

## Supplementary Materials

### Bicyclic Monoterpenoid-Based Orthopoxvirus Inhibitors: Design, Synthesis, and Biological Assessment of a Novel Amide Series

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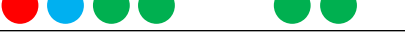













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## 1. Computational details

**Table SM1.** Phase Screen Score values for molecules calculated relative to the reference agent **3b** and the set of pharmacophoric features: A – hydrogen-bond acceptor (red circle), D – hydrogen-bond donor (blue circle), H – hydrophobic feature (green circle), R – aromatic ring (yellow circle). The numbering corresponds to the set of pharmacophores of molecule **3b** (Figure 1).

Structure ID	Phase Screen Score	pIC <sub>50</sub> (VACV)	Matching features of ligands A1.A2.D3.H4.H5.H6.H7.H8.H9.R10
13d	1.700	6.49	
15b	1.971 (RRRS) 1.711 (SSSR)	6.34	
15c	1.836 (RRRS) 1.628 (SSSR)	6.22	
17b	0.221	6.14	
15f	1.397 (SSSR) 1.220 (RRRS)	6.13	
3b	3.000	6.05	
17d	1.322	5.96	
15e	1.715 (RRRS) 1.666 (RRRS)	5.84	
19k	1.939	5.83	
12d	1.451	5.66	
15d	1.786 (RRRS) 1.715 (SSSR)	5.63	
13b	1.721	5.63	
13c	1.605	5.57	
15a	1.701 (RRRS) 1.596 (SSSR)	5.53	
17h	1.673	5.52	

17c	1.508	5.52	
19n	1.978	5.44	
19m	1.926	5.42	
13a	1.719	5.29	
13h	1.626	5.27	
12b	1.461	5.26	
17g	1.611	5.25	
17e	1.322	5.21	
13g	1.518	5.10	
17a	0.238	5.04	
12h	1.381	5.01	
12a	1.460	4.98	
12g	1.005	4.97	
11d	1.636	4.96	
11c	1.583	4.94	
12c	1.413	4.94	
17f	1.192	4.90	
19g	1.943	4.88	
21a	1.557	4.80	
20a	1.568	4.79	
11a	1.670	4.72	
19i	1.965	4.66	

11b	1.670	4.65	
13g	1.429	4.48	
20b	1.437	4.34	
12e	1.402	3.62	
11e	1.597	3.48	
19h	1.814	n/a	
13e	1.615	n/a	
20d	1.606	n/a	
13f	1.606	n/a	
11f	1.594	n/a	
20e	1.565	n/a	
21c	1.565	n/a	
20c	1.565	n/a	
21b	1.450	n/a	
12f	1.406	n/a	
21d	1.369	n/a	

**Table SM2.** Molecular docking results for phospholipase domain.

ID	pIC50 (VACV)	Docking score	$\Delta G_{\text{binds}}$ , kcal/mol	Interaction with residues		Molecular docking protocol
				H-bonds	Other interactions	
3b	6.02	-7.351	-45.74	Ser135, Asn312, Ser140	Gln310 – halogen bond Phe52, Leu118 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation (RMSD = 0.210)
11c	4.78	-6.712	-39.46	Asn133, Asn278	Leu118, Cys120 – hydrophobic with hydrocarbon ligand cage	flexible docking without ligand positional constraints
12a	4.98	-7.001	-41.02	none	Leu118, Cys120 –	positional constraints regarding

						hydrophobic with hydrocarbon ligand cage	the reference ligand with tolerance 2Å RMSD = 1.596	
12b	5.26	-5.868	-43.32		Ser135	Phe52, Leu118, Cys120 – hydrophobic with hydrocarbon ligand cage	positional constraints regarding the reference ligand with tolerance 2Å RMSD = 0.922	
12c	4.94	-5.230	-39.44		Ser135, Asn133, Ser140, Asn55	Arg89 – salt bridge, Cys52 – hydrophobic with aromatic ring	positional constraints regarding the reference ligand with tolerance 2Å RMSD = 0.795	
12d	5.66	-6.173	-43.13		Ser135, Ser140	Ile144 – hydrophobic with – CF <sub>3</sub> group	positional constraints regarding the reference ligand with tolerance 2Å RMSD = 1.717	
12h	5.01	-6.615	-36.78		Asn133, Asn312	Phe52, Leu118, Cys120 – hydrophobic with hydrocarbon ligand cage	flexible docking without ligand positional constraints	
13a	5.29	-6.386	-40.06		none	Ala134 – hydrophobic with hydrocarbon ligand cage		
13c	5.57	-5.196	-43.17		Ser135	Phe52, Leu239, Cys120, Leu118 – hydrophobic with hydrocarbon ligand cage	positional constraints regarding the reference ligand with tolerance 2Å RMSD = 0.768	
13d	6.49	-6.207	-47.50		Ser135	Phe52, Leu239, Cys120, Leu118 – hydrophobic with hydrocarbon ligand cage	positional constraints regarding the reference ligand with tolerance 2Å RMSD = 0.913	
13g	5.10	-6.567	-40.07		Ser327	none	flexible docking without ligand positional constraints	
13h	5.27	-6.055	-41.17		Ser135	Cys53, Phe52, Leu239 – hydrophobic with hydrocarbon ligand cage	positional constraints regarding the reference ligand with tolerance 2Å RMSD = 1.605	
15a	RRRS	5.42	-7.111	-41.36	-41.36 Median values	Ser135	Ile144 – hydrophobic with chlorine atoms	positional constraints regarding the reference ligand with tolerance 2Å RMSD = 0.945
	RRRS		-6.159	-36.23		Ser135	Ile144 – hydrophobic with chlorine atoms	RMSD = 0.503

	RSSS		-6.159	-41.55		Ser135	Ile144 – hydrophobic with chlorine atoms Cys120, Phe52, Leu118, Leu239 – hydrophobic with hydrocarbon ligand cage	RMSD = 0.867
	RSSS		-6.550	-41.69		Ser135	Ile144 – hydrophobic with chlorine atoms Cys120, Leu118, Leu239 – hydrophobic with hydrocarbon ligand cage	RMSD = 0.282
15b	RRRS	5.92	-6.558	-42.84	-43.21	none	Tyr335, Leu118, Cys120, Phe52 – hydrophobic with aromatic ring and bromine atom	flexible docking without ligand positional constraints
	SSSR		-6.724	-43.58		Ser135	Cys120, Phe52 Leu118, Leu239 – hydrophobic with hydrocarbon ligand cage	
15d	SSSR	5.32	-6.983	-41.11	-43.02	Ser135	Cys120, Phe52 Leu118, Leu239 – hydrophobic with hydrocarbon ligand cage	flexible docking without ligand positional constraints
	RRRS		-6.468	-44.93		Ser135	Cys120, Phe52 Leu118, Leu239 – hydrophobic with hydrocarbon ligand cage	
15e	SSSR	5.34	-6.671	-38.27	-39.58	Ser135	Leu118, Leu239 – hydrophobic with hydrocarbon ligand cage	flexible docking without ligand positional constraints
	RRRS		-7.849	-40.88		Ser135, Ser140	Cys120, Phe52 Leu118, Leu239 – hydrophobic with hydrocarbon ligand cage	
15f	SSSR	5.73	-7.812	-41.57	-41.73	Ser135	Cys120, Phe52 Leu118, Leu239 – hydrophobic with hydrocarbon ligand cage	flexible docking without ligand positional constraints
	RRRS		-7.845	-41.90		Ser135	Trp279, Ile144 – hydrophobic with cyclohexane fragment	
20b		4.83	-5,754	-36.38	Ser327, Asn133	Cys120, Phe52 Leu118, Leu239 – hydrophobic with hydrocarbon ligand cage	positional constraints regarding the reference ligand with tolerance 2Å RMSD = 1.453	
ID	pIC50 (VACV)	Docking score	$\Delta G_{\text{bind}}$ , kcal/mol	Interaction with residues				Molecular docking protocol
				H-bonds		Other interactions		

3b	6.02	-10.793	-80.19	Ser292	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
11c	4.78	-10.069	-63.17	Tyr258	pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
12a	4.98	-9.822	-69.51	Tyr258, Ser292	pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
12b	5.26	-9.306	-54.02	Tyr258	pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
12c	4.94	-10.674	-67.24	Tyr258	pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
12d	5.66	-11.368	-56.17	Tyr258	pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
12h	5.01	-10.605	-53.58	Tyr258	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
13a	5.29	-9.719	-61.96	Tyr258	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	
13c	5.57	-9.561	-58.73	Tyr258	pi-pi stacking Tyr258,	Redocking using representative

						Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
13d	6.49	-11.065	-63.73			Tyr258 pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
13g	5.10	-9.054	-57.00			Tyr258 Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
13h	5.27	-10.437	-55.85			Tyr258 Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
15a	RRRS	5.42	-9.795	-64.87	-65.41	Ser292 Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
	RSSS		-10.178	-65.95			
15b	RRRS	5.92	-9.977	-69.44	-66.65	Tyr258, Ser292 Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
	SSSR		-9.403	-63.85			
15d	SSSR	5.32	-11.373	-69.57	-67.69	Tyr258 Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with	Redocking using representative frame of MD simulation, positional constraints regarding

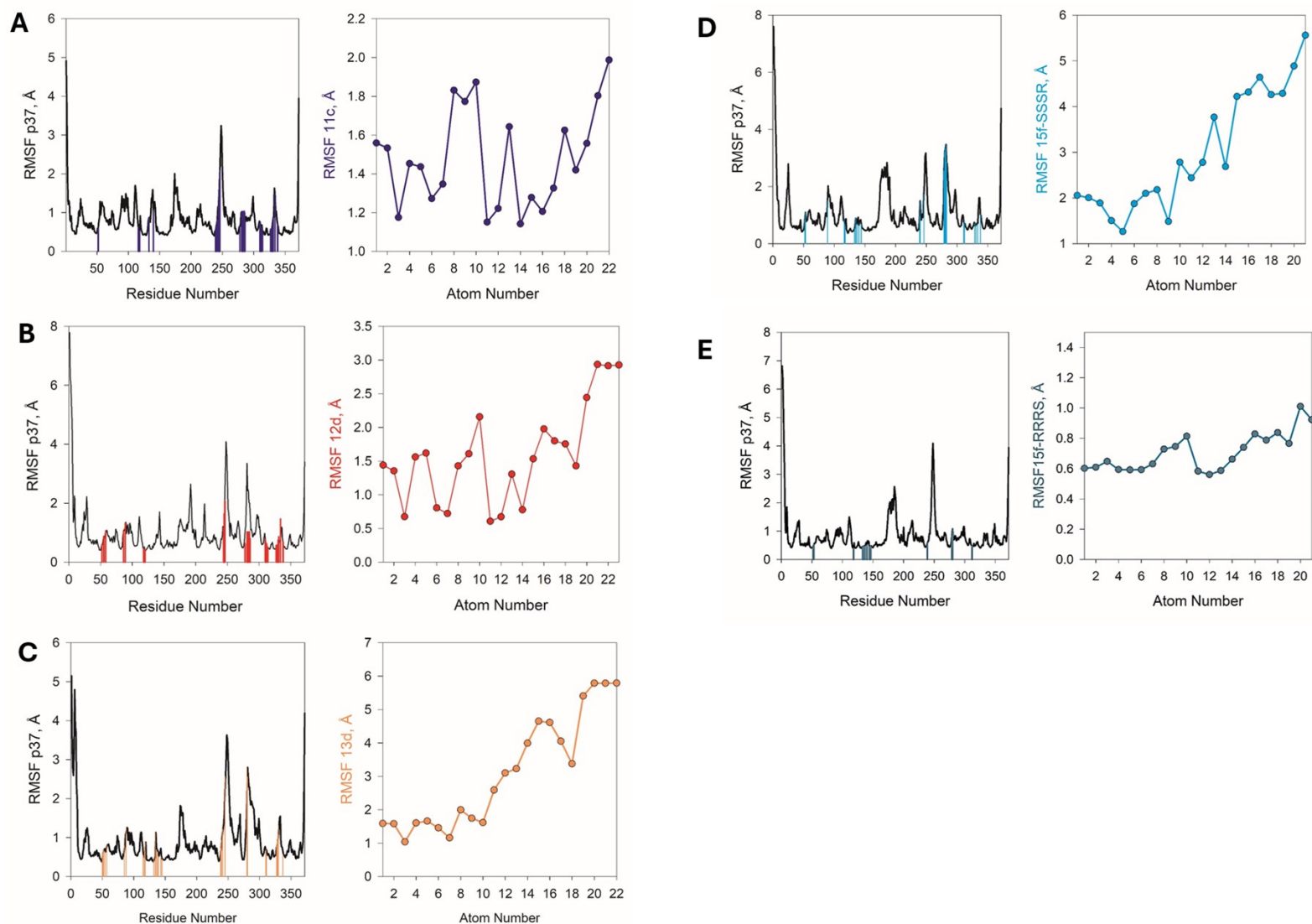
	RRRS		-11.386	-65.81		Tyr258, Ser292	hydrocarbon ligand cage Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	the reference ligand with tolerance 5Å
15e	SSSR	5.34	-9.638	-68.10	-65.24	Tyr258, Ser292	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
	RRRS		-8.916	-62.38		Tyr258, Ser292	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	
15f	SSSR	5.73	-9.972	-57.55	-64.24	Tyr258	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
	RRRS		-9.548	-70.93		Tyr258, Ser292	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	
20b		4.83	-9.457	-49.27		Tyr258	halogen bond with Tyr289, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å

**Table SM3.** Molecular docking results for homodimer.

ID	pIC <sub>50</sub> (VACV)	Docking score	$\Delta G_{\text{bind}}$ , kcal/mol	Interaction with residues		Molecular docking protocol
				H-bonds	Other interactions	
3b	6.02	-10.793	-80.19	Ser292	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
11c	4.78	-10.069	-63.17	Tyr258	pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
12a	4.98	-9.822	-69.51	Tyr258, Ser292	pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
12b	5.26	-9.306	-54.02	Tyr258	pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
12c	4.94	-10.674	-67.24	Tyr258	pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
12d	5.66	-11.368	-56.17	Tyr258	pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
12h	5.01	-10.605	-53.58	Tyr258	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with

13a		5.29	-9.719		-61.96	Tyr258	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	tolerance 5Å
13c		5.57	-9.561		-58.73	Tyr258	pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
13d		6.49	-11.065		-63.73	Tyr258	pi-pi stacking Tyr258, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
13g		5.10	-9.054		-57.00	Tyr258	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
13h		5.27	-10.437		-55.85	Tyr258	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
15a	RRRS	5.42	-9.795		-64.87	Ser292	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
	RSSS		-10.178		-65.95	Tyr258	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
15b	RRRS	5.92	-9.977		-69.44	Tyr258, Ser292	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
	SSSR		-9.403		-63.85	Tyr258, Ser292	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 –	

							hydrophobic with hydrocarbon ligand cage	
15d	SSSR	5.32	-11.373	-69.57	-67.69	Tyr258	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
	RRRS		-11.386	-65.81		Tyr258, Ser292	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	
15e	SSSR	5.34	-9.638	-68.10	-65.24	Tyr258, Ser292	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
	RRRS		-8.916	-62.38		Tyr258, Ser292	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	
15f	SSSR	5.73	-9.972	-57.55	-64.24	Tyr258	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å
	RRRS		-9.548	-70.93		Tyr258, Ser292	Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	
20b		4.83	-9.457		-49.27	Tyr258	halogen bond with Tyr289, Ala288, Tyr285, Tyr253, Leu296, Ala295, Leu293 – hydrophobic with hydrocarbon ligand cage	Redocking using representative frame of MD simulation, positional constraints regarding the reference ligand with tolerance 5Å



**Figure SM1.** MD trajectory analysis of the studied complexes: A – p37-11c, B – p37-12d, C – p37-13d, D – 15f-SSSR, and E – 15f-RRRS. The first plot in each panel shows the root mean square fluctuation (RMSF) of C $\alpha$  atoms, characterizing local flexibility along the protein chain. Protein residues that interact with the ligand are marked with colored vertical bars. The second plot of each panel represents the Ligand Root Mean Square Fluctuation (RMSF), characterizing changes in the ligand atom positions

## 2. HPLC-MS/MS conditions

The chromatographic system used in the study consisted of a Shimadzu LC-20AD Prominence chromatograph equipped with a cooled autosampler, binary gradient pump and column oven thermostated at 40°C. SCIEX 3200MD QTRAP mass spectrometer (SCIEX, USA) was used as a detector. Chromatographic separations were performed on a ProntoSil 120-5 C18 AQ column (2×75 mm, 5 μm, EcoNova, Novosibirsk, Russia) using mobile phase consisting of water acidified with 0.1% formic (v/v) as eluent A and methanol containing 0.1% formic acid (v/v) as eluent B. The gradient was as follows: 0 min – 3% B; 0.5 min – 3% B; 2 min – 95% B, 5.5 min – 95% B, the flow rate was 250 μL/min; the injection volume was 10 μL. After a chromatographic analysis, the column was equilibrated for an additional 3 min step.

The MS/MS detection was performed in MRM mode using positive electrospray ionization. Following mass spectrometer settings were applied: curtain gas (CUR) = 30 psi, collision-activated dissociation gas (CAD) = medium, ion source voltage (IS) = 5500 V, temperature (TEM) = 350°C, sprayer gas (GS1) = 25 psi, evaporator gas (GS2) = 25 psi, dwell time was 100 ms. Other parameters for the detection of compound **13d** and IS are given in Table SM4. The instruments were controlled and the data collected using Analyst 1.6.3 software (SCIEX), data processing was performed using MultiQuant 3.0.3 software (SCIEX).

**Table SM4.** Parameters for mass spectrometry detection of the compound **13d** and internal standard

Analyte and its parent ion m/z, Da (Q1)	Fragment ion m/z, Da (Q3)	CE, V <sup>C</sup>	DP, V	EP, V	CXP, V
<b>13d</b> (312.2)	173.1	31	66	10.5	4
	81.0	41	66	10.5	4
	79.1	67	66	10.5	4
2-Ad (152.2, IS)	107.2	37	21	3	8
	93.1	35	16	3	14

## 3. Whole blood sample preparation for quantification of compound **13d**

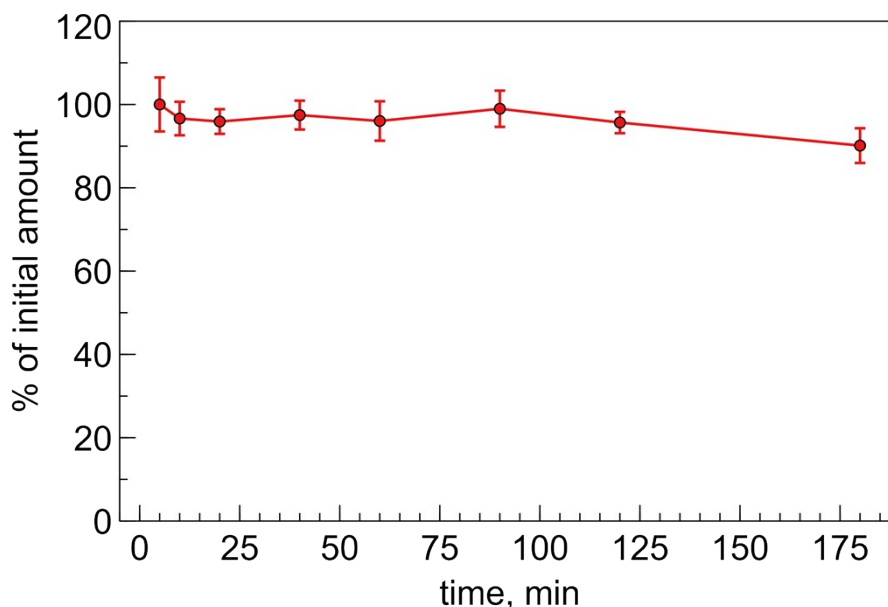
A mixture of methanol and 0.2 M zinc sulfate in water (8:2, v/v) containing 1000 ng/mL of 2-Ad as an internal standard (IS) was used as the protein precipitation solution. For sample processing, a 10 μL aliquot of the blood sample was added to 150 μL of the precipitation solution. The mixture was then vortexed for 30 seconds and incubated for 20 minutes at ambient temperature. Following

incubation, the sample was vortexed again for 30 seconds and centrifuged at 13,400g for 10 minutes (Eppendorf Centrifuge 5424). Finally, 140  $\mu\text{L}$  of the supernatant was transferred to an insert in a vial for subsequent analysis.

#### 4. Stability study of compound **13d** in whole murine blood

33  $\mu\text{L}$  aliquot of a methanolic solution of the compound (1000 ng/mL) was added to 297  $\mu\text{L}$  of murine whole blood, yielding a final concentration of 100 ng/mL. Aliquots (10  $\mu\text{L}$ ) of the spiked blood were taken 5, 10, 20, 40, 60, 90, 120, and 180 minutes after sample preparation, three samples being taken at each time point.

The samples were analyzed, and the peak areas on the chromatograms were assessed for compound **13d**. The value obtained for the sample taken at the first time point (5 min) was set at 100%. The relative amount of the compound remaining in blood was calculated as the percentage ratio of the peak area at a given time point to the initial peak area (at 5 min).



**Figure SM2.** Kinetic dependence of the compound **13d** concentration in blood.

#### 5. Stock solutions and calibration solutions

A stock solution of compound **13d** as prepared by dissolving a weighed amount of the substance in methanol to obtain a concentration of 1.0 mg/mL. This stock solution was subsequently diluted in methanol to prepare a series of working solutions with concentrations of 50, 100, 500, 1000, 5000, 10000 and 50000 ng/mL. For spiking, 33  $\mu\text{L}$  of each working solution was mixed with 297  $\mu\text{L}$  of whole murine blood stabilized with EDTA, resulting in spiked blood samples with final concentrations of 5, 10, 50, 100, 500, 1000, and 5000 ng/mL. The obtained calibrators were then processed as described above.

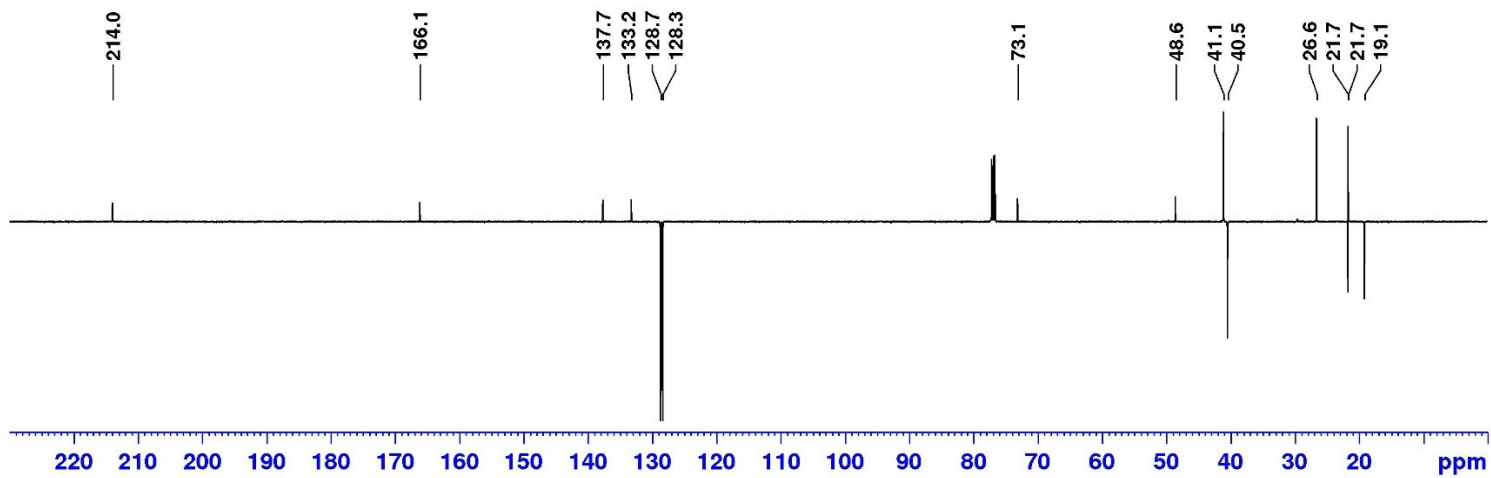
## **6. Experiments on animals**

The pharmacokinetics of compound **13d** was studied at a dose of 100 mg/kg following its intragastric administration in the form of a suspension with tween-80. Eight animals participated in the experiment. After administration of the substance, blood (10  $\mu$ L) was taken from the tail vein at 5, 10, 20, 30, 60 min, 2, 3, 4, 5, 6, 8 and 24 h after administration. The sample was mixed with a precipitating solution and processed according to the protocol described above.

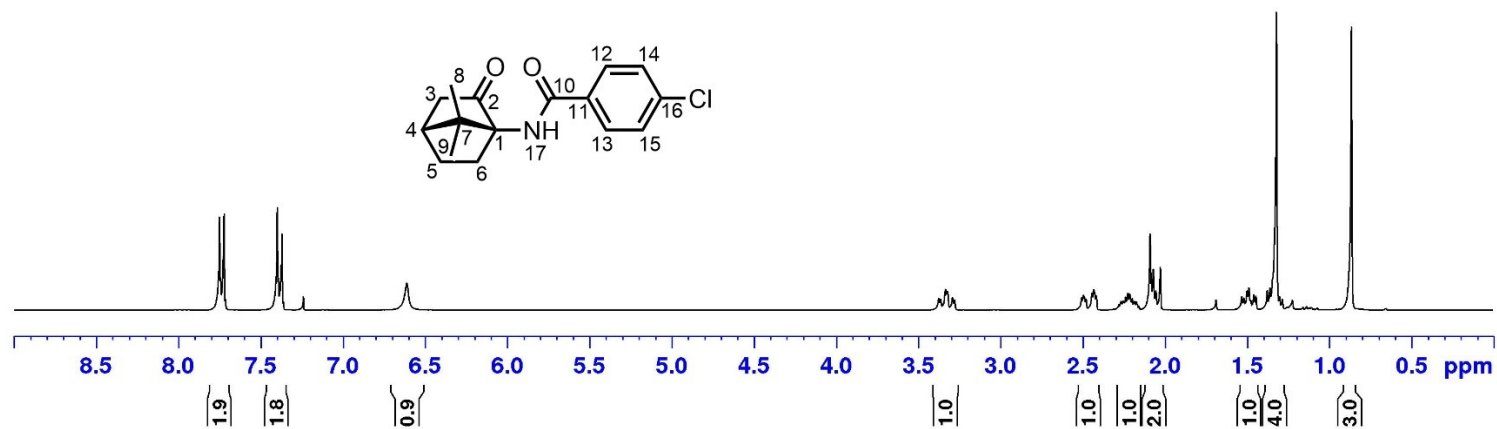
## 7. NMR $^1\text{H}$ and $^{13}\text{C}$ spectra

### Compound 11a

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

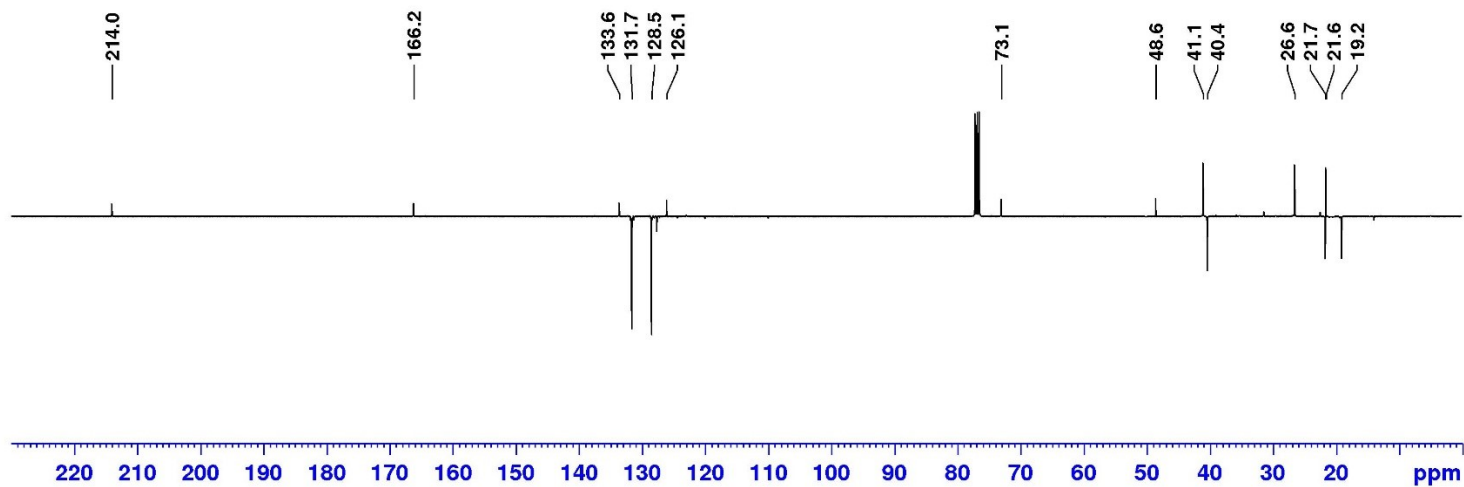


$^1\text{H}$ , 300MHz,  $\text{CDCl}_3$

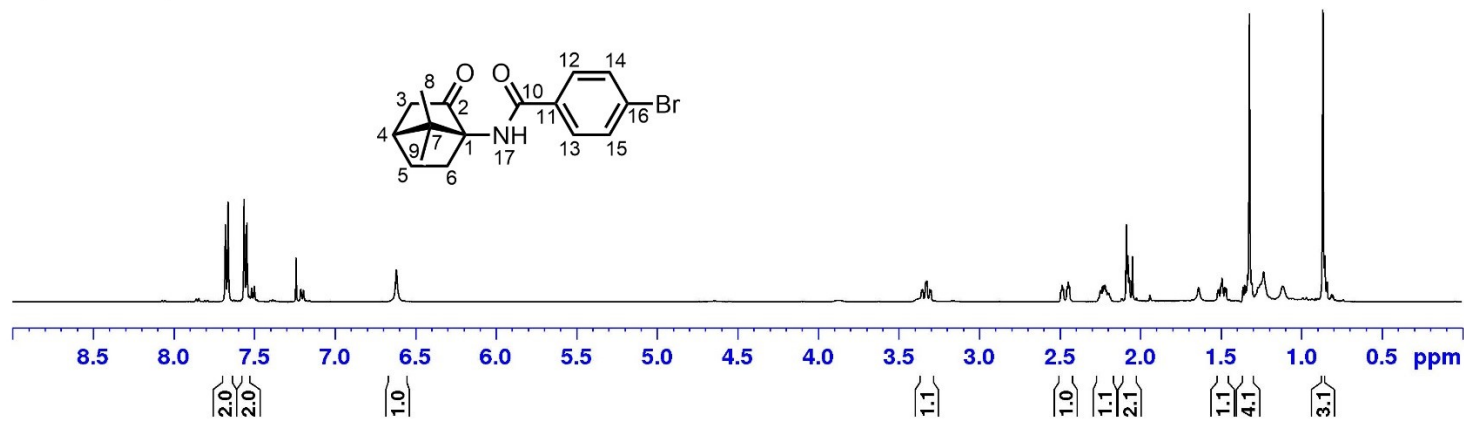


# Compound 11b

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

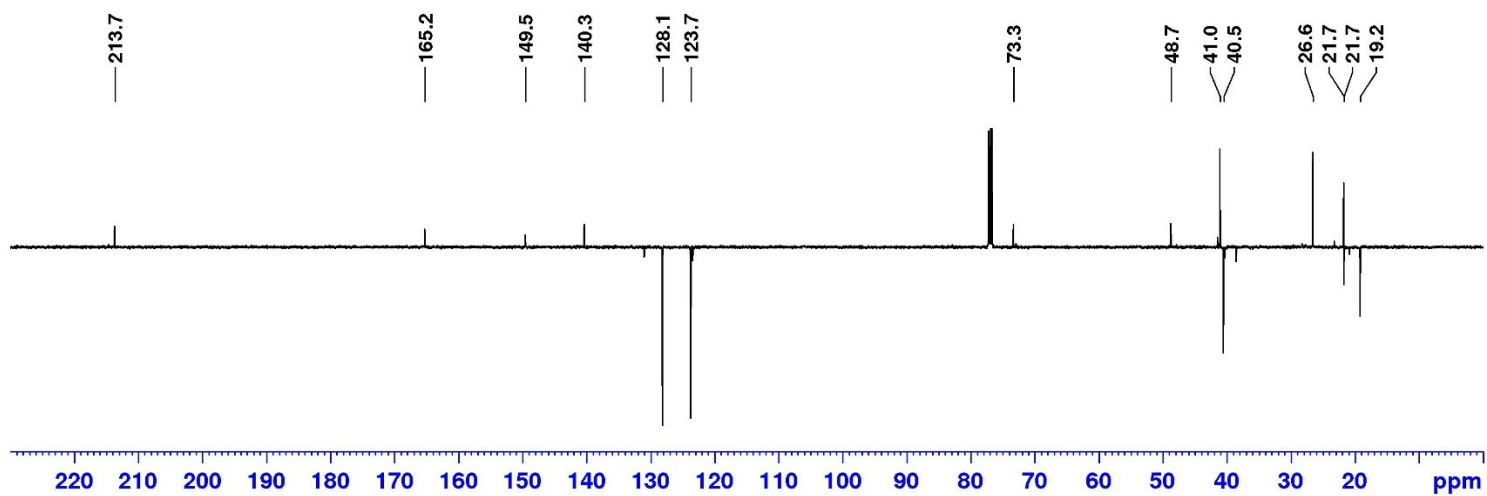


<sup>1</sup>H, 500 MHz, CDCl<sub>3</sub>

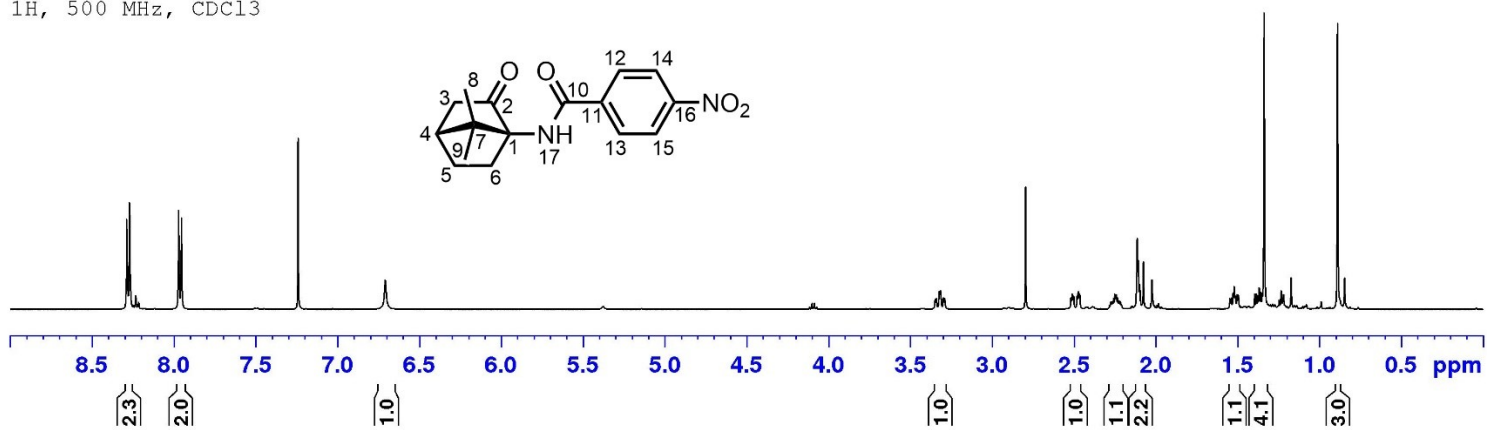


# Compound 11c

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

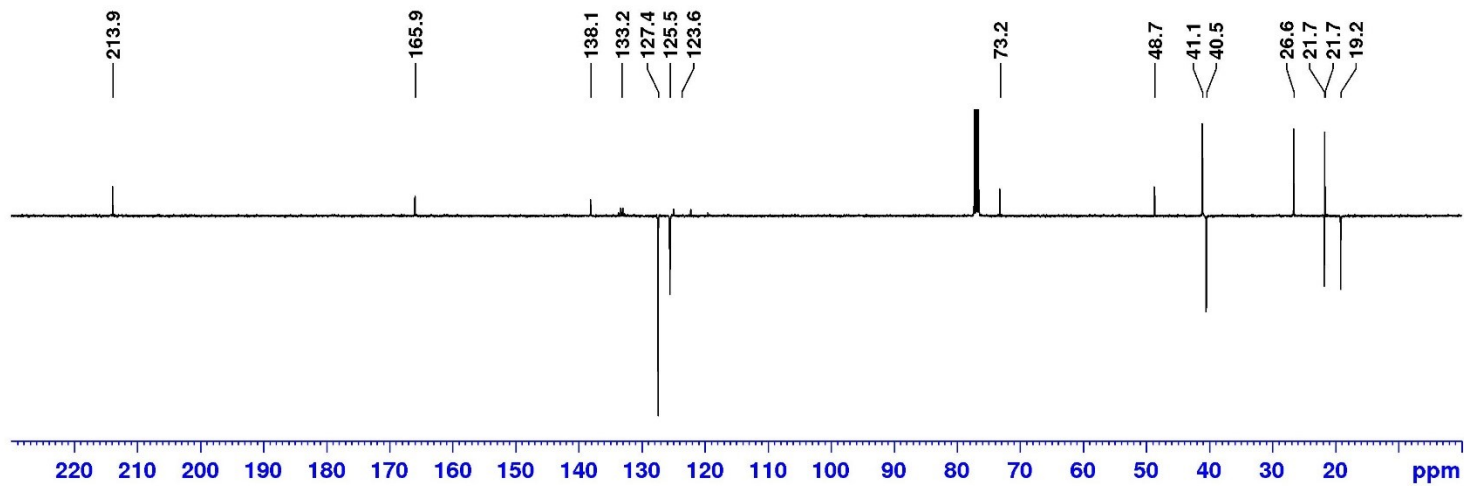


<sup>1</sup>H, 500 MHz, CDCl<sub>3</sub>

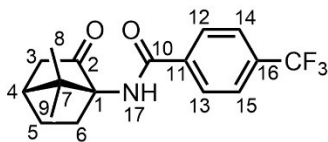
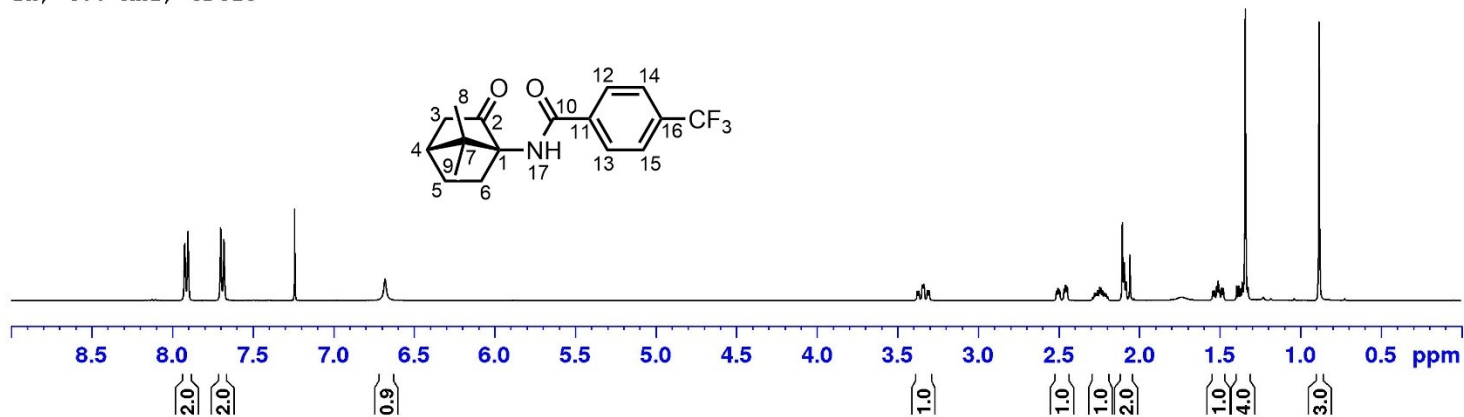


# Compound 11d

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

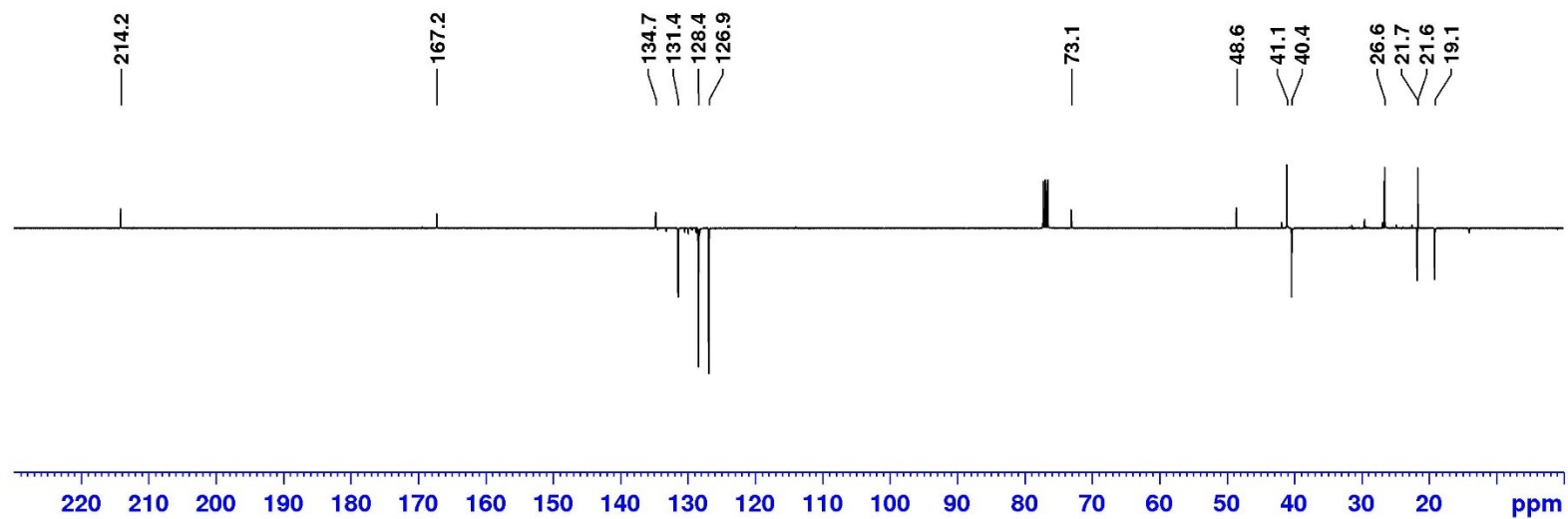


<sup>1</sup>H, 400 MHz, CDCl<sub>3</sub>

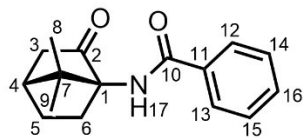
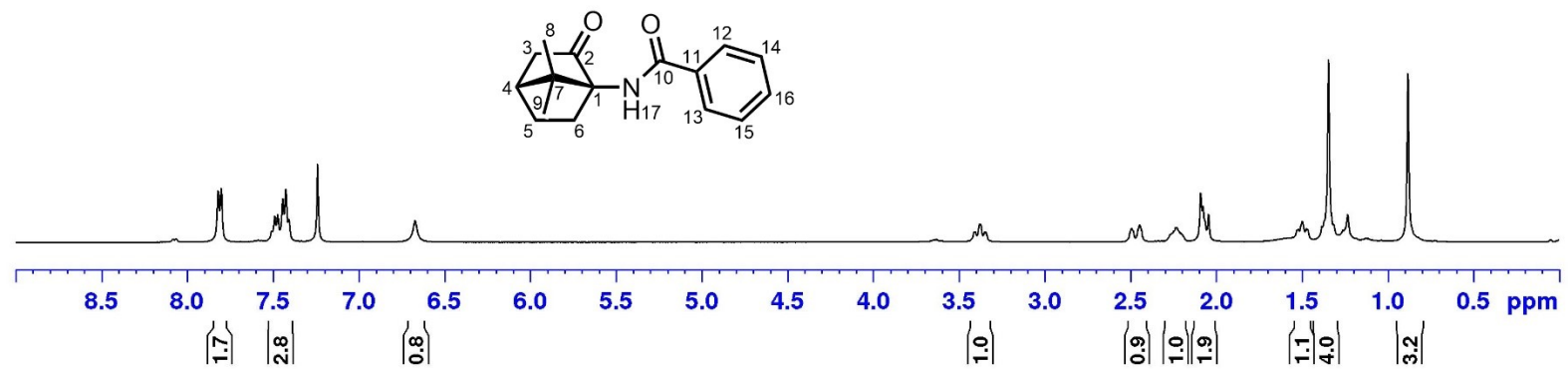


# Compound 11e

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

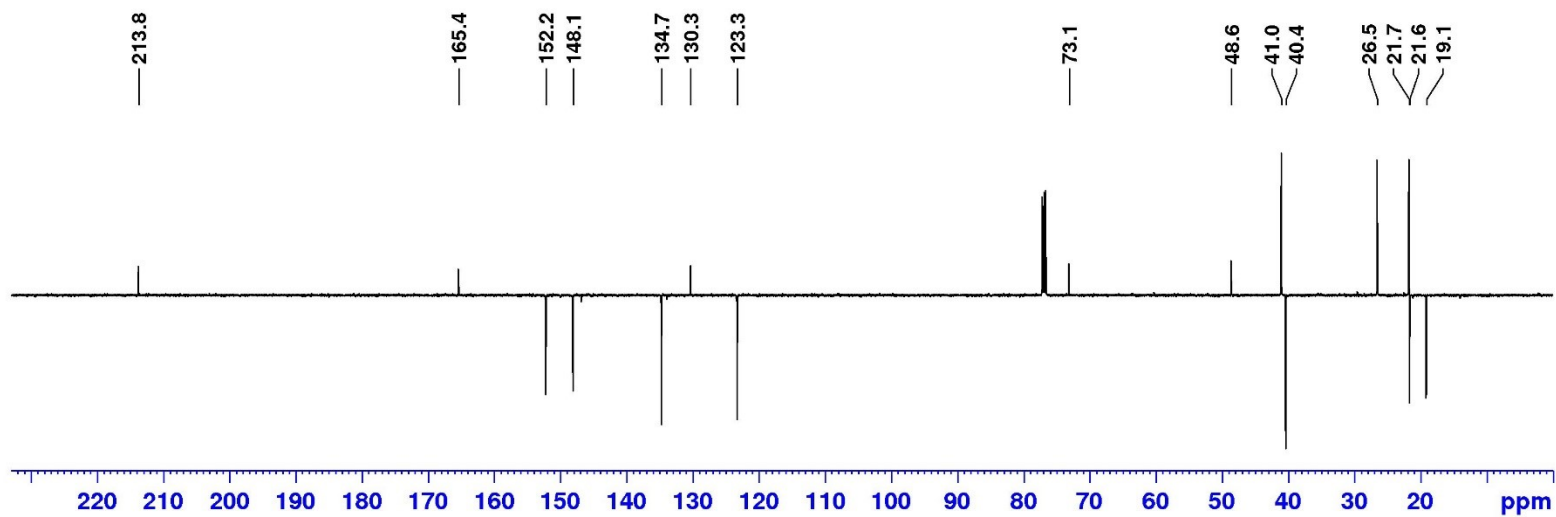


<sup>1</sup>H, 400MHz, CDCl<sub>3</sub>

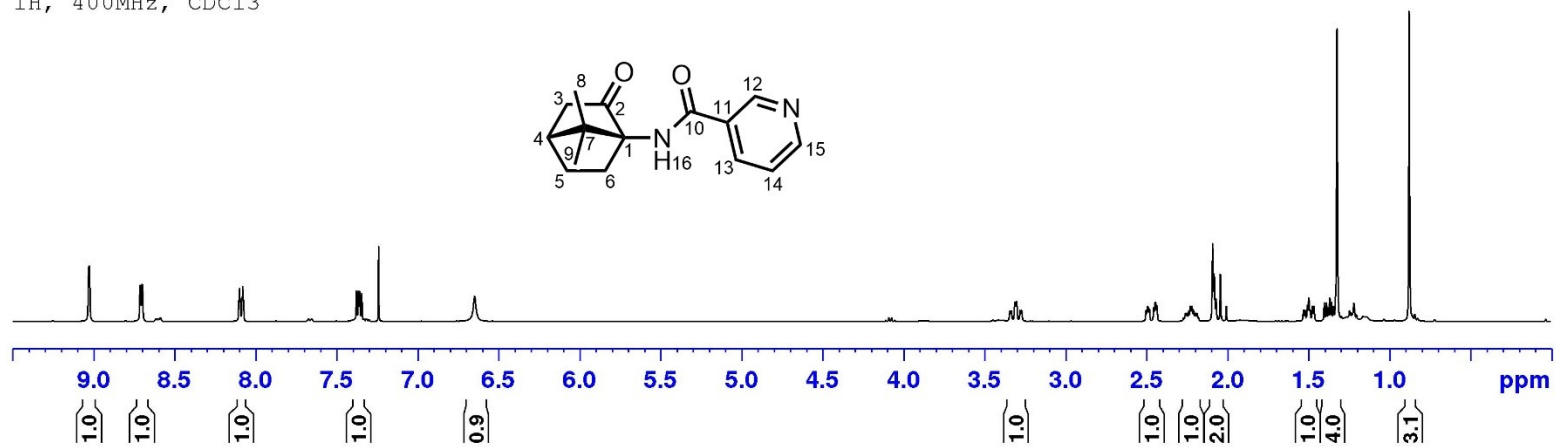


# Compound 11f

<sup>13</sup>C, JMOD, CDC13

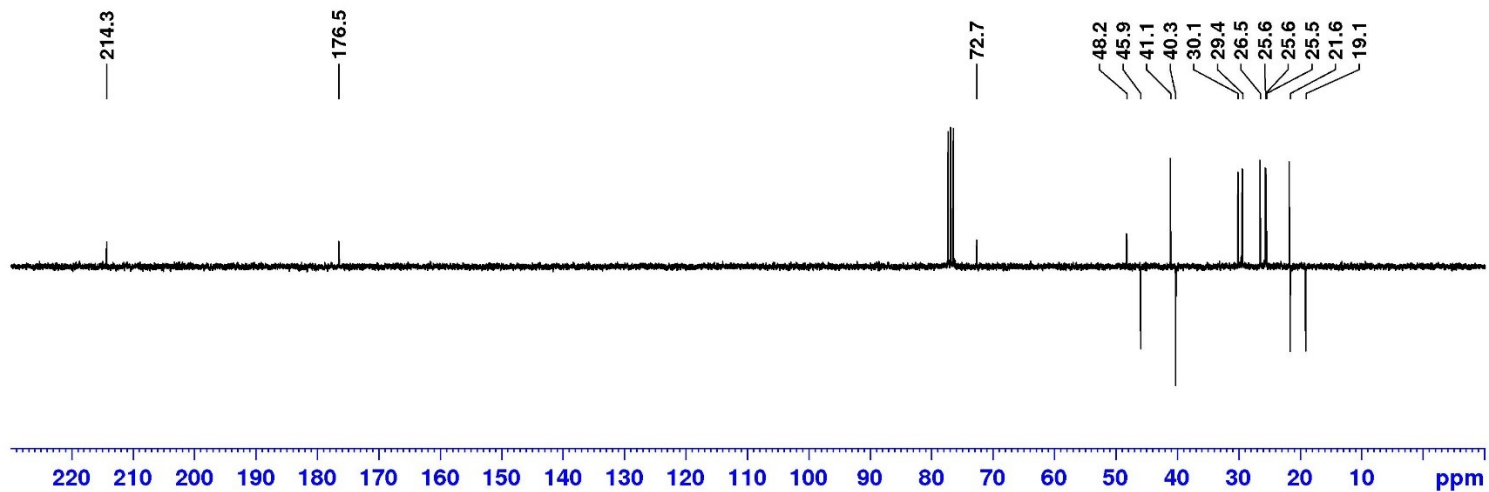


<sup>1</sup>H, 400MHz, CDC13

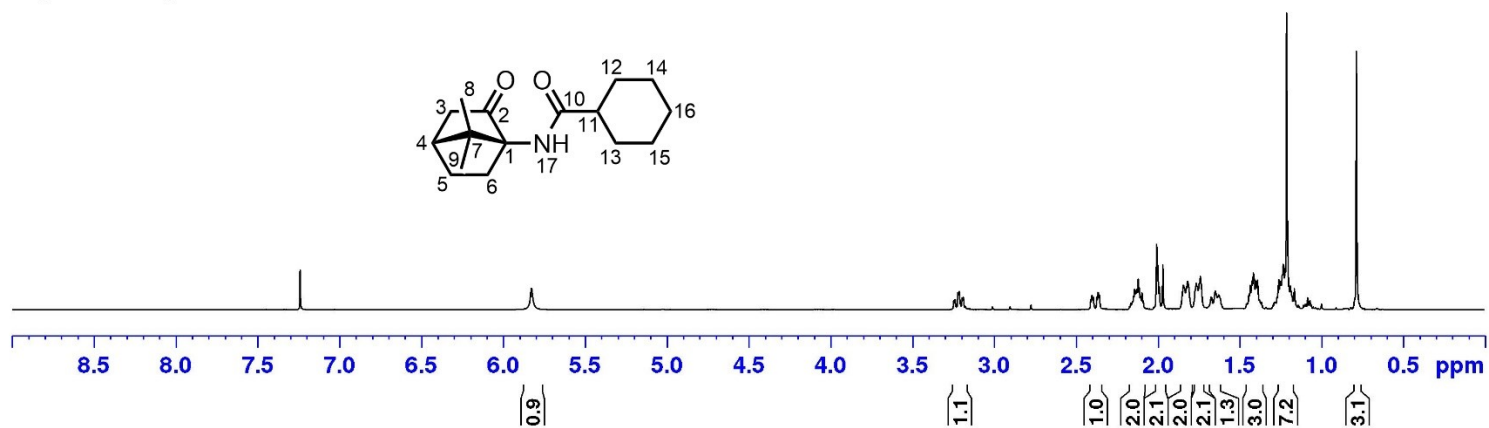


# Compound 11g

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

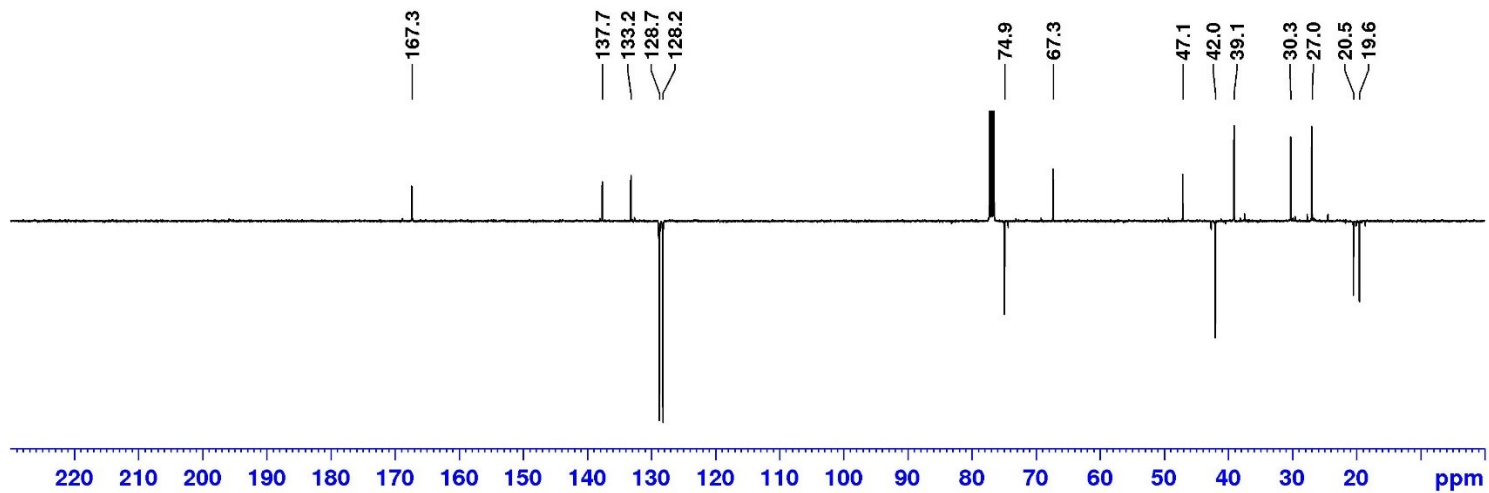


$^1\text{H}$ , 500 MHz,  $\text{CDCl}_3$

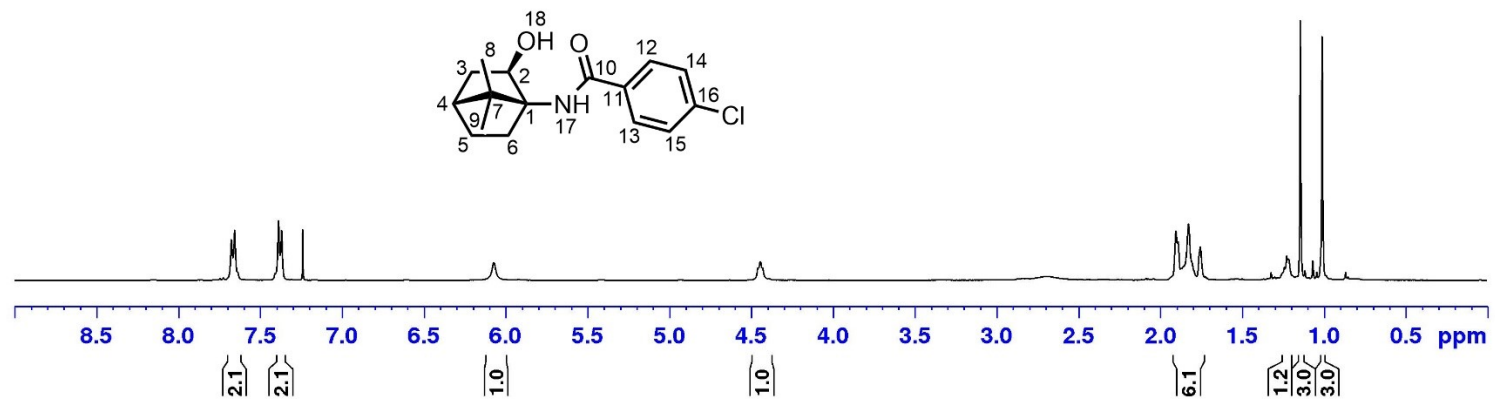


# Compound 12a

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

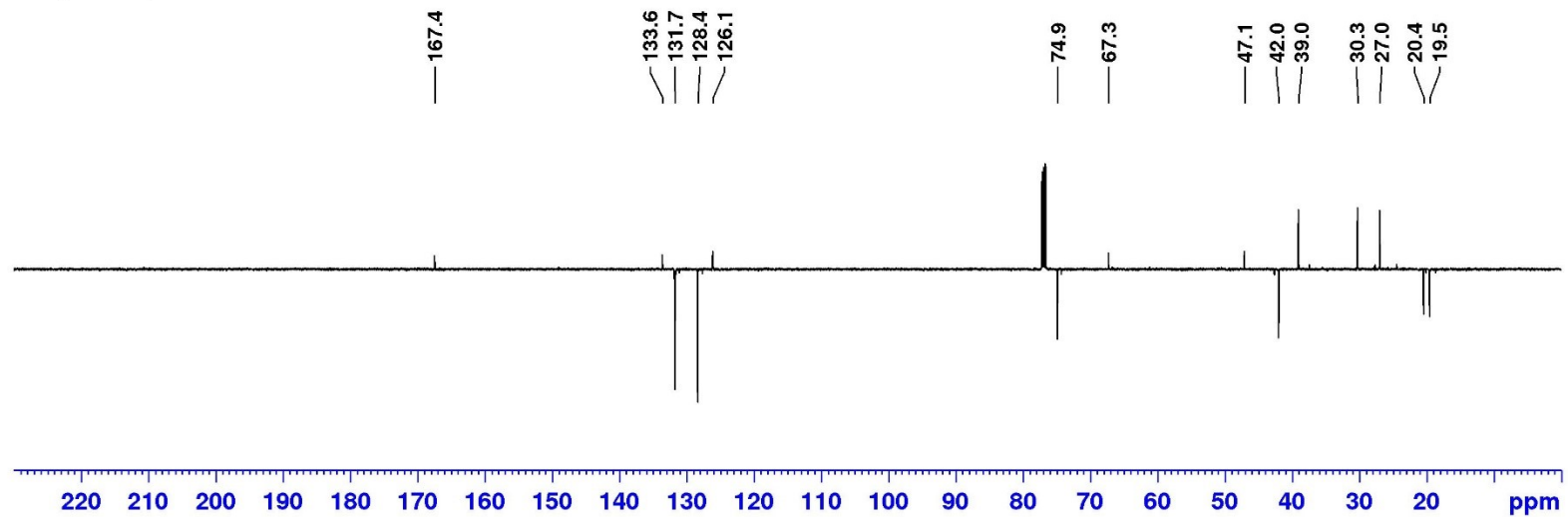


<sup>1</sup>H, 400 MHz, CDCl<sub>3</sub>

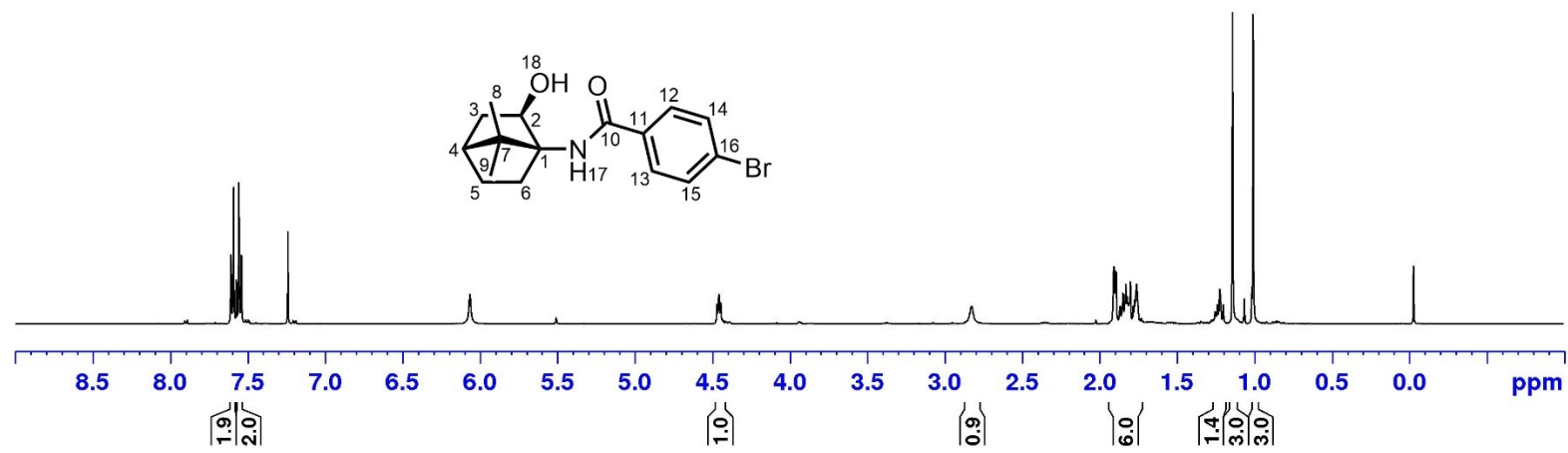


# Compound 12b

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

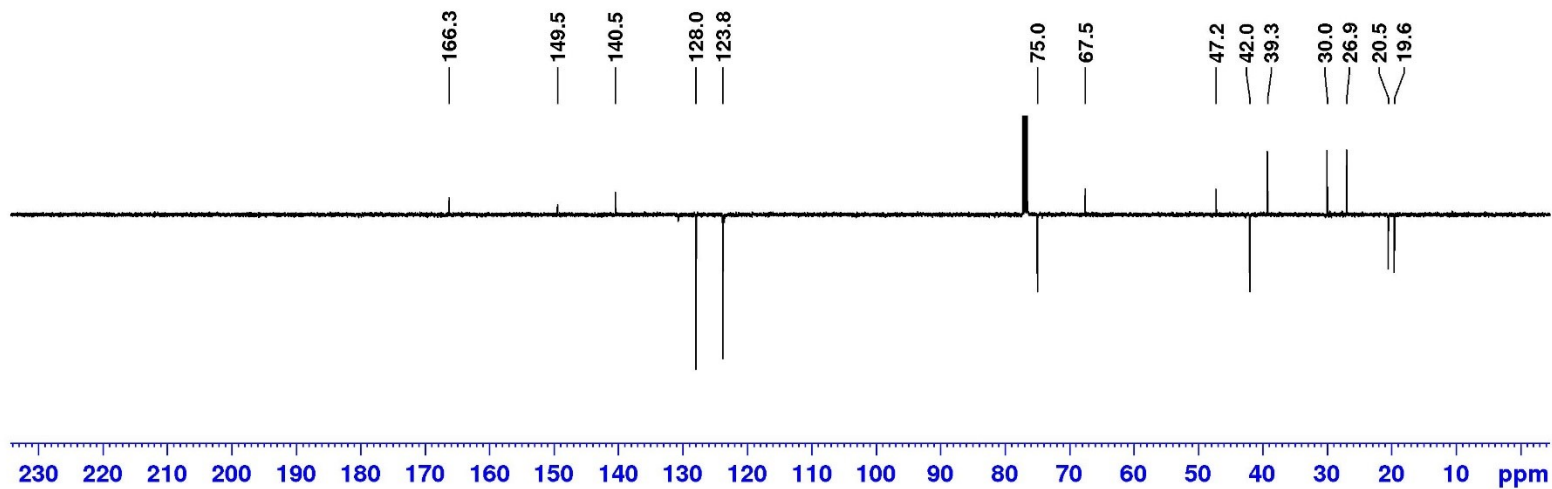


$^1\text{H}$ , 500MHz,  $\text{CDCl}_3$

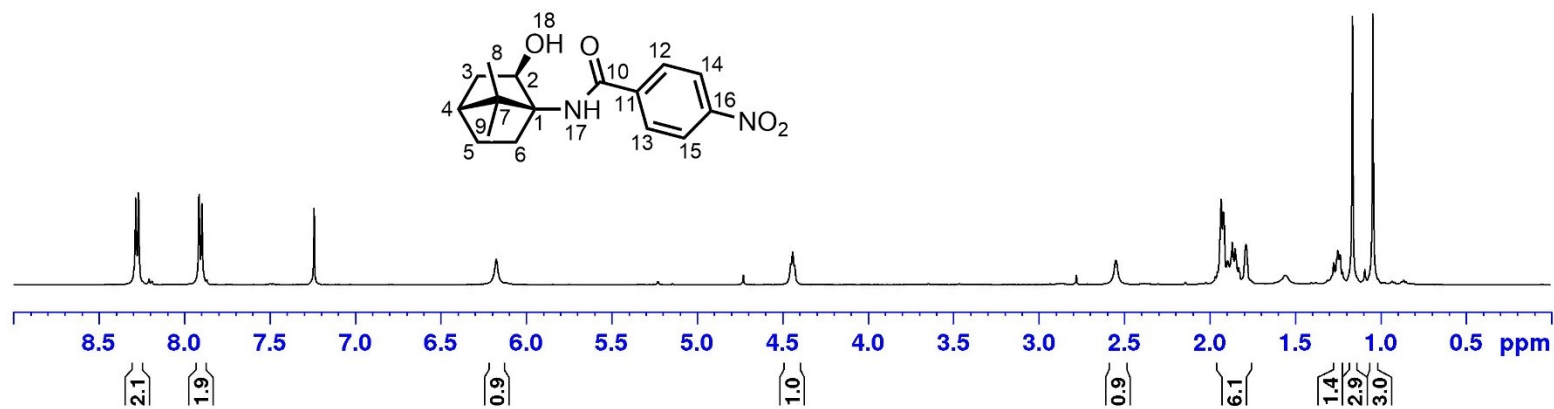


# Compound 12c

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

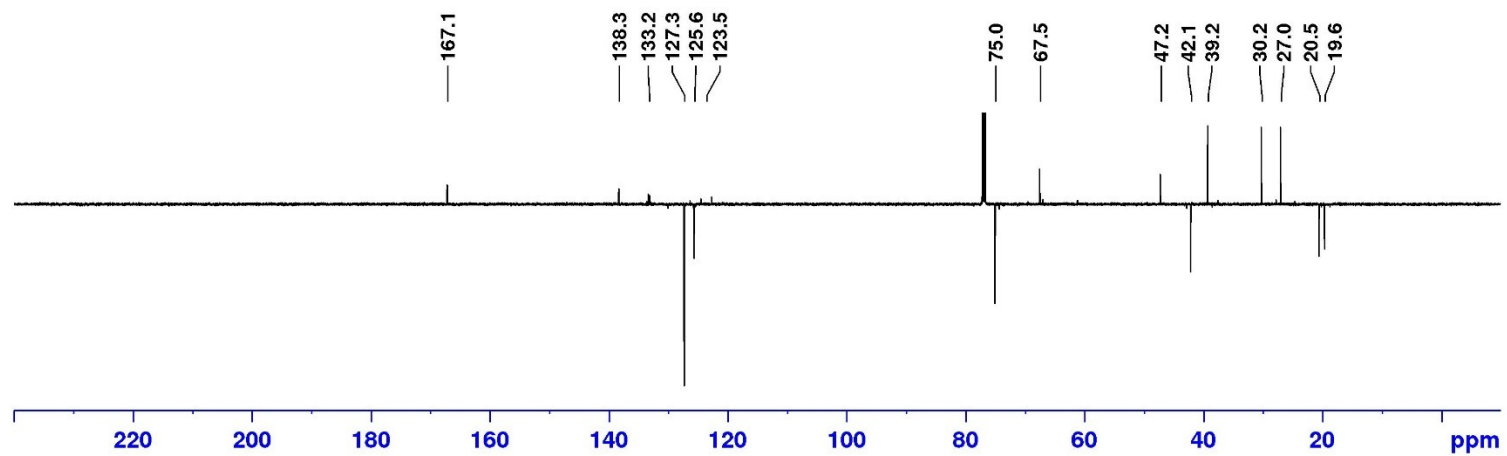


<sup>1</sup>H, 500 MHz, CDCl<sub>3</sub>

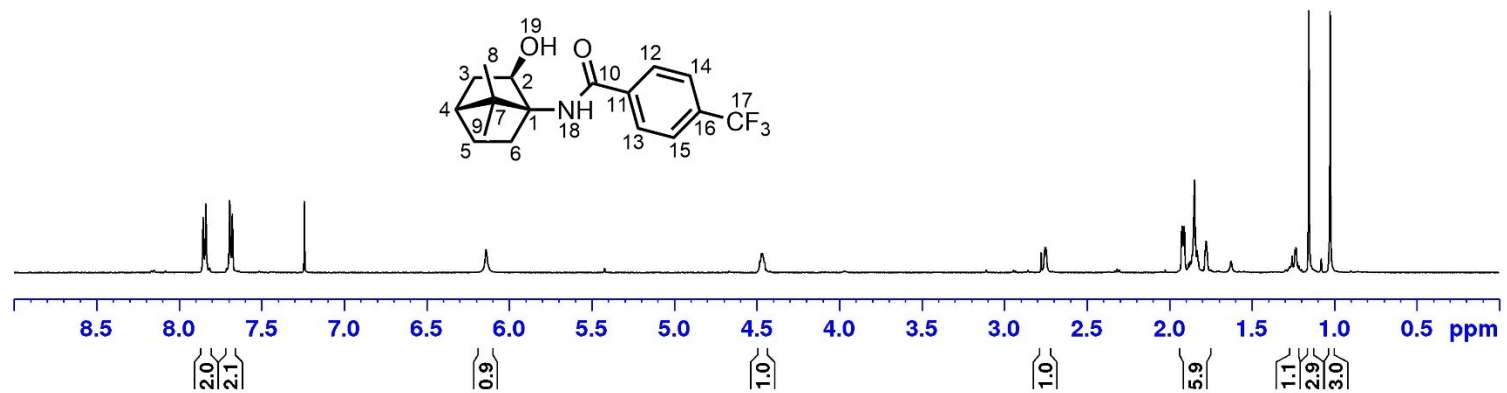


# Compound 12d

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

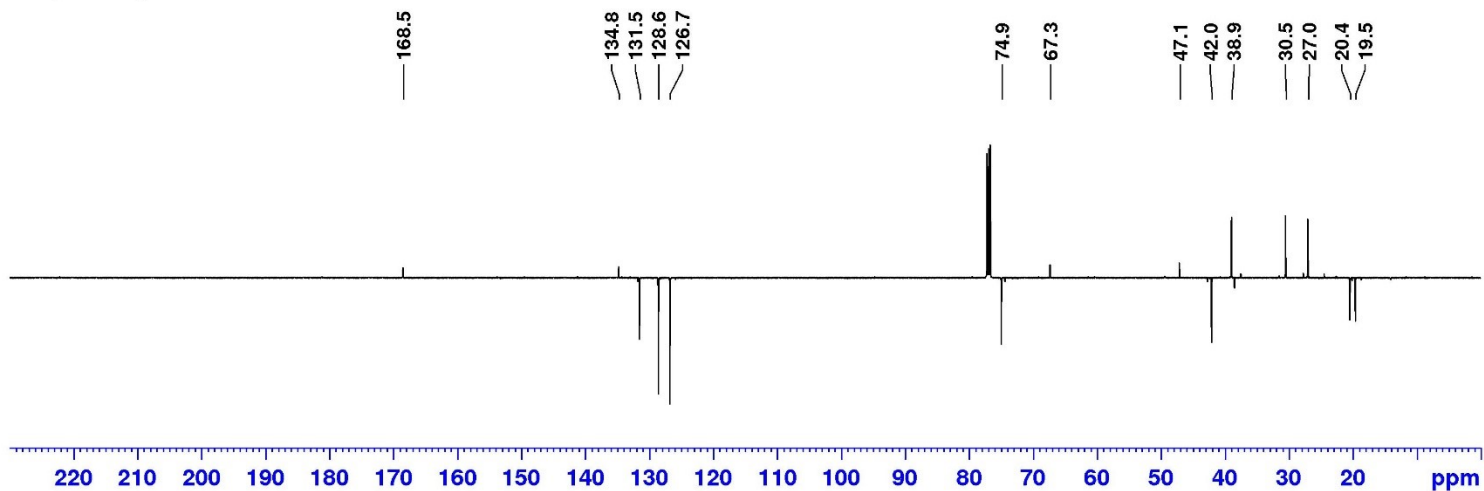


$^1\text{H}$ , 500 MHz,  $\text{CDCl}_3$

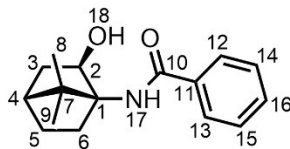
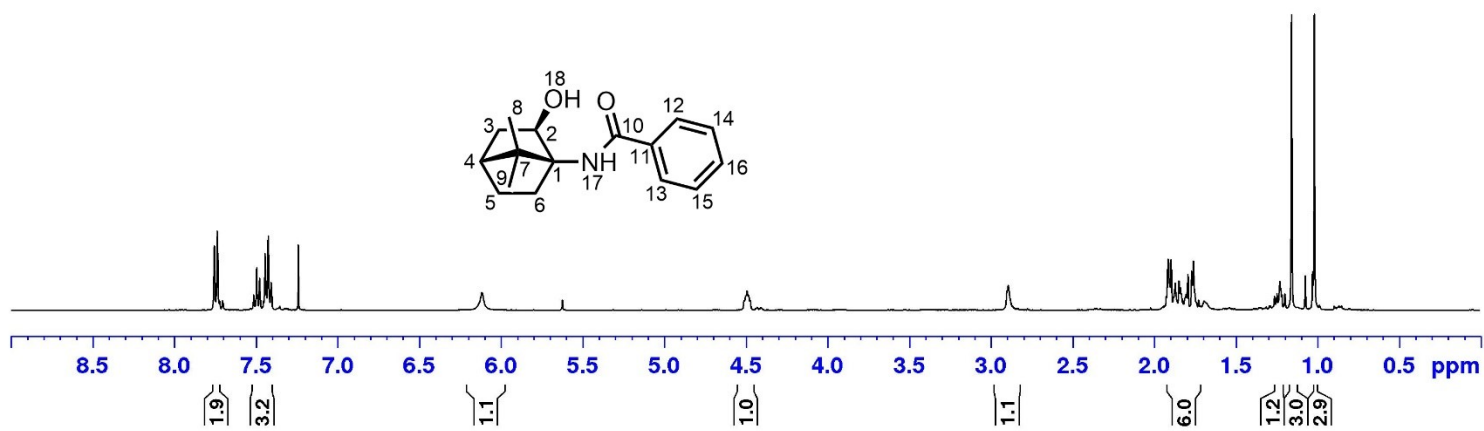


# Compound 12e

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

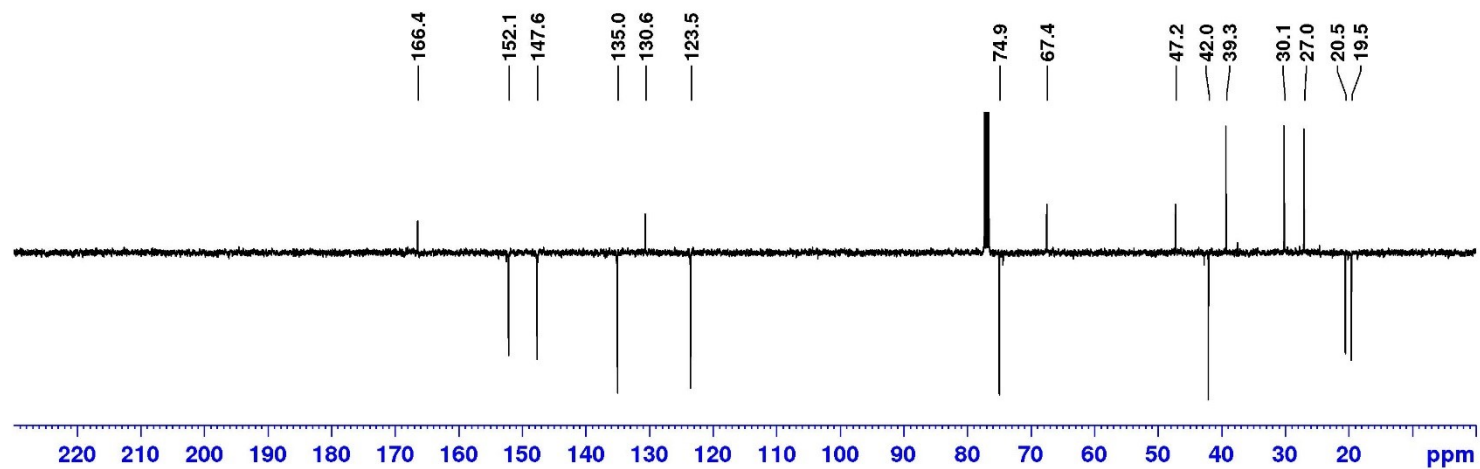


<sup>1</sup>H, 400 MHz, CDCl<sub>3</sub>

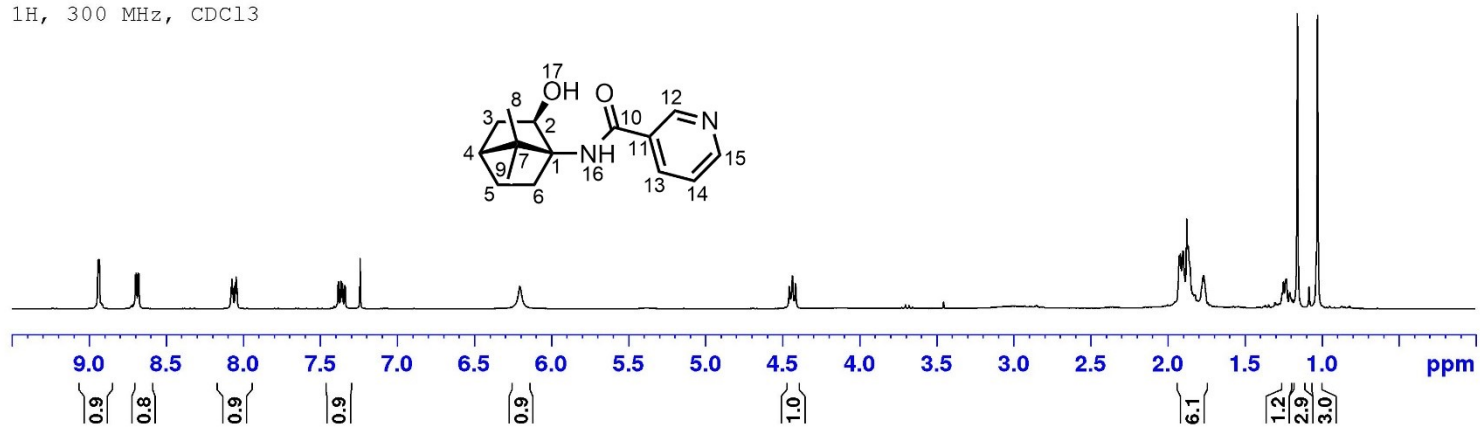


# Compound 12f

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

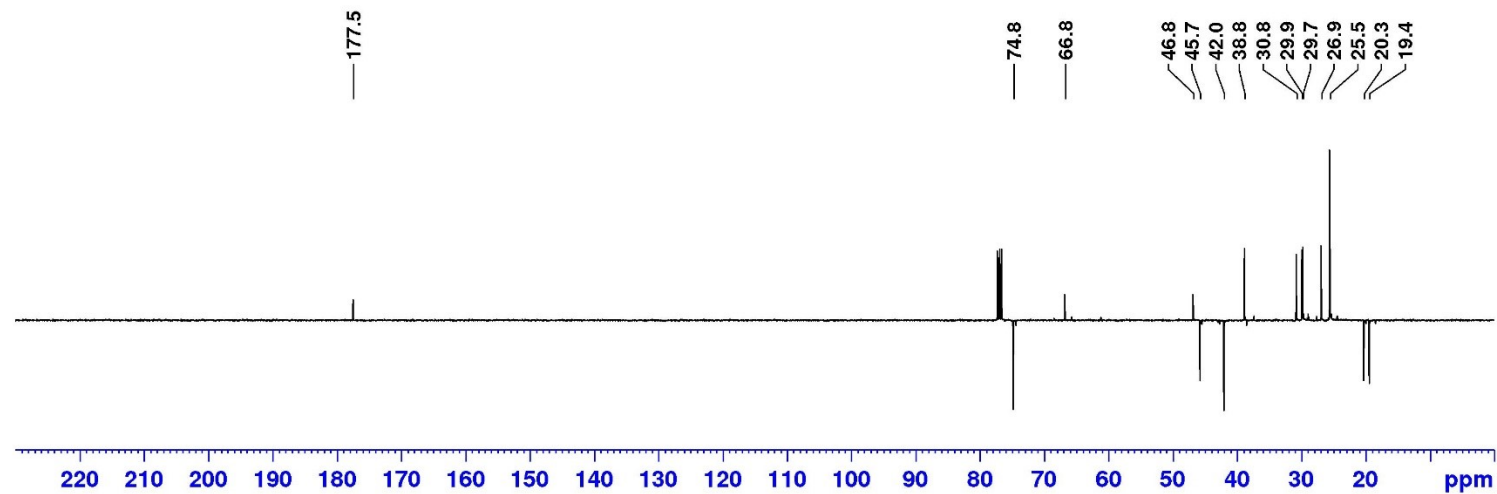


$^1\text{H}$ , 300 MHz,  $\text{CDCl}_3$

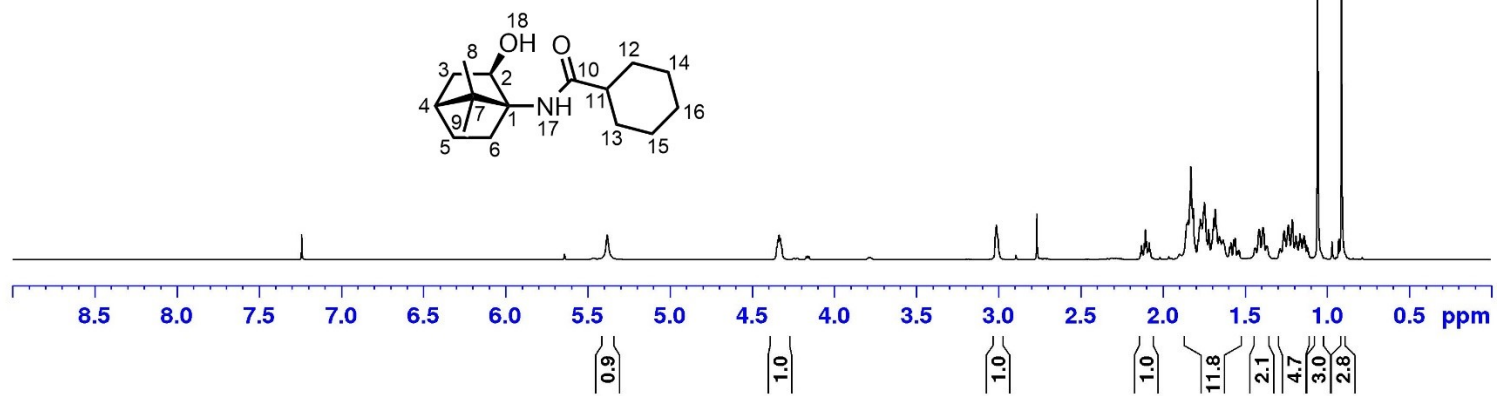


# Compound 12g

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

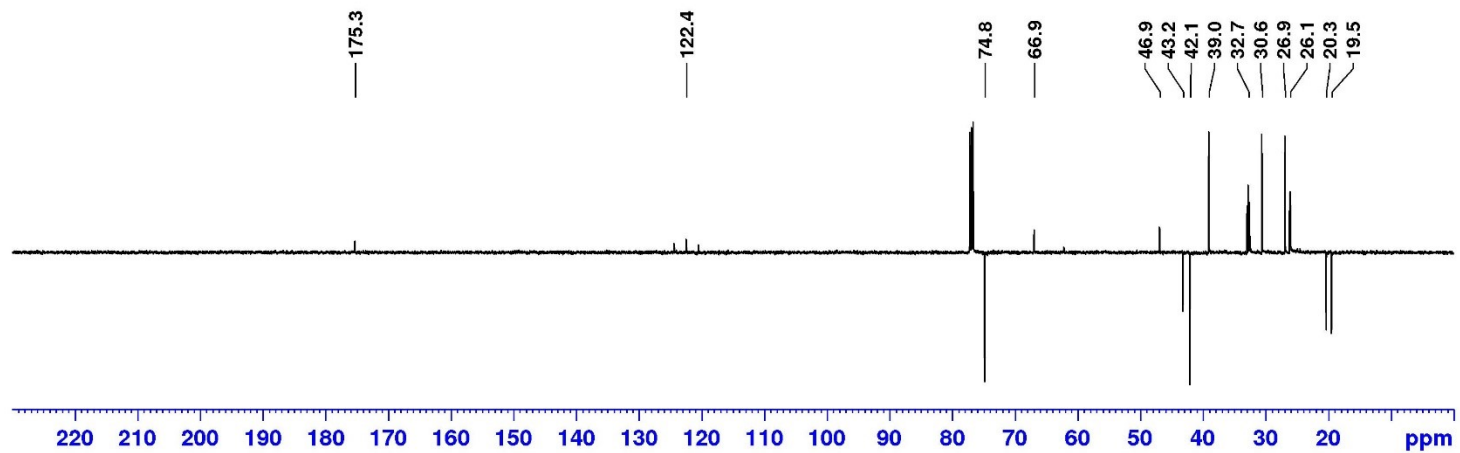


$^1\text{H}$ , 400 MHz,  $\text{CDCl}_3$

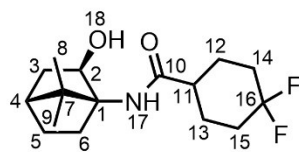
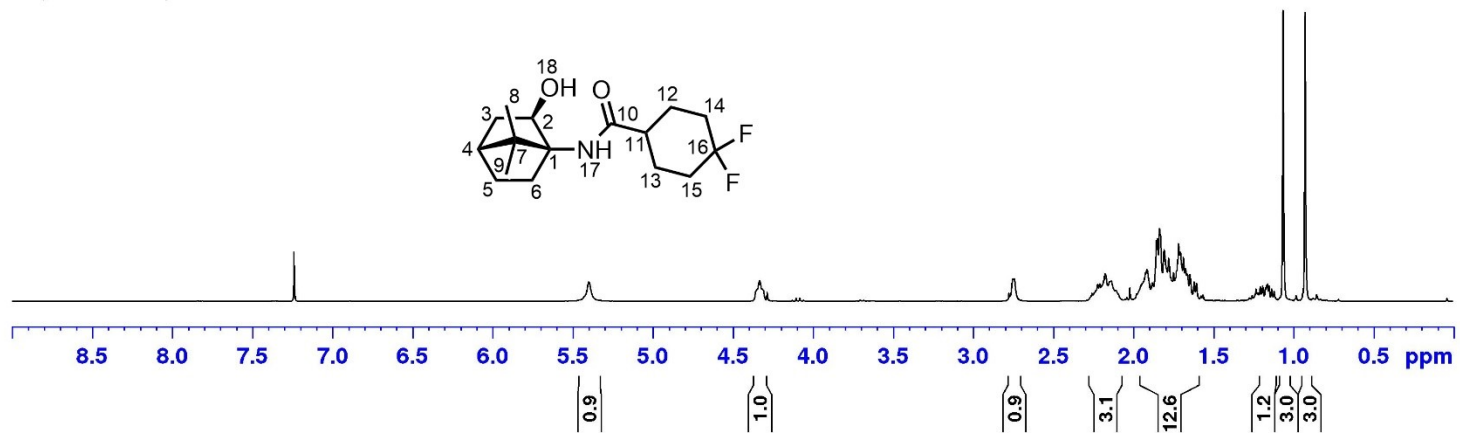


# Compound 12h

<sup>13</sup>C, JMOD, CDCL<sub>3</sub>

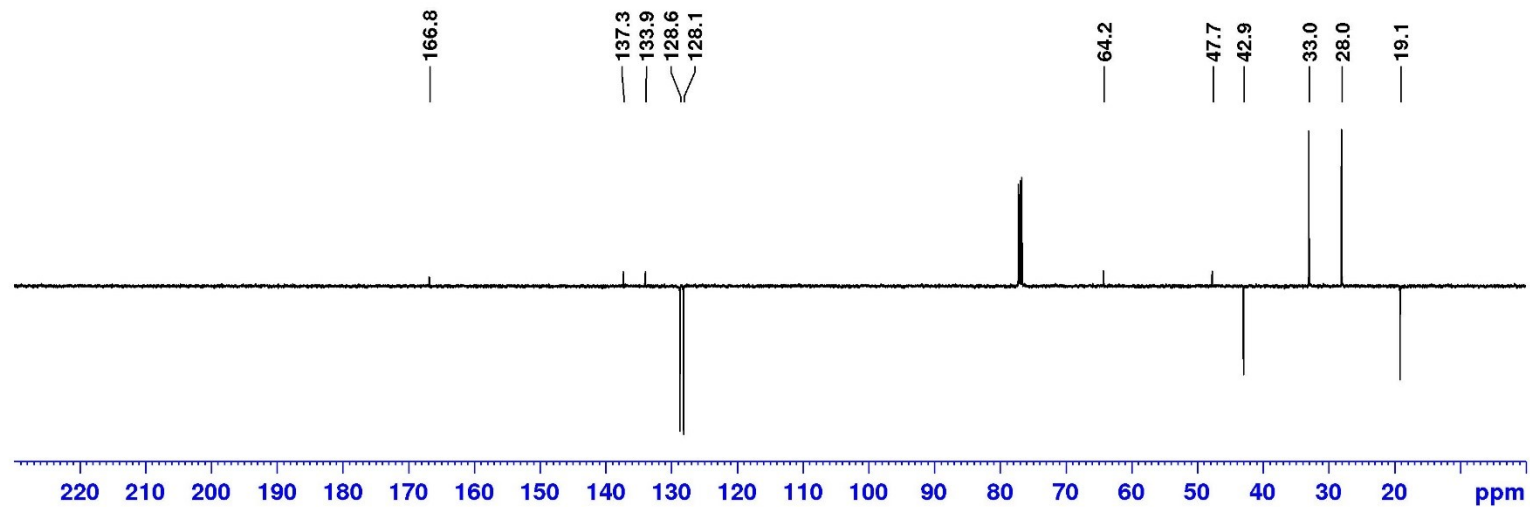


<sup>1</sup>H, 300 MHz, CDCL<sub>3</sub>

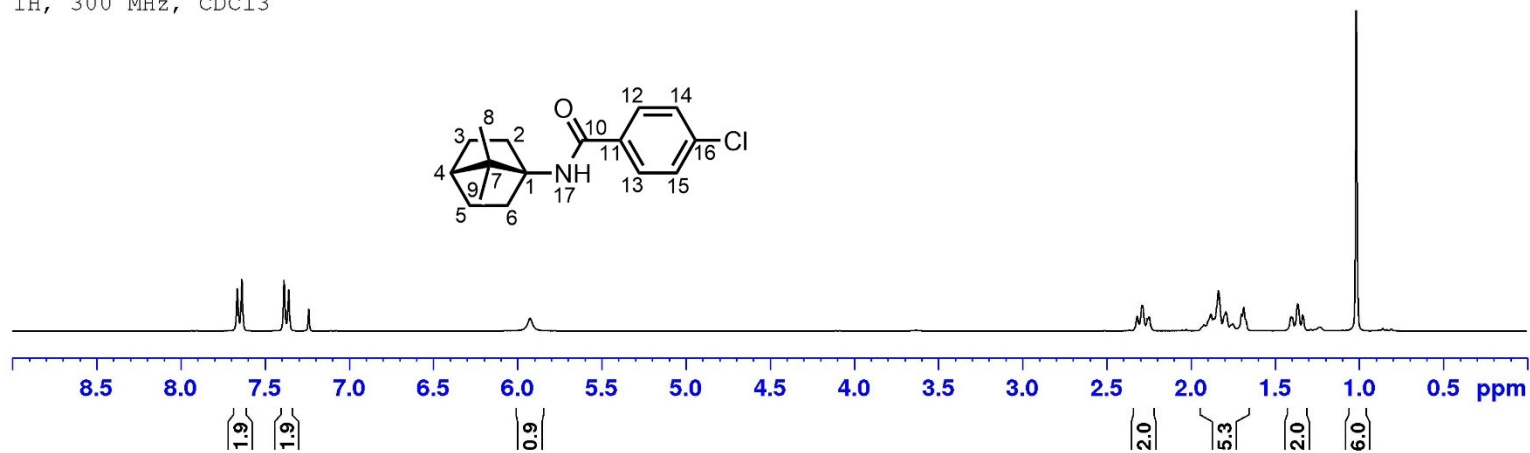


# Compound 13a

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

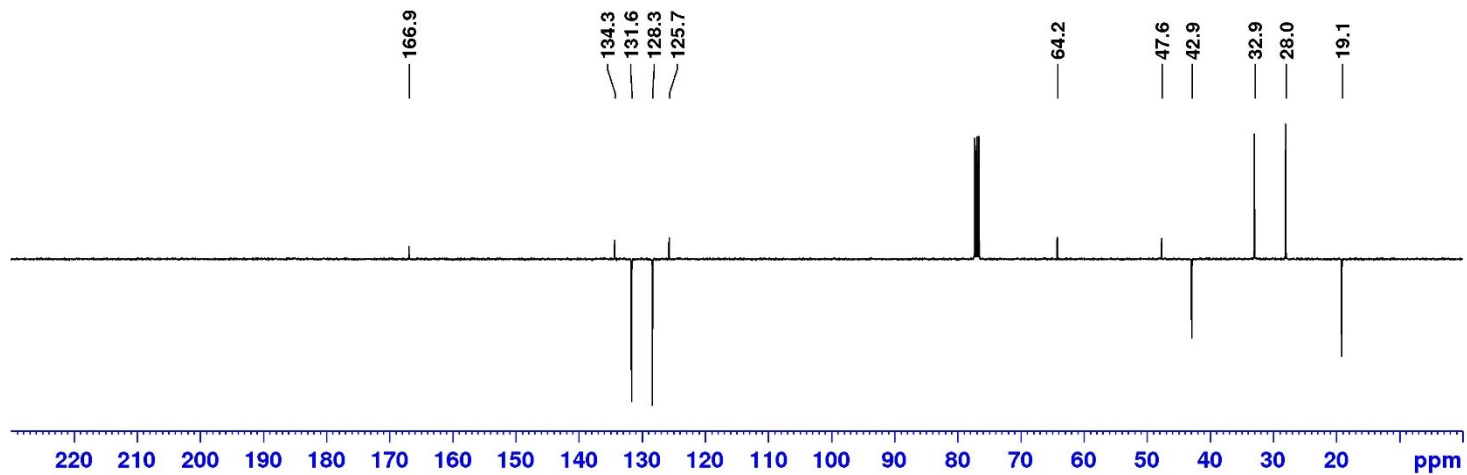


<sup>1</sup>H, 300 MHz, CDCl<sub>3</sub>

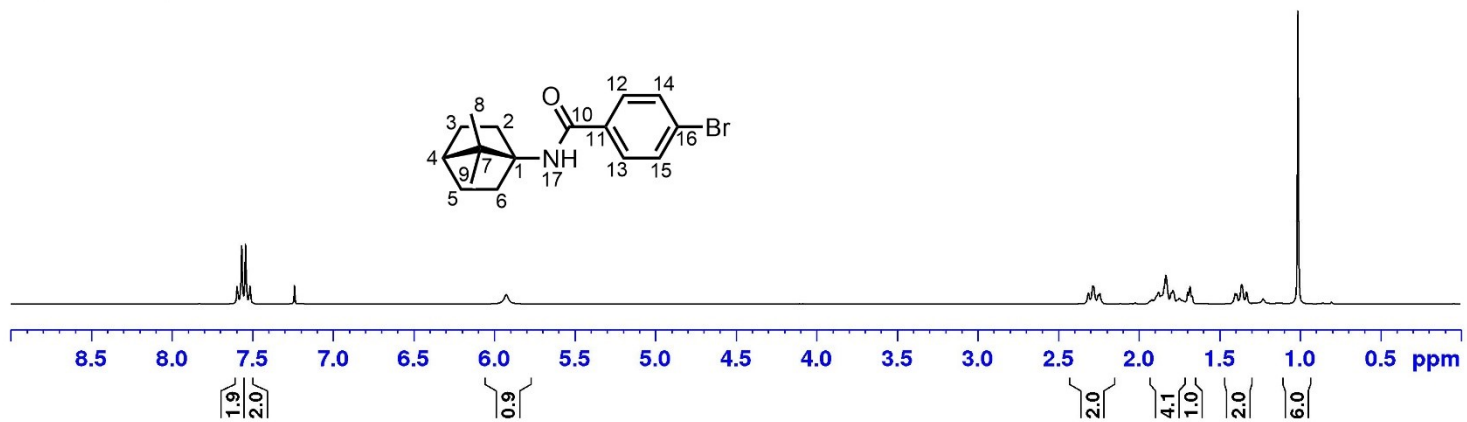


# Compound 13b

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

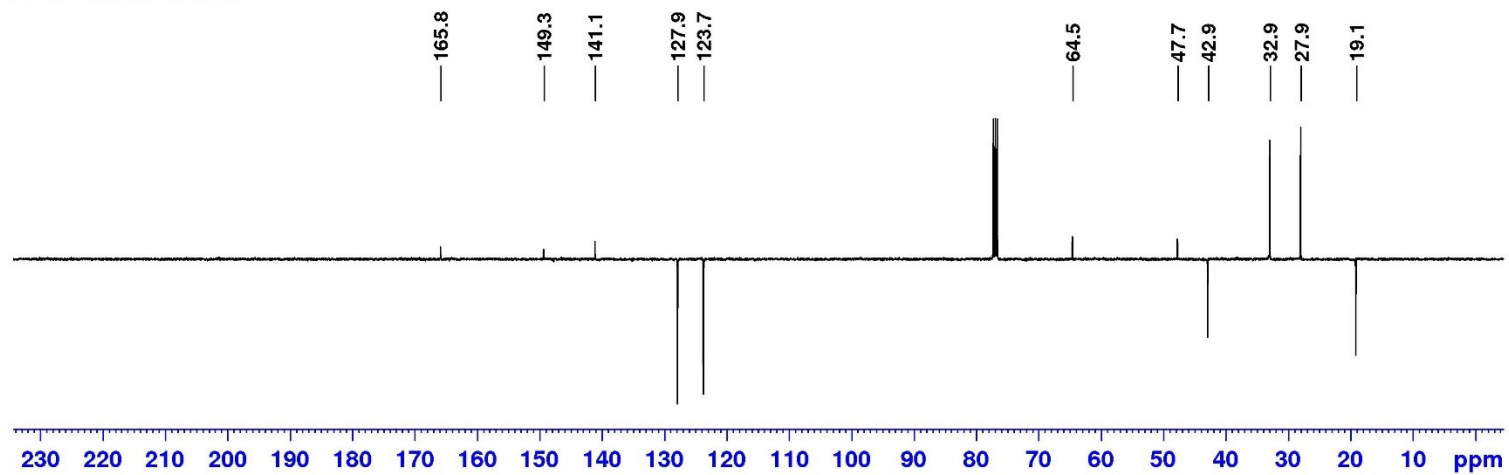


<sup>1</sup>H, 300 MHz, CDCl<sub>3</sub>

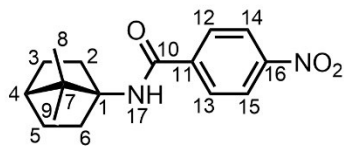
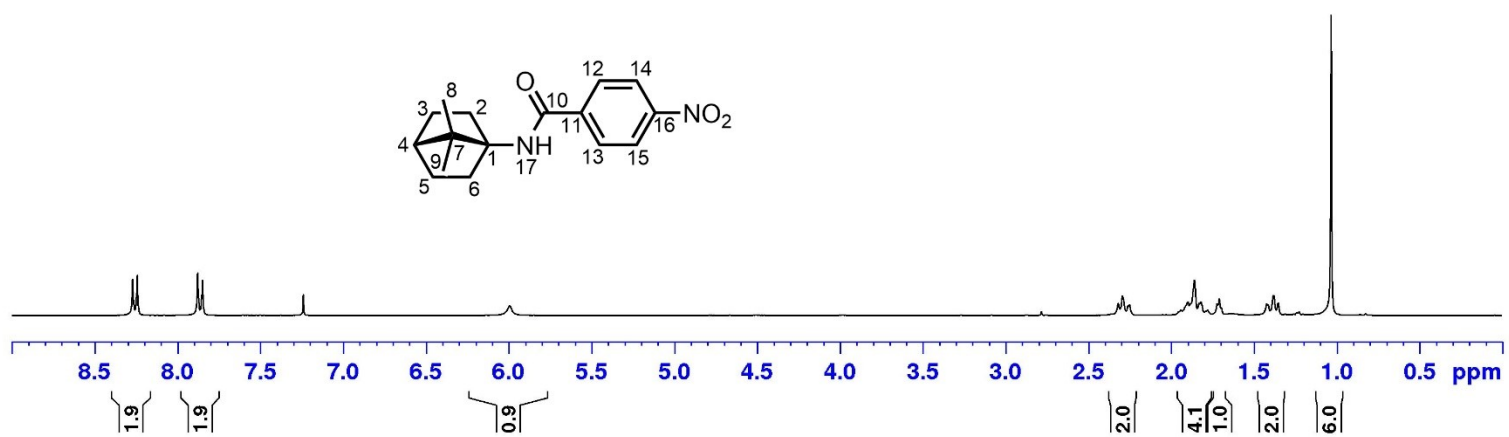


# Compound 13c

<sup>13</sup>C, JMOD, CDC13

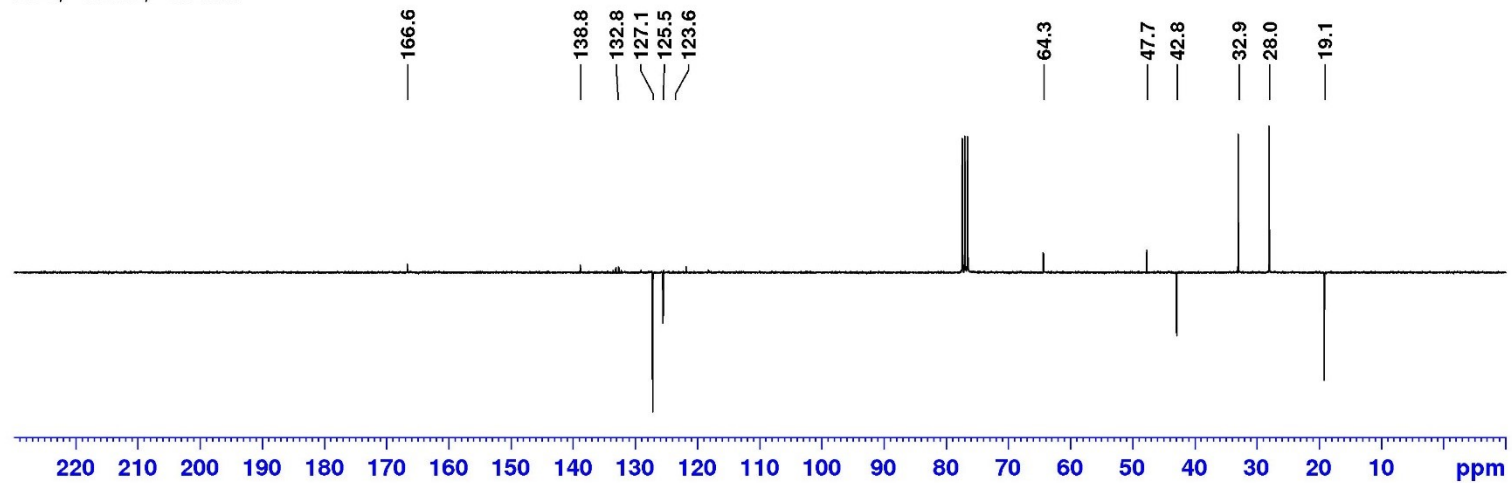


<sup>1</sup>H, 300 MHz, CDC13

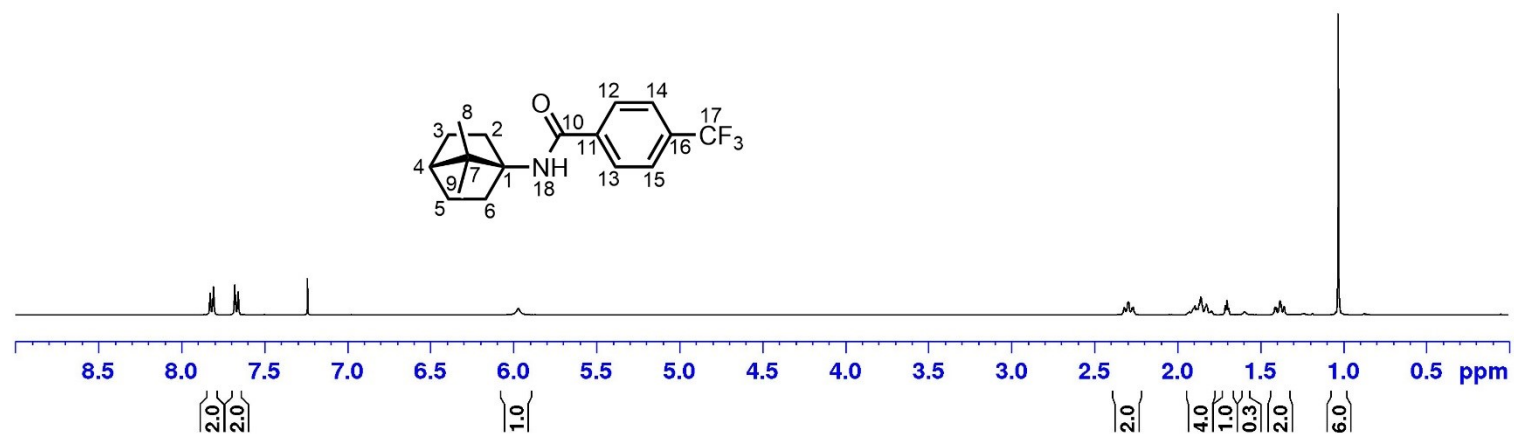


# Compound 13d

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

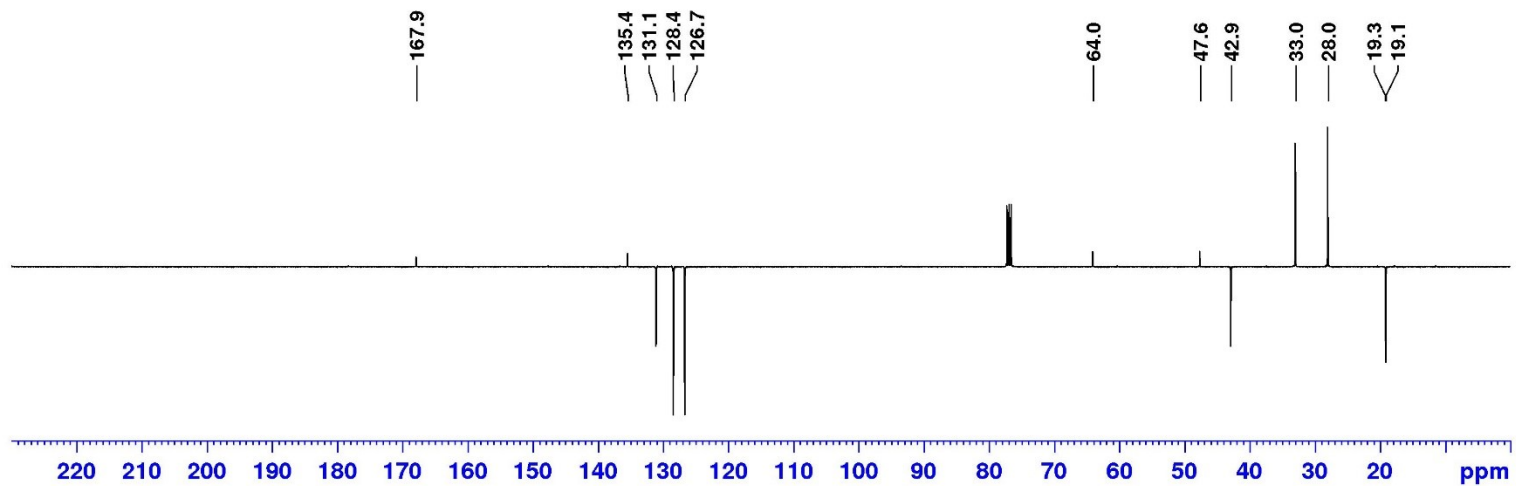


$^1\text{H}$ , 400 MHz,  $\text{CDCl}_3$

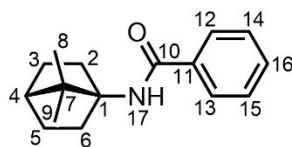
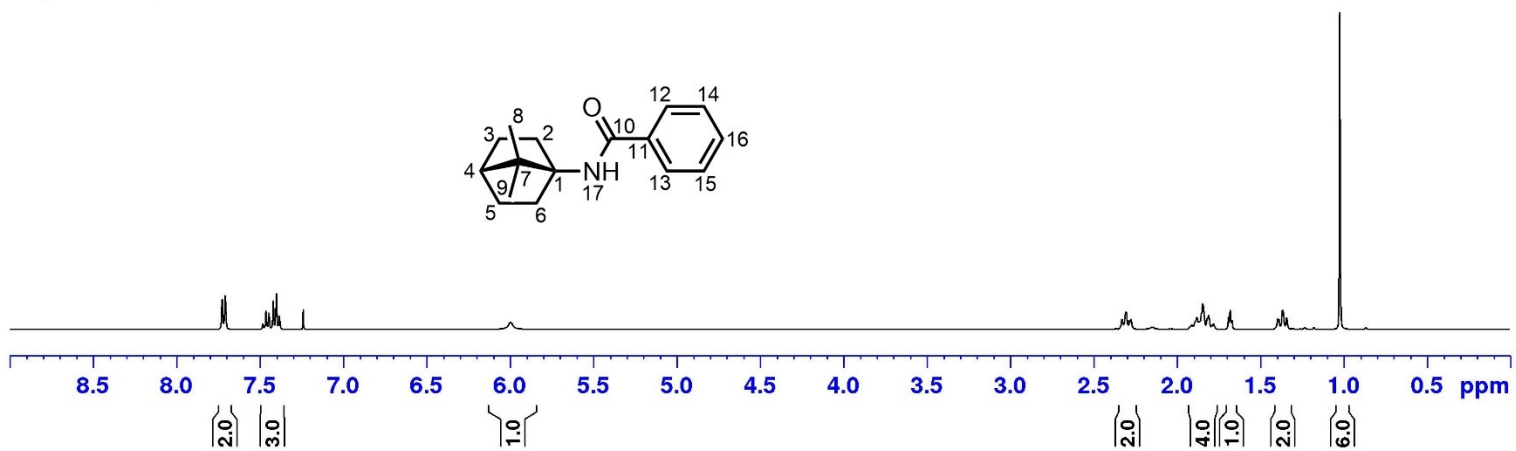


# Compound 13e

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

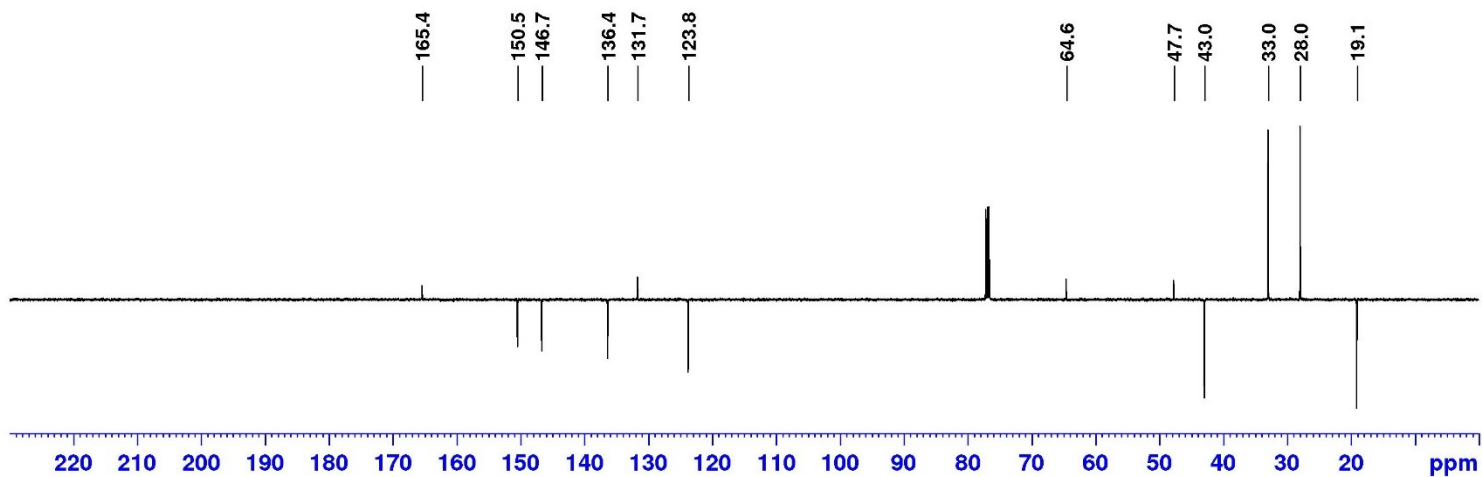


<sup>1</sup>H, 400MHz, CDCl<sub>3</sub>

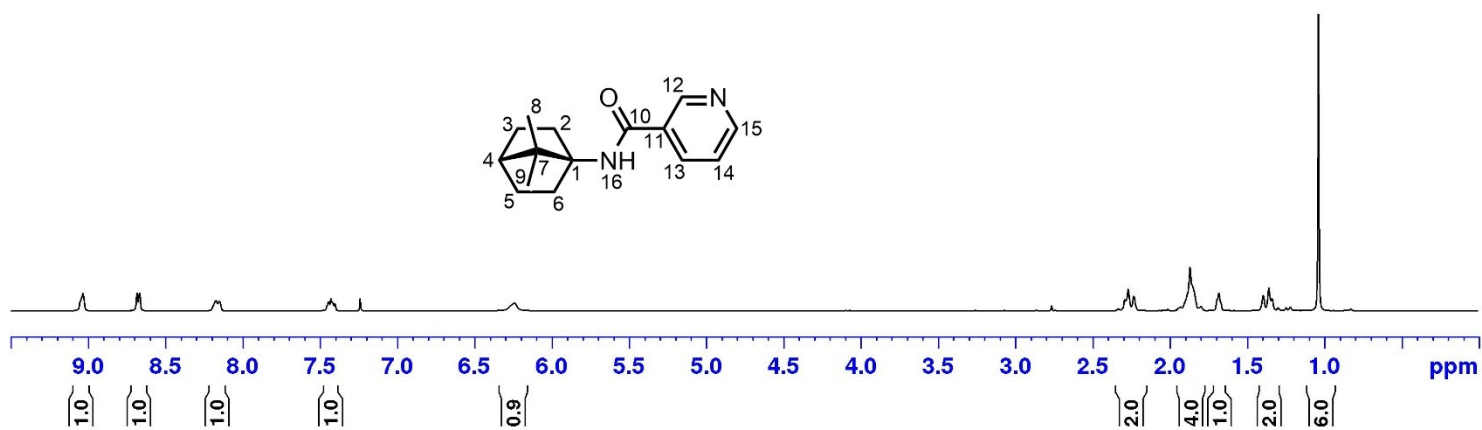


# Compound 13f

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

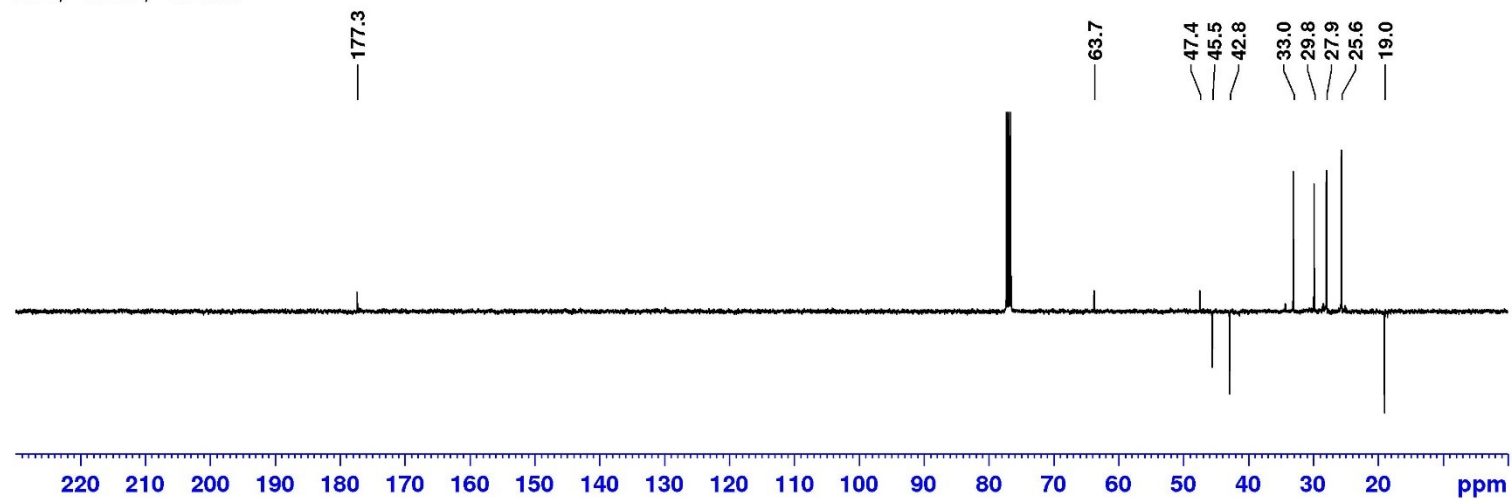


$^1\text{H}$ , 300 MHz,  $\text{CDCl}_3$

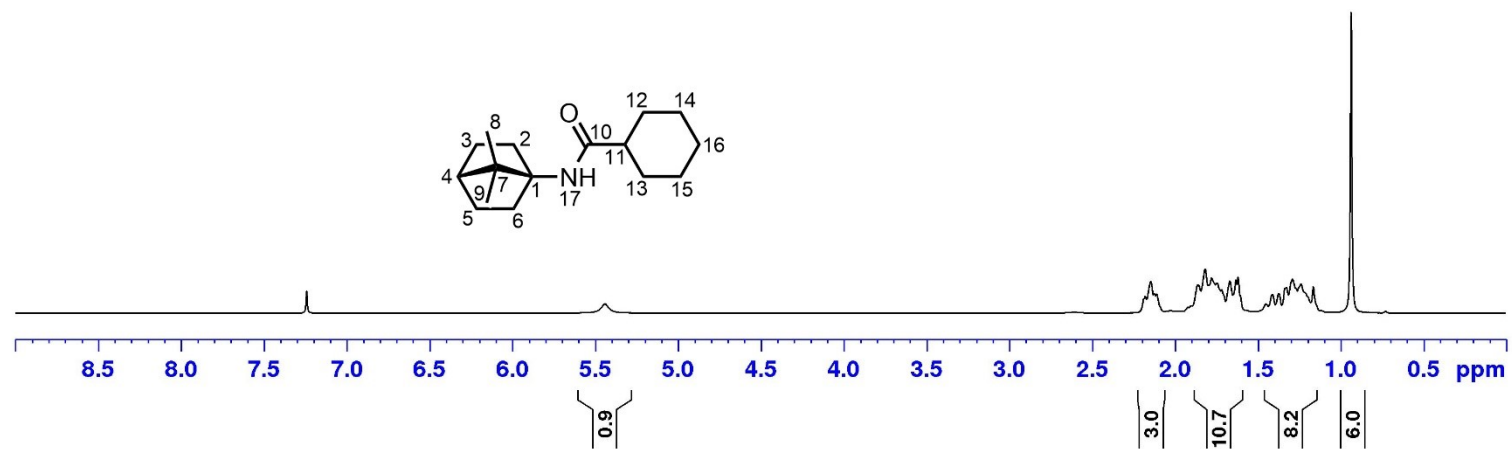


# Compound 13g

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

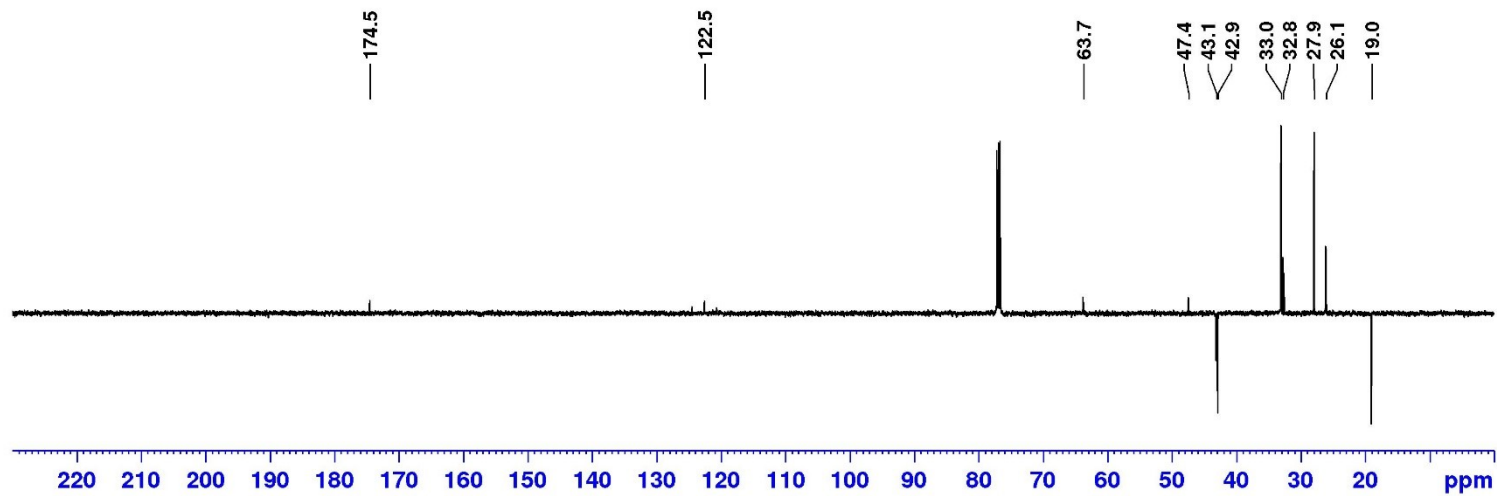


<sup>1</sup>H, 300 MHz, CDCl<sub>3</sub>

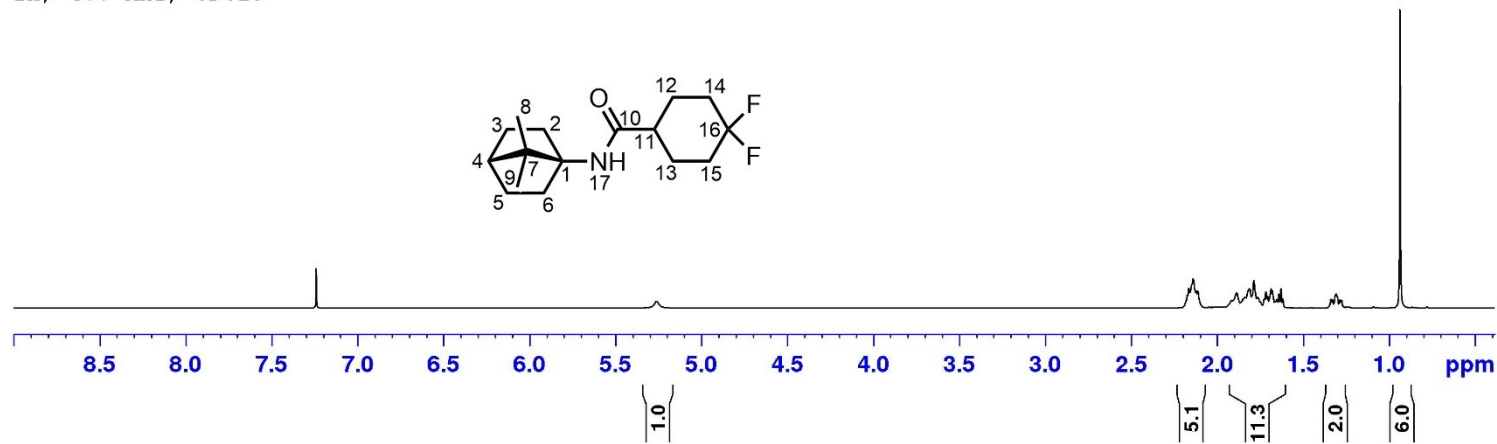


# Compound 13h

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

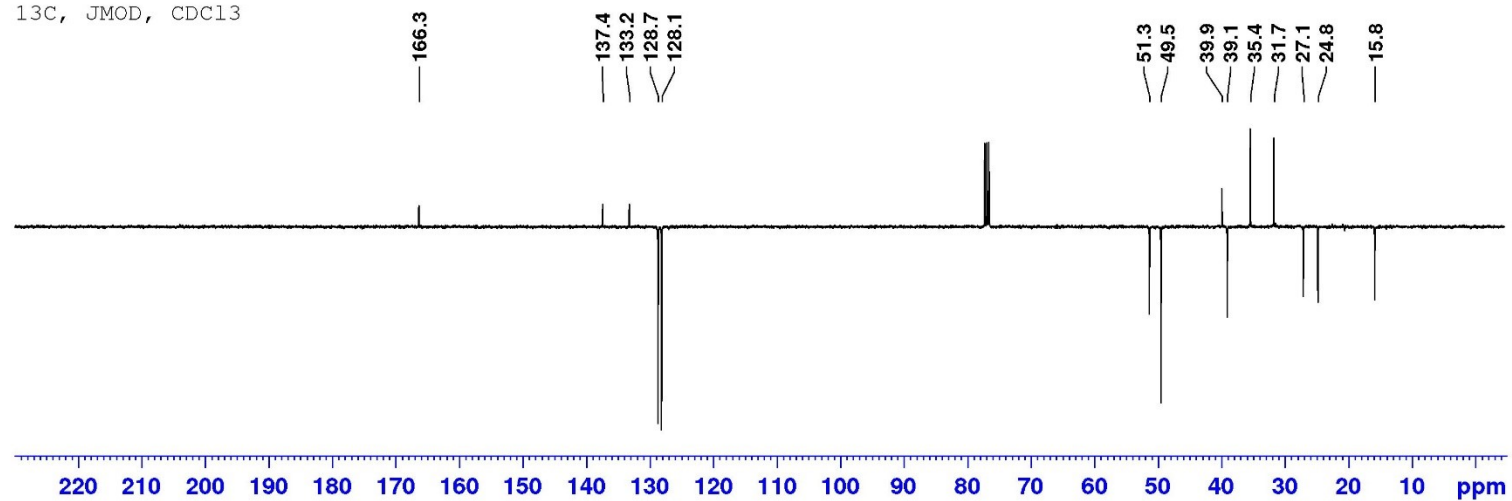


$^1\text{H}$ , 400 MHz,  $\text{CDCl}_3$

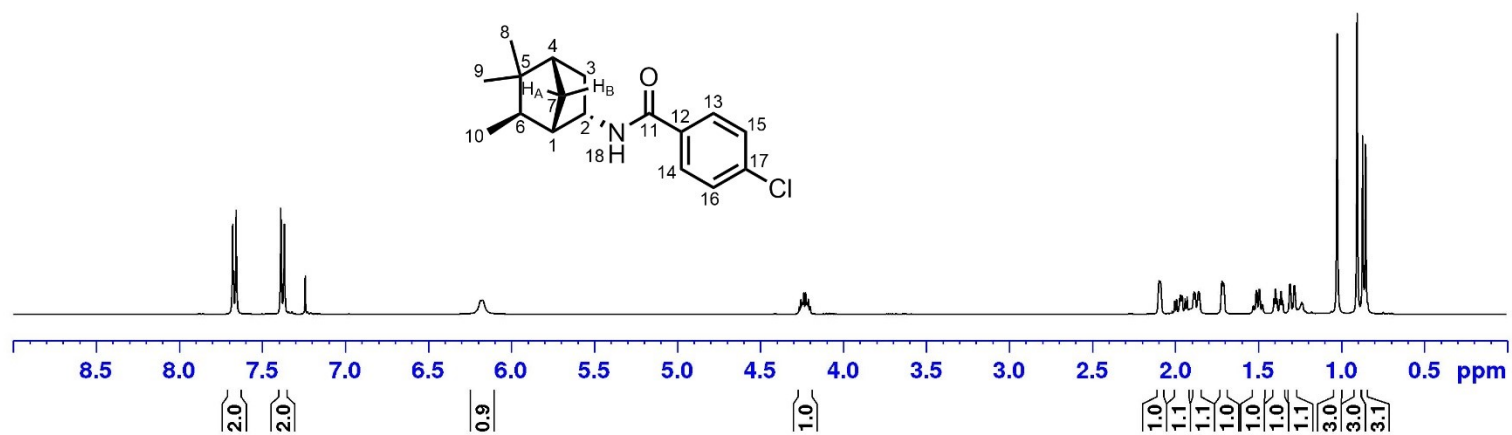


# Compound 15a

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

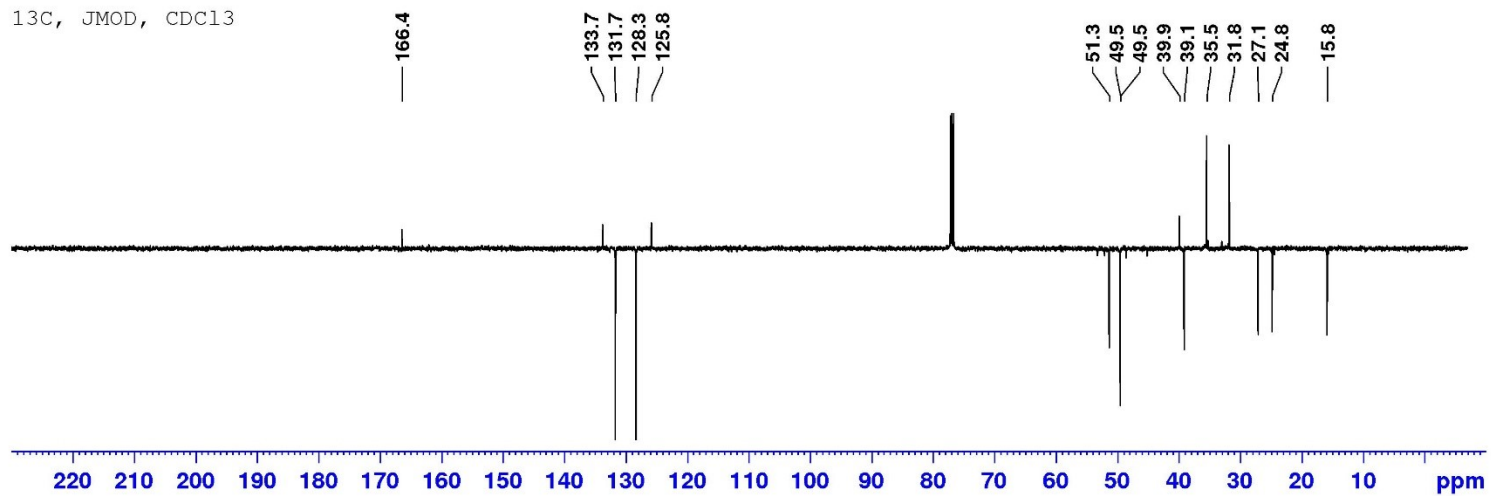


$^1\text{H}$ , 400 MHz,  $\text{CDCl}_3$

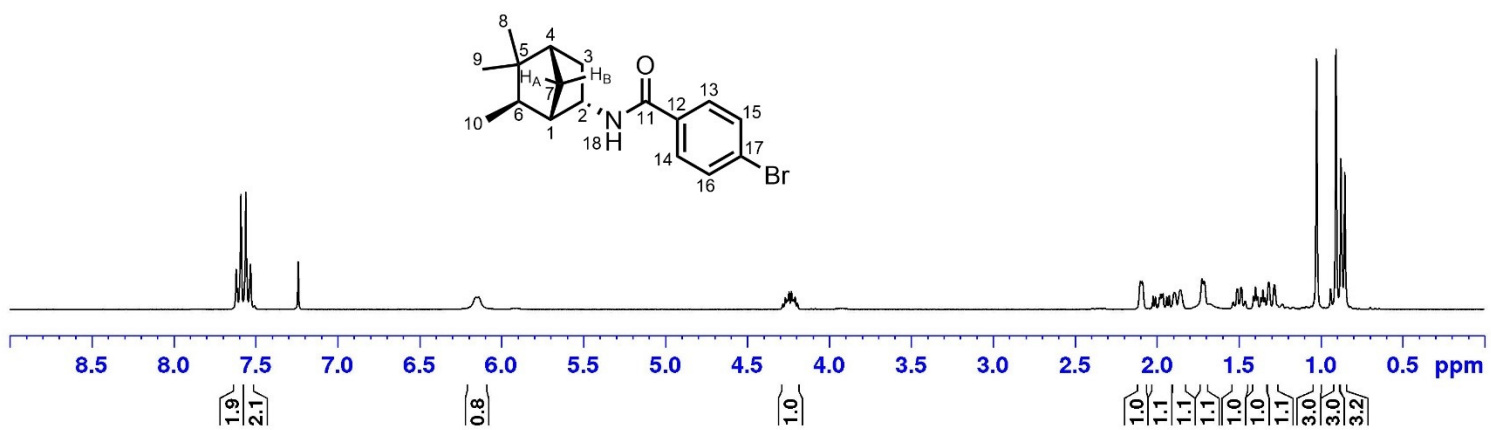


# Compound 15b

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

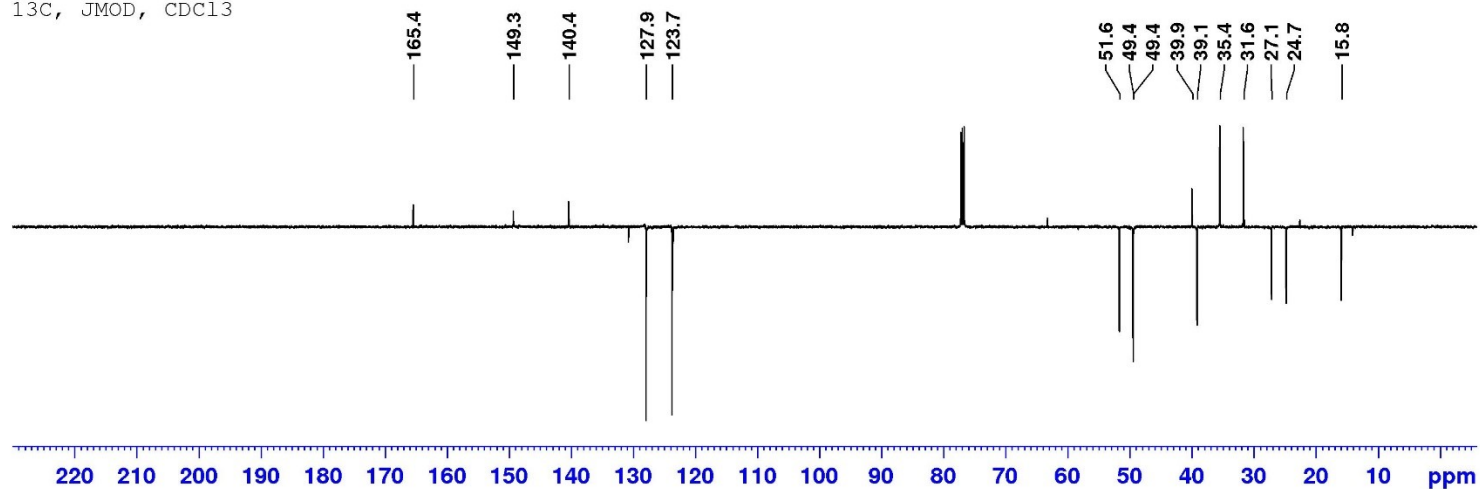


$^1\text{H}$ , 300 MHz,  $\text{CDCl}_3$

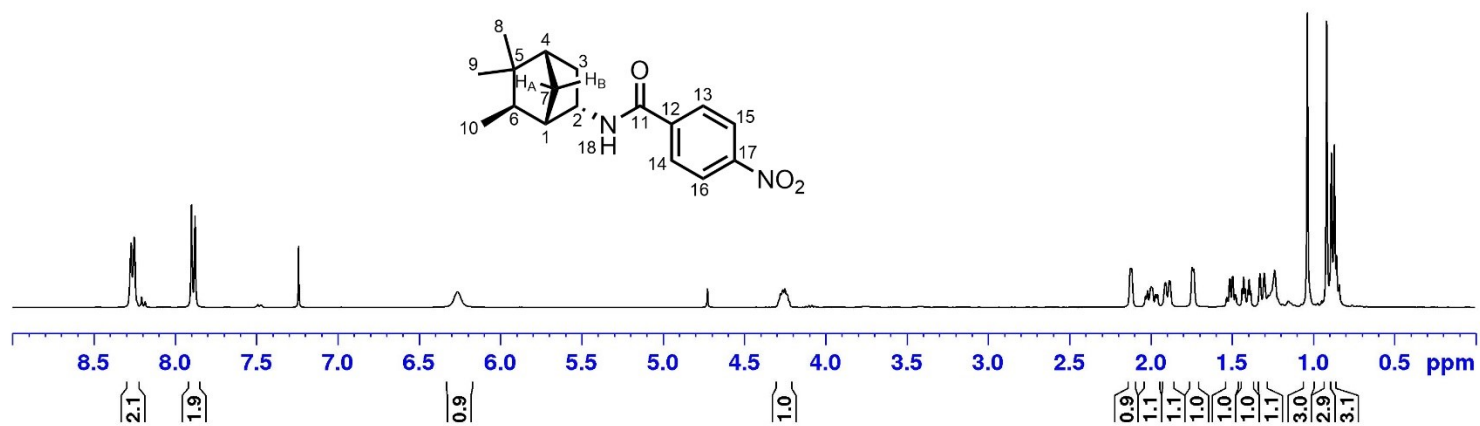


# Compound 15c

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

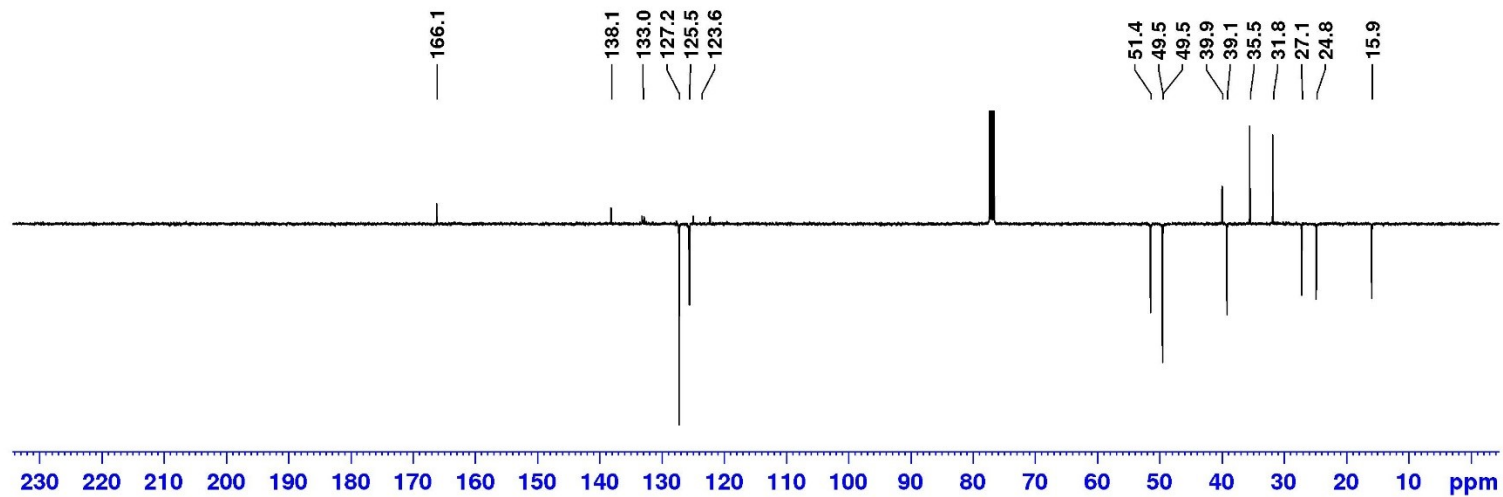


$^1\text{H}$ , 400 MHz,  $\text{CDCl}_3$

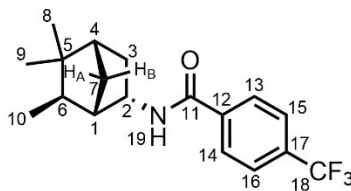
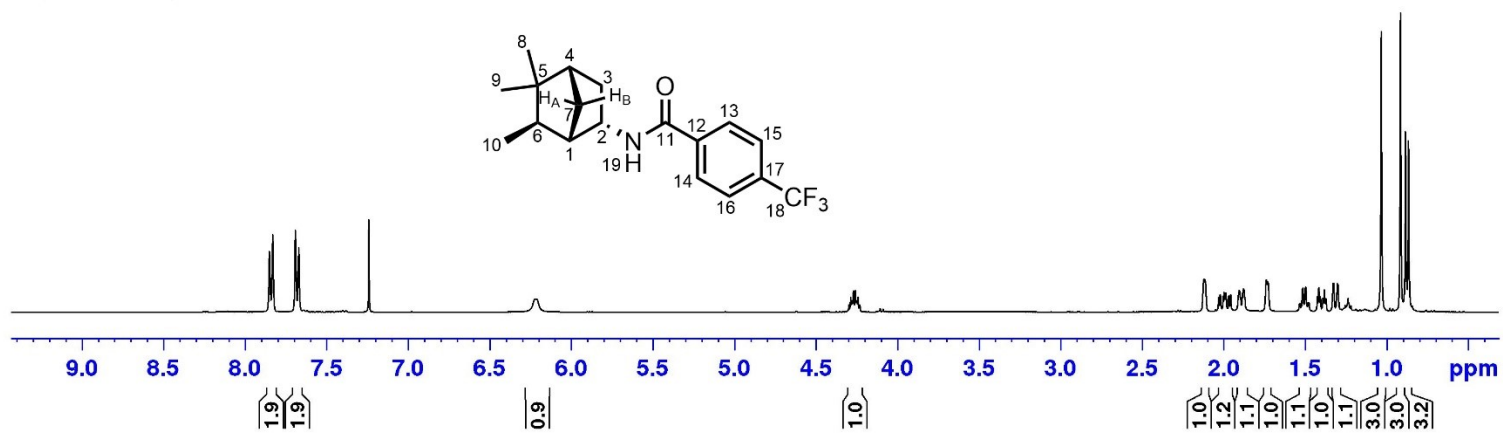


# Compound 15d

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

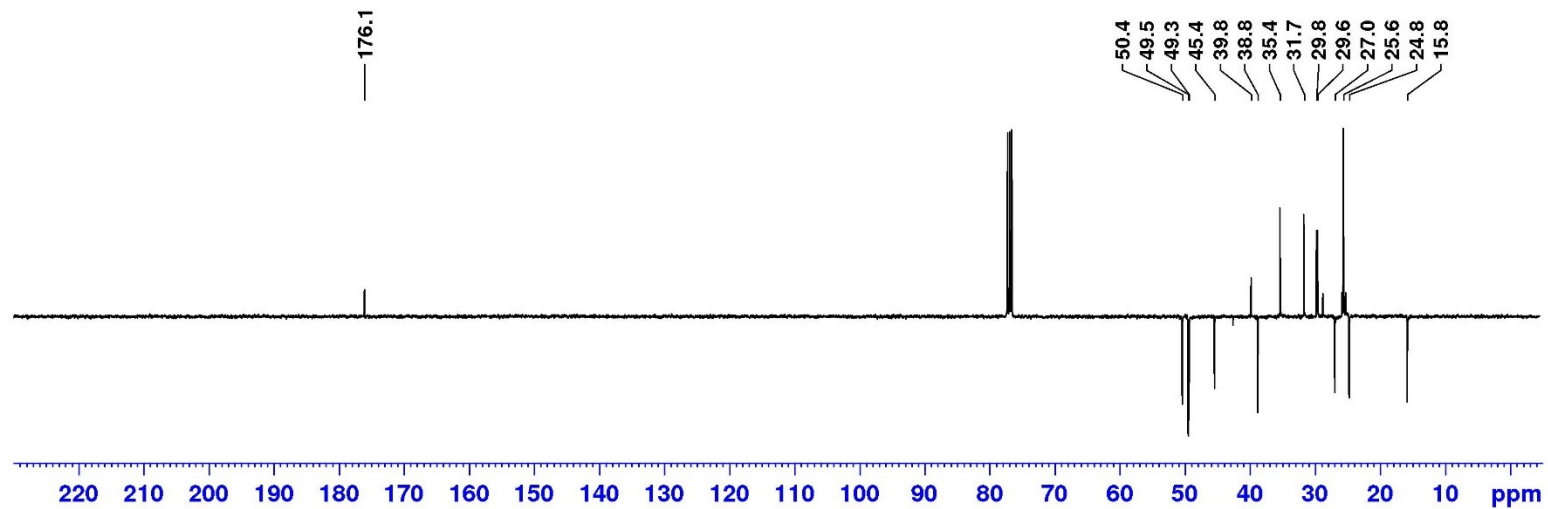


$^1\text{H}$ , 400 MHz,  $\text{CDCl}_3$

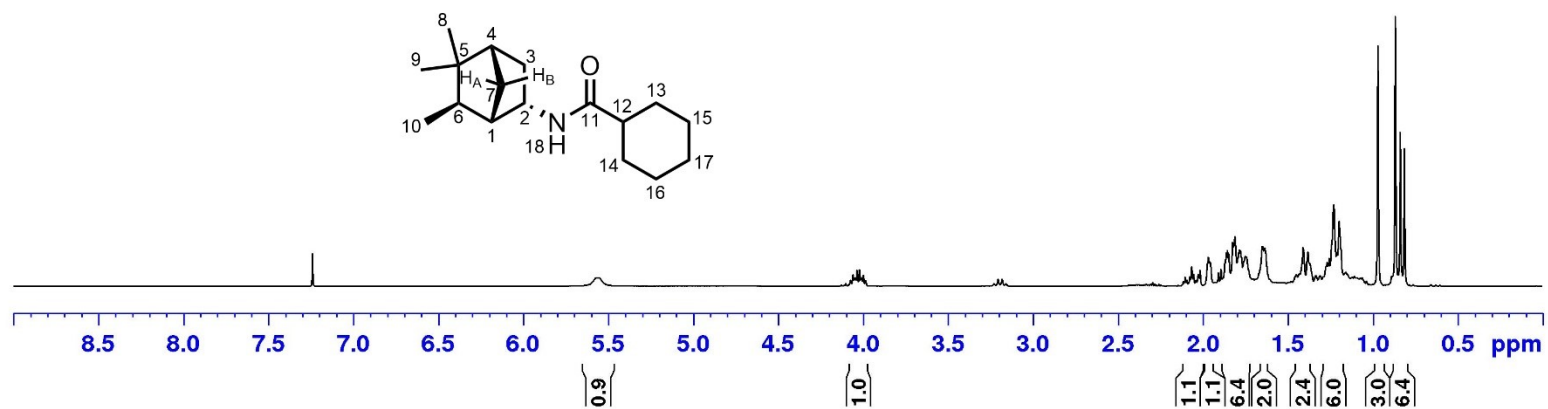


# Compound 15e

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

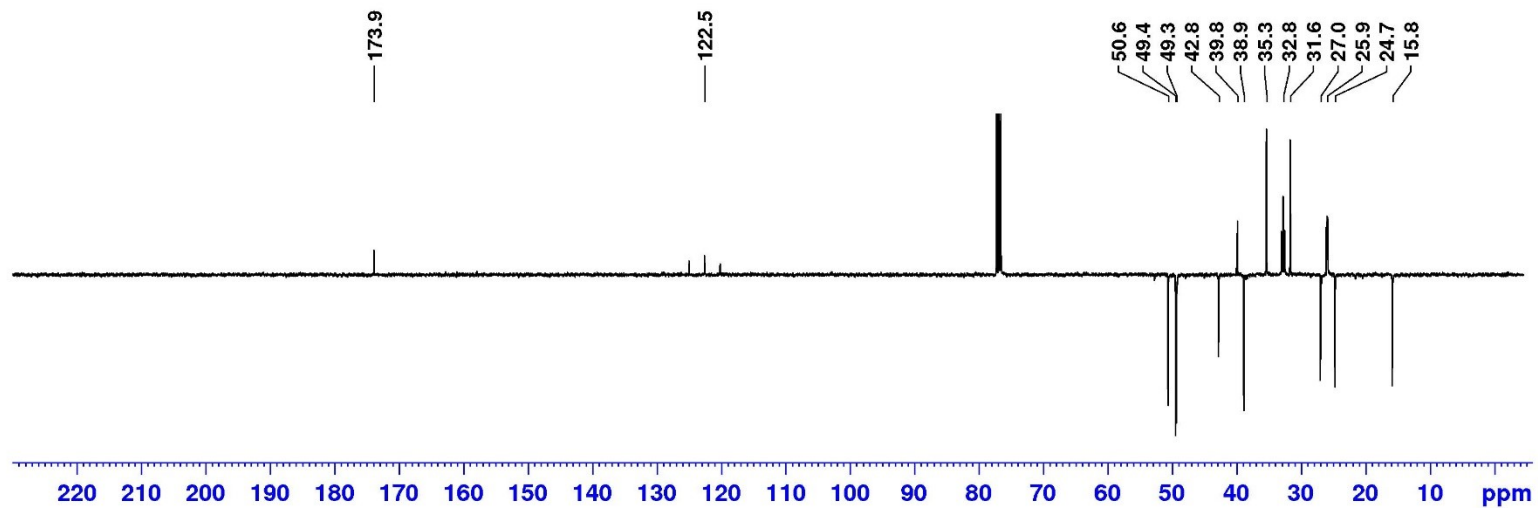


<sup>1</sup>H, 300 MHz, CDCl<sub>3</sub>

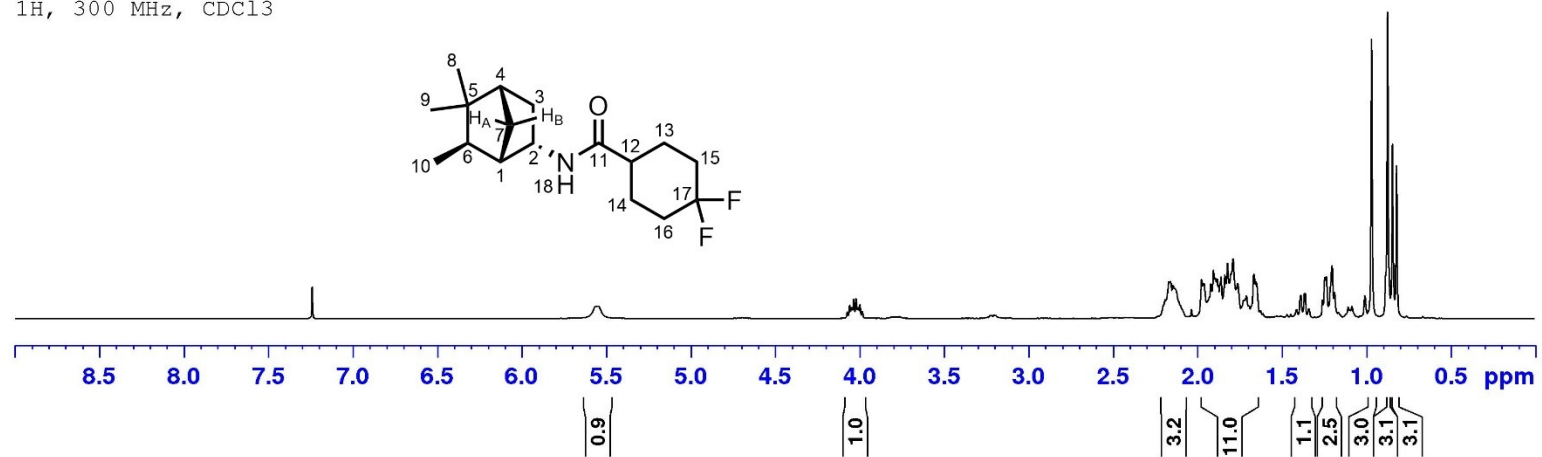


# Compound 15f

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

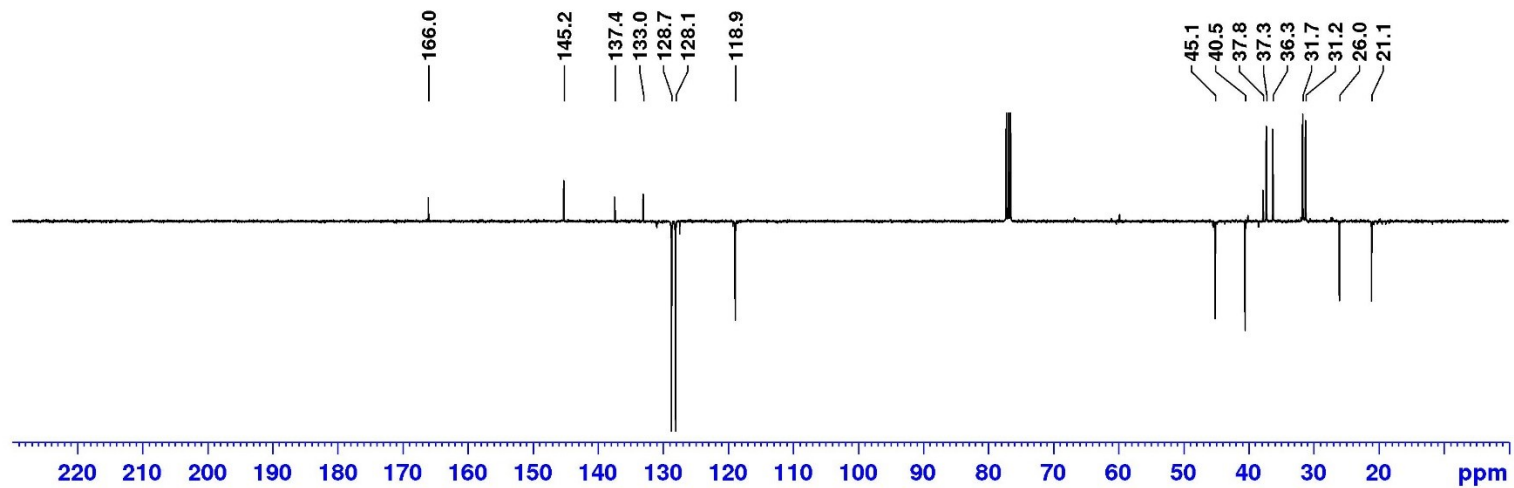


$^1\text{H}$ , 300 MHz,  $\text{CDCl}_3$

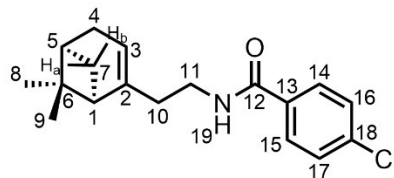
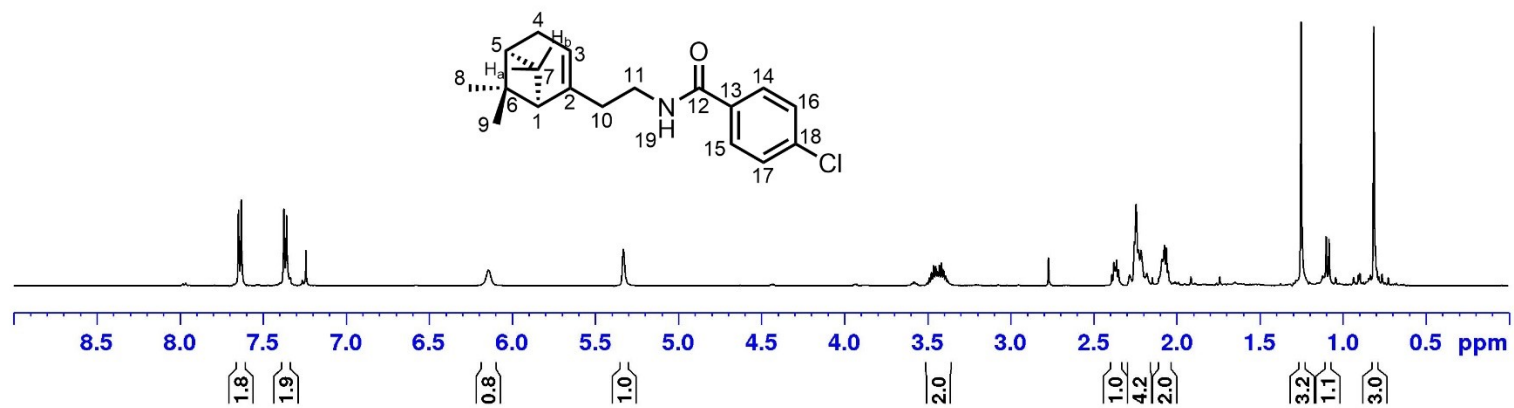


# Compound 17a

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

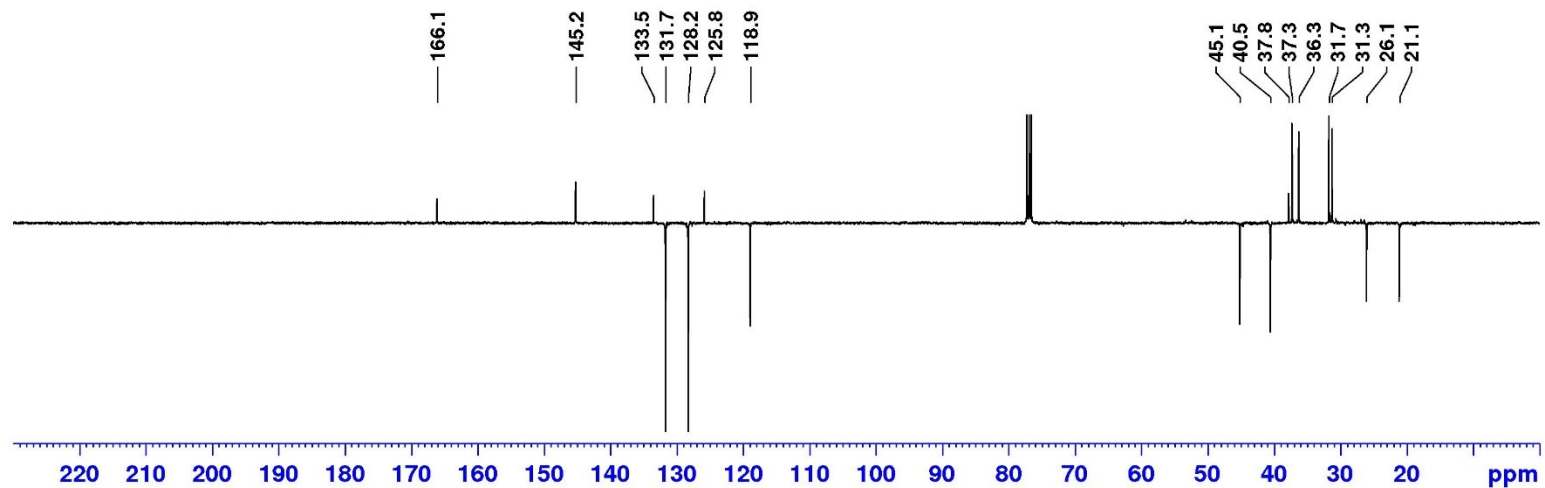


<sup>1</sup>H, 500 MHz, CDCl<sub>3</sub>

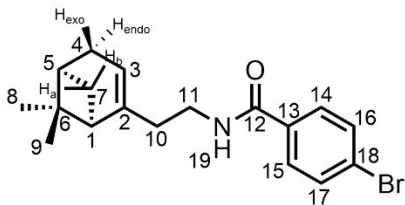
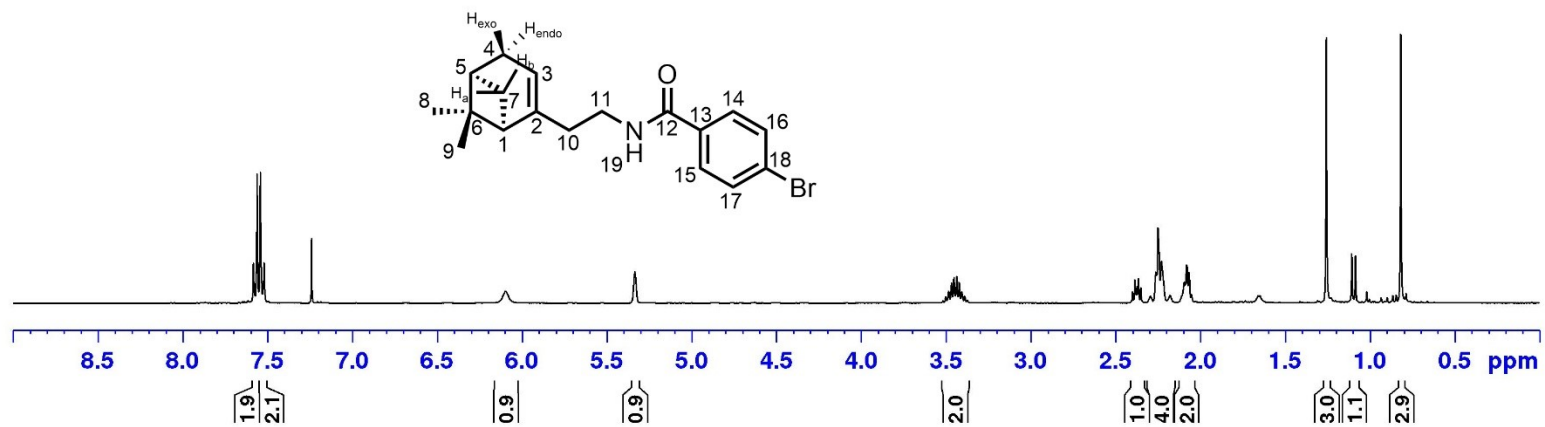


# Compound 17b

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

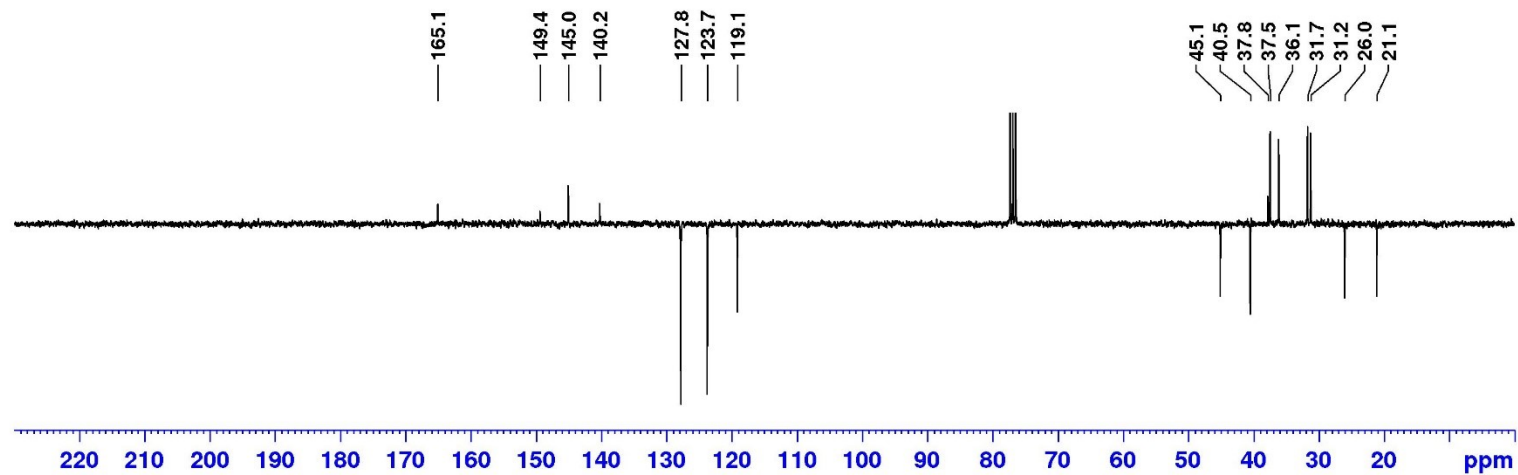


<sup>1</sup>H, 400 MHz, CDCl<sub>3</sub>

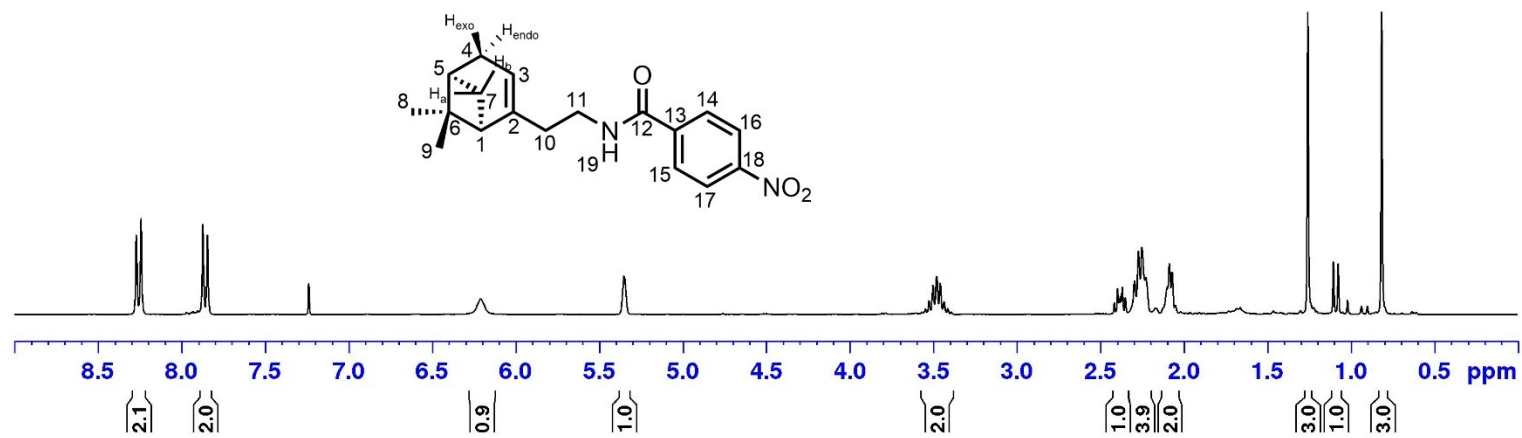


# Compound 17c

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

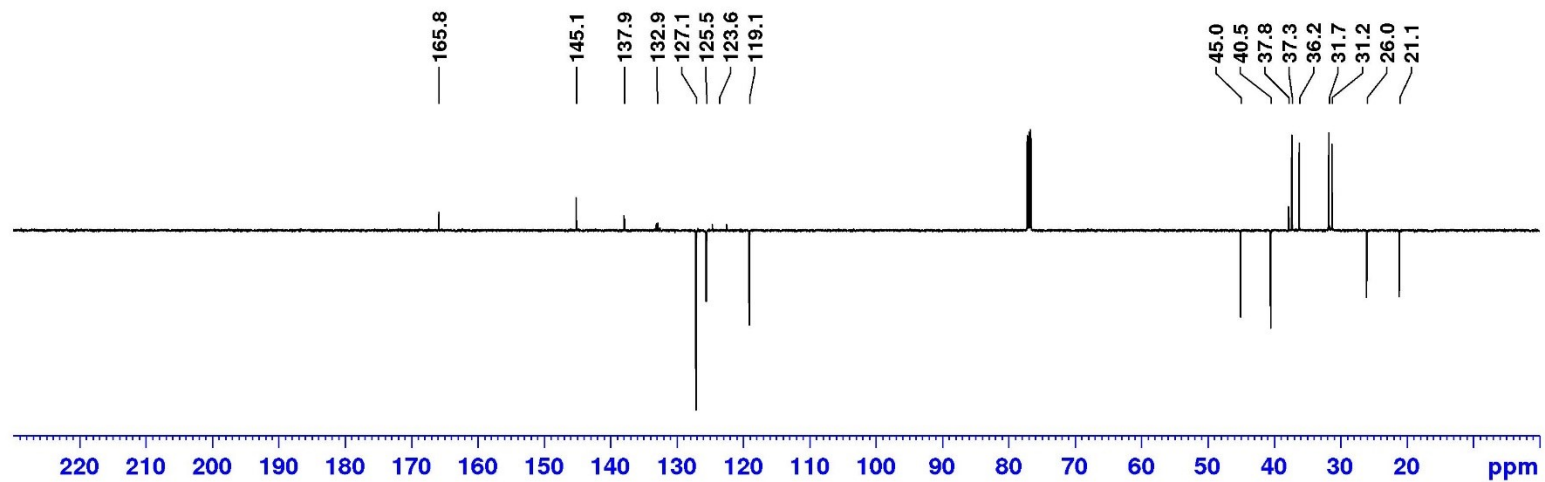


<sup>1</sup>H, 300 MHz, CDCl<sub>3</sub>

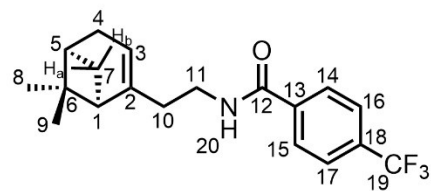
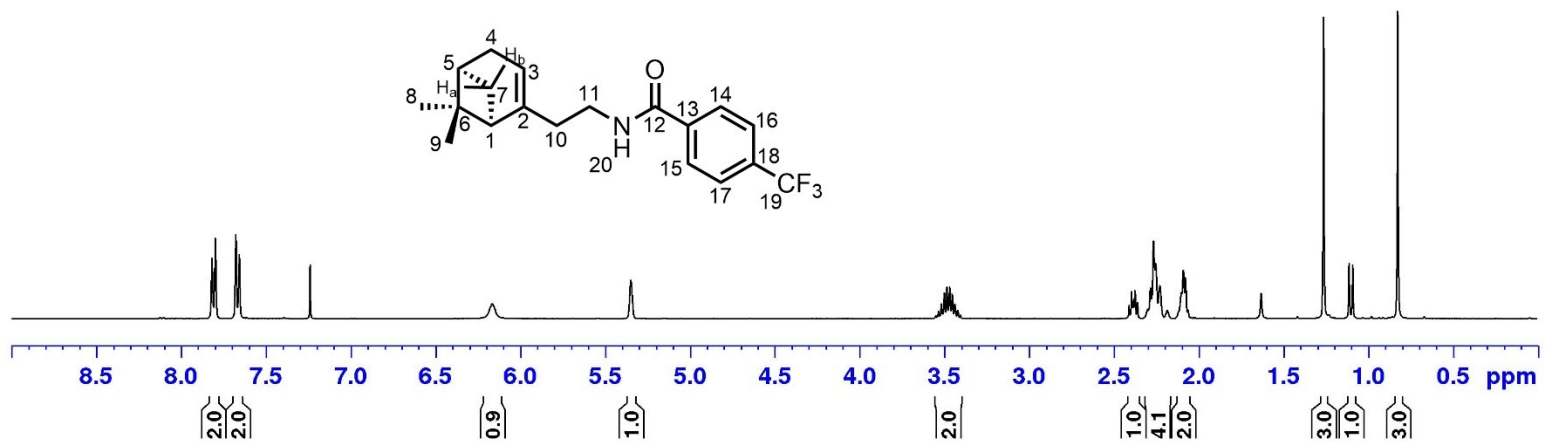


# Compound 17d

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

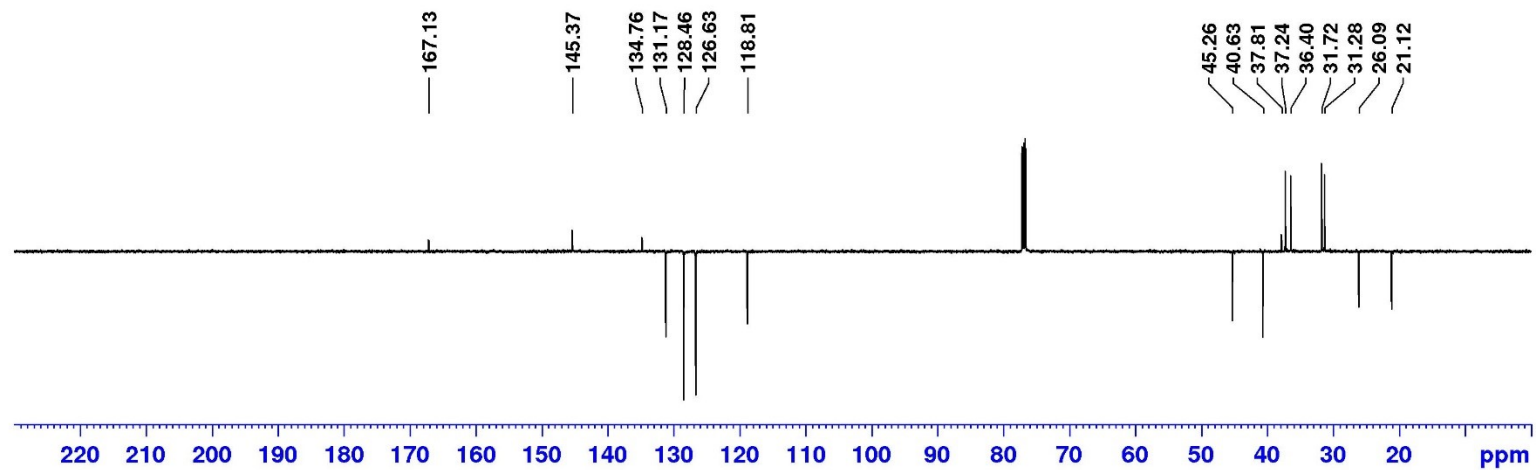


<sup>1</sup>H, 400 MHz, CDCl<sub>3</sub>

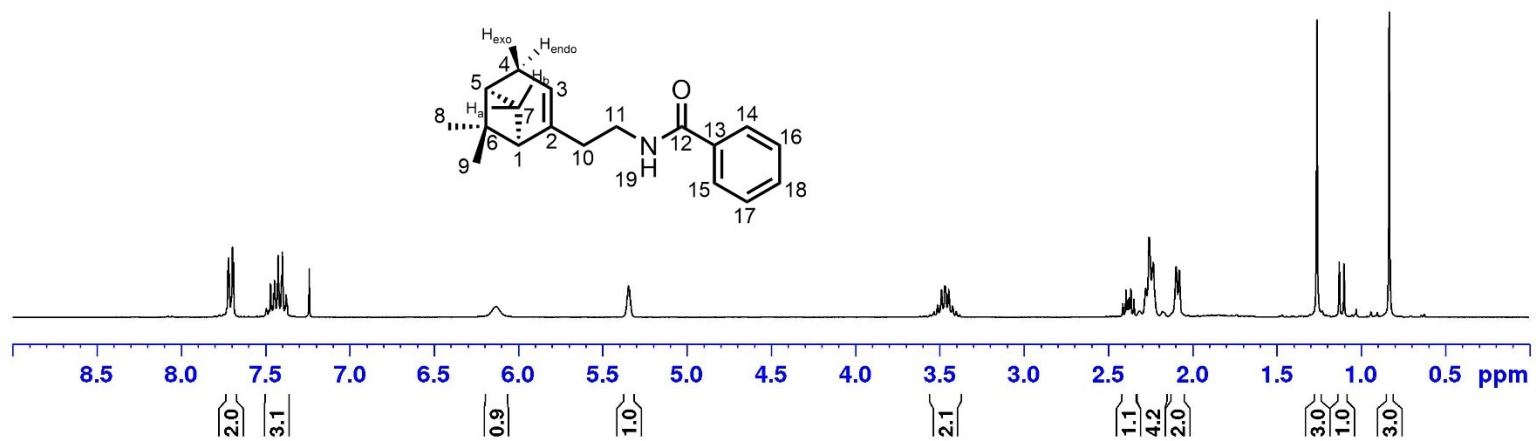


# Compound 17e

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

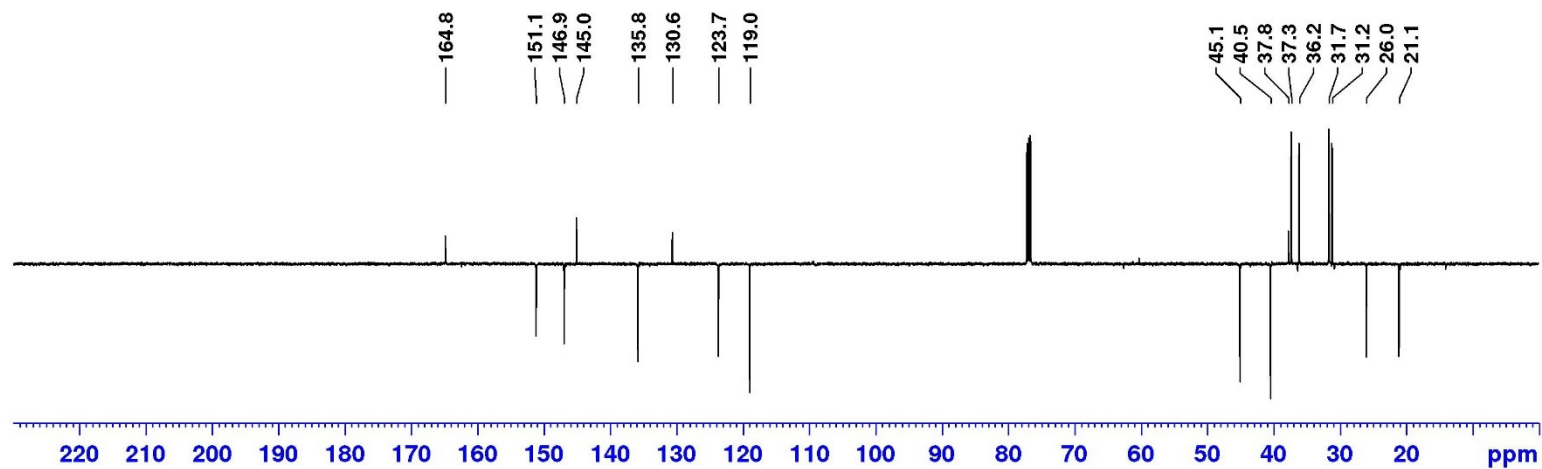


<sup>1</sup>H, 300 MHz, CDCl<sub>3</sub>

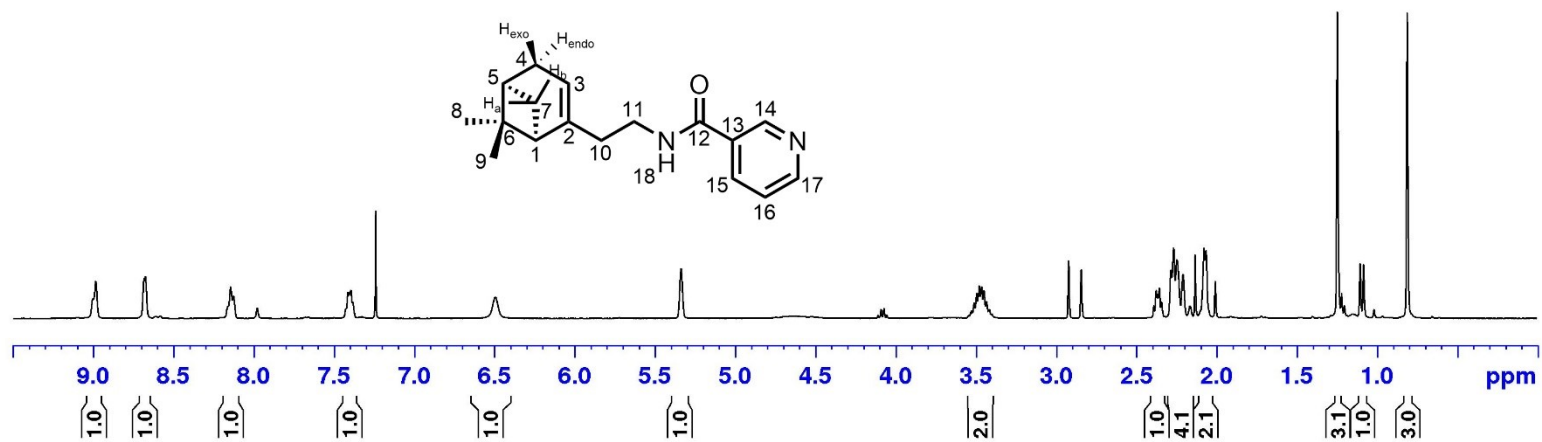


# Compound 17f

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

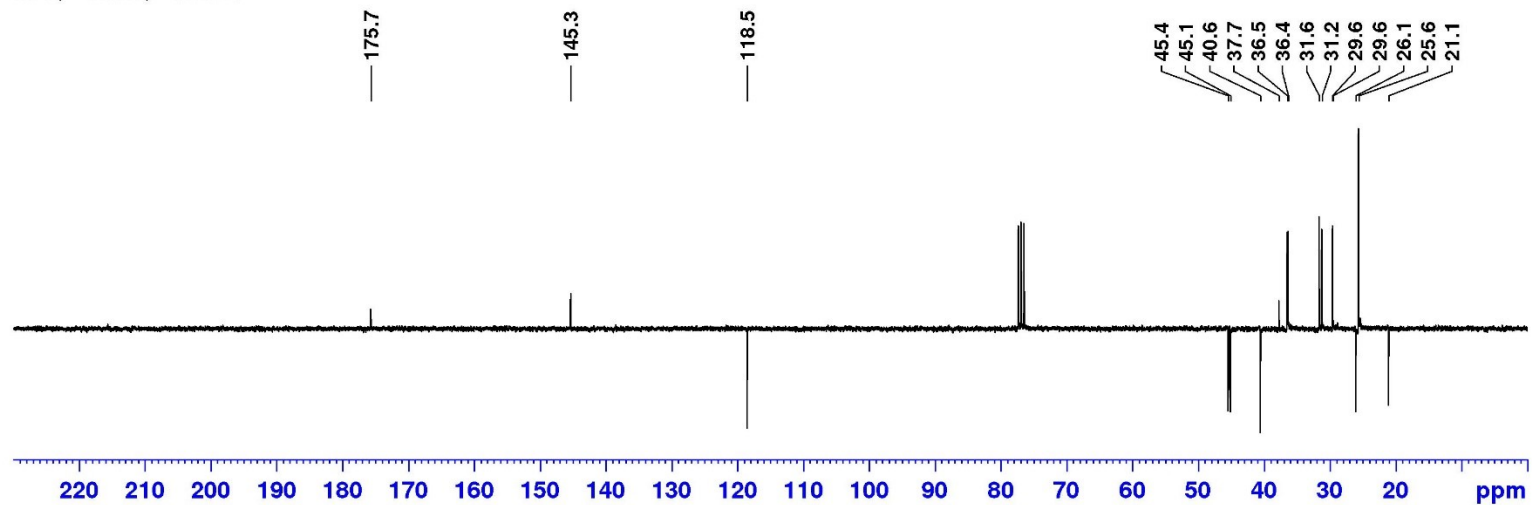


<sup>1</sup>H, 400 MHz, CDCl<sub>3</sub>

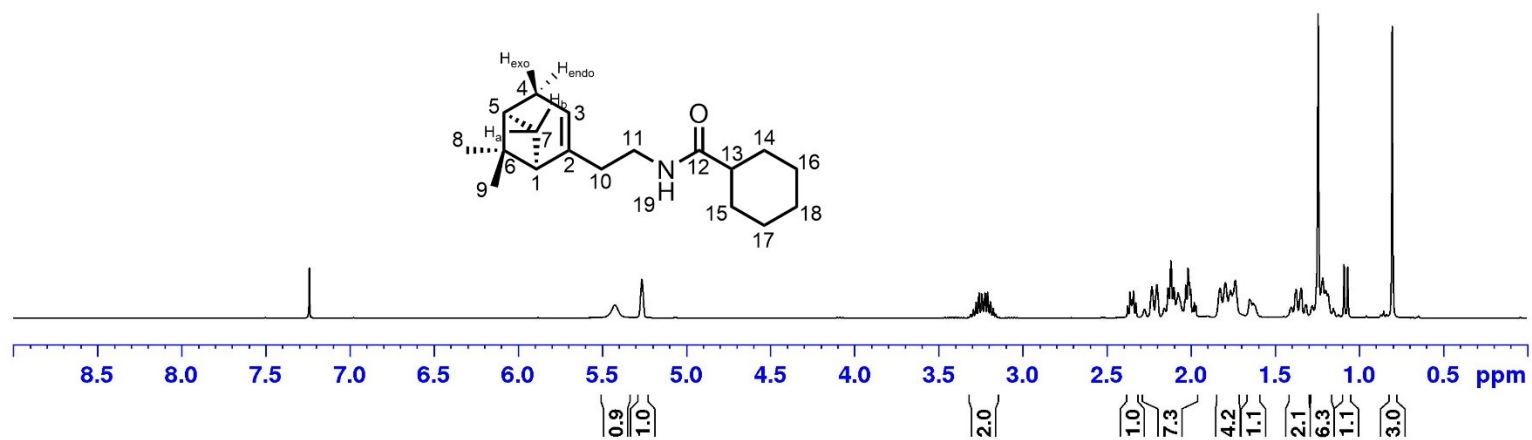


# Compound 17g

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

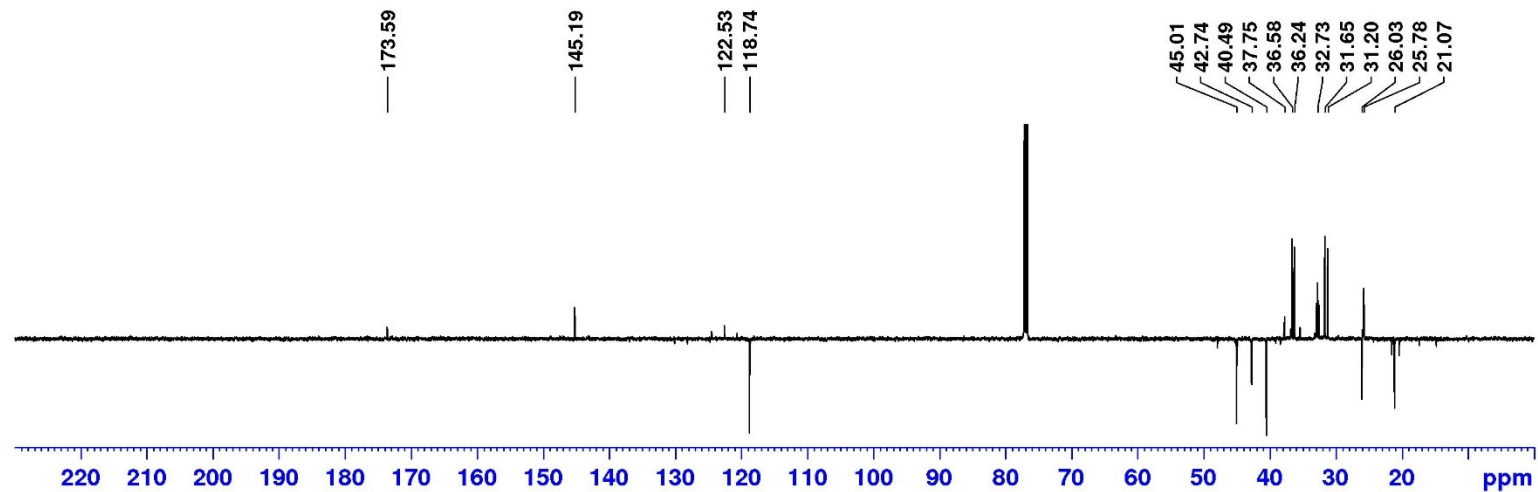


$^1\text{H}$ , 400 MHz,  $\text{CDCl}_3$

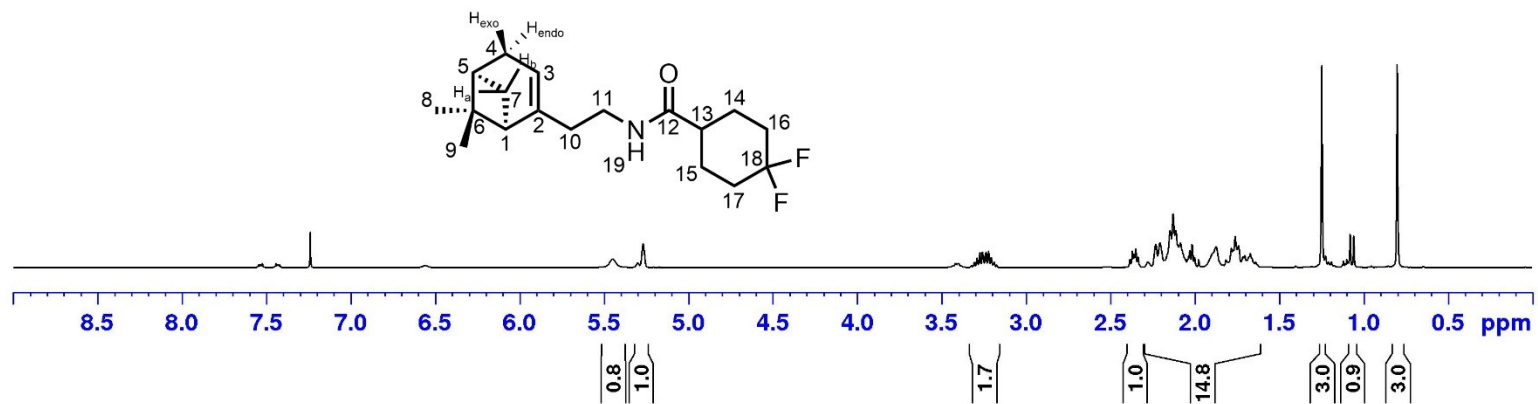


# Compound 17h

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

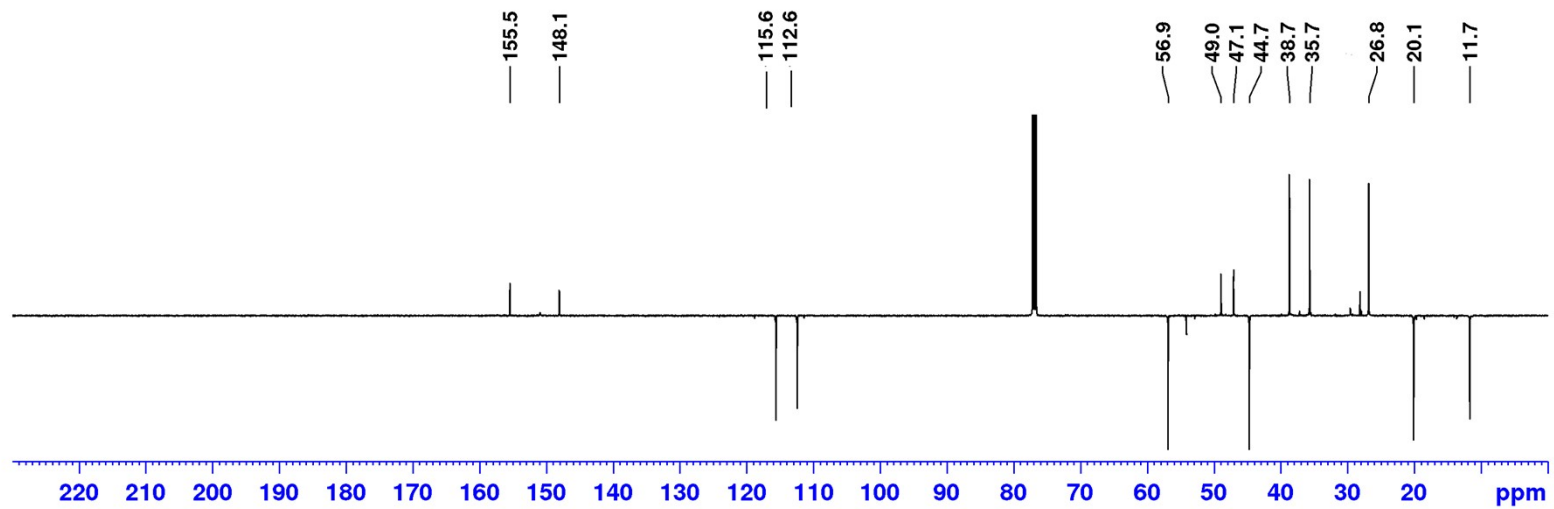


<sup>1</sup>H, 400 MHz, CDCl<sub>3</sub>

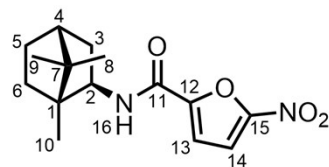
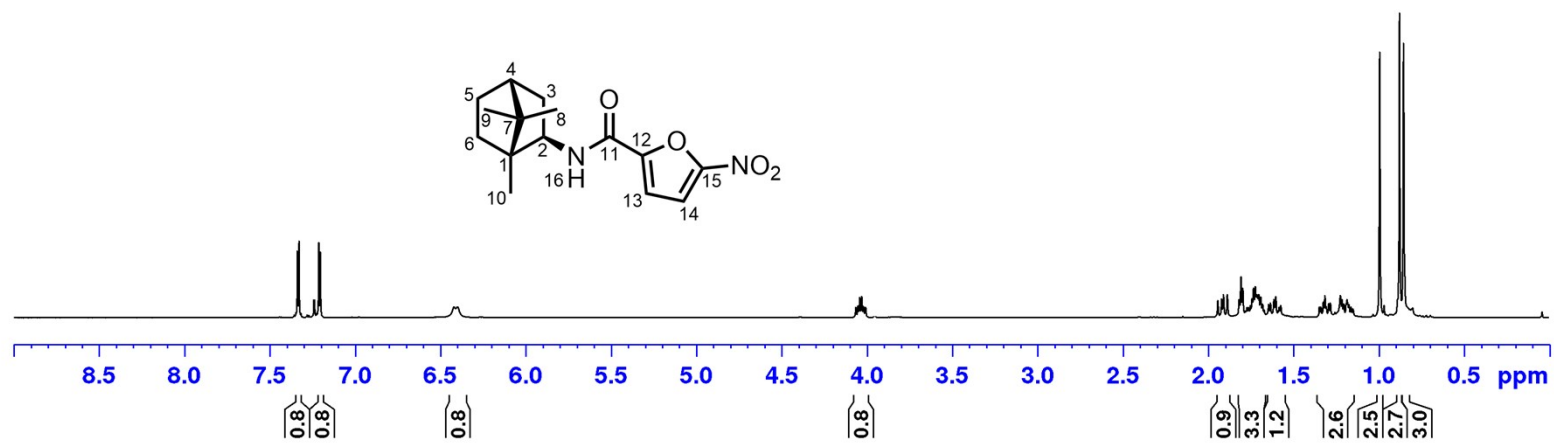


# Compound 20a

<sup>13</sup>C, JMOD, CDC13

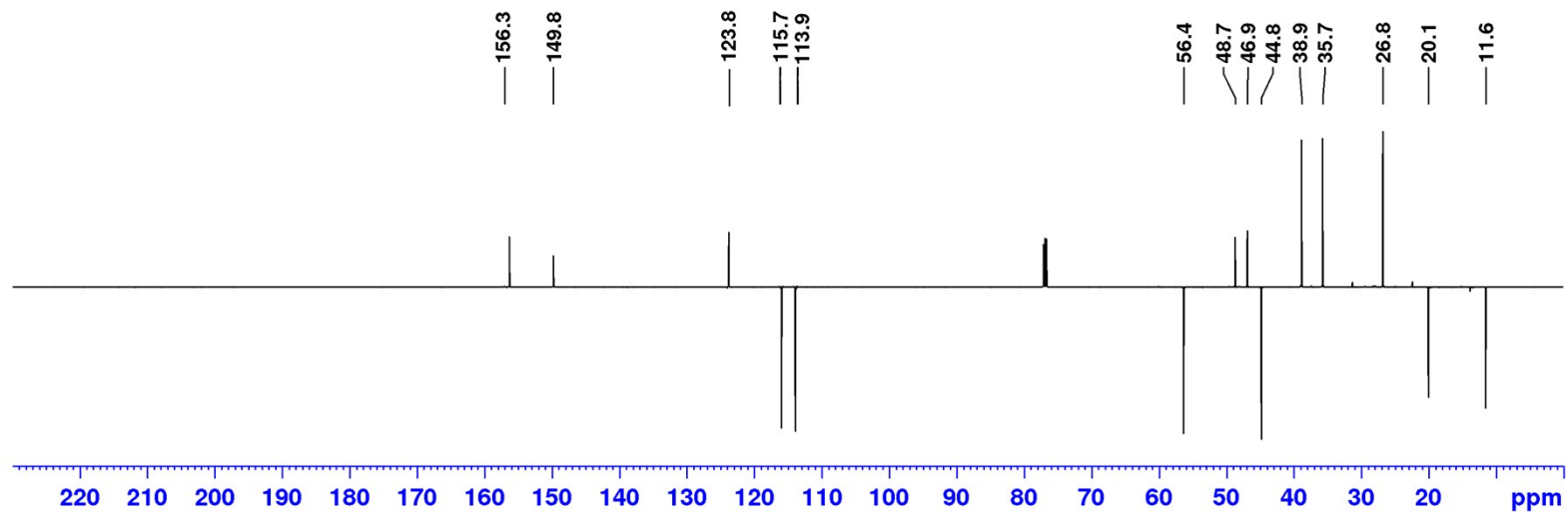


<sup>1</sup>H, 400MHz, CDC13

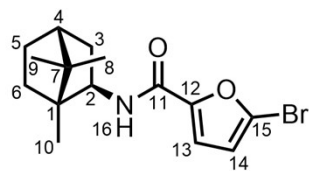
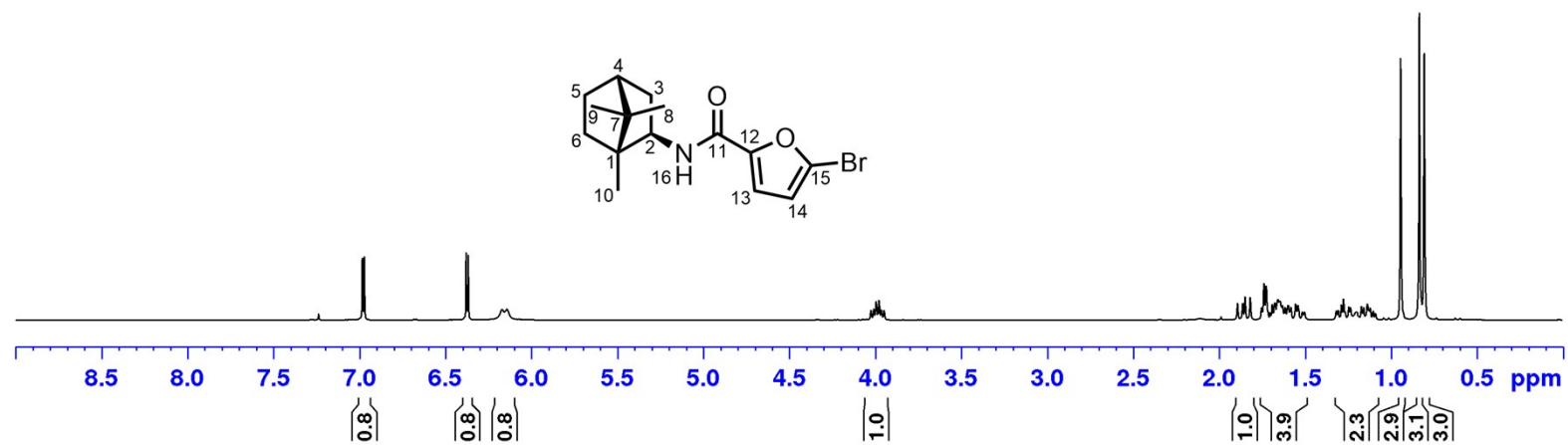


# Compound 20b

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

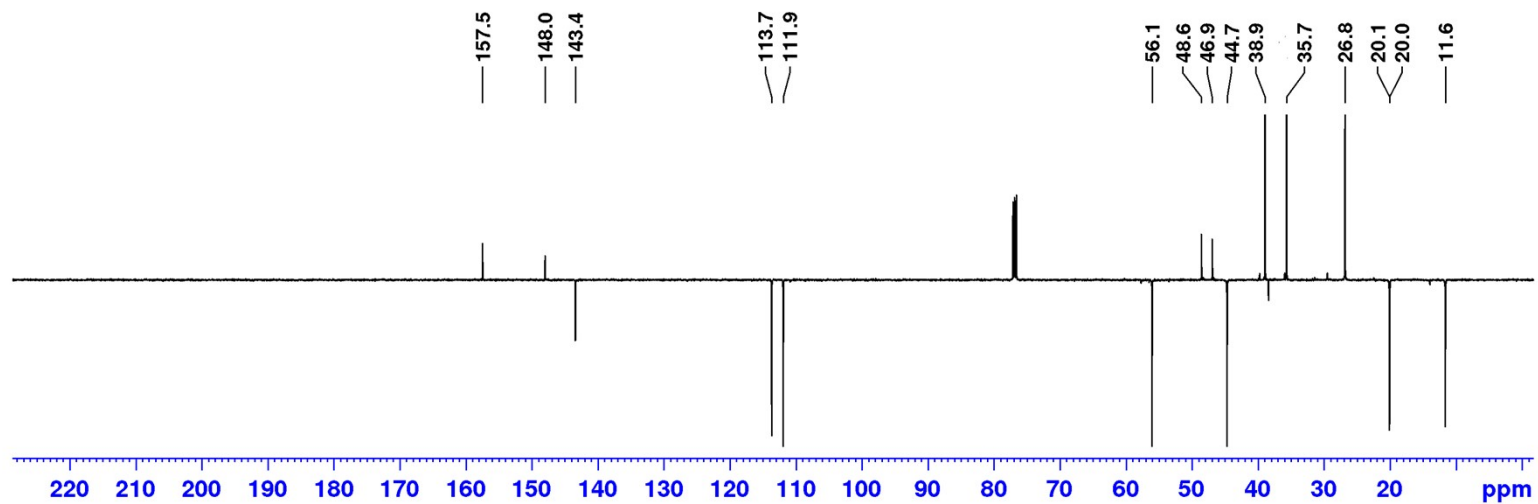


<sup>1</sup>H, 300MHz, CDCl<sub>3</sub>

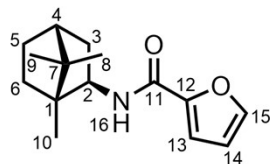
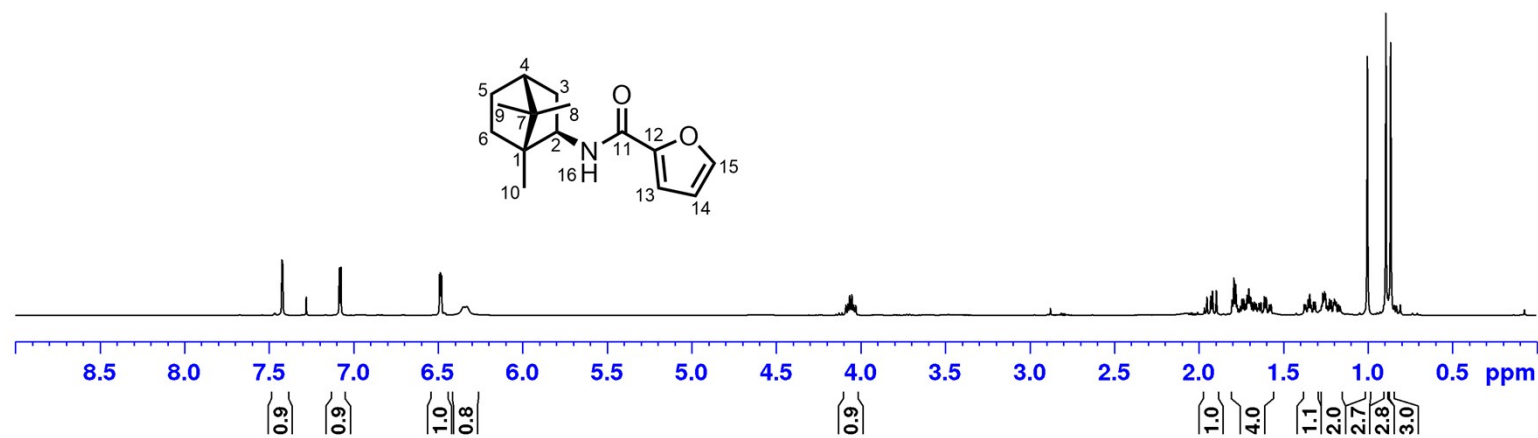


# Compound 20c

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

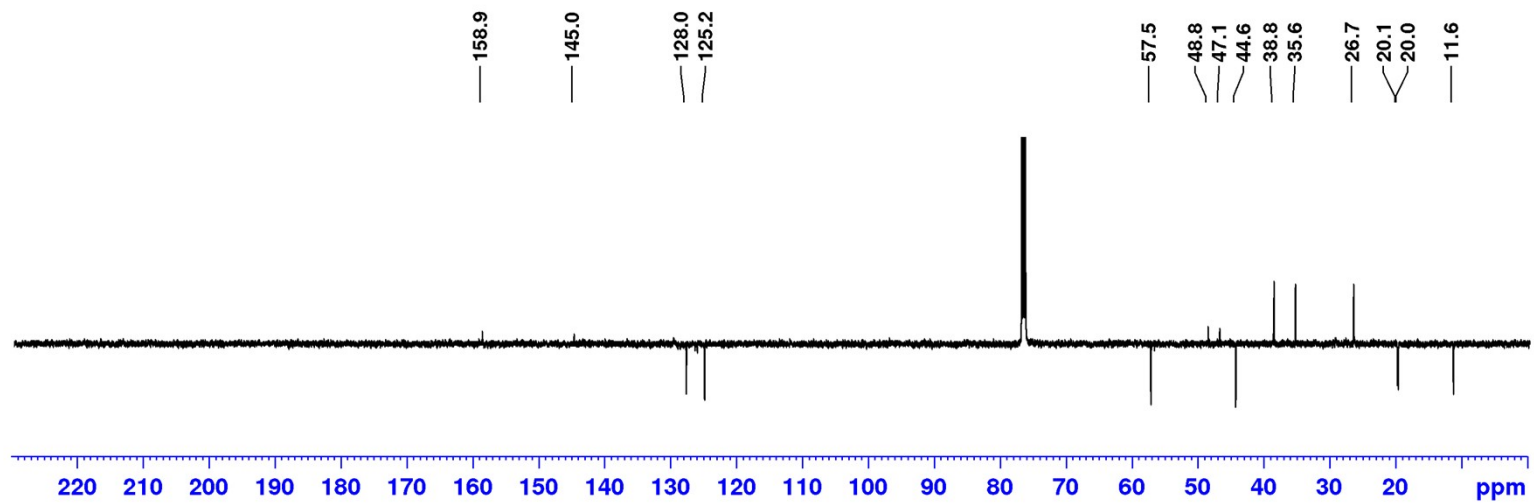


$^1\text{H}$ , 400 MHz,  $\text{CDCl}_3$

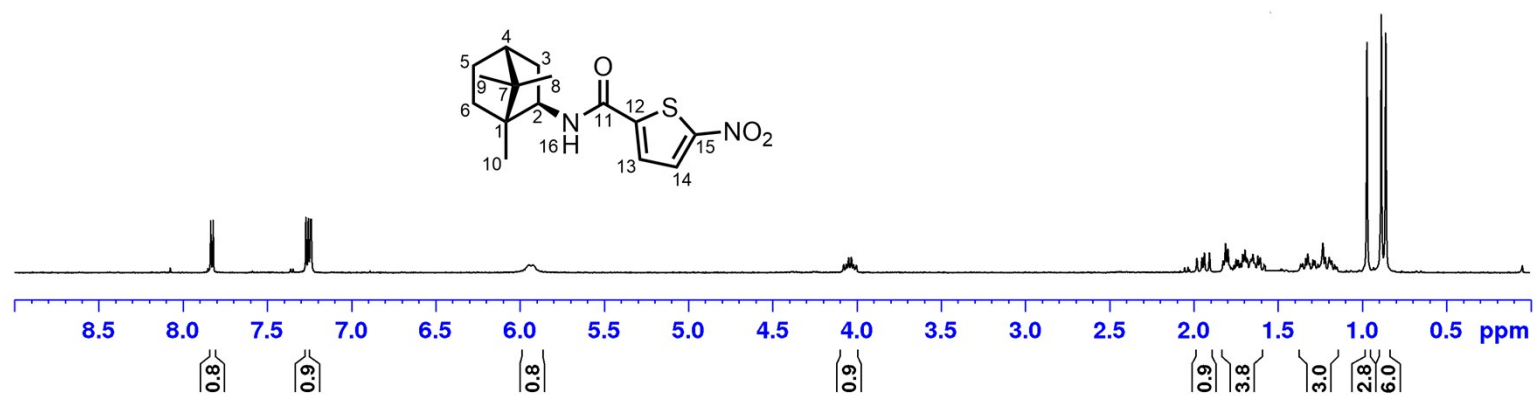


# Compound 20d

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

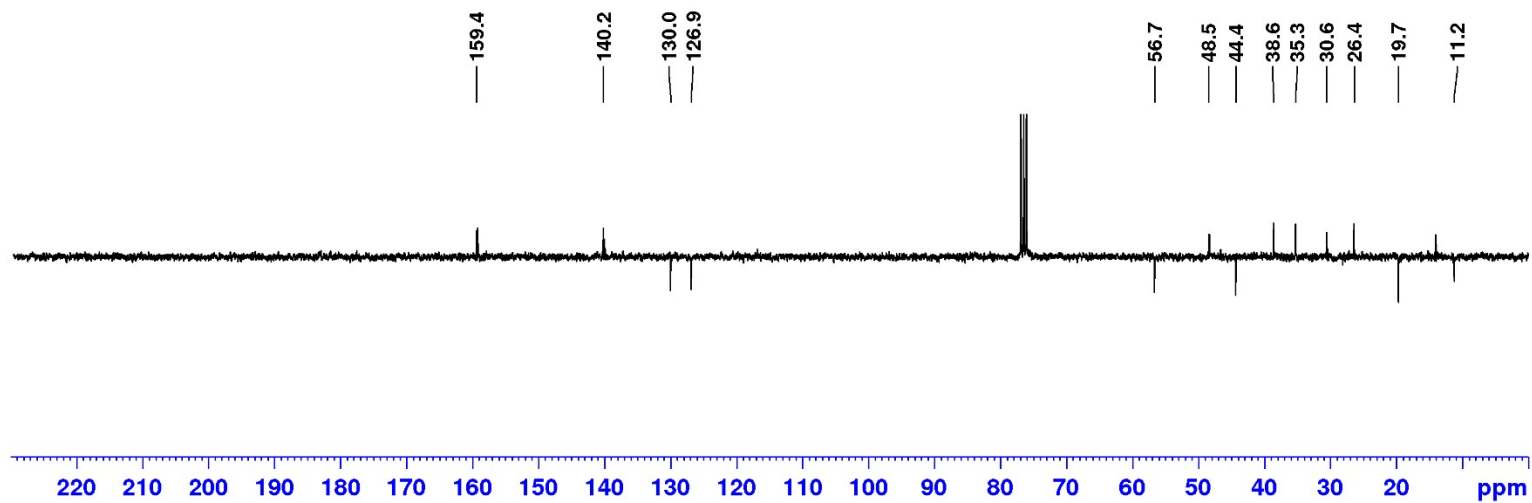


<sup>1</sup>H, 300MHz, CDCl<sub>3</sub>

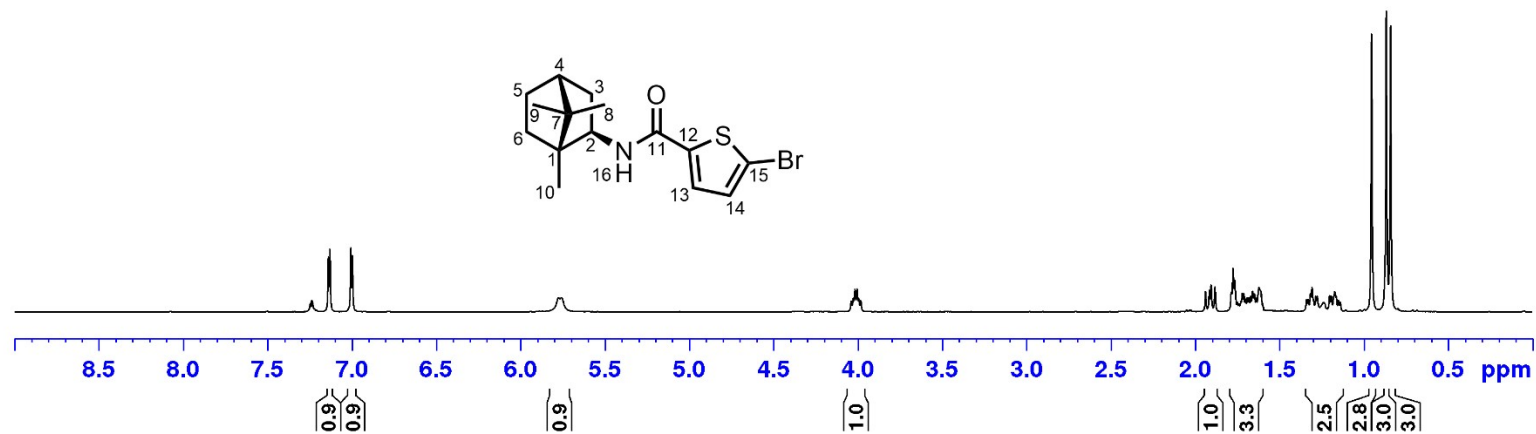


# Compound 20e

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

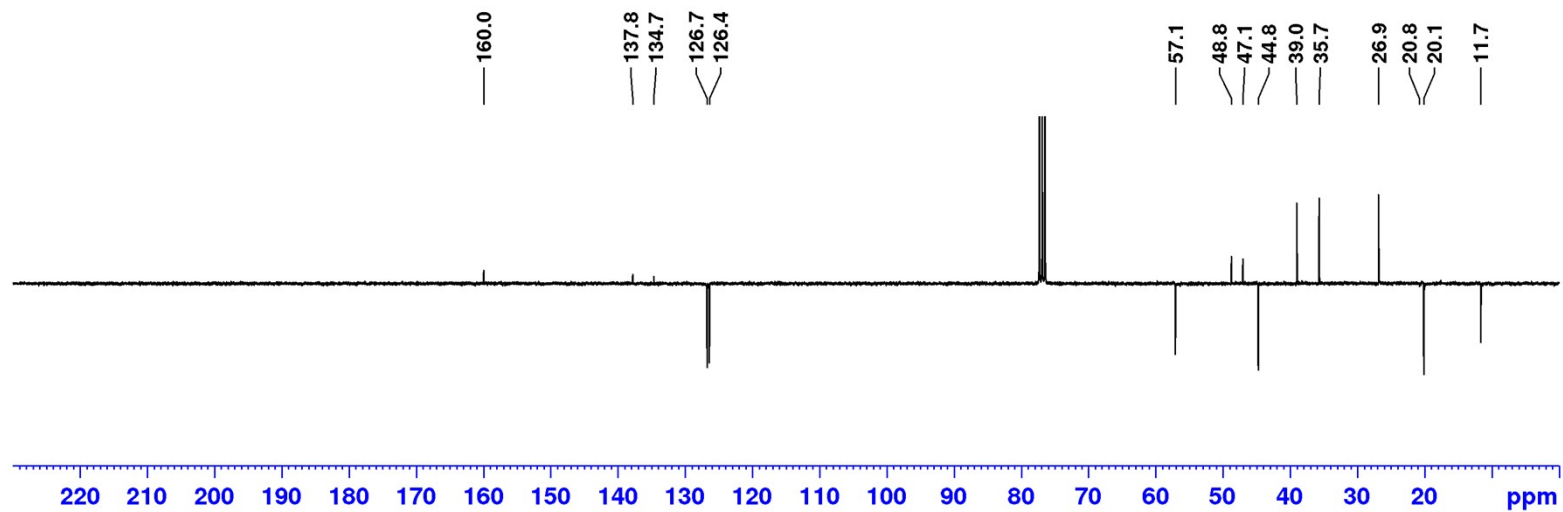


$^1\text{H}$ , 400MHz,  $\text{CDCl}_3$

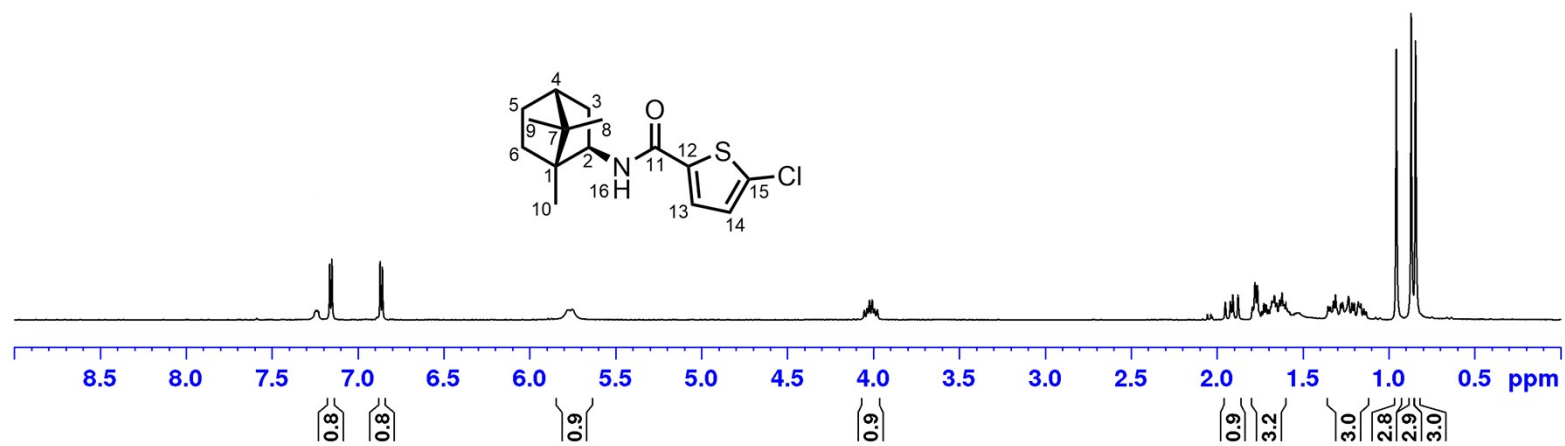


# Compound 20f

<sup>13</sup>C, JMOD, CDC13

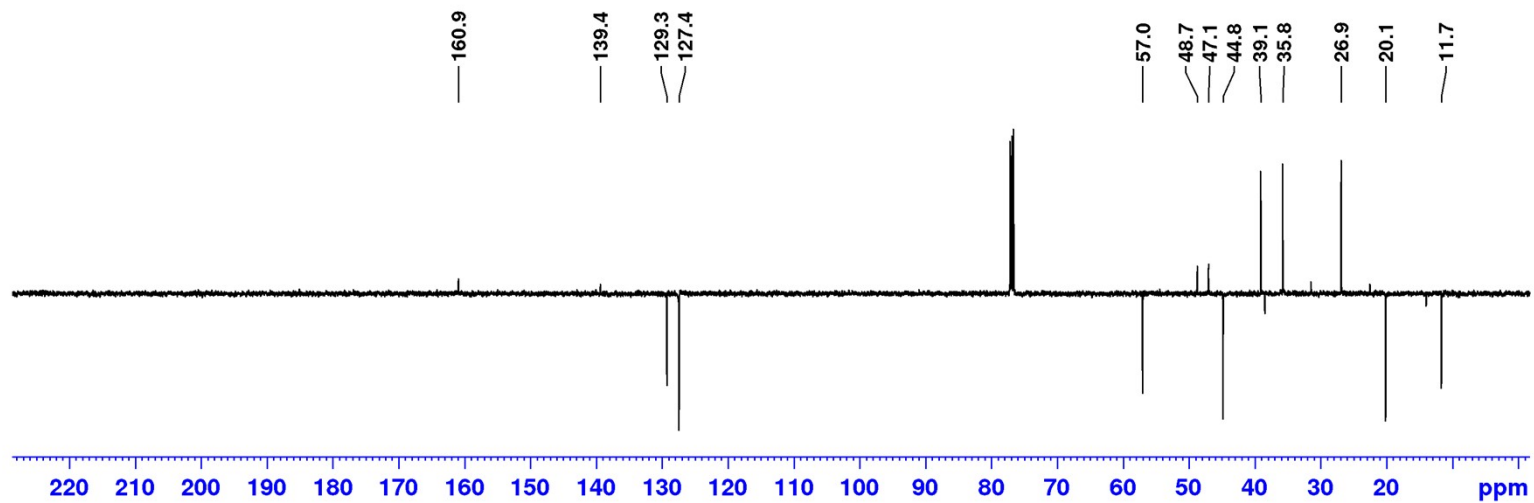


<sup>1</sup>H, 300MHz, CDC13

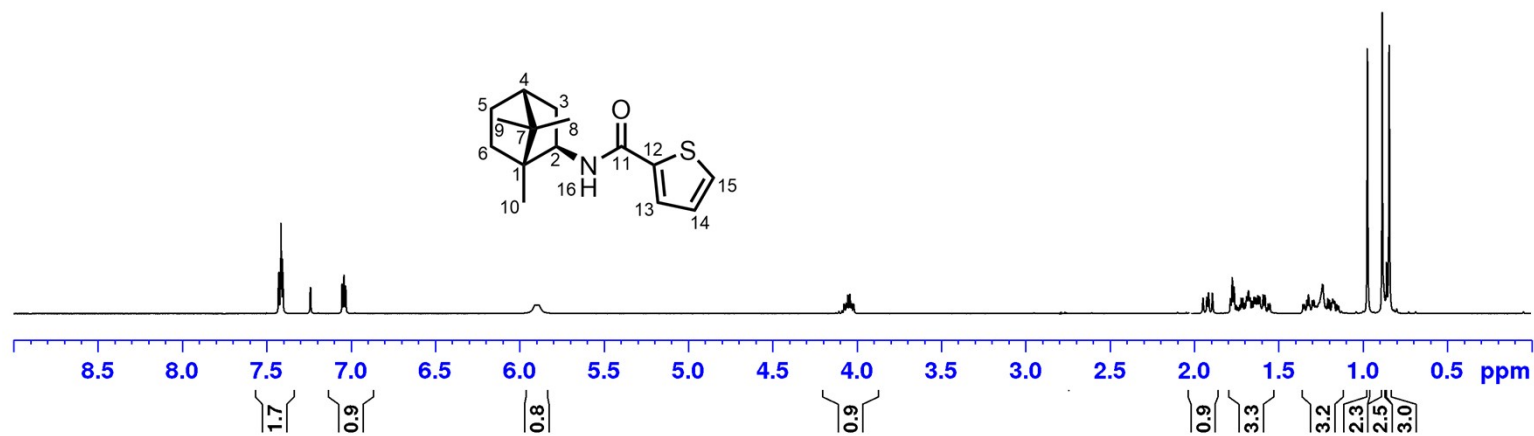


# Compound 20g

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

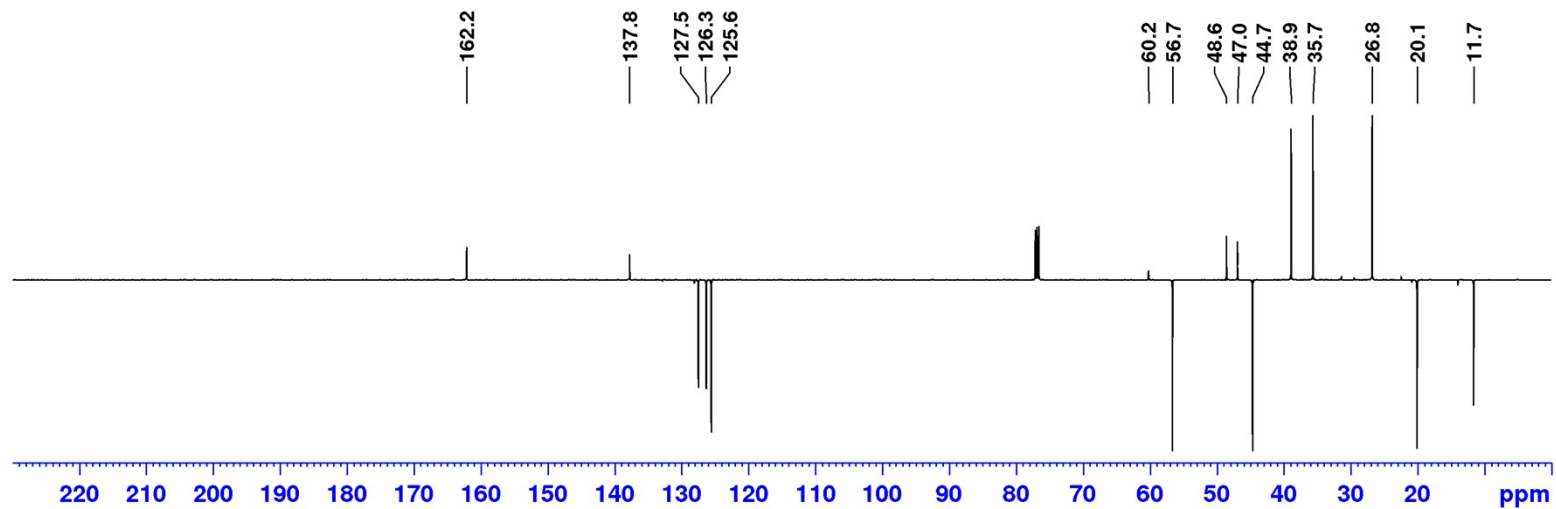


<sup>1</sup>H, 400MHz, CDCl<sub>3</sub>

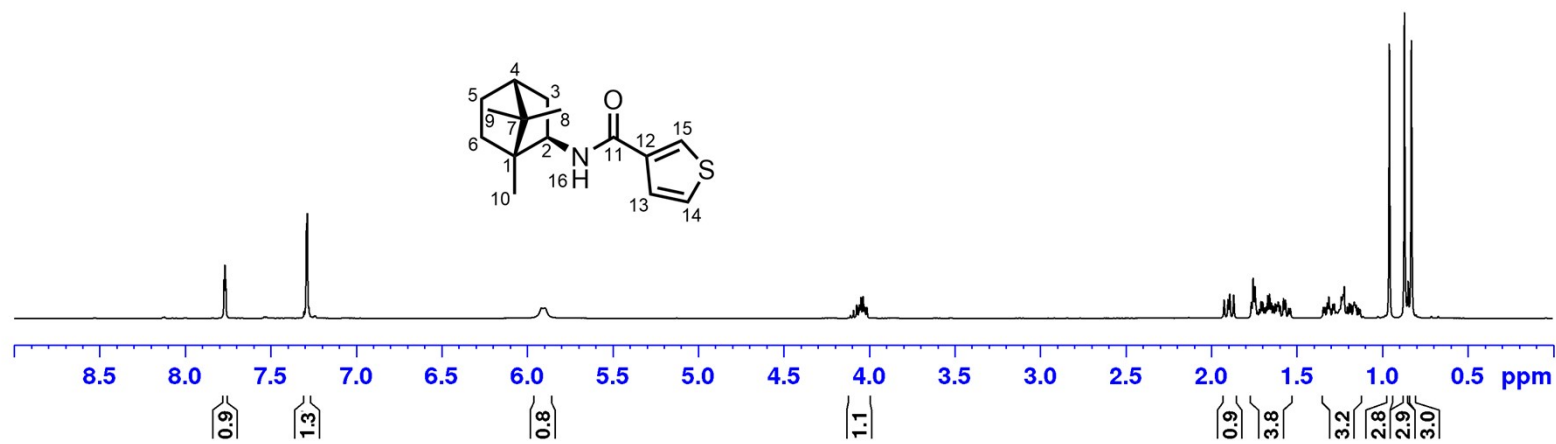


# Compound 20h

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

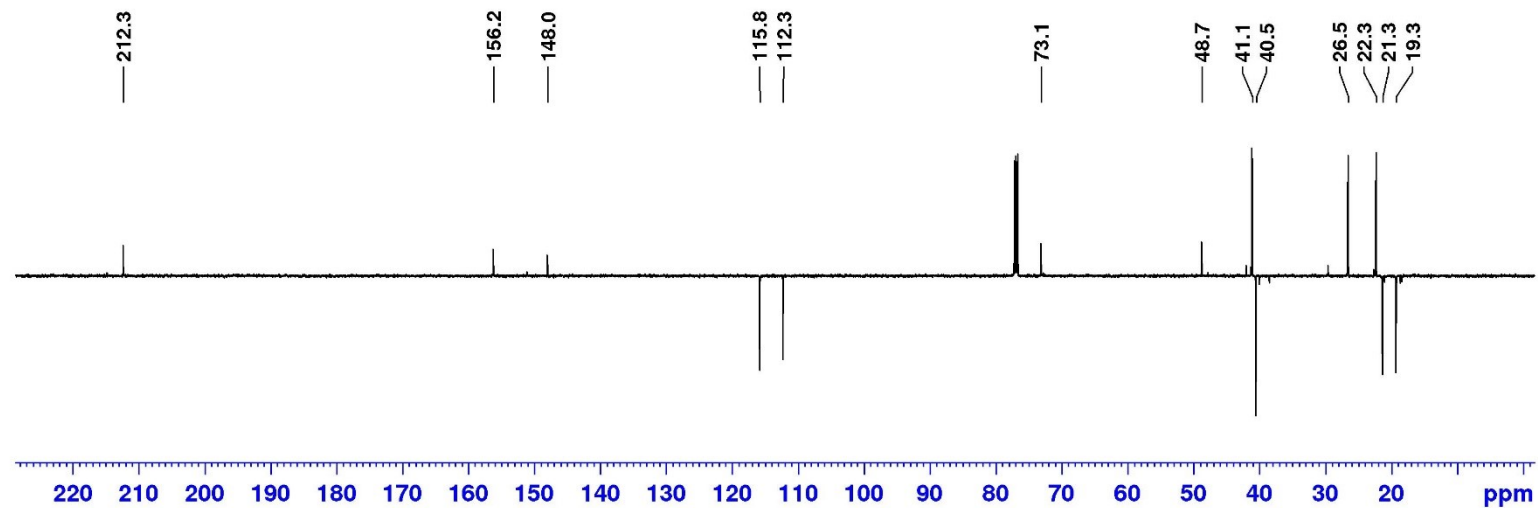


$^1\text{H}$ , 400MHz,  $\text{CDCl}_3$

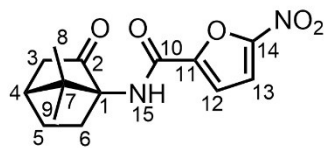
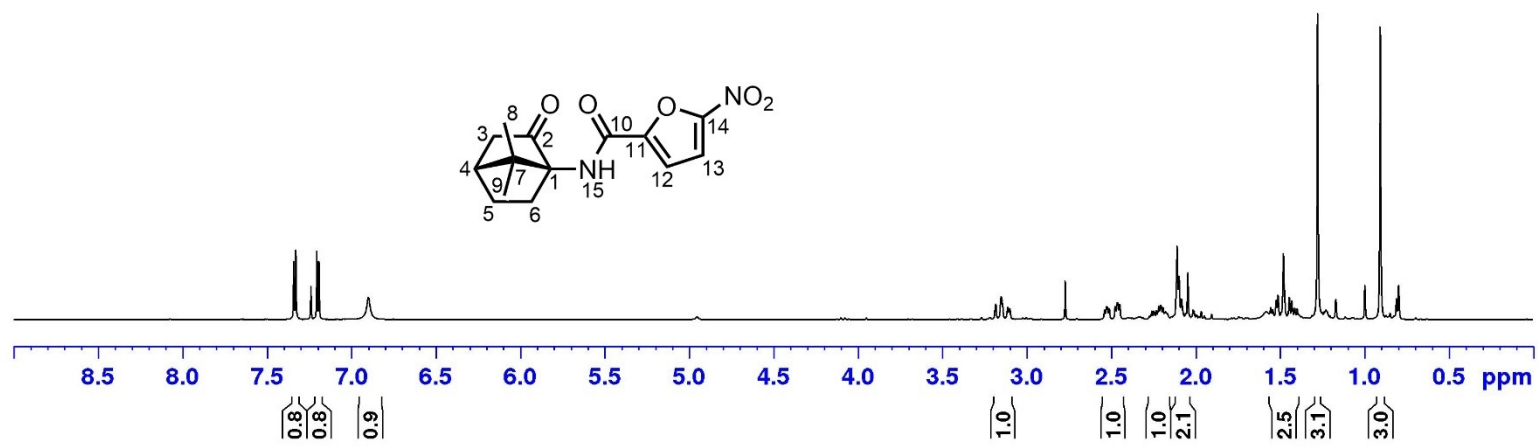


# Compound 21a

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

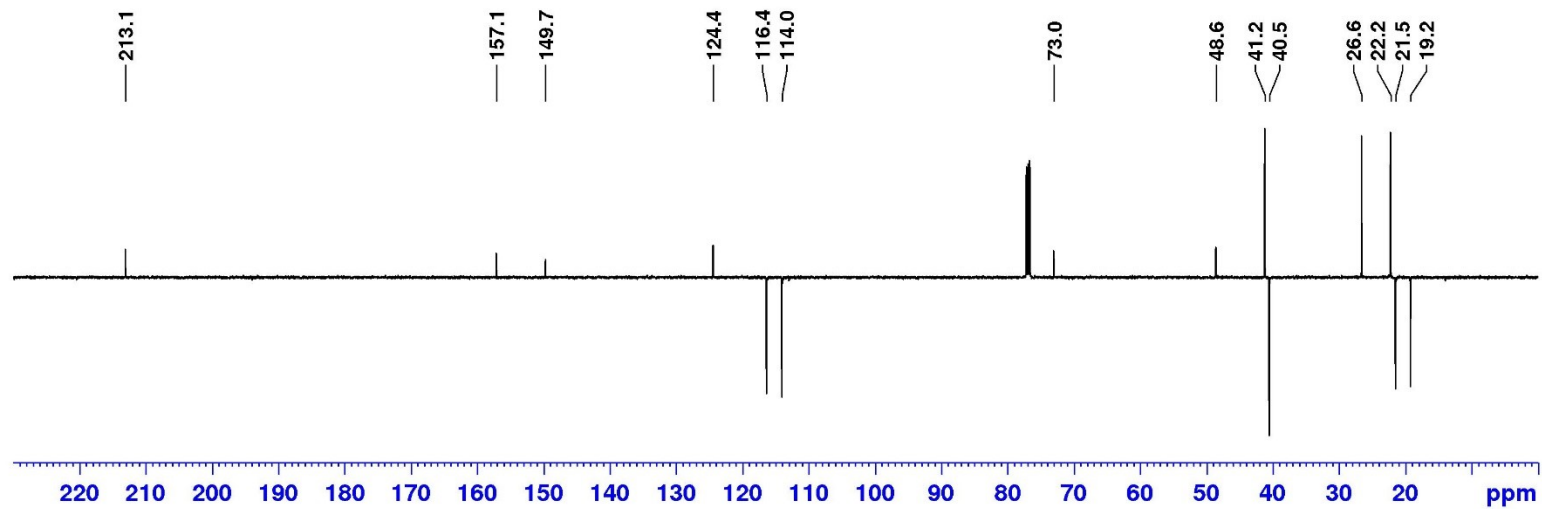


<sup>1</sup>H, 300 MHz, CDCl<sub>3</sub>

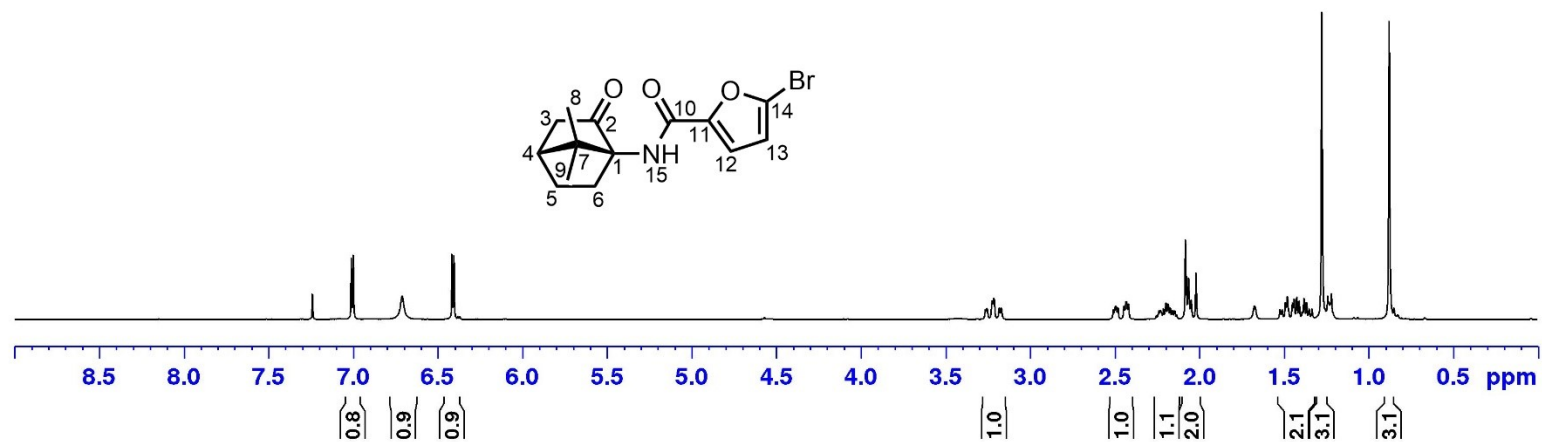


# Compound 21b

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

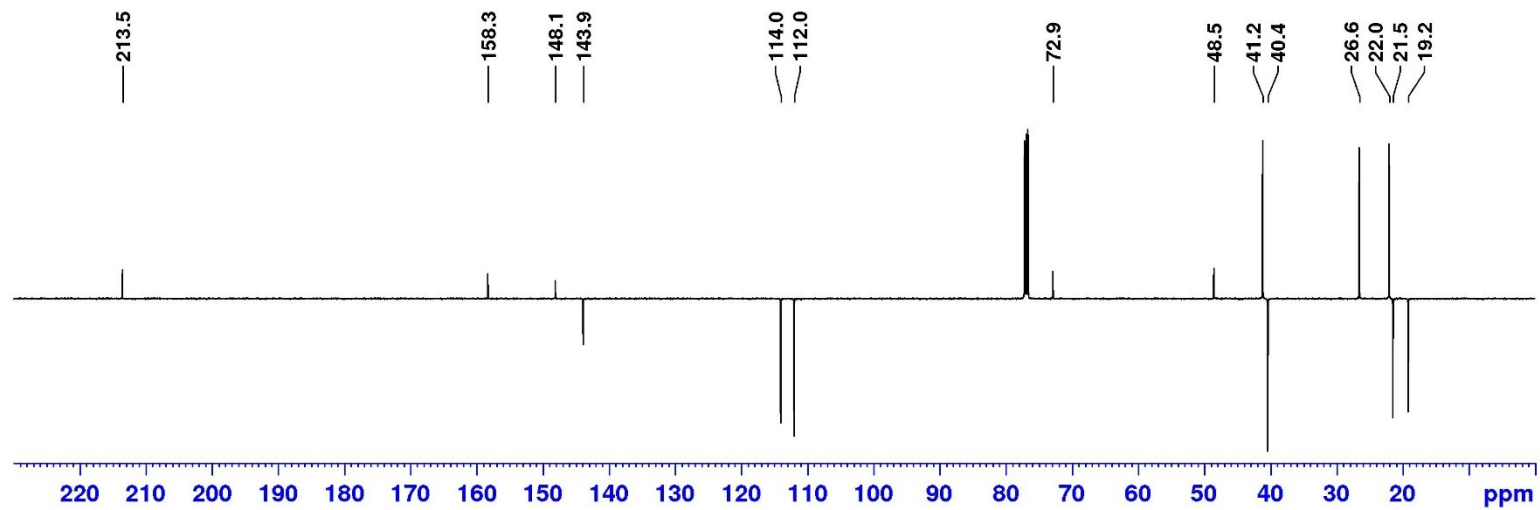


$^1\text{H}$ , 300 MHz,  $\text{CDCl}_3$

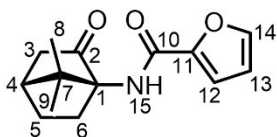
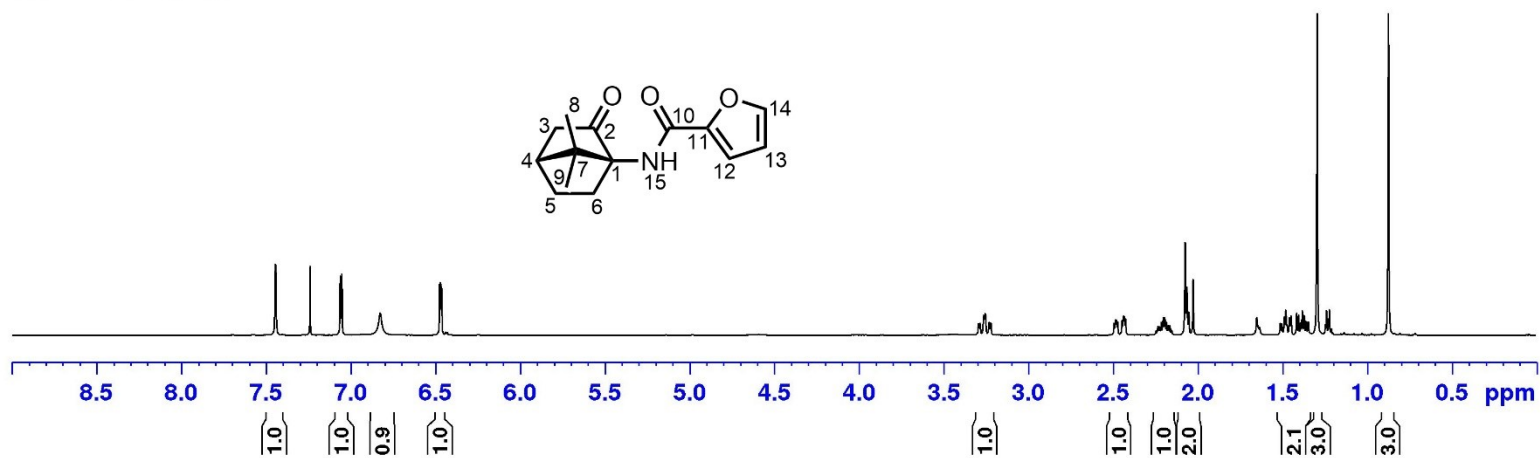


# Compound 21c

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

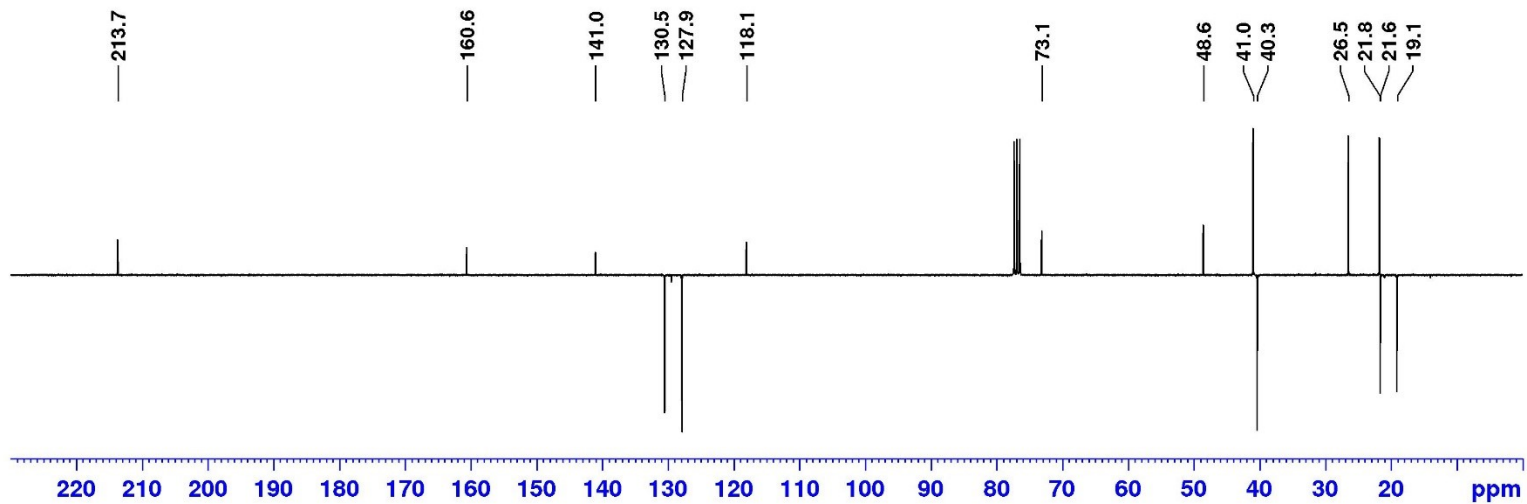


$^1\text{H}$ , 400 MHz,  $\text{CDCl}_3$

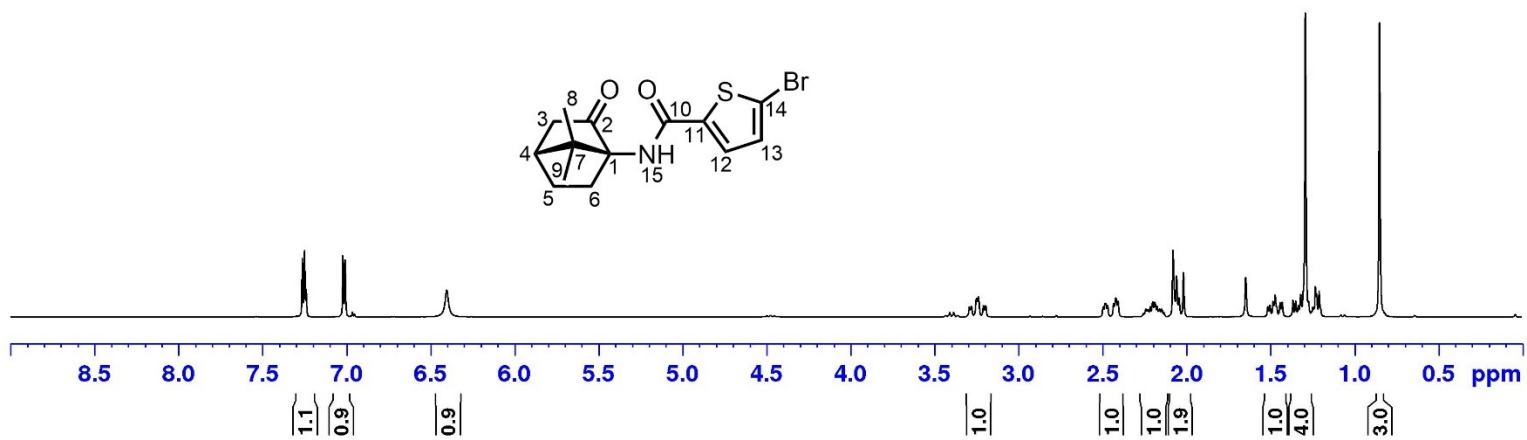


# Compound 21d

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

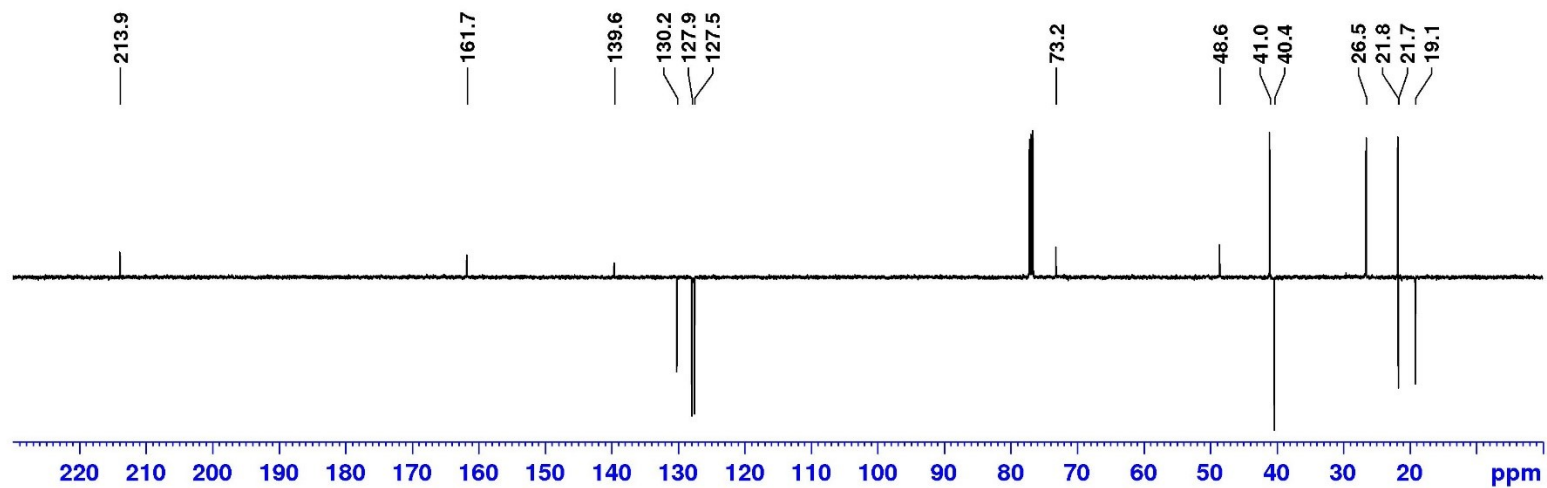


$^1\text{H}$ , 300 MHz,  $\text{CDCl}_3$

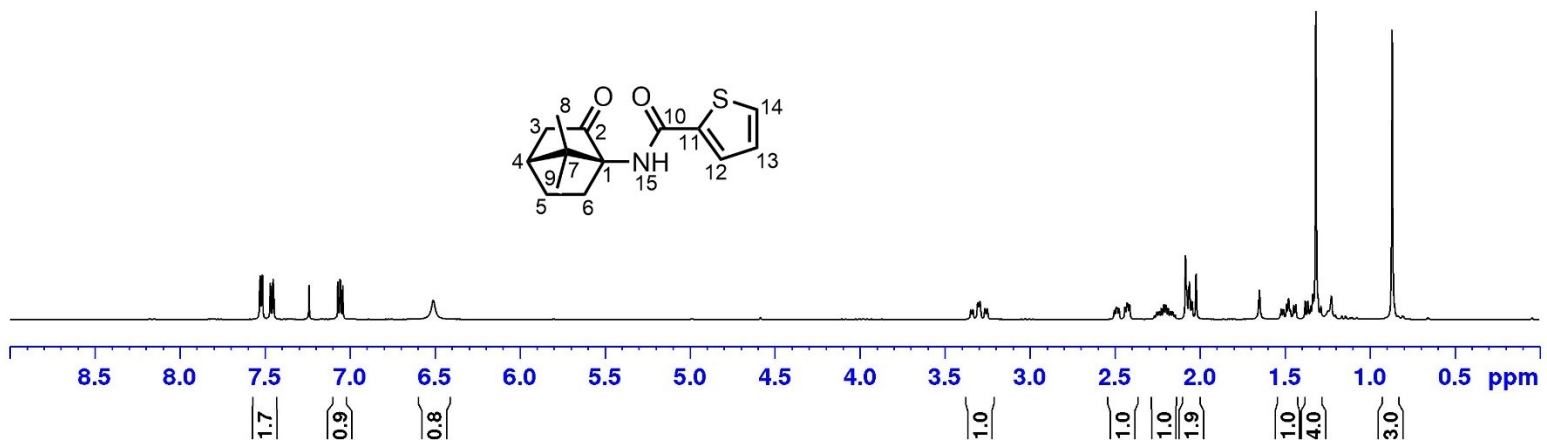


# Compound 21e

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

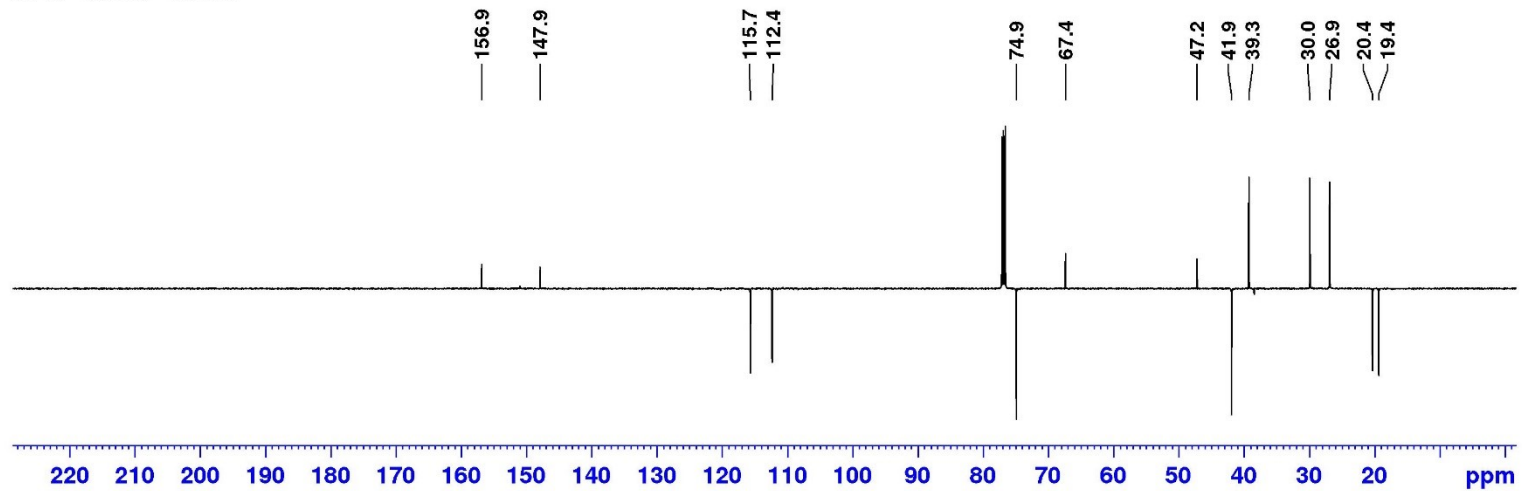


$^1\text{H}$ , 300 MHz,  $\text{CDCl}_3$

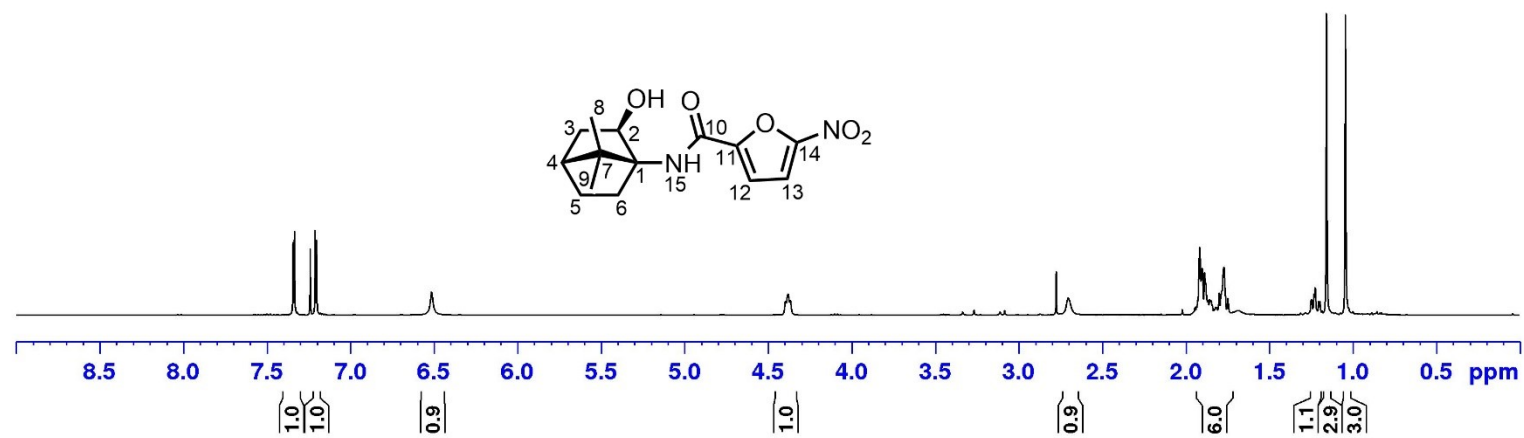


# Compound 22a

$^{13}\text{C}$ , JMOD,  $\text{CDCl}_3$

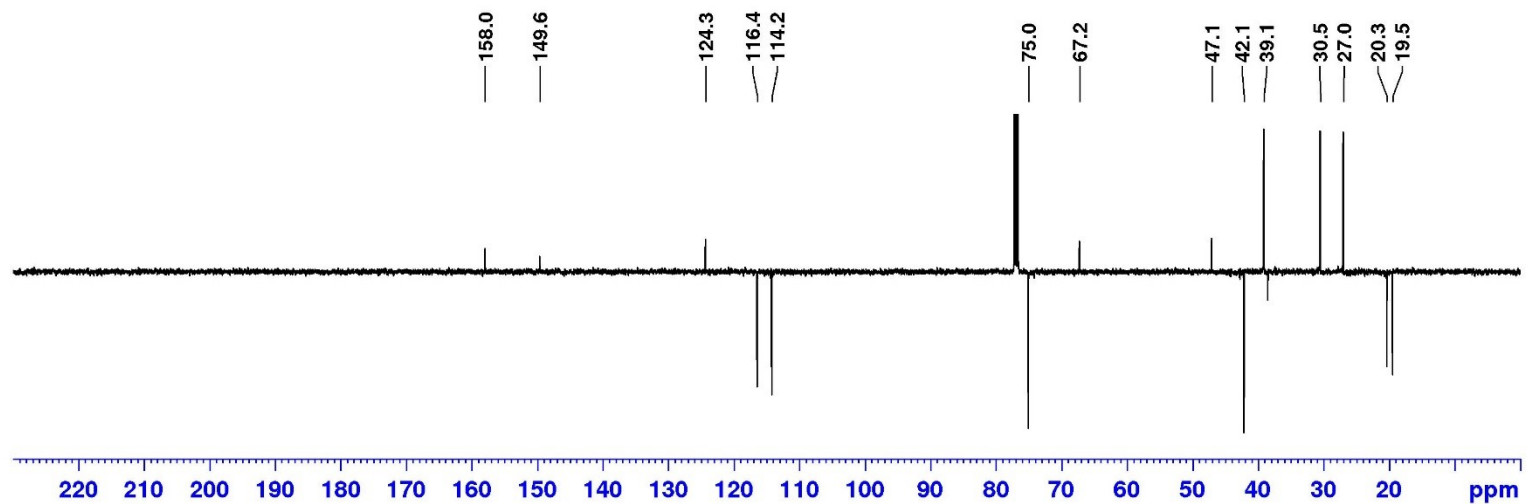


$^1\text{H}$ , 400 MHz,  $\text{CDCl}_3$

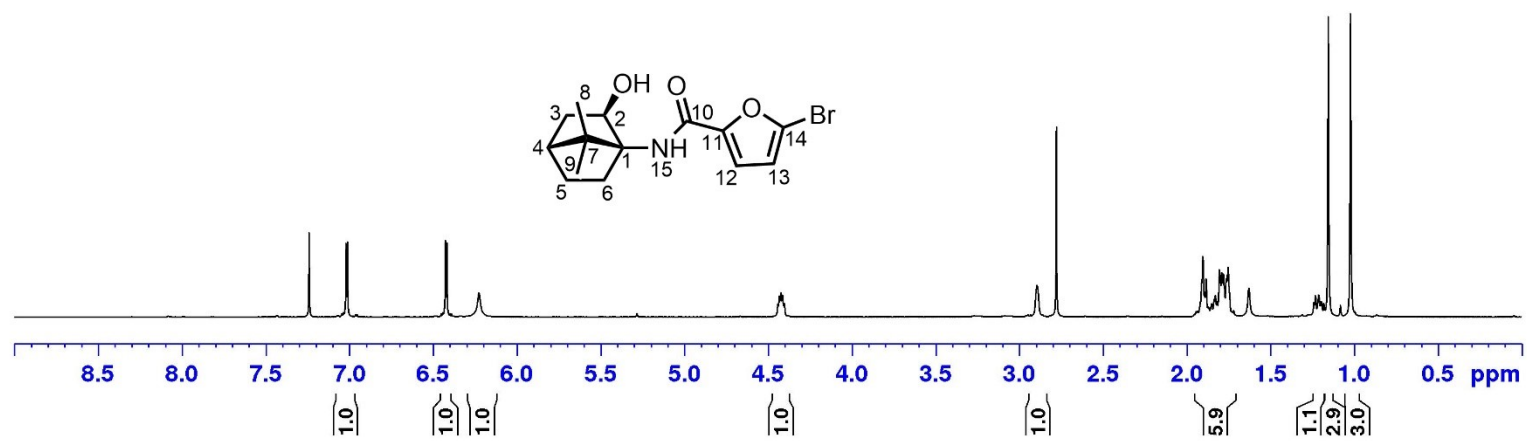


# Compound 22b

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

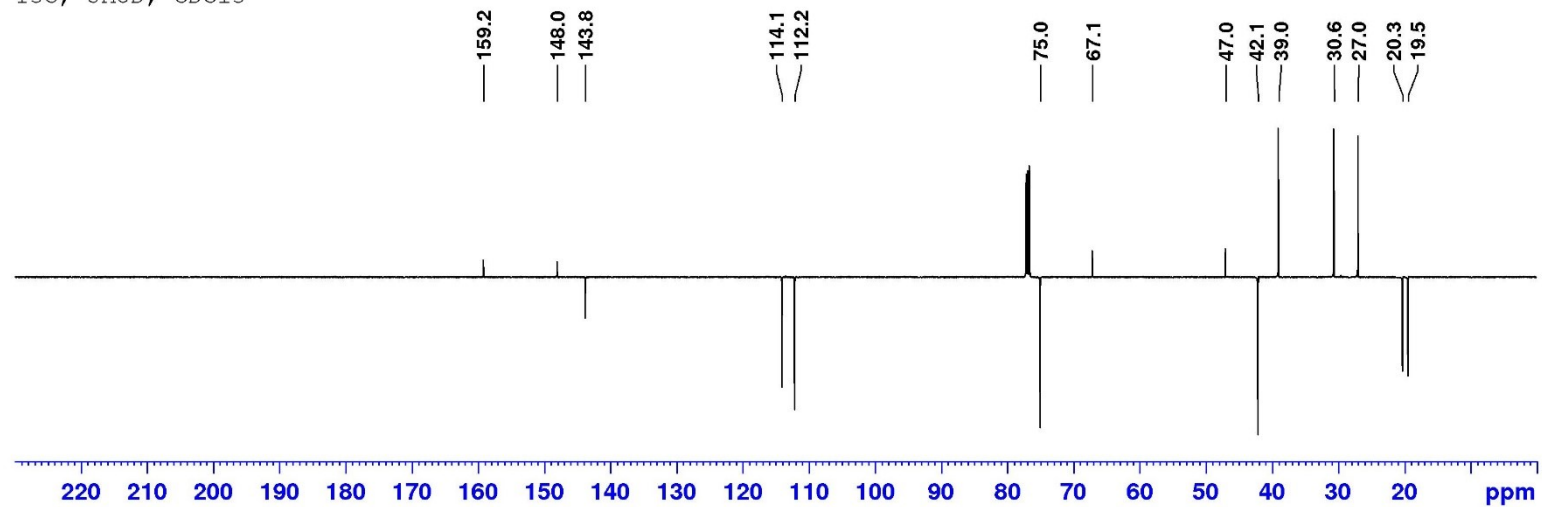


<sup>1</sup>H, 400 MHz, CDCl<sub>3</sub>

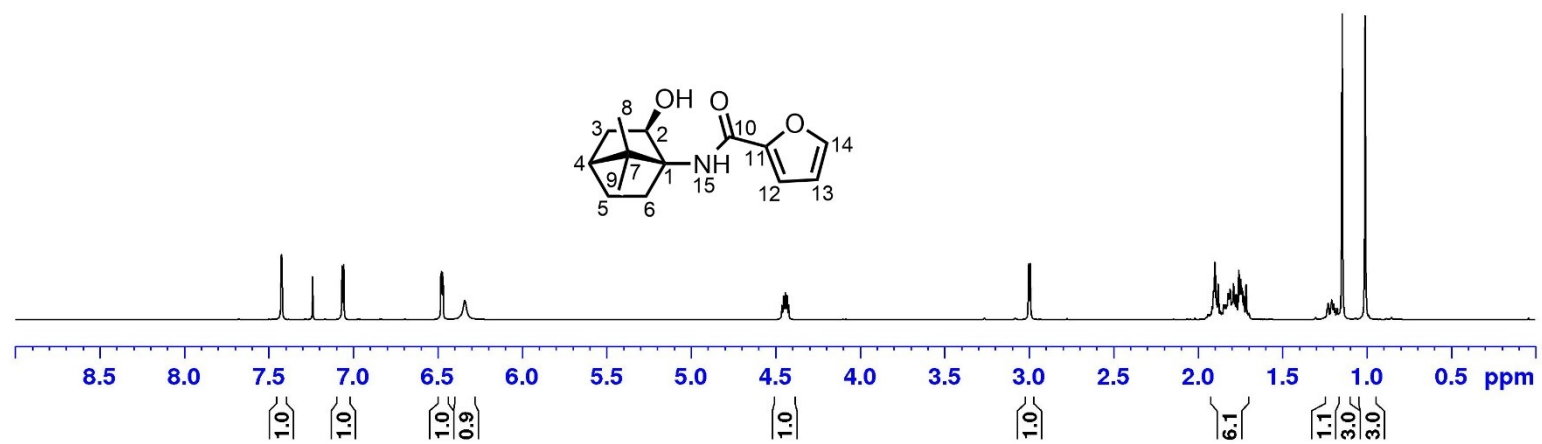


# Compound 22c

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>

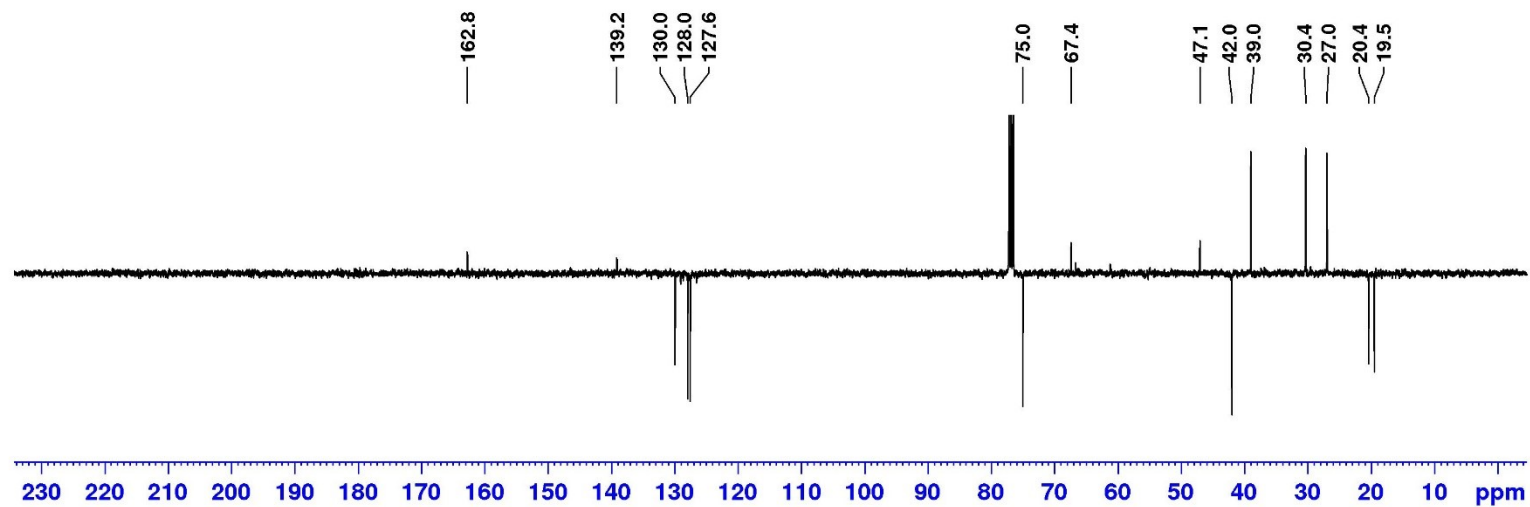


<sup>1</sup>H, 400 MHz, CDCl<sub>3</sub>



# Compound 22d

<sup>13</sup>C, JMOD, CDCl<sub>3</sub>



<sup>1</sup>H, 400 MHz, CDCl<sub>3</sub>

