Polymer-Bound Oxathiaphospholane: A Solid-Phase Reagent for Regioselective Monothiophosphorylation and Monophosphorylation of Unprotected Nucleosides and Carbohydrates

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Supporting Information

Experimental

1. General: All reactions were carried out in Bio-Rad polypropylene columns by shaking and mixing using Glass-Col small tube rotator in dry conditions at room temperature unless otherwise stated. Real-time monitoring of loading of compounds on resin beads was carried out with a Thermo-Nicolet 550 FT-IR spectrophotometer coupled with a Nic-Plan microscope using OMNIC software. The chemical structures of final products were confirmed by nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR, ^{31}P NMR) determined on a Bruker NMR spectrometer (400 MHz). ^{13}C NMR spectra were acquired using *J* modulated spin echo technique where methyl and methane carbon resonances appear as positive peaks, and methylene and quaternary carbon resonances appear as negative peaks. Chemical shifts are reported in parts per millions (ppm). The chemical structures of compounds were confirmed by a high-resolution PE Biosystems Mariner API-time of flight mass spectrometer. The substitution of the resins for each step was estimated from the weight gain of the resin. Total isolated yields for final products were calculated based on the loading of NovaSyn^{TM} Tentagel bromo resin (**1**, 0.35 mmol/g) and bromoacetamidomethyl NovaGel^{TM} resin (**4**, 0.64 mmol/g) and the amount of monophosphorylated and monothiophosphorylated products.

2. Solid-phase monophosphorylation using NovaSyn™ Tentagel resin bound-1,3,2-oxathiaphospholane (3).

Preparation of NovaSyn™ Tentagel resin-bound 2,3-dimercapto-1-propanol (2).

NovaSyn™ Tentagel bromo resin (**1**, 5.0 g, 130 μm beads, 0.35 mmol/g) was swelled in dry NMP (35 mL) and was shaken at room temperature for 15 min. 2,3-Dimercapto-1-propanol (702 μL, 7.0 mmol) and potassium carbonate (0.97 g, 7.0 mmol) were added to the swelled resin. The shaking was continued for 24 h at room temperature. The resin was filtered and washed with dry NMP (2 × 35 mL), dry DCM (2 × 35 mL), dry MeOH (3 × 35 mL), and dried under vacuum to give **2** (5.074 g, 98%, 0.34 mmol/g). IR (cm^{-1}): 3495 (OH).

Preparation of NovaSyn™ Tentagel resin-bound 2-(*N*,*N*-diisopropylamine)-1,3,2-oxathiaphospholane (3). *N*,*N*-Diisopropyl phosphoramidite dichloride (984 μL, 7.0 mmol) and pyridine (566 μL, 7.0 mmol) were added to the swelled solution of **2** (5.074 g, 0.34 mmol/g) in anhydrous NMP (35 mL). The mixture was shaken for 24 h at room temperature. The resin was collected by filtration and washed with NMP (2 × 30 mL), DCM (2 × 30 mL), and dry MeOH (3 × 30 mL), respectively, and dried under vacuum to give **3** (5.292 g, 97%, 0.32 mmol/g). IR (cm^{-1}): 1037 (P-O-C). Polymer-bound 2-(*N*,*N*-diisopropylamine)-1,3,2-oxathiaphospholane **(3)** was stable at room temperature for 24 h drying period and was kept at -20 °C for two weeks before using in the next reaction.

Preparation of NovaSyn™ Tentagel resin-bound 5′-*O*-(1,3,2-oxathiaphospholane)thymidine (7a), 5′-*O*-(1,3,2-oxathiaphospholane)uridine (7b), 5′-*O*-(1,3,2-oxathiaphospholane)adenosine (7c), 6-*O*-(1,3,2-oxathiaphospholane)-α,β-D-mannose (7d), 6′-*O*-(1,3,2-oxathiaphospholane)-6-*O*-α-D-galactopyranosyl-α,β-D-glucose (7e). Nucleosides and carbohydrates (**a-e**, 0.64 mmol) and 1-*H*-tetrazole (34 mg, 0.48 mmol) were added to **3** (500 mg, 0.32 mmol/g) in anhydrous THF (2 mL) and DMSO (2-3 mL) or in anhydrous DMSO (5 mL) in case of adenosine. The mixture was shaken for 24 h at room temperature. The resin was collected by filtration and washed with DMSO (3 × 15 mL), THF (2 × 10 mL), and MeOH (3 × 15 mL), respectively, and dried under vacuum to give **7a-e** (511-543 mg), respectively. IR (cm^{-1}): **7a**: 3514 (OH), 1037 (P-O-C); **7b**: 3515 (OH), 1033 (P-O-C); **7c**: 3517 (OH), 1025 (P-O-C); **7d**: 3518 (OH), 1034 (P-O-C); **7e**: 3419 (OH), 1034 (P-O-C).

Oxidation of NovaSyn™ Tentagel resin-bound phosphitylated precursors, 7a-e, to polymer-bound 1,3,2-oxathiaphospholane phosphotrieseter derivatives of thymidine (9a), uridine (9b), adenosine(9c), α,β-D-mannose (9d), and 6-*O*-α-D-galactopyranosyl-α,β-D-glucose (9e). *tert*-Butyl hydroperoxide in decane (5-6 M, 128 μL, 0.64 mmol) was added to the resins (**7a-e**, 511-543 mg) in THF (3 mL). After 1 h shaking at room temperature, the resins were collected by filtration and washed with DMSO (10 mL), THF (2 × 10 mL), and MeOH (3 × 15 mL), respectively, and were dried under vacuum to give **9a-e** (512-545 mg). IR (cm^{-1}): **9a**: 3417 (O-H), 1033 (P-O-C); **9b**: 3409 (O-H), 1031 (P-O-C); **9c**: 3405 (O-H), 1035 (P-O-C); **9d**: 3420 (O-H), 1032 (P-O-C); **9e**: 3415 (O-H), 1037 (P-O-C). Polymer-bound 1,3,2-oxathiaphospholane phosphotrieseter derivatives **9a-e** were stable at room temperature for 24 h drying period and were used immediately after drying for the use in the next reaction.

Preparation of thymidine-5′-*O*-monophosphate (11f), uridine-5'-*O*-monophosphate (11g), adenosine-5'-*O*-monophosphate (11h), α,β-D-mannose-6-*O*-phosphate (11i), and 6-*O*-α-D-galactopyranosyl-6′-*O*-phosphate-α,β-D-glucose (11j). To the swelled resins (**9a-e**, 512-545 mg) in anhydrous DCM (3 mL) was added DBU (64 μL, 0.64 mmol) and 3-hydroxypropionitrile (0.64 mmol, 46 μL). After 48 h shaking of the mixture at room temperature, the resins were collected by filtration and washed with DCM (2 × 10 mL), THF (2 × 10 mL), and MeOH (3 × 10 mL), respectively. The solvents of filtrate solutions were immediately evaporated at room temperature for **11f** and **11g** and at -20 °C for **11h-j**, respectively. The residues were mixed with Amberlite AG-50W-X8 (100-200 mesh, hydrogen form, 500 mg, 1.7 meq/g) in water:dioxane (70:30 v/v, 3 mL) for 30 min at room temperature for **11f** and **11g** and at -20 °C for **11h-j**, respectively. After filtration, the solvents were evaporated and the crude products were purified using C_{18} Sep-Pak using appropriate solvents. The solvents were evaporated and the residues were dried under vacuum to yield **11f-j**. The total isolated yields for **11f-j** were: **11f** (37.7 mg, 71%), **11g** (38.8 mg, 69%), **11h** (45.2 mg, 75%), **11i** (28.0, 62%), **11j** (41.8, 57%). The compounds were characterized by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and high resolution mass spectrometer (ESI-TOF). The chemical structures were confirmed with comparing their spectral properties with authentic samples purchased from Sigma-Aldrich and Acros Organics and literature. Compounds **11i**[38] and **11j**[30] have been previously characterized by ^{1}H NMR and ^{13}C NMR. Compounds **11f**,[39] **11g,**[39] and **11h**[39-41] have been previously characterized by ^{1}H NMR. ^{13}C NMR (DMSO-d_6): δ **11f**: 12.64 (5-*C*H$_3$), 39.82 (C-2'), 61.71 (C-5'), 70.83 (C-3'), 84.16, 87.61 (C-4', C-1'), 109.79 (C-5), 136.52 (C-6), 150.86 (C-2 *C*=O), 164.18 (C-4 *C*=O); ^{13}C NMR (D_2O) δ **11g**: 61.03 (C-5'), 69.74 (C-2'), 74.15 (C-3'), 84.51, 89.67 (C-4', C-1'), 102.49 (C-5), 142.14

(C-6), 151.83 (C-2 *C*=O), 166.34 (C-4 *C*=O); ^{13}C NMR (DMSO-d_6): δ **11h**: 65.98 (C-5'); 71.31 (C-2'), 74.45 (C-3'), 84.14, 87.99 (C-4', C-1'), 119.67 (C-5), 140.72 (C-8), 150.14 (C-4), 151.90 (C-2), 155.58 (C-6). ^{31}P NMR (in D_2O and H_3PO_4 85% in water as external standard): δ **11f**: 4.54 (s); **11g**: 4.56 (s); **11i**; 4.33 (s); **11j**: 4.95 (s); ^{31}P NMR (in DMSO and H_3PO_4 85% in water as external standard): δ **11h**: 4.39 (s).

3. Solid-phase monothiophosphorylation using NovaSyn^{TM} Tentagel resin bound 1,3,2-oxathiaphospholane (3).

Sulfurization of NovaSyn^{TM} Tentagel resin-bound phosphitylated precursors, 7a-e, to NovaSyn^{TM} Tentagel resin-bound 1,3,2-oxathiaphospholane thiophosphotrieseter derivatives of thymidine (12a), uridine (12b), adenosine (12c), α,β-D-mannose (12d), and 6-*O*-α-D-galactopyranosyl-α,β-D-glucose (12e). Beaucage's reagent (3*H*-1,2-benzodithiole-3-one 1,1-dioxide) (128 mL, 0.64 mmol) was added to the resins (**7a-e**, 511-543 mg) in AcCN (4 mL). After 6 h shaking at 40 °C, the resins were collected by filtration and washed with AcCN (2 ×10 mL), THF (2 × 10 mL), and MeOH (3 × 10 mL), respectively, and were dried under vacuum to give **12a-e** (515-547 mg). IR (cm^{-1}): **12a**: 3507 (O-H), 1029 (P-O-C); **12b**: 3511 (O-H), 1033 (P-O-C); **12c**: 3320 (O-H), 1026 (P-O-C); **12d**: 3506 (O-H), 1029 (P-O-C); **12e**: 3489 (O-H), 1035 (P-O-C). Polymer-bound 1,3,2-oxathiaphospholane thiophosphotrieseter derivatives **12a-e** were stable at room temperature for 24 h drying period and were used immediately after drying for the use in the next reaction.

Preparation of thymidine-5′-*O*-monothiophosphate (18k), uridine-5'-*O*-monothiophosphate (18l), adenosine-5'-*O*-monothiophosphate (18m), α,β -D-mannose-6-*O*-thiophosphate (18n), and 6-*O*-α-D-galactopyranosyl-6′-*O*-thiophosphate-α,β-D-glucose (18o). To the swelled resins (**12a-e**, 515-547 mg) in anhydrous DMSO (5 mL) was added DBU (64 μL, 0.64 mmol) and 3-hydroxypropionitrile (0.64 mmol, 46 μL). After 48 h shaking of the mixture at room temperature, the resins were collected by filtration and washed with DCM (10 mL), THF (10 mL), and MeOH (10 mL), respectively. The solvents of filtrate solutions were immediately evaporated at room temperature for **18k** and **18l** and at -20 °C for **18m-o**, respectively. The residues were mixed with Amberlite AG-50W-X8 (100-200 mesh, hydrogen form, 500 mg, 1.7 meq/g) in water:dioxane (70:30 v/v, 3 mL) for 30 min at room temperature for **18k** and **18l** and at -20 °C for **18m-o**, respectively. After filtration, the solvents were evaporated and the crude products were purified using C_{18} Sep-Pak using appropriate solvents. The solvents were evaporated and the residues were dried under vacuum to yield **18k-o**. The total isolated yields for **18k-o** were: **18k** (37.0 mg, 63%), **18l** (38.9 mg, 66%), **18m** (44.1 mg, 70%), **18n** (26.9 mg, 56%), **18o** (38.8 mg, 51%). The compounds were characterized by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and high resolution mass spectrometer (ESI-TOF).

Thymidine-5′-*O*-monothiophosphate (18k). ^{1}H NMR (D_2O): δ 1.87 (d, $J_{5\text{-}CH_3,6}$ = 1.3 Hz, 5-CH_3, 3H); 2.25-2.40 (m, H-2', H-2'', 2H), 3.65 (dd, $J_{5',5''}$ = 12.5, $J_{5',4'}$ = 4.9 Hz, H-5', 1H), 3.73 (dd, $J_{5',5''}$ = 12.5, $J_{5'',4'}$ = 3.8 Hz, H-5'', 1H), 3.95-4.04 (m, H-4', 1H), 4.41-4.47 (m, H-3',1H), 6.23 (t, J = 6.7 Hz, H-1', 1H), 7.70 (d, $J_{6,5\text{-}CH_3}$ = 1.3 Hz, H-6, 1H); ^{13}C NMR (D_2O): δ 12.65 (5-CH_3), 39.82 (C-2'), 61.71 (C-5'), 70.83 (C-3'), 84.16, 87.61 (C-4', C-1'), 109.79 (C-5), 136.52 (C-6), 150.86

(C-2 *C*=O), 164.18 (C-4 *C*=O); ^{31}P NMR (in D_2O and H_3PO_4 85% in water as external standard): δ 43.35 (s); HR-MS (ESI-TOF) (m/z) calcd. 338.2741, found 338.2669 $[M]^+$, 339.2737 $[M+H]^+$.

Uridine-5'-*O*-monothiophosphate (18l). ^{1}H NMR (D_2O): δ 3.92 (dd, $J_{5',5''}$ = 11.8, $J_{5',4'}$ = 6.0 Hz, H-5', 1H), 3.78 (dd, $J_{5',5''}$ = 11.8, $J_{5'',4'}$ = 4.9 Hz, H-5'', 1H), 4.20-4.24 (m, H-4', 1H), 4.29-4.34 (m, H-3',1H), 4.37-4.40 (m, H-2', H-2'', 2H), 5.93-5.98 (m, H-1', H-5, 2H), 8.09 (d, $J_{6,5}$ = 8.1 Hz, H-6, 1H); ^{13}C NMR (D_2O): δ 61.03 (C-5'), 69.73 (C-2'), 74.15 (C-3'), 84.50, 89.66 (C-4', C-1'), 102.48 (C-5), 142.12 (C-6), 151.83 (C-2 *C*=O), 166.33 (C-4 *C*=O); ^{31}P NMR (in D_2O and H_3PO_4 85% in water as external standard): δ 44.88 (s); HR-MS (ESI-TOF) (m/z) calcd. 340.2469, found, 341.2403 $[M+H]^+$.

Adenosine-5'-*O*-monothiophosphate (18m). ^{1}H NMR (DMSO-d_6): δ 3.94-4.02 (m, H-5', 1H), 4.02-4.10 (m, H-5'', 1H), 4.07-4.15 (m, H-4', 1H), 4.16-4.22 (m, H-3', 1H), 4.60 (dd, $J_{2',1'}$ = 5.7, $J_{2',3'}$ = 5.3 Hz, H-2', 1H), 5.95 (d, $J_{1',2'}$ = 5.7 Hz, H-1', 1H), 7.80-8.00 (br s, 6-NH_2, 2H), 8.17 (s, H-2, 1H), 8.23 (s, H-8, 1H); ^{13}C NMR (DMSO-d_6): δ 65.96 (C-5'); 71.31 (C-2'), 74.45 (C-3'), 84.19, 87.99 (C-4', C-1'), 119.67 (C-5), 140.72 (C-8), 150.14 (C-4), 151.90 (C-2), 155.58 (C-6); ^{31}P NMR (DMSO and H_3PO_4 85% in water as external standard): δ 47.45 (s); HR-MS (ESI-TOF) (m/z) calcd. 363.2868, found 364.2829 $[M+H]^+$.

α,β-D-Mannose-6-monothiophosphate (18n). ^{1}H NMR (D_2O): δ 3.32-3.38 (t, H-5β, 1H), 3.51-3.67 (m, H-4β, 1H), 3.58-3.78 (m, H-3β, H-4α, H-3α, 3H), 3.78-3.84 (m, H-2α, H-5α, H-2β, 3H) 3.84-3.93 (m, H-6α, H-6β, 4H), 4.87 (d, H-1β, $J_{1,2}$ = 1.2 Hz, 1H), 5.15 (d, H-1α, $J_{1,2}$ = 2.0 Hz, 1H); ^{31}P NMR (in D_2O and H_3PO_4 85% in water as external standard): δ 54.78 (s); ^{13}C NMR

(D_2O): δ 61.30 (C-6, α and β), 66.92 (C-4β), 67.16 (C-4α), 70.54 (C-3α), 70.98 (C-2α), 71.52 (C-3β), 72.67 (C-5α), 73.34 (C-2β), 76.42 (C-5β), 93.97 (C-1β), 94.33 (C-1α); HR-MS (ESI-TOF) (m/z) calcd. 276.2014; found: 277.2050 $[M+H]^+$. *Anal.* Calcd. P 11.21%, found 11.16%.

6-*O*-α-D-Galactopyranosyl-6′-*O*-thiophosphate-α,β-D-glucose (18o). ^1H NMR (D_2O): δ 3.13-3.20 (m, H-2β, 1H), 3.37-3.49 (m, H-4α, H-4β, H-3β, 3H), 3.52-3.70 (m, H-2α, H-2β, H-3α, H-5β, 4H), 3.72-3.82 (m, H-6α, H-6β, 4H), 3.82-3.86 (m, H-6', 4H), 3.86-3.95 (m, H-5', H-5α, H-4', H-3', H-2', 9H), 4.56-4.65 (m, H-1', 2H), 5.12 (m, H-1β, 1H); 5.18 (m, H-1α, 1H). ^{31}P NMR (in D_2O and H_3PO_4 85% in water as external standard): δ 50.17 (s). ^{13}C NMR (D_2O): δ 61.48 (C-6'), 66.15 (C-6β) 66.24 (C-6α), 68.82 (C-2'), 69.56 (C-4'), 69.76 (C-4β), 69.84 (C-3'), 69.92 (C-4α), 70.42 (C-5α), 71.27 (C-5'), 71.77 (C-2α), 73.31 (C-3α), 74.40 (C-2β), 74.66 (C-5β), 76.23 (C-3β), 92.53 (C-1α), 96,40 (C-1β), 98.49 (C-1'); HR-MS (ESI-TOF) (m/z) calcd. 438.0597, found 438.0102 $[M]^+$, 419.2013 $[M-H_2O-H]^+$. *Anal.* Calcd. P 7.07%, found 7.10%.

4. Solid-phase monophosphorylation using acetamidomethyl NovaGel™ resin bound-1,3,2-oxathiaphospholane (6).

Preparation of NovaGel™ resin-bound 2,3-dimercapto-1-propanol (5).

Bromoacetamidomethyl NovaGel™ resin (**4**, 5.0 g, 100-200 mesh, DVB (1%), 0.64 mmol/g) was swelled in dry NMP (35 mL) and was shaken at room temperature for 15 min. 2,3-Dimercapto-1-propanol (1,284 μL, 12.8 mmol) and potassium carbonate (1.77 g, 12.8 mmol) were added to the swelled resin. The shaking was continued for 24 h at room temperature. The

resin was filtered and washed with dry NMP (2 × 35 mL), dry DCM (2 × 35 mL), dry MeOH (3 × 35 mL) and dried under vacuum to give **5** (5.136 g, 98%, 0.61 mmol/g). IR (cm^{-1}): 3499 (OH).

Preparation of NovaGel™ resin-bound 2-(*N*,*N*-diisopropylamine)-1,3,2-oxathiaphospholane (6). *N*,*N*-Diisopropyl phosphoramidite dichloride (1,800 μL, 12.8 mmol) and pyridine (1,035 μL, 12.8 mmol) were added to a swelled solution of **5** (5.136 g, 0.61 mmol/g) in anhydrous NMP (35 mL). The mixture was shaken for 24 h at room temperature. The resin was collected by filtration and washed with NMP (2 × 30 mL), DCM (2 × 30 mL), and dry MeOH (3 × 30 mL), respectively, and dried under vacuum to give **6** (5.524 g, 96%, 0.55 mmol/g). IR (cm^{-1}): 3515 (OH), 1029 (P-O-C). Polymer-bound 2-(*N*,*N*-diisopropylamine)-1,3,2-oxathiaphospholane **(6)** was stable at room temperature for 24 h drying period and was kept at -20 °C for two weeks before using in the next reaction.

Preparation of NovaGel™ resin-bound 5′-*O*-(1,3,2-oxathiaphospholane)thymidine (8a), 5′-*O*-(1,3,2-oxathiaphospholane)uridine (8b), 5′-*O*-(1,3,2-oxathiaphospholane)adenosine (8c), 6-*O*-(1,3,2-oxathiaphospholane)-α,β-D-mannose (8d), and 6′-*O*-(1,3,2-oxathiaphospholane)-6-*O*-α-D-galactopyranosyl-α,β-D-glucose (8e). Nucleosides or carbohydrates (**a-e**, 1.20 mmol) and 1-*H*-tetrazole (64 mg, 0.90 mmol) were added to **6** (500 mg, 0.55 mmol/g) in anhydrous THF (2-3 mL) and DMSO (3-5 mL). The mixture was shaken for 24 h at room temperature. The resin was collected by filtration and washed with DMSO (3 × 15 mL), THF (2 × 10 mL), and MeOH (3 × 15 mL), respectively, and dried under vacuum to give **8a-e** (519-577 mg). IR (cm^{-1}):

8a: 3434 (OH), 1030 (P-O-C); **8b**:3418 (OH), 1029 (P-O-C); **8c**: 3409 (OH), 1030 (P-O-C); **8d**: 3422 (OH), 1029 (P-O-C); **8e**: 3416 (OH), 1022 (P-O-C).

Oxidation of NovaGel^{TM} resin-bound phosphitylated precursors, 8a-e, to polymer-bound 1,3,2-oxathiaphospholane phosphotrieseter derivatives of thymidine (10a), uridine (10b), adenosine(10c), α,β-D-mannose (10d), and 6-*O*-α-D-galactopyranosyl-α,β-D-glucose (10e). *tert*-Butyl hydroperoxide in decane (5-6 M) (240 μL, 1.20 mmol) was added to the resins (**8a-e**, 519-577 mg) in THF (3 mL). After 1 h shaking at room temperature, the resins were collected by filtration and washed with DMSO (10 mL), THF (2 × 10 mL), and MeOH (3 × 10 mL), respectively, and were dried under vacuum to give **10a-e** (521-579 mg). IR (cm^{-1}): **10a**: 3401 (O-H), 1029 (P-O-C); **10b**: 3388 (O-H), 1029 (P-O-C); **10c**: 3375 (O-H), 1032 (P-O-C); **10d**: 3376 (O-H), 1027 (P-O-C); **10e**: 3356 (O-H), 1033 (P-O-C). Polymer-bound 1,3,2-oxathiaphospholane phosphotrieseter derivatives **10a-e** were stable at room temperature for 24 h drying period and were used immediately after drying for the use in the next reaction.

Preparation of thymidine-5′-*O*-monophosphate (11f), uridine-5'-*O*-monophosphate (11g), adenosine-5'-*O*-monophosphate (11h), α,β-D-mannose-6-*O*-phosphate (11i), and 6-*O*-α-D-galactopyranosyl-6′-*O*-phosphate-α,β-D-glucose (11j). To the swelled resins (**10a-e**, 521-579 mg) in anhydrous DMSO (5 mL) was added DBU (120 μL, 1.20 mmol) and 3-hydroxypropionitrile (1.20 mmol, 86 μL). After 48 h shaking of the mixture at room temperature, the resins were collected by filtration and washed with DCM (2 × 10 mL), THF (2 × 10 mL), and MeOH (3 × 10 mL), respectively. The solvents of filtrate solutions were immediately evaporated at room temperature for **11f** and **11g** and at -20 °C for **11h-j**,

respectively. The residues were mixed with Amberlite AG-50W-X8 (100-200 mesh, hydrogen form, 500 mg, 1.7 meq/g) in water:dioxane (70:30 v/v, 3 mL) for 30 min at room temperature for **11f** and **11g** and at -20 °C for **11h-j**, respectively. After filtration, the solvents were evaporated and the crude products were purified using C_{18} Sep-Pak using appropriate solvents. The solvents were evaporated and the residues were dried under vacuum to yield **11f-j**. The total isolated yields for **11f-j** were: **11f** (68.5 mg, 67%), **11g** (73.0 mg, 71%), **11h** (77 mg, 70%), **11i** (52.8, 64%), **11j** (73.7, 55%). The compounds were characterized by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR and high resolution mass spectrometer (ESI-TOF). The chemical structures were confirmed with comparing their spectral properties with authentic samples purchased from Sigma-Aldrich and Acros Organics and literature. Compounds **11i**[38] and **11j**[30] have been previously characterized by ^{1}H NMR and ^{13}C NMR. Compounds **11f**,[39] **11g,**[39] and **11h**[39-41] have been previously characterized by ^{1}H NMR. ^{13}C NMR of **11f**, **11g**, and **11h** and ^{31}P NMR of **11f**, **11g**, **11h**, **11i**, and **11j** were similar to those of compounds prepared from NovaSyn™ Tentagel resin as described above.

5. Solid-phase monothiophosphorylation using NovaGel™ resin-bound 1,3,2-oxathiaphospholane (6).

Sulfurization of NovaGel™ resin-bound phosphitylated precursors, 8a-e, to NovaSyn™ Tentagel resin-bound 1,3,2-oxathiaphospholane thiophosphotrieseter derivatives of thymidine (13a), uridine (13b), adenosine (13c), α,β-D-mannose (13d), and 6-*O*-α-D-galactopyranosyl-α,β-D-glucose (13e). Beuacage's reagent (3*H*-1,2-benzodithiole-3-one 1,1-dioxide (234 mg, 1.17 mmol) was added to the resins (**8a-e**, 519-577 mg) in AcCN (4 mL). After

6 h shaking at 40 °C, the resins were collected by filtration and washed with AcCN (2 ×10 mL), THF (2 × 10 mL), and MeOH (3 × 10 mL), respectively, and were dried under vacuum to give **13a-e** (530-586 mg). IR (cm^{-1}): **13a**: 3410 (O-H), 1029 (P-O-C); **13b**: 3406 (O-H), 1033 (P-O-C); **13c**: 3390 (O-H), 1029 (P-O-C); **13d**: 3383 (O-H), 1029 (P-O-C); **13e**: 3363 (O-H), 1034 (P-O-C). Polymer-bound 1,3,2-oxathiaphospholane thiophosphotrieseter derivatives **13a-e** were stable at room temperature for 24 h drying period and were used immediately after drying for the use in the next reaction.

Preparation of thymidine-5′-*O*-monothiophosphate (18k), uridine-5'-*O*-monothiophosphate (18l), adenosine-5'-*O*-monothiophosphate (18m), α,β-D-mannose-6-*O*-thiophosphate (18n), and 6-*O*-α-D-galactopyranosyl-6′-*O*-thiophosphate-α,β-D-glucose (18o). To the swelled resins (**13a-e**, 530-586 mg) in anhydrous DCM (5 mL) was added DBU (120 µL, 1.20 mmol) and 3-hydroxypropionitrile (1.20 mmol, 86 µL)). After 48 h shaking of the mixture at room temperature, the resins were collected by filtration and washed with DCM (10 mL), THF (10 mL), and MeOH (10 mL), respectively. The solvents of filtrate solutions were immediately evaporated at room temperature for **18k** and **18l** and at -20 °C for **18m-o**, respectively. The residues were mixed with Amberlite AG-50W-X8 (100-200 mesh, hydrogen form, 500 mg, 1.7 meq/g) in water:dioxane (70:30 v/v, 3 mL) for 30 min at room temperature for **18k** and **18l** and at -20 °C for **18m-o**, respectively. After filtration, the solvents were evaporated and the crude products were purified using C_{18} Sep-Pak using appropriate solvents. The solvents were evaporated and the residues were dried under vacuum to yield **18k-o**. The total isolated yields for **18k-o** were: **18k** (69.8 mg, 65%), **18l** (75.5 mg, 70%), **18m** (84.1 mg, 73%), **18n** (49.2 mg, 56%), **18o** (73.7 mg, 53%). The compounds were characterized by ^{1}H NMR, ^{13}C NMR, ^{31}P

NMR, and high resolution mass spectrometer (ESI-TOF). The spectral properties of **18k-o** were similar to those of compounds prepared from NovaSyn^{TM} Tentagel resin as described above.

6. References for physical and spectral properties:

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