SUPPLEMENTARY MATERIAL

Metabolomics approach to chemical diversity of the Mediterranean marine sponge *Agelas oroides*

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Abstract: The Mediterranean marine sponge *Agelas oroides* (Schmidt, 1864) is known to contain a large quantity of oroidin, a deterrent, antifouling and antibiofilm pyrrole-2-aminoimidazole. In contrast with other tropical specimens, the chemical composition of Mediterranean *Agelas oroides* is surprisingly relatively poor in other related metabolites. In the course of finding novel marine natural products, LC-MS based metabolomics study of the Mediterranean *Agelas oroides*, however, revealed that next to the major compound oroidin, the sponge contains in fact a great diversity of known pyrrole-imidazole alkaloids in minute amounts. Here we describe identification of 13 known oroidin class alkaloids along with one new monobromoagelaspongin (**24**). Five betaines and one amine were also identified from the aqueous fraction. One of those compounds (-)-equinobetaine B (**30**) was found to be an enantiomer of the known natural product (+)-equinobetaine B.

Keywords: *Agelas oroides*; Agelasidae, pyrrole-2-aminoimidazole; monobromoagelaspongin, (-)-equinobetaine B.

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Supplemental file legend

Table S1. LCMS analysis of the butanolic extract from a Mediterranean *A. oroides* specimen. Sole brominated compounds were selected during this analysis.

Table S2. ^{1}H and ^{13}C NMR Data (500 and 125 MHz, resp.; CD_3OD) for monobromoagelaspongin **(24)**.

Table S3. ^{1}H-NMR of (–)-equinobetaine in different conditions and its comparison with reported data (Capon et al. 2005).

Figure S1. Structure of compounds **11-17** previously isolated from the Australian marine sponge *Agelas oroides*.

Figure S2. HPLC chromatogram ($\lambda = 270$ nm) of the 'oroidin free' fraction revealing the high Pyrrole-2-Aminoimidazole chemodiversity of a Mediterranean *Agelas oroides* specimen.

Figure S3. ^{1}H-NMR Spectrum (500 MHz, CD_3OD) of oroidin

Figure S4. ^{1}H-NMR Spectrum (500 MHz, CD_3OD) of 4,5-dibromopyrrole-2-carboxylic acid

Figure S5. ^{1}H-NMR Spectrum (500 MHz, CD_3OD) of methyl ester 4,5-dibromopyrrole-2-carbonitrile

Figure S6. ^{1}H-NMR Spectrum (500 MHz, CD_3OD) of a mixture of 4,5-dibromopyrrole-2-carboxamide and longamide B

Figure S7. ^{1}H-NMR Spectrum (500 MHz, CD_3OD) of hymenidin

Figure S8. ^{1}H-NMR Spectrum (500 MHz, CD_3OD) of dispacamide A

Figure S9. ^{1}H-NMR Spectrum (500 MHz, CD_3OD) of dispacamide C

Figure S10. ^{1}H-NMR Spectrum (500 MHz, CD_3OD) of longamide

Figure S11. ^{1}H-NMR Spectrum (500 MHz, CD_3OD) of a mixture of longamide B and 4,5-dibromopyrrole-2-carboxamide.

Table S1.

m/z [M+H]+ (Exact Mass)	m/z [M-H]$^-$ (Exact Mass)	Natural product	Rt (min)
326, 328 (326.0251)		Monobromoagelaspongin (**24**)	3.4
310, 312 (310.0255)		Hymenidin (**18**)	7.3
473, 475, 477 (472.9505)	471, 473, 475 (470.9673)	n.d	8.0
404, 406, 408 (403.9220)	402, 404, 406 (401.9323)	Dibromoagelaspongin *	9.6
450, 452, 454 (449.9262)	448, 450, 452 (447.9405)	n.d.	10.8
420, 422, 424 (419.9173)	418, 420, 422 (417.9299)	Dispacamide C (**19**)	11.2
404, 406, 408 (403.9233)	402, 404, 406 (401.9343)	Dispacamide A (**7**)	12.4
388, 390, 392 (387.9266)		Oroidin (**1**)	12.7
	434, 436, 438 (433.9629)	n.d.	13.9
	509, 511, 513 (508.9427)	Taurodispacamide (**6**) *	14.5
789, 791, 793, 795, 797 (788.8260)	787, 789, 791, 793, 795 (786.8687)	Mauritiamine (**22**)	15.6
	337, 339, 341 (336.8955)	n.d.	16.3
309, 311, 313 (308.8794)	307, 309, 311 (306.8851)	Longamide (**20**)	17.4
	265, 267, 269 (264.8726)	4,5-Dibromopyrrole-2-carboxamide (**4**)	18.3
	353, 355, 357 (352.8933)	n.d.	18.7
351, 353, 355 (350.8862)	349, 351, 353 (348.8978)	Longamide B (**21**)	18.9
267, 269, 271 (266.8678)	266, 268, 270 (265.8577)	4,5-Dibromopyrrole-2-caboxylic acid (**2**)	19.7
	311, 313, 315 (310.8798)	n.d.	19.8
	381, 383, 385 (380.9262)	n.d.	20.4
	321, 323, 325 (320.9015)	n.d.	20.9
	309, 311, 313 (308.9020)	n.d.	21.2
	293, 295, 297 (292.8696)	n.d.	21.5
365, 367, 369		Hanishin methylester (**23**)	22.0

(364.9025)			
	351, 353, 355 (350.9143)	n.d.	23.0
	395, 397, 399 (394.9433)	n.d.	23.5
	365, 367, 369 (364.9314)	n.d.	23.7
	247, 249, 251 (246.8594)	4,5-Dibromopyrrole-2-carbonitrile (**3**)	24.9

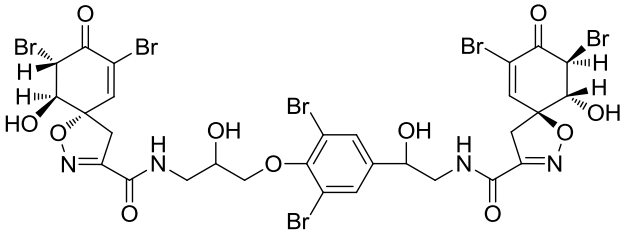
Rt: Retention time obtained on a Phenomenex Gemini C6-Phenyl; n.d.: not determined; * identification based on LCMS solely.

Table S2.

Position	**δ(C), type**	**δ(H) (*J* in Hz)**
2	159.9	–
3a	90.2	–
4a	32.7	2.07 (m)
4b	–	1.77 (*m*)
5a	18.9	2.05 (*m*)
5b	–	1.77 (*m*)
6a	35.9	4.11 (*ddd*, *J* = 13.8, 8.6, 7.2)
6b	–	3.15 (*ddd*, *J* = 13.8, 7.0, 4.5)
8	160.2	–
8a	127.1	–
9	109.7	6.68 (*d*, *J* = 1.1)
10	103.0	–
11	121.7	7.31 (*d*, *J* = 1.1)
12a	94.2	–

Table S3.

	equinobetaine B (**30**)		
Position	δ_H (+)**30.TFA**, D_2O (Capon et al. 2005)	δ_H (-)**30.TFA**, D_2O This work	δ_H (-)**30.HCOOH**, D_2O This work
1	-	-	-
2	4.78	5.23	4.89 (s)
2-OMe	3.22	3.45	3.39 (s)
3	-	-	-
4	7.31	7.60	7.47 (s)
5-N-Me	3.78	3.86	3.82 (s)
6	8.52	8.75	8.69 (s)
7-N-Me	3.63	3.89	3.87 (s)



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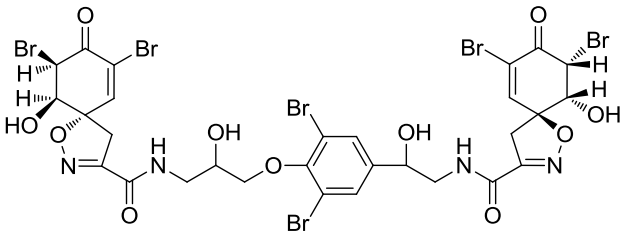

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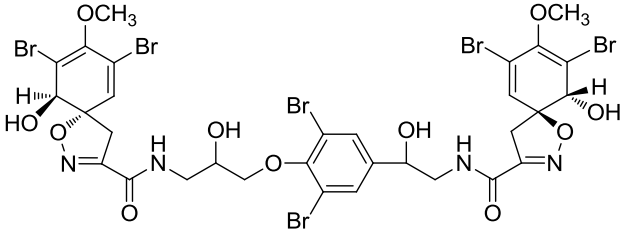

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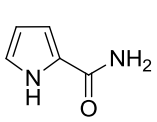

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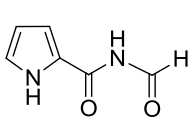

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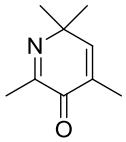

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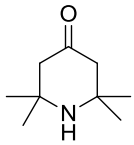

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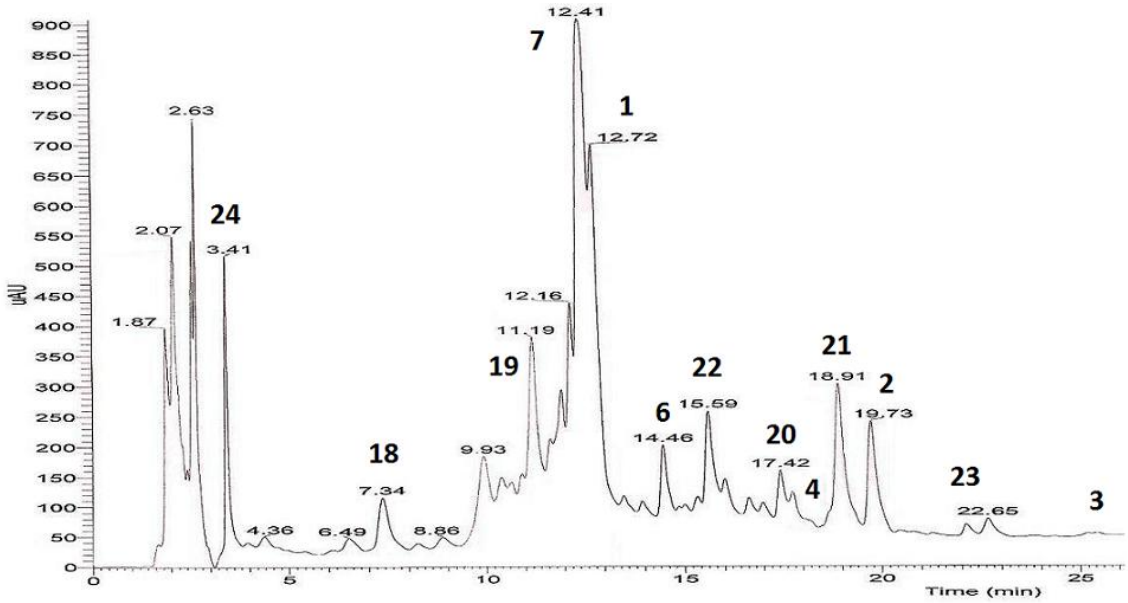
Figure S1



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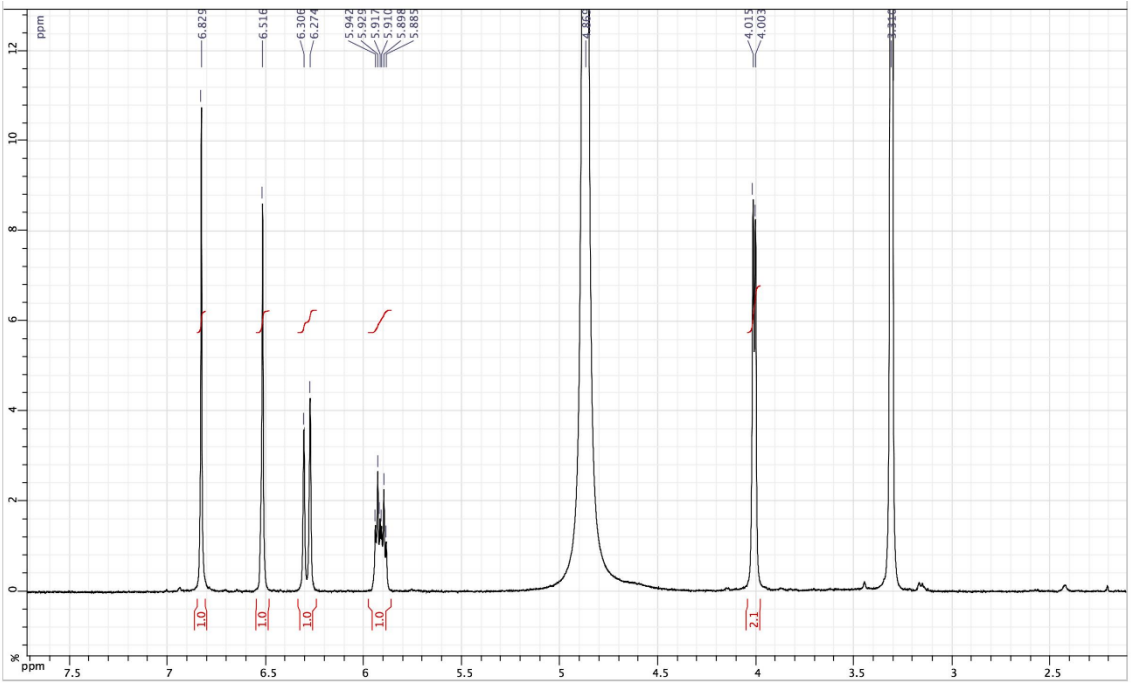

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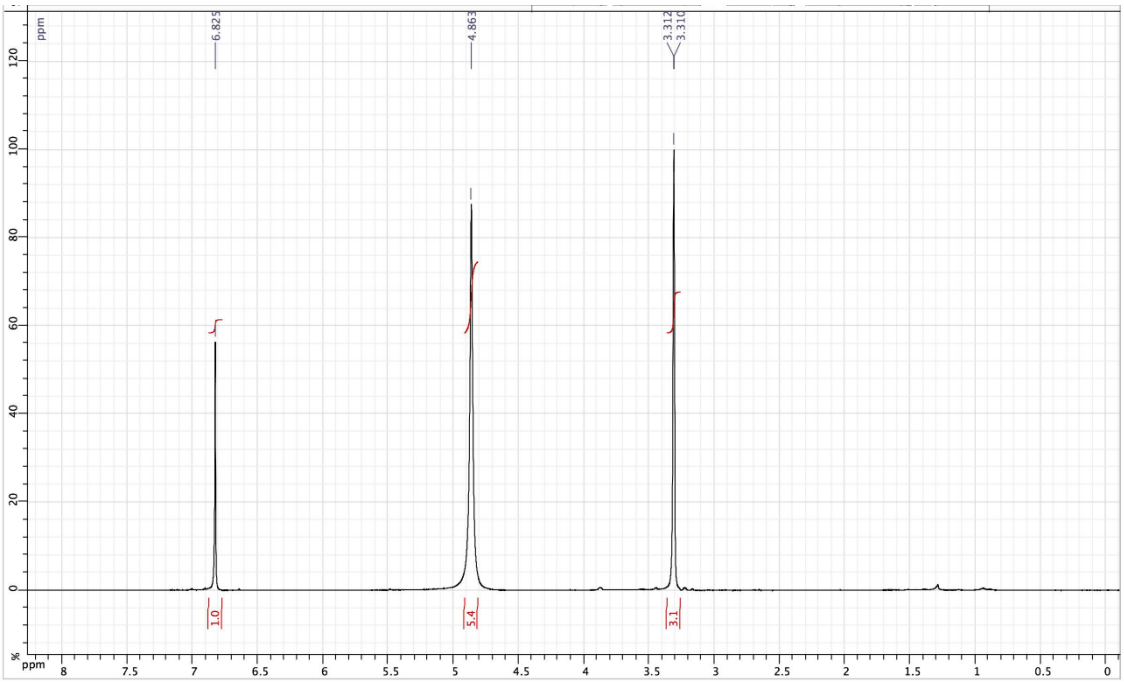

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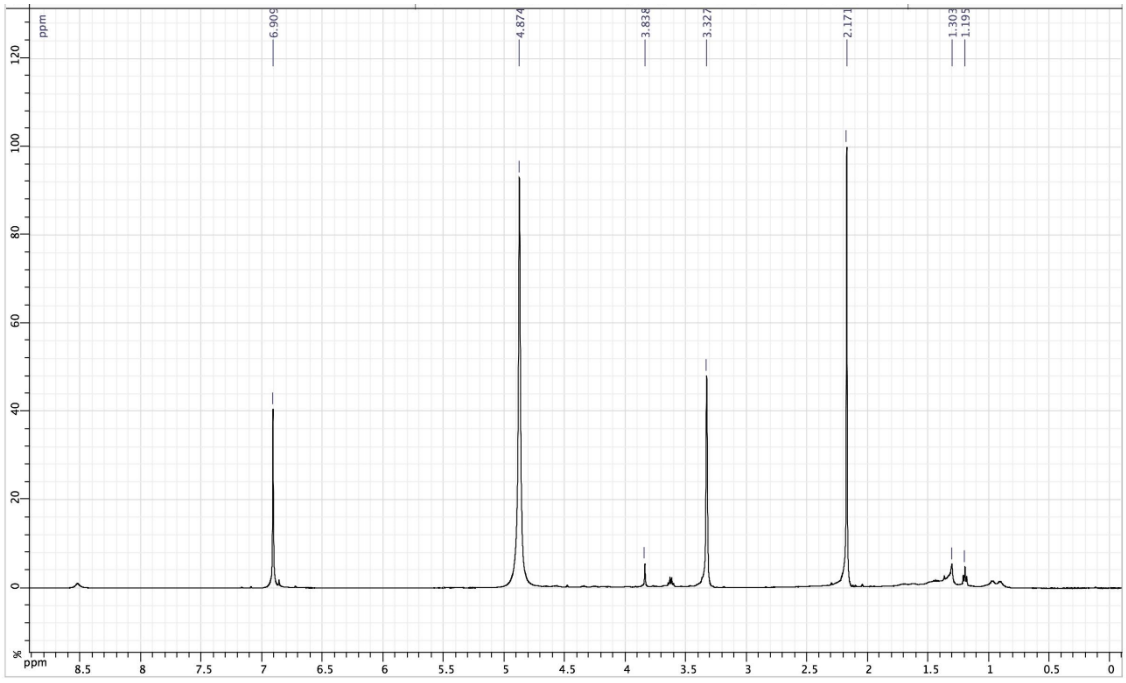

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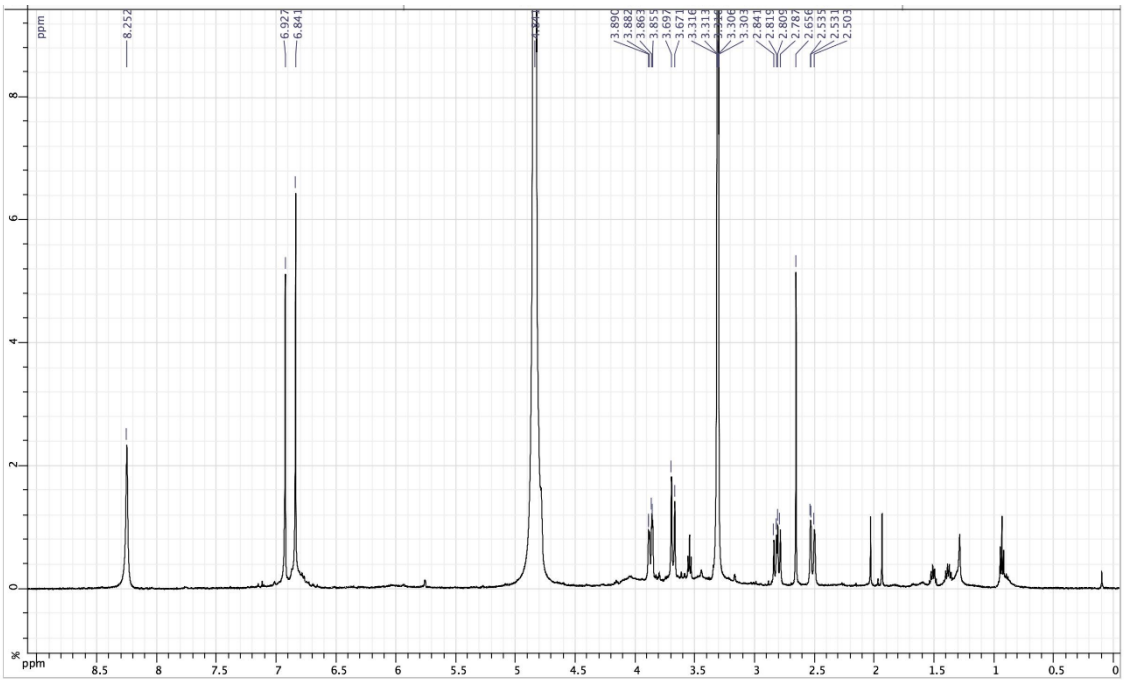

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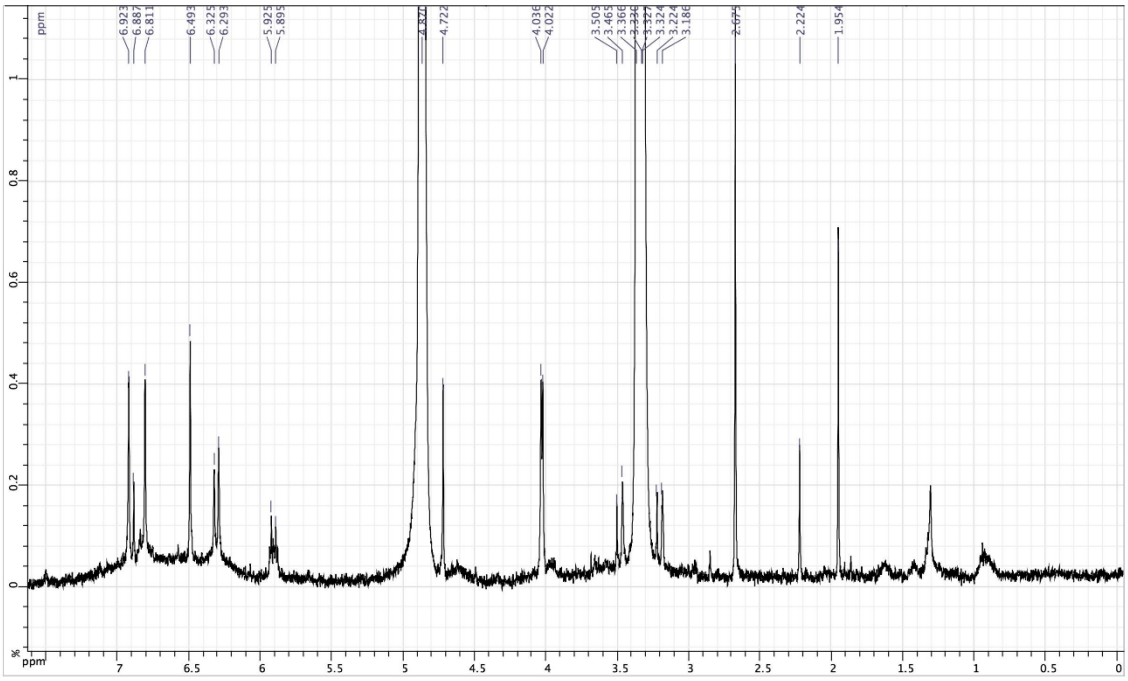

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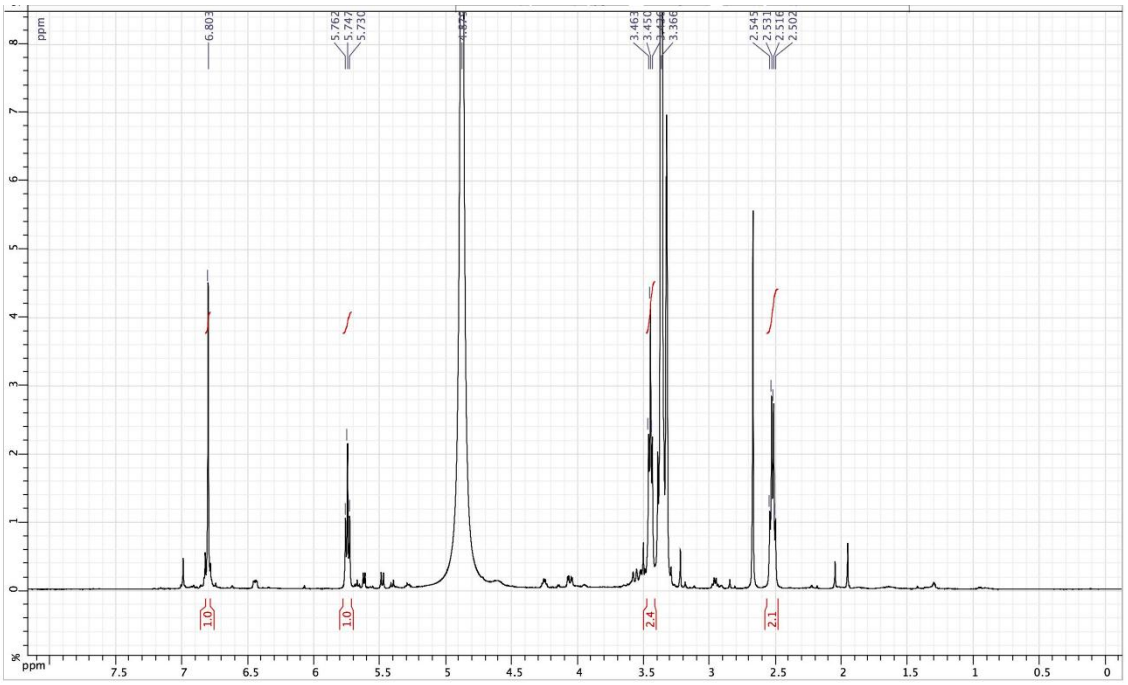

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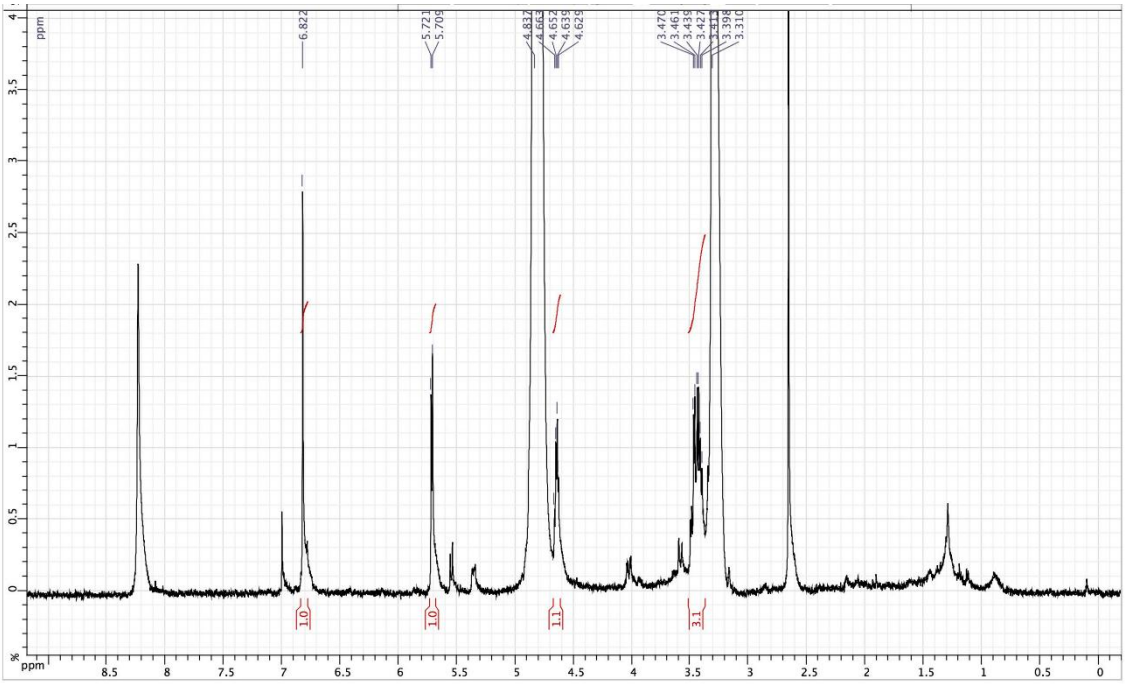

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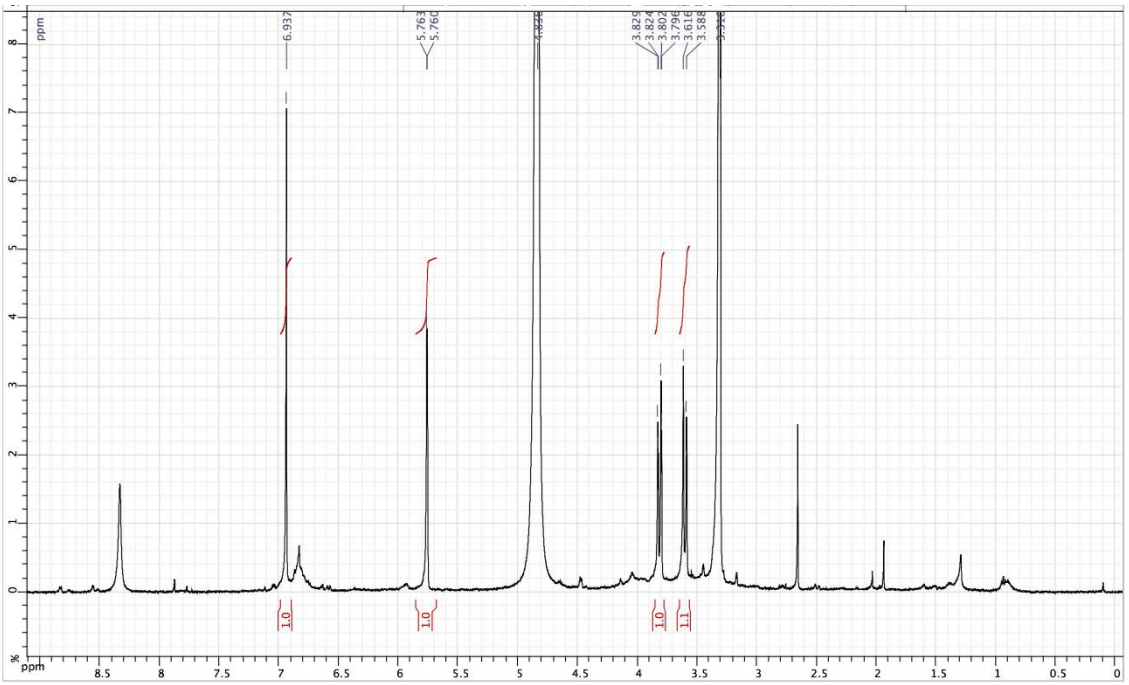

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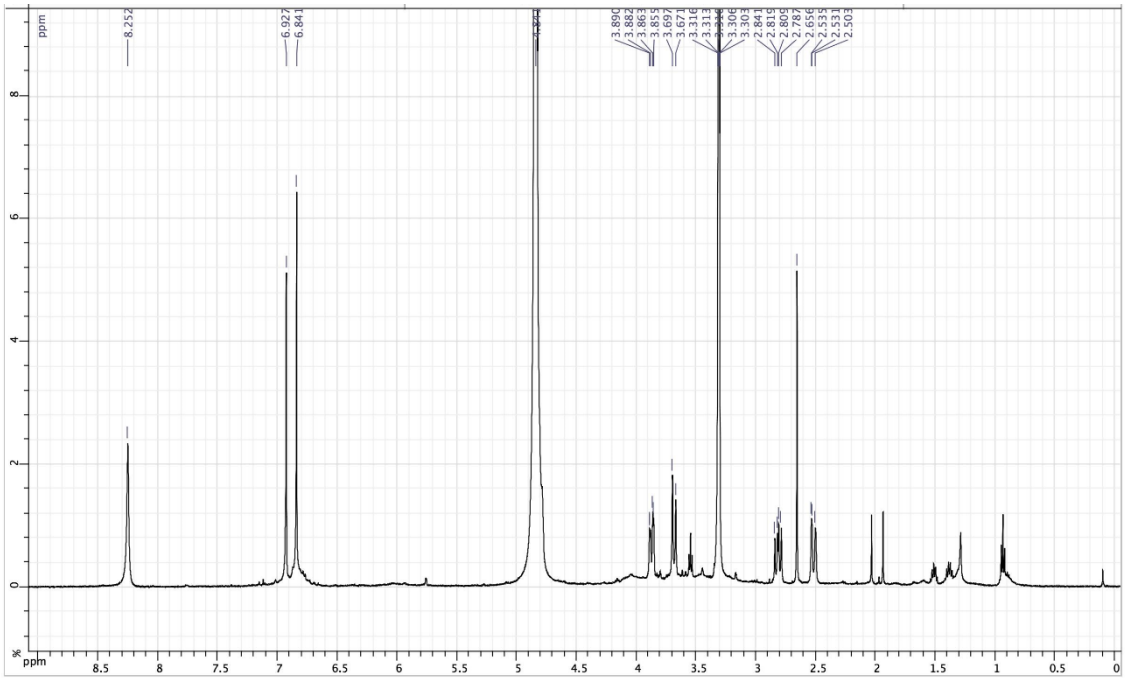

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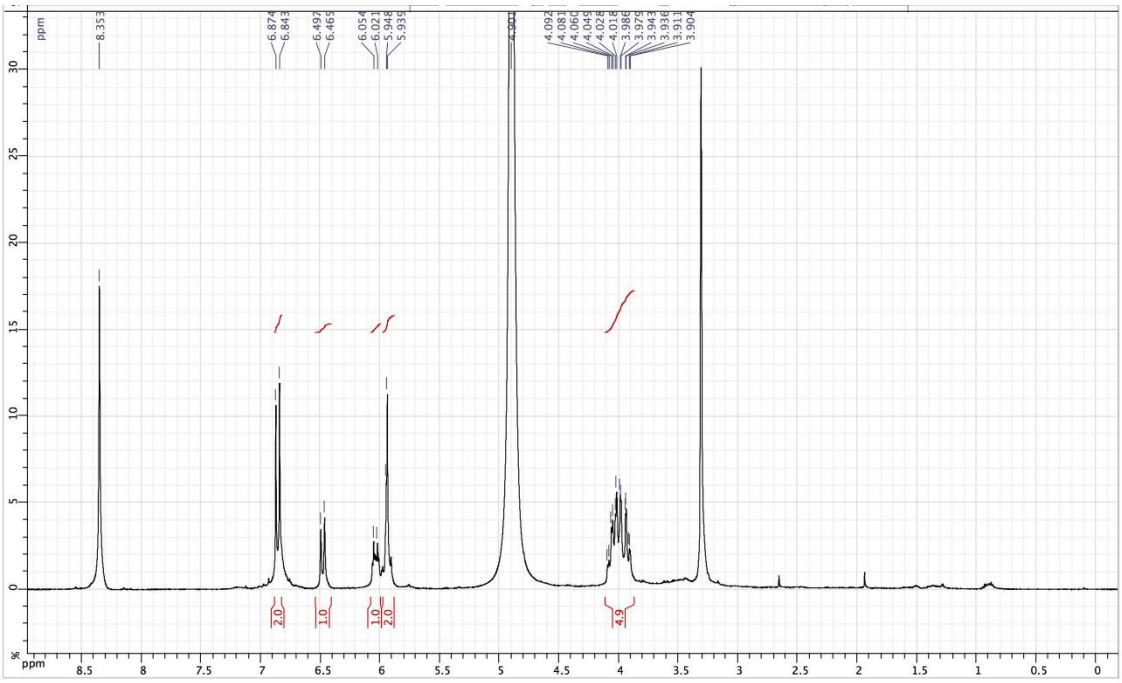

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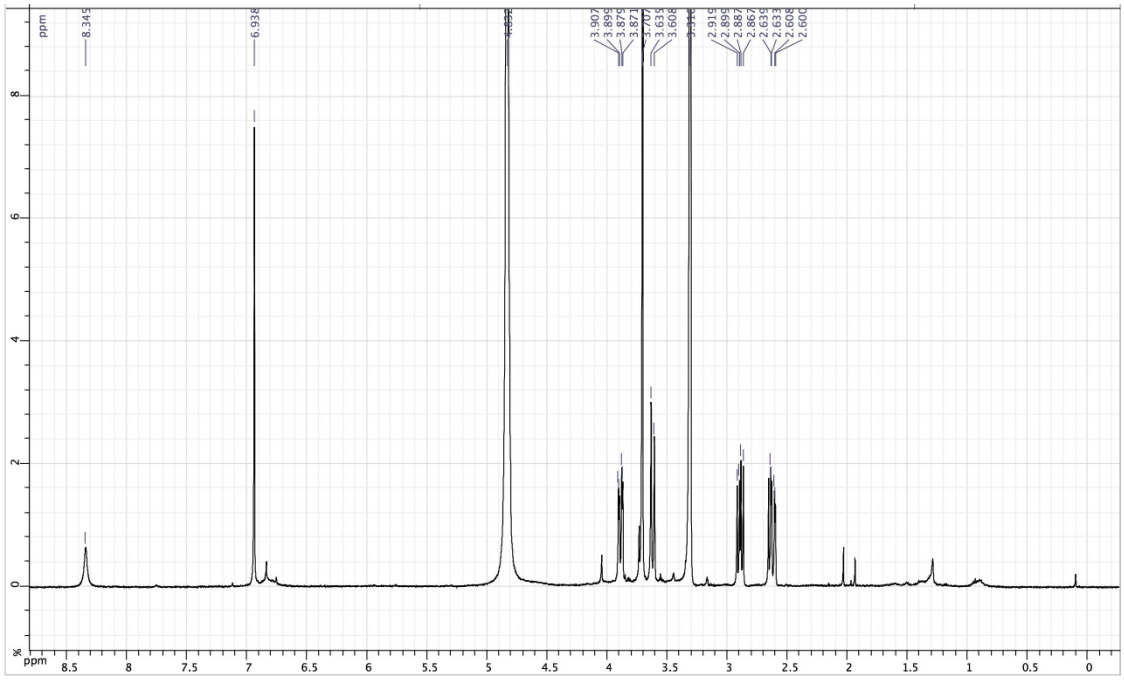

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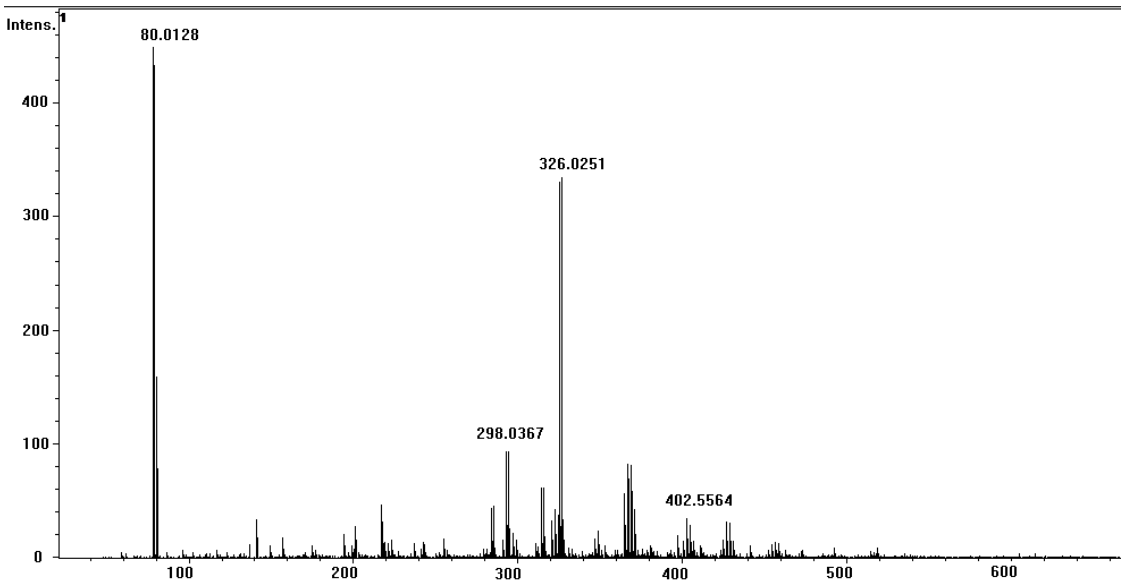
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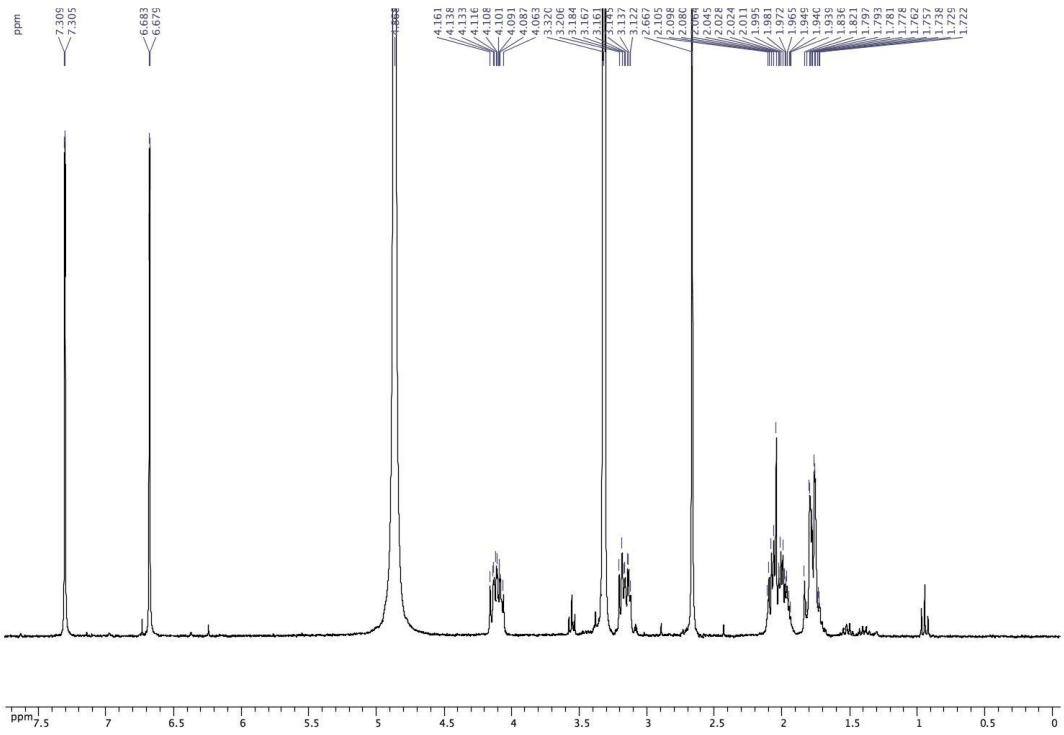
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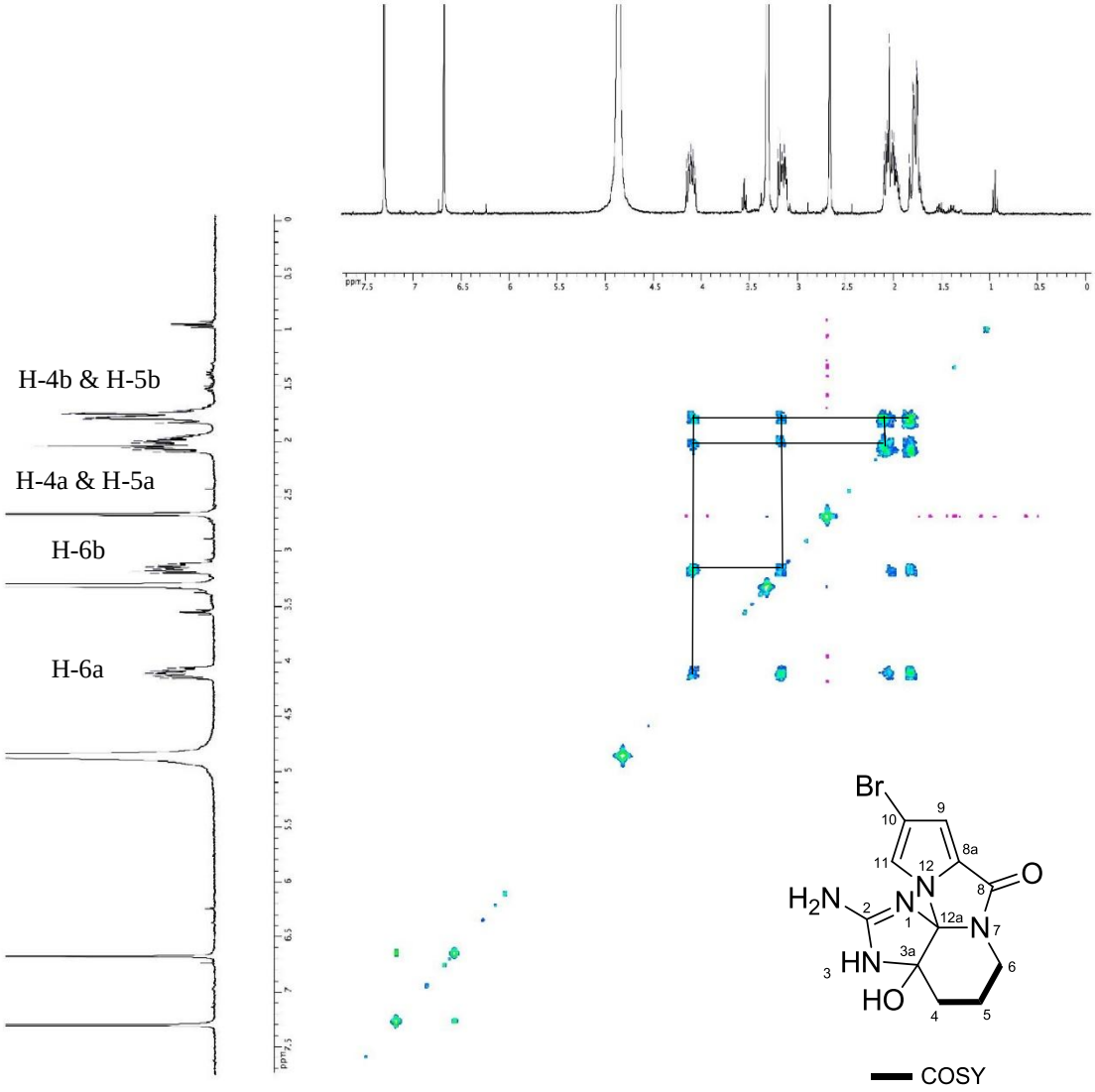

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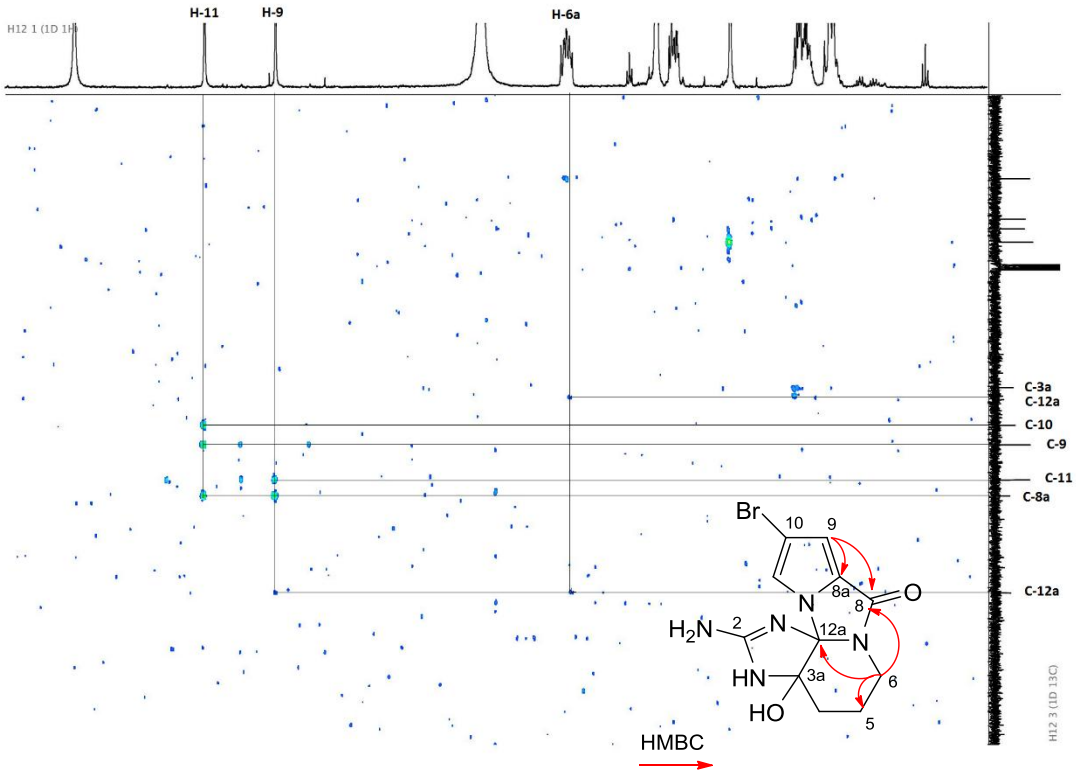

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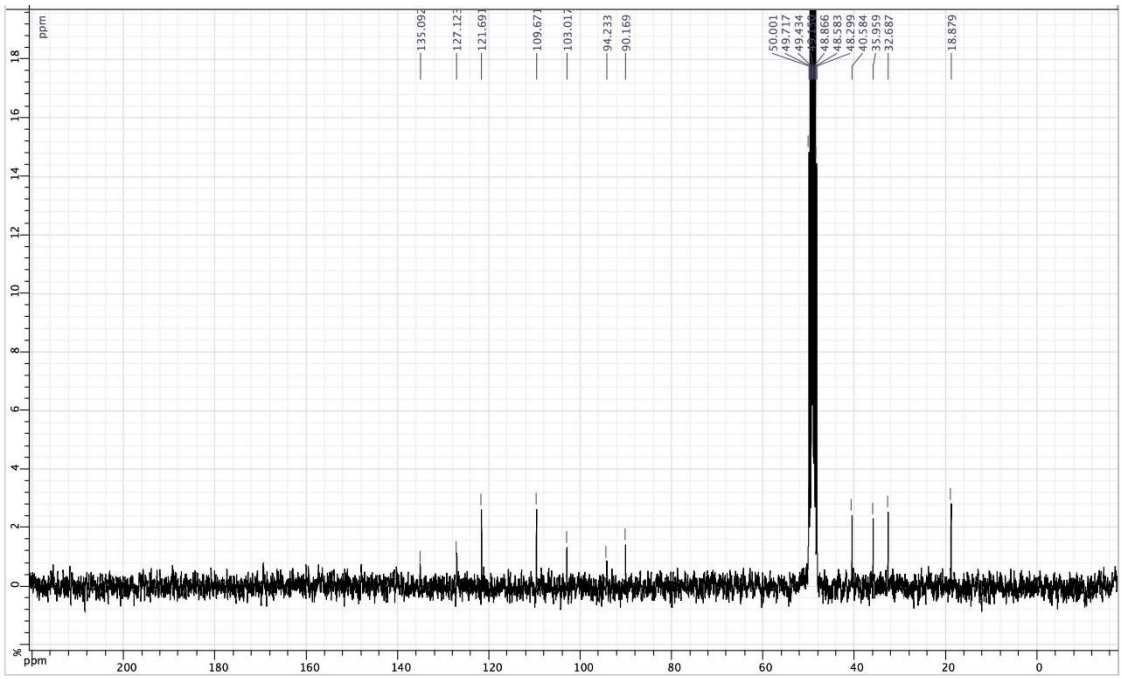

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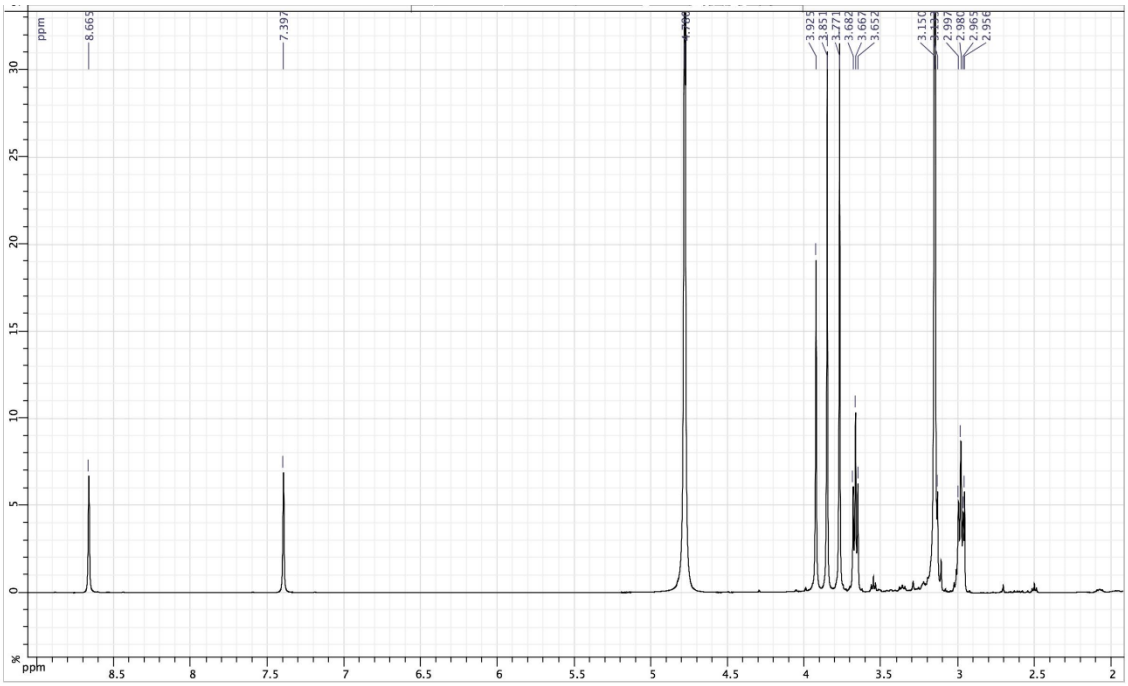

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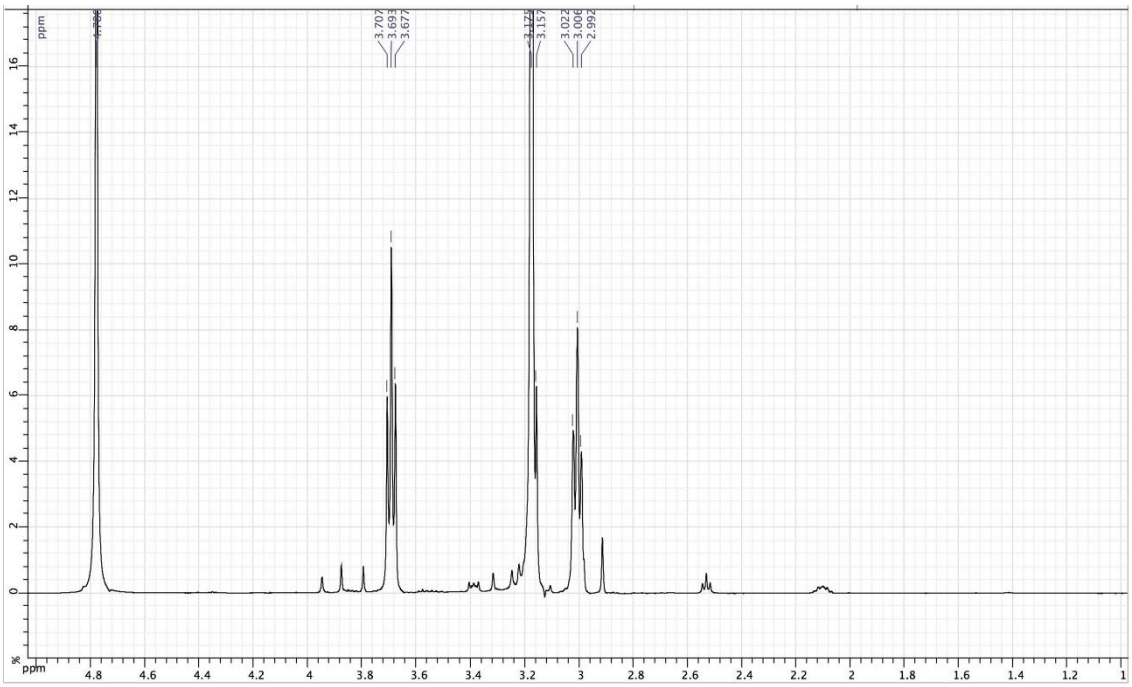

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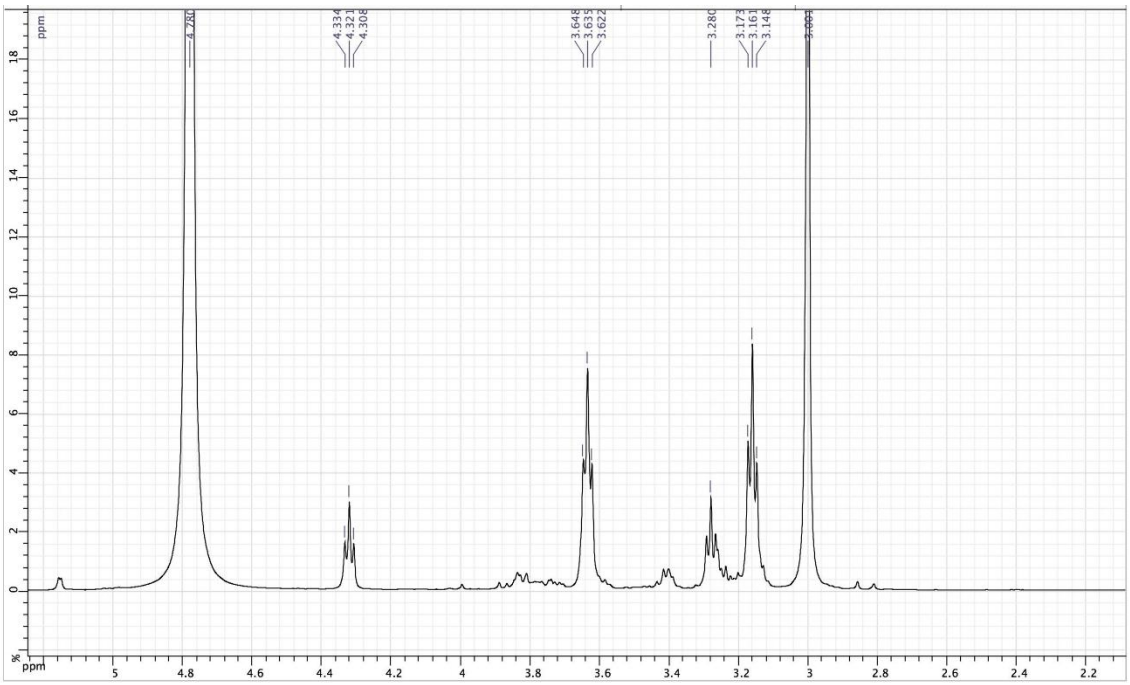

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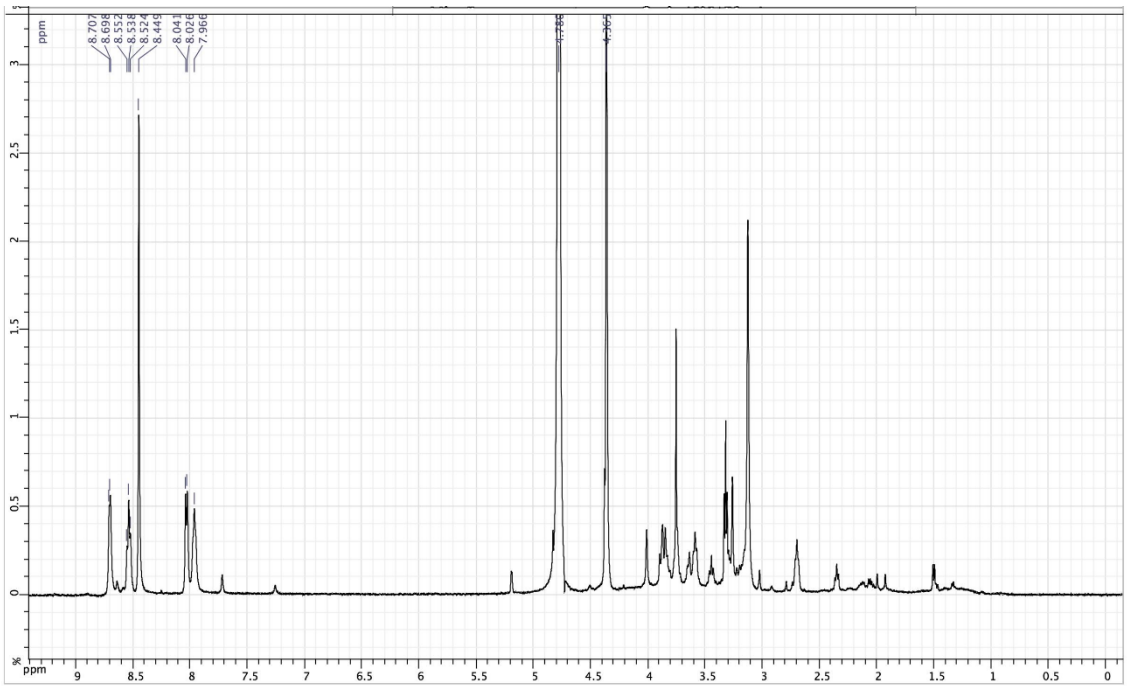

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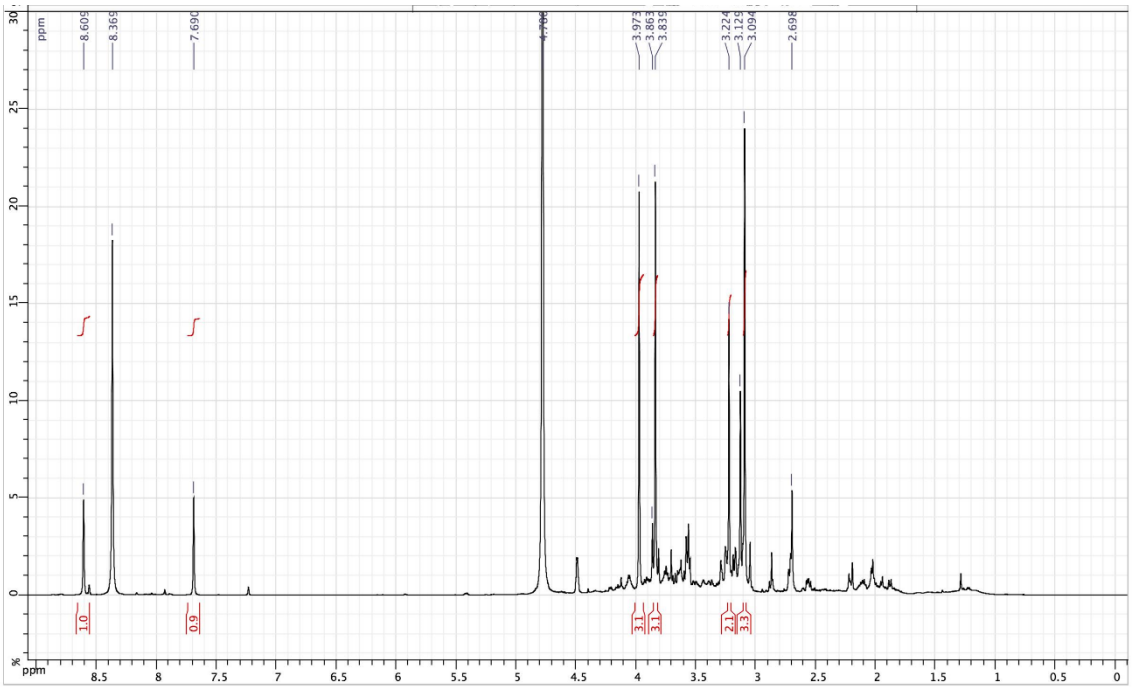

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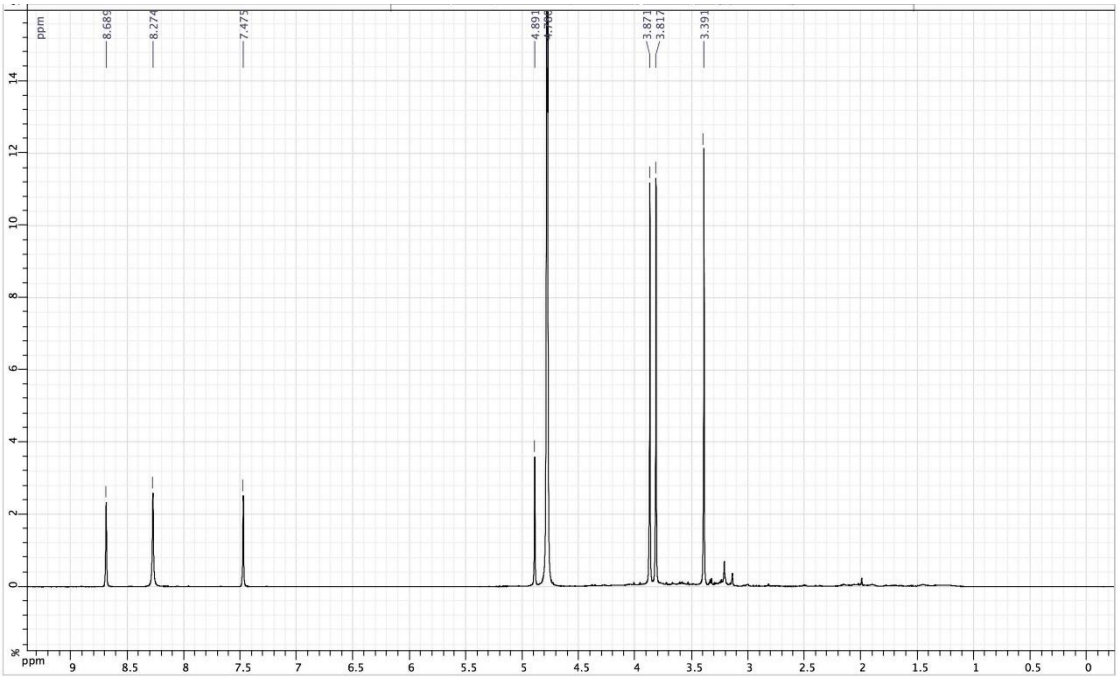

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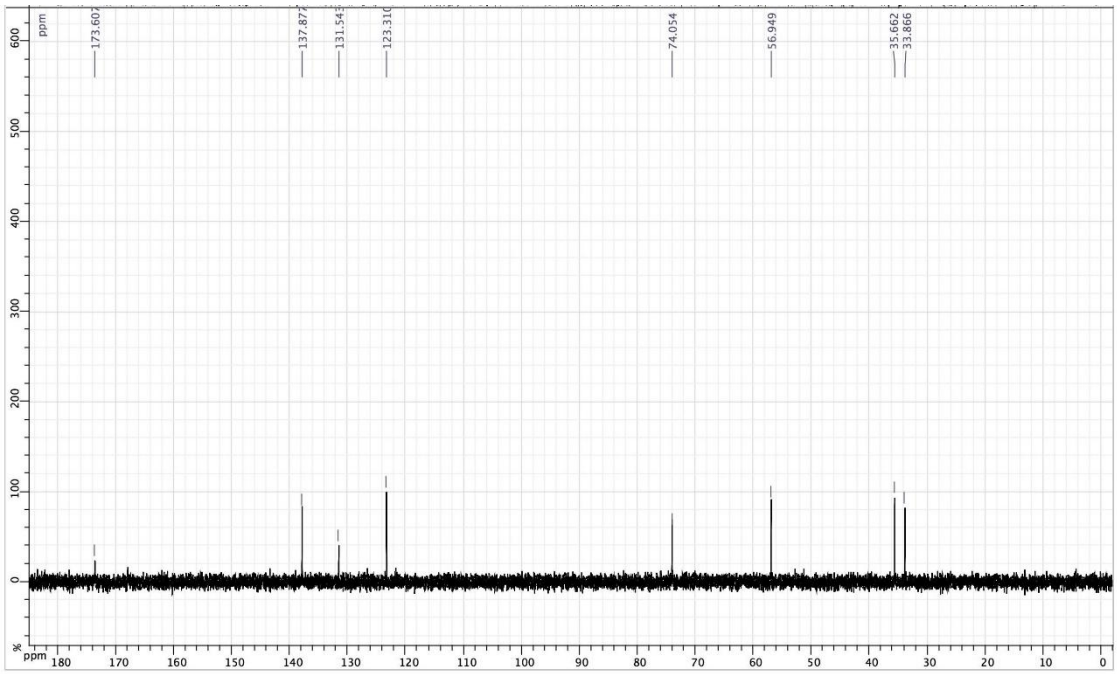
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ppm
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Figure S25