Supplementary material

#ONT	Sequence 5'→3'
1	tggacgatatcccgcaagaggcccggcagtaccggcataaccaagcctatgcctacagc
2	P-ccgaggatgacgatgagcgcattgttagatttcatacacggtgcctgactgcgttagcaatt
3	P-atccagggcgacggtg
4	catcctcggcaccgtcaccctggatgctgtaggcatag
5	aattgctaacgcagtcaggcaccgtgtatgaaatctaacaatgcgctcatcgtcatcctcgg
6	P-gctgtaggcataggcttggttatgccggtactgccgggcctcttgcgggatatcgtcca
7	P-caccgtcgccctggat
8	gcctacagcatccagggcgacggtgccgaggatg
9	gggggctcggcaccgtcaccctggatgctgtagg-P
10	gggggtcaggcaccgtgtatgaaatctaacaat-P

Table 1S**.** Sequences of oligodeoxyribonucleotides used for synthesis of 137mer non modified DNA and DNA bearing bulky adducts. Flanking ONTs 1, 2 were used to synthesize "upper" strand (strand I) via ligation using ONT 4 as template. Flanking ONTs 5, 6 were used to synthesize "bottom" strand (strand II) via ligation using ONT 8 as template. ONTs 3 and 7 were used to synthesize non-modified control strands. ONTs 9 and 10 were used in excision assay as specific and non-specific templates.

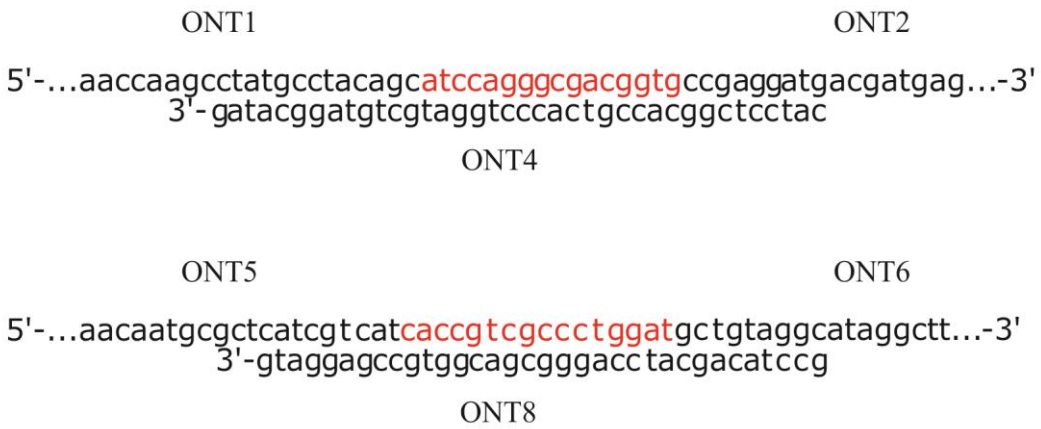

Figure 1S. Scheme of oligonucleotide annealing.

Organic synthesis

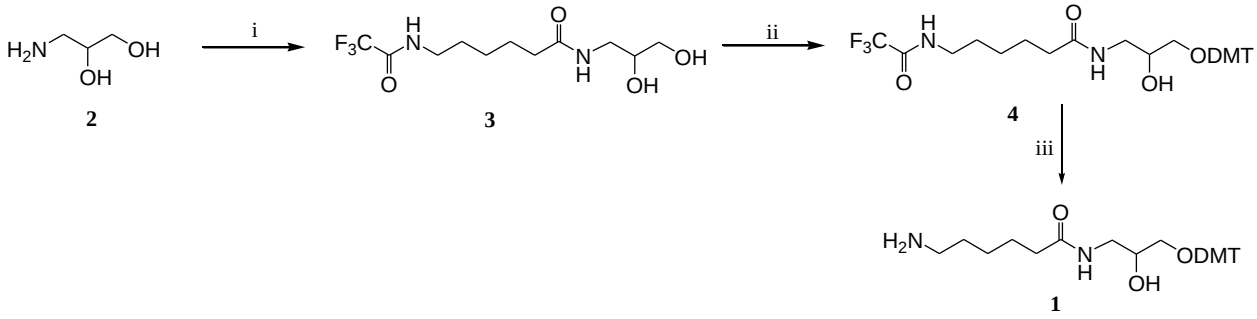

Figure 2S. Reagents and conditions: i) *N*-hydroxysuccinimide ester of TFA-NH-$(CH_2)_5$-COOH, Et_3N, DMF; ii) DMTCl, pyridine; iii) aq. NH_3 (25 %), pyridine.

1. Compound 4 (Fig. 2S)

3-amino-1,2-propanediol (700 mg, 7.68 mol) was dissolved in 5 ml DMF, 1.33 ml (9.6 mmol) of triethylamine was added. Then *N*-hydroxysuccinimide ester of TFA-NH-$(CH_2)_5$-COOH (3.11 g, 9.6 mmol) in 10 ml DMF was added and the solution was stirred for 48 h at room temperature. The solvent was removed under reduced pressure and the residue was coevaporated twice with pyridine, dissolved in dry pyridine (10 ml). To stirred solution 4,4'-dimetoxytrityl chloride (2.6 g, 7.68 mmol) in five portions at 1-2 h was added. The mixture was allowed to react for 24 h and then was concentrated to 1-2 mL in vacuo. The residue was diluted with CH_2Cl_2 (50 ml), washed with 5% $NaHCO_3$ and brine, dried over Na_2SO_4 (anh.). The solvent was removed by rotary evaporation and the crude product was purified by silica gel column chromatography (0 —>5% MeOH in CH_2Cl_2, 0.1% pyridine). Fractions containing the product were combined and evaporated in vacuo. The residue was then dissolved in dichloromethane (2 mL) and the product was precipitated with a ten-fold volume of hexane to give **4** (2.57 g, 55.5%) as a beige foam. TLC (CH_2Cl_2/EtOH 95:5) R_f 0.27.
^{1}H NMR ($CDCl_3$, δ): 7.47-7.19 [9H, m, ArH, DMTr], 6.82 [4H, bd, J 8.8, DMTr], 5.73 [1H, br t, NH], 3.87 [1H, m, CHOH], 3.77 [6H, s, OCH_3, DMT], 3.57-3.49, 3.24-3.16 [2H, m, $NCH_2CH(OH)$], 3.33 [2H, q, J 6.7, $NCH_2(CH_2)_4C(O)$], 3.12 [2H, m, CH_2O], 2.09 [2H, t, J 7.1, $N(CH_2)_4CH_2C(O)$], 1.57 [4H, m, $NCH_2CH_2CH_2CH_2CH_2C(O)$], 1.32 [2H, m, $N(CH_2)_2CH_2(CH_2)_2C(O)$]; ESI MS ($M + Na^+$): 625.9 (calcd 625.2).

2. Compound 1 (Fig. 2S)

Compound **4** (2.57 g, 4.26 mmol) was dissolved in a mixture of pyridine (10 ml) and concentrated aqueous ammonia (9 mL). The reaction mixture was stirred 3 days at room temperature. The reaction was monitored by TLC ((CH_2Cl_2/EtOH 95:5)). To stirred solution concentrated aqueous ammonia twice (x 5 ml) was added. After disappearance of the starting material the reaction mixture was evaporated in vacuo. The residue was diluted with CH_2Cl_2 (50 ml), washed with 5% $NaHCO_3$ and brine, dried over Na_2SO_4 (anh.). The solvent was removed by rotary evaporation. The residue was then dissolved in dichloromethane (2 mL) and the product was precipitated with a ten-fold volume of hexane to give **1** (1.63 g, 75.3%) as a beige foam.
^{1}H NMR ($CDCl_3$, δ): 7.46-7.22 [9H, m, Ar*H*, DMTr], 6.84 [4H, bd, *J* 8.8, DMTr], 6.01 [1H, br t, N*H*], 3.88 [1H, m, C*H*OH], 3.79 [6H, s, OCH_3, DMT], 3.63-3.52, 3.27-3.19 [2H, m,

NCH_2CH(OH)], 3.16 [2H, m, CH_2O], 2.69 [2H, m, NCH_2(CH$_2$)$_4$C(O)], 2.12 [2H, t, *J* 7.2, N(CH$_2$)$_4$CH_2C(O)], 1.59 [2H, m, NCH$_2$CH_2(CH$_2$)$_3$C(O)], 1.47 [2H, m, NCH$_2$(CH$_2$)$_2$CH_2CH$_2$C(O)], 1.32 [2H, m, N(CH$_2$)$_2$CH_2(CH$_2$)$_2$C(O)]; ESI MS (M + H$^+$): 507.0 (calcd 506.3).

3. Compound 5 (Fig. 3S)

To a magnetically stirred solution of 9-anthracenecarboxylic acid (600 mg, 2.7 mmol) and *N*-hydroxybenzotriazole (437.4 mg, 3.24 mmol) in CH_2Cl_2 (10 mL) was added a solution of DCC (667.4 mg, 3.24 mmol) in CH_2Cl_2 (4 mL), the mixture was stirred for 4 h. The *N,N'*-dicyclohexylurea formed was filtered off and washed with CH_2C1_2, and the combined organic solutions were evaporated under reduced pressure. The crude product was used in next reaction without additional purification.
Compound **1** (1.32 g, 2.6 mmol) was dissolved in 10 ml CH_2Cl_2 (+ 0.1% pyridine), triethylamine (362 µl, 2.6 mmol) and activated ester of 9-anthracenecarboxylic acid (2.7 mmol) in 10 ml CH_2Cl_2 were added. The solution was stirred for 24 h at room temperature. The reaction was monitored by TLC (system A). Then the solvent was evaporated and the residue was purified by silica gel column chromatography (0 —>5% MeOH in CH_2Cl_2, 0.1% pyridine). Fractions containing the product were combined and evaporated in vacuo. The residue was then dissolved in dichloromethane (2 mL) and the product was precipitated with a ten-fold volume of hexane to give **5** (1.53 g, 82.8%) as a beige foam. TLC (CH_2Cl_2/EtOH 9:1) R_f 0.63.
^{1}H NMR ($CDCl_3$, δ): 8.44 [1H, s, ANT-*H*^{10}], 8.04 [2H, d, *J* 8.3, ANT-*H*^{1,8}], 7.98 [2H, d, *J* 8.3, ANT-*H*^{4,5}], 7.54-7.43 [4H, m, ANT-*H*^{2,3,6,7}], 7.31-7.19 [9H, m, Ar*H*, DMT], 6.81 [4H, d, *J* 8.8, Ar*H*, DMT], 6.28 [1H, br t, N*H*], 5.80 [1H, br t, N*H*], 3.78 [1H, m, C*H*OH], 3.76 [6H, s, OCH_3], 3.67 [2H, m, NCH_2(CH$_2$)$_4$C(O)], 3.50-3.42, 3.18-3.13 [2H, m, NCH_2CH(OH)], 3.08 [2H, m, CH_2O], 2.11 [2H, t, *J* 7.3, N(CH$_2$)$_4$CH_2C(O)], 1.43 [2H, m, NCH$_2$CH_2(CH$_2$)$_3$C(O)], 1.27 [m, 4H, N(CH$_2$)$_2$(CH_2)$_2$CH$_2$C(O)]; MALDI TOF MS (M + Na$^+$): 733.6 (calcd 733.3).

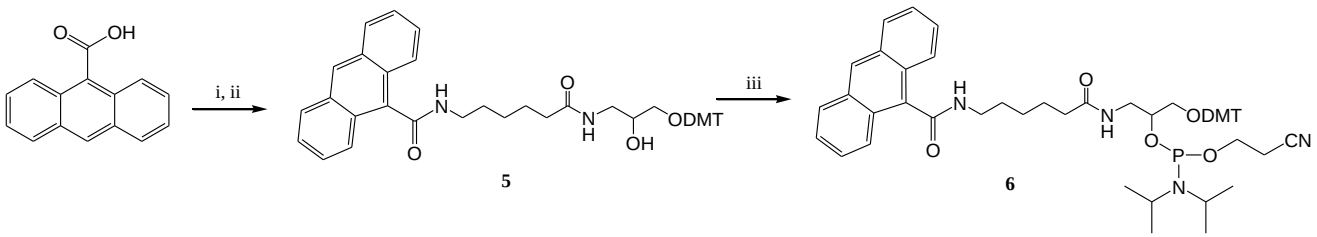

Figure 3S. Reagents and conditions: i) *N*-hydroxybenzotriazole, DCC, CH_2Cl_2, 2 h; ii) compound **1**, Et_3N, CH_2Cl_2; iii) 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphodiamidite, diisopropylammonium tetrazolide, CH_2Cl_2.

4. Compound 7 (Fig. 4S)

To a magnetically stirred solution of 5(6)-carboxy-3',6'-*O*-dipivaloylfluorescein (3 g, 5.5 mmol) and *N*-hydroxysuccinimide (695.7 mg, 6.05 mmol) in CH_2Cl_2 (20 mL) was added a solution of DCC (1.25 g, 6.05 mmol) in CH_2Cl_2 (5 mL), the mixture was ice-cooled and stirred for 2 h. The *N,N'*-dicyclohexylurea formed was filtered off and washed with CH_2C1_2, and the combined organic solutions were evaporated under reduced pressure. The crude product was used in next reaction without additional purification.
Compound **1** (2.66 g, 5.25 mmol) was dissolved in 15 ml CH_2Cl_2 (+ 0.1% pyridine), triethylamine (876 µl, 6.3 mmol) and *N*-hydroxysuccinimide ester of 5(6)-carboxy-3',6'-*O*-dipivaloylfluorescein (3.36 g, 5.25 mmol) in 10 ml CH_2Cl_2 were added. The solution was stirred for 24 h at room temperature. The reaction was monitored by TLC ((CH_2Cl_2/EtOH 95:5)). The

mixture was diluted with 25 ml CH_2Cl_2 (+ 0.1% pyridine), washed with aqueous KH_2PO_4 buffer (1M, pH 7.0, 30 ml) and brine (30 ml), dried over Na_2SO_4 (anh.). The solvent was evaporated and the residue was purified by silica gel column chromatography (0 —>10% MeOH in CH_2Cl_2, 0.1% pyridine). Fractions containing the product were combined and evaporated in vacuo. The residue was then dissolved in dichloromethane (2 mL) and the product was precipitated with a ten-fold volume of hexane to give **7** (3.12 g, 57.8%) as a beige foam. TLC (CH_2Cl_2/EtOH 95:5) R_f 0.37.

^{1}H NMR ($CDCl_3$, δ): 8.51 [0.6H, s, 5-isomer Flu-*H*^{4}], 8.35 [0.6H, d, *J* 8.1, 5-isomer Flu-*H*^{6}], 8.28 [0.4H, d, *J* 8.0, 6-isomer Flu-*H*^{5}], 8.18 [0.6H, bt, N*H*], 8.10 [0.4H, d, *J* 8.0, 6-isomer Flu-*H*^{4}], 8.01 [0.4H, bt, N*H*], 7.85 [0.4H, s, 6-isomer Flu-*H*^{7}], 7.52-7.18 [12H, m, 5-isomer Flu-*H*$^{7,4',5'}$, Ar*H*, DMT], 7.02-6.92 [4H, m, Flu-*H*$^{1',2',7',8'}$], 6.87 [4H, d, *J* 8.9, Ar*H*, DMT], 3.86 [1H, m, C*H*OH], 3.78 [6H, s, OCH_3], 3.48 [2H, m, NCH_2(CH$_2$)$_4$C(O)], 3.38-2.96 [4H, m, NCH_2CH(OH), CH_2O], 2.18 [2H, bt, N(CH$_2$)$_4$CH_2C(O)], 1.72-1.48 [4H, m, NCH$_2$CH_2CH$_2$CH_2CH$_2$C(O)], 1.36 [18H, s, C(CH_3)$_3$], 1.29 [m, 4H, N(CH$_2$)$_2$CH_2(CH$_2$)$_2$C(O)]; MALDI TOF MS (M + Na$^+$): 1055.4 (calcd 1055.4).

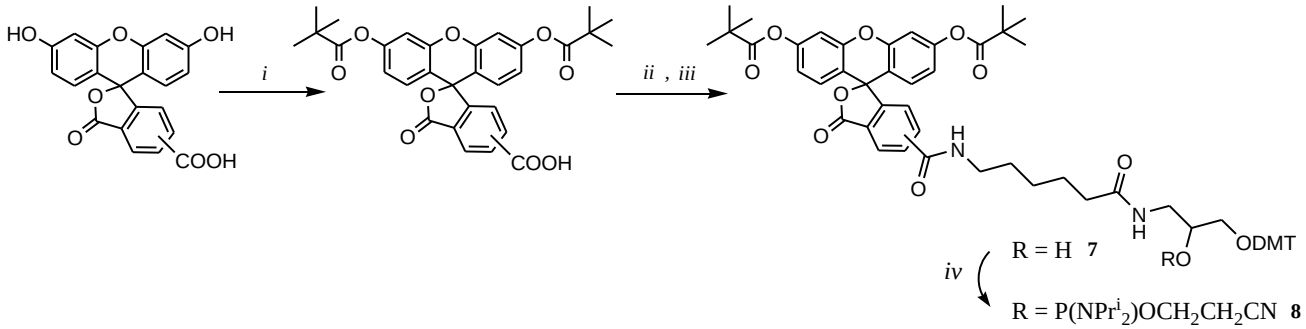

Figure. 4S. Reagents and conditions: i) Piv_2O, DIEA, DMF, 72 h; ii) *N*-hydroxysuccinimide, DCC, CH_2Cl_2, 2 h; iii) compound **1**, Et_3N, CH_2Cl_2; iv) 2-cyanoethyl-*N*,*N*,*N'*,*N'*-tetraisopropylphosphodiamidite, diisopropylammonium tetrazolide, CH_2Cl_2.

5. Synthesis of Phosphoramidites. General Procedure.

Compound **5** (**7**) (Fig. 3S/4S) (1.05 mmol) was dissolved in a freshly distilled CH_2Cl_2 (10 ml), diisopropylammonium tetrazolide (90 mg, 0.525 mmol) was added, followed by addition of *N*,*N*,*N'*,*N'*-tetraisopropyl-(2-cyano)ethyl phosphodiamidite (0.50 ml, 1.575 mmol). The reaction was monitored by TLC ((CH_2Cl_2/$(CH_3)_2CO$ 4:1)). After 2 h, the reaction mixture was evaporated, the residue was treated with hexane (30 ml), and the mixture was kept overnight at -20 ºC. Hexane was then decanted, and the residue was purified by chromatography on silica gel column with 1% Et_3N in hexane:CH_2Cl_2 (1:1). Elution with 1% Et_3N in CH_2Cl_2 gave the target fractions. Fractions containing the product were combined and evaporated in vacuo. The residue was then dissolved in dichloromethane (2 mL) and the product was precipitated with a ten-fold volume of hexane.

Compound 6. Yield **6** 74.9%, TLC (CH_2Cl_2/$(CH_3)_2CO$ 4:1) R_f 0.74. ^{1}H NMR ($CDCl_3$, δ) 8.48 [1H, s, ANT-*H*^{10}], 8.08 [2H, d, *J* 8.3, ANT-*H*^{1,8}], 8.02 [2H, d, *J* 8.3, ANT-*H*^{1,8}], 7.57-7.42 [4H, m, ANT-*H*^{2,3,6,7}], 7.31-7.19 [9H, m, Ar*H*, DMT], 6.82 [4H, m, *J* 8.8, Ar*H*, DMT]; 4.04 [1 H, m, C*H*OH], 3.77 [s, 6H, OCH_3], 3.76-3.04 [10H, m, NC*H*, POCH_2, NCH_2(CH$_2$)$_4$C(O), NHCH_2CH(OH)CH_2O], 2.58 (1H, t, *J* 6.1, CH_2CN), 2.43 (1H, t, *J* 6.3, CH_2CN), 2.15 [2H, m, N(CH$_2$)$_4$CH_2C(O)], 1.49 [2H, m, NCH$_2$CH_2(CH$_2$)$_3$C(O)], 1.29 [4H, m, N(CH$_2$)$_2$(CH_2)$_2$CH$_2$C(O)], 1.23-1.10 [12H, m, CH(CH_3)$_2$]; ^{31}P NMR ($CDCl_3$, δ) 149.38 (s), 148.93 (s); ESI MS (M + Et_3N): 1012.5 (calcd 1011.6).

Compound 8. Yield **8** 64.5%, TLC (CH_2Cl_2/$(CH_3)_2CO$ 4:1) R_f 0.81. ^1H NMR ($(CD_3)_2CO$, δ) 8.50 [0.6H, s, 5-isomer Flu-H^4], 8.34 [0.6H, d, *J* 8.1, 5-isomer Flu-H^6], 8.27 [0.4H, d, *J* 8.0, 6-isomer Flu-H^5], 8.14 [0.6H, bt, N*H*], 8.08 [0.4H, d, *J* 8.0, 6-isomer Flu-H^4], 7.98 [0.4H, bt, N*H*], 7.82 [0.4H, s, 6-isomer Flu-H^7], 7.52-7.18 [12H, m, 5-isomer Flu-$H^{7,4',5'}$, Ar*H*, DMT], 7.00-6.83 [8H m, Ar*H*, DMT, Flu-$H^{1',2',7',8'}$], 4.11 [1 H, m, C*H*OH], 3.76 [s, 6H, OCH_3], 3.69-3.05 [10H, m, NC*H*, $POCH_2$, $NCH_2(CH_2)_4C(O)$, $NHCH_2CH(OH)CH_2O$], 2.72 (1H, bt, CH_2CN), 2.62 (1H, bt, CH_2CN), 2.15 [2H, m, $N(CH_2)_4CH_2C(O)$], 1.60 [2H, m, $NCH_2CH_2(CH_2)_3C(O)$], 1.34 [18H, s, $C(CH_3)_3$], 1.20 [4H, m, $N(CH_2)_2(CH_2)_2CH_2C(O)$], 1.11 [12H, m, $CH(CH_3)_2$]; ^{31}P NMR ($(CD_3)_2CO$, δ) 150.20 (s), 149.82 (s); ESI MS (M + Et_3N): 1334.1 (calcd 1333.7).

6. Oligonucleotide synthesis

Oligonucleotide synthesis was performed on an ASM-800 DNA/RNA synthesizer (Biosset) on a 0,1 μmolar scale. Standard phosphoramidite chemistry was used [24, 25]. This consist of cycles of four chemical reactions: a) removal of the DMTr-group with 2% dichloroacetic acid in dichloromethane; b) coupling of phosphoramidite; c) capping with acetic anhydride and 1,4-dimethylaminopyridine; and d) oxidation of P(III) phosphite triester with 0,01M iodine in tetrahydrofurane/pyridine/water to stable P(V) phosphate. Purification was accomplished using RP-Cartridge (ChemGenes Inc.) according to recommended procedures. Oligonucleotide derivatives were analyzed by gel electrophoresis (20% PAGE-denaturing) and in every case demonstrated single band purity.