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Supplementary Materials: Synthesis and Antiproliferative Activity of Novel Heterocyclic Indole-Trimethoxyphenyl Conjugates

Michael M. Cahill, Kevin D. O'Shea, Larry T. Pierce, Hannah J. Winfield, Kevin S. Eccles, Simon E. Lawrence and Florence O. McCarthy

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General Procedures

Solvents were distilled prior to use as follows: dichloromethane was distilled from phosphorous pentoxide; ethyl acetate was distilled from potassium carbonate; ethanol and methanol were distilled from magnesium in the presence of iodine; toluene was distilled from sodium and benzophenone; hexane was distilled prior to use; tetrahydrofuran was freshly distilled from sodium and benzophenone. Diethyl ether was obtained pure from Riedel-de Haën. Organic phases were dried using anhydrous magnesium sulphate. All commercial reagents were used without further purification unless otherwise stated. Infrared spectra were recorded as a thin film on sodium chloride plates for liquids or potassium bromide (KBr) disc for solids on a Perkin Elmer Spectrum 100 FT-IR spectrometer. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer. ^1H (400 MHz) NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer. ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectra were recorded on a Bruker Avance III 600 MHz NMR spectrometer equipped with a dual CH cryoprobe. All spectra were recorded at room temperature ($\sim 20^\circ\text{C}$) in deuterated chloroform (CDCl_3) with tetramethylsilane (TMS) as an internal standard, or deuterated dimethylsulfoxide ($\text{DMSO}-d_6$). ^1H NMR spectra recorded in deuterated dimethylsulfoxide ($\text{DMSO}-d_6$) were assigned using the $\text{DMSO}-d_6$ peak as the reference peak. Chemical shifts (δ_{H} and δ_{C}) are expressed in parts per million (ppm) relative to the reference peak. Coupling constants (J) are expressed in Hertz (Hz). Splitting patterns in ^1H NMR spectra are designated as s (singlet), br s (broad singlet), d (doublet), br d (broad doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), ddd (doublet of doublet of doublets), ddt (doublet of doublet of triplets) and m (multiplet). Low resolution mass spectra were recorded on a Waters Quattro Micro triple quadrupole spectrometer (QAA1202) in electrospray ionisation (ESI) mode using 50% acetonitrile-water containing 0.1% formic acid as eluent. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier Time of Flight spectrometer (KD160) in electrospray ionisation (ESI) mode using 50% acetonitrile-water containing 0.1% formic acid as eluent. Melting points were measured in a uni-melt Thomas Hoover capillary melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck 60 PF254), and visualisation was achieved by UV light detection (254 nm). Single crystal data were collected on either a Bruker SMART X2S diffractometer (**54** and **55**) or a Bruker APEX II DUO diffractometer (**53**) using $\text{Mo K}\alpha$ ($\lambda = 0.7107 \text{ \AA}$) radiation, as previously described [1]. Calculations were made using the APEX2 and SHELX software [2,3].

Supplementary Chemical Data

2-(1H-Indol-3-yl)-2-oxoacetic acid (17). Indole (**16**, 10.02 g, 85 mmol) was dissolved in diethyl ether (150 mL) and was cooled to 0 °C in an ice bath. Oxalyl chloride (11.0 mL, 1.48 g/mL, 128 mmol) was carefully added drop wise over 20 min. The resulting yellow mixture was allowed to stir at room temperature for 30 min. After this time, the reaction mixture was once again cooled to 0 °C using an ice bath. Saturated, aqueous sodium bicarbonate solution (20 mL) was added very carefully and the resultant red and yellow biphasic mixture was allowed to stir overnight. Following this, the acid was isolated by vacuum filtration washed with ice-cold ether (5 mL) and allowed to air dry to yield the oxoacetic acid (**17**) as a bright yellow solid (14.80 g, 78.2 mmol, 92%); mp 216–217 °C (literature [4]: 213–214 °C), $\nu_{\max}/\text{cm}^{-1}$ (KBr) 3249, 1907, 1790, 1696, 1614, 1505; δ_{H} (400 MHz, DMSO- d_6) 7.28 (m, 2H, C-H₅, C-H₆), 7.55 (dd, 1H, J = 6.1, 1.9 Hz, C-H₇), 8.18 (dd, 1H, J = 6.6, 2.1 Hz, C-H₄), 8.42 (d, 1H, J = 3.3 Hz, C-H₂), 12.34 (brs, 1H, NH); m/z (ES⁺) 190.3 [M + H]⁺ (40%).

Potassium 2-(1H-indol-3-yl)-2-oxoacetate (18). To a solution of 2-(1H-indol-3-yl)-2-oxoacetic acid **1** (14.75 g, 77.9 mmol) in ethanol (250 mL) was added potassium hydroxide (4.37 g, 78 mmol). The resultant mixture was allowed to stir at room temperature for 4 h, after which time the product was isolated by vacuum filtration and washed with ice cold ethanol (20 mL) to yield a light pink solid (10.64 g, 46.8 mmol, 60%), m.p. > 250 °C; $\nu_{\max}/\text{cm}^{-1}$ (KBr) 3597, 3393, 2547, 1787, 1596, 1500; δ_{H} (400 MHz, DMSO- d_6) 7.14 (m, 2H, C-H₅, C-H₆), 7.46 (dd, 1H, J = 6.8, 1.4 Hz, C-H₇), 8.09 (d, 1H, J = 2.8 Hz, C-H₂), 8.17 (dd, 1H, J = 7.1, 1.6 Hz C-H₄), 12.2 (brs, 1H, NH); δ_{C} (100 MHz, DMSO- d_6) 112.1 (CH, aromatic CH), 113.9 (C, aromatic C), 121.9 (2× CH, 2× aromatic CH), 122.2 (CH, aromatic CH), 125.9 (C, aromatic C), 135.8 (C, aromatic C), 136.5 (CH, aromatic CH), 170.0 (C, C=O), 193.1 (C, C=O); m/z (ES[−]) 188.4 [M-H][−] (100%).

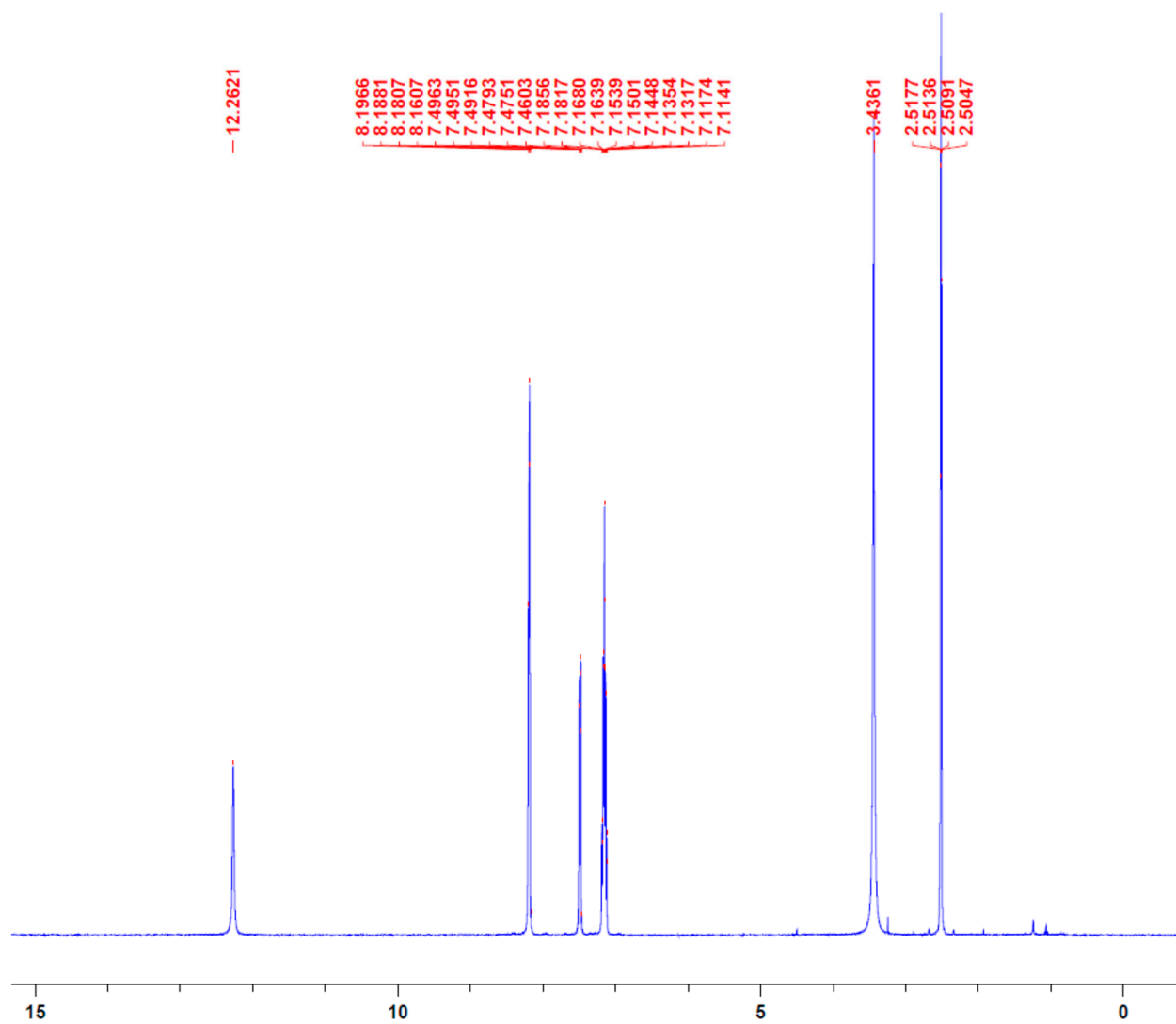
1-Methyl-1H-indole (19). To a solution of indole (10.20 g, 87.06 mmol) in dry DMF (200 mL) at 0 °C was added sodium hydride (5.20 g, 130 mmol) in a portion-wise manner. The resultant mixture was allowed to stir for 30 min, after which time a solution of methyl iodide (8.30 mL, 133.3 mmol) in dry DMF (20 mL) was added dropwise. The reaction mixture was then allowed to stir overnight. Following this, the reaction mixture was carefully poured into ice-cold water and this was then extracted with ethyl acetate (4× 50 mL). Combined ethyl acetate layers were then washed with 1 M aqueous HCl (4× 50 mL), water (5× 50 mL) and brine (4× 50 mL) before being dried over anhydrous magnesium sulphate and concentrated under reduced pressure to yield the product as a clear oil which was used without further purification (9.79 g, 74.6 mmol, 86%); $\nu_{\max}/\text{cm}^{-1}$ (NaCl) 3056, 2917, 1614, 1515; δ_{H} (400 MHz, CDCl₃) 3.76 (s, 3H, NCH₃), 6.48 (dd, 1H, J = 3.1, 0.8 Hz, C-H₃), 7.03 (d, 1H, J = 3.1 Hz, C-H₂), 7.10 (t, 1H, J = 7.0 Hz, C-H₅), 7.22 (t, 1H, J = 7.0 Hz, C-H₆), 7.31 (d, 1H, J = 8.1 Hz, C-H₇), 7.62 (d, 1H, J = 7.9 Hz, C-H₄); m/z (ES⁺) 132.3 [M + H]⁺ (50%).

2-(1-Methyl-1H-indol-3-yl)-2-oxoacetic acid (20). To a solution of 1-methyl-1H-indole **3** (9.79 g, 74.64 mmol) in diethyl ether (150 mL) cooled to 0 °C was added oxalyl chloride (9.85 mL, 1.48 g/mL, 112 mmol) dropwise over 20 min. The resultant deep yellow slurry was allowed to stir at room temperature for 30 min before saturated aqueous sodium bicarbonate solution (20 mL) was added with care. The resultant biphasic mixture was allowed to stir overnight. Following this, the product was isolated by vacuum filtration to yield the oxoacetic acid as a cream coloured solid (7.60 g, 37.4 mmol, 50%), m.p. 158–161 °C (literature [5]: 155–158 °C) $\nu_{\max}/\text{cm}^{-1}$ (KBr) 3495, 3250, 3145, 1754, 1618, 1308; δ_{H} (400 MHz, DMSO- d_6) 3.92 (s, 3H, N-CH₃), 7.34 (m, 2H, C-H₅, C-H₆), 7.61 (d, 1H, J = 7.8 Hz, C-H₇), 8.20 (d, 1H, J = 7.2 Hz, C-H₄), 8.49 (s, 1H, C-H₂), 13.91 (brs, 1H, OH); m/z (ES⁺) 204.3 [M + H]⁺ (100%).

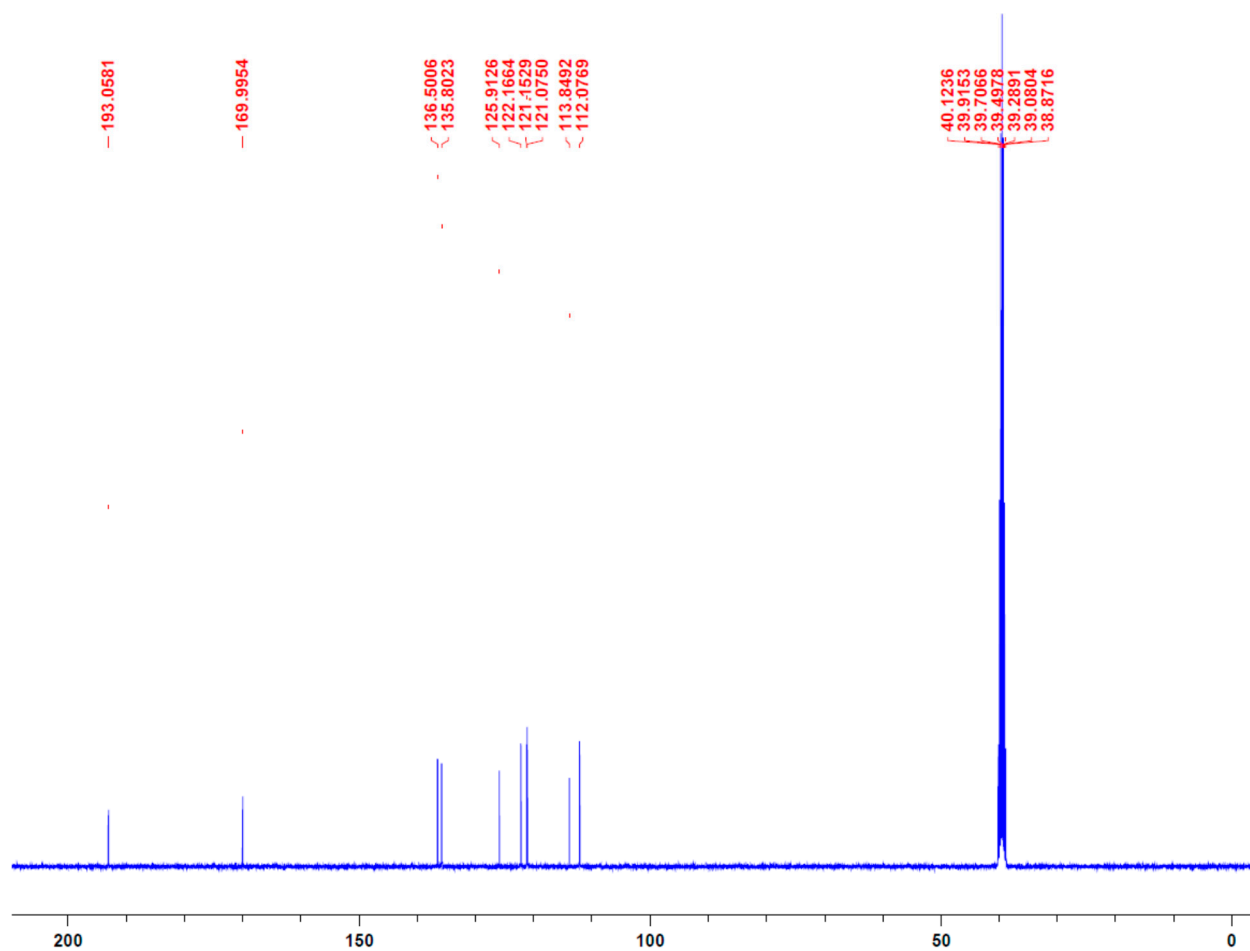
Potassium 2-(1-methyl-1H-indol-3-yl)-2-oxoacetate (21). To a solution of 2-(1-methyl-1H-indol-3-yl)-2-oxoacetic acid **4** (7.60 g, 37.4 mmol) in ethanol (100 mL) was added potassium hydroxide (2.09 g, 37.4 mmol). The resultant mixture was allowed to stir at room temperature for 4 h before the product was isolated by vacuum filtration. The product cake was then washed with ice cold ethanol (10 mL) and dried overnight to yield the potassium salt as a white solid (6.44 g, 26.7 mmol, 71 %), m.p. > 250 °C; $\nu_{\max}/\text{cm}^{-1}$ (KBr) 3416, 3048, 2908, 2535, 1930, 1717, 1650; δ_{H} (400 MHz, DMSO- d_6) 3.85 (s, 3H, N-CH₃), 7.23

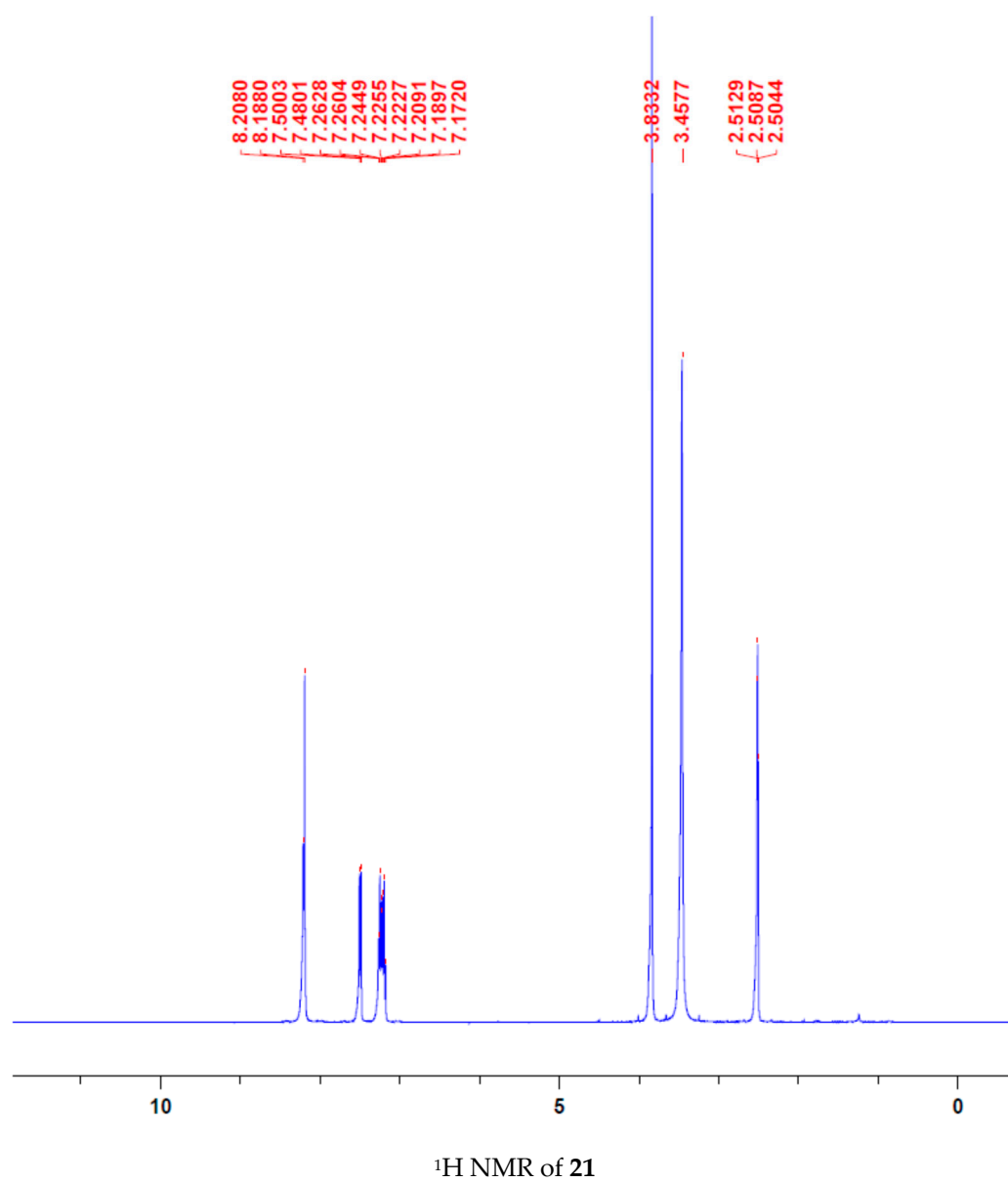
(m, 2H, C-H₅, C-H₆), 7.51 (d, 1H, $J = 8.0$ Hz, C-H₇), 8.18 (d, 1H, $J = 7.4$ Hz, C-H₄) 8.19 (s, 1H, C-H₂); δ_c (100 MHz, DMSO- d_6) 32.9 (CH₃, N-CH₃), 110.3 (CH, aromatic CH), 112.9 (C, aromatic C), 121.5 (2× CH, 2× aromatic CH), 122.3 (CH, aromatic CH), 126.3 (C, aromatic C), 137.0 (C, aromatic C), 139.3 (CH, aromatic CH), 169.7 (C, C=O), 192.7 (C=O); m/z (ES[−]) 202.4 [M-H][−] (100%).

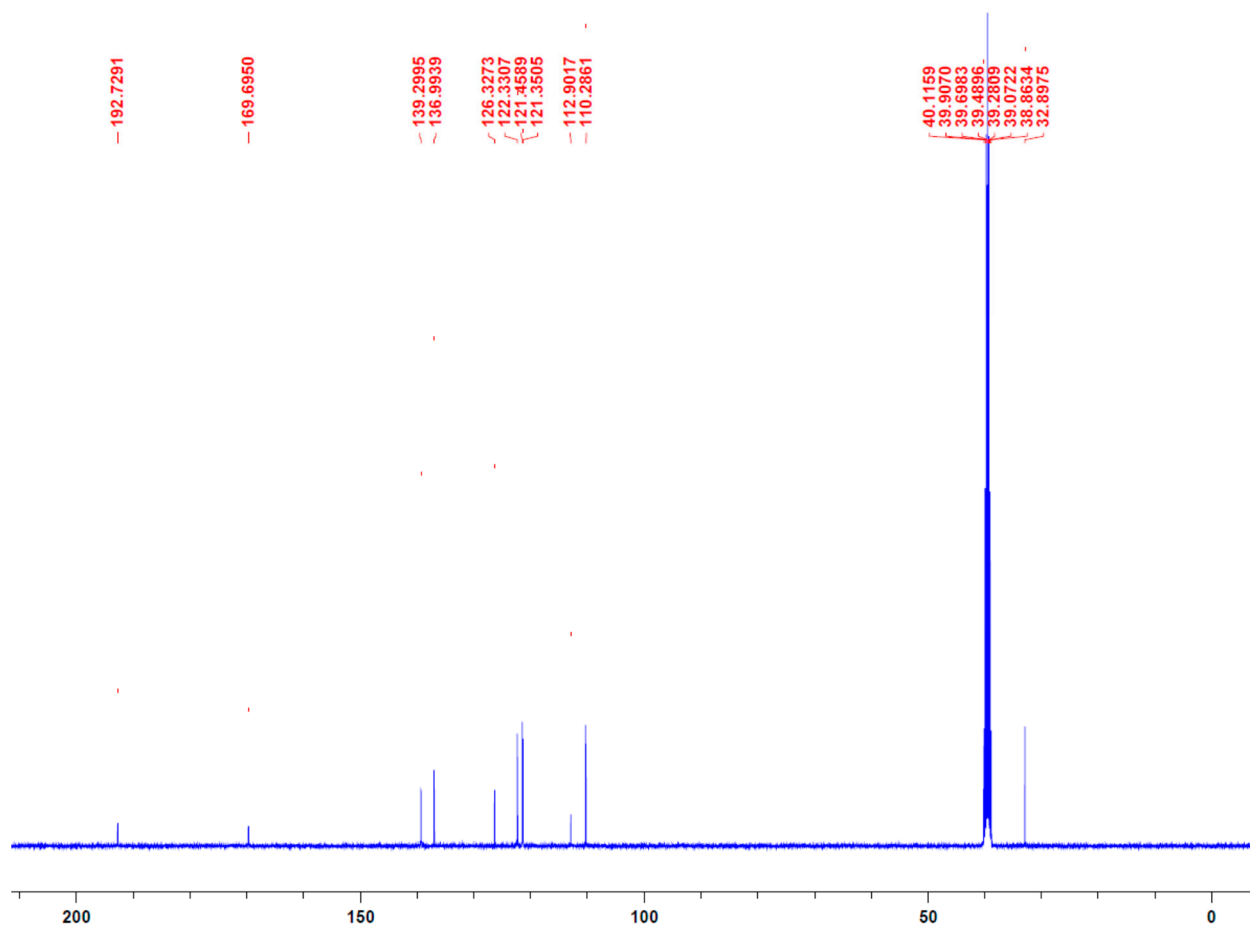
NMR Spectra

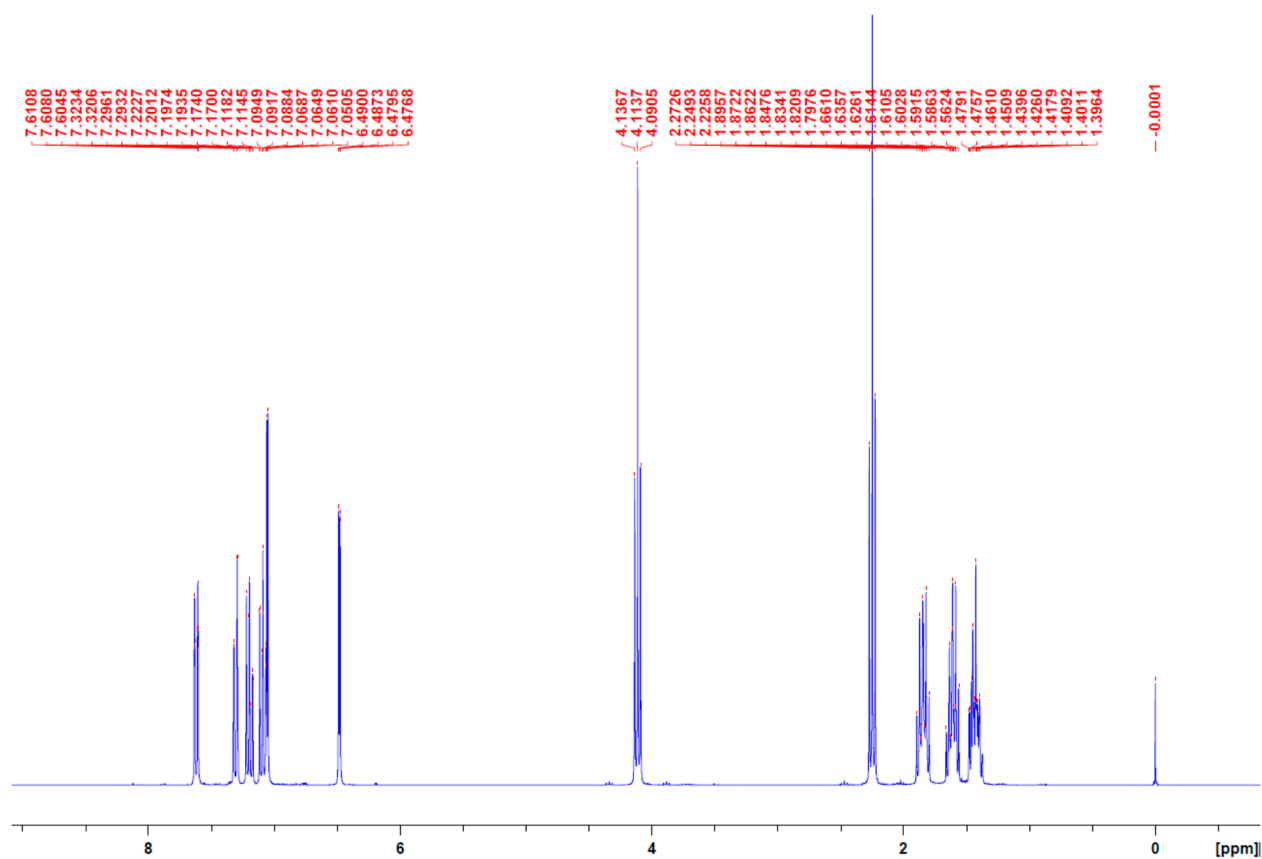


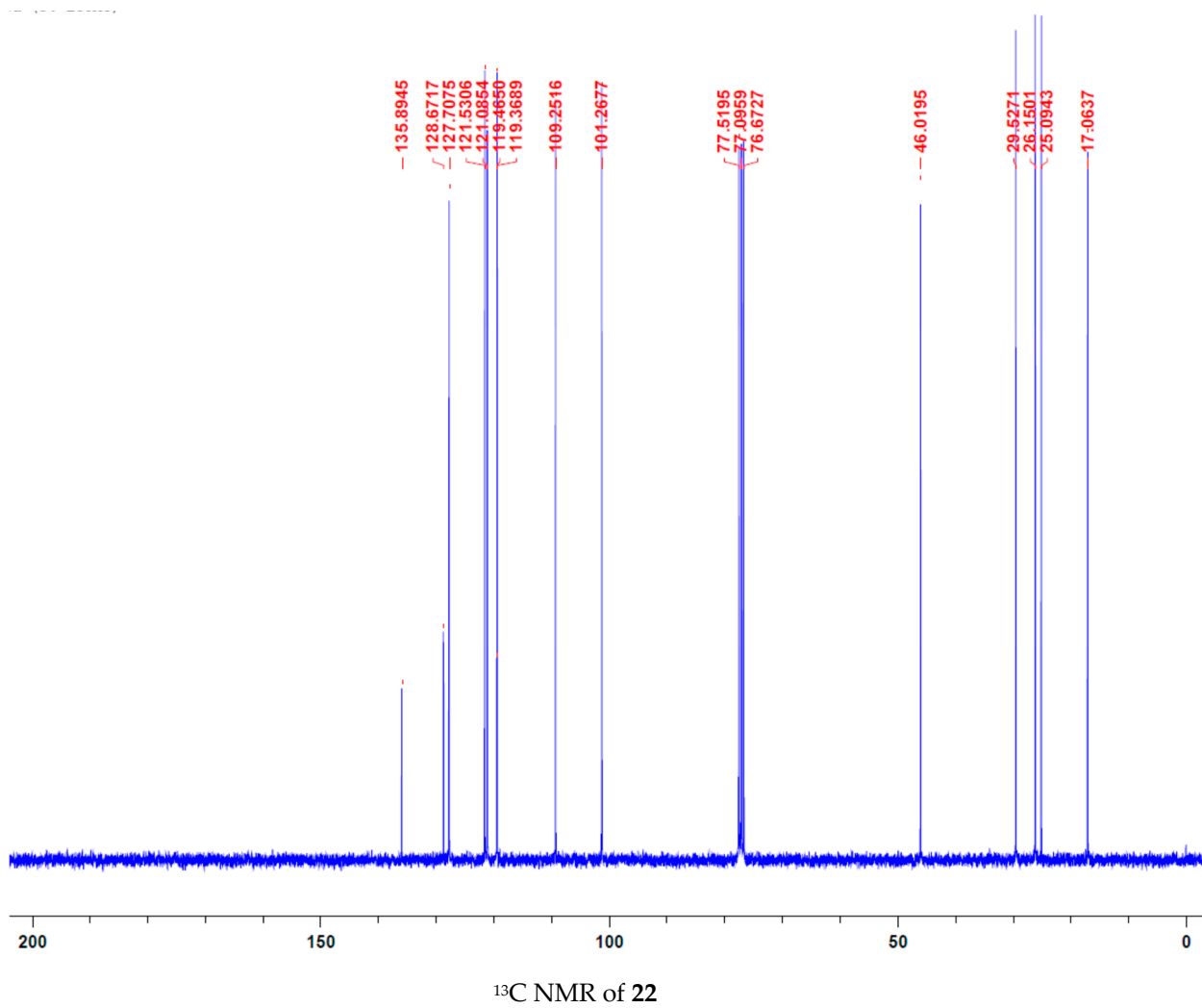
^1H NMR of 18.

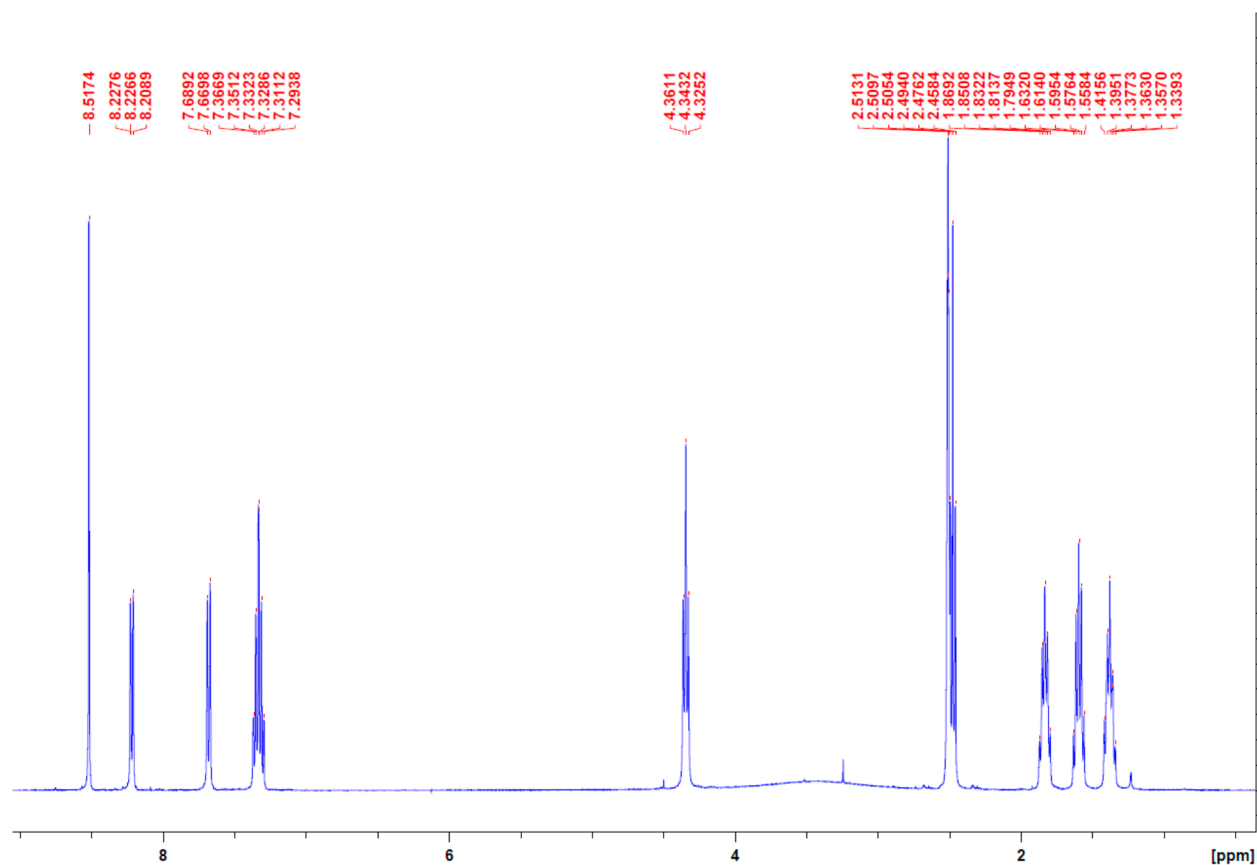
¹³C NMR of **18**

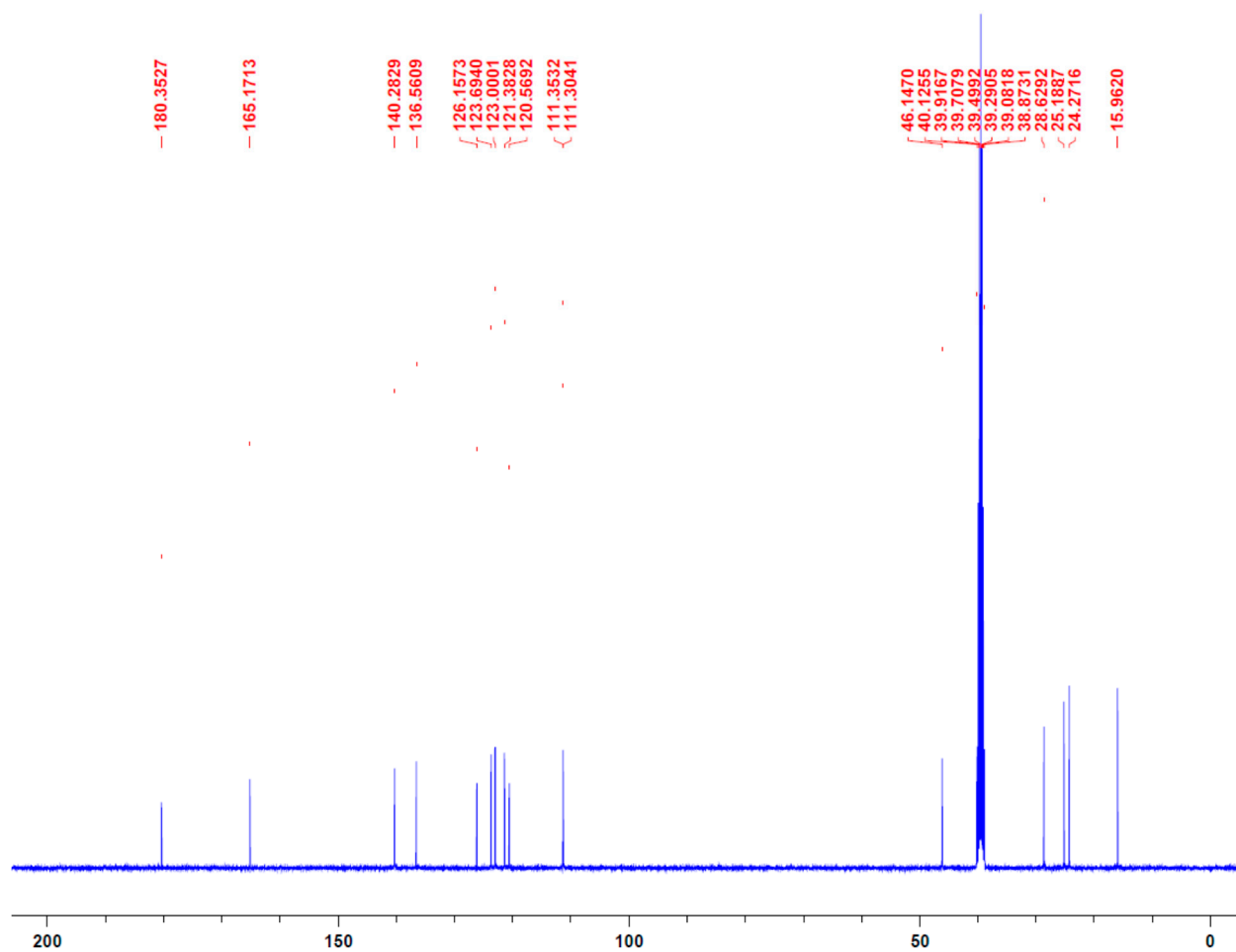


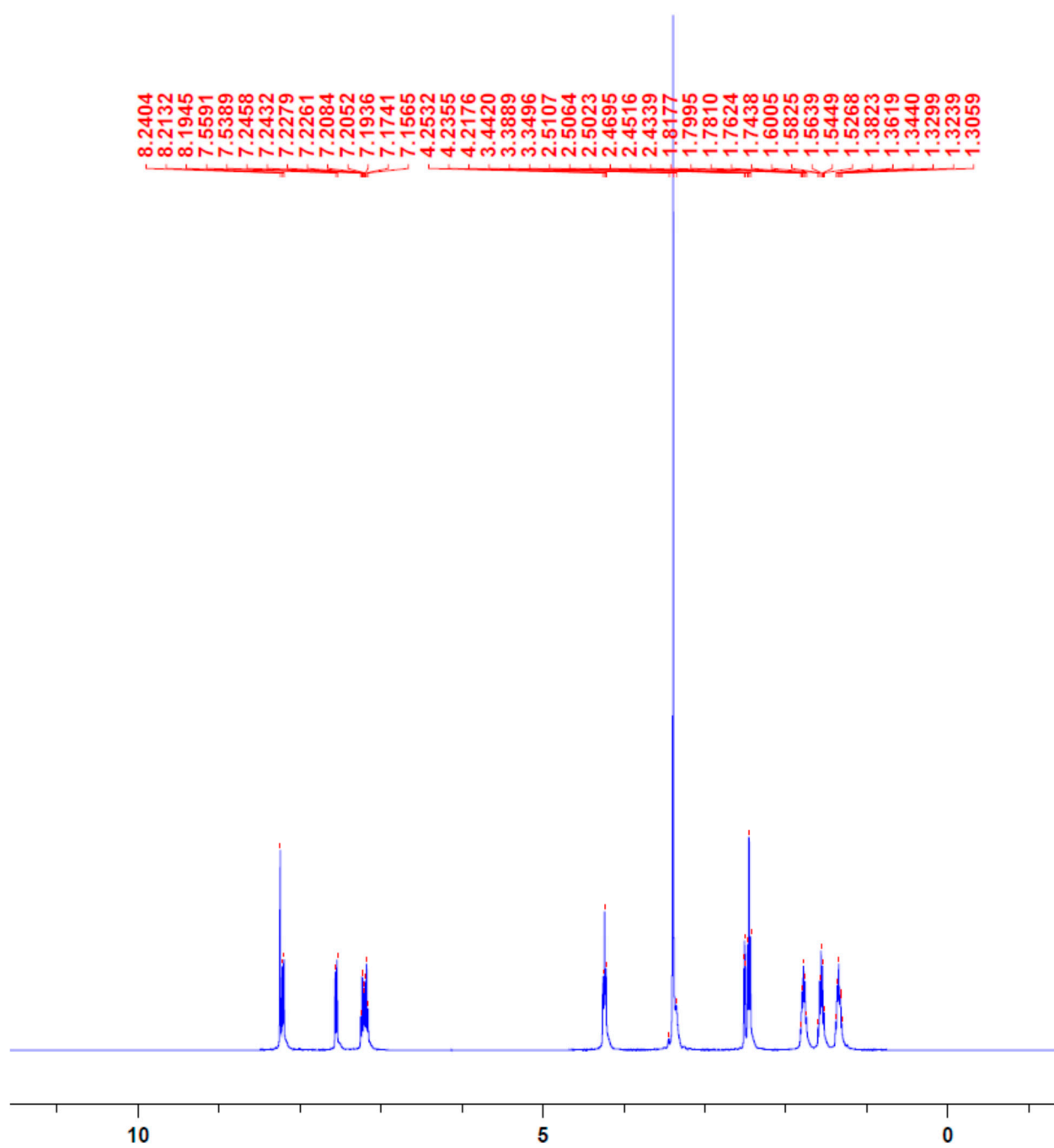
¹³C NMR of **21**

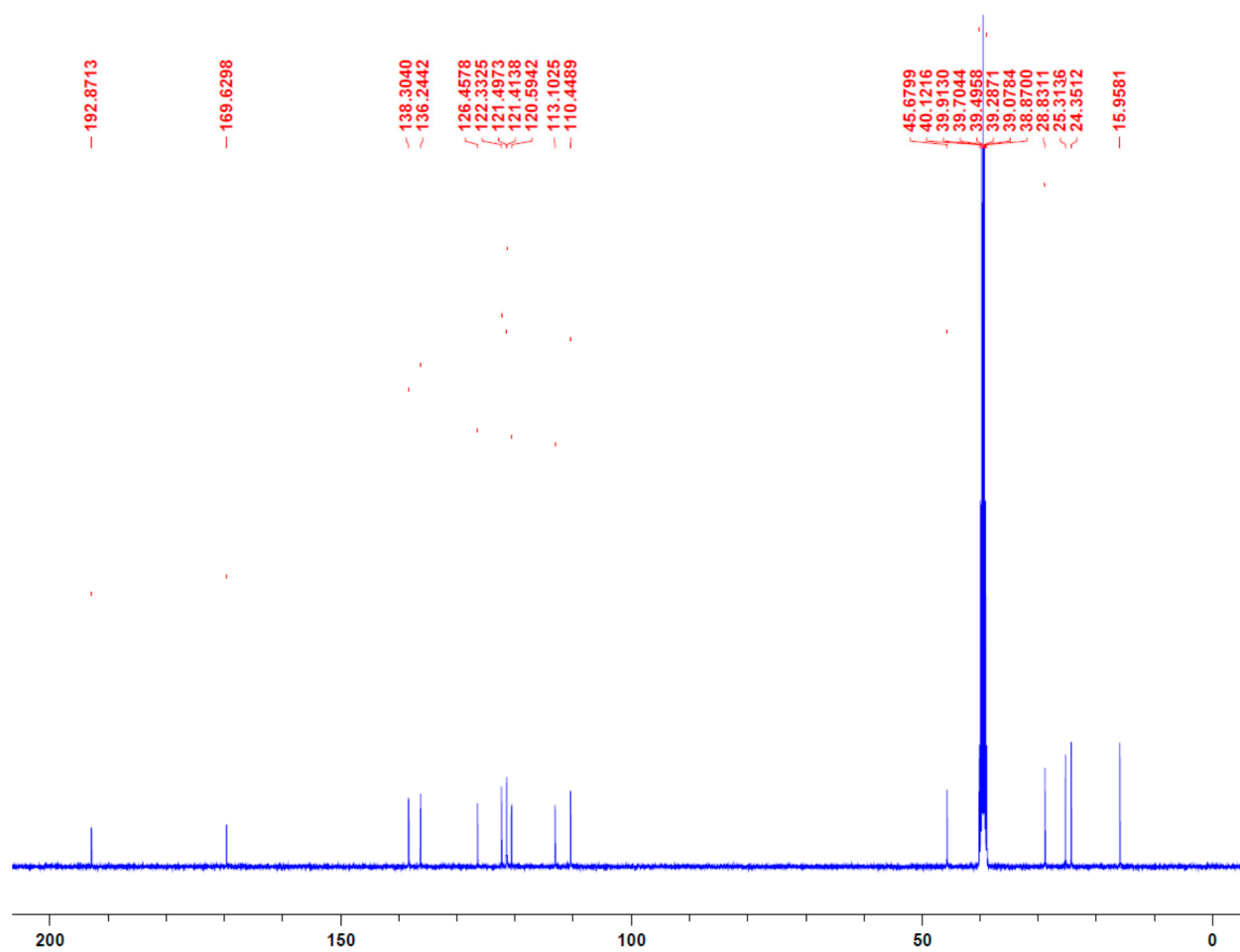
¹H NMR of 22

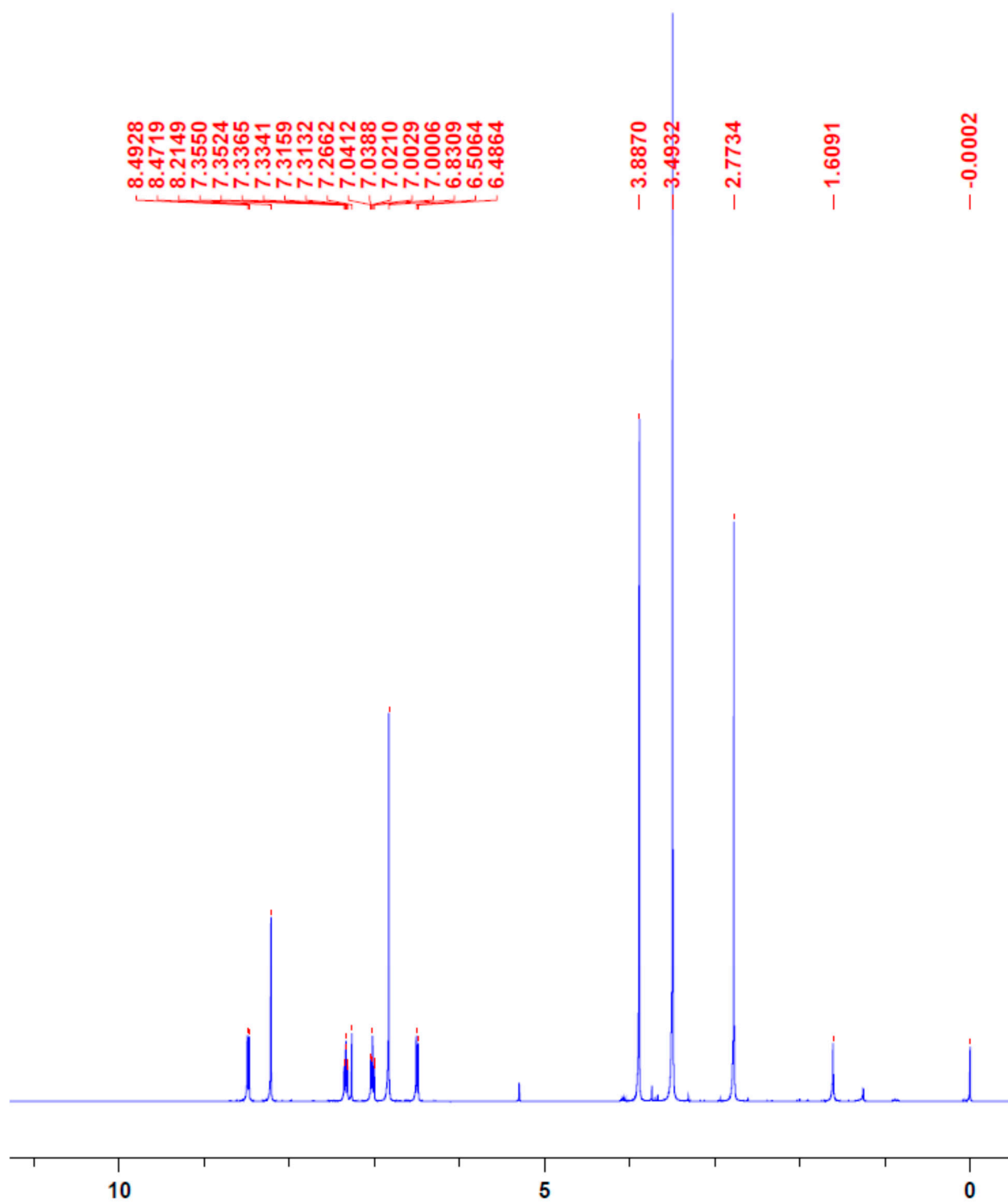


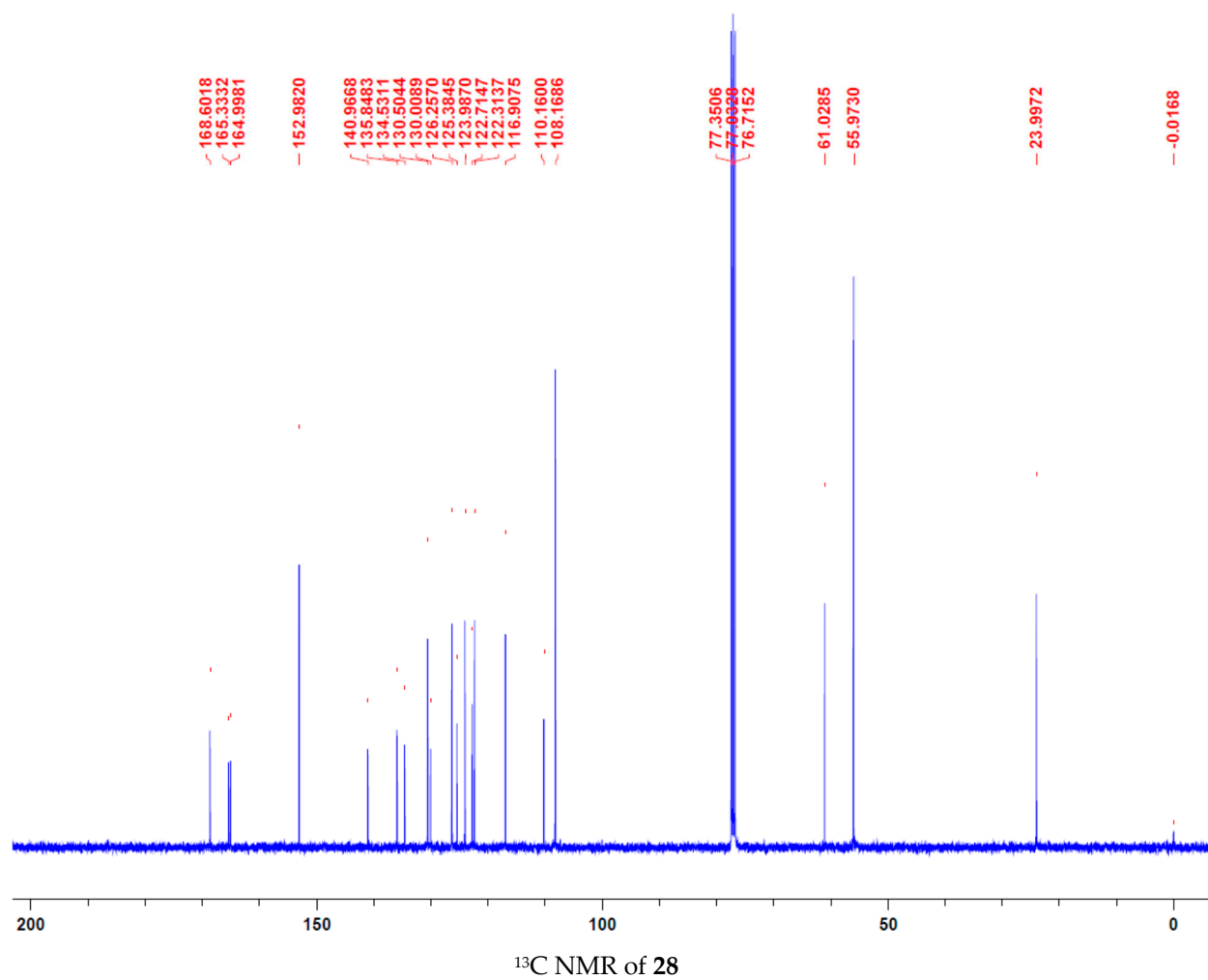
^1H NMR of 23

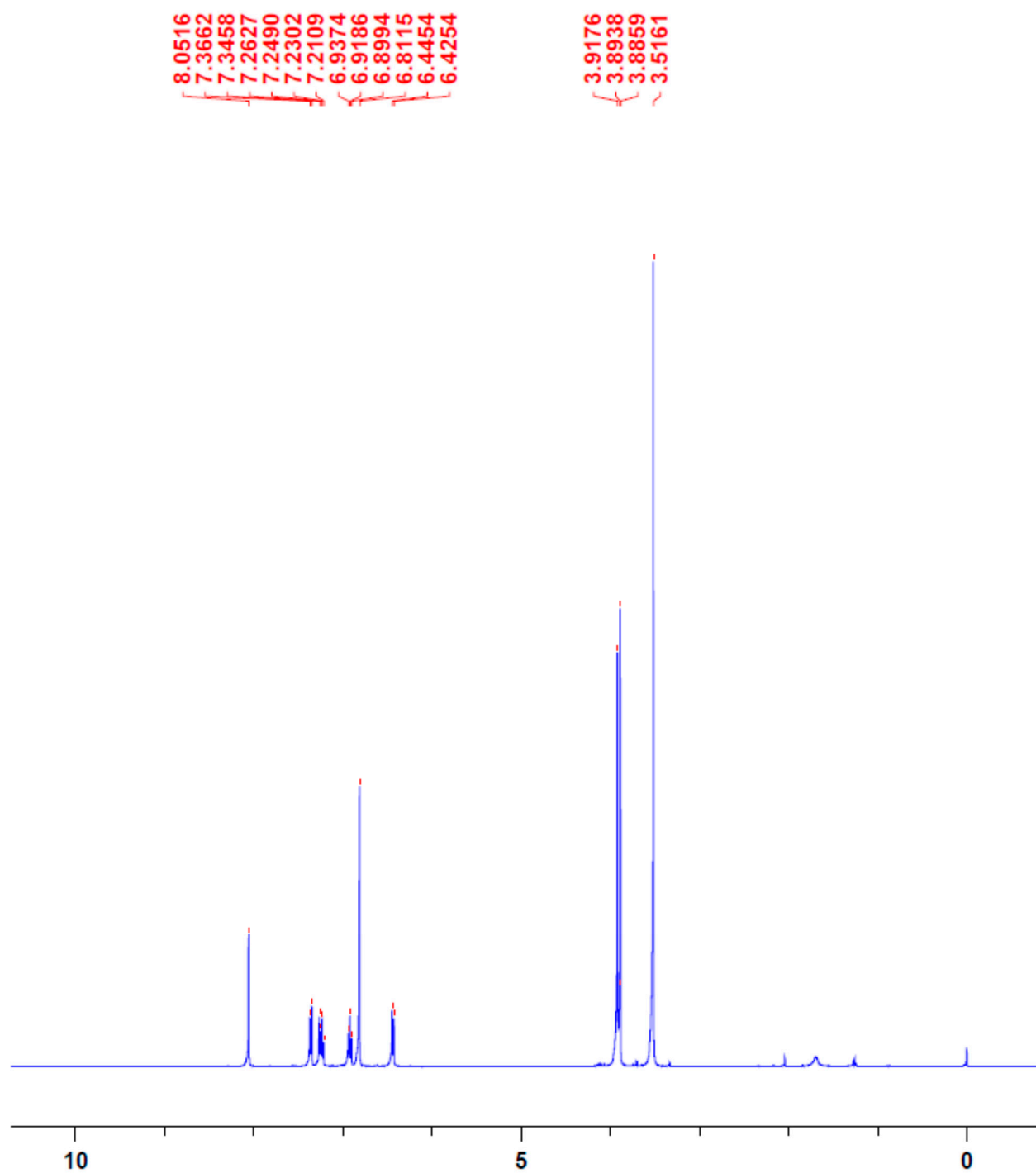
¹³C NMR of **23**

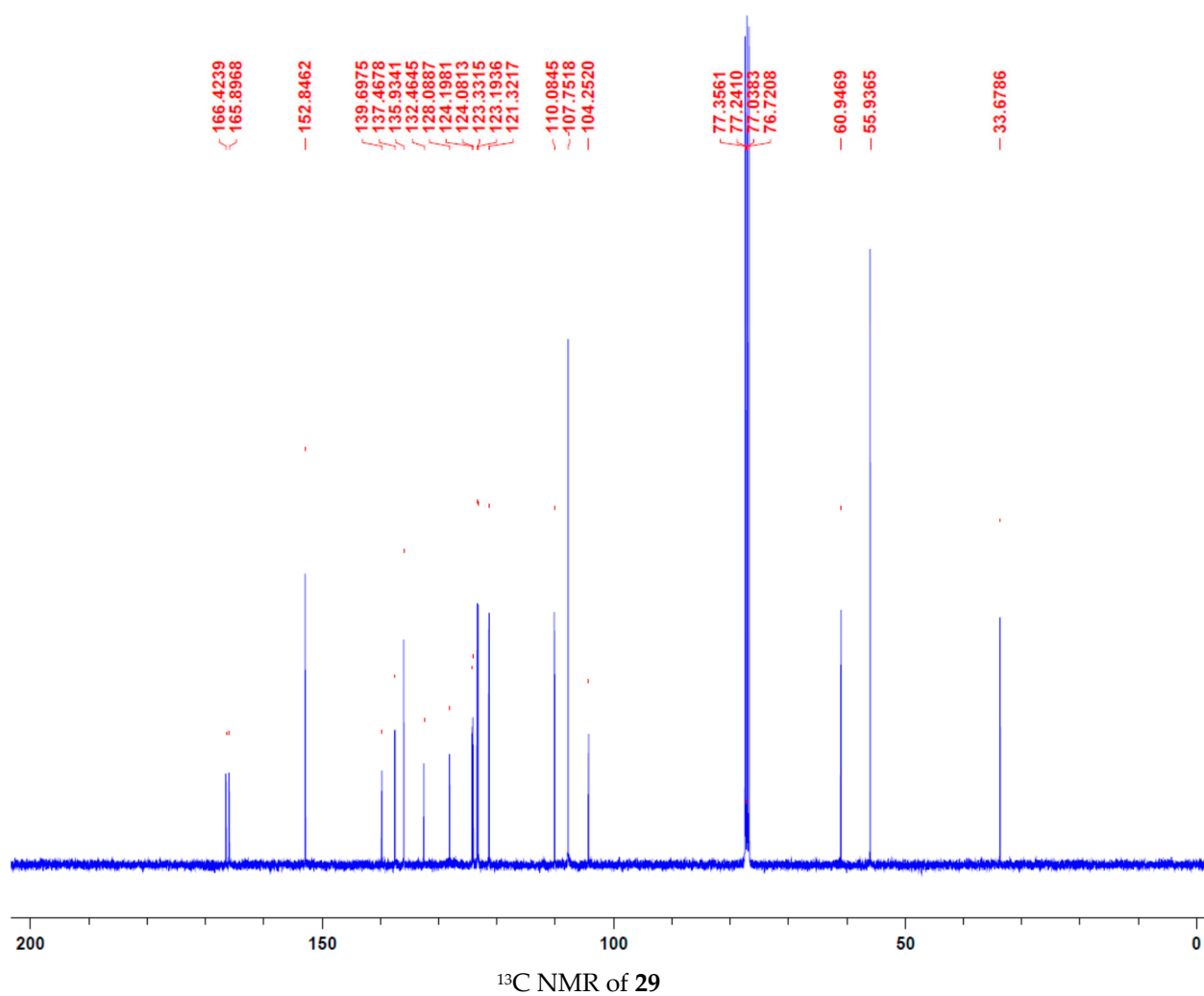
¹H NMR of 24

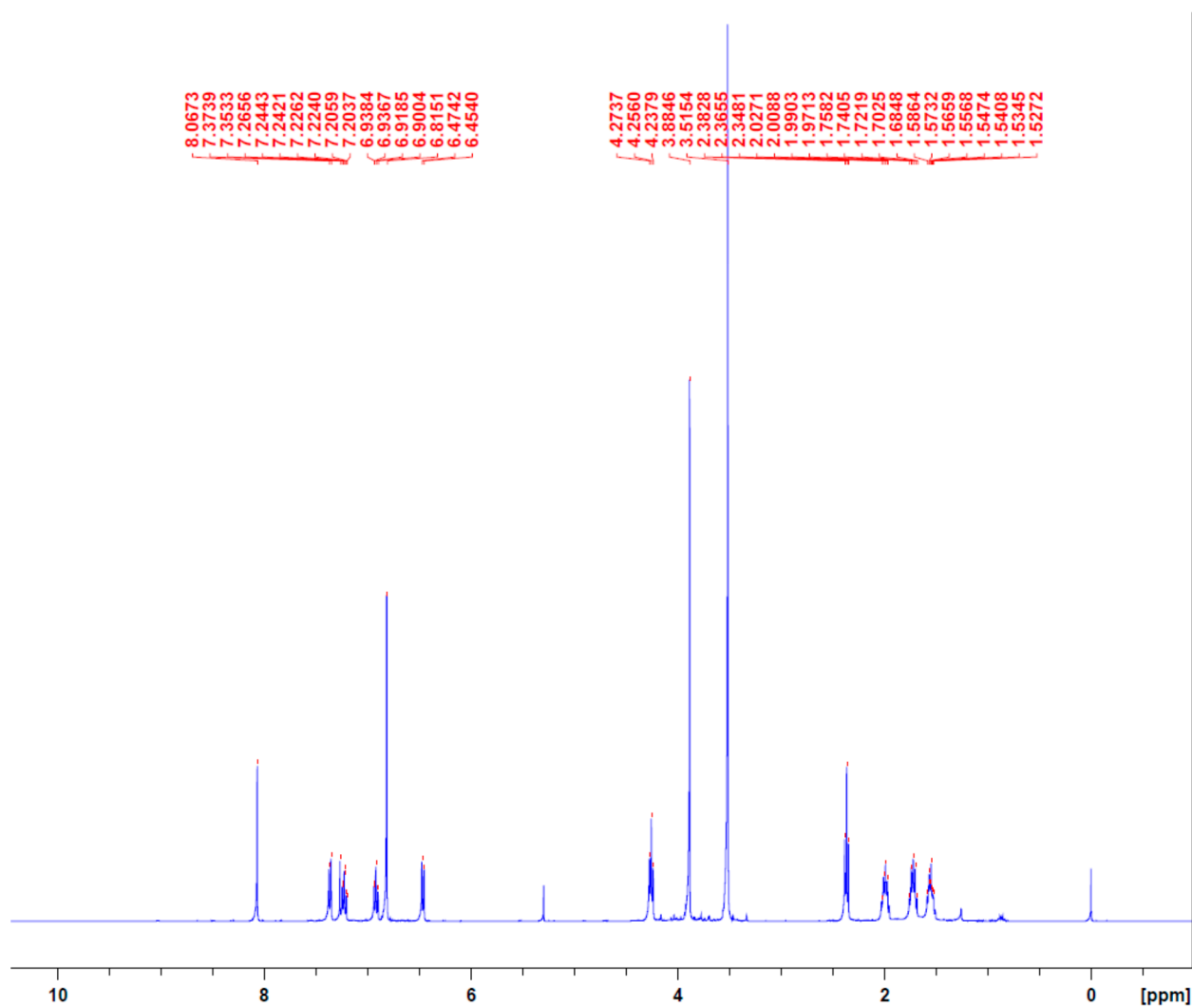
¹³C NMR of 24

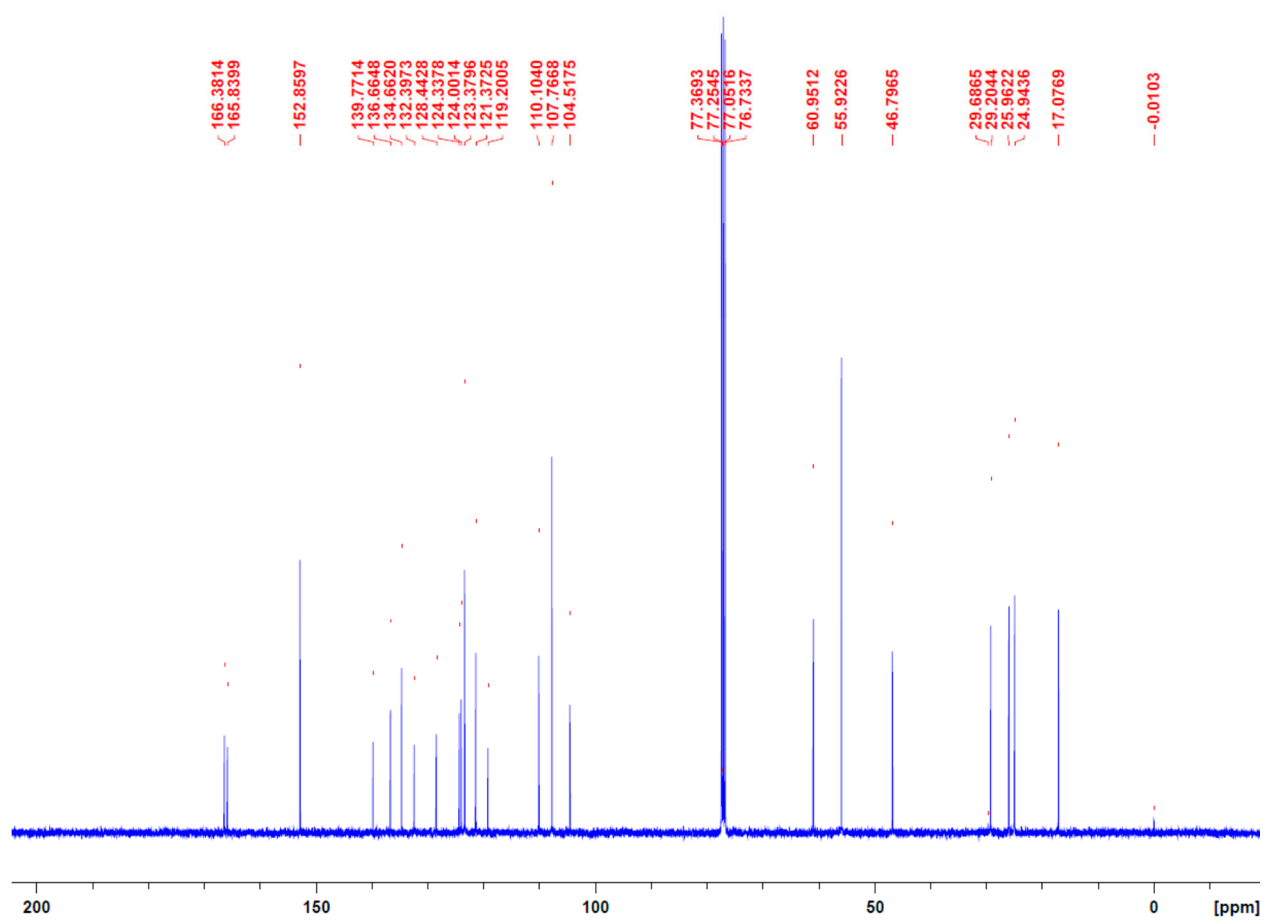
^1H NMR of 28

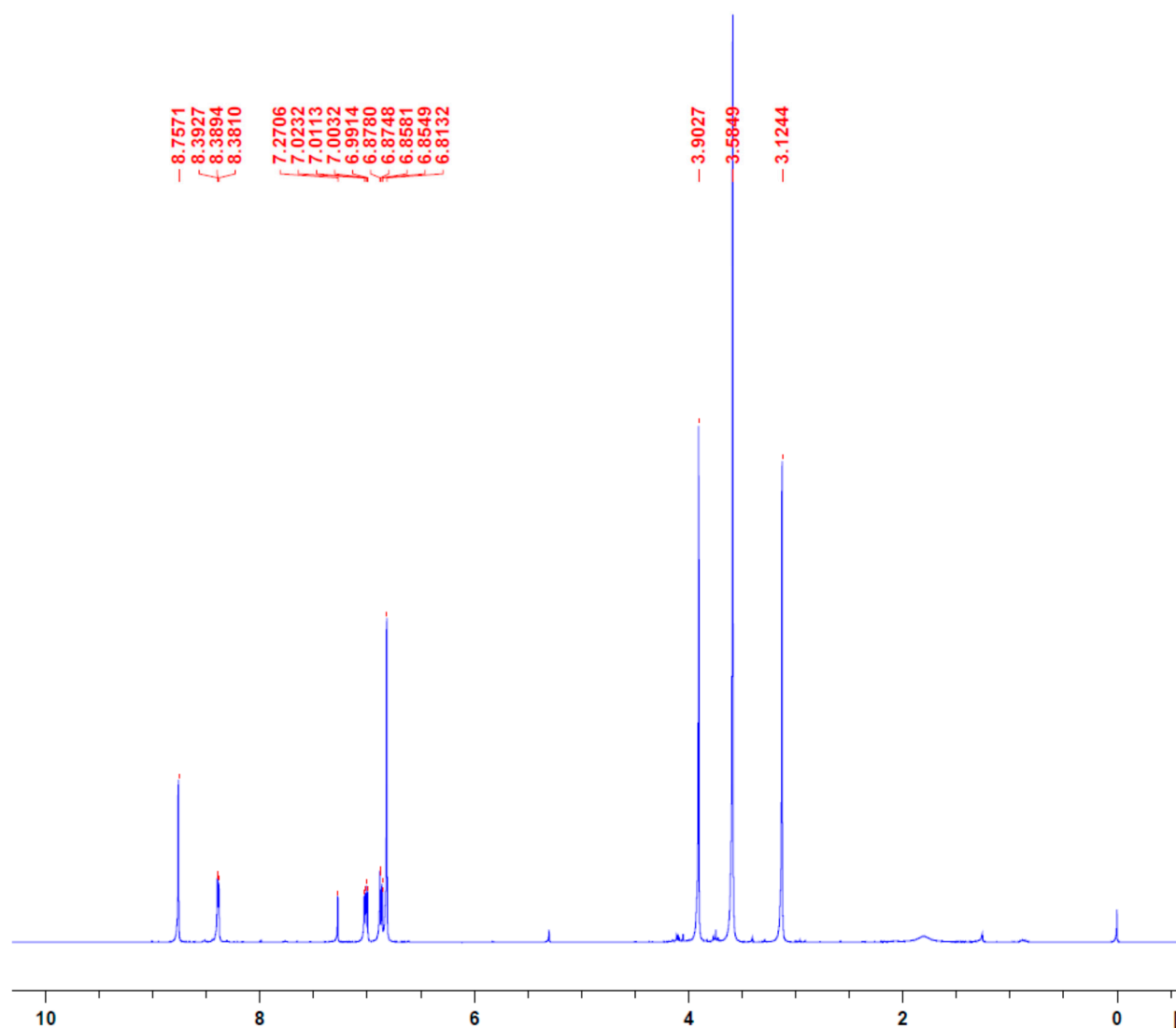


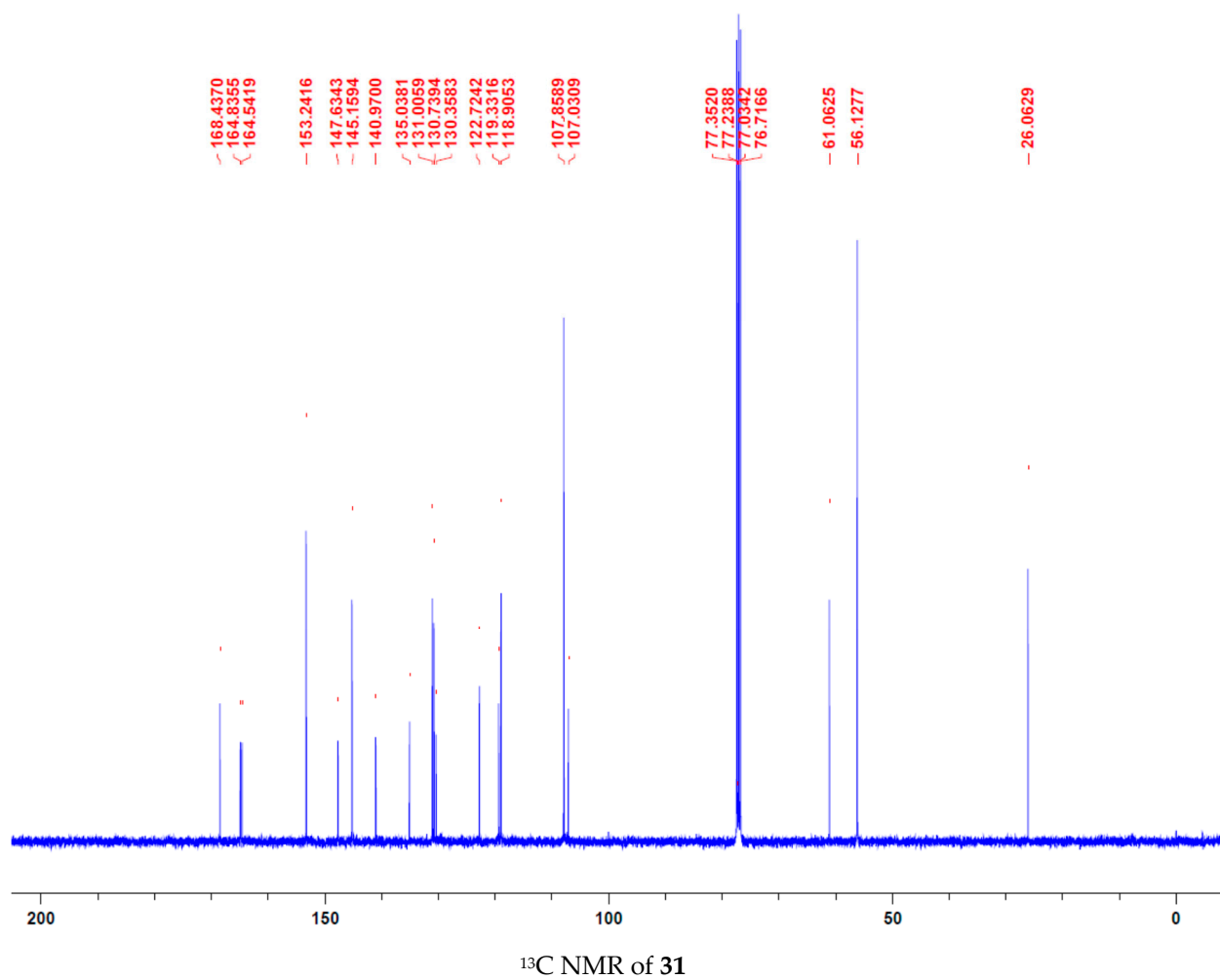
 ^1H NMR of 29

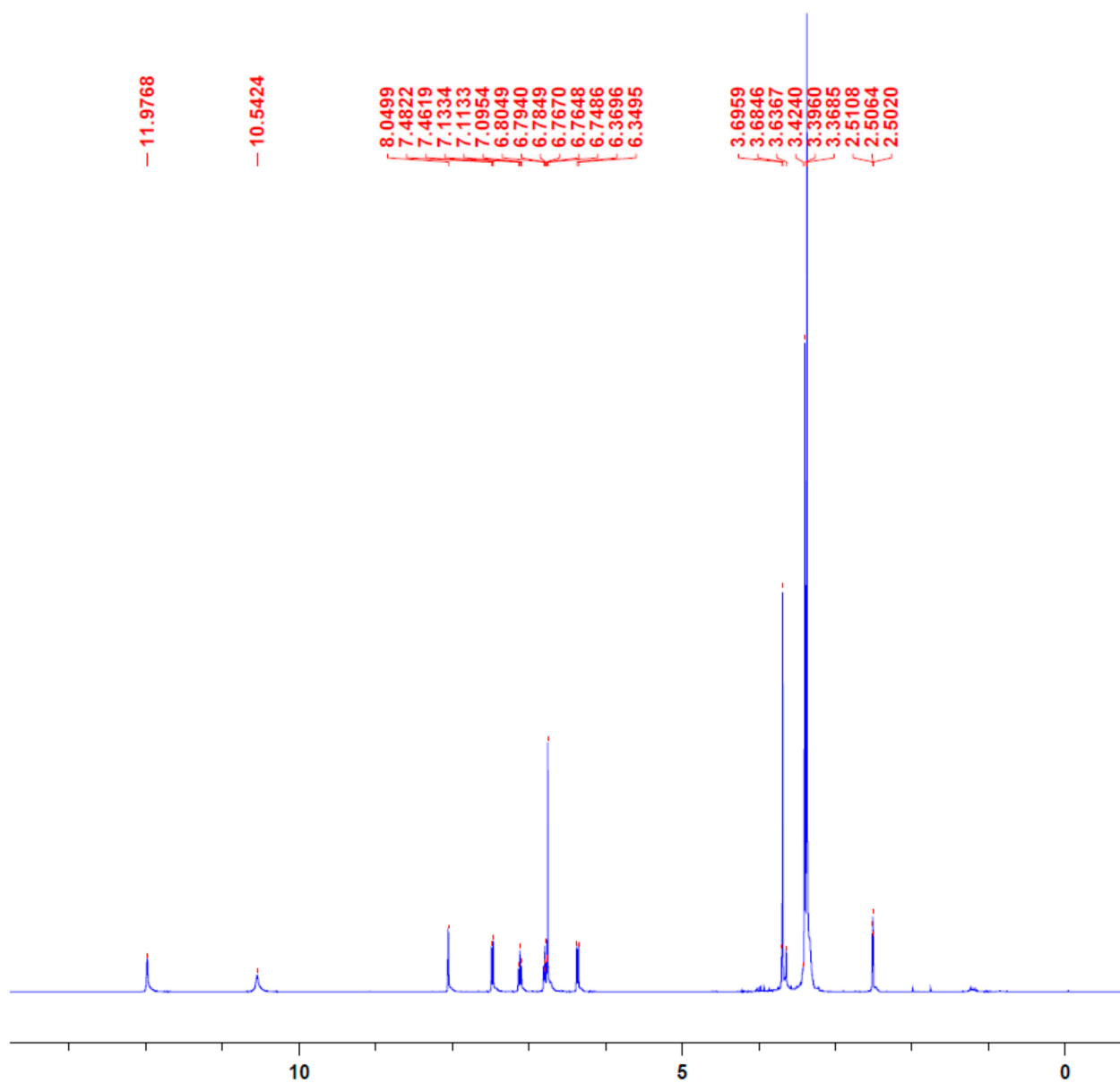


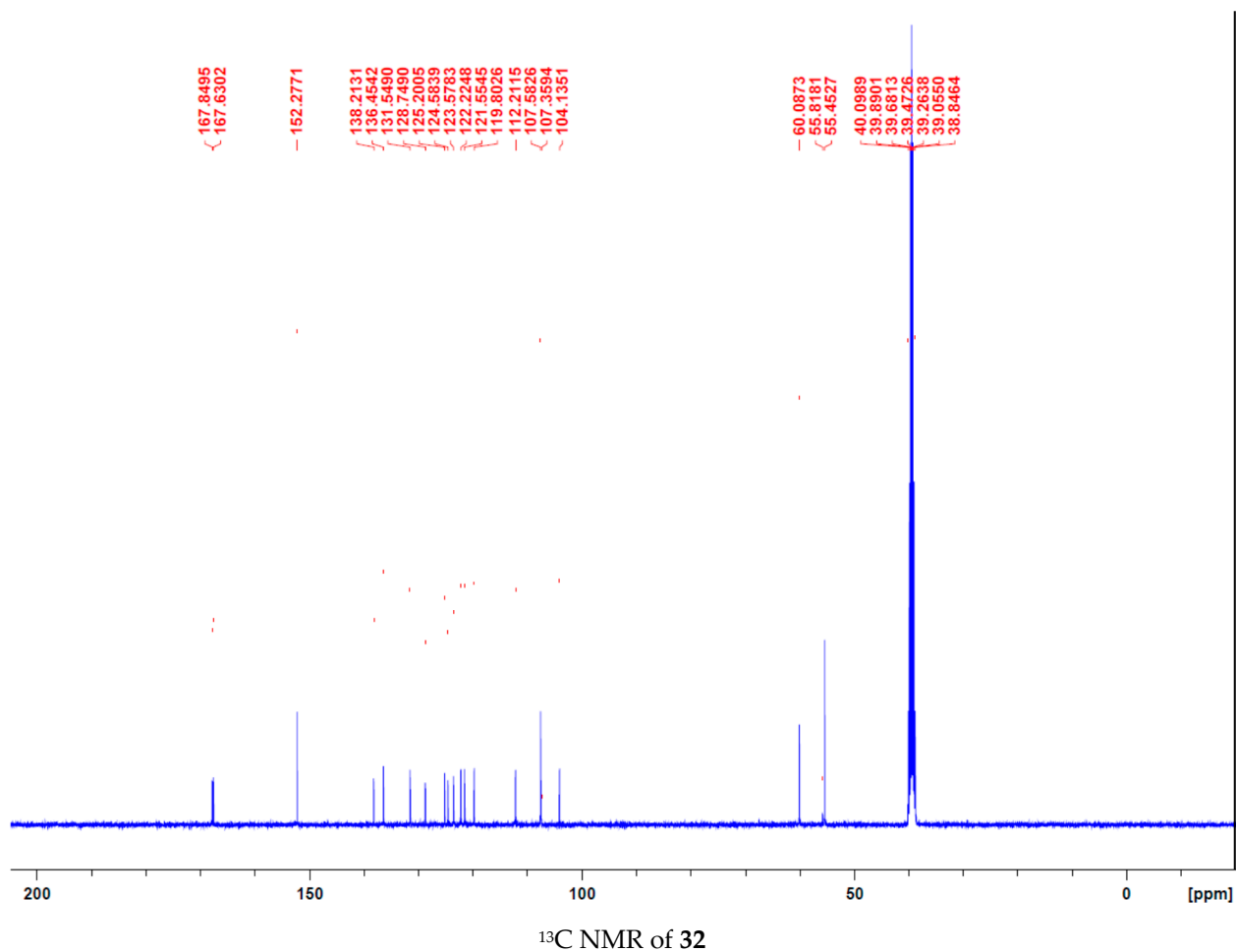
¹H NMR of 30

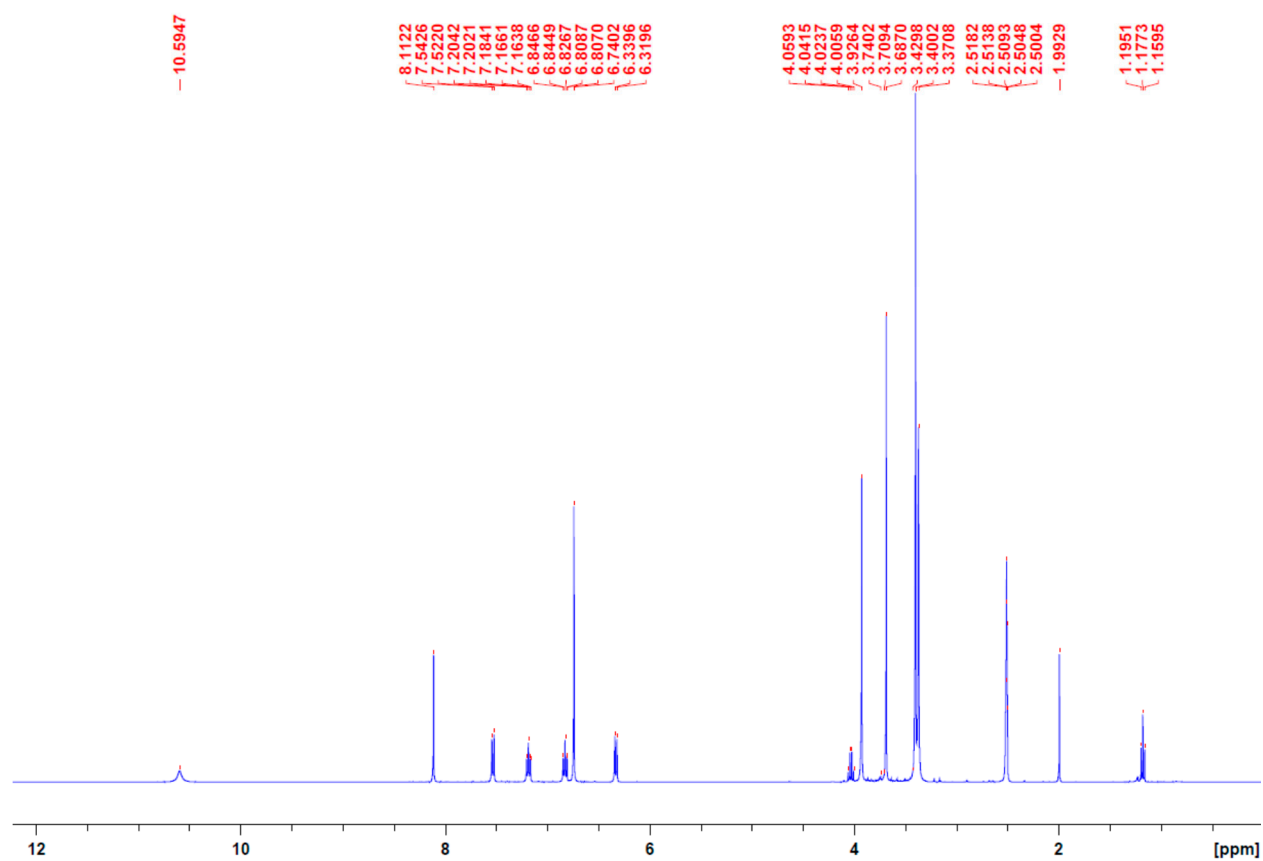
¹³C NMR of 30

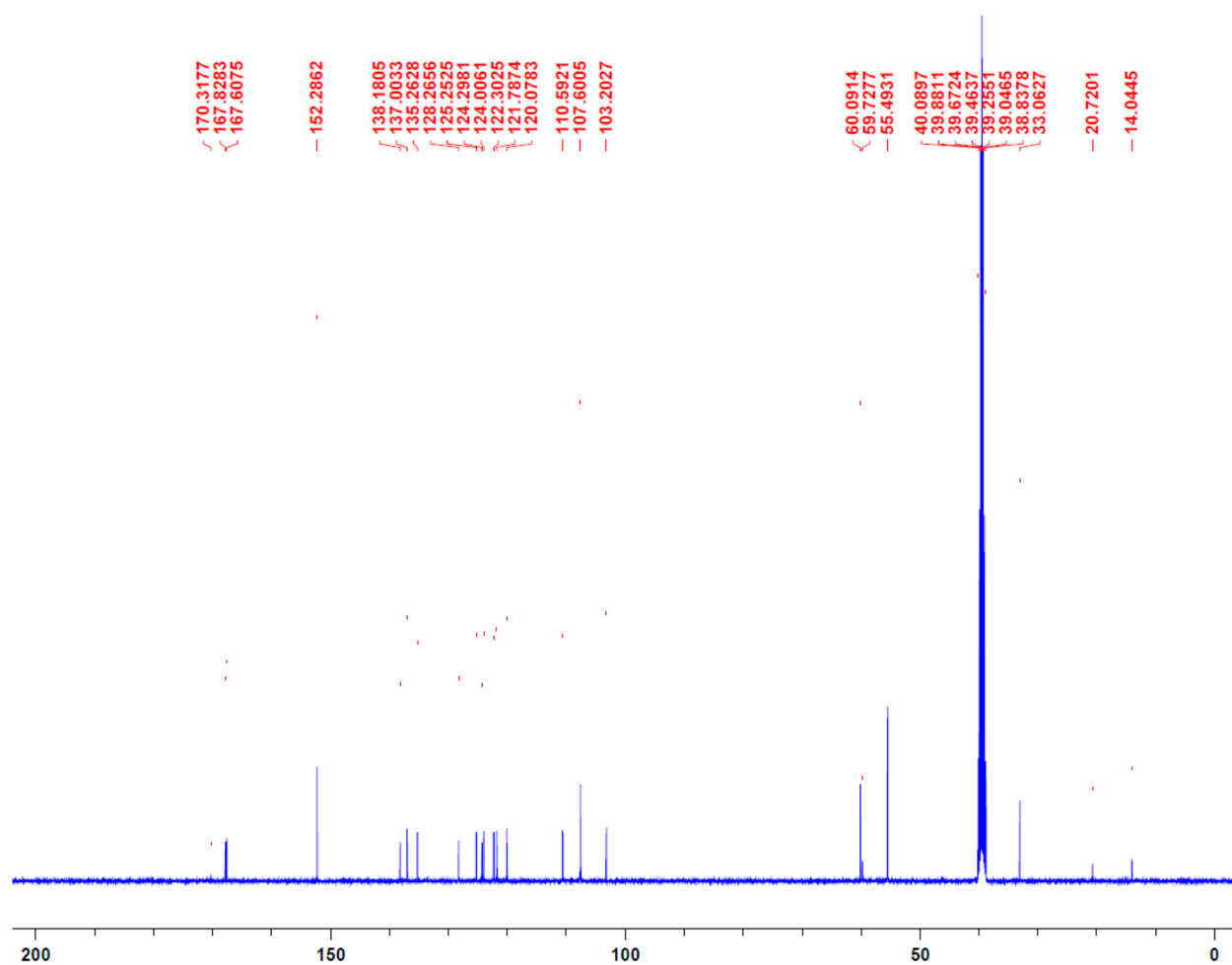
 ^1H NMR of **31**

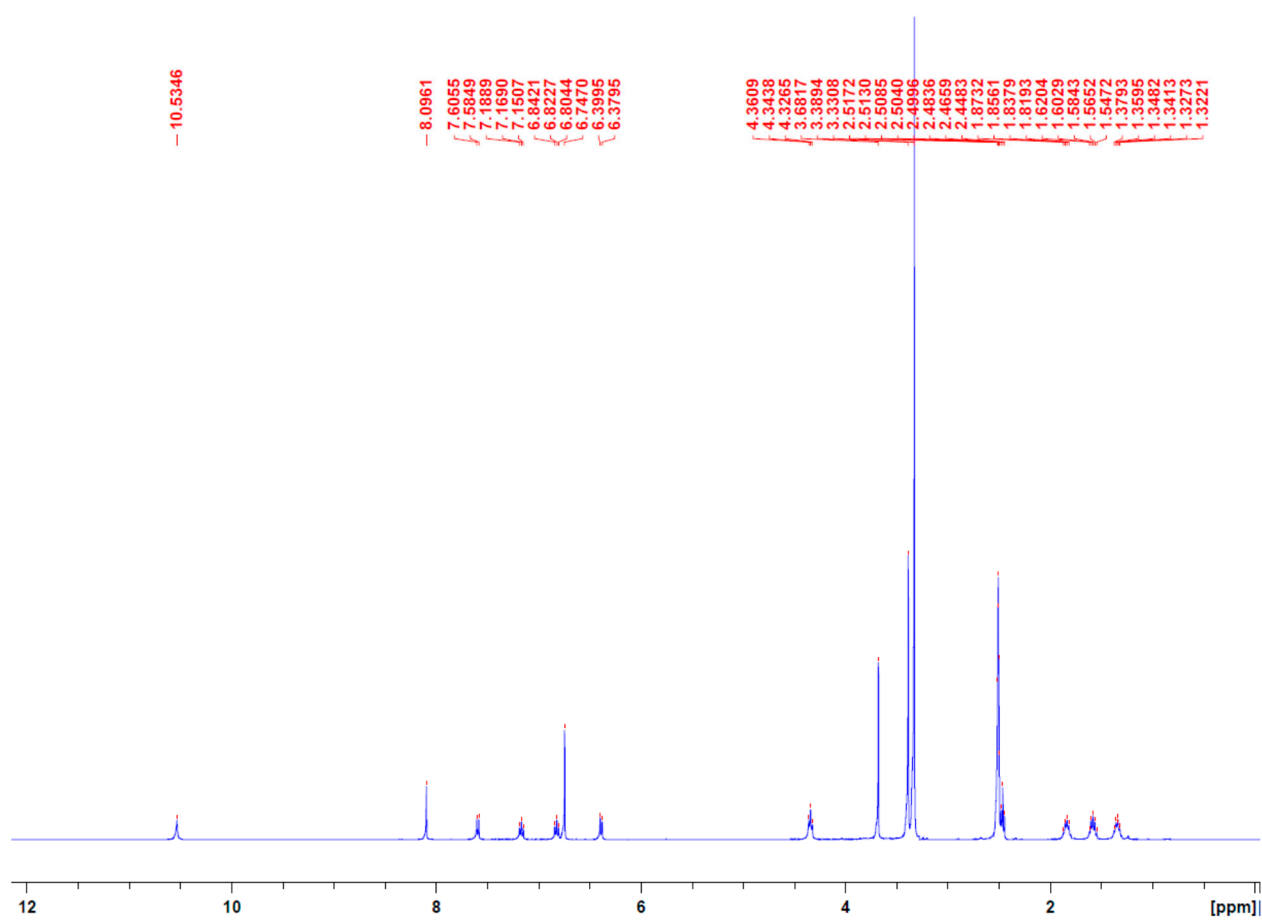


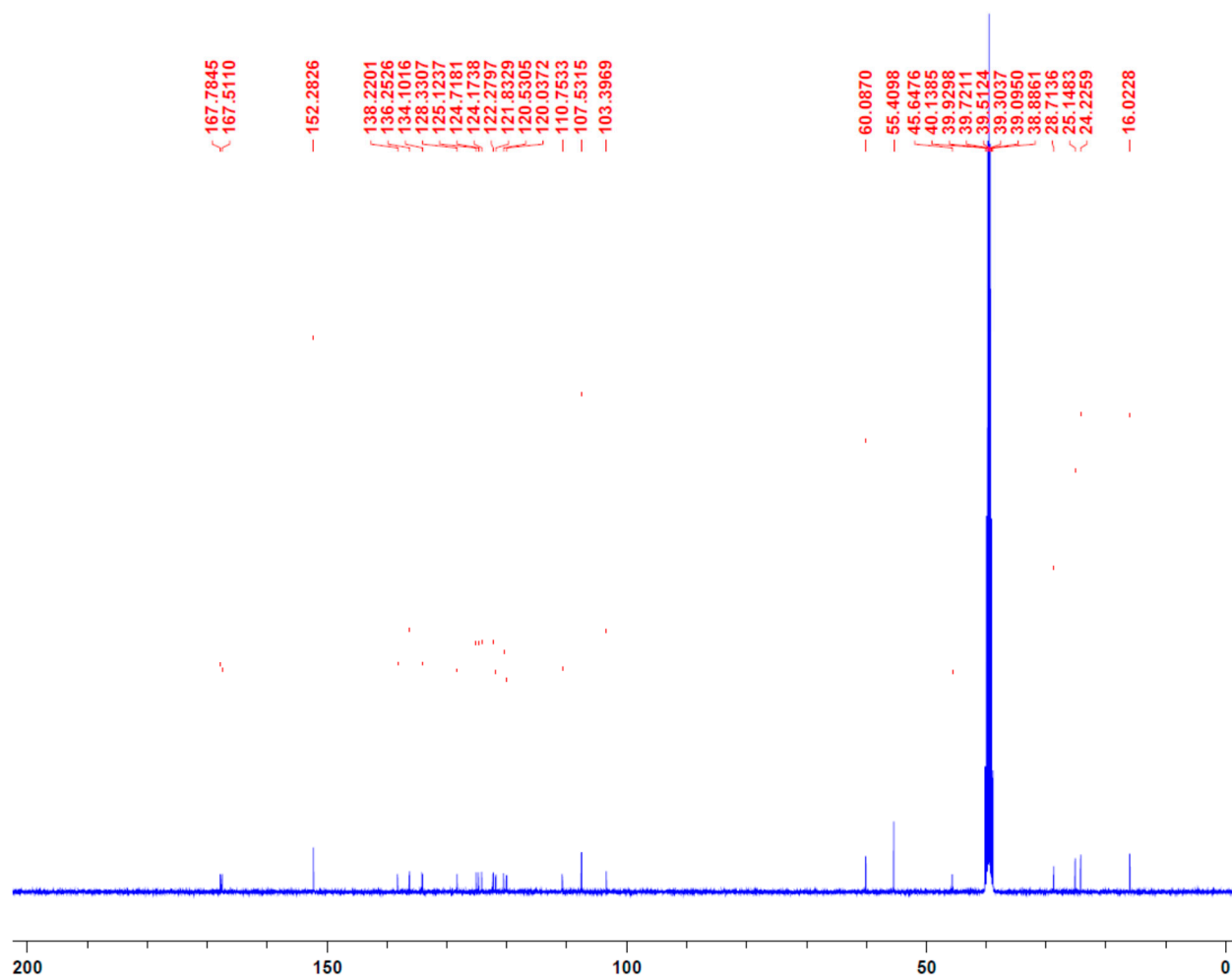
¹H NMR of 32

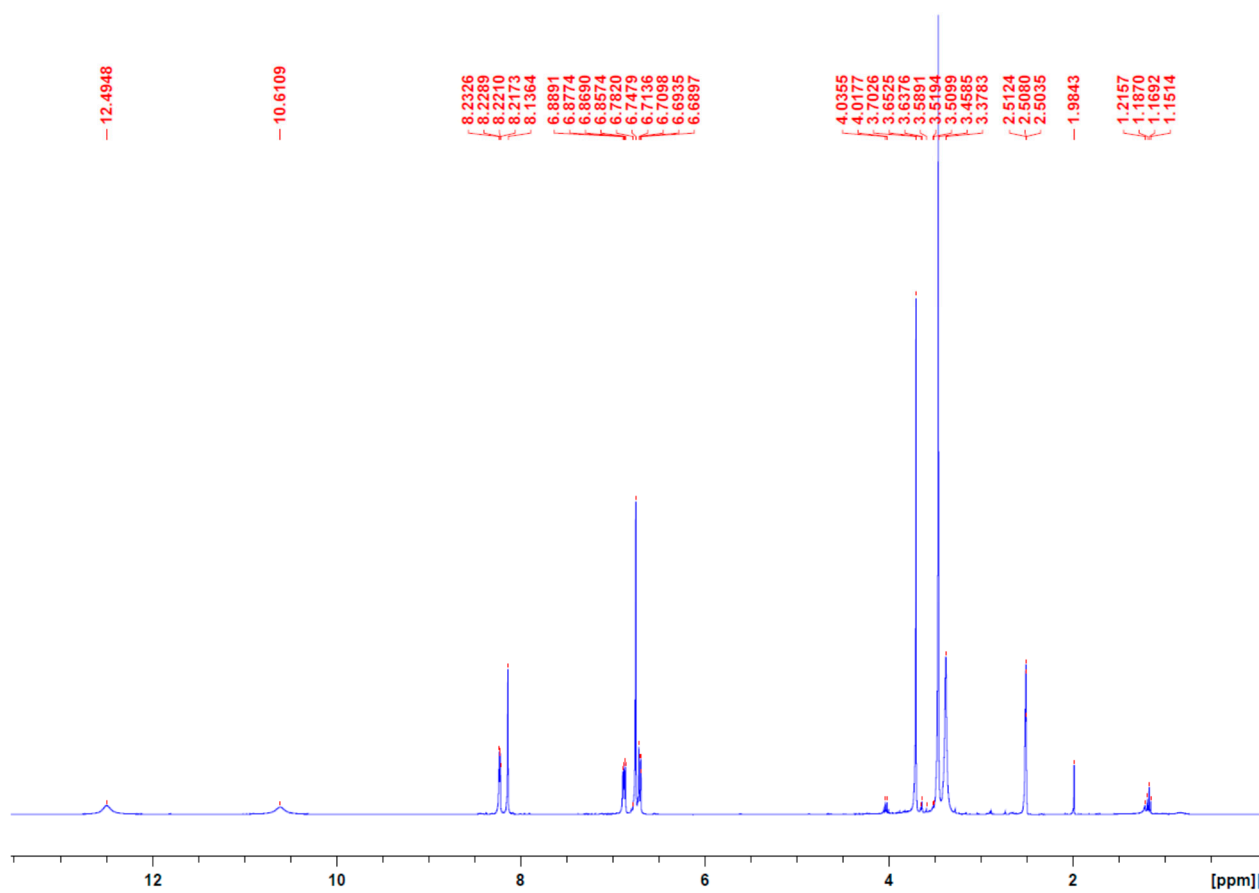


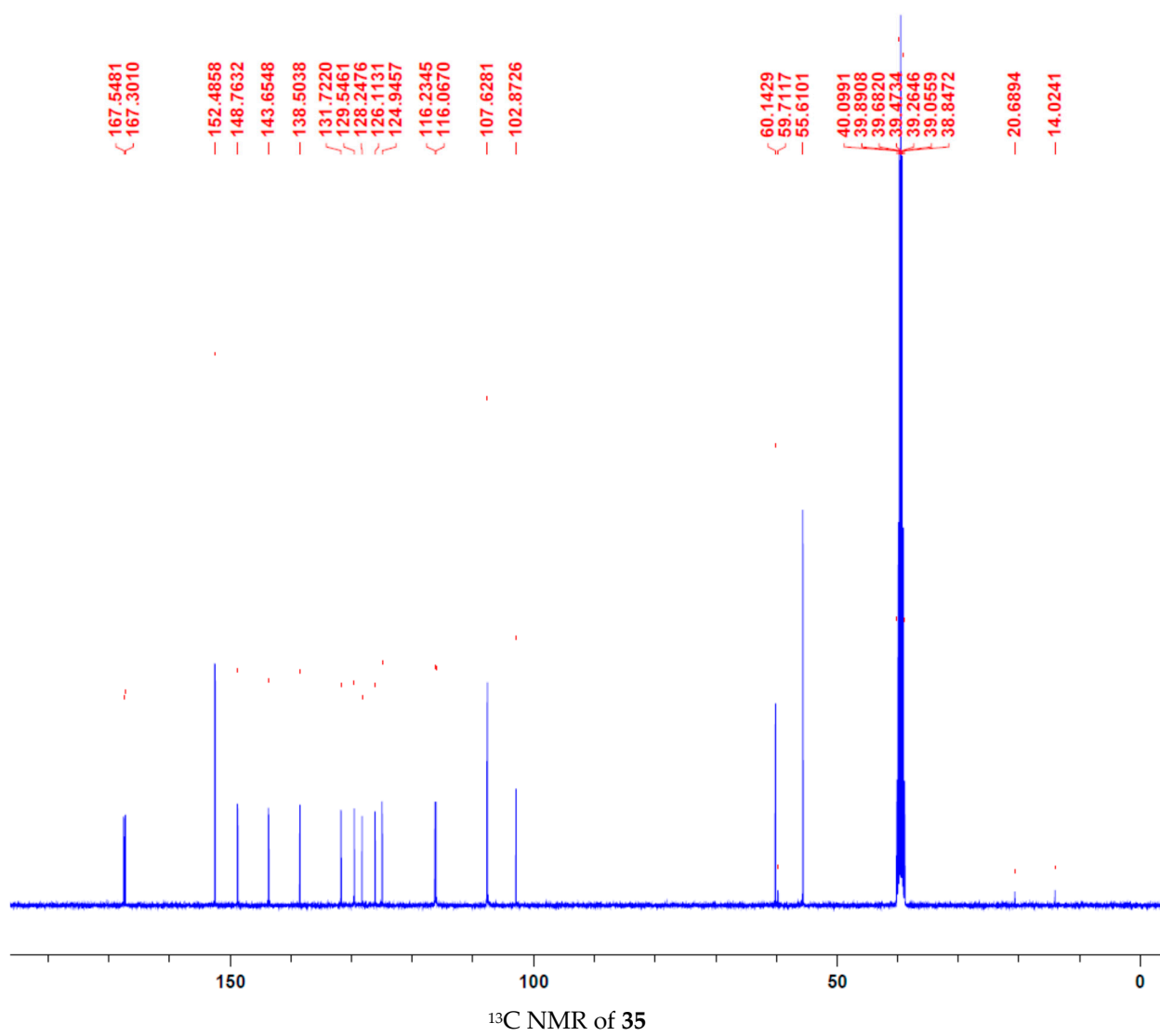
¹H NMR of 33

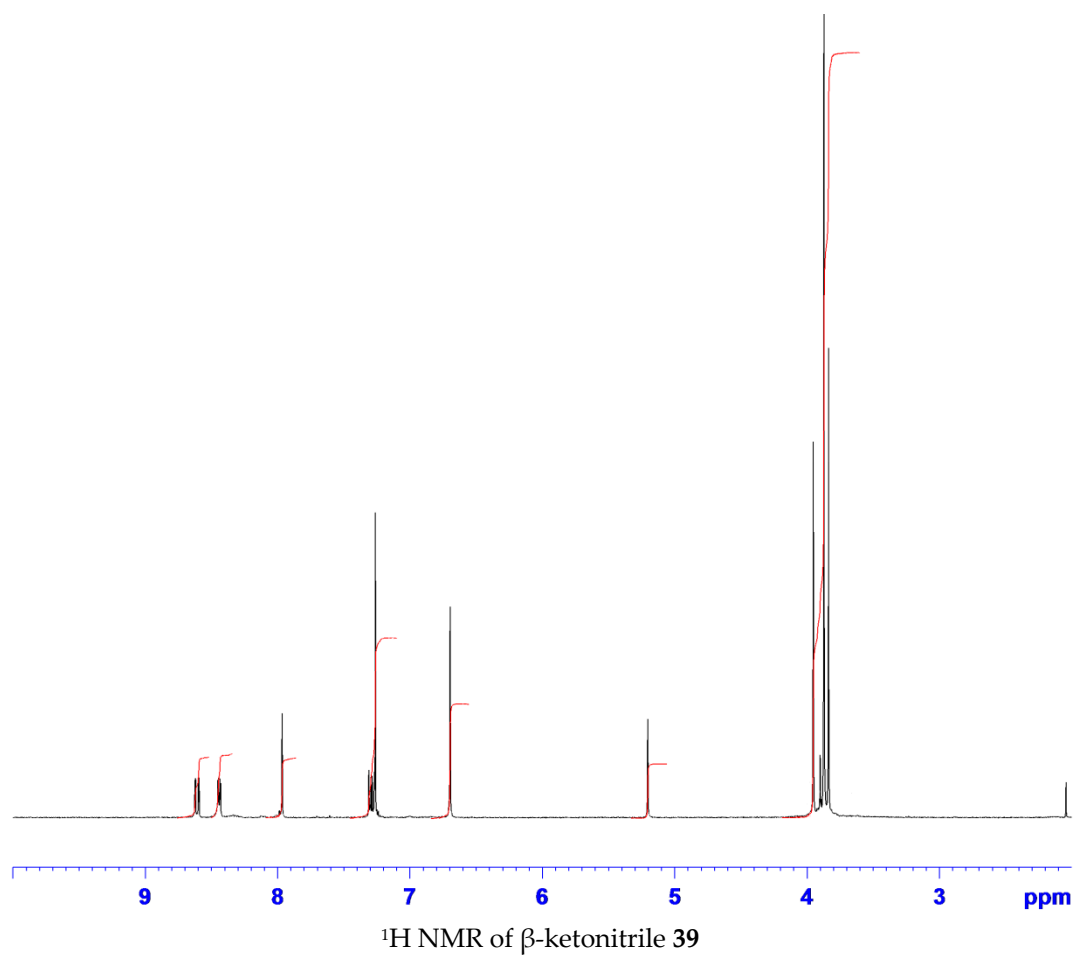
¹³C NMR of 33

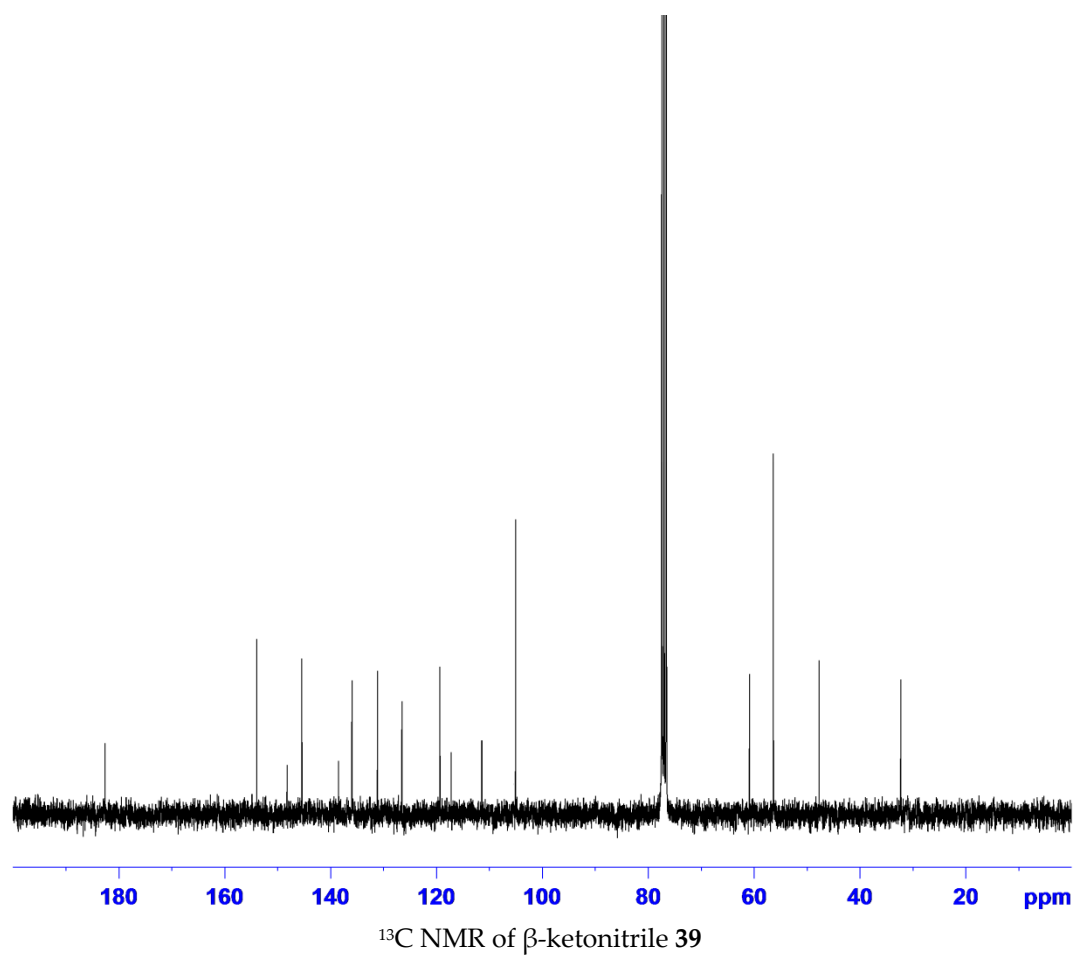
¹H NMR of 34

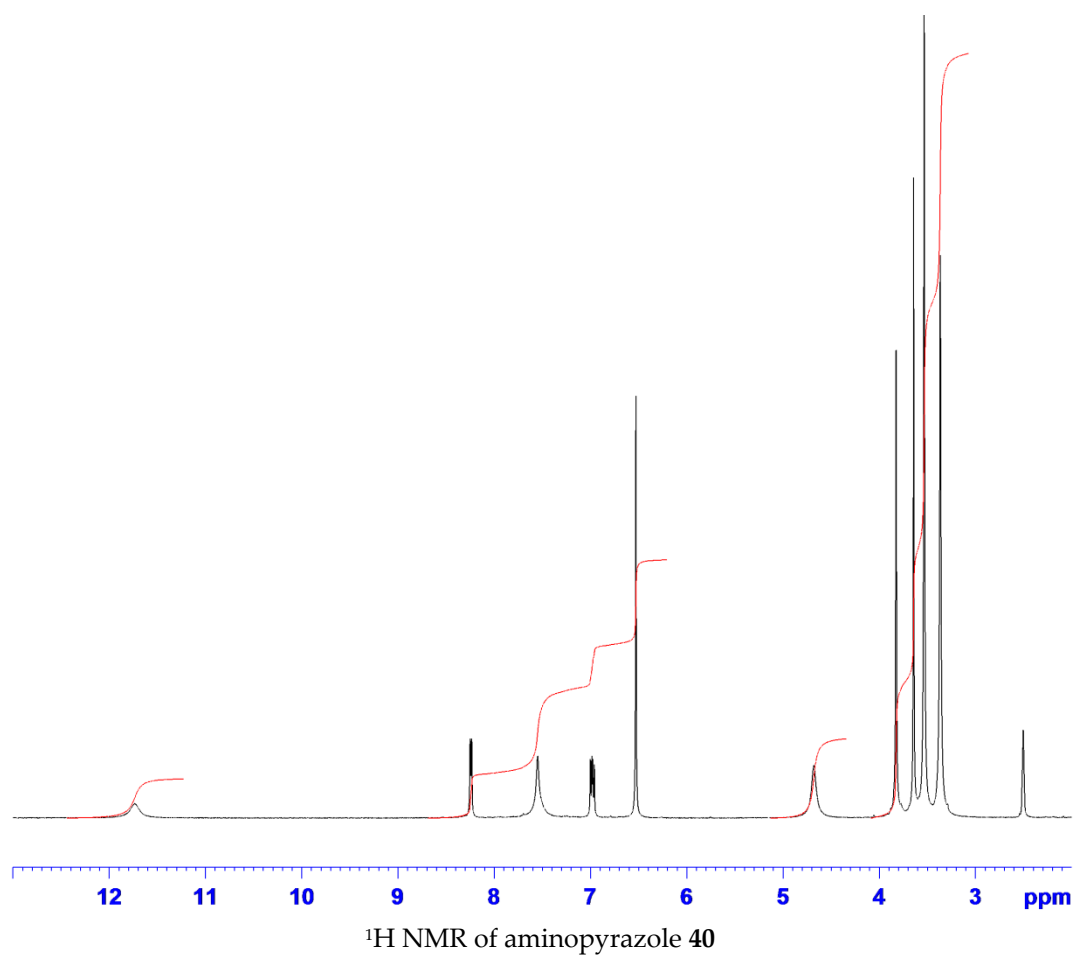
 ^{13}C NMR of **34**

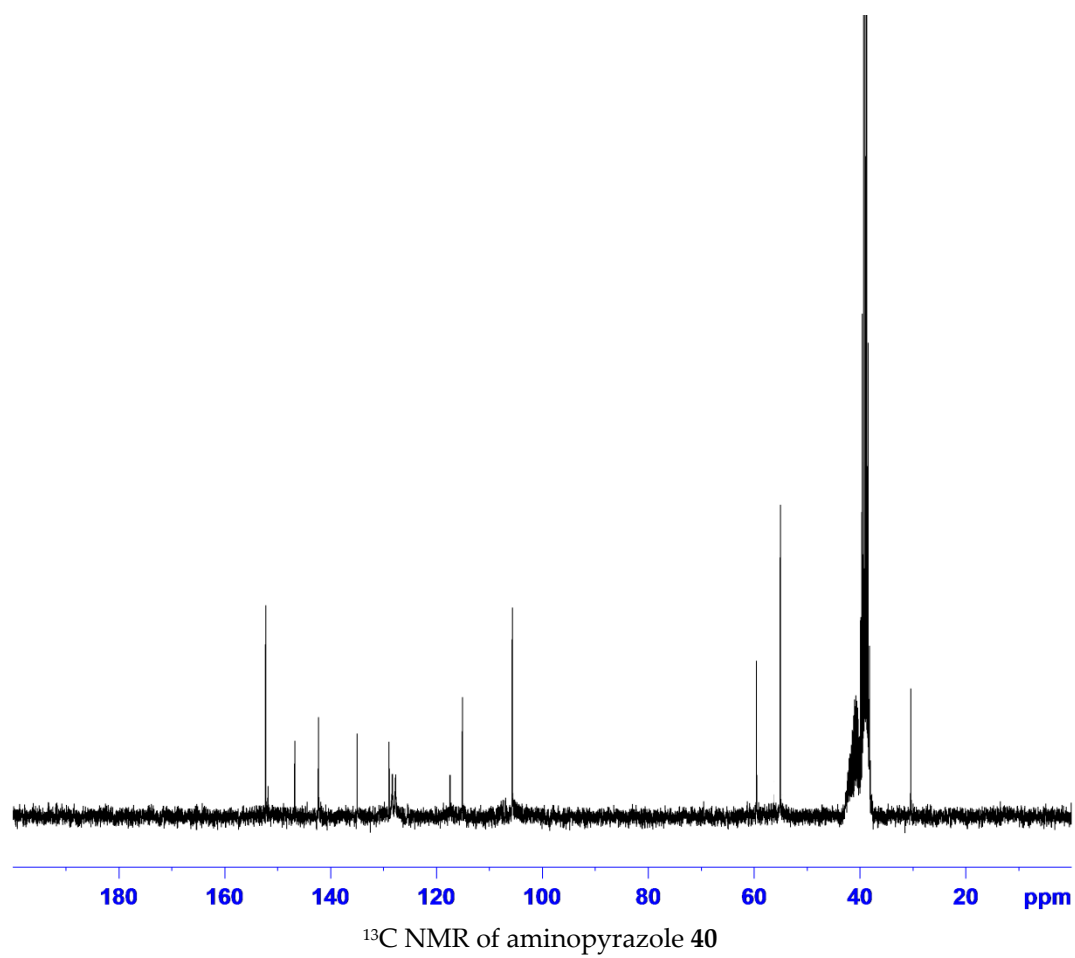
 ^1H NMR of 35

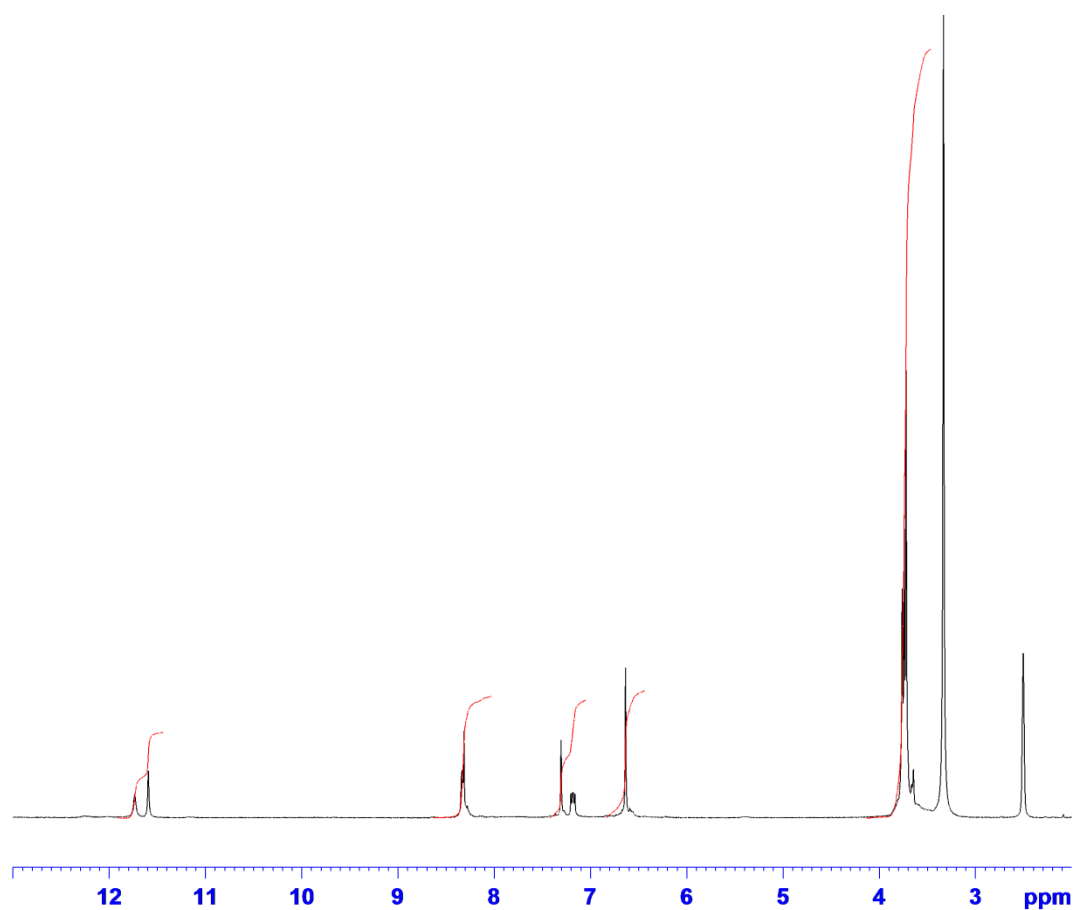


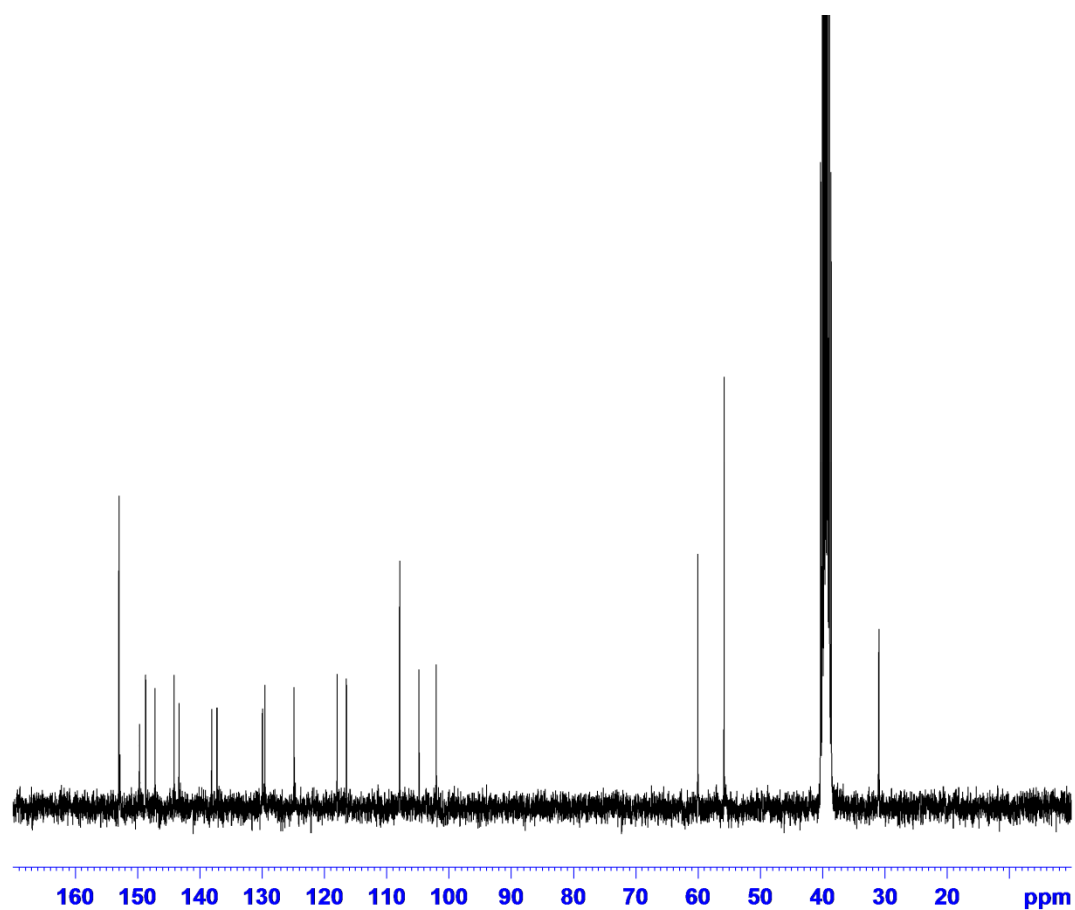


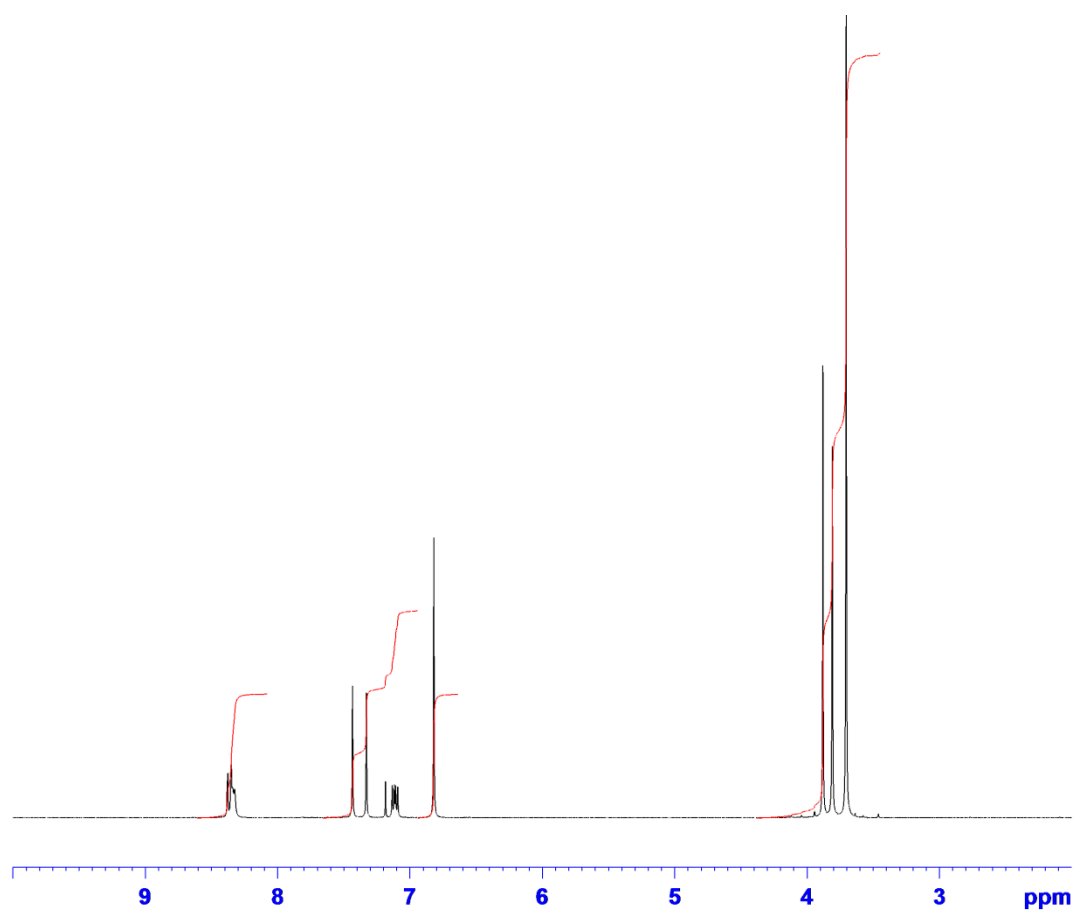


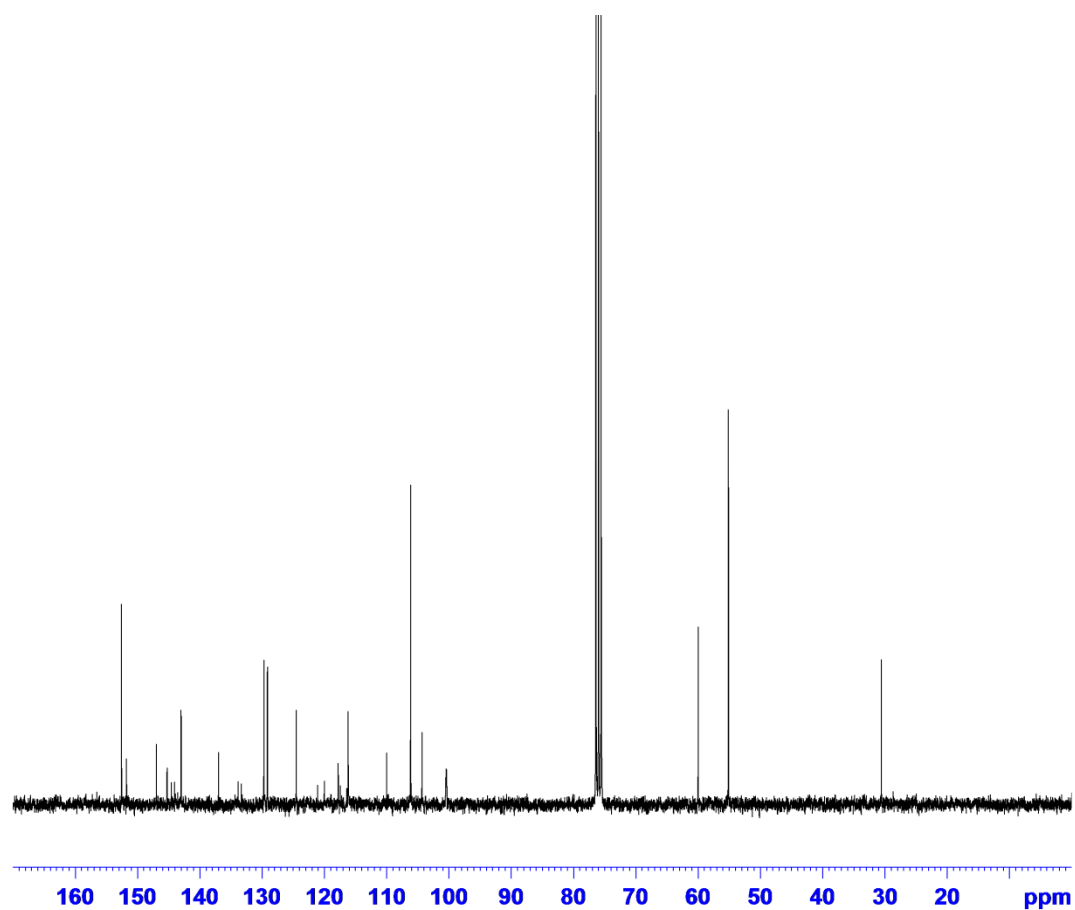




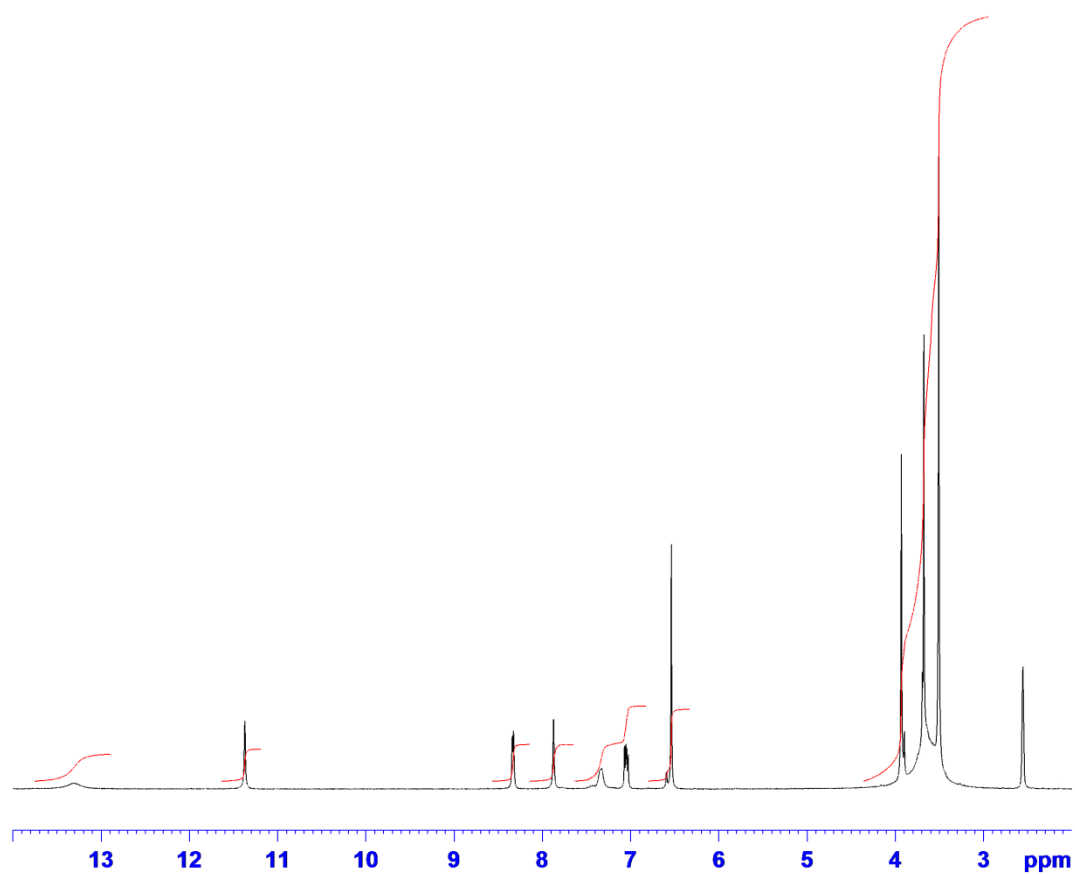
 ^1H NMR of triazinedione 41

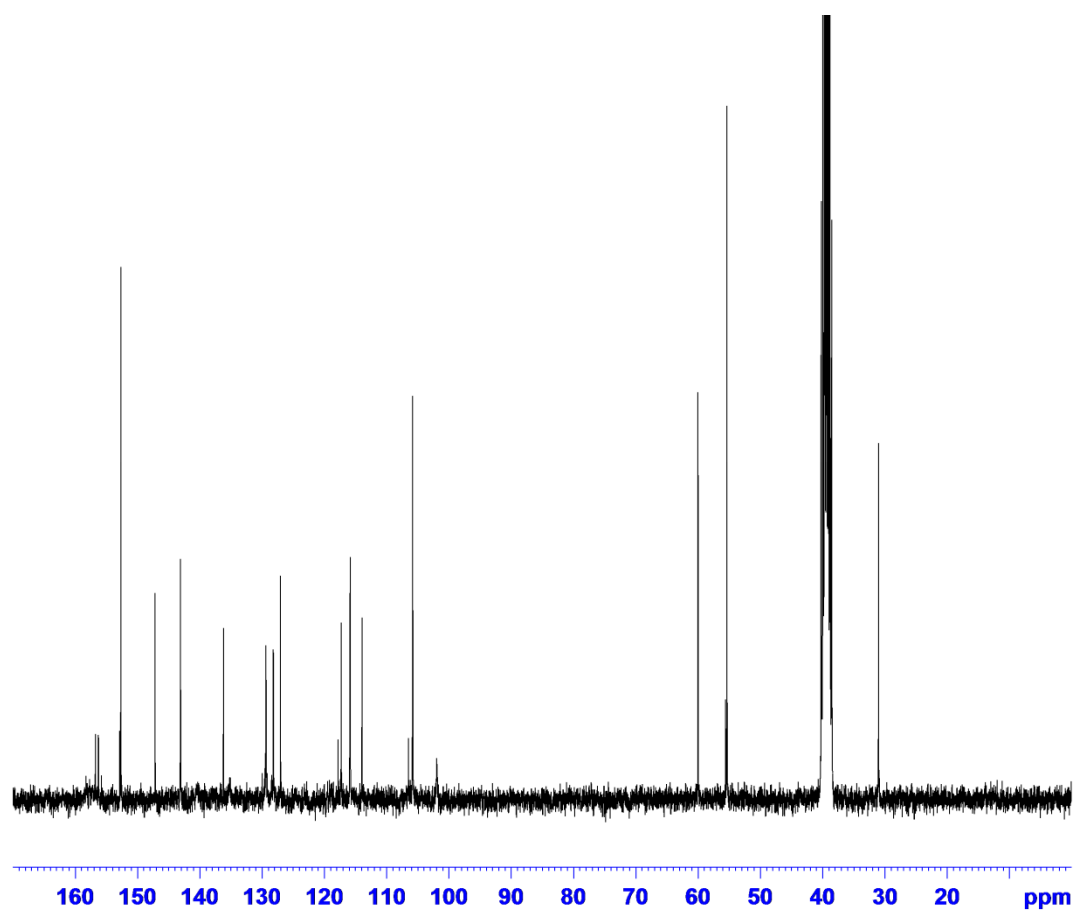
 ^{13}C NMR of triazinedione 41

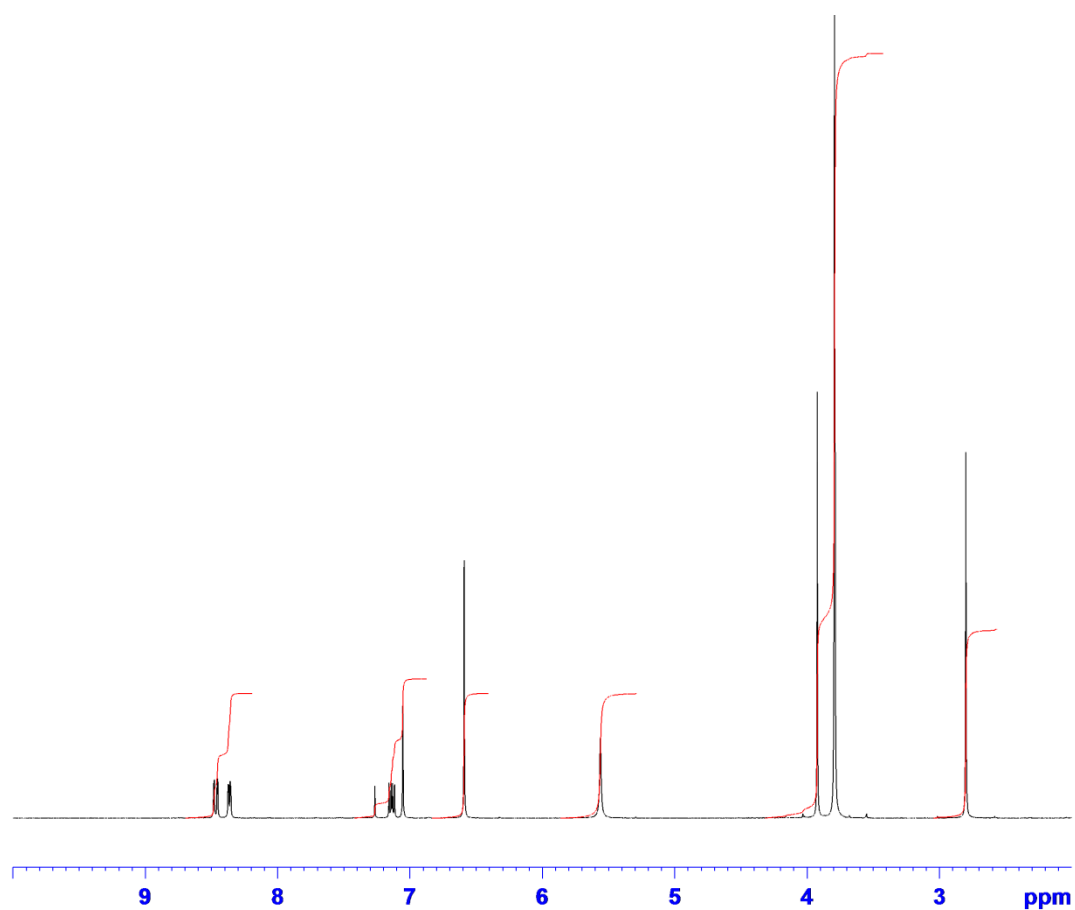
 ^1H NMR of pyrazolo[1,5-*a*]pyrimidine 42

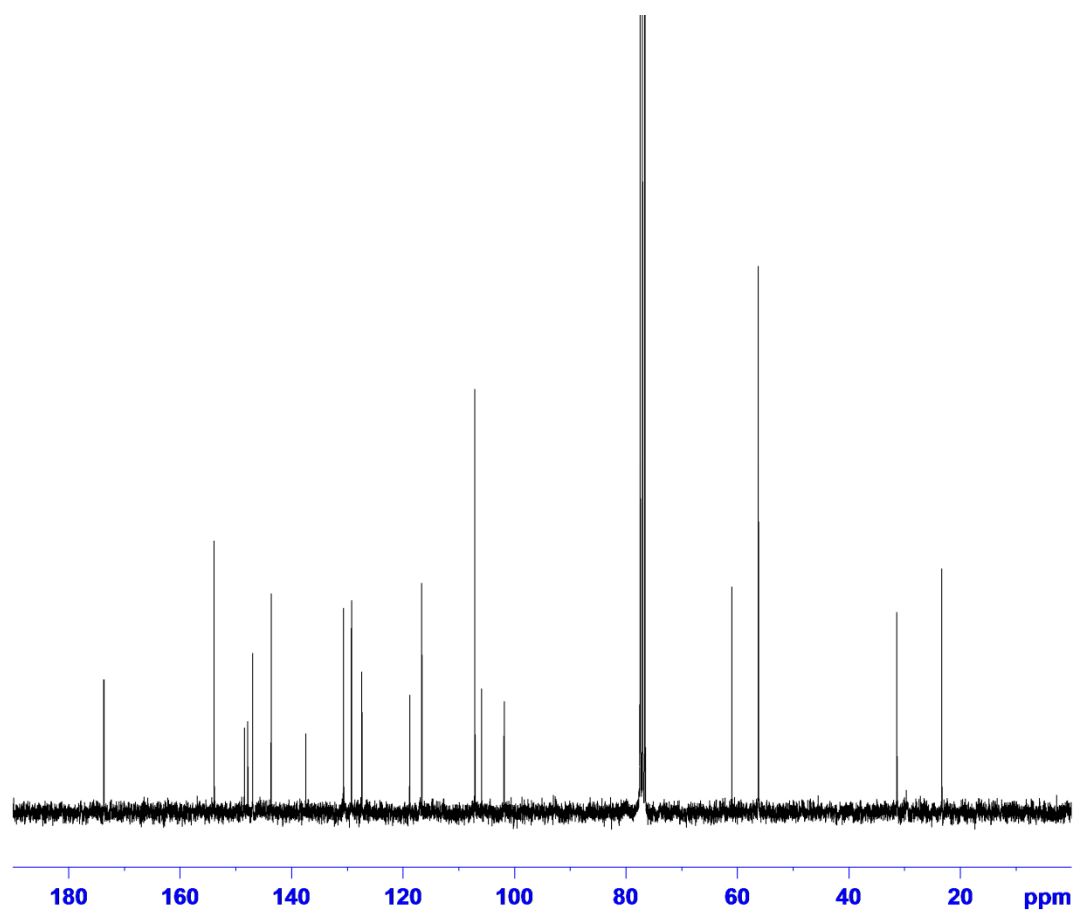


^{13}C NMR of pyrazolo[1,5-*a*]pyrimidine 42

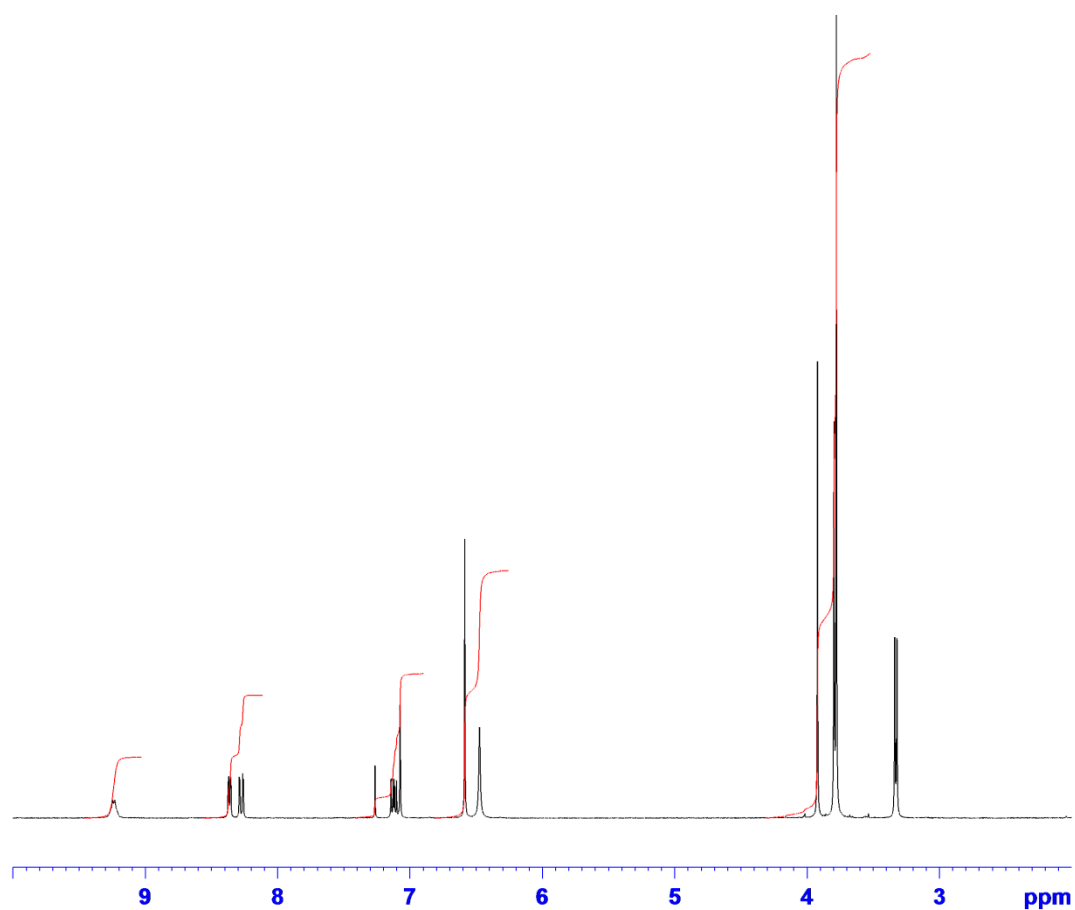
 ^1H NMR of trifluoroacetamide 43

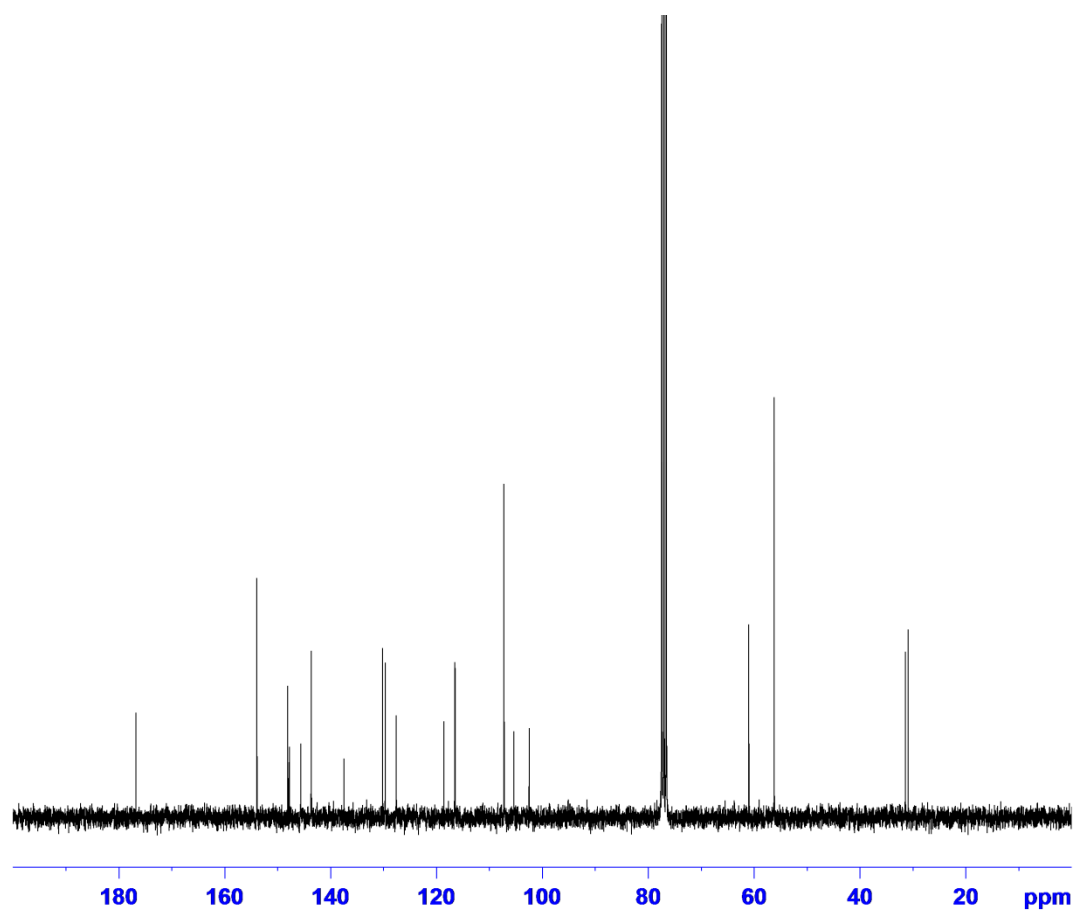
 ^{13}C NMR of trifluoroacetamide 43

 ^1H NMR of acetylated aminopyrazole **44**



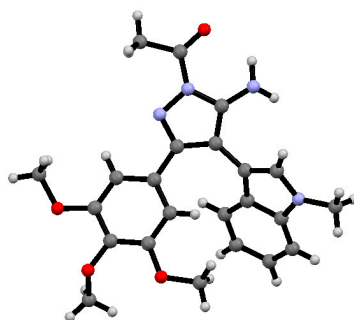
^{13}C NMR of acetylated aminopyrazole 44

 ^1H NMR of carbothioamide 45

 ^{13}C NMR of carbothioamide 45

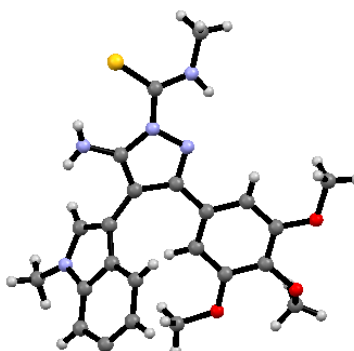
Crystallography Data

Acetylated aminopyrazole **54**:



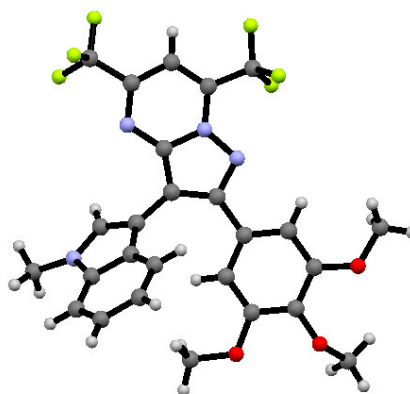
Crystal data: $C_{23}H_{24}N_4O_4$, $M = 420.46$, triclinic, $a = 10.067(6)$ Å, $b = 10.865(7)$ Å, $c = 10.939(8)$ Å, $\alpha = 89.55(2)^\circ$, $\beta = 70.960(19)^\circ$, $\gamma = 82.166(19)^\circ$, $V = 1119.6(12)$ Å³, $T = 300.0(2)$ K, space group $P-1$, $Z = 2$, 7316 reflections measured, 3803 unique ($R_{\text{int}} = 0.049$). The final R_1 values were 0.0698 (2181 reflections with $I > 2\sigma(I)$) and 0.1267 (all data). The final $wR(F^2)$ values were 0.1623 ($I > 2\sigma(I)$) and 0.2167 (all data).

Carbothioamide **55**:



Crystal data: $C_{23}H_{25}N_5O_3S$, $M = 451.54$, monoclinic, $a = 16.1044(17)$ Å, $b = 9.5801(11)$ Å, $c = 14.6361(14)$ Å, $\beta = 92.804(4)^\circ$, $V = 2255.4(4)$ Å³, $T = 300.0(2)$ K, space group $P2_1/c$, $Z = 4$, 27235 reflections measured, 5551 unique ($R_{\text{int}} = 0.049$). The final R_1 values were 0.0652 (3533 reflections with $I > 2\sigma(I)$) and 0.1059 (all data). The final $wR(F^2)$ values were 0.1804 ($I > 2\sigma(I)$) and 0.2095 (all data).

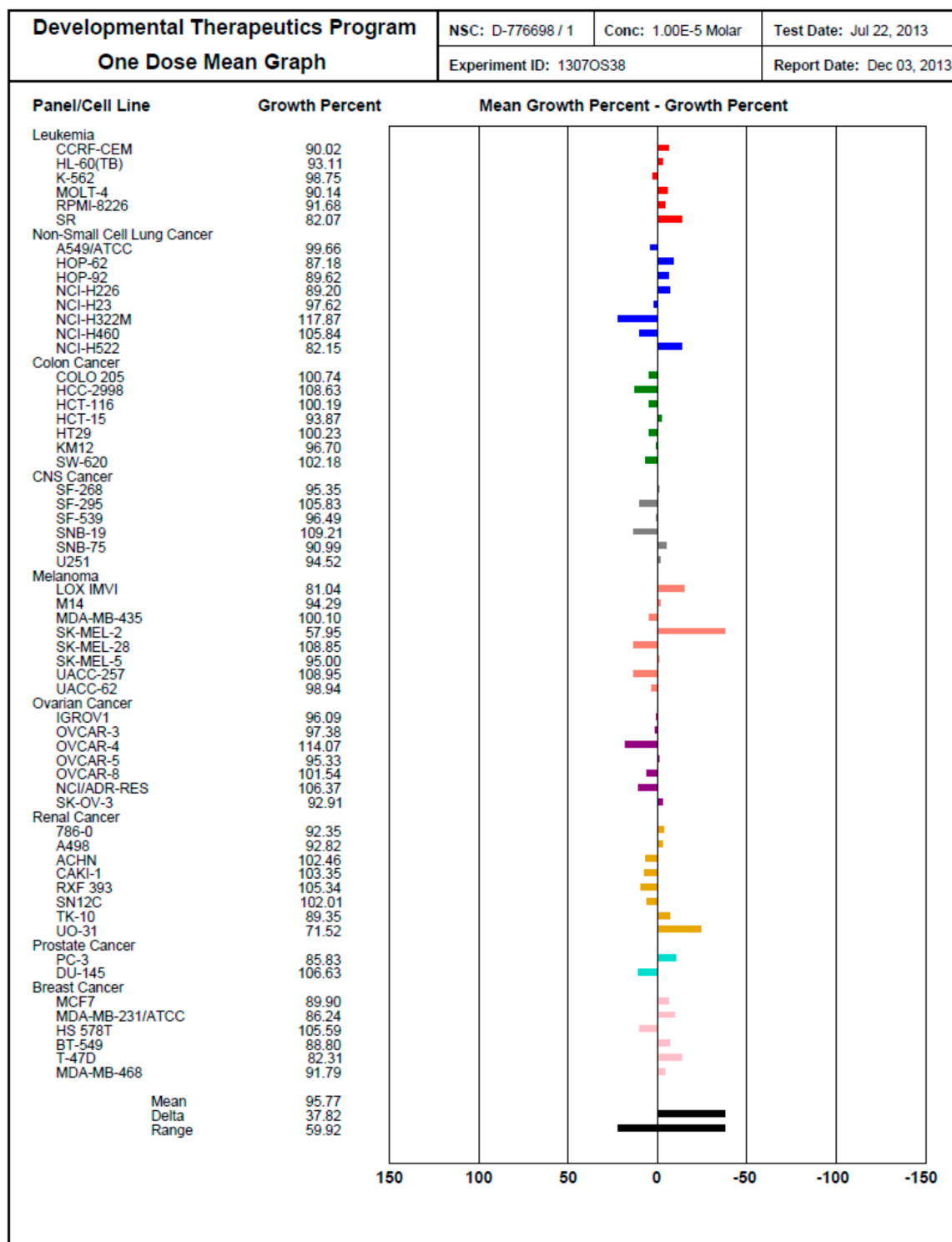
Pyrazolo[1,5-*a*]pyrimidine **53**:



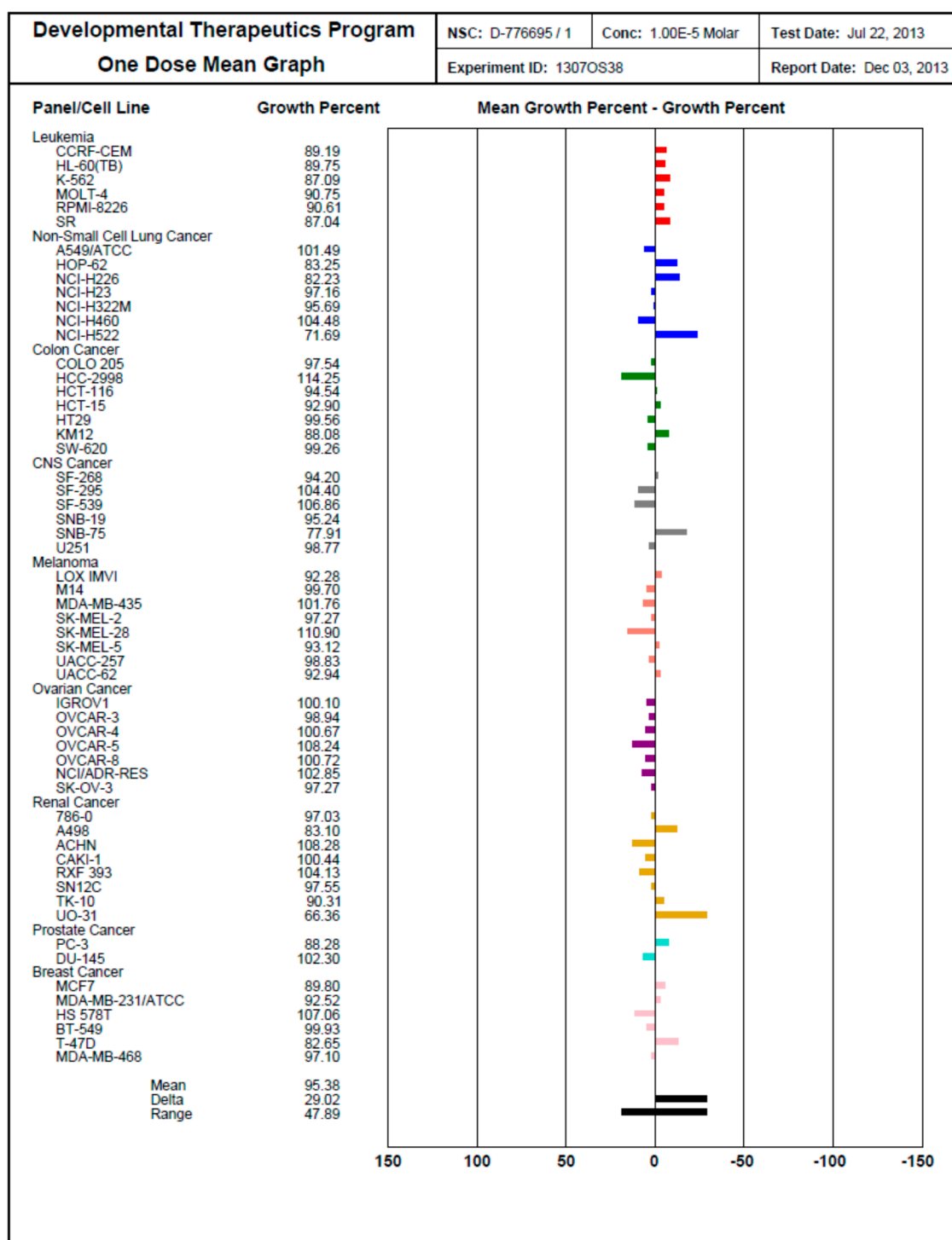
Crystal data: C₂₆H₂₀F₆N₄O₃, $M = 550.46$, triclinic, $a = 13.960(2) \text{ \AA}$, $b = 14.052(2) \text{ \AA}$, $c = 14.580(2) \text{ \AA}$, $\alpha = 114.173(3)^\circ$, $\beta = 108.342(4)^\circ$, $\gamma = 93.100(4)^\circ$, $V = 2420.8(7) \text{ \AA}^3$, $T = 100.0(2) \text{ K}$, space group $P-1$, $Z = 4$, 51437 reflections measured, 9691 unique ($R_{\text{int}} = 0.0758$). The final R_1 values were 0.0484 (5857 reflections with $I > 2\sigma(I)$) and 0.1043 (all data). The final $wR(F^2)$ values were 0.1093 ($I > 2\sigma(I)$) and 0.1411 (all data).

NCI-60 Cell Line Growth Data (One Dose 10⁻⁵ M)

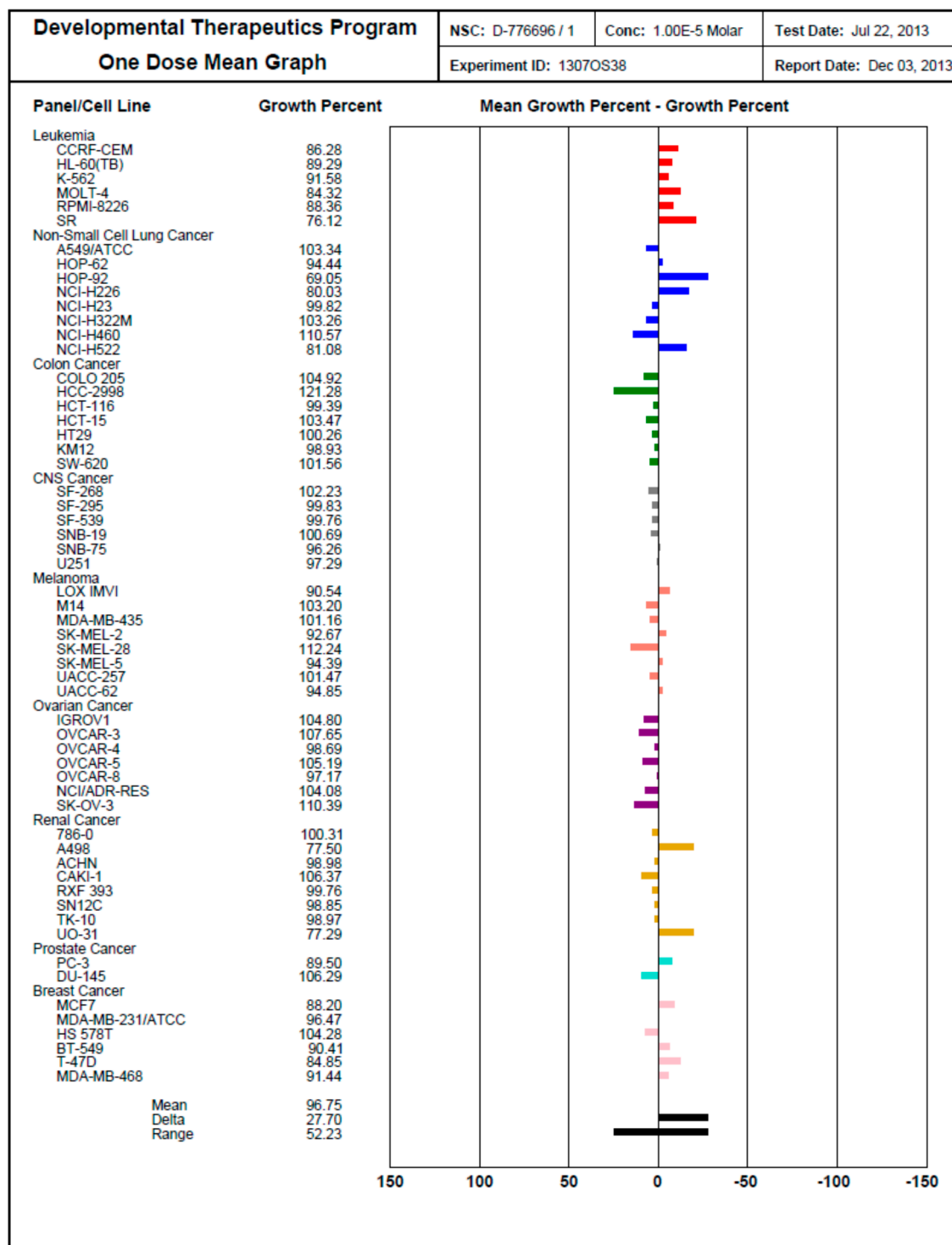
Compound 32



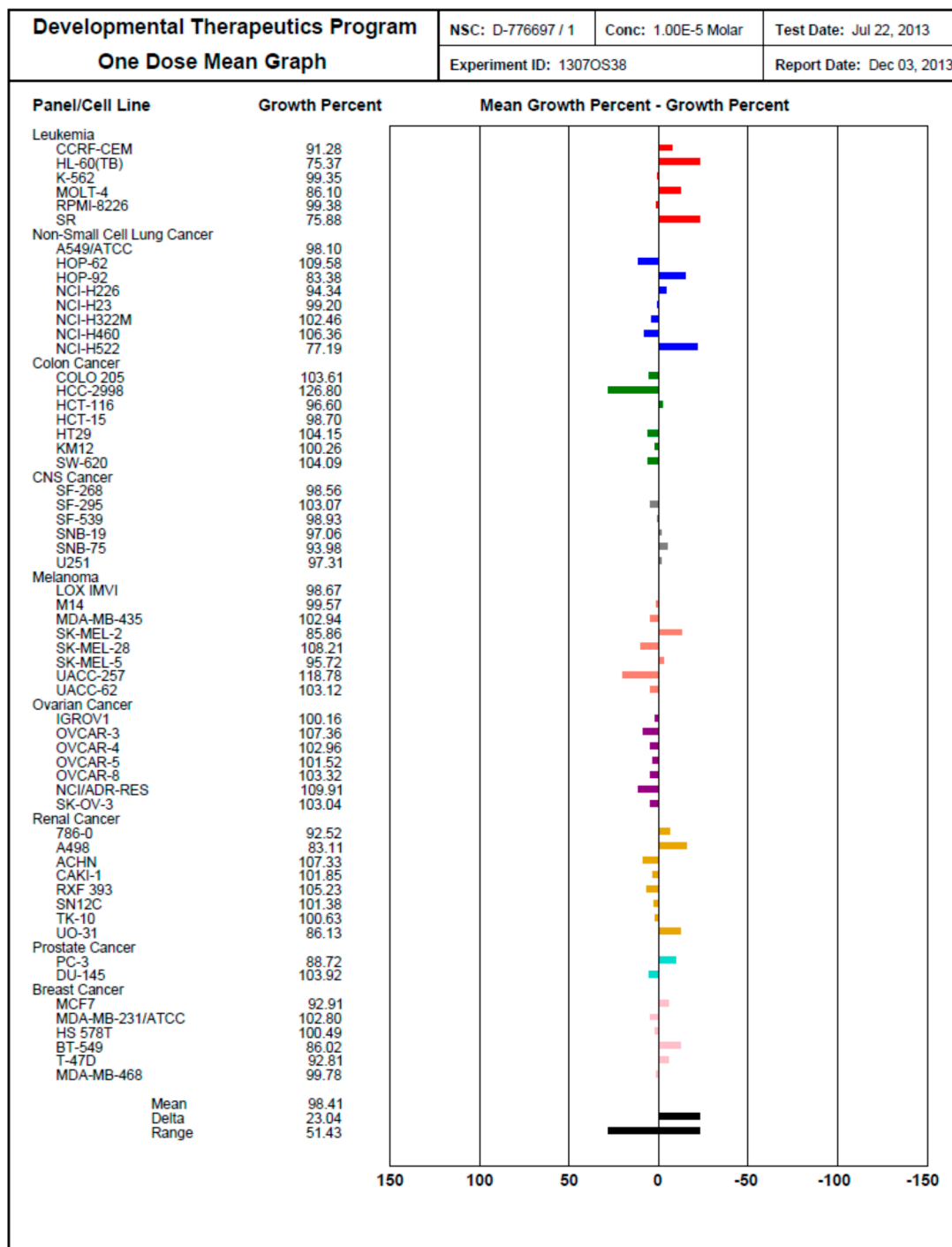
Compound 33



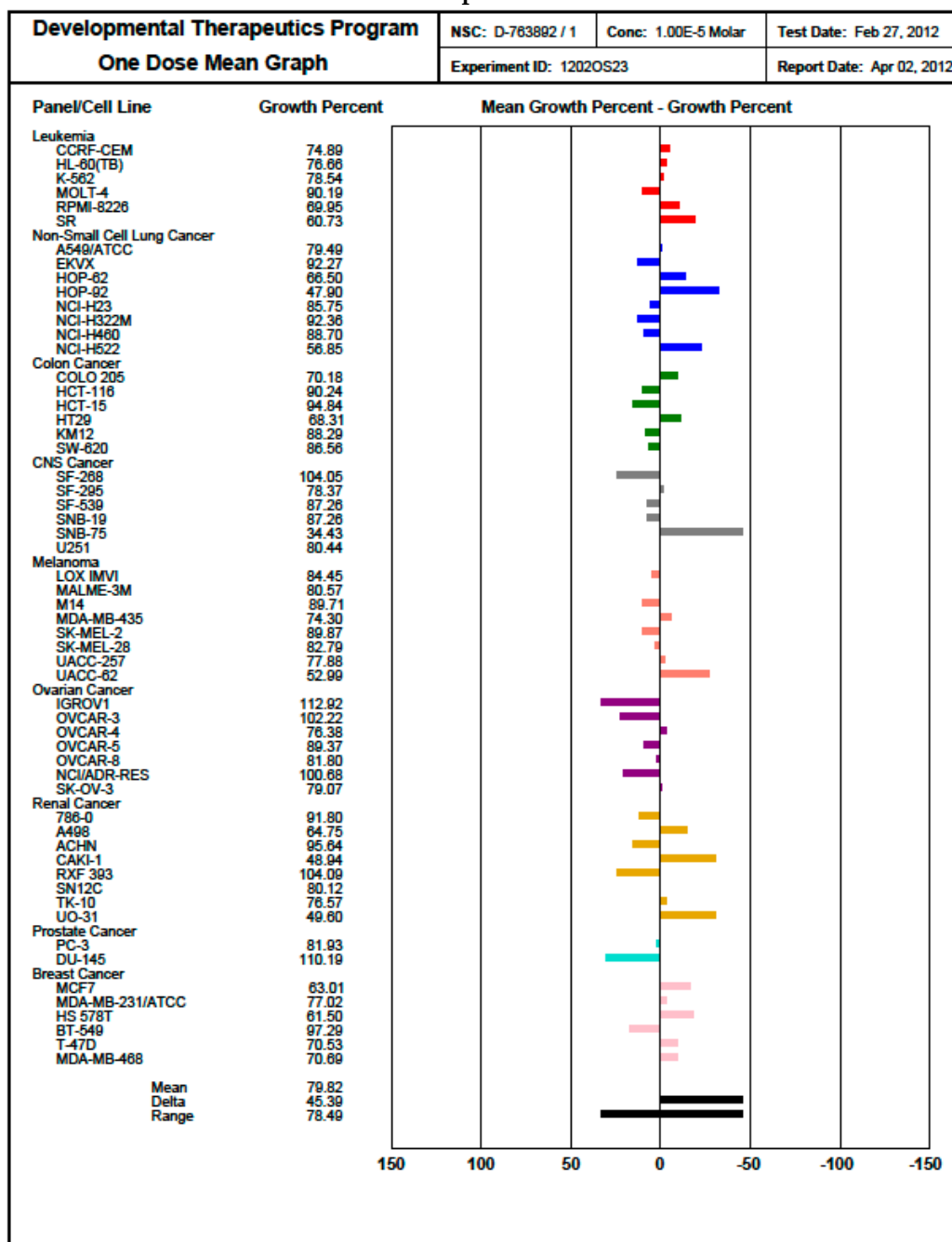
Compound 34



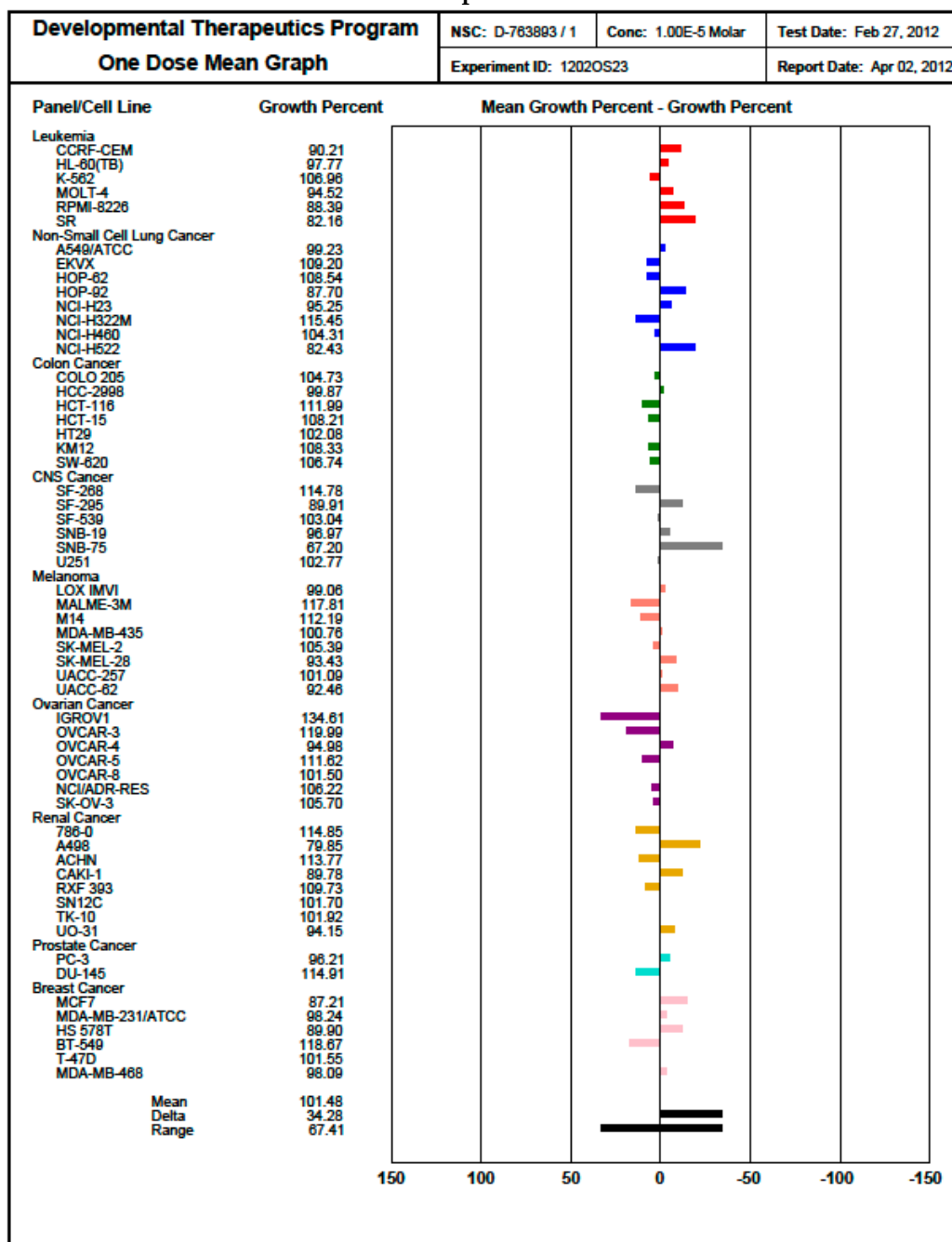
Compound 35



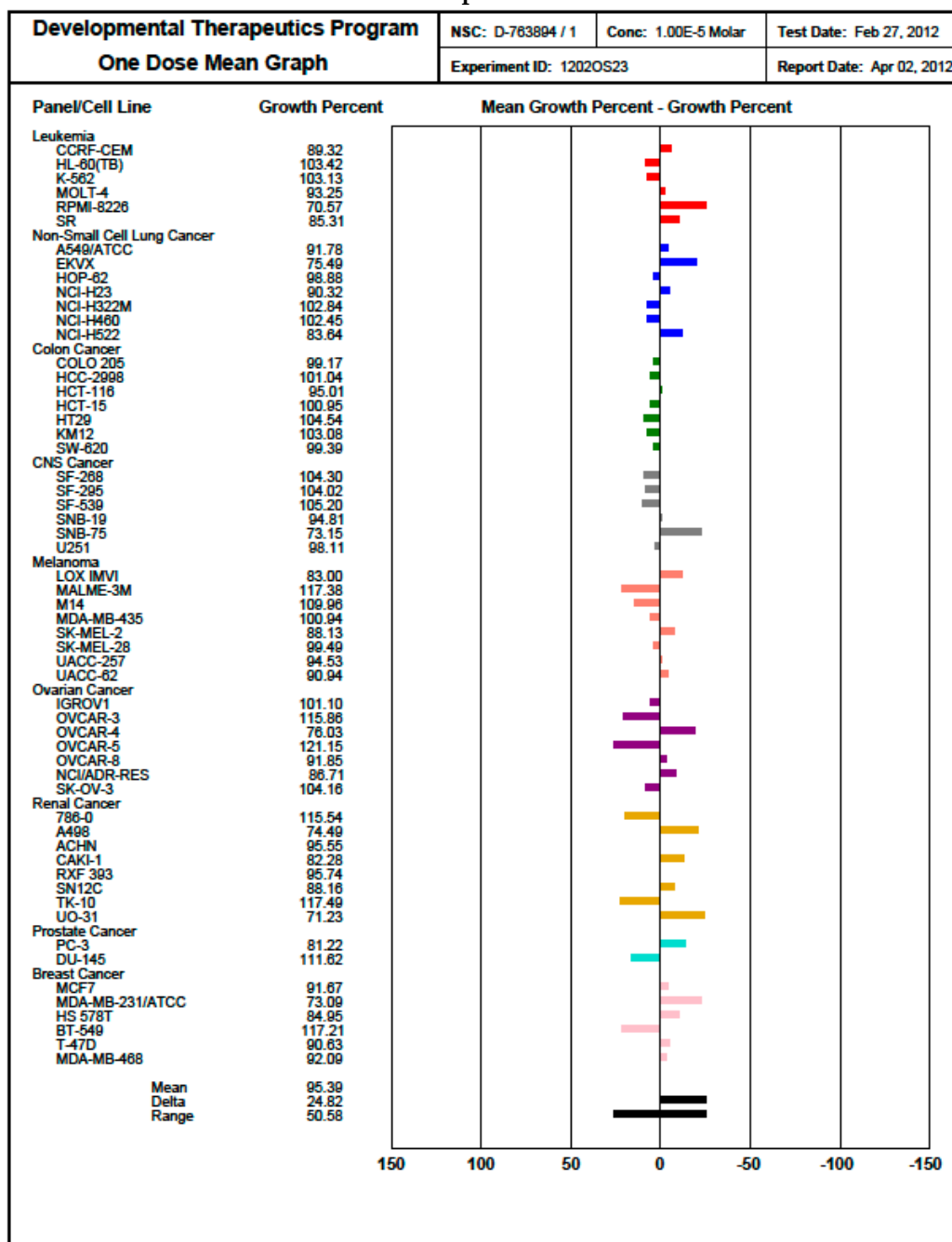
Compound 40



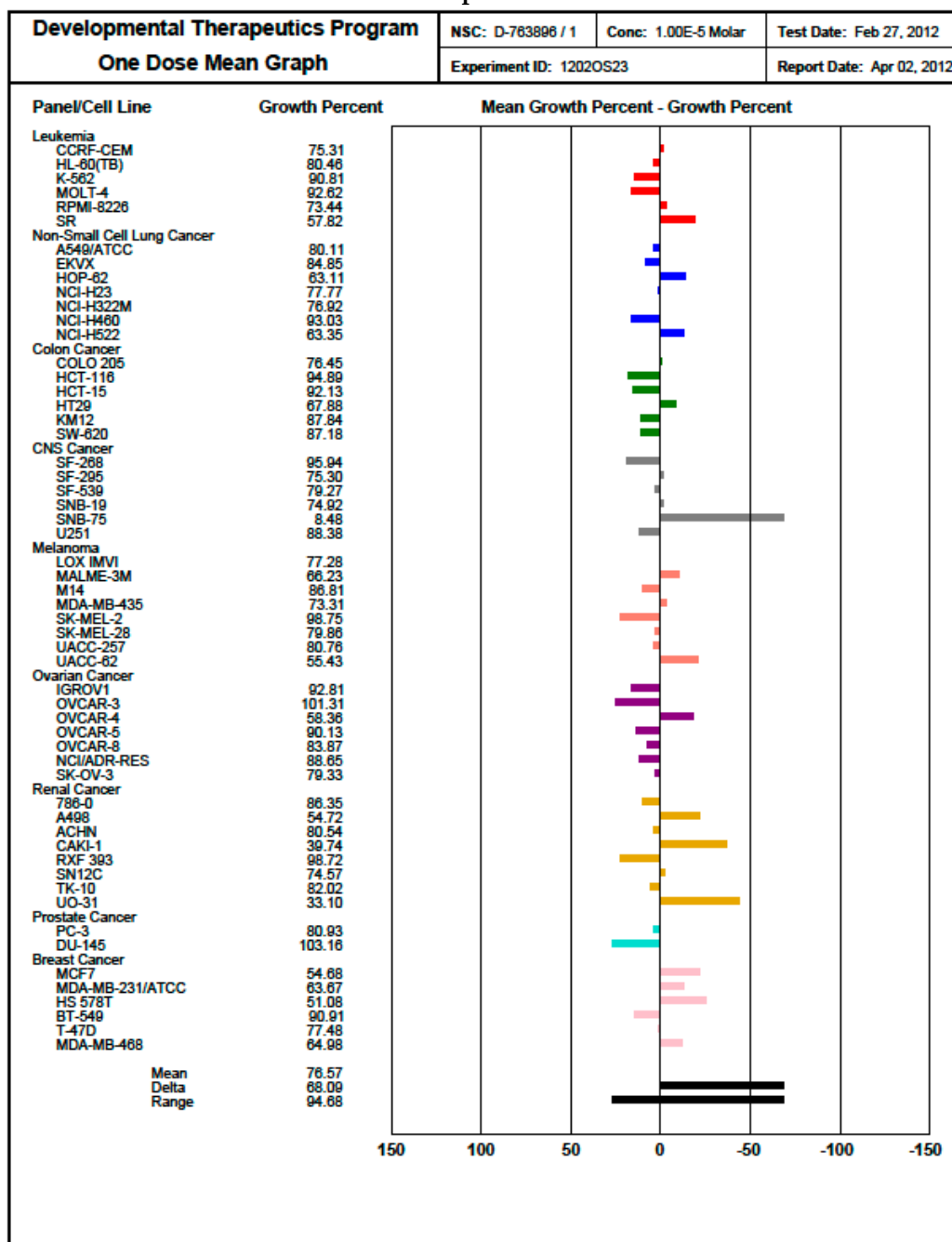
Compound 41



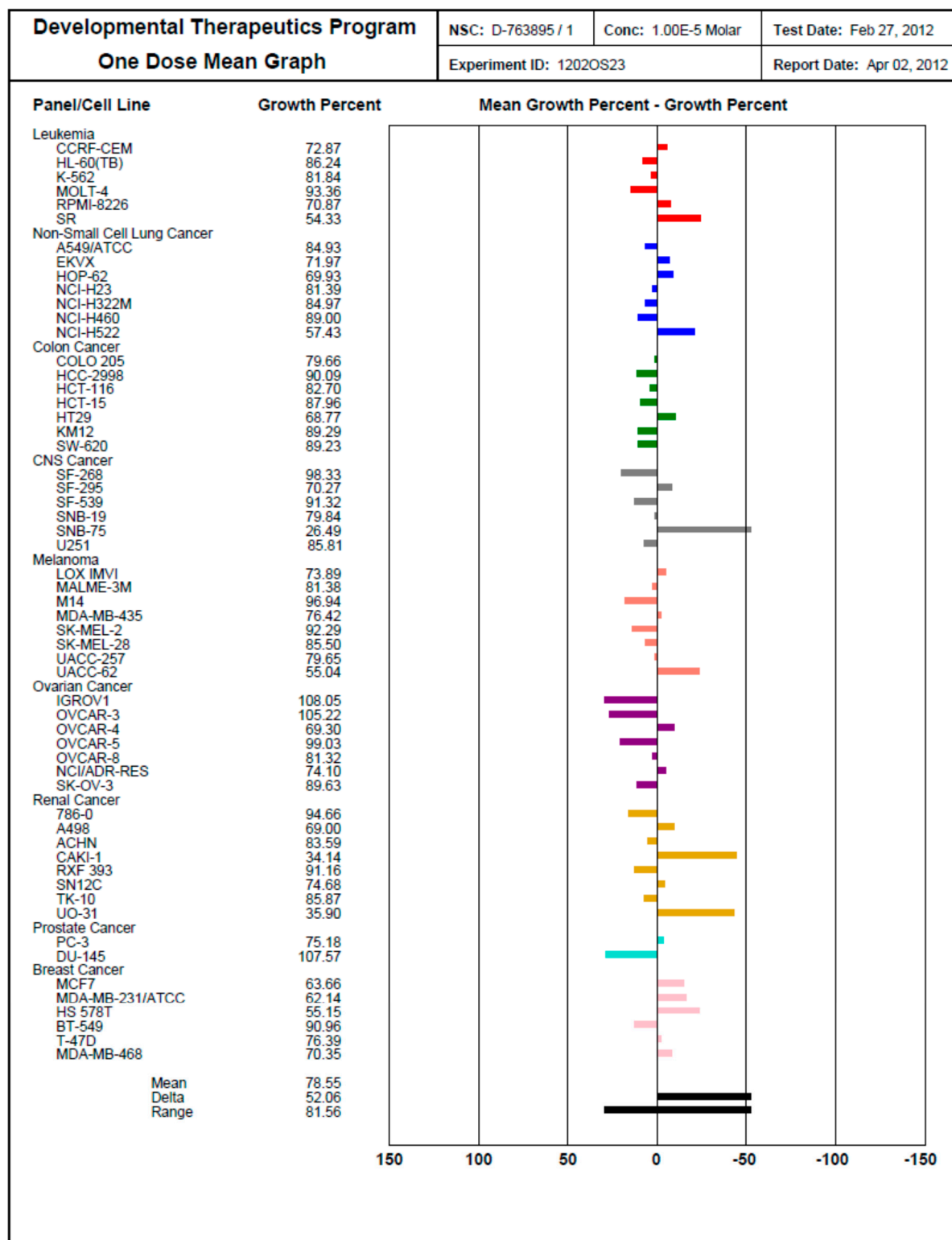
Compound 42



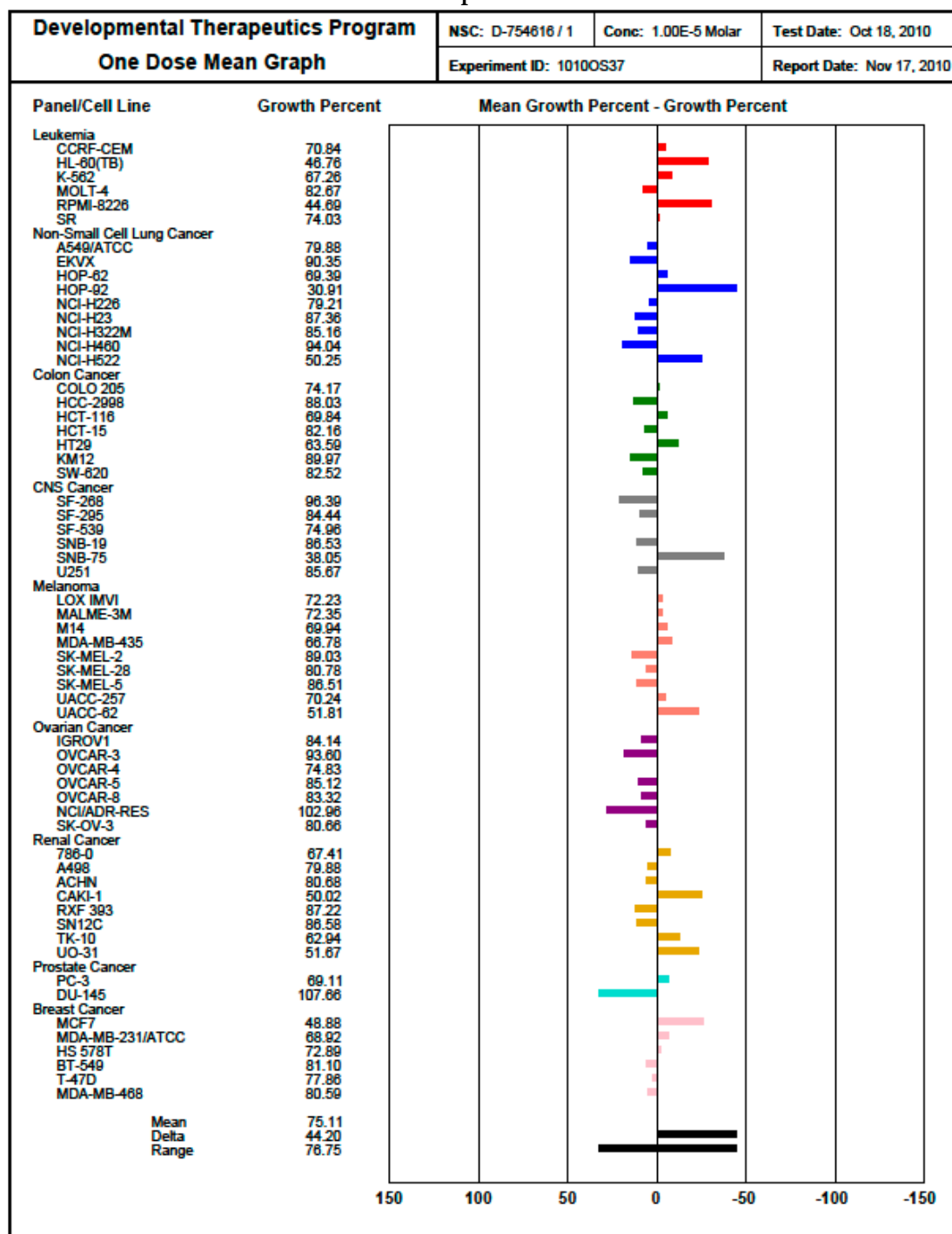
Compound 44



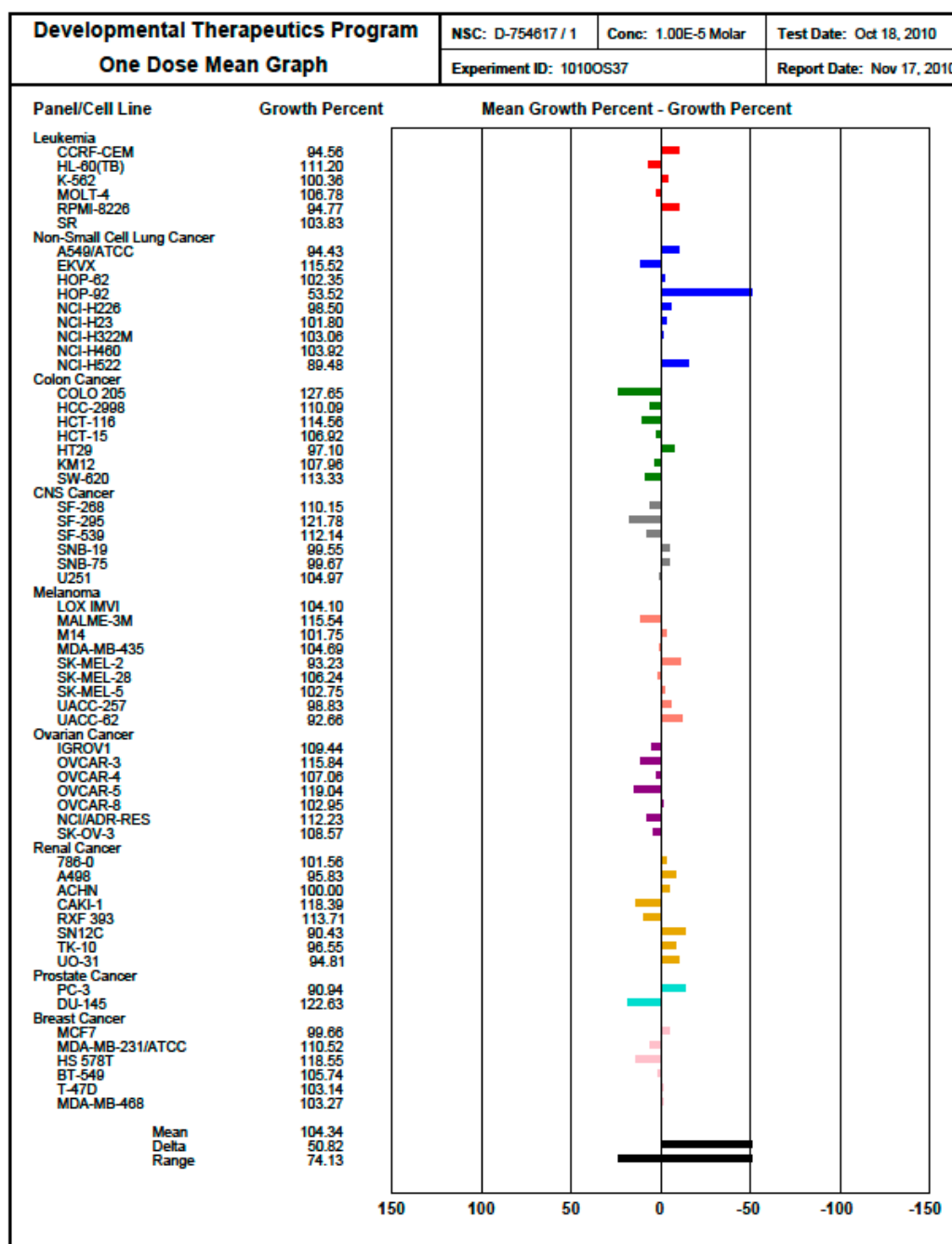
Compound 45



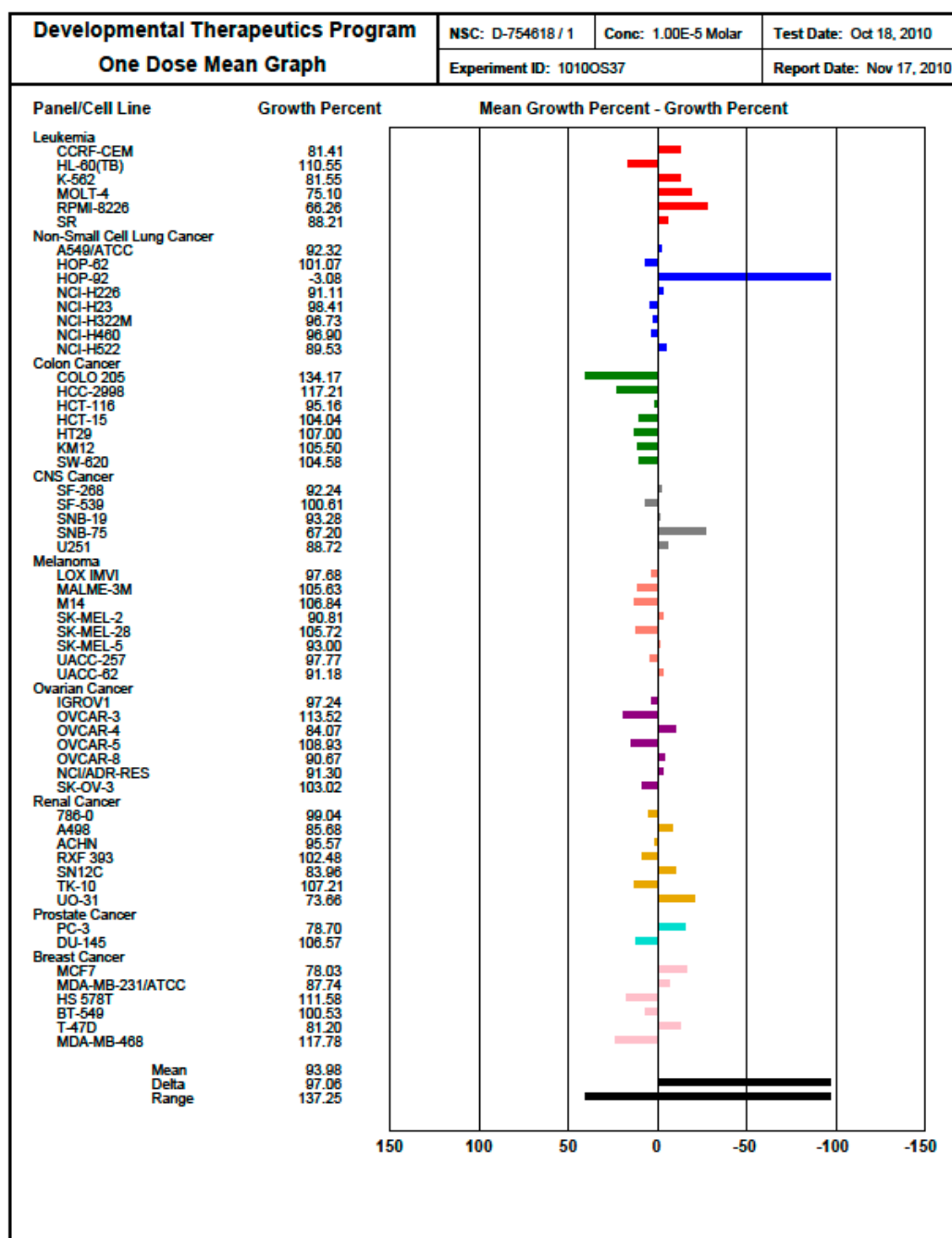
Compound 46



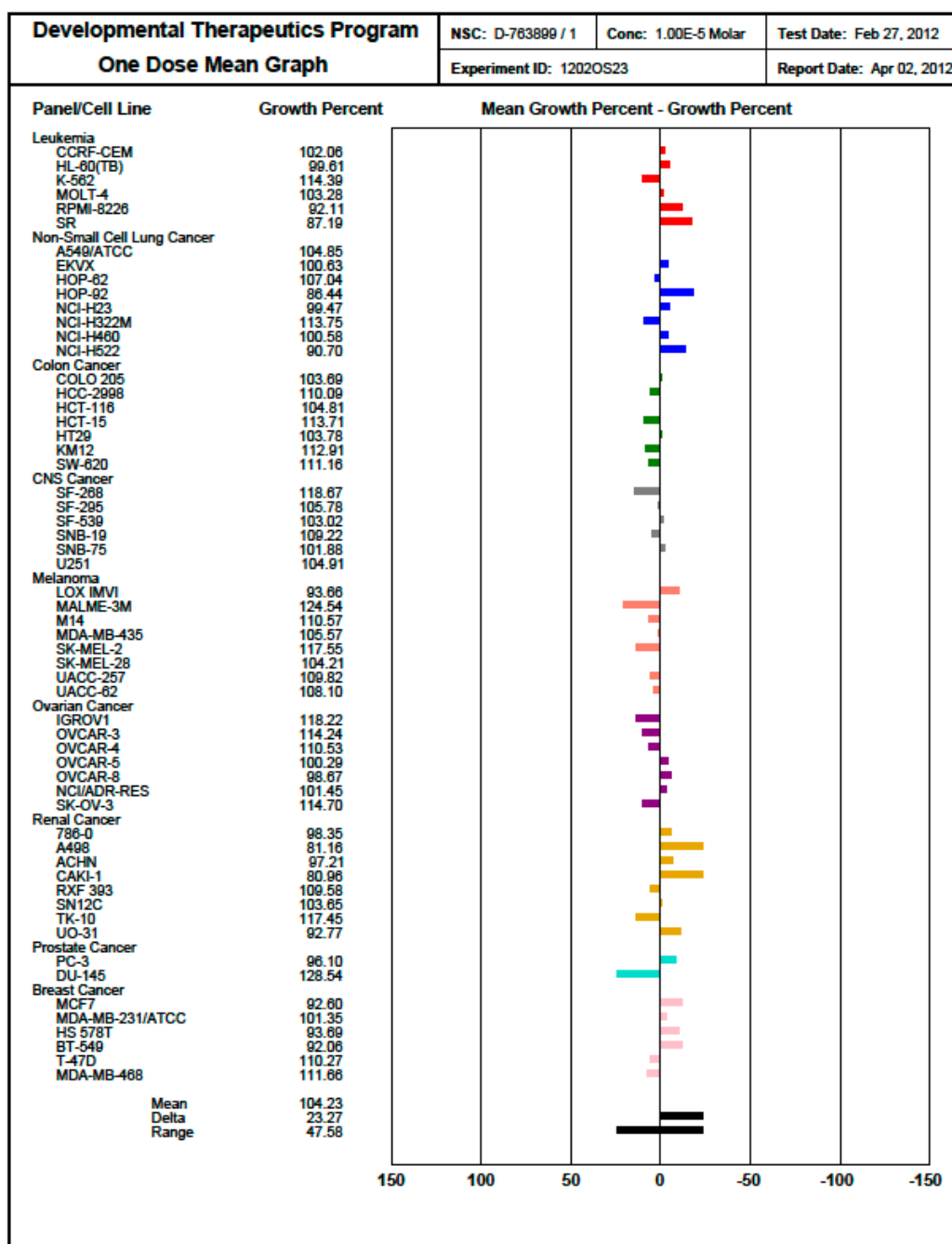
Compound 47



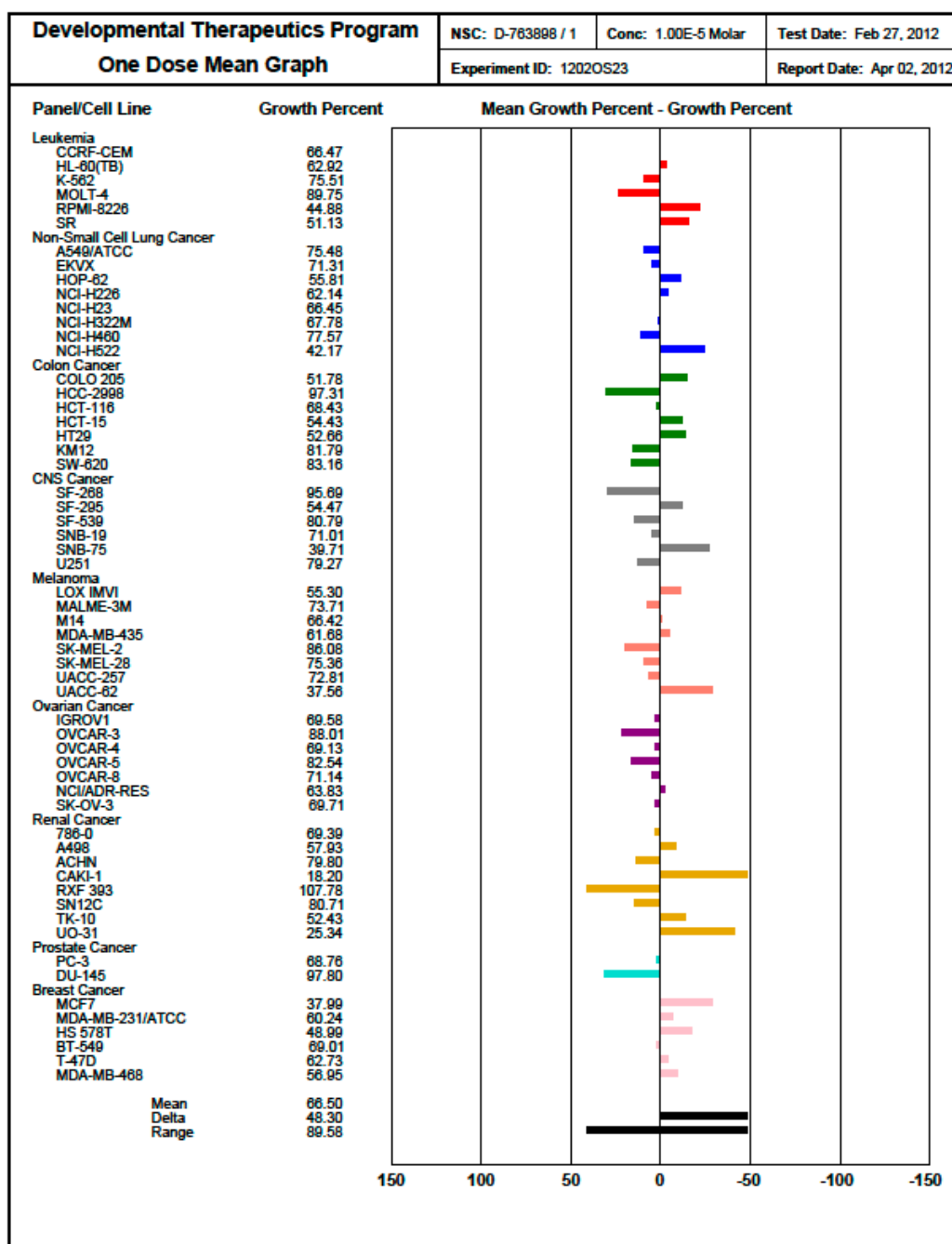
Compound 48



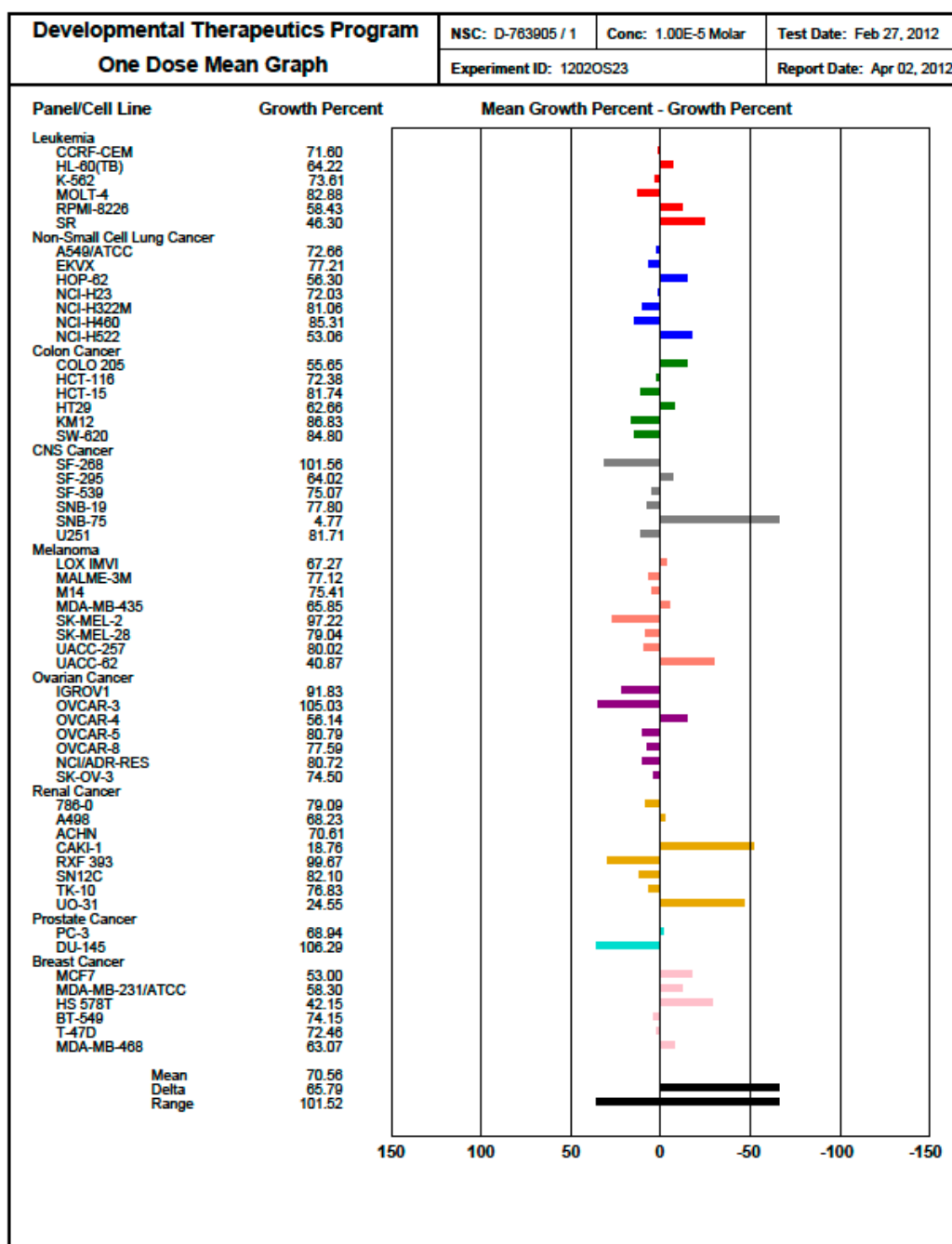
Compound 49



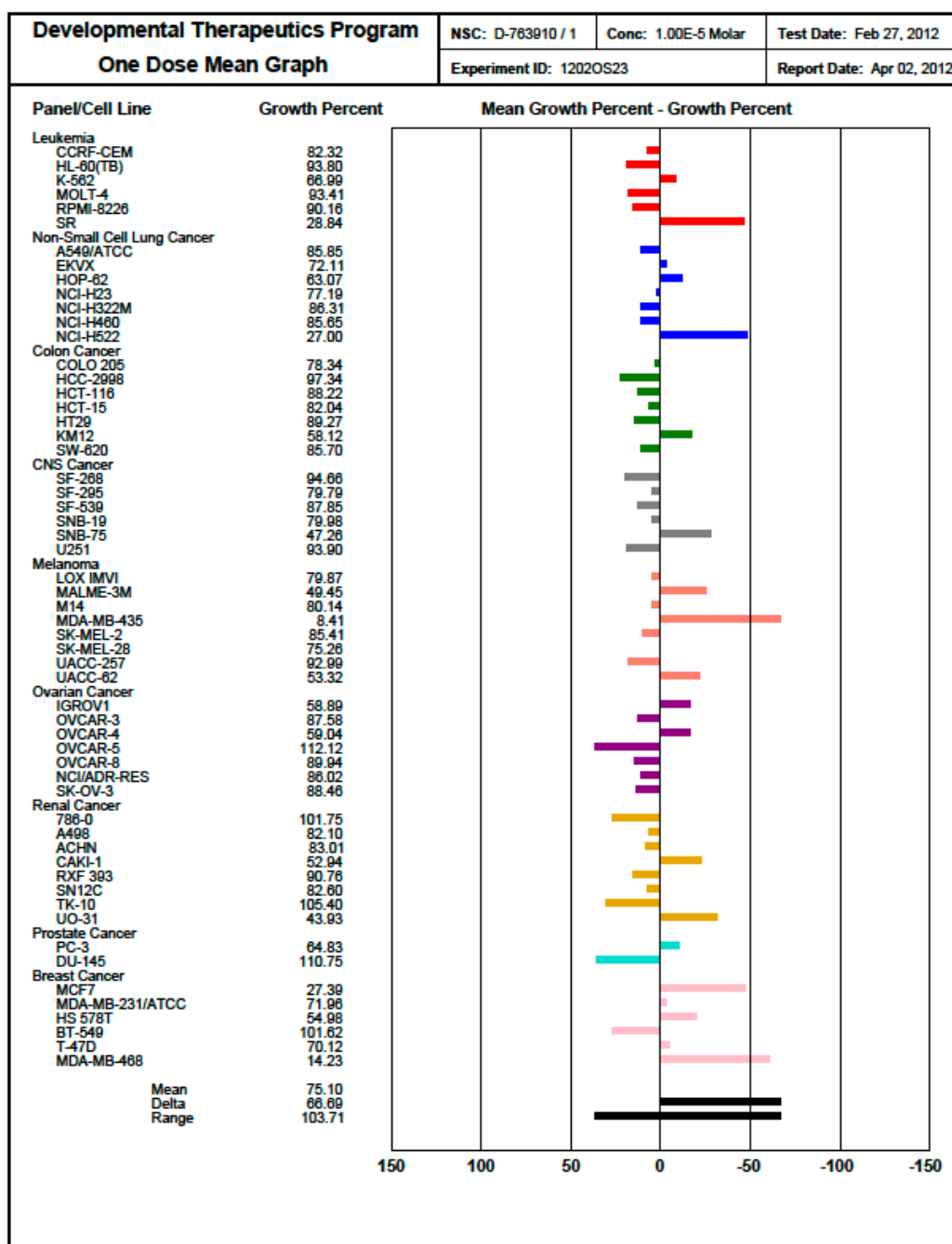
Compound 50



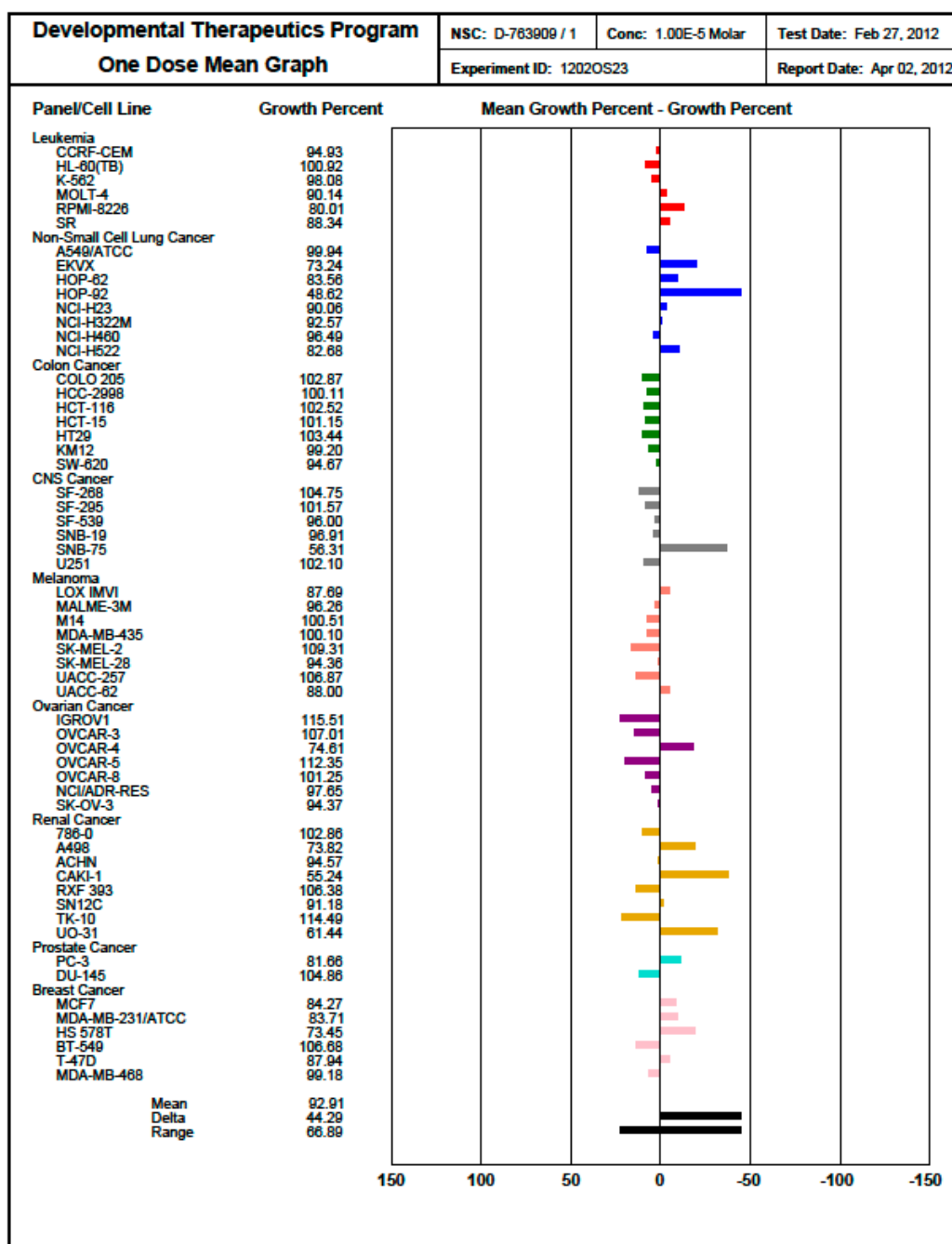
Compound 51



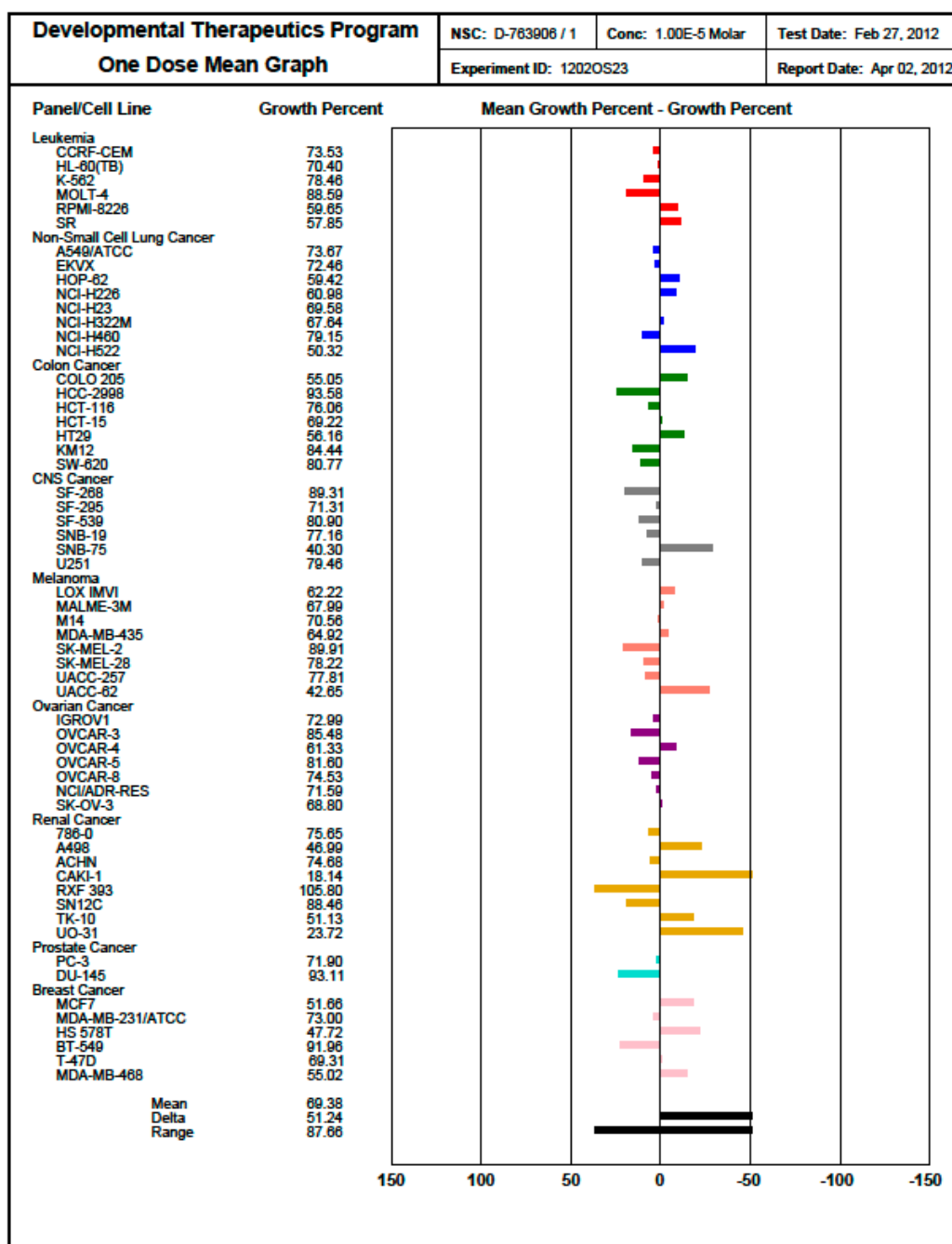
Compound 52



Compound 53



Compound 54



Compound 55

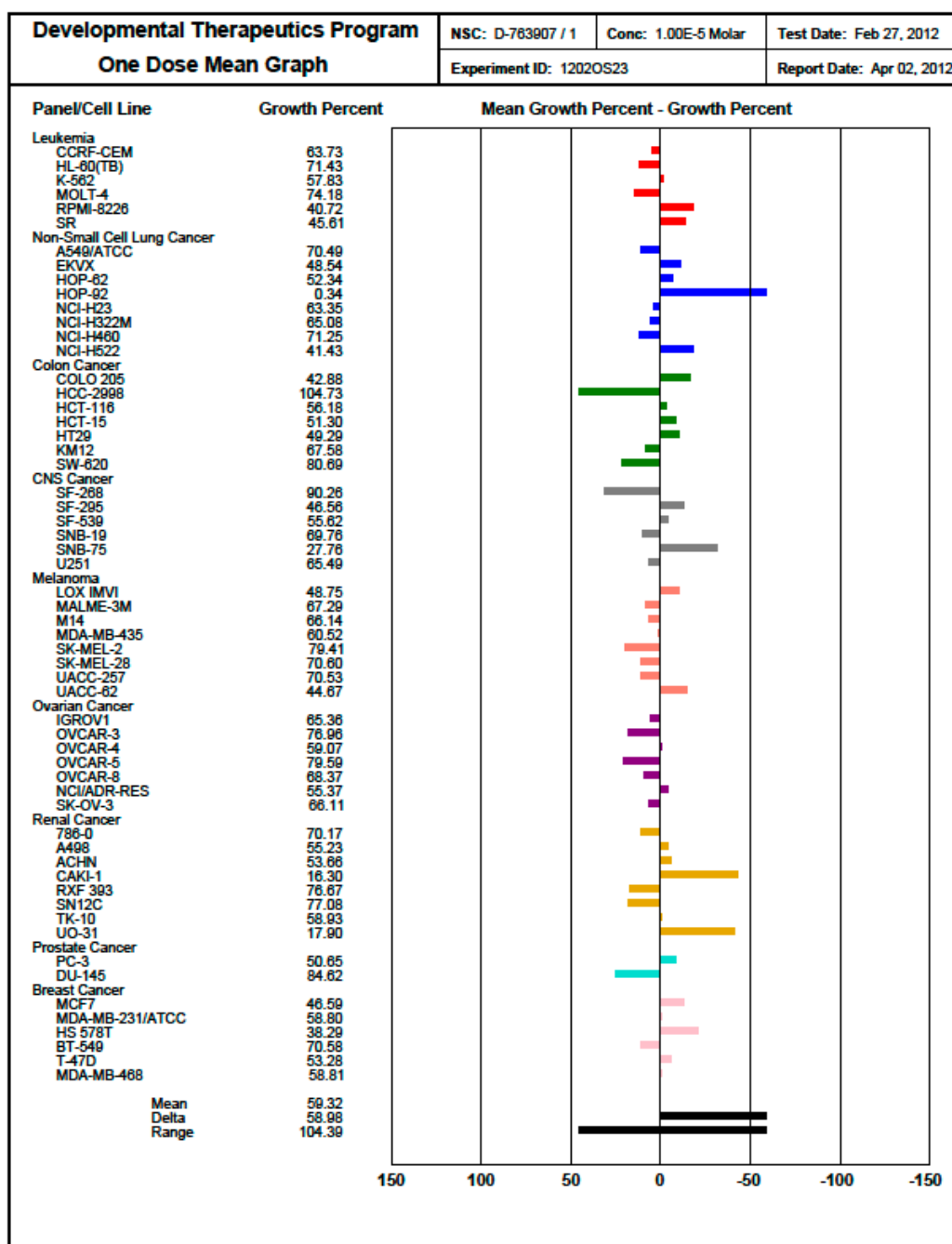
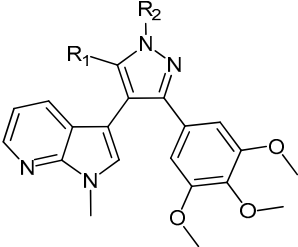


Table of activity for Topo II decatenation assay

Table 1. Topo II decatenation assay: (+) inhibition at 100 μ M; (–) no inhibition at 100, 10 and 1 μ M.

Structure	Compound	Activity
	Ellipticine	+
	46 ; R ₁ =NH ₂ , R ₂ =H	–
	47 ; -NHCONHCO-	–
	48 ; -NC(CF ₃)CHC(CF ₃)-	–
	49 ; R ₁ =NH ₂ , R ₂ =COCH ₃	–
	50 ; R ₁ =NH ₂ , R ₂ =CSNHCH ₃	–

References

1. Eccles, K.S.; Stokes, S.P.; Daly, C.A.; Barry, N.M.; McSweeney, S.P.; O' Neill, D.J.; Kelly, D.M.; Jennings, W.B.; Ní Dhubhghaill, O.M.; Moynihan, H.A.; et. al. Evaluation of the bruker smart X2S: Crystallography for the nonspecialist?. *J. Appl. Cryst.* **2011**, *44*, 213–215.
2. Sheldrick, G.M. A short history of SHELX *Acta Crystallogr.* **2008**, *A64*, 112–122.
3. APEX2, v2009.3–0; Bruker AXS: Madison, WI, USA, 2009.
4. Narumiya, S.; Takai, K.; Tokuyama, T.; Noda, Y.; Ushiro, H.; Hayaishi, O. A new metabolic pathway of tryptophan initiated by tryptophan side chain oxidase. *J. Biol. Chem.* **1979**, *254*, 7007–7015.
5. Blunt, J.W.; Erasmuson, A.F.; Ferrier, R.J.; Munro, M.H.G. Syntheses of haptens related to the benzenoid and indole portions of sporidesmin a; ¹³c nmr spectra of indole derivatives. *Aust. J. Chem.* **1979**, *32*, 1045–1054.