Supporting Information

High-Yield Solution-Phase Synthesis of Di- and Tri-nucleotide Blocks Assisted by Polymer-Supported Reagents

Cécile Dueymes,[†] Andreas Schönberger,^{†,‡} Ilaria Adamo,[†] Aude-Emmanuelle Navarro,[†] Albert Meyer,[†] Meinolf Lange,[‡] Jean-Louis Imbach,[†] Fritz Link,[‡] François Morvan,[†] and Jean-Jacques Vasseur*,[†]

ERT "Oligonucléotides: Méthodologie, Valorisation", UMR 5625 CNRS-UM2 University Montpellier II, 34095 Montpellier, France and Girindus AG, 33790 Halle-Kuensebeck/Westf, Germany

Preparation of polymer-supported reagents:

Polyvinyl-bound pyridinium *p*-toluene sulfonate **2**

Polymer-supported trimethylammonium periodate **7**

Polymer-supported trimethylammonium tetrathionate **10**

Polymer-supported sulfonic acid hydrazinium **17**

Synthesis of some dimers and trimers illustrative of the process:

5'-OH-dG^{iBu}po_{CNE}dG^{iBu}-3'-O-Lev

Overall Synthesis Scheme of 5'-OH-dG^{iBu}po_{CNE}dG^{iBu}-3'-O-Lev

5'-OH-dTp_{CNE}dG^{iBu}po_{CNE}dG^{iBu}-3'-O-Lev

5'-OH-dG^{iBu}ps_{CNE}dT-3'-O-Lev

Overall Synthesis Scheme of 5'-OH-dG^{iBu}ps_{CNE}dT-3'-O-Lev

5'-OH-dC^{Bz}ps_{CNE}dA^{Bz}-3'-O-Lev

5'-OH-dG^{iBu}ps_{CNE}dC^{Bz}-3'-O-Lev

5'-OH-dTp_{CNE}dT-3'-O-Lev

5'-OH-dA^{Bz}ps_{CNE}dT-3'-O-Lev

5'-OH-dC^{Bz}ps_{CNE}dA^{Bz}ps_{CNE}dT-3'-O-Lev

5'-O-DMTr-dC^{Bz}ps_{CNE}dTp_{CNE}dT-3'-O-Lev

5'-O-DMTr-dC^{Bz}ps_{CNE}dTp_{CNE}dT-3'-OH

5'-O-DMTr-dC^{Bz}ps_{CNE}dG^{iBu}-3'-O-Lev

5'-O-DMTr-dC^{Bz}ps_{CNE}dG^{iBu}-3'-OH

Overall Synthesis Scheme of 5'-O-DMTr-dC^{Bz}ps_{CNE}dG^{iBu}-3'-OH

Polyvinyl-bound pyridinium *p*-toluene sulfonate 2

The resin PVP sulfonate is commercially available from Aldrich.

The loading in sulfonate anion has been determined by elemental analysis:

% nitrogen: 4.59 %, % sulfur 10.70 %,

3.34 mmol of sulfonate anion per g of resin.

Polymer-supported trimethylammonium periodate 7

Amberlyst A-26 (10 g, OH⁻ form) was washed with methanol (2 x 25 ml) then with dichloromethane (2 x 25 ml). A solution of sodium periodate (10 g, 43 mmol, 1.3 eq) in deionised water (200 ml) was added to the resin and the resulting mixture was shaken for 15 hours. The resin was washed with water (1000 ml), methanol (2 x 30 ml) and dichloromethane (2 x 20 ml) before drying under reduced pressure to give 8.5 g of solid-supported periodate. The loading in periodate anion was determined by elemental analysis:

% nitrogen: 3.40 %, % iodine: 27.16 %,

2.14 mmol of periodate ion per g of resin

Polymer-supported trimethylammonium tetrathionate 10

Amberlyst A-26 (10 g, OH⁻ form) was washed with methanol (2 x 25 ml) then with dichloromethane (2 x 25 ml). A solution of potassium tetrathionate (30.35 g, 100 mmol, 3 eq) in deionised water (200 ml) was added to the resin and the resulting mixture was shaken for 20 hours. The resin was washed with water (4000 ml), methanol (2 x 100 ml) and

dichloromethane (2 x 100ml) before drying under reduced pressure to give 8.5 g of solid-supported tetrathionate. The loading in tetrathionate anion was determined by elemental analysis:

% nitrogen: 4.24 %, % sulfur: 23.25 %, less than 100 ppm of potassium

1.81 mmol of tetrathionate per g of resin.

In comparison, the loading in ammonium cation of starting Amberlyst A-26 is 3.35 mmol per g of resin (elemental analysis: % nitrogen: 4.69 %) showing that one tetrathionate ion was bound to 2 quaternary ammonium onto the resin.

After a typical sulfurization reaction (see synthesis of dimers) where 5 molar equivalents of tetrathionate were used, the resin was dried.

Elemental analysis gave % nitrogen: 4.36 %, % sulfur 21.98 % in accord with a loading of 1.80 mmol of a 4:1 mixture of tetrathionate/trithionate per g of resin.

Polymer-supported sulfonic acid hydrazinium 17

Dried commercial Amberlyst A-15 (200 g) was previously washed with water (2 x 1000 ml) then with a 2 M HCl aqueous solution (2 x 500 ml) and finally with water (12 x 500 ml) until the eluent reached pH 6. Hydrazine monohydrate (200 ml, 4.12 mol, approx. 4.5 eq with respect to supported sulfonic acid) dissolved in deionised water (1000 ml) was added to the resin and the mixture filled up with water to a total volume of 2300 ml. The mixture was shaken for 2.5 hours. The resin was filtered off and washed with deionised water (12 x 500 ml), ethanol (2 x 500 ml) and dichloromethane (2x 500 ml) before drying at 50 °C under reduced pressure (10 mbar) for 2.5 hours to give 231.9 g of solid-supported hydrazinium .

Elemental analysis gave % nitrogen: 11.14 %, % sulfur 12.57 %, % carbon 43.37% in accord with a loading of 3.98 mmole of N_2H_5^+ per g of resin

Synthesis of the dimer 5'-OH-dG^{iBu}po_{CNE}dG^{iBu}-3'-O-Lev

A. Coupling

In a dried flask, 5'-OH-dG^{iBu}-3'-O-Lev (4.35 g, 10.0 mmol, 1.0 eq.) and 5'-O-DMTr-dG^{iBu}-3'-O-cyanoethyl-phosphoramidite (10.74 g, 13.0 mmol, 1.3 eq.) were dissolved in dry acetonitrile (100 ml). The solvent was removed in vacuum. This process was repeated twice. The resulting residue was dissolved in dry dichloromethane (100 ml) under argon.

The solution of the reagents was transferred under argon to a dried flask containing poly(4-vinylpyridinium-*p*-toluenesulfonate) (30 g, 100.0 mmol tosyl-, 10 eq.). The mixture was shaken for 1 hour at room temperature. The experiment was monitored by HPLC.

After consumption of 5'-OH-dG^{iBu}-3'-O-Lev, water (0.5 ml) was added and the reaction mixture was shaken for additional 15 min until the excess of phosphoramidite was hydrolyzed.

The resin was separated by filtration and washed three times with dichloromethane (3 x 50 ml). The collected organic phase was concentrated to a volume of 100 ml. The HPLC analysis indicated a conversion of 100 % in relation of the 5'-OH-dG^{iBu}-3'-O-Lev to the requested phosphite triester dimer. This dimer was used without additional purification.

B. Oxidation

Amberlyst A-26 IO₄⁻ (8.0 g, 20.0 mmol, IO₄⁻, 2.0 eq.) was added to the dimer solution. The mixture was shaken 2 hours at room temperature. The reaction was monitored by RP-HPLC and ³¹P-NMR. After completion, the resin was filtered off and washed three times with dichloromethane (3 x 50 ml).

C. Detritylation

The solution (250 ml) of 5'-O-DMTr-dG^{iBu}-dG^{iBu}-3'-O-Lev cyanoethyl phosphate triester was diluted with methanol (107 ml) and cooled at 0°C. A solution at 0°C of 10 % *p*-

toluenesulfonic acid in dichloromethane/methanol (90 ml, 7:3, v/v) was added and the resulting mixture was stirred for 30 minutes at 0°C. After completion, water (100 ml) was added and the resulting mixture was vigorously stirred for 10 min at 0°C. A saturated NaHCO₃ aqueous solution (100 ml) was added and again, the reaction mixture was stirred strongly for 10 min at 0°C. Then, in a separating funnel, dichloromethane (100 ml) and a saturated NaHCO₃ solution (200 ml) were added at room temperature. The organic layer was washed with a solution of 0.2 M Na₂S₂O₃ and 0.5 M NaCl (300 ml). The organic layer was separated and dried over anhydrous Na₂SO₄. After filtration the solvent was evaporated under vacuum. The resulting residue was dissolved in dichloromethane (50 ml) and added to diethylether (1000 ml) at 0°C. During the addition of the dichloromethane solution, a white solid precipitated. This white solid was filtered off, washed with diethylether (500 ml) and dried under vacuum.

Yield 7.03 g (89 %, 8.9 mmol).

RP-HPLC spectrophotometric purity at 260 nm: 91%

³¹P NMR (CD₃CN): δ (ppm) -1.82, -1.93.

MALDI-TOF MS (positive mode, THAP as matrix) : [M+H]⁺ m/z_{calc} 888.1, m/z_{exp} 888.3

Overall Synthesis Scheme of 5'-OH-dG^{iBu}po_{CNE}dG^{iBu}-3'-O-Lev

Synthesis of the trimer 5'-OH-dTpo_{CNE}dG^{iBu}po_{CNE}dG^{iBu}-3'-O-Lev

A. Coupling

In a dried flask, 5'-OH-dG^{iBu}po_{CNE}dG^{iBu}-3'-O-Lev (7.03 g, 7.9 mmol, 1.0 eq.) and 5'-O-DMTr-dT-3'-O-cyanoethyl-phosphoramidite (8.26 g, 11.0 mmol, 1.4 eq) were dissolved in anhydrous acetonitrile (80 ml). The solvent was removed in vacuum. This process was repeated three times. After the last evaporation, the resulting residue was dissolved in dry dichloromethane (80 ml) under argon.

The solution was transferred under argon to a dried flask containing poly(4-vinylpyridinium-*p*-toluenesulfonate) (23.6 g, 79.0 mmol tos-, 10 eq.). After the addition, the mixture was shaken for 2.5 hour at room temperature. The experiment was monitored by HPLC.

After consumption of 5'-OH-dG^{iBu}po_{CNE}dG^{iBu}-3'-O-Lev, water (0.5 ml) was added and the reaction mixture was shaken for additional 15 min until the excess of phosphoramidite was hydrolyzed.

The resin was separated by filtration and washed three times with dichloromethane (3 x 40 ml). The collected organic phase was concentrated to a volume of 80 ml. The trimer was used without additional purification.

B. Oxidation

Amberlyst A-26 IO₄⁻ (6.3 g, 15.8 mmol, IO₄⁻, 2.0 eq.) was added to the trimer solution. The mixture was shaken 2 hours at room temperature. The reaction was monitored by RP-HPLC and ³¹P-NMR. After completion, the resin was filtered off and washed three times with dichloromethane (3 x 40 ml).

C. Detritylation

The solution (250 ml) of 5'-*O*-DMTr-dT-dG^{iBu}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphate triester was diluted with methanol (86 ml) and cooled at 0°C. A solution at 0°C of 10 % p-toluenesulfonic acid in dichloromethane/methanol (71 ml, 7:3, v/v) was added and the mixture was stirred for 30 minutes at 0°C. After completion, water (100 ml) was added and the resulting mixture was vigorously stirred for 10 min at 0°C. A saturated NaHCO₃ aqueous solution (100 ml) was added and again, the reaction mixture was stirred strongly for 10 min at 0°C. Then, in a separating funnel, dichloromethane (200 ml) and a saturated NaHCO₃ solution (200 ml) were added at room temperature. The organic layer was washed with a solution of 0.2 M Na₂S₂O₃ and 0.5 M NaCl (500 ml) then separated and dried over anhydrous Na₂SO₄. After filtration, the solvent was evaporated under vacuum. The resulting residue was dissolved in dichloromethane (60 ml) and added to diethylether (800 ml) at 0°C. During the addition of the dichloromethane solution, a white solid precipitated. This white solid was filtered off, washed with diethylether (400 ml) and dried under vacuum.

Yield 7.5 g (76 %, 6.02 mmol).

RP-HPLC spectrophotometric purity at 260 nm: 95%

³¹P NMR (CD₃OD): δ (ppm) -1.57, -1.63, -1.73, -1.78, -1.87, -1.93.

MALDI-TOF MS (positive mode, THAP as matrix) : [M+H]⁺ m/z_{calc} 1246.07, m/z_{exp} 1244.72

Synthesis of the dimer 5'-OH-dG^{iBu}ps_{CNE}dT-3'-O-Lev

A. Coupling

In a dried flask, 5'-OH-dT-3'-O-Lev (3.40 g, 10.0 mmol, 1.0 eq.) and 5'-O-DMTr-dG^{iBu}-3'-O-cyanoethyl-phosphoramidite (12.4 g, 15.0 mmol, 1.5 eq) were dissolved in dry acetonitrile (100 ml). The solvent was removed in vacuum. This process was repeated three times. The resulting residue was dissolved in dry dichloromethane (100 ml) under argon.

The solution was transferred under argon to a dried flask containing poly(4-vinylpyridinium-*p*-toluenesulfonate) (30 g, 100.0 mmol tos-, 10 eq.). The resulting mixture was shaken for 2 hours at room temperature. The experiment was monitored by HPLC.

After consumption of 5'-OH-dT-3'-O-Lev, water (0.5 ml) was added and the mixture was shaken for additional 30 min until the excess of 5'-O-DMTr-dG^{iBu}-3'-O-cyanoethyl-phosphoramidite was hydrolyzed.

The resin was separated by a filtration and washed three times with dichloromethane (3 x 50 ml). The collected organic phase was concentrated to a volume of 100 ml.

B. Sulfurization

Amberlyst A-26 S₄O₆²⁻ (26.6 g, 50.0 mmol S₄O₆²⁻, 5.0 eq.) was added to the phosphite triester dimer solution. The mixture was shaken 1 hour 45 min at room temperature. The reaction was monitored by RP-HPLC and ³¹P-NMR. After completion, the resin was filtered off and washed three times with dichloromethane (3 x 50 ml).

C. Detritylation

The solution (250 ml) of 5'-O-DMTr-dG^{iBu}-dG^{iBu}-3'-O-Lev cyanoethyl phosphate triester was diluted with methanol (107 ml) and cooled at 0°C. A solution at 0°C of 10 % *p*-toluenesulfonic acid in dichloromethane/methanol (90 ml, 7:3, v/v) was added and the resulting mixture was stirred for 20 minutes at 0°C. After completion, water (100 ml) was

added and the resulting mixture was vigorously stirred for 10 min at 0°C. A saturated NaHCO₃ aqueous solution (100 ml) was added and again, the reaction mixture was stirred strongly for 10 min at 0°C. Then, in a separating funnel, dichloromethane (200 ml) and a saturated NaHCO₃ solution (300 ml) were added at room temperature. The organic layer was washed with a 2.5% aqueous NaHCO₃ solution (3 x 500 ml) then separated and dried over anhydrous Na₂SO₄ (40g). After filtration the solvent was evaporated in vacuum. The residue was dissolved in dichloromethane (50 ml) and added to diethylether (1000 ml) at 0°C to give a white precipitate. This white solid was filtered off, washed with diethylether (500 ml) and dried under vacuum.

Yield 7.37 g (91 %, 9.1 mmol).

RP-HPLC spectrophotometric purity at 260 nm: 91%

³¹P NMR (CDCl₃): δ (ppm) 68.48, 68.09.

MALDI-TOF MS (positive mode, THAP as matrix) : [M+H]⁺ m/z_{calc} 809.77, m/z_{exp} 809.73

Overall Synthesis Scheme of 5'-OH-dG^{iBu}_{pSCNE}dT-3'-O-Lev

Synthesis of the dimer 5'-OH-dC^{Bz}ps_{CNE}dA^{Bz}-3'-O-Lev

A. Coupling

In a dried flask, 5'-OH-dA^{Bz}-3'-O-Lev (4.53 g, 10.0 mmol, 1.0 eq.) and 5'-O-DMTr-dC^{Bz}-3'-O-cyanoethyl-phosphoramidite (12.5 g, 15.0 mmol, 1.5 eq) were dissolved in dry acetonitrile (100 ml). The solvent was removed in vacuum. This process was repeated twice. The resulting residue was dissolved in dry dichloromethane (100 ml) under argon.

The solution was transferred under argon to a dried flask containing poly(4-vinylpyridinium-*p*-toluenesulfonate) (30 g, 100.0 mmol tos-, 10 eq.). The mixture was shaken for 2 hours at room temperature. The experiment was monitored by HPLC.

After consumption of 5'-OH-dA^{Bz}-3'-O-Lev, water (0.5 ml) was added and the reaction mixture was shaken for additional 30 min until the excess of 5'-O-DMTr-dG^{iBu}-3'-O-cyanoethyl-phosphoramidite was hydrolyzed.

The resin was separated by a filtration and washed three times with dichloromethane (3 x 50 ml). The collected organic phase was concentrated to a volume of 100 ml.

B. Sulfurization

Amberlyst A-26 S₄O₆²⁻ (26.6 g, 50.0 mmol S₄O₆²⁻, 5.0 eq.) was added to the phosphite triester dimer solution. The mixture was shaken 4 hours at room temperature. The reaction was monitored by RP-HPLC and ³¹P-NMR. After completion, the resin was filtered off and washed three times with dichloromethane (3 x 50 ml).

C. Detritylation

The solution (250 ml) of 5'-O-DMTr-dC^{Bz}-dA^{Bz}-3'-O-Lev cyanoethyl phosphate triester was diluted with methanol (107 ml) and cooled at 0°C. A solution at 0°C of 10 % *p*-toluenesulfonic acid in dichloromethane/methanol (90 ml, 7:3, v/v) was added and the

resulting mixture was stirred for 30 minutes at 0°C. After completion, water (100 ml) was added and the mixture was vigorously stirred for 10 min at 0°C. A saturated NaHCO₃ aqueous solution (100 ml) was added and again, the reaction mixture was stirred strongly for 10 min at 0°C. Then, in a separating funnel, dichloromethane (200 ml) and a saturated NaHCO₃ solution (300 ml) were added at room temperature. The organic layer was washed with a 2.5% aqueous NaHCO₃ solution (3 x 500 ml) then separated and dried over anhydrous Na₂SO₄ (40g) After filtration, the solvent was evaporated under vacuum. The residue was dissolved in dichloromethane (50 ml) and added to diethylether (1000 ml) at 0°C to give a white precipitate. This white solid was filtered off, washed with diethylether (500 ml) and dried under vacuum.

Yield 8.7 g (94 %, 9.4 mmol).

RP-HPLC spectrophotometric purity at 260 nm: 91%

³¹P NMR (CDCl₃): δ (ppm) 68.03, 67.80.

MALDI-TOF MS (positive mode, THAP as matrix) : [M+H]⁺ m/z_{calc} 916.88, m/z_{exp} 916.00

Synthesis of the dimer 5'-OH-dG^{iBu}ps_{CNE}dC^{Bz}-3'-O-Lev

A. Coupling

To a solution of 5'-OH-dC^{Bz}-3'-O-Lev (4.30 g, 10.0 mmol, 1.0 eq.) in hot DMF (30 ml) and anhydrous dichloromethane (30 ml) was added 10 g of molecular sieve (4 Å). Then, poly(4-vinylpyridinium-*p*-toluenesulfonate) (30 g, 100.0 mmol tos-, 10 eq.) was added and the resulting mixture was stirred overnight. 5'-O-DMTr-dG^{iBu}-3'-O-cyanoethyl-phosphoramidite (12.5 g, 15.0 mmol, 1.5 eq) dissolved in dried dichloromethane (45 ml) was added. The mixture was shaken for 2 hours at room temperature. The experiment was monitored by HPLC.

After consumption of 5'-OH-dG^{iBu}-3'-O-Lev, water (0.5 ml) was added and the reaction mixture was shaken for additional 30 min until the excess of 5'-O-DMTr-dG^{iBu}-3'-O-cyanoethyl-phosphoramidite was hydrolyzed.

The resin was separated by a filtration and washed three times with dichloromethane (3 x 50 ml). The collected organic phase was concentrated to a volume of 100 ml.

B. Sulfurization

Amberlyst A-26 S₄O₆²⁻ (27.0 g, 50.0 mmol S₄O₆²⁻, 5.0 eq.) was added to the phosphite triester dimer solution. The mixture was shaken 2 hours at room temperature. The reaction was monitored by RP-HPLC and ³¹P-NMR. After completion, the resin was filtered off and washed three times with dichloromethane (4 x 25 ml).

C. Detritylation

The solution (200 ml) of 5'-O-DMTr-dG^{iBu}-dC^{Bz}-3'-O-Lev cyanoethyl phosphate triester was diluted with methanol (107 ml) and cooled at 0°C. A solution at 0°C of 10 % *p*-toluenesulfonic acid in dichloromethane/methanol (90 ml, 7:3, v/v) was added and the mixture was stirred for 30 minutes at 0°C. After completion, water (100 ml) was added and

the resulting mixture was vigorously stirred for 10 min at 0°C. A saturated NaHCO₃ aqueous solution (100 ml) was added and again, the reaction mixture was stirred strongly for 10 min at 0°C. The mixture was poured into a separating funnel and dichloromethane (200 ml) was added. The two phases were separated. The organic layer was washed with a 2.5% aqueous NaHCO₃ solution (3 x 250 ml). The organic layer was separated and dried over anhydrous Na₂SO₄ (40g) After filtration the solvent was evaporated under vacuum. The resulting residue was dissolved in dichloromethane (50 ml) and added to diethylether (1000 ml) at 0°C to give a white precipitate. This white solid was filtered off, washed with diethylether (500 ml) and dried under vacuum.

Yield 8.7 g (94 %, 9.4 mmol).

RP-HPLC spectrophotometric purity at 260 nm: 95%

³¹P NMR (CD₃CN): δ (ppm) 68.39, 67.58.

MALDI-TOF MS (positive mode, THAP as matrix) : [M+H]⁺ m/z_{calc} 898.86, m/z_{exp} 896.90

Synthesis of the dimer 5'-OH-dTps_{CNE}dT-3'-O-Lev

A. Coupling

In a dried flask, 5'-OH-dT-3'-O-Lev (1.70g, 5.0 mmol, 1.0 eq.) and 5'-O-DMTr-dT-3'-O-cyanoethyl-phosphoramidite (6.33 g, 8.5 mmol, 1.7 eq) were dissolved in dry acetonitrile (50 ml). The solvent was removed under vacuum. This process was repeated twice. The resulting residue was dissolved in dry dichloromethane (100 ml) under exclusion of moisture and air. The solution was transferred under argon to a dried flask containing poly(4-vinylpyridinium-*p*-toluenesulfonate) (14.85 g, 50.0 mmol tos-, 10 eq.). The mixture was shaken for 2 hours at room temperature. The experiment was monitored by HPLC.

After consumption of 5'-OH-dT-3'-O-Lev, water (0.5 ml) was added and the mixture was shaken for additional 30 min until the excess of 5'-O-DMTr-dT-3'-O-cyanoethyl-phosphoramidite was hydrolyzed.

The resin was separated by a filtration then washed three times with dichloromethane (3 x 50 ml). The collected organic phase was concentrated to a volume of 40 ml.

B. Sulfurization

Amberlyst A-26 S₄O₆²⁻ (17.5 g, 25.0 mmol S₄O₆²⁻, 5.0 eq.) was added to the phosphite triester dimer solution. The mixture was shaken 17 hours at room temperature. The reaction was monitored by ³¹P-NMR. After completion, the resin was filtered off and washed three times with dichloromethane (3 x 50 ml).

C. Detritylation

The solution (190 ml) of 5'-O-DMTr-dT-dT-3'-O-Lev cyanoethyl phosphate triester was diluted with methanol (81 ml) and cooled at 0°C. A solution at 0°C of 10 % *p*-toluenesulfonic acid in dichloromethane/methanol (68 ml, 7:3, v/v) was added and the resulting mixture was stirred for 30 minutes at 0°C. Reaction was followed by tlc. After completion, water (100 ml)

was added and the resulting mixture was vigorously stirred for 10 min at 0°C. A saturated NaHCO₃ aqueous solution (100 ml) and dichloromethane (100 ml) were added at room temperature. The two phases were separated. The organic layer was washed with a 2.5% aqueous NaHCO₃ solution (4 x 150 ml) as many times as necessary to get rid of the 5'-OH-dT-3'-H-cyanoethyl phosphonate monomer. The organic layer was separated and dried over anhydrous Na₂SO₄ (40g) After filtration, the solvent was evaporated in vacuum. The residue was dissolved in dichloromethane (50 ml) and added to diethylether (500 ml) at 0°C to give a white precipitate. This white solid was filtered off, washed with diethylether (250 ml) and dried under vacuum.

Yield 2.9 g (81 %, 4.0 mmol).

RP-HPLC spectrophotometric purity at 260 nm: 96%

³¹P NMR (CDCl₃): δ (ppm) 68.12, 68.15.

MALDI-TOF MS (positive mode, THAP as matrix) : [M+H]⁺ m/z_{calc} 714.66, m/z_{exp} 714.63

Synthesis of the dimer 5'-OH-dA^{Bz}ps_{CNE}dT-3'-O-Lev

A. Coupling

In a dried flask, 5'-OH-dT-3'-O-Lev (1.70 g, 5 mmol, 1.0 eq.) and 5'-O-DMTr-dA^{Bz}-3'-O-cyanoethyl-phosphoramidite (6.40 g, 7.5 mmol, 1.5 eq.) were dissolved in dry acetonitrile (50 ml). The solvent was removed under vacuum. This process was repeated twice. After the last evaporation the residue was dissolved in dry dichloromethane (50 ml) under argon.

The solution was transferred under argon to a dried flask containing poly(4-vinylpyridinium-*p*-toluenesulfonate) (15 g, 50.0 mmol tosyl-, 10 eq.). After the addition, the mixture was shaken for 2.5 hours at room temperature. The experiment was monitored by HPLC.

After consumption of 5'-OH-dT^z-3'-O-Lev, water (0.5 ml) was added and the mixture was shaken for additional 30 min until the excess of 5'-O-DMTr-dG^{iBu}-3'-O-cyanoethyl-phosphoramidite was hydrolyzed.

The resin was separated by a filtration then washed three times with dichloromethane (3 x 50 ml). The collected organic phase was concentrated to a volume of 50 ml.

B. Sulfurization

Amberlyst A-26 S₄O₆²⁻ (13.5 g, 25.0 mmol S₄O₆²⁻, 5.0 eq.) was added to the phosphite triester dimer solution. The mixture was shaken 4 hours at room temperature. The reaction was monitored by RP-HPLC and ³¹P-NMR. After completion, the resin was filtered off and washed three times with dichloromethane (3 x 50 ml).

C. Detritylation

The solution (200 ml) of 5'-O-DMTr-dA^{Bz}-dT-3'-O-Lev cyanoethyl phosphate triester was diluted with methanol (85 ml) and cooled at 0°C. A solution at 0°C of 10 % *p*-toluenesulfonic acid in dichloromethane/methanol (71 ml, 7:3, v/v) was added and the resulting mixture was

stirred for 30 minutes at 0°C. After completion, water (100 ml) was added and the resulting mixture was vigorously stirred for 10 min at 0°C. A saturated NaHCO₃ aqueous solution (100 ml) was added and again, the mixture was stirred strongly for 10 min at 0°C. Then, in a separating funnel, dichloromethane (200 ml) and a saturated NaHCO₃ solution (300 ml) were added at room temperature. The two phases were separated. The organic layer was washed with a 2.5% aqueous NaHCO₃ solution (5 x 200 ml). Washings were performed as many times as necessary to remove the 5'-OH-dA^{Bz}-H-phosphonate diester. The organic layer was separated and dried over anhydrous Na₂SO₄ (40g) After filtration the solvent was evaporated under vacuum. The resulting residue was dissolved in dichloromethane (30 ml) and added to diethylether (500 ml) at 0°C to give a white precipitate. This white solid was filtered off, washed with diethylether (250 ml) and dried under vacuum.

Yield 3.96 g (96 %, 4.80 mmol).

RP-HPLC spectrophotometric purity at 260 nm: 88%

³¹P NMR (CD₃CN): δ (ppm) 67.96, 66.94.

MALDI-TOF MS (positive mode, THAP as matrix) : [M+H]⁺ m/z_{calc} 827.79, m/z_{exp} 826.99

Synthesis of the trimer 5'-OH-dC^{Bz}ps_{CNE}dA^{Bz}ps_{CNE}dT-3'-O-Lev

A. Coupling

In a dried flask, 5'-OH-dA^{Bz}ps_{CNE}dT-3'-O-Lev (2.0 g, 2.42 mmol, 1.0 eq.) and 5'-O-DMTr-dC^{Bz}-3'-O-cyanoethyl-phosphoramidite (3.63 g, 4.35 mmol, 1.8 eq) were dissolved in dry acetonitrile (25 ml). The solvent was removed under vacuum. This process was repeated three times. After the last evaporation, the residue was dissolved in dry dichloromethane (25 ml) under argon.

The solution was transferred under argon to a dried flask containing poly(4-vinylpyridinium-*p*-toluenesulfonate) (15 g, 50.0 mmol tos-, 10 eq.). The reaction mixture was shaken for 2;5 hours at room temperature. The experiment was monitored by HPLC.

After consumption of 5'-OH-dT^z-3'-OLev, water (0.5 ml) was added and the resulting mixture was shaken for additional 30 min until the excess of 5'-O-DMTr-dG^{iBu}-3'-O-cyanoethyl-phosphoramidite was hydrolyzed.

The resin was separated by filtration then washed three times with dichloromethane (3 x 25 ml). The collected organic phase was concentrated to a volume of 25 ml.

B. Sulfurization

Amberlyst A-26 S₄O₆²⁻ (6.54 g, 12.1 mmol S₄O₆²⁻, 5.0 eq.) was added to the phosphite triester dimer solution. The mixture was shaken 2 hours 15 min at room temperature. The reaction was monitored by RP-HPLC and ³¹P-NMR. After completion, the resin was filtered off and washed three times with dichloromethane (3 x 25 ml).

C. Detritylation

The solution (100 ml) of 5'-*O*-DMTr-dA^{Bz}-dT-3'-*O*-Lev cyanoethyl phosphate triester was diluted with methanol (43 ml) and cooled at 0°C. A solution at 0°C of 10 % p-toluenesulfonic acid in dichloromethane/methanol (36 ml, 7:3, v/v) was added and the reaction mixture was stirred for 30 minutes at 0°C. After completion, water (40 ml) was added and the resulting mixture was vigorously stirred for 10 min at 0°C. A saturated NaHCO₃ aqueous solution (70 ml) was added and again, the reaction mixture was stirred strongly for 10 min at 0°C. Then, in a separating funnel, dichloromethane (40 ml) was added. The two phases were separated. The organic layer was washed with a 2.5% aqueous NaHCO₃ solution (5 x 100 ml). Washings were performed as many times as necessary to remove the 5'-OH-dC^{Bz}-H-phosphonate diester. The organic layer was separated and dried over anhydrous Na₂SO₄ (20g). After filtration, the solvent was concentrated under vacuum. The resulting residue was dissolved in dichloromethane (5 ml) and added to diethylether (100 ml) at 0°C to give a white precipitate. This white solid was filtered off, washed with diethylether (100 ml) and dried under vacuum. Yield 2.45 g (78 %, 4.80 mmol).

RP-HPLC spectrophotometric purity at 260 nm: 91%

³¹P NMR (CD₃CN): δ (ppm) 67.97.

MALDI-TOF MS (positive mode, THAP as matrix) : [M+H]⁺ m/z_{calc} 1290.21, m/z_{exp} 1289.62

Synthesis of the trimer 5'-O-DMTr-dC^{Bz}ps_{CNE}dTps_{CNE}dT-3'-O-Lev

A. Coupling

In a dried flask, 5'-OH-dTps_{CNE}dT-3'-O-Lev (2.6 g, 3.64 mmol, 1.0 eq.) and 5'-O-DMTr-dC^{Bz}-3'-O-cyanoethyl-phosphoramidite (5.46 g, 6.56 mmol, 1.8 eq) were dissolved in dry acetonitrile (35 ml). The solvent was removed under vacuum. This process was repeated three times. After the last evaporation the residue was dissolved in dry dichloromethane (35 ml) under argon.

The solution was transferred to a dried flask under exclusion of moisture and air, which contains poly(4-vinylpyridinium-*p*-toluenesulfonate) (10.8 g, 36.4 mmol tos-, 10 eq.). After the addition, the mixture was shaken for 2.2 hours at room temperature. The experiment was monitored by HPLC.

After consumption of 5'-OH-dTps_{CNE}dT-3'-O-Lev, water (0.35 ml) was added and the mixture was shaken for additional 30 min until the excess of 5'-O-DMTr-dC^{Bz}-3'-O-cyanoethyl-phosphoramidite was hydrolyzed.

The resin was separated by filtration then washed three times with dichloromethane (3 x 35 ml). The collected organic phase was concentrated to a volume of 40 ml.

B. Sulfurization

Amberlyst A-26 S₄O₆²⁻ (12.7 g, 18.2 mmol S₄O₆²⁻, 5.0 eq.) was added to the phosphite triester dimer solution. Pyridine (5ml) was added to avoid partial detritylation. The mixture was shaken 17 hours at room temperature. The reaction was monitored by ³¹P-NMR. After

completion, the resin was filtered off and washed three times with dichloromethane (3 x 50 ml). The resulting solution was concentrated to an oil under vacuum.

C. Oxidation

The oil was dissolved in dichloromethane (19 ml) and Amberlist A26 IO_4^- was added (7.28 g, 18.2 mmol IO_4^-). The mixture was shaken for 1.5 hours, then the resin was filtered off and washed with dichloromethane (150 ml). The solution was washed with a 0.2M aqueous $\text{Na}_2\text{S}_2\text{O}_3$ and 0.5 M NaCl solution (100 ml) then dried over Na_2SO_4 (40 g) before evaporation. The residue was dissolved in dichloromethane (30 ml) and filtered on a pad of silica gel (50 g). Elution was performed with dichloromethane/methanol (96/4, v:v, 100 ml). After evaporation, the residue was dissolved in dichloromethane (35 ml) and precipitated in diethylether (350 ml) at 0°C. The white precipitate was filtered off, washed with diethylether (250 ml) and dried under vacuum.

Yield was 3.96 g (73 %, 2.67 mmol).

RP-HPLC spectrophotometric purity at 260 nm: 94%

^{31}P NMR (CDCl_3): δ (ppm) 68.52, 68.65.

MALDI-TOF MS (positive mode, THAP as matrix) : $[\text{M}+\text{H}]^+$ m/z_{calc} 1479.46, m/z_{exp} 1478.22

Synthesis of the trimer 5'-O-DMTr-dC^{Bz}ps_{CNE}dTp_{CNE}dT-3'-OH

The trimer 5'-O-DMTr-dC^{Bz}ps_{CNE}dTp_{CNE}dT-3'-O-Lev (2.0 g, 1.35 mmol) was dissolved in pyridine (32 ml) and acetic acid (8 ml), and Amberlyst A15 N₂H₅⁺ (5.48 g, 20.3 mmol, 15 eq) was added. The reaction was followed by tlc. The mixture was shaken for 5 hours at room temperature. The resin was filtered off and washed with dichloromethane (200 ml). The resulting solution was washed with a saturated NaHCO₃ aqueous solution (110 ml). The aqueous layer was extracted with dichloromethane (3 x 100 ml) and the combined organic layers were dried on Na₂SO₄ and concentrated under reduce pressure. The residue was dissolved in dichloromethane (13 ml) and precipitated in diethylether (130 ml) at 0°C. The white precipitate was washed with cold diethylether and dried under vacuum.

Yield 1.71 g (92 %, 1.24 mmol).

RP-HPLC spectrophotometric purity at 260 nm: 92%

³¹P NMR (CDCl₃): δ (ppm) ?.

MALDI-TOF MS (positive mode, THAP as matrix) : [M+H]⁺ m/z_{calc} 1381.36, m/z_{exp} 1380.76.

Synthesis of the dimer 5'-O-DMTr-dC^{Bz}ps_{CNE}dG^{iBu}-3'-O-Lev

A. Coupling

In a dried flask, 5'-OH-dG^{iBu}-3'-O-Lev (2.18 g, 5 mmol, 1.0 eq.) and 5'-O-DMTr-dC^{Bz}-3'-O-cyanoethyl-phosphoramidite (7.25 g, 8.7 mmol, 1.7 eq) were dissolved in dry acetonitrile (50 ml). The solvent was removed under vacuum. This process was repeated three times. After the last evaporation the residue was dissolved in dried dichloromethane (50 ml) under argon.

The solution was transferred under argon to a dried flask containing poly(4-vinylpyridinium-*p*-toluenesulfonate) (14.84 g, 50 mmol tos⁻, 10 eq.). The mixture was shaken for 6 hours at room temperature. The experiment was monitored by HPLC .

After consumption of 5'-OH-dG^{iBu}-3'-O-Lev, water (0.25 ml) was added and the resulting mixture was shaken for additional 30 min until the excess of 5'-O-DMTr-dC^{Bz}-3'-O-cyanoethyl-phosphoramidite was hydrolyzed.

The resin was separated by a filtration then washed three times with dichloromethane (3 x 50 ml). The collected organic phase was concentrated to a volume of 40 ml.

B. Sulfurization

Amberlyst A-26 S₄O₆²⁻ (17.5 g, 25 mmol S₄O₆²⁻, 5.0 eq.) was added to the phosphite triester dimer solution. Pyridine (5ml) was added to avoid partial detritylation. The mixture was shaken 11 hours at room temperature. The reaction was monitored by ³¹P-NMR. After completion, the resin was filtered off and washed three times with dichloromethane (3 x 50 ml). The resulting solution was concentrated to an oil under vacuum.

C. Oxidation

The oil was dissolved in dichloromethane (22 ml) and Amberlist A26 IO₄⁻ was added (10 g, 25 mmol IO₄⁻). The mixture was shaken for 1.7 hours, then the resin was filtered off and

washed with dichloromethane (200 ml). The solution was washed with a 0.2M aqueous $\text{Na}_2\text{S}_2\text{O}_3$ and 0.5 M NaCl solution (150 ml) then dried over Na_2SO_4 (40 g) before evaporation. The residue was dissolved in dichloromethane (30 ml) and filtered on a pad of silica gel (30 g). Elution was performed with dichloromethane/methanol (97/3, v:v, 100 ml). After evaporation, the residue was dissolved in dichloromethane (50 ml) and precipitated in diethylether (500 ml) at 0°C. The white precipitate was filtered off, washed with diethylether (400 ml) and dried under vacuum.

Yield 5.16 g (86 %, 4.3 mmol).

RP-HPLC spectrophotometric purity at 260 nm: 96%

^{31}P NMR (CDCl_3): δ (ppm) 68.42, 68.69.

MALDI-TOF MS (positive mode, THAP as matrix) : $[\text{M}+\text{H}]^+$ m/z_{calc} 1201.24, m/z_{exp} 1200.25

Synthesis of the dimer 5'-O-DMTr-dC^{Bz}ps_{CNE}dG^{iBu}-3'-OH

The dimer 5'-O-DMTr-dC^{Bz}ps_{CNE}dTp_{CNE}dT-3'-O-Lev (0.5 g, 0.45 mmol) was dissolved in pyridine (69ml) and acetic acid (17 ml) and Amberlyst A15 N₂H₅⁺ (11.8 g, 43.7 mmol, 15 eq) was added. The reaction was followed by tlc. The mixture was shaken for 4 hours at room temperature. The resin was filtered off and washed with dichloromethane (300 ml). The resulting solution was washed with a saturated NaHCO₃ aqueous solution (250 ml). The aqueous layer was extracted with dichloromethane (3 x 150 ml) and the combined organic layers were dried on Na₂SO₄ and concentrated under reduce pressure. The residue was dissolved in dichloromethane (30 ml) and precipitated in diethylether (300 ml) at 0°C. The white precipitate was washed with cold diethylether (200 ml) and dried under vacuum.

Yield 2.89 g (90 %, 2.62 mmol).

RP-HPLC spectrophotometric purity at 260 nm: 95%

³¹P NMR (CDCl₃): δ (ppm) ?.

MALDI-TOF MS (positive mode, THAP as matrix) : [M+H]⁺ m/z_{calc} 1103.14, m/z_{exp} 1102.50

Overall Synthesis Scheme of 5'-O-DMTr-dC^{Bz}p_{SCNE}dG^{iBu}-3'-OH