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Supporting Information

β,γ-bis-substituted PNA with configurational and conformational switch: Preferred binding to cDNA/RNA and cell-uptake

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General information:

All the non-aqueous reactions were carried out under the inert atmosphere of Nitrogen/ Argon and the chemicals used were of laboratory or analytical grade. All solvents used were dried and distilled according to standard protocols. TLCs were carried out on pre-coated silica gel 60 F_{254} (Merck). Column chromatographic separations were performed using silica gel 60- 120 mesh (Merck) or 200- 400 mesh (Merck) and using the solvent systems EtOAc/Petroleum ether and MeOH/DCM. ^{1}H and ^{13}C NMR spectra were obtained using Bruker AC-200, AC-400 and AC-500 NMR spectrometers. The chemical shifts are reported in delta (δ) values and referred to internal standard TMS for ^{1}H. High resolution mass spectra were recorded on a Thermo Fisher Scientific Q Exactive mass spectrometer. Specific rotations of the sample were procured from Bellingham Stanley ADP220 Polarimeter.

(4*S*, 5*S*)-1,4-dimethoxybutane-2,3-diamine ($2a_1$)

(4*R*, 5*R*)-1,4-dimethoxybutane-2,3-diamine ($2b_1$)

To a solution of azide (0.5g, 2.9mmol) in methanol was added 10% Pd/C (0.05g). The mixture was hydrogenated at room temperature under 50 psi pressure for 4h. The catalyst was then removed by filtration over celite. The filtrate was collected and the solvent was removed under vacuum to furnish a colorless liquid which eventually turned brown. The crude compound was carried forward for the next reaction without further purification.

(4*S*, 5*S*)-1,4-bis(benzyloxy)butane-2,3-diamine ($2a_2$)

(4*R*, 5*R*)--1,4-bis(benzyloxy)butane-2,3-diamine ($2b_2$)

To a solution of azide (0.3g, 0.9mmol) in THF (12mL), PPh_3 (0.89g, 3.4mmol) and few drops of water was added. The solution was heated to 60^{o}C for 1h, cooled down to rt. The mixture was diluted with ether (10mL) and the organic phase was extracted with AcOH (10% in H_2O).The combined aqueous extract was washed with ether and lyophilized to give amine. The crude compound was used as such for the next reaction without further purification.

(4*S*, 5*S*)-*tert*-butyl (3-amino-1,4-dimethoxybutan-2-yl)carbamate ($3a_1$)

A solution of di-*tertiary* butyl carbonate (1.5g,7.0mmol) diluted in dioxane(250mL) was added over a period of 5h to the solution of diamine(1.3g, 8.8mmol) in dioxane(75mL) at 0^{o}C.The mixture was allowed to stir for 12h at rt. The solvent was removed under vacuum and water was added to it when di-*tert*-butyl carbamate compound separated out. The aqueous layer was extracted with 10% MeOH in DCM (10x50mL). The organic layer was collected and solvent removed to get **$3a_1$** (1.4g, 52%). $[\alpha]_D^{20}$ -7.84 (c 1.02, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.55(s, 9H), 3.25-3.47(m, 5H), 3.35(s, 6H), 3.62-3.65(m, 1H), 5.31-5.35 (br s); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 28.31, 50.79, 51.27, 58.85, 73.48, 75.03, 79.16, 155.83; HRMS calcd for $C_{11}H_{25}N_2O_4$: 249.1809, Observed mass: 249.1815.

(4*R*, 5*R*)-*tert*-butyl (3-amino-1,4-dimethoxybutan-2-yl)carbamate ($3b_1$)

$[\alpha]_D^{20}$ +7.1 (c 1, $CHCl_3$)

(4*S*, 5*S*)-*tert*-butyl (3-amino-1,4-bis(benzyloxy)butan-2-yl)carbamate ($3a_2$)

A solution of di-*tertiary* butyl carbonate (0.99g, 4.5mmol) diluted in dioxane (250mL) was added over a period of 5h to the solution of diamine(1.7g, 5.7mmol) in dioxane (75mL).The mixture was allowed to stir for 12h at rt. The solvent was removed under vacuum and water was added to it when di-*tert*-butyl carbamate compound separated out. The

aqueous layer was extracted with 8% MeOH in DCM (10x50mL). The organic layer was collected and solvent removed to get **3a$_2$** (1.3g, 55%). $[\alpha]_D^{20}$-9.2(c 1.4,$CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.43(s, 9H), 3.45-3.64(m, 6H), 3.78(m, 1H), 4.49(s, 4H), 5.26-5.30(br s, 1H), 7.31(s, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 28.34, 51.10, 61.59, 69.12, 70.22, 73.23, 79.98, 127.87, 127.94, 128.44, 137.79, 156.70; HRMS calcd for $C_{23}H_{33}N_2O_4$: 401.2435, Observed mass: 401.2435

(4*R*, 5*R*)-*tert*-butyl (3-amino-1,4-bis(benzyloxy)butan-2-yl)carbamate (3b$_2$)

$[\alpha]_D^{20}$ +9.4 (c 1.0, $CHCl_3$)

(4*S*,5*S*)-ethyl 2-((3-((*tert*-butoxycarbonyl)amino)-1,4-dimethoxybutan-2-yl)amino)acetate (4a$_1$)

Compound **3a$_1$** (3.2g, 12.9mmol) was dissolved in 20mL dry ACN and TEA (3.6mL, 25.8mL) was added with stirring. The reaction mixture was cooled to 0^{o}C and ethyl bromoacetate (1.7mL, 15.5mmol) diluted in 10mL dry ACN was added drop wise. The reaction was stirred at rt for 4h after which solvent was removed under vacuo and the residue was diluted with EtOAc. The organic layer was washed with $NaHCO_3$ (2x50mL), dried over anhydrous Na_2SO_4 and concentrated to get the crude compound. The crude compound was purified by column chromatography (20% EtOAc in pet ether) to yield Compound **4a$_1$** as colorless liquid (3.2g,74%); $[\alpha]_D^{20}$ -6(c 2.0, $CHCl_3$);^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.28-1.31(t,3H,*J*=7.2Hz), 1.44(s, 9H), 2.27(br s, 1H), 2.94-3.01(m, 1H), 3.33(s, 3H), 3.34(s, 3H), 3.36-3.48(m, 6H), 3.77-3.81(m, 1H),4.13-4.24 (q, 2H, *J*=7.2Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 14.11, 28.28, 49.82, 57.79, 58.79, 60.62, 72.42, 73.33, 79.07, 155.71, 172.53; HRMS calcd for $C_{15}H_{31}N_2O_6$: 335.2177, Observed mass: 335.2183

(4*R*,5*R*)-ethyl 2-((3-((*tert*-butoxycarbonyl)amino)-1,4-dimethoxybutan-2-yl)amino)acetate (4b$_1$)

$[\alpha]_D^{20}$ +6.7(c 1.7, $CHCl_3$)

(4*S*,5*S*)-ethyl 2-((1,4-bis(benzyloxy)-3-((*tert*-butoxycarbonyl)amino)butan-2-yl)amino) acetate (4a$_2$)

Compound **3a$_2$** (0.6g, 2.4mmol) was dissolved in 5mL dry ACN and TEA (0.7mL, 4.8mmol) was added with stirring. The reaction mixture was cooled to 0^{o}C and ethyl bromoacetate (0.3mL, 2.9mmol) diluted in 1mL dry ACN was added drop wise. The reaction was stirred at rt for 4h after which solvent was removed under vacuo and the residue was diluted with EtOAc. The organic layer was washed with $NaHCO_3$ (2x30mL), dried over

anhydrous Na_2SO_4 and concentrated to get the crude compound. The crude compound was purified by column chromatography (15% EtOAc in pet ether) to yield Compound **4a₂** as colorless liquid (0.82g, 71%). $[\alpha]_D^{20}$ -10 (c 3.2, $CHCl_3$);^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.21-1.28 (t,3H,*J*=7.2Hz), 1.43(s, 9H), 2.19(br s, 1H), 3.06-3.10 (m, 1H), 3.45-3.59 (m, 6H),3.86-3.90(m, 1H), 4.1-4.2 (q, 2H, *J*=7.2Hz), 4.47-4.54 (m, 4H), 7.31(s, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 14.20, 28.41, 49.97, 57.91, 60.75, 70.09, 71.04, 73.33, 79.20, 127.7, 127.74, 128.38, 138.12, 155.81, 172.56; HRMS calcd for $C_{27}H_{39}N_2O_6$: 487.2803, Observed mass: 487.2806

(4*R*,5*R*)-ethyl 2-((1,4-bis(benzyloxy)-3-((*tert*-butoxycarbonyl)amino)butan-2-yl)amino) acetate (4b₂)

$[\alpha]_D^{20}$ +10.3 (c 3.5, $CHCl_3$)

(*4S,5S*)-ethyl 2-(*N*-(3-((*tert*-butoxycarbonyl)amino)-1,4-dimethoxybutan-2-yl)-2-chloroacetamido)acetate (5a₁)

Chloroacetyl chloride (1.4mL, 17.9mmol) was added in 2-3 portions to the solution of compound **4a₁** (1.2g, 3.6mmol) and $NaHCO_3$ (3g, 35.9mmol) in 20mL dioxane, water (1:1) at 0^{o}C. The pH of the reaction mixture was maintained between 8-9. The mixture was allowed to stir for 1/2h.After completion of the reaction the dioxane was removed under vacuum and the water was extracted with ethyl acetate (3x50mL). The organic layer was concentrated to get the crude compound. The crude compound was purified by column chromatography (15% EtOAc in Pet ether) to get colorless liquid (1.2g, 81%); $[\alpha]_D^{20}$ -11.91 (c 1.7, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.24-1.31 (m, 3H, rotameric mixture), 1.41-1.45 (9H, rotameric mixture), 2.95-3.03 (m, 1H), 3.10-3.38 (m, 6H, rotameric mixture), 3.39-3.77(m, 6H), 4.02-4.20(m, 5H) ; ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 14.2, 28.41, 49.97, 57.91, 60.75, 70.09, 71.04, 73.23, 73.33, 79.20, 127.7, 127.74, 128.38, 138.12, 155.81, 172.56 ; HRMS calcd for $C_{17}H_{31}O_7N_2ClNa$: 433.1712, Observed mass: 433.1727

(4*R*,5*R*)-ethyl 2-(*N*-(3-((*tert*-butoxycarbonyl)amino)-1,4-dimethoxybutan-2-yl)-2-chloroacetamido)acetate (5b₁)

$[\alpha]_D^{20}$ +12.79 (c 1.7, $CHCl_3$)

(4*S*,5*S*)-ethyl 2-(*N*-(1,4-bis(benzyloxy)-3-((*tert*-butoxycarbonyl)amino)butan-2-yl)-2-chloroacetamido) acetate (5a₂)

Chloroacetyl chloride (2.1mL, 26.7mmol) was added in 2-3 portions to the solution of compound **4a₂**(2.6g, 5.3mmol) and $NaHCO_3$ (4.5g, 53.4mmol) in 25mL dioxane, water(1:1)

at 0°C. The pH of the reaction mixture was maintained between 8-9. The mixture was allowed to stir for 1/2h. After completion of the reaction the dioxane was removed under vacuum and the water was extracted with ethyl acetate (3x50mL). The organic layer was concentrated to acquire the crude compound. The crude compound was purified by column chromatography (15% EtOAc in Pet ether) to get colorless liquid (2.6g, 84%).$[\alpha]_D^{20}$ -6.7 (c 3.7, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.40-1.51 (m,3H, 9H,rotameric mixture), 3.23-3.29(m, 1H), 3.48-3.60(m, 5H), 3.84-4.19(m, 3H), 4.35-4.37(m, 4H), 4.48-4.56(m, 5H),5.27-5.29(br s, 1H), 7.33(s, 10H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 14.19, 28.33, 42.50, 57.69, 60.39,69.00, 69.41, 71.04, 73.36, 73.60, 81.04, 127.80, 128.33, 128.57, 137.09, 152.69, 166.92, 171.05; HRMS calcd for $C_{29}H_{40}O_7N_2Cl$: 563.2519, Observed mass: 563.2519

(4*R*,5*R*)-ethyl 2-(*N*-(1,4-bis(benzyloxy)-3-((*tert*-butoxycarbonyl)amino)butan-2-yl)-2-chloroacetamido) acetate ($5b_2$)

$[\alpha]_D^{20}$ +6.3 (c 3.5, $CHCl_3$)

(4*S*,5*S*)-ethyl 2-(*N*-(3-((*tert*-butoxycarbonyl)amino)-1,4-dimethoxybutan-2-yl)-2-thyminyl acetate ($6a_1$)

Compound **$5a_1$** (2.1g, 5.1mmol), activated K_2CO_3 (0.85g, 6.1mmol) and Thymine (0.7g, 5.6mmol) was suspended in 20mL of dry DMF and the reaction mixture was allowed to stir for 6h at rt. The solvent was removed under vacuum and the residue was extracted with ethyl acetate (3x100mL). The organic layer was washed with brine (3x50mL) and was dried over anhydrous Na_2SO_4. Ethyl acetate was removed under vacuum to get the crude compound. The crude compound was purified by column chromatography (30% EtOAc in Pet ether) to get white solid (1.9g, 80%); $[\alpha]_D^{20}$ -6.9(c 0.6, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.23-1.32(3H, rotameric mixture), 1.4, 1.44(9H, rotameric mixture), 1.91(s, 3H), 3.18-3.40(6H, rotameric mixture), 3.52-3.76(m,4H), 4.01-4.49(m, 6H), 4.69-5.16(m,2H), 7.00,7.05(1H, rotameric mixture), 8.55,8.61(1H, rotameric mixture); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 12.28, 14.16, 28.31, 45.93, 47.88, 56.64, 59.31, 61.24, 61.78, 71.36, 72.23, 79.99, 110.57, 141.64, 151.24, 164.70, 167.93, 168.75, 170.14;HRMS calcd for $C_{22}H_{36}N_4O_9Na$: 523.2374, Observed mass: 523.2387.

(4*R*,5*R*)-ethyl 2-(*N*-(3-((*tert*-butoxycarbonyl)amino)-1,4-dimethoxybutan-2-yl)-2-thyminyl acetate ($6b_1$)

$[\alpha]_D^{20}$ +7.0(c 0.6, $CHCl_3$)

(4*S*,5*S*)-ethyl 2-(*N*-(1,4-bis(benzyloxy)-3-((*tert*-butoxycarbonyl)amino)butan-2-yl)-2-thyminyl acetate ($6a_2$)

Compound **$5a_2$** (1g, 1.8mmol), activated K_2CO_3 (0.3g, 2.1mmol) and Thymine (0.25g, 1.9mmol) was suspended in 10mL of dry DMF and the reaction mixture was allowed to stir for 6h at rt. The solvent was removed under vacuum and the residue was extracted with ethyl acetate (50mLX3). The organic layer was washed with brine (25mLx3) and was dried over anhydrous Na_2SO_4. Ethyl acetate was removed under vacuum to get the crude compound. The crude compound was purified by column chromatography (30% EtOAc in Pet ether) to get white solid (0.99g, 83%); $[\alpha]_D^{20}$ -23.8(c 5, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.10-1.26(m, 3H, rotameric mixture), 1.38, 1.44(9H, rotameric mixture), 3.47-3.93(m, 4H), 4.01-4.24(m, 6H), 4.34-4.82(m, 6H), 7.22-7.45(m, 11H), 7.96-8.08(br s, 1H), 9.09-9.15(br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 12.35, 14.19, 28.30, 45.97, 48.01, 56.83, 60.41, 61.07, 61.68, 69.14, 73.25, 79.63, 110.52, 127.18, 127.81, 128.39, 137.35, 141.01, 151.05, 155.88, 164.48, 168.57, 170.01; HRMS calcd for $C_{34}H_{44}O_9N_4Na$: 675.3001, Observed mass: 675.3010.

(4*R*,5*R*)-ethyl 2-(*N*-(1,4-bis(benzyloxy)-3-((*tert*-butoxycarbonyl)amino)butan-2-yl)-2-thyminyl acetate ($6b_2$)

$[\alpha]_D^{20}$ +24(c 5, $CHCl_3$)

(4*S*,5*S*)-ethyl 2-(*N*-(3-((*tert*-butoxycarbonyl)amino)-1,4-dimethoxybutan-2-yl)-2-(6 benzyloxy carbonyl adeninyl) acetate ($7a_1$)

Compound **$5a_1$**(1.2g, 2.9mmol), activated K_2CO_3 (0.48g, 3.5 mmol) and N^6-benzyloxycarbonyladenine (0.87g, 3.2mmol) was suspended in 15mL of dry DMF and the reaction mixture was allowed to stir for 6h at rt. The solvent was removed under vacuum and the residue was extracted with ethyl acetate (3x50mL). The organic layer was washed with brine (3x25mL) and was dried over anhydrous Na_2SO_4. Ethyl acetate was removed under vacuum to get the crude compound. The crude compound was purified by column chromatography (30% EtOAc in Pet ether) to get white solid (1.3g, 72%); $[\alpha]_D^{20}$ -19.6(c 5, $CHCl_3$);^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.21-1.34(3H, rotameric mixture), 1.39,1.41(9H, rotameric mixture), 3.19-3.44(m, 7H, rotameric mixture), 3.53-3.82(m, 3H), 4.10-4.58(m, 5H), 4.86-5.46(m, 5H), 7.34-7.42(m, 5H), 8.10-8.11(s,1H, rotameric mixture), 8.72,8.75 (1H, rotameric mixture); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 14.16, 28.23, 43.85, 56.91, 58.64, 59.41,61.12, 67.63, 71.85,79.56, 121.07, 128.40, 128.59, 135.58, 144.13,

149.43, 151.09, 152.75, 155.80, 166.73, 167.62, 169.85; HRMS calcd for $C_{30}H_{42}O_9N_7$: 644.3039, Observed mass: 644.3034.

(4*R*,5*R*)-ethyl 2-(*N*-(3-((*tert*-butoxycarbonyl)amino)-1,4-dimethoxybutan-2-yl)-2-(6 benzyloxy carbonyl adeninyl) acetate ($7b_1$)

$[\alpha]_D^{20}$ +20(c 4.8, $CHCl_3$)

(4*S*,5*S*)-ethyl 2-(*N*-(1,4-bis(benzyloxy)-3-((*tert*-butoxycarbonyl)amino)butan-2-yl)-2-(6 benzyloxy carbonyl adeninyl) acetate ($7a_2$)

Compound **$5a_2$** (1g, 1.8mmol), activated K_2CO_3 (0.3g, 2.1 mmol) and N^6-benzyloxycarbonyladenine (0.5g, 1.9mmol) was suspended in 10mL of dry DMF and the reaction mixture was allowed to stir for 6h at r.t. The solvent was removed under vacuum and the residue was extracted with ethyl acetate (3x50mL). The organic layer was washed with brine (3x25mL) and was dried over anhydrous Na_2SO_4. Ethyl acetate was removed under vacuum to get the crude compound. The crude compound was purified by column chromatography (20% EtOAc in Pet ether) to get white solid (1.1g, 76%); $[\alpha]_D^{20}$ -26.5(c 5, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.08-1.17 (3H, rotameric mixture), 1.29, 1.38 (9H, rotameric mixture), 3.43-3.75(m, 4H, rotameric mixture), 3.9-4.31(m, 7H), 4.40-4.92 (m, 5H),5.27,5.28 (2H,rotameric mixture), 7.27(m, 15H), 7.68 (1H, rotameric mixture), 7.99 (1H, rotameric mixture); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 13.92, 28.32, 47.39, 60.39, 60.84, 61.62, 68.02, 69.21, 69.28, 73.02, 73.27, 79.62, 127.41,127.59, 1258.35, 128.60, 129.10, 135.21, 135.47, 137.64, 139.44, 147.97, 148.66, 155.87, 162.03, 167.83, 167.94; HRMS calcd for $C_{42}H_{50}O_9N_7$: 796.3665, Observed mass: 796.3657

(4*R*,5*R*)-ethyl 2-(*N*-(1,4-bis(benzyloxy)-3-((*tert*-butoxycarbonyl)amino)butan-2-yl)-2-(6 benzyloxy carbonyl adeninyl) acetate ($7b_2$)

$[\alpha]_D^{20}$ +25.3(c 4.8, $CHCl_3$)

(4*S*,5*S*)-2-(*N*-(3-((*tert*-butoxycarbonyl) amino)-1, 4-dimethoxybutan-2-yl)-2- thyminyl acetic acid ($8a_1$)

The compound **$6a_1$** (0.4g, 0.8mmol) was dissolved in 1mL methanol and to it 2mL of 1N LiOH solution was added**.** The completion of the reaction was monitored by TLC. After 30mins, methanol was removed under vacuum and the aqueous layer was neutralized with Dowex H^+ resin. The resin was separated by filtration. The aqueous layer was washed with ethyl acetate (3x50mL) and the combined organic layer was lyophilized to get the free acid (0.28g, 74%). $[\alpha]_D^{20}$ +10.8(c 1.3, MeOH);^{1}H NMR (200 MHz, D_2O): δ (ppm) 1.32,1.36(9H,

rotameric mixture), 1.78,1.82 (3H, rotameric mixture), 3.21-3.34 (6H, rotameric mixture), 3.48-3.65(m, 4H), 3.91-4.51(m, 6H), 7.28, 7.34 (1H, rotameric mixture); ^{13}C NMR (50 MHz, DMSO-d6): δ (ppm) 12.16, 48.17, 49.90, 58.28, 58.47, 70.63, 77.72, 79.41, 107.81, 138.09, 151.21, 151.77, 155.75, 164.64, 165.18; HRMS calcd for $C_{20}H_{32}O_9N_4Na$: 495.2061, Observed mass: 495.2060.

(4*R*,5*R*)-2-(*N*-(3-((*tert*-butoxycarbonyl) amino)-1, 4-dimethoxybutan-2-yl)-2- thyminyl acetic acid ($8b_1$)

$[\alpha]_D^{20}$ -10.8(c 1.2, MeOH)

(4*S*,5*S*)-2-(*N*-(1, 4-bis (benzyloxy)-3-((*tert*-butoxycarbonyl) amino) butan-2-yl)-2-thyminyl acetic acid ($8a_2$)

The compound **$6a_2$** (1g, 1.5mmol) was dissolved in 2mL methanol and to it 4mL of 1N LiOH solution was added**.** The completion of the reaction was monitored by TLC. After 30mins, methanol was removed under vacuum and the aqueous layer was neutralized with Dowex H^+ resin. The resin was separated by filtration. The aqueous layer was washed with ethyl acetate (3x50mL) and the combined organic layer was lyophilized to get the free acid (0.75g, 79%). $[\alpha]_D^{20}$ +28.3(c 1.5, MeOH);^{1}H NMR (200 MHz, CD_3OD): δ (ppm) 1.29,1.32 (9H, rotameric mixture), 1.79 (s, 3H), 3.41-3.70(m, 4H), 3.74-4.13 (m, 4H), 4.17-4.65(m, 6H), 7.18-7.20(m, 1H); ^{13}C NMR (50 MHz, DMSO-d6): δ (ppm) 12.36, 28.59, 62.31, 62.55, 62.95, 68.51, 70.56, 72.28, 72.60, 78.14, 108.45, 127.87, 128.00, 128.57, 138.85, 142.72, 151.65, 156.02, 165.26, 168.03, 169.10; HRMS calcd for $C_{32}H_{40}O_9N_4Na$: 647.2687, Observed mass: 647.2687.

(4*R*,5*R*)-2-(*N*-(1, 4-bis (benzyloxy)-3-((*tert*-butoxycarbonyl) amino) butan-2-yl)-2-thyminyl acetic acid ($8b_2$)

$[\alpha]_D^{20}$ -28.0(c 1.5, MeOH)

(4*S*,5*S*)-2-(*N*-(3-((*tert*-butoxycarbonyl) amino)-1, 4-dimethoxybutan-2-yl)-2-(6 benzyloxy carbonyl adeninyl) acetic acid ($9a_1$)

The compound **$7a_1$** (1g, 1.6mmol) was dissolved in 2mL methanol and to it 4mL of 1N LiOH solution was added**.** The completion of the reaction was monitored by TLC. After 30mins, methanol was removed under vacuum and the aqueous layer was neutralized with Dowex H^+ resin. The resin was separated by filtration. The aqueous layer was washed with ethyl acetate (3x50mL) and the combined organic layer was lyophilized to get the free acid (0.68g, 71%). $[\alpha]_D^{20}$ +6.8(c 2.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.30,

1.42(9H, rotameric mixture), 3.14-3.30(6H, rotameric mixture), 3.42-3.68(m, 4H), 4.11-4.27(m,4H), 4.98-5.27(m, 4H), 7.33-7.35(m, 5H), 8.14-8.18(1H,rotameric mixture), 8.70, 8.72(1H, rotameric mixture); ^{13}C NMR (10 MHz, $CDCl_3$): δ (ppm) 28.36, 44.29, 46.34, 48.43, 49.31, 58.51, 58.95,59.15,67.68,70.98, 72.08, 79.57, 120.46, 128.46, 128.59, 135.48, 144.46, 149.15, 151.47, 152.74, 155.62, 155.83, 167.26, 172.67. HRMS calcd for $C_{28}H_{37}O_9N_7$: 616.2726, Observed mass: 616.2727

(4*R*,5*R*)-2-(*N*-(3-((*tert*-butoxycarbonyl) amino)-1, 4-dimethoxybutan-2-yl)-2-(6 benzyloxy carbonyl adeninyl) acetic acid ($9b_1$)

$[\alpha]_D^{20}$ -6.4(c 2.5, MeOH)

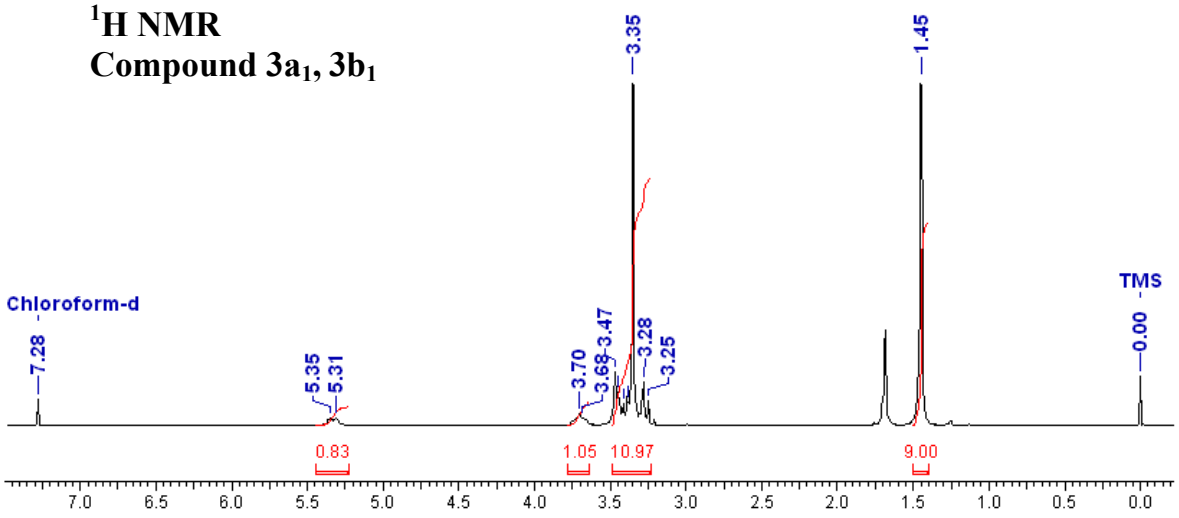
(4*S*,5*S*)-2-(*N*-(1, 4-bis (benzyloxy)-3-((*tert*-butoxycarbonyl)amino)butan-2-yl)-2-(6 benzyloxy carbonyl adeninyl) acetic acid ($9a_2$)

The compound **$7a_2$** (0.5g, 0.6mmol) was dissolved in 1mL methanol and to it 2mL of 1N LiOH solution was added**.** The completion of the reaction was monitored by TLC. After 30mins, methanol was removed under vacuum and the aqueous layer was neutralized with Dowex H^+ resin. The resin was separated by filtration. The aqueous layer was washed with ethyl acetate (3x50mL) and the combined organic layer was lyophilized to procure the free acid (0.38g, 73%). $[\alpha]_D^{20}$ +26.7 (c 2.0, MeOH); ^{1}H NMR (200 MHz, CD_3OD): δ (ppm) 1.22,1.35(9H, rotameric mixture), 3.42-3.71(m,6H), 4.09-4.27(m, 4H), 4.33-4.55(m, 6H), 7.21-7.24(m, 15H), 8.03(s, 1H), 8.10(s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 28.33, 47.18, 57.82, 58.38, 65.08, 69.57, 72.94, 73.32, 73.81, 81.16, 119.14, 126.97, 127.80, 128.36, 129.02, 135.55, 137.68, 137.75, 140.53, 149.99, 153.13, 155.56, 165.90, 168.99; HRMS calcd for $C_{40}H_{44}O_9N_7$: 766.3195, Observed mass: 766.3198

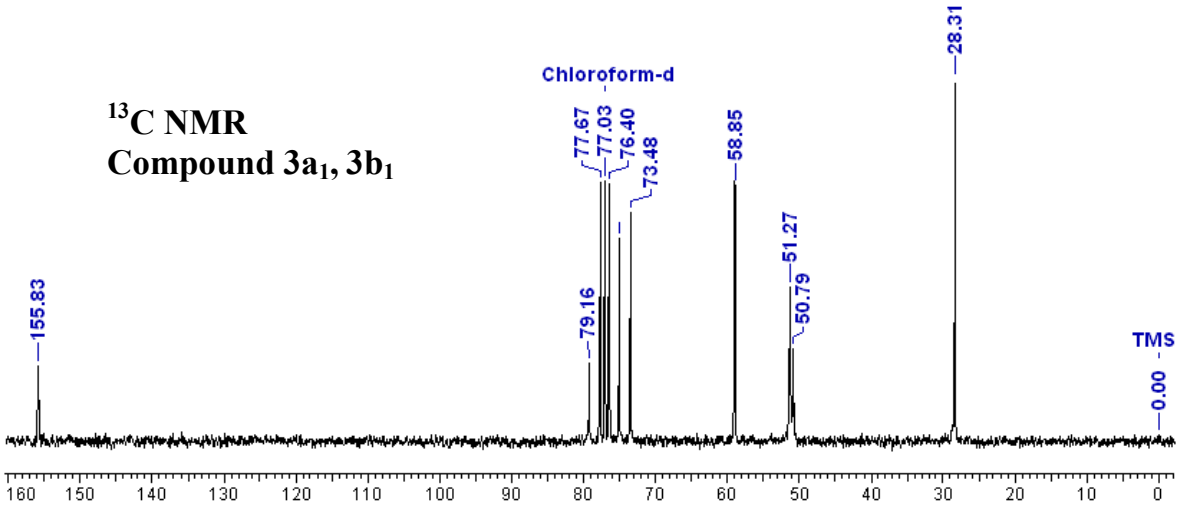
(4*R*,5*R*)-2-(*N*-(1, 4-bis (benzyloxy)-3-((*tert*-butoxycarbonyl) amino)butan-2-yl)-2-(6 benzyloxy carbonyl adeninyl) acetic acid ($9b_2$)

$[\alpha]_D^{20}$ -26.4(c 1.9, MeOH)

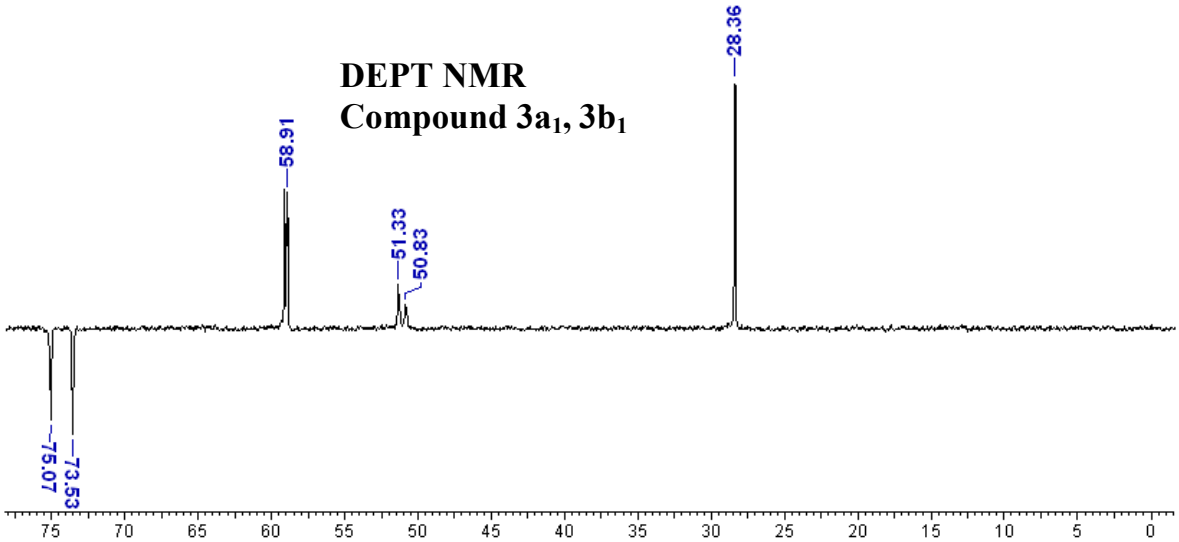
^{1}H NMR
Compound $3a_1$, $3b_1$

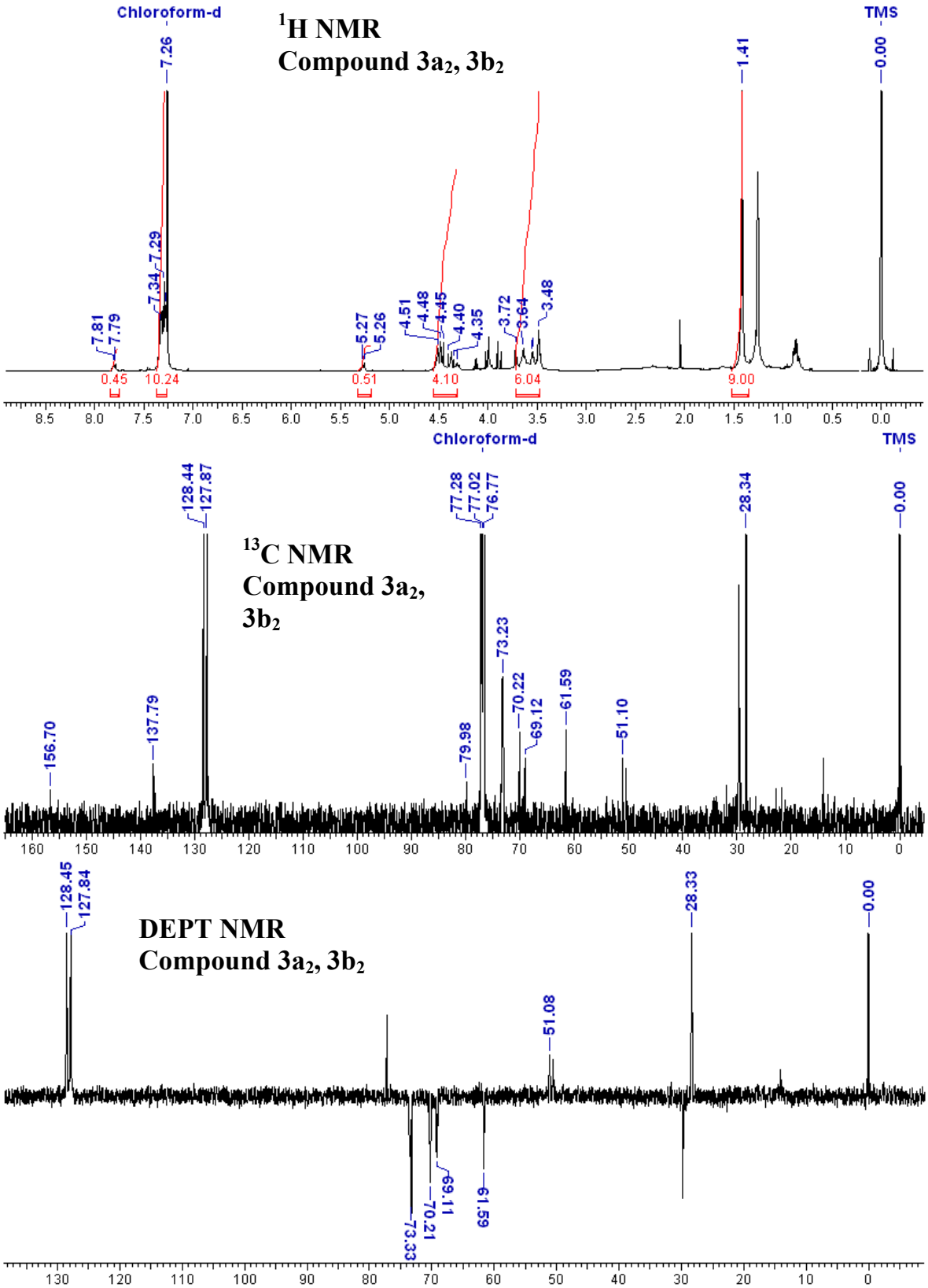


^{13}C NMR
Compound $3a_1$, $3b_1$

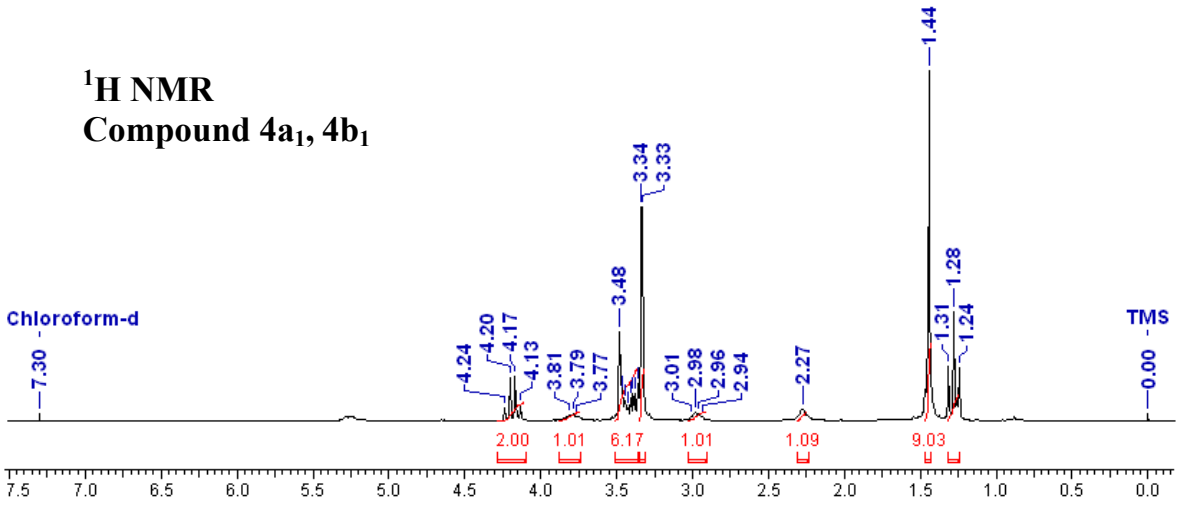


DEPT NMR
Compound $3a_1$, $3b_1$

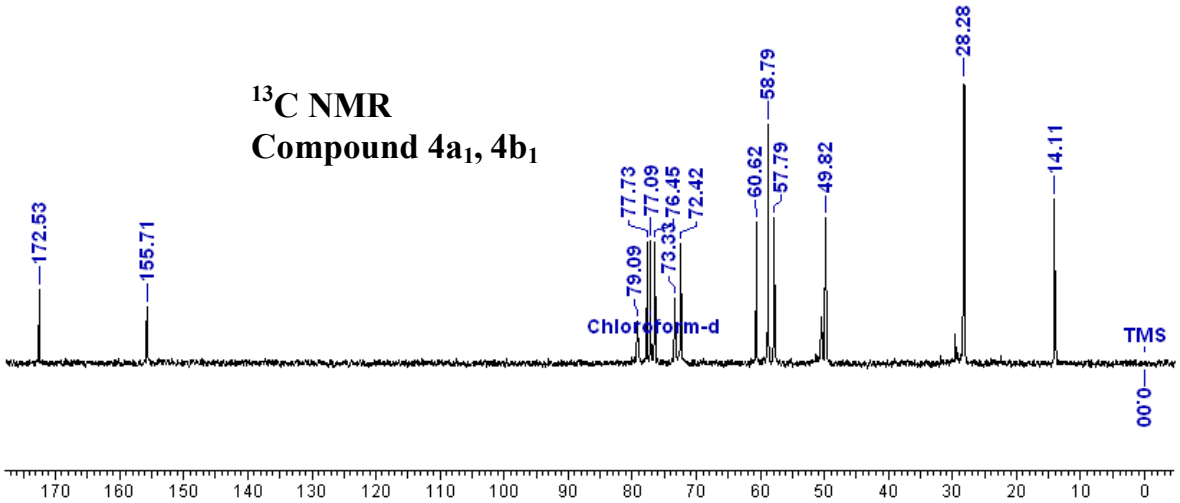



Chloroform-d
TMS
^{1}H NMR
Compound 3a$_2$, 3b$_2$
7.26
1.41
0.00
7.81
7.79
7.34
7.29
5.27
5.26
4.51
4.48
4.45
4.40
4.35
3.72
3.64
3.48
0.45
10.24
0.51
4.10
6.04
9.00
Chloroform-d
TMS
^{13}C NMR
Compound 3a$_2$,
3b$_2$
128.44
127.87
77.28
77.02
76.77
28.34
0.00
73.23
70.22
69.12
61.59
51.10
156.70
137.79
79.98
DEPT NMR
Compound 3a$_2$, 3b$_2$
128.45
127.84
28.33
0.00
51.08
73.33
70.21
69.11
61.59

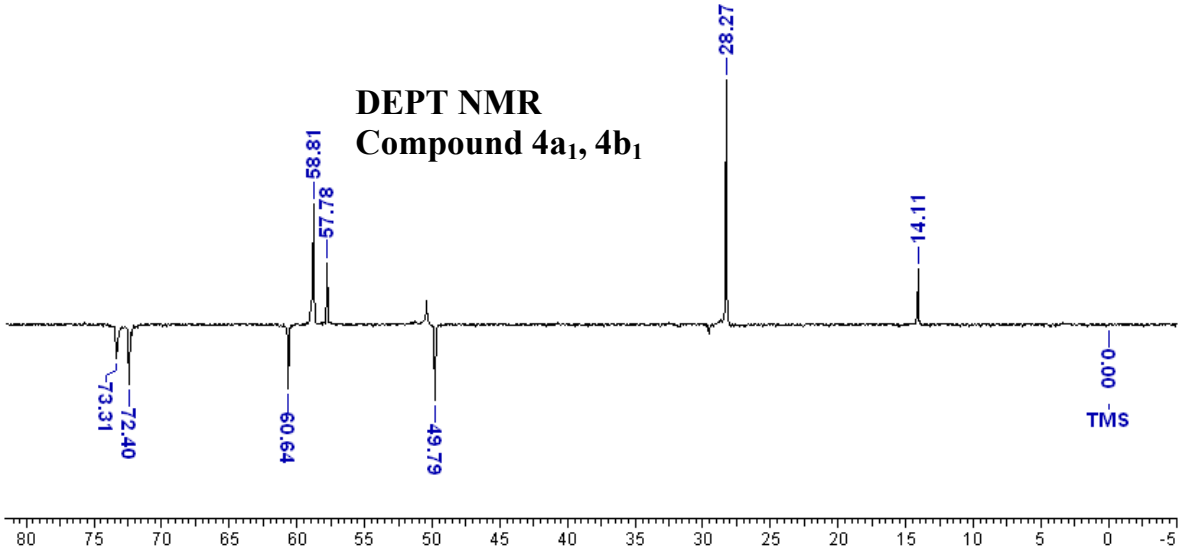
^{1}H NMR
Compound 4a$_1$, 4b$_1$



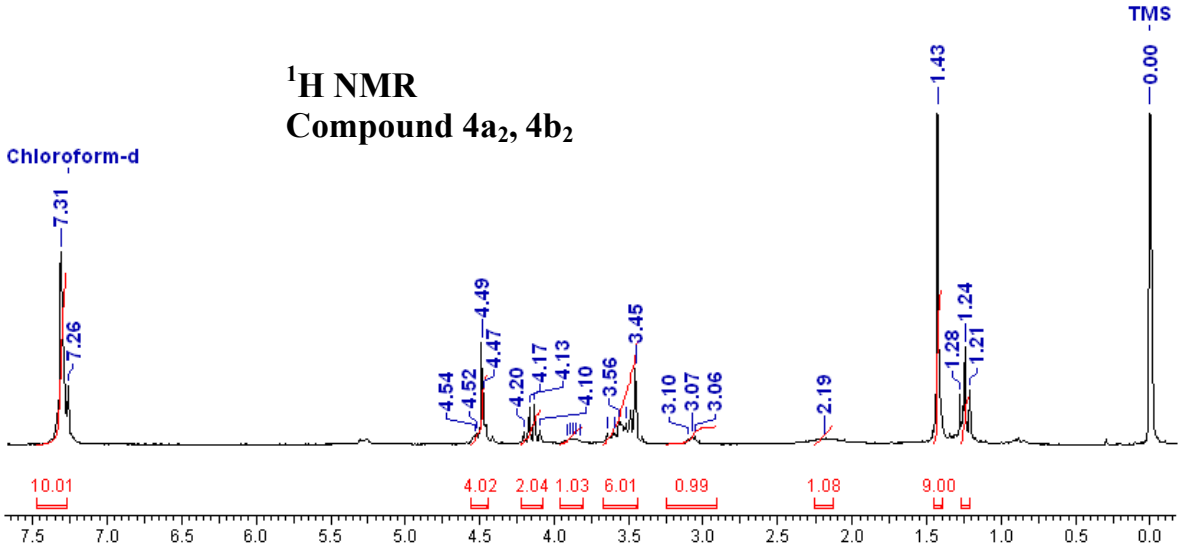
^{13}C NMR
Compound 4a$_1$, 4b$_1$



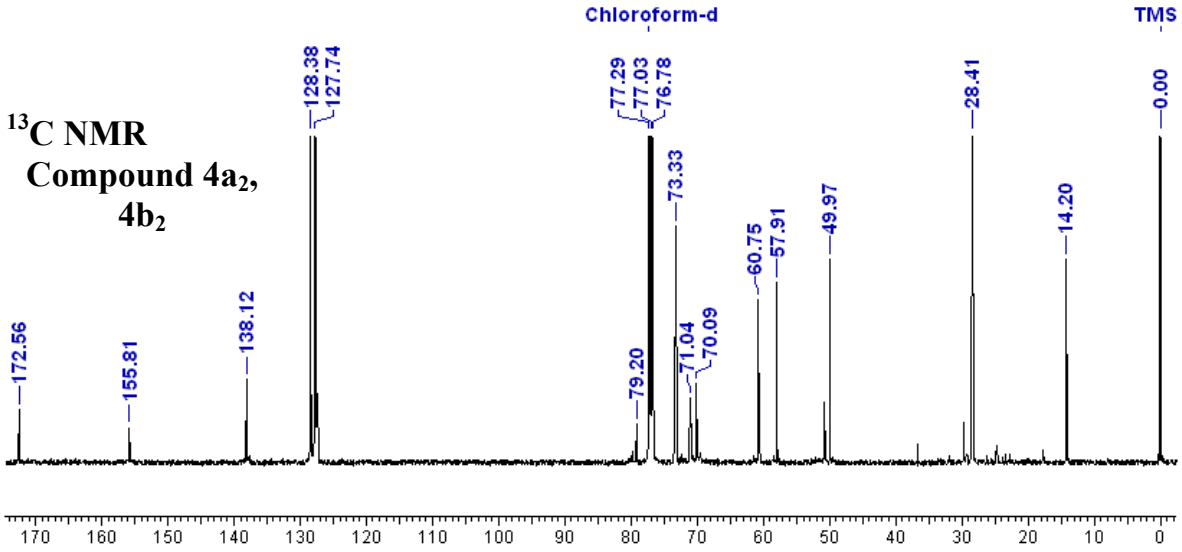
DEPT NMR
Compound 4a$_1$, 4b$_1$



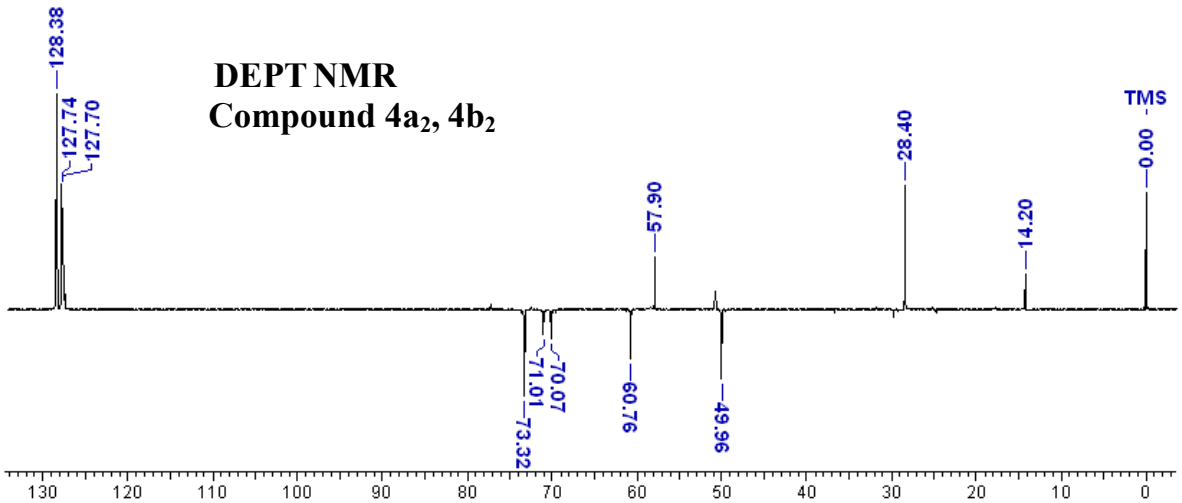
^{1}H NMR
Compound $4a_2$, $4b_2$



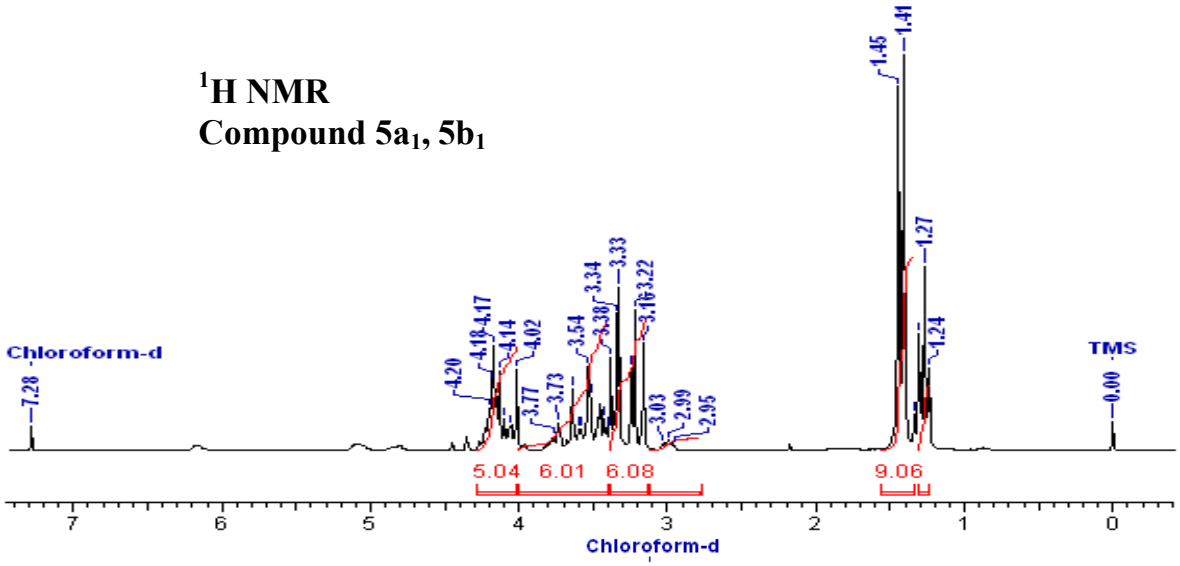
^{13}C NMR
Compound $4a_2$, $4b_2$



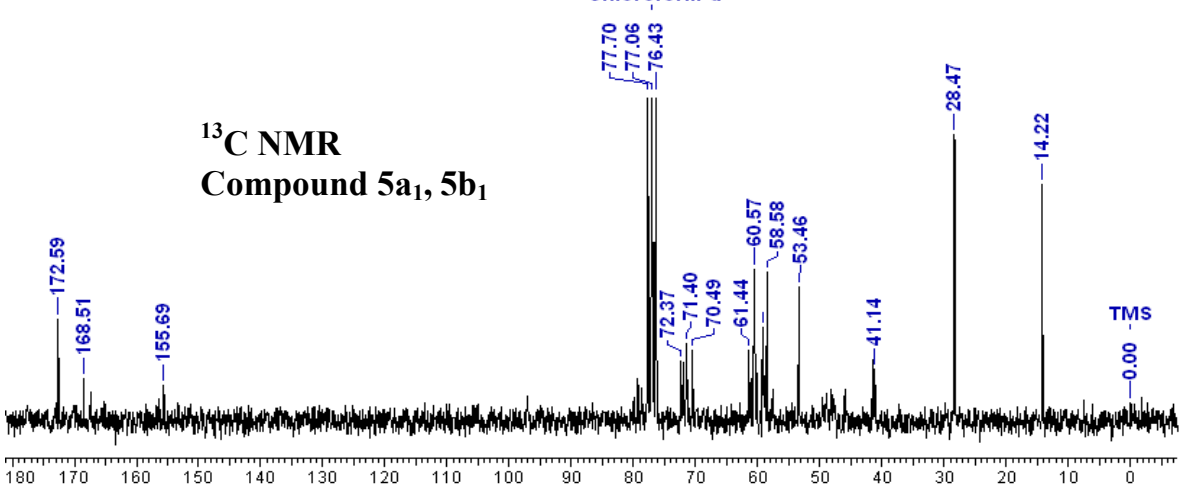
DEPT NMR
Compound $4a_2$, $4b_2$



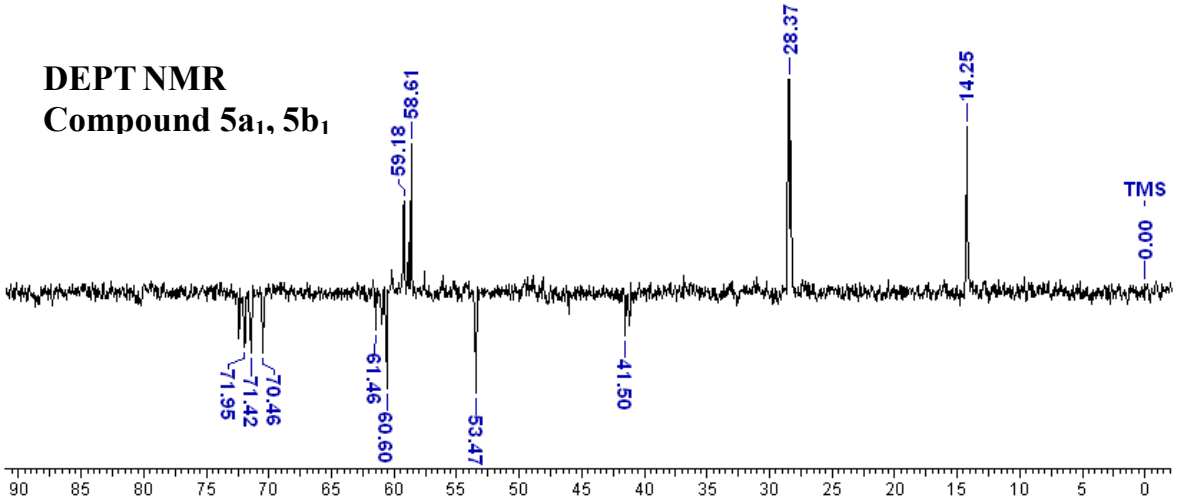
^{1}H NMR
Compound 5a$_1$, 5b$_1$

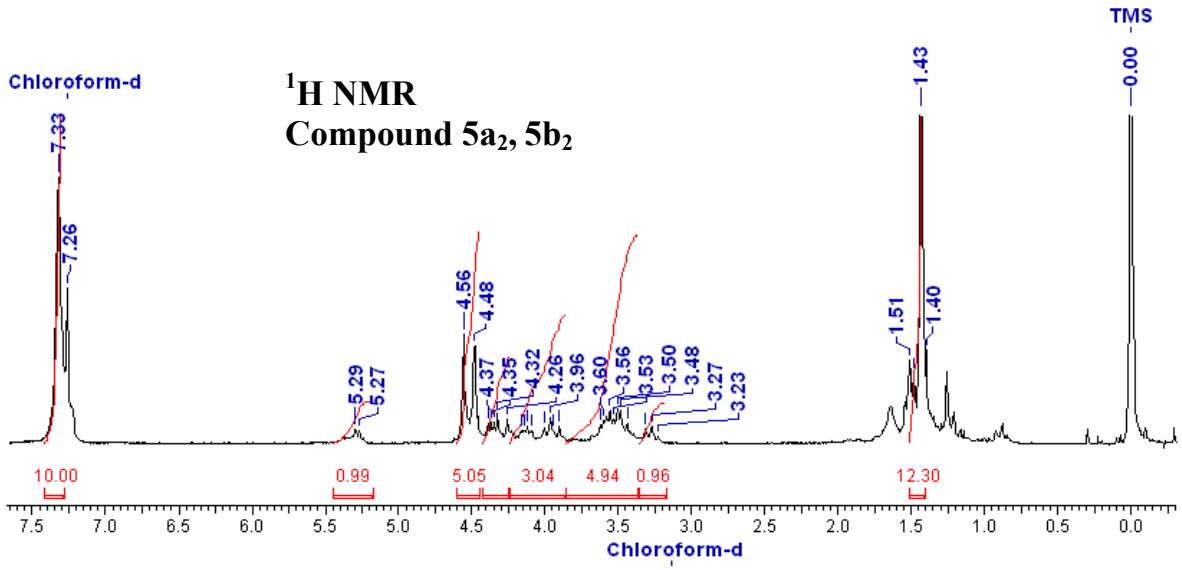


^{13}C NMR
Compound 5a$_1$, 5b$_1$

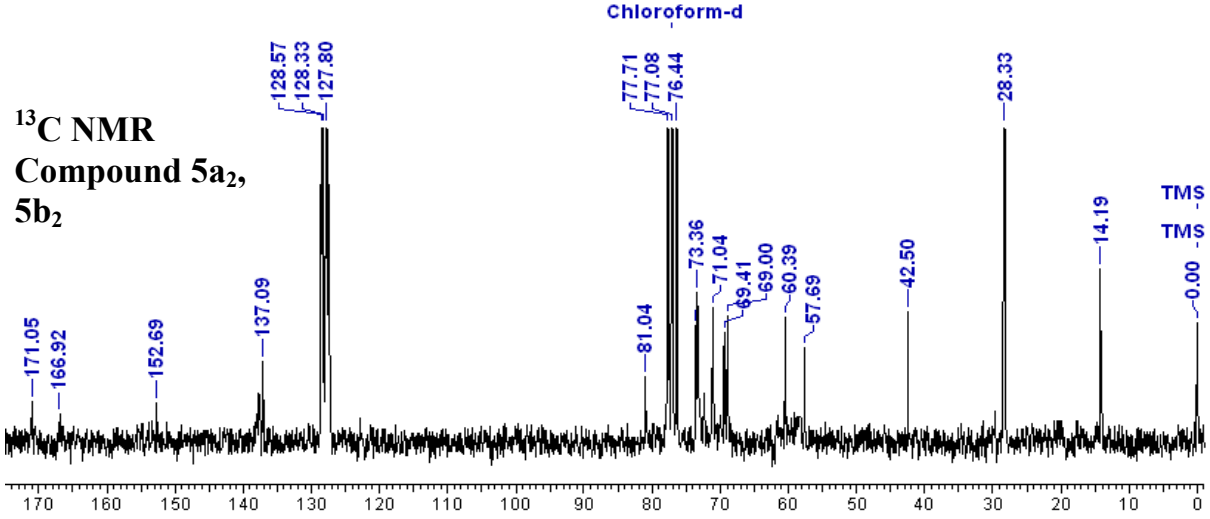


DEPT NMR
Compound 5a$_1$, 5b$_1$

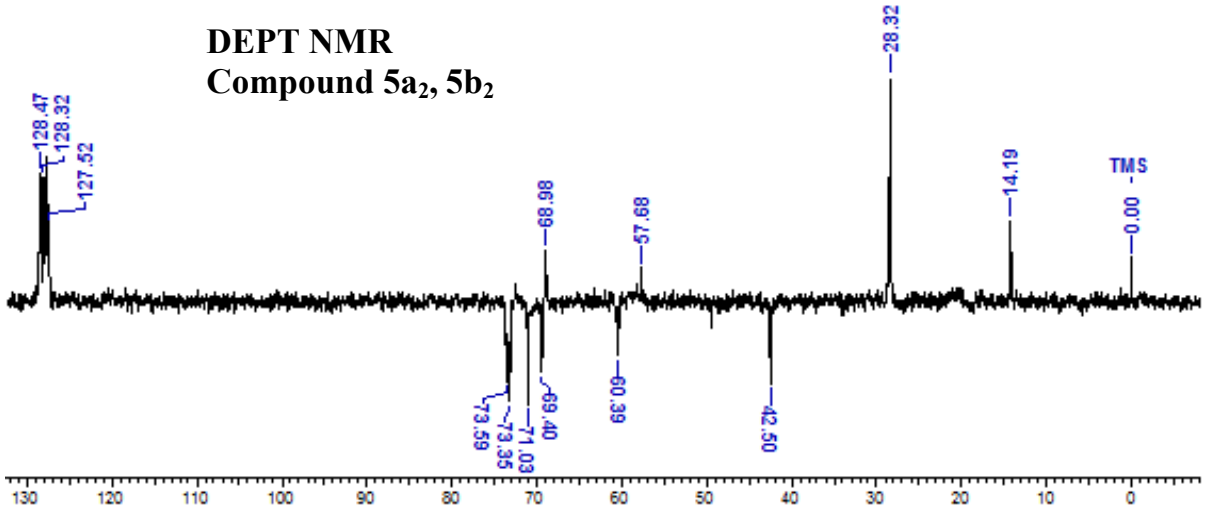




^{1}H NMR
Compound 5a$_2$, 5b$_2$

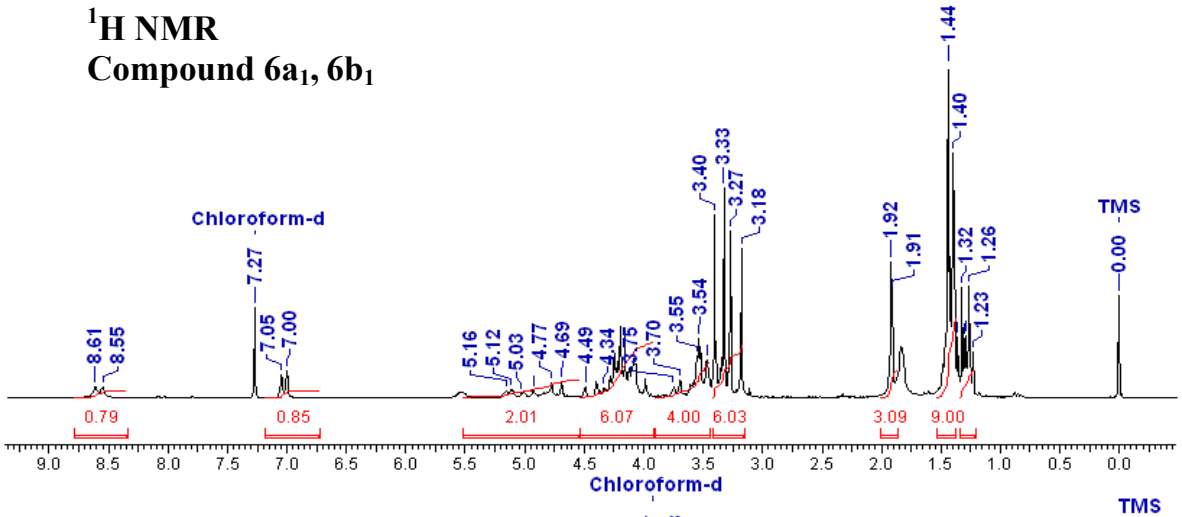


^{13}C NMR
Compound 5a$_2$, 5b$_2$

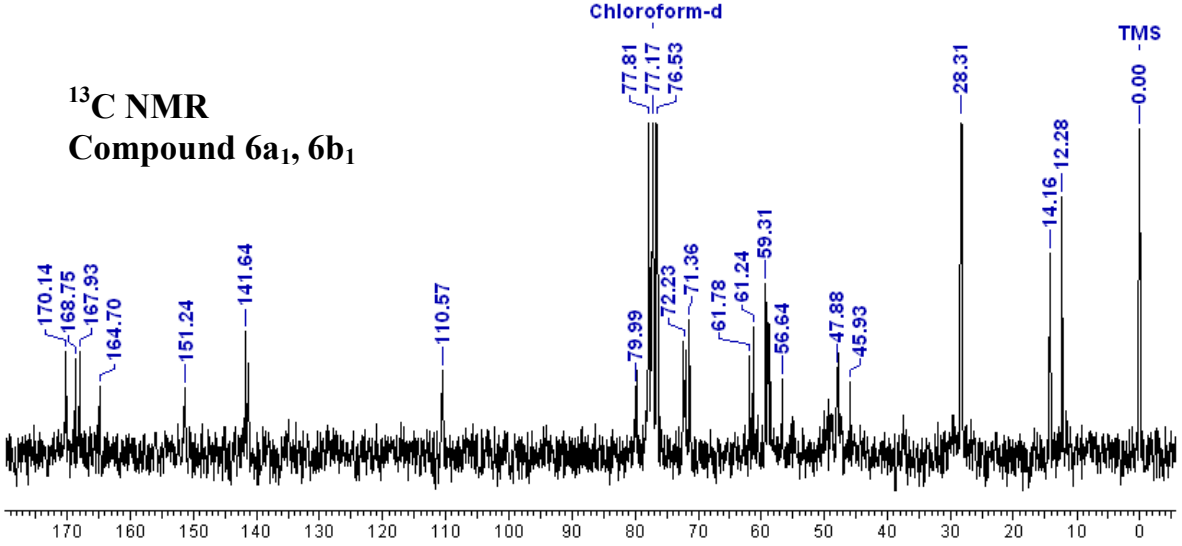


DEPT NMR
Compound 5a$_2$, 5b$_2$

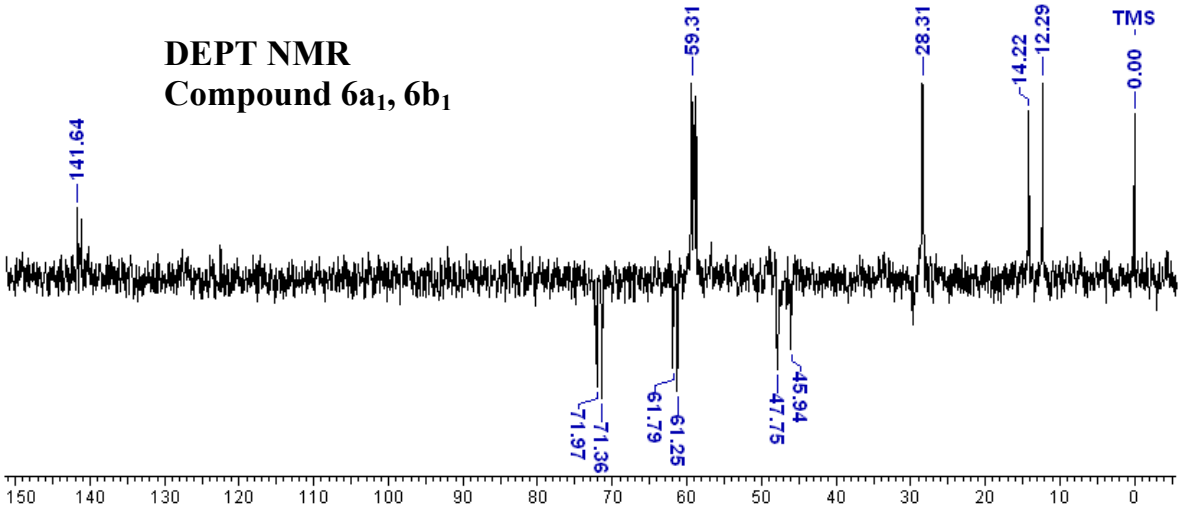
^{1}H NMR
Compound 6a$_1$, 6b$_1$



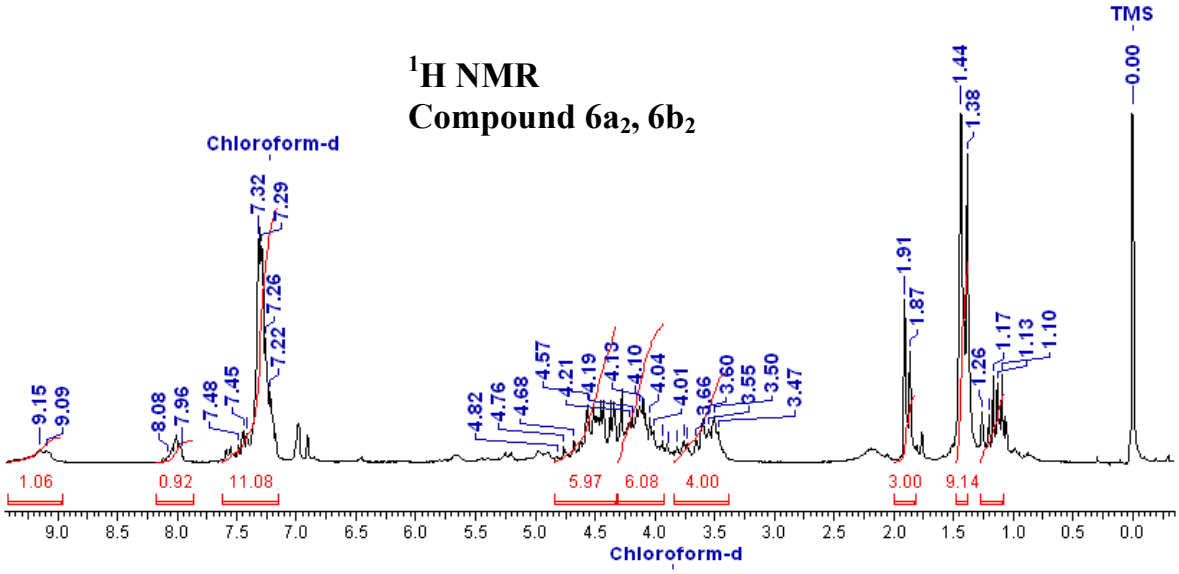
^{13}C NMR
Compound 6a$_1$, 6b$_1$



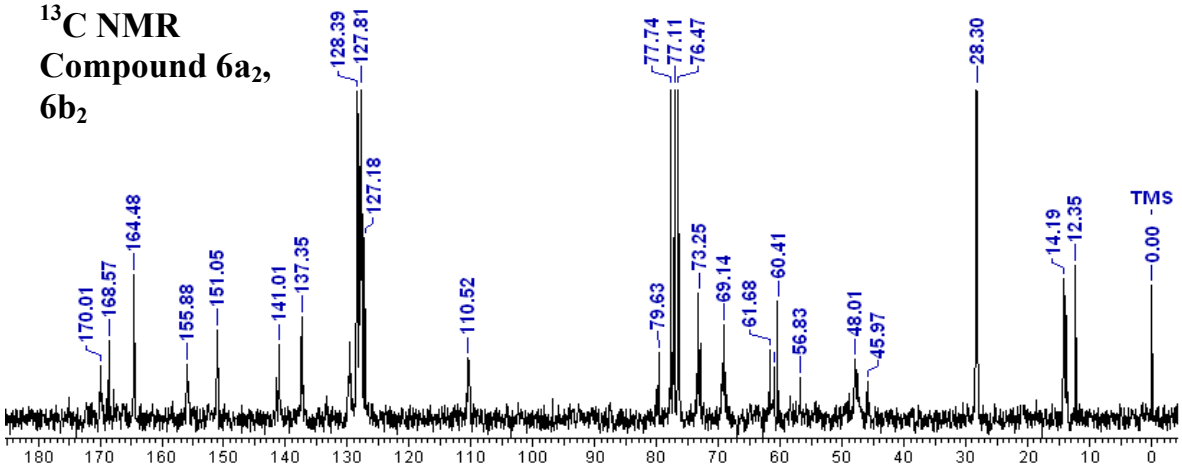
DEPT NMR
Compound 6a$_1$, 6b$_1$



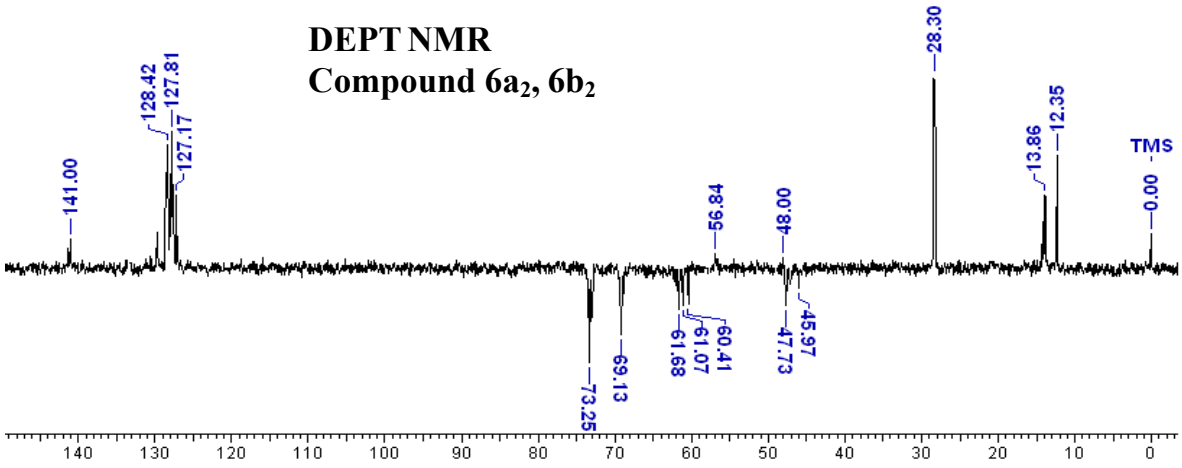
^{1}H NMR
Compound $6a_2$, $6b_2$



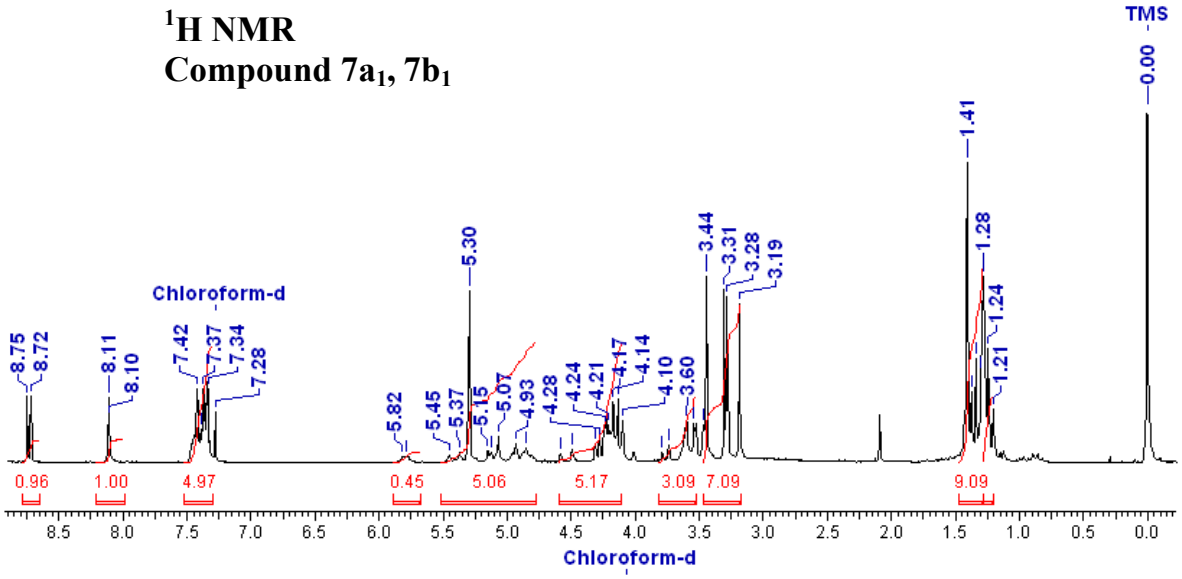
^{13}C NMR
Compound $6a_2$, $6b_2$



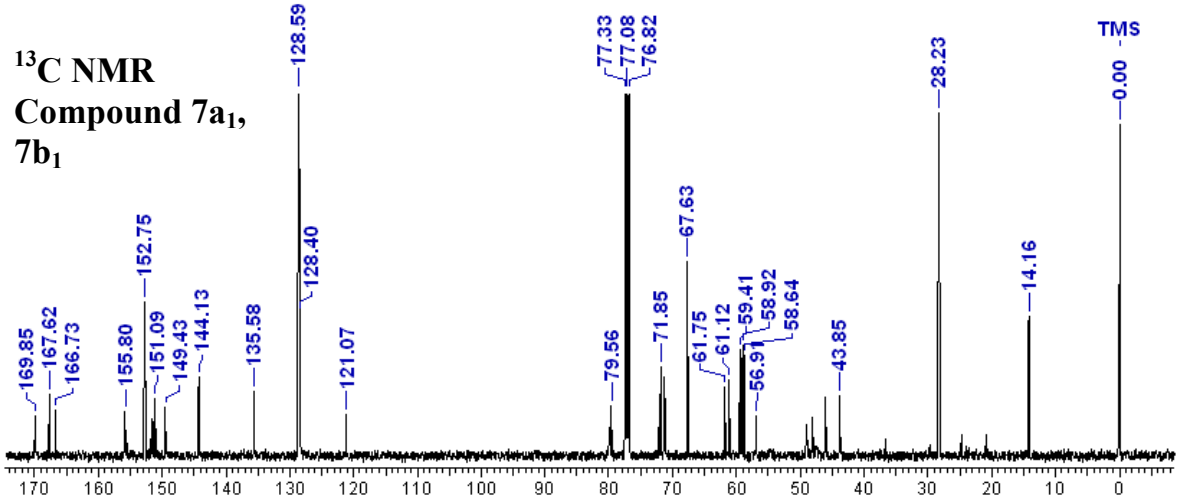
DEPT NMR
Compound $6a_2$, $6b_2$



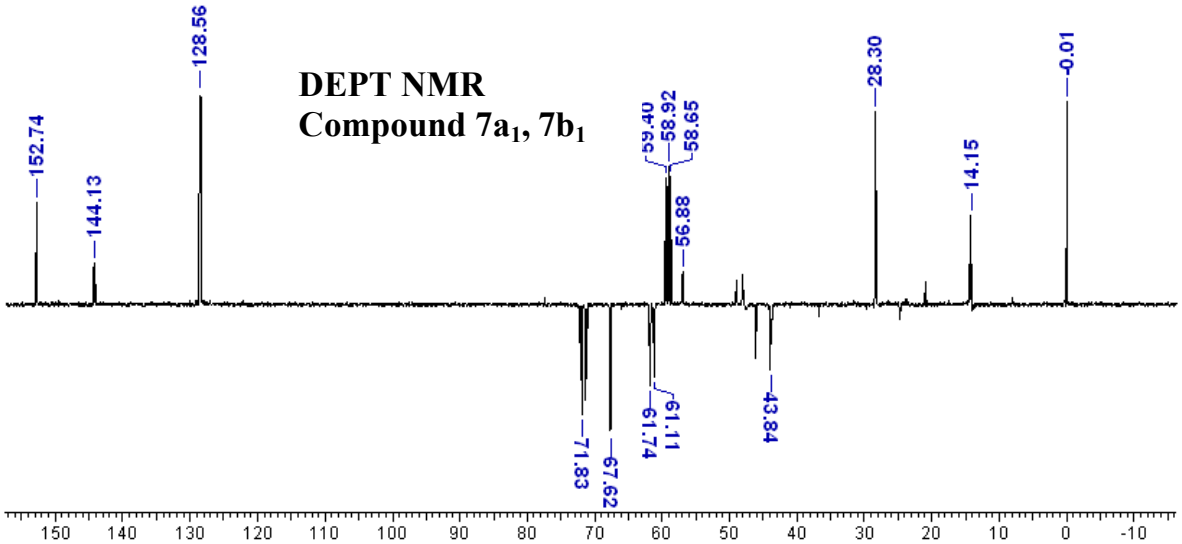
^{1}H NMR
Compound 7a$_1$, 7b$_1$



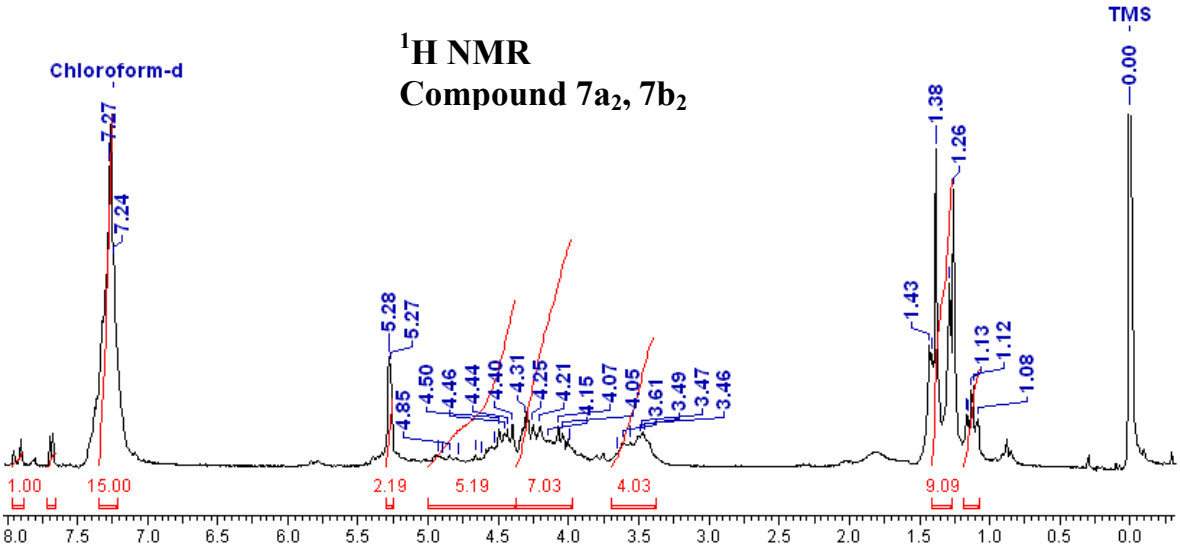
^{13}C NMR
Compound 7a$_1$, 7b$_1$



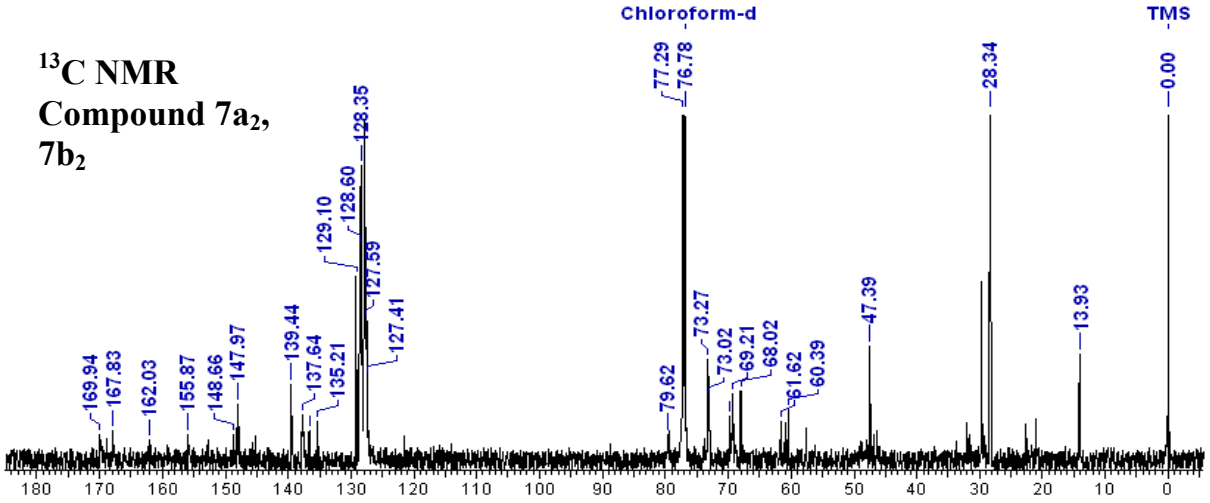
DEPT NMR
Compound 7a$_1$, 7b$_1$



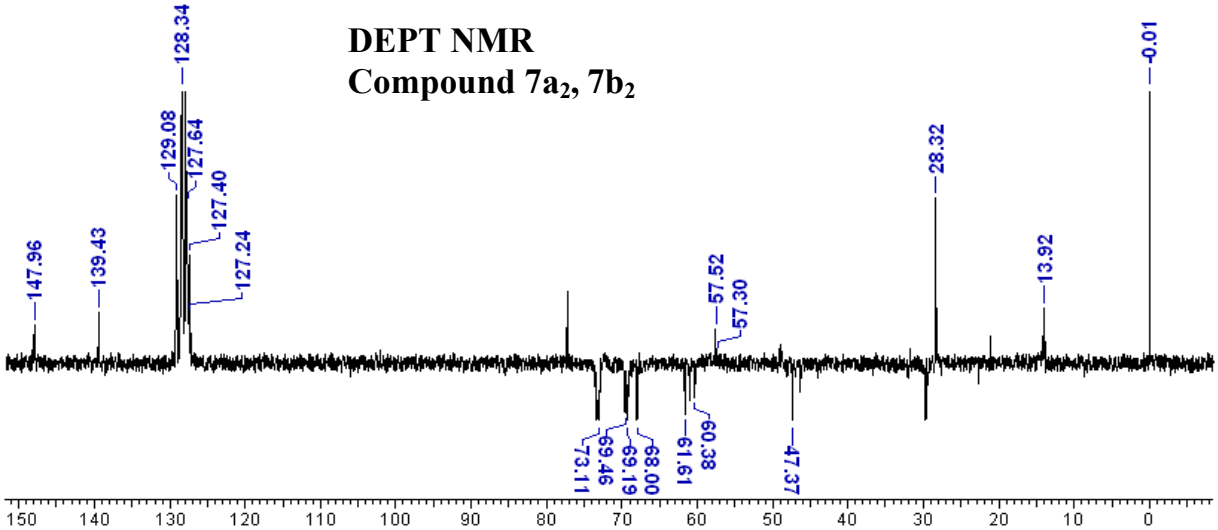
^{1}H NMR
Compound $7a_2$, $7b_2$

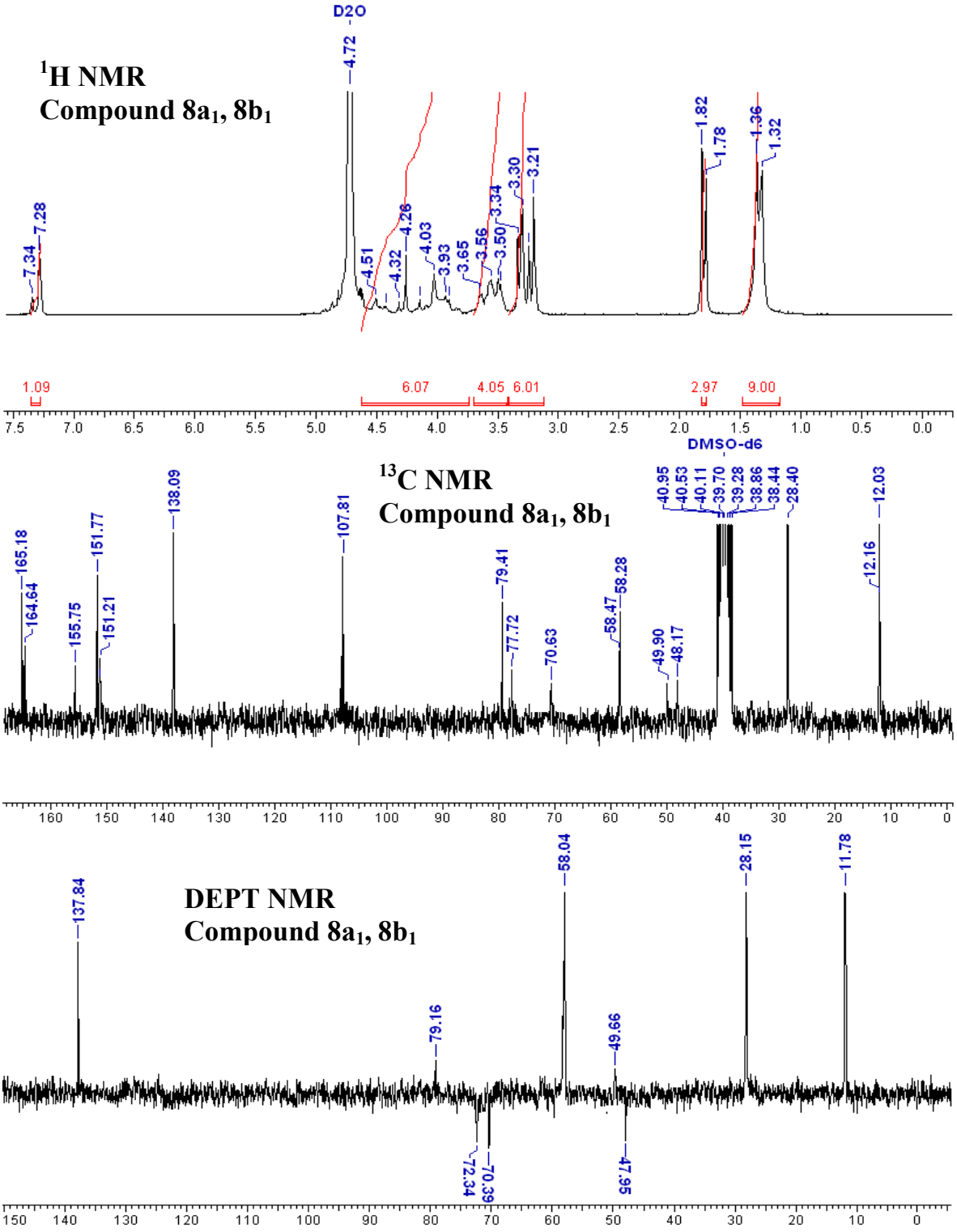


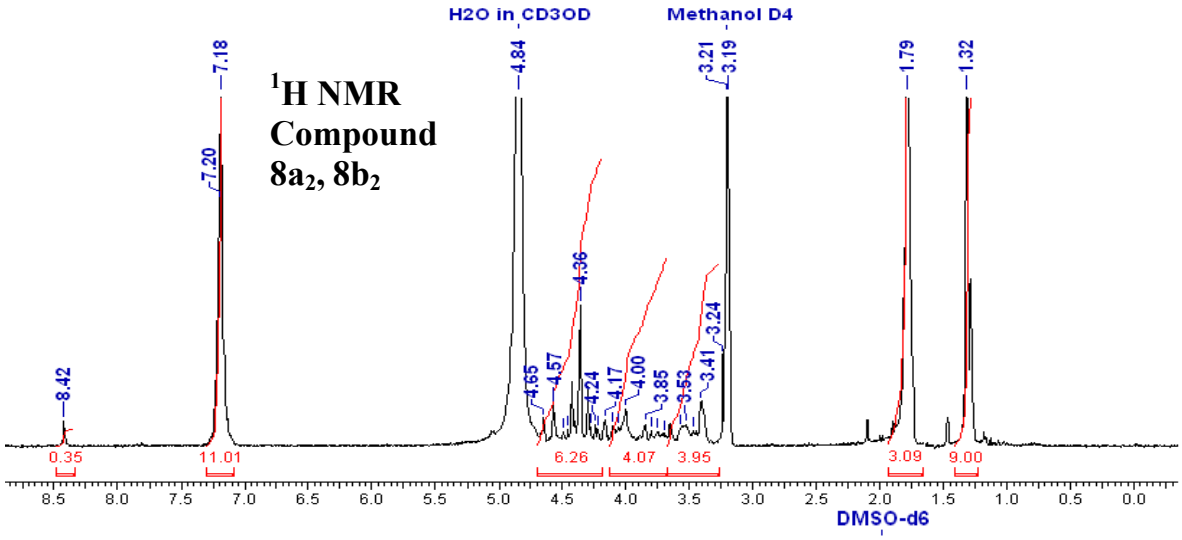
^{13}C NMR
Compound $7a_2$, $7b_2$

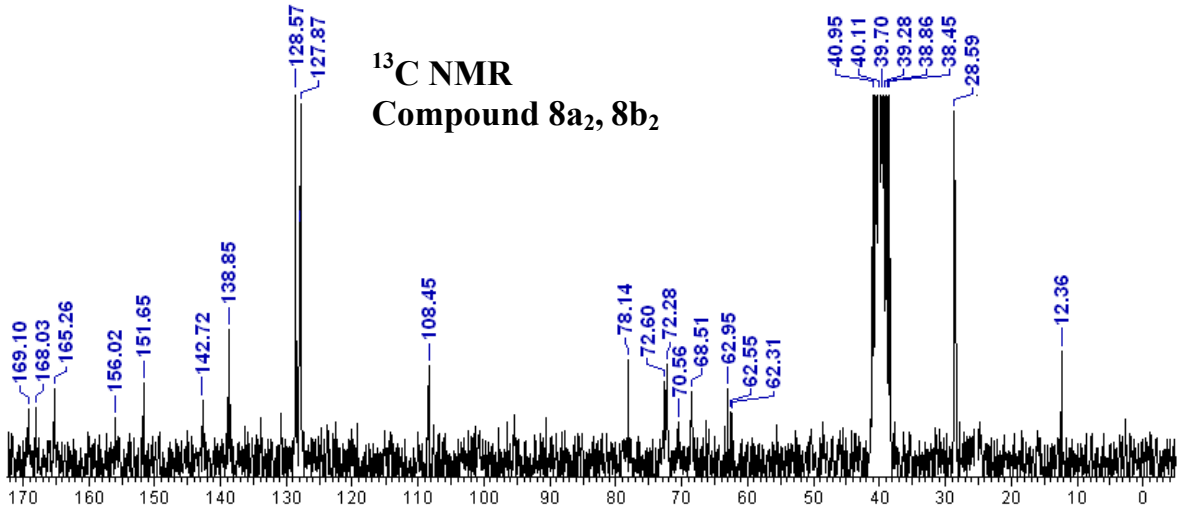


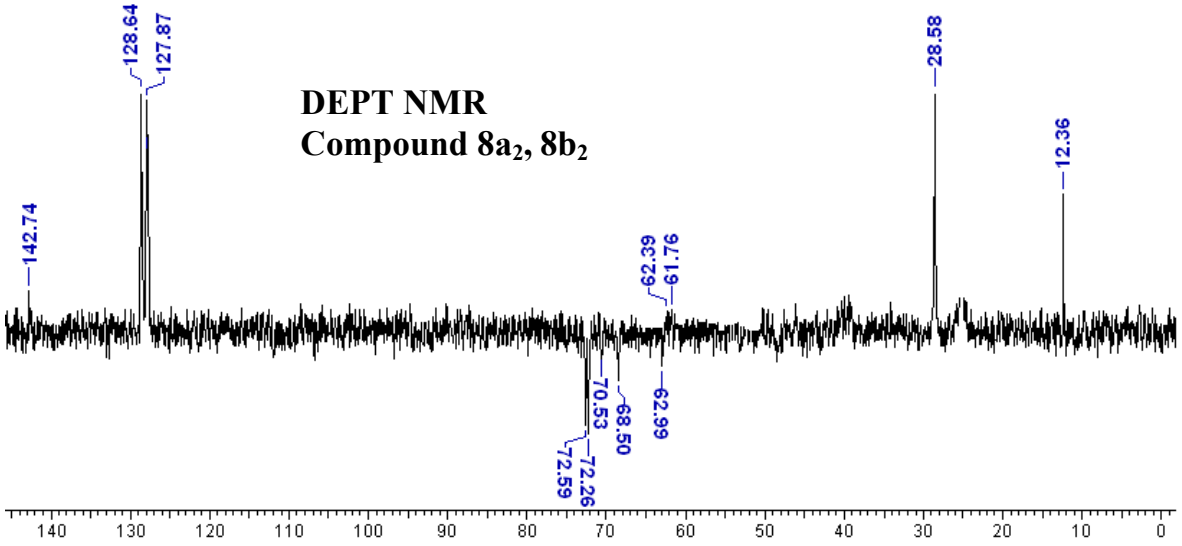
DEPT NMR
Compound $7a_2$, $7b_2$



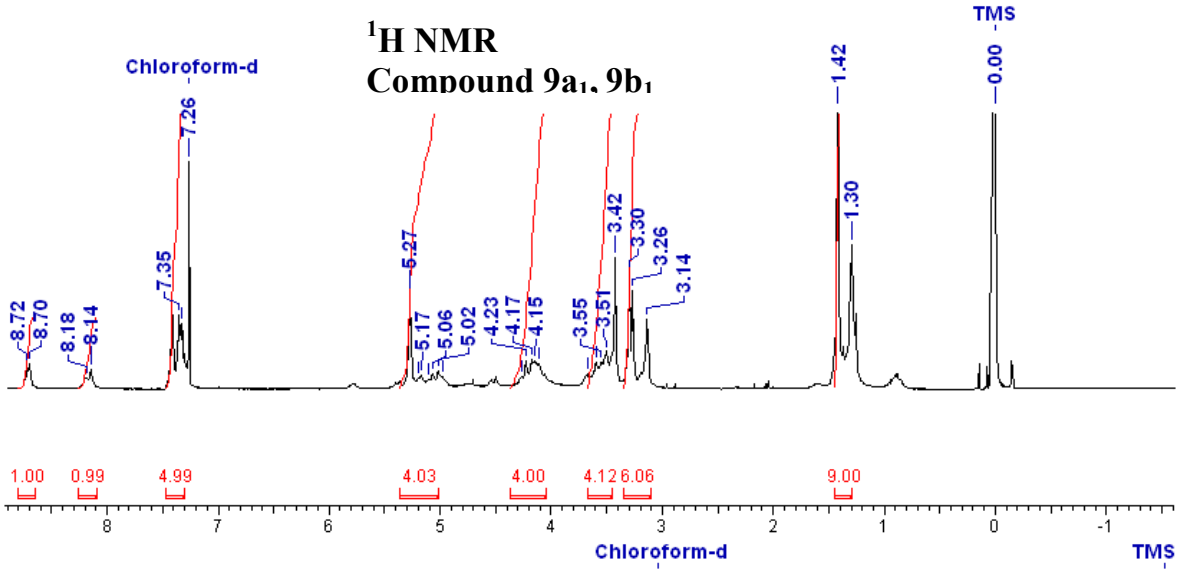

^{1}H NMR
Compound 8a$_1$, 8b$_1$
D2O
4.72
7.34
7.28
4.51
4.32
4.26
4.03
3.93
3.65
3.56
3.50
3.34
3.30
3.21
1.82
1.78
1.36
1.32
1.09
6.07
4.05
6.01
2.97
9.00
^{13}C NMR
Compound 8a$_1$, 8b$_1$
DMSO-d6
165.18
164.64
155.75
151.77
151.21
138.09
107.81
79.41
77.72
70.63
58.47
58.28
49.90
48.17
40.95
40.53
40.11
39.70
39.28
38.86
38.44
28.40
12.16
12.03
DEPT NMR
Compound 8a$_1$, 8b$_1$
137.84
79.16
72.34
70.39
58.04
49.66
47.95
28.15
11.78


H2O in CD3OD
Methanol D4
DMSO-d6
^{1}H NMR
Compound 8a$_2$, 8b$_2$

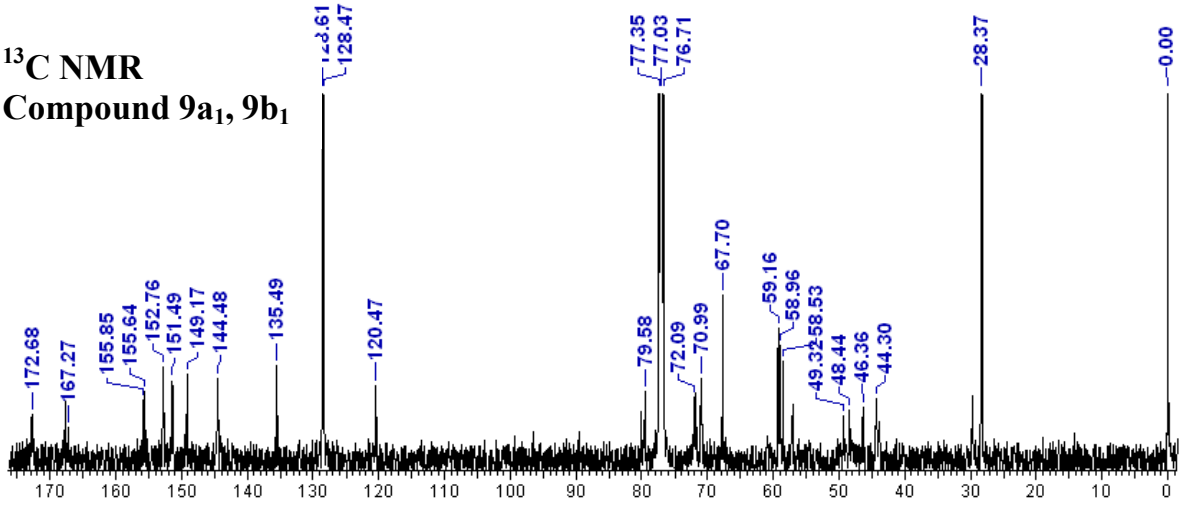

^{13}C NMR
Compound 8a$_2$, 8b$_2$


DEPT NMR
Compound 8a$_2$, 8b$_2$

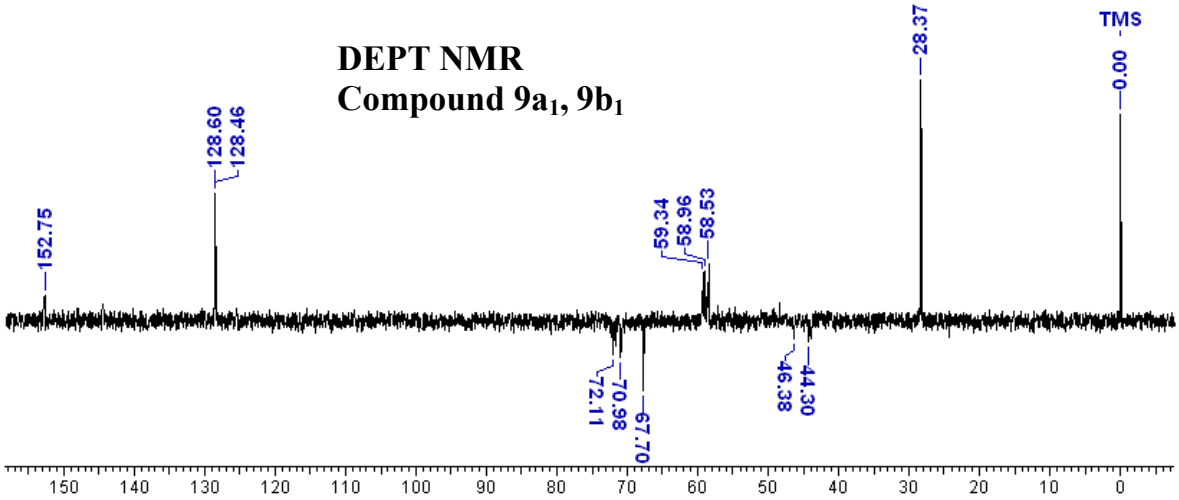
^{1}H NMR
Compound 9a$_1$, 9b$_1$

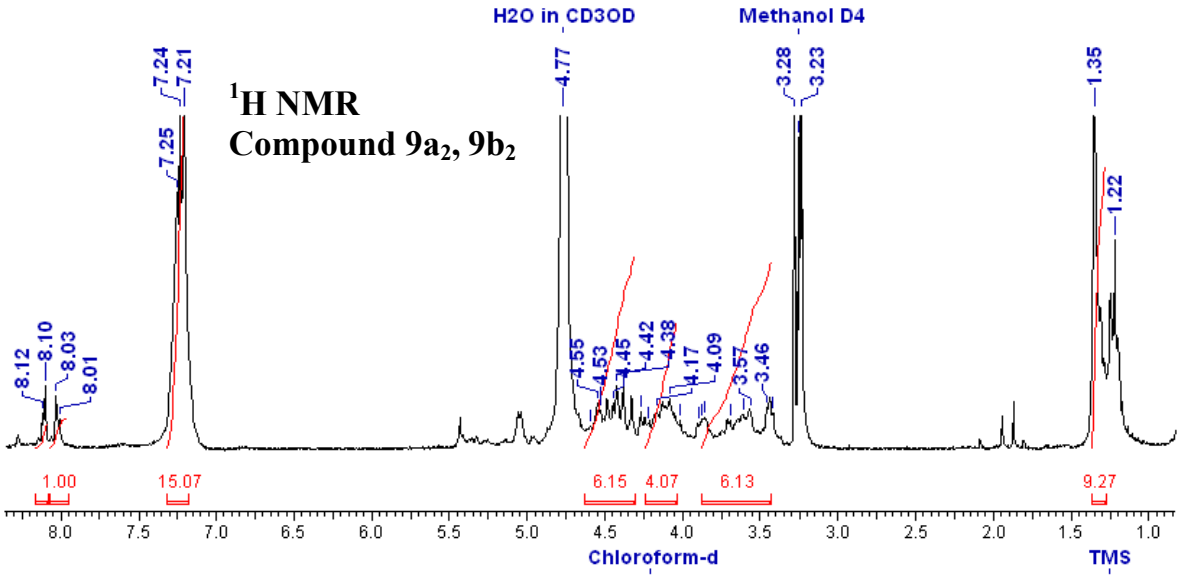


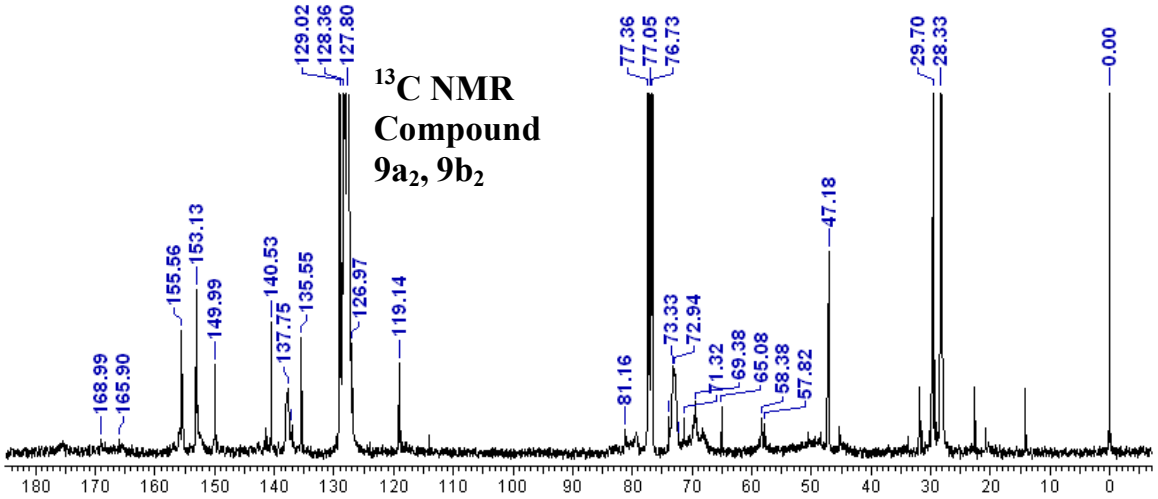
^{13}C NMR
Compound 9a$_1$, 9b$_1$

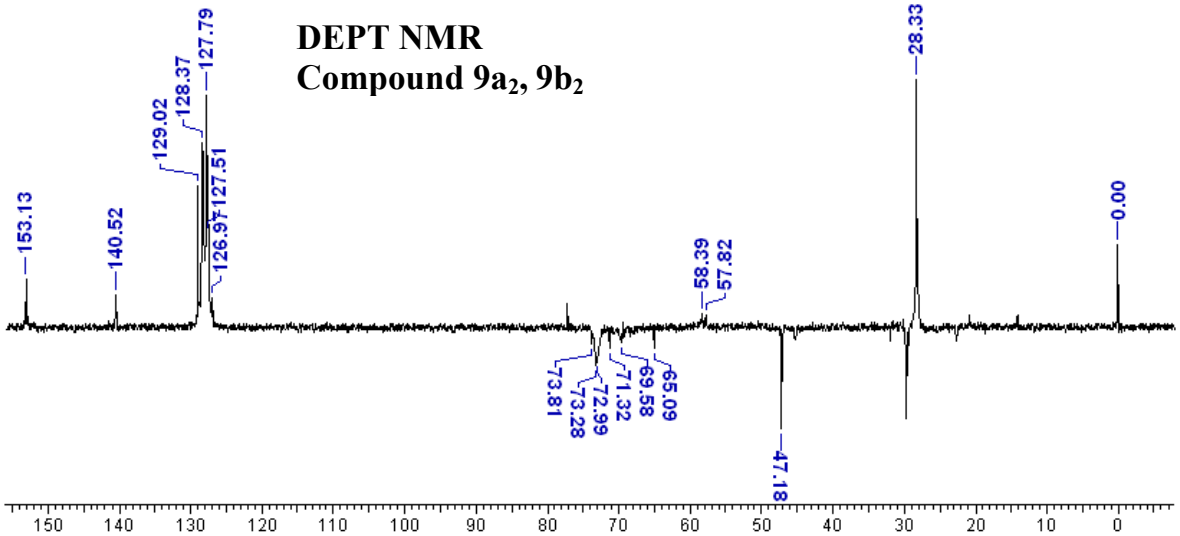


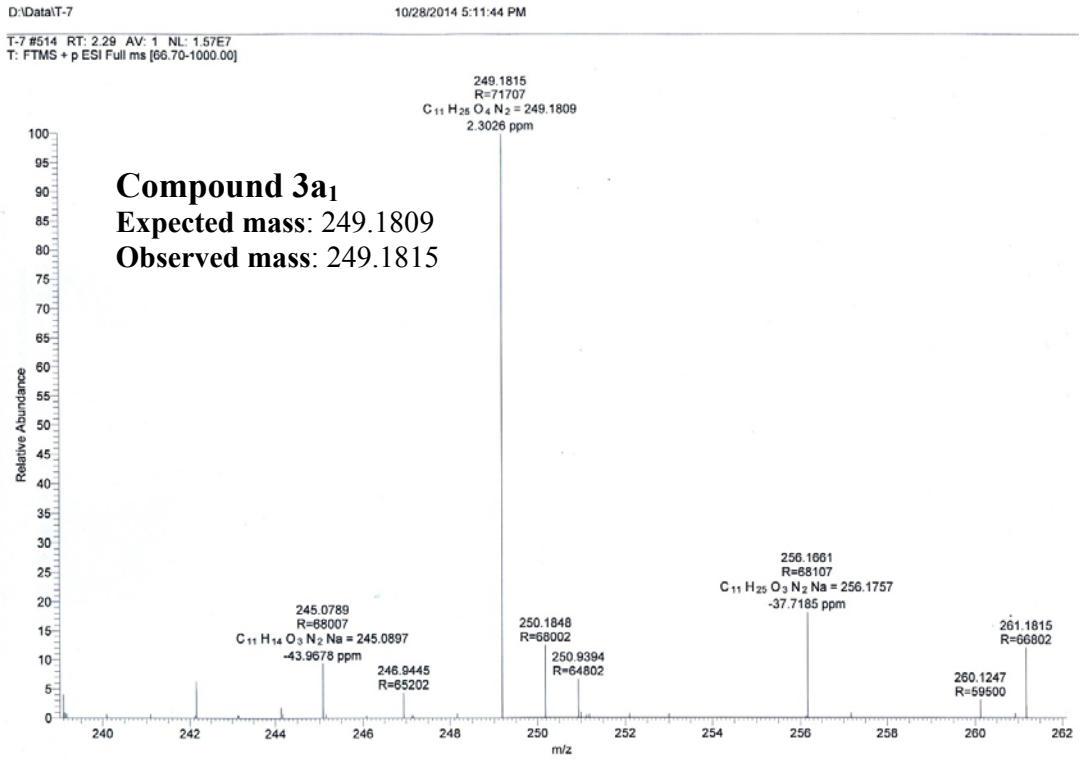
DEPT NMR
Compound 9a$_1$, 9b$_1$

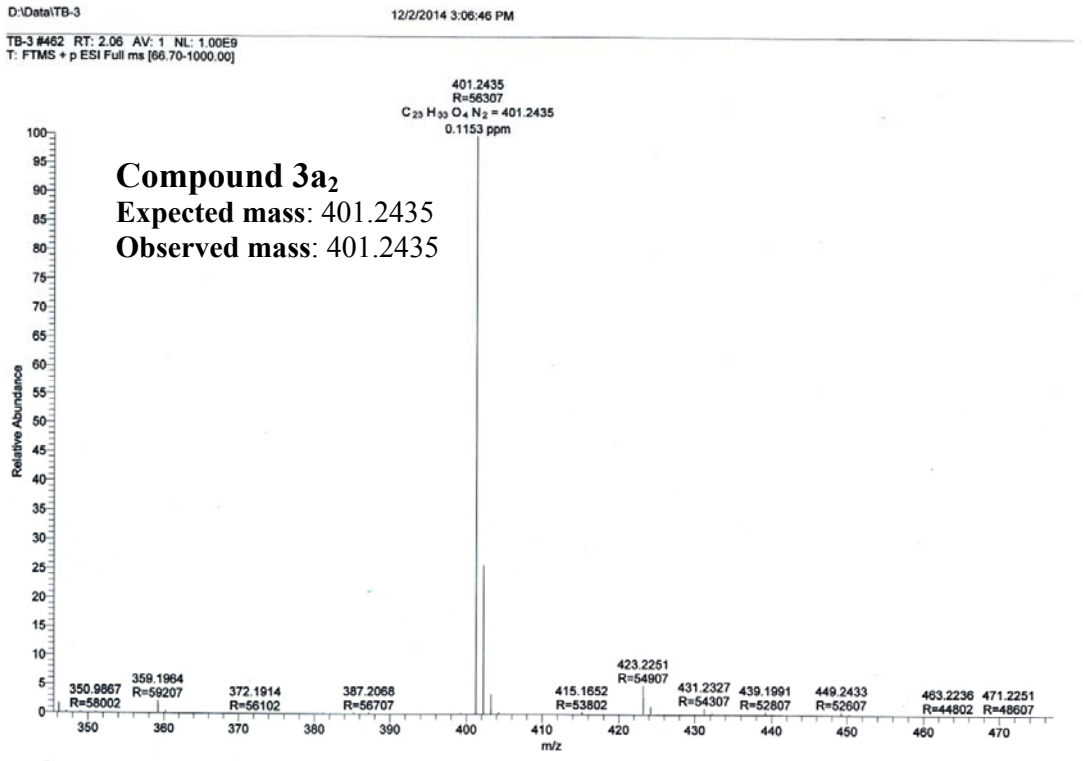


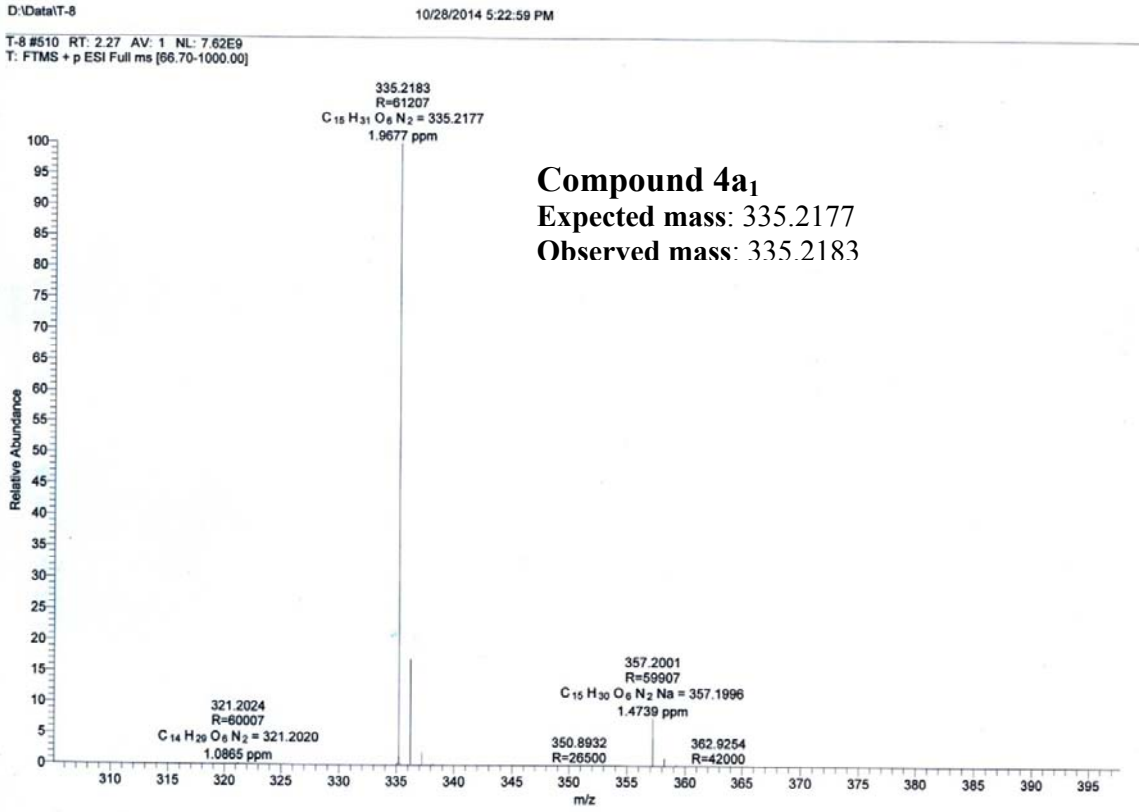

H2O in CD3OD
Methanol D4
^{1}H NMR
Compound 9a$_2$, 9b$_2$
7.24
7.21
7.25
4.77
3.28
3.23
1.35
1.22
8.12
8.10
8.03
8.01
4.55
4.53
4.45
4.42
4.38
4.17
4.09
3.57
3.46
1.00
15.07
6.15
4.07
6.13
9.27
Chloroform-d
TMS


^{13}C NMR
Compound
9a$_2$, 9b$_2$
129.02
128.36
127.80
77.36
77.05
76.73
29.70
28.33
0.00
155.56
153.13
140.53
135.55
126.97
119.14
149.99
137.75
168.99
165.90
81.16
73.33
72.94
71.32
69.38
65.08
58.38
57.82
47.18

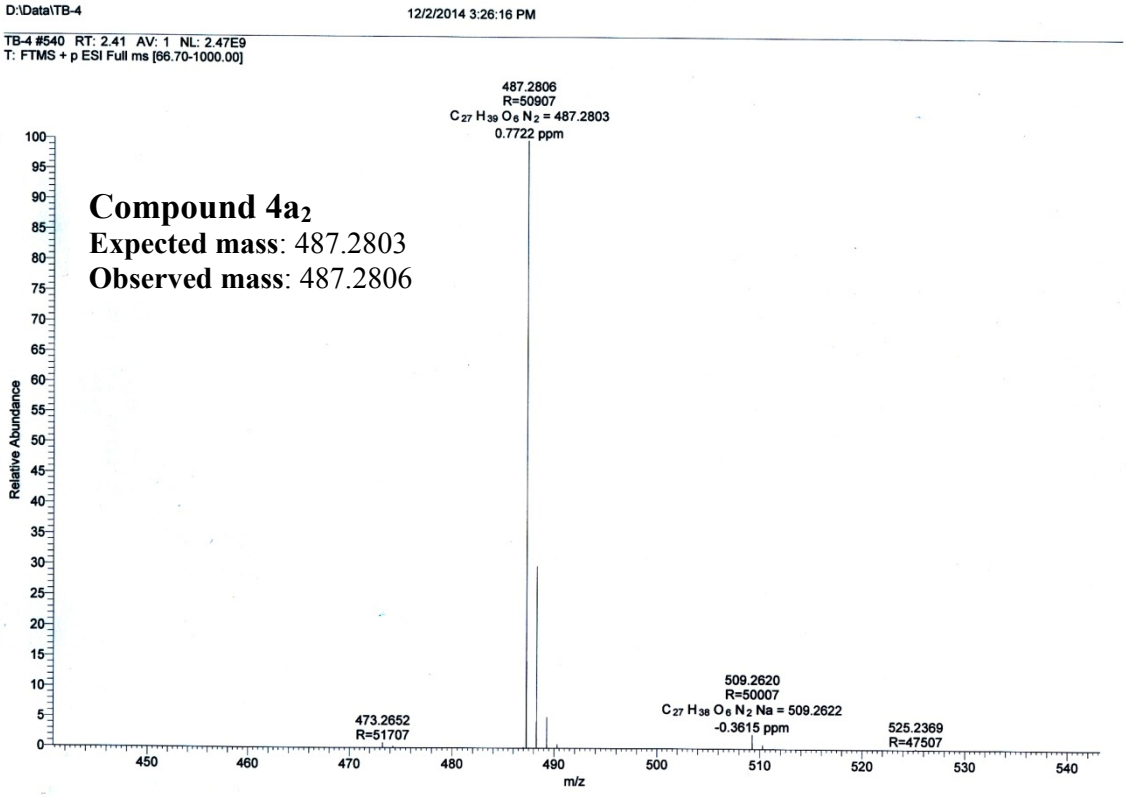

DEPT NMR
Compound 9a$_2$, 9b$_2$
129.02
128.37
127.79
127.51
126.97
153.13
140.52
28.33
0.00
58.39
57.82
73.81
73.28
72.99
71.32
69.58
65.09
47.18


D:\Data\T-7
10/28/2014 5:11:44 PM
T-7 #514 RT: 2.29 AV: 1 NL: 1.57E7
T: FTMS + p ESI Full ms [66.70-1000.00]
249.1815
R=71707
C11 H25 O4 N2 = 249.1809
2.3026 ppm
Compound 3a1
Expected mass: 249.1809
Observed mass: 249.1815
245.0789
R=68007
C11 H14 O3 N2 Na = 245.0897
-43.9678 ppm
246.9445
R=65202
250.1848
R=68002
250.9394
R=64802
256.1661
R=68107
C11 H25 O3 N2 Na = 256.1757
-37.7185 ppm
260.1247
R=59500
261.1815
R=66802
Relative Abundance
m/z

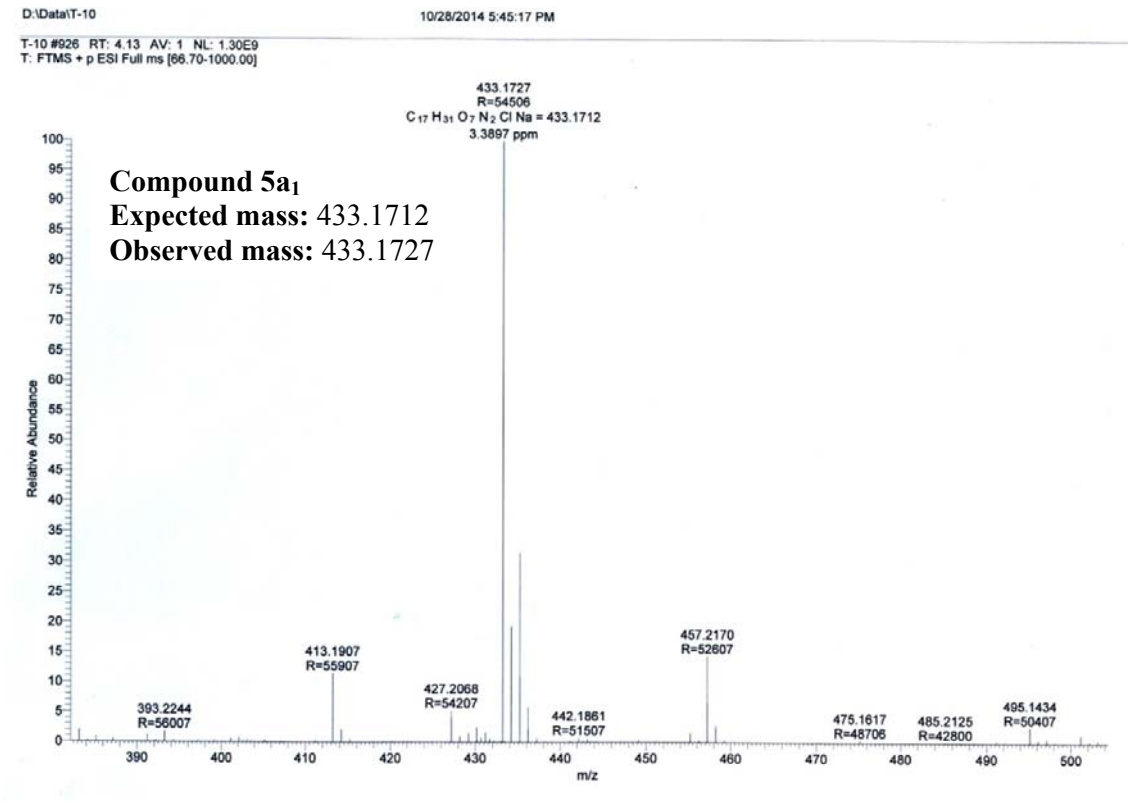

D:\Data\TB-3
12/2/2014 3:06:46 PM
TB-3 #462 RT: 2.06 AV: 1 NL: 1.00E9
T: FTMS + p ESI Full ms [66.70-1000.00]
401.2435
R=56307
C23 H33 O4 N2 = 401.2435
0.1153 ppm
Compound 3a2
Expected mass: 401.2435
Observed mass: 401.2435
350.9867
R=58002
359.1964
R=59207
372.1914
R=56102
387.2068
R=56707
415.1652
R=53802
423.2251
R=54907
431.2327
R=54307
439.1991
R=52807
449.2433
R=52607
463.2236
R=44802
471.2251
R=48607
Relative Abundance
m/z

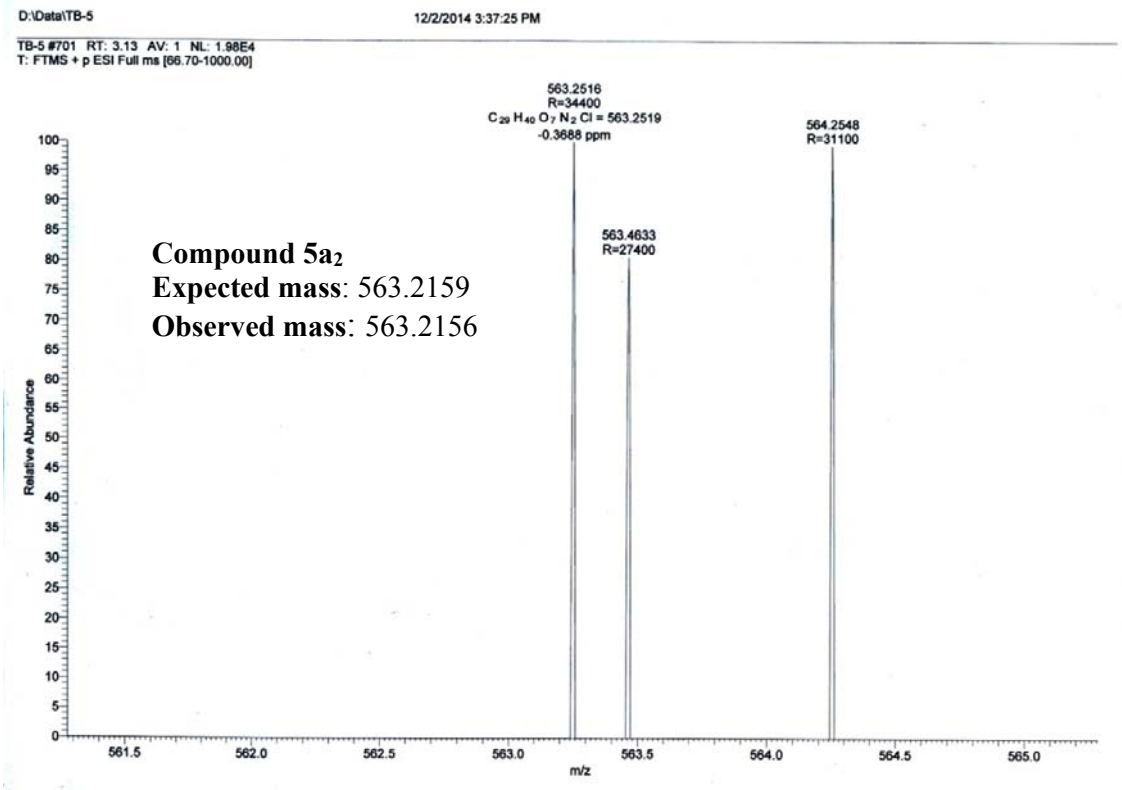


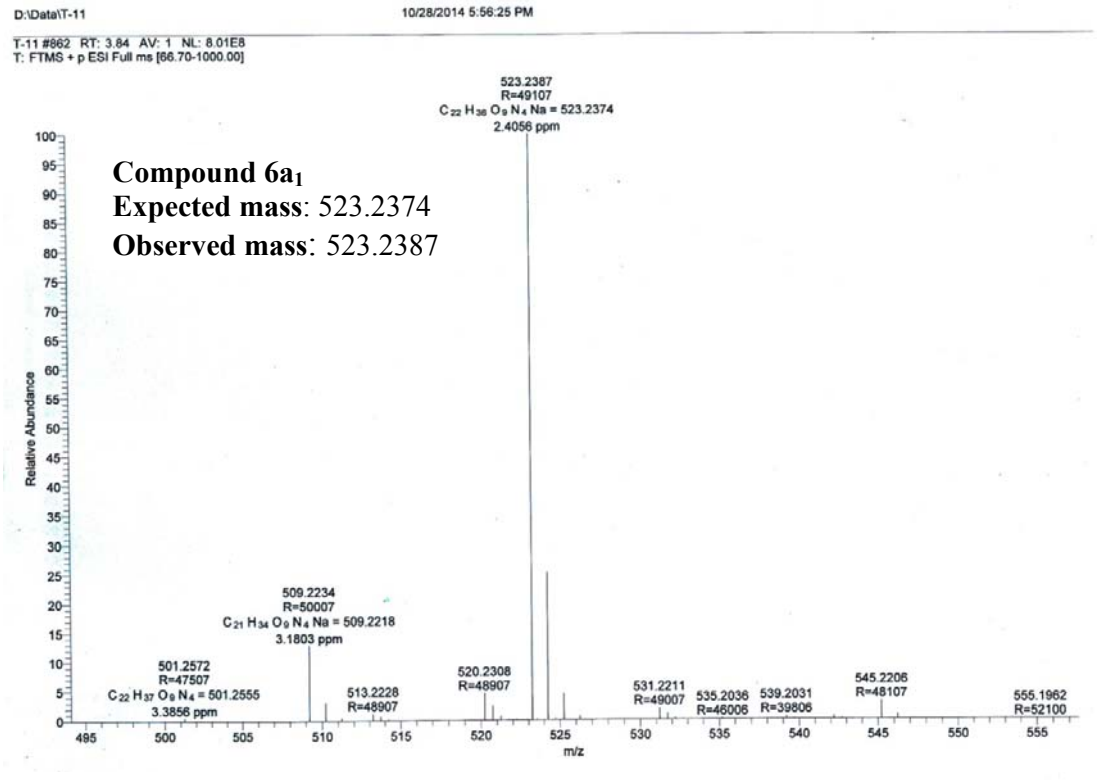
Compound 4a$_1$
Expected mass: 335.2177
Observed mass: 335.2183

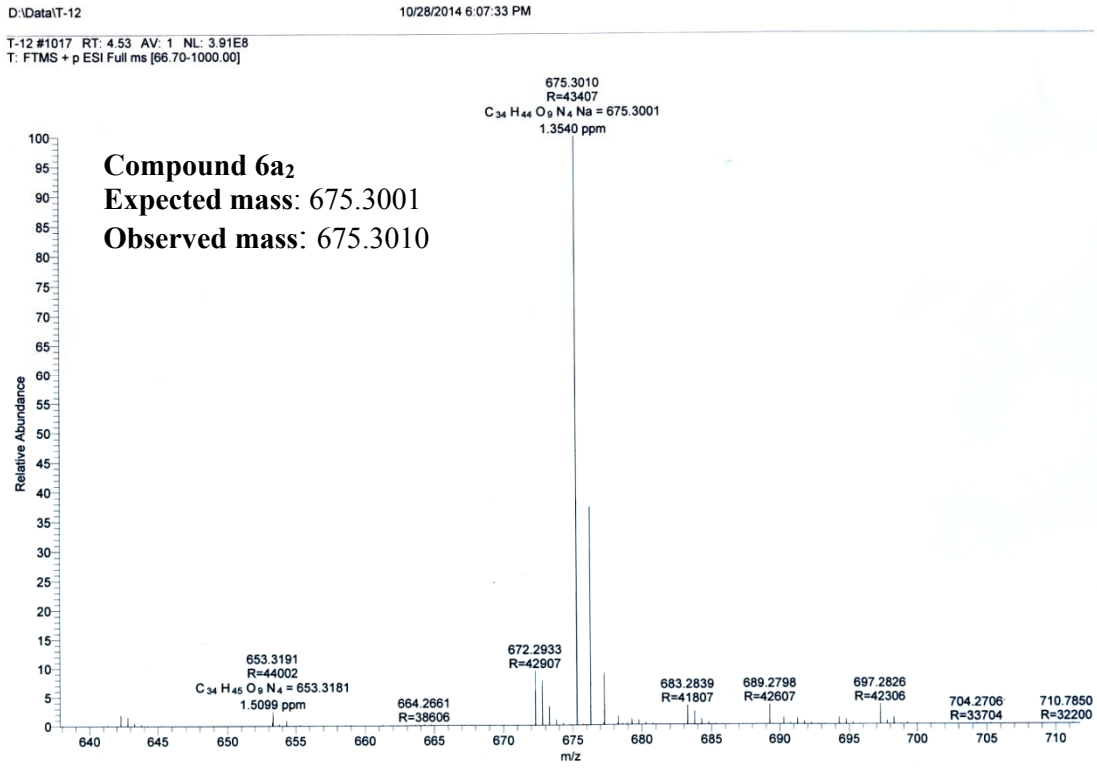


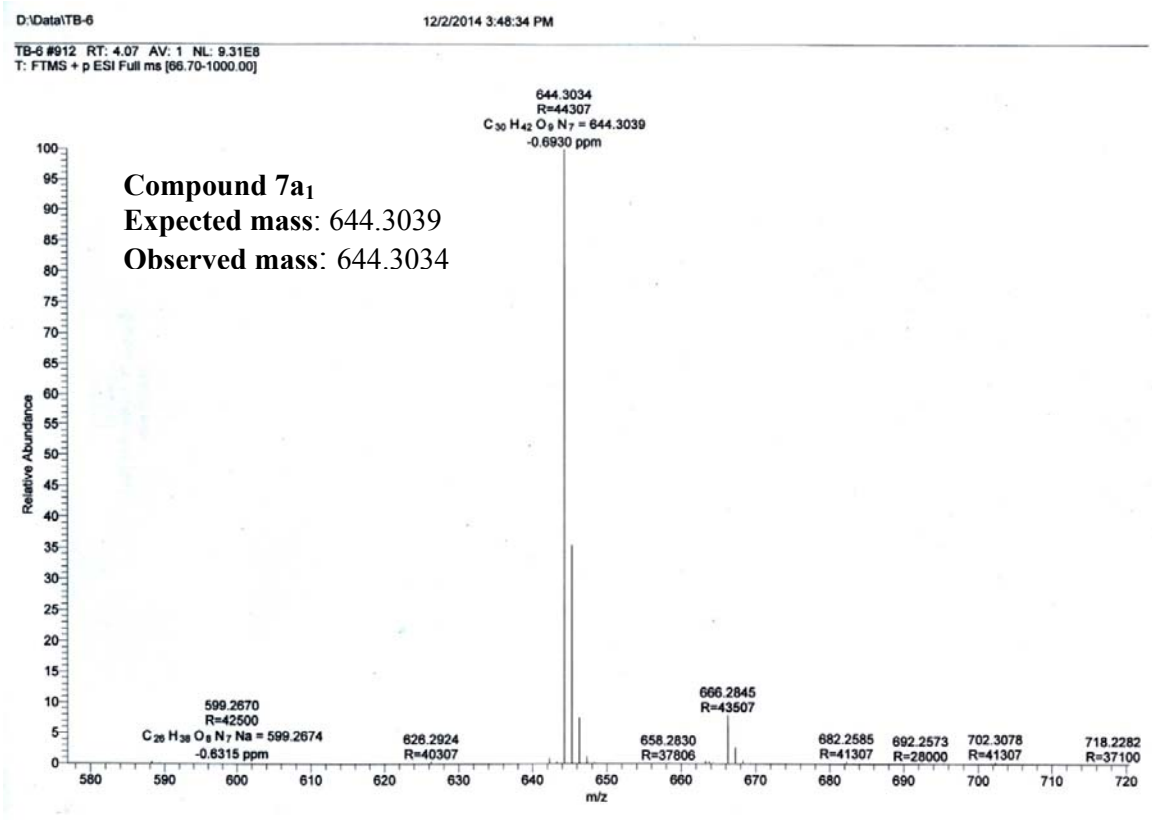
Compound 4a$_2$
Expected mass: 487.2803
Observed mass: 487.2806

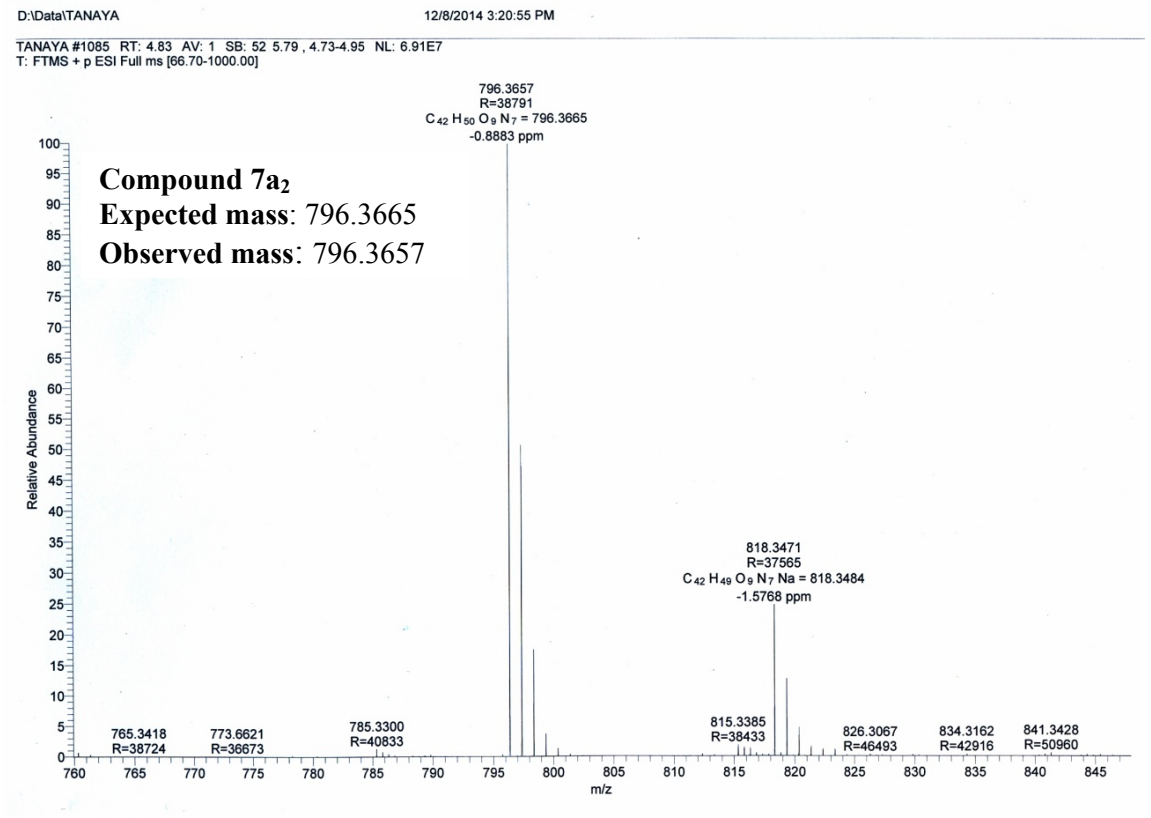

D:\Data\T-10
10/28/2014 5:45:17 PM
T-10 #926 RT: 4.13 AV: 1 NL: 1.30E9
T: FTMS + p ESI Full ms [66.70-1000.00]
433.1727
R=54506
C17 H31 O7 N2 Cl Na = 433.1712
3.3897 ppm
Compound 5a1
Expected mass: 433.1712
Observed mass: 433.1727
413.1907
R=55907
427.2068
R=54207
457.2170
R=52807
393.2244
R=56007
442.1861
R=51507
475.1617
R=48706
485.2125
R=42800
495.1434
R=50407
Relative Abundance
m/z

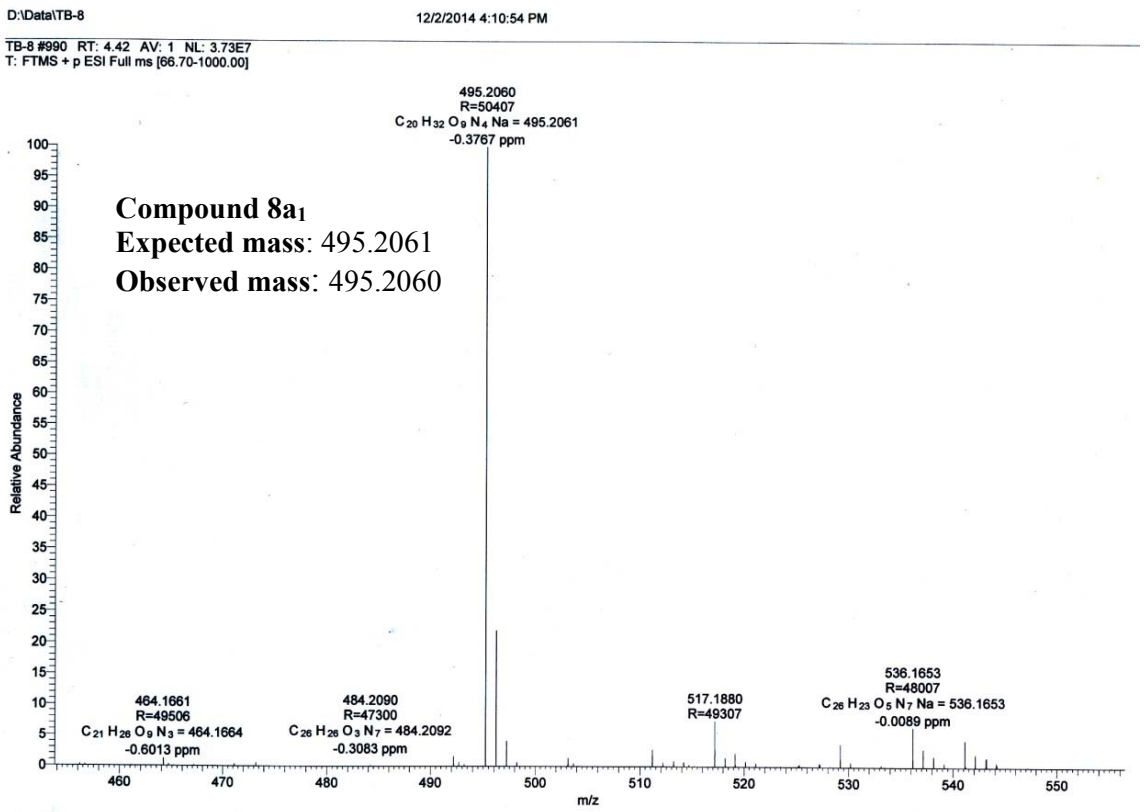

D:\Data\TB-5
12/2/2014 3:37:25 PM
TB-5 #701 RT: 3.13 AV: 1 NL: 1.98E4
T: FTMS + p ESI Full ms [66.70-1000.00]
563.2516
R=34400
C29 H40 O7 N2 Cl = 563.2519
-0.3688 ppm
564.2548
R=31100
563.4633
R=27400
Compound 5a2
Expected mass: 563.2159
Observed mass: 563.2156
Relative Abundance
m/z

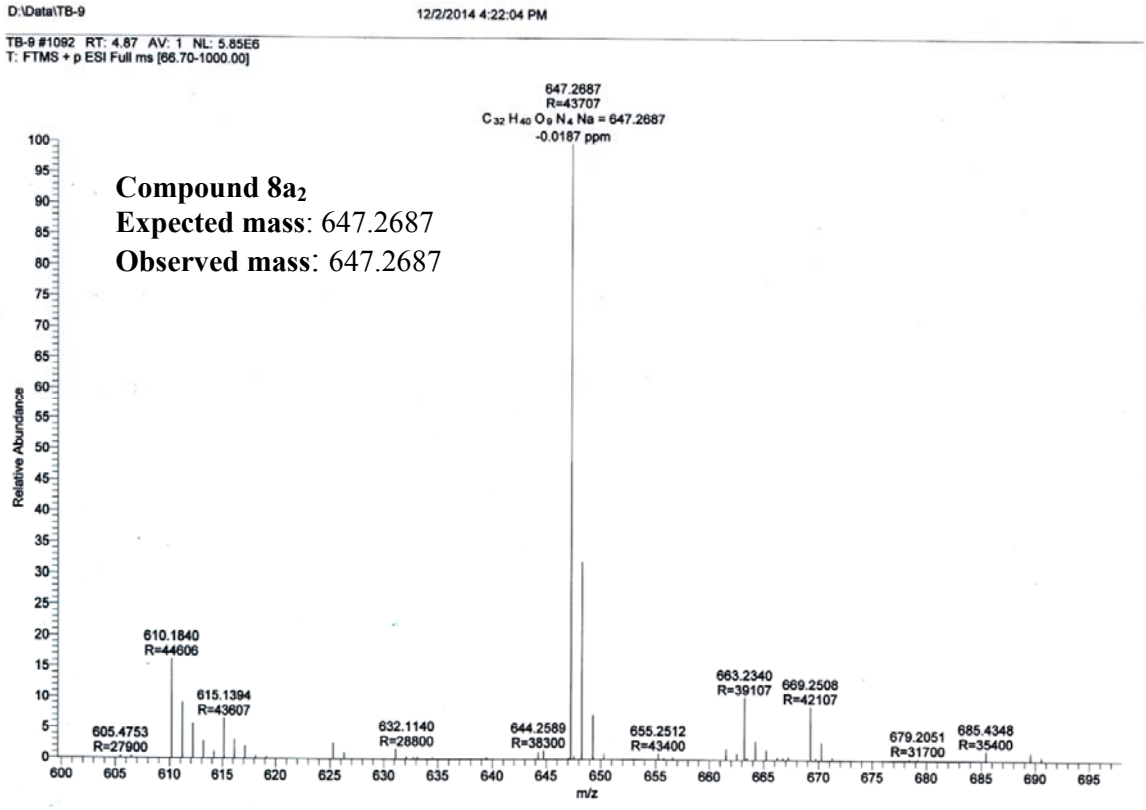

D:\Data\T-11
10/28/2014 5:56:25 PM
T-11 #862 RT: 3.84 AV: 1 NL: 8.01E8
T: FTMS + p ESI Full ms [66.70-1000.00]
523.2387
R=49107
C22 H38 O9 N4 Na = 523.2374
2.4056 ppm
Compound 6a1
Expected mass: 523.2374
Observed mass: 523.2387
509.2234
R=50007
C21 H34 O9 N4 Na = 509.2218
3.1803 ppm
501.2572
R=47507
C22 H37 O9 N4 = 501.2555
3.3856 ppm
513.2228
R=48907
520.2308
R=48907
531.2211
R=49007
535.2036
R=46006
539.2031
R=39806
545.2206
R=48107
555.1962
R=52100
Relative Abundance
m/z

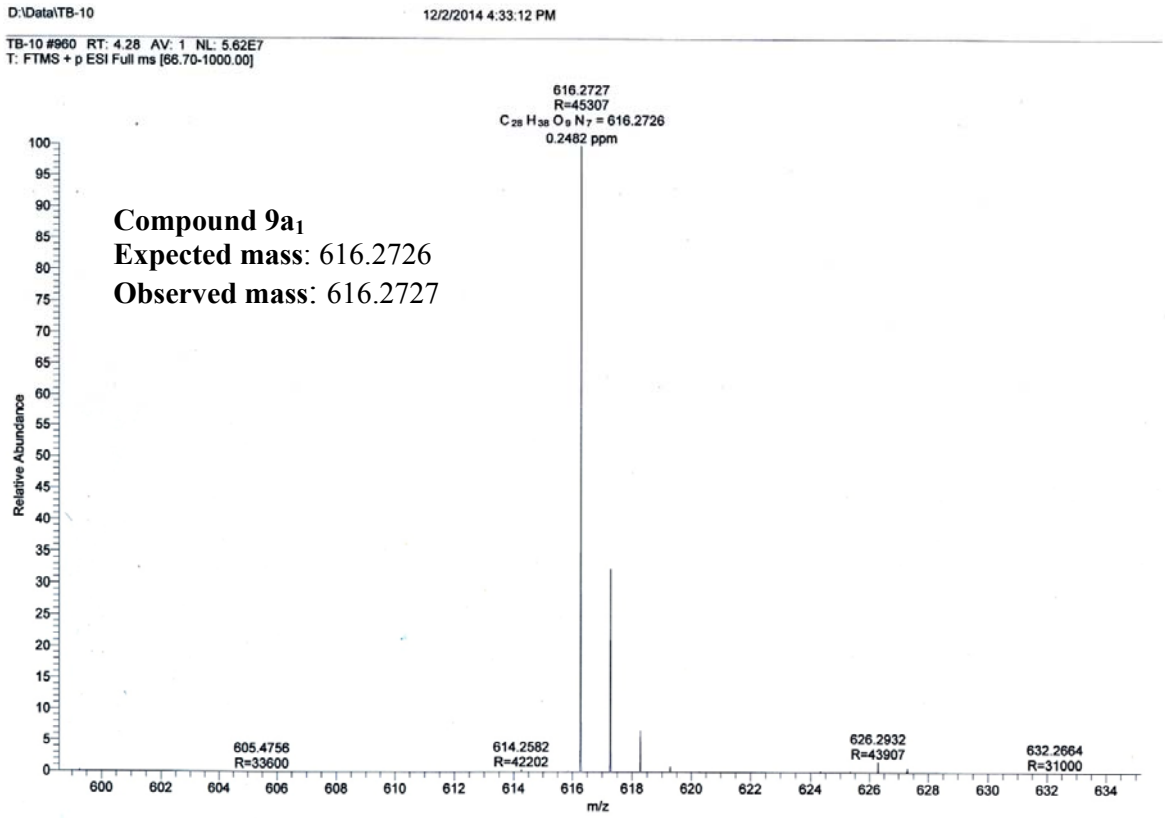

D:\Data\T-12
10/28/2014 6:07:33 PM
T-12 #1017 RT: 4.53 AV: 1 NL: 3.91E8
T: FTMS + p ESI Full ms [66.70-1000.00]
675.3010
R=43407
C34 H44 O9 N4 Na = 675.3001
1.3540 ppm
Compound 6a2
Expected mass: 675.3001
Observed mass: 675.3010
653.3191
R=44002
C34 H45 O9 N4 = 653.3181
1.5099 ppm
664.2661
R=38606
672.2933
R=42907
683.2839
R=41807
689.2798
R=42607
697.2826
R=42306
704.2706
R=33704
710.7850
R=32200
Relative Abundance
m/z

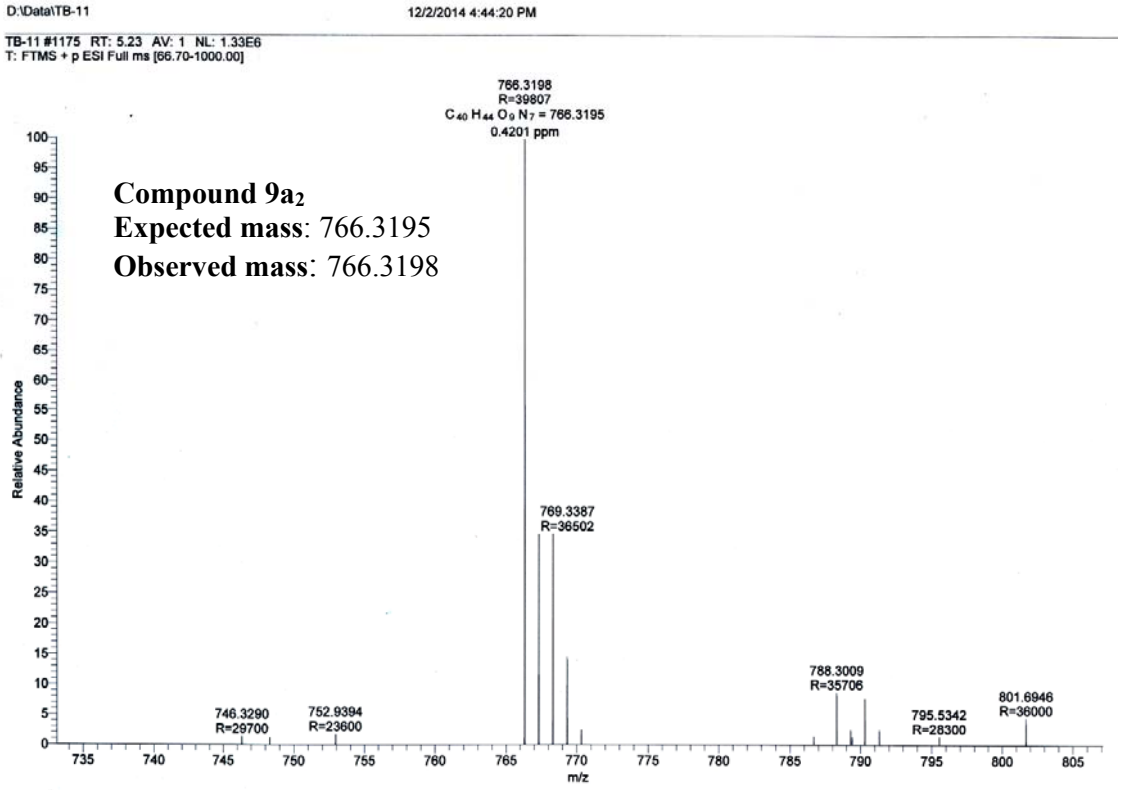

D:\Data\TB-6
12/2/2014 3:48:34 PM
TB-6 #912 RT: 4.07 AV: 1 NL: 9.31E8
T: FTMS + p ESI Full ms [66.70-1000.00]
644.3034
R=44307
C30 H42 O9 N7 = 644.3039
-0.6930 ppm
Compound 7a1
Expected mass: 644.3039
Observed mass: 644.3034
599.2670
R=42500
C26 H38 O8 N7 Na = 599.2674
-0.6315 ppm
626.2924
R=40307
658.2830
R=37806
666.2845
R=43507
682.2585
R=41307
692.2573
R=28000
702.3078
R=41307
718.2282
R=37100
Relative Abundance
m/z


D:\Data\TANAYA
12/8/2014 3:20:55 PM
TANAYA #1085 RT: 4.83 AV: 1 SB: 52 5.79 , 4.73-4.95 NL: 6.91E7
T: FTMS + p ESI Full ms [66.70-1000.00]
796.3657
R=38791
C42 H50 O9 N7 = 796.3665
-0.8883 ppm
Compound 7a2
Expected mass: 796.3665
Observed mass: 796.3657
765.3418
R=38724
773.6621
R=36673
785.3300
R=40833
815.3385
R=38433
818.3471
R=37565
C42 H49 O9 N7 Na = 818.3484
-1.5768 ppm
826.3067
R=46493
834.3162
R=42916
841.3428
R=50960
Relative Abundance
m/z


D:\Data\TB-8
12/2/2014 4:10:54 PM
TB-8 #990 RT: 4.42 AV: 1 NL: 3.73E7
T: FTMS + p ESI Full ms [66.70-1000.00]
495.2060
R=50407
C20 H32 O9 N4 Na = 495.2061
-0.3767 ppm
Compound 8a1
Expected mass: 495.2061
Observed mass: 495.2060
464.1661
R=49506
C21 H26 O9 N3 = 464.1664
-0.6013 ppm
484.2090
R=47300
C26 H29 O3 N7 = 484.2092
-0.3083 ppm
517.1880
R=49307
536.1653
R=48007
C26 H23 O5 N7 Na = 536.1653
-0.0089 ppm
Relative Abundance
m/z


D:\Data\TB-9
12/2/2014 4:22:04 PM
TB-9 #1092 RT: 4.87 AV: 1 NL: 5.85E6
T: FTMS + p ESI Full ms [66.70-1000.00]
647.2687
R=43707
C32 H40 O9 N4 Na = 647.2687
-0.0187 ppm
Compound 8a2
Expected mass: 647.2687
Observed mass: 647.2687
610.1840
R=44606
615.1394
R=43607
605.4753
R=27900
632.1140
R=28800
644.2589
R=38300
655.2512
R=43400
663.2340
R=39107
669.2508
R=42107
679.2051
R=31700
685.4348
R=35400
Relative Abundance
m/z

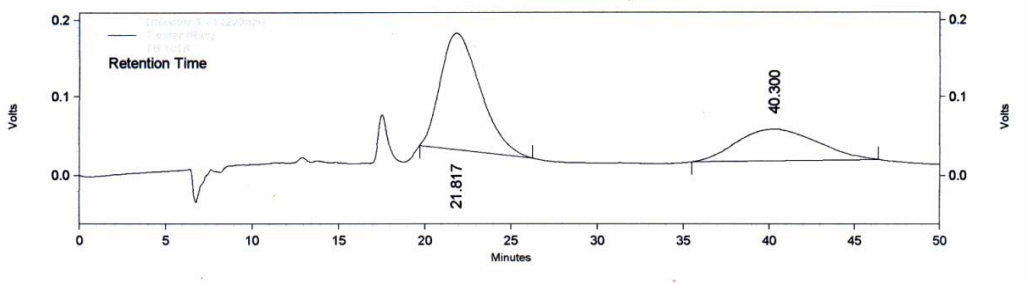

D:\Data\TB-10
12/2/2014 4:33:12 PM
TB-10 #960 RT: 4.28 AV: 1 NL: 5.62E7
T: FTMS + p ESI Full ms [66.70-1000.00]
616.2727
R=45307
C28 H38 O9 N7 = 616.2726
0.2482 ppm
Compound 9a1
Expected mass: 616.2726
Observed mass: 616.2727
605.4756
R=33600
614.2582
R=42202
626.2932
R=43907
632.2664
R=31000
Relative Abundance
m/z


D:\Data\TB-11
12/2/2014 4:44:20 PM
TB-11 #1175 RT: 5.23 AV: 1 NL: 1.33E6
T: FTMS + p ESI Full ms [66.70-1000.00]
766.3198
R=39807
C40 H44 O9 N7 = 766.3195
0.4201 ppm
Compound 9a2
Expected mass: 766.3195
Observed mass: 766.3198
769.3387
R=36502
746.3290
R=29700
752.9394
R=23600
788.3009
R=35706
795.5342
R=28300
801.6946
R=36000
Relative Abundance
m/z

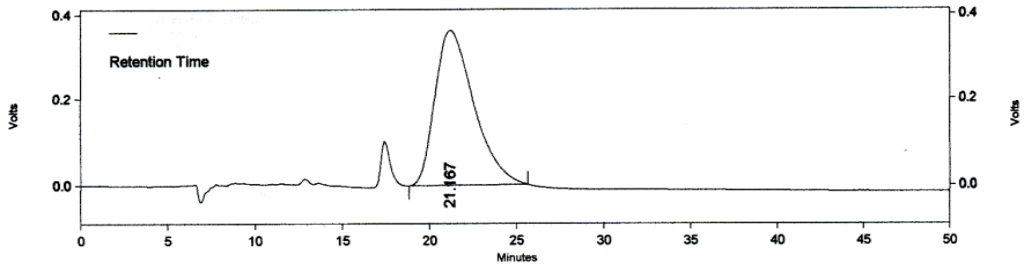
Chiral HPLC of $6a_1/6b_1$

Chiral HPLC was done on Kromasil 5-Amycoat (4.6x250mm) column in mobile phase isopropyl alcohol: Petroleum ether (30:70)

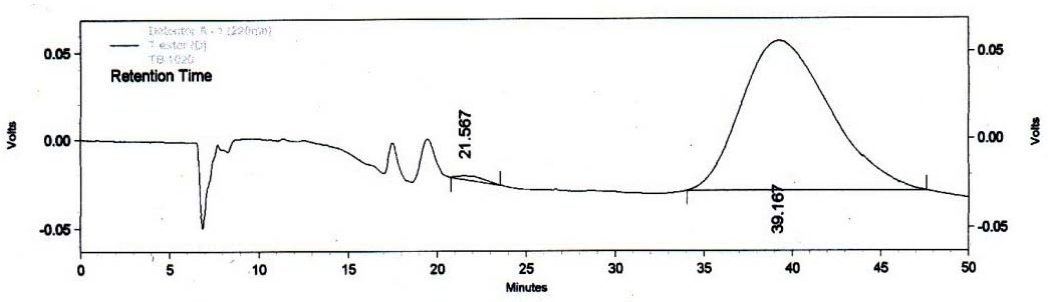
a) Chiral HPLC of **$6a_1$ + $6a_2$**



b) Chiral HPLC of **$6a_1$**



c) Chiral HPLC of **$6a_2$**



Synthesis of *(S,S)/ (R,R)*-β,γ-bis-hydroxy/ bis-methoxy substituted oligomers

Oligomers were synthesised on solid phase utilizing Boc chemistry on Lysine derivatized MBHA resin. The crude PNAs were purified on a semipreparative C18 column attached to a Waters HPLC system. A gradient elution method contained A = 5% Acetonitrile in water + 0.1% trifluoroacetic acid and B = 50% Acetonitrile in water + 0.1% trifluoroacetic acid (A to B = 100% in 20 min with a flow rate of 1.5mL/min), and the eluent was

monitored at 260 nm. The purity of the oligomers was further assessed by RP-C18 analytical HPLC column. The purities of the purified oligomers were found to be >98%. The MALDI-ToF spectra were recorded on AB SCIEX 5800 MALDI ToF ToF instrument and the matrix used for analysis was CHCA (α-Cyano-4-hydroxycinnamic acid).

HPLC purification of (*S,S)/(R,R)*-β,γ-bis-methoxy substituted oligomers

Table S1: HPLC purification of **PNA 1, PNA 2**

Seq Code	Sequences	Retention Time
PNA 1	aaccgatttcag-K	10.9
PNA 2	K_4-aaccgatttcag-K	10.9

Fig S1: HPLC chromatogram of purified **PNA 1**

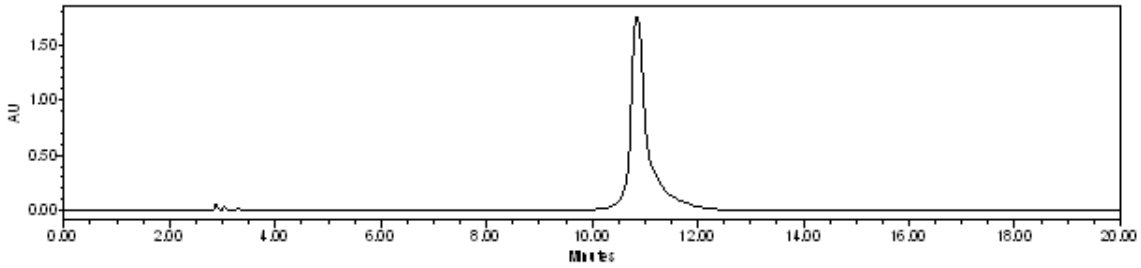
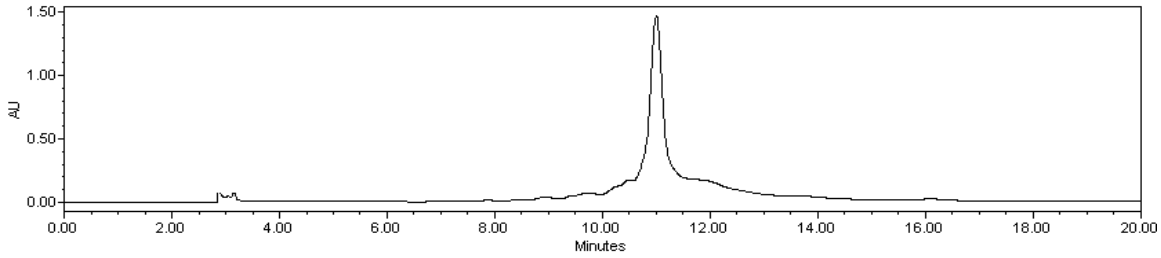
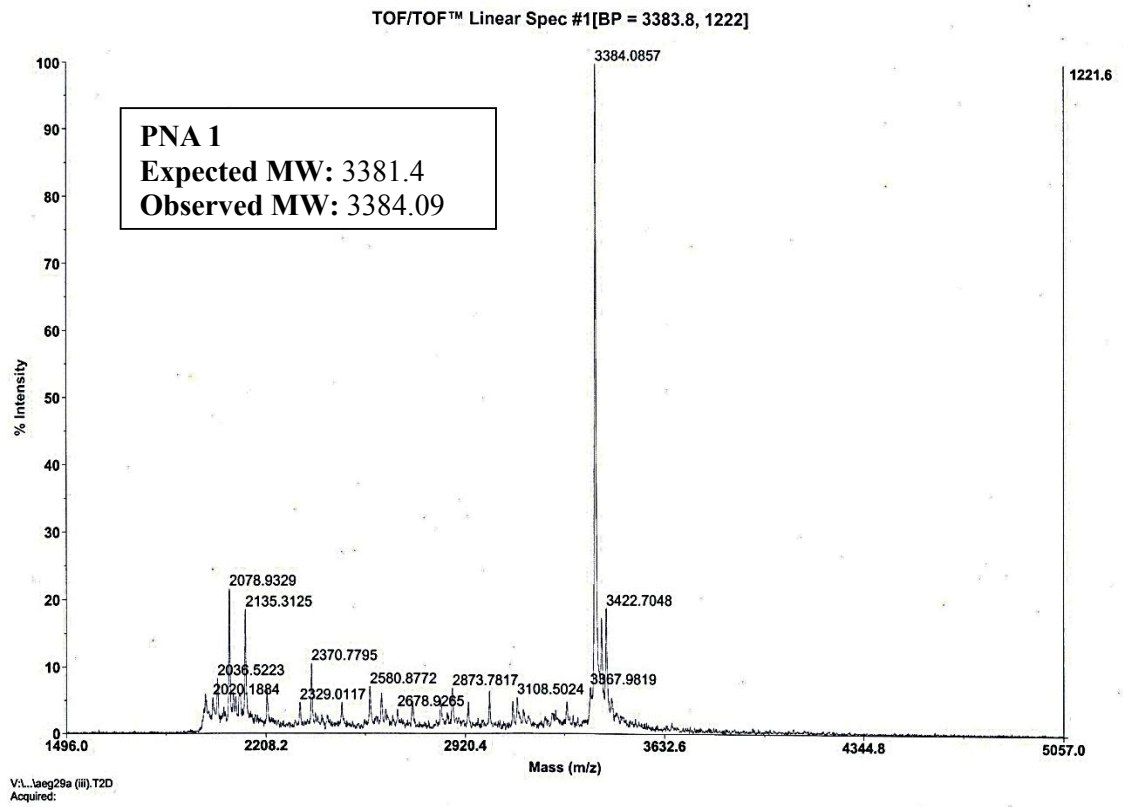


Fig S2: HPLC chromatogram of purified **PNA 2**




TOF/TOF™ Linear Spec #1[BP = 3383.8, 1222]
PNA 1
Expected MW: 3381.4
Observed MW: 3384.09
3384.0857
1221.6
2078.9329
2135.3125
3422.7048
2370.7795
2036.5223
2020.1884
2329.0117
2580.8772
2678.9265
2873.7817
3108.5024
3367.9819
% Intensity
Mass (m/z)
1496.0
2208.2
2920.4
3632.6
4344.8
5057.0
V:\...\aeg29a (ii).T2D
Acquired:

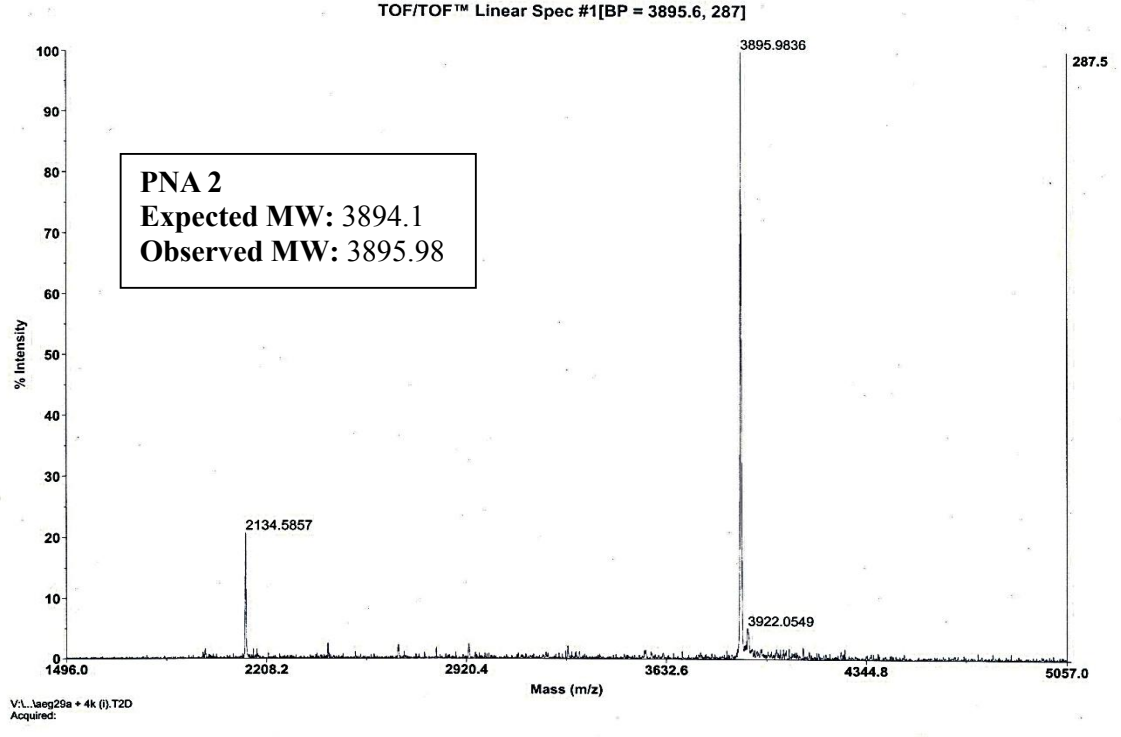

TOF/TOF™ Linear Spec #1[BP = 3895.6, 287]
PNA 2
Expected MW: 3894.1
Observed MW: 3895.98
3895.9836
287.5
2134.5857
3922.0549
% Intensity
Mass (m/z)
1496.0
2208.2
2920.4
3632.6
4344.8
5057.0
V:\...\aeg29a + 4k (i).T2D
Acquired:

Table S2: HPLC purification of aaccga$\mathbf{T^{OH}}$ttcag-K

Seq Code	Sequences	Retention time
PNA 3a	aaccga$^{(R,R)}\mathbf{T^{OH}}$ttcag-K	10.3
PNA 3b	aaccga$^{(S,S)}\mathbf{T^{OH}}$ttcag-K	10.3

Fig S3: HPLC chromatogram of purified **PNA 3a**

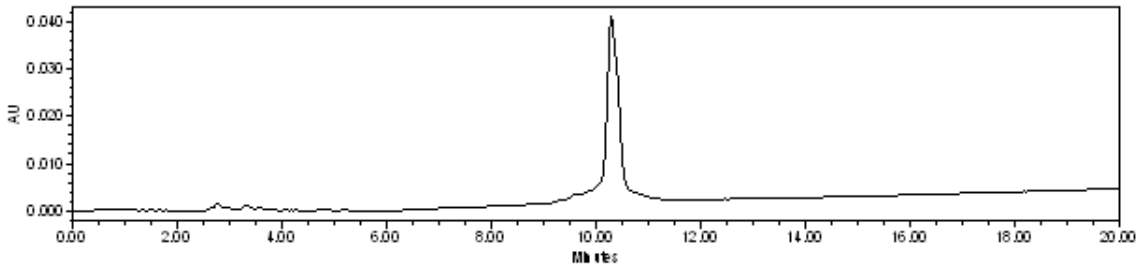
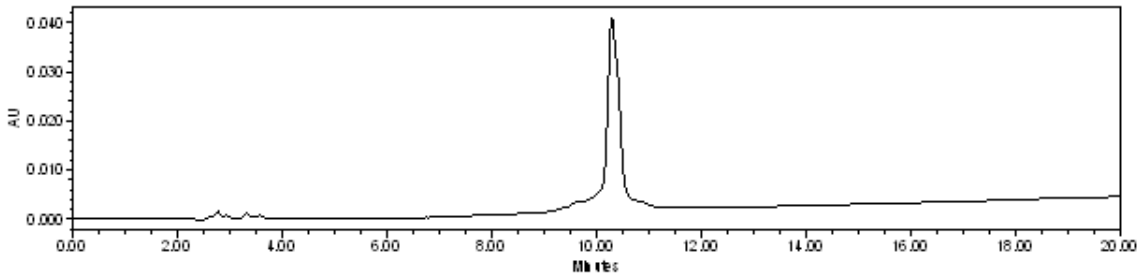
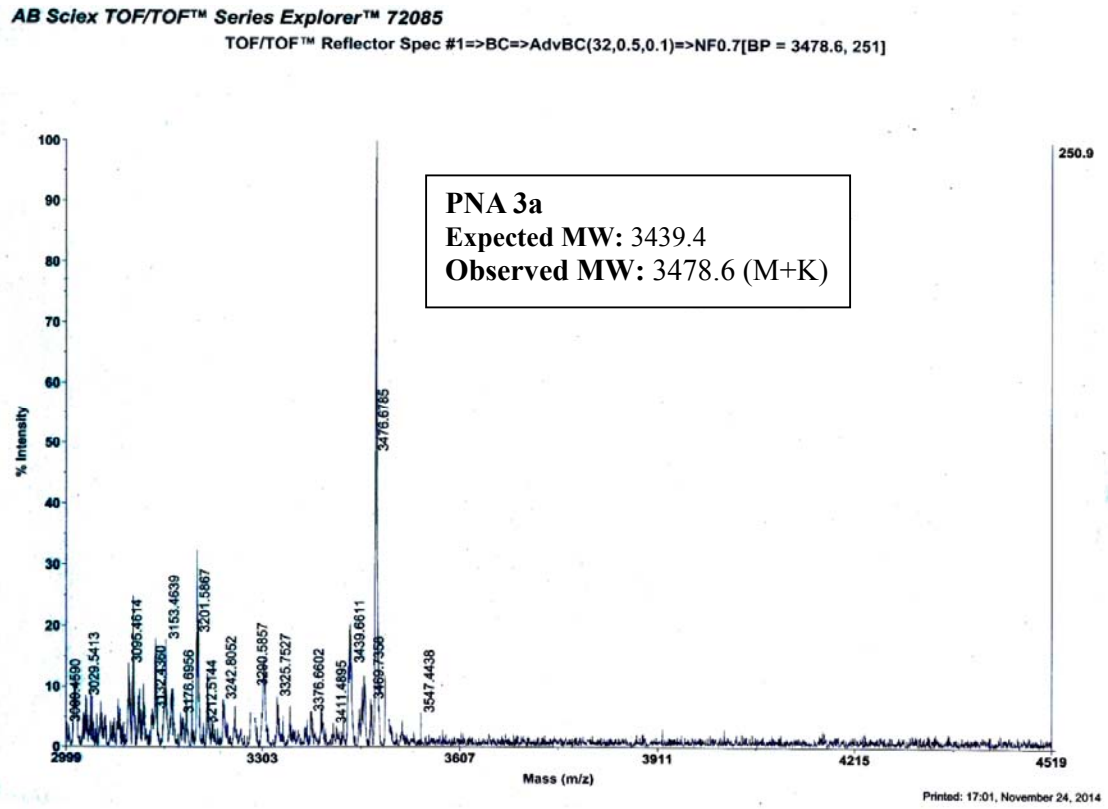
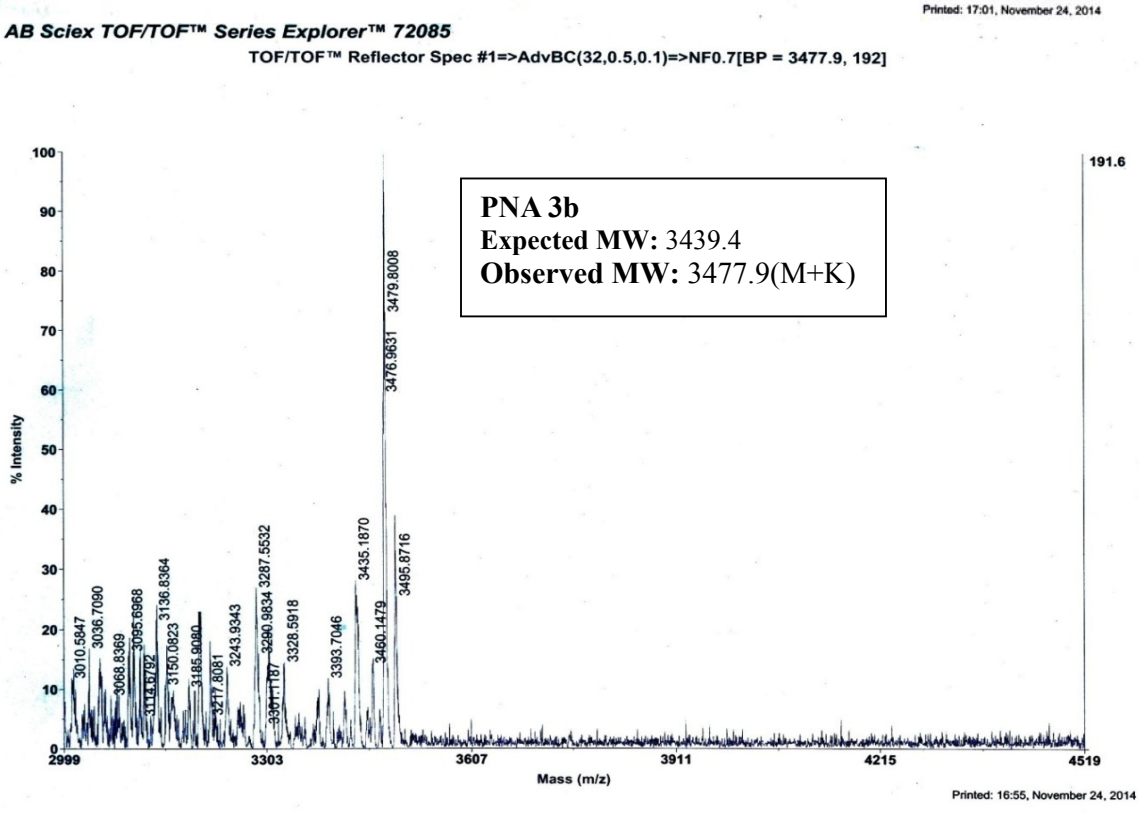


Fig S4: HPLC chromatogram of purified **PNA 3b**




AB Sciex TOF/TOF™ Series Explorer™ 72085
TOF/TOF™ Reflector Spec #1=>BC=>AdvBC(32,0.5,0.1)=>NF0.7[BP = 3478.6, 251]
PNA 3a
Expected MW: 3439.4
Observed MW: 3478.6 (M+K)
% Intensity
Mass (m/z)
Printed: 17:01, November 24, 2014


AB Sciex TOF/TOF™ Series Explorer™ 72085
TOF/TOF™ Reflector Spec #1=>AdvBC(32,0.5,0.1)=>NF0.7[BP = 3477.9, 192]
PNA 3b
Expected MW: 3439.4
Observed MW: 3477.9(M+K)
% Intensity
Mass (m/z)
Printed: 16:55, November 24, 2014

Synthesis of complementary Oligonucleotides

The complementary and parallel complementary DNA oligomers were synthesized on Bioautomation Mer-Made 4 synthesizer using standard β-cyanoethyl phosphoramidite chemistry. The oligomers were synthesized on polystyrene solid support, followed by ammonia treatment. The purity of the oligomers was ascertained by RP HPLC on a C18 column to be more than 98% and was used without further purification in the biophysical studies of PNA. The complementary RNA and mismatch oligonucleotides were obtained commercially.

List of DNA sequences

cDNA: 5'-CTGAAATCGGTT;

Parallel DNA: 5'-TTGGCTAAAGTC

List of RNA sequences

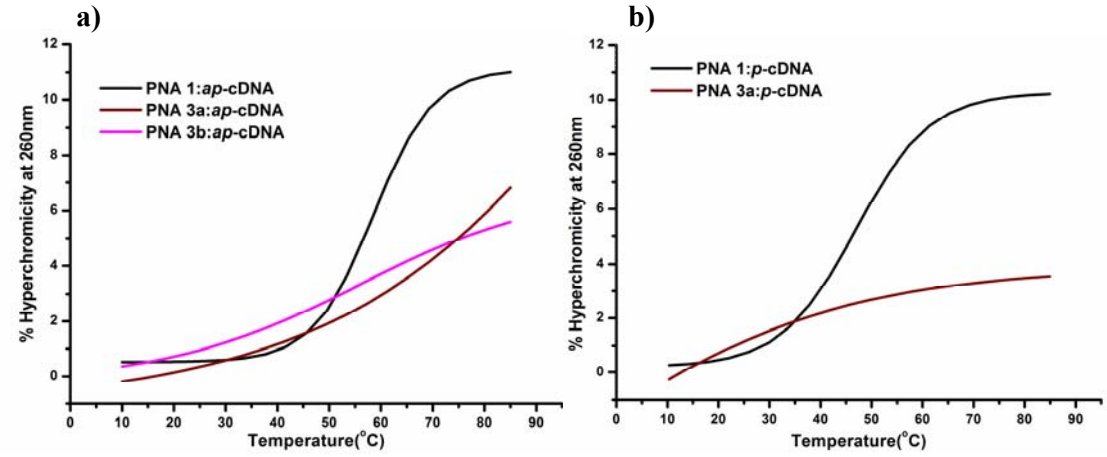
cRNA: 5'-CUGAAAUCGGUU

Mismatch RNA: CUGAA***U***UCGGUU

UV-Melting Study

UV melting experiments were performed in Analytik Jena UV/Vis Spectrophotometer. Melting temperatures were obtained from the maxima of the first derivative of the melting curves (A260 *vs* temperature) in buffer containing 10 mM sodium phosphate, 10 mM or 100 mM sodium chloride, pH 7.2 using 1.0μM concentrations of each of the two complementary strands. Each experiment was performed at least thrice.

UV melting at 10 mM NaCl concentration:



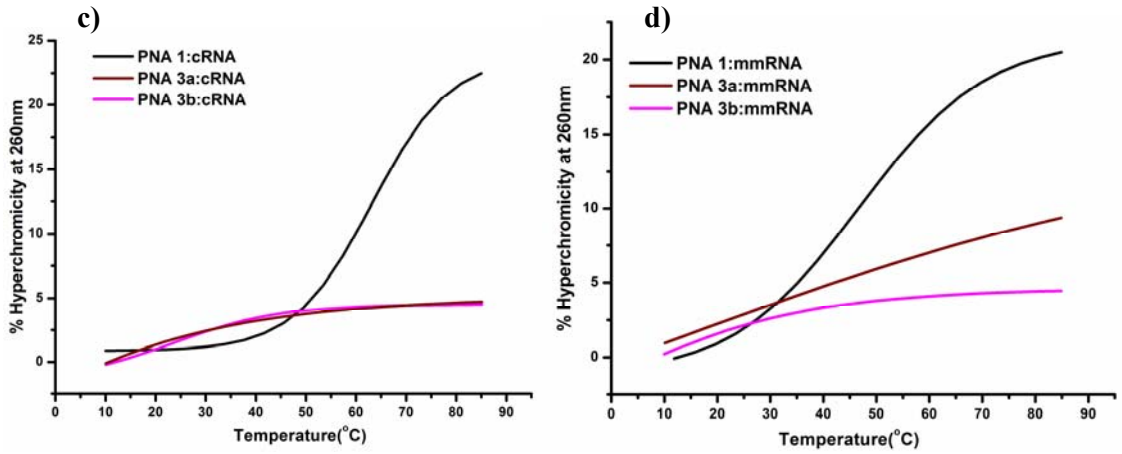


Fig S5: UV melting plots of **PNA 3a, 3b** with a) *ap*-cDNA b) *p*-cDNA c) cRNA d) mmRNA at 10 mM NaCl concentration

Table S3: UV meltings at 100 mM NaCl salt concentration.

Seq Code	Sequences	*ap*-cDNA	cRNA
PNA 1	aaccgatttcag-K	53.7	60.9
PNA 3a	aaccga$^{(R,R)}$T^{OH}ttcag-K	n.t.	n.t.
PNA 3b	aaccga$^{(S,S)}$T^{OH}ttcag-K	n.t.	n.t.

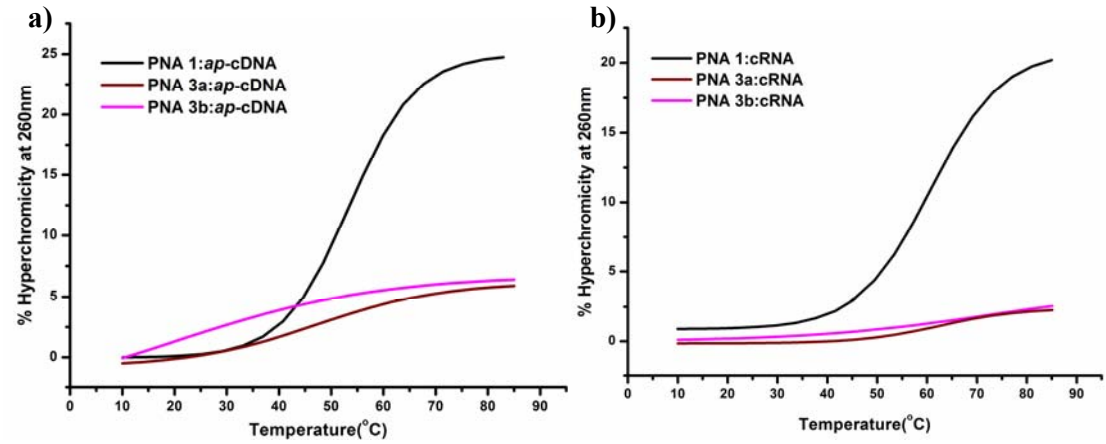


Fig S6: UV melting plots of **PNA 3a, 3b** with a) *ap*-cDNA b) cRNA at 100 mM NaCl concentration

Table S4: HPLC purification of aaccga**T**$^{\mathbf{OMe}}$ttcag-K

Seq Code	Sequences	Retention time
PNA 4a	aaccga$^{(R,R)}$**T**$^{\mathbf{OMe}}$ttcag-K	11.7
PNA 4b	aaccga$^{(S,S)}$**T**$^{\mathbf{OMe}}$ttcag-K	11.8

Fig S7: HPLC chromatogram of purified **PNA 4a**

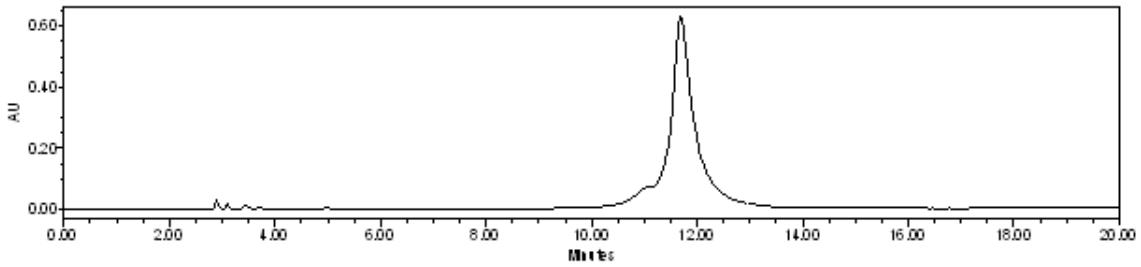
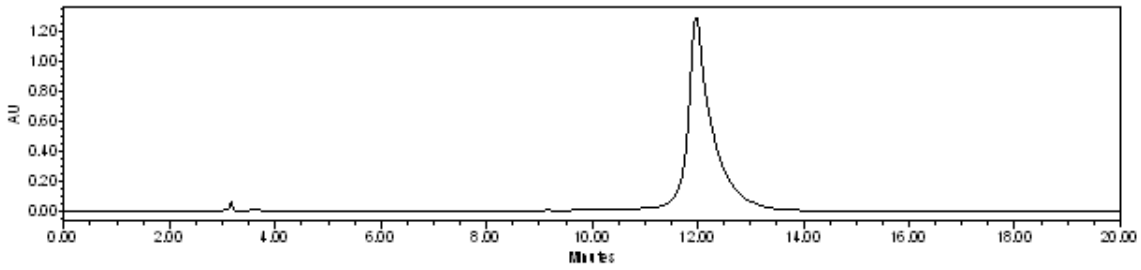
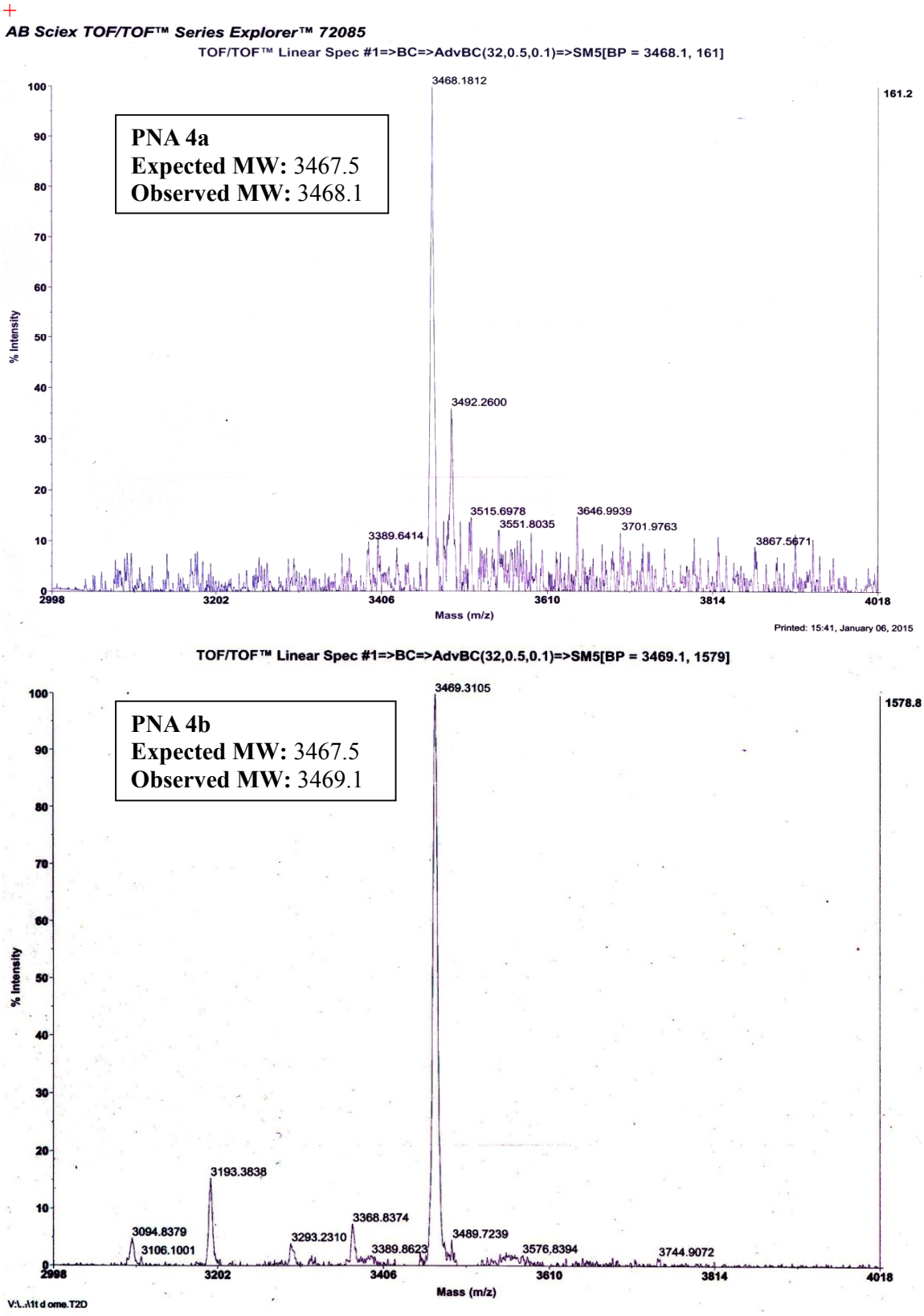


Fig S8: HPLC chromatogram of purified **PNA 4b**




AB Sciex TOF/TOF™ Series Explorer™ 72085
TOF/TOF™ Linear Spec #1=>BC=>AdvBC(32,0.5,0.1)=>SM5[BP = 3468.1, 161]
PNA 4a
Expected MW: 3467.5
Observed MW: 3468.1
3468.1812
161.2
3492.2600
3515.6978
3551.8035
3646.9939
3701.9763
3389.6414
3867.5671
% Intensity
Mass (m/z)
Printed: 15:41, January 06, 2015
TOF/TOF™ Linear Spec #1=>BC=>AdvBC(32,0.5,0.1)=>SM5[BP = 3469.1, 1579]
PNA 4b
Expected MW: 3467.5
Observed MW: 3469.1
3469.3105
1578.8
3193.3838
3094.8379
3106.1001
3293.2310
3368.8374
3389.8623
3489.7239
3576.8394
3744.9072
% Intensity
Mass (m/z)

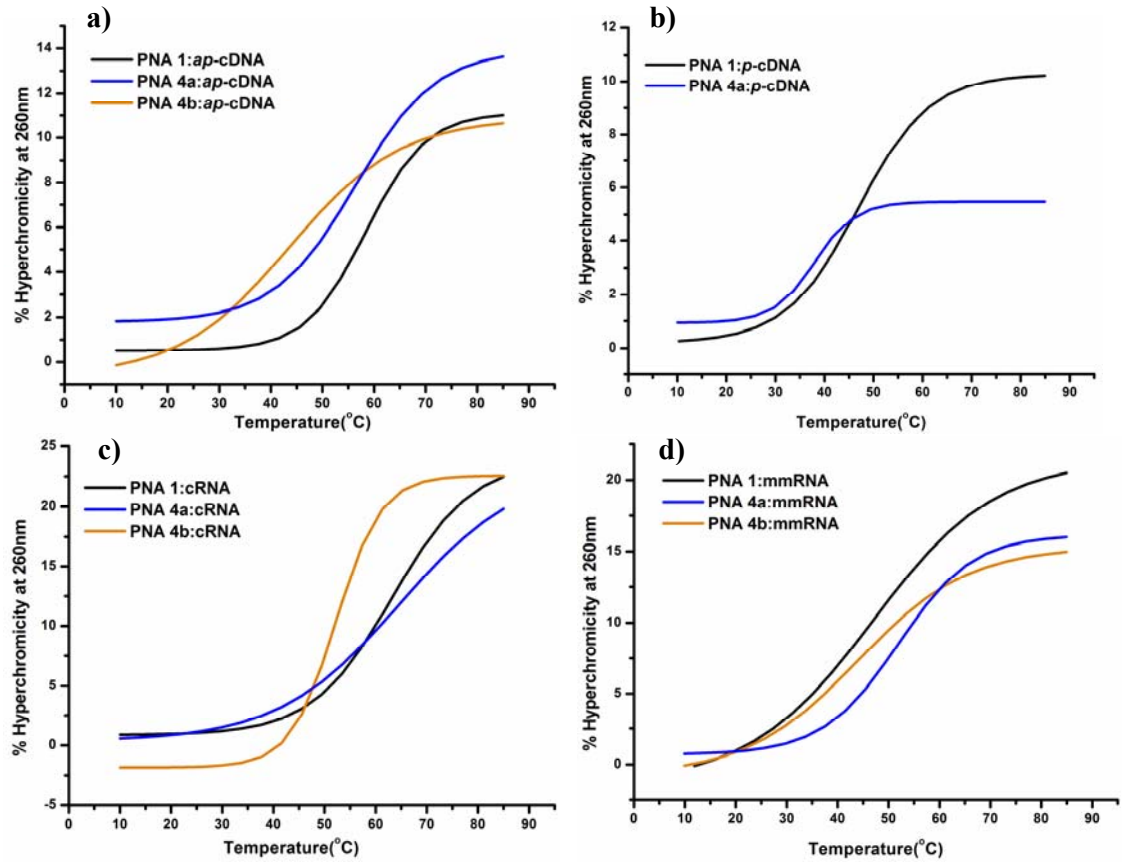


Fig S9: UV melting plots of **PNA 4a, 4b** with a) *ap*-cDNA b) *p*-cDNA c) cRNA d) mmRNA at 10 mM NaCl concentration

Table S5: UV meltings at 100 mM NaCl salt concentration.

Seq Code	Sequences	*ap*-cDNA	cRNA
PNA 1	aaccgatttcag-K	53.7	60.9
PNA 4a	aaccga$^{(R,R)}$T^{OMe}ttcag-K	53.7	59.9
PNA 4b	aaccga$^{(S,S)}$T^{OMe}ttcag-K	36.1	44.3

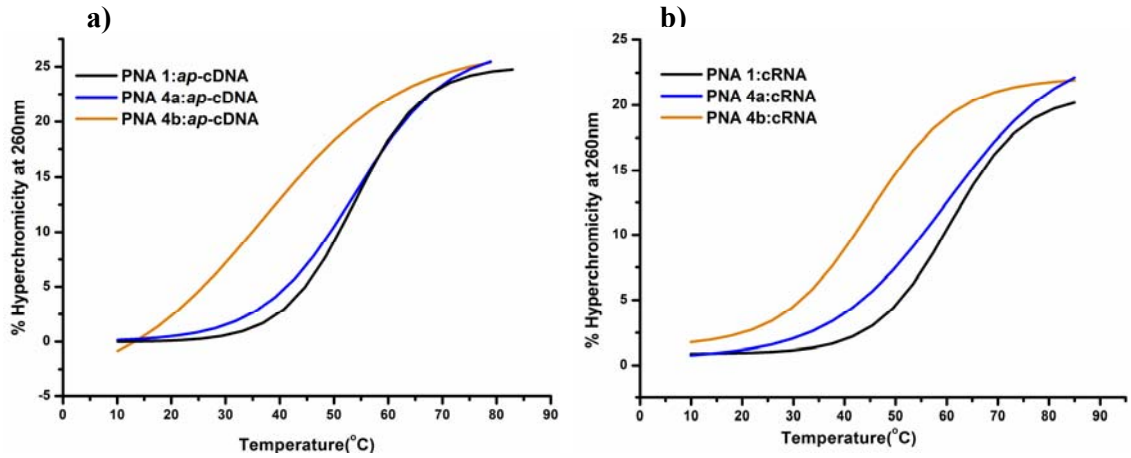


Fig S10: UV melting plots of **PNA 4a, 4b** with a) *ap*-cDNA b) cRNA at 100 mM NaCl concentration

Table S6: HPLC purification of aaccga$\mathbf{T^{OMe}}$t$\mathbf{T^{OMe}}$cag-K

Seq Code	Sequences	Retention time
PNA 5a	aaccga$^{(R,R)}\mathbf{T^{OMe}}$t$^{(R,R)}\mathbf{T^{OMe}}$CAG-K	12.6
PNA 5b	aaccga$^{(S,S)}\mathbf{T^{OMe}}$t$^{(S,S)}\mathbf{T^{OMe}}$cag-K	12.5

Fig S11: HPLC chromatogram of purified **PNA 5a**

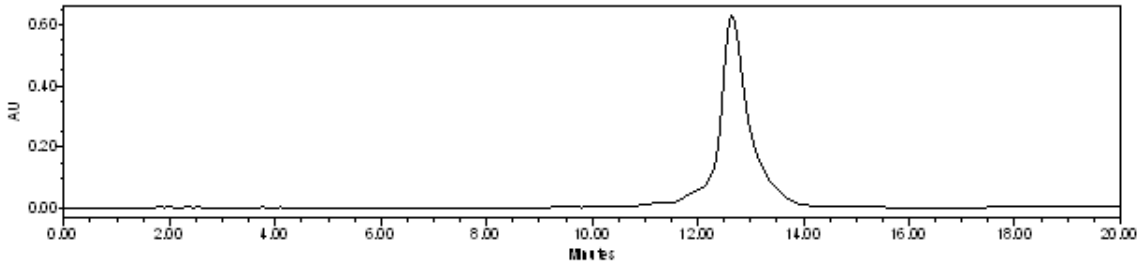
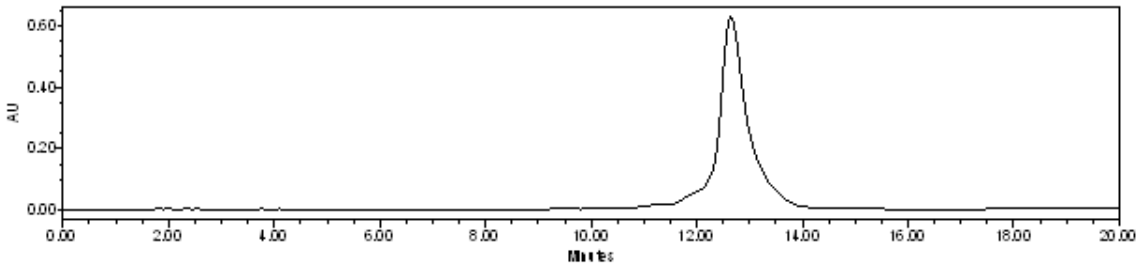
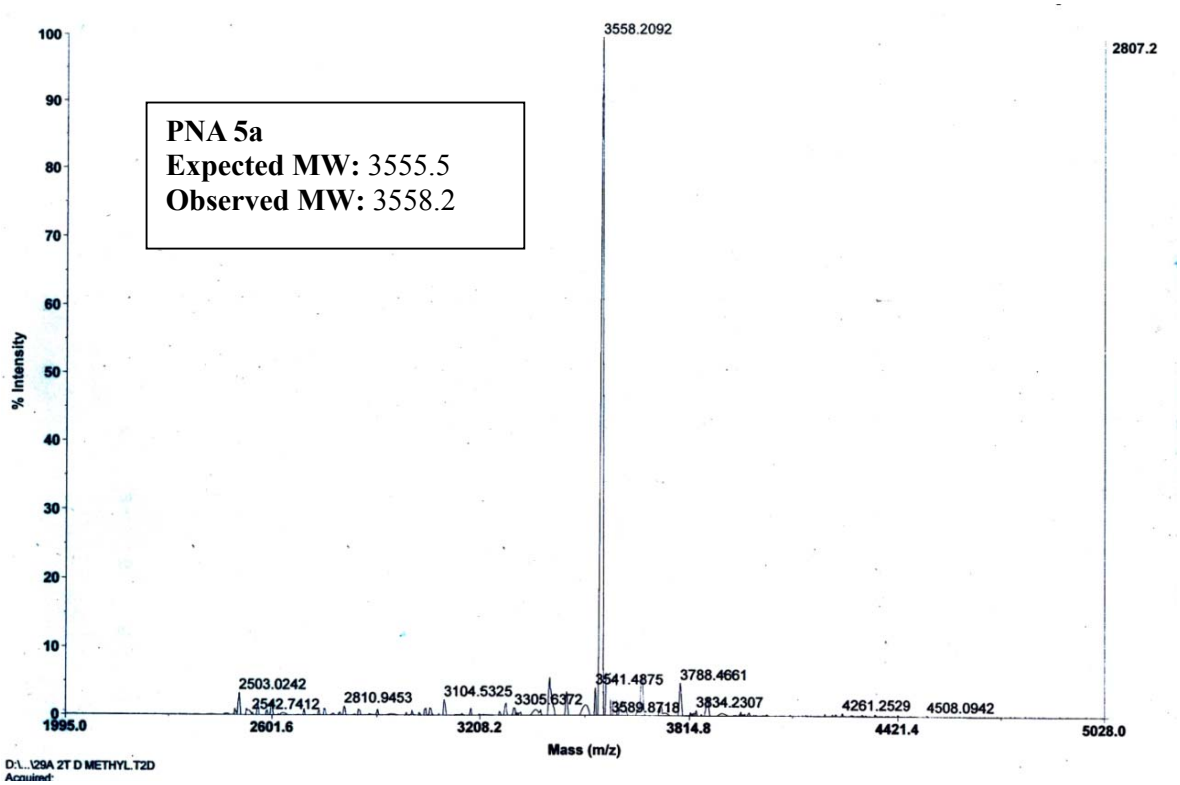
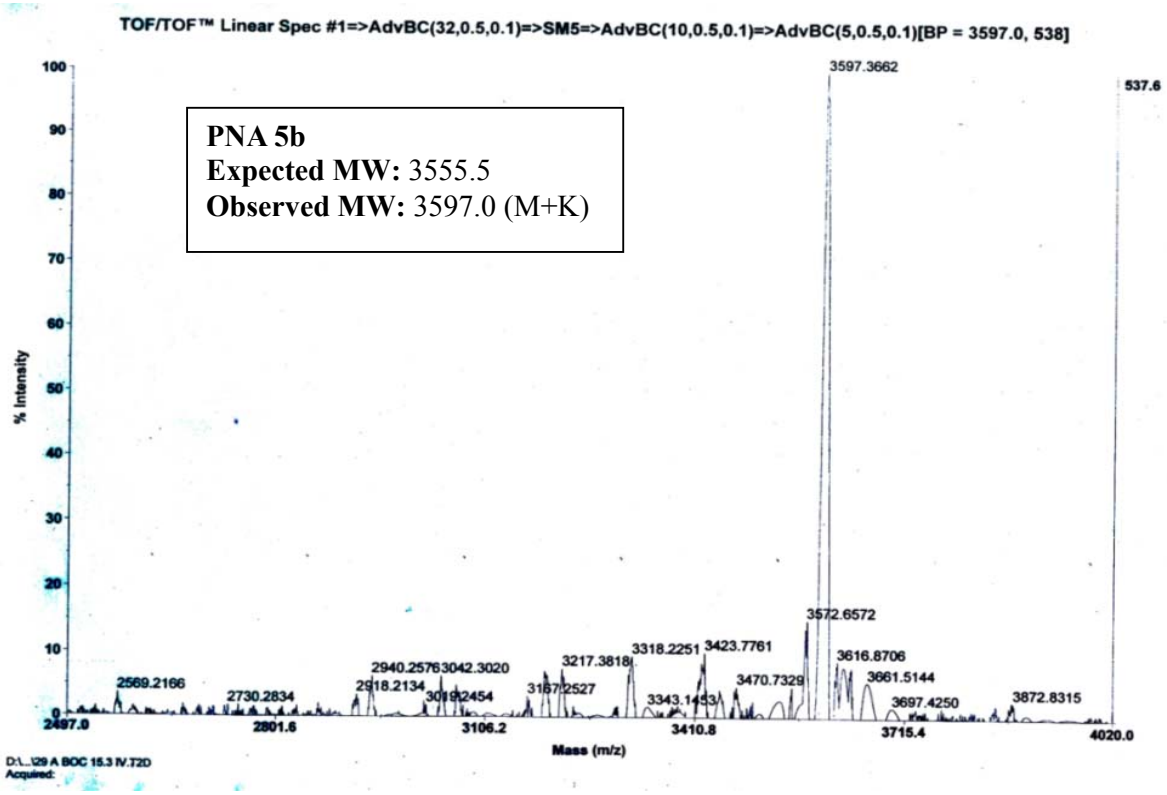


Fig S12: HPLC chromatogram of purified **PNA 5b**







UV melting at 10 mM NaCl concentration:

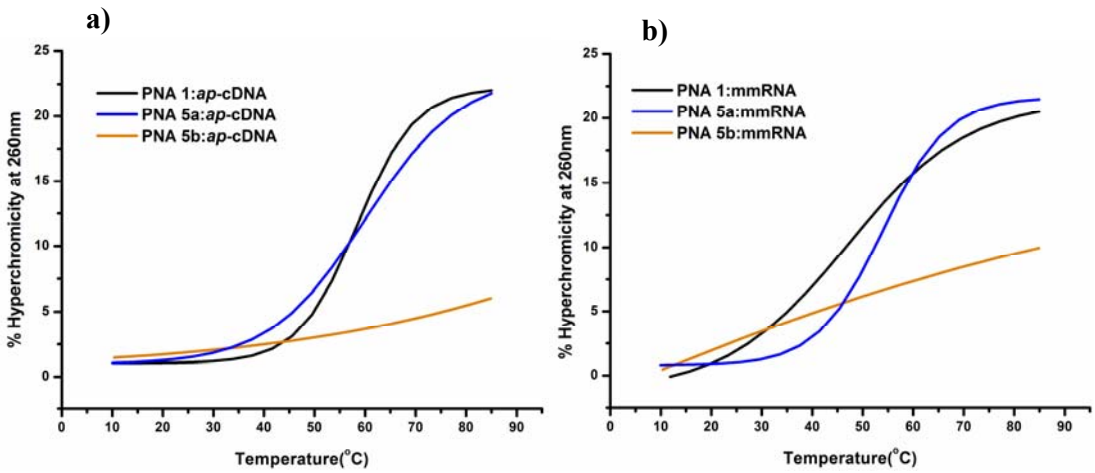


Fig S13: UV melting plots of **PNA 1, 5a, 5b** with a) *ap*-cDNA b) mmRNA at 10 mM NaCl concentration

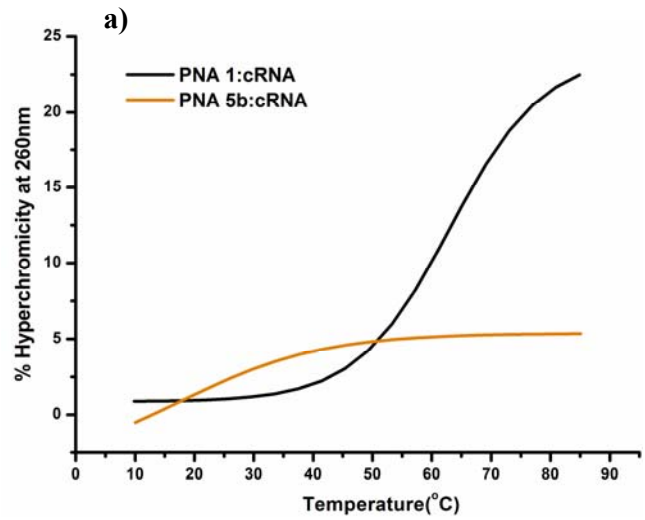


Fig S14: UV melting plots of **PNA 1, 5b** with a) cRNA at 10mM NaCl concentration

***Table S7*:** UV melting at 100 mM NaCl concentration

Seq Code	Sequences	*ap*-cDNA	cRNA
PNA 1	aaccgatttcag-K	53.7	60.9
PNA 5a	aaccga$^{(R,R)}$**T**^{OMe}t$^{(R,R)}$**T**^{OMe}cag-K	54.5	63.9
PNA 5b	aaccga$^{(S,S)}$ **T**^{OMe}t$^{(S,S)}$**T**^{OMe}cag-K	n.t.	n.t.

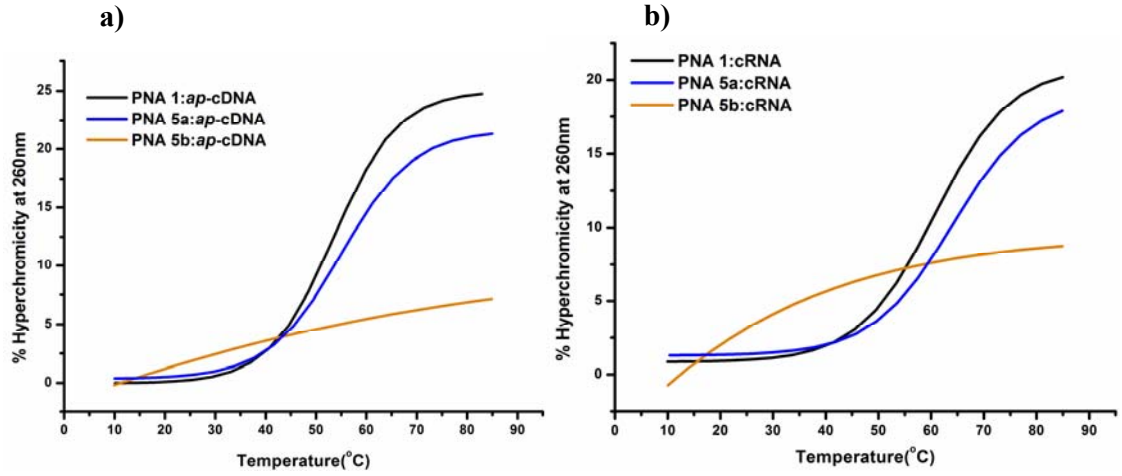


Fig S15: UV melting plots of **PNA 1, 5a, 5b** with a) *ap*-cDNA b) cRNA at 100 mM NaCl concentration

***Table S8*:** HPLC purification of aaccg$\mathbf{A^{OH}}$tttcag-K

Seq Code	Sequences	Retention time
PNA 6a	aaccg$^{(R,R)}\mathbf{A^{OH}}$tttcag-K	10.9
PNA 6b	aaccg$^{(S,S)}\mathbf{A^{OH}}$tttcag-K	10.8

Fig S16: HPLC chromatogram of purified **PNA 6a**

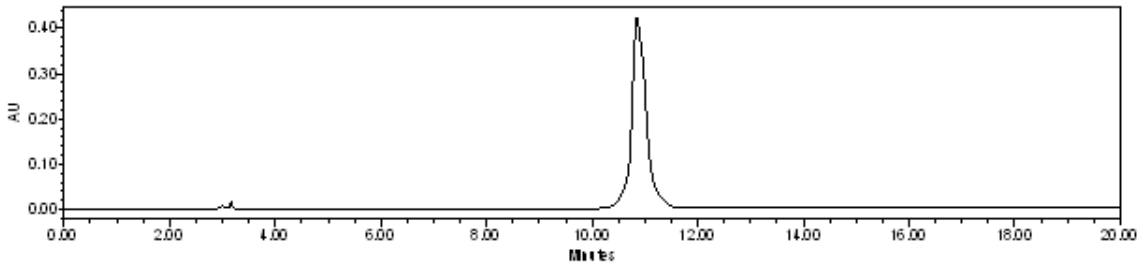
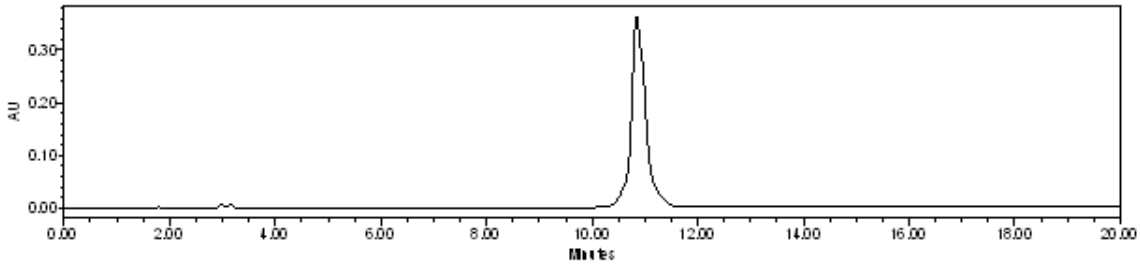
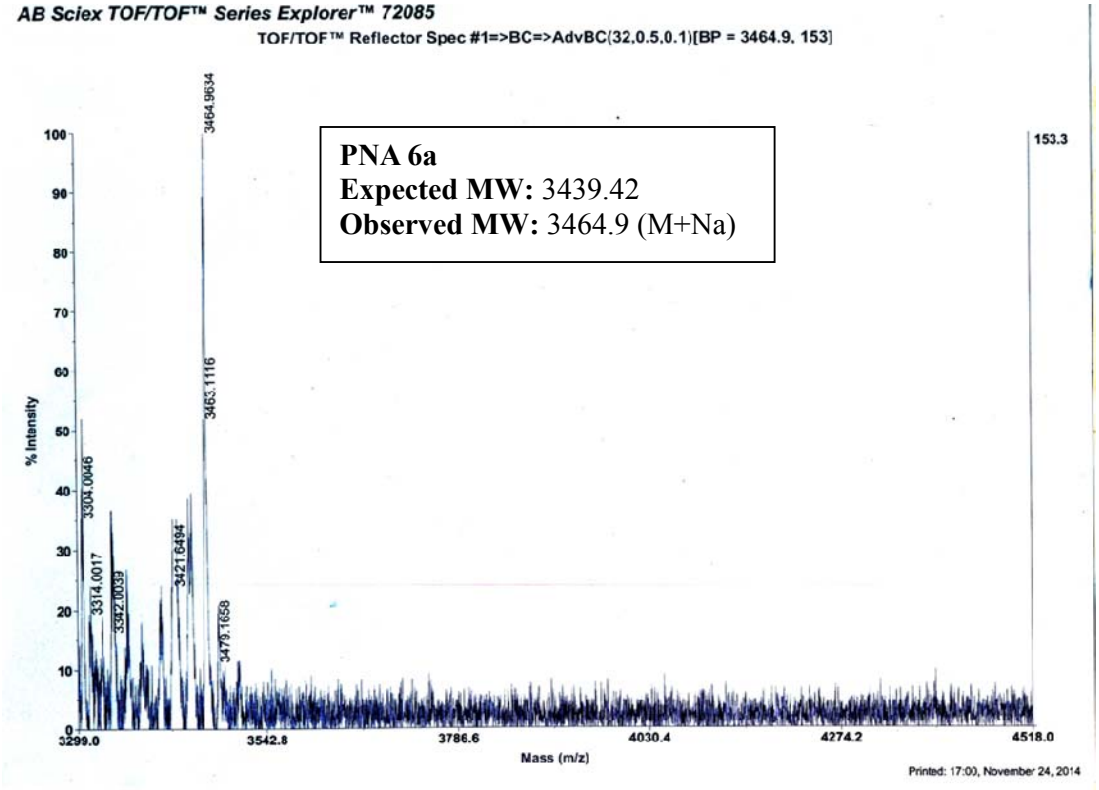
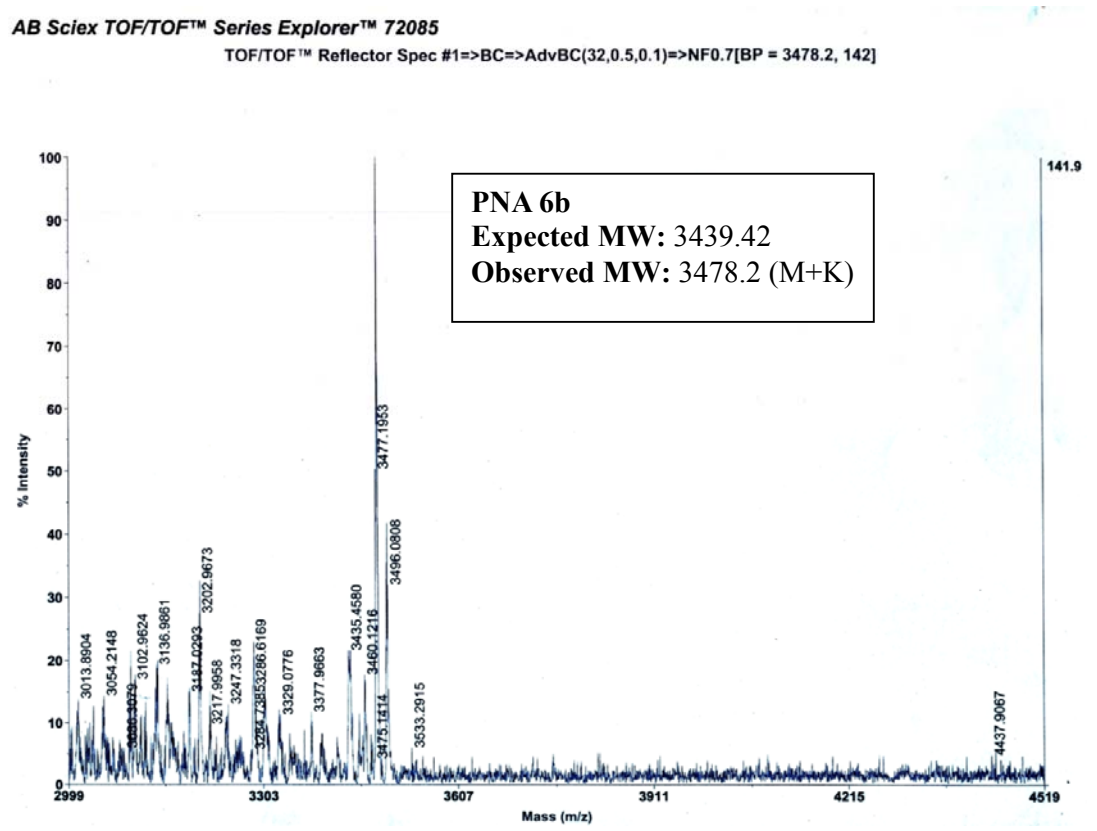


Fig S17: HPLC chromatogram of purified **PNA 6b**




AB Sciex TOF/TOF™ Series Explorer™ 72085
TOF/TOF™ Reflector Spec #1=>BC=>AdvBC(32,0.5,0.1)[BP = 3464.9, 153]
PNA 6a
Expected MW: 3439.42
Observed MW: 3464.9 (M+Na)
% Intensity
Mass (m/z)
Printed: 17:00, November 24, 2014


AB Sciex TOF/TOF™ Series Explorer™ 72085
TOF/TOF™ Reflector Spec #1=>BC=>AdvBC(32,0.5,0.1)=>NF0.7[BP = 3478.2, 142]
PNA 6b
Expected MW: 3439.42
Observed MW: 3478.2 (M+K)
% Intensity
Mass (m/z)

UV melting at 10mM salt concentration:

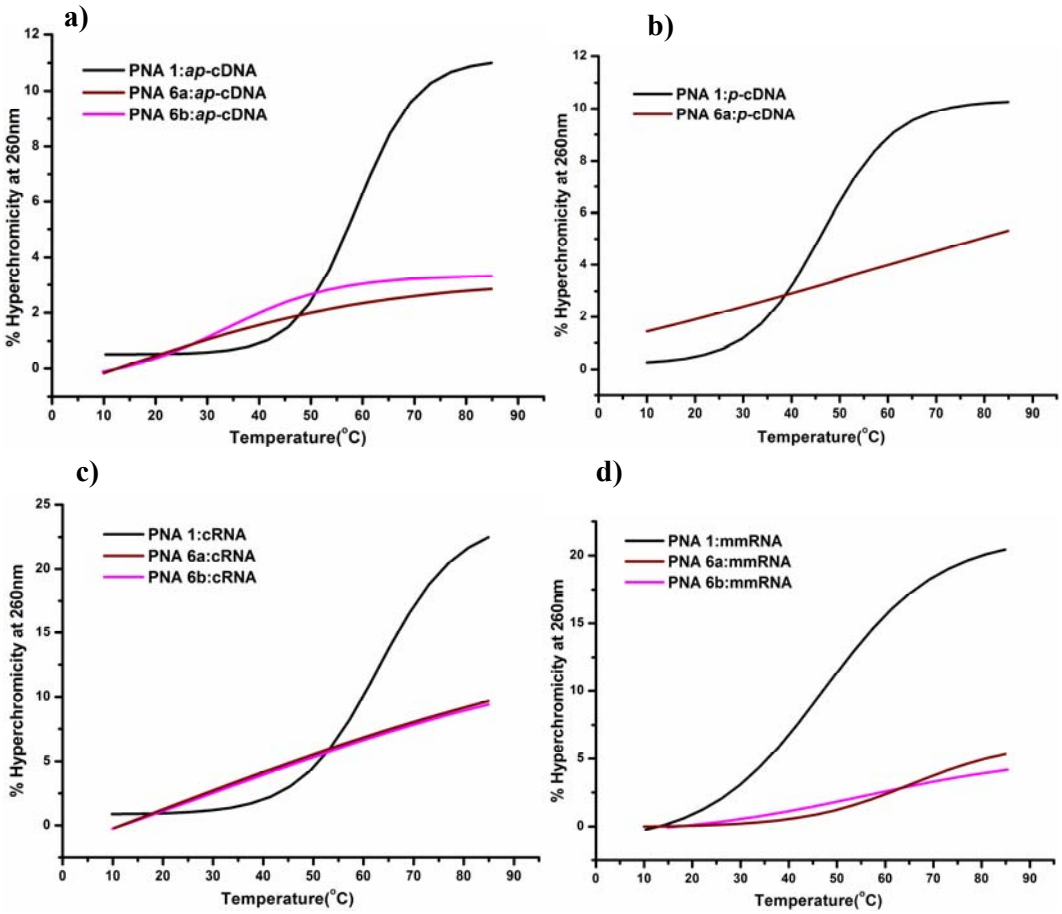


Fig S18: UV melting plots of **PNA 1, 6a, 6b** with a) *ap*-cDNA b) *p*-cDNA c) cRNA d) mmRNA at 10mM NaCl concentration

***Table S9*:** UV melting at 100 mM NaCl concentration

Seq Code	Sequences	*ap*-cDNA	cRNA
PNA 1	aaccgatttcag-K	53.7	60.9
PNA 6a	aaccg$^{(R,R)}$**A**^{OH}tttcag-K	n.t.	n.t.
PNA 6b	aaccg$^{(S,S)}$**A**^{OH}tttcag-K	n.t.	n.t.

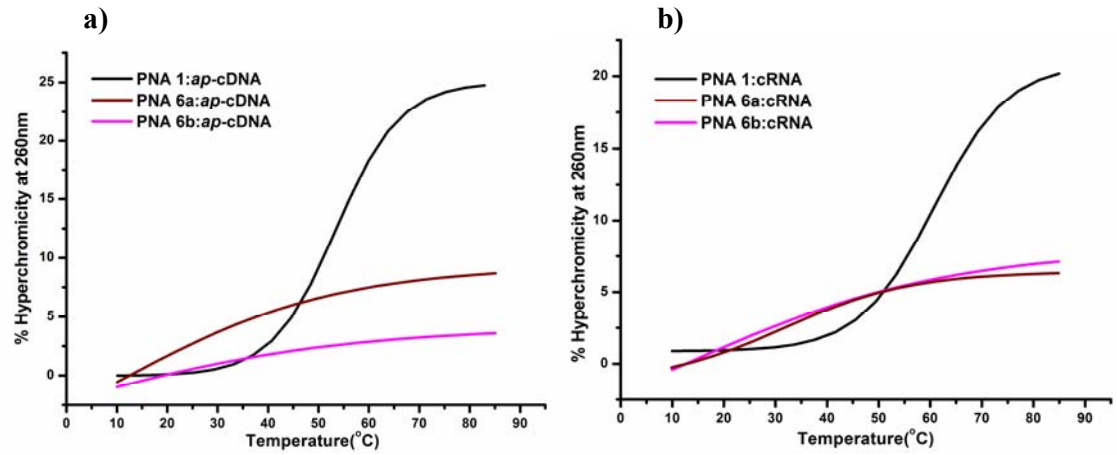


Fig S19: UV melting plots of **PNA 1, 6a, 6b** with a) *ap*-cDNA b) cRNA at 100mM NaCl concentration

***Table S10*:** HPLC purification of aaccg**A**$^{\mathbf{OMe}}$tttcag-K

Seq Code	Sequences	Retention time
PNA 7a	aaccg$^{(R,R)}$**A**$^{\mathbf{OMe}}$tttcag-K	11.4
PNA 7b	aaccg$^{(S,S)}$**A**$^{\mathbf{OMe}}$tttcag-K	11.3

Fig S20: HPLC chromatogram of purified **PNA 7a**

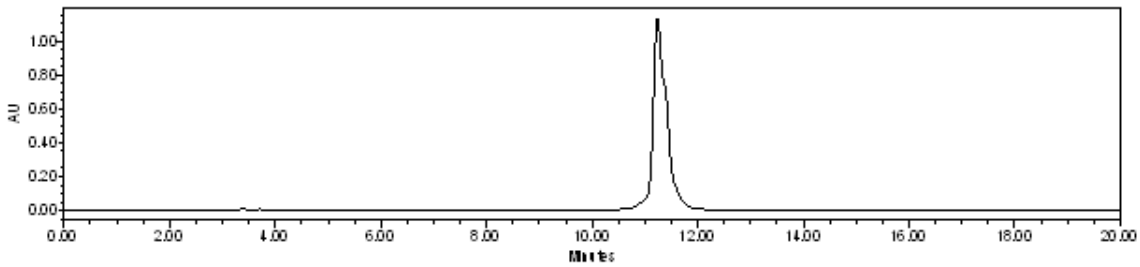
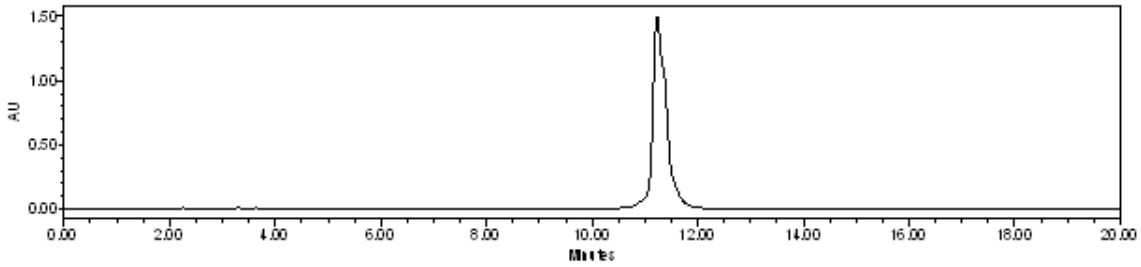
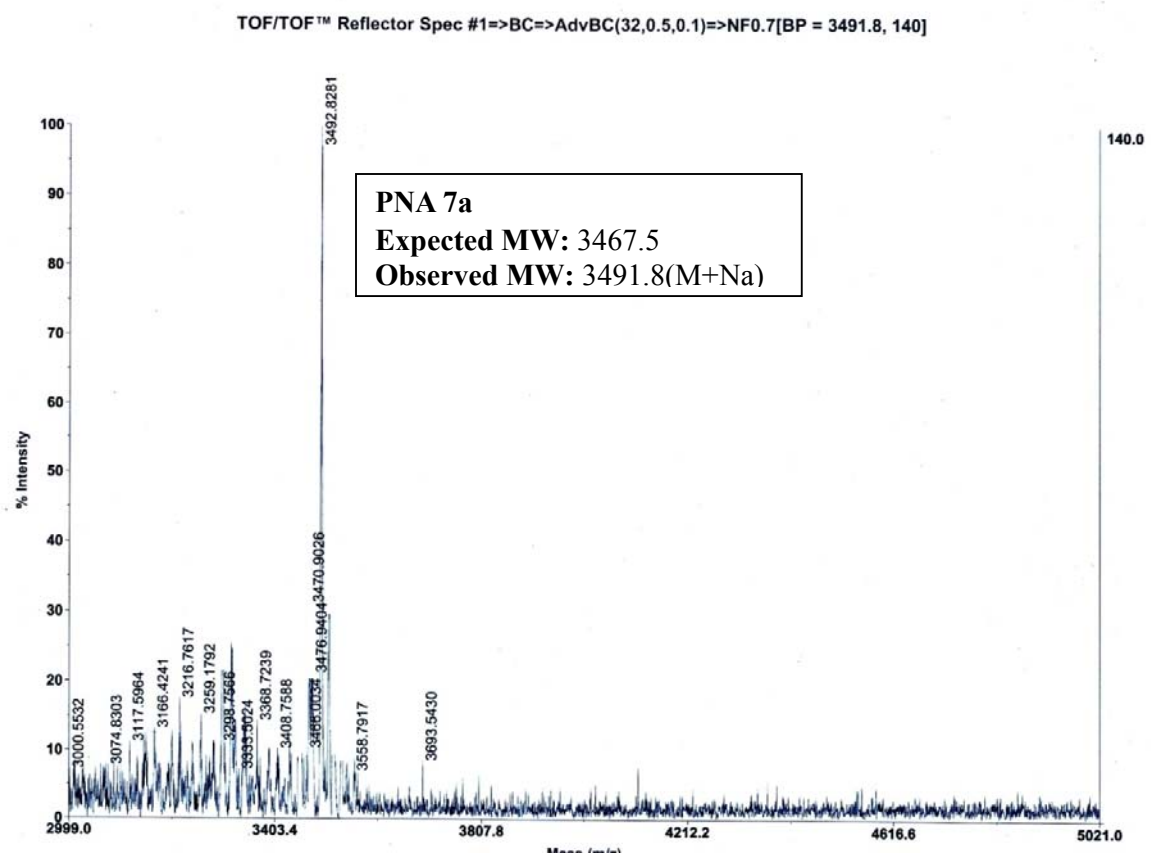
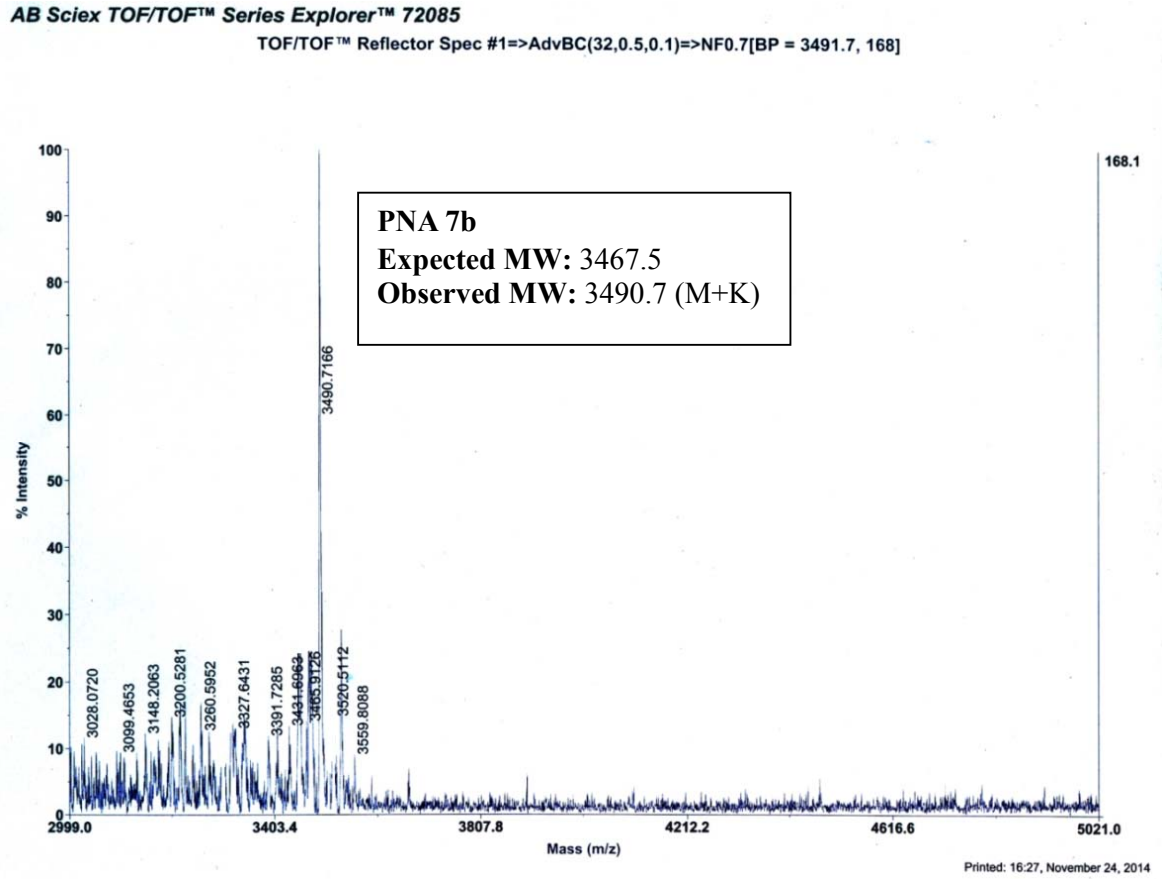


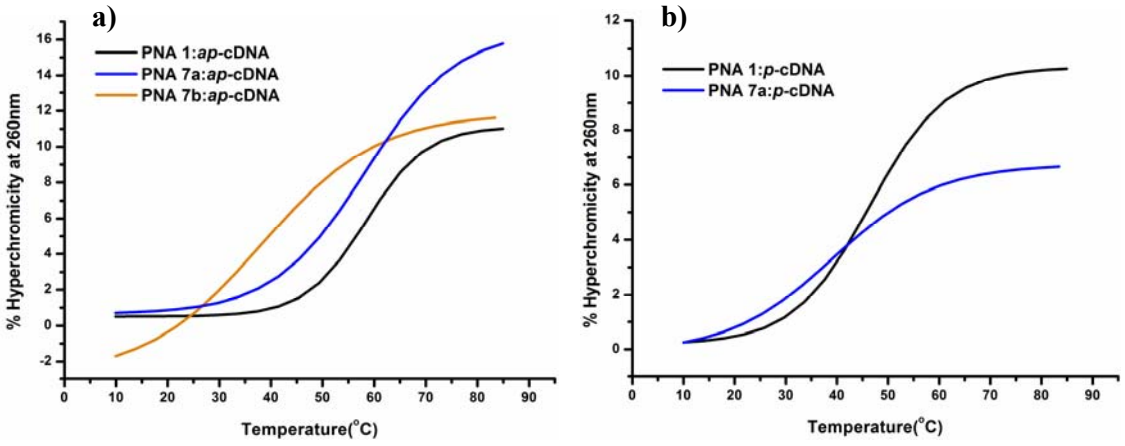
Fig S21: HPLC chromatogram of purified **PNA 7b**







UV melting at 10mM salt concentration:



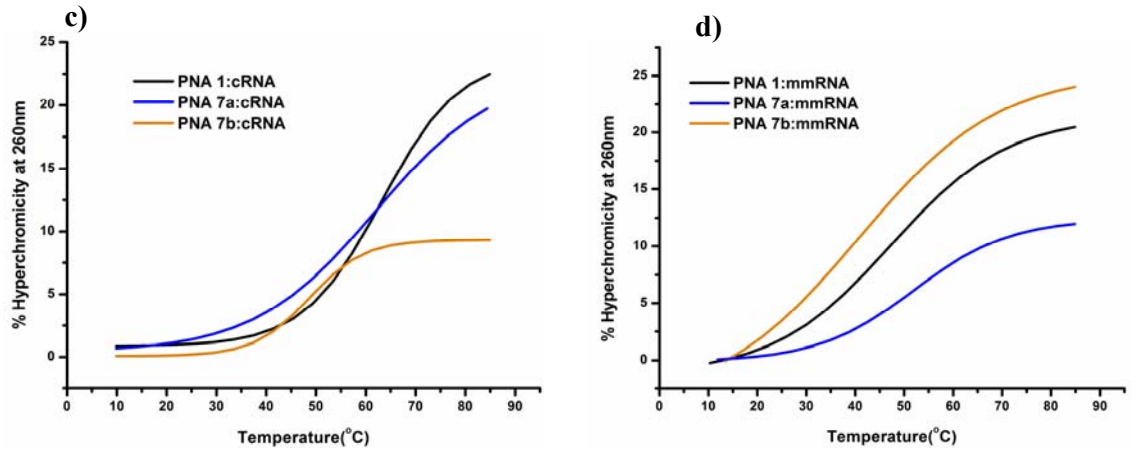


Fig S22: UV melting plots of **PNA 1, 7a, 7b** with a) *ap*-cDNA b) *p*-cDNA c) cRNA d) mmRNA at 10mM NaCl concentration

***Table S11*:** UV meltings at 100mM NaCl concentration

Seq Code	Sequences	*ap*-cDNA	cRNA
PNA 1	aaccgatttcag-K	53.7	60.9
PNA 7a	aaccg$^{(R,R)}$A^{OMe}tttcag-K	50.7	57.3
PNA 7b	aaccg$^{(S,S)}$A^{OMe}tttcag-K	28.7	41.9

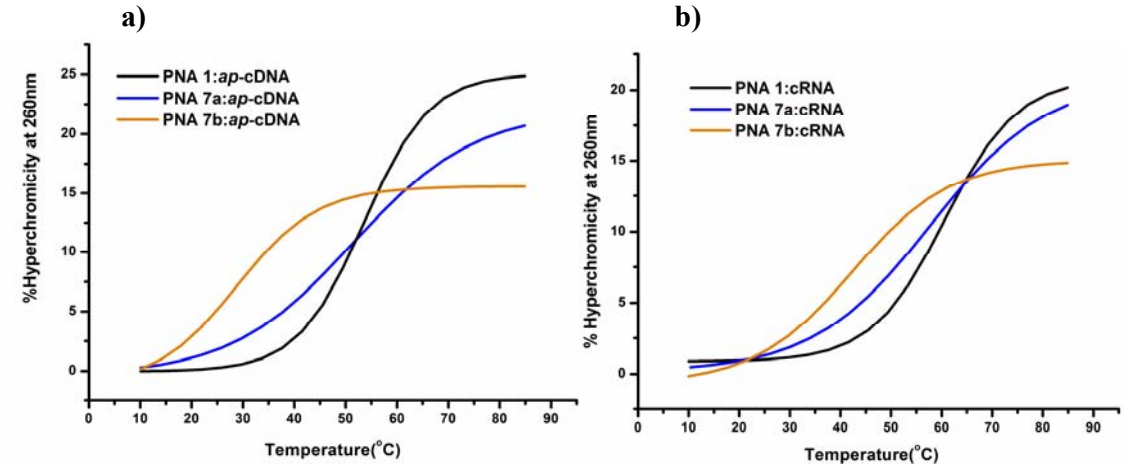


Fig S23: UV melting plots of **PNA 1, 7a, 7b** with a) *ap*-cDNA b) cRNA at 100mM NaCl concentration

***Table S12*:** HPLC purification of $\mathbf{A^{OH}A^{OH}}$ccgatttcag-K

Seq Code	Sequences	Retention Time
PNA 8a	$^{(R,R)}\mathbf{A^{OH}}\ ^{(R,R)}\mathbf{A^{OH}}$ CCGATTTCAG-K	10.9
PNA 8b	$^{(S,S)}\mathbf{A^{OH}}\ ^{(S,S)}\mathbf{A^{OH}}$CCGATTTCAG-K	10.9

Fig S24: HPLC chromatogram of purified **PNA 8a**

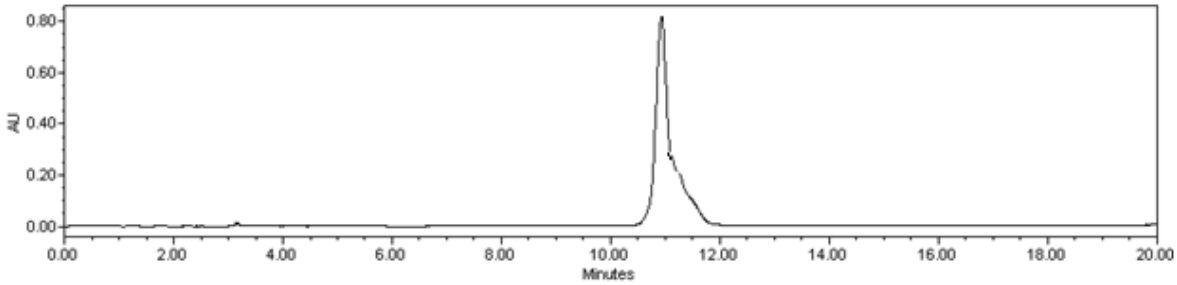
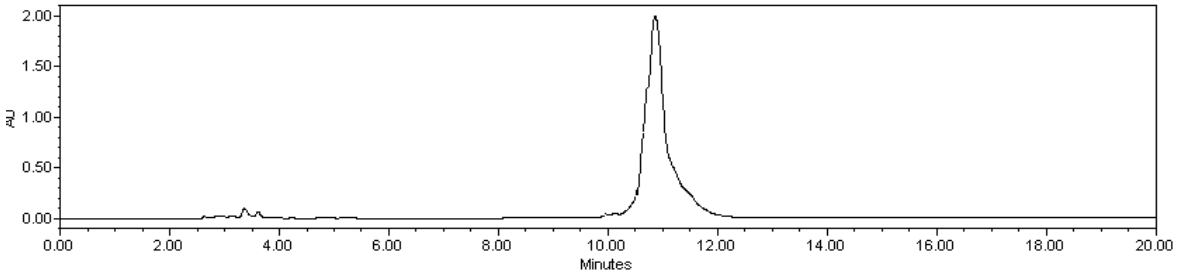
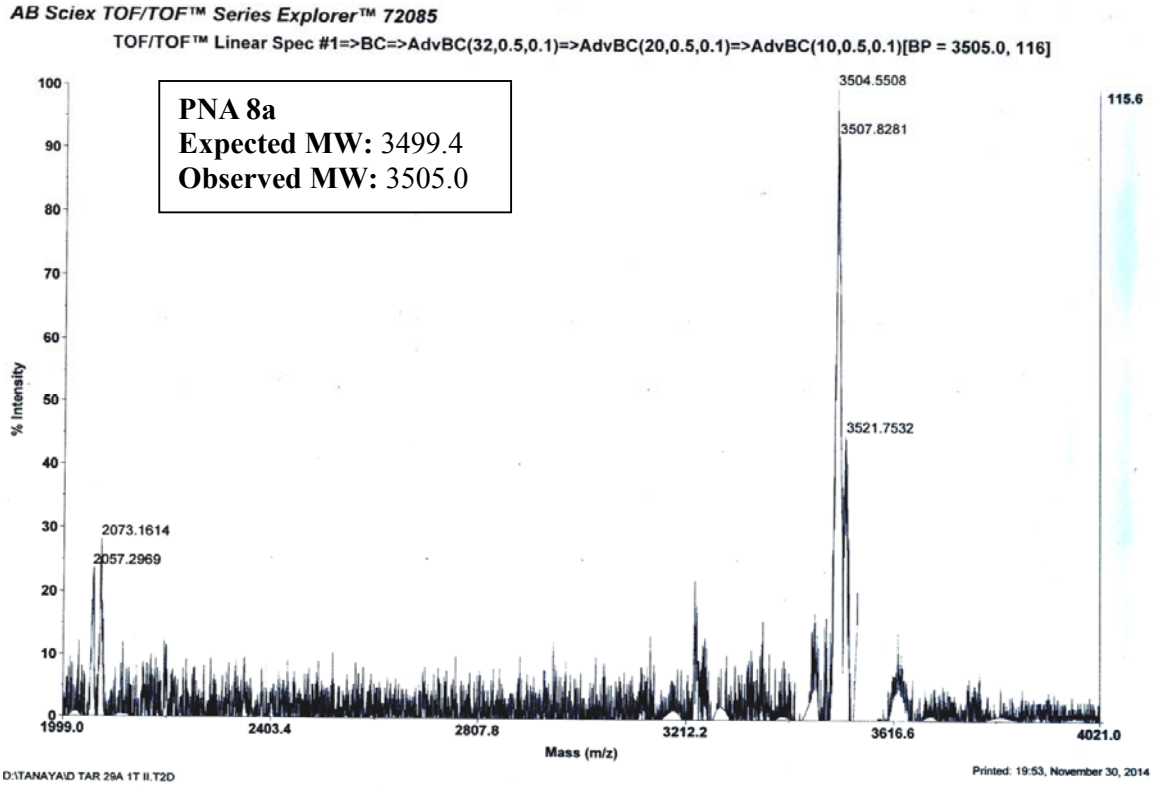
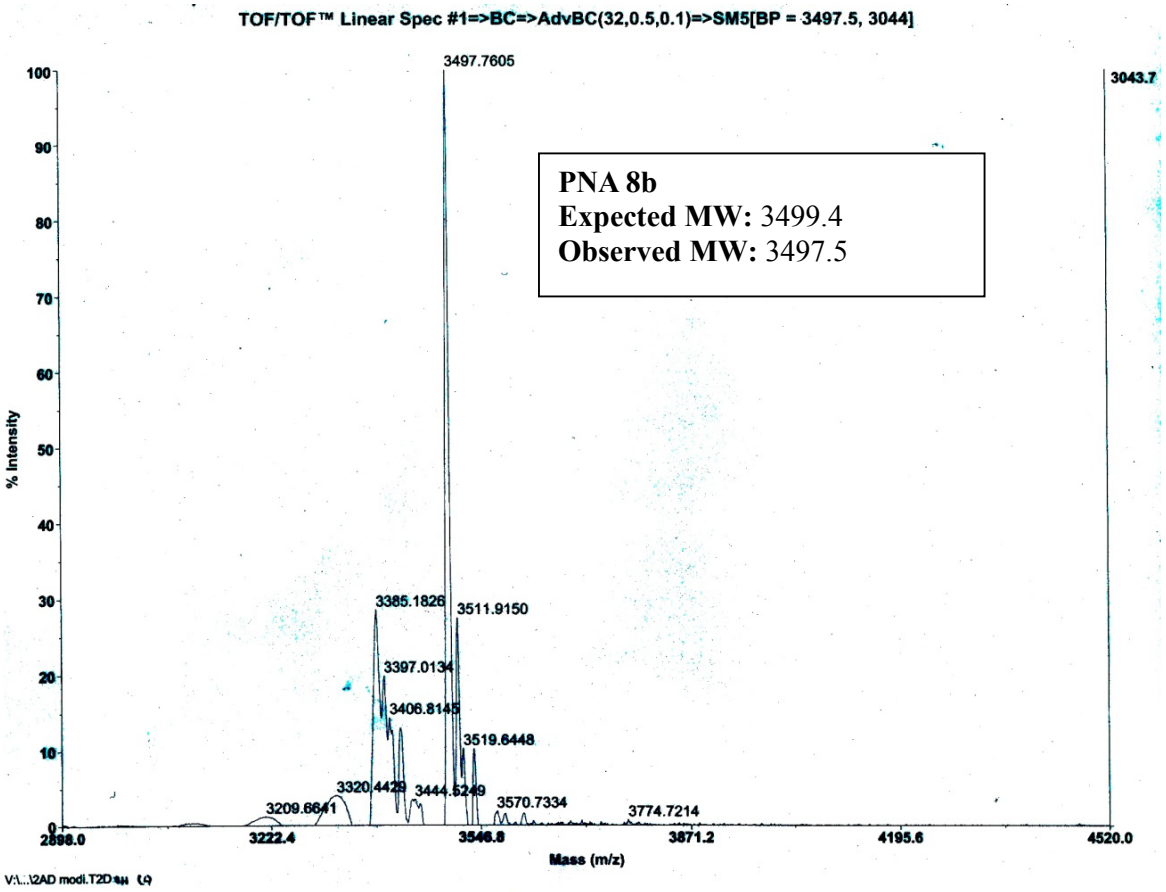


Fig S25: HPLC chromatogram of purified **PNA 8b**




AB Sciex TOF/TOF™ Series Explorer™ 72085
TOF/TOF™ Linear Spec #1=>BC=>AdvBC(32,0.5,0.1)=>AdvBC(20,0.5,0.1)=>AdvBC(10,0.5,0.1)[BP = 3505.0, 116]
PNA 8a
Expected MW: 3499.4
Observed MW: 3505.0
3504.5508
3507.8281
115.6
3521.7532
2073.1614
2057.2969
% Intensity
Mass (m/z)
1999.0
2403.4
2807.8
3212.2
3616.6
4021.0
D:\TANAYA\D TAR 29A 1T II.T2D
Printed: 19:53, November 30, 2014


TOF/TOF™ Linear Spec #1=>BC=>AdvBC(32,0.5,0.1)=>SM5[BP = 3497.5, 3044]
PNA 8b
Expected MW: 3499.4
Observed MW: 3497.5
3497.7605
3043.7
3385.1826
3511.9150
3397.0134
3406.8145
3519.6448
3320.4429
3444.5249
3209.6641
3570.7334
3774.7214
% Intensity
Mass (m/z)
3222.4
3546.8
3871.2
4195.6
4520.0

UV melting at 10mM salt concentration:

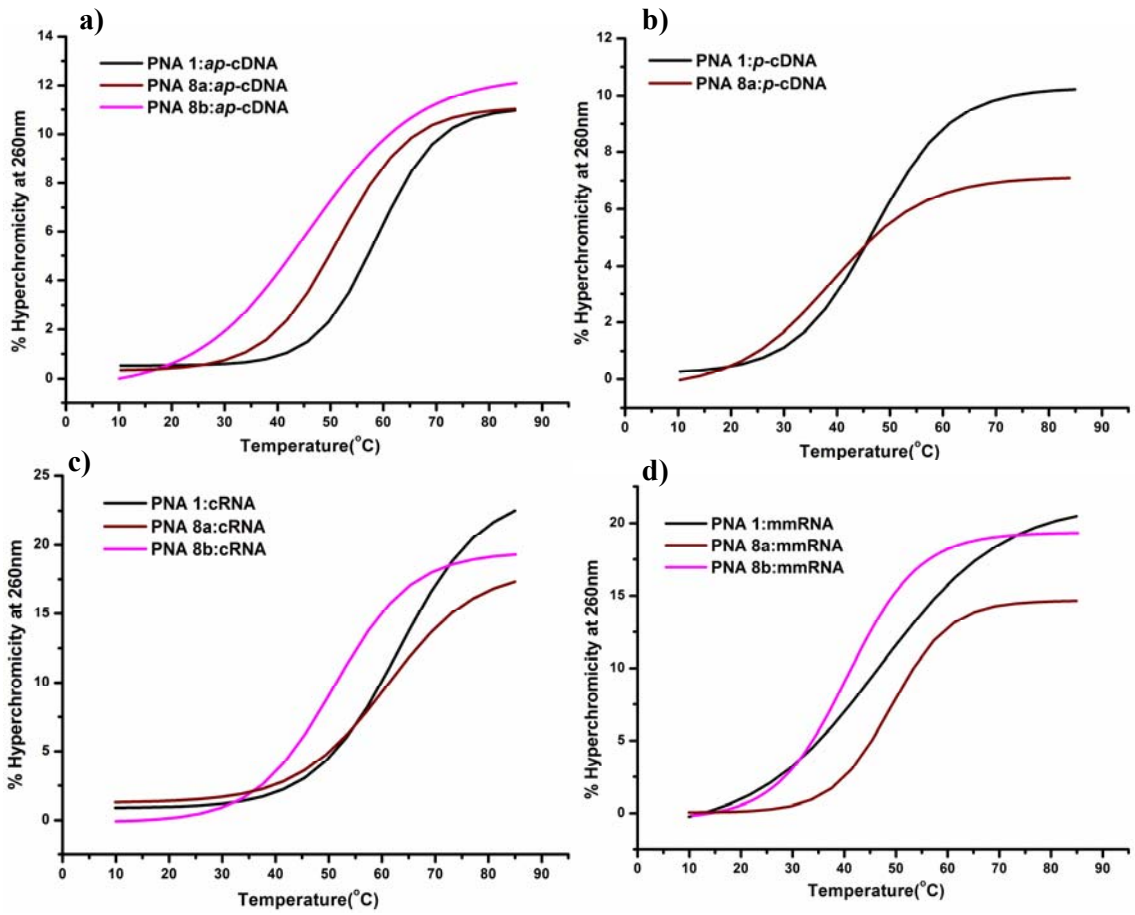


Fig S26: UV melting plots of **PNA 1, 8a, 8b** with a) *ap*-cDNA b) *p*-cDNA c) cRNA d) mmRNA at 10mM NaCl concentration

***Table S13*:** UV meltings at 100mM NaCl concentration

Seq Code	Sequences	*ap*-cDNA	cRNA
PNA 1	aaccgatttcag-K	53.7	60.9
PNA 8a	$^{(R,R)}$**A**^{OH} $^{(R,R)}$**A**^{OH} CCGATTTCAG-K	49.7	58.5
PNA 8b	$^{(S,S)}$**A**^{OH} $^{(S,S)}$**A**^{OH}CCGATTTCAG-K	42.1	46.6

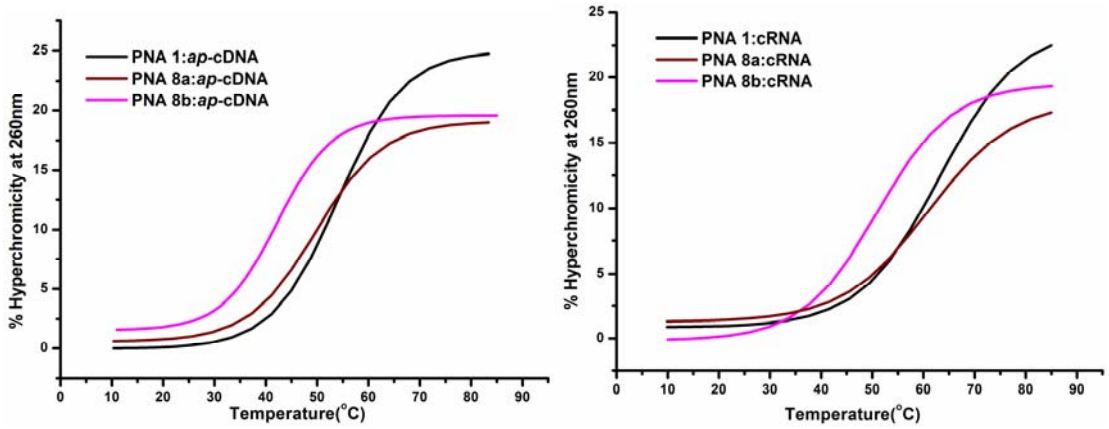


Fig S27: UV melting plots of **PNA 1, 8a, 8b** with a) *ap*-cDNA b) cRNA at 100mM NaCl concentration

***Table S14*:** HPLC purification of $\mathbf{A^{OMe}A^{OMe}}$ccgatttcag-K

Seq Code	Sequences	Retention Time
PNA 9a	$^{(R,R)}\mathbf{A^{OMe}}\,^{(R,R)}\mathbf{A^{OMe}}$ ccgatttcag-K	11.6
PNA 9b	$^{(S,S)}\mathbf{A^{OMe}}\,^{(S,S)}\mathbf{A^{OMe}}$ccgatttcag-K	11.6

Fig S28: HPLC chromatogram of purified **PNA 9a**

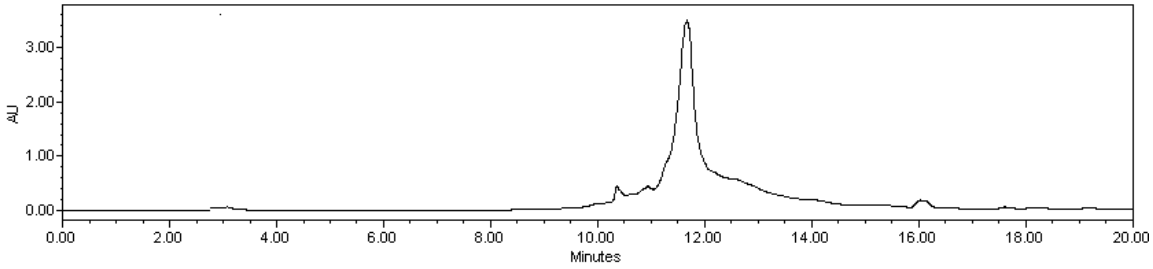
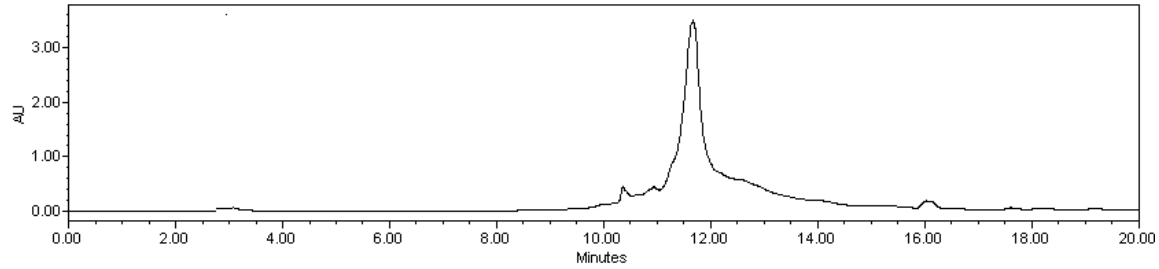
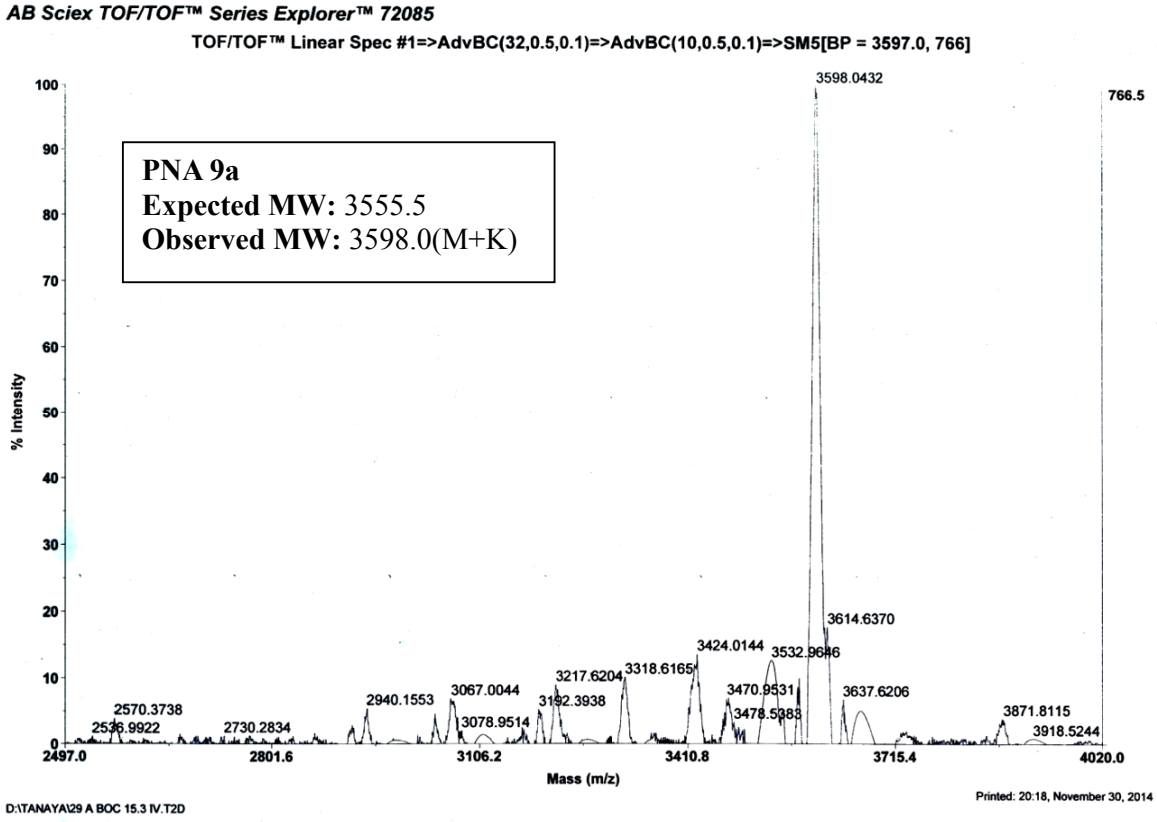
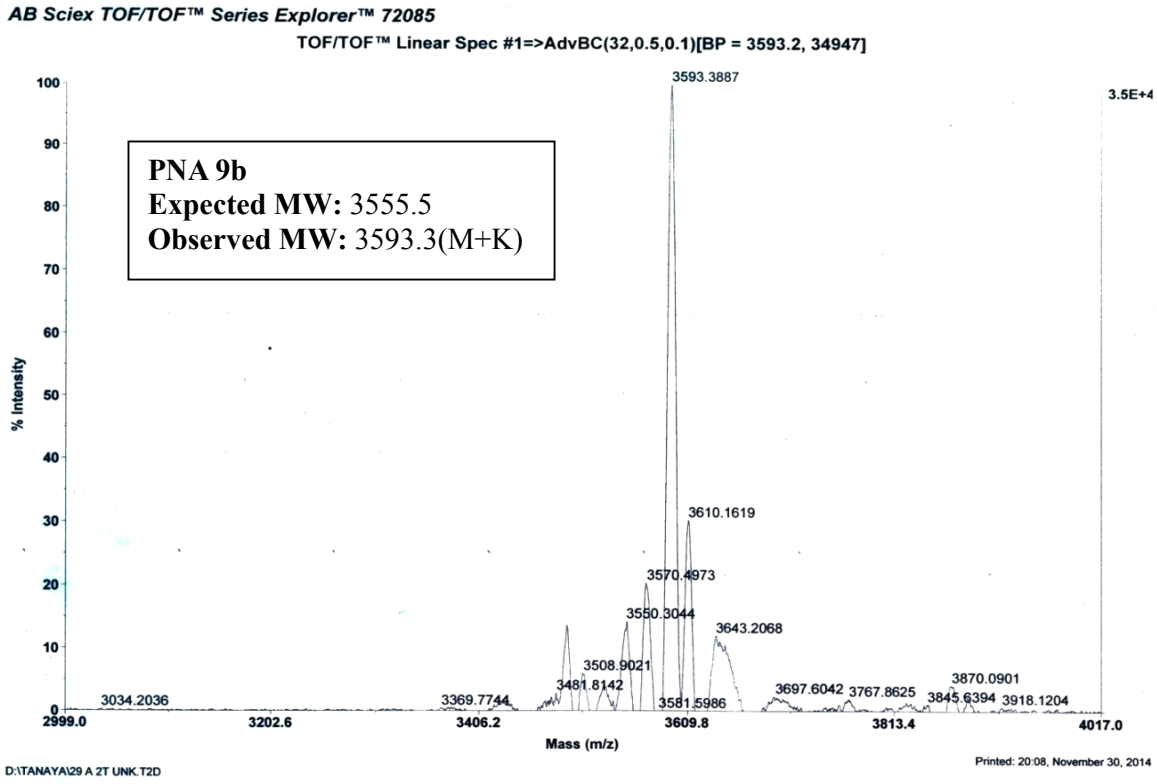
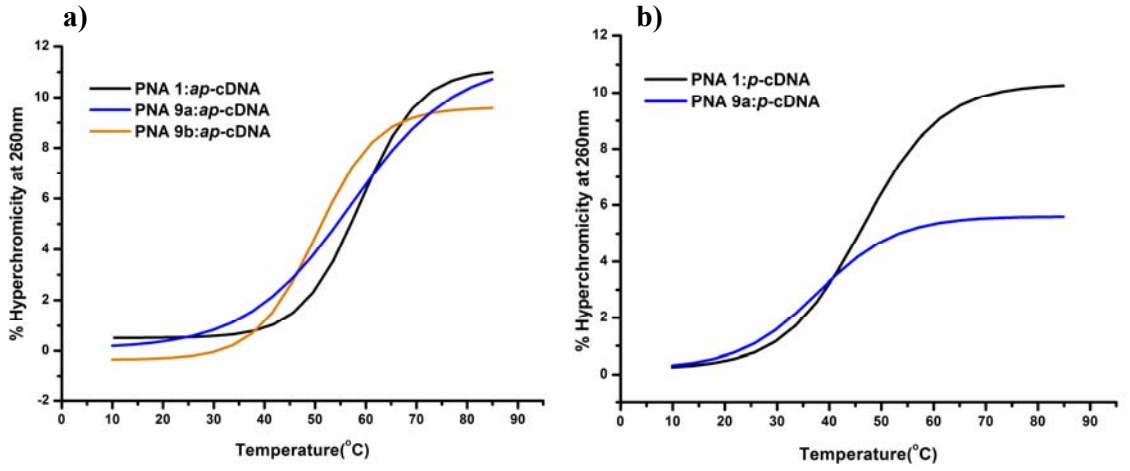


Fig S29: HPLC chromatogram of purified **PNA 9b**






AB Sciex TOF/TOF™ Series Explorer™ 72085
TOF/TOF™ Linear Spec #1=>AdvBC(32,0.5,0.1)[BP = 3593.2, 34947]
PNA 9b
Expected MW: 3555.5
Observed MW: 3593.3(M+K)
% Intensity
Mass (m/z)
3593.3887
3610.1619
3570.4973
3550.3044
3643.2068
3508.9021
3481.8142
3034.2036
3369.7744
3581.5986
3697.6042
3767.8625
3870.0901
3845.6394
3918.1204
D:\TANAYA\29 A 2T UNK.T2D
Printed: 20:08, November 30, 2014


a)
PNA 1:ap-cDNA
PNA 9a:ap-cDNA
PNA 9b:ap-cDNA
% Hyperchromicity at 260nm
Temperature(°C)
b)
PNA 1:p-cDNA
PNA 9a:p-cDNA
% Hyperchromicity at 260nm
Temperature(°C)

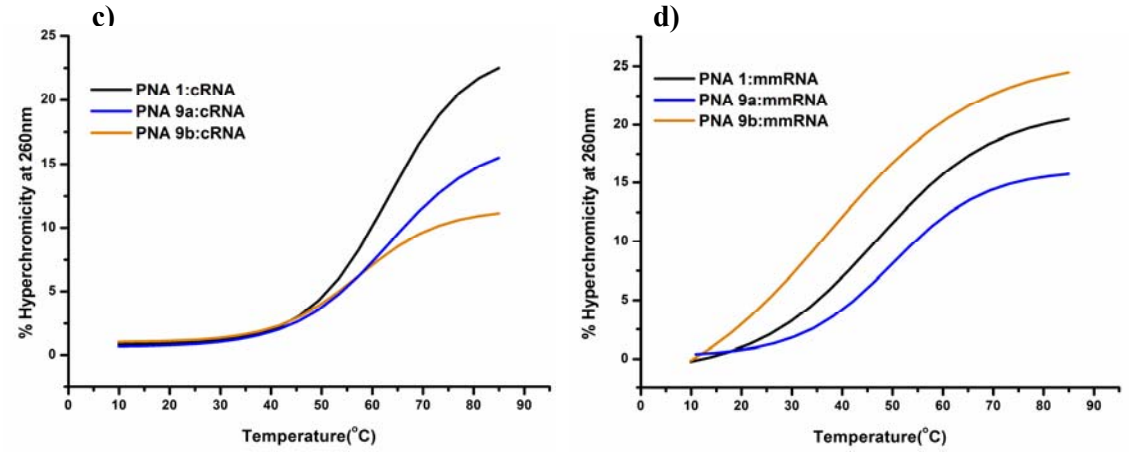


Fig S30: UV melting plots of **PNA 1, 9a, 9b** with a) *ap*-cDNA b) *p*-cDNA c) cRNA d) mmRNA at 10mM NaCl concentration

***Table S15*:** UV meltings at 100mM NaCl concentration

Seq Code	Sequences	*ap*-cDNA	cRNA
PNA 1	aaccgatttcag-K	53.7	60.9
PNA 9a	$^{(R,R)}\mathbf{A}^{\mathbf{OMe}}\,^{(R,R)}\mathbf{A}^{\mathbf{OMe}}$ ccgatttcag-K	52.1	61
PNA 9b	$^{(S,S)}\mathbf{A}^{\mathbf{OMe}}\,^{(S,S)}\mathbf{A}^{\mathbf{OMe}}$ccgatttcag-K	47	50.4

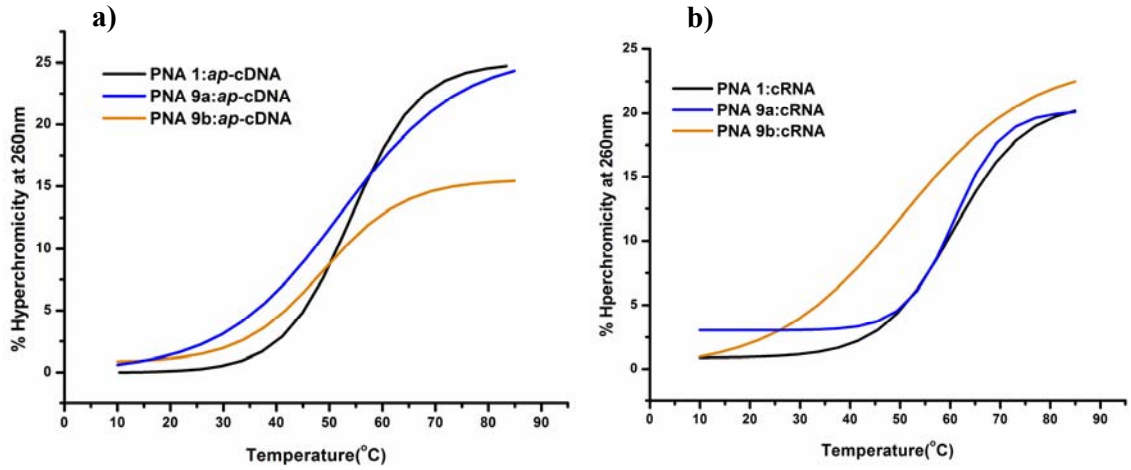
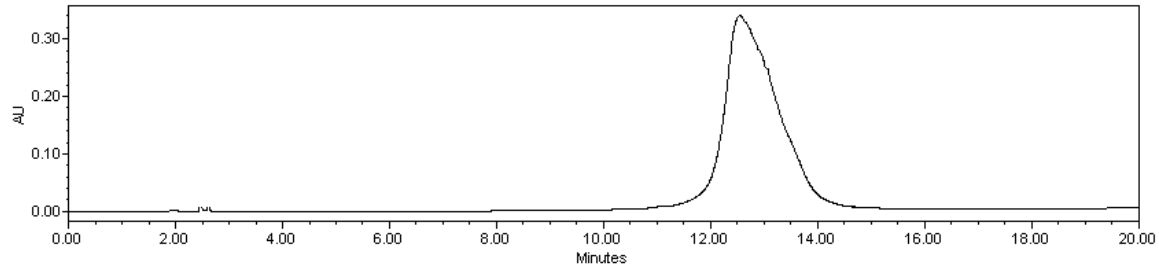


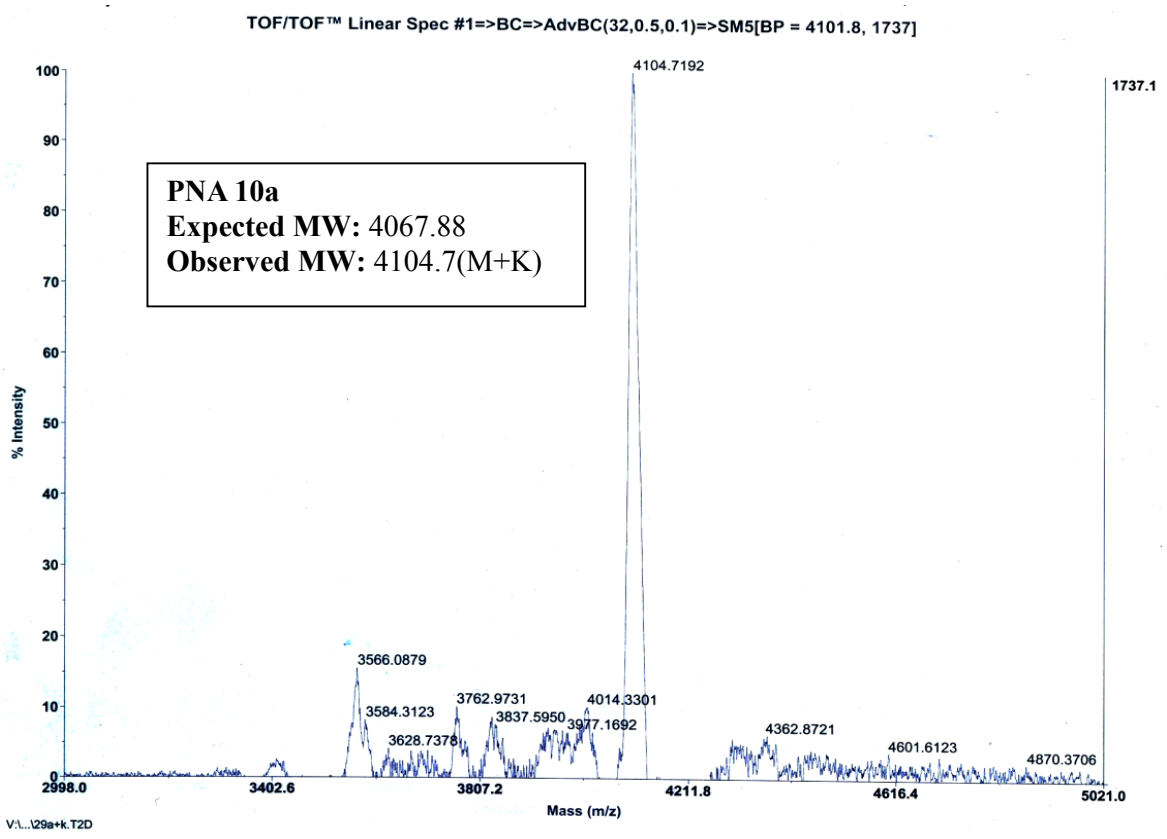
Fig S31: UV melting plots of PNA **1, 9a, 9b** with a) *ap*-cDNA b) cRNA at 100mM NaCl concentration

***Table S16*:** HPLC purification of K_4- accga$^{(R,R)}$**T**^{OMe}t$^{(R,R)}$**T**^{OMe}cag-K

Seq Code	Sequences	Retention Time
PNA 10a	K_4- accga$^{(R,R)}$**T**^{OMe}t$^{(R,R)}$**T**^{OMe}cag-K	12.5

Fig S32: HPLC chromatogram of purified **PNA 10a**





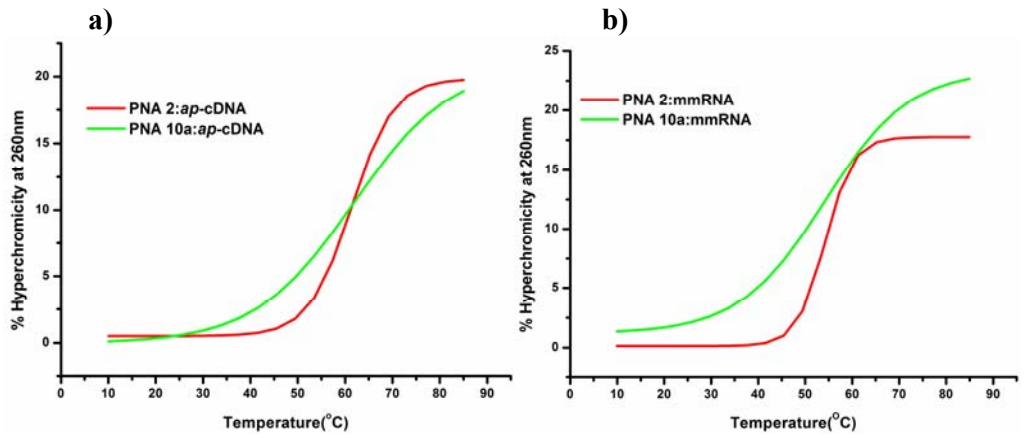


Fig S33: UV melting plots of PNA **1, 10a** with a) *ap*-cDNA b) mmRNA at 10mM NaCl concentration

***Table S17*:** UV meltings at 100mM NaCl concentration

Seq Code	Sequences	*ap*-cDNA	cRNA
PNA 2	K_4- aaccgatttcag-K	58.5	62.7
PNA 10a	K_4- accga$^{(R,R)}$**T**^{OMe}t$^{(R,R)}$**T**^{OMe}cag-K	55.1	63.1

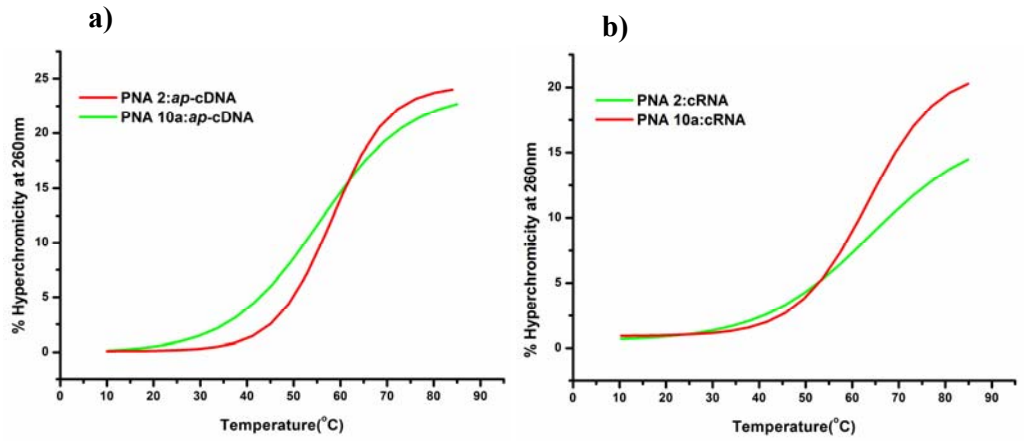


Fig S34: UV melting plots of PNA **1, 10a** with a) *ap*-cDNA b) cRNA at 100mM NaCl concentration

Cellular uptake studies:-

Carboxyfluorescein was attached to the synthesized PNA oligomers PNA 1, 2, 5a, 10a for studying the internalization of the oligomers into cells. To synthesize carboxyfluorescein attached PNA oligomers, couplings were carried out using PNA 1, 2, 5a, 10a on MBHA resin in presence of ten equivalents of 5(6)-carboxyfluorescein, HOBt, DIPCDI (Diisopropyl carbodiimide) in DMF overnight. The oligomers were cleaved from solid phase in presence of TFA, TFMSA employing the regular protocol. The crude peptide was purified by semipreparative C18 column.

***Table S18*:** HPLC purification of Carboxyfluorescein tagged PNA 1, 2, 5a, 10a and their MALDI-ToF analysis

Seq code	Sequences	R.t.	Calc. mass	Obsvd. mass
PNA 1-CF	CF-aaccgatttcag-K	14.6	3737.4	3736.0
PNA 2-CF	CF-K_4-aaccgatttcag-K	14.9	4249.8	4248.4
PNA 5a-CF	CF-aaccga$^{(R,R)}$ $\mathbf{T}^{\mathbf{OMe}}$ t$^{(R,R)}$ $\mathbf{T}^{\mathbf{OMe}}$ cag-K	15.2	3915.7	3918.3
PNA 10a-CF	CF-K_4- aaccga$^{(R,R)}$ $\mathbf{T}^{\mathbf{OMe}}$ t$^{(R,R)}$ $\mathbf{T}^{\mathbf{OMe}}$ cag-K	14.7	4428.4	4431.95

Fig S35: HPLC chromatogram of purified **PNA 1-CF**

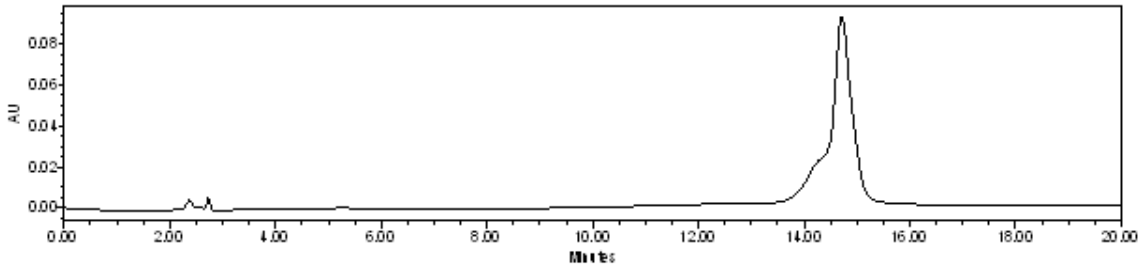


Fig S36: HPLC chromatogram of purified **PNA 2-CF**

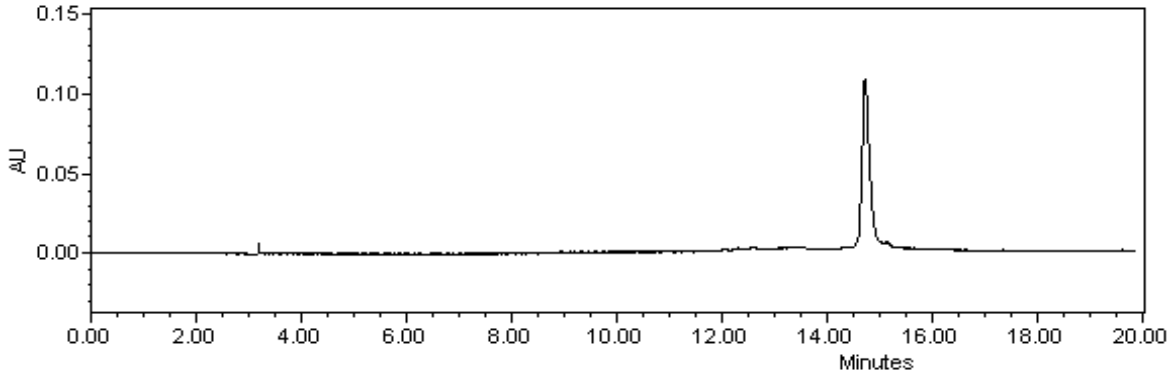


Fig S37: HPLC chromatogram of purified **PNA 5a-CF**

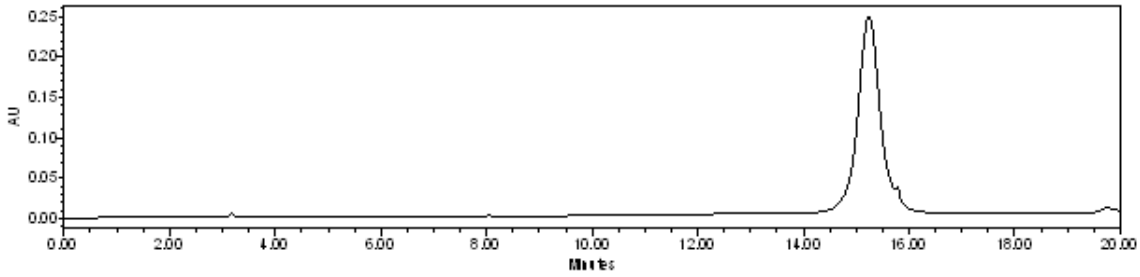
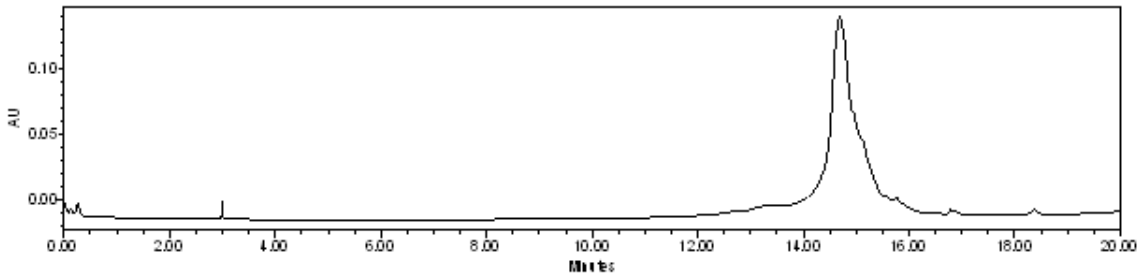
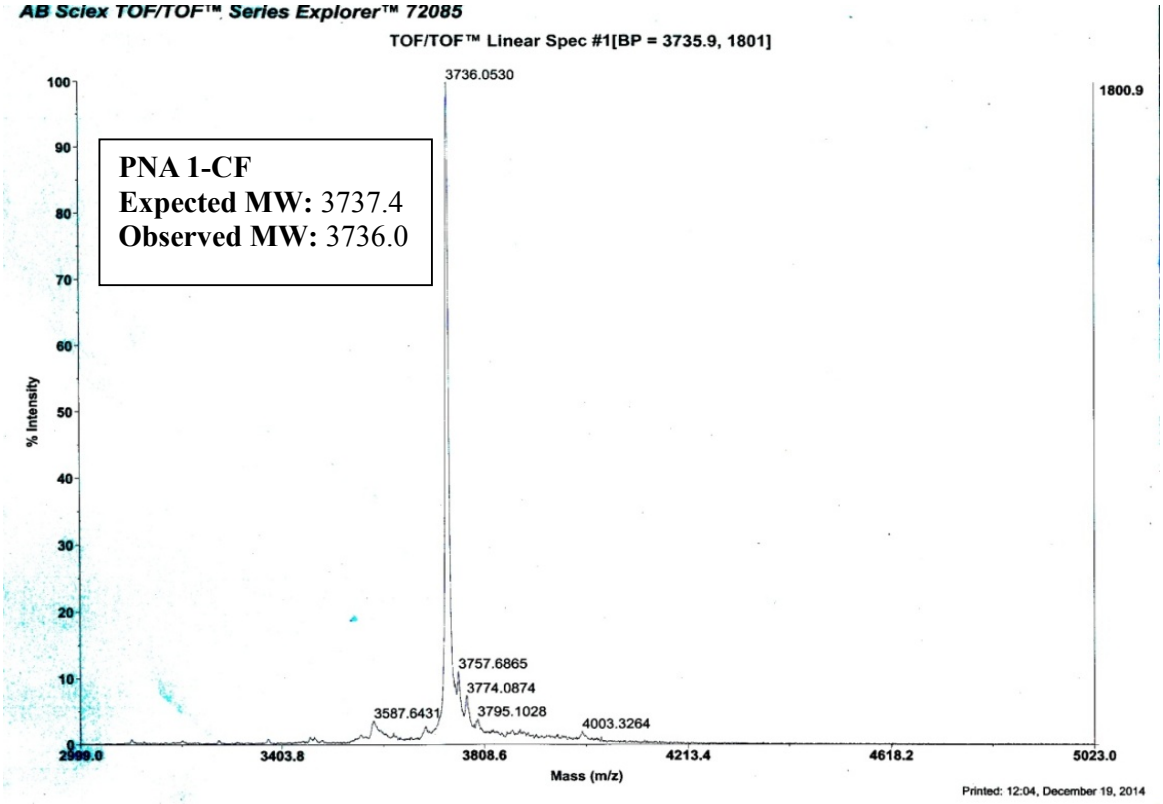
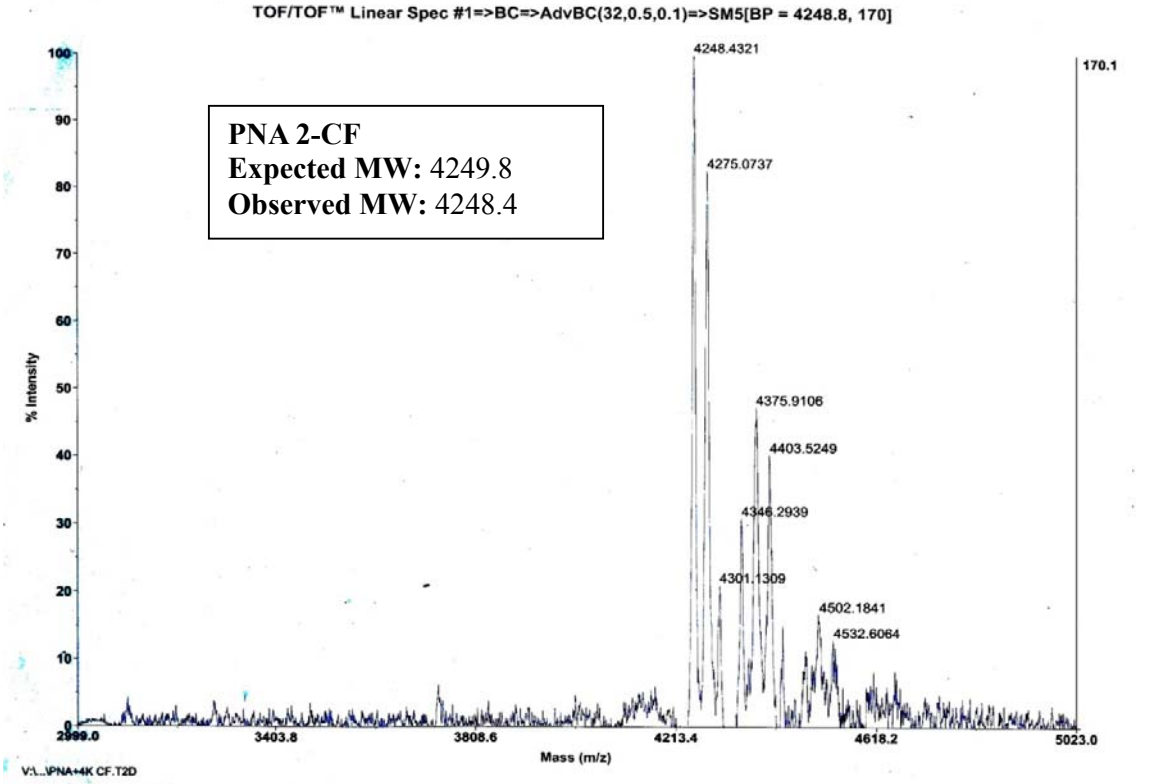
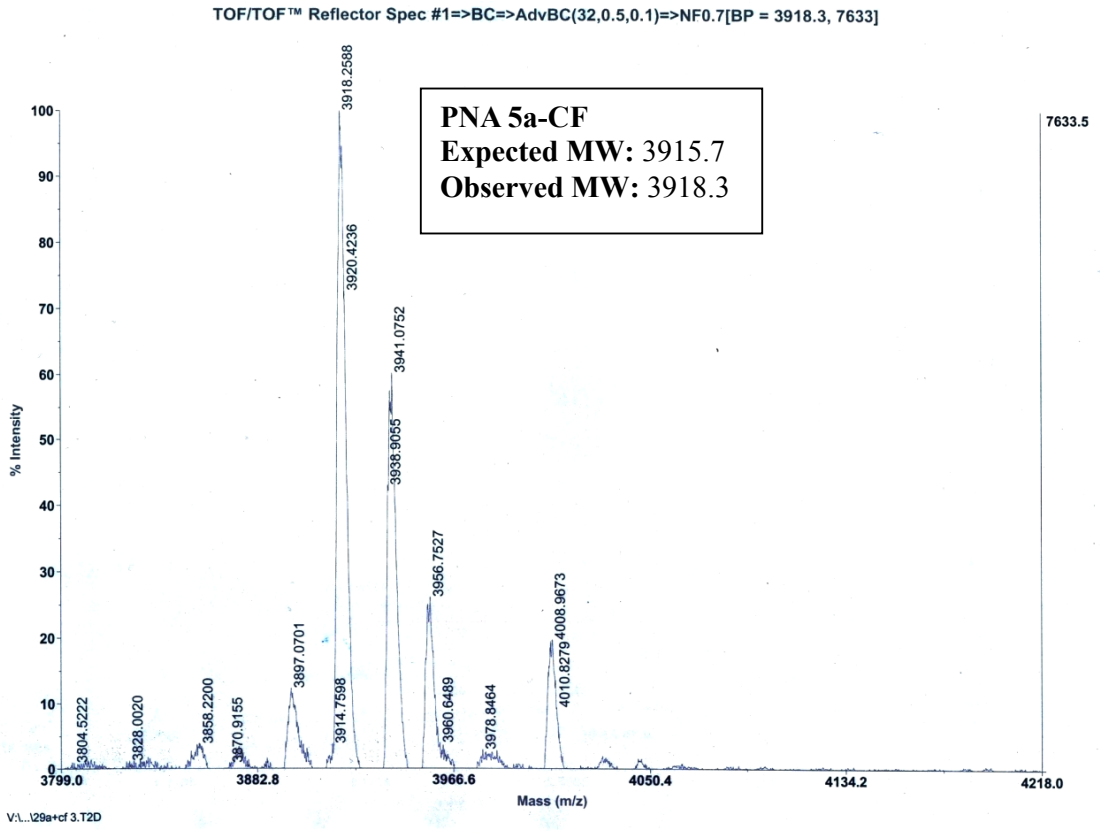


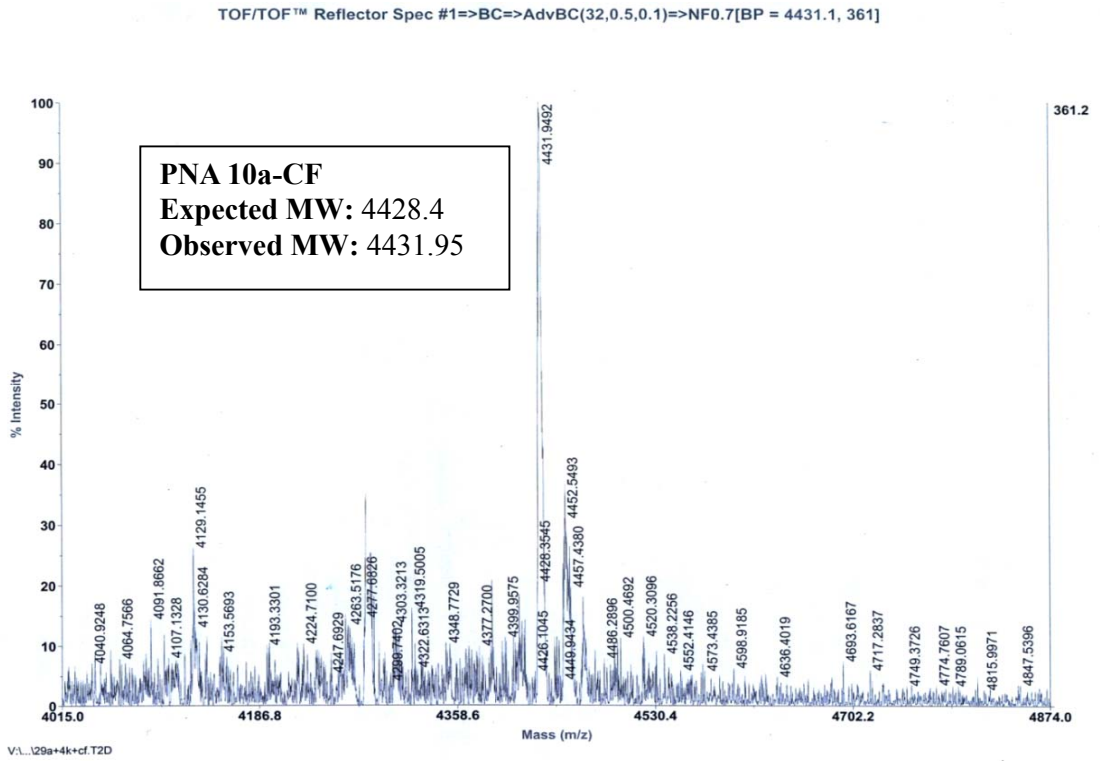
Fig S38: HPLC chromatogram of purified **PNA 10a-CF**




AB Sciex TOF/TOF™ Series Explorer™ 72085
TOF/TOF™ Linear Spec #1[BP = 3735.9, 1801]
PNA 1-CF
Expected MW: 3737.4
Observed MW: 3736.0
3736.0530
1800.9
3757.6865
3774.0874
3587.6431
3795.1028
4003.3264
% Intensity
Mass (m/z)
Printed: 12:04, December 19, 2014


TOF/TOF™ Linear Spec #1=>BC=>AdvBC(32,0.5,0.1)=>SM5[BP = 4248.8, 170]
PNA 2-CF
Expected MW: 4249.8
Observed MW: 4248.4
4248.4321
170.1
4275.0737
4375.9106
4403.5249
4346.2939
4301.1309
4502.1841
4532.6064
% Intensity
Mass (m/z)
V:\...\PNA+4K CF.T2D


TOF/TOF™ Reflector Spec #1=>BC=>AdvBC(32,0.5,0.1)=>NF0.7[BP = 3918.3, 7633]
PNA 5a-CF
Expected MW: 3915.7
Observed MW: 3918.3
% Intensity
Mass (m/z)
V:\...\29a+cf 3.T2D


TOF/TOF™ Reflector Spec #1=>BC=>AdvBC(32,0.5,0.1)=>NF0.7[BP = 4431.1, 361]
PNA 10a-CF
Expected MW: 4428.4
Observed MW: 4431.95
% Intensity
Mass (m/z)
V:\...\29a+4k+cf.T2D

Cell culture and Cellular uptake using Flow Cytometry

HCT-116 (human colorectal carcinoma) were cultured in Dulbecco's modified Eagle's medium (DMEM, GlutaMAX, 4.5 g/L D-glucose, pyruvate ; Life technologies) supplemented with 10% Fetal Bovine serum (FBS) at 37°C in 5% CO_2 atmosphere. HCT-116 cells were seeded in 12- well plates at 6 x10^4 cells per well and grown overnight until 70% confluency. Next day, media was removed and cells were washed with 1X PBS. To each well, PNA (PNA1, 2, 5a, 10a) was added in serum free media (OptiMEM, Invitrogen) at a final concentration of 1 μM and incubated for 10 hrs at 37°C/5% CO_2. Untreated cells were used as a negative control. After incubation, cells were washed with 1X PBS supplemented with Heparin (1mg/ml) to remove the cell surface bound PNA. Next, the cells were washed with 1:1 mixture of Trypan Blue and 1X PBS to further remove the extracellular fluorescent artifacts. Trypsin (0.25%) was then added, and the cells were harvested in complete media (DMEM, Life Technologies) after 5 min. The cells were centrifuged down, washed with 1X PBS twice and resuspended in 1X PBS. To see the internalisation of carboxyfluorescein-labeled PNA conjugates, intracellular fluorescence was determined by flow cytometry analysis using a FACSAria flow cytometer (Becton Dickinson). Experiments were performed in triplicate with acquisition of total of 1000 events in each sample was done. FITC (530/30nm) band pass filter was used for fluorescence analysis of the cells.

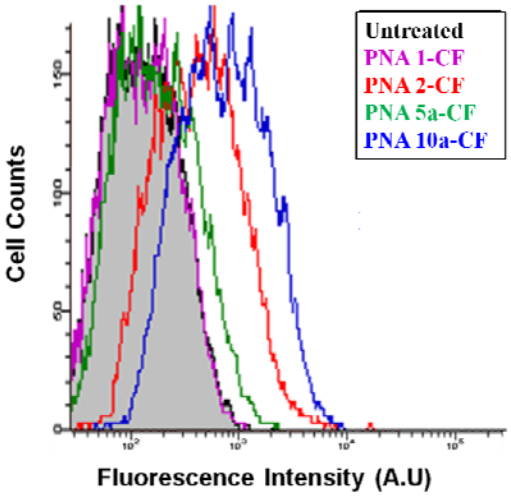


Fig S39: FACS analysis of the **PNA 1, 2, 5a, 10a** oligomers with carboxyfluorescein (CF) at 37°C

Electrophoretic gel mobility shift assay

Electrophoretic gel experiment was employed to establish that β,γ-bis-hydroxy oligomers do not bind to complementary DNA while β,γ-bis-methoxy substituted oligomers does. **PNA 1, 3a, 4a** were mixed individually with cDNA (350μM) in water. The samples were lyophilized and resuspended in 2μL sodium phosphate buffer (10Mm, pH 7.2). The samples were annealed by heating to 90°C followed by slow cooling to room temperature and refrigeration at 4°C for 6h. To this 2μLof 40% sucrose solution in TBE buffer (pH 8.0) was added and loaded on the gel. Bromophenol Blue (BPB) was used as the tracer dye separately in adjacent well. Gel electrophoresis was performed on a 15% non-denaturing polyacrylamide gel (acrylamide: bis-acrylamide, 29:1) at a constant power supply of 150V, until the BPB migrated to three-fourth of the gel length. During electrophoresis the temperature was maintained at 10°C. The spots were visualized through UV shadowing by illuminating the gel placed on a pre-coated silica gel plate (F_{254}), using UV-light.

Lane 1: *ap*-cDNA: **PNA 3a** (aaccga$^{(R,R)}$**T**^{OH}ttcag-K)

Lane 2: *ap*-cDNA: **PNA 4a (**aaccga$^{(R,R)}$**T**^{OMe}ttcag-K)

Lane 3: *ap*-cDNA: **PNA 1** (aaccgatttcag-K)

Lane 4: Single strand *ap*-**cDNA**

Lane 5: Bromophenol Blue

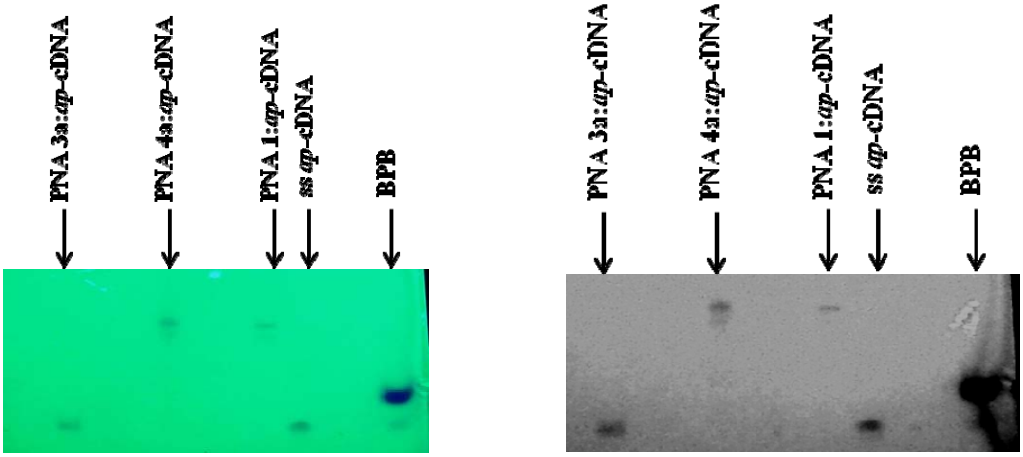


Fig S40: Electrophoretic gel mobility shift assay of **PNA 1, 3a, 4a**