Electronic Supplementary Material (ESI) for MedChemComm.

Supporting Information for

FgaPT2, a biocatalytic tool for alkyl-diversification of indole natural products

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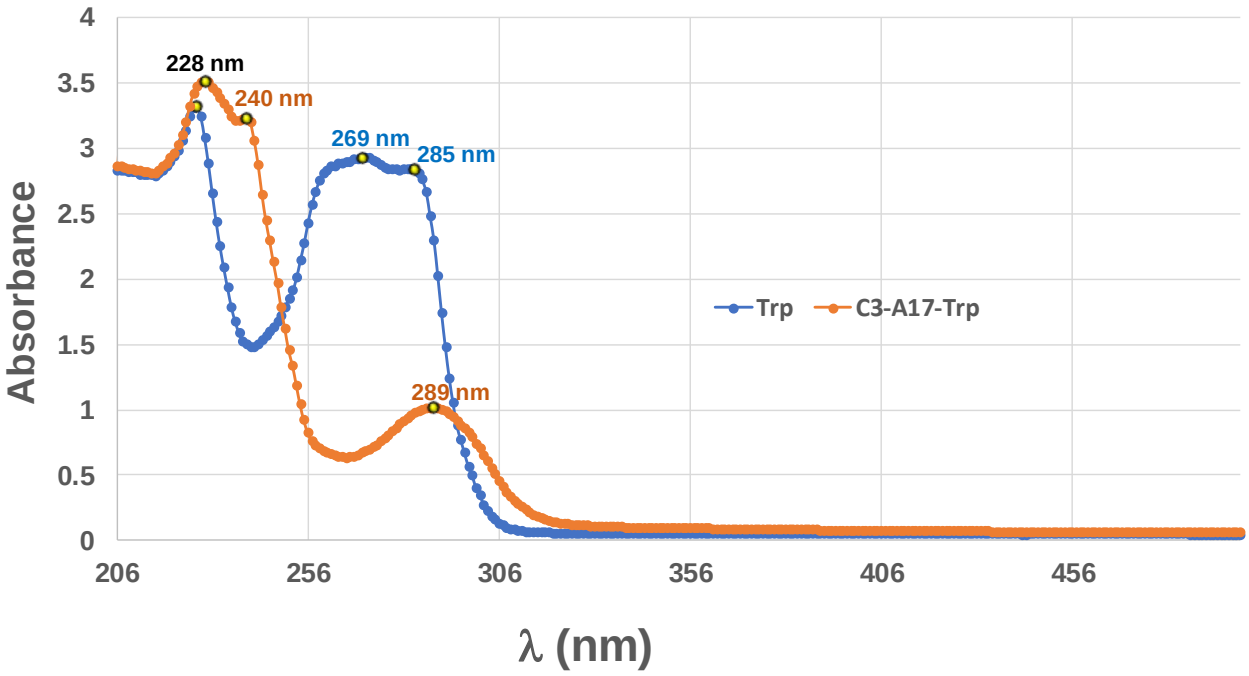


Figure S1: Comparison of UV wavelength scan of L-Trp (blue) and **17** (orange).

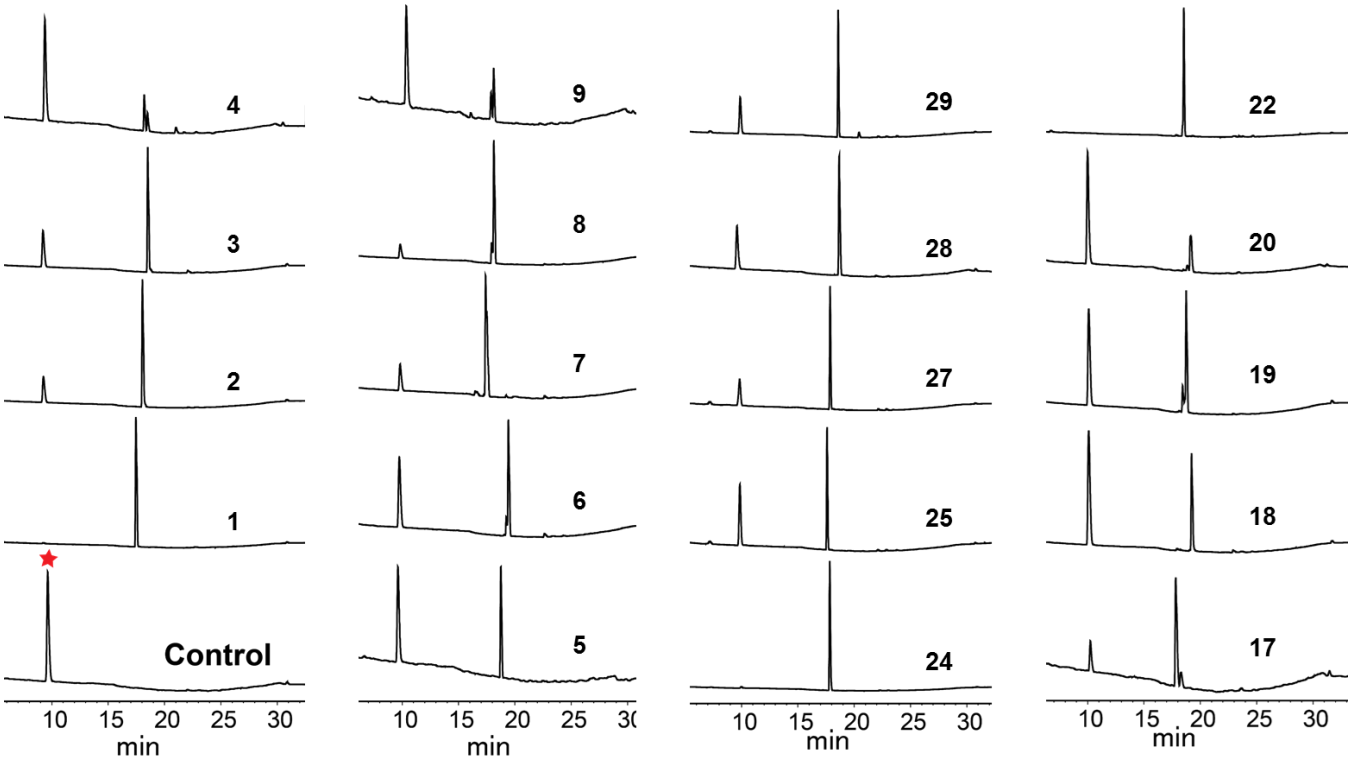


Figure S2: RP-HPLC chromatograms of FgaPT2 catalyzed reactions with L-Trp (labelled with red asterisk) and alkyl-PP analogues that led to the formation of corresponding alkyl tryptophan analogues. The alkyl-PP analogue used for the reaction is labelled on the chromatogram.

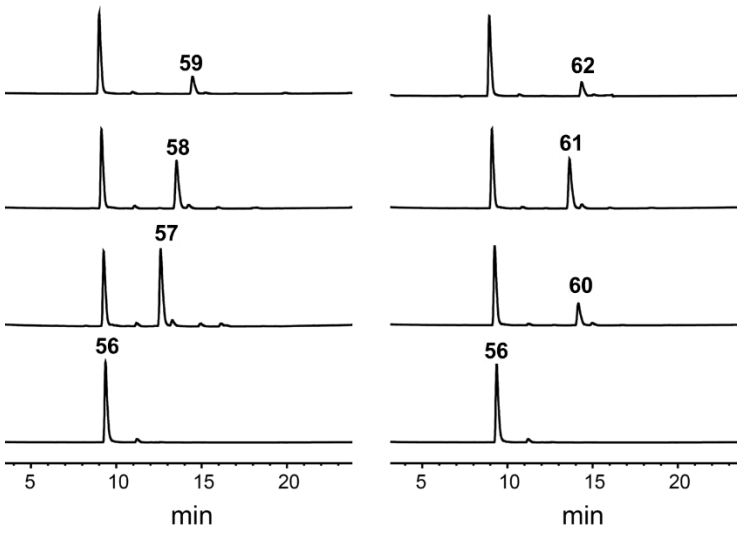


Figure S3: RP-HPLC chromatograms of FgaPT2 catalyzed reactions with 7-hydroxy-K252c (**56**) and alkyl-PP analogues that led to the formation of corresponding alkyl-7-hydroxy-K252c analogues (**57**-**62**).

Alkyl-PP analogue	Concentration of Alkyl-PP (mM)	k_{cat} (min^{-1})	K_M (mM)	k_{cat} / K_M ($mM^{-1}min^{-1}$)
1	2	65 ± 3	0.17 ± 0.04	380 ± 90
2	5	14.3 ± 0.2	0.28 ± 0.02	51 ± 4
3	5	20.80 ± 0.4	0.26 ± 0.02	78 ± 6
4	5	0.38 ± 0.01	0.18 ± 0.02	2.1 ± 0.2
6	10	5.2 ± 0.5	0.14 ± 0.07	36 ± 16
7	2	0.84 ± 0.05	0.19 ± 0.06	4 ± 1
8	5	3.0 ± 0.1	0.16 ± 0.03	18 ± 4
9	5	1.44 ± 0.08	0.33 ± 0.08	4 ± 1
17	5	33 ± 2	0.24 ± 0.06	137 ± 35
20	10	2.23 ± 0.04	0.31 ± 0.02	7.2 ± 0.5
22	5	5.1 ± 0.2	0.17 ± 0.02	29 ± 4
24	5	5.48 ± 0.09	0.22 ± 0.02	25 ± 2
25	5	0.88 ± 0.02	0.20 ± 0.02	4.3 ± 0.5
27	5	0.31 ± 0.01	0.12 ± 0.02	2.5 ± 0.4
28	5	0.123 ± 0.004	0.25 ± 0.03	0.48 ± 0.06
29	5	0.242 ± 0.006	0.25 ± 0.03	1.0 ± 0.1

Table S1: Kinetic parameters for FgaPT2 with varied concentration of L-Trp and constant concentration with representative alkyl-PP analogues in 25 mM Tris, 5 mM $MgCl_2$, 50 mM KCl, pH 7.5, 35 °C.

Table S2: Summary of HRMS data of synthetic alkyl-PP analogues.

Compound	Chemical Formula	Calculated Mass (Da)	Observed (Da)
5	$C_7H_{13}O_7P_2$ $[M-H]^-$	271.0142	271.0148
9	$C_4H_8BrO_7P_2$ $[M-H]^-$	308.8934	308.8932
22	$C_7H_9O_7P_2$ $[M-H]^-$	266.9824	266.9828
23	$C_8H_{11}O_8P_2$ $[M-H]^-$	296.9929	296.9939
24	$C_8H_{11}O_8P_2$ $[M-H]^-$	296.9929	296.9927
25	$C_8H_{11}O_8P_2$ $[M-H]^-$	296.9929	296.9926
26	$C_8H_{11}O_7P_2$ $[M-H]^-$	280.9980	280.9978
27	$C_7H_8FO_7P_2$ $[M-H]^-$	284.9729	284.9728
28	$C_7H_8ClO_7P_2$ $[M-H]^-$	300.9439	300.9428
29	$C_7H_8BrO_7P_2$ $[M-H]^-$	344.8929	344.8927
30	$C_7H_8NO_9P_2$ $[M-H]^-$	311.9674	311.9677
31	$C_9H_{13}O_9P_2$ $[M-H]^-$	327.0035	327.0048
32	$C_9H_{13}O_9P_2$ $[M-H]^-$	327.0035	327.0027
33	$C_7H_7F_2O_7P_2$ $[M-H]^-$	302.9635	302.9629
34	$C_{10}H_{15}O_{10}P_2$ $[M-H]^-$	357.0140	357.0135

Table S3: Summary of HRMS data of alkyl-Trp analogues from FgaPT2 catalyzed alkylation reaction with L-Trp and synthetic alkyl-PP analogues. The subscript '2' represent the minor product based on HPLC retention time.

Alkyl -PP used for the reaction	Enzyme Product	Chemical Formula	Calculated Mass (Da)	Observed Mass (Da)
1	**35**	$C_{16}H_{21}N_2O_2$ $[M+H]^+$	273.1603	273.1602
2	**36**	$C_{17}H_{23}N_2O_2$ $[M+H]^+$	287.1760	287.1754
3	**37**	$C_{18}H_{25}N_2O_2$ $[M+H]^+$	301.1916	301.1916
4	**42**	$C_{18}H_{23}N_2O_2$ $[M+H]^+$	299.1760	299.1756
4	**43**	$C_{18}H_{23}N_2O_2$ $[M+H]^+$	299.1760	299.1761
5	**38**	$C_{18}H_{23}N_2O_2$ $[M+H]^+$	299.1760	299.1768
6	**44**	$C_{19}H_{25}N_2O_2$ $[M+H]^+$	313.1916	313.1913
6	**6-Trp$_2$**	$C_{19}H_{25}N_2O_2$ $[M+H]^+$	313.1916	313.1911
7	**40**	$C_{15}H_{19}N_2O_2$ $[M+H]^+$	259.1447	259.1449
7	**41**	$C_{15}H_{19}N_2O_2$ $[M+H]^+$	259.1447	259.1444
8	**45**	$C_{15}H_{18}ClN_2O_2$ $[M+H]^+$	293.1057	293.1061
8	**8-Trp$_2$**	$C_{15}H_{18}ClN_2O_2$ $[M+H]^+$	293.1057	293.1062
9	**46**	$C_{15}H_{18}BrN_2O_2$ $[M+H]^+$	337.0552	337.0552
9	**9-Trp$_2$**	$C_{15}H_{18}BrN_2O_2$ $[M+H]^+$	337.0552	337.0547
10	**10-Trp**	$C_{14}H_{17}N_2O_2$ $[M+H]^+$	245.1290	245.1294
13	**13-Trp**	$C_{20}H_{21}N_2O_2$ $[M+H]^+$	321.1603	321.1601
14	**14-Trp**	$C_{21}H_{23}N_2O_3$ $[M+H]^+$	351.1709	351.1711
15	**15-Trp**	$C_{21}H_{23}N_2O_2$ $[M+H]^+$	335.1760	335.1757
16	**16-Trp**	$C_{26}H_{25}N_2O_2$ $[M+H]^+$	397.1916	397.1912
17	**53**	$C_{16}H_{19}N_2O_2$ $[M+H]^+$	271.1447	271.1442
17	**17-Trp$_2$**	$C_{16}H_{19}N_2O_2$ $[M+H]^+$	271.1447	271.1444
18	**47**	$C_{18}H_{23}N_2O_2$ $[M+H]^+$	299.1760	299.1768
19	**54**	$C_{17}H_{21}N_2O_2$ $[M+H]^+$	285.1603	285.1602
19	**19-Trp$_2$**	$C_{17}H_{21}N_2O_2$ $[M+H]^+$	285.1603	285.1605
20	**55**	$C_{18}H_{23}N_2O_2$ $[M+H]^+$	299.1715	299.1751
20	**20-Trp$_2$**	$C_{18}H_{23}N_2O_2$ $[M+H]^+$	299.1715	299.1749
21	**21-Trp**	$C_{22}H_{23}N_2O_2$ $[M+H]^+$	347.1760	347.1767
22	**48**	$C_{18}H_{19}N_2O_2$ $[M+H]^+$	295.1447	295.1430
24	**39**	$C_{19}H_{21}N_2O_3$ $[M+H]^+$	325.1552	325.1547
25	**49**	$C_{19}H_{21}N_2O_3$ $[M+H]^+$	325.1552	325.1540
27	**50**	$C_{18}H_{18}FN_2O_2$ $[M+H]^+$	313.1352	313.1338
28	**51**	$C_{18}H_{18}ClN_2O_2$ $[M+H]^+$	329.1057	329.1039
29	**52**	$C_{18}H_{18}BrN_2O_2$ $[M+H]^+$	373.0552	373.0531

Table S4: Summary of HRMS data of alkyl-7-hydroxy-K252c analogues (**57**-**62**) from FgaPT2 catalyzed alkylation reaction with 7-hydroxy-K252c (**56**) and synthetic alkyl-PP analogues.

Alkyl -PP used for the reaction	Compound	Chemical Formula	Calculated Mass (Da)	Observed Mass (Da)
-	**56**	$C_{20}H12N_3O_2$ $[M-H]^-$	326.0930	326.0934
1	**57**	$C_{25}H_{20}N_3O_2$ $[M-H]^-$	394.1556	394.1553
2	**58**	$C_{26}H_{22}N_3O_2$ $[M-H]^-$	408.1712	408.1712
3	**59**	$C_{27}H_{24}N_3O_2$ $[M-H]^-$	422.1869	422.1882
4	**60**	$C_{27}H_{22}N_3O_2$ $[M-H]^-$	420.1712	420.1727
5	**61**	$C_{27}H_{22}N_3O_2$ $[M-H]^-$	420.1712	420.1709
6	**62**	$C_{28}H_{24}N_3O_2$ $[M-H]^-$	434.1869	434.1878

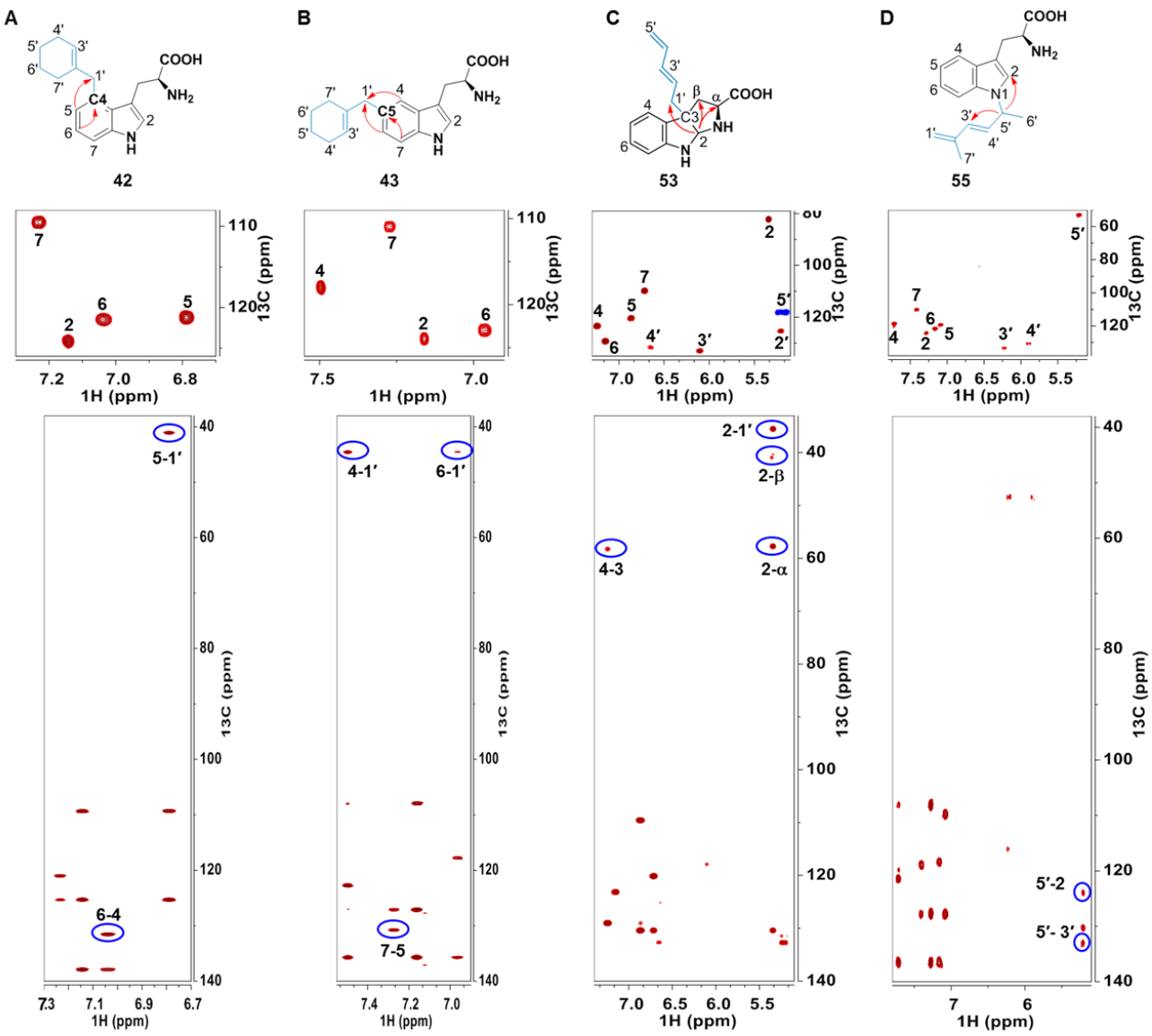


Figure S4: Representative 2D $^{1}H,^{13}C$ HSQC (top) and HMBC (bottom) demonstrating the C4 (**A**), C5 (**B**), C3 (**C**) and N1 (**D**) regio-chemical attachment of the alkyl groups on L-Trp. The blue circled HMBC peaks show a correlation between atoms with red arrows on the structure. Black and blue colored labels denote signals from the indole ring and alkyl-group, respectively.

The NMR analysis of C4-alkyl-Trp analogues

Six allylic analogues (**1, 2, 3, 4, 5,** and **7**) and an o-methoxy benzyl analogue (**24**) in FgaPT2 catalyzed L-Trp alkylation reactions produced normal C4 alkylated products. The reaction involving **1**, **2**, **3**, **5**, and **24** produced a single C4 product, whereas **4** and **7** produced the C4-isomer as the major product. Based on ^{1}H and 2D NMR analysis of the alkyl-group, all seven C4-alkyl-Trp analogues were confirmed to be normal alkylated. The ^{1}H NMR of C4-alkyl-Trp analogues when compared to that of tryptophan, showed the disappearance of the downfield shifted aromatic doublet signal corresponding to the H4 (7.58 ppm) of tryptophan. Among the four observed aromatic signals of these alkyl-tryptophan analogues, one was a singlet corresponding to H2 of the indole moiety, two doublets (H5 and H7) and one triplet (H6). Assignment of the aromatic signals was further supported by the COSY correlation peaks (H5-H6 and H6-H7) and carbon chemical shift values derived from ^{1}H-^{13}C-HSQC, that confirmed the substitution of the alkyl group to take place on the C4 position of the aromatic ring of tryptophan (top panel of **Figure S4A**, a representative ^{1}H-^{13}C-HSQC spectrum of **42**). In order to unambiguously confirm the findings, representative 2D ^{1}H-^{13}C-HMBC spectra were collected. The sequential connectivity between H5 of the indole moiety to the C1' of the alkyl group (bottom panel of **Figure S4A**, a representative ^{1}H-^{13}C-HMBC spectrum of **42**, **Table S5**) and/or H1' of the alkyl group to the C5 and C3a of the tryptophan aromatic moiety (**Table S5**) unambiguously confirmed the attachment of alkyl group at C4 position of tryptophan. Further, the NMR data of **35** and **40** matched the previously reported values,[30, 41] that formed the basis for comparison with the rest of the C4-isomers. Additional confirmation of the products was obtained from HRMS data, that displayed mass corresponding to the alkylated tryptophan product (**Table S3**). These results demonstrate the ability of FgaPT2 to catalyze C4-normal alkylation of tryptophan using unnatural alkyl donors including, *o*-methoxybenzyl and other allylic (**1**-**5**, and **7**) alkyl donors.

The NMR analysis of C5-alkyl-Trp analogues

Eleven alkyl-PP analogues (**4**, **6**, **7**, **8**, **9**, **18**, **22**, **25**, **27**, **28**, and **29**) in a FgaPT2 catalyzed alkylation reaction with tryptophan produced normal C5 alkylated products. These include single product of FgaPT2 reactions with **18**, **22**, **25**, **27**, **28**, **29**; major product of reactions involving **6**, **8**, and **9**; and minor product of reactions with **4** and **7**. Detailed ^{1}H and 2D NMR analysis of the alkyl-group of all 11 of the C5-alkyl-Trp analogues were confirmed to be normal alkylated (**Table S5**). Comparison of ^{1}H NMR and ^{1}H-^{13}C-HSQC spectra of the C5-alkyl-Trp analogues with that of tryptophan revealed that the upfield shifted triplet signal corresponding to H5 of the indole moiety (^{1}H,^{13}C =7.05 ppm, 118.1 ppm) has disappeared. Further, the presence of two singlets (H2 and H4) and two doublets (H6 and H7) and COSY correlation between H6-H7, along with their signature carbon chemical shift values derived from the combined analysis of COSY, and ^{1}H-^{13}C-HSQC spectra (top panel of **Figure S4B**, a representative ^{1}H-^{13}C-HSQC

spectrum of **43**, **Table S5**); implied the alkyl addition to take place at the C5 position of the aromatic ring of tryptophan. To confirm the C5 regio-specific attachment of the alkyl group and to identify the aromatic signals of the benzylic alkyl substituents, ^{1}H-^{13}C-HMBC spectra were collected for all new benzylic alkyl tryptophan analogues (involving **25**, **27**, **28** and **29**) and two representative allylic alkyl tryptophan analogues (involving **4** and **9**) (**Table S5**). The HMBC spectra displayed the correlation from H4 and H6 of tryptophan to the C1' of the alkyl-group (bottom panel of **Figure S4B**, a representative ^{1}H-^{13}C-HMBC spectrum of **43**, **Table S5**); and/or from H1' of the alkyl group to C4 and C6 of the tryptophan aromatic moiety (see Supporting Information, **Table S5**), confirming the C5-alkylation of tryptophan. In addition, the NMR data of **48** matched the previously reported values.[41, 42] Additional confirmation of alkyl group attachment to tryptophan was obtained via HRMS analysis (**Table S3**). These results support the ability of FgaPT2 to catalyze normal alkylation at the C5 position of unsubstituted L-tryptophan using diverse benzylic and allylic alkyl donors.

The NMR analysis of C3-alkyl-Trp analogue (**53**)

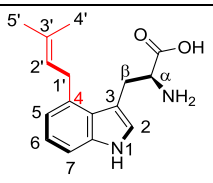
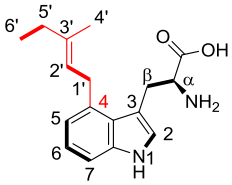
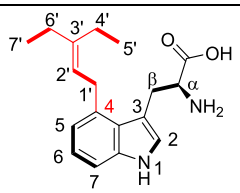
The UV spectra of the major product of the FgaPT2 catalyzed a reaction with **17** and tryptophan displayed maximum absorption at 228, 240, and 289 nm (**Figure S1**), suggesting the presence of an indoline-like structure. This was further confirmed by HRMS and NMR analysis (**Tables S3** & **S5**). The ^{1}H and 2D NMR analysis of the alkyl group suggested the alkylation to happen in a normal manner. In addition, the coupling pattern and COSY correlation of the four aromatic protons suggested the six-membered aromatic ring of the indole moiety is similar to that of tryptophan, implying the alkyl substitution is not directly on the six-membered ring of tryptophan. Further, the combined analysis of ^{1}H-^{13}C-HSQC and ^{1}H-^{13}C-HMBC spectra revealed the upfield shifted signals of H2/C2 (5.34 ppm/82.2 ppm) and C3 (58.2 ppm) of tryptophan (**Figure S4C**), clearly indicating the broken aromaticity of the pyrrole ring. The unambiguous confirmation of cyclization, that yielded a hexahydro-pyrroloindoline moiety was derived from ^{1}H-^{13}C-HMBC analysis, that displayed the connectivity between H1' of the alkyl group to C2, Cβ and C3a position; and C2 to Cα of the alkyl-pyrroloindoline moiety (bottom panel of **Figure S4C**, **Table S5**). These analyses confirmed FgaPT2 to catalyze normal C3-alkylation followed by cyclization of tryptophan in the presence of **17**.

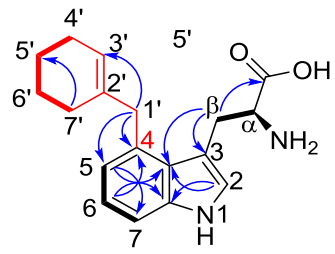
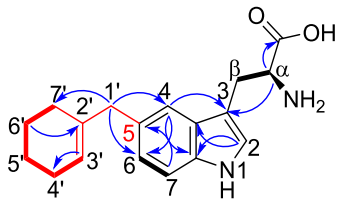
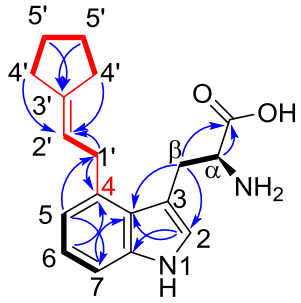
The NMR analysis of N1-alkyl-Trp analogues (**54** and **55**)

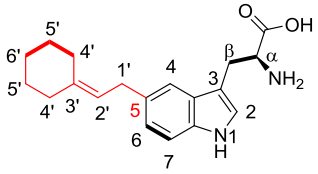
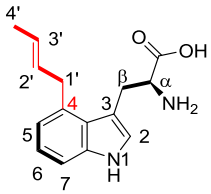
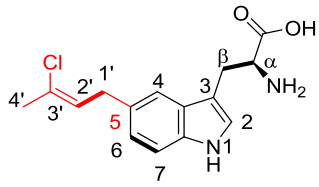
The NMR analysis of the major product of FgaPT2 catalyzed reaction using alkyl-PP analogues, **19** and **20**, confirmed them to be N1-alkylated. The detailed analysis of **54** suggested a normal alkylation based on the pattern of signals of the alkyl moiety, whereas, **55** indicated it to be reverse alkylated via the C5' (reverse$_{C5'}$) of alkyl-PP analogue, **20** (**Figure 5**). More specifically, the chemical shifts, coupling pattern

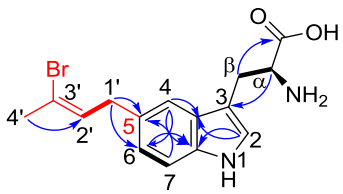
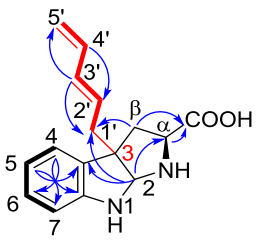
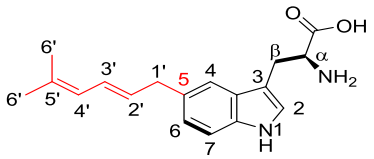
and COSY correlation of all aromatic protons (H2, H4, H5, H6, and H7) of the **54** and **55** analogues appeared similar to that of tryptophan, implying no substitution to take place directly on the carbon atom of the aromatic ring of tryptophan. This is supported by the ^{1}H and ^{13}C chemical shift assignment (top panel of **Figure S4D**, a representative ^{1}H-^{13}C-HSQC spectrum of **55**, **Table S5**) and COSY correlations (H4-H5, H5-H6, H6-H7) of the **54** and **55**. The H1' chemical shift value of the alkyl group in **54** at 5.05 ppm supported the N-alkylation of tryptophan with attachment via C1'. The pattern of signals of alkyl moiety in **55** suggested C5' of **20** was attached to N1 of the indole ring with C5'/H5' chemical shift values at 53 ppm/5.21 ppm respectively (top panel of **Figure S4D**, **Table S5**). The confirmation of the N1-alkylation and alkyl group chemical shift assignment of **55** was derived from the ^{1}H-^{13}C HMBC spectra, that displayed a correlation between H5' of the alkyl group and C2 of the tryptophan aromatic moiety (bottom panel of **Figure S4D** for **55**, **Table S5**). This is the first demonstration of FgaPT2 to carry out the reverse alkylation (although via C5' group of **20**) at N1 of tryptophan. Therefore, while the FgaPT2-catalyzed N1-normal alkylation has been previously demonstrated with tryptophan derivatives using DMAPP,[*34*] this study revealed the possibility of N1-reverse$_{C5'}$ alkylation of native tryptophan by an unnatural alkyl-donor, **20**.

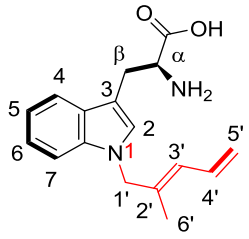
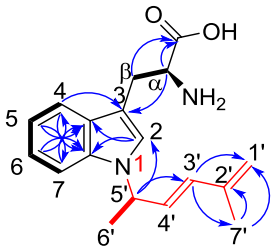
Table S5. Summary of NMR chemical shift assignment of the alkyl-L-Trp analogues (500 MHz). Where δ_H and δ_C values are in ppm and coupling constant, *J* is in Hz. a= DMSO-d_6, b= MeOH-d_4

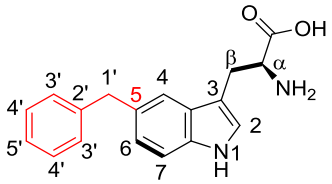
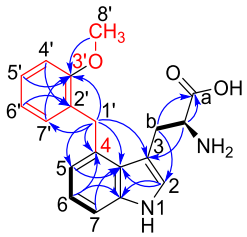
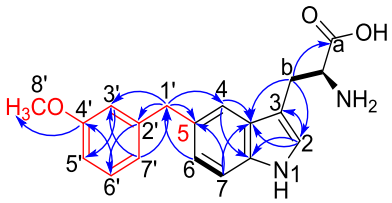
Structure / COSY HMBC Correlation / Compound	 **35[a]***		 **36[a]**		 **37[a]**	
Position	**δ_H, multi (J)**	**δ_C**	**δ_H, multi (J)**	**δ_C**	**δ_H, multi (J)**	**δ_C**
COOH	-	n.f.	-	n.f.	-	n.f.
NH	10.99, s	-	10.88, d (2.5)	-	10.89, d (2.5)	-
2	7.18, s	125.3	7.18, d (2.5)	124.8	7.18, d (2.5)	124.6
3	-	n.f.	-	n.f.	-	n.f.
3a	-	n.f.	-	n.f.	-	n.f.
4	-	n.f.	-	n.f.	-	n.f.
5	6.73, d (7.7)	119.4	6.71, d (7.6)	118.9	6.72, d (7.6)	118.7
6	6.97, t (7.7)	121.7	6.95, t (7.6)	121.5	6.95 t, (7.6)	121.5
7	7.19, d (7.7)	110.1	7.17, d (7.6)	109.9	7.17, d (7.6)	109.9
7a	-	n.f.	-	n.f.	-	n.f.
β	3.51, dd (15.6, 4.4); 3.11, dd (15.6, 10.5)	n.f.	3.57, dd (15.6, 3.3); 2.94, dd (15.6, 10.7)	n.f.	3.58, dd (15.6, 3.2); 2.95, dd (15.6, 10.6)	29.7
α	3.41	n.f.	3.39, dd (10.6, 3.2)	56.1	3.39, dd (10.6, 3.2)	55.9
1'	3.7-3.66, m	31.9	3.68, dd (16.0, 7.8) 3.74, dd (16.0, 6.0)	31.5	3.71, dd (16.0, 7.8) 3.76, dd (16.0, 7.1)	31.1
2'	5.29, dd (7.8, 6.0)	124.1	5.32 dd (7.8, 6.0)	122.7	5.29, t (7.1)	122.1
3'	-	n.f.	-	n.f.	-	n.f.
4'	1.70, s	26.1	1.71, s	16.6	2.08-2.02, m	29.2
5'	1.70, s	18.4	2.02, q (7.4)	32.4	0.98, t (7.5)	13.4
6'	-	-	0.98, t (7.4)	13.1	2.18-2.12,m	23.5
7'	-	-	-	-	0.97, t (7.5)	13.4

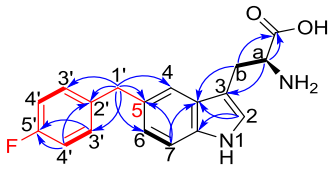
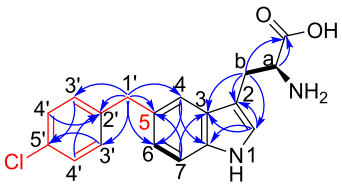
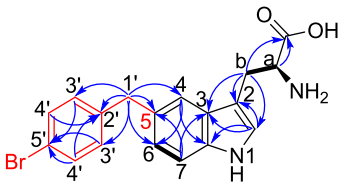
Structure / COSY HMBC Correlation / Compound	 **42**[b]		 **43**[b]		 **38**[b] (80% C4^{N} isomer + 20% unknown isomer)	
Position	**δ_H, multi (J)**	**δ_C**	**δ_H, multi (J)**	**δ_C**	**δ_H, multi (J)**	**δ_C**
COOH	-	173.0	-	173.1	-	170.5
NH	exc.	-	exc	-	exc	-
2	7.14, s	124.1	7.16, s	123.9	7.17, s	124.8
3	-	109.3	-	107.9	-	107.5
3a	-	125.3	-	127.1	-	124.4
4	-	131.5	7.50, d (1.5)	118.0	-	133.8
5	6.79, d (7.6)	121.2	-	130.8	6.85, dd, (7.1, 0.8)	119.8
6	7.04, t (7.6)	121.5	6.97, dd (8.4, 1.5)	122.9	7.04, dd, (8.2, 7.1)	121.9
7	7.23, d (7.6)	109.5	7.27, d (8.4)	110.9	7.24, dd, (8.2, 0.8)	109.7
7a	-	137.8	-	135.7	-	138.2
β	3.76, dd (15.6, 3.5) 3.02, dd (15.6,11.2)	29.3	3.53, dd (15.2, 3.9); 3.10, dd (15.2, 9.9)	27.4	3.83, dd, (15.5, 3.9); 3.19, dd, (15.5, 11.1)	28.8
α	3.83, dd (11.2, 3.5)	56.2	3.86, dd (9.9, 3,9)	55.5	4.18, dd, (11.1, 3.9)	54.3
1'	3.67, s	41.5	3.33, s	44.7	3.76 – 3.72, m	33.4
2'	-	138.1	-	138.2	5.47 – 5.41, m	119.4
3'	5.08, s	121.8	5.54-5.43, m	121.6	-	144.3
4'	1.96-1.9, m	25.1	2.08-2.00, m	25.1	2.39 – 2.25, m	33.5, 28.7
5'	1.60-1.54, m	22.6	1.64-1.52, m	22.5	1.78 – 1.70, m 1.69-1.62, m	26.4, 26.3
6'	1.69-1.63, m	23.1	1.64-1.52, m	22.5	-	-
7'	2.13-2.06, m	28.8	1.93-1.86, m	27.8	-	-

Structure / COSY HMBC Correlation / Compound	 **44[a]**		 **40[a]***		 **45[c]**
Position	**δ_H, multi (J)**	**δ_C**	**δ_H, multi (J) /**	**δ_C**	**δ_H, multi (J)**
COOH	-	n.f.	-	n.f.	-
NH	10.73, br.s	-	10.88, br. s	-	exc
2	7.14, d (1.9)	124.5	7.16, d (2.1)	124.7	7.30, s
3	-	n.f.	-	n.f.	-
3a	-	n.f.	-	n.f.	-
4	7.31, s	117.6	-/	n.f.	7.58, s
5	-	n.f.	6.73, d (7.1)	119.7	-
6	6.88, dd (8.4, 1.2)	122.3	6.97, t (7.6)	121.5	7.47, d (8.4)
7	7.24, d (8.4)	111.8	7.18, dd (7.6, 7.1)	110.4	7.18, d (8.4)
7a	-	n.f.	-	n.f.	-
β	3.33-3.25, m 2.85, dd (15.2,9.7)	27.9	3.57,dd (15.5, 3.6) 2.88,dd (15.5, 11.1)	29.7	3.47, dd (15.2, 4.8) 3.24, dd (15.2, 8.5)
α	3.39, dd, (9.7, 3.9)	55.3	3.36-3.34, m	56.1	4.04, dd (8.5, 4.8)
1'	3.36, d (7.5)	33.5	3.74, dd (15.5, 6.4) 3.62, dd (15.5, 6,1)	36.2	3.63, d (7.4)
2'	5.27, t (7.5)	121.7	5.68-5.56, m	131.7	5.92-5.81, m
3'	-	n.f.	5.52-5.42, m	125.5	-
4'	2.25, t (5.2) 2.07, t (5.2)	28.7, 37.1	1.62, d (6.1)	18.2	2.18-2.13, m
5'	1.60-1.44, m	28	-	-	-
6'	1.60-1.44, m	28	-	-	-

Structure / COSY HMBC Correlation / Compound	 **46[b]**		 **53[b]**		 **47[b]**
Position	**δ_H, multi (J)**	**δ_C**	**δ_H, multi (J)**	**δ_C**	**δ_H, multi (J)**
COOH	-	173.0		169.2	-
NH	exc	-	exc	-	exc
2	7.18, s	124.2	5.34, s	82.2	7.17, s
3	-	107.9	-	58.2	-
3a	-	127.1	-	130.5	-
4	7.53, s	122.4	7.24, d (7.6)	123.5	7.51, s
5	-	129.9	6.86, t (7.6)	120.3	-
6	6.99, d (8.3)	117.4	7.15, t (7.6)	129.2	6.98, d (8.3)
7	7.29, d (8.3)	111.3	6.71, d (7.6)	109.7	7.28, d (8.3)
7a	-	135.8	-	148.7	-
β	3.51, dd (15.3, 4.0) 3.12, dd (15.3, 9.5)	27.3	2.76, dd (13.3, 6.2) 2.44, dd (13.3, 11.8)	40.7	3.51, dd (15.3, 4,0) 3.10, dd (15.3, 9.6)
α	3.86, dd, (9.5, 4.0)	55.5	4.00, dd (11.8, 6.2)	57.9	3.85, dd (9.6, 4.0)
1'	3.57, d (7.0)	37.9	2.78-2.68, m	34.9	3.52-3.49, m
2'	5.92, t (7.0)	129.1	5.24-5.16, m	125.3	5.71, dt (14.8, 7.00)
3'	-	121.4	6.10, t (11.0)	132.7	6.31,dd(14.8, 11.0)
4'	2.32, s	27.7	6.65, ddd (16.7, 11.0, 10.6)	131.6	5.79, d (11.0)
5'	-	-	5.22, d (16,7) 5.15, d (10.6)	118.0	-
6'	-	-	-	-	1.75, (s); 1.73, (s)

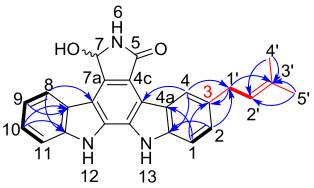
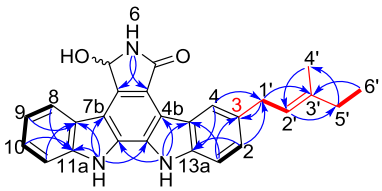
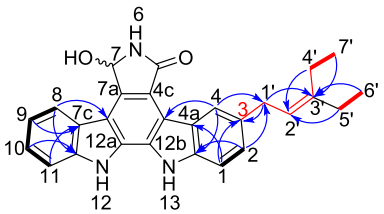
Structure / COSY HMBC Correlation/ Compound	 **54[a]**	 **55[b]**	
Position	**δ_H, multi (J)**	**δ_H, multi (J)**	**δ_C**
COOH	-	-	172.9
NH	-	-	-
2	7.19, s	7.27, s	124.2
3	-	-	108.1
3a	-	-	127.8
4	7.58, d (7.9)	7.71, d (7.8)	118.6
5	7.02, dd (7.9, 7.6)	7.08, dd (7.8, 7.6)	119.1
6	7.12, dd (8.2, 7.6)	7.16, dd (8.2, 7.6)	121.6
7	7.39, d (8.2)	7.40, d (8.2)	109.8
7a	-	-	136.5
β	3.34-3.24, m 2.93, dd (15.1, 8.8)	3.52, dd (15.2, 4.1) 3.15, dd (15.2, 9.3)	27.4
α	3.41-3.36, m	3.85, dd (9.3, 4.1)	55.6
1'	5.05-4.90, m	4.96, s 4.93, s	116.3
2'	-	-	141.3
3'	6.30, d (15.8)	6.22, d (15.8)	133.1
4'	5.80, dt (15.8, 6.1)	5.89, dd (15.8, 6.01)	130.3
5'	4.89-4.77, m	5.21, p (7.0, 6.0)	52.9
6'	1.76, s	1.68, d (7.0)	19.6
7'	-	1.81, s	17.6

Structure / COSY HMBC Correlation / Compound	 **48[a]***		 **39[b]**		 **49[b]**	
Position	**δ_H, multi (J)**	**δ_C**	**δ_H, multi (J)**	**δ_C**	**δ_H, multi (J)**	**δ_C**
COOH	-	n.f.	-	170.2	-	170.8
NH	10.77, s	-	exc	-	exc	-
2	7.15, s	124.9	7.14, s	124.9	7.18, s	124.6
3	-	n.f.	-	107.4	-	106.5
3a	-	n.f.	-	124.8	-	127.0
4	7.41, s	118.5	-	131.5	7.48, d (1,6)	117.5
5	-	n.f.	6.77, d (7.2)	121.6	-	132.0
6	6.92, dd (8.3, 1.6)	122.8	7.08, dd (8.2, 7.2)	121.9	7.00, dd (8.4, 1.6)	123.4
7	7.24, d, (8.3)	111.9	7.31, d (8.2)	110.1	7.30, d (8.4)	111.5
7a	-	n.f.	-	138.0	-	135.7
β	3.28, dd(15.2, 3.7) 2.86, dd (15.2, 9.6)	n.f.	3.52, dd (15.4, 3.9); 2.98, dd (15.4, 11.4)	28.4	3.50, dd, (15.3, 4.7) 3.27, dd (15.3, 8.4)	26.5
α	3.38, dd (9.6, 3.7)	n.f.	3.73, dd (11.4, 3.9)	53.7	4.19, dd (8.4, 4.7)	53.5
1'	3.98, s	42.3	4.42, d (16.9); 4.28, d (16.9)	33.2	4.03, s	41.7
2'	-		-	129.5	-	144.1
3'	7.29 – 7.17, m	128.9	-	157.1	6.76, t (2.1)	114.2
4'	7.29 – 7.17, m	128.9	6.96, d, (8.2)	109.9	-	159.8
5'	7.18 – 7.1, m	125.9	7.18, dd (8.2,7.4)	127.2	6.71, dd (8.2, 2.1)	110.7
6'	-	-	6.74, t (7.4)	120.0	7.15, t (7.9)	129.1
7'	-	-	6.64, d (7.4)	129.1	6.80, dd (7.9, 2.1)	121.1
8’	-	-	3.85, s	54.5	3.73 (s, 3H)	54.3

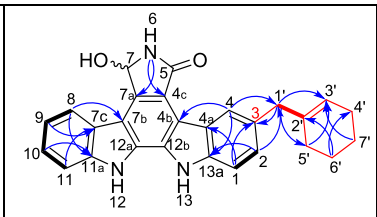
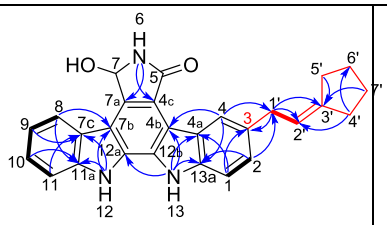
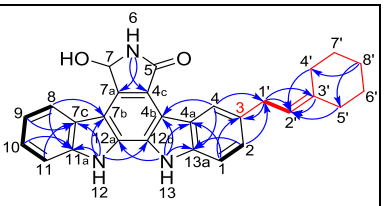
Structure / COSY HMBC Correlation / Compound	 **50**[b]		 **51**[b]		 **52**[b]	
Position	**δ_H, multi (J)**	**δ_C**	**δ_H, multi (J)**	**δ_C**	**δ_H, multi (J)**	**δ_C**
COOH	-	170.3	-	170.5	-	170.4
NH	exc	-	exc	-	exc	-
2	7.18, s	124.4	7.19, s	124.7	7.19, s	124.7
3	-	106.2	-	106.5	-	106.4
3a	-	127.1	-	127.1	-	127.1
4	7.46, d (1.4)	117.4	7.47, s	117.5	7.46, d (1.4)	117.5
5	-	131.9	-	131.5	-	131.5
6	6.98, dd (8.3, 1.4)	122.9	6.98, d (8.3)	123.0	6.98, dd (8.3, 1.4)	123.0
7	7.31, d (8.3)	111.2	7.31, d (8.3)	111.5	7.31, d (8.3)	111.5
7a	-	135.7	-	136.2	-	135.7
β	3.48, dd (15.2, 4.9) 3.30, dd (15.2, 8.1)	26.1	3.48, dd (15.3, 4.8) 3.28, dd (15.3, 8.1)	26.5	3.48, dd (15.3, 4.8) 3.28, dd (15.3, 8.1)	26.5
α	4.23, dd, (8.1, 4.9)	53.2	4.19, dd (8.1, 4.8)	53.5	4.22, dd (8.1, 4.9)	53.4
1'	4.04, s	40.8	4.04, s	41.2	4.03, s	41.0
2'	-	138.5	-	141.4	-	141.8
3'	7.21, dd (8.6, 5.5)	129.9	7.19, d (8.3)	130.1	7.14, d (8.3)	130.4
4'	6.96, dd (9.1, 8.6)	114.3	7.23, d (8.3)	128.1	7.38, d (8.3)	131.1
5'	-	161.3 d. (240.3)	-	131.1	-	119.4

*NMR assignment of these compounds has been reported previously; ▬ = COSY correlations; ↷ = HMBC correlations.

Table S6. Summary of NMR chemical shift assignment of the alkyl-7-hydroxy-K252c analogues in Acetone-d6 (500 MHz). Where δ_H and δ_C values are in ppm and coupling constant, *J* is in Hz.

Structure / COSY HMBC Correlation / Compound	 **57**		 **58**		 **59**	
Position	**δH, multi (J)**	**δC**	**δH, multi (J)**	**δC**	**δH, multi (J)**	**δC**
1	7.53, d (8.1)	111.2	7.53, d (8.0)	110.9	7.54, d (8.3)	110.9
2	7.26, dd (8.1, 1.8)	126.7	7.26, dd (8.0, 1.8)	126.3	7.28, dd (8.3, 1.8)	126.1
3	-	132.7	-	132.7	-	132.8
4	9.18, d (1.8)	125.4	9.18, d (1.8)	125.2	9.20, d (1.8)	124.9
4a	-	123.6	-	123.6	-	123.7
4b	-	116.3	-	116.3	-	116.31
4c	-	n.f.	-	135.1	-	n.f.
5	-	n.f.	-	n.f.	-	n.f.
6	7.72, s	-	7.72, s	-	7.72, s	-
7	6.57, d (10.6)	79.6	6.56, d (5.4)	79.4	6.59, d (8.6)	79.3
7-OH	5.24, d (10.6)	-	5.24, d (5.4)	-	5.24, d (8.6)	-
7a	-	n.f.	-	118.6	-	118.6
7b	-	n.f.	-	115.7	-	115.8
7c	-	122.9	-	122.9	-	122.9
8	8.49, d (7.9)	123.7	8.50, d (7.8)	123.4	8.49, d (7.9)	123.4
9	7.30-7.26, m	120.4	7.30-7.26, m	119.9	7.31-7.27, m	120.0
10	7.46-7.42, m	125.7	7.45-7.41, m	125.3	7.46-7.42, m	125.3
11	7.67, d (7.9)	111.8	7.66, d (8.0)	111.5	7.66, d (8.1)	111.5
11a	-	140.1	-	140.1	-	140.1
12	11.08, s	-	11.14, s	-	11.03, s	-
12a	-	n.f.	-	n.f.	-	n.f.
13	10.85, s	-	10.91, s	-	10.81, s	-
13a	-	138.5	-	138.6	-	138.6
1'	3.54, d (7.3)	34.9	3.55, d (7.2)	34.5	3.57, d (7.4)	33.9
2'	5.46, t (7.3)	125.4	5.48, t (7.2)	123.5	5.43, t (7.4)	122.9
3'	-	130.9	-	136.6	-	142.3
4'	1.82, s	17.3	1.82, s	15.7	2.11, q (7.6)	29.2
5'	1.76, s	25.6	2.07, q (7.4)	32.5	2.28, q (7.6)	23.1
6'	-	-	1.04, t (7.4)	12.6	1.09, t (7.6)	12.6
7'	-	-	-	-	1.04, t (7.6)	12.5

*NMR assignment of these compounds has been reported previously; ━ = COSY correlations; ↷ = HMBC correlations.

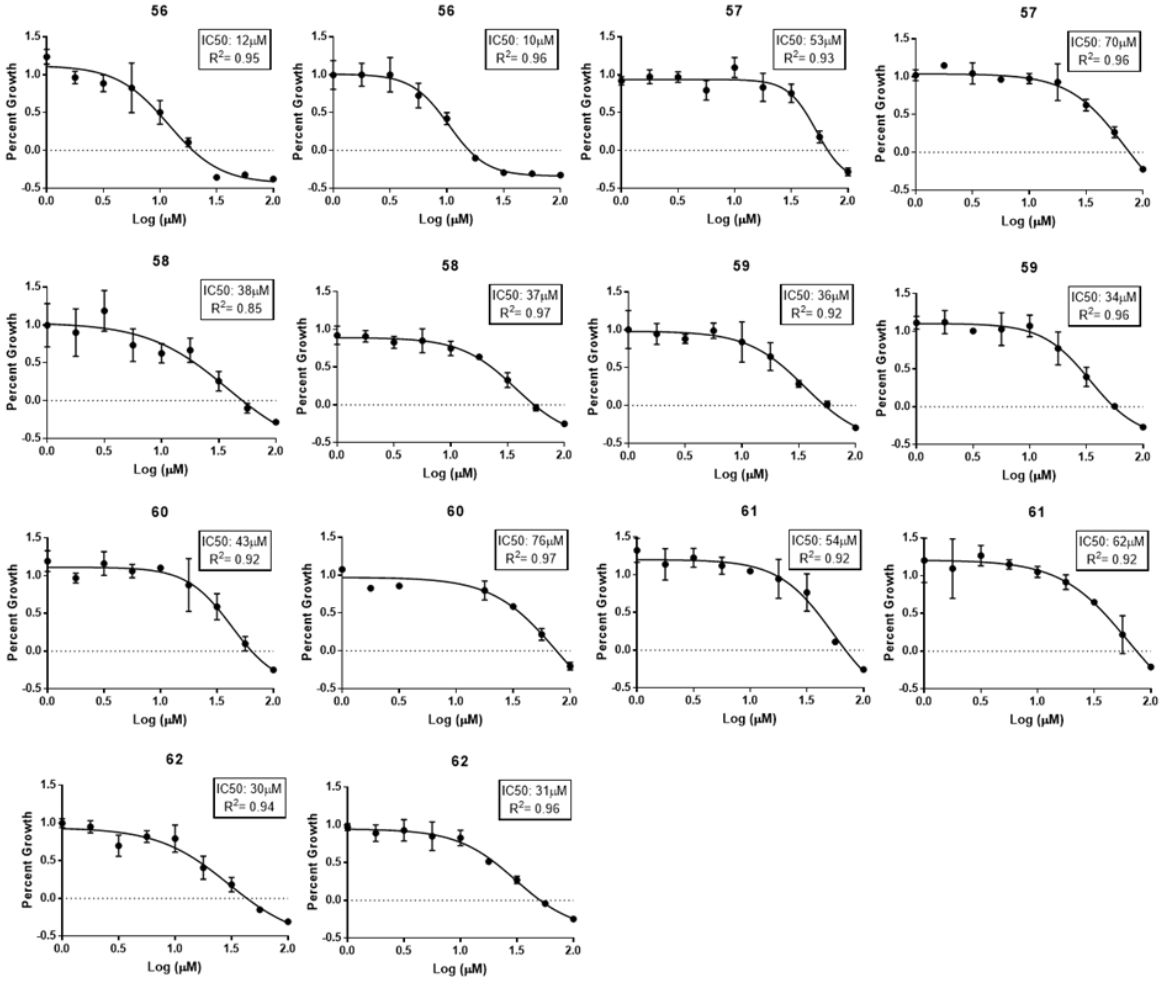
Structure / COSY HMBC Correlation / Compound	 60		 61		 62	
Position	**δH, multi (J)**	**δC**	**δH, multi (J)**	**δC**	**δH, multi (J)**	**δC**
1	7.53 (d, (8.2)	110.8	7.55, d (8.3)	111.0	7.53, d (8.2)	110.9
2	7.26 d, (8.2)	126.6	7.26, d (8.3)	125.9	7.26, d (8.2)	126.4
3	-	131.1	-	132.1	-	132.7
4	9.16 (s)	125.9	9.16, s	124.9	9.17, s	125.1
4a	-	123.5	-	123.5	-	123.6
4b	-	116.3	-	115.9	-	116.2
4c	-	135.2	-	134.3	-	135.1
5	-	n.f.	-	n.f.	-	n.f.
6	7.75 (s)	-	7.66, s	-	7.73, s	-
7	6.57 (d, (8.1)	79.4	6.55, d (8.3)	79.4	6.56, d (9.0)	79.3
7-OH	5.24 (d, (8.1)	-	5.28, d (8.3)	-	5.24, d (9.0)	-
7a	-	118.5	-	118.1	-	118.5
7b	-	115.7	-	115.3	-	115.6
7c	-	122.9	-	122.9	-	122.9
8	8.49 (d, (7.9)	123.4	8.46, d (7.9)	123.3	8.48, d (7.9)	123.3
9	7.29 (dd, (7.9, 7.6)	119.8	7.25, dd (7.9, 7.7)	119.7	7.28, t (7.9)	119.9
10	7.44 (dd, (8.1, 7.6)	125.2	7.42, dd (8.3, 7.7)	125.1	7.43, t (7.6)	125.3
11	7.66 (d, (8.1)	111.5	7.67, d (8.3)	111.6	7.66, d (8.1)	111.4
11a	-	140.1	-	140.4	-	140.1
12	11.02 (s)	-	12.24, s	-	11.16, s	-
12a	-	n.f.	-	127.3	-	126.9
12b	-	n.f.	-	n.f.	-	128.6
13	10.81 (s)	-	11.96, s	-	10.92, s	-
13a	-	n.f.	-	138.8	-	138.5
1'	3.43 (s)	44.9	3.51, d (7.3)	36.3	3.53, d (7.5)	33.6
2'	-	138.2	5.57, tp (7.3, 2.4)	120.4	5.39, t (7.5)	121.5
3'	5.52 (s)	122.2	-	142.7	-	139.1
4'	2.05 – 2.00 (m)	25.4	2.42, td (7.3, 2.4)	28.9	2.15, t (5.5)	37.3
5'	2.00 – 1.91 (m)	27.9	2.29, td (7.3, 2.4)	33.7	2.37, t (5.2)	28.6
6'	1.81 – 1.38 (m)	22.8	1.74, p (7.3, 6.9)	26.2	1.73 – 1.51, m	27.7
7'	1.81 – 1.38 (m)	22.8	1.64, p (7.3, 6.9)	26.4	1.73 – 1.51, m	28.6
8'	-	-	-	-	1.73 – 1.51, m	26.8

n.f. = Not found; exec.=exchangeable

Table S7: Values used to generate Figure 3 obtained from two experimental replicates.

Alkyl-PP analogue	% Conversion
1	94 ± 8
2	77 ±2
3	66 ± 8
4	40 ± 16
5	42 ± 4
6	52 ± 5
7	70 ±16
8	78 ± 10
9	41 ± 8
10	1 ± 0
13	1 ± 1
14	0.5 ± 0.1
15	0.9 ± 0.1
16	1 ± 0.8
17	84 ± 9
18	38 ± 11
19	54 ± 9
20	22.4 ± 0.6
21	0.7 ± 0.0
22	100.0 ± 0.0
24	94 ± 8
25	46 ± 15
27	70 ± 13
28	60 ± 2
29	61 ± 4

A.



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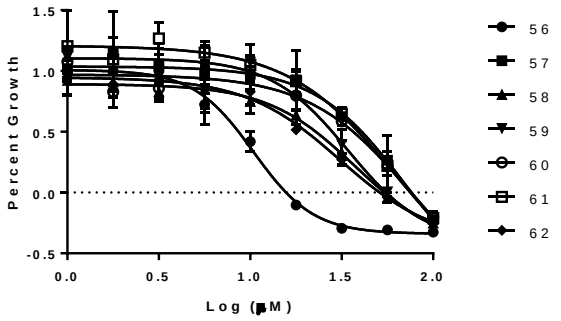

Figure S5: Anticancer activity of alkyl-7-hydroxy-K252c analogues. Individual cytotoxicity curves (duplicate) for the 7-hydroxy-K252c parent compound (**56**) and analogues (**57-62**) in HCT-116 cells. (**B**) Cytotoxicity curves for the 7-hydroxy-K252c parent compound and analogues (**56-62**) in HCT-116 cells. Error bars represent standard deviation within one independent experiment.

Supporting Information for

FgaPT2, a biocatalytic tool for alkyl-diversification of indole natural products

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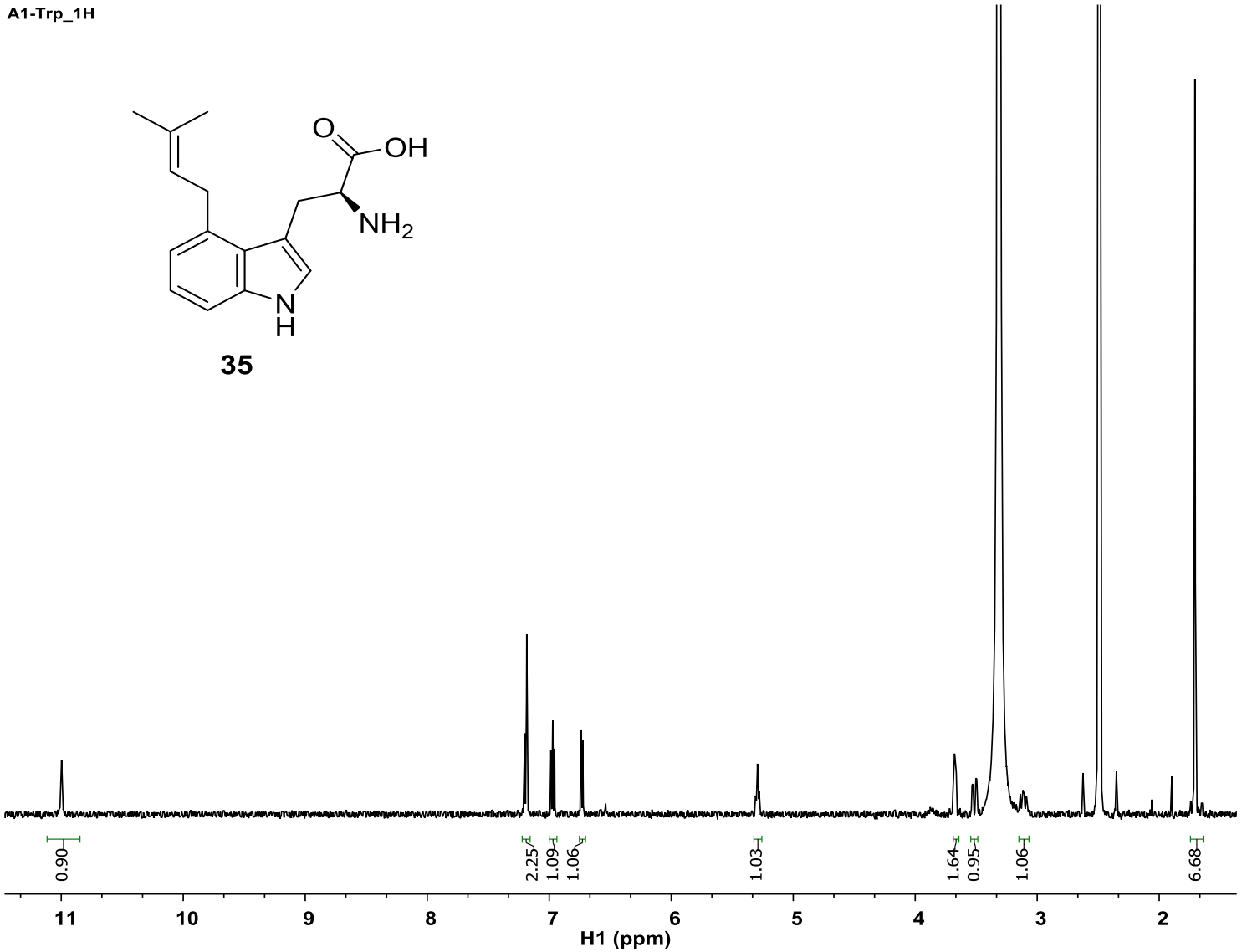
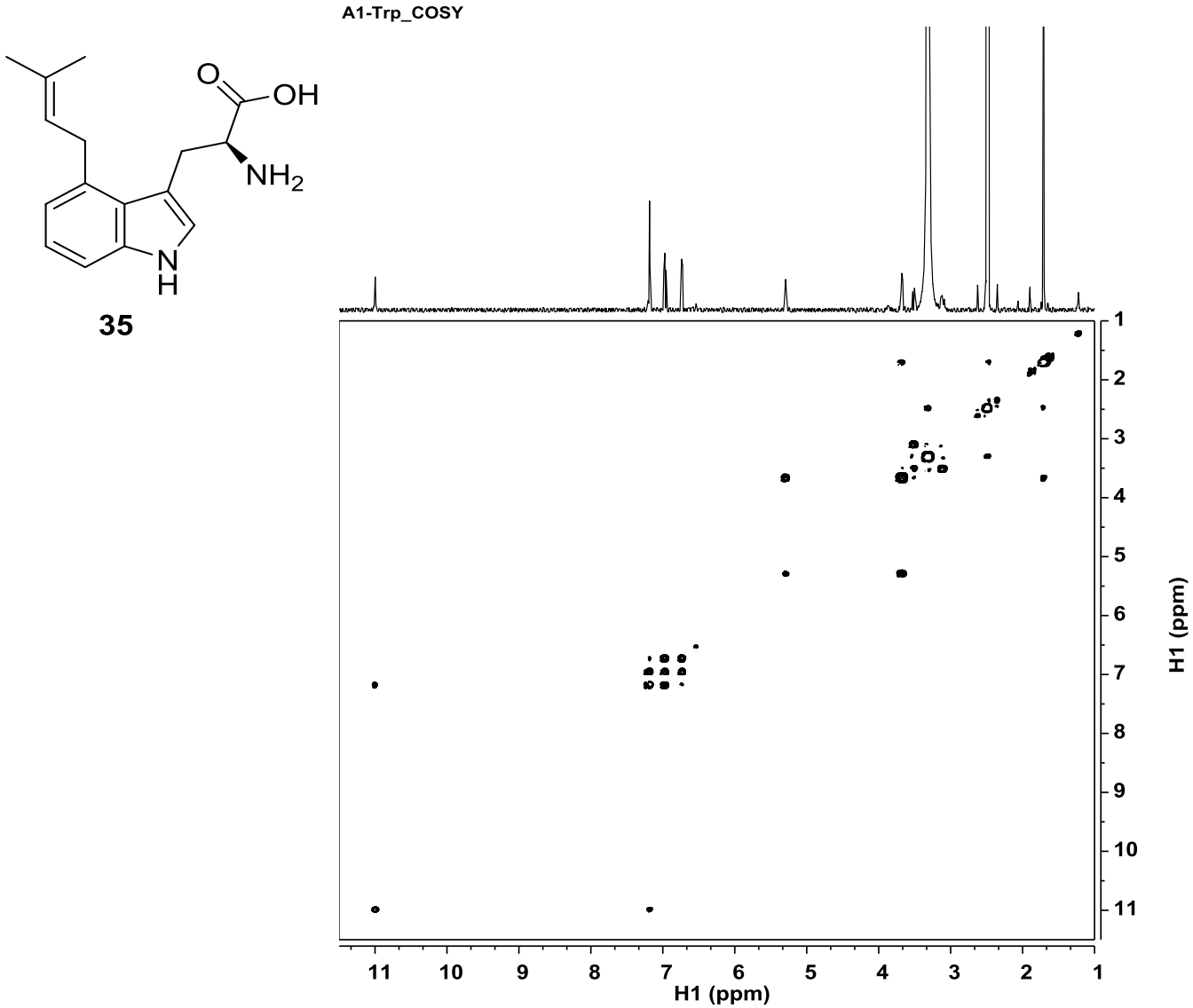
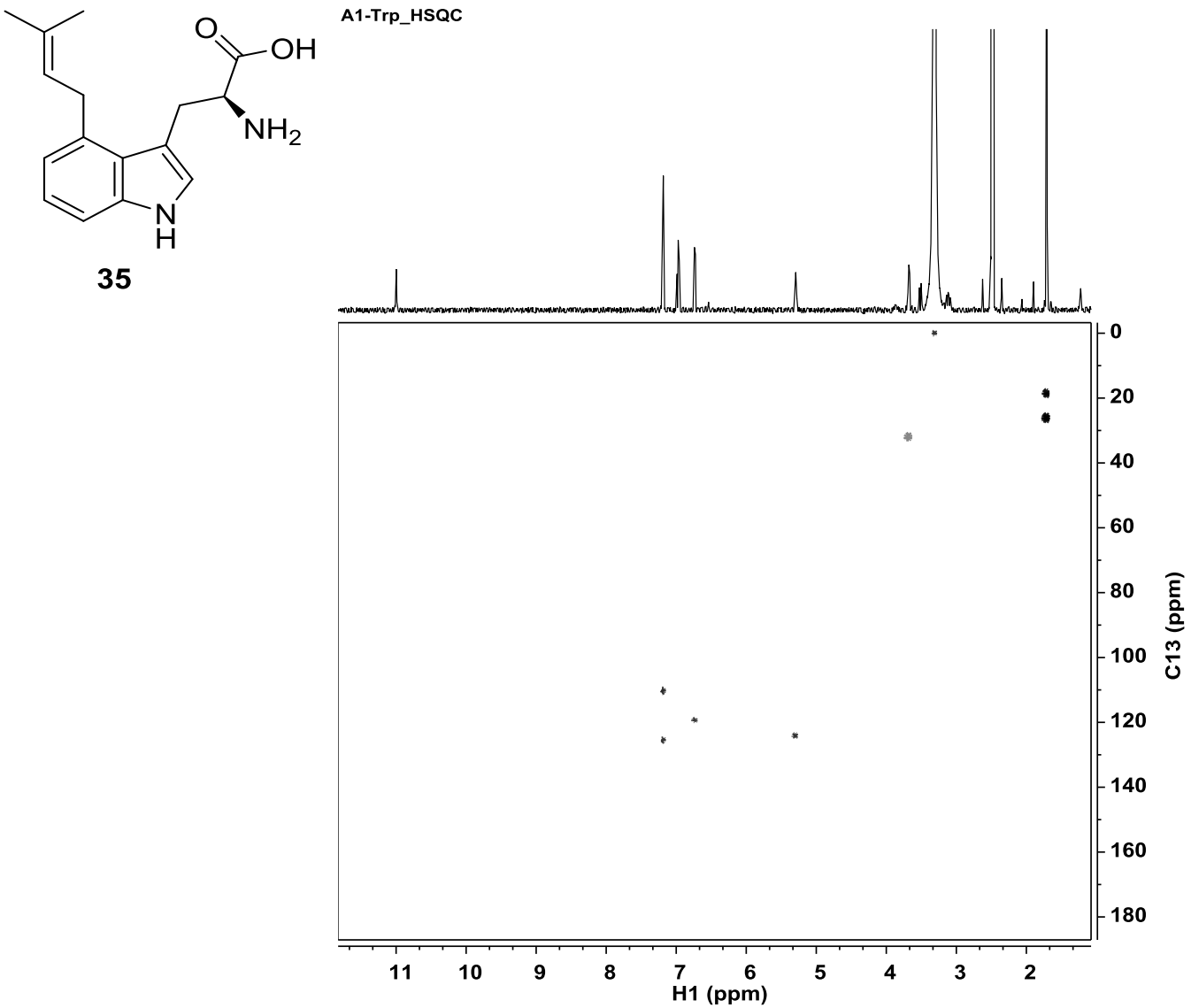


^{1}H NMR Spectrum (500 MHz) of compound **35** in DMSO-d_6



2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **35** in DMSO-d_6



2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **35** in DMSO-d_6

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Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
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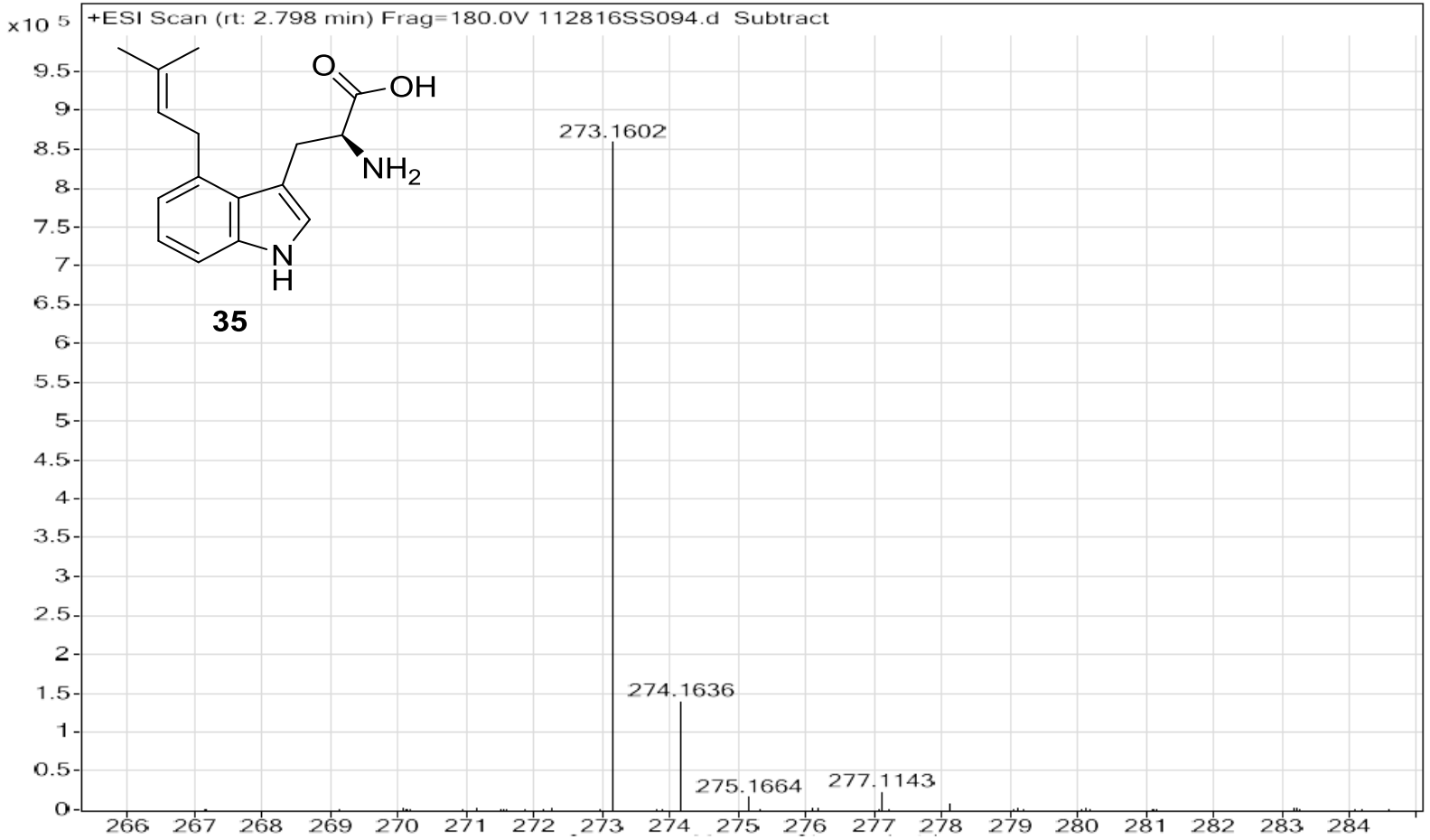
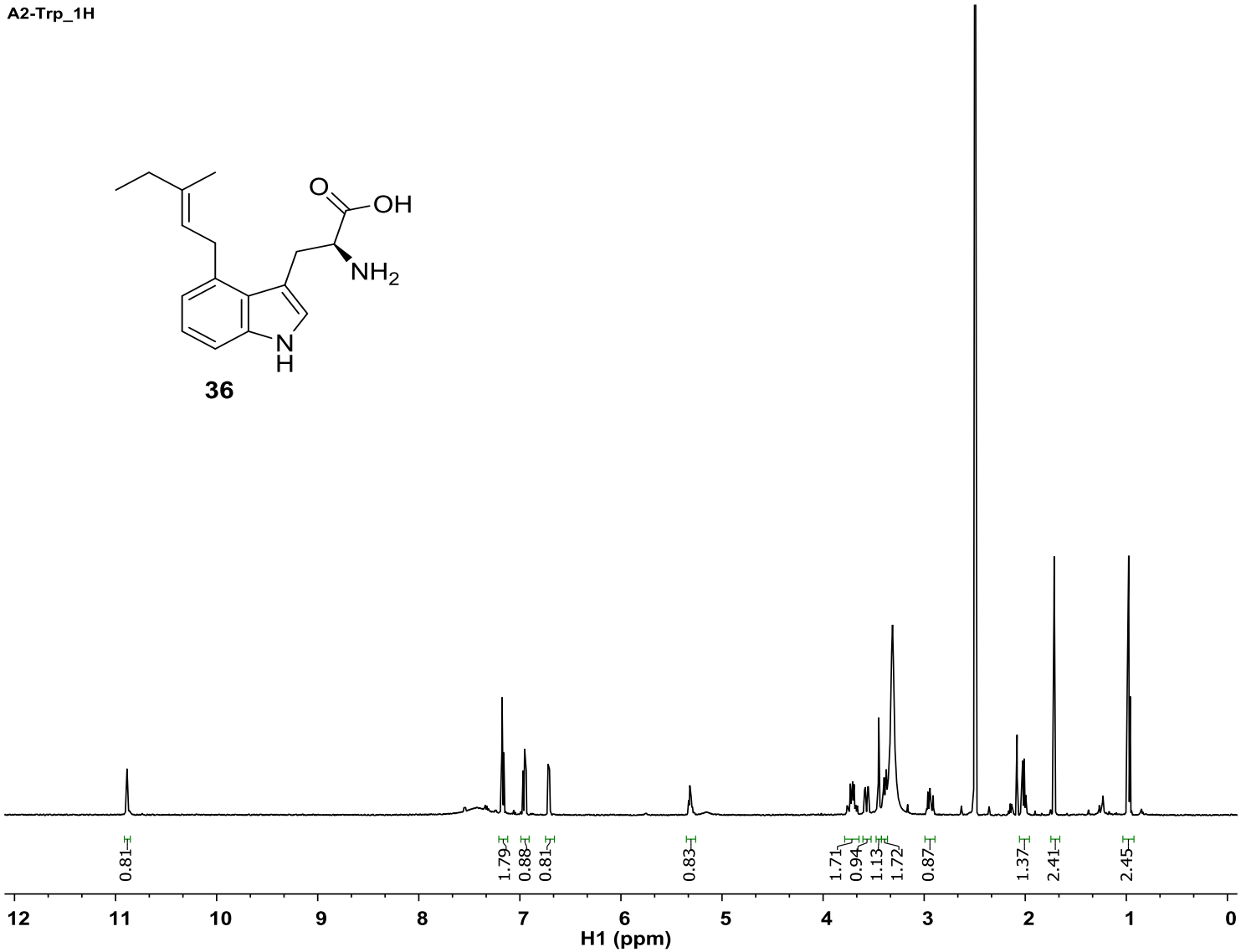
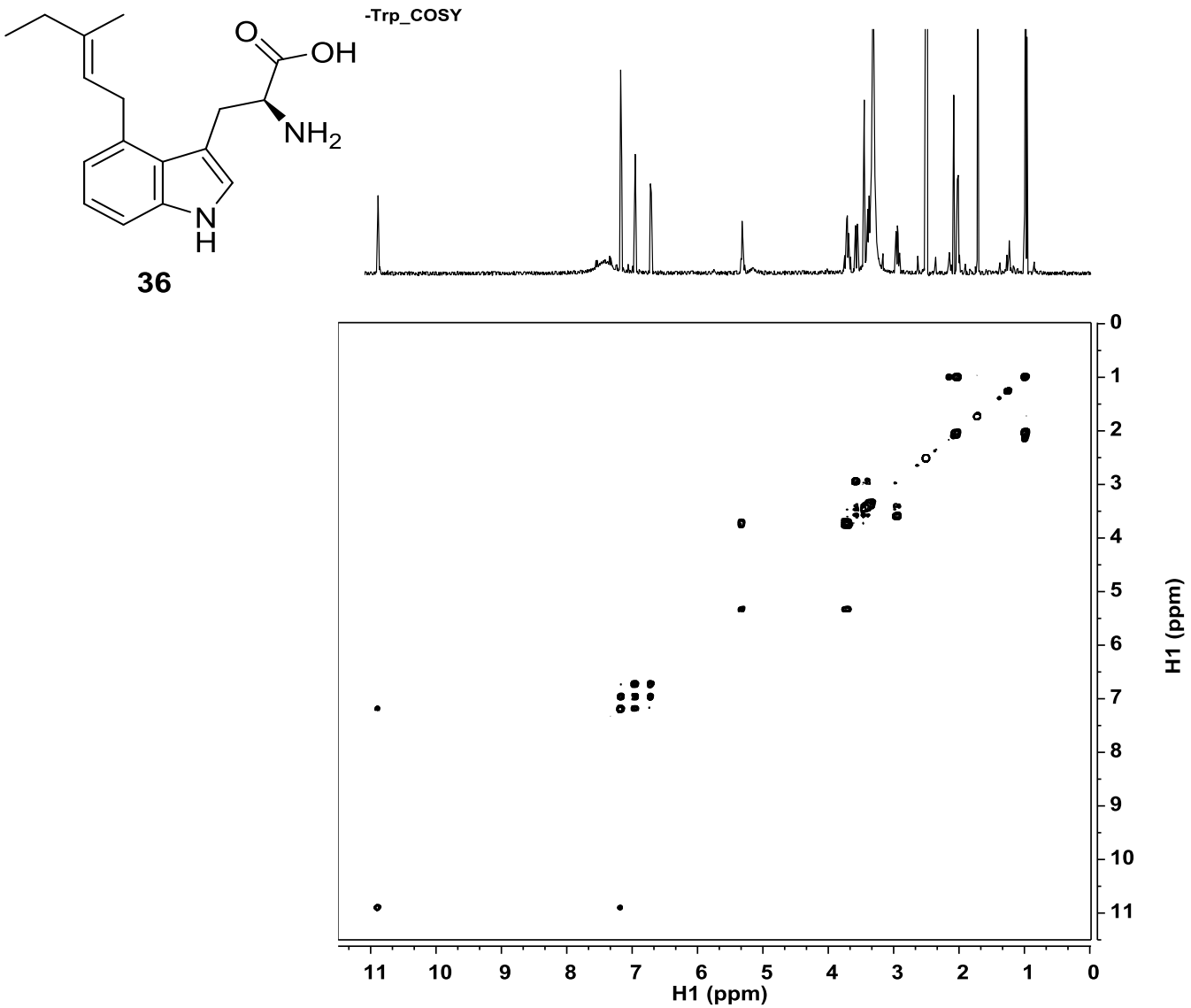


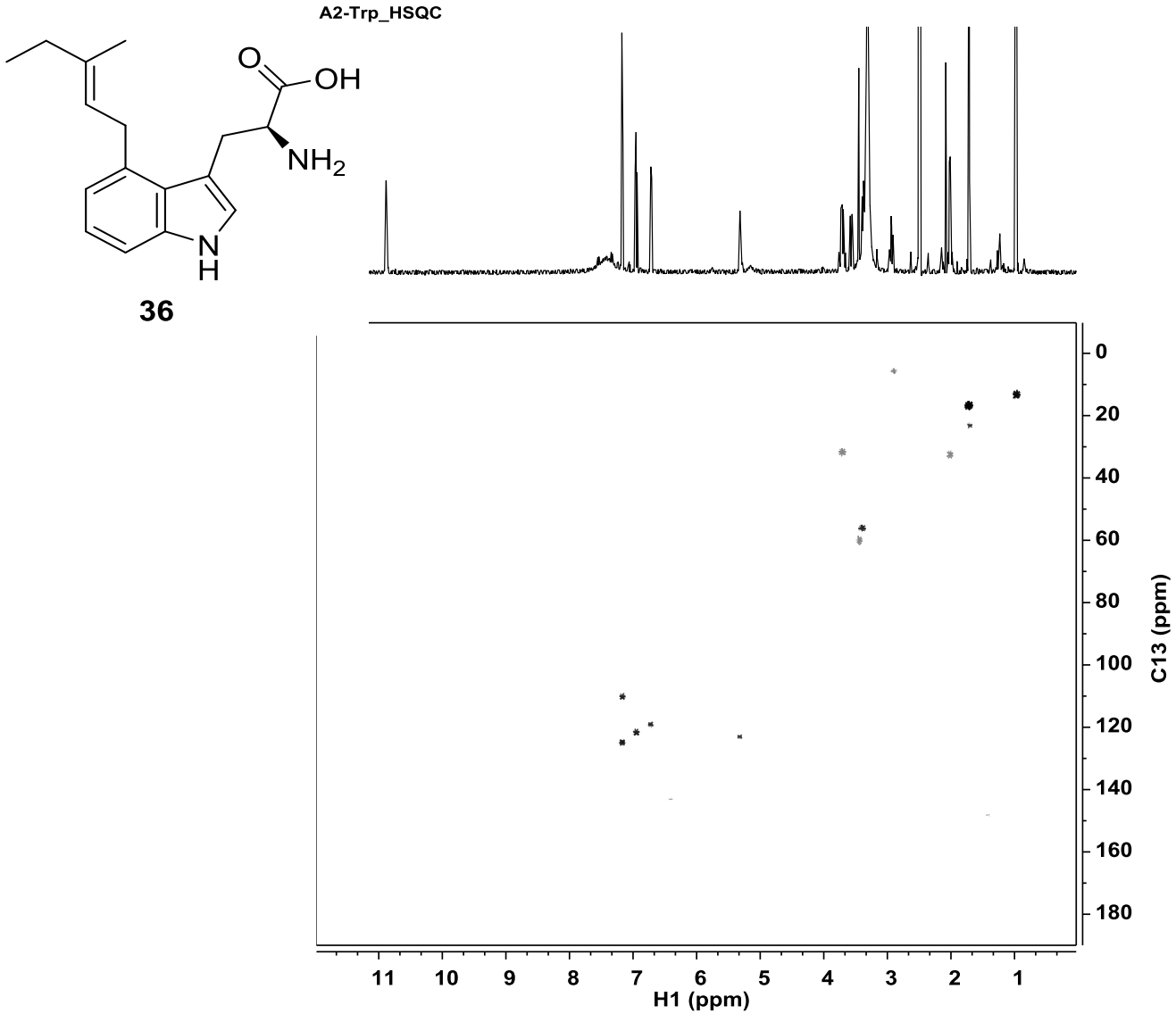
HRMS spectrum of **35**



^{1}H NMR Spectrum (500 MHz) of compound **36** in DMSO-d_6



2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **36** in DMSO-d_6



2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **36** in DMSO-d_6

Sample Name	TRP-37	**Position**	P1-E1	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
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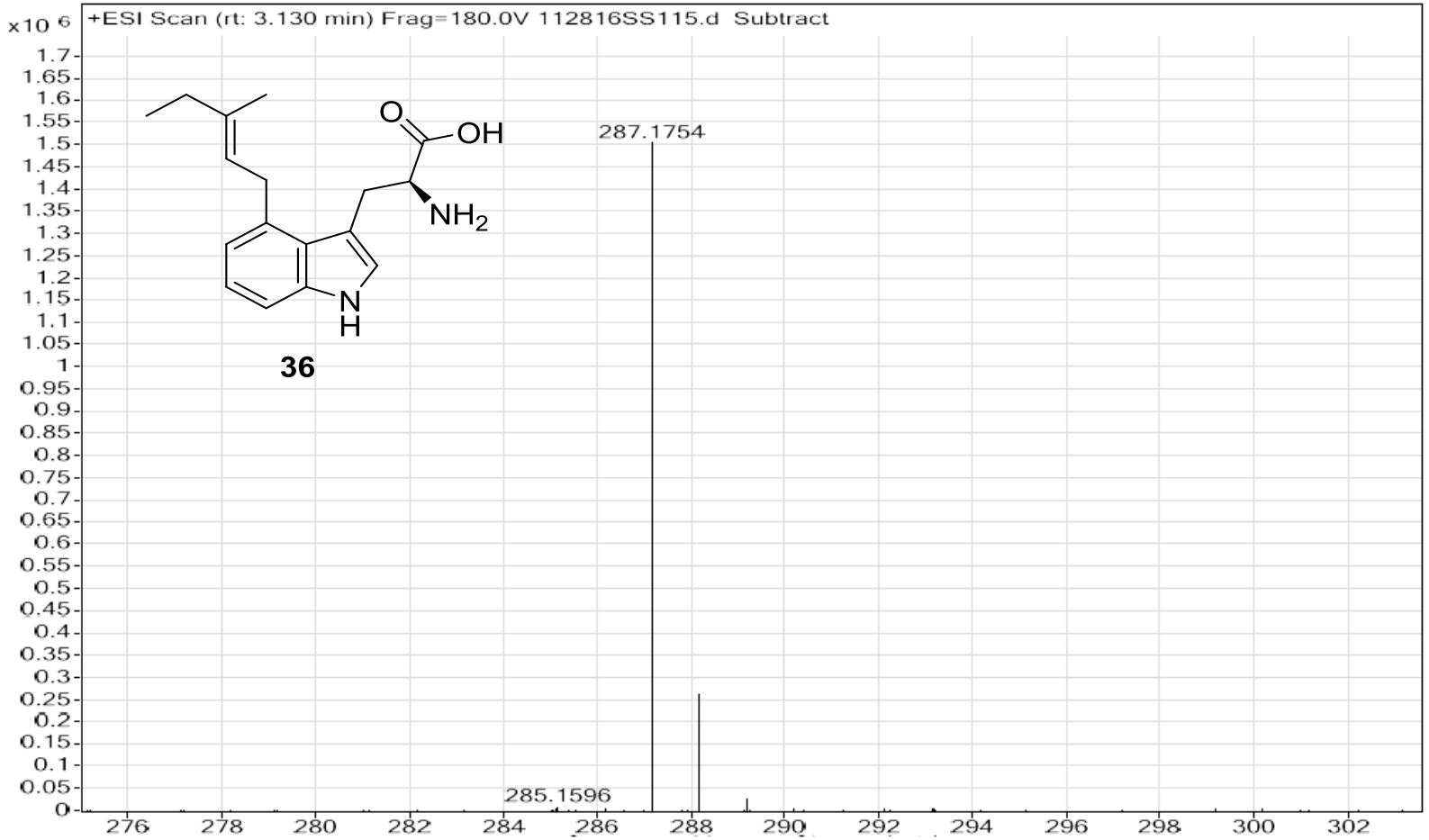
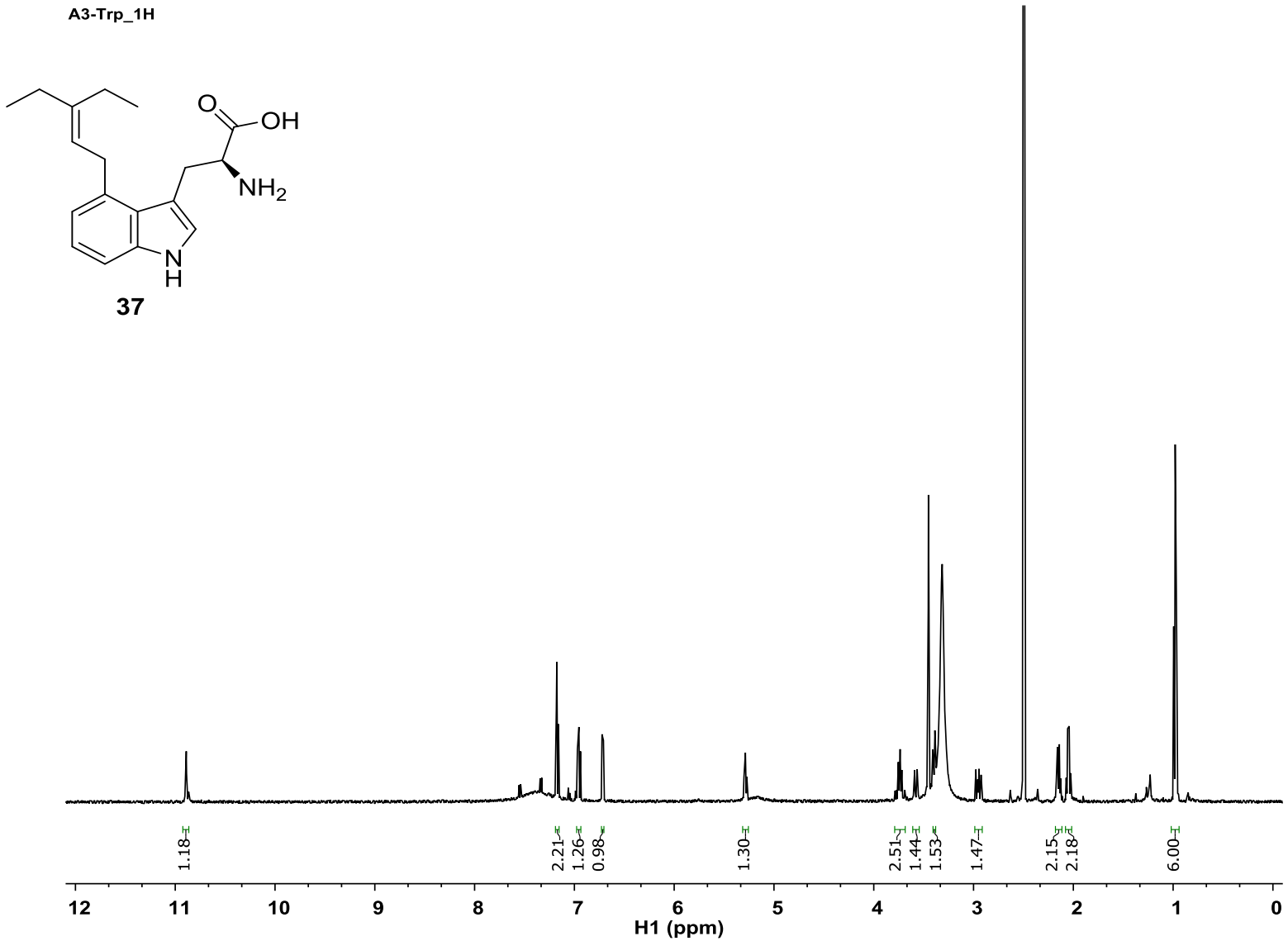
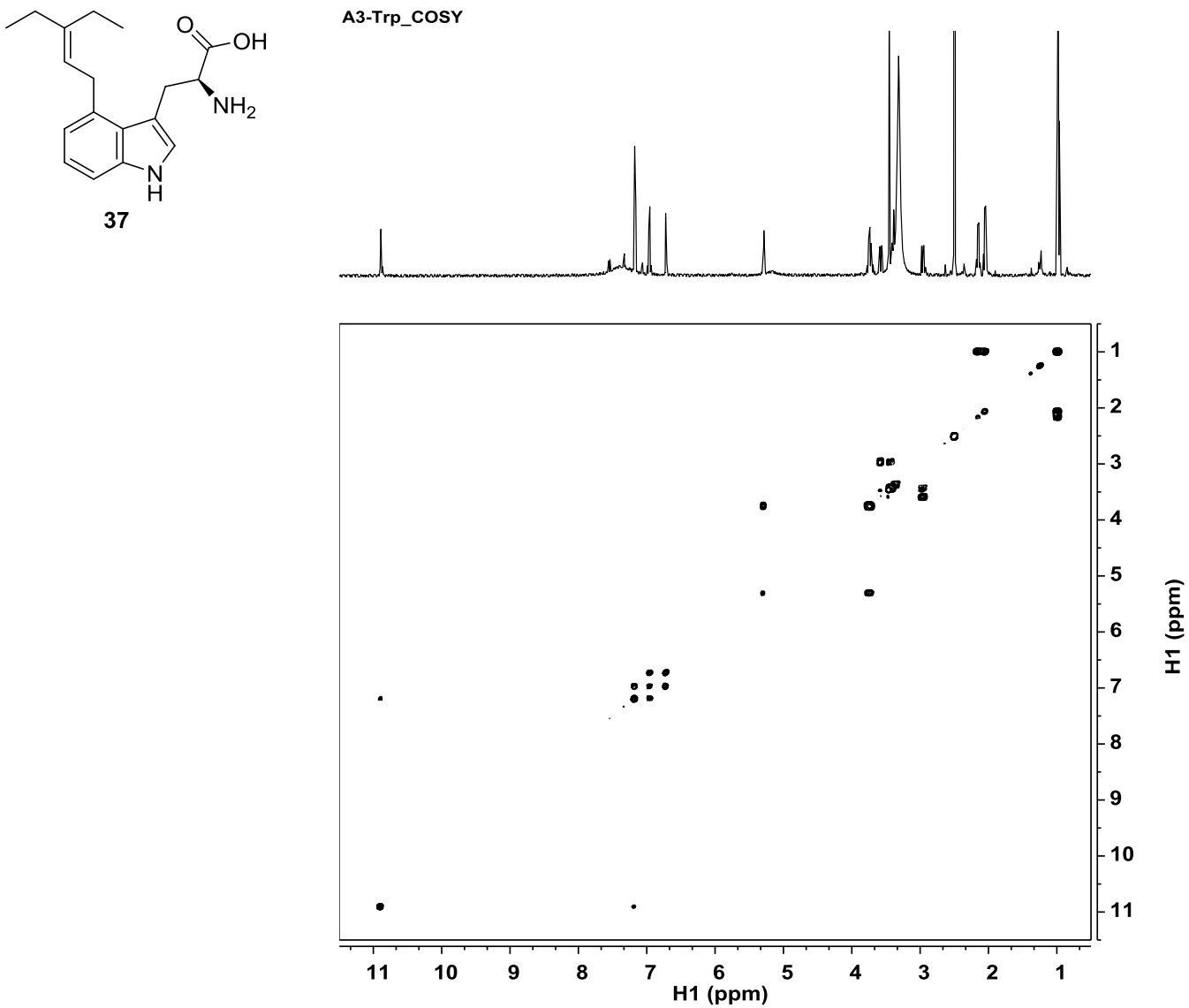


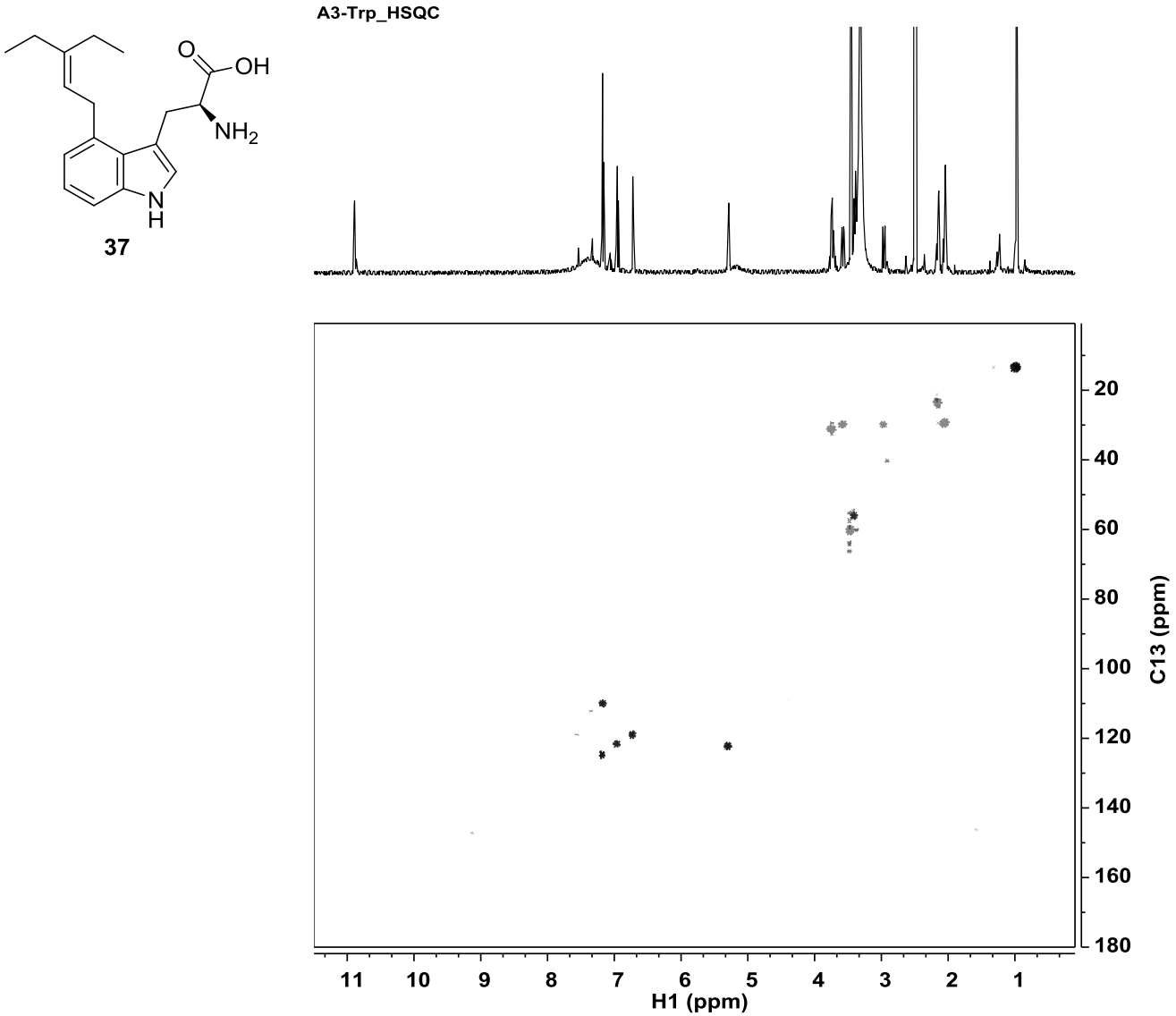
HRMS spectrum of **36**



^{1}H NMR Spectrum (500 MHz) of compound **37** in DMSO-d_6



2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **37** in DMSO-d_6



2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **37** in DMSO-d_6

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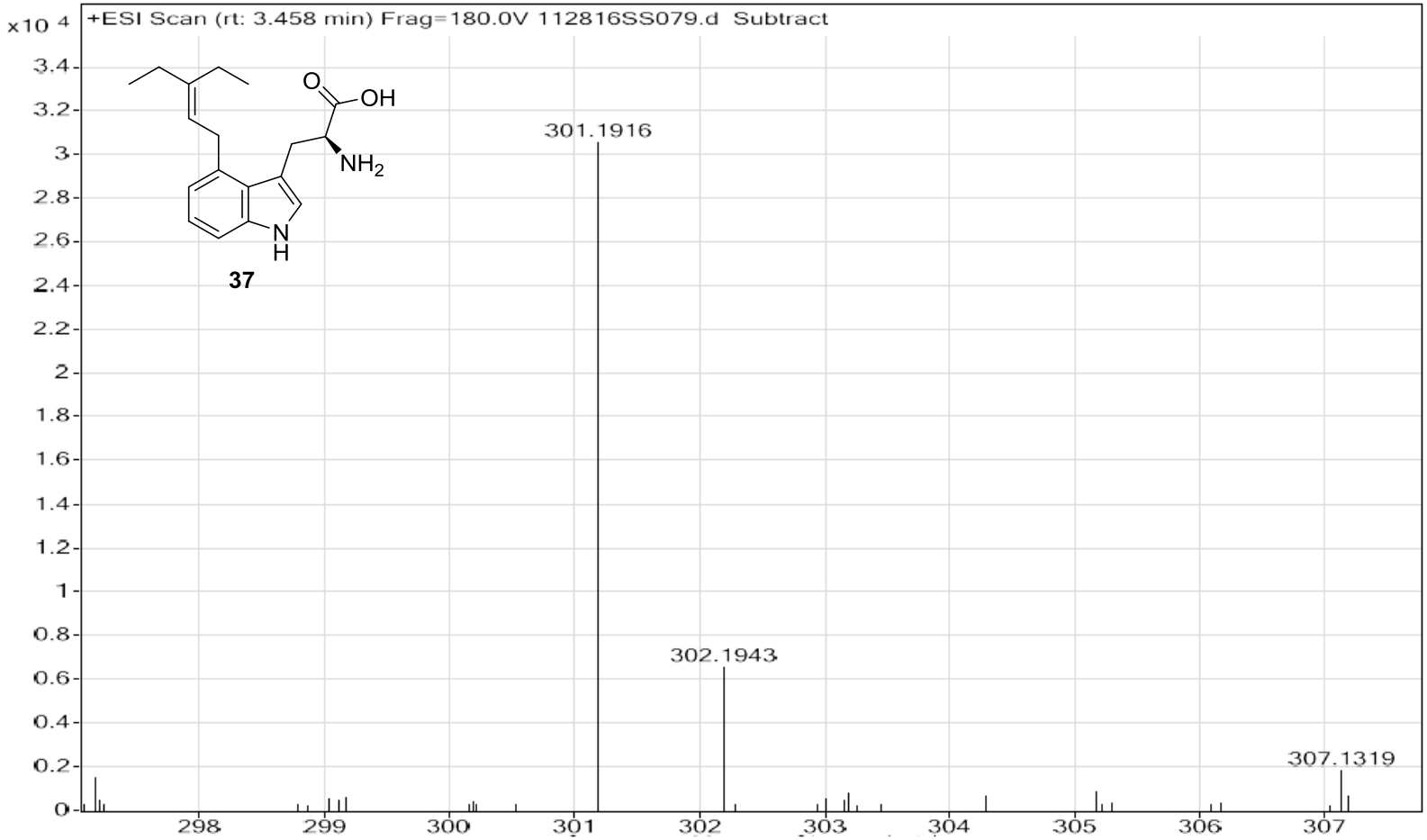
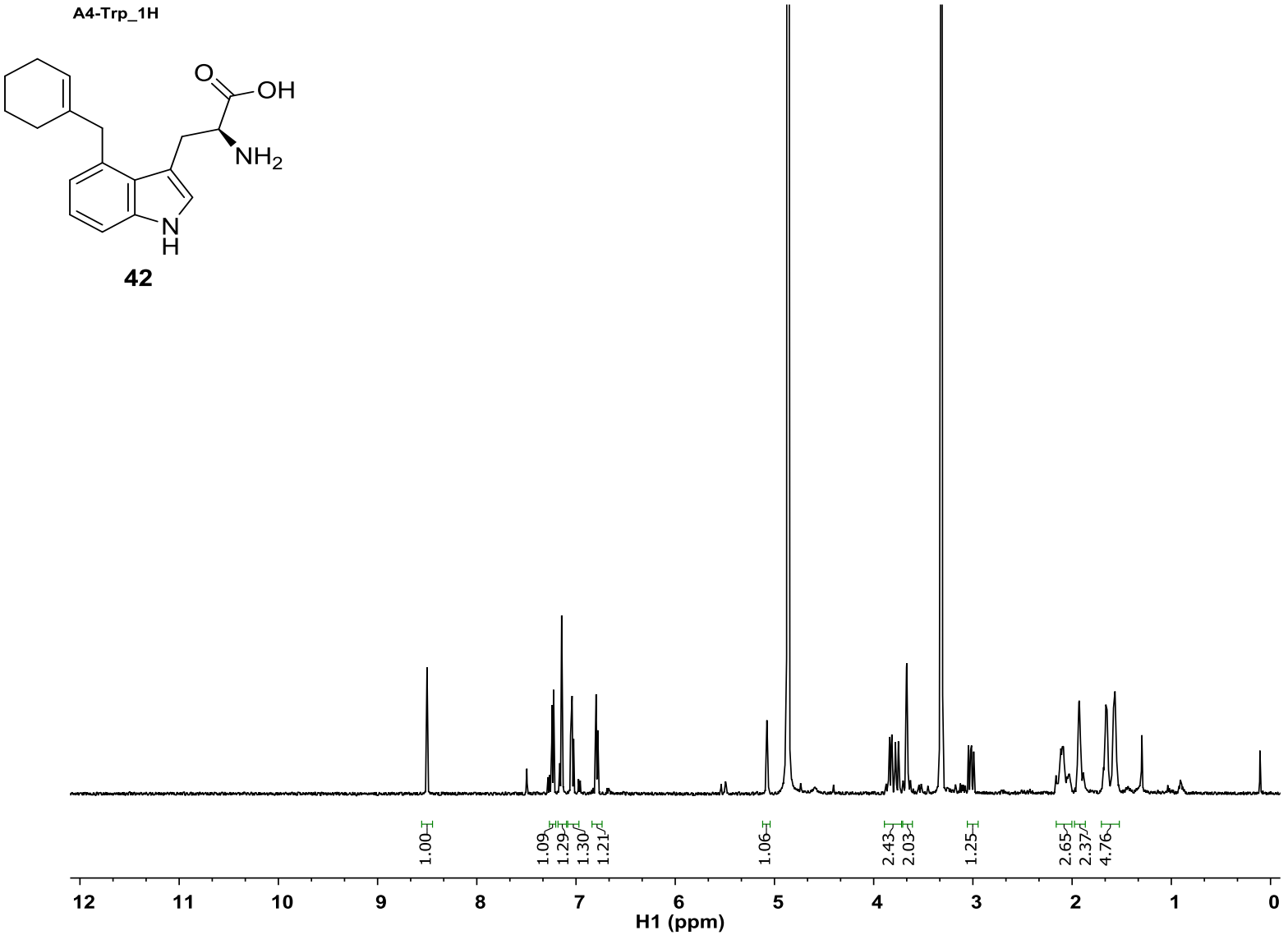
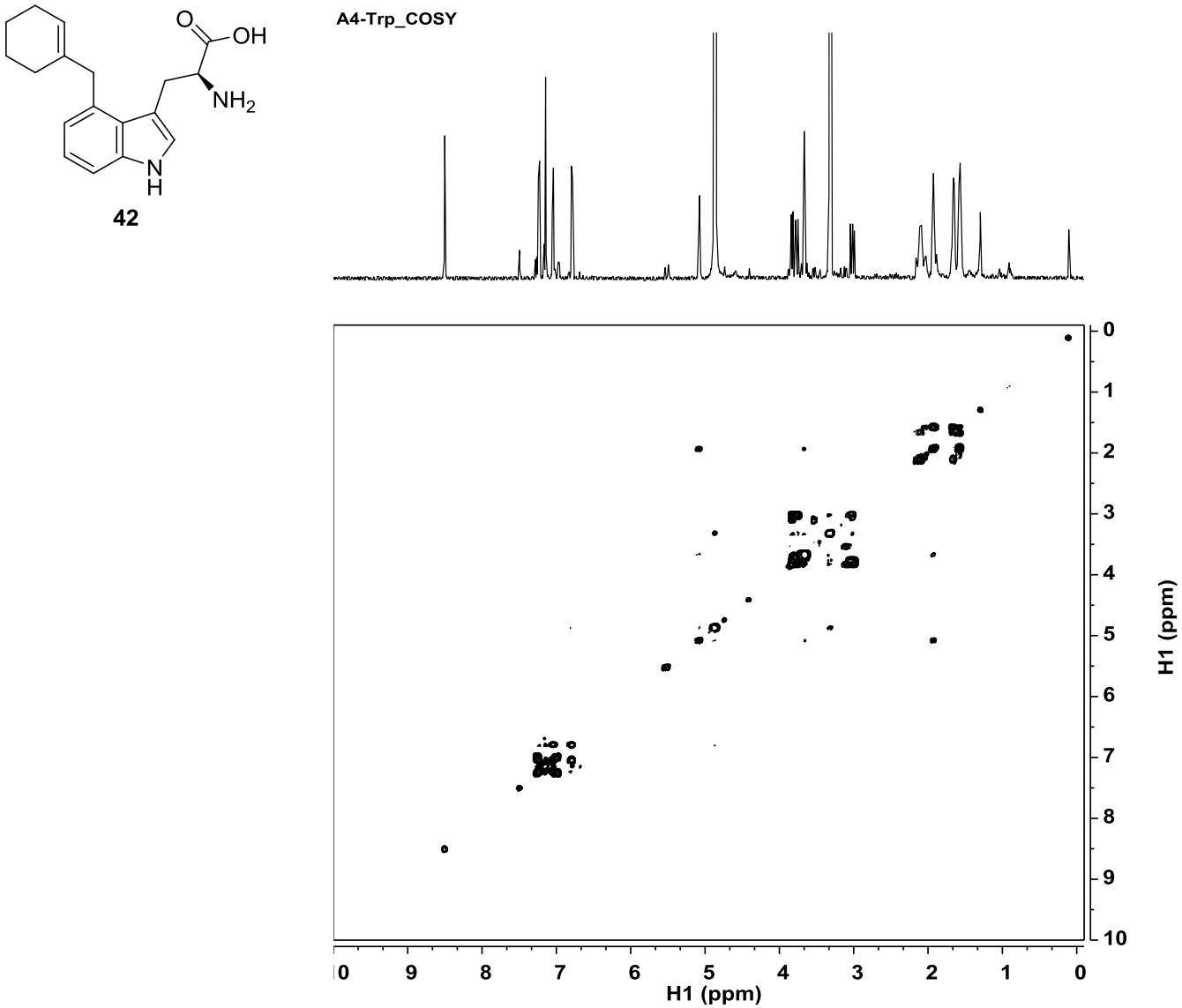


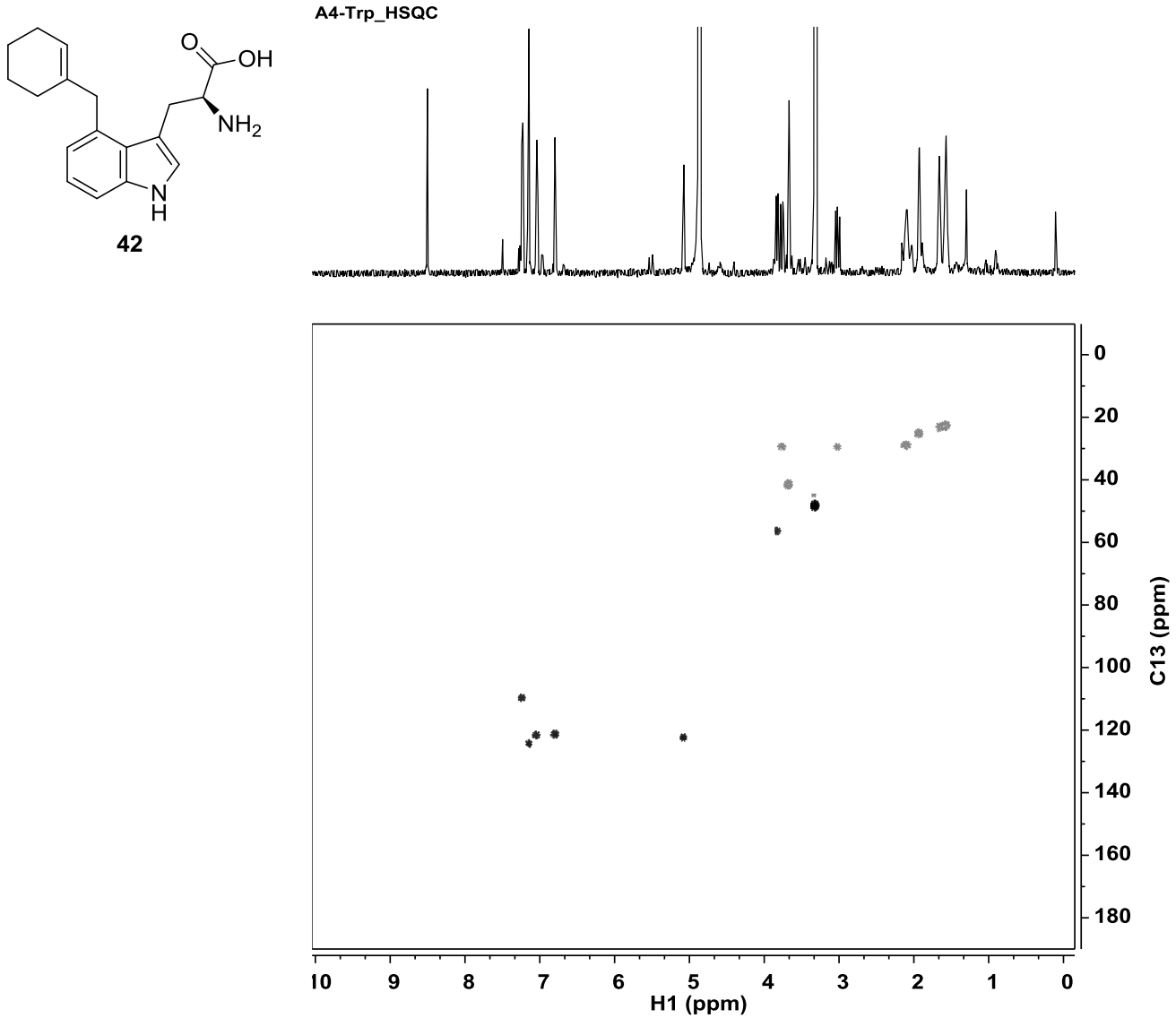
HRMS spectrum of **37**



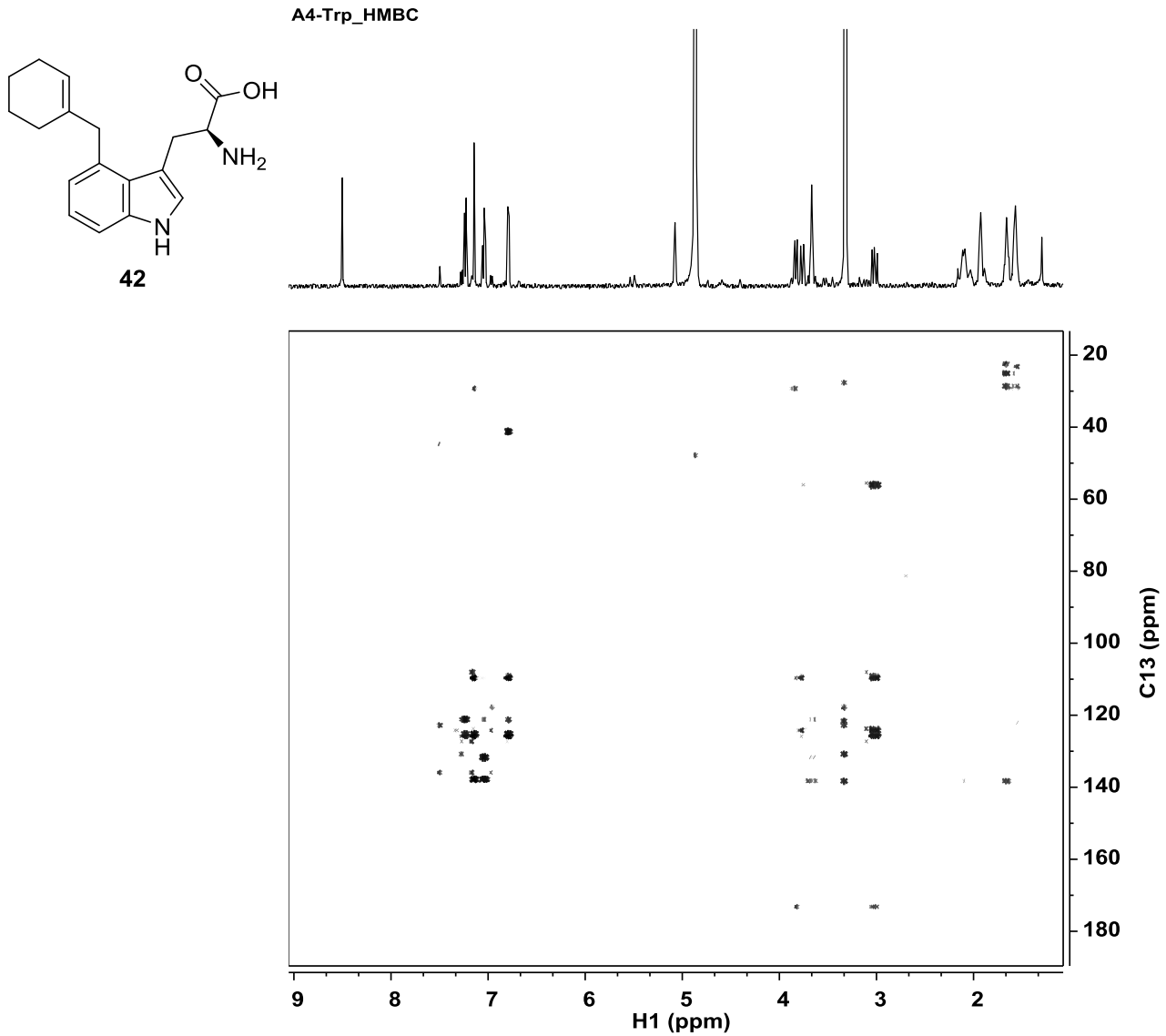
^{1}H NMR Spectrum (500 MHz) of compound **42** in MeOH-d_4



2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **42** in MeOH-d_4



2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **42** in MeOH-d_4



2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **42** in MeOH-d_4

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User Name		**Inj Vol**	5	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	112816SS106.d
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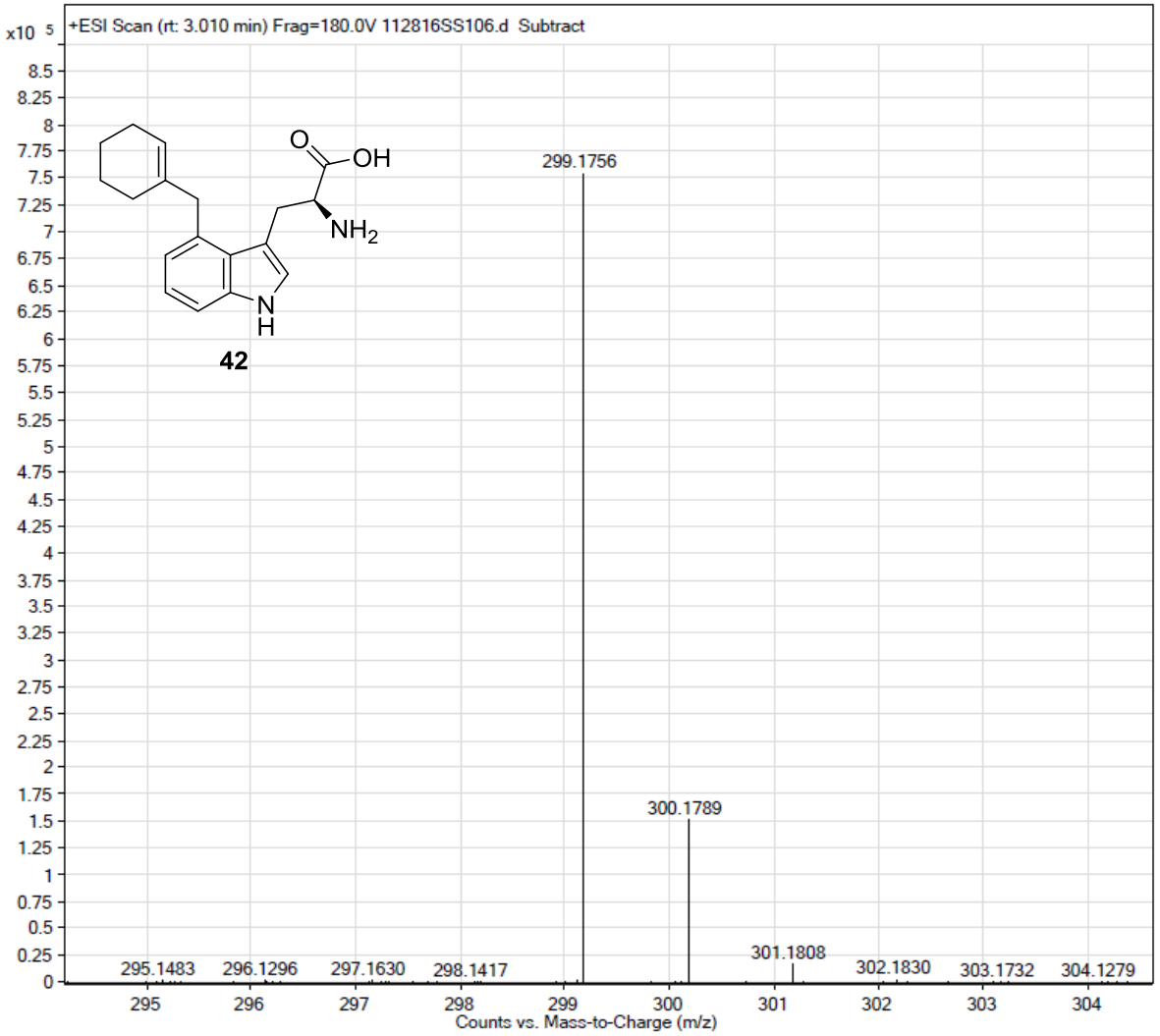
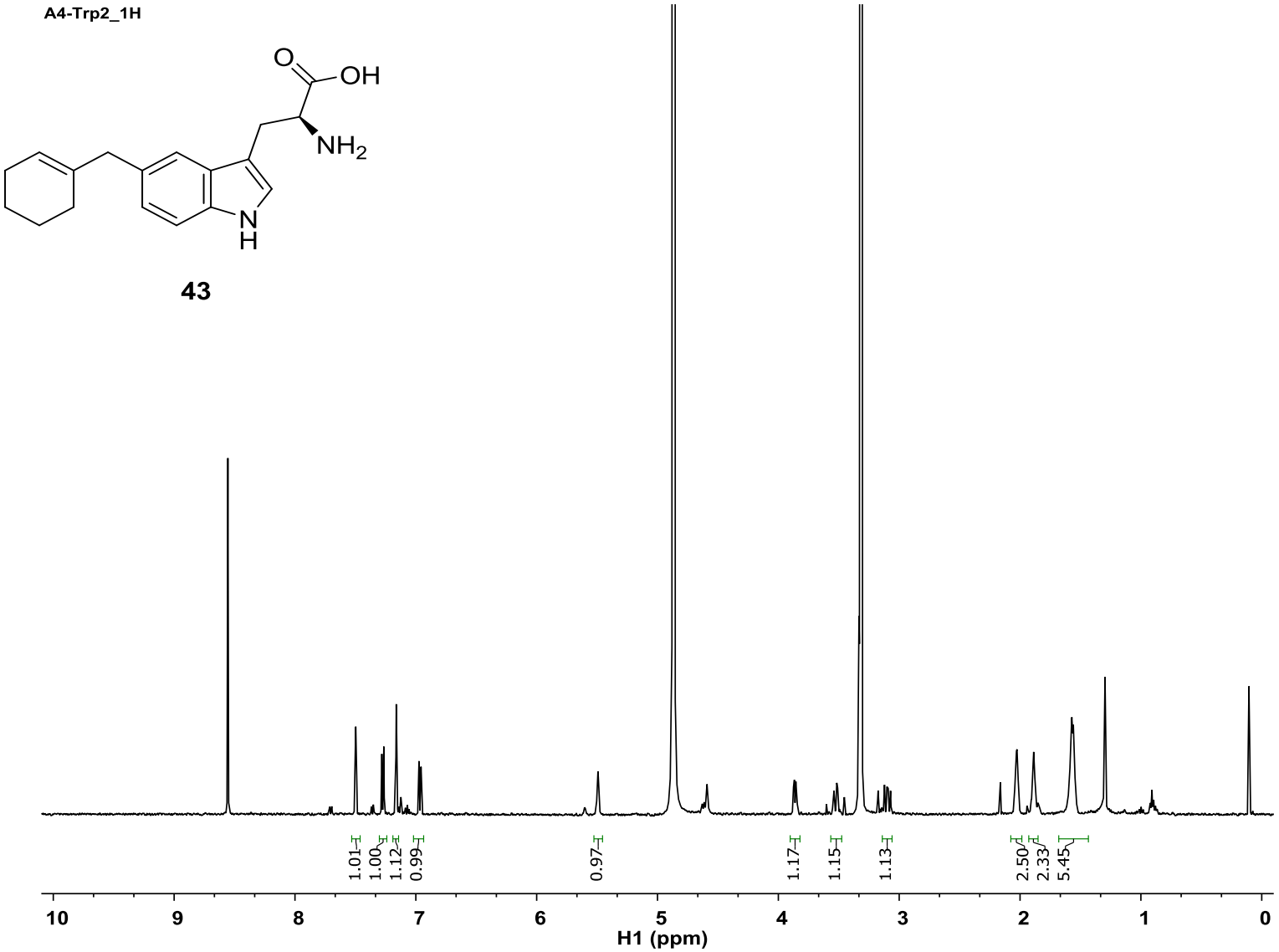
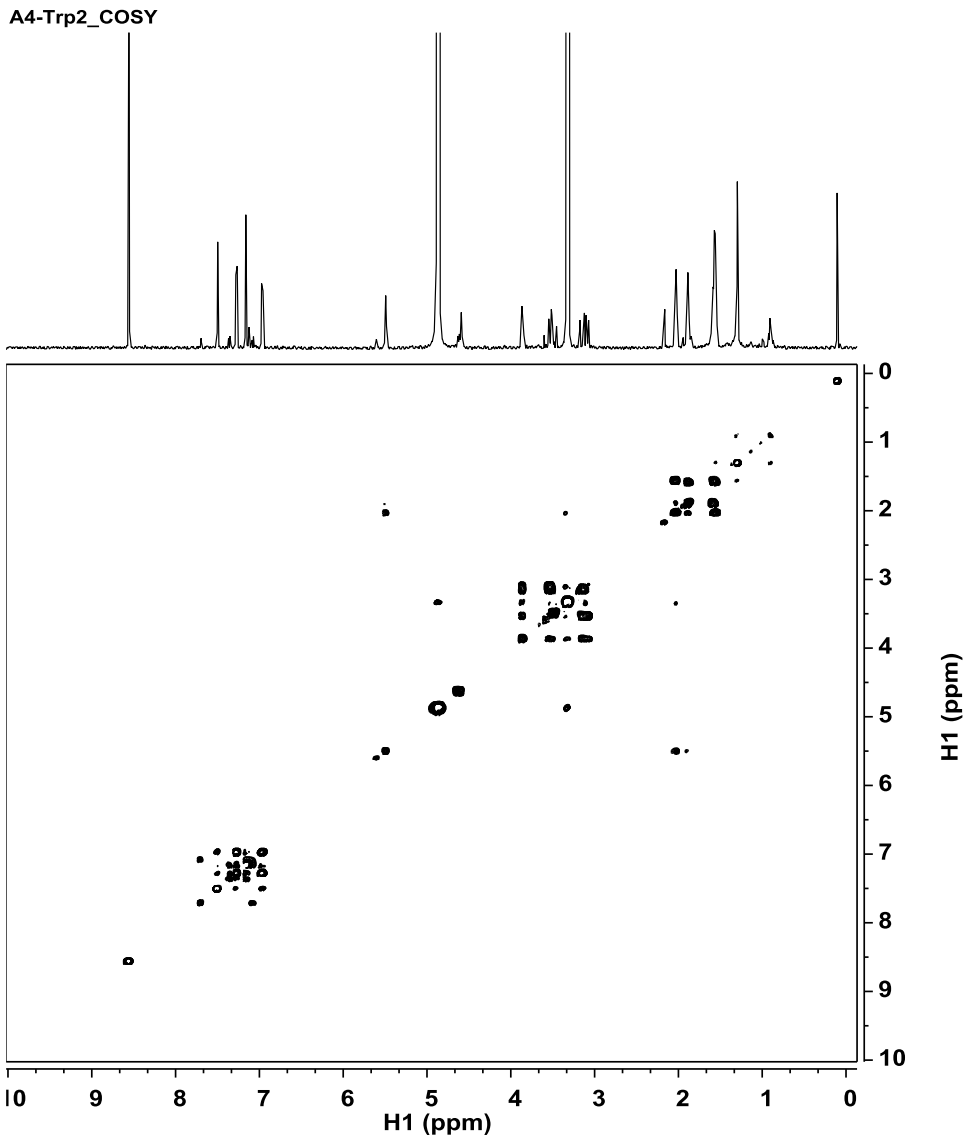


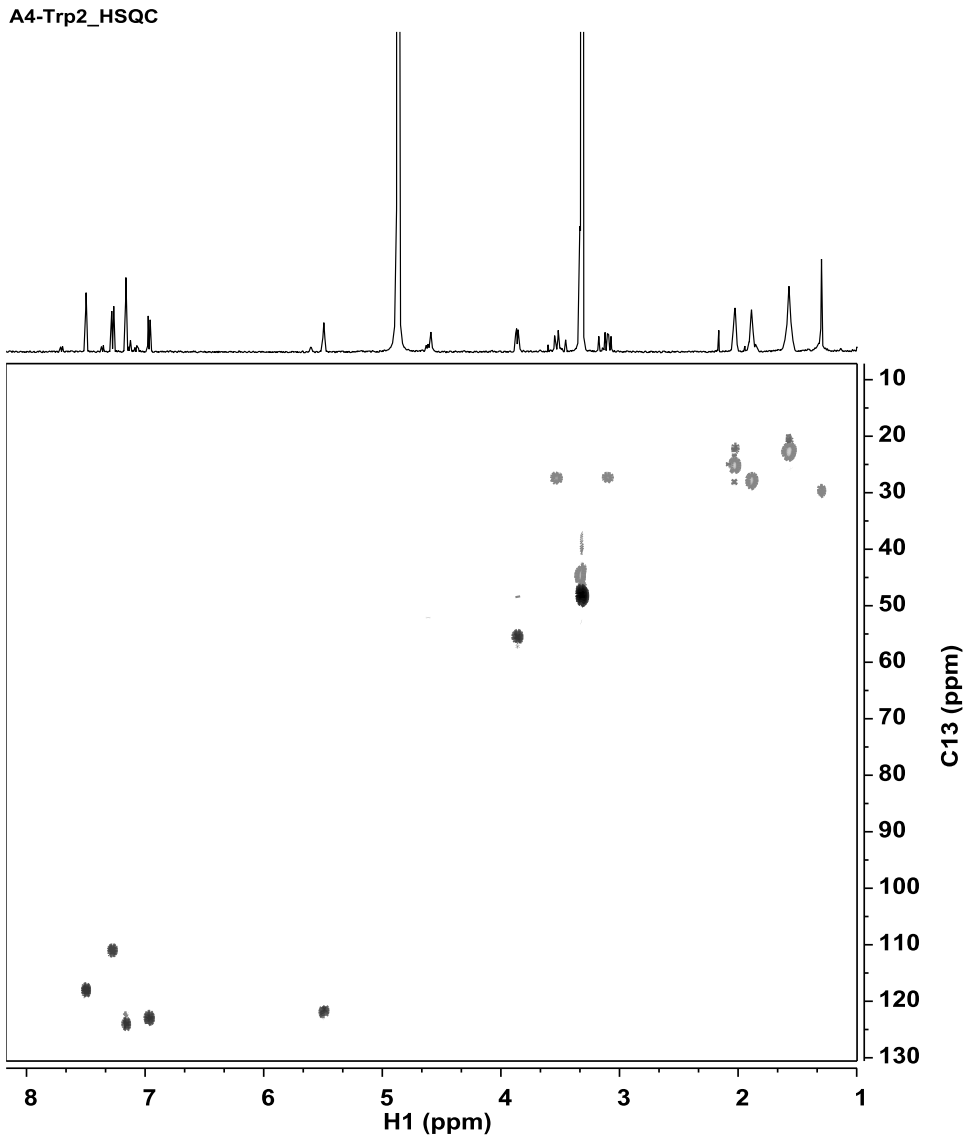
HRMS spectrum of **42**



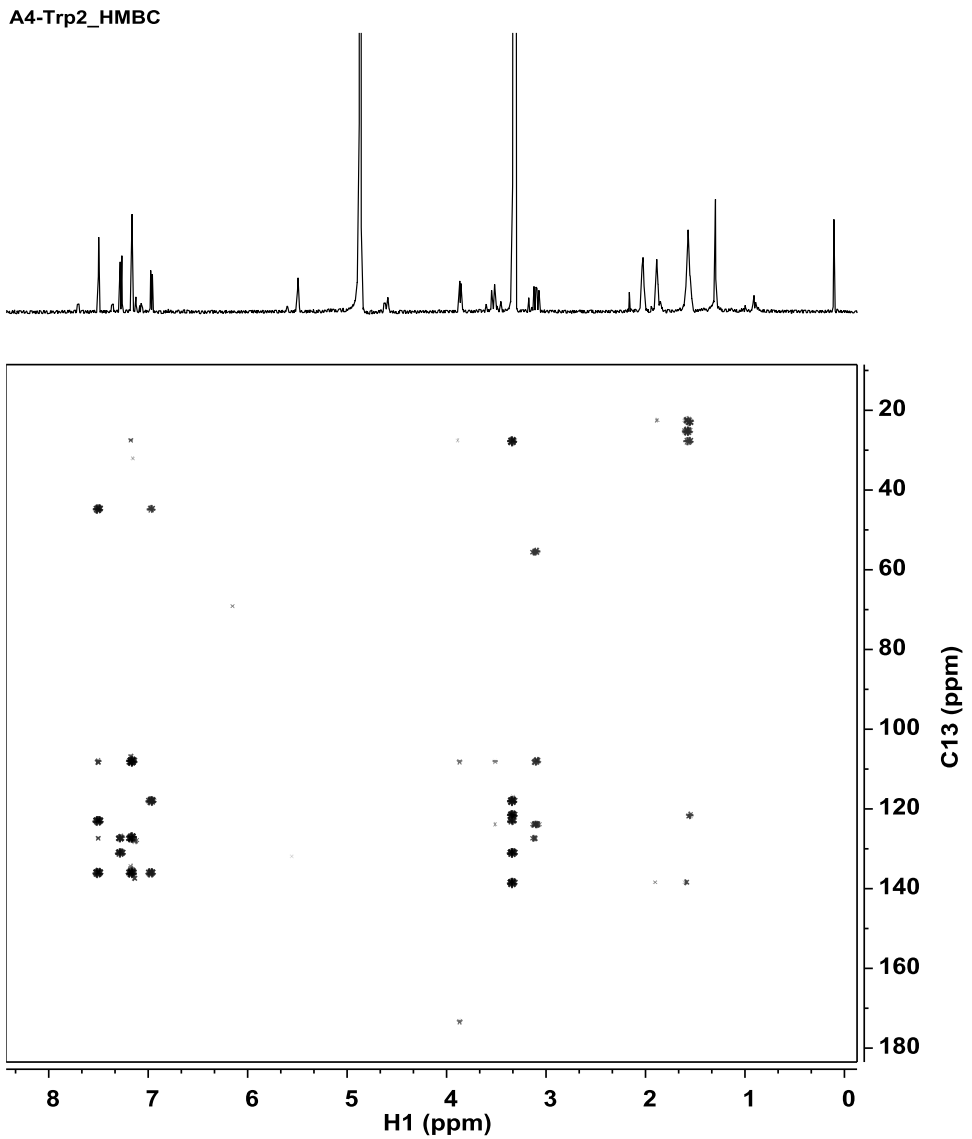
^{1}H NMR spectrum (500 MHz) of **43** in MeOH-d_4



2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **43** in MeOH-d_4



2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **43** in MeOH-d_4



2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **43** in MeOH-d_4

Sample Name	TRP-34	**Position**	P1-D7	**Instrument Name**	Instrument 1	**User Name**	
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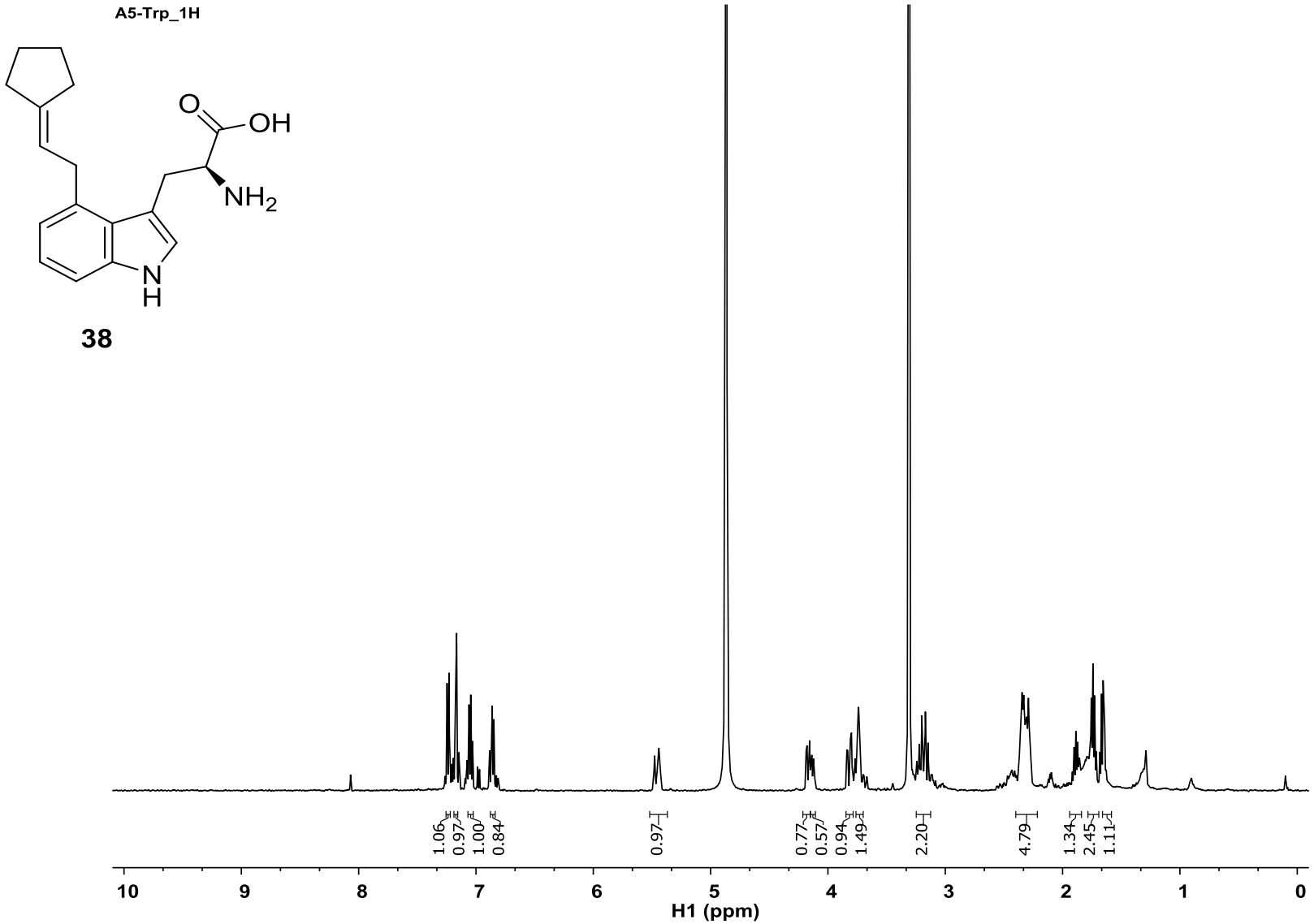
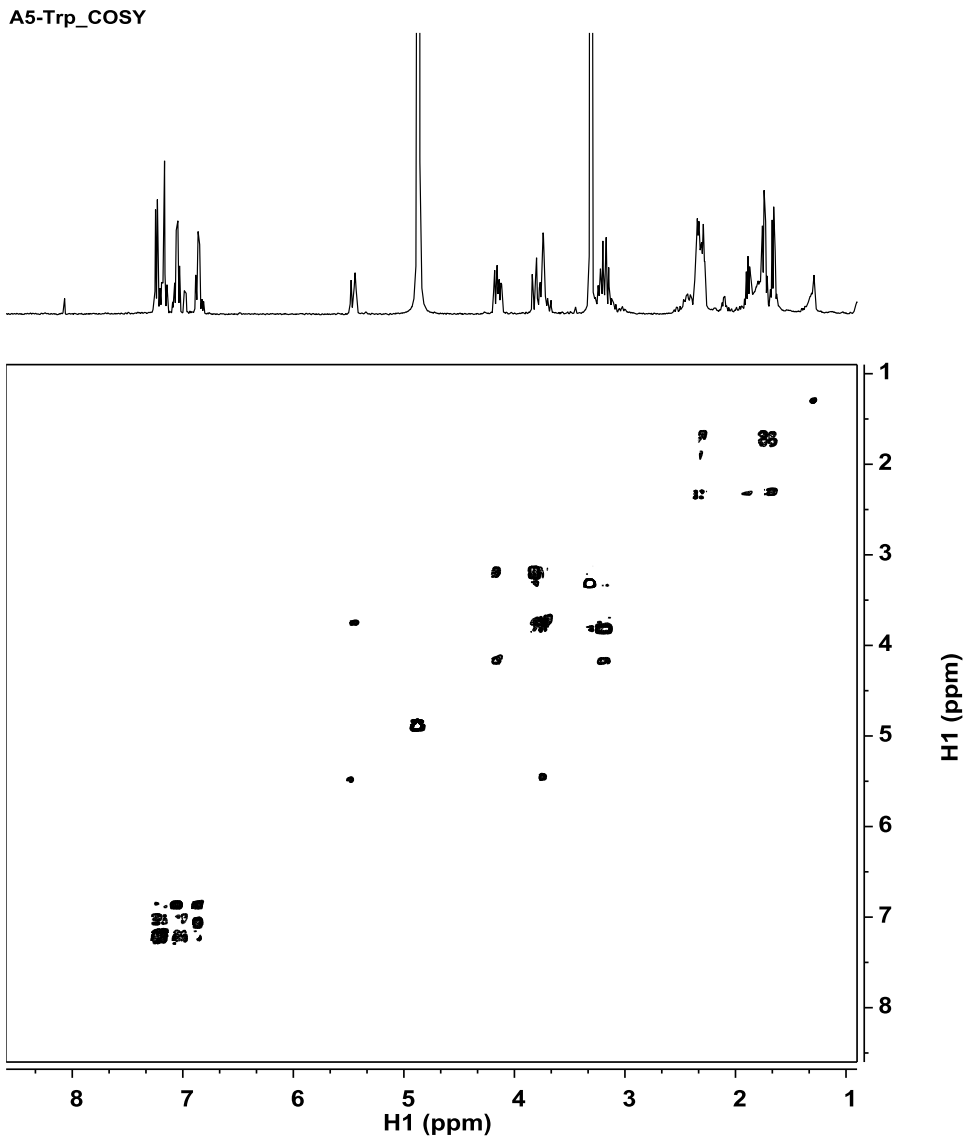
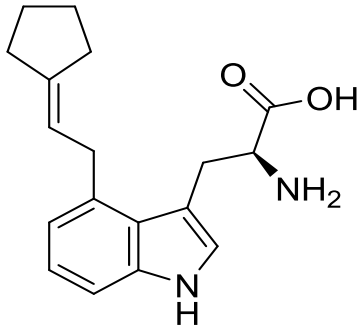
HRMS spectrum of **43**



^{1}H NMR Spectrum (500 MHz) of compound **38** in MeOH-d_4 (*C4^{N}* isomer + 20% unknown isomer)



2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of compound **38** in MeOH-d_4 (*C4*^{N} isomer + 20% unknown isomer)



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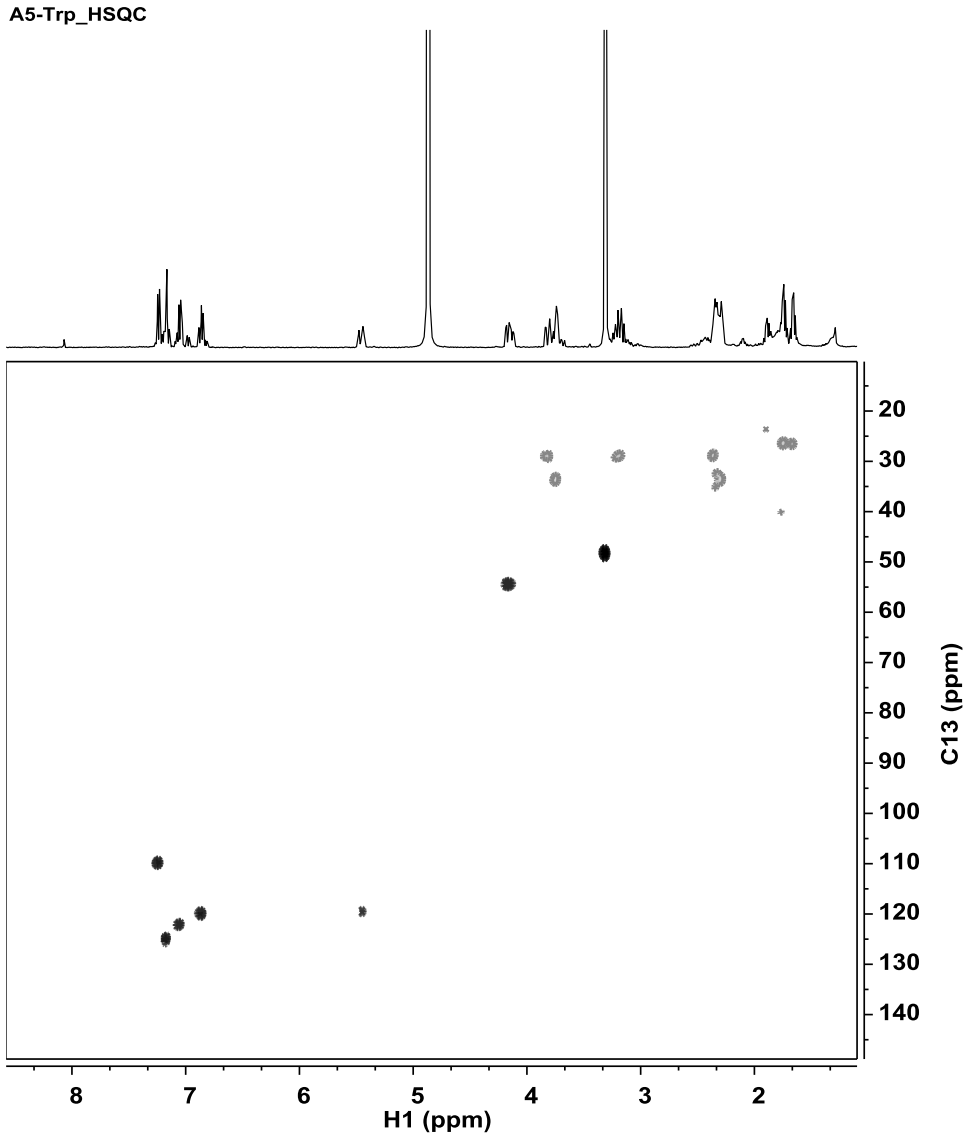
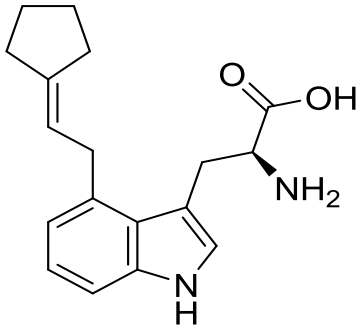


2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **38** in MeOH-d_4



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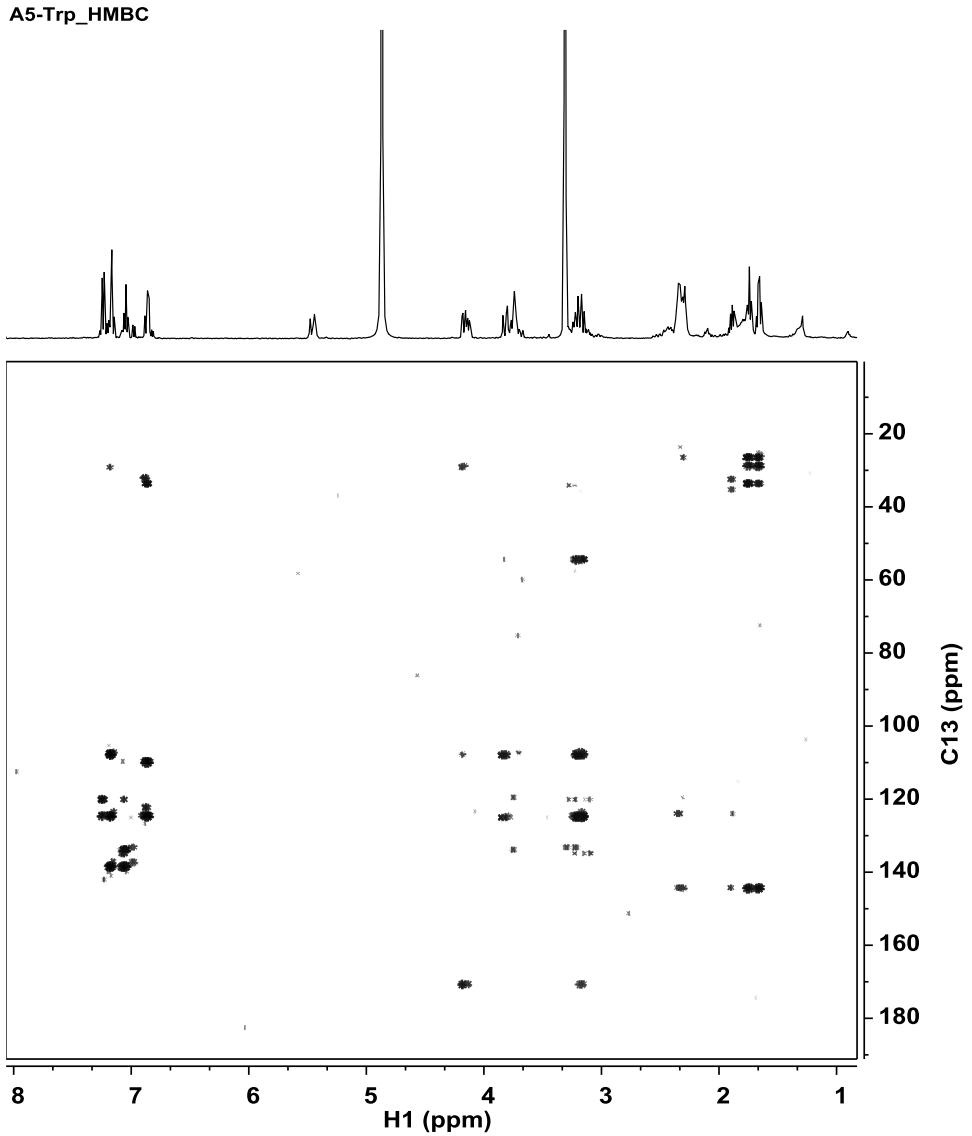
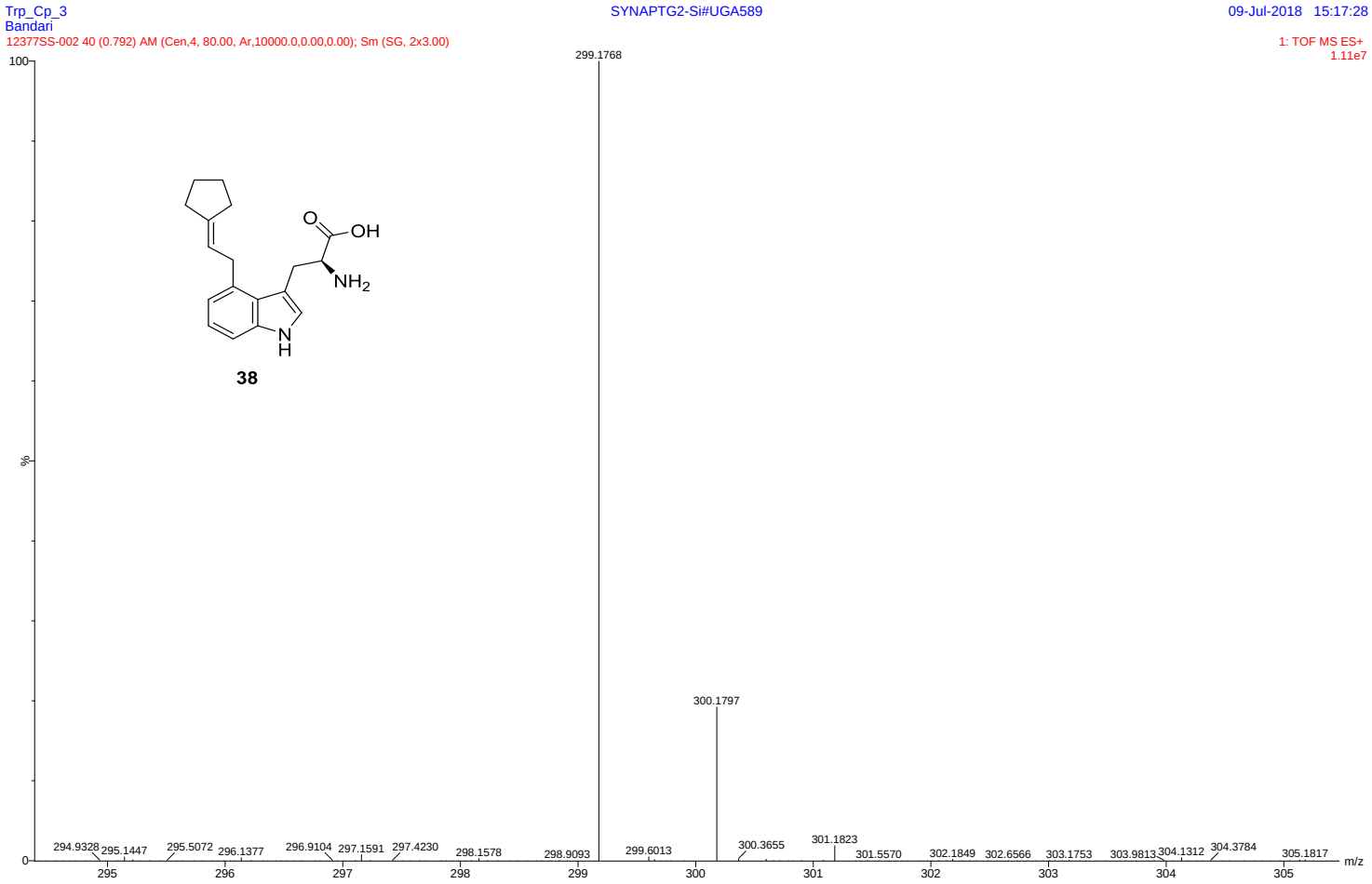
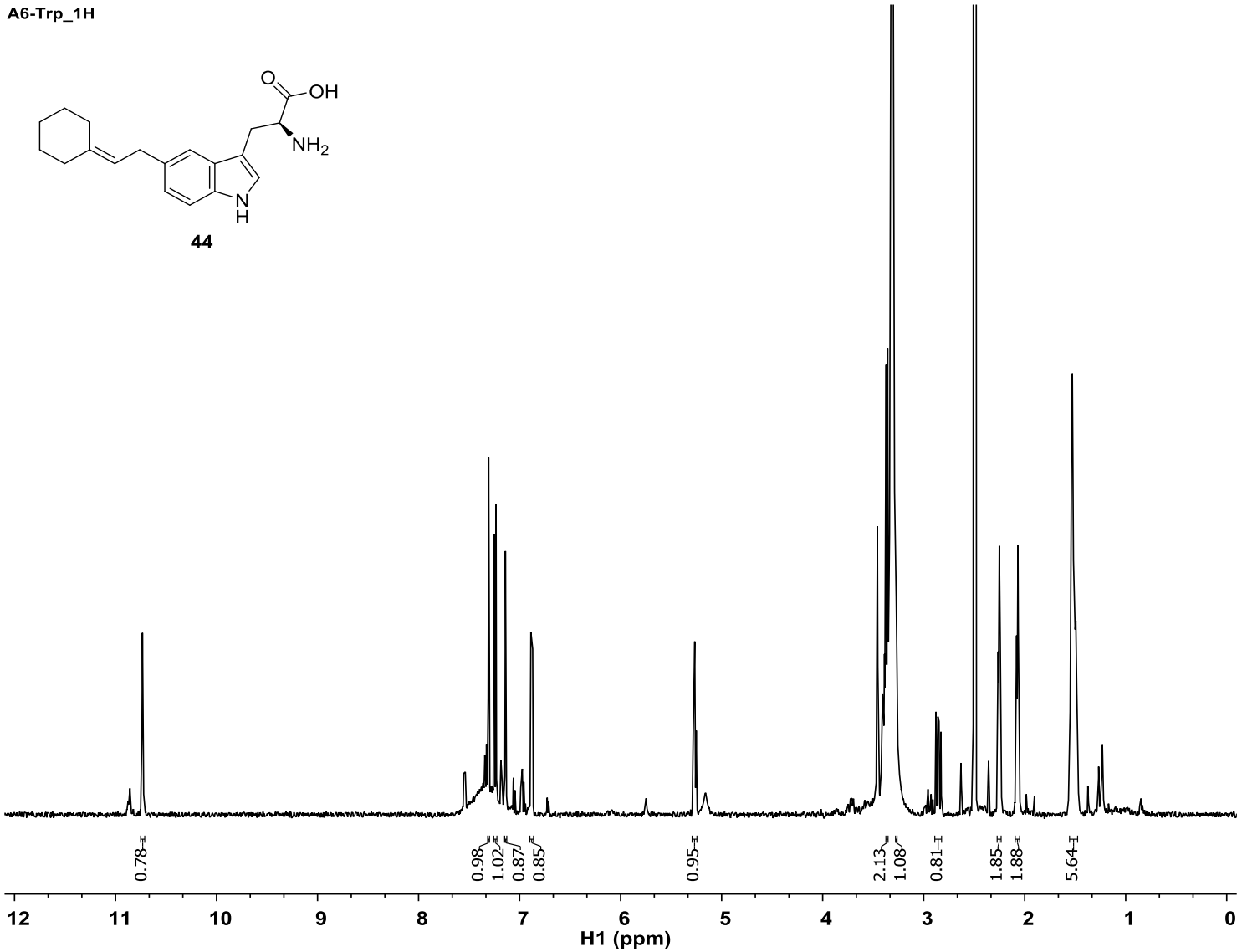


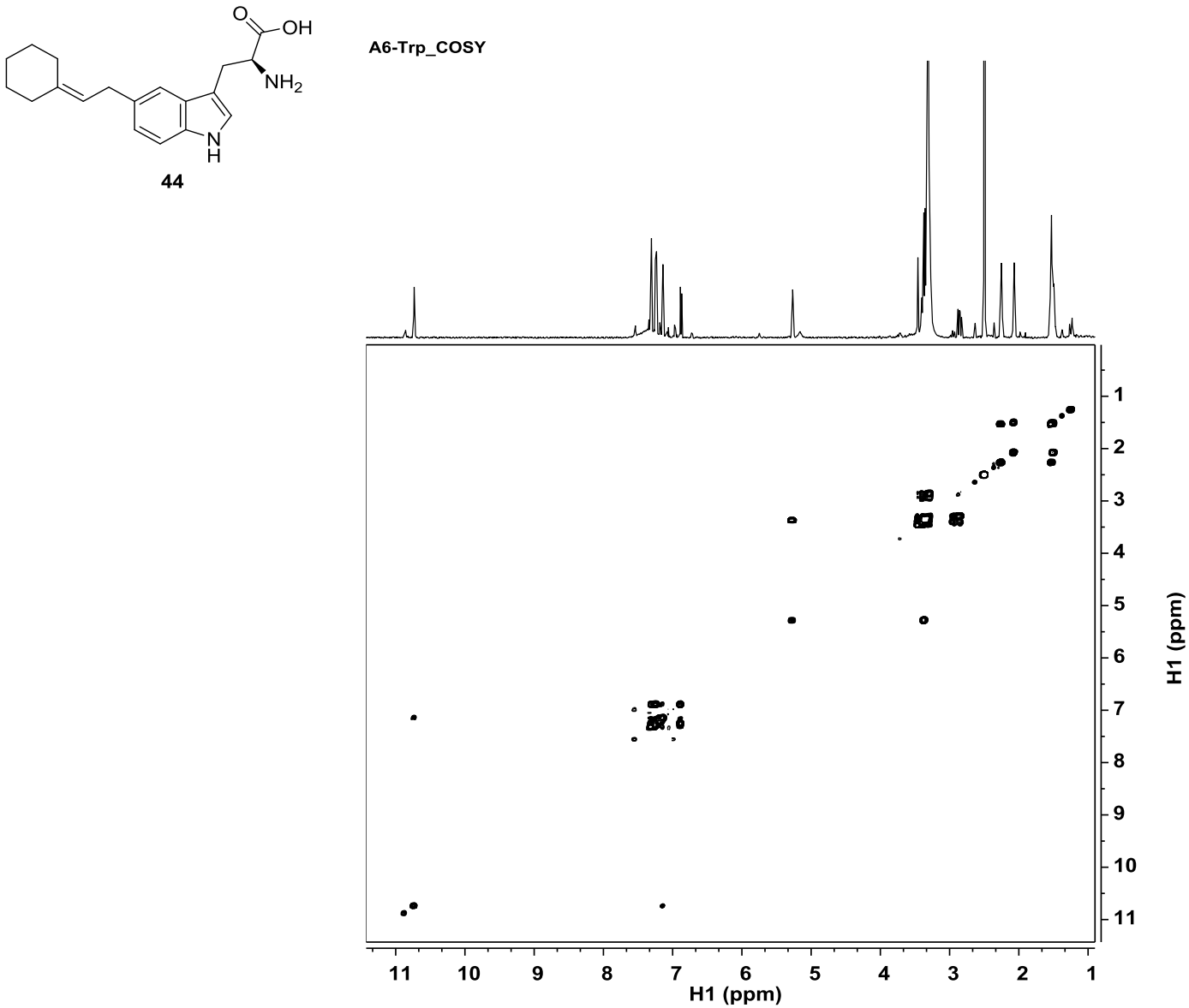
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **38** in MeOH-d_4



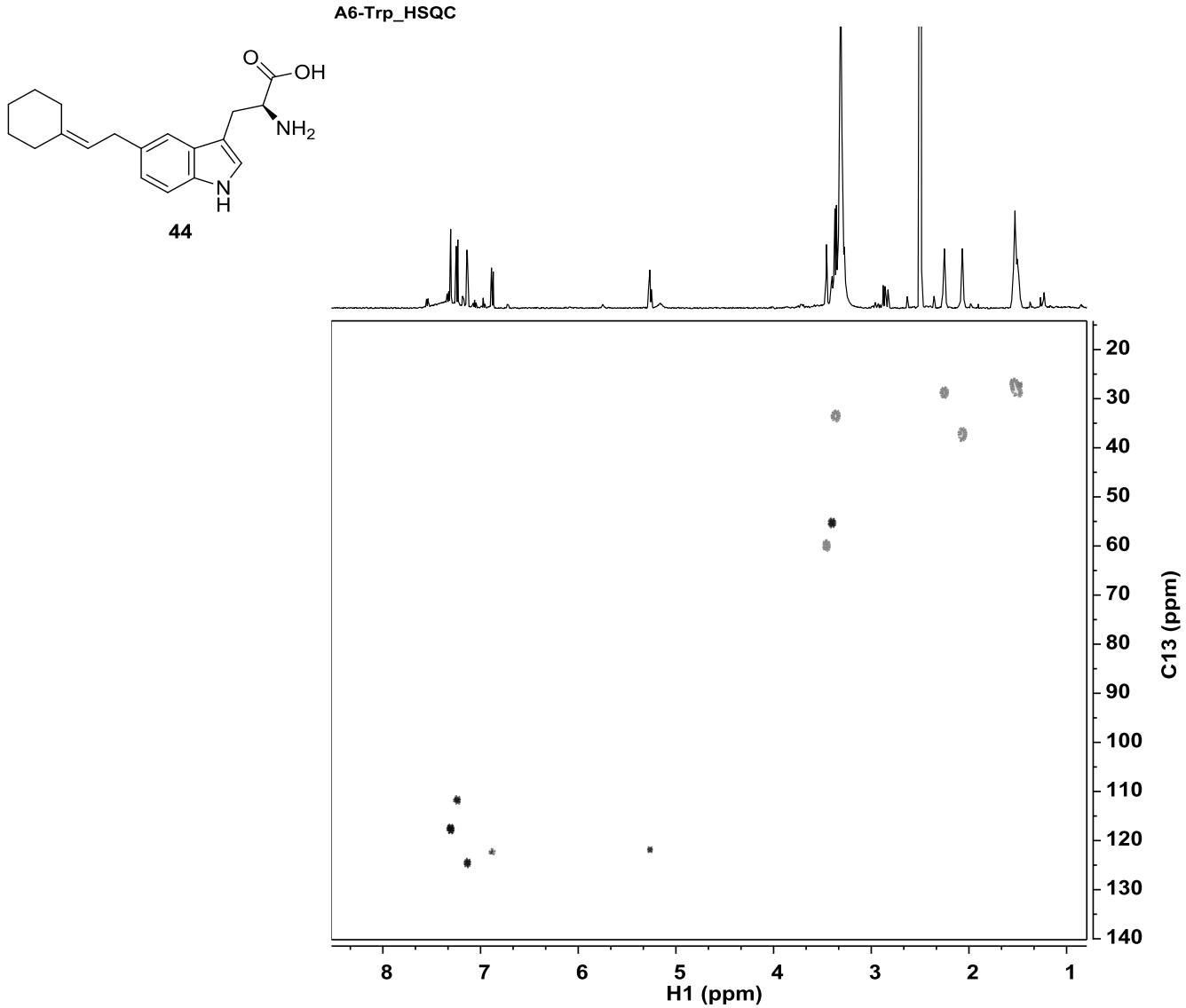
HRMS spectrum of **38**



^{1}H NMR Spectrum (500 MHz) of compound **44** in DMSO-d6



2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **44** in DMSO-d6



2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **44** in DMSO-d6

Sample Name	TRP-26	**Position**	P1-C8	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
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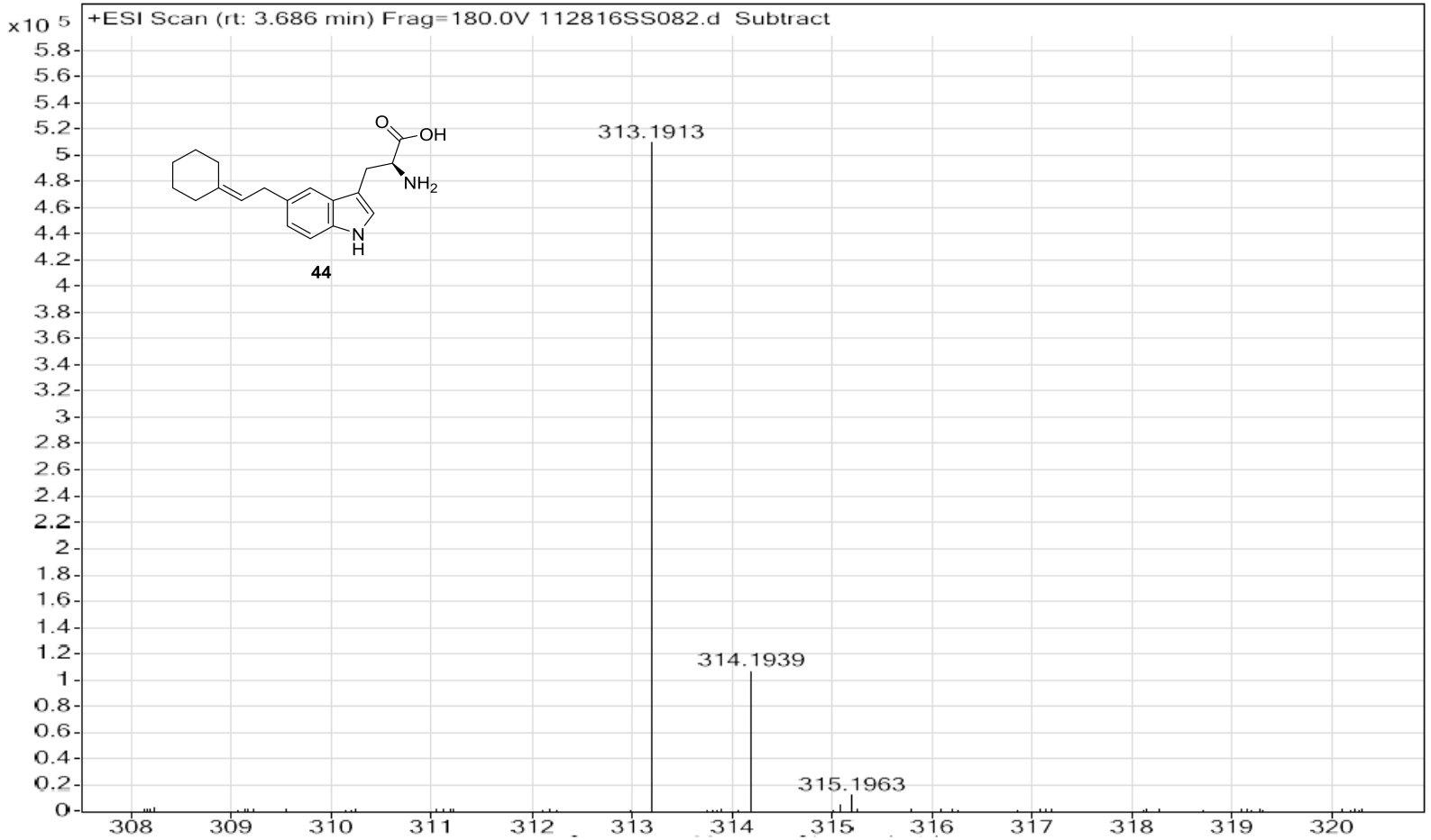
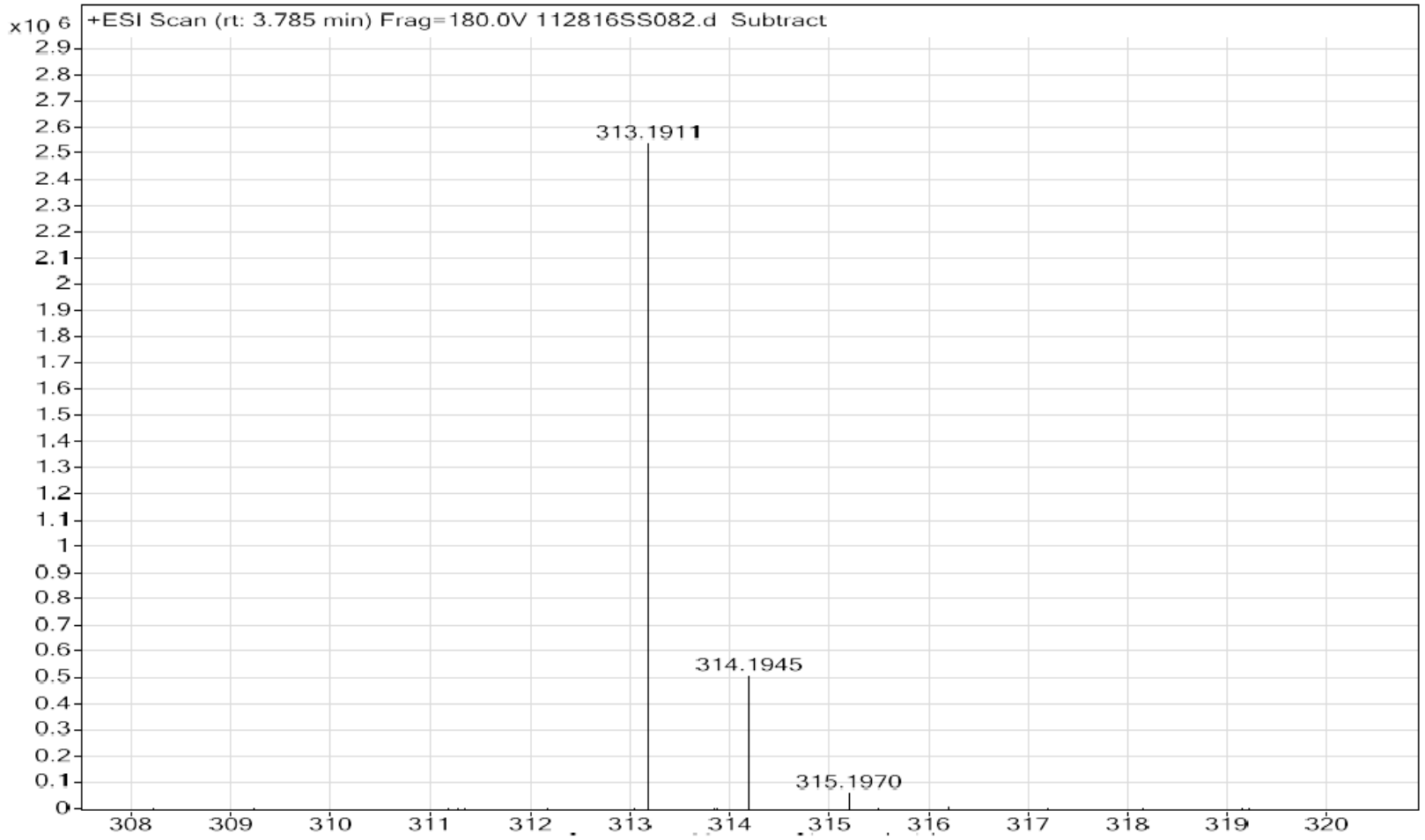
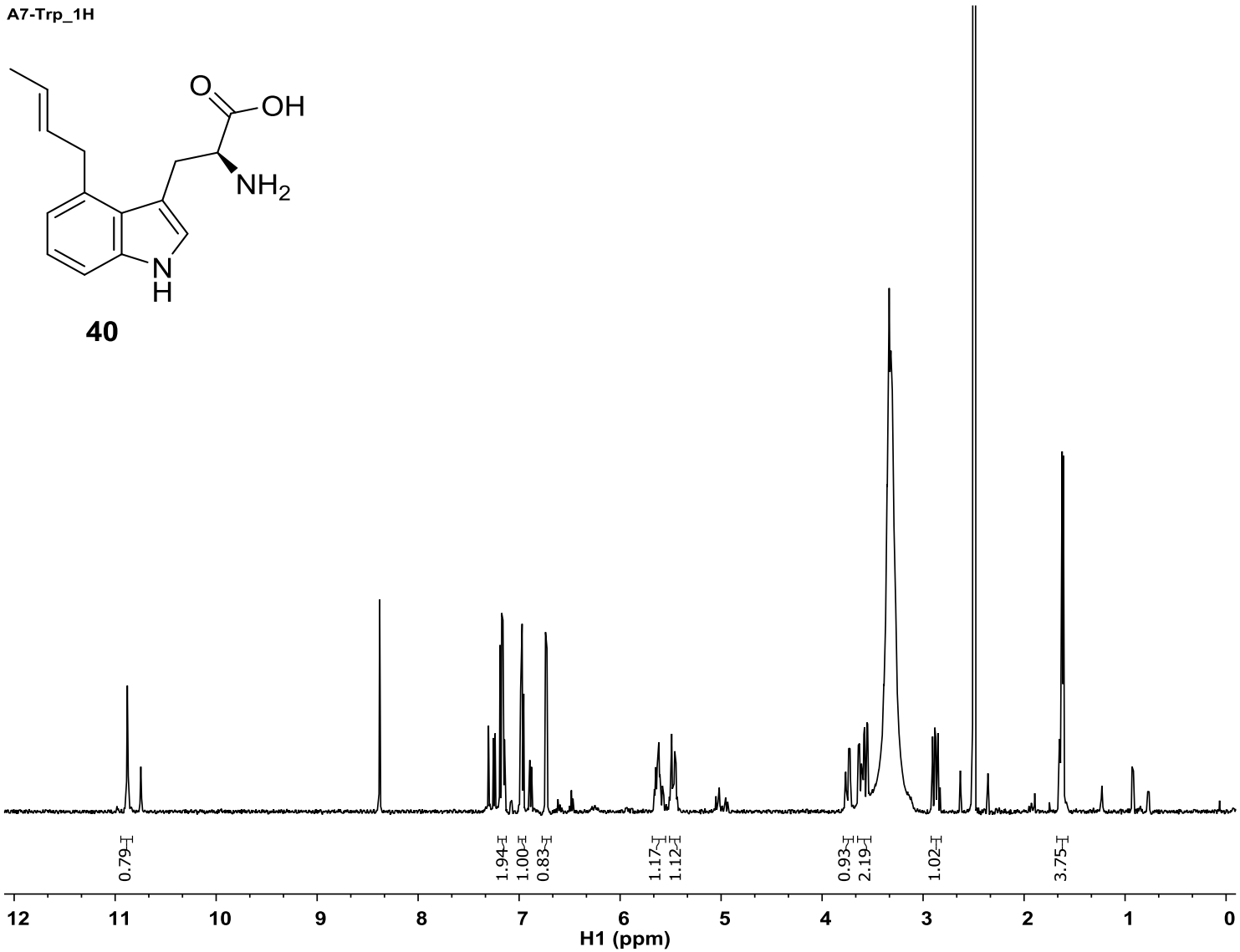


HRMS spectrum of **44**

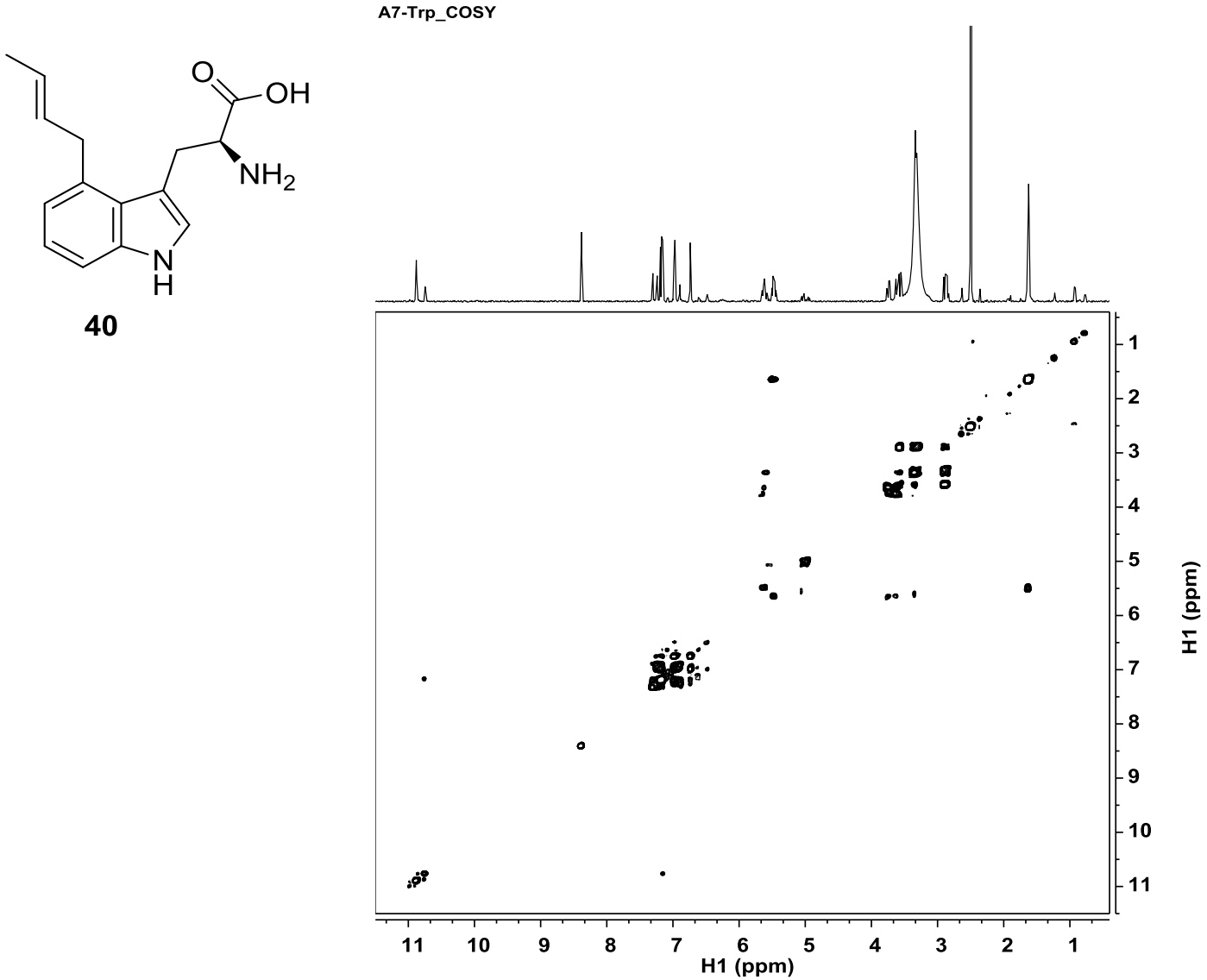
Sample Name	TRP-26	**Position**	P1-C8	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS082.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 3:20:56 AM



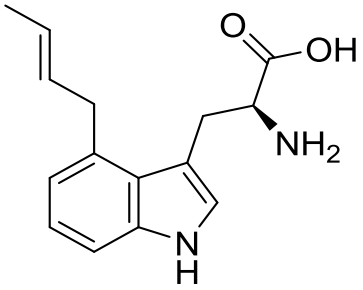
HRMS of 6-Trp$_2$



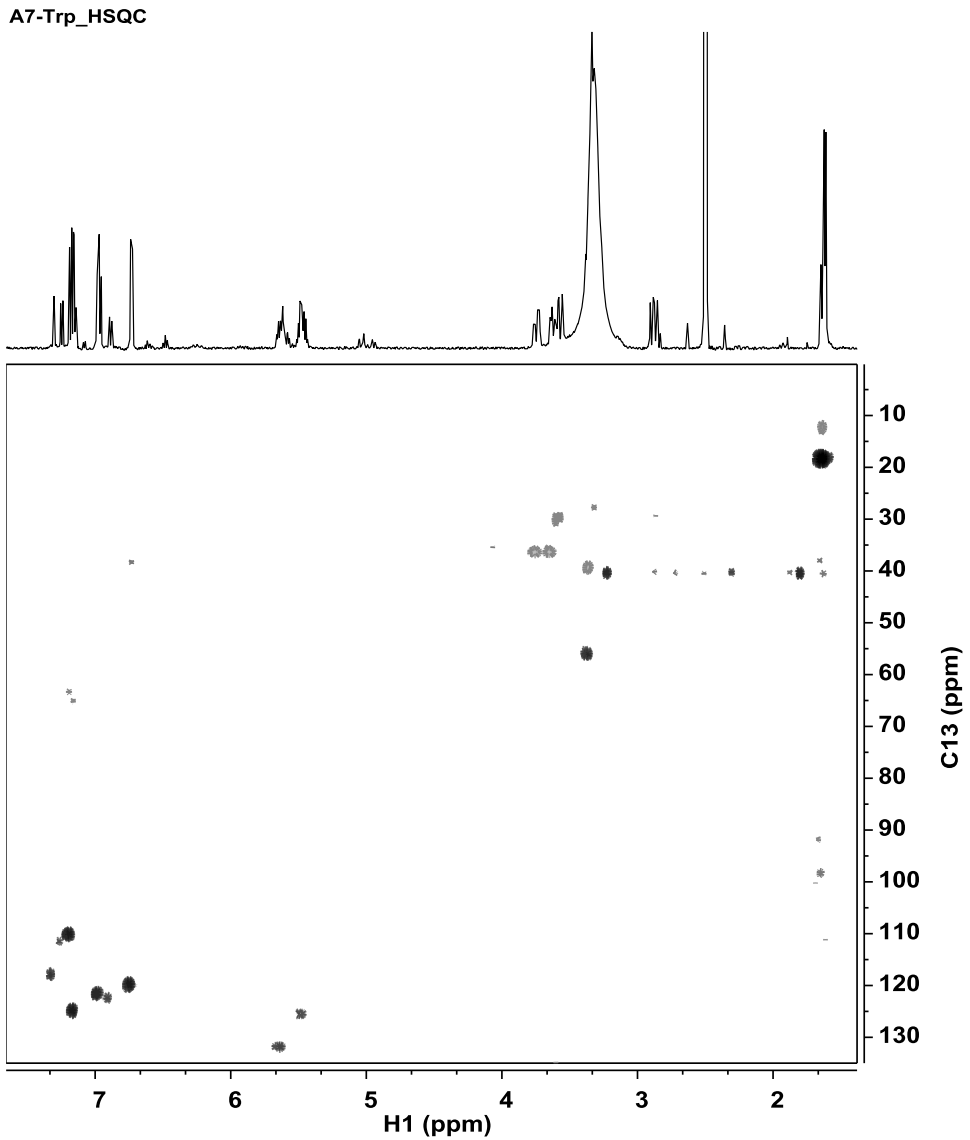
^{1}H NMR Spectrum (500 MHz) of compound **40** in DMSO-d6 (*C4*^{N} isomer + 30% *C5*^{N} isomer)



2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **40** in DMSO-d6 (*C4*^{N} isomer + 30% *C5*^{N} isomer)

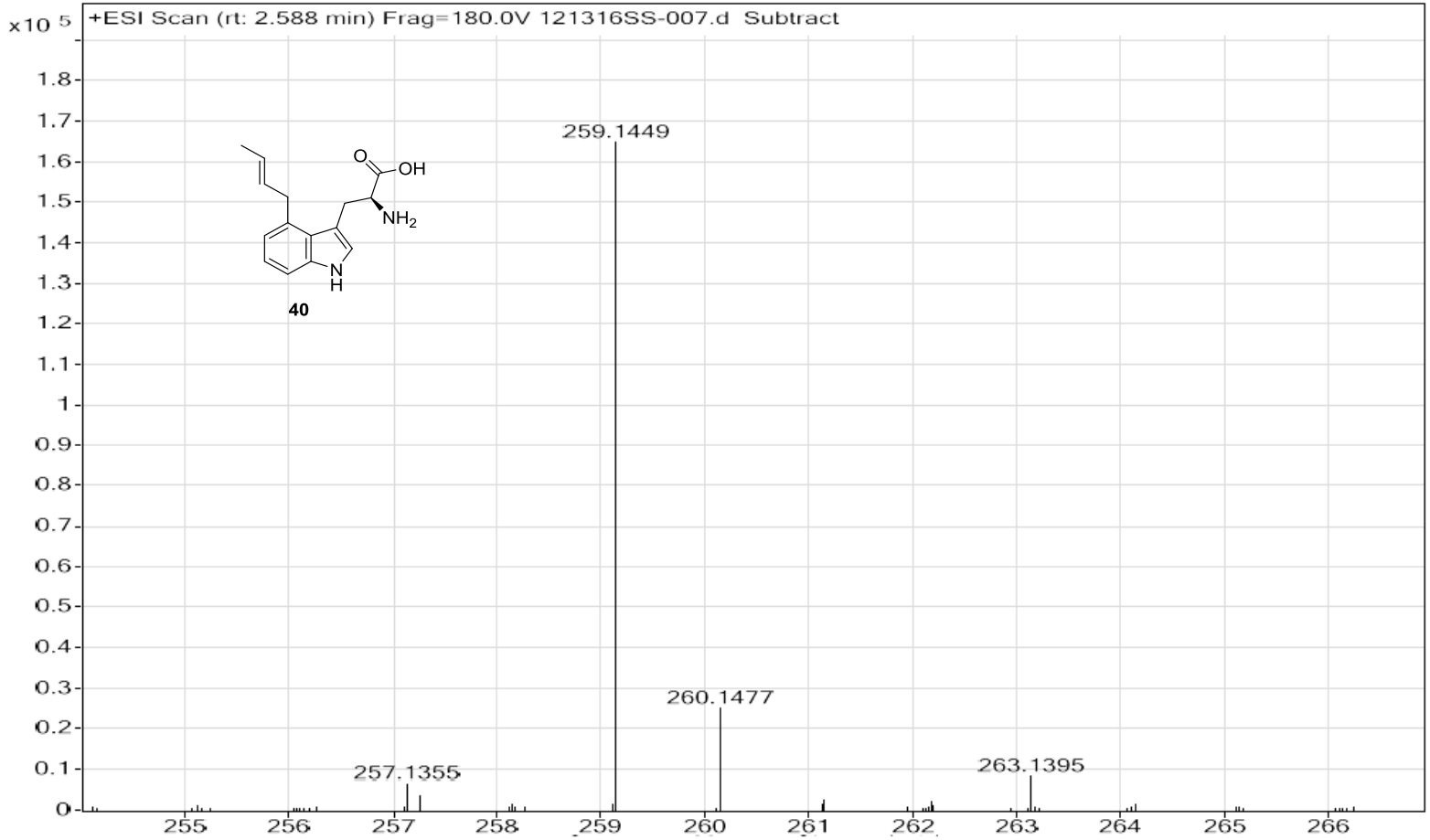


40



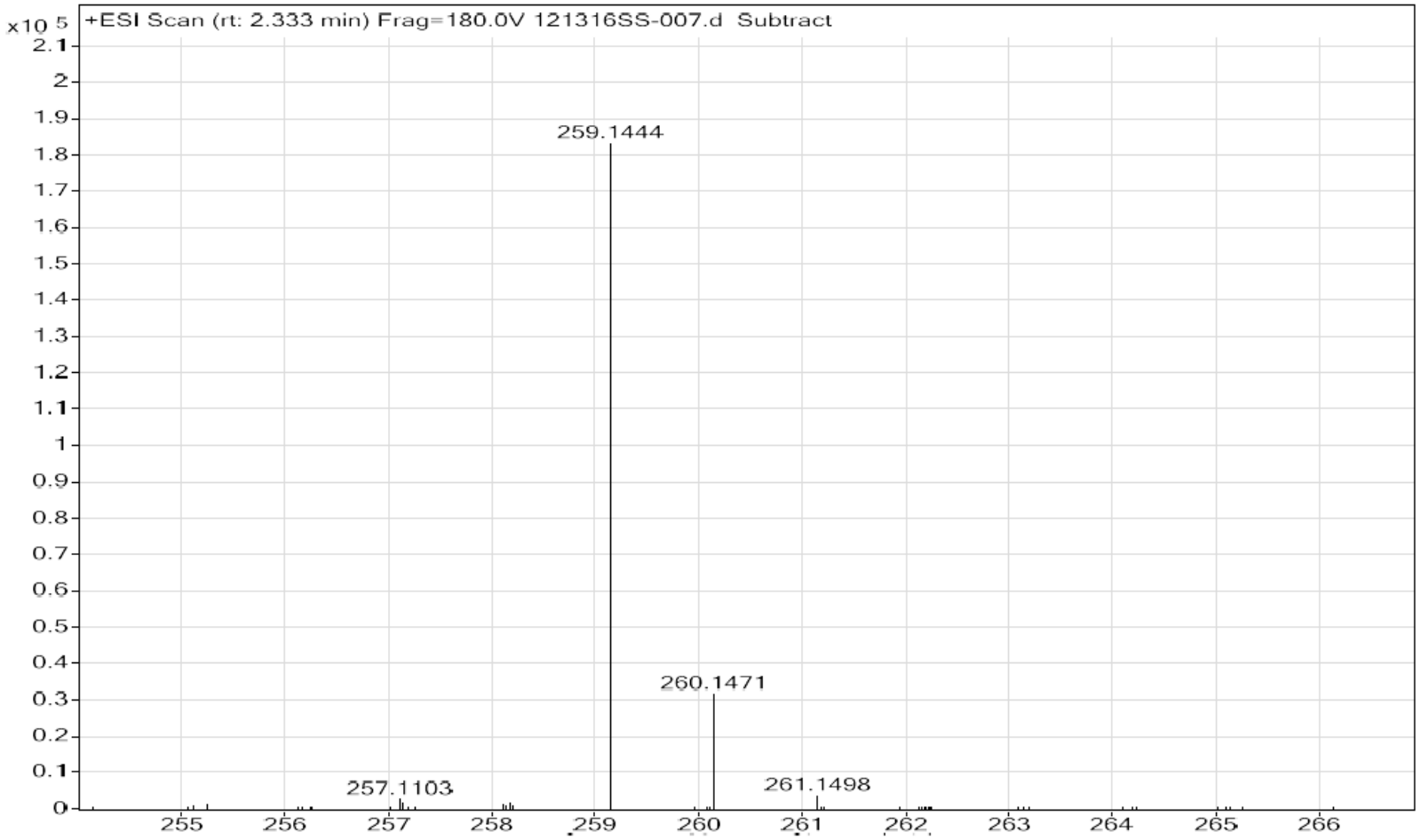
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **40** in DMSO-d6 (*C4*^{N} isomer + 30% *C5*^{N} isomer)

Sample Name	Vial-34	**Position**	P1-A2	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	121316SS-007.d	**ACQ Method**	LCMSUV-pos-short-120	**Comment**		**Acquired Time**	12/13/2016 6:02:52 PM

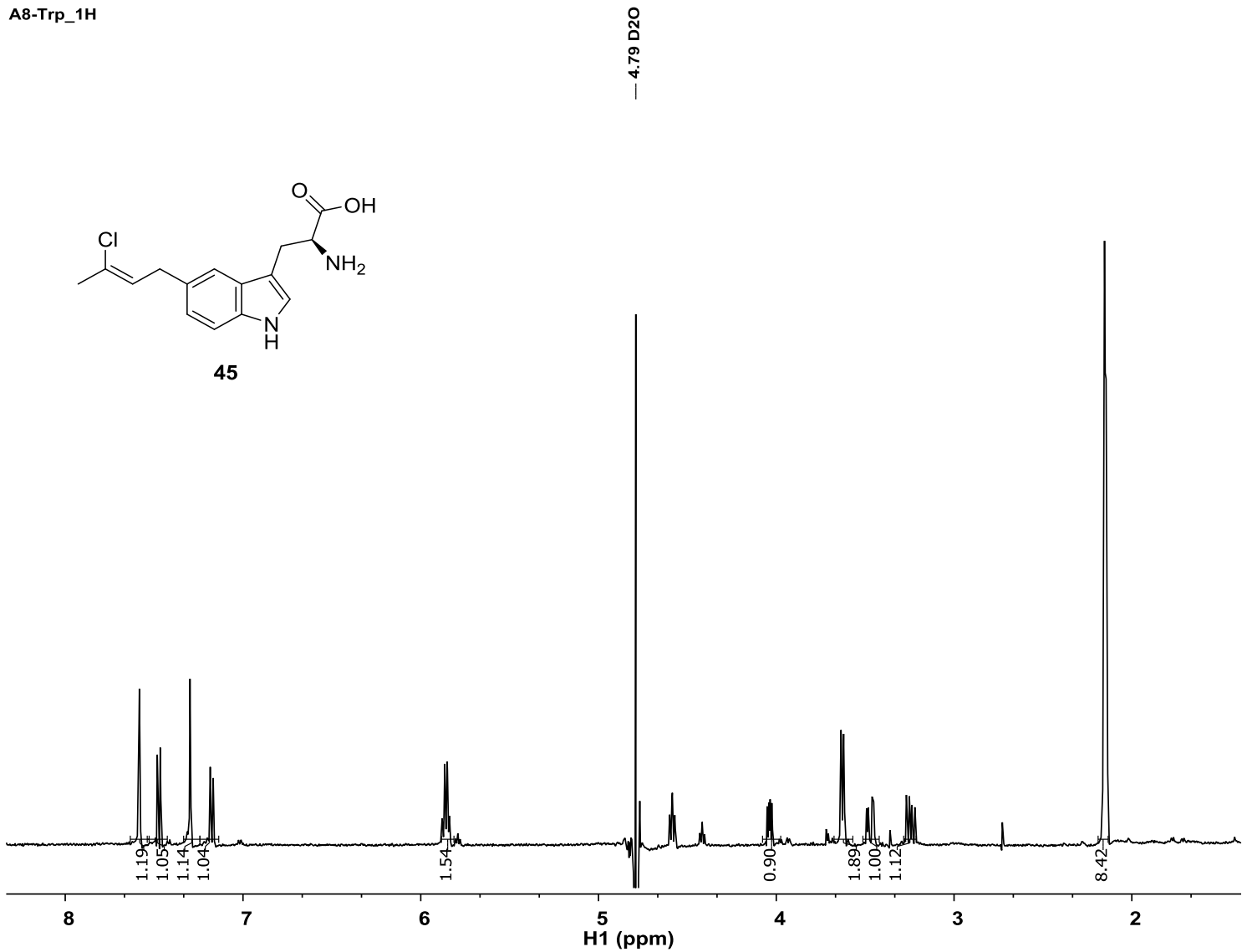


HRMS spectrum of **40**

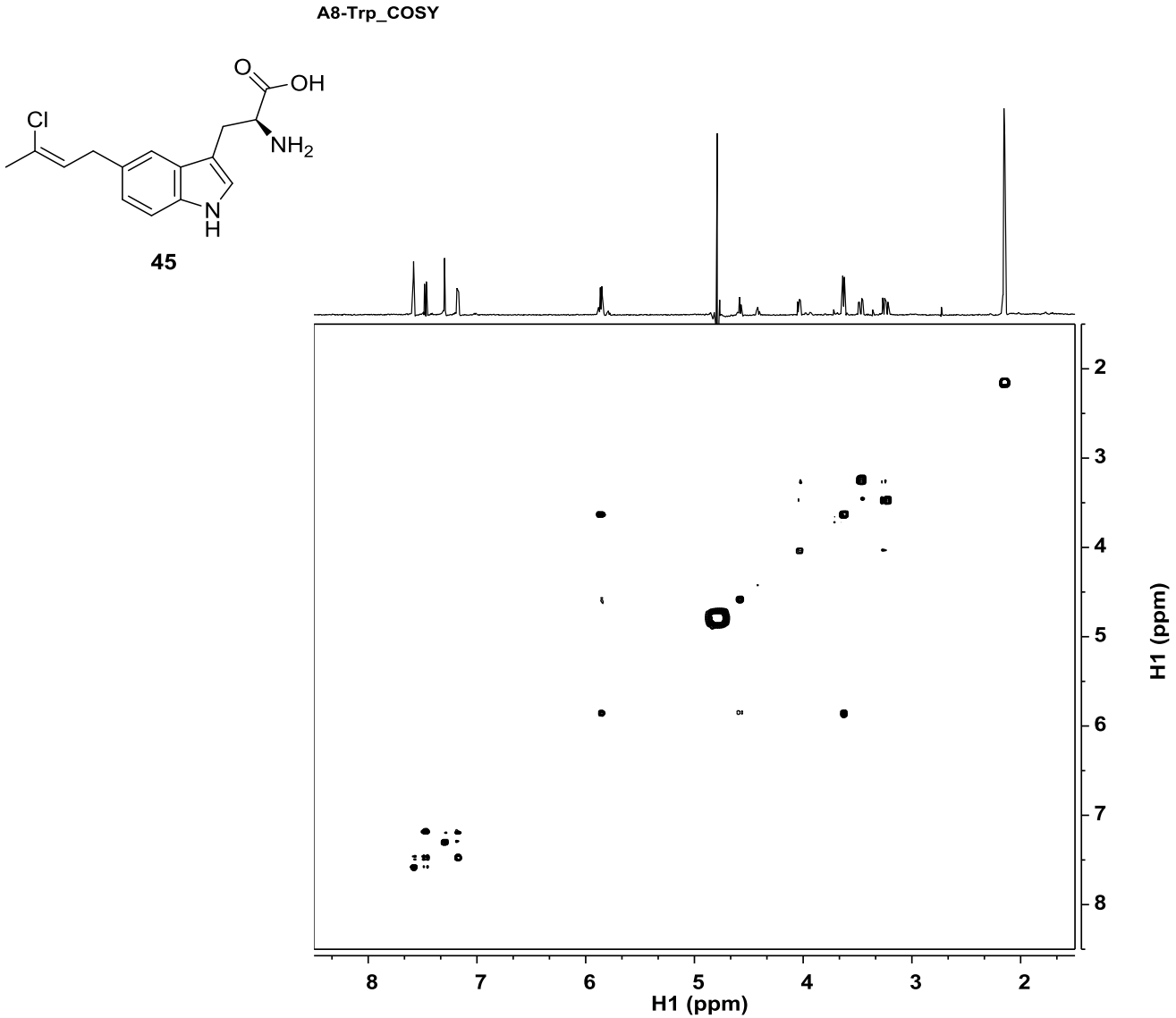
Sample Name	Vial-34	**Position**	P1-A2	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	121316SS-007.d	**ACQ Method**	LCMSUV-pos-short-120	**Comment**		**Acquired Time**	12/13/2016 6:02:52 PM



HRMS of 41

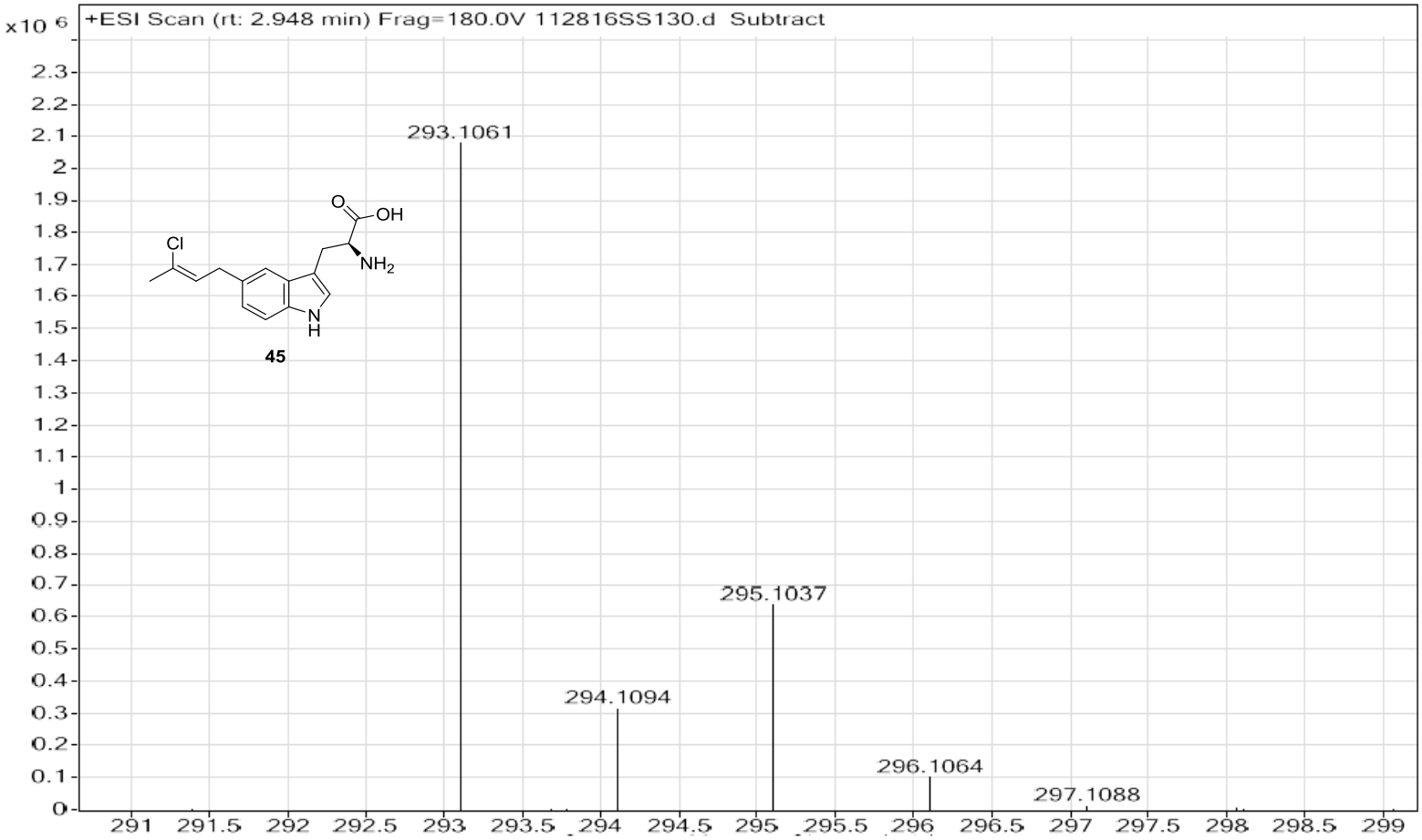


^{1}H NMR Spectrum (500 MHz) of compound **45** in D_2O



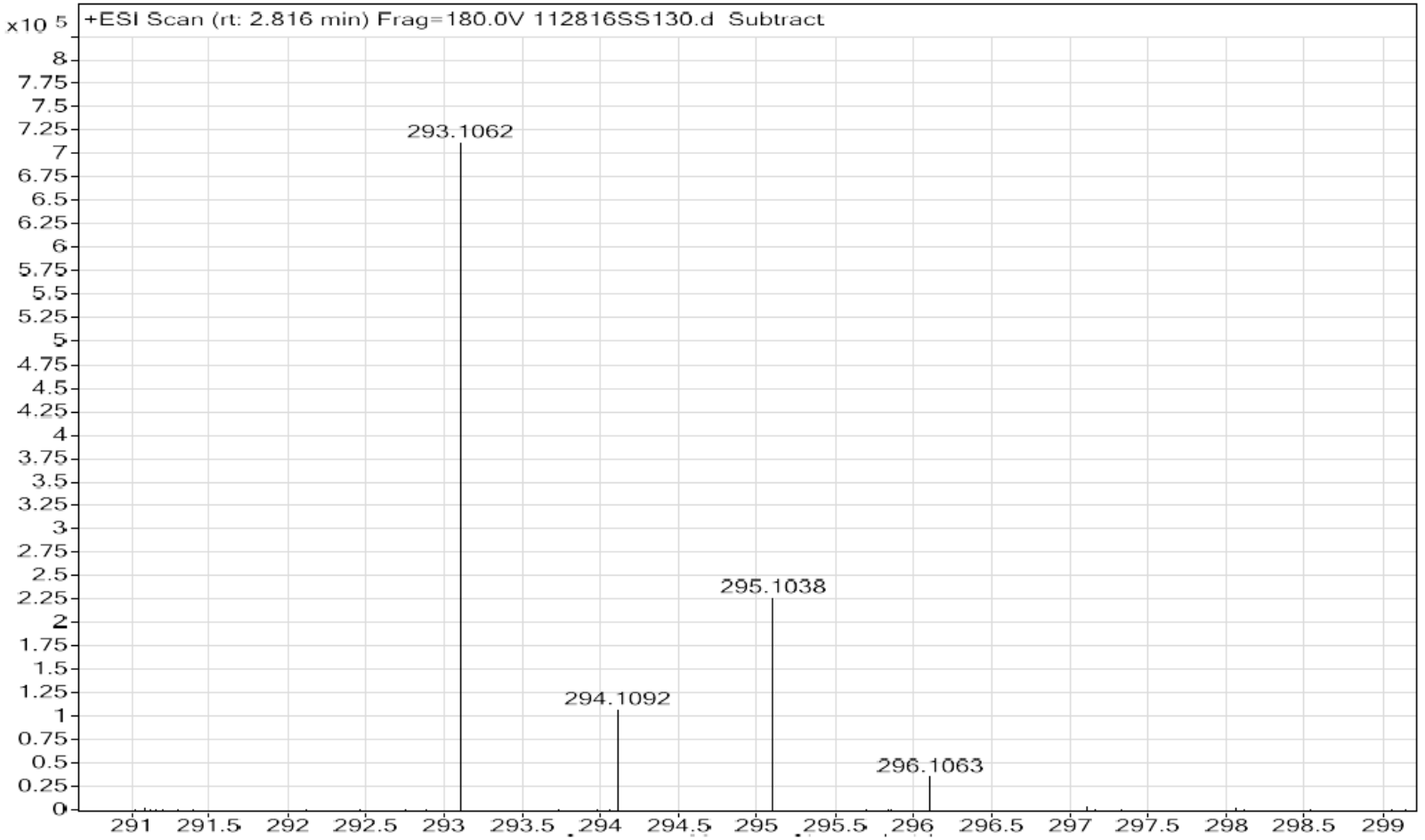
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **45** in D_2O

Sample Name	TRP-42	**Position**	P1-E6	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS130.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 12:04:09 PM

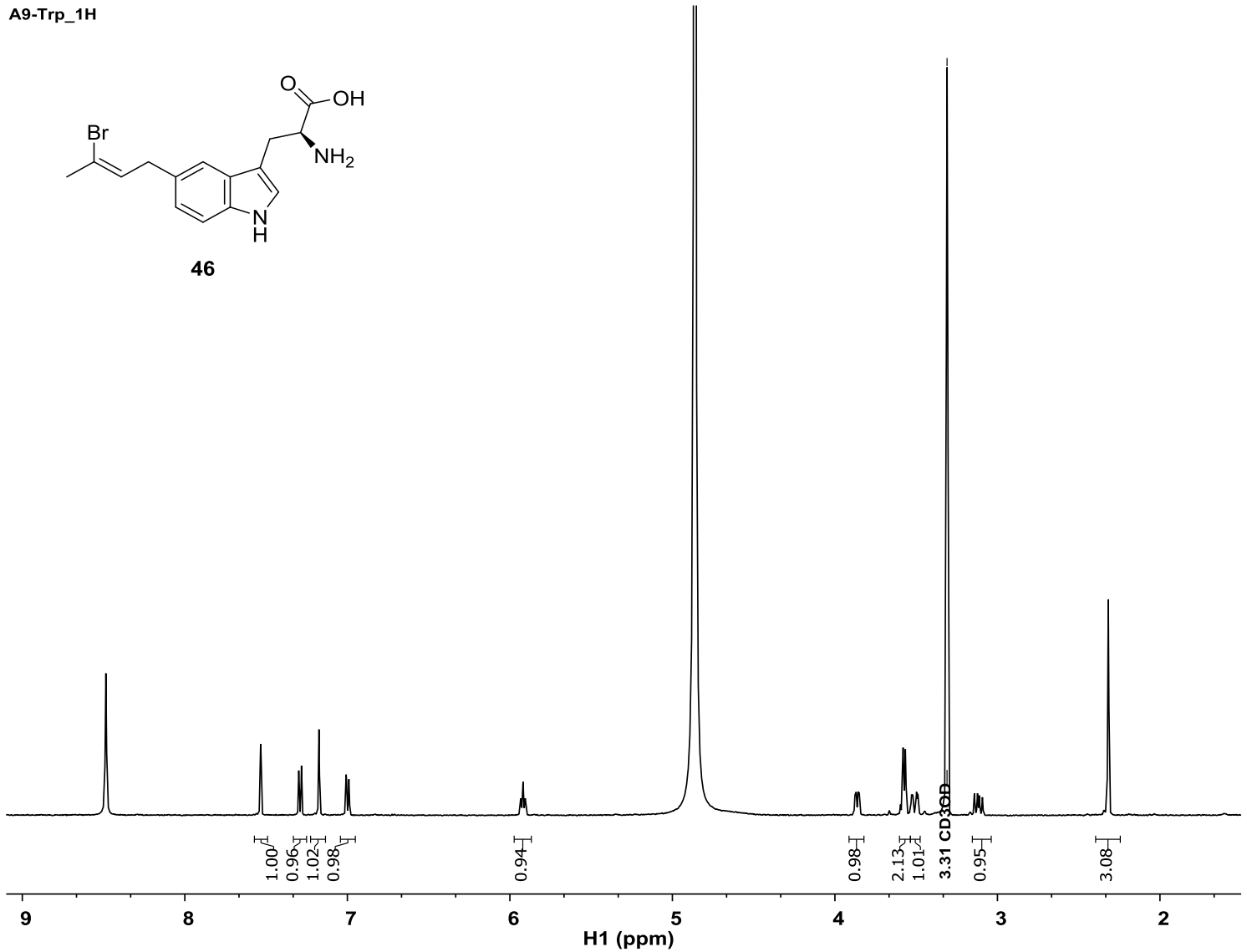


HRMS spectrum of **45**

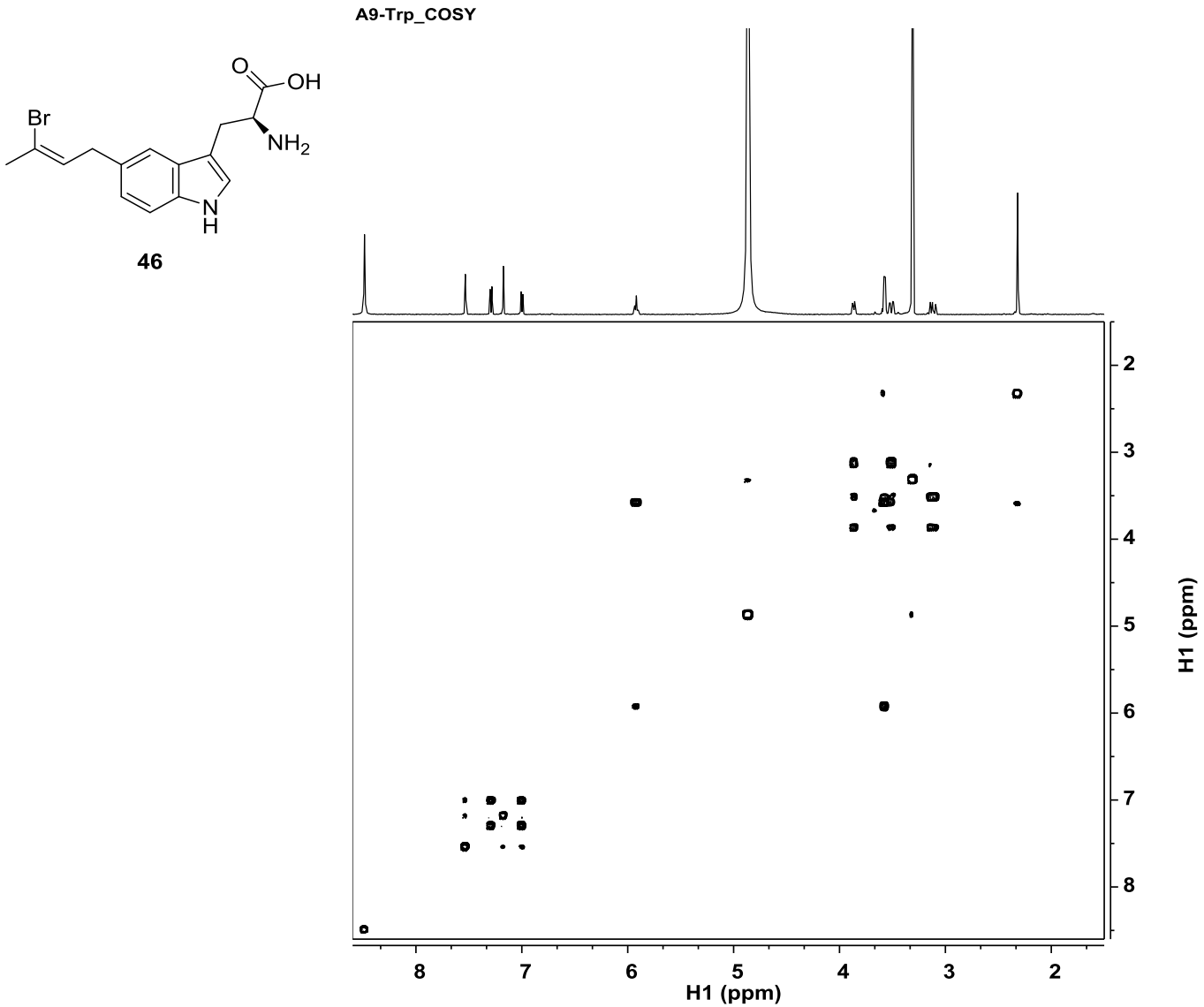
Sample Name	TRP-42	**Position**	P1-E6	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS130.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 12:04:09 PM



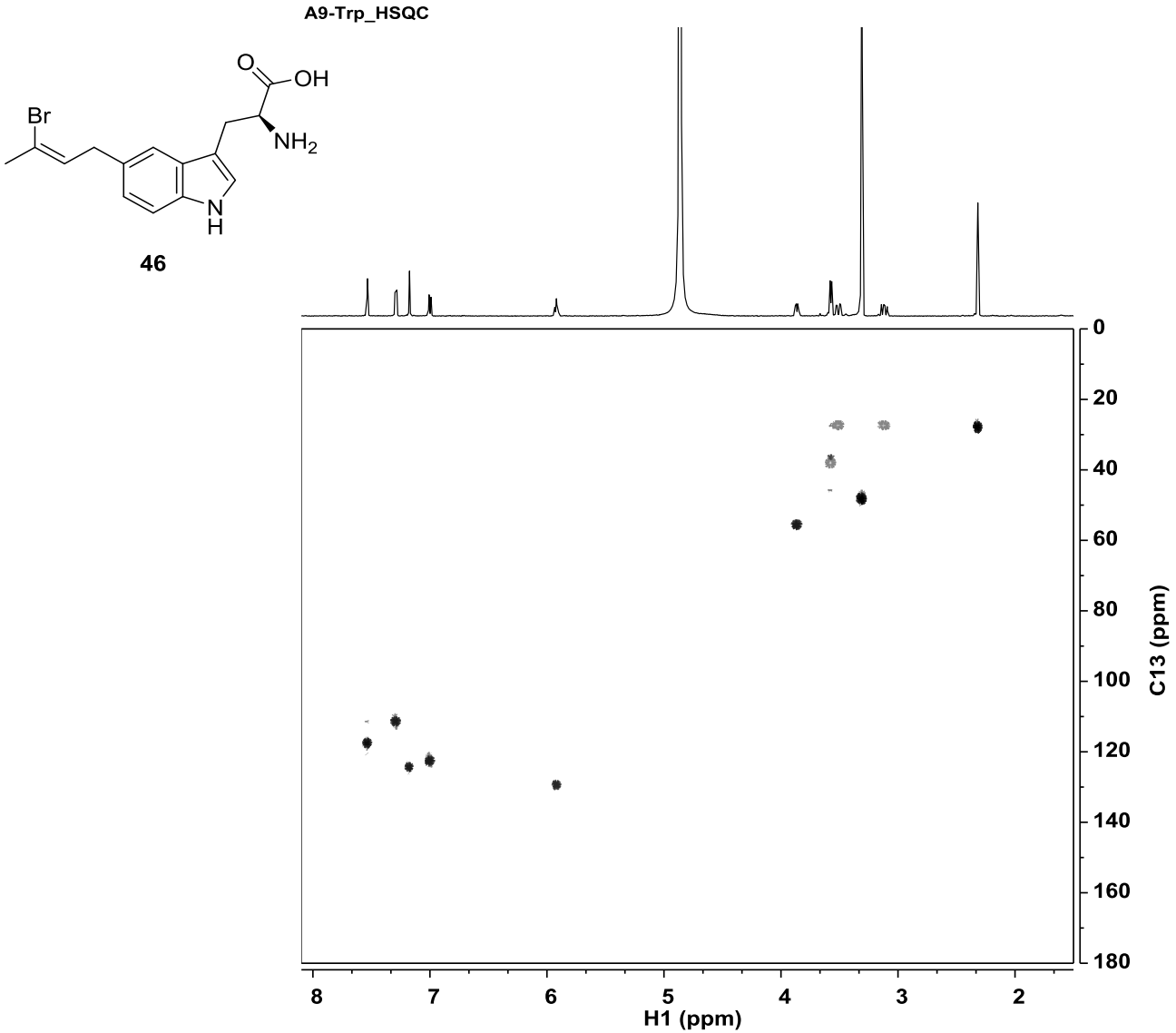
HRMS of 8-Trp$_2$



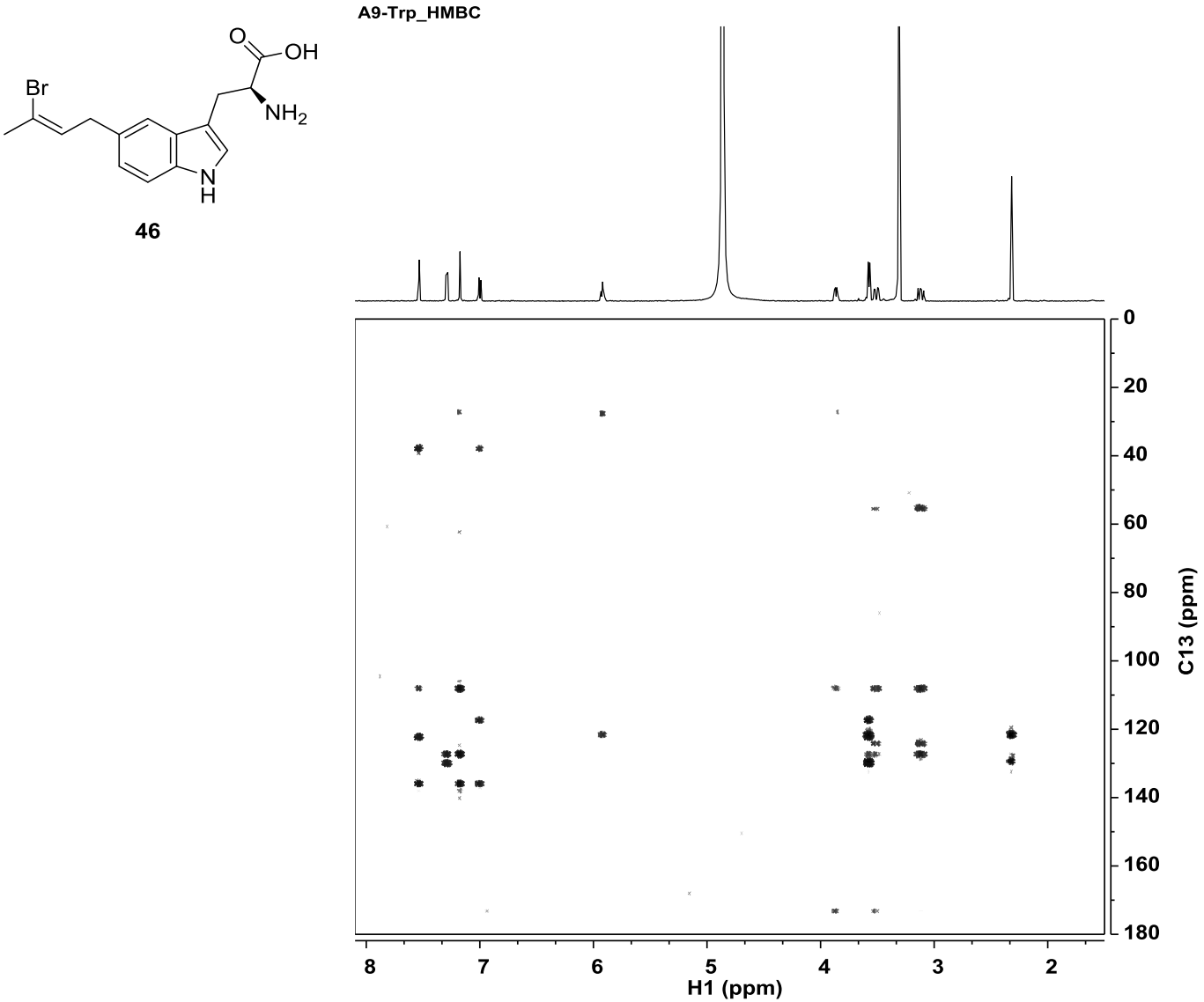
^{1}H NMR Spectrum (500 MHz) of compound **46** in MeOH d4.



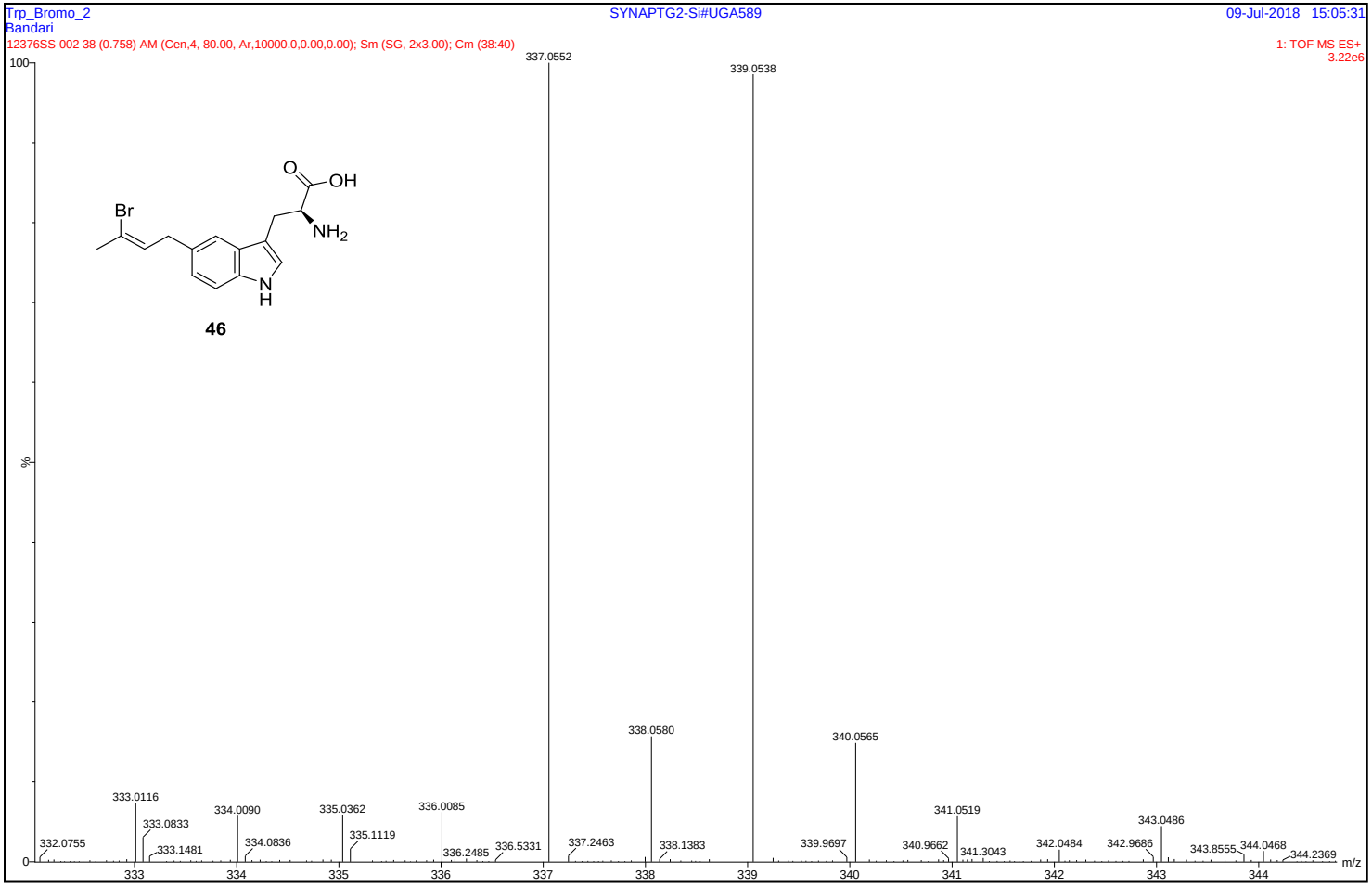
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **46** in MeOH-d_4



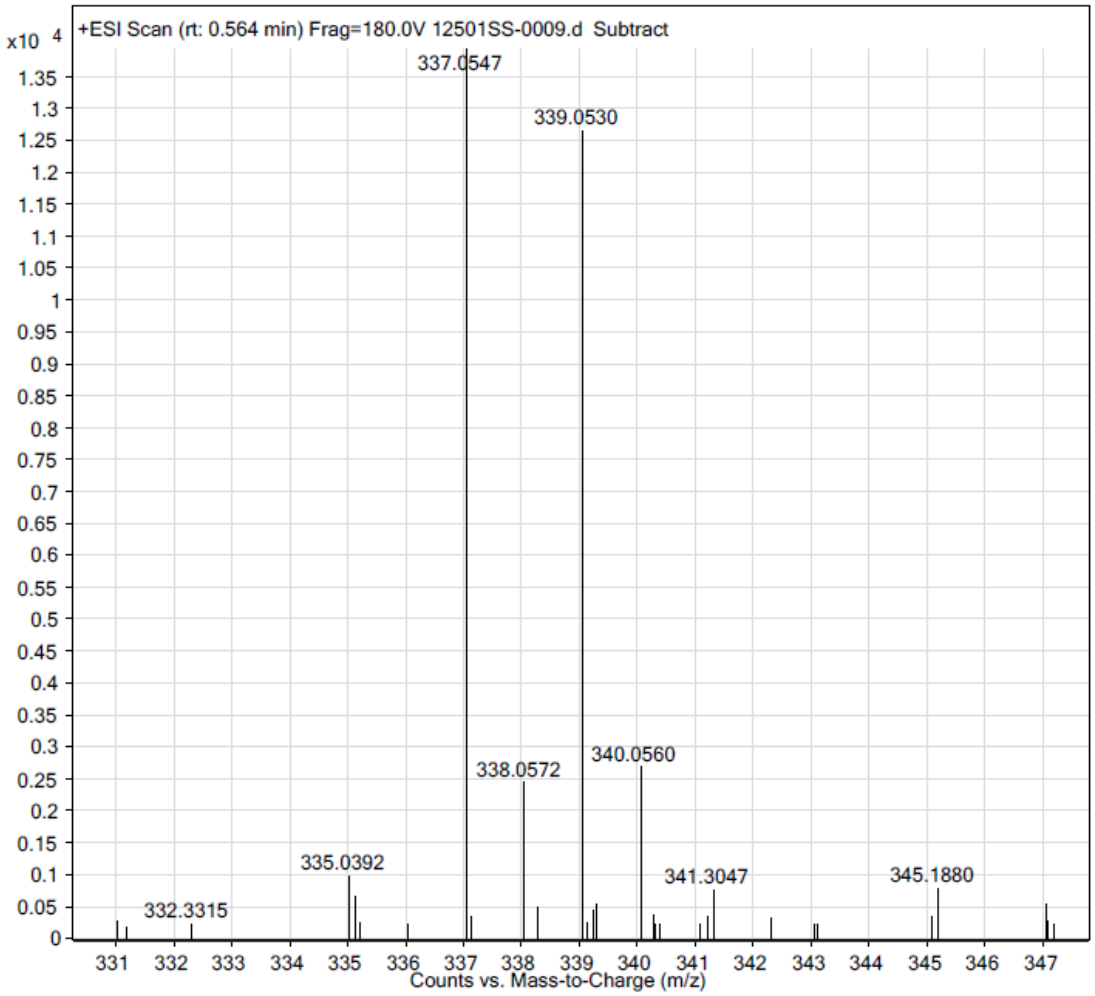
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **46** in MeOH-d_4



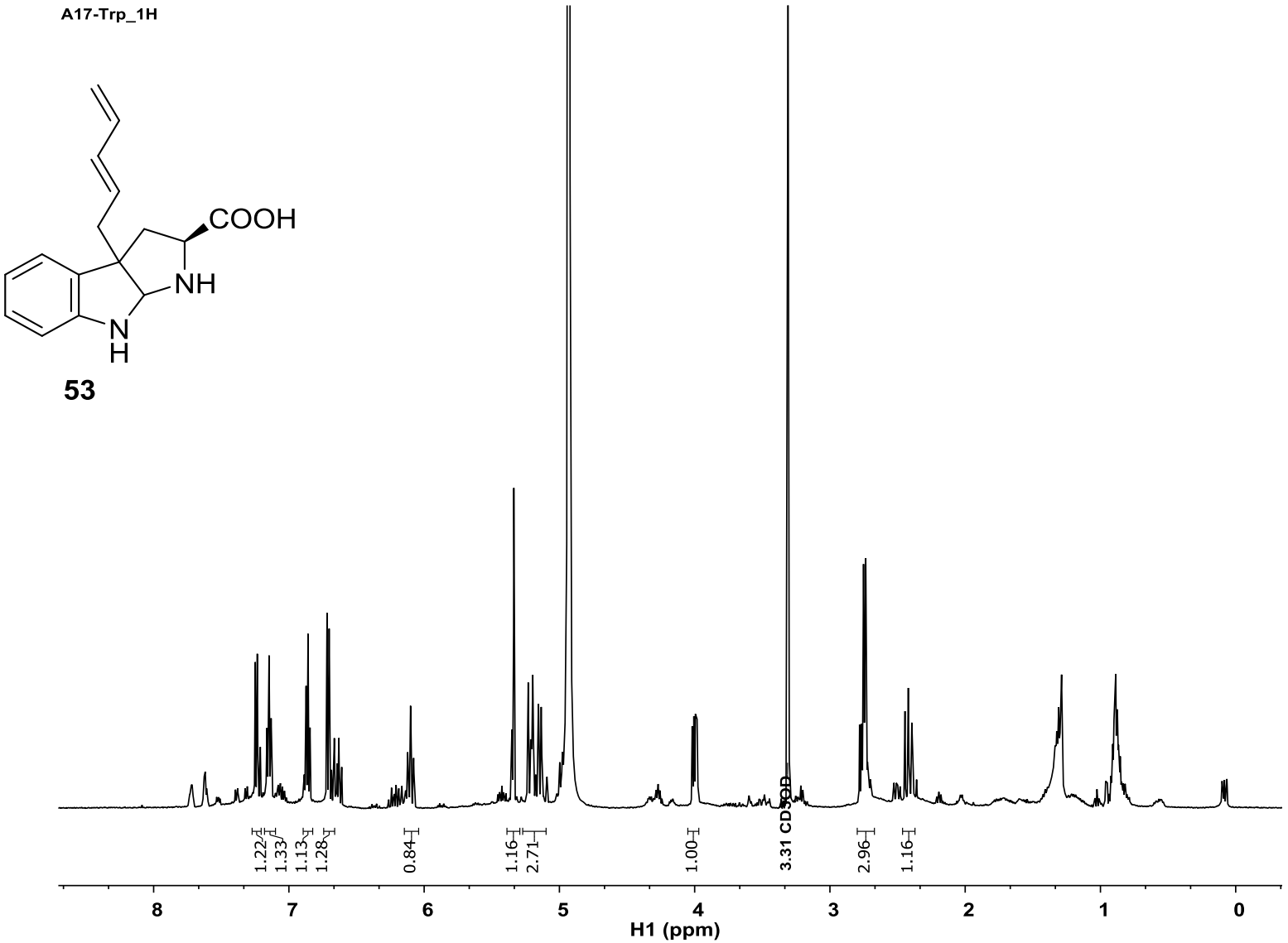
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **46-** in MeOH-d_4



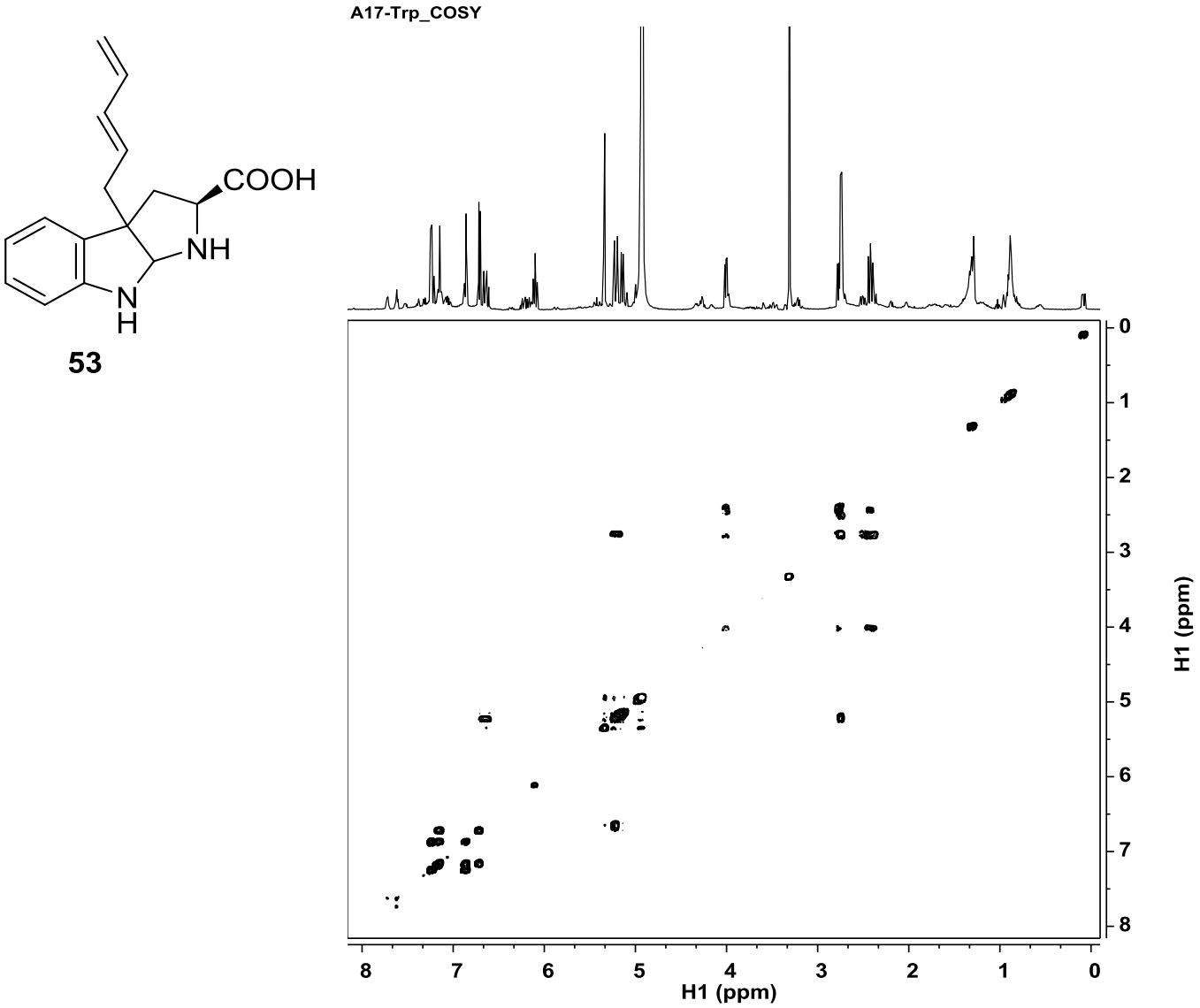
HRMS spectrum of **46**



HRMS spectrum of **9-Trp$_2$**

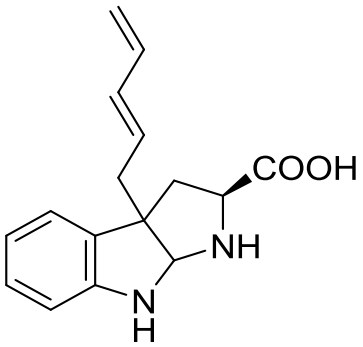


^{1}H NMR Spectrum (500 MHz) of compound **53** in MeOH-d4

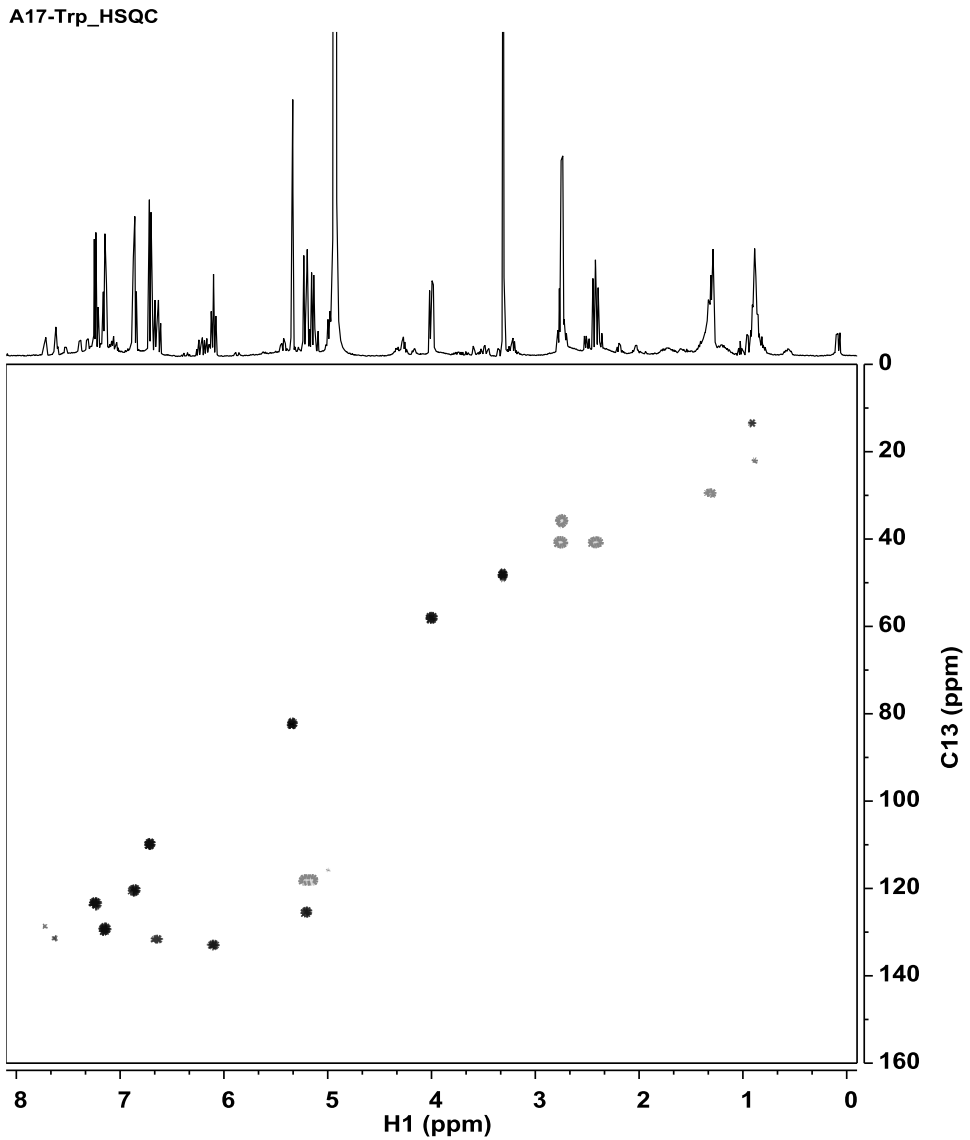


53

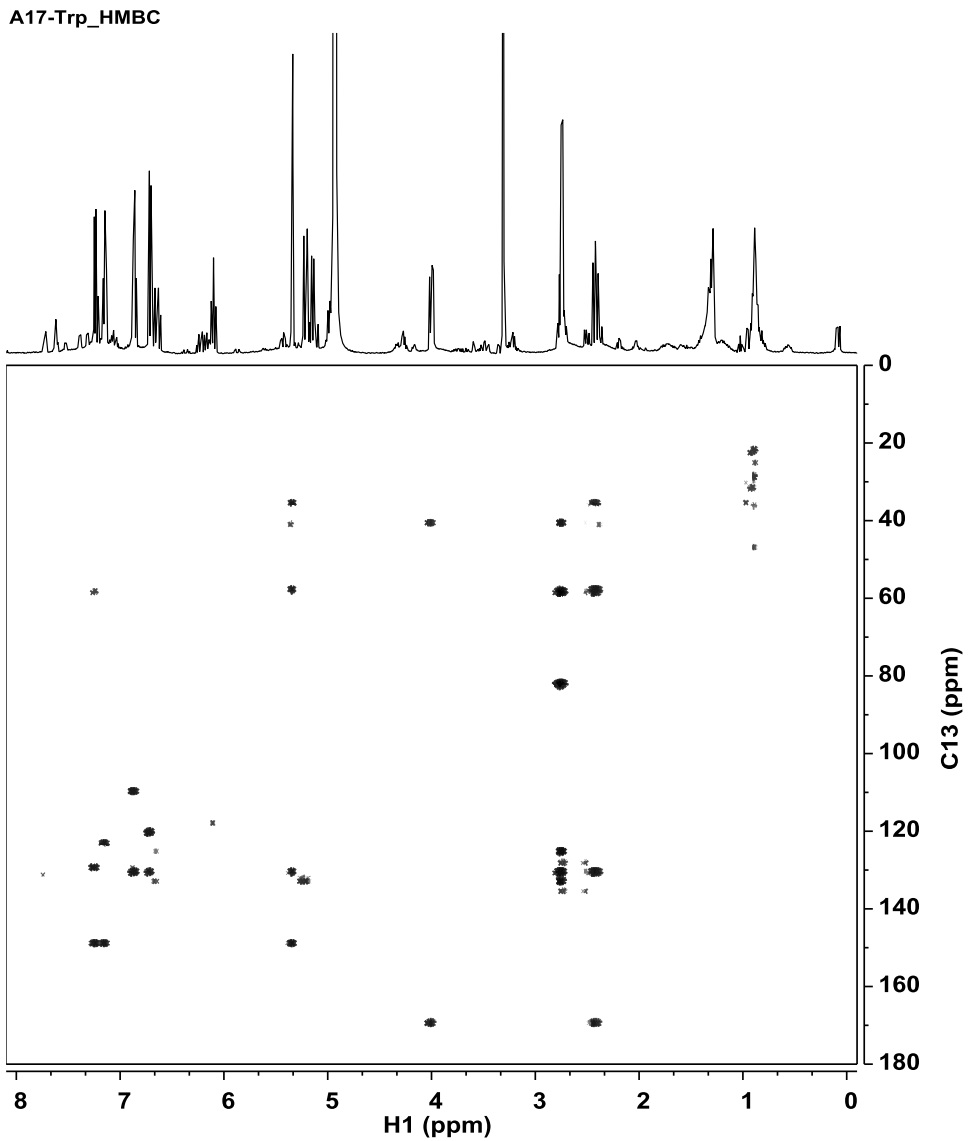
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **53** in MeOH-d4



53

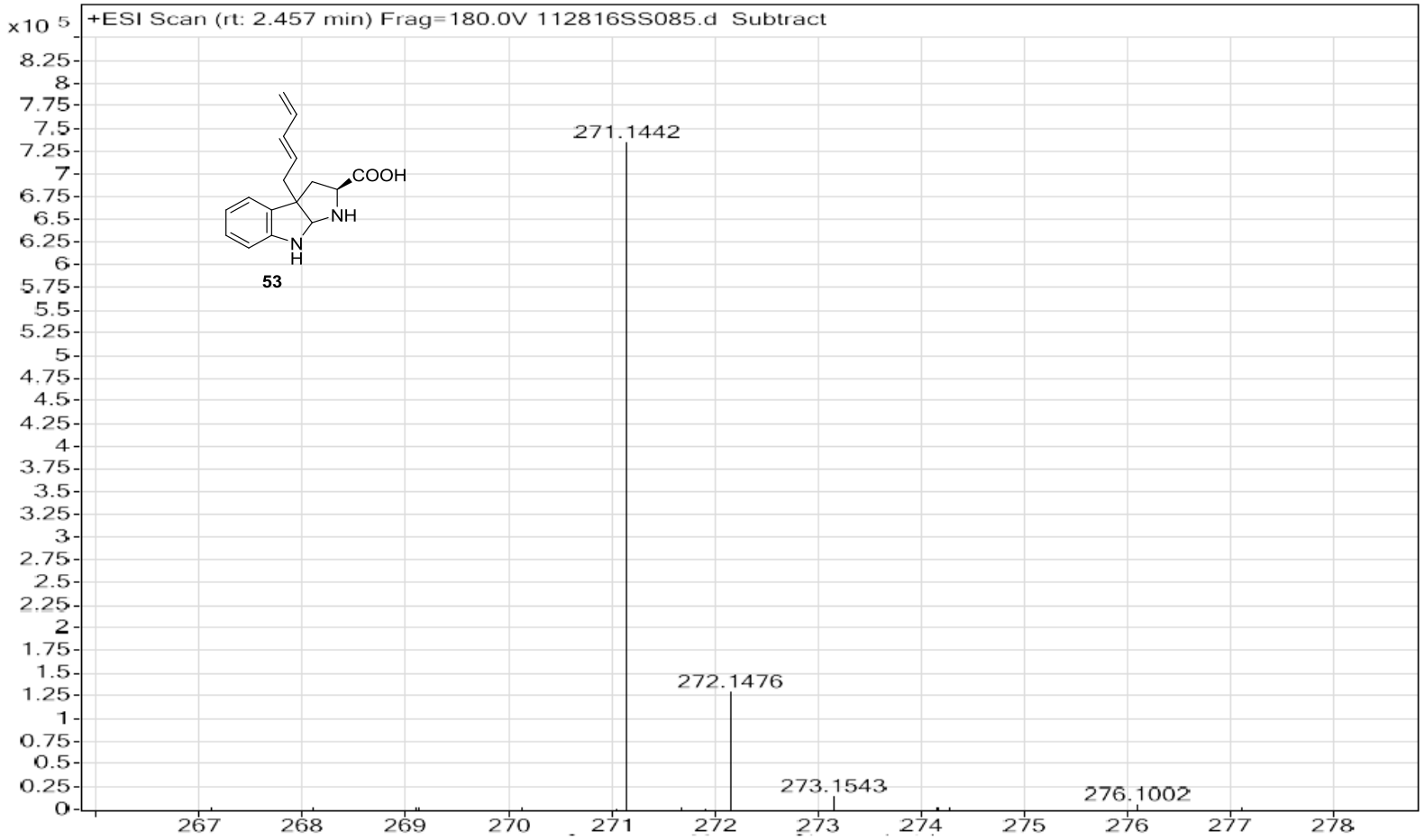


2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **53** in MeOH-d4



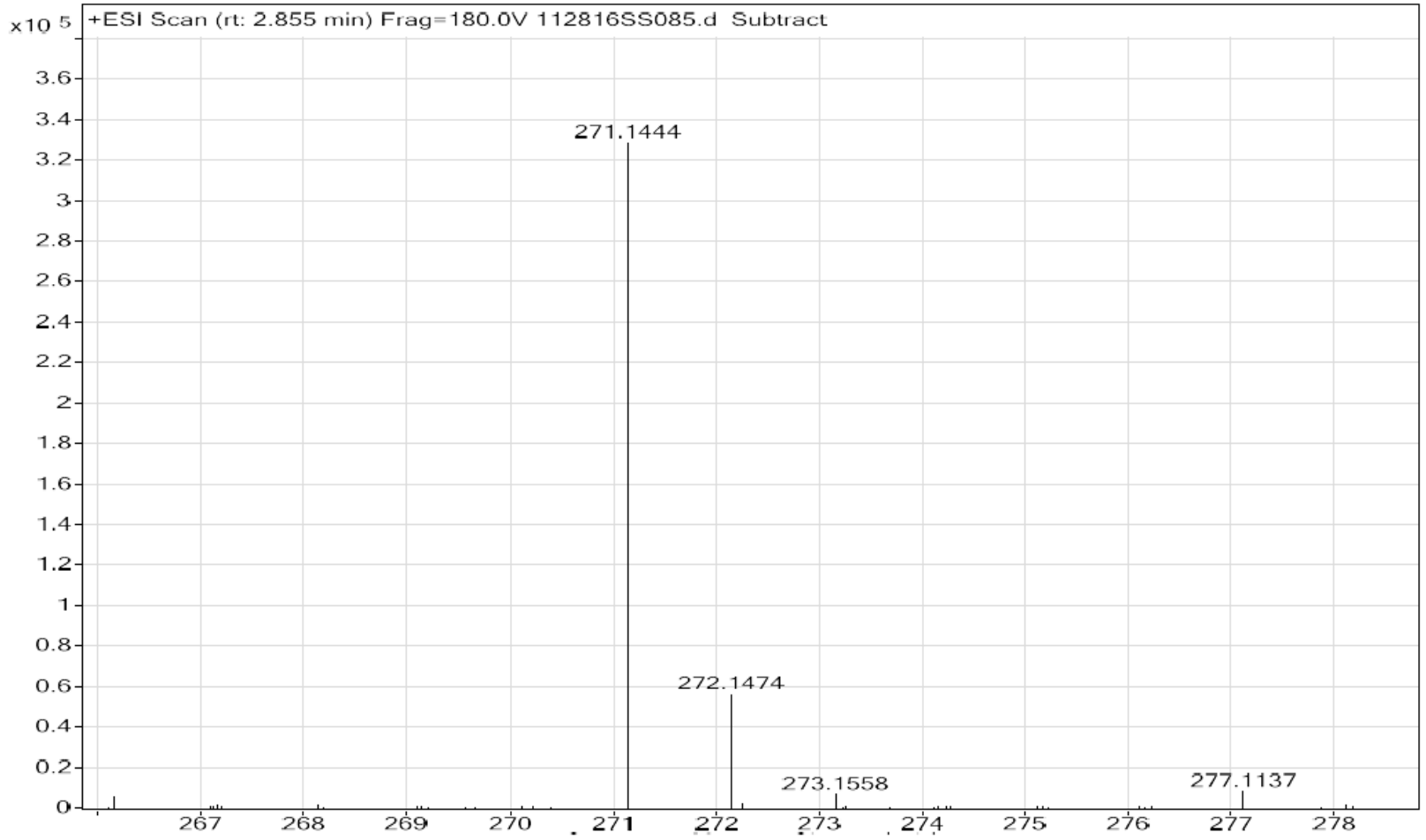
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **53** in MeOH-d4

Sample Name	TRP-27	**Position**	P1-C9	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS085.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 3:53:36 AM

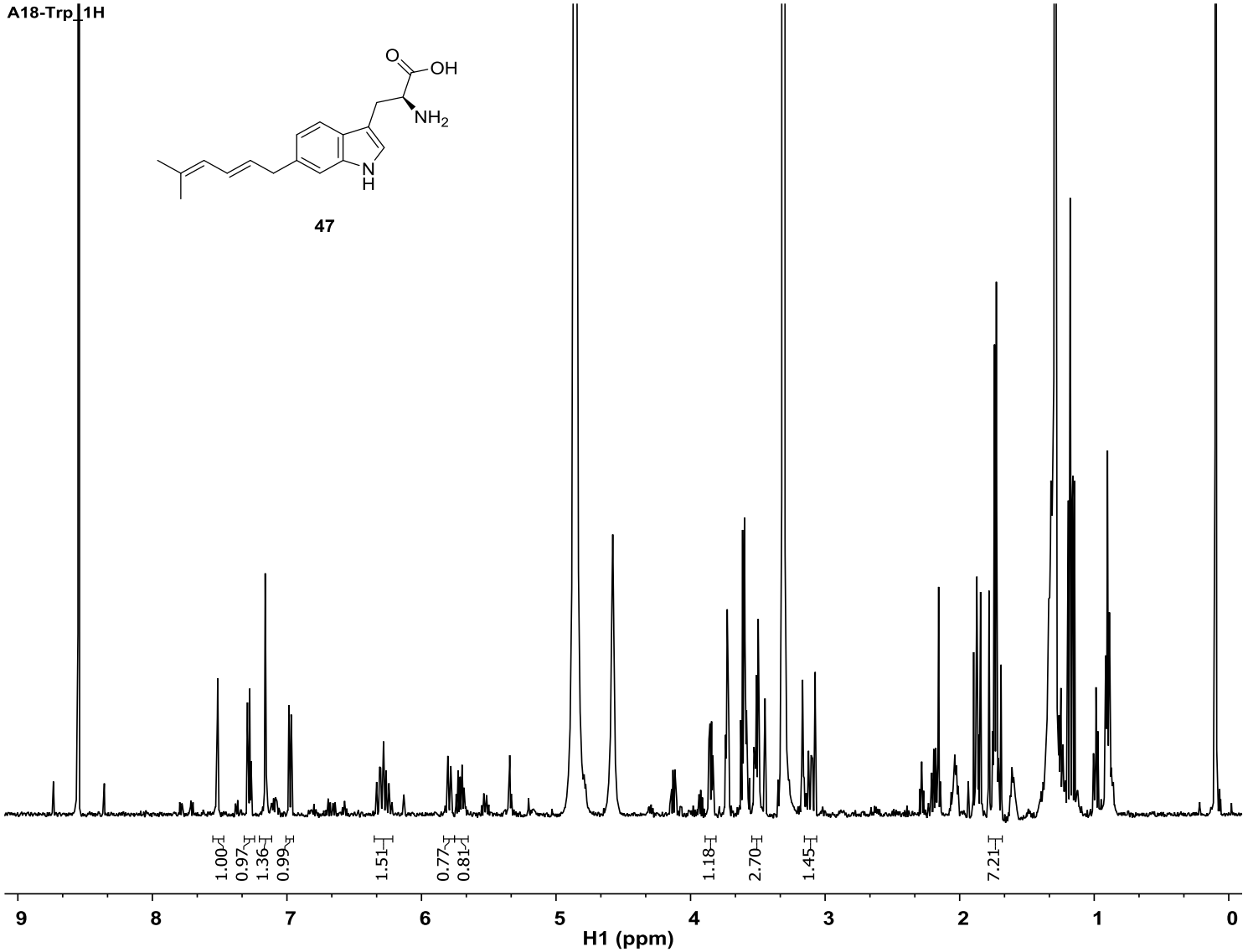


HRMS spectrum of **53**

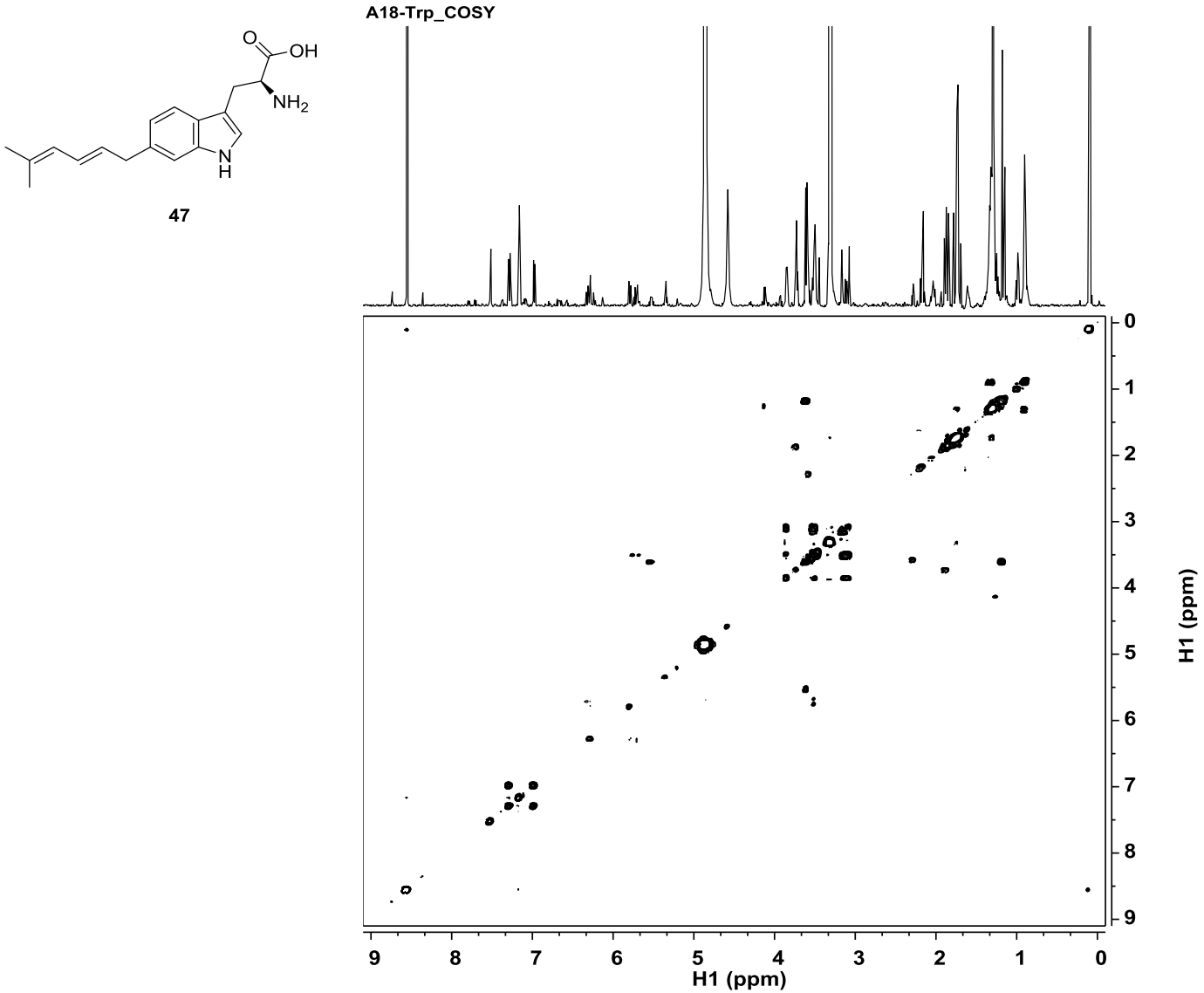
Sample Name	TRP-27	**Position**	P1-C9	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS085.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 3:53:36 AM



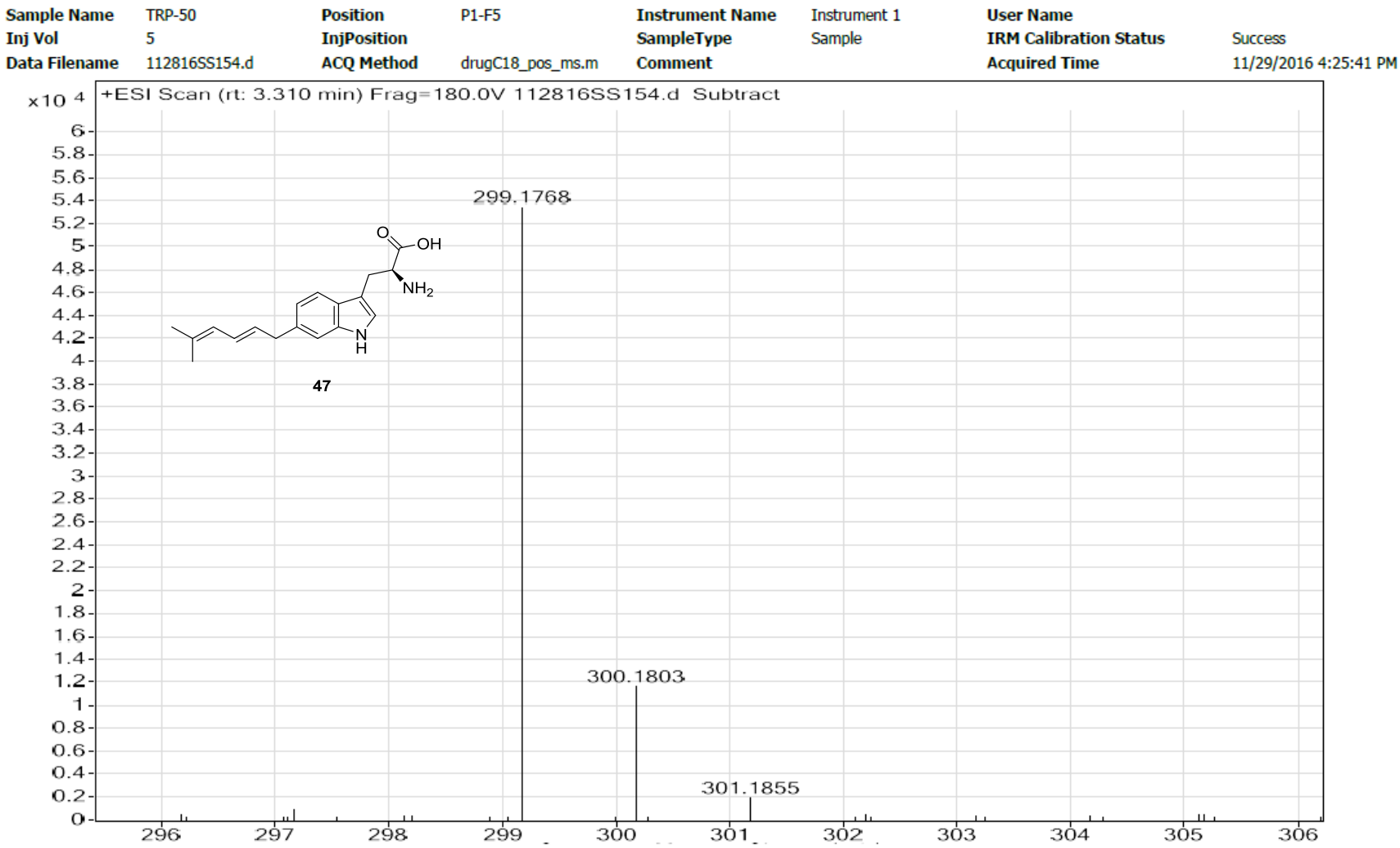
HRMS of 17-Trp$_2$



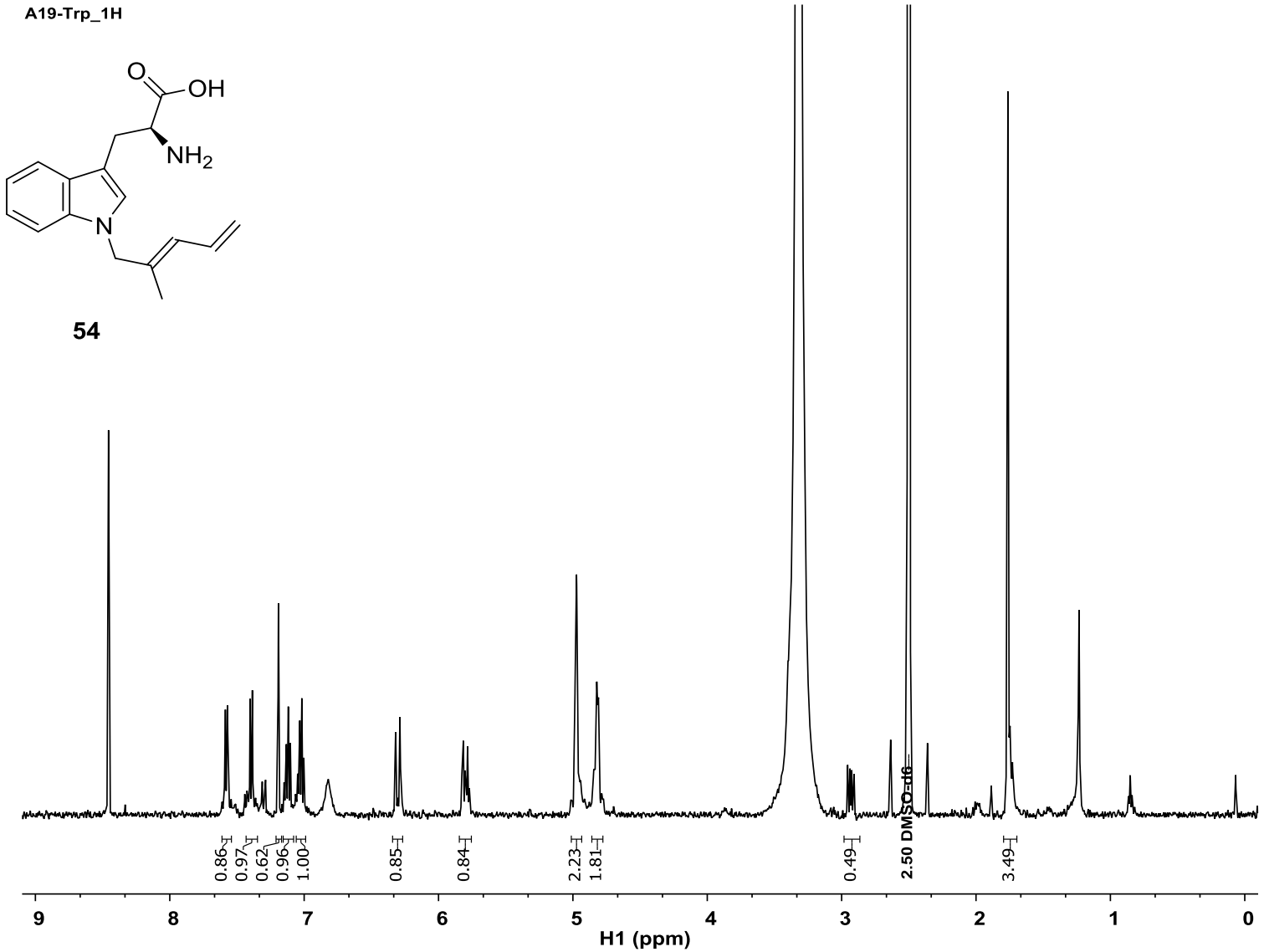
^{1}H NMR Spectrum (500 MHz) of compound **47** in MeOH-d4



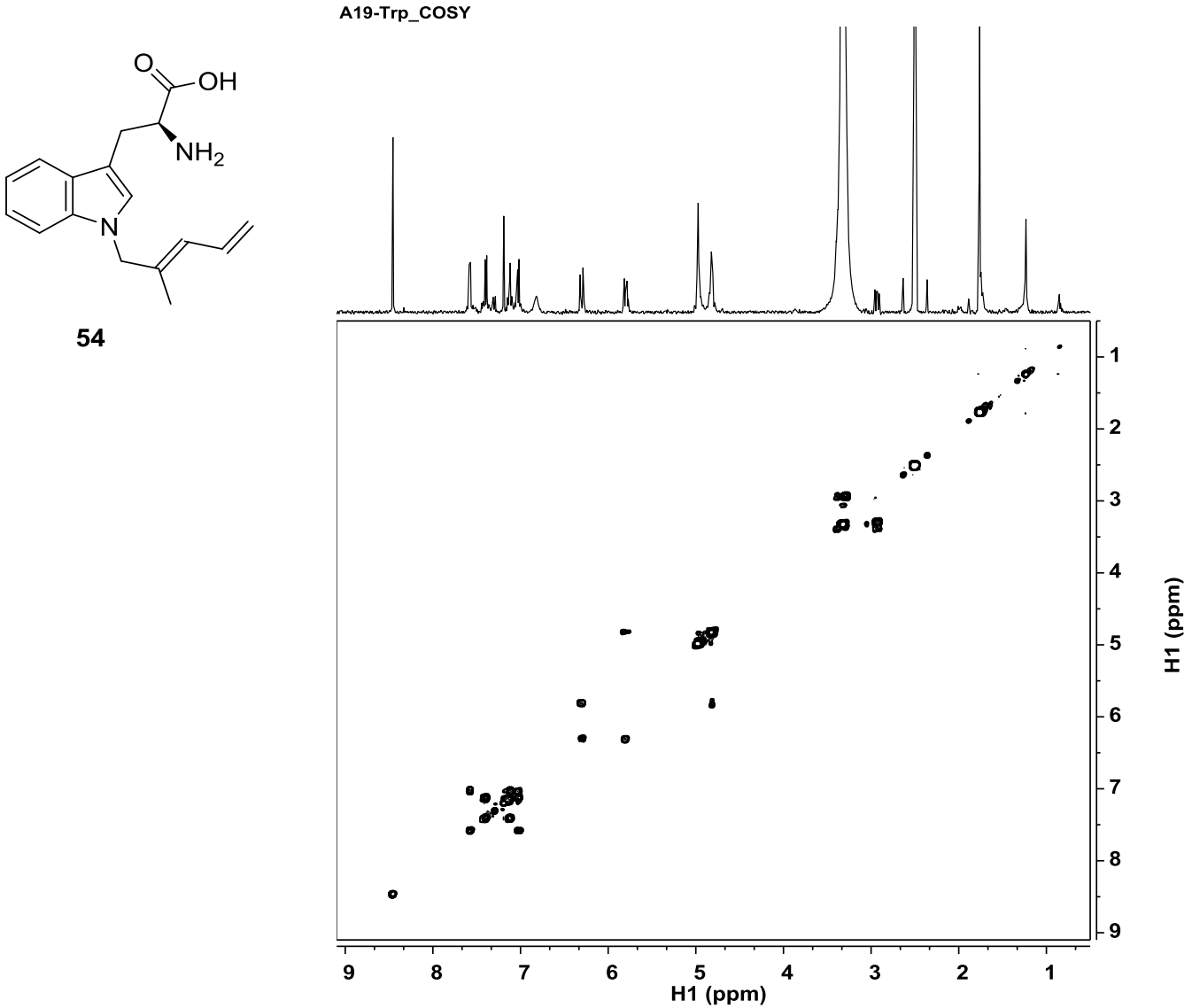
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **47** in MeOH-d4



HRMS spectrum of **47**

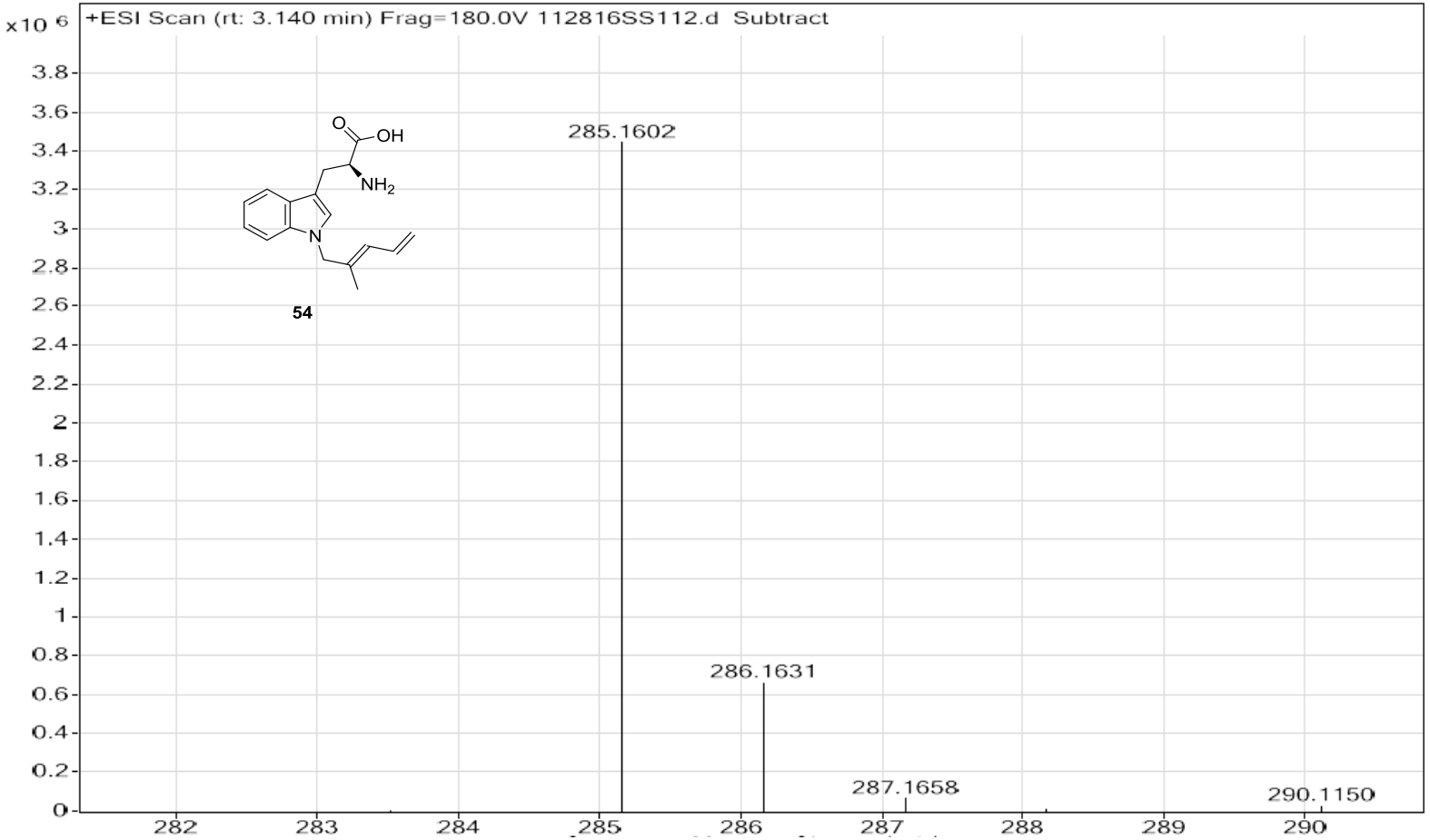


^{1}H NMR Spectrum (500 MHz) of compound **54** in DMSO-d6



2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **54** in DMSO-d6

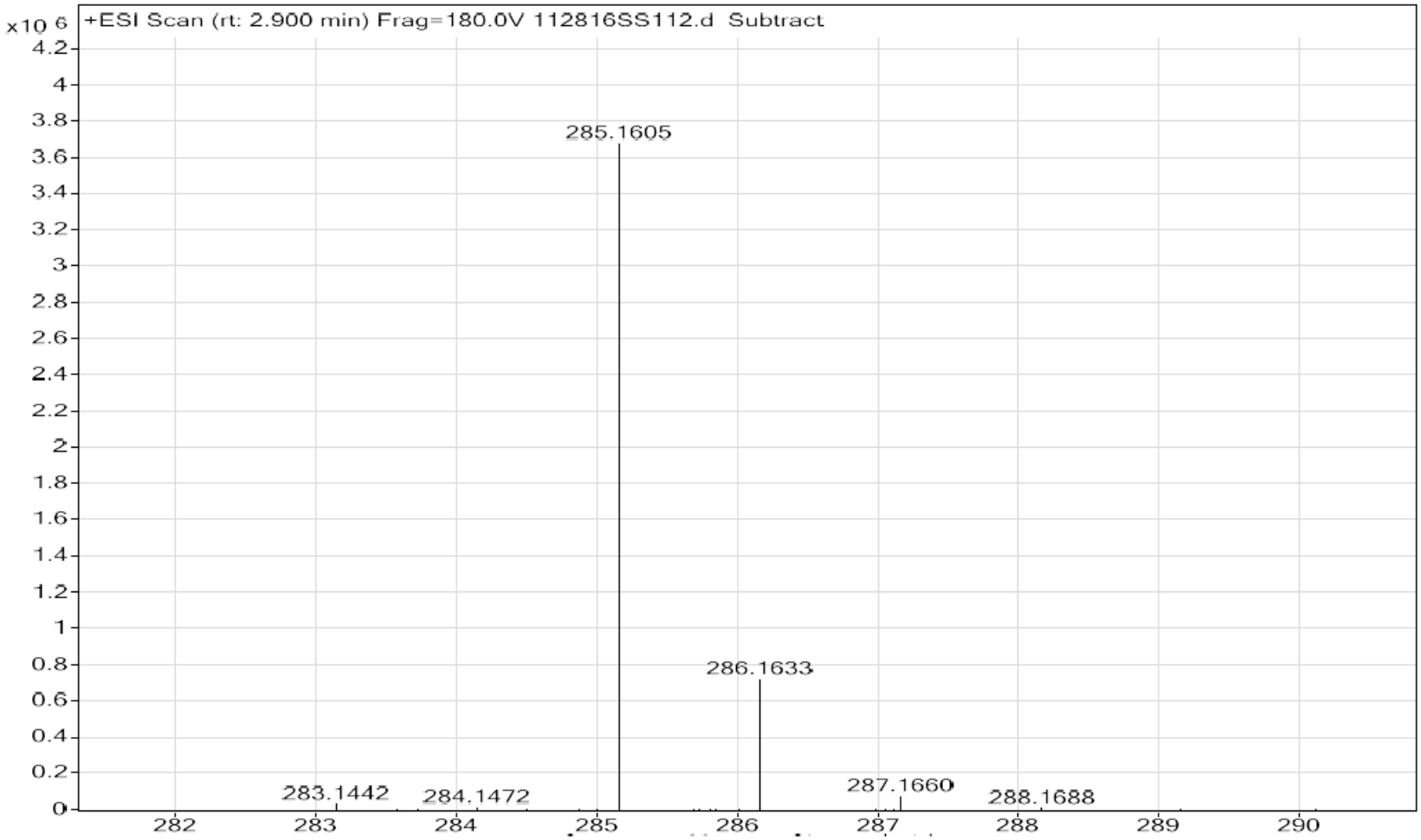
Sample Name	TRP-36	**Position**	P1-D9	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS112.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 8:47:58 AM



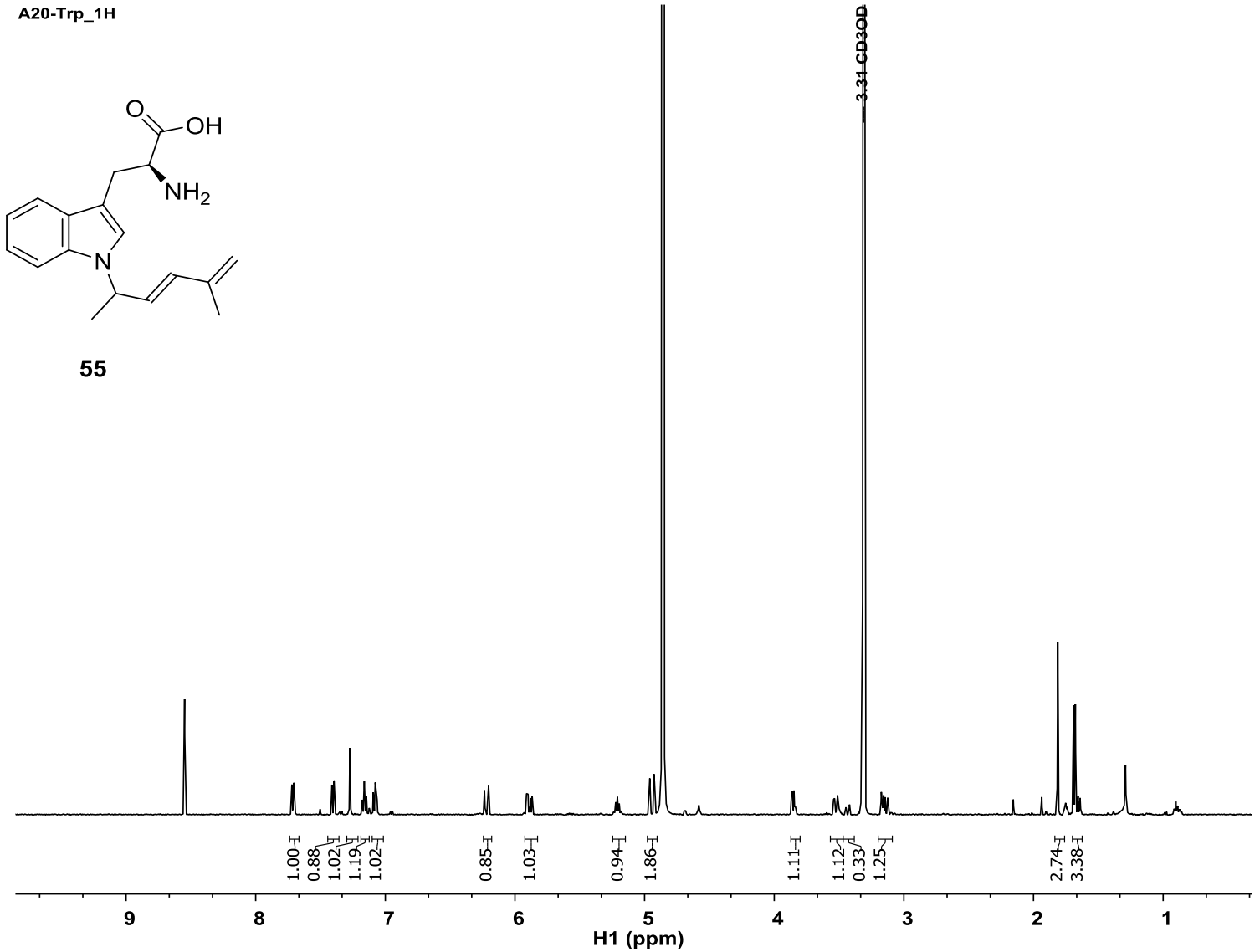
54

HRMS spectrum of **54**

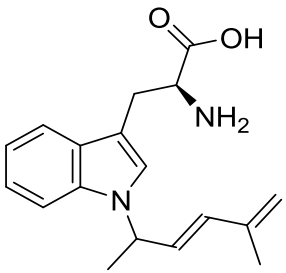
Sample Name	TRP-36	**Position**	P1-D9	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS112.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 8:47:58 AM



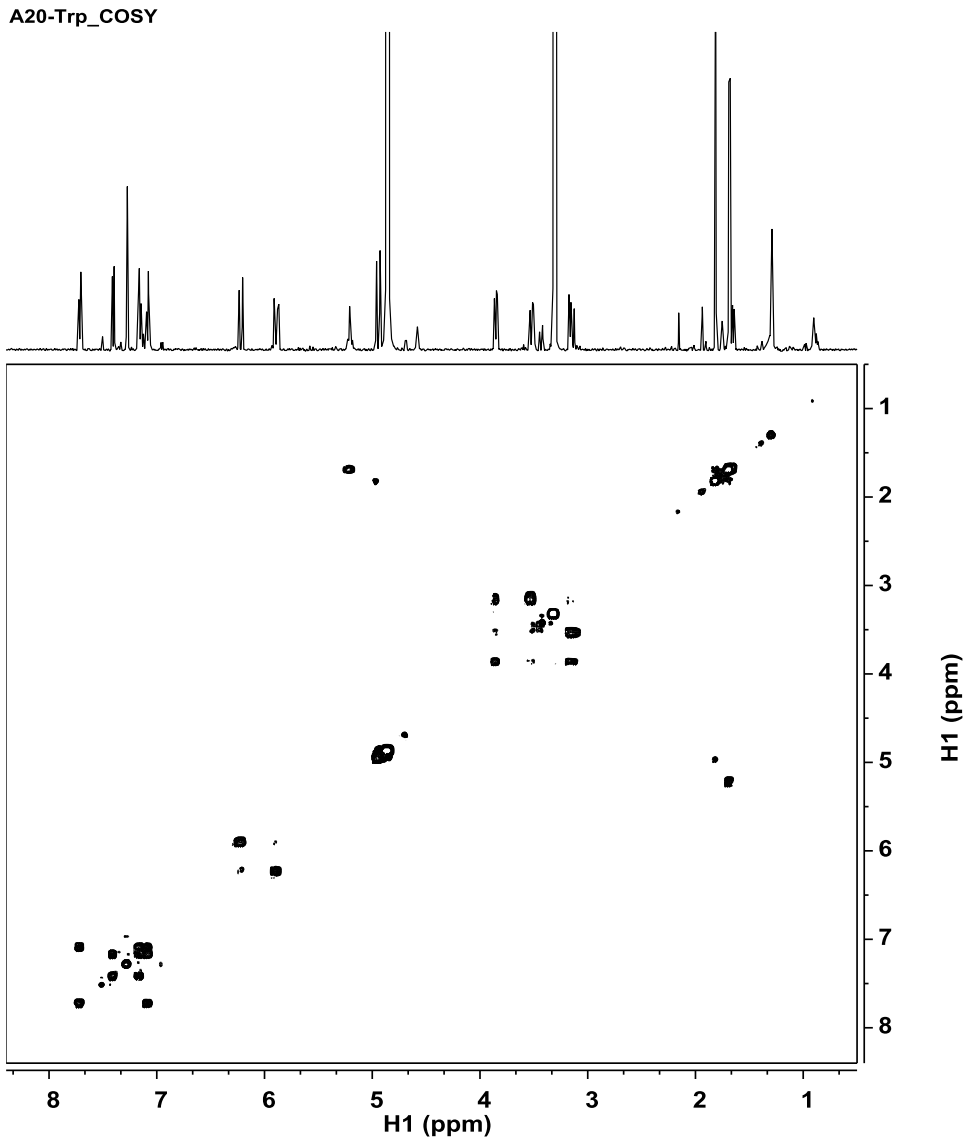
HRMS of 19-Trp$_2$



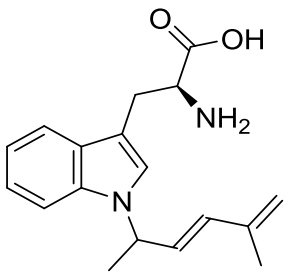
^{1}H NMR Spectrum (500 MHz) of compound **55** in MeOH-d4



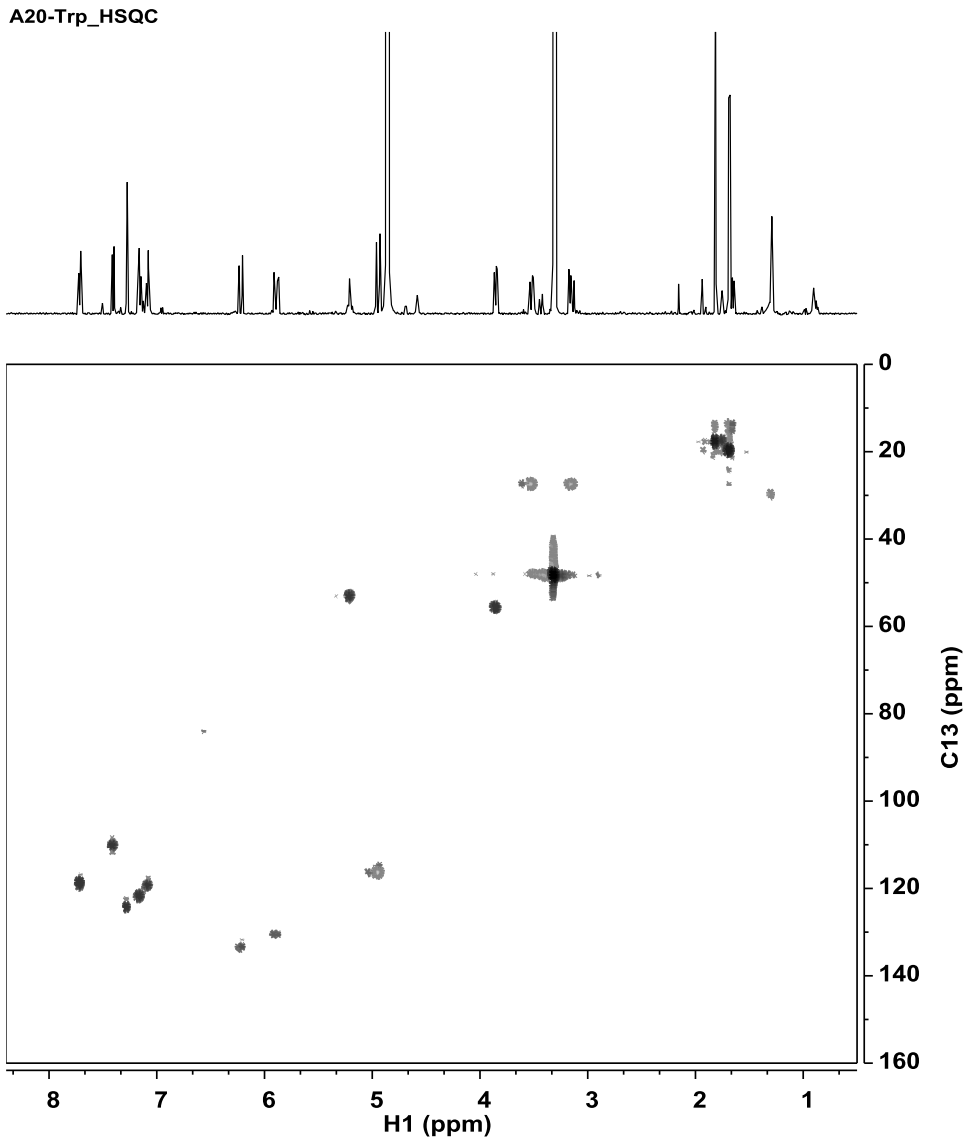
55



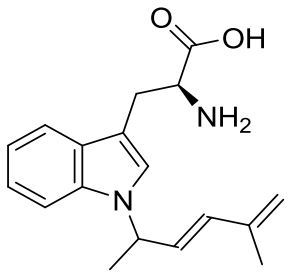
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **55** in MeOH-d4



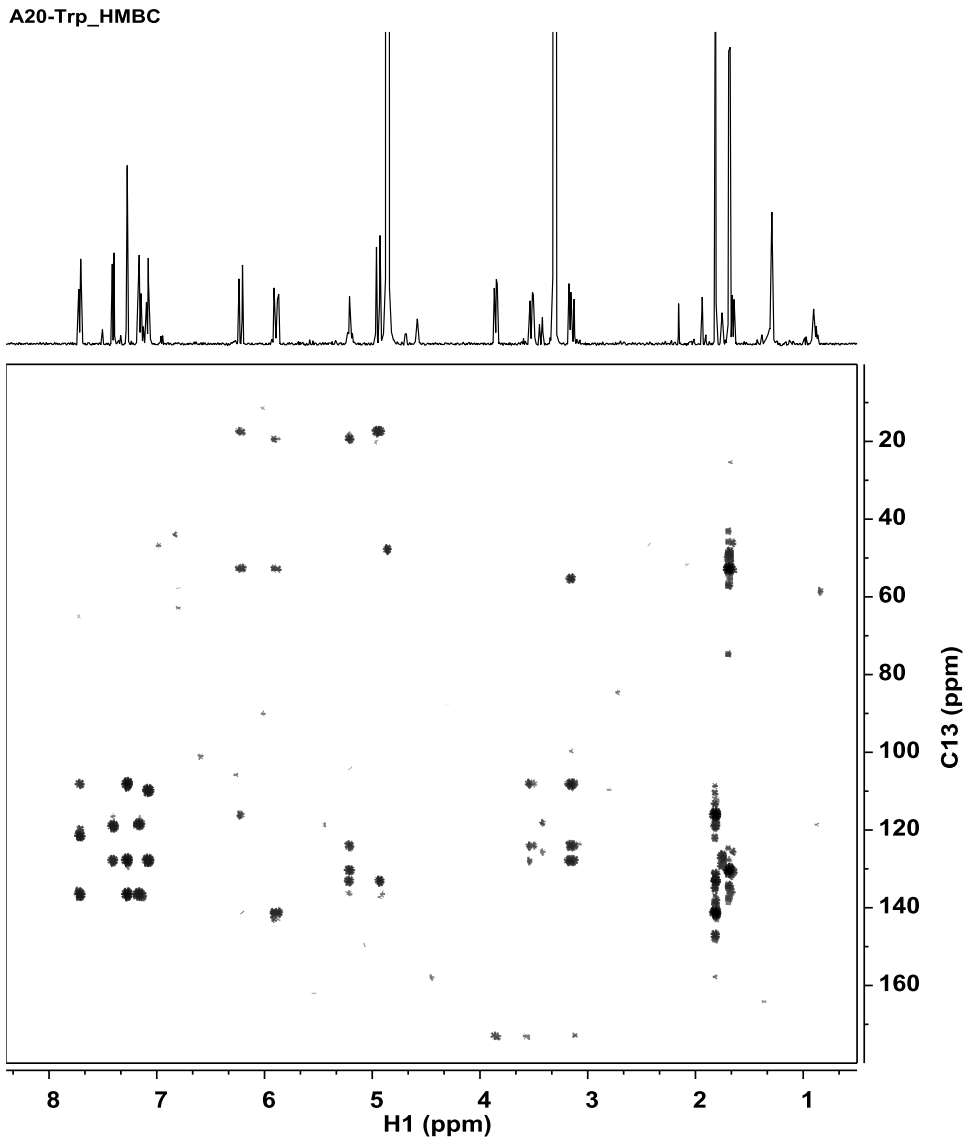
55



2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **55** in MeOH-d4

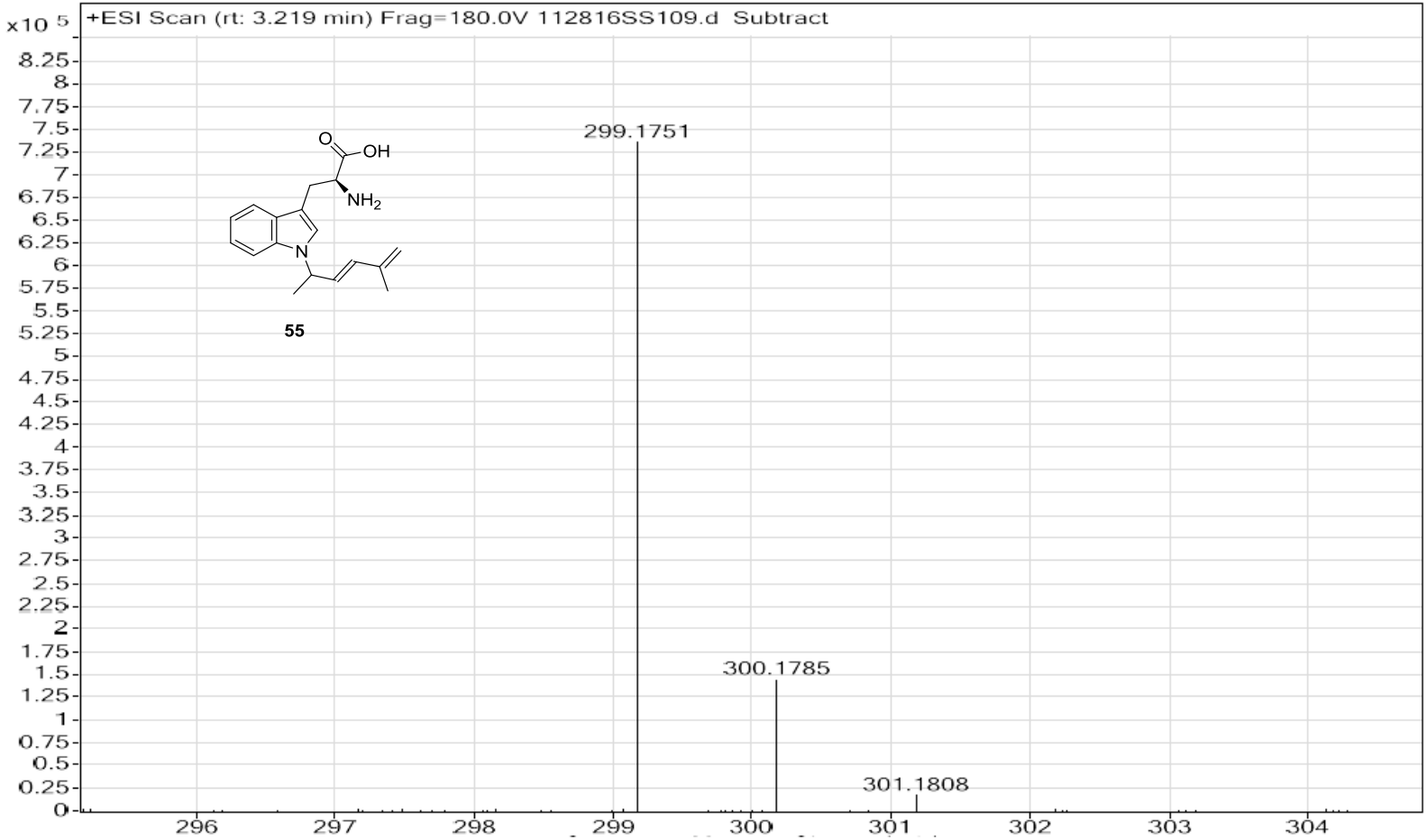


55



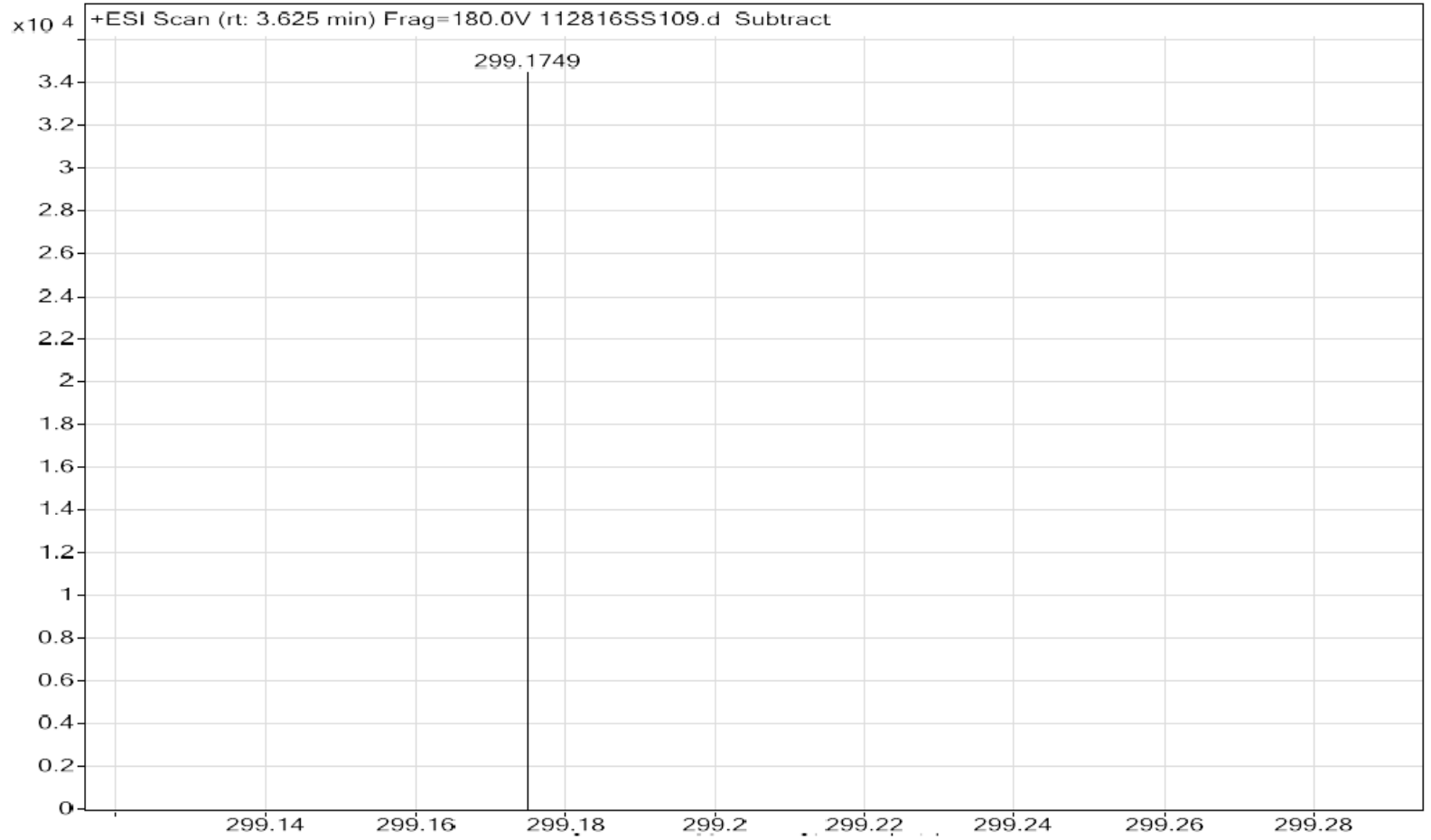
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **55** in MeOH-d4

Sample Name	TRP-35	**Position**	P1-D8	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS109.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 8:15:14 AM



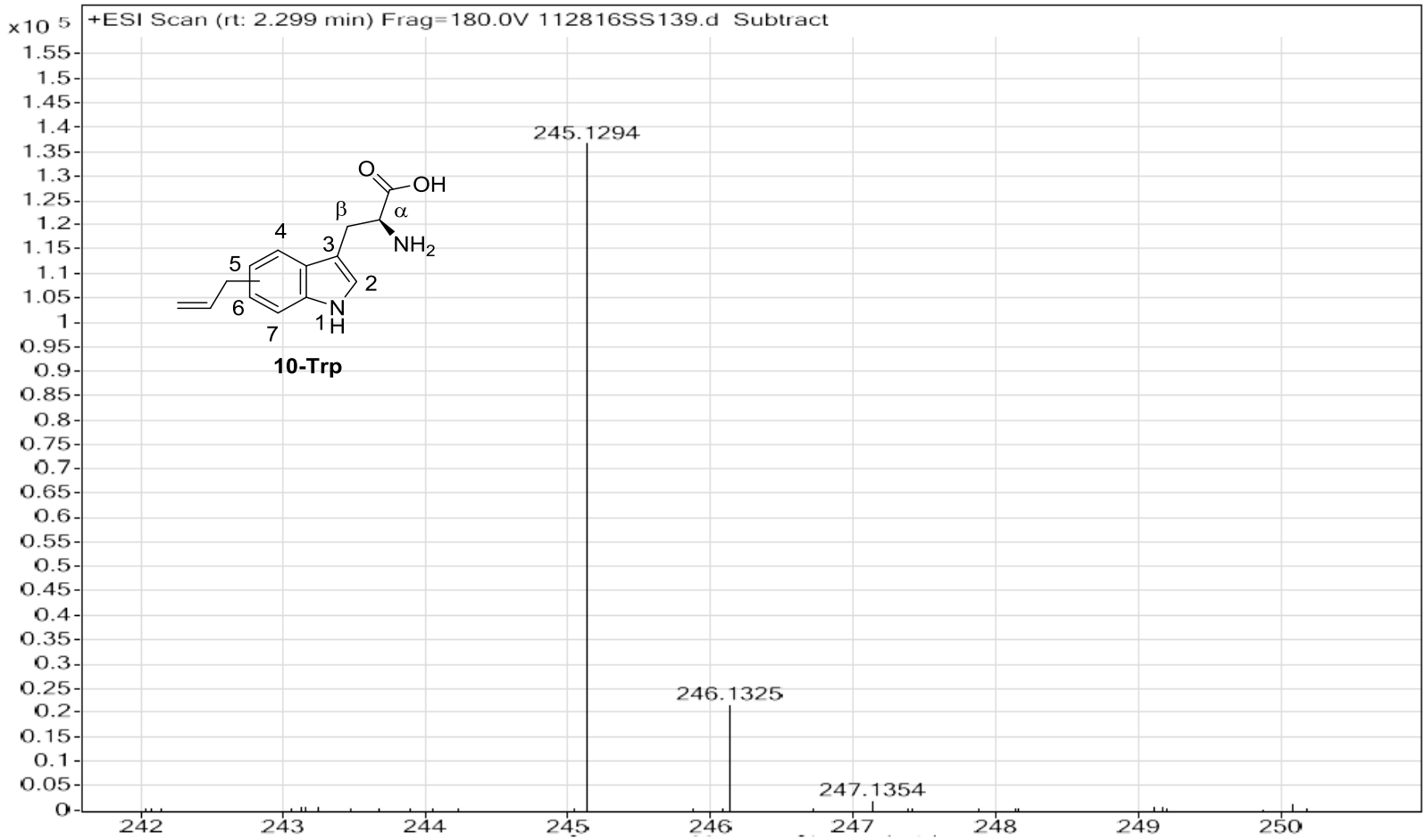
HRMS spectrum of **55**

Sample Name	TRP-35	**Position**	P1-D8	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS109.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 8:15:14 AM

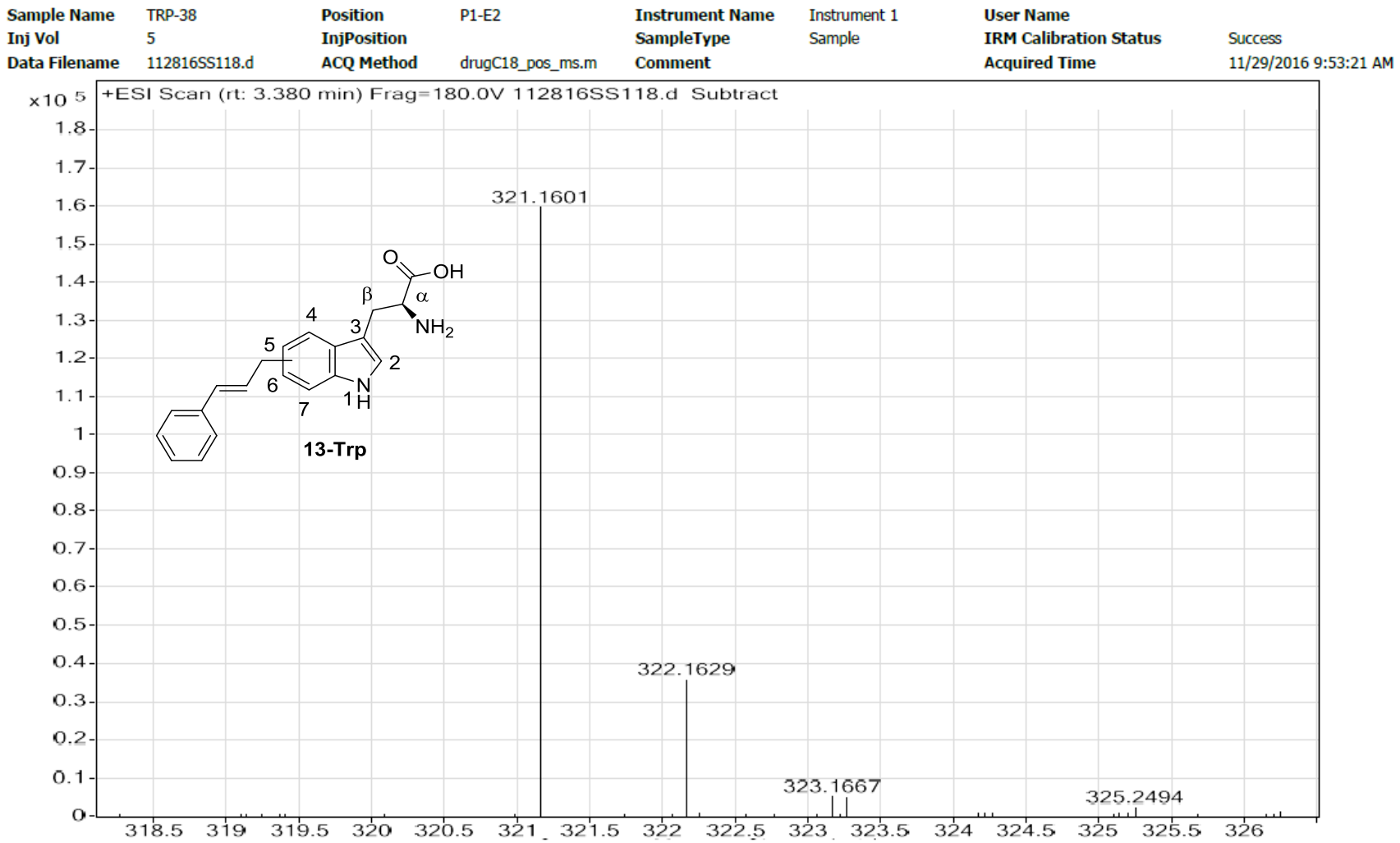


HRMS of 20-Trp$_2$

Sample Name	TRP-45	**Position**	P1-E9	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS139.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 1:42:16 PM

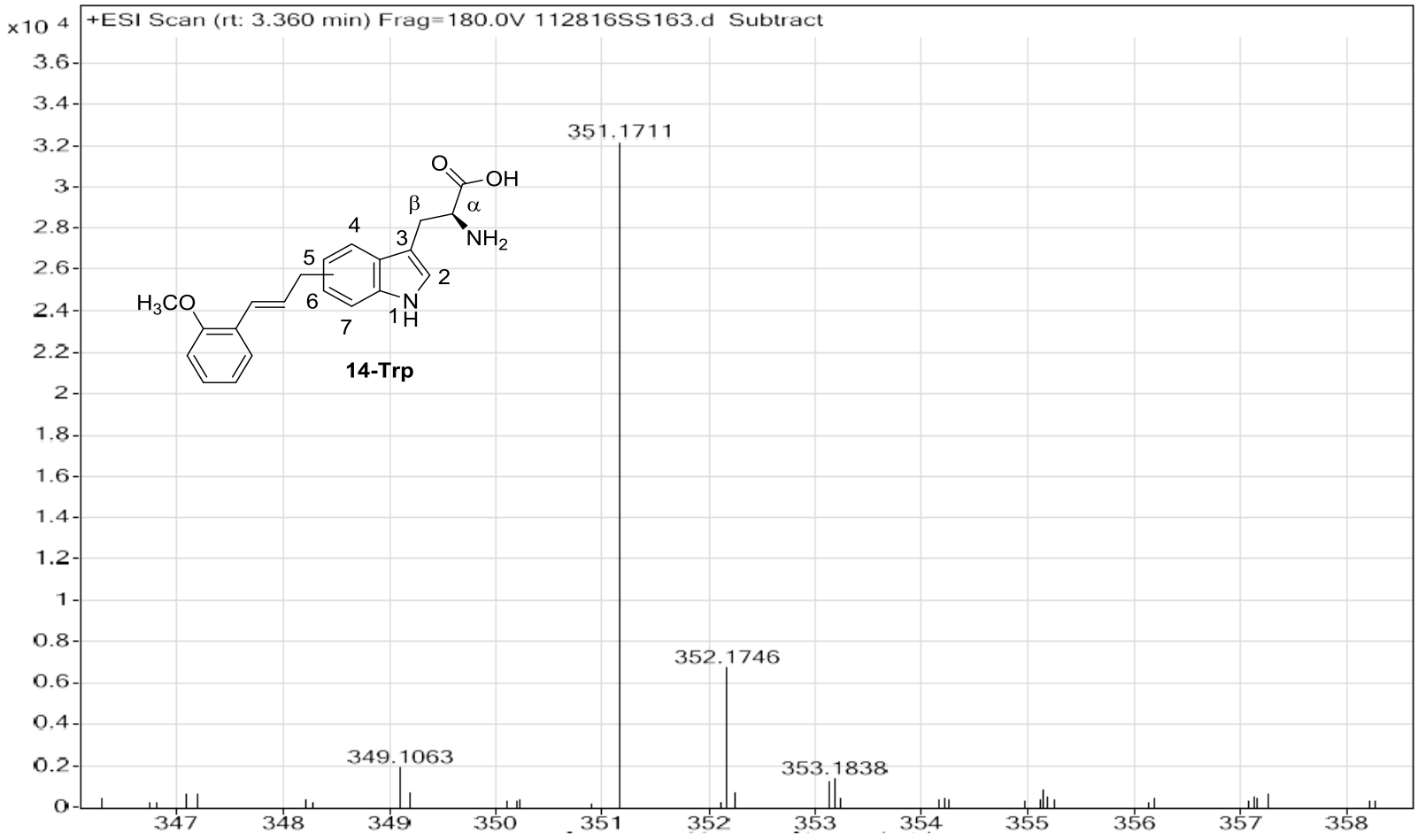


HRMS spectrum of **10-Trp**



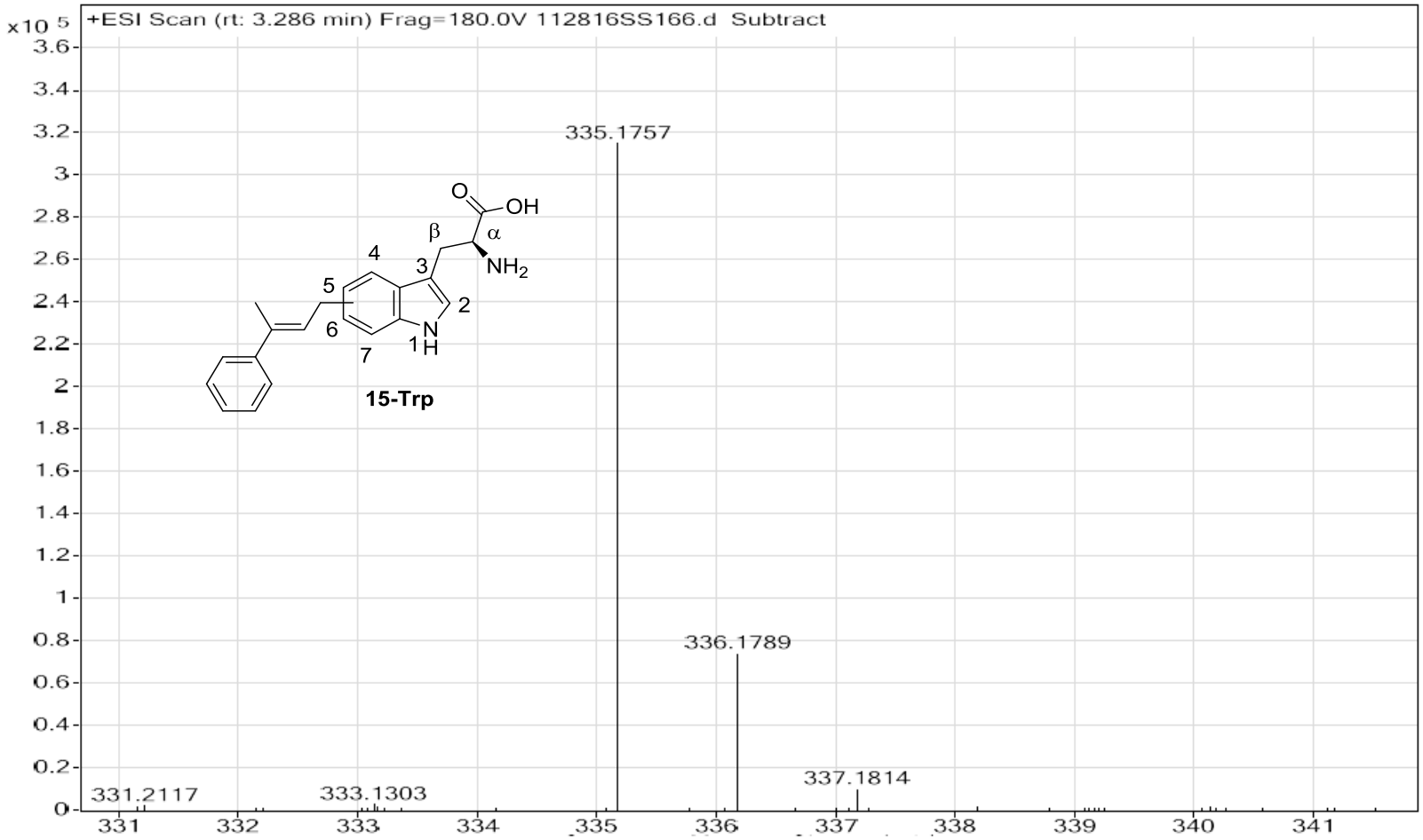
HRMS spectrum of **13-Trp**

Sample Name	TRP-53	**Position**	P1-F8	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS163.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 6:03:53 PM



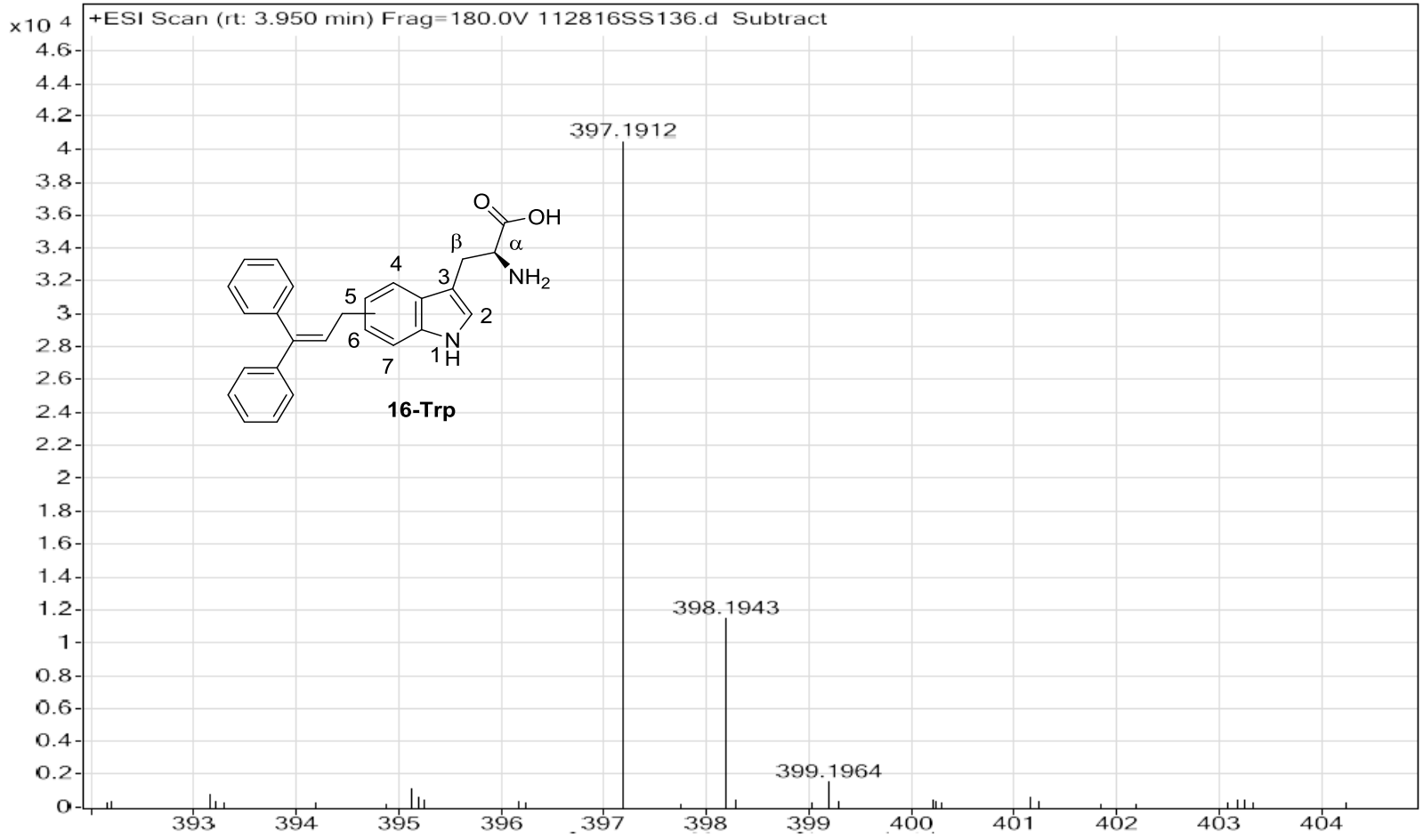
HRMS spectrum of **14-Trp**

Sample Name	TRP-54	**Position**	P1-F9	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS166.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 6:36:34 PM



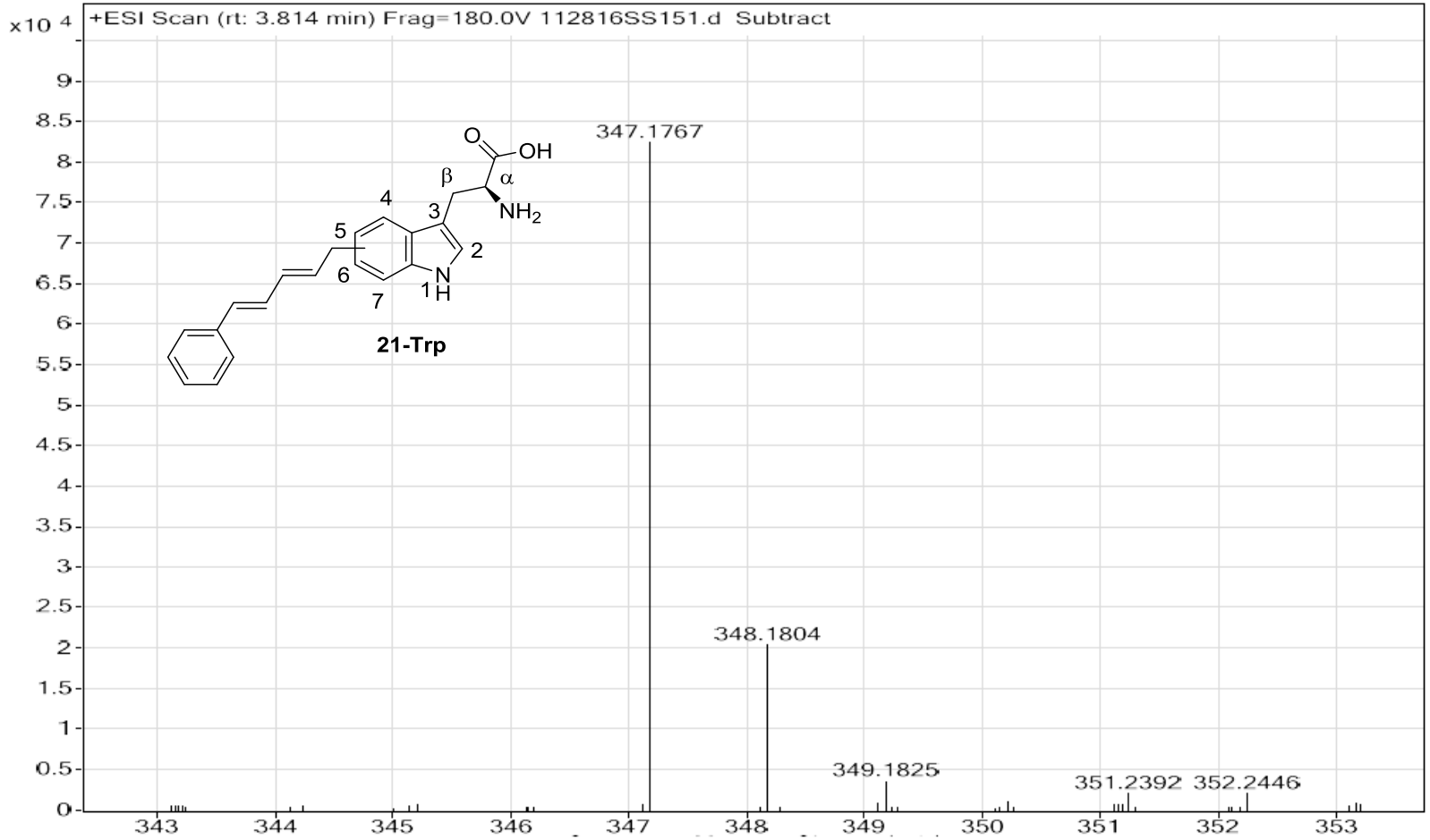
HRMS spectrum of **15-Trp**

Sample Name	TRP-44	**Position**	P1-E8	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS136.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 1:09:33 PM

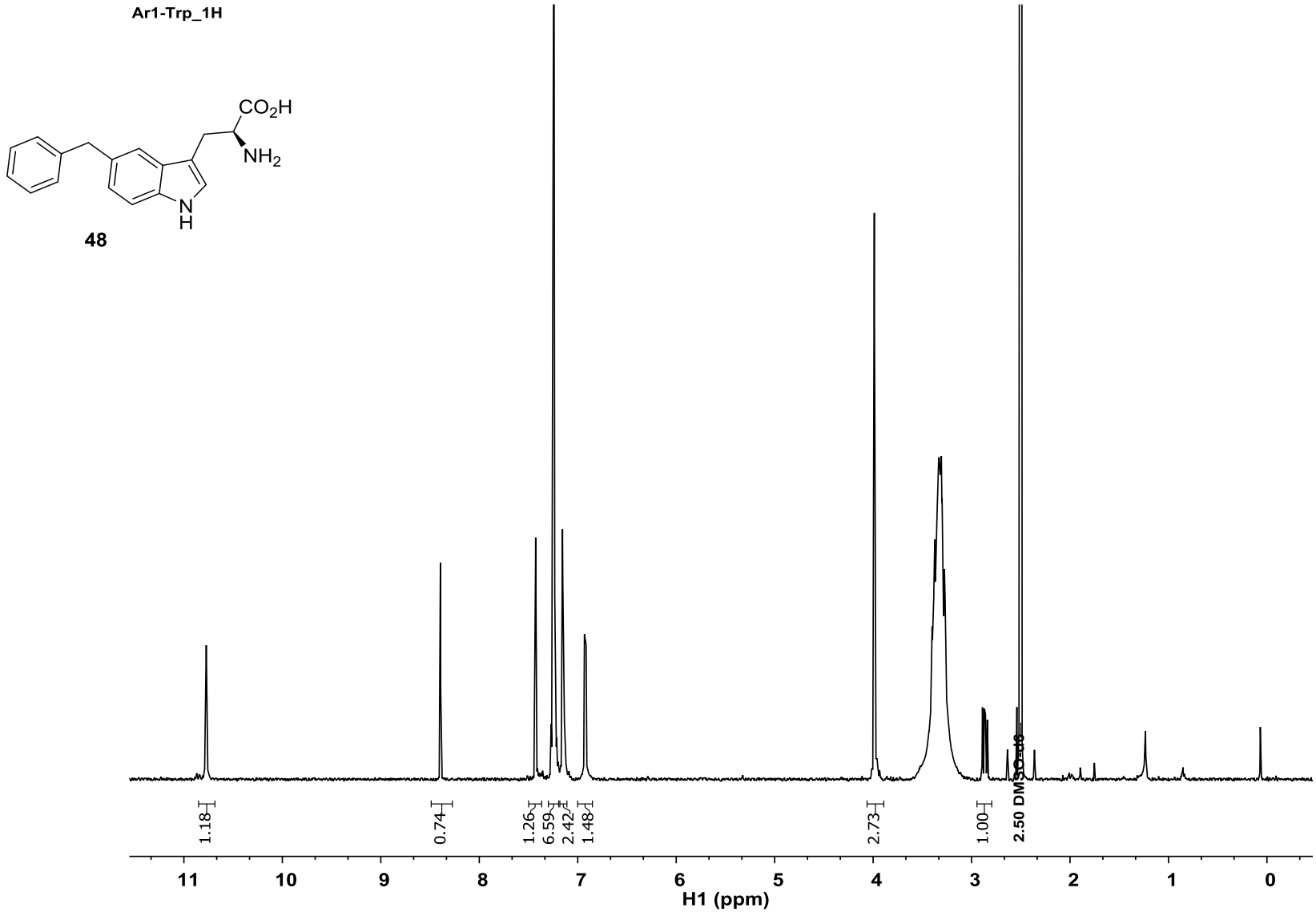


HRMS spectrum of **16-Trp**

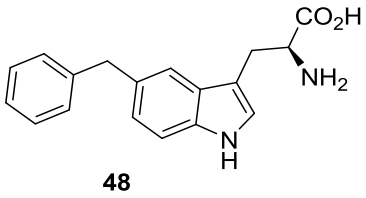
Sample Name	TRP-49	**Position**	P1-F4	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	112816SS151.d	**ACQ Method**	drugC18_pos_ms.m	**Comment**		**Acquired Time**	11/29/2016 3:53:00 PM



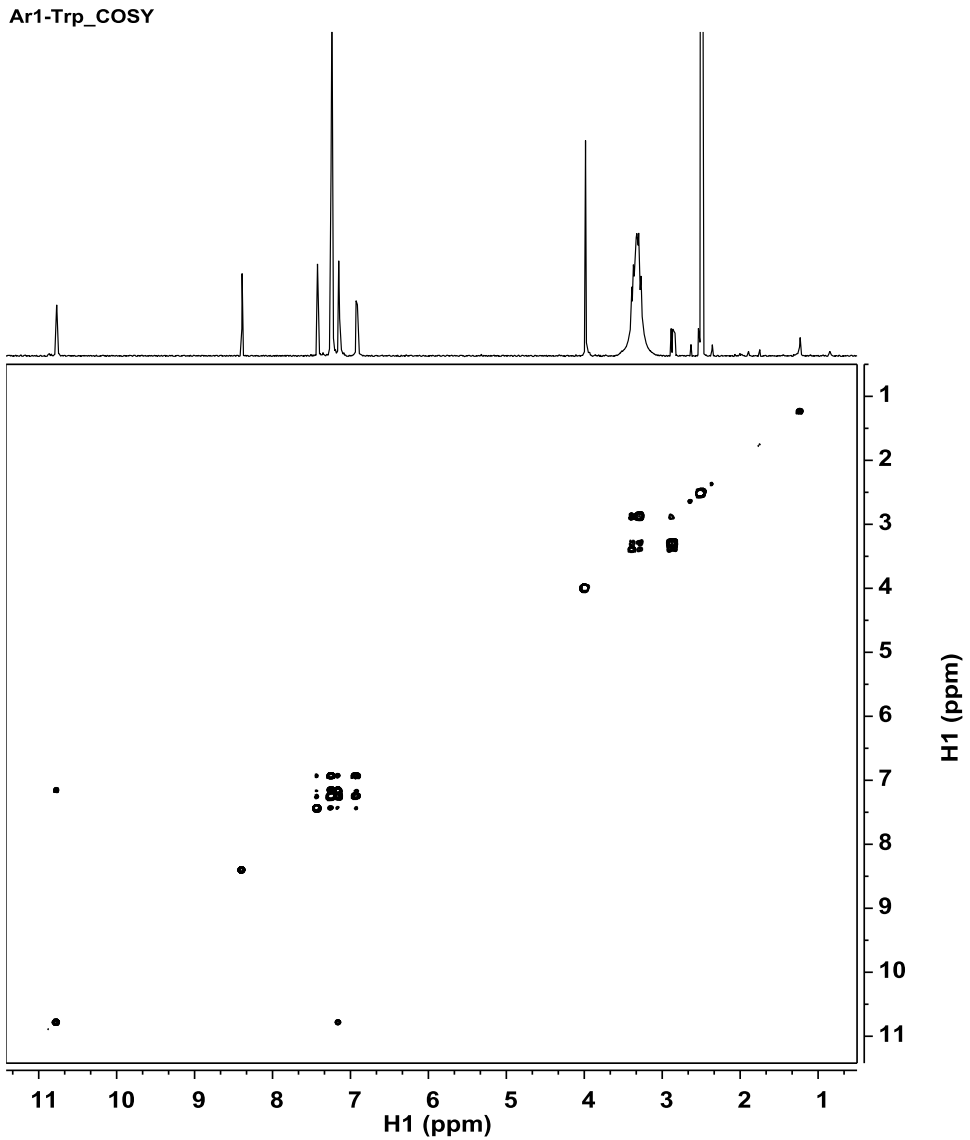
HRMS spectrum of **21-Trp**



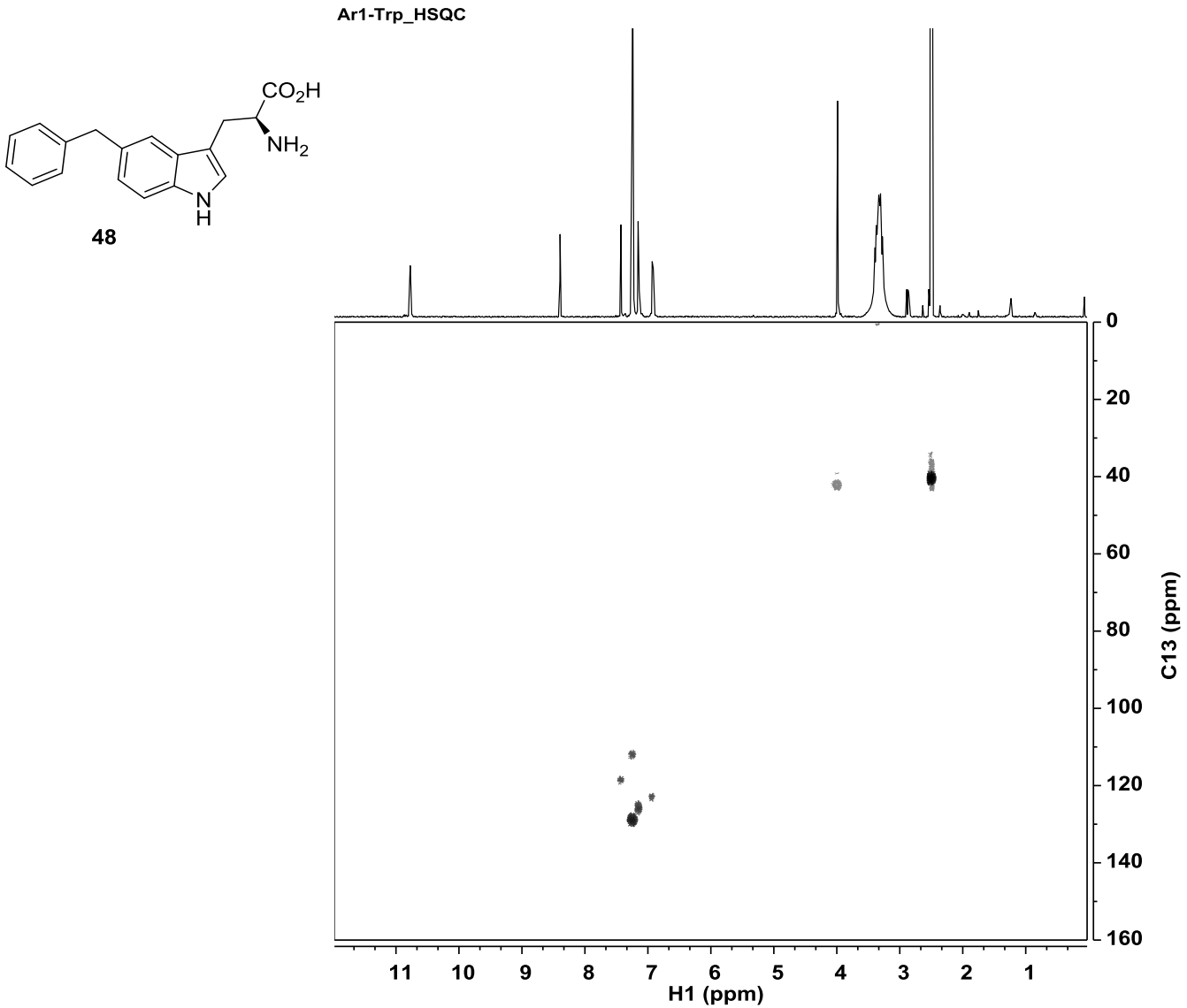
1D ^{1}H-NMR spectrum (500 MHz) of **48** in DMSO-d6



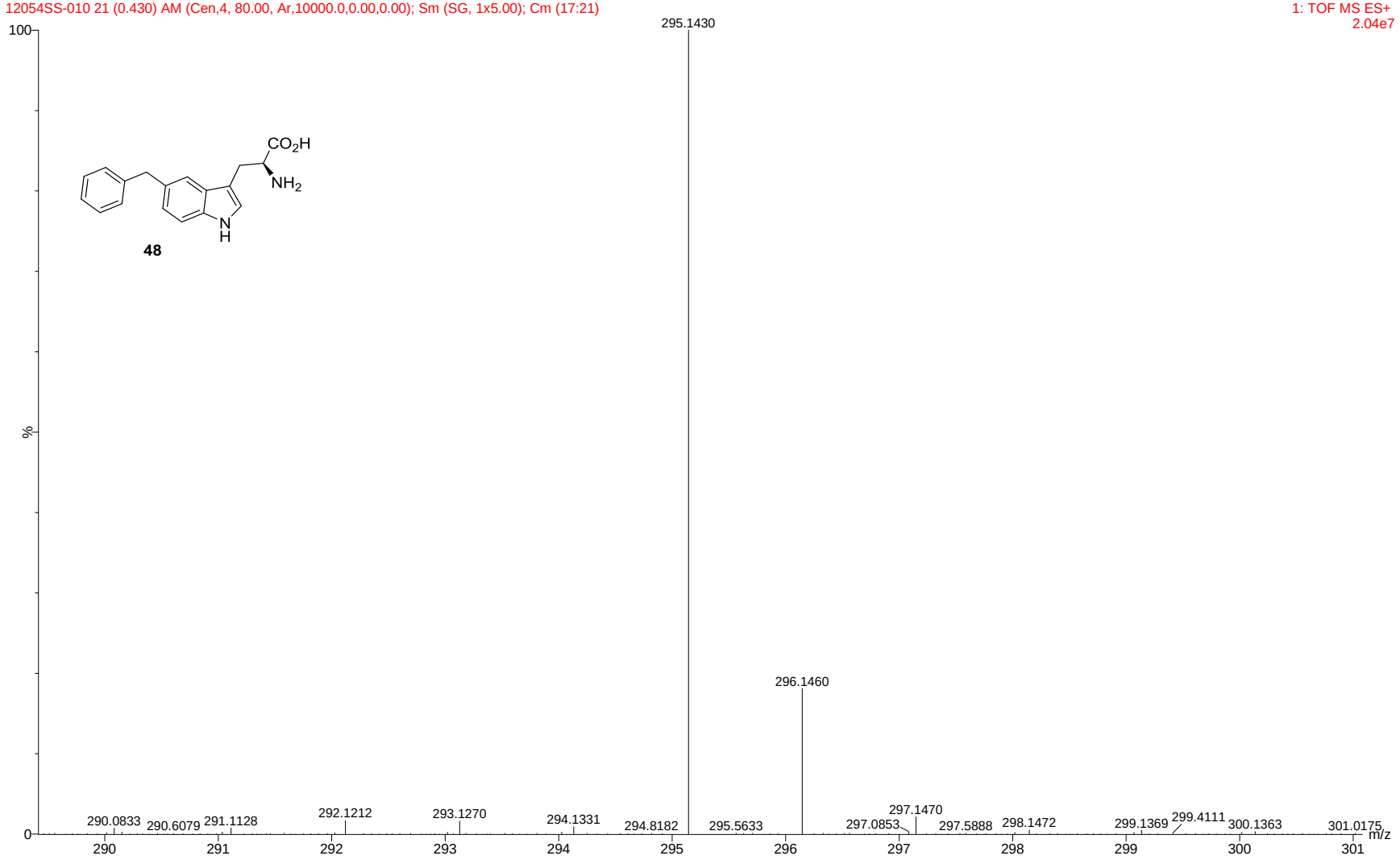
48



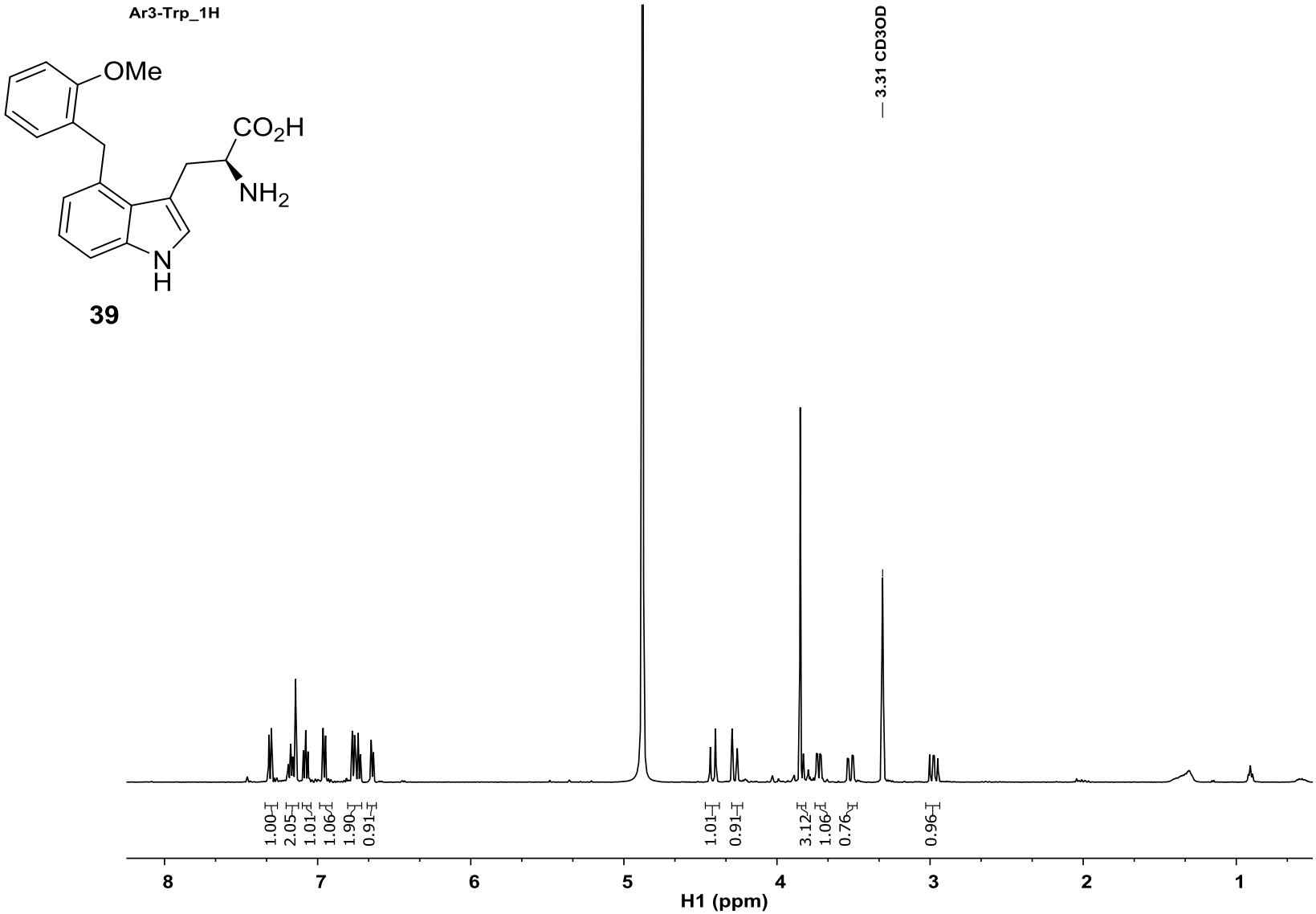
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **48** in DMSO-d6



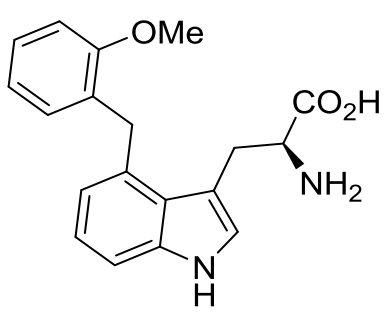
2D ^{1}H-^{13}C COSY NMR spectrum (500 MHz) of **48** in DMSO-d6



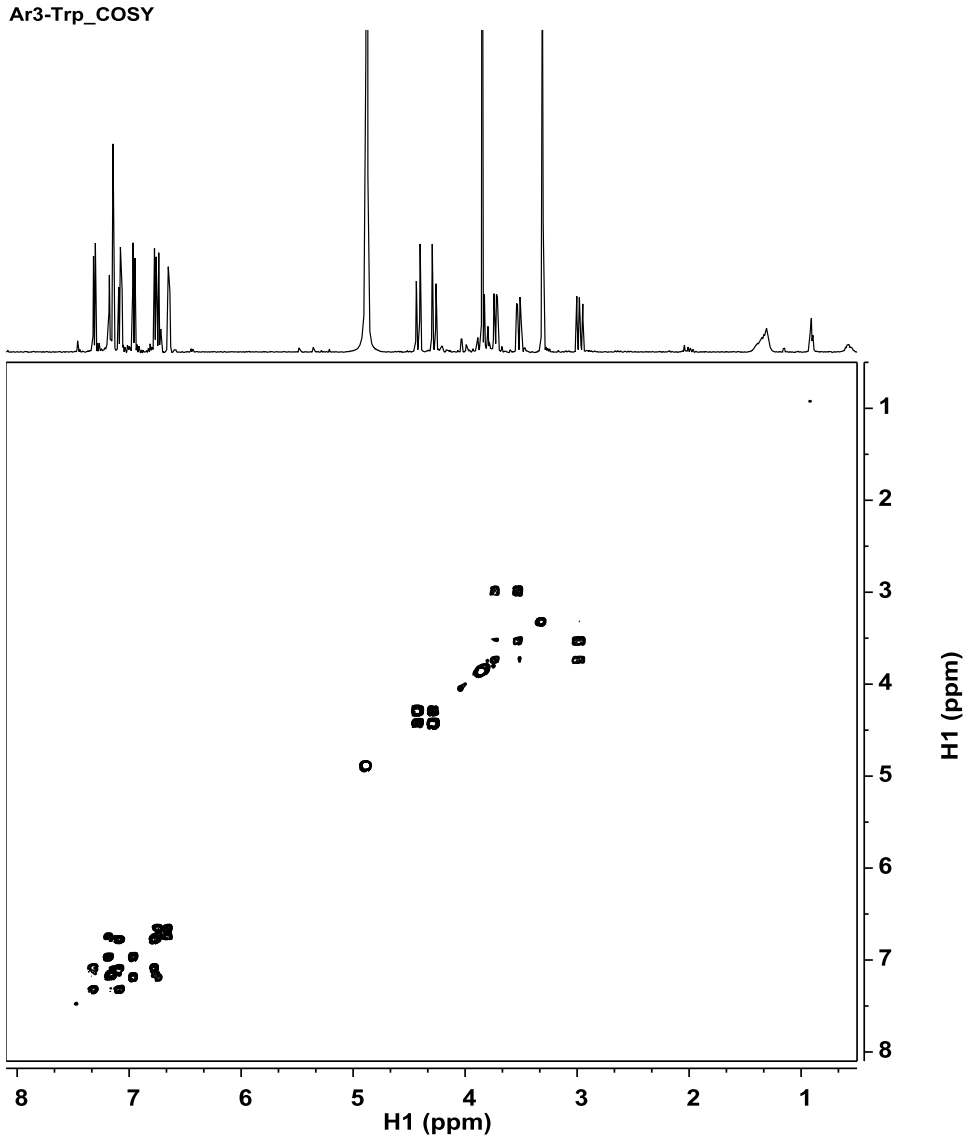
HRMS spectrum of **48**



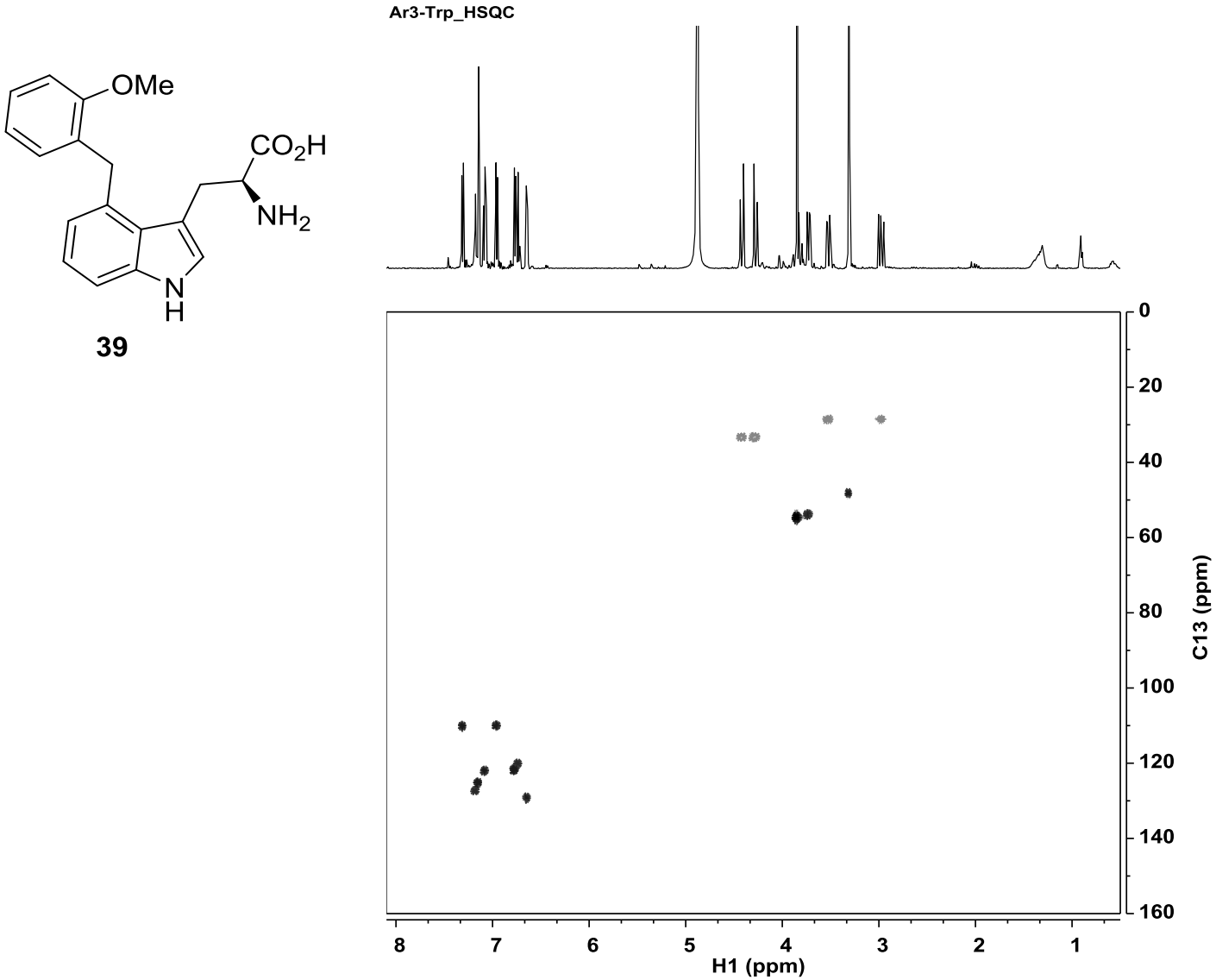
1D ^{1}H-NMR spectrum (500 MHz) of **39** in MeOH-d_4



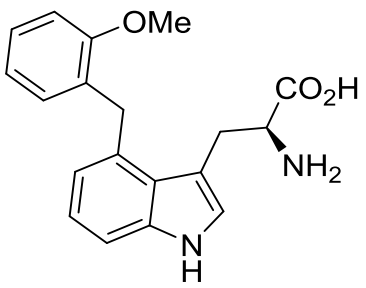
39



2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **39** in MeOH-d4



2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **39** in MeOH-d4



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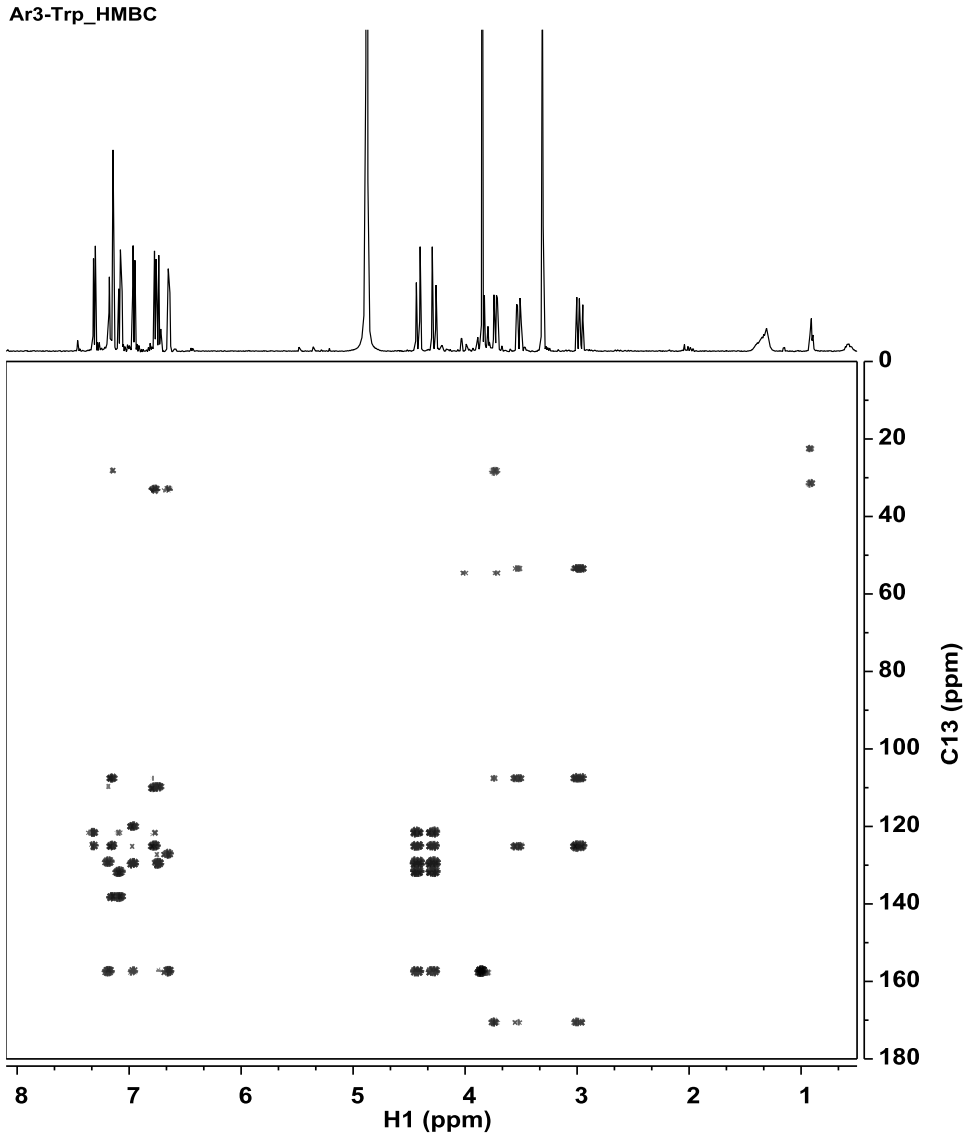
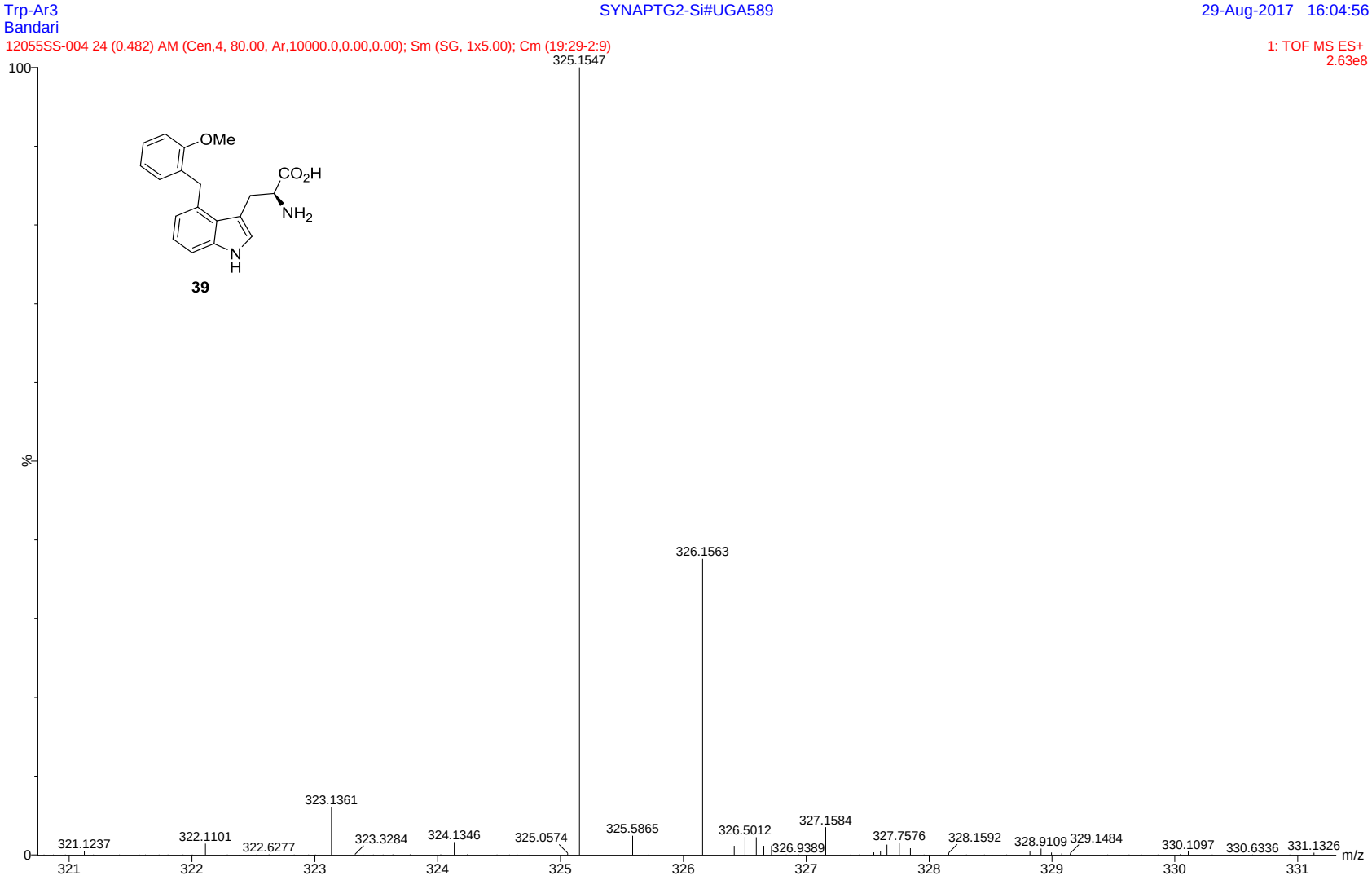
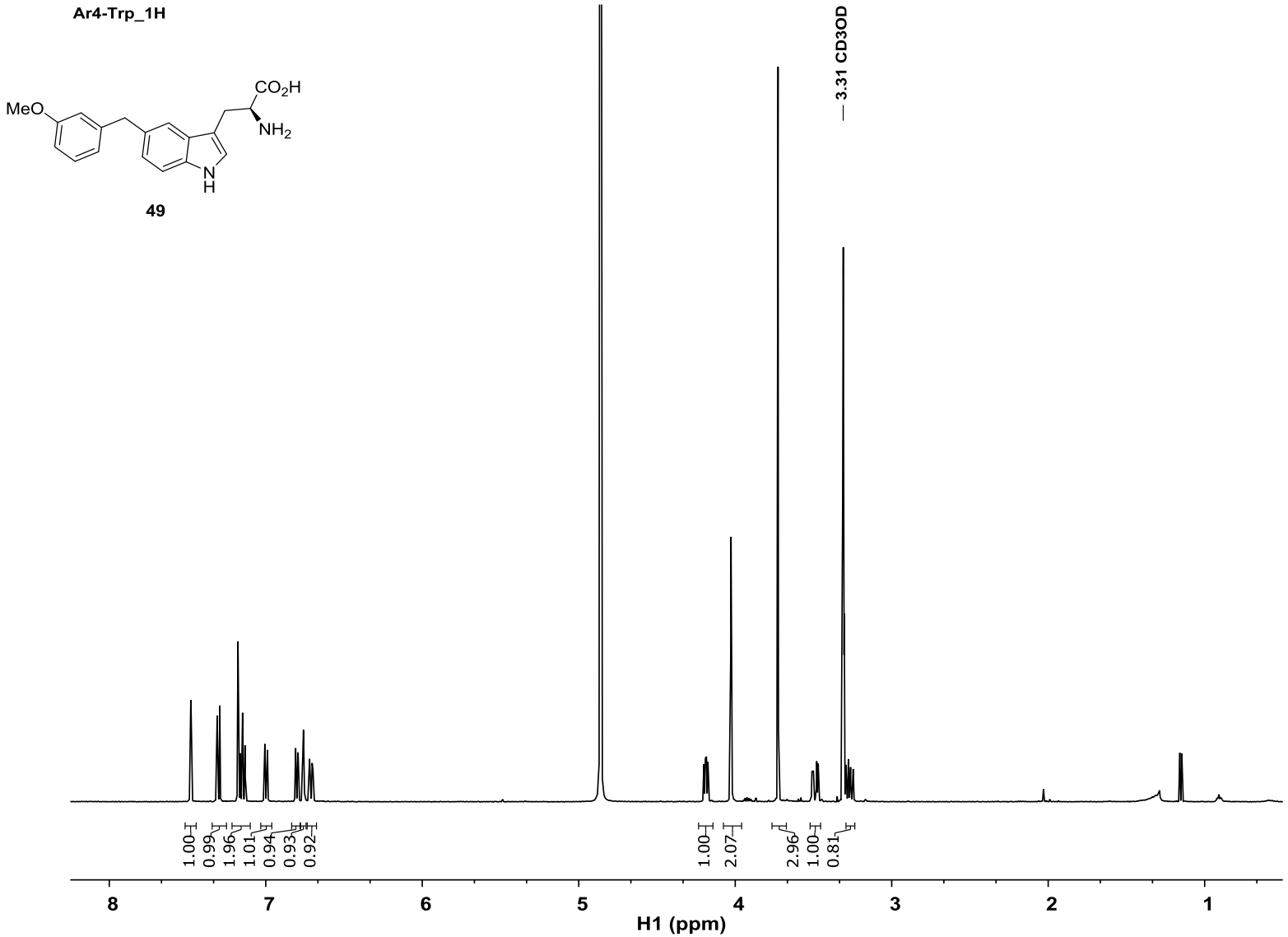


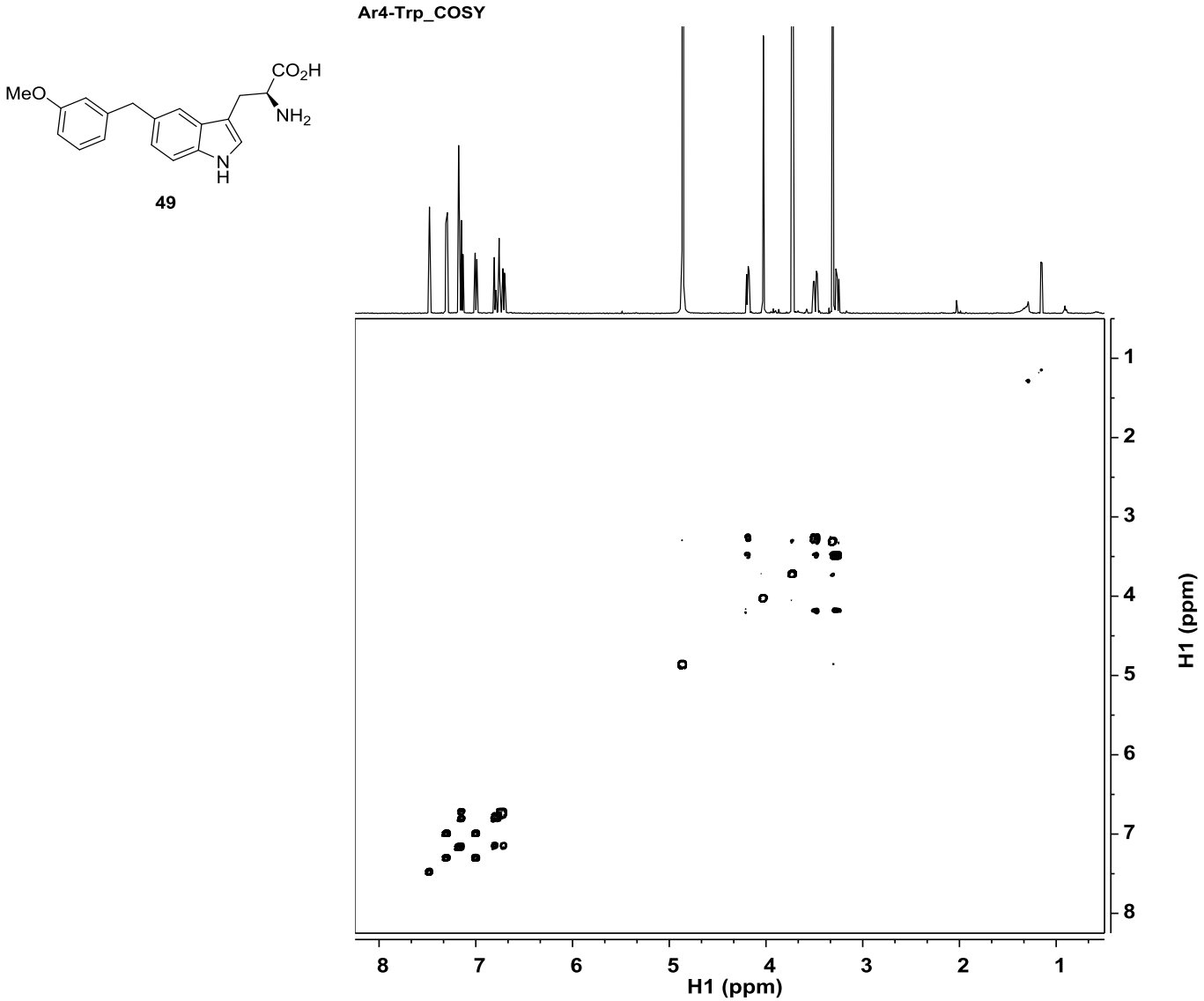
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **39** in MeOH-d4



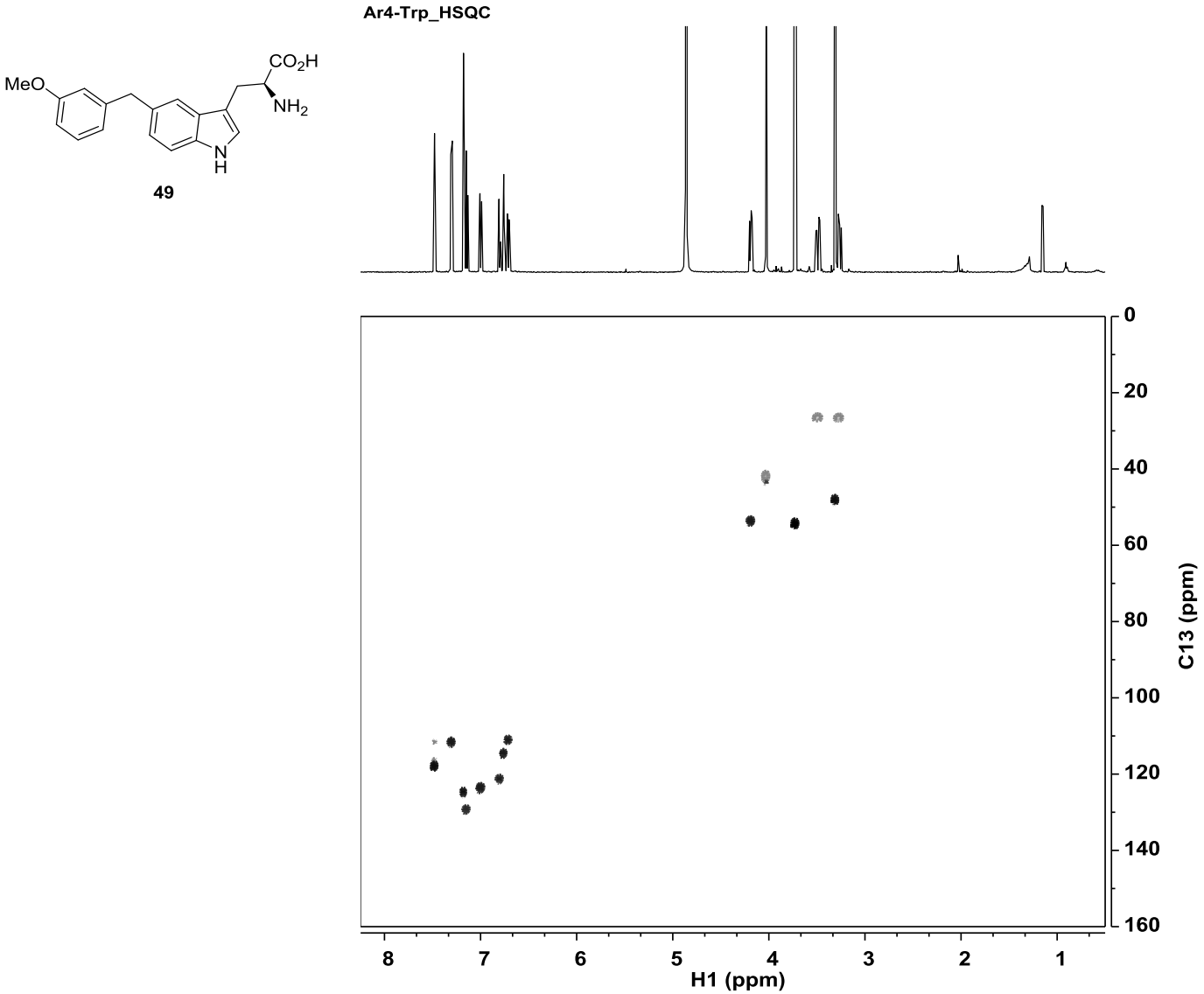
HRMS spectrum of **39**



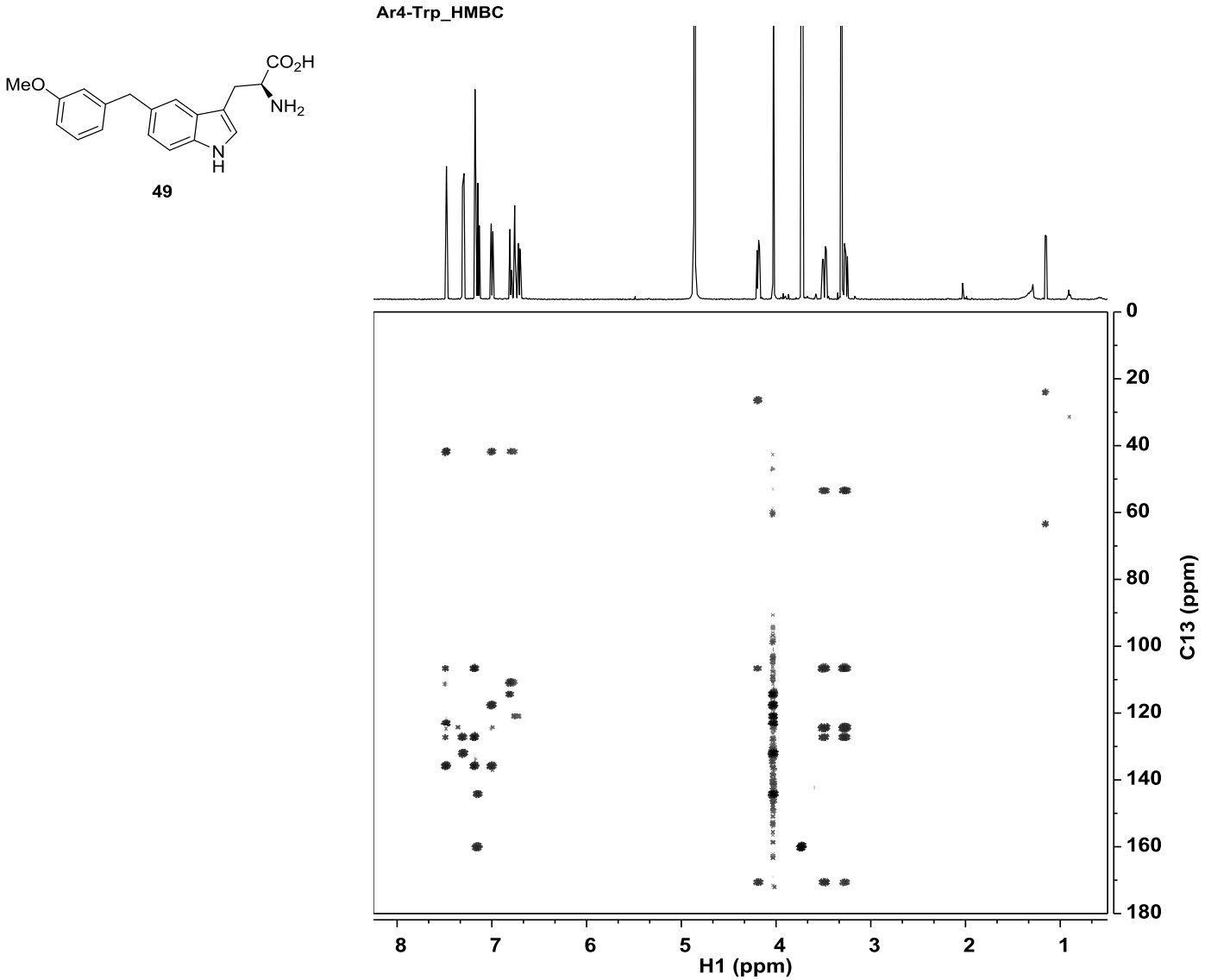
1D ^{1}H-NMR spectrum (500 MHz) of **49** in MeOH-d4



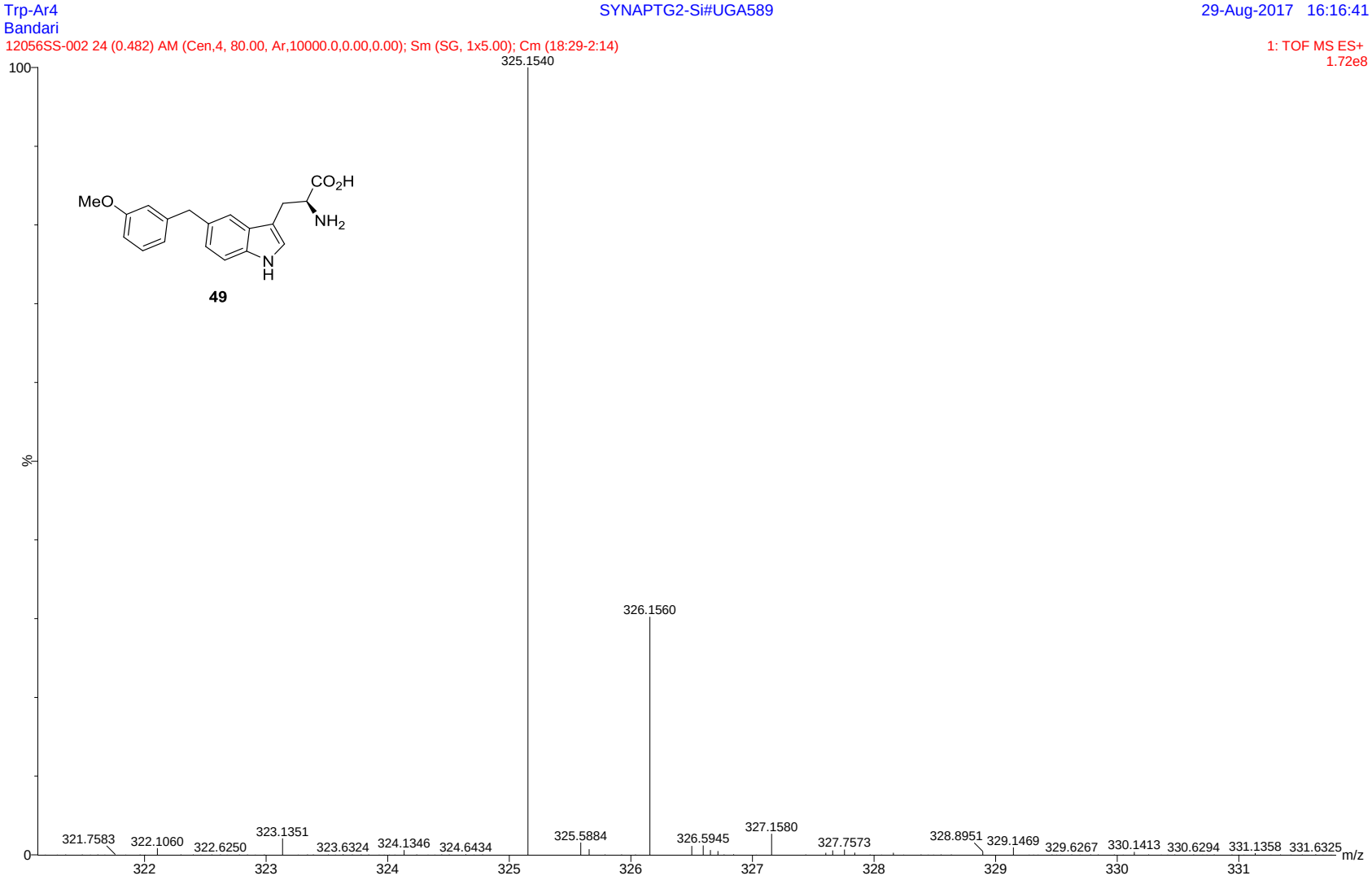
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **49** in MeOH-d4



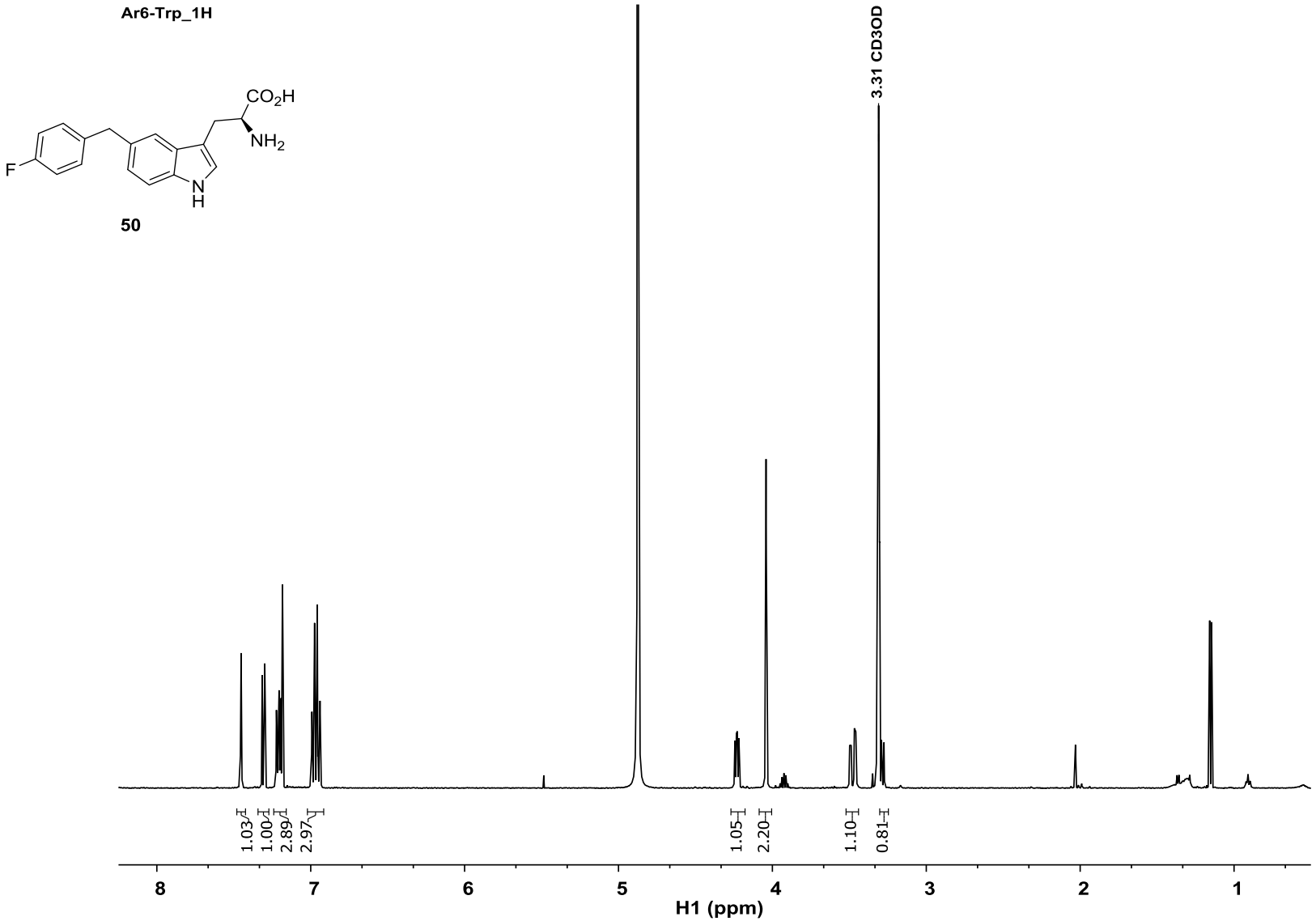
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **49** in MeOH-d4



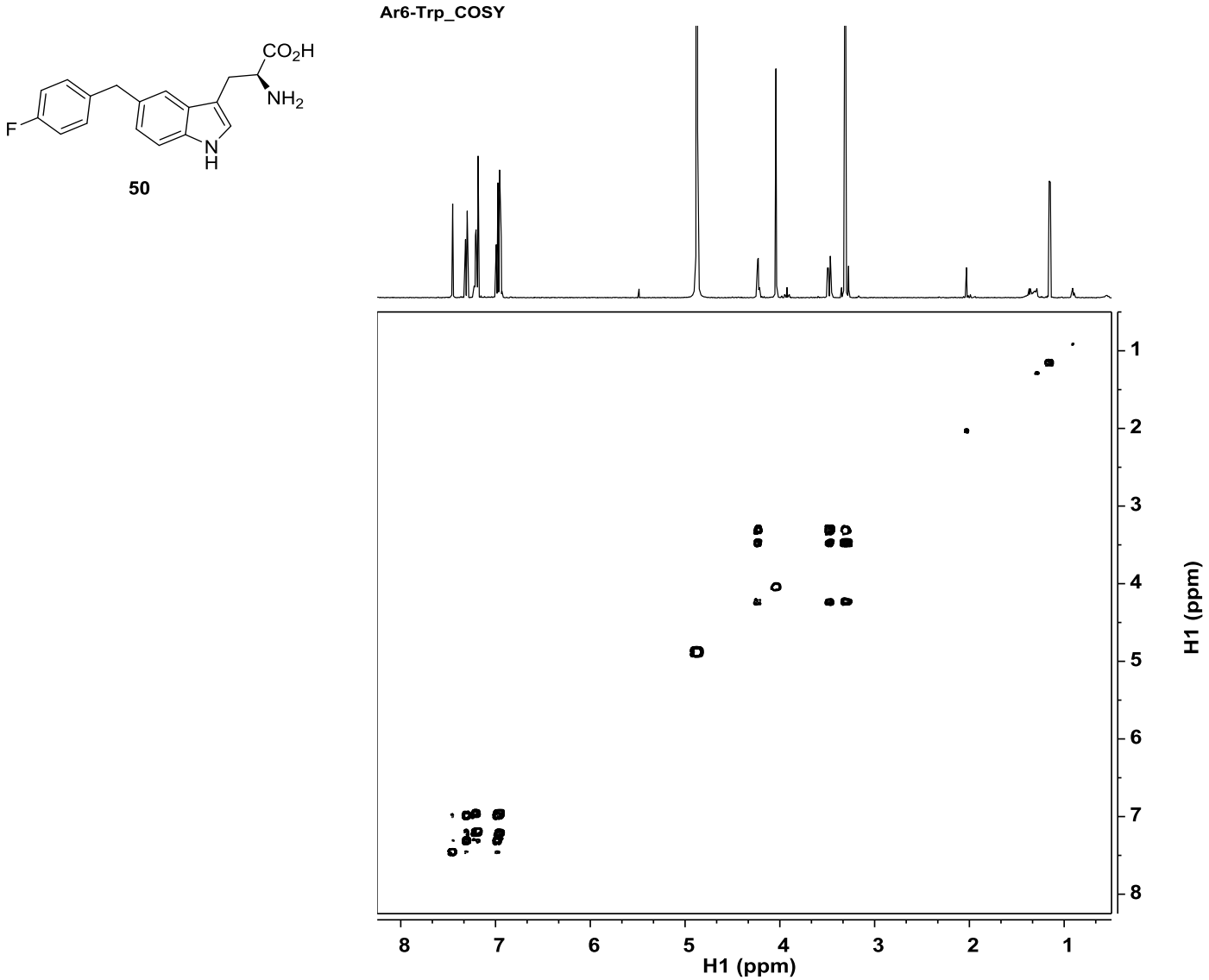
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **49** in MeOH-d4



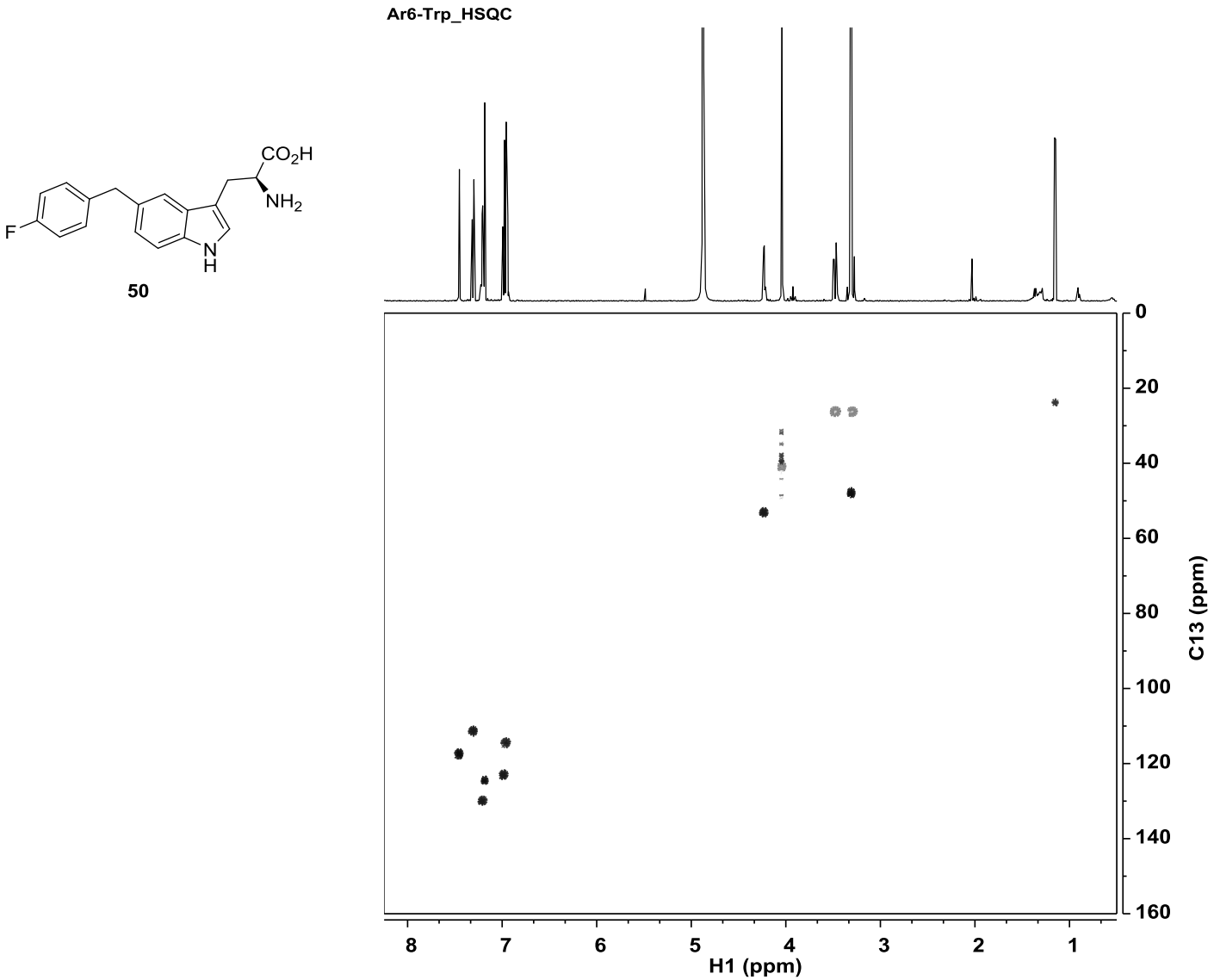
HRMS spectrum of **49**



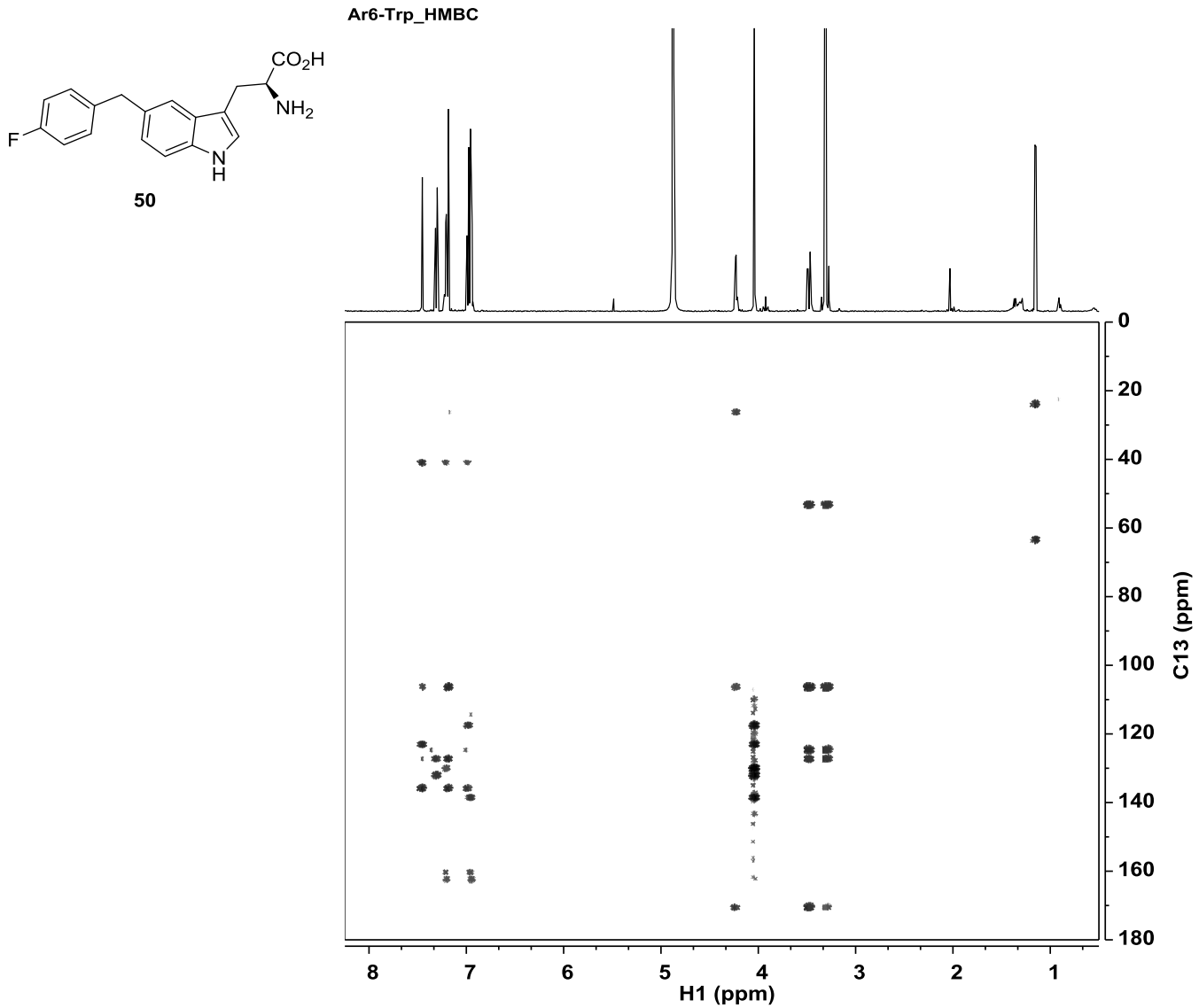
1D ^{1}H-NMR spectrum (500 MHz) of **50** in MeOH-d_4



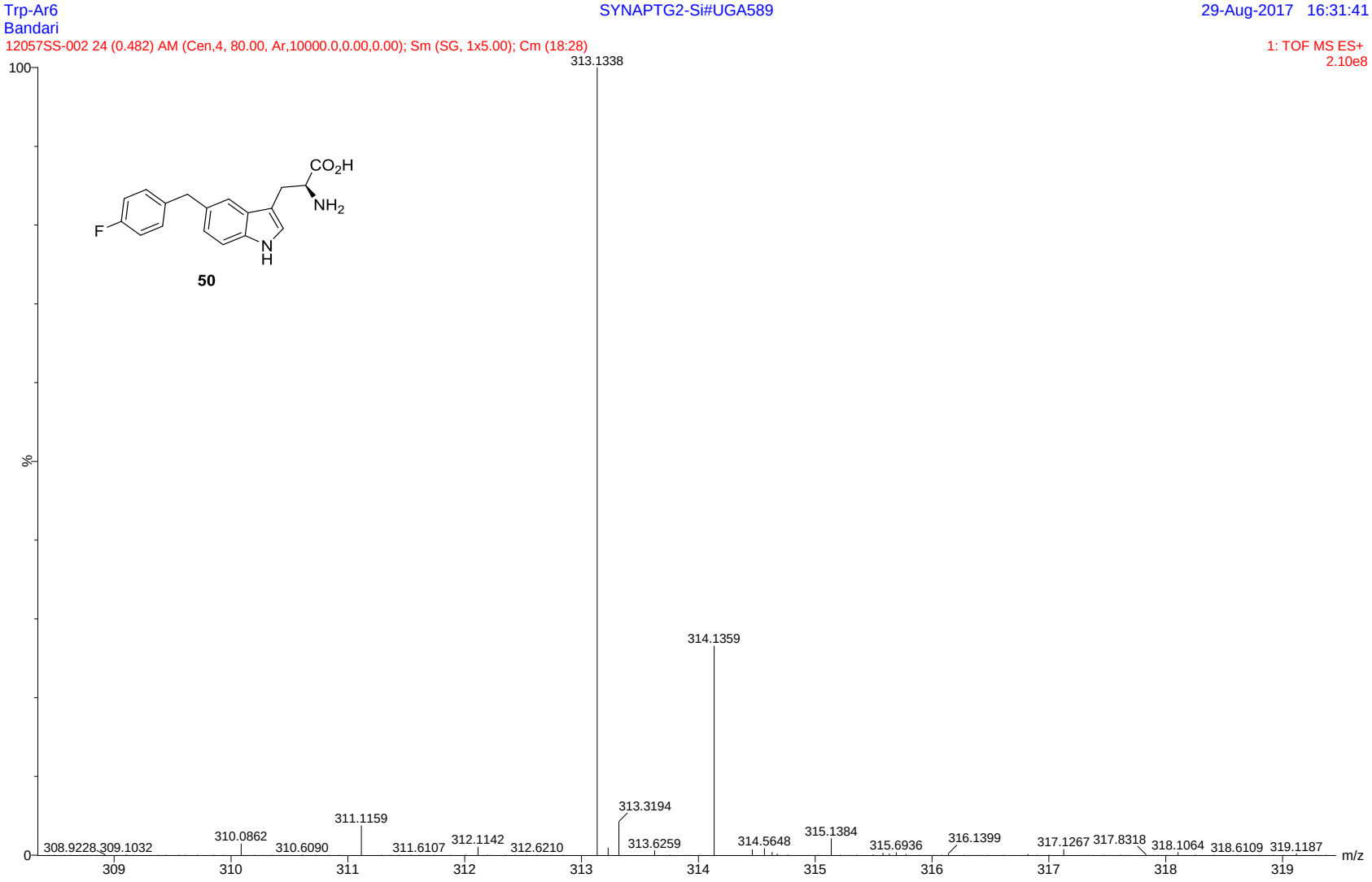
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **50** in MeOH-d4



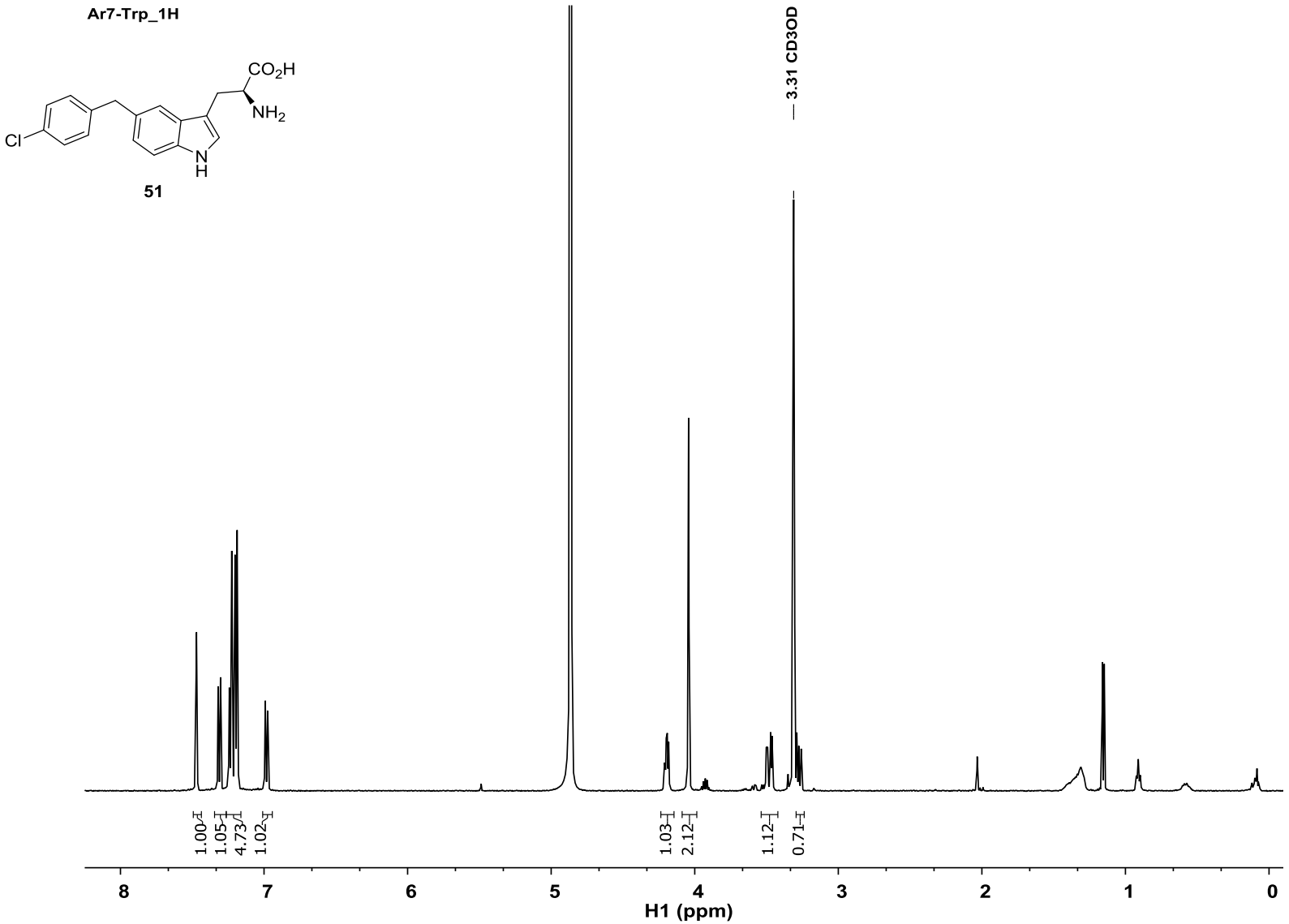
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **50** in MeOH-d4



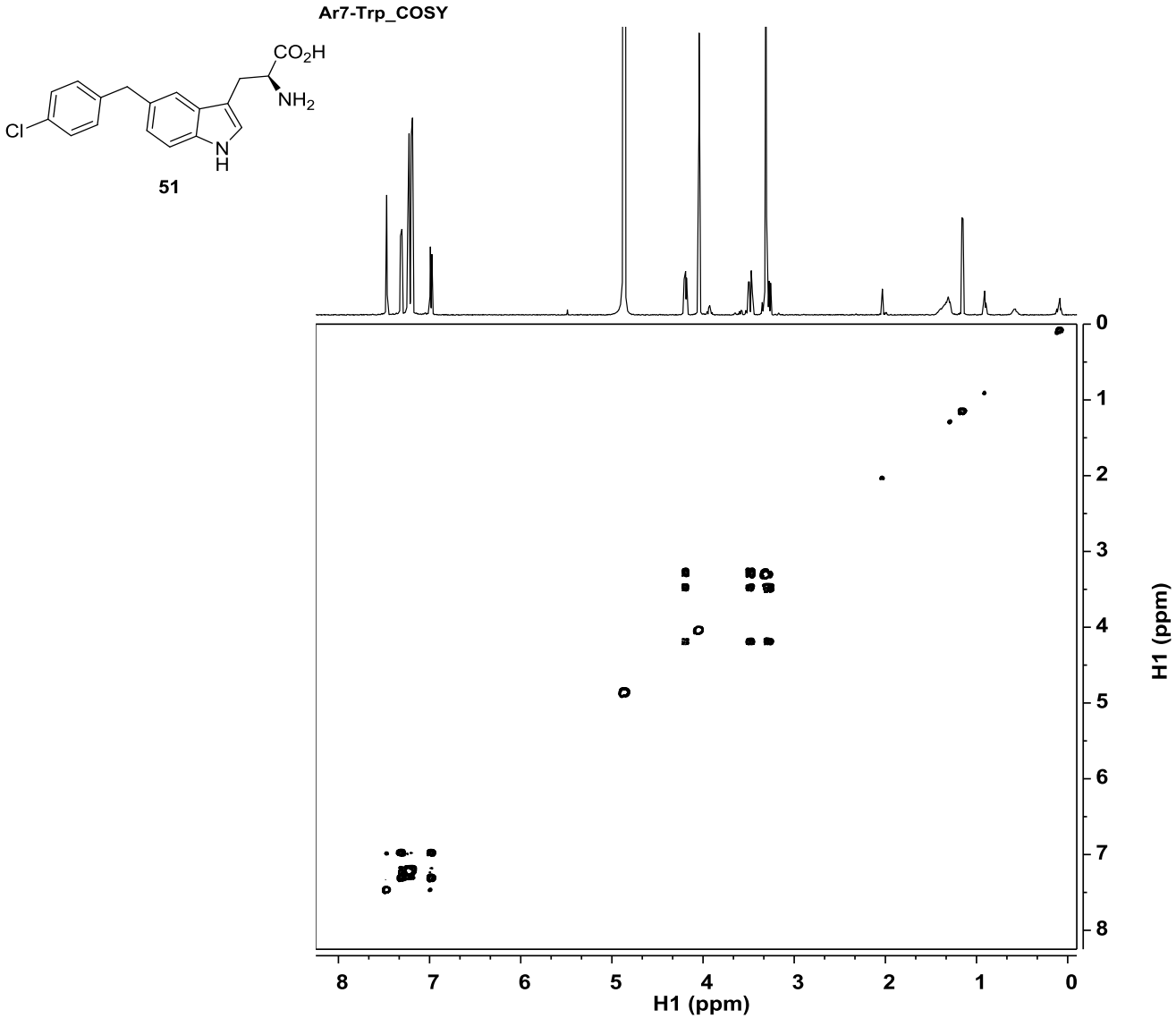
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **50** in MeOH-d4



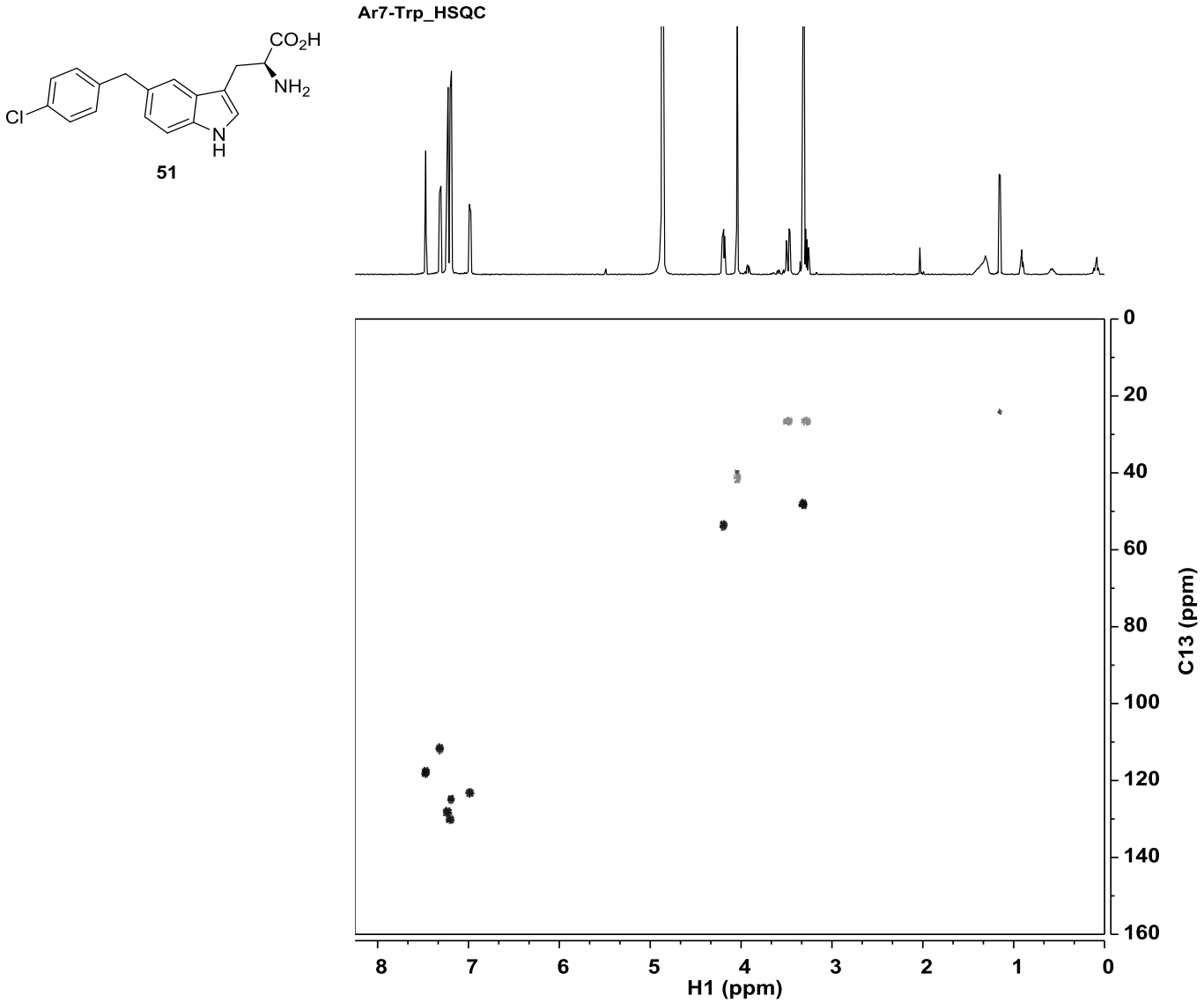
HRMS spectrum of **50**



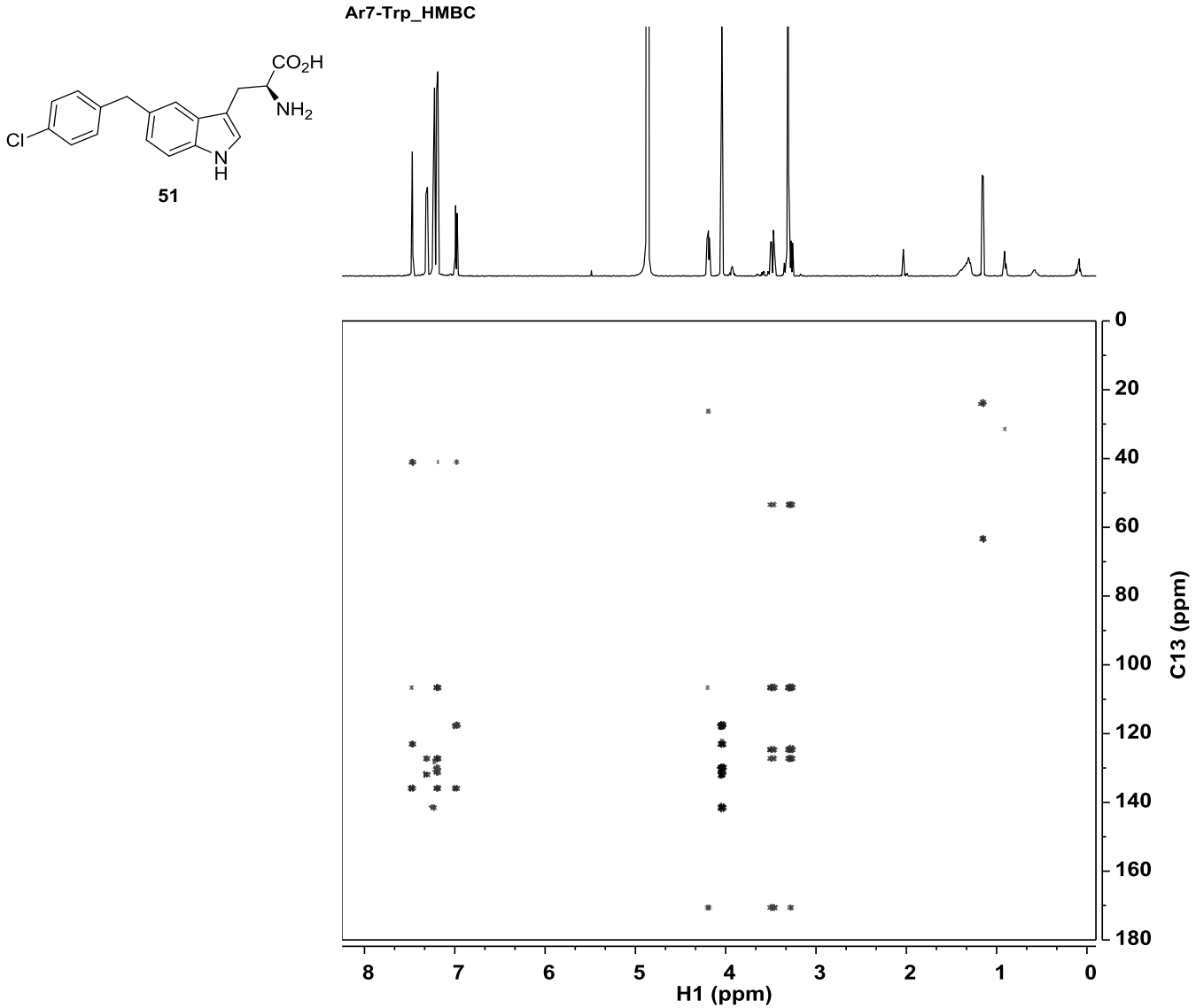
1D ^{1}H-NMR spectrum (500 MHz) of **51** in MeOH-d4



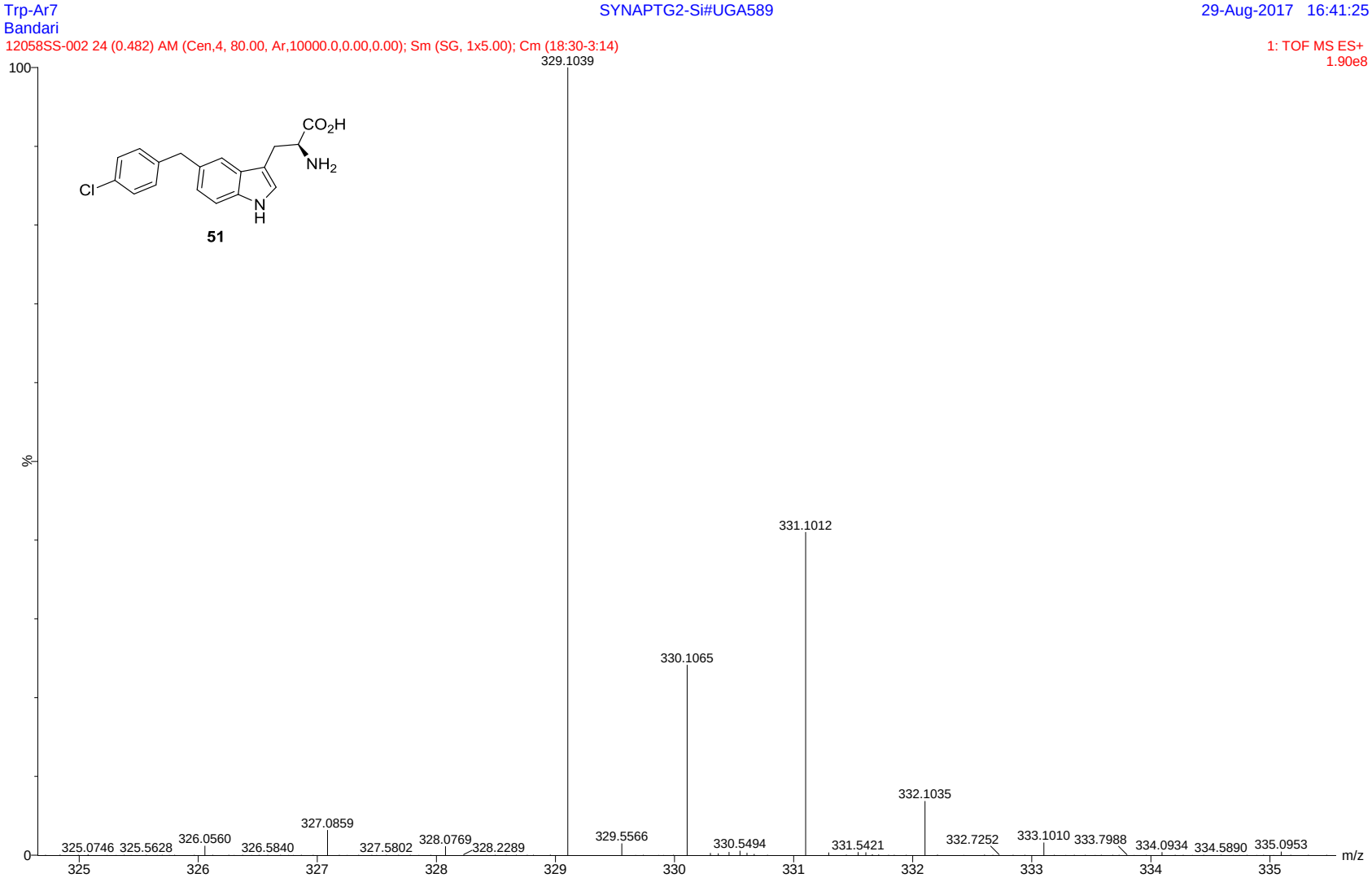
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **51** in MeOH-d4



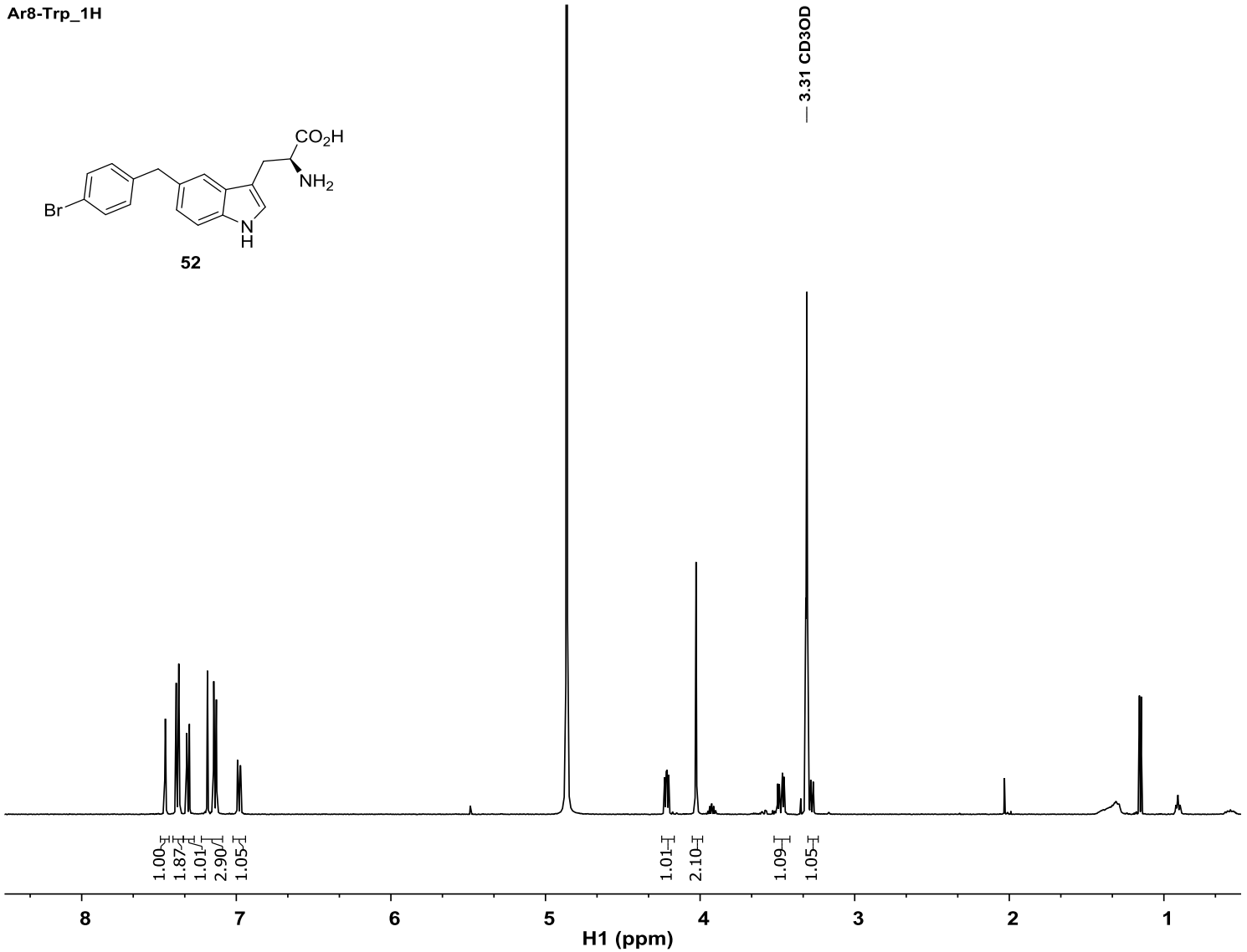
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **51** in MeOH-d4



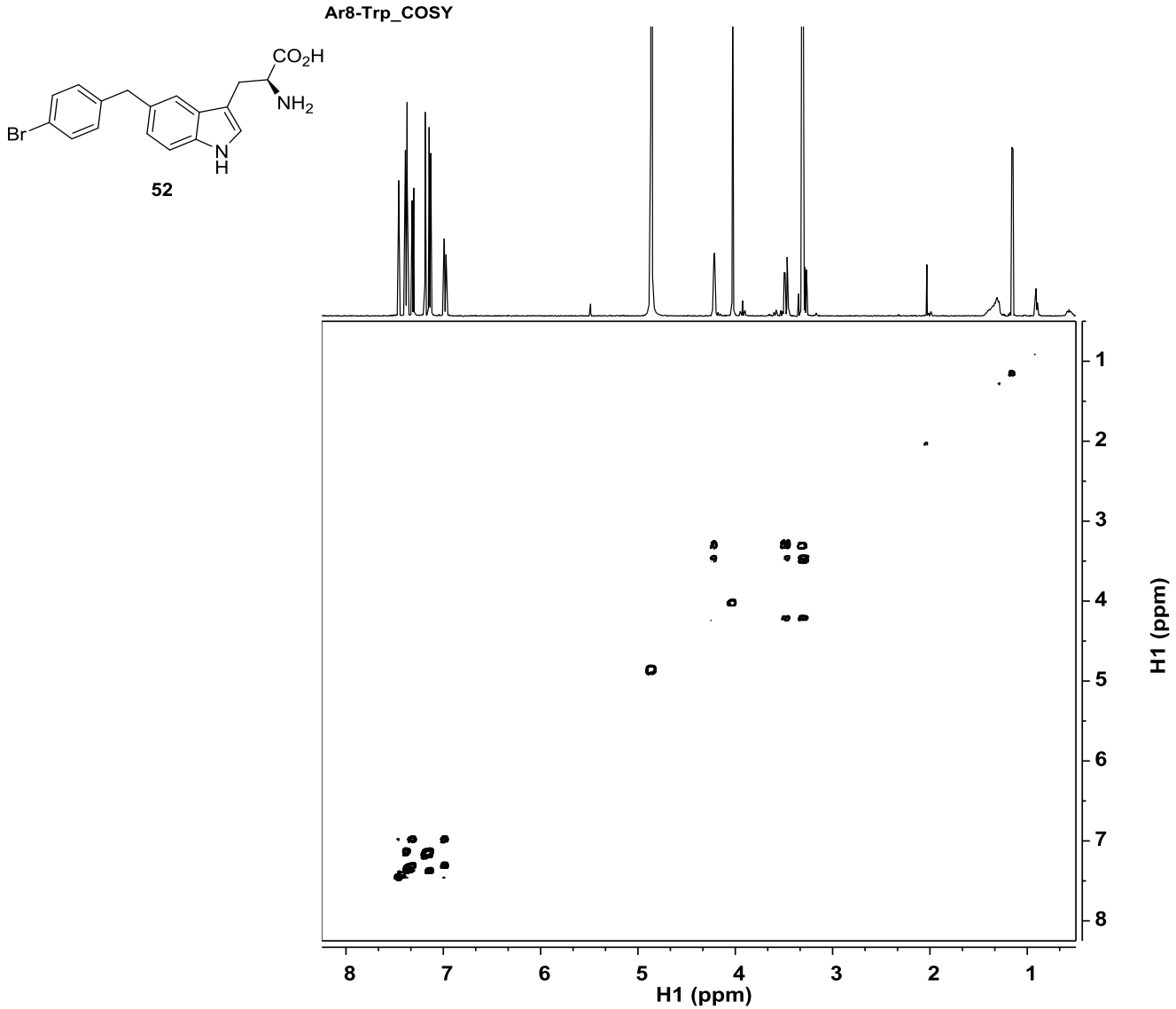
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **51** in MeOH-d4



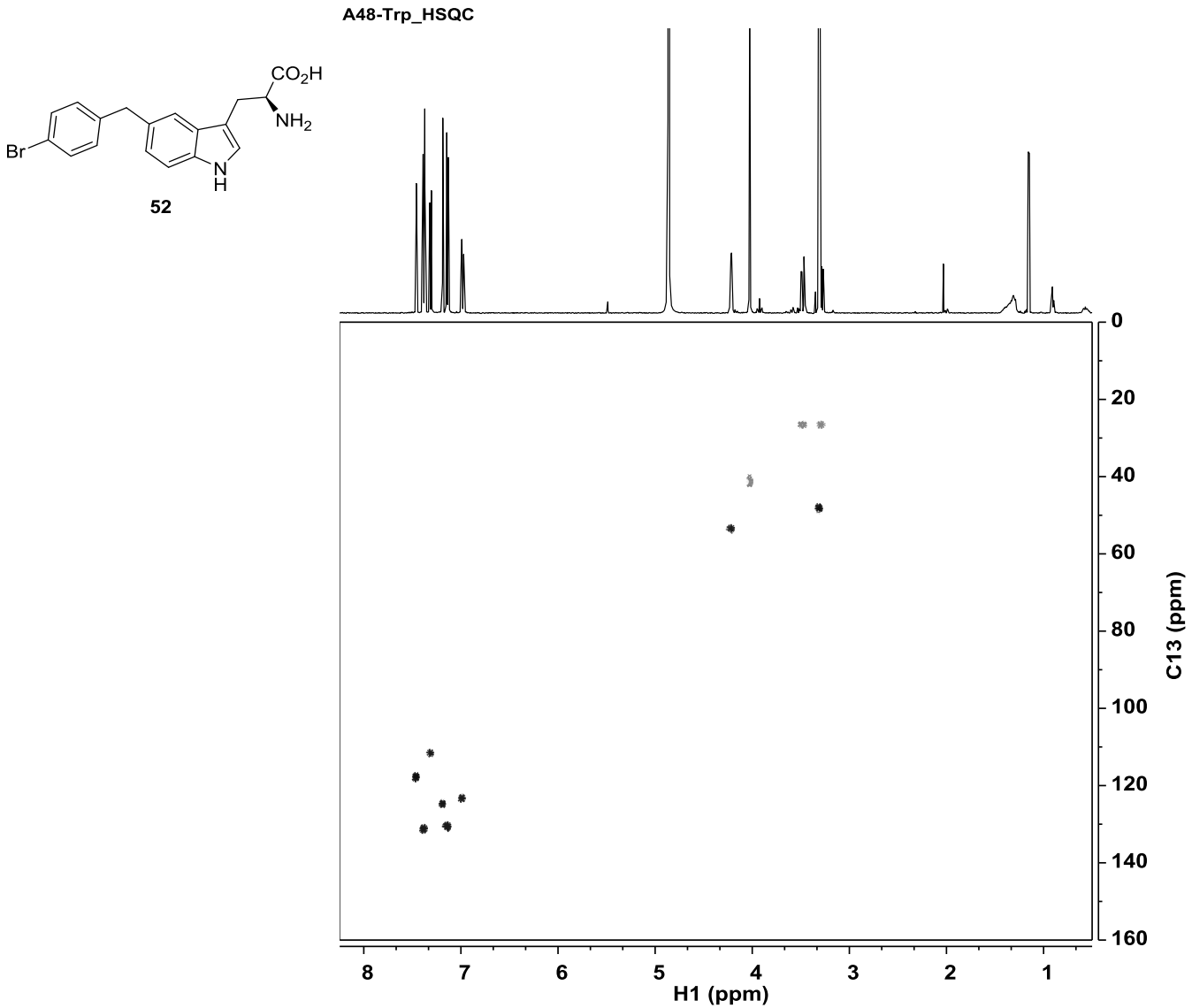
HRMS spectrum of **51**



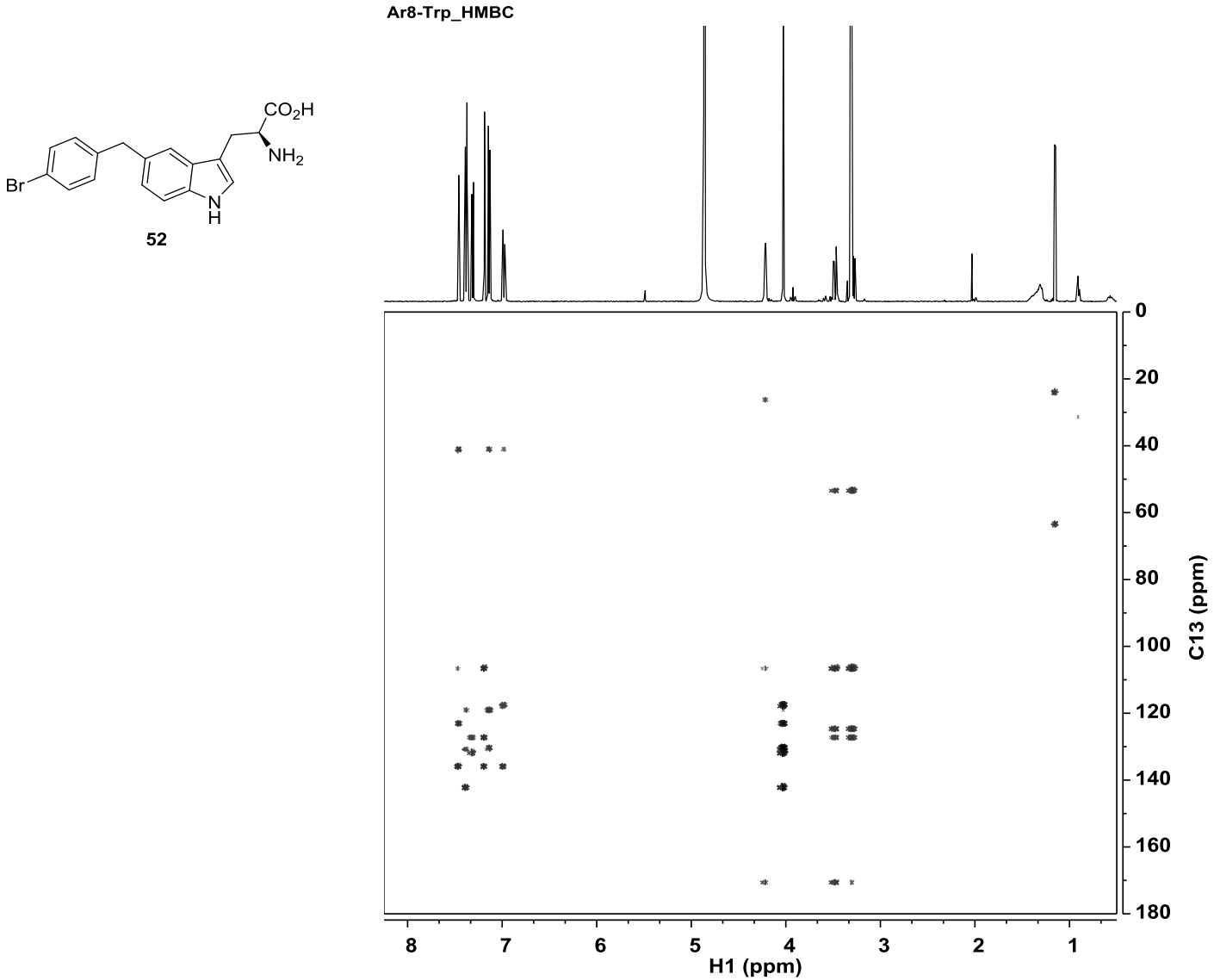
1D ^{1}H-NMR spectrum (500 MHz) of **52** in MeOH-d4



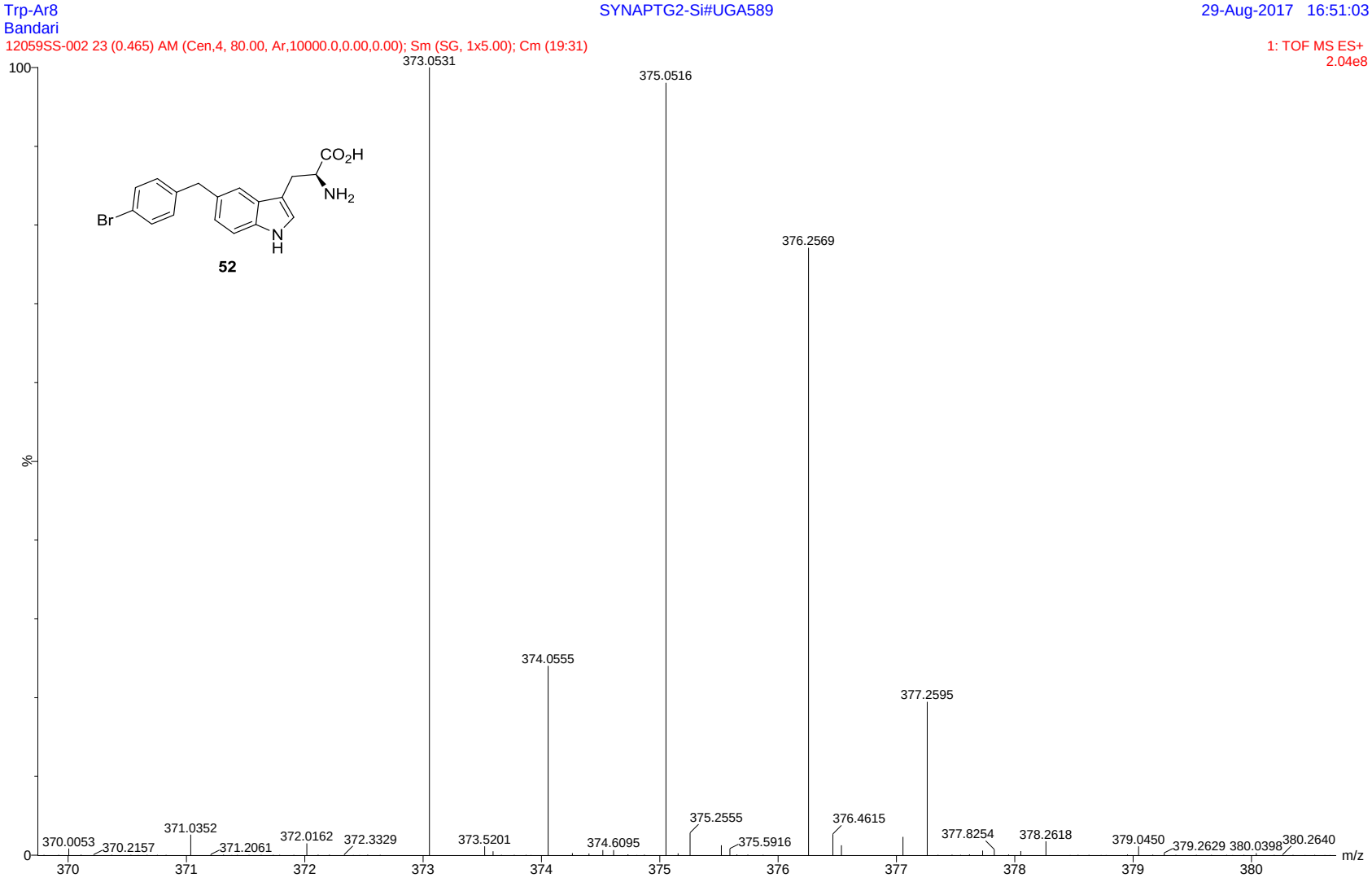
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **52** in MeOH-d4



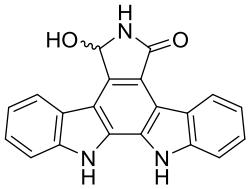
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **52** in MeOH-d4

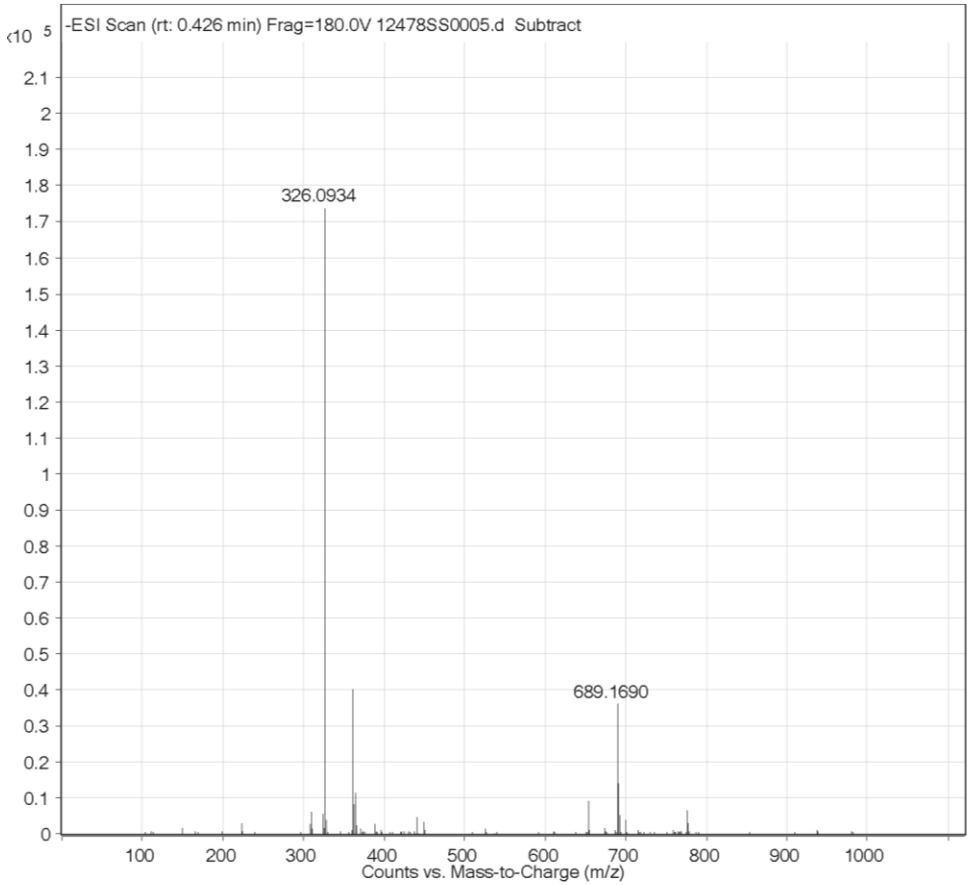


2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **52** in MeOH-d4

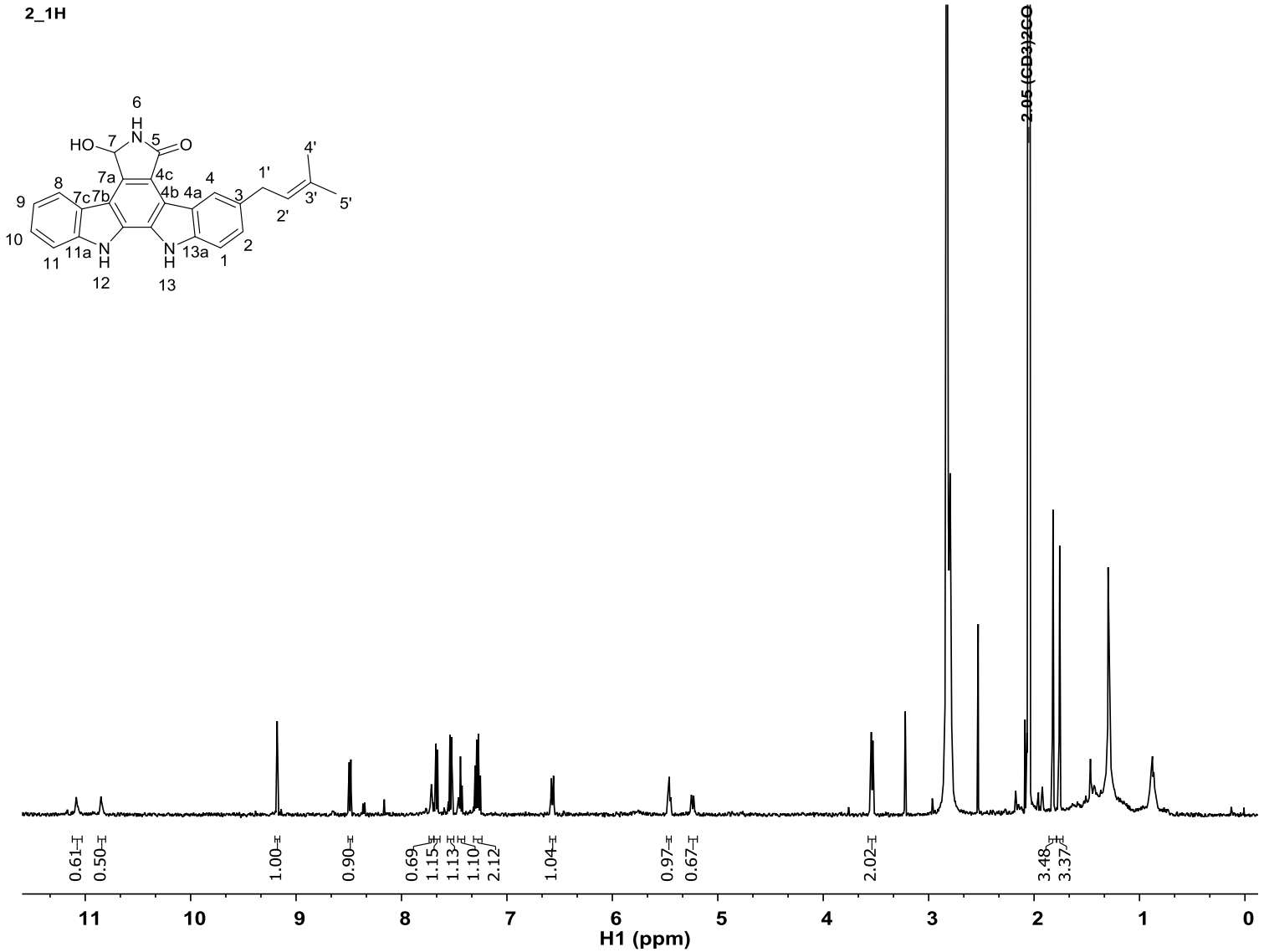


HRMS spectrum of **52**

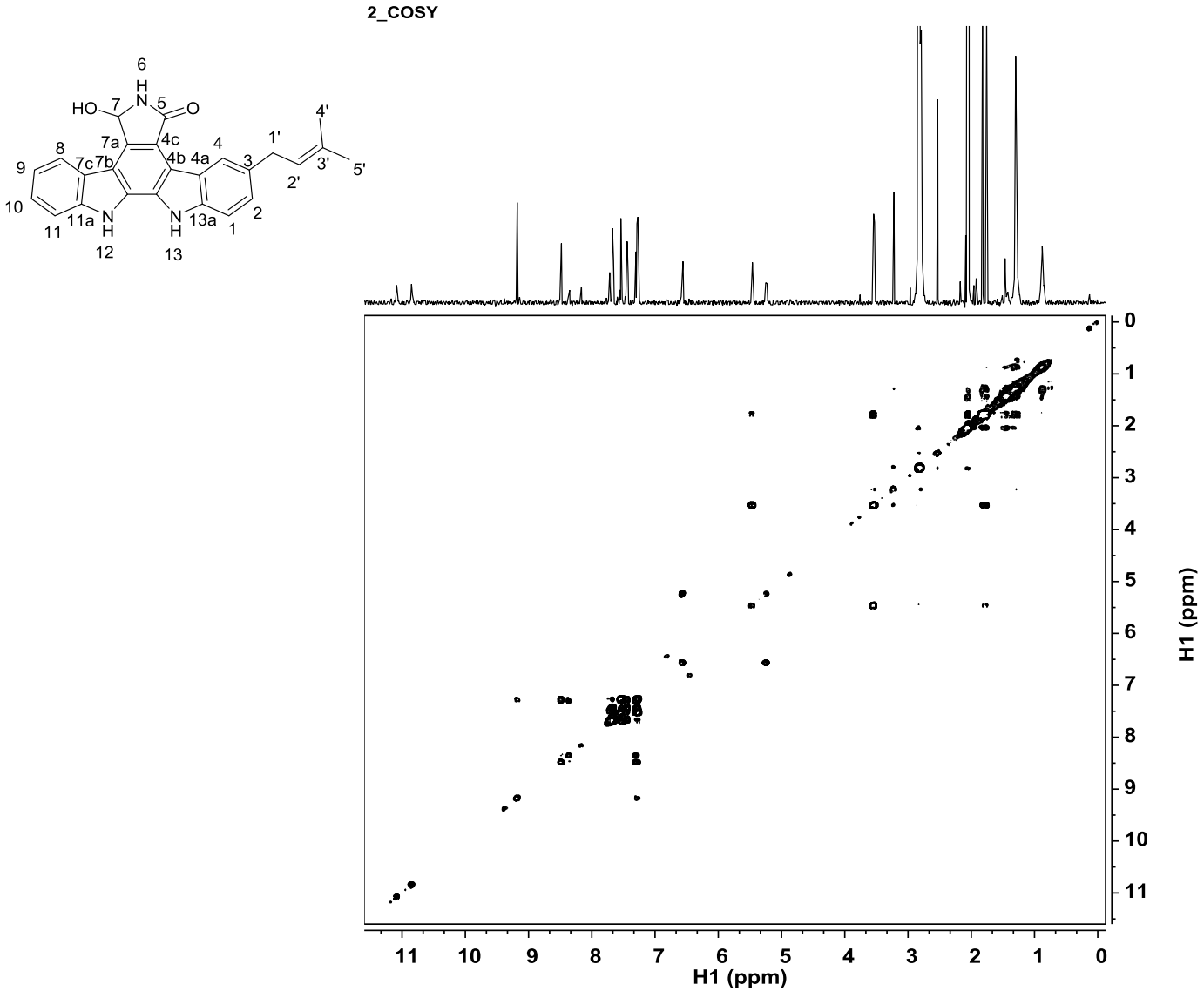




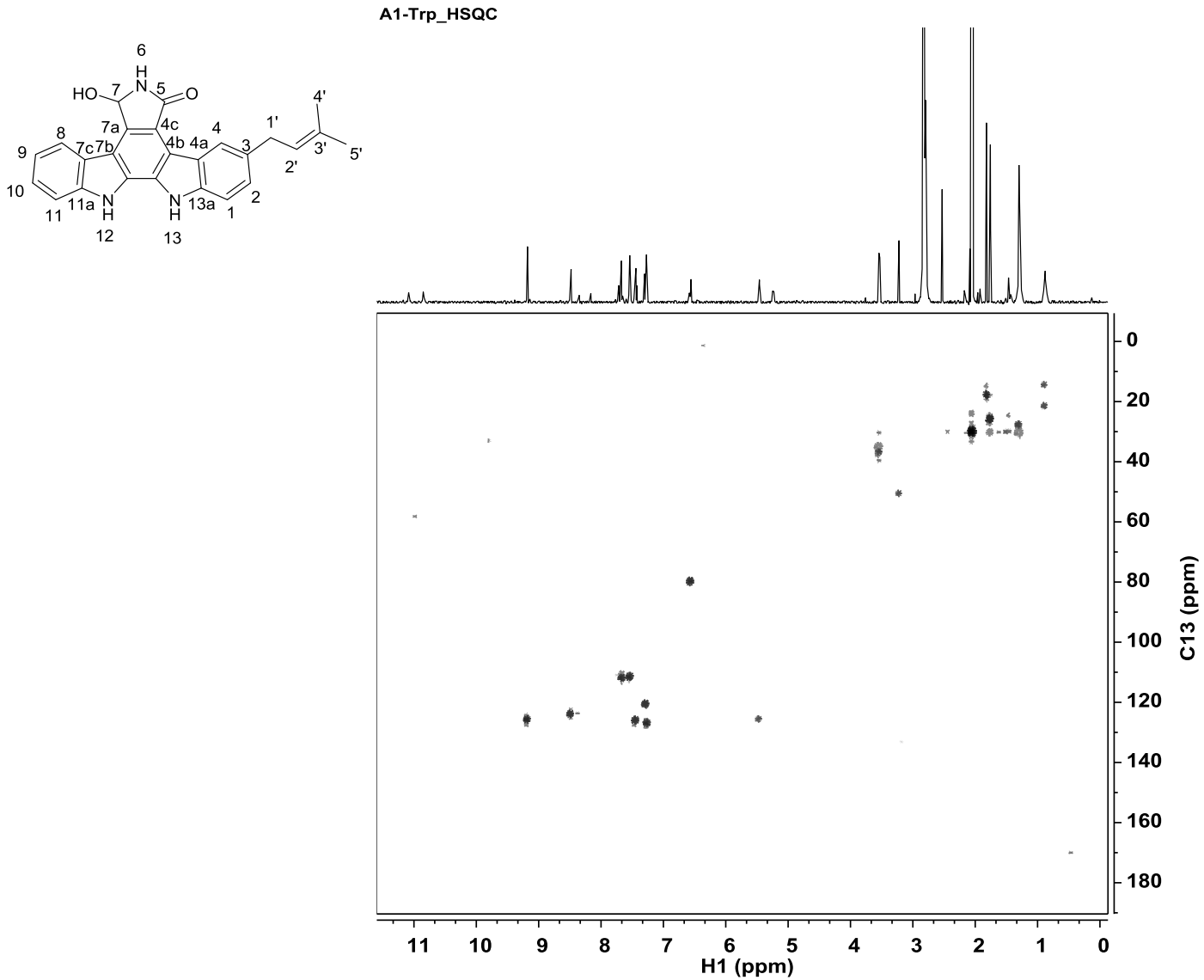
HRMS spectrum of **56**



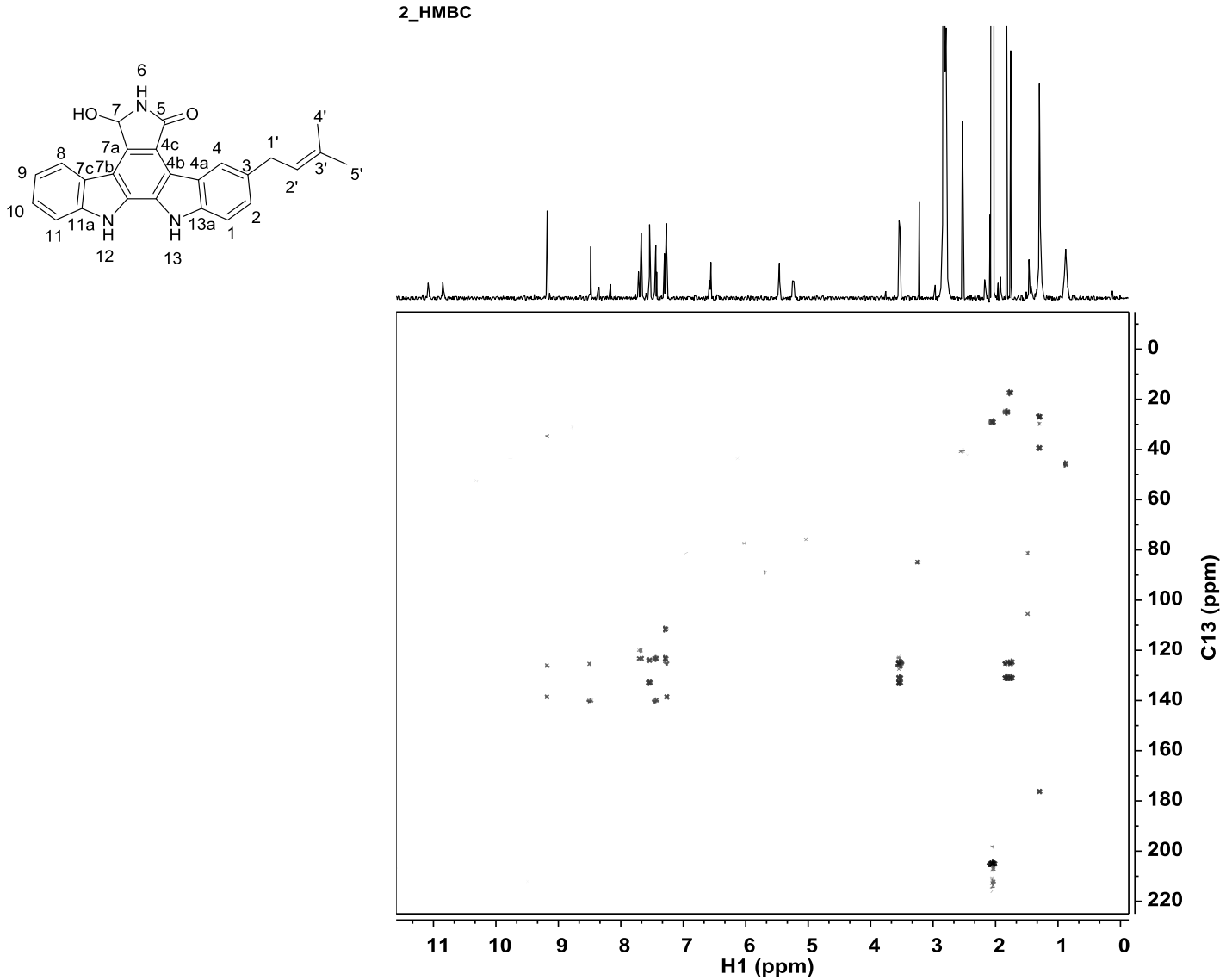
^{1}H NMR Spectrum (500 MHz) of compound **57** in Acetone-d6



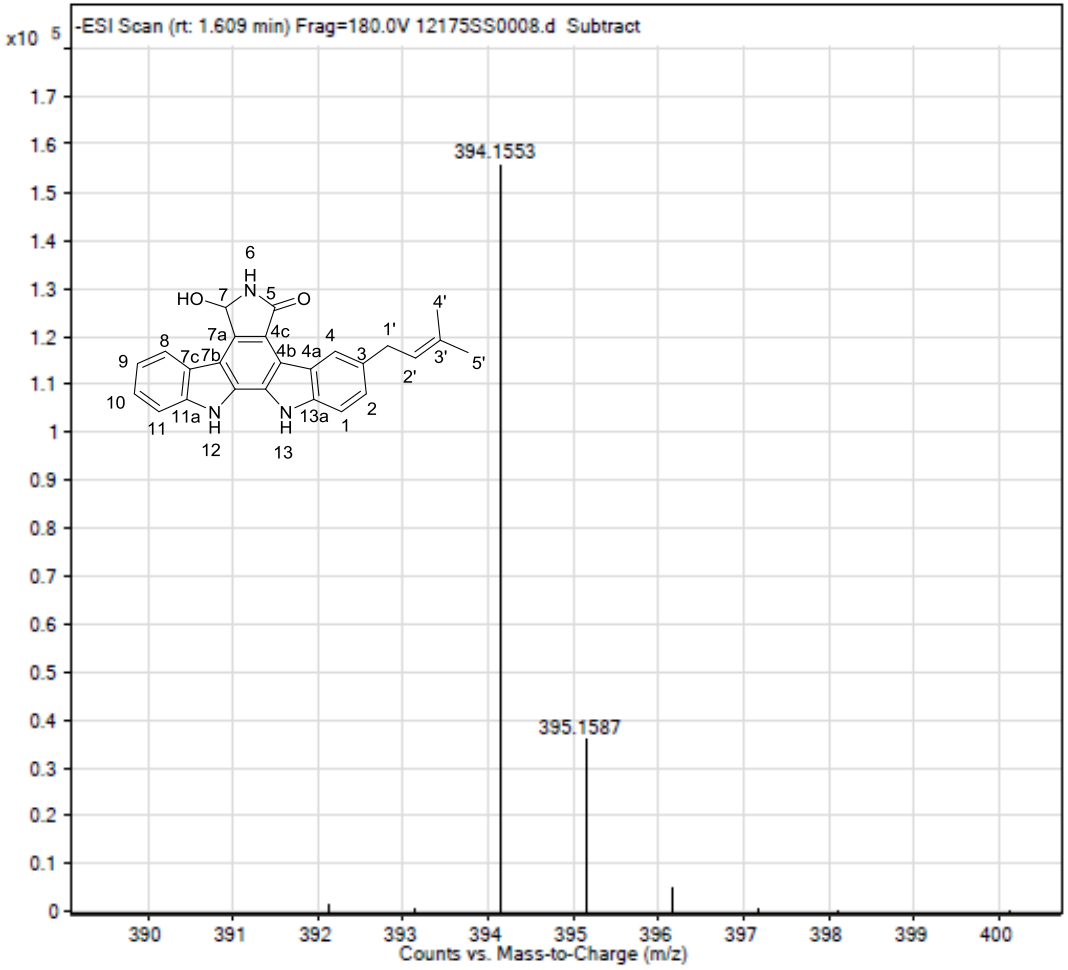
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **57** in Acetone-d6



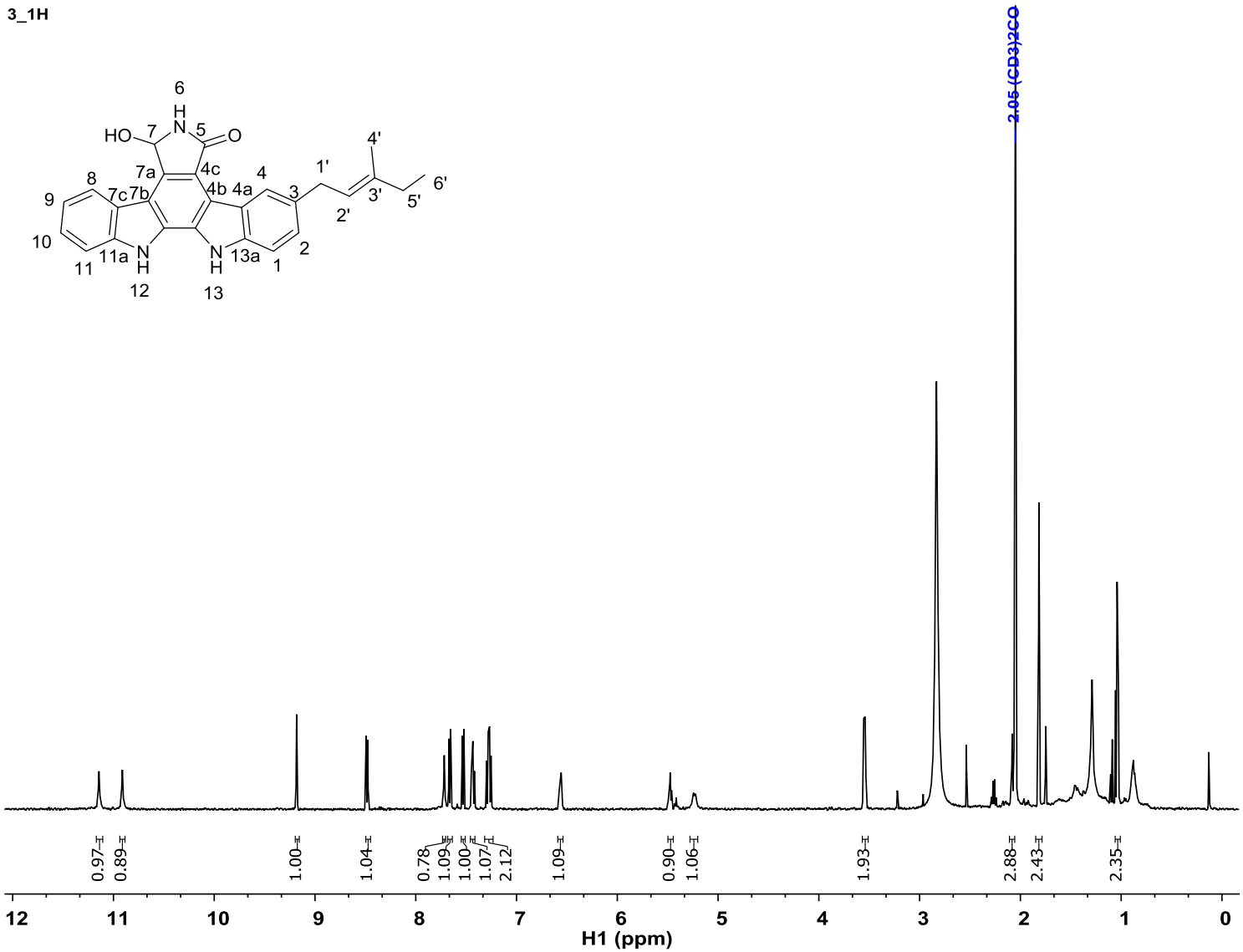
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **57** in Acetone-d6



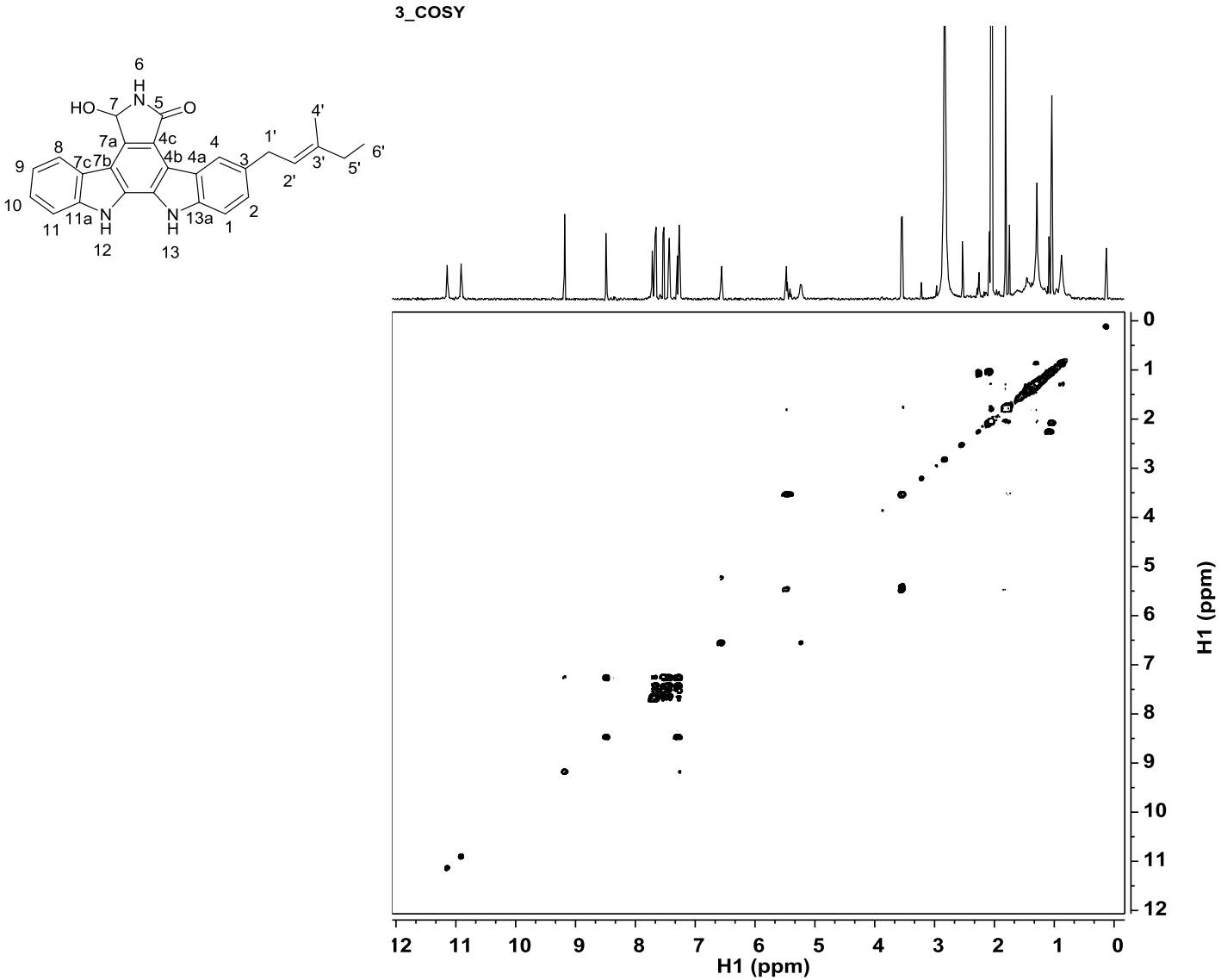
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **57** in Acetone-d6



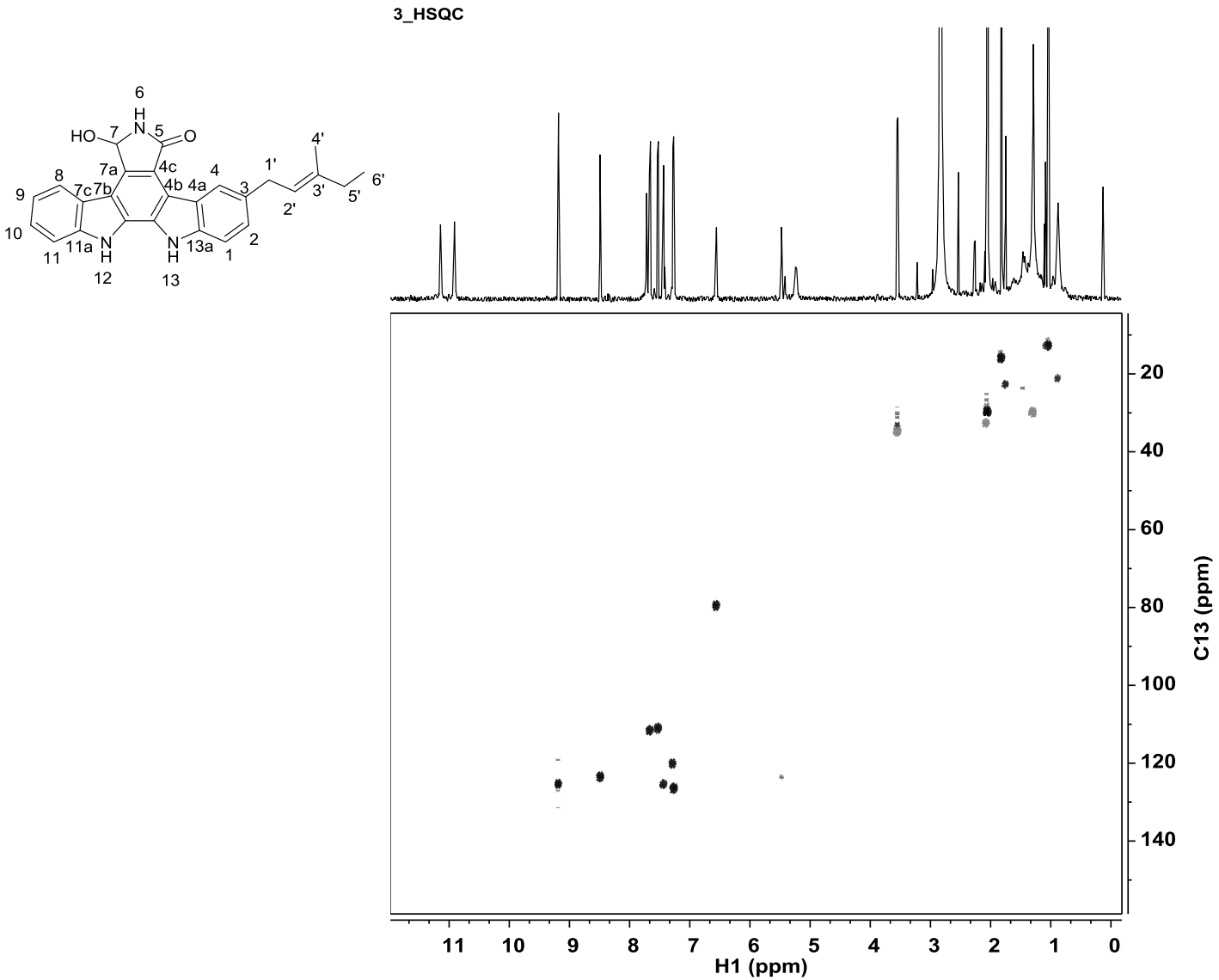
HRMS spectrum of **57**



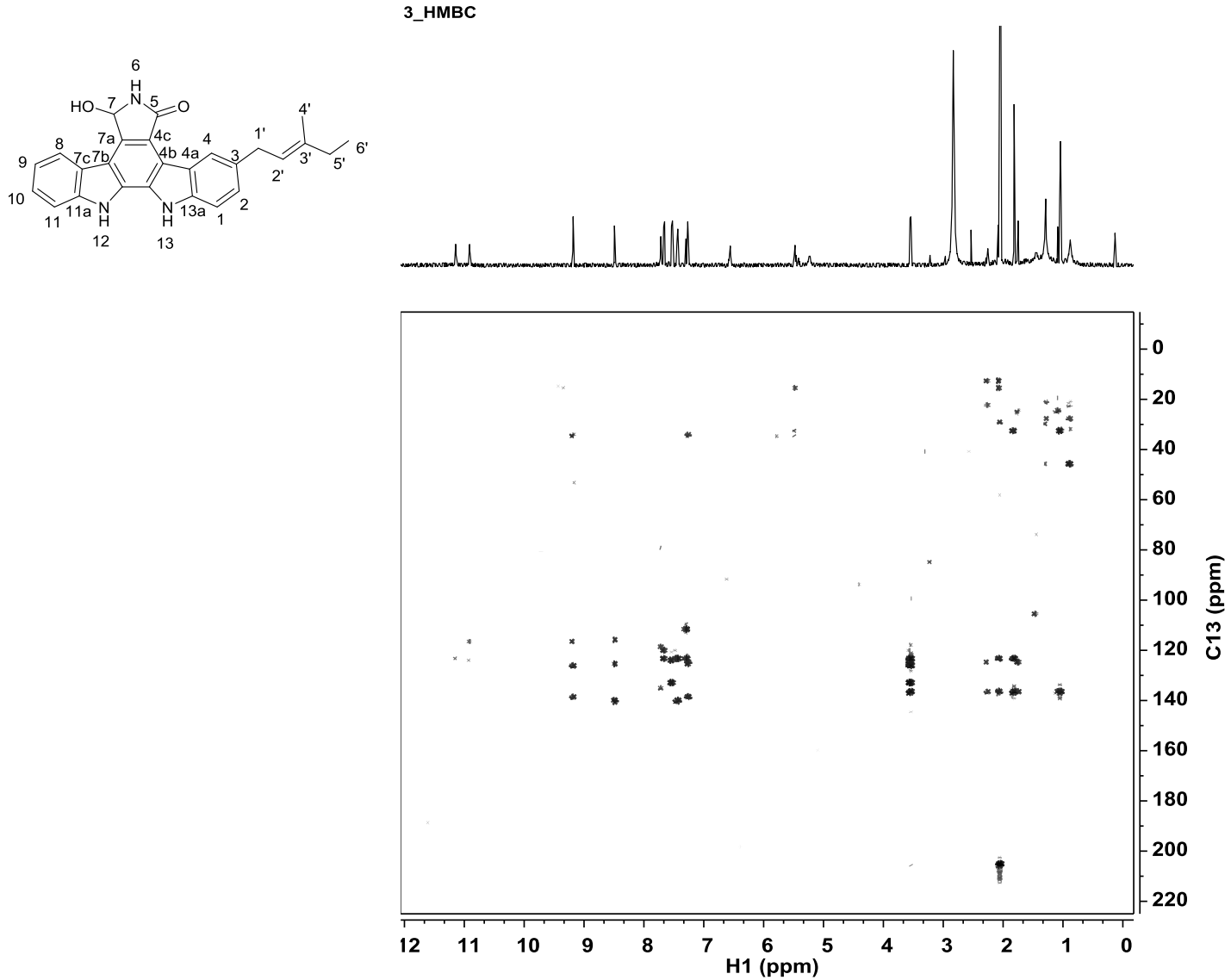
^{1}H NMR Spectrum (500 MHz) of compound **58** in Acetone-d6



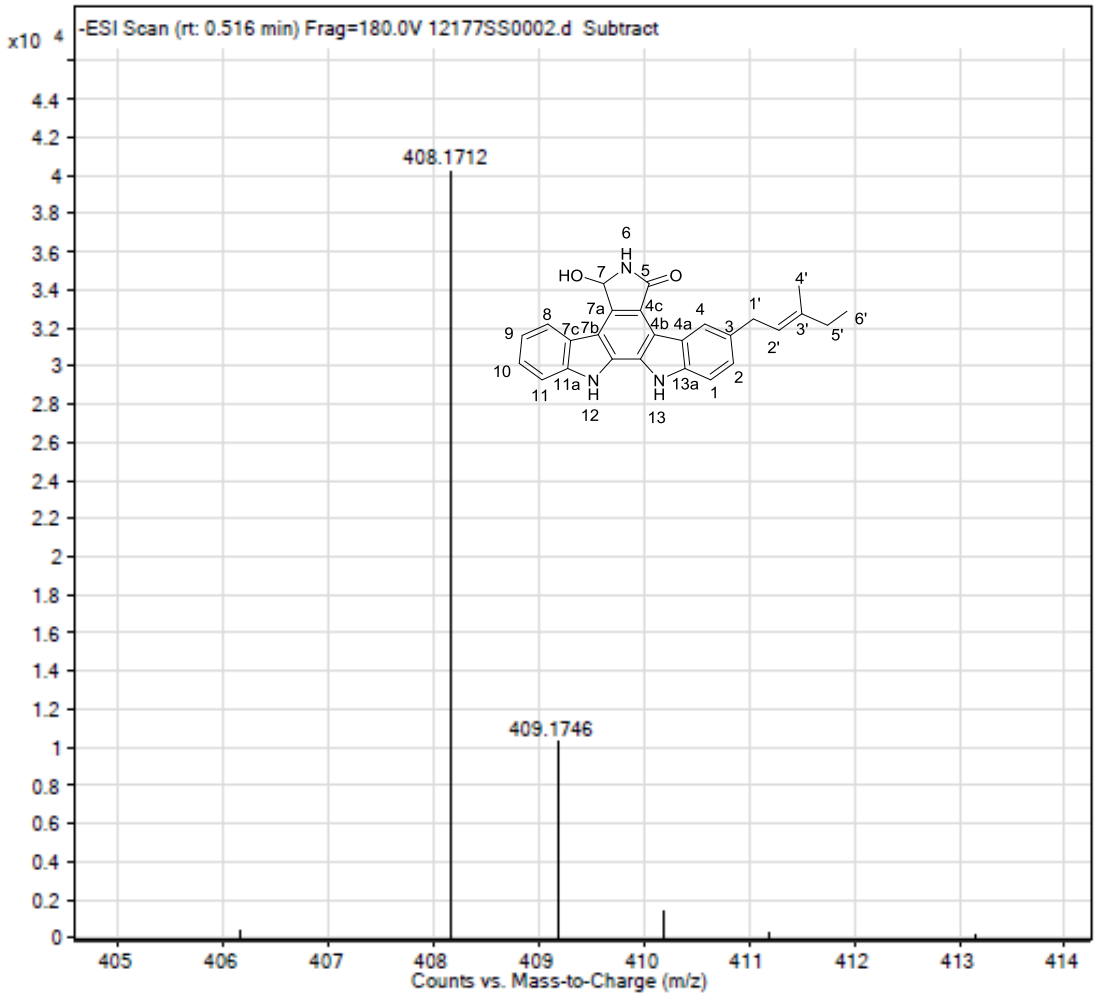
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **58** in Acetone-d6



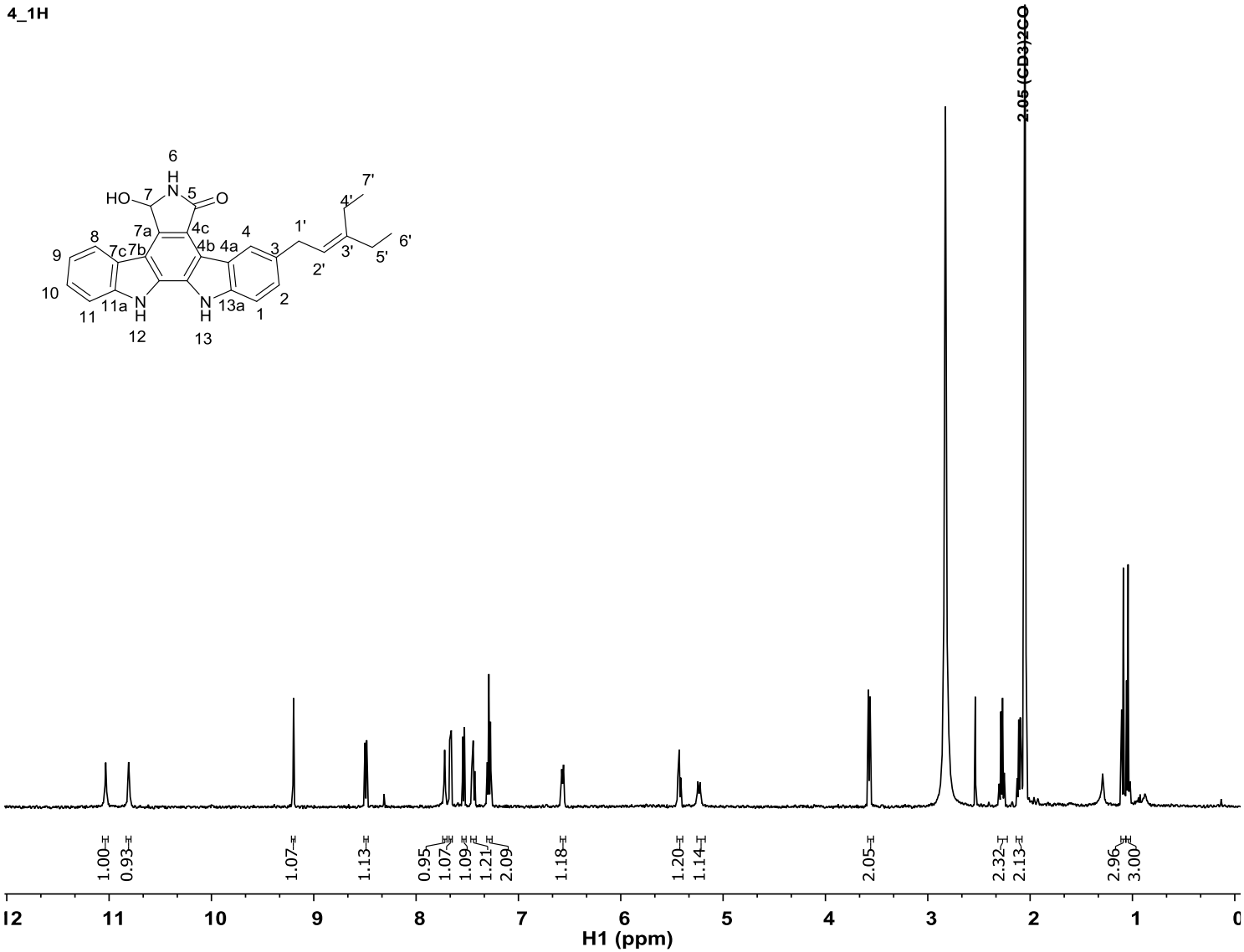
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **58** in Acetone-d6



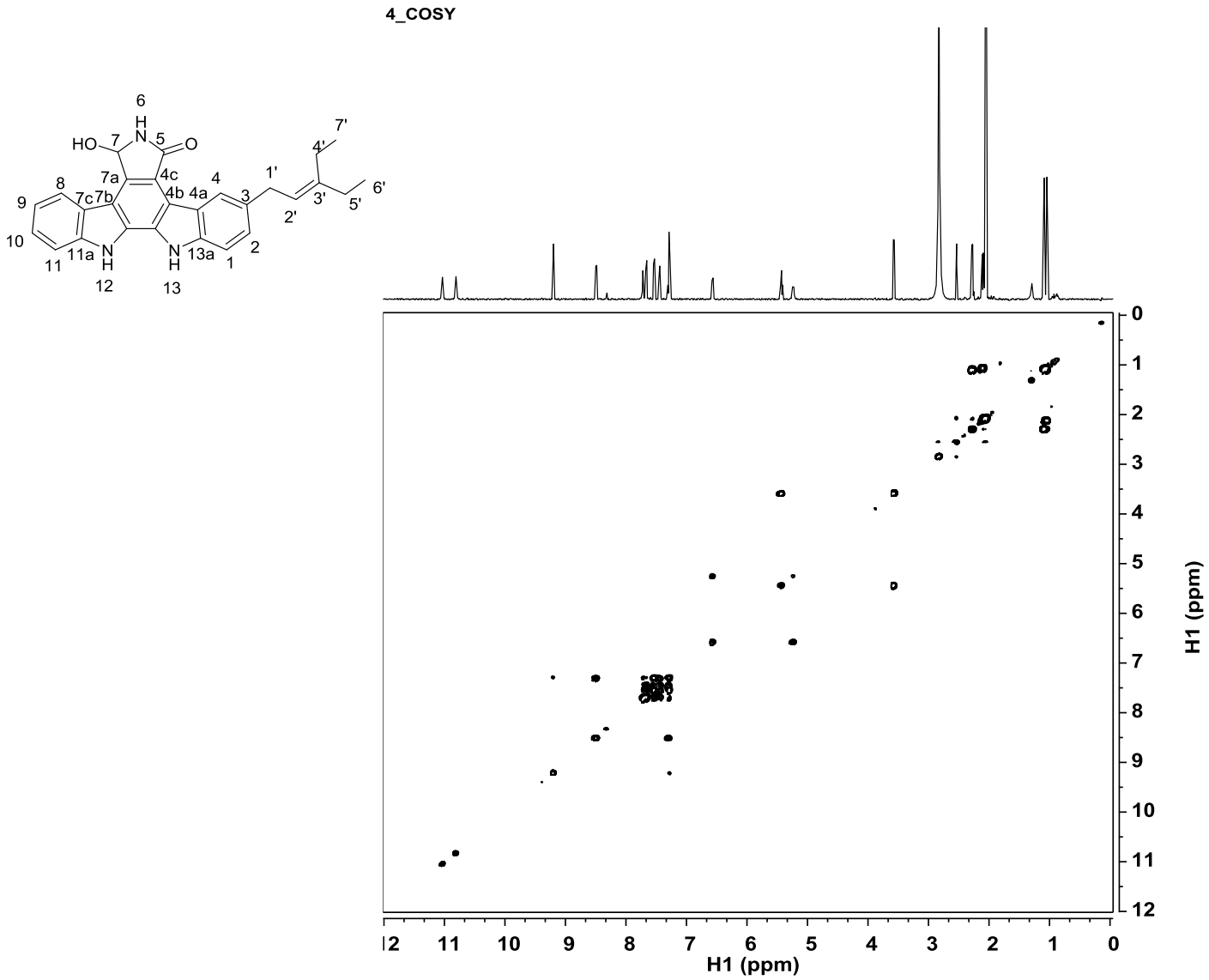
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **58** in Acetone-d6



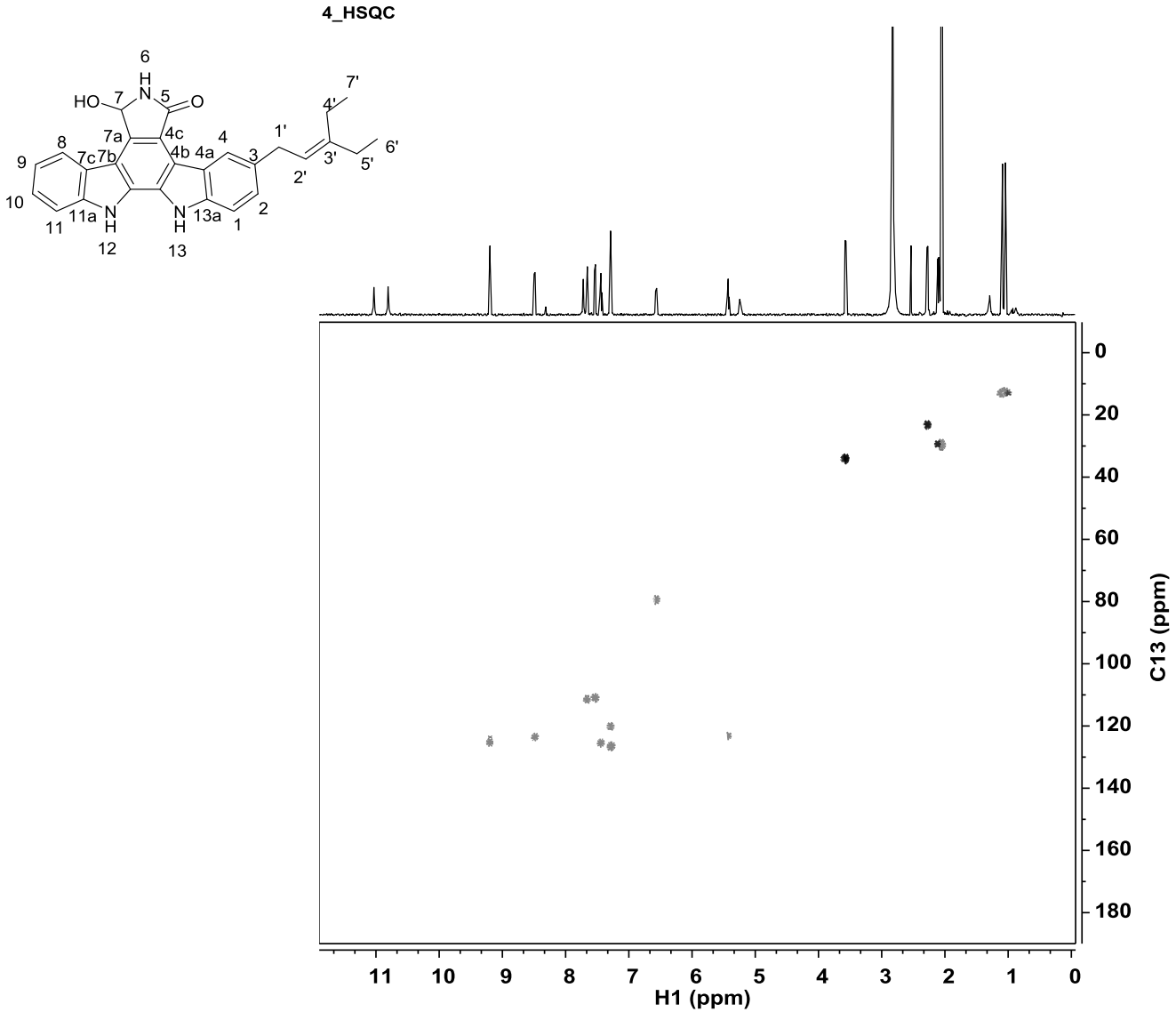
HRMS spectrum of **58**



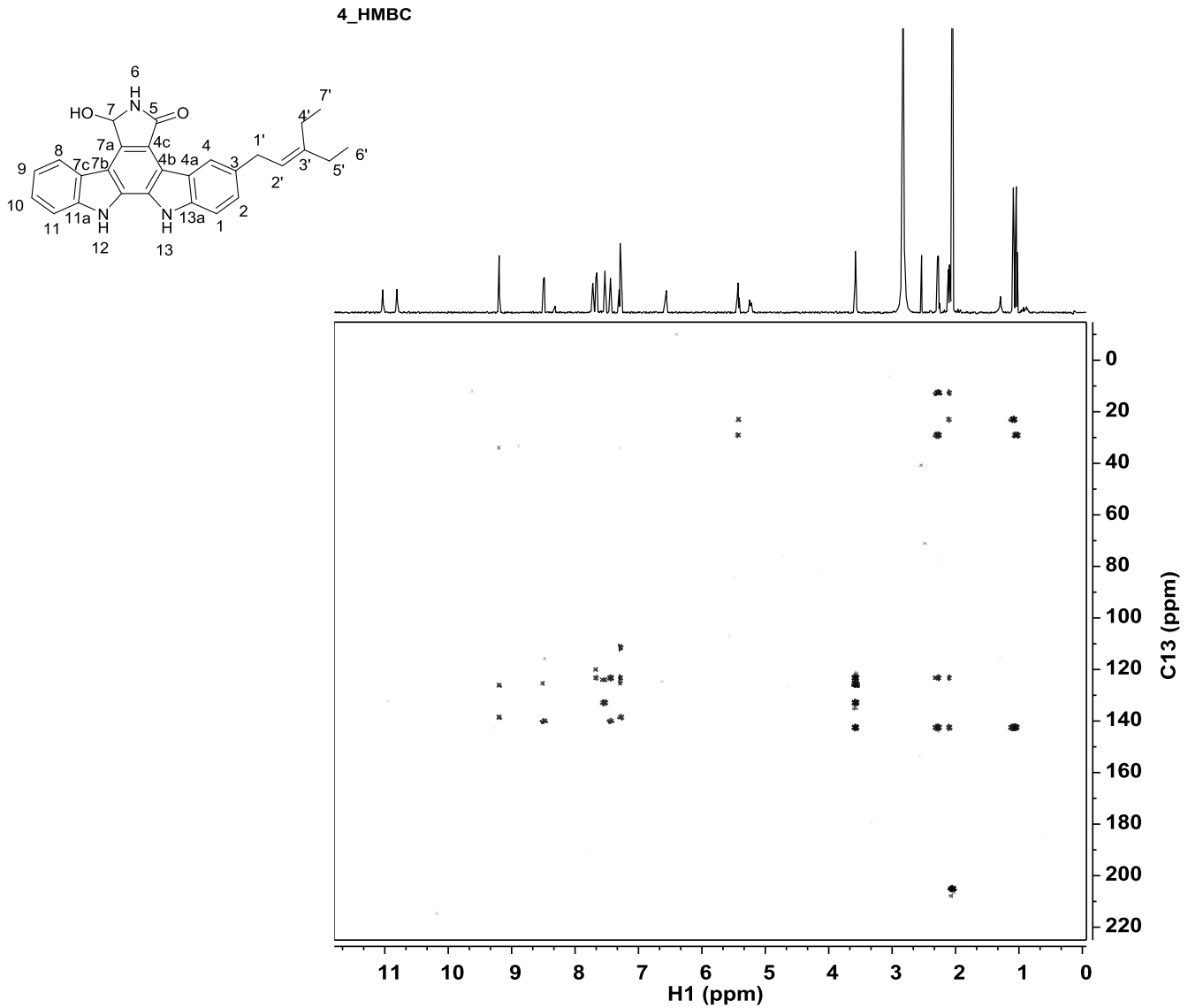
^{1}H NMR Spectrum (500 MHz) of compound **59** in Acetone-d6



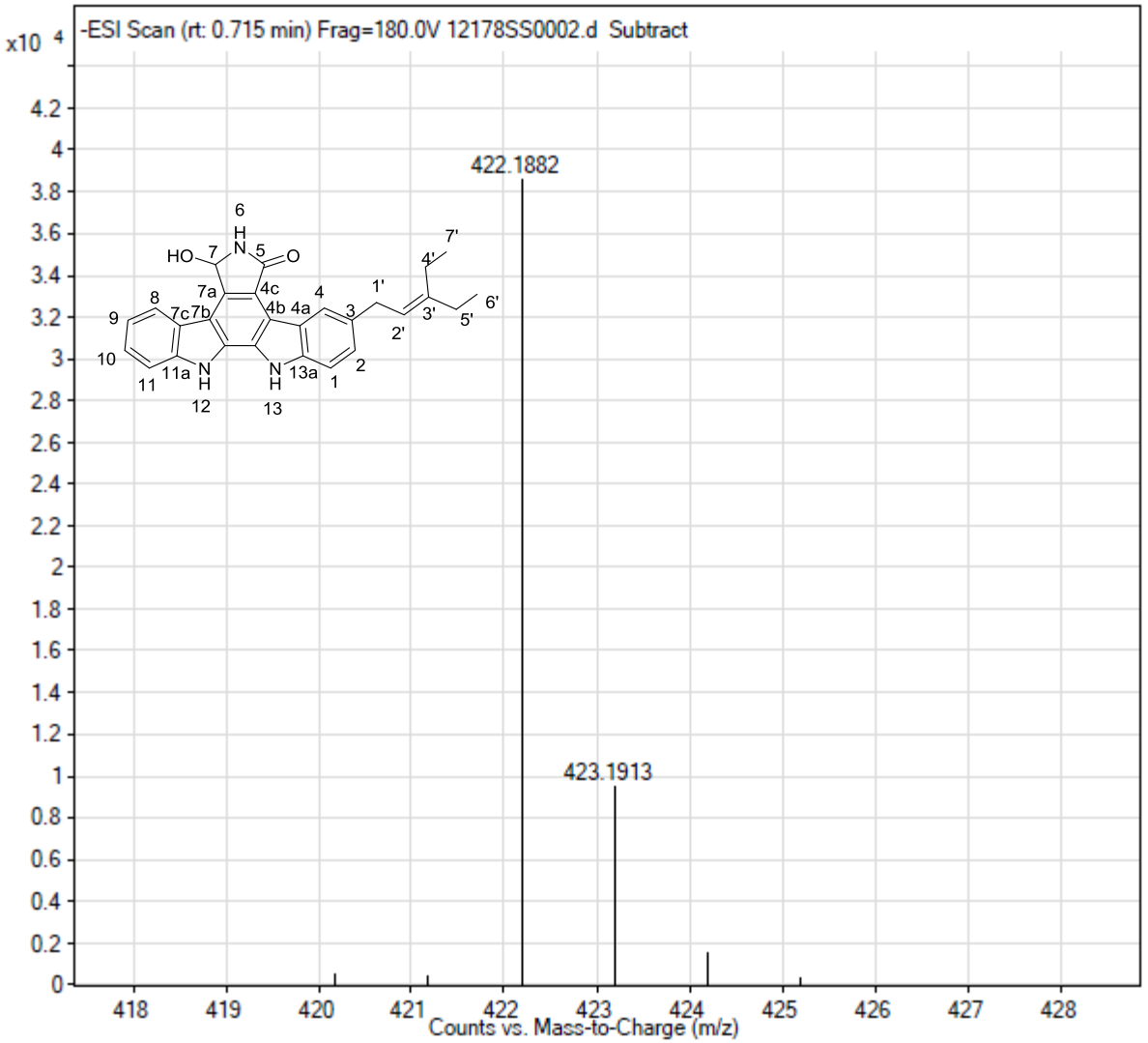
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **59** in Acetone-d6



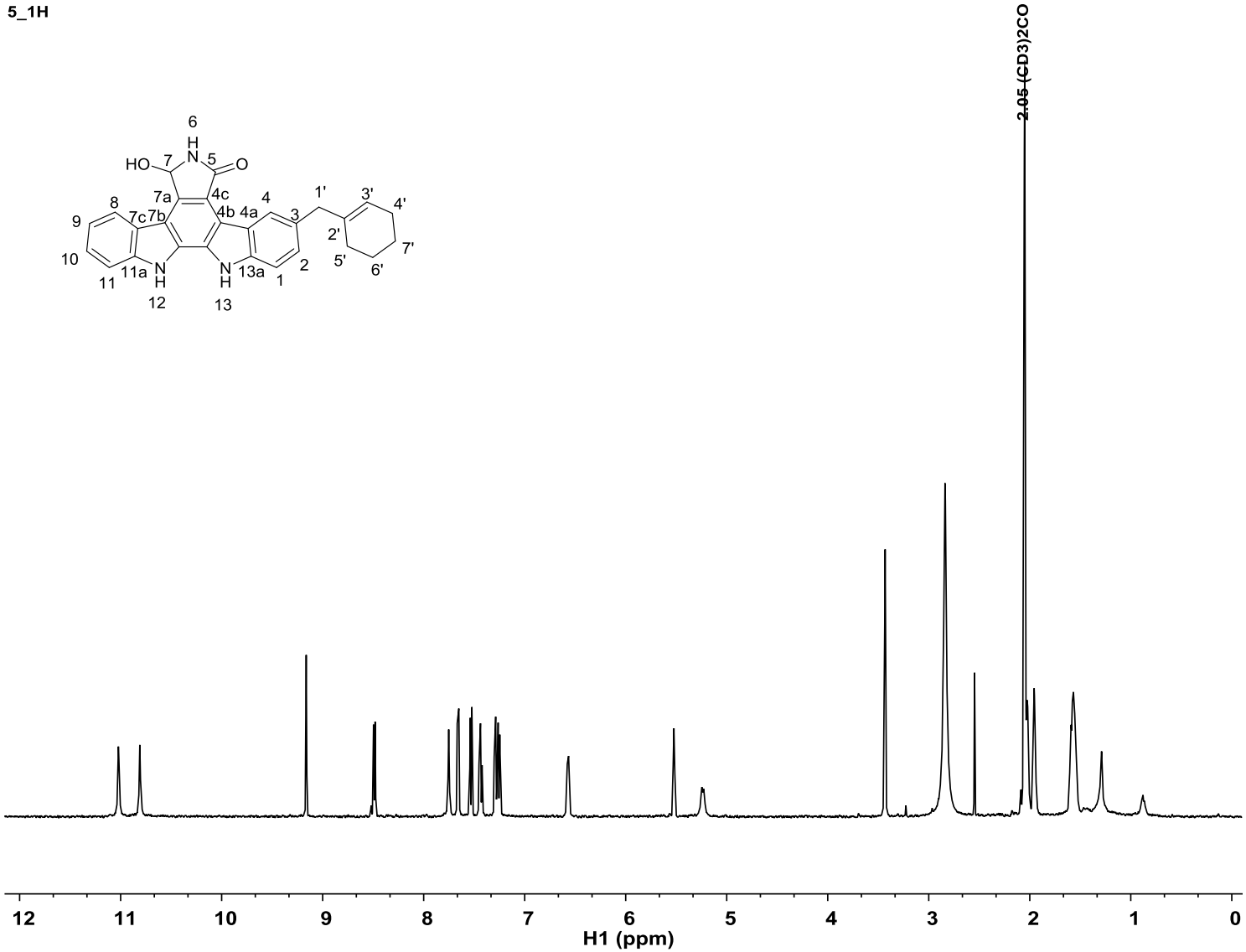
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **59** in Acetone-d6



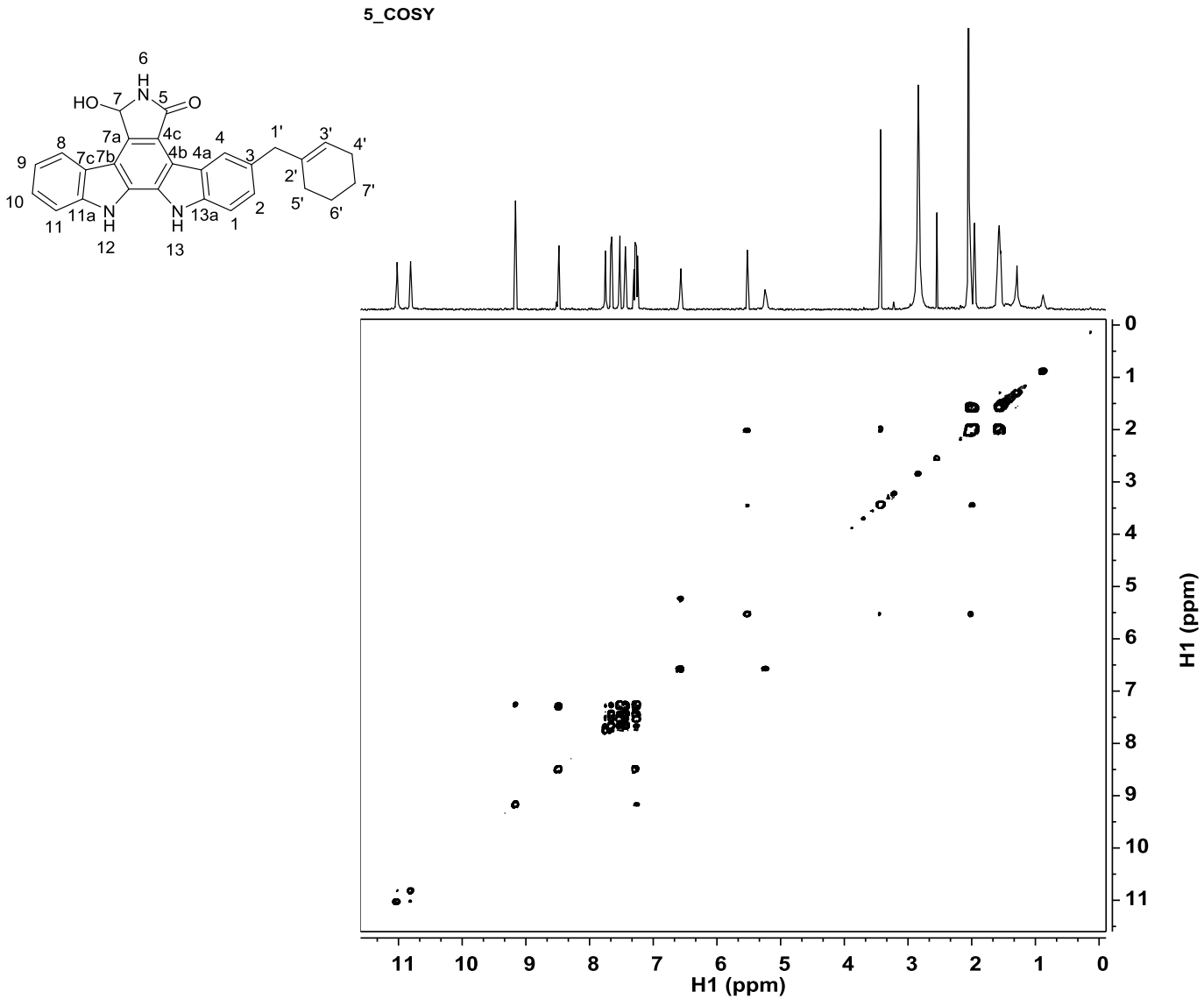
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **59** in Acetone-d6



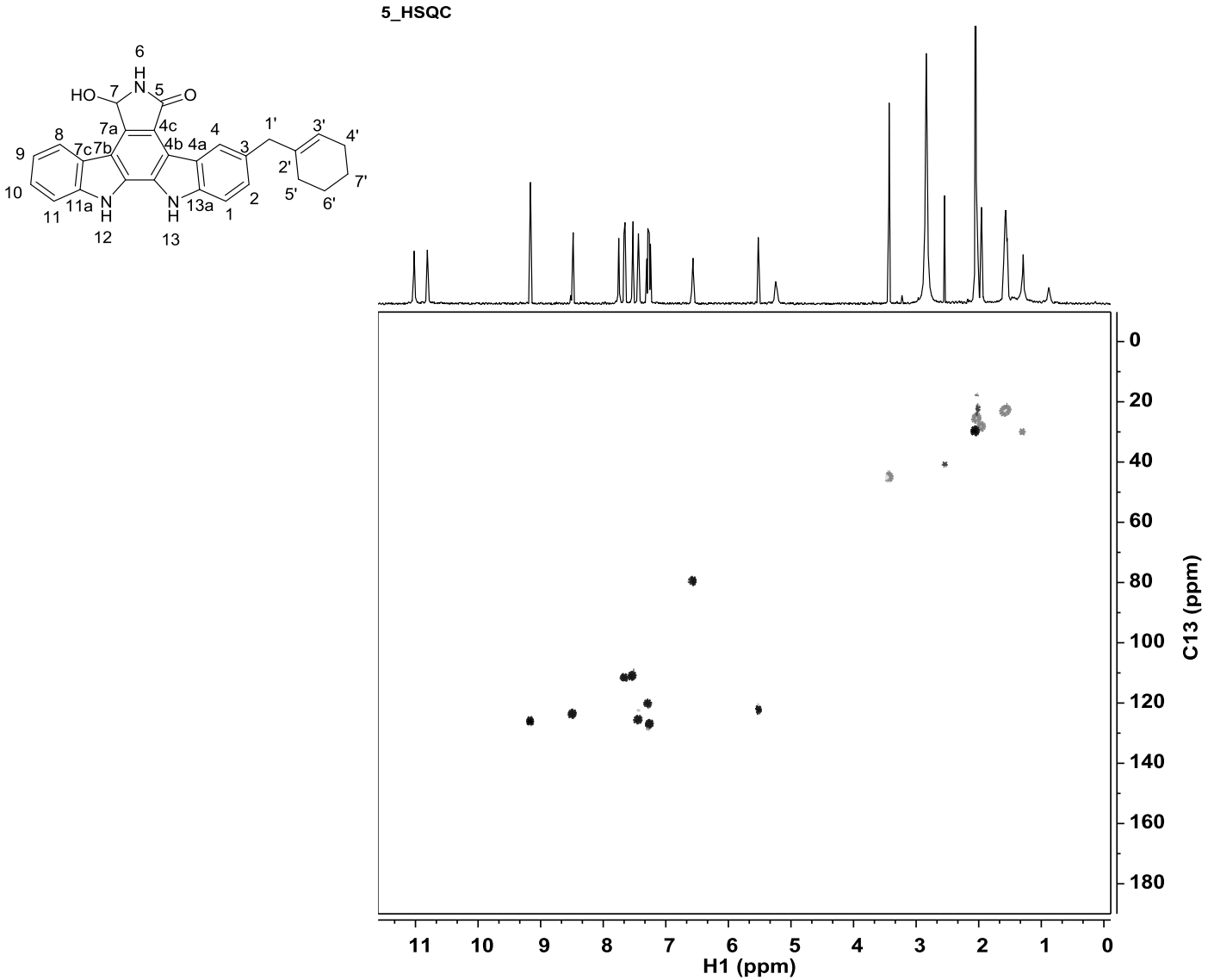
HRMS spectrum of **59**



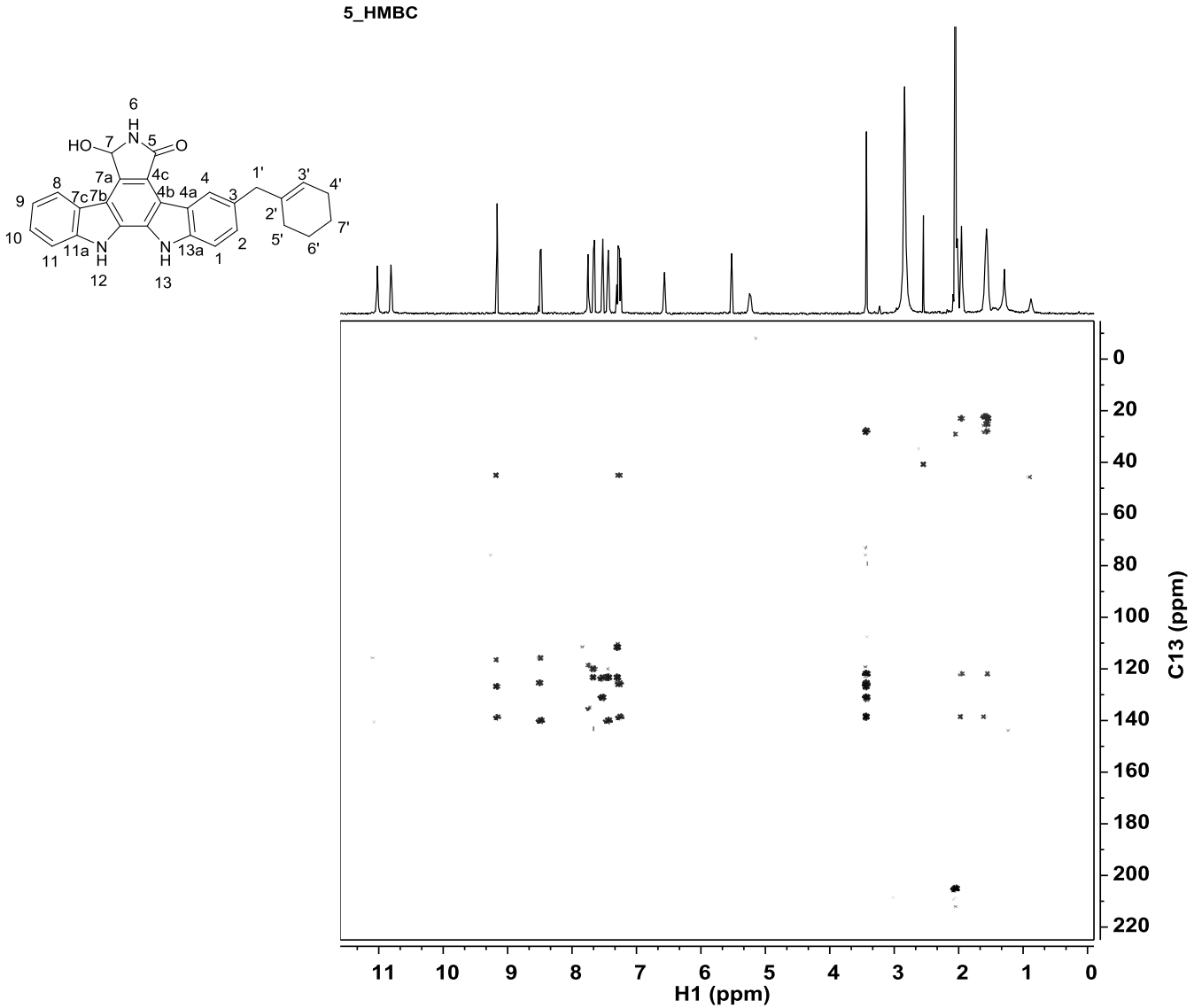
^{1}H NMR Spectrum (500 MHz) of compound **60** in Acetone-d6



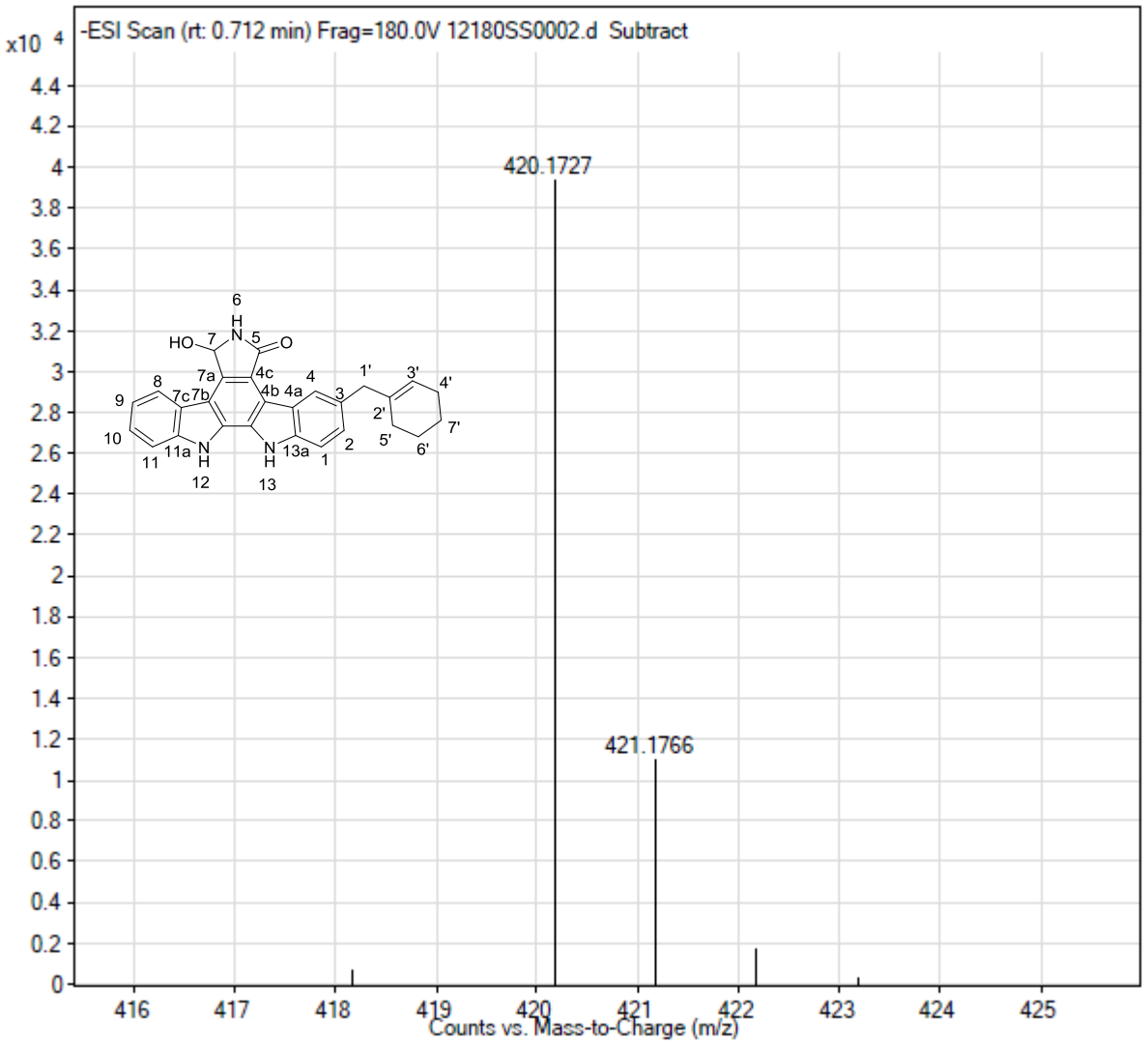
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **60** in Acetone-d6



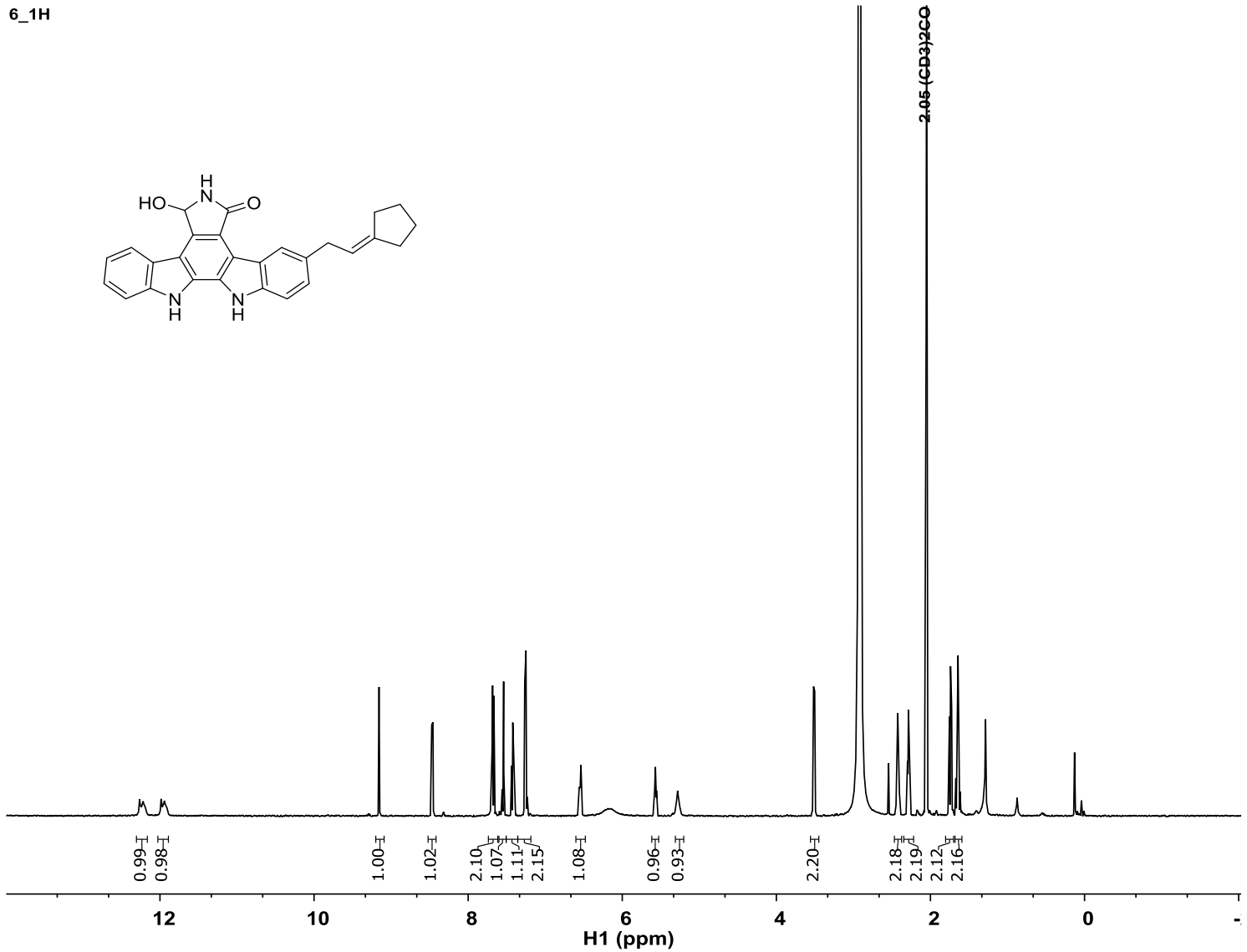
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **60** in Acetone-d6



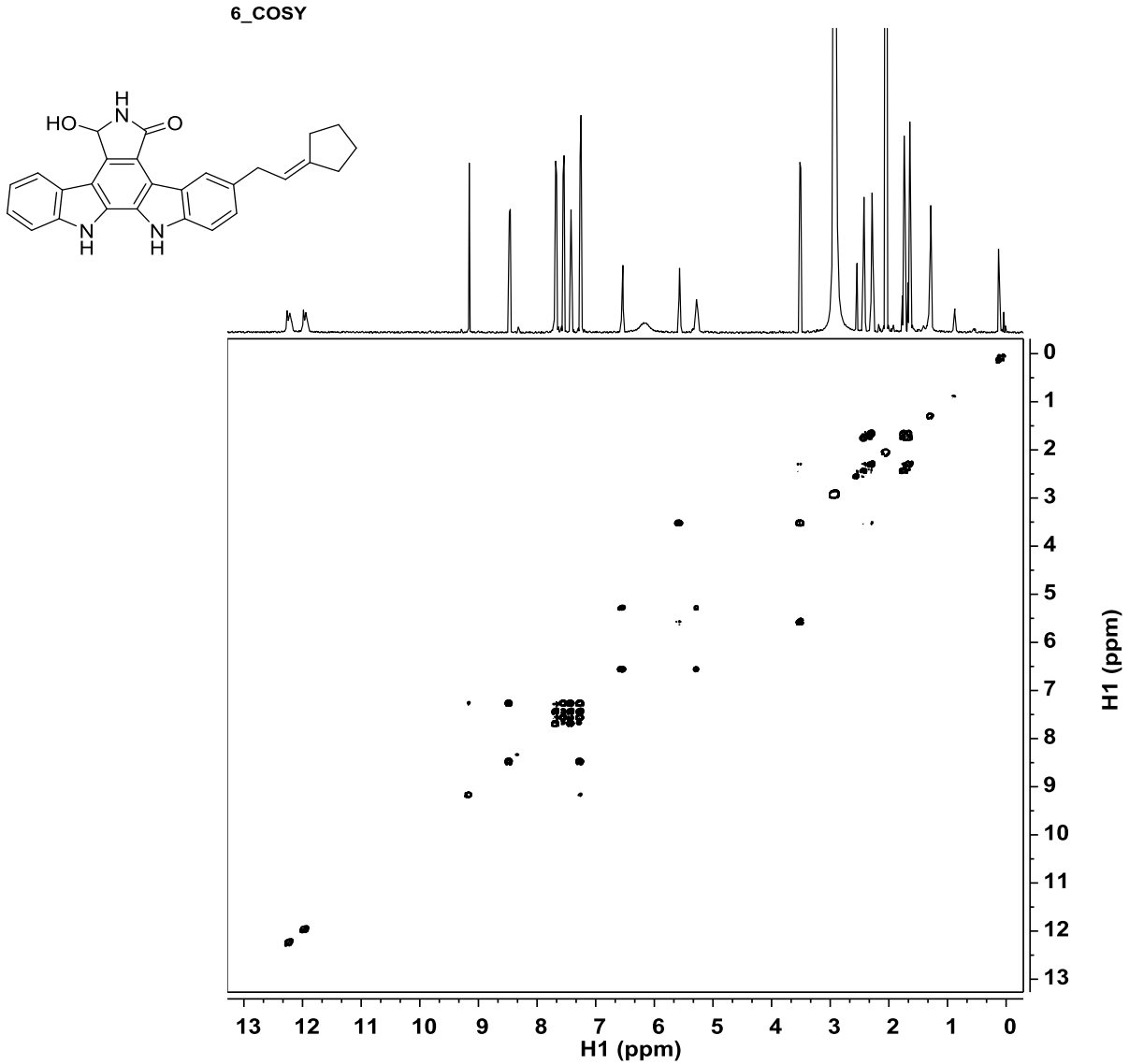
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **60** in Acetone-d6



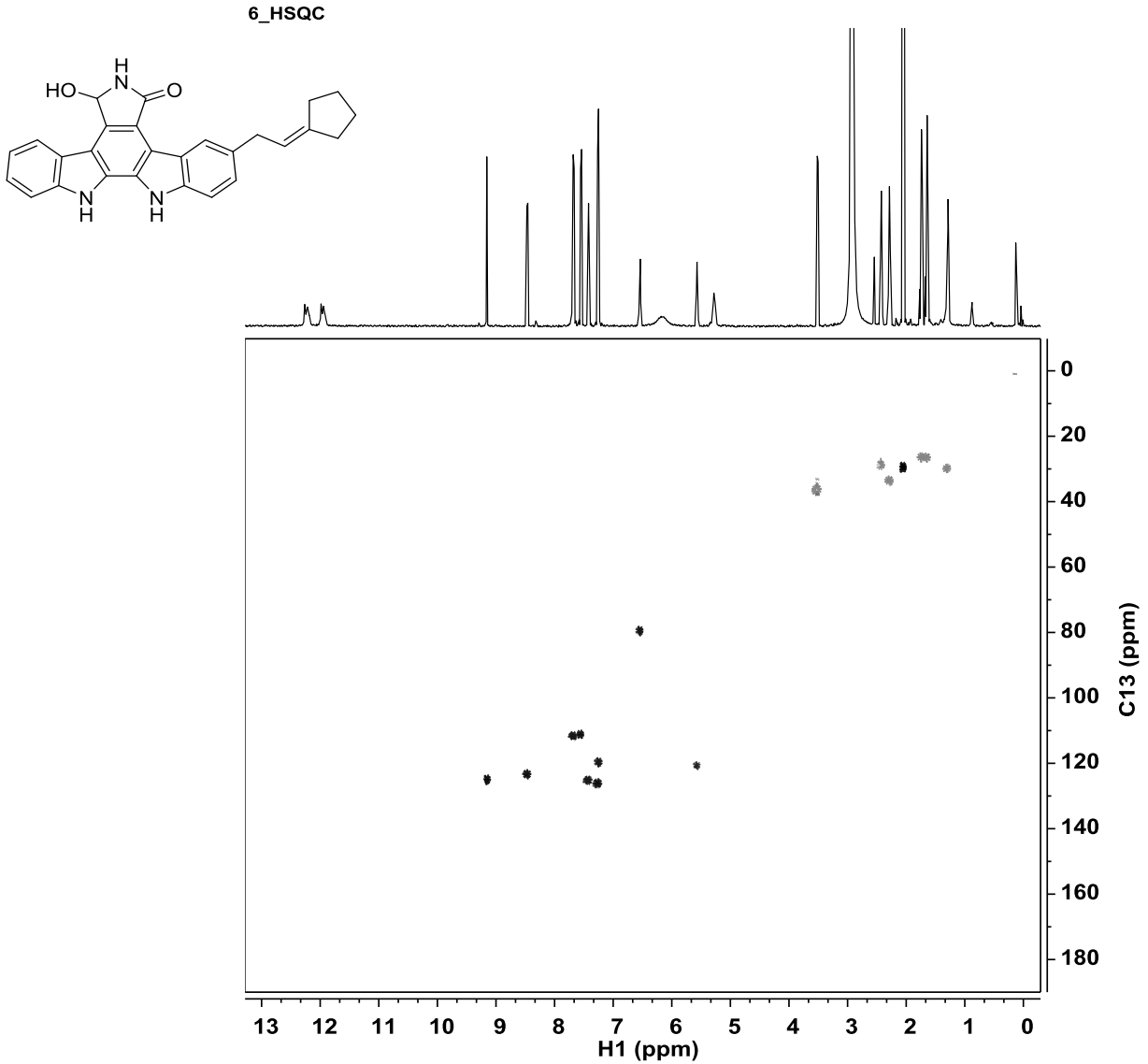
HRMS spectrum of **60**



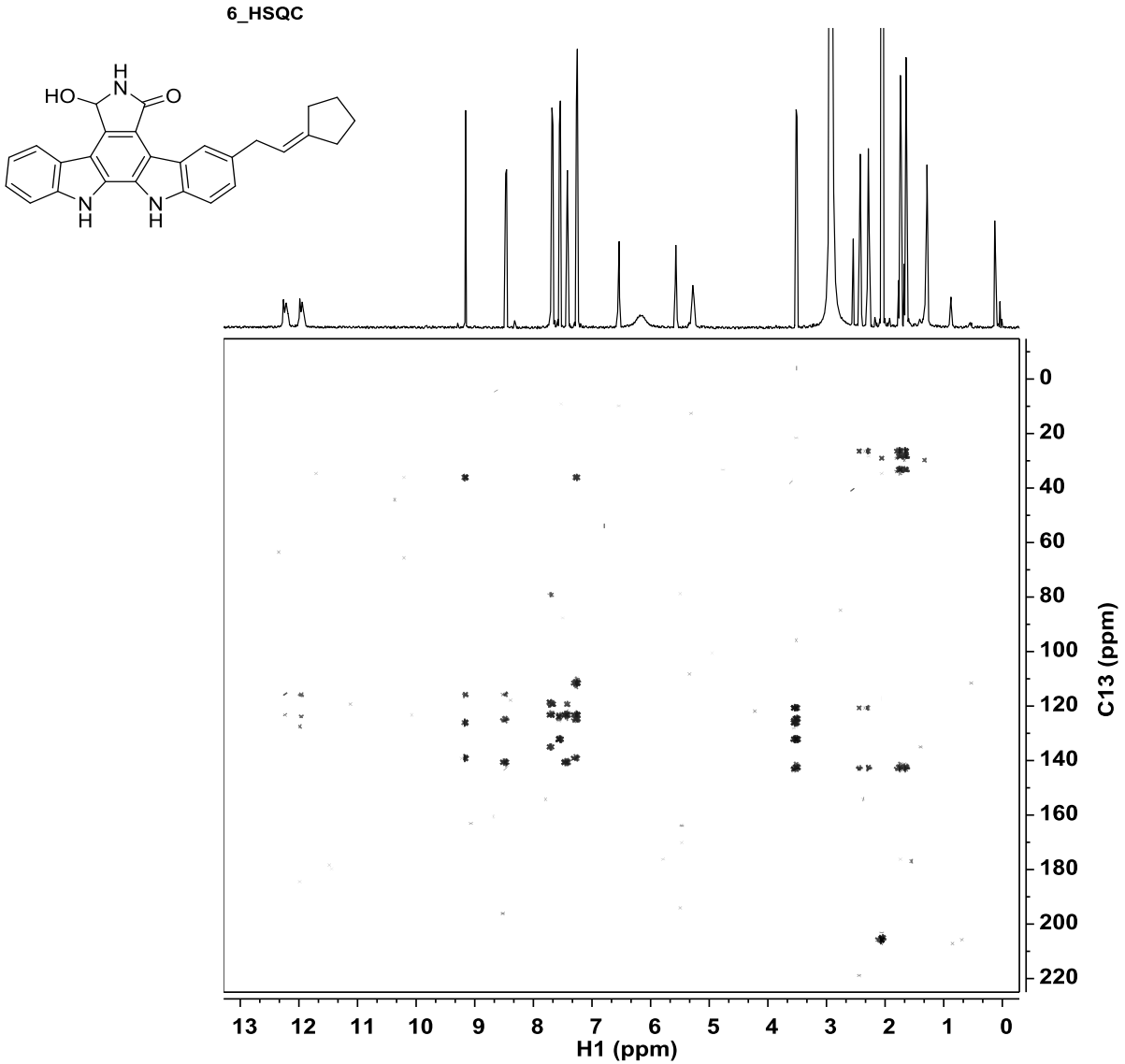
^{1}H NMR Spectrum (500 MHz) of compound **61** in Acetone-d6



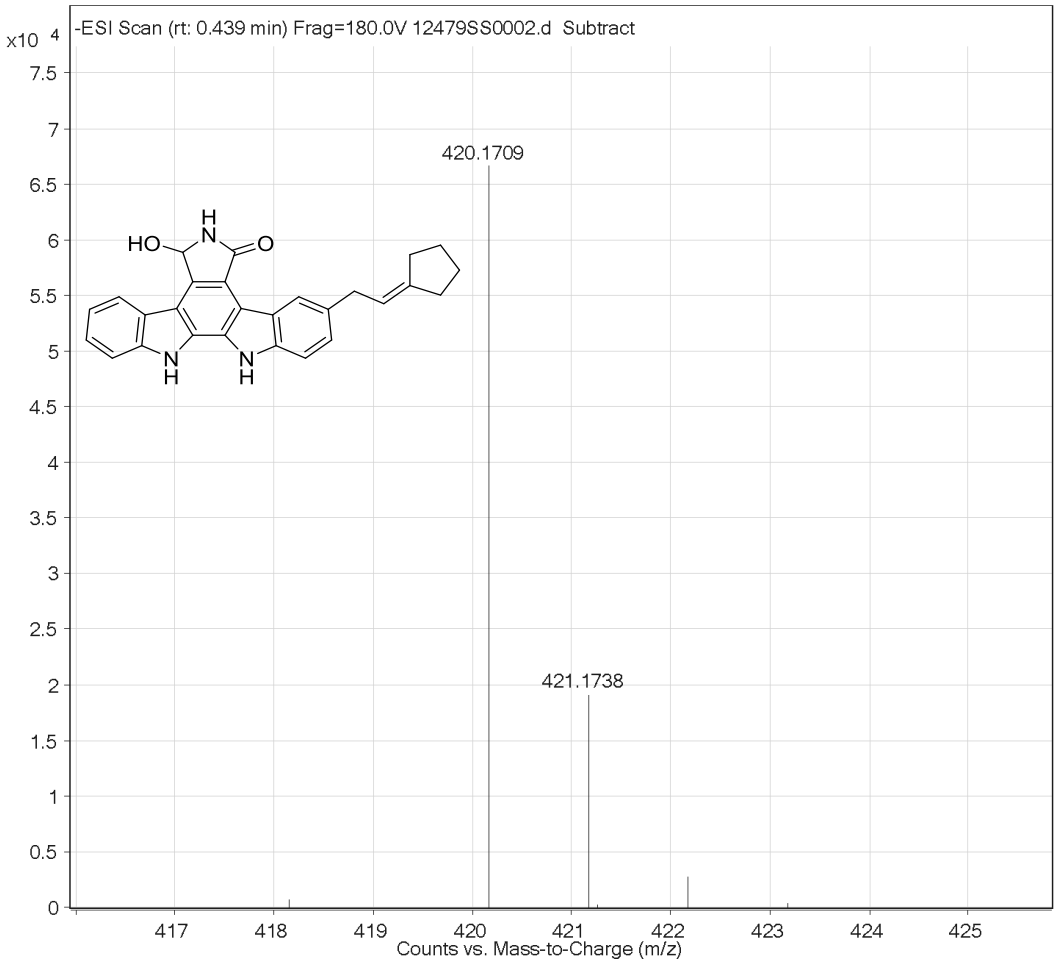
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **61** in Acetone-d6



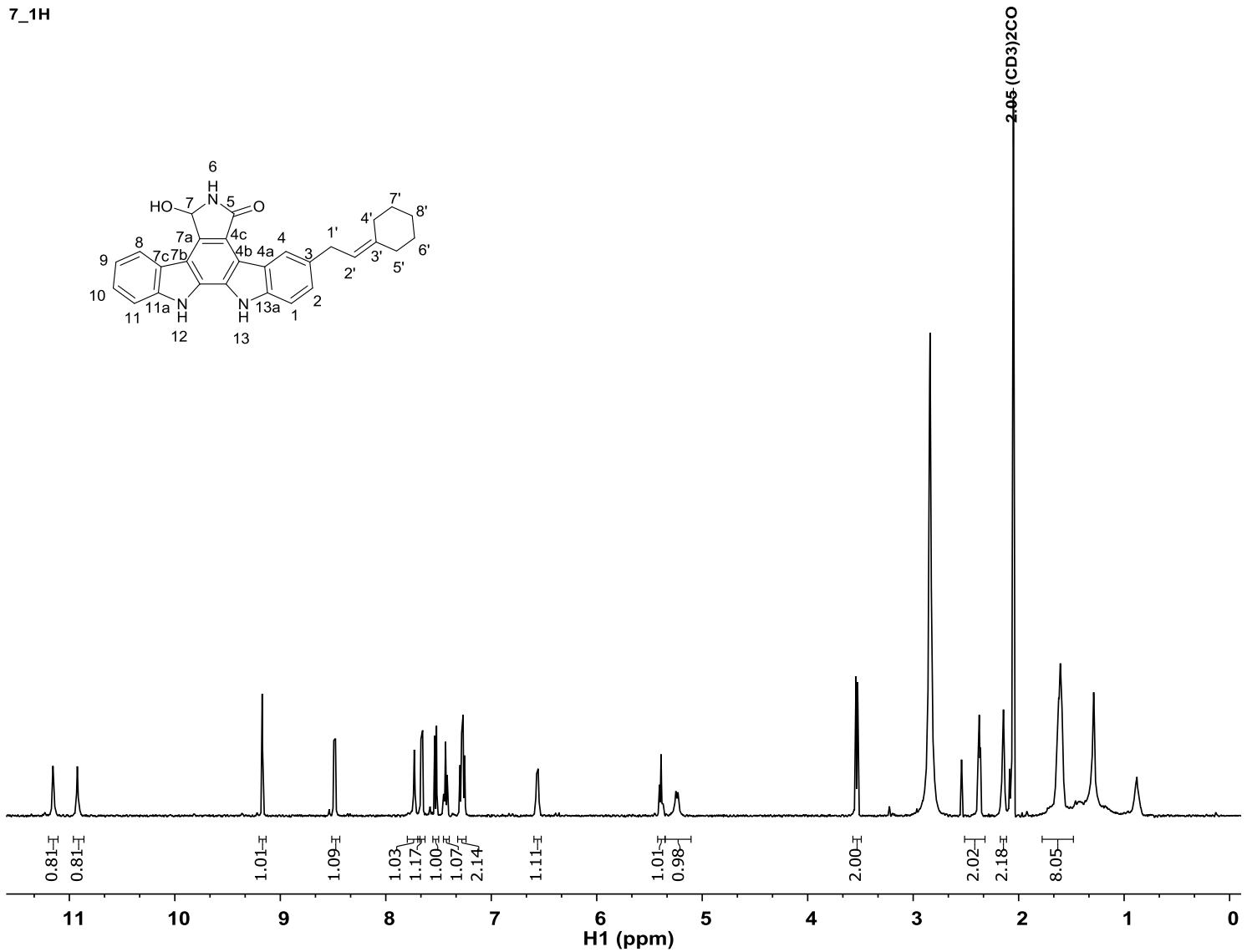
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **61** in Acetone-d6



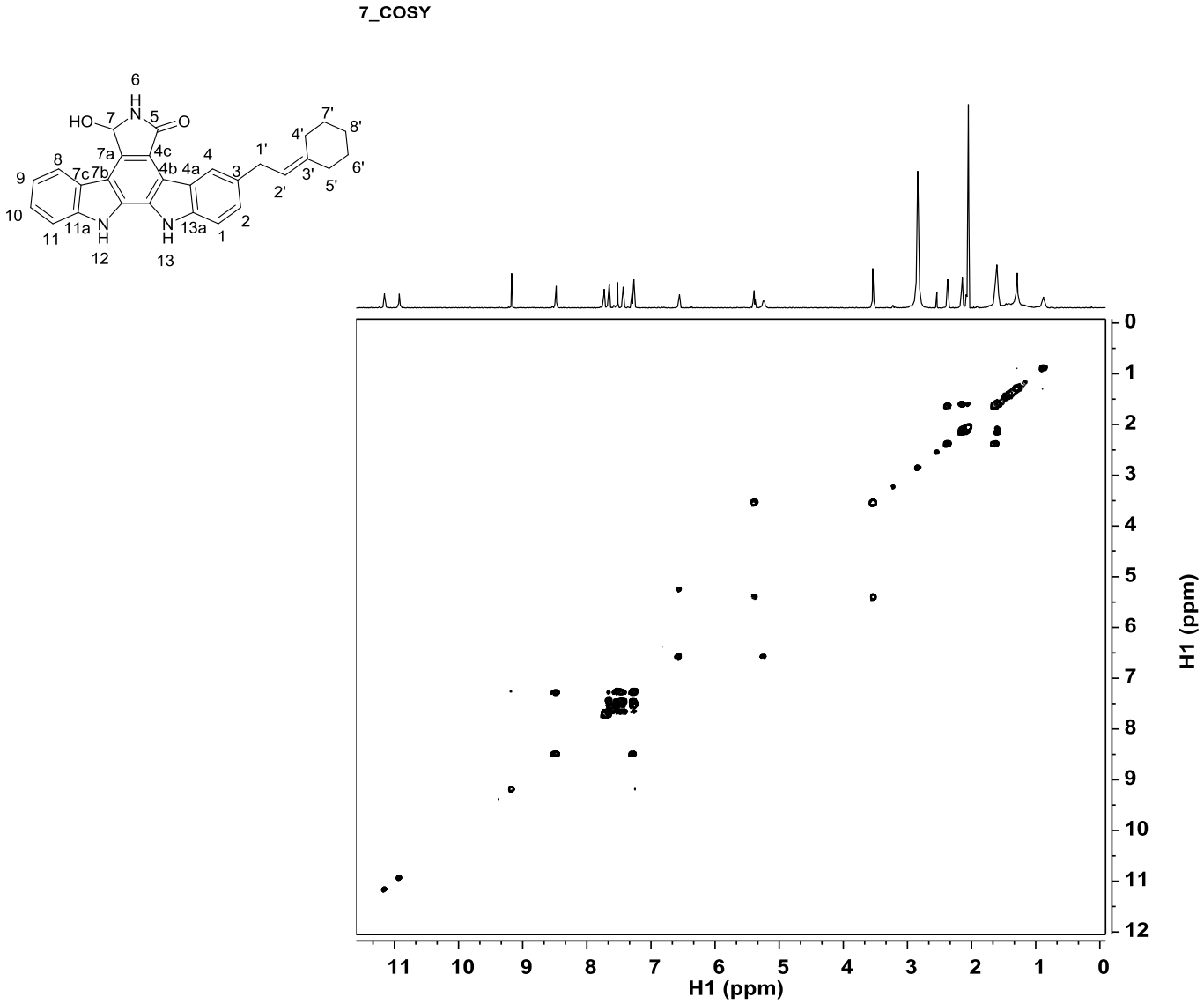
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **61** in Acetone-d6



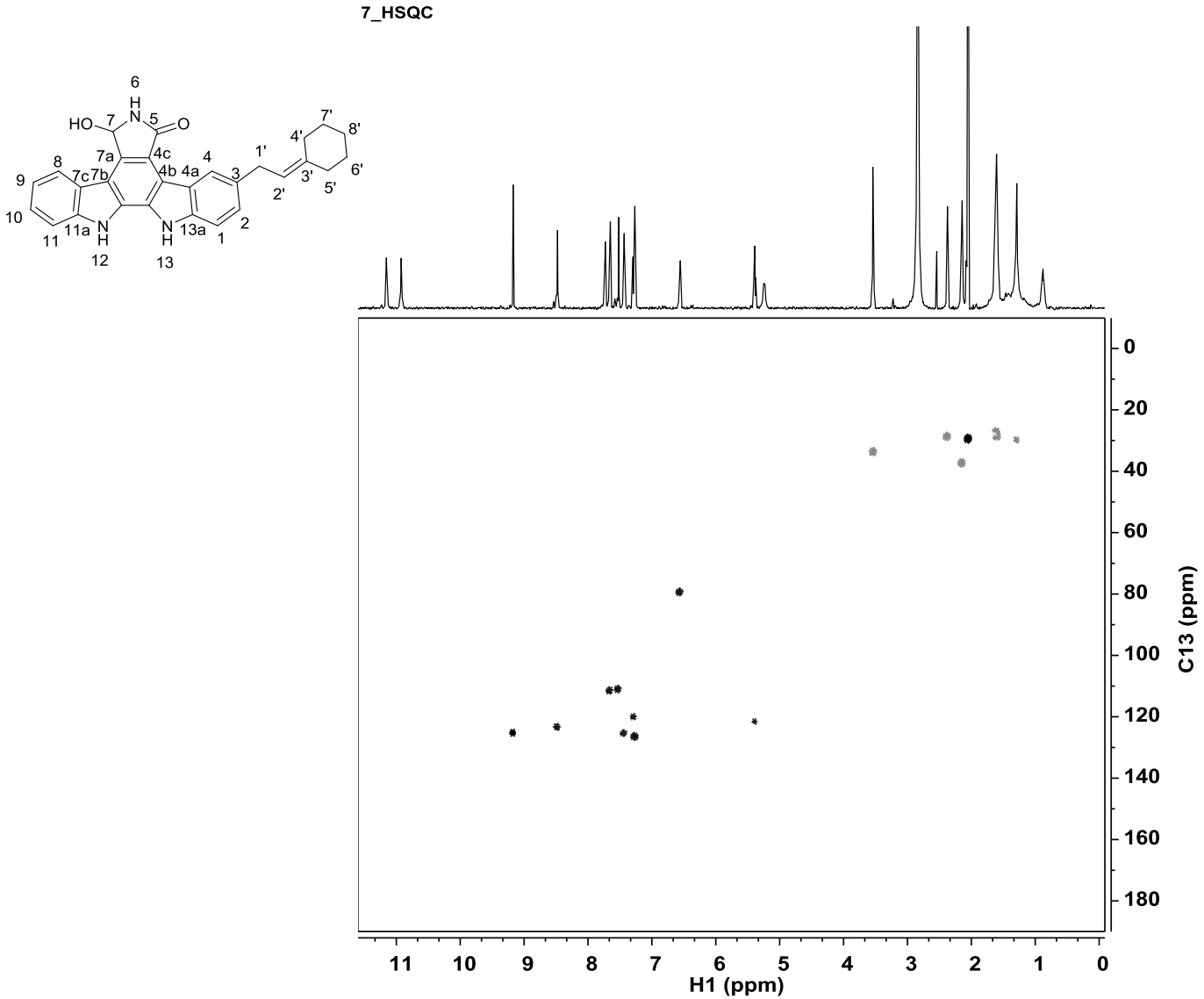
HRMS spectrum of **61**



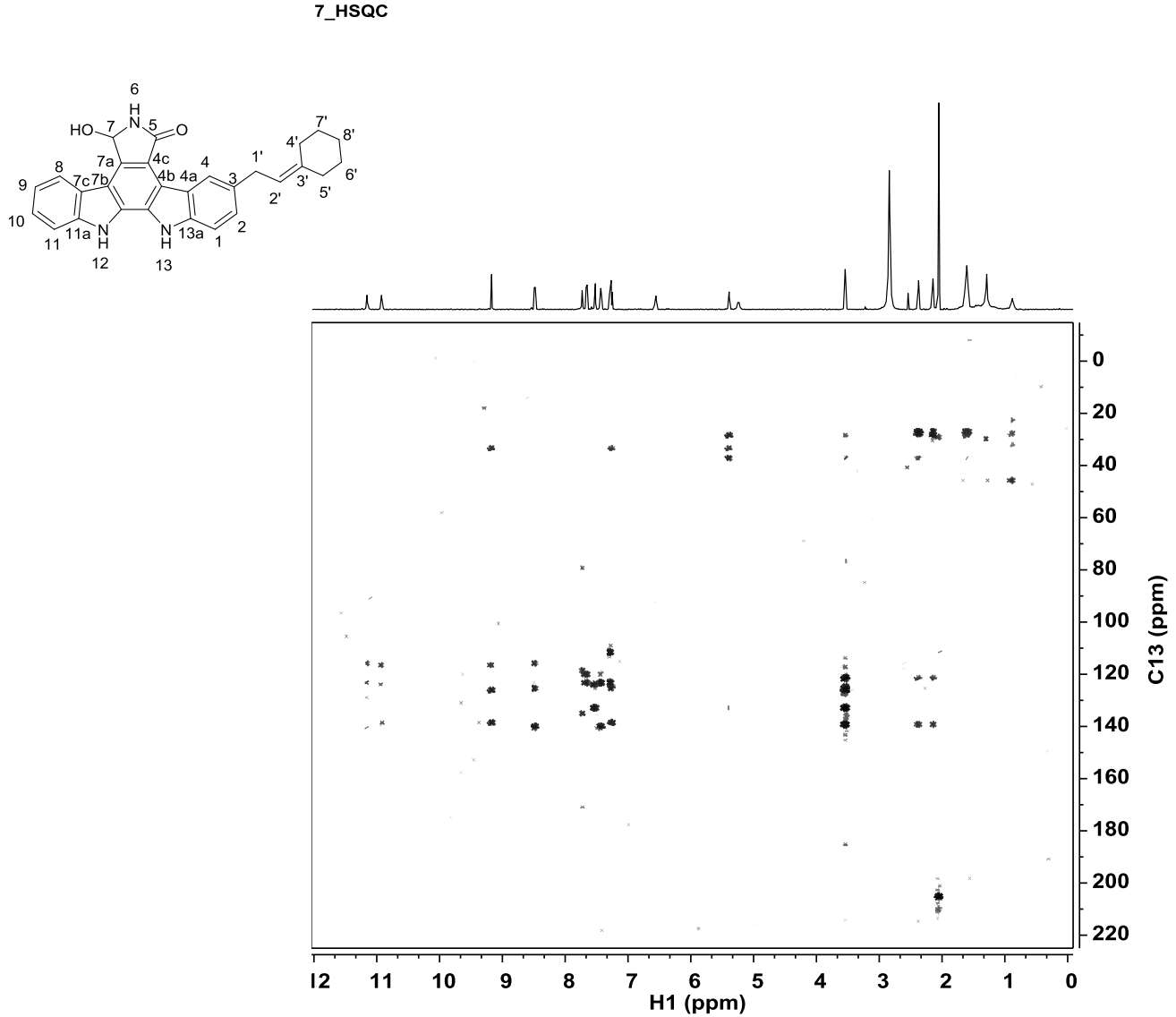
^{1}H NMR Spectrum (500 MHz) of compound **62** in Acetone-d6



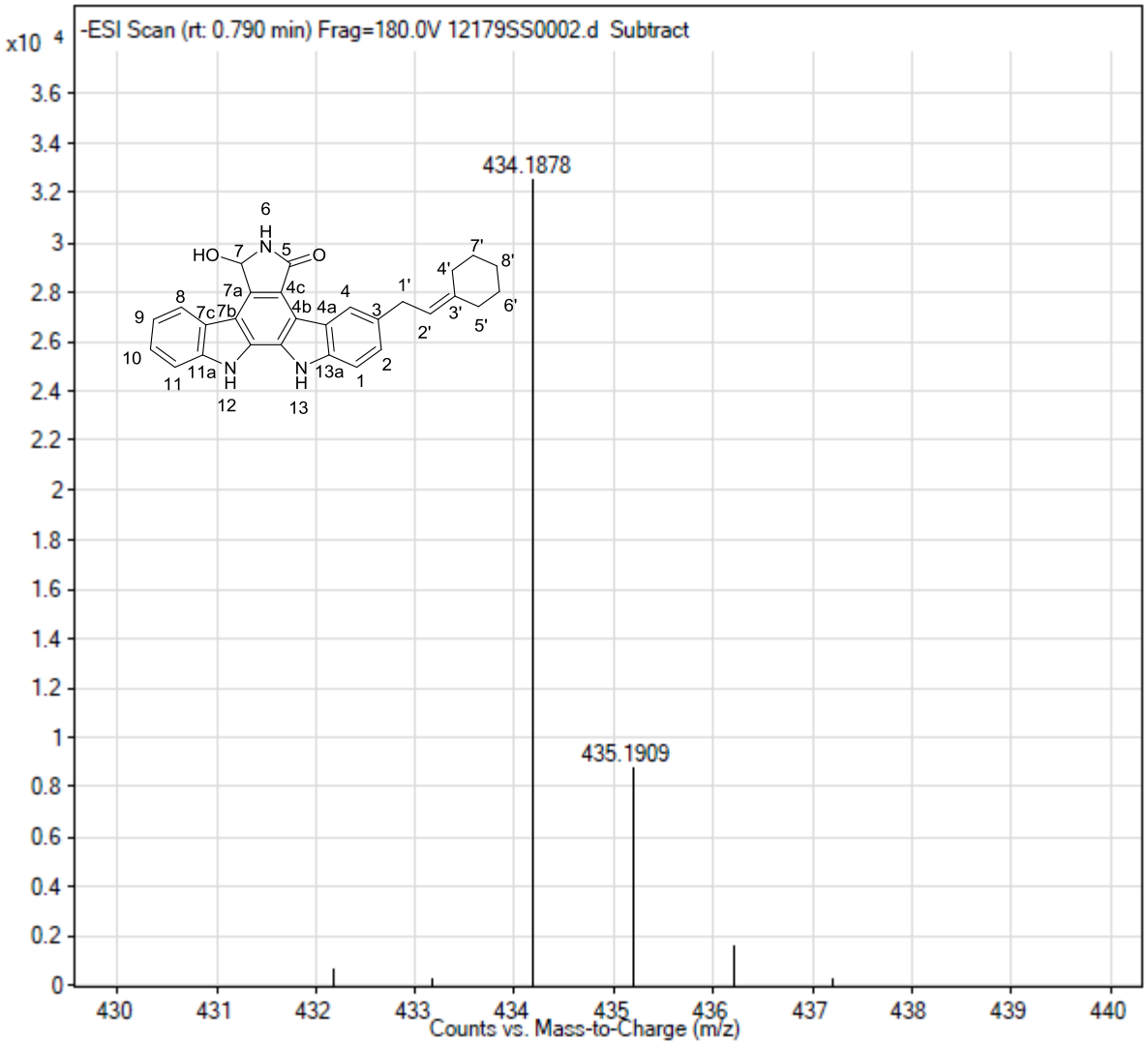
2D ^{1}H-^{1}H COSY NMR spectrum (500 MHz) of **62** in Acetone-d6



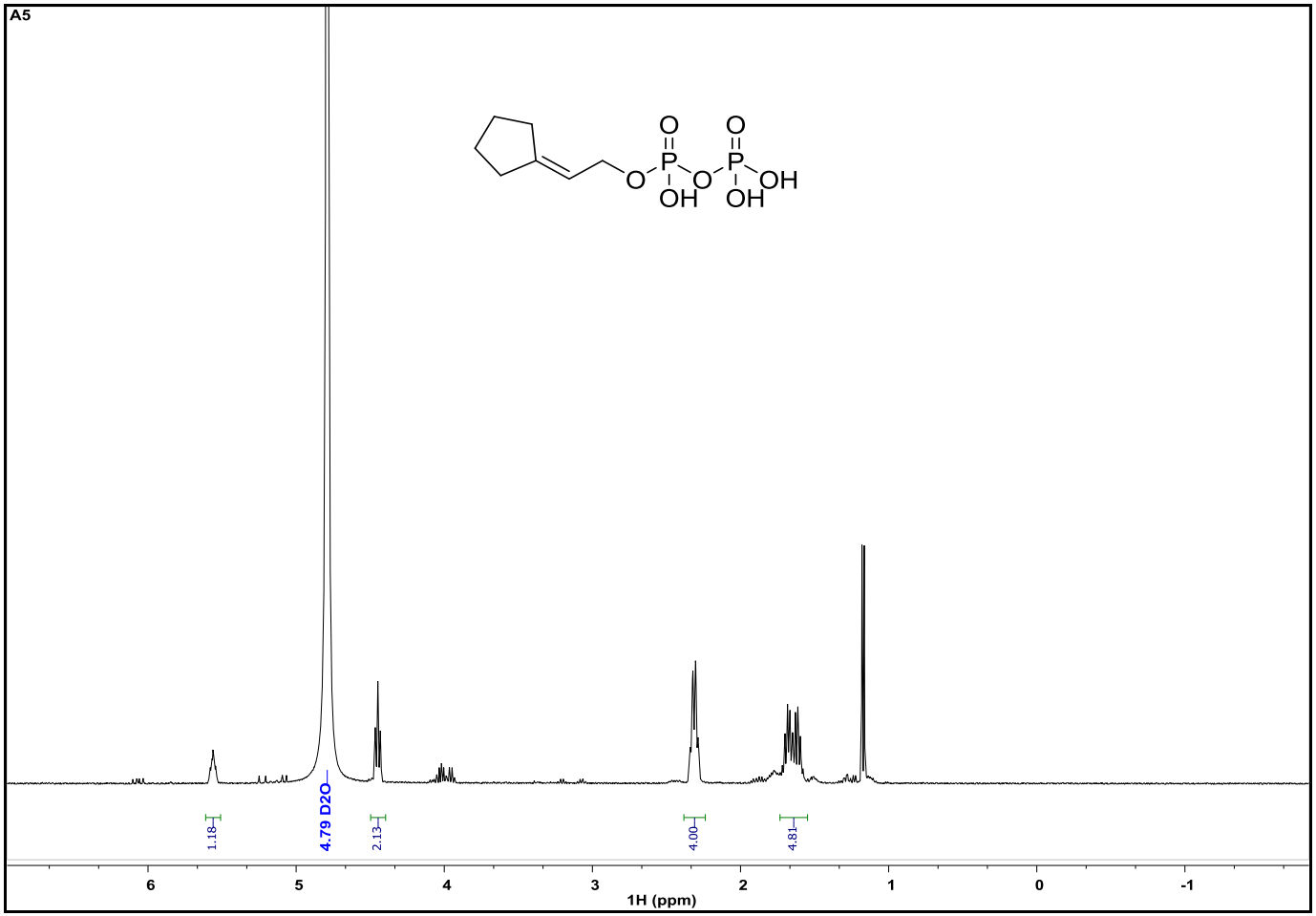
2D ^{1}H-^{13}C HSQC NMR spectrum (500 MHz) of **62** in Acetone-d6



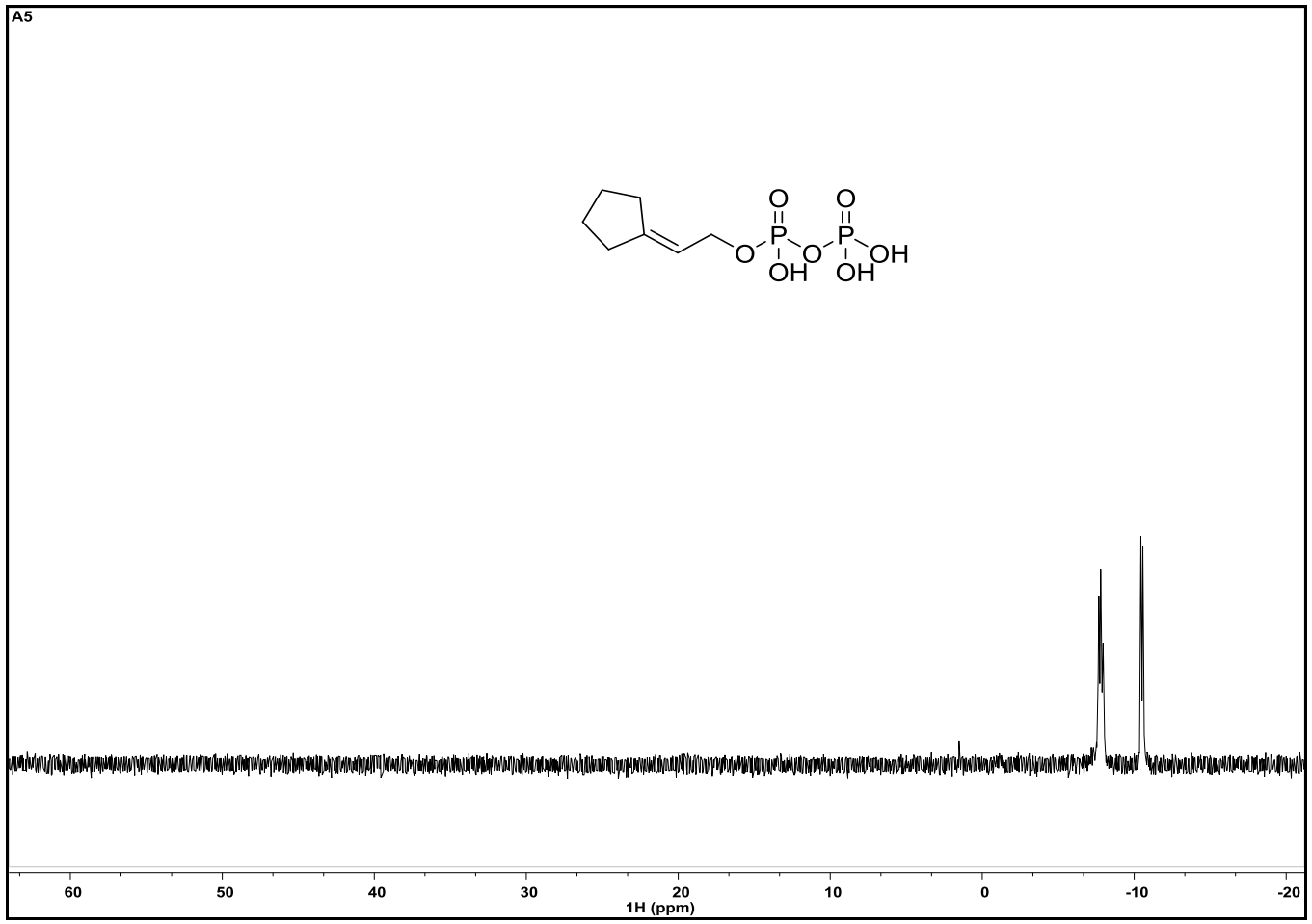
2D ^{1}H-^{13}C HMBC NMR spectrum (500 MHz) of **62** in Acetone-d6



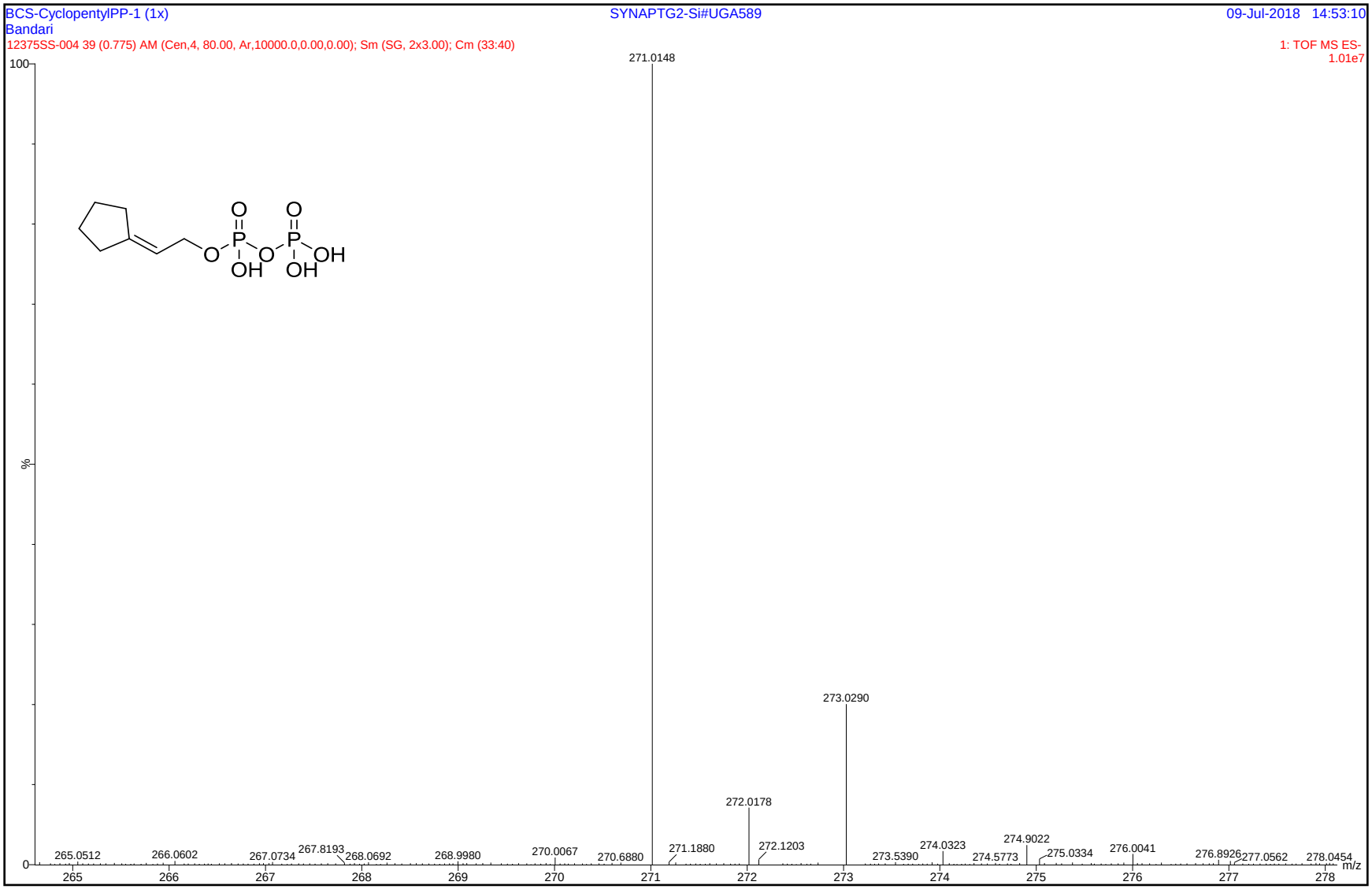
HRMS spectrum of **62**



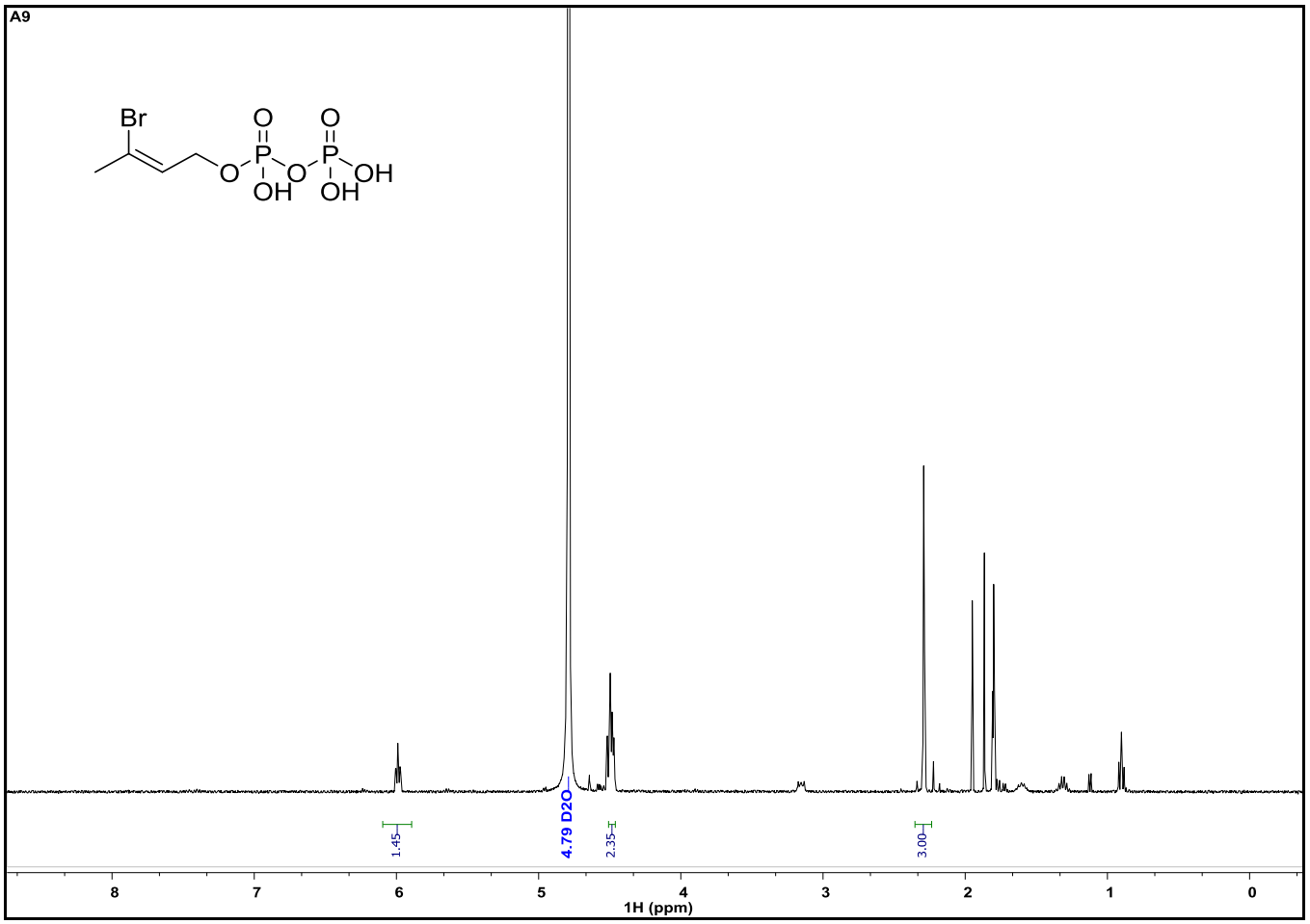
^{1}H NMR Spectrum (400 MHz) of compound **5** in D_2O



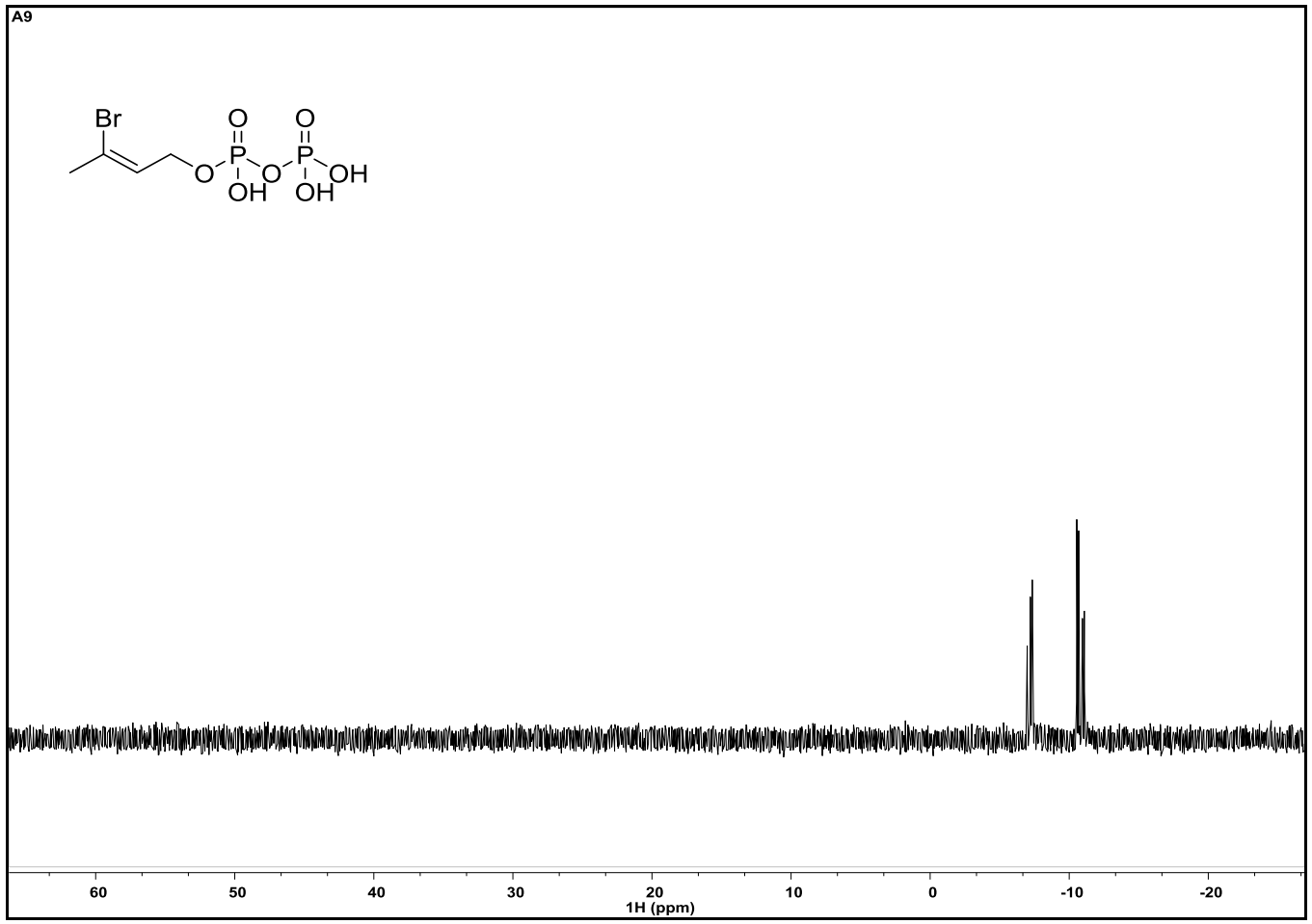
^{31}P NMR Spectrum (162 MHz) of compound **5** in D_2O



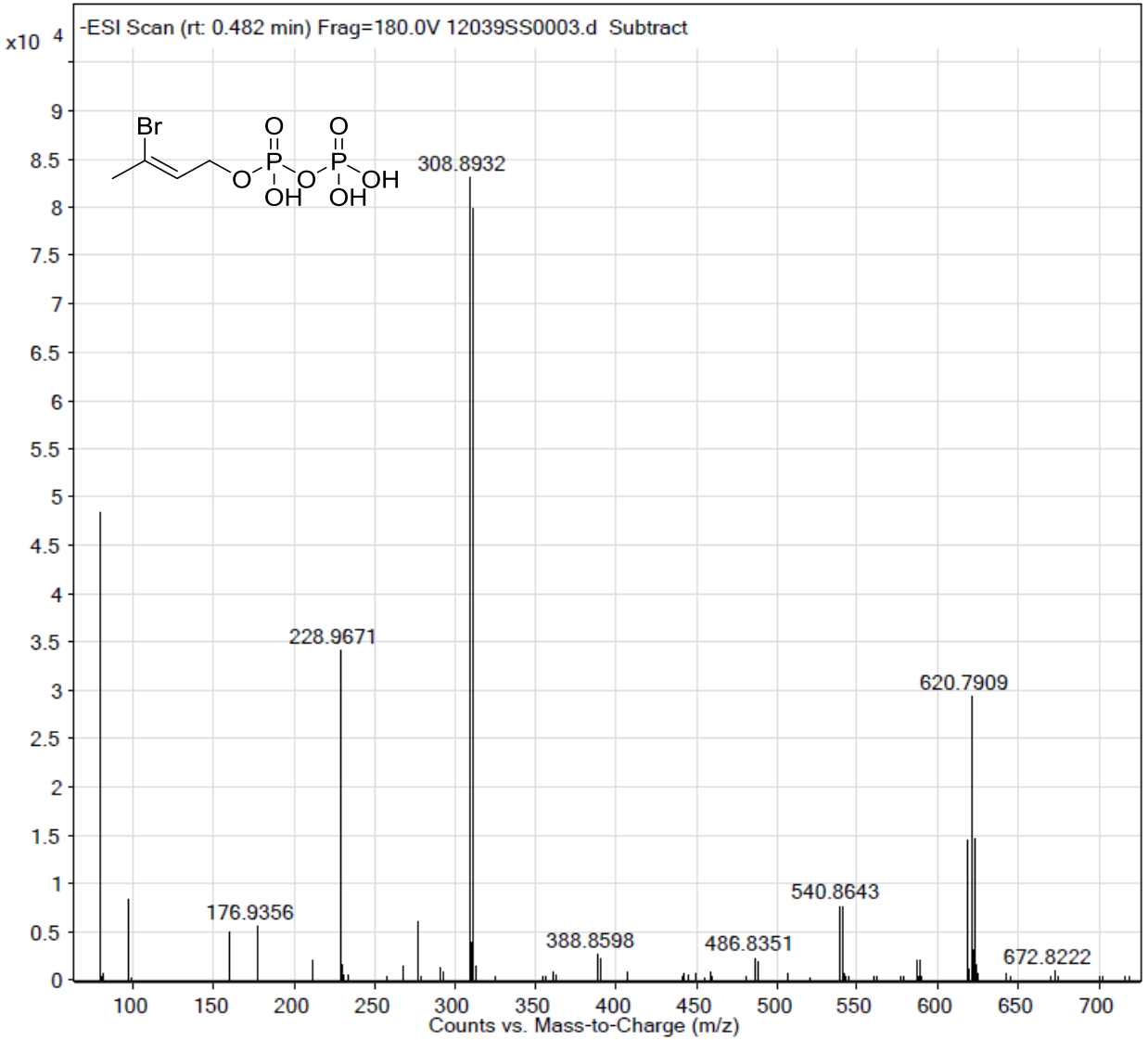
HRMS spectrum of **5**



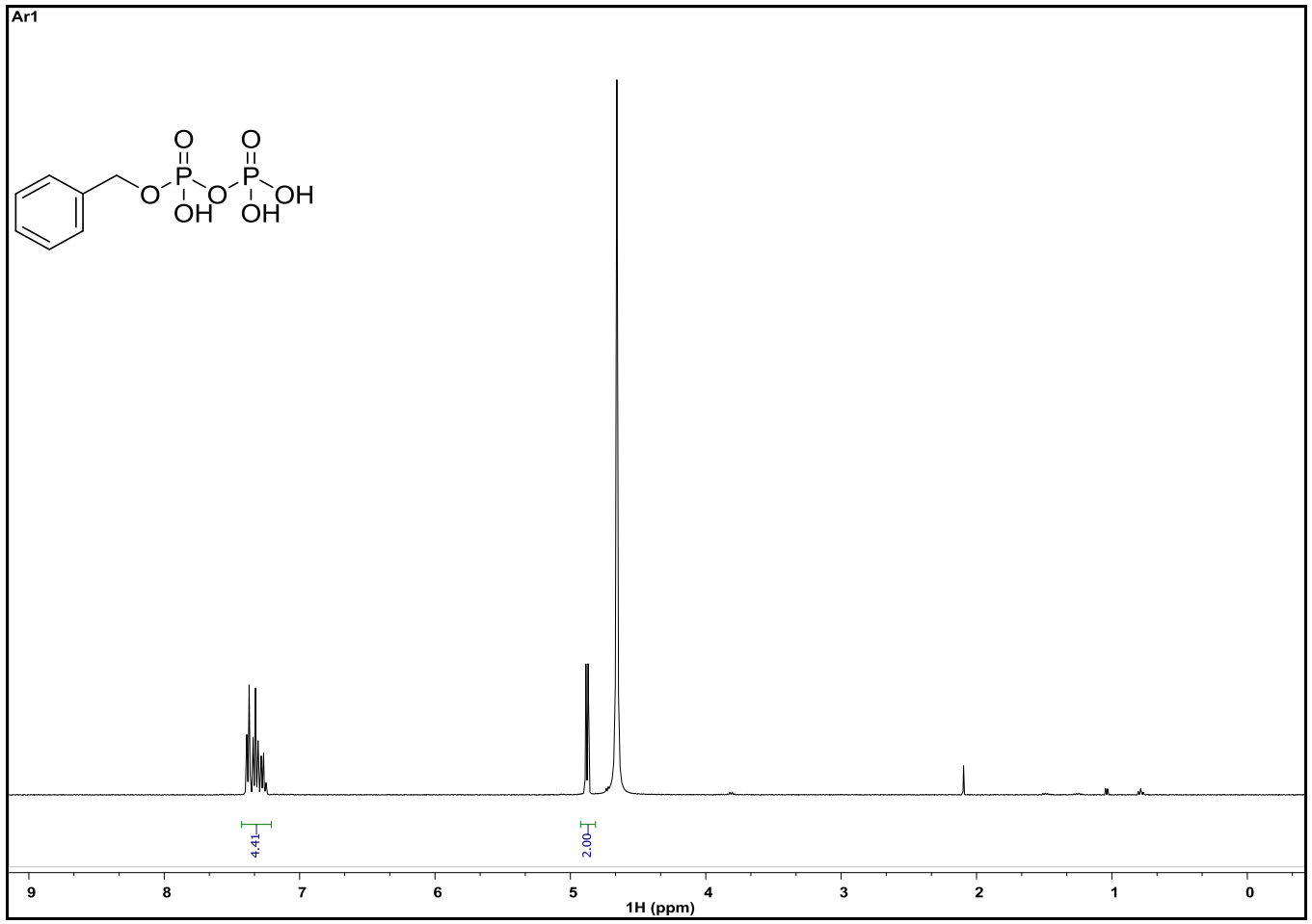
^{1}H NMR Spectrum (400 MHz) of compound **9** in D_2O



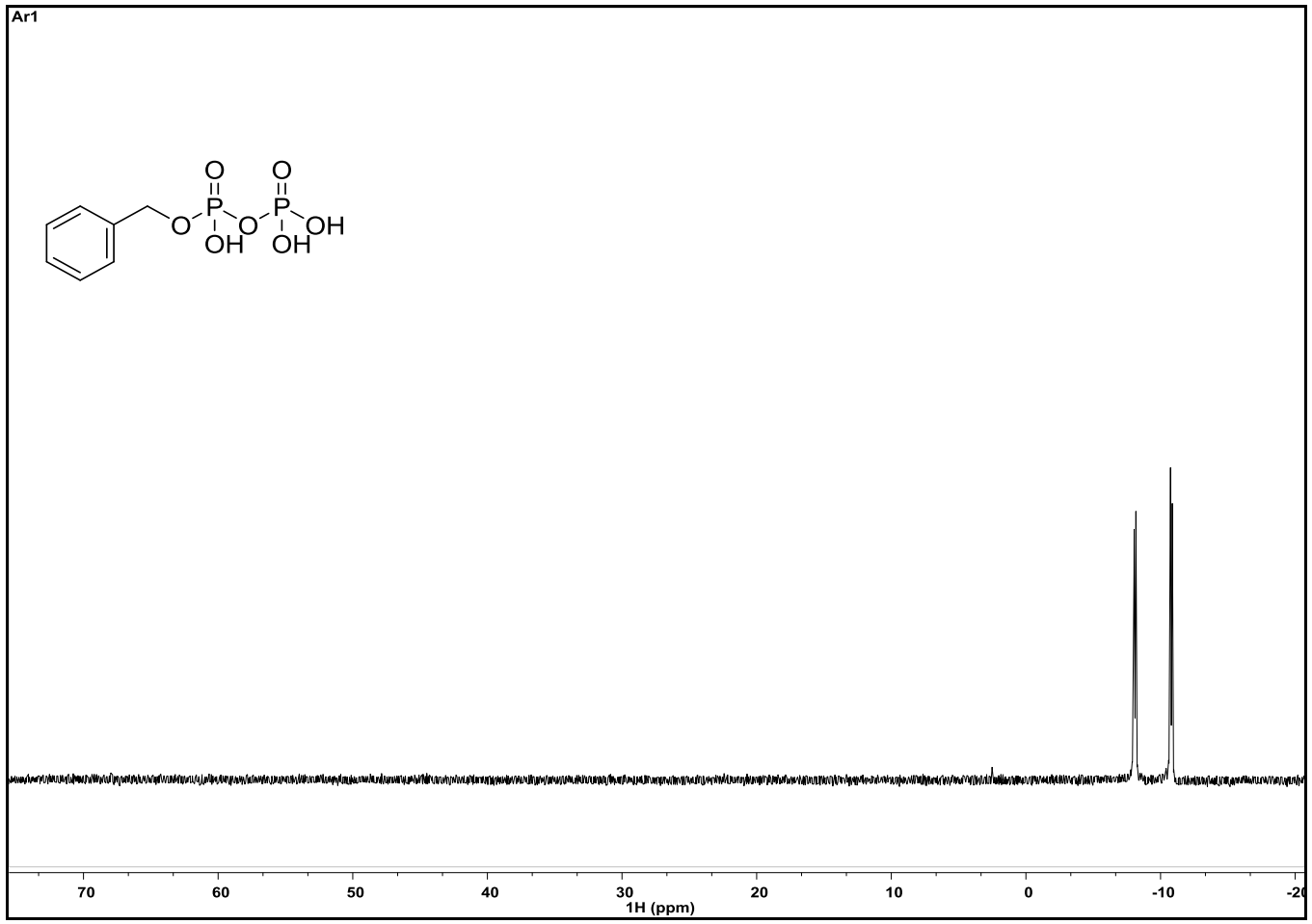
^{31}P NMR Spectrum (162 MHz) of compound **9** in D_2O



HRMS spectrum of **9**

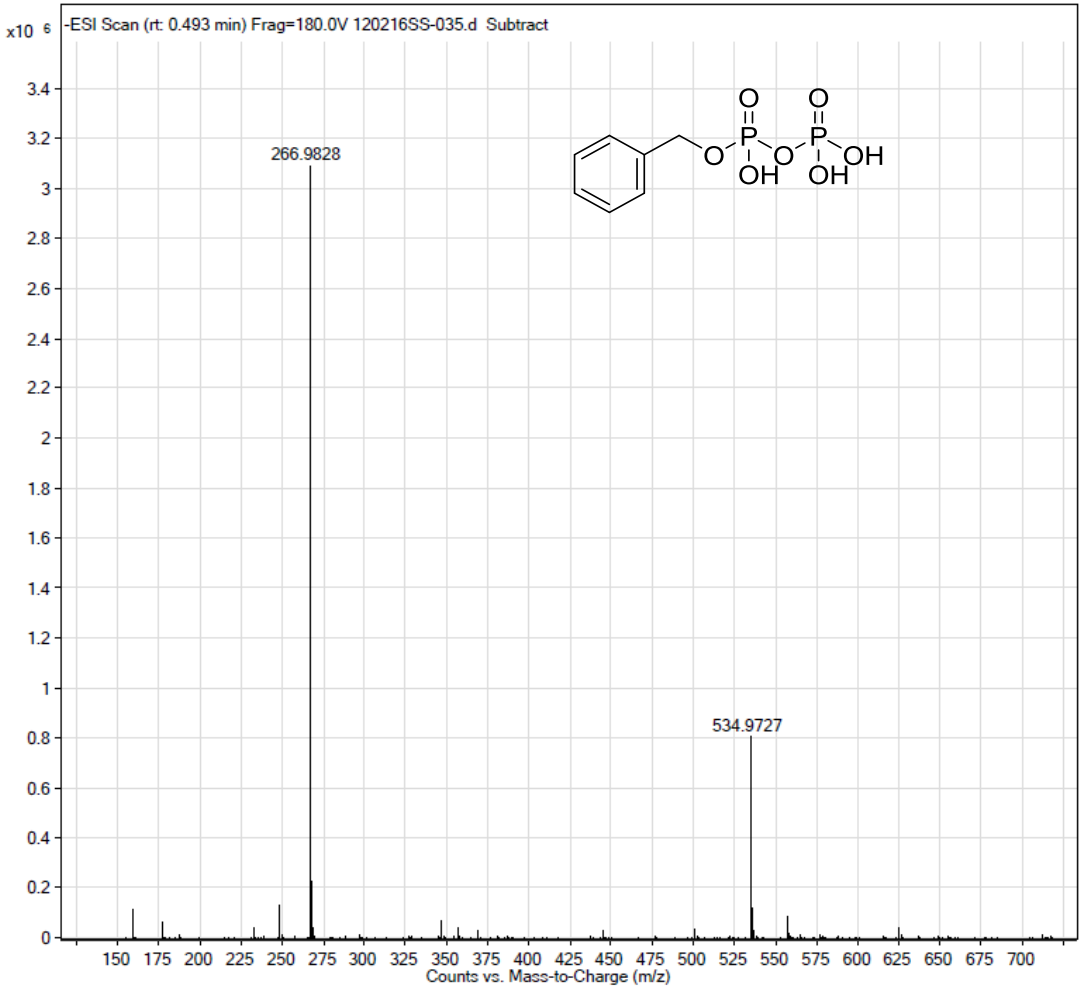


^{1}H NMR Spectrum (400 MHz) of compound **22** in D_2O

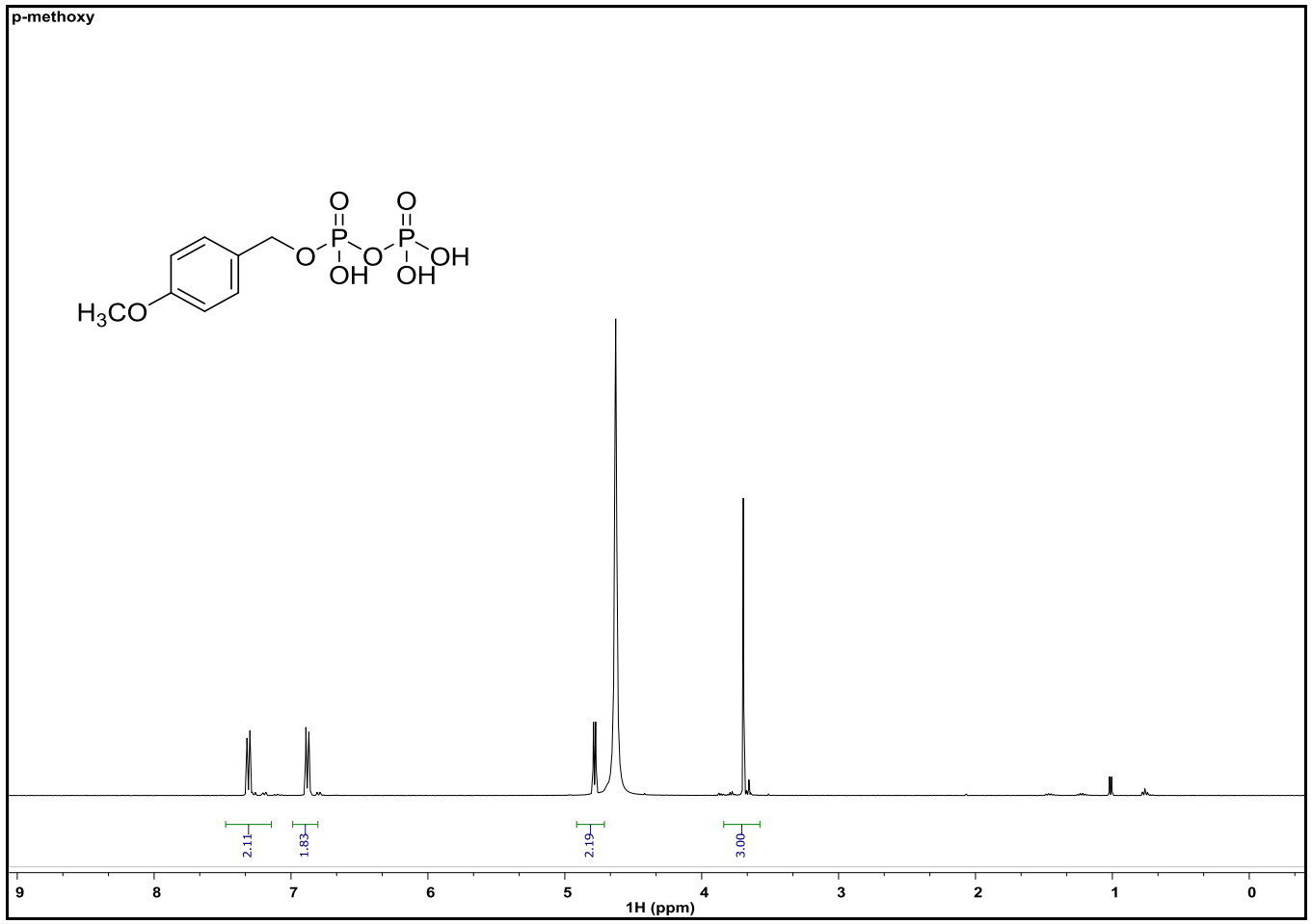


^{31}P NMR Spectrum (162 MHz) of compound **22** in D_2O

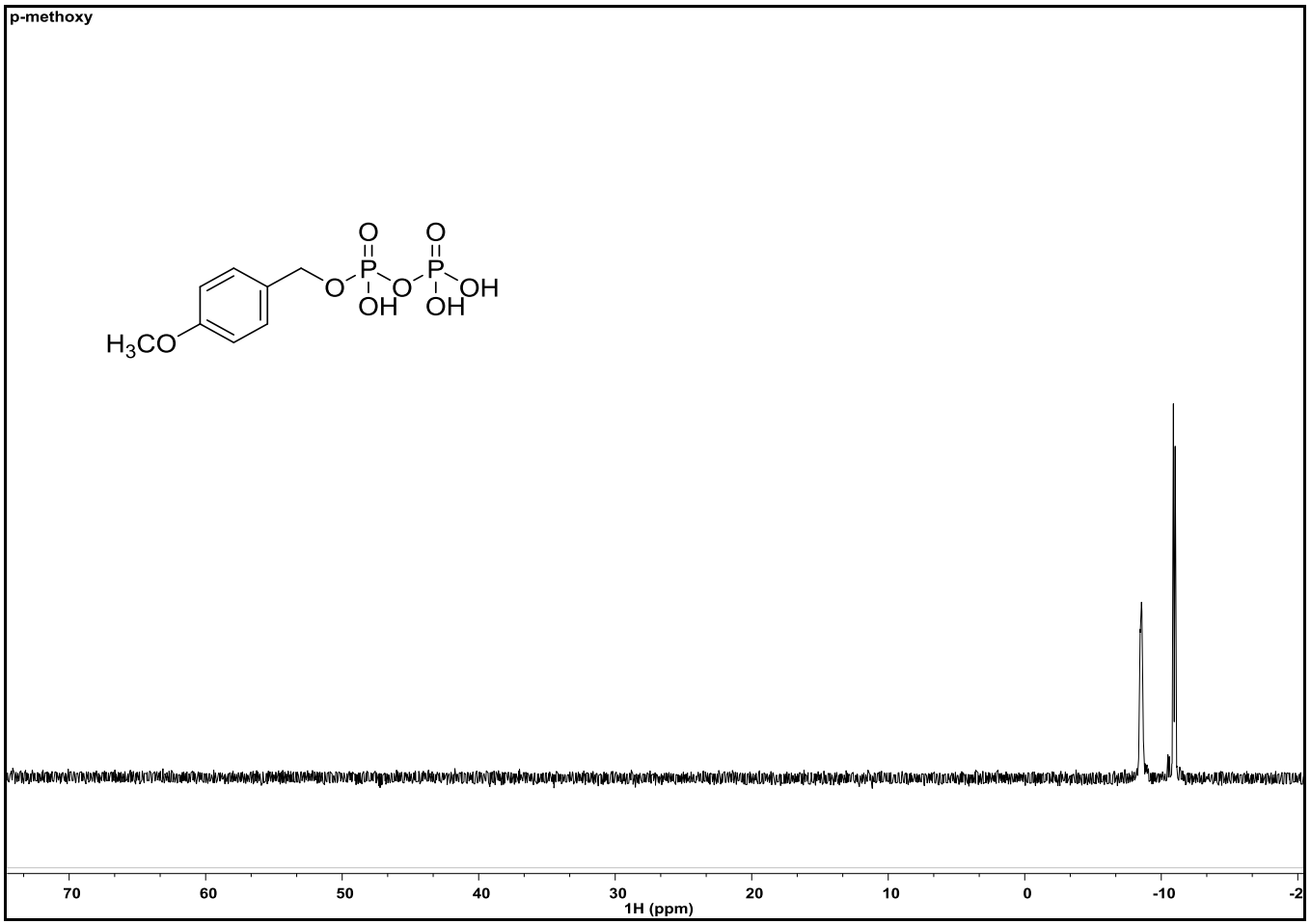
Sample Name	BCS-33	**Position**	P1-A9	**Instrument Name**	Instrument 1
User Name		**Inj Vol**	5	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	120216SS-035.d
ACQ Method	Neg-Loop-MS.m	**Comment**		**Acquired Time**	12/2/2016 6:51:09 PM



HRMS spectrum of **22**

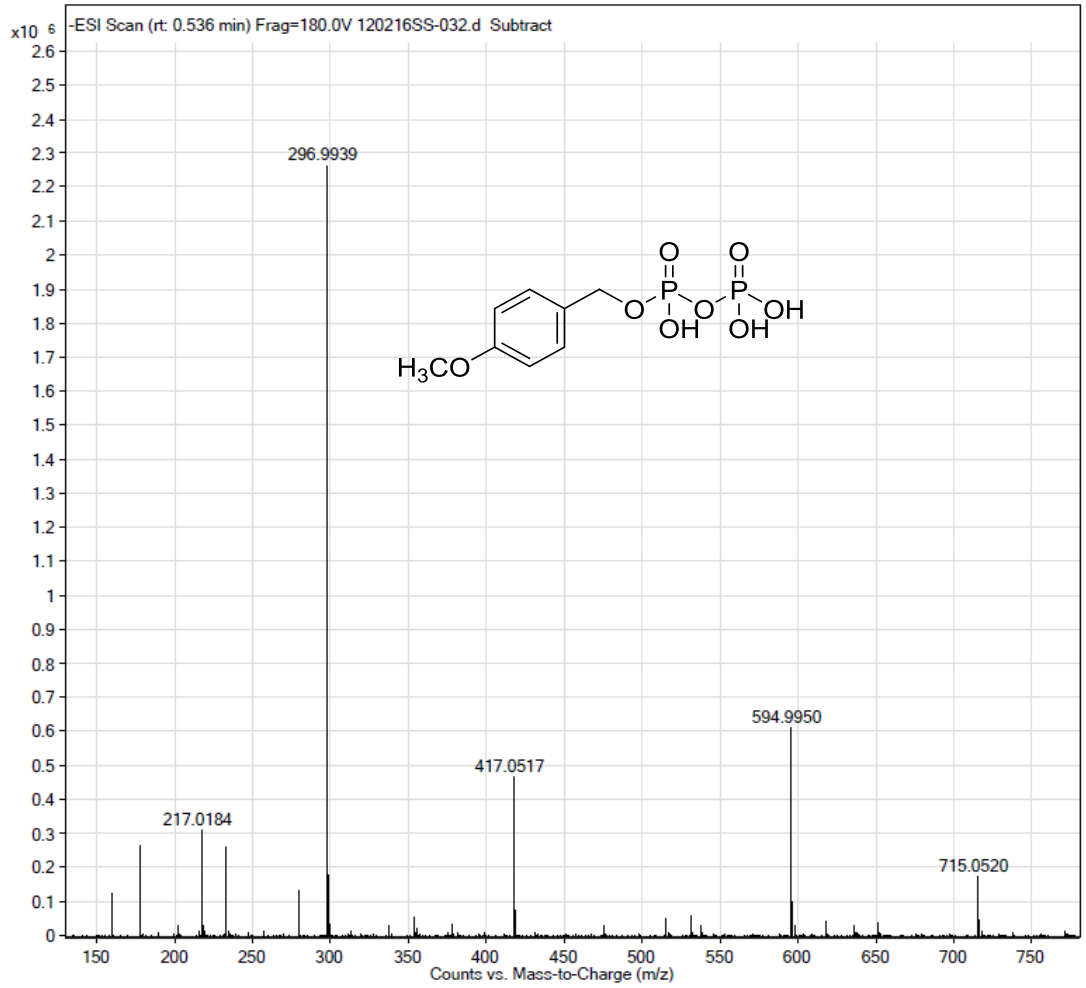


^{1}H NMR Spectrum (400 MHz) of compound **23** in D_2O

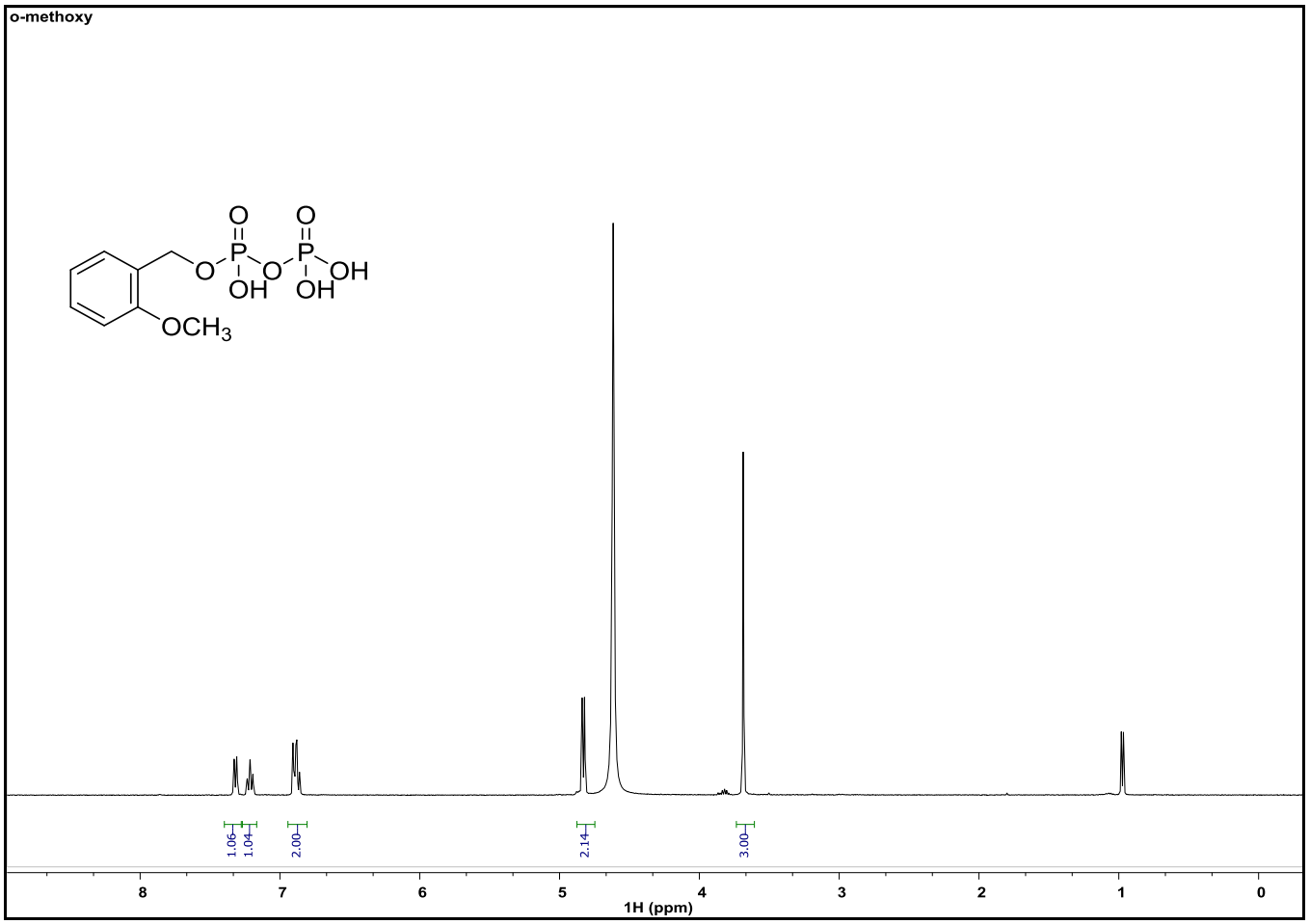


^{31}P NMR Spectrum (162 MHz) of compound **23** in D_2O

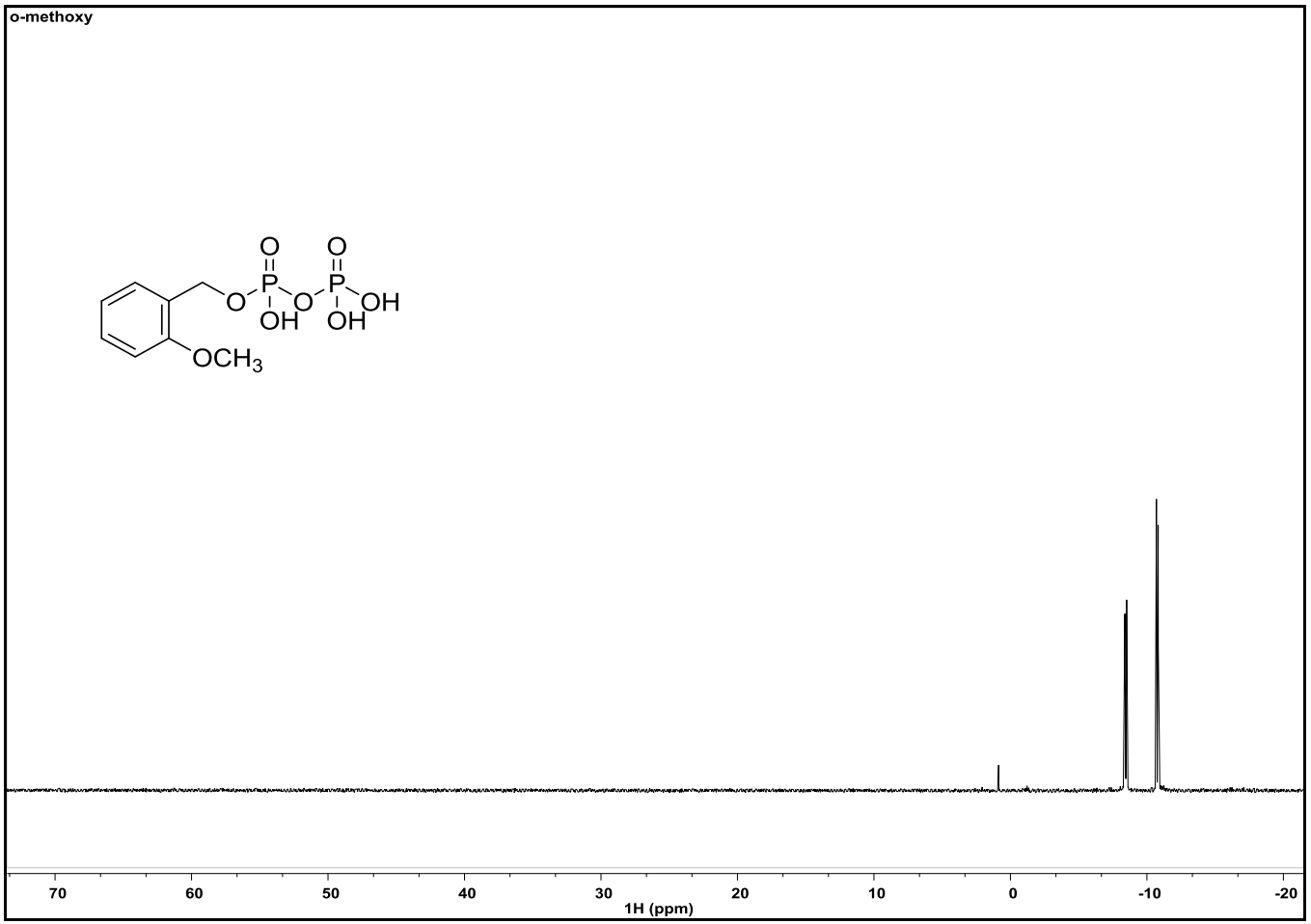
Sample Name	BCS-41	**Position**	P1-A8	**Instrument Name**	Instrument 1
User Name		**Inj Vol**	5	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	120216SS-032.d
ACQ Method	Neg-Loop-MS.m	**Comment**		**Acquired Time**	12/2/2016 6:39:13 PM



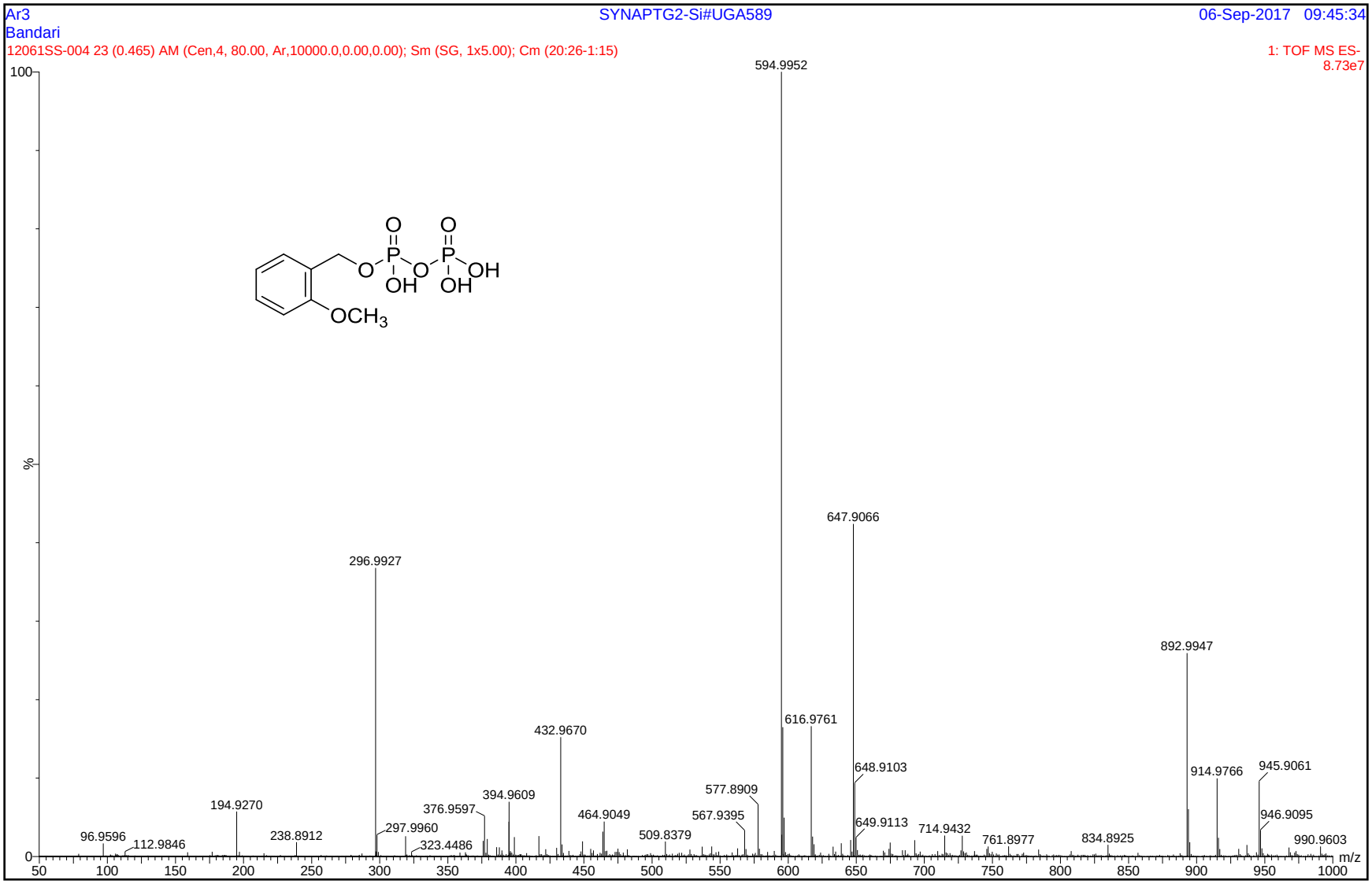
HRMS spectrum of **23**



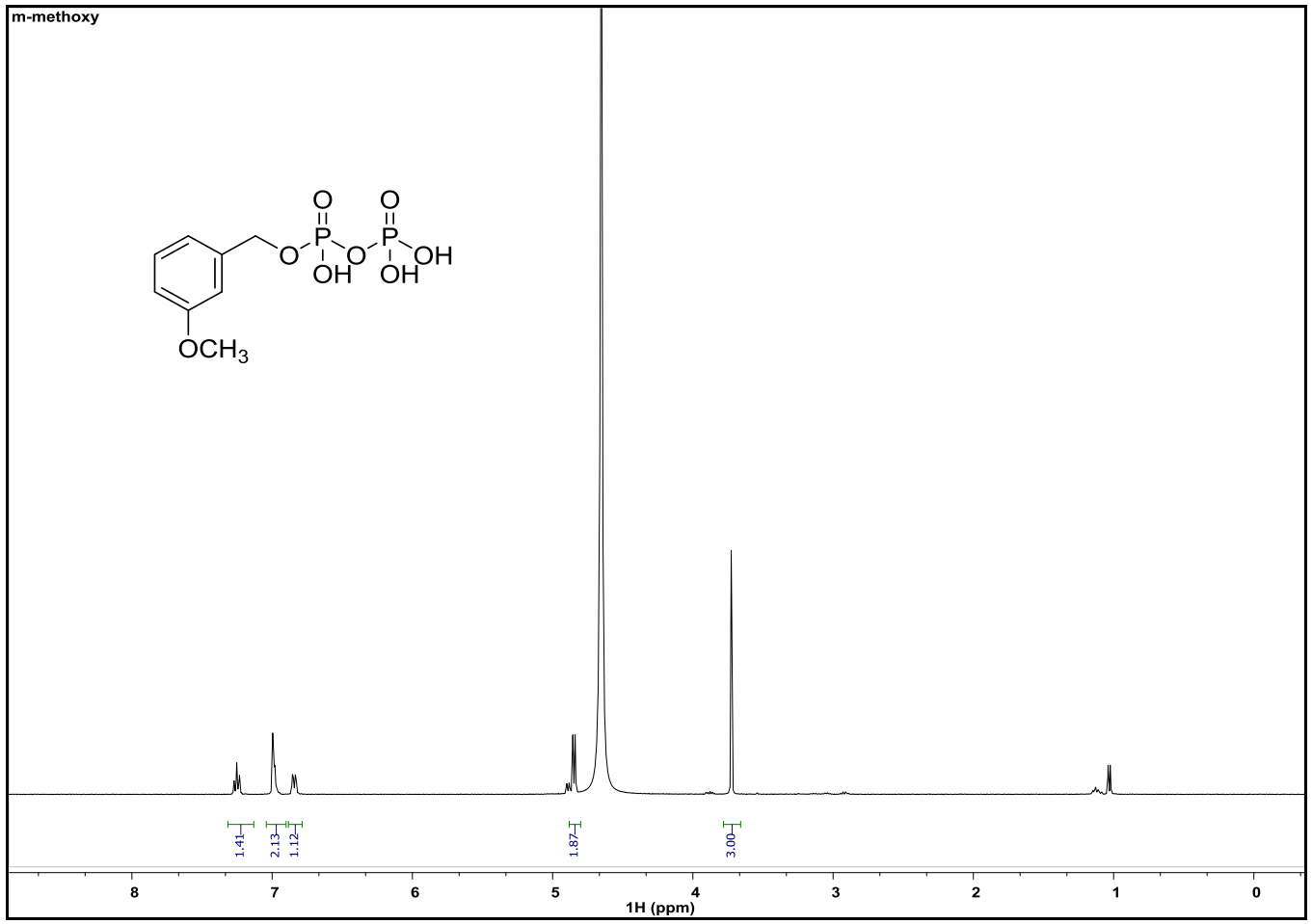
^{1}H NMR Spectrum (400 MHz) of compound **24** in D_2O



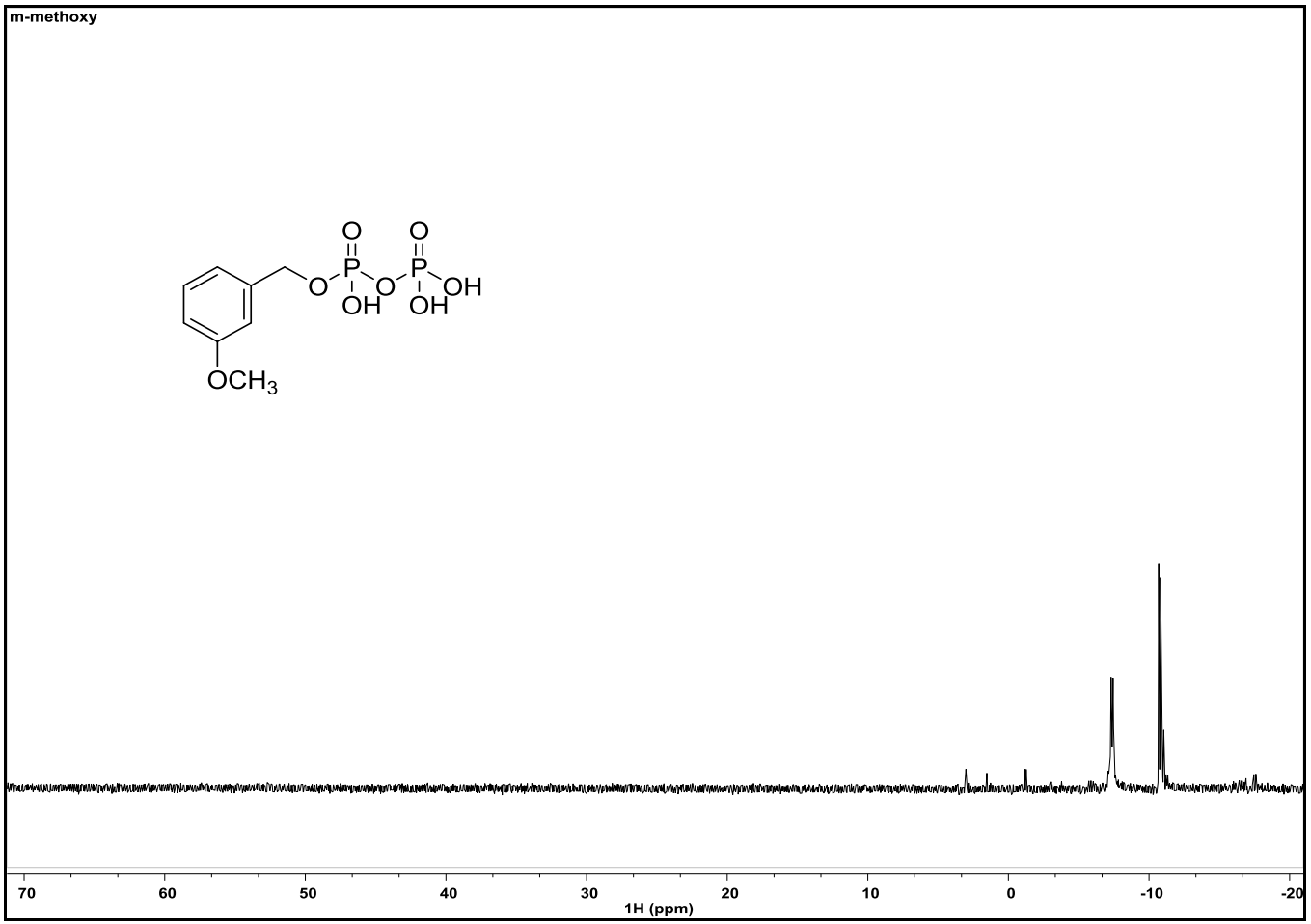
^{31}P NMR Spectrum (162 MHz) of compound **24** in D_2O



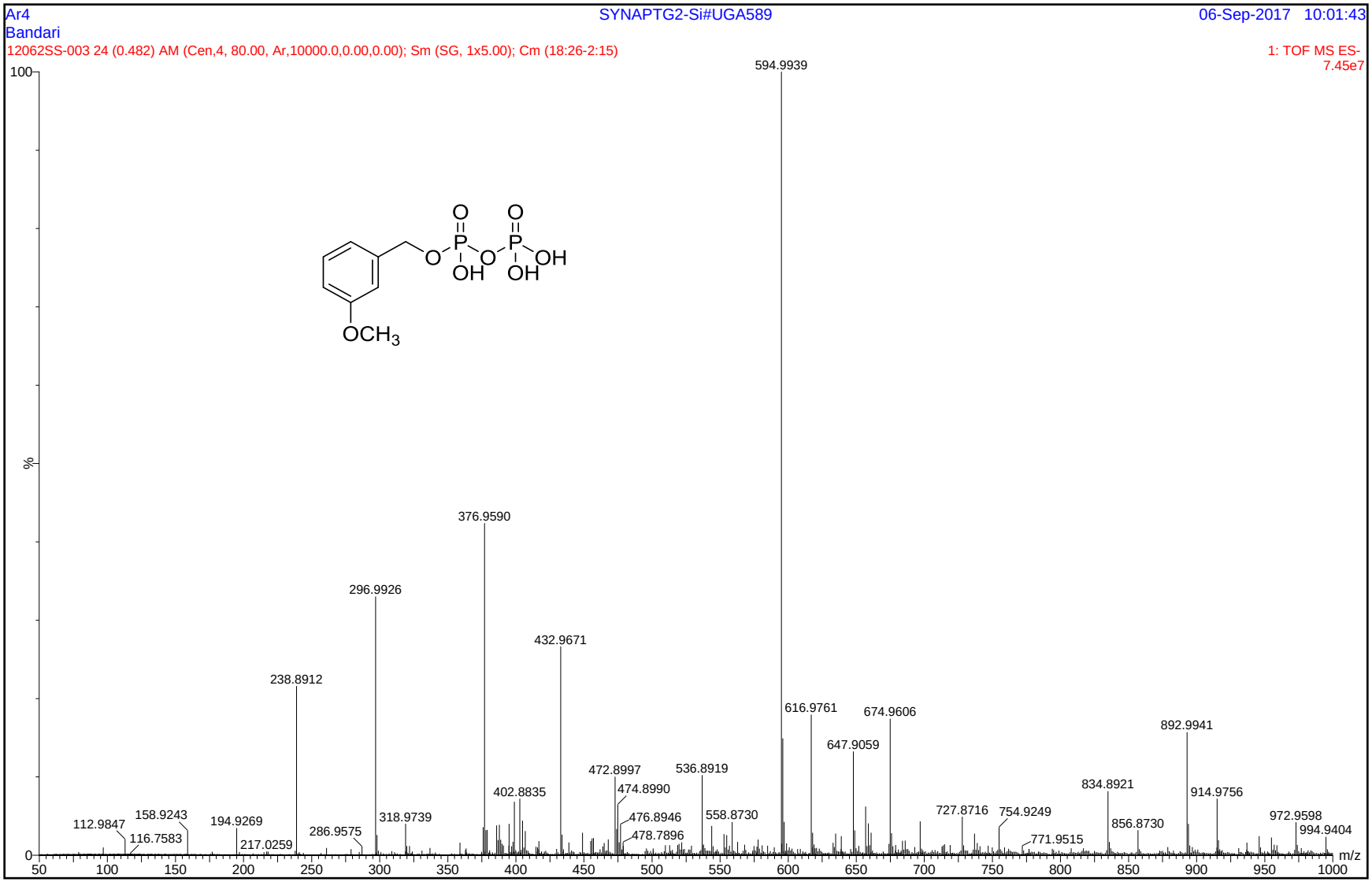
HRMS spectrum of **24**



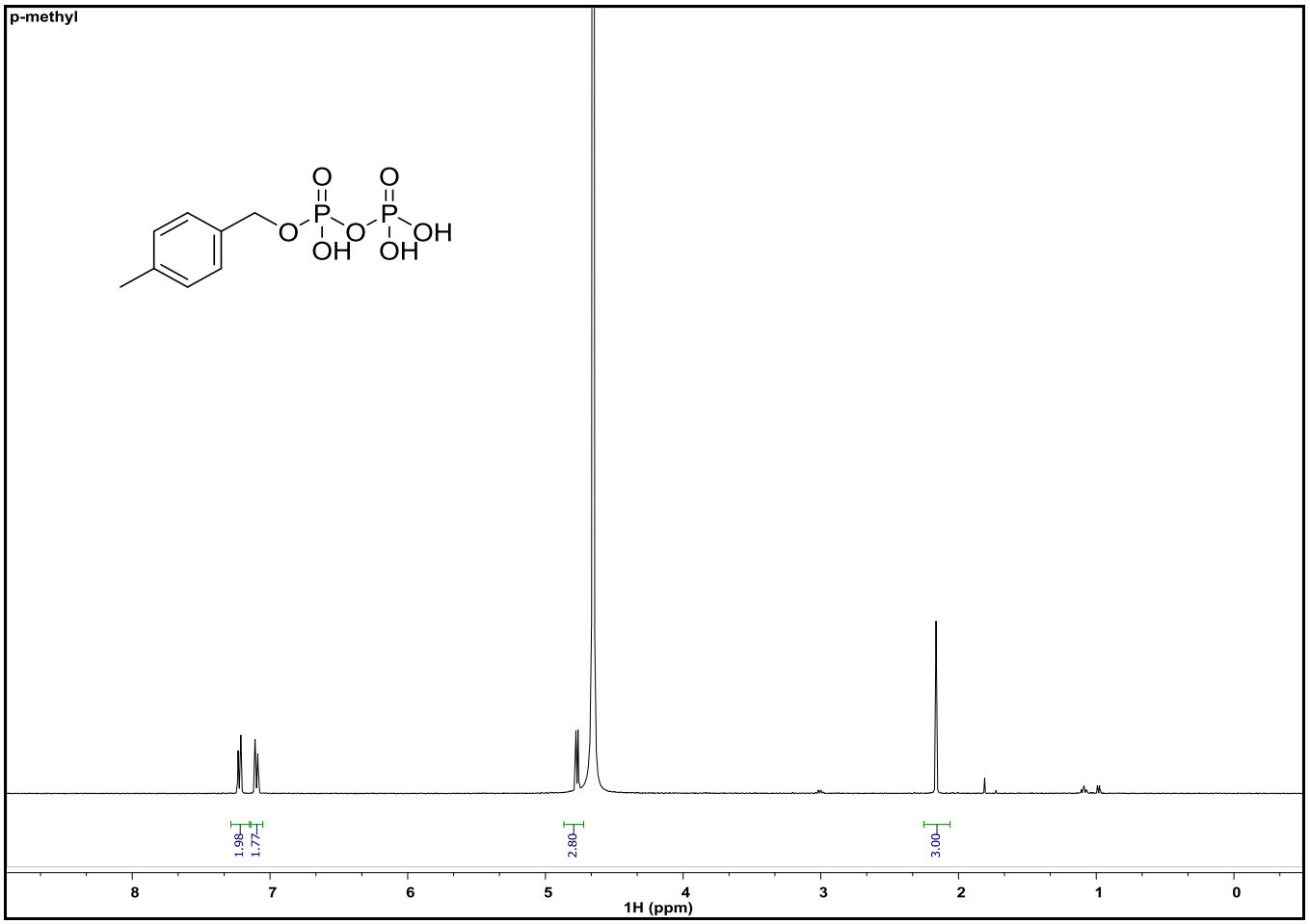
^{1}H NMR Spectrum (400 MHz) of compound **25** in D_2O



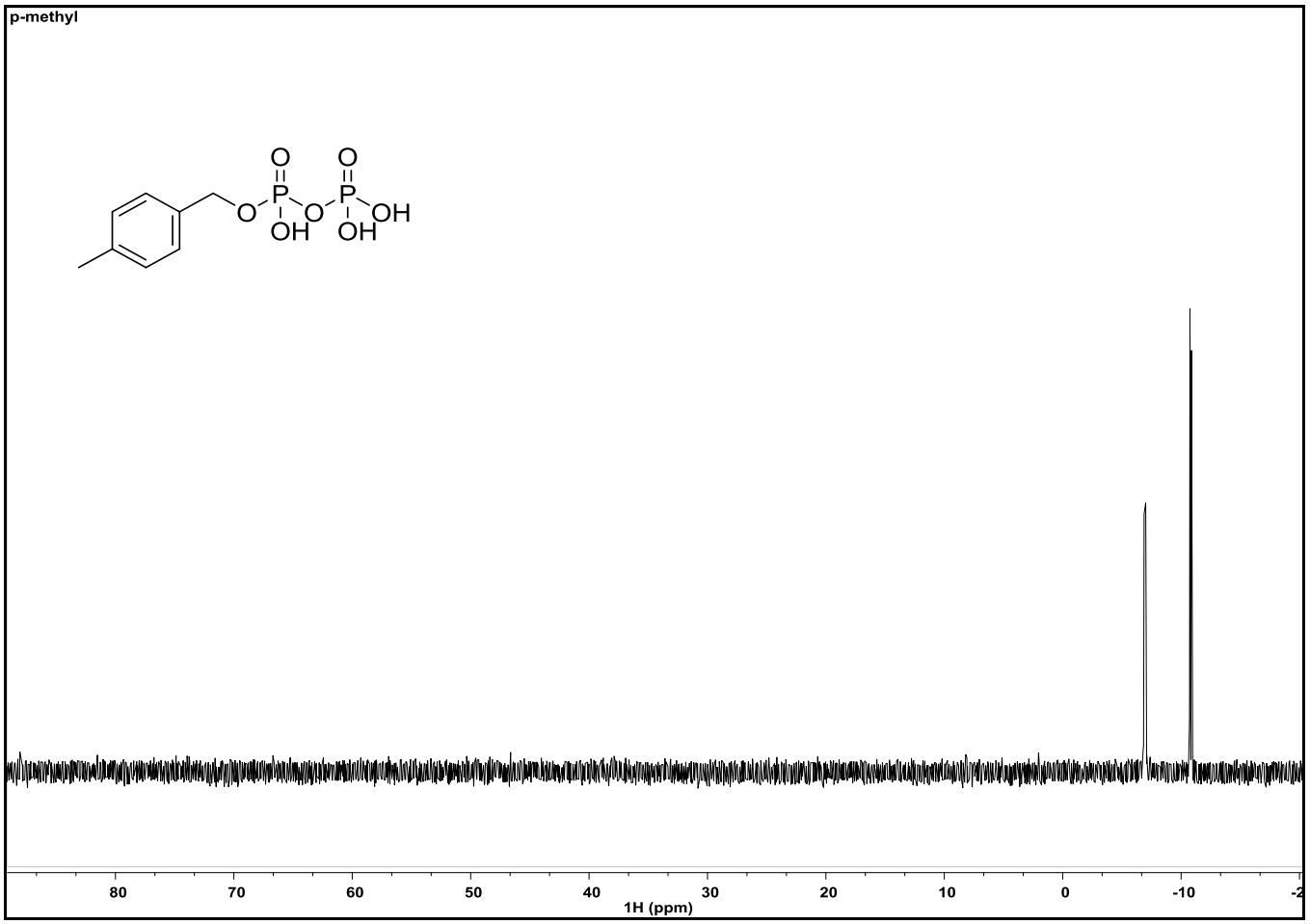
^{31}P NMR Spectrum (162 MHz) of compound **25** in D_2O



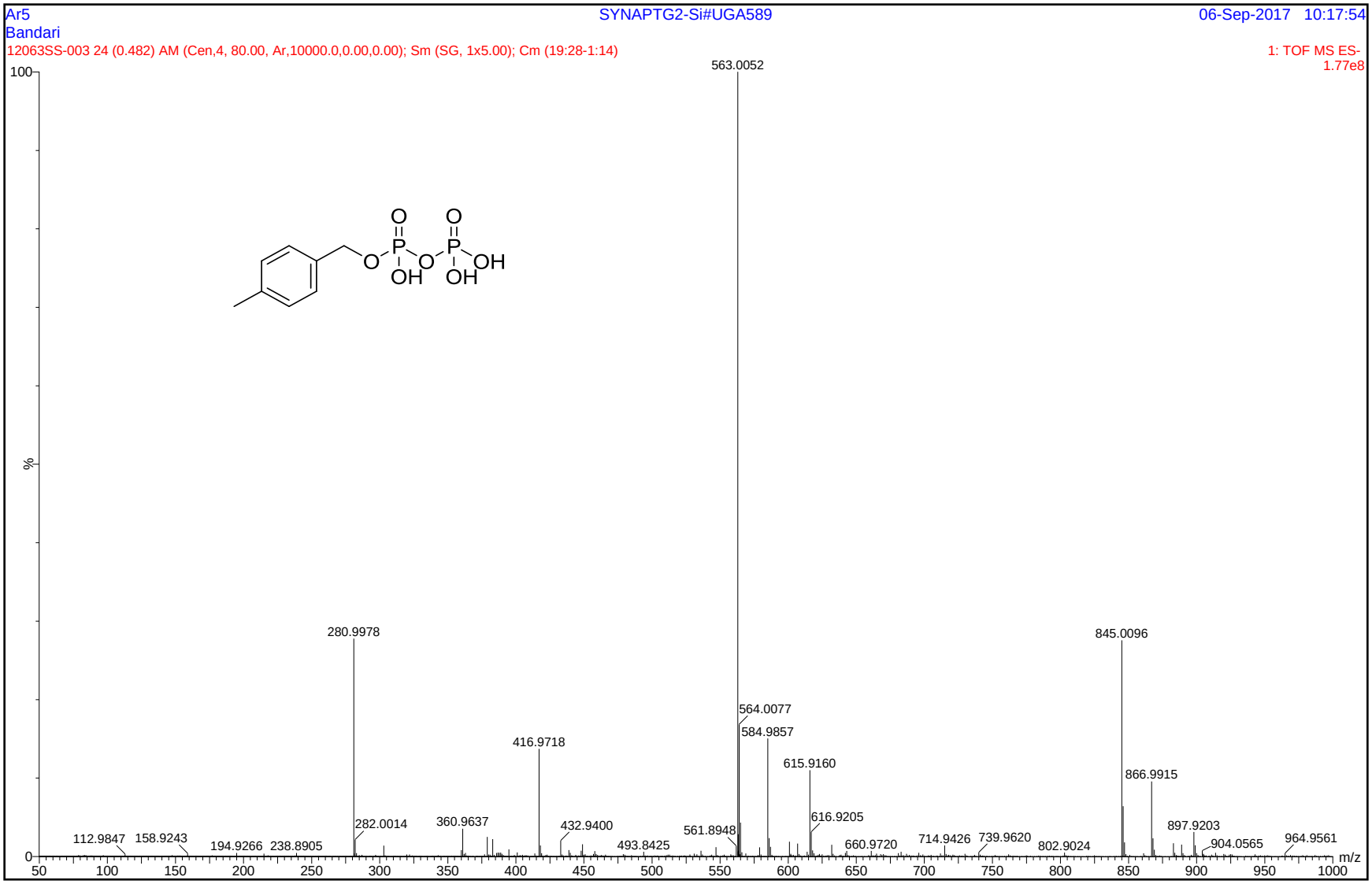
HRMS spectrum of **25**



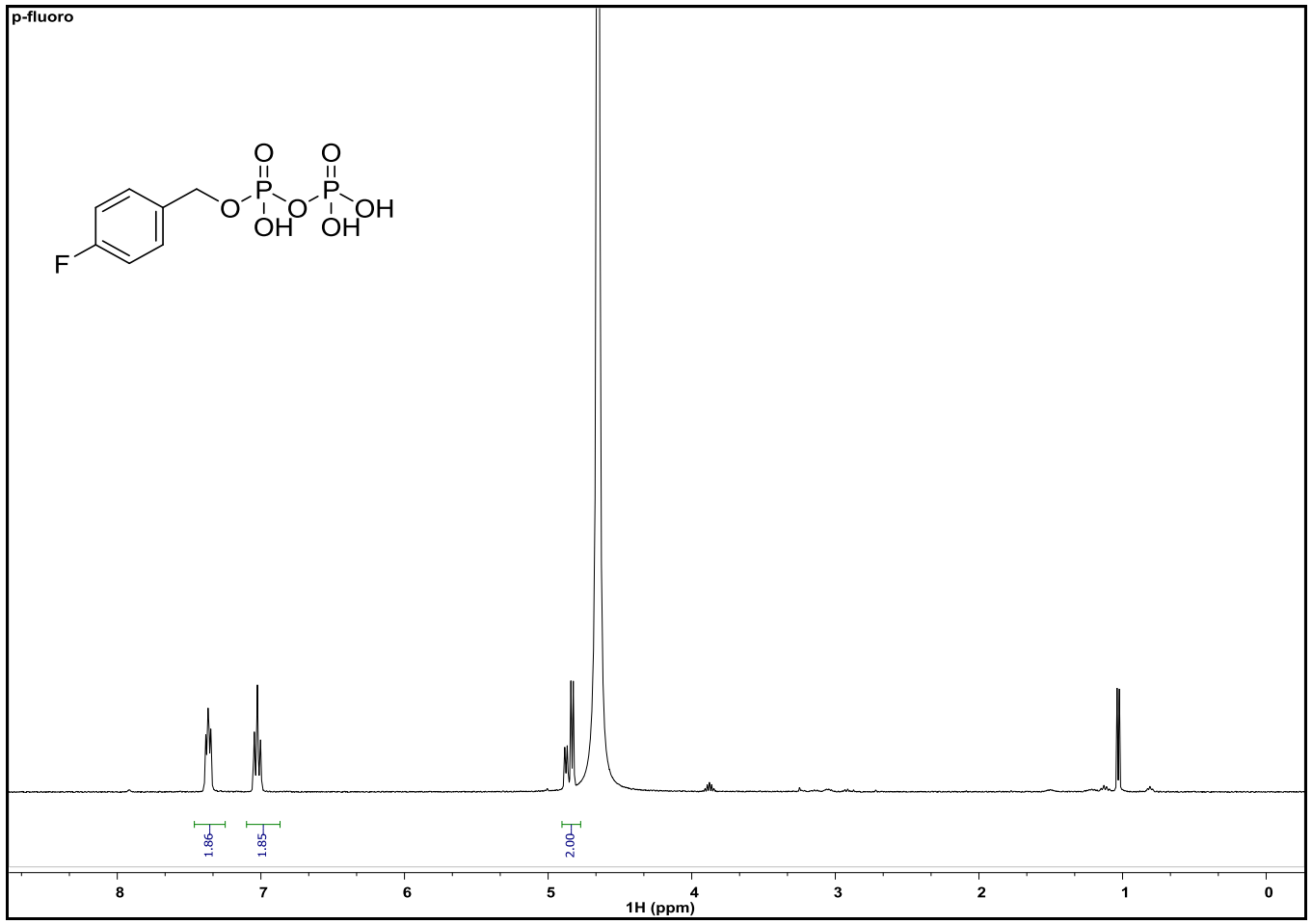
^{1}H NMR Spectrum (400 MHz) of compound **26** in D_2O



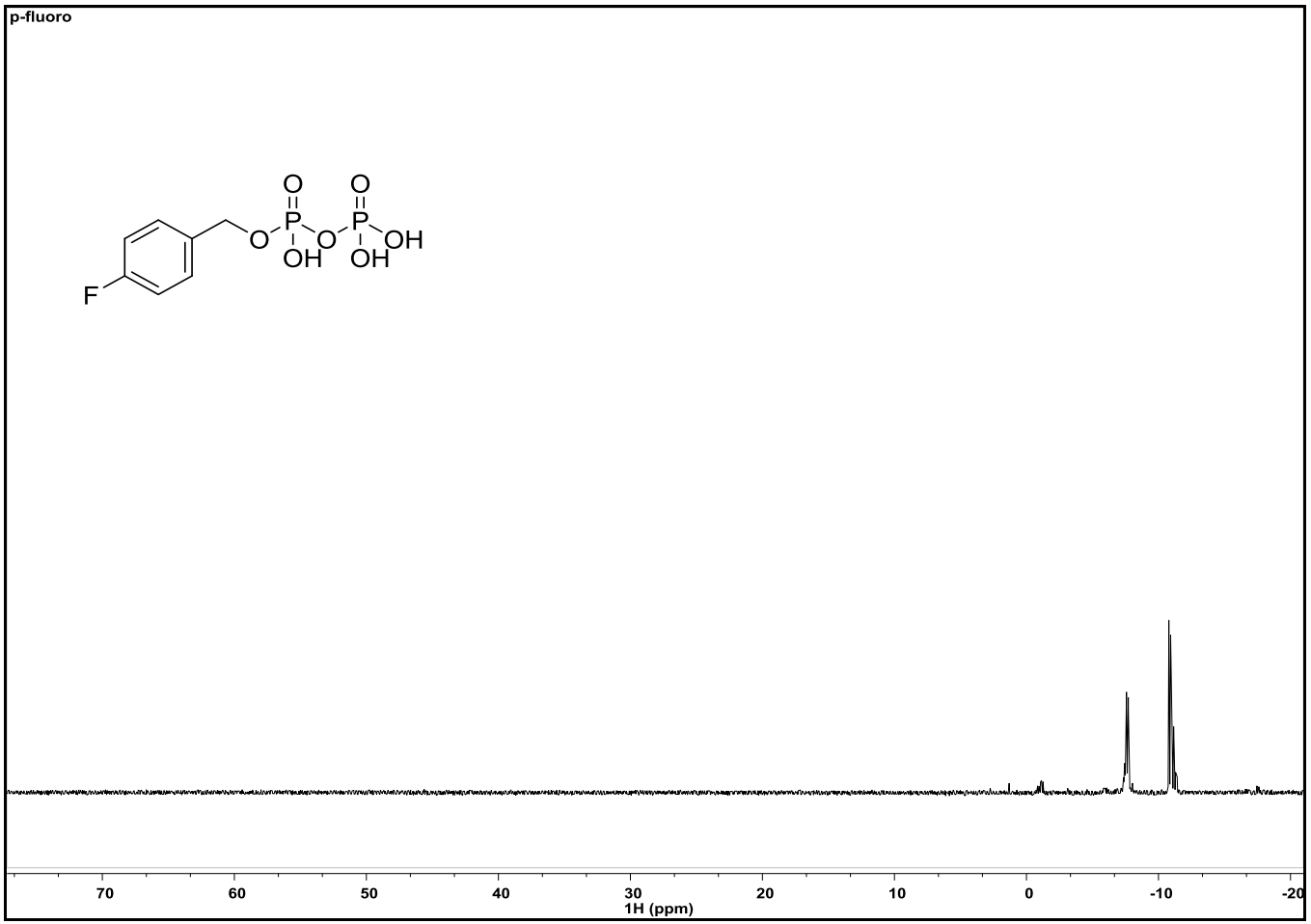
^{31}P NMR Spectrum (162 MHz) of compound **26** in D_2O



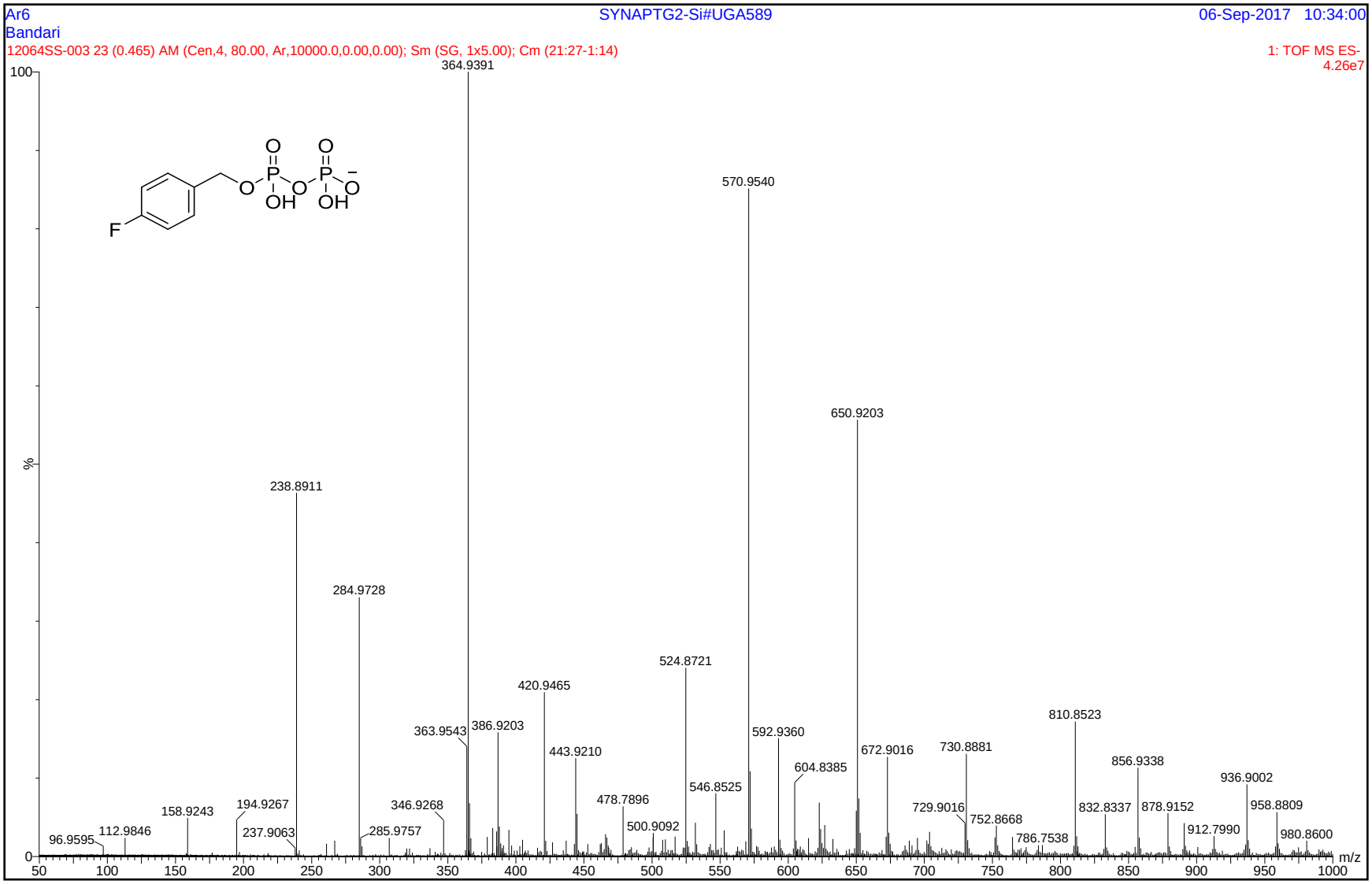
HRMS spectrum of **26**



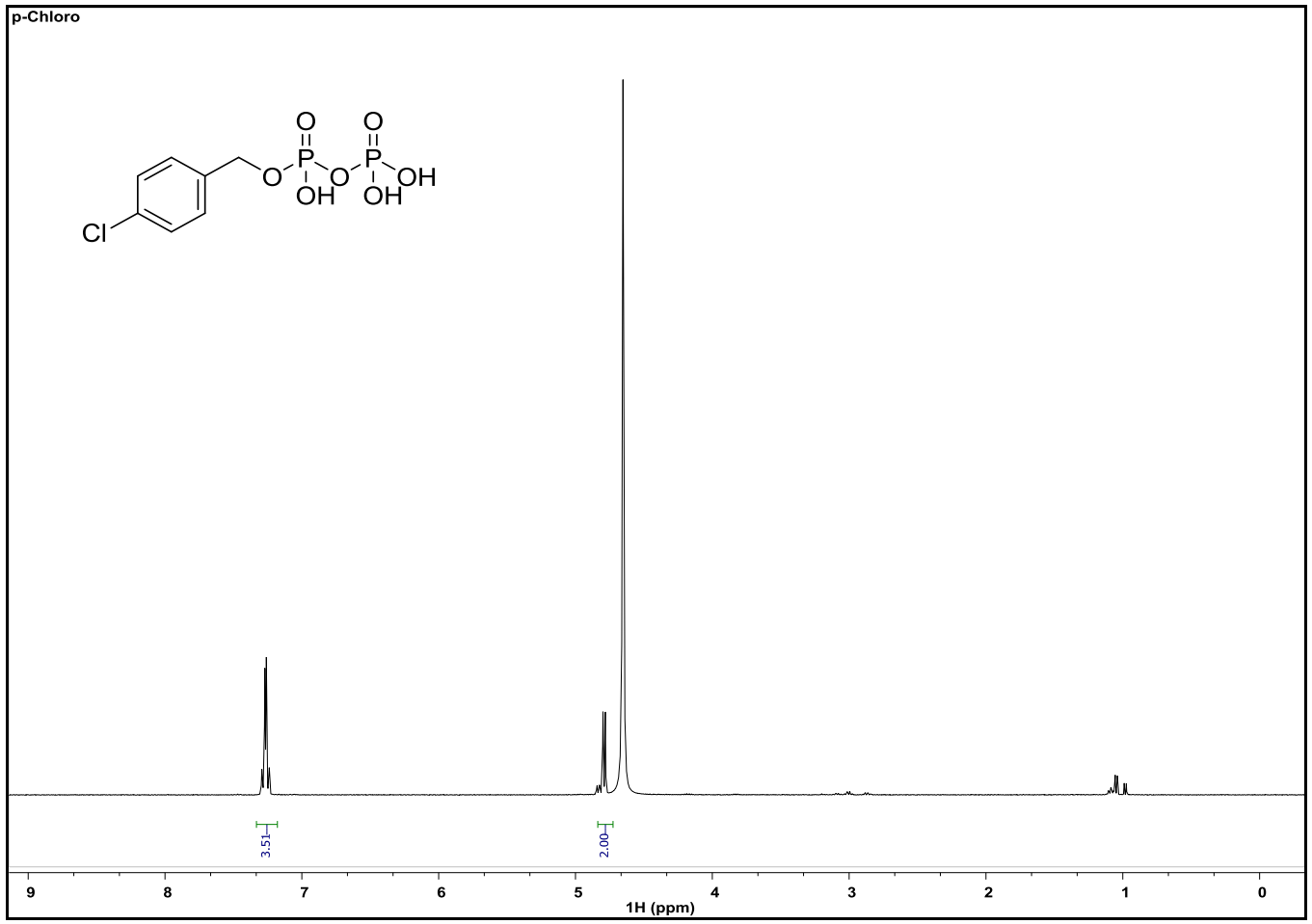
^{1}H NMR Spectrum (400 MHz) of compound **27** in D_2O



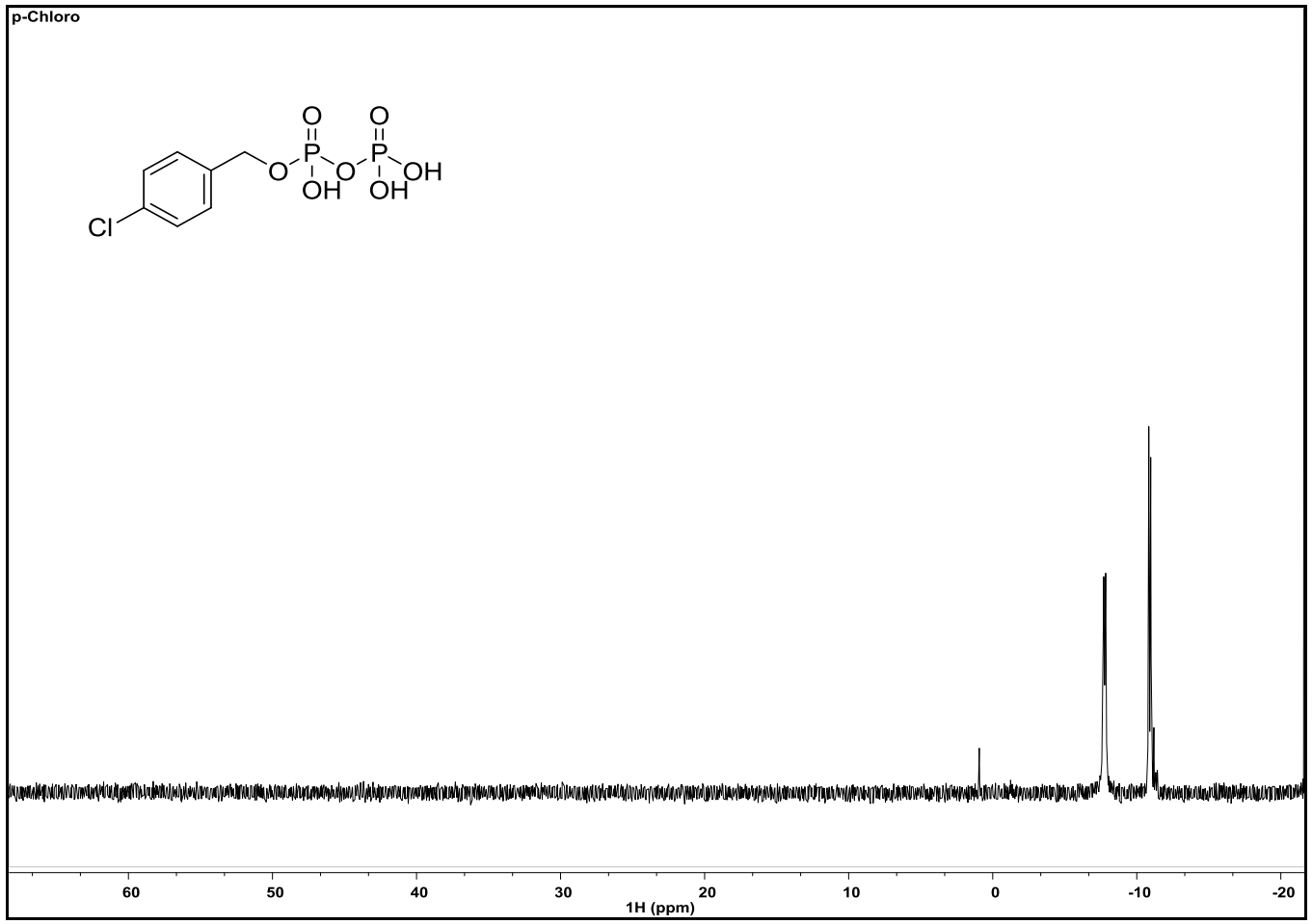
^{31}P NMR Spectrum (162 MHz) of compound **27** in D_2O



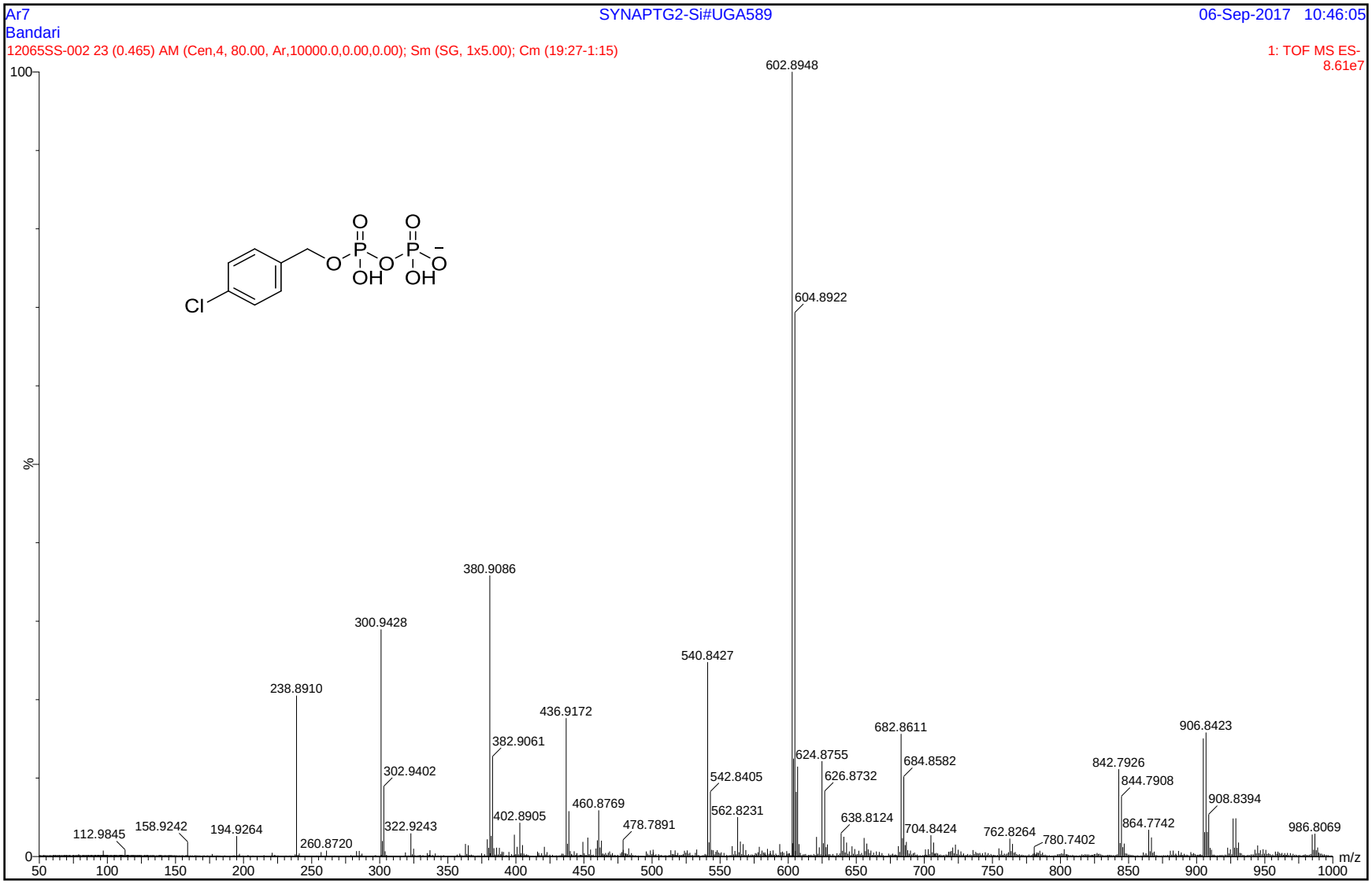
HRMS spectrum of **27**



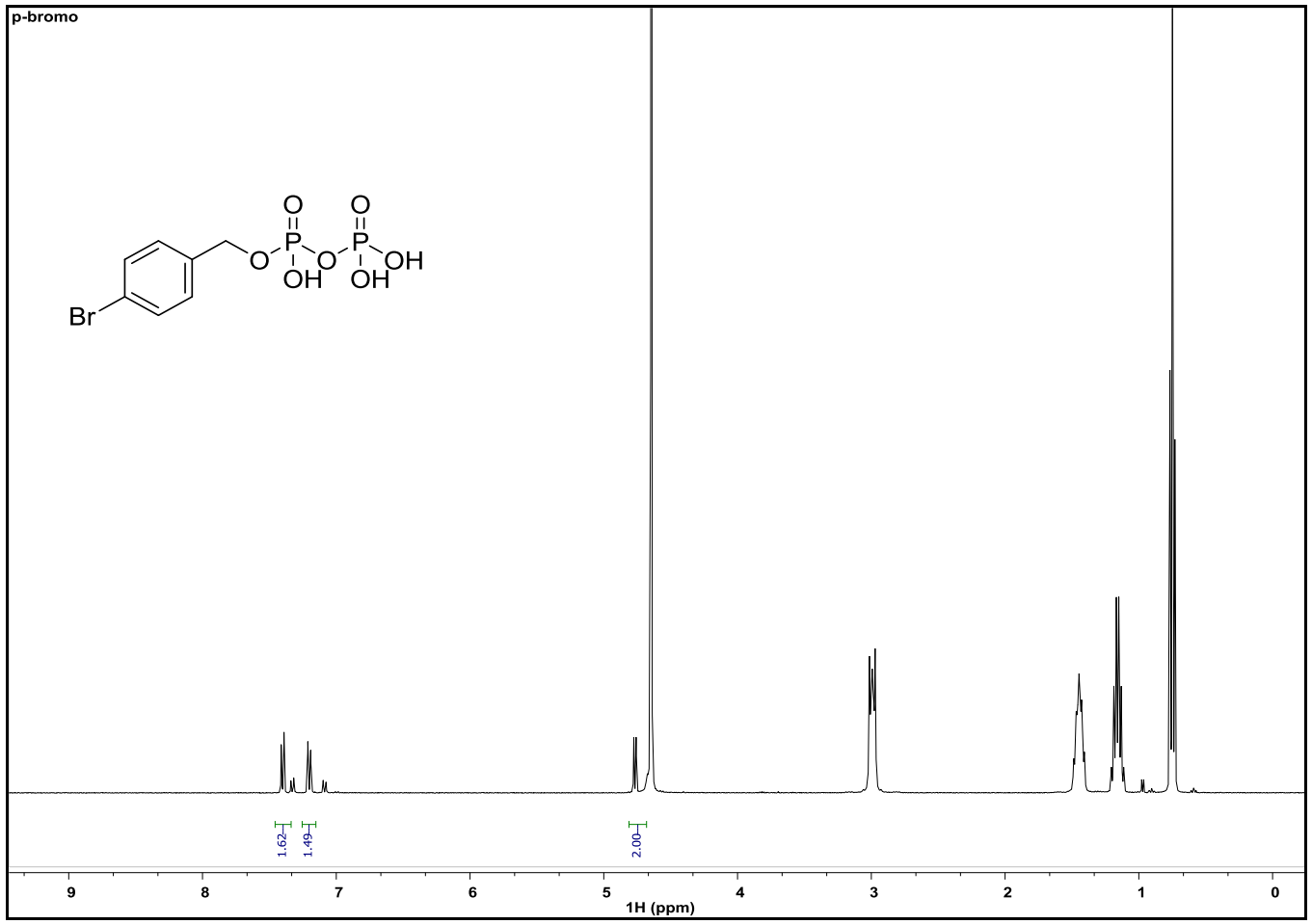
^{1}H NMR Spectrum (400 MHz) of compound **28** in D_2O



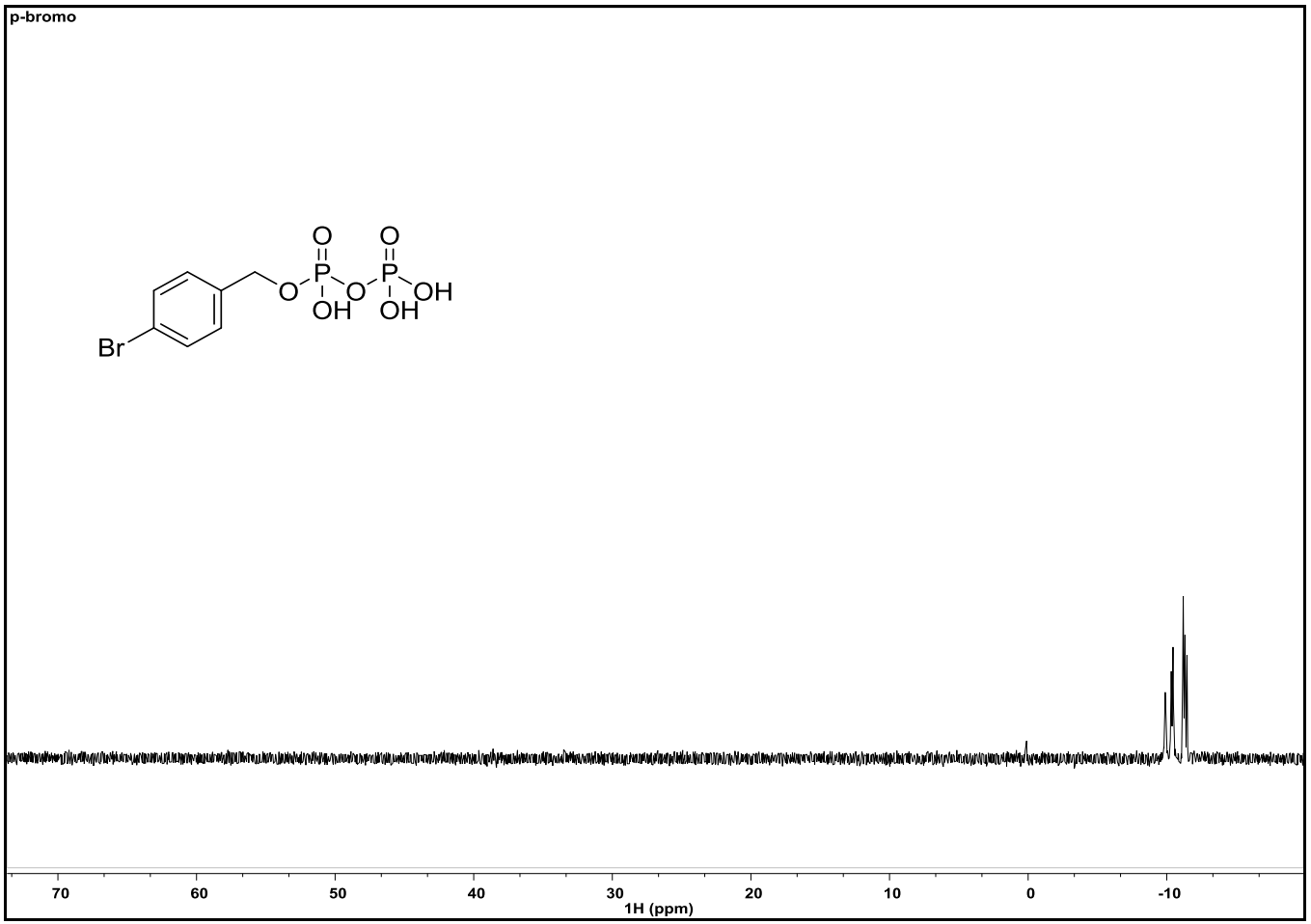
^{31}P NMR Spectrum (162 MHz) of compound **28** in D_2O



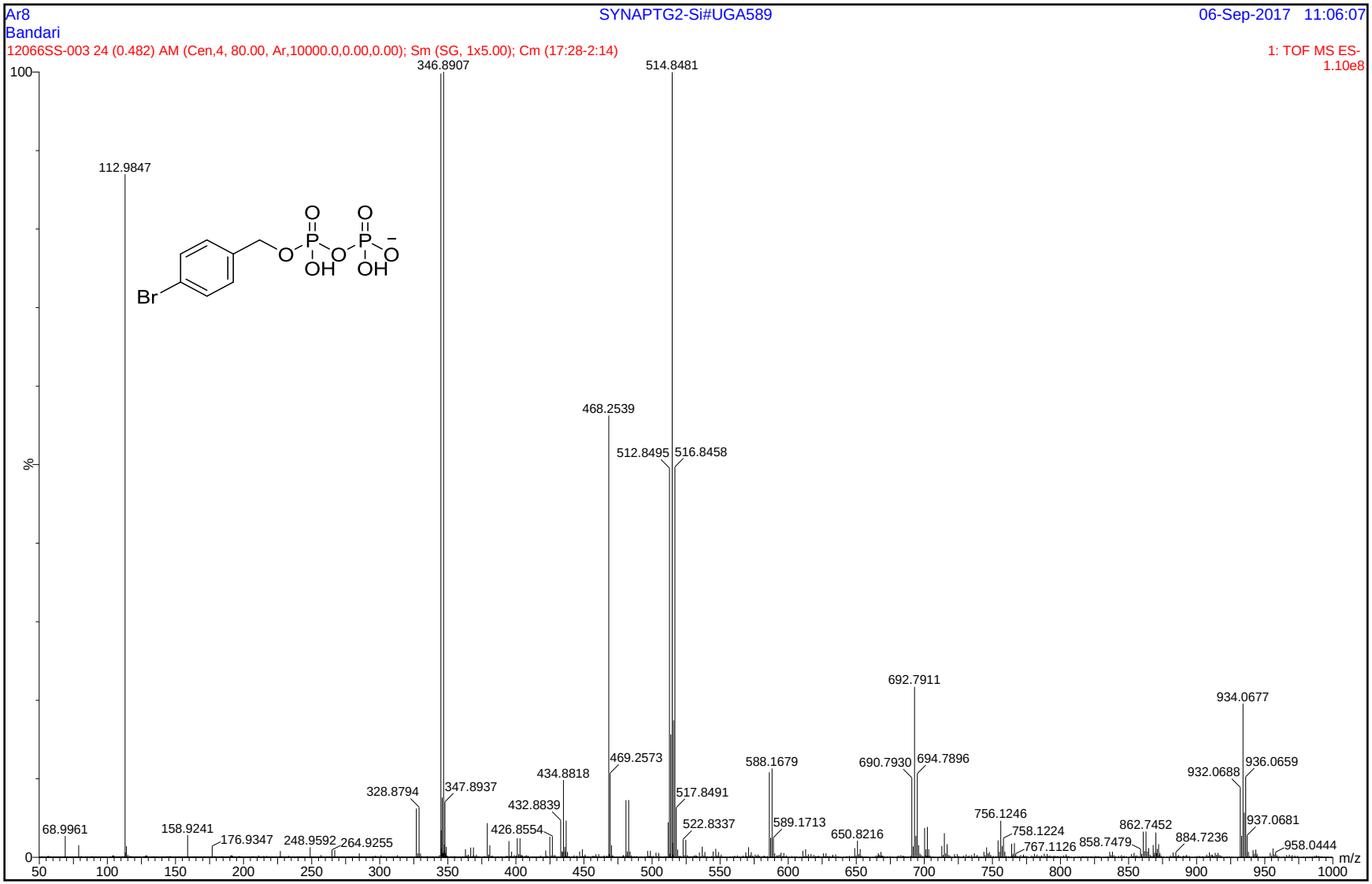
HRMS spectrum of **28**



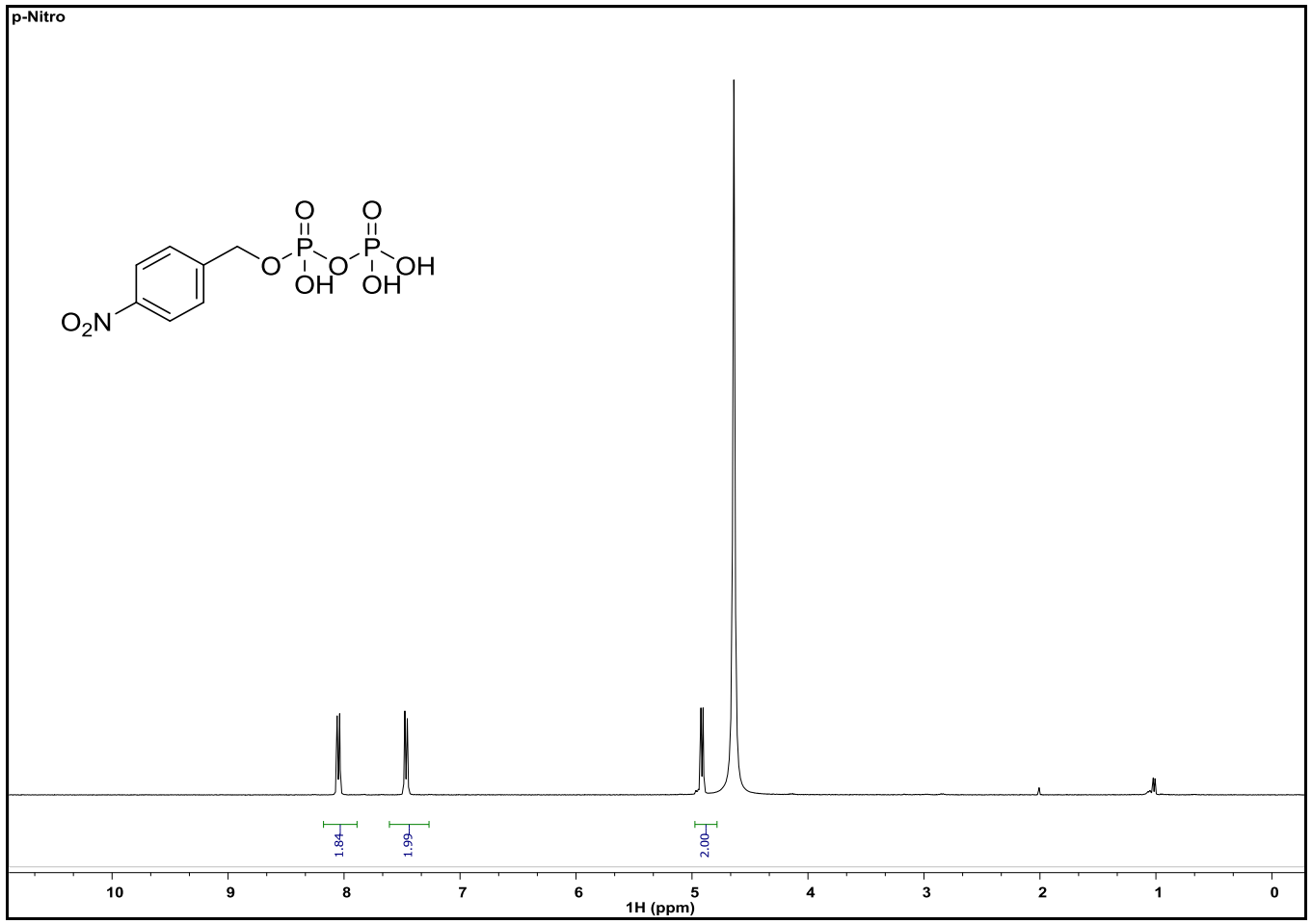
^{1}H NMR Spectrum (400 MHz) of compound **29** in D_2O



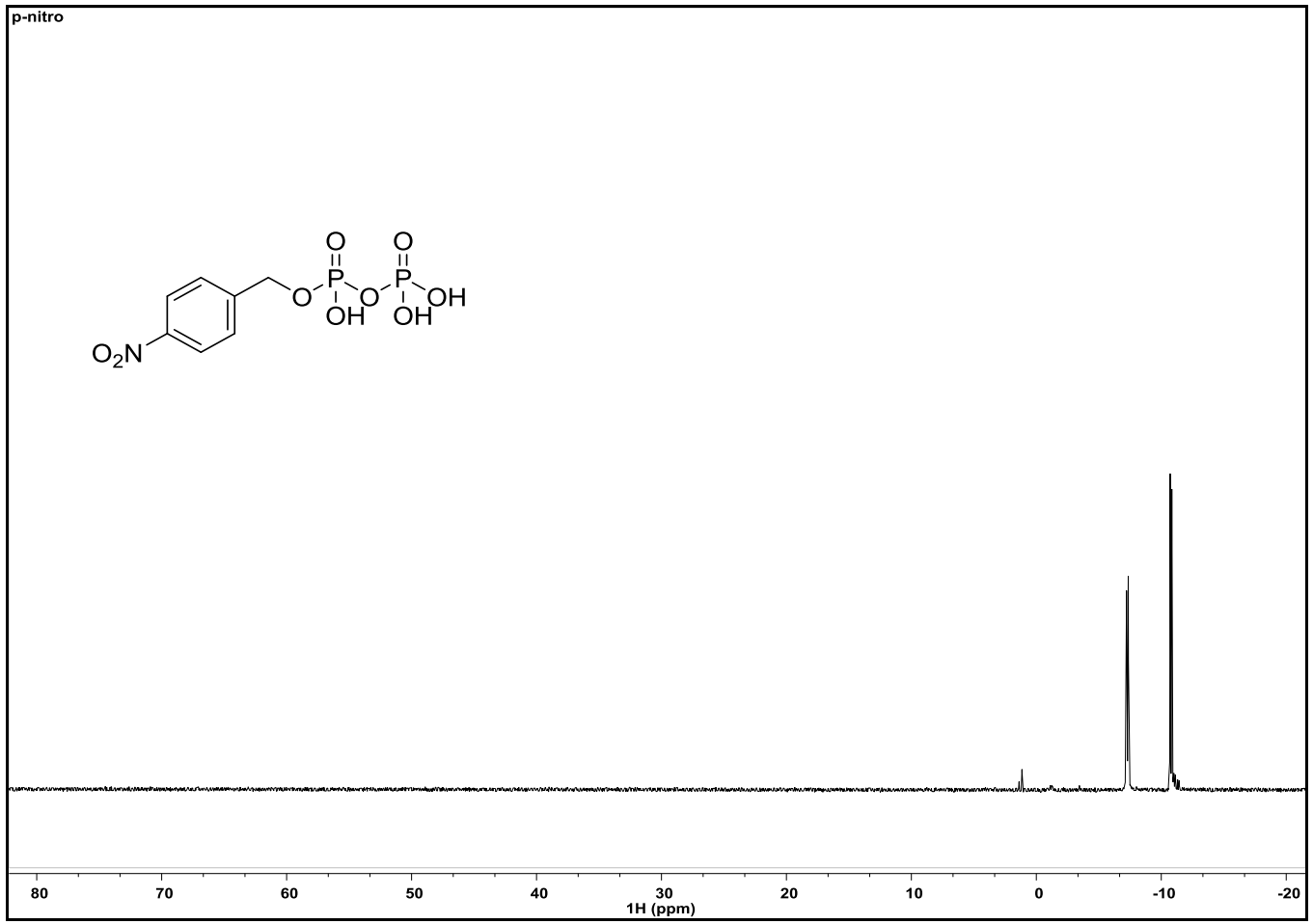
^{31}P NMR Spectrum (162 MHz) of compound **29** in D_2O



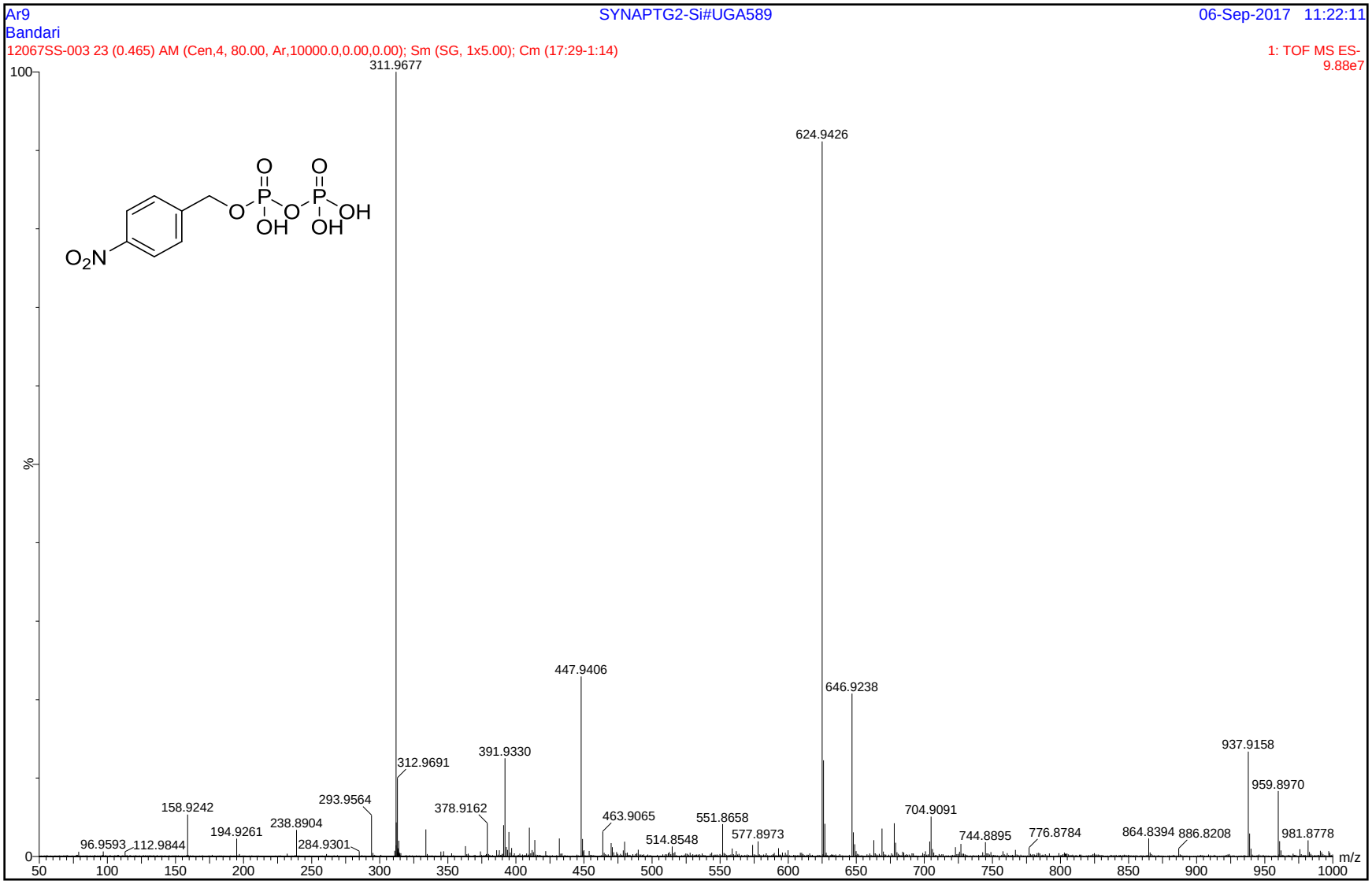
HRMS spectrum of **29**



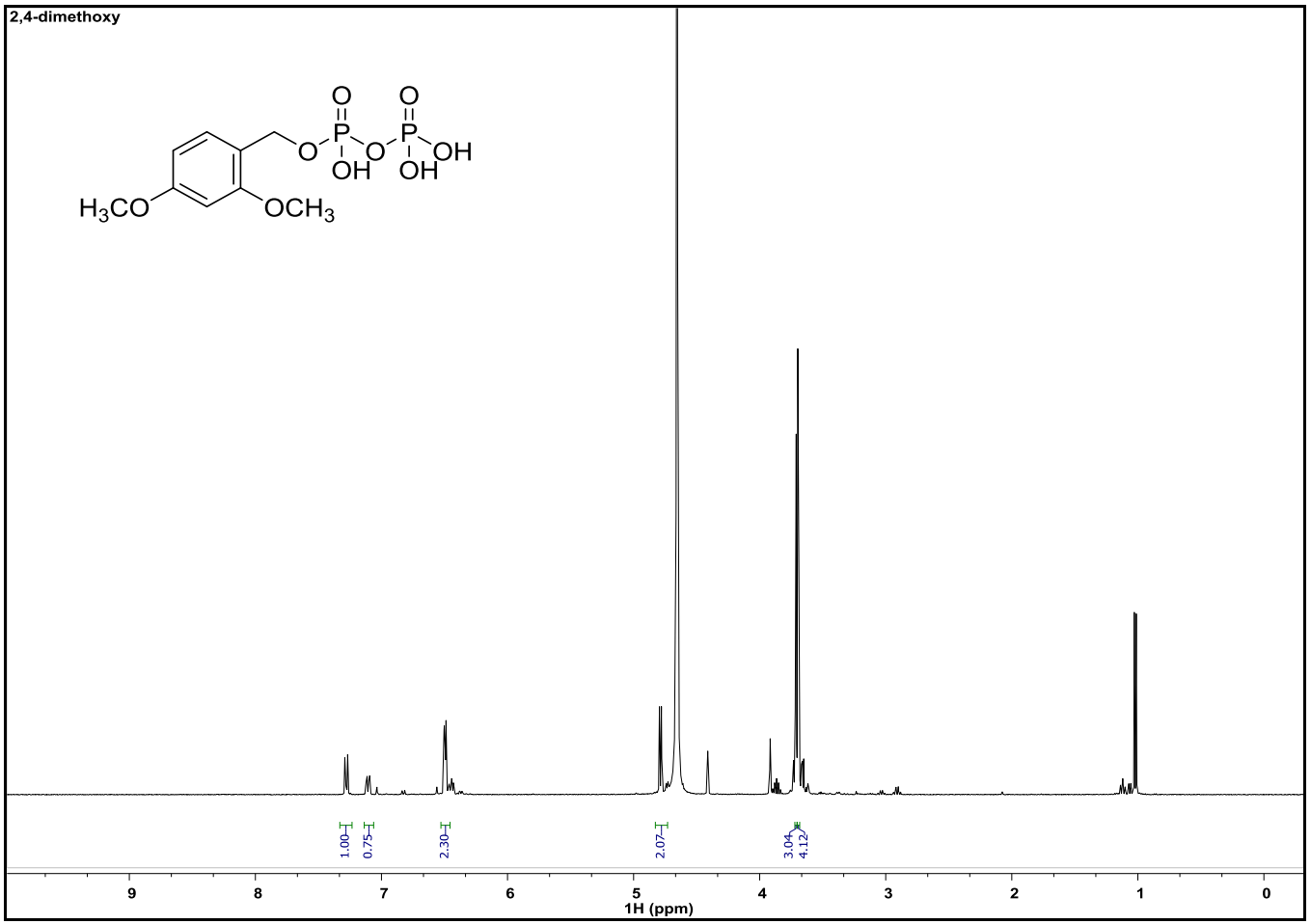
^{1}H NMR Spectrum (400 MHz) of compound **30** in D_2O



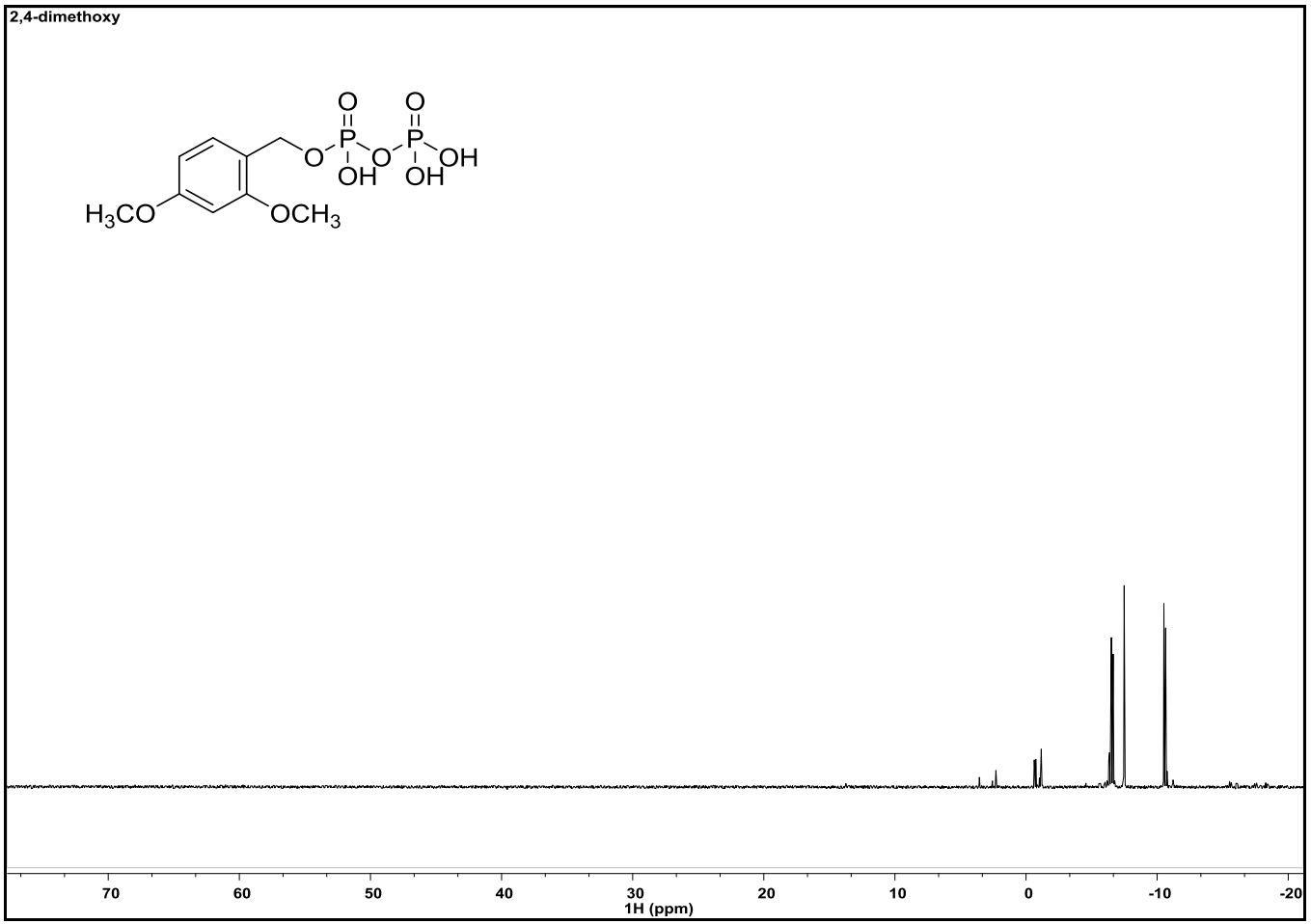
^{31}P NMR Spectrum (162 MHz) of compound **30** in D_2O



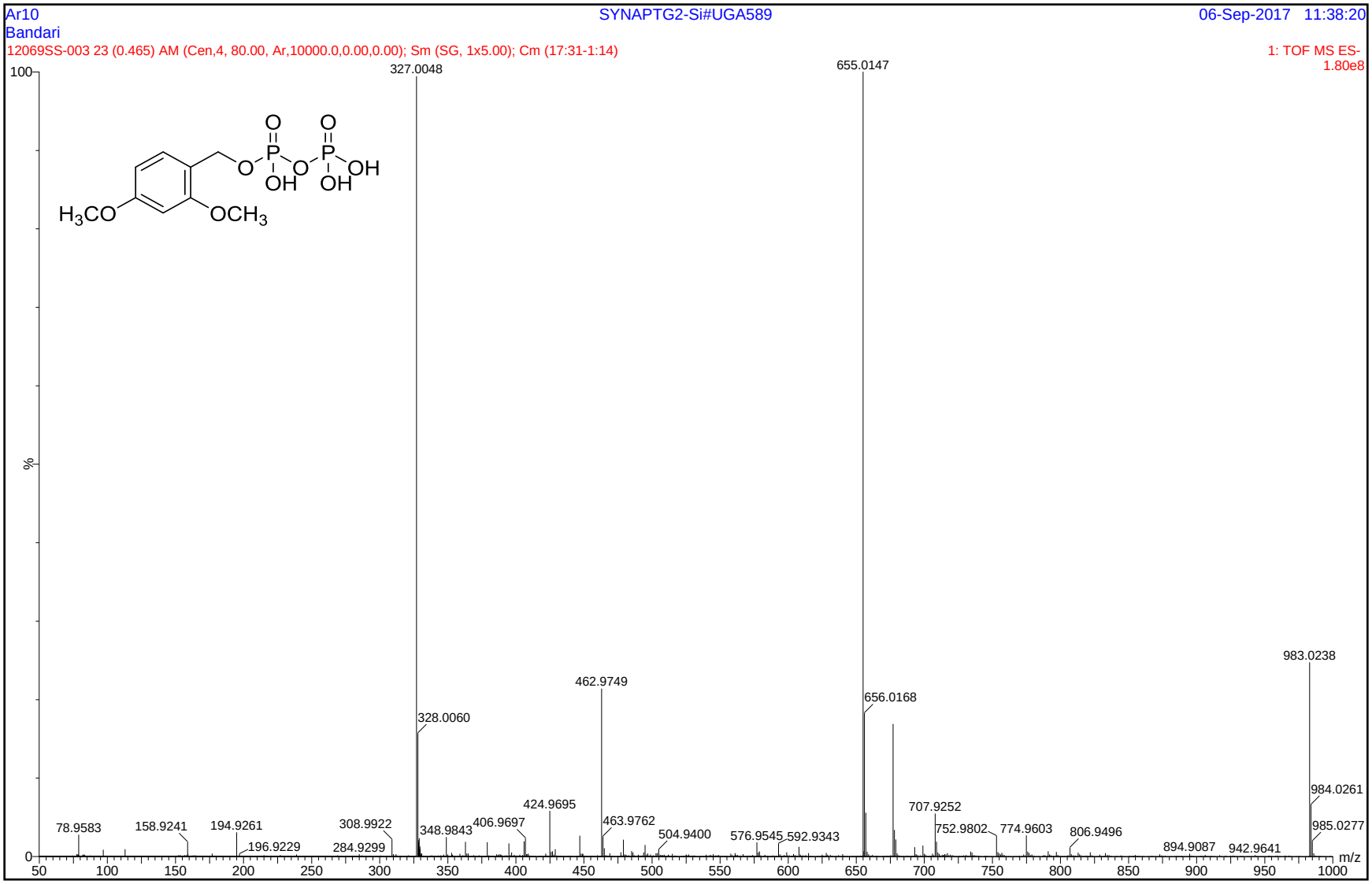
HRMS spectrum of **30**



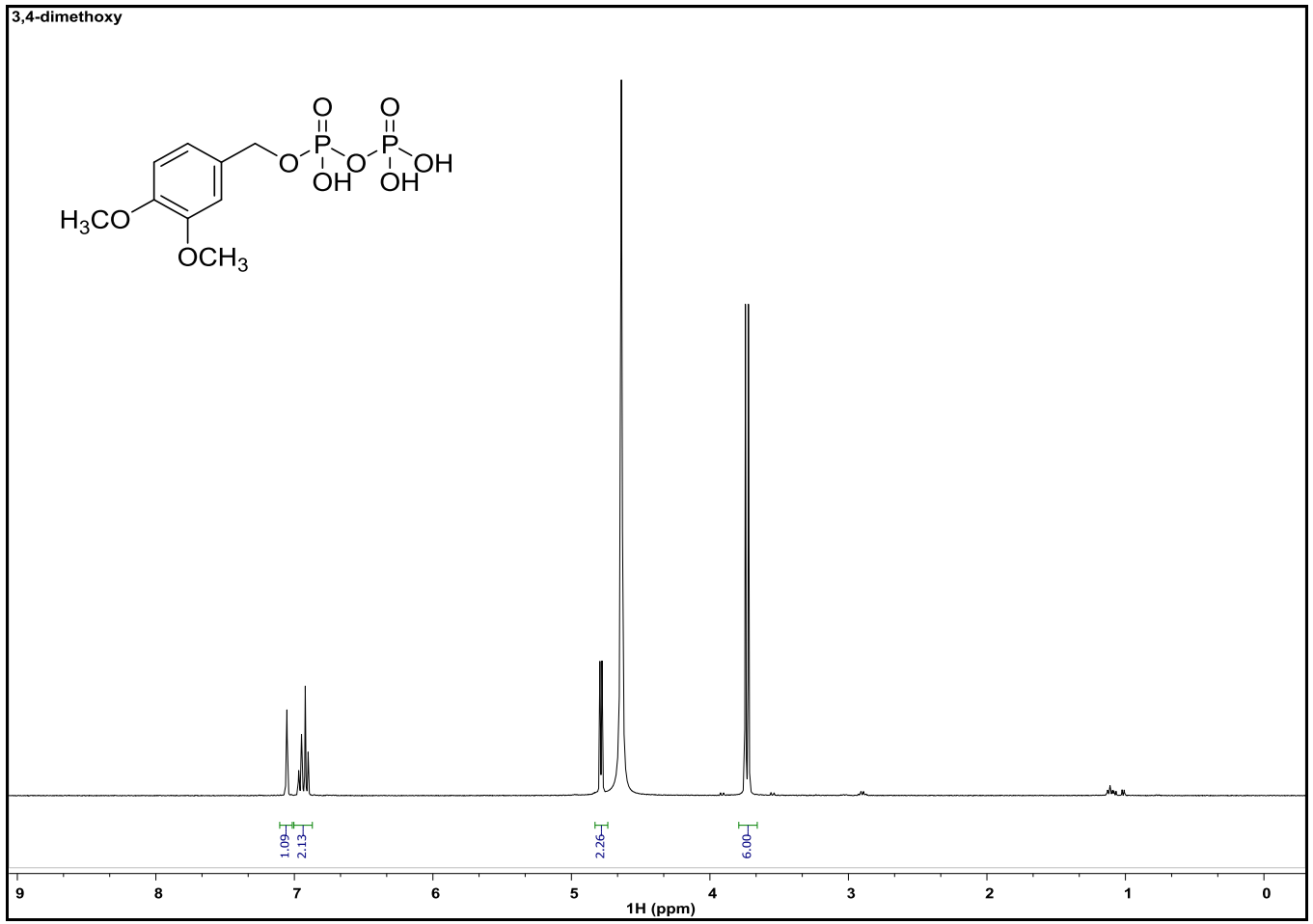
^{1}H NMR Spectrum (400 MHz) of compound **31** in D_2O



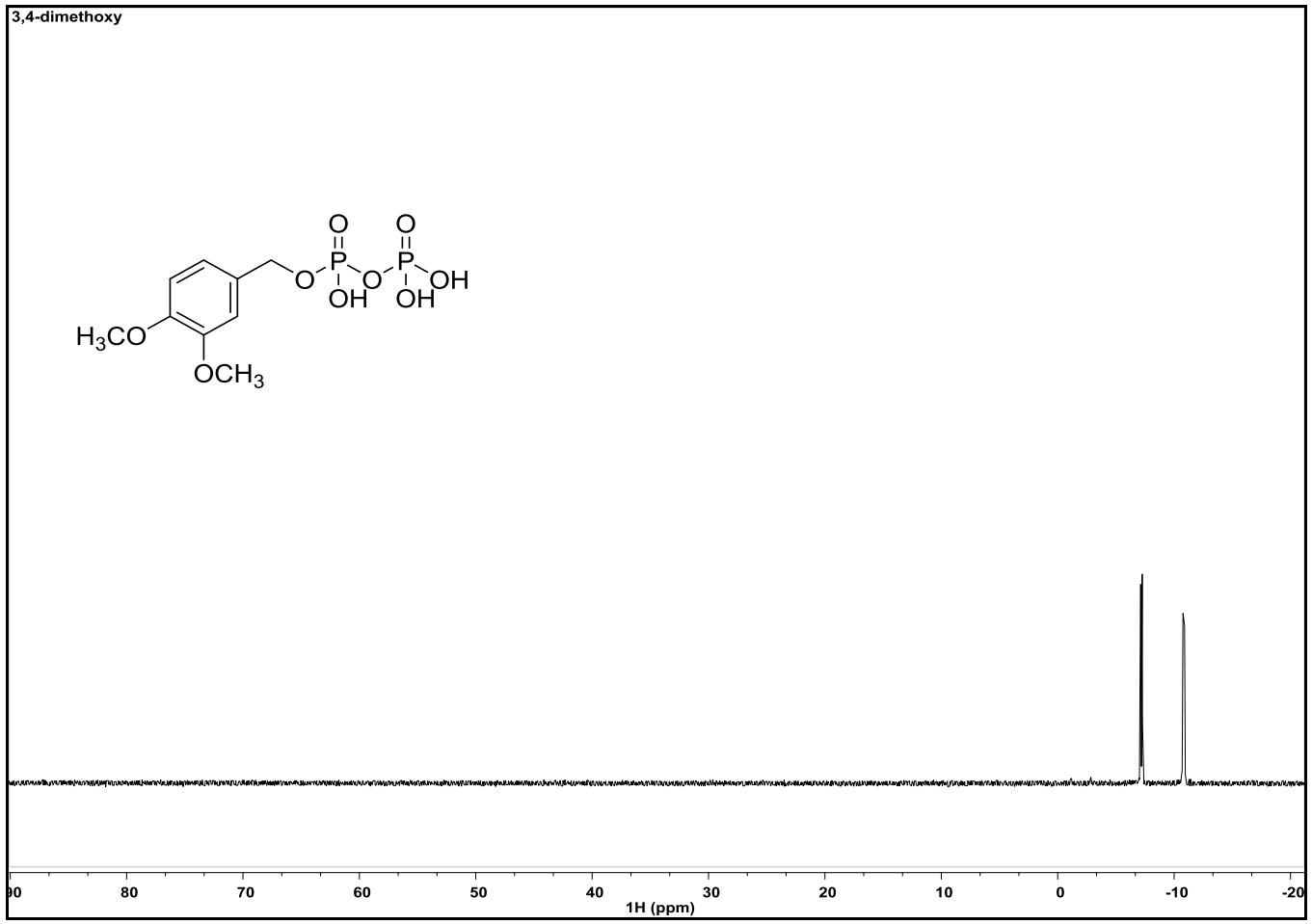
^{31}P NMR Spectrum (162 MHz) of compound **31** in D_2O



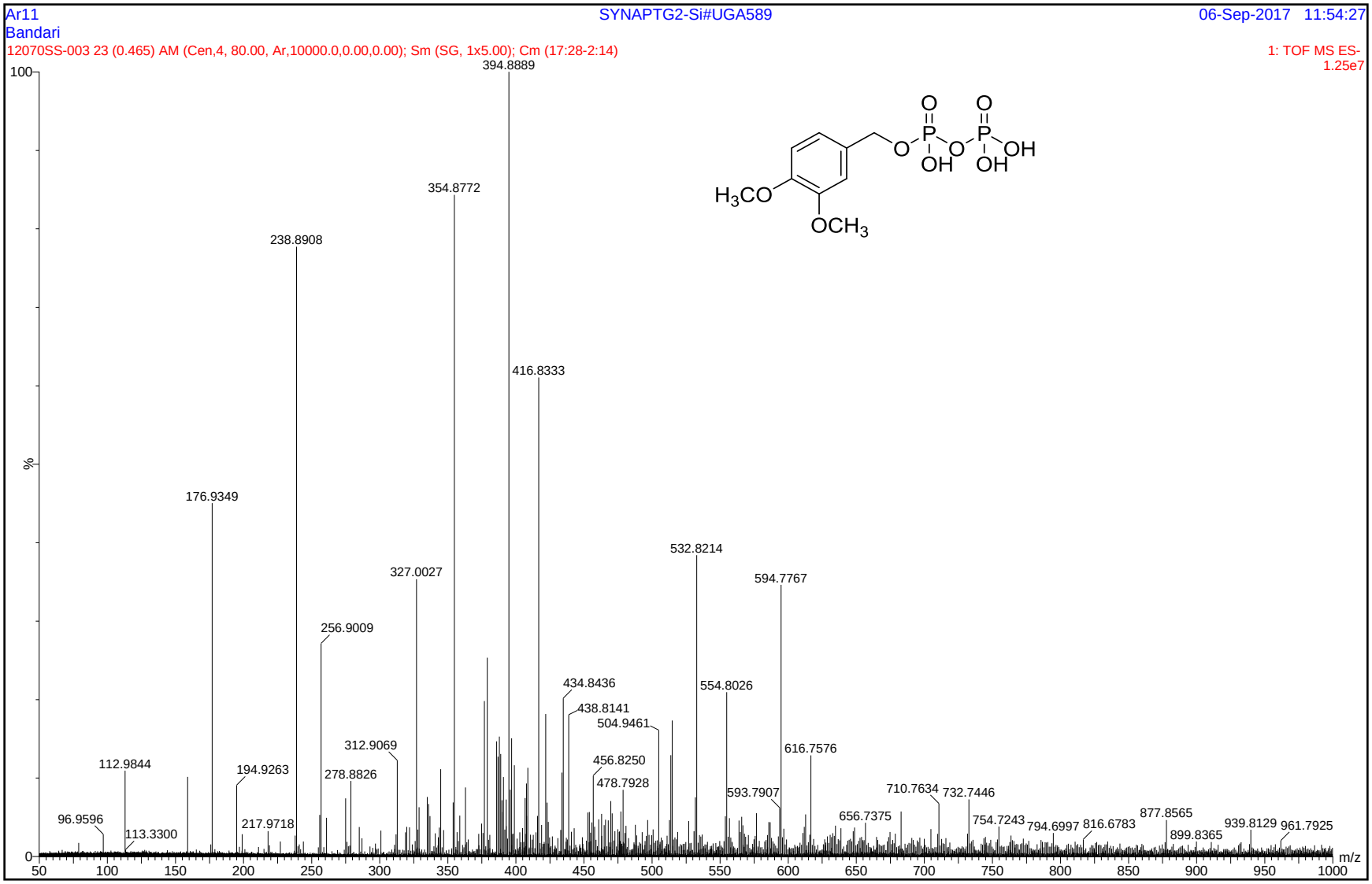
HRMS spectrum of **31**



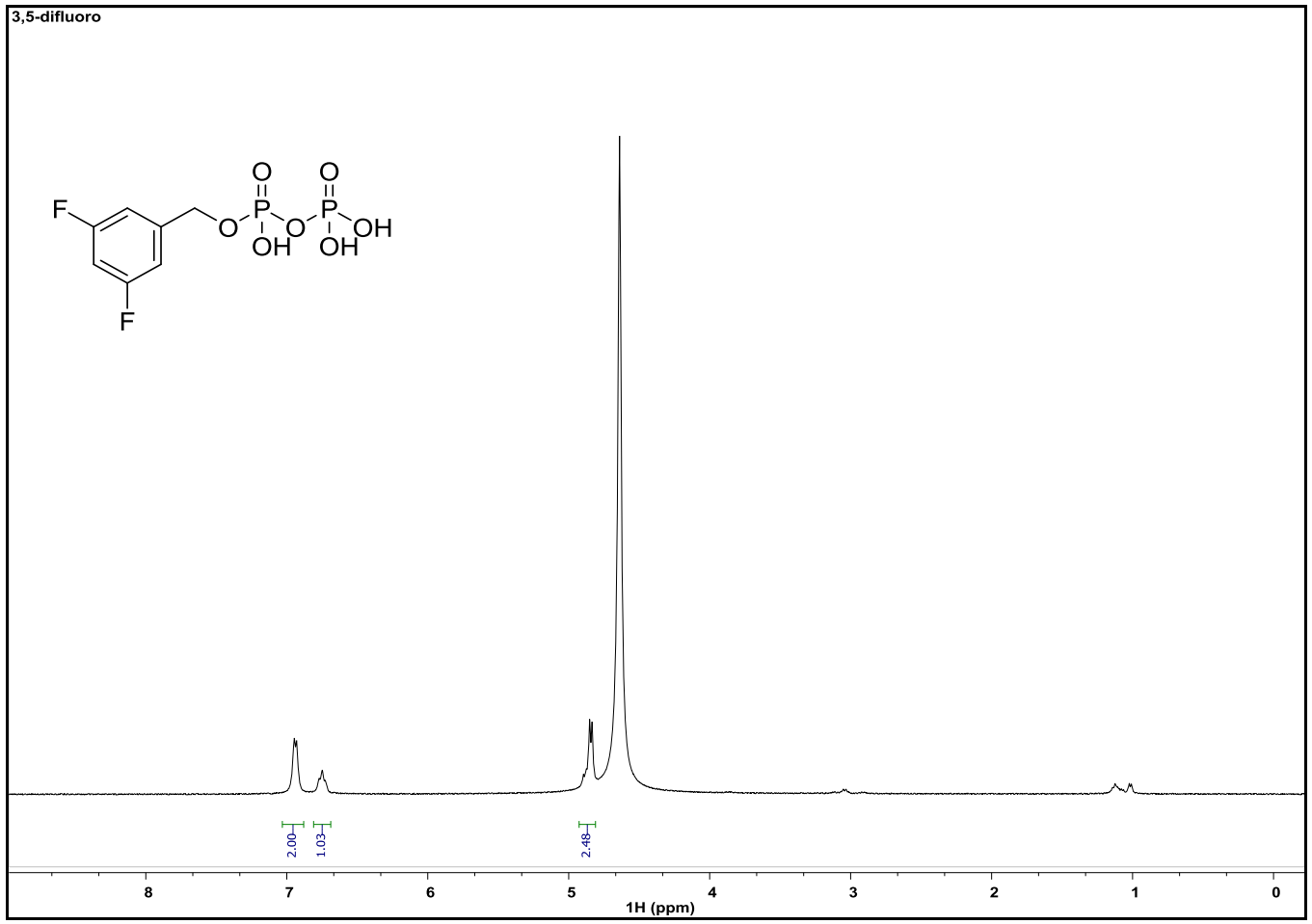
^{1}H NMR Spectrum (400 MHz) of compound **32** in D_2O



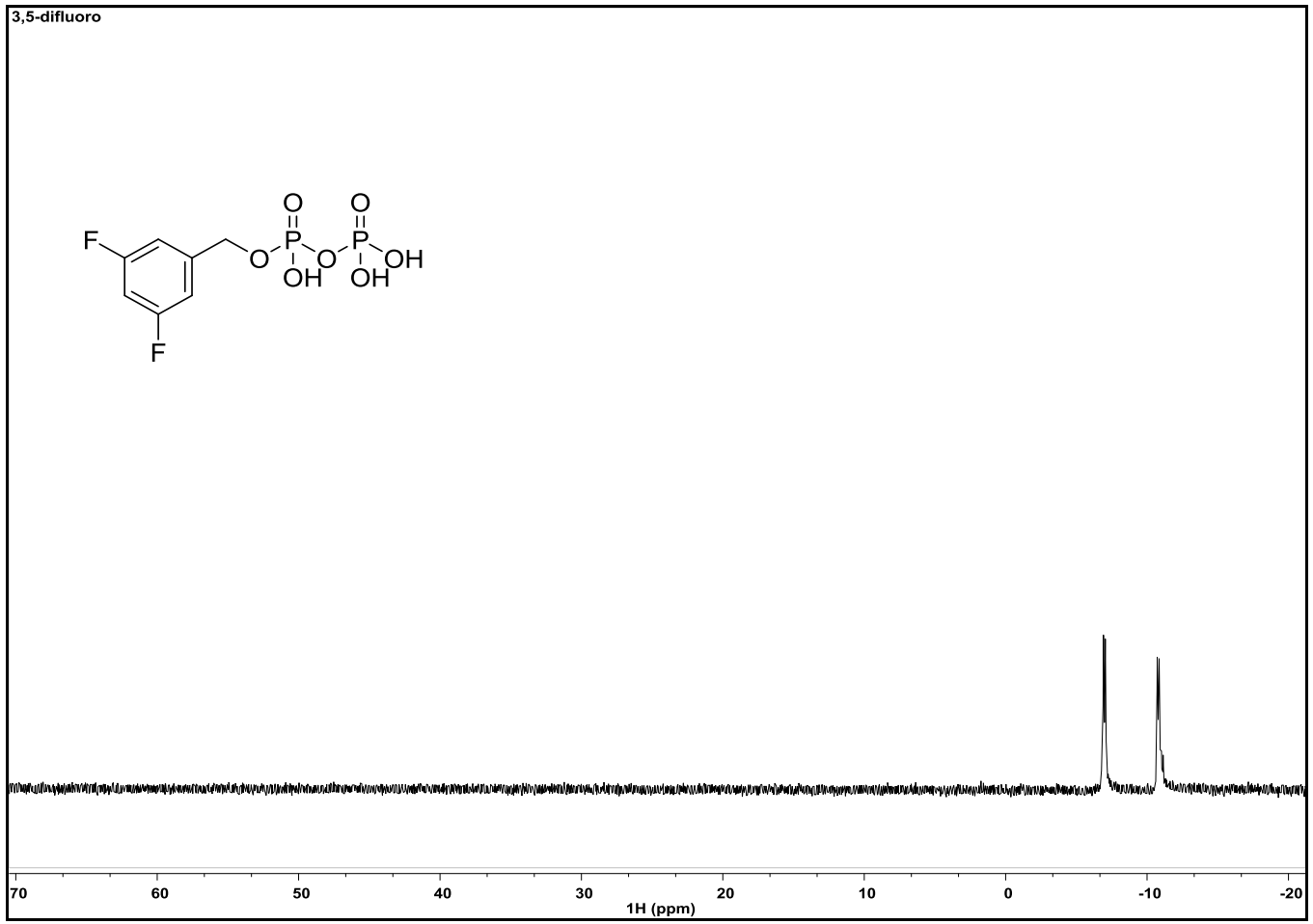
^{31}P NMR Spectrum (162 MHz) of compound **32** in D_2O



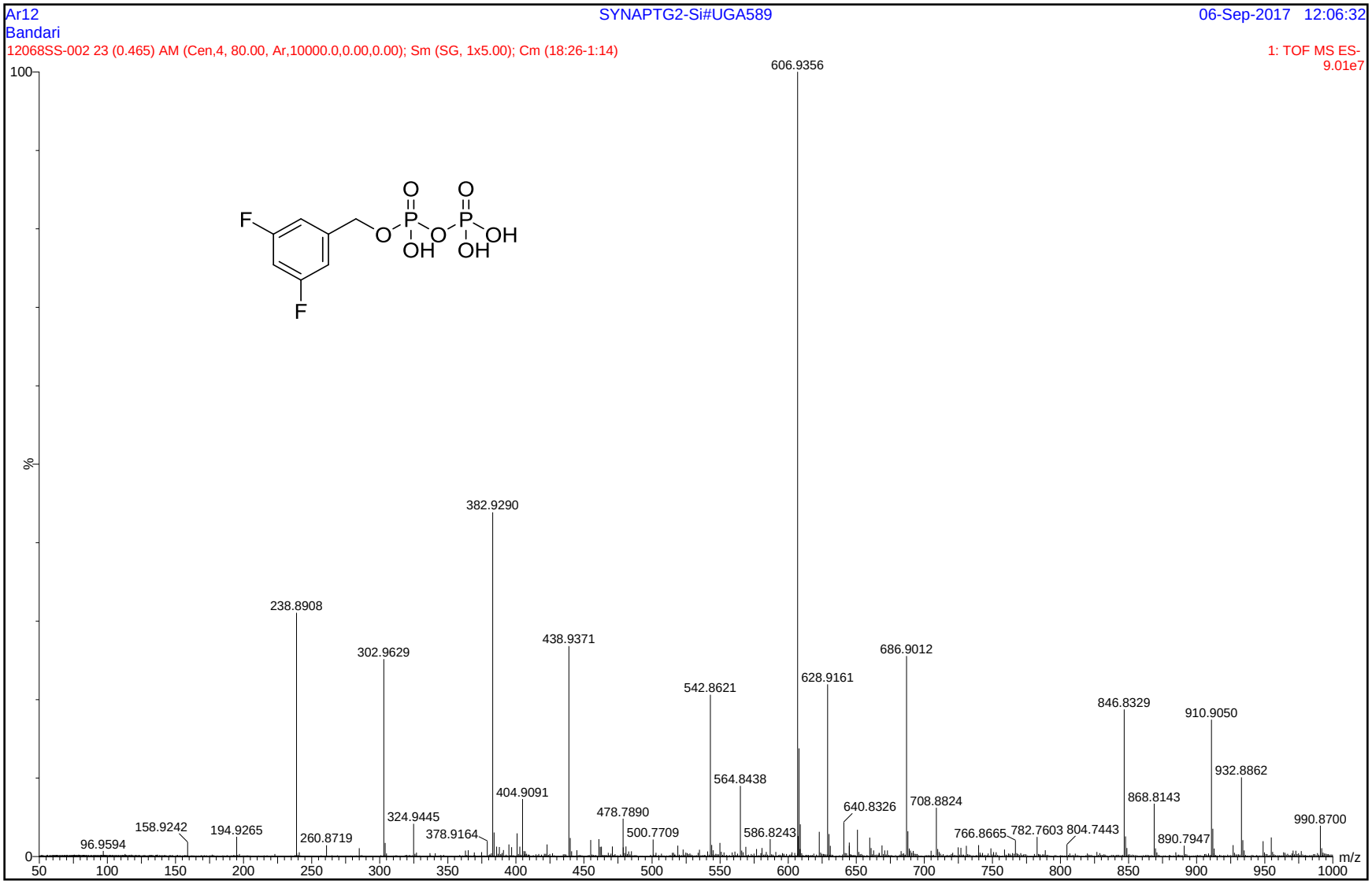
HRMS spectrum of **32**



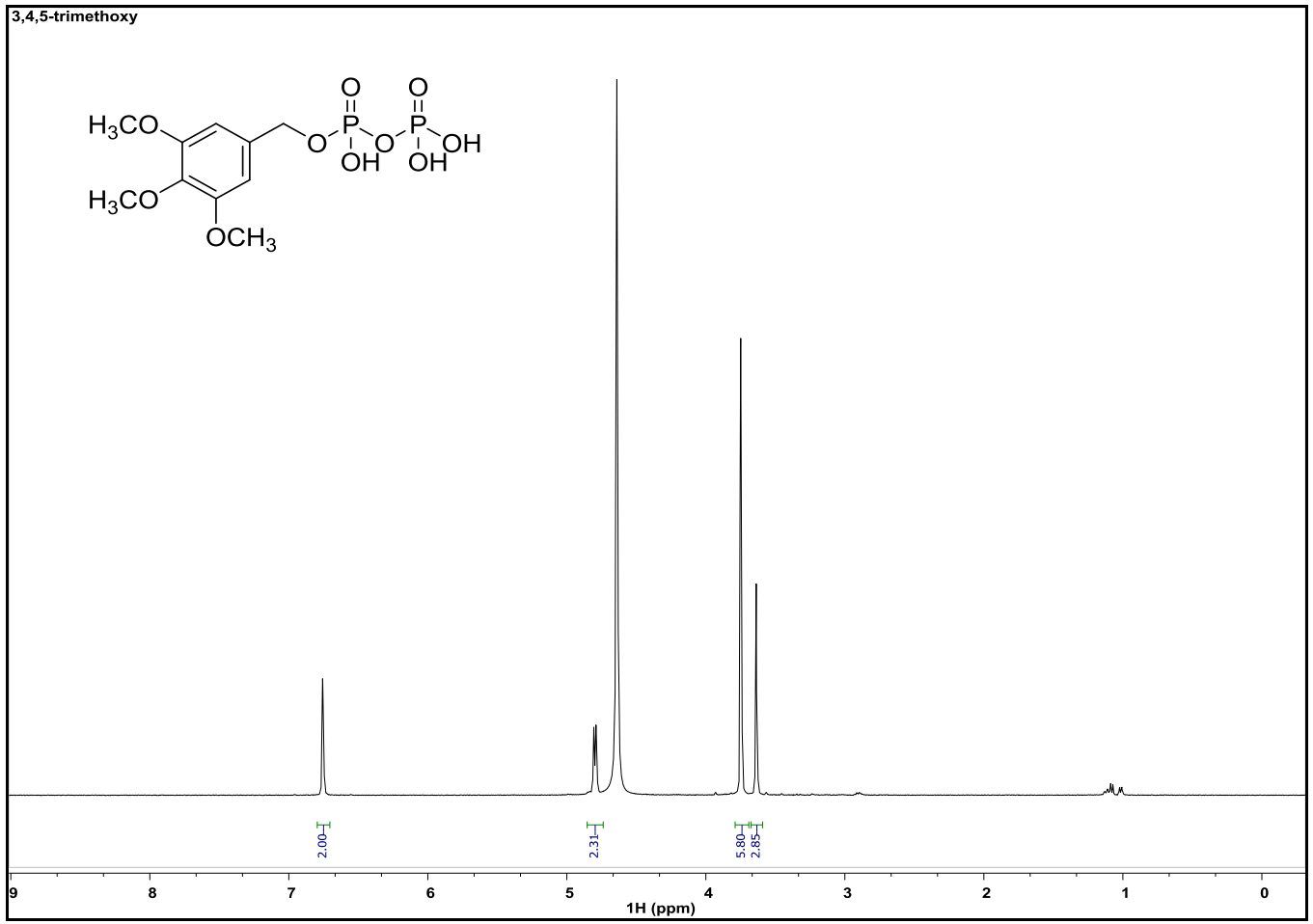
^{1}H NMR Spectrum (400 MHz) of compound **33** in D_2O



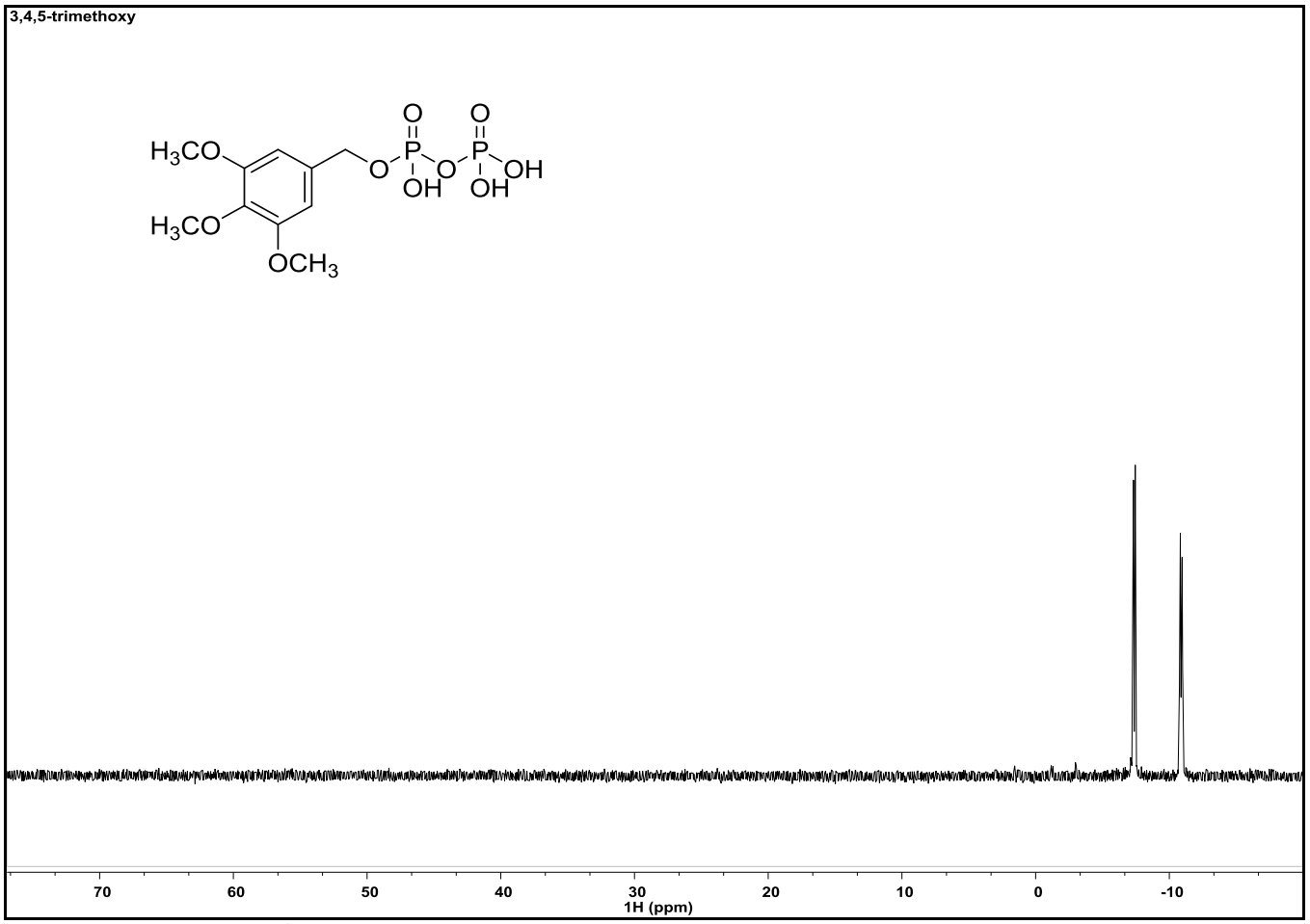
^{31}P NMR Spectrum (162 MHz) of compound **33** in D_2O



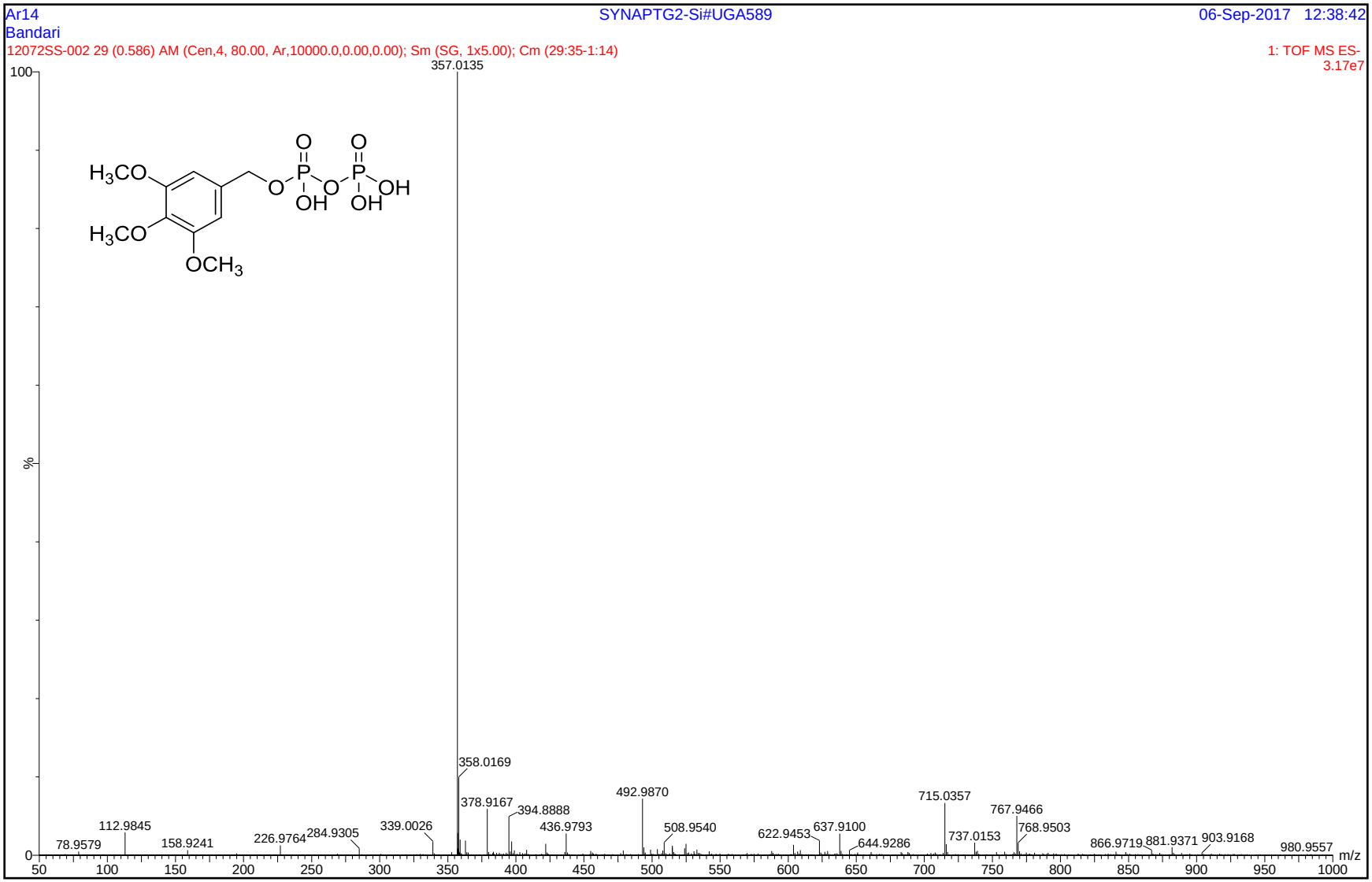
HRMS spectrum of **33**



^{1}H NMR Spectrum (400 MHz) of compound **34** in D_2O



^{31}P NMR Spectrum (162 MHz) of compound **34** in D_2O



HRMS spectrum of **34**