Supplementary Information

Cyclohexyl “base-pair” stabilizes the duplex and intensify pyrene fluorescence by shielding it from natural base-pairs

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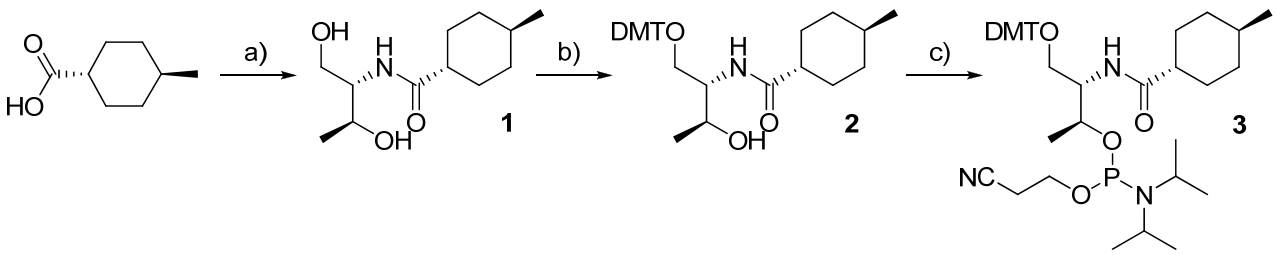


Scheme S1. Synthesis of phosphoramidite tethering biphenyl-4-carboxylic acid. Reagents and conditions: a) D-threoninol, DCC, HOBt, DMF, r.t., overnight, quant.; b) DMT-Cl, DIPEA, DMAP, CH_2Cl_2, pyridine, 0→r.t., 63 %.; c) $(i\mathrm{Pr})_2\mathrm{NP(Cl)(OCH_2CH_2CN)}$, Et_3N, THF, 0 °C → r.t., 1h, 78 %.

The phosphoramidite monomer tethering 4-methylcyclohexanecarboxylic acid was synthesized as follows:

trans-4-Methylcyclohexanecarboxylic acid (0.83 g, 5.83 mmol) was coupled with D-threoninol (0.527 g, 4.86 mmol) in the presence of *N*, *N*'-dicyclohexylcarbodiimide (1.50 g, 7.27 mmol) and 1-hydroxybenzotriazole (0.99 g, 7.32 mmol) in DMF (40 ml). After the reaction mixture was stirred at room temperature for overnight, the solvent was removed and the remained oil was subjected to silica gel column chromatography (CH_3OH : $CHCl_3$ = 1:5, R_f = 0.18) to afford compound **1** (yield quant.). ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 6.29 (d, *J* = 8 Hz, 1H, -N*H*CO-), 4.18 (m, 1H, -C*H*(CH3)OH), 3.80 (m, 3H, -C*H*$_2$OH, -CH$_2$C*H*(NHCO)CH), 3.25 (br, 2H, -CH$_2$O*H*, -CH(CH$_3$)O*H*), 2.10 (m, 1H, -COC*H*(CH$_2$)$_2$), 1.90 (m, 2H, -COCH(C*H*$_2$)$_2$), 1.77 (m, 2H, -(C*H*$_2$)$_2$CHCH$_3$), 1.49 (m, 2H, -COCH(C*H*$_2$)$_2$), 1.37 (m, 1H, -(CH$_2$)$_2$C*H*CH$_3$), 1.18 (d, 3H, *J* = 6 Hz, -CH(C*H*$_3$)OH), 0.94 (m, 2H, -(C*H*$_2$)$_2$CHCH$_3$), 0.89 (d, 3H, *J* = 6.5 Hz, -(CH$_2$)$_2$CHC*H*$_3$). HRMS(FAB) Calcd for $C_{12}H_{24}NO_3$ ($M+H^+$) 230.1756. Found 230.1753.

Dry pyridine solution (20 ml) containing **1** (1.11 g, 4.86 mmol) and *N*,*N*-diisopropylethylamine (DIPEA) (1.26 ml, 7.26 mmol) was cooled on ice under nitrogen. Then, 4,4'-dimethoxytrityl chloride (DMT-Cl) (2.46 g, 7.26 mmol) and 4-(dimethylamino)pyridine (0.07 g, 0.60 mmol) in CH_2Cl_2 (10 ml) was added to the above mixture. After 3 h of vigorous stirring, the solvent was removed by evaporation, followed by silica gel column chromatography (AcOEt : hexane : Et_3N = 50:50:3, R_f = 0.32) to afford **2** (1.62 g, yield 63 %). ^{1}H-NMR [$CDCl_3$, 500 MHz] δ = 7.37 (m, 2H, aromatic protons of DMT), 7.30-7.22 (m, 7H, aromatic protons of DMT), 6.84-6.82 (m, 4H, aromatic protons of DMT), 6.13 (d, *J* = 9 Hz, 1H, -N*H*CO-), 4.07 (m, 1H, -C*H*(CH3)OH), 3.91 (m, 1H, -CH$_2$C*H*(NHCO)CH), 3.79 (s, 6H, -OC*H*$_3$), 3.43 (dd, *J* = 4 Hz, 10 Hz, 1H, -C*H*$_2$ODMT), 3.40 (dd, *J* = 3.5 Hz, 9.5 Hz, 1H, -C*H*$_2$ODMT), 3.16 (br, 1H, -CH(CH$_3$)O*H*), 2.07 (m, 1H, -COC*H*(CH$_2$)$_2$), 1.90 (m, 2H, -COCH(C*H*$_2$)$_2$), 1.79 (m, 2H, -(C*H*$_2$)$_2$CHCH$_3$), 1.50 (m, 2H, -COCH(C*H*$_2$)$_2$), 1.39 (m, 1H, -(CH$_2$)$_2$C*H*CH$_3$), 1.11 (d, 3H, *J* = 6.5 Hz, -CH(C*H*$_3$)OH), 0.97 (m, 2H, -(C*H*$_2$)$_2$CHCH$_3$), 0.90 (d, *J* = 6.5 Hz, 3H, -(CH$_2$)$_2$CHC*H*$_3$). HRMS(FAB) Calcd for $C_{33}H_{41}NO_5$ (M^+) 531.2985. Found 531.2980.

DIPEA (1.06 ml, 4.70 mmol) and 2-cyanoethyldiisopropylchlorophosphoramidite (0.55 ml, 2.45 mmol) were added to a solution of compound **2** (0.65 g, 1.22 mmol) in THF (5 ml) at 0 °C. After 20 min of vigorous stirring on ice, the solution was stirred for 40 min at room temperature. Then, an excess of AcOEt was added to the reaction mixture and was washed with saturated aqueous solution of $NaHCO_3$ and of NaCl. After drying over $MgSO_4$, the solvent was removed by evaporation, followed by silica gel column chromatography (AcOEt : hexane : Et_3N = 30:70:3, R_f = 0.70) to afford **3** (0.70 g, yield 78 %). ^{31}P-NMR [121 MHz, $CDCl_3$] δ =148.8, 148.7. HRMS(FAB) Calcd for $C_{42}H_{58}N_3O_6PNa$ ($M+Na^+$) 754.3961. Found 754.3975.

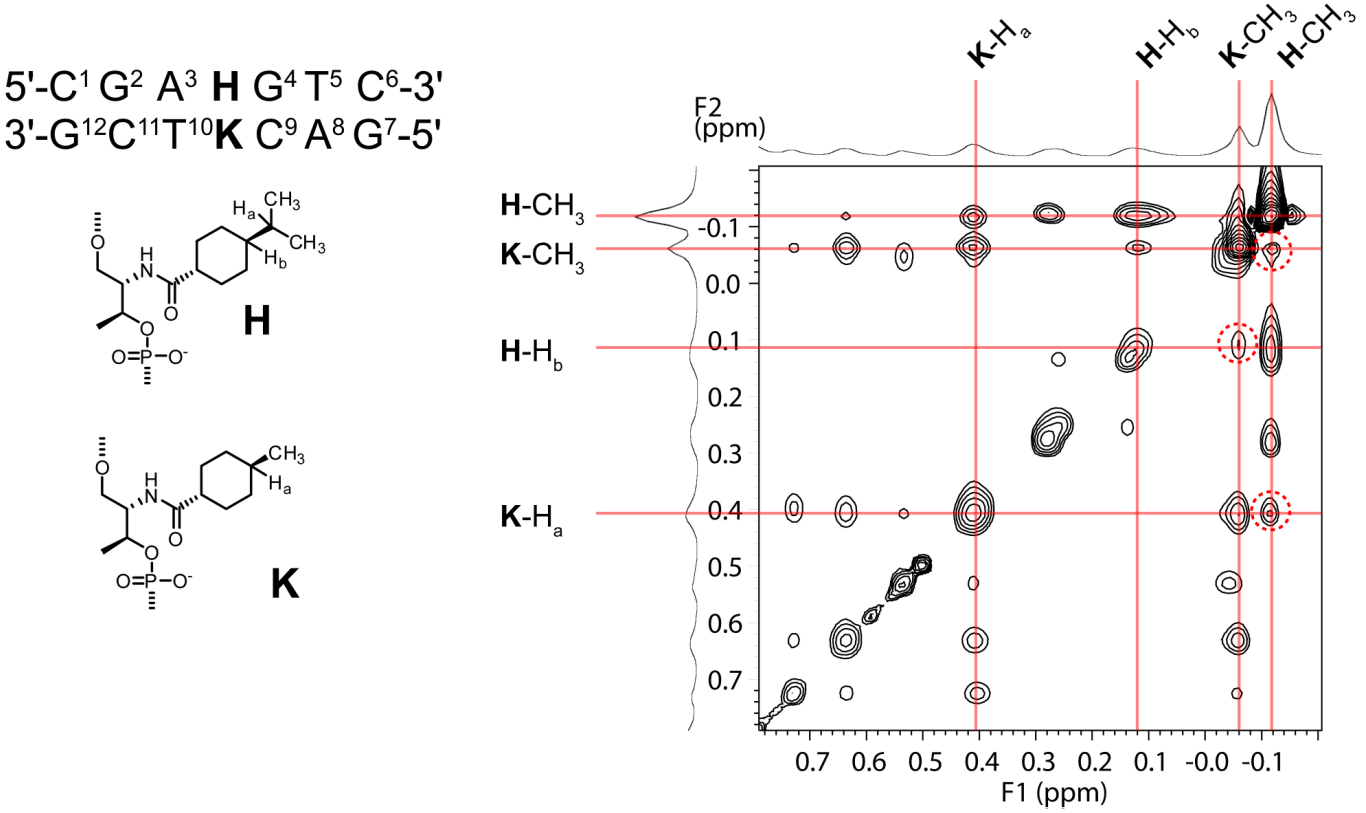


Figure S1. 2D NOESY spectra of **H1C**/**K1D** between aliphatic protons in H_2O/D_2O (9/1). 1D spectra of aliphatic protons are shown at the top and left of the chart. The residue numbers and proton numbers of **H** and **K** residues are shown at the left.

NMR samples were prepared by dissolving three-times-lyophilized DNA in an H_2O/D_2O 9:1 solution containing 10 mM sodium phosphate (pH 7.0) to give a duplex concentration of 1.7 mM. NaCl was added to give a final sodium concentration of 200 mM.

NMR spectra were measured with a Varian INOVA spectrometer (700 MHz) equipped for triple resonance at a probe temperature of 275 K. Resonances were assigned by standard methods using a combination of 1D, TOCSY (60 ms of mixing time), DQF-COSY and NOESY (150 ms of mixing time) experiments. All spectra in the H_2O/D_2O 9:1 solution were recorded using the 3-9-19 WATERGATE pulse sequence for water suppression.

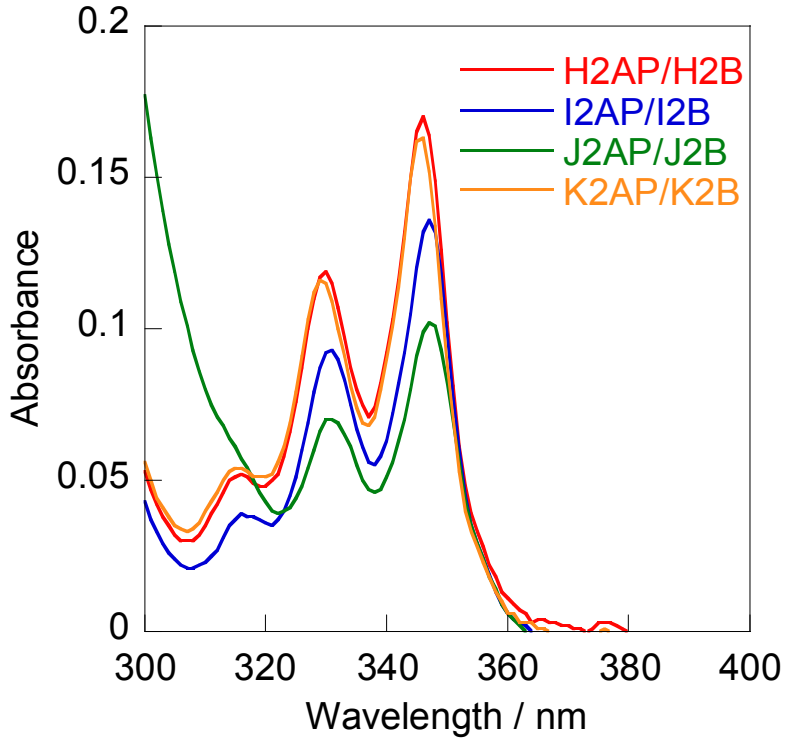


Figure S2. UV-VIS spectra of **H2AP**/**H2B**, **I2AP**/**I2B**, **J2AP**/**J2B**, and **K2AP**/**K2B** at 20 °C. Conditions: [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), [DNA] = 5.0 μM.

Sequences:

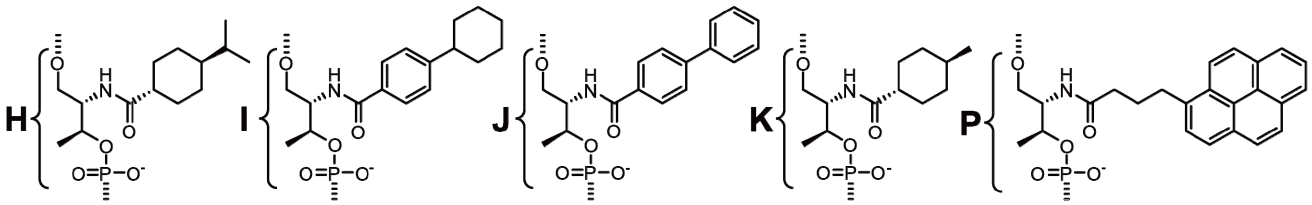
H*n*A : 5'-GGTATC**H**$_n$GCAATC-3' n = 1,2,6
H*n*B : 3'-CCATAG**H**$_n$CGTTAG-5' n = 1,2,6
I1A : 5'-GGTATC**I**GCAATC-3'
I*n*B : 3'-CCATAG**I**$_n$CGTTAG-5' n = 1,2,6
J1A : 5'-GGTATC**J**GCAATC-3'
J*n*B : 3'-CCATAG**J**$_n$CGTTAG-5' n = 1,2
K1A : 5'-GGTATC**K**GCAATC-3'
K*n*B : 3'-CCATAG**K**$_n$CGTTAG-5' n = 1,2,6

A*n*A : 5'-GGTATCA$_n$GCAATC-3' n = 1,2,6
T*n*B : 3'-CCATAGT$_n$CGTTAG-5' n = 1,2,6
G1A : 5'-GGTATCGGCAATC-3'
C1B : 3'-CCATAGCCGTTAG-5'

P0 : 5'-GGTATCGCAATC-3'
P1 : 5'-GGTATC**P**GCAATC-3'
N : 3'-CCATAGCGTTAG-5'
H1C : 5'-CGA**H**GTC-3'
K1D : 3'-GCT**K**CAG-5'

H2AP : 5'-GGTATC**HPH**GCAATC-3'
I2AP : 5'-GGTATC**IPI**GCAATC-3'
J2AP : 5'-GGTATC**JPJ**GCAATC-3'
K2AP : 5'-GGTATC**KPK**GCAATC-3'

H6AP : 5'-GGTATC**HHHPHHH**GCAATC-3'
I6AP : 5'-GGTATC**IIIPIII**GCAATC-3'
K6AP : 5'-GGTATC**KKKPKKK**GCAATC-3'



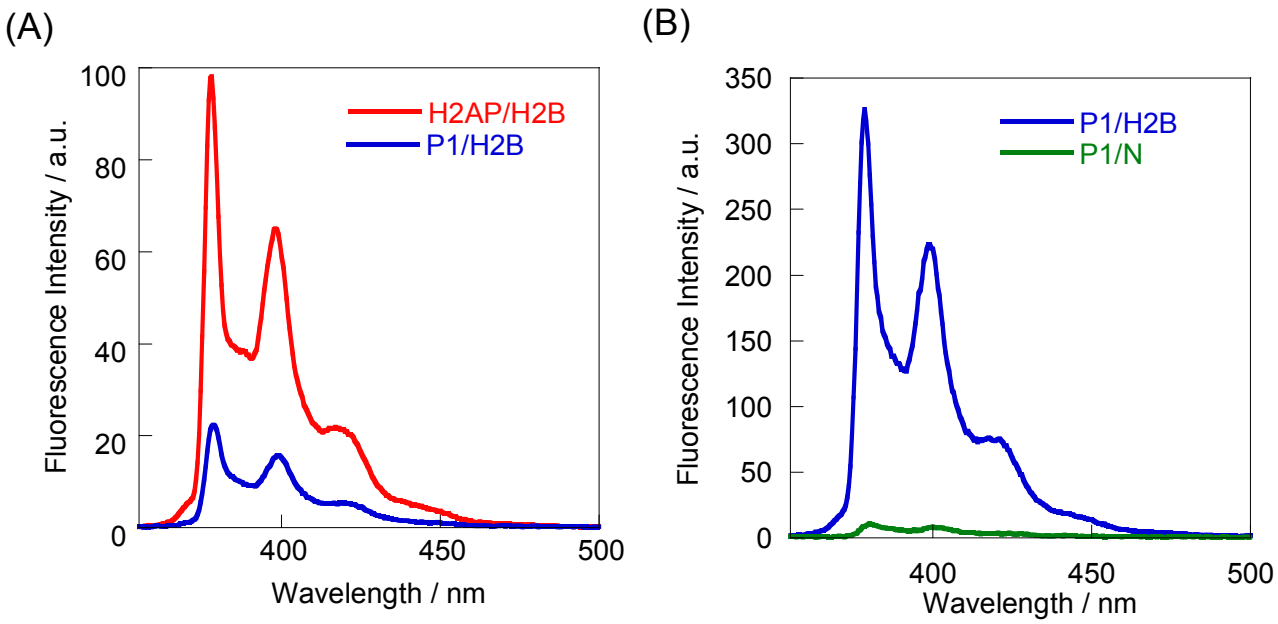


Figure S3. Fluorescence emission spectra of **H2AP**/**H2B**, **H2AP**/**N** and **P1**/**N** at 20 °C. Note that spectra in (B) were measured at high sensitivity mode whereas those in (A) were measured at medium sensitivity mode. Conditions: [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), [ODN] = 1.0 μM.

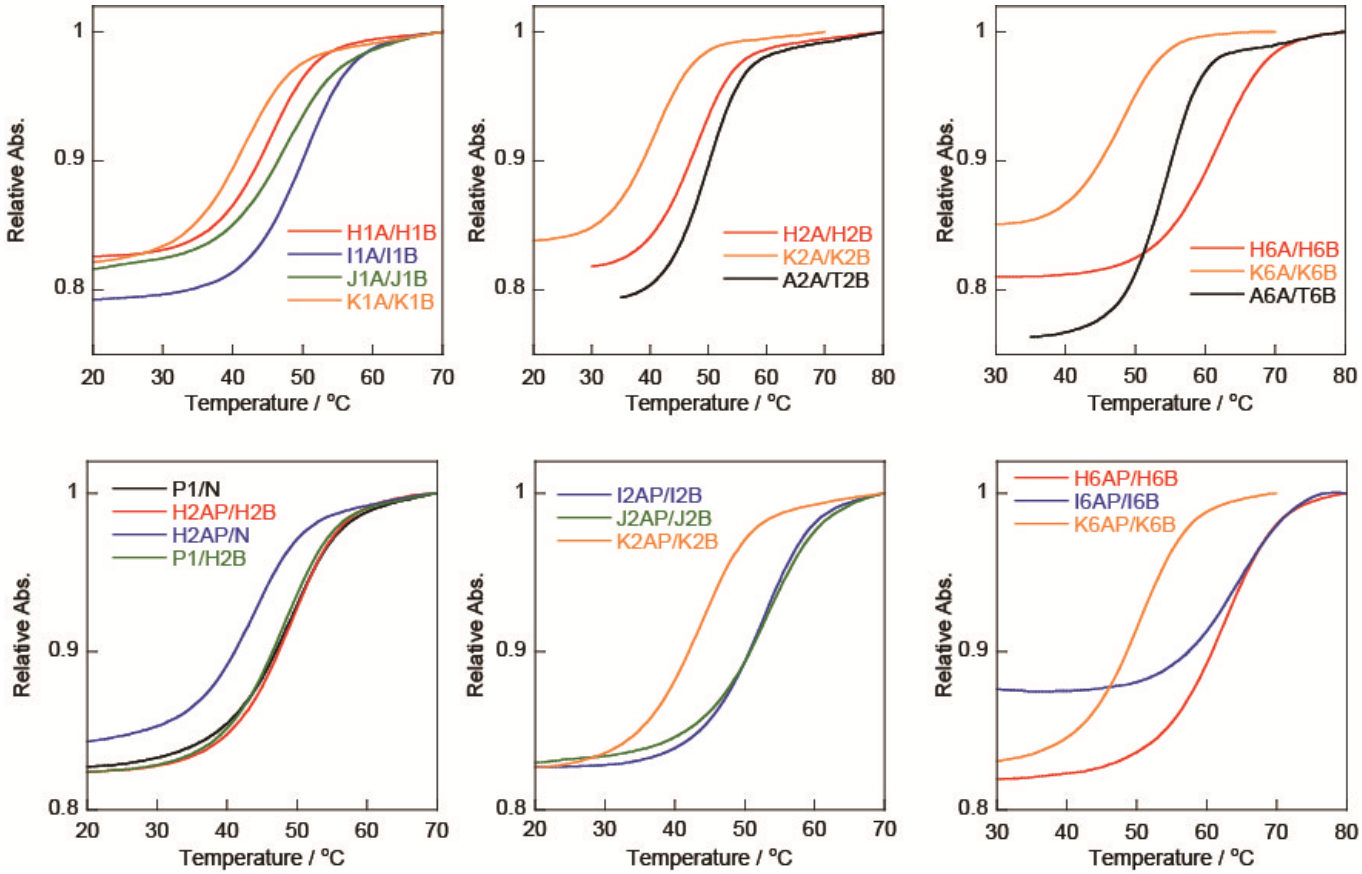


Figure S4. Melting curves of duplexes. Conditions: [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

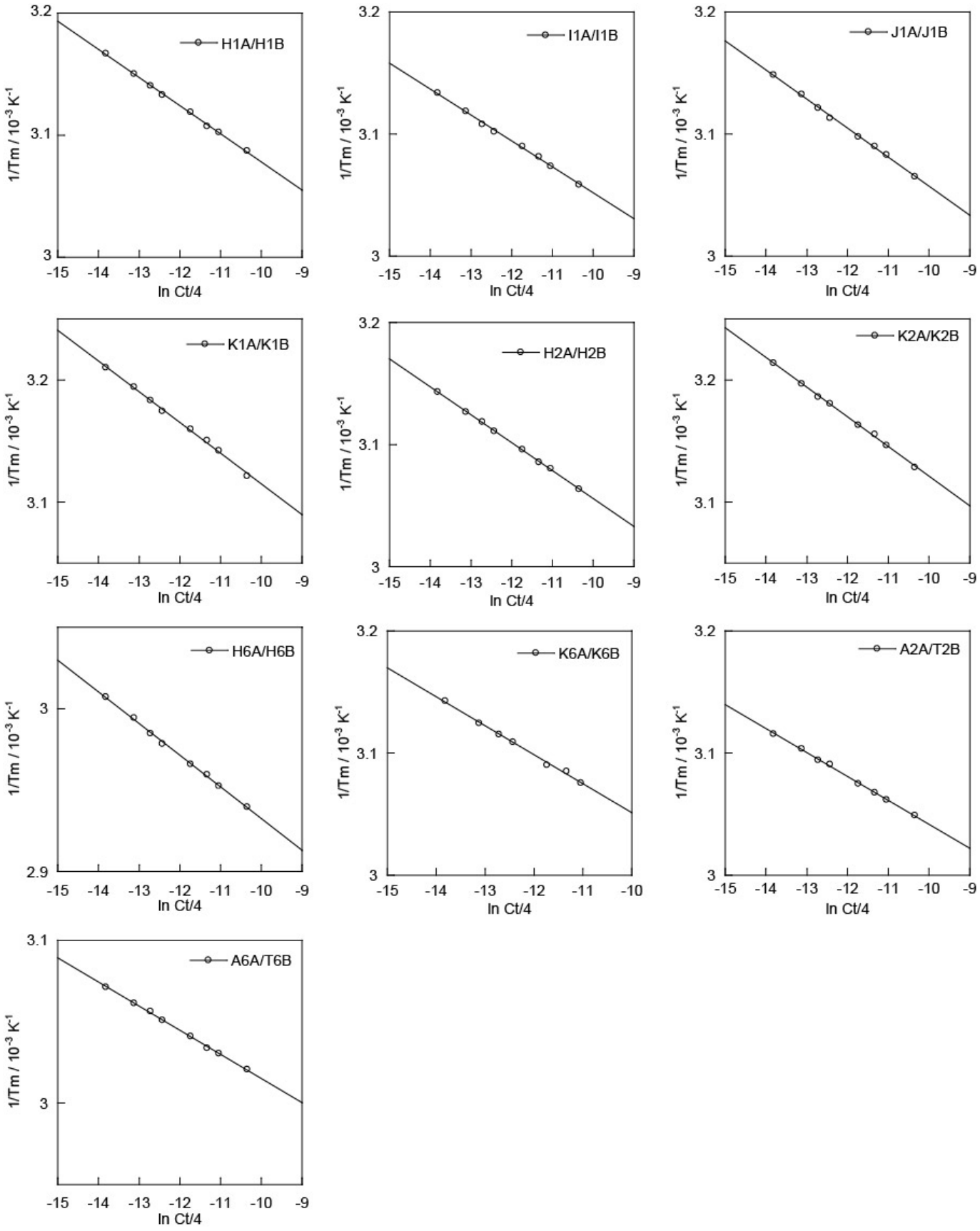
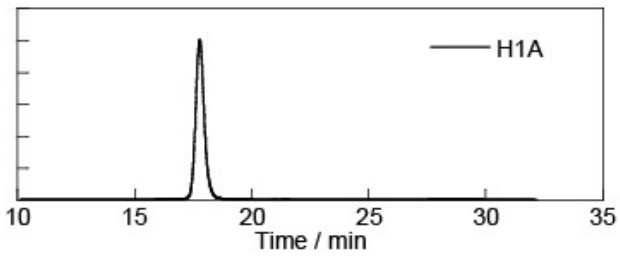
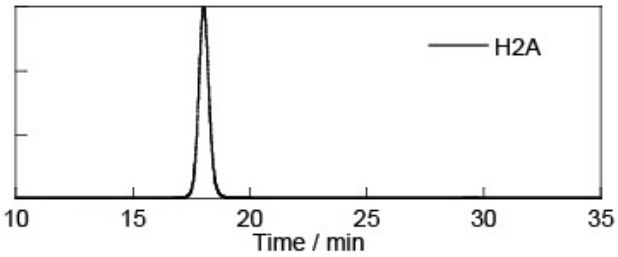
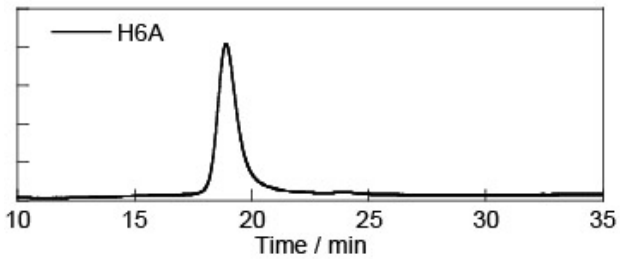
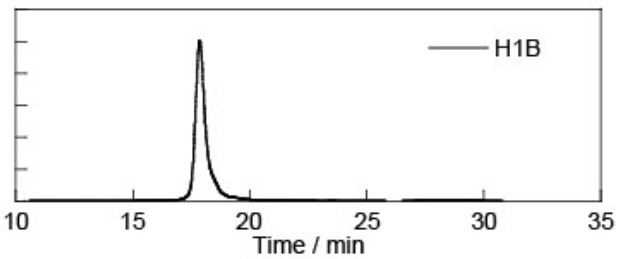


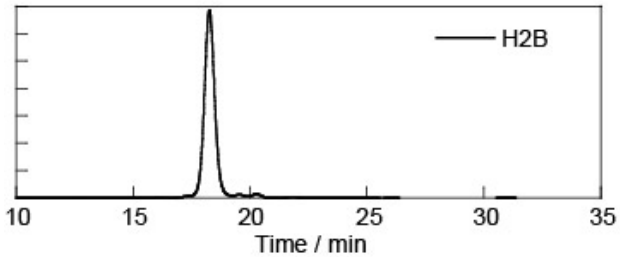
Figure S5. $1/T_m$ versus $\ln(C_T/4)$ plots of duplexes. Conditions: [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer).

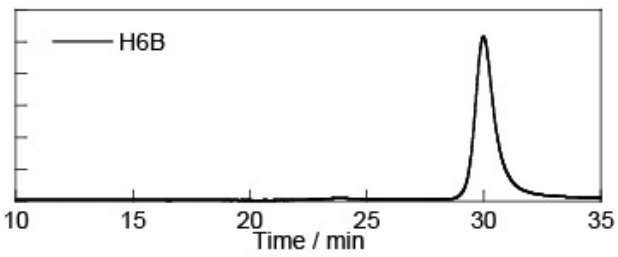


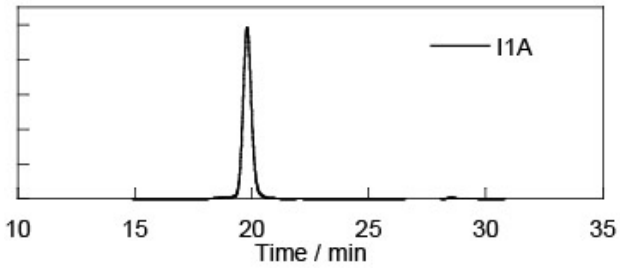


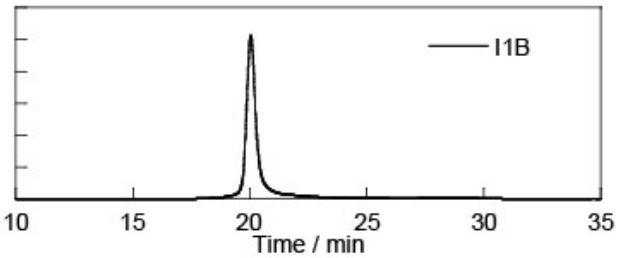


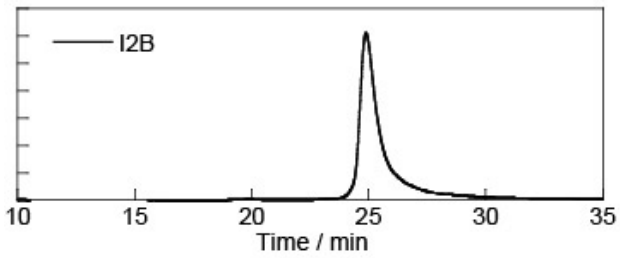


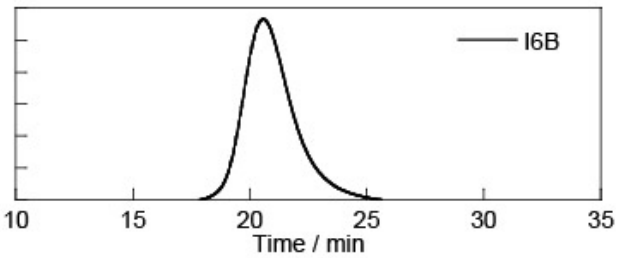


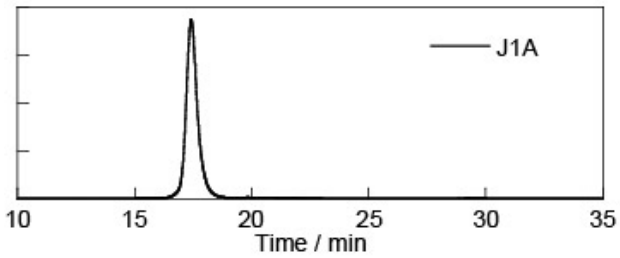


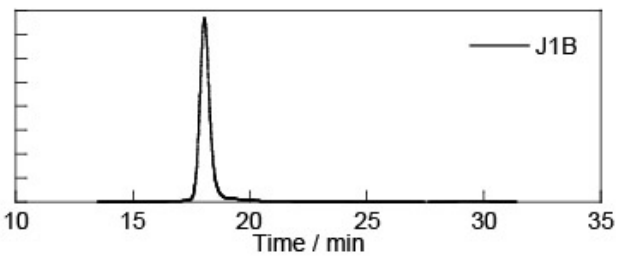


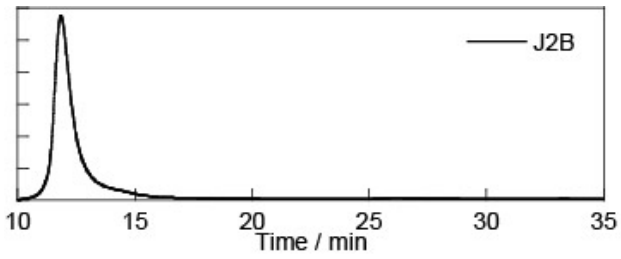


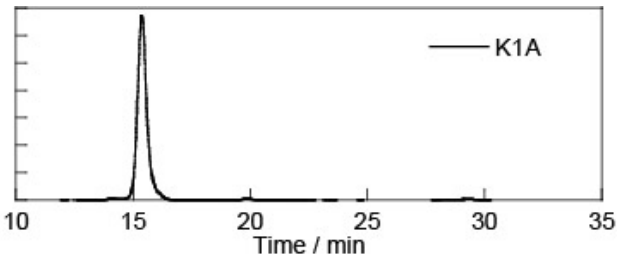


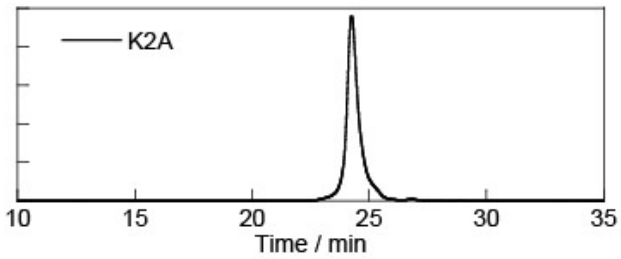


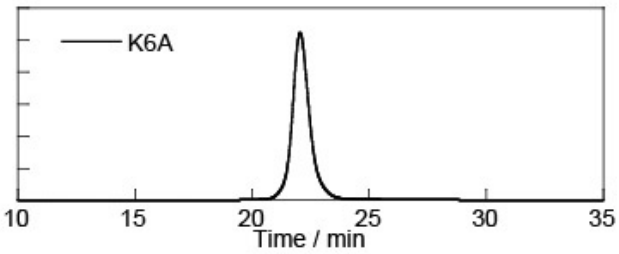


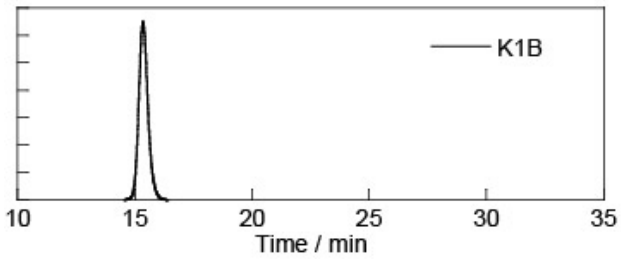


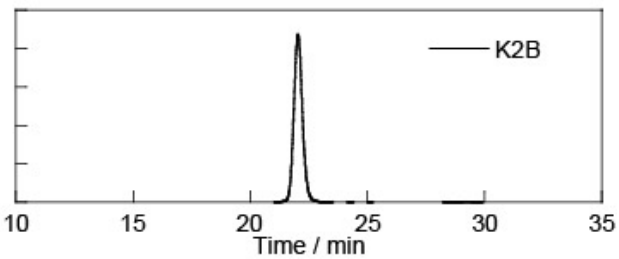


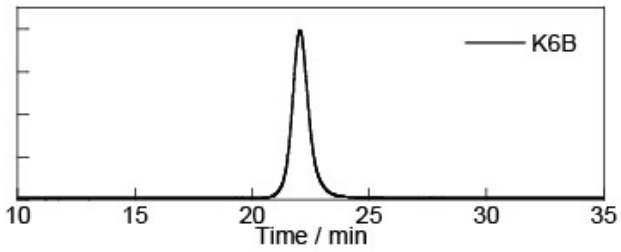


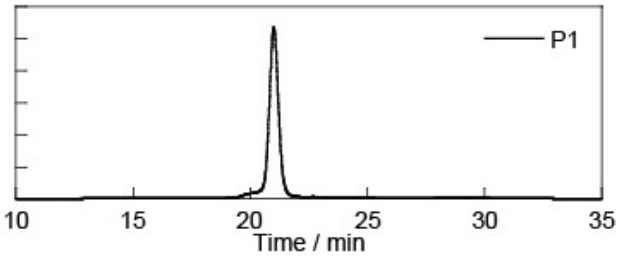


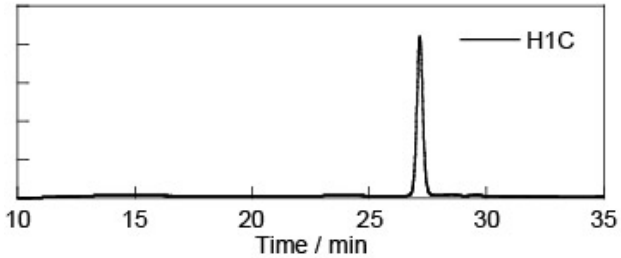


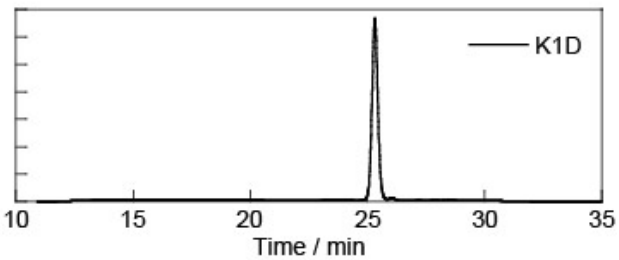


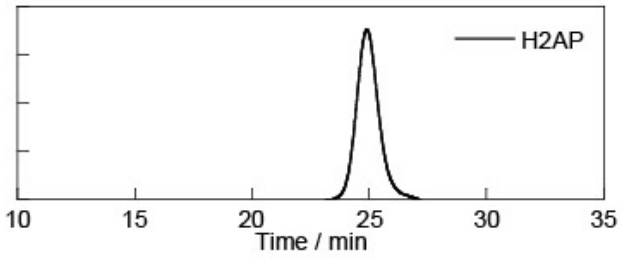


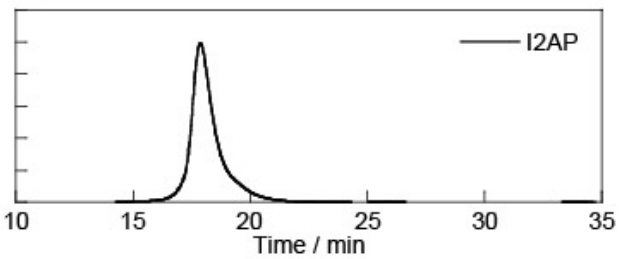












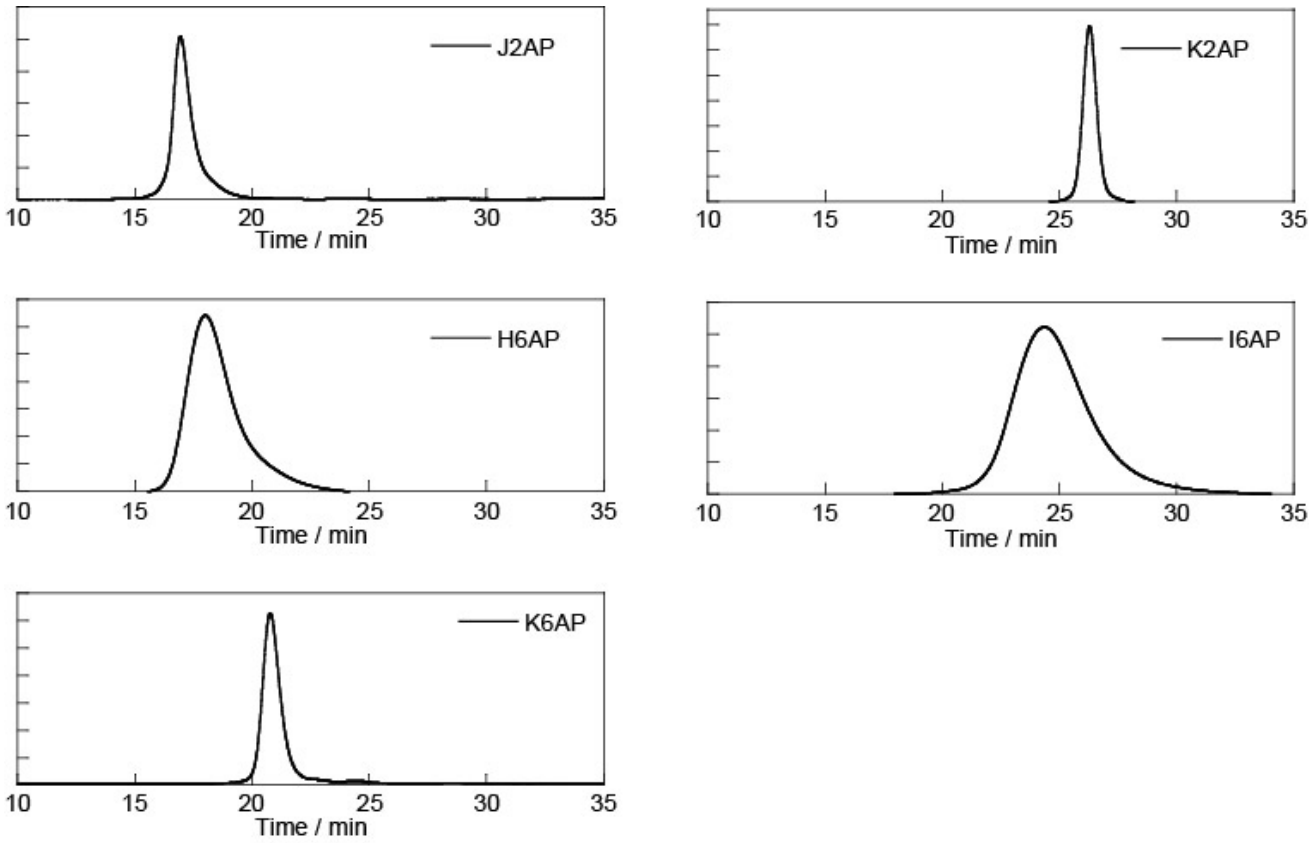


Figure S6. HPLC charts of purified ODNs. A linear gradient of acetonitrile in 50 mM ammonium formate over 30 min was used (flow rate: 0.5 mL/min). Typical gradients: 7.5-17.5 % (**X1A**, **X1B** and **P1**) 12.5-22.5 % (**X2A** and **X2B**), 30-45 % (**X6A** and **X6B**), 5-15 % (**H1C** and **K1D**), 20-30 % (**X2AP**), 40-50 % (**X6AP**). X represents **H**, **I**, **J** and **K**.