Novel 3-substituted 7-phenylpyrrolo[3,2-*f*]quinolin-9(6*H*)ones as Single Entities with Multitarget Antiproliferative Activity

D. Carta[a], R. Bortolozzi[b], E. Hamel[c], G. Basso[b], S. Moro[a], G. Viola[b], M.G. Ferlin[a]

[a] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, 35131 - Padova, Italy
[b] Laboratory of Oncohematology, Department of Women's and Children's Health, University of Padova, 35128 - Padova, Italy
[c] Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institute of Health, Frederick, 21702 Maryland, USA

Table of Contents

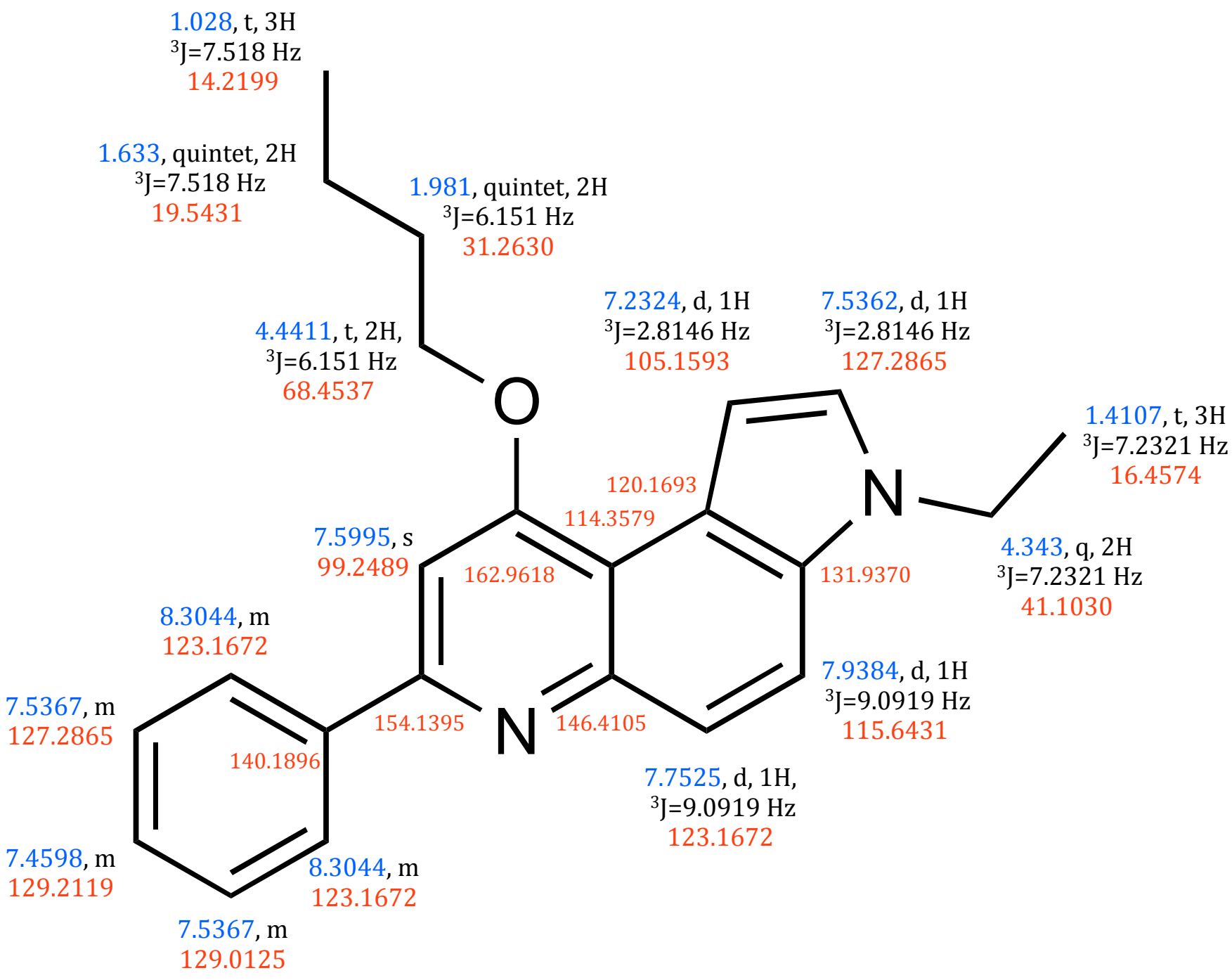


Figure S1. Complete ^{1}H and ^{13}C NMR signals assignation and structure elucidation for compound **10**.

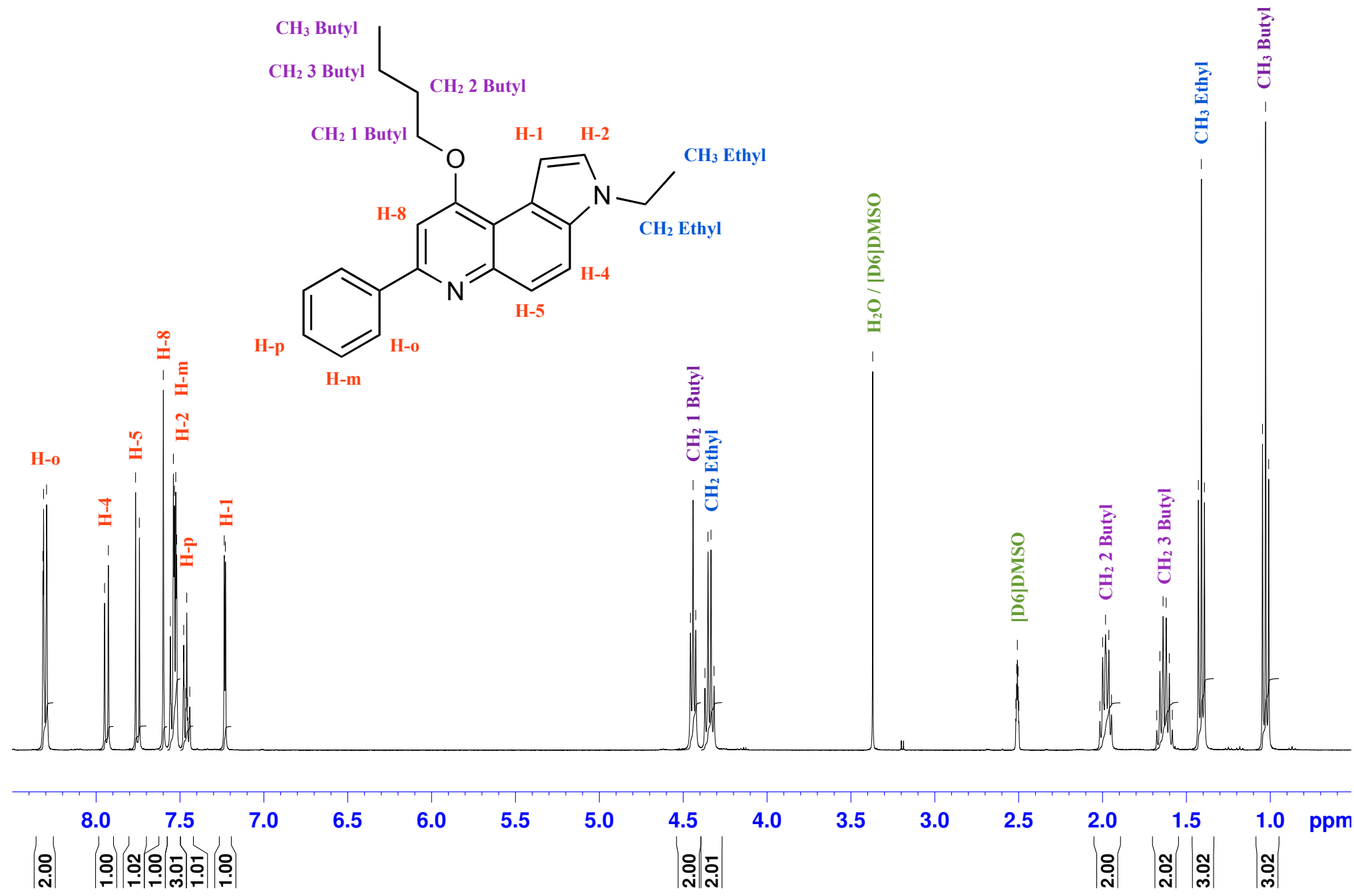


Figure S2. ^{1}H NMR spectrum (400 MHz, [D6]DMSO) of compound **10**.

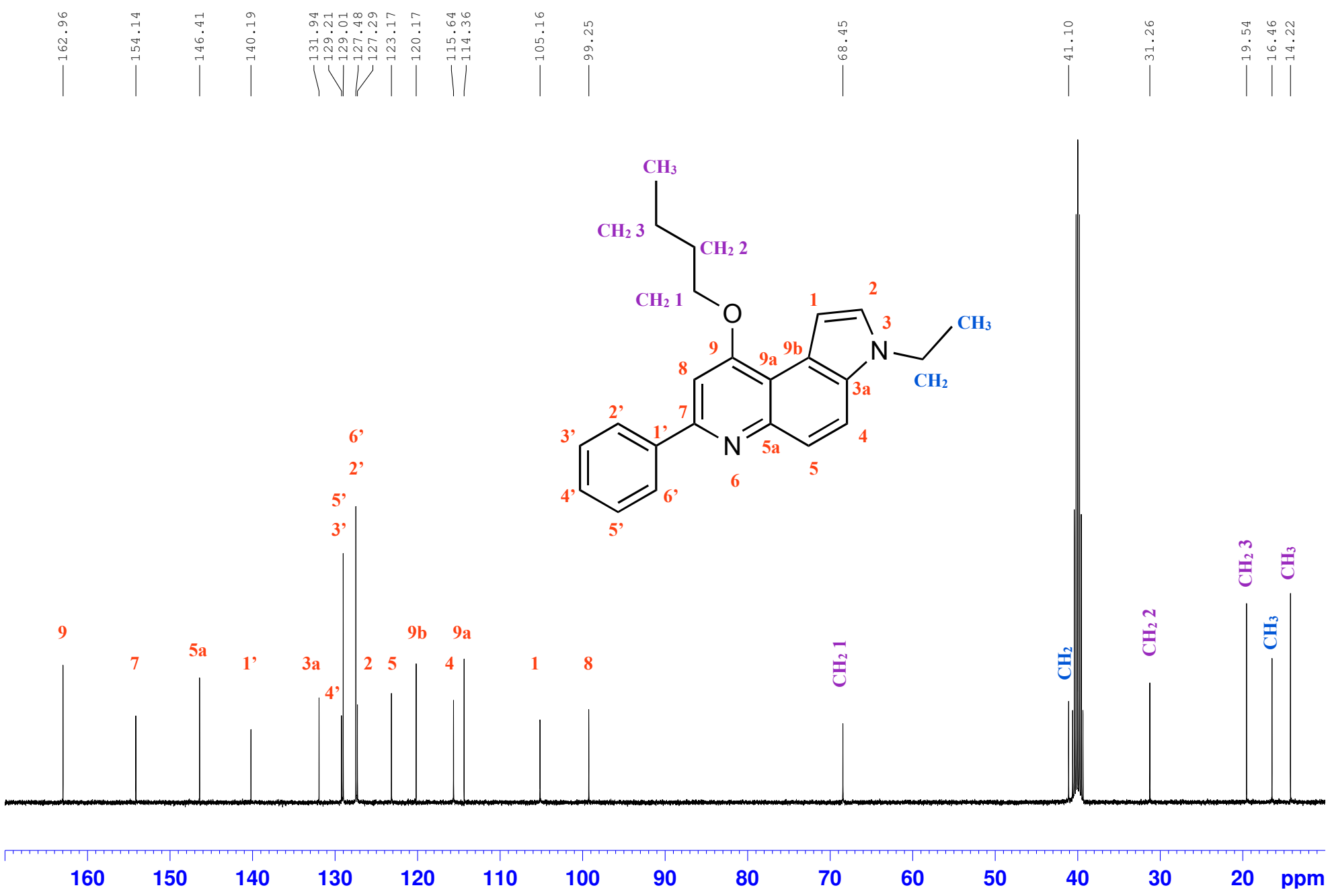
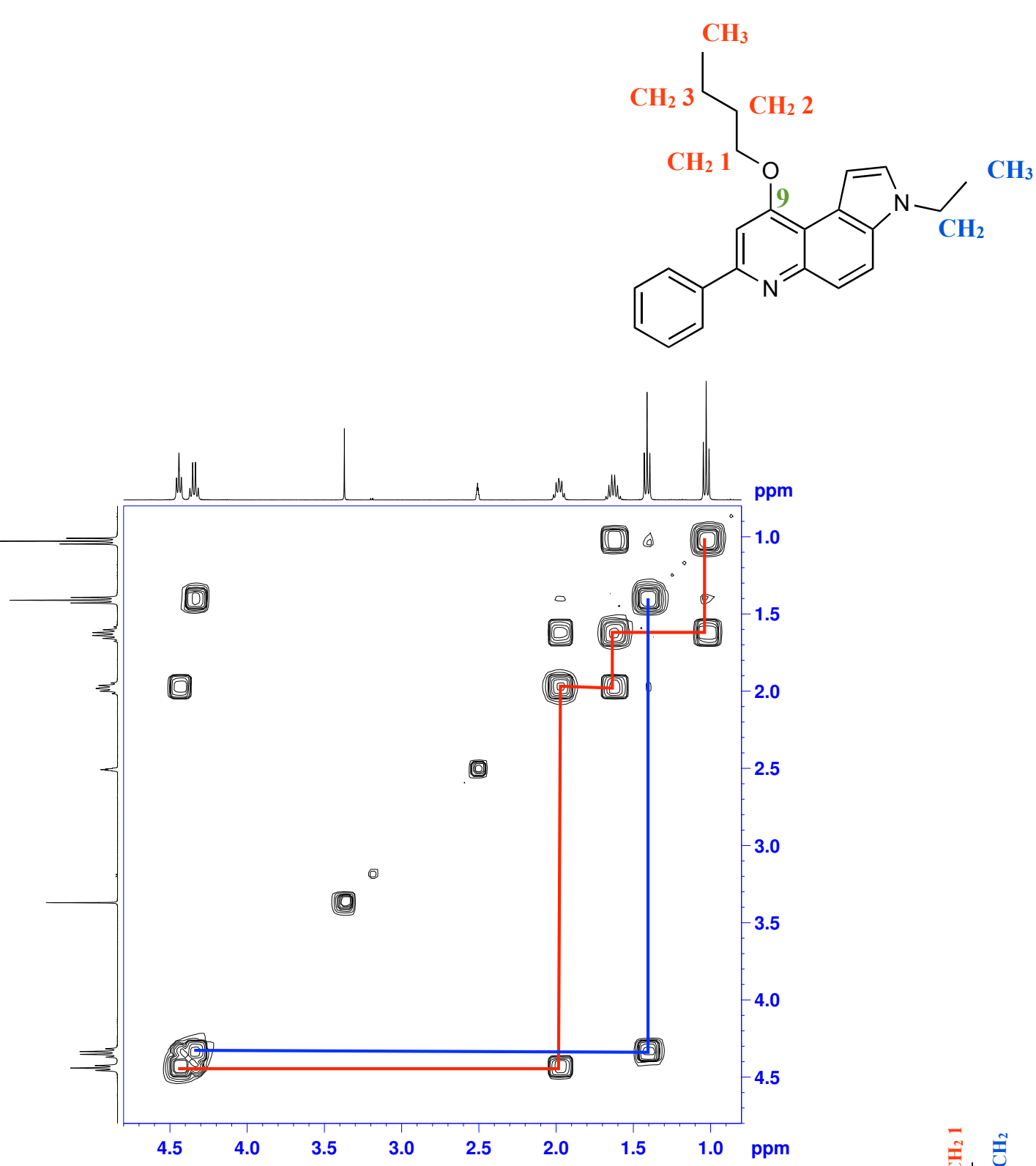


Figure S3. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, [D6]DMSO) of compound **10**.



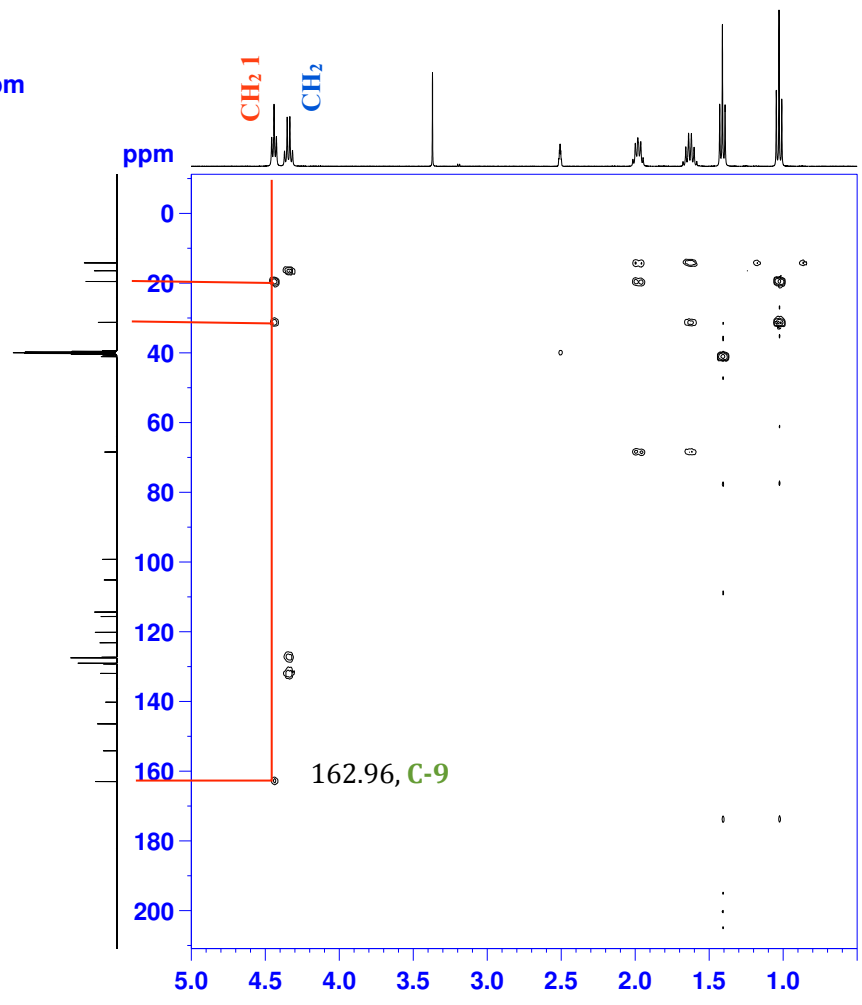


Figure S4. ^{1}H-^{1}H 2D COSY (left) and ^{1}H-^{13}C 2D HMBC (right) NMR correlation tables ([D6]DMSO) of compound **10**.

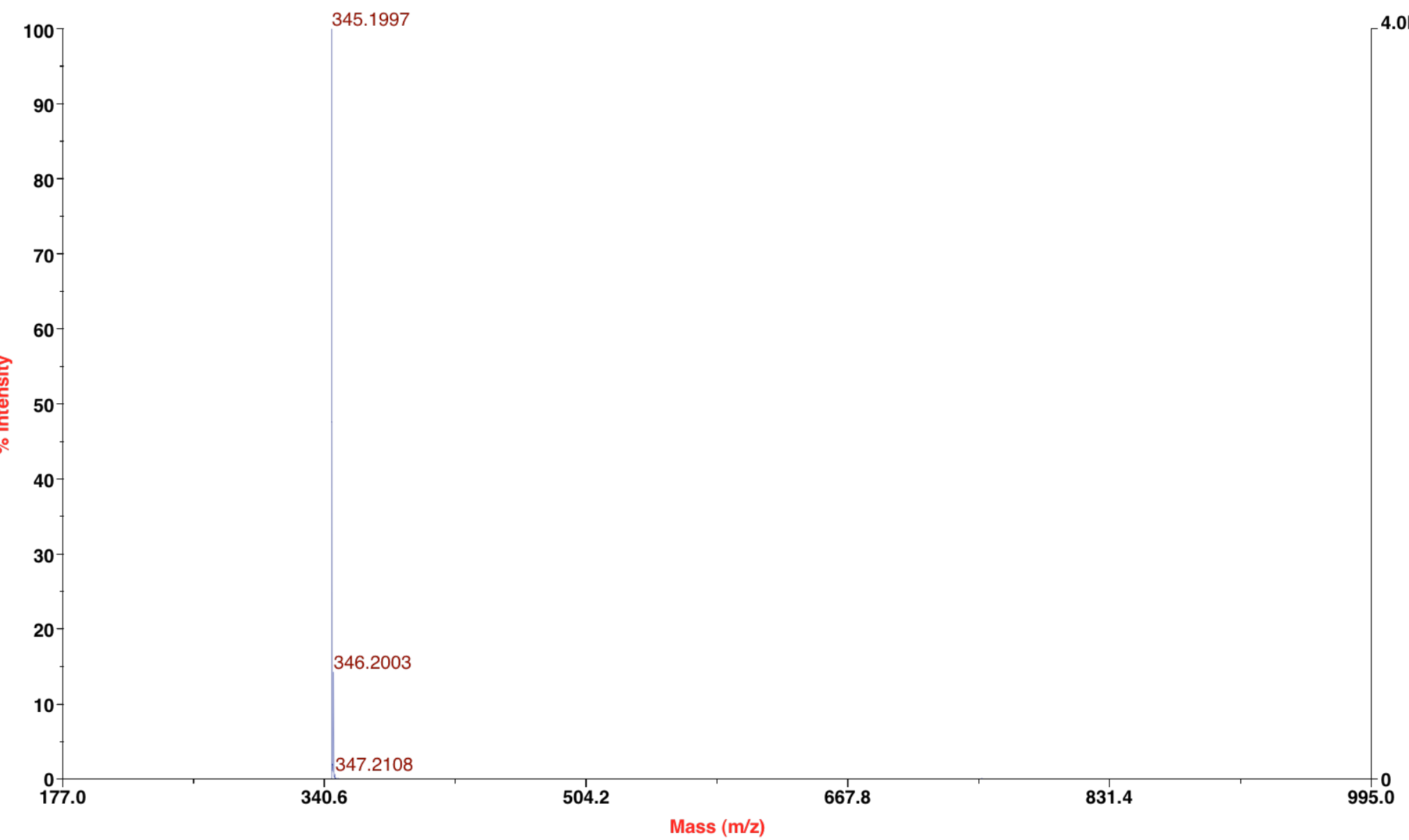


Figure S5. HRMS (ESI-MS, 140 eV) spectrum of compound **10**.

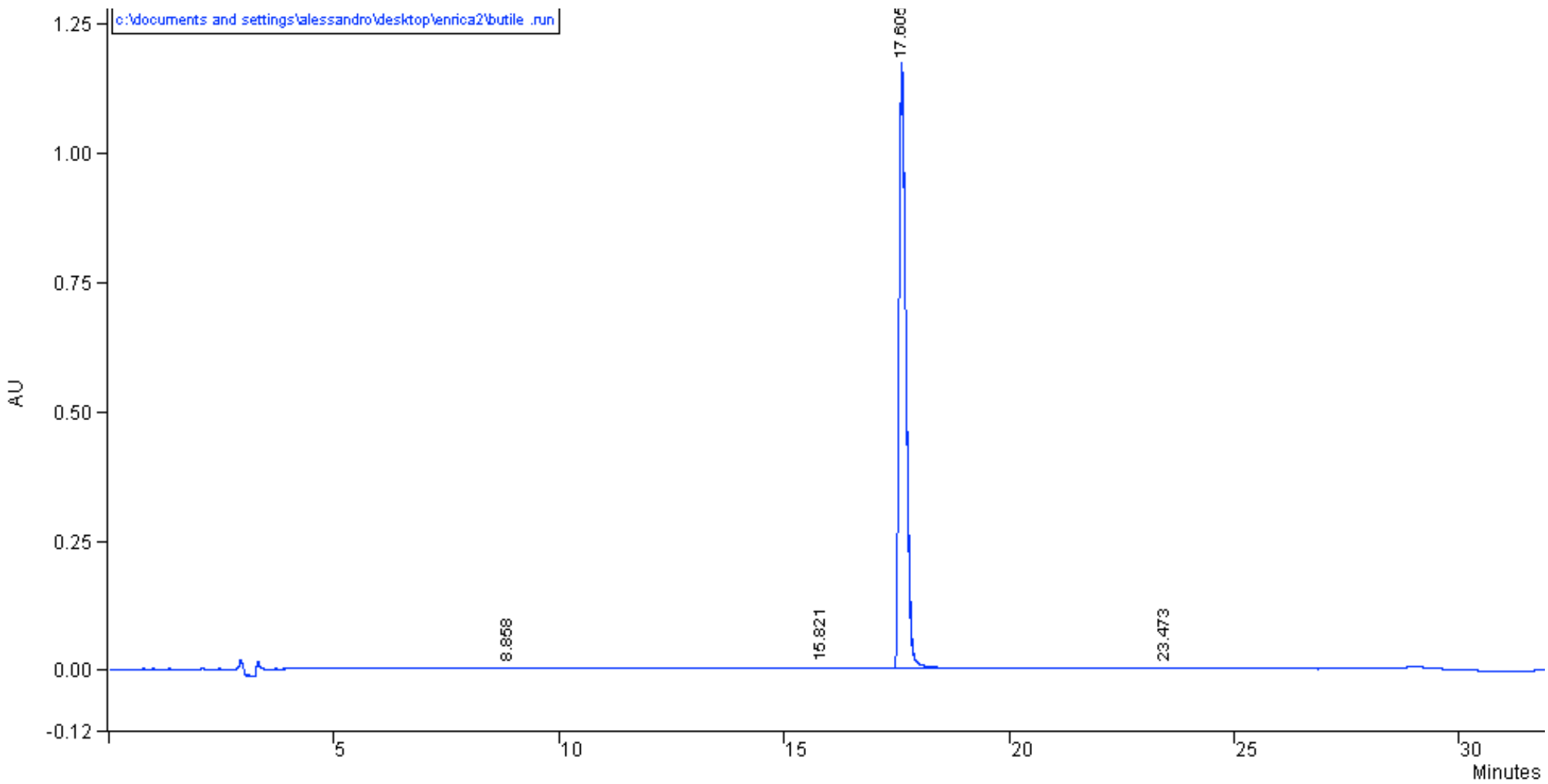


Figure S6. HPLC trace of compound **10**.

[D6]DMSO

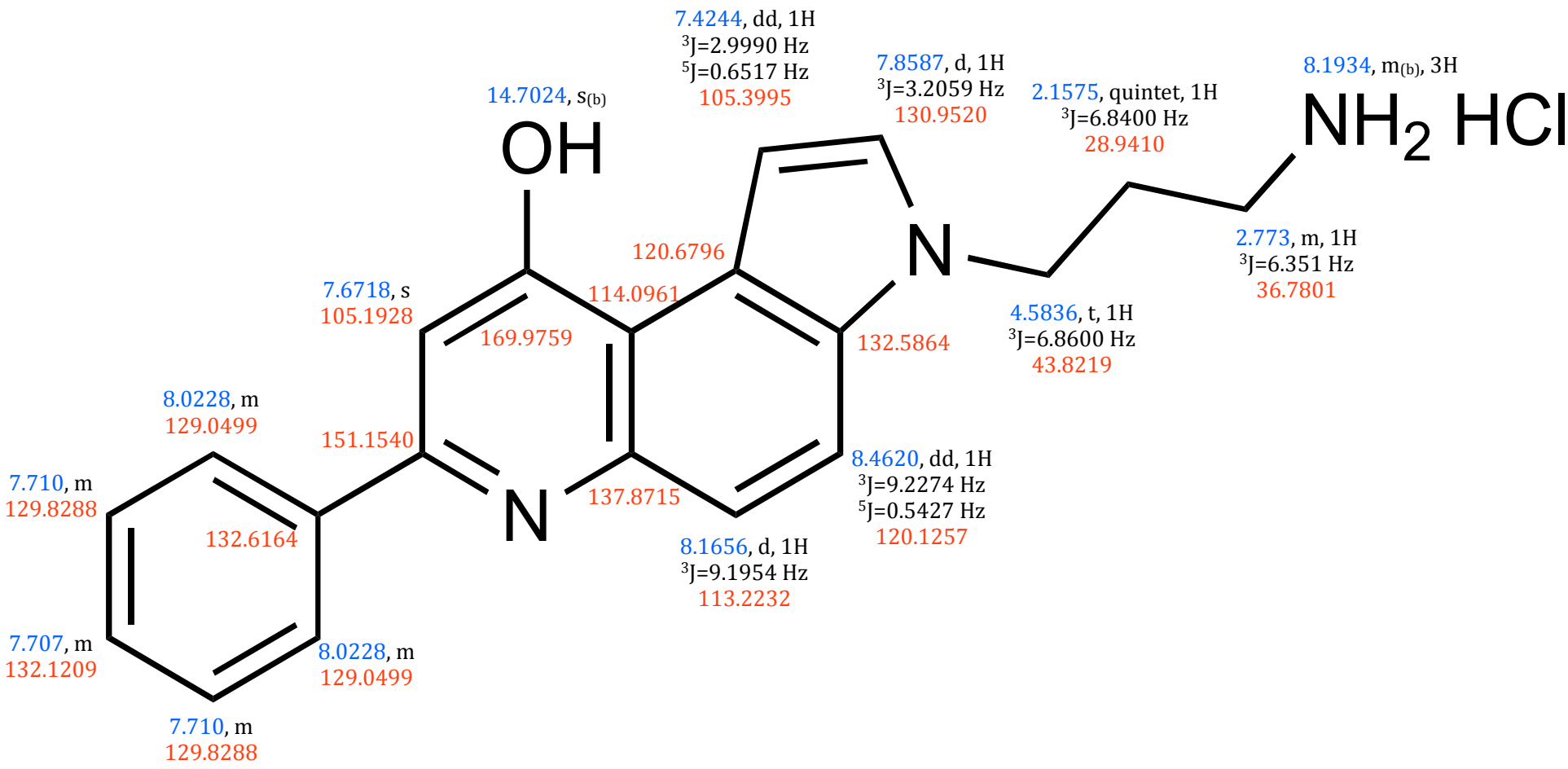


Figure S7. Complete ^{1}H and ^{13}C NMR signals assignation and structure elucidation for compound **18**.

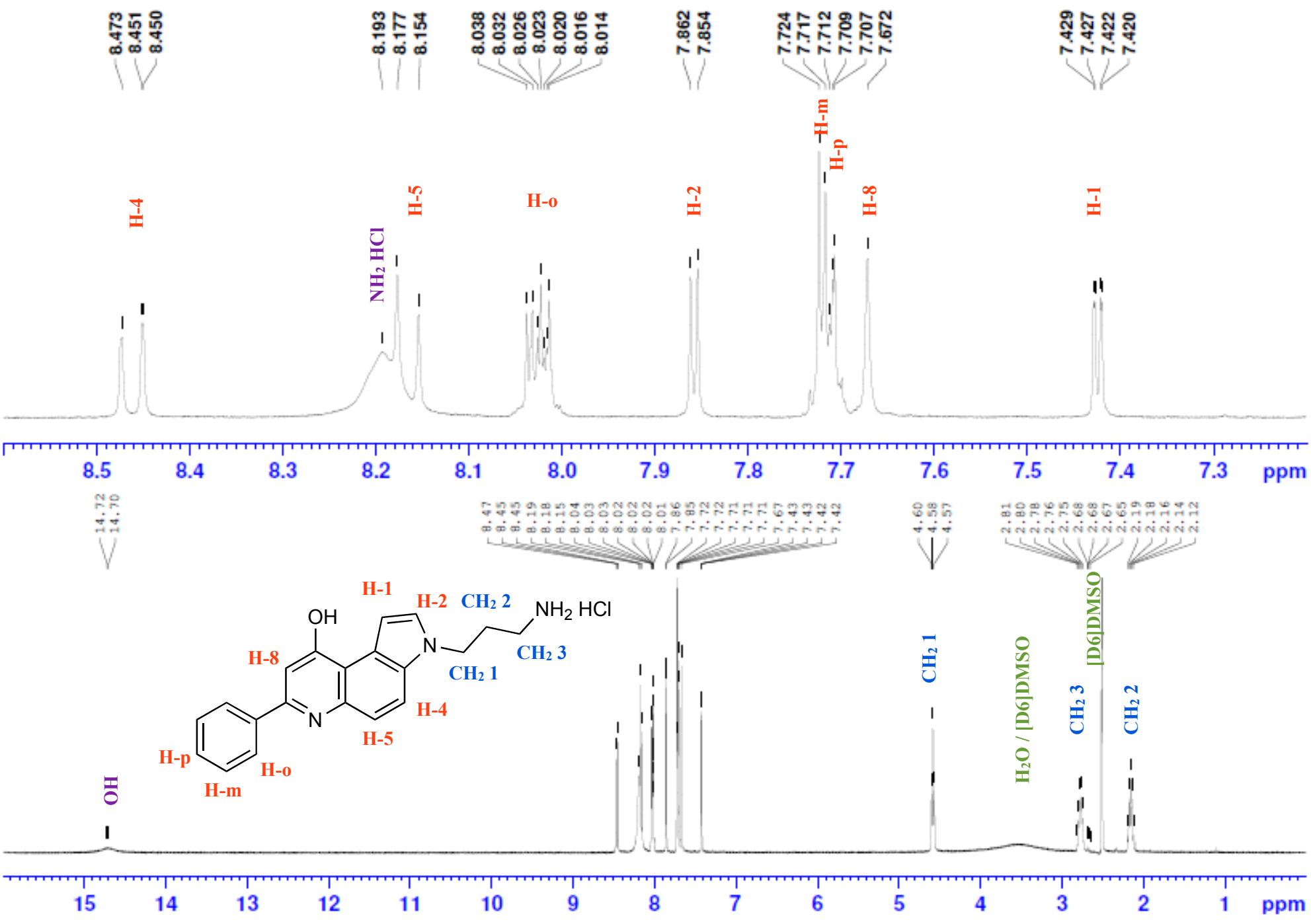


Figure S8. ^{1}H NMR spectrum (400 MHz, [D6]DMSO) of compound **18**.

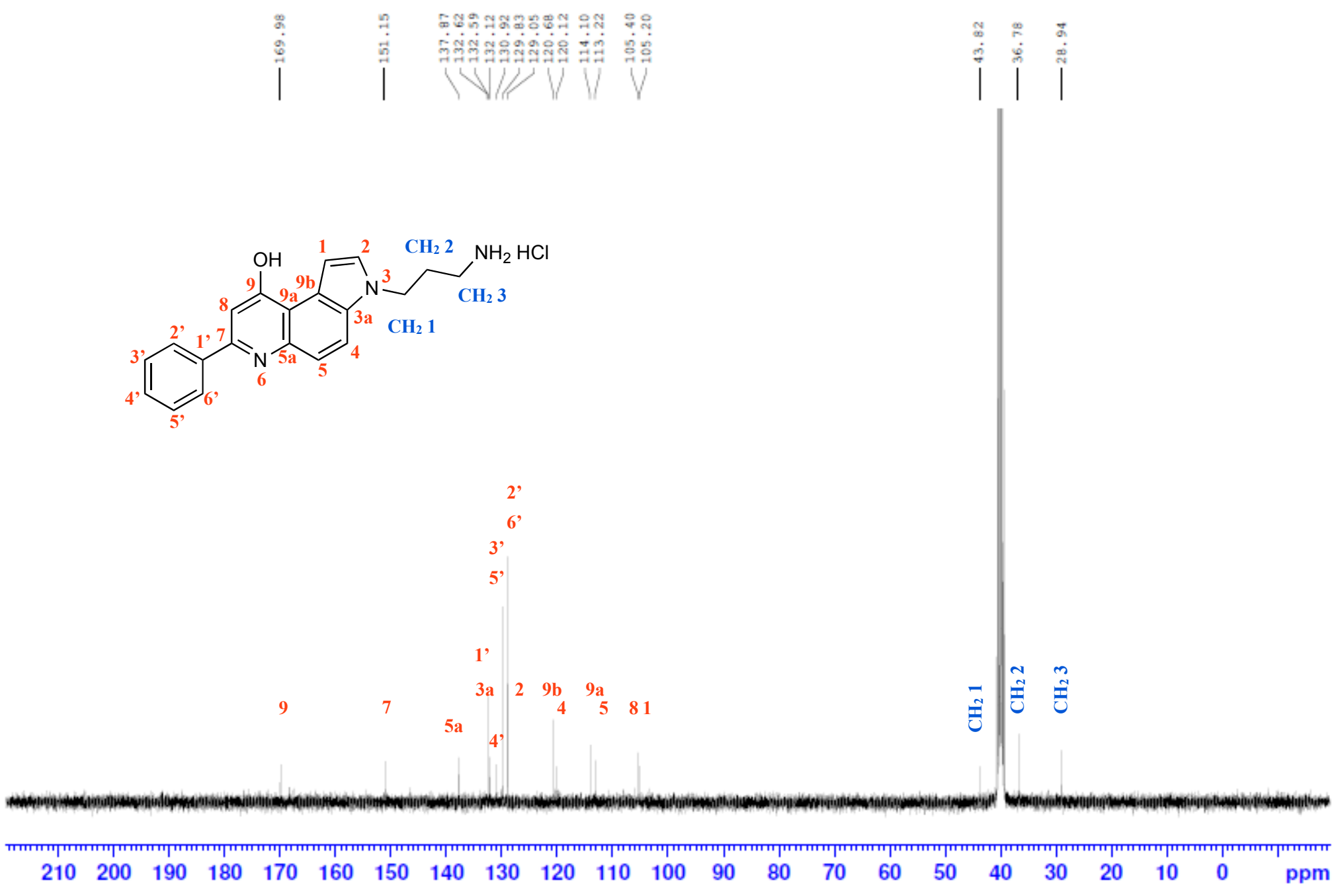


Figure S9. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, [D6]DMSO) of compound **18**.

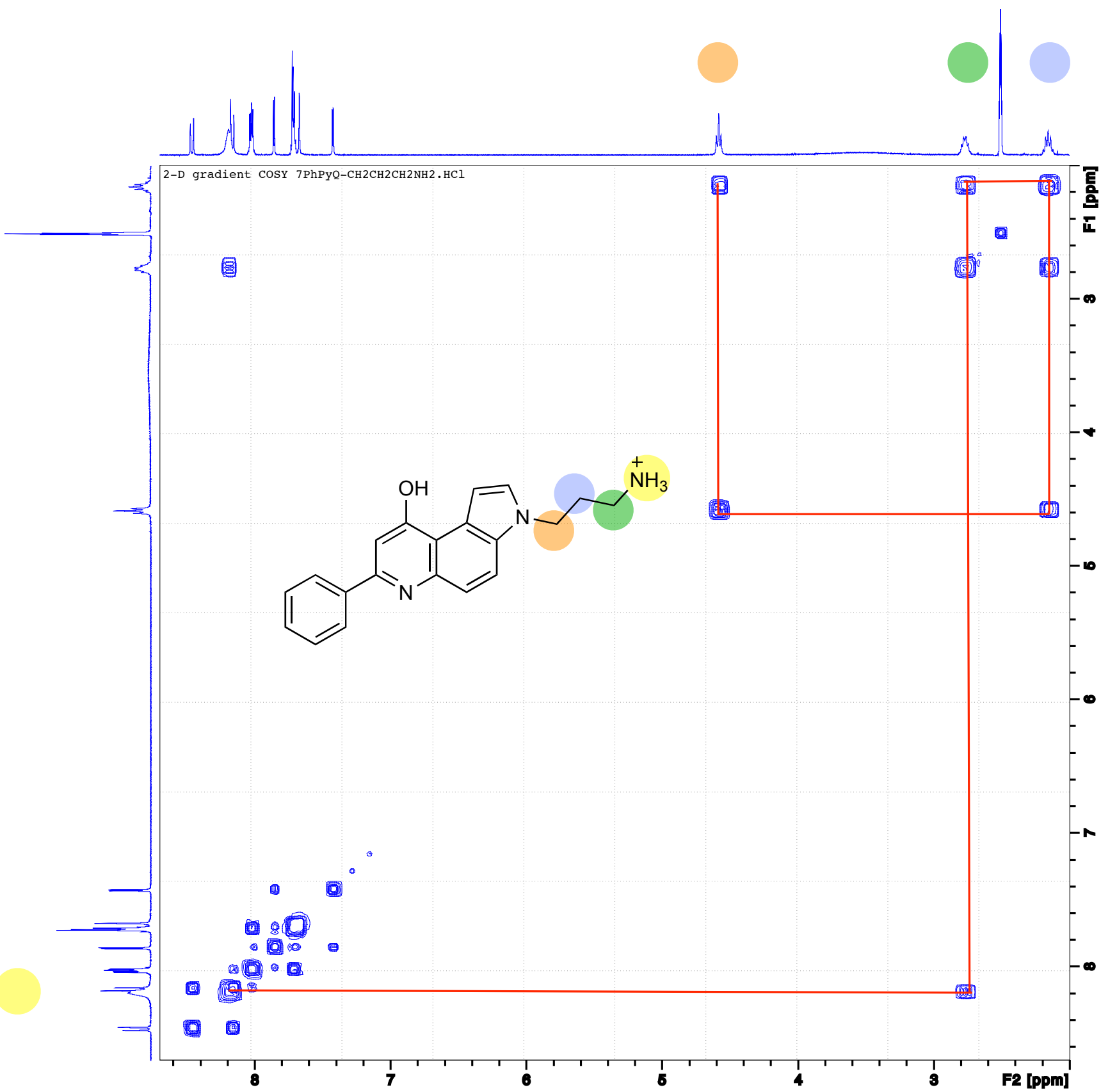


Figure S10. ^{1}H-^{1}H 2D COSY NMR correlation table ([D6]DMSO) of compound **18**.

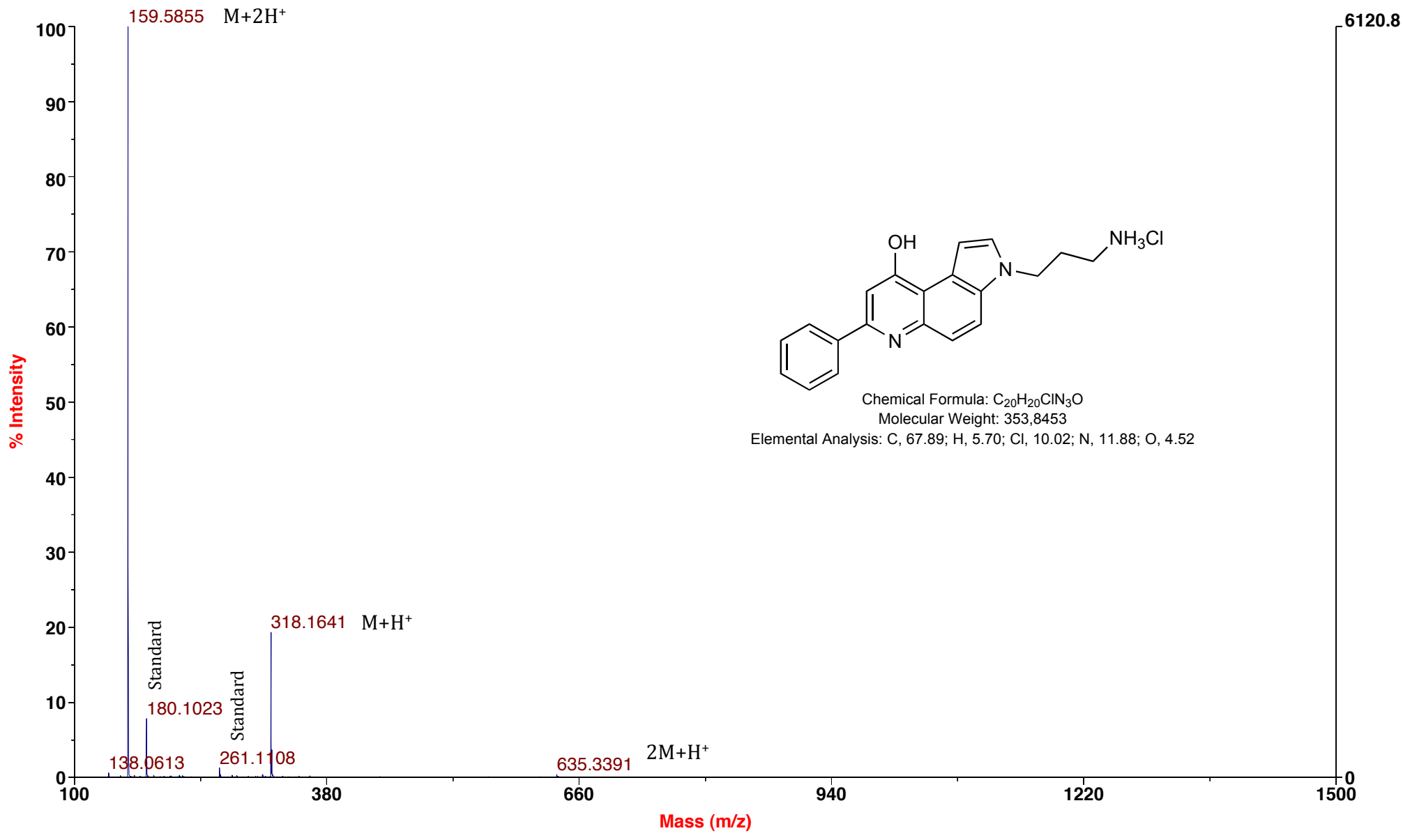


Figure S11. HRMS (ESI-MS, 140 eV) spectrum of compound **18**.

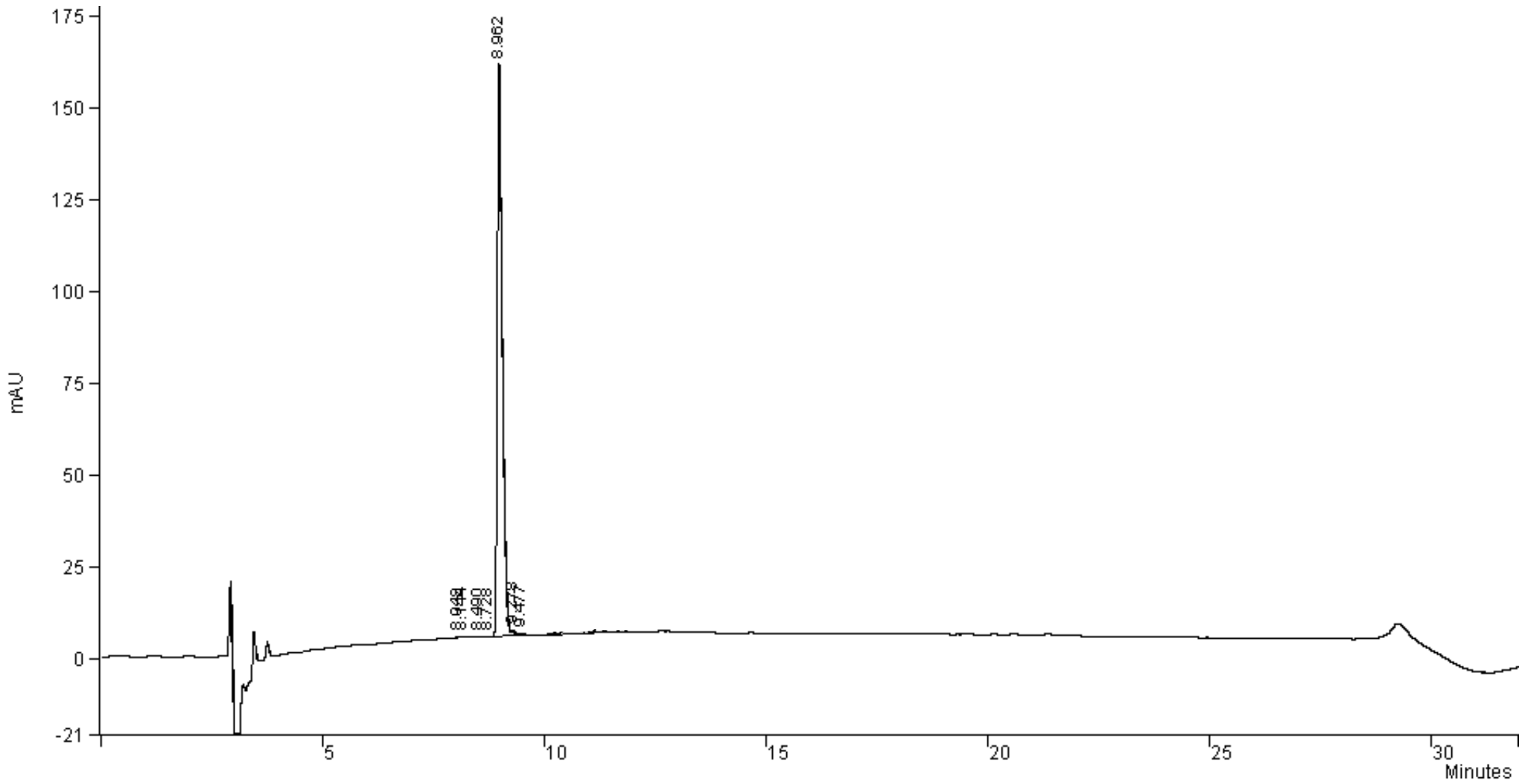


Figure S12. HPLC trace of compound **18**.

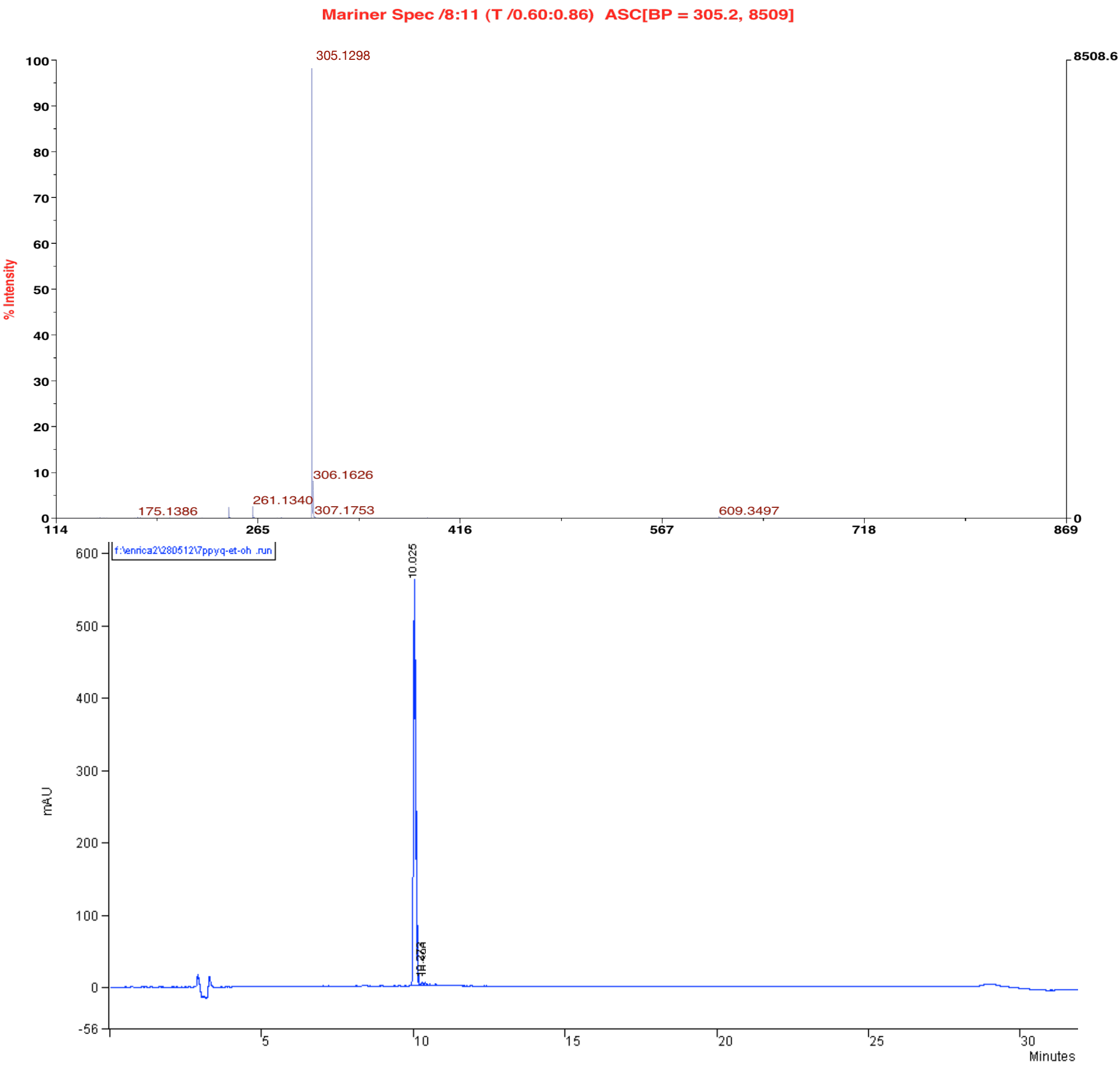


Figure S13. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **5a**.

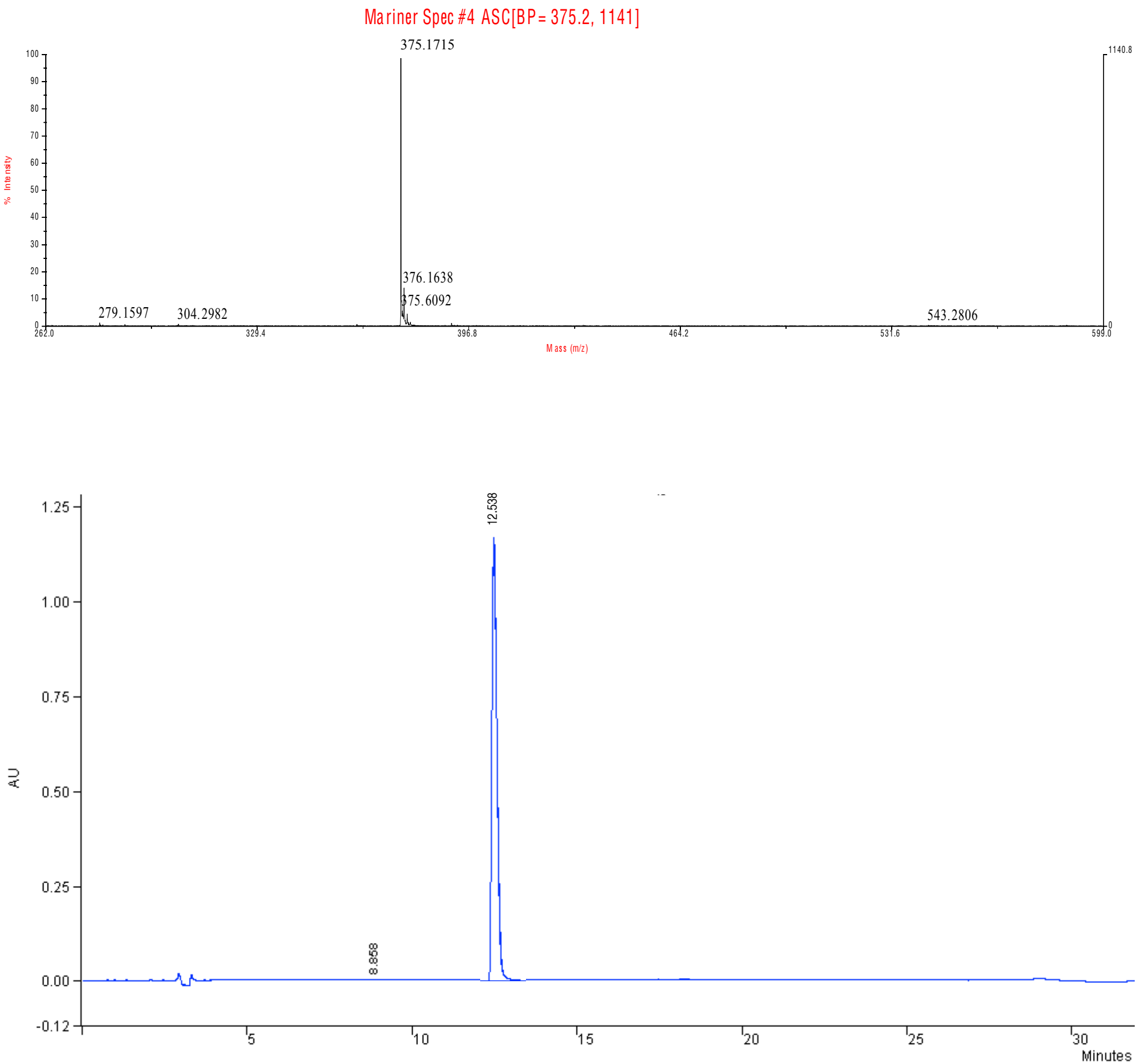
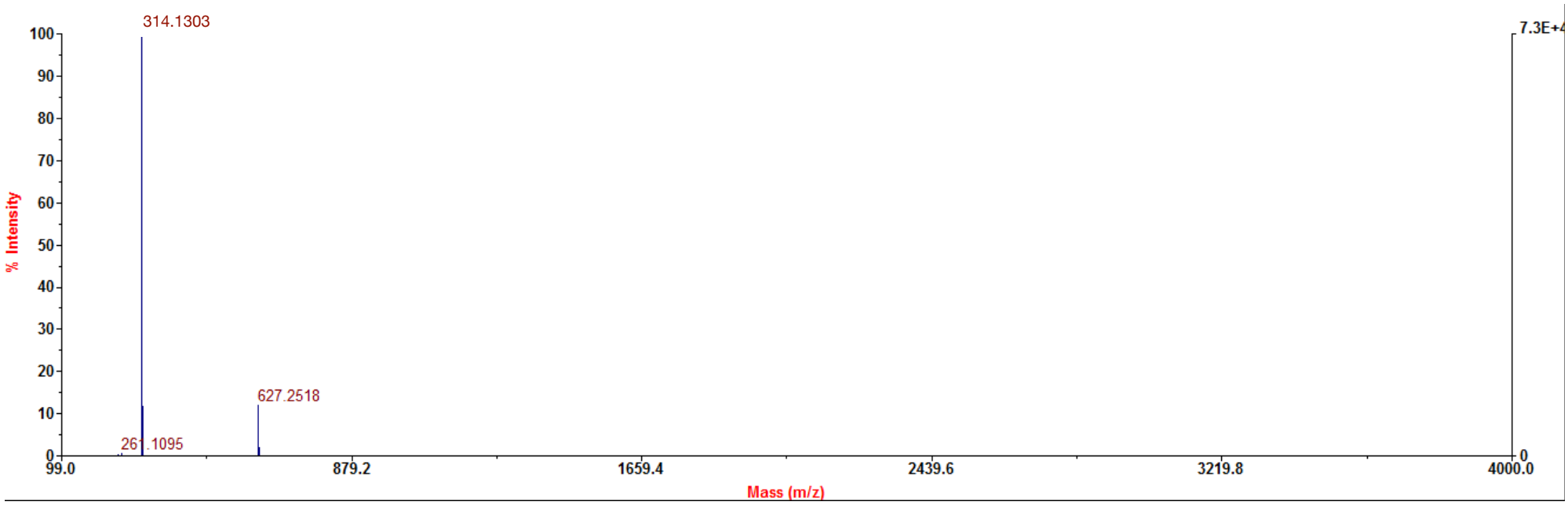


Figure S14. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **5b**.



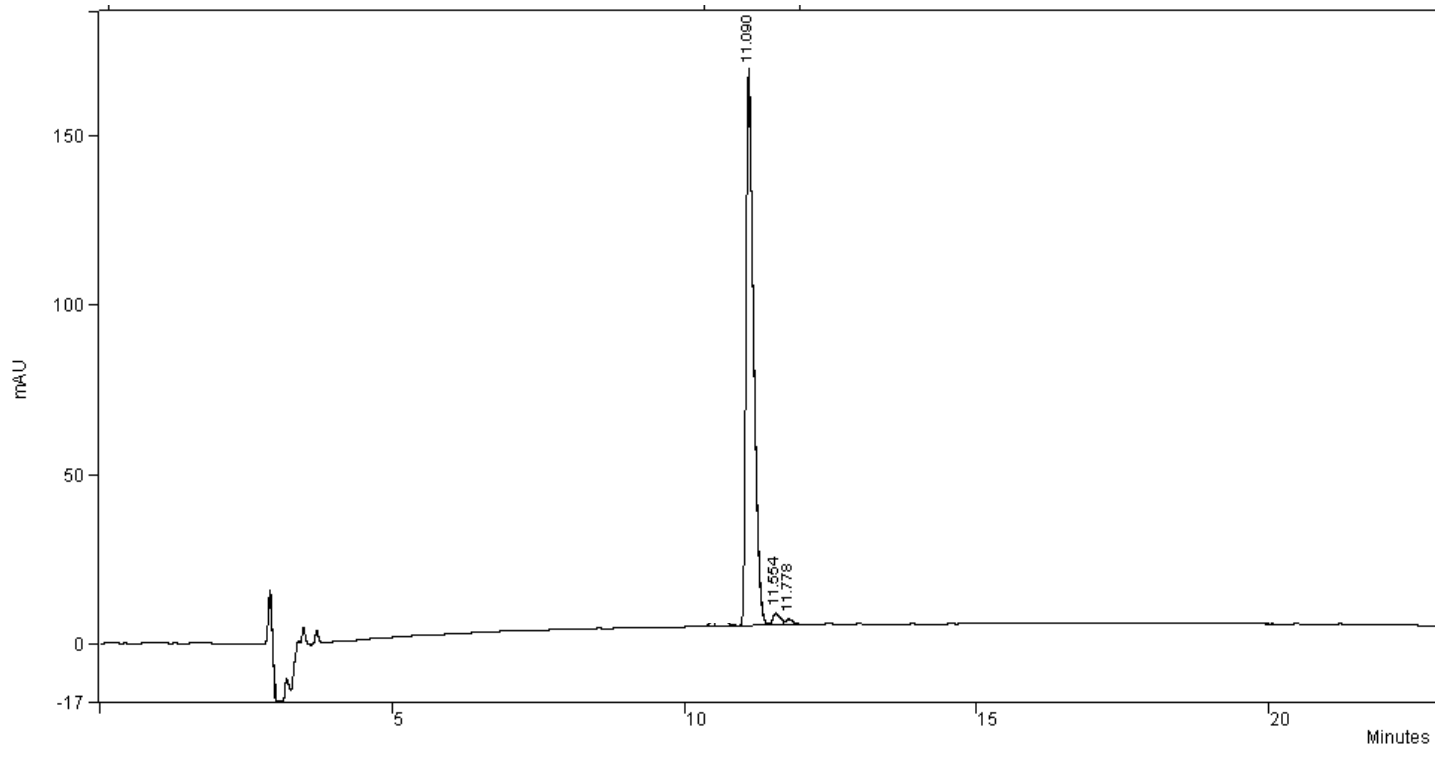
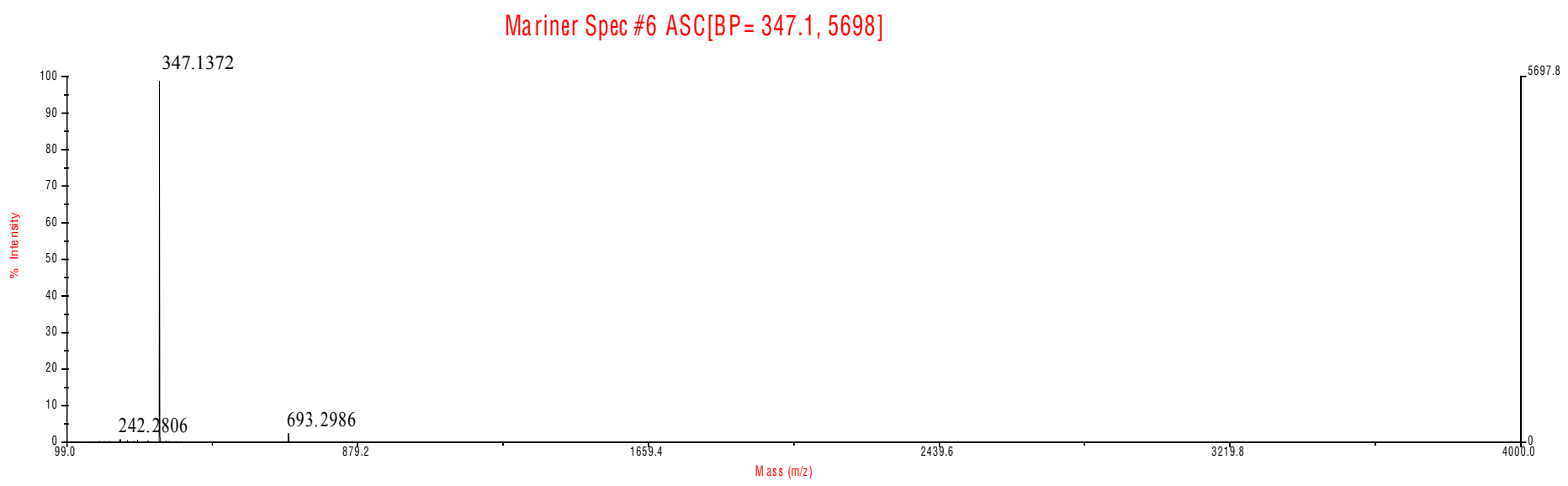


Figure S15. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **5c**.



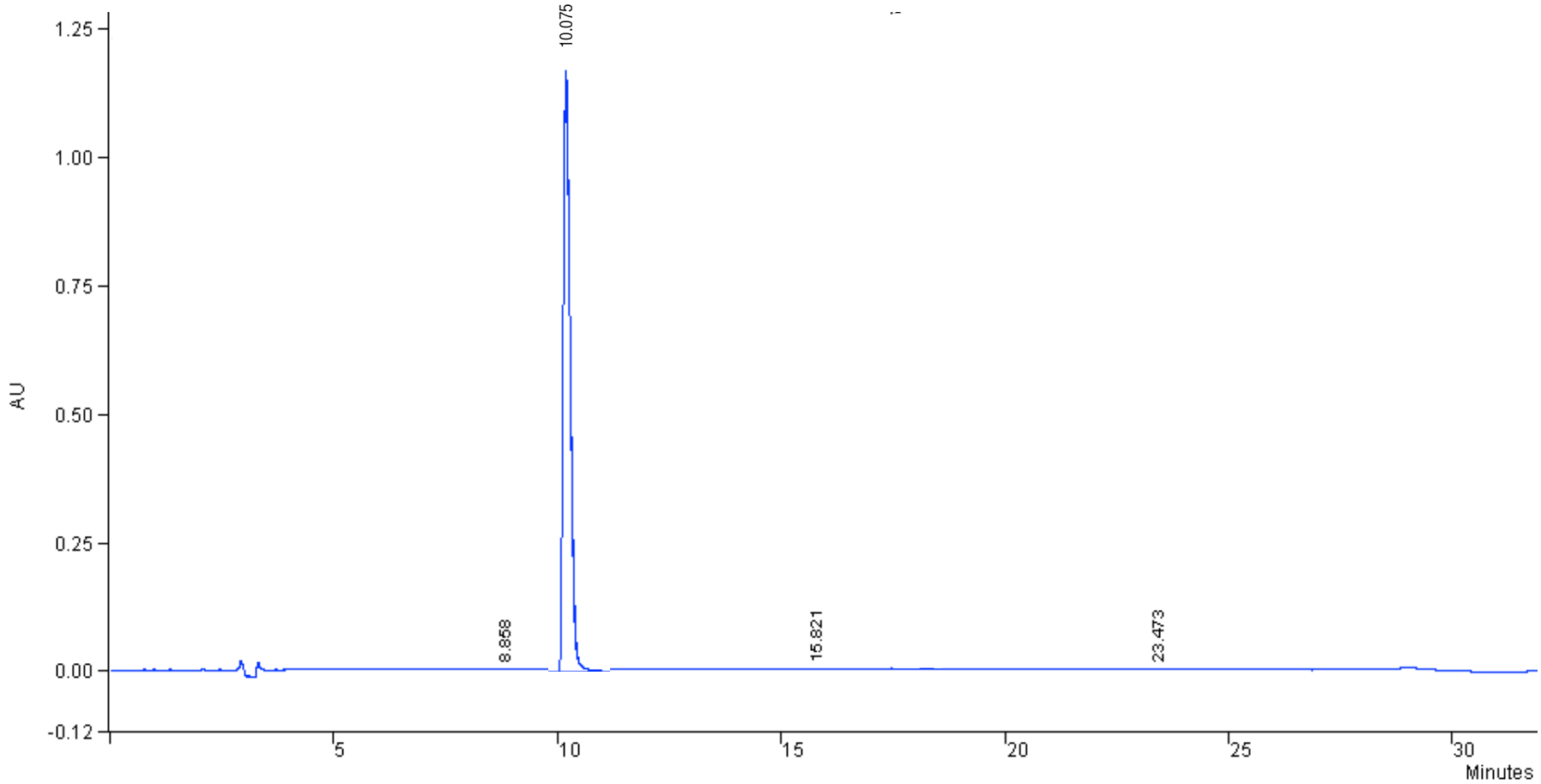


Figure S16. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **5d**.

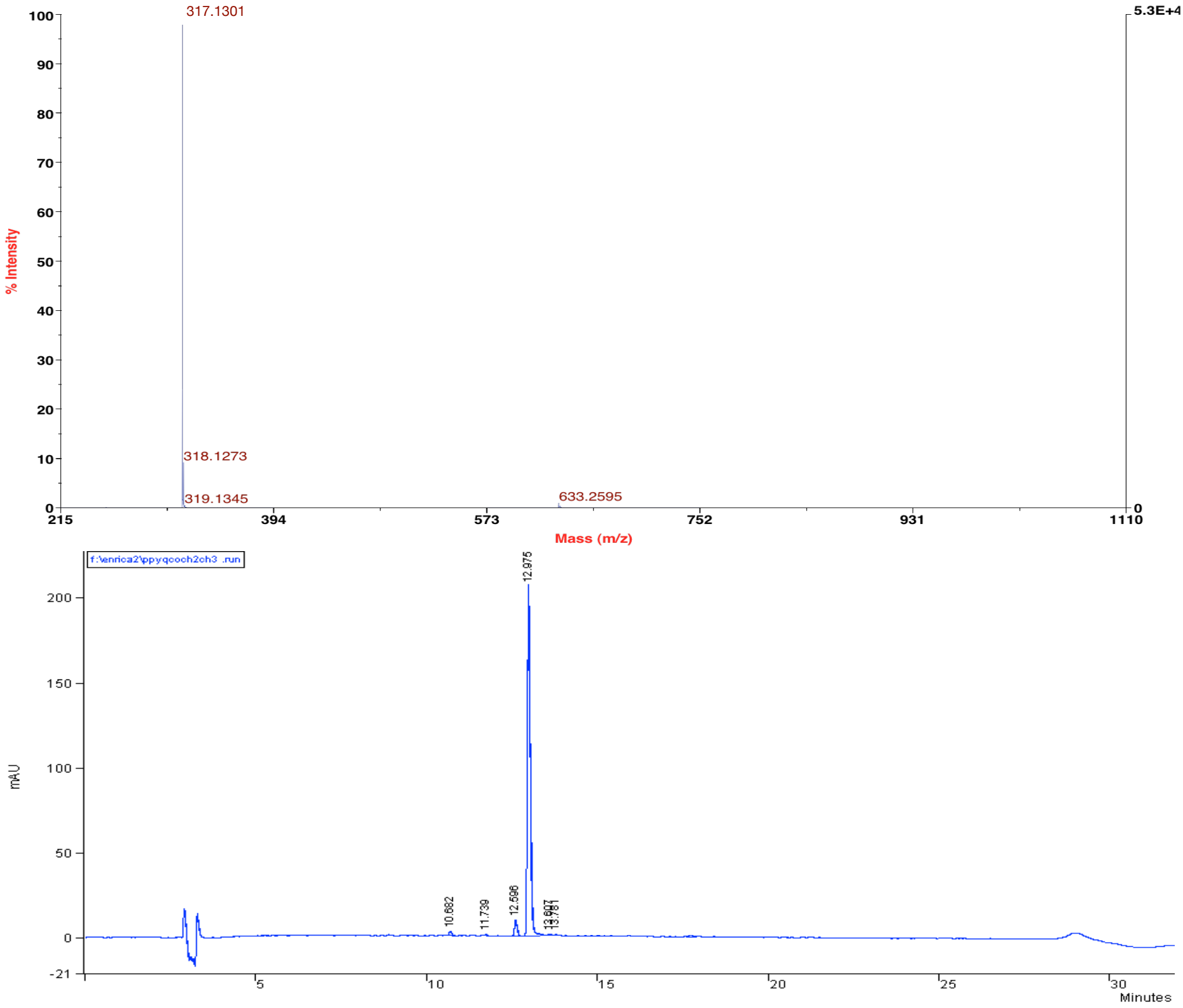
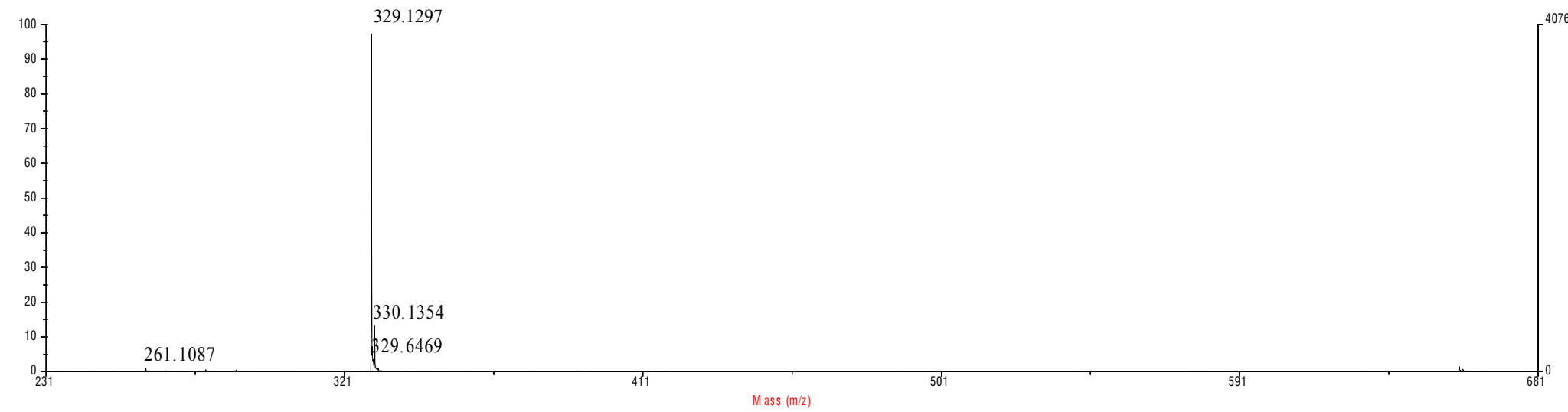


Figure S17. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **5e**.



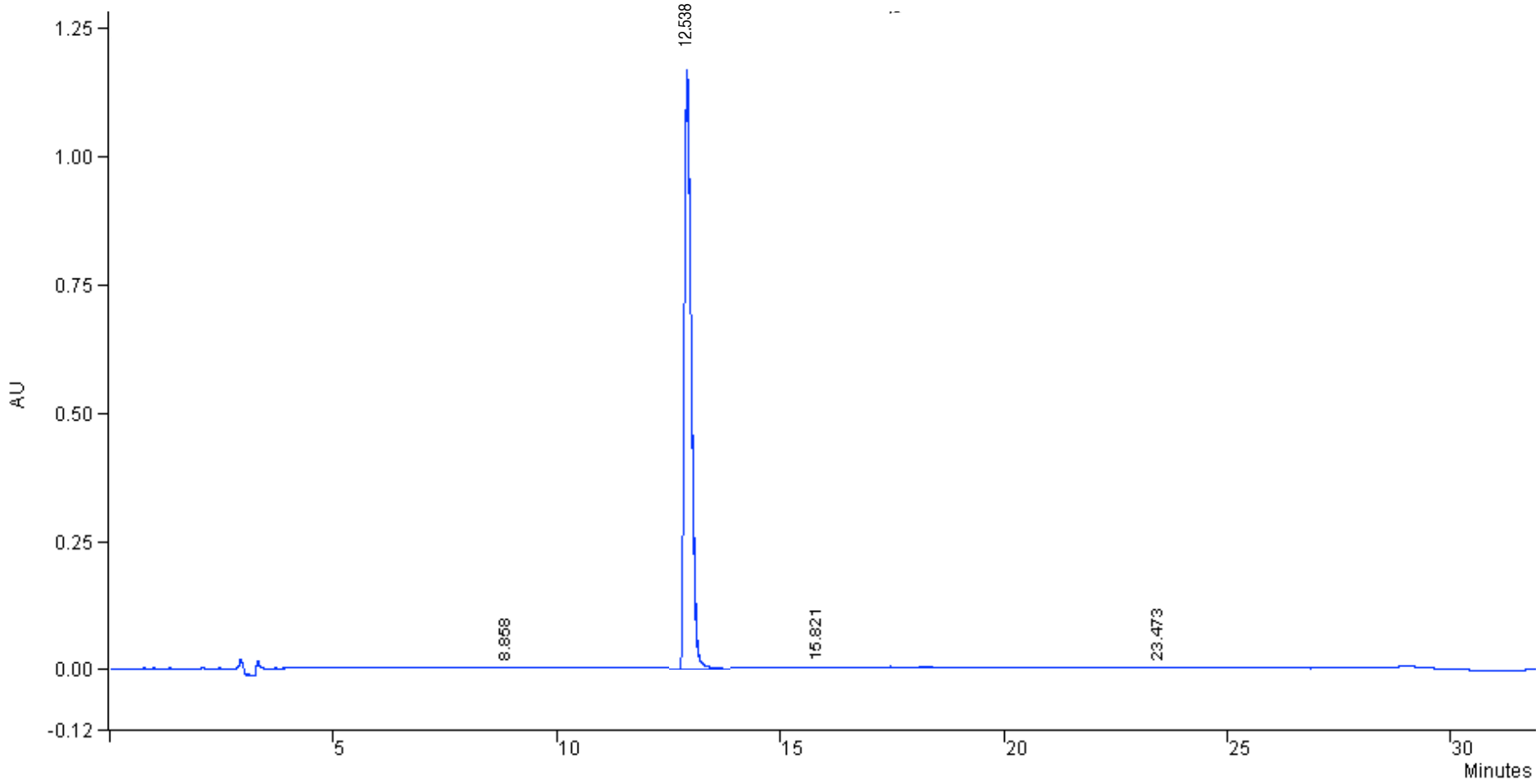


Figure S18. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **5f**.

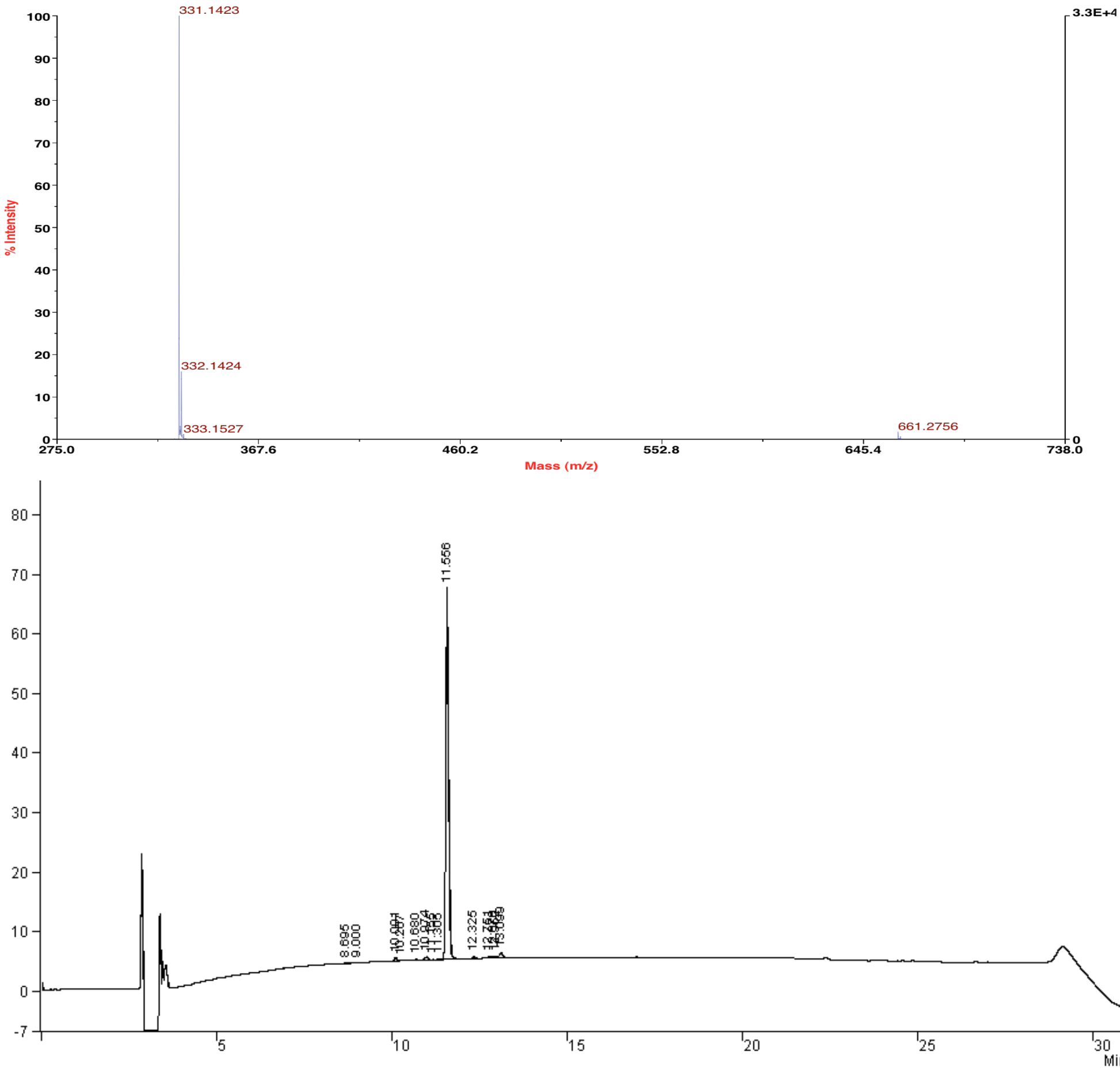


Figure S19. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **8f**.

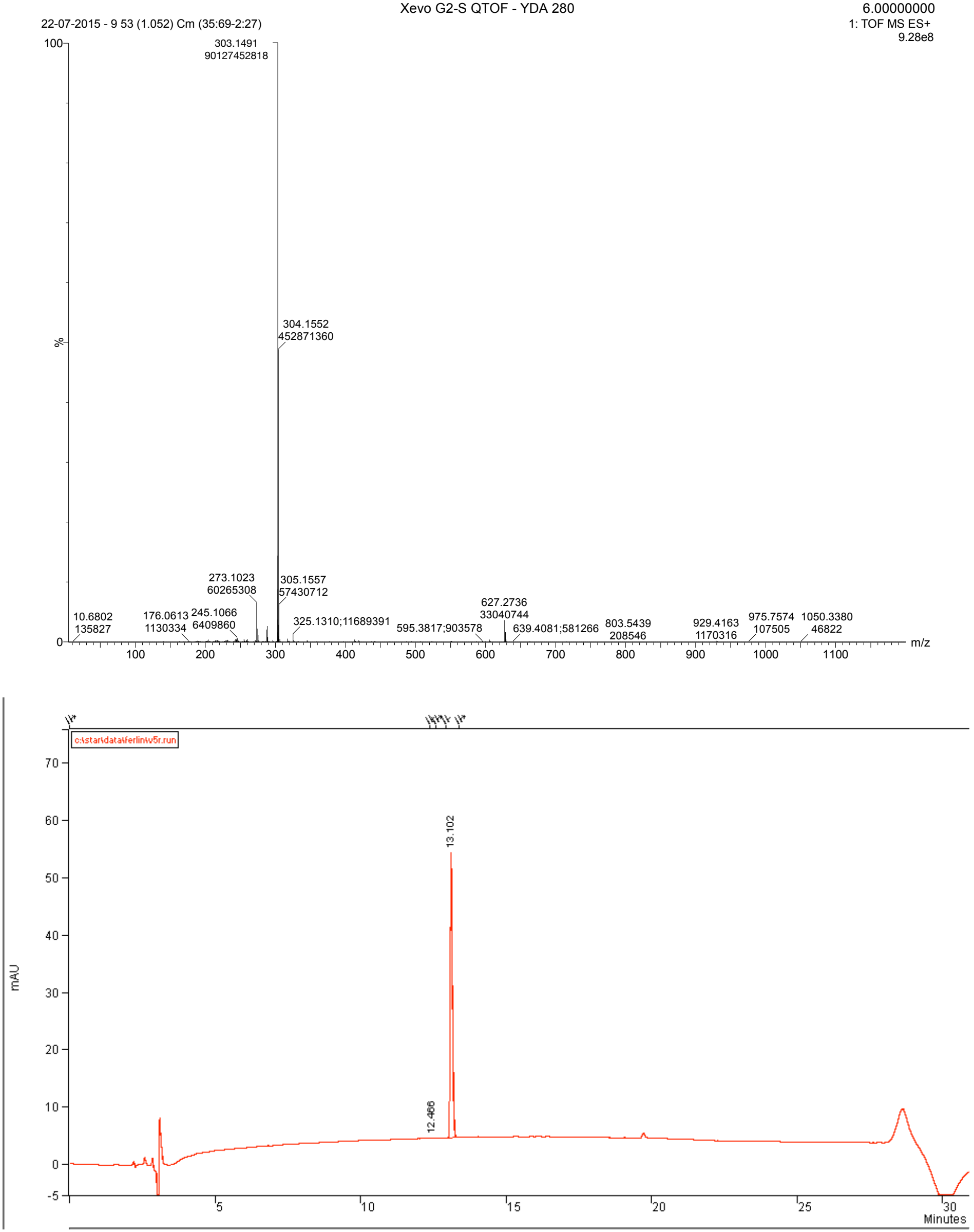


Figure S20. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **9**.

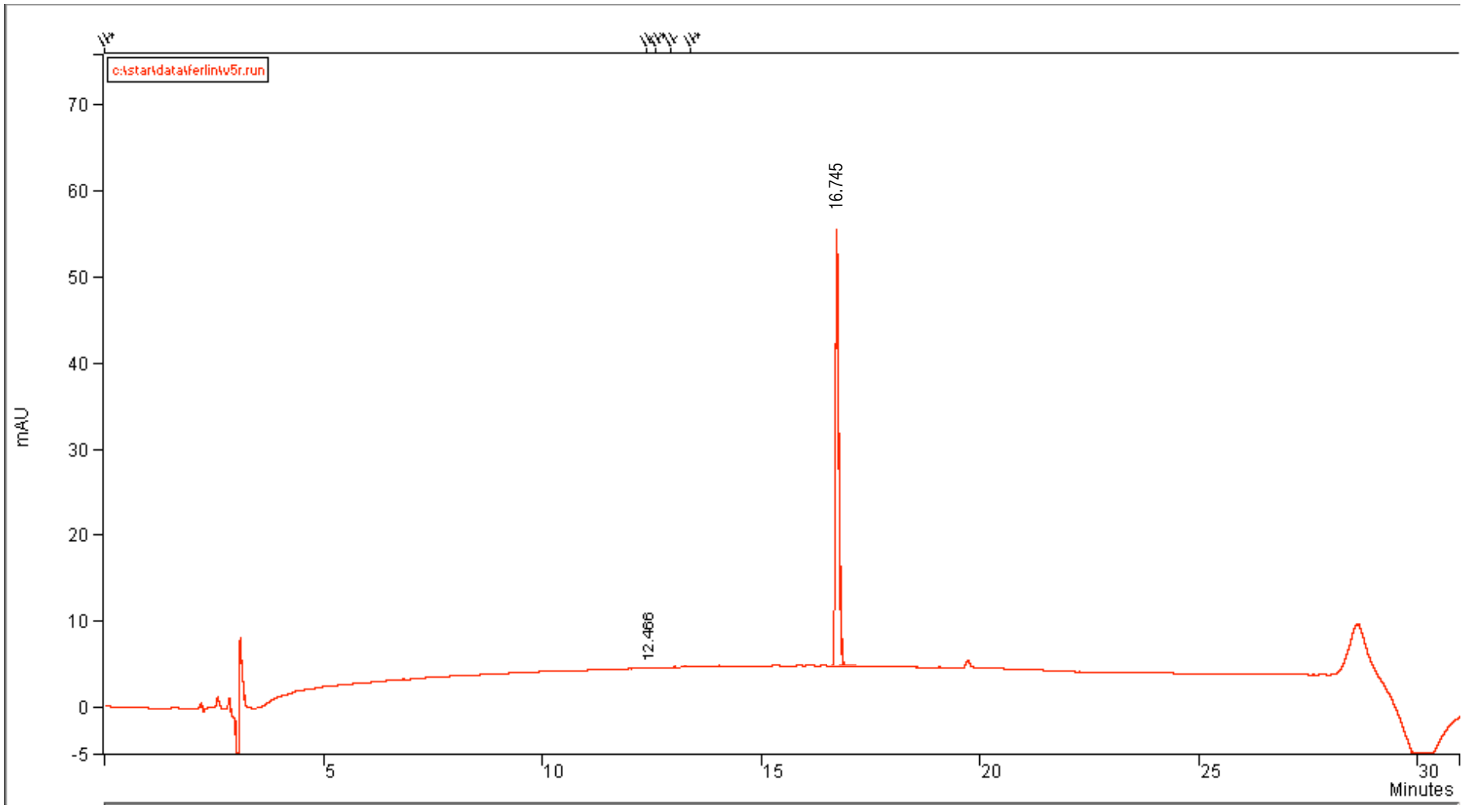


Figure S21. HPLC trace of compound **11**.

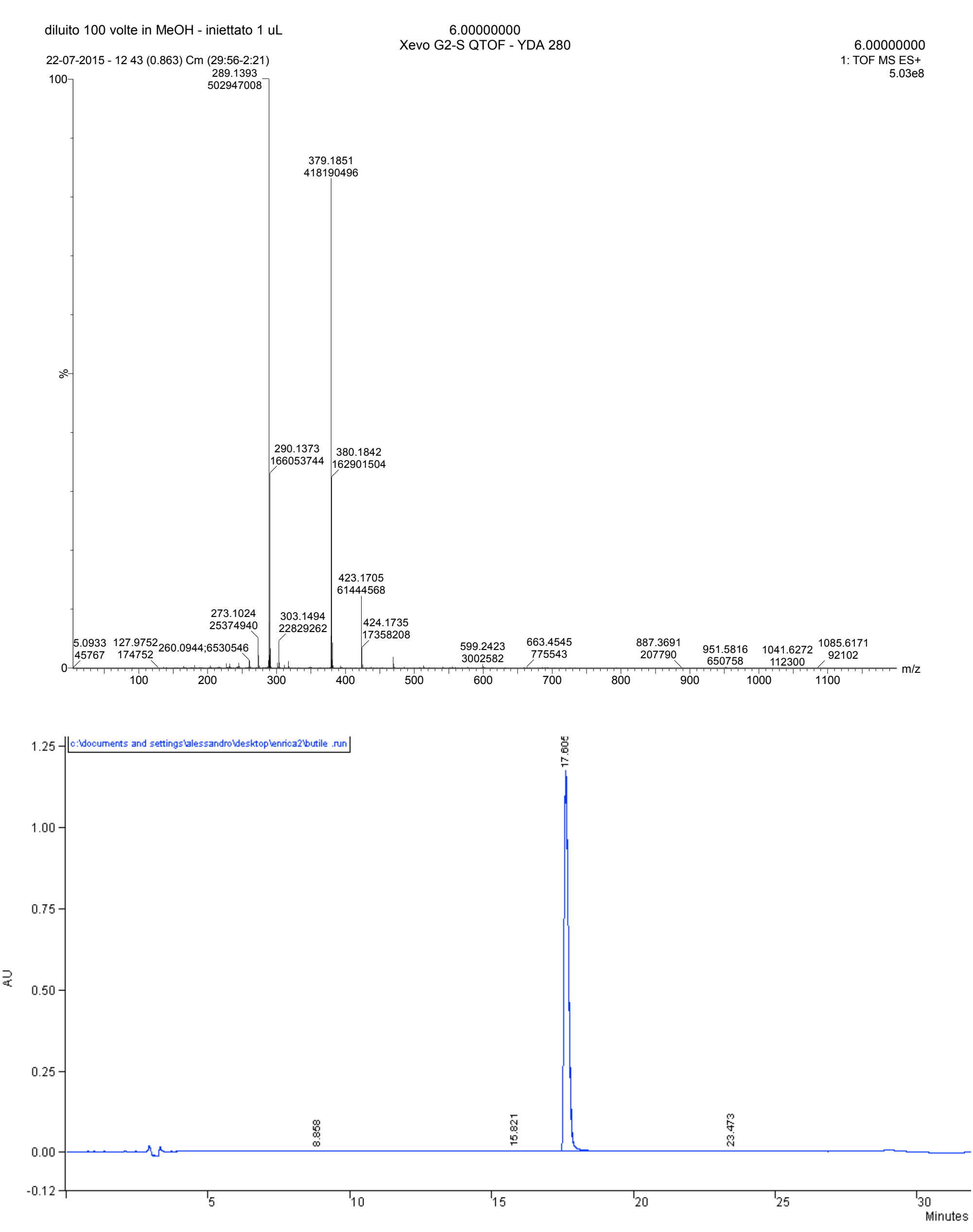
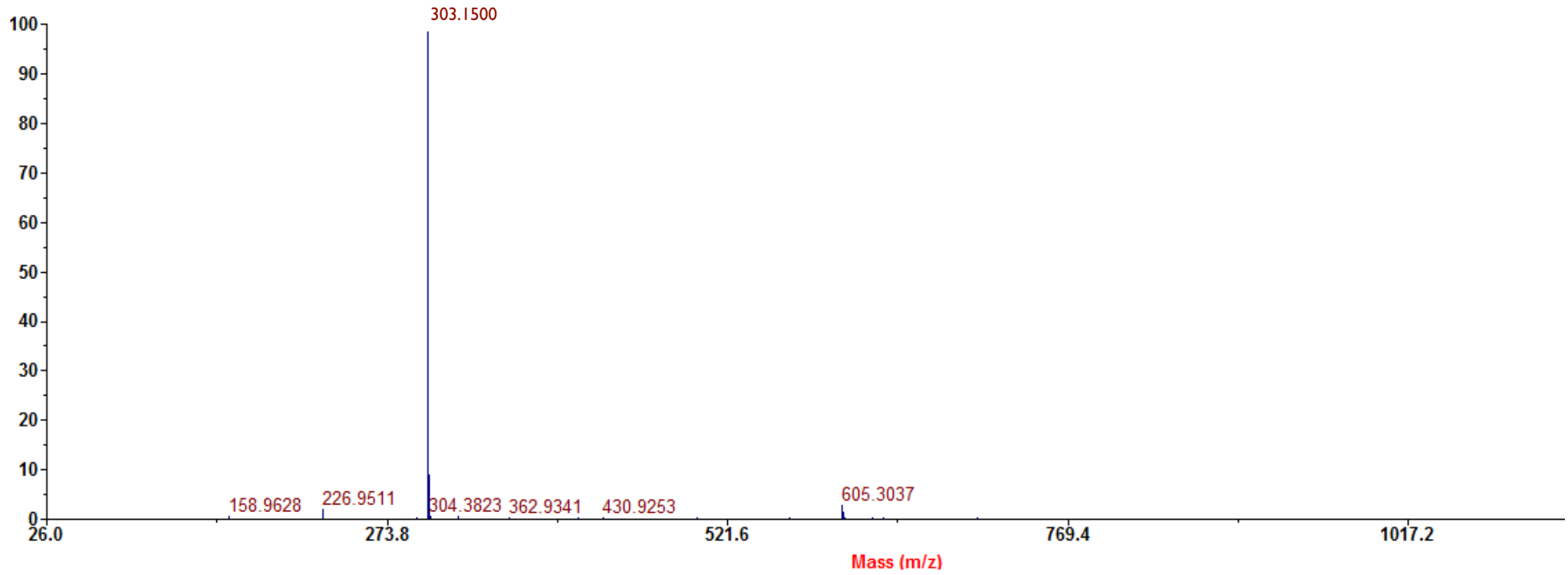


Figure S22. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **12**.



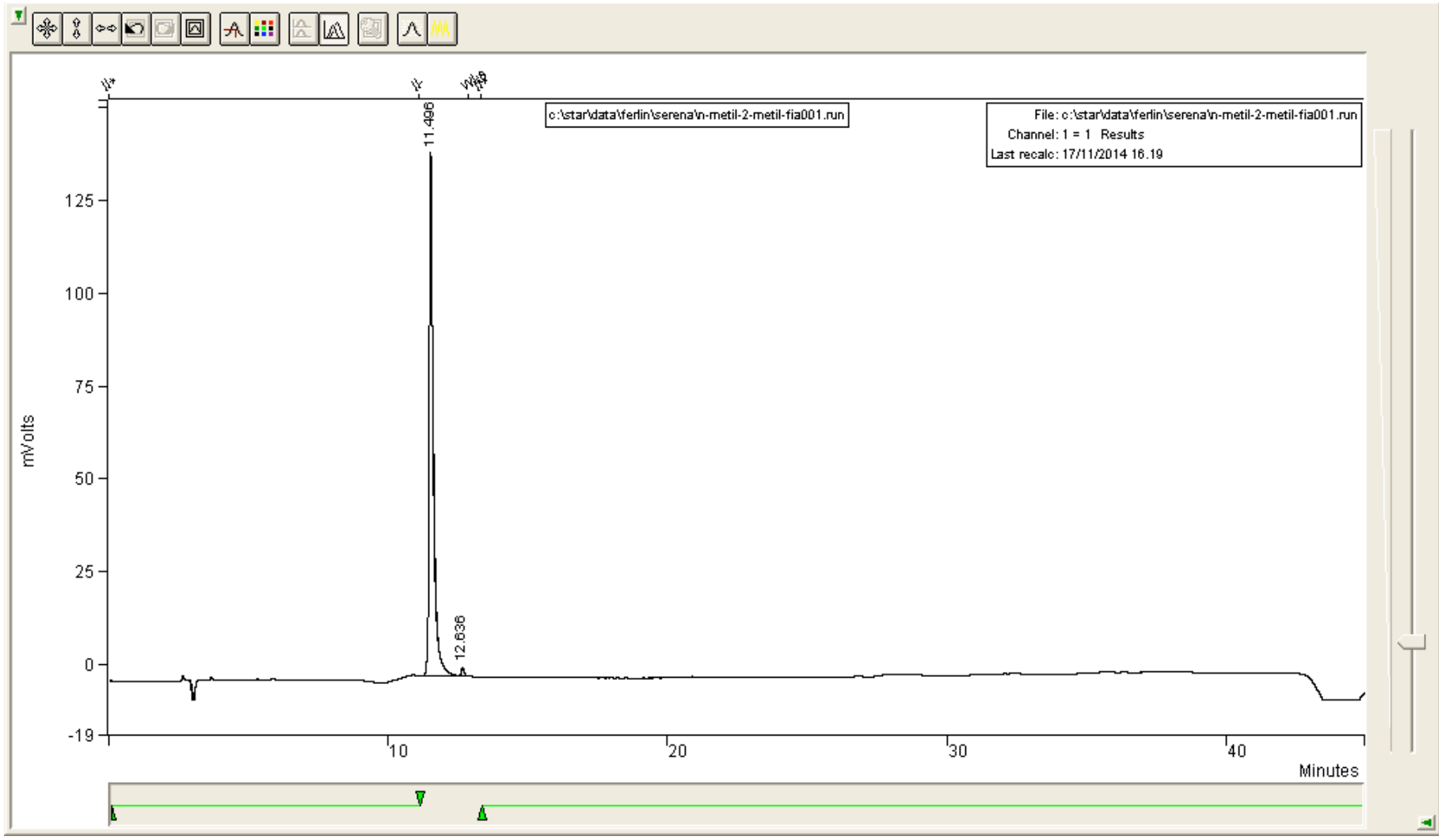


Figure S23. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **13**.

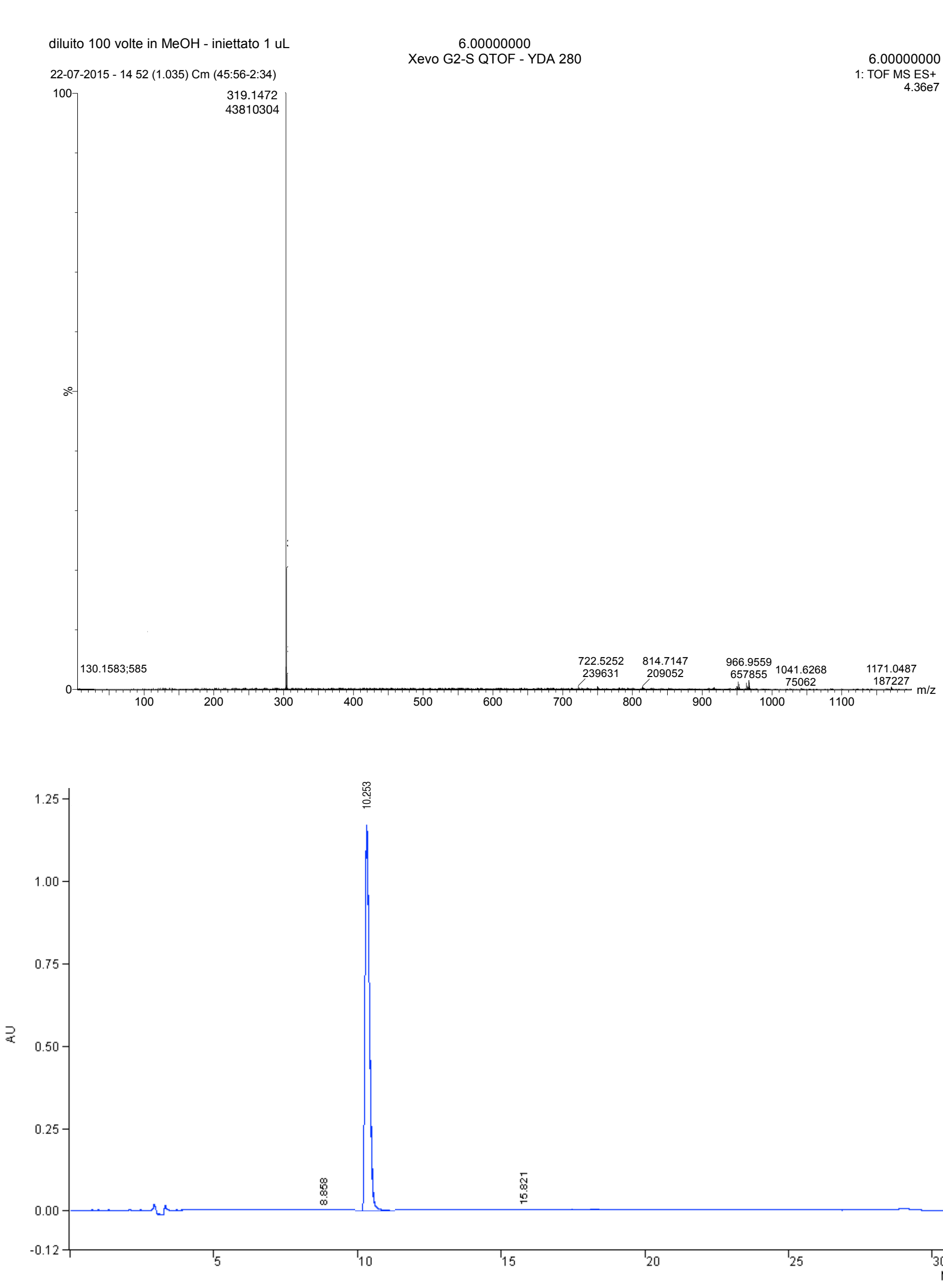


Figure S24. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **14**.

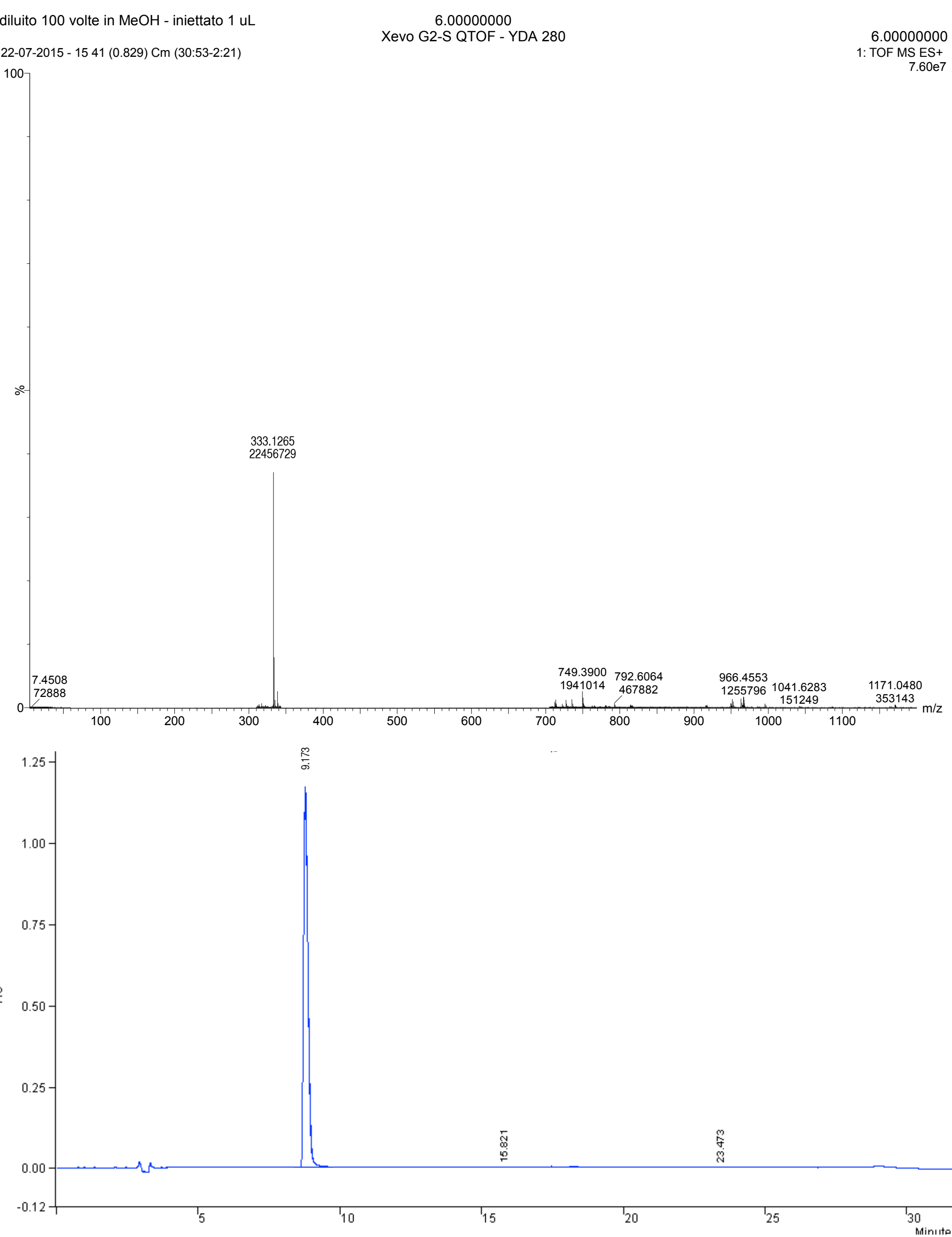


Figure S25. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **15**.

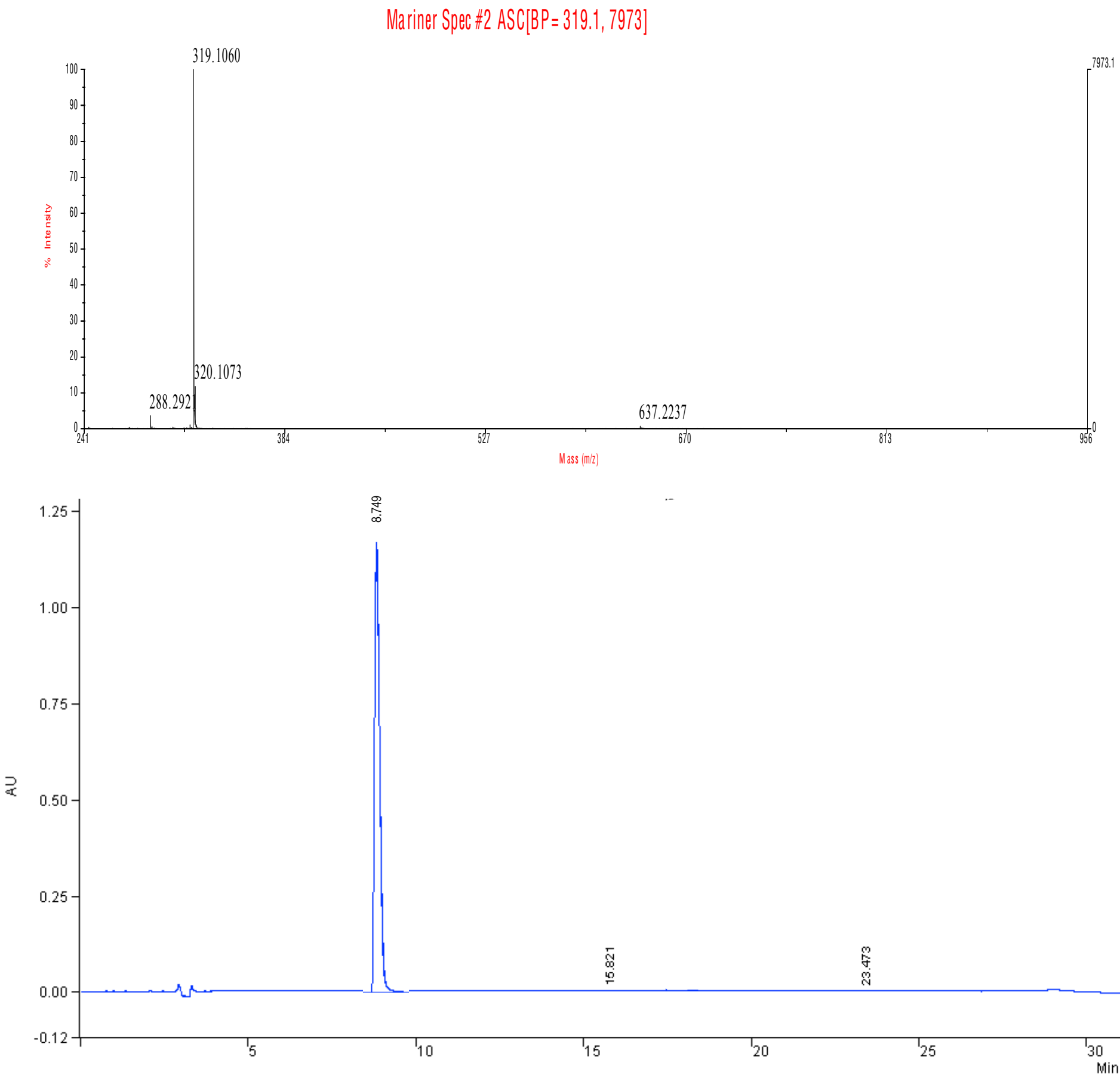


Figure S26. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **16**.

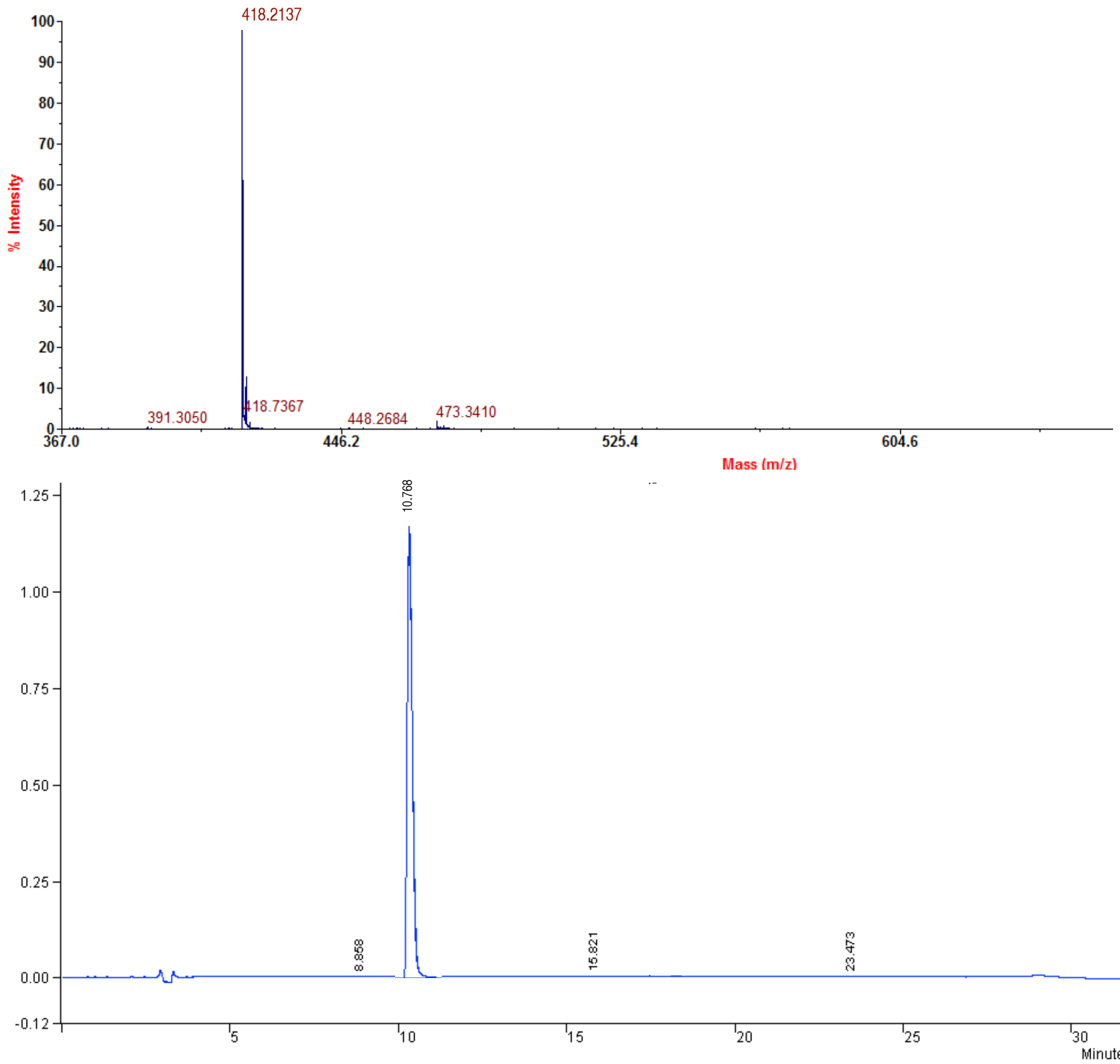
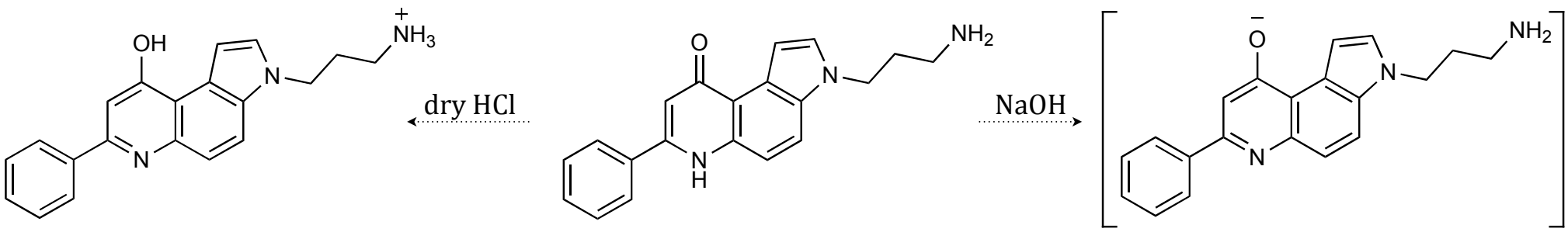


Figure S27. HRMS (ESI, 140 eV) spectrum and HPLC trace of compound **17**.

Compound **18** quinolinonic and hydroxyquinolinic isoforms interconvert in a pH dependant way



compound **18**

pH<2

1D and 2D NMR spectrometry and UV-Vis spectroscopy gave indications that compound **18** was in the form of 9-hydroxy-pyrroloquinoline, because of the treatment with HCl gas used to obtain the PPyQ hydrochloride **18**. Below pH 2 the hydroxyquinolinic form predominates.

Quinolinonic isoform

5<pH<9

In the range of pH from 5 to 9, UV spectra appeared unvaried: the quinolinonic isoform is the only tautomer present.

Quinolinic phenate

pH>10

UV spectra suggest the stabilisation of an additional tautomer at a pH value higher than 10, probably corresponding to a quinolinic phenate salt.

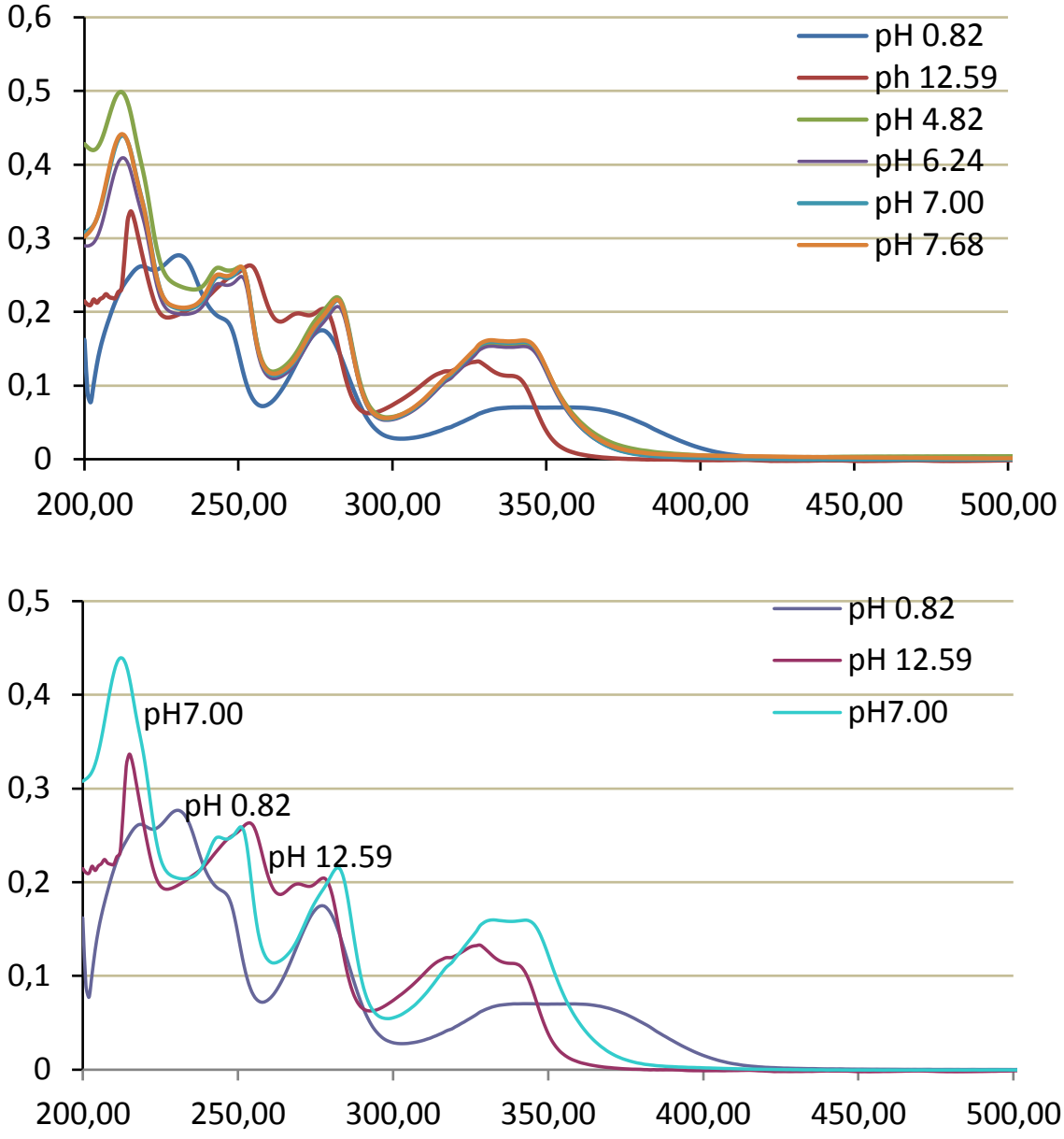


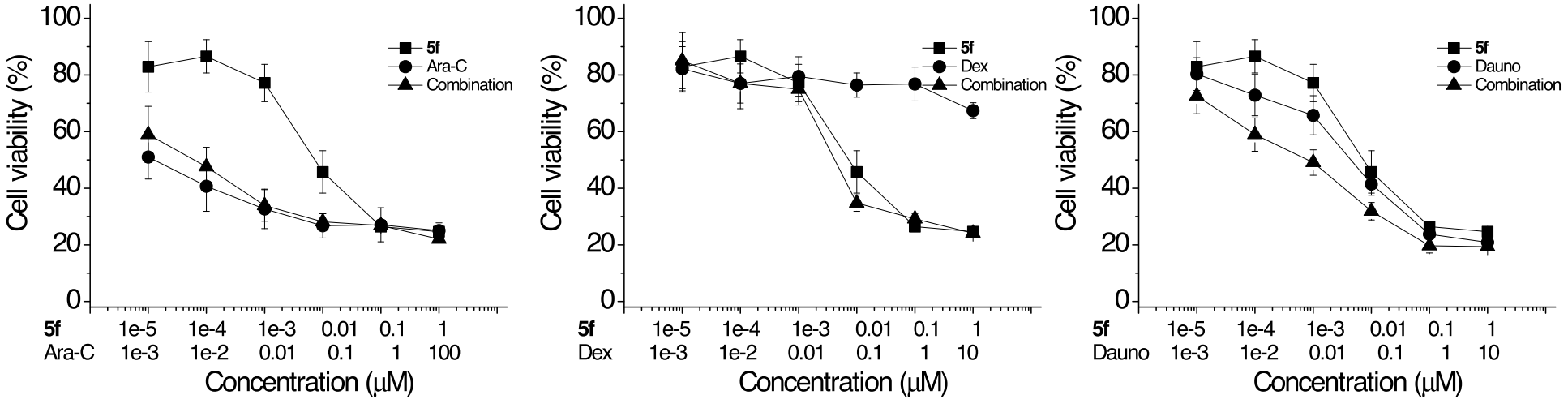
Figure S28. Behaviour of compound **18** in aqueous solution at various pH values.

Table S1. Elemental Analysis of all final compounds **5a-f**, **8f** and **9-18**.

Elemental Analysis of final tested compounds **5a-f**, **8f** and **9-18**.

	CALCULATED				EXPERIMENTAL				Error %
	C	H	N	O	C	H	N	O	
5a	74.98	5.30	9.20	10.51	74.76	5.28	9.17	10.48	0.3
5b	73.78	5.92	7.48	12.82	73.48	5.90	7.45	12.77	0.4
5c	76.66	4.82	13.41	5.11	76.57	4.81	13.39	5.10	0.12
5d	72.82	5.24	8.09	13.86	72.51	5.22	8.06	13.80	0.42
5e	75.93	5.10	8.86	10.11	75.63	5.08	8.83	10.07	0.39
5f	76.81	4.91	8.53	9.74	76.57	4.89	8.50	9.71	0.31
8f	76.34	5.49	8.48	9.69	76.03	5.47	8.45	9.65	0.40
9	79.44	6.00	9.26	5.29	79.34	5.99	9.25	5.28	0.12
10	80.20	7.02	8.13	4.64	79.70	6.98	8.08	4.61	0.62
11	82.51	5.86	7.40	4.23	82.41	5.85	7.39	4.22	0.12
12	76.76	5.25	6.63	11.36	76.51	5.23	6.61	11.32	0.32
13	79.44	6.00	9.26	5.29	79.12	5.98	9.22	5.27	0.40
14	75.45	5.70	8.80	10.05	75.31	5.69	8.78	10.03	0.19
15	72.28	4.85	8.43	14.44	72.00	4.83	8.40	14.38	0.39
16	71.69	4.43	8.80	15.08	71.38	4.41	8.76	15.02	0.43
17	71.92	6.52	10.06	11.50	71.63	6.49	10.02	11.45	0.41
18	67.89	5.70	11.88	4.52	67.73	5.69	11.85	4.51	0.23

A



B

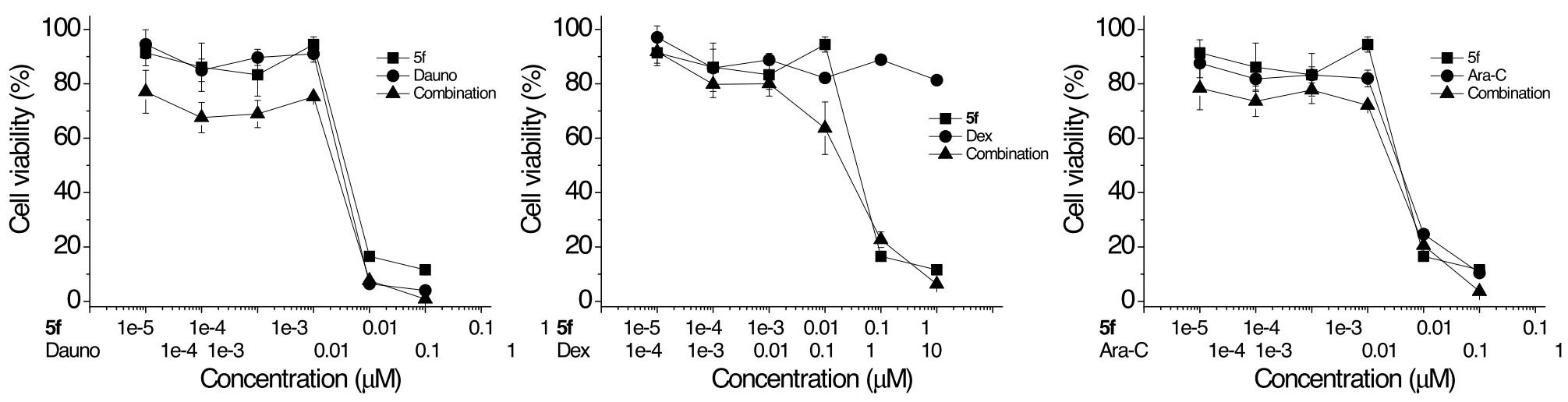


Figure S29. Combination cytotoxicity of **5f** and Daunorubucine (Dauno), Dexamethasone (Dex), Cytarabine (Ara-C). Dose-response curves of Jurkat (A) and THP1 (B) to **5f**, Dauno, Dex, Ara-C, and the combinations. Cell viability was determined after 48 hours of treatment by MTT test. Data are presented as the mean ± SE of at least three independent experiments. The CI values were calculated by Chou and Talalay method and are shown in Table 5.

Table S2. Table of the collected PDB structures and the corresponding references.

Structure	***PDB code***	Reference
Tubulin in complex with colchicine.	1SA0	1
N-terminal kinase domain of RSK2 in complex with afzelin.	4EL9	2
PLK4 Kinase in complex with the inhibitor 400631.	4JXF	*n.a.*
FMS-like tyrosine kinase 3 (FLT3)	1RJB	3
JAK1 kinase (JH1 domain) in complex with compound 49.	4E4N	4
GSK3 kinase in complex with a 5-aryl-4-carboxamide-1,3-oxazole inhibitor.	4AFJ	5

n.a. not available

References:

1. Ravelli, R.B.; Gigant, B.; Curmi, P.A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M." Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain." *Nature.* **2004**, *428*, 198-202.
2. Utepbergenov, D.; Derewenda, U.; Olekhnovich, N.; Szukalska, G.; Banerjee, B.; Hilinski, M. K.; Lannigan, D. A.; Stukenberg, P. T.; Derewenda, Z. S. Insights into the inhibition of the p90 ribosomal S6 kinase (RSK) by the flavonol glycoside SL0101 from the 1.5 Å crystal structure of the N-terminal domain of RSK2 with bound inhibitor. *Biochemistry* **2012***, 51*, 6499-6510.
3. Griffith J, Black J, Faerman C, Swenson L, Wynn M, Lu F, Lippke J, Saxena K. "The structural basis for autoinhibition of FLT3 by the juxtamembrane domain." *Mol Cell.* **2004**, *13*,169-178.
4. Kulagowski JJ, Blair W, Bull RJ, Chang C, Deshmukh G, Dyke HJ, Eigenbrot C, Ghilardi N, Gibbons P, Harrison TK, Hewitt PR, Liimatta M, Hurley CA, Johnson A, Johnson T, Kenny JR, Bir Kohli P, Maxey RJ, Mendonca R, Mortara K, Murray J, Narukulla R, Shia S, Steffek M, Ubhayakar S, Ultsch M, van Abbema A, Ward SI, Waszkowycz B, Zak M. " Identification of imidazo-pyrrolopyridines as novel and potent JAK1 inhibitors." *J Med Chem.* **2012**, *55*, 5901-5921.
5. Gentile, G.; Merlo, G.; Pozzan, A.; Bernasconi, G.; Bax, B.; Bamborough, P.; Bridges, A.; Carter, P.; Neu, M.; Yao, G.; Brough, C.; Cutler, G.; Coffin, A.; Belyanskaya, S. 5-Aryl-4-carboxamide-1,3-oxazoles: Potent and selective GSK-3 inhibitors. *Bioorg. Med. Chem. Lett.* **2012***, 22*, 1989-1994.

Table S3. Simplified molecular-input line-entry for all final compounds **5a-f**, **8f** and **9-18**.

5a	O=c3cc(c1ccccc1)[nH]c4ccc2c(ccn2CCO)c34	**11**	CCn1ccc5c1ccc4nc(c2ccccc2)cc(OCc3ccccc3)c45
5b	COc5ccc(c4cc(=O)c1c(ccc2c1ccn2CC3CC3)[nH]4)cc5OC	**12**	CCn1ccc5c1ccc4nc(c2ccccc2)cc(OC(=O)OCc3ccccc3)c45
5c	N#CCCn1ccc4c1ccc3[nH]c(c2ccccc2)cc(=O)c34	**13**	CCn1ccc2c1ccc4c2c(=O)cc(c3ccccc3)n4C
5d	CCOC(=O)Cn1ccc4c1ccc3[nH]c(c2ccccc2)cc(=O)c34	**14**	Cn4c(c1ccccc1)cc(=O)c3c2ccn(CCCO)c2ccc34
5e	CCC(=O)n1ccc4c1ccc3[nH]c(c2ccccc2)cc(=O)c34	**15**	Cn4c(c1ccccc1)cc(=O)c3c2ccn(CCC(=O)O)c2ccc34
5f	O=C(C1CC1)n2ccc5c2ccc4[nH]c(c3ccccc3)cc(=O)c45	**16**	O=C(O)Cn1ccc4c1ccc3[nH]c(c2ccccc2)cc(=O)c34
8f	O=C(C1CC1)N2CCc4c2ccc5[nH]c(c3ccccc3)cc(=O)c45	**17**	CC(C)(C)OC(=O)NCCCn1ccc4c1ccc3[nH]c(c2ccccc2)cc(=O)c34
9	CCn1ccc4c1ccc3nc(c2ccccc2)cc(OC)c34	**18**	[H]N([H])([H])(Cl)CCCn1ccc4c1ccc3[nH]c(c2ccccc2)cc(=O)c34
10	CCCCOc3cc(c1ccccc1)nc4ccc2c(ccn2CC)c34		