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Large-scale synthesis of β-L-fucopyranosyl phosphate and the preparation of GDP-β-L-fucose

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ABSTRACT

A practical 15-mmol large-scale synthesis of β-L-fucopyranosyl dicyclohexylammonium phosphate from L-fucose in 63% overall yield was developed. The synthesis took advantage of a neighboring Bz-2 group participating in a Koenigs–Knorr-like glycosylation. The sugar phosphate was transformed into the activated sugar nucleoside, guanosine diphosphate β-L-fucopyranose, on a gram scale.

INTRODUCTION

Practical syntheses of nucleoside phosphate sugars are required to make these compounds available for syntheses of oligosaccharides using Leloir-pathway enzymes[1,2]. The glycosyl transferases that use these activated sugars as glycosyl donors are increasingly available, either commercially or through cloning[3–5].

L-Fucose is the most hydrophobic of the sugars commonly encountered in mammalian oligosaccharides. Oligosaccharides containing L-fucose are known as tumor-associated antigens and recognition of these is important in certain diagnostics of cancer[6]. Oligosaccharides containing L-fucose are also known as part of human blood group H determinants; this material is the substrate for the glycosyl transferases that construct the A and B blood group antigens[7]. L-Fucose is also found in glycolipids[8], bacterial polysaccharides[9], and plasma glycoproteins[10]. Guanosine diphosphate β-L-fucopyranose (GDP-Fuc) is the substrate for the fucosyl transferases involved in the biosynthesis of these substances.

GDP-Fuc has been synthesized previously by both enzymic and chemical methods. The enzymic methods usually start from guanosine diphosphate α-D-mannopyranose (GDP-Man) and use a GDP-Man oxidoreductase system[11,12]. The utility of this method is limited by the troublesome isolation of the enzyme. The major problem with chemical methods is the lack of good anomeric control in the synthesis of fucopyranosyl phosphate. Although fucopyranose has the same overall stereochemistry as galactopyranose, for which anomeric control in glycosylation reactions can be obtained by neighboring-group participation, it is well known that

the 6-deoxy group diminish the effect of the neighbor group[13]. GDP-Fuc has been synthesized from β-L-fucopyranosyl phosphate by condensation with nucleoside 5'-phosphate activated in the form of a morpholidate[14,15], dibutylphosphinothioic anhydride[16], imidazolate[17], and diphenyl pyrophosphate[18]. Nunez et al.[19] have described one of the best syntheses of β-L-fucopyranosyl phosphate as its dicyclohexylammonium salt, with an overall yield from L-fucose of 50%. A major drawback with this synthesis is that it requires the anomeric stability of 2,3,4-tri-*O*-acetyl-β-L-fucopyranose during drying. If drying is prolonged, the compound equilibrates to an anomeric mixture with the α anomer as the preponderant conformer[19]. This complication makes the procedure less satisfactory for large-scale synthesis. In addition, the anomers must be separated by a cumbersome ion-exchange chromatography using a gradient solvent system. Schanbacher and Wilken[20] have used a simple condensation reaction between 1,2,3,4-tetra-*O*-acetyl-α-L-fucopyranose and crystalline phosphoric acid to synthesize the corresponding α-L-fucopyranosyl dicyclohexylammonium phosphate in 20% overall yield. Recently, Schmidt et al.[21] have described the synthesis of both the α and β anomer of 2,3,4-tri-*O*-acetyl-L-fucopyranosyl phosphate via trichloroacetimidate.

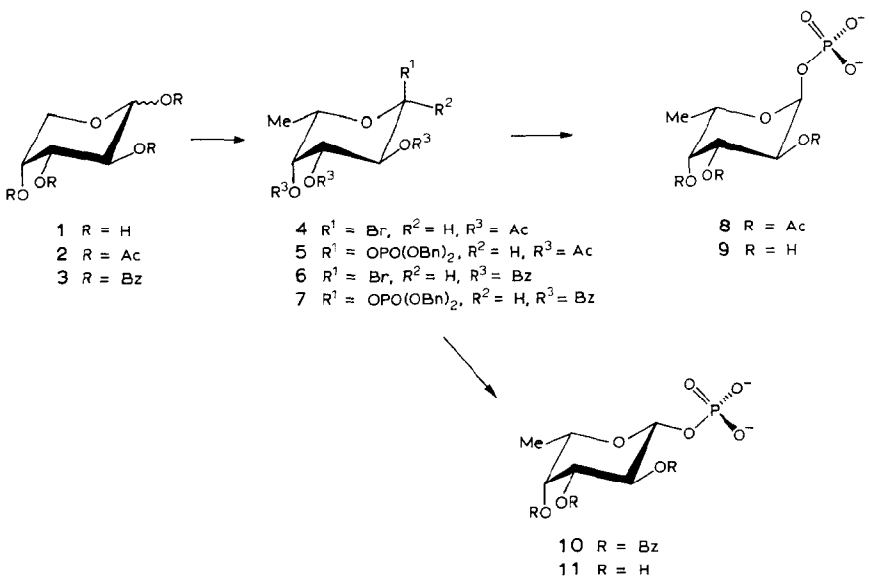
In this paper, we describe a practical, large-scale, five-step synthesis of β-L-fucopyranosyl dicyclohexylammonium phosphate in 63% overall yield with only one chromatographic purification. We also demonstrate that this compound can be converted to GDP-Fuc on a gram scale.

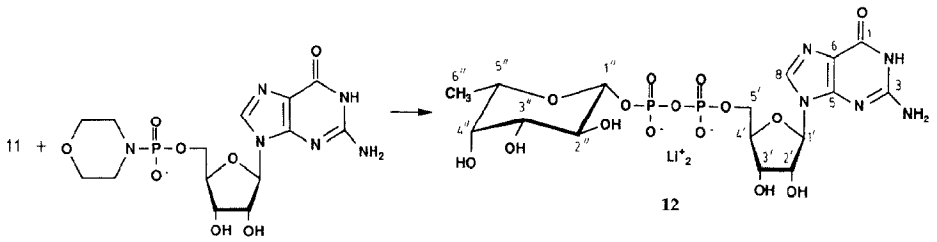
RESULTS AND DISCUSSION

Generally glycosyl phosphates have been prepared by the reaction of a free anomeric hydroxyl group with an activated phosphate precursor. In our approach, we have choosen to activate the glycosyl component since the configuration of L-fucose should make neighboring-group participation possible in glycoside synthesis, with proper choice of protection for the 2-hydroxyl group. Usually, acetyl groups are preferred for this role but reaction of **4** with triethylammonium dibenzyl phosphate in the presence of an excess of silver carbonate, under the normal conditions for a Koenigs–Knorr-type glycosylation, gave 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl dibenzylphosphate (**5**) as the only product (in small scale, it was found that **5** also could be obtained without using silver carbonate when the reaction was done in dry acetonitrile). This surprising result is a good example of the lacking neighboring-group participation in the reaction of fucose as previously observed[13]. Apparently, formation of the fucopyranosyl oxonium ion is preferred over the acetoxonium ion that can be formed between C-1 and C-2, and the attack by the phosphorus nucleophile is governed by the anomeric effect. This process generates products with the α configuration exclusively. To achieve the formation of the desired β anomer, we changed from acetyl to benzoyl protecting groups since an intermediate benzoyloxonium ion can be stabilized by delocalization of the positive charge to the benzene ring. Although the generation of the glycosyl

bromide usually is a quantitative reaction, we never achieved greater than 80–85% conversion of the perbenzoylated fucopyranose. Changing the solvent for the bromination from acetic acid to dichloromethane only decreased the yield. Reaction of the crude glycosyl bromide **6** on a small scale under the conditions described above for the corresponding acetate gave the desired 2,3,4-tri-*O*-benzoyl-β-L-fucopyranosyl dibenzyl phosphate **7**. During scale-up, only slight excesses of silver carbonate and triethylammonium dibenzylphosphate were used, which did not affect the anomeric configuration. The reaction gave a 73% overall yield after column chromatography. The phosphate group was deprotected by hydrogenolysis in a mixture of toluene, pyridine, and triethylamine using Pd–C as catalyst. The solvent system was choosen to keep the product in solution. Debenzoylation of the free phosphate was achieved by refluxing a solution of 2,3,4-tri-*O*-benzoyl-β-L-fucopyranosyl ditriethylammonium phosphate (**10**) in 1:1 methanol–cyclohexylamine. The deprotection with cyclohexylamine has the advantage over the normal deprotection with sodium methoxide or sodium hydroxide in that it directly gives the desired β-L-fucopyranosyl dicyclohexylammonium phosphate (**11**) in the crystalline form. In addition, large-scale deprotection of **10** using methoxide in methanol proved to be a very slow reaction unless an undesirably large excess of methoxide was used, because the free phosphate tends to buffer the reaction mixture. The 63% overall yield of the key intermediate **11** is an important improvement from the previously reported 50% for the large-scale synthesis[19].

Since the goal of this work was to find a convenient large-scale synthesis of GDP-Fuc (**12**), we prepared the target compound on a gram scale by coupling





excess β-L-fucopyranosyl phosphate to guanosine monophosphate morpholidate (GMP-morpholidate) following the procedure of Nunez et al.[19]. In order to make β-L-fucopyranosyl phosphate soluble in pyridine, the counterion had to be changed from cyclohexylammonium to triethylammonium. The coupling reaction was carried out under strictly anhydrous conditions for 3.5 days, and gave GDP-Fuc that could easily be purified by ion-exchange chromatography and isolated as a white powder in 32% yield, based on added GMP-morpholidate. β-L-Fucopyranosyl dilithium phosphate was recovered in 66% yield. Thus, the yield of GDP-Fuc was 64%, based on the β-L-fucopyranosyl phosphate consumed. By ^{31}P NMR spectroscopy, the GMP-morpholidate from Sigma contained several equivalents of inorganic phosphate. This contaminant might have reduced the yield. The ion-exchange chromatography gave a very good separation and proved to be very reproducible.

EXPERIMENTAL

General methods.—^{1}H NMR spectra were recorded at 400 MHz; H shifts for solutions in $CDCl_3$ are referenced to internal Me_4Si (δ 0), and for solutions in D_2O to HDO (δ 4.75). ^{13}C NMR spectra were recorded at 100 MHz (Bruker AM-400); C shifts for solutions in $CDCl_3$ are referenced to the center of the $CDCl_3$ peaks (δ 77.0), and for solutions in D_2O to external 1,4-dioxane (δ 67.4). TLC plates (EM Science) were coated with Silica Gel 60 F_{254} (0.2 mm). Column chromatography was done with Silica Gel 60 (particle size, 0.040–0.064 mm, 230–400 mesh ASTM, EM Science). All moisture-sensitive reactions were conducted under Ar. Dichloromethane and MeCN were distilled from CaH_2. Hydrogen bromide in acetic acid, Ag_2CO_3, dibenzyl phosphate, and L-fucose were purchased from Aldrich. Molecular sieves were activated by heating to > 200°C at 0.15 kPa for 3 days.

2,3,4-Tri-O-acetyl-α-L-fucopyranosyl bromide (**4**).—L-Fucose (**1**; 3.1 g, 18.9 mmol, 95% pure, Aldrich) was suspended in pyridine (20 mL) and Ac_2O (10 mL) and stirred overnight. The homogeneous mixture was poured into ice and extracted with CH_2Cl_2 (80 mL). The organic phase was successively washed with cold 4 M HCl (3 × 50 mL) and satd $NaHCO_3$ solution (3 × 40 mL), dried ($MgSO_4$), and concentrated to give 1,2,3,4-tetra-*O*-acetyl-L-fucopyranose (**2**) as a syrup (6.26 g,

quantitative). Crude **2** (3.77 g, 11.4 mmol) was dissolved in CH_2Cl_2 and cooled to 0°C. To this solution was added 30% HBr–AcOH (6 mL) and the mixture stirred at room temperature for 30 min; TLC in 1:1 EtOAc–hexane showed complete conversion. The mixture was diluted with CH_2Cl_2 (70 mL) and washed successively with ice–water and satd $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated to give **4** (4.03 g, quantitative), which crystallized from Et_2O–petrolum ether; mp 63–66°C, lit[22] mp 64–66°C. ^1H NMR ($CDCl_3$): δ 1.18 (d, J 6.5 Hz, H-6), 1.97, 2.06, and 2.13 (3 × CH_3CO), 4.36 (bq, J 6.5 Hz, H-5), 4.97 (dd, J 3.9, 10.0 Hz, H-2), 5.31 (bd, J 3.0 Hz, H-4), 5.36 (dd, J 3.1, 10.0 Hz, H-3), 6.64 (d, J 3.8 Hz, H-1). ^{13}C NMR ($CDCl_3$) δ 15.4 (C-6, s), 20.4, 20.5, and 20.6 (3 × CH_3, s), 67.8 (C-5, s), 68.4 (C-2, s), 69.8 (C-4, s), 70.0 (C-3, s), 89.3 (C-1, s), 169.7, 170.0, and 170.1 (3 × CO, s).

*Dibenzyl 2,3,4-tri-*O*-acetyl-α-*L*-fucopyranosyl phosphate* (**5**).—The crude glycosyl bromide **4** (3.6 g, 10.2 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and stirred with 3A molecular sieves for 1 h. Silver carbonate (7.33 g, 27 mmol) was mixed with 3A molecular sieves in a foil-covered flask and cooled to −78°C. A solution of triethylammonium dibenzyl phosphate (6.1 g, 17.5 mmol) in dry CH_2Cl_2 (15 mL) was added under Ar, followed by the solution of the glycosyl bromide **4** and additional CH_2Cl_2 (15 mL). The mixture was stirred overnight at room temperature. When TLC in 2:1 EtOAc–hexane showed complete conversion to a single new compound, the mixture was filtered through Celite and purified by column (2.3 × 17 cm) chromatography (silica gel; 1:1 EtOAc–hexane) to give **5** (4.05 g, 72%) as a syrup; prolonged exposure to silica gel resulted in decomposition; ^1H NMR ($CDCl_3$): δ 1.89, 1.97, 2.17 (s, 3 × OAc), 3.89 (q, J 6.5 Hz, H-5), 5.01 (d, 2H, J 7.9 Hz), 5.07 (dd, 1 H, J 7.5, 11.5 Hz), 5.10 (dd, 1 H, J 7.5, 11.5 Hz), 5.24 (dd, J 0.8, 3.9 Hz, H-4), 5.25–5.34 (m, H-2,3), 6.01 (dd, J 3.5, 6.8 Hz, H-1), and (7.28–7.37 (10 H, m). ^{13}C NMR ($CDCl_3$): δ 15.6 (s, C-6), 68.9 (d, J 8.7 Hz, C-2), 69.5, 69.6 (d, 2C, J 6.3 Hz), 69.8, 70.2, 70.9, (s, C-3,4,5), and 96.7 (d, J 4.4 Hz, C-1).

*Disodium α-*L*-fucopyranosyl phosphate* (**9**).—Compound **5** (300 mg, 0.55 mmol) was dissolved in toluene (10 mL)–pyridine (2.5 mL)–triethylamine (0.5 mL) and hydrogenated overnight with 10% activated Pd–C (100 mg) as catalyst. The resulting syrup was dissolved by adding more pyridine, then filtered through Celite and concentrated to give bis(triethylammonium) 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl phosphate **8** as a syrup. This was dissolved in MeOH (10 mL) and the solution made basic by adding 0.1 M NaOMe–MeOH (12 mL) and stirred for 3 days to complete the deprotection. The mixture was neutralized with dry ice, concentrated and washed with hot EtOH. The remaining solid was dissolved in MeOH and precipitated by addition of acetone to give **9** (145 mg, 92%); the NMR data are in agreement with literature values[23]: ^1H NMR (D_2O): δ 1.13 (d, J 6.7 Hz, H-6), 3.65 (ddd, J 3.6, 10.2, 2.1 Hz, H-2), 3.74 (bd, J 3.1 Hz, H-4), 3.85 (dd, J 10.2, 3.1 Hz, H-3), 4.20 (bq, J 6.7 Hz, H-5), and 5.36 (dd, J 3.6, 7.0 Hz, H-1); ^{13}C NMR (D_2O): δ 16.7 (C-6), 68.3 (C-5), 70.0 (d, C-2), 71.0 (C-3), 73.3 (C-4), and 95.3 (d, C-1).

*Dicyclohexylammonium β-*L*-fucopyranosyl phosphate* (**11**).—L-Fucose (**1**; 4.0 g, 24.4 mmol, 95% pure, Aldrich) was dissolved in pyridine (50 mL) and cooled to

0°C. Benzoyl chloride (17.2 g, 122 mmol) was slowly added and the heterogeneous mixture stirred overnight at room temperature. TLC in 1:2 EtOAc–hexane showed complete benzoylation. The mixture was poured into ice and diluted with CH_2Cl_2 (100 mL). The organic phase was successively washed with 4 M HCl (4 × 60 mL) and satd $NaHCO_3$ solution (3 × 50 mL), dried ($MgSO_4$) and concentrated to give 1,2,3,4-tetra-*O*-benzoyl-L-fucopyranose (**3**) as a syrup (15 g, quantitative), containing a small amount of benzoic acid. The crude product was dissolved in glacial AcOH (16 mL) and cooled in an ice bath. Saturated HBr in AcOH (16 mL) was added and the solution stirred at room temperature for 4 h. The mixture was diluted with CH_2Cl_2 (100 mL) and then washed successively with ice–water and satd $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated to give 2,3,4-tri-*O*-benzoyl-α-L-fucopyranosyl bromide **6** as a syrup. TLC in 1:2 EtOAc–hexane showed that the product contained a small amount of the starting material, but NMR showed that it was sufficiently pure (> 80%) for the next step; ^1H NMR ($CDCl_3$): δ (d, *J* 6.5, 3 H-6), 4.50 (dqt, *J* 1.0, 6.5 Hz, H-5), 5.63 (dd, *J* 4.0, 10.4 Hz, H-2), 5.85 (dd, *J* 3.4, 1.0 Hz, H-4), 6.02 (dd, *J* 10.5, 3.5 Hz, H-3), and 6.94 (d, *J* 4.0 Hz, H-1). ^{13}C NMR ($CDCl_3$): δ 15.8 (C-6), 68.6 (C-2), 69.2, 70.4, 70.8 (C-3,4,5), and 89.4 (C-1).

Crude **6** (15 g) was dissolved in dry CH_2Cl_2 (50 mL) and stirred with 3A molecular sieves (2 g) for 10 min. A solution of triethylammonium dibenzyl phosphate (25.2 mmol) in dry CH_2Cl_2 (15 mL) was added together with Ag_2CO_3 (8.2 g, 29.7 mmol). (Triethylammonium dibenzyl phosphate was prepared by mixing dibenzyl phosphate with excess triethylamine at 0°C, followed by concentration in a high vacuum. It was then dissolved in CH_2Cl_2 and stirred with 3A molecular sieves for > 2 h.) The resulting mixture was stirred in a foil-covered flask under Ar for 15 h and then filtered through Celite and concentrated. The resulting syrup (17.8 g) was purified by chromatography on a silica gel column (6.5 × 28 cm) with elution with 1:2 → 2:1 EtOAc–hexane to give pure 2,3,4-tri-*O*-benzoyl-β-L-fucopyranosyl dibenzyl phosphate **7** (12.1 g, 73% overall) as a syrup; the compound decomposed within days at 0°C and, therefore, had to be used immediately; ^1H NMR ($CDCl_3$): δ 1.35 (d, *J* 6.5 Hz, 3 H-6), 4.21 (dqt, *J* 1.1, 6.5 Hz, H-5), 4.77, 4.86 (dd, 2 H, *J* 7.2, 11.7 Hz), 5.10, 5.14 (dd, 2 H, *J* 7.6, 11.7 Hz), 5.57 (dd, *J* 10.4, 3.4 Hz, H-3), 5.67 (dd, *J* 7.2, 8.0 Hz, H-1), 5.75 (dd, *J* 3.5, 1.0 Hz, H-4), and 5.89, (dd, *J* 8.0, 10.4 Hz, H-2). ^{13}C NMR ($CDCl_3$): δ 16.2 (s, C-6), 69.4 (d, 1 C, *J* 5.1 Hz), 69.6 (d, 1 C, *J* 5.9 Hz), 69.8 (d, *J* 9.4 Hz, C-2), 70.7 (s, C-4), 70.9 (s, C-3), 71.8 (s, C-5), and 97.1 (d, *J* 4.8 Hz, C-1).

Compound **7** (12.1 g, 16.4 mmol) was dissolved in toluene (100 mL), and pyridine (20 mL) and triethylamine (15 mL) were added. The solution was hydrogenated in an H_2 atmosphere with 10% activated Pd–C (700 mg) as catalyst. When TLC in 6:3:1 propanol–25% $NH_3 \cdot H_2O$–H_2O showed quantitative reaction, the mixture was filtered and the filtrate concentrated to give bis(triethylammonium) 2,3,4-tri-*O*-benzoyl-β-L-fucopyranosyl phosphate (**10**) as a dry foam (12.2 g, 98%); ^1H NMR ($CDCl_3$): δ 1.28 (d, 3 H-6), 4.08 (bqt, H-5), 5.44–5.55 (m, H-1,3),

and 5.63–5.77 (m, H-2,4); ^{13}C NMR: δ 16.2 (s, C-6), 22.3, 43.7 ($CH_3CH_2NH^+$), 69.7 (s, C-4), 70.5 (d, J 8.0 Hz, C-2), 71.2 (s, C-3), 72.2 (C-5,6), and 95.9 (d, J 4.1 Hz, C-1). Compound **10** (12.2 g) was dissolved in MeOH (40 mL) and cyclohexylamine (40 mL) and heated at reflux for 4 h. The reaction was monitored by TLC in 6:3:1 propanol–25% $NH_3 \cdot H_2O$–H_2O. When quantitative debenzoylation was achieved, the mixture was concentrated and fractionated between water and $CHCl_3$. The water phase was washed three times with $CHCl_3$ and then concentrated to dryness by coevaporating MeOH. The product was dissolved in hot EtOH and precipitated by addition of acetone. The product was separated by filtration to give **11** as a white solid (6.3 g, 63% overall yield); mp 177–179°C (brown color developed before melting); the NMR data are in agreement with published data[19]: ^1H NMR (D_2O, pH 7.0): δ 1.07 (m, 2 H), 1.16 (d, J 6.8 Hz, 3 H-6), 1.24 (m, 8 H), 1.55 (m, 2 H), 1.70 (m, 4 H), 1.88 (m, 4 H), 3.04 (m, 2 H), 3.41 (dd, J 7.8, 9.9 Hz, H-2), 3.57 (dd, J 9.9, 3.5 Hz, H-3), 3.62 (dd, J 3.5, 0.9 Hz, H-4), 3.71 (dqt, J 0.9, 6.8 Hz, H-5), and 4.73 (t, J 7.8 Hz, H-1); ^{13}C NMR (D_2O, pH 7.0): δ 17.1 (s, C-6), 25.4 (s, 2 NH_3 $^+C_6H_{11}$) 25.9, 31.9, 51.9, 72.9 (s, C-4), 72.7 (s, C-5), 73.5 (d, J 5.1 Hz, C-2), 74.4 (s, C-3), and 99.1 (d, J 4.3 Hz, C-1). Anal. Calcd for $C_{18}H_{39}N_2O_8P \cdot 0.5H_2O$: C, 47.88; H, 8.93; N, 6.20; P, 7.08. Found: C, 47.40; H, 8.72; N, 6.04; P, 7.14.

Guanosine diphosphate β-L-fucopyranose, dilithium salt (**12**).—Compound **11** (3.7 g, 8.4 mmol) was dissolved in water (40 mL), applied to a Dowex 50 column (Et_3N^+; 20 × 2 cm), and eluted with water (200 mL). The combined fractions were concentrated, and the residual solvent was coevaporated with pyridine six times at < 35°C and reduced pressure, keeping the flask moisture-free by using Ar to bring the pressure back to normal. The dry **11** was dissolved in dry pyridine and added to a suspension of guanosine monophosphate morpholidate and 4-morpholine-*N*,*N*'-dicyclohexylcarboxamidine (4.3 g, 5.9 mmol, ~ 95% pure, Sigma) in pyridine (10 mL). The activated nucleoside monophosphate was previously dried before reaction by coevaporating dry pyridine three times. After coevaporating the mixture with pyridine two more times it was dissolved (suspended) in dry pyridine (80 mL) and vigorously stirred under Ar for 3.5 days. The reaction was monitored by phosphorus NMR (121 MHz). The product was concentrated, dissolved in a minimum amount of water and applied to a Dowex 1 column (Cl^-; 22 × 2 cm, 200–400 mesh). The column was eluted with a LiCl gradient (0.0 → 0.5 M) made of water (1 L) and 0.5 M LiCl (1 L) in two connected vessels, and samples were collected in 20 mL fractions. The appropriate fractions were concentrated and desalted on a Sephadex G-10 column (50 × 2.5 cm) to give the Li salt of GDP-Fuc (1.44 g, 43%) as a syrup. By reference to an internal standard of a known amount of acetonitrile, the product was established by ^1H NMR to be free of LiCl. Dissolution of the syrup in water (5–7 mL) followed by addition of acetone (45 mL) gave **12** as a white solid (1.12 g); ^1H NMR (D_2O): δ 1.14 (d, J 6.7 Hz, H-6″), 3.48 (dd, J 7.9, 10.0 Hz, H-2″), 3.58 (dd, J 10.0, 3.4 Hz, H-3″), 3.63 (bd, J 3.2 Hz, H-4″), 3.69 (bq, J 6.7 Hz, H-5″), 4.16 (m, 2 H-5′), 4.27 (m, H-4′), 4.44 (dd, J 4.0

Hz, H-3′), 4.65 (t, *J* 5.5 Hz, H-2′), 4.84 (t, *J* 8.0 Hz, H-1″), 5.81 (d, *J* 5.8 Hz, H-1′), and 8.0 (s, H-8); ^{13}C NMR (D_2O): δ 16.5 (s, C-6″), 66.3 (d, *J* 5.2 Hz, C-5′) 71.4 (s, C-3′), 72.1 (d, *J* 8.0 Hz, C-2″), 72.5, 72.2 (s, C-4″,5″), 73.5 (s, C-3″), 74.9 (s, C-2′) 84.5 (d, *J* 9.2 Hz, C-4′), 87.9 (s, C-1′), 99.5 (d, *J* 5.9 Hz, C-1″), 117.0 (s, C-5), 138.4 (s, C-8), 152.6 (s, C-4), 154.8 (s, C-2), and 159.7 (C-6,6′). FABMS: *m/z* 594 $[M - Li]^-$. In addition, dilithium β-L-fucopyranosyl phosphate (1.34 g, 5.2 mmol) could be recovered as a second fraction from the ion-exchange chromatography.

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