Hydrogenphosphonate synthesis of sugar phosphomonoesters

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A highly efficient procedure for the phosphorylation of sugar hydroxy derivatives has been developed. A four-step sequence comprising H-phosphonate formation, pivaloyl chloride-mediated coupling with fluoren-9-ylmethanol, oxidation, and cleavage of the fluoren-9-ylmethyl ester led to the sugar monophosphate derivatives **4a**–**g** in 83–93% yield.

Introduction

The key roles that carbohydrate phosphates play in Nature, both as components of nucleic acids and various coenzymes, and in the biosynthesis and metabolism of sugars, make novel methods for their preparation of great value. There is an abundant literature on phosphorylation procedures[1] including reports of chemical syntheses of sugar phosphomonoesters from hydroxylic derivatives using phosphomonoester (phosphoryl trichloride–*N*-ethylmorpholine),[2] phosphodiester (β-cyanoethyl phosphate–condensing agent, *e.g.* DCC, TPSCl, *etc.*),[3] phosphotriester [diphenyl phosphorochloridate,[4] dibenzyl phosphorochloridate,[5] or bis(2,2,2-trichloroethyl) phosphorochloridate[6] in pyridine] and phosphoramidite (dibenzyl diisopropylphosphoramidite–1*H*-tetrazole followed by oxidation)[7] methods with moderate to high efficiency. In addition, sugar phosphates can be prepared enzymically.[8]

The highly efficient hydrogenphosphonate (H-phosphonate) synthesis of phosphoric diesters (involving the formation of H-phosphonic monoesters[9,10] from alcohols followed by esterification with the second alcohol to form H-phosphonic diesters[11] and oxidation with iodine–pyridine–water system[12]) is well established and widely used in many synthetic projects towards oligonucleotides[13] and sugar phosphodiesters.[10,14–17] The principal advantages of the H-phosphonate approach are both high efficiency and high reaction rate for all three chemical steps involved.

It is worthy to note that, unlike the rapid oxidation of the H-phosphonic diesters to phosphoric diesters with iodine, the transformation of the H-phosphonic monoesters to phosphates requires trimethylsilylation (to form the corresponding bis-trimethylsilyl alkyl phosphites) prior to the oxidation to phosphoric triesters, followed by hydrolysis of the trimethylsilyl groups.[12,18] The latter approach did not find widespread application for a preparative synthesis of organic phosphates, probably because the efficiency of the presilylation can be monitored by ^{31}P NMR only.

Results and discussion

The above findings urged us to examine the applicability of the H-phosphonate approach for the phosphorylation of primary, secondary and anomeric HO-groups of carbohydrates using a fluoren-9-ylmethyl ester as P-protecting group[19–21] to facilitate the oxidation step. The protecting group could then be easily removed with piperidine to provide the desired phosphomonoesters. We now report a novel, efficacious method for *O*-phosphorylation of the model hydroxylic derivatives **1a**–**1g** (Scheme 1) to produce the phosphomonoesters **4a**–**4g**, respectively, *via* the consecutive preparation of the H-phosphonates **2a**–**2g** and the monosaccharide fluoren-9-ylmethyl phosphodiesters **3a**–**3g**.

The monohydroxylic carbohydrate derivatives **1a**–**1g** were first H-phosphonylated by reaction (30 min) with triimidazolylphosphine (prepared *in situ* from PCl_3, imidazole and Et_3N) followed by mild hydrolysis to give the H-phosphonates **2a**–**2g**, respectively, in excellent 93–100% yield. Signals characteristic of the H-phosphonate group (δ_P 1.87–5.79; δ_H 6.67–7.14; $^1J_{P,H}$ 624–650 Hz) were present in the ^{31}P and ^{1}H NMR spectra of the compounds. The H-phosphonic monoesters **2a**–**2g** were then converted to the fluoren-9-ylmethyl phosphodiesters **3a**–**3g** (91–97%; δ_P between −1.85 and 0.74), respectively, by esterification (30 min) with fluoren-9-ylmethanol (2.5 equiv.) in pyridine in the presence of trimethylacetyl chloride followed by *in situ* oxidation (30 min) of the resulting H-phosphonic diesters with iodine in aq. pyridine. The targeted phosphomonoesters **4a**–**4g** were prepared from the diesters **3a**–**3g**, respectively, by mild cleavage (30 min) of the fluoren-9-ylmethyl group with piperidine in dichloromethane (1 : 5 v/v) in superior 94–100% yield.

Because of the high efficiency of each step, the whole reaction sequence benefits from the absence of any chromatographic isolation for the purification of both the intermediates **2a**–**2g** and **3a**–**3g**, and the final products **4a**–**4g** (see Experimental section). All the chemical transformations could be easily monitored by TLC.

The described procedure led smoothly to the sugar phosphates **4a**–**g** in 83–93% total yield starting from the corresponding monohydroxylic derivatives. All the reactions proceeded rapidly and very efficiently, including transformation of compounds **1b**, **1f** and **1g** containing sterically hindered hydroxy groups at C-4. Similar phosphorylation of the diol **1h** provided the corresponding 2,3-diphosphate **4h** in 71% yield. It should be noted that the fluoren-9-ylmethyl P-protecting group could be cleaved under extremely mild conditions which do not affect *O*-benzyl, *O*-benzylidene, *O*-benzoyl or *O*-acetyl protecting groups. In contrast, cleavage of the most widely used P-protecting groups[1,3–5,7] requires either hydrogenation (for benzyl, dibenzyl and diphenyl phosphates), or basic treatment (for phenyl, 2- and 4-chlorophenyl and β-cyanoethyl phosphates), which may not be compatible with generic synthetic strategy.

The structures of the sugar phosphates **4a**–**4h** were confirmed by NMR and mass spectrometry data (see Experimental section). Signals in the ^{31}P NMR spectra appeared as a triplet ($^3J_{P,H}$ 5.6 Hz) for compound **4a** and as a doublet ($^3J_{P,H}$ 6–11

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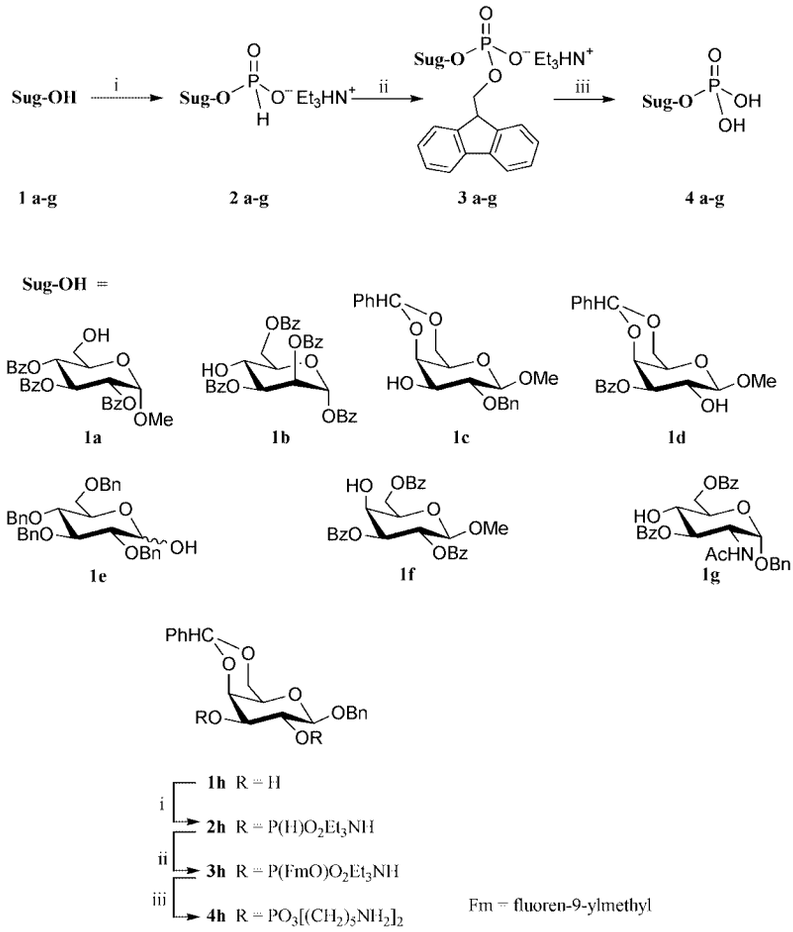

Scheme 1 *Reagents*: i, (a) triimidazolylphosphine, MeCN; (b) Et_3NHHCO_3, water (pH 7); ii, (a) fluoren-9-ylmethanol, pivaloyl chloride, pyridine; (b) I_2, pyridine–water; iii, piperidine, CH_2Cl_2.

Hz) for the derivatives **4b**–**h** at δ_P between 0.28 and 4.19 and were characteristic of phosphomonoesters. The position of the phosphate group (*O*-1, -2, -3, -4, or -6) was clearly indicated by the signals of the corresponding H-atoms in the ^1H NMR spectra. These signals were shifted as a result of the phosphorylation and were coupled with phosphorus. The signals in the ES(−) mass spectra of the monophosphates **4a**–**4g** corresponded to the pseudo-molecular ion $[M - H]^-$ for the compounds. The signals in the FAB(+) mass spectrum of the 2,3-diphosphate **4h** were consistent with the molecular mass of the derivative.

In conclusion, we report a novel, fast and simple method for the phosphorylation of sugar hydroxylic derivatives based on H-phosphonate chemistry.[9–14] The method can be considered as a useful alternative to the traditional procedures,[1–8] because it appears to be (1) equally highly efficient for all three major types of HO-group (primary, secondary and anomeric) present in carbohydrates and (2) fully compatible with principal types of *O*-protecting groups.

Experimental

General procedures

Optical rotations were measured with a Perkin-Elmer 141 polarimeter; $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. NMR spectra (^1H at 300 MHz and ^{31}P at 121 MHz) were recorded with a Bruker DPX-300 spectrometer for solutions in $CDCl_3$. Chemical shifts (δ in ppm) are given relative to those for Me_4Si (for ^1H) and external aq. 85% H_3PO_4 (for ^{31}P); *J*-values are given in Hz. ES mass spectra were recorded with a Micromass Quattro system (Micromass Biotech, UK). FAB mass spectra were recorded with a VG 70–250 SE mass spectrometer using an Ion-tech xenon gun. Filtration of solutions through a silica gel pad was performed on Kieselgel 60 (0.040–0.063 mm) (Merck). TLC was performed on Kieselgel 60 F_{254} (Merck) with *A*, dichloromethane–methanol (3 : 1) and *B*, dichloromethane–methanol (9 : 1). Dichloromethane, acetonitrile and pyridine were freshly distilled from CaH_2. Solutions worked up were concentrated under reduced pressure at <40 °C. Petroleum spirit refers to that fraction with distillation range 60–80 °C.

Methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside 6-phosphate, dipiperidinium salt 4a

To a stirred solution of imidazole (538 mg, 7.90 mmol, 7 equiv.) in MeCN (15 cm^3) at 0 °C was added phosphorus trichloride (0.18 cm^3, 2.07 mmol, 5.5 equiv.) and then triethylamine (1.18 cm^3, 8.46 mmol, 7.5 equiv.). The mixture was stirred for 15 min, after which a solution of compound **1a** (192 mg, 0.376 mmol) in MeCN (6 cm^3) was added dropwise during 10 min at 0 °C. The mixture was stirred at rt for 20 min and quenched with 1 mol dm^{-3} triethylammonium (TEA) hydrogen carbonate (pH 7; 6 cm^3). The clear solution was stirred for 15 min, CH_2Cl_2 (70 cm^3) was added, and the organic layer was washed in turn with ice–water and cold 0.5 mol dm^{-3} TEA hydrogen carbonate, dried by filtration through cotton wool, and concentrated to give the pure H-phosphonate **2a** (254 mg, 100%) as an amorphous solid, δ_P 4.95 (dt, $^1J_{P,H}$ 623.8, $^3J_{P,H}$ 7.5); δ_H 6.93 (d, HP).

The H-phosphonate **2a** (214 mg, 0.317 mmol) was dissolved in pyridine (5 cm^3), fluoren-9-ylmethanol (156 mg, 0.793 mmol, 2.5 equiv.) was added followed by the addition of pivaloyl chloride (0.156 cm^3, 1.27 mmol, 4 equiv.), and the mixture was stirred at rt for 30 min, whereafter a freshly prepared solution of iodine (160 mg, 0.63 mmol, 2 equiv.) in pyridine–water (95 : 5; 3 cm^3) was added. After 30 min, CH_2Cl_2 was added and

the solution was washed successively with ice-cold 1 mol dm^{-3} aq. $Na_2S_2O_3$ and cold 0.5 mol dm^{-3} aq. TEA hydrogen carbonate, dried by filtration through cotton wool, and concentrated. A solution of the residue in toluene–ethyl acetate (7:3) was passed through a silica gel pad using, first, the same solvent and then dichloromethane–methanol (9:1) for the elution. The DCM–MeOH fraction was washed with 0.5 mol dm^{-3} aq. TEA hydrogen carbonate and concentrated to produce the phosphodiester **3a** (265 mg, 97%) as a chromatographically homogeneous amorphous solid, δ_P −0.54 (quintet, $J_{P,H}$ 4.7).

The phosphodiester **3a** (50 mg, 0.058 mmol) was dissolved in CH_2Cl_2 (2.0 cm^3), piperidine (0.4 cm^3) was added, and the mixture was kept at rt for 30 min and then concentrated at 0.01 mmHg (oil-pump). A solution of the residue in methanol–petroleum spirit (1:2; 50 cm^3) was extracted with water (20 cm^3) and the aqueous layer was concentrated to give the phosphomonoester **4a** (41 mg, 95%; 92% based on compound **1a**) as a chromatographically pure amorphous solid, $[\alpha]_D^{24}$ +36 (*c* 1, $CHCl_3$); R_f 0.38 (solvent *A*); δ_H 1.55 (4 H, br, 2 × CH_2-CH_2CH_2N), 1.75 (8 H, br, 4 × CH_2CH_2N), 3.00 (8 H, br, 4 × CH_2N), 3.46 (3 H, s, OMe), 3.95 (1 H, dt, $J_{5,6a} = J_{6a,P} = 5.6$, 6-H^a), 4.03 (1 H, ddd, $J_{6b,P}$ 5.6, $J_{6a,6b}$ 11.3, 6-H^b), 4.26 (1 H, ddd, $J_{5,6b}$ 2.1, 5-H), 5.16 (1 H, d, $J_{1,2}$ 3.5, 1-H), 5.21 (1 H, dd, $J_{2,3}$ 10.1, 2-H), 5.52 (1 H, t, $J_{3,4} = J_{4,5} = 9.7$, 4-H), 6.12 (1 H, dd, 3-H) and 7.20–8.00 (15 H, m, 3 × Ph); δ_P 3.18 (t, *J* 5.6); ES-MS(−) *m*/*z* 584.89 (100%, $[M - H]^-$) (free acid $C_{28}H_{27}O_{12}P$ requires *M*, 586.12).

1,2,3,6-Tetra-*O*-benzoyl-α-D-mannopyranose 4-phosphate, bis(triethylammonium) salt 4b

The tetrabenzoate **1b** (100 mg) was first converted to the H-phosphonate **2b** [117 mg, 93%; δ_P 4.71 (dd, $^1J_{P,H}$ 632.6, $^3J_{P,H}$ 11.3); δ_H 6.99 (d, HP)] and then to the phosphodiester **3b** [139 mg, 95%; δ_P −1.11 (dt, J_{P,CH_2} 5.4, $J_{P,4\text{-}H}$ 9.7)] as described in the preparation of the phosphate **4a**.

The phosphodiester **3b** (29 mg, 0.031 mmol) was dissolved in CH_2Cl_2 (0.5 cm^3), piperidine (0.1 cm^3) was added and the mixture was kept at rt for 30 min, then diluted with CH_2Cl_2, washed with 0.5 mol dm^{-3} HCl, dried by filtration through cotton wool, and concentrated. A solution of the residue in toluene–ethyl acetate (7:3) was passed through a silica gel pad using, first, the same solvent and then dichloromethane–methanol (9:1) for the elution. The DCM–MeOH fraction was washed with 0.5 mol dm^{-3} aq. TEA hydrogen carbonate and concentrated to produce the phosphomonoester **4b** (25 mg, 94%; 83% based on compound **1b**) as a chromatographically pure amorphous solid, $[\alpha]_D^{24}$ +11 (*c* 1, $CHCl_3$); R_f 0.08 (solvent *B*); δ_H 0.98 (18 H, t, *J* 7.2, 6 × CH_3CH_2N), 2.59 (12 H, q, 6 × CH_3CH_2N), 4.45 (1 H, ddd, $J_{5,6a}$ 6.4, $J_{4,5}$ 9.8, 5-H), 4.64 (1 H, dd, $J_{6a,6b}$ 11.9, 6-H^a), 4.85 (1 H, dd, $J_{5,6b}$ 1.5, 6-H^b), 5.51 (1 H, q, $J_{3,4} = J_{4,5} = J_{4,P} = 9.8$, 4-H), 5.78 (1 H, dd, $J_{1,2}$ 1.9, $J_{2,3}$ 3.4, 2-H), 5.83 (1 H, dd, 3-H), 6.45 (1 H, d, 1-H) and 7.00–8.20 (20 H, m, 4 × Ph); δ_P 1.26 (d, *J* 9.8); ES-MS(−) *m*/*z* 675.16 (100%, $[M - H]^-$) (free acid $C_{34}H_{29}O_{13}P$ requires *M*, 676.13).

Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside 3-phosphate, dipiperidinium salt 4c

This compound was prepared from compound **1c** (55 mg) *via* the consecutive formation of the H-phosphonate **2c** [73 mg, 94%; δ_P 5.79 (dd, $^1J_{P,H}$ 630.7, $^3J_{P,H}$ 10.7); δ_H 7.05 (d, HP)] and the phosphodiester **3c** [94 mg, 94%; δ_P −0.79 (dt, J_{P,CH_2} 4.4, $J_{P,3\text{-}H}$ 8.0)] followed by P-deprotection of the derivative **3c** (20 mg) as described for the preparation of the phosphate **4a**. This produced the phosphomonoester **4c** (17 mg, 100%; 88% based on compound **1c**) as a chromatographically pure amorphous solid, $[\alpha]_D^{24}$ +24 (*c* 1, $CHCl_3$); R_f 0.40 (solvent *A*); δ_H 1.52 (4 H, br, 2 × C$H_2CH_2CH_2N$), 1.78 (8 H, br, 4 × CH_2CH_2N), 3.00 (8 H, br, 4 × CH_2N), 3.24 (1 H, s, 5-H), 3.60 (3 H, s, OMe), 3.62 (1 H, dd, 2-H), 3.90 and 4.12 (AB, J_{gem} 12.2, 6-H^a and 6-H^b), 4.31 (1 H, dt, $J_{2,3} = J_{3,P} = 9.6$, 3-H), 4.44 (1 H, d, $J_{1,2}$ 7.6, 1-H), 4.52 (1 H, d, $J_{3,4}$ 3.1, 4-H), 4.76 and 4.86 (AB, J_{gem} 11.2, PhCH_2), 5.54 (1 H, s, PhC*H*) and 7.10–7.60 (10 H, m, 2 × Ph); δ_P 2.14 (d, *J* 9.6); ES-MS(−) *m*/*z* 451.03 (100%, $[M - H]^-$) (free acid $C_{21}H_{25}O_9P$ requires *M*, 452.12).

Methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside 2-phosphate, dipiperidinium salt 4d

This compound was prepared from compound **1d** (100 mg) *via* the consecutive formation of the H-phosphonate **2d** [134 mg, 96%; δ_P 3.29 (dd, $^1J_{P,H}$ 641.8, $^3J_{P,H}$ 10.8); δ_H 6.95 (d, HP)] and the phosphodiester **3d** [177 mg, 97%; δ_P −1.41 (dt, J_{P,CH_2} 5.9, $J_{P,2\text{-}H}$ 10.3)] followed by P-deprotection of the derivative **3d** (40 mg) as described for the preparation of the phosphate **4a**. This produced the phosphomonoester **4d** (34 mg, 100%; 93% based on compound **1d**) as a chromatographically pure amorphous solid, $[\alpha]_D^{24}$ +52 (*c* 1, $CHCl_3$); R_f 0.43 (solvent *A*); δ_H 1.52 (4 H, br, 2 × C$H_2CH_2CH_2N$), 1.78 (8 H, br, 4 × CH_2CH_2N), 3.00 (8 H, br, 4 × CH_2N), 3.55 (3 H, s, OMe), 3.62 (1 H, s, 5-H), 4.07 and 4.34 (AB, J_{gem} 12.5, 6-H^a and 6-H^b), 4.49 (1 H, $J_{1,2}$ 7.8, 1-H), 4.52 (1 H, d, 4-H), 4.55 (1 H, dt, $J_{2,3} = J_{2,P} = 9.8$, 2-H), 5.15 (1 H, dd, $J_{3,4}$ 3.5, 3-H), 5.47 (1 H, s, PhC*H*) and 7.10–8.20 (10 H, m, 2 × Ph); δ_P 1.39 (d, *J* 9.8); ES-MS(−) *m*/*z* 464.99 (100%, $[M - H]^-$) (free acid $C_{21}H_{23}O_{10}P$ requires *M*, 466.10).

2,3,4,6-Tetra-*O*-benzyl-α,β-D-glucopyranosyl phosphate, monopiperidinium salt 4e

This compound was prepared from compound **1e** (100 mg; anomeric mixture, α:β ≈ 3.4:1) *via* the consecutive formation of the H-phosphonate **2e** [254 mg, 99%; δ_P 1.87 (dd, $^1J_{P,H}$ 649.0, $^3J_{P,H}$ 8.3, P$^\alpha$) and 2.29 (dd, $^1J_{P,H}$ 650.4, $^3J_{P,H}$ 9.1, P$^\beta$); δ_H 7.08 (d, HP$^\alpha$) and 7.14 (d, HP$^\beta$); α:β ≈ 3.4:1] and the phosphodiester **3e** [303 mg, 93%; δ_P −1.85 (dt, J_{P,CH_2} 5.6, $J_{P,1\text{-}H}$ 8.2, P$^\beta$) and −1.34 (dt, J_{P,CH_2} 4.9, $J_{P,1\text{-}H}$ 8.0, P$^\alpha$); α:β ≈ 3.4:1] followed by the P-deprotection of the derivative **3e** (64 mg) as described for the preparation of the phosphate **4a**. This produced the phosphomonoester **4e** (48 mg, 94%; 87% based on compound **1e**) as an amorphous solid, R_f 0.50 (solvent *A*); δ_H (*inter alia*) 1.40 (2 H, br, C$H_2CH_2CH_2N$), 1.65 (4 H, br, 2 × CH_2CH_2N), 3.03 (4 H, br, 2 × CH_2N), 5.20 (dd, $J_{1,2}$ 9.8, $J_{1,P}$ 8.0, 1-H$^\beta$) and 5.83 (dd, $J_{1,2}$ 2.4, $J_{1,P}$ 6.0, 1-H$^\alpha$); δ_P 0.28 (d, *J* 8.0, P$^\beta$) and 0.44 (d, *J* 6.0, P$^\alpha$); α:β ≈ 3.4:1; ES-MS(−) *m*/*z* 619.1 (100%, $[M - H]^-$) (free acid $C_{34}H_{37}O_9P$ requires *M*, 620.22).

Methyl 2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside 4-phosphate, dipiperidinium salt 4f

This compound was prepared from compound **1f** (160 mg) *via* the consecutive formation of the H-phosphonate **2f** [208 mg, 100%; δ_P 3.84 (dd, $^1J_{P,H}$ 631.7, $^3J_{P,H}$ 11.6); δ_H 6.98 (d, HP)] and the phosphodiester **3f** [260 mg, 96%; δ_P −0.59 (dt, J_{P,CH_2} 5.5, $J_{P,4\text{-}H}$ 9.9)] followed by P-deprotection of the derivative **3f** (60 mg) as described for the preparation of the phosphate **4a**. This produced the phosphomonoester **4f** (51 mg, 97%; 93% based on compound **1f**) as a chromatographically pure amorphous solid, $[\alpha]_D^{24}$ +28 (*c* 1, $CHCl_3$); R_f 0.38 (solvent *A*); δ_H 1.60 (4 H, br, 2 × C$H_2CH_2CH_2N$), 1.80 (8 H, br, 4 × CH_2CH_2N), 3.06 (8 H, br, 4 × CH_2N), 3.44 (3 H, s, OMe), 4.16 (1 H, dd, $J_{5,6a}$ 2.6, $J_{5,6b}$ 8.8, 5-H), 4.47 (1 H, dd, $J_{6a,6b}$ 12.1, 6-H^a), 4.62 (1 H, d, $J_{1,2}$ 7.8, 1-H), 4.66 (1 H, dd, 6-H^b), 4.86 (1 H, dd, $J_{3,4}$ 3.2, $J_{4,P}$ 11.0, 4-H), 5.31 (1 H, dd, $J_{2,3}$ 10.4, 3-H), 5.63 (1 H, dd, 2-H) and 7.20–8.00 (15 H, m, 3 × Ph); δ_P 2.01 (d, *J* 11.0); ES-MS(−) *m*/*z* 585.07 (100%, $[M - H]^-$) (free acid $C_{28}H_{27}O_{12}P$ requires *M*, 586.12).

Benzyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside 4-phosphate, dipiperidinium salt 4g

This compound was prepared from compound **1g** (60 mg) *via* the consecutive formation of the H-phosphonate **2g** [78 mg,

99%; δ_P 5.65 (dd, $^1J_{P,H}$ 629.0, $^3J_{P,H}$ 11.4); δ_H 6.67 (d, HP)] and the phosphodiester **3g** [91 mg, 91%; δ_P 0.74 (dt, J_{P,CH_2} 6.3, $J_{P,4\text{-}H}$ 8.6)] followed by P-deprotection of the derivative **3g** (91 mg) as described for the preparation of the phosphate **4a**. For these transformations, the following quantities of reagents were required: PCl_3 (9.9 equiv.), imidazole (11.9 equiv.), Et_3N (14.5 equiv.), fluoren-9-ylmethanol (4.4 equiv.), trimethylacetyl chloride (7.1 equiv.) and iodine (3.45 equiv.). This produced the phosphomonoester **4g** (80 mg, 100%; 90% based on compound **1g**) as a chromatographically pure amorphous solid, $[\alpha]_D^{24}$ +39 (*c* 1, $CHCl_3$); R_f 0.39 (solvent *A*); δ_H 1.40 (4 H, br, 2 × CH_2CH_2-CH_2N), 1.60 (8 H, br, 4 × CH_2CH_2N), 1.86 (3 H, s, Ac), 2.82 (8 H, br, 4 × CH_2N), 3.86 (1 H, dt, $J_{5,6a} = J_{5,6b}$ = 3.3, 5-H), 4.23 (1 H, ddd, $J_{2,NH}$ 4.9, 2-H), 4.36 (1 H, dd, $J_{6a,6b}$ 12.0, 6-H^a), 4.48 (1 H, dd, 6-H^b), 4.45 and 4.66 (AB, J_{gem} 11.6, PhCH_2), 4.52 (1 H, q, $J_{3,4} = J_{4,5} = J_{4,P}$ = 10.0, 4-H), 5.30 (1 H, t, $J_{2,3}$ 10.0, 3-H), 5.52 (1 H, d, $J_{1,2}$ 3.0, 1-H) and 7.20–8.20 (15 H, m, 3 × Ph); δ_P 4.19 (d, *J* 10.0); ES-MS(−) *m*/*z* 598.1 (100%, $[M - H]^-$) (free acid $C_{29}H_{30}NO_{11}P$ requires *M*, 599.15).

Benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside 2,3-diphosphate, tetrapiperidinium salt 4h

This compound was prepared from the diol **1h** (50 mg) *via* the consecutive formation of the 2,3-di(H-phosphonate) **2h** [δ_P 5.88 (dd, $^1J_{P,H}$ 633.3, $^3J_{P,H}$ 10.1, P) and 5.99 (dd, $^1J_{P,H}$ 646.6, $^3J_{P,H}$ 10.5, P′); δ_H 6.96 (d, $^1J_{P,H}$ 633.3, HP) and 6.98 (d, $^1J_{P,H}$ 646.6, HP′)] and the 2,3-bisphosphodiester **3h** [107 mg, 71% based on compound **1h**; δ_P 0.54 (dt, J_{P,CH_2} 4.7, $J_{P,H}$ 9.4) and 1.65 (dt, J_{P,CH_2} 4.4, $J_{P,H}$ 9.0)] followed by P-deprotection of the derivative **3h** (53 mg) as described for the preparation of the phosphate **4a**. The following quantities of the reagents per HO-group were used: PCl_3 (6.1 equiv.), imidazole (7.3 equiv.), Et_3N (8.6 equiv.), fluoren-9-ylmethanol (2.7 equiv.), trimethylacetyl chloride (4.3 equiv.), and iodine (2.1 equiv.). This produced the 2,3-diphosphate **4h** (42 mg, 100%; 71% based on compound **1h**) as a chromatographically pure amorphous solid, $[\alpha]_D^{24}$ +27 (*c* 1, $CHCl_3$); R_f 0.21 (solvent *A*); δ_H 1.52 (8 H, br, 4 × CH_2CH_2-CH_2N), 1.78 (16 H, br, 8 × CH_2CH_2N), 3.00 (16 H, br, 8 × CH_2N), 3.53 (1 H, s, 5-H), 3.90 (1 H, dt, $J_{2,3} = J_{2,P}$ = 9.7, 2-H), 4.18 and 4.27 (AB, J_{gem} 12.3, 6-H^a and 6-H^b), 4.20 (1 H, dt, $J_{3,P}$ 9.7, 3-H), 4.37 (1 H, d, $J_{3,4}$ 3.1, 4-H), 4.46 (1 H, d, $J_{1,2}$ 7.7, 1-H), 4.68 and 4.97 (AB, J_{gem} 12. 0, PhCH_2), 5.55 (1 H, s, PhC*H*) and 7.10–7.60 (10 H, m, 2 × Ph); δ_P 4.06 (d, *J* 9.7); FAB-MS(+) *m*/*z* 279 (60%, $[M + K + H]^{2+}$), 328 (20, $[M + (CH_2)_5NH + 3NH_3 + 2H]^{2+}$), 364 (100, $[M + 2 (CH_2)_5NH + K + H]^{2+}$) and 524 (30, $[M + 6 (CH_2)_5NH + NH_3 + 2 H]^{2+}$) (free acid $C_{20}H_{24}O_{12}P_2$ requires *M*, 518.07).

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