A Solid Phase Reagent for the Phosphorylation of Carbohydrates and Nucleosides

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Supporting Information

Preparation of polymer-bound benzoyl chloride (2). Carboxypolystyrene (**1**, 5 g, 100-200 mesh, 1% DVB, 3.9 mmol/g) was suspended in dry toluene (50 mL), stirred slowly and heated at 120 °C for 1 h. Oxalyl chloride (14 mL, 32.9 mmol) was added and refluxing was continued for 24 h. The resin was filtered under argon and washed with dry toluene (100 mL), dry ether (50 mL), and dry toluene (100 mL), successively ending with dry ether (50 mL) and dried under vacuum for 5 h to give **2** (5.5 g, 80%, 3.11 mmol/g). IR (cm^{-1}): 1773 (C=O, Acid chloride), 1737 (C=O); Anal. Found (%): Cl: 11.0.

Preparation of polymer-bound *p*-hydroxybenzaldehde (3). *p*-Hydroxybenzaldehyde (12 g, 98.0 mmol) and triethylamine (15 mL) were added to a swelled solution of resin **2** (5 g, 3.1 mmol/g) in dry dichloromethane (10 mL) under argon. After addition of DMAP (100 mg, 0.8 mmol), the loading was monitored by U.V. (267 nm) over a 24 h period taking 100 μL aliquots from the solution. The first 10 h assay indicated maximum disappearance of the *p*-hydroxybenzaldehyde. The reaction was allowed to proceed further by adding dicyclohexylcarbodiimide. After 12 h methanol (1 mL) was added to neutralize unreacted benzoyl chloride or benzoic acid. After 12 h further stirring, the resin was filtered and washed successively with CH_2Cl_2 (100 mL), THF (50 mL), MeOH (50 mL), THF (50 mL) and dry ether (50 mL) and dried under vacuum for 1 h to give **3** (6.8 g, 98%, 3.06 mmol/g). IR (cm^{-1}): 1701 (C=O, aldehyde), 1739 (C=O, ester).

Preparation of polymer-bound *p*-hydroxybenzyl alcohol (4). Sodium borohydride (1.13 g, 30 mmol) and isopropyl alcohol (6 mL) were added to a swelled solution of **3** (5 g, 3.06 mmol/g) in THF (35 mL). After stirring for 4 h, the resin was collected by filtration and washed successively with THF (100 mL), 3% v/v acetic acid/dioxane (100 mL), THF (50 mL), MeOH (50 mL), THF (50 mL) and dry ether (50 mL) and dried under vacuum for 1 h to give **4** (5 g, 90%, 2.74 mmol/g). IR (cm^{-1}): 3415 (O-H), 1734 (C=O, ester).

Preparation of polymer-bound cyanoethoxy *N*,*N*-diisopropylamine phosphine (5). 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (1.4 mL, 6 mmol) was added dropwise to a stirred solution of **4** (1 g, 2.74 mmol/g) and diisopropylethylamine (1.56 mL, 9 mmol) in anhydrous THF (10 mL) and stirred for 24 h at room temperature under argon atmosphere. The resin was collected by filtration and washed successively with THF (100 mL), MeOH (50 mL), THF (50 mL) and dry ether (50 mL) and dried under vacuum for 1 h to give **5** (1.3 g, 56%, 1.54 mmol/g for total nitrogen content). IR (cm^{-1}): 2252 (CN), 1737 (C=O), 1014 (P-O-C); Anal. Found (%): N: 4.3.

Preparation of polymer-bound 2-cyanoethyl 1,2:3,4-di-*O*-isopropylidene-D-galactopyranosyl-6-phosphite triester (6A). 1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose (**6**, 234 mg, 0.9 mmol) and 1*H*-tetrazole (42 mg, 0.6 mmol) were added to **5** (200 mg, 1.53 mmol/g) in anhydrous THF (4 mL) and DMSO (1 mL) and stirred for 24 h at room temperature under argon atmosphere. The resin was collected by filtration and washed successively with THF (50 mL), MeOH (20 mL), THF (50 mL) and dry ether (20 mL) and dried under vacuum for 1 h to afford **6A** (265 mg). IR (cm^{-1}): 2252 (CN), 1736 (C=O), 1014 (P-O-C).

Preparation of polymer-bound 2-cyanoethyl α-D-galactopyranosyl 1-phosphite triester (7A). Benzylamine (65 µL, 0.8 mmol) was added to a solution of galactose pentaacetate (**7**, 333 mg, 0.9 mmol) in anhydrous THF (5 mL). After 24 h stirring, **5** (200 mg, 1.53 mmol/g) and 1*H*-tetrazole (42 mg, 0.6 mmol) were added and the solution was stirred for 24 h at room temperature under argon atmosphere. The resin collected by filtration and washed successively with THF (50 mL), MeOH (20 mL), THF (50 mL) and dry ether (20 mL) and dried under vacuum for 1 h to give **7A** (287 mg). IR (cm^{-1}): 2254 (CN), 1736 (C=O), 1014 (P-O-C).

Preparation of polymer-bound 2-cyanoethyl uridine-5'-phosphite triester (8A). Uridine (**8**, 198 mg, 0.9 mmol) and 1*H*-tetrazole (42 mg, 0.6 mmol) were added to **5** (200 mg, 1.53 mmol/g)) in anhydrous THF (4 mL) and DMSO (1 mL) and stirred for 24 h at room temperature under an argon atmosphere. The resin was collected by filtration and washed successively with THF (50 mL), MeOH (20 mL), THF (50 mL) and dry ether (20 mL) and dried under vacuum for 1 h to give **8A** (228 mg). IR (cm^{-1}): 3414 (OH), 2254 (CN), 1736 (C=O), 1014 (P-O-C).

Preparation of polymer-bound 2-cyanoethyl 1,2:3,4-di-*O*-isopropylidene-D-galactopyranosyl-6-phosphate triester (6B), 2-cyanoethyl α-D-galactopyranosyl 1-phosphate triester (7B) and 2-cyanoethyl uridine-5'-monophosphate triester (8B) using peroxide oxidation. *t*-Butyl hydroperoxide in decane (5-6 M) (100 µL, 0.6 mmol) was added to the resin (**6A-8A**, 228-287mg, ~1.53 mmol/g) in THF (5 mL). After 1 h stirring at room temperature, the resins were collected by filtration and washed successively with THF (50 mL), MeOH (20 mL), THF (50 mL) and dry ether (20 mL) and dried under vacuum for 1 h to give **6B-8B**.

Preparation of polymer-bound 1,2:3,4-di-*O*-isopropylidene-D-galactopyranosyl-6-phosphodiester (6C), α-D-galactopyranosyl 1-phosphodiester (7C) and 2-cyanoethyl uridine-5'-monophosphodiester (8C) by decyanoethylation. To a stirred solution of resins **6B**, **7B**, or **8B** (228-287mg, ~1.53 mmol/g) in THF (5 mL) was added DBU (60 µL, 0.6 mmol). After 48 h stirring at room temperature, the resins were collected by filtration and washed successively with THF (50 mL), MeOH (20 mL), THF (50 mL) and dry ether (20 mL) and were dried under vacuum for 1 h to give **6C-8C**. IR (cm^{-1}) for **6C**: 3300 (O-H), 1725 (C=O), 1070 (P-O-C).

1,2:3,4-Di-*O*-isopropylidene-D-galactopyranosyl-6-phosphate (6E) α-D-galactopyranosyl 1-phosphate (7E) and uridine-5'-monophosphate (8E) disodium salts. To resins (**6C-8C**, 228-287mg, ~1.53 mmol/g) in anhydrous dioxane (5 mL) and MeOH (1 mL) was added anhydrous sodium methoxide (60 mg, 1.1 mmol). After 24 h stirring at room temperature, water (1 mL) was added and stirring was continued for another 1 h. Amberlite AG-50W-X8 (H^+) was added until the pH became acidic. The products were eluted from the resins which were washed successively with dioxane (5 mL), water (1 mL), dioxane (5 mL) and water (1 mL). The solution containing the product was directly lyophilized. The purity of compounds was above 90% after initial lyophilization of products. The ^1H NMR in this step indicated the presence of the *p*-hydroxymethyl or *p*-methoxymethylphenol. The impurities were separated from the final products using C_{18} Sep-Pak or reverse phase HPLC (RP-HPLC). After evaporation of solvents, the resulting white solids were dissolved in H_2O and filtrated through a syringe containing Amberlite AG-50W-X8 (Na^+) (~200 mg) and fitted with a Gelman nylon acrodisc 0.2 µm microfilter. The products were lyophilized to give **6E** (61.7 mg, 79%), **7E** (36.9 mg, 78%) and **8E** (49 mg, 67%).

1,2:3,4-Di-*O*-isopropylidene-D-galactopyranosyl-6-phosphate disodium salt **(6E)**: ^{1}H NMR (500 MHz, D_2O) δ 5.62 (d, H-1, $J_{1,2}$ = 4.9 Hz, 1H), 4.78 (dd, H-3, $J_{3,4}$ = 8.0, $J_{3,2}$ = 2.5 Hz, 1H), 4.53 (dd, H-2, $J_{2,3}$ = 2.5, $J_{2,1}$ = 4.9 Hz, 1H), 4.47 (dd, H-4, $J_{4,3}$ = 8.0, $J_{4,5}$ = 2.0 Hz, 1H), 4.14 (ddd, H-5, $J_{5,6b}$ = 5.0, $J_{5,6a}$ = 1.0, $J_{5,4}$ = 2.0 Hz, 1H), 4.00 (m, H-6b, 1H), 3.93 (m, H-6a, 1H), 1.49, 1.40 (2s, 6H, CMe_2), 1.43, 1.40 (2s, 6H, CMe_2); Electrospray MS 363 (M + Na^+), 379 (M + K^+).

α-D-Galactopyranosyl 1-phosphate disodium salt **(7E)**: ^{1}H NMR (500 MHz, D_2O) δ 5.50 (dd, H-1, $J_{1,2}$ = 3.6, $J_{1,P}$ = 7.4 Hz, 1H), 4.16 (ddd, H-5, $J_{5,6a}$ = 7.1, $J_{5,6b}$ = 4.9, $J_{5,4}$ = 1.0 Hz, 1H), 4.10 (dd, H-4, $J_{4,3}$ = 3.4, $J_{4,5}$ = 1.0 Hz, 1H), 3.89 (dd, H-3, $J_{3,2}$ = 10.2, $J_{3,4}$ = 3.4 Hz, 1H), 3.78 (ddd, H-2, $J_{2,4}$ = 1.7, $J_{2,1}$ = 3.6, $J_{2,3}$ = 10.2 Hz, 1H), 3.78 (dd, H-6a, $J_{6a,b}$= 11.7, $J_{6a,5}$ = 7.1 Hz, 1H), 3.73 (dd, H-6b, $J_{6b,a}$ = 11.7 Hz, $J_{6b,5}$ = 4.9 Hz, 1H); Electrospray MS 261 (M + H^+), 305 (M + $2Na^+$-H^+), 327 (M + $2Na^+$-$2H^+$).

Uridine-5'-monophosphate disodium salt **(8E)**: ^{1}H NMR (500 MHz, D_2O) δ 7.91 (d, H-6, $J_{6,5}$ = 8.1 Hz, 1H), 5.94 (d, H-1’, $J_{1',2'}$ = 4.2 Hz, 1H), 5.87 (d, H-5, $J_{5,6}$ = 8.1 Hz, 1H), 4.25 (m, H-2’, H-3’, 2H), 4.15 (m, H-4’, 1H), 4.05 (m, H-5’a, 1H), 3.94 (m, H-5’b, 1H); Electrospray MS 325 (M + H^+), 347 (M + Na^+), 369 (M + $2Na^+$-H^+).