Oxidative Substitutions of BoranephosphonateDiesters as a Route to Post Synthetically Modified DNA

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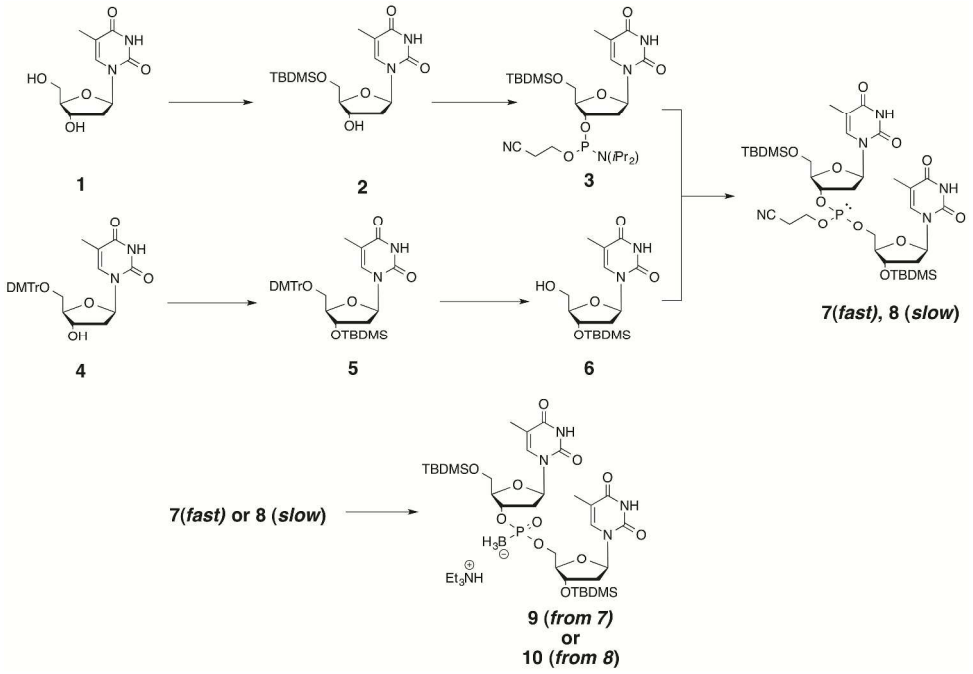
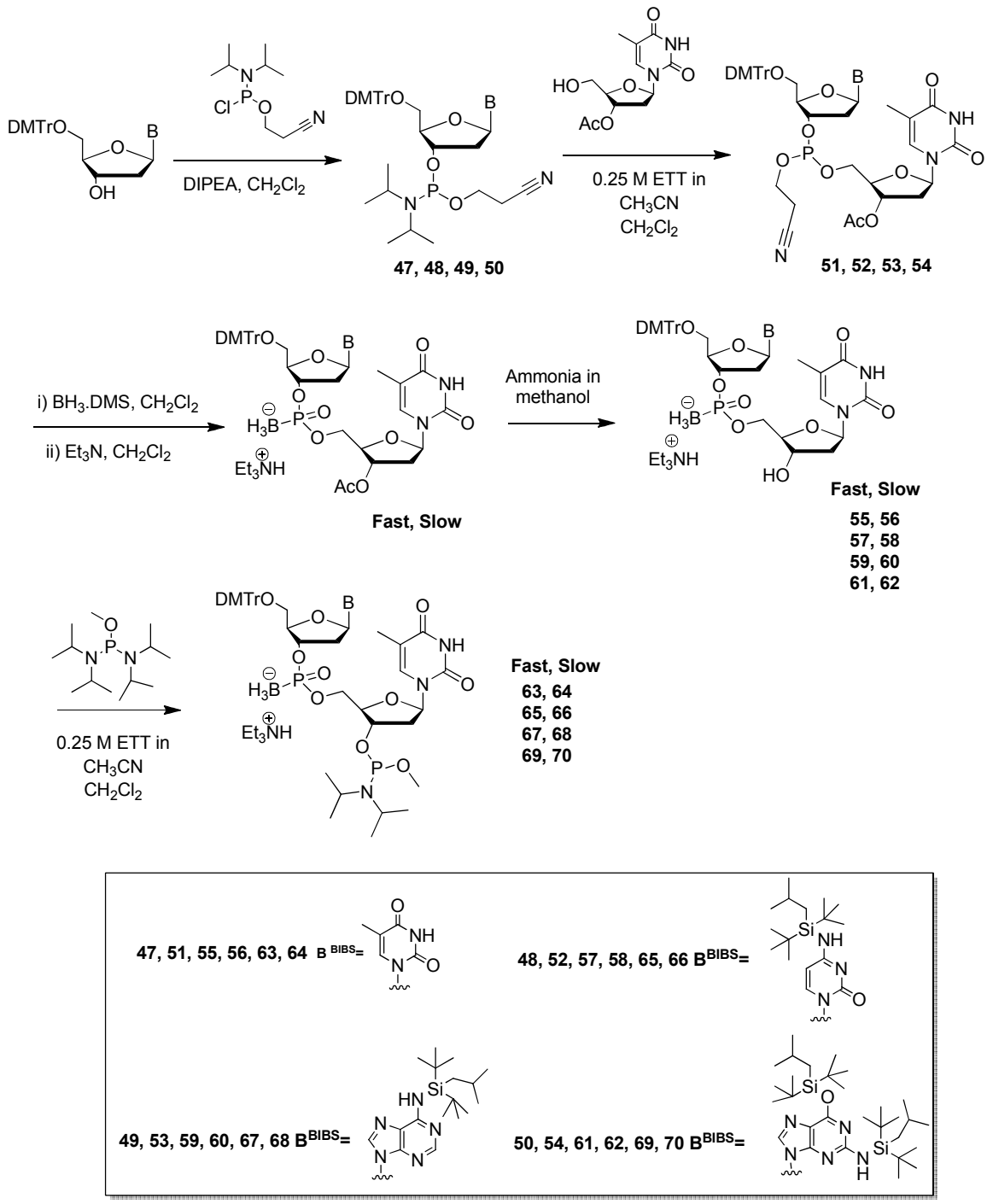

HO
OH
1
TBDMSO
OH
2
TBDMSO
NC
N(iPr2)
3
DMTrO
OH
4
DMTrO
OTBDMS
5
HO
OTBDMS
6
TBDMSO
NC
OTBDMS
7(fast), 8 (slow)
7(fast) or 8 (slow)
TBDMSO
H3B
Et3NH
OTBDMS
9 (from 7)
or
10 (from 8)

Scheme S1



Scheme S2

Experimental

General. All chemicals were purchased from Sigma-Aldrich unless otherwise noted. Standard reagents used in automated DNA synthesis as well as 5'-dimethoxytrityl-2'-

deoxythymidine 3'-[(methyl)-(N,N-diisopropylamino)]-phosphoramiditewere purchased from Glen Research. 2'-Deoxynuceosides were purchased from ChemGenes Corporation. Deuterated solvents were purchased from Cambridge Isotopes.

NMR experiments were carried out on a BrukerAvance-III 300 (^{1}H = 300.13 MHz., and/or a BrukerAvance-III 400 (^{1}H=400.13 MHz). Chemical shifts are given in ppm with positive shifts downfield: all ^{1}H and ^{13}C chemical shifts were referenced relative to the signal from residual protons of a lock solvent. For ^{1}H 3.31 ppmforMeOD and 5.32ppm for CD_2Cl_2 and for ^{13}C, 49.15 ppm forMeOD and 54.00 ppm for CD_2Cl_2 were used. ^{31}P Chemical shifts are referenced to 0.0 ppm in the ^{1}H NMR spectrum according to the standard IUPAC method.[i] In order to measure NOE correlations to the BH_3 protons, 2-dimensional NOESY with ^{11}B decoupling (NOESY{^{11}B}) experiments were performed using broadband (GARP1) decoupling of the ^{11}B frequency to eliminate ^{1}H relaxation of the BH_3 protons due to coupling to the quadrupolar^{11}B center. ^{11}B Decoupling was performed during both the evolution (t1) and acquisition (t2) time period in the NOESY pulse sequence. NOE mixing times of 0.6 seconds were used for all 2D-NOESY experiments presented herein.

Analytical Polyacrylamide Gel Electrophoresis.20% polyacrylamide gels (7.5 cm in length) containing 7 M urea were run at 100 V. The 2'-deoxyoligonucleotides were dissolved in a Tris-EDTA buffer containing 7.0 M urea and heated to 95^{o} C followed by cooling on ice prior to loading on the gel. After electrophoresis, the gels were stained using SYBR GOLD nucleic acid stain.

LC-MSanalyses were carried out on an Agilent 6530 series Q-TOF LC/MS spectrometer. A waters ACQUITY UPLC BEH C18, 1.7 μm,2.1 X 100 nm column was used with a gradient of 0-100% of buffer B in 50 min with a flow rate of 0.2 mL/min at 75^{o}C (buffer A wasa1:80:9.5:9.5mixture of 500mM dibutylammoniumacetate:water: isopropanol:acetonitrile and buffer Bwas a 1:10:44.5:44.5mixture of 500mM dibutylammonium acetate: water: isopropanol:acetonitrile).

MALDI-TOF analysis. MALDI-TOF was performed on a PerSeptiveBiosystems Voyager-DE STR Biospectrometry Workstation. 2'-deoxyoligonucleotides were

dissolved in water at a concentration of 100-200 μM. 10 μL of this solution was treated with the ammonium form of a Dowexcation exchange resin. The matrix was prepared by adding 0.2 mmol of 2,4,6-trihydroxyacetophenone monohydrate (45 mg) and 8.2 μmol of ammonium citrate (2 mg) to 500 μL of acetonitrile/water (1:1), which forms a supersaturated solution (a cloudy suspension). 5 μL each of the matrix solution and the2'-deoxyoligonucleotide solution were mixed and 2 μL was pippetted onto a spot on theMALDI-plate.

Thermal Denaturing Studies.UV-vis measurements were performed on a CARY 100 Bio UV-visible spectrophotometer equipped with a thermoelectrically controlled multicell holder. Samples containing phosphoramidate DNA and RNA were prepared in a buffer containing 1 M NaCl, 10 mM sodium phosphate, pH 6.8. The solutions were heated to 90 °C for 5 min followed by cooling to 15 °C at a rate of 1 °C min^{-1}, equilibrated for 5 min, and then heated back to 90 °C at the same rate. The absorbance at 260 nm was recorded throughout at intervals of 0.5 °C. The derivative of the heating curve was calculated and the temperature corresponding to the maximum of the derivative curve was determined to be the melting temperature.

Synthesis

5′-*tert*-butyldimethylsilyloxy-2′-deoxythymidine (2).

In a round bottomed flask 2'-deoxythymidine (**1**) (2.92 g, 12.05 mmol) was coevaporatedtwice with pyridine (30 mL) and dried overnight under vacuum. N, N-dimethlaminopyridine (DMAP) (0.147 g, 1.20 mmol) was added and the flask was flushed repeatedly with argon.Subsequently anhydrous pyridine (30 mL) and *tert*-butyldimethylsilyl chloride (2.1 g, 13.9 mmol) were added. The reaction mixture was stirred overnight at room temperature under argon while the progress of the reaction was monitored using TLC (9.5:0.5; $CHCl_3$:CH_3OH). The reaction was stopped by removal of pyridine *in vacuo* followed by co-evaporation with toluene (2 x 30 mL). The crude reaction mixture was diluted with ethylacetate (100 mL) and washed first with saturated sodiumbicarbonate (50 mL) and then brine (50 mL). The ethylacetate layer was dried

over sodium sulfate, filtered and evaporated to dryness. The resulting dry crude mixture was dissolved in a mixture of ethylacetate:hexanes (1:1) and purified by flash chromatography on a silica gel column. The silica gel slurry was initially prepared in a mixture of ethylacetate:hexanes (1:1) and poured into the column. The desired product (**2**) was eluted using a gradient of 1:1 ethylacetate:hexanes to 7:3 ethylacetate:hexanes and isolated as a white foam (3.5 g, 83% yield). ^{1}H NMR (CD_2Cl_2, 400 MHz)δ: 8.58 (1H, bs, NH), 7.50 (1H, s, H-6), 6.35-6.39 (1H, dd, *J*= 8.0 Hz, H-1′), 4.48 (1H, m, H-3′), 4.03 (1H, m, H-4′), 3.87 (2H, m, H-5′), 2.37 (1H, m, H-2′), 2.10 (1H, m, H-2′), 1.92 ((3H, s, CH_3-5), 0.92 (18H, m, ^{t}Bu-TBDMS), 0.11 (12H, Me-TBDMS). ^{13}C NMR ($CDCl_3$)δ: -5.45, -5.36, 12.56, 18.37, 25.93, 41.14, 63.66, 85.08, 87.42, 111.00, 135.54, 150.67, 164.07. ESI-MS (m/z): 379.2 $(M+Na)^{+}$.

5′-*tert*-butyldimethylsilyloxy-2′-deoxythymidine3′-O-cyanoethyl-N,N-diisopropylphosphoramidite (3):

Compound (**2**) (0.342 g, 0.96 mmol) was dried overnight under vacuum in a round bottomed flask. The flask was then flushed repeatedly with argon.Anhydrous dichloromethane (10 mL), N,N-diisopropylethylamine (0.67 mL, 3.84 mmol) and N,N-diisopropylamino-cyanoethylphosphoamidic chloride (0.25 g, 1.05 mmol) were added to the flask. The reaction mixture was stirred at room temperature for one hour under argon. At this time TLC (7:3; ethylacetate:hexanes) showed complete disappearance of starting material. The reaction mixture was diluted with dichloromethane and extracted twice with a saturated solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The crude product was dissolved in a mixture of ethylacetate:hexane (1:1) and purified by flash chromatography on a silica column. The silica gel slurry was prepared with the starting eluant mixture containing an additional 5% triethylamine. After pouring thesilica gel slurry, the column was washed with two column volumes of the starting solvent mixture containing no triethylamine. Compound (3) was purified using a gradient of ethylacetate:hexanes (1:1) to ethylacetate:hexanes(7:3) and isolated as a white foam (0.48 g, 91%). ^{31}P NMR (CD_2Cl_2)δ: 148.12.^{1}H NMR (CD_2Cl_2, 400 MHz)δ: 9.00 (1H, bs, NH), 7.49 (1H, d, *J*= 16.0 Hz, H-6), 6.31 (1H, m, H-1′), 4.52 (1H, m, H-3′), 4.18 (0.5H, m, H-4′), 4.10 (0.5H,

m, H-4′), 3.39-3.93 (4H, m, H-5′, OCH_2-CNEt), 3.56 (2H, HN^iPr_2), 2.64 (2H, m, CH_2CN-CNEt), 2.35-2.50 (1H, m, H-2′), 2.09 (1H, m, H-2′), 1.89 (3H, s, CH_3-5), 1.17-1.20 (9H, m, $^iPr_2NH$), 0.92 (18H, m, ^{t}Bu-TBDMS), 0.12 (12H, Me-TBDMS). ^{13}C NMR ($CDCl_3$)δ: -5.62, -5.47, -5.34, -5.19, 12.52, 18.34, 20.37, 20.44, 24.44, 24.52, 24.58, 24.65, 25.92, 40.11, 40.16, 43.16, 43.28, 58.12, 58.30, 63.39, 73.85, 74.02, 76.76, 84.97, 86.83, 86.87, 110.88, 117.52, 135.37, 150.44, 164.02. ESI-MS (m/z): 557.3 $(M+H)^+$, 579.2 $(M+Na)^+$.

5′-hydroxy-3′-*tert*-butyldimethylsilyloxy-2′-deoxythymidine (6):

5'-dimethoxytritylthymidine (**4**) (2.09 g, 3.84 mmol) was placed in a round bottom flask and coevaporatedtwice with pyridine (20 mL) and dried overnight under vacuum. N, N-dimethlaminopyridine (DMAP) (0.094 g, 0.76 mmol) was added and the flask was repeatedly flushed with argon.Anhydrous pyridine (20 mL) and*tert*-butyldimethylsilyl chloride (0.86 g, 5.76 mmol) were added to the flask. The reaction mixture was stirred at room temperature under argon for two days while being monitored using TLC (9.5:0.5; $CHCl_3$:CH_3OH). The reaction was stopped by removal of pyridine *in vacuo*followed by co-evaporation with toluene (2 x 30 mL). The crude reaction mixture was suspended inethylacetate (100 mL) and then washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL). The ethylacetate layer was dried over sodium sulfate, filtered and the solvent removed by evaporation to dryness to yield compound (**5**) as a white foam. This compound was used for the next step without further purification.

Compound (**5**) was dissolved in 30 mL of dichloromethane and *p*-toluenesulfonic acid (1.0 g in 10 mL methanol) added dropwise until the orange color persisted. The reaction mixture was stirred at room temperature for 3 h. At this time TLC (9.5:0.5; $CHCl_3$:CH_3OH) showed complete disappearance of starting material. Pyridine was added dropwise to the mixture to quench the excess acid. The crude reaction mixture was evaporated to dryness, diluted with ethylacetate (100 mL) and washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL). The ethylacetate layer was dried over sodium sulfate, filtered and evaporated to dryness. The crude product was dissolved in chloroform and loaded onto a silica gel column for purification by flash chromatography. The silica gel slurry was initially prepared in chloroform and poured into the column. The

desired product (**6**) was eluted using a gradient of 100% chloroform to chloroform:methanol (9.5:0.5), and isolated as a white foam (1.01 g, 74% after two steps). ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 8.45 (1H, bs, NH), 7.35 (1H, s, H-6), 6.15 (1H, t, *J*= 8.0 Hz, H-1′), 4.49 (1H, m, H-3′), 3.92 (2H, m, H-5′, H-4′), 3.75 (1H, m, H-5′), 2.36 (1H, m, H-2′), 2.21 (1H, m, H-2′), 1.91 (3H, s, CH_3-5), 0.89 (18H, m, ^{t}Bu-TBDMS), 0.08 (12H, Me-TBDMS). ^{13}C NMR ($CDCl_3$) δ: -4.88, -4.73, 12.43, 17.92, 25.70, 40.63, 53.48, 61.74, 71.57, 76.80, 86.26, 87.63, 110.83, 137.07, 150.61, 164.42.ESI-MS (m/z): 379.2 $(M+Na)^{+}$.

5′-O-(TBDMS)thymidin-3′-yl 3′-O-(TBDMS)thymidin-5′-yl cyanoethylphosphite: (**7**)and(**8**):

Compound (**3**) (0.58 g, 1.04 mmol) and compound (**6**) (0.41 g, 1.15 mmol)were dried overnight under vacuum in two round bottomed flasks. The flask containing compound (**3**) was flushed repeatedly with argon. Anhydrous dichloromethane (7.0 mL) and compound (**6**) dissolved in 5.0 mL dichloromethane were added to the flask containing compound (**3**). This was followed by dropwise addition of 2.6 mL of 1-H tetrazole (0.4 M in acetonitrile; Glen Research) to the reaction flask.After stirring for 2 h at room temperature, TLC (9.5:0.5; $CHCl_3$:CH_3OH) showed complete disappearance of starting material. The crude reaction mixture was evaporated to dryness, diluted with ethylacetate (20 mL), and then washed with saturated $NaHCO_3$ (10 mL) and brine (10 mL). The ethylacetate layer was dried over sodium sulfate, filtered and evaporated to dryness. The crude mixture was dissolved in a solution of ethylyacetate:hexane (1:1)andpurified by flash chromatography on a silica column. Compound (**7**) followed by (**8**) were eluted using aslowgradient of ethylacetate-hexanes (1:1) to ethylacetate-hexanes (8:2).Thesediastereomers and subsequent compounds derived from them were labeled as the “fast” and “slow” isomers respectively. The two compounds (**7**) and (**8**) were obtainedaswhitefoams(0.36 g, 35% and 0.33 g, 32% respectively).

Compound 7: ^{31}P NMR (CD_2Cl_2) δ: 139.65.^{1}H NMR (CD_2Cl_2, 400 MHz): 7.46 (1H, s, ^{5}H-6), 7.37 (1H, s, ^{3}H-6), 6.30 (1H, m, ^{5}H-1′), 6.24 (1H, m, ^{3}H-1′), 4.83 (1H, t, ^{5}H-3′), 4.41 (1H, m, ^{3}H-3′), 4.15 (1H, m, ^{5}H-4′), 4.03 (5H, m, 5H-5′, ^{3}H-4′, OCH_2-CNEt), 3.86

(2H, m, ^{3}H-5′), 2.67 (2H, m, CH_2CN-CNEt), 2.41 (1H, m, ^{5}H-2′), 2.26 (1H, m, ^{5}H-2′), 2.21 (2H, m, ^{3}H-2′), 1.90 (6H, m, $^{3}CH_3$C-5, $^{5}CH_3$C-5), 0.91 (18H, m, ^{t}Bu-TBDMS), 0.12 (12H, Me-TBDMS). ESI-MS (m/z): 812.3 $(M+H)^+$, 834.3 $(M+Na)^+$.

Compound 8: ^{31}P NMR (CD_2Cl_2) δ: 139.30.^{1}H NMR (CD_2Cl_2, 400 MHz): 7.47 (1H, s, ^{5}H-6), 7.32 (1H, s, ^{3}H-6), 6.32 (1H, m, ^{5}H-1′), 6.22 (1H, m, ^{3}H-1′), 4.90 (1H, t, ^{5}H-3′), 4.38 (1H, m, ^{3}H-3′), 4.16 (1H, m, ^{5}H-4′), 3.94 (5H, m, 5H-5′, ^{3}H-4′, OCH_2-CNEt), 3.86 (2H, m, ^{3}H-5′), 2.68 (2H, m, CH_2CN-CNEt), 2.44 (1H, m, ^{5}H-2′), 2.33 (1H, m, ^{5}H-2′), 2.28 (2H, m, ^{3}H-2′), 1.91 (6H, m, $^{3}CH_3$C-5, $^{5}CH_3$C-5), 0.92 (18H, m, ^{t}Bu-TBDMS), 0.11 (12H, Me-TBDMS). ESI-MS (m/z): 812.3 $(M+H)^+$, 834.3 $(M+Na)^+$.

5′-O-(TBDMS)thymidin-3′-yl 3′-O-(TBDMS)thymidin-5′-yl boranophosphate: (**9**)and(**10**):

Compound (**7**) and (**8**) (0.8 g each, 1 mmol) were each dissolved in anhydrous dichloromethane followed by dropwise addition of $BH_3.Me_2S$ (0.2 mL, 10.0-10.2 M BH_3) to the starting material solutions. The reaction mixtures were stirred at room temperature and monitored by ^{31}P NMR. After 1h,the ^{31}P NMR spectra showed complete conversion of starting materialstothebroadproduct peak at 116.8 ppm. Excess BH_3-Me_2S in the reaction mixtures wasquenched by addition of a few drops of methanol andthesolventremoved under reduced pressure in a rotaryevaporator. The products were further dried overnight under high vacuum.

These crude reaction mixtureswere each dissolved in ethanol (5.0 mL) followed by addition of30% NH_4OH (2.5 mL). The reaction mixtures werethen stirred overnight.TLC (9:0.5:0.5; $CHCl_3$:CH_3OH:Et_3N) and ^{31}P NMR showed complete disappearance of starting material. The reaction mixtures were then evaporated to dryness. The resulting crude mixtures were dissolved in a solution of chloroform:triethylamine (9.5:0.5), loaded onto silica columnsandthe products were purified using silica gel flash column chromatography. Silica gel slurries were prepared with the starting eluant mixture containing an additional 5% triethylamine. After pouring the silica gel slurries into columns, they were each washed with two column volumes of the starting solvent mixture. Compounds (**9**) and (**10**) were purified using a gradient of

9.5:0.5; $CHCl_3$:Et_3N to 9:0.5:0.5; $CHCl_3$:CH_3OH:Et_3N and isolated as white foams (0.6 g each, 68%).

Compound 9: ^{31}P NMR (CD_2Cl_2) δ: 94.19.^1H NMR ($CDCl_3$, 400 MHz)δ: 7.70 (1H, s, ^5H-6), 7.56 (1H, s, ^3H-6), 6.35-6.45 (2H, m, ^5H-1′, ^3H-1′), 4.78-4.82 (1H, m, ^5H-3′), 4.50-4.52 (1H, m, ^3H-3′), 4.18-4.24 (2H, m, ^5H-4′, ^3H-4′), 3.83-4.01 (4H, m, 5H-5′, ^3H-5′), 2.96-2.98 (6H, m, CH_2-Et_3N), 2.30-2.34 (1H, m, ^5H-2′), 2.12-2.15 (2H, m, ^3H-2′), 1.93-2.05 (4H, m, ^5H-2′, $^3CH_3$C-5), 1.89 (3H, s, $^5CH_3$C-5), 1.21-1.28 (9H, m, CH_3-Et_3N), 0.82-0.89 (18H, m, ^{t}Bu-TBDMS), 0.34 (3H, m, BH_3), 0.06 (12H, Me-TBDMS).^{13}C NMR ($CDCl_3$)δ: -5.41, -4.70, -4.68, 9.14, 12.45, 12.59, 17.90, 18.31, 25.74, 25.94, 39.75, 39.79, 40.69, 45.71, 61.59, 61.66, 63.83, 73.32, 75.32, 84.80, 84.90, 86.85, 86.91, 87.24, 87.27, 111.07, 111.37, 135.23, 136.20, 150.78, 150.85, 164.08, 164.24. ESI-MS (m/z): 873.5 $(M+Et_3NH)^+$.

Compound 10:^{31}P NMR (CD_2Cl_2) δ: 94.42.^1H NMR (CD_2Cl_2, 400 MHz):^1H NMR (CD_2Cl_2, 400 MHz) δ: 12.33 (1H, bs, $HNEt_3$), 8.76 (1H, bs, NH), 8.69 (1H, bs), 7.68 (1H, s, ^5H-6), 7.55 (1H, s, ^3H-6), 6.31 (2H, m, ^5H-1′, ^3H-1′), 4.89 (1H, m, ^5H-3′), 4.46 (1H, m, ^3H-3′), 4.20 (1H, m, ^5H-4′), 4.05 (3H, m, 5H-5′, ^3H-4′), 3.80 (2H, m, ^3H-5′), 3.01 (6H, m, CH_2-Et_3N), 2.45 (1H, m, ^5H-2′), 2.40 (2H, m, ^3H-2′), 2.20 (1H, m, ^5H-2′), 1.96 (3H, s, $^3CH_3$C-5), 1.88 (3H, s, $^5CH_3$C-5), 1.30 (9H, m, CH_3-Et_3N), 0.91 (18H, m, ^{t}Bu-TBDMS), 0.37 (3H, m, BH_3), 0.10 (12H, Me-TBDMS). ^{13}C NMR ($CDCl_3$) δ: -5.42, -5.41, -4.76, -4.72, -4.69, 8.66, 12.46, 17.90, 18.31, 21.92, 25.72, 25.76, 25.91, 38.14, 40.33, 40.76, 45.61, 57.55, 62.22, 62.27, 63.85, 72.85, 75.12, 84.78, 84.89, 85.02, 86.51, 86.58, 86.64, 86.68, 110.88, 110.96, 111.16, 118.60, 135.38, 136.25, 150.73, 150.86, 164.22, 164.41. ESI-MS (m/z): 873.5 $(M+Et_3NH)^+$.

5′-O-(TBDMS)thymidin-3′-yl-3′-O-(TBDMS)thymidin-5′-yl methylphosphoramidate: (**11)** and **(12)**:

Compound (**9**) and (**10**) (0.24 g each, 0.31 mmol) were dissolved in anhydrous tetrahydrofuran (5.0 mL). Iodine (0.24 g, 0.93 mmol) immediately followed by methylamine (1.6 mL, 2.0 M in THF, 3.1 mmol)were added to each reaction mixture. These reaction mixtures were stirred at room temperature under argon and monitored

using TLC (9:1; $CHCl_3$:CH_3OH) and ^{31}P NMR. After completion (1 h) the reaction mixtures were concentrated to minimum volume, diluted with ethylacetate (10 mL) and washed with saturated sodium thiosulfate (5 mL) and brine (5 mL). The ethylacetate layers were dried over Na_2SO_4, filtered and evaporated to dryness. The crude products were purified by flash chromatography on a silica column. The silica gel slurries were prepared in $CHCl_3$. Compound (**11**) and (**12**) weredissolved in chloroform, loaded onto silica columns and purified using a gradient of $CHCl_3$ to $CHCl_3$:CH_3OH(9.5:0.5).Both (**11**) and (**12**) were isolated as white foams in 78% and 72% yield respectively.

Compound 11: ^{31}P NMR (CD_2Cl_2) δ: 9.99.^{1}H NMR (CD_2Cl_2, 400 MHz): 7.49-7.50 (2H, m, ^{5}H-6, ^{3}H-6), 6.37-6.40 (1H, m, ^{5}H-1′), 6.29-6.32 (1H, m, ^{3}H-1′), 5.00-5.03 (1H, m, ^{5}H-3′), 4.47-4.51 (1H, m, ^{3}H-3′), 4.29-4.30 (1H, m, ^{5}H-4′), 4.19-4.26 (2H, m, 5H-5′), 4.06-4.07 (1H, m, ^{3}H-4′), 3.86-3.92 (2H, m, ^{3}H-5′), 3.58-3.62 (1H, m, ^{5}H-2′), 2.60-2.65 (3H, m, NHMe), 2.28-2.30 (1H, m, ^{5}H-2′), 2.15-2.22 (2H, m, ^{3}H-3′), 1.92-1.93 (6H, m, $^{5}CH_3C$-5, $^{3}CH_3C$-5), 0.2-0.95 (18H, m, ^{t}Bu-TBDMS), 0.13-0.16 (12H, Me-TBDMS).^{13}C NMR (CD_2Cl_2) δ:-5.85, -5.78, -5.71, -5.13, -4.97, 12.22, 12.30, 17.79, 18.19, 18.76, 25.46, 25.49, 25.69, 27.21, 27.22, 39.24, 39.28, 40.44, 44.52, 45.80, 63.38, 65.15, 65.20, 71.68, 77.47, 77.52, 84.63, 85.09, 85.23, 85.31, 85.72, 85.77, 110.92, 110.94, 135.12, 135.75, 150.63, 150.66, 164.33, 164.37.ESI-MS (m/z): 788.3 $(M+H)^+$, 810.3 $(M+Na)^+$.

Compound 12: ^{31}P NMR (CD_2Cl_2) δ: 10.50.^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 7.55 (1H, s, ^{5}H-6), 7.41 (1H, s, ^{3}H-6), 6.37-6.40 (1H, m, ^{5}H-1′), 6.28-6.32 (1H, m, ^{3}H-1′), 4.96-4.99 (1H, m, ^{5}H-3′), 4.46-4.48 (1H, m, ^{3}H-3′), 4.35 (1H, m, ^{5}H-4′), 4.19-4.22 (2H, m, 5H-5′), 4.08-4.09 (1H, bs, ^{3}H-4′), 3.93 (2H, m, ^{3}H-5′), 3.78-3.81 (1H, m, ^{5}H-2′), 2.62-2.67 (3H, m, NHMe), 2.30-2.33 (1H, m, ^{5}H-2′), 2.12-2.19 (2H, m, ^{3}H-2′), 1.93 (6H, s, $^{3}CH_3C$-5, $^{5}CH_3C$-5), 0.93-0.94 (18H, m, ^{t}Bu-TBDMS), 0.14-0.15 (12H, Me-TBDMS).^{13}C NMR (CD_2Cl_2) δ: -5.78, -5.12, 12.31, 17.78, 18.20, 25.48, 25.71, 27.24, 39.28, 40.46, 45.76, 63.43, 65.64, 72.02, 77.65, 84.77, 85.25, 86.31, 110.94, 135.10, 135.62, 150.80, 164.46. ESI-MS (m/z): 788.3 $(M+H)^+$, 810.3 $(M+Na)^+$.

General method for the synthesis of 5'-dimethoxytrityl-N-BIBS protected 2'-deoxynucleoside 3'-O-cyanoethyl-N,N-diisopropylphosphoramidites:

The 5'-dimethoxytrityl-N-BIBS protected 2'-deoxynucleosides[ii] were added to a round bottom flask which was then flushed with argon. Anhydrous dichloromethane and diisopropyl ethylamine (4.0 equiv.) were added. Chloro-cyanoethyl-N,N-isopropylphosphorodiamidite (1.2 equivalents) was dissolved in anhydrous dicholoromethaneand added dropwise*via* syringe. The reaction was stirred at room temperature for 2-3 hours at which time TLC showed complete disappearance of the starting material. The reaction mixture was diluted in dichloromethane and extracted twice with a saturated solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. The product was purified by flash chromatography on a silica column. The silica gel slurry was prepared with the starting eluant mixture containing an additional 5% triethylamine. After pouring the slurry, thecolumn was washed with two column volumes of the starting solvent mixture containing no triethylamine. Compound **48, 49** were purified using a gradient of 3:7 ethylacetate-hexanes to 1:1 ethylacetate-hexanes. **50**was purified using a 7:3 hexanes-diethylether mixture. Compound **47** was obtained from ChemGenes Corporation.

N^2-di(tert-butyl)isobutylsilyl-5'-dimethoxytrityl-2'-deoxycytidine3'-O-cyanoethyl-N,N-diisopropylphosphoramidite (48): 87% yield. ^{31}P NMR (CD_2Cl_2) δ: 148.56, 148.80. ^1H NMR (CD_2Cl_2, 400 MHz) δ: 7.84 (1H, dd, H-6), 7.47 (2H, m, DMT), 7.28-7.38 (7H, m, DMT), 6.87-6.90 (4H, m, DMT), 6.29-6.35 (1H, m, H-1′), 5.53-5.54 (1H, d, *J*= 4.0 Hz, H-5), 4.65 (1H, bs, H-3′), 4.50 (1H, bs, H-4′), 4.19 (1H, bs, H-5′), 4.10-4.13 (1H, m, HN^iPr_2), 3.82 (6H, s, DMT), 3.62-3.71 (2H, m, HN^iPr_2, H-5′), 3.39-3.51 (2H, m, CH_2CN-CNEt), 2.65-2.68 (1H, m, -CHCN-CNEt), 2.49-2.52 (1H, m, -CHCN-CNEt), 2.25-2.30 (1H, m, H-2′), 2.05-2.10 (1H, m, H-2′), 1.14-1.29 (30H, $^iPr_2NH$, BIBS), 0.98-1.03 (9H, m, BIBS). ^{13}C NMR (CD_2Cl_2) δ: 20.18, 20.24, 20.32, 20.39, 24.28, 24.34, 24.40, 24.76, 26.12, 26.14, 28.62, 40.52, 40.74, 43.23, 43.35, 55.20, 58.30, 58.49, 62.68, 62.96, 72.48, 72.65, 73.14, 73.32, 84.94, 85.17, 85.80, 85.85, 96.02, 96.08, 113.15, 117.53, 117.68, 126.92, 127.87, 128.13, 128.18, 130.08, 130.14, 135.51, 135.61, 140.66, 144.69, 155.06, 158.75, 168.13. ESI-MS (m/z): 928.4 $(M+H)^+$.

N^2-di(tert-butyl)isobutylsilyl-5'-dimethoxytrityl-2'-deoxyadenosine3'-O-cyanoethyl-N,N-diisopropylphosphoramidite (49): 85% yield. ^{31}P NMR (CD_2Cl_2) δ: 148.53, 148.52. ^1H NMR (CD_2Cl_2, 400 MHz) δ: 8.23 (1H, s, H-8), 7.95-7.97 (1H, d, *J*= 8.0 Hz, H-2), 7.42-7.46 (2H, DMT), 7.32-7.26 (4H, m, DMT), 7.23-7.30 (3H, m, DMT), 6.81-6.87 (4H, m, DMT), 6.42-6.45 (6.42-6.45, 1H, t, *J*= 12.0, 4.0 Hz, H-1′), 4.77-4.83 (1H, m, H-3′), 4.28-4.31 (1H, m, H-4′), 3.85-3.91 (1H, m, HN^iPr_2), 3.81 (6H, DMT), 3.72-3.78 (1H, m, HN^iPr_2), 3.59-3.72 (2H, m, H-5'), 3.31-3.46 (2H, m, -CH_2CN-CNEt), 2.93-3.00 (1H, m, -CHCN-CNEt), 2.65-2.68 (1H, m, -CHCN-CNEt), 2.51-2.54 (1H, m, H-2′), 2.10-2.17 (1H, m, H-2′), 1.15-2.19 (30H, m, $^iPr_2$NH, BIBS), 0.99-1.08 (9H, m, BIBS). ^{13}C NMR (CD_2Cl_2) δ: 20.02, 20.03, 20.04, 20.08, 21.01, 24.29, 24.30, 24.33, 24.36, 24.53, 24.78, 26.19, 26.23, 28.60, 38.98, 39.02, 39.06, 43.17, 43.30, 55.16, 55.18, 58.25, 58.35, 58.44,58.53, 63.53, 63.57, 73.38, 73.55, 84.26, 84.32, 85.38, 85.44, 85.61, 85.65, 86.31, 113.04, 117.62, 117.74, 122.27, 126.72, 126.75, 127.76, 128.03, 128.08, 129.99, 130.03, 130.07, 130.10, 135.67, 135.71, 135.85, 138.70, 144.85, 149.18, 149.21, 152.36, 158.09, 158.62, 158.64. ESI-MS (m/z): 974.4 $(M+Na)^+$.

N^2,O^6-bis[di(tert-butyl)isobutylsilyl]-5'-dimethoxytrityl-2'-deoxyguanosine3'-O-cyanoethyl-N,N-diisopropylphosphoramidite (50): 79% yield. ^{31}P NMR ($CDCl_3$) δ: 149.28, 148.37. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.87, 7.80 (1H, s, H-8), 7.45 (2H, m, DMT), 7.20−7.37 (7H, m, DMT), 6.85 (4H, m, DMT), 6.37 (1H, m, H-1'), 4.58 (1H, m, H-3'), 4.50 (1H, s, N-2), 4.28 (1H, m, H-4'), 3.73-3.86 (1H, m, HN^iPr_2), 3.81 (6H, d, DMT), 3.55-3.67 (3H, m, HN^iPr_2, H-5'), 3.29-3.44 (1H, m, -CHCN-CNEt), 2.49-2.63 (2H, m, -CH_2CN-CNEt), 2.36-2.44 (1H, m, -CHCN-CNEt), 2.13-2.22 (1H, m, H-2'), 2.01-2.08 (1H, m, H-2'), 1.18-1.21 (9H, m, amidite-CH_3), 1.17 (18H, s, BIBS), 1.14, 1.13 (18H, s, BIBS), 1.02-1.05 (2H, m, BIBS), 0.93-0.99 (14H, m, BIBS). ^{13}C NMR ($CDCl_3$) δ:20.09, 20.16, 20.31, 20.38, 20.68, 20.70, 20.88, 20.89, 20.93, 20.95, 21.52, 22.04,24.51, 24.55, 24.56, 24.58, 24.62, 24.65, 24.72, 24.88, 26.42, 26.46, 26.53, 26.60, 28.36, 28.94, 28.96, 28.98, 41.16, 43.18, 43.24, 43.30, 43.37, 55.22, 55.24, 58.05, 58.17, 58.24, 58.36, 63.25, 63.62, 73.61, 74.18, 83.75, 83.84, 85.07, 85.12, 85.38, 86.40, 86.44, 113.20, 117.11, 117.34, 126.88, 126.92, 127.90, 127.91, 128.10, 128.17, 130.04, 130.06, 130.07,

130.11, 135.61, 135.64, 135.67, 136.36, 144.49, 154.03, 154.09, 158.53, 159.32, 160.62. ESI-MS (m/z): 1166.6 $(M+H)^+$, 1188.6 $(M+Na)^+$.

General method for the synthesis of dinucleosidetriesters:

3'-Acetyl 2'-deoxythymidine (1.2 equiv) was dried underhighvacuum for 3-4 h in a round bottom flask and then flushed with argon. The 5'-dimethoxytrityl-N-BIBS protected 2'-deoxynucleoside 3'-O-cyanoethyl-N,N- diisopropylphosphoramidites(**47** to **50)** were dissolved separately in anhydrous dichloromethane andadded *via* syringe. Subsequently 1.0 equivalent of ethylthiotetrazole (0.25 M in CH_3CN obtained from Glen Research) was added dropwise to this solution over one-half hour while stirring. The reaction was then allowed to stir overnight at room temperature.At this time TLC showed complete disappearance of the starting material. Reaction mixtures were evaporated to dryness, diluted in dichloromethane and extracted twice with a saturated solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered andevaporated to dryness. The products (**51** to **54**) were purified by flash chromatography on a silica column. The silica gel column was prepared inchloroform containing 2% triethylamineand poured into the column. The crude mixture was suspended in a small volume of this same solutionand loaded onto the column followed by elution using a gradient of chloroform:Et_3N(98:2) tochloroform:Et_3N:CH_3OH (95:2:3).

Compound 51:87% yield. ^{31}P NMR (CD_2Cl_2) δ: 139.24, 139.02.^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 7.62 (1H, m, H-6), 7.46-7.50 (1H, m, DMT), 7.35-7.41 (7H, m, DMT, H-6), 7.28-7.32 (1H, m, DMT), 6.89-6.93 (4H, m), 6.44-6.48 (1H, m, ^{5}H-1′), 6.38-6.42 (1H, m, ^{3}H-1′), 5.27-5.34 (1H, m, ^{3}H-3′), 5.04-5.12 (1H, m, ^{5}H-3′), 4.25-4.26 (1H, m, ^{5}H-4′), 4.12-4.21 (3H, m, ^{3}H-4′, -CH_2-CNEt), 4.01-4.08 (2H, m, ^{3}H-5′), 3.83 (6H, s, DMT), 3.43-3.55 (2H, m, ^{3}H-5′), 2.64-2.71 (2H, m, CH_2-CNEt), 2.57-2.62 (1H, m, ^{3}H-2′), 2.43-2.51 (2H, m, ^{3}H-2′, ^{5}H-2′), 2.22-2.32 (1H, m, ^{5}H-2′), 2.13 (3H, s, OAc), 1.95 (3H, s, $^3CH_3$C-5), 1.55 (3H, s, $^5CH_3$C-5).). ^{13}C NMR (CD_2Cl_2) δ: 11.76, 12.49, 20.23, 20.27, 20.32, 20.81, 37.16, 39.78, 55.29, 57.61, 57.72, 57.94, 58.08, 62.46, 63.02, 73.46, 73.57, 73.72, 73.85, 74.20, 77.77, 83.33, 83.42, 84.62, 84.67, 84.74, 85.13, 85.17, 85.23, 86.98,

87.01, 111.28, 111.35, 113.28, 117.48, 127.16, 128.04, 130.13, 135.35, 135.56, 144.53, 150.94, 158.86, 164.38, 164.52, 170.47, 170.53. ESI-MS (m/z): 950.3 $(M+Na)^+$

Compound 52: 81% yield. ^{31}P NMR (CD_2Cl_2) δ: 139.05, 139.18. ^1H NMR (CD_2Cl_2, 400 MHz) δ: 7.80 (1H, m, H-6), 7.44-7.46 (2H, m, DMT), 7.40 (1H, m, H-5), 7.32-7.36(7H, m, DMT), 6.87-6.90 (4H, m, DMT), 6.31-6.36 (2H, m, ^{5}H-1′, ^{3}H-1′), 5.36 (1H, m, NHBIBS), 5.30-5.32 (1H, m, ^{5}H-3′), 5.23-5.24 (1H, m, ^{3}H-3′), 4.94-5.00 (2H, m, ^{5}H-4′, ^{3}H-4′), 4.16-4.22 (2H, m, -CH_2-CNEt), 3.97-4.00 (2H, m,^{5}H-5′), 3.82 (6H, s, DMT), 3.49-3.51 (1H, m, -CH-CNEt), 3.39-3.43 (1H, m, -CH-CNEt), 2.56-2.60 (3H, m, ^{3}H-5′, ^{5}H-2′), 2.31-2.38 (2H, m, ^{5}H-2′), 2.12 (3H, s, OAc), 1.91 (3H, s, $^3CH_3$C-5), 1.13 (16H, BIBS), 0.99-1.07 (9H, m, BIBS). ^{13}C NMR (CD_2Cl_2) δ: 9.42, 11.26, 12.39, 20.17, 20.29, 20.67, 20.72, 20.74, 20.95, 24.77, 26.09, 26.16, 28.59, 37.12, 40.56, 46.10, 55.21, 57.36, 57.45, 57.74, 57.87, 62.37, 62.56, 71.30, 72.91, 73.17, 73.33, 74.23, 83.33, 84.68, 84.81, 84.78, 84.88, 85.79, 85.82, 86.78, 86.81, 96.32, 111.20, 113.19, 117.29, 127.00, 128.08, 130.07, 135.14, 135.39, 140.42, 144.53, 150.47, 155.06, 158.77, 163.67, 168.21, 170.31.ESI-MS (m/z): 1111.4 $(M+H)^+$.

Compound 53: 81% yield. ^{31}P NMR (CD_2Cl_2) δ: 139.38, 139.13. ^1H NMR (CD_2Cl_2, 400 MHz) δ: 8.27-8.30 (1H, m, ^{5}H-8), 7.93-7.96 (1H, m, ^{5}H-2), 7.43-7.45 (3H, m, DMT), 7.32-7.35 (4H, m, DMT), 7.23-7.30 (3H, m, DMT), 6.83-6.87 (3H, m, DMT), 6.43-6.47 (1H, td, *J*= 6.0, 4.0 Hz, ^{5}H-1′), 6.31-6.36 (1H, m, ^{3}H-1′), 5.39 (1H, bs, NHBIBS), 5.32-5.34 (1H, m, ^{5}H-3′), 5.27-5.30 (1H, m, ^{3}H-3′), 4.28-4.33 (1H, m, ^{5}H-4′), 4.12-4.24 (3H, m, ^{3}H-4′, OCH2-CNEt)**,** 4.01-4.07 (2H, m,^{5}H-5′), 3.81 (6H, s, DMT), 3.44-3.48 (1H, m, -CH-CNEt), 3.36-3.41 (1H, m, -CH-CNEt), 3.01-3.12 (1H, m, ^{3}H-5′), 2.60-2.67 (3H, m, ^{3}H-5′, ^{5}H-2′), 2.37-2.44 (1H, m, ^{5}H-2′), 2.21-2.34 (1H, m, ^{5}H-2′), 2.10 (3H, s, OAc), 1.92 (3H, s, $^3CH_3$C-5), 1.18 (16H, BIBS), 1.07-1.08 (3H, m, BIBS), 0.99-1.03 (8H, m, BIBS). ^{13}C NMR (CD_2Cl_2) δ: 11.58, 12.38, 12.41, 20.19, 20.24, 20.28, 20.33, 20.72, 20.78, 20.79, 21.00, 24.79, 26.23, 28.60, 37.17, 38.64, 38.83, 46.19, 55.19, 57.51, 57.87, 58.01, 62.32, 62.38, 63.20, 73.83, 73.95, 74.12, 74.22, 74.28, 74.32, 83.35, 83.41, 83.44, 83.49, 84.27, 84.37, 84.88, 84.98, 85.20, 85.24, 85.27, 86.06, 86.48, 86.53, 111.22, 113.11, 117.28, 122.30, 126.85, 127.83, 128.02, 130.00, 130.06, 135.16, 135.28, 135.55,

135.59, 135.66, 135.69, 138.67, 138.80, 144.70, 144.75, 149.11, 150.40, 152.42, 158.16, 158.70, 163.54, 170.38. ESI-MS (m/z): 1157.4 $(M+Na)^+$.

Compound 54:73% yield. ^{31}P NMR ($CDCl_3$) δ: 139.02, 138.48. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.57 (1H, br s, T N-3), 7.85, 7.84 (1H, s, G C-8), 7.19-7.44 (9H, m, DMT), 6.82-6.87 (4H, m, DMT), 6.35-6.40 (2H, m, ^{5}H-1′, ^{3}H-1′), 5.26 (1H, m, ^{5}H-3′), 4.93 (1H, m, ^{3}H-3′), 4.96 (1H, m, ^{3}H-4′), 4.90 (1H, m, ^{5}H-4′), 4.52 (1H, s, G N-2), 4.27−3.90 (4H, m, ^{3}H-5′, -OCH2-CNEt), 3.79 (6H, s, DMT), 3.34-3.43 (2H, m, ^{5}H-5′), 2.49-2.61 (2H, m, -OCH2-CNEt), 2.34-2.43 (1H, m, ^{5}H-2′), 2.12-2.24 (2H, m, ^{3}H-2′), 2.09-2.11 (3H, s, T 3'-OAc), 2.05 (3H, s, T-Me), 1.16 (18H, s, BIBS), 1.11−1.14 (18H, s, BIBS), 1.03 (2H, d), 0.91−1.01 (14H, m, BIBS).^{13}C NMR ($CDCl_3$) δ:12.71, 14.22, 21.7, 20.22, 20.25, 20.30, 20.74, 20.92, 20.94, 21.06, 21.21, 21.46, 21.48, 21.51, 21.81, 22.04, 24.03, 24.71, 24.84, 26.48, 26.54, 26.55, 26.57, 26.60, 28.18, 28.30, 28.35, 28.91, 28.93, 28.95, 37.21, 37.37, 40.67, 41.27, 55.22, 55.24, 57.40, 57.51, 57.63, 57.69, 57.83, 60.39, 62.20, 62.90, 73.40, 73.53, 73.67, 74.17, 74.21, 83.28, 83.32, 83.36, 83.54, 84.51, 84.55, 84.70, 84.74, 86.64, 86.69, 99.98, 111.60, 111.63, 112.89, 113.27, 113.29, 116.90, 117.07, 117.11, 127.00, 127.04, 127.96, 127.98, 128.07, 130.05, 134.94, 135.04, 135.39, 135.43, 135.48, 136.14, 136.22, 144.33, 144.38, 150.56, 150.58, 154.00, 154.03, 158.60, 158.63, 19.39, 160.69, 163.69, 170.34, 170.47. ESI-MS (m/z): 1349.6 $(M+H)^+$, 1371.6 $(M+Na)^+$.

General procedure for boronation and removal of cyanoethyl groups:

Compounds **51** to **54** were dissolved in anhydrous dicholoromethane followed by dropwise addition of borane-dimethylsulfide complex (1.2 equivalents) solution under argon atmosphere. The reaction mixtures were stirred at room temperature for 1 h. Reaction was monitored by ^{31}P NMR and excess borane was quenched by adding a few drops of methanol upon completion. Reaction mixtures were evaporated to dryness and left underhigh vacuum for 2-3 h. Subsequently, the crude reaction mixtureswere suspended in dichloromethane and triethylamine (3.0 equiv.) was added to themand left to stir overnight at room temperature. Reaction was monitored by phosphorus NMR. Reaction mixtures were evaporated to dryness and used for the next step without further purification.

General method for removal of acetyl groups and separation of diastereomers:

After boronation and removal of the cyanoethyl group, the compounds were dissolved in methanol.An equal volume of 2.0 M ammonia in methanol was added and the reaction mixtures were stirred for 4-5 hours. TLC (8.5:1.0:0.5 chloroform:methanol:triethylamine) showed complete disappearance of starting material. Reaction mixtures were evaporated to dryness. The resulting crude mixtures obtained from **51** to **53**were dissolved in solutions of chloroform:triethylamine (9.5:0.5), loaded onto silica columns and the products were purified using flash column chromatography. Silica gel slurries were prepared with the starting eluant mixture containing an additional 5% triethylamine. After pouring the silica gel slurries into columns, they were each washed with two column volumes of the starting solvent mixture. Each diastereomers(**55** to **60)**were purified using a gradient of 9.5:0.5; $CHCl_3$:Et_3N to 8.5:1.0:0.5; $CHCl_3$:CH_3OH:Et_3N and isolated as white foams. Compounds **61** and **62** were purified using a gradient of $CHCl_3$ to 9.5:0.5; $CHCl_3$:Et_3N. The compounds were isolated from 47% to 60% overall yield after three steps.These diastereomers were labeled as the "fast" and "slow" isomers respectively on basis of their mobility on the column and used further.

Compound 55: ^{31}P NMR (CD_2Cl_2) δ: 95.14. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 7.59 (2H, d, ^{5}H-6, ^{3}H-6), 7.46 (2H, d, DMT), 7.34 (7H, m, DMT), 7.28 (1H, m, DMT), 6.89 (3H, m, DMT), 6.39-6.43 (1H, t, *J*= 4.0 Hz, ^{5}H-1′), 6.32-6.35 (1H, t, *J*= 4 Hz, ^{3}H-1′), 5.12 (1H, br s, ^{5}H-3′), 4.45 (1H, br s, ^{3}H-3′), 4.30 (1H, m, ^{3}H-4′), 4.03 (3H, m, ^{5}H-4′, ^{3}H-5′), 3.81 (6H, s, DMT), 3.41 (2H, m, ^{5}H-5′), 2.78-2.80 (6H, m, CH_2-Et_3N), 2.57 (1H, m, ^{5}H-2′), 2.36-2.41 (1H, m, ^{3}H-2′), 2.27-2.30 (1H, m, ^{5}H-2′), 2.15-2.20 (1H, m, ^{3}H-2′), 1.94 (3H, s, $^{3}CH_3$C-5), 1.48 (3H, s, $^{5}CH_3$C-5), 1.14-1.18 (9H, m, CH_3-Et_3N), 0.40-0.45 (3H, d, BH_3). ^{13}C NMR (CD_2Cl_2) δ: 9.90, 11.52, 11.60, 12.25, 12.33, 22.38, 39.88, 39.97, 45.89, 53.17, 53.44, 55.25, 62.59, 62.64, 63.97, 71.66, 74.43, 74.46, 84.52, 84.76, 85.42, 85.46, 85.70, 85.77, 86.93, 110.98, 111.20, 113.22, 127.02, 127.97, 128.08, 130.08, 130.11,135.33, 135.52, 135.58, 136.02, 144.59, 150.72, 150.93, 158.77, 158.78, 164.08, 164.21. ESI-MS (m/z): 948.3 $(M+H+Et_3NH)^+$.

Compound 56: ^{31}P NMR (CD_2Cl_2) δ: 95.58. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 7.64 (1H, s, ^{5}H-6), 7.58 (1H, s, ^{3}H-6), 7.45-7.47 (2H, d, DMT), 7.35 (7H, m, DMT), 7.25-7.29 (1H, m, DMT), 6.88 (3H, m, DMT), 6.38-6.42 (1H, dd, *J*= 8.0, 4.0 Hz, ^{5}H-1′), 6.32-6.35 (1H, t, *J*= 8.0 Hz, ^{3}H-1′), 5.13 (1H, br s, ^{5}H-3′), 4.53 (1H, br s, ^{3}H-3′), 4.29 (1H, m, ^{3}H-4′), 4.00 (3H, m, ^{3}H-4′, ^{3}H-5′), 3.81 (6H, s, DMT), 3.36-3.48 (2H, m, ^{5}H-5′), 2.80-2.85 (6H, m, CH_2-Et_3N), 2.53-2.57 (1H, m, ^{5}H-2′), 2.34-2.41 (2H, m, ^{3}H-2′, ^{5}H-2′), 2.21-2.27 (1H, m, ^{3}H-2′), 1.95 (3H, s, $^{3}CH_3$C-5), 1.44 (3H, s, $^{5}CH_3$C-5), 1.16-1.20 (9H, m, CH_3-Et_3N), 0.40-0.45 (3H, d, BH_3). ^{13}C NMR (CD_2Cl_2) δ: 9.66, 11.52, 11.60, 12.26, 12.23, 23.22, 39.53, 39.73, 40.07, 45.81, 52.89, 53.17, 55.24, 70.78, 71.65, 74.99, 84.32, 84.74, 85.60, 85.66, 85.81, 85.85, 86.97, 110.55, 110.95, 110.97, 111.18, 113.22, 127.02, 127.97, 128.10, 130.08, 130.10, 135.32, 135.52, 135.60, 136.06, 144.57, 150.71, 150.88, 158.79, 164.06, 164.19. ESI-MS (m/z): 948.3 $(M+H+Et_3NH)^+$.

Compound 57: ^{31}P NMR (CD_2Cl_2) δ: 95.33. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 7.74-7.76 (1H, s, ^{5}H-6), 7.61 (1H, m, ,^{3}H-6), 7.45-7.47 (2H, m, DMT), 7.32-7.36 (7H, m, DMT), 6.86-6.89 (4H, m, DMT), 6.27-6.36 (2H, m, ^{5}H-1′, ^{3}H-1′), 5.02-5.06 (1H, bs, ^{5}H-3′), 4.50-4.54 (1H, m, ^{5}H-4′), 4.31 (1H, bs, ^{3}H-3′), 4.21-4.27 (1H, m, ^{3}H-4′), 4.00-4.06 (1H, m, ^{5}H-5′), 3.96-3.97 (1H, m, ^{5}H-5′), 3.81 (6H, s, DMT), 3.36-3.45 (2H, m, ^{3}H-5′), 2.78-2.83 (6H, m, CH_2-Et_3N), 2.67-2.72 (1H, m, ^{5}H-4′), 2.33-2.39 (1H, m, ^{5}H-2′), 2.16-2.27 (2H, m, ^{5}H-2′, ^{3}H-2′), 2.05-2.08 (1H, m, ^{3}H-2′), 1.94 (3H, s, $^{3}CH_3$C-5), 1.16-1.20 (26H, m, BIBS, CH_3-Et_3N), 1.13 (3H, m, BIBS), 0.99-1.00 (7H, m, BIBS), 0.42-0.46 (3H, d, BH_3). ^{13}C NMR (CD_2Cl_2) δ: 9.87, 12.32, 20.66, 20.95, 24.77, 26.09, 28.57, 39.62, 40.36, 45.95, 55.20, 61.16, 61.22, 63.72, 70.25, 74.90, 84.32, 85.62, 85.72, 86.22, 86.73, 96.46, 110.71, 113.13, 126.90, 127.89, 128.12, 130.09, 135.40, 135.57, 140.62, 144.62, 150.33, 155.37, 158.69, 163.77, 168.23. ESI-MS (m/z): 1131.5 $(M+Et_3NH)^+$.

Compound 58: ^{31}P NMR (CD_2Cl_2) δ: 94.87. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 7.74-7.76 (1H, s, ^{5}H-6), 7.53 (1H, m, ^{3}H-6), 7.44-7.46 (2H, m, DMT), 7.31-7.35 (7H, m, DMT), 6.86-6.89 (4H, m, DMT), 6.31-6.35 (2H, m, ^{5}H-1′, ^{3}H-1′), 5.06 (1H, bs, ^{5}H-3′), 4.51-4.54 (1H, m, ^{5}H-4′), 4.43-4.46 (1H, m, ^{3}H-3′), 4.31-4.32 (1H, m, ^{3}H-4′), 3.99-4.06 (2H, m, ^{5}H-5′), 3.81 (6H, s, DMT), 3.39-3.40 (2H, m, ^{3}H-5′), 2.90-2.96 (6H, m, CH_2-Et_3N), 2.73-2.79 (1H, m, ^{5}H-4′), 2.27-2.33 (1H, m, ^{5}H-2′), 2.12-2.17 (2H, m, ^{5}H-2′, ^{3}H-2′),

2.03-2.08 (1H, m, ^{3}H-2′), 1.94 (3H, s, $^{3}CH_3$C-5), 1.24-1.28 (26H, m, BIBS, CH_3-Et_3N), 1.13 (3H, m, BIBS), 0.99-1.00 (7H, m, BIBS), 0.42-0.45 (3H, d, BH_3). ^{13}C NMR (CD_2Cl_2) δ: 9.17, 12.27, 20.65, 20.96, 24.77, 26.09, 28.57, 39.90, 40.79, 45.80, 55.22, 62.86, 62.91, 71.37, 74.08, 74.12, 84.57, 85.27, 85.32, 85.99, 86.05, 86.27, 86.68, 96.49, 110.84, 113.16, 126.90, 127.89, 128.08, 130.06, 135.41, 135.54, 135.79, 140.52, 144.66, 150.34, 155.37, 158.69, 163.70, 168.26.ESI-MS (m/z): 1131.5 $(M+Et_3NH)^+$.

Compound 59: ^{31}P NMR (CD_2Cl_2) δ: 95.77. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 8.29 (1H, s, ^{5}H-8), 7.96 (1H, s, ^{5}H-2), 7.61 (1H, s, ^{3}H-6), 7.44-7.46 (3H, m, DMT), 7.34-7.36 (4H, m, DMT), 7.23-7.30 (3H, m, DMT), 6.83-6.86 (3H, m, DMT), 6.47-6.50 (1H, t, *J*= 4.0, ^{5}H-1′), 6.28-6.31 (1H, t, *J*= 4.0, ^{3}H-1′), 5.40 (1H, bs, NHBIBS), 5.12-5.14 (1H, m, ^{5}H-3′), 4.53-4.47 (1H, m, ^{5}H-4′), 4.41 (1H, bs, ^{3}H-3′), 4.23-4.28 (1H, m, ^{3}H-4′), 4.00-4.06 (1H, m, ^{5}H-5′), 3.95-3.96 (1H, m, ^{5}H-5′), 3.81 (6H, s, DMT), 3.39-3.40 (2H, m, ^{3}H-5′), 2.85-2.91 (6H, m, CH_2-Et_3N), 2.68-2.72 (1H, m, ^{5}H-2′), 2.23-2.39 (2H, m, ^{5}H-2′, ^{3}H-2′), 2.10-2.17 (1H, m, ^{3}H-2′), 1.93 (3H, s, $^{3}CH_3$C-5), 1.18-1.24 (26H, m, BIBS, CH_3-Et_3N), 1.07-1.09 (3H, m, BIBS), 0.99-1.00 (7H, m, BIBS), 0.41-0.46 (3H, d, BH_3). ^{13}C NMR (CD_2Cl_2) δ: 9.41, 12.29, 20.78, 24.78, 26.22, 28.59, 39.35, 39.84, 45.88, 55.18, 61.55, 64.08, 70.47, 74.68, 84.26, 84.36, 85.41, 85.77, 85.82, 86.45, 110.78, 113.08, 122.07, 126.77, 127.78, 128.09, 130.12, 135.63, 135.90, 138.27, 144.79, 149.18, 150.33, 152.36, 158.16, 158.64, 163.70. ESI-MS (m/z): 1155.5 $(M+Et_3NH+H)^+$.

Compound 60: ^{31}P NMR (CD_2Cl_2) δ: 94.72. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 8.29 (1H, s, ^{5}H-8), 7.96 (1H, s, ^{5}H-2), 7.59 (1H, s, ^{3}H-6), 7.44-7.46 (3H, m, DMT), 7.33-7.36 (4H, m, DMT), 7.23-7.30 (3H, m, DMT), 6.83-6.86 (3H, m, DMT), 6.47-6.50 (1H, t, *J*= 4.0, ^{5}H-1′), 6.29-6.33 (1H, t, *J*= 4.0, ^{3}H-1′), 5.39 (1H, bs, NHBIBS), 5.09-5.12 (1H, m, ^{5}H-3′), 4.49-4.52 (1H, m, ^{5}H-4′), 4.40 (1H, bs, ^{3}H-3′), 4.04-4.07 (2H, m, ^{3}H-4′, ^{5}H-5′), 3.99-4.01 (1H, m, ^{5}H-5′), 3.81 (6H, s, DMT), 3.39-3.40 (2H, m, ^{3}H-5′), 2.84-2.88 (6H, m, CH_2-Et_3N), 2.69-2.73 (1H, m, ^{5}H-2′), 2.10-2.32 (3H, m, ^{5}H-2′, ^{3}H-2′), 1.95 (3H, s, $^{3}CH_3$C-5), 1.18-1.24 (26H, m, BIBS, CH_3-Et_3N), 1.07-1.09 (3H, m, BIBS), 0.99-1.02 (7H, m, BIBS), 0.41-0.46 (3H, d, BH_3). ^{13}C NMR (CD_2Cl_2) δ: 9.54, 12.24, 20.78, 21.01, 24.77, 26.17, 26.22, 28.59, 39.54, 40.14, 45.88, 55.19, 62.20, 62.25, 64.13, 71.30, 84.20, 84.41, 85.53, 86.45, 110.86, 113.09, 126.77, 127.78, 128.07, 130.13, 135.62, 135.87,

138.32, 144.83, 149.20, 150.28, 152.37, 158.17, 158.65, 163.54. ESI-MS (m/z): 1155.5 $(M+Et_3NH+H)^+$.

Compound 61: ^{31}P NMR ($CDCl_3$) δ: 96.07, 95.12. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 (1H, s, G H-8), 7.61 (1H, s, T N-3), 7.43 (2H, m, DMT), 7.33 (4H, m, DMT), 7.25 (2H, m, DMT), 7.20 (1H, m, DMT), 6.81 (4H, m, DMT), 6.37 (1H, t, ^{5}H-1′), 6.30 (1H, t, ^{3}H-1′), 4.99-5.03 (1H, m, ^{5}H-3′), 4.45-4.49 (2H, m, ^{5}H-4′, ^{3}H-4′), 4.30-4.32 (1H, q, ^{3}H-3′), 4.24-4.30 (1H, t, ^{3}H-5′), 3.93−3.96 (1H, m, ^{3}H-5′), 3.88−3.90 (1H, m, ^{5}H-5′), 3.78 (6H, s, DMT), 3.37 (2H, d, ^{3}H-5′, ^{5}H-2′), 2.76-2.82 (6H, m, CH_2-Et_3N), 2.57−2.62 (1H, dq, ^{5}H-2′), 2.47−2.54 (1H, m, ^{3}H-2′), 2.32−2.38 (1H, m, ^{3}H-2′), 2.13−2.23 (2H, m), 1.95 (6H, s, T-Me), 1.15 (18H, s, BIBS), 1.11 (27H, s, BIBS, CH_3-Et_3N), 1.02 (2H, d, BIBS), 0.96−0.91 (14H, m, BIBS).^{13}C NMR ($CDCl_3$) δ:7.79, 12.62, 14.85, 20.73, 20.91, 21.05, 21.50, 22.06, 24.70, 24.85, 26.51, 26.56, 26.59, 28.30, 28.34, 28.79, 28.95, 39.68, 40.64, 52.83, 55.21, 60.73, 63.84, 70.04, 74.00, 83.79, 84.07, 85.25, 86.44, 111.18, 111.19, 117.12, 126.79, 127.85, 128.14, 130.11, 130.15, 135.62, 135.71, 135.87, 136.48, 144.55, 150.38, 154.08, 158.44, 158.46, 159.30, 160.06, 163.85. ESI-MS (m/z): 1369.7 $(M+H+Et_3NH)^+$.

Compound 62:^{31}P NMR ($CDCl_3$) δ: 95.35, 94.31. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (1H, s, G H-8), 7.55 (1H, s, T N-3), 7.41 (2H, m, DMT), 7.30 (4H, m, DMT), 7.23 (2H, m, DMT), 7.15 (1H, m, DMT), 6.79 (4H, m, DMT), 6.31-6.37 (2H, m, ^{5}H-1′, ^{3}H-1′), 4.92-4.96(1H, m, ^{5}H-3′), 4.47 (1H, s, NH), 4.34-4.37 (1H, m, ^{3}H-4′), 4.29-4.32 (1H, m, ^{5}H-4′), 3.91-3.96(3H, m, ^{3}H-3′, ^{3}H-5′), 3.75 (6H, s, DMT), 3.51−3.57 (1H, q, ^{5}H-5′), 3.29−3.38 (1H, m, ^{3}H-2′), 2.82-2.87 (6H, m, CH_2-Et_3N),2.61−2.66 (1H, dq, ^{5}H-5′), 2.49−2.56 (1H, m, ^{3}H-2′), 2.20−2.26 (1H, m, ^{5}H-2′), 2.10−2.18 (1H, m, ^{5}H-2′), 1.92 (3H, d, T-Me), 1.13 (18H, m, BIBS,), 1.09 (27H, m, BIBS, CH_3-Et_3N), 1.00 (2H, d, BIBS), 0.93 (14H, m, BIBS).^{13}C NMR ($CDCl_3$) δ: 7.97, 8.00, 12.51, 14.82, 20.67, 20.89, 20.90, 21.48, 22.03, 24.67, 24.84, 26.47, 26.49, 26.55, 26.56, 28.26, 28.32, 28.42, 28.93, 29.67, 34.41, 40.11, 40.78, 52.91, 55.21, 62.13, 63.19, 63.90, 71.32, 73.60, 83.58, 84.19, 84.89, 84.93, 85.25, 85.31, 86.42, 111.05, 113.19, 117.07, 126.79, 127.86, 128.10, 130.08, 130.14, 135.60, 135. 66, 135.99, 136.55, 144.58, 150.47, 154.18, 158.43, 158.45, 159.27, 160.62, 163.91.ESI-MS (m/z): 1369.7 $(M+H+Et_3NH)+$.

General method for the synthesis of 3'O-methyl phosphoramidites:

Compounds **55** to **62** were added separately to round bottom flasks flushed with argon. Anhydrous dichloromethane and methyl N,N,N′,N′-tetraisopropylphosphorodiamidite (1.2 equivalents) were added via syringe. Subsequently 1.0 equivalent of ethylthiotetrazole (0.25 M in CH_3CN obtained from Glen Research) was added dropwise to each solution over one-half hour while stirring. The reactions were stirred at room temperature for 2-3 hours at which time TLC showed complete disappearance of starting materials. The reaction mixture were evaporated to dryness and purified by flash chromatography on a silica column. Silica gel slurries were prepared in the starting eluant mixture containing an additional 5% triethylamine. After pouring the silica gel slurries into columns, they were washed with two column volumes of the starting solvent mixture. Compounds (**63** to **68**) were purified using a gradient of 9.5:0.5; $CHCl_3$:Et_3N to 9.0:0.5:0.5; $CHCl_3$:CH_3OH:Et_3N whereas compound **69** and **70** were purified using a gradient starting from $CHCl_3$ to 9.5:0.5; $CHCl_3$:Et_3N. All the compounds were isolated as white foams.

Compound 63:81% yield. ^{31}P NMR (CD_2Cl_2) δ: 149.26, 148.93, 94.97. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 7.71 (1H, s, ^{5}H-6), 7.61 (1H, s, ^{3}H-6), 7.45-7.47 (2H, m DMT), 7.32-7.36 (7H, m, DMT), 7.25-7.29 (1H, m, DMT), 6.86-6.89 (3H, dd, DMT), 6.34-6.43 (2H, m, ^{3}H-1′, ^{5}H-1′), 5.19 (1H, br s, ^{5}H-3′), 4.50 (1H, br s, ^{3}H-3′), 4.27 (1H, br s, ^{3}H-4′), 4.15-4.19 (1H, m, ^{5}H-4′), 3.96-4.06 (2H, m, ^{3}H-5′), 3.81 (6H, m, DMT), 3.57-3.63 (2H, m, ^{5}H-5′), 3.44 (1H, m, HN^{i}Pr$_2$), 3.39 (1H, m, HN^{i}Pr$_2$), 3.32-3.36 (3H, dd, *J*= 13.0, 2.5 Hz, OMe), 2.78-2.84 (6H, m, CH_2-Et_3N), 2.54-2.58 (1H, m, ^{5}H-2′), 2.26-2.43 (2H, m, ^{3}H-2′, ^{5}H-2′), 2.09-2.16 (1H, m, ^{3}H-2′), 1.96 (3H, s, $^{3}CH_3$C-5), 1.43 (3H, s, $^{3}CH_3$C-5), 1.12-1.20 (21H, m, CH_3-Et_3N, N^{i}Pr$_2$). ^{13}C NMR (CD_2Cl_2) δ: 9.79, 11.51, 12.19, 24.27, 24.32, 24.35, 24.39, 24.42, 39.45, 40.04, 42.91, 42.93, 43.04, 43.05, 45.87, 50.09, 50.26, 53.16, 53.36, 55.21, 62.65, 63.93, 73.96, 74.13, 74.33, 84.67, 84.70, 85.34, 85.47, 85.51, 85.56, 86.94, 110.92, 110.97, 113.21,113.23, 127.01, 127.97, 128.08, 130.06, 130.08,

135.29, 135.56, 135.60, 136.11, 136.15, 144.56, 150.49, 158.77, 158.78, 163.88, 163.97, 163.99. ESI-MS (m/z): 1008.3 $(M+2H)^+$,1109.4 $(M+H+Et_3NH)^+$.

Compound 64:78% yield. ^{31}P NMR (CD_2Cl_2) δ: 149.23, 149.03, 93.74. ^1H NMR (CD_2Cl_2, 400 MHz) δ: 7.76 (1H, s, ^{5}H-6), 7.57 (1H, s, ^{3}H-6), 7.45-7.47 (2H, m DMT), 7.32-7.35 (7H, m, DMT), 7.26-7.29 (1H, m, DMT), 6.86-6.89 (3H, dd, DMT), 6.41-6.44 (2H, m, ^{3}H-1′, ^{5}H-1′), 5.12 (1H, br s, ^{5}H-3′), 4.64-4.70 (1H, m, ^{3}H-3′), 4.28 (1H, br s, ^{3}H-4′), 4.23-4.26 (1H, m, ^{5}H-4′), 4.19-4.22 (1H, m, ^{3}H-5′), 3.91-3.97 (2H, m, ^{3}H-5′), 3.81 (6H, m, DMT), 3.60-3.67 (2H, m, ^{5}H-5′), 3.46-3.48 (1H, m, HN^iPr_2), 3.36-3.40 (4H, m, HN^iPr_2, OMe), 2.7-2.78 (6H, m, CH_2-Et_3N), 2.47-2.52 (1H, m, ^{5}H-2′), 2.31-2.40 (2H, m, ^{3}H-2′, ^{5}H-2′), 2.20-2.26 (1H, m, ^{3}H-2′), 1.92 (3H, s, $^3CH_3$C-5), 1.40 (3H, s, $^3CH_3$C-5), 1.14-1.23 (21H, m, CH_3-Et_3N, N^iPr_2). ^{13}C NMR (CD_2Cl_2) δ: 10.01, 11.45, 12.24, 24.28, 24.31, 24.35, 24.38, 24.42, 39.37, 39.48, 42.96, 43.08, 43.11,45.92, 50.03, 50.06, 50.19, 50.22, 52.88, 53.15, 53.42, 55.22, 61.91, 63.97, 74.72, 74.78, 74.91, 75.11, 84.60, 84.72, 84.76, 85.67, 85.79, 85.83, 86.95, 110.01, 111.07, 111.09, 113.20, 127.01, 127.95, 128.03, 128.12, 130.08, 130.10, 135.32, 135.55, 135.60, 136.21, 136.25, 144.57, 150.54, 150.58, 150.62, 158.78, 158.79, 163.90, 163.96, 163.97. ESI-MS (m/z): 1008.3 $(M+2H)^+$, 1109.4 $(M+H+Et_3NH)^+$.

Compound 65: 70% yield. ^{31}P NMR (CD_2Cl_2) δ: 149.16, 148.98, 93.38. ^1H NMR (CD_2Cl_2, 400 MHz) δ: 7.73-7.77 (2H, m, ^{5}H-6, ^{3}H-6), 7.44-7.46 (3H, m, DMT), 7.23-7.29 (7H, m, DMT), 6.86-6.88 (3H, m, DMT), 6.34-6.43 (2H, m, ^{3}H-1′, ^{5}H-1′), 5.46-5.49 (1H, m, ^{5}H-5), 5.01 (1H, bs, ^{5}H-3′), 4.66-4.72 (1H, m, ^{3}H-3′), 4.46 (1H, bs, ^{5}H-4′), 4.18-4.32 (3H, m, ^{3}H-5′, ^{3}H-4′), 3.88-3.92 (1H, m, ^{3}H-5′), 3.81 (6H, s, DMT), 3.60-3.67 (2H, m, ^{5}H-5′, HN^iPr_2), 3.39-3.41 (4H, s, HN^iPr_2, -OMe), 3.36-3.37 (1H, m, ^{5}H-5′), 2.86-2.91 (6H, m, CH_2-Et_3N), 2.18-2.38 (2H, m, ^{3}H-2′), 2.03-2.10 (2H, m, ^{5}H-2′), 1.89 (3H, s, $^3CH_3$C-5), 1.19-1.26 (26H, m, BIBS, CH_3-Et_3N), 1.12 (3H, m, BIBS), 0.99-1.00 (7H, m, BIBS). ^{13}C NMR (CD_2Cl_2) δ: 9.32, 12.23, 20.65, 20.96, 24.36, 24.42, 24.77, 26.09, 26.15, 28.58, 39.25, 40.49, 45.83, 50.04, 50.20, 55.18, 63.74, 74.45, 75.04, 75.23, 75.43, 84.82, 85.64, 85.68, 86.11, 86.72, 96.19, 111.01, 113.13, 126.88, 127.87, 128.14, 130.09, 135.42, 135.59, 136.31, 136.35, 140.78, 144.64, 150.39, 155.36, 158.67, 163.64, 168.11. ESI-MS (m/z): 1292.6 $(M+H)^+$.

Compound 66: 79% yield. ^{31}P NMR (CD_2Cl_2) δ: 149.19, 148.90, 94.54. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 7.79-7.80 (1H, d, *J*= 8.0 Hz, ^{5}H-6), 7.72 (1H, s, ^{3}H-6), 7.45-7.47 (3H, m, DMT), 7.31-7.35 (7H, m, DMT), 6.86-6.88 (3H, m, DMT), 6.31-6.38 (2H, m, ^{3}H-1′, ^{5}H-1′), 5.49-5.51 (1H, d, *J*= 8.0 Hz, ^{5}H-5), 5.11 (1H, bs, ^{5}H-3′), 4.49-4.53 (1H, m, ^{3}H-3′), 4.28 (1H, bs, ^{5}H-4′), 4.15-4.20 (1H, m, ^{3}H-4′), 3.97-4.04 (2H, m, ^{3}H-5′), 3.81 (6H, s, DMT), 3.57-3.64 (2H, m, ^{5}H-5′), 3.39-3.45 (2H, m, HN^{i}Pr$_2$), 3.33-3.36 (3H, dd, -OMe), 2.76-2.78 (6H, m, CH_2-Et_3N), 2.20-2.34 (2H, m, ^{3}H-2′), 2.05-2.14 (2H, m, ^{5}H-2′), 1.95 (3H, s, $^{3}CH_3$C-5), 1.14-1.20 (26H, m, BIBS, CH_3-Et_3N), 1.13 (3H, m, BIBS), 0.99-1.00 (7H, m, BIBS). ^{13}C NMR (CD_2Cl_2) δ: 10.04, 12.19, 20.65, 20.96, 24.27-24.39, 26.08, 26.15, 28.58, 39.38, 41.05, 45.93, 50.09, 50.26, 55.17, 62.43, 63.58, 74.23, 74.40, 84.67, 85.29, 86.13, 86.72, 95.07, 110.97, 113.16, 126.88, 127.89, 128.11, 130.08, 135.39, 135.58, 136.10, 140.75, 144.64, 150.32, 155.14, 158.67, 163.61, 168.12. ESI-MS (m/z): 1292.6 $(M+H)^+$.

Compound 67: 83% yield..^{31}P NMR (CD_2Cl_2) δ: 149.19, 148.99, 93.75. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 8.28 (1H, s, ^{5}H-8), 7.93 (1H, s, ^{5}H-2), 7.74 (1H, s, ^{3}H-6), 7.44-7.46 (3H, m, DMT), 7.33-7.35 (4H, m, DMT), 7.23-7.29 (3H, m, DMT), 6.82-6.85 (3H, m, DMT), 6.45-6.48 (1H, t, *J*= 4.0, ^{5}H-1′), 6.36-6.40 (1H, t, *J*= 4.0, ^{3}H-1′), 5.10-5.14 (1H, bs, ^{5}H-3′), 4.63-4.68 (1H, bs, ^{3}H-3′), 4.38-4.40 (1H, m, ^{5}H-4′), 4.23 (1H, m, ^{5}H-4′), 4.18-4.21 (1H, m, ^{3}H-5′), 3.92-3.98 (1H, m, ^{3}H-5′), 3.80 (6H, s, DMT), 3.77 (1H, m, ^{5}H-5′), 3.59-3.67 (3H, m, ^{5}H-5′, HN^{i}Pr$_2$), 3.35-3.39 (3H, d, -OMe), 2.77-2.90 (6H, m, CH_2-Et_3N), 2.64-2.68 (1H, m, ^{3}H-2′), 2.06-2.36 (3H, m, ^{3}H-2′, ^{5}H-2′), 1.92 (3H, s, $^{3}CH_3$C-5), 1.18-1.21 (26H, m, BIBS, CH_3-Et_3N), 1.12 (3H, m, BIBS), 0.99-1.01 (7H, m, BIBS).^{13}C NMR (CD_2Cl_2) δ: 9.61, 9.78, 12.22, 20.78, 21.02, 24.26, 24.34, 24.78, 26.18, 28.60, 39.28, 42.96, 43.10, 45.88, 50.03, 50.20, 55.16, 62.18, 64.15, 74.54, 74.93, 84.28, 85.01, 85.86, 86.40, 111.00, 113.05, 122.10, 126.73, 127.75, 128.11, 130.11, 135.67, 135.84, 136.28, 138.40, 144.82, 149.32, 150.34, 152.37, 158.06, 158.62, 163.56. ESI-MS (m/z): 1316.6 $(M+Et_3NH+H)^+$.

Compound 68:86% yield. ^{31}P NMR (CD_2Cl_2) δ: 149.23, 148.86, 94.71. ^{1}H NMR (CD_2Cl_2, 400 MHz) δ: 8.27 (1H, s, ^{5}H-8), 7.95-7.96 (1H, d,*J*= 4.0 Hz,^{5}H-2), 7.74-7.75 (1H, m, ^{3}H-6), 7.43-7.46 (3H, m, DMT), 7.33-7.35 (4H, m, DMT), 7.22-7.28 (3H, m,

DMT), 6.82-6.85 (3H, m, DMT), 6.45-6.49 (1H, m, ^{5}H-1′), 6.36-6.39 (1H, m, ^{3}H-1′), 5.12-5.16 (1H, bs, ^{5}H-3′), 4.54-4.59 (1H, bs, ^{3}H-3′), 4.36-4.39 (1H, m, ^{5}H-4′), 4.19 (1H, m, ^{5}H-4′), 4.03-4.05 (2H, m, ^{3}H-5′), 3.80 (6H, s, DMT), 3.56-3.65 (2H, m, ^{5}H-5′), 3.39-3.40 (2H, m, HN^{i}Pr$_2$), 3.33-3.37 (3H, d, -OMe), 2.79-2.83 (6H, m, CH_2-Et$_3$N), 2.65-2.71 (1H, m, ^{3}H-2′), 2.28-2.39 (1H, m, ^{3}H-2′), 2.09-2.19 (2H, m, ^{5}H-2′), 1.95 (3H, s, $^{3}CH_3$C-5), 1.18-1.20 (26H, m, BIBS, CH_3-Et$_3$N), 1.06-1.08 (3H, m, BIBS), 0.99-1.02 (7H, m, BIBS).^{13}C NMR (CD_2Cl_2) δ: 9.58, 12.19, 20.78, 21.01, 24.42, 24.79, 26.23, 27.33, 28.60, 39.46, 39.59, 42.92, 43.06, 45.83, 50.10, 50.28, 55.15, 62.84, 63.01, 73.95, 74.25, 84.36, 84.74, 85.68, 86.37, 110.95, 113.06, 122.14, 126.73, 127.76, 128.10, 130.12, 135.67, 135.85, 136.10, 138.52, 144.83, 149.30, 150.31, 152.34, 158.05, 158.59, 163.54. ESI-MS (m/z): 1316.6 (M+Et$_3$NH+H)$^{+}$.

Compound 69: 81% yield. ^{31}P NMR ($CDCl_3$) δ: 149.10, 148.70, 93.90. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79, 7.79 (1H, s, G H-8), 7.77 (1H, m, T N-3), 7.43 (2H, m, DMT), 7.33 (4H, m, DMT), 7.25 (2H, m, DMT), 7.19 (1H, m, DMT), 6.81 (4H, m, DMT), 6.47 (1H, dt, ^{5}H-1′), 6.38 (1H, dt, ^{3}H-1′), 4.99-5.04 (1H, m, ^{3}H-3′), 4.62−4.67 (1H, m, ^{5}H-3′), 4.49 (1H, s, NH), 4.34 (1H, m, ^{3}H-4′), 4.16−4.23 (2H, m, ^{5}H-4′, HN^{i}Pr$_2$), 3.84−3.88 (1H, m, HN^{i}Pr$_2$), 3.78 (6H, s, DMT), 3.55−3.65 (2H, m, ^{3}H-5′), 3.33-3.36 (3H, d, -OMe), 2.70-2.76 (6H, m, CH_2-Et$_3$N), 2.59−2.61 (1H, m, ^{5}H-5′), 2.47−2.53 (1H, m, ^{5}H-5′), 2.28−2.38 (1H, m, ^{3}H-2′), 2.14−2.22 (2H, m, ^{3}H-2′, ^{5}H-2′), 1.99−2.06 (1H, m, ^{5}H-2′), 1.93 (3H, s, T-Me), 1.15 (18H, s, BIBS), 1.14 (14H, s, N^{i}Pr$_2$, BIBS), 1.11 (27H, s, BIBS, Et$_3$N), 1.03 (2H, d, BIBS), 0.95 (14H, m, BIBS).^{13}C NMR ($CDCl_3$) δ:12.47, 14.86, 20.73, 20.89, 20.93, 21.51, 22.06, 24.51, 24.57, 24.59, 24.62, 24.65, 24.70, 24.84, 26.47, 26.51, 26.58, 26.60, 28.30, 28.35, 28.92, 28.94, 34.45, 39.52, 40.64, 42.95, 43.01, 43.08, 43.13, 50.10, 50.26, 55.19, 61.86, 63.88, 73.87, 73.96, 75.21, 75.40, 83.71, 84.7, 84.82, 85.27, 85.66, 86.41, 111.44, 111.47, 113.17, 117.10, 126.78, 127.84, 128.17, 130.10, 130.14, 135.67, 135.74, 136.28, 136.31, 136.51, 144.57, 150.46, 150.51, 154.15, 158.45, 158.46, 159.29, 160.59, 163.74. ESI-MS (m/z): 1530.7 (M+H+Et$_3$NH)$^{+}$.

Compound 70: 75% yield. ^{31}P NMR ($CDCl_3$) δ: 149.15, 148.62, 95.11.^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.82 (1H, s, G H-8), 7.76 (1H, s, T N-3), 7.42 (2H, m, DMT), 7.32 (4H, m, DMT), 7.24 (2H, m, DMT), 7.17 (1H, m, DMT), 6.80 (4H, m, DMT), 6.37−6.44 (2H,

m, ^{5}H-1′, ^{3}H-1′), 4.99−5.03 (1H, m, ^{3}H-3′), 4.49-4.53 (1H, m, ^{5}H-3′), 4.49 (1H, s, NH), 4.30−4.33 (1H, q, ^{3}H-4′), 4.19, 4.15 (1H, s, ^{5}H-4′), 3.95−3.99 (1H, m, ^{3}H-5′), 3.77 (6H, s, DMT), 3.56−3.62 (2H, m, HN^{i}Pr$_2$), 3.29−3.39 (5H, m, ^{3}H-5′, ^{5}H-5′, OMe), 2.84-2.86 (6H, m, -CH$_2$Et$_3$N), 2.58−2.65 (1H, m, ^{5}H-5′), 2.48−2.55 (1H, m, ^{3}H-2′), 2.25−2.37 (1H, m, ^{3}H-2′), 2.17 (1H, m, ^{5}H-2′), 1.98-2.10 (1H, m, ^{5}H-2′), 1.95 (3H, s, T-Me), 1.16 (18H, d, BIBS), 1.15 (14H, s, BIBS, N^{i}Pr$_{2,}$), 1.11 (27H, s, BIBS, Et$_3$N), 1.02 (2H, d, BIBS), 0.93−0.97 (2H, m, BIBS), 0.90−0.92 (14H, m, BIBS).^{13}C NMR ($CDCl_3$) δ: 8.09, 12.43, 20.71, 20.91, 21.51, 22.05, 24.52, 24.57, 24.60, 24.64, 24.67, 24.70, 24.84, 26.50, 26.58, 28.34, 28.93, 29.69, 39.59, 41.03, 42.88, 43.01, 43.05, 50.29, 50.45, 55.18, 63.84, 73.42, 83.66, 84.71, 85.07, 86.42, 111.12, 113.17, 117.07, 126.76, 127.84, 128.18, 130.09, 130.13, 135.68, 135.72, 136.35, 136.64, 144.57, 150.31, 150.33, 154.20, 158.44, 159.28, 160.59, 163.69.ESI-MS (m/z): 1530.7 $(M+H+Et_3NH)^{+}$.

Synthesis of Trimethylphosphite-borane(TMPB):10g (80.5 mmoles) of trimethylphosphite was dissolved in 100 mL of anhydrous CH_2Cl_2. To thismixture 8.5mL (85 mmoles) of borane-dimetylsulfide complex (10-10.2 M in BH_3) was added drop wise over a half hour while stirring. The reaction mixture was allowed to stir for an additional 2 hours. At this time ^{31} P NMR analysis of the crude mixture showed complete disappearance of the starting phosphite. The solvent and excess borane-dimethylsulfidewere removed by concentrating the mixture *in vacuo* to yield the product as a colorless oil (10.1 g, 91% yield). ^{31} P NMR ($CDCl_3$): δ 118.9 (q). ^{1}H {^{11}B} NMR ($CDCl_3$): δ 3.72 (9H, dd, OCH_3), 0.02-0.79 (3H, qd, BH_3). ^{11}B NMR ($CDCl_3$): δ -45.41 (pentet). ^{13}C NMR ($CDCl_3$): δ -53.22 (d).

Solid phase synthesis using 5'-O-dimethoxytrityl dideoxynucleotideboranephosphonate 3'-phosphoramidites.

Synthesis of 2'-deoxyoligonucleotides containing only phosphate linkages was carried out on an ABI 394 Synthesizer. All syntheses were performed at a 0.2 μmol scale using a low volume polystyrene solid support having a 5'-dimethoxytrityl-2'-deoxythymidine joined *via* a succinate linkage to the support. In addition to compounds **63** to **66,** 5'-dimethoxytrityl2'-deoxythymidine3'-O-methyl-N,N-diisopropylphosphoramidite (Glen Research) was used. A standard 0.2 μmole synthesis

cycle with an increased coupling time of 900 s was used for dimerphosphoramidite coupling. Compounds **63** to **66** and commercially obtained 5'-O-dimethoxytrityl-2'-deoxythymidine 3'-O-methyl N,N-diisopropylaminophosphoramidite(Glen Research) were dissolved in anhydrous CH_3CN at concentrations of 0.1 M. Other reagents were: 1. Activator (0.25 M ethylthiotetrazole), 2. Cap A (THF/Pyridine/Ac_2O), 3. Cap B (16% 1-methylimidazole in THF), 4.Oxidizing Solution (1.0 Mtert-butyl hydroperoxide in CH_2Cl_2) and 5. Deblock: 10% TMPB 0.5% solution of trifluoroacetic acid in anhydrous $CHCl_3$. All of these reagents except the TFA solution and the tert-butyl hydroperoxide solutionswere purchased from Glen Research. Thesereagentswere prepared fresh prior to use. Deprotection and cleavage of synthetic, unmodified DNA oligomers was carried out as described for bpDNA synthesis in the methods section.

Step	**Reagent/conditions**	**Time**
Detritylation	10% TMPB + 0.5% TFA in $CHCl_3$	Flow 45 s
Wash	MeOH	Flow 20 s, wait 10 s, flow 10 s.
Condensation	0.1 M phosphoramidite **63** to **66** (in CH_3CN) + Activator* (0.25 M ethylthiotetrazole in CH_3CN).	Wait 900 s
Capping	Cap A* (THF/Pyridine/Ac_2O) + Cap B * (16% 1-methylimidazole in THF)	Flow 10 s, wait 5 s
Wash	Anhydrous CH_3CN	Flow 10s
Oxidation	1.0 M tert-butyl hydroperoxide in CH_2Cl_2	Flow 8 s, wait 15 s
Wash	Anhydrous CH_3CN	Flow 10 s
Wash	Anhydrous CH_2Cl_2	Flow 25 s

Table S1: Synthesis cycle of bpDNAoligomer from bp-dimerphosphoramidites (**63** to **66**). * Standard reagents were purchased from Glen research.

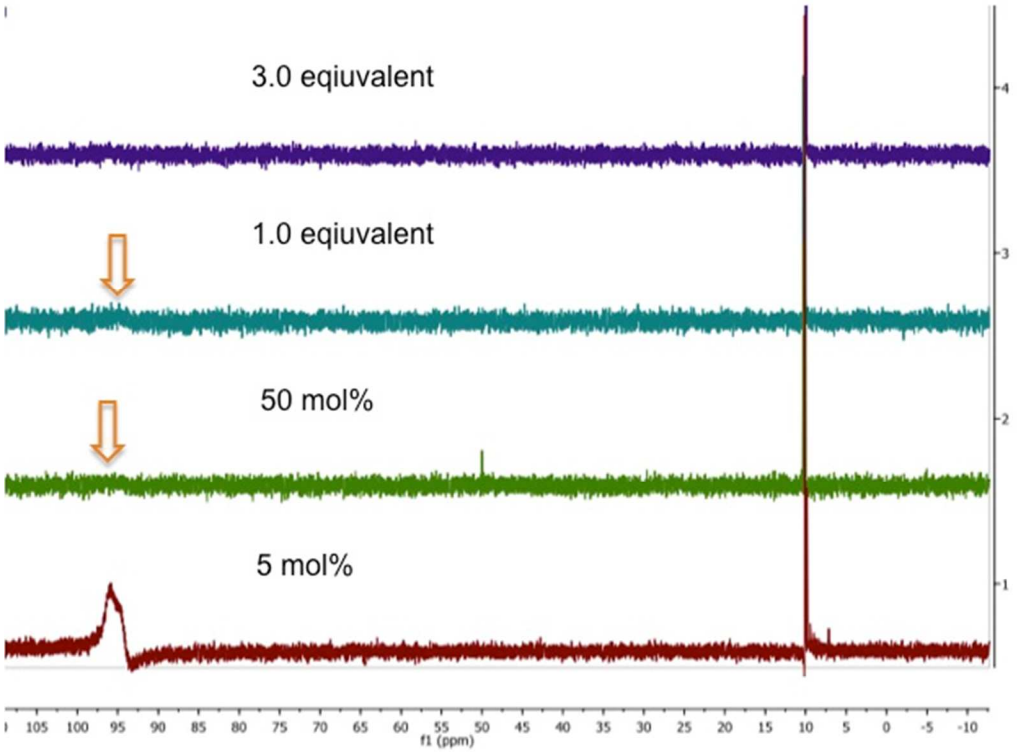


Figure S1. The reaction of **9** with methylamine using different iodine concentrations. The arrow shows the position of the boranephosphonatediester peak of the starting material.

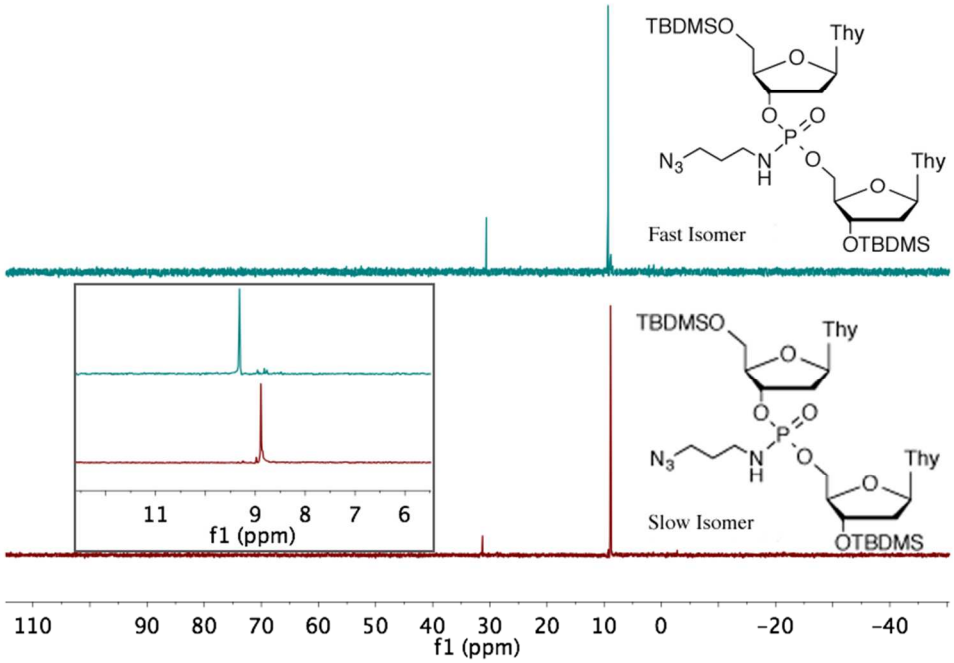


Figure S2. ^{31}P NMR spectra of the crude reaction mixtures,obtained from the reactions of boranephosphonatedimers **9** (top) and **10**(bottom) with 3-azidopropylamineand and iodine,show clean conversion to the phosphoramidate.

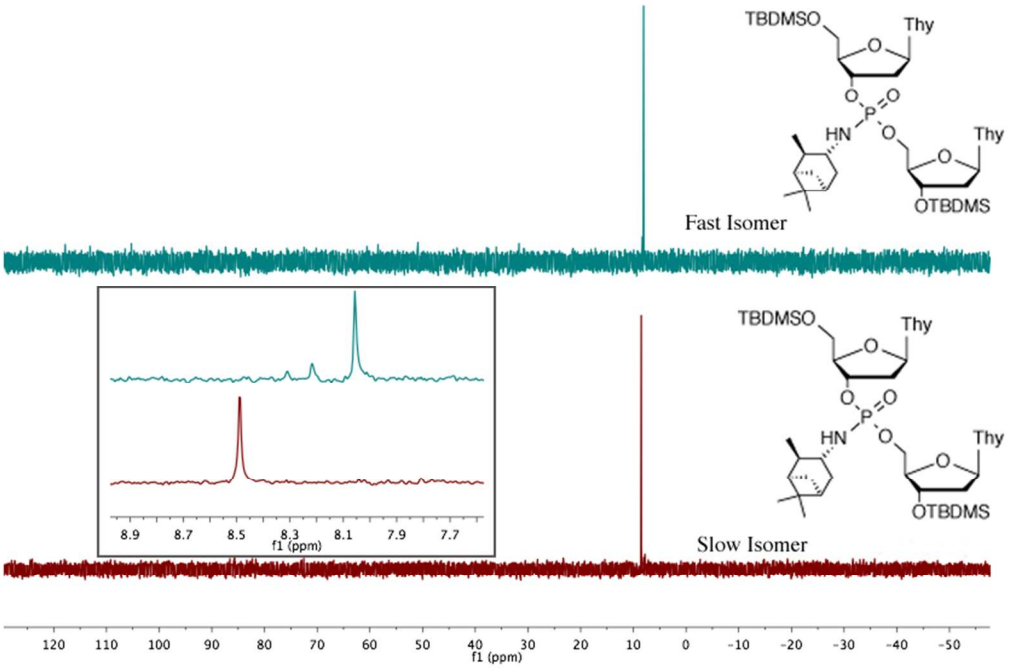


Figure S3. ^{31}P NMR of the crude reaction mixtures, obtained from the reactions of boranephosphonatedimers**9**(top) and **10**(bottom) with the hindered camphenylamineand iodine,show clean conversion to the phosphoramidate.

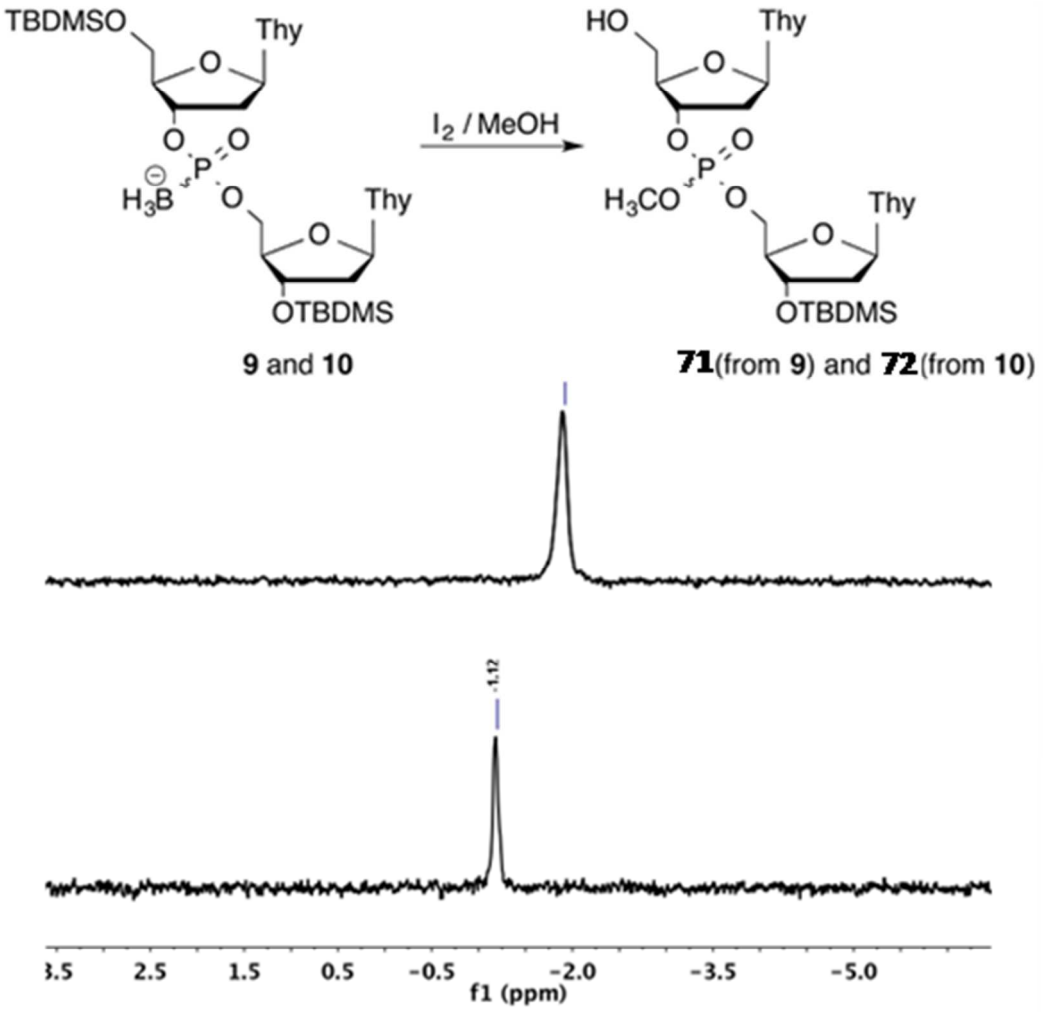


Figure S4. Formation of methlyphosphotriesters **71** and 72 upon reaction of boranephosphonate dimers **9** and **10** respectively with iodine in methanol.

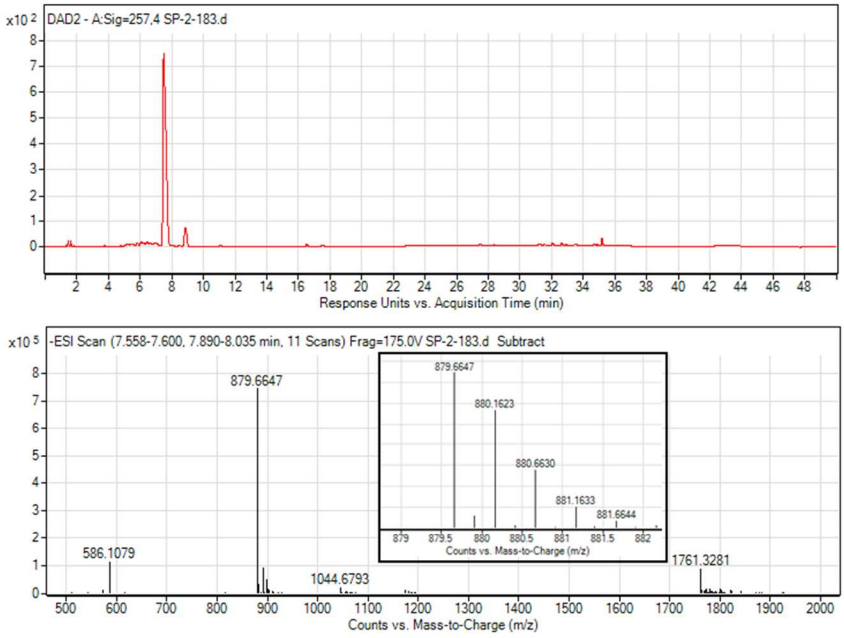


Figure S5. LC-MS analysis of ODN1.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the spectrum corresponds to the -2 charged peak.

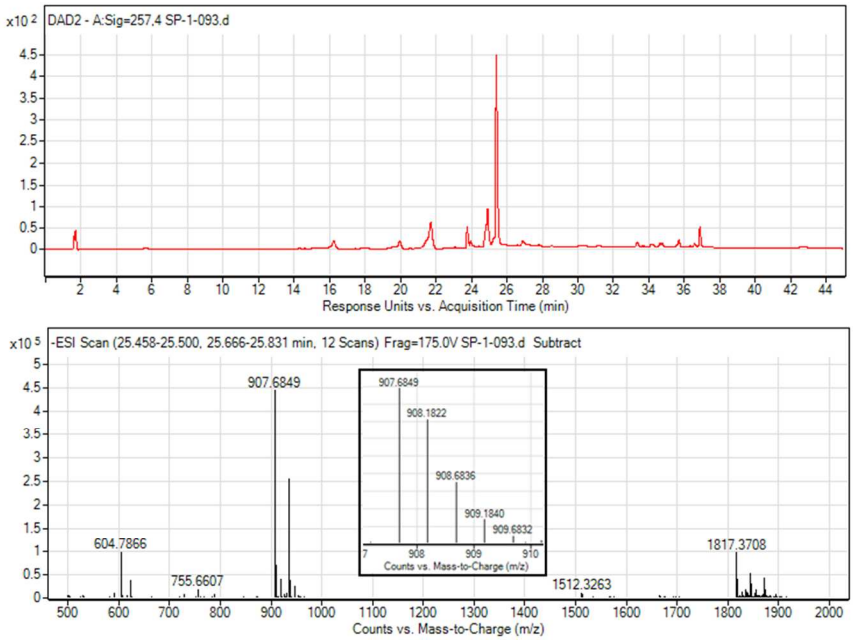


Figure S6. LC-MS analysis of ODN2.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak.

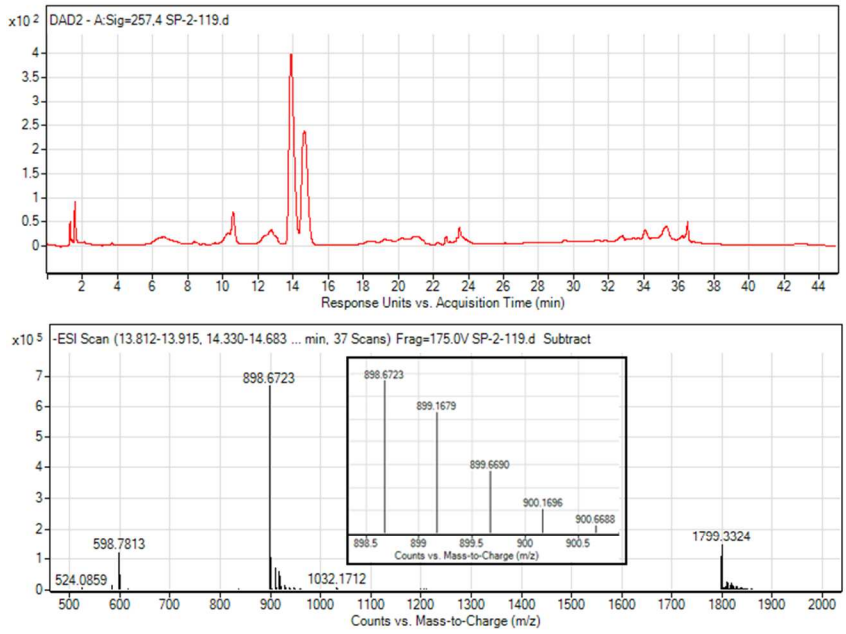


Figure S7. LC-MS analysis of ODN3.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak. The two peaks observed in the LC trace correspond to two diastereomers.

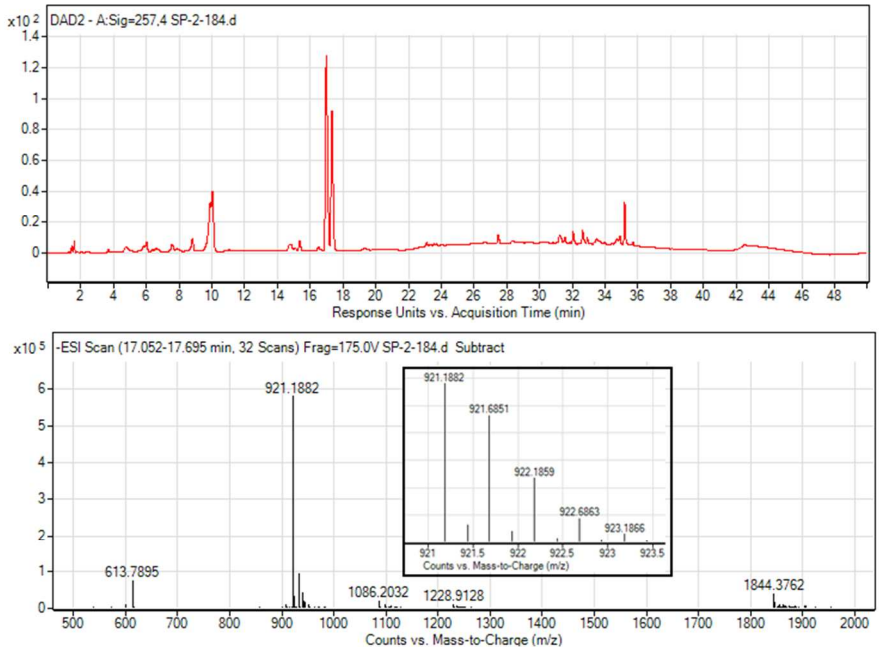


Figure S8. LC-MS analysis of ODN4.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak. The two peaks observed in the LC trace correspond to two diastereomers.

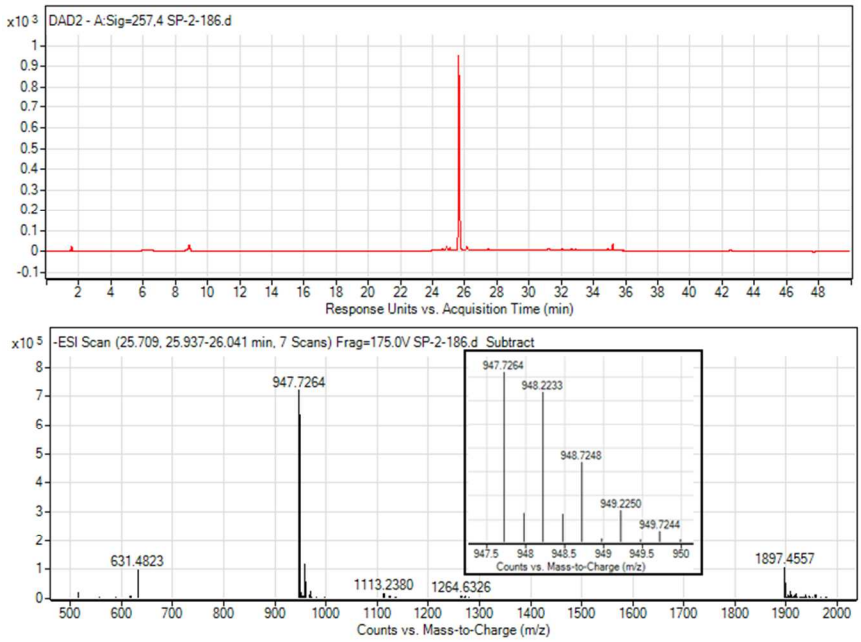


Figure S9. LC-MS analysis of ODN5.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak.

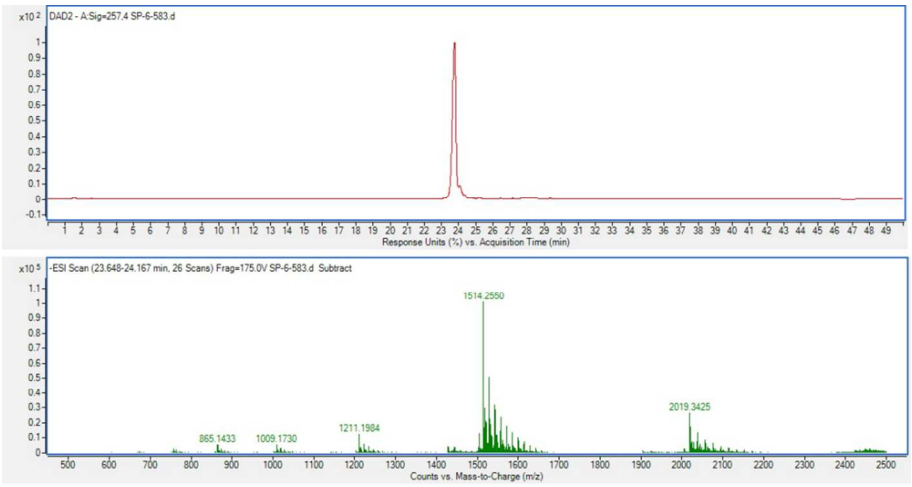


Figure S10. LC-MS analysis of ODN 6.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to -4 charged peak.

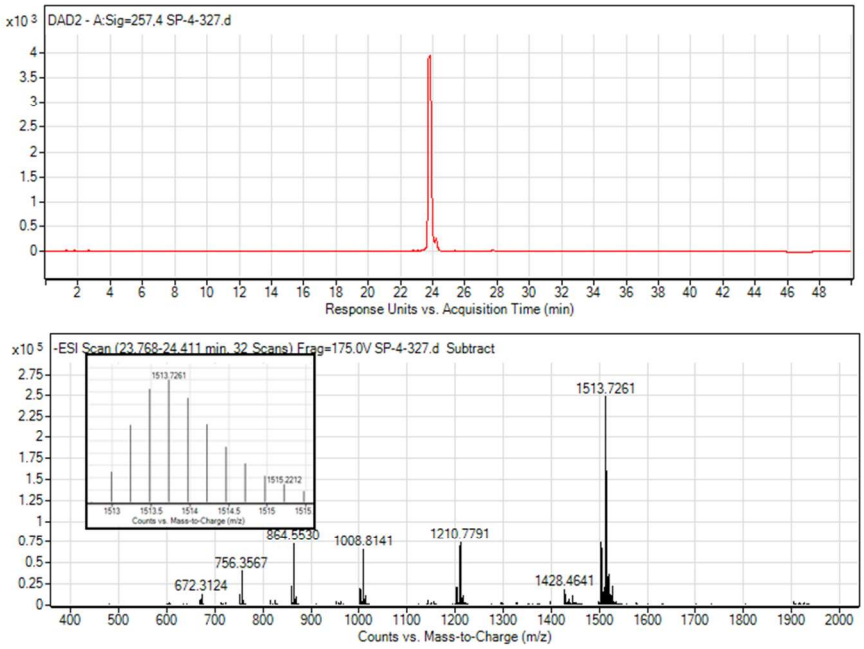


Figure S11. LC-MS analysis of ODN 7.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to -4 charged peak.

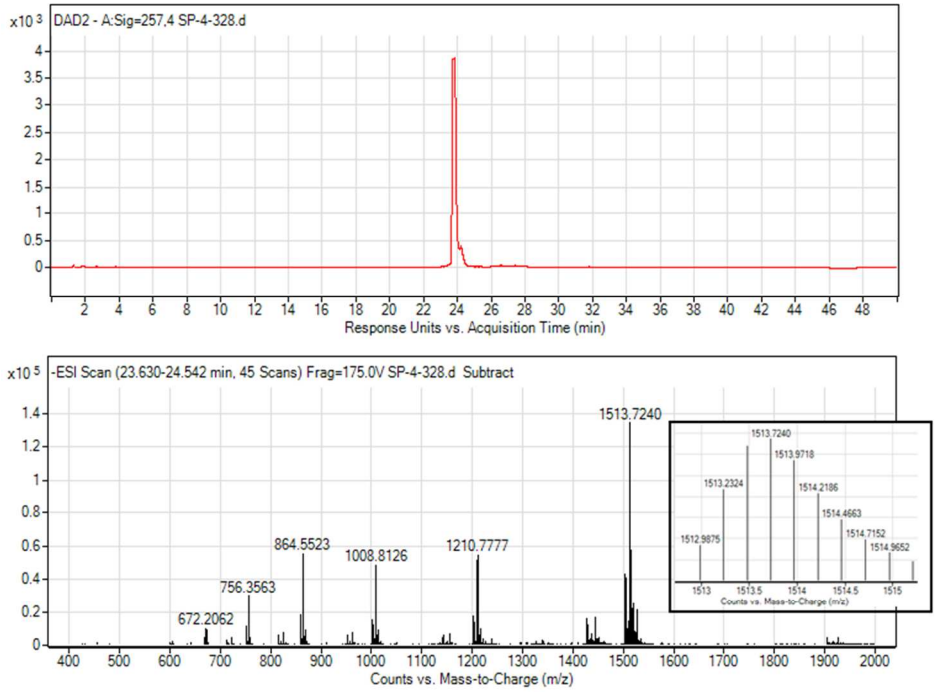


Figure S12. LC-MS analysis of ODN 8.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to -4 charged peak.

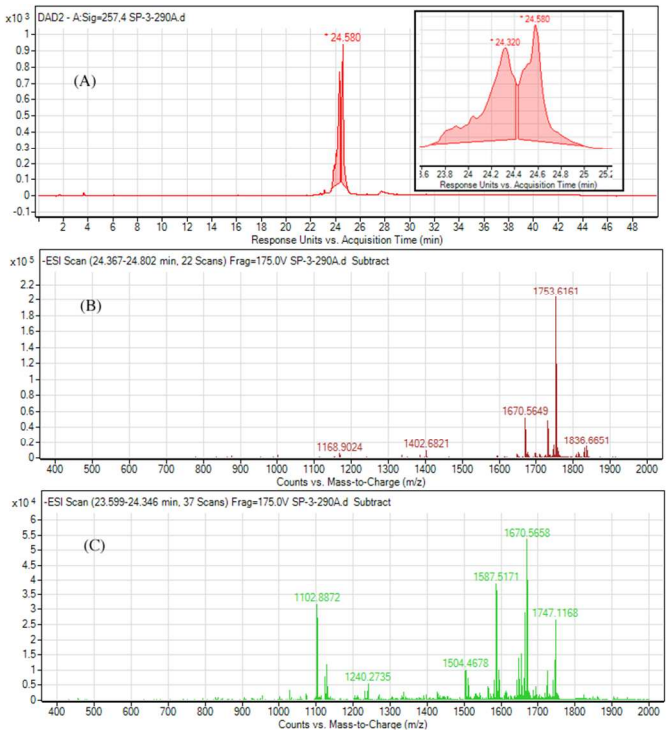


Figure S13. LC-MS analysis of ODN 9.The base peak in both mass spectra corresponds to the -4 charged peak. The mass spectrum in (B) corresponds to integration over the peak eluting at 24.58 min while the spectrum in (C) corresponds to the peak at 24.3 min.

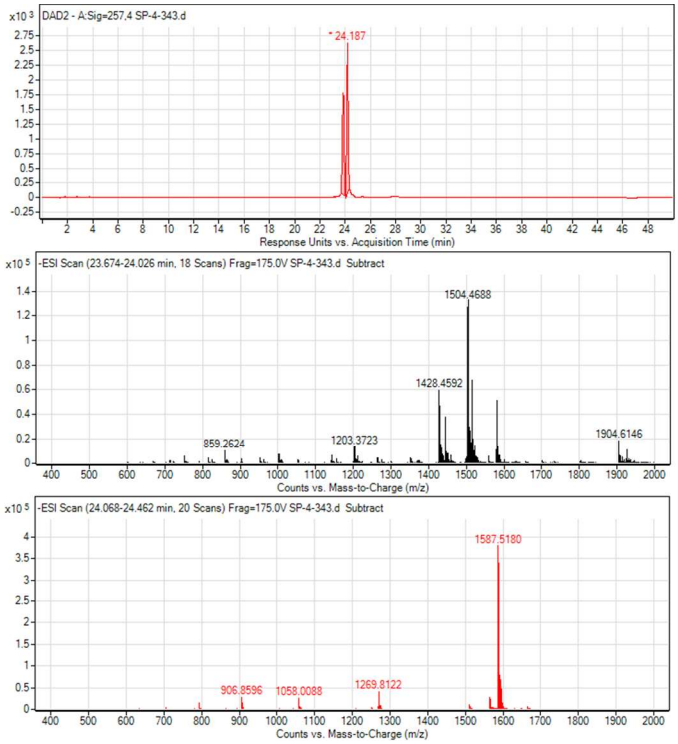


Figure S14. LC-MS analysis of ODN 10. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged

peak. The two mass spectra correspond to the earlier (middle spectra) and later (bottom spectra) eluting compounds.

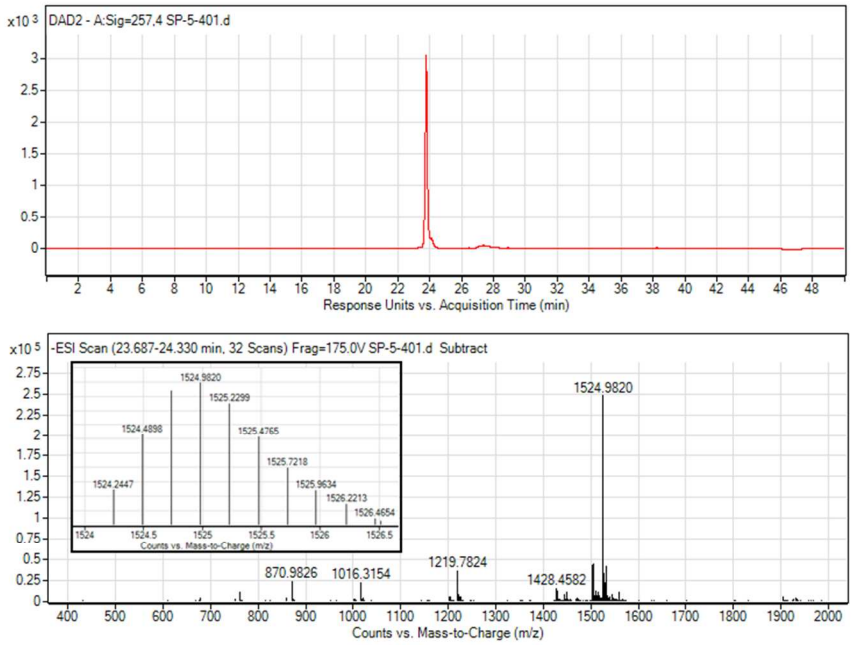


Figure S15. LC-MS analysis of ODN 11.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak.

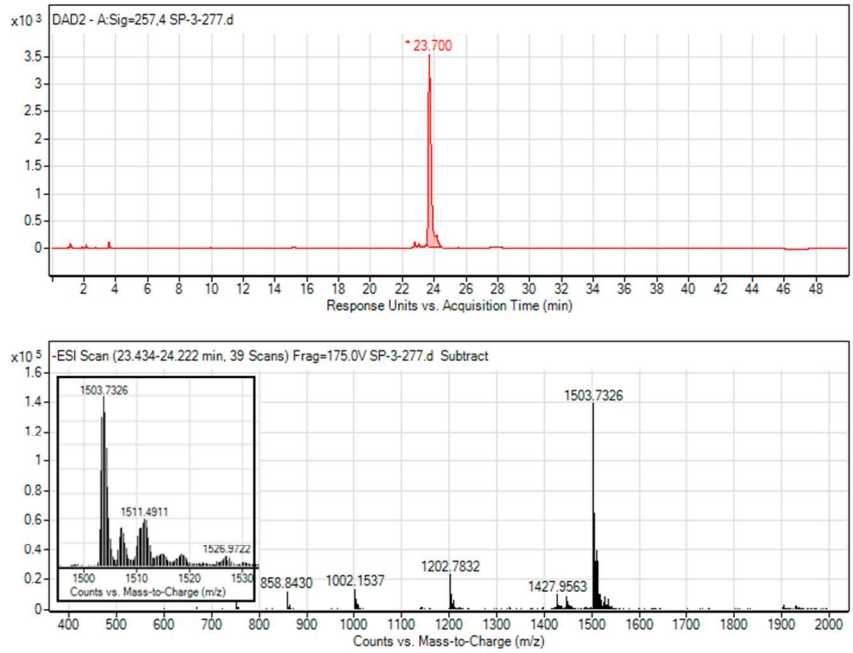


Figure S16. LC-MS analysis of ODN 12.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the-4 charged peak.

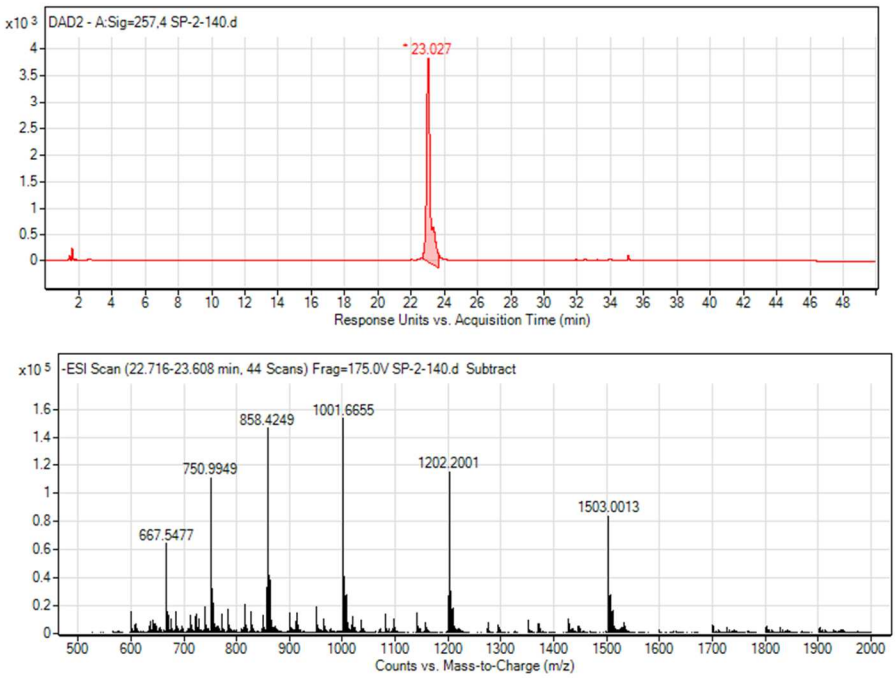


Figure S17. LC-MS analysis of ODN 13.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak.

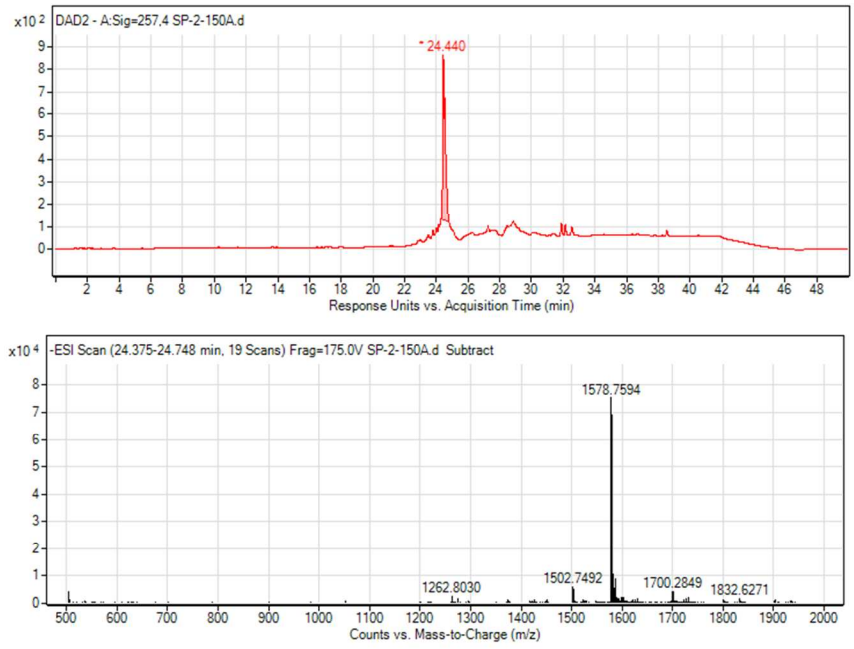


Figure S18. LC-MS analysis of ODN 14.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak.

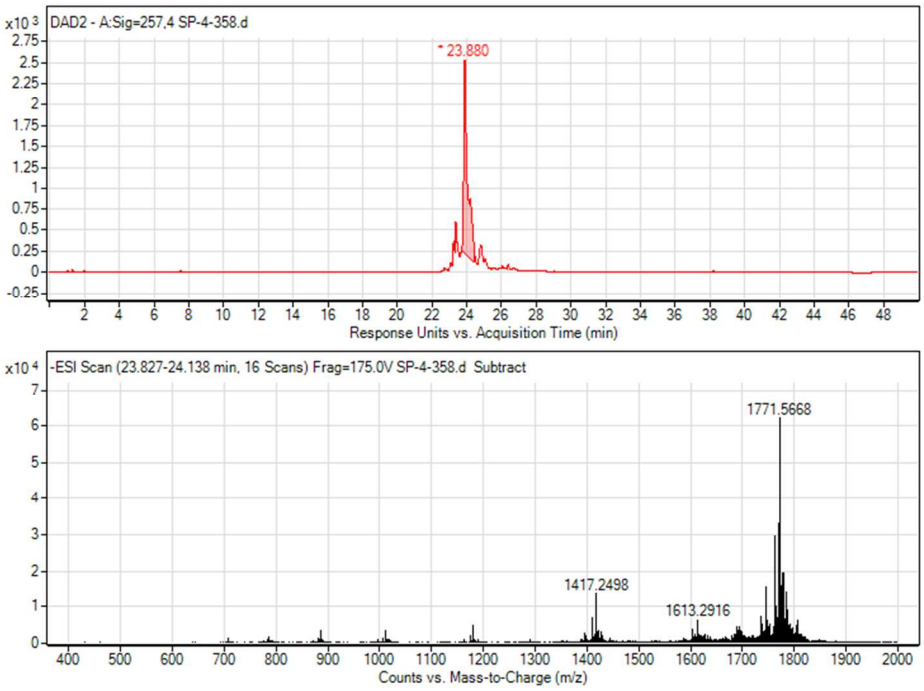


Figure S19. LC-MS analysis of ODN 15.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to -4 charged peak.

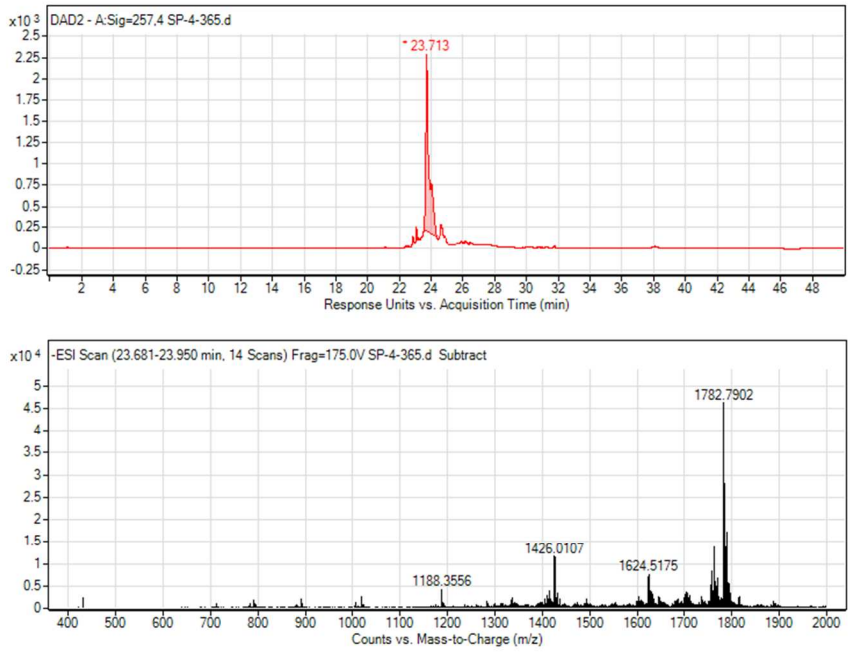


Figure S20. LC-MS analysis of ODN 16.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak.

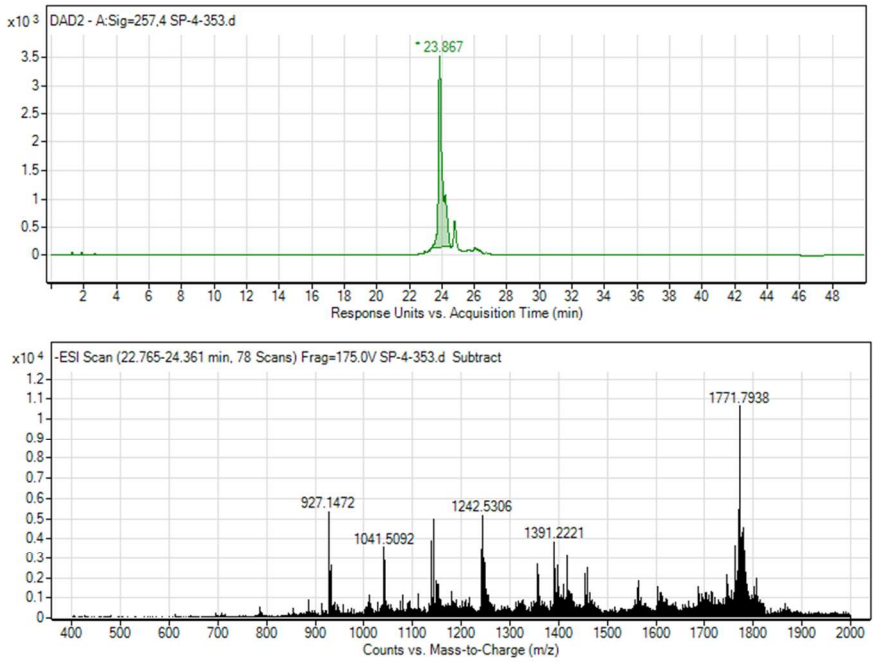


Figure S21. LC-MS analysis of ODN 17.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak.

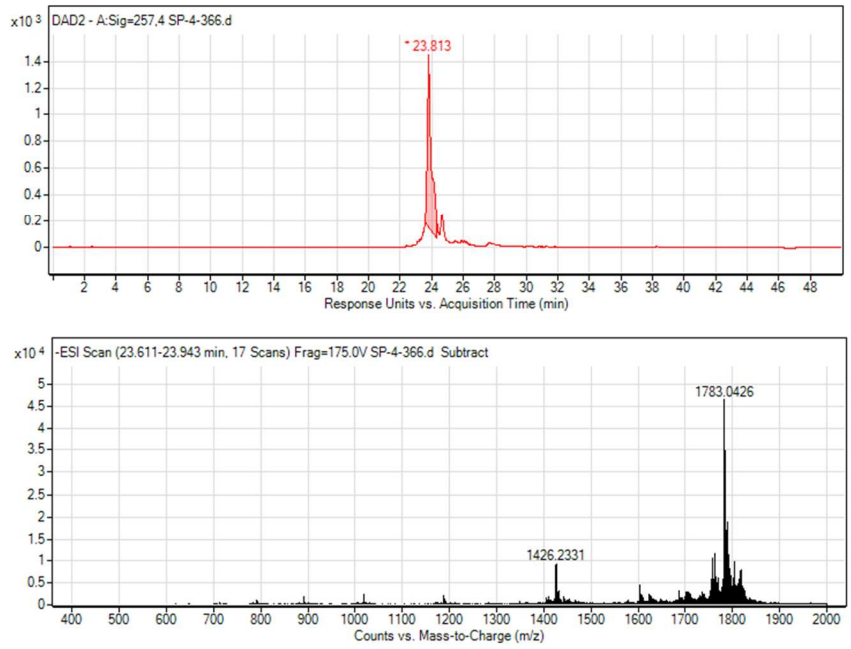


Figure S22. LC-MS analysis of ODN 18.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak.

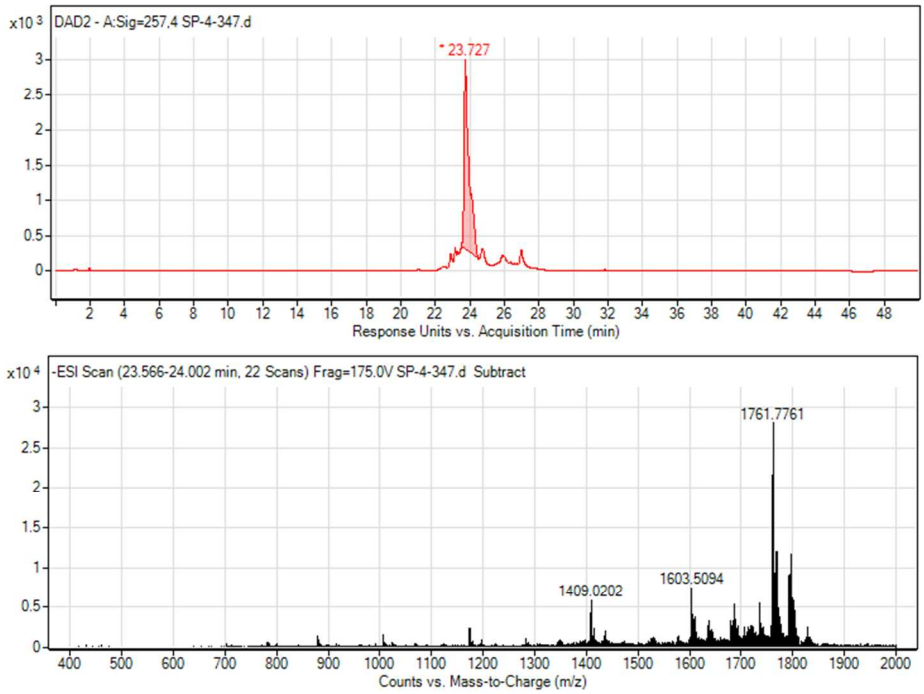


Figure S23. LC-MS analysis of ODN 19.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak.

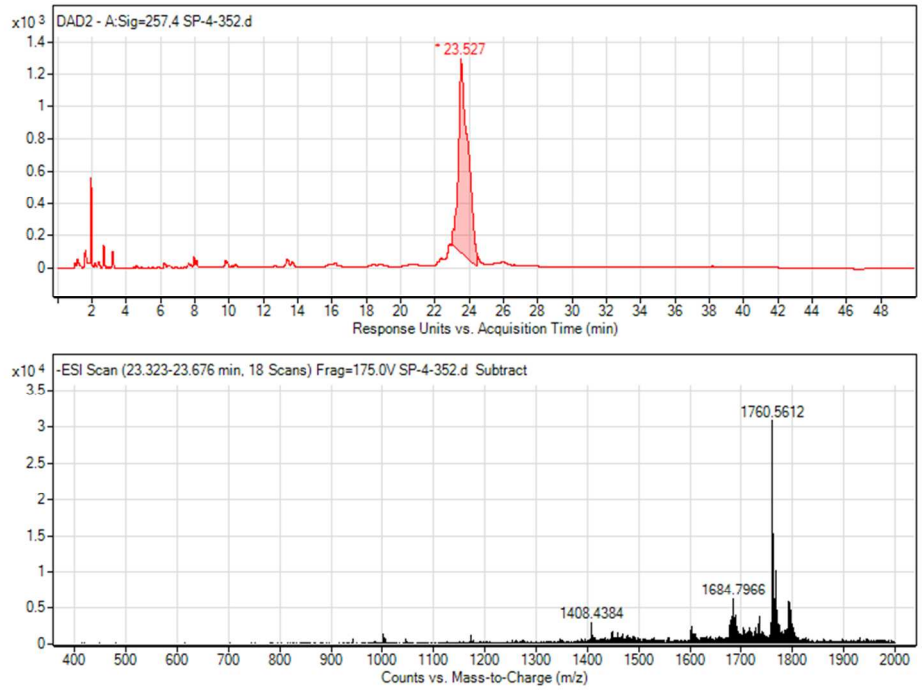


Figure S24. LC-MS analysis of ODN 20.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak.

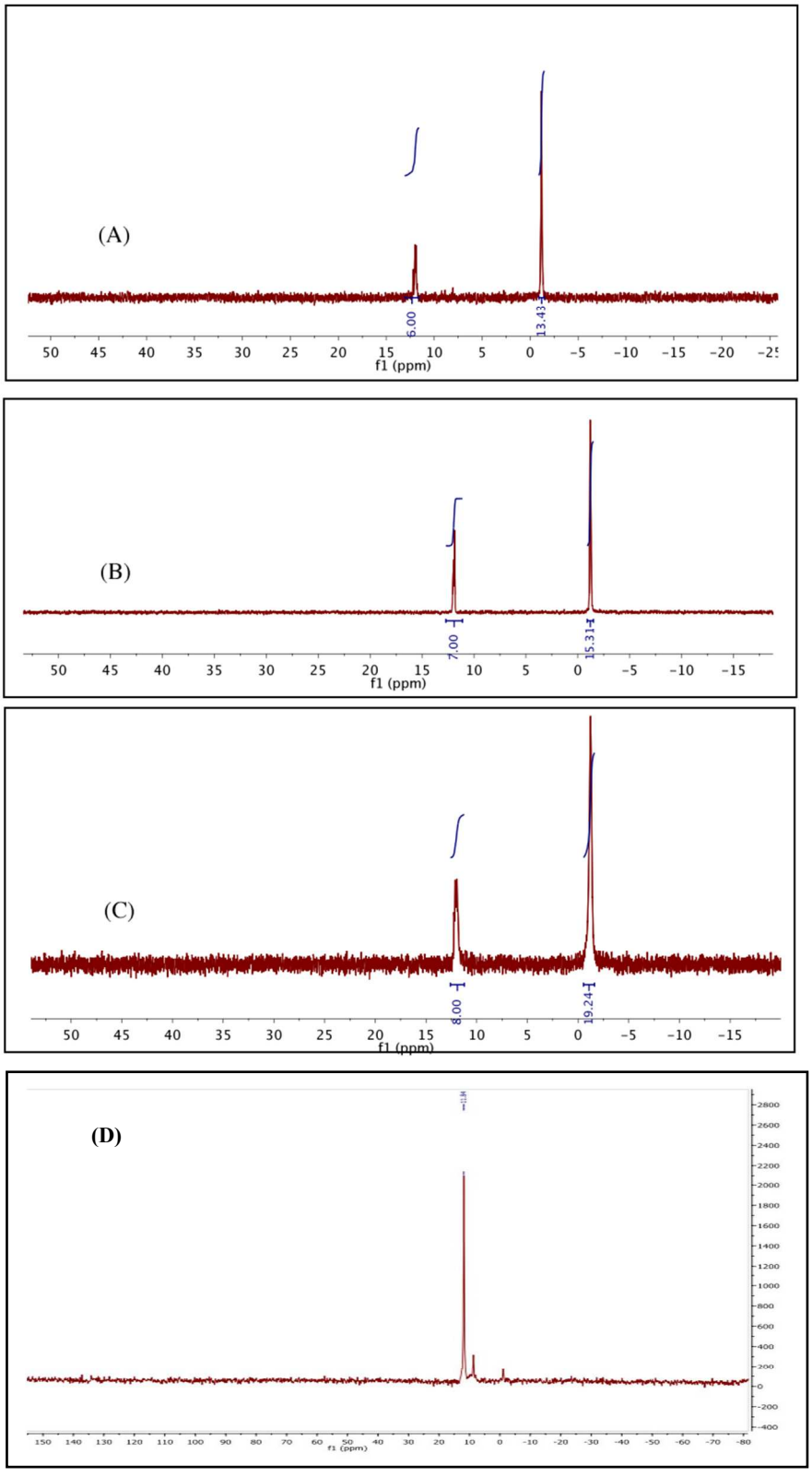


Figure S25. ^{31}P NMR of the crude reaction mixtures of ODN 12(A),ODN13(B), ODN 14 (C) and ODN 21 (D).

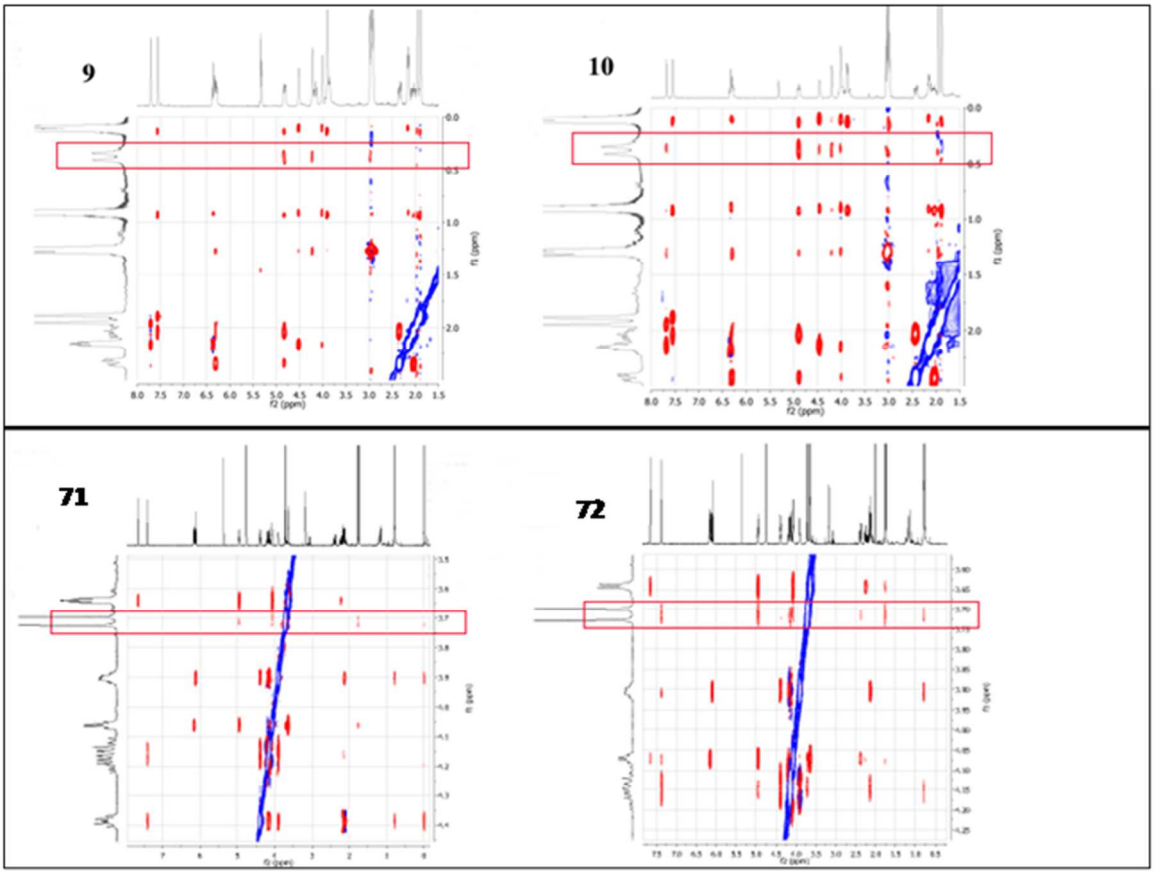


Figure S26. NOESY{11 B} spectra of **9** and **10** in CD_2Cl_2 (top) and NOESY spectra of **71** and **72** in Methanol-d4 (bottom). The red rectangles highlight the through space contacts between the BH_3 protons (in **9** or **10**) or OCH_3 protons (**71** or **72**) and the other protons in the respective molecules.

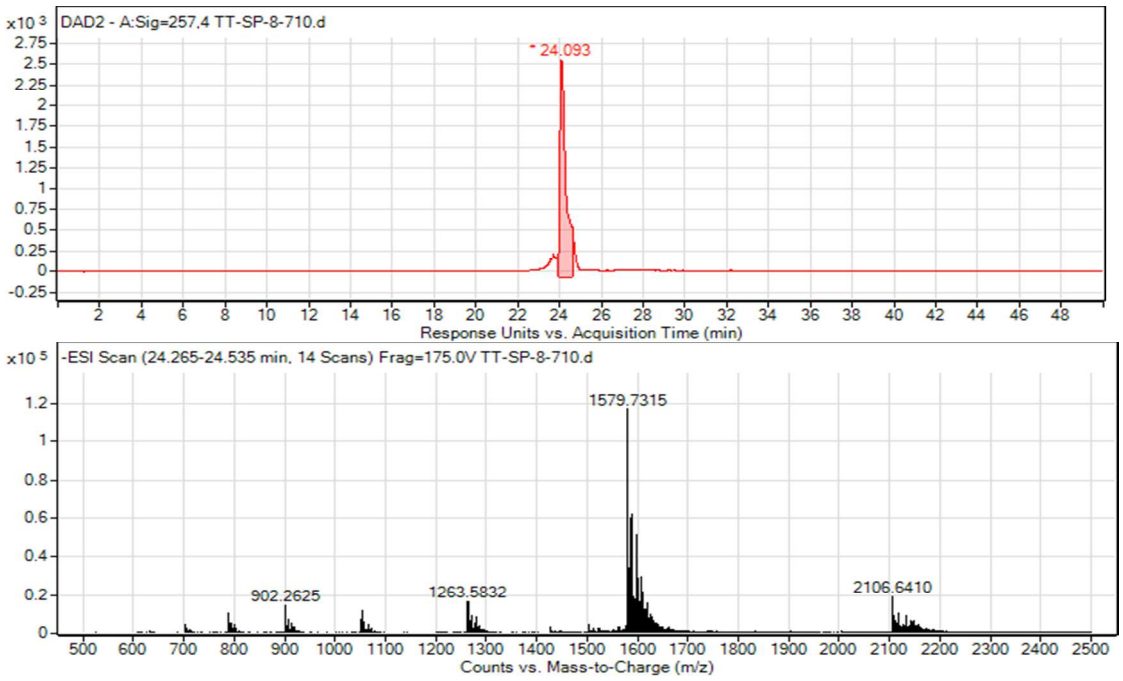


Figure S27. LC-MS analysis of ODN 22.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak.

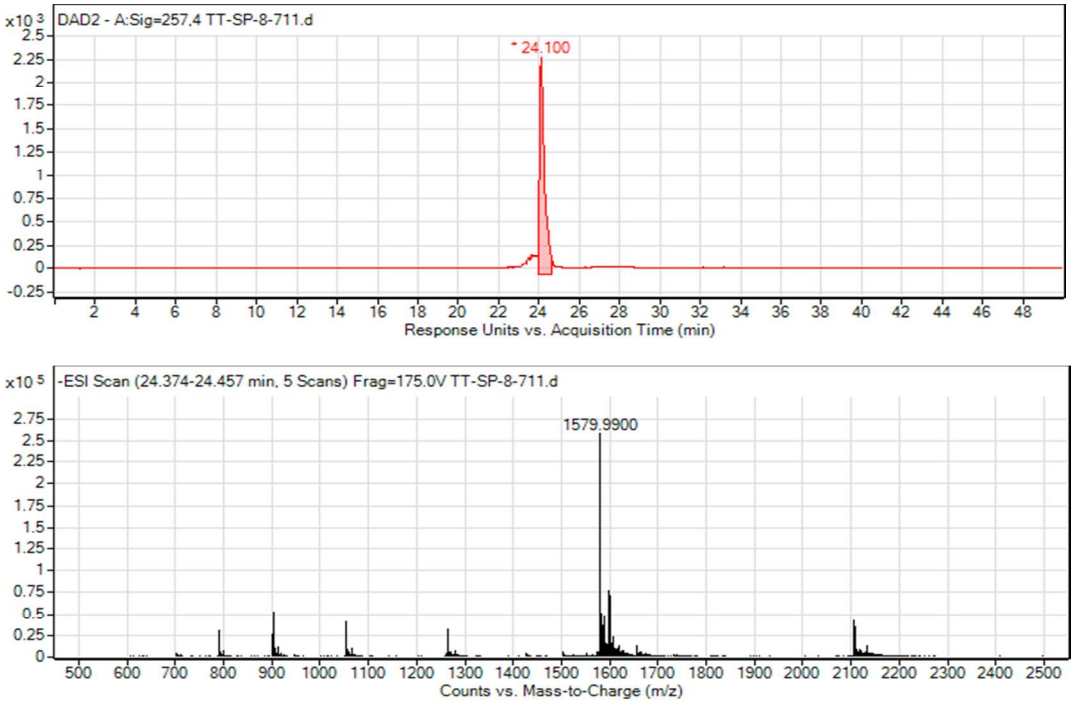


Figure S28. LC-MS analysis of ODN 23.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak.

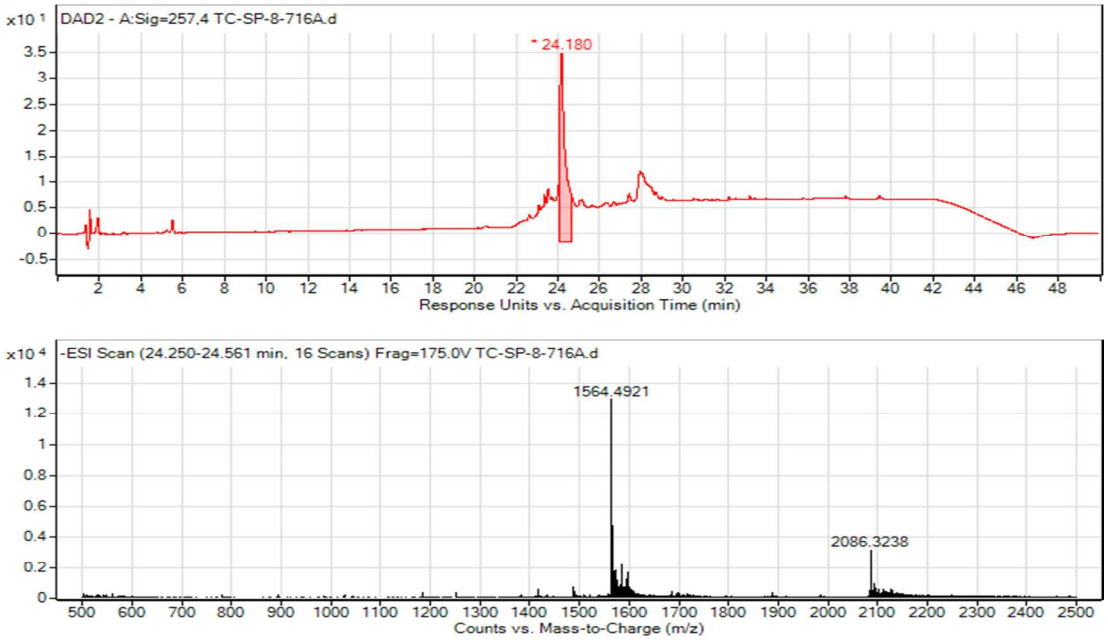


Figure S29. LC-MS analysis of ODN 24.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak.

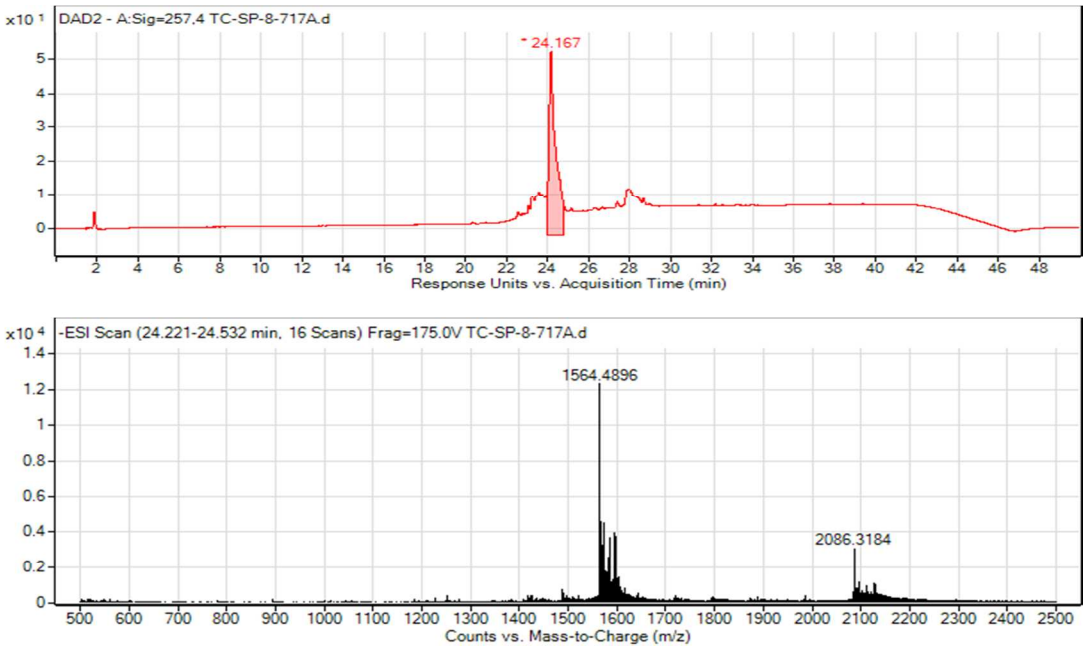


Figure S30. LC-MS analysis of ODN 25.UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak.

References

[i] R.K. Harris, E.D. Becker, S.M. Cabral De Menezes, P.Granger, R.E. Hoffman, and K.W. Zilm, "Further Conventions for NMR Shielding and Chemical Shifts," *Pure and Applied Chemistry*, 80(1), 59-84 (2008).
[ii] Roy, S.; Olesiak, M.; Shang, S.; Caruthers, M. H. *J. Am. Chem. Soc.*, **2013**, *135*, 6234-6241.