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Supplementary Material

NMR peak assigments

5'-*O*-(4,4'-dimethoxytrityl)-riboflavin 4

^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.45 (1H, s, N*H*), 8.03 (1H, s, Ar*H*), 7.86 (1H, s, Ar*H*), 7.25 (9H, m, Ar*H* trityl), 6.78 (4H, m, Ar*H* trityl), 5.04 (2H, m, 1'-CH_2 or 5'-CH_2), 4.45 (1H, s, O*H*), 4.17 (1H, m, 2'-C*H* or 4'-C*H*), 3.95 (1H, m, 4'-C*H* or 2'-C*H*), 3.75 (6H, s, 2 OCH_3), 3.68 (2H, m, 3'-C*H* and O*H*), 3.45 (3H, m, 5'-CH_2 or 1'-CH_2 and O*H*), 2.45 (6H, s, 2 CH_3).

2',3',4'-*O*-acetyl-5'-(4,4'-dimethoxytrityl)-riboflavin 5

^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.00 (1H, s, N*H*), 7.53 (1H, s, Ar*H*), 7.35 (1H, s, Ar*H*), 7.23 (9H, m, Ar*H* trityl), 6.75 (4H, d, Ar*H* trityl), 5.60 (2H, m, 1'-CH_2 or 5'-CH_2), 5.32 (2H, m, 2 C*H*), 4.78 (1H, m, C*H*), 3.75 (6H, s, 2 OCH_3), 3.28 (2H, m, 5'-CH_2 or 1'-CH_2), 2.50 (2H, s, CH_2), 2.41 (2H, s, CH_2), 2.32 (2H, s, CH_2), 1.97 (3H, s, CH_3), 1.65 (3H, s, CH_3).

Synthesis of isoalloxazines 9a-b

- *N*-(6'-hydroxyhexyl)-3,4-dimethylaniline 6b: ^{1}H NMR (200 MHz, $CDCl_3$) δ = 6.90 (1H, d, J = 8Hz, Ar*H*), 6.40 (1H, d, J < 2Hz, Ar*H*), 6.34 (1H, dd, J = 8Hz, J < 2Hz, Ar*H*), 3.62 (2H, t, CH_2-OH or CH_2-NH), 3.35 (2H, m, O*H* and N*H*), 3.05 (2H, t, CH_2-NH or CH_2-OH), 2.16 (3H, s, CH_3), 2.12 (3H, s, CH_3), 1.55 (4H, m, 2 CH_2), 1.38 (4H, m, 2 CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 146.6, 137.2, 130.2 (Ar*C*H), 125.1, 114.6 (Ar*C*H), 110.2 (Ar*C*H), 62.8 (*C*H_2-OH or *C*H_2-NH), 44.2 (*C*H_2-NH or *C*H_2-OH), 32.6 (*C*H_2), 29.6 (*C*H_2), 27.0 (*C*H_2), 25.5 (*C*H_2), 20.0 (*C*H_3), 18.6 (*C*H_3).

- **6-[*N*-(6'-hydroxyhexyl)-3,4-xylidino]uracil 7b**: ^{1}H NMR (200 MHz, DMSO-*d*6) δ = 10.30 (1H, br s, N*H*), 10.10 (1H, br s, N*H*), 7.20 (1H, d, *J* = 4Hz, Ar*H*), 7.00 (1H, s, Ar*H*), 6.95 (1H, d, *J* = 4Hz, Ar*H*), 4.30 (1H, t, O*H*), 4.10 (1H, s, 5-C*H*), 3.55 (2H, t, 1'-CH_2 or 6'-CH_2), 3.35 (2H, t, 6'-CH_2 or 1'-CH_2), 2.20 (6H, s, 2 CH_3), 1.35 (4H, m, 2 CH_2), 1.25 (4H, m, 2 CH_2); ^{13}C NMR (75 MHz, DMSO-*d*6) δ = 164.3 (*C*=O), 154.6 (*C*=O), 151.3 (C_6), 139.7, 138.5, 136.4, 131.1 (Ar*C*H), 128.8 (Ar*C*H), 125.2 (Ar*C*H), 76.1 (5-*C*H), 60.8 (6'-*C*H_2 or 1'-*C*H_2), 51.3 (1'-*C*H_2 or 6'-*C*H_2), 32.5 (*C*H_2), 27.5 (*C*H_2), 25.9 (*C*H_2), 25.5 (*C*H_2), 19.7 (*C*H_3), 19.3 (*C*H_3).

- **Isoalloxazine 5-oxide 8b**:

^{1}H NMR (200 MHz, DMSO-*d*6) δ = 11.00 (1H, br s, N*H*), 8.07 (1H, s, Ar*H*), 7.76 (1H, s, Ar*H*), 4.75 (1H, br s, O*H*), 4.49 (2H, m, 1'-CH_2), 3.38 (2H, m, 6'-CH_2), 2.48 (3H, s, CH_3), 2.37 (3H, s, CH_3), 1.65 (2H, m, CH_2), 1.40 (6H, m, 3 CH_2).

- ***N*-(6'-hydroxyhexyl)isoalloxazine 9b**:

^{1}H NMR (300 MHz, DMSO-*d*6) δ = 11.24 (1H, br s, N*H*), 7.90 (1H, s, Ar*H*), 7.77 (1H, s, Ar*H*), 4.56 (2H, t, 1'-CH_2), 4.31 (1H, m, O*H*), 3.39 (2H, m, 6'-CH_2), 2.49 (3H, s, CH_3), 2.39 (3H, s, CH_3), 1.71 (2H, m, CH_2), 1.42 (6H, m, 3 CH_2); ^{13}C NMR (75 MHz, DMSO-*d*6) δ = 159.9, 155.6, 150.0, 146.5, 139.9, 135.7, 133.7, 130.9 (Ar*C*H), 130.7, 115.9 (Ar*C*H), 60.5 (6'-*C*H_2 or 1'-*C*H_2), 44.1 (1'-*C*H_2 or 6'-*C*H_2), 32.3 (*C*H_2), 26.46 (*C*H_2), 26.0 (*C*H_2), 25.2 (*C*H_2), 20.5 (*C*H_3), 18.7 (*C*H_3).

Flavin phosphoramidates 11a-b

Compound **11a**: ^{1}H NMR (200 MHz, CDCl3) δ = 8.40 (1H, br s, N*H*), 8.00 (1H, s, Ar*H*), 7.60 (1H, s, Ar*H*), 4.90 (2H, m, 1'-CH_2), 4.21 (4H, m, POCH_2 and 2 NC*H* [(iPr)$_2$]), 3.42 (2H, m, 3'-CH_2), 2.81 (2H, m, CH_2CN), 2.54 (3H, s, CH_3), 2.42 (3H, s, CH_3), 1.61 (2H, m, CH_2), 1.24 (12H, dd, 4 CH_3 [(iPr)$_2$]).

Compound **11b**: ^{1}H NMR (300 MHz, MeOD) δ = 8.10 (1H, br s, Ar*H*), 7.90 (1H, s, Ar*H*), 4.95 (2H, m, 1'-CH_2), 4.15 (4H, m, POCH_2 and 2 NC*H* [(iPr)$_2$]), 3.30 (2H, m, 6'-CH_2), 2.90 (2H, m, CH_2CN), 2.60 (3H, s, CH_3), 2.48 (3H, s, CH_3), 2.10 (2H, m, CH_2), 1.90 (2H, m, CH_2), 1.80 (4H, m, 2 CH_2), 1.20 (12H, dd, 4 CH_3 [(iPr)$_2$]).

Flavin H-phosphonates 12a-b

Compound **12a**: ^{1}H NMR (200 MHz, DMSO-*d*6) δ = 11.27 (1H, s, N*H*), 8.58 (d, PyH$^+$), 7.88 (1H, s, Ar*H*), 7.79 (1H, s, Ar*H*), 7.77 (m, PyH$^+$), 7.39 (m, PyH$^+$), 6.76 (1H, d, $J_{H\text{-}P}$ = 657 Hz, P*H*), 4.65 (2H, t, 1'-CH_2), 4.05 (2H, m, 3'-CH_2), 2.49 (3H, s, CH_3), 2.39 (3H, s, CH_3), 2.07 (2H, m, 2'-CH_2).

Compound **12b**: ^{1}H NMR (200 MHz, DMSO-*d*6) δ = 11.28 (1H, s, N*H*), 8.56 (d, PyH$^+$), 7.88 (1H, s, Ar*H*), 7.79 (1H, s, Ar*H*), 7.41 (m, PyH$^+$), 7.38 (m, PyH$^+$), 6.69 (1H, d, $J_{H\text{-}P}$ = 656 Hz, P*H*), 4.56 (2H, t, 1'-CH_2), 3.90 (2H, m, 6'-CH_2), 2.49 (3H, s, CH_3), 2.39 (3H, s, CH_3), 1.65 (2H, m, CH_2), 1.60 (2H, m, CH_2), 1.56 (4H, m, 2 CH_2); ^{13}C NMR (75 MHz, D$_2$O) δ = 163.3 (*C* PyH$^+$), 160.3, 153.5, 151.5, 150.1 (*C*PyH$^+$), 144.1 (*C* pyH$^+$), 142.1, 136.3, 136.2, 133.3 (Ar*C*H), 133.2, 130.3, 119.0 (Ar*C*H), 67.2 (6'-*C*H$_2$ or 1'-*C*H$_2$), 48.8 (1'-*C*H$_2$ or 6'-*C*H$_2$), 32.7 (*C*H$_2$), 29.3 (*C*H$_2$), 28.2 (*C*H$_2$), 27.6 (*C*H$_2$), 23.6 (*C*H$_3$), 21.8 (*C*H$_3$).

Flavin-thymidine H-phosphodiester 13

^{1}H NMR (500 MHz, CDCl3) two diastereoisomers A and B: δ = 8.67 (2H, s, 2 N*H*), 8.60 (2H, s, 2 N*H*), 8.05 (2H, s, 2 Ar*H*), 7.50 (1H, s, T$_A$6-*H* or T$_B$6-*H*), 7.44 (1H, s, T$_B$6-*H* or T$_A$6-*H*), 7.38 (2H, s, 2 Ar*H*), 6.90 (1H, d, $J_{H\text{-}P}$ = 708 Hz, P*H*), 6.89 (1H, d, $J_{H\text{-}P}$ = 708 Hz, P*H*), 6.35 (1H, dd, *J* = 9Hz, *J* = 5.6 Hz, T$_A$1'-*H*), 6.32 (1H, dd, *J* = 9Hz, *J* = 5.6 Hz, T$_B$1'-*H*), 5.25 (2H, m, 2 T3'-*H*), 4.67 (4H, br m, 2 F1'-CH_2), 4.35 (4H, m, 2 T5'-CH_2), 4.16 (2H, m, 2 T4'-*H*), 4.11 (4H, m, 2 F6'-CH_2), 2.55 (6H, s, 2 F-CH_3), 2.45 (6H, s, 2 F-CH_3), 2.43 (1H, m, T$_A$2'-*H*), 2.41 (1H, m, T$_B$2'-*H*), 2.21 (1H, m, T$_A$2'-*H*), 2.19 (1H, m, T$_B$2'-*H*), 2.08 (6H, s, 2 CH_3CO), 1.84 (4H,

m, 2 F2'-CH_2), 1.72 (4H, m, 2 F5'-CH_2), 1.57 (6H, s, 2 T-CH_3), 1.54 (4H, m, 2 F3'-CH_2), 1.51 (4H, m, 2 F4'-CH_2); H-phosphonate doublet: 200 MHz δ_A = 7.61, 6.19 (J_{HP} = 708 Hz), δ_B = 7.60, 6.19 (J_{HP} = 704 Hz); 300 MHz δ_A= 8.08, 5.72 (J_{HP} = 708 Hz), δ_B = 8.06, 5.72 (J_{HP} = 704 Hz); 500 MHz δ_A = 8.67, 5.13 (J_{HP} = 708 Hz), δ_B = 8.65, 5.13 (J_{HP} = 704 Hz).

Flavin-thymidine phosphodiester 15

Acetylated intermediate **14**: ^{1}H NMR (300 MHz, D_2O) δ = 7.62 (1H, s, Ar*H*), 7.58 (1H, s, Ar*H*), 7.35 (1H, s, T6-*H*), 5.82 (1H, m, T1'-*H*), 5.23 (1H, m, T3'-*H*), 4.45 (2H, brm, F1'-CH_2), 4.00 (2H, m, T5'-CH_2), 3.90 (1H, m, T4'-*H*), 3.86 (2H, m, F6'-CH_2), 2.51 (3H, s, F-CH_3), 2.39 (3H, s, F-CH_3), 2.24 (1H, m, T2'-*H* or T2"-*H*), 2.12 (4H, m, CH_3Ac and T2'-*H* or T2"-*H*), 1.74 (4H, m, 2 F-CH_2), 1.66 (3H, s, T-CH_3), 1.56 (4H, br m, 2 F-CH_2).

Compound **15**: ^{1}H NMR (500 MHz, D2O) δ = 7.74 (1H, s, Ar*H*), 7.66 (1H, s, Ar*H*), 7.33 (1H, d, T6-*H*), 5.86 (1H, q, T1'-*H*), 4.45 (2H, brm, F1'-CH_2), 4.03 (1H, m, T3'-*H*), 3.92 (1H, m, T4'-*H*), 3.90 (4H, m, T5'-CH_2 and F6'-CH_2), 2.54 (3H, s, F-CH_3), 2.43 (3H, s, F-CH_3), 2.20 (1H, m, T2'-*H* or T2"-*H*), 2.05 (1H, m, T2"*H* or T2'*H*), 1.64 (3H, s, T-CH_3), 1.80 (2H, m, F-CH_2), 1.71 (2H, m, F-CH_2), 1.58 (2H, m, F-CH_2), 1.40 (2H, m, F-CH_2); ^{13}C NMR-DEPT (75 MHz, D_2O) δ = 139.5 (T-*C*H), 133.6 (F-*C*H), 119.0 (F-*C*H), 88.7 (T-*C*H), 87.6 (T-*C*H), 74.0 (T-*C*H), 69.0 (*C*H_2), 68.0 (*C*H_2), 48.8 (F-*C*H_2), 42.0 (T-*C*H_2), 32.1 (F-*C*H_2), 29.5 (F-*C*H_2), 28.0 (F-*C*H_2), 27.0 (F-*C*H_2), 23.6 (F-*C*H_3), 21.6 (F-*C*H_3), 14.6 (T-*C*H_3).

Flavin-thymidine phosphorothioate 16

^{1}H NMR (300 MHz, D_2O) two diastereomers A and B: δ = 7.55 (4H, s, 4 Ar*H*), 7.36 (1H, s, T_A6-*H* or T_B6-*H*), 7.39 (1H, s, T_B6-*H* or T_A6-*H*), 5.95 (2H, m, 2 T1'-*H*), 4.67 (2H, m, 2 T3'-*H*), 4.47 (4H, brm, 2 F1'-CH_2), 4.10 (2H, m, 2 T4'-*H*), 3.97 (4H, m, 2 T5'-CH_2), 3.93 (4H, m, 2 F6'-CH_2), 2.50 (6H, s, 2 F-CH_3), 2.38 (6H, s, 2 F-CH_3), 2.20 (2H, m, 2 T2'-*H*), 2.10 (2H, m, 2 T2'-*H*), 1.71 (3H, s, T-CH_3), 1.69 (8H,

m, 4 F-CH_2), 1.68 (3H, s, T-CH_3), 1.56 (8H, m, 4 F-CH_2); ^{13}C NMR-DEPT (75 MHz, D_2O) δ = 139.7 (T-*C*H), 133.4 (F-*C*H), 119.0 (F-*C*H), 88.7 (T-*C*H), 87.8 (T-*C*H), 74.3 (T-*C*H), 68.8 (*C*H_2), 67.7 (*C*H_2), 48.8 (F-*C*H_2), 42.0 (T-*C*H_2), 32.0 (F-*C*H_2), 29.5 (F-*C*H_2), 28.2 (F-*C*H_2), 27.4 (F-*C*H_2), 23.6 (F-*C*H_3), 21.6 (F-*C*H_3), 14.6 (T-*C*H_3).

Flavin-thymidine methylphosphotriester 17

^{1}H NMR (300 MHz, CDCl3) two diastereomers A and B: δ = 8.60 (2H, br s, 2 N*H*), 8.05 (2H, s, 2 Ar*H*), 7.43 (1H, s, T_A6-*H* or T_B6-*H*), 7.39 (1H, s, T_B6-*H* or T_A6-*H*), 7.37 (2H, s, 2 Ar*H*), 6.32 (2H, m, 2 T1'-*H*), 4.67 (4H, brm, 2 F1'-CH_2), 4.54 (2H, m, 2 T3'-*H*), 4.30 (2H, m, 2 T4'-*H*), 4.12 (4H, m, 2 T5'-CH_2), 3.80 (4H, m, 2 F6'-CH_2), 2.56 (6H, s, 2 F-CH_3), 2.44 (6H, s, 2 F-CH_3), 2.18 (4H, m, 4 T2'-*H*), 1.93 (3H, s, T-CH_3), 1.92 (3H, s, T-CH_3), 1.85 (4H, m, 2 F-CH_2), 1.72 (4H, m, 2 F-CH_2), 1.52 (8H, m, 4 F-CH_2).

Synthesis of the flavin-oligonucleotide adducts

Oligo 1 Fl-C6-p-T11 (11 mer)

^{1}H NMR (500 MHz, D_2O) δ = 7.94 (1H, s, F6-*H*), 7.72 (1H, d, T6-*H*), 7.71 (1H, s, F9-*H*), 7.70-7.65 (8H, m, 8 T6-*H*), 7.63 (1H, s, T6-*H*), 7.55 (1H, s, T6-*H*), 6.37-6.22 (10H, m, 10 T1'-*H*), 6.08 (1H, m, T1'-*H*), 4.92 (11H, m, 11 T3'-*H*), 4.78-4.60 (2H, m, F1'-CH_2), 4.35 (10H, m, 10 T4'-*H*), 4.25 (1H, m, T4'-*H*), 4.20-4.00 (22H, m, 11 T5'-CH_2), 3.91 (2H, m, F6'-CH_2), 2.57 (3H, s, F8-CH_3), 2.60-2.50 (11H, m, 11 T2'-*H*), 2.46 (3H, s, F7-CH_3), 2.43-2.10 (11H, m, 11 T2'*H*), 1.93-1.86 (34H, m, 10 T5-CH_3 and 2 F-CH_2), 1.85-1.40 (4H, m, 2 F-CH_2), 1.79 (3H, s, T5-CH_3).

Oligo 2 Fl-C6-p(T11)$_2$ (22 mer)

^{1}H NMR (500 MHz, D_2O) δ = 7.94 (1H, s, F6-*H*), 7.72 (2H, s, 2 T6-*H*), 7.70-7.64 (19H, m, 18 T6-*H* and F9-*H*), 7.39 (2H, s, 2 T6-*H*), 6.36-6.22 (20H, m, 20 T1'-*H*), 6.18 (2H, m, 2 T1'-*H*), 4.92 (22H, m, 22 T3'-*H*), 4.70-4.60 (2H, m, F1'-CH_2), 4.34

(22H, m, 22 T4'-*H*), 4.20-4.10 (46H, m, 22 T5'-C*H*$_2$ and F6'-C*H*$_2$), 2.60-2.48 (25H, m, F8-C*H*$_3$ and 22 T2'-*H*), 2.45 (3H, s, F7-C*H*$_3$), 2.43-2.22 (22H, m, 22 T2'*H*), 1.96-1.85 (64H, m, 20 T5-C*H*$_3$ and 2 F-C*H*$_2$), 1.75-1.40 (4H, 3 m, 2 F-C*H*$_2$), 1.79 (3H, s, T5-C*H*$_3$), 1.76 (3H, s, T5-C*H*$_3$).

Oligo 3 Fl-C6-p-T16 (16 mer)

^{1}H NMR (500 MHz, D_2O) δ = 8.02 (1H, s, F6-*H*), 7.82 (2H, s, 2 T6-*H*), 7.81 (1H, s, F9-*H*), 7.80-7.72 (12H, m, 12 T6-*H*), 7.63 (2H, s, 2 T6-*H*), 6.40-6.30 (15H, m, 15 T1'-*H*), 6.16 (1H, m, T1'-*H*), 5.02 (16H, m, 16 T3'-*H*), 4.84-4.70 (2H, m, F1'-C*H*$_2$), 4.44 (15H, m, 15 T4'-*H*), 4.35 (1H, m, T4'-*H*), 4.32-4.10 (32H, m, 16 T5'-C*H*$_2$), 4.00 (2H, m, F6'-C*H*$_2$), 2.66 (3H, s, F8-C*H*$_3$), 2.70-2.62 (16H, m, 16 T2'-*H*), 2.55 (3H, s, F7-C*H*$_3$), 2.50-2.38 (16H, m, 16 T2'*H*), 2.20-1.74 (49H, m, 15 T5-C*H*$_3$ and 2 F-C*H*$_2$), 1.87 (3H, s, T5-C*H*$_3$), 1.82-1.60 (4H, 2 m, 2 F-C*H*$_2$).

Oligo 5-6 (16 mers)

^{1}H NMR (500 MHz, D_2O) δ = 7.96 (1H, s, F6-*H*), 7.76 (1H, m, F9-*H*), 4.88 - 4.60 (2H, m, F1'-C*H*$_2$), 3.97 (2H, m, F6'-C*H*$_2$), 2.61 (3H, s, F8-C*H*$_3$), 2.50 (3H, s, F7-C*H*$_3$), 2.10-1.82 (4H, m, 2 F-C*H*$_2$), 1.90 - 1.40 (4H, m, 2 F-C*H*$_2$).