Furan-PNA: a mildly inducible irreversible interstrand crosslinking system targeting single and double stranded DNA

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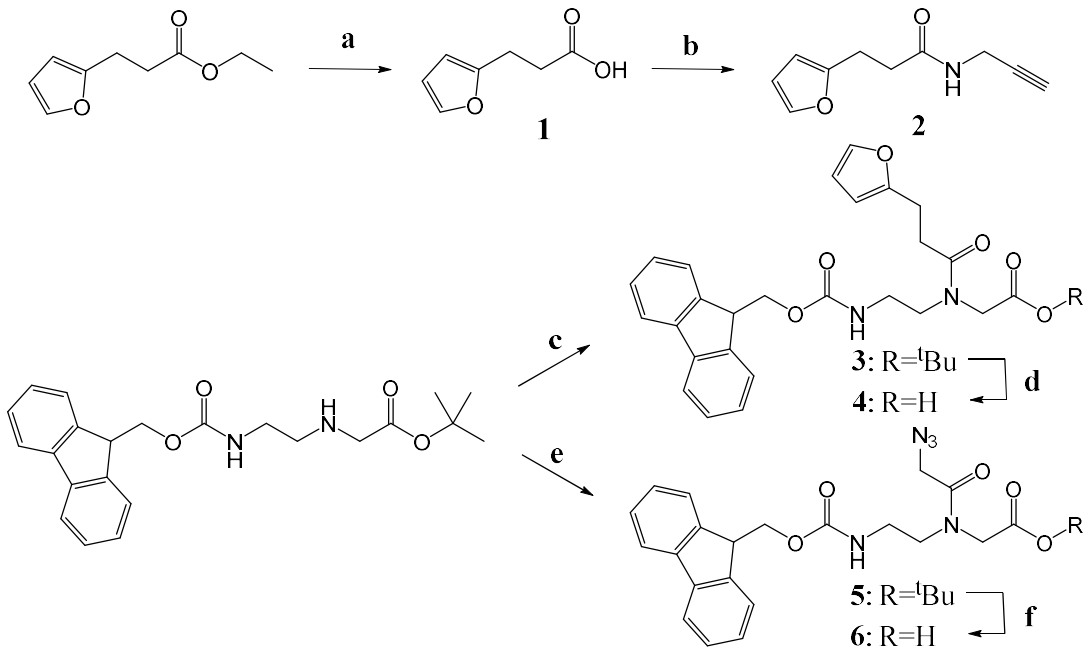
General

All solvents and chemical reagents were purchased from Sigma-Aldrich in the highest purity available. Non-modified DNA sequences were purchased from Eurogentec (Seraing, Belgium).

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 300 or a Brucker DRX 500 spectrometer operating at room temperature. Chemical shifts are reported in parts per million relative to the residual solvent peak. Multiplicities are reported as singlet (s), doublet (d), doublet of doublets (dd), triplet (t) or multiplet (m).

LC-MS data were collected on an Agilent 1100 Series instrument with a Phenomenex Kinetex C18 100Å column (150 x 4.6 mm, 5 µm at 35 °C) connected to an ESMSD type VL mass detector (quadrupole ion trap mass spectrometer) with a flow rate of 1.5 ml/min was used with the following solvent systems: (A): 0.1% HCOOH in H_2O and (B) MeCN. The column was flushed with 100% A for 2 min, then a gradient from 0 to 100% B over 6 min was used, followed by 2 min of flushing with 100% B. UPLC-ESI-Q data were collected on a Waters Acquity UPLC system equipped with a Waters Acquity UPLC BEH C18 column (12.1x50 mm, 1.7 µm) at 35°C. A flow rate of 0.25 ml/min was used with the following solvent systems: (A): 0.2% FA in H_2O and (B): 0.2% FA in MeCN. The column was flushed for 0.9 min with solvent A, then a gradient from 0 to 50% B in 5.7 min. RP-HPLC analyses were recorded on an Agilent 1100 Series instrument with a Phenomenex Luna C18 column (250 x 4.6 mm, 5 µm) at 35 °C. A flow rate of 1 ml/min was used with the following solvent systems: (A): 0.1% TFA in H_2O and (B): MeCN. The column was flushed for 5 min with solvent A, then a gradient from 0 to 50% B in 30 min and to 100% B (HPLC1) or recorded on an Agilent 1200 system equipped with an Aeris Widepore column (150 x 4,6 mm, 3.6 µm) at 60°C. A flow rate of 0.8 mL/min was used with following solvent systems: (A): 0.1 M TEAA-buffer (with 5% MeCN) and (B): MeCN. The column was flushed for 2 min with solvent A, then a gradient from 0 to 12% B in 13 min and to 99% B in 1 min was used, followed by a flush of 5 min with 99% B (HPLC2). MALDI-TOF spectra were acquired on an ABI Voyager DE-STR MALDI-TOF with a high performance nitrogen laser (337 nm), using the positive and reflector mode with delayed extraction. The matrix solution utilized was prepared as follows: 10 mg 2,5-Dihydroxybenzoic acid in 100 µL mQ/MeCN 2:1 with 1% TFA. Concentrations of DNA- and PNA-solutions were measured with a Trinean DropSense96 UV/VIS droplet reader. Thermal denaturation experiments were recorded on a Varian Cary 300 Bio instrument equipped with a six-cell thermostatted cell holder. Densitometric evaluation was performed using the program ImageJ 1.50.b, indication of the mean density is provided, together with the minimum and maximum value of intensity (0-255 range).

Probe synthesis



Scheme S1: synthesis of the PNA monomers used in this study.

3-(furan-2-yl)-propanoic acid (1): in a round bottom flask ethyl 3-(furan-2-yl)-propanoate (990.3mg, 5.89mmol, 1eq) was dissolved in 20mL THF. The solution was then cooled to 0°C with an ice bath before the addition of LiOH (988.5mg, 23.56mmol, 4eq) dissolved in 20mL water. After 5 minutes the solution was allowed to warm to r.t. and to react for 2h before removing the organic solvent under reduced pressure. The pH of the solution was lowered to 3 with concentrated HCl and the precipitation was favored for 2h at 4°C. The precipitate was then collected through Buchner filtration, and a second aliquot of product was obtained by extraction of the solution with AcOEt (2x20mL). Combining the two fractions **1** was obtained as a white solid in 85.6% yield (706.5mg). **TLC (AcOEt) Rf:** 0.40; **^{1}H NMR (DMSO-d^6, 400MHz) δ(ppm):** 12.20 (s, 1H), 7.50 (dd, *J* = 1.8, 0.8 Hz, 1H), 6.34 (dd, *J* = 3.1, 1.9 Hz, 1H), 6.09 (ddd, *J* = 2.9, 1.8, 0.9 Hz, 1H), 2.83 (t, *J* = 7.4 Hz, 2H), 2.54 (t, *J* = 7.4 Hz, 2H); **^{13}C NMR (DMSO-d^6, 100MHz) δ(ppm):** 173.8, 154.8, 141.8, 110.8, 105.6, 32.4, 23.4; **HR-MS (ESI, MeOH):** *m/z* calcd for $[C_7H_7O_3]^-$: 139.04007, found: 139.04041.

3-(furan-2-yl)-N-(prop-2-yn-1-yl)propanamide (2): in a round bottom flask **1** (56.1mg, 0.400mmol, 1eq) and HBTU (159.4mg, 0.420, 1.05eq) were dissolved in 2mL DMF. The solution was then cooled to 0°C with an ice bath before the addition of DIPEA (135.63µL, 0.821mmol, 2.05eq). The reaction was left to stir at 0°C for 15 minutes followed by 15 minutes at r.t.. Finally, propargylamine (51.28µL, 0.801mmol, 2eq) was added to the mixture and left to react for 5h. The reaction was then diluted with AcOEt (50mL) and washed with 0.1M HCl (2x50mL), saturated aq. $NaHCO_3$ (2x50mL) and brine (50mL). The organic layer was dried over Na_2SO_4 and the solvent evaporated under reduced pressure to give **2** as a brownish solid in 88.5% yield (62.8mg). **TLC (AcOEt) Rf:** 0.21; **^{1}H NMR ($CDCl_3$, 300MHz) δ(ppm):** 7.23 (dd, *J* = 1.8, 0.8 Hz, 1H), 6.21 (dd, *J* = 3.1, 1.9 Hz, 1H), 5.59 (br s, 1H), 5.59 (br s, 1H), 3.97 (dd, *J* = 5.2, 2.6 Hz, 1H), 2.93 (t, *J* = 7.5 Hz, 2H), 2.46 (t, *J* = 7.5 Hz, 2H), 2.15 (t, *J* = 2.6 Hz, 1H); **^{13}C NMR ($CDCl_3$, 75MHz) δ(ppm):** 171.3, 154.1, 141.3, 110.3, 105.6, 79.4, 71.6, 34.7, 29.2, 23.8; **MS (ESI, MeOH):** *m/z* calcd for $C_{10}H_{11}NO_2$ [M]: 177.07898, found: 178.2 $[M+H]^+$, 377.2 $[2M+Na]^+$, 176.1 $[M-H]^-$; **HR-MS (ESI, MeOH):** *m/z* calcd for $[C_{10}H_{12}NO_2Na]^+$: 178.08626, found: 178.0859.

Fmoc-PNA-Furan-O^{t}Bu (3): in a round bottom flask **1** (64.7mg, 0.462mmol, 2eq) and DhBtOH (75.4mg, 0.462mmol, 2eq) were dissolved in 2mL DMF and cooled to 0°C with an ice bath. EDC·HCl (88.5mg, 0.462mmol, 2eq) and DIPEA (114.5µL, 0.693mmol, 3eq) were then added and the mixture was left to react 10 minutes at 0°C and subsequently 10 minutes at r.t.. Finally, Fmoc-PNA-backbone-O^{t}Bu (100.6mg, 0.232mmol, 1eq) was added and the mixture was left to react for 4h. The reaction was then diluted with AcOEt (100mL) and washed with 0.1M HCl (2x100mL), saturated aq. $NaHCO_3$ (2x100mL) and brine (100mL). The organic layer was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The crude was then purified through percolation over silica (elution from hexane to hexane:AcOEt 1:1) to obtain **3** as a foamy solid in 85.3% yield (102.7mg). **TLC (AcOEt) Rf:** 0.64; **^{1}H NMR ($CDCl_3$, 300MHz, major rotamer) δ(ppm):** 7.69 (d, J = 7.5 Hz, 2H), 7.50 (d, J = 7.1 Hz, 2H), 7.32 (t, J = 7.2 Hz, 2H), 7.23 (t, J = 7.2 Hz, 2H), 7.13 (s, 1H), 6.12 (br s, 1H), 5.95 – 5.80 (m, 2H), 4.28 (d, J = 7.0 Hz, 2H), 4.14 (t, J = 6.8 Hz, 1H), 3.84 (s, 2H), 3.42 (t, J = 5.8 Hz, 2H), 3.30 (t, J = 5.5 Hz, 2H), 2.87 (t, J = 7.0 Hz, 2H), 2.60 (t, J = 7.0 Hz, 2H), , 1.40 (s, 9H); **^{13}C NMR ($CDCl_3$, 75MHz, major rotamer) δ(ppm):** 171.3, 168.7, 155.6, 153.6, 142.8, 140.3, 126.6, 126.05, 124.1, 118.9, 110.6, 109.2, 104.3, 81.3, 66.0, 48.9, 48.3, 46.2, 38.4, 30.3, 27.0, 22.5; **MS (ESI, MeOH):** *m/z* calcd for $C_{30}H_{34}N_2O_6$ [M]: 518.24169, found: 463.2 [M-i-butene+H]$^+$, 519.4 [M+H]$^+$, 541.3 [M+Na]$^+$, 553.2 [M+Cl]$^-$; **HR-MS (ESI, MeOH):** *m/z* calcd for $[C_{30}H_{35}N_2O_6]^+$: 519.24896, found: 519.2487.

Fmoc-PNA-Furan-OH (4): in a round bottom flask **3** (92.9mg, 0.179mmol, 1eq) was dissolved in 20mL DCM and cooled to 0°C with an ice bath. To this mixture 5mL TFA was added dropwise and the solution was left to react for 1h at 0°C and then 2h at r.t.. When conversion was complete the solvents were evaporated in presence of MeOH (20mL, color change from deep blue to yellow/green). Remaining TFA was finally co-evaporated with $CHCl_3$ to obtain **4** as a brownish solid in a quantitative yield. **TLC (hexane:AcOEt 1:1) Rf:** 0.07; **^{1}H NMR (DMSO-d^6, 300MHz, major rotamer) δ(ppm):** 12.59 (s, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.50 – 7.23 (m, 5H), 6.35 – 6.30 (m, 1H), 6.11 – 6.02 (m, 1H), 4.29 (d, *J* = 6.3 Hz, 2H), 4.19 (t, *J* = 7.4 Hz, 1H), 3.94 (s, 2H), 3.46 – 3.22 (m, 2H), 3.20 – 3.05 (m, 2H), 2.82 – 2.75 (m, 2H), 2.70 – 2.60 (m, 2H); **^{13}C NMR (DMSO-d^6, 75MHz, major rotamer) δ(ppm):** 171.6, 170.9, 156.2, 154.7, 143.8, 140.7, 127.6, 127.0, 125.0, 120.1, 110.3, 105.0, 65.4, 47.3 (x2), 46.7, 39.1, 30.0, 23.1; **MS (ESI, MeOH):** *m/z* calcd for $C_{26}H_{26}N_2O_6$ [M]: 462.17909, found: 463.2 [M+H]$^+$, 461.2 [M-H]$^-$, 923.4 [2M-H]$^-$; **HR-MS (ESI, MeOH):** *m/z* calcd for $[C_{26}H_{27}N_2O_6]^+$: 463.18636, found: 463.1863.

Fmoc-PNA-N$_3$-O^{t}Bu (5): in a round bottom flask 2-azidoacetic acid (38.28µL, 0.511mmol, 2eq) and DhBtOH (83.4mg, 0.511mmol, 2eq) were dissolved in 2mL DMF and cooled to 0°C with an ice bath. EDC·HCl (98.0mg, 0.511mmol, 2eq) and DIPEA (126.8µL, 0.767mmol, 3eq) were added and the mixture was left to react 10 minutes at 0°C and then 10 minutes at r.t.. Finally, Fmoc-PNA-backbone-O^{t}Bu (110.7mg, 0.256mmol, 1eq) was added and the mixture was left to react for 3h. The reaction was then diluted with AcOEt (100mL) and washed with 0.1M HCl (2x100mL), saturated aq. $NaHCO_3$ (2x100mL) and brine (100mL). The organic layer was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The crude was then purified through percolation over silica (elution from hexane:AcOEt 9:1 to hexane:AcOEt 1:1) to obtain **5** as a yellowish oil in 97.8% yield (119.9mg). **TLC (AcOEt) Rf:** 0.63; **^{1}H NMR ($CDCl_3$, 300MHz, major rotamer) δ(ppm):** 7.70 (d, *J* = 7.4 Hz, 2H), 7.52 (d, *J* = 7.5 Hz, 2H), 7.34 (t, *J* = 7.4 Hz, 2H), 7.25 (t, *J* = 7.4 Hz, 2H), 5.74 (br s, 1H), 4.32 (d, *J* = 7.0 Hz, 2H), 4.15 (t, *J* = 6.8 Hz, 1H), 3.88 (s, 2H), 3.85 (s, 2H), 3.40 – 3.25 (m, 4H), 1.41 (s, 9H); **^{13}C NMR ($CDCl_3$, 75MHz, major rotamer) δ(ppm):** 169.0, 168.1, 156.6, 143.8, 141.3, 127.8, 127.1, 125.0, 120.0, 82.7, 67.0, 50.0, 49.8, 48.6, 47.2, 39.2, 28.0; **MS (ESI, MeOH):** *m/z* calcd for $C_{25}H_{29}N_5O_5$ [M]: 479.21687, found: 424.2 [M-i-butene+H]$^+$, 502.2 [M+Na]$^+$, 514.1 [M+Cl]$^-$; **HR-MS (ESI, MeOH):** *m/z* calcd for $[C_{25}H_{29}N_5O_5Na]^+$: 502.20609, found: 502.2052.

Fmoc-PNA-N_3-OH (6): in a round bottom flask **5** (113.0mg, 0.236mmol, 1eq) was dissolved in 10mL DCM and cooled to 0°C with an ice bath. To this mixture 4mL TFA was added dropwise and the solution was left to react for 5 minutes at 0°C and subsequently 30 minutes at r.t.. When conversion was complete the solvents were evaporated in presence of MeOH (10mL). Remaining TFA was finally co-evaporated with $CHCl_3$ to obtain **6** as a yellowish solid in a quantitative yield. **TLC (AcOEt) Rf:** 0.50; **^{1}H NMR (DMSO-d^6, 300MHz, major rotamer) δ(ppm):** 12.73 (s, 1H), 7.89 (d, *J* = 7.4 Hz, 2H), 7.67 (d, *J* = 7.4 Hz, 2H), 7.44 – 7.27 (m, 5H), 4.31 (t, *J* = 7.6 Hz, 2H), 4.22 (d, *J* = 7.6 Hz, 1H), 4.16 (s, 2H), 3.96 (s, 2H), 3.38 – 3.23 (m, 2H), 3.22 – 3.05 (m, 2H).; **^{13}C NMR (DMSO-d^6, 75MHz, major rotamer) δ(ppm):** 170.4, 167.9, 156.3, 143.8, 140.7, 127.6, 127.0, 125.1, 120.1, 65.4, 49.5, 49.1, 46.7, 45.7,38.8; **MS (ESI, MeOH):** *m/z* calcd for $C_{21}H_{21}N_5O_5$ [M]: 423.15427, found: 424.2 $[M+H]^+$, 422.1 $[M-H]^-$, 845.2 $[2M-H]^-$; **HR-MS (ESI, MeOH):** *m/z* calcd for $[C_{21}H_{22}N_5O_5]^+$: 424.16155, found: 424.1610.

PNA synthesis: the synthesis of all the PNAs was performed with standard Fmoc-based manual synthesis protocol using **Fmoc-PNA-T(N_3)-OH**, **4** and **6** in addition to standard monomers, on a Rink amide ChemMatrix resin loaded with Fmoc-Gly-OH as first monomer (0.2 mmol/g), using HBTU/DIPEA as activating mixture. Cleavage of the resin was performed using a TFA/m-cresol 9:1 solution for all the PNA strands except for **PNA F** which was cleaved with a TFA/m-cresol/tioanisole 8:1:1 solution. The cleavage step was carried out for 1h, twice.

Comparison of cleavage cocktails for the protection of furan: few beads (about 1mg) of a test resin containing a furan moiety (Fmoc-AFGATCT-Gly-Res) were placed in an eppendorf tube, then 50 µL of cleavage cocktail (a- TFA/m-cresol 9:1, b- TFA/m-cresol/tioanisole 8:1:1, c- TFA/TIS/m-cresol 8:1:1, d- TFA/TIS/tioanisole 8:1:1) was added and left to react. After 1h30' ethyl ether was added and the precipitate was collected by centrifugation. The crudes were then analyzed by HPLC-UV and the identity of the peaks was confirmed by purification and MALDI analysis.

General protocol for click reaction: different solutions were prepared: 200 mM solution of **2** in MeOH, 200 mM solution of copper sulfate in H_2O, 200 mM solution of sodium ascorbate in H_2O. Reaction was carried out with a final PNA concentration (from crude PNA) of 2 mM or 5 mM using a molar ratio alkyne/ascorbate/Cu(II) of 2:4:2. The mixture was then left to react for 2h before the purification (no significant variation of the HPLC profile observed between 15 minutes and 1h).

UPLC-MS characterization: PNA T: R_t: 2.90 min, MW: 3090.98, *m/z found:* 1031.4 $[M+3H]^{3+}$, 773.6 $[M+4H]^{4+}$, 619.2 $[M+5H]^{5+}$, 516.1 $[M+6H]^{6+}$; **PNA T(f):** R_t: 3.19 min, MW: 3351.23, *m/z found:* 1187.3 $[M+3H]^{3+}$, 838.8 $[M+4H]^{4+}$, 671.2 $[M+5H]^{5+}$, 599.6 $[M+6H]^{6+}$; **PNA f:** R_t: 3.07 min, MW: 3227.13, *m/z found:* 1176.8 $[M+3H]^{3+}$, 807.8 $[M+4H]^{4+}$, 646.5 $[M+5H]^{5+}$, 538.9 $[M+6H]^{6+}$; **PNA F:** R_t: 3.13 min, MW: 3089.00, *m/z found:* 1030.8 $[M+3H]^{3+}$, 773.3 $[M+4H]^{4+}$, 618.8 $[M+5H]^{5+}$, 516.1 $[M+6H]^{6+}$.

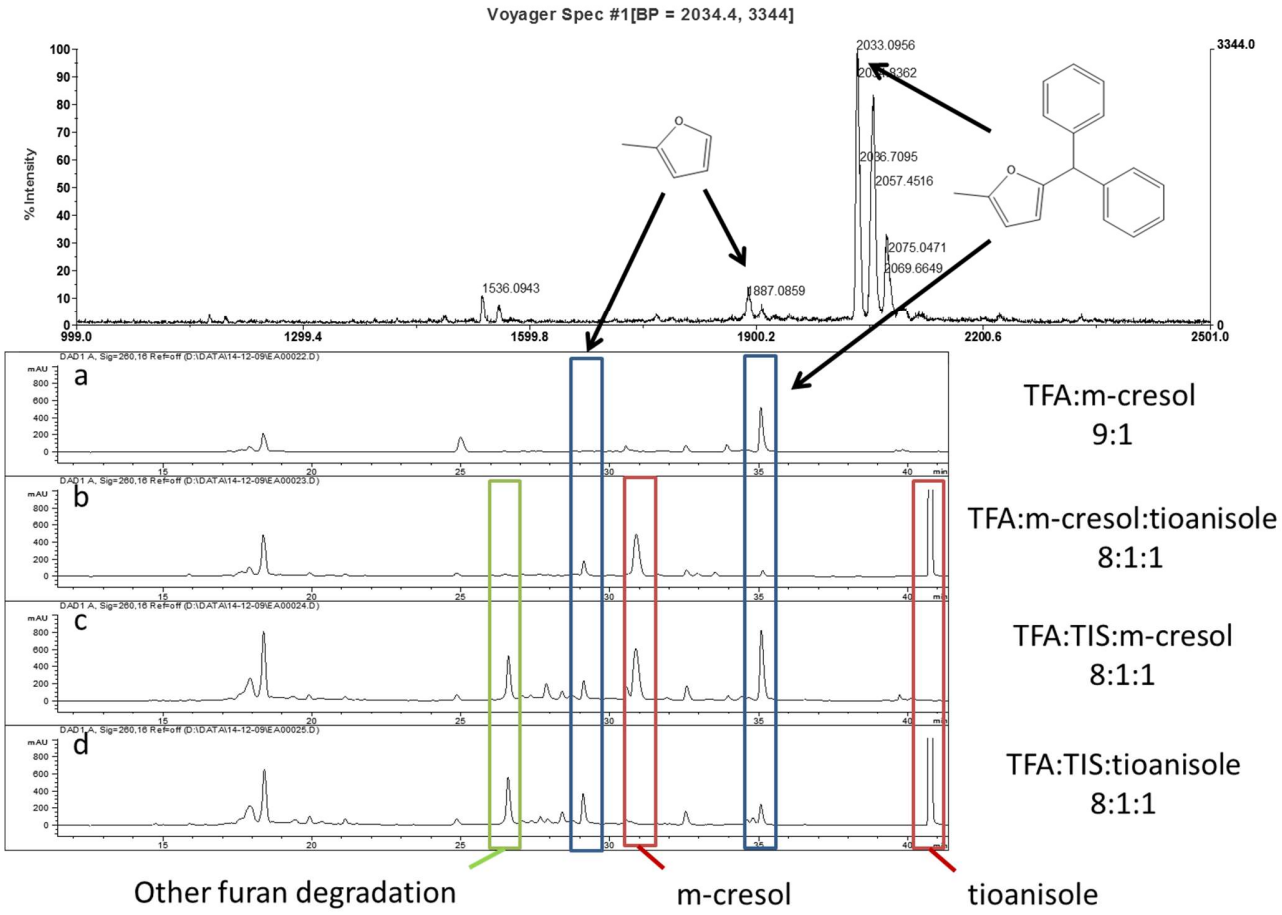


Fig. S1: MALDI spectrum of the crude from test a (top) and HPLC1-UV profiles of the crude obtained from the different cleavage cocktails.

Measurements of T_m values: thermal denaturation profiles were measured by monitoring the absorbance at 260nm from 18°C to 90°C and from 90°C to 18°C with a heating rate of 1°C/min and recording every 0.1°C (3 cycles). Measurement condition: strand concentration = 5μM in pH 7.0 PBS buffer (100 mM NaCl, 10 mM NaH_2PO_4). Melting temperatures were calculated from the first derivative of the heating curves using the Cary 300 Bio software.

Table S1: melting temperature (°C) of the PNA:DNA complexes; PNA X: Ac-GGGCA**X**GATCT-Gly-NH_2, DNA Y: 5'-AGATC**Y**TGCCC-3', DNA as: 5'-GGGCATGATCT-3'. Number inside parentheses indicate the hysteresis of the processes, i.e. the differences between the melting and annealing temperature, obtained by the heating and cooling curves respectively.

		DNA			
		A	C	G	T
PNA	0	68.94 (4.78)	49.97 (4.10)	55.44 (3.63)	56.84 (4.21)
	f	49.98 (3.08)	49.81 (3.02)	59.52 (3.28)	51.24 (2.13)
	T(f)	69.03 (2.99)	51.00 (2.62)	55.79 (2.78)	57.85 (3.11)
	F	47.37 (2.98)	47.21 (3.28)	47.96 (3.18)	47.22 (3.60)
	DNA T	50.52 (3.33)	33.86 (3.38)		

General protocol for crosslink reactions: a solution was prepared at 10 µM concentration of each strand in PBS with a total volume of 50 µL solution. The complexes were slowly annealed from 90° to room temperature (in about 2h). During the crosslink reaction, temperature was kept constant in an Eppendorf thermomixer comfort at 25°C (unless otherwise mentioned). A stock solution of NBS (0.5 nmol/2 µL) was freshly prepared and to start the reaction, 1 equiv (=0.5 nmol) of NBS was added. This was repeated every 15 min until 4 equiv of NBS were added. The reactions were monitored by HPLC2.
For strand displacement experiments dsDNA was annealed at 20 µM probe concentration, then PNA was added to a final probe concentration of 10 µM, the solution was then left to equilibrate at controlled temperature (25°C or 37°C) for 3 hours.

General protocol for PAGE: a 20% polyacrylamide gel (acrylamide:bisacrylamide 19:1) for short oligonucleotide probe, or a 16% polyacrylamide gel (acrylamide:bisacrylamide 37.5:1) for long oligonucleotide probes, were prepared in 1x Tris-Borat-EDTA (TBE) buffer containing 7 M urea. The temperature of the gel was stabilized with a Julabo F12 at 25°C. The power supply used for gel electrophoresis was a consort EV202 and a voltage of 260 V was used to run the gels. Gels were stained with GelRed (VWR) or SYBR gold (Thermo Fisher Scientific, Life Technologies) and pictures were taken with an Autochemi imaging system (UVP). 4µL of the crosslink solution (10µM) were mixed with 16 µL formamide and from this mixture 8 µL was loaded on the gel.

PAGE experiments

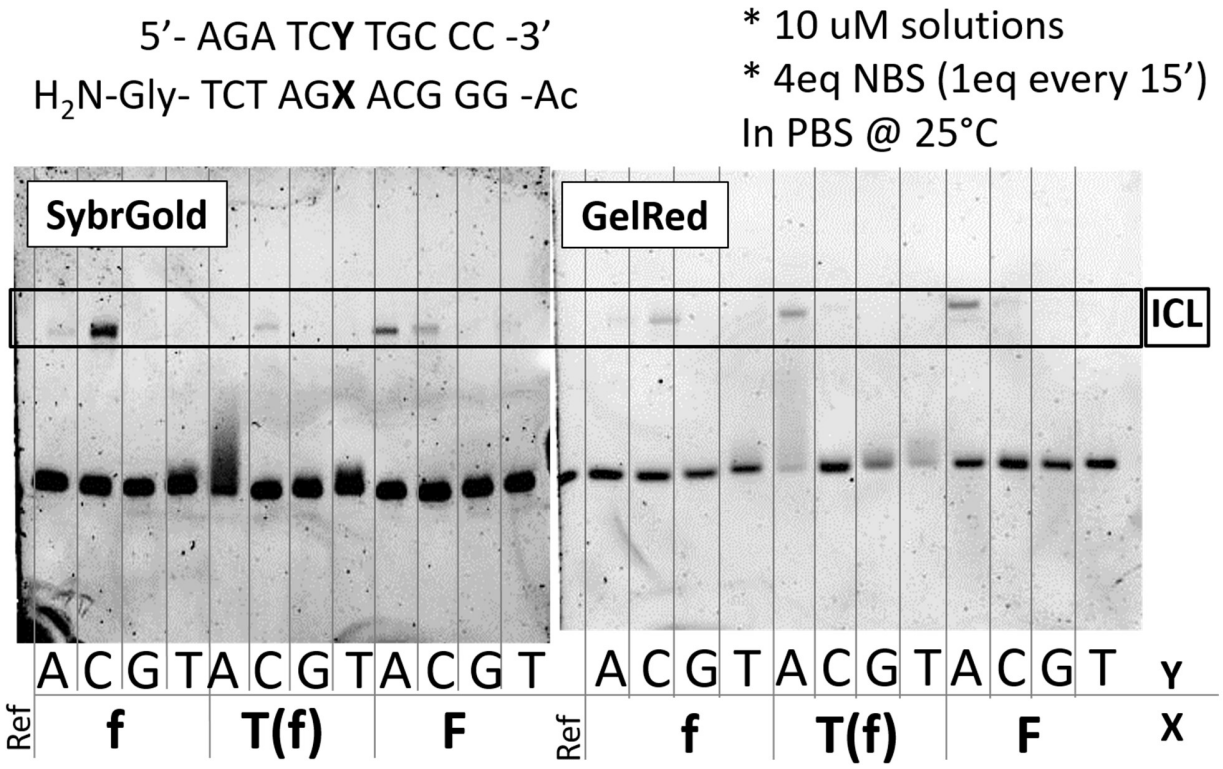


Fig. S2: full denaturing PAGE picture of the PNA:DNA crosslink experiment.

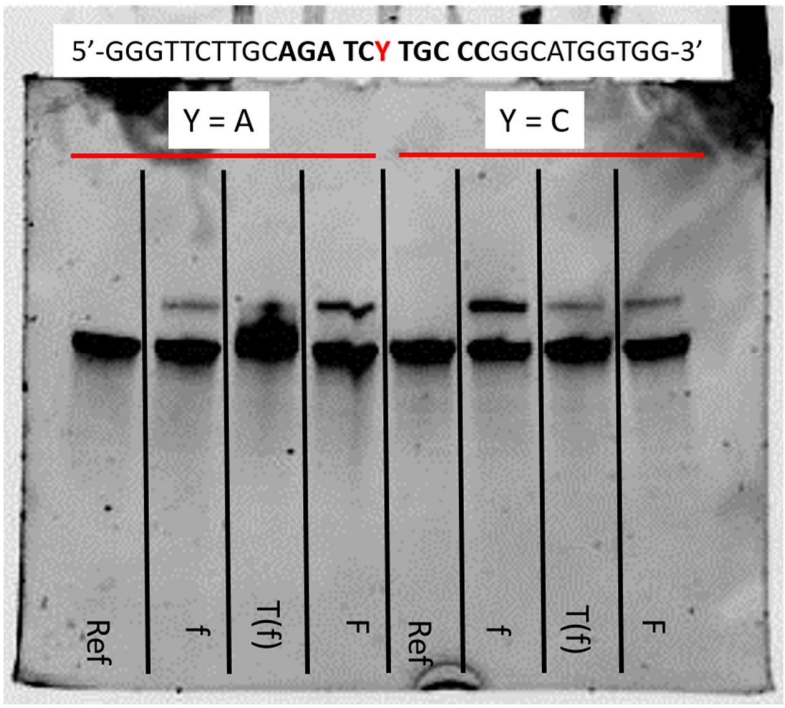


Fig. S3: full denaturing PAGE picture of the PNA:DNA crosslink experiment with long sequences. GelRed staining was used.

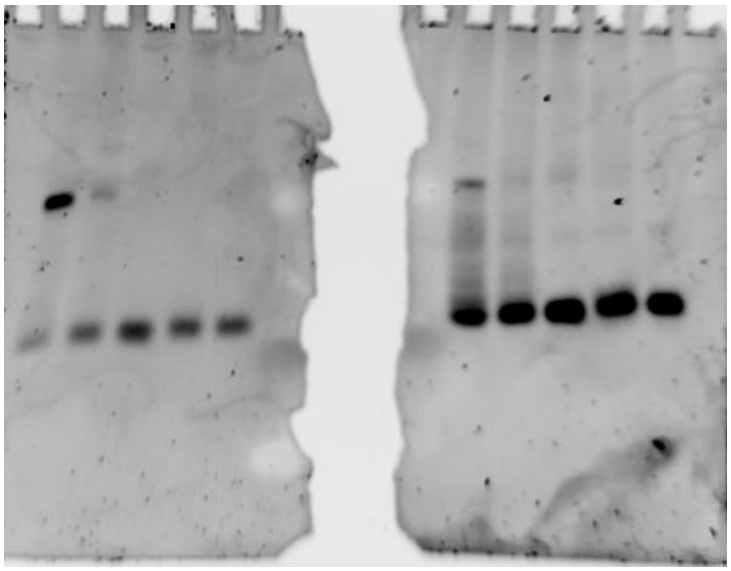


Fig. S4: denaturing PAGE experiments for PNA T(f) probe. Lanes (from left to right, each gel): 1) no NBS activation; 2) DNA A; 3) DNA C; 4) DNA G; 5) DNA T. Left gel was stained with GelRed, right gel was stained with SybrGold.

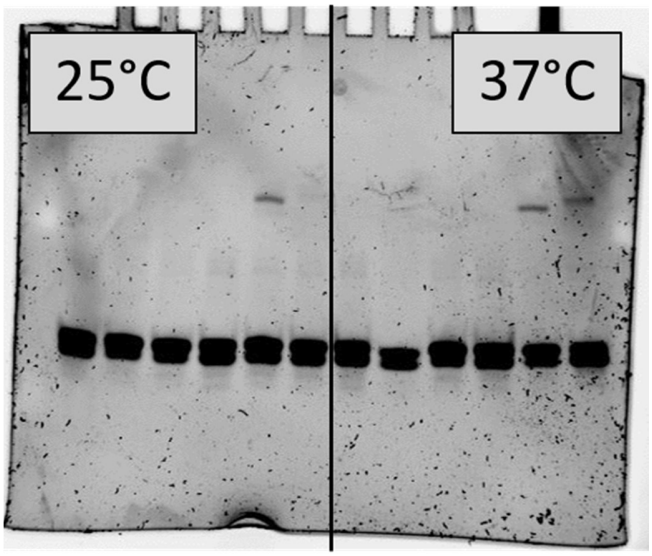


Fig. S5: full denaturing PAGE picture of the strand invasion crosslink experiments. Lanes (from left to right, each section): 1) Ref dsDNA A; 2) + PNA f; 3) + PNA F; 4) Ref dsDNA C; 5) + PNA f; 6) + PNA F. Stain: SybrGold.

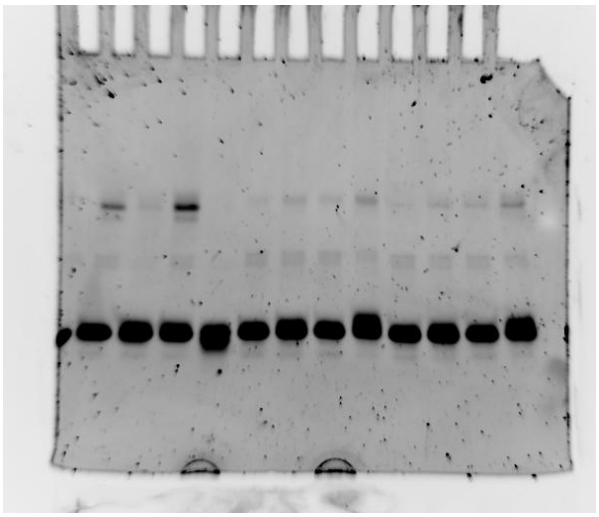


Fig. S6: effect of 2% DMF in strand displacement crosslink experiments. Displacement was carried out at 37°C for 12h. Lanes (from left to right): 1) Ref; 2) PNA f + dsDNA A; 3) PNA f + dsDNA A + 2% DMF; 4) PNA f + dsDNA C; 5) PNA f + dsDNA C + 2% DMF; 6) PNA F + dsDNA A; 7) PNA F + dsDNA A + 2% DMF; 8) PNA F + dsDNA F; 9) PNA F + dsDNA C + 2% DMF; 10) PNA T(f) + dsDNA A; 11) PNA T(f) + dsDNA A + 2% DMF; 12) PNA T(f) + dsDNA C; 13) PNA T(f) + dsDNA C+ 2% DMF; 14) color.

Table S2: densitometric evaluation of the ICL regions of the PAGE experiments shown in Fig. 3 and Fig. S2.[a]

Lane	PNA	DNA	SybrGold			GelRed		
			Mean	Min	Max	Mean	Min	Max
1	Ref lane		57.475	53	64	64.699	60	70
2	f	A	61.761	50	115	68.558	59	89
3		C	99.507	52	189	81.486	64	103
4		T	55.362	50	67	67.793	58	103
5		G	54.362	42	86	72.5	66	81
6	T(f)	A	54.489	43	64	93.341	68	140
7		C	57.764	45	77	71.243	65	79
8		T	56.565	37	205	67.935	64	91
9		G	56.036	48	97	69.96	67	76
10	F	A	74.38	52	120	105.46	72	168
11		C	69.986	57	87	76.123	66	94
12		T	60.264	49	104	65.159	62	69
13		G	61.312	53	71	62.406	55	66

[a] As it cannot be assumed that the response factor for DNA and PNA-DNA is identical, the densitometric absolute values are given only for the ICL band region.

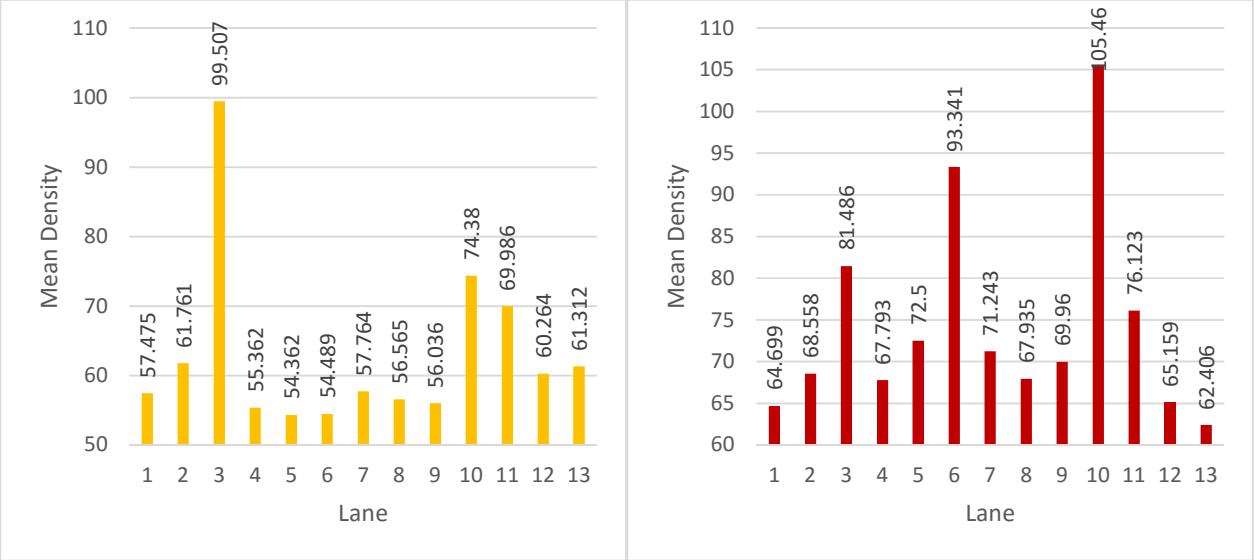


Fig. S7: histogram representation of the data presented in Table S2. Left: gel stained with SybrGold; right: gel stained with GelRed.

Table S3: densitometric evaluation of the ICL region of PAGE shown in Fig. 5 and Fig. S5.[a]

Lane	PNA	DNA	Mean	Min	Max	
1	Ref dsDNA A		80.486	51	102	25°C
2	f	dsDNA A	79.372	68	102	
3	F		76.793	68	102	
4	Ref dsDNA C		65.128	51	102	
5	f	dsDNA C	118.003	68	238	
6	F		85.293	51	119	
7	Ref dsDNA A		76.559	51	153	37 °C
8	f	dsDNA A	87.462	68	170	
9	F		83.417	68	153	
10	Ref dsDNA C		82.479	68	102	
11	f	dsDNA C	135.062	85	187	
12	F		175.803	136	238	

[a] As it cannot be assumed that the response factor for DNA and PNA-DNA is identical, the densitometric absolute values are given only for the ICL.

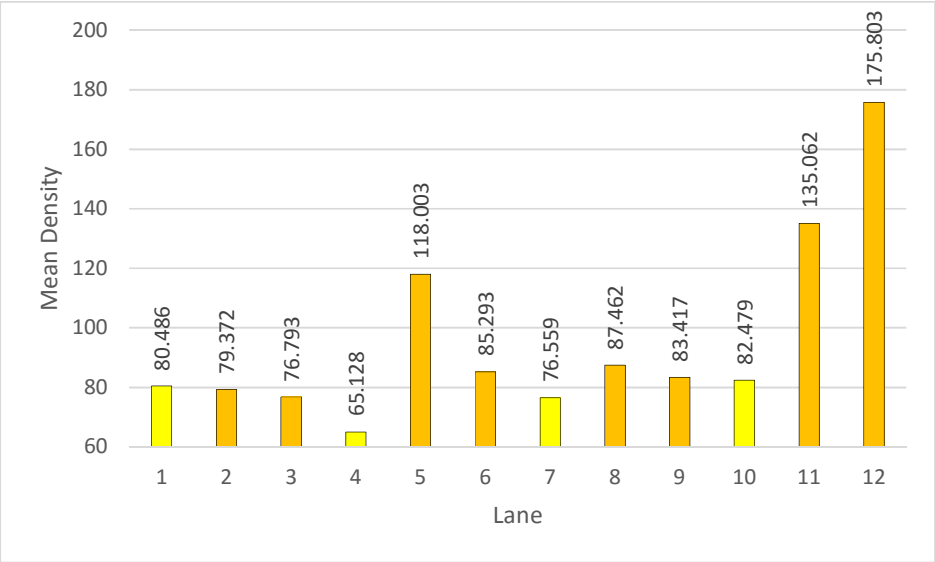


Fig. S8: histogram representation of the data presented in Table S3. Brighter bars correspond to reference lanes.

Identification of the ICL products

HPLC analysis of the ICL experiments was performed at 0 eq, 2eq and 4 eq of NBS added. Samples were prepared diluting 2 µL of sample with 18µL of milliQ water, sample analysis was performed using HPLC2 conditions with 15 µL of sample.

DNA and starting PNA retention times (respectivelly 10 minutes and 13 minutes regions) were determined by injection of reference samples. ICL was identified with the peaks appearing before the PNA after the addition of NBS. This peaks were isolated, freeze-dried, and the identity was analized with both PAGE experiments and MALDI-TOF analysis.

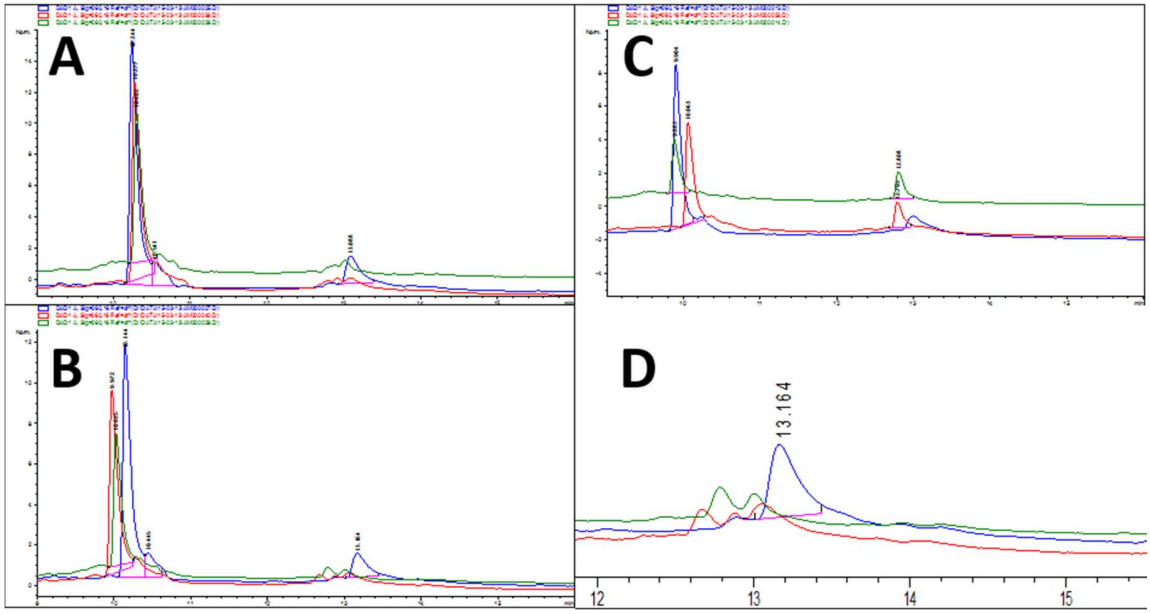


Fig. S9: example of HPLC trace of the ICL experiments at 0 eq (blue), 2 eq (red) and 4 eq NBS (green); (A) PNA F + DNA A, (B) PNA F + DNA C, (C) PNA f + DNA C, (D) zoom of the 12-15 minutes region of the PNA F + DNA C experiments.

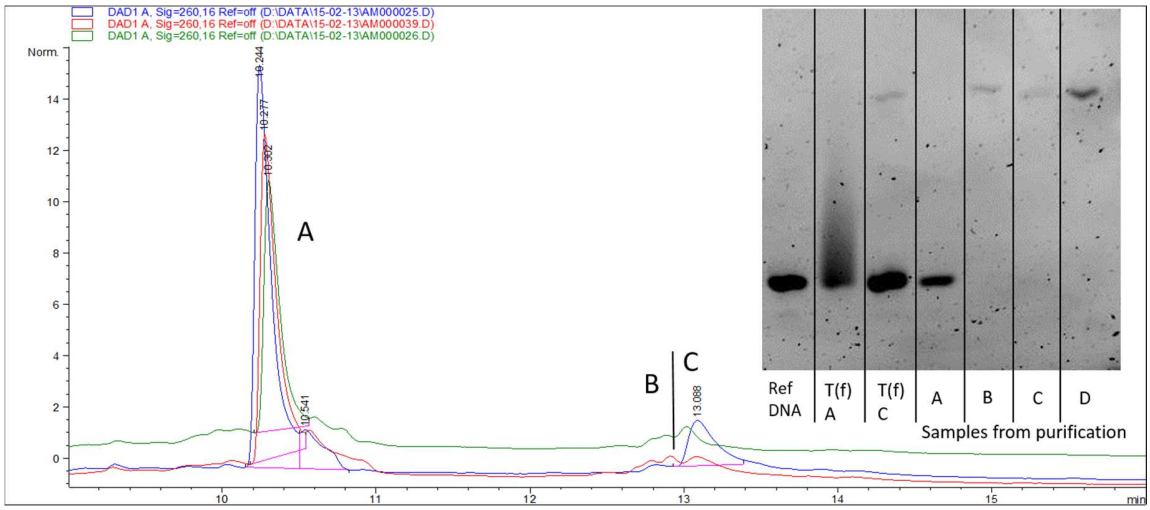


Fig. S10: HPLC trace of purified regions of a PNA F+ DNA A sample. In the insert the denaturing PAGE gel shift of the purified peaks in comparison with DNA A and PNA T(f) ICL experiments. Lane D in the gel corresponds to the B peak of a PNA F + DNA C ICL experiment. SybrGold staining was used.

For MALDI-TOF analysis, the collected products were directly dissolved in 2 µL of matrix solution and spotted on the MALDI plate. Both DNA and PNA matrixes were tested, but signals were obtained only when PNA matrix was used (2,5-Dihydroxybenzoic acid). Examples of MALDI analysis are reported in Fig. S11, analysis of the MALDI peaks was carried out with a weighted average of the signals in order to reduce noise and to obtain the mean m/z associated to the signal (Table S4).

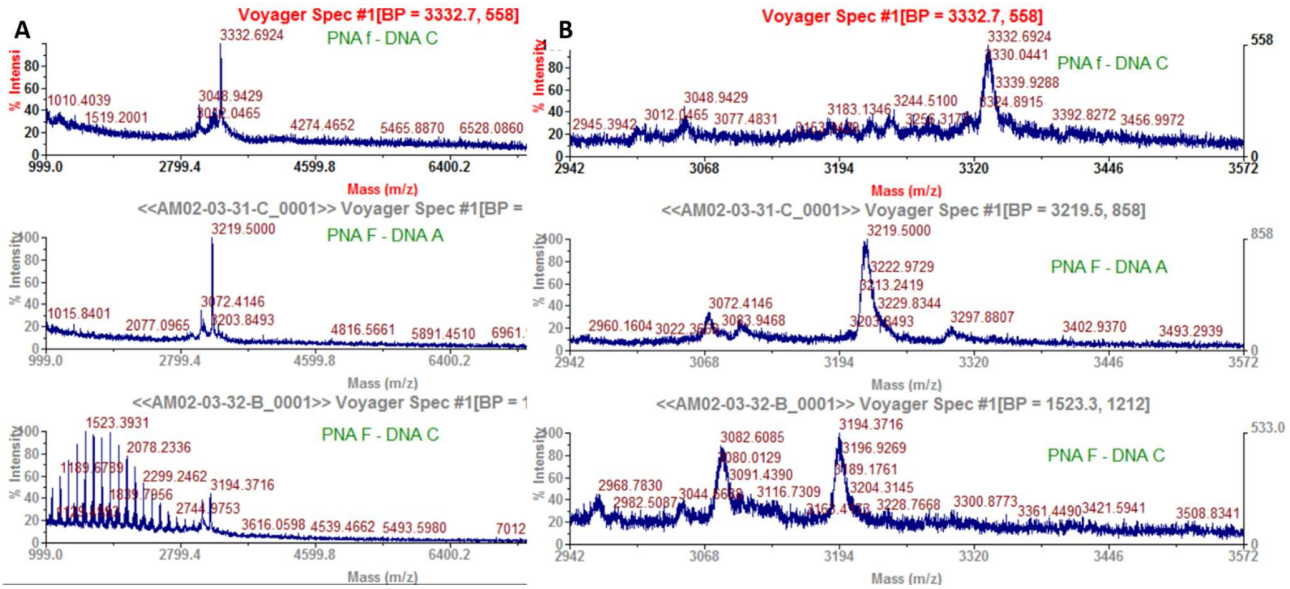


Fig. S11: MALDI spectra of the isolated peaks (A), and zoom of the region used for the peak analysis (B).

Table S4: weighted average of the ICL MALDI peaks. Δ mass calculated in respect the MW of the furan-containing PNA.

		DNA		Δ mass	
		A	C	A	C
PNA	f	-	3333.4	-	106.3
	F	3219.1	3194.9	130.1	105.9

NMR spectra

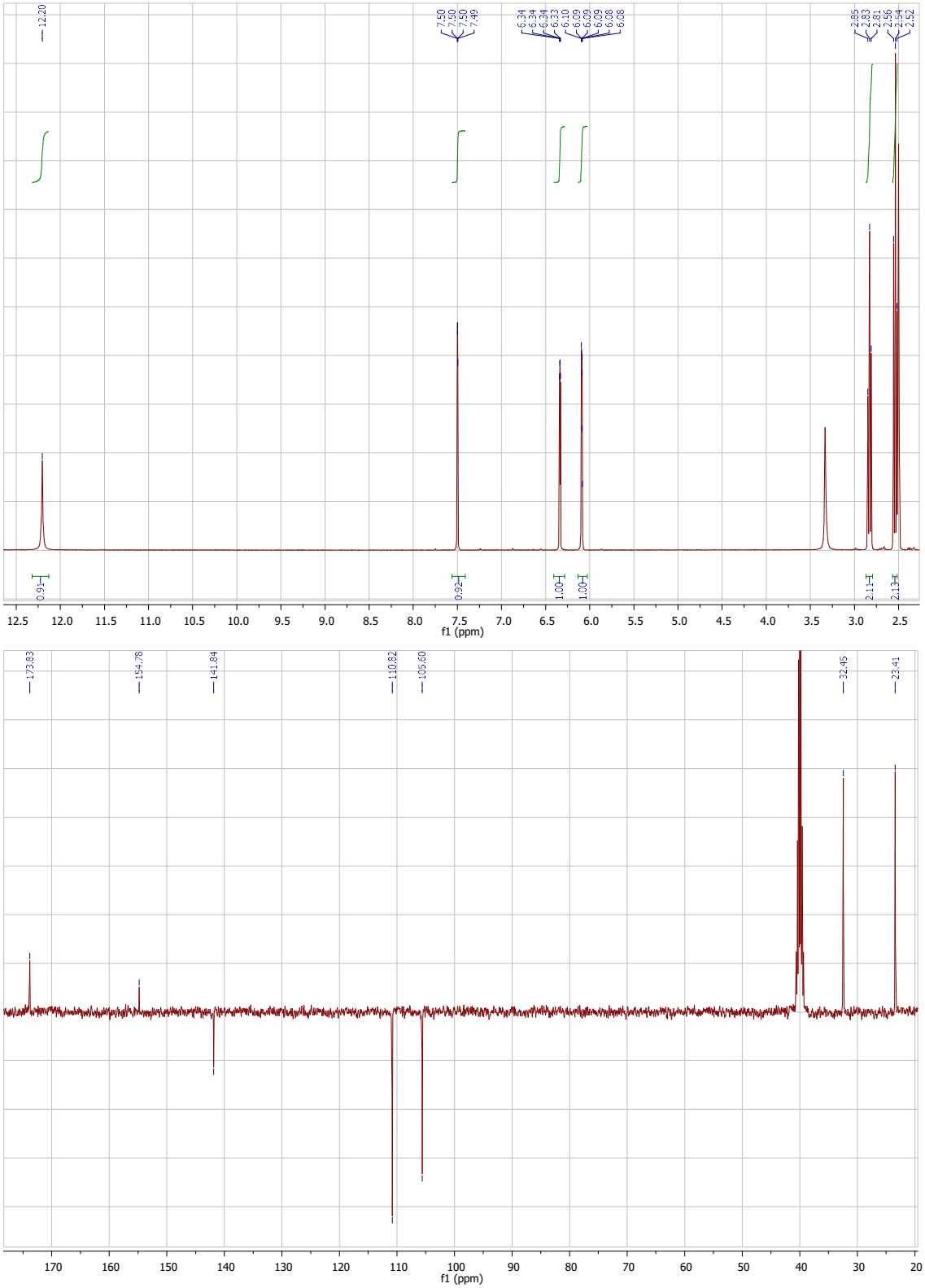


Fig. S12: ^{1}H-NMR and ^{13}C-NMR (APT) of compound **1**

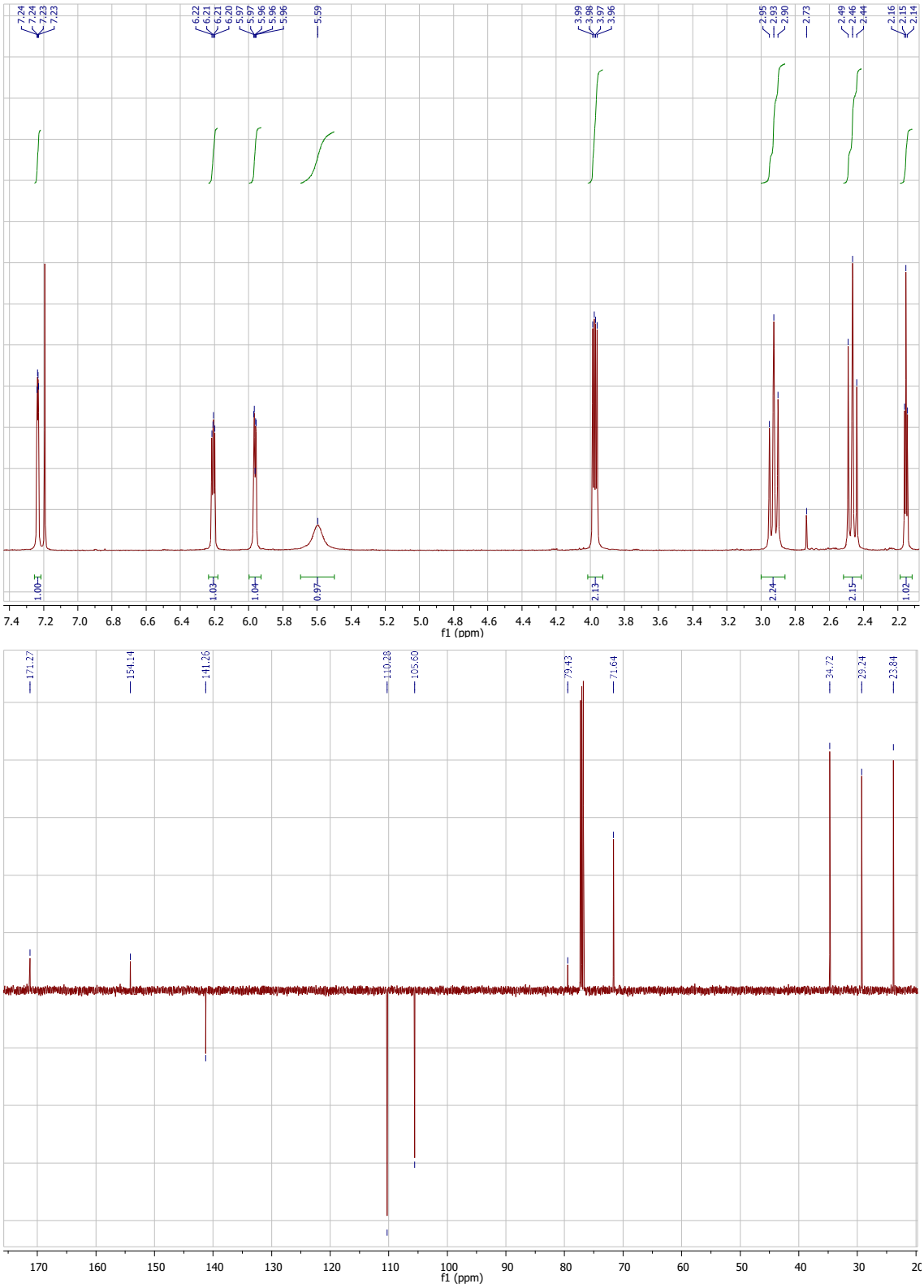


Fig. S13: ^{1}H-NMR and ^{13}C-NMR (APT) of compound **2**

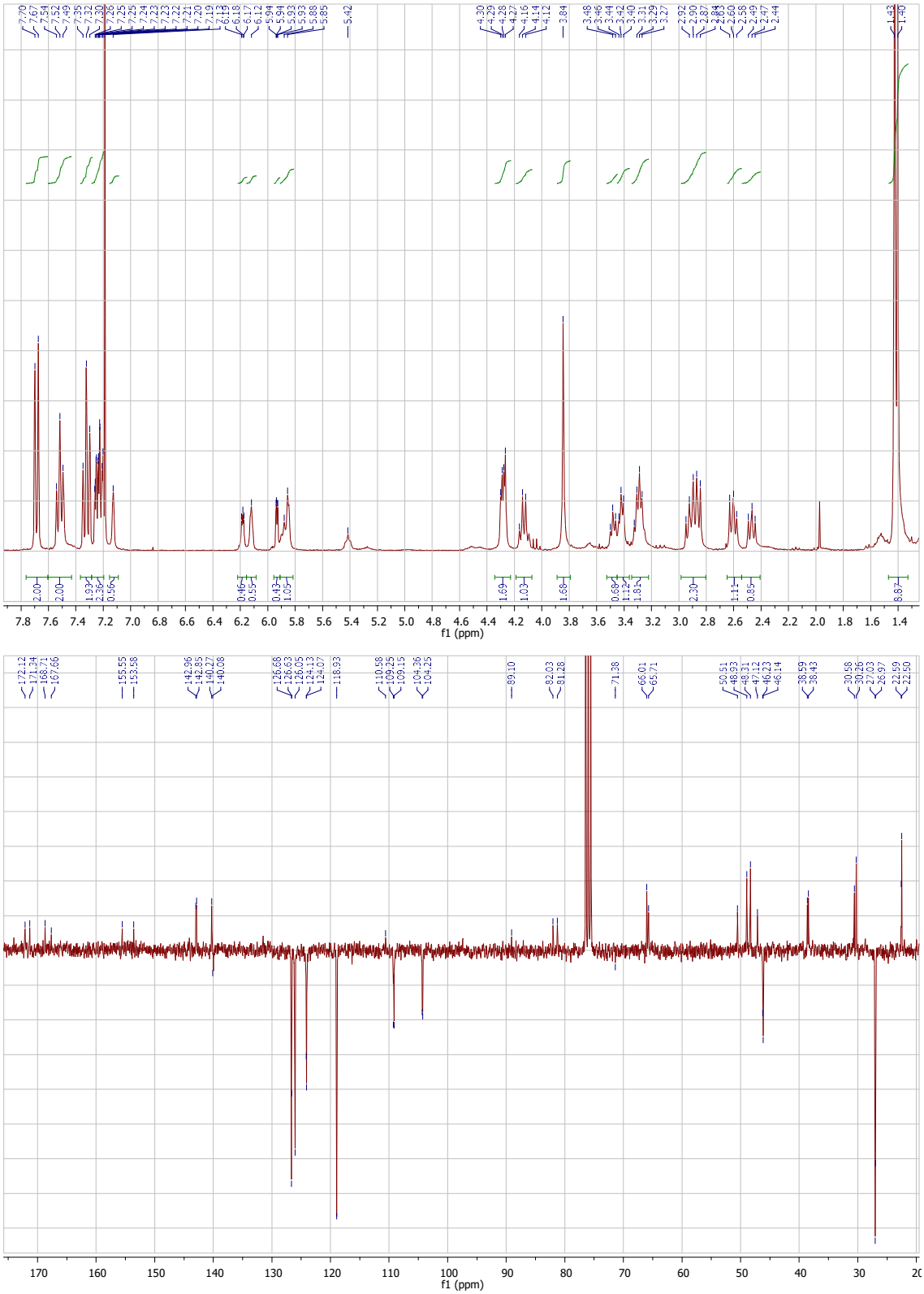


Fig. S14: ^{1}H-NMR and ^{13}C-NMR (APT) of compound **3**

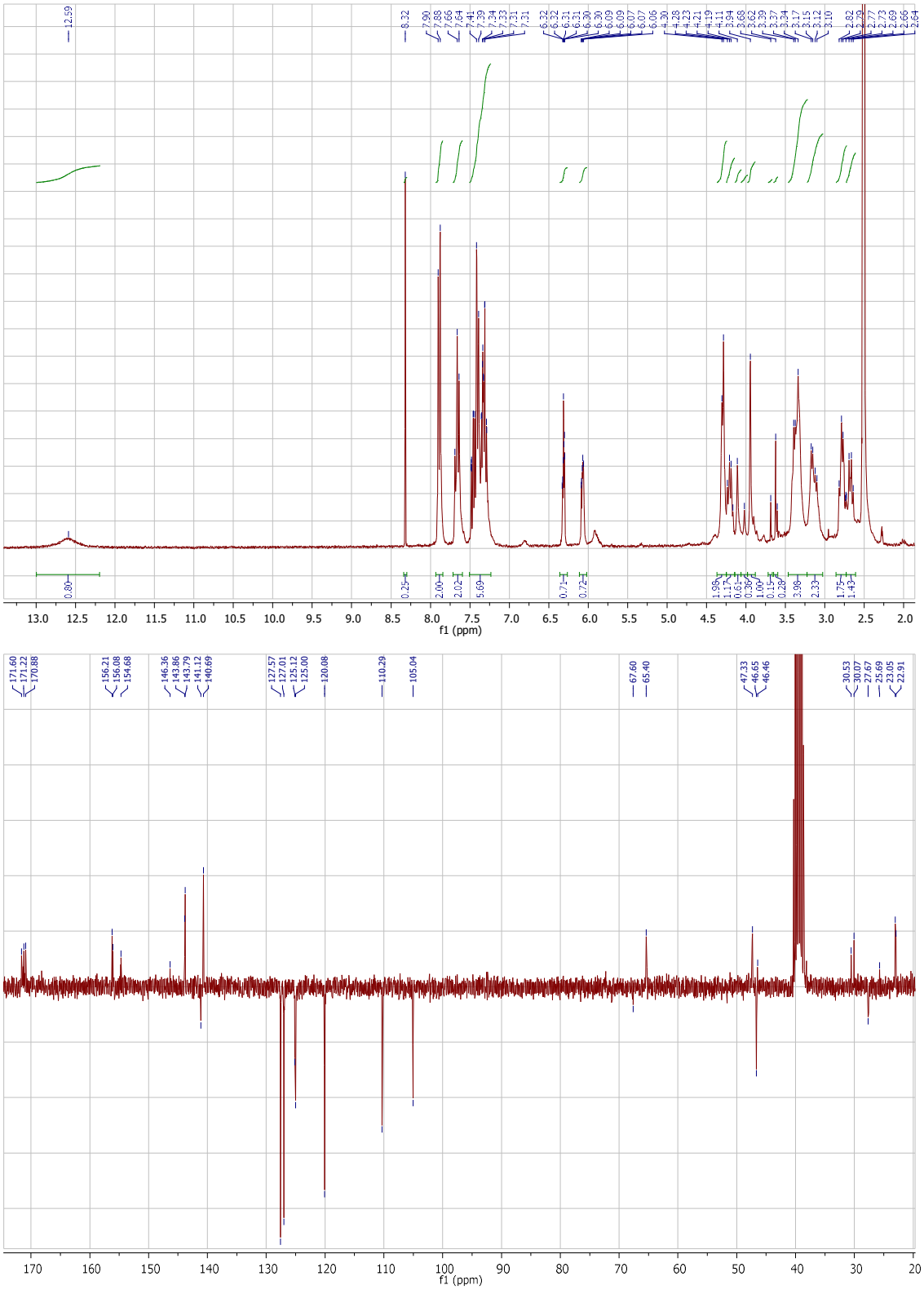


Fig. S15: ^{1}H-NMR and ^{13}C-NMR (APT) of compound **4**

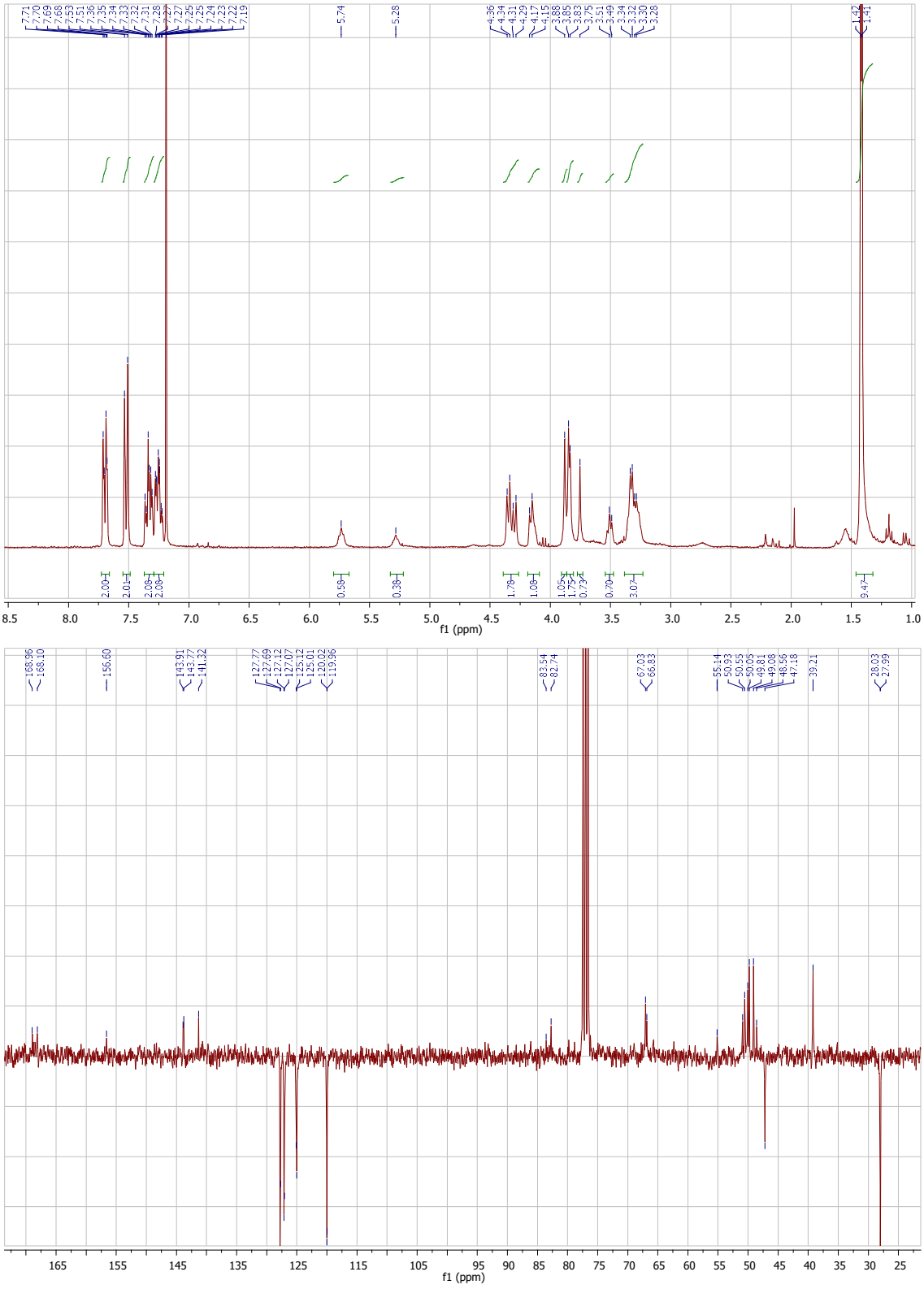


Fig. S16: ^{1}H-NMR and ^{13}C-NMR (APT) of compound **5**

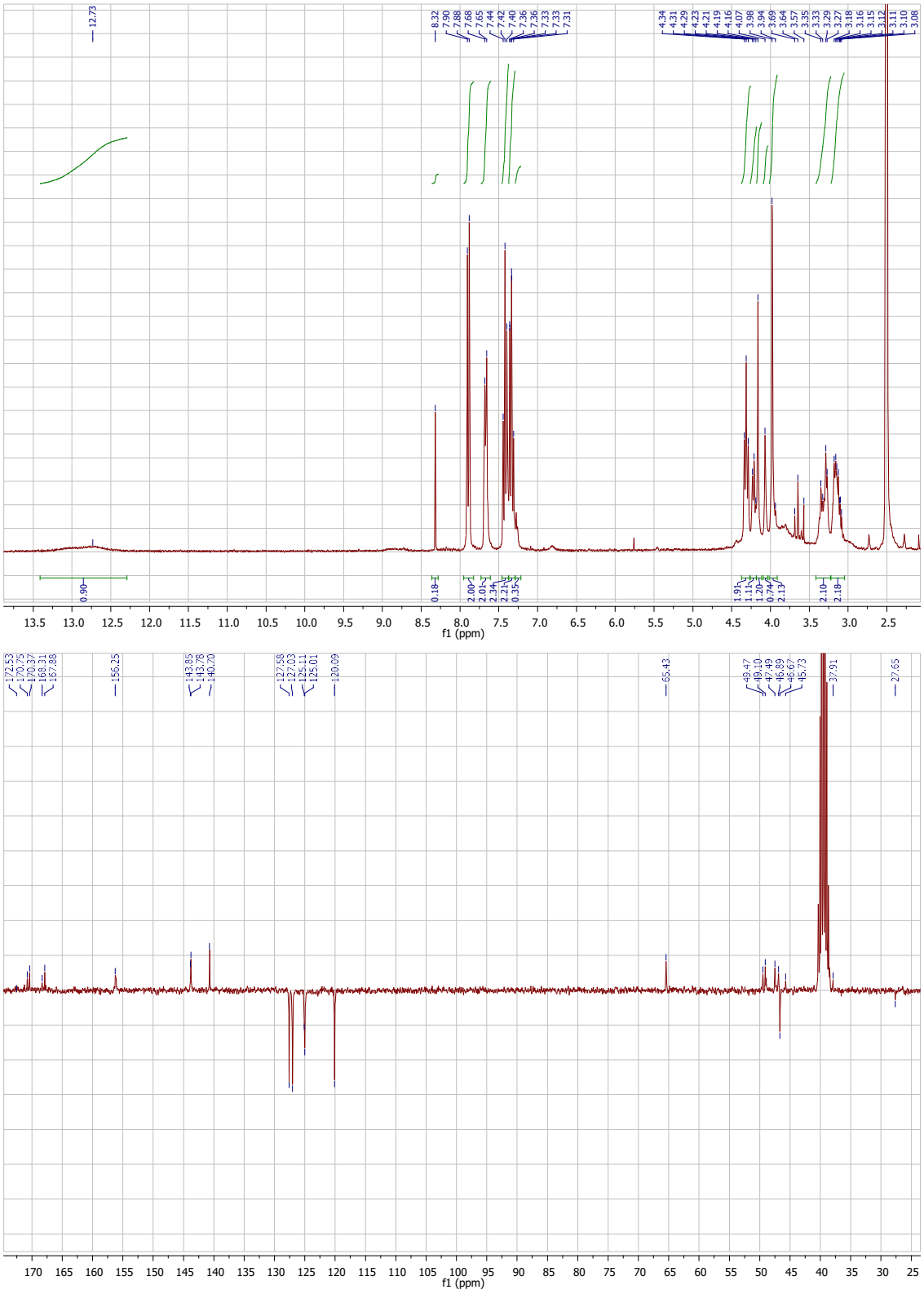


Fig. S17: ^{1}H-NMR and ^{13}C-NMR (APT) of compound **6**

UPLC-MS chromatograms

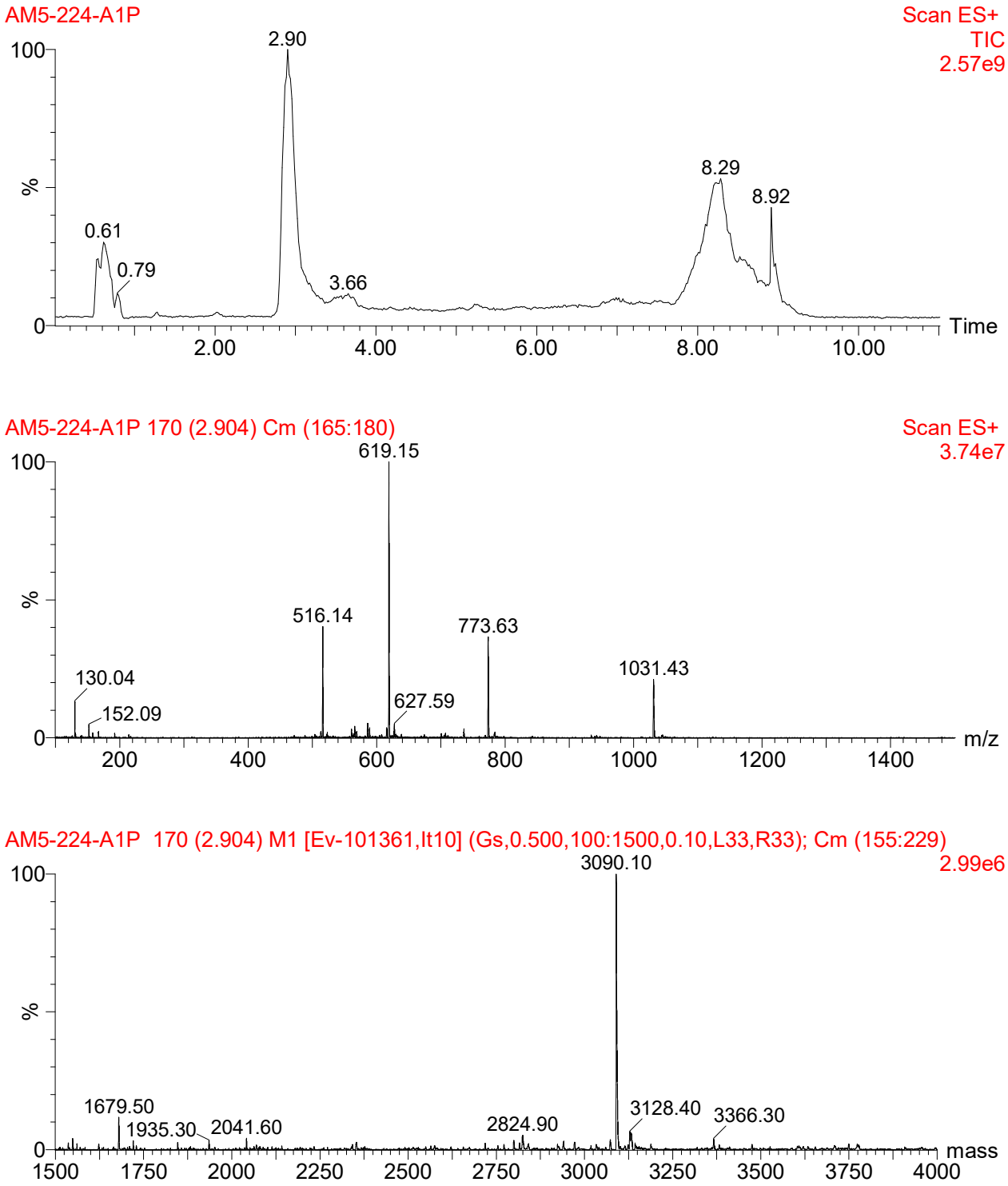


Fig. 18: UPLC-MS of **PNA T**: UPLC-MS trace (top), MS spectrum of the corresponding peak at 2.90 min (center) and mathematical deconvolution of the multicharged signals (bottom).

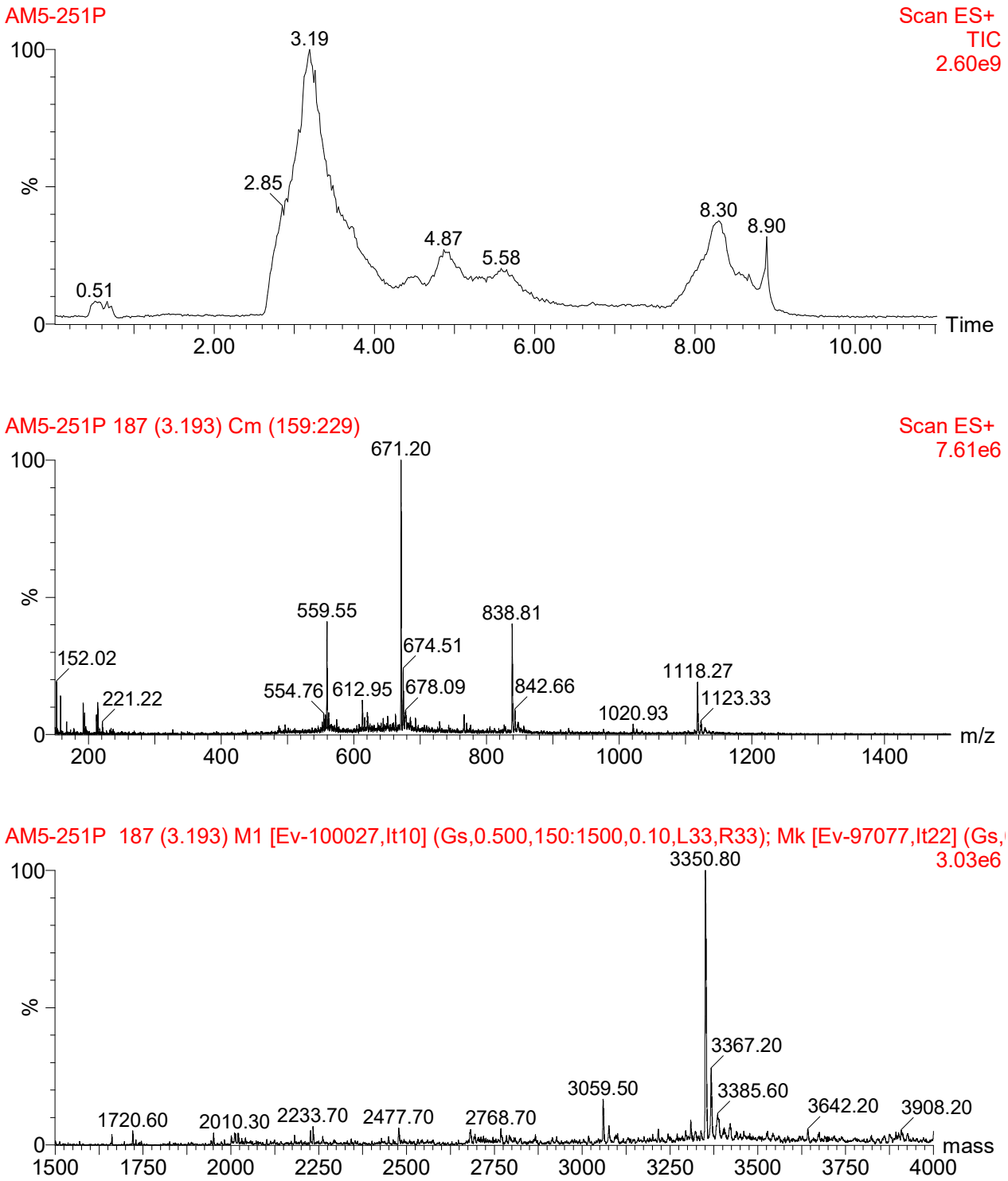


Fig. 19: UPLC-MS of **PNA T(f)**: UPLC-MS trace (top), MS spectrum of the corresponding peak at 3.19 min (center) and mathematical deconvolution of the multicharged signals (bottom).

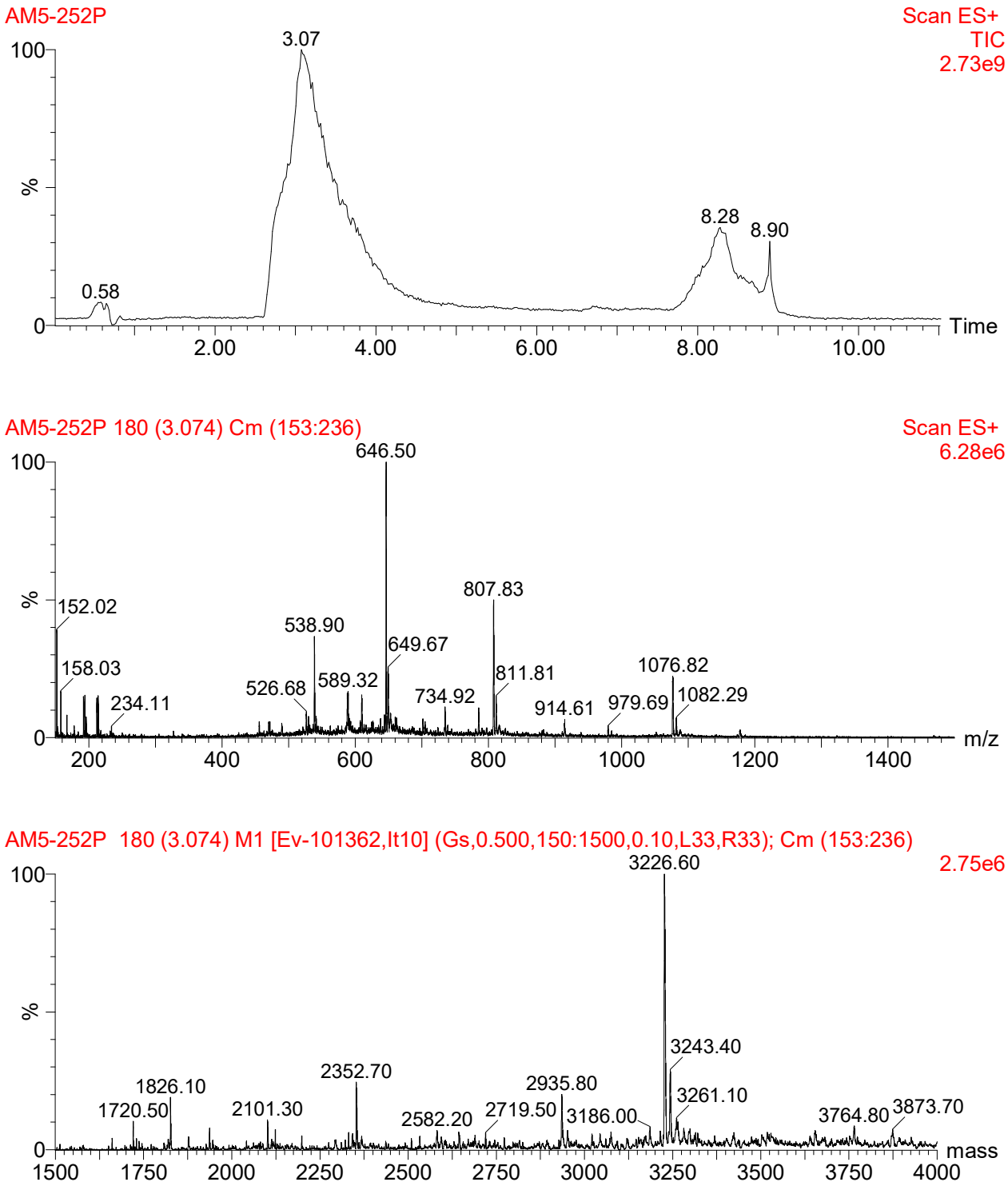


Fig. 20: UPLC-MS of **PNA f**: UPLC-MS trace (top), MS spectrum of the corresponding peak at 3.07 min (center) and mathematical deconvolution of the multicharged signals (bottom).

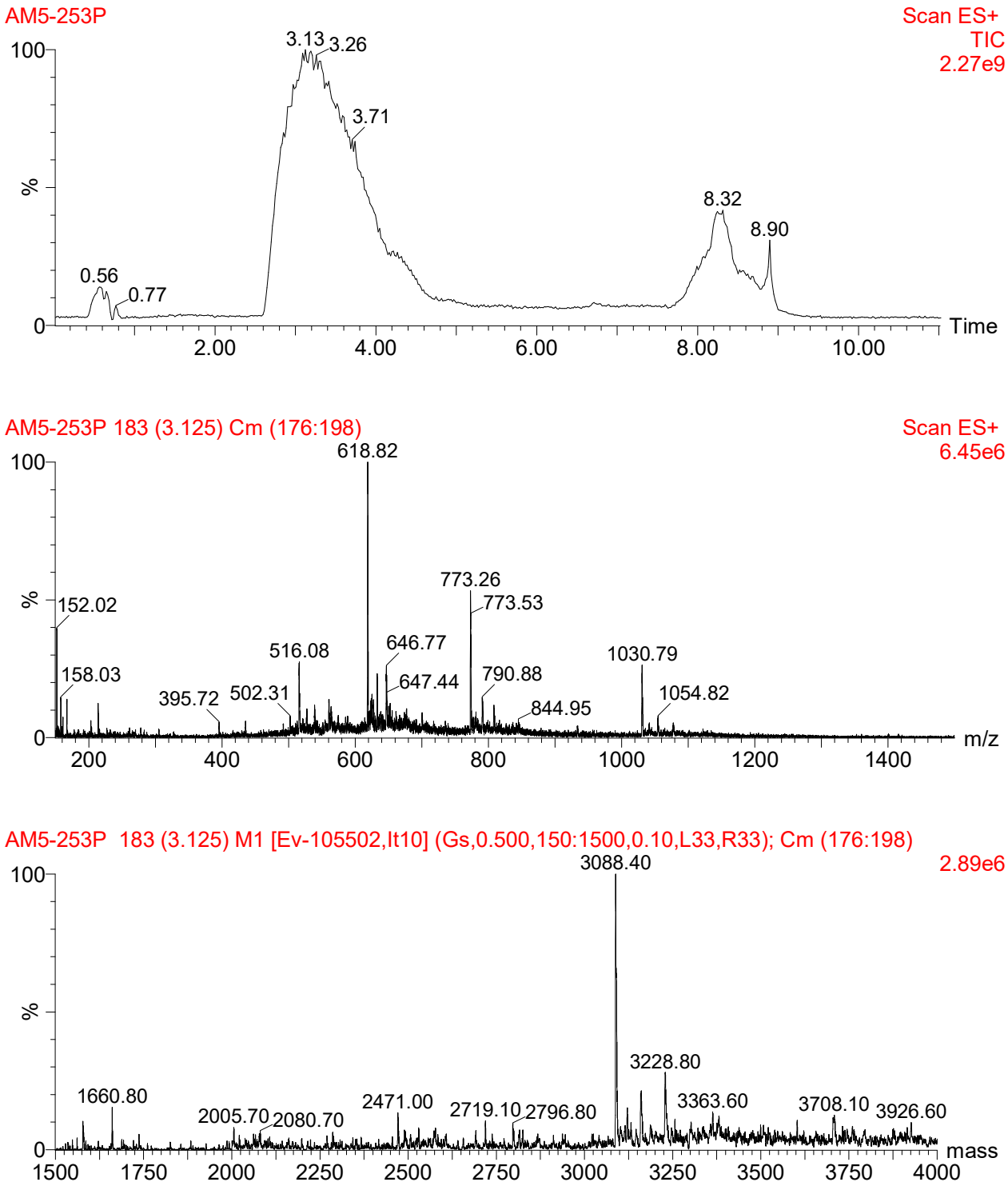


Fig. S21: UPLC-MS of **PNA F**: UPLC-MS trace (top), MS spectrum of the corresponding peak at 3.13 min (center) and mathematical deconvolution of the multicharged signals (bottom).