Supporting information

Four levels of wavelength selective uncaging for oligonucleotides

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Syntheses

All reactions were performed using solvents of p.a. quality or higher. NMR spectra were recorded on Bruker AM 250, AV 300 and AV 400 MHz instruments. To assign proton shifts additional ^{1}H-^{1}H-COSY and ^{13}C-HSQC experiments were performed. For flash chromatography silica gel 60 by Machery-Nagel was used. TLC analyses were performed on aluminum plates coated with silica gel 60 F 254 (Merck).

Synthesis of dT^{NpHP} phosphoramidite

5`-*O*-(4,4`-Dimethoxytrityl)-2`-deoxythymidin (4) was synthesized as previously described.[1]

1-(4-triispropylsilyloxy)acetophenone (2)

p-Hydroxyacetophenone (8.0 g, 58.8 mmol, 1.0 eq.) and imidazole (12 g, 176.3 mmol, 3.0 eq.) were dissolved in 25 ml dry DMF under argon atmosphere. Triisopropylsilylchloride (15.3 g, 79.5 mmol, 1.3 eq) were added and the mixture was stirred at room temperature for 10 min and then quenched with ethanol. The solvent was evaporated and the residue dissolved in ethyl acetate. The organic phase was washed consecutively with 1 N HCl and water then dried with $MgSO_4$. The solvent was evaporated and the residue was purified via column chromatography with dichloromethane/cyclohexane 2:1. A colourless oil was obtained.

Yield: 17.2 g (quantitative)

TLC (dichloromethane/cyclohexane 2:1): R_f = 0.44

^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.87 (d, 2H, *J* = 8.9, H_{ar}, 2 x CH), 6.90 (d, 2H, *J* = 8.9, H_{ar}, 2 x CH), 2.54 (s, 3H, CH_3), 1.33-1.20 (m, 3H, 3 x CH TIPS), 1.10 (d, 18H, *J* = 7.0, 6 x CH_3 TIPS) ppm.

^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 196.9, 160.8, 130.65, 119.8, 26.4, 18.0, 12.8 ppm.

MALDI-HRMS: *m/*z calcd. for $C_{17}H_{28}BrO_2Si$ $[M+H]^+$ 293.19313, found 293.19343 (Δm 0.00030, error 1.0 ppm)

2-bromo-1-(4-triisopropylsilyloxy)acetophenone (3)

$CuBr_2$ (26.1 g, 117.0 mmol, 2.0 eq.) was suspended in 100 ml ethyl acetate and a solution of compound **2** (17.1 g, 58.7 mmol, 1.0 eq.) in 100 ml chloroform was added. The mixture was stirred under reflux overnight. After cooling to room temperature and filtration the solvent was concentrated under reduced conditions. Purification with flash chromatography (dichloromethane/n-hexane 1:1) afforded a yellowish oil.

Yield: 15.6 g (72 %)

TLC (dichloromethane/cyclohexane 2:1): R_f = 0.65

^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.91 (d, 2H, *J* = 9.0, H_{ar}, 2 x CH), 6.92 (d, 2H, *J* = 9.0, H_{ar}, 2 x CH), 2.54 (s, 3H, CH_2Br), 1.35-1.21 (m, 3H, 3 x CH TIPS), 1.10 (d, 18H, *J* = 6.8, 6 x CH_3 TIPS) ppm.

^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 196.9, 160.8, 130.65, 119.8, 26.4, 18.0, 12.8 ppm.

MALDI-HRMS: *m/*z calcd. for $C_{17}H_{28}BrO_2Si$ $[M+H]^+$ 371.10365, found 371.10379 (Δm 0.00014, error 0.4 ppm)

5`-*O*-(4,4`-Dimethoxytrityl)-N^3[*p*-triisopropylsilyloxyphenacyl]-2`-deoxythymidin (5)

Nucleoside **4** (4.0 g, 7.35 mmol, 1.0 eq.) was dissolved in 40 ml methanol and potassium carbonate (2.0 g, 14.7 mmol, 2.0 eq.) was added. The mixture was stirred

for 30 minutes and then a solution of compound **3** (4.5 g, 12.1 mmol, 1.6 eq) in 20 ml methanol was added. The reaction mixture turns immediately yellowish and was stirred at room temperature overnight, then diluted with 30 mL ethyl acetate and washed with sat. aq. NaCl. The organic layer was dried over $MgSO_4$ and after filtration the solvent was removed under reduced pressure. The residue was purified by flash chromatography (cyclohexane/ethyl acetate 3:2). The column was packed with the initial solvent containing 0.5 % NEt_3. A yellowish foam was obtained.

Yield: 2.25 g (37 %)

TLC (cyclohexane/acetone 2:1): R_f = 0.40

^{1}H NMR (250 MHz, DMSO-d_6): δ = 8.02 (d, 2H, *J* = 8.7, H_{ar}, 2 x CH NpHP), 7.53 (s, 1H, CH dT), 7.43-7.24 (m, 9H, 9 x H_{ar} DMTr), 7.02 (d, 2H, *J* = 8.8, H_{ar}, 2 x CH NpHP), 6.93-6.90 (m, 4H, 4 x H_{ar} DMTr), 6.26 (t, 1H, *J* = 6.6, 1´-H), 5.29 (s, 2H, NCH_2CO), 4.38-4.34 (m, 1H, 3´-H), 3.93-3.92 (m, 1H, 4´-H), 3.74 (s, 6H, 2 x OCH_3), 3.25-3.22 (m, 2H, 5´-H), 2.37-2.16 (m, 2H, 2´-H), 1.50 (s, 3H, CH_3 dT), 1.39-1.22 (m, 3H, 3 x CH TIPS), 1.08 (d, 18H, *J* = 7.1, 6 x CH_3 TIPS) ppm.

^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 190.7, 162.3, 160.5, 158.2, 157.8, 150.2, 148.3, 144.6, 140.2, 135.4, 135.3, 130.5, 129.7, 128.9, 128.0, 127.9, 127.7, 127.4, 119.8, 113.3, 112.7, 108.7, 108.4, 85.9, 85.7, 84.8, 79.9, 70.4, 55.0, 26.3, 17.6, 12.2 ppm.

MALDI-HRMS: *m/*z calcd. for $C_{48}H_{58}N_2O_9Si$ $[M+Na]^+$ 857.38038, found 857.37962 (Δm 0.00076, error 0.9 ppm)

3`-*O*-(2-Cyanoethoxy-*N*,*N*`-diisopropylamin)phosphine-5`-*O*-(4,4`-Dimethoxytrityl)-N^3[*p*-triisopropylsilyloxyphenacyl]-2`-deoxythymidin (6)

Nucleoside **5** (200 mg, 0.24 mmol, 1 eq.) was dissolved in 5 mL dry dichloromethane under argon atmosphere. 209 µL N-ethyldiisopropylamine (1.12 mmol, 5eq.) were added followed by 160 µL 2-cyanoethoxy-*N,N*-diisopropylaminochlorophosphine (0.72 mmol, 3 eq.). The reaction mixture was stirred at room temperature for 10 minutes then diluted with 15 mL dichloromethane and washed with sat. aq. $NaHCO_3$. The organic layer was dried over $MgSO_4$ and after filtration the solvent was removed

under reduced pressure. The residue was purified by flash chromatography (cyclohexane/acetone 3:1). The column was packed with the initial solvent containing 0.5 % NEt_3. A yellowish foam was obtained.
Yield: 248 mg (79 %)

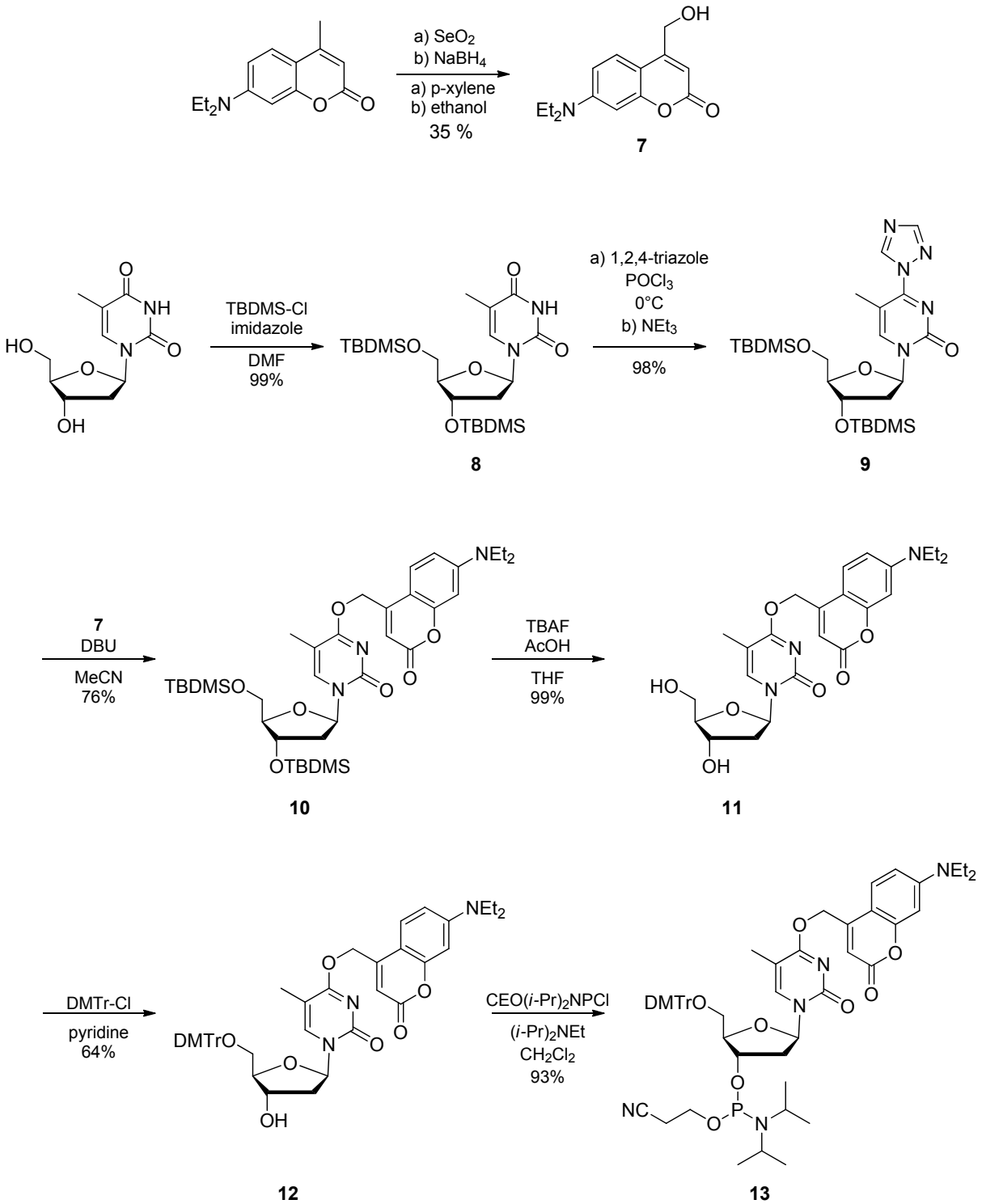
TLC (cyclohexane/acetone 2:1): R_f = 0.49

^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.01 (d, 2H, *J* = 8.4, H_{ar}, 2 x CH NpHP), 7.69 (d, 1H, *J* = 3.1, CH dT), 7.43-7.24 (m, 9H, 9 x H_{ar} DMTr), 7.02 (d, 2H, *J* = 8.6, H_{ar}, 2 x CH NpHP), 6.93-6.88 (m, 4H, 4 x H_{ar} DMTr), 6.29-6.23 (m, 1H, 1´-H), 5.28 (s, 2H, NCH_2CO), 4.62-4.56 (m, 1H, 3´-H), 4.10-4.03 (m, 1H, 4´-H), 3.74 (s, 6H, 2 x OCH_3), 3.67-3.46 (m, 4H, OCH_2, 2 x $NCH(iPr)_2$), 3.30-3.27 (m, 2H, 5´-H), 2.78-2.62 (m, 1H, 2´-H), 2.45-2.41 (m, 2H, CH_2CN), 1.54 (d, 3H, CH_3 dT), 1.36-1.26 (m, 3H, 3 x CH TIPS), 1.16-1.07 (m, 30H, 6 x CH_3 TIPS, 4 x $NCHCH_3$) ppm.

^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 190.6, 162.3, 160.5, 158.2, 150.2, 150.1, 144.5, 135.3, 135.1, 130.5, 129.7, 127.9, 127.7, 127.6, 119.8, 118.9, 118.7, 113.2, 108.9, 108.8, 86.1, 86.0, 58.4, 58.1, 55.0, 42.7, 42.5, 24.3, 24.2, 24.1, 19.8, 19.7, 17.8, 17.6, 12.2, 12.0 ppm.

^{31}P NMR (162 MHz, DMSO-d_6): δ = 147.8, 147.7, 147.4, 147.4ppm.

Synthesis of dT^{DEACM} phosphoramidite



7-Diethylamino-4-hydroxymethylcoumarin (7) was synthesized as previously described.[2]

3´-5´-Bis-*O*-(*tert*-butyldimethylsilyl)-2´-desoxythymidin (8) was synthesized in analogy to P. Potier et al..[3]

4-(1,2,4-Triazole-1-yl)-3´-5´-bis-*O*-(*tert*-butyldimethylsilyl)-2´-desoxythymidine (9) was synthesized as previously described.[4]

3´-5´-Bis-*O*-(*tert*-butyldimethylsilyl)-O^4-[7-diethylamino-4-ylmethylcoumarin]-2´-desoxythymidine (10)

Nucleoside **9** (6.5 g, 12.46 mmol, 1.1 eq.) and 7-diethylamino-4-hydroxymethyl-coumarin (2.8 g, 11.3 mmol, 1 eq.) were dissolved in 60 ml dry acetonitrile. 1,8-Diazabicyclo[5.4.0]undec-7-en (1.86 ml, 12.46 mmol, 1.1 eq.) was added and the reaction mixture was stirred at room temperature for 12 hours. The solvent was evaporated and the residue was purified via column chromatography with cyclohexane/ethyl acetate 1:1. A orange foam was obtained.

Yield: 6.01 g (76%)

TLC (cyclohexane/ethyl acetate 1:1): R_f = 0.57

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 0.08 (12 H, s, CH_3, 2*TBDMS), 0.87 (18H, s, 2* *t*-Bu), 1.10-1.13 (6H, t, CH_3, NEt_2 coumarin), 1.98 (3H, s, CH_3, C-5), 2.10-2.15 (1H, m, H-2´), 2.21-2.25 (1H, m, H-2´), 3.41-3.45 (4H, q, CH_2, NEt_2 coumarin), 3.72-3.75 (1H, m, H-5´), 3.78-3.81 (1H, m, H-5´), 3.87-3.89 (1H, m, H-4´), 4.35-4.37 (1H, m, H-3´), 5.55-5.56 (2H, m, CH_2, coumarin), 5.97 (1H, s, CH coumarin), 6.12-6.14 (1H, t, J_1=6.43, H-1´), 6.55(1H, d, CH_{ar}, coumarin), 6.69-6.72 (1H, dd, CH_{ar}, coumarin), 67.48-7.50 (1H, d, CH_{ar}, coumarin), 7.82 (1H, s, H-6) ppm

^{13}C NMR (62.5 MHz, $CDCl_3$): δ = -5.03, -4.47, -4.30, 0.63, 12.77, 18.17, 18.44, 26.13, 26.20, 44.47, 63.06, 63.89, 72.53, 86.21, 87.81, 97.33, 103.40, 105.34, 105.69, 109.25, 125.95, 141.62, 150.96, 151.05, 154.70, 156.29, 161.08, 169.29, ppm.

MALDI-HRMS: *m/*z calcd. for $C_{36}H_{57}N_3O_7Si_2$ $[M+Na]^+$ 722.36272, found 722.36177 (Δm 0.00096, error 1.3 ppm)

O^4-[7-Diethylamino-4-ylmethylcoumarin]-2´-desoxythymidine (11)

Nucleoside **10** (6 g, 8.59 mmol, 1 eq.) was dissolved in 20 ml tetrahydrofuran. 5 ml acetic acid was added and the reaction mixture was stirred at room temperature for 10 minutes. Afterwards 1 M tetrabutylammonium fluoride solution (in tetrahydrofuran; 25.3 ml, 25.26 mmol, 3 eq.) was added and the mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was purified via column chromatography with dichloromethane/methanol 9:1. A orange foam was obtained.

Yield: 4.05 g (quantitative)

TLC (cyclohexane/ethyl acetate 1:1): R_f = 0.38

^{1}H-NMR (250 MHz, DMSO): δ = 1.13 (6H, t, CH_3, NEt_2 coumarin), 1.98 (3H, s, CH_3, C-5), 2.03-2.08 (1H, m, H-2´), 2.17-2.26 (1H, m, H-2´), 3.40-3.48 (4H, q, CH_2, NEt_2 coumarin), 3.58-3.64 (2H, m, 2*OH), 3.80-3.84 (1H, m, H-5´), 4.20-4.26 (1H, m, H-5´), 5.07-5.11 (1H, t, H-4´), 5.23-5.25 (1H, d, H-3´), 5.56 (2H, s, CH_2, coumarin), 5.98 (1H, s, CH coumarin), 6.11-6.17 (1H, t, J_1=6.62, H-1´), 6.56-6.57 (1H, d, CH_{ar}, coumarin), 6.70-6.74 (1H, dd, CH_{ar}, coumarin), 7.48-7.55 (1H, d, CH_{ar}, coumarin), 8.13 (1H, s, H-6) ppm.

^{13}C NMR (62.5 MHz, DMSO): δ = 11.85, 12.32, 40.64, 44.02, 60.96, 63.33, 69.96, 85.64, 87.67, 96.86, 102.83, 104.79, 105.23, 108.81, 125.44, 141.83, 150.50, 150.67, 154.36, 155.82, 160.65, 168.77 ppm.

MALDI-HRMS: *m/*z calcd. for $C_{24}H_{29}N_3O_7$ $[M+H]^+$ 471.20055, found 471.20021 (Δm 0.00034, error 0.7 ppm)

5´-*O*-(4,4´-Dimethoxytrityl)- *O*^{4}-[7-diethylamino-4-ylmethylcoumarin]-2´-desoxy thymidine (12)

Compound **11** (4 g, 8.59 mmol, 1eq.) was co-evaporated in 100 ml pyridine. The residue was dissolved in 75 ml dry pyridine under argon atmosphere and the mixture was cooled down to 0°C. Afterwards 4,4´-dimethoxytrityl chloride (3.46 g, 10.31 mmol, 1.2 eq.) was added and the reaction mixtures was stirred at 0 °C for 1 hour and then at room temperature overnight. The reaction mixture was quenched with 20

ml ethanol and stirred at room temperature for further 25 minutes. The solvent was evaporated and the residue was dissolved in dichloromethane. The organic layer was washed consecutively with aq. citric acid (5%) and sat. aq. $NaHCO_3$. The aqueous layer was washed with dichloromethane. The combined organic phase was dried with $MgSO_4$. The solvent was evaporated and the residue was purified via column chromatography with dichloromethane/methanol 95:5 (initial solvent for packing column contained 0.5 % NEt_3). A yellow foam was obtained.

Yield: 4.23 g (64%)

TLC (dichloromethane/methanol 95:5): R_f = 0.37

^{1}H-NMR (250 MHz, DMSO): δ = 1.17 (6H, t, CH_3, NEt_2 coumarin), 1.63 (3H, s, CH_3, C-5), 2.16-2.33 (2H, m, H-2´), 3.40-3.44 (4H, q, CH_2, NEt_2 coumarin), 3.7 (6H, s, 2*OCH_3), 3.40-3.95-3.97 (1H, m, H-5´), 4.28-4.36 (1H, m, H-5´), 5.32-5.34 (1H, d, H-3´), 5.56 (2H, s, CH_2, coumarin), 5.76 (1H, s, H-3´), 5.97 (1H, s, CH coumarin), 6.17-6.19 (1H, t, J_1=6.28, H-1´), 6.55 (1H, d, CH_{ar}, coumarin), 6.69-6.71 (1H, dd, CH_{ar}, coumarin),6.89-6.91 (4H, DMTr-arom. H), 7.23-7.32 (9H, DMTr-arom. H), 7.48-7.50 (1H, d, CH_{ar}, coumarin), 7.91 (1H, s, H-6) ppm.

^{13}C NMR (62.5 MHz, DMSO): δ = 11.40, 12.30, 44.01, 63.41, 70.11, 85.63, 85.92, 96.87, 103.03, 104.89, 105.24, 108.79, 113.29, 123.90, 125.46, 126.82m 127.67, 127.95, 129.75, 135.25, 135.44, 141.21, 144.74, 149.62, 150.57, 154.24, 155.84, 158.18, 160.63, 168.81 ppm.

MALDI-HRMS: *m/*z calcd. for $C_{45}H_{47}N_3O_9$ $[M+Na]^+$ 796.32045, found 796.31963 (Δm 0.00082, error 1.0 ppm)

5´-*O*-(4,4´-Dimethoxytrityl)- *O*^{4}-[7-diethylamino-4-ylmethylcoumarin]-2´-desoxy thymidine-3´-(cyanoethyl-N,N-diisopropylphosphoramidite (13)

Compound **12** (300 mg, 400 µmol, 1 eq.) was dissolved in dry dichloromethane and *N*-ethyl-*N,N*-diisopropylamine (340 µl, 2 mmol, 5 eq.) was added. The reaction mixture was stirred a room temperature for 15 minutes. Subsequently 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (178 µl, 800 µmol, 2 eq) was added and the reaction mixture was stirred at room temperature for further 2 hours. The solution

was diluted with dichloromethane and washed with sat. aq. $NaHCO_3$. The organic layer was dried with $MgSO_4$. The solvent was evaporated and the residue was purified via column chromatography with cyclohexane/acetone 1:1. A yellow solid was obtained.

Yield: 362 mg (93%)

TLC (cyclohexane/acetone 95:5): R_f = 0.63

^{1}H-NMR (300 MHz, DMSO): δ = 1.08-1.15 (18H, m, 12H iPr-CH_3 + 6H CH_3, NEt_2 coumarin), 1.46-1.53 (1H, m, H-2´), 1.65-1.68 (3H, d, CH_3, C-5), 2.25-2.29 (1H, m, H-2´), 2.72-2.78 (m, 2H, cyanoethyl-CH_2), 3.41-3.48 (4H, q, CH_2, NEt_2 coumarin), 3.72 (6H, s, 2 OCH_3), 4.06-4.13 (1H, m, H-5´), 4.52-4.57 (1H, d, H-3´), 5.57 (2H, s, CH_2, coumarin), 5.98 (1H, s, CH coumarin), 6.17-6.22 (1H, t, J_1=8.15, H-1´), 6.55-6.57 (1H, d, CH_{ar}, coumarin), 6.69-6.71 (1H, dd, CH_{ar}, coumarin) ,6.89-6.91 (4H, DMTr-arom. H), 7.23-7.32 (9H, DMTr-arom. H), 7.38-7.40 (3H, m, CH_3), 7.49-7.50 (1H, d, CH_{ar}, coumarin), 7.91 (1H, s, H-6) ppm.

^{13}C NMR (62.5 MHz, DMSO): δ = 11.39, 12.30, 13.80, 18.85, 19.74, 19.79, 19.85, 24.21, 24.30, 24.36, 31.37, 42.54, 42.64, 44.01, 58.19, 58.26, 58.34, 58.40, 60.25, 63.45, 69.99, 72.03, 85.64, 85.70, 86.05, 96.87, 103.14, 103.23, 104.92, 105.24, 108.78, 112.75, 113.25, 118.76, 118.94, 125.47, 126.84, 127.64, 127.91, 128.92, 129.73, 135.10, 135.27, 144.59 150.49, 150.52, 154.17, 155.83, 158.22, 160.62, 168.87 ppm.

^{31}P NMR (121 MHz, DMSO): δ = 147.34, 147.68 ppm

DNA synthesis and purification

Solid phase synthesis of 15mer oligonucleotides was accomplished on an ABI 392 DNA/RNA synthesizer on ABI LV200 polystyrene columns using. The modified oligonucleotides were synthesized using standard synthesis protocols in DMTr-on mode. Deprotection was performed with 33% NH_4OH over night at room temperature. After evaporation of the solvent the crude product was purified by reversed-phase HPLC was performed on a Nucleosil 100-5 C18 column (eluent A: 0.1 M triethylammonium acetate buffer pH 7, eluent B: acetonitrile; gradient A: 5% B

for 2 min, 5% to 40% B in 33min, flow 1mL/min). The solvent was evaporated and the dimethoxytrityl group was cleaved off over 20 min with 80% aqueous acetic acid. After removing the acid the resulting oligonucleotide was purified again by RP-HPLC using gradient A. Purity and identity were confirmed by HPLC-ESI-MS and analytical RP-HPLC using gradient B: 5% B for 2 min, 5% to 26.3% B in 22 min, flow 1 mL/min. The unmodified oligonucleotide was purchased HPLC purified from Metabion.

Supplementary Table 1: ESI-MS data of synthesized oligonucleotides used for this study

Sequence	**Exact Mass calcd**.[Da]	**Mass found** [Da]
5'-GCA TAA ATA AAG GTG-3'	4646.8	4646.8
5'-GCA TAA AT^{NpHP}A AAG GTG-3'	4781.8	4781.2
5'-GCA TAA AT^{DEACM}A AAG GTG-3'	4876.0	4875.9
5'-GCA TAA AT^{NPP}A AAG GTG-3'	4809.9	4810.0
5'-GCA TAA ACA AAG GTG-3'	4631.8	4631.8
5'-GCA TAA AC^{NDBF}A AAG GTG-3'	4870.9	4871.0
5´-GCA TAT AT^{NpHP}A TAC GTG-3´	4723.8	4723.1
5´-CAC GTA TAT ATA TGC-3´	4548.8	4548.8

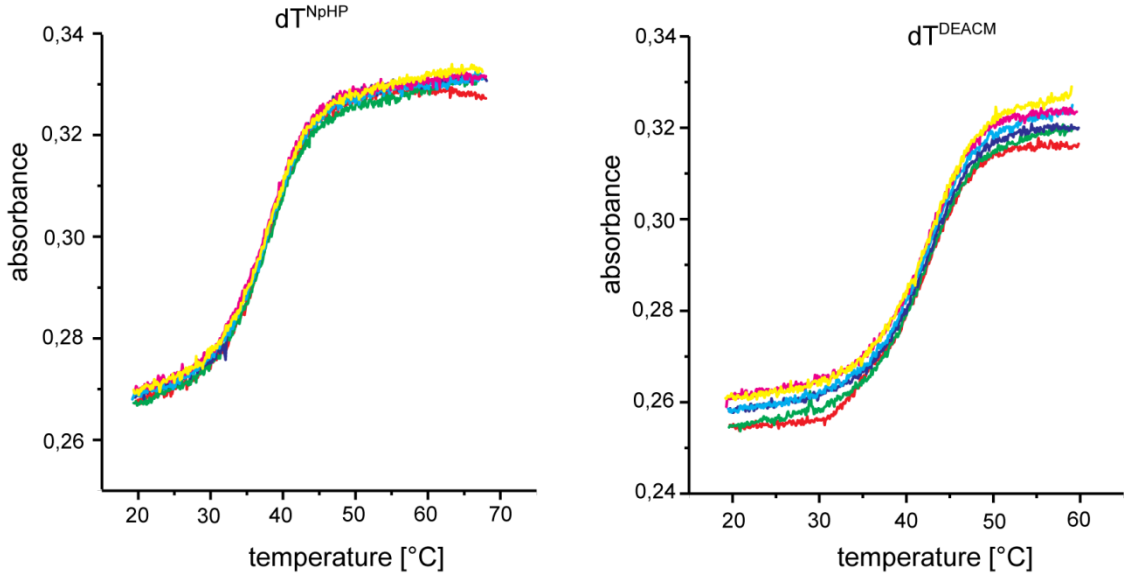
Melting points

Melting points, UV/vis spectra, concentrations and extinction coefficients were measured with an Evolution 300 spectrophotometer (ThermoFisher). For determination of melting points 1 mL of a solution containing 1 µM of each DNA (see Table) and 1 µM of the corresponding counter strand in 1x PBS buffer (pH 7.4, 10 mM phosphate, 2.7 mM KCl, 137 mM NaCl) was used. Supplementary Table 2: melting points of duplexes with different caged residues in one strand with respect to the unmodified duplex (ΔT_M).

Supplementary Table 2: melting points of duplexes with different caged residues in one strand with respect to the unmodified duplex (ΔT_M)

Sequence	**T_M [°C]**	**ΔT_M [°C]**
5'-GCA TAA ATA AAG GTG-3'	48.9	-
5'-GCA TAA AT^{NpHP}A AAG GTG-3'	39.4	9.5
5'-GCA TAA AT^{DEACM}A AAG GTG-3'	42.8	6.1
5'-GCA TAA AT^{NPP}A AAG GTG-3'	39.3	9.6
5'-GCA TAA ACA AAG GTG-3'	54.3	-
5'-GCA TAA AC^{NDBF}A AAG GTG-3'	38.2	16.1

UV/VIS spectra for determination of extinction coefficients were recorded using 70 µL of a solution containing 4 µM DNA oligonucleotide in the range of 200-300 nm and 40 µM DNA oligonucleotide for the measurement at wavelengths higher than 300 nm in 1x PBS. Obtained curves were smoothed by the spectrometer software.

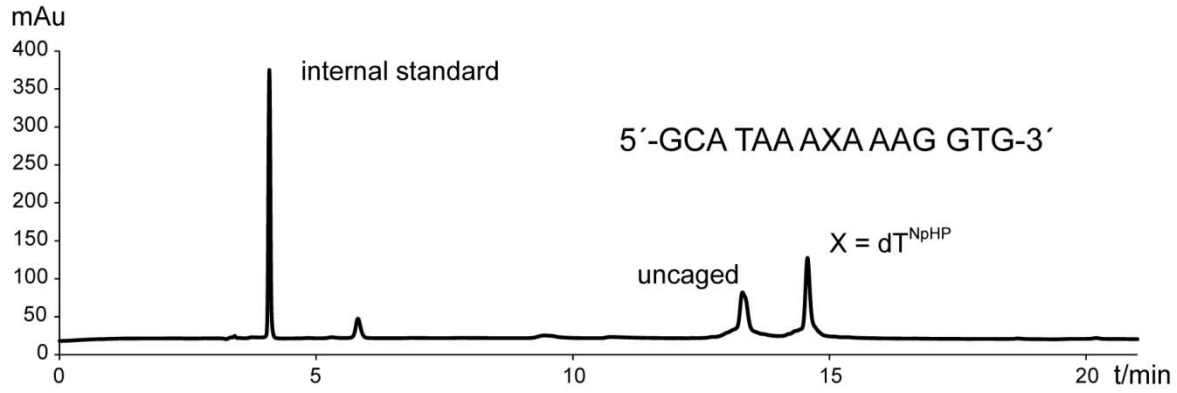


Supplementary Figure 1. Melting curves of 5'-GCA TAA AXA AAG GTG-3' and the complementary strand. Left: DNA duplex with X = dT^{NpHP}. Right: DNA duplex with X = dT^{DEACM}.

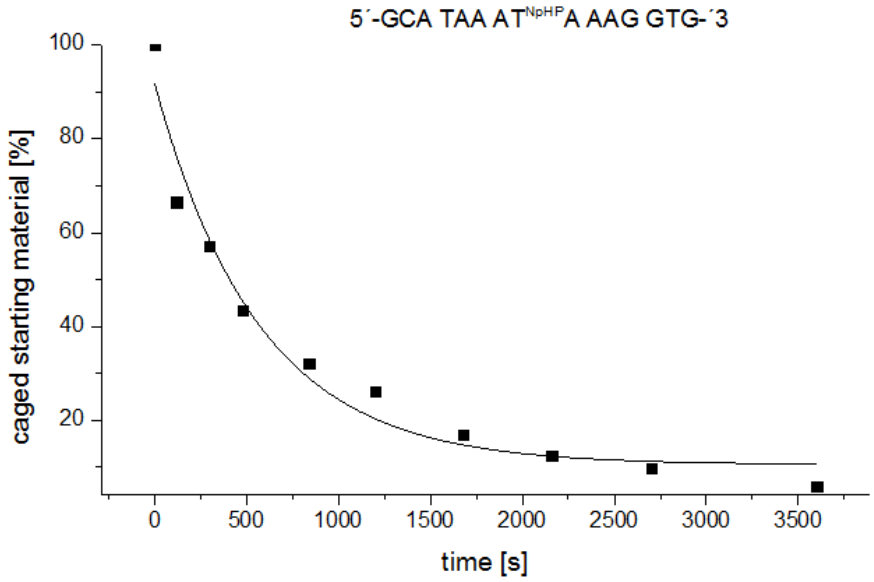
Quantum yield determination

Quantum yields were determined using dimethoxynitrobenzene (DMNB) actinometry according to Zhang et al. 13 µL samples were irradiated in a cuvette using a 100 W HG short arc-lamp (LSB610), which is attached to a monochromator, at λ = 300 nm.

The irradiation samples contained 20 µM oligonucleotide and 0.5 µL of an internal standard (uridine/uracil) in 1x PBS buffer. After irradiation the samples were analyzed with RP-HPLC using gradient B. Each experiment was done in triplicates. The initial slope was determined by differentiation of the exponential fit of the deprotection kinetics. The quantum yield was determined by calculating the ratio between initial slope and absorbed photons achieved by actinometry.



Supplementary Figure 2. Example of an HPLC spectrum for quantification of dT^{NpHP} deprotection (20 µM, irradiation for 480 s)

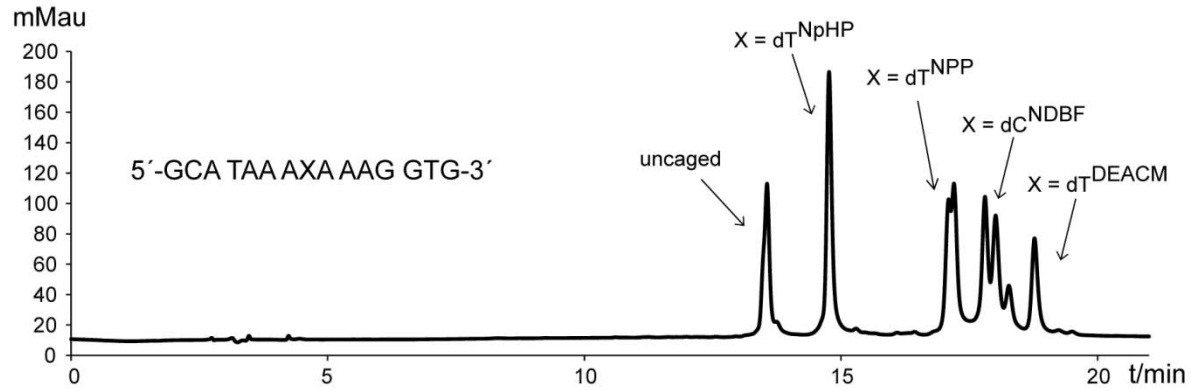


Supplementary Figure 3. Deprotection kinetics and exponential fit of dT^{NpHP}-containing oligonucleotide at 300 nm (20 µM, 100 W HG short arc-lamp, attached to a monochromator)

Wavelength-selective deprotection

Experiments were performed with LEDs ordered from Roithner Lasertechnik installed into a multi LED device with λ_{max} = 505 nm (P_{max} = 20 cd, H2A1-H505), λ_{max} = 440 nm (P_{max} = 350 mW, VL440-EMITTER), λ_{max} = 365 nm (P_{max} = 250 mW, UVLED-365-250-SMD) or with an 100 W Hg short arc lamp (LSB610) with a band pass filter at 313 nm from Lot Oriel (313FS25-50). Each sample (60 µL) contained 4 oligonucleotides in 3 µM concentration in PBS buffer. Each irradiation experiment was performed with RP-HPLC as for quantum yield determination.

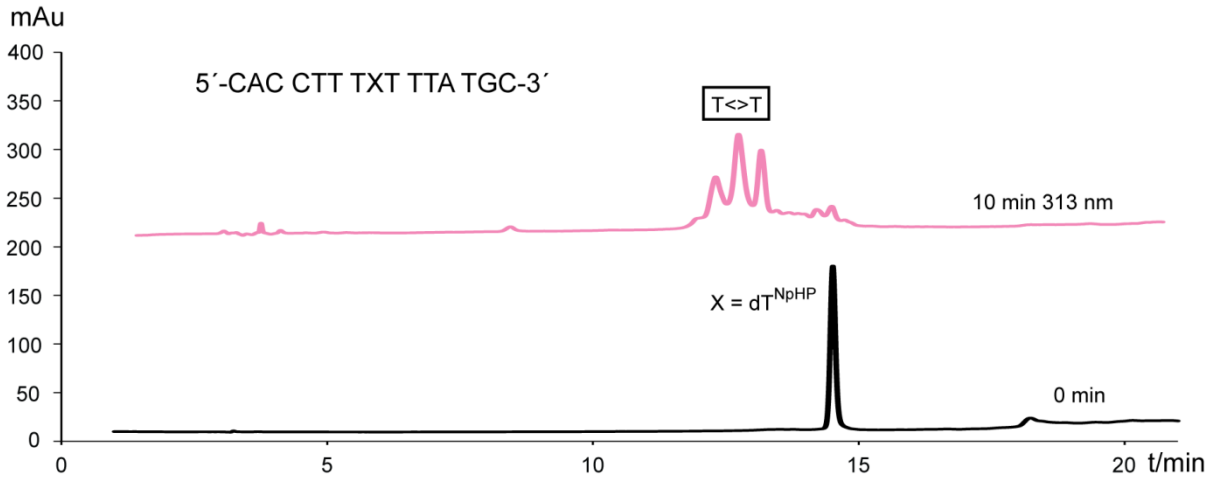
To quantify the relative powers of the light sources at the location of the respective sample ferrioxalate actinometry was used:[5] 4 mW (313 nm), 54 mW (365 nm), 11 mW (440 nm), 3 mW (505 nm).



Supplementary Figure 4. Example of an HPLC trace after irradiating a sample at 505 nm for 60min (60 µL of a 3 µM solution of the indicated oligonucleotide).

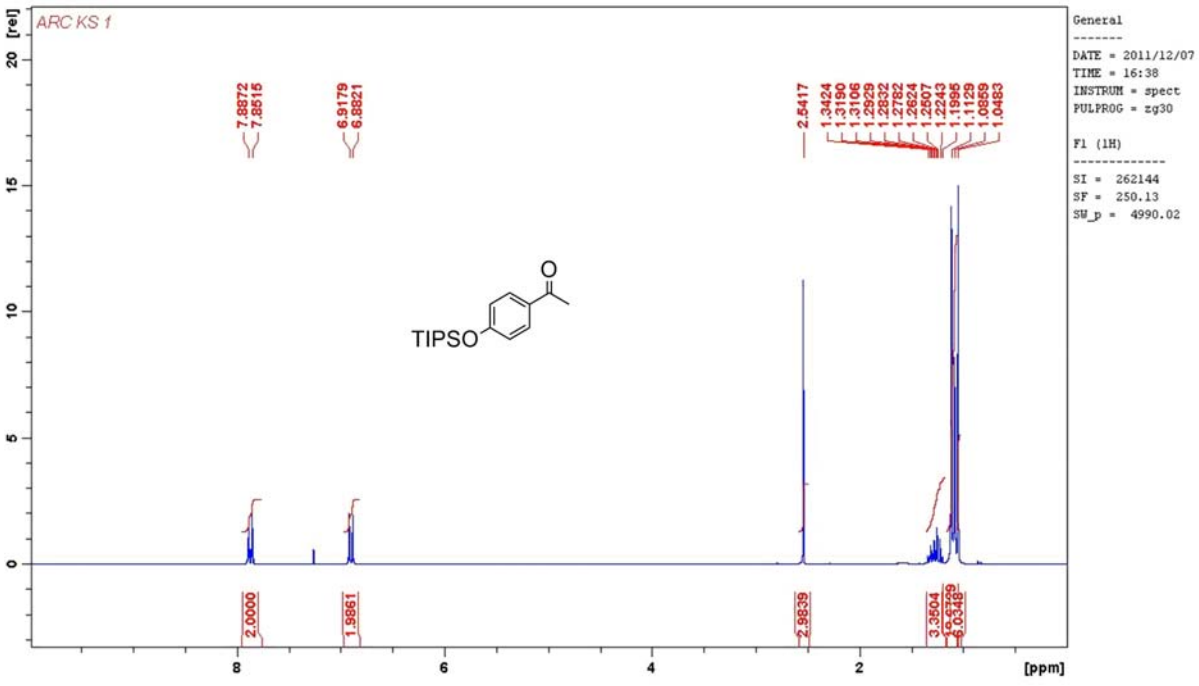
Supplementary Table 3. Ratio of caged oligonucleotides after irradiation.

	DEACM	NDBF	NPP	NpHP
0 min	100 %	100 %	100 %	100 %
120 min, 505 nm	10 %	80 %	98 %	100 %
5 min, 440 nm	-	6 %	93 %	100 %
20 sec, 365 nm	-	-	6 %	100 %

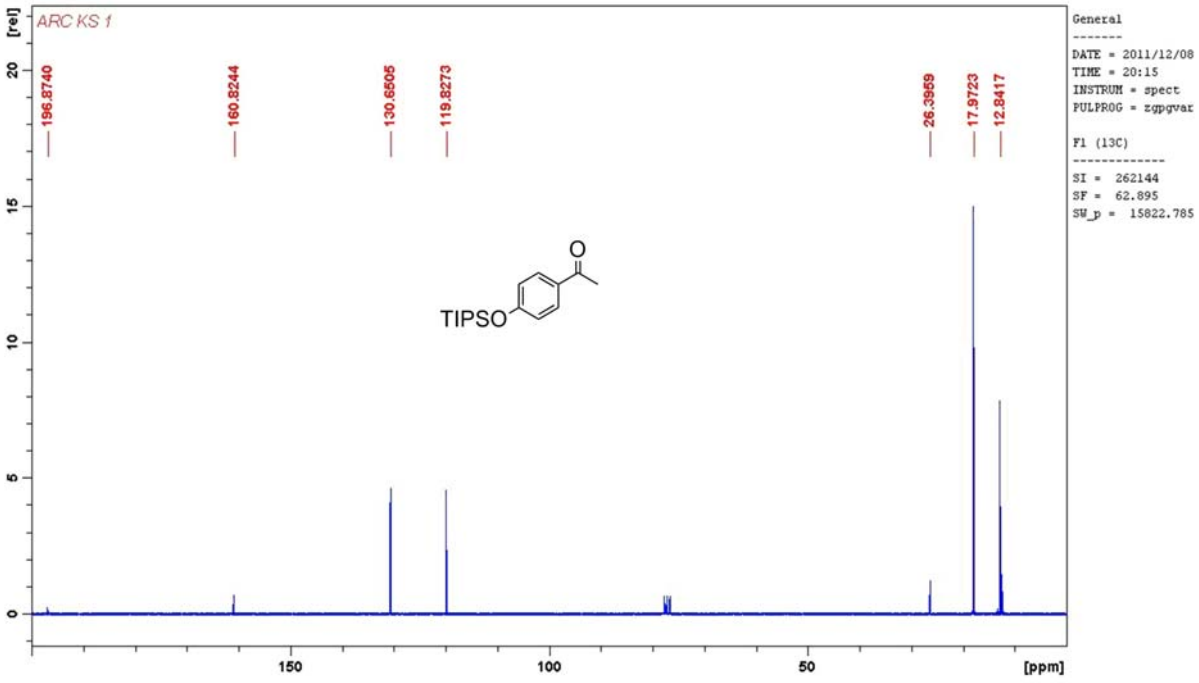


Supplementary Figure 5. HPLC trace of the Sequence 5´-CAC CTT TT^{NpHP}T TTA TGC-3´ before (black line) and after 10 min irradiation at 313 nm (pink line). (60 μL of a 3 μM solution in PBS buffer)

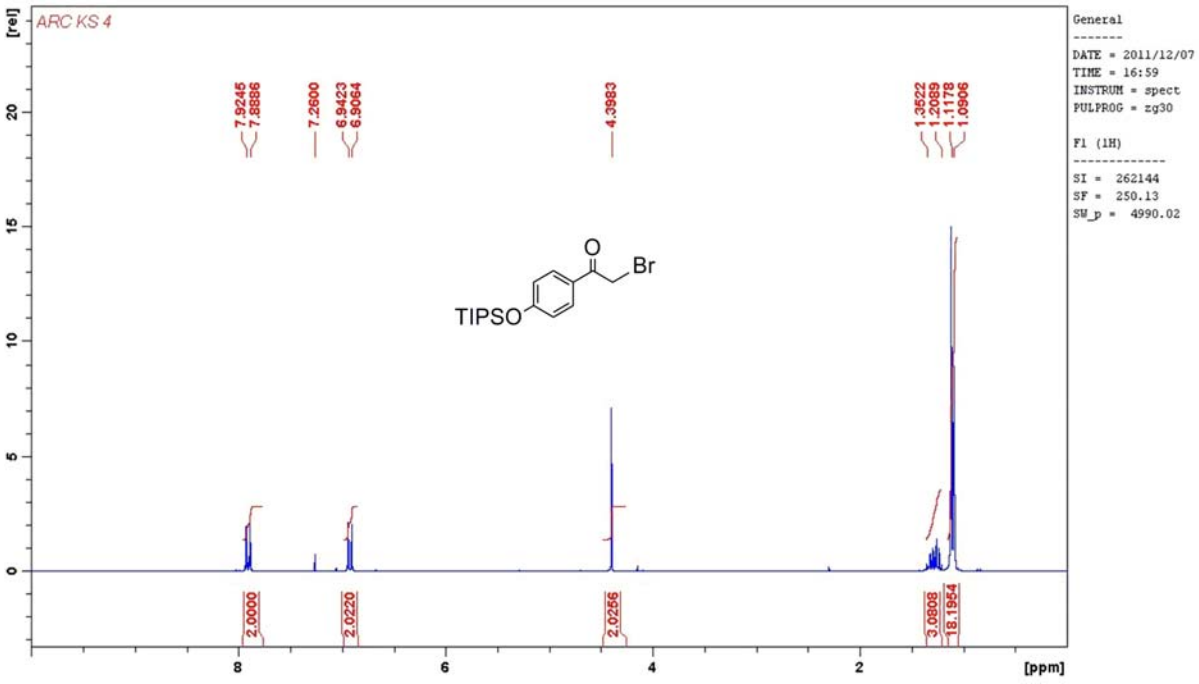
NMR spectra



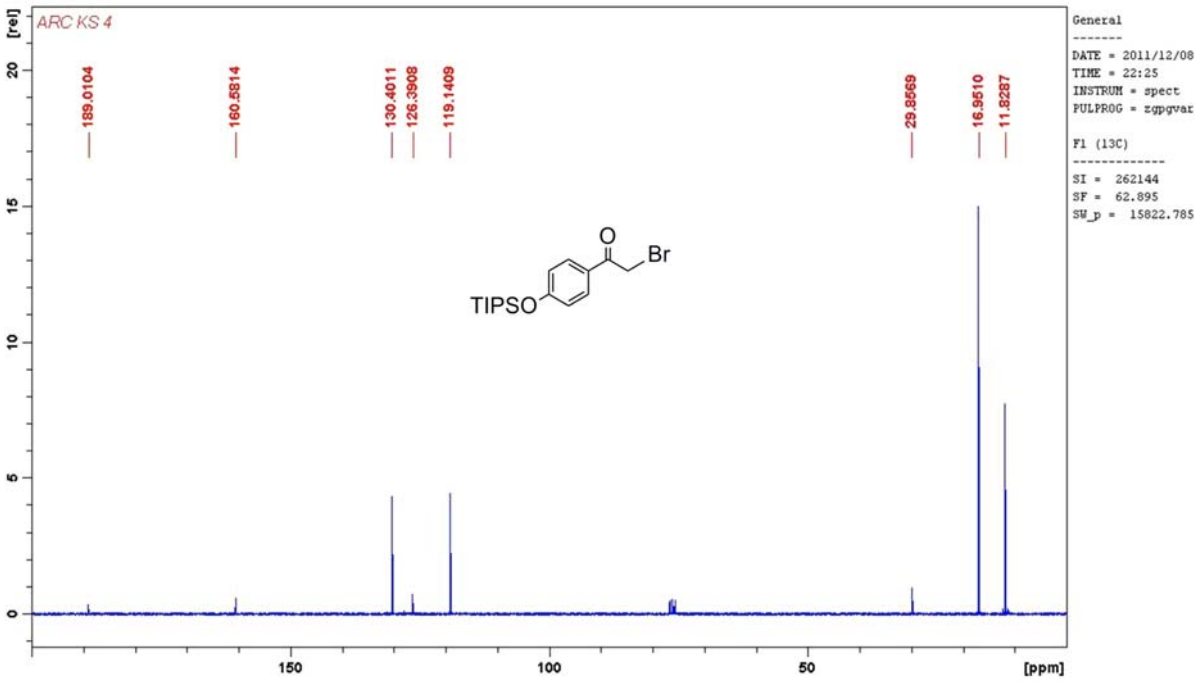
^{1}H NMR of compound **2**



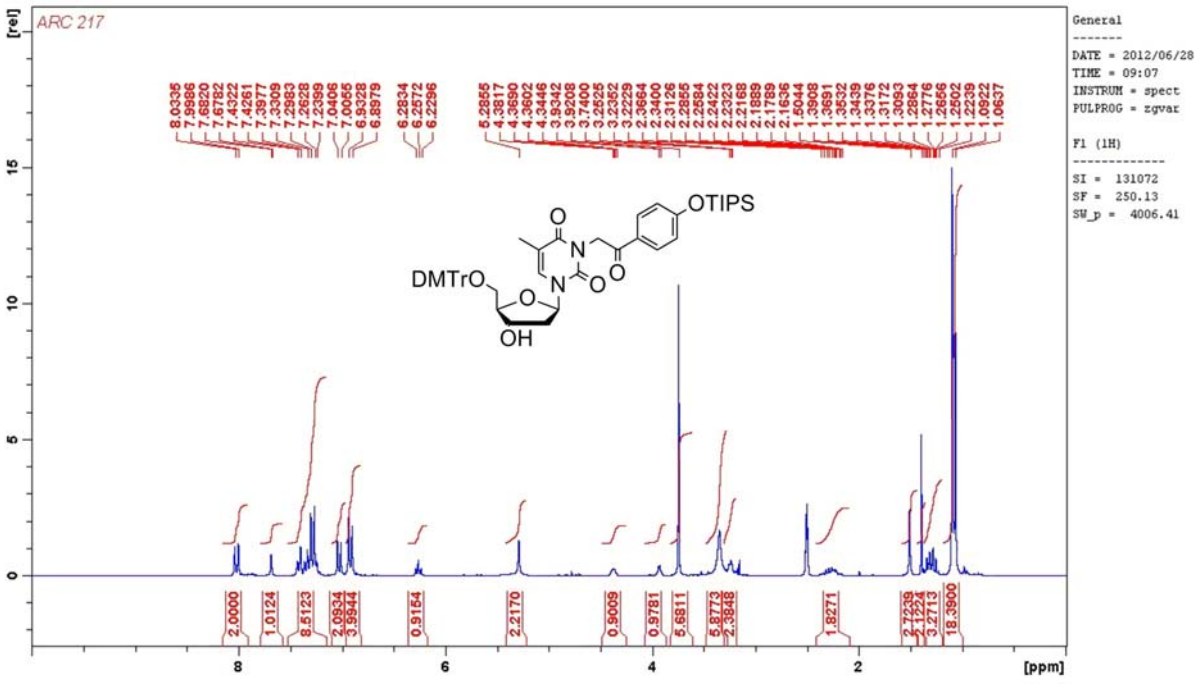
^{13}C NMR of compound **2**



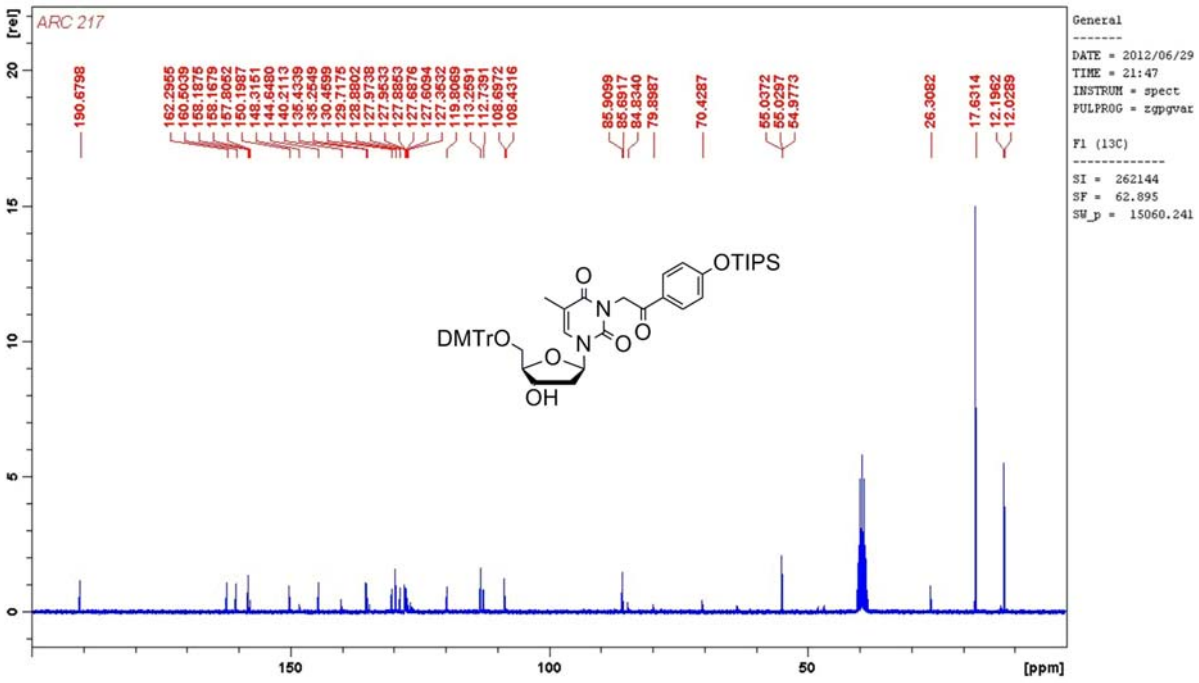
^{1}H NMR of compound **3**



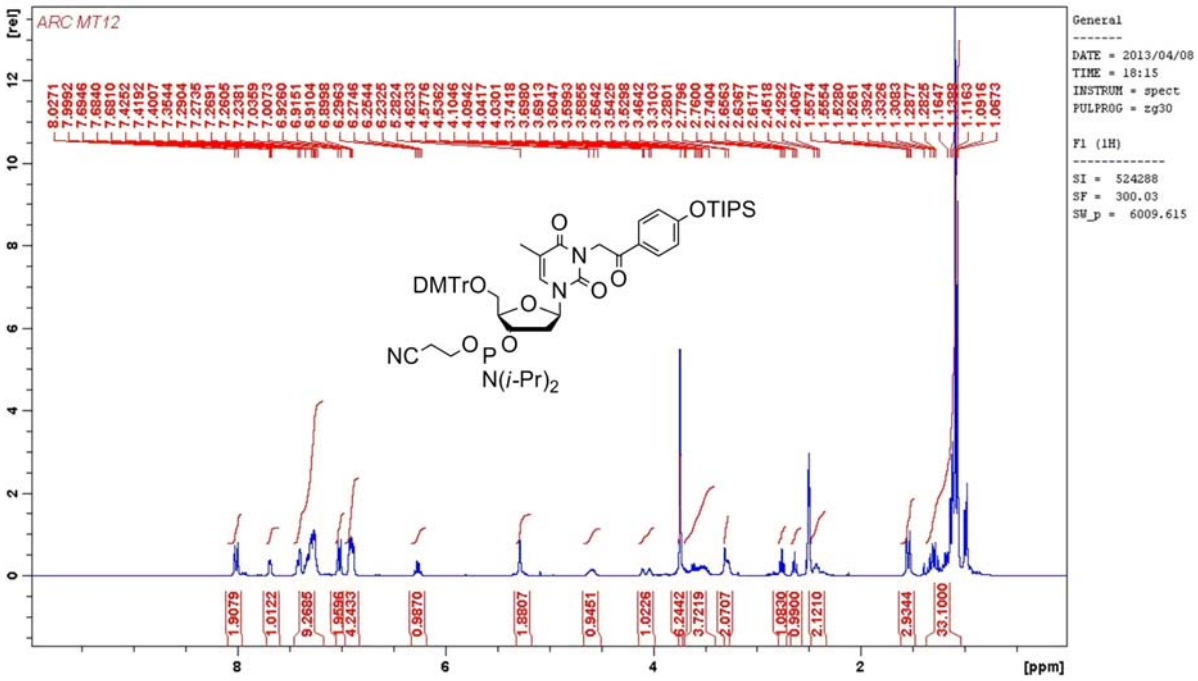
^{13}C NMR of compound **3**



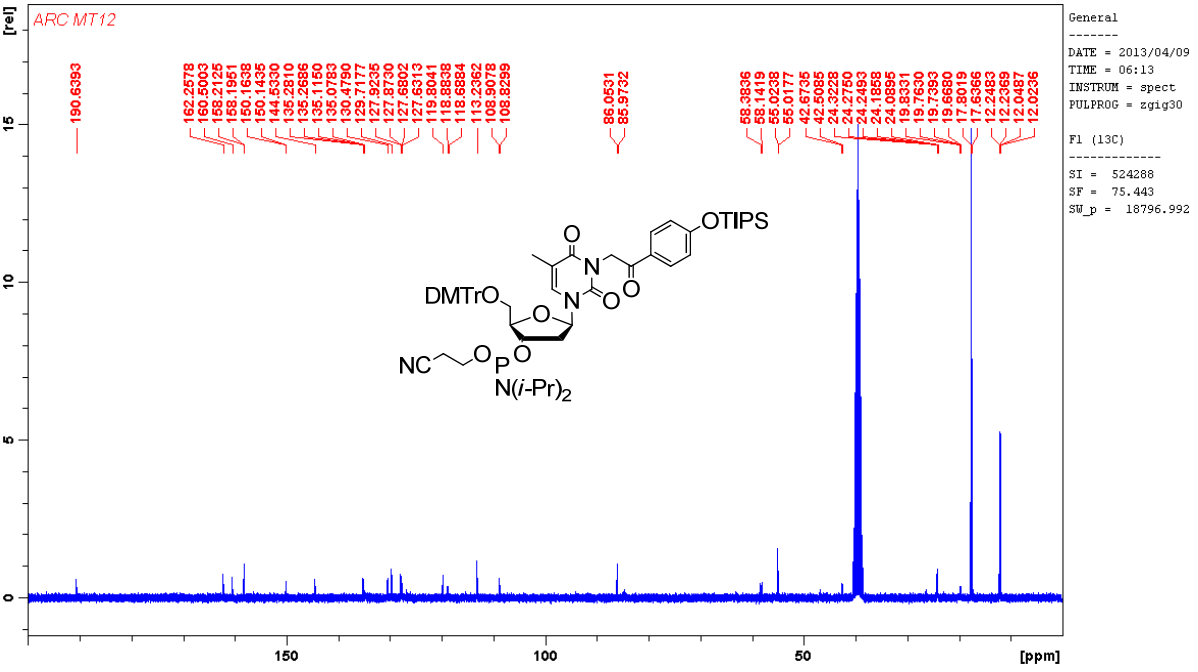
^{1}H NMR of compound **5**



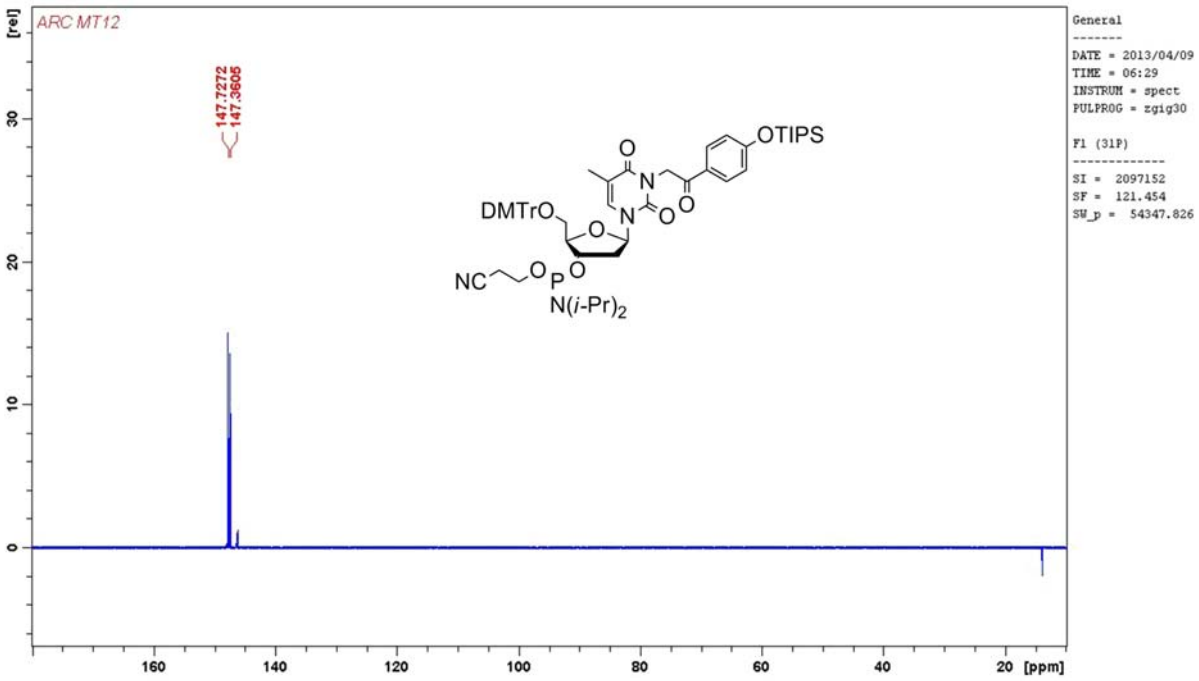
^{13}C NMR of compound **5**



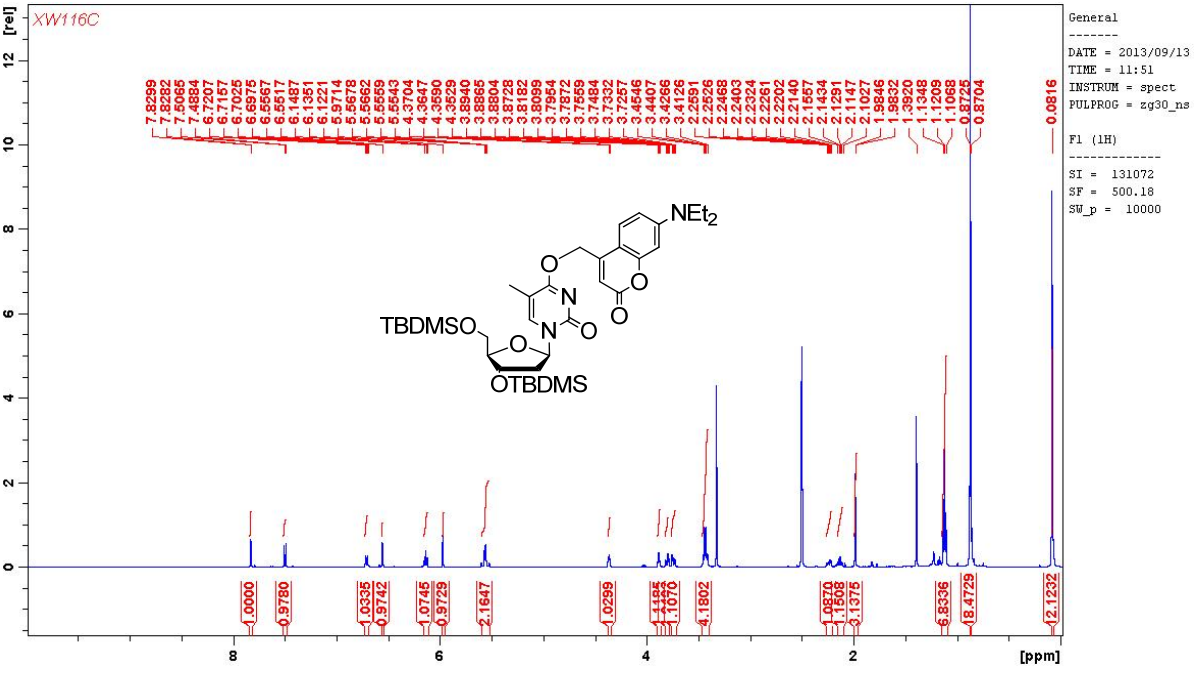
^{1}H NMR of compound **6**



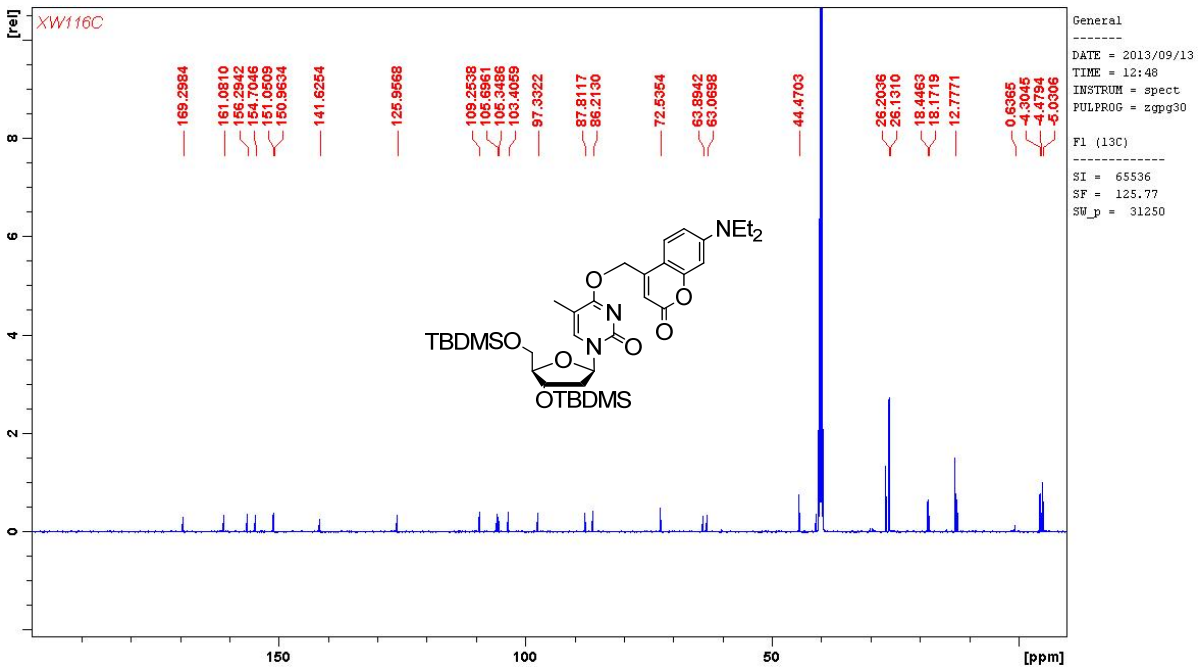
^{13}C NMR of compound **6**



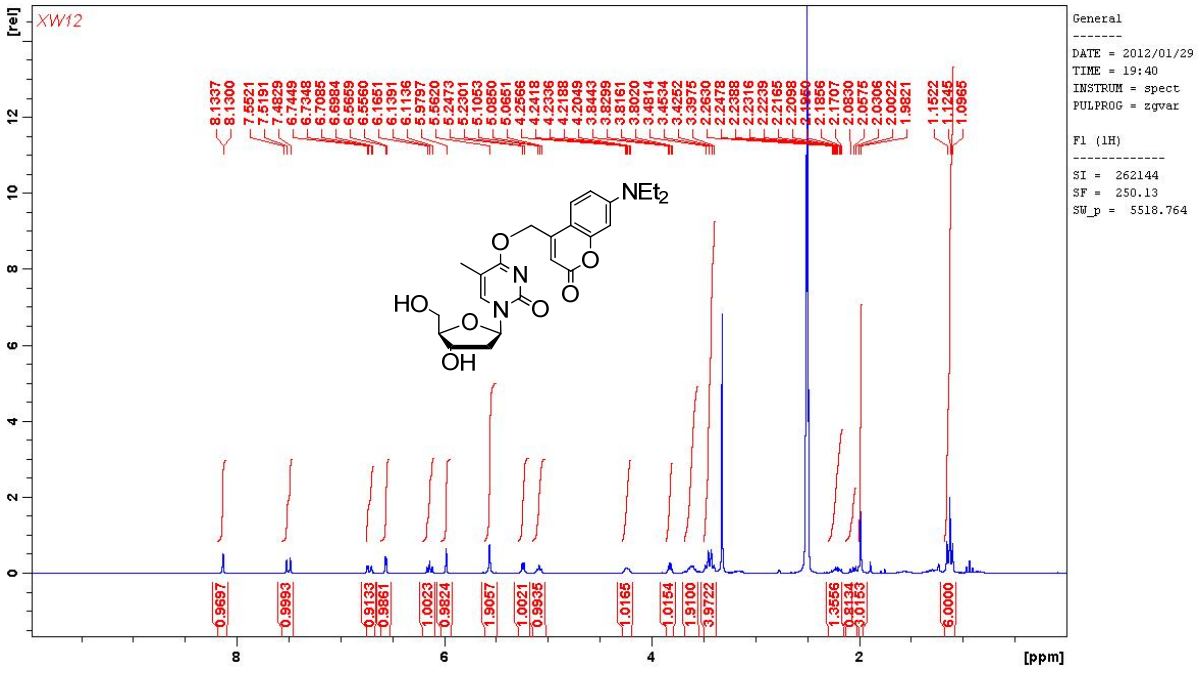
^{31}P NMR of compound **6**



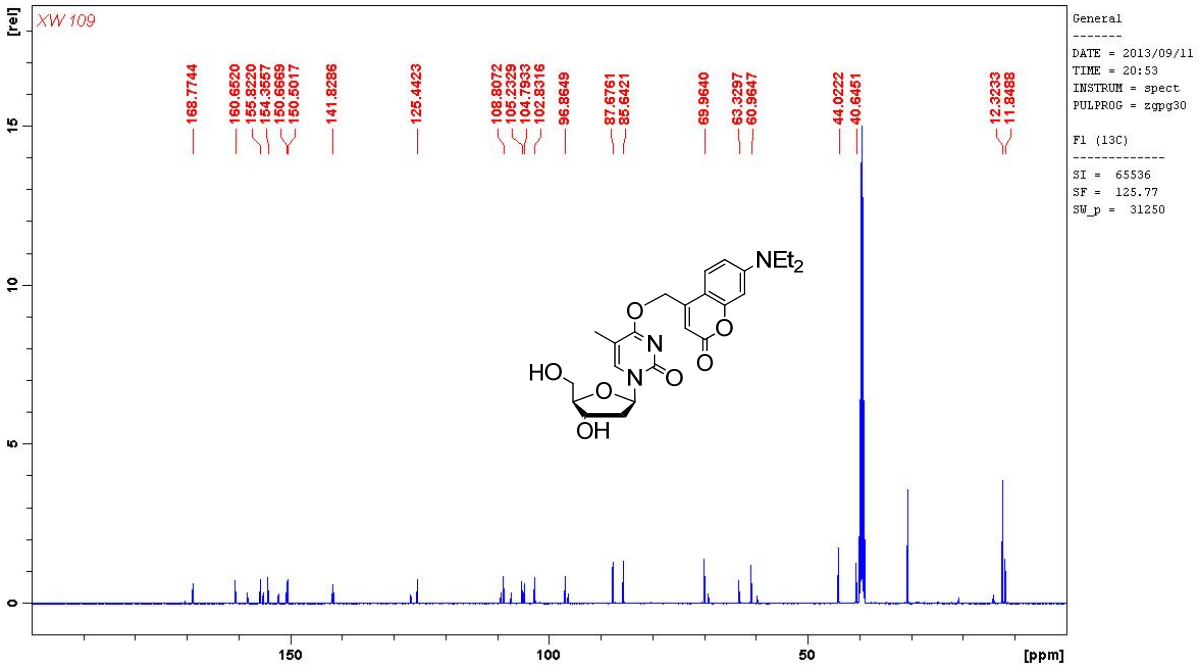
^{1}H NMR of compound **10**



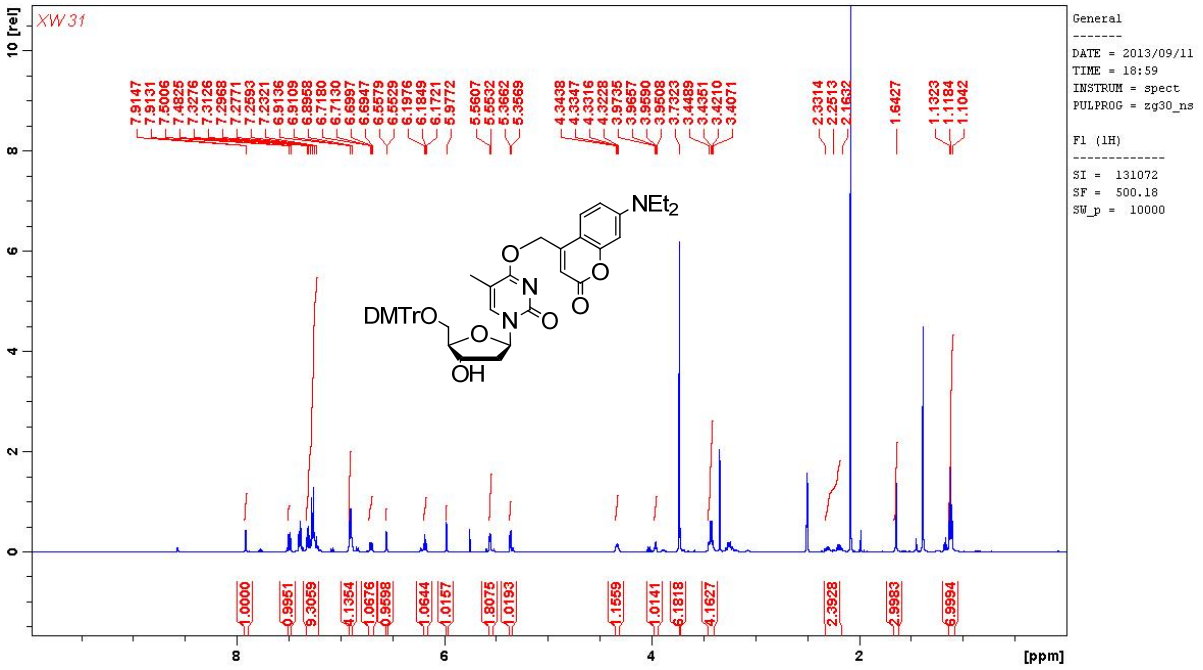
^{13}C NMR of compound **10**



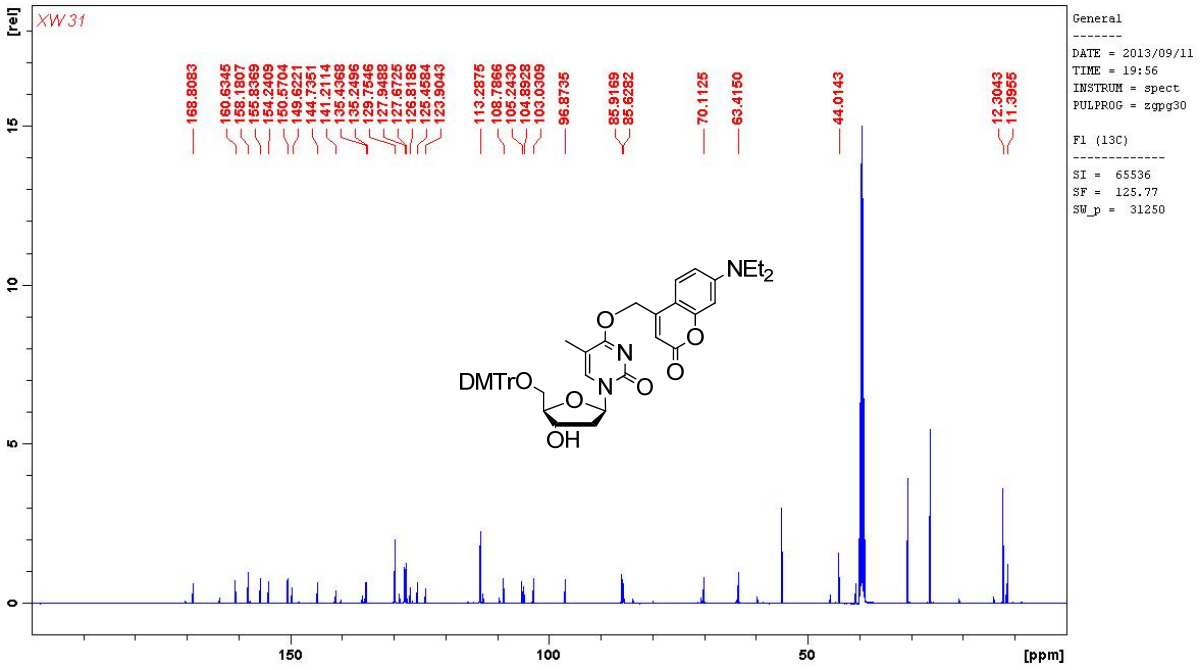
^{1}H NMR of compound **11**



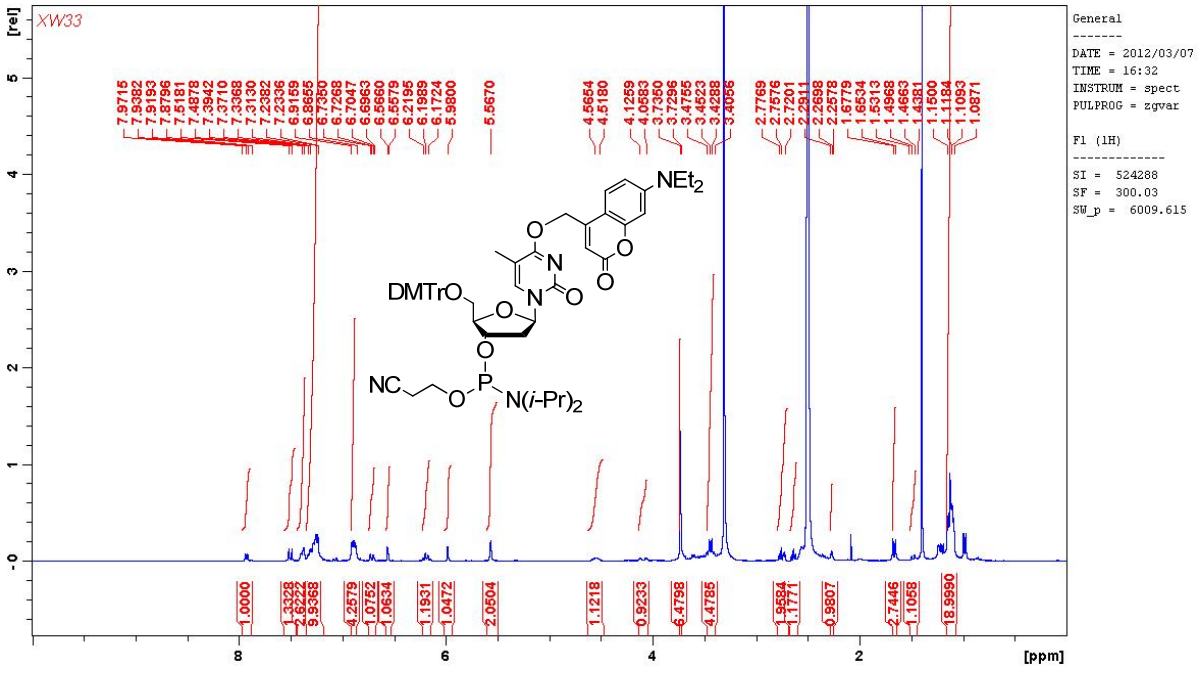
^{13}C NMR of compound **11**



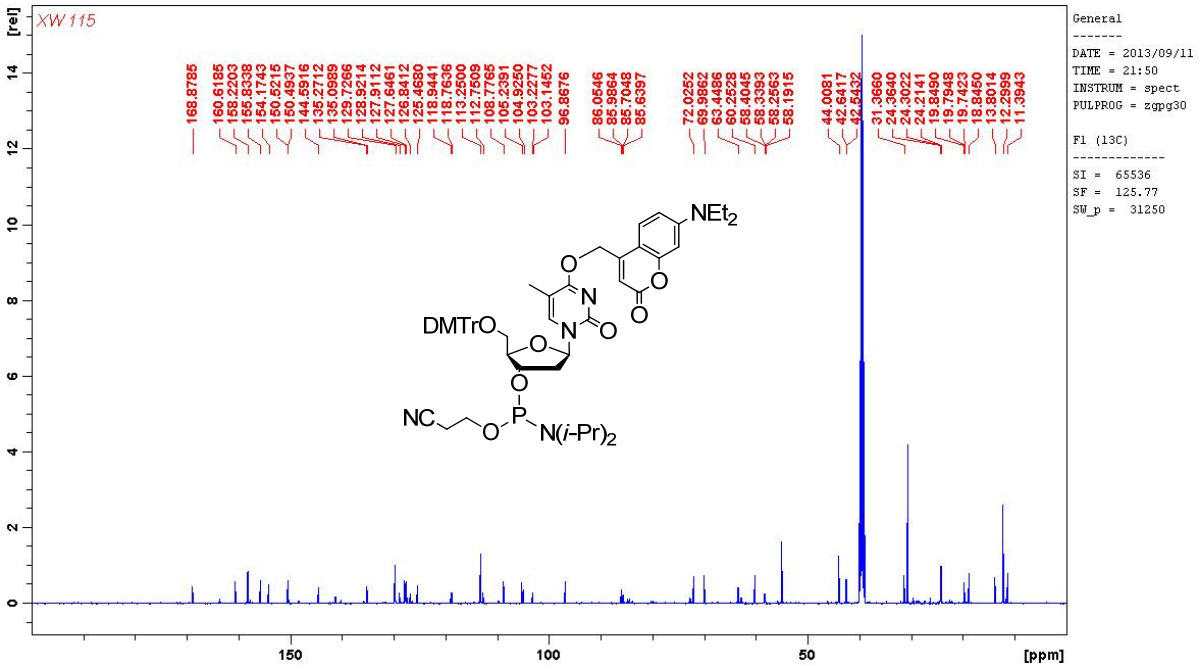
^{1}H NMR of compound **12**



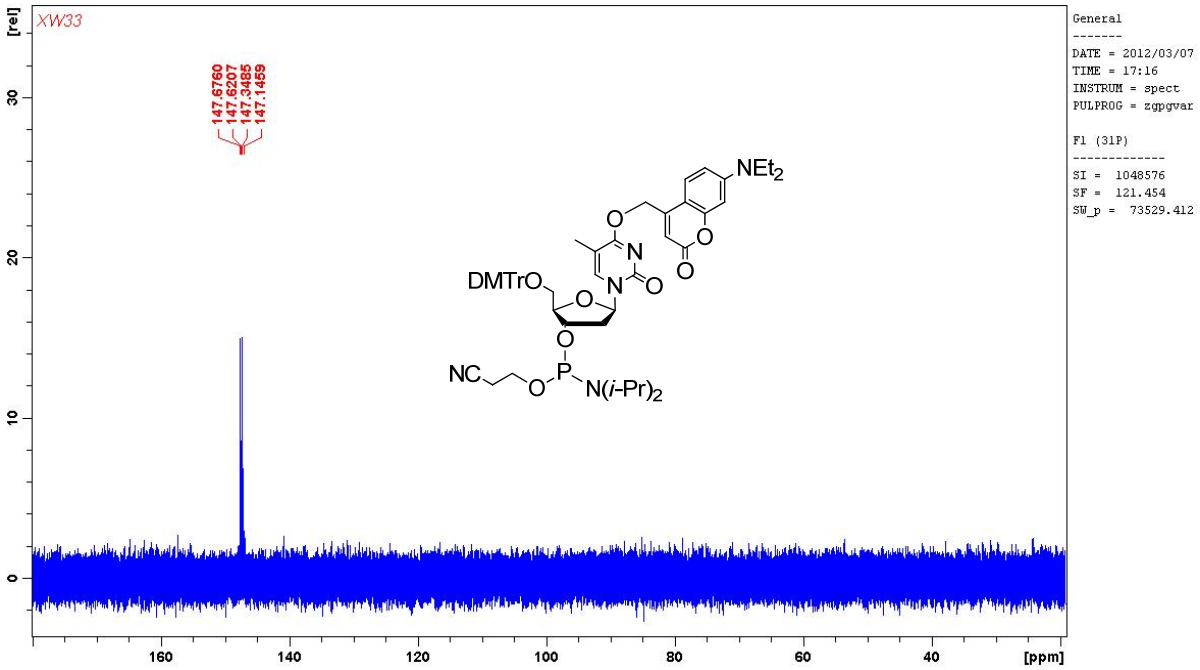
^{13}C NMR of compound **12**



^{1}H NMR of compound **13**

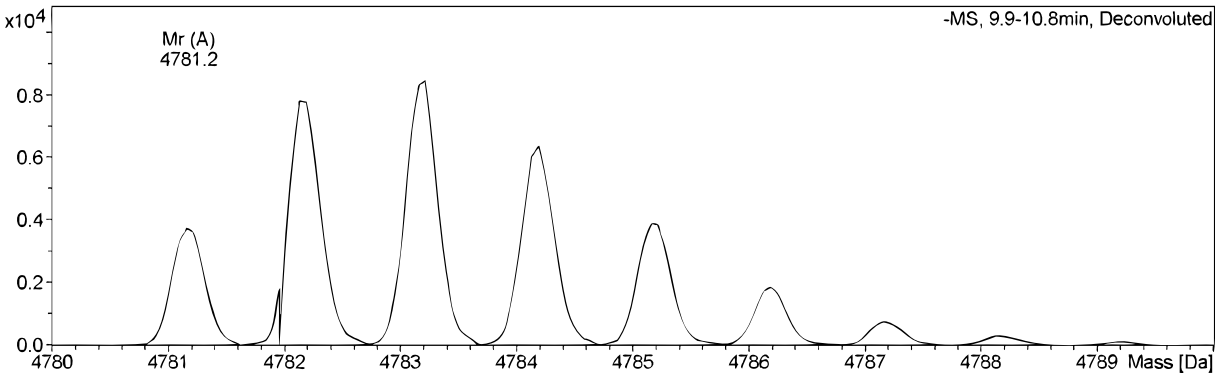


^{13}C NMR of compound **13**

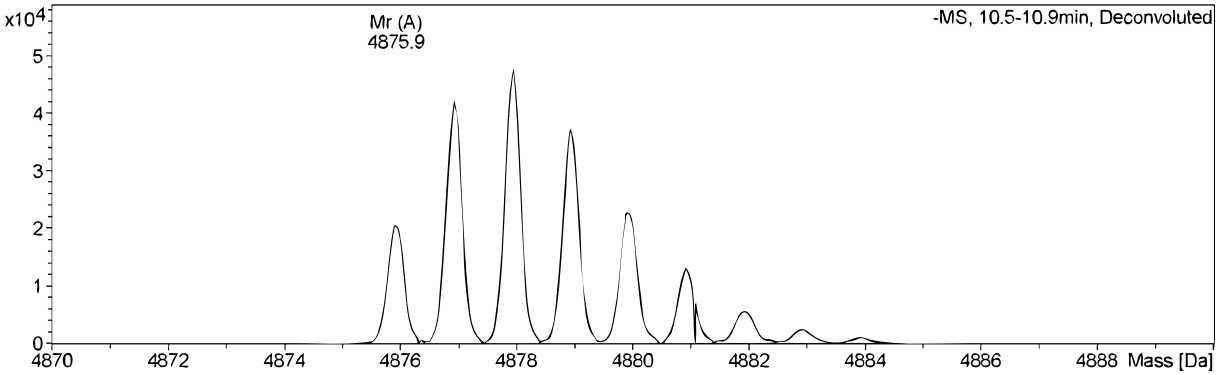


^{31}P NMR of compound **13**

Mass spectra of caged oligonucleotides



5’-GCA TAA AT^{NpHP}A AAG GTG-3’



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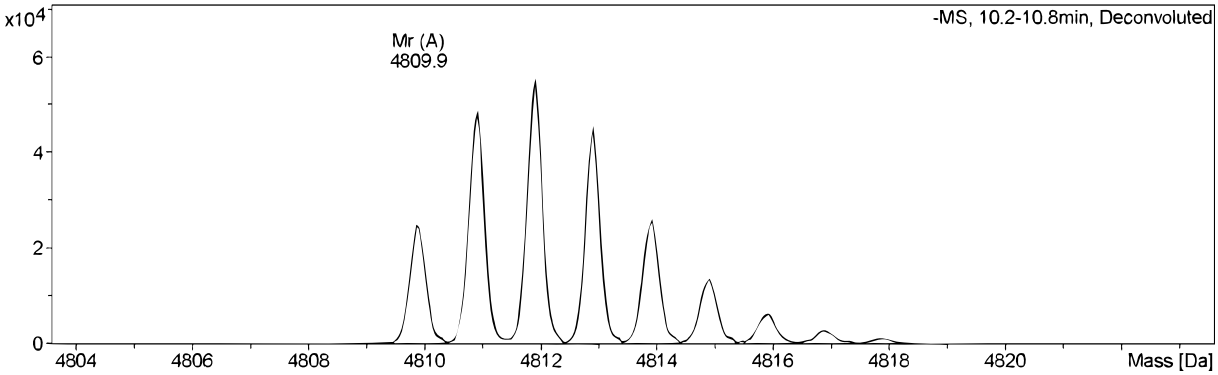

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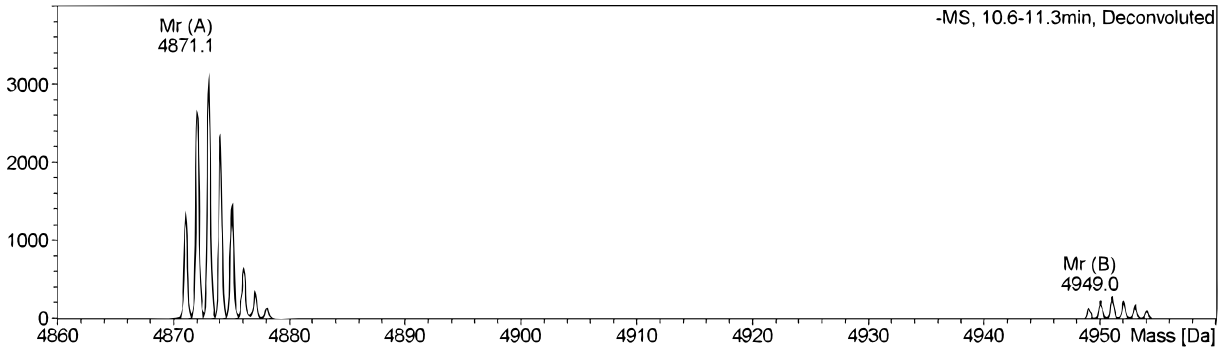

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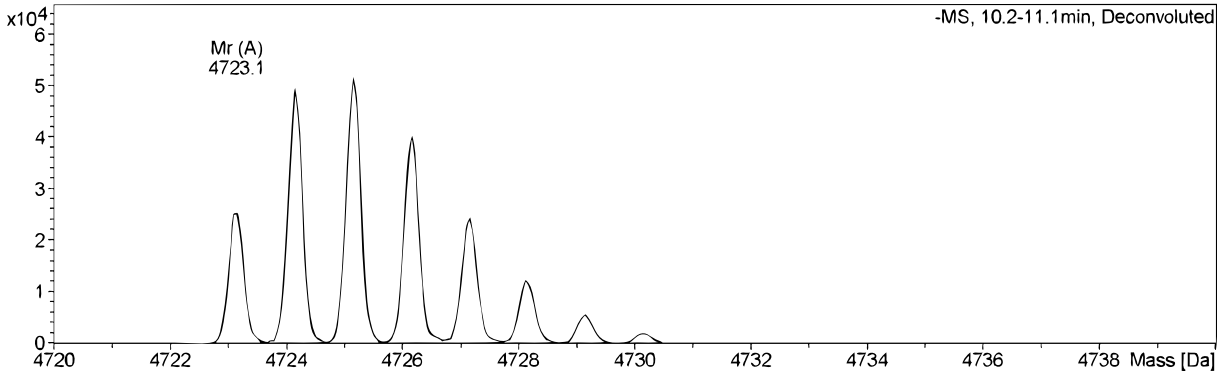

5´-GCA TAT AT^{NpHP}A TAC GTG-3´

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