Electronic Supplementary Information for

Dissecting metabolic puzzles through isotope feeding: a novel amino acid in the biosynthetic pathway of cruciferous phytoalexins rapalexin A and isocyalexin A

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General experimental

All solvents were HPLC grade and used as such, except for THF (dried over sodium). Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Flash column chromatography (FCC): silica gel, grade 60, 230-400 μm. Organic extracts were dried over Na_2SO_4 and the solvents were removed using a rotary evaporator.

NMR spectra were recorded on Bruker Avance 500 MHz spectrometers. For ^{1}H NMR (500 MHz) and ^{13}C NMR (125.8 MHz) spectra, the chemical shifts (δ) are reported in parts per million (ppm) relative to TMS. Fourier transform infrared (FTIR) data were acquired on a spectrometer and spectra were measured by the diffuse reflectance method.

HPLC-DAD analysis was carried out with Agilent 1100 series HPLC systems equipped with quaternary pump, autosampler, diode array detector (DAD, wavelength range 190-600 nm), and degasser; Zorbax Eclipse XDB-C18 column (5 μm particle size silica, 150 × 4.6 mm I.D.), equipped with an in-line filter, with the mobile phase H_2O-CH_3OH from 50:50 to 0:100, linear gradient for 25 min, and a flow rate of 0.75 mL/min. Samples were dissolved in CH_3OH.

HPLC-DAD-ESI-MS analysis was carried out with an Agilent 1100 series HPLC system equipped with an autosampler, binary pump, degasser, and a diode array detector connected directly to a mass detector (Agilent G2440A MSD-Trap-XCT ion trap mass spectrometer) with an electrospray ionization (ESI) source. Chromatographic separations were carried out at room temperature using an Eclipse XDB-C-18 column (5 μm particle size silica, 150 mm × 4.6 mm I.D.). The mobile phase consisted of a linear gradient of: method A (applied to non-polar extracts), in H_2O (with 0.2% HCO_2H) -CH_3CN (with 0.2% HCO_2H) from 75:25 to 25:75 in 25 min, to 0:100 in 5 min and a flow rate of 1.0 mL/min; method B (applied to polar extracts), H_2O (with 0.2% HCO_2H) - CH_3CN (with 0.2% HCO_2H) from 90:10 to 50:50 in 25 min and a flow rate of 1.0 mL/min. Data acquisition was carried out in positive and negative polarity modes in a single LC run, and data processing carried out with Agilent Chemstation Software. Samples were dissolved in CH_3CN or CH_3CN-H_2O.

HR-EI-MS spectral data were obtained using a VG 70 SE mass spectrometer using a solids probe. HPLC-HRMS-ESI was performed on an Agilent HPLC 1100 series directly connected to a QSTAR XL Systems Mass Spectrometer (Hybrid Quadrupole-TOF LC/MS/MS) with turbo spray ESI source. Chromatographic separation was carried out at room temperature using a Hypersil ODS C-18 column (5 μm particle size silica, 200 × 2.1 mm I.D.) or a Hypersil ODS C-18 column (5 μm particle size silica, 100 × 2.1 mm I.D.). The mobile phase consisted of a linear gradient of: Method A, H_2O (with 0.2% HCO_2H) - CH_3CN (with 0.2% HCO_2H) from 75:25 to 25:75 in 25 min, to 0:100 in 5 min

and a flow rate of 0.25 ml/min. Data acquisition was carried out in either positive or negative polarity mode per LC runs. Data processing was carried out by Analyst QS Software. Samples were dissolved in CH_3CN or CH_3OH-H_2O (1:1).

Tables

The percentage of deuterium incorporation into each metabolite was established from analysis of data obtained by HPLC-ESI-MS (positive or negative ion modes) using the expressions shown in footnotes to each table (e.g., % of ^2H incorporation = $\{[M \pm 1 + n]^{+/-}/([M \pm 1]^{+/-} + [M \pm 1 + n]^{+/-})\} \times 100$ (n = 3, 4, 6), where n is the number of deuterium atoms, and M is the peak intensity of the quasi-molecular ion peak $[M \pm 1]^{+/-}$); the peak intensities of deuterated and non-deuterated compounds were identical; unless otherwise stated, the ions $[M \pm 1 + n]^{+/-}$ were not detected in natural abundance samples.

Table S1 Metabolites of (*S*)-[$^{13}C_{11}$,$^{15}N_2$]Trp in UV–elicited rutabaga roots.

Metabolites detected (**#a**)[a]	% Incorporation±Std
[1,2',3',3a',4',5',6',7',7a'-$^{13}C_9$,$^{15}N_2$]rapalexin A (**10**)	25.9±1.4[b]
[1,2',3',3a',4',5',6',7',7a'-$^{13}C_9$,$^{15}N_2$]isocyalexin A (**12**)	17.0±6.4[b]
[2',3',3a',4',5',6',7',7a'-$^{13}C_8$,^{15}N]isalexin (**13**)	12.1±1.1[c]
[1,2, 2',3',3a',4',5',6',7',7a'-$^{13}C_{10}$,$^{15}N_2$]cyclobrassinin (**4**)	15.5±4.7[c]
[1,2,2',3',3a',4',5',6',7',7a'-$^{13}C_{10}$,$^{15}N_2$]spirobrassinin (**7**)	11.2±0.1[c]
[1,2,2',3',3a',4',5',6',7',7a'-$^{13}C_{10}$,$^{15}N_2$]rutalexin (**9**)	13.0±0.3[b]
[1,2',3',3a',4',5',6',7',7a'-$^{13}C_9$,^{15}N]-4-methoxyindole-3-carboxaldehyde (**24**)	16.2±0.7[c]
[1,2,2',3',3a',4',5',6',7',7a'-$^{13}C_{10}$,$^{15}N_2$]glucobrassicin (**14**)	31.3±0.7[b]
[1,2,2',3',3a',4',5',6',7',7a'-$^{13}C_{10}$,$^{15}N_2$]-4-methoxyglucobrassicin (**16**)	10.3±1.5[b]
[1,2,2',3',3a',4',5',6',7',7a'-$^{13}C_{10}$,$^{15}N_2$]-1-methoxyglucobrassicin (**15**)	10.0±2.0[b]
(*S*)-[$^{13}C_{11}$,$^{15}N_2$]Trp	74.0±3.0[b]

[a] ^{13}C, ^{5}N-Labelled compounds are referred to by the number used for naturally-occurring compounds used in the manuscript.

[b] Negative ion mode. Incorporations calculated from HPLC-ESI-MS (normalized peak intensities); % of $^{13}C^{15}N$ incorporation = $\{[M - 1 + n]^-/([M - 1]^- + [M - 1 + n]^-)\} \times 100$ ± Std (standard deviation), where n=number of ^{13}C plus ^{15}N atoms (9-12); standard deviations calculated from experiments conducted in triplicate.

[c] Positive ion mode. Incorporations calculated from HPLC-ESI-MS (normalized peak intensities); % of $^{13}C^{15}N$ incorporation = $\{[M + 1 + n]^+/([M + 1]^+ + [M + 1 + n]^+)\} \times 100$ ± Std (standard deviation), where n=number of ^{13}C plus ^{15}N atoms (9-12); standard deviations calculated from experiments conducted in triplicate.

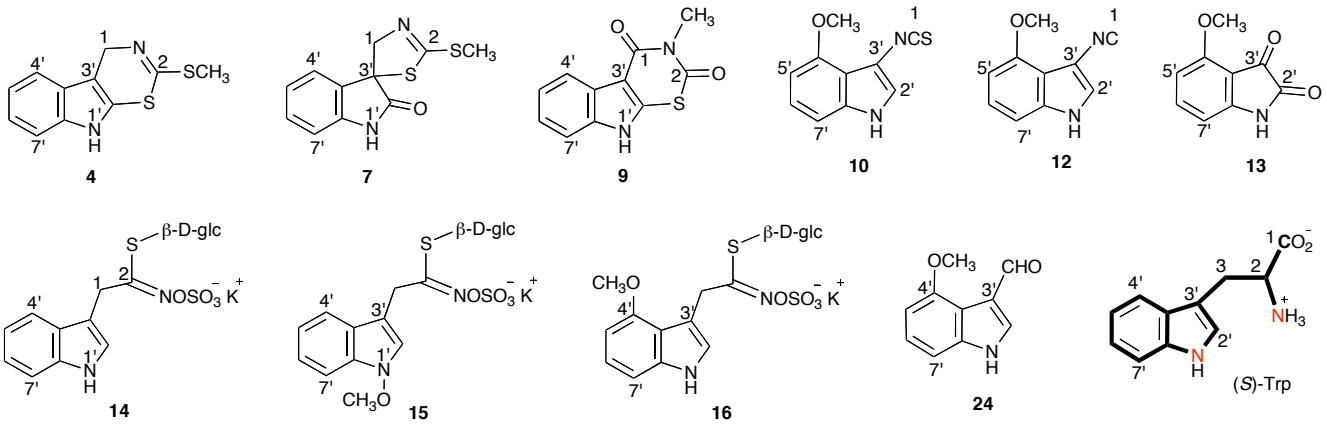


Table S2 Metabolites of (*R,S*)-[$^2H_3$CO,5',6',7'-$^2H_3$]-4'-methoxyindolyl-3'-glycine (19a), [$^2H_3$CO,5',6',7'-$^2H_3$]-4'-methoxyindolyl-3'-carboxaldehyde oxime (20a) and [$^2H_3$CO,5',6',7'-$^2H_3$]desulfoglucorapassicin (27a) in UV–elicited rutabaga roots.

Metabolites detected (**#a**)[a]	% Incorporation ± Std		
	Amino ac **19a**	Oxime **20a**	Desulfo **27a**
[$^2H_3$CO,5',6',7'-$^2H_3$]rapalexin A (**10a**)	4.2±0.7[b]	3.4±2.5[b]	6.2±4.2[b]
[$^2H_3$CO,5',6',7'-$^2H_3$]isocyalexin A (**12a**)	12.8±3.4[b]	9.0±6.0[b]	NI[d]
[$^2H_3$CO,5,6,7-$^2H_3$]isalexin (**13a**)	5.0±2.6[c]	2.5±0.6[c]	7.1±2.9[c]
[^2H_n]cyclobrassinin (**4a**)	NI[d]	NI[d]	NI[d]
[^2H_n]spirobrassinin (**7a**)	NI[d]	NI[d]	NI[d]
[^2H_n]rutalexin (**9a**)	NI[d]	NI[d]	NI[d]
[$^2H_3$CO,5',6',7'-$^2H_3$]-4'-methoxyindolyl-3'-glycine (**19a**)	100[b,c]	ND[d]	ND[d]
[$^2H_3$CO,5',6',7'-$^2H_3$]-4'-methoxyindole-3'-carboxaldehyde oxime (**20a**)	ND[d]	100	ND[d]
[$^2H_3$CO,5',6',7'-$^2H_3$]-4'-methoxyindole-3'-carbonitrile (**42a**)	ND[d]	69.0±2.7[b]	93.0±1.0[b]
[$^2H_3$CO,5',6',7'-$^2H_3$]-4'-methoxyindole-3'-carboxaldehyde (**24b**)	66.0±2.0[c]	52.0±1.0	ND[d]
[$^2H_3$CO,5',6',7'-$^2H_3$]desulfoglucorapassicin (**27a**)	ND[d]	ND[d]	100
[^2H_n]glucobrassicin (**14a**)	NI[d]	NI[d]	NI[d]
[^2H_n]-4'-methoxyglucobrassicin (**16a**)	NI[d]	NI[d]	NI[d]
[^2H_n]-1'-methoxyglucobrassicin (**15a**)	NI[d]	NI[d]	NI[d]

[a] Deuterated compounds are referred to by a number followed by the letters **a** or **b**.

[b] Negative ion mode. Incorporations calculated from HPLC-ESI-MS (normalized peak intensities); % of ^2H incorporation = $\{[M\text{-}1 + n]^-/([M\text{-}1]^- + [M\text{-}1 + n]^-)\} \times 100$ (n = 4, 5 or 6) ± Std (standard deviation), where n = number of deuterium atoms.

[c] Positive ion mode. Incorporations calculated from HPLC-ESI-MS (normalized peak intensities); % of ^2H incorporation = $\{[M + 1 + n]^+/([M + 1]^+ + [M + 1 + n]^+)\} \times 100$ (n = 3, 4, 5 or 6) ± Std (standard deviation), where n = number of deuterium atoms.

[d] NI=no incorporation implies ≤0.1% deuterium; ND=not detected.

Table S3 Metabolites of [2,2,4',5',6',7'-$^2H_6$]glucobrassicin (14a) and [4',5',6',7'-$^2H_4$]desulfoglucobrassicin (34a) in UV–elicited rutabaga roots.

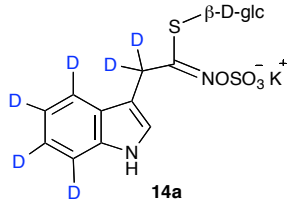
Metabolites detected (**#a**)[a]	% Incorporation ± Std		Metabolites detected (**#a**)[a]
	Glucobrassicin (**14a**)	Desulfoglucobrass. (**34a**)	
[5',6',7'-$^2H_3$]rapalexin A (**10a**)	3.9±1.1[b]	4.0±1.7[b]	[5',6',7'-$^2H_3$]rapalexin A (**10a**)
[5',6',7'-$^2H_3$]isocyalexin A (**12a**)	2.4±0.8[b]	3.0±0.3[b]	[5',6',7'-$^2H_3$]isocyalexin A (**12a**)
[5,6,7-$^2H_3$]isalexin (**13a**)	3.2±1.1[c]	≤0.5[c]	[5,6,7-$^2H_3$]isalexin (**13a**)
[2,2,4',5',6',7'-$^2H_6$]cyclobrassinin (**4b**)	1.9±0.7[c]	2.4±0.3[b,c]	[4',5',6',7'-$^2H_4$]cyclobrassinin (**4a**)
[2,2,4',5',6',7'-$^2H_6$]spirobrassinin (**7b**)	2.6±0.6[b,c]	2.0±0.6[b,c]	[4',5',6',7'-$^2H_4$]spirobrassinin (**7a**)
[4',5',6',7'-$^2H_4$]rutalexin (**9a**)	NI[d]	1.8±0.3[b,c]	[4',5',6',7'-$^2H_4$]rutalexin (**9a**)
[2,2,4',5',6',7'-$^2H_6$]glucobrassicin (**14a**)	18.8±5.1[b]	10.1±2.0[b]	[4',5',6',7'-$^2H_4$]glucobrassicin (**14a**)
[2,2,5',6',7'-$^2H_5$]-4'-methoxyglucobrassicin (**16a**)	5.0±0.4[b]	3.8±2.0[b]	[5',6',7'-$^2H_3$]-4'-methoxyglucobrassicin (**16a**)
[2,2,4',5',6',7'-$^2H_6$]-1'-methoxyglucobrassicin (**15a**)	1.1±0.2[b]	2.8±0.5[b]	[4',5',6',7'-$^2H_4$]-1'-methoxyglucobrassicin (**15a**)
[4',5',6',7'-$^2H_4$]desulfoglucobrassicin (**34a**)	NI[d]	64.0±0.5[b]	[4',5',6',7'-$^2H_4$]desulfoglucobrassicin (**34a**)

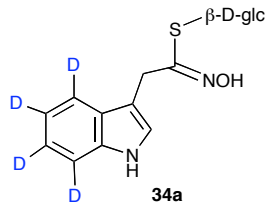
[a] Deuterated compounds are referred to by a number followed by the letters **a** or **b**.

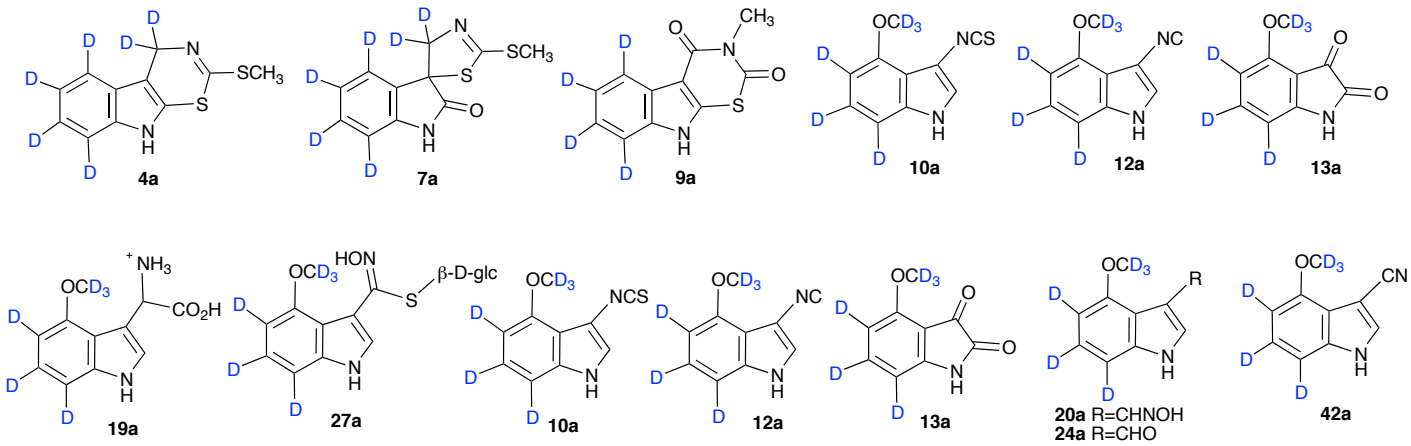
[b] Negative ion mode. Incorporations calculated from HPLC-ESI-MS (normalized peak intensities); % of ^2H incorporation = $\{[M-1+n]^-/([M-1]^- + [M-1+n]^-)\} \times 100$ (n = 3, 4, 5 or 6) ± Std (standard deviation), where n = number of deuterium atoms.

[c] Positive ion mode. Incorporations calculated from HPLC-ESI-MS (normalized peak intensities); % of ^2H incorporation = $\{[M+1+n]^+/([M+1]^+ + [M+1+n]^+)\} \times 100$ (n = 3, 4, 5 or 6) ± Std (standard deviation), where n = number of deuterium atoms.

[d] NI=no incorporation implies ≤0.1% deuterium; ND=not detected.

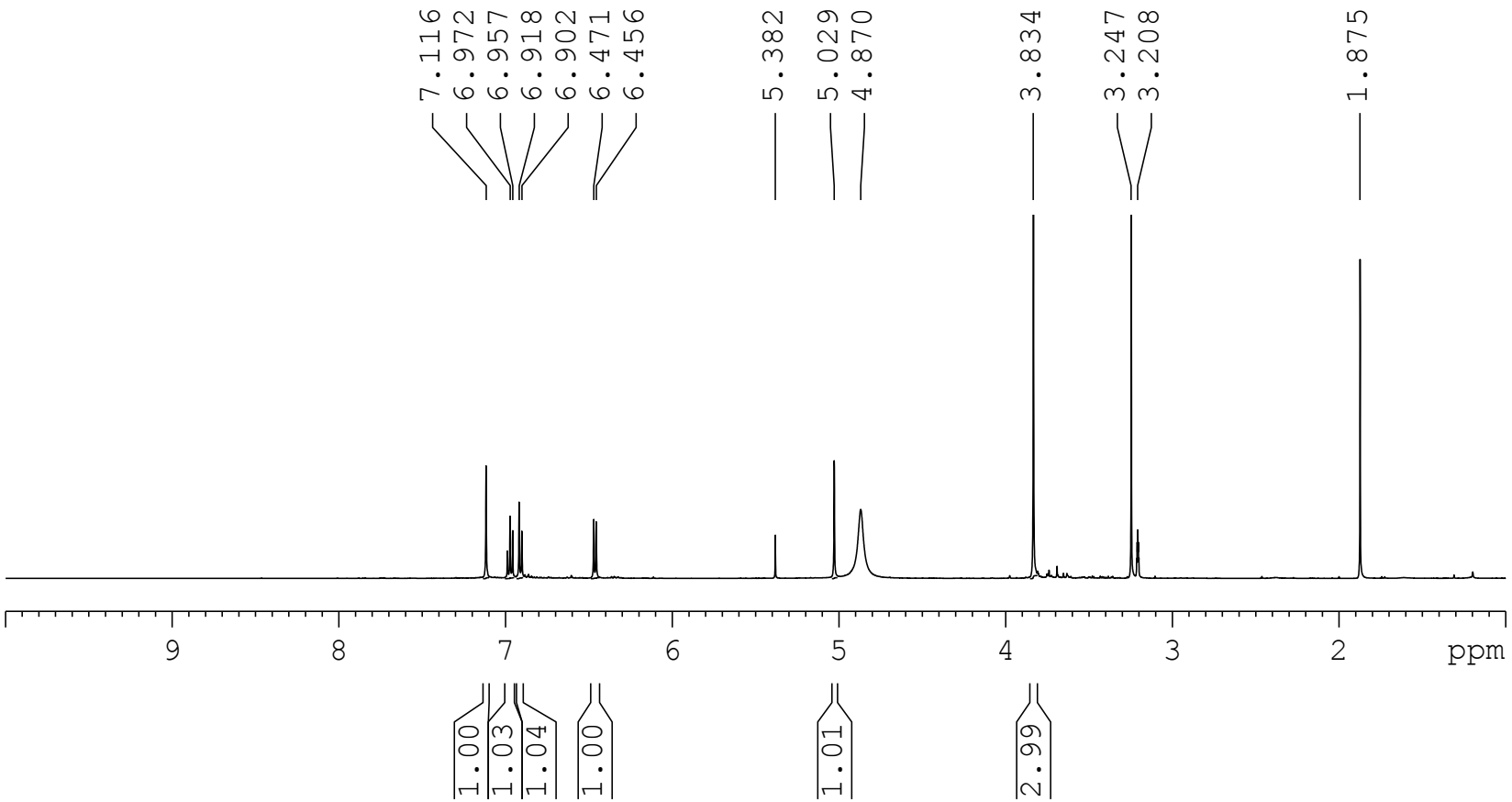






^{1}H and ^{13}C NMR spectra of compounds 19, 19a, 20, 20a, 24, 24b, 27, 27a, 29, 29a, 30, 30a, 31a, 32a, 33a, 34, and 34a.

^{1}H NMR of **19**



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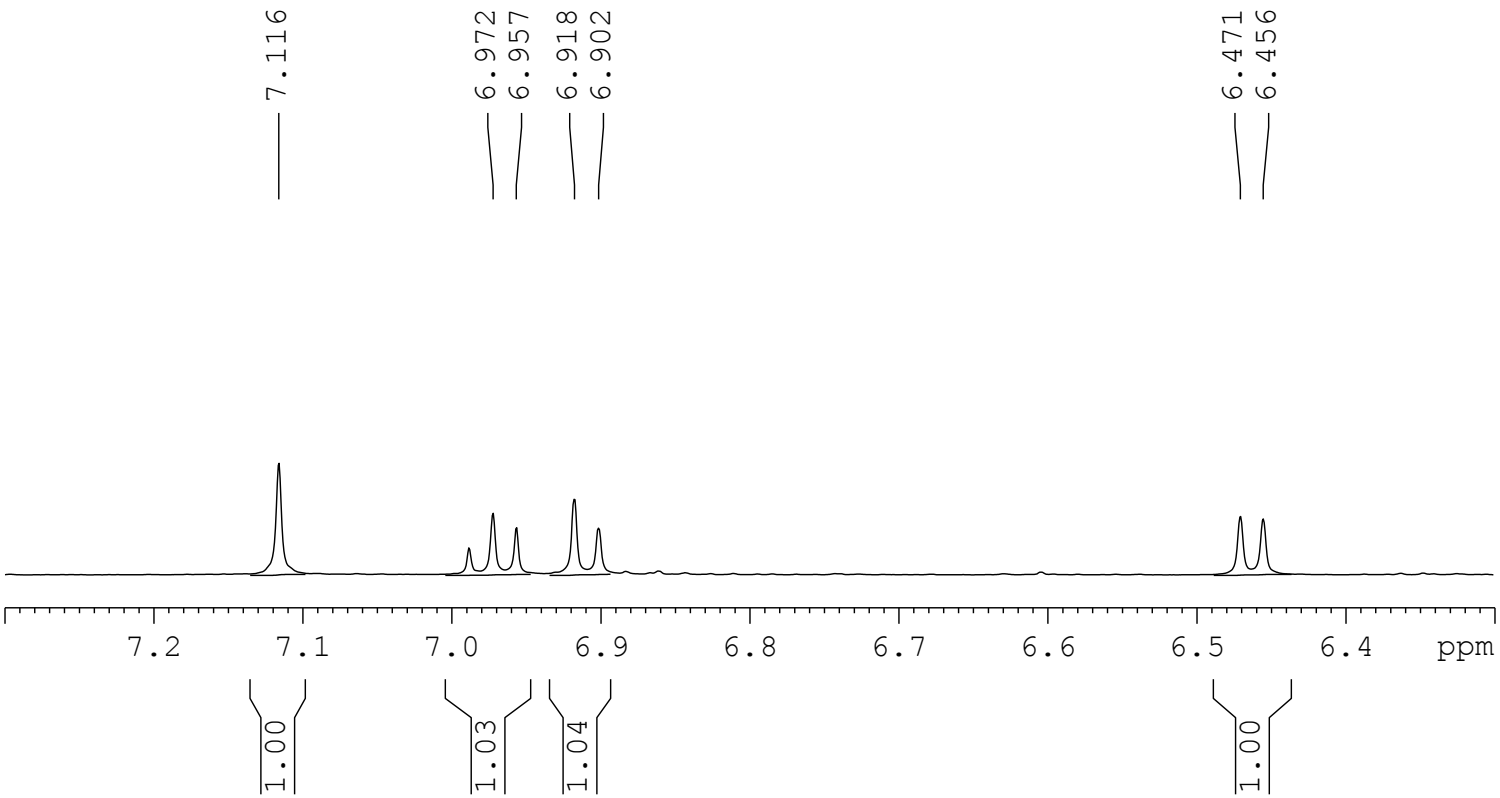

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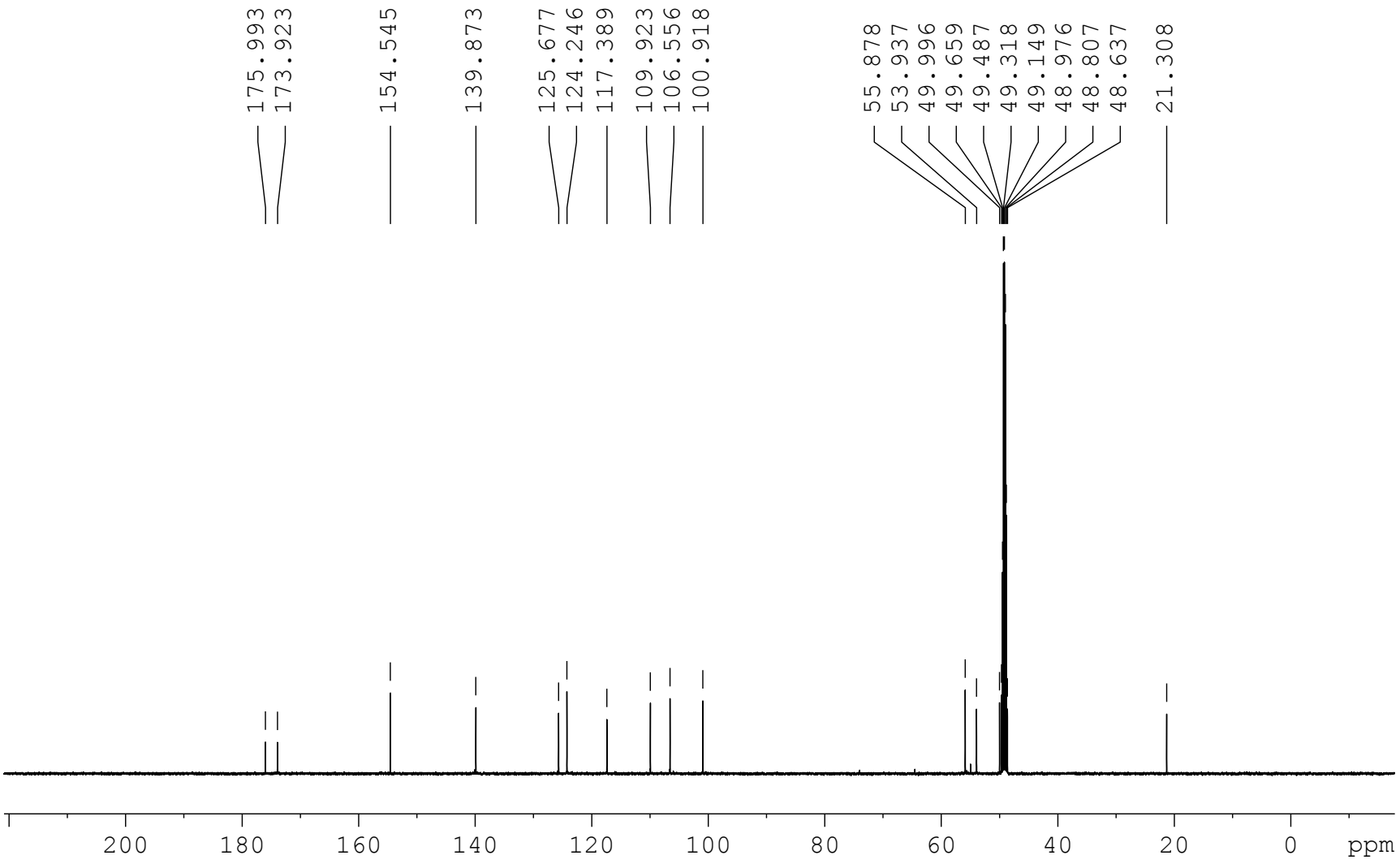

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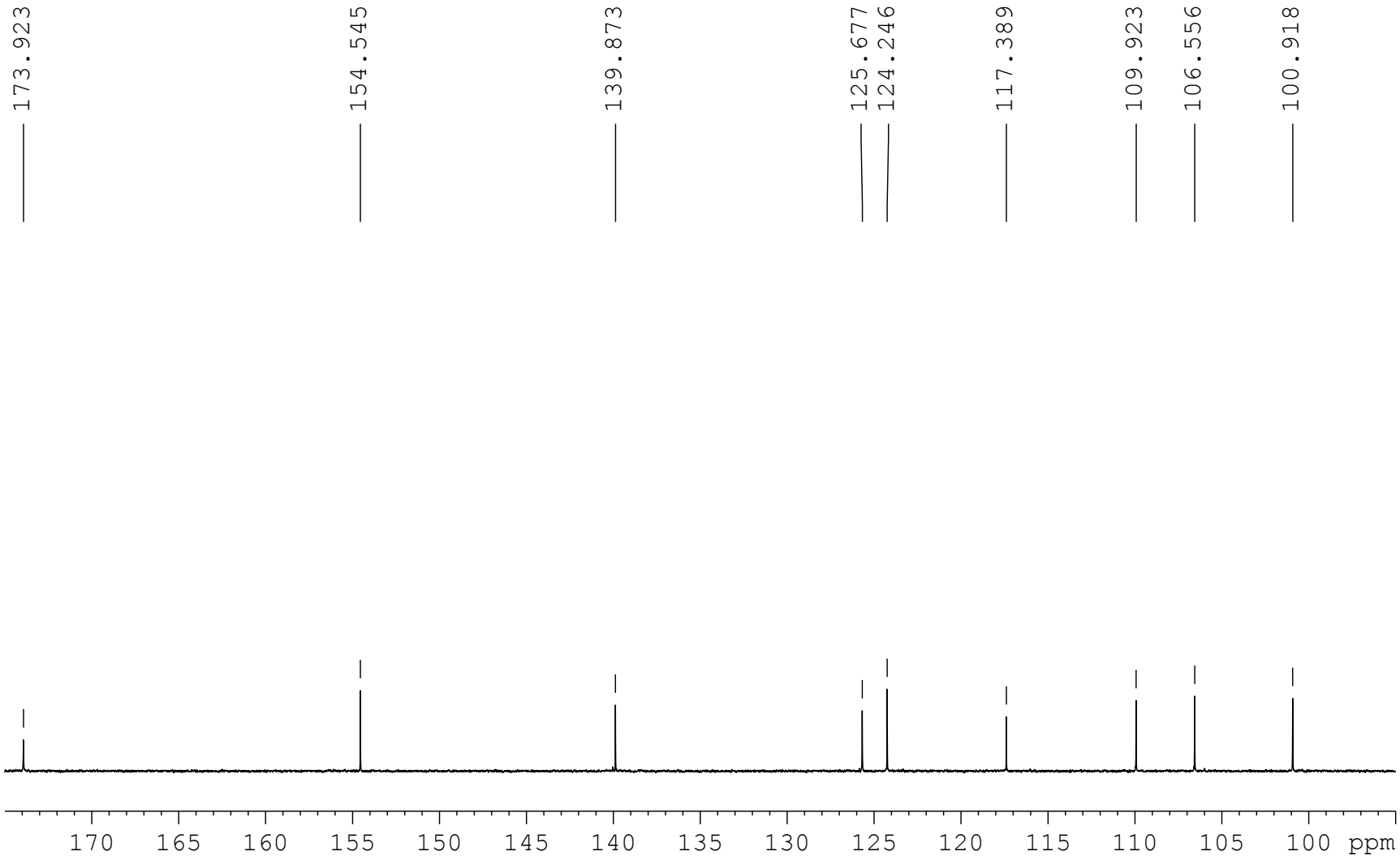
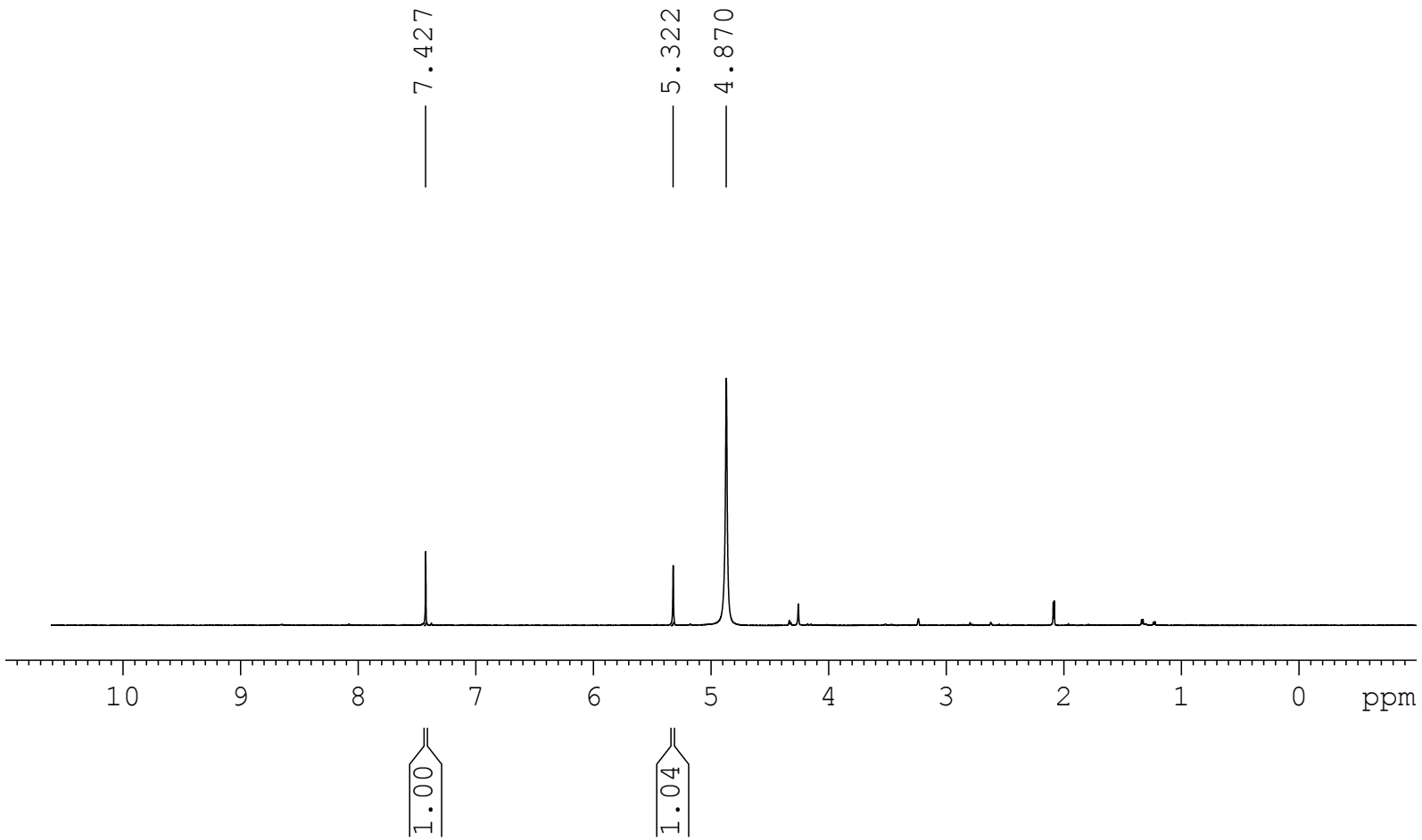


^{1}H NMR of **19a**



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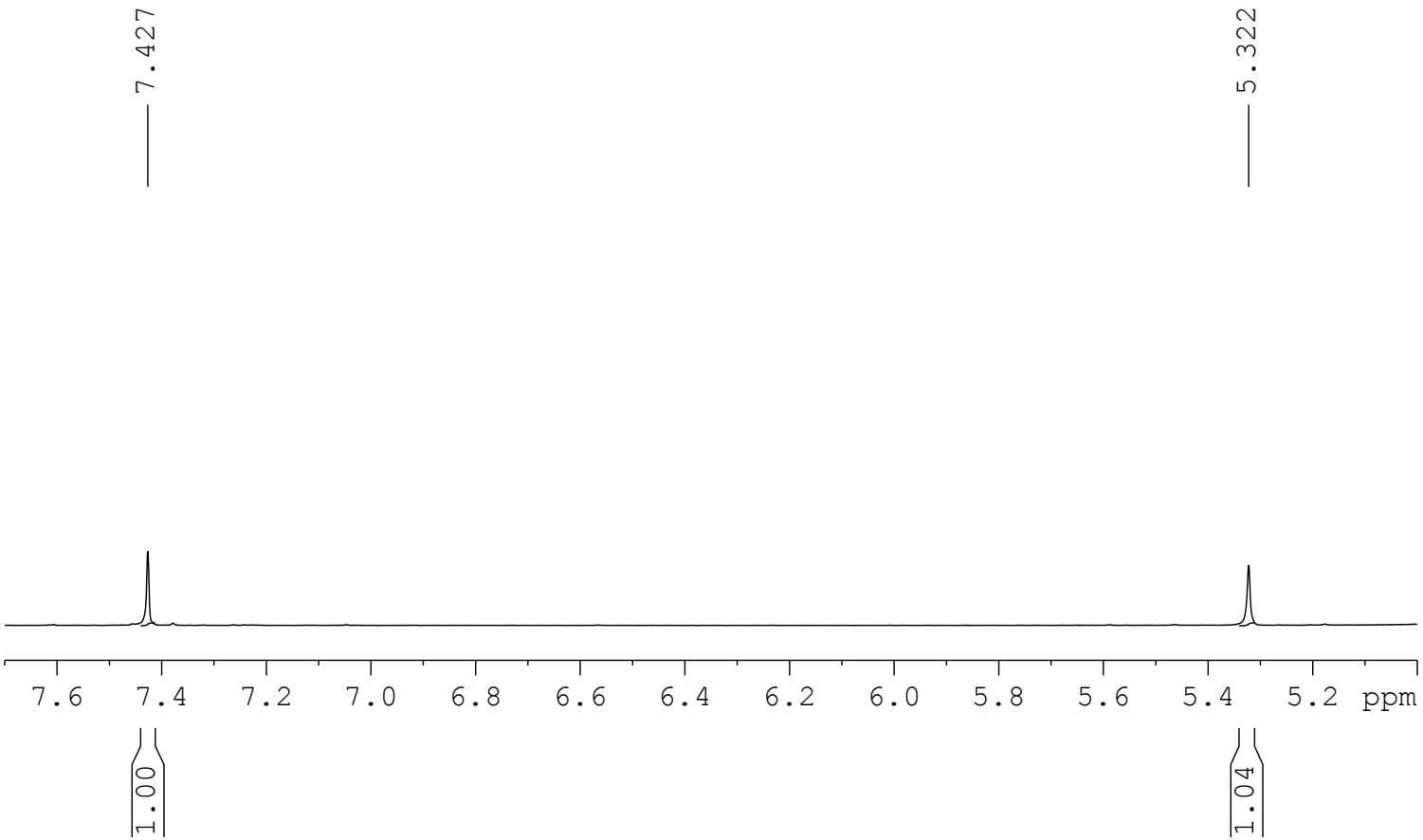

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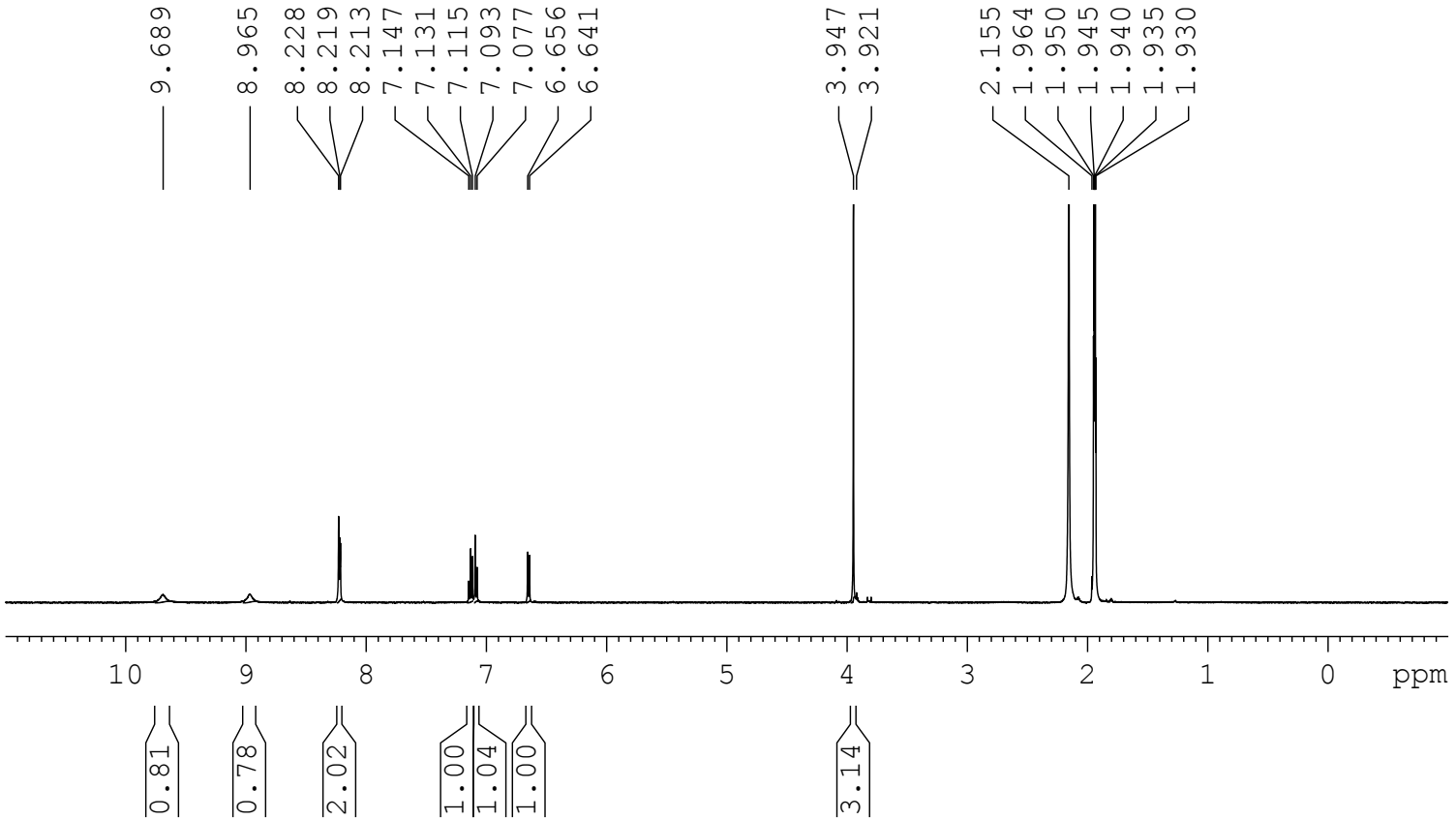

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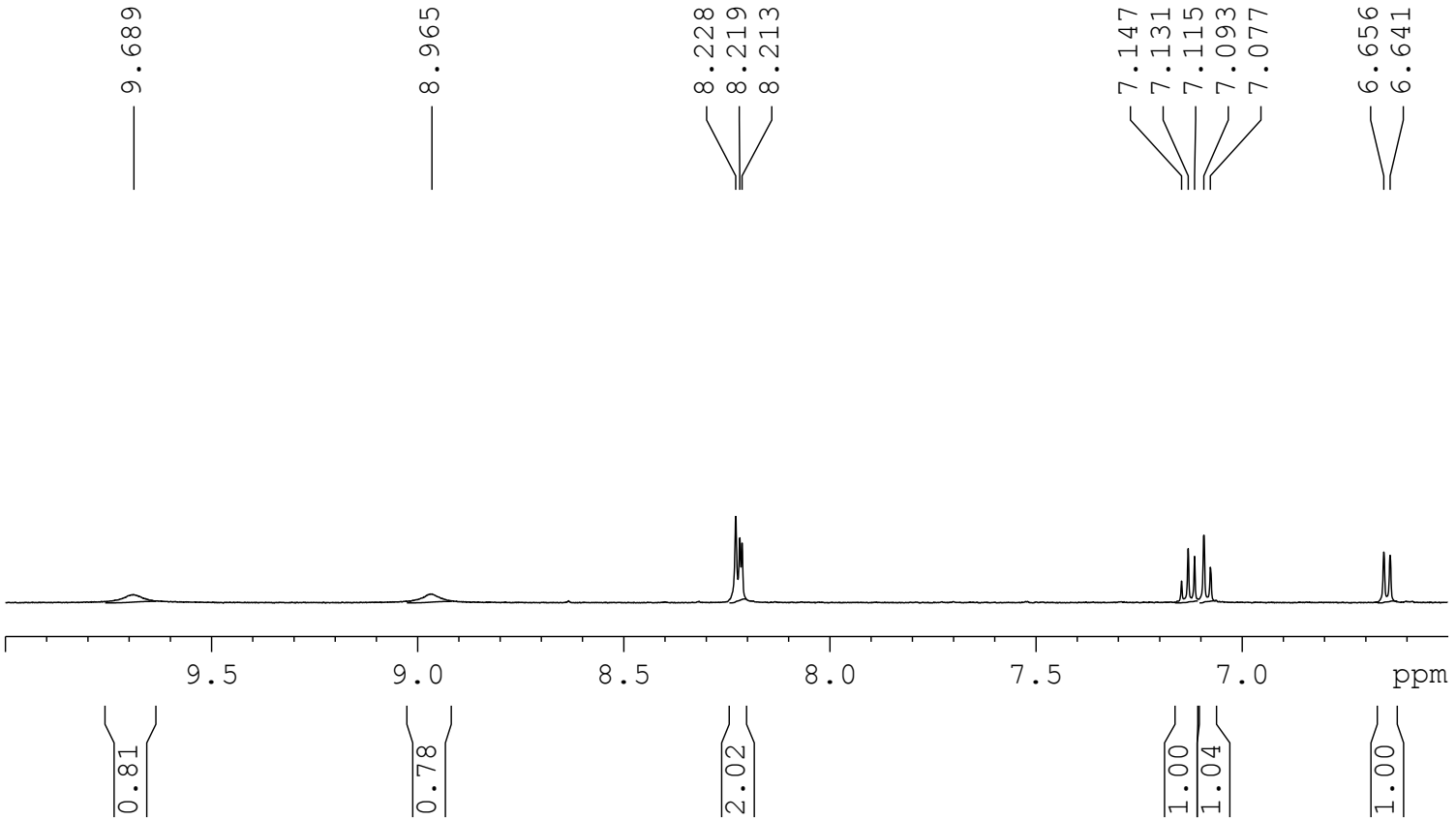

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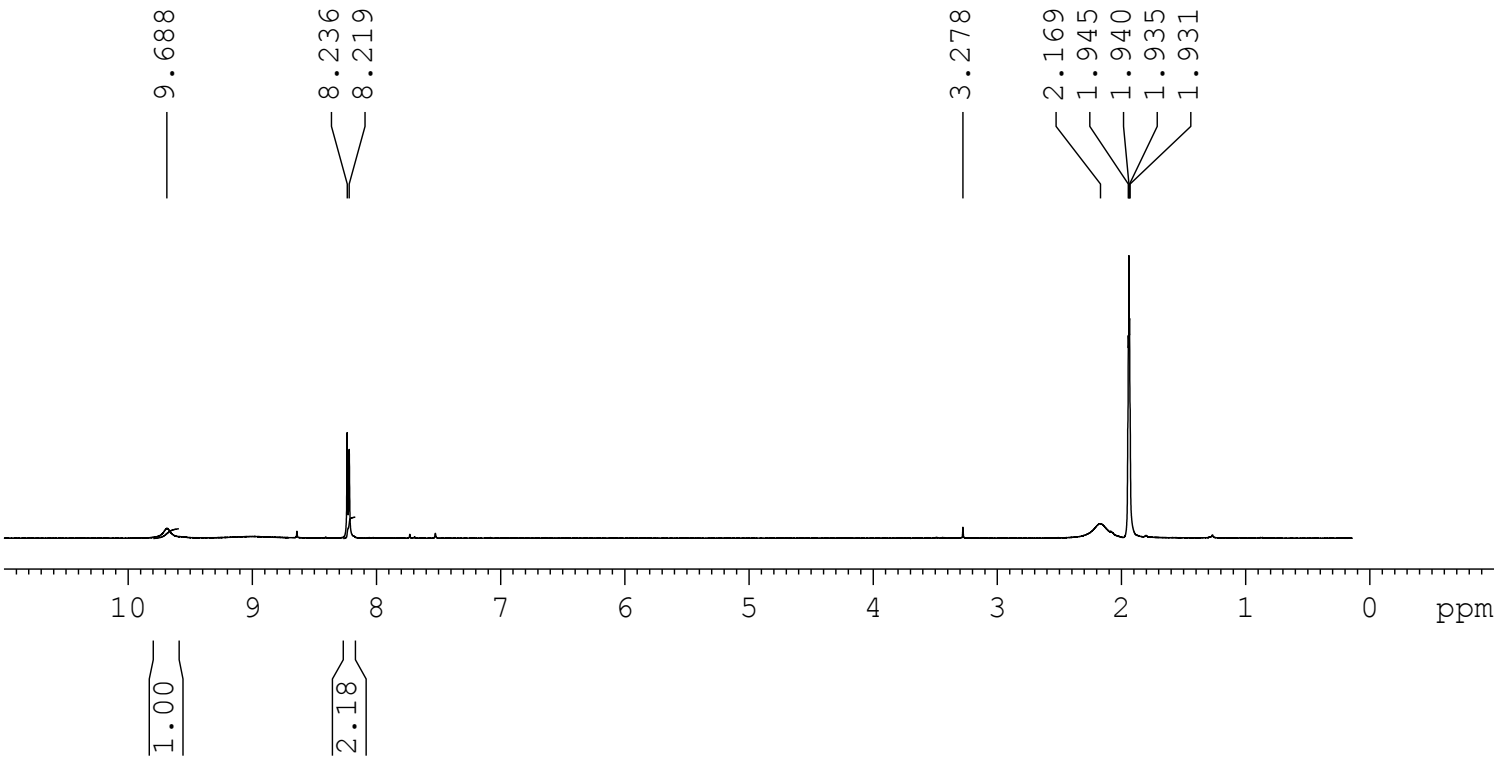

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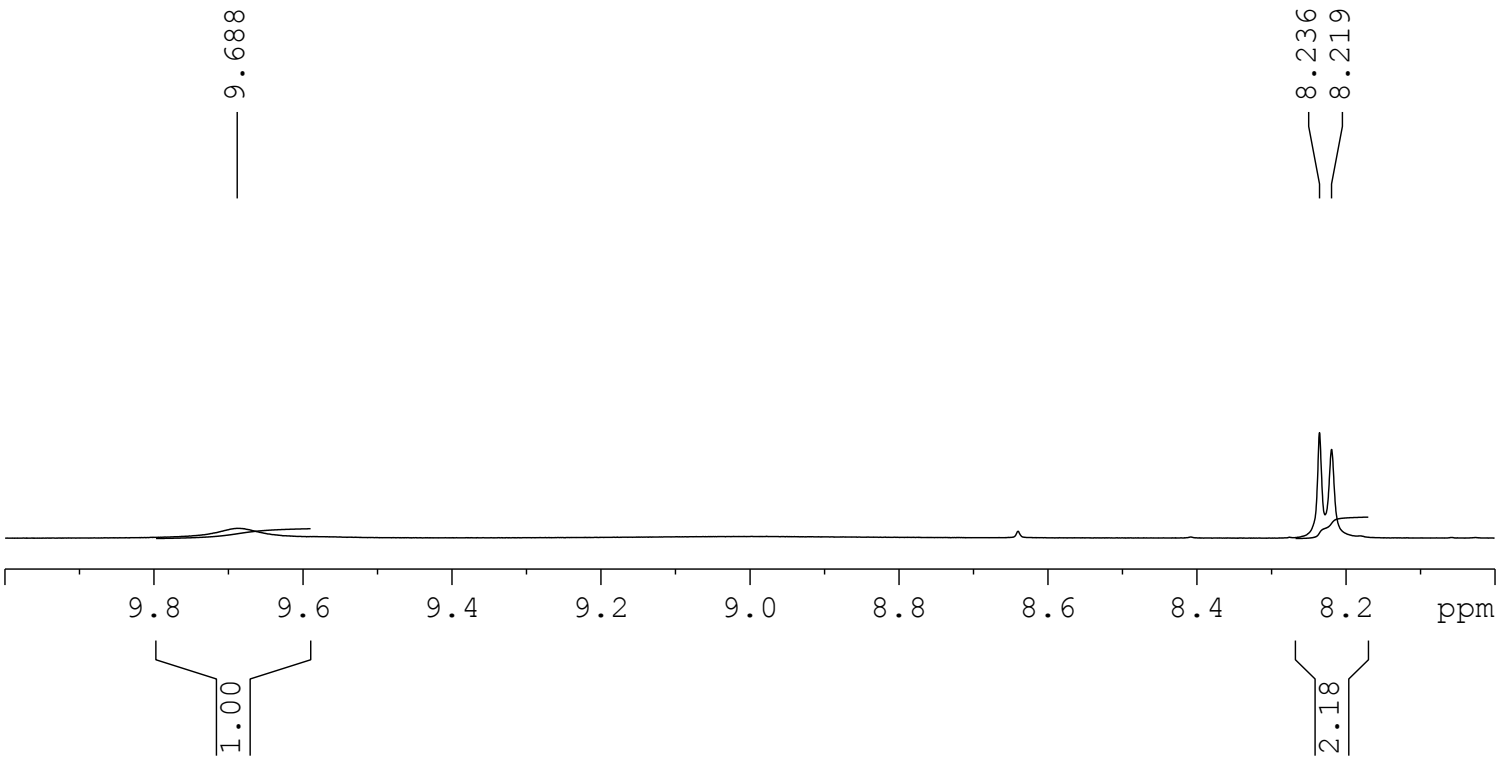

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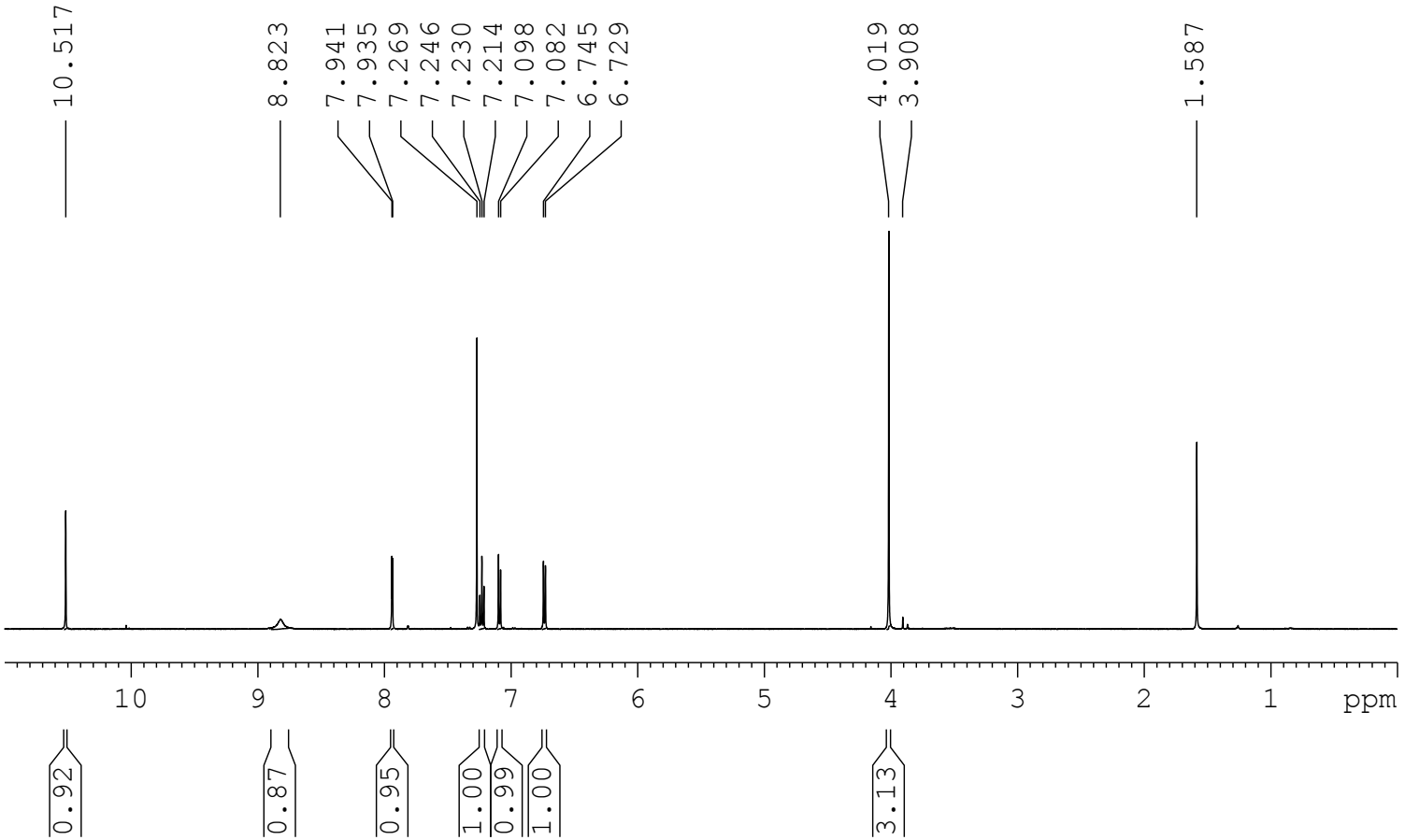

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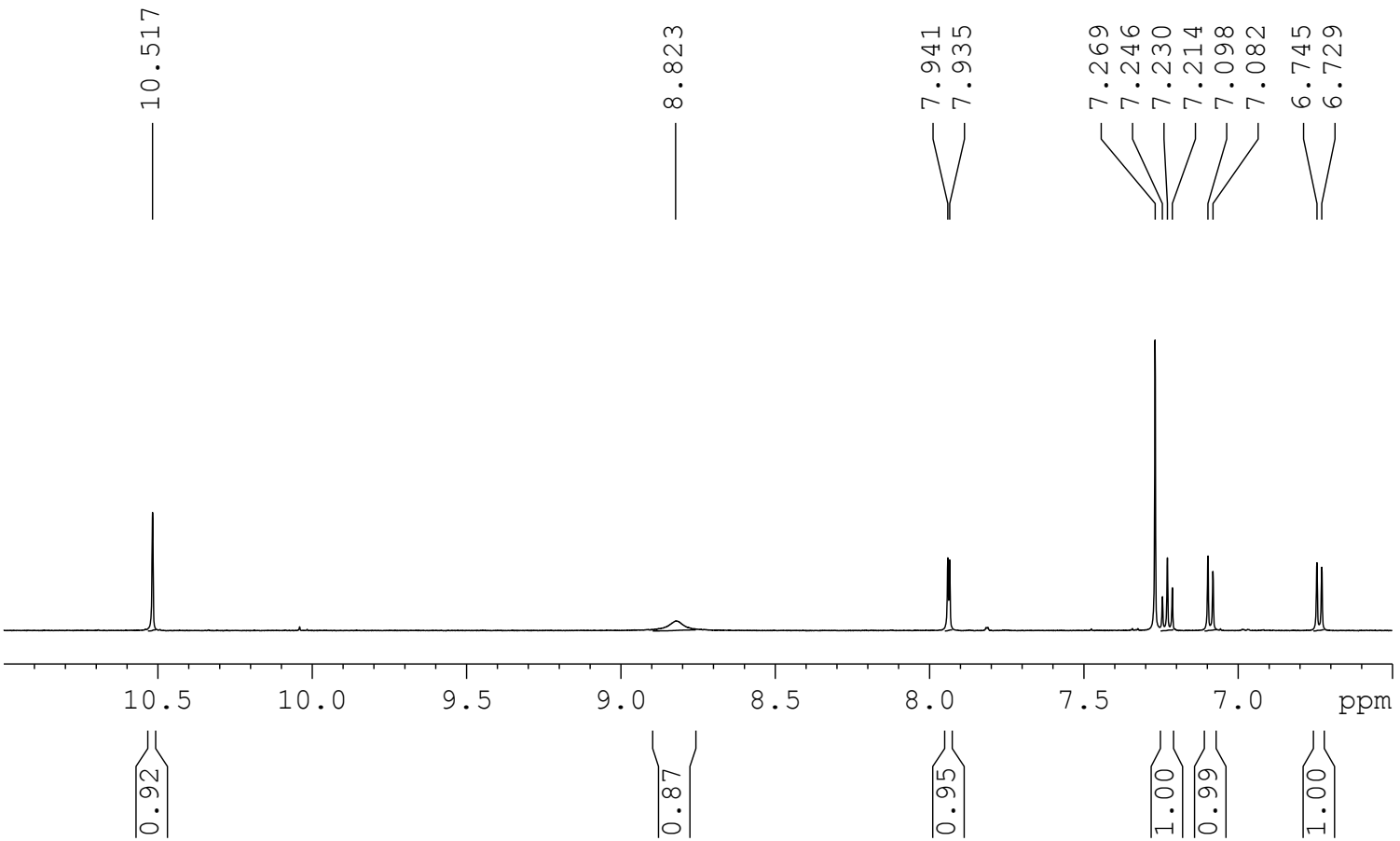

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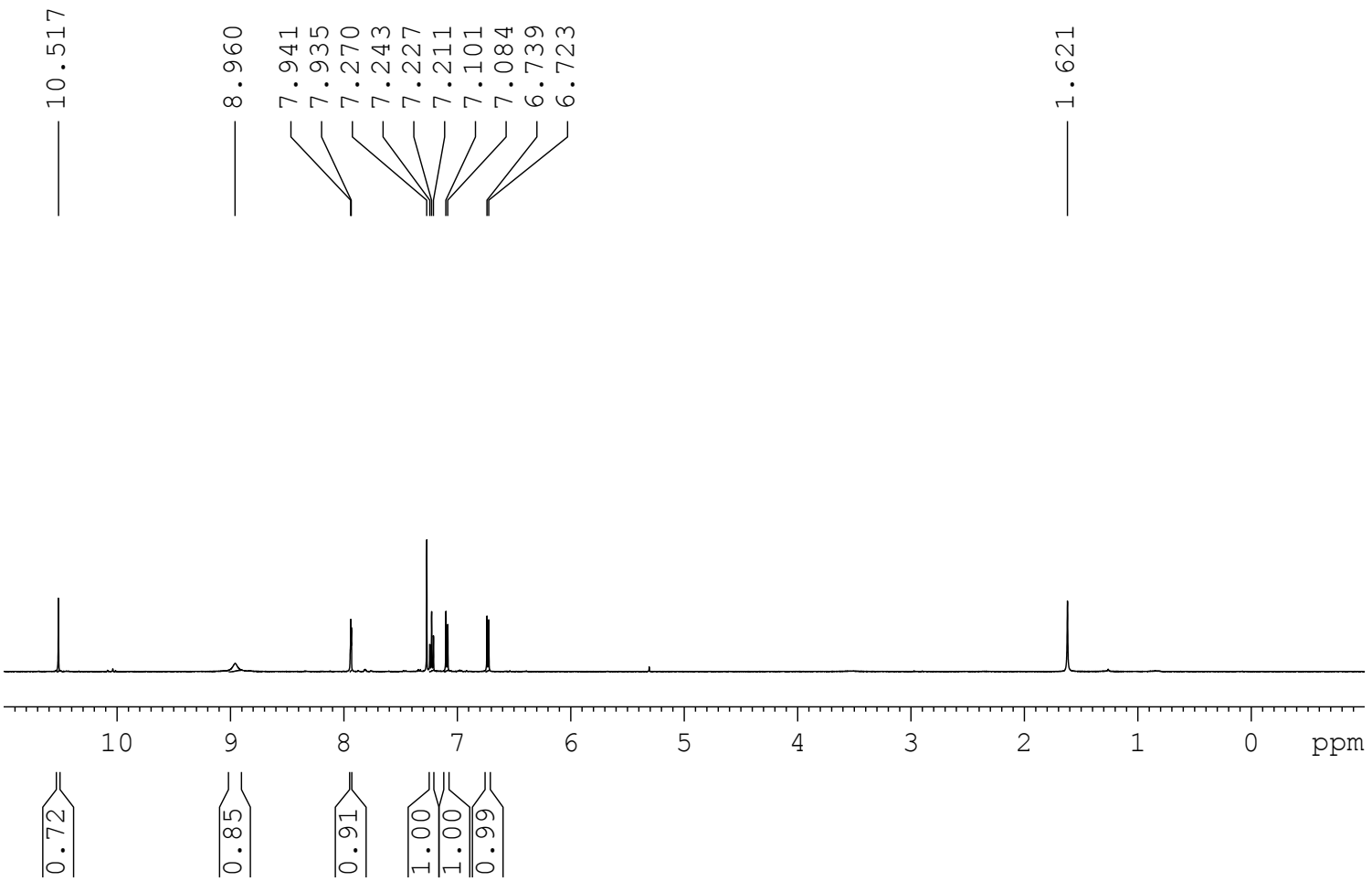

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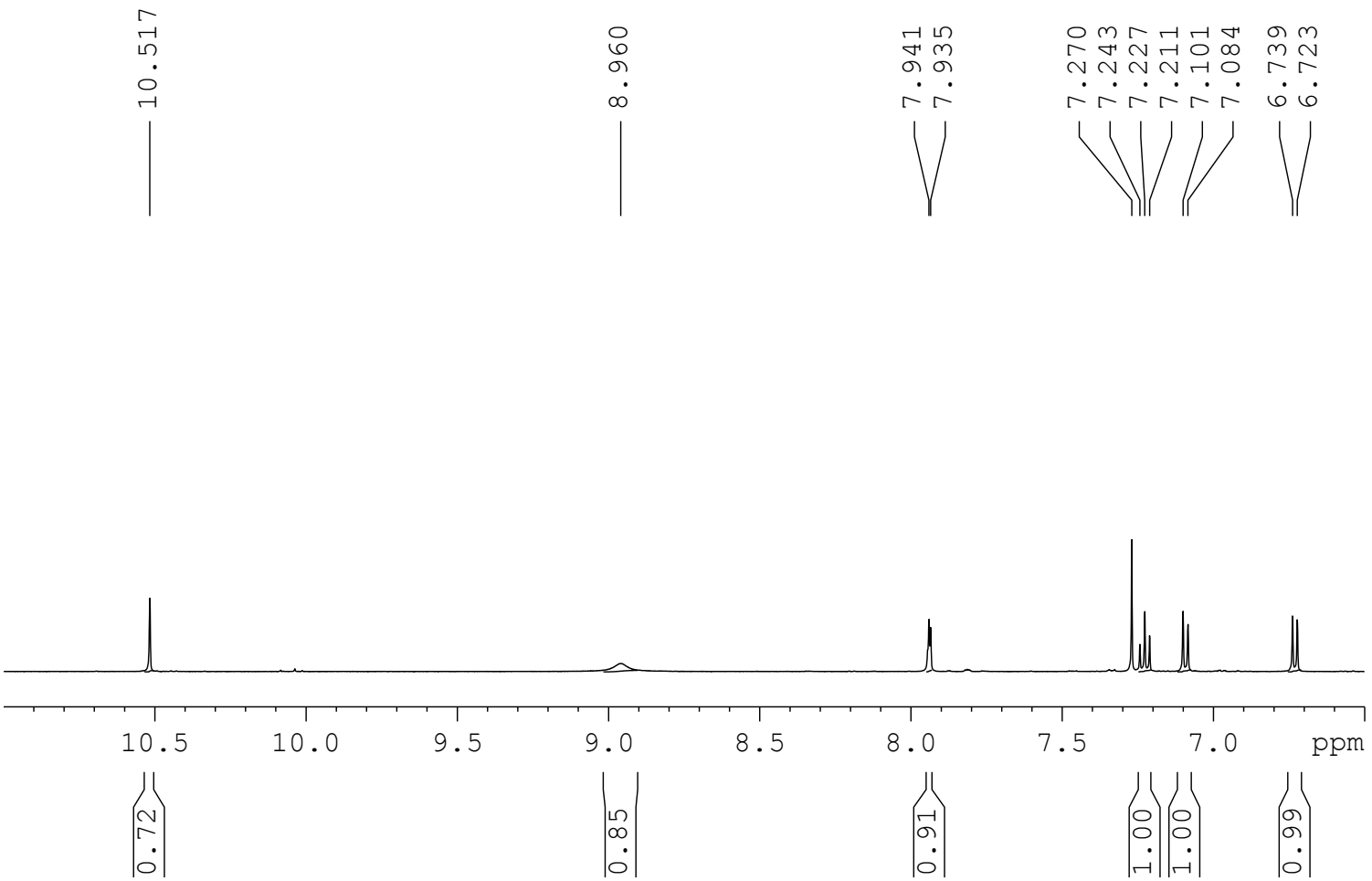

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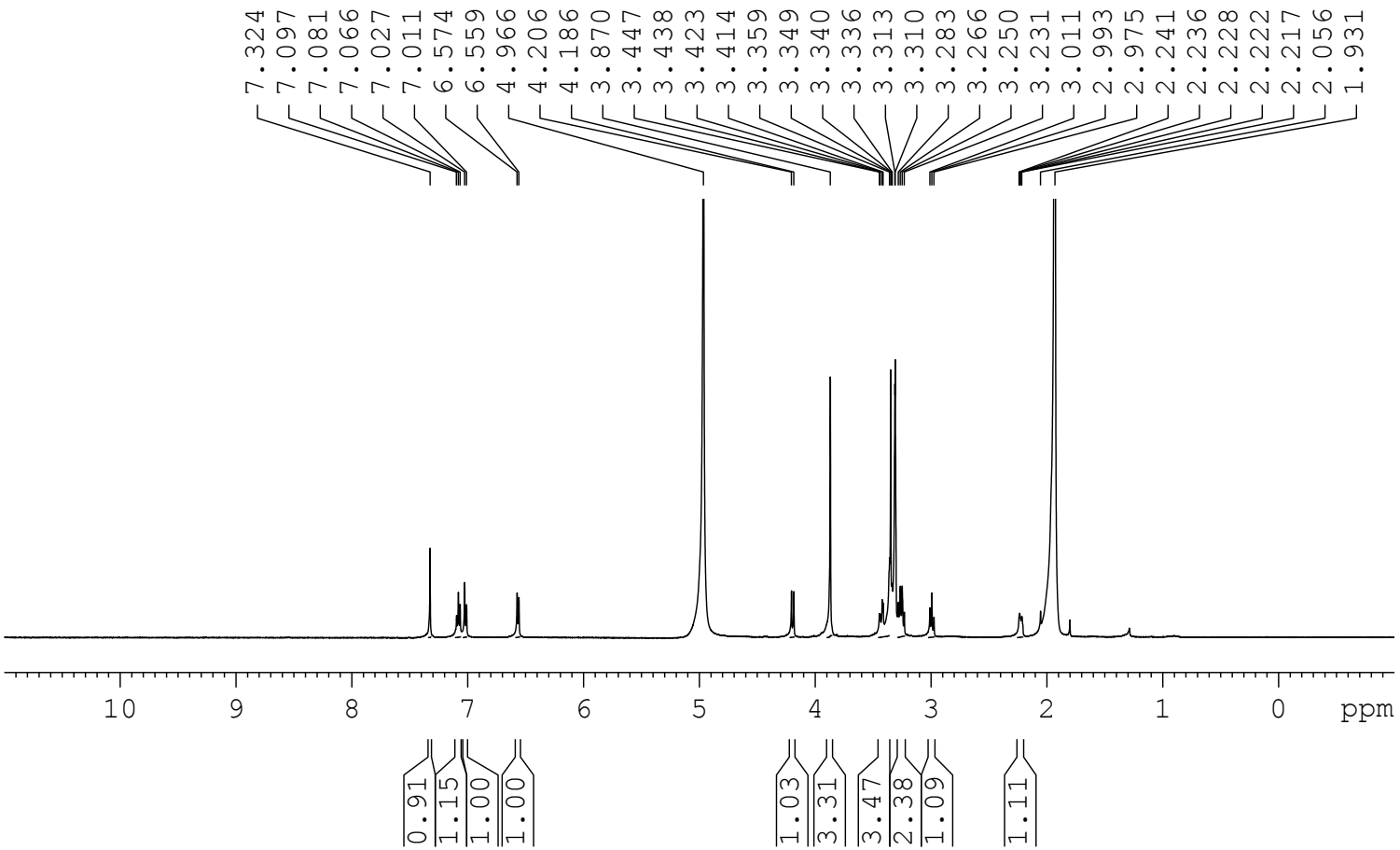

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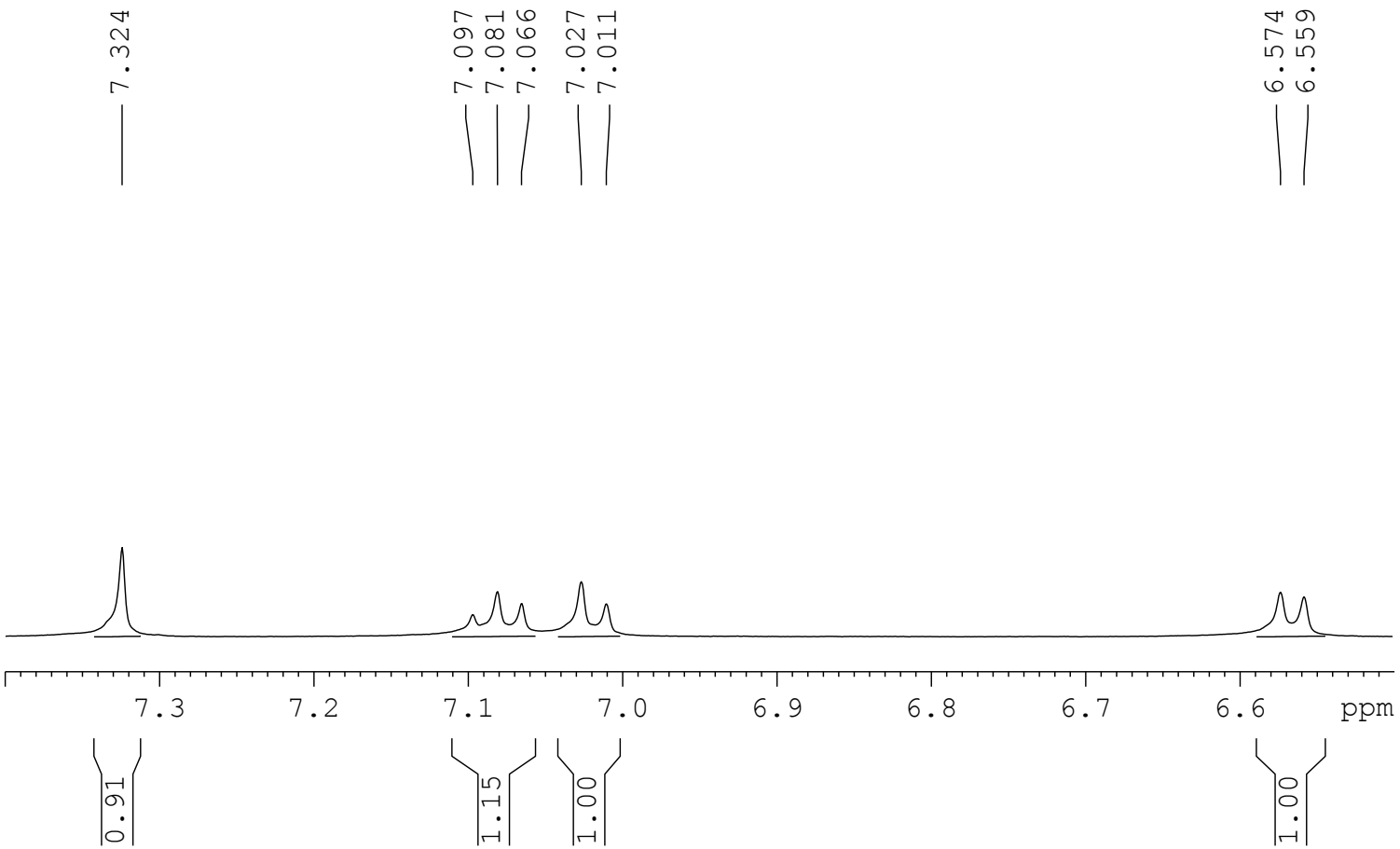

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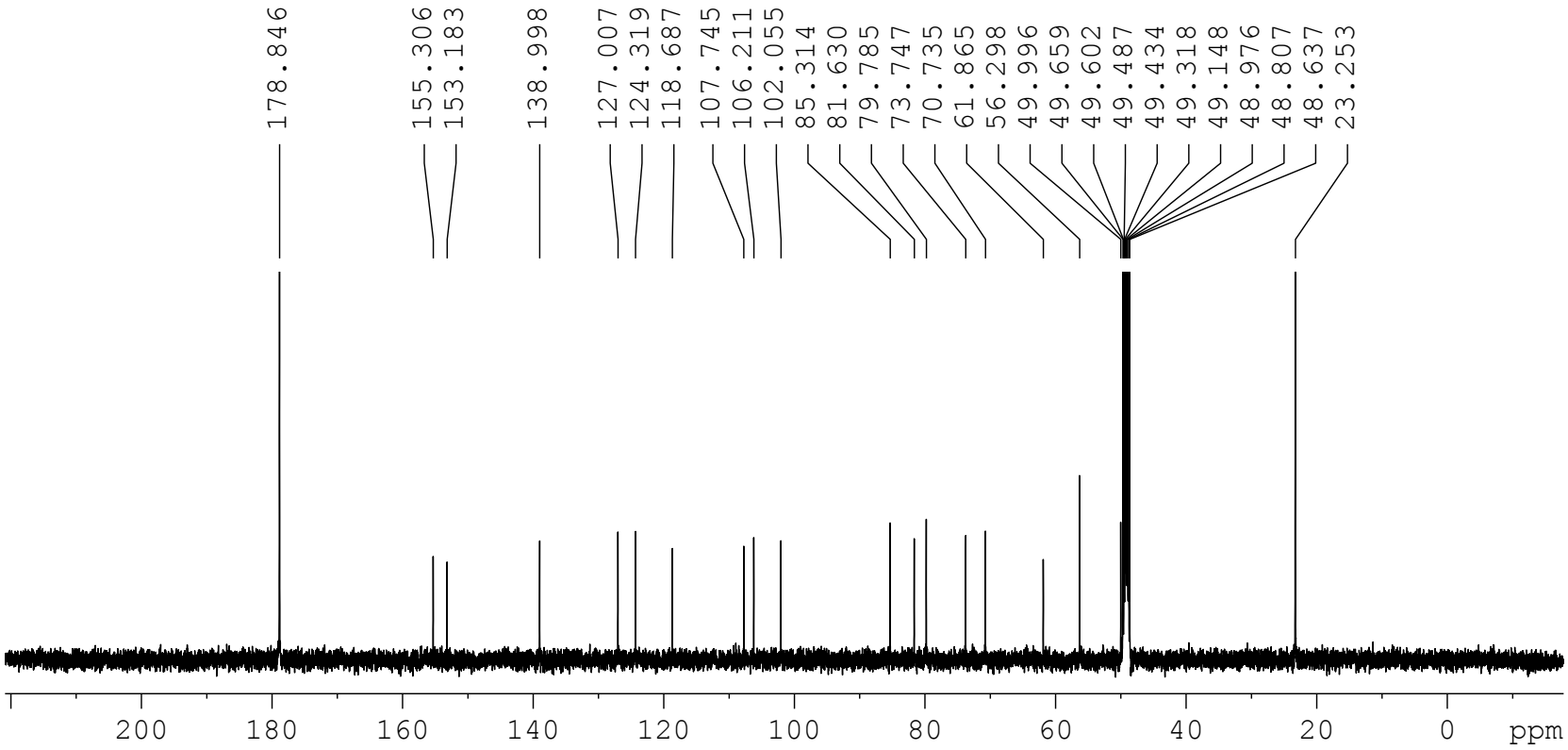

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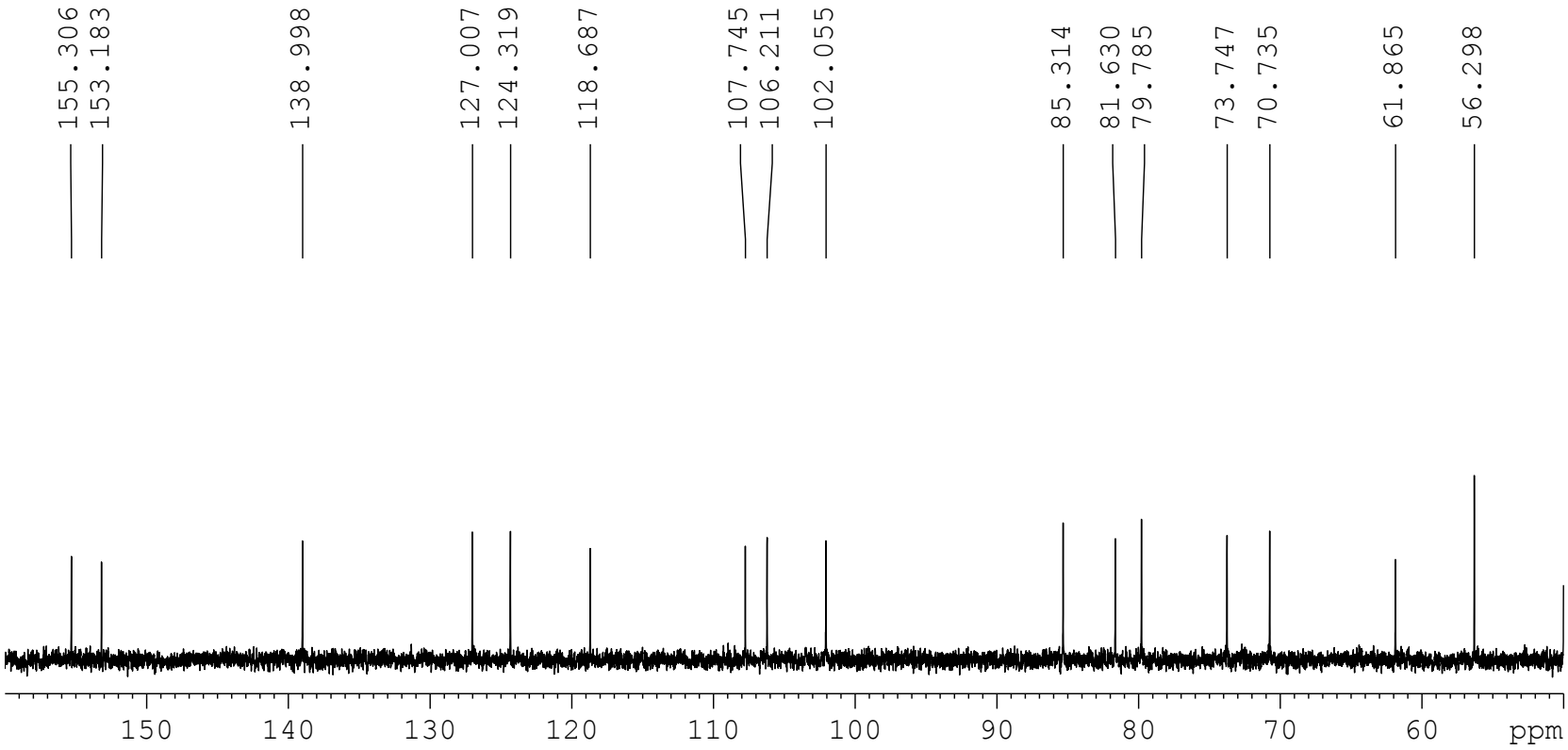

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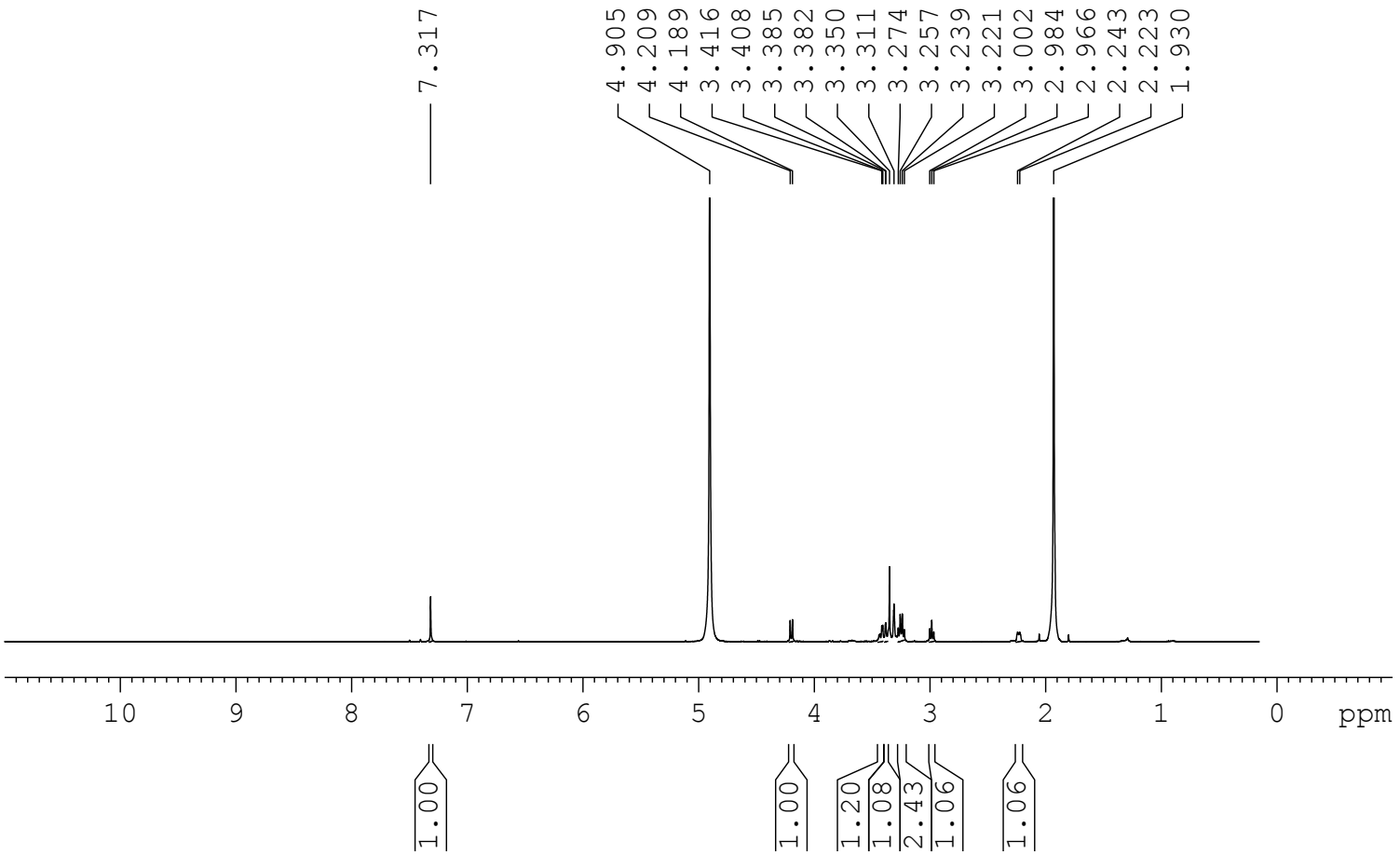
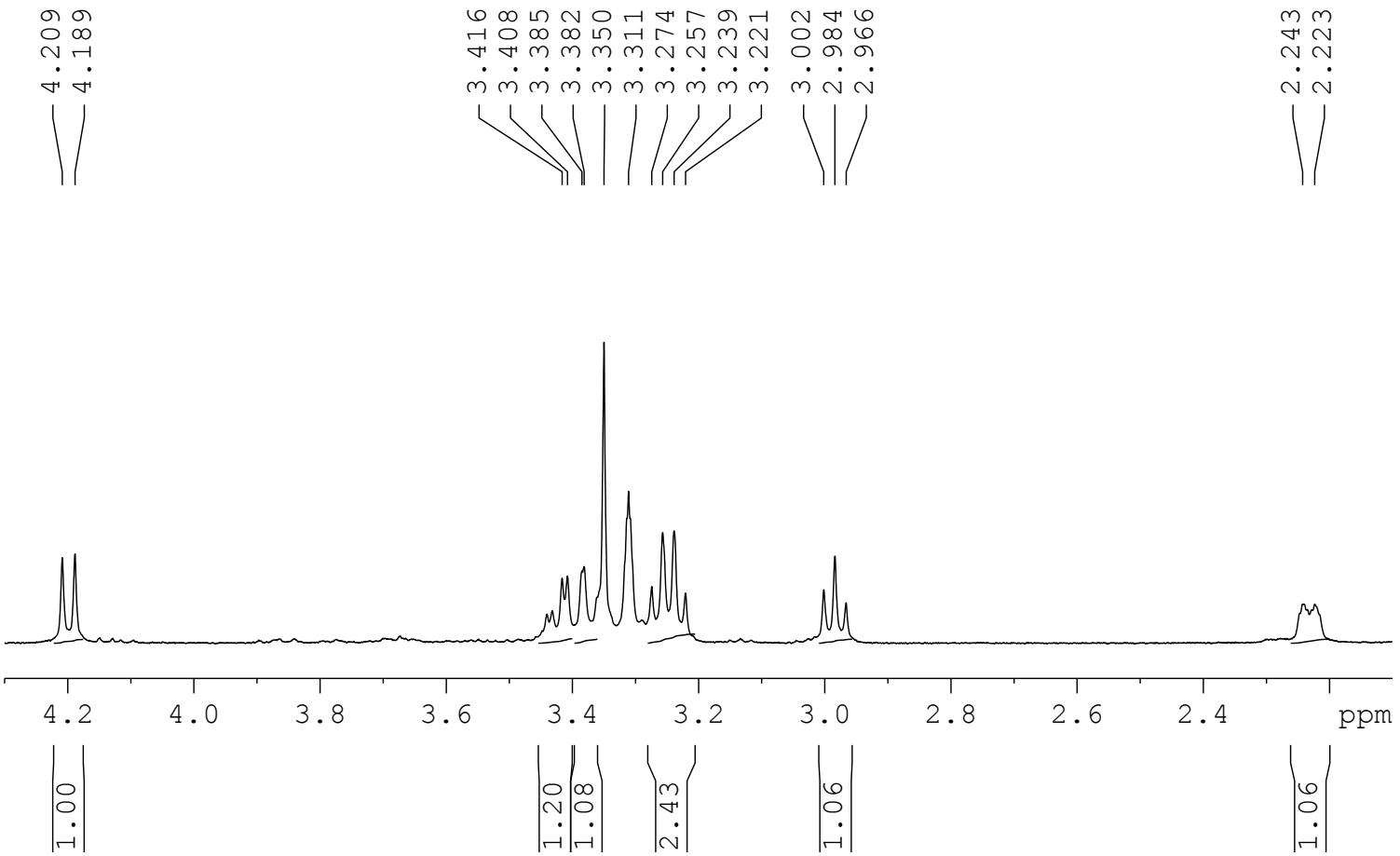
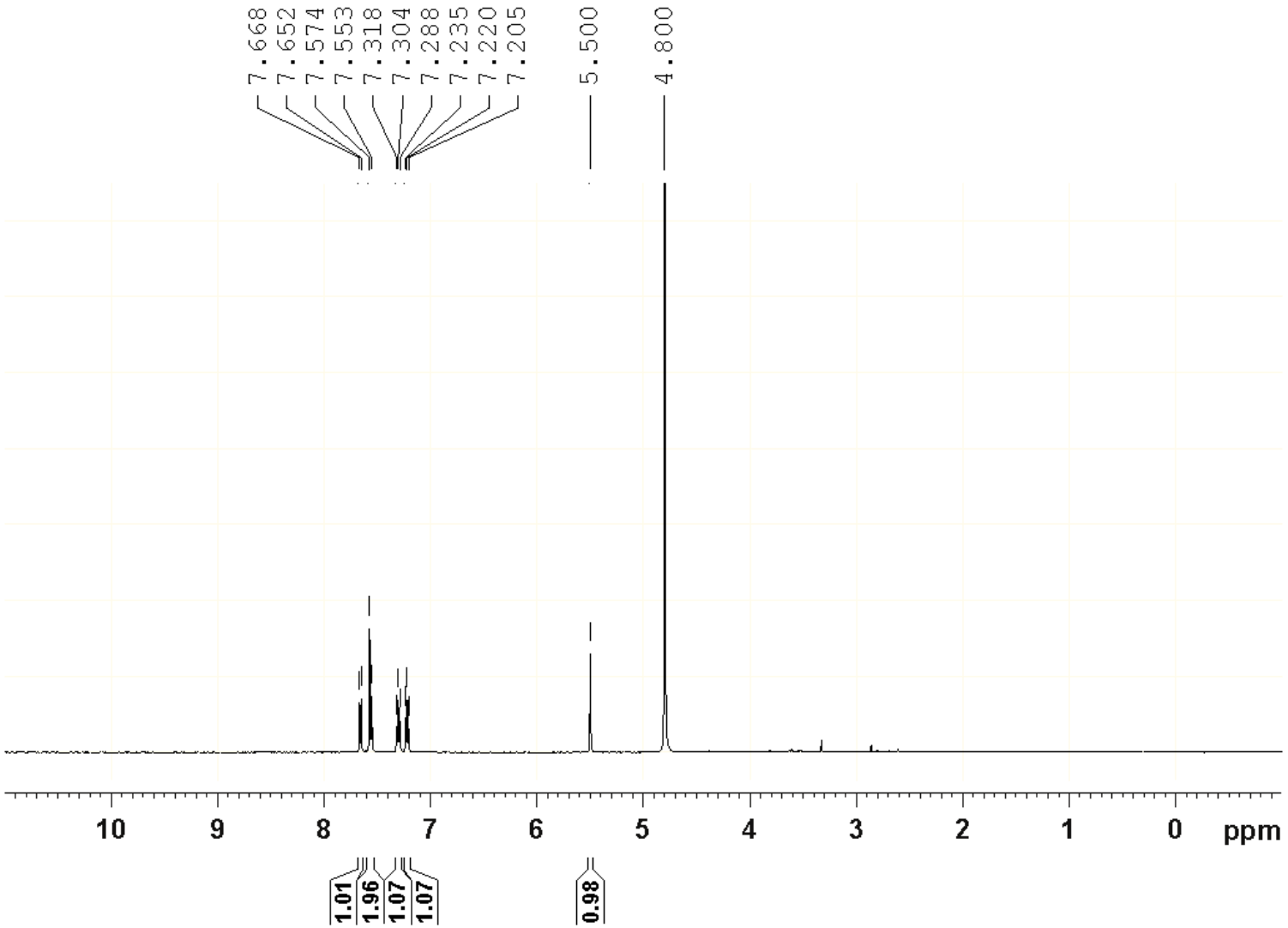


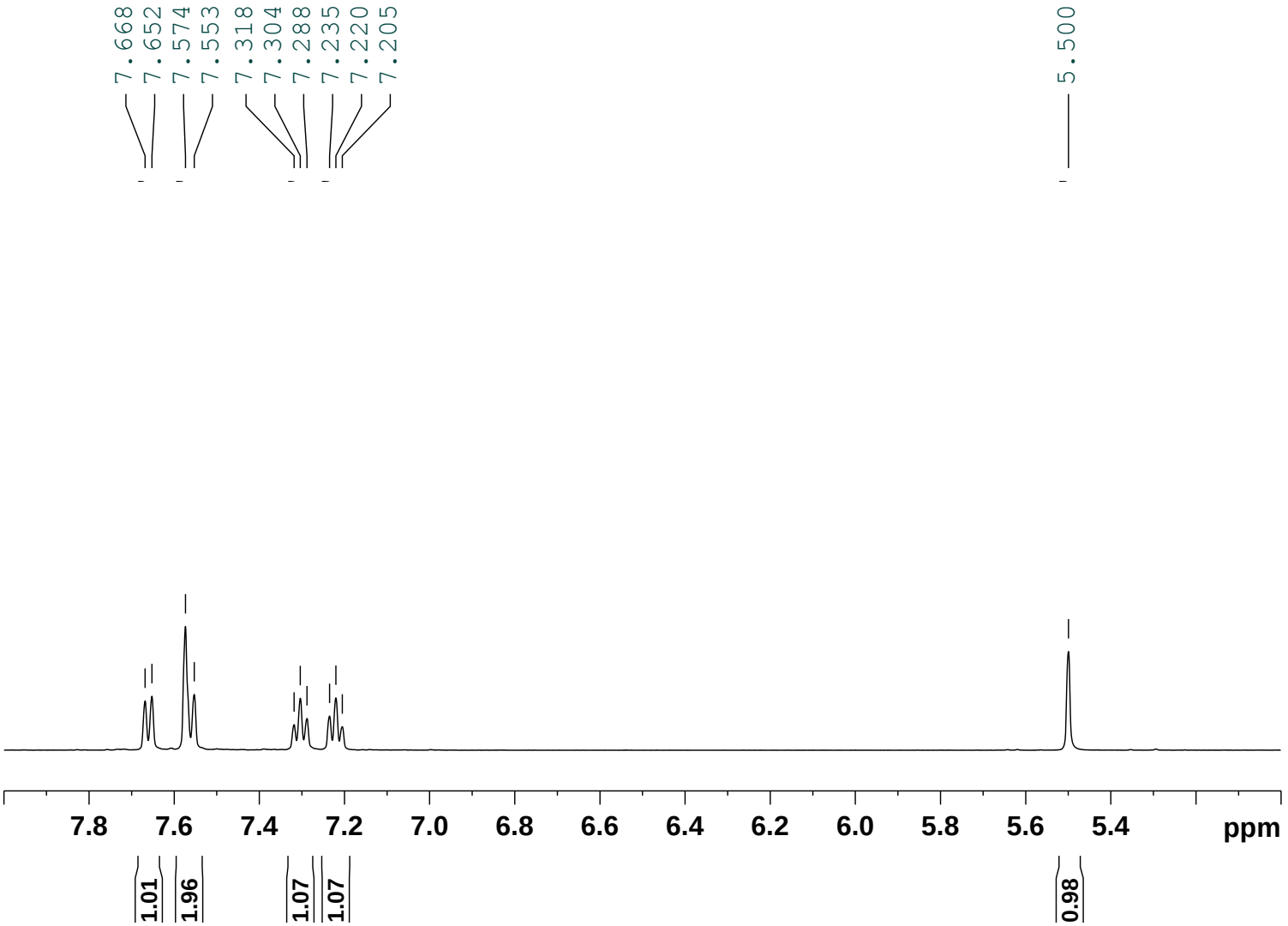
^{1}H NMR of **27a**



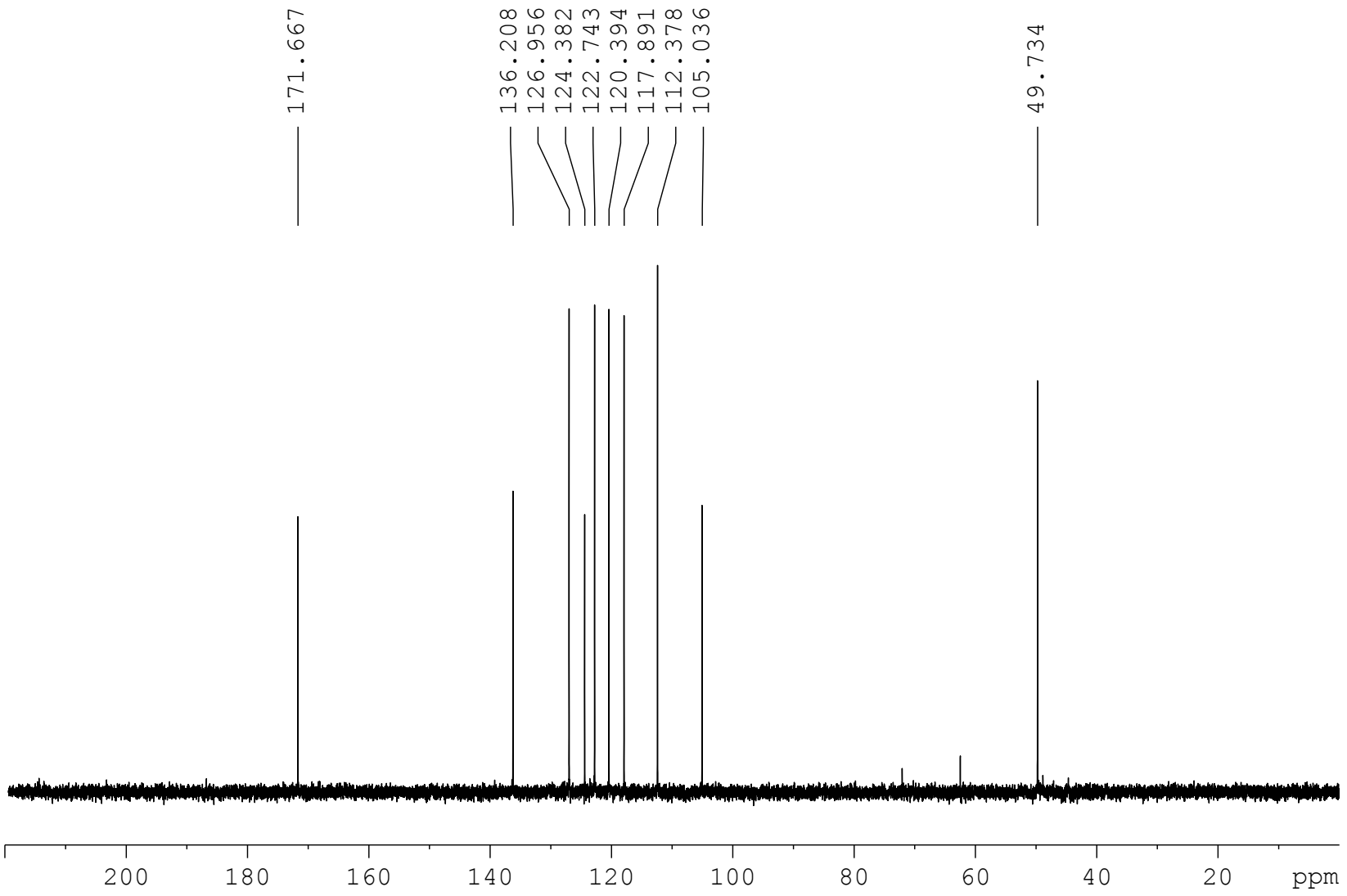
^{1}H NMR of **29**



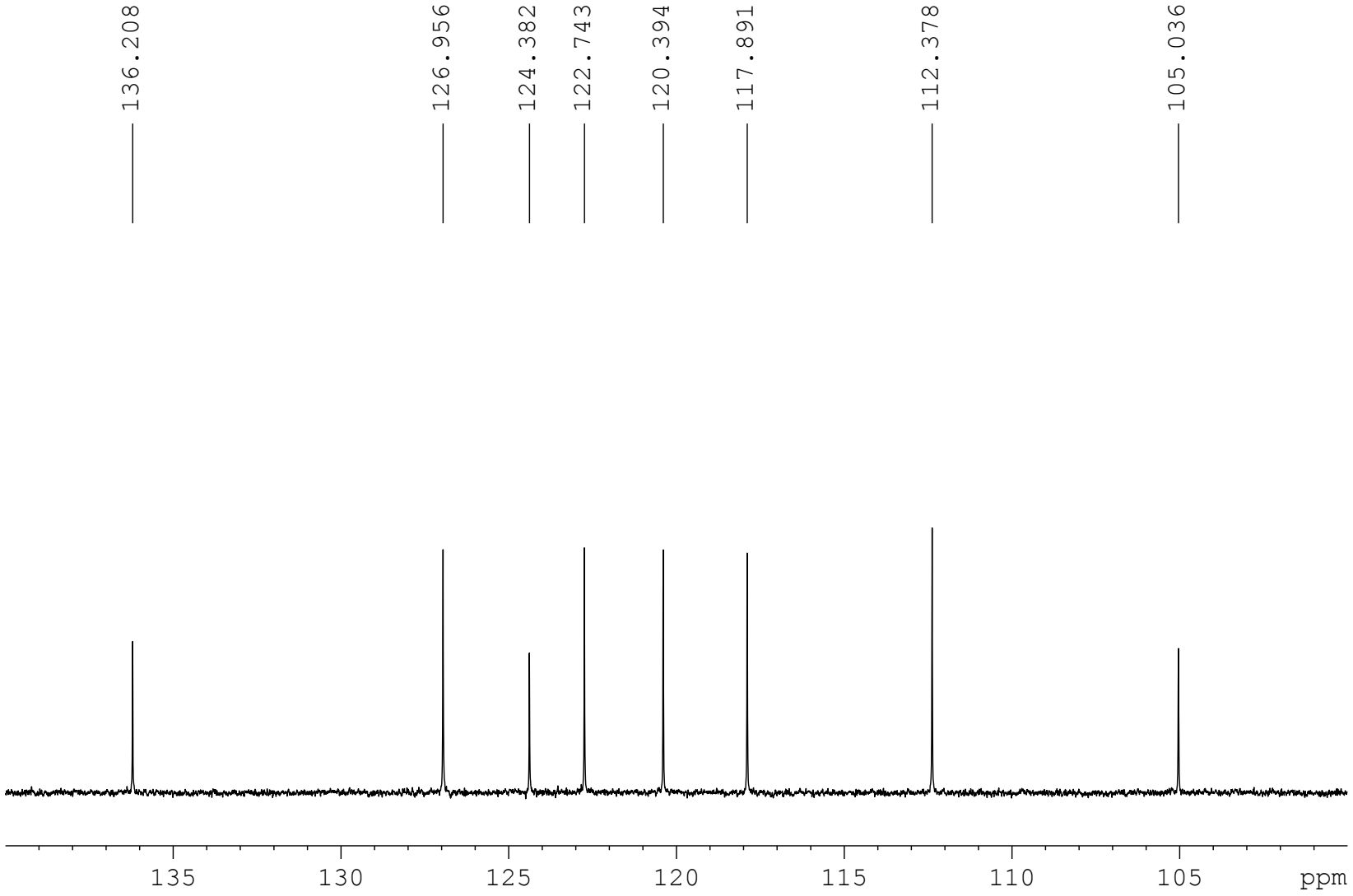
^{1}H NMR of **29**



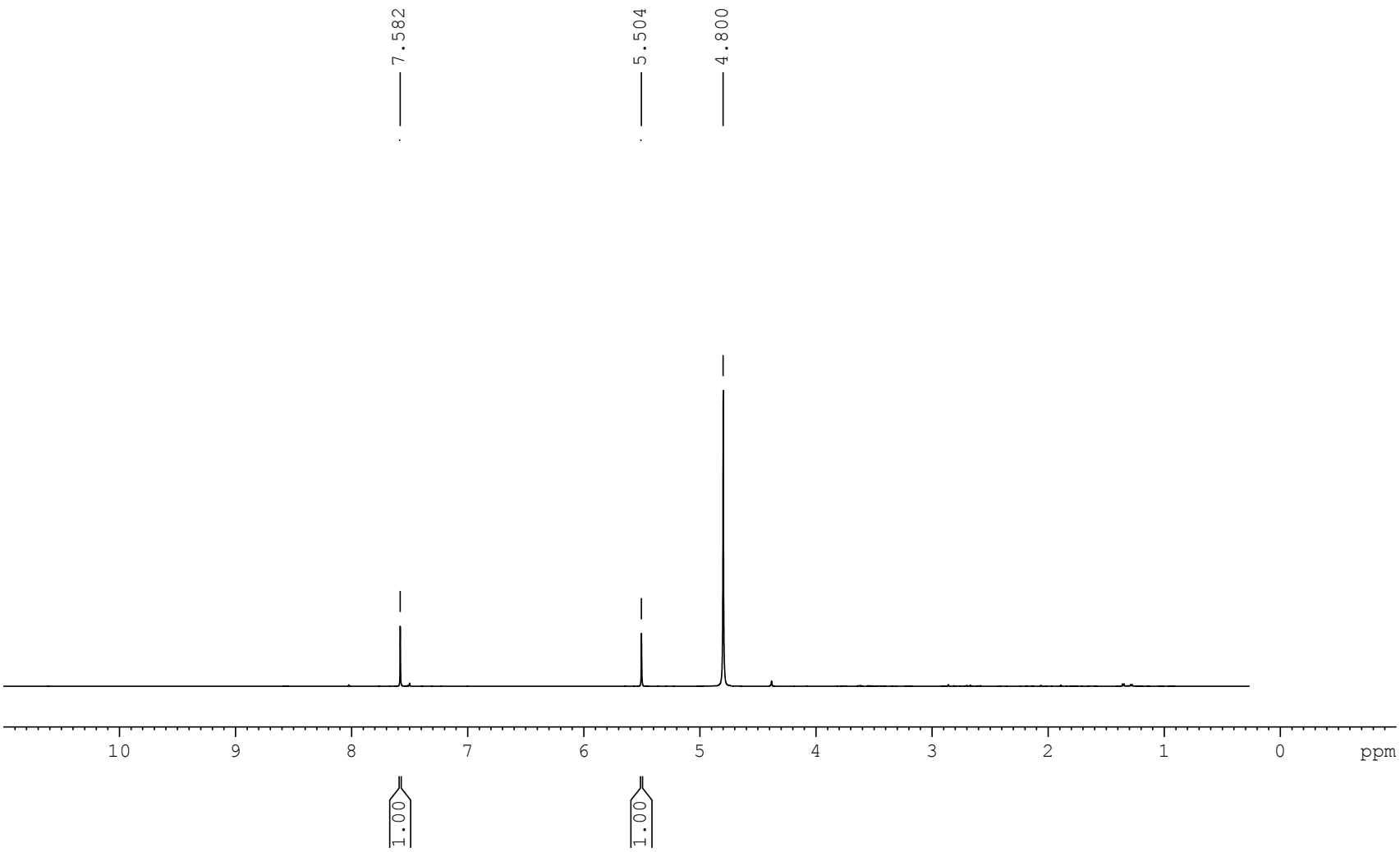
^{13}C NMR of **29**



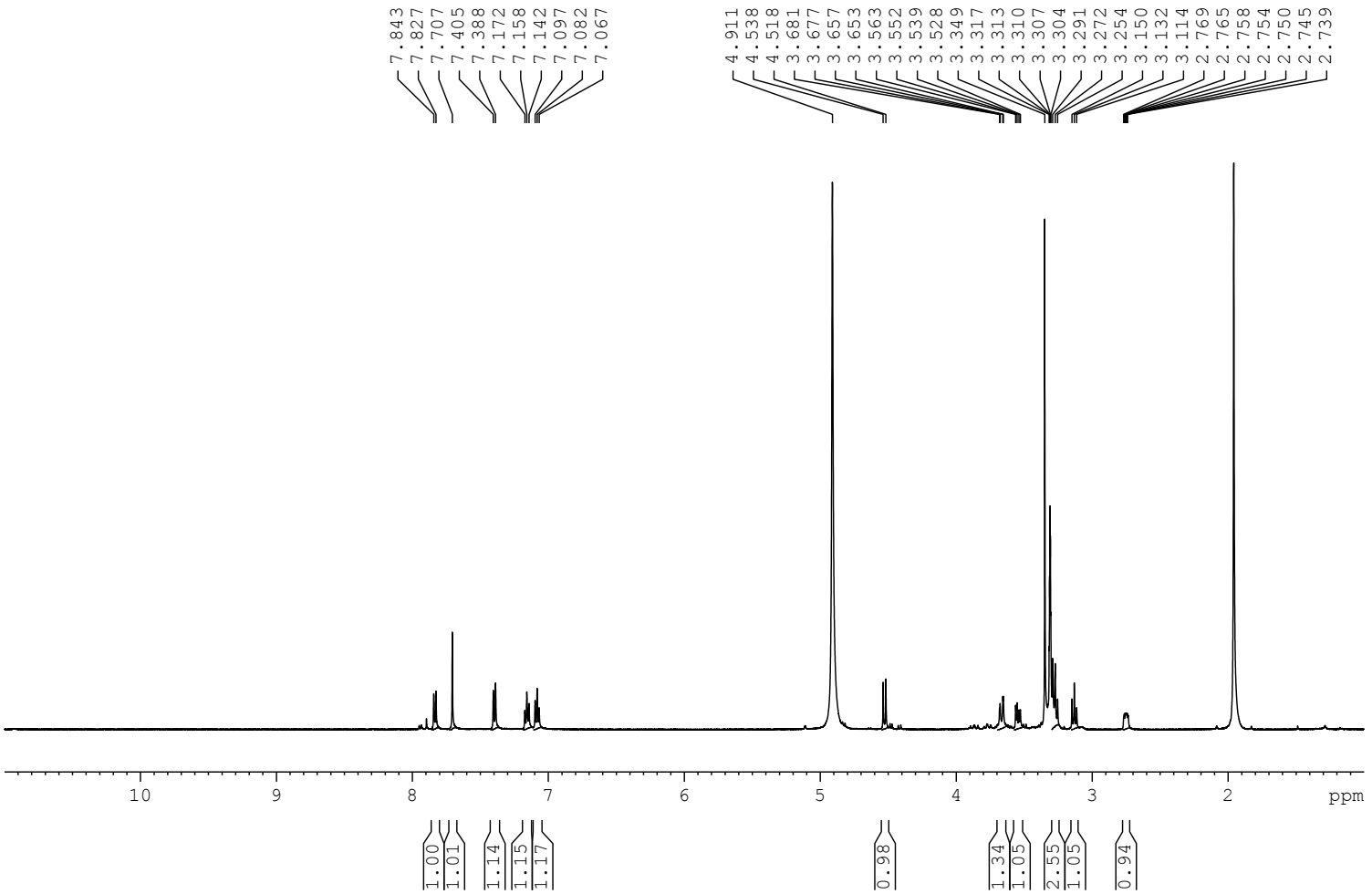
^{13}C NMR of **29**



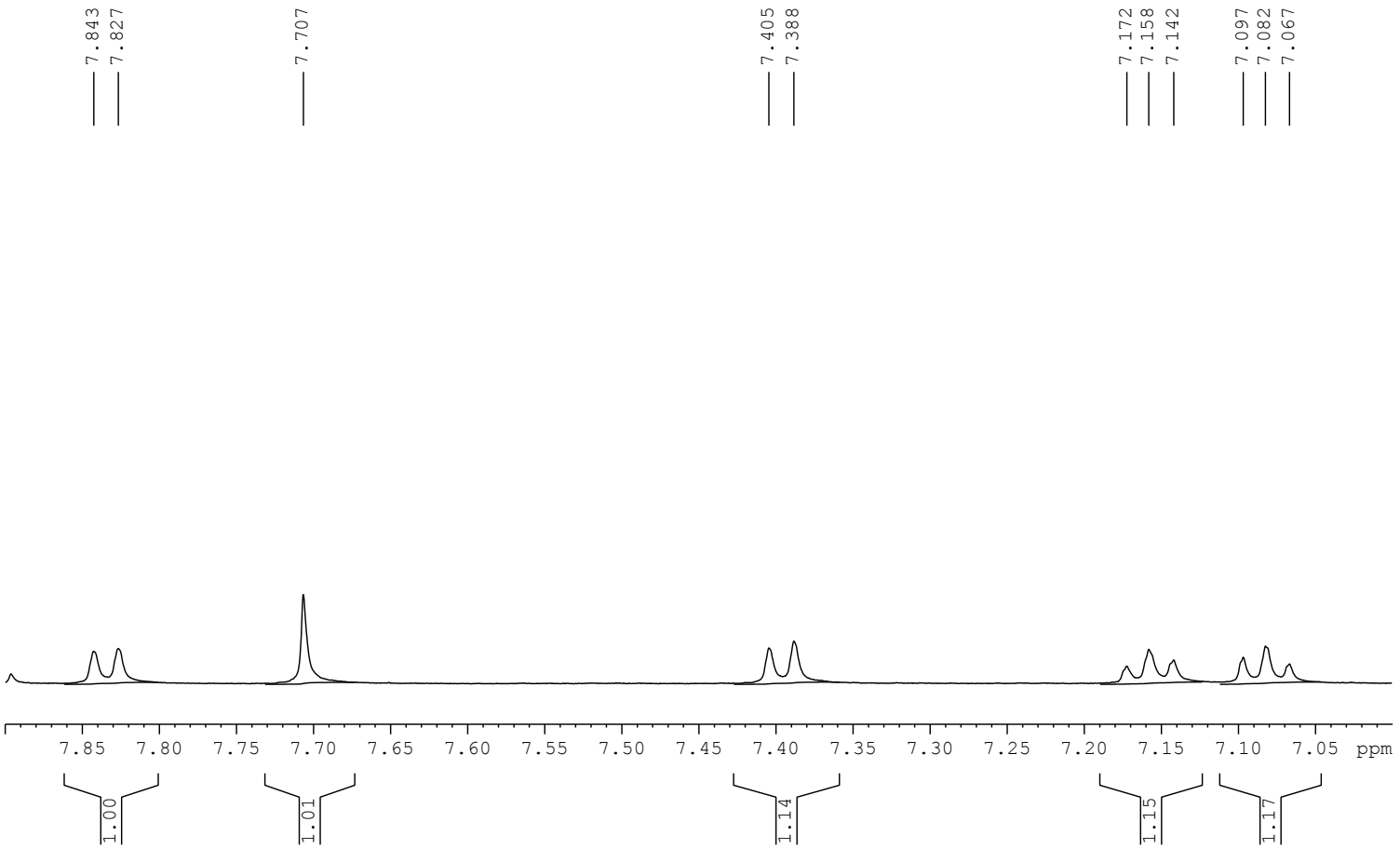
^{1}H NMR of **29a**



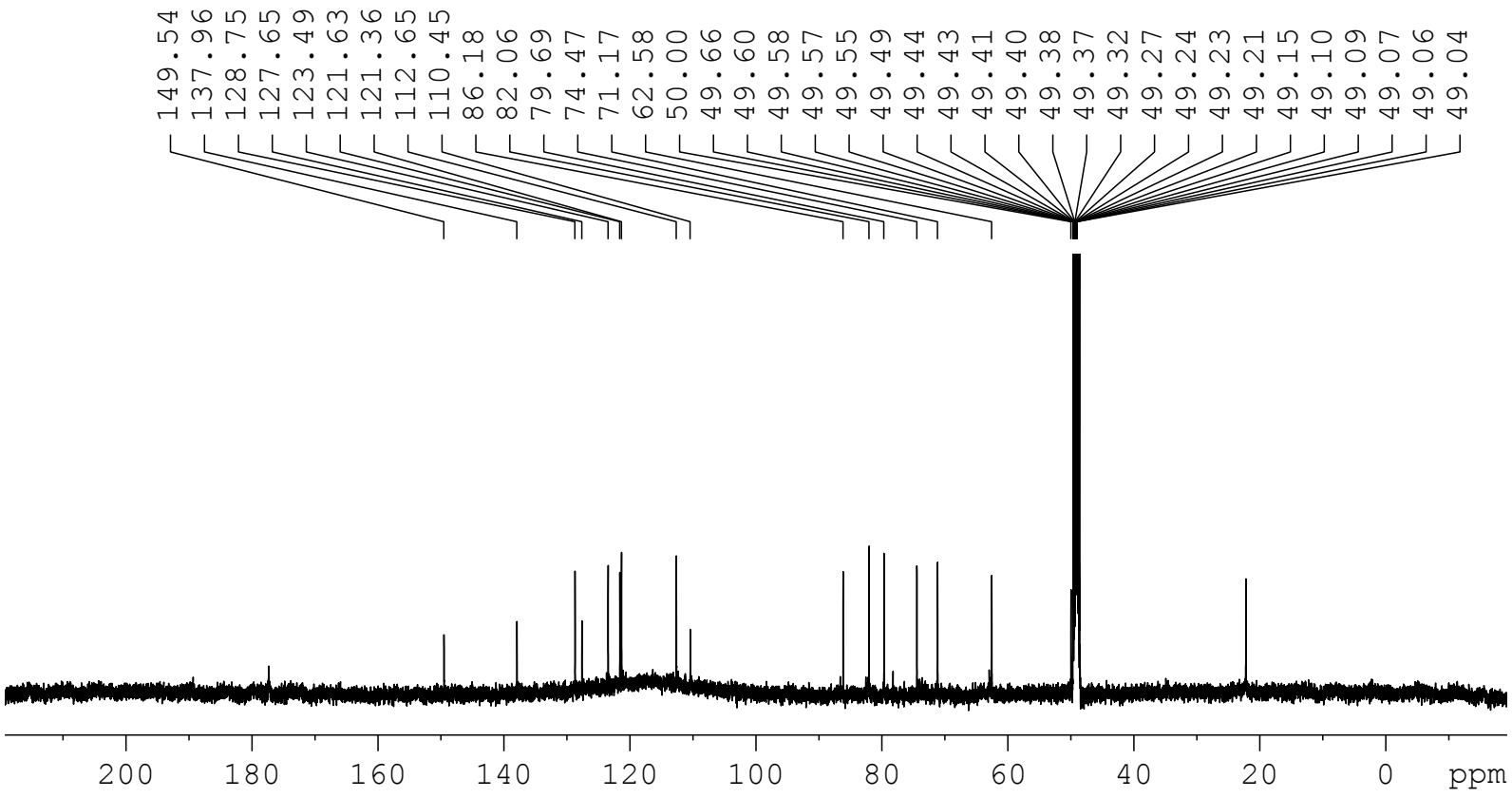
^{1}H NMR of **30**



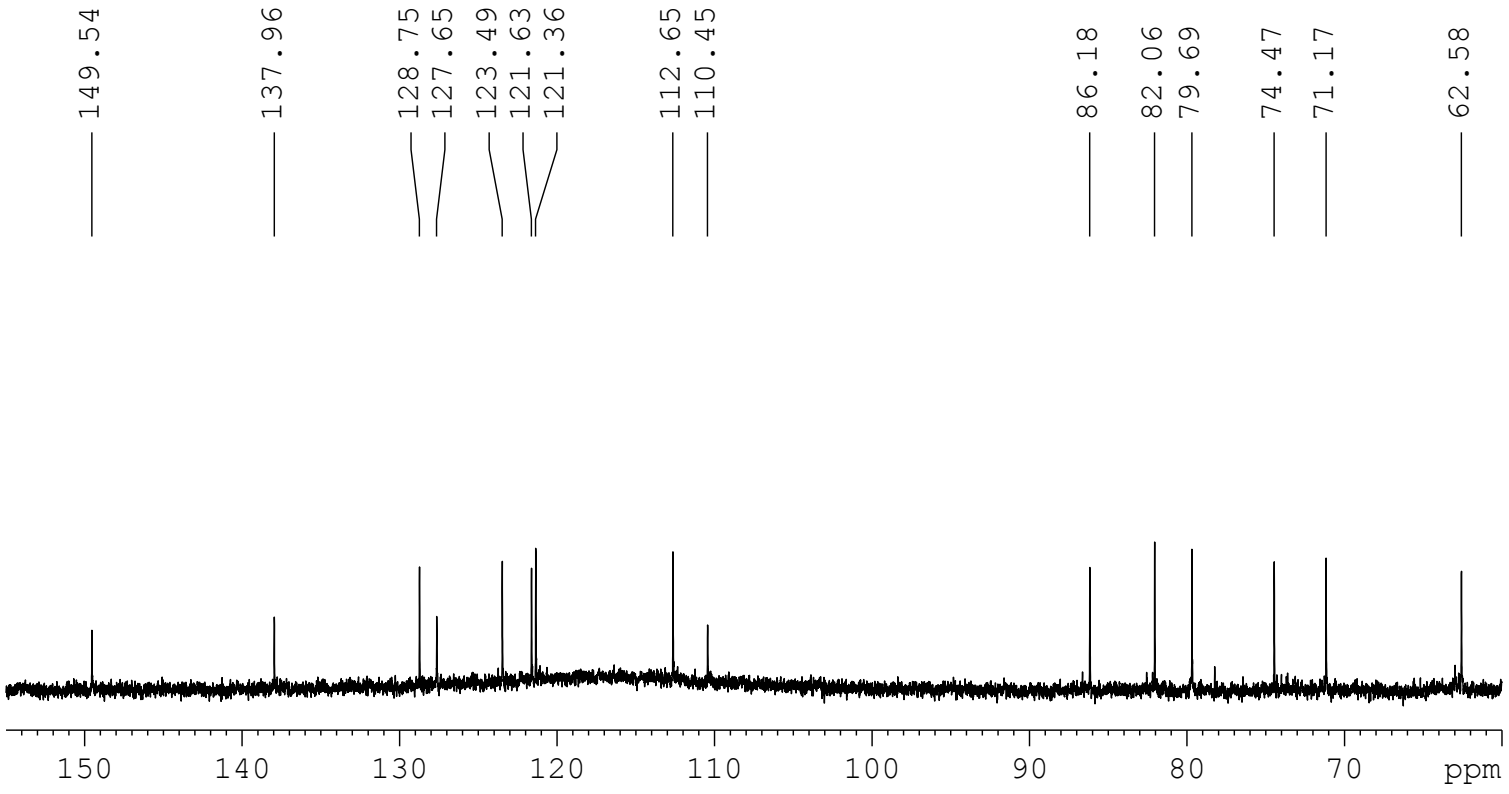
^{1}H NMR of **30**



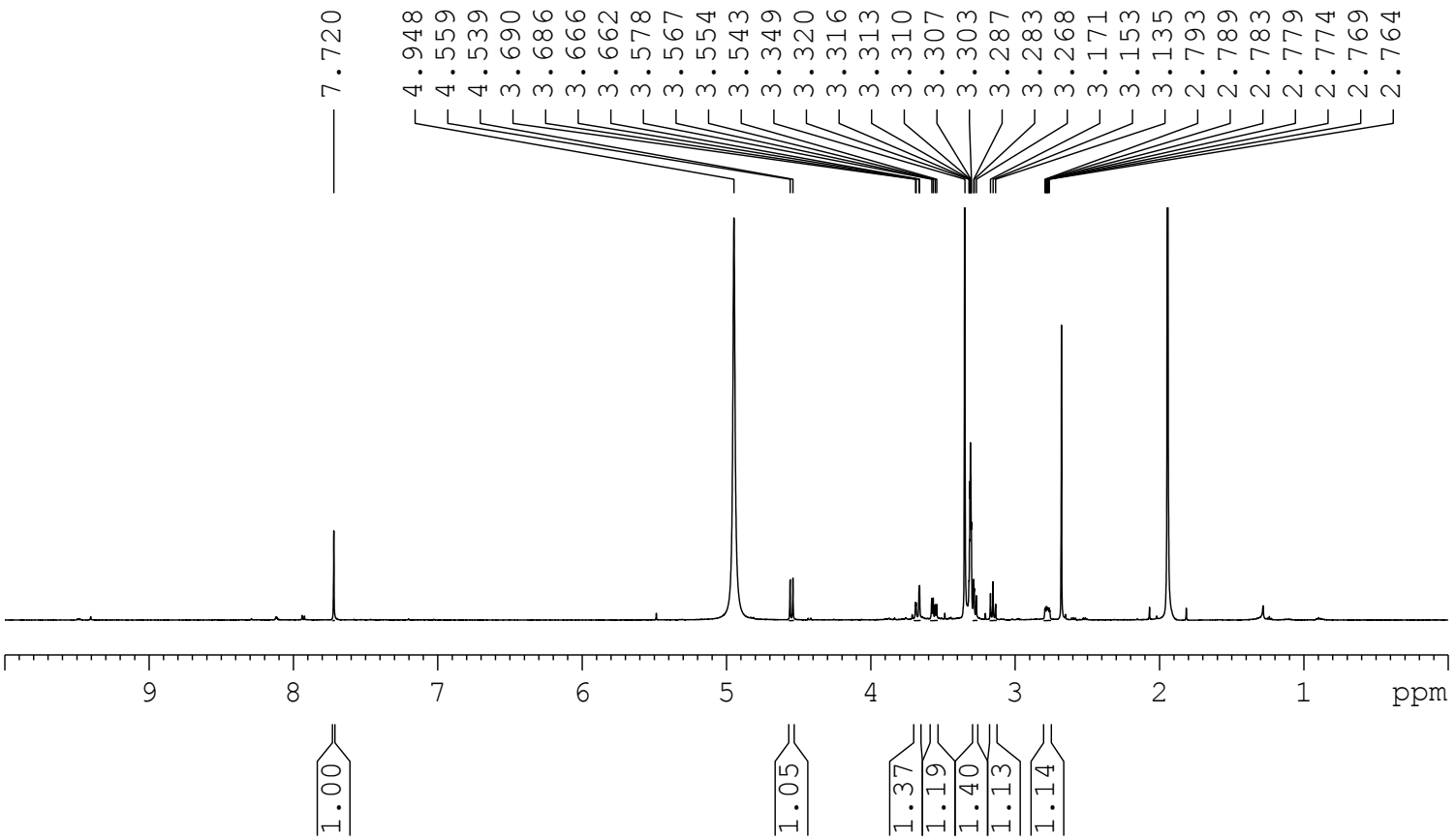
^{13}C NMR of **30**



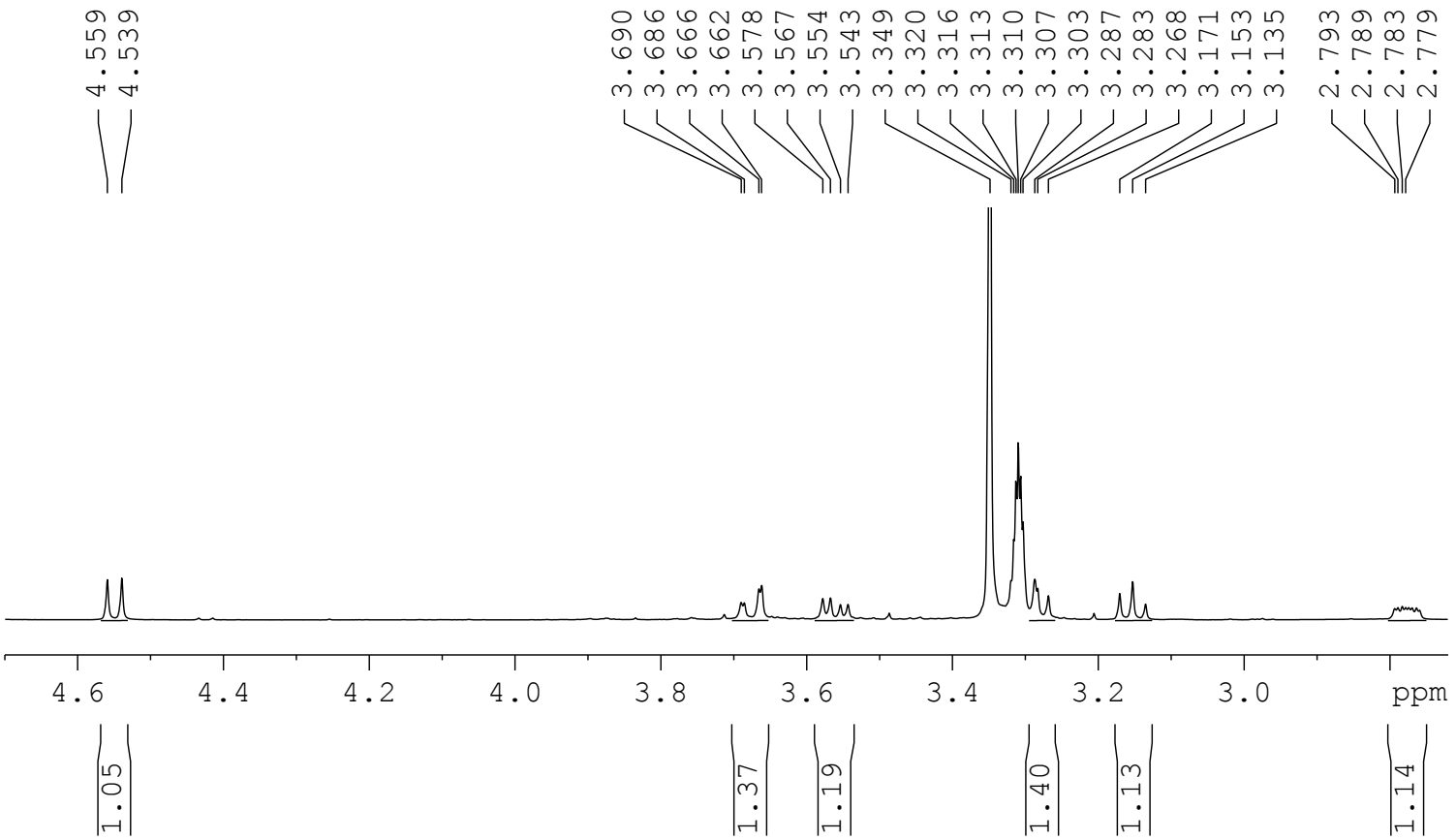
^{13}C NMR of **30**



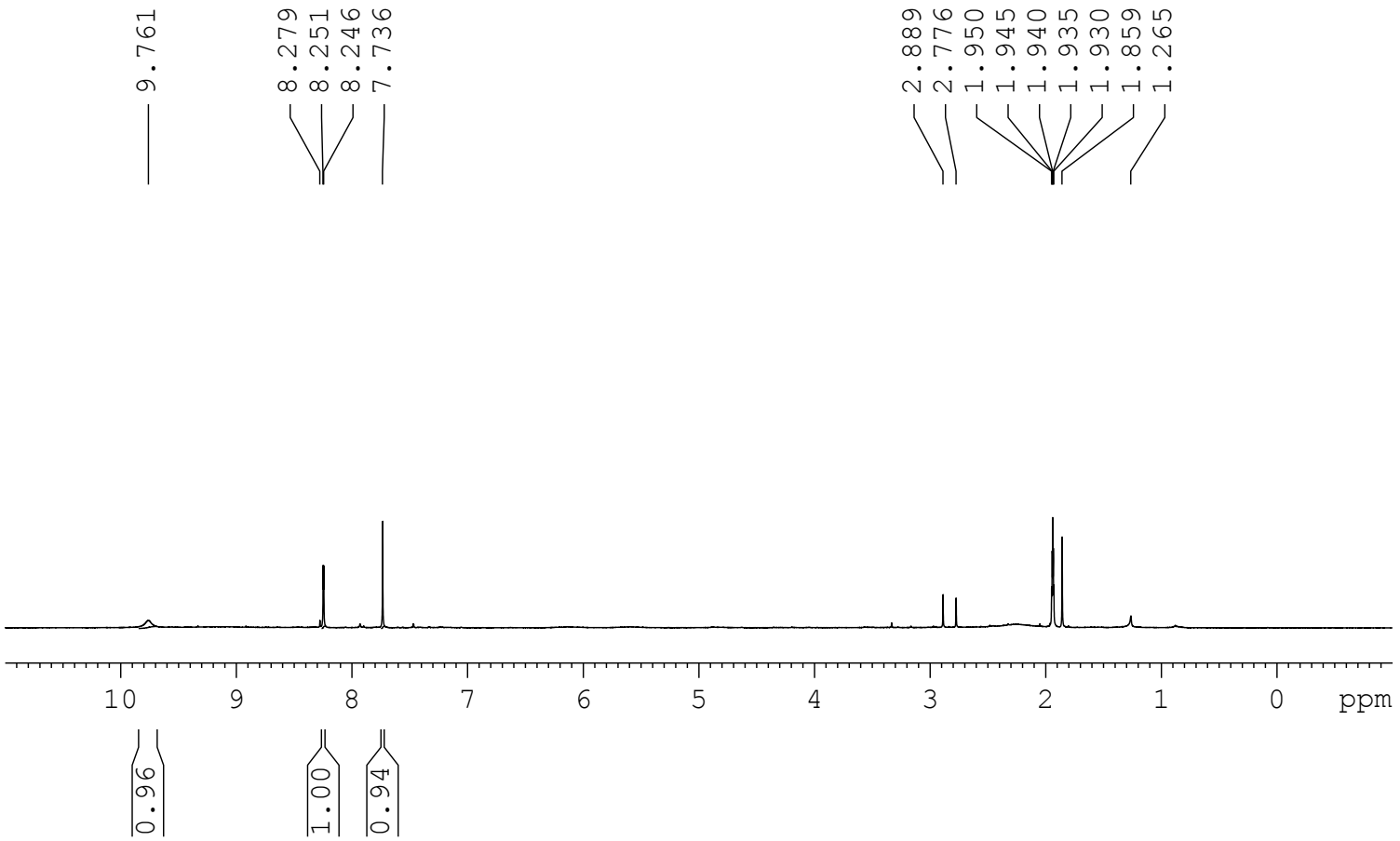
^{1}H NMR of **30a**



^{1}H NMR of **30a**

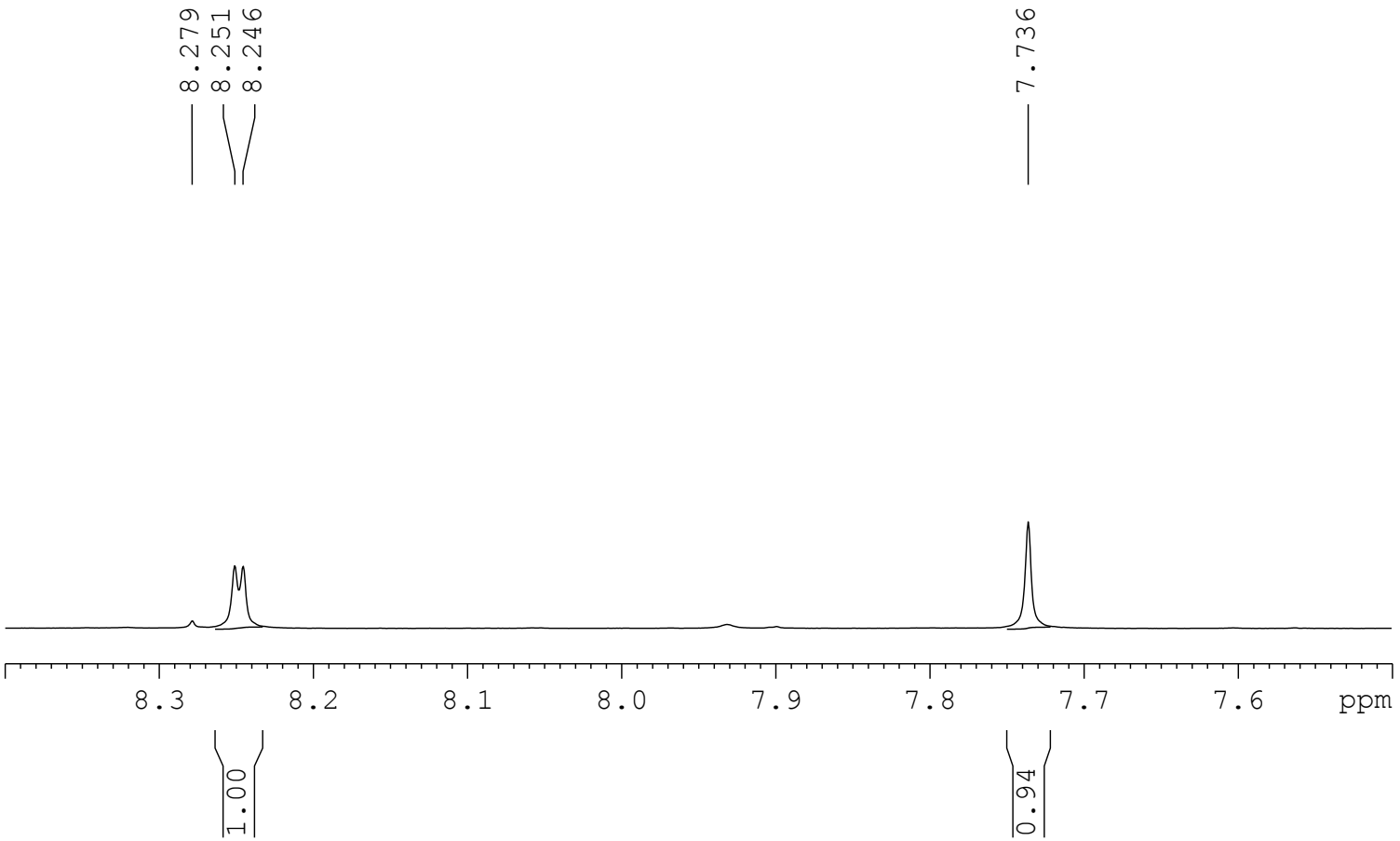


^{1}H NMR of **31a**

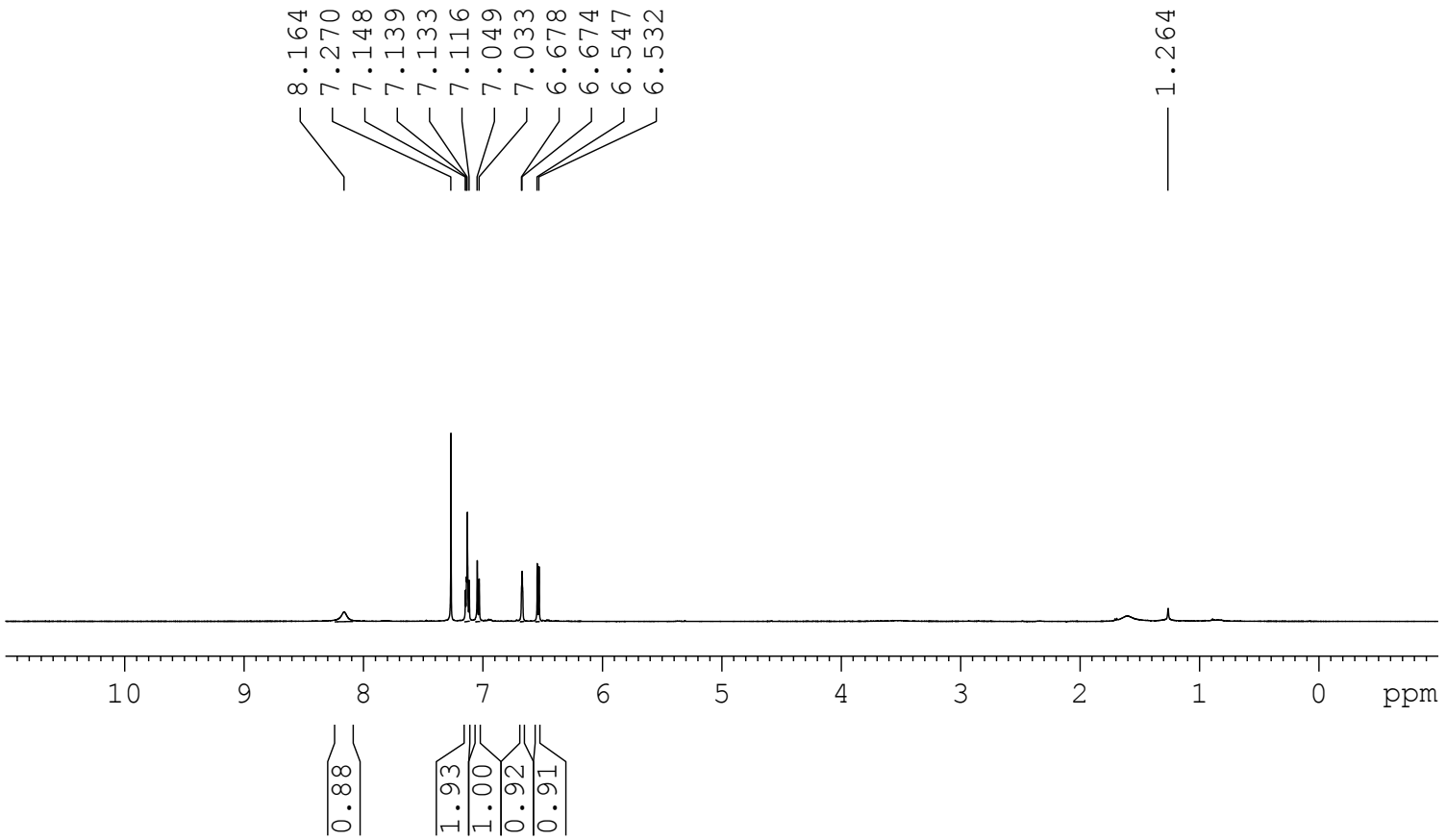


Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

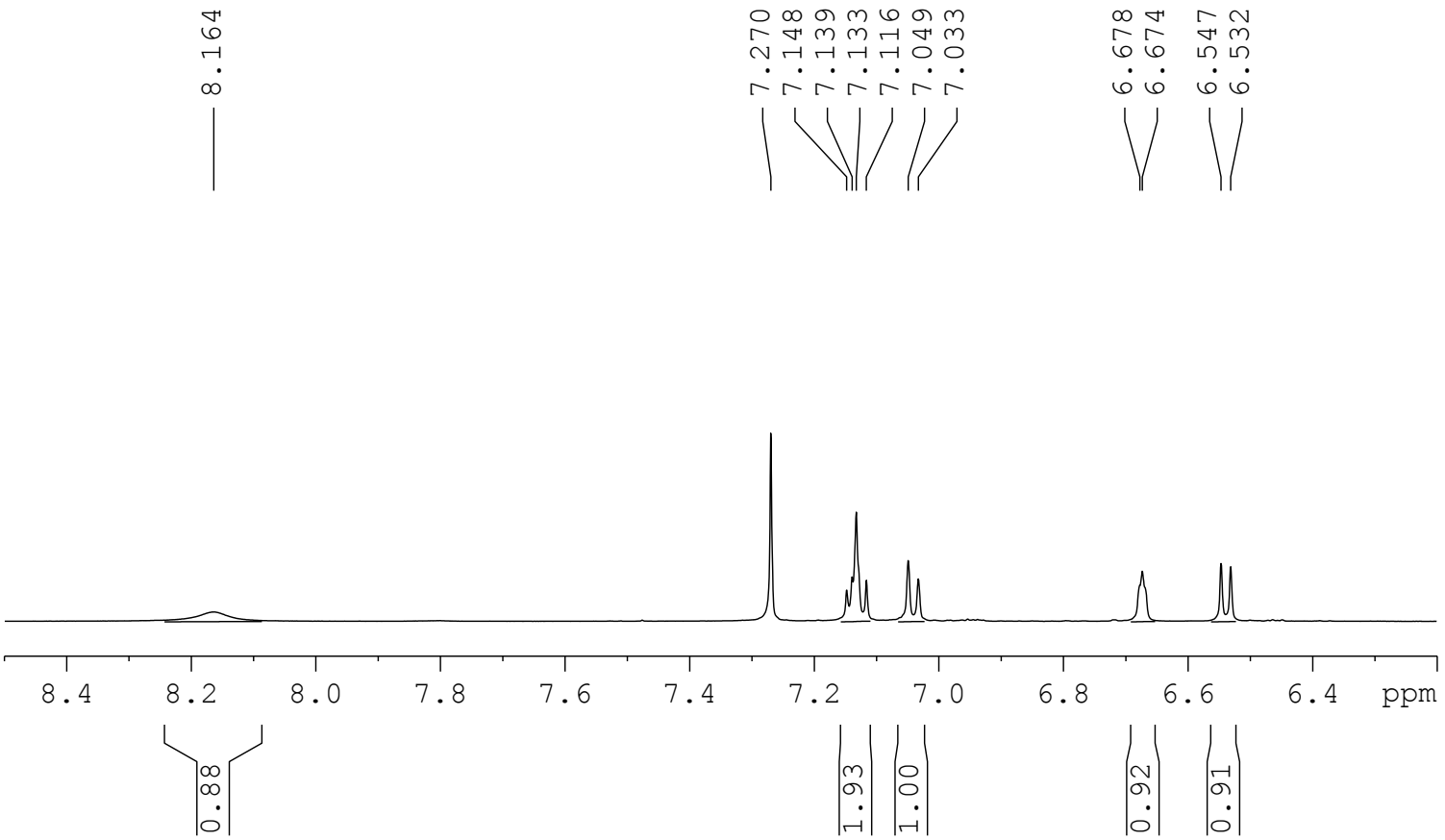
^{1}H NMR of **31a**



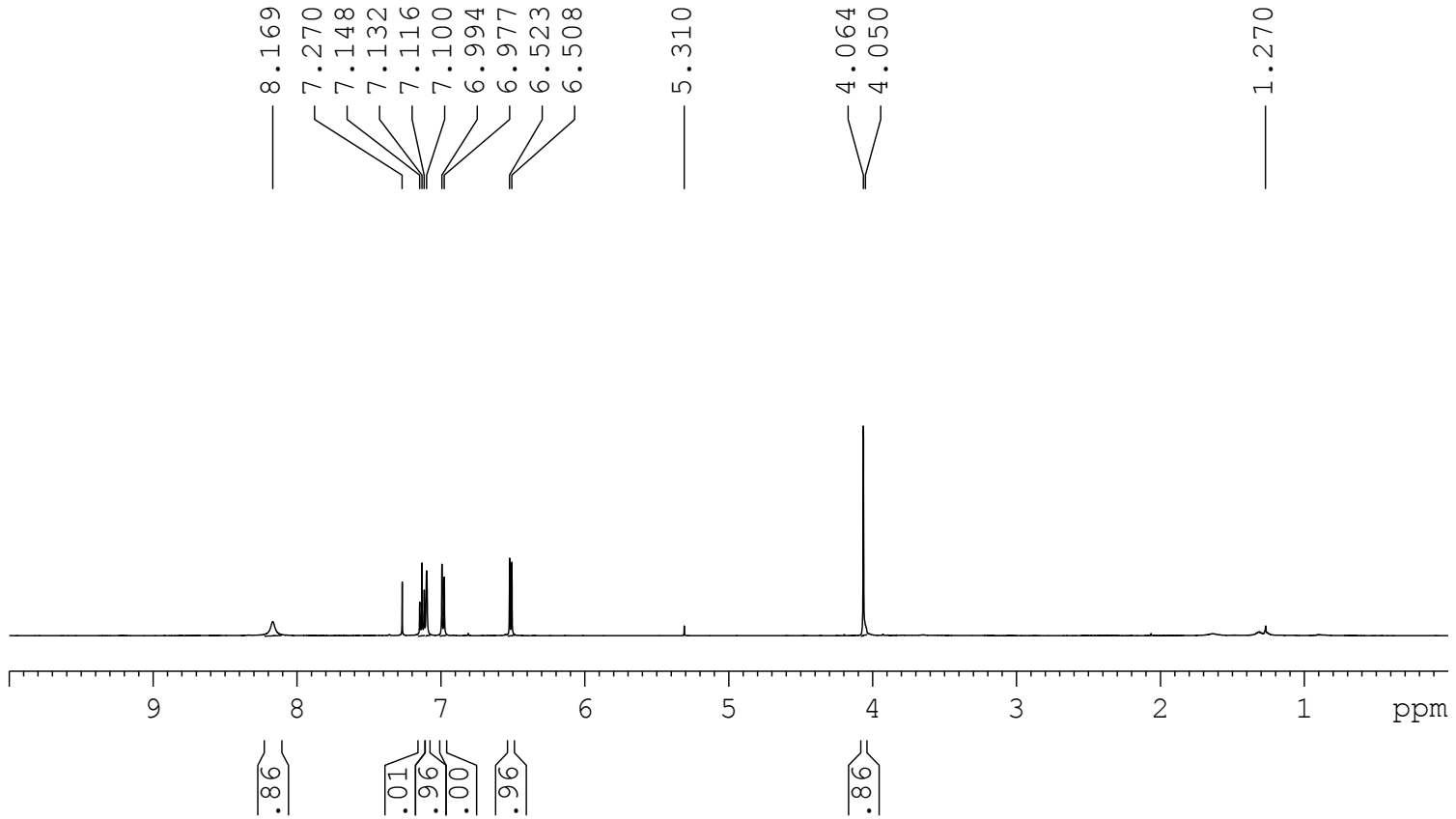
^{1}H NMR of **32a**



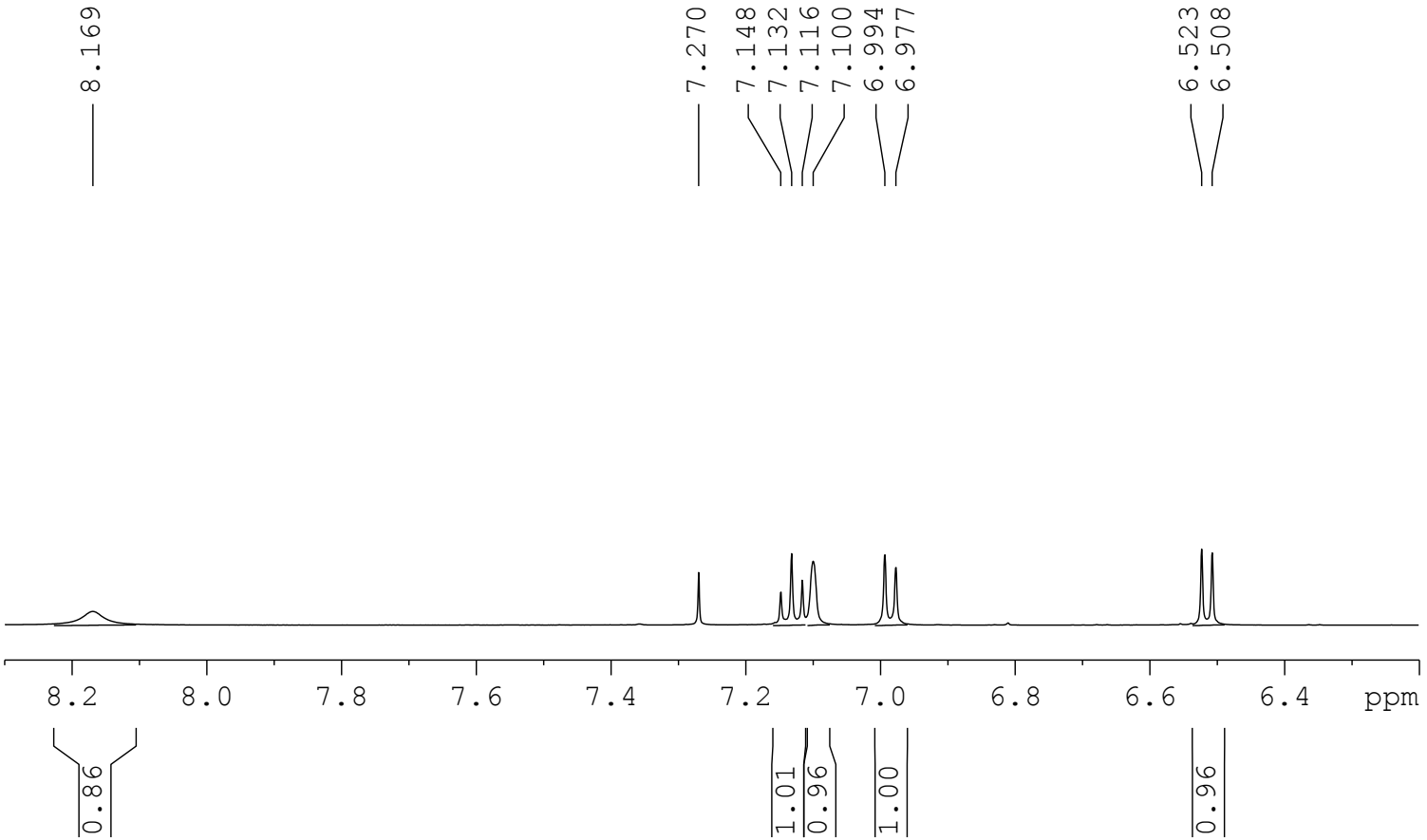
^{1}H NMR of **32a**



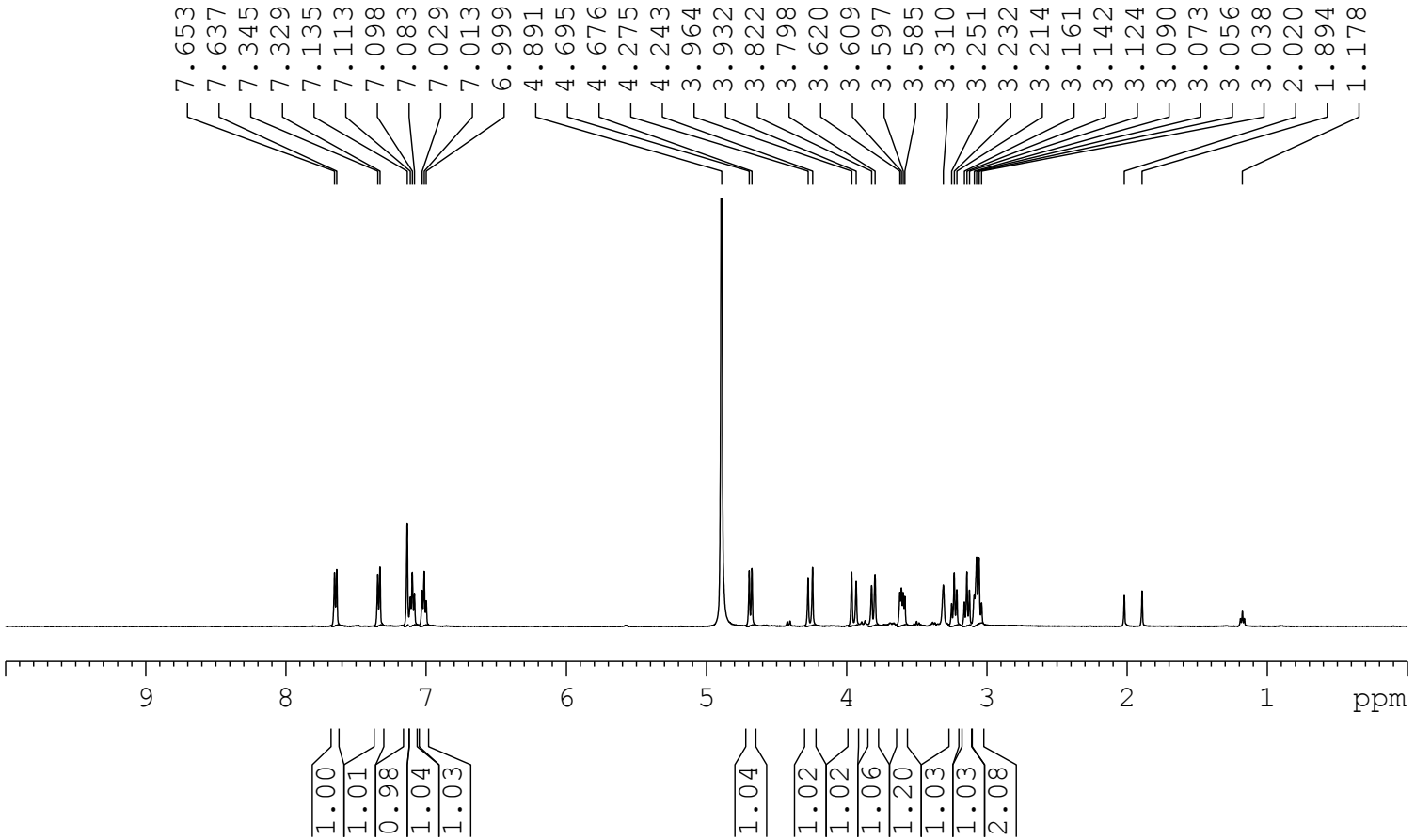
^{1}H NMR of **33a**



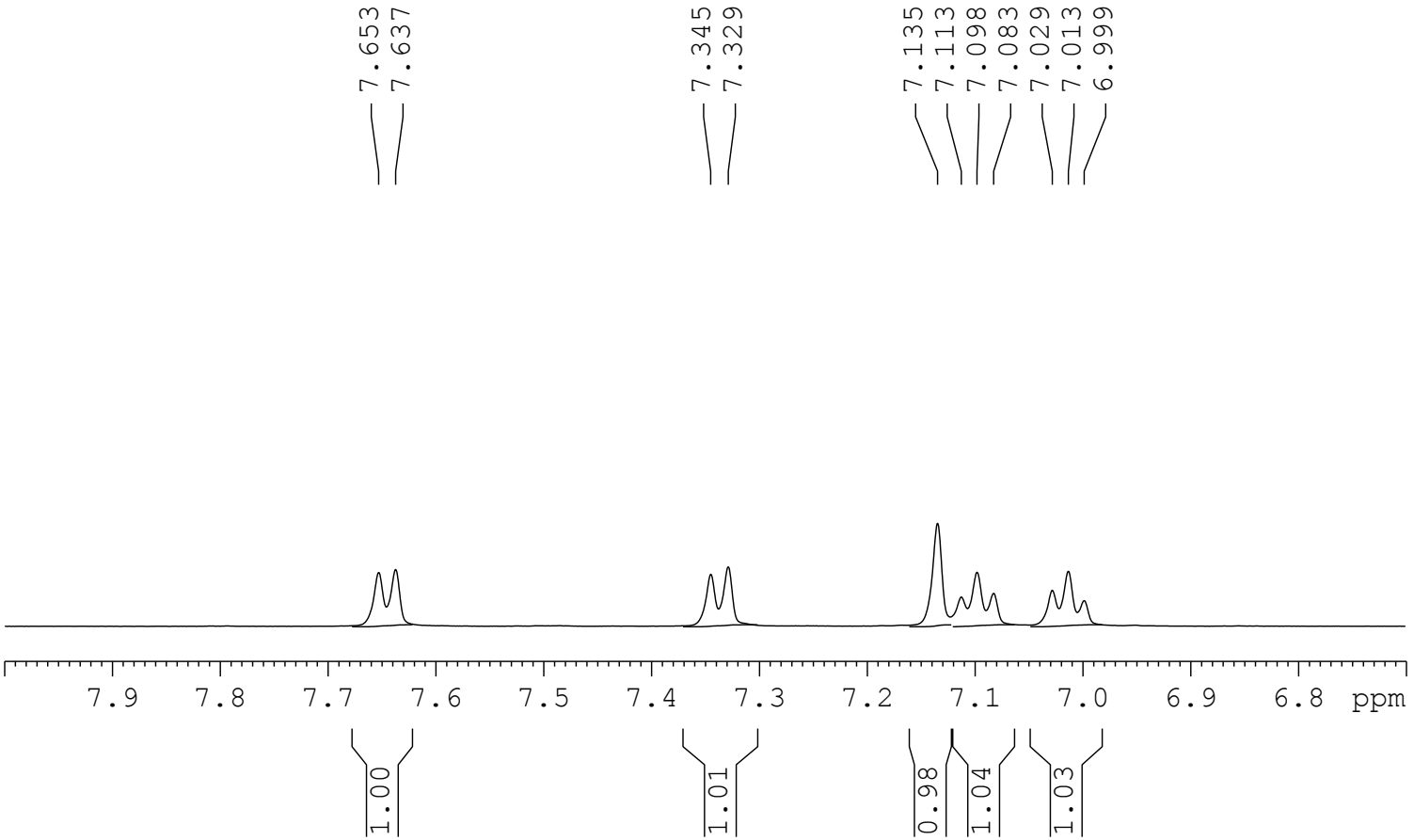
^{1}H NMR of **33a**



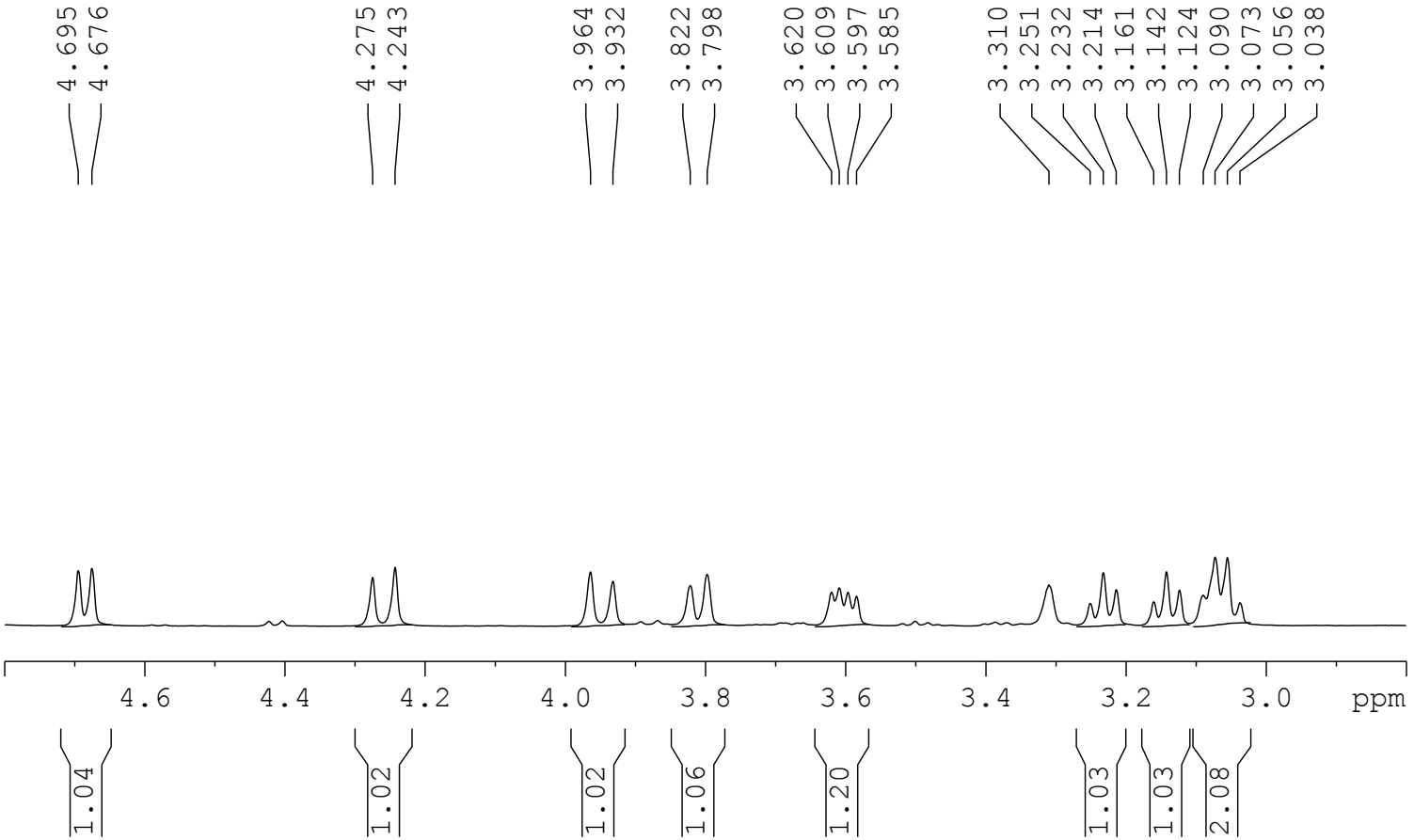
^{1}H NMR of **34**



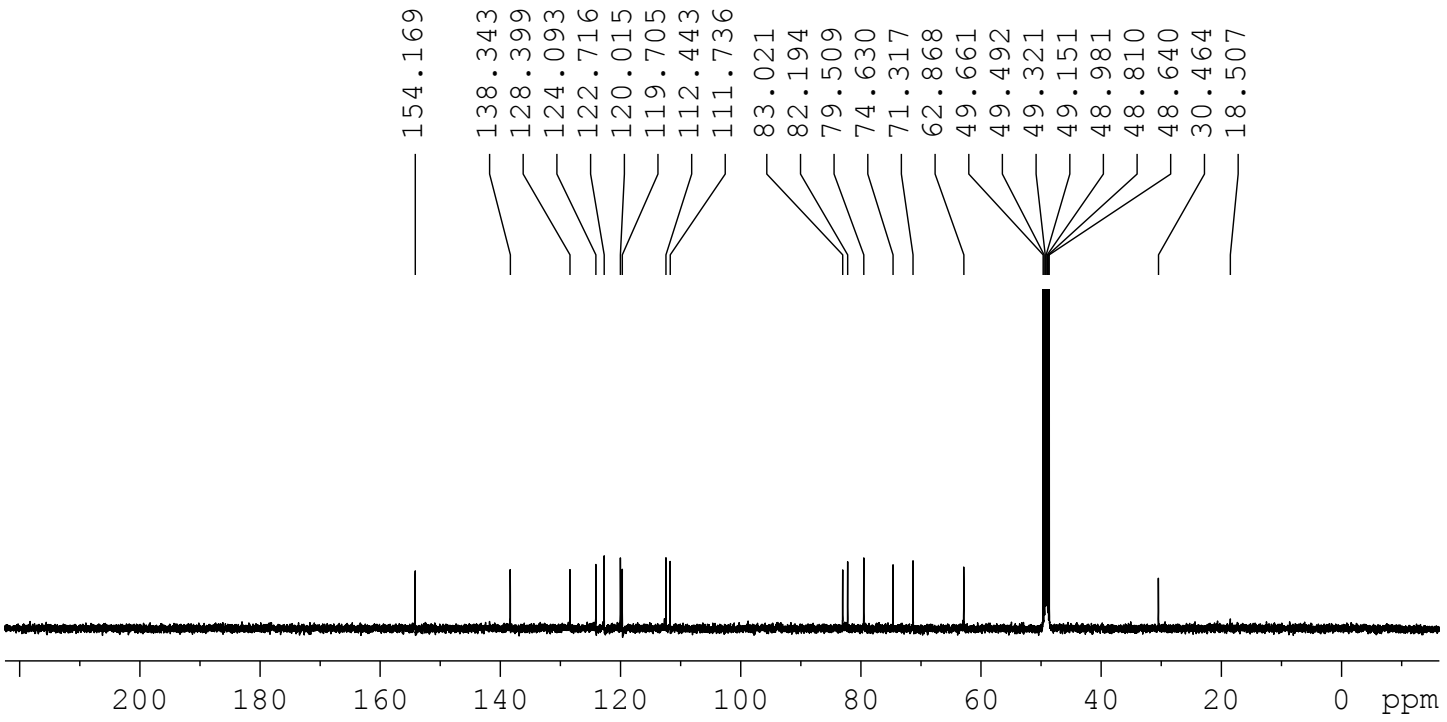
^{1}H NMR of **34**



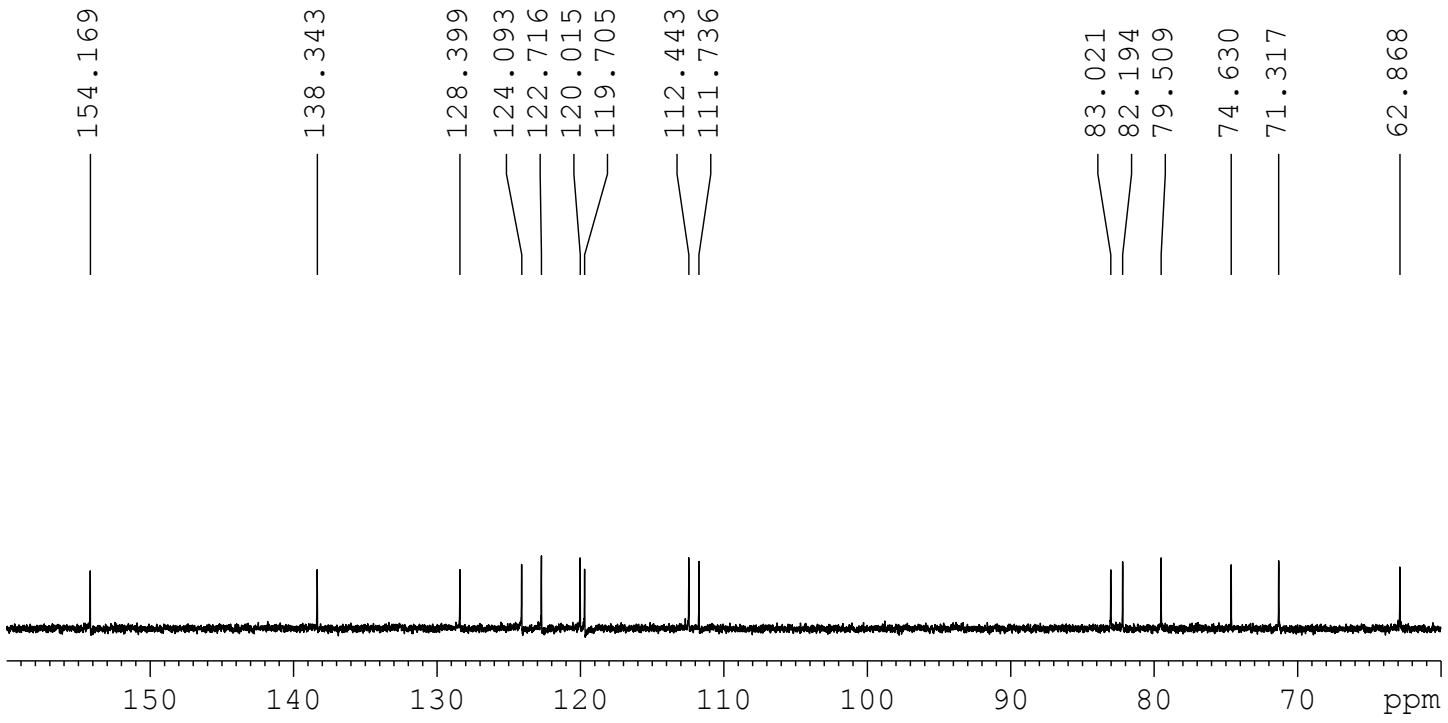
^{1}H NMR of **34**



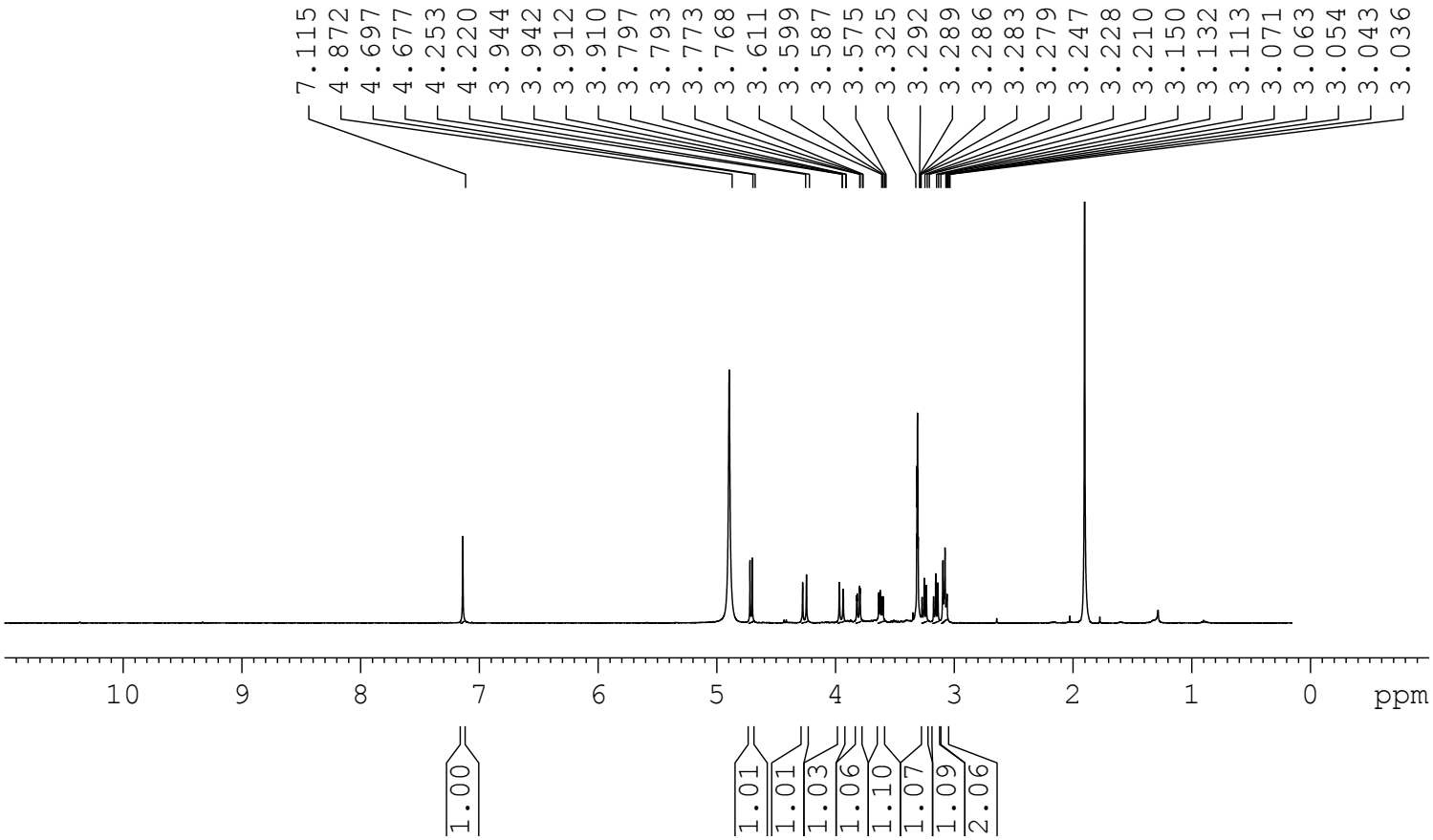
^{13}C NMR of **34**



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



^{1}H NMR of **34a**



^{1}H NMR of **34a**