Supporting Information for *Angew. Chem. Int. Ed.* Z 17607

Experimental part

Melting point are reported uncorrected. Chemical shifts are reported in ppm relative to the residual signal of the solvent, except for the ^{31}P-NMR chemical shifts, which are relative to the signal of orthophosphoric acid as external reference. Satisfactory elemental analysis(C, H, N) were obtained for the intermediates **5**, **6** and **7**.

Synthesis of the 5-deazaflavin derivatives

***N*-(6-hydroxyhexyl)aniline 4**: A mixture of aniline (60 g, 644 mmol) and 6-chlorohexanol (10 g, 73.2 mmol) was stirred at 120°C for 20 h. After cooling and addition of dichloromethane (100 mL), the solution was washed with aqueous Na_2CO_3 (10%, 40 mL). The aqueous layer was extracted with dichloromethane (2 x 100 mL). The combined organic extracts were dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel in dichloromethane for obtaining pure compound **4** (10.4 g, 53.9 mmol, 74%). This compound can be purified by distillation under reduced pressure. Mp: 46-47 °C; ^{1}H NMR (300 MHz, $CDCl_3$) *d* = 7.25-7.10 (2H, m, ArH), 6.75-6.50 (3H, m, 3ArH), 3.63 (2H, t, CH_2), 3.10 (2H, t, CH_2), 2.46 (1H, br s), 1.60 (4H, m, 2CH_2), 1.40 (4H, m, 2CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) *d* = 148.2; CH: 129.0, 117.0, 112.7; CH_2: 62.3, 43.8, 32.4, 29.3, 26.7, 25.4; LRMS [FAB+, NBA] *m/z* = 193 $[M+H]^+$.

6-[*N*-(6-hydroxyhexyl)anilino]uracil 5: To a solution of compound **4** (16.92 g, 88 mmol) in ethanol/water (2:1; 150 mL) refluxed under argon, 6-chlorouracil (5 g, 34 mmol) was added with stirring. After 38 h reflux, the solution was concentrated and then refluxed again for 6 h. After evaporation of ethanol , aqueous NaOH (10%) was added to reach pH 11. The resulting solution was extracted with dichloromethane (3x100 mL) for removing unreacted starting compound **4**.

Aqueous HCl was added to the aqueous layer to reach pH 5. The resulting precipitate was dissolved after addition of dichloromethane (300 mL) and the aqueous layer was extracted with a dichloromethane/ethanol mixture (9:1; 3 x 50 mL). After evaporation of the organic extracts, pure compound 2 was obtained (6.65 g, 21.9 mmol, 64%). Mp: decomposition; ^{1}H NMR (200 MHz, $CDCl_3$) ***d*** = 7.50 (2H, m, 2ArH), 7.42 (1H, m, ArH), 7.29 (2H, m, 2ArH), 4.64 (1H, s, U-H5), 3.66 (2H, t, CH_2), 3.51 (2H, t, CH_2), 1.63 (2H, m, CH_2), 1.50 (2H, m, CH_2), 1.35 (4H, m, $2CH_2$); LRMS [FAB+, glycerol] *m/z* = 304 $[M+H]^+$.

5-deazaflavin 6:

- Acetylation of derivative 5: To a solution of compound **5** (4 g, 13.2 mmol) in pyridine (25 mL) cooled at 0°C, acetic anhydride (11 mL) was added. The solution was stirred at room temperature for 24 h and then water was added (50 mL) at 0°C. The solution was extracted with dichloromethane (200 mL and then 2x75 mL). The combined organic extracts were dried over $MgSO_4$ and evaporated. Residual pyridine was removed by three coevaporations with toluene: LRMS [FAB+, glycerol] *m/z* = 346 $[M+H]^+$.

- Cyclisation: To a solution in DMF (25 mL) of the crude acetylated compound obtained previously, phosphorus oxychloride was added (7 mL). The solution was stirred 30 min at room temperature and then 30 min at 100°C in the dark. After cooling, the resulting mixture was poored in a water-ice mixture and concentrated aqueous ammonia was added to reach pH 6. The acetylated 5-deazaflavin derivative which precipitated was collected by filtration, washed with water and dried: ^{1}H NMR (300 MHz, MeOH-*d*4) ***d*** = 9.03 (1H, s, H5), 8.11 (1H, m, ArH), 7.98 (2H, m, 2ArH), 7.58 (1H, m, ArH), 4.83 (2H, m, CH_2), 4.07 (2H, m, CH_2), 2.00 (3H, s, CH_3), 1.87 (2H, m, CH_2), 1.67 (2H, m, CH_2), 1.57-1.48 (4H, m, $2CH_2$); LRMS [FAB+, glycerol] *m/z* = 356 $[M+H]^+$.

- Deacetylation, 5-deazaflavin 6: The acetylated 5-deazaflavin derivative obtained previously was dissolved in ethanolic KOH (0.1 M, 500 mL) and the solution was stirred at room temperature for 2 h in the dark. A saturated aqueous ammonium chloride solution was added to reach pH 6. After evaporation, the solid was washed with a minimal amount of cold water. A first fraction of the 5-deazaflavin **6** was obtained after crystallization from water/ethanol (50:50, 80 mL) and then a second fraction after evaporation of the filtrate and crystallization (2.25 g, 7.19 mmol, 55% for the three steps). Mp: 234-235 °C; ^{1}H NMR (300 MHz, DMSO-*d*6) *d* = 11.0 (1H, s, NH), 8.98 (1H, s, H5), 8.17 (1H, m, ArH); 7.94-7.92 (2H, m, 2ArH), 7.53 (1H, m, ArH), 4.69 (2H, m, CH_2), 4.31 (1H, t, OH), 3.37 (2H, m, CH_2), 1.71 (2H, m, CH_2), 1.44 (6H, m, 3 CH_2); LRMS [FAB+, glycerol] *m/z* = 314 $[M+H]^+$.

Flavin H-phosphonate 7

Compound **6** (300 mg, 0.957 mmol) was dissolved in dry pyridine (10 mL) under argon in the dark. To the solution, were added phosphorous acid (1.3 g, 12.3 mmol) and then the 2,4,6-triiospropylbenzenesulfonyl chloride (0.160 mmol; **a**: *para*-toluenesulfonyl chloride, 30.5 mg; **b**: 2,4,6-triisopropylbenzenesulfonyl chloride (1.3 g, 4.18 mmol). The mixture was stirred at room temperature for 48 h and then methanol was added (5 mL) and then dichloromethane (50 mL). Compound **7** was extracted with water (20 mL and then 2x10 mL). After evaporation, the residue was chromatographed on a short column of C_{18} reversed phase in water-methanol (80:20) to yield compound **7** (acid form; 305 mg, 0.81 mmol, 85%). Mp 131-132°C (dec.); ^{31}P NMR (122 MHz, D_2O) *d* = 7.76 (d, $J_{P\text{-}H}$ = 635 Hz); ^{1}H NMR (300 MHz, D_2O) *d* = 8.91 (1H, s, H5), 8.0-7.8 (3H, m, 3ArH), 7.47 (1H, m, ArH), 6.29 (1H, d, $J_{H\text{-}P}$ = 635 Hz), 4.67 (2H, m, CH_2), 3.69 (2H, m, CH_2), 1.70 (2H, m, CH_2), 1.48 (2H, m, CH_2), 1.30 (4H, m, 2CH_2); ^{1}H NMR (300 MHz, DMSO-*d*6) *d* = 11.1 (1H, s, NH); LRMS [FAB+, NBA] *m/z* = 378 $[M+H]^+$.

Synthesis of the deazaflavin-oligonucleotide conjugates 1 and 2

Columns containing the starting oligonucleotides were obtained from Eurogentec S.A. (phosphoramidite method, 1µmol scale, DMTr protecting group removed). The solid support was dried by flushing argon through the column and was put in a test tube which was stopped with a rubber septum. A mixture of the H-phosphonate **7** (0.052 mmol; mixture of acid and its pyridinium salt dried by coevaporation with toluene) and 1-adamantanecarboxylic acid chloride

(10.4 mg, 0.058 mmol) in dry pyridine was added to the solid support. The mixture was stirred at room temperature for 30 min. The solid support was isolated by filtration and washed with pyridine and then acetonitrile. After successive treatments with oxidizing reagents: I_2 (0.2 *M*) in THF for 5 min, I_2 (0.2 *M*) in *N*-methylmorpholine-water-THF (1:1:8) for 10 min and then I_2 (0.2 *M*) in triethylamine-water-THF (1:1:8) for 3 min, the support was washed with acetonitrile. The oligonucleotide was cleaved from the support with 0.2 *M* sodium hydroxide in methanol-water 50:50 with stirring at room temperature. The supernatant was evaporated (neutralization with aqueous HCl after deprotection with NaOH). The residue was chromatographed on a short column of reversed phase (C_{18}-Sep-Pak® Waters, 1g cartridge) in water-methanol. The oligonucleotides were purified by two chromatographies (detection at 254 nm and 390 nm): first, on C_{18} reversed phase using a linear gradient of methanol (0% initially, then increasing 1.25%/min up to 35%) in 20 mM sodium phosphate buffer pH 6 at flow rate of 1mL/min; secondly, on polyanionic resin (MonoQ® Pharmacia) using a linear gradient of potassium chloride (20% initially, then increasing 0.9%/min up to 60%) in a mixture of sodium dihydrogenphosphate 20 mM pH 6-acetonitrile (80:20) at flow rate 1.5 mL/min. Finally, desalting (C_{18}-Sep-Pak® Waters, 1g cartridge) yielded oligonucleotide (conjugate **1**: 60%, conjugate **2**: 53%).

Conjugate 1, dFl-C_6-p-T_{11} (11 mer): ^{1}H NMR (500 MHz, D_2O) *d* = 8.83 (1H, s, dF-H5), 7.65-7.95 (4H, m, dF-ArH), 7.44 (11H, m, Thd-H6), 7.71 (1H, s, Thd-H6), 6.0 (10H, m, Thd-H1'), 5.72 (1H, m, Thd-H1'). ^{31}P NMR (122 MHz, D_2O) *d* = 1.4 (1P, s), -.2 (10 P, s). MS (ammonium salt), ESI and MALDI TOF: 3659 $[M+H]^+$.

Conjugate 2 (20 mer): MS (ammonium salt), ESI and MALDITOF: 6204 $[M+H]^+$.