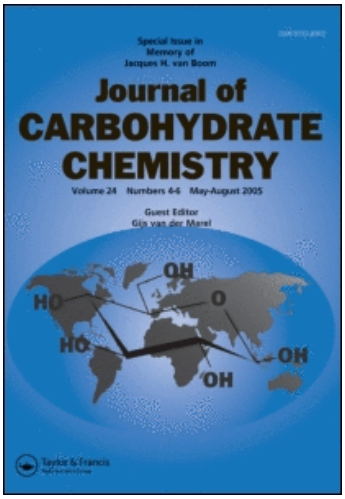

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SYNTHESIS OF GUANOSINE 5'-(β-L-FUCOPYRANOSYL)-DIPHOSPHATE REVISITED

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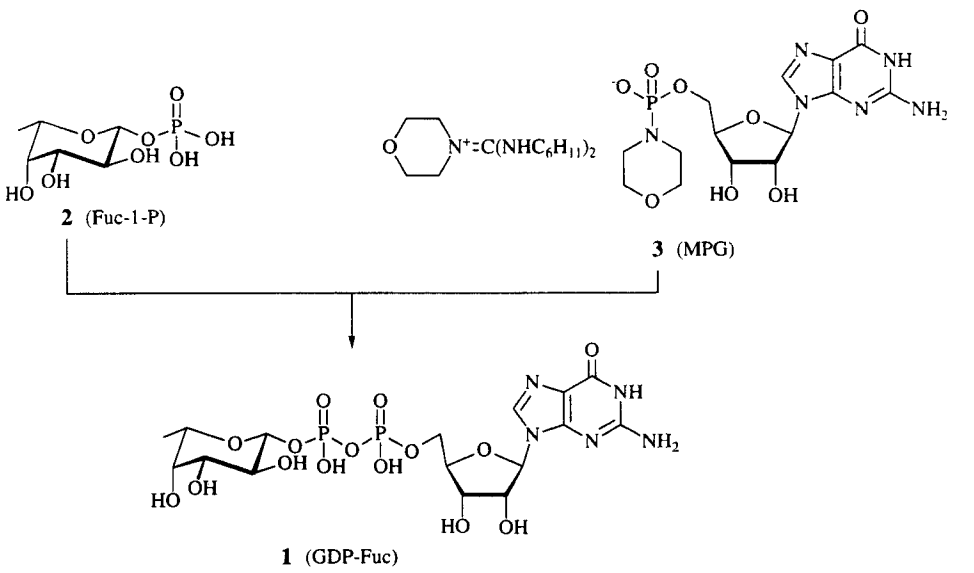
ABSTRACT

It will be demonstrated that a successful synthesis of β-L-fucopyranose-1-phosphate (**2**), a key intermediate in the preparation of guanosine 5'-(β-L-fucopyranose)-diphosphate (**1**), strongly depends on the nature of the acyl protecting groups for the non-anomeric hydroxyl functions. Thus, the perbenzoylated, instead of peracetylated, α-L-fucopyranosyl trichloroacetimidate (**11**) or the corresponding ethyl β-thiofucopyranoside proved to be a convenient starting compound for the preparation of **2**. Further, condensation of *N,N'*-dicyclohexyl-4-morpholinecarboxamidinium guanosine 5'-morpholidophosphate with excess **2** gave the title compound without concomitant formation of bisguanosine-5'-diphosphate (**16**).

INTRODUCTION

In the past few years glycosyltransferases have been applied in the construction of various glycoconjugates and oligosaccharides.[1] For instance, the enzymatic preparation of sialyl Lewis^x (SLe^x: NeuAcα2→3Galβ1→4[Fucα1→3] GlcNAc), a ligand for E-selectin[2] has recently been reported by several groups.[3] The last step in the enzymatic synthesis of SLe^x, the introduction of a fucose moiety by

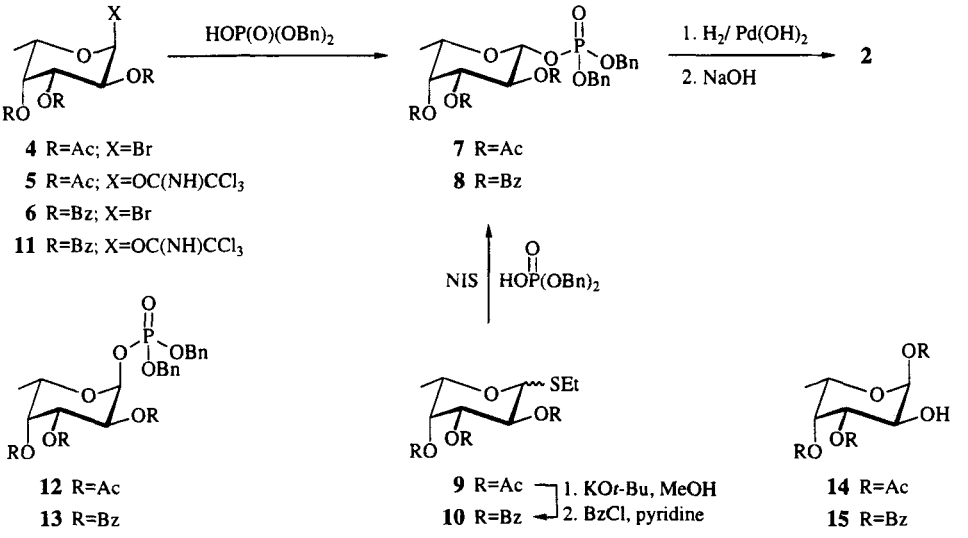


Scheme 1. Nunez approach to GDP-Fuc.

α(1→3)fucosyl-transferase, is hampered by the limited availability of the donor substrate for fucosyltransferases, *i.e.* guanosine 5'-(β-L-fucopyranose)-diphosphate (GDP-Fuc, compound **1** in Scheme 1). Up to now, enzymatic procedures towards GDP-Fuc, starting from either GDP-mannose[4] or L-fucopyranose,[5] have only been applied in small scale preparations. Consequently, much effort[6-11] has been directed towards the chemical synthesis of GDP-Fuc. We now report in detail[9] on the use of thioethyl fucopyranosyl donors in the preparation of β-L-fucopyranose 1-phosphate and the conversion of the latter compound into GDP-Fuc.

RESULTS AND DISCUSSION

A common approach to GDP-Fuc entails, as originally devised by Nunez *et al.*[6], the coupling of β-L-fucopyranose 1-phosphate (Fuc-1-P, **2**) with the activated guanosine 5'-morpholidophosphate **3** (MPG, Scheme 1). It is evident that the



Scheme 2. Possible routes to phosphotriester precursors 7, 8 of Fuc-1-P (2).

availability of anomerically pure Fuc-1-P is an essential element in a successful synthesis of the target molecule GDP-Fuc.

One route of synthesis of anomerically pure Fuc-1-P comprises, as portrayed in Scheme 2, the condensation of dibenzyl phosphate (DBP) with peracetylated L-fucopyranosyl donors **4-5**, having an α-oriented leaving group, and subsequent deprotection of the resulting β-phosphotriester intermediate (**7**). It was expected that the stereoselective introduction of the requisite 1,2-*trans* linkage would be promoted by a participating 2-*O*-acyl group in the fucopyranosyl donors. However, initial studies revealed that a 2-*O*-acetyl group was not completely satisfactory in serving this purpose. For instance, Hindsgaul *et al.*[7] observed that treatment of 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide (**4**) with DBP (tetrabutylammonium salt) was accompanied by the formation of the unwanted α-isomer **12**. Interestingly, Whitesides *et al.*[11] reported that coupling of the α-bromide **4** with DBP, in the absence or presence of silver carbonate, furnished exclusively the α-linked phosphotriester **12**. On the other hand, Schmidt *et al.*[8] obtained the anomerically pure β-anomer **7** by condensing the α-trichloroacetimidate **5** with recrystallized DBP. The

outcome of the latter glycosylation is in sharp contrast with the result obtained in the *N*-iodosuccinimide (NIS) promoted condensation of recrystallized DBP with ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside[12] (**9**). Analysis of the reaction mixture revealed the presence of two products, the minor of which was in all aspects identical with the β-phosphotriester derivative **7**. The identity of the major compound was, as evidenced by spectral data, in accord with 1,3,4-tri-*O*-acetyl-α-L-fucopyranose **14**. It was also established that **7**, dissolved in dichloromethane, was completely transformed into **14** within nine hours at 20 °C. In this respect it is of interest to note that the migration of a 2-*O*-acetyl group to the anomeric centre has also been observed in glycosylations with peracetylated glycosyl bromides[13,14] or diphenyl phosphates.[15] The failure to prepare **7** from the β-thiofucoside **9** urged us to repeat the original trichloroacetimidate procedure of Schmidt *et al.*. The results of this study can be summarized as follows. Despite many attempts we failed to suppress, as gauged by TLC analysis, the occurrence of the unwanted product **14**. However, it is now well established that deacylation on O-2 can be overcome by using the more effectively participating benzoyl group. The beneficial effect of the benzoyl group was nicely demonstrated by Whitesides *et al.*,[11] and also independently by Wong *et al.*,[10] in the successful preparation of the benzoylated β-phosphotriester derivative **8**, starting from the α-fucosyl bromide **6**. It was therefore not surprising that the perbenzoylated α-trichloroacetimidate **11** as well as the corresponding ethyl β-thiofucoside **10** could be converted stereoselectively into **8**. Thus, coupling of **11**, obtained in 95% yield by the reaction of 2,3,4-tri-*O*-benzoyl-α/β-L-fucopyranose and trichloroacetonitrile in the presence of DBU, with DBP afforded homogeneous **8** in 60% yield. A similar yield of β-phosphotriester **8** was obtained by NIS assisted condensation of **10β** with DBP. Apart from this, it was observed that both glycosylations proceeded without the occurrence of 1,3,4-tri-*O*-benzoyl-α-L-fucopyranose (**15**), indicating that the presence of benzoyl groups also increases the stability of **8** compared to the corresponding acetylated derivative **7**. The latter is endorsed by the fact that the transformation of **8** into **15** is extremely slow (*e.g.* 45% of **15** was isolated after leaving a solution of **8** in dichloromethane for seven days at 20 °C). On the basis of the aforementioned results it was anticipated that the α-anomer of **10** would also be a suitable substrate for the preparation of **8**.

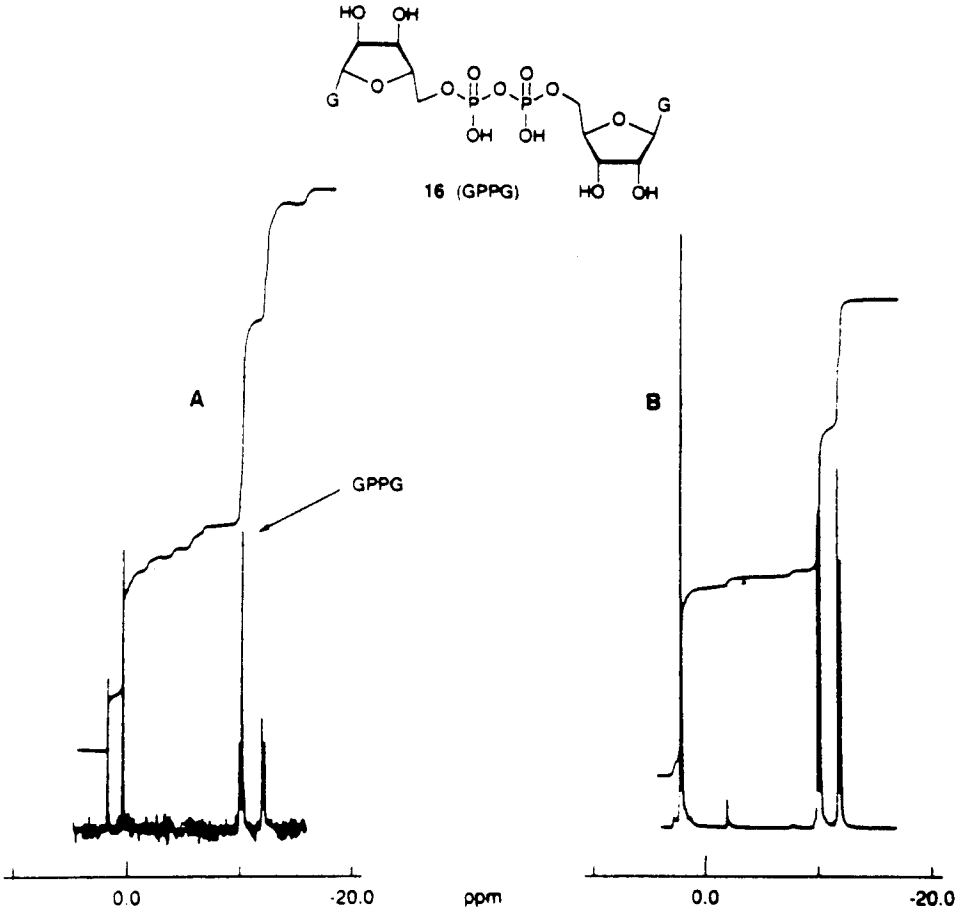


Figure 1. ^{31}P NMR spectrum (80.7 MHz) of crude GDP-Fuc (**1**), obtained *via* condensation of Fuc-1-P (**2**) with MPG (**3**), in a ratio of 1/1 (**A**) of 2/1 (**B**).

Surprisingly, no trace of the expected β-phosphotriester **8** could be detected, as evidenced by TLC analysis and NMR spectroscopy, in the NIS assisted coupling of **10**α with DBP. Instead, a major non-phosphorus containing compound, contaminated with an impurity which could not be removed by extensive silica gel chromatography, was isolated.

At this stage, attention was directed towards the preparation of GDP-Fuc (**1**). Hydrogenolysis of **8** and subsequent saponification gave, after further processing, the triethylammonium salt of Fuc-1-P (**2**) in 90% yield. Preliminary experiments

indicated that the condensation of equimolar amounts of the reactants *N,N'*-dicyclohexyl-4-morpholinecarboxaminidinium guanosine 5'-morpholidophosphate (**3**, MPG) and the tris-*n*-octylammonium salt of Fuc-1-P in DMF at 20 °C for six days or at 50 °C for 14 hours, was accompanied (see Figure 1) by the formation of bisguanosine 5'-diphosphate[16] (**16**, GPPG). However, it turned out that the occurrence of GPPG could be nullified (see Figure 1) using excess Fuc-1-P (two equivalents). Work-up and purification (Fractogel TSK HW-40) afforded homogeneous GDP-Fuc (**1**) in 48% yield (based on recovered Fuc-1-P).

In conclusion, the results presented in this paper show that a successful synthesis of Fuc-1-P (**2**), *via* a phosphotriester intermediate, is not only governed by the stability of the acyl protective groups and their proclivity to stabilize the transient oxocarbenium ion, but also by the orientation and nature of the leaving group at the anomeric centre. The latter is illustrated by the failure to prepare the perbenzoylated β-phosphotriester **8** from the corresponding L-fucose derivative **10** containing an α-oriented ethylthio group. In addition, the use of excess Fuc-1-P in the condensation with MPG (**3**) has a beneficial effect on the yield and ease of purifying the target compound **1**.

EXPERIMENTAL

General procedures. 1,2-Dichloroethane was distilled from P_2O_5 (5 g/L). Acetonitrile and pyridine were dried by refluxing overnight with CaH_2 (5 g/L) and then distilled. *N,N*-Dimethylformamide (DMF) was stirred overnight with CaH_2 (5 g/L) and then distilled under reduced pressure. Methanol was dried by refluxing with magnesium methoxide and then distilled. Tetrahydrofuran (THF) was distilled from $LiAlH_4$. Trichloroacetonitrile was distilled before use. Methanol was stored on 0.3 nm and all other solvents on 0.4 nm molecular sieves. Schleicher and Schüll DC Fertigfolien F 1500 LS 254 were used for TLC analysis. Compounds were visualized by UV light (254 nm) and by charring with 20% sulfuric acid in methanol. Column chromatography was performed on silica gel 60, 230-400 mesh (Merck). The petroleum ether used for elution during chromatography was light boiling (40-60 °C). Gel filtration was performed on Sephadex LH-20 (Pharmacia) or Fractogel TSK

HW 40. ^1H NMR (200 MHz), ^{13}C NMR (50.1 MHz) and ^{31}P NMR spectra (80.7 MHz) were recorded using a Jeol JNM-FX 200 spectrometer, unless stated otherwise. ^1H NMR (400 MHz), ^{13}C NMR (100 MHz) and ^{31}P NMR (162 MHz) spectra were recorded using a Bruker MSL-400 spectrometer. ^1H and ^{13}C Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard, ^{31}P chemical shifts are given in ppm relative to 85% H_3PO_4 as external standard.

Ethyl 2,3,4-Tri-*O*-benzoyl-1-thio-α/β-L-fucopyranoside (10). KO-*t*-Bu (750 mg, 6.7 mmol) was added to a solution of ethyl 2,3,4-tri-*O*-acetyl-1-thio-α/β-L-fucopyranoside[13] (**9**, 28.5 g, 85.0 mmol) in methanol (80 mL). After 2 h the reaction mixture was neutralized with Dowex 50 W X 4 [H^+], filtered and concentrated *in vacuo*. Residual methanol was removed by evaporation with pyridine (3 × 25 mL) and the residue was dissolved in pyridine (150 mL). Benzoyl chloride (31.5 mL, 268 mmol) was added at 0 °C over a period of 15 min. The mixture was allowed to warm to room temperature and stirring was continued for 1 h, when TLC analysis indicated completion of the reaction. The reaction was quenched with methanol and stirred for an additional 30 min. The mixture was diluted with dichloromethane and washed with water and saturated aqueous $NaHCO_3$, dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by column chromatography (diethyl ether/petroleum ether, 0/1 to 1/1, v/v). First **10α** was eluted (5.1 g, 11.6%). R_f 0.79 (diethyl ether/petroleum ether, 2/1, v/v); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 14.5 ($SCH_2\underline{C}H_3$), 15.9 (C-6), 24.7 ($S\underline{C}H_2CH_3$), 69.0, 77.2, 82.7, 83.0 (C-2, C-3, C-4, C-5), 87.5 (C-1), 127.6-132.9 ($CH_{arom.}$), 128.5, 129.5 ($C_{arom.}$), 165.0 (C=O).

Next **10β** was isolated in 79% yield (35.1 g). R_f 0.75 (diethyl ether/petroleum ether, 2/1, v/v); ^1H NMR ($CDCl_3$) δ 1.33 (t, 3 H, $SCH_2C\underline{H}_3$), 1.35 (d, 3 H, H-6, $J_{5,6}$ = 6.2 Hz), 2.86 (m, 2 H, $SC\underline{H}_2CH_3$), 4.13 (q, 1 H, H-5), 4.81 (d, 1 H, H-1, $J_{1,2}$ = 9.8 Hz), 5.62 (dd, 1 H, H-3, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.1 Hz), 5.78 (d, 1 H, H-4), 5.84 (t, 1 H, H-2, J = 10.0 Hz), 7.12-8.15 (m, 15 H, $H_{arom.}$); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 14.8 ($SCH_2\underline{C}H_3$), 16.6 (C-6), 24.0 ($S\underline{C}H_2CH_3$), 68.0, 71.2, 73.1, 73.7 (C-2, C-3, C-4, C-5), 83.5 (C-1), 128.2-133.4 ($CH_{arom.}$), 128.8-129.2 ($C_{arom.}$), 165.3, 165.8 (C=O).

2,3,4-Tri-*O*-benzoyl-α-L-fucopyranosyl Trichloroacetimidate (11). Anhydrous LiCl (42 mg, 1 mmol), DBU (119 mg, 0.78 mmol) and trichloroacetonitrile (12 mL, 12 mmol) were added to a solution of 2,3,4-tri-*O*-

benzoyl-α-L-fucopyranose[8] (476 mg, 1.00 mmol) in acetonitrile. The mixture was stirred for 16 h, the suspension was concentrated *in vacuo* and the crude product was purified by column chromatography (dichloromethane). Pure **11** was obtained in 95% yield (589 mg). R_f 0.80 (acetone/dichloromethane, 1/99, v/v); ^{1}H NMR ($CDCl_3$) δ 1.33 (d, 3 H, H-6, $J_{5,6}$ = 6.4 Hz), 4.66 (brq, 1 H, H-5), 5.88 (dd, 1 H, H-4 $J_{4,5}$ = 1.4 Hz), 5.92 (dd, 1 H, H-2, $J_{2,3}$ = 10.6 Hz), 6.04 (dd, 1 H, H-3, $J_{3,4}$ = 3.2 Hz), 6.83 (d, 1 H, H-1, $J_{1,2}$ = 3.2 Hz), 7.22-8.10 (m, 15 H, $H_{arom.}$), 8.60 (s, 1 H, NH); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 16.0 (C-6), 67.8, 67.9, 68.6, 71.2 (C-2, C-3, C-4, C-5), 90.8 (CCl_3), 94.0 (C-1), 128.1-133.4 ($CH_{arom.}$), 128.6, 128.9, 129.0 ($C_{arom.}$), 160.5 (C=NH), 165.4, 165.5, 165.7 (C=O).

Dibenzyl 2,3,4-Tri-*O*-benzoyl-β-L-fucopyranosyl Phosphate (8). *Method A:* A solution of recrystallized DBP (2.1 g, 7.5 mmol), previously dried by repeated evaporation with 1,2-dichloroethane (3 × 5 mL), and *N*-iodosuccinimide (1.68 g, 7.5 mmol) in dry 1,2-dichloroethane (50 mL) and freshly distilled THF (5 mL) was added to a mixture of **10β** (2.6 g, 5 mmol) and molecular sieves (0.3 g, 0.4 nm) in 1,2-dichloroethane (50 mL). After 15 min, TLC analysis indicated the completion of the reaction. The mixture was filtered and the filtrate was immediately extracted with a 1 M $Na_2S_2O_3$ solution and a 2 M TEAB solution. The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by flash column chromatography (acetone/dichloromethane, 2/98, v/v) gave homogeneous **8** (2.2 g) in 60% yield. R_f 0.20 acetone/dichloromethane, 3:97, v/v). ^{1}H NMR ($CDCl_3$) δ 1.34 (d, 3 H, H-6, $J_{5,6}$ = 6.4 Hz), 4.22 (dq, 1 H, H-5, $J_{4,5}$ = 1.1 Hz), 4.80, 5.13 (2 × ABM, 2 H, CH_2 benzyl), 5.56 (dd, 1 H, H-3, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.3 Hz), 5.66 (dd, 1 H, H-1, $J_{1,2}$ = 8.0 Hz, $J_{1,P}$ = 7.2 Hz), 5.75 (dd, 1 H, H-4), 5.89 (dd, 1 H, H-2), 6.99-8.13 (m, 25 H, $H_{arom.}$); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 15.9 (C-6), 69.1, 69.3 (2 × d, 2 × CH_2 benzyl), 69.5 (C-2, $J_{2,P}$ = 8.9 Hz), 70.4, 70.5, 71.5 (C-3, C-4, C-5), 96.7 (d, C-1, $J_{1,P}$ = 4.4 Hz), 127.1-135.3 ($CH_{arom.}$), 165.0, 165.1, 165.5 (C=O); $^{31}P\{^1H\}$ NMR ($CDCl_3$) δ -2.28.

Anal. Calcd for $C_{41}H_{37}O_8P$ (688.72): C 71.50, H 5.42, P 4.50; Found: C 71.42, H 5.35, P 4.41%.

Method B: A solution of recrystallized DBP (235 mg, 0.85 mmol), previously dried by repeated evaporation with 1,2-dichloroethane (3 × 5 mL), in 1,2-

dichloroethane (8 mL) was added to a mixture of **11** (500 mg, 0.81 mmol) and molecular sieves (0.3 g, 0.4 nm) in 1,2-dichloroethane (8 mL). After 5 min, TLC analysis indicated the completion of the reaction. The mixture was extracted with a 2 M TEAB solution and water, the organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification as above gave **11** in 60% yield (360 mg). All physical data were in full accord with those reported for **11** prepared *via Method A*.

1,3,4-Tri-*O*-acetyl-α-L-fucopyranose (14). A solution of dibenzyl 2,3,4-tri-*O*-acetyl-β-L-fucopyranosyl phosphate (**7**, 138 mg, 0.25 mmol) in dichloromethane was left at room temperature for 9 h, when TLC analysis indicated disappearance of the starting material. The solution was concentrated *in vacuo* and the crude product was purified by column chromatography (acetone/toluene, 0/1 to 1/9, v/v) to give 64 mg (80%) of **14**. R_f 0.15(acetone/toluene, 5/95, v/v); ^1H NMR (400 MHz) ($CDCl_3$) δ 1.14 (d, 3 H, H-6, $J_{5,6}$ = 6.4 Hz), 1.22 (s, 3 H, $C(O)CH_3$), 1.23 (s, 6 H, 2 × $C(O)CH_3$), 2.40 (bs, 1 H, OH), 4,14 (dd, 1 H, H-2, $J_{1,2}$ =3.9 Hz, $J_{2,3}$ = 10.5 Hz), 4.21 (dq, 1 H, H-5, $J_{4,5}$ = 0.8 Hz), 5.19 (dd, 1 H, H-3, Hz, $J_{3,4}$ = 3.4 Hz), 5.29 (dd, 1 H, H-4), 6.26 (d, 1 H, H-1); $^{13}C\{^1H\}$ NMR (100 MHz CH COSY) ($CDCl_3$) δ 15.8 (C-6), 20.5, 20.9 (2 × $C(O)\underline{C}H_3$), 65.8 (C-2), 67.0 (C-5), 70.7 (C-4, C-5), 92.2 (C-1), 165.3, 165.8 (C=O). *m/z* $[M+Na]^+$ 313.

Bis(triethylammonium) β-L-Fucopyranosyl Phosphate (2). 10% Pd/C (600 mg) was added to a solution of **8** (1.8 g, 2.5 mmol) and sodium acetate (470 mg, 5.7 mmol) in ethyl acetate/2-propanol/water (120 mL, 4/6/2, v/v). The suspension was shaken with hydrogen at 0.4 MPa at room temperature. After 24 h, the mixture was filtered and concentrated *in vacuo*. The residue was dissolved in methanol (75 mL) and 1 M NaOH (12 mL) and left at room temperature. After 2 h, the mixture was treated with Dowex 50 W X 4 $[NH_4^+]$, filtered and concentrated *in vacuo*. The residue was redissolved in water, extracted with dichloromethane (5 × 25 mL) and concentrated *in vacuo*. The crude product was purified by Sephadex LH-20 gel filtration (methanol/water, 4/1, v/v). After concentration and lyophilization bis(ammonium) β-l-fucopyranosyl phosphate was isolated in 90% yield (630 mg) as an amorphous white solid. ^1H NMR (D_2O) δ 1.20 (d, 3 H, H-6, $J_{5,6}$ = 6.4 Hz), 3.40 (dd, 1 H, H-2, $J_{1,2}$ = 7.5 Hz, $J_{2,3}$ = 9.8 Hz), 3.60 (dd, 1 H, H-3, $J_{2,3}$ = 3.3 Hz), 3.66

(d, 1 H, H-4), 3.73 (q, 1 H, H-5), 4.74 (dd, 1 H, H-1, $J_{1,2}$ = 7.9 Hz, $J_{1,P}$ = 7.7 Hz); $^{13}C\{^1H\}$ NMR (D_2O) δ 16.6 (C-6), 72.3, 72.4, 74.0 (C-3, C-4, C-5), 72.7 (C-2, $J_{2,P}$ = 5.9 Hz), 98.1 (d, C-1, $J_{1,P}$ = 4.4 Hz), 127.1-135.3 ($CH_{arom.}$), 165.0, 165.1, 165.5 (C=O); $^{31}P\{^1H\}$ NMR (D_2O) δ 0.25.

Anal. Calcd for $C_{18}H_{43}N_2O_8P$ (446.53): C 48.42, H 9.71, P 6.94; Found: C 48.40, H 9.58, P 6.83%.

The latter compound was dissolved in water (10 mL), applied to a column of Dowex 50 W X 4 [$TEAH^+$] which was eluted with water. Concentration and lyophilization of the filtrate gave the title compound in a quantitative yield.

Bis(sodium) Guanosine 5'-β-L-Fucopyranosyl-diphosphate (1). Tri-*n*-octylamine (0.88 mL, 2.0 mmol) was added to a solution of **2** (894 mg, 2 mmol) in freshly distilled DMF (40 mL). The mixture was concentrated *in vacuo* and the resulting tri-*n*-octylammonium salt was dried by evaporation with dioxane (3 × 5 mL) under reduced pressure and dissolved in dry DMF (50 mL). A solution of *N,N'*-dicyclohexyl-4-morpholinecarboxaminidinium guanosine 5'-morpholido-phosphate (726 mg, 1.0 mmol), previously dried by evaporation with dioxane (3 × 5 mL), in dry DMF (2 mL) was added. After 14 h at 50 °C under an argon atmosphere the mixture was concentrated *in vacuo* to dryness. The residue was redissolved in 0.05 M TEAB (4 mL, adjusted to pH 7.3 with acetic acid) and water (20 mL) and extracted with dichloromethane (5 × 10 mL). The aqueous fraction was concentrated *in vacuo*, the remaining TEAB was removed by evaporation with water (5 × 25 mL).

Crude **1** was lyophilized and purified by HW-40 gel filtration (water/methanol/2 M TEAB, 85/10/5, v/v, R_t 111 min; R_t(**2**) 104 min). The appropriate fractions were concentrated *in vacuo*, applied to a Dowex 50 X W 4 [Na^+] column, which was eluted with water. Concentration *in vacuo* of the resulting solution and lyophilization of the residue gave **1** (270 mg, 0.42 mmol) in 48% yield, based on recovered **2** (490 mg, 1.12 mmol, $TEAH^+$ salt). ^1H NMR data were in full accord with those reported[8,9,12]. $^{13}C\{^1H\}$ NMR (100 MHz) (D_2O) δ 16.6 (Fuc: C-6), 66.2 (Rib: C-5), 71.3, 71.9, 72.0, 72.3, 73.4, 74.6 (Fuc: C-2 C-3, C-4, C-5, Rib: C-2, C-3), 84.6 (Rib: C-4, $J_{4,P}$ = 9 Hz), 87.7 (Rib: C-1), 99.3 (d, Fuc: C-1, $J_{1,P}$ = 5 Hz),

117.0 (G: C-5), 138.4 (G: C-8), 152.2 (G: C-4), 154.7 (G: C-2), 159.7 (G: C-6); $^{31}P\{^{1}H\}$ NMR (D_2O) δ -12.5, -10.8 (2 × d, 2 P, J = 19.3 Hz).

Anal. Calcd for $C_{16}H_{23}N_5Na_2O_{15}P_2$ (633.31): C 30.35, H 3.66, P 9.78; Found: C 30.27, H 3.58, P 9.71%.

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