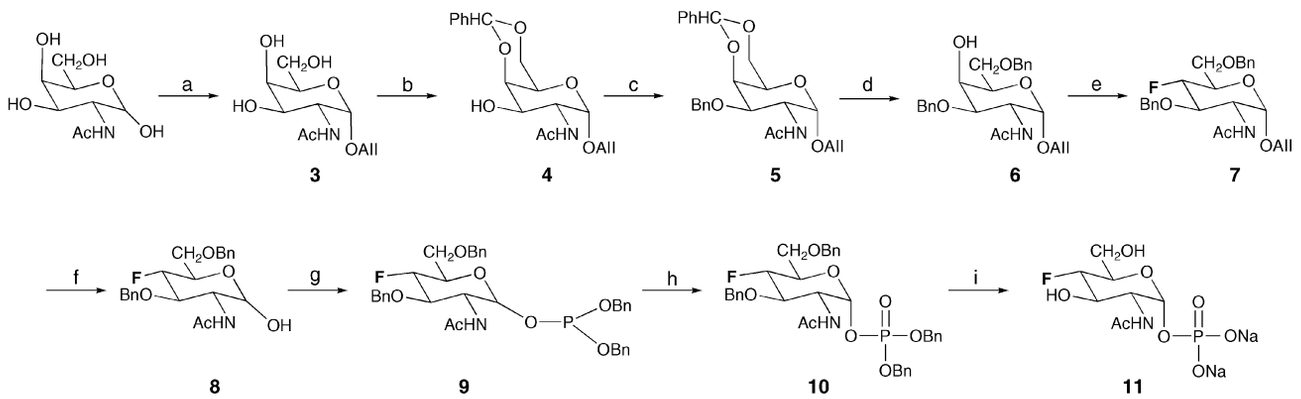


Scheme 1 Synthesis of UDP-GlcNAc from GlcNAc-1-P with UDP-GlcNAc pyrophosphorylase.

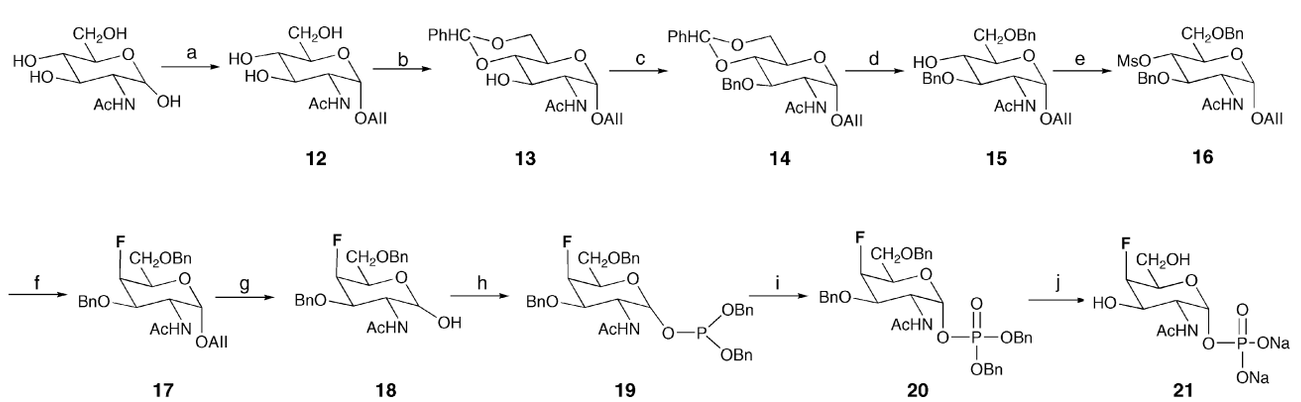
DOI: 10.1039/b402860k



Scheme 2 Enzymatic synthesis of UDP-4-FGlcNAc **1** and UDP-4-FGalNAc **2**.



Scheme 3 *Reagents and conditions*: a) $BF_3{\cdot}Et_2O$, allyl alcohol, 70 °C, 2 h, 80%; b) $PhCH(OMe)_2$, CSA, DMF, room temp., 12 h, 75%; c) BaO, $Ba(OH)_2$, BnBr, DMF, room temp., 12 h, 85%; d) $NaBH_3CN$, HCl–Et_2O, THF, room temp., 1 h, 64%; e) DAST, pyridine, CH_2Cl_2, −20 °C ⟶ room temp., 3 h, 44%; f) $PdCl_2$, NaOAc, 95% AcOH, room temp., 24 h, 87%; g) $(BnO)_2PNEt_2$, 1,2,4-triazole, CH_2Cl_2, reflux, 45 min, 54%; h) Bu^tOOH, THF, −10 °C, 1.5 h, 57%; i) 5% Pd/C, H_2 gas, EtOH–10% $NaHCO_3$, room temp., 1 d, 82%.



Scheme 4 *Reagents and conditions*: a) $BF_3{\cdot}Et_2O$, allyl alcohol, 130 °C, 2 h, 88%; b) $PhCH(OMe)_2$, CSA, DMF, room temp., 12 h, 81%; c) BaO, $Ba(OH)_2$, BnBr, DMF, room temp., 12 h, 76%; d) $NaBH_3CN$, HCl–Et_2O, THF, room temp., 1 h, 69%; e) MsCl, pyridine, −13 °C ⟶ 0 °C, 4 h, 92%; f) TBAF, CH_3CN, 100 °C, 60 h, 71%; g) $PdCl_2$, NaOAc, 90% AcOH, room temp., 13 h, 63%; h) $(BnO)_2PNEt_2$, 1,2,4-triazole, CH_2Cl_2, reflux, 45 min, 70%; i) Bu^tOOH, THF, −10 °C, 1.5 h, 54%; j) 5% Pd/C, H_2 gas, EtOH–10% $NaHCO_3$, room temp., 1 d, 87%.

uridine 5′-triphosphate (UTP) in the presence of UDP-GlcNAc pyrophosphorylase would greatly accelerate the practical synthesis of target novel sugar nucleotides **1** and **2**.

In order to develop an efficient enzyme-assisted synthetic route to fluorinated sugar nucleotides, we established chemical synthetic routes to two key intermediates, 4-FGlcNAc-1-P (**11**) and 4-FGalNAc-1-P (**21**) as their substrates (Schemes 3 and 4).

Using Lewis acid $BF_3{\cdot}Et_2O$ as a catalyst, heating the commercially available D-GalNAc or D-GlcNAc in allyl alcohol at 70 °C or 130 °C, respectively, for 2 h gave the desired α-isomer **3** or **12**[8] in good yields. We found the most suitable temperature for **3** was 70 °C. At all other temperatures the compounds decomposed resulting in low yields. Subsequent regioselective benzylidenation of the C-4 and C-6 positions was performed by using benzaldehyde dimethyl acetal and a catalytic amount of camphor-10-sulfonic acid (CSA) in dimethylformamide (DMF) to produce the partially unprotected saccharides **4** and **13** with a free 3-hydroxyl group. The fully protected derivatives **5** and **14** with a C-3 benzyl group were prepared following the previously reported procedures.[9] In order to introduce a free 4-hydroxyl

group, the benzylidene rings of **5** and **14** were opened regioselectively with sodium cyanotrihydroborate and hydrogen chloride in tetrahydrofuran[10] to afford the desired 6-*O*-benzyl ether having either a free axial (**6**) or equatorial (**15**) hydroxyl group in good yields without the formation of the respective isomeric 4-*O*-benzyl ethers. Interconversion reactions between gluco and galacto configurations at the C-4 position of **6** and **16** were selected to introduce fluorine atoms at these C-4 positions, with both axial and equatorial forms. Here we noticed that although the reaction of **6** using (diethylamino)sulfur trifluoride (DAST)[11] in the presence of pyridine in dichloromethane proceeded and gave **7** with an equatorial fluorine group in a satisfactory yield, fluorination of **15** could not be performed under similar conditions. Therefore, the 4-*O*-mesylation of **15** led to glycoside **16** with an appropriate leaving group. The subsequent nucleophilic attack by a fluorine ion generated from tetrabutylammonium fluoride (TBAF) against **16** was then carried out in refluxing acetonitrile to afford compound **17**, which had a fluorine atom with the desired axial configuration. As anticipated, the allyl moieties in compounds **7** and **17** remained intact throughout the synthetic manipulation described above, and were then deprotected using palladium chloride and sodium acetate in an aqueous acetic acid solution[12] to give 1-OH type intermediates **8** and **18** ($\alpha : \beta = 6 : 1$). The phosphorylation reaction of these hemiacetals with dibenzyl diethylphosphoramidite (DDP)[13] and triazole in dichloromethane yielded corresponding dibenzyl phosphites as anomeric mixtures **9** and **19** ($\alpha : \beta = 1 : 1$). They were then treated with *tert*-butylhydroperoxide (TBHP) in tetrahydrofuran at −10 °C to rapidly isomerize into the desired α-anomers of 1-phosphates **10** (57%) and **20** (54%), having coupling constants between the anomeric and C-2 protons of 3.20 and 3.36 Hz, respectively. This oxidation was unsuccessful when performed with hydrogen peroxide (H_2O_2) because of the labile O–P bond. We finally obtained the targeted substrates **11** and **21** as sodium salts in high yields by using de-*O*-benzylation of **10** and **20** under the usual conditions for hydrogenation.

Having fluorine-containing precursors **11** and **21** in hand, we focused our interest on their enzymatic transformation into the fluorinated sugar nucleotides **1** and **2**. In the present study, we utilized the UDP-GlcNAc pyrophosphorylase produced in *E. coli* JM109 following the method previously reported.[14] As anticipated, we found that compound **11**, which mimicked GlcNAc-1-P, was accepted as a substrate by this enzyme. After incubating **11** and UDP-GlcNAc pyrophosphorylase in 50 mM of Tris-HCl (pH 7.5) in the presence of excess UTP-3Na at 37 °C for 1 hour, we isolated compound **1** as a sodium salt from the reaction mixture by using a preparative HPLC and G-10 gel filtration (23%). Surprisingly, we discovered that compound **21**, bearing an axial C–F group at the C-4 position, could be transformed into compound **2** in 18% yield. This suggests that the present synthetic route using UDP-GlcNAc pyrophosphorylase reaction could become a convenient and versatile method for the preparation of a variety of fluorinated 2-*N*-acetamidosugar nucleotides.

Conclusion

We have developed a practical procedure for the preparation of fluorine-containing sugar nucleotides **1** and **2** by employing UDP-GlcNAc pyrophosphorylase. This method would have wide applications in the synthesis of non-natural sugar nucleotides for use as carbohydrate-based drugs.

Experimental

General procedures

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. $CHCl_3$, CH_2Cl_2, tetrahydrofuran, EtOH and MeOH were stored over 4 Å molecular sieves (MS) before use. Pyridine was stored over NaOH pellets. The 4 Å MS was dried under reduced pressure at 100 °C overnight before being used. NMR spectra were recorded at 400 MHz (JEOL λ) or 600 MHz (Bruker ADVANCE) for ^{1}H and 100 MHz for ^{13}C in a DMSO-d_6, D_2O, $CDCl_3$ solution with tetramethylsilane ($\delta_H = 0$) as an internal standard. Assignment of the ring-protons was made by first-order analysis of the spectra, and confirmed by H–H COSY spectra. Optical rotations were measured with a Perkin-Elmer Polarimeter 343 at 25 °C. Samples were dried *in vacuo* over P_2O_5 power before elemental analysis. Coupling constants are expressed in Hz. FAB and ESI-HR Mass analyses were carried out on JEOL JMS-HX110 and JMS-700TZ mass spectrometers. Chromatographic purifications were carried out using Wakogel C-200 (100–200 mesh) eluted with the given solvent mixture. Chemical reactions were monitored by thin-layer chromatography (TLC) on precoated plates of Merck $60F_{254}$ Silica Gel (layer thickness, 0.25 mm), and compounds were detected by spraying the plates with 10% H_2SO_4 in EtOH, and heating.

Enzyme preparation

E. coli JM109 cells carrying pTrc-glmU (JM109[pTrc-glmU]) were cultivated in 2×TY medium containing 0.1 mM isopropyl-β-D-galactopyranoside (IPTG). Cells were harvested by centrifugation and suspended in a buffer of 10 mM Tris-HCl (pH 7.5) containing 1 mM $MgCl_2$. Cells were disrupted by sonic oscillation, and the crude extract (CE) was prepared by removal of cell debris with centrifugation.

Enzyme assay

0.2 ml of assay mixture containing 50 mM Tris-HCl (pH 7.5), 1 mM UDP-GlcNAc, 5 mM $MgCl_2$, 3 mM sodium pyrophosphate, and 0.05–0.20 µl of CE was incubated at 37 °C for 5 min. The reaction mixture was heated in a boiling water bath for 3 min. The formed UTP in the reaction mixture was measured by HPLC. One unit of enzyme activity was defined as the amount forming 1 µmol of UTP per min under these conditions.

Allyl 2-acetamido-2-deoxy-α-D-galactopyranoside 3

To a solution of *N*-acetyl-D-galactosamine (442 mg, 2 mmol) in allyl alcohol (8 cm^3) was added a BF_3 diethyl ether complex (0.25 cm^3, 2 mmol), and the mixture was stirred for 2 h at 70 °C. Cooled to rt, the solvent was evaporated under reduced pressure. EtOH (5 cm^3) was added to dissolve the crude product, then diisopropyl ether was added to the flask to precipitate the product **3** (417 mg, 80%) as a white powder; $[\alpha]^{25}_D$ +199.8° (*c* 0.1 in MeOH); mp 191–192 °C (EtOH) (lit.,[9a] 193–194 °C); δ_H (400 MHz, DMSO-d_6) 7.58 (1H, d, *J* 8.39, NH), 5.93–5.84 (1H, m, CH=C), 5.30 (1H, dd, *J* 1.83, 17.24, C=CH_2), 5.14 (1H, dd, *J* 1.83, 10.53, C=CH_2), 4.69 (1H, d, *J* 3.51, H-1), 4.11–4.02 (2H, m, H-2, CH_2-C=C), 3.94–3.88 (1H, m, CH_2-C=C), 3.73 (1H, br d, *J* 2.90, H-4), 3.63 (1H, dd, *J* 2.90, 10.97, H-3), 3.59 (1H, br d, *J* 5.61, H-5), 3.53 (1H, dd, *J* 5.61, 10.53, H-6a), 3.49 (1H, dd, *J* 6.56, 10.53, H-6b), 3.39 (3H, br s, OH), 1.83 (3H, s, $COCH_3$); FAB-HRMS, *m/z* found $M+H^+$ 262.1317, $C_{11}H_{20}NO_6$ requires 262.1291.

Allyl 2-acetamido-2-deoxy-4,6-*O*-benzylidene-α-D-galactopyranoside 4

To a cooled solution (0 °C) of **3** (849 mg, 3.25 mmol) in DMF (11 cm^3) was added (±)-camphor-10-sulfonic acid (76 mg, 0.33 mmol) and benzaldehyde dimethyl acetal (0.70 cm^3, 4.55 mmol) dropwise. The solution was stirred for 12 h at rt, then the solvent was evaporated under reduced pressure to yield a pale yellow oil. $CHCl_3$–MeOH (6 : 1) was poured into the flask to dissolve the crude product, and 10 cm^3 water was added

to the flask and stirred for 5 min. Then 100 cm^3 hexane was added slowly to precipitate the product **4** (852 mg, 75%) as a white solid; $[\alpha]^{25}_D$ +168.5° (*c* 0.1 in MeOH); mp 220–221 °C (EtOH) (lit.,[9a] 223–225 °C); δ_H (400 MHz, DMSO-d_6) 7.69 (1H, d, *J* 8.06, NH), 7.51–7.48 (2H, m, ArH), 7.41–7.35 (3H, m, ArH), 5.93–5.84 (1H, m, CH=C), 5.59 (1H, s, PhCH), 5.32 (1H, dd, *J* 1.71, 17.33, C=CH_2), 5.16 (1H, dd, *J* 1.71, 10.50, C=CH_2), 4.82 (1H, d, *J* 3.42, H-1), 4.67 (1H, br s, OH-3), 4.18 (1H, br d, *J* 3.17, H-4), 4.14–3.93 (5H, m, H-2, H-6a, H-6b, CH_2-C=C), 3.85 (1H, dd, *J* 3.17, 11.23, H-3), 3.64 (1H, br s, H-5), 1.84 (3H, s, $COCH_3$); FAB-HRMS, *m*/*z* found $M+H^+$ 350.1586, $C_{18}H_{24}NO_6$ requires 350.1604.

Allyl 2-acetamido-2-deoxy-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranoside 5

To a cooled solution (0 °C) of **4** (2.14 g, 6.14 mmol) in DMF (40 cm^3) was added barium oxide (1.88 g, 12.3 mmol), barium hydroxide (1.94 g, 6.14 mmol) and benzyl bromide (1.46 cm^3, 12.3 mmol) dropwise. The solution was stirred for 12 h at rt. After filtration through a Celite pad, the solvent was evaporated under reduced pressure to yield a pale yellow oil. $CHCl_3$–MeOH (6 : 1) was poured into the flask to dissolve the crude product and then 100 cm^3 hexane was added slowly to precipitate the product **5** (2.29 g, 85%) as a white solid; $[\alpha]^{25}_D$ +158.0° (*c* 0.1 in MeOH); mp 235–237 °C (EtOH) (lit.,[9a] 238–241 °C); δ_H (400 MHz, DMSO-d_6) 7.86 (1H, d, *J* 8.55, NH), 7.45–7.23 (10H, m, ArH), 5.96–5.86 (1H, m, CH=C), 5.62 (1H, s, PhCH), 5.35 (1H, dd, *J* 1.71, 17.33, C=CH_2), 5.19 (1H, dd, *J* 1.71, 10.50, C=CH_2), 4.82 (1H, d, *J* 3.36, H-1), 4.65 (1H, d, *J* 11.60, $PhCH_2$), 4.53 (1H, d, *J* 11.60, $PhCH_2$), 4.48 (1H, br d, *J* 3.20, H-4), 4.26 (1H, ddd, *J* 3.36, 8.55, 11.44, H-2), 4.17–4.12 (1H, m, CH_2-C=C), 4.08 (2H, br s, H-6a,H-6b), 4.03–3.97 (1H, m, CH_2-C=C), 3.85 (1H, dd, *J* 3.20, 11.44, H-3), 3.67 (1H, br s, H-5), 1.85 (3H, s, $COCH_3$); FAB-HRMS, *m*/*z* found $M+H^+$ 440.2079, $C_{25}H_{30}NO_6$ requires 440.2073.

Allyl 2-acetamido-2-deoxy-3,6-di-*O*-benzyl-α-D-galactopyranoside 6

To a solution of **5** (878 mg, 2 mmol) in THF (24 cm^3) was added 4 Å MS (1 g) and $NaBH_3CN$ (2 g, 32 mmol), and the solution was stirred for 20 min at rt. Cooled to 0 °C, 2.0 M HCl–diethyl ether solution was added dropwise until the mixture attained pH 3 and then stirred for 1 h at rt. After filtration through a Celite pad, the solution was diluted with diethyl ether, washed with H_2O, aq. $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the product as a pale yellow oil, which was purified by silica chromatography (25 : 1 chloroform–MeOH) to give **6** (564 mg, 64%) as a white solid; $[\alpha]^{25}_D$ + 87.1° (*c* 0.1 in $CHCl_3$); mp 117–118 °C (ethyl acetate–hexane) (lit.,[9a] 120–122 °C); δ_H (400 MHz, DMSO-d_6) 7.75 (1H, d, *J* 8.85, NH), 7.38–7.23 (10H, m, ArH), 5.96–5.87 (1H, m, CH=C), 5.30 (1H, dd, *J* 1.71, 17.33, C=CH_2), 5.15 (1H, dd, *J* 1.71, 10.50, C=CH_2), 4.73 (1H, br s, OH-4), 4.71 (1H, d, *J* 3.66, H-1), 4.66 (1H, d, *J* 11.60, $PhCH_2$-3), 4.52 (2H, br s, $PhCH_2$-6), 4.45 (1H, d, *J* 11.60, $PhCH_2$-3), 4.28 (1H, ddd, *J* 3.66, 8.85, 11.29, H-2), 4.13–4.07 (1H, m, CH_2-C=C), 4.02 (1H, br s, H-4), 3.98–3.93 (1H, m, CH_2-C=C), 3.84 (1H, br t, *J* 5.95, H-5), 3.66–3.60 (2H, m, H-3, H-6a), 3.55 (1H, dd, *J* 6.87, 10.07, H-6b), 1.85 (3H, s, $COCH_3$); FAB-HRMS, *m*/*z* found $M+H^+$ 442.2207, $C_{25}H_{32}NO_6$ requires 442.2230.

Allyl 2-acetamido-2,4-dideoxy-3,6-di-*O*-benzyl-4-fluoro-α-D-glucopyranoside 7

To a cooled solution (−20 °C) of DAST (1.1 cm^3, 8.2 mmol) in dichloromethane (1 cm^3) was added dropwise a solution of **6** (454 mg, 1 mmol) in dichloromethane (2 cm^3). After stirring for 0.5 h at −20 °C, pyridine (663.2 cm^3, 8.2 mmol) was added and the mixture was stirred for 3 h at rt. Cooling to −10 °C, EtOH was added slowly. The solution was then diluted with ethyl acetate, washed with H_2O, aq. $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the crude product as a dark yellow oil, which was purified by silica chromatography (12 : 1 chloroform–ethyl acetate) to give **7** (204 mg, 44%) as a white solid; $[\alpha]^{25}_D$ +86.7° (*c* 0.1 in $CHCl_3$); mp 124–125 °C (ethyl acetate–hexane); (Found: C, 67.6; H, 6.7; N, 3.2. $C_{25}H_{31}NO_5F$ requires C, 67.7; H, 6.8; N, 3.1%); δ_H (400 MHz, $CDCl_3$) 7.38–7.27 (10H, m, ArH), 5.91–5.81 (1H, m, CH=C), 5.28 (1H, d, *J* 9.28, NH), 5.25 (1H, dd, *J* 1.71, 17.33, C=CH_2), 5.20 (1H, dd, *J* 1.71, 10.50, C=CH_2), 4.88 (1H, d, *J* 12.45, $PhCH_2$-3), 4.85 (1H, d, *J* 3.42, H-1), 4.64 (1H, ddd, *J* 8.42, 9.89, 50.78, H-4), 4.66–4.55 (3H, m, $PhCH_2$-6, $PhCH_2$-3), 4.28–4.14 (2H, m, H-2, CH_2-C=C), 4.00–3.87 (2H, m, H-5, CH_2-C=C), 3.77–3.68 (3H, m, H-3, H-6a, H-6b), 1.90 (3H, s, $COCH_3$); δ_C (100 MHz, $CDCl_3$) 169.7 (C=O), 127.8 (C-Ar), 96.5 (C-1), 90.7 (d, *J* 182.2, C-4), 69.3 (C-3), 68.4 (C-5), 60.9 (C-6), 51.6 (C-2), 23.4 (CH_3); FAB-HRMS, *m*/*z* found $M+H^+$ 444.2191, $C_{25}H_{31}NO_5F$ requires 444.2186.

2-Acetamido-2,4-dideoxy-3,6-di-*O*-benzyl-4-fluoro-D-glucopyranose 8

To a solution of **7** (221.5 mg, 0.5 mmol) in 95% aq. CH_3COOH (2.5 cm^3) was added CH_3COONa (443.3 mg, 2.5 mmol) and $PdCl_2$ (205 mg, 2.5 mmol). The mixture was then stirred for 24 h at rt, filtrated through a Celite pad, diluted with ethyl acetate, then washed with H_2O, aq. $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the crude product as a yellow oil, which was purified by silica chromatography (8 : 1 chloroform–MeOH) to give **8** (176 mg, 87%) as an α–β (6 : 1) mixture; δ_H (400 MHz, $CDCl_3$) (α isomer): 7.36–7.26 (10H, m, ArH), 5.37 (1H, d, *J* 8.70, NH), 5.20 (1H, br t, *J* 3.36, H-1), 4.87 (1H, d, *J* 12.21, $PhCH_2$-3), 4.60 (1H, d, *J* 12.36, $PhCH_2$-6), 4.57 (1H, d, *J* 12.36, $PhCH_2$-6), 4.55 (1H, d, *J* 12.21, $PhCH_2$-3), 4.50 (1H, ddd, *J* 8.39, 9.92, 50.78, H-4), 4.15–4.03 (2H, m, H-2, H-5), 3.84–3.75 (1H, m, H-3), 3.71 (1H, dt, *J* 10.83, 2.29, H-6a), 3.65 (1H, ddd, *J* 1.83, 5.65, 10.83, H-6b), 1.86 (3H, s, $COCH_3$), 1.60 (1H, br s, OH-1); FAB-HRMS, *m*/*z* found $M+H^+$ 404.1895, $C_{22}H_{27}NO_5F$ requires 404.1873.

Dibenzyl 2-acetamido-2,4-dideoxy-3,6-di-*O*-benzyl-4-fluoro-D-glucopyranosyl phosphite 9

To a solution of **8** (385 mg, 0.96 mmol) in dichloromethane (9 cm^3) was added 1,2,4-triazole (264 mg, 3.8 mmol) and dibenzyl *N*,*N*-diethylphosphoramidite (0.7 cm^3, 2.4 mmol), and the mixture was then refluxed for 45 min. After cooling to rt, the solution was diluted with diethyl ether, washed with H_2O, $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the white solid product **9** (336 mg, 54%) as an α–β (1 : 1) mixture, which was not further purified here; δ_H (400 MHz, DMSO-d_6) 8.12 (1H, d, *J* 8.70, NH-β), 8.10 (1H, d, *J* 7.93, NH-α), 7.36–7.24 (20H, m, ArH), 5.48 (1H, dt, *J* 8.54, 3.20, H-1-α), 5.15 (1H, t, *J* 8.39, H-1-β), 1.82 (3H, s, $COCH_3$-α), 1.76 (3H, s, $COCH_3$-β).

Dibenzyl 2-acetamido-2,4-dideoxy-3,6-di-*O*-benzyl-4-fluoro-α-D-glucopyranosyl phosphate 10

To a cooled solution (−10 °C) of **9** (60 mg, 0.093 mmol) in THF (2.5 cm^3) was added *tert*-butylhydroperoxide (TBHP) (0.1 cm^3, 0.46 mmol), and the mixture was stirred for 1.5 h at −10 °C. The solution was then diluted with diethyl ether, washed with aq. $Na_2S_2O_3$, aq. $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the crude product, which was purified by silica chromatography (1 : 1 hexane–ethyl acetate) to give **10** (35 mg, 57%) as a colorless syrup. **8** was also recovered in about 20% yield; δ_H (400 MHz, DMSO-d_6) 8.27 (1H, d, *J* 7.78, NH), 7.39–7.26 (20H, m,

ArH), 5.58 (1H, dt, *J* 6.56, 3.20, H-1), 5.13–5.06 (4H, m, 2 × CH_2Ph), 4.73 (2H, br s, CH_2Ph-6), 4.62 (1H, ddd, *J* 8.70, 9.92, 50.96, H-4), 4.52 (1H, d, *J* 12.06, CH_2Ph-3), 4.47 (1H, d, *J* 12.06, CH_2Ph-3), 4.15–3.87 (3H, m, H-2, H-3, H-5), 3.66–3.55 (2H, m, H-6a, H-6b), 1.81 (3H, s, $COCH_3$).

Disodium 2-acetamido-2,4-dideoxy-4-fluoro-α-D-glucopyranosyl phosphate 11

To a solution of **10** (35 mg, 0.053 mmol) in EtOH (1.6 cm^3) and 10% aq. $NaHCO_3$ (1 cm^3) was added 25 mg 5% Pd/C. After stirring for 1 d at rt under a hydrogen atmosphere, the mixture was filtrated through a Celite pad. The solvent was evaporated under reduced pressure to yield the crude product, which was purified by G-10 gel chromatography (H_2O) to give **11** (15 mg, 82%) as a colorless foamy solid (freeze-dried); $[\alpha]^{25}_D$ +36.2° (*c* 0.1 in H_2O); δ_H (400 MHz, D_2O) 5.35 (1H, dt, *J* 7.63, 3.20, H-1), 4.42 (1H, ddd, *J* 8.70, 9.92, 50.96, H-4), 4.03–4.14 (2H, m, H-2, H-3), 3.96 (1H, dt, *J* 9.92, 1.98, H-5), 3.87 (1H, dt, *J* 12.66, 1.98, H-6a), 3.78 (1H, ddd, *J* 1.98, 4.27, 12.66, H-6b), 2.05 (3H, s, $COCH_3$); δ_C (100 MHz, D_2O) 175.6 (C=O), 93.4 (C-1), 90.1 (d, *J* 179.3, C-4), 70.7 (C-3), 70.3 (C-5), 60.9 (C-6), 54.5 (C-2), 22.9 (CH_3); FAB-HRMS, *m*/*z* found $M+H^+$ 348.0215, $C_8H_{14}NO_8Na_2PF$ requires 348.0236.

Disodium uridine 5′-(2-acetamido-2,4-dideoxy-4-fluoro-α-D-glucopyranosyl) diphosphate 1

A reaction mixture (2.4 cm^3) containing 50 mM Tris-HCl (pH 7.5), 5 mM UTP-3Na, 5 mM $MgCl_2$, 0.01 cm^3 UDP-GlcNAc pyrophosphorylase (2.4 units) and **11** (4.2 mg, 0.012 mmol) was incubated at 37 °C for 1 h and the reaction was stopped by boiling for 3 min. Quantitative determination of nucleotides and bases was carried out by HPLC with a YMC-packed column Hydrosphere C-18 (4.5 × 150 mm, Eishin Chemical, Japan) at 30 °C with detection at 260 nm. The mobile phase was 0.2 M triethylamine containing phosphoric acid (pH 6.0) and the flow rate was 0.6 cm^3 min^{-1}. Further purification was carried out by G-10 gel chromatography (H_2O) to afford pure **1** (1.8 mg, 23%) as a white solid (freeze-dried); $[\alpha]^{25}_D$ +47.9° (*c* 0.1 in H_2O); δ_H (600 MHz, D_2O) 7.60 (1H, d, *J* 8.14, uridine-H″-6), 5.67 (1H, d, *J* 8.14, uridine-H″-5), 5.66 (1H, d, *J* 5.00, rib-H′-1), 5.21 (1H, dt, *J* 7.33, 3.28, H-1), 4.18 (1H, ddd, *J* 7.78, 9.96, 50.58, H-4), 4.06 (1H, t, *J* 5.00, rib-H′-2), 4.01 (1H, t, *J* 5.00, rib-H′-3), 4.00–3.97 (1H, m, rib-H′-4), 3.94 (1H, ddd, *J* 2.27, 4.77, 11.70, rib-H′-5a), 3.86 (1H, ddd, *J* 4.07, 5.33, 11.70, rib-H′-5b), 3.82–3.73 (3H, m, H-5, H-3, H-2), 3.58–3.47 (2H, m, H-6a, H-6b), 1.78 (3H, s, $COCH_3$); δ_C (100 MHz, D_2O) 175.6 (C=O), 167.0 (uridine-C″-2), 152.6 (uridine-C″-4), 142.4 (uridine-C″-6), 103.4 (uridine-C″-5), 92.2 (C-1), 90.6 (rib-C′-1), 90.0 (d, *J* 180.2, C-4), 83.9 (rib-C′-4), 74.6 (rib-C′-2), 73.9 (rib-C′-3), 71.4 (C-3), 70.4 (C-5), 65.8 (C-6), 60.5 (rib-C′-5), 54.0 (C-2), 22.9 (CH_3); ESI-HRMS, *m*/*z* found $M-2Na^++H^+$ 608.0718, $C_{17}H_{25}N_3O_{16}P_2F$ requires 608. 0833.

Allyl 2-acetamido-2-deoxy-α-D-glucopyranoside 12

To a solution of *N*-acetyl-D-glucosamine (22.1 g, 0.1 mol) in allyl alcohol (400 cm^3) was added a BF_3 diethyl ether complex (2 cm^3, 0.2 mmol), and the mixture was refluxed for 2 h at 130 °C. Cooled to rt, the solvent was evaporated under reduced pressure. EtOH (200 cm^3) was added to dissolve the crude product, then diisopropyl ether was added to the flask to precipitate the product **12** (23 g, 88%) as a white powder; $[\alpha]^{25}_D$ +175.8° (*c* 0.1 in MeOH); mp 169–171 °C (EtOH) (lit.,[8] 172–174 °C); δ_H (400 MHz, DMSO-d_6) 7.73 (1H, d, *J* 8.34, NH), 5.91–5.82 (1H, m, CH=C), 5.30 (1H, dd, *J* 1.71, 17.32, C=CH_2), 5.13 (1H, dd, *J* 1.71, 10.37, C=CH_2), 4.97 (1H, d, *J* 6.09, OH-3), 4.69 (1H, d, *J* 5.54, OH-4), 4.66 (1H, d, *J* 3.74, H-1), 4.49 (1H, t, *J* 6.09, OH-6), 4.07 (1H, dd, *J* 5.24, 13.68, CH_2-C=C-), 3.90 (1H, dd, *J* 6.41, 13.68, CH_2-C=C-), 3.68–3.60 (2H, m, H-2, H-4), 3.51–3.43 (2H, m, H-6a, H-6b), 3.38–3.34 (1H, m, H-5), 3.12 (1H, dt, *J* 6.09, 9.73, H-3), 1.82 (3H, s, $COCH_3$); FAB-HRMS, *m*/*z* found $M+H^+$ 262.1311, $C_{11}H_{20}NO_6$ requires 262.1291.

Allyl 2-acetamido-2-deoxy-4,6-*O*-benzylidene-α-D-glucopyranoside 13

To a cooled solution (0 °C) of **12** (10.4 g, 40 mmol) in DMF (130 cm^3) was added (±)-camphor-10-sulfonic acid (929 mg, 4 mmol) and benzaldehyde dimethyl acetal (7.8 cm^3, 52 mmol) dropwise. The solution was stirred for 12 h at rt, then the solvent was evaporated under reduced pressure to yield a pale yellow oil. $CHCl_3$–MeOH (6 : 1) was poured into the flask to dissolve the crude product, and 400 cm^3 water was added to the flask, and stirred for 5 min. Then 2000 cm^3 hexane was added slowly to precipitate the product **13** (11.3 g, 81%) as a white solid; $[\alpha]^{25}_D$ +67.3° (*c* 0.1 in MeOH); mp 208–210 °C (EtOH); δ (400 MHz, DMSO-d_6) 7.91 (1H, d, *J* 8.34, NH), 7.46–7.36 (5H, m, ArH), 5.94–5.84 (1H, m, CH=C), 5.61 (1H, s, PhCH), 5.33 (1H, dd, *J* 1.71, 17.32, C=CH_2), 5.17 (1H, dd, *J* 1.71, 10.37, C=CH_2), 5.15 (1H, d, *J* 6.09, OH-3), 4.75 (1H, d, *J* 3.74, H-1), 4.17 –4.11 (2H, m, H-6a, CH_2-C=C), 3.95 (1H, dd, *J* 6.41, 13.68, CH_2-C=C), 3.84 (1H, ddd, *J* 3.74, 8.34, 10.25, H-2), 3.75–3.61 (3H, m, H-3, H-5, H-6), 3.48 (1H, t, *J* 9.73, H-4), 1.84 (3H, s, $COCH_3$); FAB-HRMS, *m*/*z* found $M+H^+$ 350.1589, $C_{18}H_{24}NO_6$ requires 350.1604.

Allyl 2-acetamido-2-deoxy-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside 14

To a cooled solution (0 °C) of **13** (1.74 g, 5 mmol) in DMF (36 cm^3) was added barium oxide (1.53 g, 10 mmol), barium hydroxide (1.58 g, 5 mmol) and benzyl bromide (1.2 cm^3, 10 mmol) dropwise. The solution was stirred for 12 h at rt. After filtration through a Celite pad, the solvent was evaporated under reduced pressure to yield a pale yellow oil. $CHCl_3$–MeOH (6 : 1) was poured into the flask to dissolve the crude product and then 90 cm^3 hexane was added slowly to precipitate the product **14** (1.66 g, 76%) as a white solid; $[\alpha]^{25}_D$ + 41.9° (*c* 0.1 in MeOH); mp 224–225 °C (EtOH); δ_H (400 MHz, $CDCl_3$) 7.52–7.28 (10H, m, ArH), 5.91–5.81 (1H, m, CH=C), 5.60 (1H, s, PhCH), 5.34 (1H, d, *J* 9.16, NH), 5.28–5.19 (2H, m, C=CH_2), 4.92 (1H, d, *J* 12.36, $PhCH_2$), 4.86 (1H, d, *J* 3.66, H-1), 4.64 (1H, d, *J* 12.36, $PhCH_2$), 4.32–4.26 (2H, m, H-2, H-6a), 4.15 (1H, ddt, *J* 5.34, 12.97, 1.38, CH_2-C=C), 3.96 (1H, ddt, *J* 6.26, 12.97, 1.22, CH_2-C=C), 3.89–3.71 (4H, m, H-3, H-4, H-5, H-6), 1.91 (3H, s, $COCH_3$); FAB-HRMS, *m*/*z* found $M+H^+$ 440.2066, $C_{25}H_{30}NO_6$ requires 440.2073.

Allyl 2-acetamido-2-deoxy-3,6-di-*O*-benzyl-α-D-glucopyranoside 15

To a solution of **14** (878 mg, 2 mmol) in THF (24 cm^3) was added 4 Å MS (1 g) and $NaBH_3CN$ (2 g, 32 mmol), and then the mixture was stirred for 20 min at rt. Cooled to 0 °C, 2.0 M HCl–diethyl ether solution was added dropwise until the mixture attained pH 3 and it was then stirred for 1 h at rt. After filtration through a Celite pad, the solution was diluted with diethyl ether, washed with H_2O, aq. $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the product as a pale yellow oil, which was purified by silica chromatography (25 : 1 chloroform–MeOH) to give **15** (611 mg, 69%) as a white solid; $[\alpha]_D$ +30.7° (*c* 0.1 in $CHCl_3$); mp 102–103 °C (ethyl acetate–ether); δ_H (400 MHz, $CDCl_3$) 7.37–7.28 (10H, m, ArH), 5.92–5.83 (1H, m, CH=C), 5.41 (1H, d, *J* 9.28, NH), 5.28–5.18 (2H, m, C=CH), 4.83 (1H, d, *J* 3.66, H-1), 4.76 (1H, d, *J* 11.96, $PhCH_2$-3), 4.70 (1H, d, *J* 11.96, $PhCH_2$-6), 4.62 (1H, d, *J* 11.96, $PhCH_2$-6), 4.55 (1H, d, *J* 11.96, $PhCH_2$-3), 4.29–4.14 (2H, m, H-2, CH_2-C=C), 3.98–3.93 (1H, m, CH_2-C=C), 3.79–3.58 (5H, m, H-3, H-4, H-5, H-6a, H-6b), 2.70 (1H, br s, 4-OH), 1.91 (3H, s, $COCH_3$); FAB-HRMS, *m*/*z* found $M+H^+$ 442.2226, $C_{25}H_{32}NO_6$ requires 442.2230.

Allyl 2-acetamido-2-deoxy-3,6-di-*O*-benzyl-4-mesyl-α-D-glucopyranoside 16

To a cooled solution (−13 °C) of **15** (611 mg, 1.38 mmol) in pyridine (5.5 cm^3) was added MsCl (0.32 cm^3, 4.2 mmol). After stirring for 10 min at −13 °C, then for 4 h at 0 °C, the solution was diluted with ethyl acetate, washed with 1 M HCl, aq. $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the product as a pale yellow oil, which was purified by silica chromatography (40 : 1 chloroform–ethyl acetate) to give **16** (622 mg, 92%) as a white solid; $[\alpha]^{25}_D$ +78.2° (*c* 0.1 in $CHCl_3$); mp 155–156 °C (ethyl acetate–ether); δ_H ($CDCl_3$) 7.38–7.27 (10H, m, ArH), 5.92–5.84 (1H, m, CH=C), 5.45 (1H, d, *J* 9.61, NH), 5.30–5.21 (2H, m, C=CH_2), 4.85 (1H, d, *J* 3.66, H-1), 4.76–4.69 (3H, m, H-4, $PhCH_2$-3, $PhCH_2$-6), 4.63 (1H, d, *J* 11.75, $PhCH_2$-3), 4.56 (1H, d, *J* 11.90, $PhCH_2$-6), 4.44 (1H, ddd, *J* 3.66, 9.61, 10.5, H-2), 4.19 (1H, ddt, *J* 5.34, 12.97, 1.38, CH_2-C=C), 4.02–3.93 (2H, m, H-5, CH_2-C=C), 3.86 (1H, dd, *J* 9.31, 10.53, H-3), 3.81 (1H, dd, *J* 2.29, 10.97, H-6), 3.72 (1H, dd, *J* 5.04, 10.97, H-6), 2.86 (3H, s, SO_2CH_3), 1.89 (3H, s, $COCH_3$); FAB-HRMS, *m*/*z* found M+H^+ 520.1984, $C_{26}H_{34}NO_8S$ requires 520.2005.

Allyl 2-acetamido-2,4-dideoxy-3,6-di-*O*-benzyl-4-fluoro-α-D-galactopyranoside 17

To a solution of **16** (354 mg, 0.68 mmol) in acetonitrile (8 cm^3) was added tetrabutylammonium fluoride (TBAF) (2.6 g, 8.2 mmol). It was then refluxed for 60 h, cooled to rt, and the solution was evaporated under reduced pressure to yield a pale yellow oil. The resulting oil was dissolved with 200 cm^3 ethyl acetate, washed with H_2O, brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the product **17** (213 mg, 71%) as a white solid; $[\alpha]^{25}_D$ +109.1° (*c* 0.1 in $CHCl_3$); mp 144–145 °C (ethyl acetate–ether); δ_H (400 MHz, $CDCl_3$) 7.39–7.28 (10H, m, ArH), 5.89–5.80 (1H, m, CH=C), 5.30 (1H, d, *J* 9.00, NH), 5.24–5.16 (2H, m, C=CH_2), 4.95 (1H, dd, *J* 2.29, 50.35, H-4), 4.94 (1H, d, *J* 3.66, H-1), 4.77 (1H, d, *J* 12.21, $PhCH_2$-3), 4.61–4.53 (3H, m, $PhCH_2$-6, H-2), 4.49 (1H, d, *J* 12.21, $PhCH_2$-3), 4.14 (1H, ddt, *J* 5.34, 12.97, 1.38, CH_2-C=C), 3.99–3.86 (2H, m, H-5, CH_2-C=C), 3.73 (1H, dd, *J* 7.48, 9.31, H-6a), 3.63 (1H, dd, *J* 1.37, 9.31, H-6), 3.61 (1H, ddd, *J* 2.29, 10.99, 27.90, H-3), 1.94 (3H, s, $COCH_3$); FAB-HRMS, *m*/*z* found M+H^+ 444.2165, $C_{25}H_{31}NO_5F$ requires 444.2186.

2-Acetamido-2,4-dideoxy-3,6-di-*O*-benzyl-4-fluoro-D-galacto-pyranose 18

To a solution of **17** (88.6 mg, 0.2 mmol) in 90% aq. CH_3COOH (2 cm^3) was added CH_3COONa (65.6 mg, 0.8 mmol) and $PdCl_2$ (71 mg, 0.4 mmol). After stirring for 13 h at rt, the mixture was filtrated through a Celite pad and diluted with ethyl acetate, washed with H_2O, aq. $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the crude product as a yellow oil, which was purified by silica chromatography (10 : 1 chloroform–MeOH) to give **18** (51 mg, 63%) as an α–β (6 : 1) mixture; δ_H (400 MHz, DMSO-d_6) (α isomer) 7.83 (1H, d, *J* 8.85, NH), 7.38–7.25 (10H, m, ArH), 6.79 (1H, d, *J* 3.66, OH-1), 5.00 (1H, br t, *J* 3.66, H-1),), 4.99 (1H, dd, *J* 2.14, 51.27, H-4), 4.67 (1H, d, *J* 11.60, $PhCH_2$-3), 4.55 (1H, d, *J* 11.60, $PhCH_2$-3), 4.52 (2H, br s, $PhCH_2$-6), 4.20–4.09 (2H, m, H-2, H-5), 3.79 (1H, ddd, *J* 2.14, 11.29, 28.84, H-3), 3.63 (1H, dd, *J* 5.95, 9.77, H-6a), 3.52 (1H, ddd, *J* 0.92, 6.87, 9.77, H-6b), 1.85 (3H, s, $COCH_3$); FAB-HRMS, *m*/*z* found M+H^+ 404.1879, $C_{22}H_{27}NO_5F$ requires 404.1873.

Dibenzyl 2-acetamido-2,4-dideoxy-3,6-di-*O*-benzyl-4-fluoro-D-galactopyranosyl phosphite 19

To a solution of **18** (173 mg, 0.43 mmol) in dichloromethane (7 cm^3) was added 1,2,4-triazole (118.6 mg, 1.72 mmol) and dibenzyl *N*,*N*-diethylphosphoramidate (0.3 cm^3, 1.1 mmol), and it was refluxed for 45 min. After cooling to rt, the solution was diluted with diethyl ether, washed with H_2O, aq. $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the white solid product **19** (194 mg, 70%) as an α–β (1 : 1) mixture, which was not further purified here; δ_H (400 MHz, DMSO-d_6) 8.00 (1H, d, *J* 9.00, NH-β), 7.97 (1H, d, *J* 7.91, NH-α), 7.39–7.27 (20H, m, ArH), 5.55 (1H, dd, *J* 3.36, 8.55, H-1-α), 5.11 (1H, t, *J* 8.09, H-1-β), 5.10 (1H, dd, *J* 2.14, 50.96, H-4-α), 4.25–4.13 (2H, m, H-2-α, H-5-α), 3.81 (1H, ddd, *J* 1.83, 11.29, 29.30, H-3-β), 3.52 (1H, dd, *J* 6.87, 9.92, H-6-α), 1.81 (3H, s, $COCH_3$-α), 1.76 (3H, s, $COCH_3$-β); ESI-HRMS (β isomer), *m*/*z* found M+Na^+ 670.2322, $C_{36}H_{39}NO_7FPNa$ requires 670.2346.

Dibenzyl 2-acetamido-2,4-dideoxy-3,6-di-*O*-benzyl-4-fluoro-α-D-galactopyranosyl phosphate 20

To a cooled solution (−10 °C) of **19** (32 mg, 0.05 mmol) in THF (1.5 cm^3) was added TBHP (0.05 cm^3, 0.25 mmol), and it was stirred for 1.5 h at −10 °C. The solution was diluted with diethyl ether, washed with aq. $Na_2S_2O_3$, aq. $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the crude product, which was purified by silica chromatography (1 : 1 hexane–ethyl acetate) to give **20** (18 mg, 54%) as a colorless syrup. **18** was also recovered in about 20% yield; δ_H (400 MHz, DMSO-d_6) 8.15 (1H, d, *J* 7.78, NH), 7.38–7.26 (20H, m, ArH), 5.66 (1H, dd, *J* 3.36, 6.10, H-1), 5.20–5.03 (5H, m, H-4, CH_2Ph-4, CH_2Ph-6), 4.74–4.54 (4H, m, 2 × CH_2Ph-P), 4.29–4.18 (2H, m, H-2, H-5), 3.94 (1H, ddd, *J* 2.29, 11.60, 27.92, H-3), 3.63 (1H, dd, *J* 6.10, 9.92, H-6a), 3.52 (1H, dd, *J* 6.71, 9, H-6b), 1.81 (3H, s, $COCH_3$); ESI-HRMS, *m*/*z* found M+Na^+ 686.2311, $C_{36}H_{39}NO_8FPNa$ requires 686.2295.

Disodium 2-acetamido-2,4-dideoxy-4-fluoro-α-D-galacto-pyranosyl phosphate 21

To a solution of **20** (66.3 mg, 0.1 mmol) in EtOH (3 cm^3) and 10% aq. $NaHCO_3$ (2 cm^3) was added 50 mg 5% Pd/C. After stirring for 1 d at rt under a hydrogen atmosphere, the mixture was filtrated through a Celite pad. The solvent was evaporated under reduced pressure to yield the crude product, which was purified by G-10 gel chromatography (H_2O) to give **21** (29.8 mg, 87%) as a colorless foam (freeze-dried); $[\alpha]^{25}_D$ +75.6° (*c* 0.1 in H_2O); δ_H (400 MHz, D_2O) 5.40 (1H, dd, *J* 3.21, 7.48, H-1), 4.92 (1H, dd, *J* 2.29, 50.81, H-4), 4.31–4.20 (2H, m, H-2, H-5), 4.04 (1H, ddd, *J* 2.29, 10.99, 29.75, H-3), 3.80–3.75 (2H, m, H-6a, H-6b), 2.05 (3H, s, $COCH_3$); δ_C (100 MHz, D_2O) 175.7 (C=O), 93.8 (C-1), 90.0 (d, *J* 177.5, C-4), 70.3 (C-3), 67.8 (C-5), 61.0 (C-6), 51.2 (C-2), 23.0 (CH_3); FAB-HRMS, *m*/*z* found M+H^+ 348.0262, $C_8H_{14}NO_8Na_2PF$ requires 348.0236.

Disodium uridine 5′-(2-acetamido-2,4-dideoxy-4-fluoro-α-D-galactopyranosyl) diphosphate 2

A reaction mixture (10 cm^3) containing 50 mM Tris-HCl (pH 7.5), 5 mM UTP-3Na, 5 mM $MgCl_2$, 0.25 cm^3 UDP-GlcNAc pyrophosphorylase (60 units) and **21** (18 mg, 0.052 mmol) was incubated at 37 °C for 2 h and the reaction was stopped by boiling for 3 min. Quantitative determination of nucleotides and bases was carried out by HPLC with a YMC-packed column Hydrosphere C-18 (4.5 × 150 mm, Eishin Chemical, Japan) at 30 °C with detection at 260 nm. The mobile phase was 0.2 M triethylamine containing phosphoric acid (pH 6.0) and the flow rate was 0.6 cm^3 min^{-1}. Further purification was carried out by G-10 gel chromatography (H_2O) to afford pure **2** (6.1 mg, 18%) as a white solid (freeze-dried); $[\alpha]^{25}_D$ +31.1° (*c* 0.1 in H_2O); δ_H (600 MHz, D_2O) 7.72 (1H, d, *J* 8.13, uridine-H″-6), 5.78 (1H, d, *J* 5.13, rib-H′-1), 5.76 (1H, d, *J* 8.13, uridine-H″-5), 5.39 (1H, dd, *J* 3.32, 7.21, H-1), 4.18 (1H, t, *J* 5.13, rib-H′-2), 4.14 (1H, t, *J* 5.13, rib-H′-3), 4.10–4.02 (4H, m, H-2, H-5, rib-H′-4, rib-H′-5a), 3.97 (1H, ddd, *J* 3.61, 5.49, 11.51,

rib-H′-5b), 3.89 (1H, ddd, *J* 2.48, 10.96, 29.55, H-3), 3.63–3.60 (2H, m, H-6a, H-6b), 1.88 (3H, s, $COCH_3$); δ_C (100 MHz, D_2O) 174.7 (C=O), 165.8 (uridine-C″-2), 151.7 (uridine-C″-4), 141.7 (uridine-C″-6), 102.7 (uridine-C″-5), 92.4 (C-1), 90.2 (rib-C′-1), 89.4 (d, *J* 178.4, C-4), 88.4 (rib-C′-4), 73.7 (rib-C′-2), 72.8 (rib-C′-3), 72.1 (C-3), 69.6 (C-5), 68.3 (C-6), 61.0 (rib-C′-5), 51.0 (C-2), 22.0 (CH_3); ESI-HRMS, *m*/*z* found $M-2Na^+ + H^+$ 608.0721, $C_{17}H_{25}N_3O_{16}P_2F$ requires 608. 0833.

Acknowledgements

This work was supported by a grant for the "Glycocluster Research & Development of Controlling Biomolecules" from NEDO. We thank Ms. Oka for mass measurement at the Center for Instrumental Analysis of Hokkaido University.

References

1 A. Varki, *Glycobiology*, 1993, **3**, 97; W. J. Dennis, S. Laferte, C. Waghorne, L. M. Breitman and S. R. Kerbel, *Science*, 1987, **236**, 582.
2 T. J. Grier and J. R. Rasmussen, *J. Biol. Chem.*, 1984, **259**, 1027.
3 A. M. Schultz and P. T. Mora, *Carbohydr. Res.*, 1975, **40**, 119.
4 C.-L. Schengrund and P. Kovac, *Carbohydr. Res.*, 1999, **319**, 24; M.-C. Chapeau and P. A. Frey, *J. Org. Chem.*, 1994, **59**, 6994; M. D. Burkart, S. P. Vincent, A. Duffels, B. W. Murray, S. V. Ley and C.-H. Wong, *Bioorg. Med. Chem.*, 2000, **8**, 1937; L. R. Thomas, S. A. Abbas and K. L. Matta, *Carbohydr. Res.*, 1988, **184**, 77; A. Burton, P. Wyatt and G.-J. Boons, *J. Chem. Soc., Perkin Trans. 1*, 1997, **16**, 2375.
5 T. Hayashi, B. W. Murray, R. Wang and C.-H. Wong, *Bioorg. Med. Chem.*, 1997, **5**, 497.
6 M. C. T. Hartman and J. K. Coward, *J. Am. Chem. Soc.*, 2002, **124**, 10036.
7 D. Kostrewa, A. D'Arcy, B. Takacs and M. Kamber, *J. Mol. Biol.*, 2001, **305**, 279.
8 R. T. Lee and Y. C. Lee, *Carbohydr. Res.*, 1974, **37**, 193.
9 M. A. Nashed, R. I. El-Sokkary and L. Rateb, *Carbohydr. Res.*, 1984, **131**, 47; M. Sofia, N. Allanson, A. Chen, D. Liu and T. Y. Chan, *PCT Int. Appl.*, 1997, 125.
10 P. J. Garegg and H. Hultberg, *Carbohydr. Res.*, 1981, **93**, C10; M. Oikawa, A. Wada, H. Yoshizaki, K. Fukase and S. Kusumoto, *Bull. Chem. Soc. Jpn.*, 1997, **70**, 1435; T. Zhu and G.-J. Boons, *Carbohydr. Res.*, 2000, **329**, 709.
11 M. J. Robins and S. F. Wnuk, *J. Org. Chem.*, 1993, **58**, 3800; A. Berkin, W. A. Szarek and R. Kisilevsky, *Carbohydr. Res.*, 2000, **326**, 250.
12 M. Hori and F. Nakatsubo, *Macromolecules*, 2001, **34**, 2476; Y. Zhu, L. Chen and F. Kong, *Carbohydr. Res.*, 2002, **337**, 207.
13 K.-L. Yu and B. Fraser-Reid, *Tetrahedron Lett.*, 1988, **29**, 979; M. M. Sim, H. Kondo and C.-H. Wong, *J. Am. Chem. Soc.*, 1993, **115**, 2260.
14 H. A. Nunez and R. Barker, *Biochemistry*, 1980, **19**, 489; C.-H. Wong, A. Pollak, S. D. McCurry, J. M. Sue, J. R. Knowles and G. M. Whitesides, *Methods Enzymol.*, 1982, **89**, 108; K. Okuyama, T. Hamamoto, K. Ishige, K. Takenouchi and T. Noguchi, *Biosci. Biotechnol. Biochem.*, 2000, **64**, 386.