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Supporting Information

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Supporting Information

for

Detection of RNA Hybridization by Pyrene-Labeled Probes

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Part 1: Synthesis and Characterization of pyrene-labeled adenine nucleosides

Column chromatography (CC) was performed on silica gel Kieselgel 60 (0.063-0.200 mm, Merck). (0.040-0.063 mm), TLC was carried out on Kieselgel 260 F (Merck) with detection by UV and the following solvent systems (compositions expressed as *v*/*v*): methylene chloride –ethanol 95:5 (A); methylene chloride –ethanol 8:2 (B); hexane: acetone: triethylamine 49:49:2 (C) [for amidites]; detection by UV light. NMR Spectra: Bruker Avance 300 NMR and Bruker Avance II 500 NMR spectrometers; at 300 K. Chemical shifts δ in ppm were measured relative to the solvent signals (^{1}H and ^{13}C) and rela-tive external reference = H_3PO_4 capil. (^{31}P). The coupling constants (J) are given in Hz. The signals were assigned using double resonance techniques and COSY experiments. Mass spectrometry and exact mass measurements of the nucleoside intermediates were performed on a quadrupole/ orthogo-nal-acceleration time-of-flight tandem mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) equipped with a standard electrospray ionization (ESI) interface. ESI spectra for the modified ONs were obtained by coupling the Q-Tof-2 to a capillary HPLC (CapLC, Waters, Milford, MA). Masses were obtained by deconvolution of the spectra using the MaxEnt 1 algorithm (MassLynx 3.4, Micro-mass, Manchester, UK).

1,5-Anhydro-4,6-*O*-benzylidene-2-(N^6-benzoyladenin-9-yl)-2-deoxy-D-*altro*-hexitol (2).

Nucleoside **1** (1.11 g, 3 mmol) was dried by evaporation of pyridine (2 x 20 mL), and suspended in dry pyridine (50 mL). Trimethylchlorosilane (1.0 mL, 7.9 mmol) was added and the mixture was stirred at room temperature for 30 min, then benzoyl chloride (1.2 mL, 10 mmol) was added and the reaction was maintained at room temperature for 2 h. The mixture was then cooled in an ice bath, and water (5 mL) was added. After 5 min 25% aqueous ammonia (10 mL) and 70% hydrogen fluoride in pyridine (1 mL) were added. The reaction mixture was kept at room temperature for 2 h, evaporated in vacuo to near dryness and ethanol (50 mL) and water (20 mL) were added. The formed precipitate was fil-tered off, washed with ethanol-water (1:1) and dried. Crystallization from ethanol-water (2:1) afforded to the title compound **2** as a white powder. Yield 968 mg (68%). R_f 0.30 (A). ^{1}H-NMR ($CDCl_3$) 9.46 brs (1H, NH), 8.74 s (1H, H8); 8.55 s (1H, H2); 8.05-7.31 m (10H, Bz, Ph), 5.51 s (1H, C<u>H</u>Ph), 4.87 brs (1H, H2'), 4.52-4.48 m (2H, H3', H1'a), 4.42 dd (1H, $J_{6'a,5'}$ = 4.9, $J_{6'a,6'b}$= -10.5, H6'a), 4.21 d (1H, $J_{1'a,1'b}$ =-14.4, H1b), 4.17 dd (1H, $J_{5',6'b}$ = 10.0, $J_{5',4'}$ = 5.1, H5'), 3.74 dd (1H, H6'b), 3.64 dd (1H, $J_{4',3'}$ = 7.2, H4'). ^{13}C-NMR ($CDCl_3$) 164.87 (C=O), 152.68 (C6), 152.12 (C8), 149.76 (C4), 141.96 (C2), 136.74-125.97 (Bz, Ph), 122.68 (C5), 102.15 (Ph<u>C</u>H), 76.63 (C4'), 68.91 (C6'), 67.12 (C5'), 66.21 (C3'), 64.99 (C1'), 55.36 (C2'). LSI-MS: ($C_{25}H_{23}N_5O_5$ + H^+) 474.1774, Calc. 474.1777.

1,5-Anhydro-4,6-*O*-benzylidene-2-(N^6-benzoyladenin-9-yl)-3-*O*-(pyren-1-ylmethyl)-2-deoxy-D-*altro*-hexitol (3).

A 60% dispersion of sodium hydride in mineral oil (56 mg, 1.4 mmol) was added to a suspension of **2** (350 mg, 0.74 mmol) in DMF (10 mL) at -5℃ under ni trogen. The suspension was stirred at -5℃ for 20 min and 1-(chloromethyl)pyrene (160 mg, 0.63 mmol) was added. The mixture was stirred at -5℃ for 4 h and quenched with water (2 mL) and acetic acid (0.1 mL). The formed precipitate was filtered,

washed with ethanol-water (1:1) and dried. Crystallization from ethanol afforded the title compound **3** as a white powder. Yield 183 mg (42 %). R_f 0.42 (A). ^{1}H-NMR ([D_6]DMSO): 11.28 brs (1H, NH), 8.74 s (1H, H8), 8.58 s (1H, H2), 8.07-7.30 m (19H, Ph), 5.64 s (1H, CHPh), 5.63 d (1H, *J*=-12.0, OCHH), 5.59 d (1H, OCHH), 5.08 dd (1H, $J_{2',1'b}$=2.5, $J_{2',3'}$=2.0, H2'), 4.55 d (1H, $J_{3',4'}$=2.8, H3'), 4.47 d (1H, $J_{1'a,1'b}$=-13.2, H1'a), 4.35 dd (1H, H1'b), 4.29 dd (1H, $J_{6'a,5'}$=5.3, $J_{6'a,6'b}$=-10.1, H6'a), 4.09 ddd (1H, $J_{5',4'}$=10.0, $J_{5',6'b}$=9.6, H5'), 3.86 dd (1H, H4'), 3.83 dd (1H, H6'b). ^{13}C-NMR ([D_6]DMSO): ^{13}C-NMR ($CDCl_3$) 165.81 (C=O), 152.51 (C6), 151.66 (C8), 150.51 (C4), 142.78 (C2), 137.45-124.91 (Bz, Ph), 123.93 (C5), 101.03 (PhCH), 75.91 (C4'), 72.68 (C6'), 71.36 (OCH_2), 68.19 (C5'), 67.24 (C3'), 64.76 (C1'), 53.64 (C2'). LSI-MS: ($C_{42}H_{33}N_5O_5$+ H^+) 688.2571, Calc. 688.2560.

1,5-Anhydro-2-(*N*^{6}-benzoyladenin-9-yl)-3-*O*-(pyren-1-ylmethyl)-2-deoxy-D-*altro*-hexitol (4).

Nucleoside **3** (260 mg, 0.38 mmol) was dissolved in dichloromethane (5 mL) at 0℃ and trifluoroacetic acid (0.2 mL, 1.8 mmol) was added. A suspension was stirred at 0℃ for 40 min and pH was adjusted to neutrality with triethylamine. The precipitate formed was filtered, washed with water (5 mL) and dried. The title compound **4** was obtained as a white powder. Yield 138 mg (61%). R_f 0.12 (A). ^{1}H-NMR ([D_6]DMSO): 11.14 brs (1H, NH), 8.62 s (1H, H8), 8.61 s (1H, H2), 8.33-7.57 m (14H, Ph), 5.52 d (1H, *J* = -11.6, OCHH), 5.34 d (1H, OCHH), 5.14 d (1H, $J_{OH,4'}$=5.5, OH4'), 5.00 ddd (1H, $J_{2',1'a}$=4.5, $J_{2',1'b}$=3.4, $J_{2',3'}$=5.9, H2'), 4.78 t (1H, $J_{OH,6'}$=5.8, OH6'), 4.39 dd (1H, $J_{3',4'}$=2.5, H3'), 4.23 dd (1H, $J_{1'a,1'b}$=-12.3, H1'a), 4.09 dd (1H, H1'b), 3.95 m (1H, H5'), 3.81 m (1H, H4'), 3.73 m (2H, H6'a, H6'b). ^{13}C-NMR ([D_6]DMSO): ^{13}C-NMR ($CDCl_3$) 165.70 (C=O), 152.52 (C6), 151.49 (C8), 150.28 (C4), 143.76 (C2), 133.60-124.83 (Bz, Ph), 123.56 (C5), 78.71 (C4'), 76.28 (C6'), 70.34 (OCH_2), 63.67 (C5'), 63.37 (C3'), 59.77 (C1'), 53.13 (C2'). LSI-MS: ($C_{35}H_{29}N_5O_5$+ H^+) 600.2255, Calc. 600.2247.

1,5-Anhydro-2-(*N*^{6}-benzoyladenin-9-yl)-3-*O*-(pyren-1-ylmethyl)-6-*O*-monomethoxytrityl-2-deoxy-D-*altro*-hexitol (5).

Nucleoside **4** (138 mg, 0.23 mmol) was dried by evaporation with pyridine (2 x 10 mL). The residue was dissolved in dry pyridine (20 mL), monomethoxytrityl chloride (110 mg, 0.35 mmol) was added and the resulted solution was kept in the dark for 16 h at 20°C. Methanol (0.5 mL) was added and af-ter 30 min the mixture was concentrated *in vacuo* to near dryness. The residue was dissolved in meth-ylene chloride (50 mL), washed with 10% aqueous solution of sodium bicarbonate (20 mL) and water (2 × 20 mL). The organic layer was dried over Na_2SO_4, evaporated in vacuo, evaporated with toluene (2 × 10 mL) and the residue was purified by column chromatography on silica gel (40 g). The column was washed with methylene chloride (200 mL) and then eluted with methylene chloride–ethanol 99:1 to give **5** as foam. Yield 150 mg (74 %). R_f 0.33 (A). ^{1}H-NMR ($CDCl_3$): 9.78 brs (1H, NH), 8.64 s (1H, H8), 8.38-7.24 m (27H, H2, Ph), 6.81 d (2H, *J* = 8.9, PhOMe), 5.54 d (1H, *J*= -10.5, OCHH), 5.47 d (1H, OCHH), 4.93 m (1H, H2'), 4.29 m (1H, H3'), 4.22 m (2H, H1'a, H1'b), 3.89 m (2H, H4', H5'), 3.76 s (3H, OMe), 3.45 dd (1H, $J_{6'a,5'}$= 1.2, $J_{6'a,6'b}$= -11.3, H6'a), 3.37 dd (1H, $J_{6'b,5'}$ = 2.5, H6'b), 2.26 d (1H, $J_{OH,4'}$ = 5.5, OH4'). ^{13}C-NMR ($CDCl_3$): ^{13}C-NMR ($CDCl_3$) 164.20 (C=O), 158.57 (PhOMe), 152.42 (C6), 151.64 (C8), 149.17 (C4), 142.41 (C2), 135.44-123.46 (Bz, Ph), 124.96 (C5), 113.15 (PhOMe), 86.59 (Ph_3C), 77.00

(C4'), 74.27 (C6'), 71.26 (O<u>C</u>H_2), 64.80 (C5'), 64.19 (C3'), 62.90 (C1'), 55.18 (OMe), 52.83 (C2'). LSI-MS: ($C_{55}H_{45}N_5O_6$+ H^+) 872.3462, Calc. 872.3448.

1,5-Anhydro-2-(*N*^{6}-benzoyladenin-9-yl)-3-*O*-(pyren-1-ylmethyl)-5-*O*-((2-cyanoethyl)(diisopropyl-amino)phosphanyl)-6-*O*-monomethoxytrityl-2-deoxy-D-*altro*-hexitol (6).

Starting from 143 mg (0.164 mmol) of **5** the amidite **6** was prepared in 61% yield (107 mg, 0.1 mmol). R_f 0.38 (solvent C); ^{31}P NMR ($CDCl_3$): 148.93, 150.79; LSI-MS: ($C_{64}H_{62}N_7O_7P_1$ +H^+) 1072.4485; calcd: 1072.4526.

9-(5-*O*-trityl-β-D-ribofuranosyl)-*N*^{6}-trityladenine (7) .

R_f 0.21 (A). ^{1}H-NMR ($CDCl_3$): 8.07 s (1H, H8), 7.98 s (1H, H2), 7.40-7.20 m (31H, NH, Ph), 5.91 d (1H, $J_{1',2'}$ = 5.7, H-1′), 4.72 dd (1H, $J_{2',3'}$ = 5.3, H2′), 4.41 ddd (1H, $J_{4',3'}$ = 1.9, $J_{4',5a'}$ = 3.3, $J_{4',5b'}$ = 3.2, H4′), 4.30 dd (1H, H3′), 3.48 dd (1H, $J_{5'a,5'b}$=-10.5 H5′a), 3.21 dd (1H, H5′b). ^{13}C-NMR ($CDCl_3$): 154.38 (C6), 151.67 (C8), 148.03 (C4), 144.91, 143.47 (Ph), 138.23 (C2), 129.12, 128.67, 128.06, 128.02, 127.33, 127.13 (Ph), 121.31 (C5), 91.19 (Ph_3<u>C</u>O), 87.19 (C1'), 86.53 (C4'), 76.22 (C2'), 73.06 (Ph_3<u>C</u>N), 71.63 (C3'), 63.84 (C5'). LSI-MS: ($C_{48}H_{41}N_5O_4$ + H^+) 752.3227; calcd: 752.3237.

9-[2-*O*-(Pyren-1-ylmethyl)-5-*O*-trityl-β-D-ribofuranosyl]-*N*^{6}-trityladenine (8).

A 60% dispersion of sodium hydride in mineral oil (60 mg, 1.5 mmol) was added to a suspension of **7** (752 mg, 1.0 mmol) in DMF (30 mL) at 0°C under nitrogen. The mixture was stirred at 0°C for 30 min, and 1-(chloromethyl)pyrene (250 mg, 1.0 mmol) was added. The reaction mixture was warmed to room temperature, stirred for 3 h, quenched with water (5 mL) and acetic acid (0.1 mL) and evaporat-ed in vacuo to dryness. The residue was dissolved in methylene chloride (50 mL), washed with 10% aqueous solution of sodium bicarbonate (20 mL) and water (2 × 20 mL). The organic layer was dried over Na_2SO_4, evaporated *in vacuo* to dryness. According to TLC-data, the residue contained the mix-ture of the 2′- and 3′-*O*-(Pyren-1-ylmethyl)-derivatives (R_f 0.32 and 0.30 respectively) in a ratio 5:1 as main products and traces of the starting nucleoside **2** and 2′,3′-*O*-bis-(Pyren-1-ylmethyl)-derivative. The residue was purified by column chromatography on silica gel (40 g). The column was washed with methylene chloride (200 mL) and then eluted with methylene chloride–ethanol 99:1 to give **8** as yel-lowish powder. Yield 460 mg (48%). R_f 0.32 (A). ^{1}H-NMR ($CDCl_3$): 8.31-7.64 m (10H, H8, Ph), 7.72 s (1H, H2), 7.42-7.17 m (30H, Ph), 6.91 s (1H, NH), 6.13 d (1H, $J_{1',2'}$ = 4.4, H1′), 5.55 d (1H, *J*=-12.1, OC<u>H</u>H), 5.32 d (1H, OCH<u>H</u>), 4.87 t (1H, $J_{2',3'}$ = 4.4, H2′), 4.11 m (2H, H3′, H4′), 3.42 dd (1H, $J_{5a',4'}$ = 2.3, $J_{5'a,5'b}$=-10.2, H5′a), 3.23 dd (1H, $J_{5b',4'}$= 3.5, H5′b), 2.55 d (1H, $J_{OH,3'}$=5.0 OH3′). ^{13}C-NMR ($CDCl_3$): 154.09 (C6), 152.31 (C8), 148.53 (C4), 145.03, 143.53 (Ph), 138.54 (C2), 131.19-124.45 (Ph), 122.67 (C5), 87.10 (Ph_3<u>C</u>O), 87.04 (C1'), 83.84 (C4'), 80.83 (C2'), 72.04 (O<u>C</u>H_2), 71.45 (Ph_3<u>C</u>N), 70.27 (C3'), 63.28 (C5'). LSI-MS: ($C_{65}H_{51}N_5O_4$ + H^+) 966.4031; calcd: 966.4019.

9-(2-*O*-(Pyren-1-ylmethyl)-β-D-ribofuranosyl)adenine (9).

The suspension of **8** (2.13 g, 2.2 mmol) in 80% acetic acid (50 mL) was refluxed for 30 min. The solution was cooled to room temperature and then 20 mL of water was added. The precipitated tritylcarbinol was filtered, filtrate was concentrated *in vacuo* to near dryness and 30 mL of ethanol-water (2:1) was added. Aqueous solution of sodium bicarbonate was added untill pH 7 and the formed precipitate was filtered off, washed with water (20 mL), diethyl ether (20 mL) and dried. The title compound **9** was obtained as a white powder. Yield 698 mg (66%). R_f 0.20 (B). ^{1}H-NMR ([D$_6$]DMSO): 8.34 s (1H, H8), 8.30-7.95 m (10H, H2, Ph), 7.32 brs (2H, NH_2), 6.15 d (1H, $J_{1',2'}$= 6.2, H1′), 5.53 d (1H, $J_{OH,3'}$ = 5.1, OH3′), 5.46-5.41 m (2H, OH5′, OC<u>H</u>H), 5.18 d (1H, J = -11.6, OCH<u>H</u>), 4.82 dd (1H, $J_{2',3'}$ = 5.0, H2′), 4.53 ddd (1H, $J_{3',4'}$ = 2.5, H3′), 4.10 ddd (1H, $J_{4',5a'}$ = 3.6, $J_{4',5b'}$ = 3.4, H4′), 3.74 ddd (1H, $J_{5'a,5'b}$= -12.3, H5′a), 3.64 ddd (1H, H5′b). ^{13}C-NMR ([D$_6$]DMSO): 156.09 (C6), 152.31 (C4), 148.91 (C8), 139.64 (C2), 131.17-123.39 (Ph), 119.30 (C5), 86.73 (C1'), 86.12 (C4'), 80.79 (C2'), 69.91 (O<u>C</u>H$_2$), 69.19 (C3'), 61.55 (C5'). LSI-MS: ($C_{27}H_{23}N_5O_4$ + H^+) 482.1823; calcd: 482.1828.

9-(2-*O*-(Pyren-1-ylmethyl)-β-D-ribofuranosyl)-*N*^{6}-benzoyladenine (10).

Nucleoside **9** (915 mg, 1.9 mmol) was dried by evaporation of pyridine (2 x 20 mL) and suspended in dry pyridine (50 mL). Trimethylchlorosilane (1.2 mL, 9.5 mmol) was added and the mixture was stirred at room temperature for 30 min, after then benzoyl chloride (1.1 mL, 9.5 mmol) was added and the reaction was maintained at room temperature for 2 h. The mixture was then cooled in an ice bath, and water (1 mL) was added. After 5 min 25% aqueous ammonia (5 mL) was added, and the mixture was stirred at room temperature for 1 h. The reaction mixture was then evaporated in vacuo to near dryness and ethanol (50 mL) and water (20 mL) were added. The formed precipitate was filtered off, washed with ethanol-water (1:1) and dried. Crystallization from ethanol-water (2:1) afforded the title compound **10** as a white powder. Yield 756 mg (68%). R_f 0.12 (A). ^{1}H-NMR ([D$_6$]DMSO): 11.09 brs (1H, NH), 8.61 s (1H, H8), 8.56 s (1H, H2), 8.30-7.52 m (14H, Ph), 6.25 d (1H, $J_{1',2'}$ = 6.0, H1′), 5.58 d (1H, $J_{OH,3'}$ = 5.3, OH3′), 5.48 d (1H, J= -11.8, OC<u>H</u>H), 5.22 d (1H, OCH<u>H</u>), 5.19 t (1H, $J_{OH,5'}$ = 5.3, OH5′), 4.85 dd (1H, $J_{2',3'}$ = 5.1, H2′), 4.55 ddd (1H, $J_{3',4'}$ = 3.2, H3′), 4.11 ddd (1H, $J_{4',5a'}$ = 3.9, $J_{4',5b'}$ = 3.7, H4′), 3.71 ddd (1H, $J_{5'a,5'b}$ = -12.0, H5′a), 3.64 ddd (1H, H5′b). ^{13}C-NMR ([D$_6$]DMSO): 165.73 (C=O), 151.82 (C6), 151.38 (C8), 150.30 (C4), 142.80 (C2), 133.42-125.27 (Ph), 123.05 (C5), 86.70 (C1'), 85.89 (C4'), 80.77 (C2'), 70.12 (O<u>C</u>H$_2$), 69.00 (C3'), 61.30 (C5'). LSI-MS: ($C_{34}H_{27}N_5O_5$ + H^+) 586.2077; calcd: 586.2090.

9-[2-*O*-(Pyren-1-ylmethyl)-5-*O*-(4-methoxytrityl)-β-D-ribofuranosyl]-*N*^{6}-benzoyladenine (11).

Nucleoside **10** (710 mg, 1.2 mmol) was dried by evaporation with pyridine (2 x 10 mL). The residue was dissolved in dry pyridine (20 mL), monomethoxytrityl chloride (530 mg, 1.7 mmol) was added and the resulted solution was kept in the dark for 16 h at 20°C. Methanol (0.5 mL) was added and after 30 min the mixture was concentrated *in vacuo* to near dryness. The residue was dissolved in methylene chloride (50 mL), washed with 10% aqueous solution of sodium bicarbonate (20 mL) and water (2 × 20

mL). The organic layer was dried over Na_2SO_4, evaporated *in vacuo*, evaporated with toluene (2 × 10 mL) and purified by column chromatography on silica gel (40 g). The column was washed with methylene chloride (200 mL) and then eluted with methylene chloride–ethanol 99:1 to give **11** as a foam. Yield 864 mg (83 %). R_f 0.31 (A). ^{1}H-NMR ($CDCl_3$): 10.55 brs (1H, NH), 8.43 s (1H, H8), 8.35-7.15 m (27H, H2, Ph), 6.78 d (2H, *J*= 8.8, PhOMe), 6.01 d (1H, $J_{1',2'}$ = 5.9, H1′), 5.55 d (1H, *J* = -12.5, OCHH), 5.19 d (1H, OCHH), 4.96 dd (1H, $J_{2',3'}$=5.6, H2′), 4.35 ddd (1H, $J_{3',4'}$=3.5, $J_{OH,3'}$=3.1, H3′), 4.23 ddd (1H, $J_{4',5a'}$=3.9, $J_{4',5b'}$=4.0, H4′), 3.77 s (3H, OMe), 3.44 dd (1H, $J_{5'a,5'b}$ = -11.4, H5′a), 3.26 dd (1H, H5′b), 2.84 d (1H, OH3′). ^{13}C-NMR ($CDCl_3$): 163.76 (C=O), 158.78 (PhOMe), 152.42 (C6), 151.05 (C8), 148.66 (C4), 144.26, 144.18 (Ph), 141.26 (C2), 135.28-124.26 (Ph), 122.86 (C5), 113.31 (PhOMe), 86.95 (Ph_3C), 86.75 (C1'), 84.78 (C4'), 79.19 (C2'), 72.44 (OCH_2), 70.11 (C3'), 63.35 (C5'), 55.38 (OMe). LSI-MS: ($C_{54}H_{43}N_5O_6$ + H^+) 858.3261; calcd: 858.3292.

9-{2-*O*-(Pyren-1-ylmethyl)-3-*O*-[(2-cyanoethyl)(diisopropylamino)phosphanyl]-5-*O*-(4-methoxytrityl)-β-D-ribofuranosyl}-*N*^{6}-benzoyladenine (12).

The methoxytritylated derivative **11** (810 mg, 0.94 mmol) was dissolved in 6 mL dichloromethane un-der argon and ethyl(diisopropyl)amine (491 µL, 2.82 mmol) and 2-cyanoethyl diisopropylphoramidochloridite (419 µL, 1.88 mmol) were added and, after stirring the solution for 2 h, TLC indicated complete reaction. A 10% aqueous solution of sodium hydrogencarbonate (2 mL) was added, the solution was stirred for 10 min. and partitioned between CH_2Cl_2 (50 mL) and aqueous $NaHCO_3$ (30 mL). The organic phase was washed with aqueous sodium chloride (2x30 mL) and the aqueous phases were back extracted with CH_2Cl_2 (20 mL). Evaporation of the organics left an oil which was flash-purified on 50 g of silica gel (hexane: acetone: TEA, 66:33:1) to afford the product as a foam after coevaporation with dichloromethane. The foam was dissolved in 2 mL of dichloromethane and precipitated in 150 mL cold (-70 °C) hexane to afford 892 mg (0.84 mmol, 89 %) of the title product as a white powder. R_f 0.45 (hexane: acetone: TEA, 49:49:2 – solvent C). ^{31}P NMR ($CDCl_3$): 150.59; LSI-MS: ($C_{63}H_{60}N_7O_7P_1$ +H^+) 1058.4382; calcd: 1058.4370.

9-{2-*O*-[(2-(2,2,2-Trifluoroacetamido)ethoxy)methyl]-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl}adenine (15a).

To a cooled solution (–15 ℃) under nitrogen of nucleoside **13** (510 mg, 1.0 mmol) and ((2-(2,2,2-trifluoroacetamido)ethoxy)methylacetate (**14a**) (460 mg, 2.0 mmol) in 1,2-dichloroethane (20 mL), tin tetrachloride (0.22 mL, 1.8 mmol) was added and the solution was kept at –12 ℃ for 40 min. A 10% aqueous solution of sodium hydrogencarbonate (10 mL) and methylene chloride (20 mL) were added and the suspension was stirred at 0 ℃ for 20 min. The suspension was filtered through Hyflo Super Cel, organic layer was separated, washed with water (20 mL), dried over anhydrous sodium sulphate and evaporated to dryness. The residue was purified by column chromatography on silica gel (30 g). The column was washed with methylene chloride (200 mL), methylene chloride/ethanol 99:1 (200 mL) and then eluted with methylene chloride–ethanol 98:2 to give **15a** as foam. Yield 380 mg (56%). R_f 0.23 (A).

^{1}H- NMR ($CDCl_3$): 8.28 s (1H, H-8), 8.20 s (1H, H-2), 8.10 brt (1H, $NHCOCF_3$), 6.07 s (1H, H-1'), 5.86 s (2H, NH_2), 5.02 d (1H, *J* = -7.1, OC<u>H</u>HO), 4.98 d (1H, OCH<u>H</u>O), 4.59 dd (1H, $J_{3',2'}$ = 4.4, $J_{3',4'}$ = 9.5, H-3'), 4.38 d (1H, H-2'), 4.29 d (1H, $J_{5'a,5'b}$ = -13.5, H-5'a), 4.16 dd (1H, $J_{4',5'b}$ = 1.9, H-4'), 4.02 dd (1H, H-5'b), 3.96-3.89 m (1H, OC<u>H</u>H$CH_2NHCOCF_3$), 3.85-3.78 m (1H, OCH<u>H</u>CH_2NH-$COCF_3$), 3.74-3.65 m (1H, OCH_2C<u>H</u>H$NHCOCF_3$), 3.62-3.56 m (1H, OCH_2CH<u>H</u>$NHCOCF_3$), 1.11-0.95 m (28H, iPr). ^{13}C- NMR ($CDCl_3$): 157.65 q (*J* = 36.8, <u>C</u>OCF_3), 155.70 (C6), 153.02 (C8), 148.82 (C4), 138.38 (C2), 120.42 (C5), 116.04 q (*J* = 286.5, <u>C</u>F_3), 95.94 (O<u>C</u>H_2O), 88.75 (C1'), 81.82 (C4'), 79.57 (C2'), 68.69 (C3'), 68.06 (O<u>C</u>$H_2CH_2NHCOCF_3$), 59.69 (C5'), 40.30 (OCH_2<u>C</u>$H_2NHCOCF_3$), 17.57, 17.52, 17.41, 17.25, 17.11, 17.08, 13.52, 13.05, 12.72 (iPr). LSI-MS: ($C_{27}H_{45}F_3N_6O_7Si_2$ + H^+) 679.2906; calcd: 679.2913.

9-{2-*O*-[(3-(2,2,2-Trifluoroacetamido)propoxy)methyl]-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl}adenine (15b).

HNAlogous analogous (see preparation of **15a**) condensation of (3-(2,2,2-trifluoroacetamido)propoxy)methylacetate (**14b**) (490 mg, 2.0 mmol) in the presence of tin tetrachloride (0.22 mL, 1.8 mmol) with nucleoside **13** (510 mg, 1.0 mmol) in 1,2-dichloroethane (15 mL) at –12 ℃ for 40 min gave **15b** as a foam. Yield 374 mg (54%). R_f 0.23 (A). ^{1}H-NMR ($CDCl_3$): 8.24 s (1H, H-8), 8.20 s (1H, H-2), 7.77 brt (1H, $NHCOCF_3$), 6.04 s (1H, H-1'), 5.92 s (2H, NH_2), 5.02 d (1H, *J* = -7.0, OC<u>H</u>HO), 4.93 d (1H, OCH<u>H</u>O), 4.61 dd (1H, $J_{3',2'}$ = 4.4, $J_{3',4'}$ = 9.0, H-3'), 4.41 d (1H, H-2'), 4.28 d (1H, $J_{5'a,5'b}$ = -13.4, H-5'a), 4.16 dd (1H, $J_{4',5'b}$ = 2.5, H-4'), 4.02 dd (1H, H-5'b), 3.95 m (1H, OC<u>H</u>H$CH_2CH_2NHCOCF_3$), 3.65 m (1H, OCH<u>H</u>$CH_2CH_2NHCOCF_3$), 3.60-3.43 m (2H, OCH_2CH_2C<u>H</u>$_2$NH$COCF_3$), 2.08-1.82 m (2H, OCH_2C<u>H</u>$_2$-$CH_2NHCOCF_3$), 1.15-0.86 m (28H, iPr). ^{13}C-NMR ($CDCl_3$): 157.51 q (*J* = 36.9, <u>C</u>OCF_3), 155.79 (C6), 152.96 (C8), 148.97 (C4), 138.59 (C2), 120.54 (C5), 115.86 q (*J* = 286.1, <u>C</u>F_3), 95.36 (O<u>C</u>H_2O), 88.78 (C1'), 81.68 (C4'), 78.93 (C2'), 69.28 (C3'), 66.67 (O<u>C</u>$H_2CH_2CH_2NHCOCF_3$), 59.97 (C5'), 38.33 (OCH_2CH_2<u>C</u>$H_2NHCOCF_3$), 28.02 (OCH_2<u>C</u>$H_2CH_2NHCOCF_3$), 17.59, 17.49, 17.43, 17.34, 17.24, 17.08, 16.92, 13.59, 13.27, 13.08, 12.88 (iPr). LSI-MS: ($C_{28}H_{47}F_3N_6O_7Si_2$ + H^+) 693.3089. Calc. 693.3069.

9-{2-*O*-[(2-(Pyrene-1-carboxamido)ethoxy)methyl]-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl}adenine (16a).

Nucleoside **15a** (1.56 g, 2.3 mmol) was dissolved in 8 M methylamine in ethanol (70 mL) and kept at 35℃ for 6 hours. After disappearance of the starti ng material (TLC control), the solution was evaporated to dryness. The residue was dissolved in 1,2-dichloroethane (50 mL), the *N*-hydroxysuccinimidyl-pyrene-1-carboxylate (1.03 g, 3 mmol) and DBU (0.5 mL, 3.3 mmol) were added. The solution was kept at room temperature for 4 hours and concentrated *in vacuo* to near dryness. The residue was partitioned between methylene chloride (120 mL) and water (50 mL), the organic layer was washed successively with 10% aqueous solution of sodium bicarbonate (50 mL), water (2 × 50 mL), dried over Na_2SO_4 and evaporated to dryness. The residue was applied to a column of silica gel (60 g). The column was washed with methylene chloride (300 mL), methylene chloride–ethanol 99:1 (500 mL) and then eluted with methylene chloride–ethanol 98:2 to give **16a** as a foam. Yield 1.45 g (78%). R_f 0.25 (A). ^{1}H- NMR ($CDCl_3$): 8.39-7.72 m (12H, H-2, H-8, N<u>H</u>COPyr, Ph) 5.88 s (1H, H-1′), 5.03 m (4H, NH_2,

OCH_2O), 4.41 m (2H, H-2′, H-3′), 4.20-4.08 m (4H, H-4′, H-5′a, $OC\underline{H}_2CH_2N$), 3.95-3.90 m (2H, H-5′b, $OCH_2C\underline{H}HN$), 3.80 m (1H, $OCH_2CH\underline{H}N$), 1.07-0.92 m (28H, iPr). ^{13}C- NMR ($CDCl_3$): 170.55 ($\underline{C}OPyr$), 154.74 (C6), 152.35 (C8), 147.96 (C4), 137.97 (C2), 132.21, - 124.23 (Ph), 119.74 (C5), 96.32 ($O\underline{C}H_2O$), 88.89 (C1′), 81.65 (C4′), 80.08 (C2′), 68.67 (C3′), 68.47 ($O\underline{C}H_2CH_2N$), 59.81 (C5′), 40.35 ($OCH_2\underline{C}H_2N$), 17.58 - 12.77 (iPr). LSI-MS: ($C_{42}H_{54}N_6O_7Si_2$ + H^+) 811.3636. Calc. 811.3665

9-{2-*O*-[(3-(Pyrene-1-carboxamido)propoxy)methyl]-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl}adenine (16b).

Analogous conversion of **15b** (692 mg, 1.0 mmol) yielded **16b** as a foam. Yield 620 mg (75%). R_f 0.25 (A). ^{1}H- NMR ($CDCl_3$): 8.49-7.92 m (11H, H-2, H-8, Ph) 7.24 t (1H, *J* = 5.4, N$\underline{H}$COPyr), 5.87 s (1H, H-1′), 5.79 s (2H, NH_2), 5.00 d (1H, *J* = -7.4, OC$\underline{H}$HO), 4.97 d (1H, OCH$\underline{H}$O), 4.50 dd (1H, $J_{3',2'}$ = 4.3, $J_{3',4'}$ = 9.2, H-3′), 4.38 d (1H, H-2′), 4.11-4.01 m (3H, H-4′, H-5′a, OC$\underline{H}$HCH_2CH_2N), 3.91-3.64 m (4H, H-5′b, OCH$\underline{H}CH_2C\underline{H}_2N$), 2.15-2.05 m (2H, $OCH_2C\underline{H}_2CH_2N$), 1.04-0.82 m (28H, iPr). ^{13}C NMR ($CDCl_3$): 170.28 ($\underline{C}OPyr$), 155.38 (C6), 152.79 (C8), 148.59 (C4), 138.31 (C2), 132.29 - 124.28 (Ph), 120.10 (C5), 95.47 ($O\underline{C}H_2O$), 88.65 (C1′), 81.50 (C4′), 78.95 (C2′), 69.02 (C3′), 66.86 ($O\underline{C}H_2CH_2CH_2N$), 59.76 (C5′), 38.20 ($OCH_2CH_2\underline{C}H_2N$), 29.06 ($OCH_2\underline{C}H_2CH_2N$), 17.54 - 12.88 (iPr). LSI-MS: ($C_{43}H_{56}N_6$-O_7Si_2 + H^+) 825.3813. Calc. 825.3822

9-{2-*O*-[(2-(Pyrene-1-carboxamido)ethoxy)methyl]-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl}-*N*^{6}-benzoyladenine (17a).

Nucleoside **16a** (1.40 g 1.7 mmol) was dried by evaporation of pyridine (2 x 15 mL) and suspended in dry pyridine (40 mL). Benzoyl chloride 1.0 mL (9 mmol) was added and the reaction was maintained at room temperature for 2 h. The mixture was then cooled in an ice bath, and water (1 mL) was added. After 5 min 25% aqueous ammonia (5 mL) was added, and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated *in vacuo* to near dryness. The residue was dissolved in methylene chloride (50 mL), washed with 10% aqueous solution of sodium bicarbonate (20 mL) and water (2 × 20 mL). The organic layer was dried over Na_2SO_4, evaporated in vacuo, evaporated with toluene (2 × 10 mL) and purified by column chromatography on silica gel (50 g). The column was washed with methylene chloride (200 mL) and then eluted with methylene chloride / ethanol 99:1 to give **17a** as a foam. Yield 1.15 g (73 %). R_f 0.34 (A). ^{1}H- NMR ($CDCl_3$): 8.53 brs (1H, NHBz), 8.37-7.53 m (17H, H-2, H-8, N$\underline{H}$COPyr, Ph), 5.92 s (1H, H-1′), 5.05 d (1H, *J* = -7.2, OC$\underline{H}$HO), 5.01 d (1H, OCH$\underline{H}$O), 4.44 d (1H, $J_{2',3'}$ = 4.1, H-2′), 4.37 dd (1H, $J_{3',4'}$ = 9.2, H-3′), 4.18-4.09 m (4H, H-4′, H-5′a, $OC\underline{H}_2CH_2N$), 3.95-3.90 m (2H, H-5′b, $OCH_2C\underline{H}HN$), 3.77 m (1H, $OCH_2CH\underline{H}N$), 1.05-0.90 m (28H, iPr). ^{13}C- NMR ($CDCl_3$): 170.40 ($\underline{C}OPyr$), 163.94 ($\underline{C}OBz$), 152.08 (C6), 149.51 (C8), 148.85 (C4), 140.37 (C2), 133.81 - 124.01 (Ph), 122.75 (C5), 96.29 ($O\underline{C}H_2O$), 89.04 (C1′), 81.80 (C4′), 79.06 (C2′), 68.64 (C3′), 68.26 ($O\underline{C}H_2CH_2N$), 59.75 (C5′), 40.25 ($OCH_2\underline{C}H_2N$), 17.35 - 12.72 (iPr). LSI-MS: ($C_{49}H_{58}N_6O_8$-Si_2 + H^+) 915.3891. Calc. 915.3927.

9-{2-*O*-[(3-(Pyrene-1-carboxamido)propoxy)methyl]-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl}-N^6-benzoyladenine (17b).

Analogous benzoylation of **16b** (590 mg, 0.72 mmol) yielded **17b** as a foam. Yield 510 mg (77%). R_f 0.34 (A). ^{1}H- NMR ($CDCl_3$): 8.79 brs (1H, NHBz), 8.57 s (1H, H-8), 8.49-7.45 m (15H, , H-2, Ph), 6.97 t (1H, *J* = 5.3, NHCOPyr), 5.93 s (1H, H-1′), 5.00 s (2H, OCH_2O), 4.53 dd (1H, $J_{3',2'}$ = 5.5, $J_{3',4'}$ = 9.0, H-3′), 4.46 d (1H, H-2′), 4.12-4.03 m (3H, H-4′, H-5′a, OCHHCH_2CH_2N), 3.92-3.64 m (4H, H-5′b, OCHH-CH_2CH_2N), 2.09-2.04 m (2H, OCH_2CH_2CH_2N), 1.05-0.86 m (28H, iPr). ^{13}C- NMR ($CDCl_3$): 170.21 (COPyr), 164.44 (COBz), 152.52 (C6), 150.44 (C8), 149.32 (C4), 140.96 (C2), 133.71 - 124.39 (Ph), 123.43 (C5), 95.55 (OCH_2O), 88.85 (C1′), 81.72 (C4′), 78.70 (C2′), 69.09 (C3′), 66.97 (OCH_2CH_2-CH_2N), 59.74 (C5′), 38.27 (OCH_2CH_2CH_2N), 29.19 (OCH_2CH_2CH_2N), 17.54 - 12.70 (iPr). LSI-MS: ($C_{50}H_{60}N_6O_8Si_2$ + H^+) 929.4073; calcd: 929.4084.

9-{2-*O*-[(2-(Pyrene-1-carboxamido)ethoxy)methyl]-β-D-ribofuranosyl}-N^6-benzoyladenine (18a).

Nucleoside **17a** (1.10 g, 1.2 mmol) was dissolved in 0.5 M tetrabutylammonium fluoride trihydrate in tetrahydrofuran (6 mL), kept for 10 min at 20^{O}C, evaporated to dryness, evaporated with chloroform (10 mL) and applied on a column with silica gel (20 g). The column was washed with methylene chlor-ide (100 mL), methylene chloride –ethanol 98:2 (200 mL), methylene chloride / ethanol 96:4 (200 mL), and then eluted with methylene chloride –ethanol 94:6 to give **18a** as a foam. Yield 733 mg (91%). R_f 0.10 (A). ^{1}H- NMR ($CDCl_3$): 8.91 brs (1H, NHBz), 8.60 s (1H, H-8), 8.43-7.30 m (15H, H-2, Ph), 6.94 t (1H, *J* = 5.2, NHCOPyr), 5.98 d (1H, $J_{1',2'}$ = 6.8, H-1′), 5.62 brs (1H, OH), 4.90 dd (1H, $J_{2',3'}$ = 4.6, H-2′), 4.70 d (1H, *J* = -6.7, OCHHO), 4.60 d (1H, OCHHO), 4.50 dd (1H, $J_{3',4'}$ = 1.6, H-3′), 4.24 dd (1H, $J_{4',5'a}$ = 1.4, H-4′), 3.90 dd (1H, $J_{5'a,5'b}$ = -12.9, H-5′a), 3.72-3.49 m (5H, H-5′b, OCH_2CH_2N), 3.39 brs (1H, OH). ^{13}C-NMR ($CDCl_3$): 170.52 (COPyr), 164.67 (COBz), 152.15 (C6), 150.82 (C8), 150.13 (C4), 143.20 (C2), 132.97 - 124.36 (Ph, C5), 96.28 (OCH_2O), 89.33 (C1′), 87.60 (C4′), 80.63 (C2′), 71.32 (C3′), 67.61 (OCH_2CH_2N), 62.83 (C5′), 39.91 (OCH_2CH_2N). LSI-MS: ($C_{37}H_{32}N_6O_7$ + H^+) 673.2383; calcd: 673.2405.

9-{2-*O*-[(3-(Pyrene-1-carboxamido)propoxy)methyl]-β-D-ribofuranosyl}-N^6-benzoyladenine (18b).

Analogous desilylation of **17b** (490 mg, 0.52 mmol) yielded **18b** as a foam. Yield 310 mg (85%). R_f 0.10 (A). ^{1}H- NMR ($CDCl_3$): 9.22 brs (1H, NHBz), 8.63 s (1H, H-8), 8.33-7.08 m (16H, H-2, Ph, NHCO-Pyr), 5.92 d (1H, $J_{1',2'}$ = 6.9, H-1′), 5.86 brs (1H, OH), 4.92 dd (1H, $J_{2',3'}$= 4.6, H-2′), 4.58 d (1H, *J* = -6.1, OCHHO), 4.49 d (1H, OCHHO), 4.46 d (1H, H-3′), 4.24 brs (1H, H-4′), 3.90 d (1H, $J_{5'a,5'b}$ = -12.4, H-5′a), 3.66 m (2H, H-5′b, OCHHCH_2CH_2N), 3.22 m (4H, OCHHCH_2CH_2N, OH), 1.55 m (2H, OCH_2CH_2-CH_2N). ^{13}C- NMR ($CDCl_3$): 170.39 (COPyr), 165.02 (COBz), 151.97 (C6), 150.97 (C8), 150.15 (C4), 143.26 (C2), 132.66 - 124.20 (Ph, C5), 95.73 (OCH_2O), 88.14 (C1′), 87.66 (C4′), 79.96 (C2′), 71.32 (C3′), 66.69 (OCH_2CH_2CH_2N), 62.94 (C5′), 39.37 (OCH_2CH_2CH_2N), 29.49 (OCH_2CH_2CH_2N). LSI-MS: ($C_{38}H_{34}N_6O_7$ + H^+) 687.2554. Calc. 687.2562.

9-{2-*O*-[(2-(Pyrene-1-carboxamido)ethoxy)methyl]-5-*O*-(4-methoxytrityl)-β-D-ribofuranosyl}-N^6-benzoyladenine (19a).

Nucleoside **18a** (705 mg, 1.05 mmol) was dried by evaporation with pyridine (2 x 10 mL). The residue was dissolved in dry pyridine (20 mL), monomethoxytrityl chloride (0.63 g, 2 mmol) was added and the resulted solution was kept in the dark for 16 h at 20°C. Methanol (0.5 mL) was added and after 30 min the mixture was concentrated *in vacuo* to near dryness. The residue was dissolved in methylene chloride (50 mL), washed with 10% aqueous solution of sodium bicarbonate (20 mL) and water (2 × 20 mL). The organic layer was dried over Na_2SO_4, evaporated *in vacuo*, evaporated with toluene (2 × 10 mL) and purified by column chromatography on silica gel (40 g). The column was washed with methylene chloride (200 mL), methylene chloride - ethanol 99:1 (200 mL) and then eluted with methylene chloride –ethanol 98:2 to give **19a** as a foam. Yield 760 mg (77 %). R_f 0.30 (A). ^{1}H- NMR ($CDCl_3$): 8.52 brs (1H, NHBz), 8.41 s (1H, H-8), 8.36-7.17 m (28H, H-2, Ph, NHCOPyr), 6.79 d (2H, *J*= 8.8, PhOMe), 6.17 d (1H, $J_{1',2'}$ = 3.3, H-1′), 4.94 d (1H, *J* = -7.0, OCHHO), 4.88 d (1H, OCHHO), 4.69 dd (1H, $J_{2',3'}$= 4.7, H-2′), 4.39 ddd (1H, $J_{3',4'}$ = 5.3, $J_{3',OH}$ = 6.2, H-3′), 4.22 ddd (1H, $J_{4',5'a}$ = 2.5, $J_{4',5'b}$ = 4.1, H-4′), 3.96 m (1H, OCHHCH$_2$N), 3.78 m (2H, OCH$_2$CH$_2$N), 3.74 s (3H, OMe), 3.60 m (1H, OCHHCH$_2$N), 3.49 dd (1H, $J_{5'a,5'b}$ = -10.8, H-5′a), 3.37 dd (1H, H-5′b), 2.88 d (1H, OH). ^{13}C- NMR ($CDCl_3$): 170.53 (COPyr), 164.18 (COBz), 158.82 (PhOMe), 152.40 (C6), 150.85 (C8), 149.03 (C4), 144.07, 144.01 (Ph), 141.05 (C2), 135.15 - 124.32 (Ph), 123.19 (C5), 113.38 (PhOMe), 96.43 (OCH$_2$O), 87.48 (C1′), 87.10 (Ph$_3$C), 83.63 (C4′), 80.94 (C2′), 70.07 (C3′), 67.87 (OCH$_2$CH$_2$N), 63.02 (C5′), 55.34 (OMe), 39.94 (OCH$_2$-CH$_2$N). LSI-MS: ($C_{57}H_{48}N_6O_8$ + H^+) 945.3568 Calc. 945.3606.

9-{2-*O*-[(3-(Pyrene-1-carboxamido)propoxy)methyl]-5-*O*-(4-methoxytrityl)-β-D-ribofuranosyl}-N^6-benzoyladenine (19b).

Analogous tritylation of nucleoside **18b** (290 mg, 0.42 mmol) yielded **19b** as a foam. Yield 330 mg (82%). R_f 0.30 (A). ^{1}H- NMR ($CDCl_3$): 8.79 brs (1H, NHBz), 8.54 s (1H, H-8), 8.44-7.18 m (27H, H-2, Ph), 6.86 t (1H, *J* = 5.7, NHCOPyr), 6.79 d (2H, *J*= 8.9, PhOMe), 6.20 d (1H, $J_{1',2'}$ = 5.2, H-1′), 4.95 dd (1H, $J_{2',3'}$= 4.9, H-2′), 4.83 s (2H, OCH$_2$O), 4.48 ddd (1H, $J_{3',4'}$ = 4.1, $J_{3',OH}$ = 4.2, H-3′), 4.25 ddd (1H, $J_{4',5'a}$ = 2.8, $J_{4',5'b}$ = 4.1, H-4′), 3.73 s (3H, OMe), 3.63-3.44 m (5H, OCH$_2$H$_2$CH$_2$N, H-5′a), 3.37 dd (1H, $J_{5'a,5'b}$ = -10.7, H-5′b), 3.18 d (1H, OH), 1.78 m (2H, OCH$_2$CH$_2$CH$_2$N). ^{13}C- NMR ($CDCl_3$): 170.34 (COPyr), 164.74 (COBz), 155.78 (PhOMe), 152.60 (C6), 151.93 (C8), 149.32 (C4), 144.09, 144.02 (Ph), 141.76 (C2), 135.17 - 124.44 (Ph), 123.73 (C5), 113.34 (PhOMe), 95.91 (OCH$_2$O), 87.06 (Ph$_3$C), 86.81 (C1′), 84.20 (C4′), 79.93 (C2′), 70.46 (C3′), 67.15 (OCH$_2$CH$_2$CH$_2$N), 63.36 (C5′), 55.32 (OMe), 37.65 (OCH$_2$CH$_2$CH$_2$N), 29.73 (OCH$_2$CH$_2$CH$_2$N). LSI-MS: ($C_{58}H_{50}N_6O_8$ + H^+) 959.3757 Calc. 959.3763.

9-{2-*O*-[(2-(Pyrene-1-carboxamido)ethoxy)methyl]-3-*O*-[(2-cyanoethyl)(diisopropylamino) phosphanyl]-5-*O*-(4-methoxytrityl)-β-D-ribofuranosyl}-N^6-benzoyladenine (20a).

Starting from 750 mg (0.794 mmol) of **19a** the amidite **20a** was prepared in 82% yield (747 mg, 0.65 mmol) as described for the synthesis of **6**. R_f 0.32 (solvent C); ^{31}P NMR ($CDCl_3$): 149.188, 149.976; LSI-MS: ($C_{66}H_{65}N_8O_9P_1$ +H^+) 1145.4637; calcd: 1145.4690.

9-{2-*O*-[(3-(Pyrene-1-carboxamido)propoxy)methyl]-3-*O*-[(2-cyanoethyl)(diisopropylamino) phosphanyl]-5-*O*-(4-methoxytrityl)-β-D-ribofuranosyl}-N^6-benzoyladenine (20b).

Starting from 327 mg (0.34 mmol) of **19b** the amidite **20b** was prepared in 67% yield (265 mg, 0.23 mmol) as described for the synthesis of **6**. R_f 0.32 (solvent C); ^{31}P NMR ($CDCl_3$): 148.500, 148.797; LSI-MS: ($C_{67}H_{67}N_8O_9P_1$ +H^+) 1159.4791; calcd: 1159.4847.

Synthesis and purification of oligonucleotides

The standard RNA assembly protocol was used with a 12 min coupling time, using either 0.07 M of the respective hexitol (HNA) or ribonucleoside phosphoramidites (“fast deprotecting” amidites or tac amidites from Proligo) with 0.25 M 5-(ethylsulfanyl)-tetrazole (ETT) as the activator. For incorporation of the pyrenyl containing analogues (either the altritol or ribonucleoside analogue) the same protocol was followed with 0.08 M of the incoming amidite. Oligoribonucleotides were assembled on commer-cial CPG supports, loaded with the required ribonucleoside (Glen Research), for assembly of the hexi-tol oligonucleotides, CPG was functionalized with 1,3-propanediol as “universal support” as described before.[41]

The oligomers were deprotected and cleaved from the solid support by treatment with a 1:1 mixture of 40% aqueous methylamine and conc. aqueous ammonia (AMA reagent) for 30 min at RT and 2 h at 30℃. For RNA sequences, the supernatant was lyoph ilized and the residue was further treated with 1 mL of a TEA.3HF solution (1.5 mL NMP + 0.750 mL TEA + 1 mL TEA.3HF) for 3u 30 op 55℃. The mixture was neutralized with 1 mL of a 1.5 M solution of NH_4OAc, slightly concentrated and desalted on a NAP-25® column (Sephadex G25-DNA grade; Pharmacia). The hexitol sequences were desalted on NAP-25® columns immediately following the basic treatment. The crude product was analyzed on a Mono-Q® HR 5/5 anion exchange column, then purified on a Mono-Q® HR 10/10 column (Pharmacia) with the following gradient system (A = 10 mM $NaClO_4$; B = 600 mM $NaClO_4$, both in aqueous 20 mM Tris-HCl, 15 % CH_3CN, 0.1 mM EDTA). The low-pressure liquid chromatography system consisted of a Merck-Hitachi L 6200 A intelligent pump, a Uvicord SII 2138 UV detector (Pharmacia-LKB) and a recorder. The product-containing fraction was desalted on a NAP-25® column and lyophilized. The amount of isolated material was determined by UV absorption at 260 nm. Measurements were carried out at 80℃ to allow for unstacking of the bases an d take into account the absorption of the individual heterocyclic bases.

Structure determination of oligonucleotides

Oligonucleotides were characterized and their purity was checked by HPLC/MS on a capillary chromatograph (CapLC, Waters, Milford, MA). Columns of 150 mm x 0.3 mm length (LCPackings, San Francisco, CA) were used. Oligonucleotides were eluted with an acetonitrile gradient in 50 mM triethylammonium adjusted to pH 8.0 with 1,1,1,3,3,3-hexafluoropropan-2-ol. The flow rate was 5 µL/min. Electrospray spectra were acquired on an orthogonal acceleration / time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) in the negative ion mode. The scan time used was 2 s. The combined spectra from a chromatographic peak were deconvoluted using the MaxEnt algorithm of the software (Masslynx 3.4, Micromass, Manchester, UK). Theoretical oligonucleotide molecular weights were calculated using the monoisotopic atomic weights.

	MS calcd.	MS found	Total Yield (nanomol)
HNA-skeleton:			
HA1-1	4994.0	4994.2	56.1
HA1-2	5224.1	5224.4	45.1
HA1-3	5454.2	5454.5	19.9
H0	4763.9	4764.8	135.0
RNA-skeleton:			
RA2-1	4869.7	4870.3	202.6
RA2-2	5083.8	5084.3	214.8
RA2-3	5297.9	5298.5	203.2
RA3-2	5257.8	5257.7	241.2
RA4-2	5285.9	5285.7	273.8
R0	4793.6	4794.1	259.9
RNA targets:			
HP	8187.2	8187.8	148.5
TAR	4959.7	4960.1	213.6

Part 2: Temperature-dependent relative absorbance for the single strands.

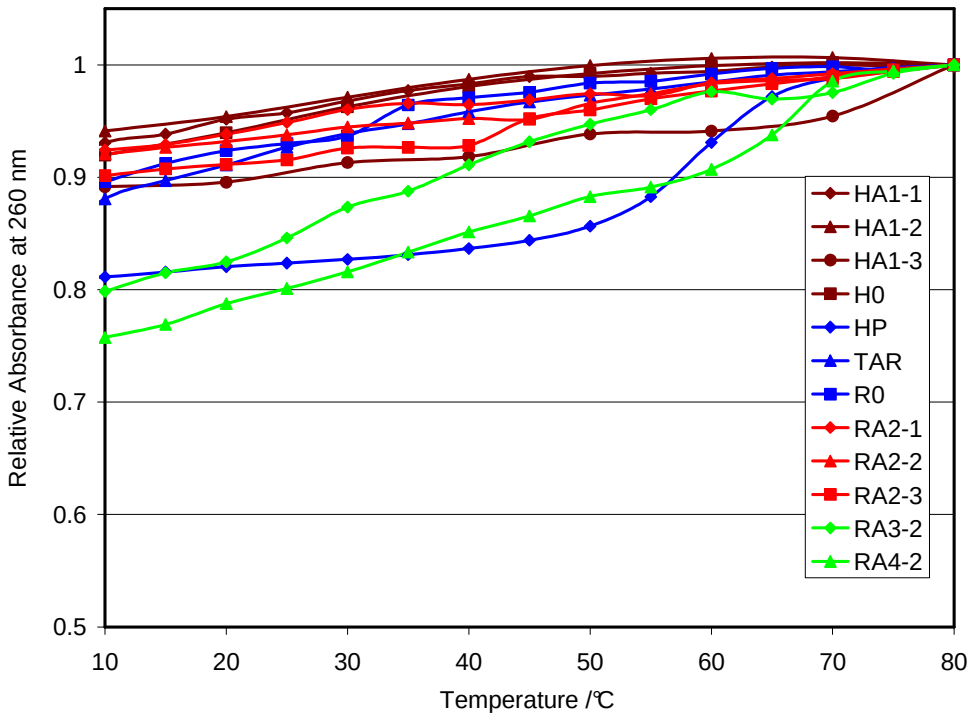

Figure S1 Temperature-dependent relative absorbance monitored at 260 nm for the single oligo-nucleotides used in this work: *HA1-1* (1 μM), *HA1-2* (1 μM), *HA1-3* (0.5 μM), *H0* (3 μM), *HP* (1 μM), *TAR* (1 μM), *R0* (4 μM), *RA2-1* (1 μM), *RA2-2* (1 μM), *RA2-3* (1 μM), *RA3-2* (1 μM), *RA4-2* (1 μM). The measurements were carried out using a 1 cm path length cell.

Part 3: Temperature-dependent absorption spectra.

Temperature-dependent absorption spectra of HNA-skeleton probes and their complexes.

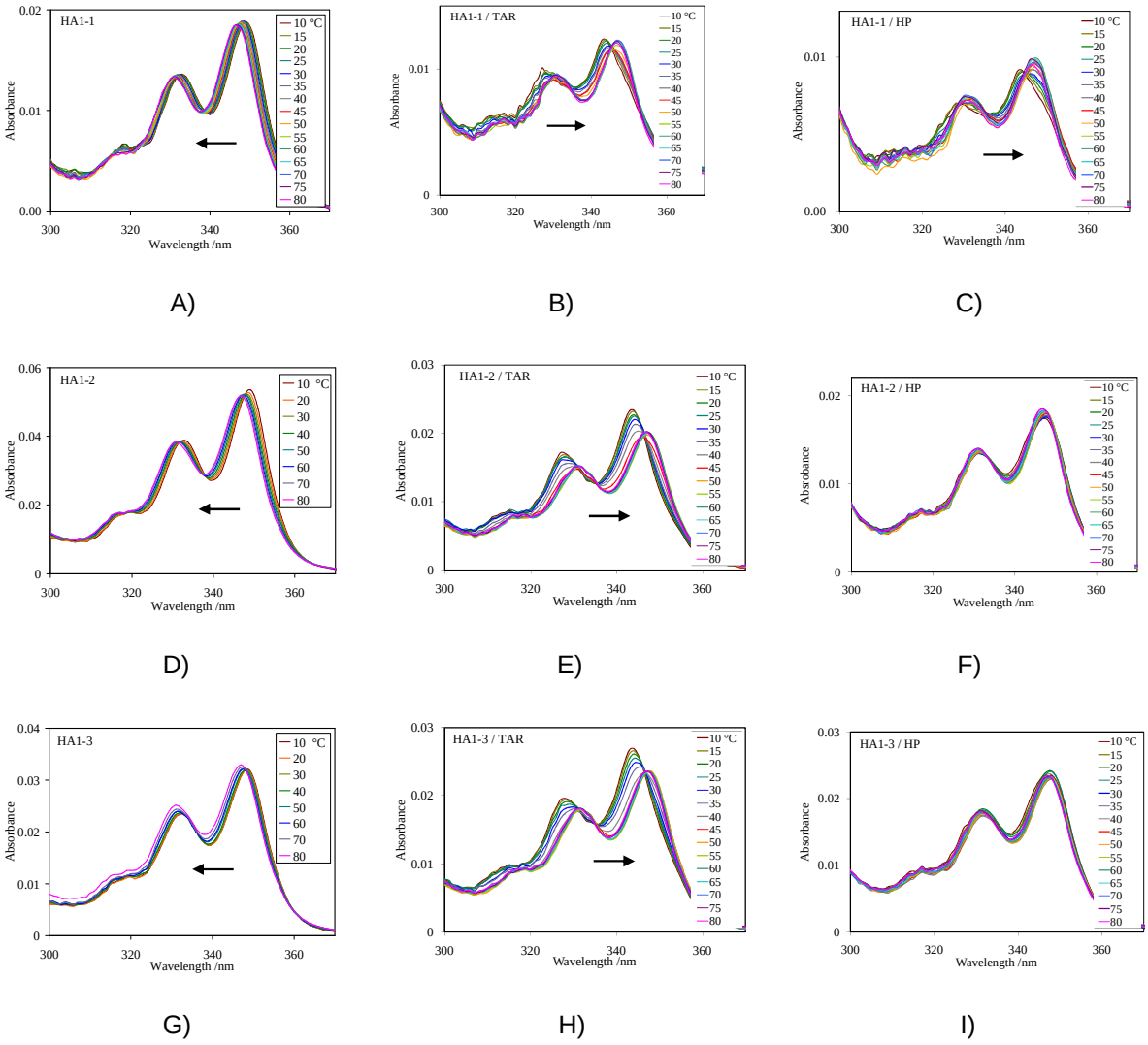

Figure S2. Temperature-dependent absorption spectra of HNA-skeleton probes and their complexes. (A) HA1-1 (0.7 μM); (B) HA1-1/TAR (4 μM); (C) HA1-1/HP (4 μM); (D) HA1-2 (1 μM); (E) HA1-2/TAR (4 μM); (F) HA1-2/HP (4 μM); (G) HA1-3 (0.5 μM); (H) HA1-3/TAR (4 μM); (I) HA1-3/HP (4 μM). Spectra of the probes and their mixture with TAR and HP were recorded using optical cells with a path length of 1 cm and 1 mm, respectively. The arrow indicates the spectral shift upon increasing the temperature.

Temperature-dependent absorption spectra of RNA-skeleton probes and their complexes.

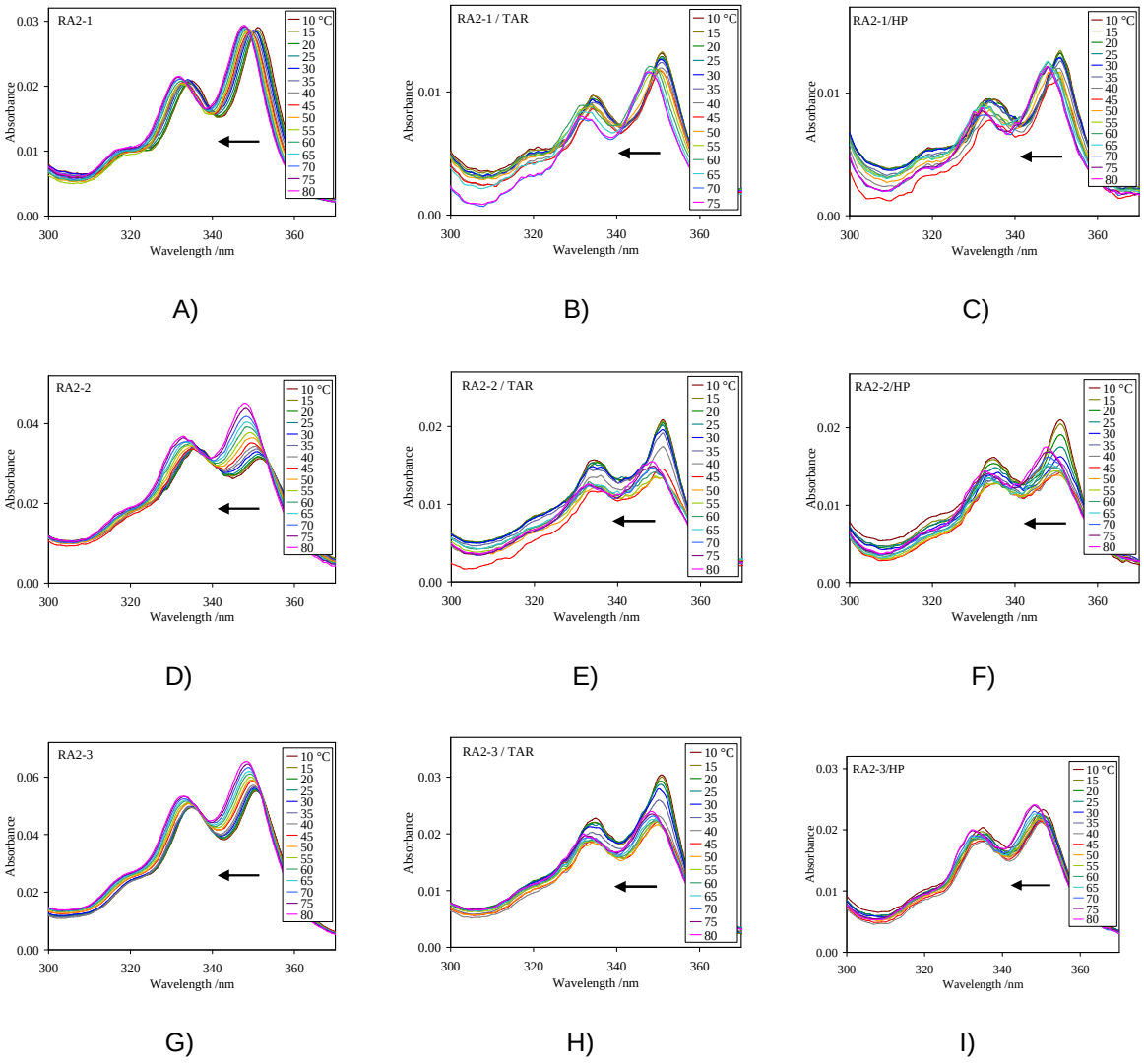

Figure S3. Temperature-dependent absorption spectra of RNA-skeleton probes and their complexes. (A) RA2-1); (B) RA2-1/TAR; (C) RA2-1/HP; (D) RA2-2; (E) RA2-2/TAR; (F) RA2-2/HP; (G) RA2-3; (H) RA2-3/TAR; (I) RA2-3/HP. Spectra of the probes and their mixture with TAR and HP were recorded using optical cells with a path length of 1 cm and 1 mm, respectively. The arrow indicates the spectral shift upon increasing the temperature.

Temperature-dependent absorption spectra of RNA-skeleton probes with pyrene-carboxaldehyde chromophores and their complexes

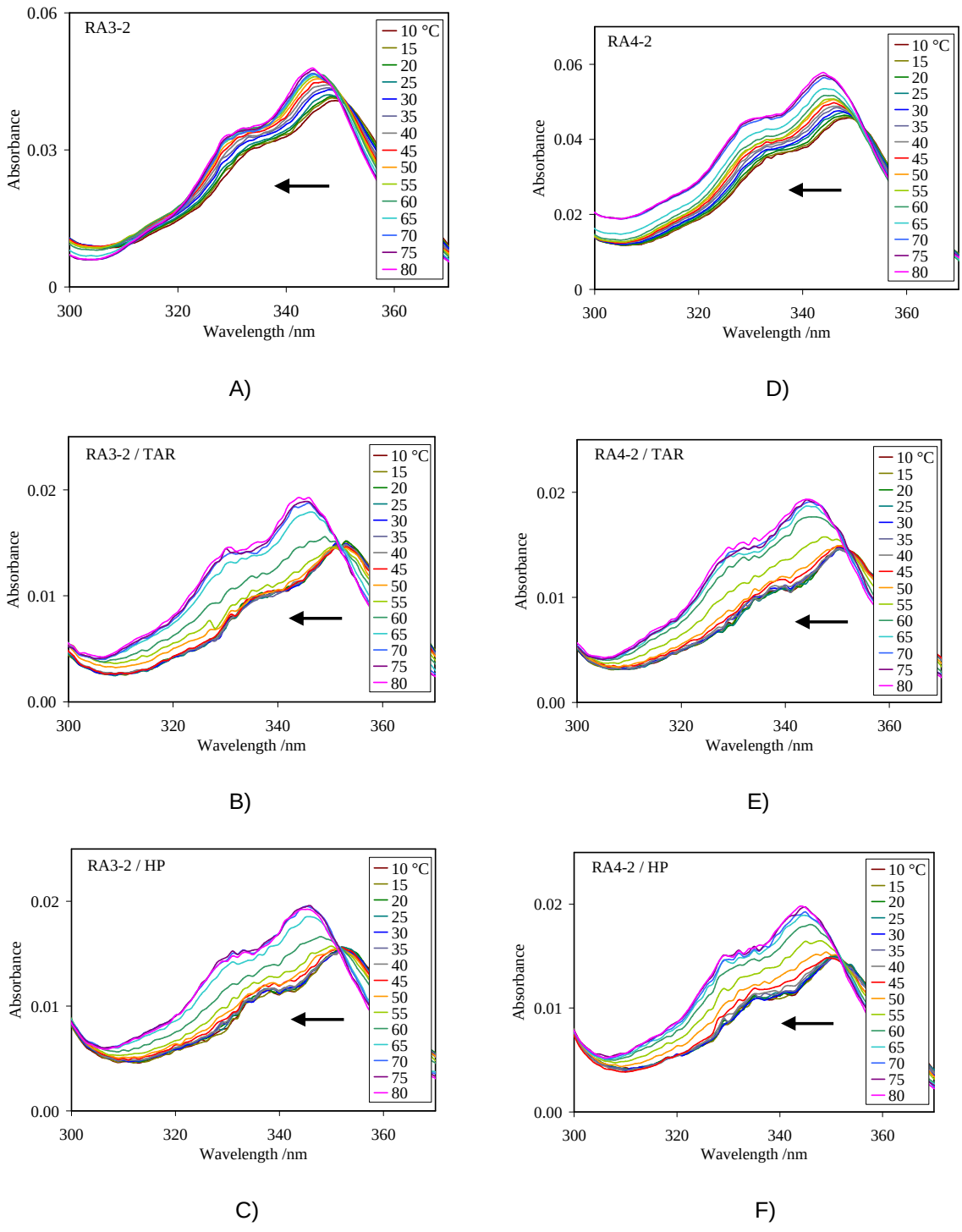

Figure S4. Temperature-dependent absorption spectra of RNA-skeleton probes with pyrene-carboxaldehyde chromophores and their complexes at the region between 300 and 370 nm. A) RA3-2; B) RA3-2/TAR; C) RA3-2/HP; D) RA4-2; E) RA4-2/TAR; F) RA4-2/HP. Spectra of the probes and their mixture with TAR and HP were recorded using optical cells with a path length of 1 cm and 1 mm, respectively. The arrow indicates the spectral shift upon increasing the temperature.

Part 4: Temperature-dependent fluorescence spectra.

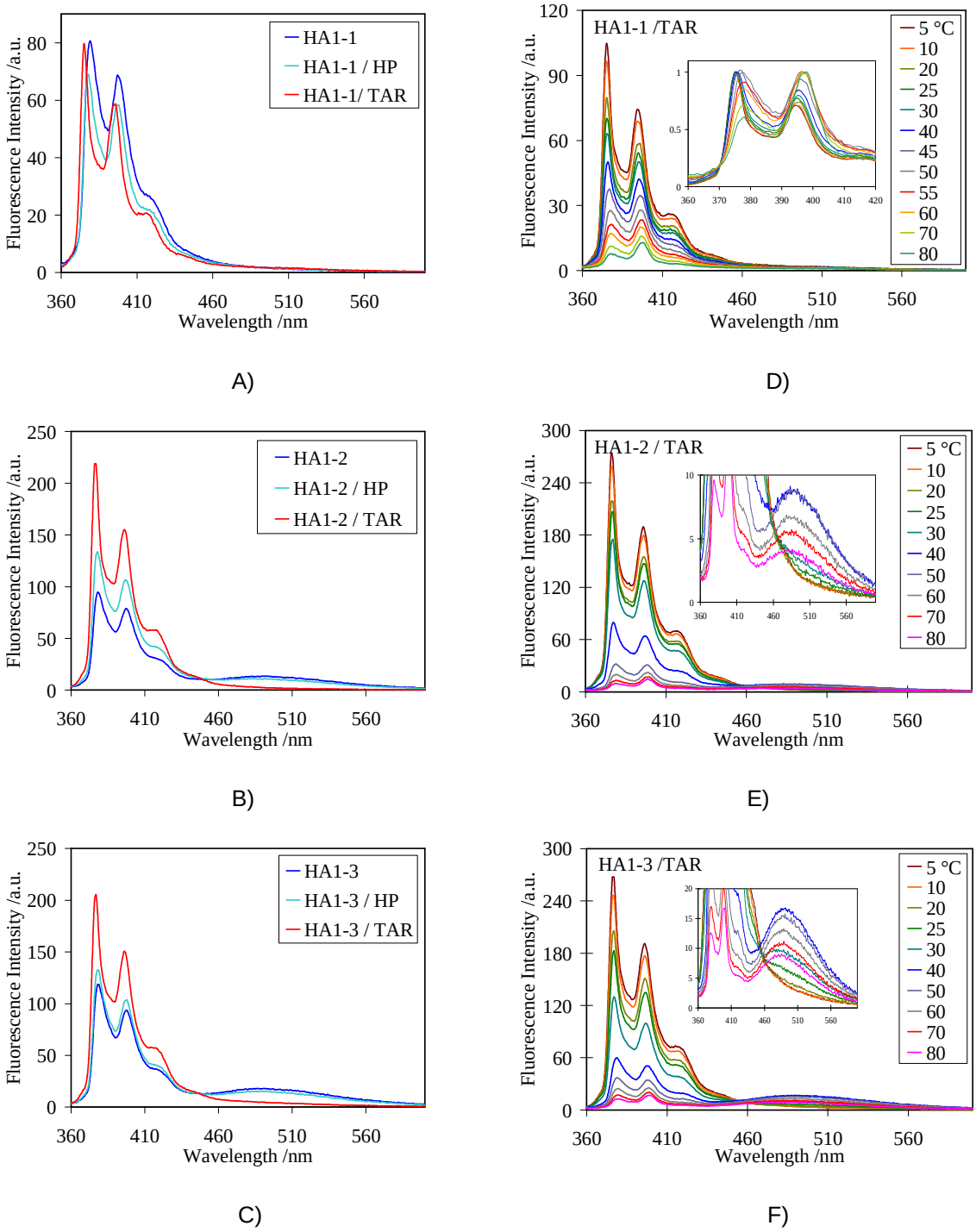

Figure S5. A) Fluorescence spectra of HNA-skeleton probe HA1-1 and the complexes with RNA targets TAR and HP at RT. B) Fluorescence spectra of HNA-skeleton probe HA1-2 and its mixture with RNA targets TAR and HP at RT. C) Fluorescence spectra of HNA-skeleton probe HA1-3 and its mixture with RNA targets TAR and HP at RT. D) Spectra were recorded in 0.16 μM solution upon excitation at 350 nm.

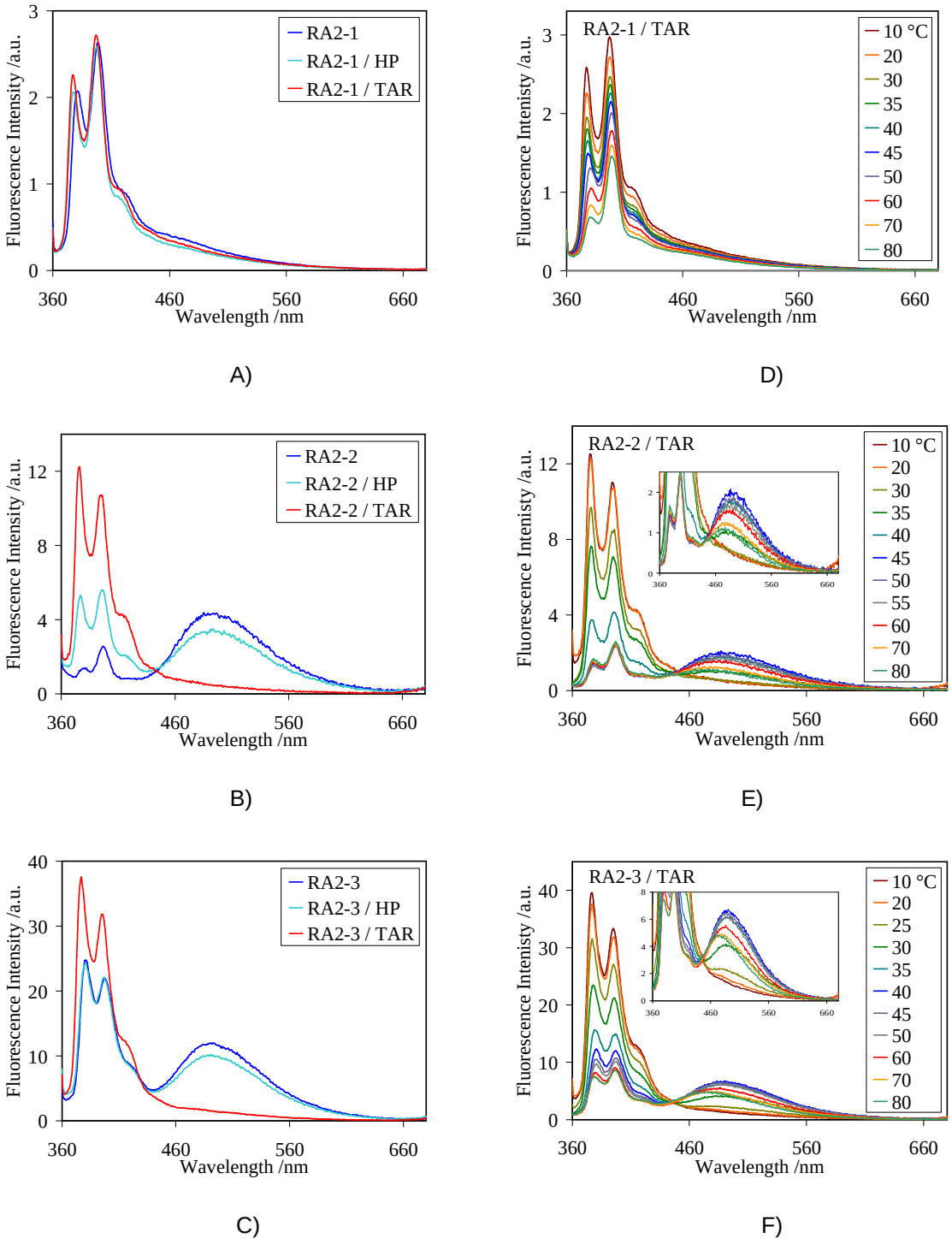

Figure S6. A) Fluorescence spectra of RNA-skeleton probe RA2-1 and the complexes with RNA targets TAR and HP at RT. B) Fluorescence spectra of RNA-skeleton probe RA2-2 and the complexes with RNA targets TAR and HP at RT. C) Fluorescence spectra of RNA-skeleton probe RA2-3 and the complexes with RNA targets TAR and HP at RT. D) Temperature-dependent fluorescence spectra of RA2-1/TAR. E) Temperature-dependent fluorescence spectra of RA2-2/TAR. F) Temperature-dependent fluorescence spectra of RA2-3/TAR. Spectra were recorded at 0.16 μM at 20°C upon excitation at 350 nm.

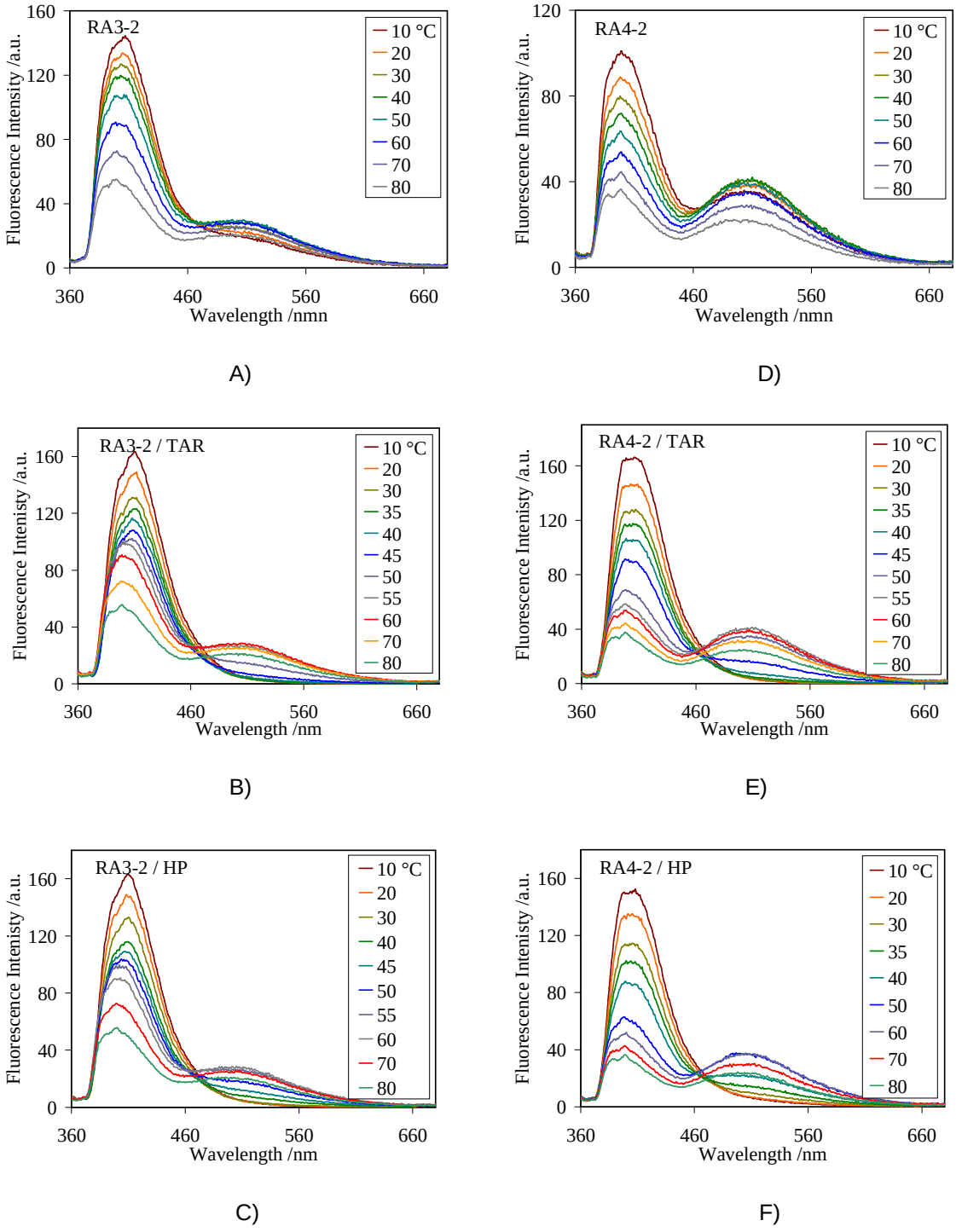

Figure S7. Temperature-dependent fluorescence spectra of RNA-skeleton probes RA3-2 and RA4-2 and their mixtures. A) RA3-2; B) RA3-2/TAR; C) RA3-2/HP; D) RA4-2; E) RA4-2/TAR; F) RA4-2/HP. Spectra were recorded in 0.16 μM solution upon excitation at 350 nm.

Part 5: Melting curves

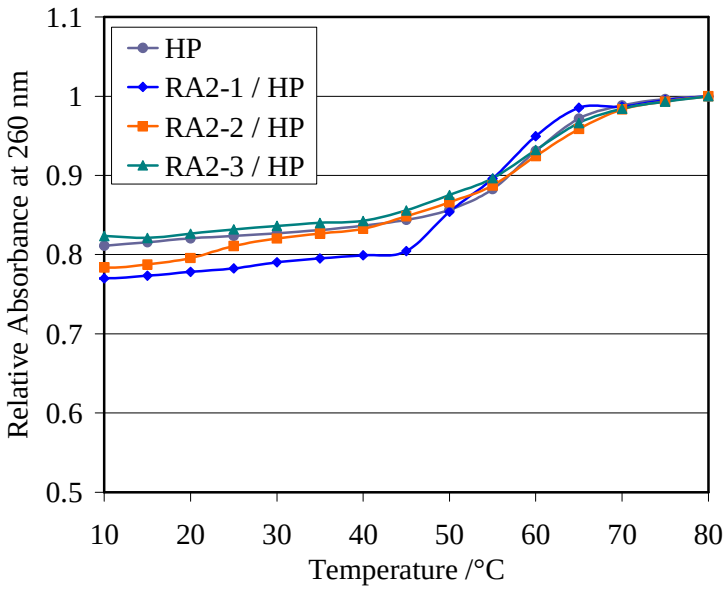

Figure S8. UV-melting curves monitored at 260 nm for RNA-skeleton probes/HP. Concentration is ~ 4 µM.

Part 6: Excitation spectra

Excitation spectra support the description "aggregate/excimer" emission. The different environment of the pyrenes yielding monomer emission and those yielding excimer/aggregate emission is indicated by the excitation spectra of RA4-2 and RA3-2: the excitation spectra show that the main maximum is broadened towards longer wavelengths and a shoulder is observed around 380 nm. For HA1-2, RA2-2, and RA2-3 the relative intensity of the 0-1 band is increased significantly compared to that of the 0-0 transition. Furthermore, we observe a redshift of the 0-0 transition.

Those effects clearly suggest that the emission beyond 420 nm is due to excitation of pyrenes which already show exciton interaction in the ground state. The long-wavelength emission is however due to species that show a much larger exciton coupling than the ground state pyrenes. This suggests indeed that after excitation of the "aggregates" significant relaxation occurs. Hence the emission beyond 420 nm is due to species that show as well characteristics of aggregates as of excimers.

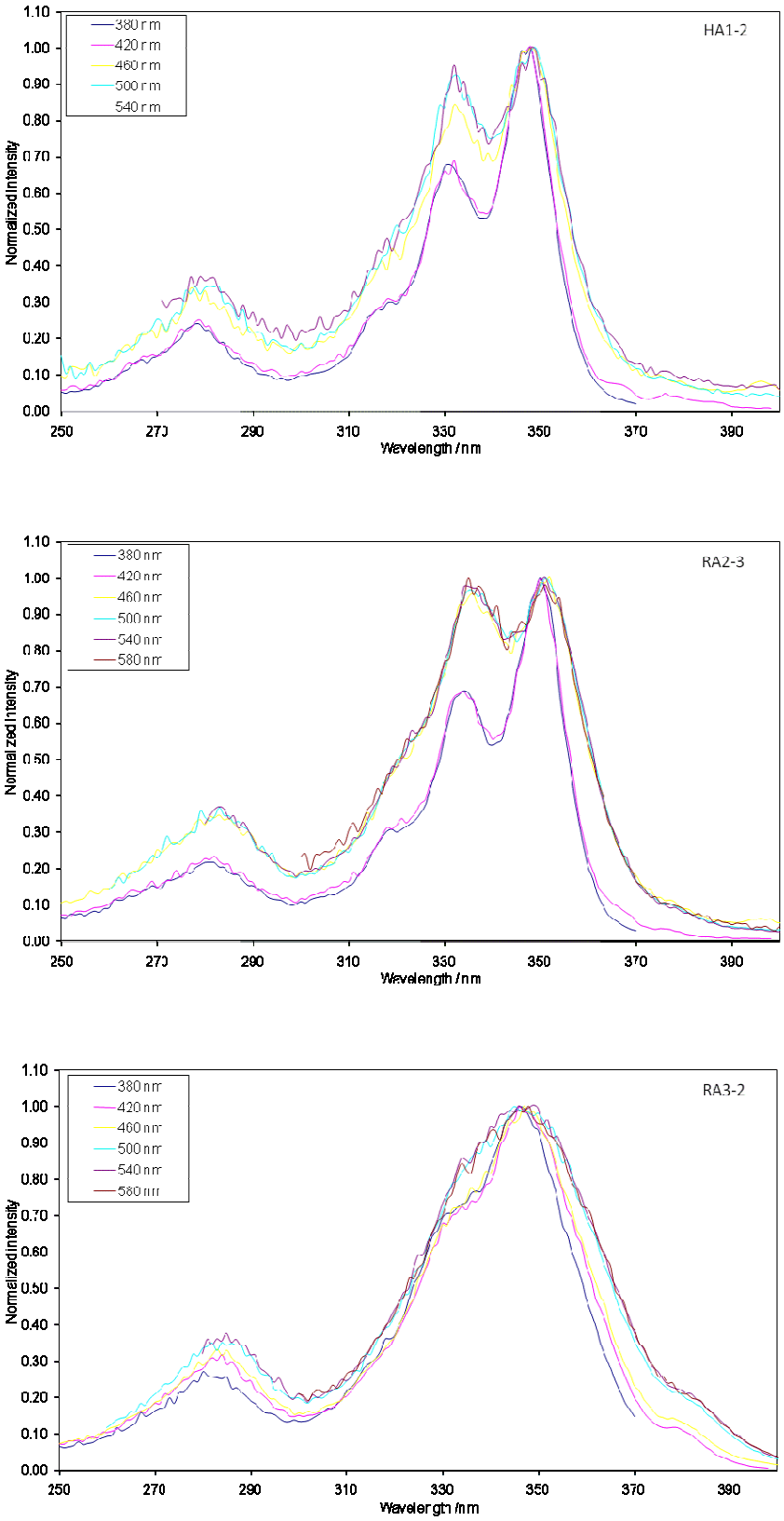

Figure S9. Excitation spectra of HA1-2, RA2-3, and RA3-2. The emission wavelengths are indicated in the inset.