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Convenient Chemoenzymatic Synthesis of β-Purine-diphosphate Sugars (GDP-fucose-analogues)

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Abstract—A series of peracetylated β-sugar-1-phosphates with L-fuco configuration are efficiently prepared chemically and coupled in high yields to purine monophosphate bases via imidazolide activation. The resulting purine diphosphate sugars are deacetylated completely by a mild treatment with commercial acetylesterase (EC 3.1.1.6) to give donor-substrates for fucosyltransferases. © 1997, Elsevier Science Ltd. All rights reserved.

Introduction

In recent years an increasing amount of knowledge has been accumulated concerning the importance of glycosides in various biological recognition processes.[1,2] Academia and the pharmaceutical industry have both tackled the synthesis of this class of biomolecules to gain insight into adhesion phenomena and/or to develop antiadhesion drugs.[3,4] The demanding synthesis of natural as well as non-natural glycosides is best accomplished enzymatically with the help of glycosyltransferases.[5,6] The merits of this method are its minimal need for protection and deprotection steps and the regio- and stereochemical unambiguity of glycosyltransfer. There are, however, two severe drawbacks to the approach. The availability of transferases, which may be obtained by isolation from natural sources or by cloning and microbial overproduction,[7,8] is still limited. In addition, the lack of a versatile synthesis, especially of non-natural donor substrates, impedes the progress of this methodology.[9]

Results and discussion

As part of our program directed toward the synthesis of fucosylated oligosaccharides as selectin ligands,[10] we needed large quantities of guanosine 5′-(β-L-fucopyranosyl)-diphosphate (GDP-fuc) and derivatives thereof (Table 1).

Although enzymatic fucosylations with in situ generation of GDP-fuc and recycling of the liberated GDP in a multi-enzyme system were reported on an analytical scale,[11] this pathway is not well elaborated yet. In addition, the practicability of this pathway, with its array of interdependent enzymes seemed unlikely for non-natural substrates. So we decided to prepare various activated donor-sugars separately, prior to the enzymatic transfer.

The chemical synthesis of the parent GDP-fuc, starting from fucose was first reported by Barker et al.[12] This cumbersome procedure was gradually improved by other groups,[13–15] but overall yields have never exceeded 30%, and the whole synthesis still remains quite demanding.[14] Especially the formation of the diphosphate bridge and the subsequent tedious separation of the desired GDP-fuc **1** from other polar side-products gave rise to low overall yields.

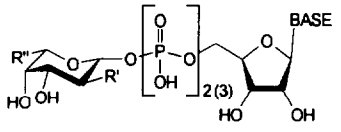
Those two steps have been successfully improved by us, and a general chemoenzymatic protocol was developed for the large scale synthesis of GDP-fuc and some analogues (Schemes 1 and 2).

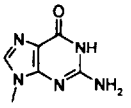
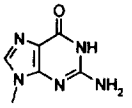
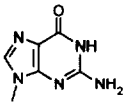
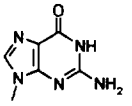
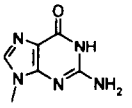
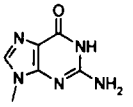
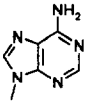
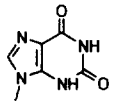
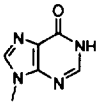
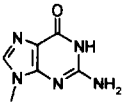
Commercially available fucose **11.1** was first peracetylated at low temperature to compound **12.1**,[16] which was subsequently treated with hydrogen bromide in acetic acid to give fucosyl bromide **13.1**. The product of this reaction is fairly clean and needs no purification. Earlier attempts to purify compound **13.1** from furanose side-products by chromatography resulted in substantial losses of material.[12] The bromide prepared this way could be stored at −80 °C for at least one month without any detectable decomposition (^{1}H NMR) but rapidly deteriorates at room temperature. Bromide **13.1** was coupled to dibenzylphosphoric acid in the presence of silver carbonate and 3 Å molecular sieves. The resulting phosphate **14.1** could be stored in the freezer at −20 °C for several months without decomposition, but decomposed in solution at room temperature (ca. 40% in 10 d, ^{1}H NMR). Subsequent hydrogenation of **14.1** in dioxane in the presence of two equivalents of triethylamine removed the benzyl groups and led to the mono(triethylammonium) salt after lyophilization from dioxane. This salt proved to be the best form in which to store β-L-fucopyranosyl phosphate **15.1** (stored at −20 °C). The free acid of **15.1** could be obtained from the triethylammonium salt via ion exchange treatment. In contrast to previous reports,[13,15] the free acid **15.1**, which has not been well characterized before, could also be stored at −20 °C for several months as a white powder when lyophilized from dioxane:water. It proved to be the best form of

β-fucosylphosphate for executing the ensuing coupling reaction.

Extensive experimentation made us quit the literature procedures for the diphosphate formation, which used morpholidate-activated guanosine-5′-monophosphate (GMP).[13–15] This sluggish reagent caused prolonged reaction times and generated unacceptably large amounts of side-products that were difficult to remove. Instead, we treated the tributylammonium salt of GMP with carbonyldiimidazole to generate in situ a highly reactive mono-imidazolyl phosphate,[17] which was subsequently reacted with the less hygroscopic acetylated fucosylphosphate **15.1**. The progress of the diphosphate coupling could easily be monitored by ^{31}P NMR of samples of the reaction mixture. The reaction was terminated and passed over a Dowex-resin (Na^+-form) when the ^{31}P NMR signal (ca −8.2 ppm) of the imidazolyl intermediate had disappeared. Minor phosphomonoester impurities were removed by digestion with calf intestine alkaline phosphatase (CIAP, EC 3.1.3.1).[18,19] Because of the base-lability of compound **16.1**[13] attempted chemical deacetylations gave only small amounts of the desired product. So we screened several esterases and lipases to remove all the acetates in one step.[20]

Table 1. Purine 5′-β-L-sugar-di-(tri)phosphates



Compd	abbreviation	R′	R″	base
1	GDP-fuc	OH	CH_3	
2	GDP-2NH_2-fuc	NH_2	CH_3	
3	GDP-2-F-fuc	F	CH_3	
4	GDP-ara	OH	H	
5	GDP-L-gal	OH	CH_2OH	
6	GDP-L-glc	OH, 4-epi-OH	CH_2OH	
7	ADP-fuc	OH	CH_3	
8	XDP-fuc	OH	CH_3	
9	IDP-fuc	OH	CH_3	
10	GTP-fuc	OH	CH_3	

Commercially available (Sigma) citrus acetylesterase (EC 3.1.1.6)[21] from orange-peels surprisingly accepted the highly polar, quite unusual peracetylated fucosylphosphate **16.1** as a substrate and removed all the acetates in one step. Precipitation with ethanol and a final ultrafiltration via Amicon YM 10000 yielded GDP-fuc **1** of spectroscopically pure quality[13] for further use in enzymatic fucosylations with different fucosyltransferases.

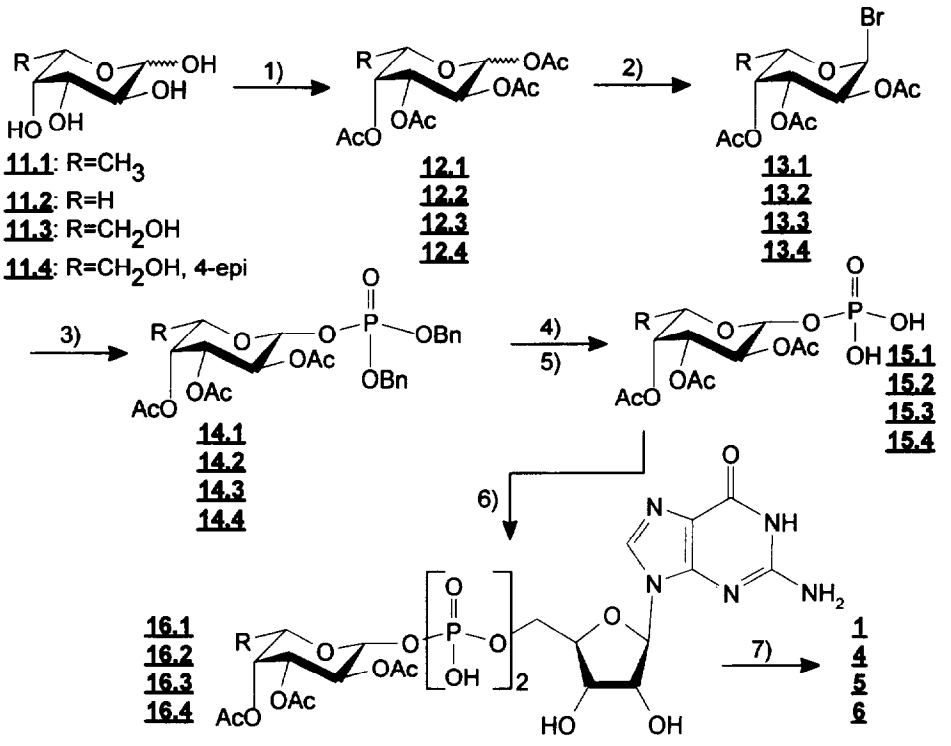
The same reaction sequence as oulined in Scheme 1 for GDP-fuc could be applied for the preparation of other purine-5′-(β-L-sugarpyranosyl)di- and triphosphates (Table 1).

The synthesis of compounds **2** and **3** is outlined in Scheme 2. The common precursor was the fucal **17**, which was obtained in high yield via tin-reduction from the bromide **13.1** in the presence of 1-methylimidazole according.[22] The fucal was subjected to an azidonitration reaction[23] giving an anomeric mixture of nitrates **18**, which were subsequently transformed into bromide **19**.[24]

Fucal **17** could also be fluorinated with xenondifluoride to give an anomeric mixture of fluorides. This mixture was first defluorinated at the 1-position and then reacetylated to give the triacetate intermediate **22** ready for bromination with hydrogen bromide to yield the activated sugar **23**. Both sugars **19** and **23** were then processed to their corresponding GDP-glycosides **2** and **3** according to the reaction pathway outlined in Scheme 1 for GDP-fuc **1**.

Conclusion

In summary, we have developed an efficient multigram procedure for the synthesis of GDP-fuc. The versatile protocol was used to prepare a series of purine di- and triphosphate-activated glycosyl donors like GDP-2NH_2-fuc **2** or GDP-2-F-fuc **3**. The key improvements comprise the use of easily handleable and stable acetylated sugar-1-phosphates, which were coupled to highly reactive purine-5′-imidazolylphosphates, resulting in high and reproducible yields of the desired purine-diphosphate-sugars. Subsequent digestion by alkaline phosphatase removed minor monophosphate impurities from the coupling reaction. The final products



Scheme 1. (1) According to lit.[12]; (2) modified from lit.[15]; (3) modified form lit.[14]; (4) dioxane, H_2–Pd/C, 2 equiv. NEt_3, (5) H_2O, Amberlite IRC-50 (weakly acidic); (6) a DMF, GMP × NBu_3 and CDI, then Dowex (Na^+-form), b. H_2O, pH 7.5, CIAP; (7) acetylesterase (EC 3.1.1.6), pH 6.8, 0.1 N NaOH.

were obtained by mild enzymatic deacetylation using acetylesterase from orange-peels, followed by Amicon-filtration to remove the protein, and finally by precipitation from water:ethanol mixtures. The reaction sequence benefits from the absence of any chromatographic purification step for all the phosphates reported.

The phosphate coupling and esterase-deacetylation protocols were likewise applicable to α-sugar phosphates and pyrimidine-5′-monophosphates (to be reported elsewhere). Some of our activated donors have already been used successfully in enzymatic fucosylation reactions with non-natural acceptors (paper in preparation). This strategy allows a rapid creation of valuable oligosaccharide libraries for biological screenings.

Experimental

^1H, ^{13}C, ^{31}P, and ^{19}F NMR (external standard: $CFCl_3$) were recorded on a Bruker AC 250 spectrometer with a multi-probe head. UV spectra were recorded on Shimadzu UV-160A spectrometer. TLC was performed on silica gel 60F254 glass sheets (Merck), and sugars were stained with *p*-anisaldehyde sulfuric acid (Pernod-mixture). Flash chromatography was carried out with silica gel 60, 0.040–0.063 mm (Merck). Acidifications and sodium exchange were done with Amberlite IRC 50 (H^+-form), 16–50 mesh (Fluka) and sodium exchanger Dowex 50W × 8 (Na^+-form), 20–50 mesh (Fluka). Dialysis was carried out with molecular porous membrane tube 25 mm, M_w cut off 12000–14000 (Spectra/por) and ultrafiltrations with Amicon filters YM-10 in an appropriate Amicon-cell.

The solvents used were of commercial quality. Calf intestine alkaline phosphatase (EC 3.1.3.1) (CIAP) was purchased from Boehringer (no 108146, 7500 U/498 μL), and acetylesterase (EC 3.1.1.6) was purchased from Sigma (A-4530) as an ammonium sulfate suspension. All new compounds gave correct mass spectra.

General procedures

Preparation of tri-*n*-butylammonium purine-5′-monophosphates

One equivalent of commercially available purine-5′-monophosphoric acid (Fluka, Sigma) was suspended in deionized water (1:15, w:v) and one equivalent tri-*n*-butylamine (Fluka) was dropped in with vigorous stirring. The mixture cleared rapidly and was stirred for an additional 15 min at room temperature before lyophilization. The resulting white powder was stored at 40 °C in a vacuum dessiccator over P_4O_{10} prior to use.

For large scale synthesis, the guanosine-5′-monophosphoric acid was produced by treatment of the much cheaper sodium salt of GMP (Fluka) with Dowex 50 × 8 (H^+-form) and then processed as described above.

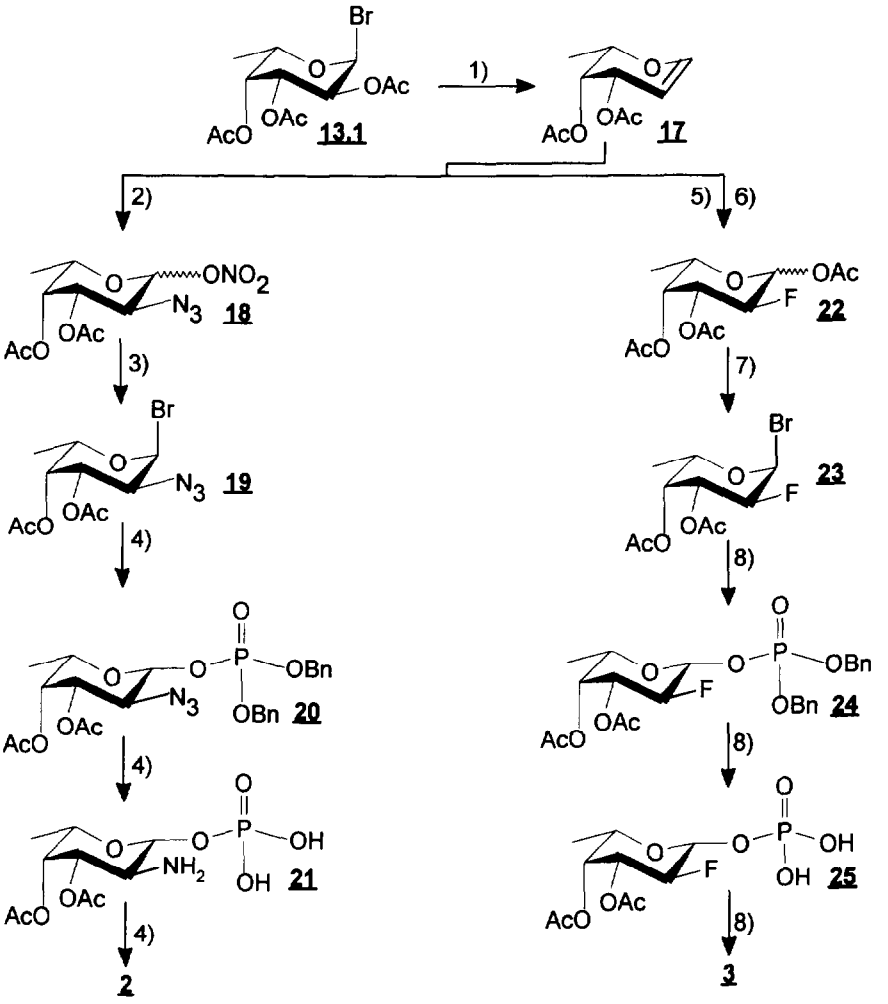
General protocol for the diphosphate couplings

Tri-*n*-butylammonium purine-5′-monophosphate (1 mmol) was clearly dissolved in 6.5 mL of dry DMF at room temperature in an argon atmosphere. Carbonyldiimidazole (2.1 mmol) was added and the mixture stirred for ca. 40 min. Dry MeOH (35.5 μL) was then injected and stirred for a further 20 min in order to destroy excess carbonyldiimidazole. Nonreacted methanol was removed by a 15 min high-vacuum pump. Subsequently, acetylated sugar-1-phosphate (1.0–1.2 mmol), dissolved in dry DMF (8 mL), was dropped to the activated purine-5′-phosphate. Stirring was continued at room temperature for about 40–50 h, until a ^{31}P NMR-spectrum of a sample of the reaction mixture showed the complete consumption of monoimidazolyl phosphate intermediates (^{31}P NMR signal ~ −8.2 ppm). Heat or ultrasound did not speed up the reaction significantly.

After complete consumption, the solvent was removed at about 40 °C in vacuum, the residue given in water and passed slowly over Dowex (Na^+-form) to remove the ammonium salts. Sugar containing fractions were combined and lyophilized. The resulting white powders were dissolved in 40 mL water and treated with ~380 U (25 μL) CIAP at room temperature overnight at pH 7.7. The mixture was then concd to about 9 mL at 40 °C in vacuum and the resulting clear, slightly yellow soln stirred with 45 mL EtOH. The precipitates, which contained the desired products, were centrifuged, washed with additional 18 mL EtOH, and finally lyophilized from dioxane:water.

Enzymatic deacetylations

Pretreatment of acetylesterase (EC 3.1.1.6). Acetylesterase from orange-peels is commercially available only as an ammonium sulfate suspension. As we noticed, ammonium salts severely impeded a simple purification procedure of the final nucleotide diphosphate sugars. The esterase suspension (250 U) was therefore put into about 10 mL deionized water, enclosed in a dialysis tube and dialysed six times against 1 L deionized water (~3.5 h overall) at room



Scheme 2. (1) THF, Sn, methylimidazole, 80%; (2) lit.[23]; (3) according to lit.[24], LiBr, 80%); (4) following steps 3–7 in Scheme 1, overall 63%; (5) lit.[25], 42%; (6) 0.1 N H_2SO_3 then Ac_2O, pyr, 69%; (7) HBr, AcOH, Ac_2O, 93%; (8) following seps 3–7 in Scheme 1, overall 80%.

temperature. The salt-free enzyme (final volume ~10 to 12 mL) can be stored at 4 °C up to 1 week without significant loss of activity.

General protocol for the enzymatic deacetylations. Acetylated nucleotide-diphosphate sugar (1 mmol) was dissolved in deionized water (4.5 mL) and incubated with 63–75 U (~3 mL) pretreated acetylesterase at 37 °C. The pH of the reaction mixture was controlled by a pH-automate and kept at pH 6.8 with 0.1 N NaOH and a pH-dosimeter. After consumption of about the theoretical amount of NaOH (usually ca. 90%), the reaction mixture was poured into 250 mL EtOH. The resulting precipitate was centrifuged off, dissolved in 40 mL water and passed through an Amicon-filter. The flow-through was lyophilized, and gave the fluffy sugar-nucleotides ready for use in enzymatic glycosylations.

2,3,4-Tri-*O*-acetyl-α-L-fucopyranosylbromide (13.1). Tetra-*O*-acetyl-L-fucose **12.1** (476.1 g, 1.43 mol) was dissolved in dry DCM (2.4 L) and cooled to 0 °C. Subsequently, acetic anhydride (240 mL) was added and 1020 mL hydrogenbromide in acetic acid (33% HBr) was dropped to this solution at 0 °C. The mixture was stirred for an additional 2 h at 0 °C and then poured onto ice-water and extracted with EtOAc. The organic phase was separated and successively washed with 3 × 4.5 L water, 5 × satd $NaHCO_3$-soln, and finally 3 × brine. The organic phase was dried over $MgSO_4$. Removal of the solvent on a rotary evaporator at room temperature left 506.0 g (quant) of a colourless syrup, which could be stored at −80 °C for at least a month without any decomposition according inspections of the ^{1}H NMR spectra of drawn samples. ^{1}H NMR ($CDCl_3$): δ 1.24 (d, J=6.8 Hz, 3H), 2.06 (s, 3H), 2.15 (s, 3H), 2.20 (s, 3H), 4.47 (bq, J=6.8 Hz, 1H), 5.08 (dd, J=11.6, 3.7 Hz, 1H), 5.41 (bd, J=3.0 Hz, 1H), 5.46 (dd, J=11.6, 3.0 Hz, 1H), 6.70 (d, J=3.7 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 15.50; 20.51; 20.57; 20.73; 67.78; 68.75; 69.91; 89.24; 169.75; 170.07; 170.20. These data were in agreement with lit. reports.[15]

2,3,4-Tri-*O*-acetyl-β-L-fucopyranosyl dibenzylphosphate (14.1). Dibenzylphosphate (6.90 g, 24.8 mmol) was dissolved under an argon atmosphere in a dry solvent mixture of DCM:Et_2O:MeCN (80 mL:80 mL:80 mL) containing 28 g freshly activated 3 Å molecular sieves at 0 °C. 4.90 g (13.9 mmol) of the bromide **13.1**, dissolved in DCM (15 mL), were added with vigorous stirring at 0 °C. Subsequently, $AgCO_3$ (7.02 g, 25.5 mmol) was added and the resulting mixture stirred overnight at room temperature (~21 h). The slurry was then filtered over a celite pad and the organic phase evapd at 40 °C to yield 7.59 g (99%) of a colourless syrup, which could be stored at −20 °C for several weeks without decomposition, but decayed slowly at room temperature in soln.

Compound **14.1**, virtually one spot on TLC (EtOAc:petrol ether, 1:1; R_f 0.32), has not been obtained yet in such an easy way in this quality.[13,15] This reaction could be scaled-up to about 800 g of product (quant). ^{1}H NMR ($CDCl_3$): δ 1.24 (d, J=6.9 Hz, 1H), 1.94 (s, 3 H), 2.03 (s, 3 H), 2.23 (s, 3 H), 3.94 (bq, J=6.9 Hz, 1 H), 5.08 (m, 5 H), 5.31 (m, 3 H), 7.39 (m, 10 H). ^{13}C NMR ($CDCl_3$): δ 13.85, 18.52 (2 × C), 18.60, 66.74 (d, $J_{C\text{-}P}$=9.1 Hz), 67.52 (d, $J_{C\text{-}P}$=4.7 Hz), 67.68, 68.19, 68.81, 68.83, 94.73 (d, $J_{C\text{-}P}$=4.7 Hz), 125.74, 125.87, 126.47, 126.53, 126.56, 133.18, 133.31, 133.40, 133.52, 167.54, 167.93, 168.44. ^{31}P NMR ($CDCl_3$): δ −3.31.

2,3,4-Tri-*O*-acetyl-β-L-fucopyranosyl-1-phosphate (15.1). Benzylester **14.1** (167.6 g; 0.30 mol) was dissolved in 2.2 L dry dioxane at room temperature. Dry triethylamine (42.3 mL, 2 equiv) and 10% Pd/C (30.0 g) were added and the mixture hydrogenated with vigorous stirring until TLC indicated the consumption of all the starting material (3–8 h). The mixture was then filtered over a pad of silica gel, prewashed with dioxane, extensively washed with dioxane and finally eluted with MeOH. The methanol phase was evapd and the resulting residue lyophilized from dioxane to yield 133.5 g (93%) of highly pure mono(triethylammonium) phosphate **15.1** as a white powder. This salt proved to be an easy handleable and fairly stable compound. We usually stored it at room temperature but for a prolonged storage refrigeration is recommended.

This compound has not been described previously. Extensive lyophilization may lead to further loss of triethylamine, which is, however, not detrimental at all. ^{1}H NMR ($CDCl_3$): δ 1.18 (d, J=6.9 Hz, 3 H), 1.30 (t, J=7.4 Hz, 9 H), 1.94 (s, 3 H), 2.06 (s, 3 H), 2.15 (s, 3 H), 3.19 (q, J=7.4 Hz, 6 H), 3.99 (bq, J=6.9 Hz, 1 H), 5.17 (m, 4 H). ^{13}C NMR ($CDCl_3$): δ 9.13 (3 × C), 16.29, 20.52 (2 × C), 20.94, 47.53 (3 × C), 70.61, 70.66 (d, $J_{C\text{-}P}$=8.1 Hz), 71.93, 72.82, 96.87 (d, $J_{C\text{-}P}$=4.6 Hz), 171.49, 171.75, 172.27. ^{31}P NMR ($CDCl_3$): δ −0.97.

The triethylamine was quantitatively removed prior to the coupling step by filtration over a weakly acidic Amberlite IRC-50 resin in water. Lyophilization yielded the free acid **15.1** as a white powder, which is stored at −20 °C or over P_4O_{10} at room temperature in a desiccator. ^{1}H NMR (CD_3OD): δ 1.19 (d, J=6.9 Hz, 3 H), 1.94 (s, 3 H), 2.05 (s, 3 H), 2.16 (s, 3 H), 4.04 (bq, J=6.9 Hz, 1 H), 5.19 (m, 4 H). ^{13}C NMR (CD_3OD): δ 16.31, 20.53 (2 × C), 21.01, 70.73, 71.04 (d, $J_{C\text{-}P}$=8.0 Hz), 71.82, 72.60, 97.15 (d, $J_{C\text{-}P}$=4.0 Hz), 171.46, 171.83, 172.27. ^{31}P NMR (CD_3OD): δ −3.68.

Guanosine-5′-(2,3,4-tri-*O*-acetyl-β-L-fucopyranosyl)-diphosphate (16.1). According to the general coupling-protocol tri-*n*-butyl-GMP (7.6 g, 13.8 mmol) was treated with fucosylphosphate **15.1** (6.4 g, 17.3 mmol) to give the title compound (9.8 g, 94%). ^{1}H NMR (D_2O): δ 1.19 (d, J=6.9 Hz, 3 H), 1.99 (s, 3 H), 2.14 (s, 3 H), 2.23 (s, 3 H), 4.11 (bq, J=6.9 Hz, 1 H), 4.25 (m, 2 H), 4.36 (m, 1 H), 4.51 (t, J=4.5 Hz; 1 H), 4.75 (t, J=7.0 Hz, 1 H), 5.10 (m, 2 H), 5.28 (m, 2 H), 5.95 (d, J=7.0 Hz, 1 H), 8.12 (s, 1 H). ^{13}C NMR (D_2O): δ 18.52, 23.50 (2 × C), 23.96, 68.80 (d, $J_{C\text{-}P}$=4.0 Hz), 73.40 (d, $J_{C\text{-}P}$=8.4 Hz), 73.64, 73.86, 74.25, 74.91, 77.48, 87.02 (d, $J_{C\text{-}P}$=9.2 Hz), 90.62, 99.00 (d, $J_{C\text{-}P}$=4.5 Hz), 119.57, 141.06, 155.11, 157.41, 162.28, 176.24, 176.76,

177.18. ^{31}P NMR (D_2O): δ −11.18 (d, J=20 Hz), −13.02 (d, J=20 Hz).

Guanosine-5′-(β-L-fucopyranosyl)-diphosphate (GDP-fuc) (1). According to the general protocol for the esterase deacetylation of compound **16.1** (5.2 g, 6.9 mmol) was treated with acetylesterase to give the title compound (4.0 g, 92%, contains 97% according to UV_{246nm}). The spectroscopic data were in good agreement with those reported.[13]

Adenosine-5′-(β-L-fucopyranosyl)-diphosphate (ADP-fuc) (7). According to the general coupling and deacetylation protocols, fucosylphosphate **15.1** (0.60 g, 1.6 mmol) was coupled to tri-*n*-butylammonium adenosine-5′-monophosphate (0.86 g, 1.6 mmol) and the resulting diphosphate deacetylated to give the title compound (0.71 g, 71% overall, contains 96% according to UV_{259nm}). ^{1}H NMR (D_2O): δ 1.19 (d, J=8.2 Hz, 3 H), 3.51 (bq, J=8.2 Hz, 1 H), 3.64 (m, 3 H), 4.18 (m, 2 H), 4.36 (m, 1 H), 4.49 (m 1 H), 4.73 (m, 1 H), 4.84 (m, 1 H), 6.02 (d, J=6.9 Hz, 1 H), 8.07 (s, 1 H), 8.39 (s, 1 H). ^{13}C NMR (D_2O): δ 16.09, 65.96 (d, $J_{C\text{-}P}$=5.4 Hz), 71.47, 71.83 (d, $J_{C\text{-}P}$=9.3 Hz), 72.11, 72.37, 73.10, 74.09, 84.48 (d, $J_{C\text{-}P}$=9.3 Hz), 87.46, 99.07 (d, $J_{C\text{-}P}$=6.0 Hz), 118.97, 140.31, 149.49, 153.34, 155.96. ^{31}P NMR (D_2O): δ −10.88 (d, J=20.3 Hz), −12.72 (d, J=20.3 Hz).

Xanthosine-5′-(β-L-fucopyranosyl)-diphosphate (XDP-fuc) (8). According to the general coupling and deacetylation protocols fucosylphosphate **15.1** (0.45 g, 1.2 mmol) was coupled to tri-*n*-butylammonium xanthosine-5′-monophosphate (0.67 g, 1.2 mmol) and the resulting diphosphate deacetylated to give the title compound (0.43 g, 56% overall, contains 99% according to UV_{249nm}). ^{1}H NMR (D_2O): δ 1.23 (d, J=8.2 Hz, 3 H), 3.69 (m, 4 H), 4.21 (m, 2 H), 4.37 (m, 1 H), 4.52 (m 1 H), 4.68 (m, 1 H), 4.90 (dd, J=8.3, 2.7 Hz, 1 H), 5.93 (d, J=6.9 Hz, 1 H), 8.06 (s, 1 H). ^{13}C NMR (D_2O): δ 15.34, 65.31 (d, $J_{C\text{-}P}$=5.3 Hz), 70.28, 70.92 (d, $J_{C\text{-}P}$=8.0 Hz), 71.02, 71.36, 72.32, 73.95, 83.39 (d, $J_{C\text{-}P}$=9.0 Hz), 86.00, 98.26 (d, $J_{C\text{-}P}$=5.7 Hz), 114.63, 136.07, 153.52, 159.64, 160.87. ^{31}P NMR (D_2O): δ −11.12 (d, J=19.5 Hz), −12.98 (d, J=19.5 Hz).

Inosine-5′-(β-L-fucopyranosyl)-diphosphate (IDP-fuc) (9). According to the general coupling and deacetylation protocols, fucosylphosphate **15.1** (0.45 g, 1.2 mmol) was coupled to tri-*n*-butylammonium xanthosine-5′-monophosphate (0.67 g, 1.2 mmol) and the resulting diphosphate deacetylated to give 0.44 g (59% overall) of the title compound (contains 97% according to UV_{248nm}). ^{1}H NMR (D_2O): δ 1.20 (d, J=7.6 Hz, 3 H), 3.56 (dq, J=7.6, 1.5 Hz, 1 H), 3.72 (m, 3 H), 4.22 (m, 2 H), 4.40 (m, 1 H), 4.53 (m 1 H), 4.91 (dd, J=7.6, 2.7 Hz, 1 H), 6.19 (d, J=6.9 Hz, 1 H), 8.17 (s, 1 H), 8.42 (s, 1 H). ^{13}C NMR (D_2O): δ 16.19, 65.95 (d, $J_{C\text{-}P}$=5.0 Hz), 70.96, 71.74 (d, $J_{C\text{-}P}$=8.1 Hz), 71.83, 72.18, 73.15, 75.28, 84.53 (d, $J_{C\text{-}P}$=9.0 Hz), 88.20, 99.12 (d, $J_{C\text{-}P}$=5.7 Hz), 124.13, 140.21, 147.13, 149.26, 159.11. ^{31}P NMR (D_2O): δ −11.13 (d, J=18.6 Hz), −12.93 (d, J=18.6 Hz).

Guanosine-5′-(β-L-fucopyranosyl)-triphosphate (GTP-fuc) (10). According to the general coupling and deacetylation protocols fucosylphosphate **15.1** (0.22 g, 0.6 mmol) was coupled to tri-*n*-butylammonium guanosine-5′-diphosphate (0.30 g, 0.5 mmol) and the resulting triphosphate deacetylated to give the title compound (0.13 g, 35% overall, contains 93% according to UV_{252nm}). ^{1}H NMR (D_2O): δ 1.26 (d, J=7.6 Hz, 3 H), 3.66 (m, 3 H), 3.71 (bq, J=7.6 Hz, 1 H), 4.27 (m, 2 H), 4.49 (m, 1 H), 4.58 (m 1 H), 4.68 (m, 1 H), 4.97 (dd, J=8.3, 2.7 Hz, 1 H), 5.92 (d, J=6.9 Hz, 1 H), 8.01 (s, 1 H). ^{13}C NMR (D_2O): δ 15.72; 65.63 (d, $J_{C\text{-}P}$=5.3 Hz), 70.65, 71.25 (d, $J_{C\text{-}P}$=8.0 Hz), 71.43, 71.76, 72.73, 74.06, 84.00 (d, $J_{C\text{-}P}$=9.0 Hz), 86.96, 98.40 (d, $J_{C\text{-}P}$=5.7 Hz), 119.33, 136.88, 151.50, 153.60, 159.00. ^{31}P NMR (D_2O): δ −11.53 (d, J=17.6 Hz), −13.09 (d, J=16.4 Hz), −22.8 (t, J=16.8 Hz).

2,3,4-Tri-*O*-acetyl-α-D-arabinopyranosyl-1-dibenzylphosphate (14.2). Freshly prepared bromide **13.2** (5.5 g, 16.2 mmol),[13] dissolved in 15 mL dry DCM, was dropped to dibenzylphosphate (8.0 g, 28.7 mmol) in DCM:Et_2O:MeCN (100 mL:100 mL:100 mL), containing 30 g freshly activated 3 Å molecular sieves at −10 °C in an argon atmosphere. Silver carbonate (8.2 g, 30.0 mmol) was added and the mixture vigorously stirred at 0 °C overnight. The reaction mixture was filtered over celite and evapd to dryness to give the title compound (7.3 g, 84%) as a colourless syrup, which was stored at −20 °C. ^{1}H NMR ($CDCl_3$): δ 1.95 (s, 3 H), 2.01 (s, 3 H), 2.12 (s, 3 H), 3.69 (dd, J=13.0, 2.8 Hz, 1 H), 4.06 (dd, J=13.0, 4.8 Hz, 1 H), 5.04 (m, 5 H), 5.27 (m, 3 H), 7.32 (m, 10 H). ^{13}C NMR ($CDCl_3$): δ 20.50, 20.53, 20.78, 62 98, 66.57, 69.57 (d, $J_{C\text{-}P}$=9.5 Hz), 69.15, 69.51 (d, $J_{C\text{-}P}$=5.5 Hz), 69.59 (d, $J_{C\text{-}P}$=5.5 Hz), 97.14 (d, $J_{C\text{-}P}$=4.8 Hz), 127.79, 127.88, 128.48, 128.52, 128.58, 135.26, 135.31, 135.43, 169.26, 169.80, 170.01. ^{31}P NMR ($CDCl_3$): δ −3.13.

2,3,4-Tri-*O*-acetyl-α-D-arabinopyranosyl-1-phosphate (15.2). Compound **14.2** (5.3 g, 9.3 mmol) was dissolved in 150 mL dry dioxane, containing dry triethylamine (2.75 mL, 19.8 mmol) and 10% Pd/C (290 mg), and hydrogenated with vigorous stirring overnight. The mixture was subsequently passed through a pad of silica gel, prewashed with dioxane, washed with petrol ether:EtOAc (1:3), and then eluted with MeOH. The MeOH was evapd, the residue treated with Amberlite and then lyophilized from dioxane (3 days) to yield 2.9 g (82%) of the free acid **15.2.** ^{1}H NMR (CD_3OD): δ 1.98 (s, 3 H), 2.08 (s, 3 H), 2.15 (s, 3 H), 3.84 (dd, J=14.4, 3.4 Hz, 1 H), 4.03 (dd, J=14.4, 4.2 Hz, 1 H), 5.10 (m, 2 H), 5.22 (m, 1 H), 5.32 (m, 1 H). ^{13}C NMR (CD_3OD): δ 20.59, 20.75, 20.92, 64.69, 69.25, 71.21 (d, $J_{C\text{-}P}$=8.6 Hz), 71.60, 97.22 (d, $J_{C\text{-}P}$=4.0 Hz), 171.56; 171.65; 171.85. ^{31}P NMR ($CDCl_3$): δ −1.27.

Guanosine-5′-(α-D-arabinopyranosyl)-1-diphosphate (GDP-ara) (4). According to the general coupling and deacetylation protocols arabinosylphosphate **15.2** (0.37 g, 1.0 mmol) was coupled to tri-*n*-butylammonium guanosine-5′-monophosphate (0.45 g, 0.9 mmol) and the resulting diphosphate deacetylated to give the title compound (0.42 g, 77% overall, contains 99% according to UV_{250nm}). All NMR data were in good agreement with those reported.[13]

2,3,4,6-Tetra-*O*-acetyl-α-L-galactopyranosyl-1-bromide (13.3). Commercial L-galactose **11.3** (2.0 g, 11.1 mmol) was acetylated as described for L-fucose[14] to yield 4.3 g (100%) of an anomeric mixture (α:β,47:53) of the tetra-acetate **12.3**. This tetra-acetate was treated at 0 °C overnight with HBr and then worked up as described for compound **13.1** to give the bromide **13.3** (4.6 g, 100%) as a colourless syrup. ^{1}H NMR ($CDCl_3$): δ 1.96 (s, 3 H), 2.03 (s, 3 H), 2.06 (s, 3 H), 2.09 (s, 3 H), 4.09 (m, 2 H), 4.43 (bt, J=7.5 Hz, 1 H), 4.98 (dd, J=11.0, 4.8 Hz, 1 H), 5.33 (dd, J=11.0, 3.4 Hz, 1 H), 5.47 (m, 1 H), 6.63 (d, J=4.2 Hz, 1 H). ^{13}C NMR ($CDCl_3$): δ 20.49, 20.57, 20.67 (2×C), 60.77, 66.89, 67.67, 67.90, 70.95, 88.02, 169.75, 169.89, 170.04, 170.33.

2,3,4,6-Tetra-*O*-acetyl-β-L-galactopyranosyl-1-dibenzyl-phosphate (14.3). Bromide **13.3** (4.6 g, 11.0 mmol) was reacted with dibenzyl phosphate and silver carbonate as decribed for compound **14.1** to give the title phosphate (6.2 g, 93%). ^{1}H NMR ($CDCl_3$): δ 1.90 (s, 3 H), 19.4 (s, 3 H), 19.6 (s, 3 H), 2.14 (s, 3 H), 3.99 (m, 1 H), 4.09 (m, 2 H), 5.00 (m, 5 H), 5.30 (m, 2 H), 5.48 (m, 1 H), 7.31 (m, 10 H). ^{13}C NMR ($CDCl_3$): δ 20.48 (2×C), 20.58 (2×C), 61.00, 66.58, 68.91 (d, $J_{C\text{-}P}$=9.1 Hz), 64.96 (d, $J_{C\text{-}P}$=5.3 Hz, 2×C), 70.37, 71.62, 96.71 (d, $J_{C\text{-}P}$=4.0 Hz), 127.29, 127.74, 127.83, 128.05, 128.54, 135.05, 135.15, 169.86, 170.03, 170.22. ^{31}P NMR ($CDCl_3$): δ −3.20.

2,3,4,6-Tetra-*O*-acetyl-β-L-galactopyranosyl-1-phosphate (15.3). Benzylester **14.3** (6.2 g, 10.2 mmol) was hydrogenated and subsequently converted to the title phosphate (3.5 g, 80%) as described for compound **15.1**. ^{1}H NMR (CD_3OD): δ 1.95 (s, 3 H), 2.01 (s, 3 H), 2.08 (s, 3 H), 2.14 (s, 3 H), 4.22 (m, 3 H), 5.10 (dd, J=8.3, 2.8 Hz, 1 H), 5.26 (m, 2 H), 5.47 (d, J=3.4 Hz, 1 H). ^{13}C NMR (D_2O): δ 21.92 (2×C), 22.08, 22.24, 63.66, 69.76, 71.80 (d, $J_{C\text{-}P}$=7.6 Hz), 72.85, 72.94, 97.39 (d, $J_{C\text{-}P}$=4.1 Hz), 174.60, 174.97, 175.17, 175.42. ^{31}P NMR (CD_3OD): δ −0.73.

Guanosine-5′-(β-L-galactopyranosyl)-1-diphosphate (GDP-L-gal) (5). According to the general coupling and deacetylation protocols galactosylphosphate **15.3** (1.00 g, 2.3 mmol) was coupled to tri-*n*-butylammonium guanosine-5′-monophosphate (1.01 g, 2.3 mmol) and the resulting diphosphate deacetylated to give the title compound (1.05 g, 70% overall, contains 99% according to UV_{251nm}). ^{1}H NMR (D_2O): δ 3.63 (m, 5 H), 3.92 (d, J=2.8 Hz, 1 H), 4.27 (m, 2 H), 4.49 (m, 1 H), 4.58 (m, 1 H), 4.95 (t, J=7.5 Hz, 1 H), 5.95 (d, 6.9 Hz, 1 H), 8.12 (s, 1 H). ^{13}C NMR (D_2O): δ 63.30; 67.45 (d, $J_{C\text{-}P}$=4.6 Hz), 70.70, 72.48, 73.72 (d, $J_{C\text{-}P}$=8.6 Hz), 74.36, 75.92, 77.96, 85.80 (d, $J_{C\text{-}P}$=9.2 Hz), 88.88, 100.58 (d, $J_{C\text{-}P}$=6.2 Hz), 118.19, 139.53, 153.77, 155.94, 160.91. ^{31}P NMR (D_2O): δ −11.19 (d, J=20.2 Hz), −12.89 (d, J=20.2 Hz).

2,3,4,6-Tetra-*O*-acetyl-α-L-glucopyranosyl-1-bromide (13.4). Commercial L-glucose **11.4** (2.0 g, 11.1 mmol) was acetylated as described for L-fucose[14] to yield 4.3 g (100%) of an anomeric mixture of the tetra-acetate **12.4**. This tetra-acetate was treated at 0 °C overnight with HBr and then worked up, as described for compound **13.1**, to give bromide **13.4** (4.5 g, 98%) as a colourless syrup. ^{1}H NMR ($CDCl_3$): δ 2.06 (s, 3 H), 2.08 (s, 3 H), 2.13 (s, 6 H), 4.14 (m, 1 H), 4.32 (m, 2 H), 4.87 (dd, J=11.0, 4.8 Hz, 1 H), 5.18 (t, J=10.3 Hz, 1 H), 5.58 (bt, J=11.0 Hz, 1 H), 6.63 (d, J=4.2 Hz, 1 H). ^{13}C NMR ($CDCl_3$): δ 20.50 (2×C), 20.61 (2×C), 60.86, 67.06, 70.07, 70.51, 72.05, 86.50, 169.42, 169.75, 169.81, 170.46.

2,3,4,6-Tetra-*O*-acetyl-β-L-glucopyranosyl-1-dibenzyl-phosphate (14.4). Bromide **13.4** (4.5 g, 10.9 mmol) was reacted with dibenzyl phosphate and silver carbonate as decribed for compound **14.1** to give the title phosphate (6.3 g, 94%). ^{1}H NMR (CD_3OD): δ 1.92 (s, 3 H), 1.98 (s, 6 H), 2.03 (s, 3 H), 4.05 (m, 1 H), 4.15 (dd, J=11.6, 2.2 Hz, 1 H), 4.28 (dd, J=11.6, 5.5 Hz, 1 H), 5.09 (m, 6 H), 5.36 (t, J=7.7 Hz, 1 H), 5.47 (t, J=7.7 Hz, 1 H), 7.38 (m, 10 H). ^{13}C NMR ($CDCl_3$): δ 20.31, 20.47 (2×C), 20.52, 61.35, 67.62, 69.57 (d, $J_{C\text{-}P}$=4.5 Hz, 2×C), 71.02 (d, $J_{C\text{-}P}$=9.2 Hz), 72.19, 72.49, 96.14 (d, $J_{C\text{-}P}$=4.5 Hz), 127.68, 127.83, 128.47, 128.60, 135.64, 169.19, 169.28, 169.91, 170.39. ^{31}P NMR ($CDCl_3$): δ −3.18.

2,3,4,6-Tetra-*O*-acetyl-β-L-glucopyranosyl-1-phosphate (15.4). Benzylester **14.4** (6.3 g, 10.3 mmol) was hydrogenated and subsequently converted to the title phosphate (3.3 g, 75%) as described for compound **15.1**. ^{1}H NMR (D_2O): δ 2.01 (s, 3 H), 2.05 (s, 3 H), 2.08 (s, 6 H), 4.06 (m, 1 H), 4.18 (dd, J=10.4, 3.4 Hz, 1 H), 4.38 (dd, J=12.4, 4.1 Hz, 1 H), 4.90 (t, J=8.3 Hz, 1 H), 5.19 (t, J=8.5, 1 H), 5.22 (bt, J=8.5 Hz, 1 H), 5.36 (bt, J=8.5 Hz, 1 H). ^{13}C NMR (D_2O): δ 22.08 (2×C), 22.15, 22.45, 63.68, 70.00, 73.53, 73.98 (d, $J_{C\text{-}P}$=8.1 Hz), 74.83, 97.09 (d, $J_{C\text{-}P}$=4.2 Hz), 174.78, 175.07 (2×C), 175.69. ^{31}P NMR (D_2O): δ −2.15.

Guanosine-5′-(β-L-glucopyranosyl)-1-diphosphate (GDP-L-glc) (6). According to the general coupling and deacetylation protocols glucosylphosphate **15.4** (0.50 g, 1.2 mmol) was coupled to tri-*n*-butylammonium guanosine-5′-monophosphate (0.51 g, 0.9 mmol) and the resulting diphosphate deacetylated to give the title compound (0.29 g, 46% overall, contains 100% according UV_{251nm}). ^{1}H NMR (D_2O): δ 3.34 (m, 2 H), 3.46 (m, 2 H), 3.67 (dd, J=12.4, 6.2 Hz, 1 H), 3.85 (dd, J=12.4, 1.4 Hz, 1 H), 4.21 (m, 2 H), 4.43 (m, 1 H), 4.51 (m, 1 H), 4.75 (t, J=5.5 Hz, 1 H), 4.94 (t, J=6.9 Hz, 1 H), 5.87 (d, 6.9 Hz, 1 H), 8.06 (s, 1 H). ^{13}C NMR

(D_2O): δ 63.28, 67.84 (d, $J_{C\text{-}P}$=4.6 Hz), 71.93, 72.93, 76.13 (d, $J_{C\text{-}P}$=8.6 Hz), 76.27, 77.67, 79.01, 86.26 (d, $J_{C\text{-}P}$=9.2 Hz), 89.32, 100.42 (d, $J_{C\text{-}P}$=6.2 Hz), 118.68, 140.03, 154.23, 156.39, 161.40. ^{31}P NMR (D_2O): δ −11.19 (d, J=20.2 Hz), −12.89 (d, J=20.2 Hz).

3,4-Di-*O*-acetyl-fucal (17). Bromide **13.1** (15.5 g, 43.8), dissolved in 45 mL THF, was added to zinc (17.0 g, 262 mmol), pretreated as given in lit.,[22] and 1-methylimidazole (0.35 mL, 43.8 mmol) in dry THF (230 mL). The mixture was refluxed for 1 h, cooled down, filtered over celite, and carefully evapd to give a slowly crystallizing syrup (8.5 g, 80%). The fucal proved to be quite volatile. ^{1}H NMR ($CDCl_3$): δ 1.21 (d, J=6.7 Hz, 3 H), 1.95 (s, 3 H), 2.09 (s, 3 H), 4.14 (bq, J=6.7 Hz, 1 H), 4.59 (dt, J=6.0, 1.2 Hz, 1 H), 5.22 (bd, J=6.0 Hz, 1 H), 6.40 (dd, J=6.0, 1.0 Hz, 1 H). ^{13}C NMR ($CDCl_3$): δ 16.46, 20.65, 20.80, 65.00, 66.24, 71.48, 98.22, 146.06, 170.35, 170.65.

2-Azido-3,4-di-*O*-acetyl-β-L-fucopyranosyl-1-dibenzylphosphate (20). According to known protocols[23,24] fucal **17** was converted to bromide **19** via the anomeric nitrates **18**. Bromide **19** (1.90 g, 5.80 mmol) was immediately coupled to dibenzyl phosphate according the general phosphorylation procedure to give the title compound (3.0 g, 97%), which was processed further immediately. ^{1}H NMR ($CDCl_3$): δ 1.21 (d, J=6.9 Hz, 3 H), 2.03 (s, 3 H), 2.15 (s, 3 H), 3.73 (dd, J=7.6, 2.0 Hz, 1 H), 3.80 (bq, J=6.9 Hz, 1 H), 4.80 (dd, J=11.7, 4.1 Hz, 1 H), 5.09 (m, 5 H), 5.18 (bd, J=4.1 Hz, 1 H), 7.31 (m, 10 H). ^{13}C NMR ($CDCl_3$): δ 15.66, 20.33 (2 × C), 60.56 (d, $J_{C\text{-}P}$=9.6 Hz), 68.90, 69.12 (d, $J_{C\text{-}P}$=4.1 Hz), 69.16 (d, $J_{C\text{-}P}$=4.3 Hz), 69.95, 71.29, 97.10 (d, $J_{C\text{-}P}$=5.1 Hz), 127.60, 128.28, 128.32, 135.11, 135.25, 135.38, 169.38, 170.05. ^{31}P NMR ($CDCl_3$): δ −2.75.

2-Amino-3,4-di-*O*-acetyl-β-L-fucopyranosyl-1-phosphate (21). Dibenzyl phosphate **20** (1.25 g, 2.3 mmol) was hydrogenated in the absence of triethylamine, as described for compound **15.1**, to give the title phosphate (0.75 g, 97%) as an internal salt, which was not treated with an ion-exchange resin. ^{1}H NMR (D_2O): δ 1.20 (d, J=6.9 Hz, 3 H), 2.06 (s, 3 H), 2.18 (s, 3 H), 3.56 (bd, J=8.9 Hz, 1 H), 4.10 (bq, J=6.9 Hz, 1 H), 5.25 (m, 5 H). ^{13}C NMR (D_2O): δ 15.01, 19.92, 20.06, 51.70 (d, $J_{C\text{-}P}$=9.5 Hz), 69.41, 69.93, 70.28, 94.09 (d, $J_{C\text{-}P}$=4.8 Hz), 170.50, 173.54. ^{31}P NMR (D_2O): δ −1.56.

Guanosine-5′-(β-L-2-amino-fucopyranosyl)-1-diphosphate (GDP-2-NH$_2$-fuc) (2). According to the general coupling and deacetylation protocols fucosylphosphate **21** (0.30 g, 0.9 mmol) was coupled to tri-*n*-butylammonium guanosine-5′-monophosphate (0.40 g, 0.8 mmol) and the resulting diphosphate deacetylated to give the title compound (0.25 g, 53% overall, contains 95% according UV_{252nm}). ^{1}H NMR (D_2O): δ 1.22 (d, J=7.6 Hz, 3 H), 3.17 (dd, J=10.3, 8.3 Hz, 1 H), 3.68 (m, 3 H), 4.20 (m, 2 H), 4.50 (m, 1 H), 4.71 (m, 1 H), 5.17 (t, J=6.9 Hz, 1 H), 5.88 (d, 6.9 Hz, 1 H), 8.06 (s, 1 H). ^{13}C NMR (D_2O): δ 15.72, 54.07 (d, $J_{C\text{-}P}$=8.6 Hz), 65.69 (d, $J_{C\text{-}P}$=5.6 Hz), 70.19, 70.46, 70.67, 72.28, 74.06, 83.97 (d, $J_{C\text{-}P}$=9.1 Hz), 87.24, 95.57 (d, $J_{C\text{-}P}$=4.6 Hz), 116.59, 137.88, 152.13, 154.34, 159.39. ^{31}P NMR (D_2O): δ −11.19 (d, J=20.3 Hz), −13.57 (d, J=20.3 Hz).

3,4-Di-*O*-acetyl-2-fluoro-β-L-fucopyranosyl-1-bromide (23). The known fluoro sugar **22** (0.58 g , 2.0 mmol)[25] was treated with HBr, as described for compound **13.1**, to give the labile title bromide (0.58 g, 93%), which was processed immediately. ^{1}H NMR ($CDCl_3$): δ 1.18 (d, J=6.9 Hz, 3 H), 2.01 (s, 3 H), 2.13 (s, 3 H), 4.39 (bq, J=6.9 Hz, 1 H), 4.71 (ddd, $J_{H\text{-}F}$=49.5 Hz, J=10.3, 4.8 Hz, 1 H), 5.35 (m, 1 H), 5.42 (dt, J=10.3, 4.8 Hz, 1 H), 6.56 (bd, J=4.8 Hz, 1 H). ^{19}F NMR ($CDCl_3$): δ −195.6.

3,4-Di-*O*-acetyl-2-fluoro-β-L-fucopyranosyl-1-dibenzylphosphate (24). Bromide **23** (0.53 g, 1.7 mmol) was phosphorylated according compound **14.1** to give the title phosphate (0.82 g, 92%). ^{1}H NMR ($CDCl_3$): δ 1.20 (d, J=6.9 Hz, 3 H), 2.04 (s, 3 H), 2.15 (s, 3 H), 3.91 (bq, J=6.9 Hz, 1 H), 4.60 (ddd, $J_{H\text{-}F}$=51.6 Hz, J=10.3, 6.9 Hz, 1 H), 5.10 (m, 5 H), 5.27 (m, 1 H), 5.35 (dt, J=6.9, 4.1 Hz, 1 H), 7.31 (m, 10 H). ^{13}C NMR ($CDCl_3$): δ 15.70, 20.46, 20.50, 68.76 (d, J=5.2 Hz), 68.83 (d, J=4.1 Hz), 69.63 (d, J=8.2 Hz), 70.20 (d, J=18.2 Hz), 70.25, 85.58 (dd, J=179.4, 4.0 Hz), 95.54 (dd, J=28.6, 4.6 Hz), 127.84, 127.90, 128.03, 128.49, 169.73, 170.19. ^{19}F NMR ($CDCl_3$): δ −208.4. ^{31}P NMR ($CDCl_3$): δ −2.99.

3,4-Di-*O*-acetyl-2-fluoro-β-L-fucopyranosyl-1-phosphate (25). Dibenzylester **24** (0.75 g, 1.5 mmol) was hydrogenated, as described for compound **15.1**, to give the title compound (0.48 g, 92%) after Amberlite-treatment (H^+-form). ^{1}H NMR (D_2O): δ 1.22 (d, J=6.9 Hz, 3 H), 2.11 (s, 3 H), 2.23 (s, 3 H), 4.18 (bq, J=6.9 Hz, 1 H), 4.64 (dt, $J_{H\text{-}F}$=51.6 Hz, J=7.6 Hz, 1 H), 5.30 (m, 3 H). ^{13}C NMR (D_2O): δ 18.10, 23.15, 23.34, 73.22, 74.71, 74.80 (d, J=29.0 Hz), 91.83 (bd, J=187.5 Hz), 98.17 (bd, J=24.0 Hz), 176.14, 176.86. ^{19}F NMR ($CDCl_3$): δ −200.8. ^{31}P NMR ($CDCl_3$): δ −1.81.

Guanosine-5′-(β-L-2-fluoro-fucopyranosyl)-1-diphosphate (GDP-2-F-fuc) (3). According to the general coupling and deacetylation protocols fucosylphosphate **25** (0.15 g, 0.4 mmol) was coupled to tri-*n*-butylammonium guanosine-5′-monophosphate (0.20 g, 0.4 mmol) and the resulting diphosphate deacetylated to give the title compound (0.19 g, 80% overall, contains 100% according to UV_{251nm}). ^{1}H NMR (D_2O): δ 1.21 (d, J=7.6 Hz, 3 H), 3.79 (m, 2 H), 4.00 (m, 1 H), 4.21 (m, 2 H), 4.26 (m, 1 H), 4.35 (m, 1 H), 450 (m, 2 H), 5.16 (m, 1 H), 5.91 (d, J=6.9 Hz, 1 H), 8.09 (s, 1 H). ^{13}C NMR (D_2O): δ 65.61 (d, J=4.8 Hz), 70.78, 71.53 (d, J=53.5 Hz), 71.99, 72.05, 74.00, 84.10 (d, J=6.4 Hz), 87.13; 90.94 (dd, J=184.0, 10.7 Hz), 95.78 (dd, J=24.2, 4.8 Hz), 116.48, 137.85, 152.04, 154.29, 159.31. ^{19}F NMR (D_2O): δ −205.8. ^{31}P NMR (D_2O): δ −11.29 (d, J=19.5 Hz), −13.23 (d, J=19.5 Hz).

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