Retargeting Gram-positive-only adarotene-derived antibacterials to broad-spectrum antibiotics

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Table S1. *In vitro* antimicrobial activity of derivatives **7**, **12**, **17** against Gram-negative bacteria Pag. S1

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Table S1. *In vitro* antimicrobial activity of derivatives 7, 12, 17 against Gram-negative bacteria

Strain	MIC (μM)		
	7	**12**	**17**
A. baumannii ATCC 19606	>128	>128	128
E. coli MG1655	>128	>128	64
K. pneumoniae ATCC 27736	>128	>128	64
P. aeruginosa PAO1	>128	>128	128

Table S2. Bacterial strains used in this study

Strains	Relevant characteristics	Reference/source
A. baumannii		
ATCC 19606^T	Type strain.	Janssen *et al*., 1997
ACICU	MDR clinical isolate.	Iacono *et al*., 2008
E. cloacae		
ATCC 13047	Type strain.	Hormaeche and Edwards, 1960
E. coli		
ATCC 25922	Reference strain.	ATCC
MG1655	K-12 ATCC 47076, type strain.	Bachman, 1996
K. pneumoniae		
ATCC 27736	Type strain.	ATCC
P. aeruginosa		
ATCC 27853	Reference strain.	ATCC
PAO1	ATCC 15692 type strain.	ATCC
PAO1 col^{R}1	*In vitro* evolved colistin-resistant PAO1 derivative.	Lo Sciuto and Imperi, 2018
PAO1 col^{R}3	*In vitro* evolved colistin-resistant PAO1 derivative.	Lo Sciuto and Imperi, 2018
PAO1 col^{R}5	*In vitro* evolved colistin-resistant PAO1 derivative.	Lo Sciuto and Imperi, 2018
PAO1 *lptE*	*lptE* conditional mutant of PAO1, carrying a deletion of the *lptE* gene and an arabinose-inducible copy of *lptE* inserted in the *attB* site of the chromosome.	Lo Sciuto *et al*., 2018

PAO1 *lptH*	*lptH* conditional mutant of PAO1, carrying a deletion of the *lptH* gene and an arabinose-inducible copy of *lptH* inserted in the *attB* site of the chromosome.	Fernández-Piñar *et al.*, 2015
PAO1-KP	PAO1 wild type strain gently provided by Prof. K. Poole (Queen's University, Kingston, Canada).	Morita *et al*., 2006
PAO1-KP Δ*efflux*	PAO1-KP mutant strain carrying mutations in the efflux pumps MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY. Gently provided by Prof. K. Poole (Queen's University, Kingston, Canada).	Morita *et al*., 2006
S. aureus		
ATCC 25923	Reference clinical strain.	ATCC

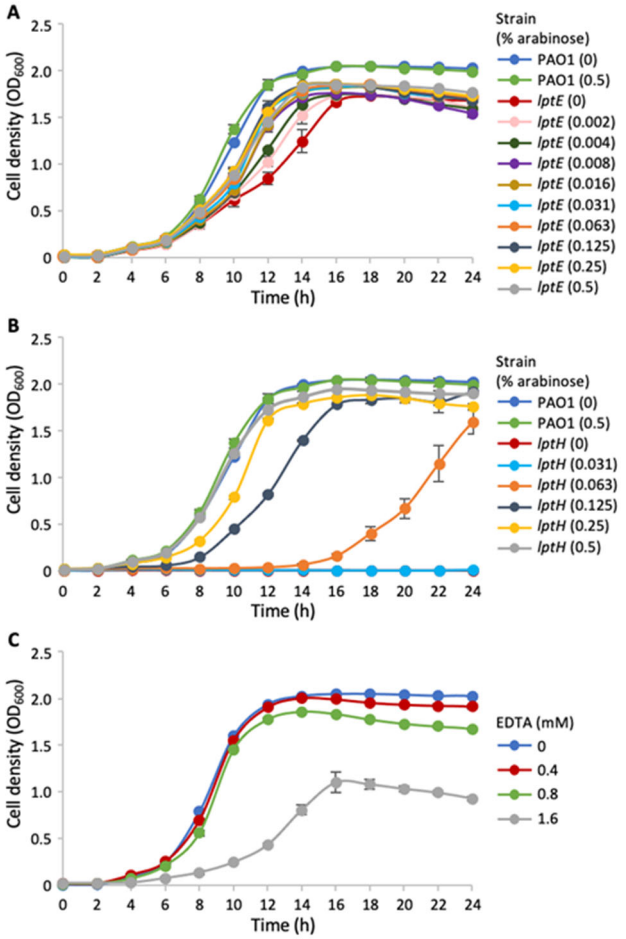


Figure S1. Growth curves of *P. aeruginosa* strains with arabinose or EDTA. **(A-B**) Growth of PAO1 and (**A**) the PAO1 *lptE* conditional mutant or (**B**) the PAO1 *lptH* conditional mutant in MHB-II supplemented with increasing concentrations of arabinose (0-0.5%). (**C**) Growth of PAO1 in MHB-II supplemented with increasing concentrations of EDTA (0-1.6 mM). Data are mean values from three independent biological replicates with standard deviations.

PAO1

SPL207 (µM) \ Colistin (µg/mL)	0	0.125	0.25	0.5	1	2	4	8	16	32	64
0											
2											
4											
8											
16											
32											
64											

PAO1 col^{R}1

SPL207 (µM) \ Colistin (µg/mL)	0	0.125	0.25	0.5	1	2	4	8	16	32	64
0											
2											
4											
8											
16											
32											
64											

PAO1 col^{R}3

SPL207 (µM) \ Colistin (µg/mL)	0	0.125	0.25	0.5	1	2	4	8	16	32	64
0											
2											
4											
8											
16											
32											
64											

PAO1 col^{R}5

SPL207 (µM) \ Colistin (µg/mL)	0	0.125	0.25	0.5	1	2	4	8	16	32	64
0											
2											
4											
8											
16											
32											
64											

Figure S2. Checkerboard assays with SPL207 and colistin. Schematic representation of the checkerboard plates containing cultures of the indicated *P. aeruginosa* strains and different concentrations of SPL207 and/or colistin. Grey boxes indicate bacterial growth. White boxes indicate growth inhibition. Representative data from at least three independent experiments are shown.

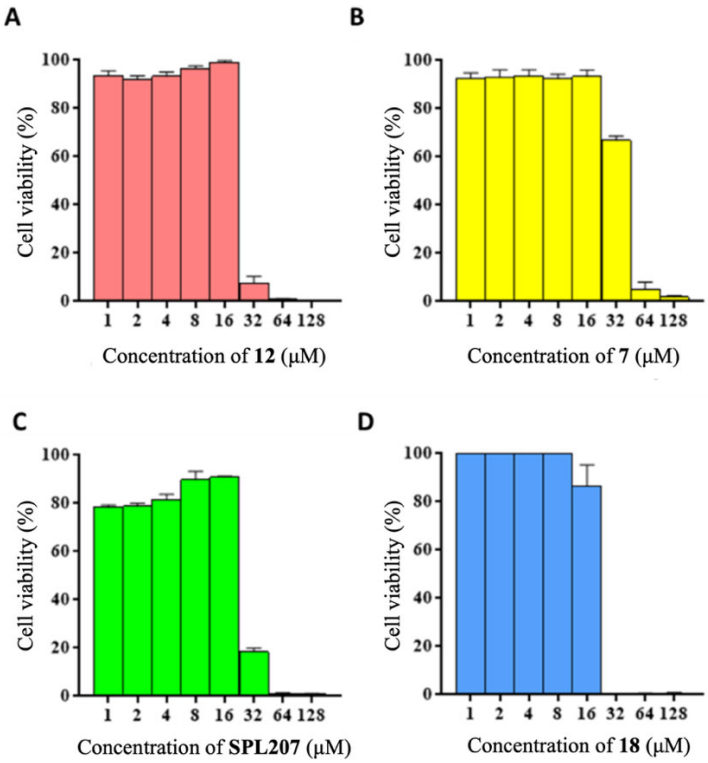


Figure S3. Cytotoxicity assays on A549 cells. Effect of compounds **12** (A), **7** (B), **SPL207** (C), **18** (D) on the viability of A549 cells, expressed as a percentage relative to control cells maintained in DMEM medium containing 1% DMSO. Each concentration was analyzed in triplicate and data are presented as the mean of three independent experiments ± standard error of the mean (SEM).

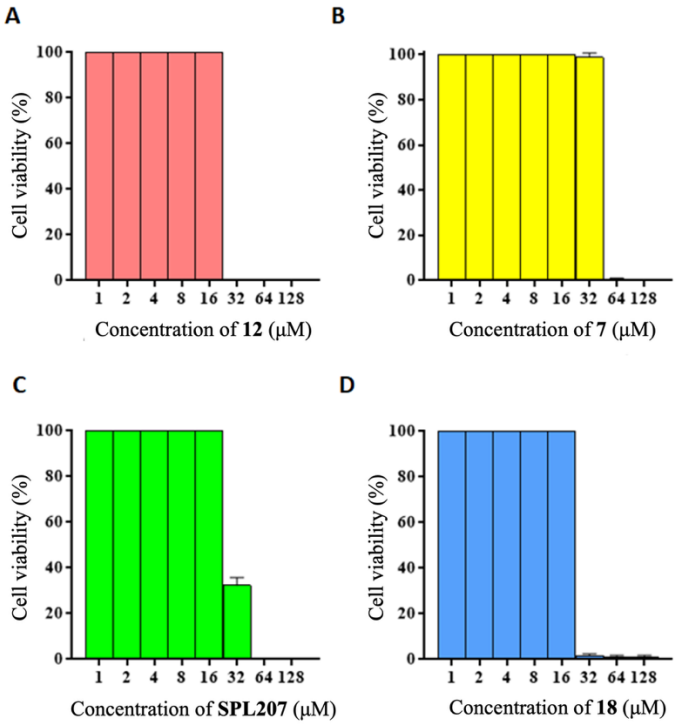
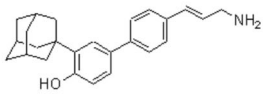
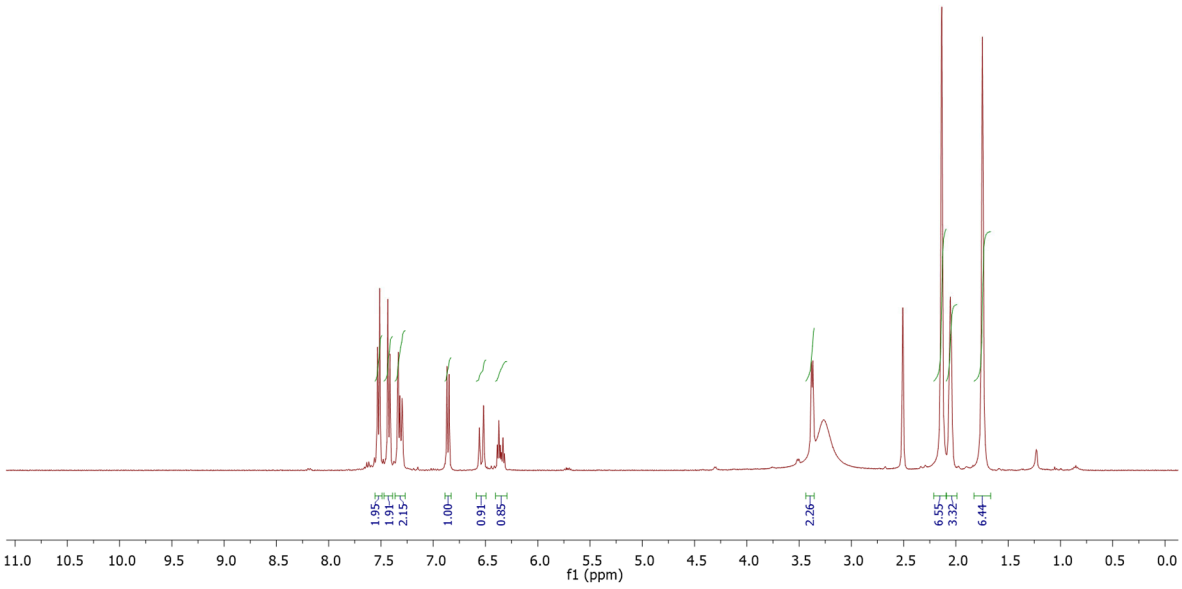


Figure S4. Cytotoxicity assays on HaCaT cells. Effect of compounds **12** (A), **7** (B), **SPL207** (C), and **18** (D) on the viability of HaCaT cells, expressed as a percentage relative to control cells maintained in DMEM medium containing 1% DMSO. Each concentration was analyzed in triplicate and data are presented as the mean of three independent experiments ± standard error of the mean (SEM).

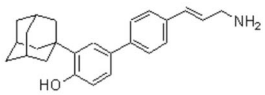
^{1}H-NMR (400 MHz, DMSO-d_6) compound **7**.

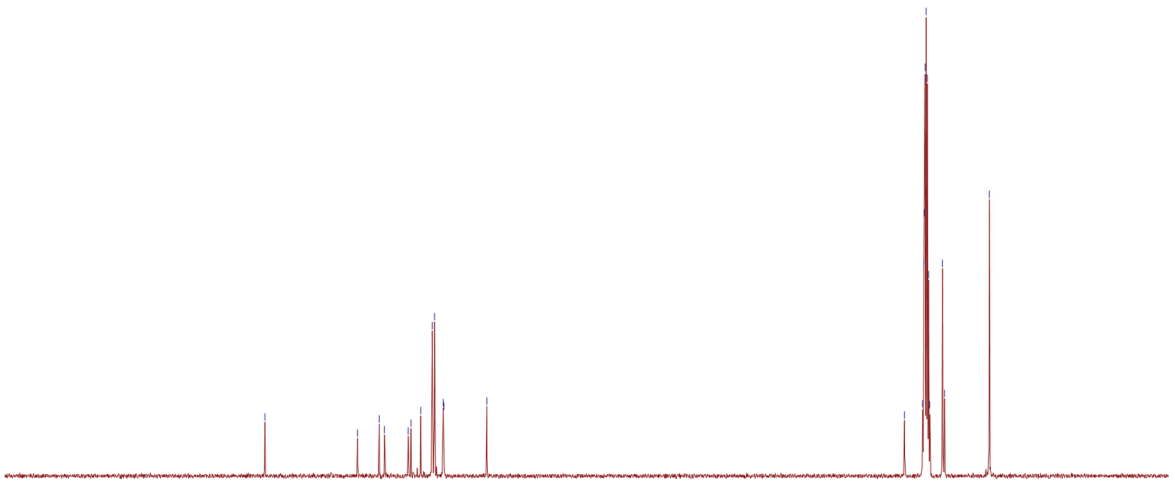




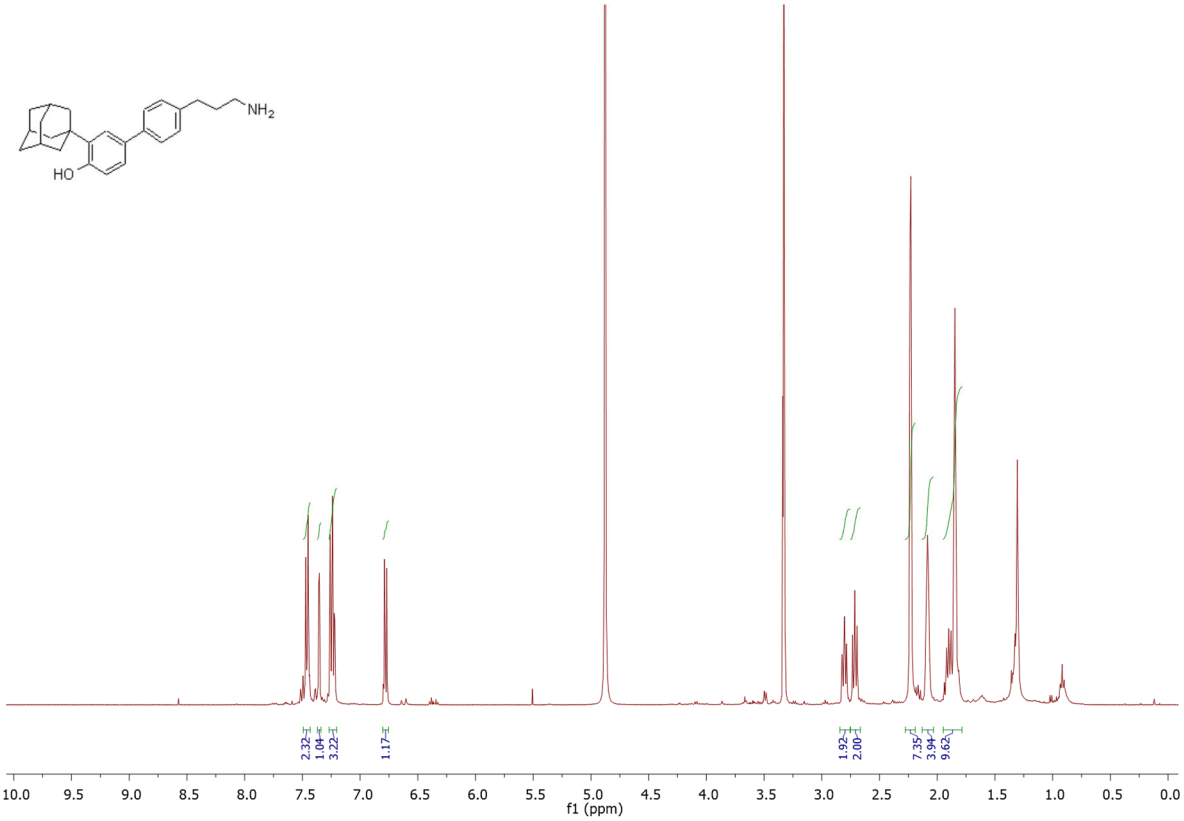
^{13}C-NMR (100 MHz, DMSO-d_6) compound **7**.



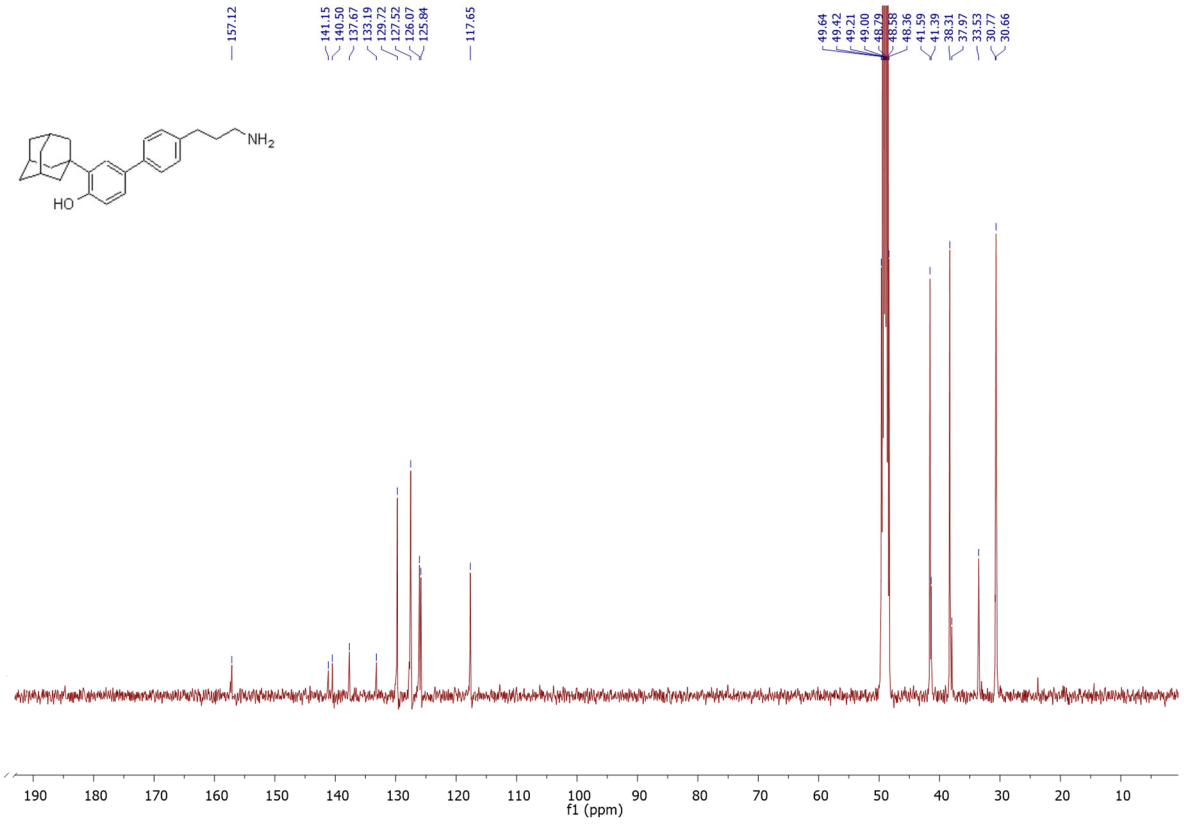




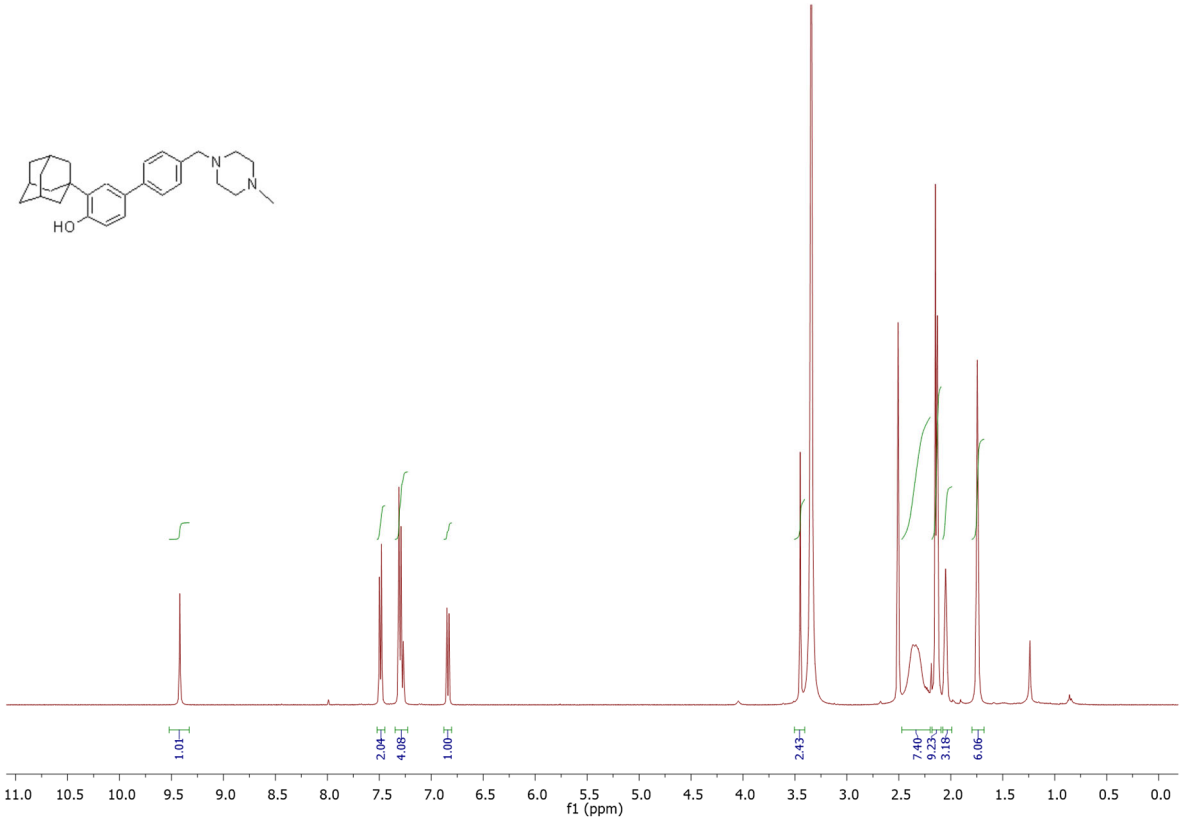
^{1}H-NMR (400 MHz, CH_3OH-d_4) compound **8**.



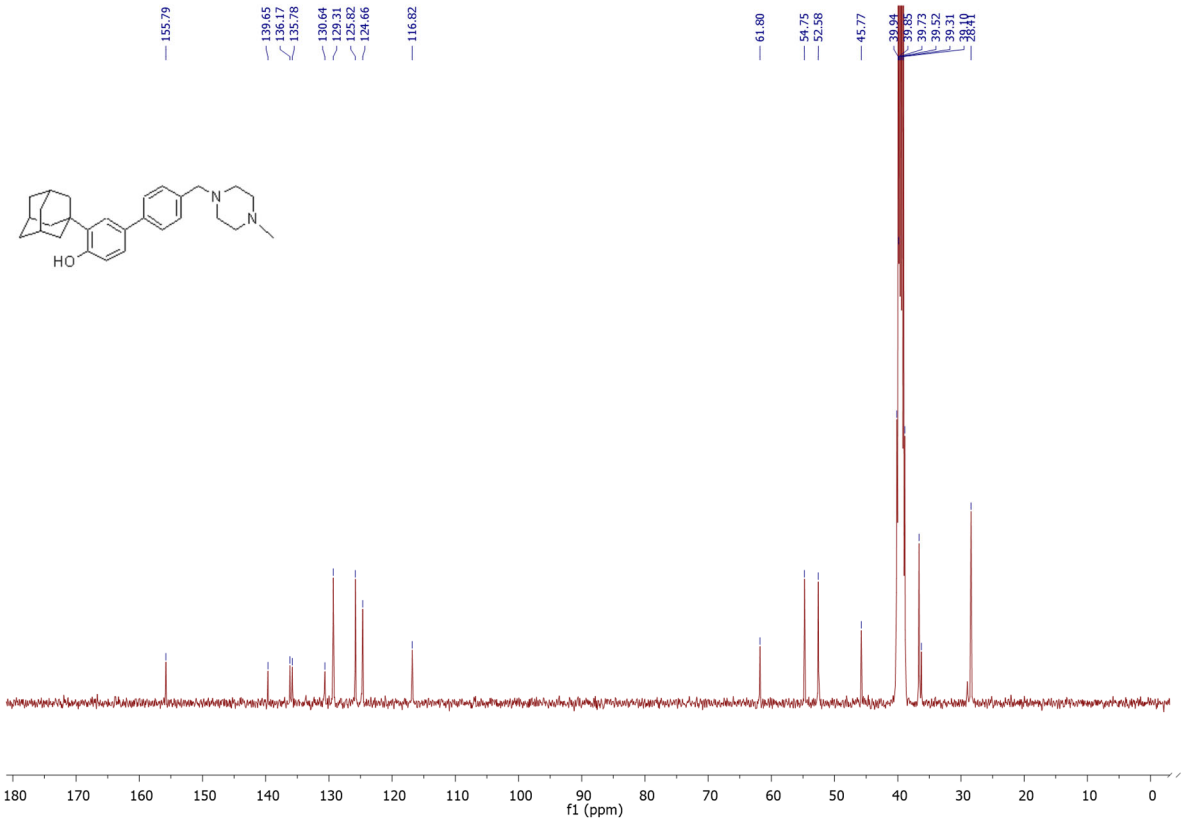
^{13}C-NMR (100 MHz, CH_3OH-d_4) compound **8**.



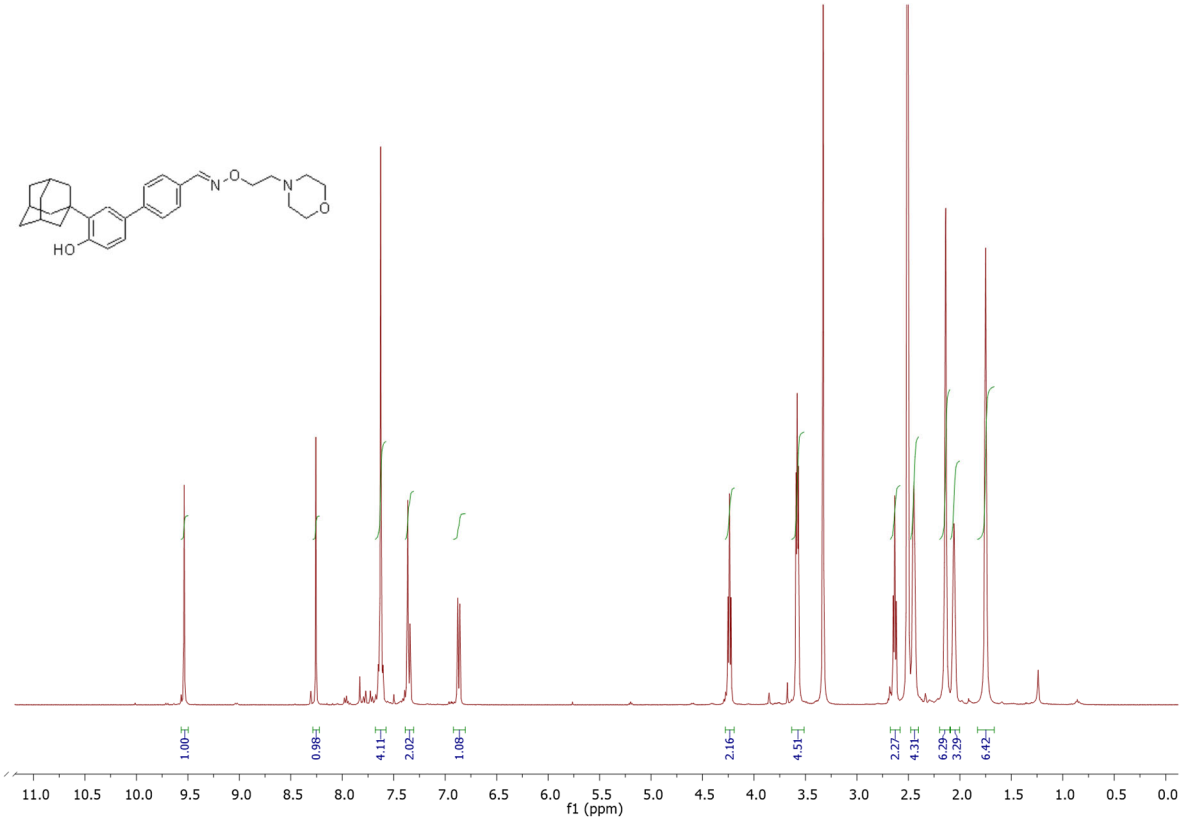
^{1}H-NMR (400 MHz, DMSO-d_6) compound **9**.



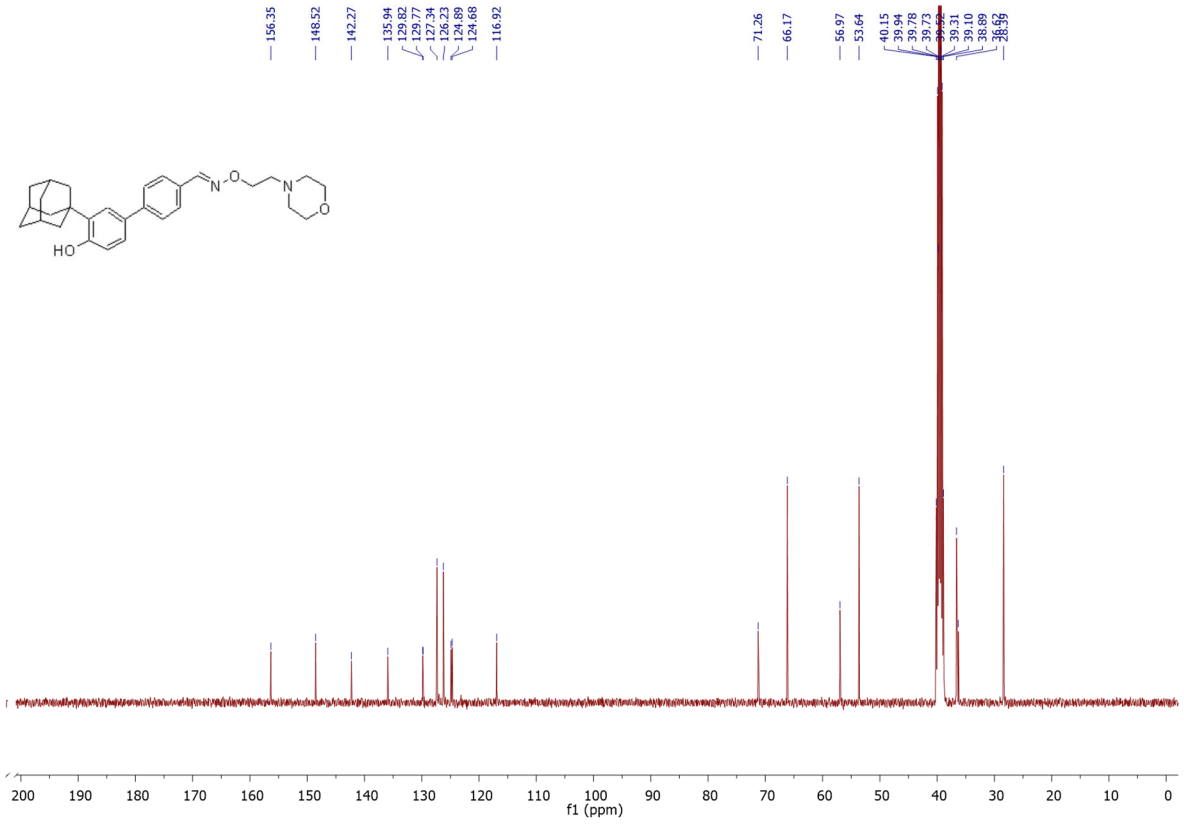
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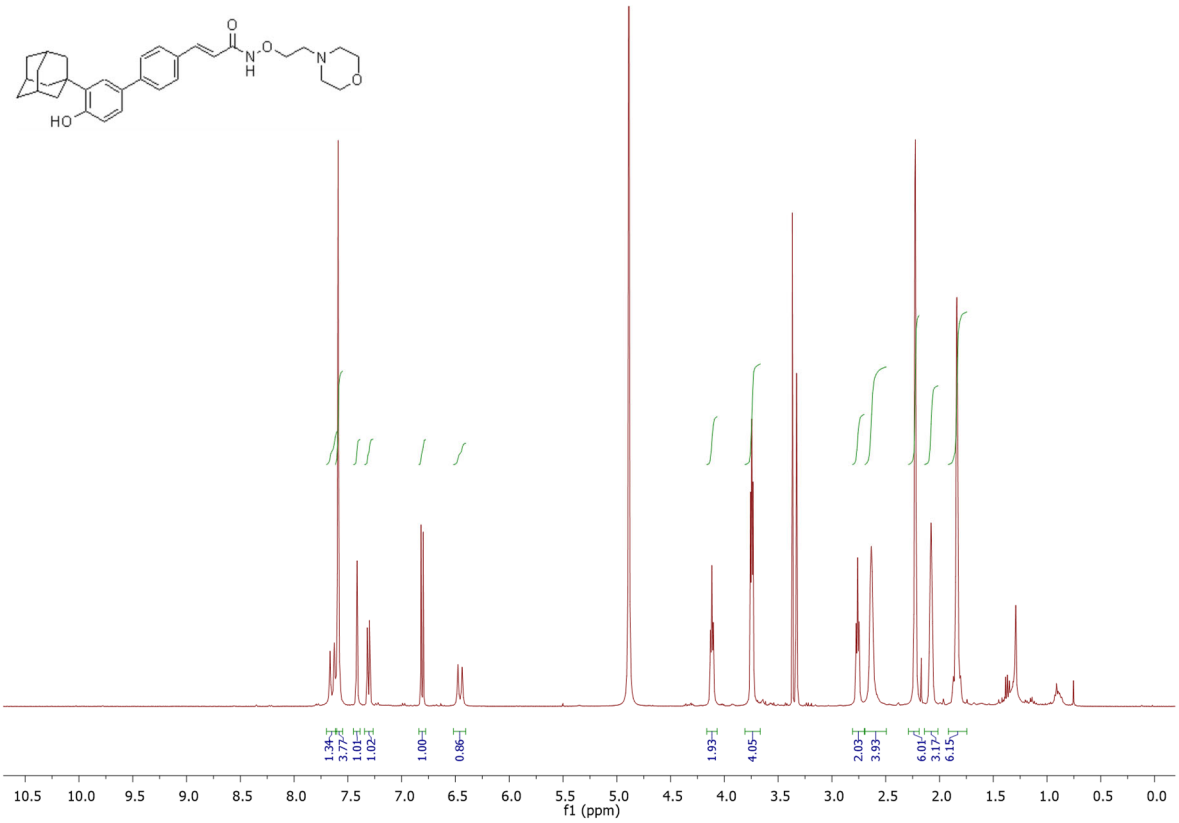
^{1}H-NMR (400 MHz, DMSO-d_6) compound **10**.



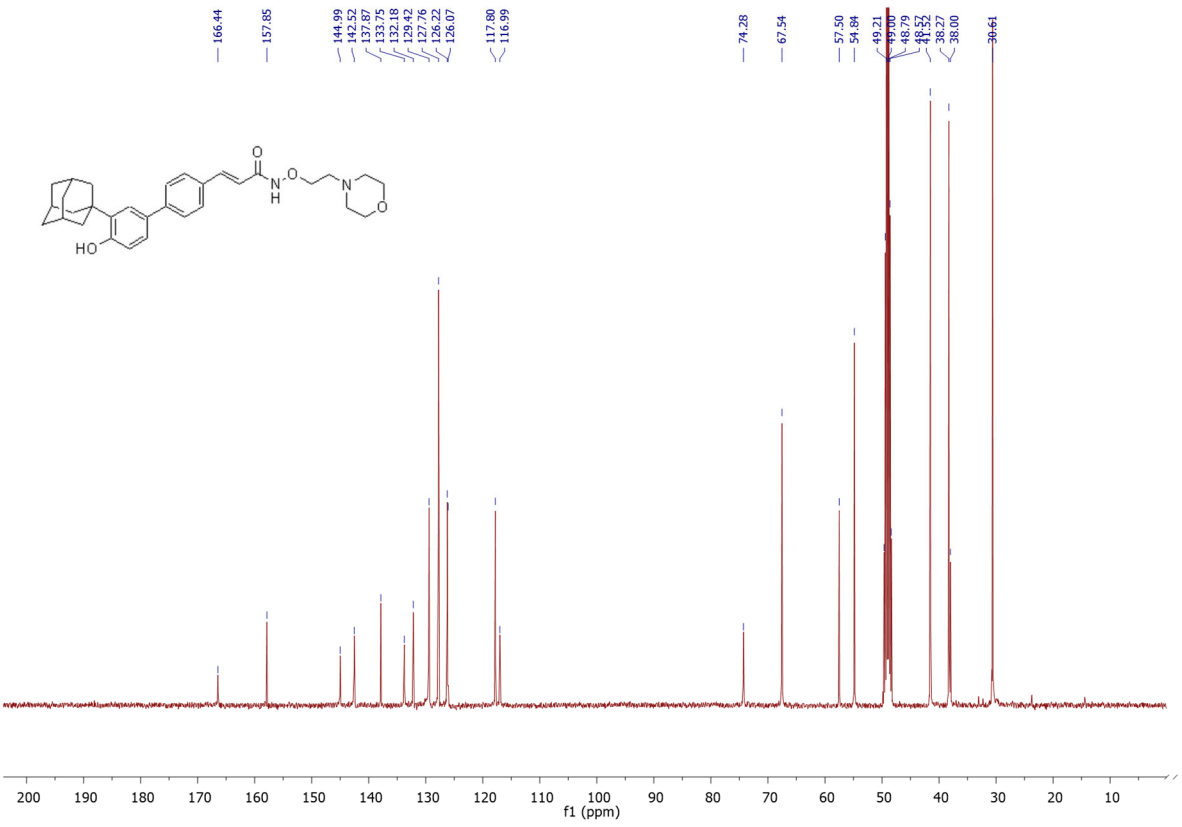
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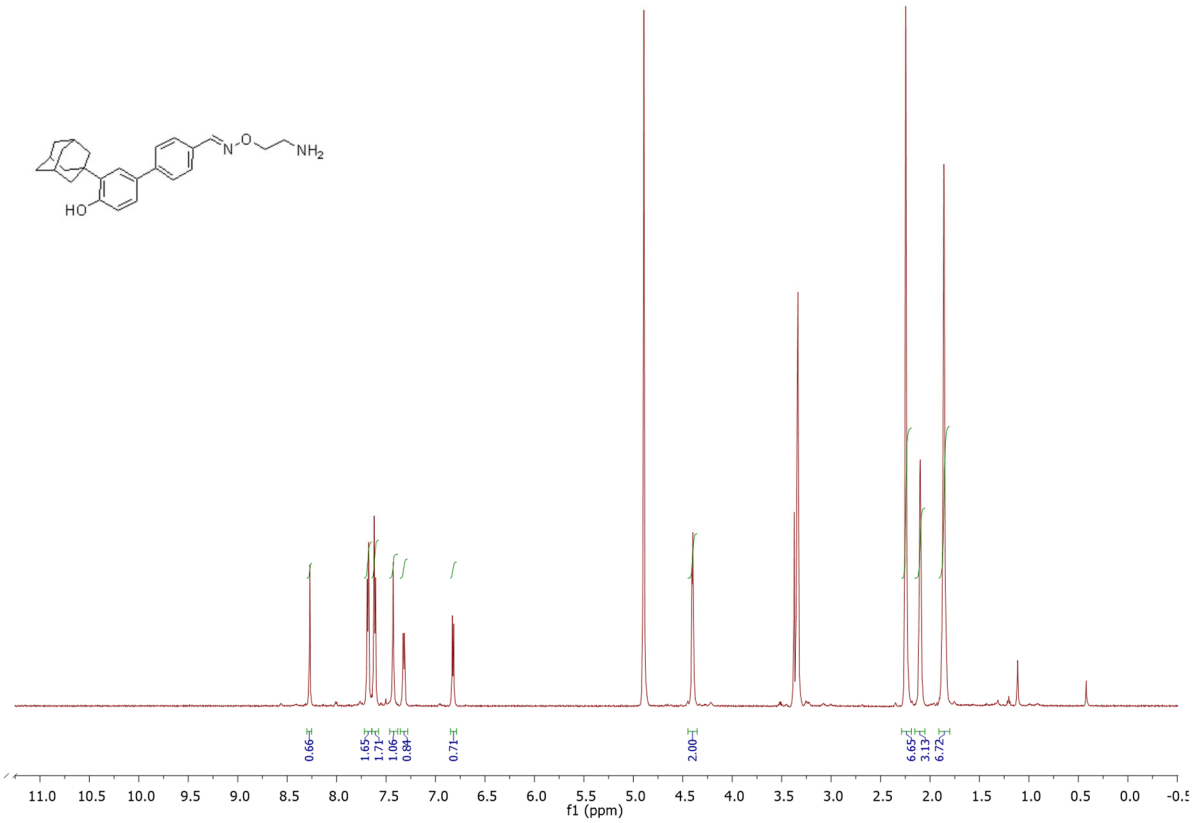
^{1}H-NMR (600 MHz, CH_3OH-d_4) compound **11**.



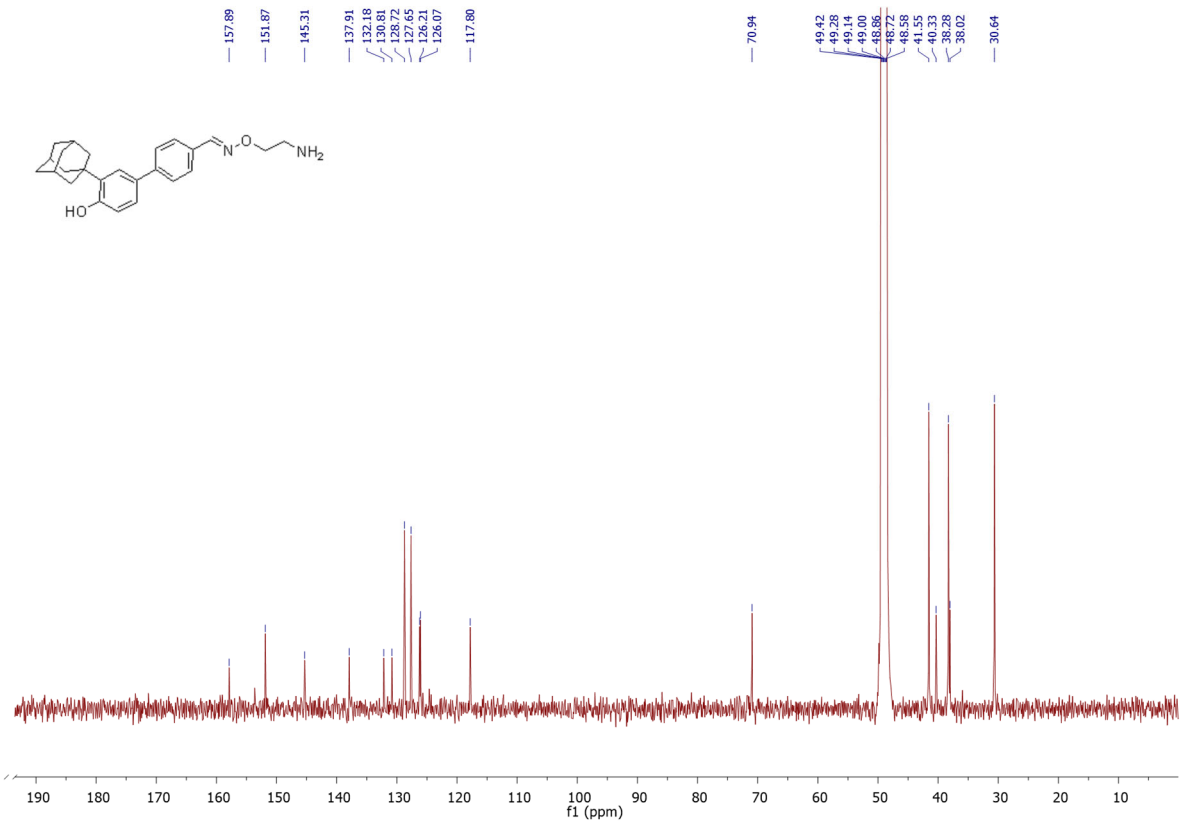
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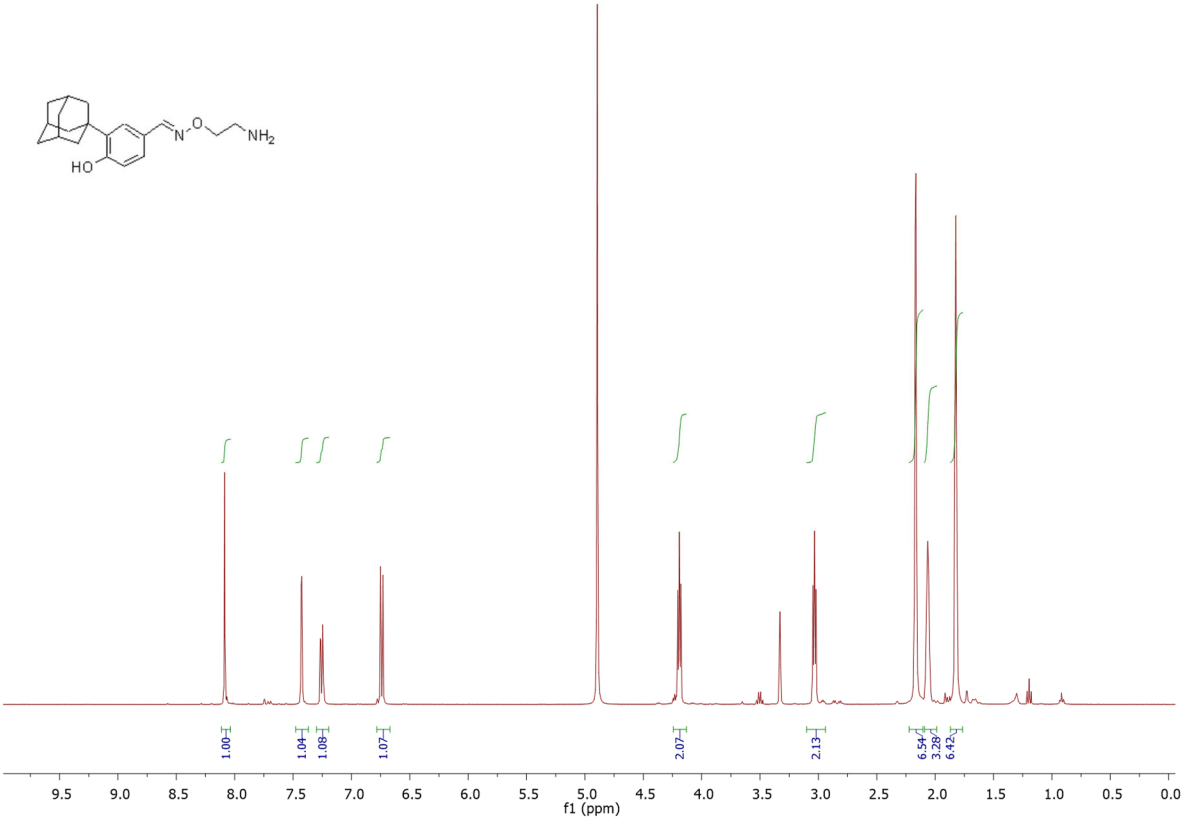
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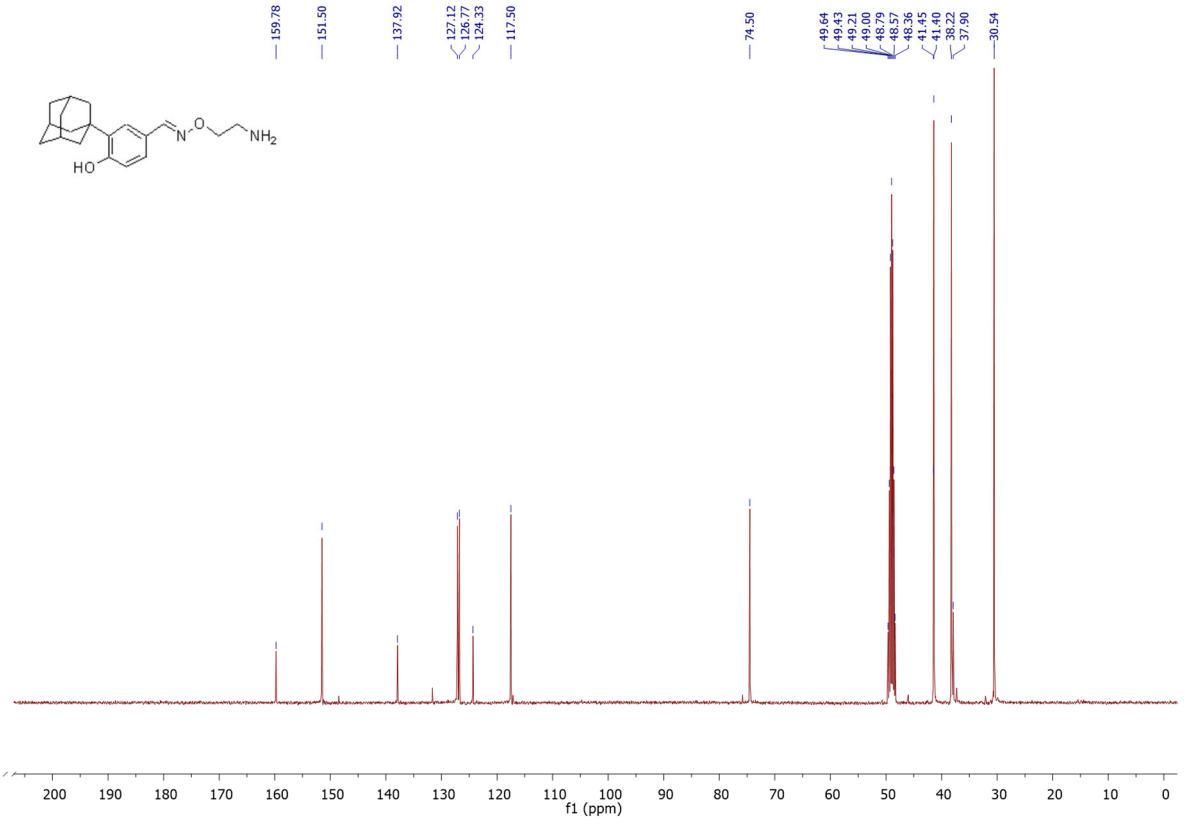
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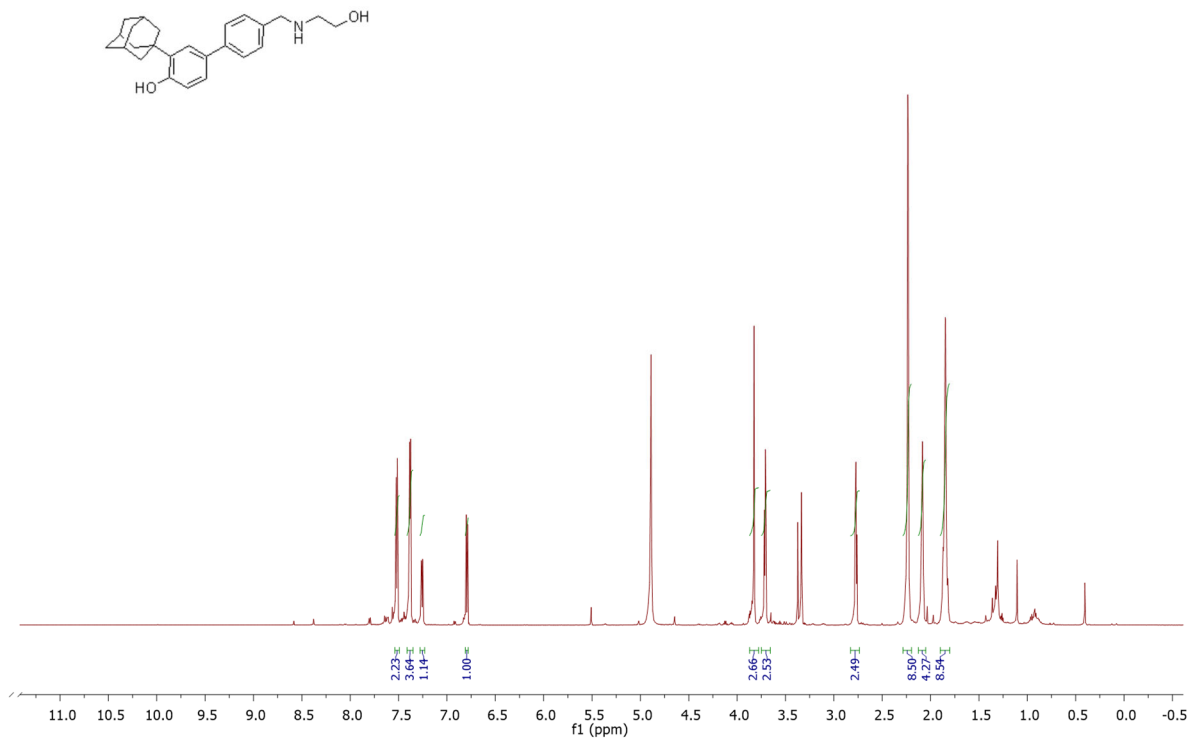
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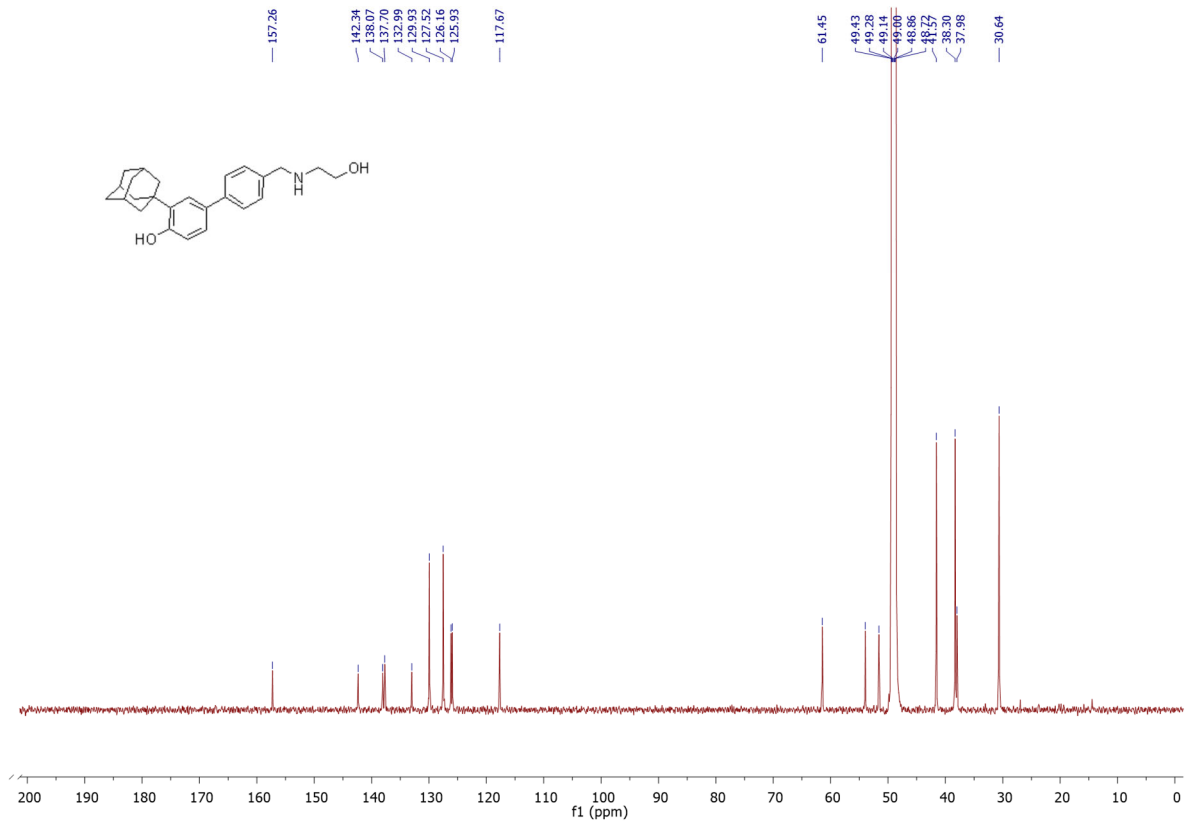
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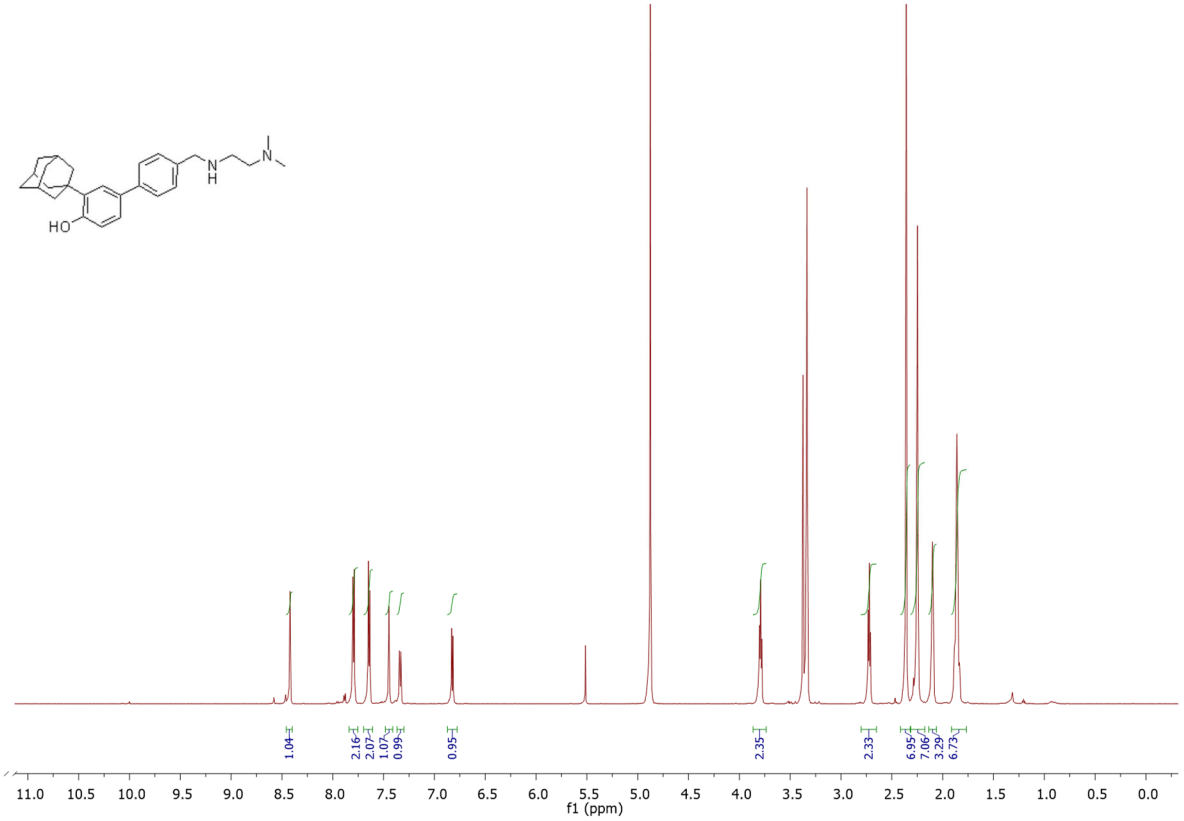
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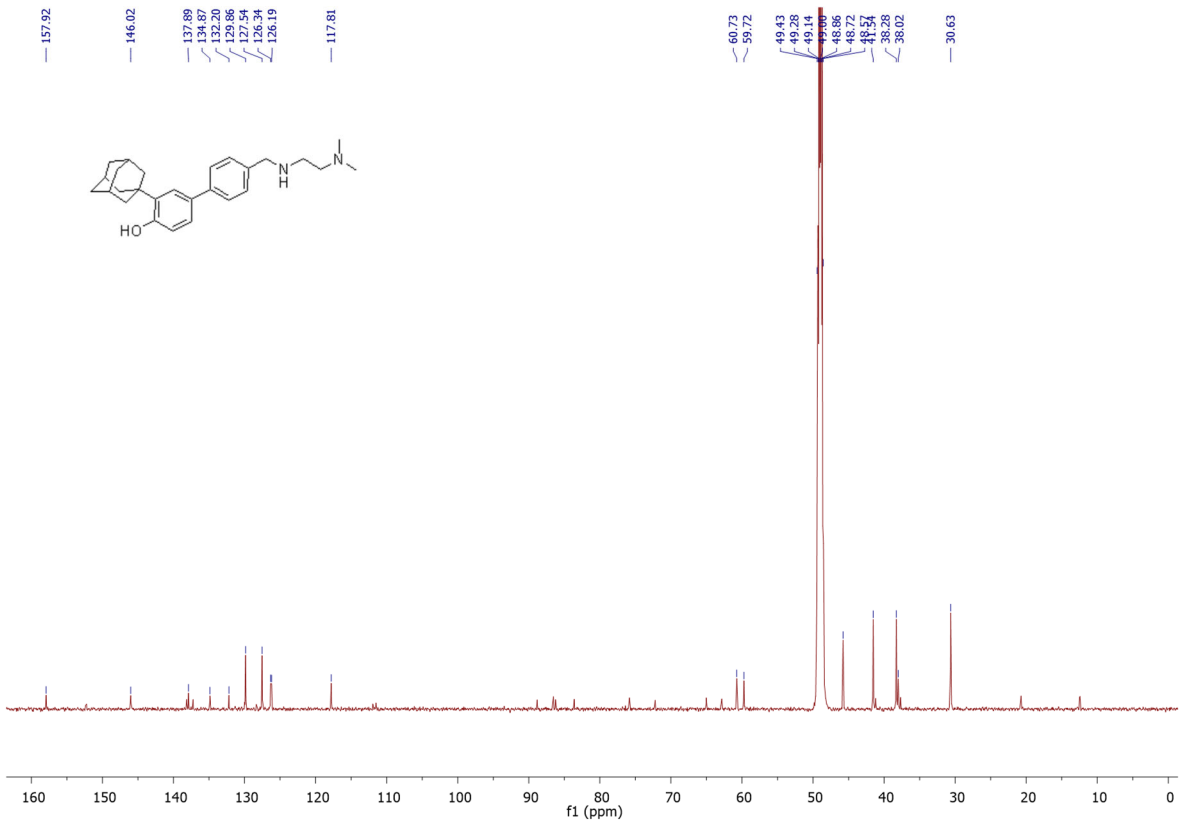
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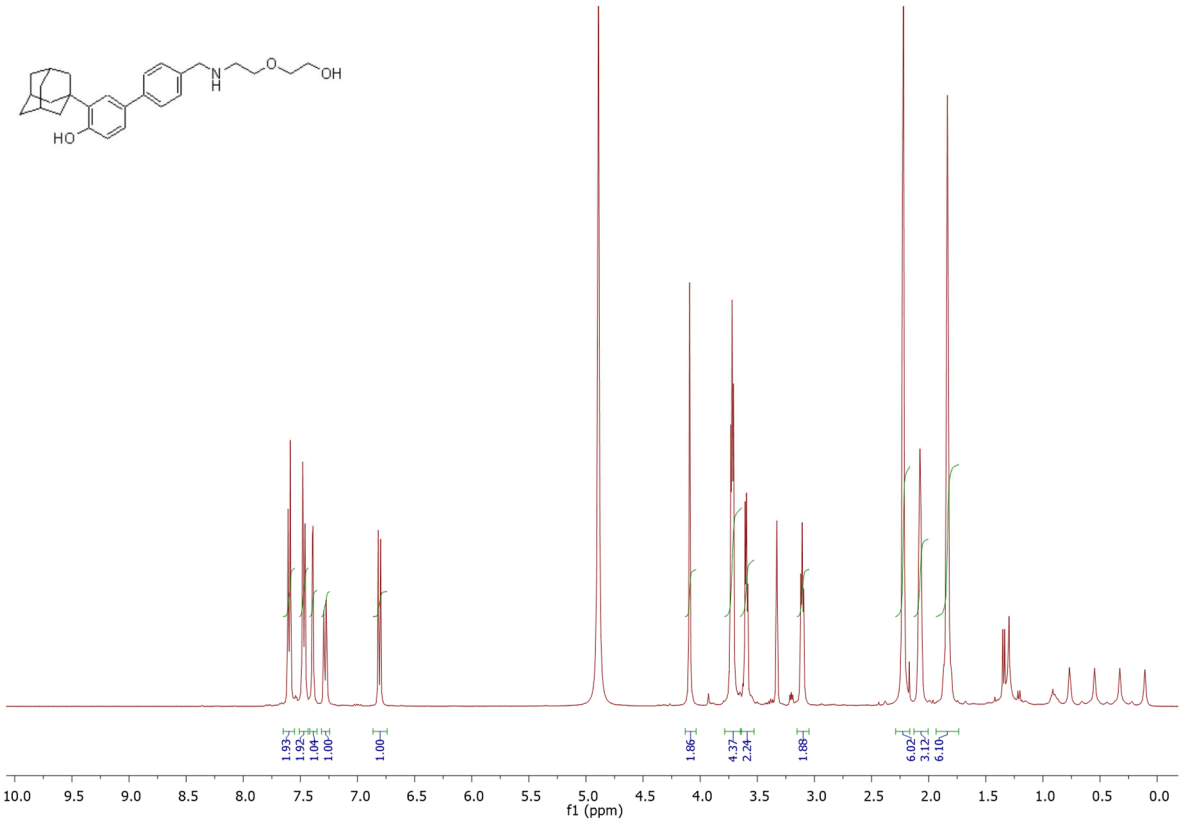
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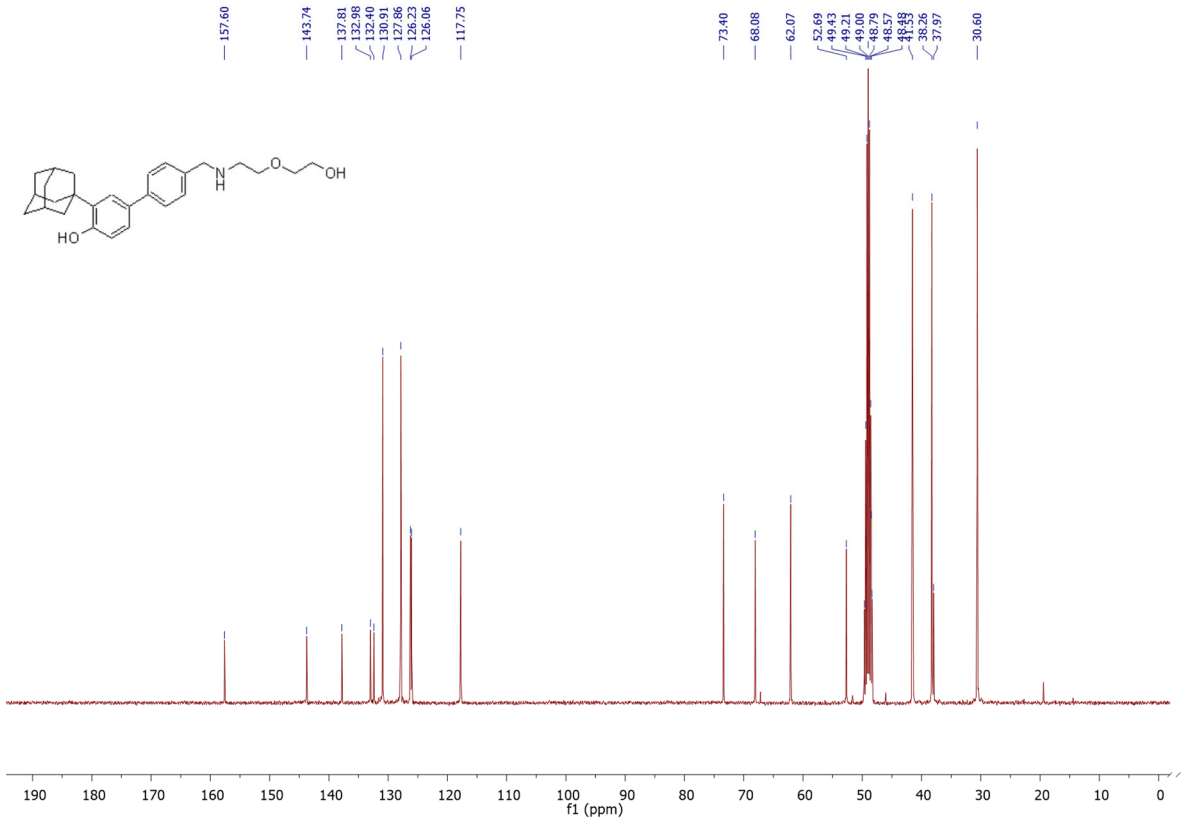
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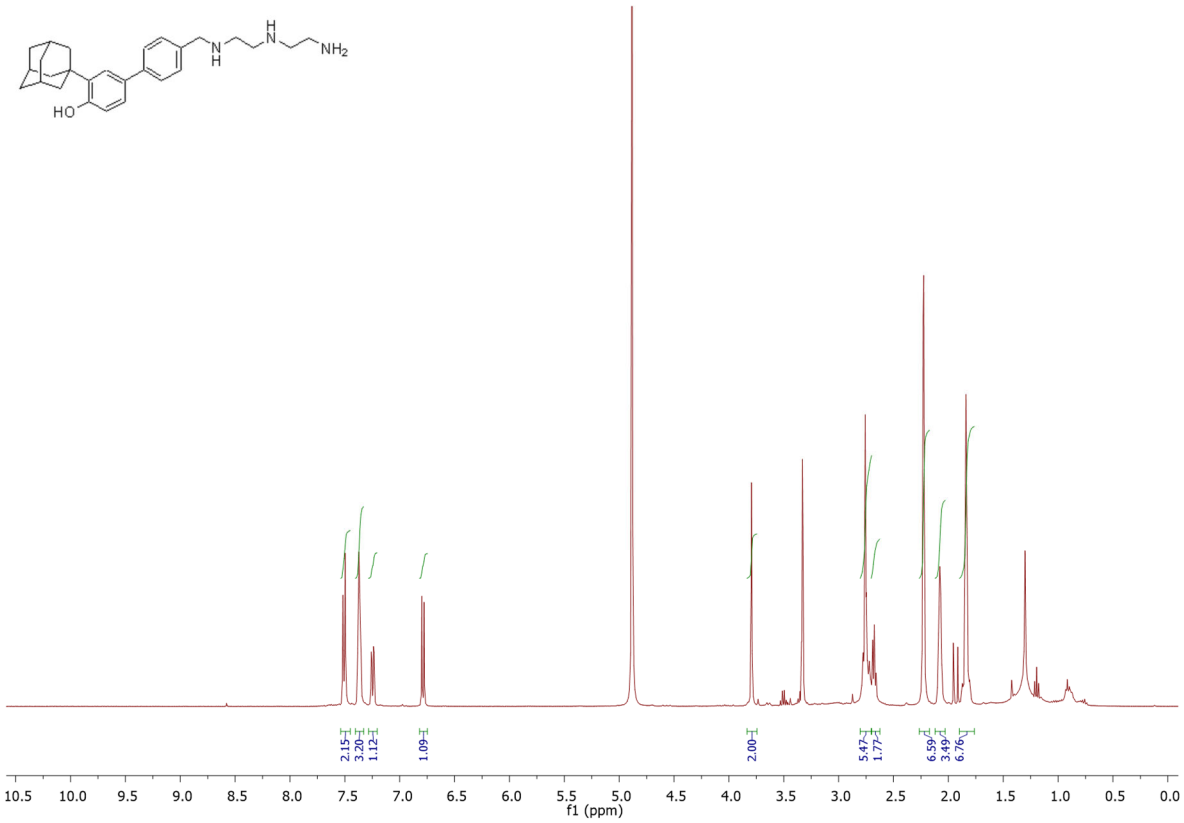
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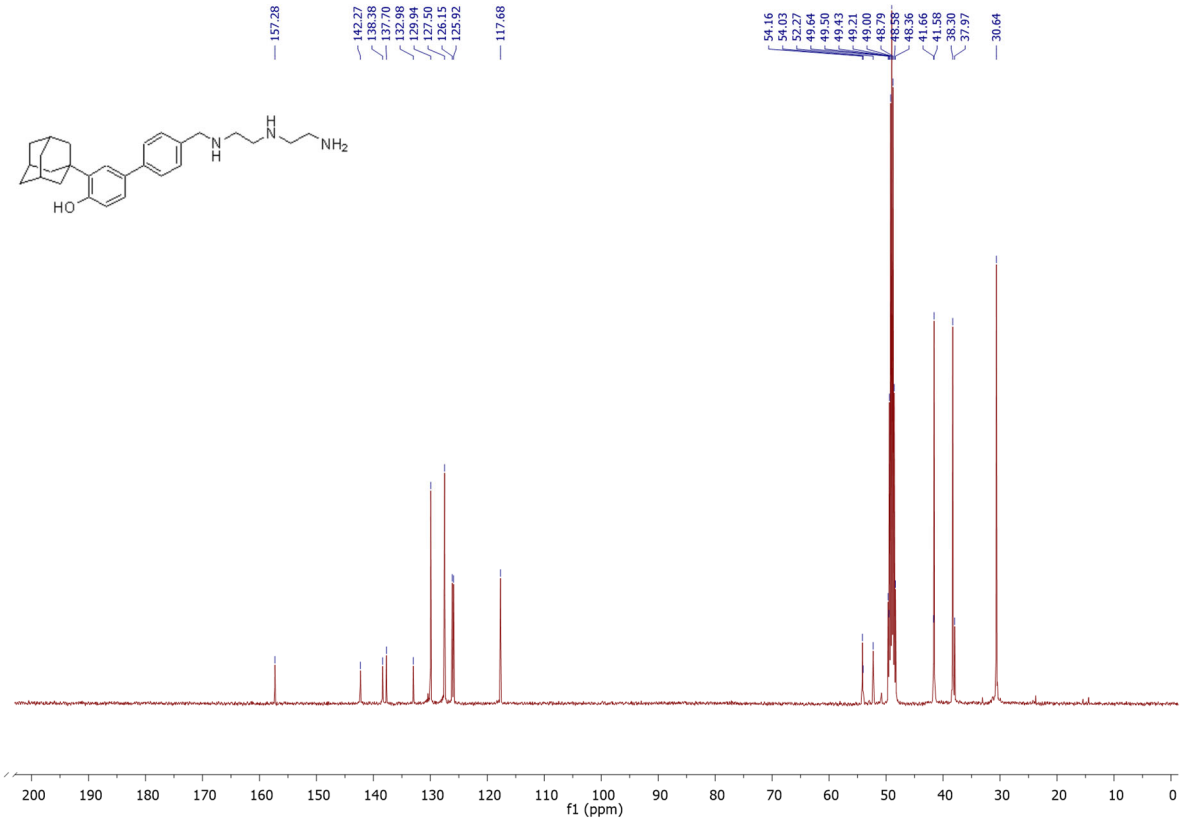
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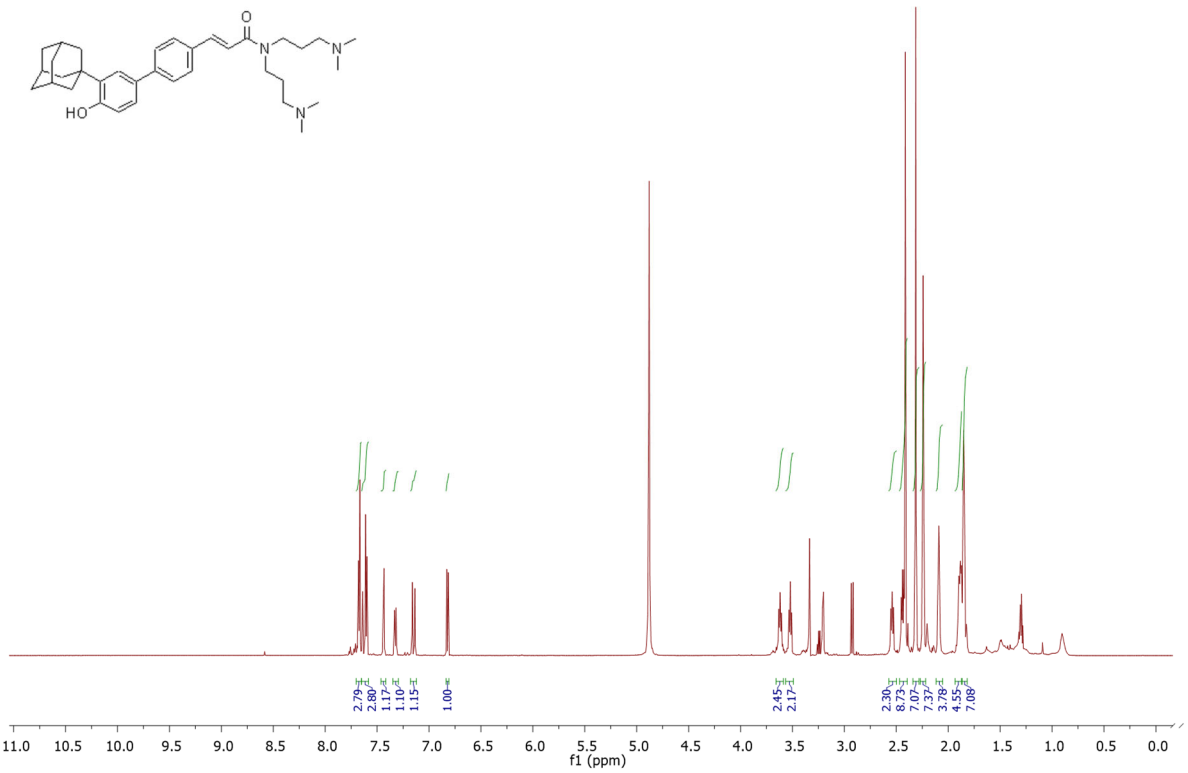
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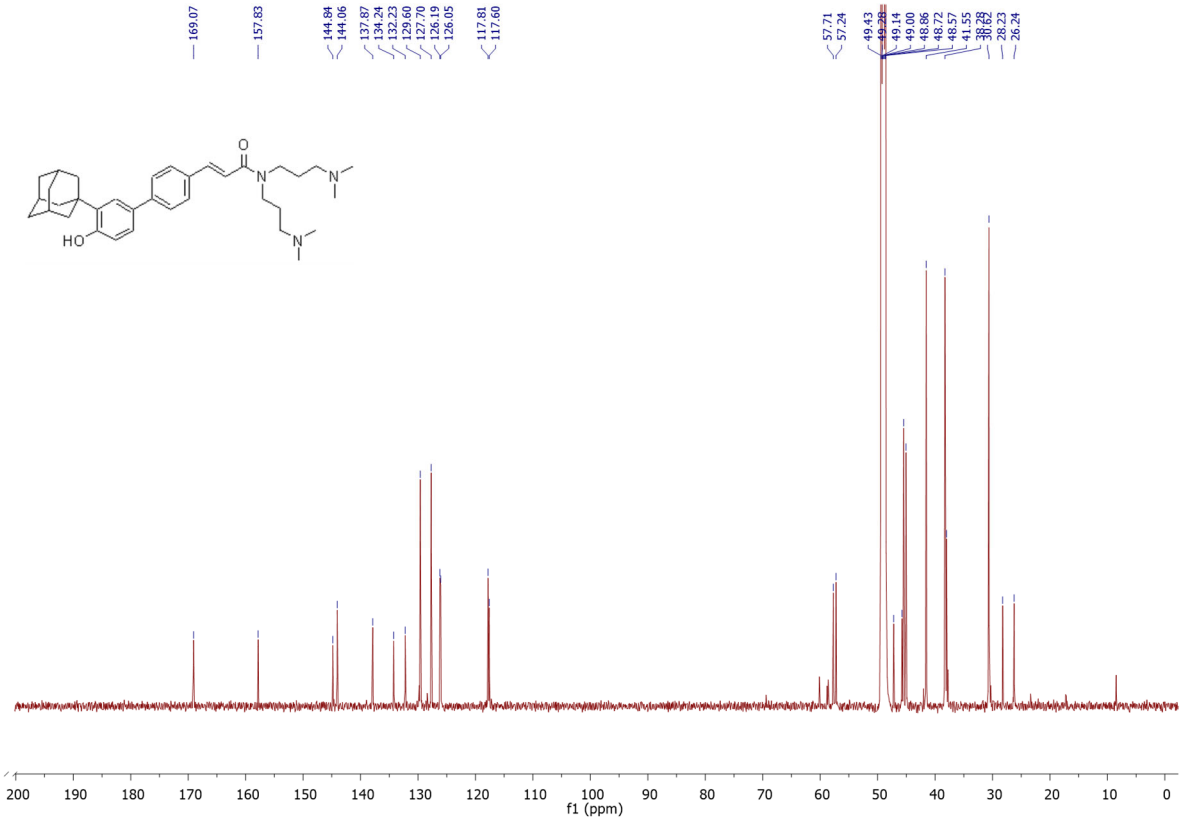
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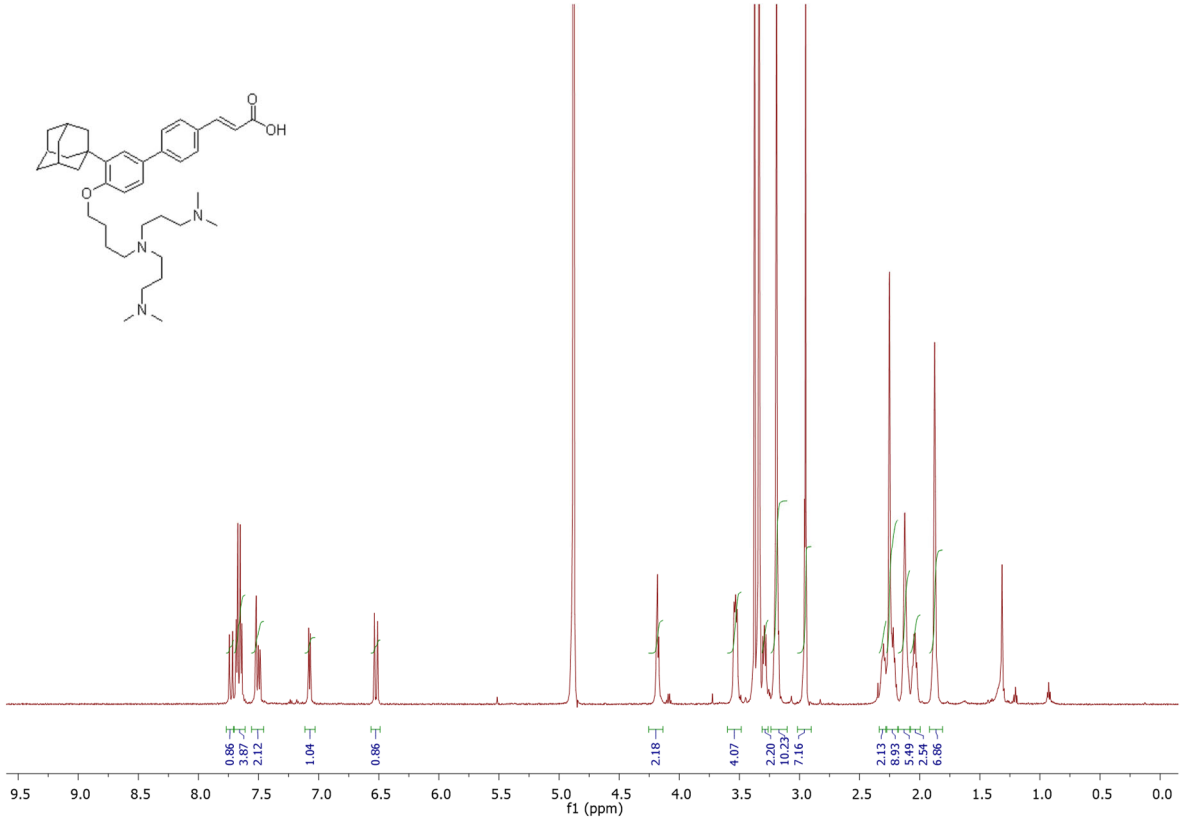
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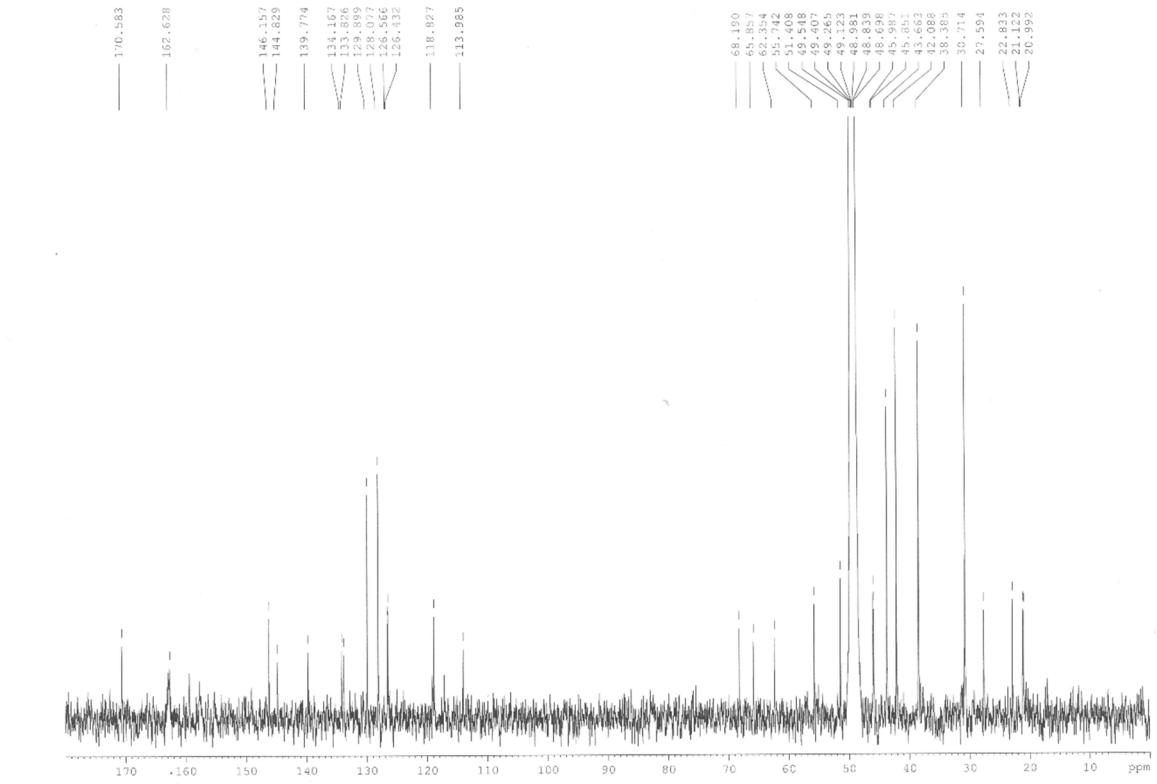
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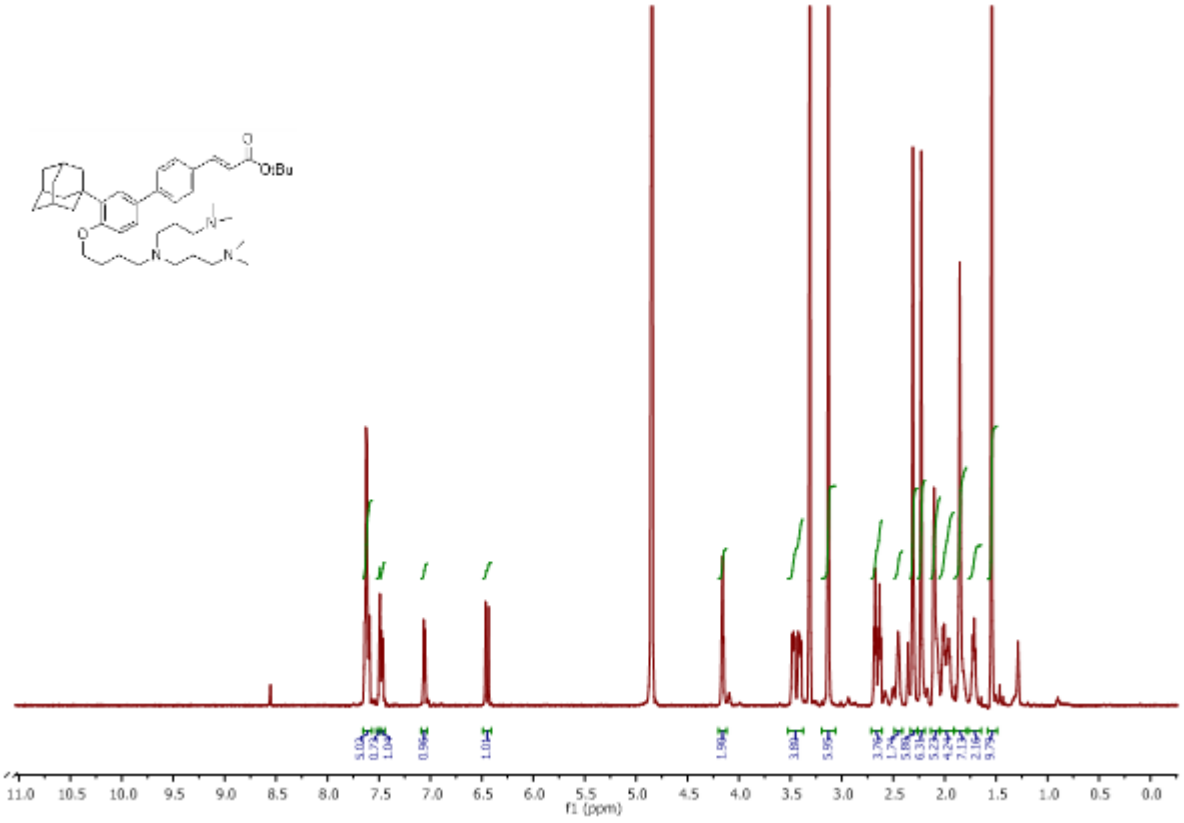
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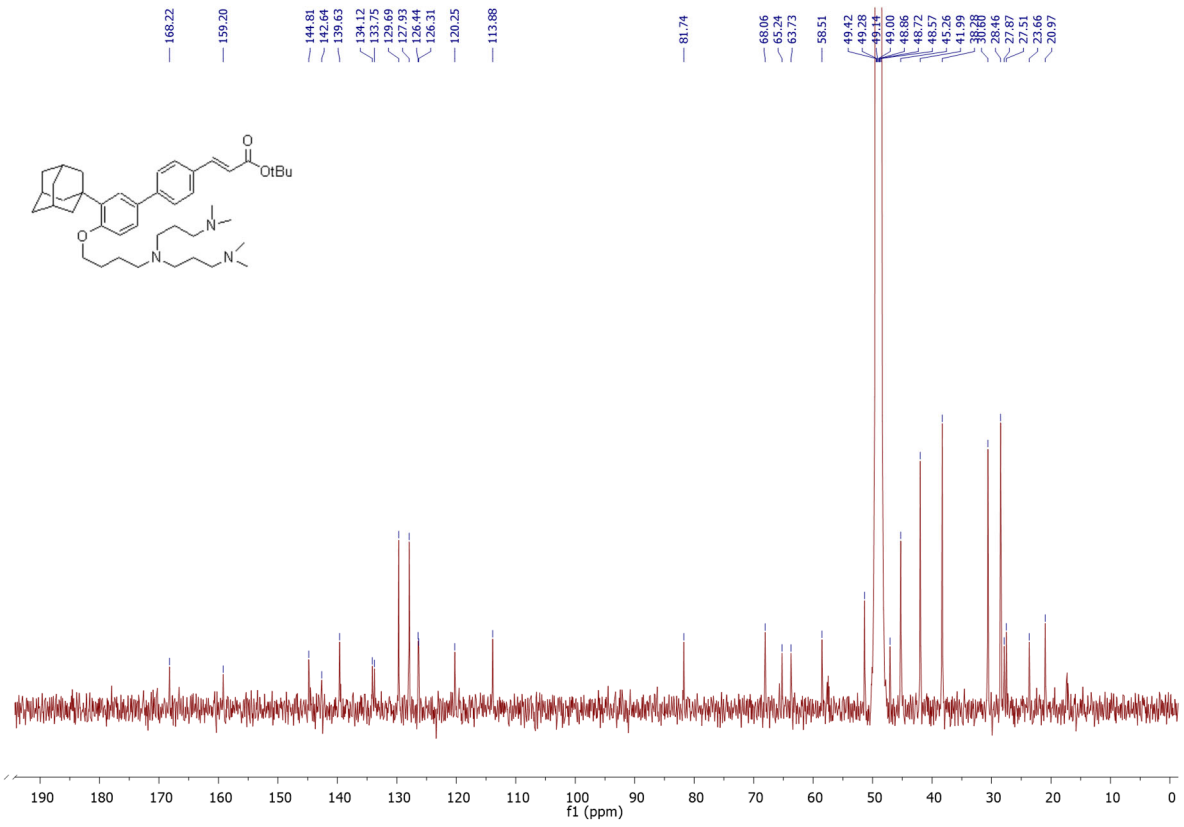
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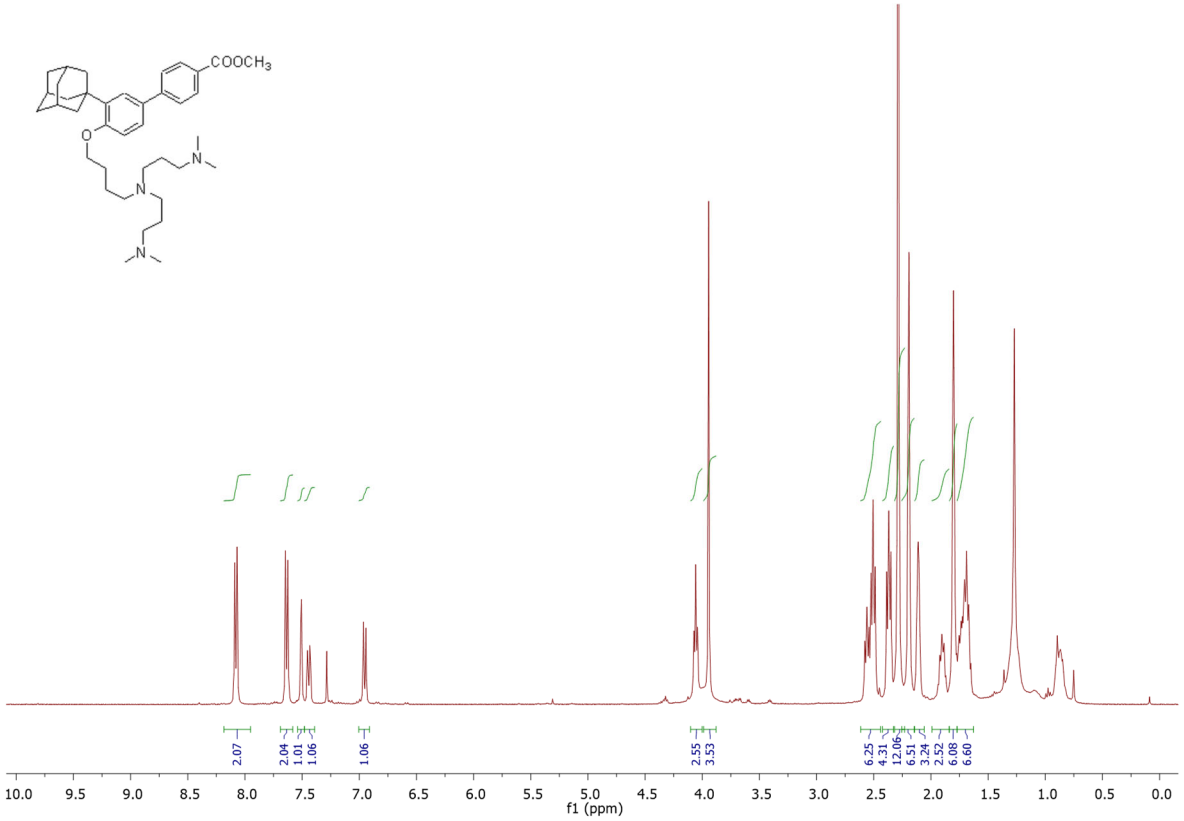
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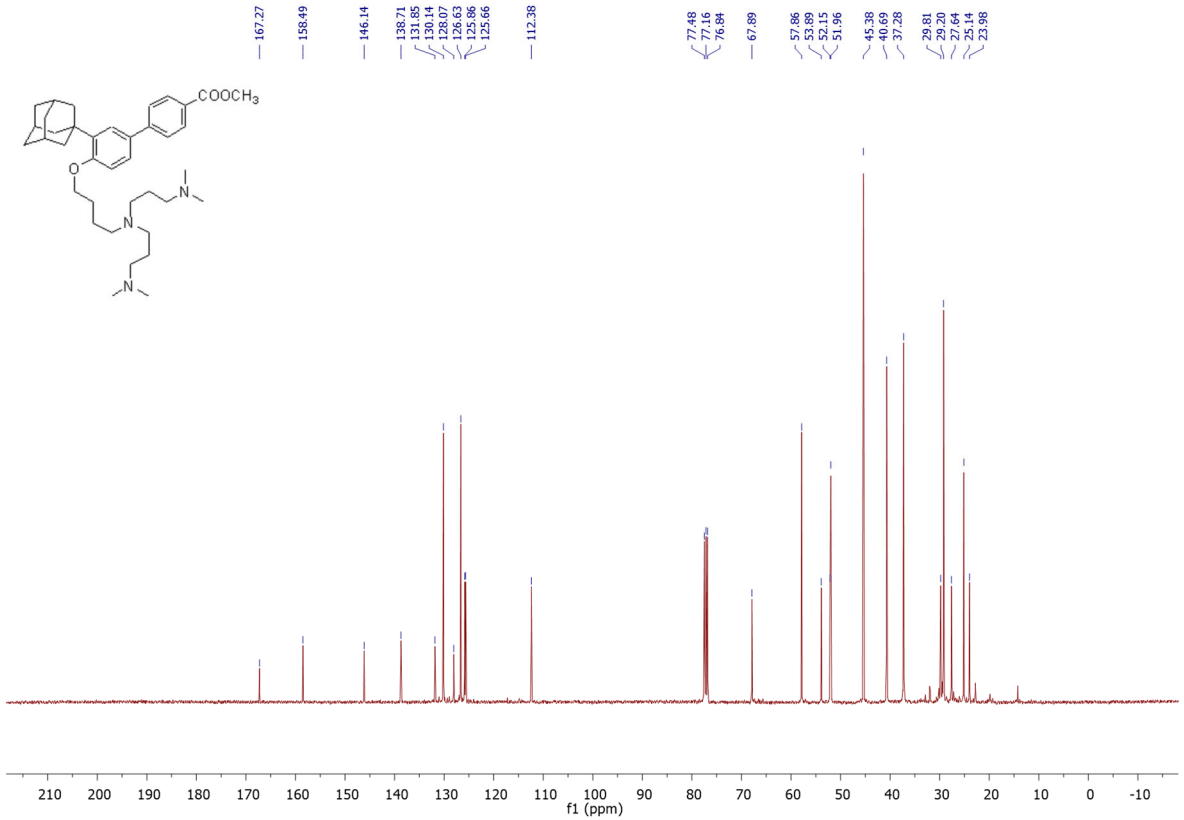
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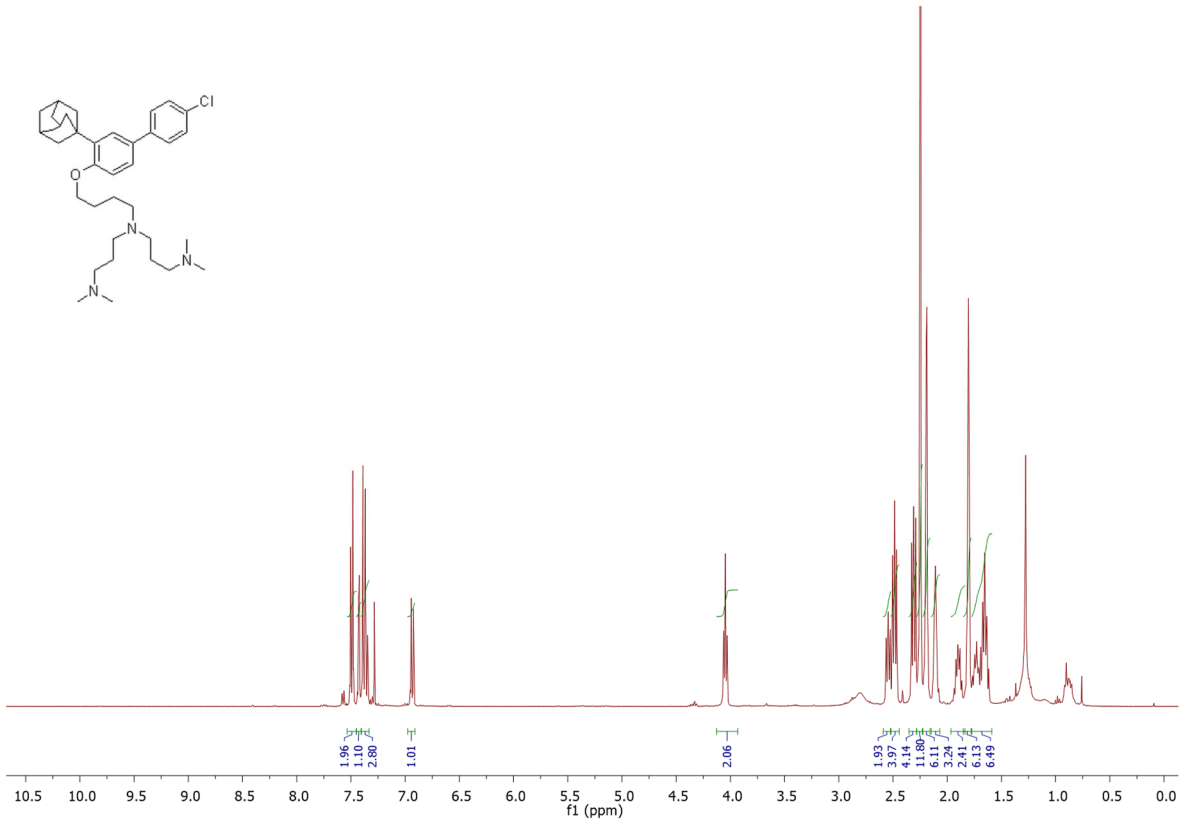
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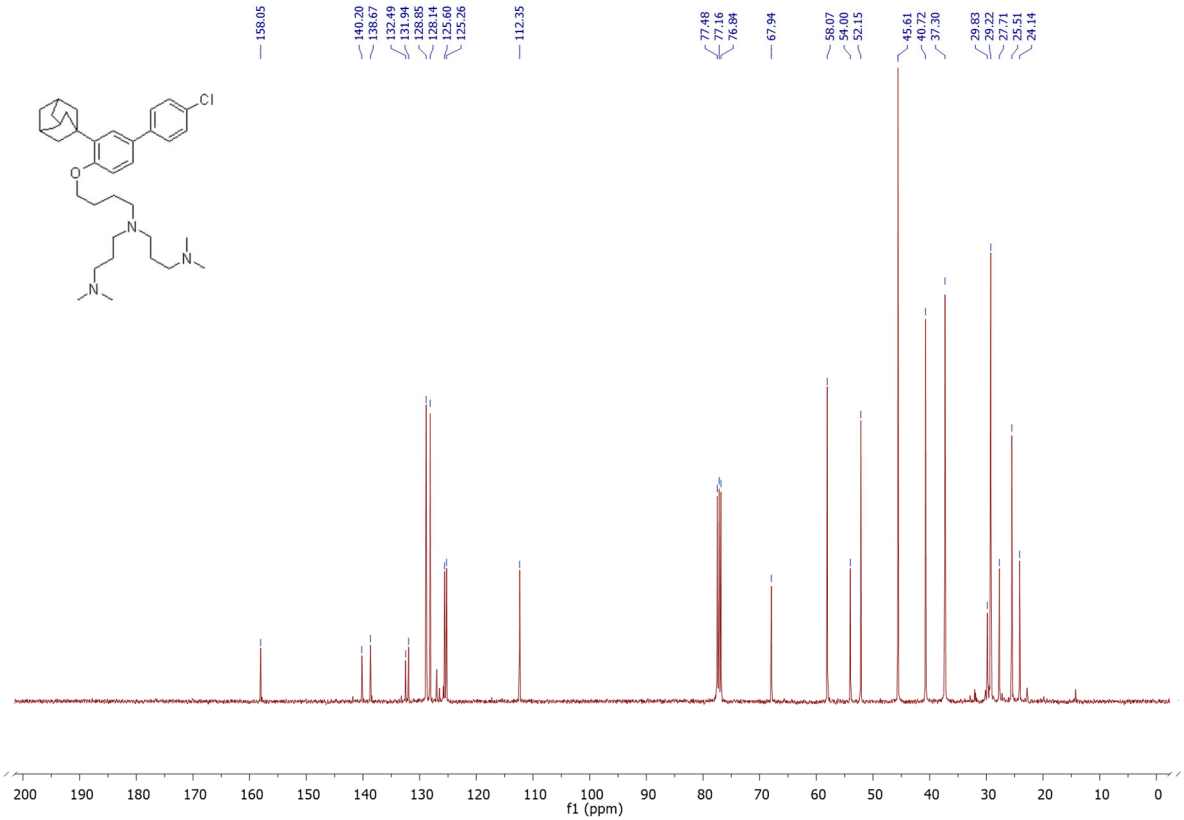
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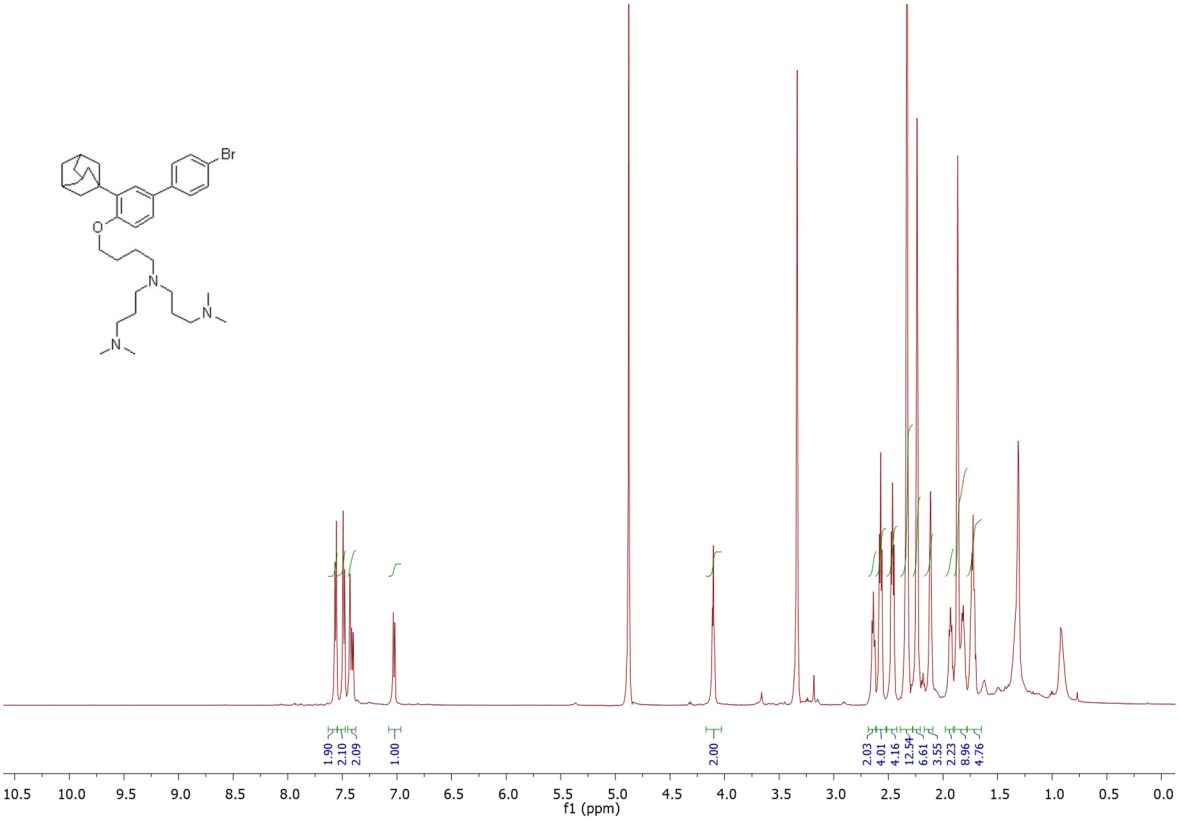
^{1}H-NMR (600 MHz, $CDCl_3$) compound **22**.



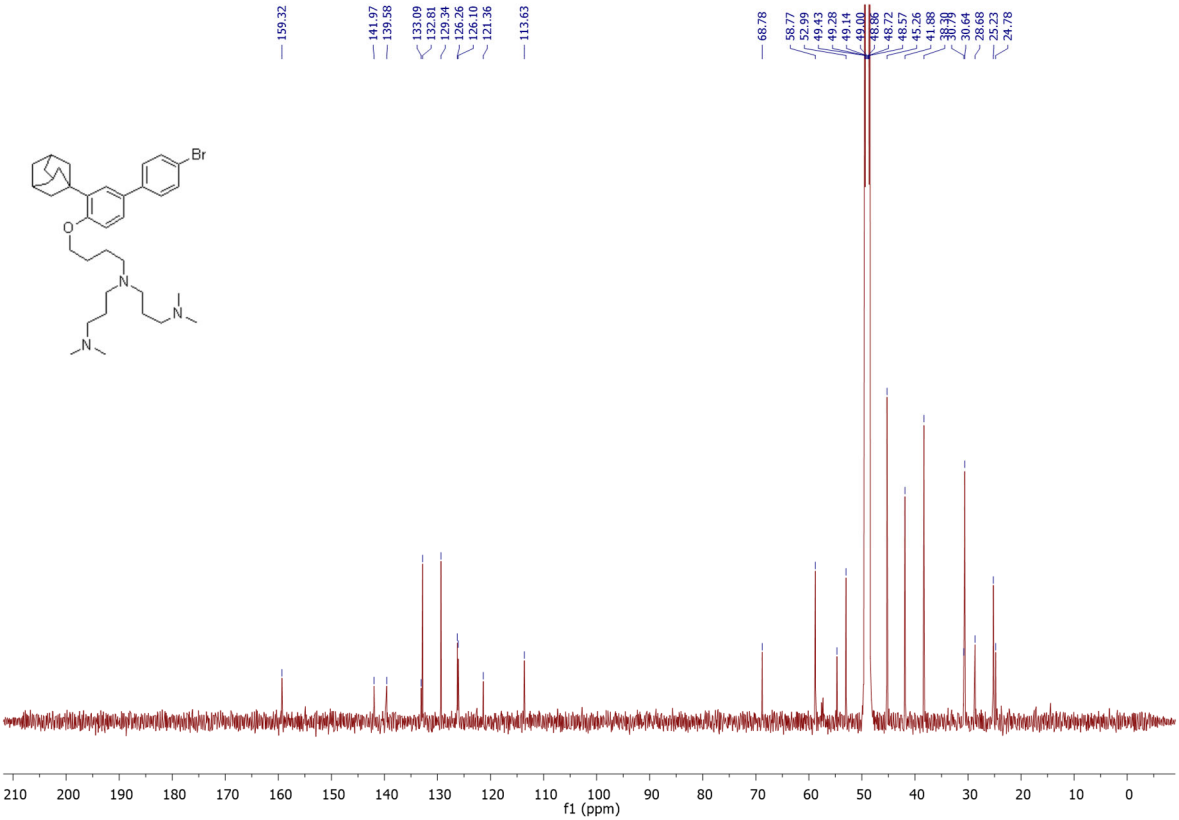
^{13}C-NMR (150 MHz, $CDCl_3$) compound **22**.



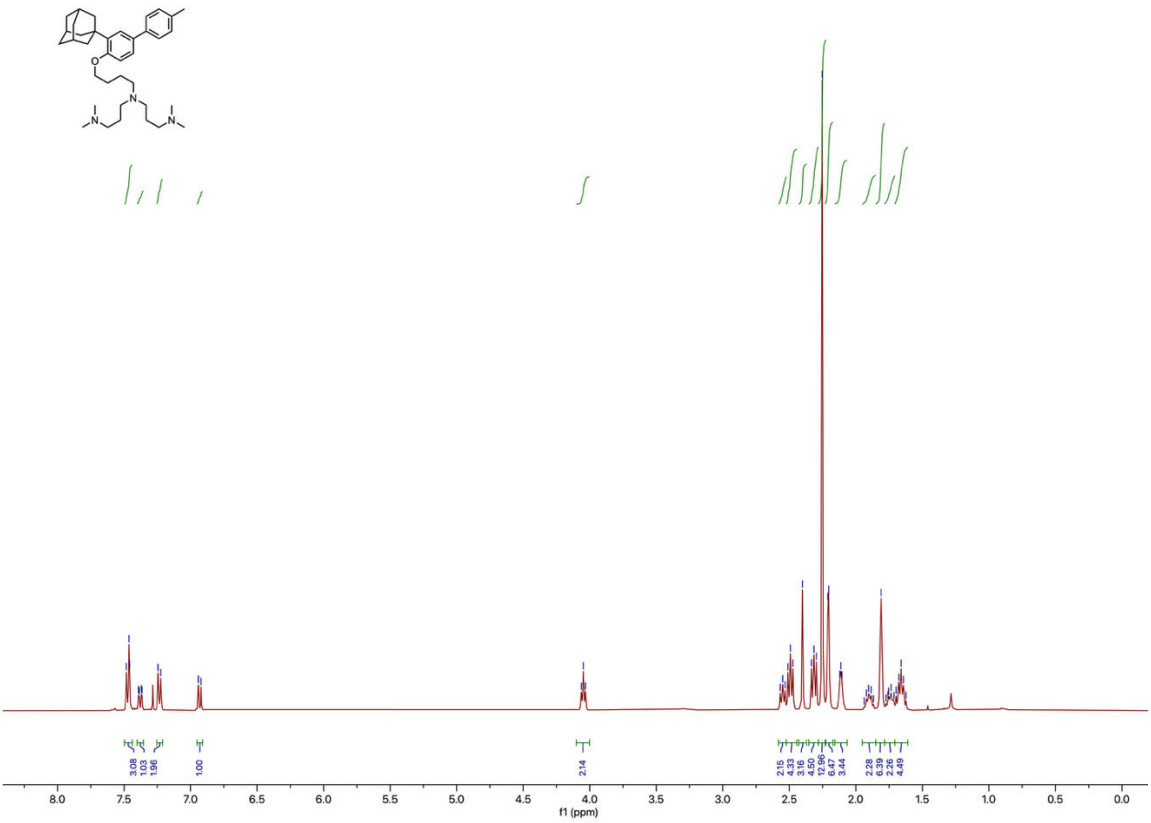
^{1}H-NMR (600 MHz, CH_3OH-d_4) compound **23**.



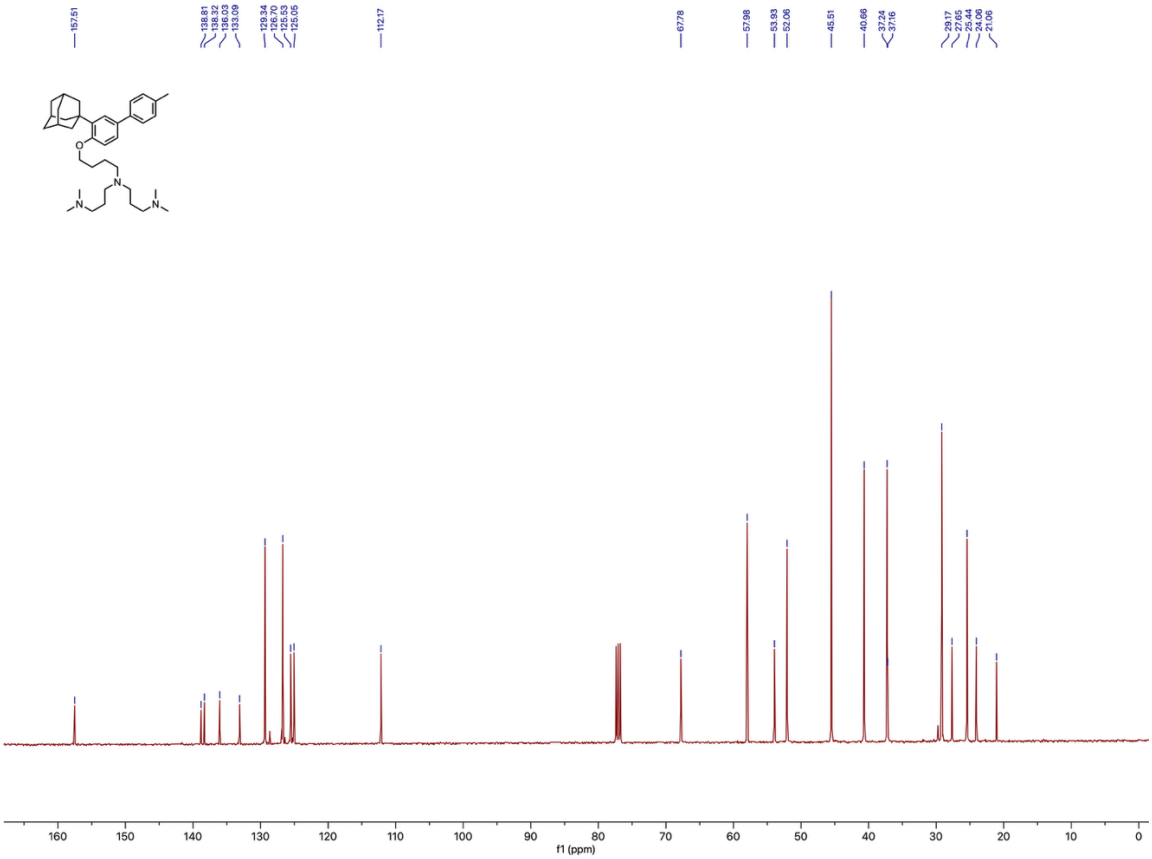
^{13}C-NMR (150 MHz, CH_3OH-d_4) compound **23**.



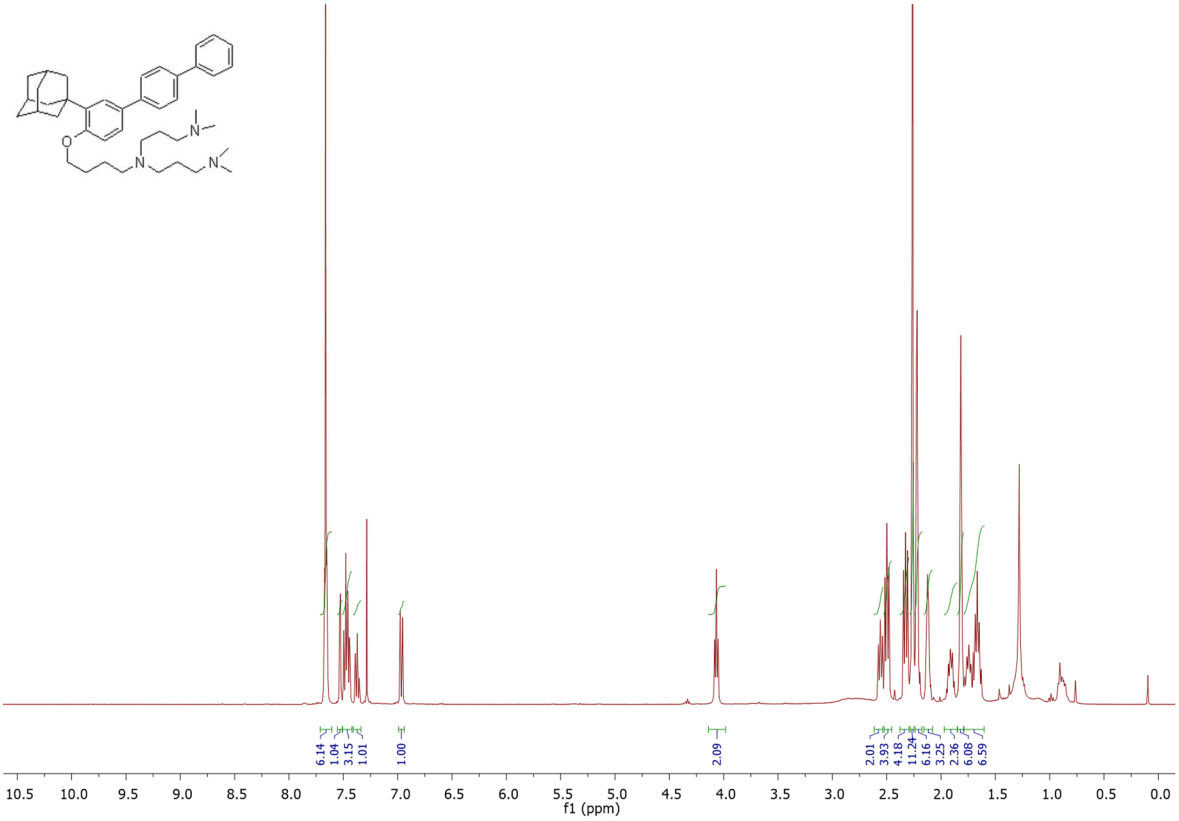
^{1}H-NMR (600 MHz, $CDCl_3$) compound **24**.



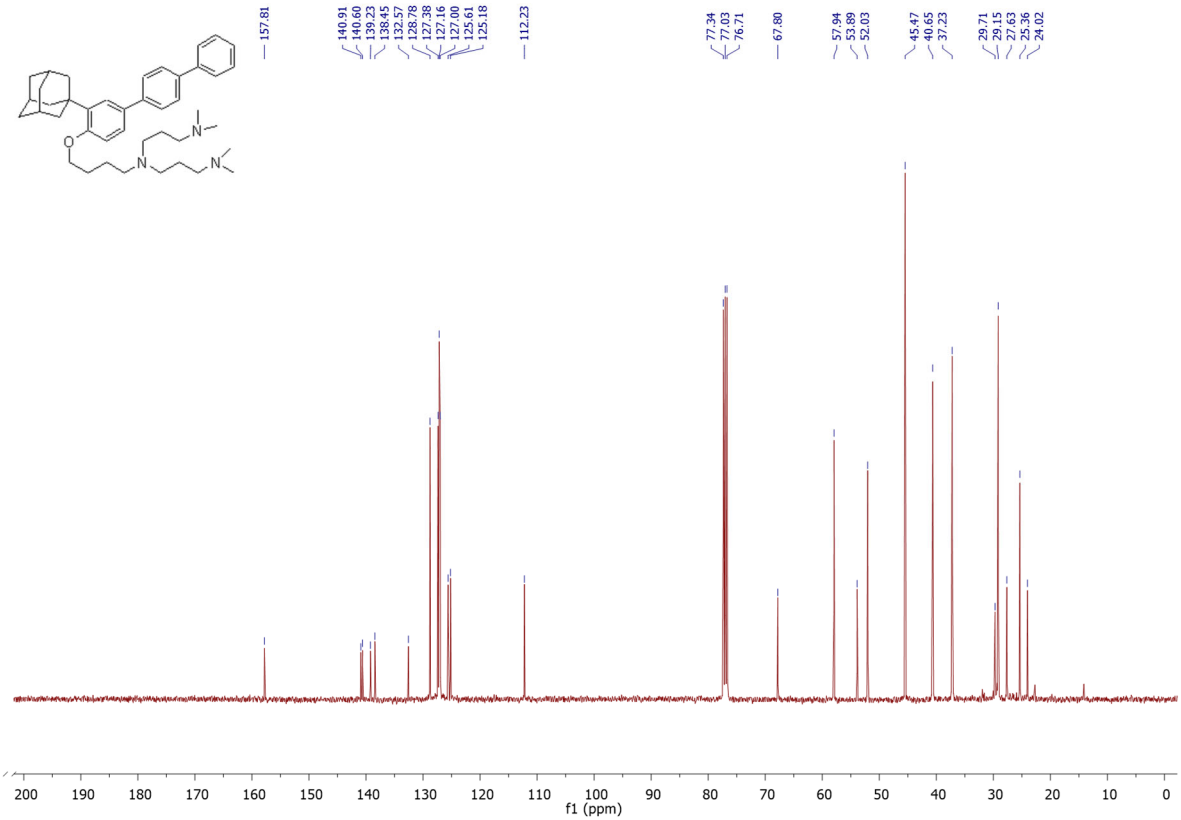
^{13}C-NMR (100 MHz, $CDCl_3$) compound **24**.



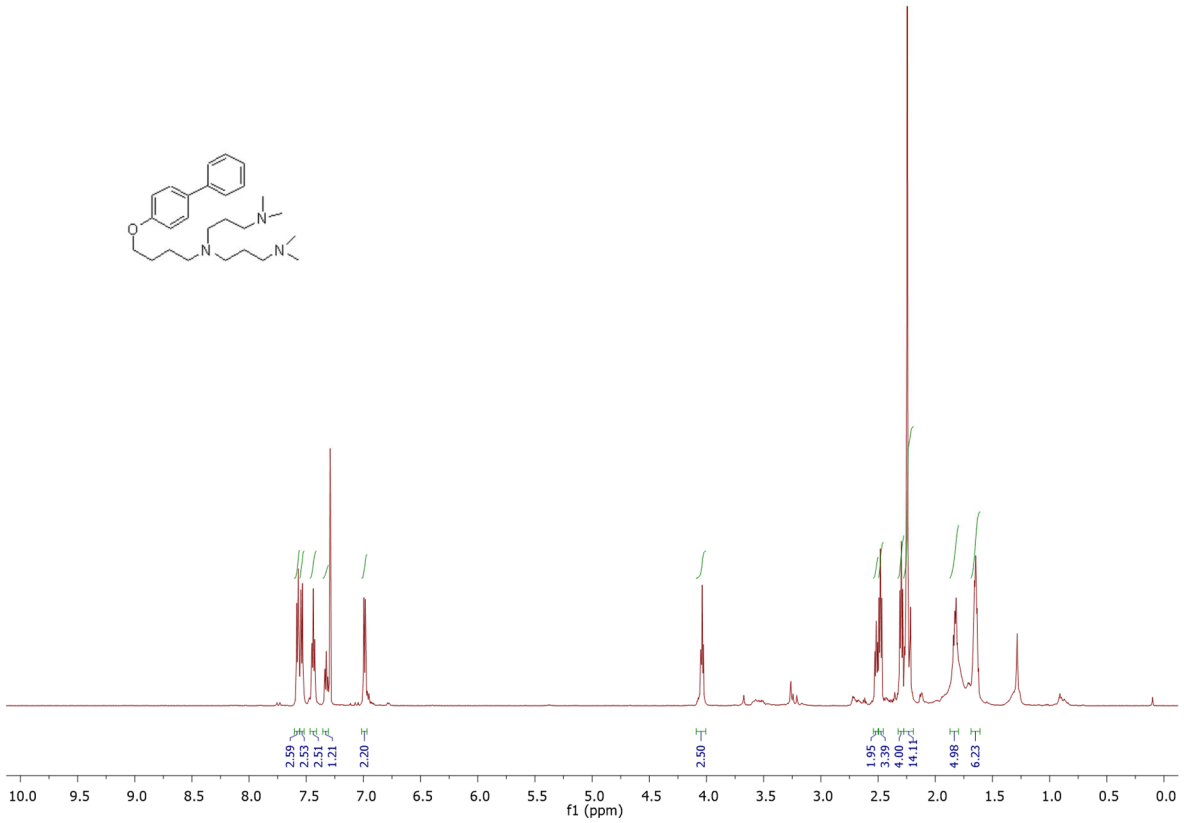
^{1}H-NMR (400 MHz, $CDCl_3$) compound **25**.



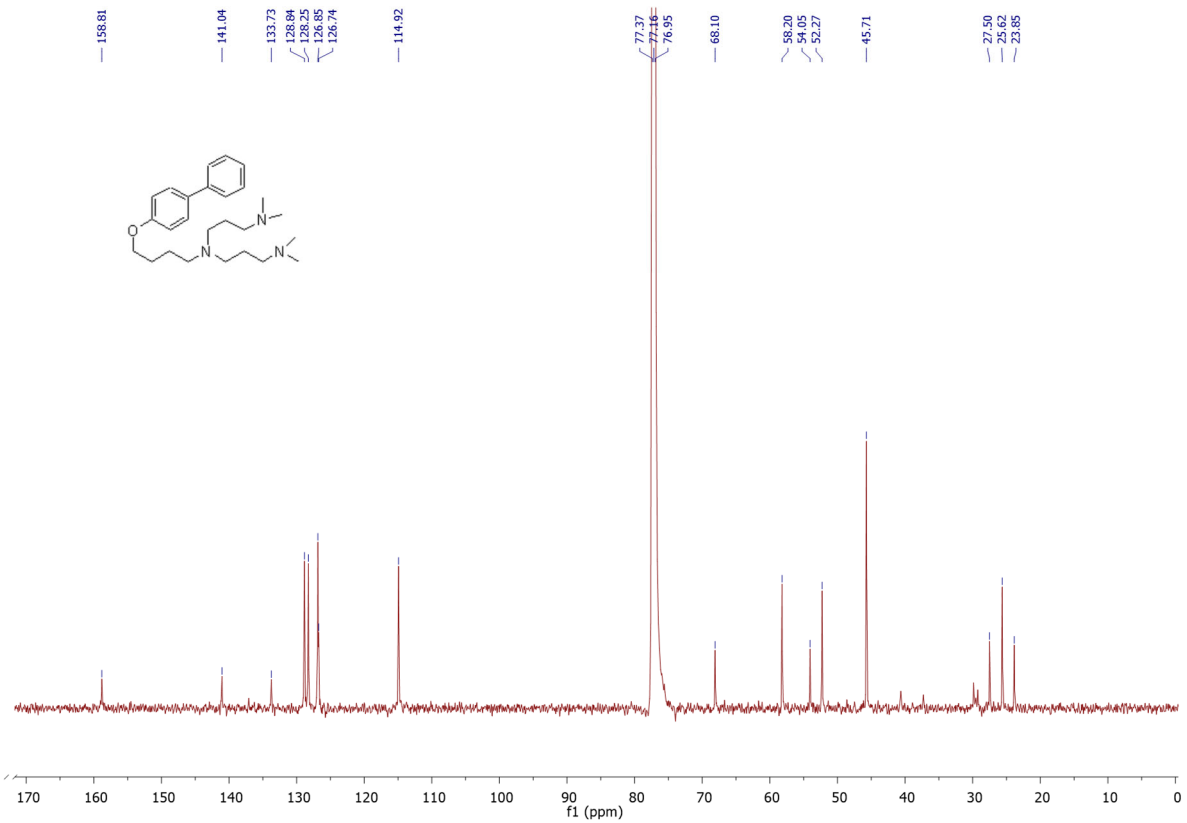
^{13}C-NMR (100 MHz, $CDCl_3$) compound **25**.



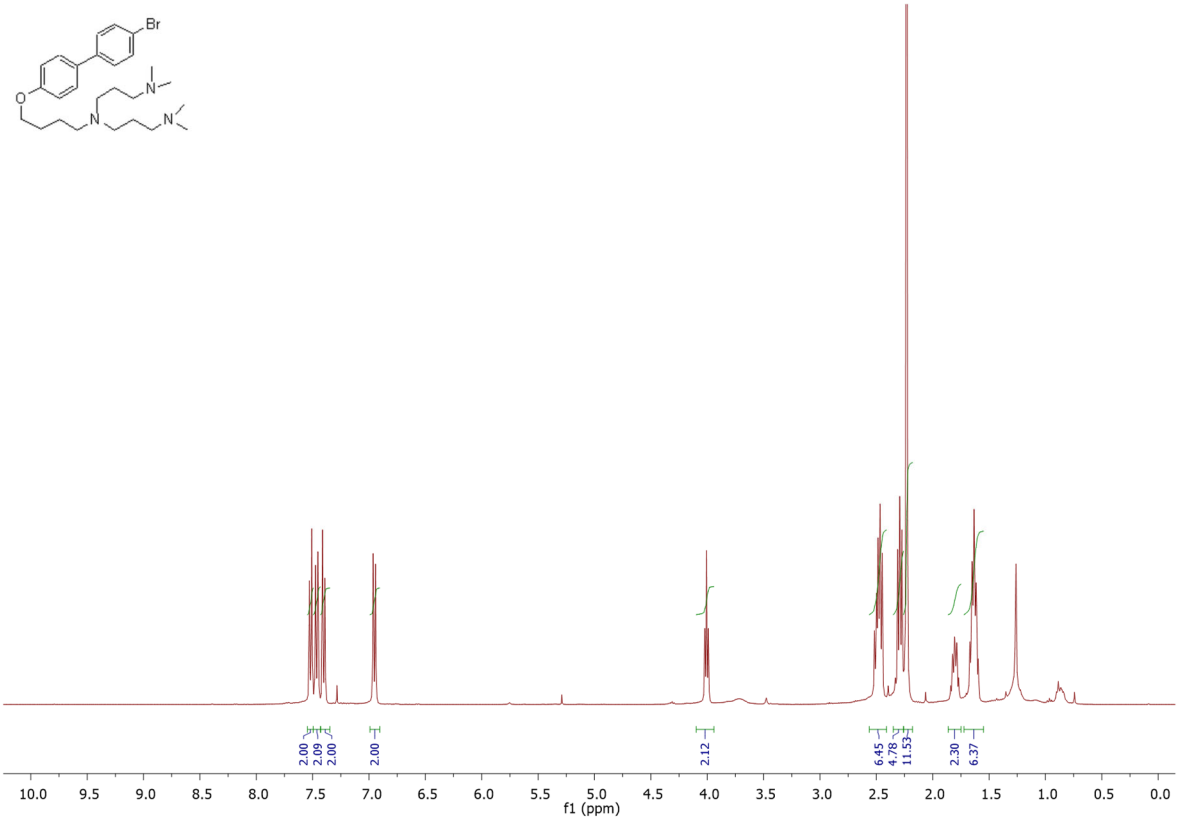
^{1}H-NMR (600 MHz, $CDCl_3$) compound **26**.



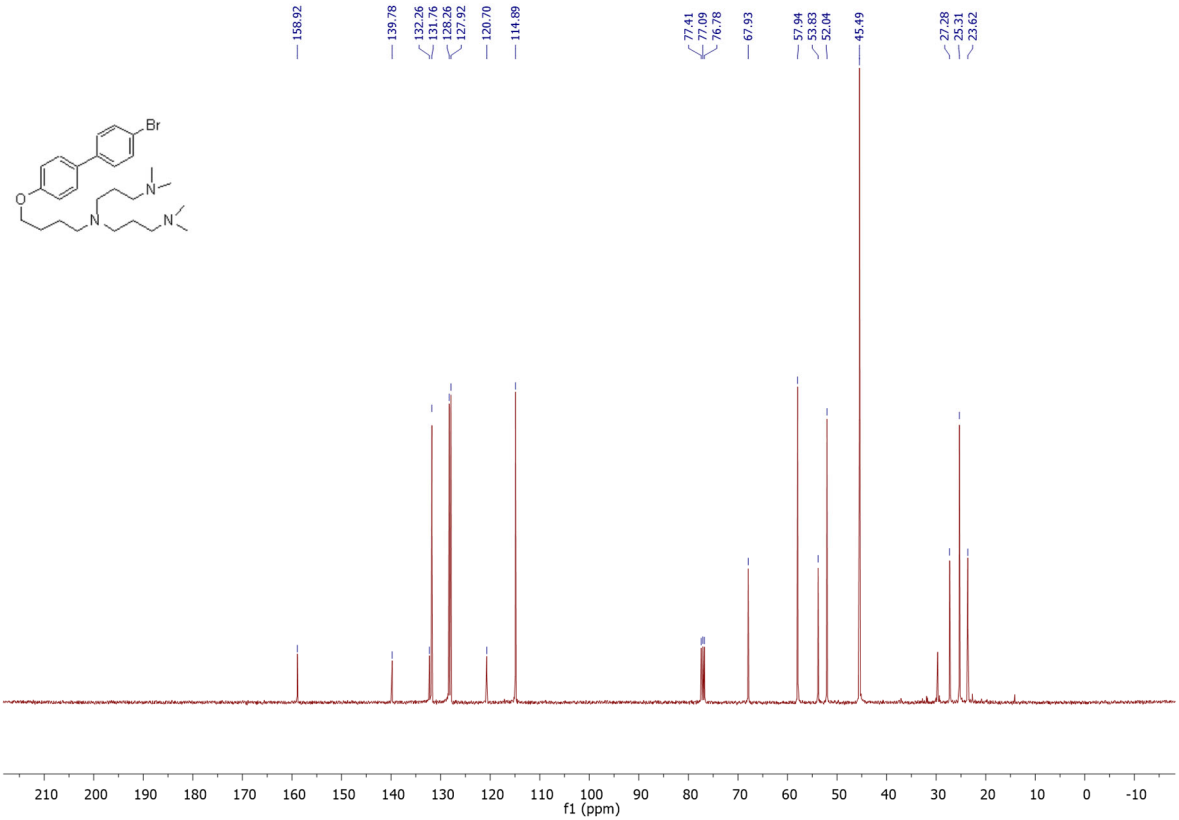
^{13}C-NMR (150 MHz, $CDCl_3$) compound **26**.



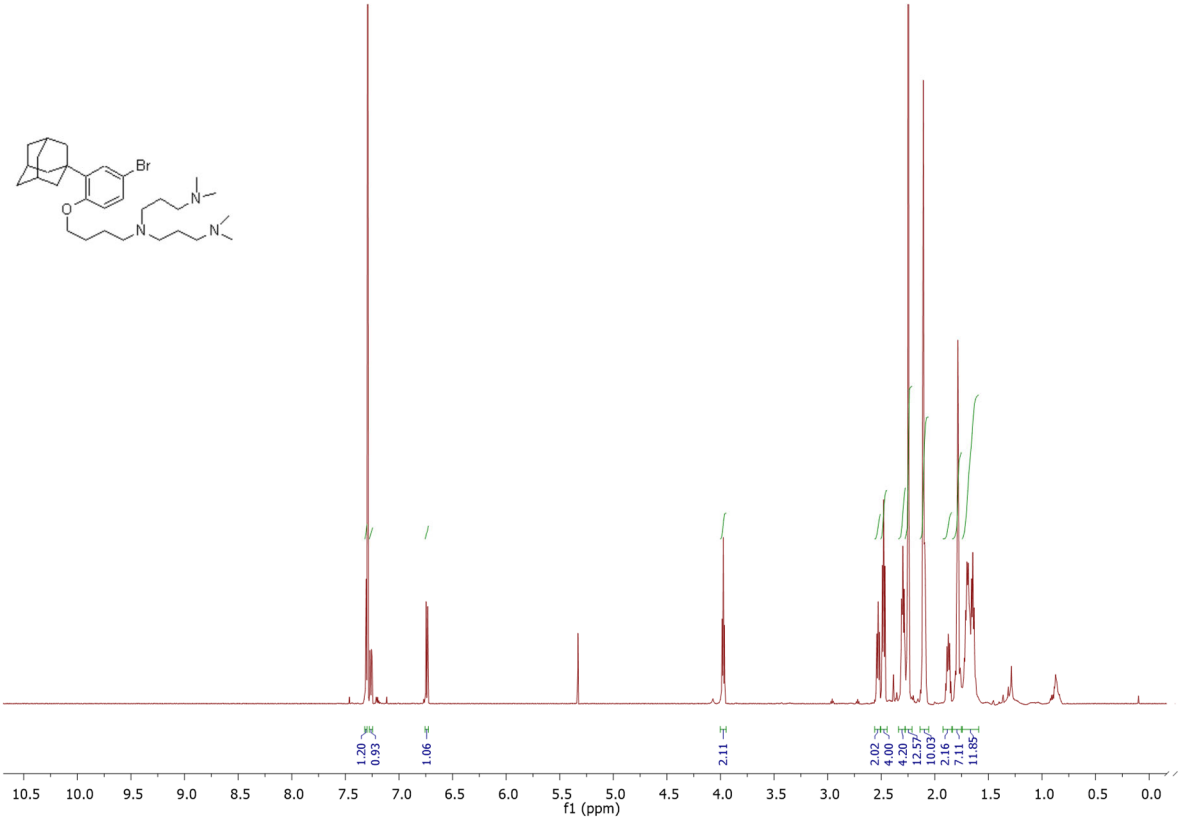
^{1}H-NMR (400 MHz, $CDCl_3$) compound **27**.



^{13}C-NMR (100 MHz, $CDCl_3$) compound **29**.



^{1}H-NMR (600 MHz, $CDCl_3$) compound **28**.



^{13}C-NMR (150 MHz, $CDCl_3$) compound **28**.

