Supporting Information for:

Selective Phosphitylation of the Primary Hydroxyl Group in Unprotected Carbohydrates and Nucleosides

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Experimental Section

General. Acetonitrile was distilled from CaH_2. Carbohydrates **1a** and **1b** (Chapeau, M.-C.; Marnett, L.J. *J. Org. Chem.* **1993**, *58*, 7258) and phosphoramidite **3a (**Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta.* **1987**, *70*, 175; Pederson, R.L.; Esker, J.; Wong, C.-H. *Tetrahedron*. **1991**, *47*, 2643) were prepared as described. The synthesis of nucleoside **1d** will be reported in due course (Graham, S.M.; Spinnato, D.S. Unpublished results.). All other carbohydrates and reagents were commercially available. The starting carbohydrate and tetrazole were dried together by evaporating three times from dry acetonitrile. All reactions were performed under an atmosphere of dry nitrogen; solutions of phosphoramidites in CH_3CN were added to the reaction using a syringe pump and gastight syringes. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were obtained in $CDCl_3$ solvent and referenced to internal tetramethylsilane or the residual solvent peak unless otherwise stated; ^{31}P NMR spectra were referenced to external 85% H_3PO_4 in D_2O. The proton and carbon assignments for **2b** were made with the aid of DQF COSY and HMQC spectra (not shown); the assignments for the remaining compounds are in accord with these assignments but should be regarded as tentative. In all cases, identification of the primary hydroxyl group as the site of phosphorylation was confirmed using the Attached Proton Test as described in the article text.

bis(2-Cyanoethyl) (Pent-4-enyl β-D-ribofuranos-5-yl) Phosphate (2b). To a stirred solution of pent-4-enyl β-D-ribofuranoside (**1b**, 91 mg, 0.42 mmol) and 5-(*p*-nitrophenyl-1*H*-tetrazole (5-NPT, **4b**, 185 mg, 0.97 mmol) in CH_3CN (17 mL) at -36 °C was added dropwise (0.02 mmol/min) a 0.45 M solution of bis(2-cyanoethyl) N,N-diisopropylaminophosphoramidite (**3a**, 0.73 mL, 0.33 mmol) in CH_3CN, and the resulting solution was stirred for 20 minutes. A 0.95 M solution of *tert*-butyl hydroperoxide (TBHP, 4.0 mL, 3.8 mmol) in CH_2Cl_2 was then added, and the reaction was warmed to room temperature with a water bath. After stirring for 20 minutes, the reaction solution was evaporated to a small volume (~2-4 mL) and diluted with an equal volume of CH_2Cl_2. The crude product was then loaded onto a silica gel column and eluted with a step gradient of 60:40 CH_2Cl_2:ethyl acetate (MC:EA) to 100:0 MC:EA, followed by 90:10 EA:EtOH, yielding phosphate **2b** (0.20 mmol, 59 % based on limiting phosphoramidite; 81% based on total recovered carbohydrate) and recovered starting material (**1b**, 0.14 mmol). ^1H-NMR (δ, 600 MHz): 5.807 (ddt, *J* = 17.1, 13.2, 6.6 Hz, 1H, H4), 5.028 (dq, *J* = 17.1, 1.6 Hz, 1H, H5), 4.976 (ddt, *J* = 10.3, 2.0, 1.3 Hz, 1H, H5), 4.942 (s, 1H, H1'), 4.328 (dt, *J* = 8.1, 6.1 Hz, 2H, $-\mathit{CH}_2CH_2CN$), 4.325 (dt, *J* = 8.1, 6.1 Hz, 2H, $-\mathit{CH}_2CH_2CN$), 4.290 (ddd, *J* = 10.9, 8.2, 4.5 Hz, 1H, H5' or H5"), 4.262 (t, *J* = 5.3 Hz, 1H, H3'), 4.216 (ddd, *J* = 10.9, 9.1, 5.9 Hz, 1H, H5' or H5"), 4.145 (app. q, *J* ~ 6.2 Hz, 1H, H4'), 4.030 (d, *J* = 4.9 Hz, 1H, H2'), 3.719 (dt, *J* = 9.6, 6.6 Hz, 1H, H1), 3.563 (s, 1H, -OH), 3.409 (dt, *J* = 9.6, 6.6 Hz, 1H, H1), 3.209 (s, 1H, -OH), 2.807 (td, *J* = 6.1, 1.0 Hz, 4H, 2 x $-CH_2\mathit{CH}_2CN$), 2.100 (q, *J* = 7.0 Hz, 2H, H3), 1.655 (quin, *J* = 6.6 Hz, 2H, H2). ^{13}C-NMR (δ): 138.097 (C4), 116.578 ($-CH_2CH_2\mathit{C}N$), 115.045 (C5), 107.366 (C1'), 80.992 (d, $^3J_{\mathrm{POCC}}$ = 6.9 Hz, C4'), 75.027 (C2'), 71.931 (C3'), 69.701 (d, $^2J_{\mathrm{POC}}$ = 6.0 Hz, C5'), 67.561 (C1), 62.469 (d, $^2J_{\mathrm{POC}}$ = 5.7 Hz, -CH_2CH$_2$CN), 30.062 (C3), 28.575 (C2), 19.583 (d, $^3J_{\mathrm{POCC}}$ = 8.0 Hz, -CH$_2$$CH_2$CN). ^{31}P-NMR (δ, 162 MHz): -0.550.

bis(2-Cyanoethyl) (Pent-4-enyl α-D-ribofuranos-5-yl) Phosphate (2a). To a stirred solution of pentenyl α-D-riboside **1a** (71.9 mg, 0.33 mmol) and 5-NPT (**4b**, 151.2 mg, 0.79 mmol) in CH$_3$CN (17 mL) at -36 °C was added dropwise (0.02 mmol/min) a 0.47 M solution of phosphoramidite **3a** (530 µL, 0.25 mmol) in CH$_3$CN. After the addition the resulting solution was stirred for an additional 20 minutes at -36 °C. A 0.95 M TBHP solution (1.3 mL, 1.2 mmol) in CH$_2$Cl$_2$ was then added, and the reaction temperature was raised to RT and stirred for 20 minutes. The reaction solution was then evaporated to a minimum volume (~2-4 mL) and diluted with an equal volume of CH$_2$Cl$_2$. The crude product was then loaded onto a silica gel column and eluted as described above, yielding phosphate **2a** (0.14 mmol, 55% based on limiting phosphoramidite; 92% based on total recovered carbohydrate) and recovered starting material (**1a**, 0.17 mmol). ^{1}H-NMR (δ): 5.792 (ddt, *J* = 17.0, 13.2, 6.6 Hz, 1H, H4), 5.06-4.95 (m, 3H, =CH$_2$ and H1'), 4.33-4.23 (m, 6H, H5', H5" 2 x -CH_2CH$_2$CN), 4.13-4.06 (m, 2H, H4', H3'), 3.951 (dd, *J* = 6.6, 4.4 Hz, 1H, H2'), 3.813 (dt, *J* = 9.8, 6.6 Hz, 1H, H1), 3.520 (dt, *J* = 9.6, 6.6 Hz, 1H, H1), 3.02 (bs, 2H, 2 x OH), 2.785 (tt, *J* = 6.1, 1.0 Hz, 4H, 2 x -CH$_2$$CH_2$CN), 2.114 (app. q, *J* ~ 6.6 Hz, 2H, H3), 1.713 (app. quint., *J* ~ 6.7 Hz, 2H, H2). ^{13}C-NMR (δ): 137.900 (C4), 116.441 (-CH$_2$CH$_2$*C*N), 115.167 (C5), 101.569 (C1'), 81.917 (d, $^3J_{\mathrm{CCOP}}$ = 6.8 Hz, C4'), 70.899 (C2'), 70.186 (C3'), 68.077 (C1), 67.872 (d, $^2J_{\mathrm{COP}}$ = 5.7 Hz, C5'), 62.364 (d, $^2J_{\mathrm{COP}}$ = 5.7 Hz, -CH_2CH$_2$CN), 30.077 (C3), 28.484 (C2), 19.522 (d, $^3J_{\mathrm{CCOP}}$ = 8.0 Hz, -CH$_2$$CH_2$CN). ^{31}P-NMR (δ,162 MHz): -4.6.

bis(2-Cyanoethyl) (*n*-Octyl β-D-glucopyranos-5-yl) Phosphate (2c). To a stirred solution of *n*-octyl β-D-glucopyranoside (**1c**, 115.2 mg, 0.39 mmol) and 5-NPT (**4b**, 182.4 mg, 0.95 mmol) in CH$_3$CN (30 mL) at -36 °C was added dropwise (0.02 mmol/min) a 0.43 M solution of phosphoramidite **3a** (760 µL, 0.33 mmol) in CH$_3$CN. After the addition the resulting solution was stirred for an additional 20 minutes at -36 °C. A 0.88 M solution of TBHP (1.8 mL, 1.4 mmol) in CH$_2$Cl$_2$ was then added, and the reaction temperature was raised to RT and stirred for 20 minutes. The reaction solution was then evaporated to a minimum volume (~2-4 mL) and diluted with an equal volume of CH$_2$Cl$_2$. The crude product was then loaded onto a silica gel column and eluted as described above, yielding phosphate **2c** (0.18 mmol, 54% based on limiting phosphoramidite; 91% based on total recovered carbohydrate) and recovered starting material (**1c**, 0.18 mmol). ^{1}H-NMR (δ, DMSO-d$_6$ + D$_2$O): 4.296 (ddd, *J* = 11.0, 6.3, 1.6 Hz, 1H, H6' or H6"), 4.26-4.19 (m, 5H, -CH_2CH$_2$CN and H1'), 4.131 (ddd, *J* = 11.0, 7.9, 5.6 Hz, 1H, H6' or H6"), 3.732 (dt, *J* = 9.6, 6.8 Hz, 1H, -CH_2(CH$_2$)$_6$CH$_3$), 3.457 (dt, *J* = 9.6, 6.8 Hz, 1H, -CH_2(CH$_2$)$_6$CH$_3$), 3.41-3.37 (m, 1H, H5'), 3.208 (t, 1H, *J* = 9.0 Hz), 3.119 (app. t, *J* ~ 9.4 Hz), and 2.990 (dd, *J* = 9.0, 7.9 Hz) (3H, H2', H3', and H4'), 2.925 (t, *J* = 5.6 Hz, 4H, 2 x -CH$_2$CN), 1.508 (quint, *J* = 7.1 Hz, 2H, -CH$_2$$CH_2$(CH$_2$)$_5CH_3$), 1.274 (bs, 10H, -CH$_2CH_2$($CH_2$)$_5CH_3$), 0.861 (t, 3H, *J* = 6.6 Hz, -CH$_3$). In a sample without D$_2$O the following were observed: 5.225 (bs, 1H, -OH), 5.066 (bs, 2H, 2 x -OH). ^{13}C-NMR (δ, DMSO-d$_6$): 118.778 (CN), 103.355 (C1'), 76.751 (C3'), 74.573 (d, $^3J_{\mathrm{CCOP}}$ = 6.0 Hz, C5'), 73.785 (C4'), 69.840, 69.688 (C2' and -CH_2(CH$_2$)$_6$CH$_3$), 68.004 (d, $^2J_{\mathrm{COP}}$ = 5.0 Hz, C6'), 63.184 (d, $^2J_{\mathrm{COP}}$ = 5.0 Hz, -CH_2CH$_2$CN), 63.181 (d, $^2J_{\mathrm{COP}}$ = 5.0 Hz, -

$\mathit{CH_2}CH_2CN$), 31.924, 29.891, 29.512, 29.337, 26.136, 22.783 (octyl CH_2's), 19.692 (d, $^3J_{CCOP}$ = 6.9 Hz, -$\mathit{CH_2}CN$), 14.643 (CH_3). ^{31}P-NMR (δ, 162 MHz, DMSO-d_6): 0.317.

Dibenzyl (4-*O*-(2,6-Dimethylphenyl)-2'-deoxyuridin-5'-yl) Phosphate (2d). To a stirred solution of 4-*O*-(2,6-dimethylphenyl)-2'-deoxyuridine (**1d**, 166.8 mg, 0.50 mmol) and 5-NPT (**4b**, 198 mg, 1.04 mmol) in CH_3CN (40 mL) at -36 °C was added dropwise (0.02 mmol/min) a 0.47 M solution of dibenzyl N,N-diisopropylaminophosphoramidite (**3b**, 890 μL, 0.42 mmol) in CH_3CN. After the addition the resulting solution was stirred for an additional 20 minutes at -36 °C. A 0.77 M solution of TBHP (2.3 mL, 1.7 mmol) in CH_2Cl_2 was then added, and the reaction temperature was raised to RT and stirred for 20 minutes. The reaction solution was then evaporated to a minimum volume (~2-4 mL) and diluted with an equal volume of CH_2Cl_2. After the suspended solid was removed by filtration through a bed of sand the crude product was loaded onto a silica gel column and eluted with a step gradient of 98:2 to 90:10 $CHCl_3$ EtOH, yielding phosphate **2d** (0.29 mmol, 69% based on limiting phosphoramidite; 75% based on total recovered carbohydrate) and recovered starting material (**1b**, 0.09 mmol). ^{1}H-NMR (δ, 400 MHz): 8.007 (d, J = 7.4 Hz, 1H, H6), 7.39-7.32 (m, 10H, 2 x $CH_2\mathit{Ph}$), 7.036 (s, 3H, $Me_2Ar\mathit{H_3}$), 6.191 (t, J = 6.1 Hz, 1H, H1'), 5.880 (d, J = 7.4 Hz, 1H, H5), 5.10-5.00 (m, 4H, 2 x -$\mathit{CH_2}Ph$), 4.24-4.12 (m, 3H, H3', H5', H5"), 4.043 (app. quintet, J ~ 3.3 Hz, 1H, H4'), 3.4-3.0 (bs, 1H, OH), 2.507 (ddd, J = 13.8, 6.0, 4.7 Hz, 1H, H2' or H2"), 2.101 (s, 6H, $ArMe_2$) 1.911 (dt, J = 13.8, 6.4 Hz, 1H, H2' or H2"). ^{13}C-NMR (δ, 100 MHz): 170.655, 155.544, 148.990, 143.885 (C6), 135.924 (d, $^3J_{CCOP}$ = 6.9 Hz, Benzyl C1), 130.116, 128.857, 128.834, 128.690, 128.675, 128.045, 128.008 (Ar CH), 125.868 (DMP C4), 94.303 (C5), 86.801 (C1'), 84.784 (d, $^3J_{CCOP}$ = 7.6 Hz, C4'), 70.097 (C3'), 69.728 (d, $^2J_{COP}$ = 5.3 Hz, CH_2Ph), 69.699 (d, $^2J_{COP}$ = 5.3 Hz, CH_2Ph), 66.558 (d, $^2J_{COP}$ = 5.3 Hz, C5'), 41.179 (C2'), 16.389 ($Ar\mathit{Me_2}$). ^{31}P-NMR (δ, 162 MHz): -2.92.

Dibenzyl (Pent-4-enyl β-D-ribofuranos-5-yl) Phosphate (2e). To a stirred solution of pentenyl β-D-riboside **1b** (80.8 mg, 0.37 mmol) and 5-NPT (**4b**, 167.7 mg, 0.88 mmol) in CH_3CN (17 mL) at -36 °C was added dropwise (0.02 mmol/min) a 0.38 M solution of dibenzyl N,N-diisopropylaminophosphoramidite (**3b**, 650 μL, 0.25 mmol) in CH_3CN. After the addition the resulting solution was stirred for an additional 20 minutes at -36 °C. A 0.95 M solution of TBHP (675 μL, 0.64 mmol) in CH_2Cl_2 was then added, and the reaction temperature was raised to RT and stirred for 20 minutes. The reaction solution was then evaporated to a minimum volume (~2-4 mL) and diluted with an equal volume of CH_2Cl_2. The crude product was then loaded onto a silica gel column and eluted with a step gradient of 90:10 to 0:100 MC:EA, yielding phosphate **2e** (0.17 mmol, 70% based on limiting phosphoramidite; 80% based on total recovered carbohydrate) and recovered starting material (**1b**, 0.12 mmol). ^{1}H-NMR (δ): 7.4-7.2 (m, 10H, 2 x Ph), 5.758 (ddt, J = 16.9, 13.3, 6.7 Hz, 1H, H4), 5.047 (d, J = 8.2 Hz, 2H, -$\mathit{CH_2}Ph$), 5.043 (d, J = 8.1 Hz, 2H, -$\mathit{CH_2}Ph$), 5.02-4.92 (m, 2H, =CH_2, overlap with -CH_2Ph and H1'), 4.918 (s, 1H, H1'), 4.219 (t, 1H, J = 5.4 Hz, H3'), 4.10-4.07 (m, 3H, H4', H5', H5"), 4.014 (d, J = 4.7 Hz, 1H, H2'), 3.881 (bs, 1H, OH) 3.647 (dt, J = 9.6, 6.6 Hz, 1H, H1), 3.344 (dt, J = 9.6, 6.6 Hz, 1H, H1), 3.130 (bs, 1H, OH) 2.031 (qt, J = 6.6, 1.2 Hz, 2H, H3), 1.583 (quin, J = 6.6 Hz, 2H, H2). ^{13}C-NMR (δ): 138.036 (C4), 135.411 (d, $^3J_{CCOP}$ = 5.7 Hz, benzyl C1), 128.779, 128.703, 128.066, 124.348 (Ar CH), 114.909 (C5), 107.215 (C1'), 81.098 (d, $^3J_{CCOP}$ = 7.6 Hz, C4'), 75.103 (C2'), 71.947 (C3'), 69.693 (d, $^2J_{COP}$ = 5.7 Hz, C5'), 68.790 (d, $^2J_{COP}$ = 6.9 Hz, CH_2Ph), 67.318 (C1), 30.112 (C3), 28.572 (C2). ^{31}P-NMR (δ, 162 MHz): -3.438.

Preparation of Phosphate 2b using 5-Methylthio-1*H*-tetrazole (5-MTT, 4c). To a stirred solution of pentenyl β-D-riboside **1b** (91.2 mg, 0.42 mmol) and 5-MTT (117.3 mg, 1.01 mmol) in CH_3CN (20.5 mL) at -36 ^oC was added dropwise (0.02 mmol/min) a 0.40 M solution of phosphoramidite **3a** (825 μL, 0.33 mmol) in CH_3CN. After the addition the resulting solution was stirred for an additional 20 minutes at -36 ^oC. A 1.1 M solution of TBHP (3.5 mL, 3.85 mmol) in CH_2Cl_2 was then added, and the reaction temperature was raised to RT and stirred for 20 minutes. The reaction solution was then evaporated to a minimum volume (~2-4 mL) and diluted with an equal volume of CH_2Cl_2. The crude product was then loaded onto a silica gel column and eluted as described above, yielding phosphate **2b** (0.20 mmol, 62% based on limiting phosphoramidite; 87% based on total recovered carbohydrate) and recovered starting material (**1b**, 0.16 mmol).

Preparation of Phosphate 2b using 1*H*-Tetrazole (4a). To a stirred solution of pentenyl β-D-riboside **1b** (68.8 mg, 0.32 mmol) and tetrazole (**4a**, 55.1 mg, 0.79 mmol) in CH_3CN (20.5 mL) at -36 ^oC was added dropwise (0.02 mmol/min) a 0.40 M solution of phosphoramidite **3a** (625 μL, 0.25 mmol) in CH_3CN. After the addition the resulting solution was stirred for an additional 50 minutes at -36 ^oC. A 0.94 M solution of TBHP (0.6 mL, 0.6 mmol) in CH_2Cl_2 was then added, and the reaction temperature was raised to RT and stirred for 20 minutes. The reaction solution was then evaporated to a minimum volume (~2-4 mL) and diluted with an equal volume of CH_2Cl_2. The crude product was then loaded onto a silica gel column and eluted as described above, yielding phosphate **2b** (0.08 mmol, 31% based on limiting phosphoramidite; 86% based on total recovered carbohydrate) and recovered starting material (**1b**, 0.20 mmol).

Preparation of Phosphate 2b, large scale. To a stirred solution of pentenyl β-D-riboside **1b** (1.02 g, 4.7 mmol) and 5-NPT (**4b**, 2.13 g, 11.1 mmol) in CH_3CN (230 mL) at -36 ^oC was added dropwise (0.2 mmol/min) a 0.5 M solution of phosphoramidite **3a** (7.0 mL, 3.5 mmol) in CH_3CN. After the addition the resulting solution was stirred for an additional 20 minutes at -36 ^oC. A 0.88 M solution of TBHP (18.8 mL, 16.5 mmol) in CH_2Cl_2 was then added, and the reaction was warmed to room temperature with a water bath. After stirring for 20 minutes, the reaction solution was evaporated to a small volume (~15-20 mL) and diluted with an equal volume of CH_2Cl_2. After the suspended solid was removed by filtration through a bed of sand the crude product was loaded onto a silica gel column and eluted as described above, yielding phosphate **2b** (2.29 mmol, 65% based on limiting phosphoramidite; 80% based on total recovered carbohydrate) and recovered starting material (**1b**, 1.43 mmol).