# Supporting Information

## Pyrrolidinyl peptide nucleic acid with α/β-peptide backbone: A conformationally constrained PNA with unusual hybridization properties

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### *N-tert*-Butoxycarbonyl-*cis*-4-hydroxy-L-proline diphenylmethyl ester (1a)

*N-tert*-Butoxycarbonyl-*trans*-4-hydroxy-L-proline diphenylmethyl ester (1.21 g, 3.0 mmol), triphenylphosphine (1.00 g, 3.6 mmol) and formic acid (0.12 mL, 3.0 mmol) were dissolved in dry THF (10 mL) in a dried 100 mL round bottom flask equipped with a magnetic bar and then cool down to 0 °C in an ice-salt bath. The solution was stirred under nitrogen balloon and DIAD (0.70 mL, 3.6 mmol) was added dropwise over 15 min and the reaction mixture was allowed to stir at 30 °C for 8 h. Chromatographic purification afforded the *cis*-4-formate ester intermediate (1.78 g, quantitative yield). $^1$H NMR (400 MHz, $CDCl_3$, TMS): $\delta$=1.32 and 1.51 (2×s, 9H; C$\underline{H}_3$ Boc rotamers), 2.32–2.39 [m, 1H; 1×C$\underline{H}_2$(3′)], 2.45–2.50 [m, 1H; 1×C$\underline{H}_2$(3′)], 3.52–3.79 [m, 2H; C$\underline{H}_2$(5′)], 4.53–4.68 [2×m, 1H; C$\underline{H}$(2′)], 5.25–5.33 [m, 1H; C$\underline{H}$(4′)], 6.95 and 6.99 (2×s, 1H; C$\underline{H}$Ph$_2$ rotamers), 7.24–7.37 (m, 10H, Ar C$\underline{H}$); $^{13}$C NMR (100 MHz, $CDCl_3$, TMS): $\delta$=28.1 and 28.4 (C$\underline{H}_3$ Boc rotamers), 35.4 and 36.3 [C$\underline{H}_2$(3′) rotamers], 52.0 and 52.3 [C$\underline{H}_2$(5′) rotamers], 57.6 and 57.9 [C$\underline{H}$(2′) rotamers], 71.4 and 72.5 [C$\underline{H}$(4′) rotamers], 77.6 and 77.7 (C$\underline{C}$H$_3$ Boc rotamers), 80.4 and 80.5 (C$\underline{H}$ Dpm rotamers), 127.1–128.5 (C$\underline{H}$ Ar Dpm rotamers) 139.7 (C$\underline{C}$ Dpm), 153.6 and 153.9 (C$\underline{O}$ Boc rotamers), 159.9 (C$\underline{H}$O) 170.5 and 170.7 (C$\underline{O}$ ester rotamers).

A portion of this formate ester (1.21 g, 3.0 mmol) was dissolved in methanol (10 mL) and concentrated aqueous ammonia solution (1 mL) was added. After 1 h, the ammonolysis was completed as indicated by TLC analysis. The solvent was removed under reduced pressure and the oily residue was purified by column chromatography on silica gel using ethyl acetate:hexanes (1:2) as eluent to give **1a** as a clear viscous oil (1.78 g, quantitative yield). $[\alpha]^{25}_D$=–14.6 (*c*=3.28 in $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, TMS) (Figure S3): $\delta$=1.27 and 1.49 (2×s, 9H, C$\underline{H}_3$ Boc), 2.09 [t, *J*=7.6 Hz, 1H; 1×C$\underline{H}_2$(3′)], 2.28–2.42 [m, 1H; 1×C$\underline{H}_2$(3′)], 3.56–3.70 [m, 2H; C$\underline{H}_2$(5′)], 4.31–4.38 [br, 1H; C$\underline{H}$(4′)], 4.45 and 4.57 [2×m, 1H; C$\underline{H}$(2′)], 6.91 and 6.99 (2×s, 1H; C$\underline{H}$Ph$_2$ rotamers), 7.29–7.37 (m, 10H, Ar C$\underline{H}$); $^{13}$C NMR (100 MHz, $CDCl_3$, TMS) (Figure S4): $\delta$=28.0 and 28.3 (C$\underline{H}_3$ Boc rotamers), 37.7 and 38.6 [C$\underline{H}_2$(3′) rotamers], 55.2 and 55.6 [C$\underline{H}_2$(5′) rotamers], 58.1 [C$\underline{H}$(2′)], 69.9 and 70.8 [C$\underline{H}$(4′) rotamers], 77.9 and 78.4 (C$\underline{C}$H$_3$ Boc rotamers), 80.2 and 80.4 (C$\underline{H}$ Dpm rotamers) 126.9–128.5 (C$\underline{H}$ Ar Dpm), 139.5 (C$\underline{C}$ Dpm), 153.8 and 154.3 (C$\underline{O}$ Boc rotamers), 173.3 and 173.6 (C$\underline{O}$ ester rotamers); elemental analysis calcd (%) for $C_{23}H_{27}NO_5$: C 69.50, H 6.85, N 3.52; found: C 69.51, H 6.68, N 3.51.

### *N-tert*-Butoxycarbonyl-(2'*S*,4'*R*)-(*N*$^3$-benzoylthymin-1-yl)-proline diphenyl methyl ester (2a)

*N-tert*-Butoxycarbonyl-*cis*-4-hydroxy-L-proline diphenylmethyl ester (**1a**) (1.21 g, 3.04 mmol), triphenylphosphine (1.23 g, 4.56 mmol) and *N*$^3$-benzoylthymine (1.05

mL, 4.56 mmol) were dissolved in dry THF (20 mL) and then cool down to 0 °C in an ice-salt bath. The solution was stirred under nitrogen balloon and diisopropyl azodicarboxylate (DIAD) (0.88 mL, 4.56 mmol) was added dropwise over a period of 15 min. The reaction mixture was allowed to stir at 30 °C for 8 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography through silica gel using ethyl acetate:hexanes (1:2) as eluent to give **2a** as a clear viscous oil (1.12 g, 60% yield). m.p.=103–105 °C; $[\alpha]^{25}_{D}$=–36.0 (*c*=1.06 in $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, TMS) (Figure S5): $\delta$=1.31 and 1.50 (2×s, 9H, C$\underline{H}_3$ Boc rotamers), 1.98 (s, 3H; C$\underline{H}_3$ Thymine), 2.32–2.44 [m, 1H; 1×C$\underline{H}_2$(3′)], 2.53–2.66 [m, 1H; 1×C$\underline{H}_2$(3′)], 3.61–3.82 [2×m, 1H; 1×C$\underline{H}_2$(5′)], 3.92–4.00 [m, 1H; 1×C$\underline{H}_2$(5′)], 4.57–4.72 [2×m, 1H; C$\underline{H}$(4′)], 5.11–5.19 [m, 1H; C$\underline{H}$(2′)], 6.91 and 6.96 (2×s, 1H; C$\underline{H}$Ph$_2$), 7.05 [s, 1H; C(6)$\underline{H}$ Thymine], 7.29–7.37 [m, 10H, Ar Dpm], 7.52 [t, *J*=7.7 Hz, 2H; C$\underline{H}$(3,5) Bz] 7.68 [t, *J*=7.3 Hz, 1H; C$\underline{H}$(4) Bz], 7.94 [d, *J*=7.5 Hz, 2H; C$\underline{H}$(2,6) Bz]; $^{13}$C NMR (100 MHz, $CDCl_3$, TMS) (Figure S6): $\delta$=12.7 (C$\underline{H}_3$ Thymine), 28.0 and 28.3 (C$\underline{H}_3$ Boc rotamers), 33.4 and 35.0 [C$\underline{H}_2$(3′) rotamers], 49.1 and 49.6 [C$\underline{H}_2$(5′) rotamers], 53.8 and 54.4 [C$\underline{H}$(4′) rotamers], 57.8 and 58.0 [C$\underline{H}$(2′) rotamers], 77.9 and 78.1 (C$\underline{C}$H$_3$ Boc rotamers), 81.1 and 81.2 (C$\underline{H}$ Dpm rotamers), 111.6 (C$\underline{5}$ Thymine), 126.8–128.6 (C$\underline{H}$ Ar Dpm), 129.2 (C$\underline{4}$ Bz), 130.4 (C$\underline{2}$ and C$\underline{6}$ Bz), 131.3 (C$\underline{3}$ and C$\underline{5}$ Bz), 135.2 and 136.2 (C$\underline{1}$ Bz), 149.6 (C$\underline{}$ Dpm), 153.4 and 153.8 (C$\underline{2}$ Thymine), 162.5 (C$\underline{4}$ Thymine), 168.9 (C$\underline{O}$ Boc), 170.8 (C$\underline{O}$ ester); elemental analysis calcd (%) for $C_{35}H_{35}N_3O_7$: C 68.95, H, 5.79, N, 6.89; found: C 68.97, H, 5.80, N 6.89.

**_N_-Fluoren-9-ylmethoxycarbonyl-(2'*S*,4'*R*)-(thymin-1-yl)-proline (3a)**

*N-tert*-Butoxycarbonyl-(2'*S*,4'*R*)-4-(*N*$^3$-benzoylthymin-1-yl)proline diphenylmethyl ester (**2a**) (0.80 g, 1.32 mmol) was treated with 10% anisole in trifluoroacetic acid (5 mL) at room temperature overnight. The solvents were removed under a stream of nitrogen. After washing with diethyl ether, the residue was dissolved in a minimum amount of 1:1 aqueous acetonitrile and was treated with solid $NaHCO_3$ until pH=8 followed by addition of FmocOSu (0.49 g, 1.45 mmol). After stirring at room temperature overnight, the reaction mixture was extracted with diethyl ether and the aqueous phase acidified with conc HCl. The titled compound was obtained as a white solid after filtration and washing with water (0.33 g, 53% yield). $[\alpha]^{25}_{D}$=+1.80 (*c*=1.00 in DMF); $^1$H NMR (400 MHz, DMSO-$d_6$, TMS) (Figure S7): $\delta$=1.80 (s, 3H; C$\underline{H}_3$ Thymine), 2.20–2.33 [m, 1H; 1×C$\underline{H}_2$(3′)], 2.59–2.74 [m, 1H; 1×C$\underline{H}_2$(3′)], 3.46–3.50 [m, 1H; 1×C$\underline{H}_2$(5′)], 3.73–3.83 [m, 1H; 1×C$\underline{H}_2$(5′)], 4.24–4.31 (m, 3H; C$\underline{H}$ and C$\underline{H}_2$ Fmoc), 4.41–4.57 [2×m, 1H; C$\underline{H}$(2′) rotamers], 5.04–5.12 [m, 1H; C$\underline{H}$(4′)], 7.31–7.36 (m, 2H; C$\underline{H}$ Fmoc), 7.41–7.46 (m, 2H; Ar C$\underline{H}$ Fmoc), 7.62 [s, 1H; C(6)$\underline{H}$ Thymine], 7.59–7.68 (m, 2H; Ar C$\underline{H}$ Fmoc), 7.90–7.92 (m, 2H; Ar C$\underline{H}$ Fmoc) 11.36, 11.39 (2×s, 1H; COO$\underline{H}$ rotamers); $^{13}$C NMR (100 MHz, DMSO-$d_6$, TMS) (Figure S8): $\delta$=12.6 (C$\underline{H}_3$ Thymine), 32.6 and 33.7 [C$\underline{H}_2$ (3′) rotamers], 46.9 and 47.0 (C$\underline{H}$ Fmoc rotamers), 49.2 and 49.4 [C$\underline{H}_2$(5′) rotamers], 52.2 and 53.2 [C$\underline{H}$(4′) rotamers], 57.9 and 58.1 [C$\underline{H}$(2′) rotamers], 67.3 and 67.6 [C$\underline{H}_2$ Fmoc rotamers], 109.9 (C$\underline{5}$ Thymine), 120.5 and 120.6 (C$\underline{H}$ Ar Fmoc rotamers), 125.5–125.7 (C$\underline{H}$ Ar Fmoc), 127.6 (C$\underline{H}$ Ar Fmoc), 128.1 (C$\underline{H}$ Ar Fmoc), 138.0 and 138.1 [C$\underline{(6)}$H Thymine rotamers], 141.1 (C$\underline{}$ Ar Fmoc), 144.0 (C$\underline{}$ Ar Fmoc), 151.3 (C$\underline{2}$ Thymine), 153.9 and 154.2 (C$\underline{O}$ Fmoc rotamers), 164.1 (C$\underline{4}$ Thymine), 173.5 and 173.8

(COOH rotamers); HRMS (FAB$^+$): *m/z* calcd for $C_{25}H_{24}O_6N_3$: 462.1665 [*M*+H]$^+$; found: 462.1646.

***N*-Fluoren-9-ylmethoxycarbonyl-(2'*R*,4'*S*)-(thymin-1-yl)-proline (3b)**

Synthesis of the titled compound was accomplished in the same way as described for compound **3a** above starting from the known intermediate *N-tert*-butoxycarbonyl--(2'*R*,4'*S*)-4-($N^3$-benzoylthymin-1-yl)proline diphenylmethyl ester (**2b**)[2] (1.57 g, 2.57 mmol) to afford the compound **3b** as a white solid (0.97 g, 82% yield). $[\alpha]^{25}_D$=–2.90 (*c*=1.00 in DMF)$^1$H NMR (400 MHz, DMSO-$d_6$, TMS) (Figure S9): $\delta$=1.80 (s, 3H; C$H_3$ Thymine), 2.21–2.33 [m, 1H; 1×C$H_2$(3′)], 2.60–2.75 [m, 1H; 1×C$H_2$(3′)], 3.45–3.53 [m, 1H; 1×C$H_2$(5′)], 3.74–3.83 [m, 1H; 1×C$H_2$(5′)], 4.18–4.41 (m, 3H; CH Fmoc and C$H_2$ Fmoc), 4.41 and 4.57 [2×m, 1H; CH(2′) rotamers], 5.05–5.12 [m, 1H; CH(4′)], 7.32–7.36 (m, 2H; CH Ar Fmoc), 7.40–7.46 (m, 2H; CH Ar Fmoc), 7.62 [m, 1H; C(6)H Thymine], 7.65–7.67 (m, 2H; CH Ar Fmoc), 7.89–7.92 (m, 2H; CH Ar Fmoc), 11.36 and 11.39 (2×s, 1H; $CO_2$H rotamers); $^{13}$C NMR (100 MHz, DMSO-$d_6$, TMS) (Figure S10): $\delta$=12.6 (C$H_3$ Thymine), 32.6 and 33.7 [C$H_2$(3′) rotamers], 46.9 (CH Fmoc), 49.2 and 49.4 [C$H_2$(5′) rotamers], 52.2 and 53.2 [CH(4′) rotamers], 57.8 and 58.0 [CH(2′) rotamers], 67.3 and 67.6 (C$H_2$ Fmoc rotamers), 109.9 (C5 Thymine), 120.5 (CH Ar Fmoc), 125.6 (CH Ar Fmoc), 127.6 (CH Ar Fmoc), 128.1 (CH Ar Fmoc), 138.0 [C(6)H Thymine], 141.1 (CH Ar Fmoc), 144.0 and 144.1 (C Ar Fmoc rotamers), 151.3 (C2 Thymine), 153.9 and 154.2 (CO Fmoc rotamers), 164.1 (C4 Thymine), 173.5 and 173.8 (COOH rotamers); HRMS (FAB$^+$): *m/z* calcd for $C_{25}H_{24}O_6N_3$: 462.1665 [*M*+H]$^+$; found: 462.1665.

***N*-Fluoren-9-ylmethoxycarbonyl-(2'*S*,4'*S*)-(thymin-1-yl)-proline (3c)**

Synthesis of the titled compound was accomplished in the same way as described for compound **3a** above starting from the known intermediate *N-tert*-butoxycarbonyl-(2'*S*,4'*S*)-($N^3$-benzoylthymin-1-yl)-proline diphenylmethyl ester (**2c**)[2] (1.10 g, 1.80 mmol to afford the compound **3c** as a white solid (0.59 g, 72% yield). $[\alpha]^{25}_D$=–9.80 (*c*=1.00 in DMF); $^1$H NMR (400 MHz, DMSO-$d_6$, TMS) (Figure S11): $\delta$=1.79 (s, 3H; C$H_3$ Thymine), 2.15–2.31 [m, 1H; 1×C$H_2$(3′)], 2.60–2.77 [2×m, 1H; 1×C$H_2$(3′)], 3.47–3.55 [m, 1H; 1×C$H_2$ (5′)], 3.86–3.93 [m, 1H; 1×C$H_2$(5′)], 4.18–4.50 [2×m, 1H; CH(2′)], 4.24–4.34 (m, 3H; C$H_2$ Fmoc and CH Fmoc), 4.94–5.00 [m, 1H; CH(4′)], 7.35 (t *J*=7.2 Hz, 2H; CH Ar Fmoc), 7.43 (t *J*=7.6 Hz, 2H; CH Ar Fmoc), 7.61 [s, 1H; C(6)H Thymine], 7.69 (d, *J*=7.3 Hz, 2H; CH Ar Fmoc), 7.90 (d *J*=7.4 Hz, 2H; CH Ar Fmoc), 11.36 (s, 1H; $CO_2$H); $^{13}$C NMR (100 MHz, DMSO-$d_6$, TMS) (Figure S12): $\delta$=12.5 (C$H_3$ Thymine), 33.1 and 34.3 [C$H_2$(3′) rotamers], 47.0 and 47.1 [CH Fmoc rotamers], 48.8 and 49.4 [C$H_2$(5′) rotamers], 52.7 and 53.2 [CH(4′) rotamers], 57.4 and 57.8 [CH(2′) rotamers], 67.4 and 67.7 (C$H_2$ Fmoc rotamers), 109.5 and 109.6 (C5 Thymine rotamers), 120.6 (CH Ar Fmoc), 125.6 (CH Ar Fmoc), 125.8 (CH Ar Fmoc), 127.6 (CH Ar Fmoc), 128.2 (CH Ar Fmoc), 138.3 [C(6)H Thymine], 141.1 (C Ar Fmoc), 144.1 (C Ar Fmoc), 151.4 (C2 Thymine), 154.4 (CO Fmoc), 164.2 (C4 Thymine), 173.1 and 173.6 (COOH rotamers); HRMS (FAB$^+$): *m/z* calcd for $C_{25}H_{24}O_6N_3$: 462.1665 [*M*+H]$^+$; found: 462.1648.

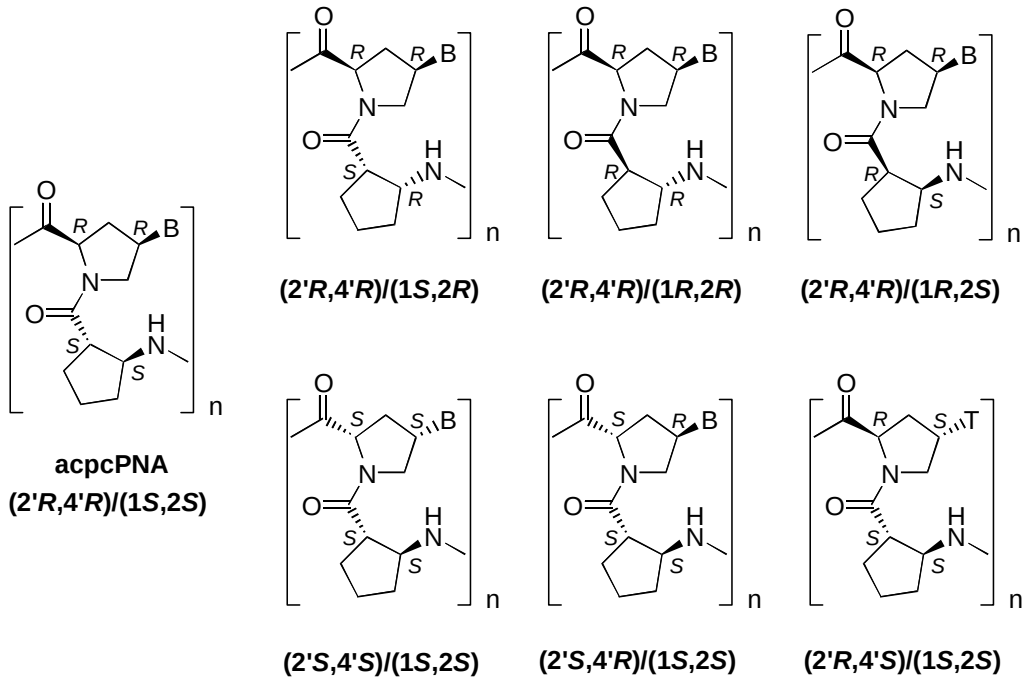
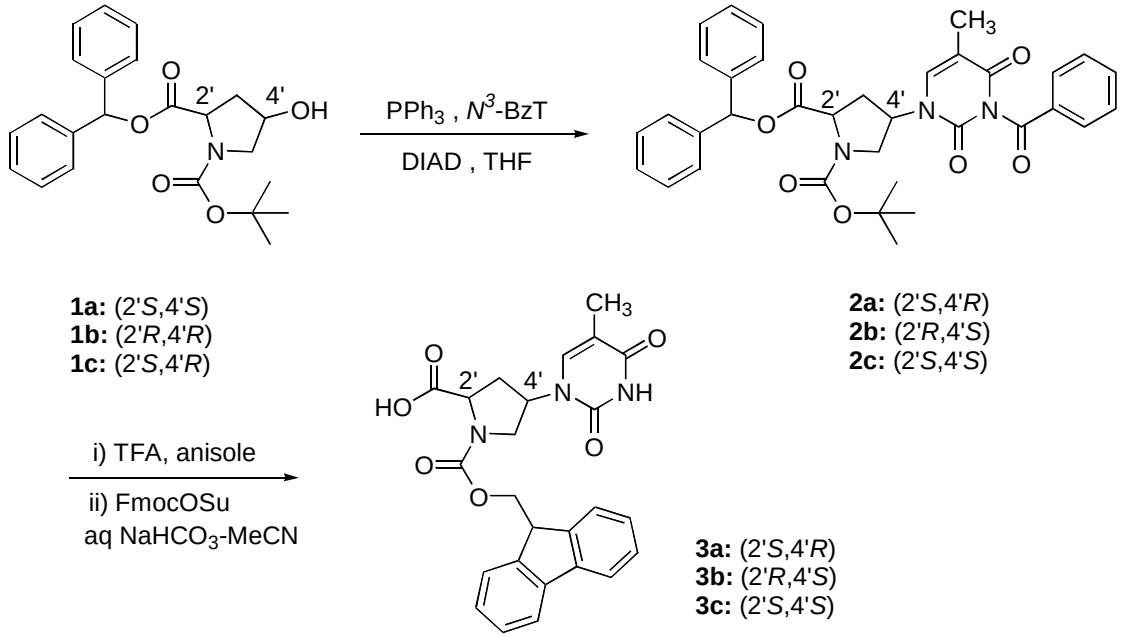



**Figure S1.** Structures of stereoisomers of acpcPNA that have been evaluated for DNA binding properties in this work. All PNA sequences have B=thymine, n=5 and *C*-terminal lysine capped. The *N*-termini were either free [(2'*R*,4'*R*)/(1*S*,2*S*), (2'*R*,4'*R*)/(1*S*,2*R*), (2'*R*,4'*R*)/(1*R*,2*R*) and (2'*R*,4'*R*)/(1*R*,2*S*)] or acetylated (2'*S*,4'*S*)/(1*S*,2*S*), (2'*S*,4'*R*)/(1*S*,2*S*), (2'*R*,4'*R*)/(1*S*,2*S*) and (2'*R*,4'*S*)/(1*S*,2*S*)].




**1a:** (2'*S*,4'*S*)
**1b:** (2'*R*,4'*R*)
**1c:** (2'*S*,4'*R*)

**2a:** (2'*S*,4'*R*)
**2b:** (2'*R*,4'*S*)
**2c:** (2'*S*,4'*S*)

**3a:** (2'*S*,4'*R*)
**3b:** (2'*R*,4'*S*)
**3c:** (2'*S*,4'*S*)

**Figure S2.** Synthesis of pyrrolidine PNA monomers carrying thymine base (T) with different configurations on the proline ring. Syntheses and characterization data of the (2'*R*,4'*R*)-T, as well as C, G and A monomers have already been reported.[1]

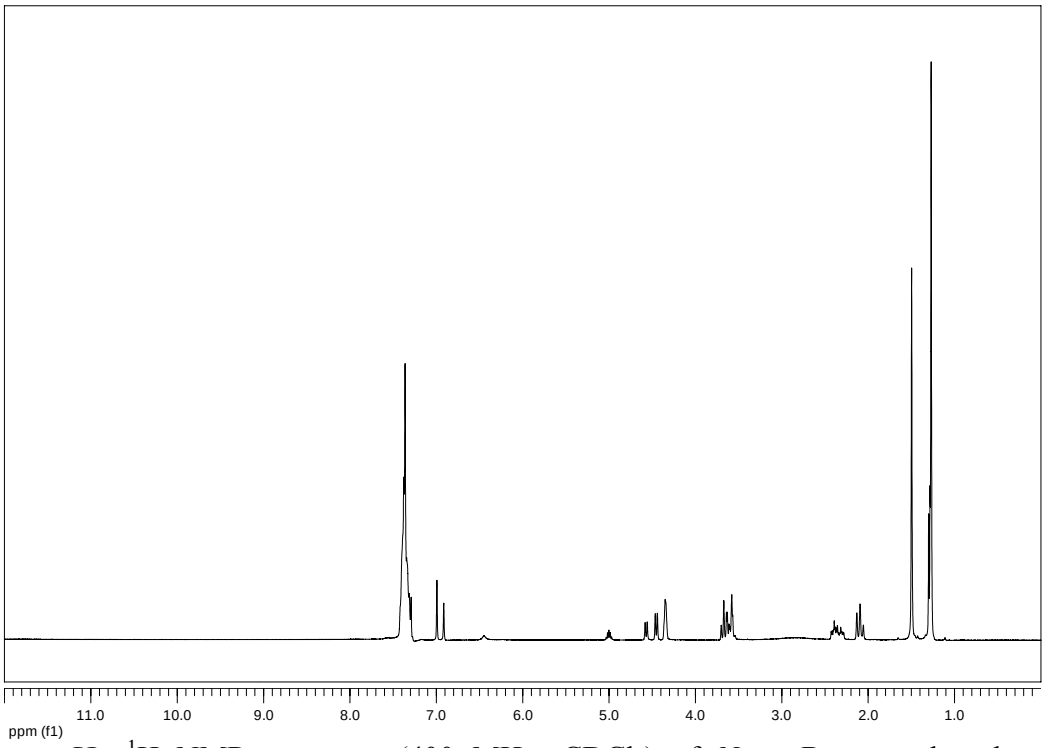



**Figure S3.** $^{1}H$ NMR spectrum (400 MHz, $CDCl_3$) of *N*-*tert*-Butoxycarbonyl-*cis*-4-hydroxy-L-proline diphenylmethyl ester (**1a**)

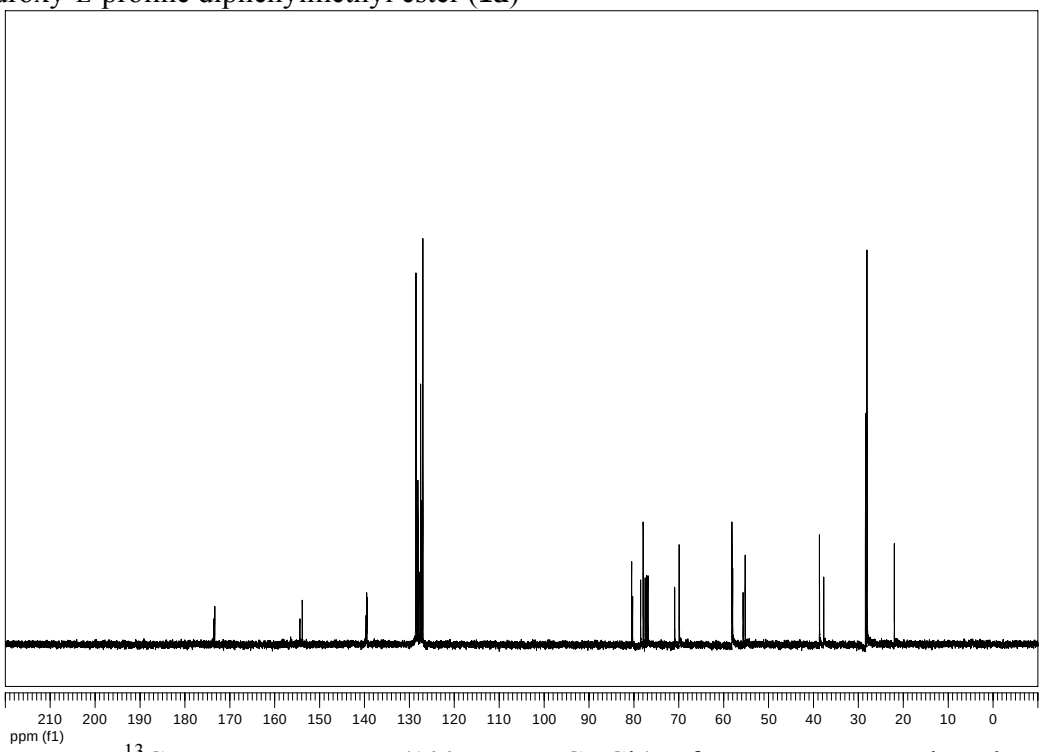



**Figure S4.** $^{13}C$ NMR spectrum (100 MHz, $CDCl_3$) of *N*-*tert*-Butoxycarbonyl-*cis*-4-hydroxy-L-proline diphenylmethyl ester (**1a**)

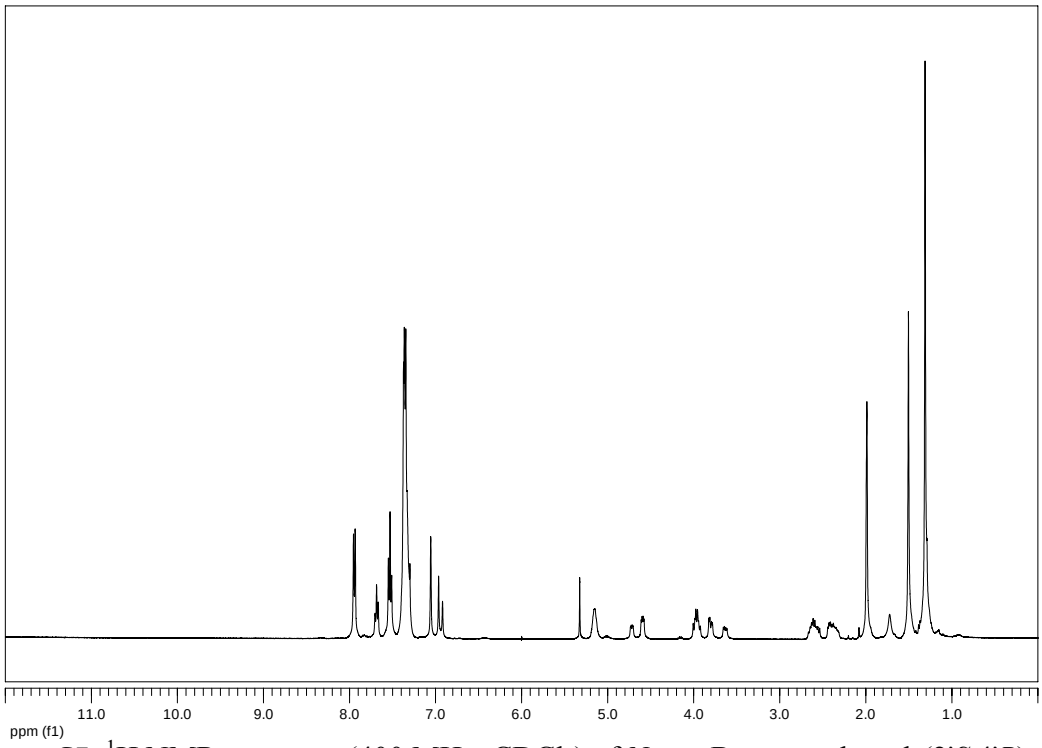



**Figure S5.** $^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of *N*-*tert*-Butoxycarbonyl-(2'*S*,4'*R*)-($N^3$-benzoylthymin-1-yl)-proline diphenyl methyl ester (**2a**)

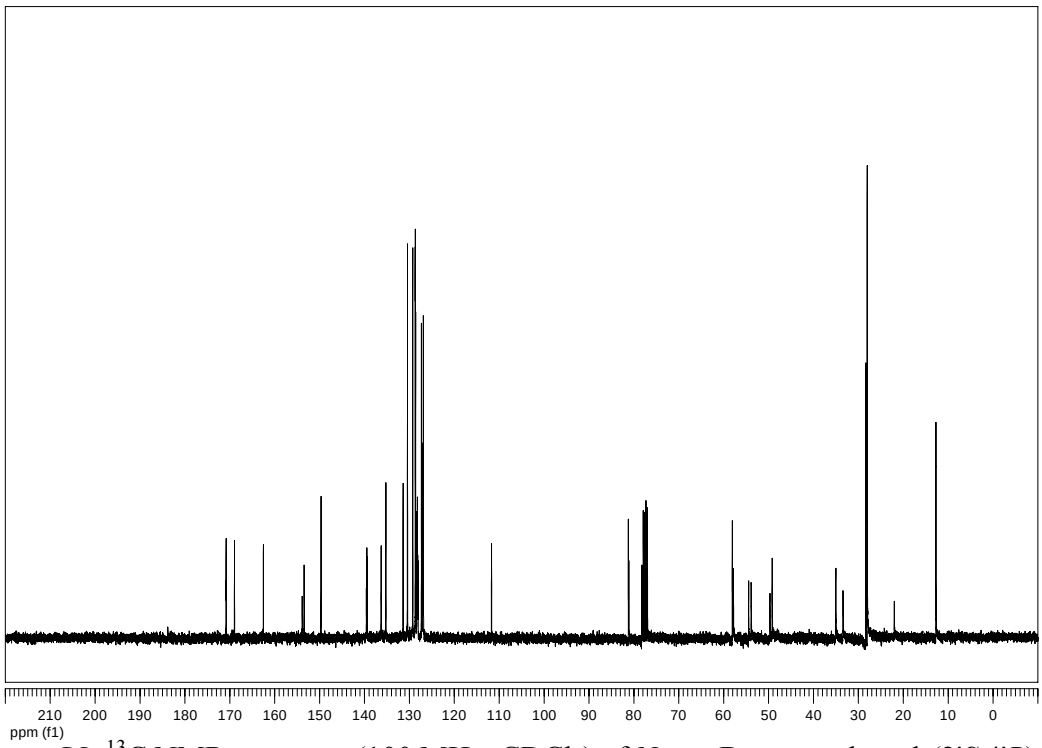



**Figure S6.** $^{13}$C NMR spectrum (100 MHz, $CDCl_3$) of *N*-*tert*-Butoxycarbonyl-(2'*S*,4'*R*)-($N^3$-benzoylthymin-1-yl)-proline diphenyl methyl ester (**2a**)

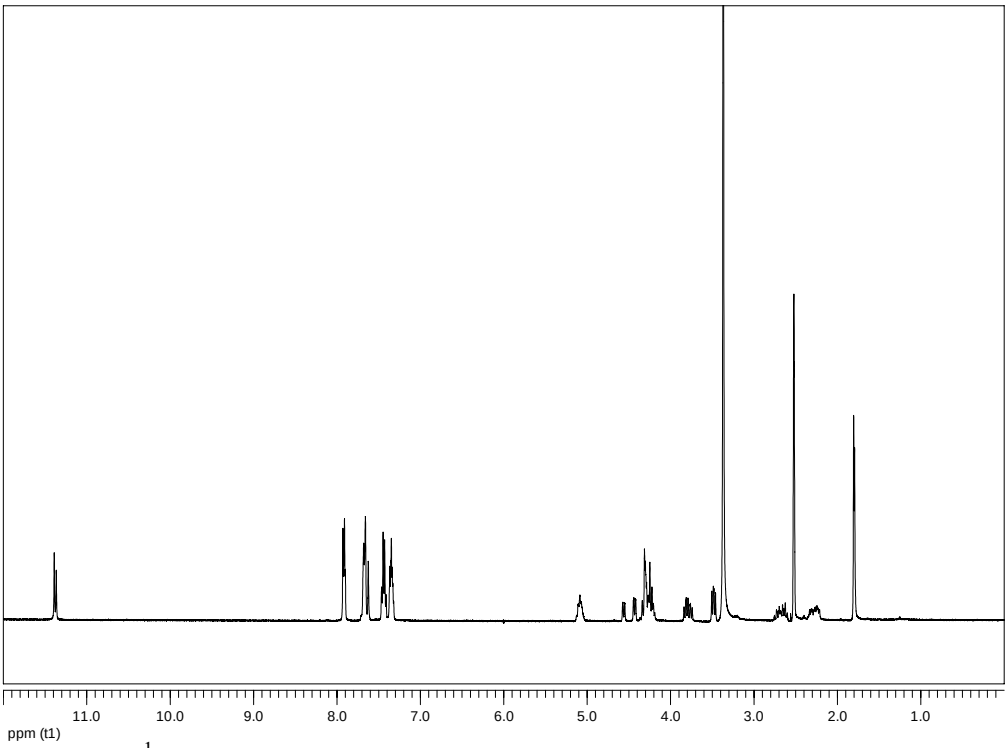



**Figure S7.** $^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$) of *N*-Fluoren-9-ylmethoxycarbonyl-(2'*S*,4'*R*)-(thymin-1-yl)-proline (**3a**)

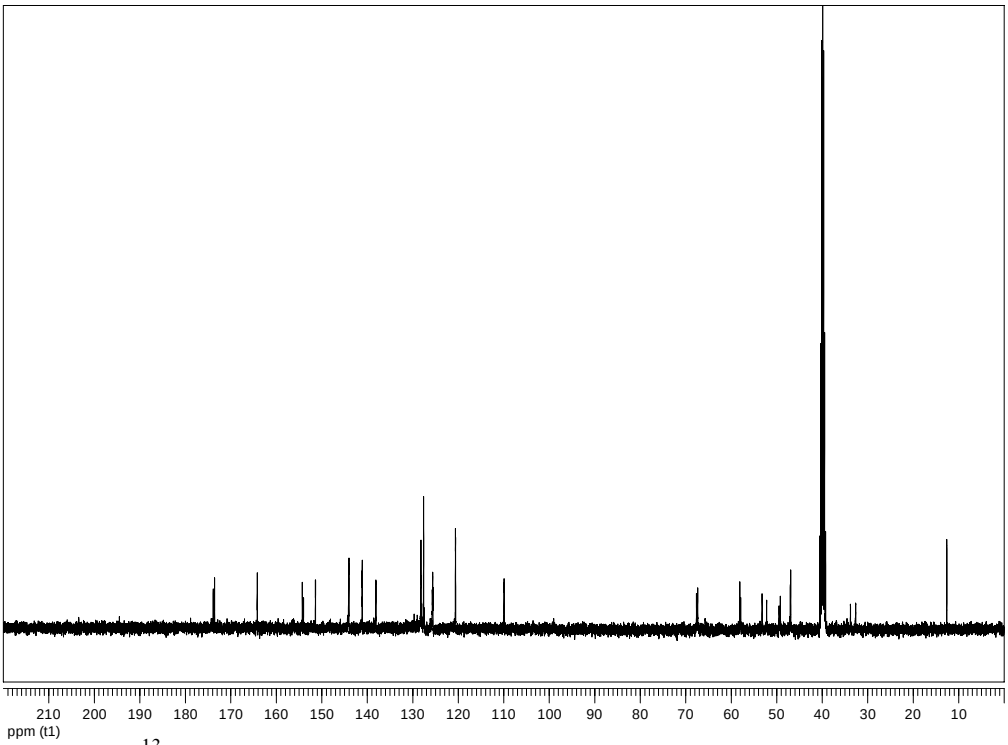



**Figure S8.** $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$) of *N*-Fluoren-9-ylmethoxycarbonyl-(2'*S*,4'*R*)-(thymin-1-yl)-proline (**3a**)

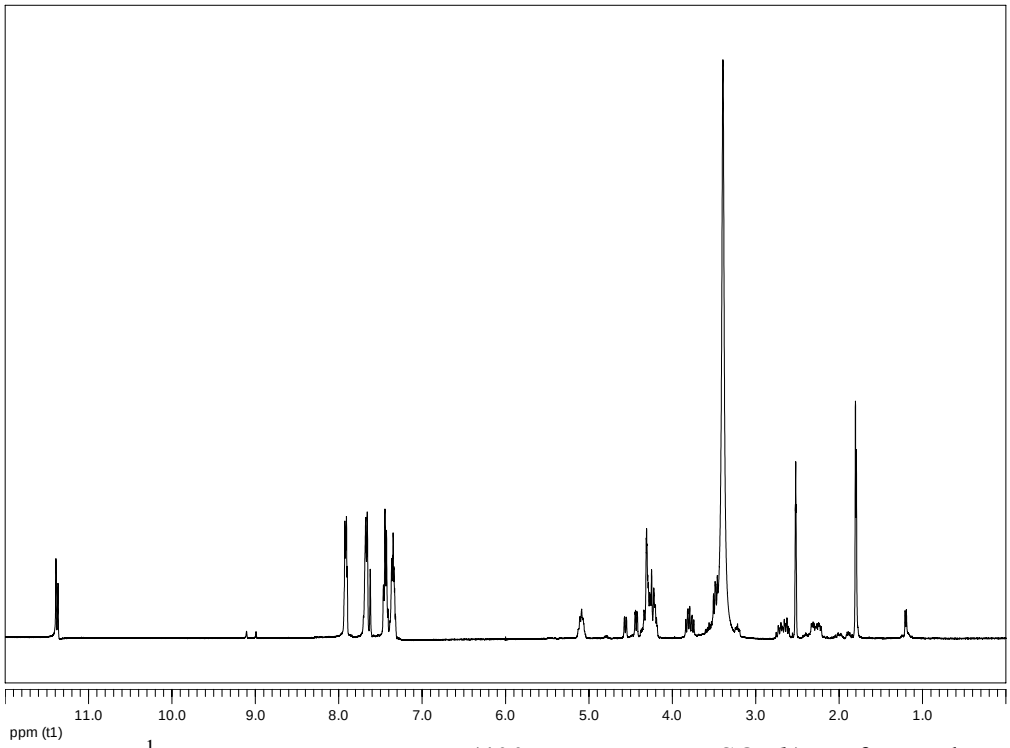



**Figure S9.** $^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$) of *N*-Fluoren-9-ylmethoxycarbonyl-(2'*R*,4'*S*)-(thymin-1-yl)-proline (**3b**)

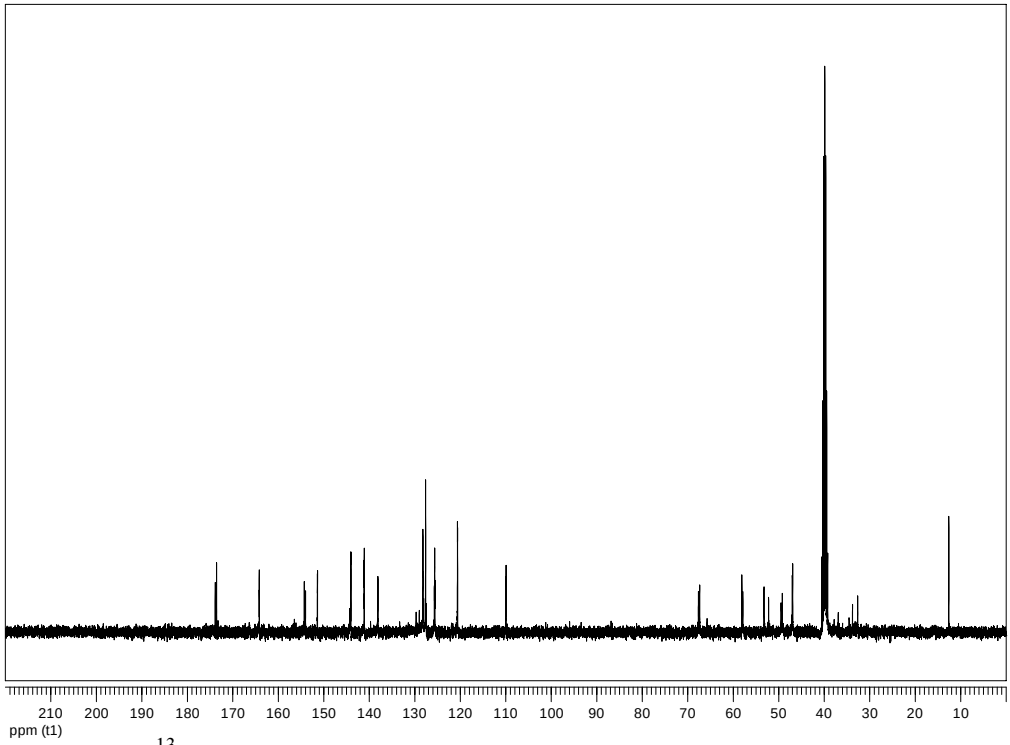



**Figure S10.** $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$) of *N*-Fluoren-9-ylmethoxycarbonyl-(2'*R*,4'*S*)-(thymin-1-yl)-proline (**3b**)

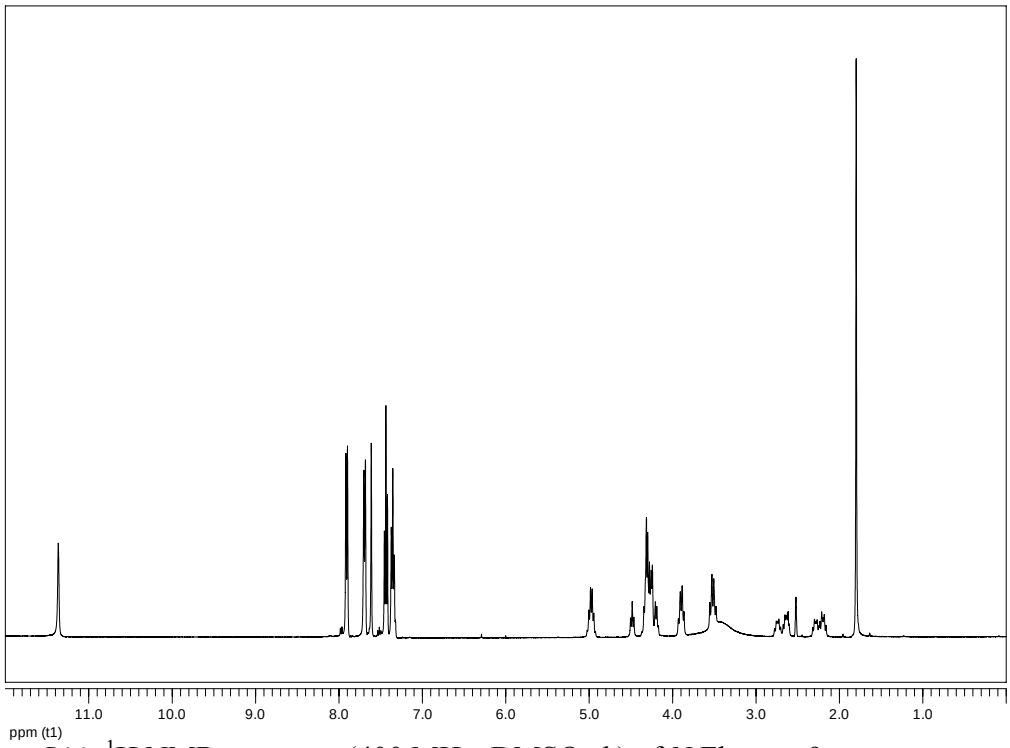



**Figure S11.** $^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$) of *N*-Fluoren-9-ylmethoxycarbonyl-(2'*S*,4'*S*)-(thymin-1-yl)-proline (**3c**)

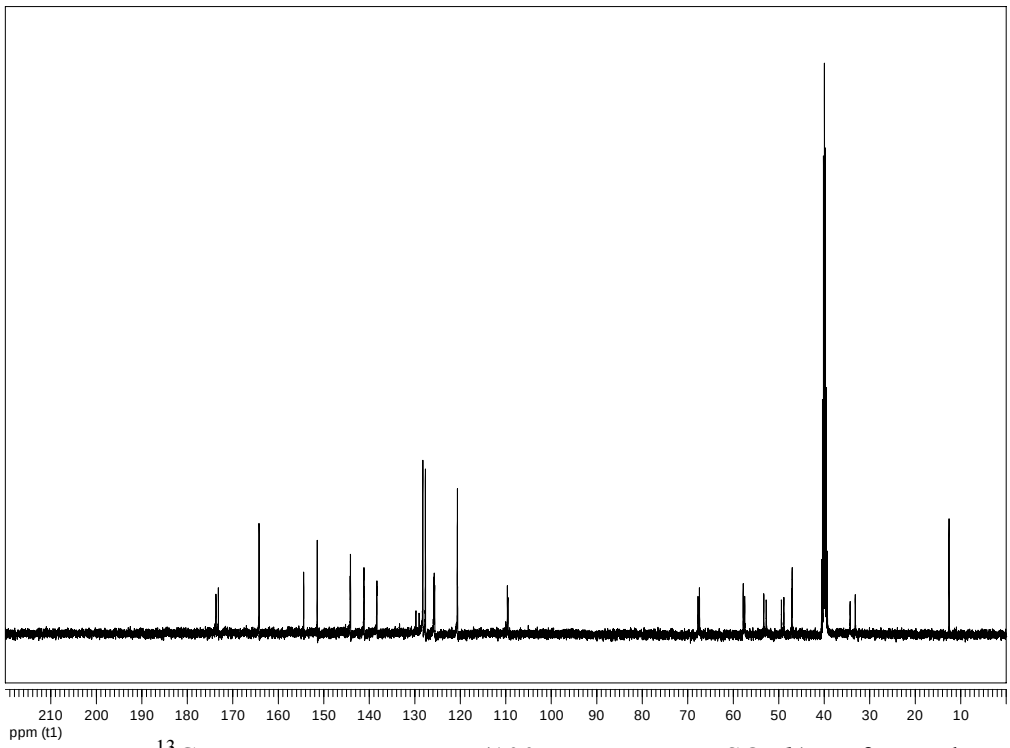



**Figure S12.** $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$) of *N*-Fluoren-9-ylmethoxycarbonyl-(2'*S*,4'*S*)-(thymin-1-yl)-proline (**3c**)

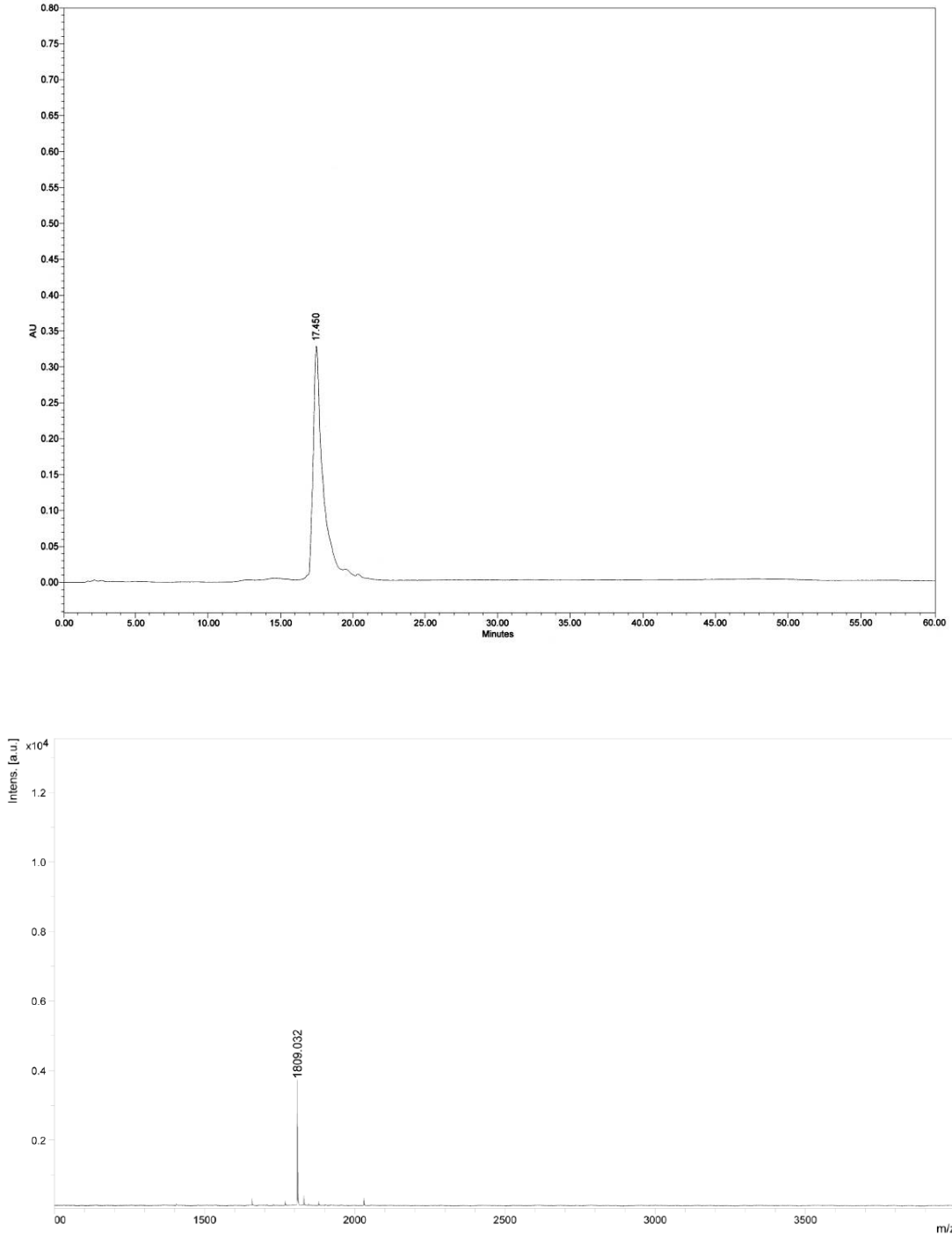



**Figure S13.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA H-$T_5$-Lys$NH_2$ (CCA matrix, Reflectron mode) (calcd for $[M+H]^+$: *m/z*=1806.7)

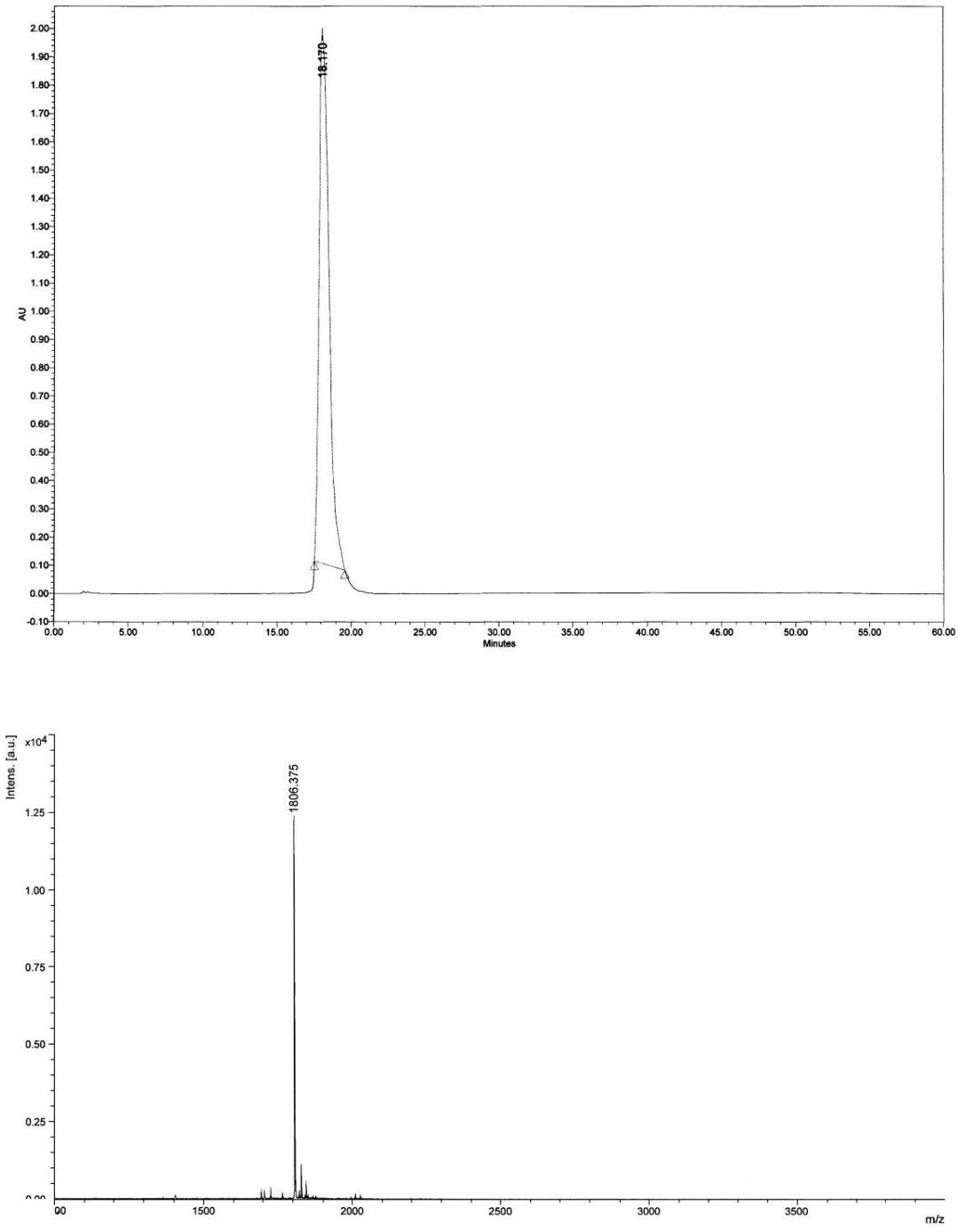



**Figure S14.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*R*)-acpcPNA H-$T_5$-Lys$NH_2$ (CCA matrix, Reflectron mode) (calcd for $[M+H]^+$: *m/z*=1806.7)

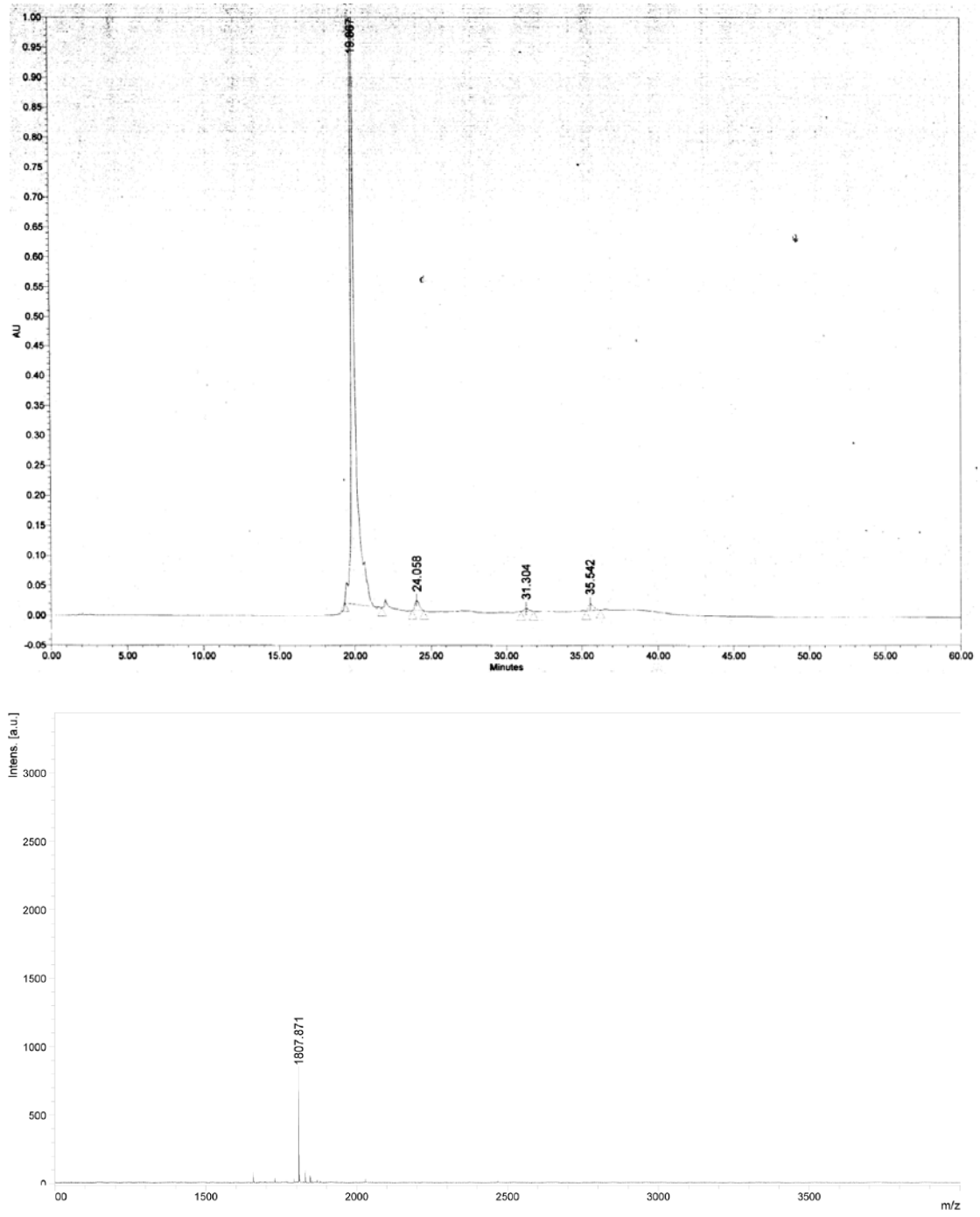
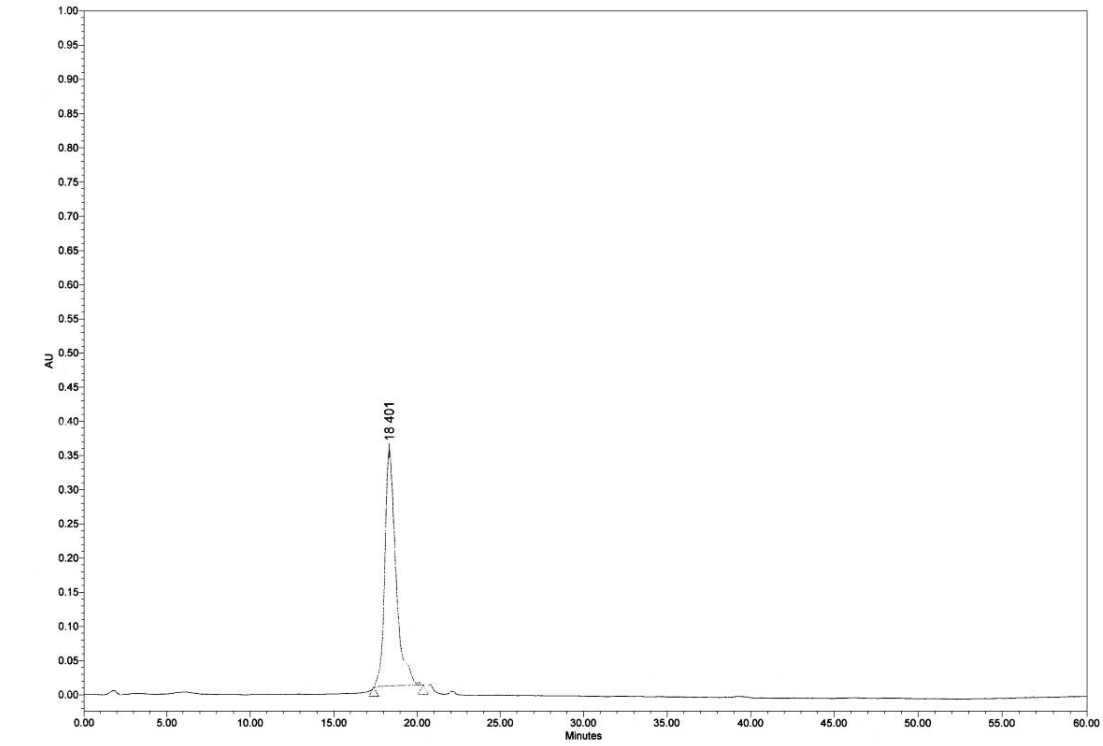



**Figure S15.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*R*,2*R*)-acpcPNA H-$T_5$-Lys$NH_2$ (CCA matrix, Reflectron mode) (calcd for $[M+H]^+$: *m/z*=1806.7)



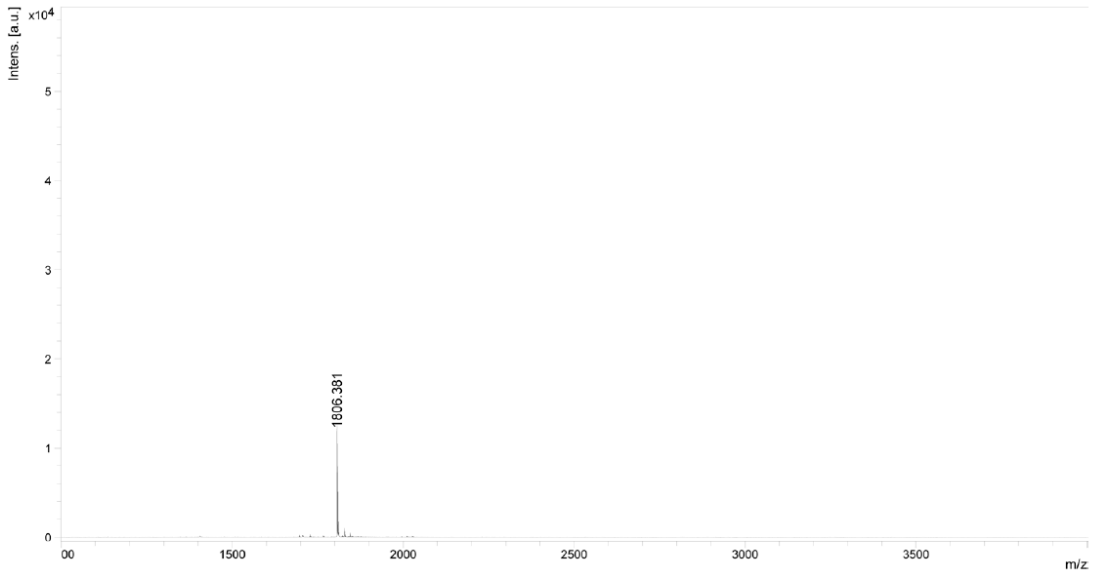




**Figure S16.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*R*,2*S*)-acpcPNA H-$T_5$-Lys$NH_2$ (CCA matrix, Reflectron mode) (calcd for $[M+H]^+$: *m/z*=1806.7)

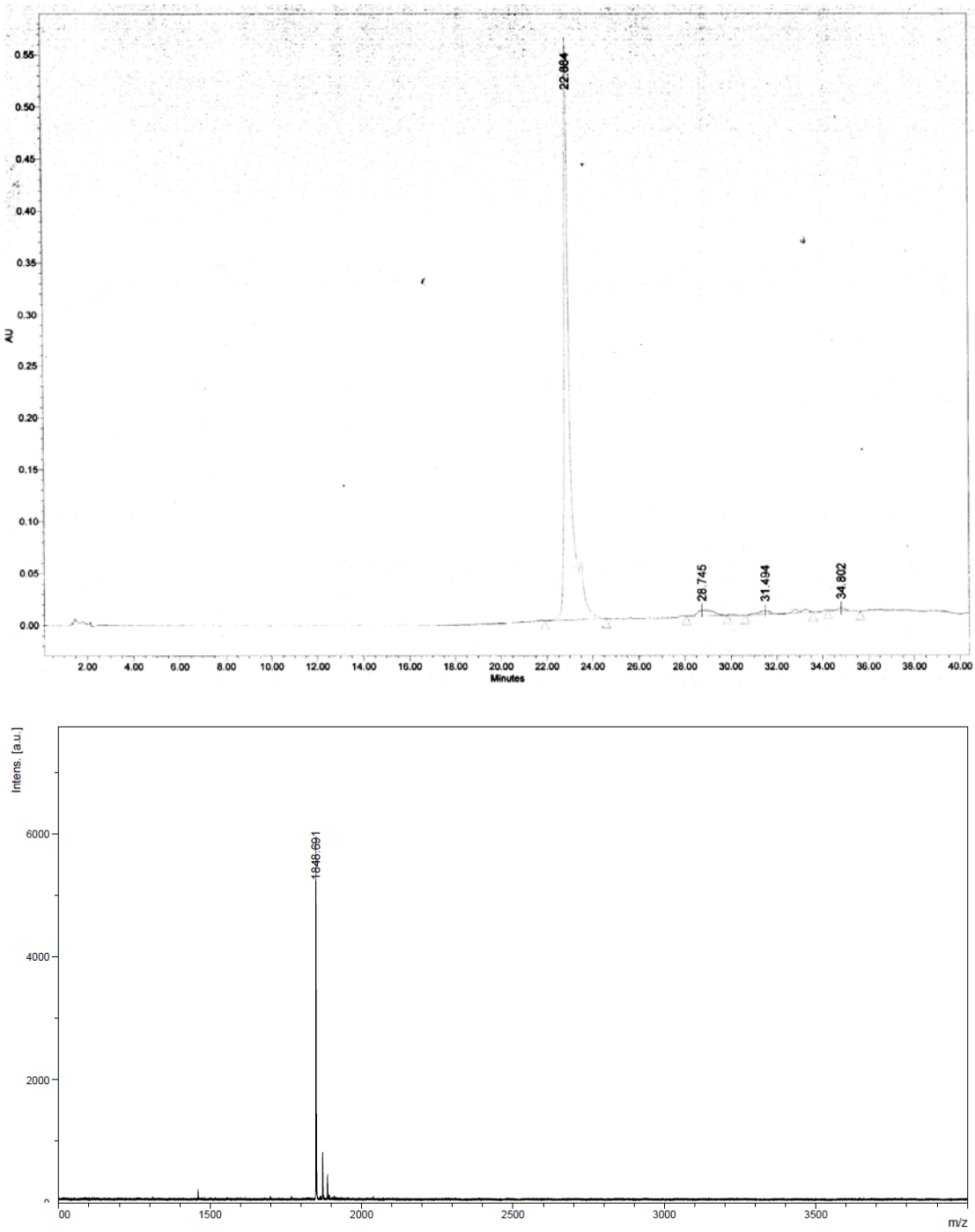



**Figure S17.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*S*,4'*S*)-pro/(1*S*,2*S*)-acpcPNA Ac-$T_5$-Lys$NH_2$ (CCA matrix, Reflectron mode) (calcd for $[M+H]^+$: *m/z*=1848.9)

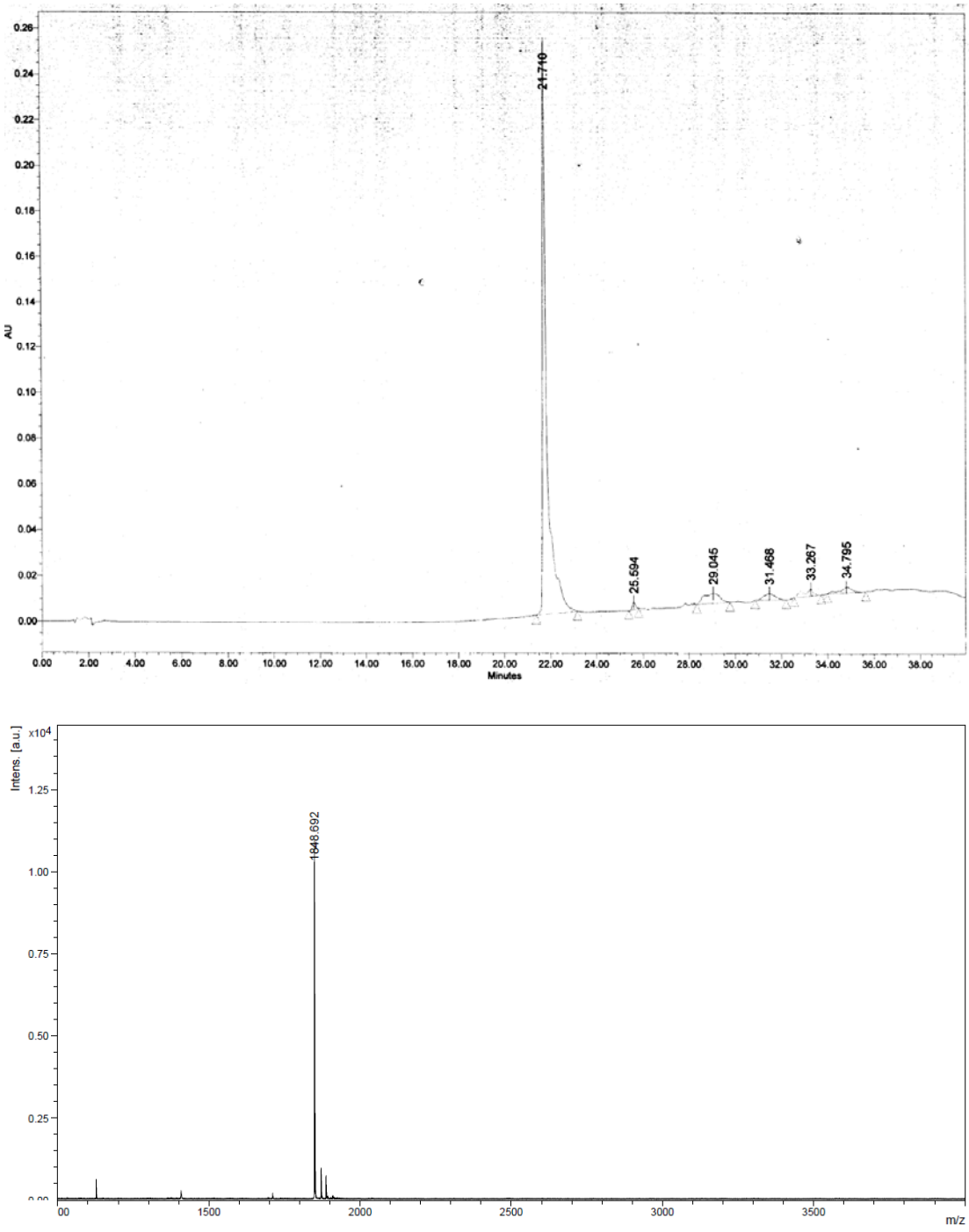



**Figure S18.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*S*)-pro/(1*S*,2*S*)-acpcPNA Ac-$T_5$-Lys$NH_2$ (**T5**) (CCA matrix, Reflectron mode) (calcd for $[M+H]^+$: *m/z*=1848.9)

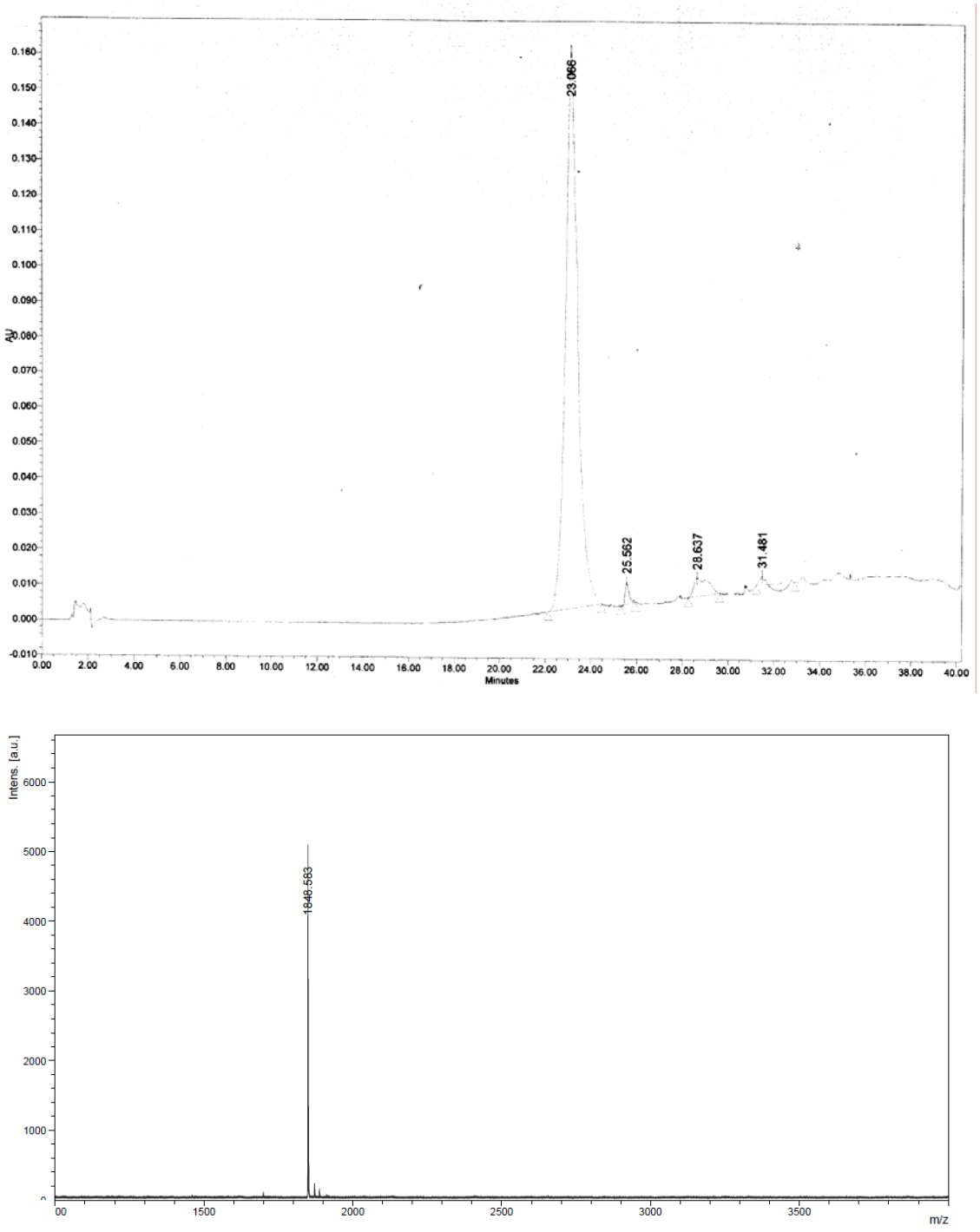



**Figure S19.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*S*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac-$T_5$-Lys$NH_2$ (CCA matrix, Reflectron mode) (calcd for $[M+H]^+$: *m/z*=1848.9)

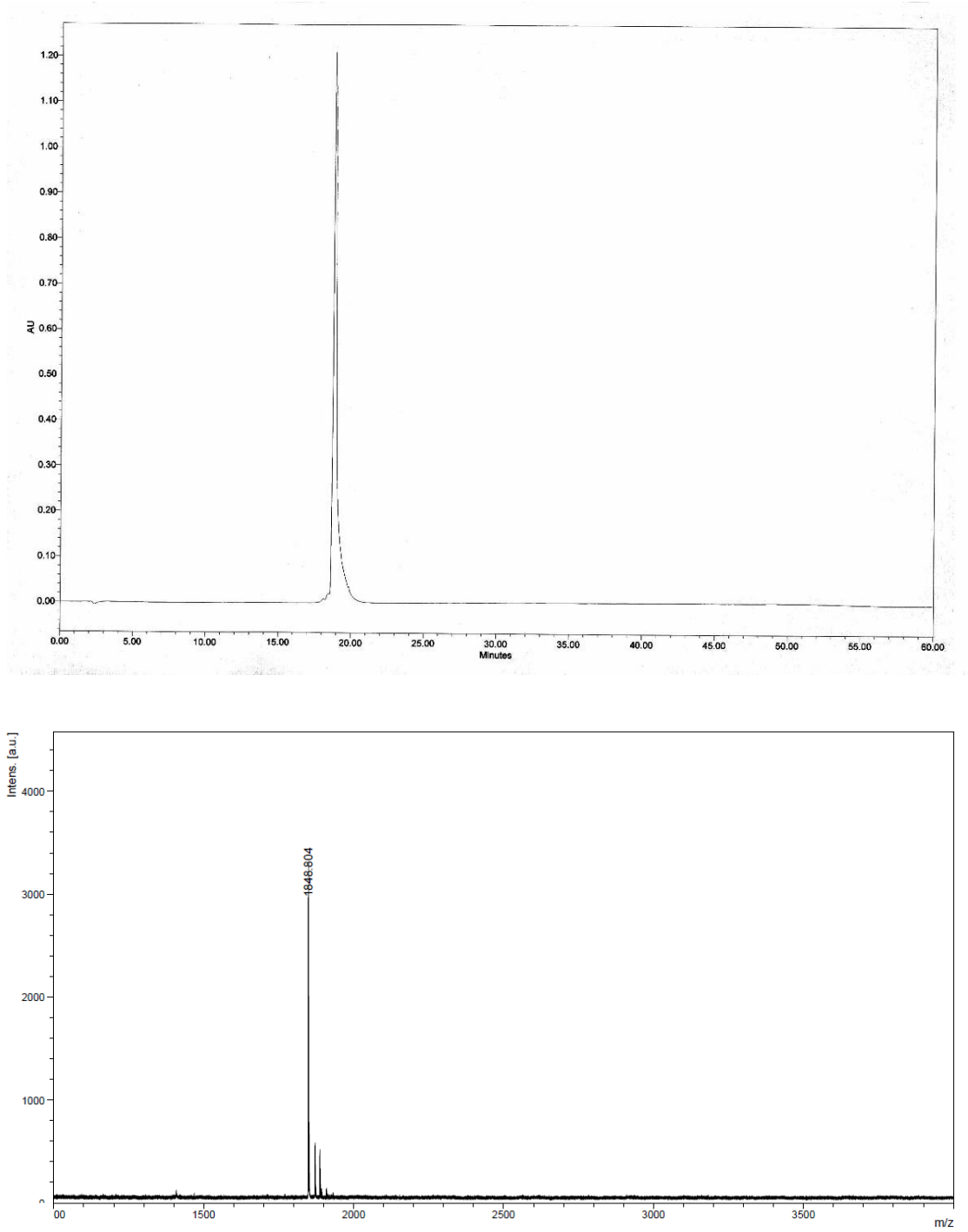



**Figure S20.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpc Ac-$T_5$-Lys$NH_2$ (**T5**) (CCA matrix, Reflectron mode) (calcd $M{\cdot}H^+$: *m/z*=1848.9)

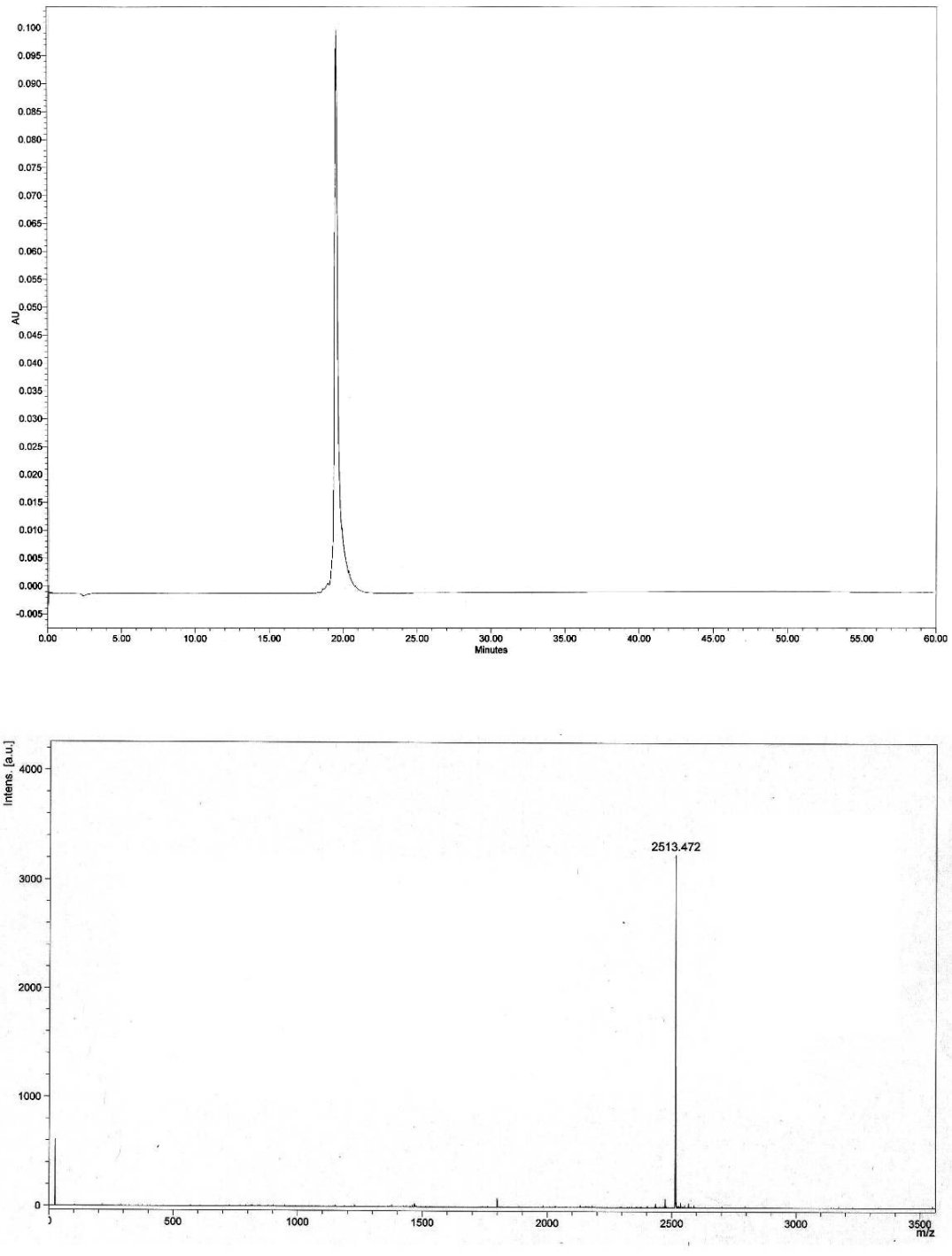



**Figure S21.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac-$T_7$-Lys$NH_2$ (**T7**) (CCA matrix, Reflectron mode) (calcd for $[M+H]^+$: *m/z*=2513.2)

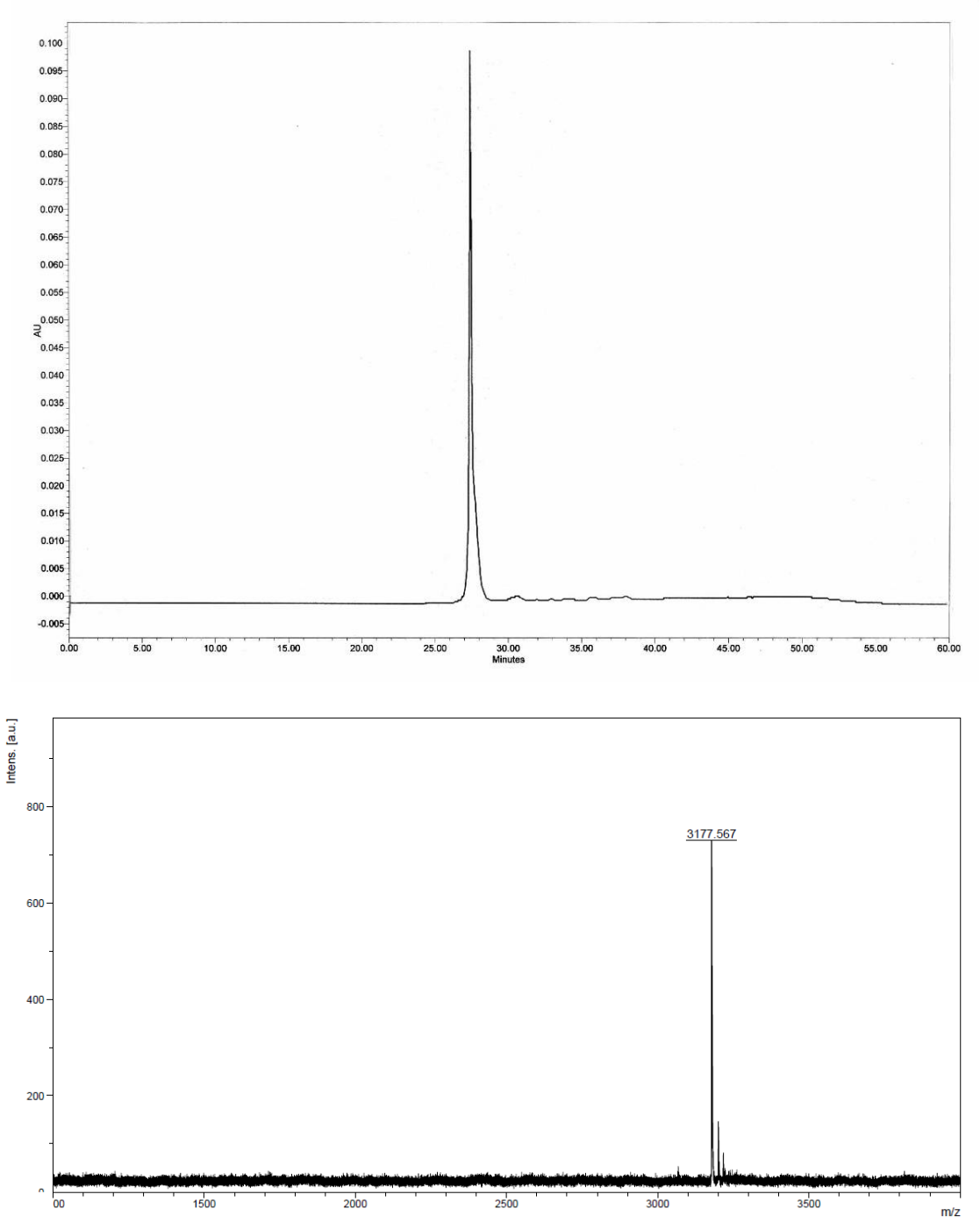



**Figure S22.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac-$T_9$-Lys$NH_2$ (**T9**) (CCA matrix, Reflectron mode) (calcd for $[M+H]^+$: *m/z*=3177.5)

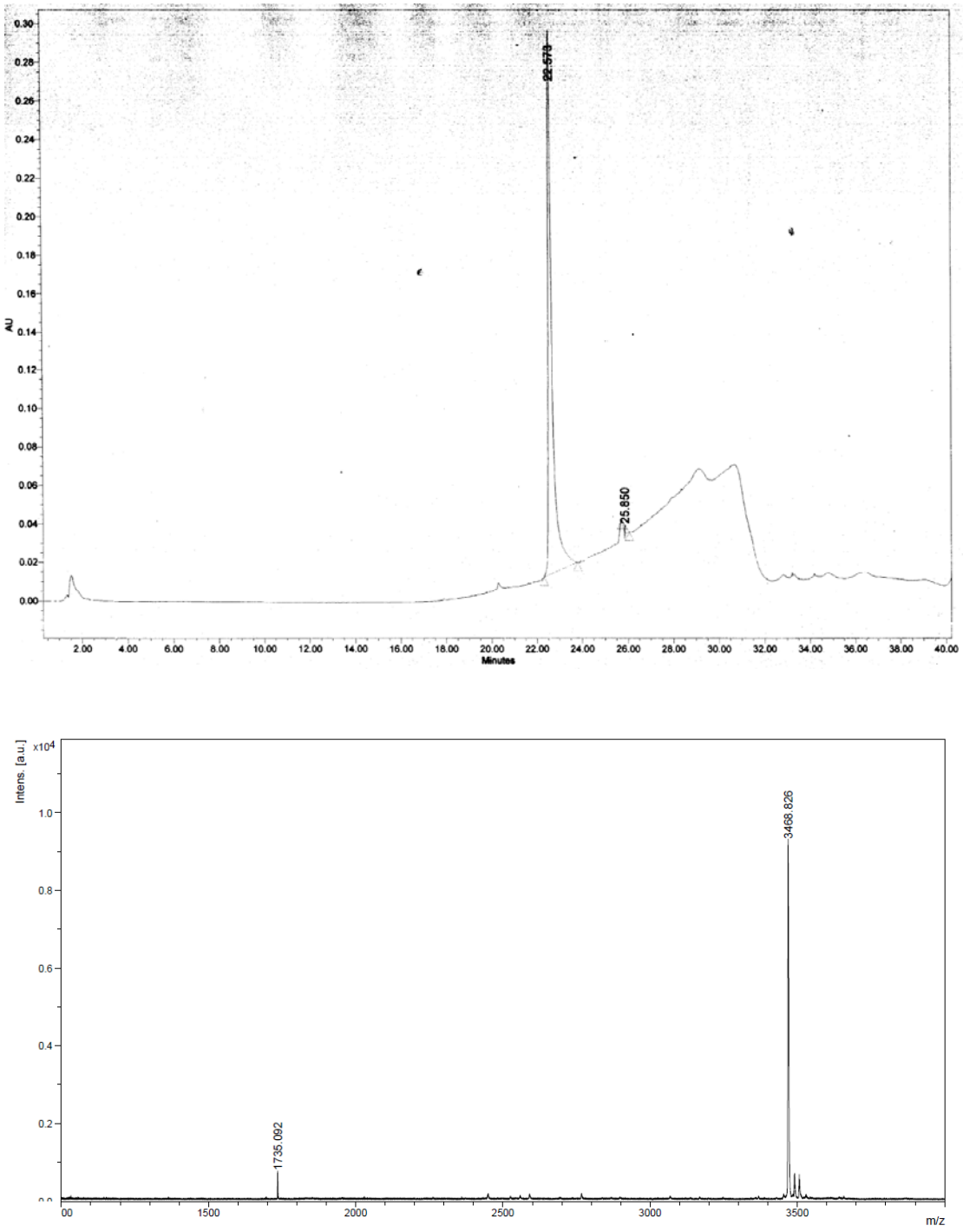



**Figure S23.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA H-$T_{10}$-Lys$NH_2$ (**T10**) (CCA matrix, Reflectron mode) (calcd for $[M+H]^+$: *m/z*=3467.6)

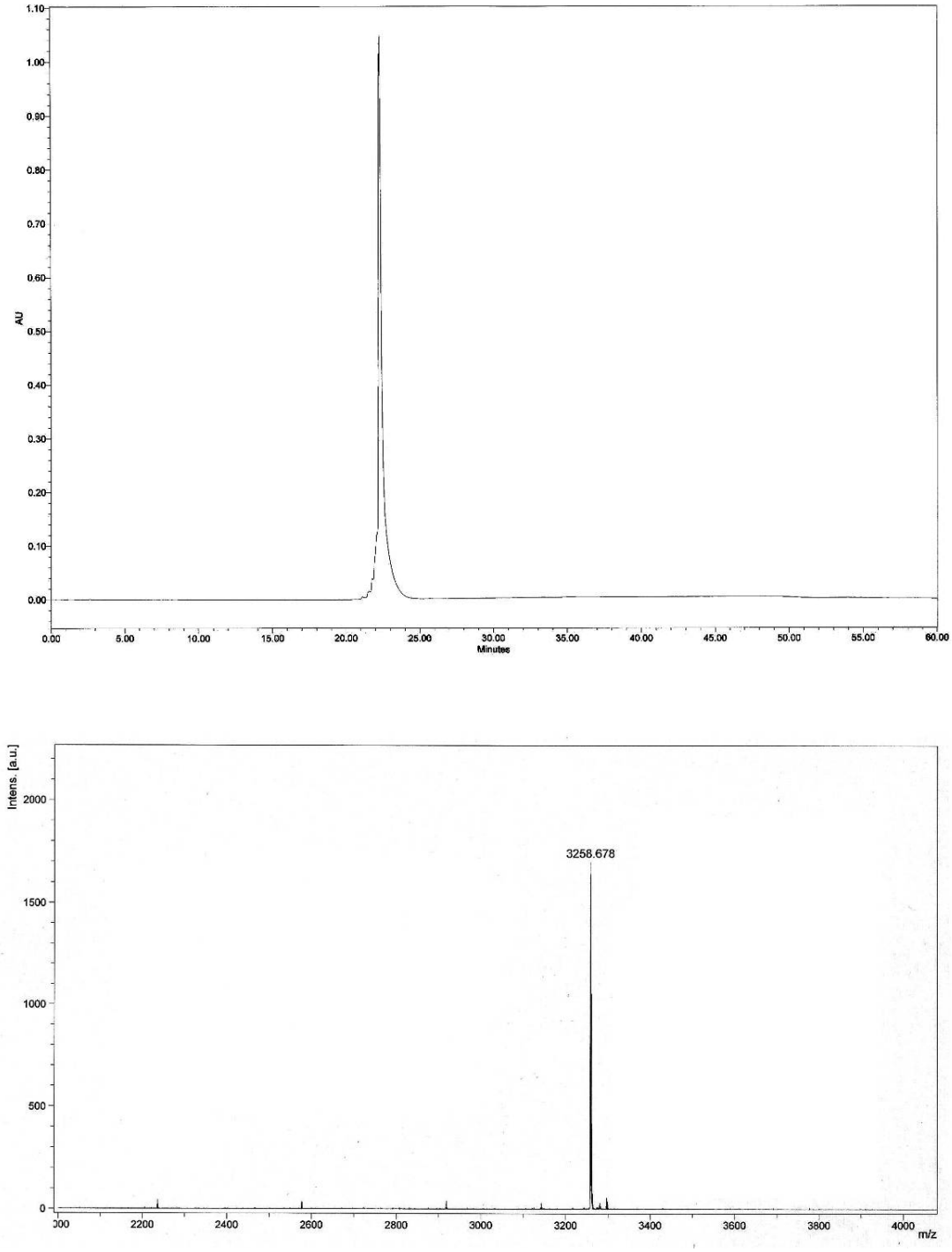



**Figure S24.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac-$A_9$-Lys$NH_2$ (**A9**) (CCA matrix, Reflectron mode) (calcd for $[M+H]^+$: *m/z*=3258.6)

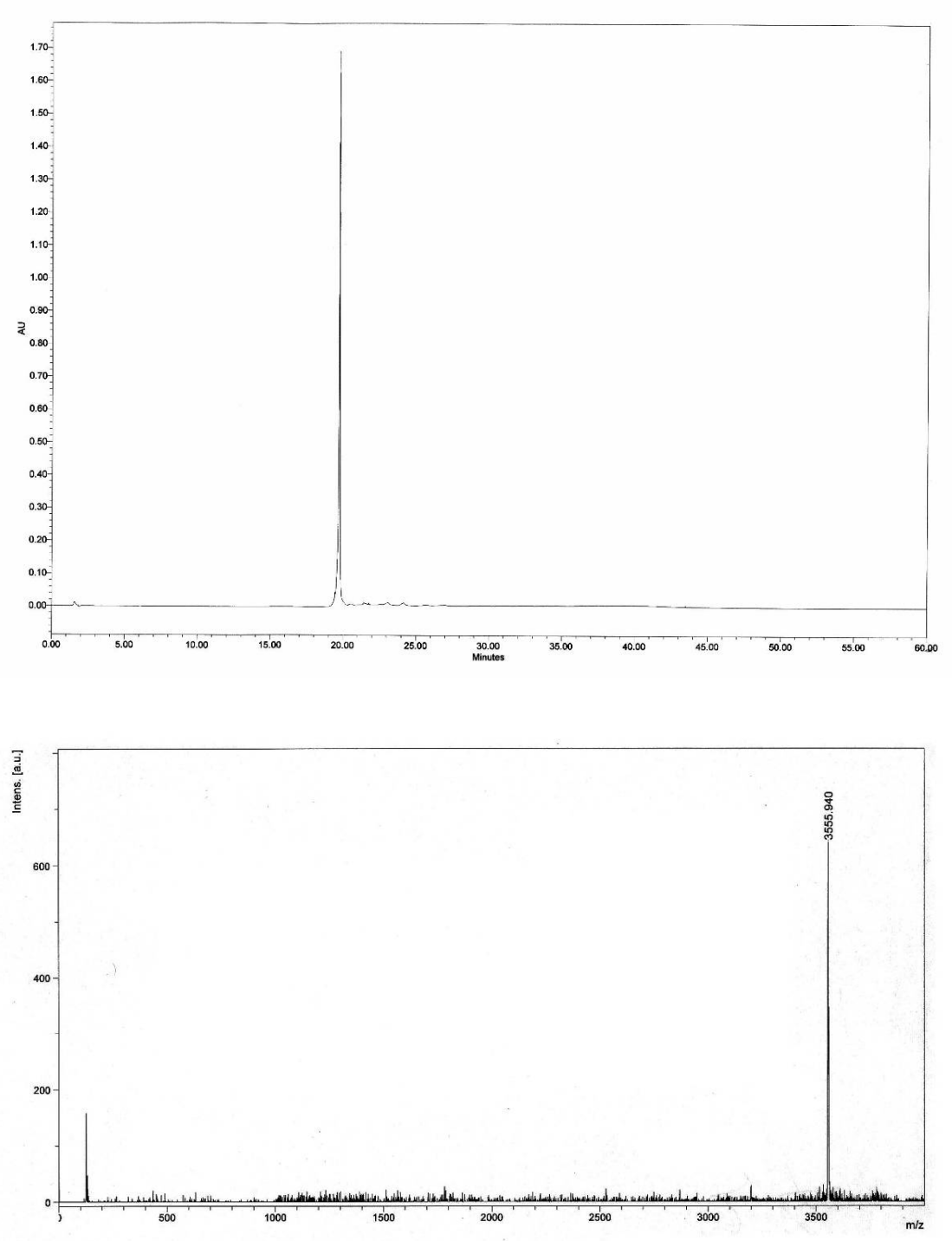



**Figure S25.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac-GTAGATCACT-Lys$NH_2$ (**M10a**) (calcd for $[M+H]^+$: *m/z*=3556.7)

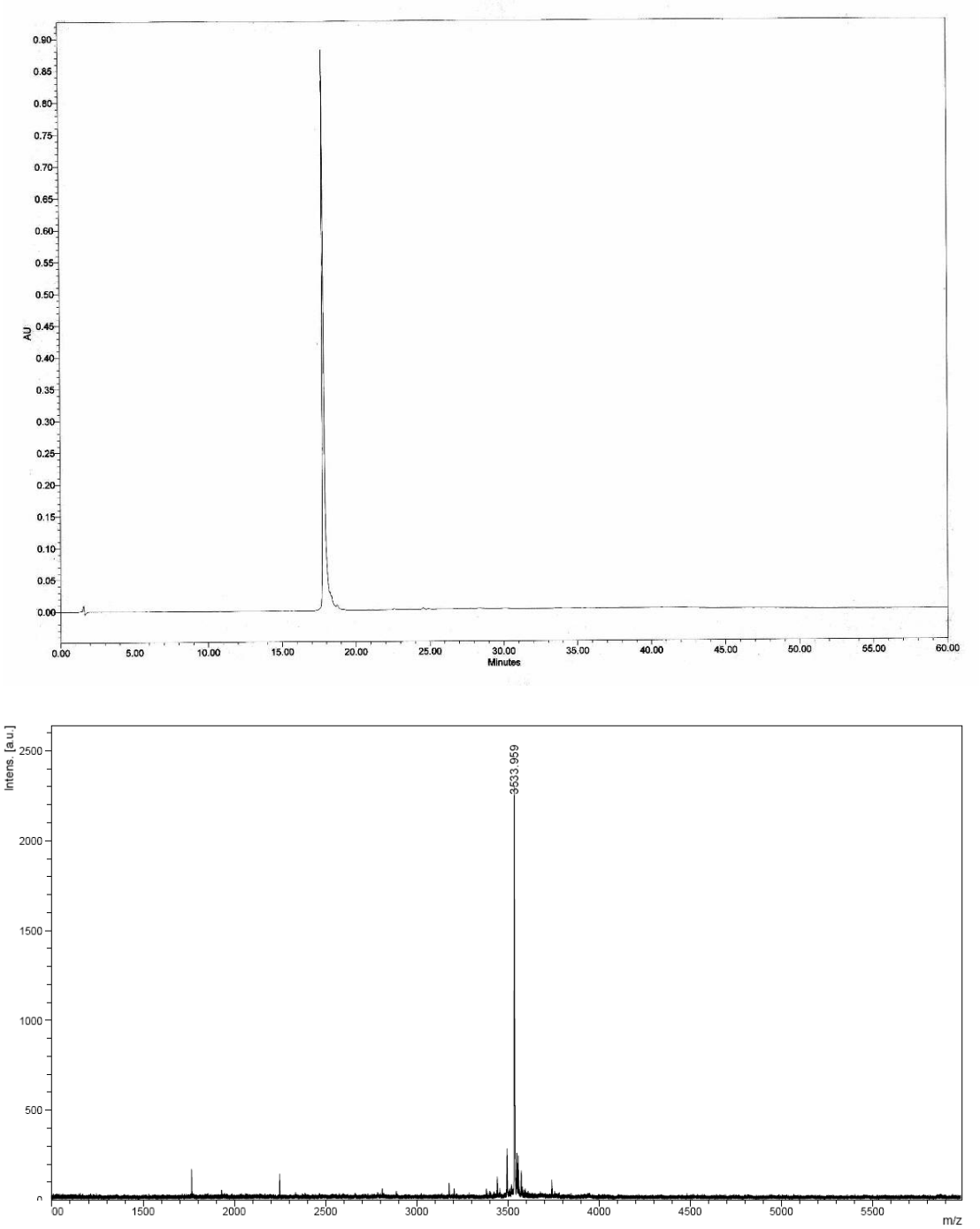



**Figure S26.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac- GCTACGTCGC-Lys$NH_2$ (**M10b**) (calcd for $[M+H]^+$: *m/z*=3533.7)

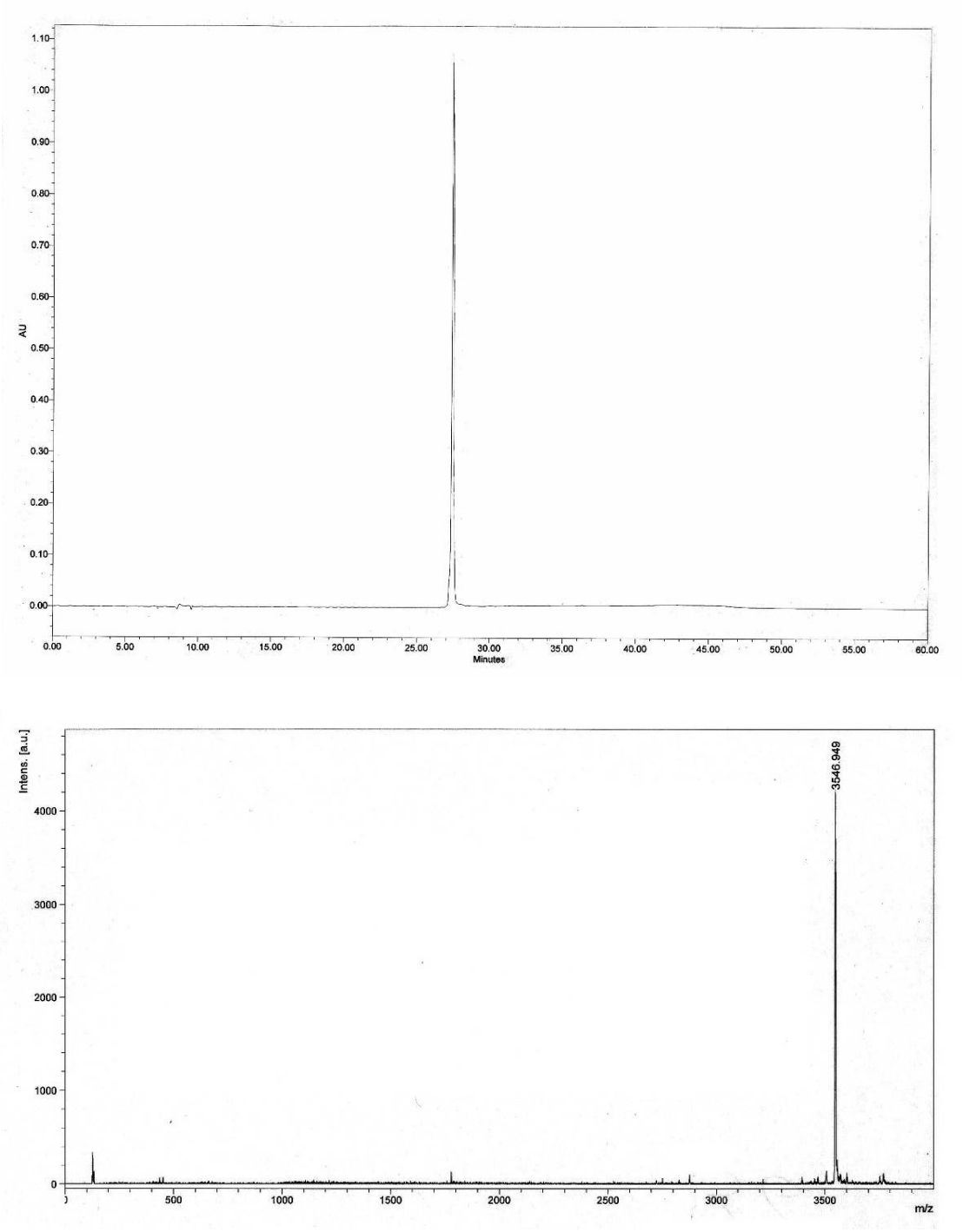



**Figure S27.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac-TATGTACTAT-LysNH2 (**M10c**) (calcd for $[M+H]^+$: *m/z*=3546.7)

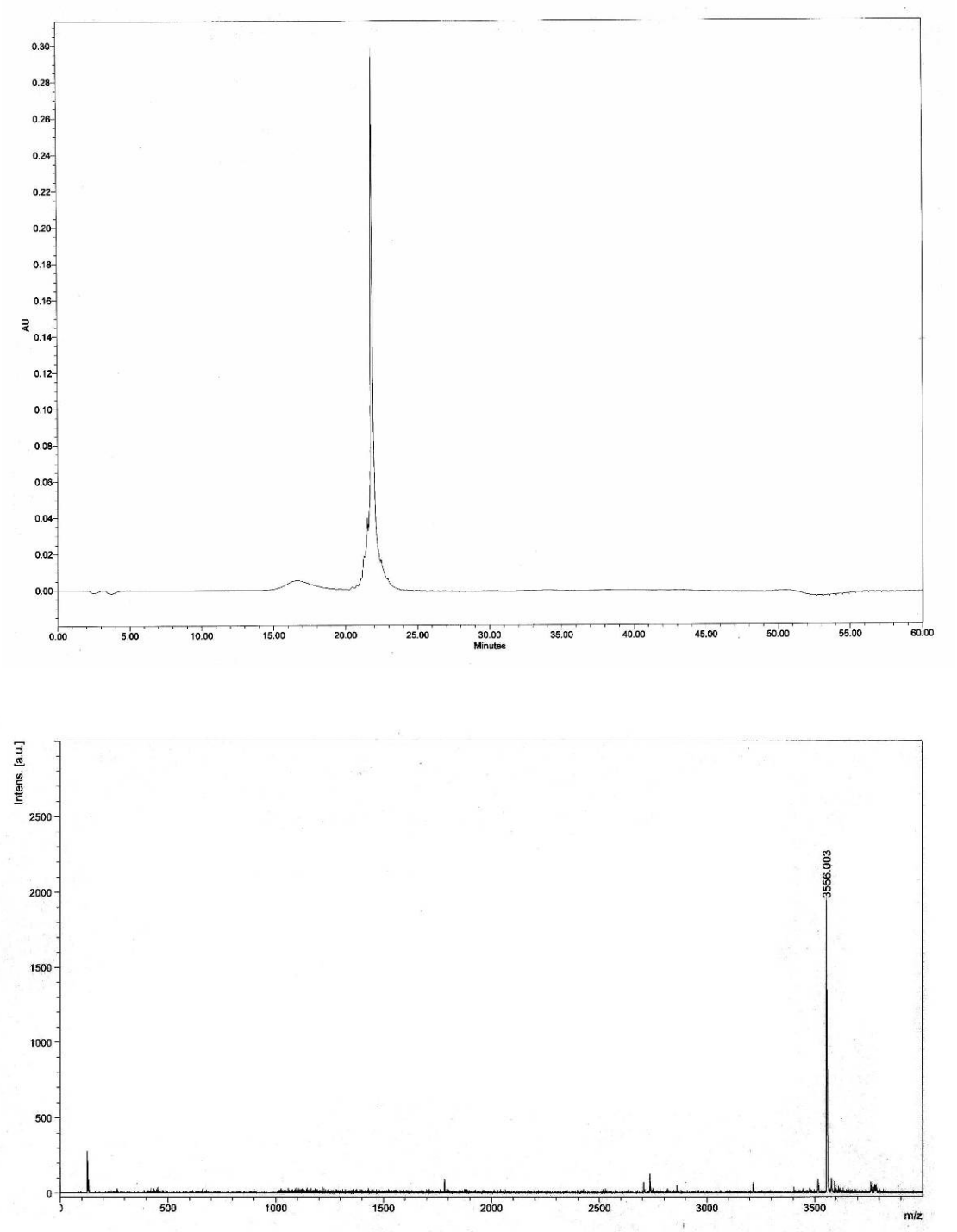



**Figure S28.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac-AGTGATCTAC-Lys$NH_2$ (**M10d**) (calcd for $[M+H]^+$: *m/z*=3556.7)

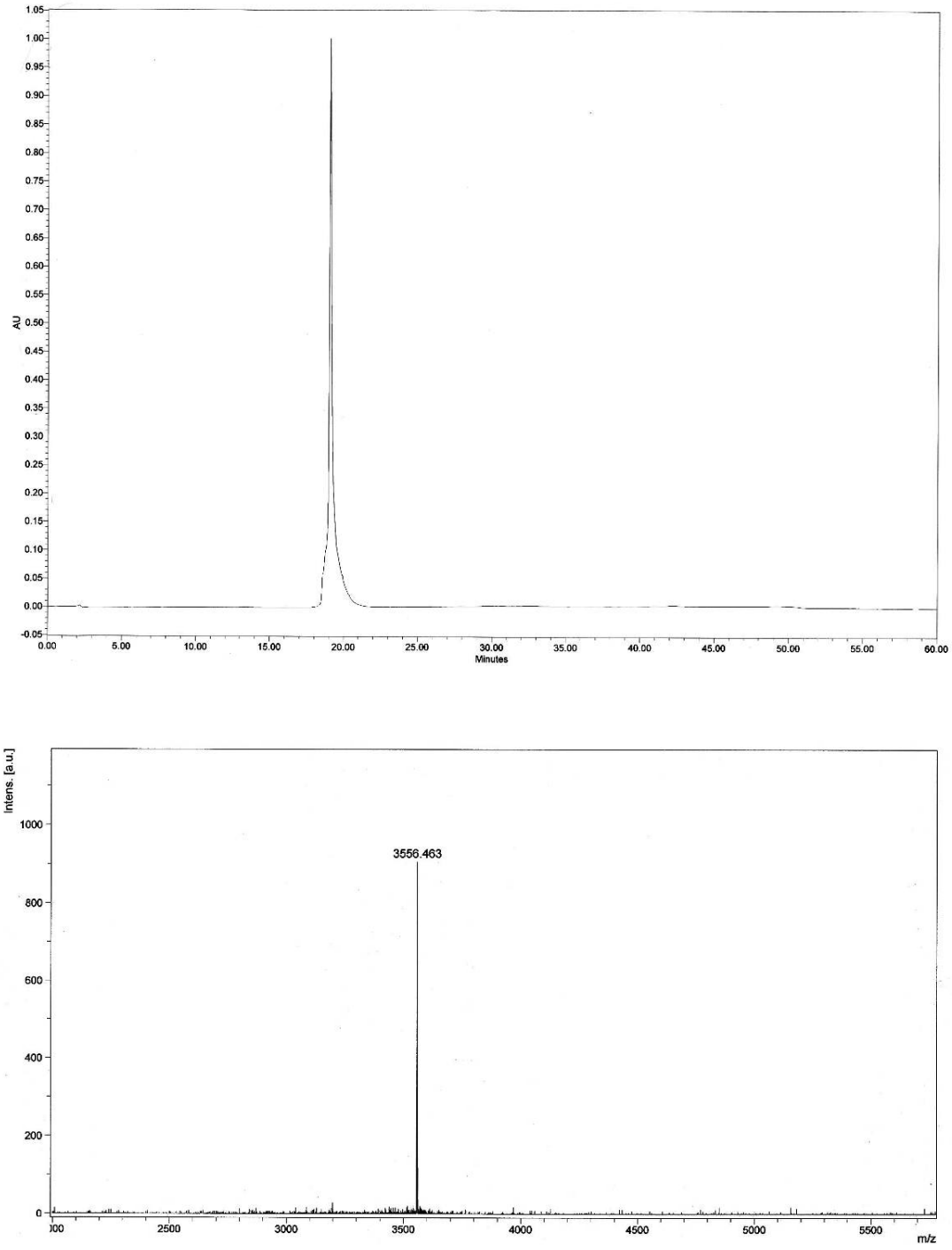



**Figure S29.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac-CATCTAGTGA-Lys$NH_2$ (**M10e**) (calcd for $[M+H]^+$: *m/z*=3556.7)

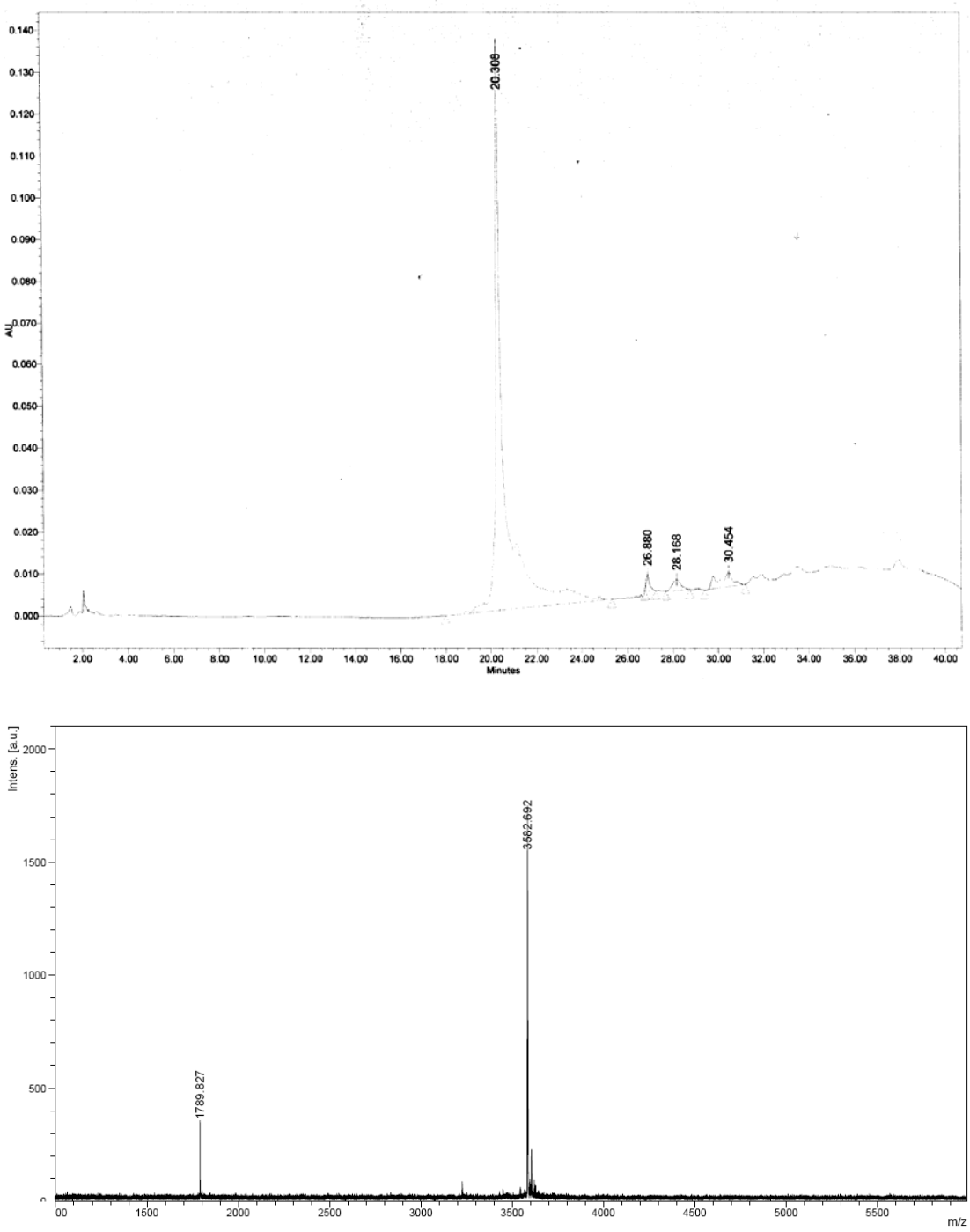



**Figure 30.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac-GCGACGTAGC-LysNH$_2$ (**M10f**) (calcd for $[M+H]^+$: *m/z*=3582.7)

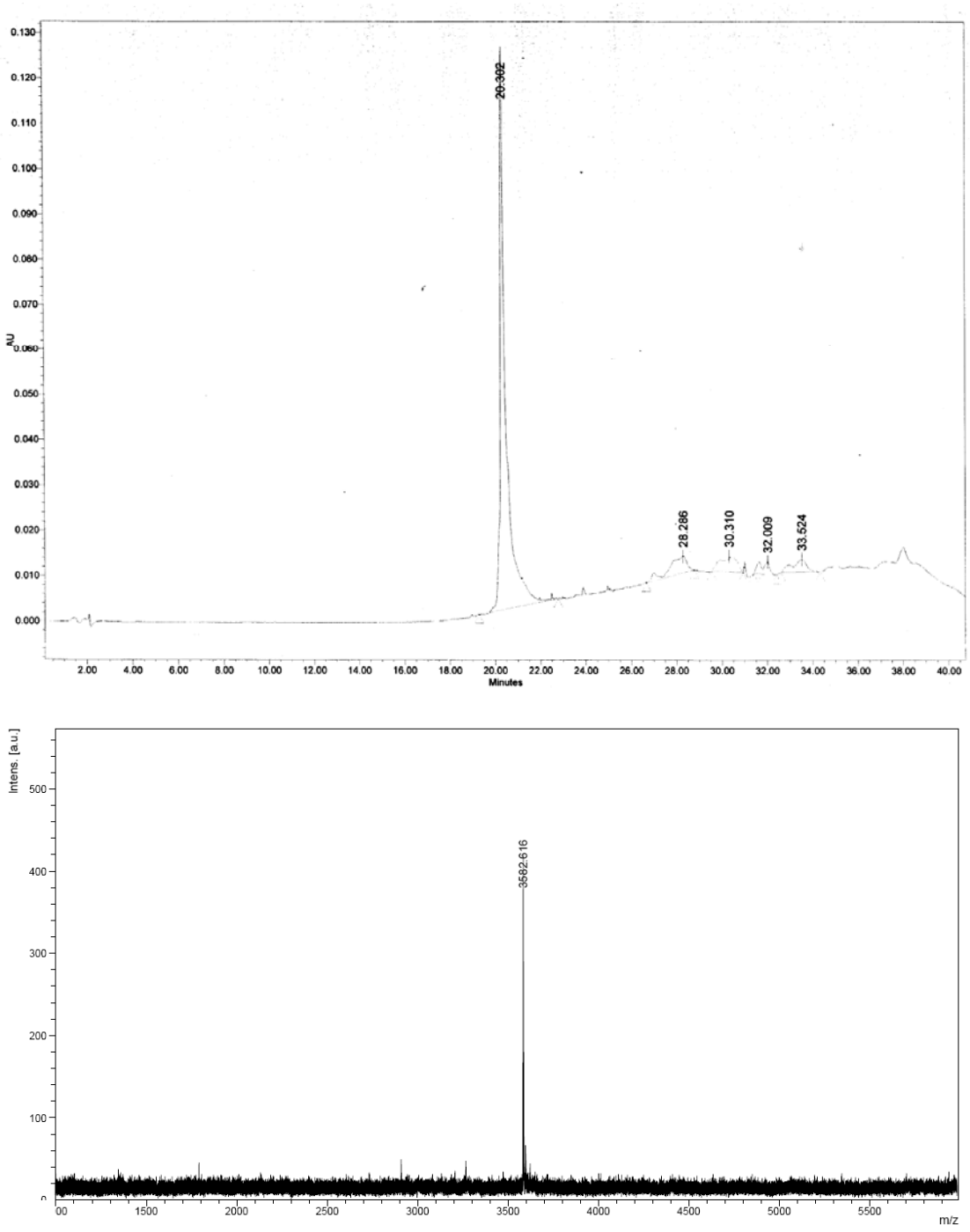



**Figure S31.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac-CGATGCAGCG-Lys$NH_2$ (**M10g**) (calcd for $[M+H]^+$: *m/z*=3582.7)

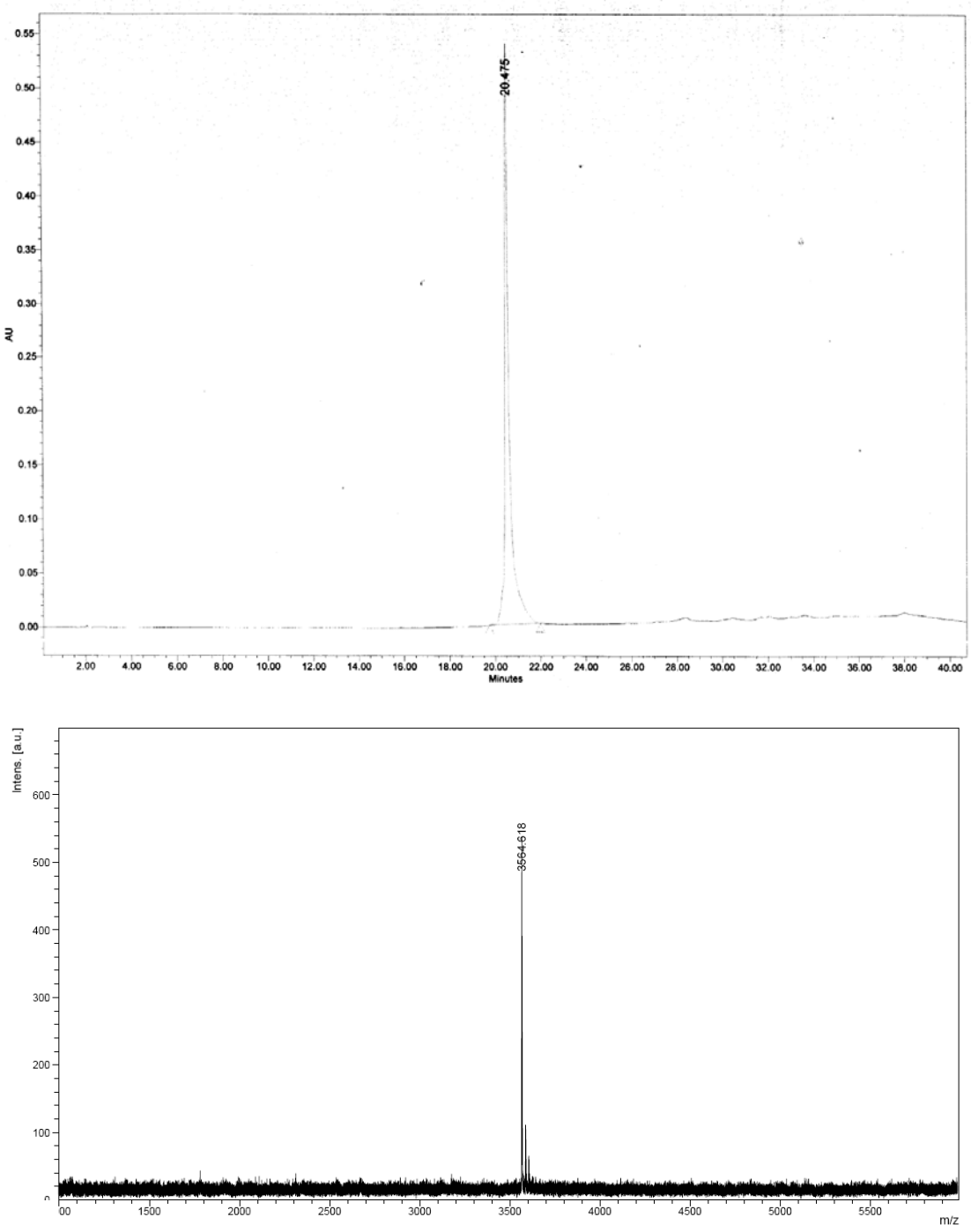



**Figure S32.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottom) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac-ATAGTACATA-Lys$NH_2$ (**M10h**) (calcd for $[M+H]^+$: *m/z*=3564.7)

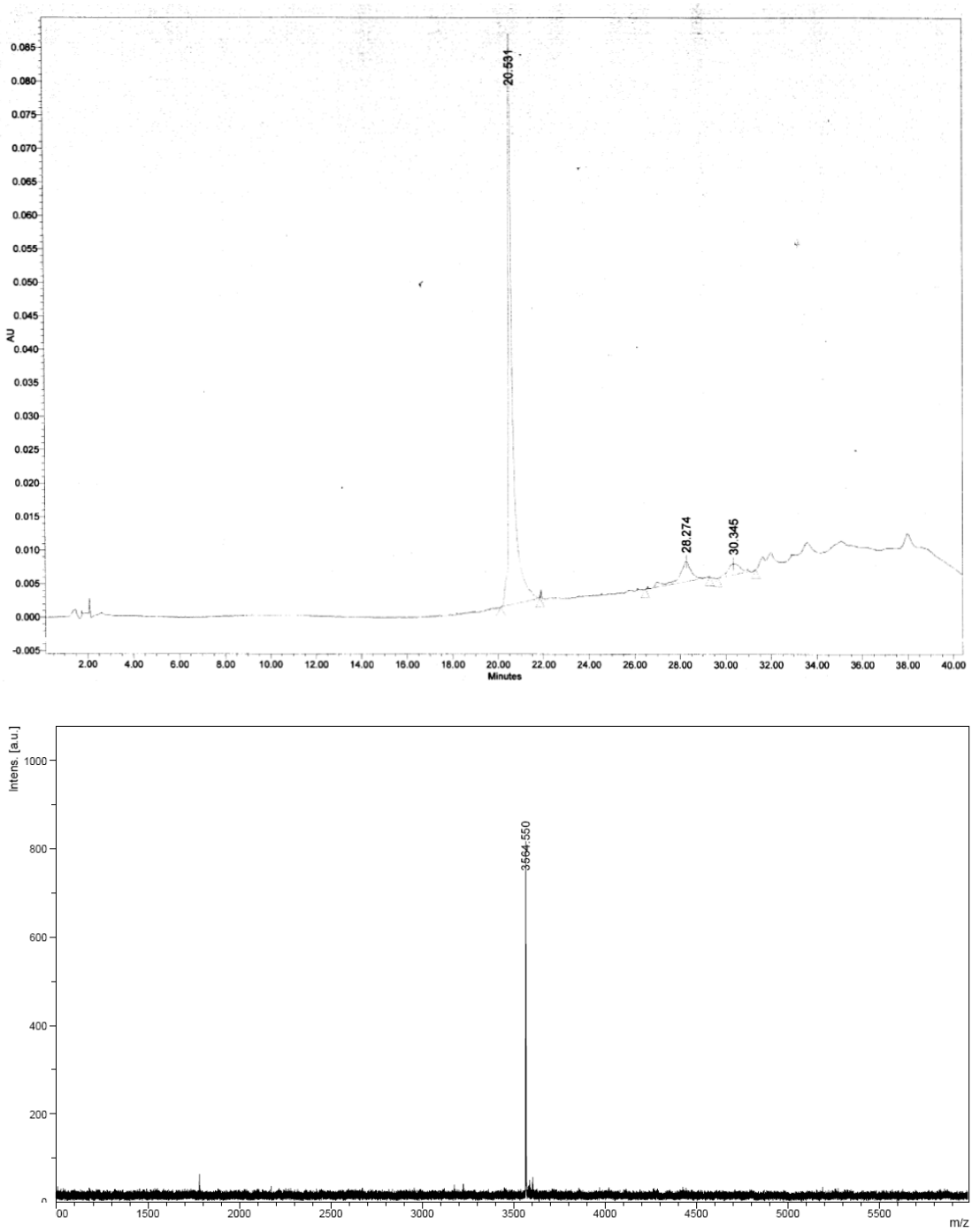



**Figure S33.** HPLC chromatogram (top) and MALDI-TOF mass spectrum (bottm) of (2'*R*,4'*R*)-pro/(1*S*,2*S*)-acpcPNA Ac-ATACATGATA-Lys$NH_2$ (**M10i**) (calcd for $[M+H]^+$: *m/z*=3564.7)

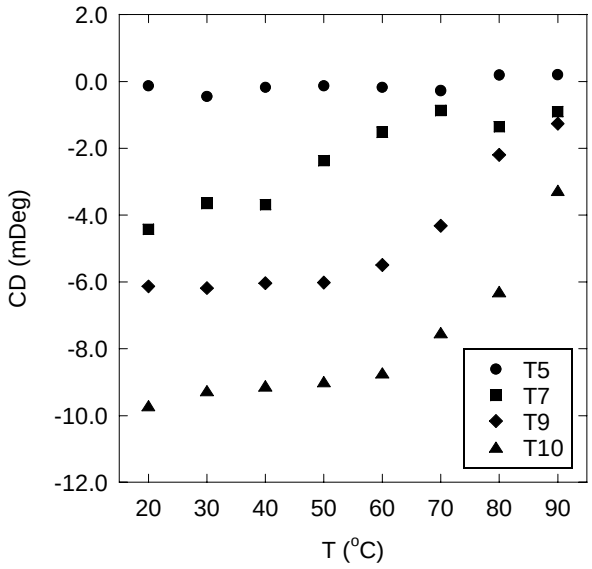



**Figure S34.** $T_m$ curves of homothymine acpcPNA hybrids: $\mathbf{T_n}$·$dA_n$ obtained from CD experiments (Figure S36). The melting experiments were carried out at concentrations of acpcPNA and DNA of 1.0 μM each, and in 10 mM sodium phosphate buffer pH 7.0.

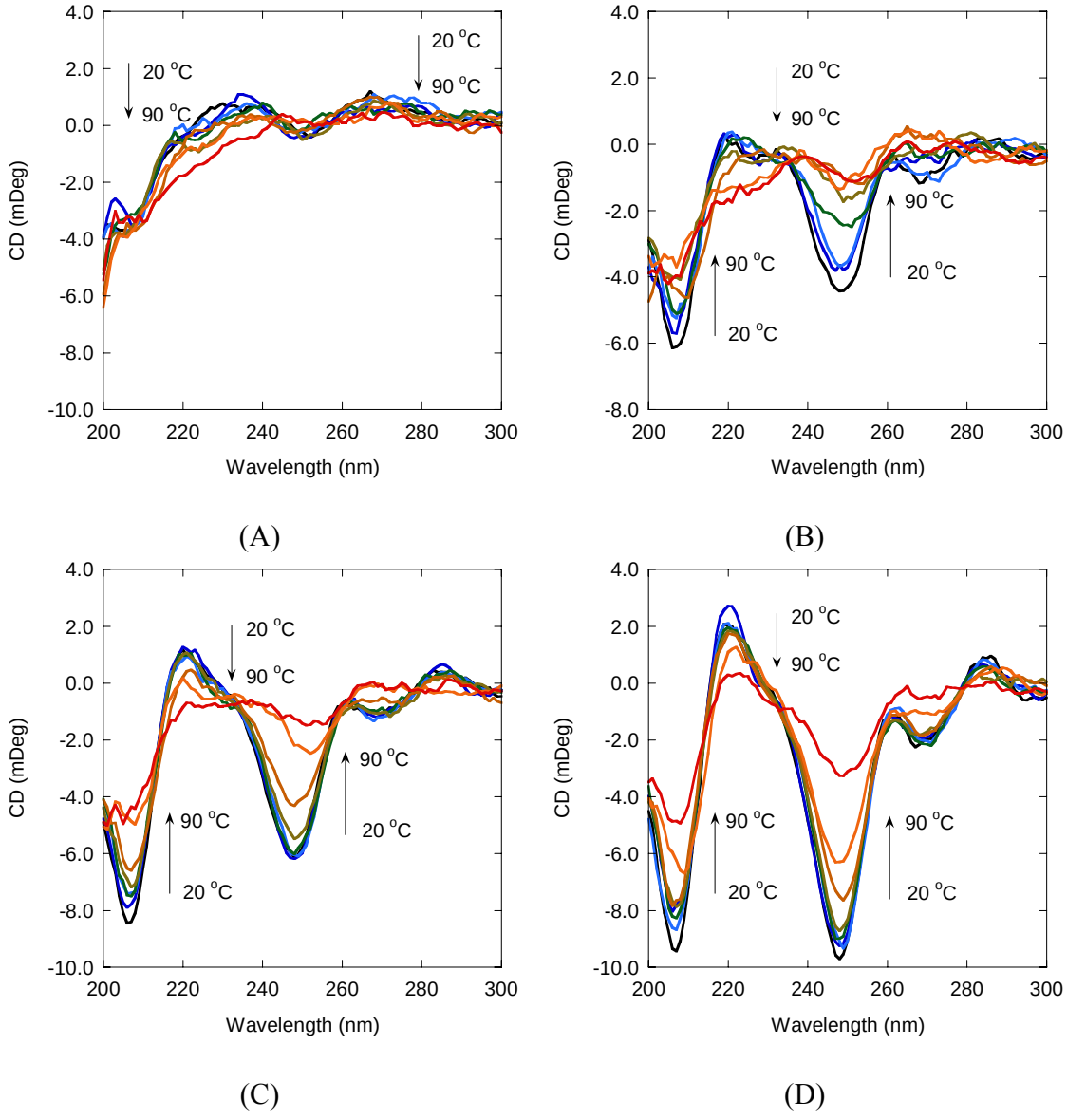



**Figure S35.** Variable CD spectra of homothymine acpcPNA hybrids: **T5**·$dA_5$ (A) **T7**·$dA_7$ (B) **T9**·$dA_9$ (C) and **T10**·$dA_{10}$ (D). The CD spectra were recorded between 20 – 90 ºC concentrations of acpcPNA and DNA of 1.0 μM each, and in 10 mM sodium phosphate buffer pH 7.0.

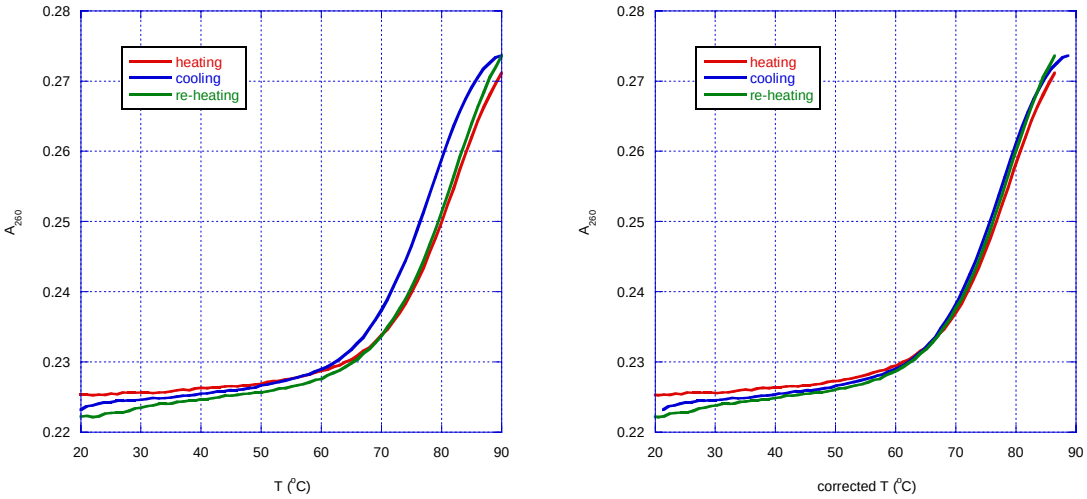



**Figure S36.** A typical $T_m$ curve obtained from heating, cooling and reheating of the acpcPNA·DNA hybrid **T7**·$dA_7$ at a heating and cooling rate of 1.0 ºC/min and a hold time at the end of each cycle of 10 min, without (A) and with (B) temperature correction. The melting experiment was performed at concentrations of acpcPNA and DNA of 1.0 μM each, and in 10 mM sodium phosphate buffer pH 7.0.

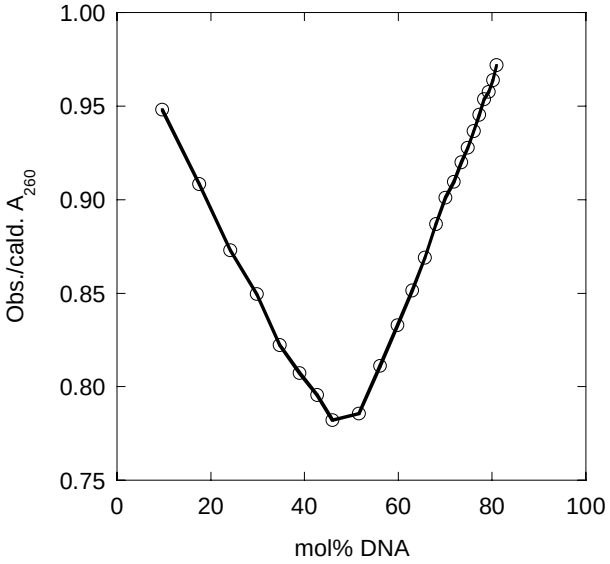



**Figure S37.** UV titration curve of acpcPNA **T9** (analyte) (initial concentration=2.3 μM) with DNA $dT_9$ (38.5 μM) as titrant. The titration was carried out in 10 mM sodium phosphate pH 7.0 containing 100 mM NaCl at 25 ºC. The plot shows the ratio of observed/calculated $A_{260}$ after each addition.[3] The UV titration revealed one inflection point at the acpcPNA:DNA ratio of 1:1.

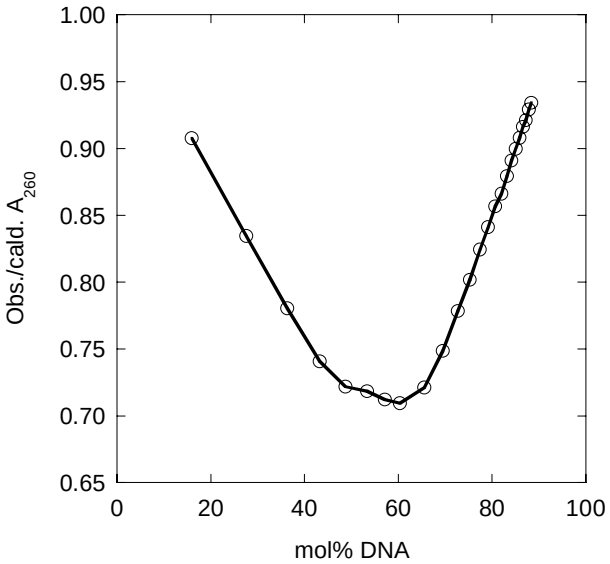
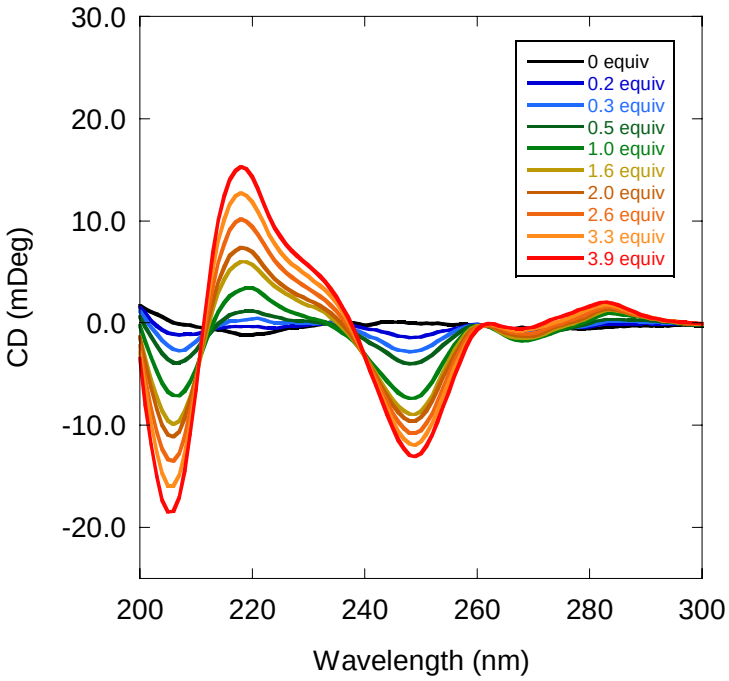
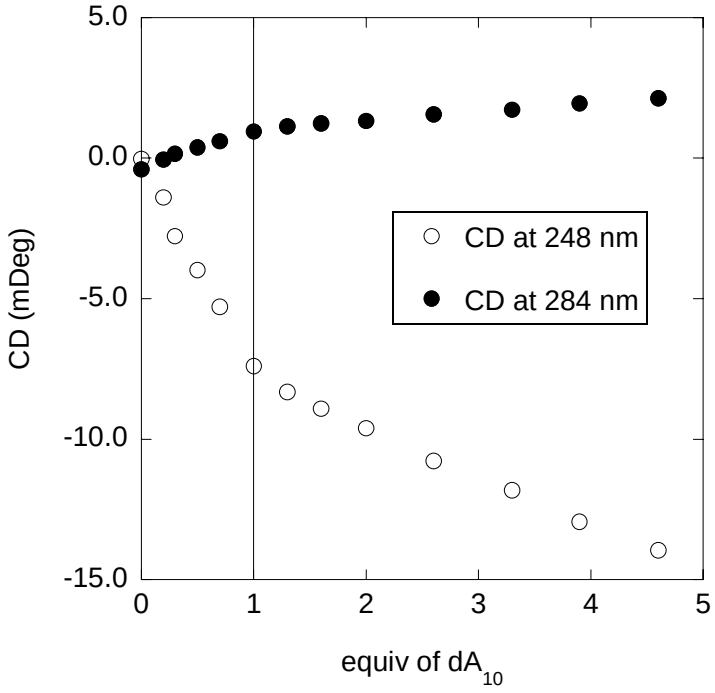



**Figure S38.** UV titration curve of acpcPNA **A9** (analyte) (initial concentration=1.3 μM) with DNA $dT_9$ (39.4 μM) as titrant. The titration was carried out in 10 mM sodium phosphate pH 7.0 containing 100 mM NaCl at 25 ºC. The plot shows the ratio of observed/calculated $A_{260}$ after each addition.[3] The UV titration revealed two inflection points at acpcPNA:DNA ratio of 1:1 and 1:2 respectively.




(A)




(B)

**Figure S39.** CD spectra (A) and CD titration curves at 248 and 284 nm (B) of acpcPNA **T10** (analyte) (initial concentration=1.1 μM) with $dA_{10}$ (173.0 μM) as titrant. The titration was carried out in 10 mM sodium phosphate pH 7.0 at 25 ºC.

**References:**

1. Vilaivan T, Srisuwannaket C. Hybridization of pyrrolidinyl peptide nucleic acids and DNA: Selectivity, base-pairing specificity, and direction of binding. Org Lett 2006; 8:1897–900.
2. Lowe G, Vilaivan T. Dipeptides bearing nucleobases for synthesis of novel peptide nucleic acids. J Chem Soc Perkin Trans 1 1997; 547–54.
3. Vilaivan T, Lowe G. A novel pyrrolidinyl PNA showing high sequence specificity and preferential binding to DNA over RNA. J Am Chem Soc 2002; 124:9326–7.