PNAs grafted with (α/γ, *R*/*S*)-aminomethylene pendants: Regio and stereospecific effects on DNA binding and improved cell uptake

Roopa Mitra[a] *and Krishna N. Ganesh* [*a,b]

[a] *Division of Organic Chemistry, National Chemical Laboratory, Dr. Homi Bhabha Road Road, Pune 411008, India.* [b] *Indian Institute of Science Education and Research, 900, NCL Innovation Park, Dr. Homi Bhabha Road, Pune 411008, India.*

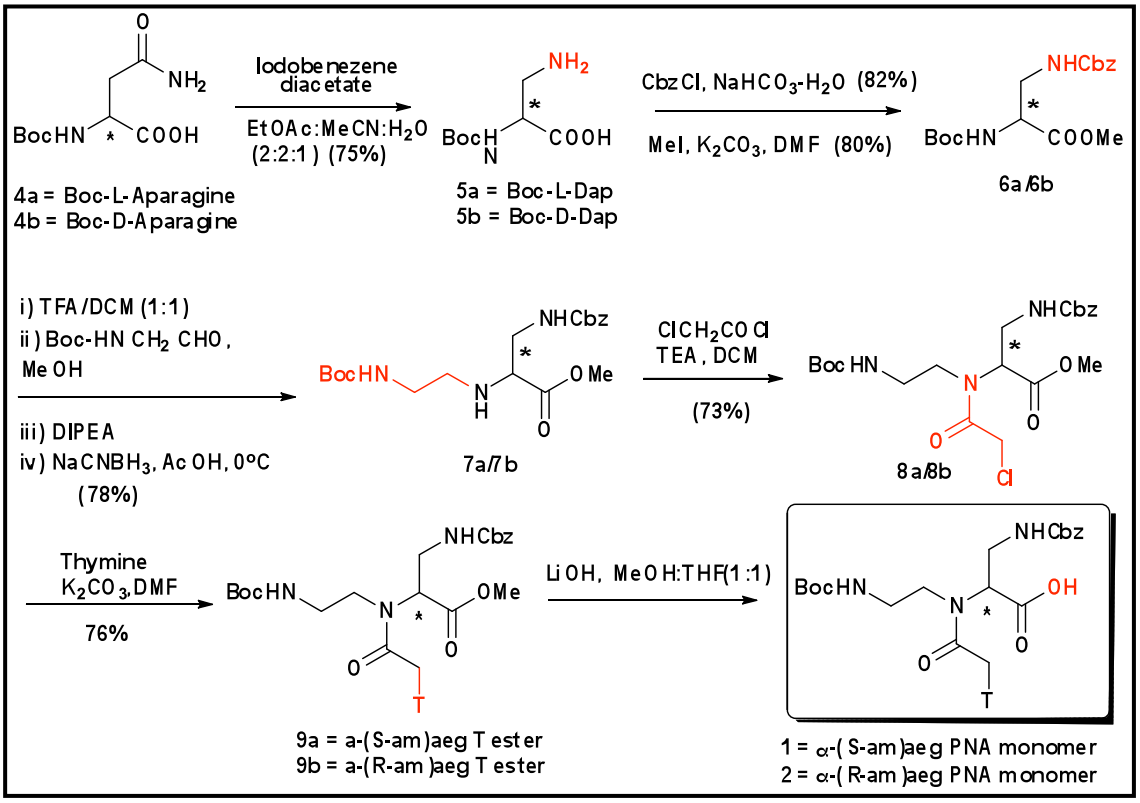
Email: kn.ganesh@iiserpune.ac.in

Supporting Information

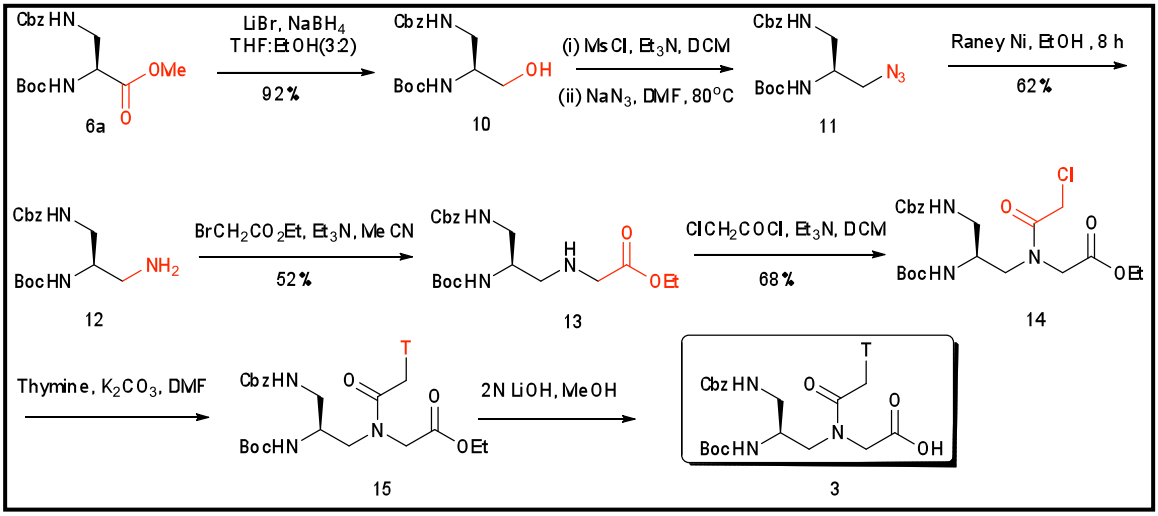
Table of contents

S1: General information

Melting points of samples were determined in open capillary tubes and are uncorrected. IR spectra were recorded on an infrared Fourier Transform spectrophotometer using Chloroform, Nujol and KBr pellets. Column chromatographic separations were performed using silica gel 60-120 mesh,and 230-400 mesh, solvent systems EtOAc/Pet ether and pure MeOH/DCM. ^{1}H and ^{13}C were obtained using Bruker AC-200 (200 MHz) and 500 MHz NMR spectrometers. The chemical shifts are reported in delta (δ) values. The optical rotations were recorded in an ADP220 Polarimeter. Mass spectra were obtained either by LCMS and MALDI-TOF mass spectrometry techniques. Oligomers were purified and analyzed by RP HPLC (C-18) and MALDI-TOF mass spectrometry.



Scheme 1. Synthesis of α-(*S/R-am*)PNA monomers 1 and 2.



Scheme 2. Synthesis of γ-(*S-am*)PNA monomer 3.

Experimental Procedures:

***N*α-Boc-L-diaminopropionic acid (5a).** A slurry of Boc-L-asparagine (**4a**) (5.0 g, 21.5 mmol) in ethyl acetate (24 mL), acetonitrile (24 mL), water (12 mL) and iodosobenzene diacetate (8.32 g, 25.8 mmol) was cooled and stirred at 16 °C for 30 min. The temperature was raised to 20 °C and the reaction was stirred until completion (4 h approx). The reaction mixture was cooled down to 0 °C and filtered under vacuum. The filter cake was washed with ethyl acetate and dried in vacuum at 65 °C to obtain the **5a** (3.88 g, 88% yield). mp 210 °C. IR (KBr) *v* (cm^{-1}) 3528, 3507, 3349, 3033-2581, 2968, 1688, 1660, 1534, 1461, 1404, 1364, 1016 cm^{-1}. ^1H NMR (200 MHz, MeOH-*d*) δ_H 7.81-7.77 (m, 1H), 7.25-7.17 (m, 1H), 4.17-4.11 (m, 1H), 3.25-3.22 (m, 2H), 1.54 (s, 9H) ppm. ESI-MS (*m/z*) Calcd: 204.2237 $[M]^+$, 227.2134 $[M+Na]^+$; Obsvd: 205.2667 $[M+H]^+$, 227.2656 $[M+Na]^+$.

***N*α-Boc-D-diaminopropionic acid (5b).** This compound was prepared from Boc-D-asparagine (**4b**) using the similar procedure as for **5a**. mp 202-208 °C. $[\alpha]_D^{25}$ -8.88, (*c* 0.225, MeOH). IR (KBr) *v* (cm^{-1}) 3528, 3507, 3349, 3033-2581, 2968, 1688, 1660, 1534, 1461, 1404, 1364, 1016 cm^{-1}. ^1H NMR (200 MHz, MeOH-*d*) δ_H 7.81-7.77 (m, 1H), 7.25-7.17 (m, 1H), 4.17-4.11 (m, 1H), 3.25-3.22 (m, 2H), 1.54 (s, 9H) ppm. ESI-MS (*m/z*)

Calcd: 204.2237 $[M]^+$, 227.2134 $[M+Na]^+$; Obsvd: 205.2853 $[M+H]^+$, 227.2629 $[M+Na]^+$.

***N*α-Boc, *N*β-Cbz amino-L-alanine methyl ester (6a).** To an ice-cold stirred solution of **5a** (330 mg, 1.6 mmol) in water (3.3 mL) was added $NaHCO_3$ (340 mg, 4 mmol) slowly in portions. To this mixture, CbzCl (330 mg, 1.9 mmol, 0.7 mL of a 50% solution in toluene) was added in one stretch and stirred vigorously for 8 h at ambient temperature. Toluene was removed under reduced pressure and the aqueous layer was washed with ether (10 mL × 2). The ethereal layer was discarded and the aqueous layer was acidified to pH 2 with citric acid. The compound was extracted into ethyl acetate as sticky oil to which in DMF (50 mL) was added activated K_2CO_3 (2.3 g, 16 mmol). After stirring for 15 min in an ice-water bath, MeI (3.8 mL, 59 mmol) was added to the white suspension and the stirring was continued at rt for 8 h. The solvent was removed from the reaction mixture and the residue was partitioned between ethyl acetate (15 mL) and water (15 mL). The organic phase was washed with brine, dried with $MgSO_4$, filtered and concentrated. The residue was purified on silica gel using light petroleum and ethyl acetate to afford **6a** as pale amber oil which on subsequent cooling solidifies into shiny white compound (3.8 g, 71.4% yield). mp 38-43 °C. $[\alpha]_D^{25}$ +25.64 (*c* 1.092, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$) δ_H 7.25 (s, 5H), 5.41 (b, 1H), 5.14 (b, 1H), 5.08 (s, 2H), 4.34 (b, 1H), 3.72 (s, 3H), 3.58 (t, 2H), 1.42 (s, 9H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 173.28, 157.22, 155.83, 136.06, 128.27, 127.84, 80.03, 66.78, 54.07, 42.50, 28.08 ppm. ESI-MS (*m/z*) Calcd: 352.3813 $[M]^+$, 375.3710 $[M+Na]^+$, 391.4796 $[M+K]^+$; Obsvd: 353.5086 $[M+H]^+$, 375.5088 $[M+Na]^+$, 391.5032 $[M+K]^+$.

***N*α-Boc, *N*β-Cbz amino-D-alanine methyl ester (6b).** This compound was prepared from **5b** using the similar procedure as for **6a**. It was isolated in the form of white solid compound. mp 42-46.4 °C. $[\alpha]_D^{25}$ -24.13 (*c* 0.663, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$) δ_H 7.25 (s, 5H), 5.47 (b, 1H), 5.20 (b, 1H), 5.07 (s, 2H), 4.35 (b, 1H), 3.72 (s, 3H), 3.57 (m, 2H), 1.42 (s, 9H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 173.28, 157.22, 155.83, 136.06, 128.27, 127.84, 80.03, 66.78, 54.07, 42.50, 28.08 ppm. ESI-MS (*m/z*) Calcd: 352.3813 $[M]^+$, 375.3710 $[M+Na]^+$; Obsvd: 353.4982$[M+H]^+$, 375.5036 $[M+Na]^+$.

***N*-(2-Boc-aminoethyl)-*N*-(3-Cbz-amino-L-alanyl) methyl ester (7a).** Compound **6a** (500 mg) was dissolved in DCM (1 mL) and TFA (1 mL) was added to it at 0 °C. The mixture was stirred for 1 h. Toluene (5 mL) was added and volatiles were removed under vacuum. After sufficient removal of volatiles, TFA salt of compound (760 mg) was obtained as crude oil. Without further purification, this residue was dissolved in methanol (10 mL) and Boc-aminoacetaldehyde (248 mg, 1.6 mmol) diluted in MeOH (2.5 mL) was added to it while continuous stirring at 0 °C. DIPEA (0.7 mL, 4.3 mmol) was added to this stirred solution drop wise very slowly. After stirring for 30 min, glacial acetic acid (0.2 mL) and $NaCNBH_3$ (134 mg, 2.1 mmol) were added sequentially. The reaction mixture was stirred for 3 h. All volatiles were removed under vacuum and the residue was dissolved in ethyl acetate and extracted with saturated aqueous $NaHCO_3$. The organic phase, after drying with $MgSO_4$, was removed under vacuum and the residue was purified by flash chromatography eluting with ethyl acetate-Pet ether. **7a** was obtained as yellow oil (470 mg, 83.8% yield). $[\alpha]_D^{25}$ +3.23 (*c* 0.5, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 7.34 (m, 5H), 5.28 (b, 1H), 5.09 (s, 2H), 4.91 (b, 1H), 3.71 (s, 3H), 3.49 (m, 1H), 3.34 (m, 2H), 3.16 (m, 2H), 2.75-2.58 (m, 2H), 1.42 (s, 9H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 173.55, 156.45, 156.14, 136.41, 128.54, 128.54, 79.34, 66.90, 60.63, 52.28, 47.54, 42.82, 40.45, 28.40 ppm. ESI-MS (*m/z*) Calcd: 418.4399 $[M+Na]^+$, 434.5485 $[M+K]^+$; Obsvd: 410.3781 $[M+Na]^+$; 432.3668 $[M+K]^+$.

***N*-(2-Boc-aminoethyl)-*N*-(3-Cbz-amino-D-alanyl) methyl ester (7b).** This compound was prepared from **6b** using the similar procedure as for **7a**. $[\alpha]_D^{25}$ -3.46 (*c* 0.578, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 7.33 (m, 5H), 5.34 (b, 1H), 5.08 (s, 2H), 4.95 (b, 1H), 3.71 (s, 3H), 3.48 (m, 1H), 3.35 (m, 2H), 3.16 (m, 2H), 2.75-2.59 (m, 2H), 1.41 (s, 9H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 172.75, 155.77, 155.45, 135.65, 127.77, 127.41, 78.58, 66.11, 59.85, 51.55, 46.74, 42.02, 39.57, 27.65 ppm. ESI-MS (m/z) Calcd: 395.4501 $[M]^+$, 418.4399 $[M+Na]^+$; Obsvd: 396.5209 $[M+H]^+$, 418.5118 $[M+Na]^+$.

***N*-(2-Boc-aminoethyl)-*N*-(chloroacetyl)-*N*-(3-Cbz-amino-L-alanyl) methyl ester (8a).** To a stirred solution of **7a** (2.8 g, 7 mmol) in DCM (30 mL) and Et_3N (3.9 mL, 28 mmol) cooled to 0 °C, was added chloroacetyl chloride (0.7 mL, 9.1 mmol). After 30 min, DCM was removed under reduced pressure and the residue was extracted with DCM

(2 × 20 mL) followed by drying under Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was purified with column chromatography (Pet ether/ethyl acetate) affording chloro compound **8a** as yellow oil (2.546 g, 77 % yield). $[\alpha]_D^{25}$ -12.35 (*c* 0.648, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 7.33 (s, 5H), 5.37 (b, 1H), 5.15-4.98 (q, 2H), 4.03 (m, 2H), 3.83 (m, 2H), 3.74 (s, 3H), 3.57 (m, 2H), 3.31 (m, 1H), 3.18 (m, 2H), 1.42 (s, 9H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 170.17, 167.58, 156.76, 156.14, 136.26, 128.54, 128.18, 79.89, 66.89, 60.36, 52.71, 49.84, 41.17, 39.92, 38.65, 28.33 ppm. ESI-MS (*m/z*) Calcd: 471.9389 $[M]^+$, 511.0372 $[M+K]^+$ Obsvd: 508.4978 $[M+K]^+$.

***N*-(2-Boc-aminoethyl)-*N*-(chloroacetyl)-*N*-(3-Cbz-amino-D-alanyl) methyl ester (8b).** This compound was prepared from **7b** using the similar procedure as for **8a**. $[\alpha]_D^{25}$ +14.8 (*c* 0.135, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 7.34 (s, 5H), 5.39 (b, 1H), 5.28 (b, 1H), 5.16-4.99 (q, 2H), 4.03 (m, 2H), 3.83 (m, 2H), 3.75 (s, 3H), 3.57 (m, 2H), 3.29 (m, 1H), 3.18 (m, 2H), 1.42 (s, 9H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 170.17, 167.65, 156.73, 156.15, 136.21, 128.58, 128.22, 79.97, 66.98, 60.36, 52.77, 49.89, 41.09, 39.97, 38.63, 28.35 ppm. ESI-MS (*m/z*) Calcd: 471.9389 $[M]^+$, 494.9286 $[M+Na]^+$, Obsvd: 472.5432 $[M+H]^+$, 494.5393 $[M+Na]^+$.

***N*-(2-Boc-aminoethyl)-*N*-(thymine-1-acetyl)-*N*-(3-Cbz-amino-L-alanyl)methyl ester (9a).** A mixture of thymine (685 mg, 5.4 mmol) and anhydrous K_2CO_3 (750 mg, 5.4 mmol) in dry DMF (25 ml) under nitrogen condition was heated at 65 °C while continuous stirring for 1.5 h. The mixture was allowed to come to room temperature and then kept in ice bath. To this was added drop wise **8a** (2.3 mg, 4.9 mmol) diluted in DMF (15 mL). The reaction mixture was stirred for 8 h. DMF was removed under vacuum and the residue was partitioned between ethyl acetate and water. The organic phase was washed with brine, dried with $MgSO_4$, filtered and concentrated. The residue was purified on silica gel using light petroleum and ethyl acetate to afford **9a** as a foamy solid (2.2 g, 78% yield). mp 69-72 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 9.19 (s, 1H), 7.33 (s, 5H), 6.86 (s, 1H), 5.79 (m, 1H), 5.54 (m, 1H), 5.09-5.06 (q, 2H), 4.72-4.64 (maj) (d, 1H), 4.19-4.11 (min) (d, 1H), 3.88-3.79 (m, 2H), 3.72 (s, 3H), 3.54 (m, 2H), 3.34 (m, 1H), 3.18-3.13 (m, 2H), 1.87 (s, 3H), 1.43 (s, 9H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$) δ_C

170.09, 167.32, 164.45, 156.90, 156.12, 151.38, 140.94, 136.52, 128.51, 128.24 ppm. ESI-MS (*m/z*) Calcd: 561.5844 $[M]^+$, 584.5741 $[M+Na]^+$, Obsvd: 562.3598 $[M+H]^+$, 584.3475 $[M+Na]^+$.

***N*-(2-Boc-aminoethyl)-*N*-(thymine-1-acetyl)-*N*-(3-Cbz-amino-D-alanyl)methyl ester (9b).** This compound was prepared from **8b** using the similar procedure as for **9a**. mp 70-79 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 9.44 (s, 1H), 7.33 (s, 5H), 6.86 (bs, 1H), 5.88 (m, 1H), 5.57 (m, 1H), 5.15-5.00 (q, 2H), 4.72-4.64 (maj) (d, 1H), 4.19-4.12 (min) (d, 1H), 3.88 (m, 2H), 3.72 (s, 3H), 3.57 (m, 2H), 3.33 (m, 1H), 3.18 (m, 2H), 1.86 (s, 3H), 1.42 (s, 9H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 170.39, 167.65, 164.80, 157.19, 156.42, 151.65, 141.23, 136.83, 128.81, 128.55, 111.23, 80.23, 67.11, 61.27, 53.04, 49.74, 48.85, 39.94, 39.07, 28.68, 12.71 ppm. ESI-MS (m/z) Calcd: 561.5844 $[M]^+$, 584.5741 $[M+Na]^+$, Obsvd: 562.3567 $[M+H]^+$, 584.3448 $[M+Na]^+$.

***N*-(2-Boc-aminoethyl)-*N*-(thymine-1-ylacetyl)-*N*-(3-Cbz-amino)-L-alanine (1).** To compound **9a** suspended in THF, a solution of 0.5 M LiOH in water was added and the mixture was stirred for 30 min. THF was removed under vacuum and the aqueous layer was washed with DCM. The aqueous layer was then neutralized with activated Dowex H^+ resin till pH of the solution turned 4.0-5.0. The resin was removed by filtration and the filtrate was concentrated to obtain the resulting Boc-protected monomer **1** in excellent yield (> 85%). ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 7.39 (m, 5H), 7.28 (bs, 1H), 5.13 (s, 2H), 4.73-4.65 (maj) (d, 1H), 4.52-4.44 (min) (d, 1H), 4.09-4.01 (t, 1H), 3.76-3.73 (d, 2H), 3.55-3.42 (m, 2H), 3.30-3.29 (m, 2H), 1.9 (s, 3H), 1.49 (s, 9H) ppm. ESI-MS (*m/z*) Calcd: 547.5575 $[M]^+$, 570.5472 $[M+Na]^+$, 586.6558 $[M+K]^+$; Obsvd: 570.5063 $[M+Na]^+$, 586.4932 $[M+K]^+$.

***N*-(2-Boc-aminoethyl)-*N*-(thymine-1-ylacetyl)-*N*-(3-Cbz-amino)-D-alanine(2).** The hydrolysis procedure as described for compound **1** was followed to afford **2** in good yield. ^{1}H NMR (200 MHz, MeOD) δ_H 7.39 (m, 5H), 7.29 (bs, 1H), 5.13 (s, 2H), 4.73-4.65 (maj) (d, 1H), 4.52-4.43 (min) (d, 1H), 4.09-4.01 (t, 1H), 3.78-3.74 (d, 2H), 3.36 (m, 2H), 3.30 (m, 2H), 1.9 (s, 3H), 1.49 (s, 9H) ppm. ESI-MS (m/z) Calcd: 547.5575 $[M]^+$, 586.6558 $[M+K]^+$; Obsvd: 585.9543 $[M+K]^+$.

***N*α-Boc, *N*β-Cbz-aminoalanine-ol (10).** To an ice-cooled solvent mixture of dry THF (15 mL) and absolute ethanol (10 mL) containing $NaBH_4$ (269 mg, 7.1 mmol) in a three necked flask, LiBr (617 mg, 7.1 mmol) was added slowly for about 30 min. The above solution was stirred for 1.0 h and the appearance of turbid milky solution indicates the formation of $LiBH_4$ *in situ*. To the above ice cooled solution was added **6a** (500 mg, 1.4 mmol) from a dropping funnel over a period of 30 min under N_2 atmosphere and the reaction mixture was stirred overnight at rt. The pH was then adjusted to 7.0 by adding saturated solution of NH_4Cl. The solvent mixture was removed under vacuum and the residue was extracted into ethyl acetate (10 mL × 3). The organic layer was washed with water followed by brine solution, dried over anhydrous Na_2SO_4 and concentrated to afford a white solid product **10** (360 mg. 78% yield, R_f =0.65, ethyl acetate: petroleum ether 1:1). mp 77.2-80.2 °C. $[\alpha]_D^{25}$ +12.6 (*c* 0.317, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 7.33 (s, 5H), 5.45 (b, 1H), 5.17 (b, 1H), 5.09 (s, 2H), 3.63 (m, 2H), 3.54 (m, 1H), 3.30 (m, 2H), 1.40 (s, 9H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 158.04, 155.98, 136.14, 128.57, 128.12, 79.85, 67.19, 61.72, 52.19, 41.05, 28.34 ppm. ESI-MS (*m/z*) Calcd: 324.3773 $[M]^+$, 347.3670 $[M+Na]^+$; Obsvd: 325.2679 $[M+H]^+$, 347.2411 $[M+Na]^+$.

(*S*)-benzyl *t*-butyl (3-azidopropane-1,2-diyl) dicarbamate (11). The compound **10** (0.3 g, 0.7 mmol) was dissolved in dry DCM (5 mL) and cooled to 0 °C. Et_3N (0.3 mL, 2 mmol) was drop wise added to it and stirred for next 10 min. To this ice cooled solution was added freshly distilled mesyl chloride (0.1 mL, 1 mmol) from a dropping funnel over a period of 10 min under N_2 atmosphere. The reaction mixture was stirred for 30 min. DCM was removed under reduced pressure and the residue was extracted into ethylacetate (5 mL × 2), washed with water, dried over Na_2SO_4 and concentrated to yield almost pure (single spot on TLC) sticky mesylate derivative (0.20 g, 90% crude yield, R_f= 0.7, ethyl acetate: petroleum ether1:1) which was immediately dissolved in DMF (2 mL) and NaN_3 (350 mg, 5.4 mmol) was added. The reaction mixture was stirred at 55 °C for 8 h. The solvent was removed under reduced pressure and the residue was extracted into ethyl acetate (5 mL × 3). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and then concentrated to yield **11** (104 mg, 83% yield R_f = 0.3; 40% ethyl acetate: petroleum ether). mp 105.8-106.9 ^oC. $[\alpha]_D^{25}$ -6.17 (*c* 0.324, $CHCl_3$). IR

($CHCl_3$) ν (cm^{-1}) 3440.22, 3019.66, 2957.18, 2927.72, 2856.13, 2400.76, 2106.59, 1712.59, 1514.91, 1455.80, 1368.47, 1215.60, 1163.85, 1064.64, 755.63 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 7.33 (s, 5H), 5.20 (b, 1H), 5.09 (s, 2H), 3.78 (m, 1H), 3.45-3.42 (d, 2H), 3.35-3.29 (t, 2H), 1.42 (s, 9H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 157.05, 155.57, 136.15, 128.51, 128.09, 80.03, 66.99, 52.20, 50.57, 42.57, 28.24 ppm. ESI-MS (*m/z*) Calcd.: 349.3851 $[M]^+$, 372.3748 $[M+Na]^+$; Obsvd: 350.1430 $[M+H]^+$, 372.1436 $[M+Na]^+$.

(*R*)-benzyl *t*-butyl (3-aminopropane-1,2-diyl)dicarbamate (12). To a solution of **11** (100 mg) in MeOH (10 mL) taken in hydrogenation flask was added Raney Nickel (5 mL). The reaction mixture was hydrogenated in a Parr apparatus for 3.5 h at rt and H_2 pressure of 35-30 psi. The catalyst was filtered off and the solvent was removed under reduced pressure to yield a residue of compound **12** as colorless oil (65 mg, 61 % yield). This compound was used for next reaction without any further purification.

γ-*am*-(Cbz)-PNA backbone ethyl ester (13). The compound **12** (0.05 g, 0.2 mmol) was treated with ethylbromoacetate (15.5 μL, 0.14 mmol) in acetonitrile (1 mL) in the presence of Et_3N (65 μL, 0.5 mmol) and the mixture was stirred at ambient temperature for 5 h. The solid that separated was removed by filtration and the filtrate was evaporated to obtain the alkylated derivative **13** (0.05 g, 79 % yield; R_f=0.63, 70% EtOAc:Pet Ether) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 7.33 (s, 5H), 5.50 (b, 1H), 5.26 (b, 1H), 5.08 (s, 2H), 4.21-4.11 (q, 2H), 3.70-3.60 (m, 1H), 3.37 (s, 2H), 3.32-3.19 (m, 2H), 2.80-2.57 (m, 2H), 1.41 (s, 9H), 1.25 (t, 3H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 172.39, 157.06, 156.12, 136.53, 128.51, 128.08, 79.66, 66.80, 60.87, 50.87, 50.32, 43.40, 28.36, 14.22 ppm. ESI-MS (*m/z*) Calcd: 409.4768 $[M]^+$, Obsvd: 410.4262 $[M+H]^+$.

γ-*am*-(Cbz)-(N-chloroacetyl)-PNA backbone ethyl ester (14). Compound **13** (500 mg, 1.2 mmol) was dissolved in DCM (5 mL) under ice cooled condition and Et_3N (0.7 mL, 4.8 mmol) was added to it. The reaction mixture was stirred for few minutes and then chloroacetyl chloride (0.1 mL, 1.6 mmol) was added drop wise with vigorous stirring. The reaction was complete within 0.5 h. The compound was partitioned between organic layer and aqueous phase and the product was extracted into the organic phase. It was then purified on silica gel using column chromatography technique to obtain **14** as

colorless oil in good yield (400 mg, 67.4 % yield; R_f = 0.6; 40 % EtOAc: Pet Ether). ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 7.32 (s, 5H), 5.89 (b, 1H), 5.47 (b, 1H), 5.15-5.01 (q, 2H), 4.27-4.23 (m, 1H), 4.20-4.14 (m, 2H), 3.96 (s, 2H), 3.81-3.61 (m, 2H), 3.45-3.34 (m, 2H), 3.15-2.96 (m, 2H), 1.40 (s, 9H), 1.28-1.25 (t, 3H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 169.02, 168.59, 157.69, 155.85, 136.38, 128.51, 128.04, 79.71, 66.95, 62.23, 61.52, 51.14, 50.55, 50.29, 40.96, 28.35, 14.09 ppm. ESI-MS (*m/z*) Calcd.: 486.0054 $[M]^+$, 508.9951 $[M+Na]^+$; Obsvd: 486.2656 $[M]^+$, 508.3486 $[M+Na]^+$.

γ-*am*-(Cbz)-(*N*-thymine-1-ylacetyl)-PNA backbone ethyl ester (15). A mixture of **14** (1 g, 2 mmol), thymine (286 mg, 26 mmol) and anhydrous K_2CO_3 (313 mg, 26 mmol) in dry DMF (10 mL) under N_2 atmosphere was heated with stirring at 65 °C for 5 h. After cooling, the solvent was removed under reduced pressure to leave a residue, which was extracted into DCM (2 × 25 mL) and dried over $NaSO_4$. The solvent was evaporated and the crude compound was purified by column chromatography (MeOH/DCM) to afford a pale white solid of **15** (935 mg, 79 % yield, R_f = 0.8, 80% EtOAc:Pet Ether). mp 141.9-144.1 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 10.10 (maj) & 9.70 (min) (s, 1H), 7.31 (s, 5H), 7.02 (maj) & 6.95 (min) (br s, 1H), 6.30 (maj) & 6.07 (min) (br s, 1H), 5.88 (maj) & 5.61 (min) (br s, 1H), 5.13-5.01 (m, 2H), 4.40-4.32 (m, 2H,), 4.25-4.22 (q, 2H), 4.16-4.11 (m, 1H), 3.91-3.75 (m, 2H), 3.58 (m, 2H), 3.40 (m, 2H), 1.87 (s, 3H), 1.40 (maj) & 1.33 (min) (s, 9H), 1.24-1.20 (t, 3H) ppm. ^{13}C NMR (400 MHz, $CDCl_3$) δ_C 169.33 (maj) & 169.10 (min), 168.70 (maj) & 167.52 (min), 164.48, 157.77, 156.01(min) & 155.85 (maj), 151.76 (min) & 151.29 (maj), 141.43 (min) & 140.96 (maj), 136.47 (min) & 136.34 (maj), 128.50, 128.14, 110.98, 79.78, 66.95, 62.37, 61.49, 50.52, 48.03, 41.47, 28.36, 14.09, 12.34 ppm. ESI-MS (*m/z*) Calcd.: 575.6109 $[M]^+$, 598.6006 $[M+Na]^+$; Obsvd: 576.4506 $[M+H]^+$, 598.4256 $[M+Na]^+$.

γ-*am*-(Cbz)-(*N*-thymine-1-ylacetyl)-PNA monomer (3). The usual hydrolysis procedure as is described for compound **8** was followed to afford the monomer **3** in good yield. ^{1}H NMR (200 MHz, DMSO) δ_H 11.34 (br s, 1H), 7.36 (s, 5H), 6.93 (maj) & 6.69 (min) (s, 1H), 5.06 (s, 2H), 4.75-4.47 (m, 2H), 4.16 (m, 1H), 4.0-3.85 (m, 2H), 3.53-3.34 (m, 2H), 3.15 (m, 2H), 1.77 (s, 3H), 1.38 (s, 9H) ppm. ESI-MS (*m/z*) Calcd.: 547.5575

$[M]^+$, 570.5472 $[M+Na]^+$, 586.6558 $[M+K]^+$, Obsvd: 548.45.4579 $[M+H]^+$, 570.4857 $[M+Na]^+$, 586.4633 $[M+K]^+$.

Circular Dichroism: CD spectra were recorded on JASCO J-715 spectro-polarimeter. The CD spectra of the PNA: DNA complexes and the relevant single strands were recorded in 10 mM sodium phosphate buffer, 10 mM NaCl (pH 7.2) as an accumulation of 5 scans from 300 to 190 nm using 1 cm cell, a resolution of 0.1 nm, band-width of 1.0 nm, sensitivity of 2 m deg, response of 2 sec and a scan speed of 50 nm/min. The concentration of the samples were calculated on the basis of absorbance from the molar extinction coefficients of the corresponding nucleobases (i.e., T = 8.8 $cm^2/\mu mol$; C = 7.3 $cm^2/\mu mol$; G = 11.7 $cm^2/\mu mol$ and A = 15.4 $cm^2/\mu mol$). The samples were annealed by keeping the samples at 90 °C for 5 min followed by slow cooling to room temperature. Then the samples were cooled by keeping at 4 °C overnight. The CD spectra of all the samples were recorded at 10°C at the range of 190-300 nm. The ellipticity value was plotted as a function of the PNA mole fraction at 256nm.

Job's Plot: For the binding stoichiometry determination, eleven mixtures of PNA:DNA with different ratios to each other such as 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10 and 100:0; all of the same total strand concentration (2 μM) in sodium phosphate buffer (10 mM NaCl, pH 7.2). The samples were annealed and their CD spectra were recorded at 10°C at the range of 190-300 nm. The ellipticity value was plotted as a function of the PNA mole fraction at 256nm where the CD plots show an isodichroic point at around 260-265 nm. The ellipticity values of all the mixtures at 262 nm (for P3) and 266 nm (for P4) are plotted against mole fraction of PNA. The ellipticity of PNA readily increases, reaches maxima and then again decreases. The stoichiometry of the paired strands was obtained from the intersecting point of this mixing curve, in which the optical property at a given wavelength is plotted as a function of mole fractions of each strand. The data were processed using Microcal Origin 6.0.

Absorbance spectra at 260 nm was also recorded for the mixtures of PNA:DNA in different proportions as mentioned above. A mixing curve was plotted, absorbance at

fixed wavelength (λ_{max} 260 nm) against mole fractions of PNA. The minima of intersect corresponds to the binding stoichiometric molar ratio.

UV-T_m measurements: The complexes were prepared in 10 mM sodium phosphate buffer, pH 7.2 containing NaCl (10 mM). Absorbance versus temperature profiles were obtained by monitoring at 260 nm with Varian Cary 300 UV-spectrophotometer equipped with a Peltier temperature programmer and Julabo water circulator, scanning from 10 to 85 °C with temperature increment of 0.5 °C per minute. The data were processed using Microcal Origin 6.0 and T_m values derived from the derivative curves.

Fluorescence Microscopy: Hela cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 in D-MEM containing 2 mM L-glutamine, 10% FBS and 4 μg/L gentamycin solution. They were allowed to grow for the next 24 h after which the culture medium containing PNA was discarded. The cells were washed twice with PHEM buffer (60 mM Pipes, 25 mM Hepes, 10 mM EGTA, 2 mM $MgCl_2.6H_20$; pH 6.9) for 10 min at room temperature and then gently fixed for 10 min using 3.5% paraformaldehyde and 0.05% glutaraldehyde in PHEM buffer as the fixing agent. After fixation, the cells were washed thoroughly three more times for 10 min with PHEM. The coverslips were then mounted using mounting medium that comprised of 90% glycerol, 0.5% *N*-propyl gallate (or propyl 3,4,5-trihydroxybenzoate) and 20 mM Tris-Cl (pH 8.0) containing 0.0004 mg/mL DAPI (or 4', 6-diamidino-2-phenylindole) on objective glass slides. The distribution of fluorescence was examined by fluorescence microscopy on Zeiss Imager.Z1 microscope with 60× objective. Micrographs were viewed and analysed using AXIO-VISION software. Images were acquired with the same camera settings for all cells with PNAs.

Flow Cytometry Analysis. Screening of cellular uptake was carried out on HeLa cells grown continuously as adherent monolayers in D-MEM culture media supplemented with 10% fetal calf serum and PS (100 units/mL penicillin and 0.1 mg/mL streptomycin). The cells were seeded into 60 mM plates at a density of $1\sim2\times10^6$/dish in 3 mL media and grown in a humidified 5% CO_2 atmosphere at 37 °C and 4 °C separately. The cells were

then incubated with the fluorescently labeled PNA oligomers (*cf* P **1**-**4**) at 1 μM for 24 h. Following incubation, the cell suspension was centrifuged at 300 *g*. The cell pellet was washed twice with 1xPBS (pH 7.4) at 300x*g* for 5 min. Cells were washed once more with PBS and were finally resuspended in PBS (500 μL). For each experiment, a control of cells that were not incubated with PNA was also analyzed. Fluorescence analysis for both the live cell lines was performed with FACS Calibur Flow Cytometer (Becton Dickinson) using FL1 laser for excitation of carboxyfluorescein at 488 nm. Cell Quest software was used to analyze the data. A minimum of 20,000 events per sample was analyzed.

Cell Viability Assay. To determine the cellular toxicity of the *am*-PNAs, they were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability experiment. HeLa cells were seeded into 96-well plates at a density of 50000 cells/mL in a medium as reported above. For the assay, 10 μL of 5 mg/mL MTT solution was added in each well containing cell monolayer having various concentrations of *am*-PNAs (0.1, 0.5, 1 and 2 μM) in 100 μL culture medium in dark. For each experiment, a control of cells which were not incubated with PNAs was also analyzed. Plates were incubated at 37 °C for 4 h, followed by removal of MTT/media solution. The precipitated crystals were dissolved in 100 μL of DMSO and the solution absorbance was read at 570 nm using Varioskan Flash multimode plate reader.

Figure S2: Dosage-dependent cytotoxicity profile of PNA oligomers (*cf*P 2-*cf*P 4) at 37 °C on the basis of MTT Assay.

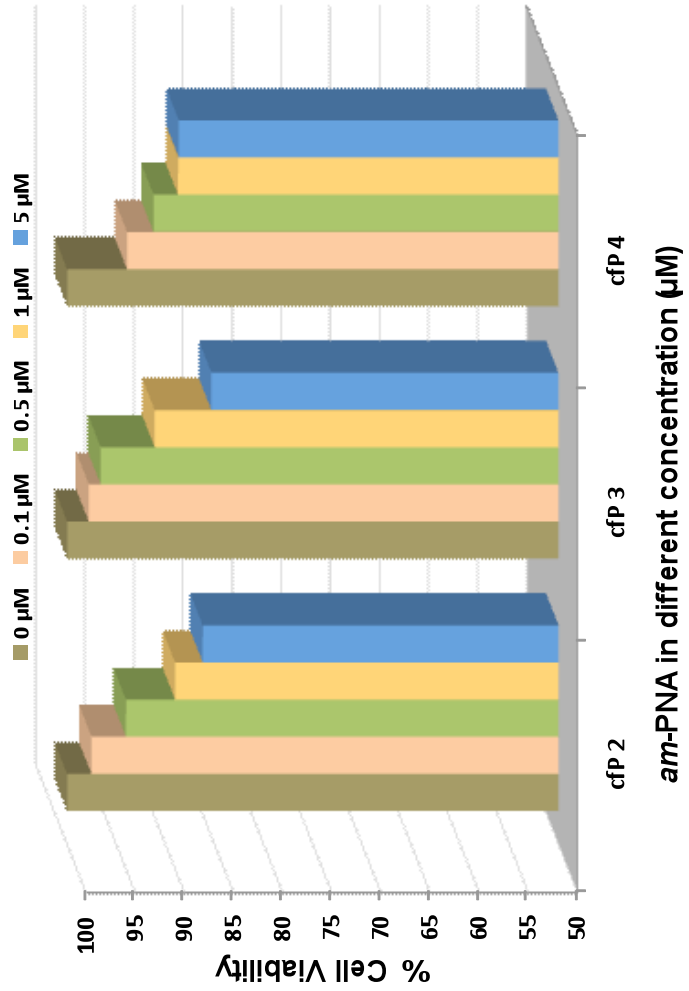
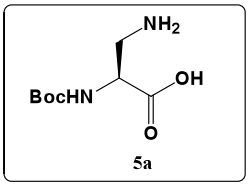
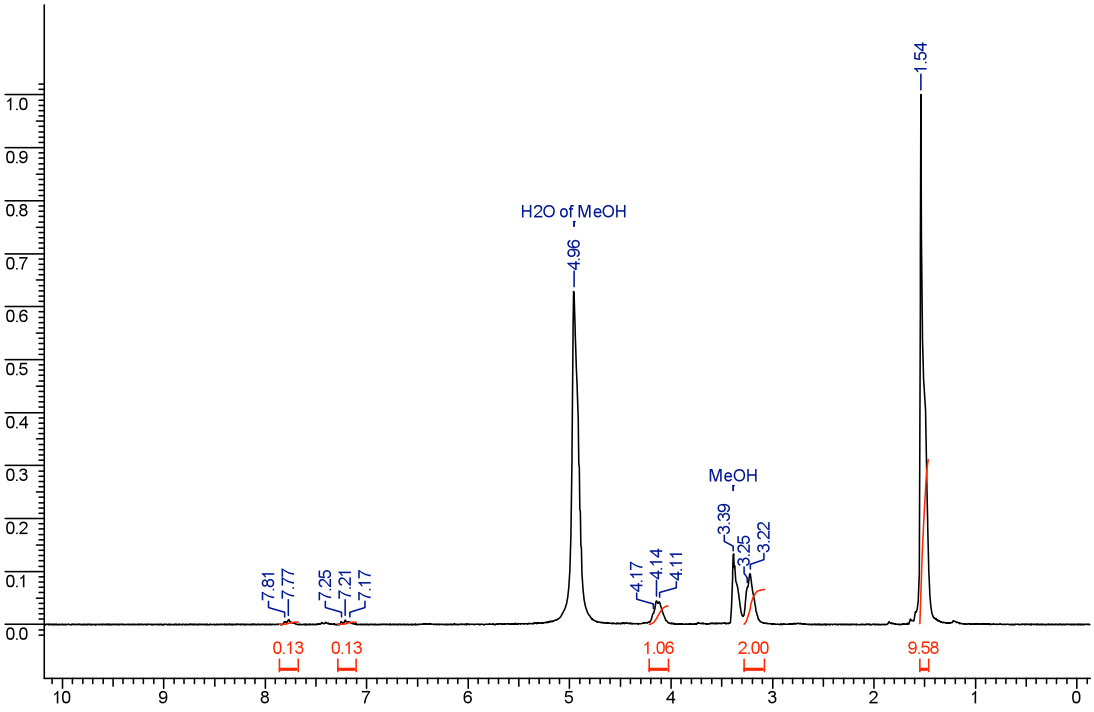


Figure S3: ^{1}H NMR spectra and LC-MS of compound 5a (solvent MeOD)



5a



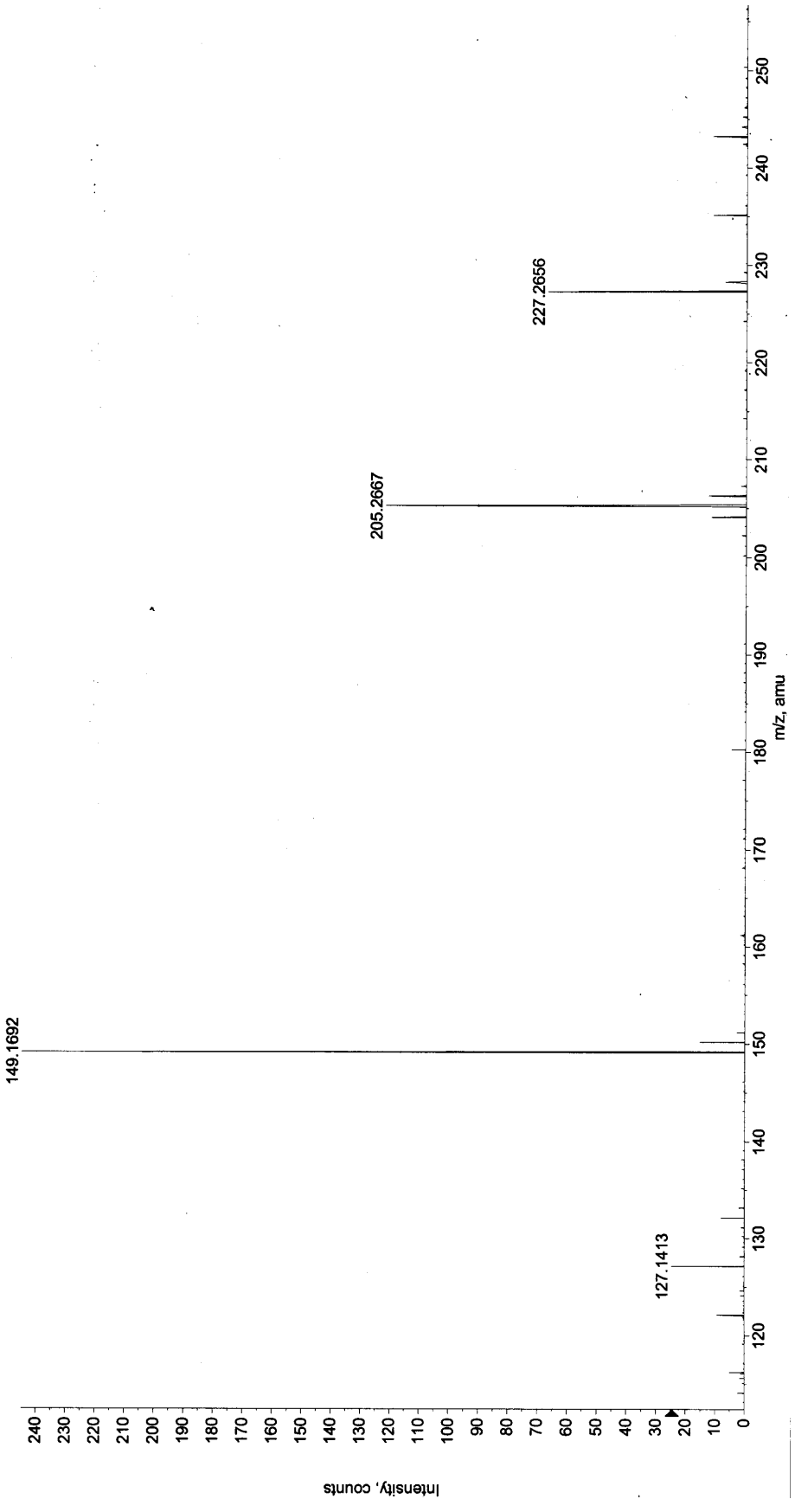
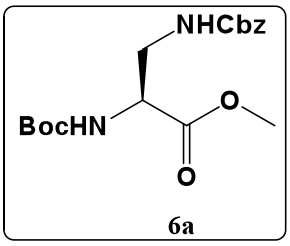
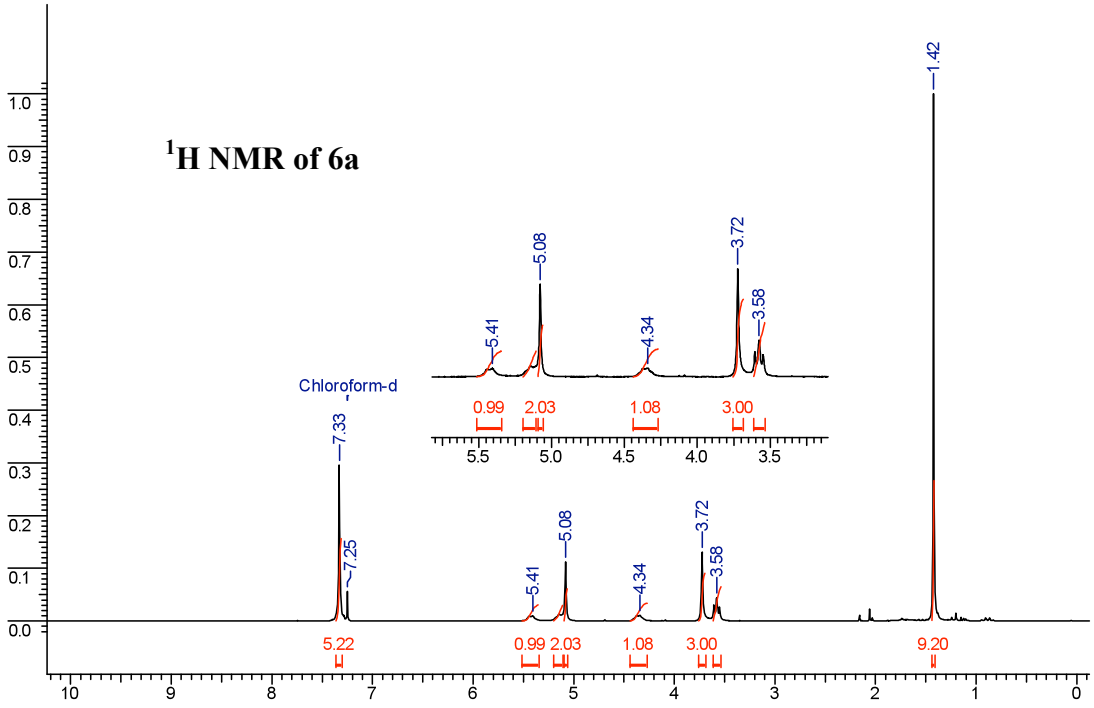
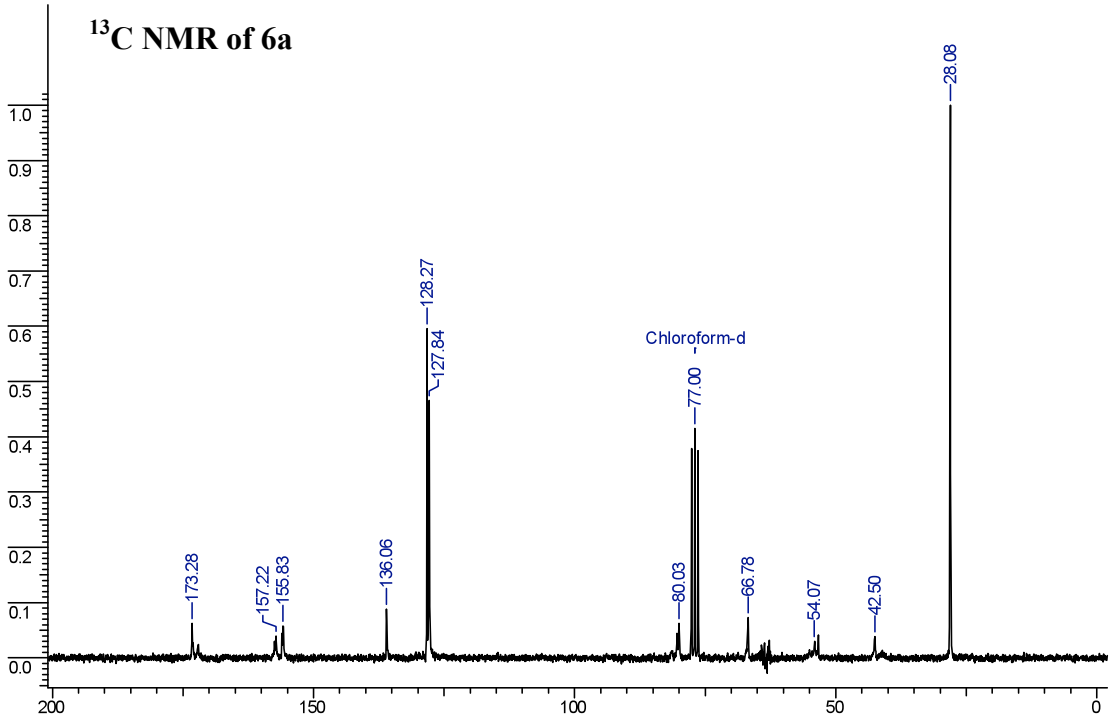

149.1692
127.1413
205.2667
227.2656
Intensity, counts
m/z, amu

Figure S4: ^{1}H ^{13}C NMR, DEPT NMR spectra of compound 6a (solvent $CDCl_3$)





^{13}C NMR of 6a



DEPT NMR of 6a

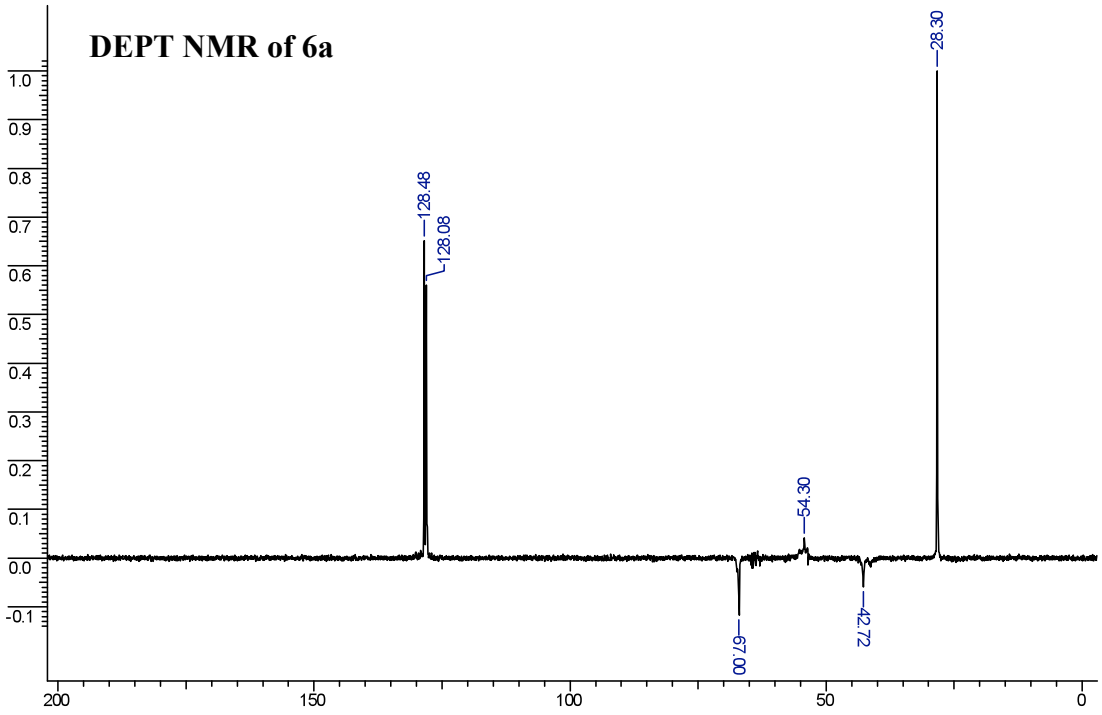
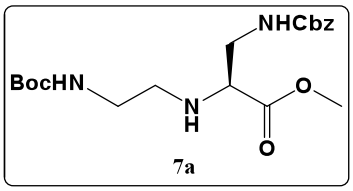
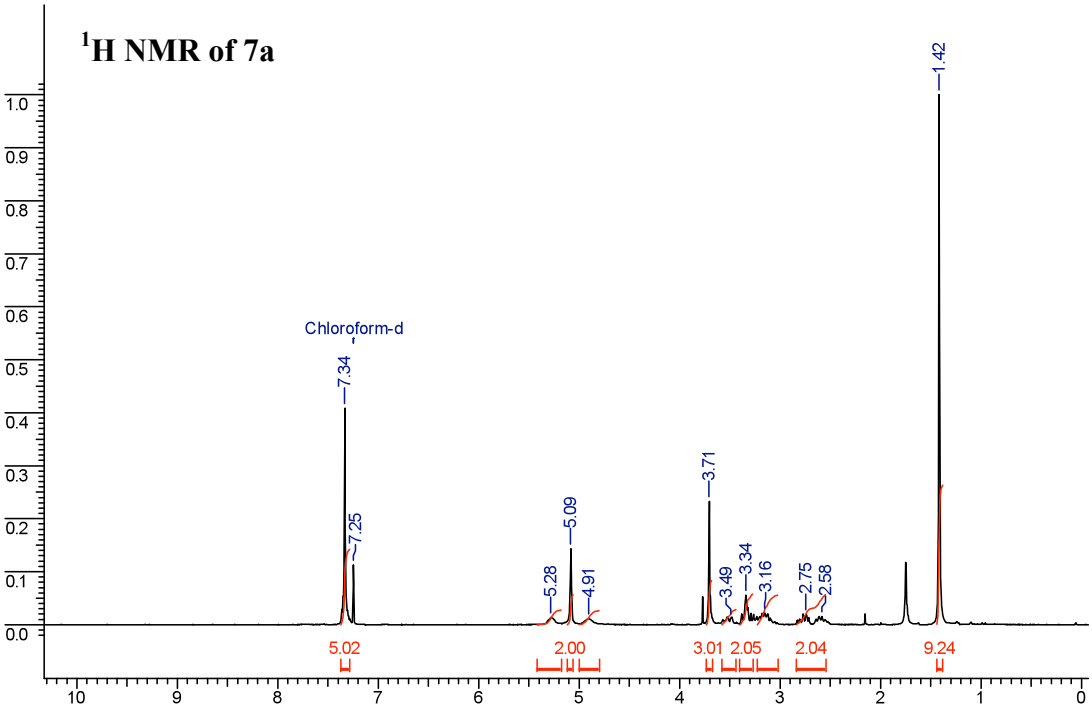
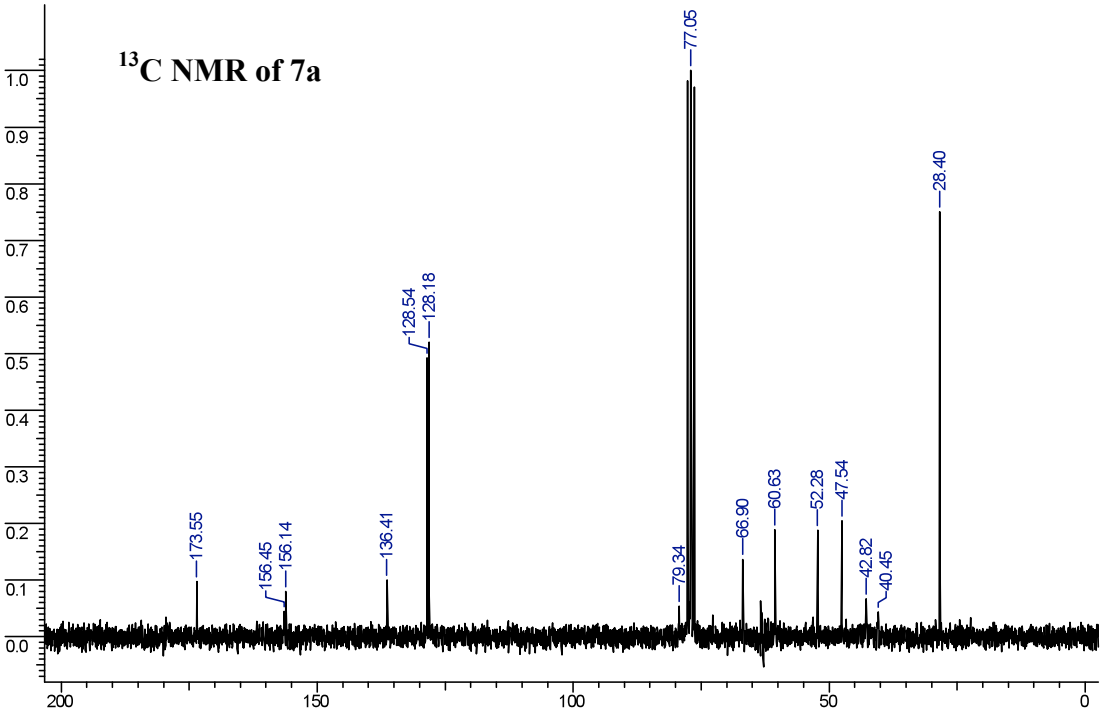


Figure S5: ^{1}H ^{13}C NMR, DEPT NMR spectra of compound 7a (solvent $CDCl_3$)



7a




^{13}C NMR of 7a
77.05
28.40
128.54
128.18
173.55
156.45
156.14
136.41
79.34
66.90
60.63
52.28
47.54
42.82
40.45
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
200
150
100
50
0

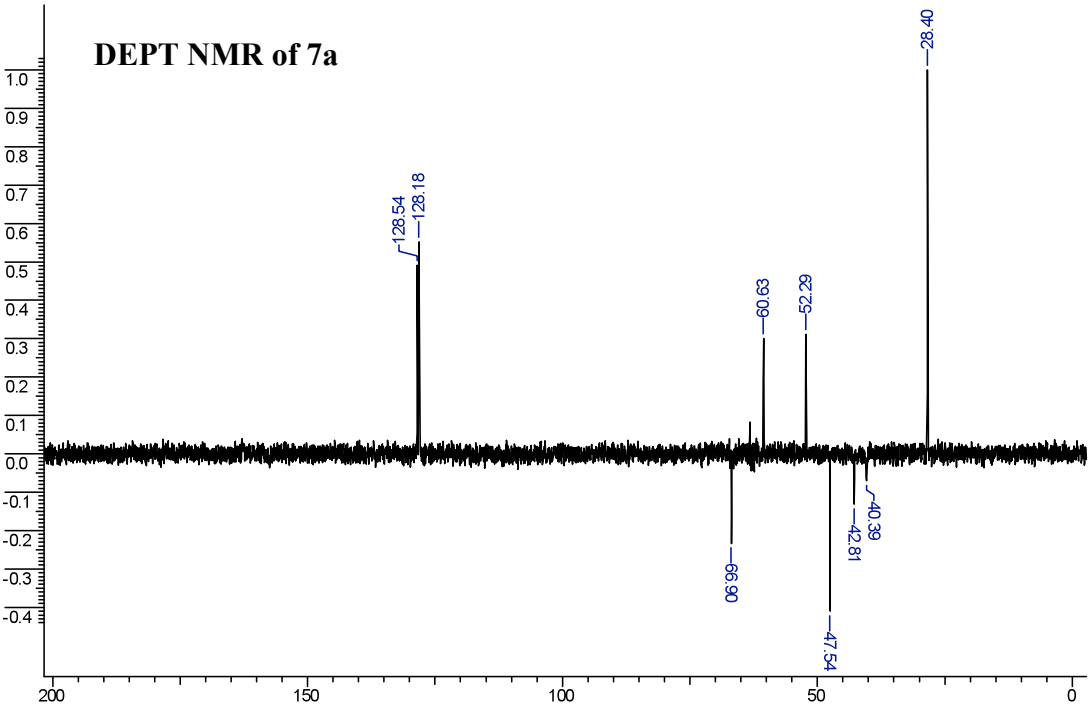
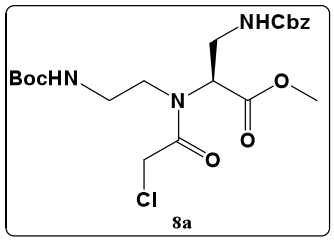
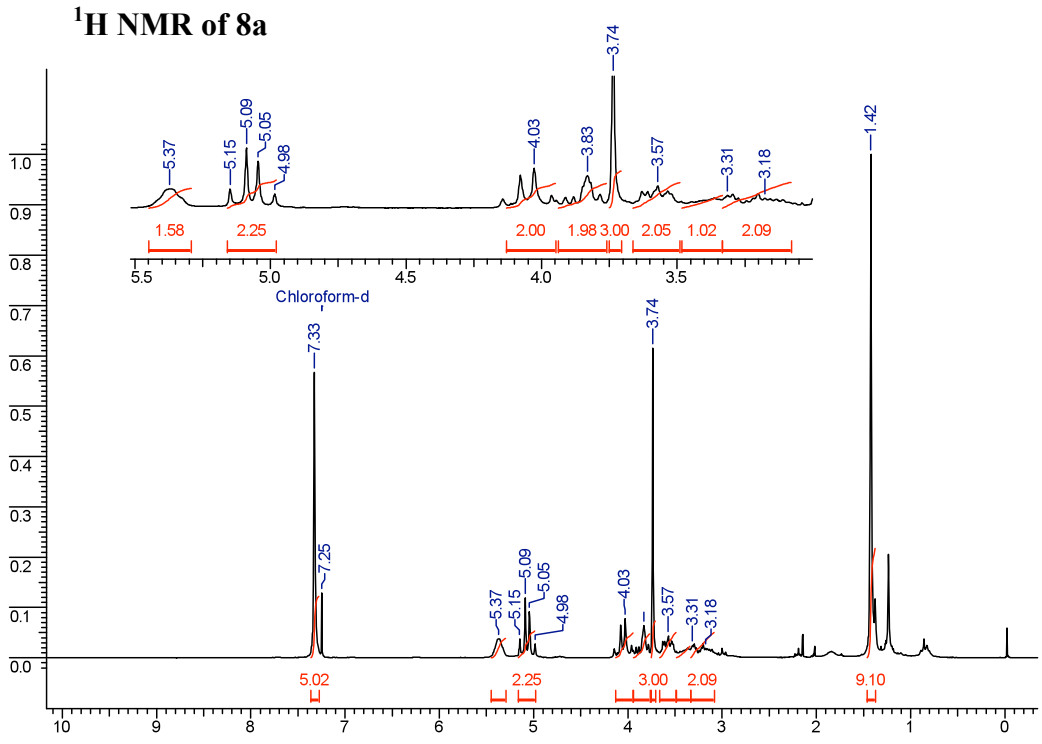
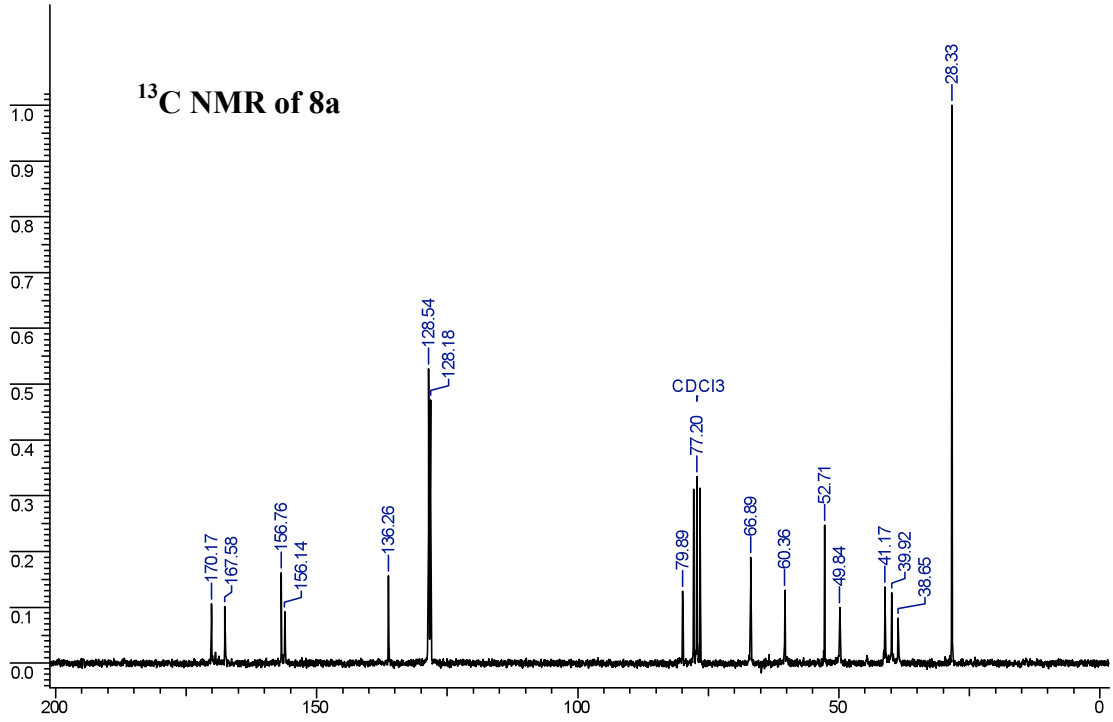

DEPT NMR of 7a
28.40
128.54
128.18
60.63
52.29
66.90
47.54
42.81
40.39
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
200
150
100
50
0

Figure S6: ^{1}H ^{13}C NMR, DEPT NMR spectra of compound 8a (solvent $CDCl_3$)






13C NMR of 8a
28.33
128.54
128.18
CDCl3
77.20
170.17
167.58
156.76
156.14
136.26
79.89
66.89
60.36
52.71
49.84
41.17
39.92
38.65
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
200
150
100
50
0

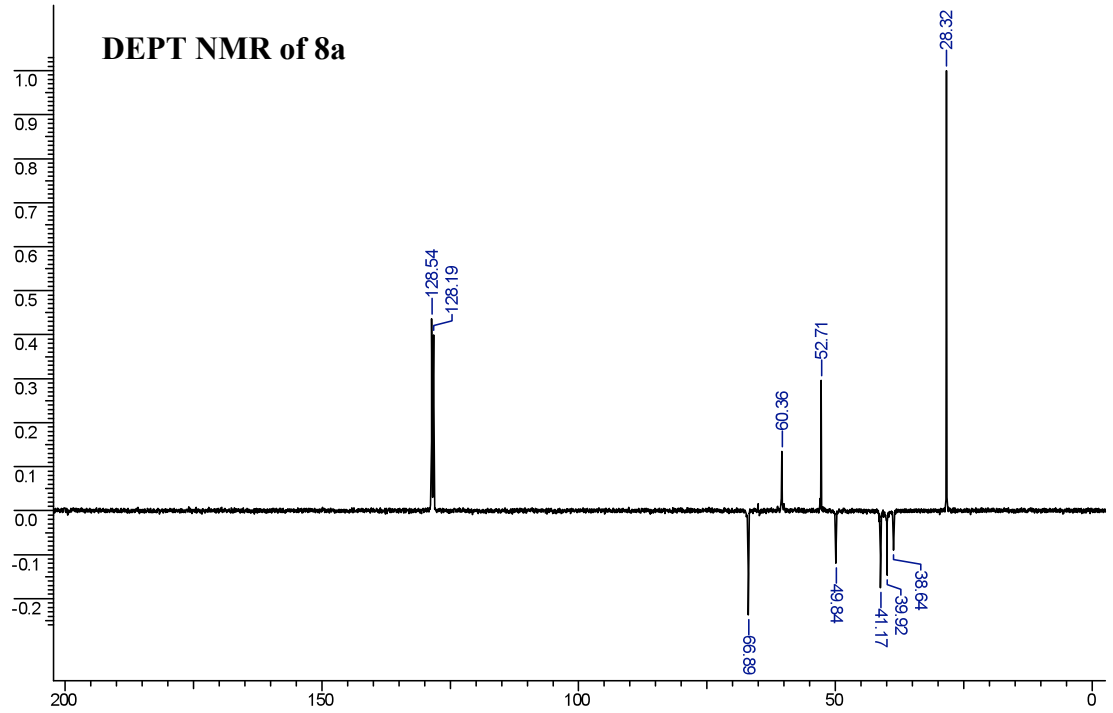
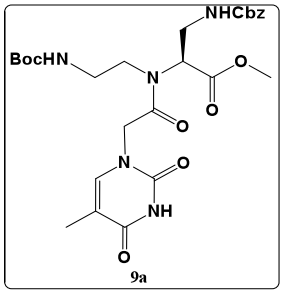
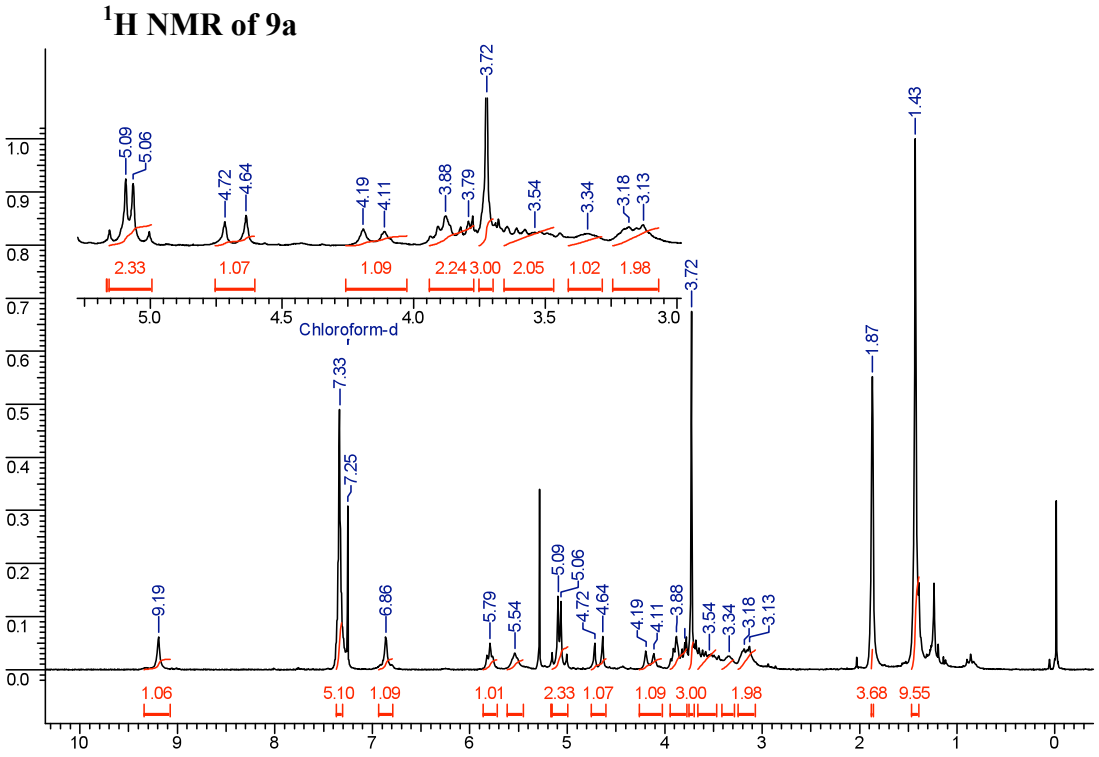
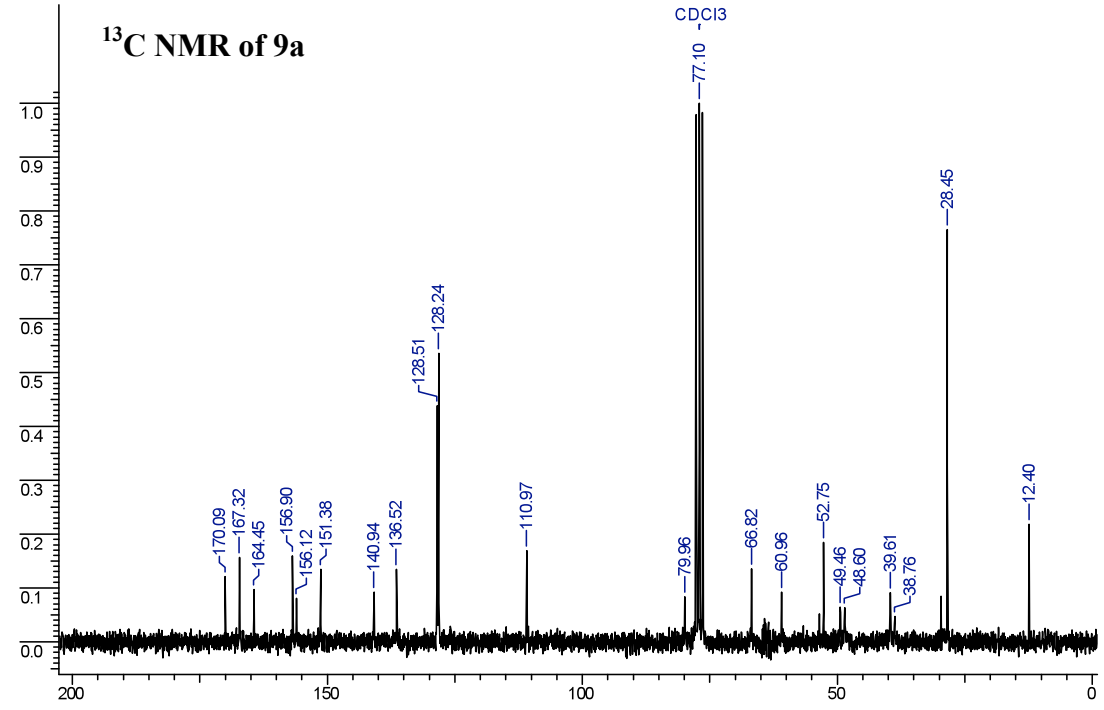

DEPT NMR of 8a
28.32
128.54
128.19
52.71
60.36
68.99
49.84
41.17
39.92
38.64
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
200
150
100
50
0

Figure S7: ^{1}H ^{13}C NMR, DEPT NMR spectra of compound 9a (solvent $CDCl_3$)






13C NMR of 9a
CDCl3
77.10
28.45
128.24
128.51
170.09
167.32
164.45
156.90
156.12
151.38
140.94
136.52
110.97
79.96
66.82
60.96
52.75
49.46
48.60
39.61
38.76
12.40

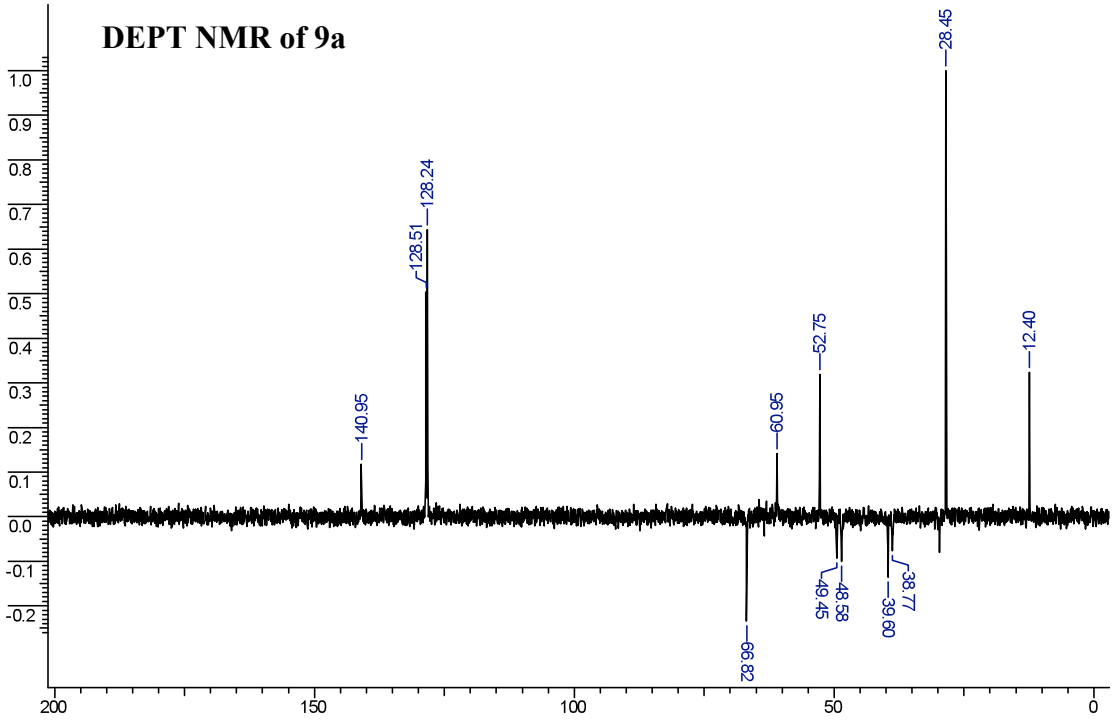
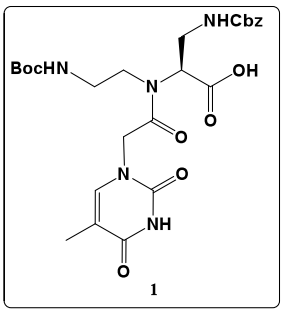
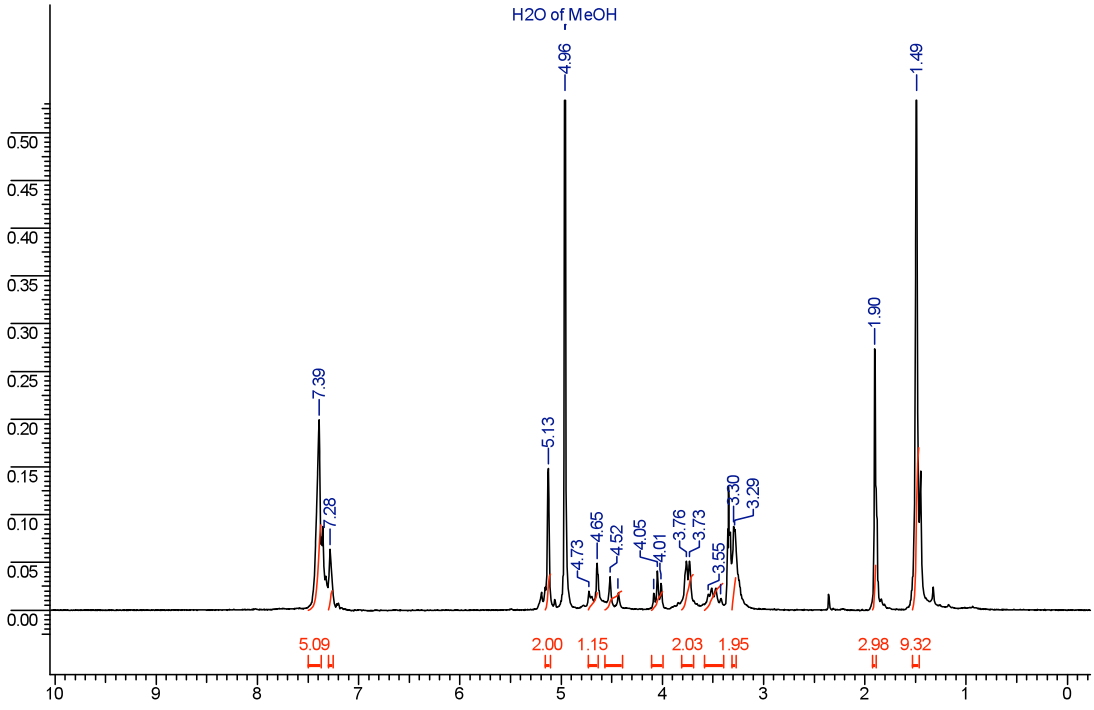

DEPT NMR of 9a
28.45
128.24
128.51
52.75
12.40
140.95
60.95
66.82
49.46
48.58
39.60
38.77

Figure S8: ^{1}H NMR and LC-MS of compound 1 (solvent MeOD)



^{1}H NMR of 1



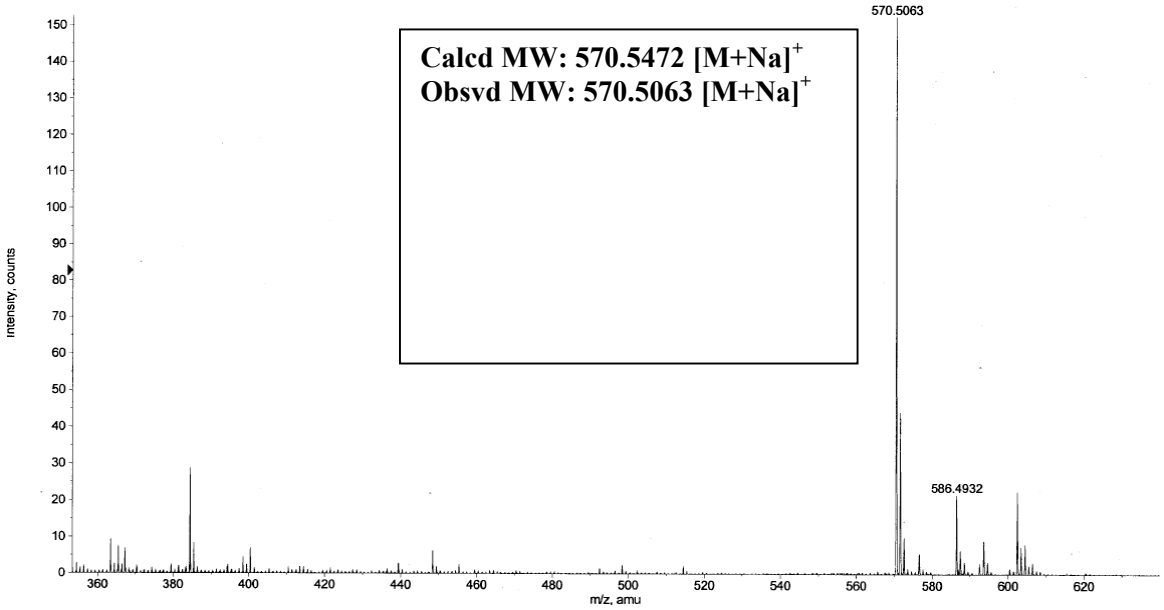
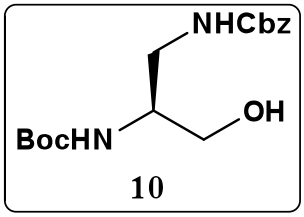
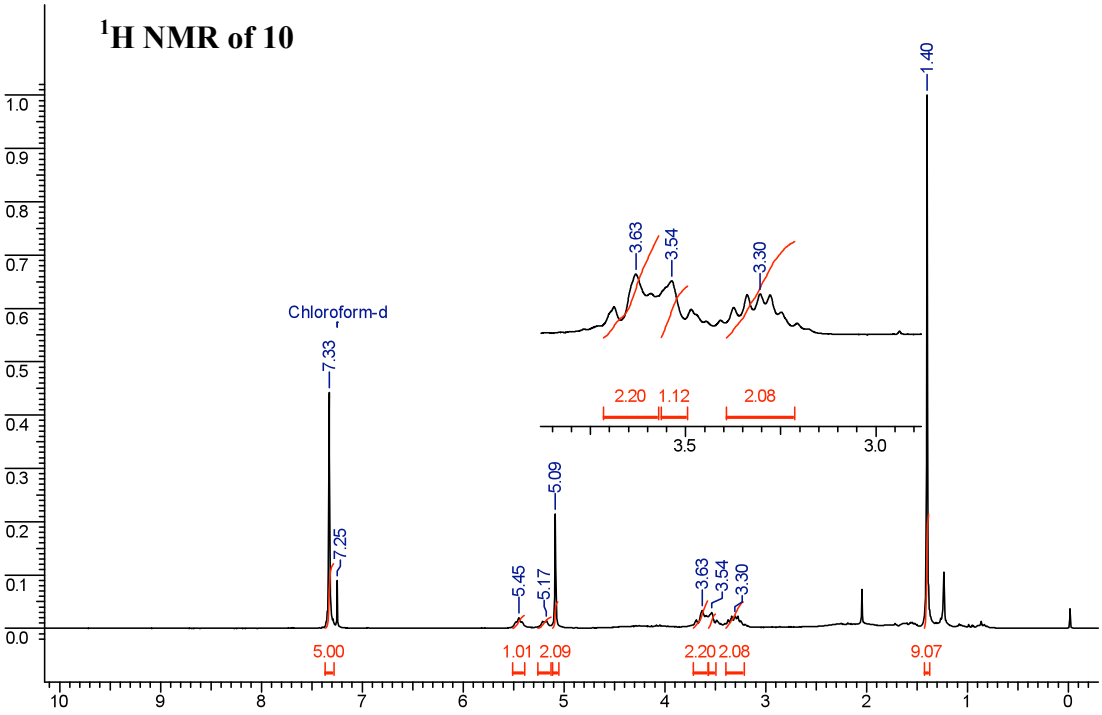
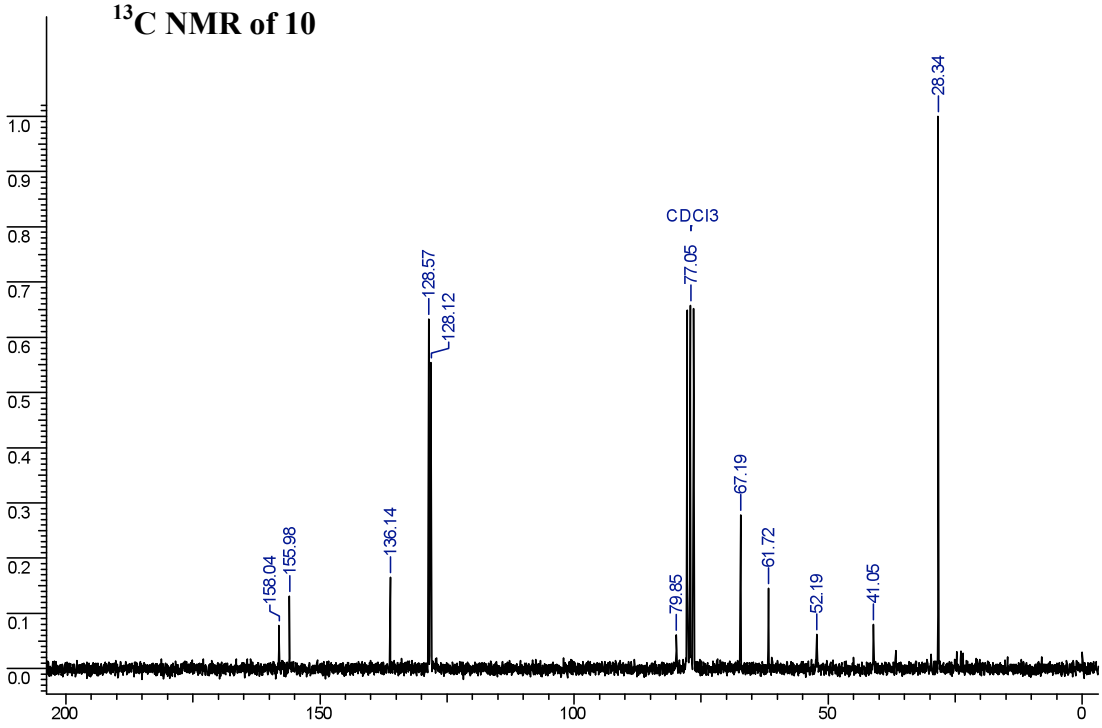

Calcd MW: 570.5472 [M+Na]+
Obsvd MW: 570.5063 [M+Na]+
570.5063
586.4932
Intensity, counts
m/z, amu

Figure S9: ^{1}H ^{13}C NMR, DEPT NMR spectra of compound 10 (solvent $CDCl_3$)





^{13}C NMR of 10



DEPT NMR of 10

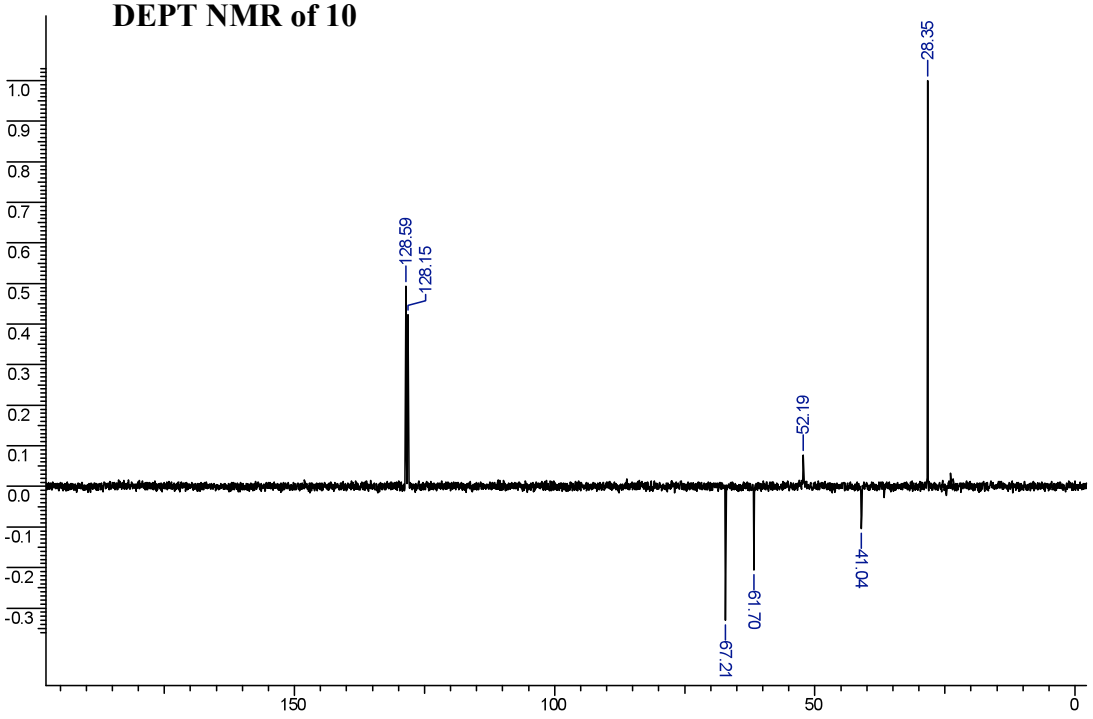
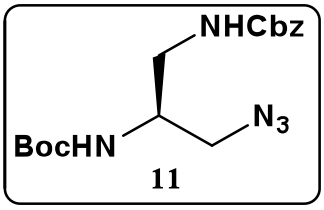
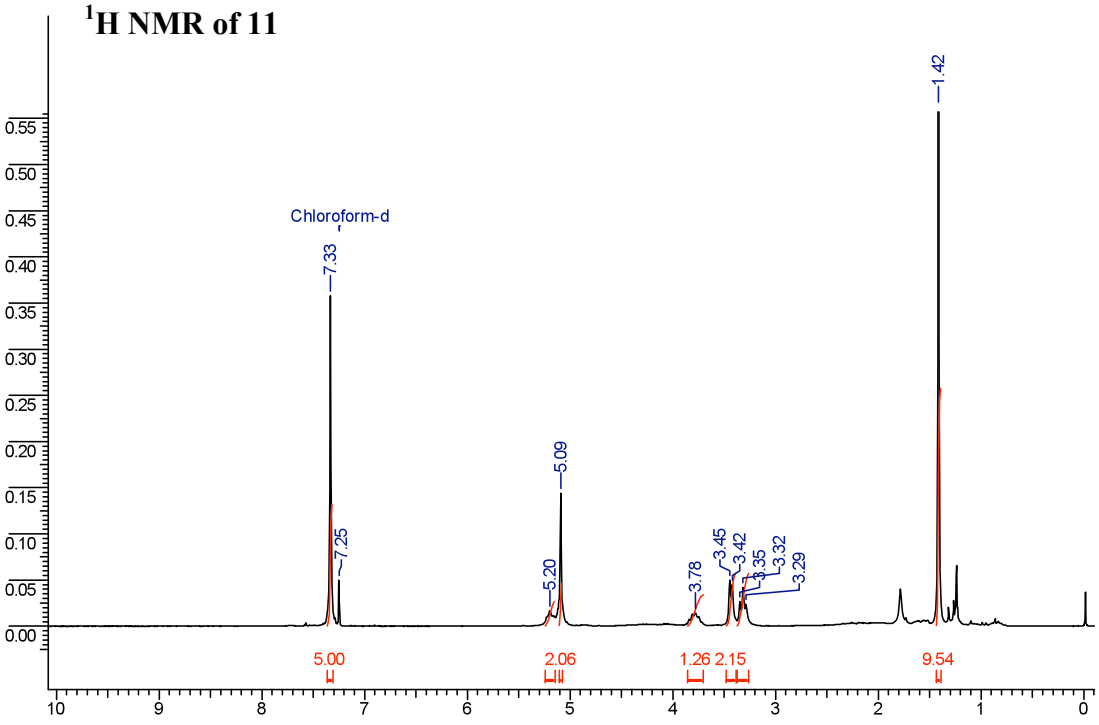
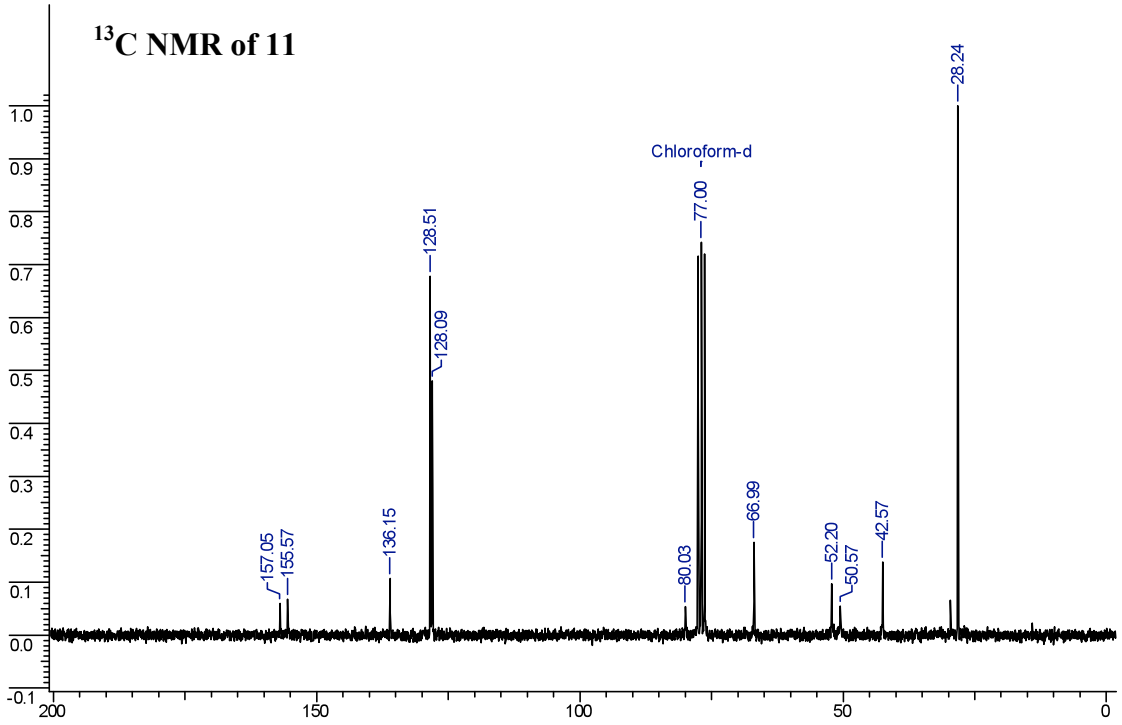


Figure S10: ^{1}H, ^{13}C NMR, DEPT NMR spectra of compound 11 (solvent $CDCl_3$)





^{13}C NMR of 11



DEPT NMR of 11

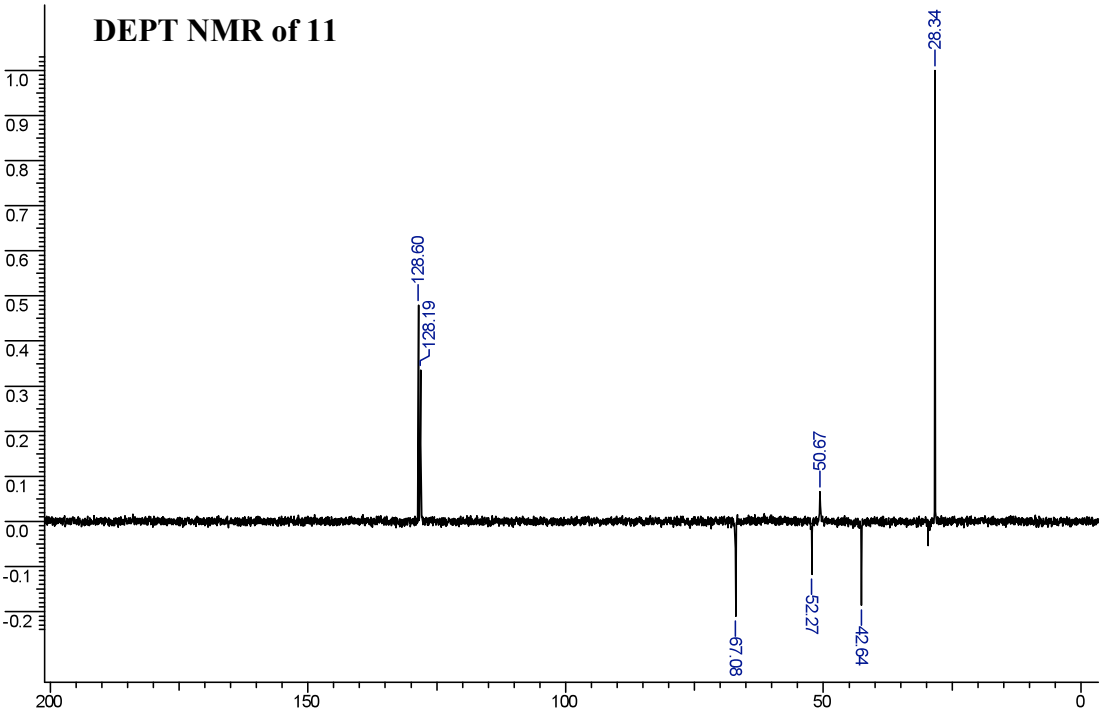
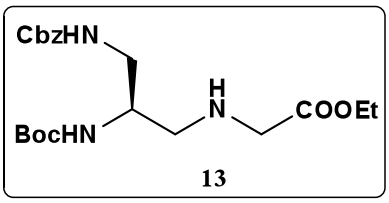
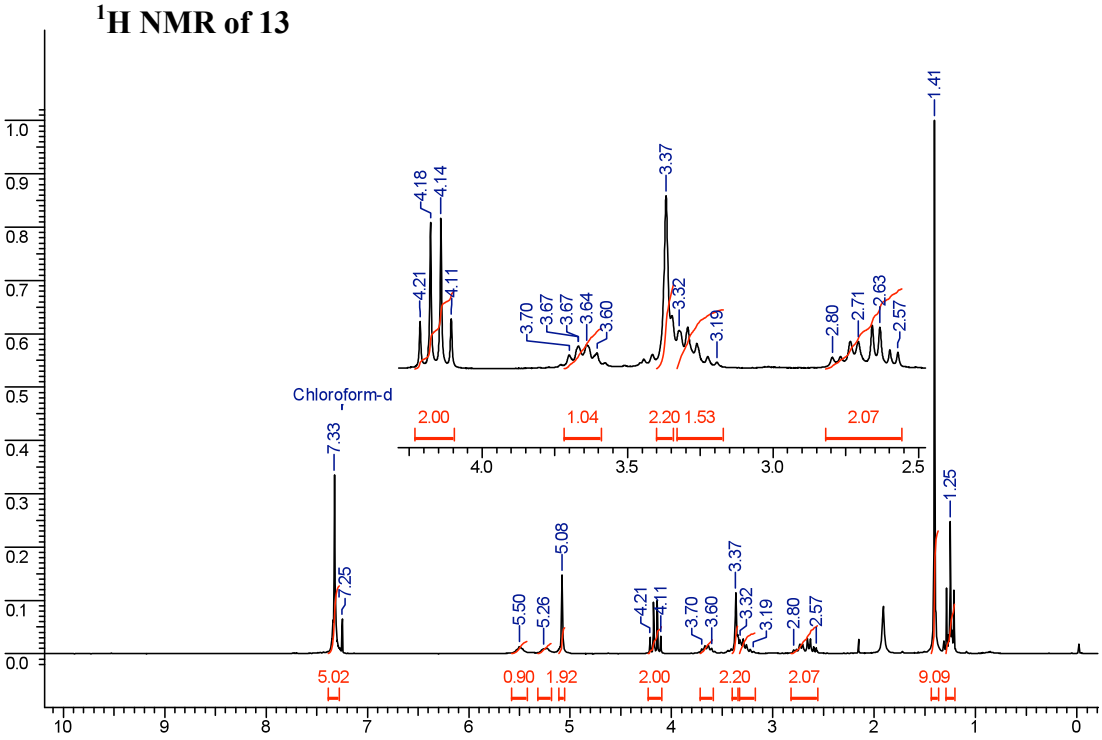
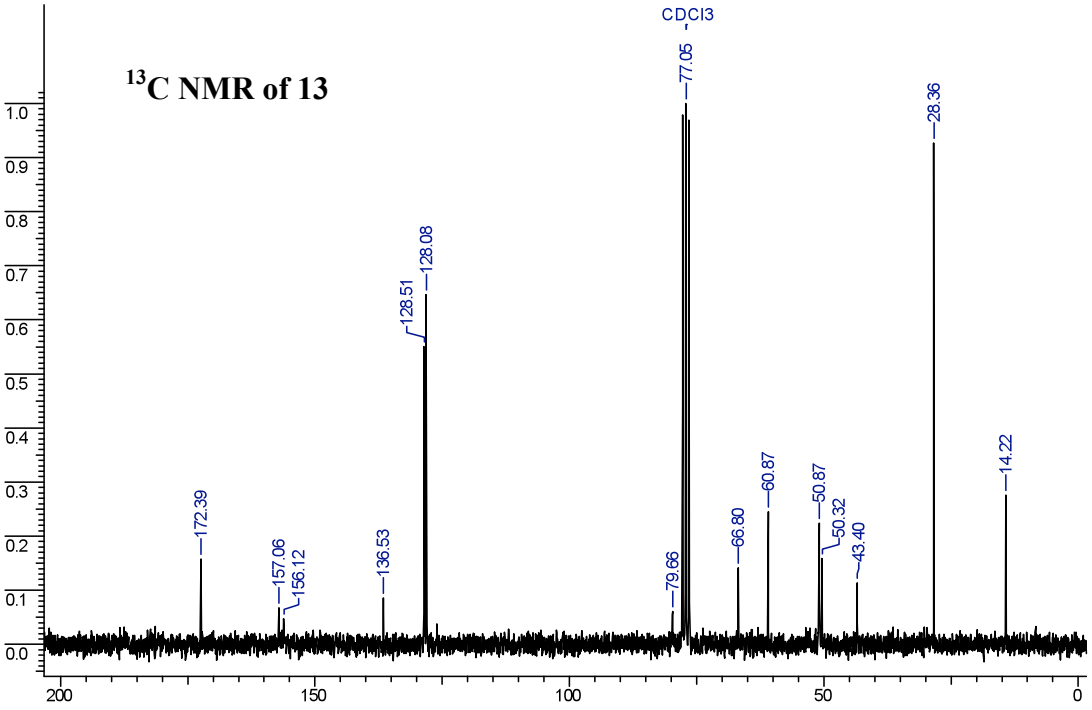


Figure S11: ^{1}H, ^{13}C NMR, DEPT NMR spectra of compound 13 (solvent $CDCl_3$)






^{13}C NMR of 13
CDCl3
77.05
28.36
128.51
128.08
172.39
157.06
156.12
136.53
79.66
66.80
60.87
50.87
50.32
43.40
14.22
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
200
150
100
50
0

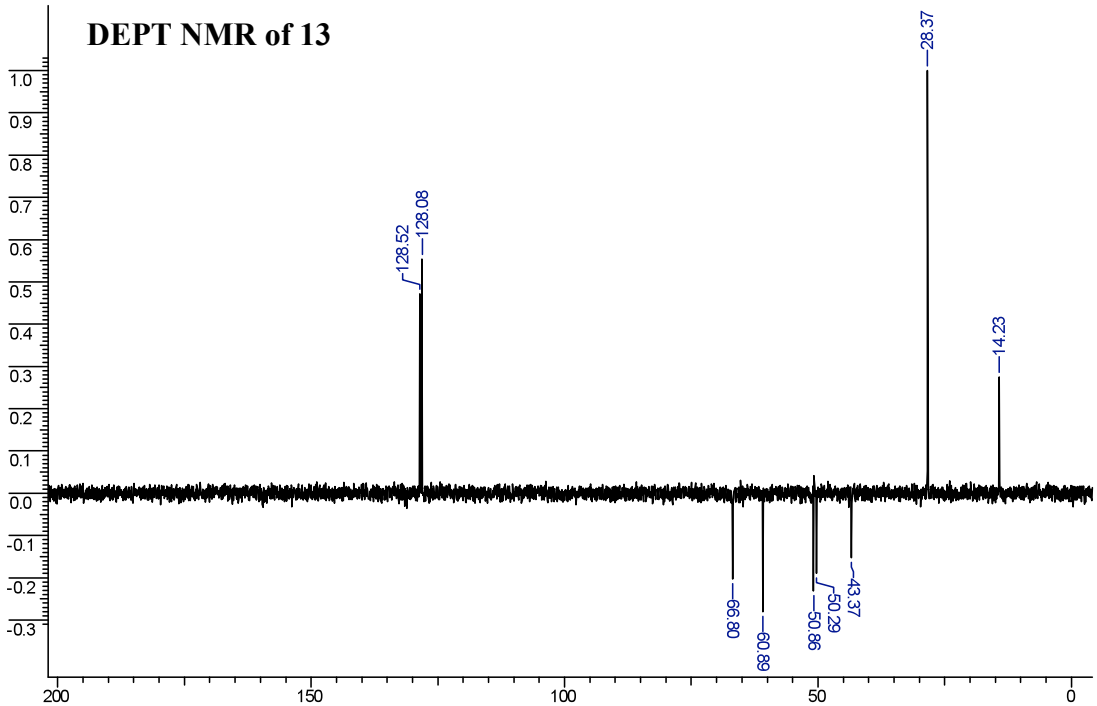
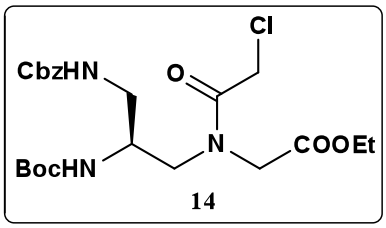
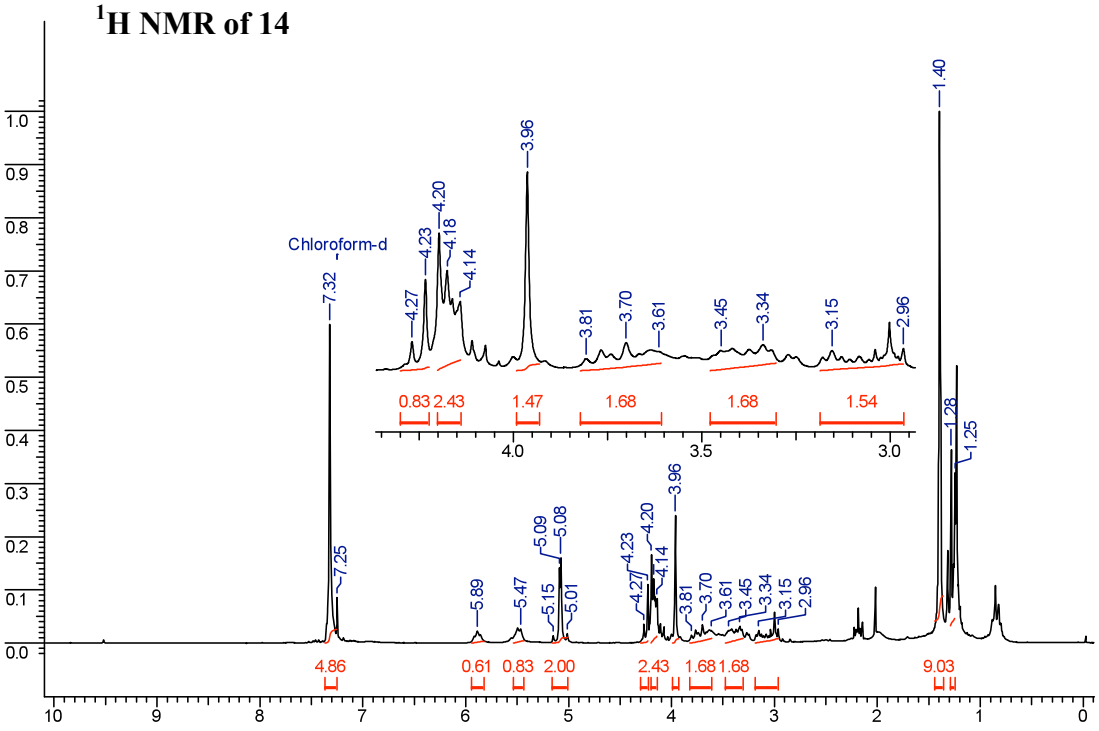
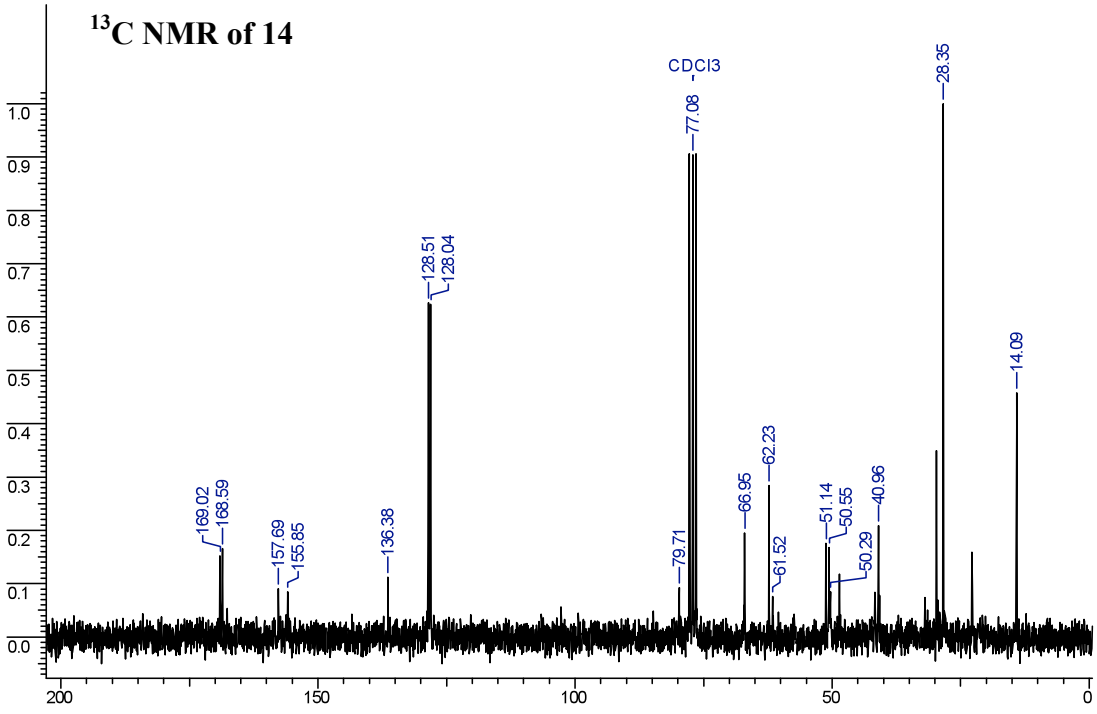

DEPT NMR of 13
28.37
128.52
128.08
14.23
66.80
60.89
50.86
50.29
43.37
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
200
150
100
50
0

Figure S12: ^{1}H, ^{13}C NMR, DEPT NMR spectra of compound 14 (solvent $CDCl_3$)






^{13}C NMR of 14
CDCl3
77.08
28.35
128.51
128.04
14.09
169.02
168.59
157.69
155.85
136.38
79.71
66.95
62.23
61.52
51.14
50.55
50.29
40.96
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
200
150
100
50
0

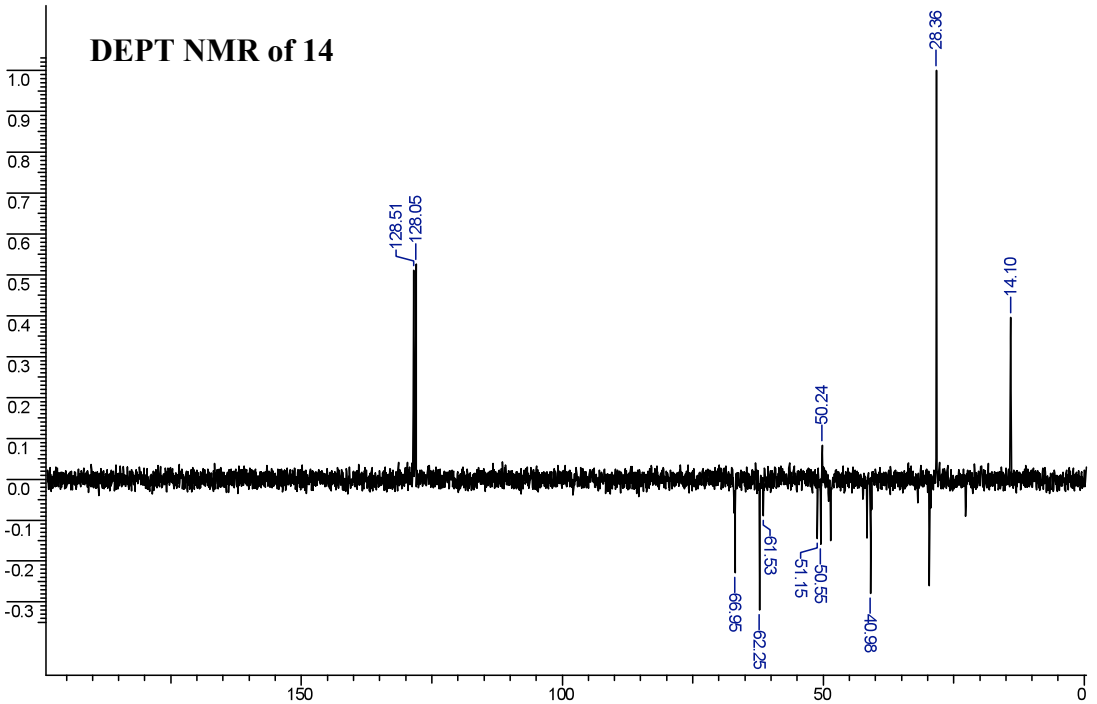
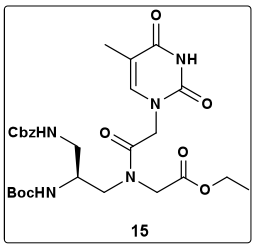
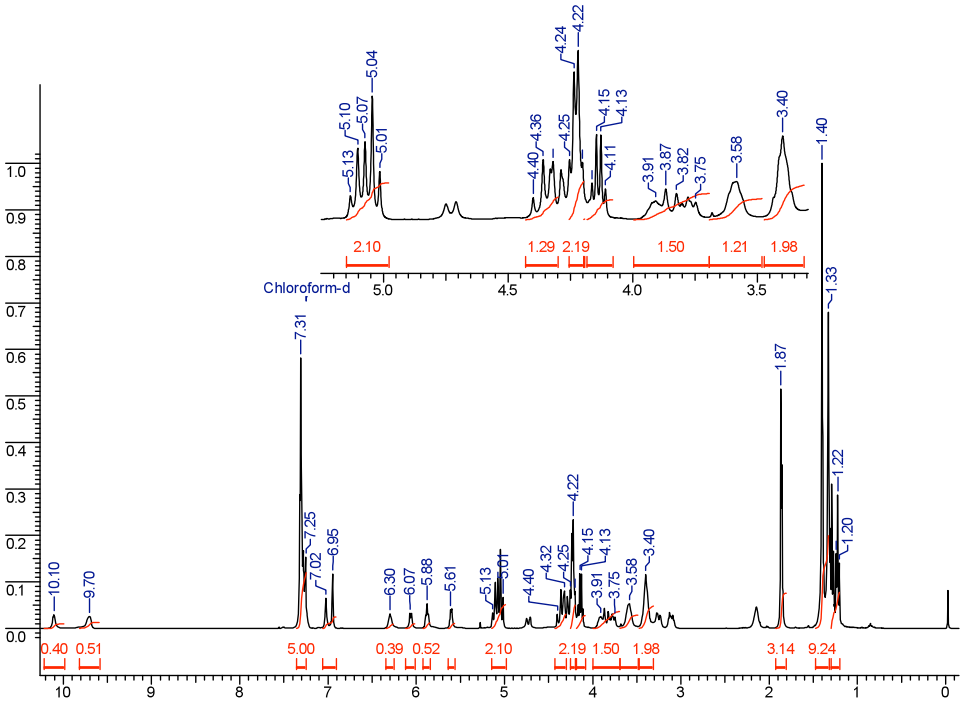

DEPT NMR of 14
28.36
128.51
128.05
14.10
50.24
66.96
62.25
61.53
51.15
50.55
40.98
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
150
100
50
0

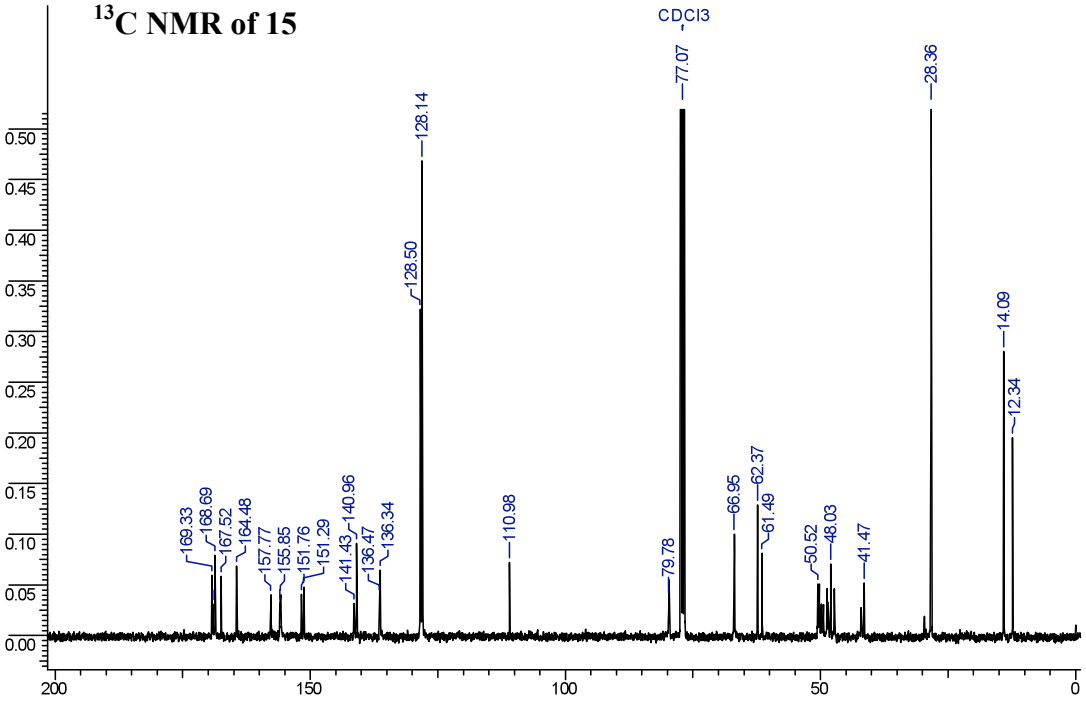
Figure S13: ^{1}H ^{13}C NMR, DEPT NMR spectra of compound 15 (solvent $CDCl_3$)



^{1}H NMR of 15



^{13}C NMR of 15



DEPT NMR of 15

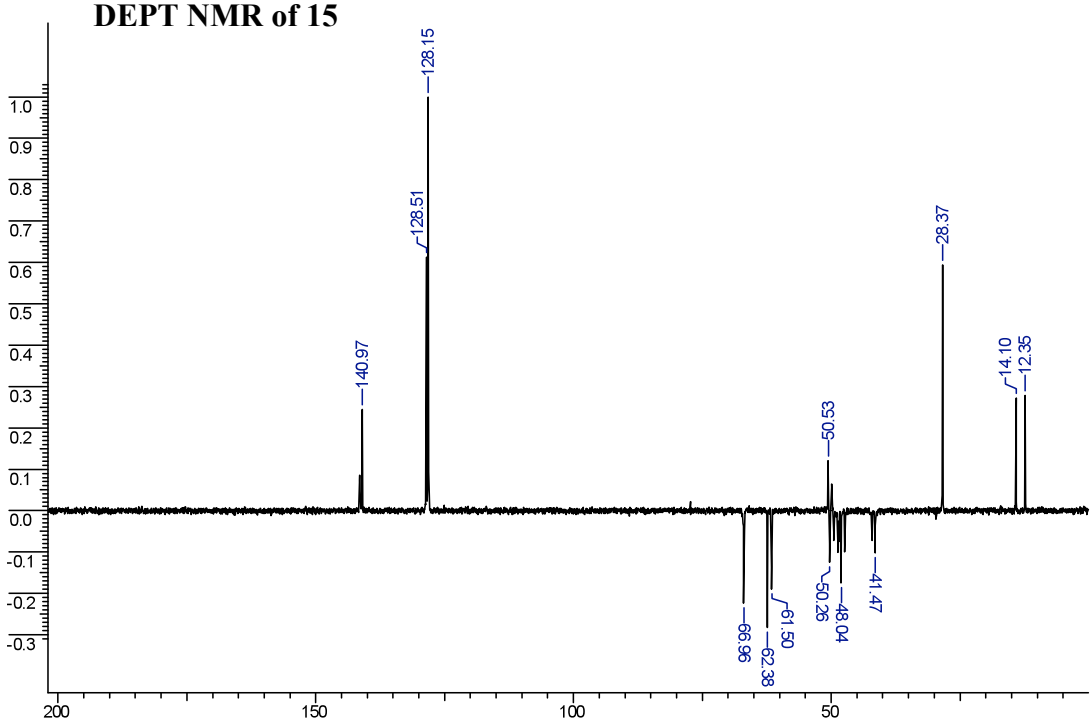
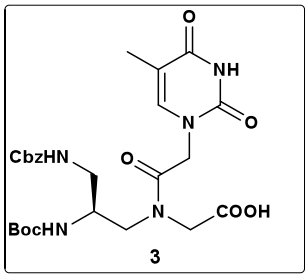
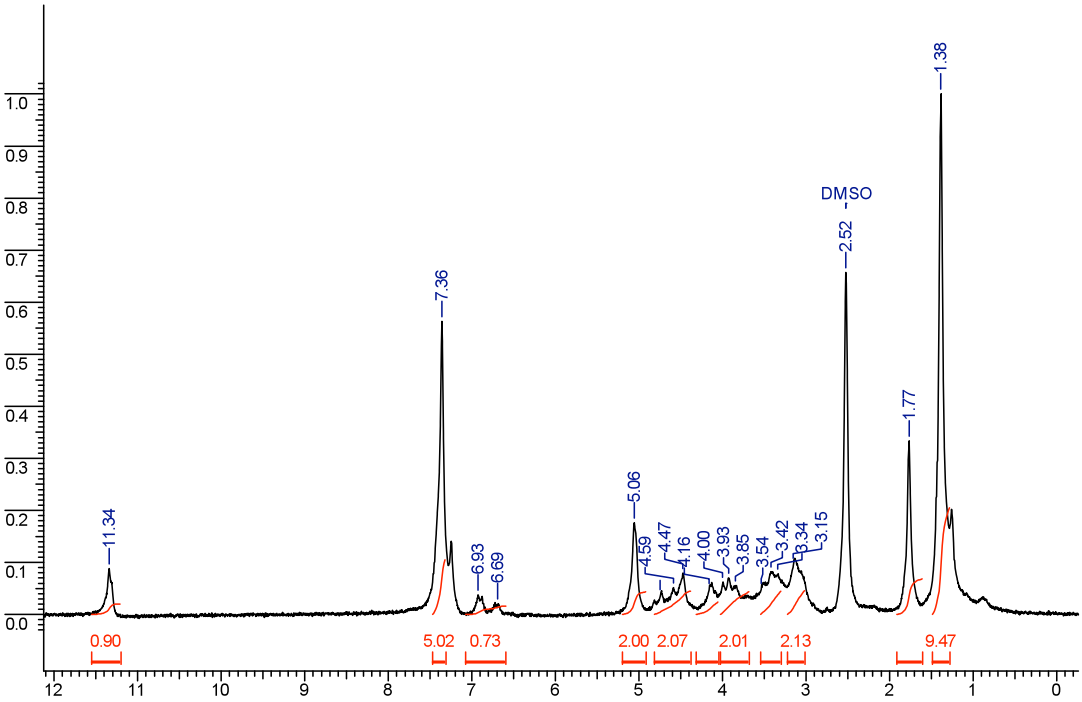


Figure S14: ^{1}H NMR and LC-MS spectra of compound 3 (solvent DMSO)



^{1}H NMR of 3



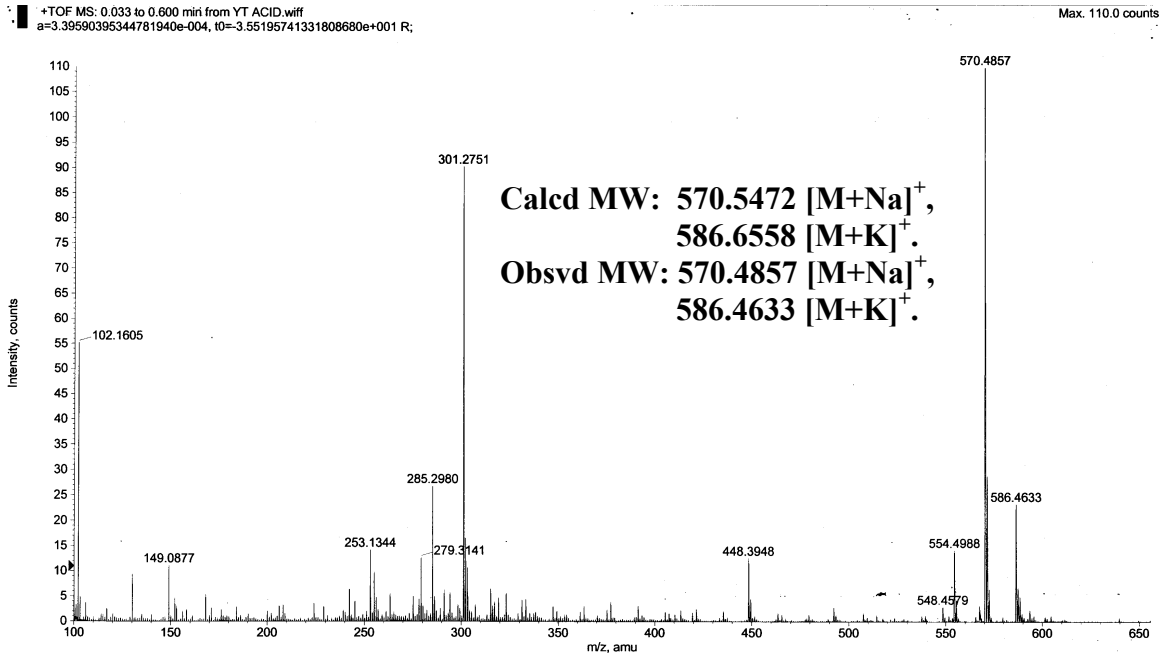
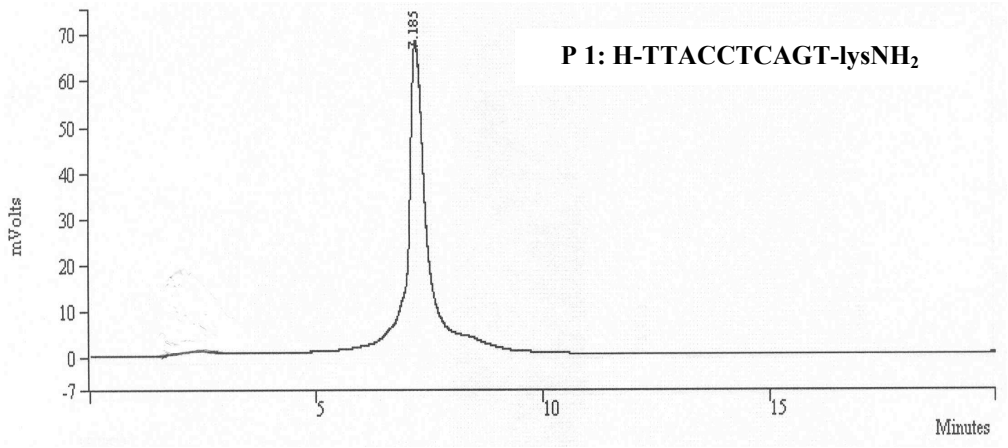
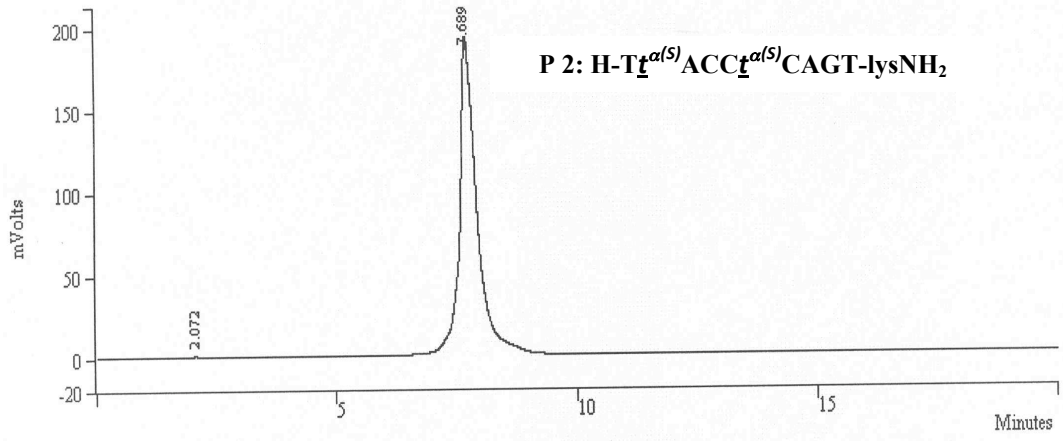

+TOF MS: 0.033 to 0.600 min from YT ACID.wiff
a=3.3959039534478194 0e-004, t0=-3.55195741331808680e+001 R;
Max. 110.0 counts
Intensity, counts
m/z, amu
570.4857
301.2751
102.1605
285.2980
586.4633
253.1344
279.3141
149.0877
448.3948
554.4988
548.4579
Calcd MW: 570.5472 [M+Na]+,
586.6558 [M+K]+.
Obsvd MW: 570.4857 [M+Na]+,
586.4633 [M+K]+.

Figure S15: HPLC Profile of P1, P2 and P3 oligomers





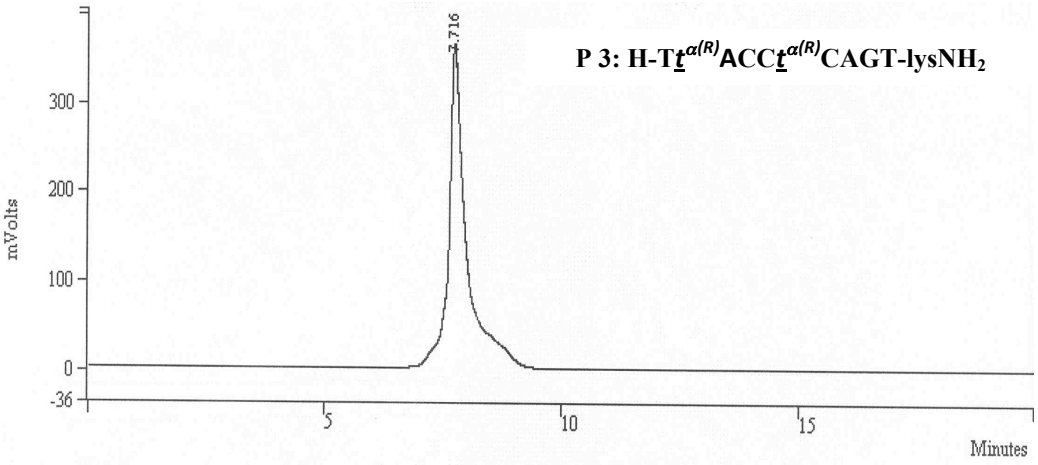


Figure S16: HPLC Profile of P4, *cf* P1 and *cf* P2 oligomers

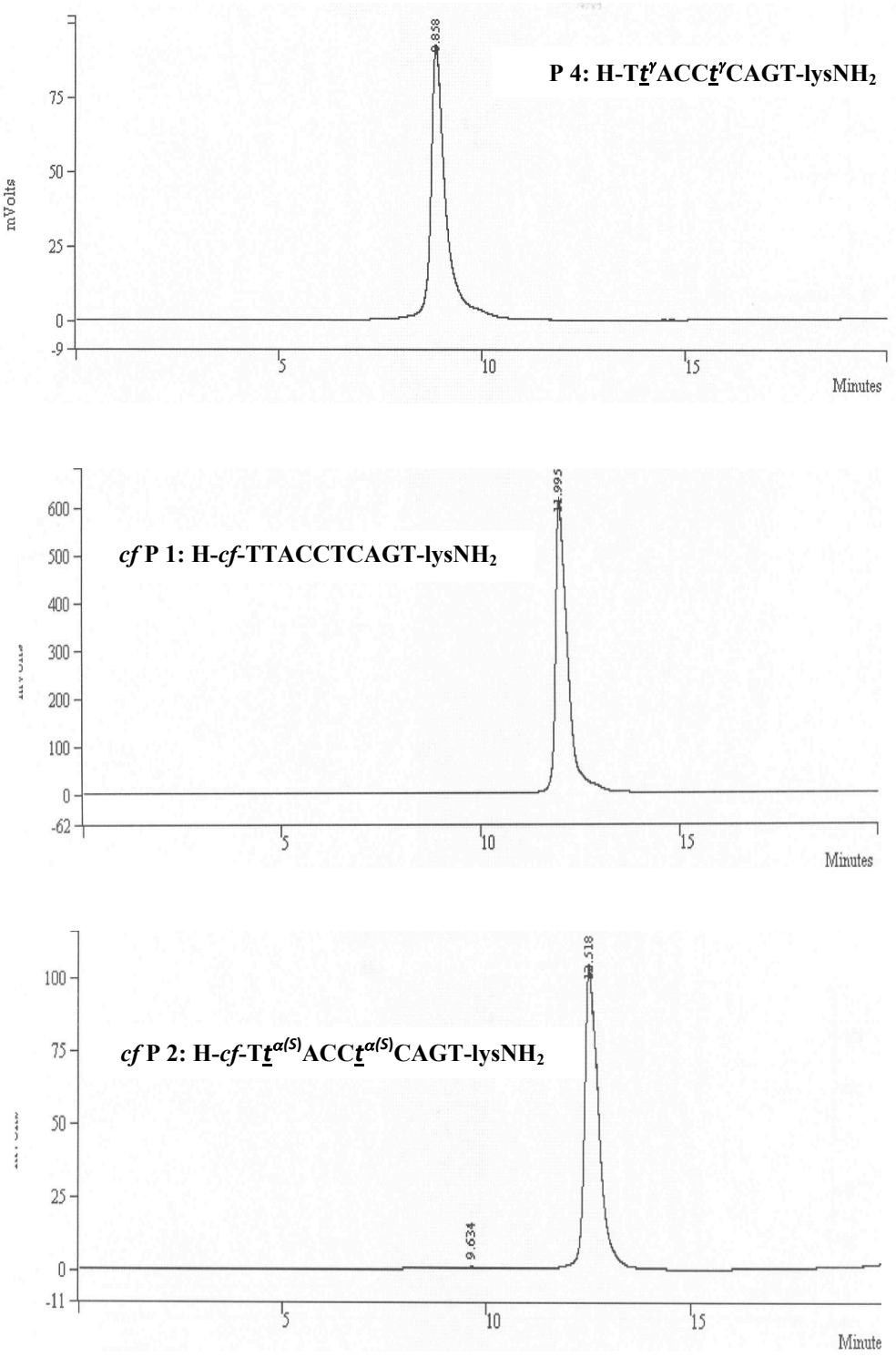
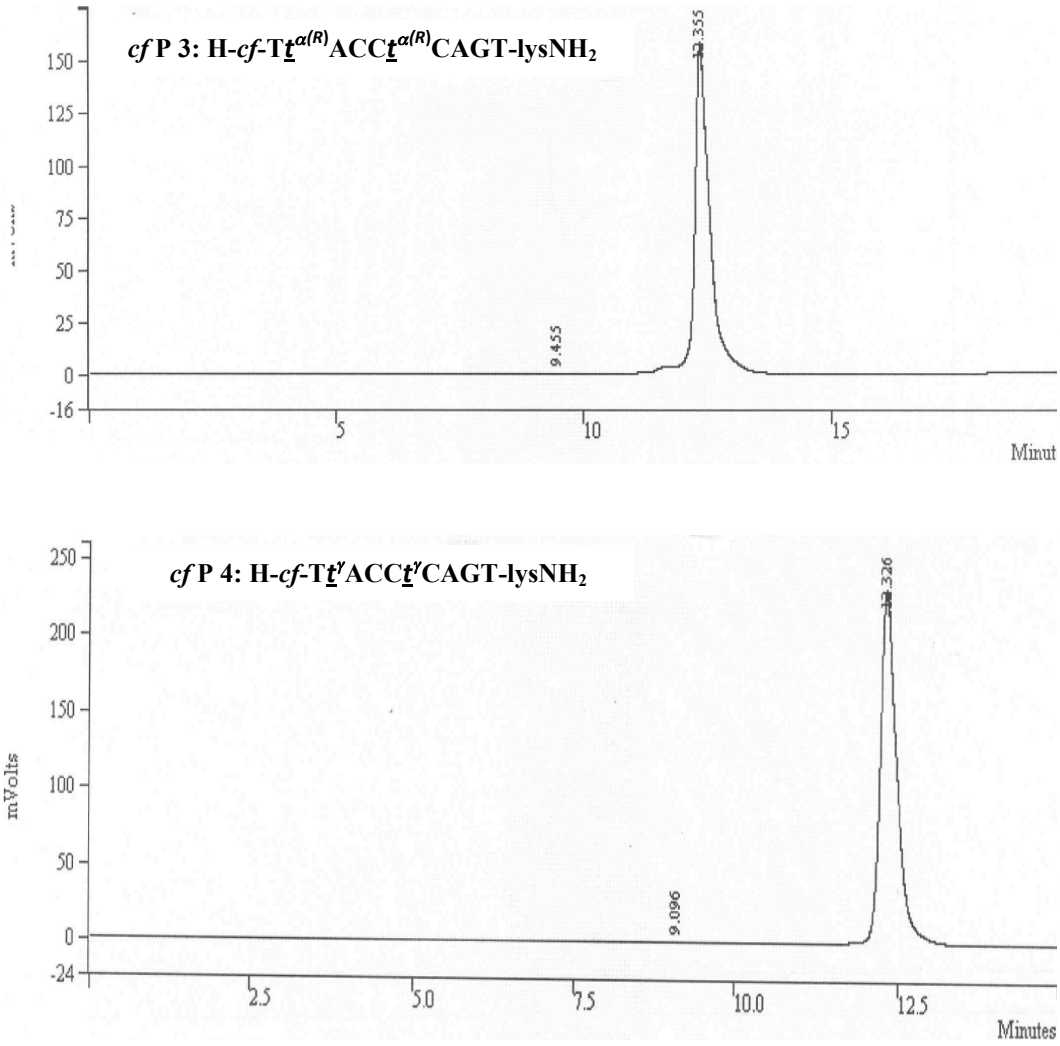
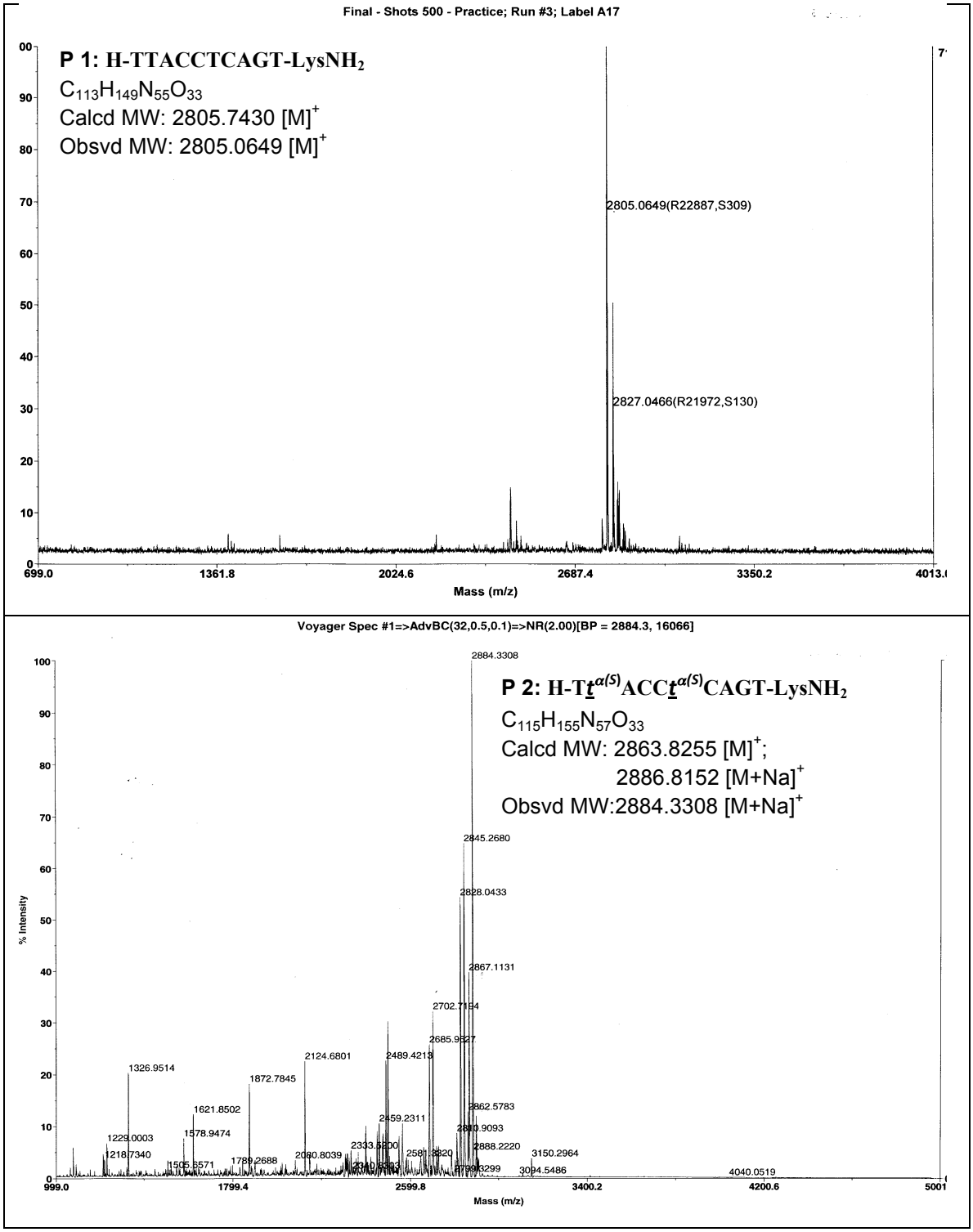


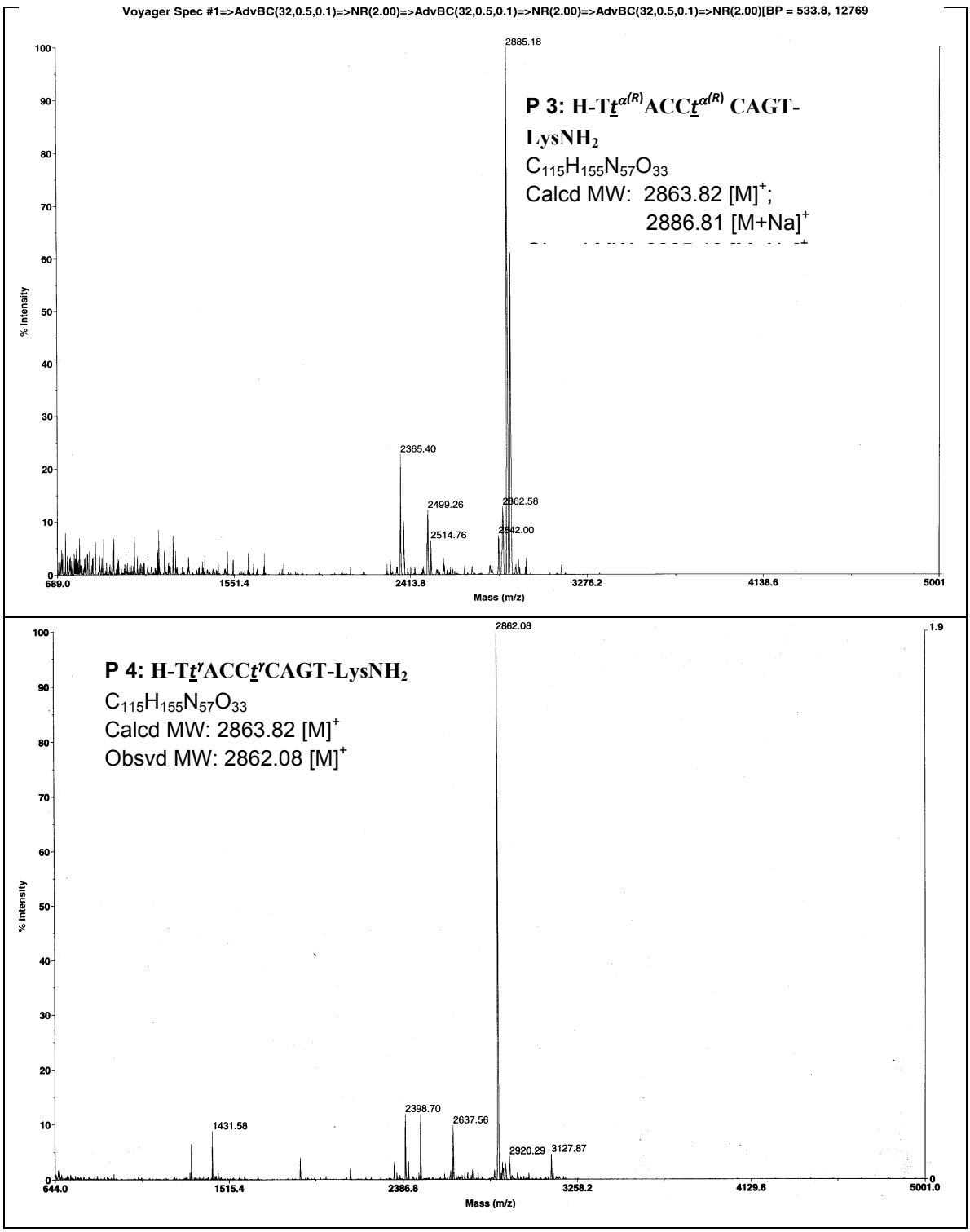
Figure S17: HPLC Profile of *cf* P3 and *cf* P4 oligomers

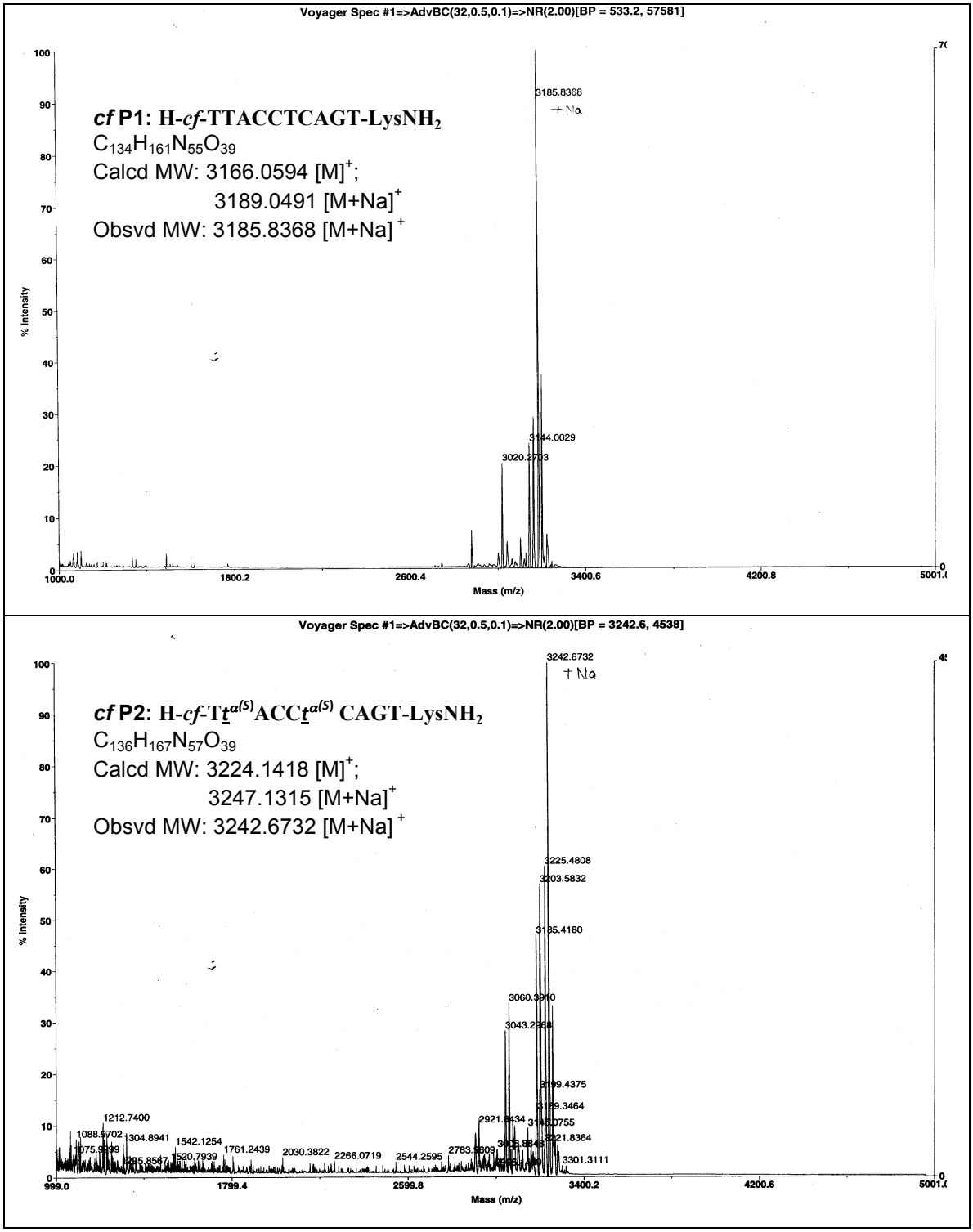


Purity of oligomers found to be more than 90% in all the cases.

Figure S18: MALDI-TOF spectra of P1-P4 and *cf* P1- P4 oligomers




Voyager Spec #1=>AdvBC(32,0.5,0.1)=>NR(2.00)=>AdvBC(32,0.5,0.1)=>NR(2.00)=>AdvBC(32,0.5,0.1)=>NR(2.00)[BP = 533.8, 12769
2885.18
P 3: H-Tt^α(R)ACCt^α(R) CAGT-LysNH2
C115H155N57O33
Calcd MW: 2863.82 [M]+;
2886.81 [M+Na]+
2365.40
2499.26
2514.76
2862.58
2642.00
% Intensity
Mass (m/z)
689.0
1551.4
2413.8
3276.2
4138.6
5001
P 4: H-Tt^γACCt^γCAGT-LysNH2
C115H155N57O33
Calcd MW: 2863.82 [M]+
Obsvd MW: 2862.08 [M]+
2862.08
1.9
2398.70
2637.56
1431.58
2920.29
3127.87
644.0
1515.4
2386.8
3258.2
4129.6
5001.0


Voyager Spec #1=>AdvBC(32,0.5,0.1)=>NR(2.00)[BP = 533.2, 57581]
cf P1: H-cf-TTACCTCAGT-LysNH2
C134H161N55O39
Calcd MW: 3166.0594 [M]+;
3189.0491 [M+Na]+
Obsvd MW: 3185.8368 [M+Na] +
3185.8368
+Na
3144.0029
3020.2703
% Intensity
Mass (m/z)
1000.0
1800.2
2600.4
3400.6
4200.8
Voyager Spec #1=>AdvBC(32,0.5,0.1)=>NR(2.00)[BP = 3242.6, 4538]
cf P2: H-cf-Tt(α(S))ACCt(α(S)) CAGT-LysNH2
C136H167N57O39
Calcd MW: 3224.1418 [M]+;
3247.1315 [M+Na]+
Obsvd MW: 3242.6732 [M+Na] +
3242.6732
+Na
3225.4808
3203.5832
3185.4180
3060.3910
3043.2360
3199.4375
3159.3464
3140.0755
3221.8364
3301.3111
1212.7400
1088.9702
1304.8941
1542.1254
1075.9299
1520.7939
1761.2439
2030.3822
2266.0719
2544.2595
2783.5609
2921.8134
% Intensity
Mass (m/z)
999.0
1799.4
2599.8
3400.2
4200.6

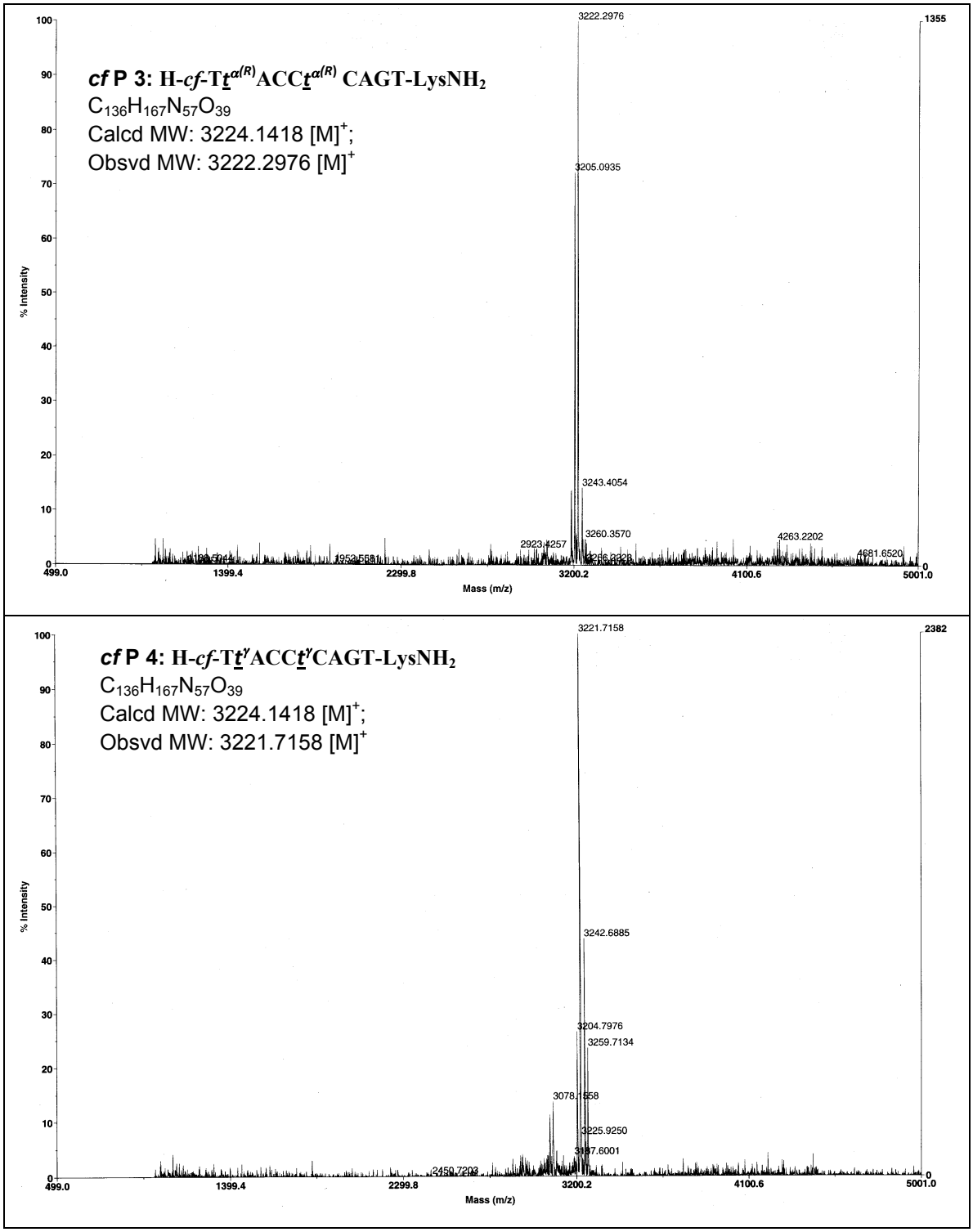
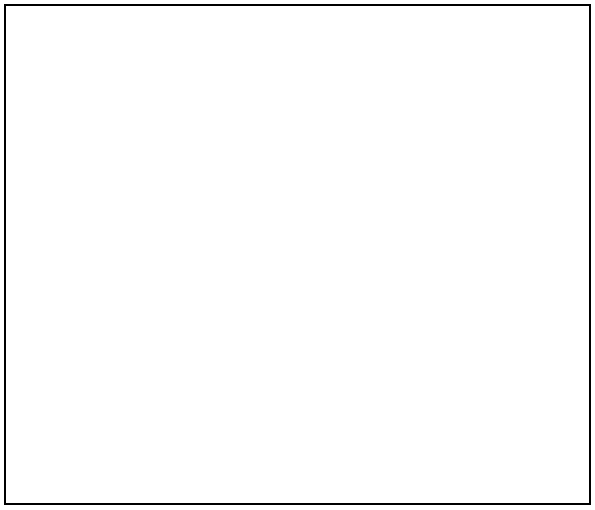

cf P 3: H-cf-Tt^α(R)ACCt^α(R) CAGT-LysNH2
C136H167N57O39
Calcd MW: 3224.1418 [M]+;
Obsvd MW: 3222.2976 [M]+
3222.2976
1355
3205.0935
3243.4054
3260.3570
2923.4257
4263.2202
4681.6520
% Intensity
Mass (m/z)
499.0
1399.4
2299.8
3200.2
4100.6
5001.0
cf P 4: H-cf-Tt^γACCt^γCAGT-LysNH2
C136H167N57O39
Calcd MW: 3224.1418 [M]+;
Obsvd MW: 3221.7158 [M]+
3221.7158
2382
3242.6885
3204.7976
3259.7134
3225.9250
2459.7203
% Intensity
Mass (m/z)

Figure S19: UV-Jobs plot of doubly modified *am*-PNA:DNA duplexes (P**2**, P**3** and P**4**) with complementary antiparallel DNA **1**



[10/20/2010 22:51 "/Graph1" (0-50)]
Linear Regression for Data1_B:
Y = A + B * X

Parameter	Value	Error
A	3.07238	0.02518
B	-0.01223	8.31685E-4

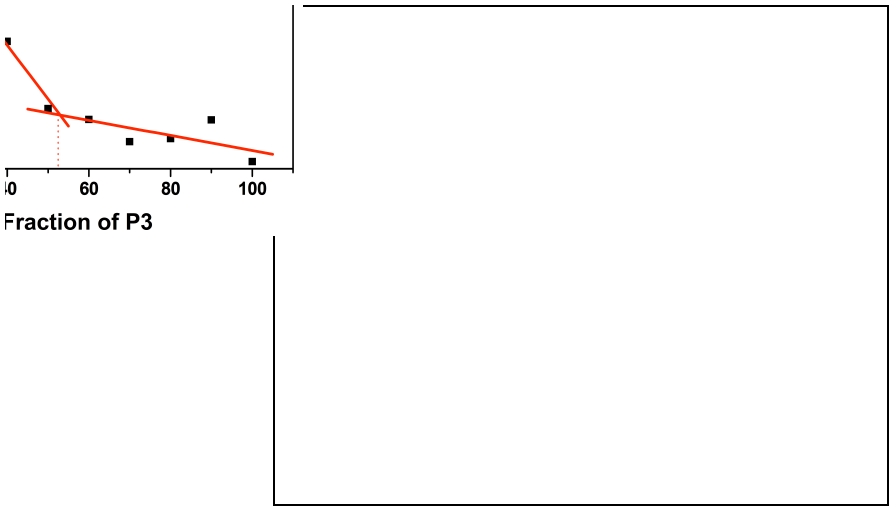
R	SD	N	P
-0.99088	0.03479	6	1.24512E-4

[10/20/2010 22:58 "/Graph2" (50-100]
Linear Regression for Data1_B:
Y = A + B * X

Parameter	Value	Error
A	0.63952	0.12898
B	0.03803	0.00168

R	SD	N	P
0.99613	0.07015	6	<0.0001

Mole Fraction	0	10	20	30	40	50	60	70	80	90	100
Absorbance at 260 nm	3.10	2.95	2.79	2.71	2.55	2.50	2.91	3.32	3.80	4.02	4.4



[10/20/2010 22:09 "/Graph1" (0-50)]
Linear Regression for Data1_B:
Y = A + B * X

Parameter	Value	Error
A	0.15796	0.00342
B	-0.0011	1.12927E-4

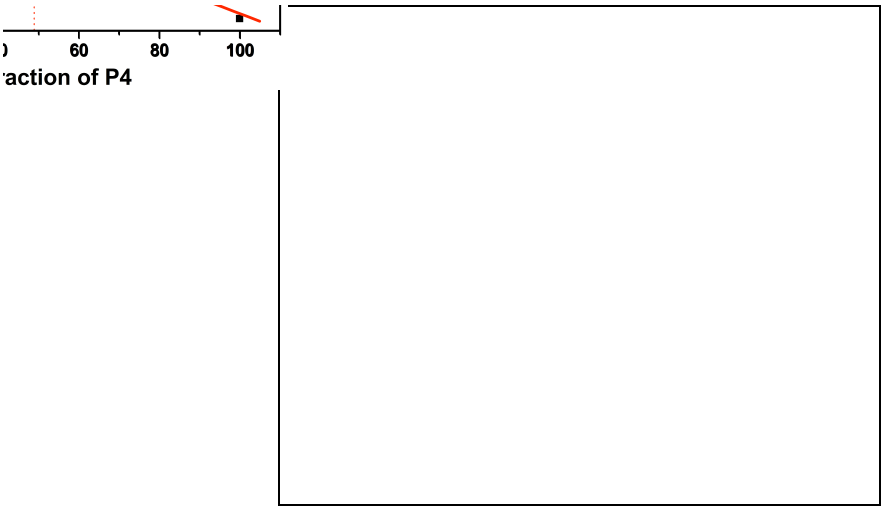
R	SD	N	P
-0.97939	0.00472	6	6.32819E-4

[10/20/2010 22:18 "/Graph2" (50-100)]
Linear Regression for Data1_B:
Y = A + B * X

Parameter	Value	Error
A	0.10802	0.00552
B	-1.53429E-4	7.17862E-5

R	SD	N	P
-0.73017	0.003	6	0.09939

Mole Fraction	0	10	20	30	40	50	60	70	80	90	100
Absorbance at 260 nm	0.1519	0.1528	0.1394	0.1233	0.1149	0.1012	0.0990	0.0945	0.0951	0.0989	0.0904



[10/20/2010 23:11 "/Graph1" (0-50)]
Linear Regression for Data1_D:
Y = A + B * X

Parameter	Value	Error
A	0.15585	0.00217
B	-9.49429E-4	7.16667E-5

R	SD	N	P
-0.9888	0.003	6	1.87608E-4

[10/20/2010 23:16 "/Graph2" (50-100)]
Linear Regression for Data1_D:
Y = A + B * X

Parameter	Value	Error
A	0.12357	0.00423
B	-2.84286E-4	5.50362E-5

R	SD	N	P
-0.93254	0.0023	6	0.00667

Mole Fraction	0	10	20	30	40	50	60	70	80	90	100
Absorbance at 260 nm	0.1519	0.1496	0.1390	0.1285	0.1160	0.1077	0.1098	0.1020	0.1000	0.0998	0.0942

Figure S20: CD-Jobs plot of doubly modified *am*-PNA:DNA duplexes (P**3** and P**4**) with complementary antiparallel DNA **1**

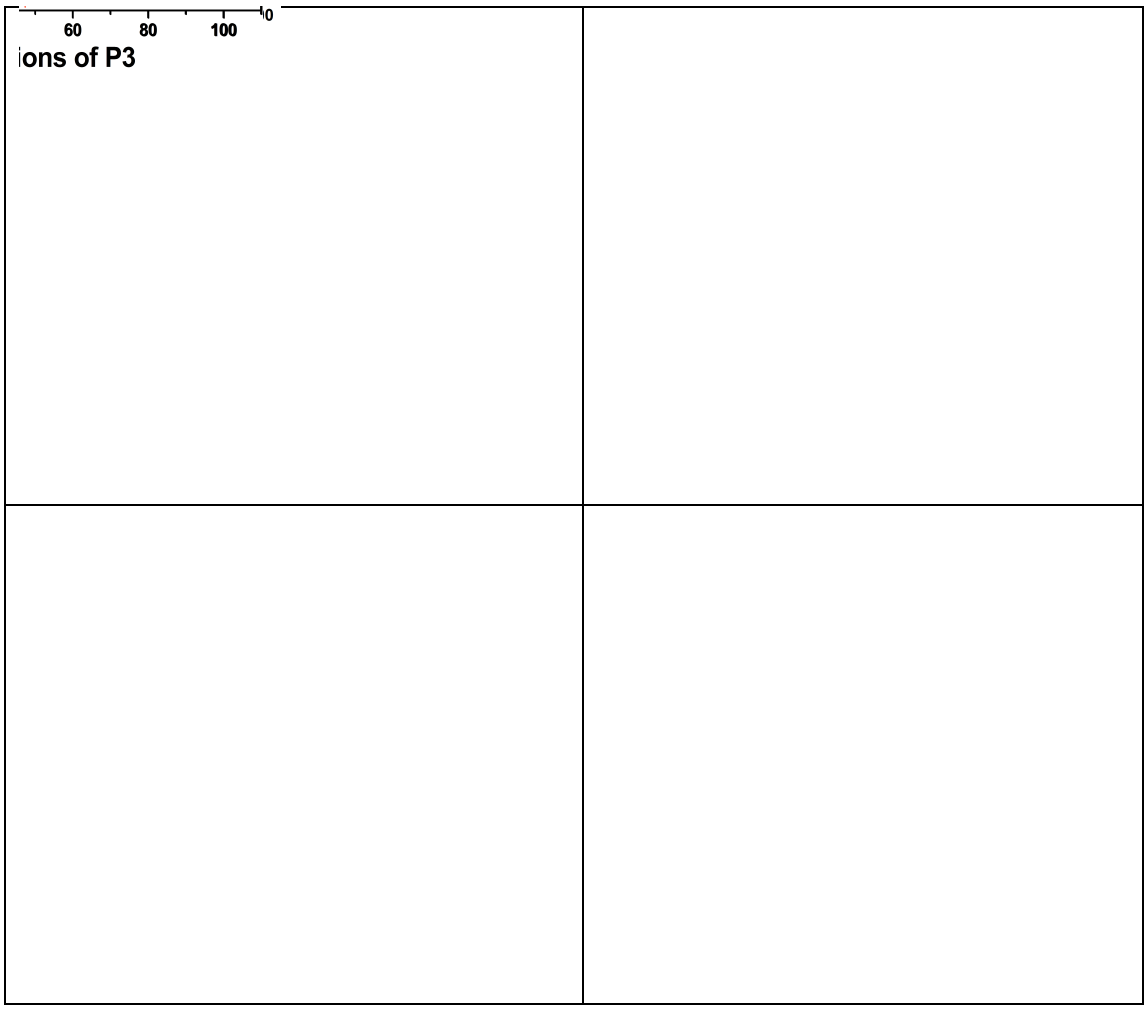


Figure S21: CD spectra of aeg PNA P1, α-(*S-am*)*aeg*-PNA P2; α-(*R-am*)*aeg*-PNA P3 and γ-(*S-am*)*aeg*-PNA P4 each at 2 μM strand concentration in 10 mM sodium phosphate (pH 7.4) with 10 mM NaCl

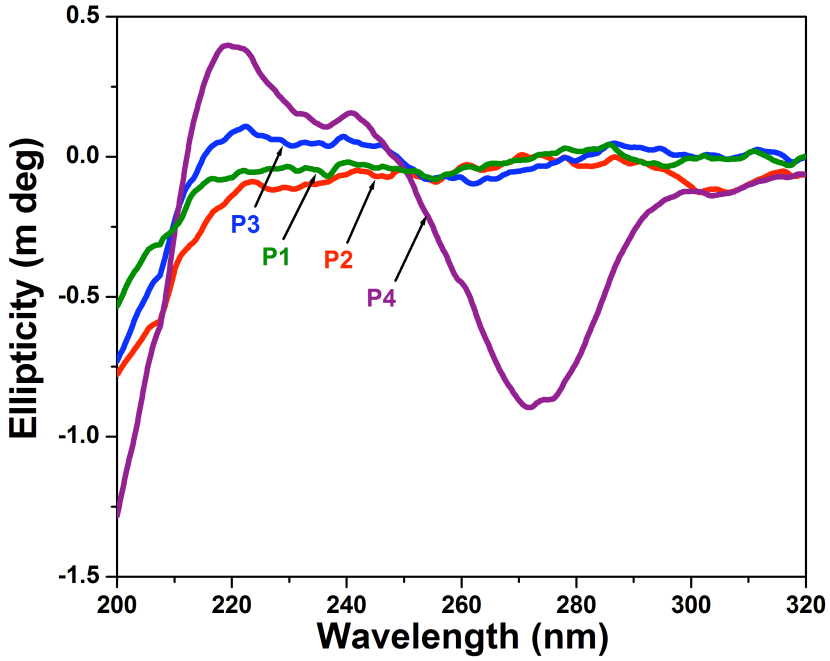


Figure S22: UV-Melting profile of PNA:DNA duplexes of P**1**, P**2**, P**3** and P**4** with complementary antiparallel DNA **1**, parallel DNA **2** and mismatch DNA **3**

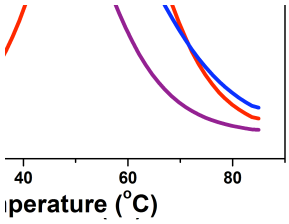

40
60
80
perature (°C)

Figure S23: UV-Absorbance spectra of cf**P 1-4** and their corresponding Fluorescence emission spectra at 490 nm