Supplementary Information

Synthesis and effects of conjugated tocopherol analogues on peptide nucleic acid hybridisation

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GENERAL PROCEDURES

NMR spectra were recorded on a Bruker Avance-300 Spectrophotometer (^{1}H at 300.13 MHz and ^{13}C at 75.47 MHz). All PNA and PNA conjugates were analysed by Matrix Assisted Desorption Ionisation Time of Flight mass spectrometry (MALDI-TOF-MS) using an Ultraflex III instrument (Bruker Daltonics, Germany) and α-cyano-4-hydroxy cinnamic acid as the matrix. Electrospray ionisation (ESI) mass spectrometry was carried out using an Esquire^{6000} ion trap mass spectrometer (Bruker Daltonics, Germany). The samples were introduced at a flow rate of 4 μL/min and a mass range of 50 – 3000 m/z was recorded. A scan rate of 5500 m/z/second was used with the temperature set at 300 °C. Liquid Secondary Ion (LSI) mass spectrometry was operated with 2 KV accelerating voltage and ~10 KV primary Cs ion energy. The proton donor was Meta Nitro Benzyl Alcohol used on a direct insertion probe with peak match resolution at ~6000+ ppm across a 500 ppm window. Window references were MNBA/caffeine and MNBD/cortisone. The molecular ion peaks (m/z) were denoted MH^+**.** TLC was performed using Merck Kieslgel 60 F_{254} plates. Analytical HPLC purity analysis was performed on a Phenomenex Luna 250 x 4.6 mm C8 10u column detection at 254 nm and flow rate of 1 mL/min with a gradient of 0-5' 40% ACN/H_2O (0.1% TFA), 5-20' 95% ACN/H_2O (0.1% TFA). Drying and purification methods for solvents and reagents were followed by directions from Armarego and Chai.[1] Melting points were collected on hot stage Reichert "Thermopan" apparatus. The DNA sequence used in the thermodynamic experiments was purchased from Sigma Aldrich.

SYNTHETIC PROCEDURES

4-Pentynoic acid: 4-Pentynoic acid was prepared using previously described procedure from 4-penty-1-nol[2] in 48% yield, mp 40-44 ºC (lit.[2] 42-46 °C); δ_H (300 MHz, $CDCl_3$); 10.06 (1H, bs), 2.63-2.58 (2H, m), 2.54-2.46 (2H, m), 1.99-1.95 (1H, m); δ_C (75 MHz, $CDCl_3$); 117.5, 81.7, 69.9, 32.8, 13.7.

Ethyl-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate (1c): A solution of Trolox® or (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carbox-ylic acid (300 mg, 1.20 mmol) in 4 ml dry ethanol and H_2SO_4 (80 μL) was refluxed in the presence of molecular sieves (3Å) for 4 hours. The molecular sieves were filtered off and the solvent removed *in vacuo*. The residue was dissolved in ether (25 mL) and washed with water (25 mL), brine (25 mL), then dried with $MgSO_4$ and evaporated to dryness to give **1c** as an off-white solid (330 mg, 99%). A small amount of the product was recrystallised from ethyl acetate, mp 125-126 °C (lit.[3] 124-126 °C); δ_H (300 MHz, DMSO); 7.41 (1H, s), 4.02 (2H, q, 6.9 Hz,), 2.57-2.46 (1H, m), 2.39-2.31 (2H, m), 2.02 (3H, s), 2.00 (3H, s), 1.94 (3H, s), 1.82-1.68 (1H, m), 1.47 (3H, s), 1.06 (3H, t, *J* 7.5); δ_C (75 MHz, DMSO); 173.3, 146.2, 145.2, 123.1, 121.4, 120.6, 116.9, 76.8, 61.0, 30.6, 25.5, 20.9, 14.4, 13.2, 12.3, 12.2; LR MS (ESI) *m/z* $(M+H)^+$ 279.2; HR MS

(ESI) *m/z* Calcd for $C_{16}H_{22}O_4Na$ requires $(M+Na)^+$ 301.1410, Found 301.1405.

Compounds **3a, 4a and 5a** were synthesised according to procedures found in the literature.[4]

5a-Bromo-α-tocopherol (3a): orange oil, 89%. δ_H (300 MHz, $CDCl_3$); 4.74 (2H, s), 2.76 (2H, t, *J* = 6.8Hz), 2.14 (3H, s), 2.10 (3H, s), 1.53-1.06 (26H, m), 0.89-0.82 (12H, m); δ_C (75 MHz, $CDCl_3$); 145.7, 145.1, 126.8, 121.9, 119.0, 117.1, 74.6, 39.5, 39.0, 37.1, 36.9, 32.5, 32.3, 31.2, 27.6, 27.2, 24.5, 24.1, 23.4, 22.4, 22.3, 22.3, 20.6, 19.4, 19.3, 18.9 13.8, 11.9, 11.7.

Ethyl-5-(bromomethyl)-6-hydroxy-2,7,8-trimethylchroman-2-carboxylate (3b): To a stirred solution of **1c** (781 mg, 2.80 mmol) in anhydrous DCM (40 mL), a solution of bromine (168 µL, 3.28 mmol) in DCM (5 mL) was drop wise over a period of 15 minutes. After the addition was complete, the reaction was stirred for 12 hours. The solvent and remaining HBr was removed to produce **3b** as an orange solid (994 mg, 99%) which was used directly in the next step with a small amount recrystallised, mp 113-114 °C. δ_H (300 MHz, $CDCl_3$); 5.28 (OH, s), 4.57 (2H, ABq, *J* 9.9, CH_2Br), 4.10 (2H, q, *J* 7.2), 2.86-2.79 (1H, m), 2.78-2.64 (1H, m), 2.48-2.40 (1H, s), 2.18 (3H, s), 2.14 (1H, m), 1.94-1.84 (1H, s), 1.59 (1H, s), 1.16 (3H, s, *J* 7.2); δ_C (75 MHz, $CDCl_3$); 173.5, 146.2, 127.2, 122.3, 119.1, 117.2, 77.2, 61.2, 30.2, 27.3, 25.3, 19.5, 14.1, 12.3, 12.1.

Bromo-α-tocopheryl acetate (4a): Colourless plates, 97%. δ_H (300 MHz, $CDCl_3$); 4.38 (2H, bs), 2.76 (2H, t, *J* 6), 2.37 (3H, s), 2.10 (3H, s), 2.00 (3H, s), 1.89-1.72 (2H, m), 1.57-1.03 (26H, m), 0.86-0.82 (12H, m); δ_C (75 MHz, $CDCl_3$); 169.1, 149.5, 140.4, 127.6, 127.0, 124.0, 117.4, 75.2, 39.0, 37.1, 37.0, 36.9, 32.4, 32.3, 30.3, 27.6, 25.3, 24.4, 24.1, 22.4, 22.3, 20.6, 20.3, 19.4, 19.3, 18.8, 12.8, 11.9.

Ethyl 6-acetoxy-5-(bromomethyl)-2,7,8-trimethylchroman-2-carboxylate (4b): A solution of **3b** (994 mg, 2.78 mmol) acetic anhydride (398 µL, 4.17 mmol), H_2SO_4 (1 drop) in dry DCM (100 mL) was stirred overnight. The reaction was quenched with ice cold water and stirred for a further 2 hours. The two layers were separated and the aqueous phase was repeatedly washed with DCM. The organic layers were pooled, dried with $MgSO_4$ and evaporated to dryness and purified by column chromatography (5:1 hexane:EtOAc) to afford **4b** as a yellow oil (964 mg, 88%). δ_H (300 MHz, $CDCl_3$); 4.37-4.25 (2H, m(b), hindered rotation), 4.12 (2H, q, *J* 7.2), 2.87-2.81 (1H, m), 2.80-2.63 (1H, m), 2.61-2.43 (1H, m), 2.36 (3H, s), 2.18 (3H, s), 2.00 (3H, s), 1.91-1.85 (1H, m), 1.60 (3H, s), 1.16 (3H, t, *J* 3.3); δ_C (75 MHz, $CDCl_3$); 173.2, 169.2, 149.8, 141.5, 128.4, 127.4, 124.3, 117.6, 77.5, 61.3, 60.4, 29.9, 25.3, 20.6, 19.5, 14.1, 13.1, 12.3; Analytical HPLC: t_R = 18.86 min; LR MS (ESI) *m/z* $(M+ NH_4)^+$ 416.0, 418.0 ; HR MS (ESI) *m/z* Calcd. for $C_{18}H_{27}O_5K$ requires $(M+NH_4)^+$ 416.1067, 418.1052 Found 416.1070, 418.1050.

5a-Azido-tocopheryl acetate (5a): Pale yellow oil 95%. δ_H (300 MHz, $CDCl_3$); 4.18 (2H, s), 2.72 (2H, t, *J* 6.6), 2.35 (3H, s), 2.12 (3H, s), 2.02 (3H, s), 1.88-1.71 (2H, m), 1.58-1.07 (26H, m), 0.86-0.82 (12H, m); δ_C (75 MHz, $CDCl_3$); 169.4, 149.5, 140.7, 127.4, 126.5, 121.7, 117.6, 75.2, 45.9, 39.8, 39.0, 37.1, 37.0, 36.9, 32.4, 32.3, 30.5, 27.6, 24.4, 24.1, 23.6, 22.4, 22.3, 20.6, 20.2, 19.6, 19.4, 1939, 12.8, 11.9.

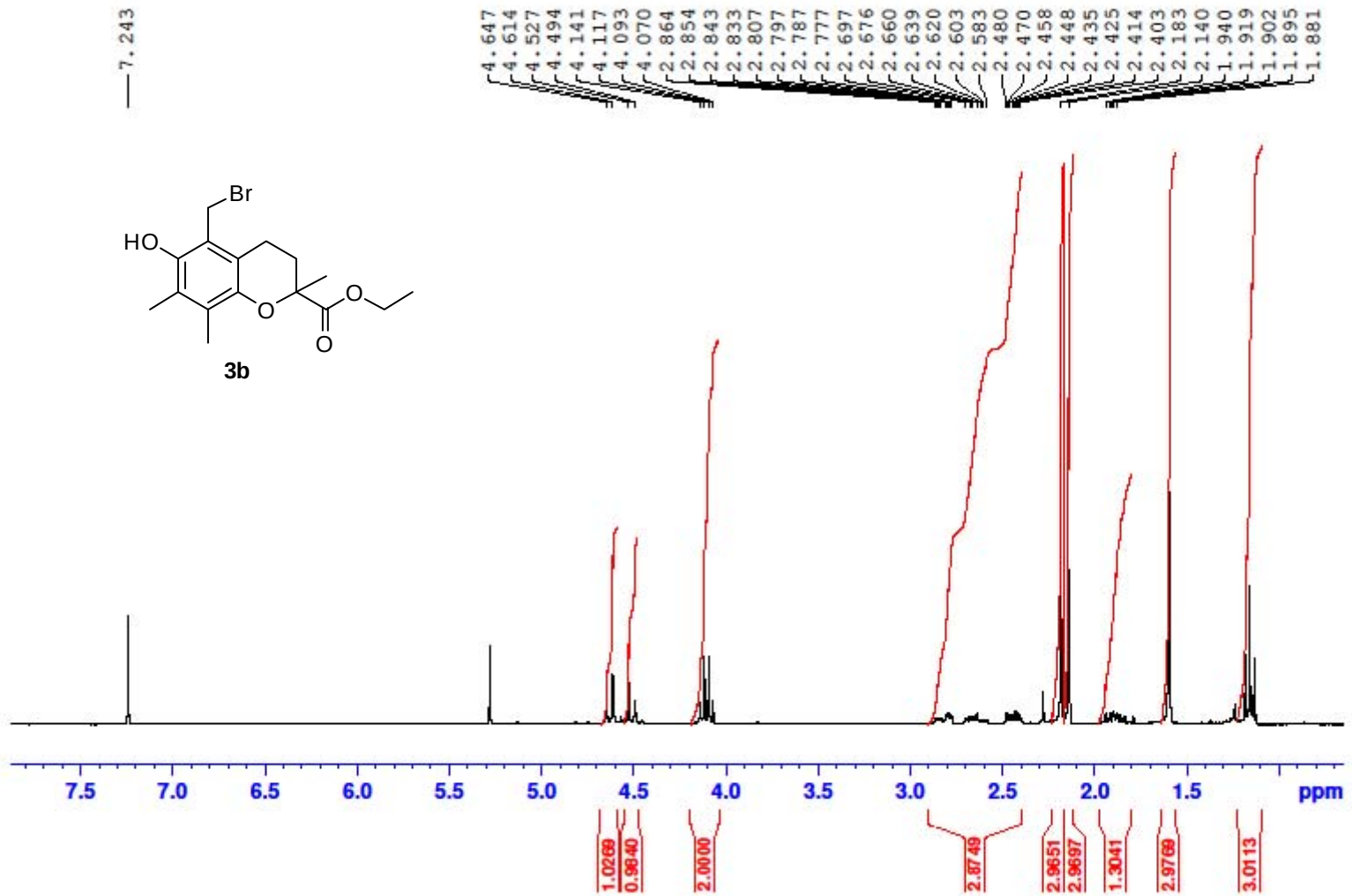
Ethyl 6-acetoxy-5-(azidomethyl)-2,7,8-trimethylchroman-2-carboxylate (5b): To a solution of **4b** (957 mg, 2.40 mmol) was stirred in acetonitrile (96 mL). To this sodium azide (220 mg, 3.60 mmol) was added and the solution refluxed for 3 hours. The solution was slowly cooled, the solid material was then removed by filtration and the remaining solvent removed under pressure to give **5b** as a dark yellow/orange oil (836 mg, 97%). δ_H (300 MHz, $CDCl_3$); 4.146-4.075 (4H, m), 2.74-2.64 (2H, m), 2.48-2.38 (1H, m), 2.33 (3H, s), 2.19 (3H, s), 2.02 (3H, s), 1.91-1.81 (1H, m), 1.60 (3H, s), 1.16 (3H, t, *J* 7.2); δ_C (75 MHz, $CDCl_3$); 173.3, 169.6, 149.8, 141.9, 128.2, 127.1, 122.0, 117.8, 77.5, 61.3, 46.2, 30.0, 25.2, 20.6, 20.2, 14.0, 13.2, 12.2; Analytical HPLC: t_R =

18.18 min; LR MS (ESI) *m/z* $(M+NH_4)^+$ 379.2; HR MS (ESI) *m/z* calcd. for $C_{36}H_{58}N_3O_5$ requires $(M+ NH_4)^+$ 379.1976 Found 379.1974.

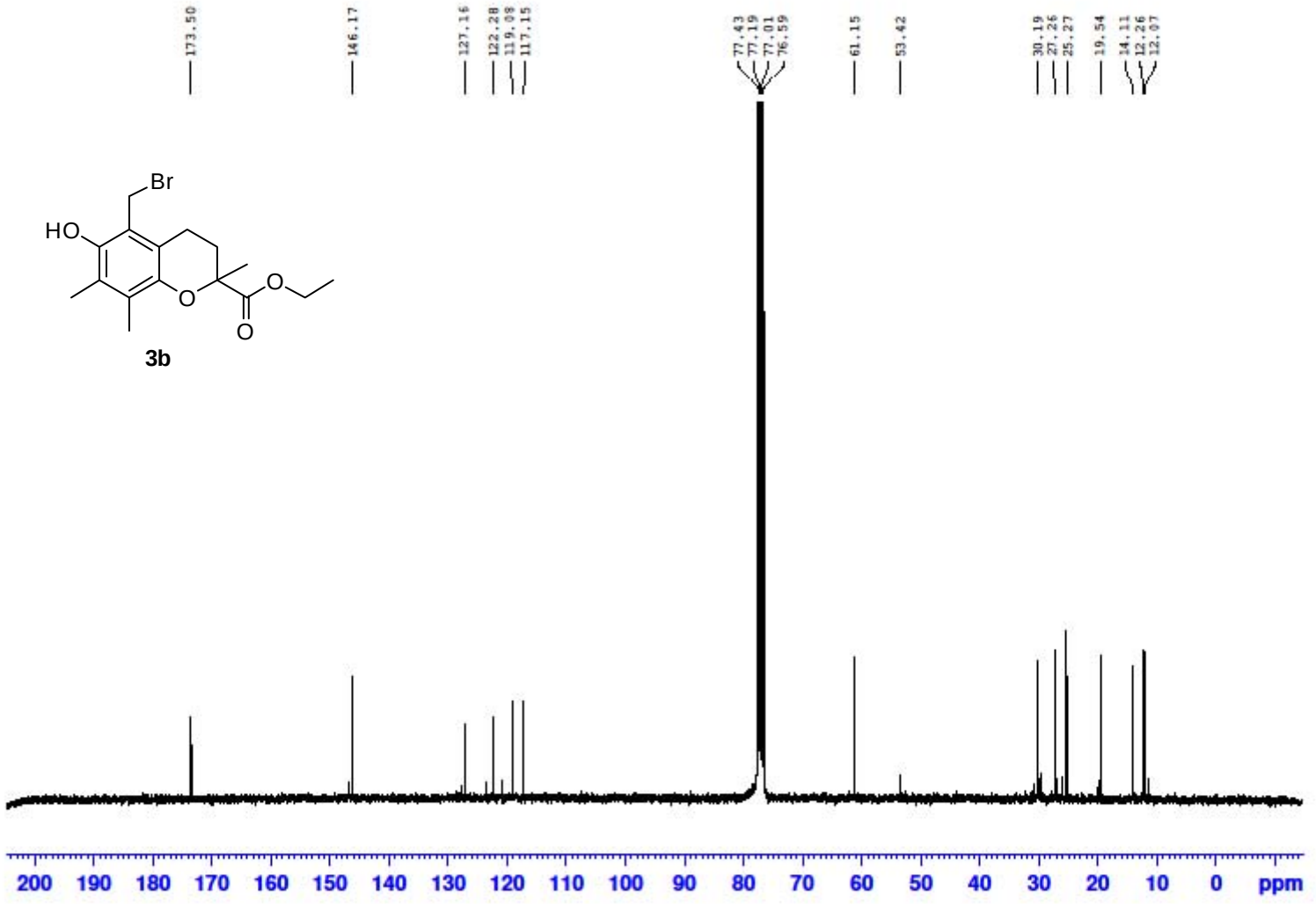
3-(1-((6-Acetoxy-2-hexadecyl-2,7,8-trimethylchoman-5-yl)methyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (6a): 5a-Azido-α-tocopheryl acetate **5a** (1.11 g, 2.16 mmol) and 4-pentynoic acid (340 mg, 3.46 mmol) were dissolved in 2:1 mixture of $^tBuOH:H_2O$ (10.8 ml) and stirred. Copper (1 g as mesh) was added and the solution stirred for 12 hours. Dichloromethane (5 ml) was added and the solution filtered through Celite®, dried with $MgSO_4$, evaporated to dryness and purified by flash column chromatography (20:1 hexane:EtOAc). The product was then recrystallised in hexane to afford **6a** as white shimmering crystals (700 mg, 53%), mp 89-91 °C. δ_H (300 MHz, MeOD); 7.46 (1H, s), 5.37 (2H, s), 2.95 (2H, bt), 2.66-2.56 (4H, m), 2.26 (3H, s), 2.10 (3H, s), 1.97 (3H, s), 1.77-1.76 (2H, m), 1.55-1.07 (26H, m), 0.86-0.83 (12H, m); δ_C (75 MHz, MeOD); 174.1, 169.5, 149.4, 141.0, 127.6, 126.7, 121.3, 118.1, 103.5, 101.6, 75.0, 46.4, 39.1, 38.8, 36.8, 36.8, 36.7, 32.5, 32.2, 32.0, 30.3, 29.1, 27.4, 24.2, 23.7, 22.4, 21.4, 21.3, 20.2, 20.0, 19.0, 18.8, 18.5, 18.5, 17.1, 16.7, 15.8, 13.9, 11.6, 10.7; LR MS (ESI) *m/z* $(M+H)^+$ 612.6; HR MS (LSI) *m/z* calcd. for $C_{36}H_{58}N_3O_5$ requires $(M+H)^+$ 612.44 Found 612.43.

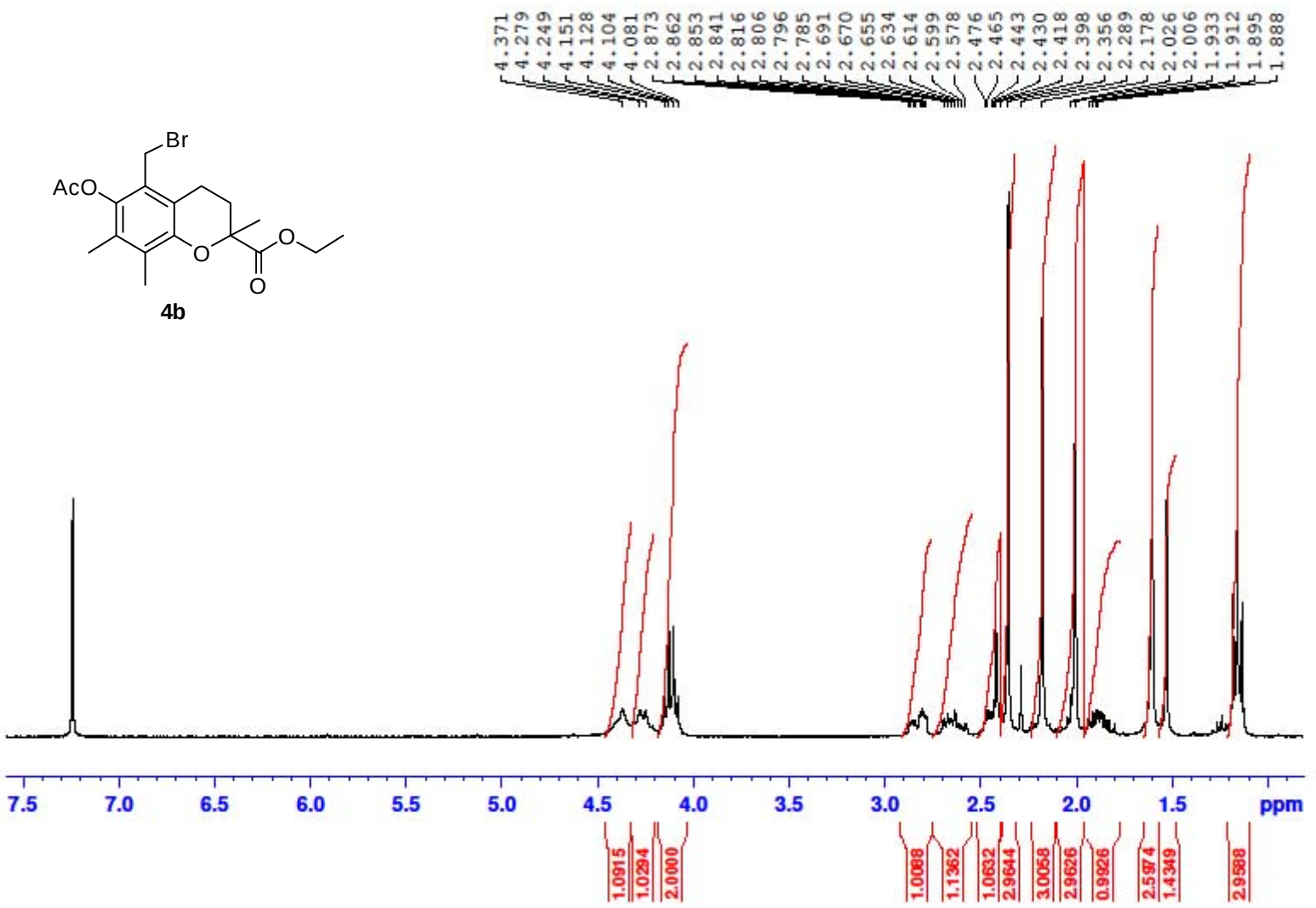
3-(1-((6-Acetoxy-2-(ethoxycarbonyl)-2,7,8-trimethylchroman-5-yl)methyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (6b): A solution of **5a** (816 mg, 2.30 mmol) was stirred in ^tBuOH (5 mL). To this a solution of 4-pentynoic acid (244 mg, 2.50 mmol) in H_2O (2 mL) was added, followed by the addition of copper metal (500 mg as mesh) and the reaction was stirred overnight. Dichloromethane (20 mL) was added to the solution and the copper was filtered off. The organic layer was washed with H_2O (25 mL), 3M HCl (6 x 30 mL), dried with $MgSO_4$ and evaporated to dryness to give **6b** as a dark yellow viscous oil (769 mg, 55%). δ_H (300 MHz, CD_3OD); 7.37 (1H, s), 5.38 (2H, s), 4.07 (2H, q, *J* 7.2), 2.87 (2H, t, *J* 7.2), 2.79-2.34 (3H, m), 2.58 (2H, t, *J* 7.2), 2.27, (3H, s), 2.17 (s, 3H), 2.00 (s, 3H), 1.91-1.78 (m, 1H), 1.58 (s, 3H), 1.09 (t, 3H, *J* 7.2); δ_C (75 MHz, CD_3OD); 174.5, 173.1, 169.8, 149.9, 142.0, 128.3, 127.1, 121.8, 121.5, 118.3, 77.5, 61.1, 45.3, 32.9, 29.7, 24.1, 20.4, 19.7, 19.1, 13.0, 11.8, 11.0; Analytical HPLC: t_R = 12.63 min; LR MS (ESI) *m/z* $(M+H)^+$ 460.1; HR MS (APCI) *m/z* Calcd for $C_{23}H_{28}N_3O_7$ requires $(M-H)^-$ 458.1933, Found 458.1940.

1H & 13 C NMR SPECTRA

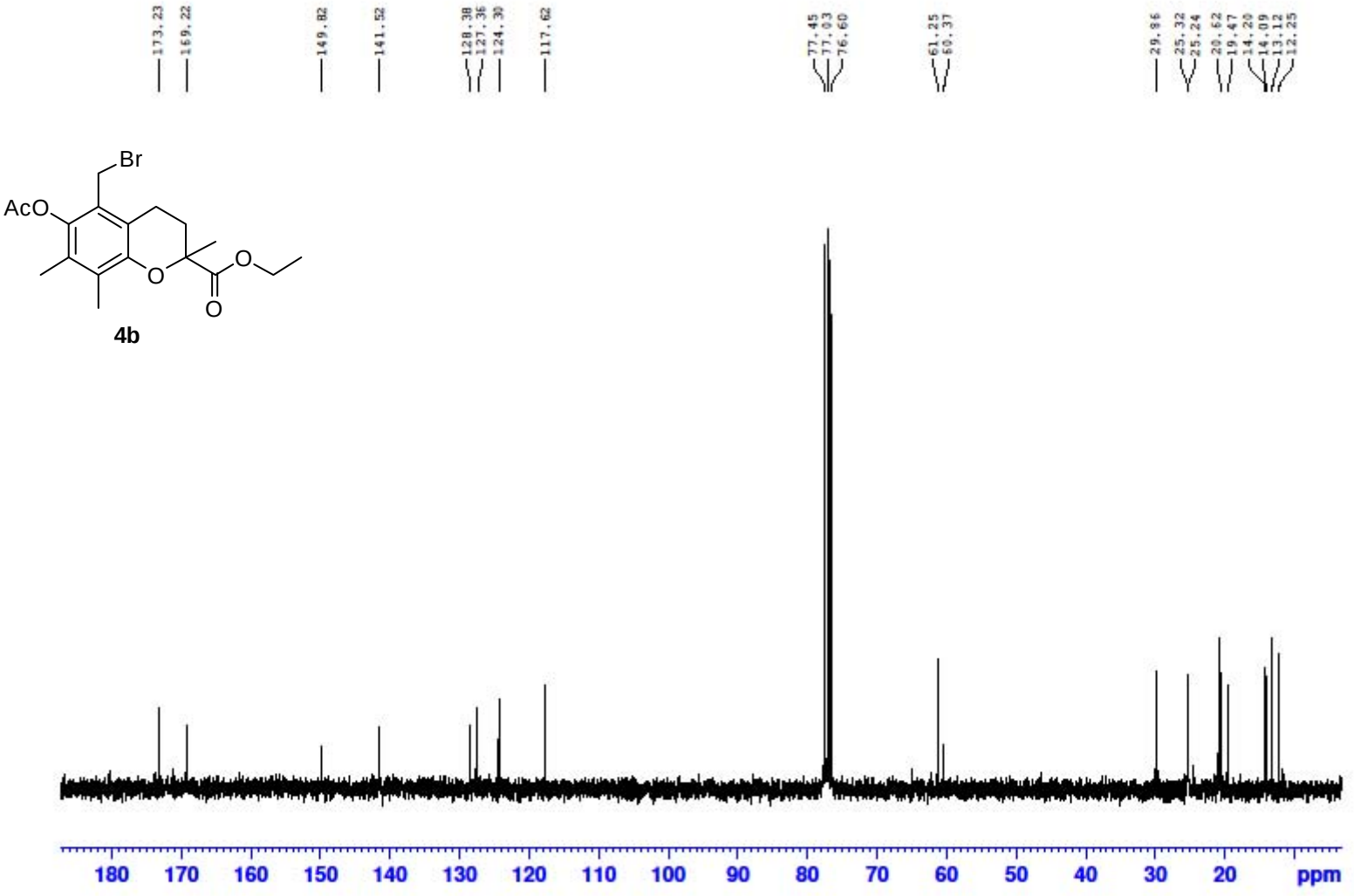


3b





4b



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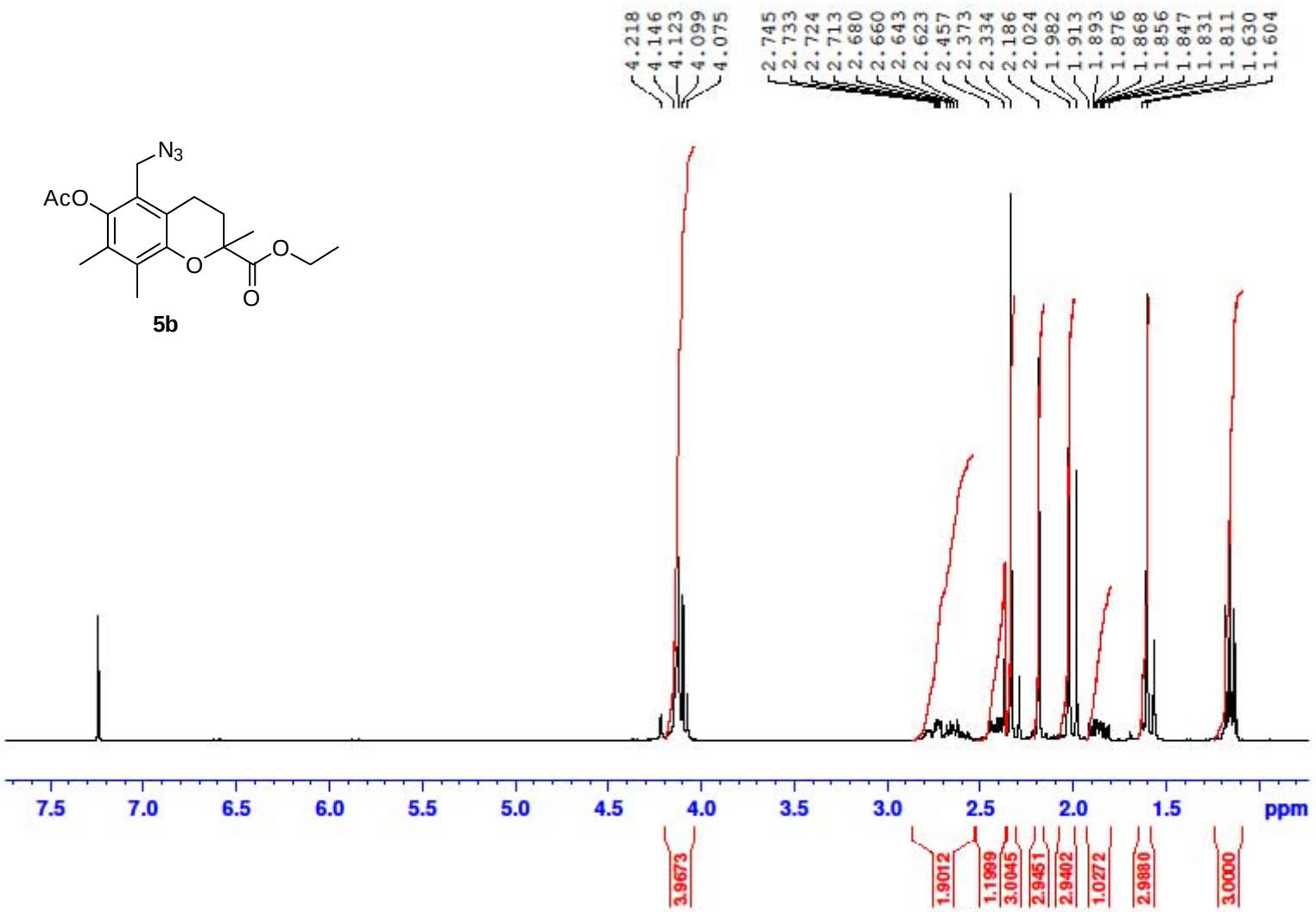

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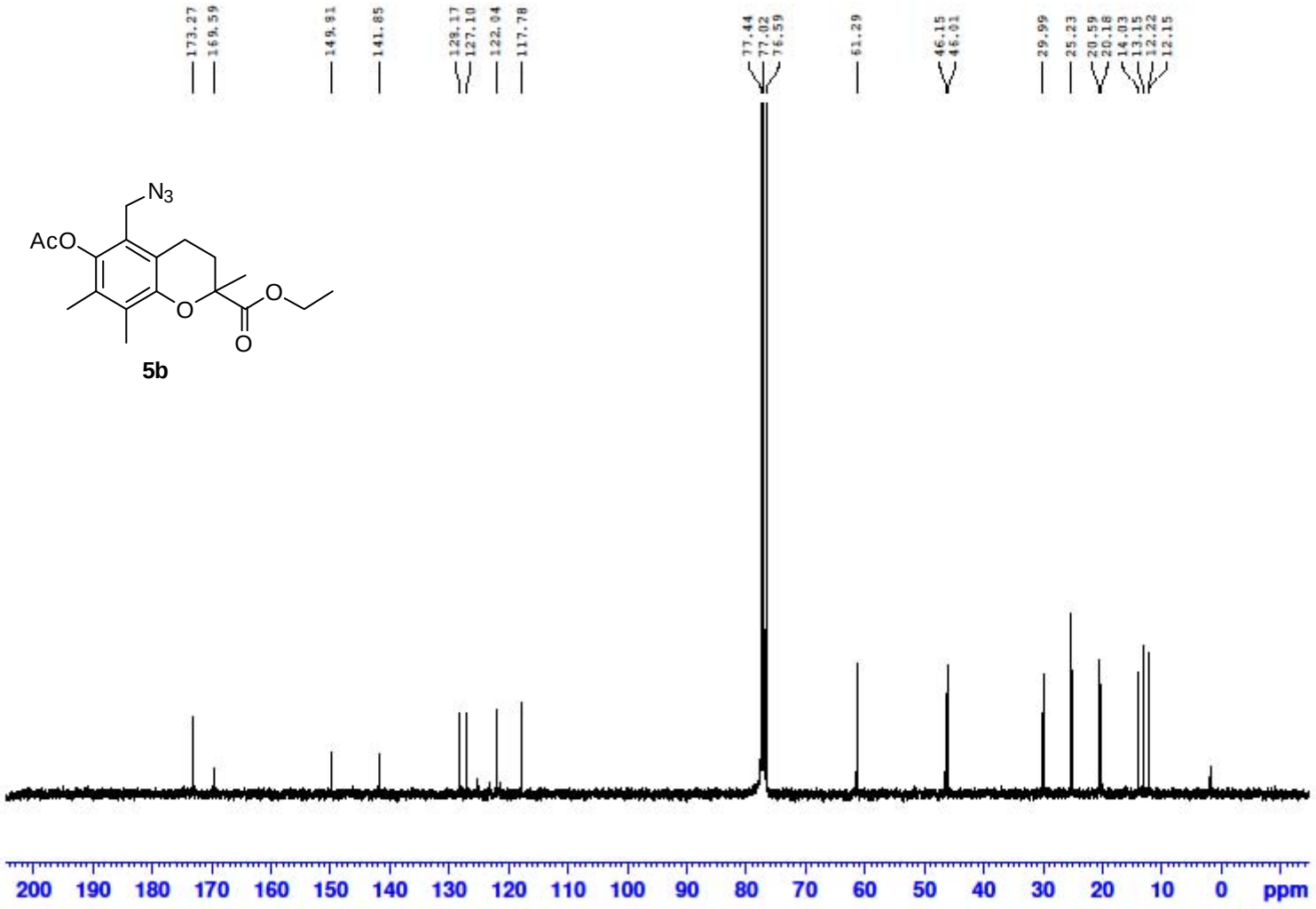

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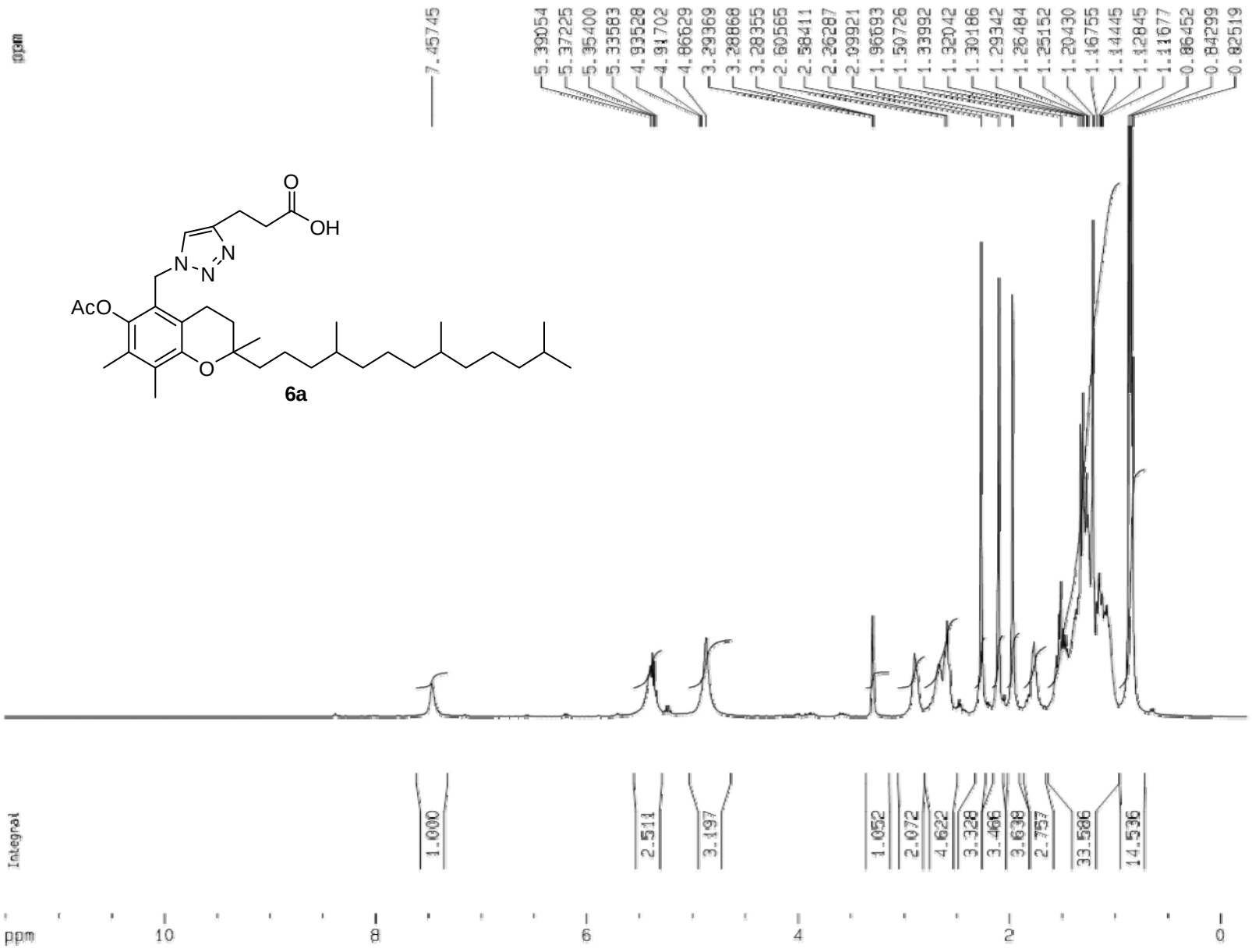
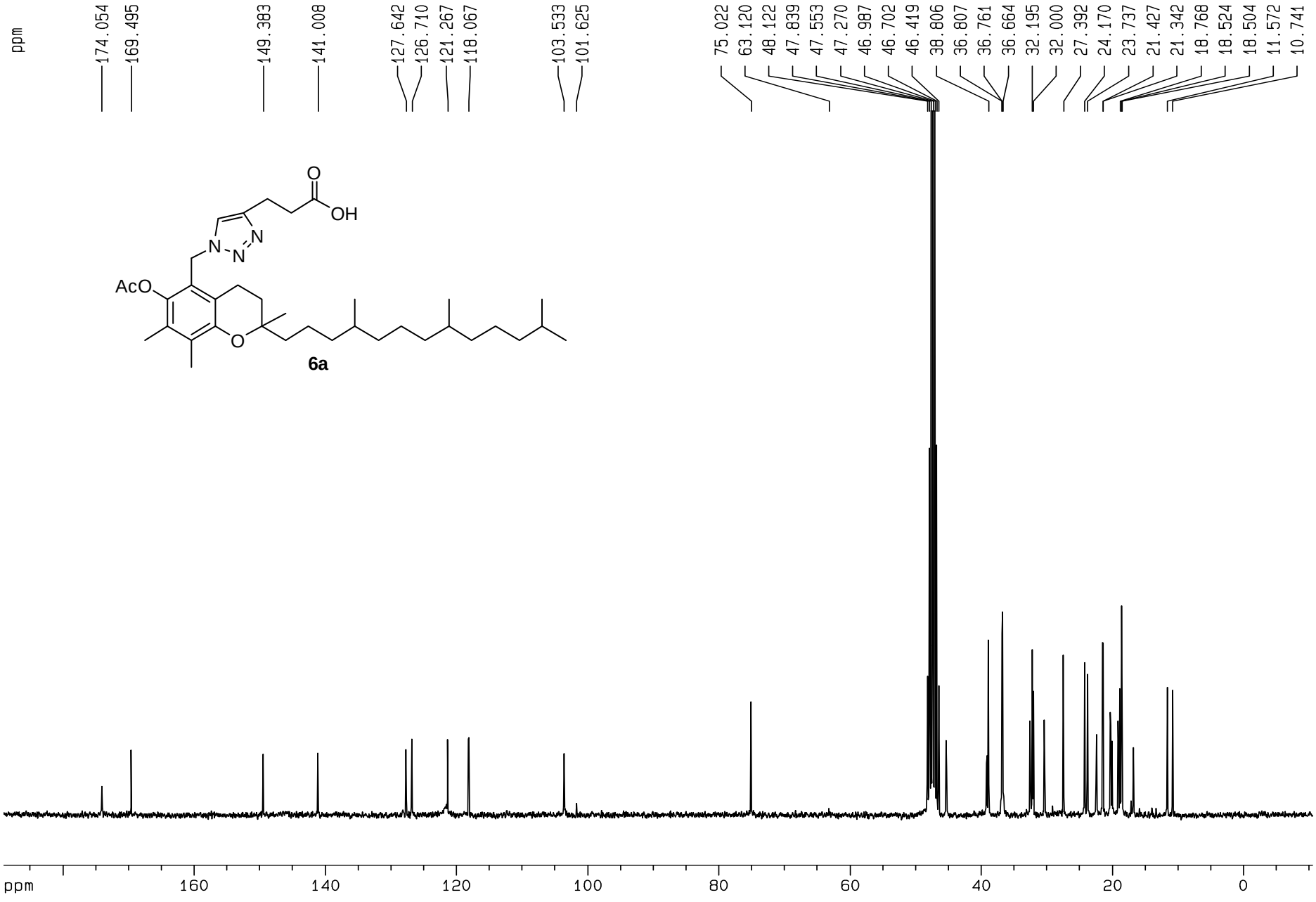
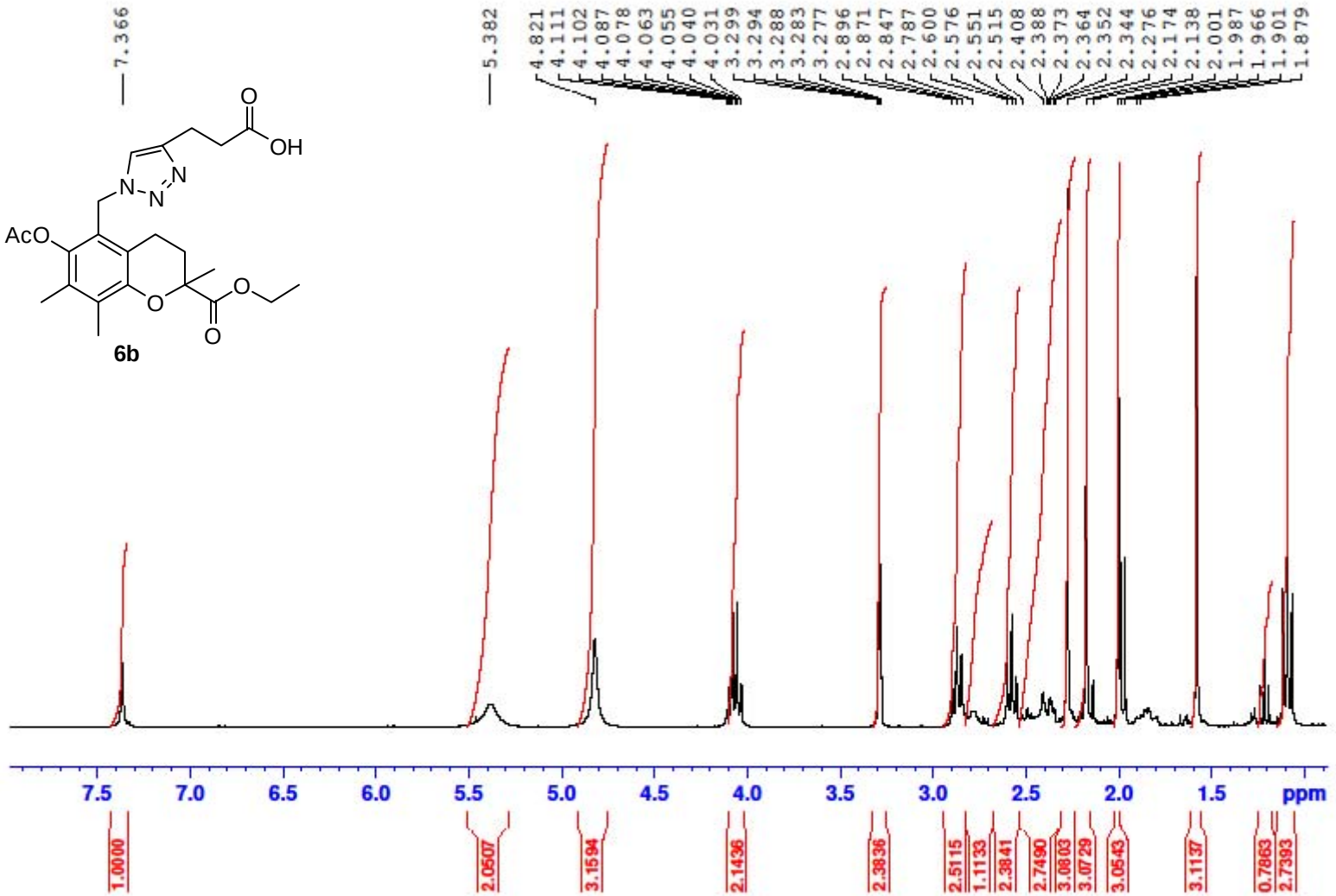
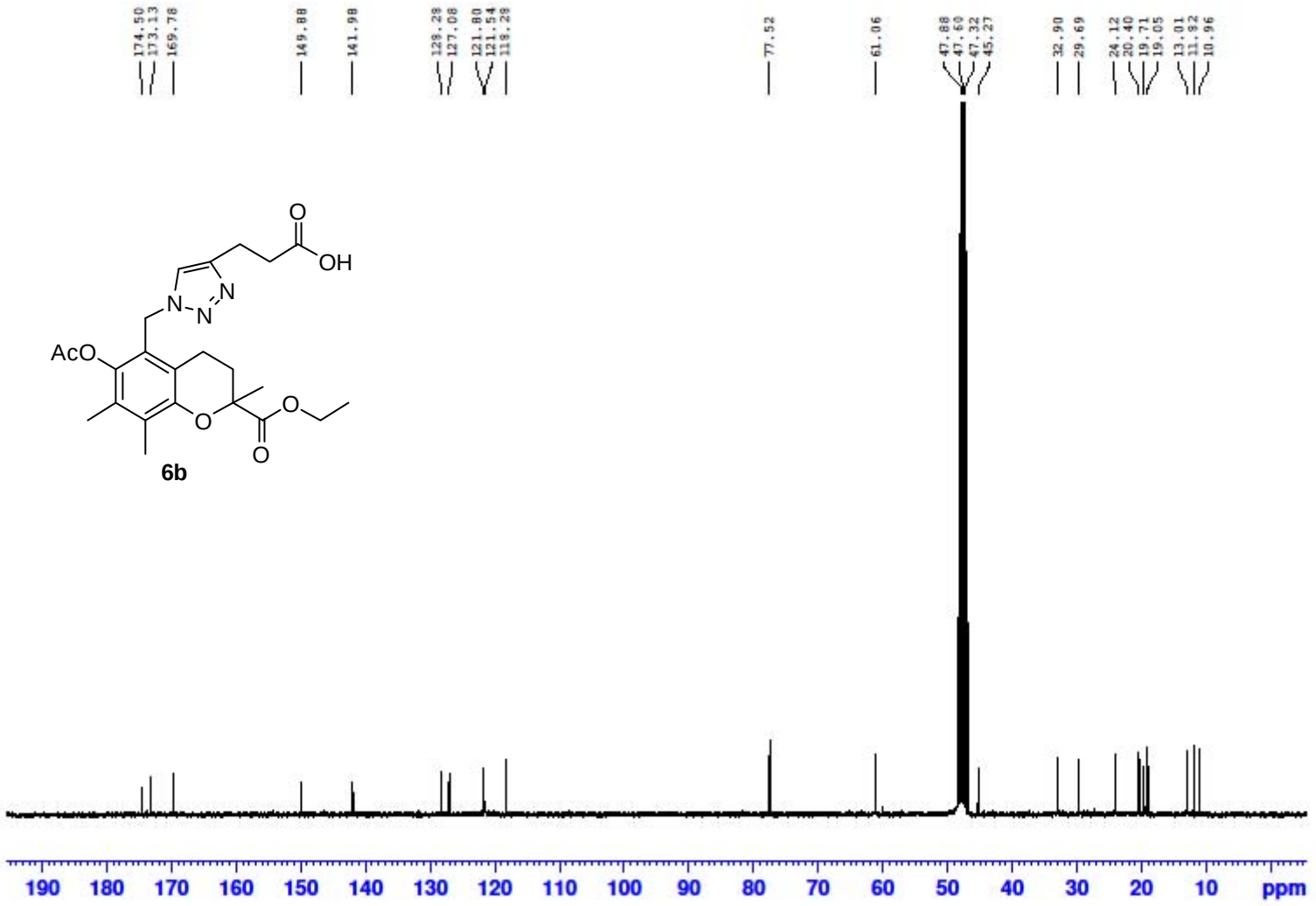


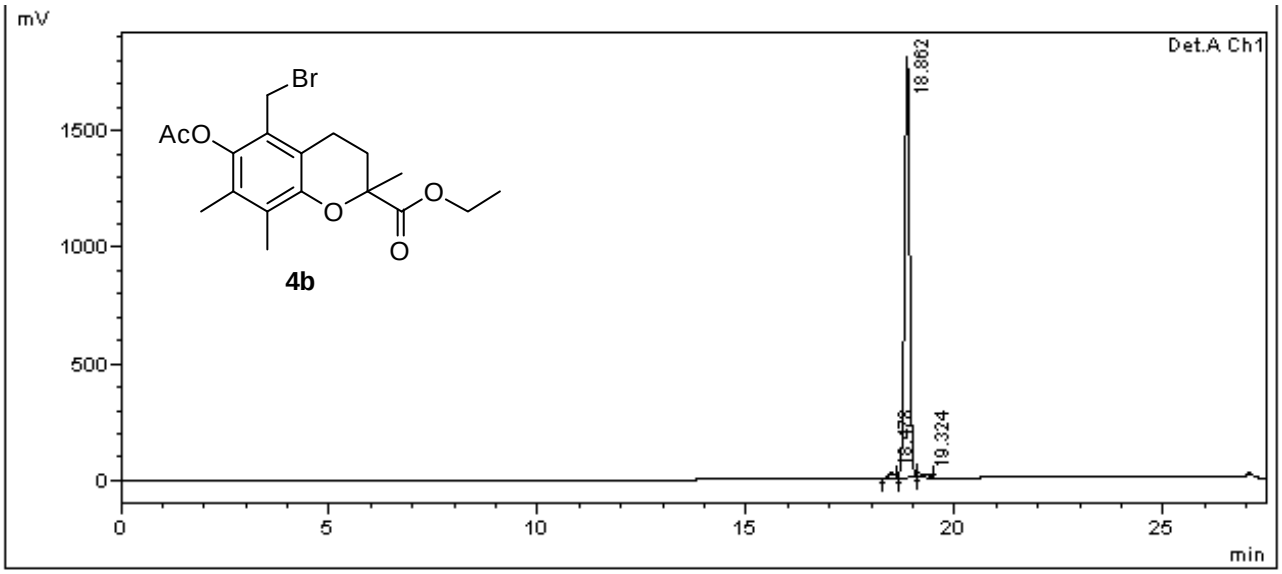






6b

HPLC Purity Trace

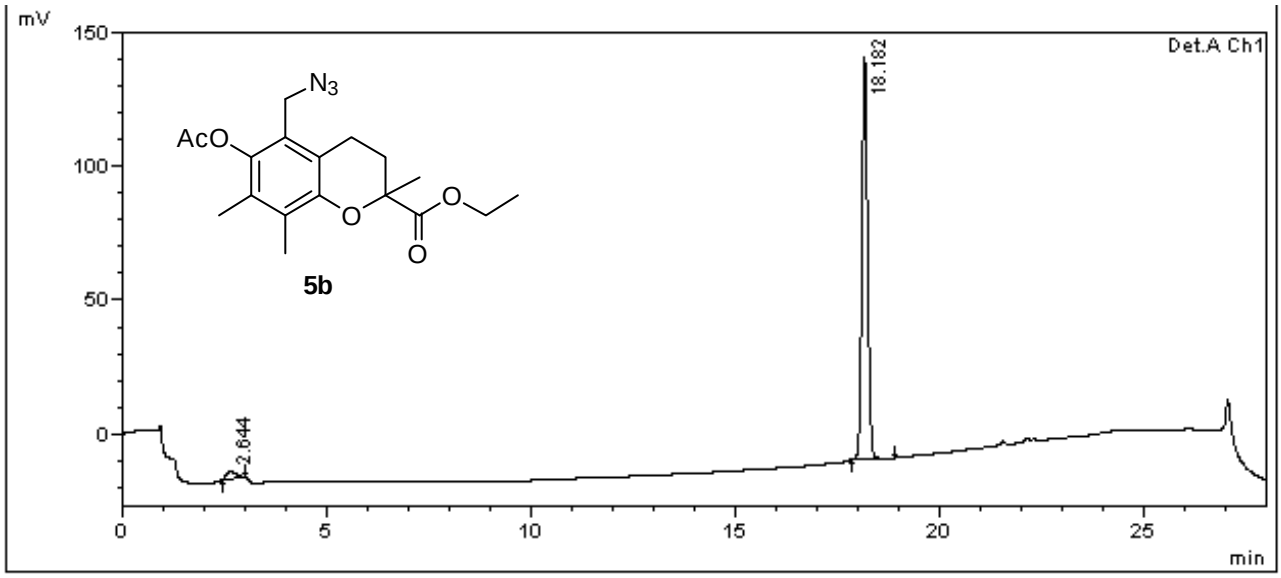


4b

PeakTable

Detector A Ch1 254nm

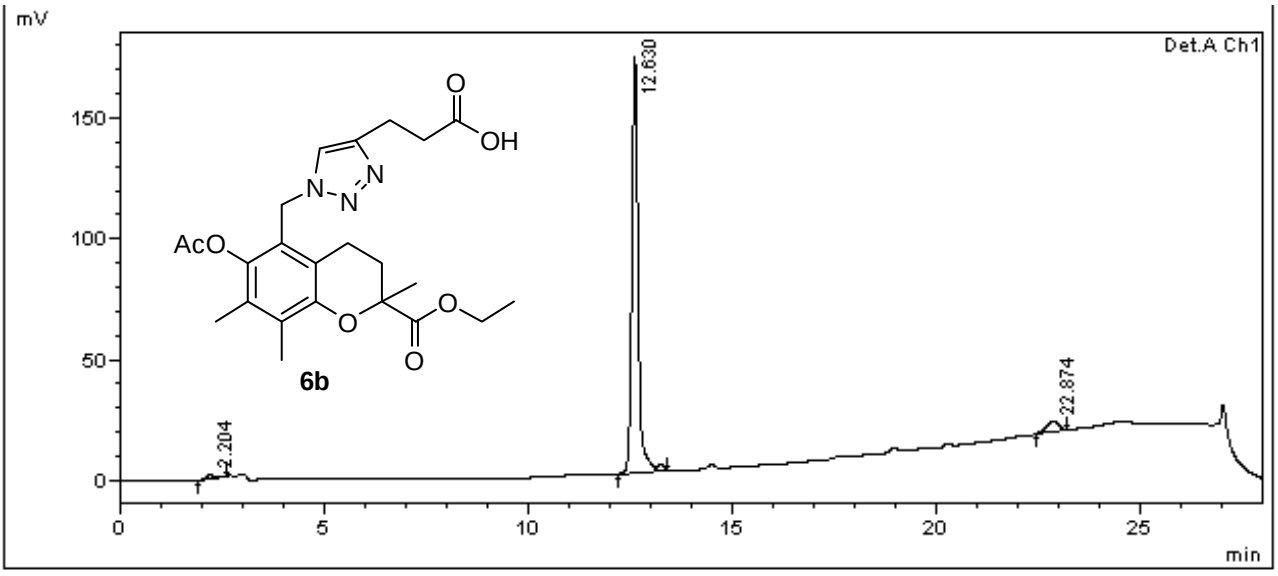
Peak#	Ret. Time	Area	Height	Area %	Height %
1	18.478	230561	20798	1.450	1.132
2	18.862	15497975	1803012	97.491	98.124
3	19.324	168322	13672	1.059	0.744
Total		15896858	1837481	100.000	100.000



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.644	49425	2976	3.316	1.940
2	18.182	1441081	150396	96.684	98.060
Total		1490506	153371	100.000	100.000



Peak Table

Detector A Ch1 254nm

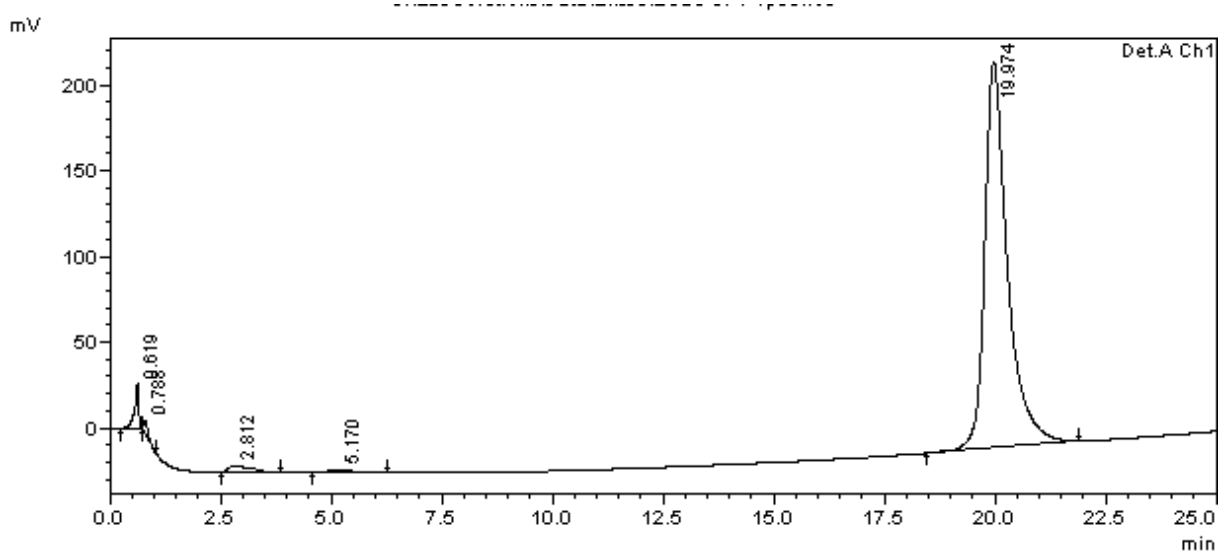
Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.204	20926	1626	1.067	0.915
2	12.630	1850920	171755	94.332	96.642
3	22.874	90281	4342	4.601	2.443
Total		1962128	177723	100.000	100.000

PNA OLIGOMER SYNTHESIS

Bhoc- and Fmoc-protected PNA monomers (A, C, G and T) and 2-aminoethoxy-2-ethoxyacetic acid (AEEA) were purchased from ASM Research Chemicals and were used without further purification. Automated synthesis was performed on an Expedite 8909 nucleic acid synthesiser, on a 2μmol scale using Fmoc-PAL-PEG-PS resin (0.19mmol/g) from Applied Biosystems, following the manufacturer's protocol. The PNA was cleaved from the resin using TFA/*m*-cresol (4:1) then precipitated and washed with ice cold ether and dried. The crude PNA was purified using a Phenomenex Jupiter C18 10 μm, 250mm x 10mm column, with gradient elution using water (Eluent A) and acetonitrile (Eluent B) with 0.1% TFA. The pure PNA fractions were collected, lyophilised and characterised by MALDI-TOF and ESI mass spectroscopy as appropriate.

CONJUGATED-PNA HPLC PURITY TRACES

7a-PNA1



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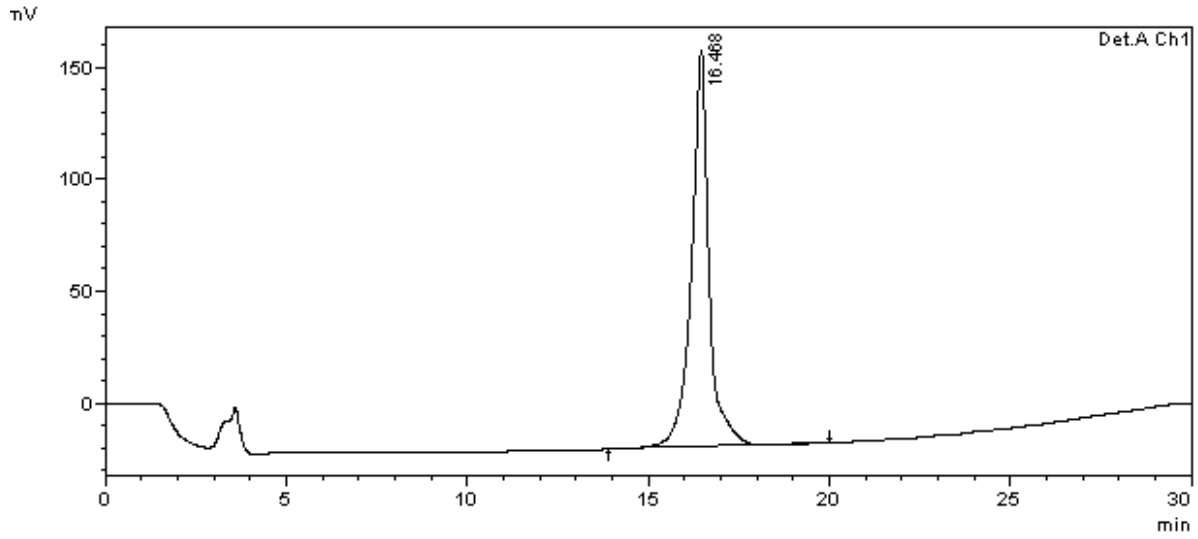

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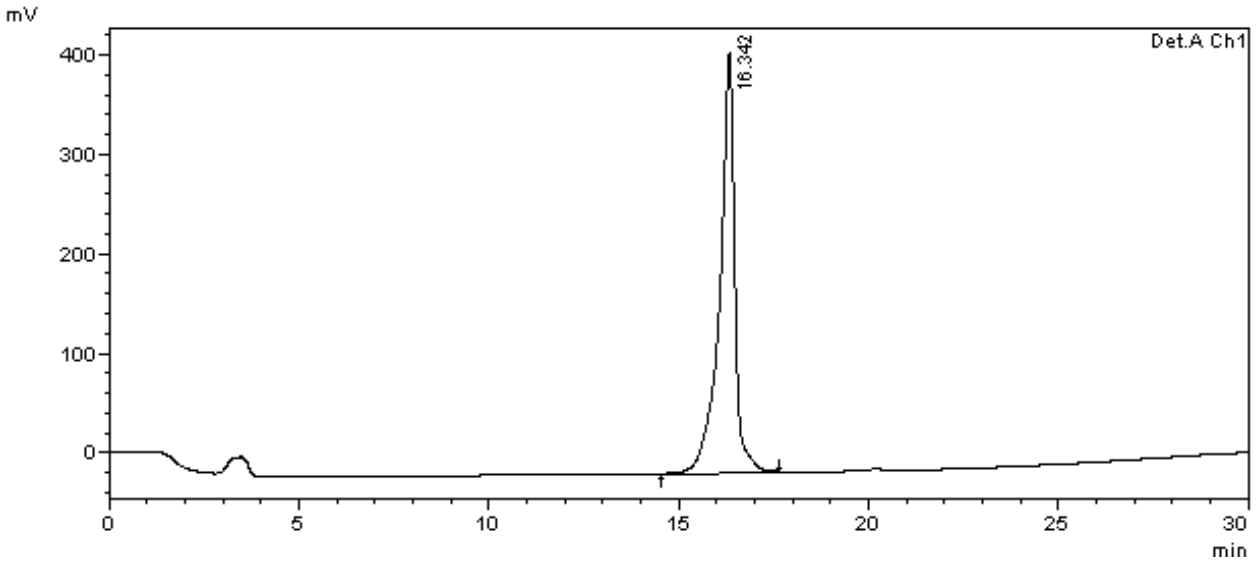
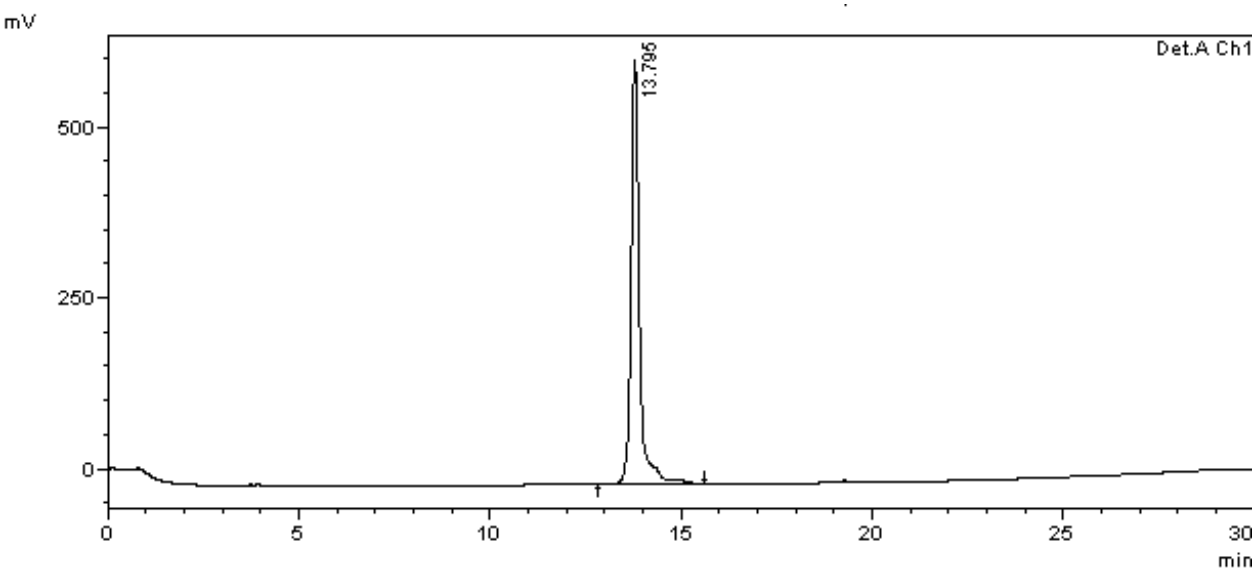
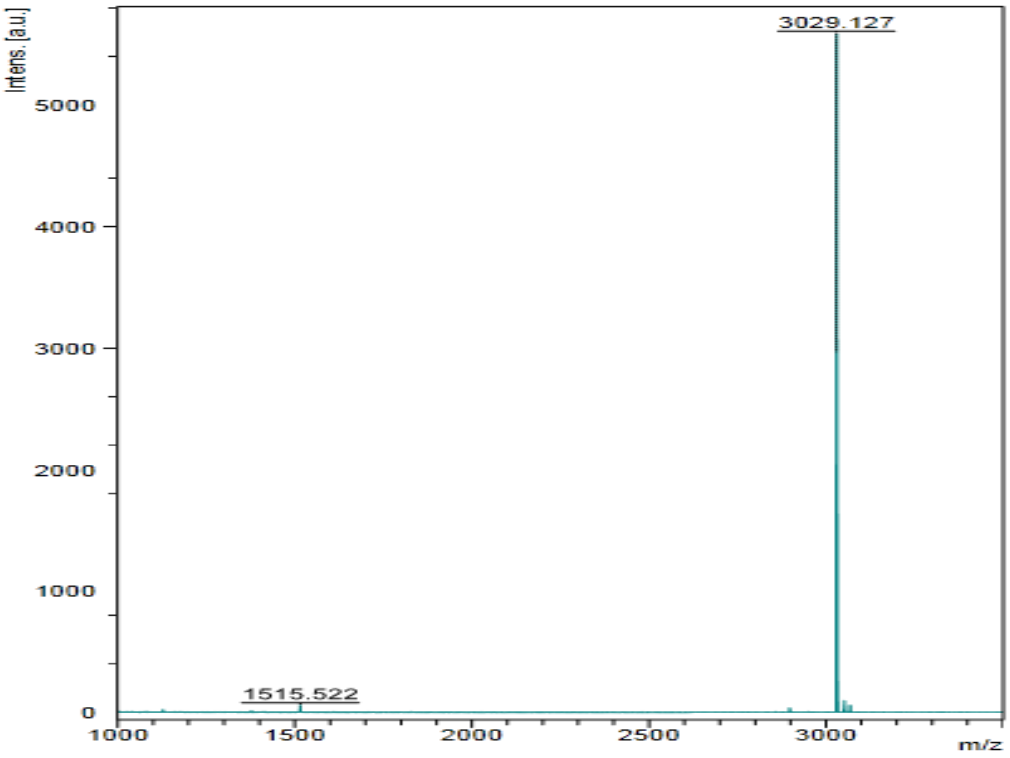


9-PNA1

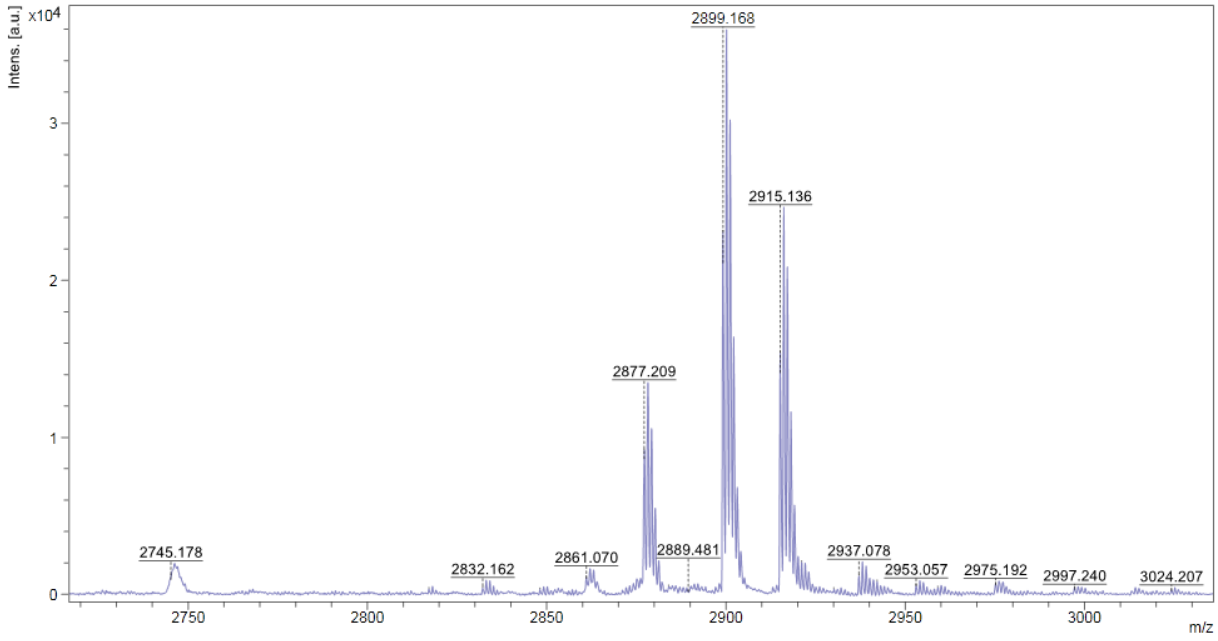


CONJUGATED-PNA MALDI SPECTRA

7a-PNA1



7b-PNA1



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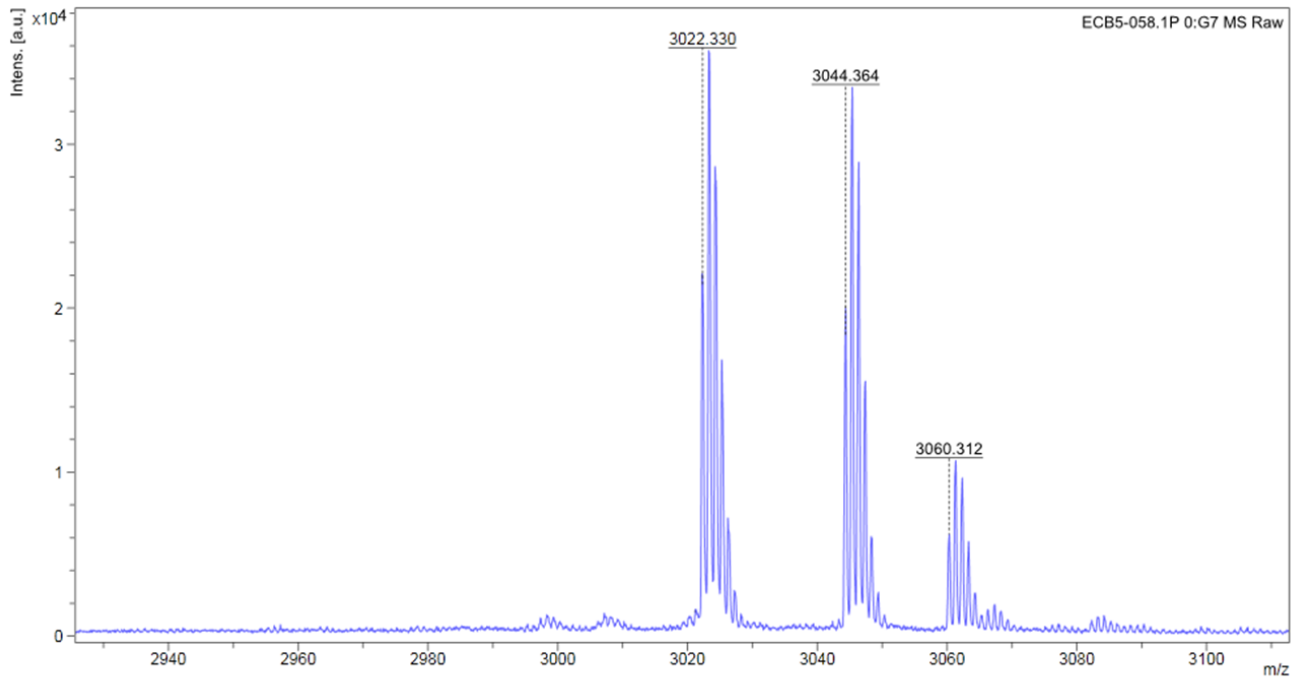
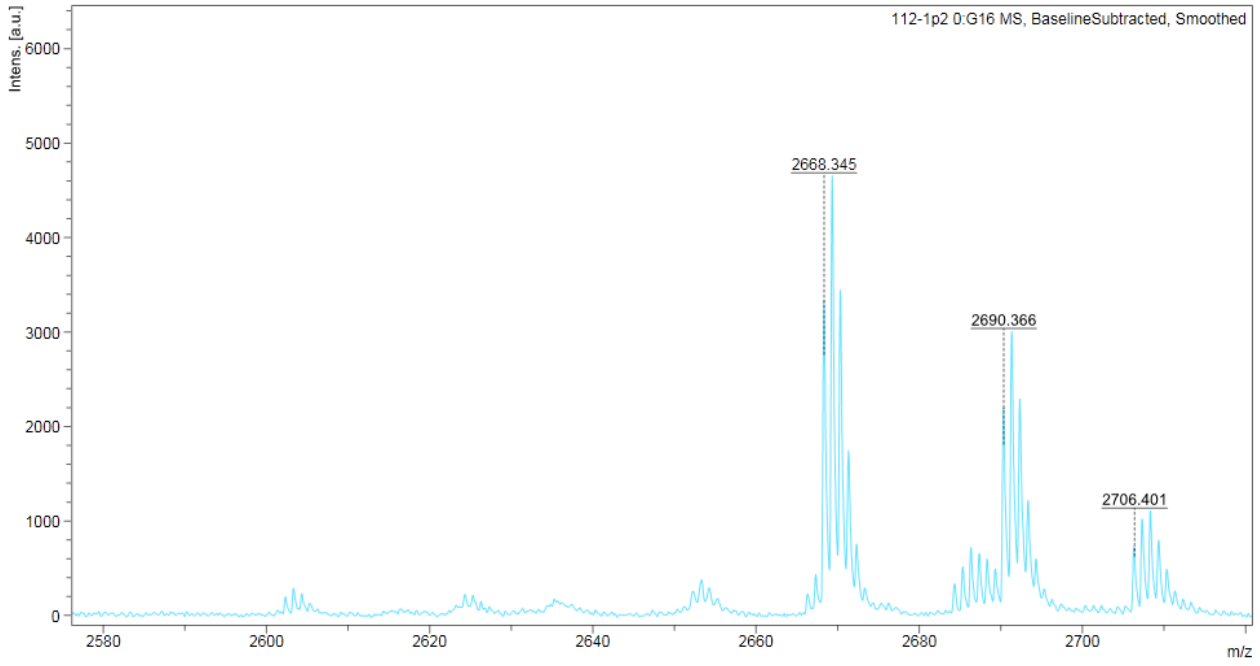


9-PNA1



DETERMINATION OF SOLUTION CONCENTRATION

All experiments were carried out in 10mM sodium phosphate buffer (pH 7.0). The concentrations of both PNA and DNA strands were determined by UV absorption at a wavelength of 260 nm at 80 ºC, using quartz cells with a 1 cm path length. The following extinction coefficients were used; $\varepsilon_{DNA:A}$ = 15300 M^{-1} cm^{-1}, $\varepsilon_{DNA:G}$ = 12220 M^{-1} cm^{-1}, $\varepsilon_{DNA:C}$ = 7600 M^{-1} cm^{-1}, $\varepsilon_{DNA:T}$ = 8700 M^{-1} cm^{-1}, $\varepsilon_{PNA:A}$ = 13700 M^{-1} cm^{-1}, $\varepsilon_{PNA:G}$ = 11700 M^{-1} cm^{-1}, $\varepsilon_{PNA:C}$ = 6600 M^{-1} cm^{-1}, ε_{PNAT} = 8600 M^{-1} cm^{-1}.[5]

UV MELTING EXPERIMENTS

Melting curves were performed on a Varian Cary 100 Bio UV-Vis spectrophotometer with a Cary temperature controller. The duplexes formed during the ITC experiments were used directly to obtain the melting curves, as previously undertaken in the literature.[6] Samples were prepared by heating to 80 ºC for 5 min, cooling to 20 ºC over 20 min and holding at 20 ºC for a further 20 min. The melting curves were measured at 260 nm, with the temperature increasing from 20 ºC to 80 ºC at a rate of 0.5 ºC/min, with data collection occurring every 0.2 ºC. Each duplex melting curve was performed in triplicate, at a minimum.

DETERMINATION OF THERMODYNAMIC PARAMETERS via UVM

The melting temperature (T_m) is dependent upon α, which is the fraction of the single strand in a duplex state, as described by Marky and Breslaur[7] is shown by equation (A) below:

$$\alpha = \frac{(A_s - A)}{(A_s - A) + (A - A_d)} \qquad (A)$$

where A is absorbance at a given T and A_s and A_d is the absorbance from the single strand and the duplex respectively. The T_m of the duplex is determined where α = 0.5.

In order to calculate the van't Hoff enthalpy, the equilibrium constant (K) must be determined and expressed in terms of α for a non-complementary association, as shown in equation (B), where C_T is the total strand concentration and *n* is the number of strands associated with the complex.

$$K = \frac{\alpha}{(C_T/n)^{n-1}\,(1-\alpha)^n} \qquad (B)$$

Thus, the Gibbs free energy change can be determined (equation C) where a plot of ln (K) vs 1/T will determine both the enthalpy and entropy of the system (equations D and E respectively).

$$\Delta G_{vH}^\circ = -RT \ln(K) = \Delta H^\circ - T\Delta S^\circ \qquad (C)$$

$$\Delta H_{vH}^\circ = \text{slope } (\ln(K) \text{ vs } 1/T)\ R \qquad (D)$$

$$\Delta S_{vH}^\circ = \text{intercept } (\ln(K) \text{ vs } 1/T)\ R \qquad (E)$$

ISOTHERMAL TITRATION CALORIMETRY

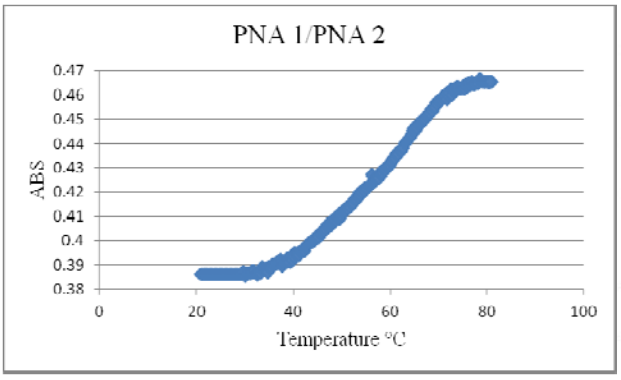
Calorimetric experiments were performed on a CSC 5300 Nano-ITC 111 instrument at 25 ºC, where one of the oligomer strands

(~0.1 mM, 100 µL) was titrated into 1.4 mL of the complementary strand (~5 µM). Each injection was 4 or 5 µL at 5 min intervals for a total of 25 injections. Stirrer speed was set to 250 rpm. Solutions were thoroughly degassed by sonification and absolute concentrations determined as outlined above. The reference cell was filled with degassed and deionised water. Isotherms were examined using the software NanoAnalyze v2.0, whereby the binding constant (K_b), intrinsic molar enthalpy change (ΔH_b^o) and stoichiometry of binding (*n*) were determined by means of best fit (independent model) of the calorimetric data. The data was corrected by subtracting the heat of dilution from the experiment. Each duplex was titrated in triplicate, at a minimum.

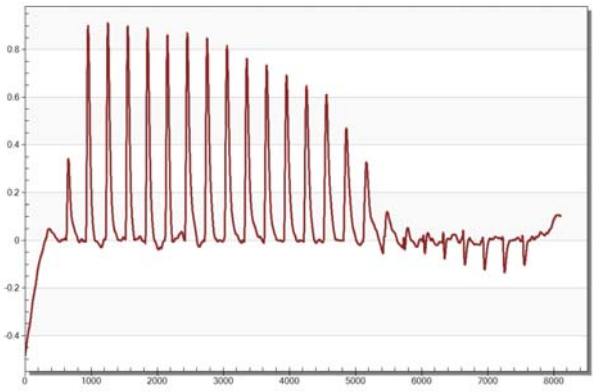
REPRESENTATIVE EXAMPLES OF THERMODYNAMIC EXPERIMENTAL RESULTS FROM UVM AND ITC

PNA1/PNA2

A. UVM

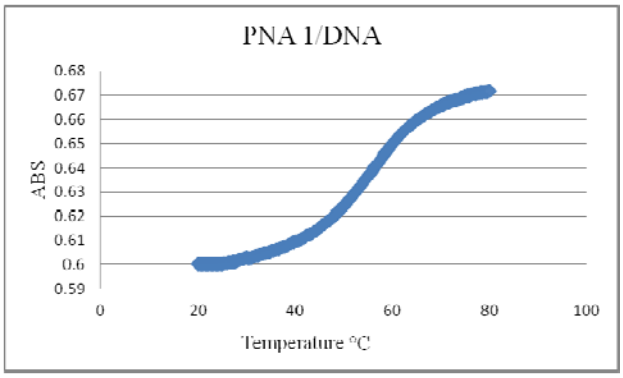


B. ITC

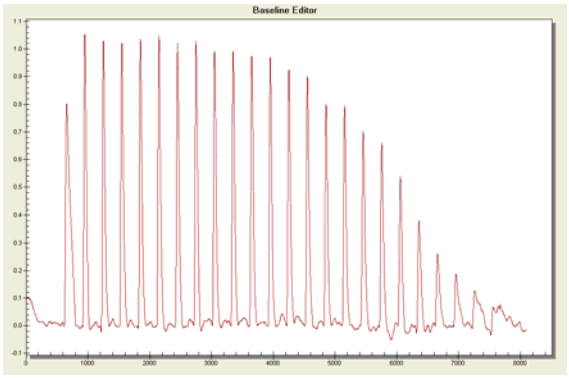


PNA 1/DNA

A. UVM

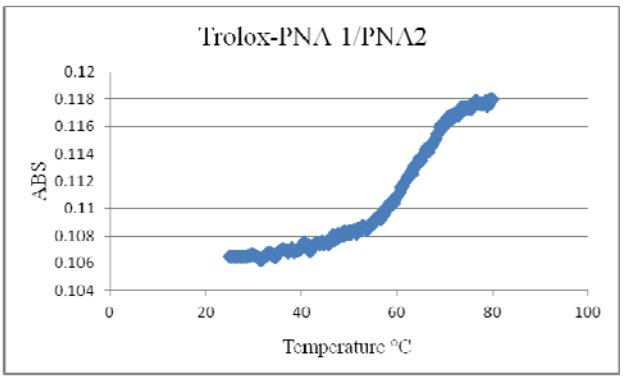


B. ITC

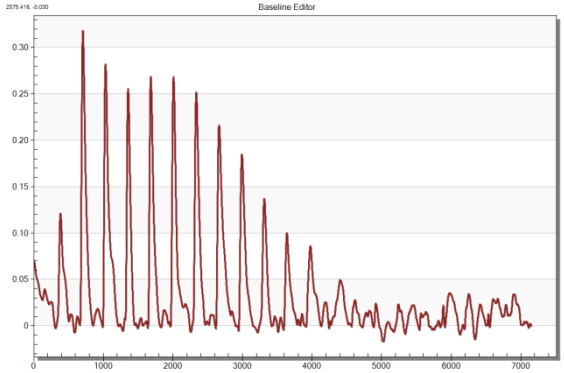


7b-PNA1/PNA 2

A. UVM

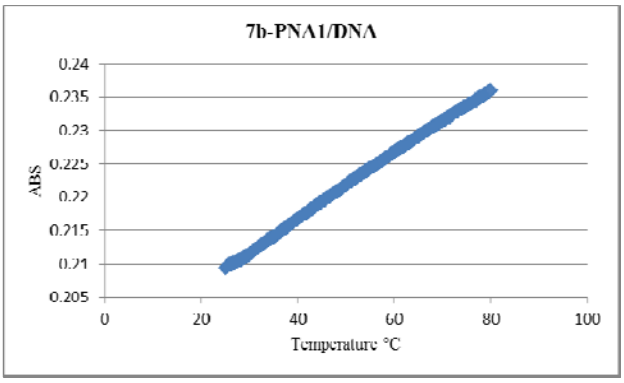


B. ITC

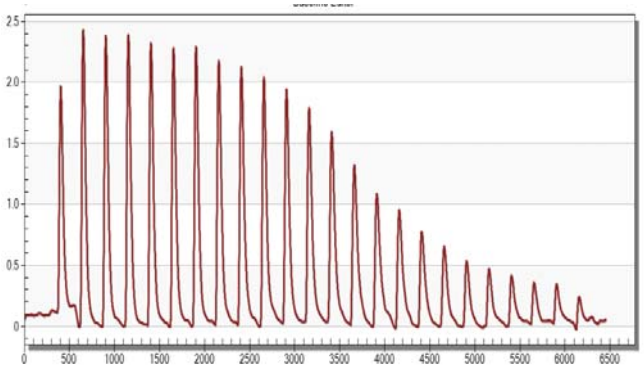


`7b-PNA1/DNA

A. UVM

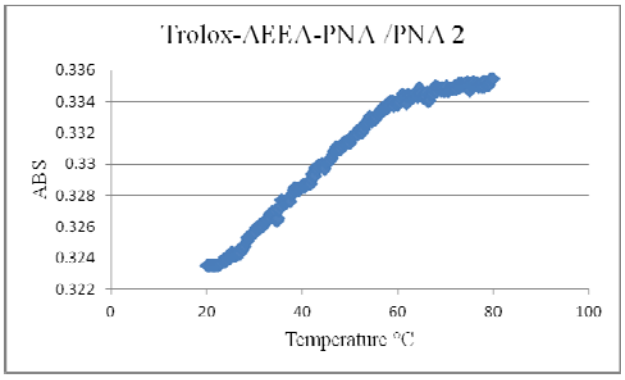


B. ITC

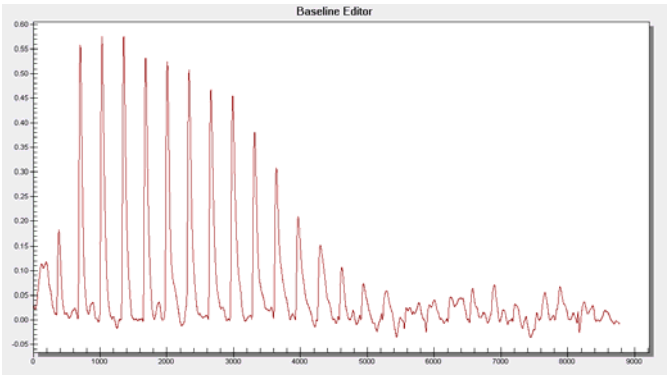


8b-PNA1/PNA 2

A. UVM

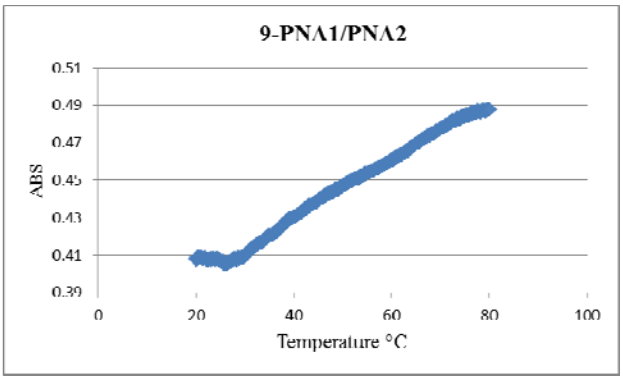


B. ITC

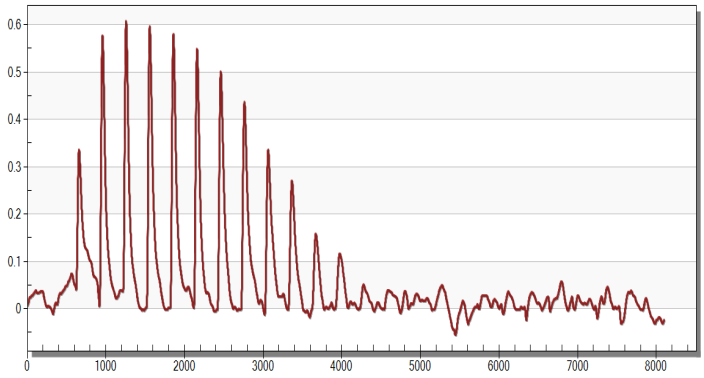


9-PNA1/PNA2

A. UVM

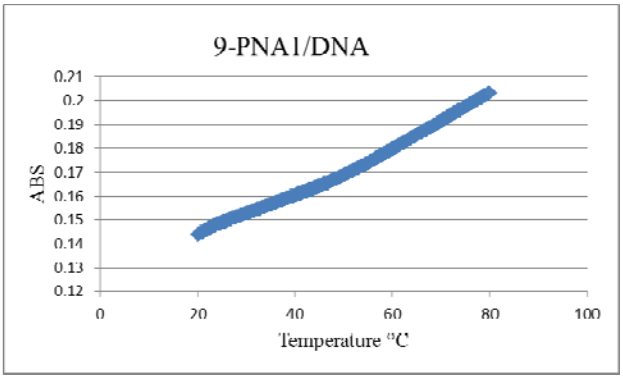


B. ITC

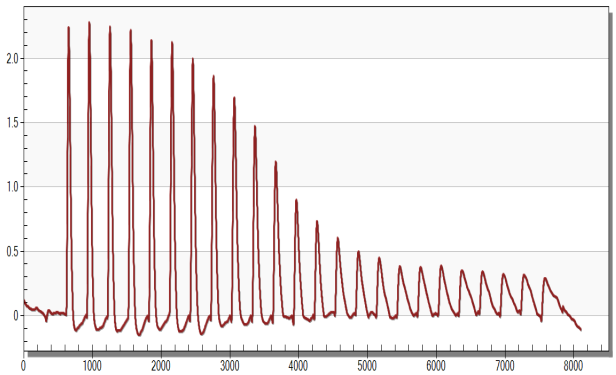


9-PNA1/DNA

A. UVM



B. ITC



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