Glycosyl Imidates, 47[1]

Stereospecific Synthesis of α- and β-L-Fucopyranosyl Phosphates and of GDP-Fucose via Trichloroacetimidate

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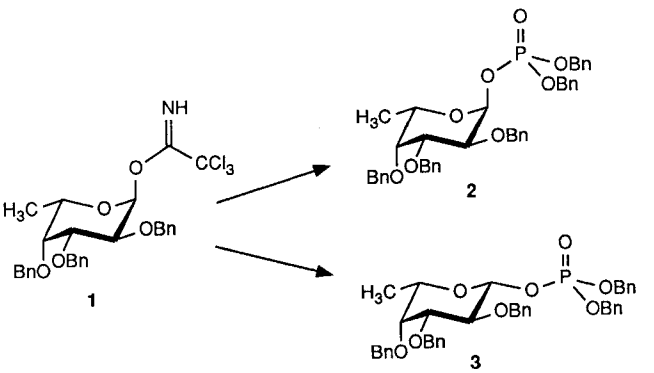
Reaction of the benzyl- and acetyl-protected α-trichloroacetimidates **1** and **6α** with dialkyl and diaryl phosphates in the presence of traces of acid affords stereoelectively the thermodynamically more stable α-L-fucopyranosyl phosphates **2**, **7** and **8**, respectively, in high yields. The use of very pure, recrystallized dibenzyl phosphate results in the stereoeletive formation of the β-L-fucopyranosyl phosphates **3** and **9**. In each case separation of the anomers is not required because of the very high stereoselectivity of the reactions. After deprotection the fucose 1-phosphate **12** is coupled with GMP morpholidate **10** to yield GDP-fucose **13**. After the development of a new purification procedure for GDP-fucose **13** we have obtained a very pure compound suitable for biochemical investigations. Analytical and preparative HPLC has been performed on reversed-phase columns using a volatile buffer system (triethylammonium hydrogen carbonate) as the eluant.

L-Fucose is included in bacterial lipopolysaccharides and in blood-group substances[2,3] or other mammalian glycosphingolipids[4,5]. Fucosylated glycolipids have antigenic properties and play an important role in cell-growth regulation and cell differentiation[6]. Therefore, the metabolism, development during cell growth, and oncogenic transformation of glycolipds especially in tumor cells have been intensively studied[2,6]. GDP-fucose is the substrate for fucosyl transferases involved in the biosynthesis of fucose-containing oligosaccharides. Although much GDP-fucose is required for these biochemical investigations, but only one chemical synthesis has been reported[7]. Enzymatic syntheses[8-10] proceeding via fucose 1-phosphate or via GDP-mannose are suitable only for the preparation of very small quantities.

The key intermediate in the chemical synthesis of GDP-fucose is fucose 1-phosphate, whose availability is the limitating factor. α-Fucopyranosyl phosphate can be readily synthesized according to the chlorophosphoamidite method[11]. The reported syntheses, however, of β-fucopyranosyl phosphate[12,7] proceed with low yields and low stereoselectivity, probably due to its increased lability against acids[13]. So far, enzymatic syntheses[8,14] of β-fucopyranosyl phosphate are not suitable for large-scale preparations. Owing to the good results obtained by use of trichloroacetimidates in the synthesis of glycosyl phosphates[15,16] we have developed new syntheses for α- and β-fucopyranosyl phosphates operating with high stereoselectivity. The trichloroacetimidates of fucose are also useful as glycosyl donors in the chemical synthesis of fucose-containing oligosaccharides[17].

Using the easily available benzyl-protected trichloroacetimidate **1**[17], we have synthesized the benzyl-protected fucosyl phosphates **2** and **3** in high yields. Reaction of **1** with dibenzyl phosphate in the presence of traces of acid yields stereoselectively the thermodynamically more stable α anomer **2**. The use of very pure recrystallized dibenzyl phosphate resulted in the stereoselective formation of the β anomer **3**. The anomeric configurations of **2** and **3** have been determined by ^{1}H-NMR analysis. The coupling constants of **2** ($J_{1,2}$ = 3.4, $J_{2,P}$ = 3.0 Hz) are characteristic of α anomers, and the corresponding values of **3** ($J_{1,2}$ = 7.7, $J_{2,P} \approx$ 0 Hz) confirm the β configuration.



Starting from tetra-*O*-acetyl-L-fucose **4** the synthesis of tri-*O*-acetyl-L-fucose **5** is reported[7,18] to proceed via the halogenose and then silver-catalysed halogen substitution. We have replaced this procedure by a one-step process by applying the hydrazine acetate method for the direct cleavage of the anomeric acetyl group. The resulting anomeric mixture of **5** is used for the synthesis of glycosyl imidates according to reported procedures[19,20]. Depending on the employed base, it is possible to control the stereoselectivity of the imidate formation. Reaction of **5** with trichloroaceto-

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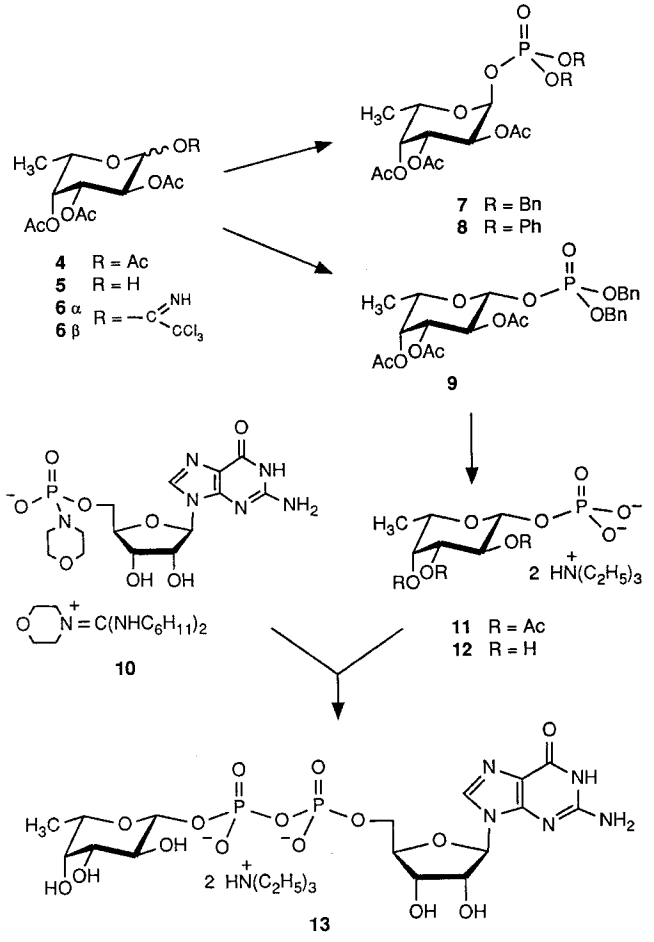
nitrile and DBU gives the anomers **6α** and **6β** in a ratio of 6.7:1 in high yield (total 92%). The use of sodium hydride affords stereoelectively **6α** (71%), and the use of potassium carbonate a mixture of **6α** and **6β** in a ratio of 1:1.3 (total 76%). The anomeric configuration has been ascertained by the coupling constants $J_{1,2}$ (**6α**: 3.4 Hz, **6β**: 8.2 Hz). The trichloroacetimidates **6α** and **6β** are easily separated by liquid chromatography in order to have available anomerically pure glycosyl donors for the synthesis of glycosyl phosphates or fucose-containing oligosaccharides.



The acetyl-protected fucosyl phosphates are synthesized according to the procedure used for preparation of the benzyl-protected derivatives. The thermosynamically more stable α anomer **7** is prepared by reaction of **6α** with dibenzyl phosphate containing traces of acid. The use of diphenyl phosphate leads to the formation of the phosphate derivative **8**. With very pure, recrystallized dibenzyl phosphate the β anomer **9** is obtained stereoselectively in high yield. The coupling constants of **7** ($J_{1,2}$ = 3.4, $J_{2,P}$ = 2.6 Hz) and of **8** ($J_{1,2}$ = 3.4, $J_{2,P}$ = 2.9 Hz) are characteristic of α anomers. The β configuration of **9** has been ascertained after deprotection. Cleavage of the benzyl ester groups by catalytic hydrogenation yields the triethylammonium salt **11**. Deacetylation with triethylamine in aqueous methanol furnishes the unprotected β-L-fucosyl phosphate **12**. The ^{1}H-NMR data of **12** ($J_{1,2}$ = 7.6, $J_{1,P} \approx 0$ Hz) confiirm the β configuration and the retention of the anomeric configuration during the deprotection steps.

For the synthesis of nucleoside 5′-diphosphorous acid derivatives several methods are reported[21] using different activated GMP derivatives. According to the reported synthesis[7] of GDP-fucose we have chosen the condensation of β-L-fucosyl phosphate **12** with GMP 5′-morpholidophosphate **10**[22,23]; however, improvements and changes especially in the purification procedure are required. The solubility is increased by use of the tris-*n*-octylammonium salt of the β-L-fucosyl phosphate. The condensation with GMP morpholidophosphate **10** is monitored by analytical HPLC. The resulting GDP-fucose **13** is isolated and purified by preparative HPLC on reversed-phase columns. Using a volatile buffer system (triethylammonium hydrogen carbonate) as eluant, we have easily obtained a product free of buffer salts by evaporation and subsequent lyophilisation. Because of its very high purity the product is suitable for biochemical investigations. The NMR data are in good agreement with the reported data[7] and prove the retention of the β configuration of the fucose moiety ($J_{1,2}$ = 7.7, $J_{2,P} \approx 0$ Hz).

Experimental

Melting points are uncorrected. – ^{1}H NMR and ^{13}C NMR (internal standard tetramethylsilane): Bruker WM 250 Cryospec and Jeol GNM-GX 400; ^{31}P NMR (internal standard phosphoric acid): Jeol GNM-GX 400. – Optical rotations: Perkin-Elmer 241 MC polarimeter. – Thin-layer chromatography (TLC): DC-Plastikfolien Kieselgel 60 F_{254} (Merck), detection by UV light (254 nm) or 10% H_2SO_4 and heating to 110°C. – Column chromatography: Kieselgel 60 (Merck; Korngröße 0.063–0.200 mm). – HPLC: LiChrospher RP 18 (Merck, 125 × 4 mm, 5 µm); 0.05 M triethylammonium hydrogen carbonate (adjusted to pH = 7.1 with acetic acid) containing 3.5% acetonitrile was used as eluant (0.5 ml/min); t_R = 3.24 min (GMP), 3.85 min (GDP-fucose), 6.27 min (GPPG), 12.7 min (GMP morpholidate). For preparative HPLC (Merck, LiChrosorb RP 18, 250 × 10 mm, 7 µm) the same eluant as above (3.0 ml/min) was used. Because of the use of a volatile buffer, the product can be isolated very easily by evaporation and subsequent lyophilization.

Dibenzyl 2,3,4-Tri-O-benzyl-α-L-fucopyranosyl Phosphate (**2**): A solution of **1**[17] (0.50 g, 0.86 mmol) in dry dichloromethane (20 ml) and commercially available dibenzyl phosphate (0.24 g, 0.86 mmol) is stirred under nitrogen at room temp. for 2 h. If the dibenzyl phosphate is very pure, it is necessary to add a small amount of diethyl ether–boron trifluoride to achieve the formation of the α-glucosyl phosphate **2**. Evaporation and chromatography (toluene/acetone, 9:1) yields **2** (0.54 g, 90%) as a colourless oil; R_f = 0.60 (toluene/acetone, 7:1) $[\alpha]_D^{29}$ = −68.2 (*c* = 1, chloroform), $[\alpha]_{578}^{29}$ = −70.7 (*c* = 1, chloroform). – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.00 (d, $J_{5,6}$ = 6.4 Hz, 3H, 6-H), 3.57 (d, $J_{3,4}$ = 2.3 Hz, 1H, 4-H), 3.79 (dd, $J_{2,3}$ = 10.0, $J_{3,4}$ = 2.3 Hz, 1H, 3-H), 3.91 (q, $J_{5,6}$ = 6.4 Hz, 1H, 5-H), 4.04 (ddd, $J_{2,P}$ = 3.0, $J_{1,2}$ = 3.4, $J_{2,3}$ = 10.0 Hz, 1H, 2-H), 4.54–4.99 (m, 10H, $CH_2C_6H_5$), 5.85 (dd, $J_{1,2}$ = 3.4, $J_{1,P}$ = 6.1 Hz, 1H, 1-H), 7.13–7.30 (m, 25H, C_6H_5). – ^{31}P NMR (161.7 MHz, $CDCl_3$): δ = −1.56.

$C_{41}H_{43}O_8P$ (694.7) Calcd. C 70.88 H 6.24
Found C 71.00 H 6.30

Dibenzyl 2,3,4-Tri-O-benzyl-β-L-fucopyranosyl Phosphate (**3**): A solution of **1**[17] (0.50 g, 0.86 mmol) in dry dichloromethane (20 ml)

and recrystallized dibenzyl phosphate (0.24 g, 0.86 mmol) is stirred under nitrogen at room temp. for 1 h. Evaporation ad chromatography (toluene/acetone, 7:1) yield **3** (0.57 g, 95%) as a colourless oil; R_f = 0.45 (toluene/acetone, 7:1), $[\alpha]_D^{24}$ = −9.7 (*c* = 1, chloroform), $[\alpha]_{578}^{24}$ = −10.2 (*c* = 1, chloroform). – ^{1}H NMR 400 MHz, $CDCl_3$): δ = 1.18 (d, $J_{5,6}$ = 6.4 Hz, 3H, 6-H), 3.57−3.61 (m, 3H, 3-H, 4-H, 5-H), 3.92 (dd, $J_{1,2}$ = 7.7, $J_{2,3}$ = 9.5 Hz, 1H, 2-H), 4.67−5.10 (m, 10H, $CH_2C_6H_5$), 5.19 (dd, $J_{1,2}$ = 7.7, $J_{1,P}$ = 7.1 Hz, 1H, 1-H), 7.18−7.37 (m, 25H, C_6H_5). – ^{31}P NMR (161.7 MHz, $CDCl_3$): δ = −1.65.

$C_{41}H_{43}O_8P$ (694.7) Calcd. C 70.88 H 6.24
Found C 71.00 H 6.34

2,3,4-Tri-O-acetyl-L-fucopyranose[7,18]: (**5**): A solution of 1,2,3,4-tetra-*O*-acetyl-α-L-fucopyranose[7,18] (**3α** (6.00 g, 18.0 mmol) and hydrazinium acetate (1.99 g, 21.6 mmol) in dry dimethyl formamide (20 ml) is stirred at 50°C for 4.5 h. After addition of ethyl acetate, the mixture is extracted twice with aqueous sodium chloride. Evaporation and chromatography (petroleum ether/ethyl acetate, 1:1) yield **5** [4.15 g, 79%, α:β = 3:1 (on the basis of ^{1}H NMR)]; R_f = 0.47 (petroleum ether/ethyl acetate, 1:1). – ^{1}H NMR (400 MHz, $CDCl_3$) of **5α**: δ = 1.15 (d, $J_{5,6}$ = 6.6 Hz, 3H, 6-H), 2.00, 2.10, 2.18 (3s, 9H, 3 CH_3CO), 3.98 (br. s, 1H, OH), 4.43 (q, $J_{5,6}$ = 6.6 Hz, 1H, 5-H), 5.14 (dd, $J_{2,3}$ = 10.9, $J_{3,4}$ = 3.0 Hz, 1H, 3-H), 5.31 (d, $J_{3,4}$ = 3.0 Hz, 1H, 4-H), 5.41 (dd, $J_{1,2}$ = 3.4, $J_{2,3}$ = 10.9 Hz, 1H, 2-H), 5.46 (d, $J_{1,2}$ = 3.4 Hz, 1H, 1-H); of **5β**: δ = 1.23 (d, $J_{5,6}$ = 6.6 Hz, 3H, 6-H), 2.00, 2.09, 2.19 (3s, 9H, CH_3CO), 3.88 (q, $J_{5,6}$ = 6.6 Hz, 1H, 5-H), 4.25 (br. s, 1H, OH), 4.70 (d, $J_{1,2}$ = 7.6 Hz, 1H, 1-H), 5.03 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 2.9 Hz, 1H, 3-H), 5.10 (dd, $J_{1,2}$ = 7.6, $J_{2,3}$ = 10.5 Hz, 1H, 2-H), 5.25 (d, $J_{3,4}$ = 2.9 Hz, 1H, 4-H).

2,3,4-Tri-O-acetyl-α-L-fucopyranosyl Trichloroacetimidate (**6α**) *and 2,3,4-Tri-O-acetyl-β-L-fucopyranosyl Trichloroacetimidate* (**6β**). – *Procedure (a):* To a mixture of **5** (2.00 g, 6.89 mmol) and trichloroacetoitrile (7.0 ml, 70 mmol) in dry dichloromethane (20 ml) is added sodium hydride (0.25 g, 10.9 mmol) at room temp. under nitrogen. After stirring for 12 h the mixture is filtered through Celite, and the solvents are evaporated. Chromatography (petroleum ether/ethyl acetate, 3:1) of the residue yields **6α** (2.13 g, 71%).

Procedure (b): A mixture of **5** (0.50 g, 1.72 mmol), lithium chloride (73 mg, 1.72 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (0.20 ml, 1.34 mmol) and trichloroacetonitrile (2.0 ml, 1.34 mmol) and trichloroacetonitrile (2.0 ml, 20 mmol) in dry acetontirile (20 ml) is stirred under nitrogen at room temp. for 12 h. Evaporation and separation by chromatography (petroleum ether/diethyl ether, 2:5) yield **6α** (0.60 g, 80%) and **6β** (89 mg, 12%).

Procedure (c): A mixture of **5** (0.85 g, 2.93 mmol), trichloroacetonitrile (3 ml, 30 mmol) and potassium carbonate (2.73 g, 19.7 mmol) in dry dichloromethane (12 ml) is stirred under nitrogen at room temp. for 4 h. After filtration through Celite the solvent is evaporated. Separation of the residue by chromatography (petroleum ether/diethyl ether, 2:5) yields **6α** (.42 g, 33%) and **6β** (0.54 g, 43%).

6α: $[\alpha]_D^{23}$ = −116.0 (*c* = 1, chloroform), $[\alpha]_{578}^{23}$ = −119.5 (*c* = 1, chloroform), R_f = 0.54 (petroleum ether/ethyl acetate, 2:1), m.p. 107−109°C (from petroleum ether/diethyl ether). – IR: $\tilde{\nu}$ = 3340 cm^{-1} (NH), 1751 (CO), 1677 (CN). – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19 (d, $J_{5,6}$ = 6.4 Hz, 3H, 6-H), 2.02, 2.03, 2.20 (3s, 9H, CH_3CO), 4.38 (q, $J_{5,6}$ = 6.4 Hz, 1H, 5-H), 5.35 (dd, $J_{1,2}$ = 3.4, $J_{2,3}$ = 10.6 Hz, 1H, 2-H), 5.41 (d, $J_{3,4}$ = 2.7 Hz, 1H, 4-H), 5.44 (dd, $J_{2,3}$ = 10.6, $J_{3,4}$ = 2.7 Hz, 1H, 3-H), 6.55 (d, $J_{1,2}$ = 3.4 Hz, 1H, 1-H), 8.63 (s, 1H, NH).

6β: $[\alpha]_D^{23}$ = −24.5 (*c* = 1, chloroform), $[\alpha]_{578}^{23}$ = −25.5 (*c* = 1, chloroform), R_f = 0.34 (petroleum ether/ethyl acetate, 2:1), m.p. 60−63°C (from petroleum ether/diethyl ether). – IR: = 3300 cm^{-1} (NH), 1750 (CO), 1675 (CN). – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.27 (d, $J_{5,6}$ = 6.4 Hz, 3H, 6-H), 2.01, 2.02, 2.21 (3s, 9H, CH_3CO), 4.02 (q, $J_{5,6}$ = 6.4 Hz, 1H, 5-H), 5.12 (dd, $J_{2,3}$ = 10.2, $J_{3,4}$ = 3.2 Hz, 1H, 3-H), 5.30 (d, $J_{3,4}$ = 3.2 Hz, 1H, 4-H), 5.48 (dd, $J_{1,2}$ = 8.2, $J_{2,3}$ = 10.2 Hz, 1H, 2-H), 5.82 (d, $J_{1,2}$ = 8.2 Hz, 1H, 1-H), 8.69 (s, 1H, NH).

$C_{14}H_{18}Cl_3NO_8$ (434.7) Calcd. C 38.68 H 4.17 N 3.22
6α: Found C 38.69 H 4.15 N 3.18
6β: Found C 38.48 H 4.24 N 3.21

Dibenzyl 2,3,4-Tri-O-acetyl-α-L-fucopyranosyl Phosphate (**7**): A solution of **6α** (0.20 g, 0.46 mmol) in dry dichloromethane (7 ml) and commercially available dibenzyl phosphate (0.13 g, 0.47 mmol) is stirred under nitrogen at room temp. for 2.5 h. If the dibenzyl phosphate is very pure, it is necessary to add a small amount of diethyl ether−boron trifluoride to achieve the formation of the α-glycosyl phosphate **7**. Evaporation, chromatography (toluene/acetone, 7:1) and rechromatography (chloroform/diethyl ether, 20:1) yields **7** (0.23 g, 91%) as a colourless oil, R_f = 0.38 (toluene/acetone/triethylamine, 84:15:1), $[\alpha]_D^{24}$ = −83.7 (*c* = 1, chloroform), $[\alpha]_{578}^{24}$ = −87.3 (*c* = 1, chloroform). – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.05 (d, $J_{5,6}$ = 6.6 Hz, 3H, 6-H), 1.91, 2.00, 2.16 (3s, 9H, CH_3CO), 4.16 (q, $J_{5,6}$ = 6.6 Hz, 1H, 5-H), 5.05−5.09 (m, 4H, $CH_2C_6H_5$), 5.20 (ddd, $J_{1,2}$ = 3.4, $J_{2,P}$ = 2.6 Hz, $J_{2,3}$ = 10.9 Hz, 1H, 2-H), 5.27 (d, $J_{3,4}$ = 2.8 Hz, 1H, 4-H), 5.33 (dd, $J_{2,3}$ = 10.9, $J_{3,4}$ = 2.8 Hz, 1H, 3-H), 5.91 (dd, $J_{1,2}$ = 3.4, $J_{1,P}$ = 6.6 Hz, 1H, 1-H), 7.35 (m, 10H, C_6H_5). – ^{31}P NMR (161.7 MHz, $CDCl_3$): δ = −2.38.

$C_{26}H_{31}O_{11}P$ (550.5) Calcd. C 56.72 H 5.68
Found C 56.48 H 5.69

Diphenyl 2,3,4-Tri-O-acetyl-α-L-fucopyranosyl Phosphate (**8**): A solution of **6α** (0.50 g, 1.15 mmol) in dry dichloromethane (25 ml) and commercially available diphenyl phosphate (0.29 g, 1.16 mmol) is stirred under nitrogen at room temp. for 6.5 h. If the diphenyl phosphate is very pure, it is necessay to add a small amount of diethyl ether−boron trifluoride to achieve the formation of the α-glycosyl phosphate **8**. Evaporation and chromatography (petroleum ether/diethyl ehter, 1:5) yield **8** (0.38 g, 63%) as a colourless oil; R_f = 0.41 (petroleum ether/diethyl ehter, 1:5). – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.04 (d, $J_{5,6}$ = 6.4 Hz, 1H, 6-H), 1.87, 2.01, 2.17 (3 s, 9H, CH_3CO), 4.20 (q, $J_{5,6}$ = 6.4 Hz, 1H, 6-H), 1.87, 2.01, 2.17 (3 s, 9H, CH_3CO), 4.20 (q, $J_{5,6}$ = 6.4 Hz, 1H, 5-H), 5.23 (dd, $J_{2,3}$ = 10.83, $J_{3,4}$ = 2.8 Hz, 1H, 3-H), 5.30 (d, $J_{3,4}$ = 2.8 Hz, 1H, 4-H), 5.37 (ddd, $J_{1,2}$ = 3.4, $J_{2,3}$ = 10.8, $J_{2,P}$ = 2.9 Hz, 1H, 2-H), 6.09 (dd, $J_{1,2}$ = 3.4, $J_{1,P}$ = 6.1 Hz, 1H, 1-H), 7.17−7.40 (m, 10H, C_6H_5). – ^{31}P NMR (161.7 MHz, $CDCl_3$): δ = −13.3.

$C_{24}H_{27}O_{11}P$ (522.4) Calcd. C 55.17 H 5.21
Found C 54.97 H 5.25

Dibenzyl 2,3,4-Tri-O-acetyl-β-L-fucopyrnaosyl Phosphate (**9**): A solution of **6α** (0.30 g, 0.69 mmol) in dry dichloromethane (12 ml) and recrystallized dibenzyl phosphate (0.19 g, 0.68 mmol) is stirred under nitrogen at room temp. for 1 h. Evaporation, chromatography (toluene/acetone, 7:1) and rechromatography (petroleum ether/ether, 1:5) yield **9** (0.36 g, 86%) as a colourless oil; R_f = 0.30 (toluene/acetone, 7:1), $[\alpha]_D^{25}$ = +0.5 (*c* = 1, chloroform) $[\alpha]_{578}^{25}$ = +0.7 (*c* = 1, chloroform). – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.21 (d, $J_{5,6}$ = 6.4 Hz, 3H, 6-H), 1.91, 1.99, 2.19 (3s, 9H, CH_3CO), 3.91 (q, $J_{5,6}$ = 6.4 Hz, 1H, 5-H), 5.01−5.05 (m, 4H, $CH_2C_6H_5$), 5.10 (dd, $J_{2,3}$ = 7.45, $J_{3,4}$ = 2.4 Hz, 1H, 3-H), 5.26 (d, $J_{3,4}$ = 2.4 Hz, 1H, 4-H), 5.28−5.34 (m, 2H, 1-H, 2-H), 7.27 (m, 10H, C_6H_5). – ^{31}P NMR (161.7 MHz, $CDCl_3$): δ = −2.41.

$C_{26}H_{31}O_{11}P$ (550.5) Calcd. C 56.72 H 5.68
Found C 56.52 H 5.71

Bis(triethylammonium) 2,3,4-Tri-O-acetyl-β-L-fucopyranosyl Phosphate (**11**): A mixture of **9** (72 mg, 0.13 mmol) and palladium (4 mg) in 10 ml of dry tetrahydrofuran/ethyl acetate (1:1) is shaken under hydrogen. When TLC (R_f = 0.19; chloroform/methanol, 60:40) indicates the end of the reaction, the mixture is filtered and evaporated. Chromatography (chloroform/methanol/triethylamine, 60:39:1) and lyophilization in dioxane yields **11** (67 mg, 89%) as an amorphous powder. – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.19 (d, $J_{5,6}$ = 6.4 Hz, 3H, 6-H), 1.36 (t, J = 7.2 Hz, 18H, NCH_2CH_3), 1.97, 2.09, 2.16 (3s, 9H, CH_3CO), 3.09 (q, J = 7.2 Hz, 12H, NCH_2CH_3), 3.96 (q, $J_{5,6}$ = 6.4 Hz, 1H, 5-H), 4.75–5.25 (m, 4H, 1-H, 2-H, 3-H, 4-H), 12.06 (br. s, 2H, NH).

$C_{24}H_{49}N_2O_{11}P \cdot H_2O$ (590.6) Calcd. C 48.80 H 8.70 N 4.74
Found C 48.98 H 8.78 N 4.64

Bis(triethylammonium) β-L-Fucopyranosyl Phosphate (**12**): To a solution of **11** (80.0 mg, 0.135 mmol) in 3 ml of methanol/water (1:1) is added triethylamine (0.5 ml). After 18 h the mixture is concentrated. Lyophilisation of the residue from water yields **12** (73.8 mg, 100%) as an hygroscopic white powder (containing 18% water), which is used for the next step without further purification. – ^{1}H NMR (250 MHz, CD_3OD): δ = 1.25 (d, $J_{5,6}$ = 6.4 Hz, 3H, 6-H), 1.28 (t, J = 7.3 Hz, 18H, NCH_2CH_3), 3.13 (q, J = 7.3 Hz, 12H, NCH_2CH_3), 3.48–3.61 (m, 3H, 2-H, 3-H, 4-H), 3.67 (q, $J_{5,6}$ = 6.4 Hz, 1H, 5-H), 4.85 (dd, $J_{1,2}$ = $J_{1,P}$ = 7.6 Hz, 1H, 1-H).

Bis(triethylammonium) β-L-Fucopyranosyl Guanosine 5′-Pyrophosphate[7] (**13**): To a solution of **12** (73.8 mg, 0.135 mmol) in dry pyridine (5 ml) is added tri-*n*-octylamine (0.117 ml, 0.135 mmol). The mixture is concentrated and the resulting tri-*n*-octylammonium salt is dried by repeated evaporation with pyridine at 10^{-2} Torr and transferred to a round-bottomed flask with molecular sieves, which was dried by heating with a Bunsen burner at 10^{-3} Torr. After addition of *N,N′*-dicyclohexyl-4-morpholinecarboxaminidinum guanosine 5′-morpholidophosphate[22,23] (**10**) (109 mg, 0.15 mmol) in pyridine/dimethylformamide (1:1) (6 ml), which is proviously dried by repeated evaporation with pyridine/dimethylformamide (1:1) (3 × 10 ml) at 10^{-2} Torr, the reaction is monitored by analytical HPLC. After standing for 5 d [68% yield (on the basis of HPLC analysis)] the mixture is evaporated to dryness. Dissolution in 0.05 M triethylammonium hydrogen carbonate (adjusted to pH = 7.4 with acetic acid), removal of tri-*n*-octylamine by extraction with diethyl ether, and separation from the byproducts by preparative HPLC (t_R = 9.4 min) yields **13** (23.3 mg, 22%) as an amorphous solid. – ^{1}H NMR (400 MHz, D_2O): δ = 1.06 (d, $J_{5'',6''}$ = 6.4 Hz, 3H, 6″-H), 1.11 (t, J = 7.3 Hz, 18H, NCH_2CH_3), 3.03 (q, J = 7.3 Hz, 12H, NCH_2CH_3), 3.38 (dd, $J_{2'',3''}$ = 9.9, $J_{1'',2''}$ = 7.7 Hz, 1H, 2″-H), 3.49 (dd, $J_{2'',3''}$ = 9.9, $J_{3'',4''}$ = 3.4 Hz, 1H, 3″-H), 3.54 (d, $J_{3'',4''}$ = 3.4 Hz, 1H, 4″-H), 3.60 (q, $J_{5'',6''}$ = 6.4 Hz, 1H, 5″-H), 4.04 (m, 2H, 5′$_A$-H, 5′$_B$-H), 4.18 (br. s, 1H, 4′-H), 4.36 (dd, $J_{2',3'}$ = 5.0, $J_{3',4'}$ = 3.2 Hz, 1H, 3′-H), 4.65 (br. s, HOD, 2′-H), 4.75 (dd, $J_{1'',2''}$ = 7.7, $J_{1'',P}$ = 8.1 Hz, 1H, 1″-H), 5.76 (d, $J_{1',2'}$ = 6.4 Hz, 1H, 1′-H), 7.95 (s, 1H, 8-H).

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