Supporting Information

Solid Phase Enzymatic Synthesis of Oligonucleotides

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Typical procedures.

Synthesis of adenosine(5')diphospho(5')deoxycitidine-3-phosphate (4): The 3',5'-deoxycytidine biphosphate ammonium salt (20 mg; 0.05 mMol) was dissolved in 1.5 mL of water and stirred with 2 g of an ion-exchange resin (DOWEX 50 WX8, H+ form) for 1 h. The solution was decanted and the resin was washed 5 times with 10 mL portions of water. Evaporation of the aqueous solution at reduced pressure provided the free acid as amorphous powder. The free acid was suspended in a mixture of MeOH/H_2O (1/1) (1 mL), tri-*n*-octylamine (176.8 mg; 215 mL) was added and the mixture was refluxed until the solid dissolved (1 h). The solution was cooled and evaporated. The residue was dried by repeated addition of dry pyridine (3 x 5 mL). Adenosine 5'-monophosphomorpholidate (23 mg; 0.033 mMol) was added to the trioctylammonium salt of 2'-deoxycytidene 3',5'-diphosphate and the mixture was dried by repeated addition of dry pyridine (2 x 5 mL) and then of dry toluene (2 x 5 mL). The crude mixture was dissolved in anhydrous DMF (3.5 mL) and heated at 50 °C for 48 h. DMF was removed in vacuo at 40 °C. The crude mixture was purified by HPLC, but contains some of the 3'-adenylated deoxycytidine phosphate isomer.

Attachment of the primer to the solid support: To 120 mg of resin in 0.1 M *N*-methylimidazole buffer (pH 6) containing 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (3 mL) was added 5'-phosphorylated primer (500 μg; 0.625 μMol). The suspension was stirred at room temperature for 48 h. The beads were then washed with H_2O (3 x 1 mL).

Solid phase synthesis of pdN(pdN)$_n$pdNp:

ATP + pdNp: The reactions were performed in an Eppendorf tube in a mixture containing 50 mM Tris HCL, pH = 8, 10 mM $MnCl_2$, 20 mM DTT, 0.4 mM ATP, 5 mM phospocreatine, 8 mM spermine, 170 Myokinase U/mL, 175 U/mL Creatine Kinase, 10 mg/mL BSA, 8 mM pdNp, 0.5 mM of the primer on the resin (10 mg) or 0.5 mM dNp(dNp)$_n$dN and 3300 U/mL of T4 RNA ligase. The reaction was incubated at 17 °C for 48 h (Table 1).

AppdCp: The reactions were performed in an Eppendorf tube in a mixture containing 50 mM Tris HCl, pH = 8, 10 mM $MnCl_2$, 20 mM DTT, 10 mg/mL BSA, 5 mM AppdCp, 0.5 mM primer on resin (10 mg) or 0.5 mM dNp(dNp)$_n$dN and 3300 U/mL of T4 RNA ligase. The reaction was incubated at 17 °C for 48 h. The beads were washed with H_2O, (2 x 200 μL) and Tris HCl 50 mM pH = 8 (5 x 300 μL) (Table 2).

Cleavage of the oligonucleotide from the solid support:

Chemical cleavage: A solution of 6.95 g $NH_2OH \cdot HCl$ in 45 mL of 2 N NaOH was carefully brought to pH 4.5 by addition of 4 N HCl after which the total volume of the solution was brought to 50 mL by the addition of H_2O. The suspension of 10 mg of the oligonucleotide-containing resin in a solution of NH_2OH/H_2O (1/1) (30 μL) was heated at 37 °C for 1 h. The solution containing the oligonucleotide including the primer was analyzed by capillary electrophoresis and the solution was subsequently desalted on Biogel P6 microspin column (BioRad) and analyzed by MALDI-TOF-MS.

Enzymatic cleavage: The suspension of the oligonucleotide containing resin (10 mg) in 20 μL of 50 mM Tris HCl buffer pH 8, 10 mM $MgCl_2$ was treated with RNase A (800 ng = 4 μL) at 37 °C for 1 h. The solution containing the synthesized oligonucleotide was analyzed by capillary electrophoresis (Table 3).

Cleavage of the phosphate blocking group at the 3'-end of the synthesized oligonucleotide: The suspension of the oligonucleotide-containing resin (10 mg) in 15 μL of 50 mM Tris HCl pH 8, 10 mM $MgCl_2$ buffer was treated with 3 U of alkaline phosphatase at 37 °C for 1 h. The solution was heated at 95 °C for 5 min, the beads were washed with H_2O (2 x 200 μL) and 50 mM Tris HCl pH 8 (5 x 300 μL).