Supporting information

Synthesis of PDE IV Inhibitors.* First Asymmetric Synthesis of Two GlaxoSmithKline's Highly Potent Rolipram Analogues

Petr A. Zhmurov,[a] Alexey Yu. Sukhorukov,*[a] Vladimir I. Chupakhin,[b] Yulia V. Khomutova,[a] Sema L. Ioffe and Vladimir A. Tartakovsky[a]

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* Part 4

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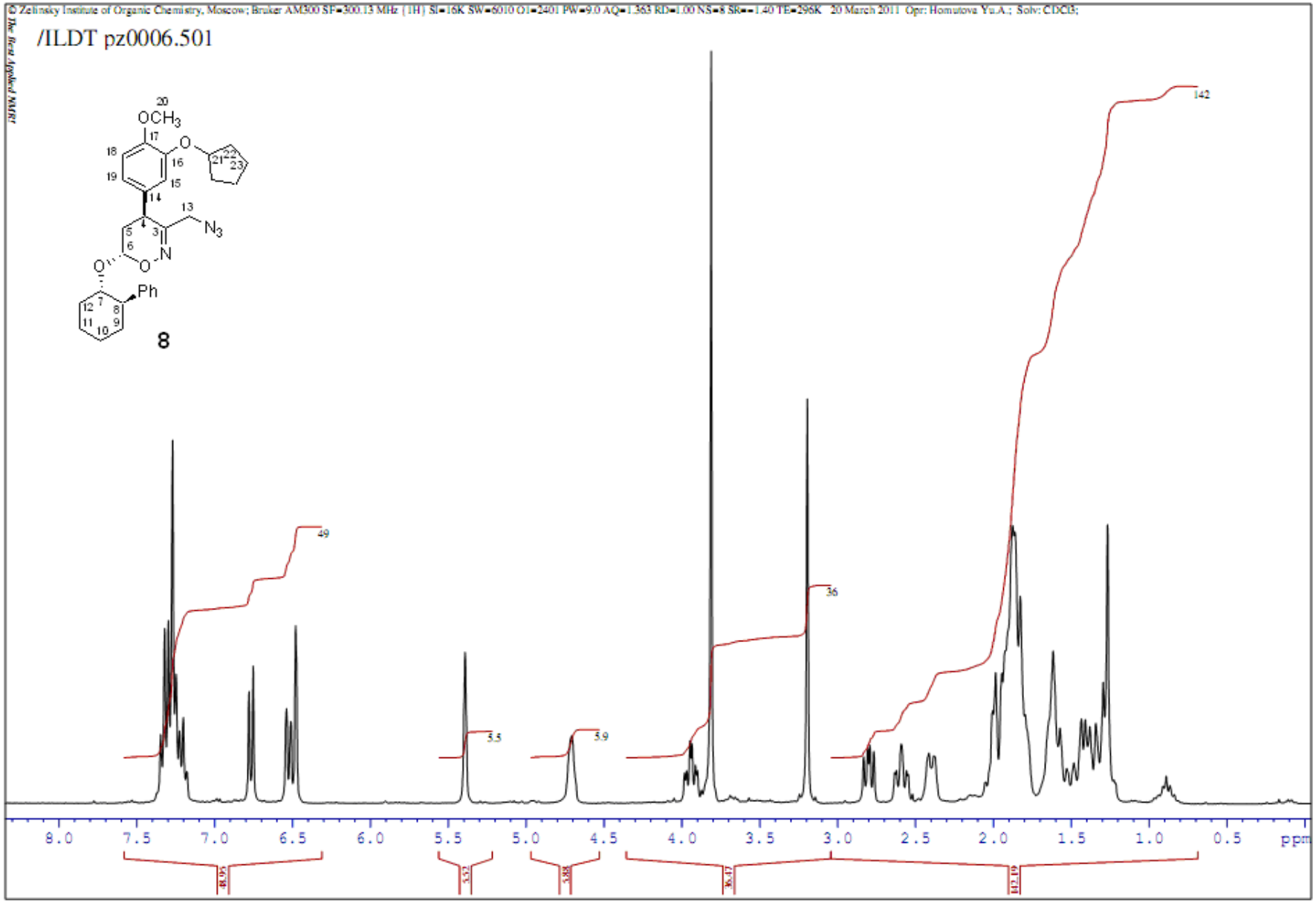

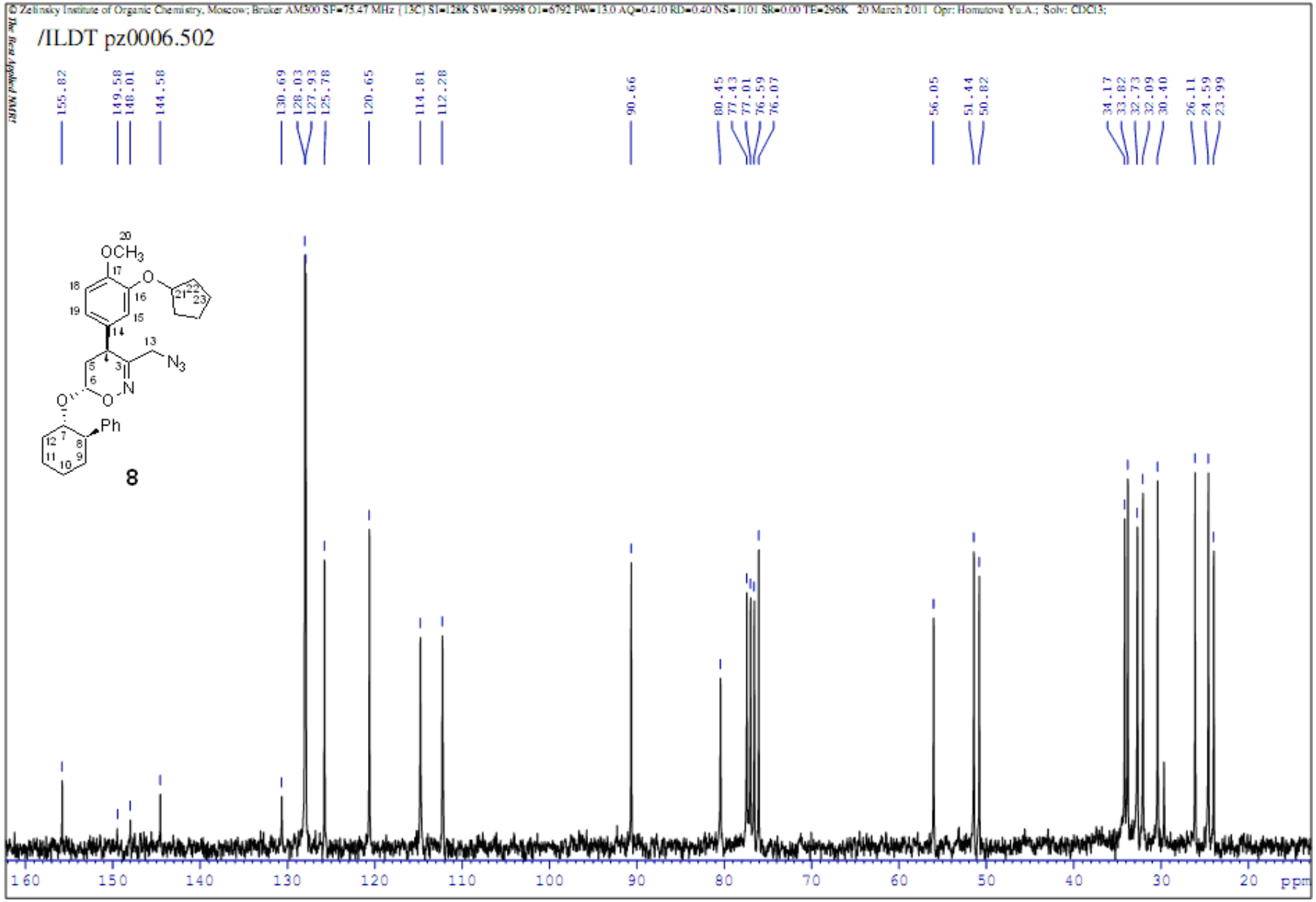

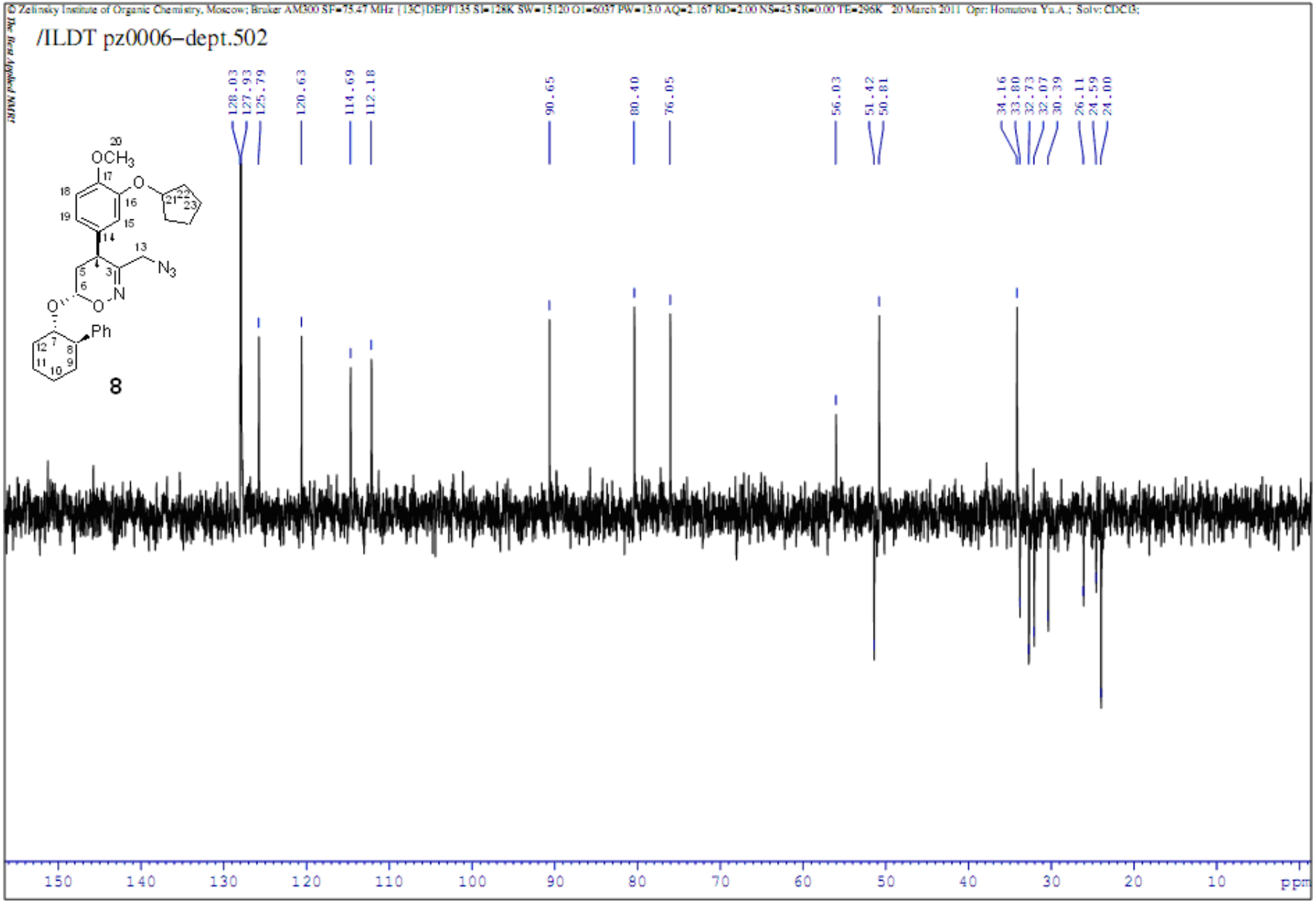

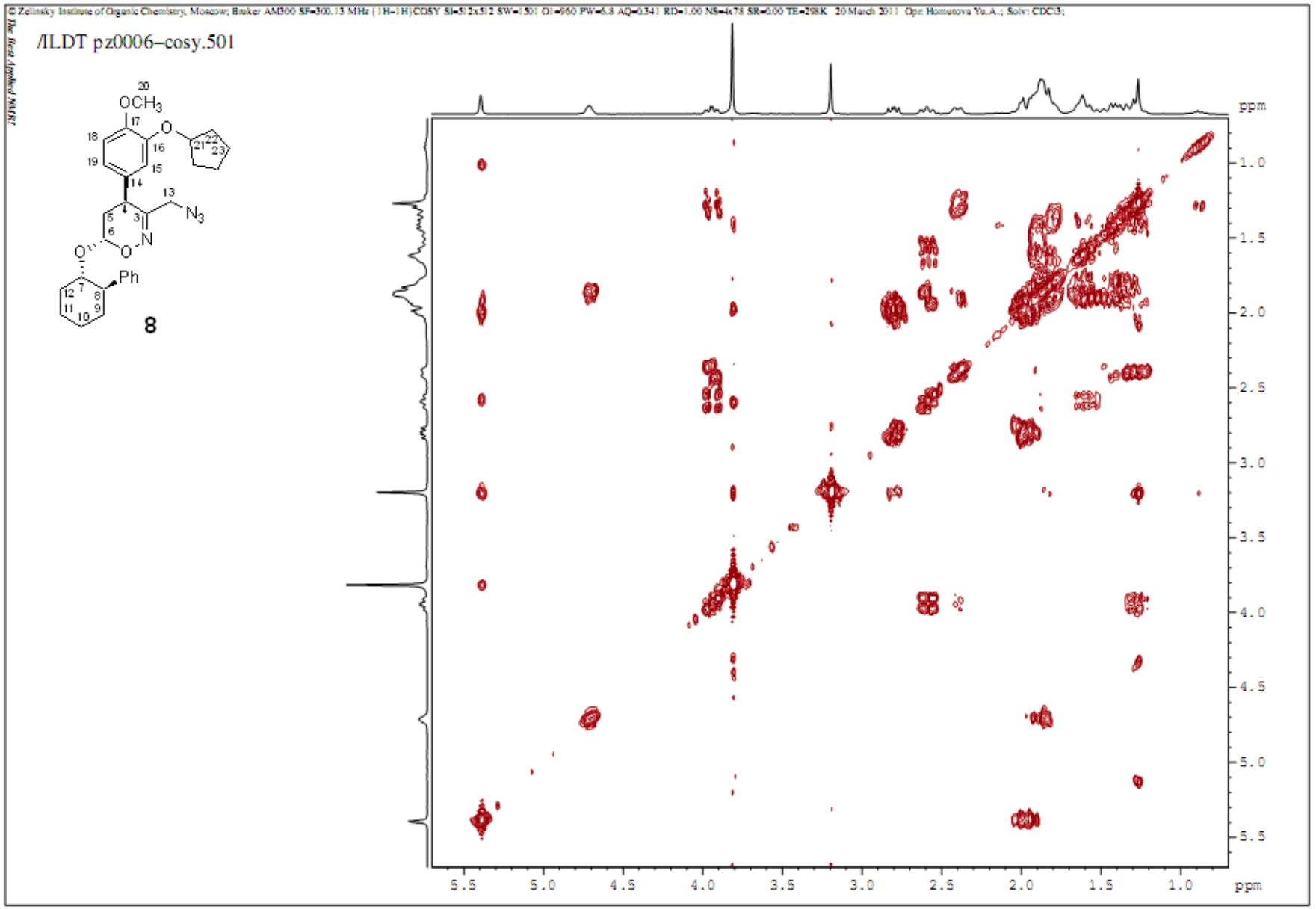
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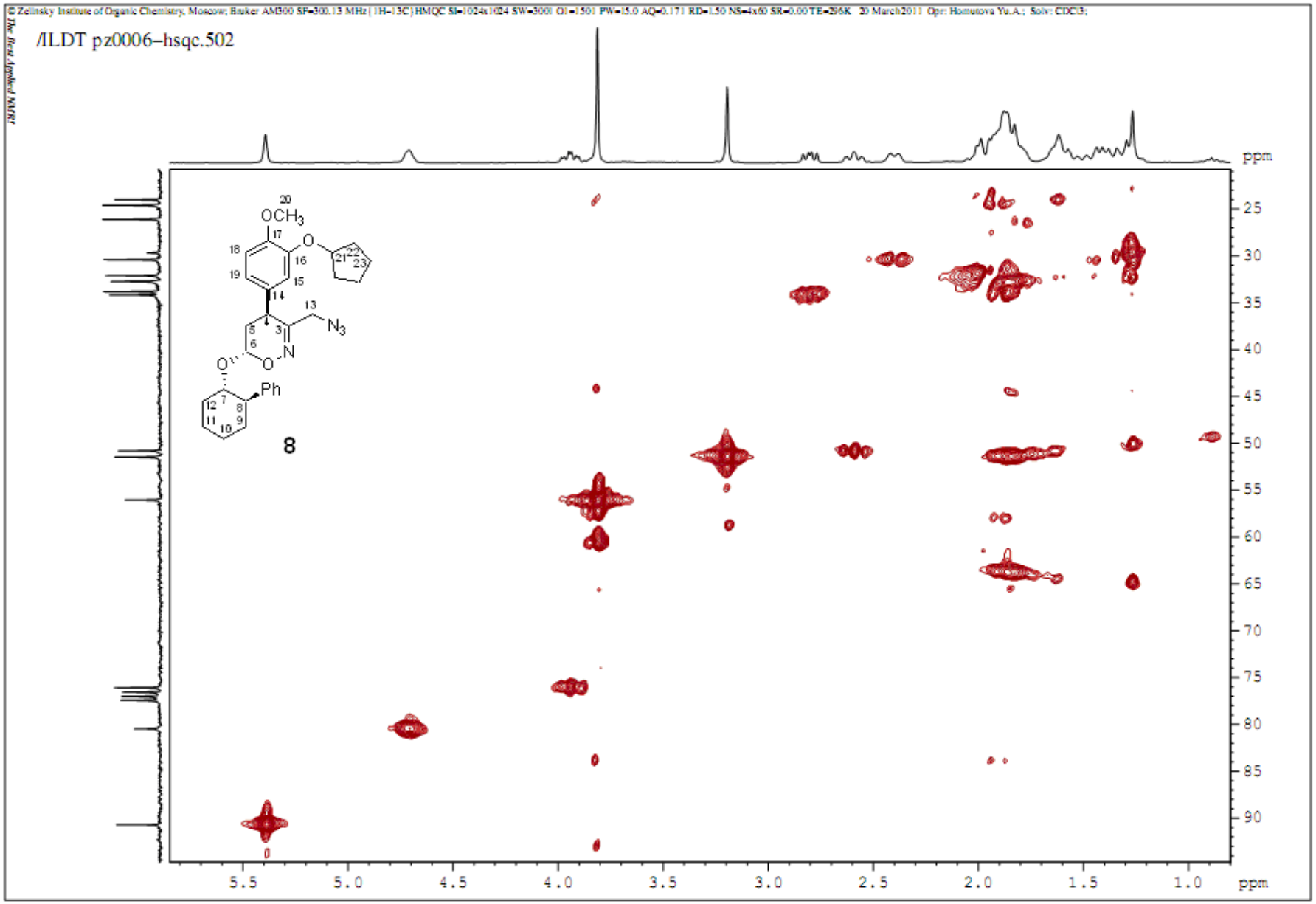

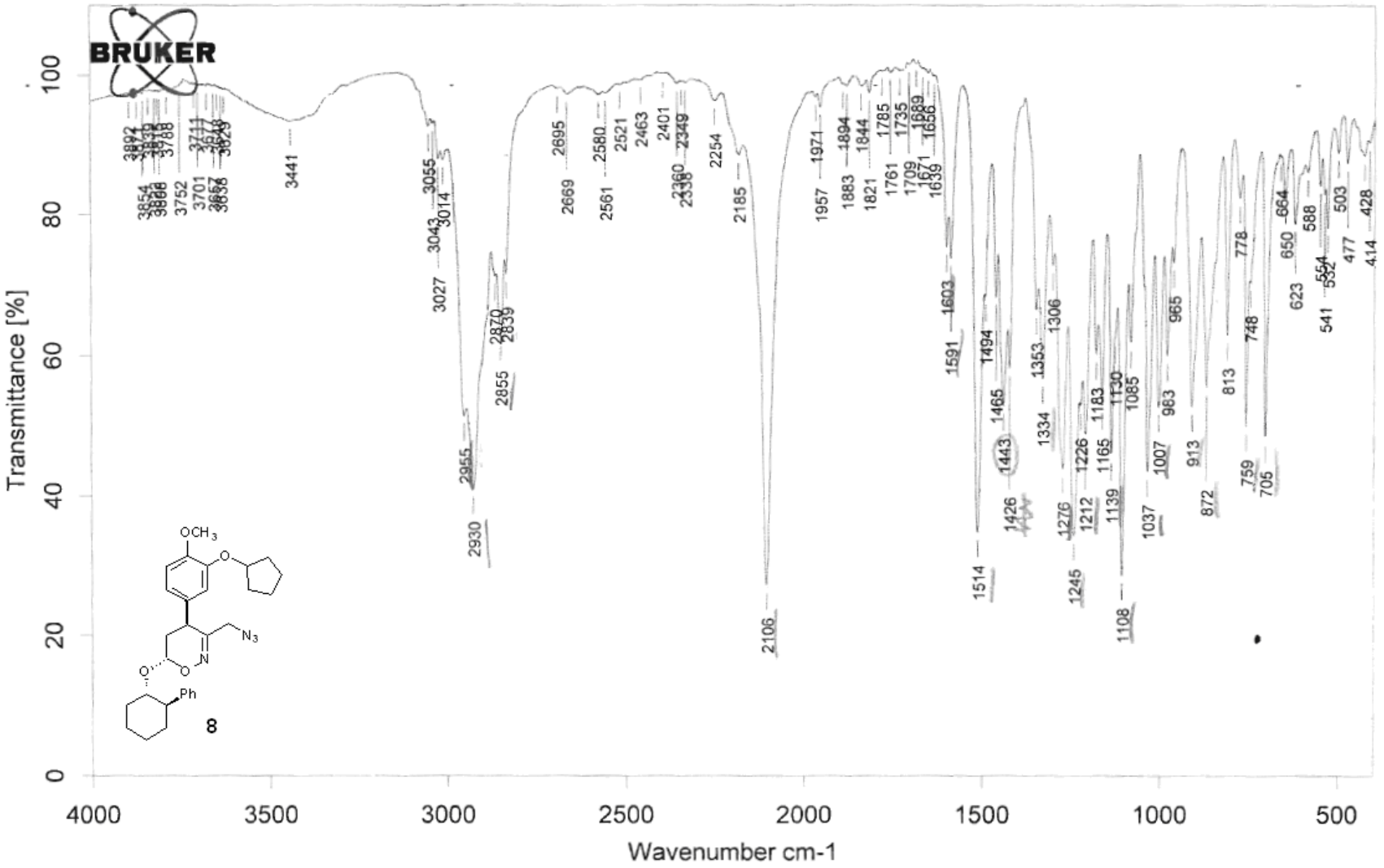

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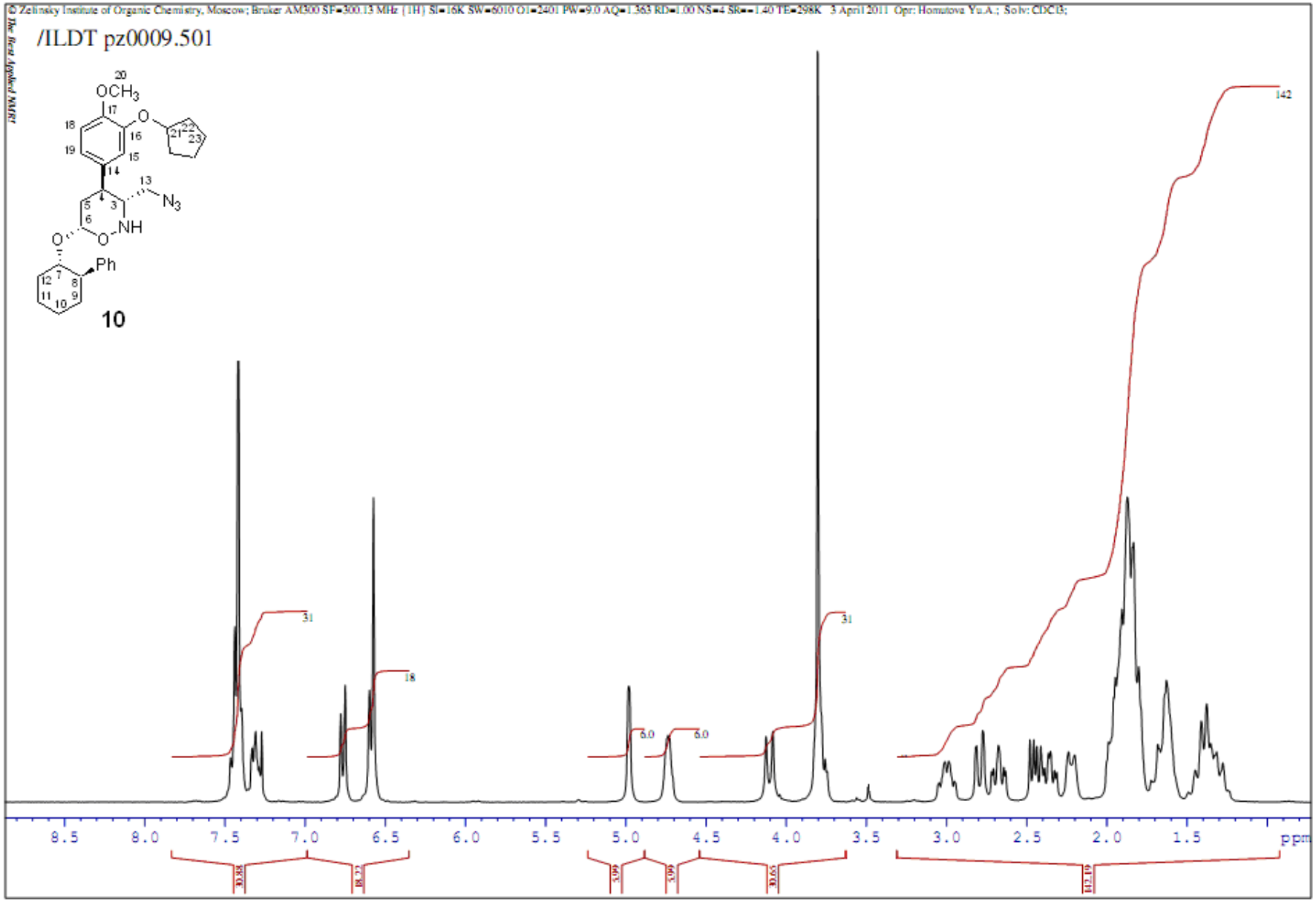

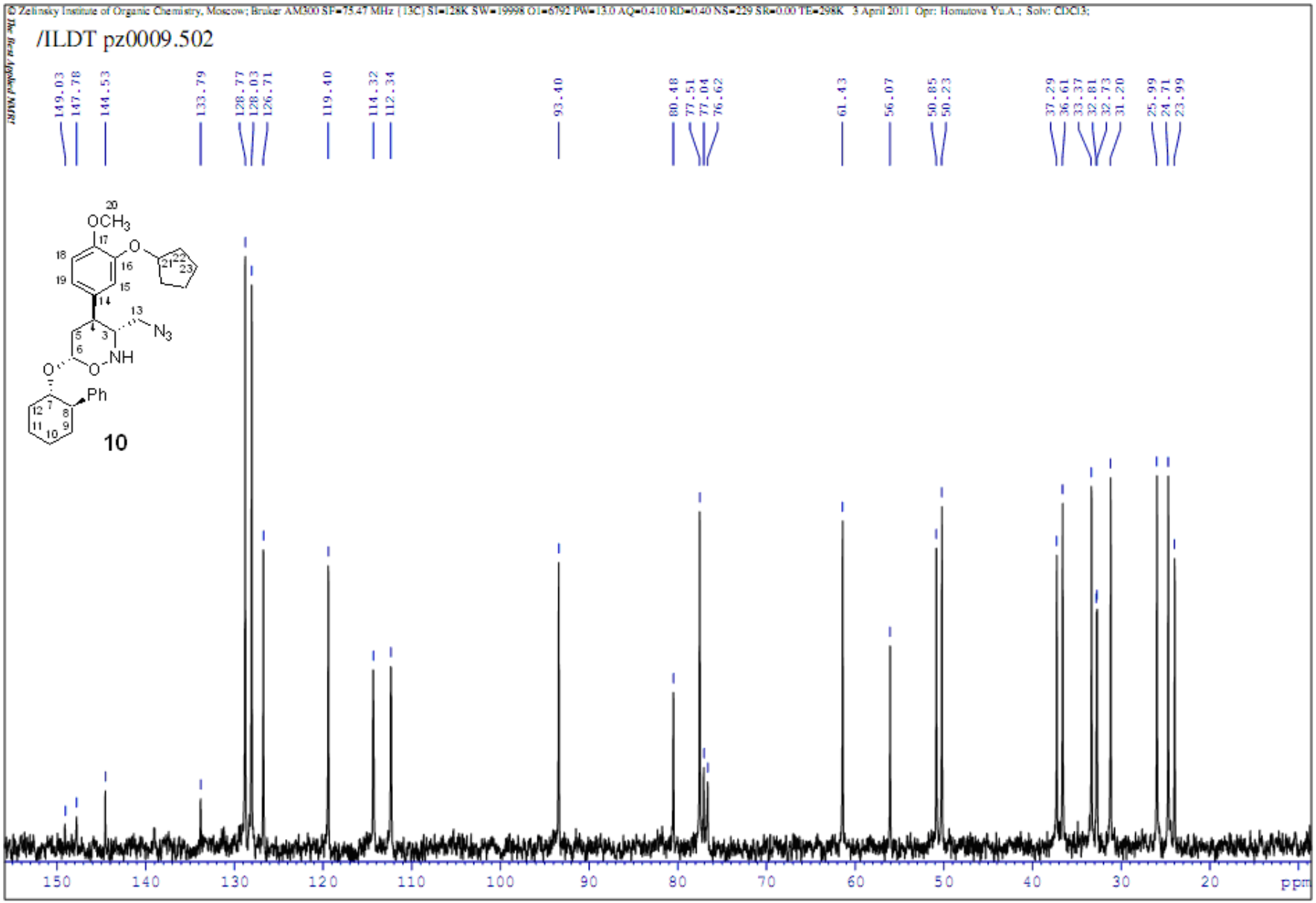

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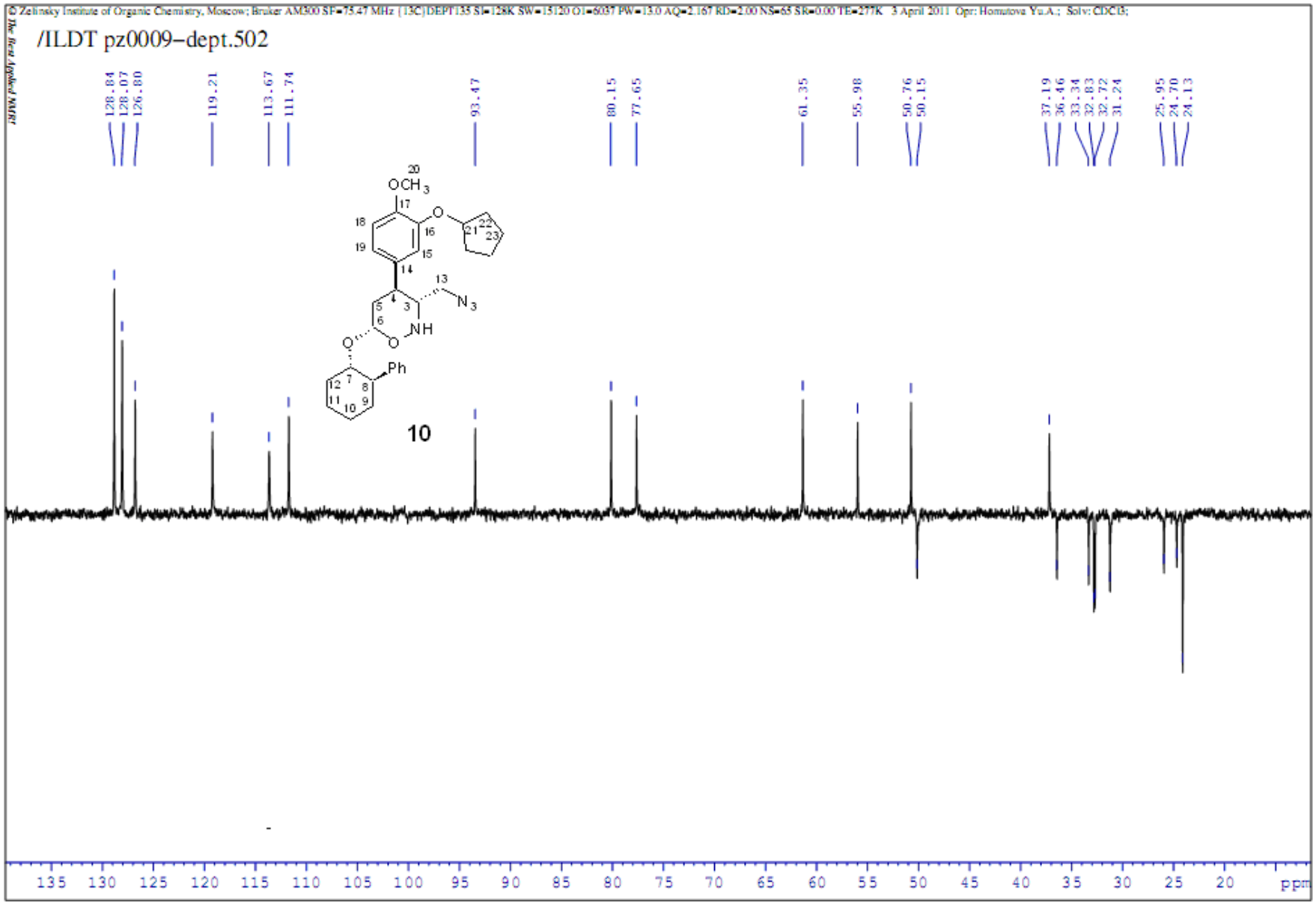
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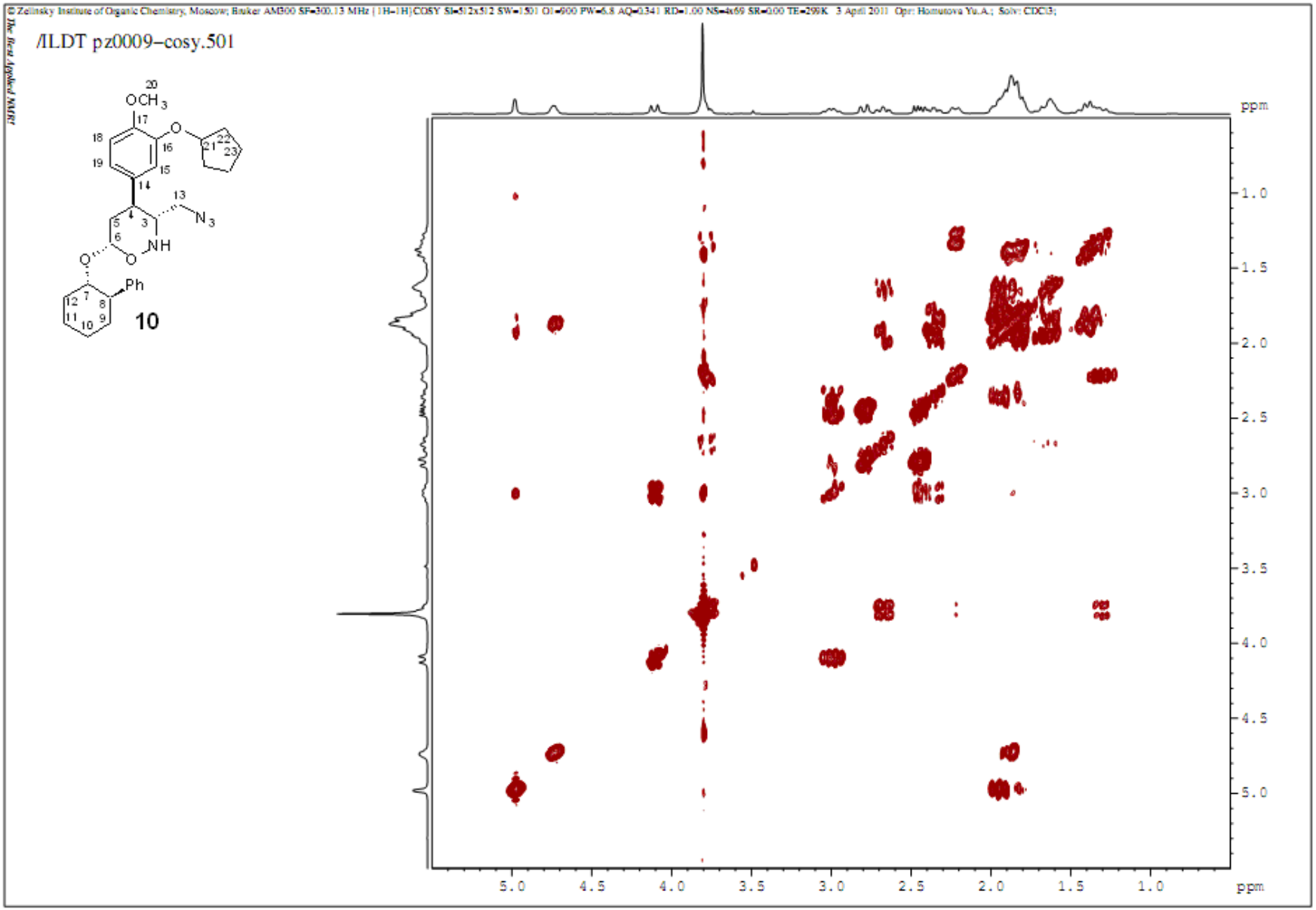

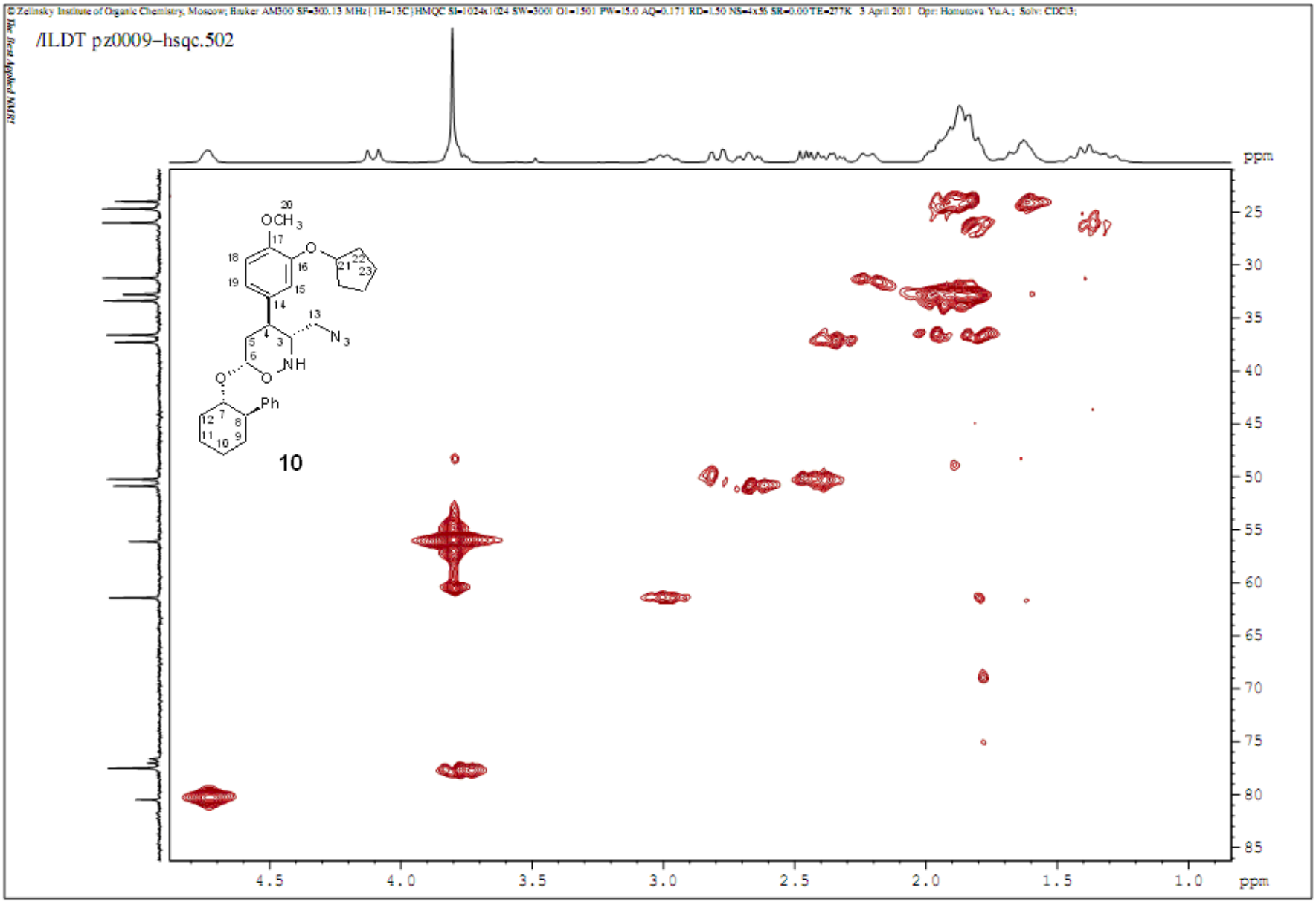

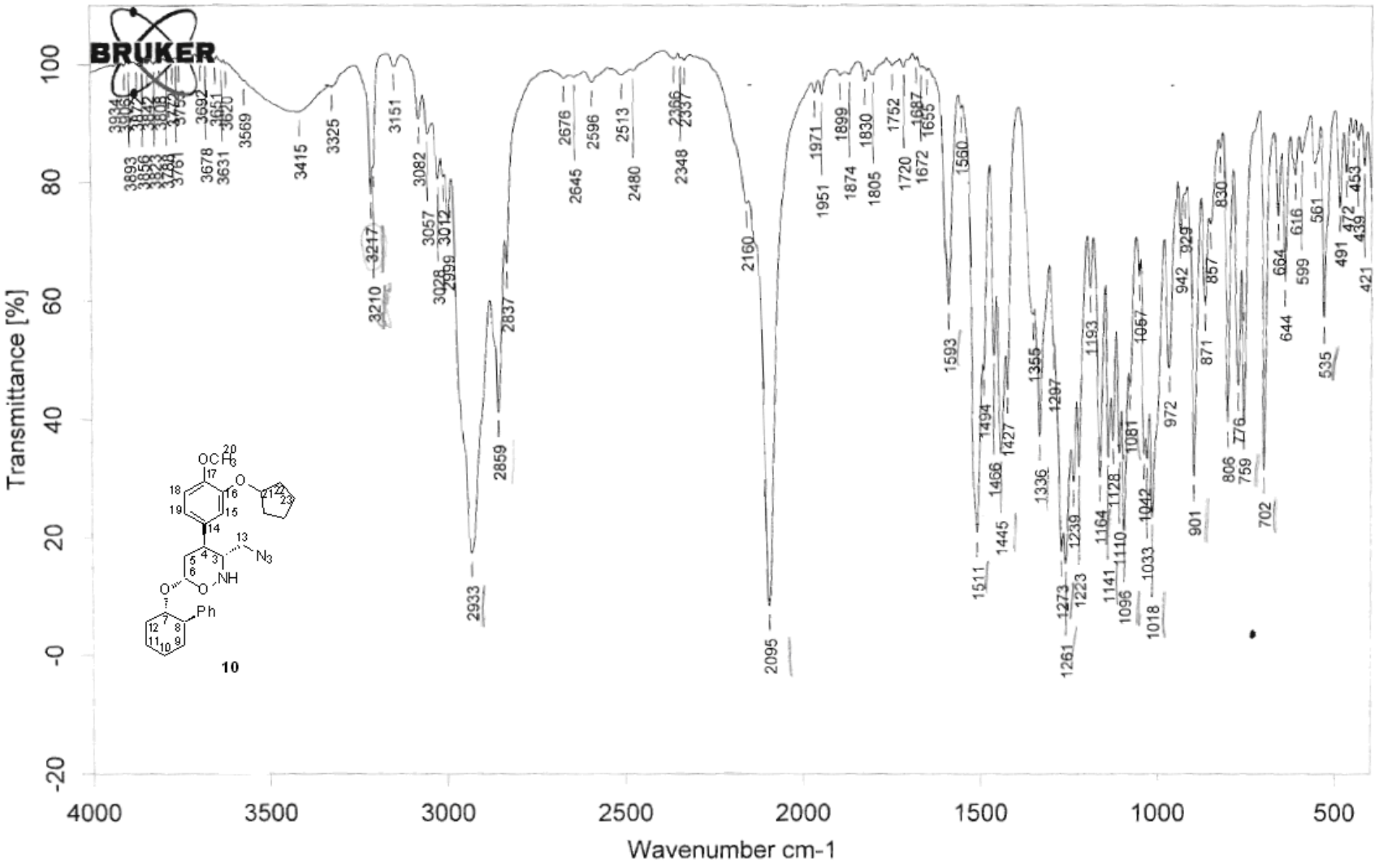

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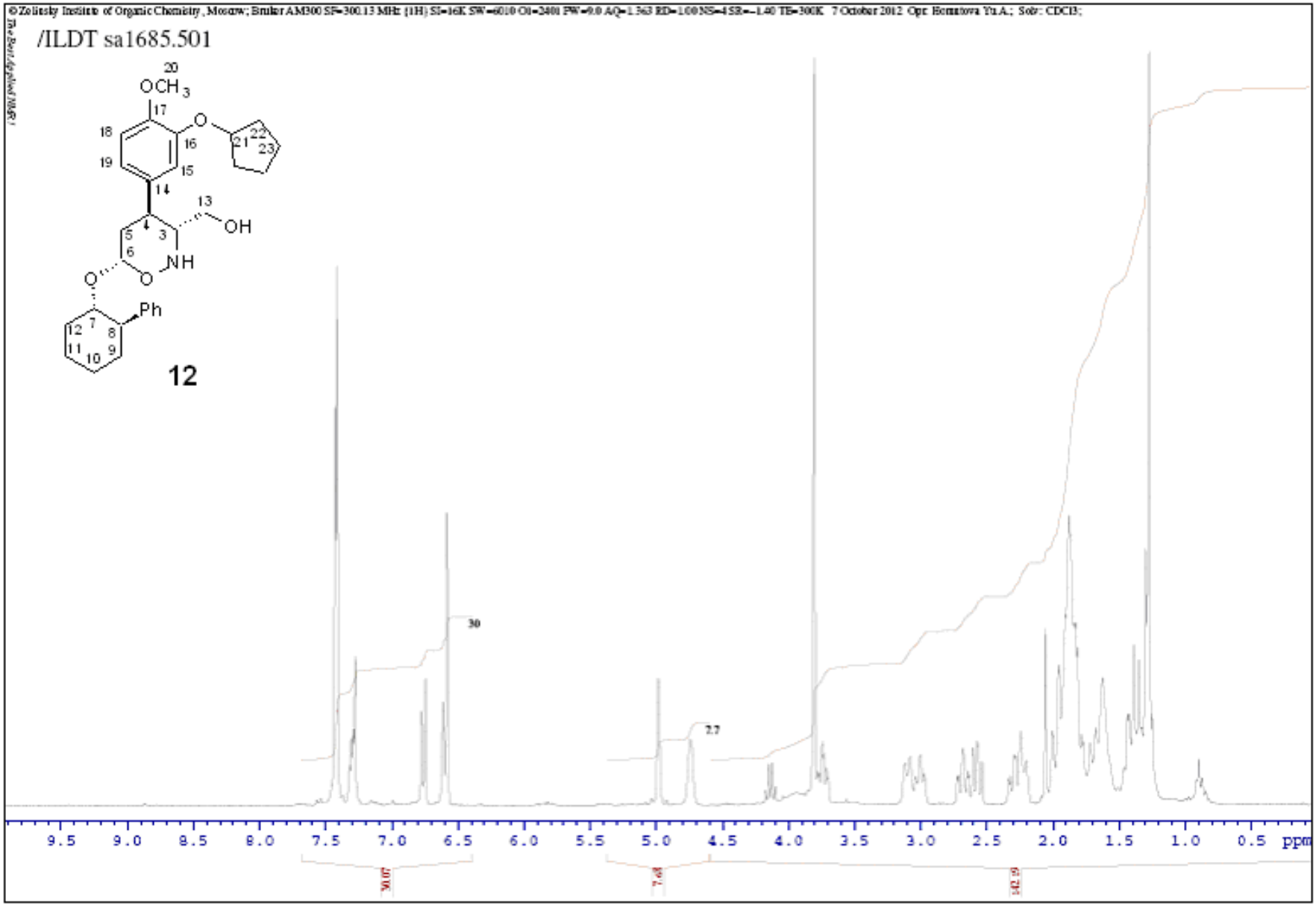

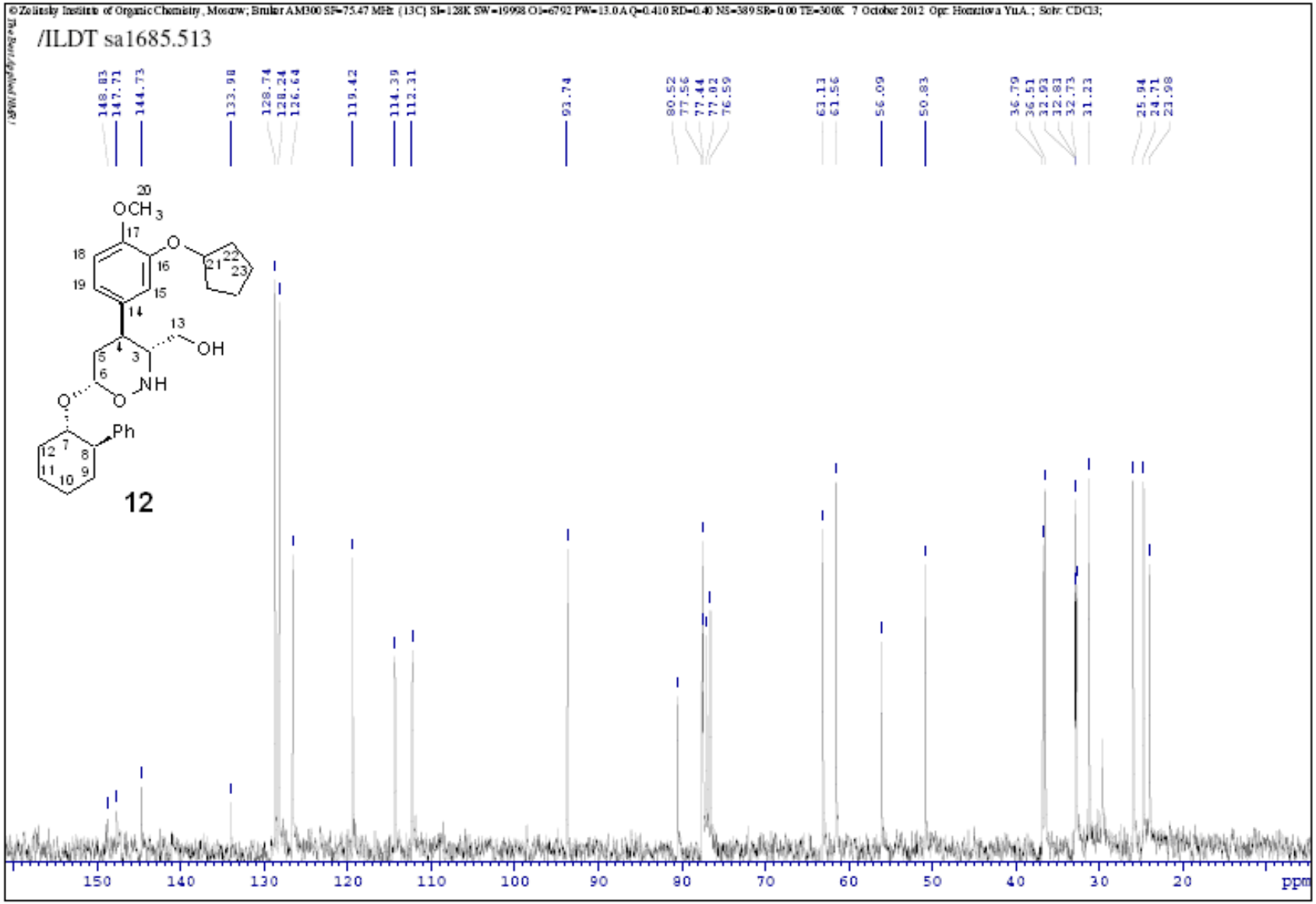
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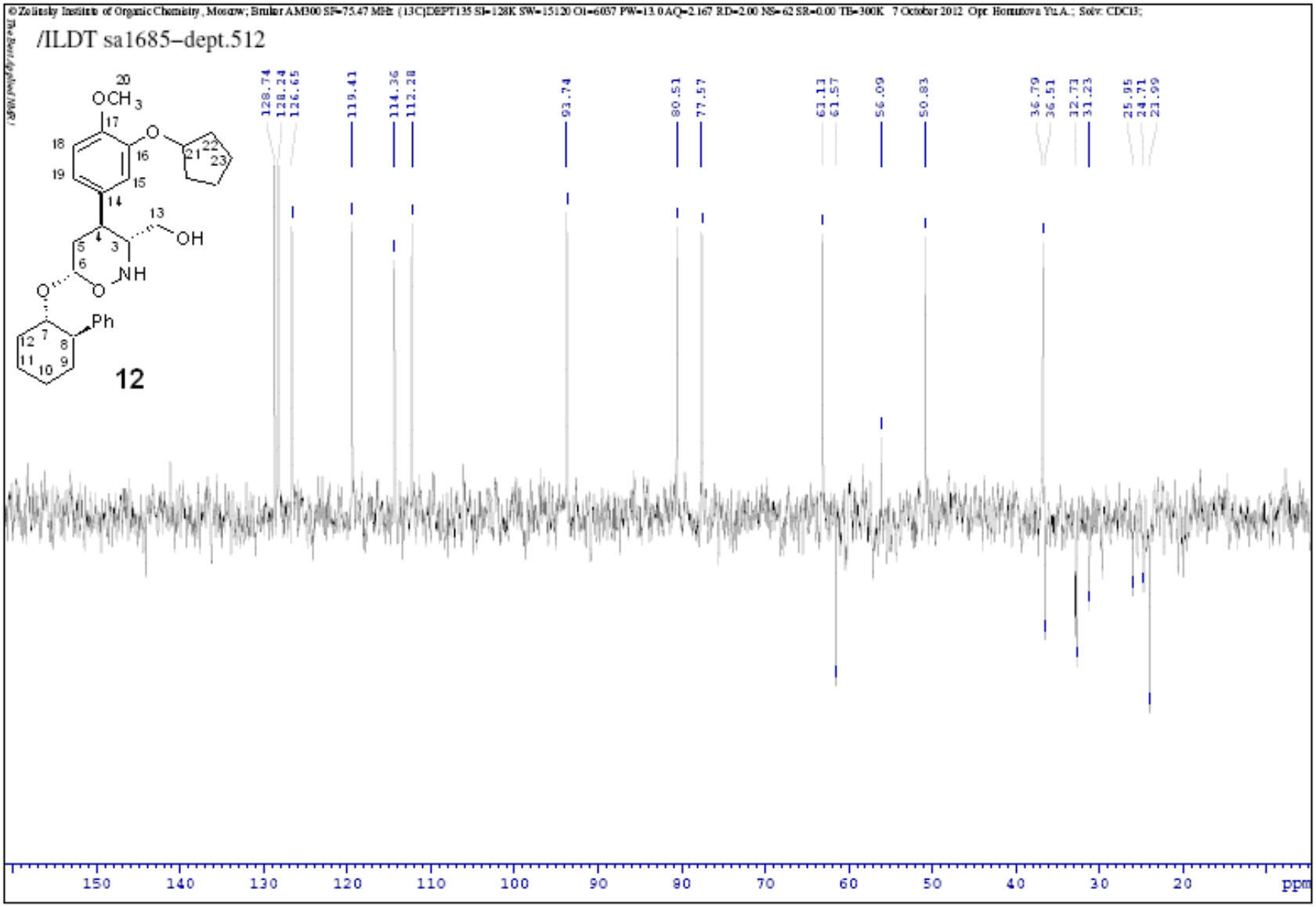

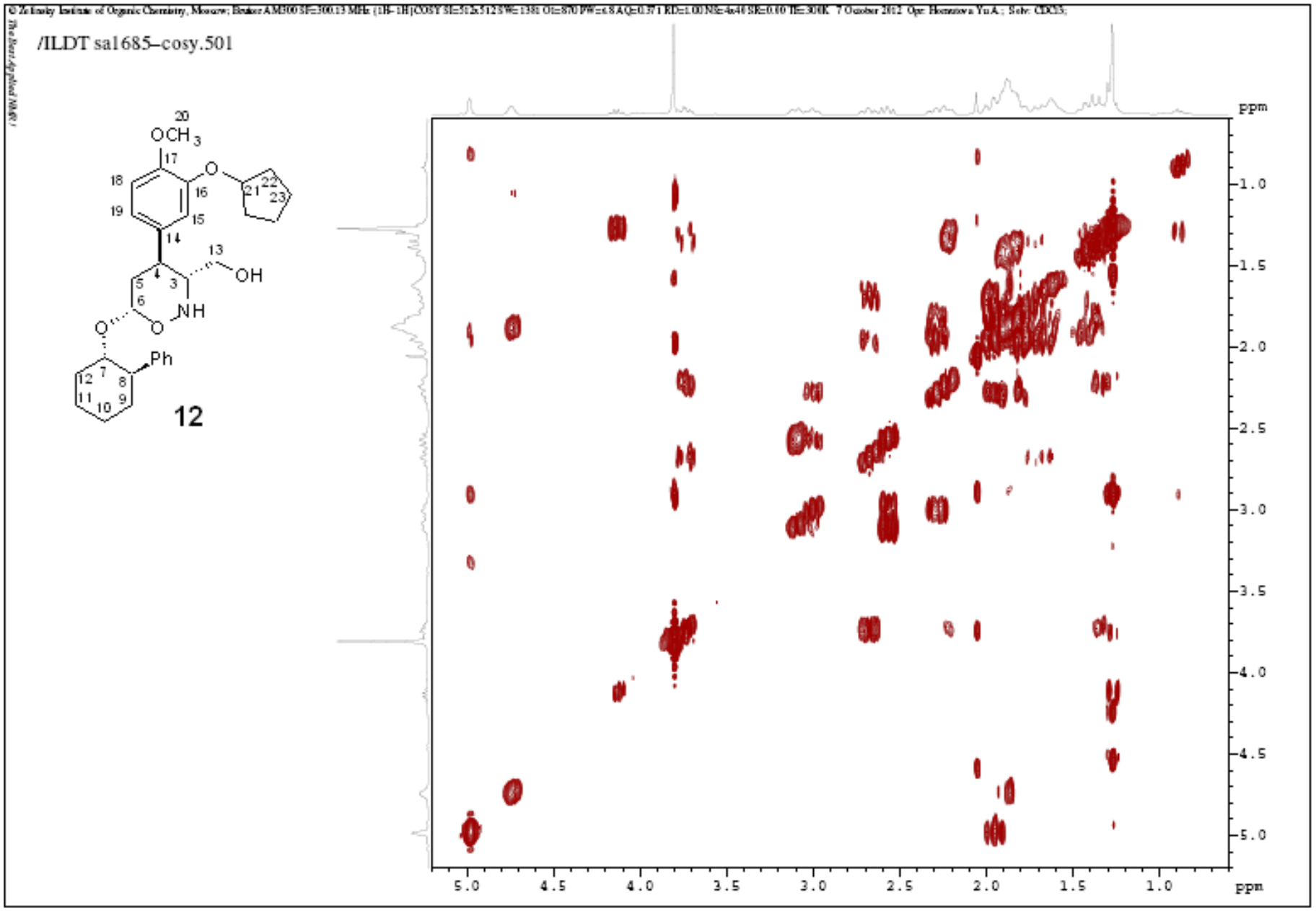

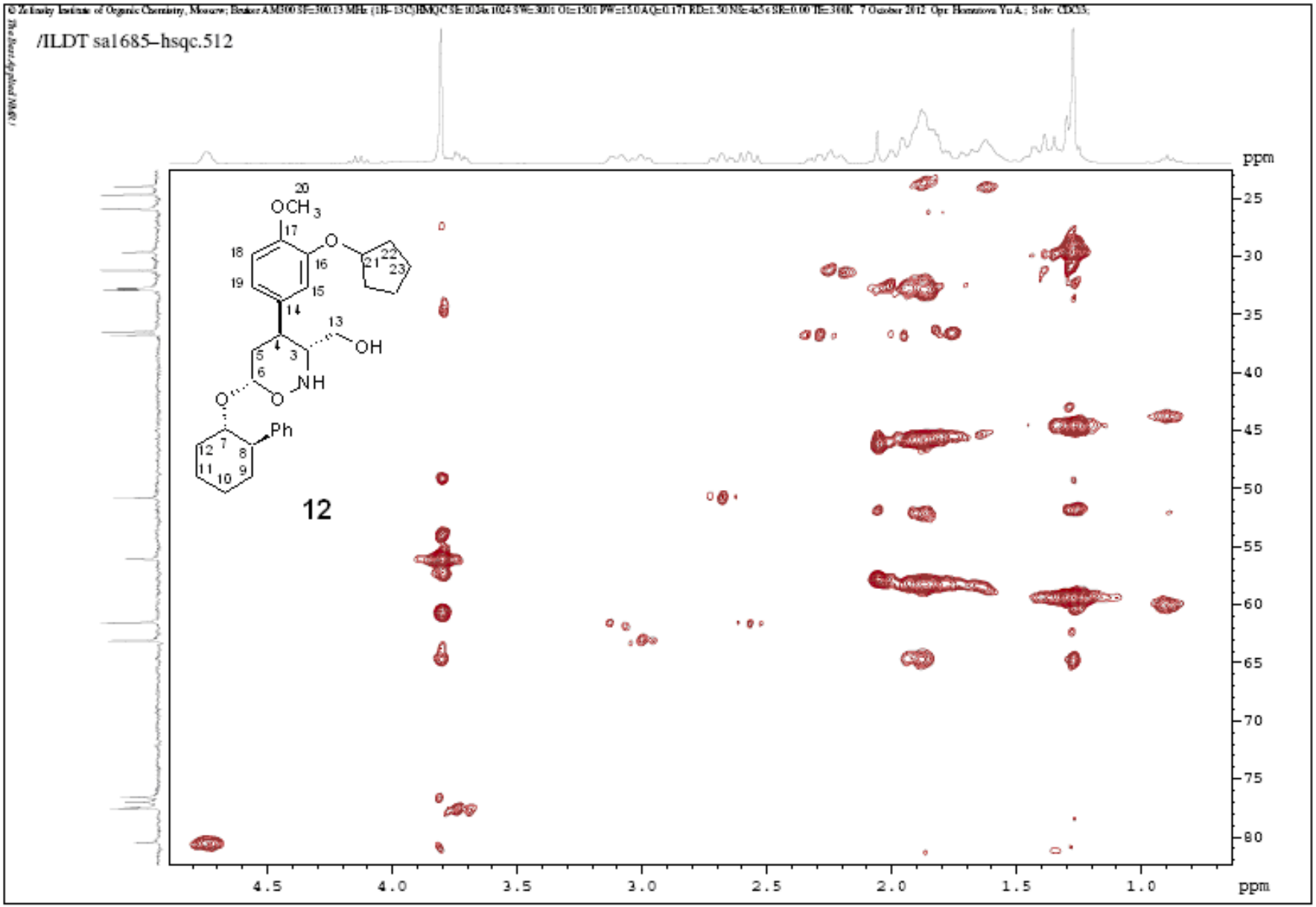

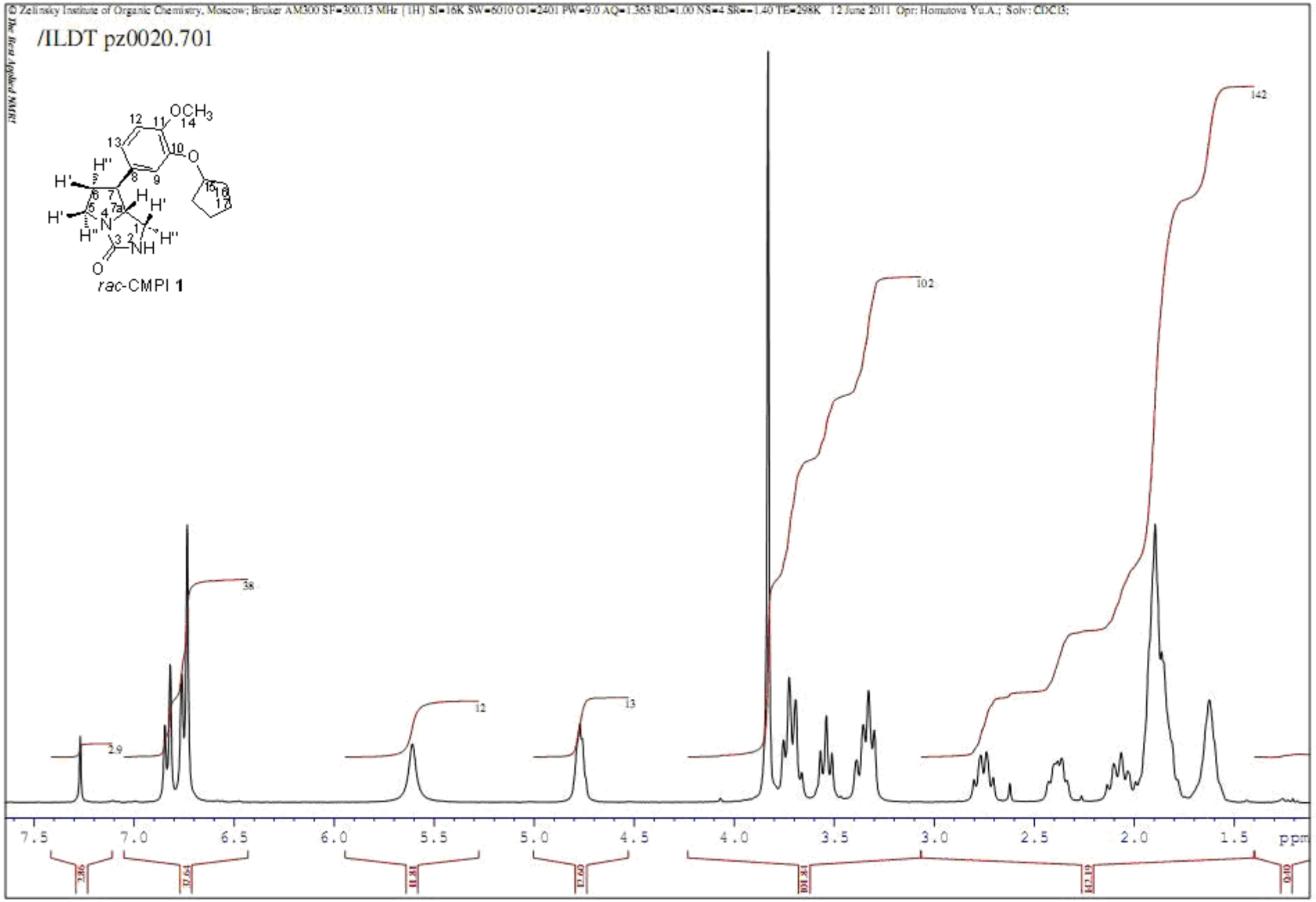

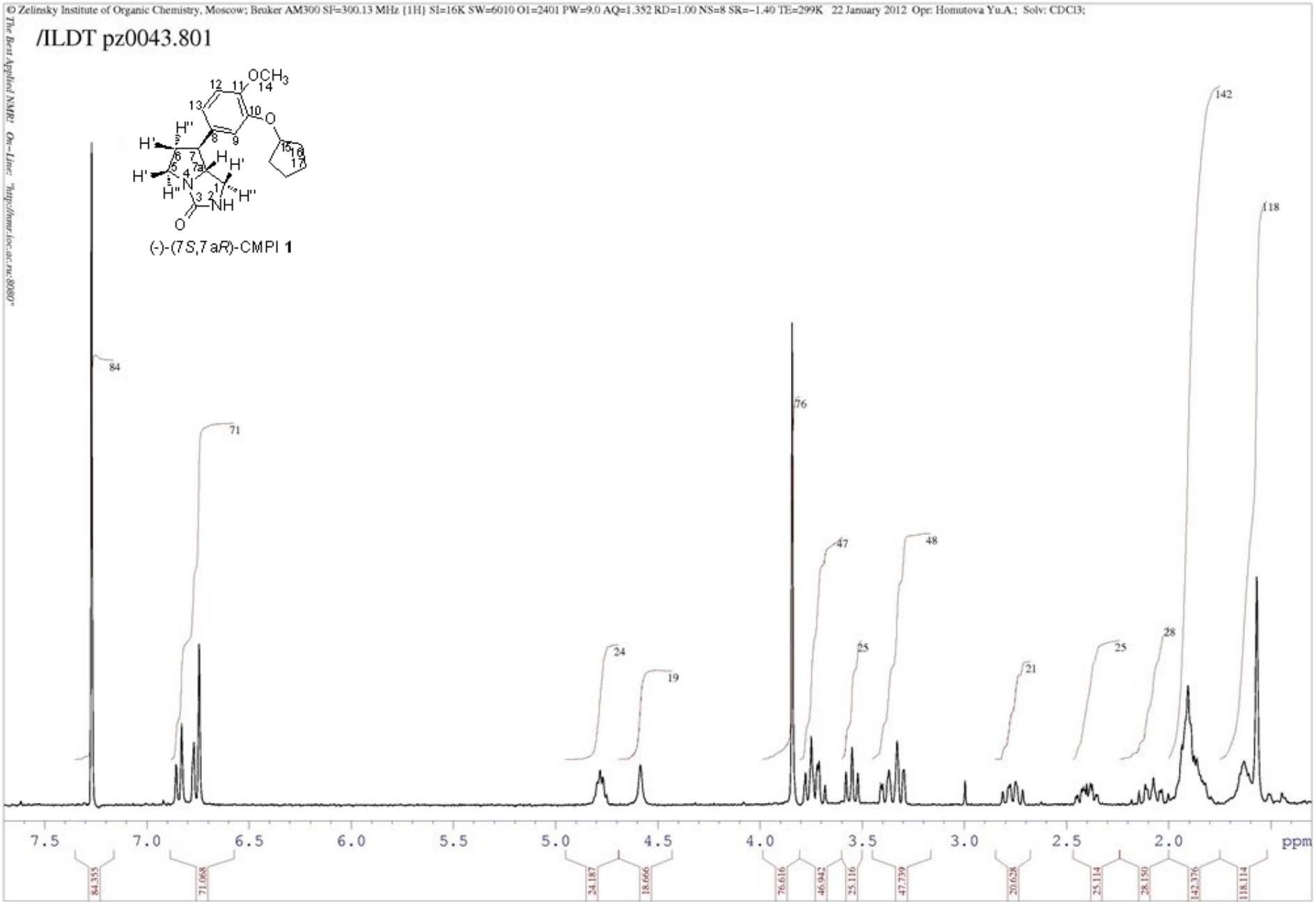
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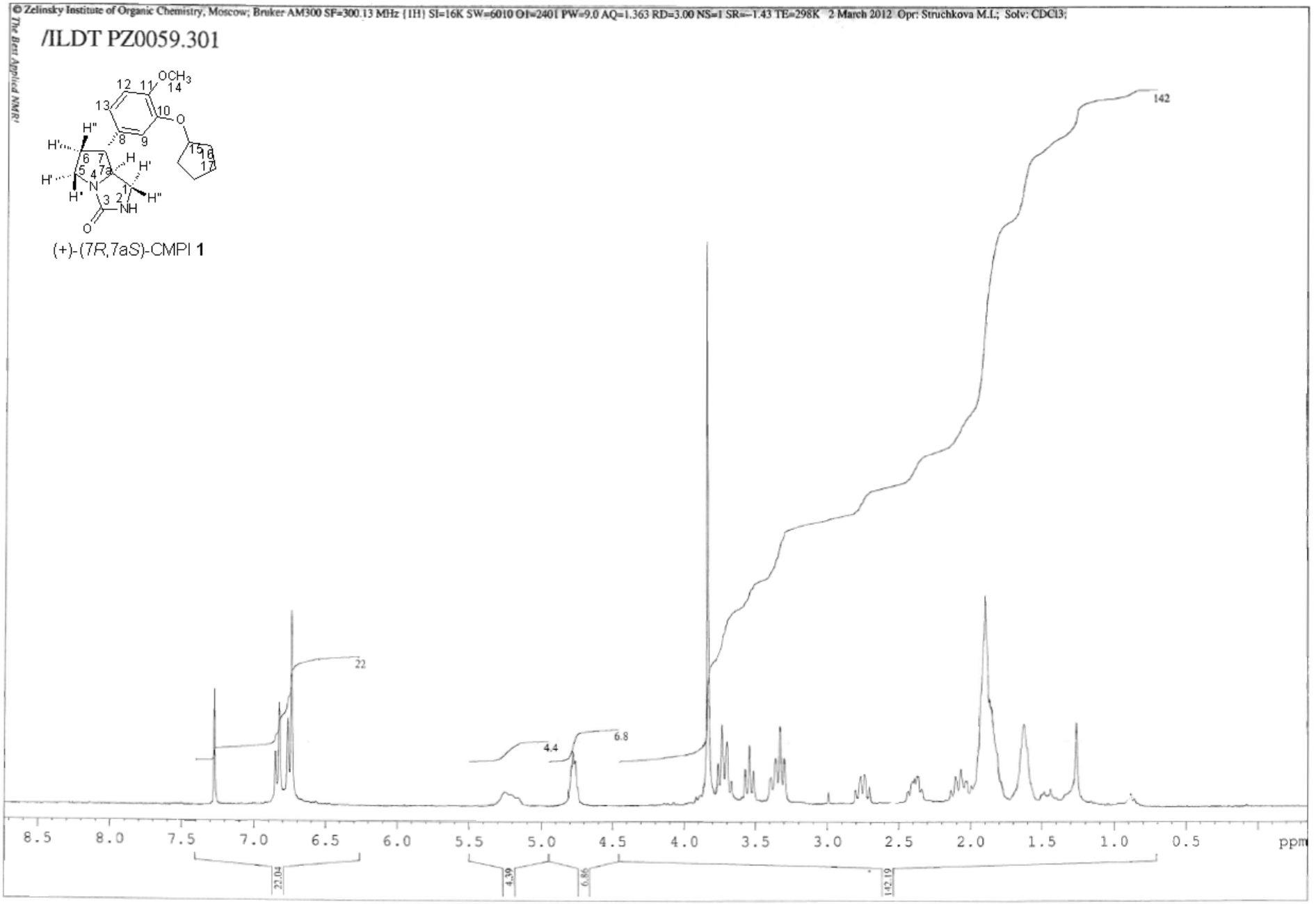

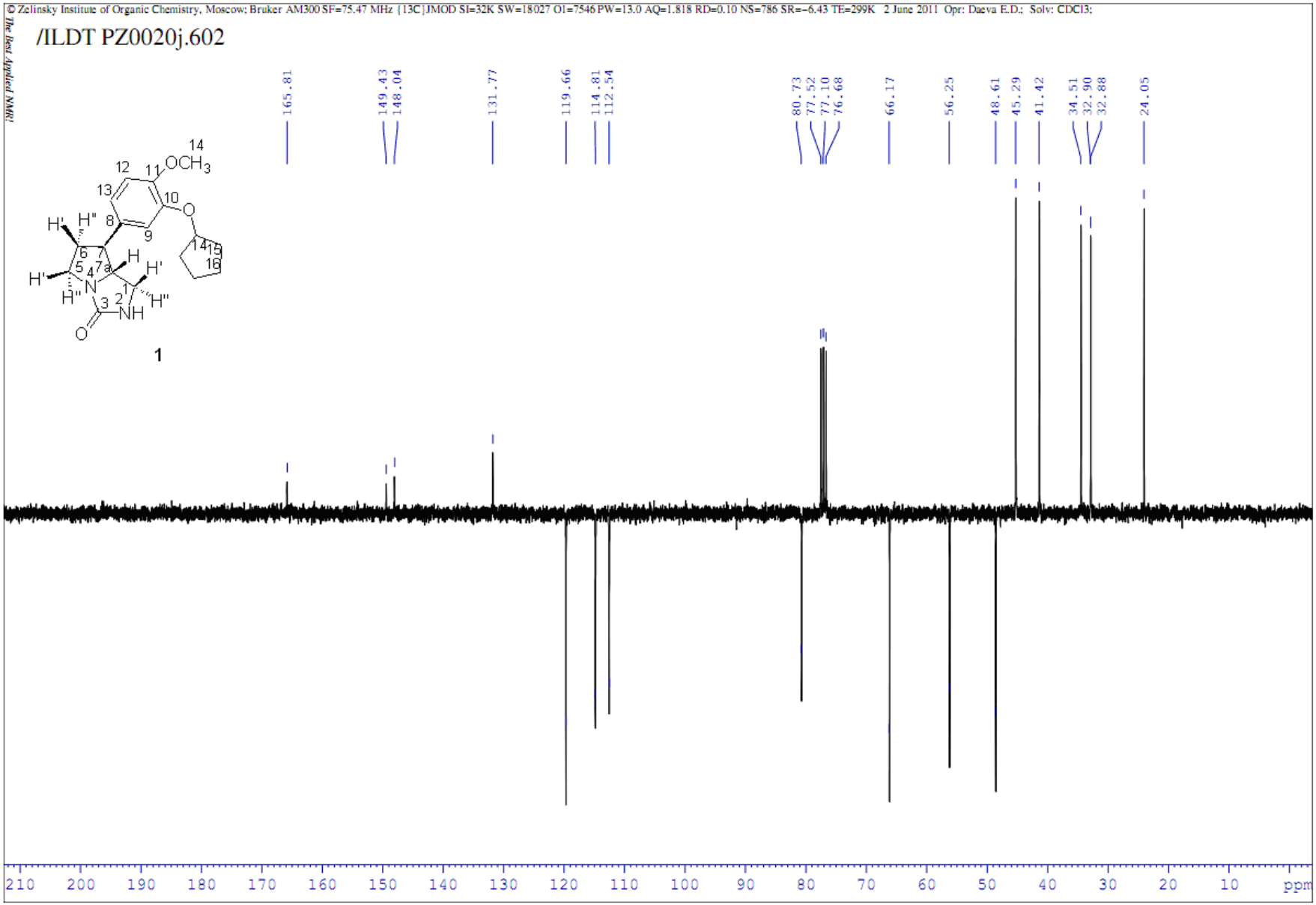
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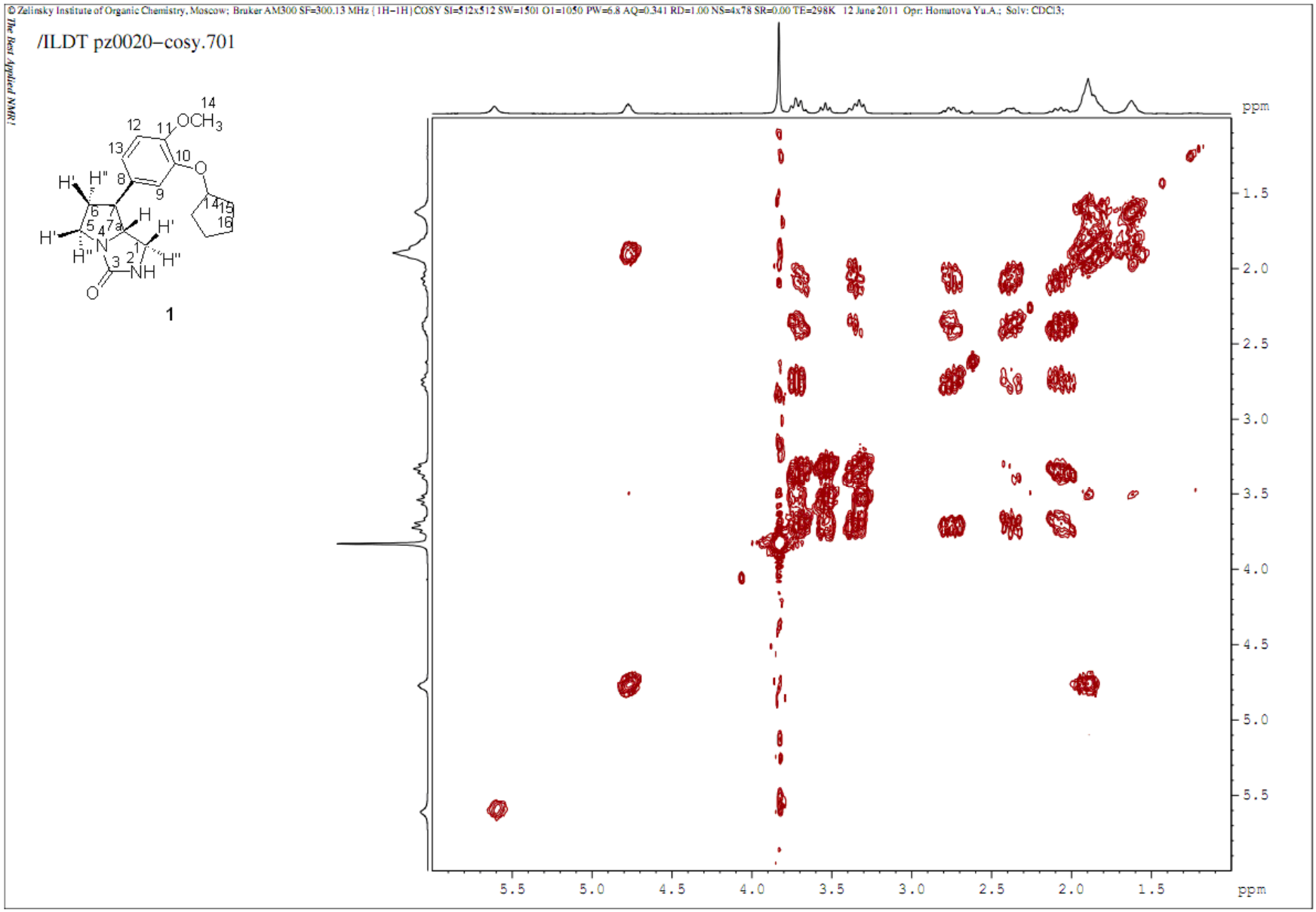

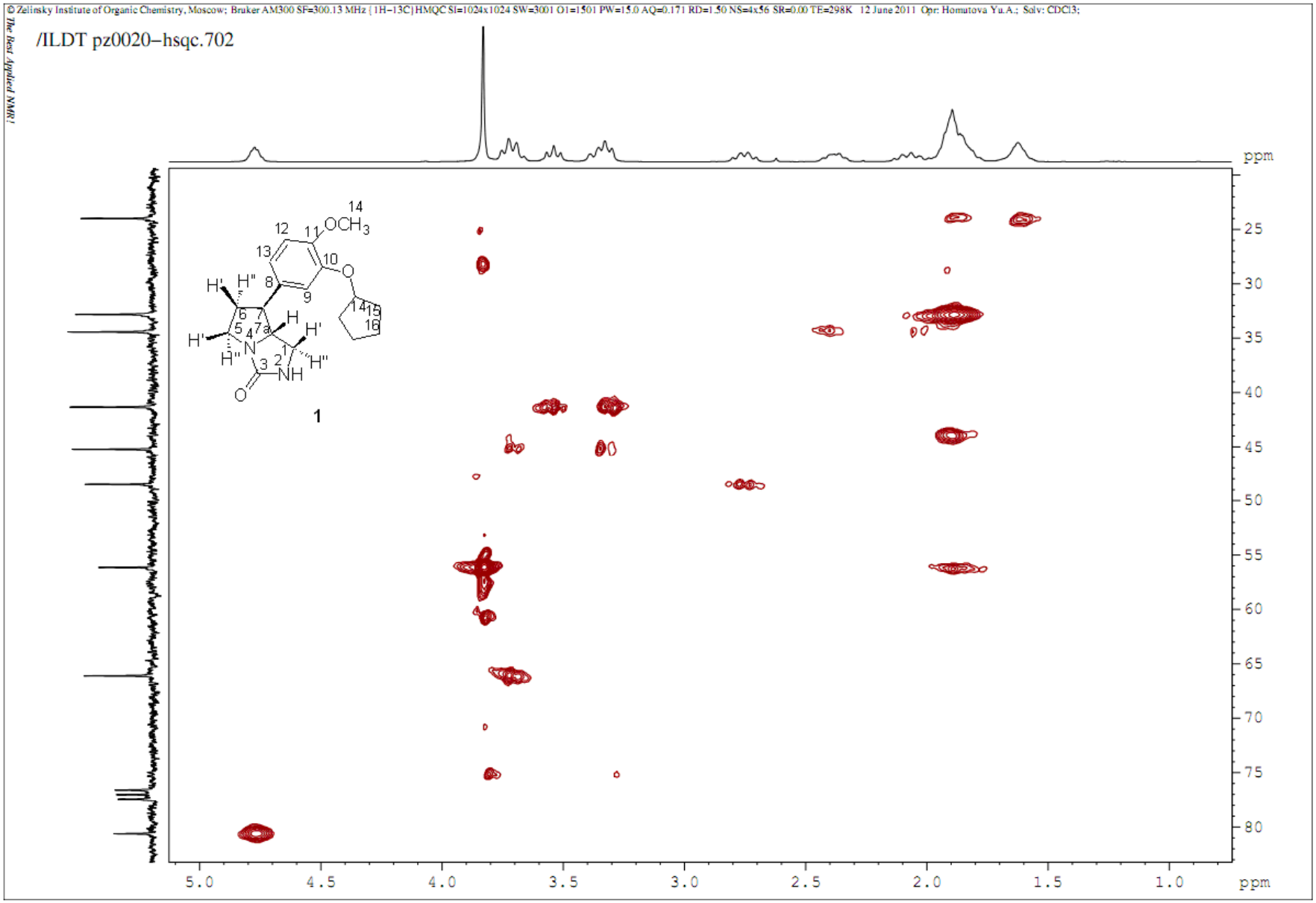
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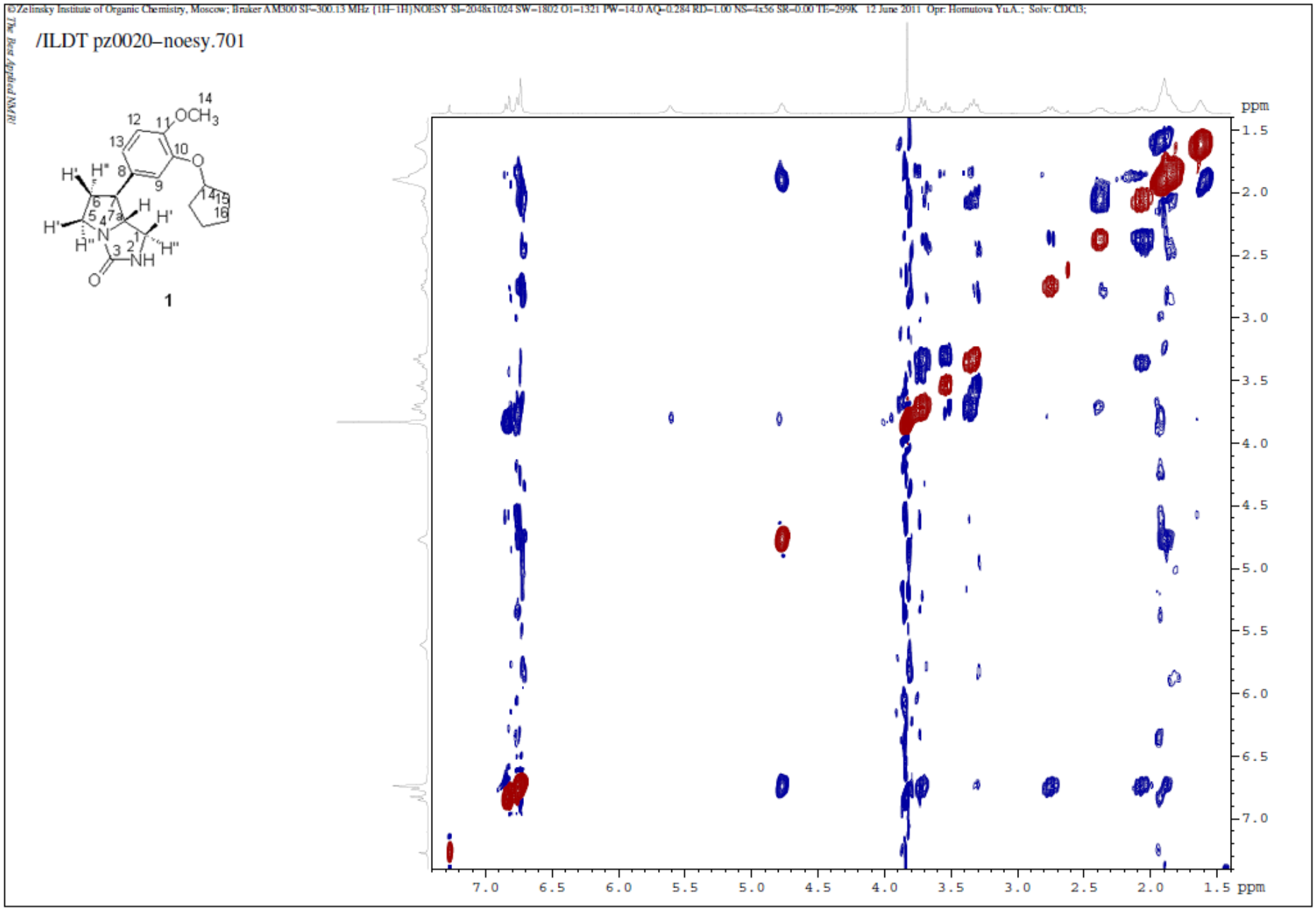

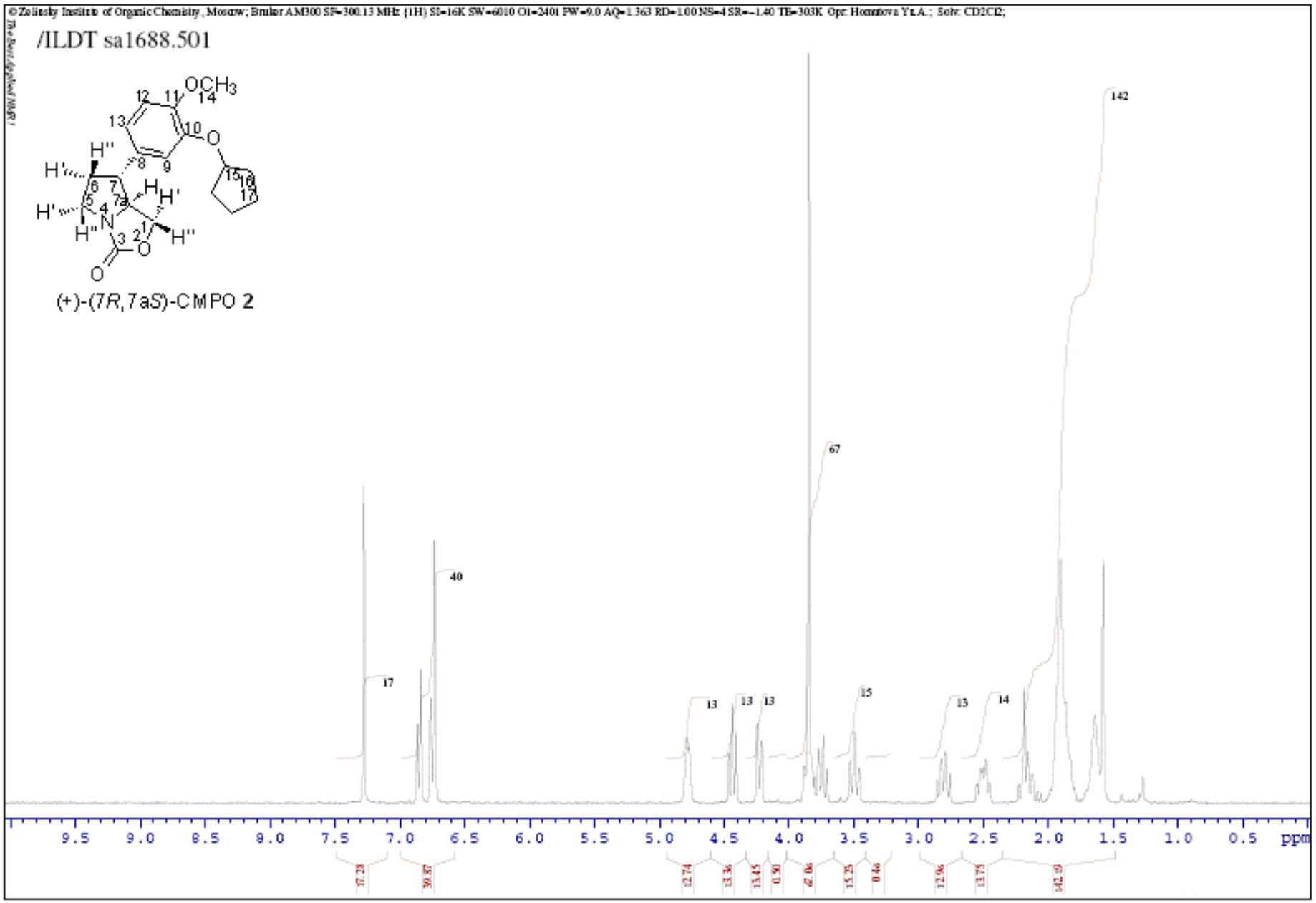

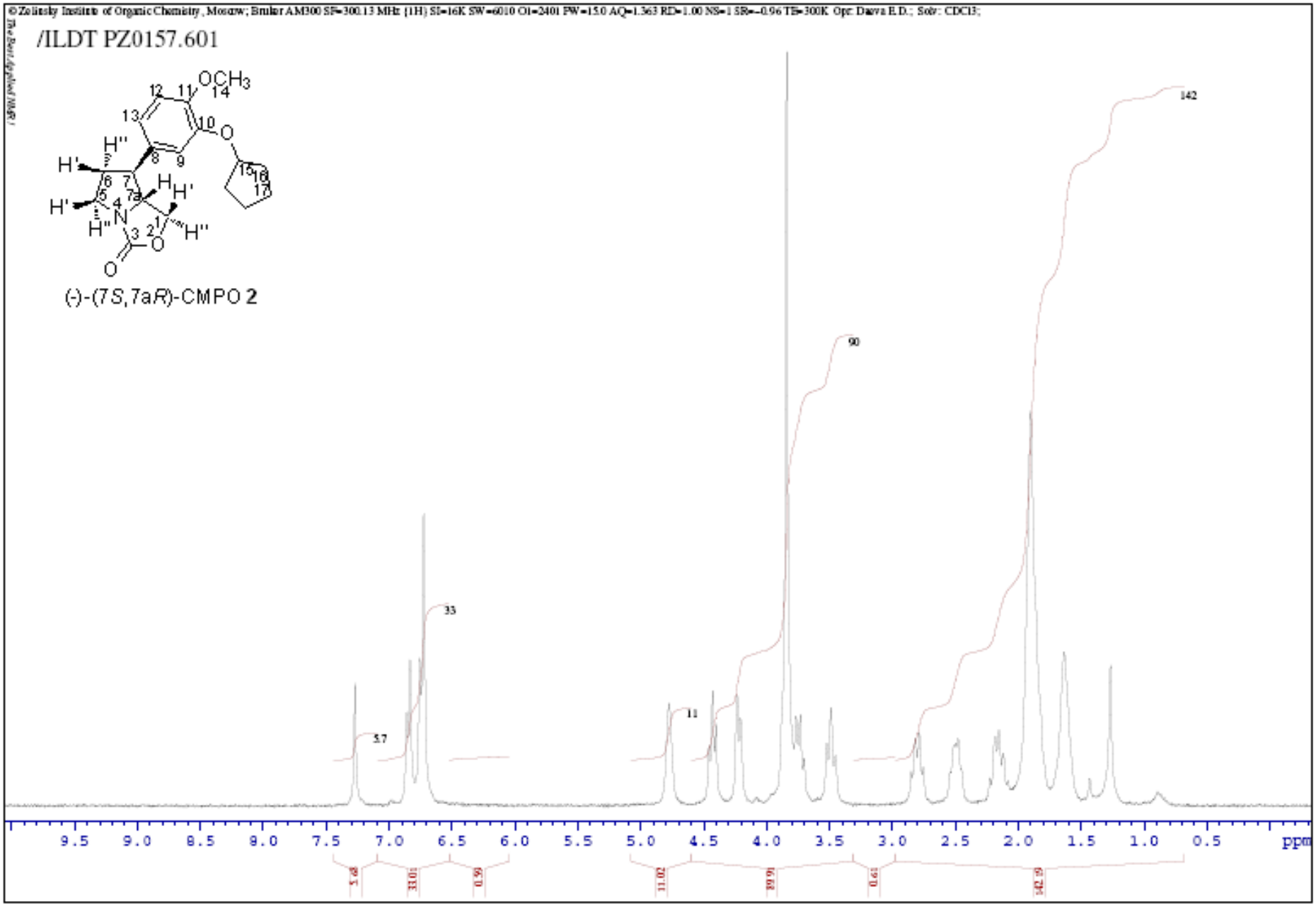

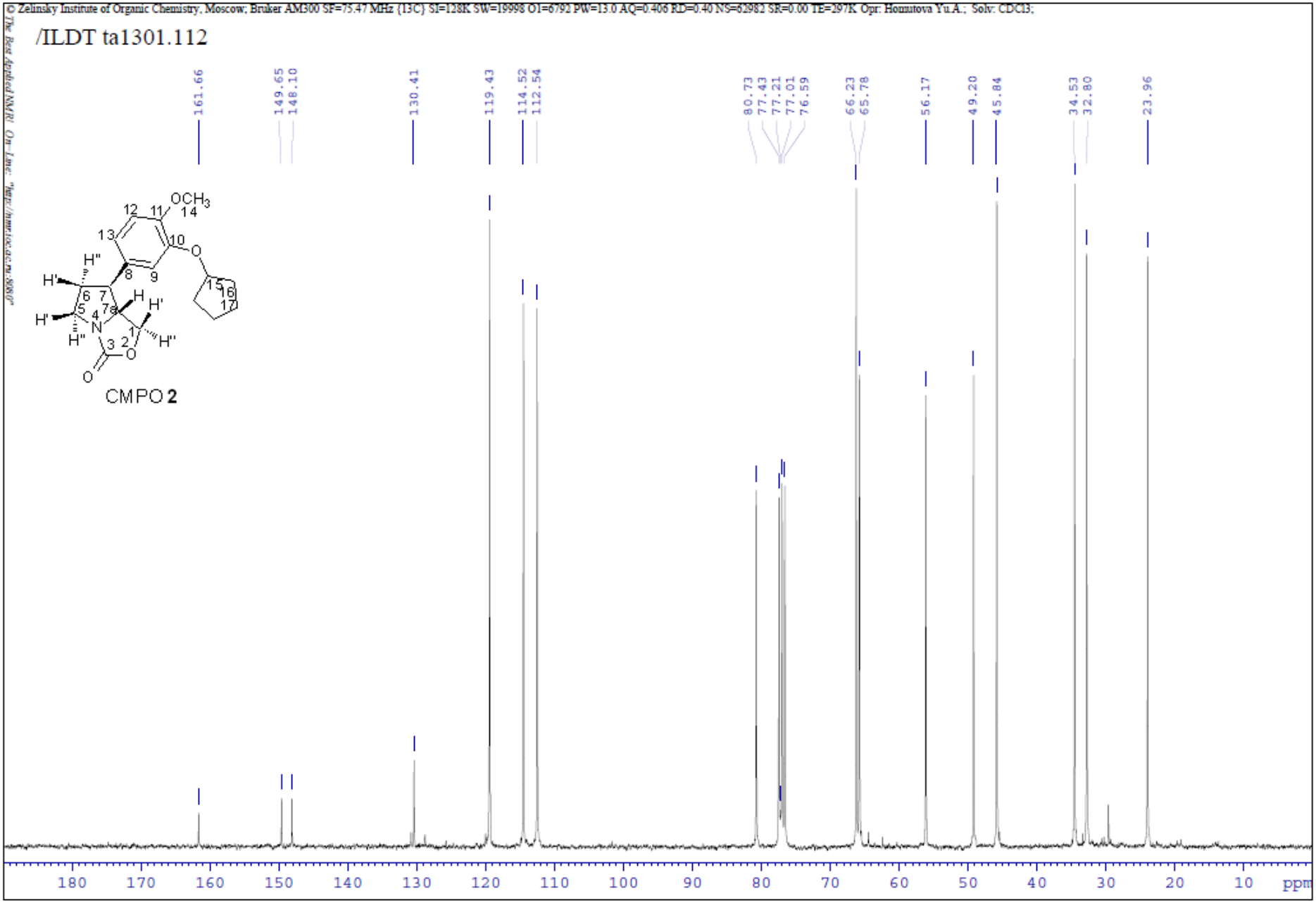
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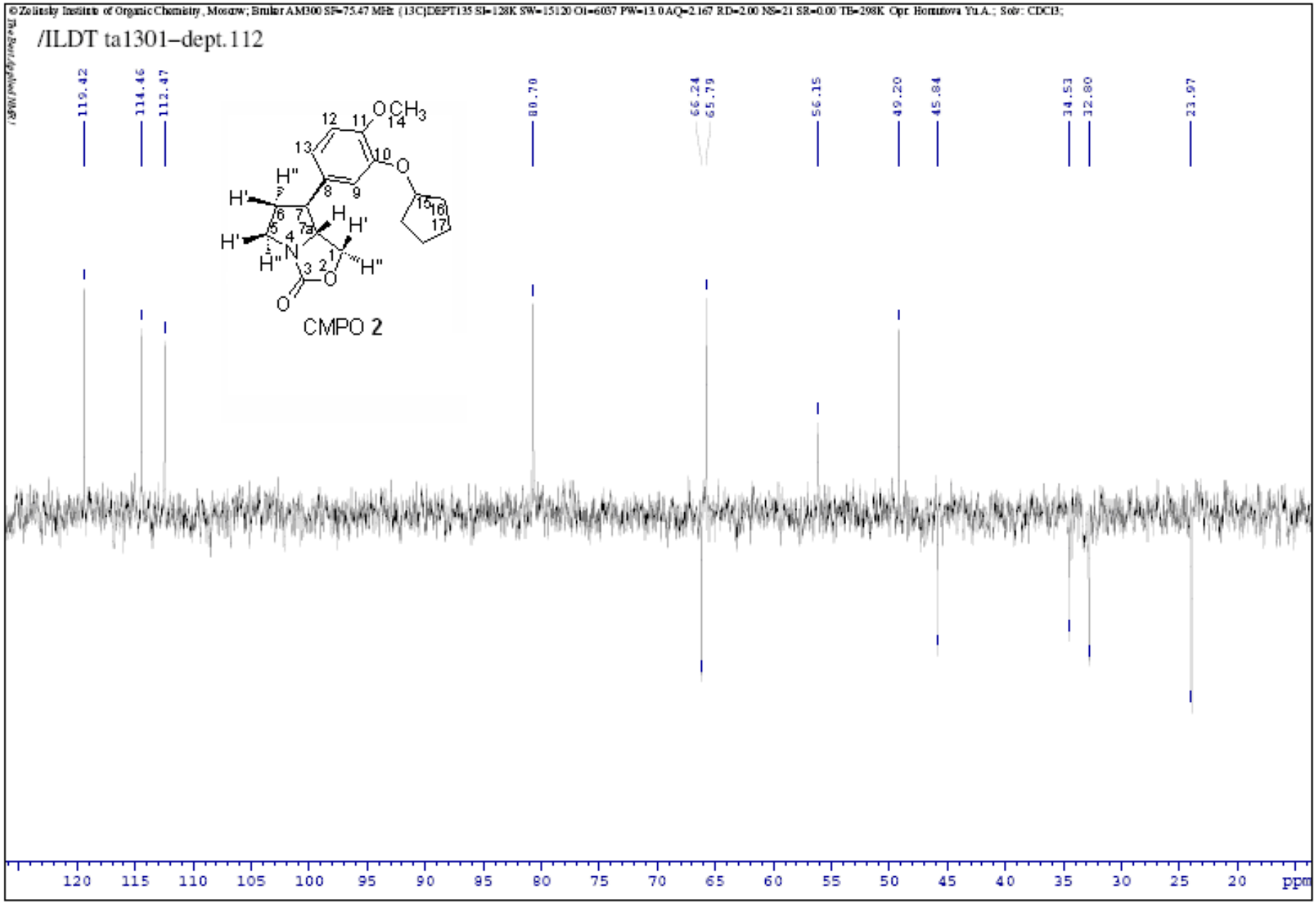

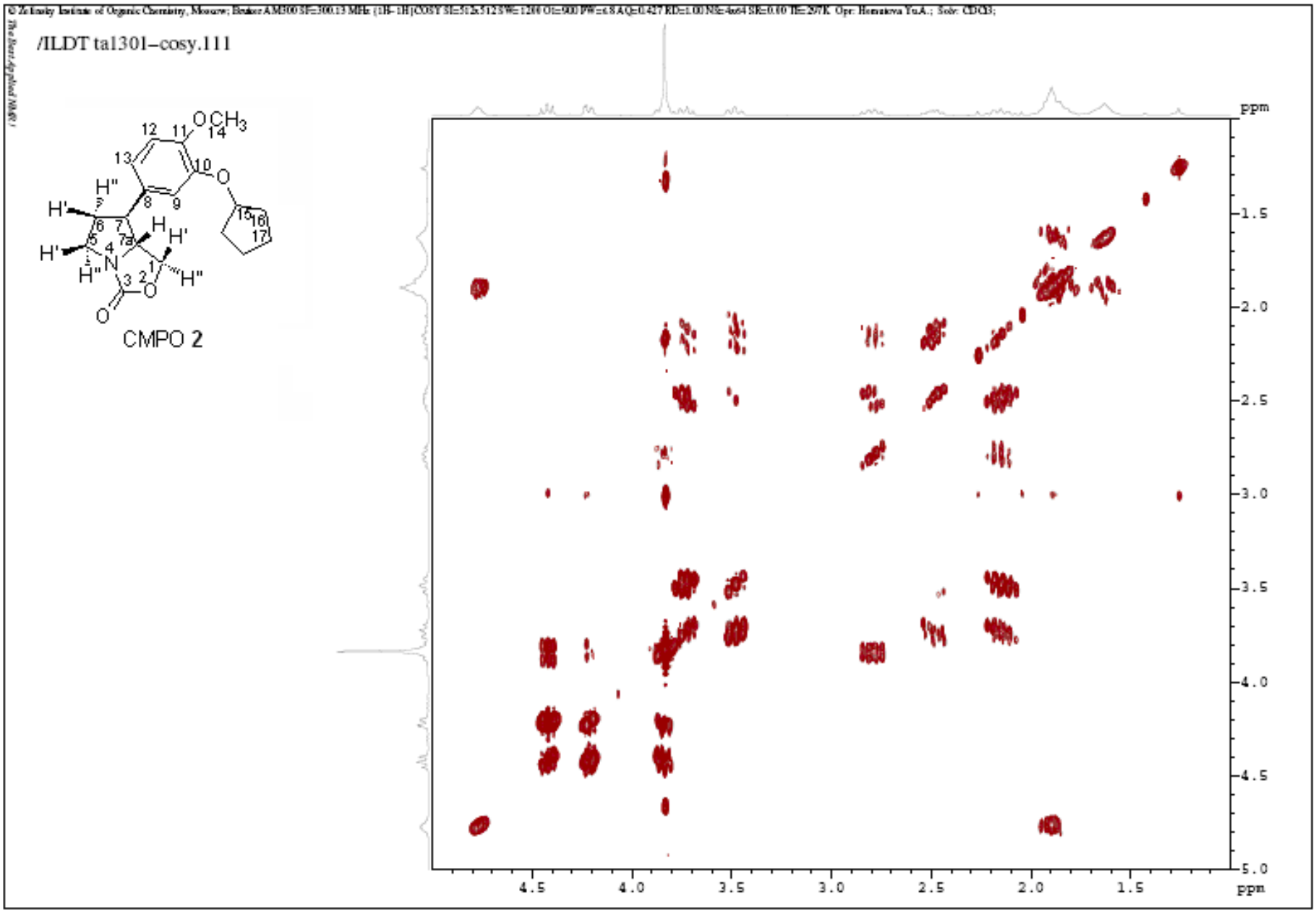

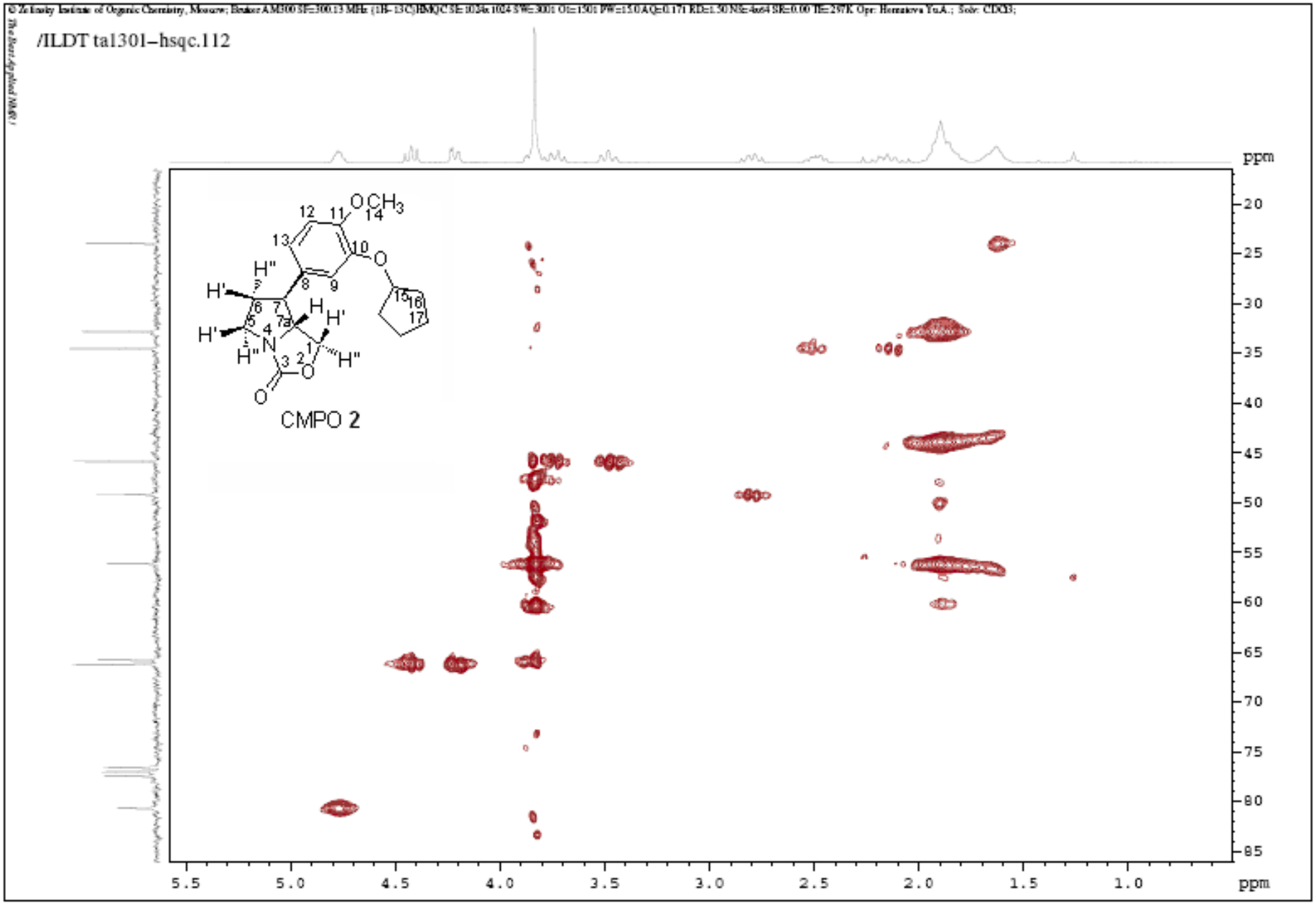

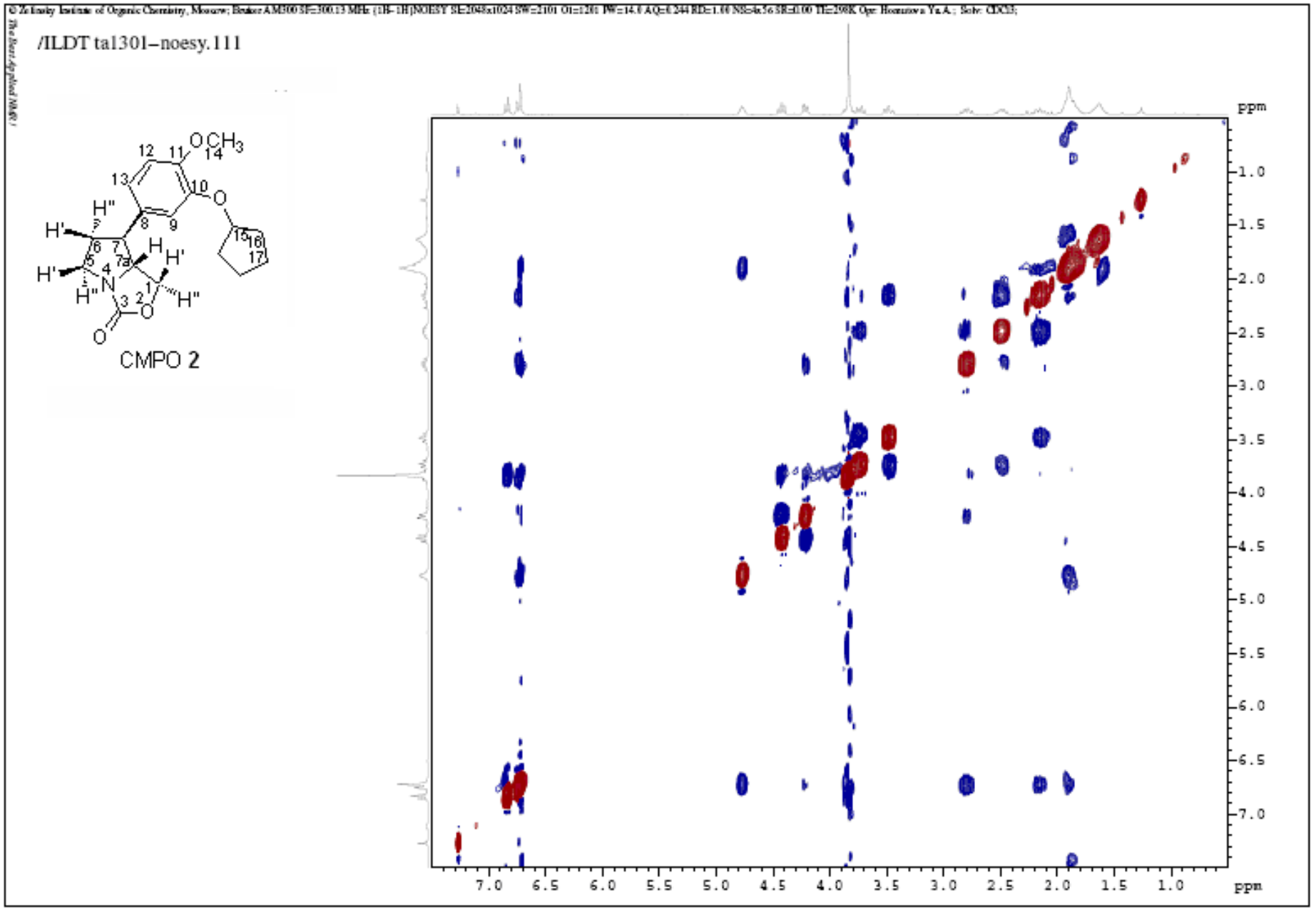

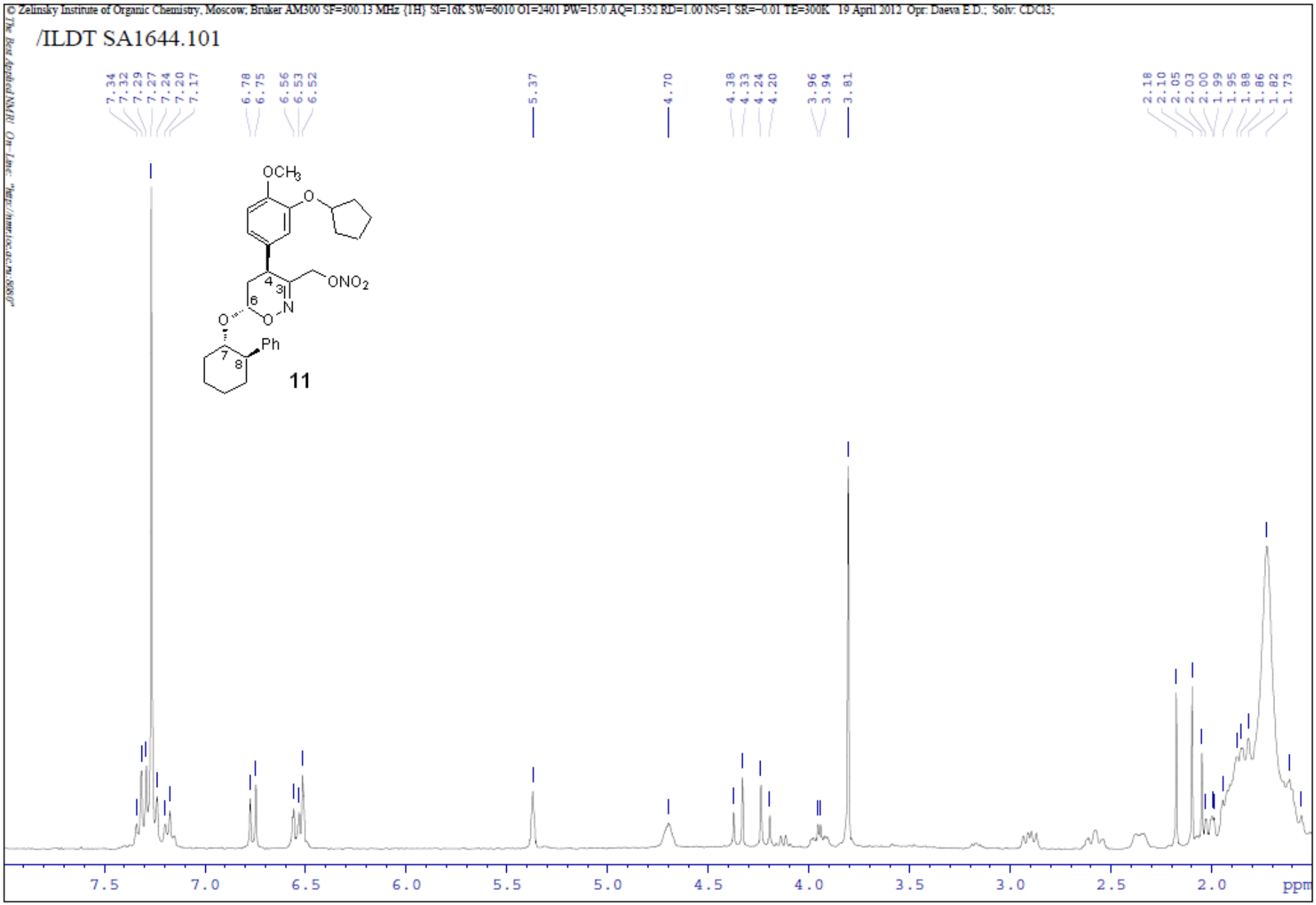

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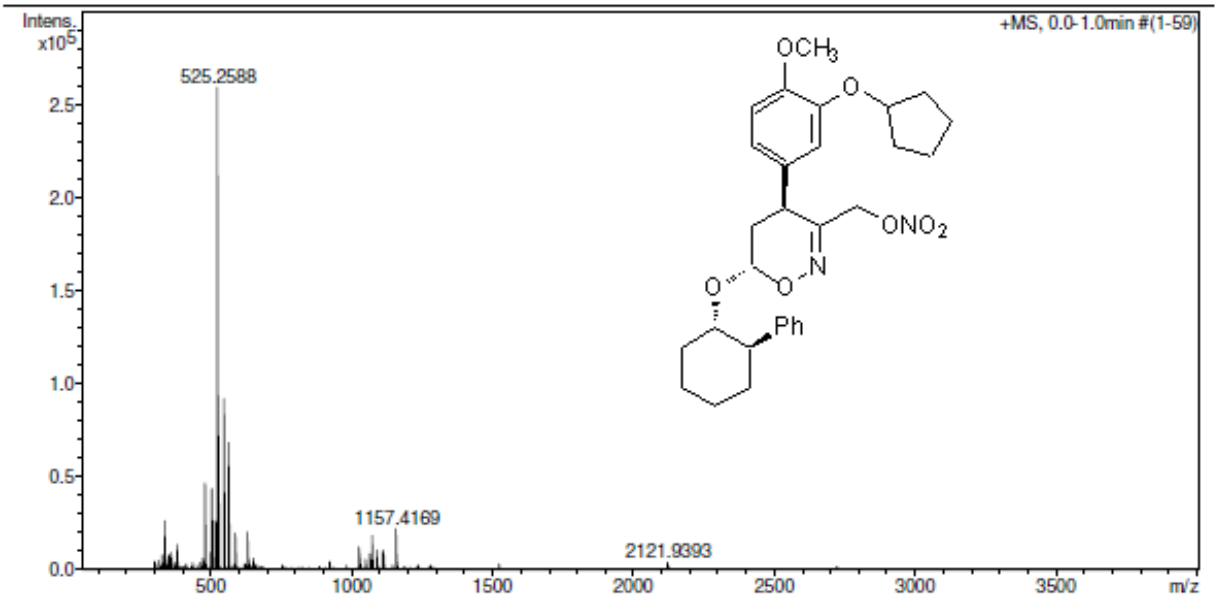

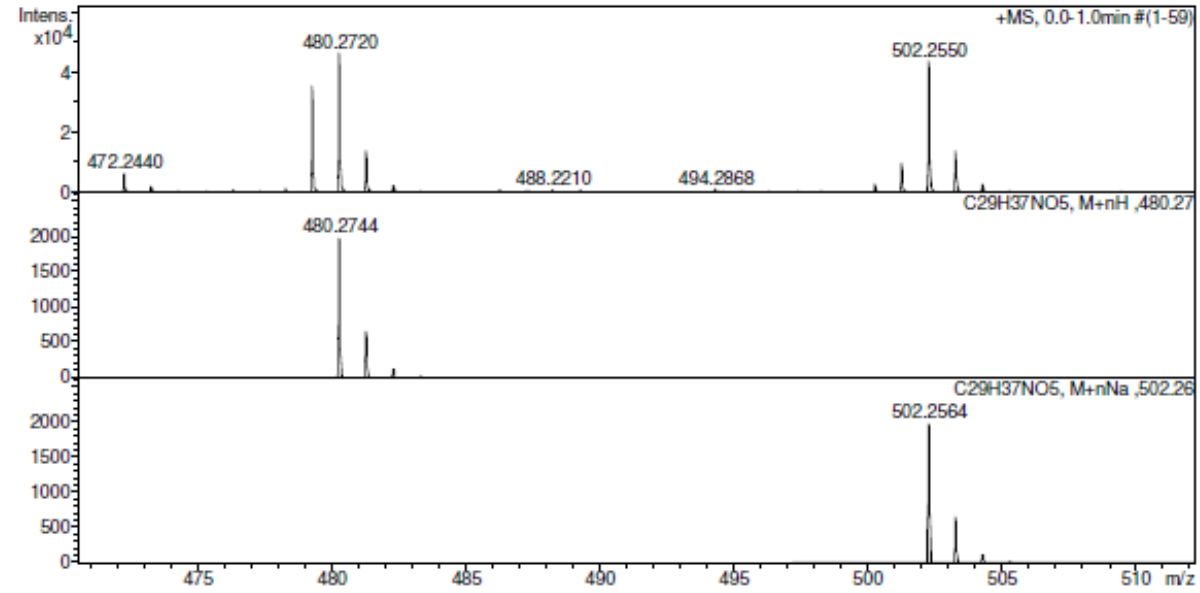

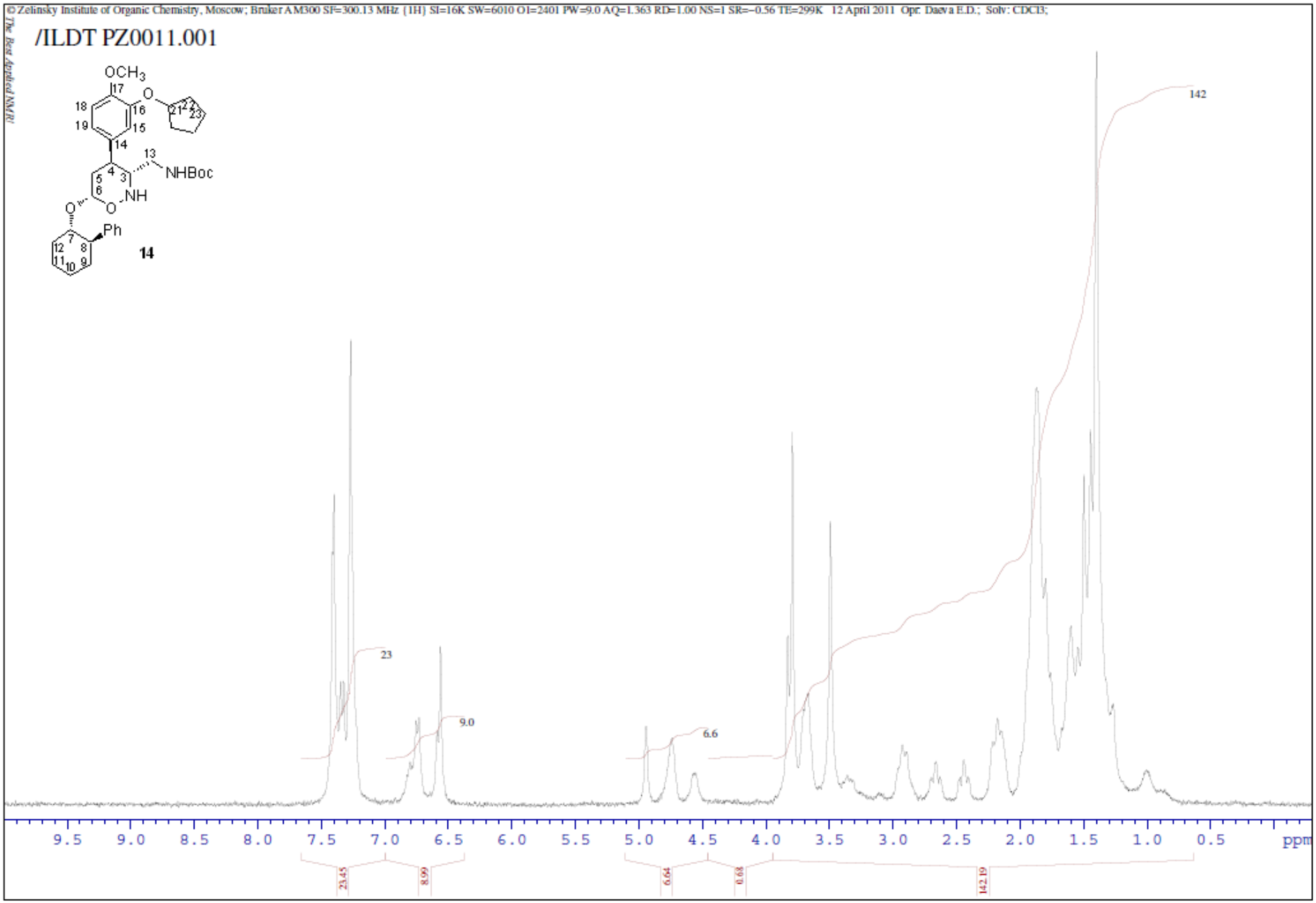
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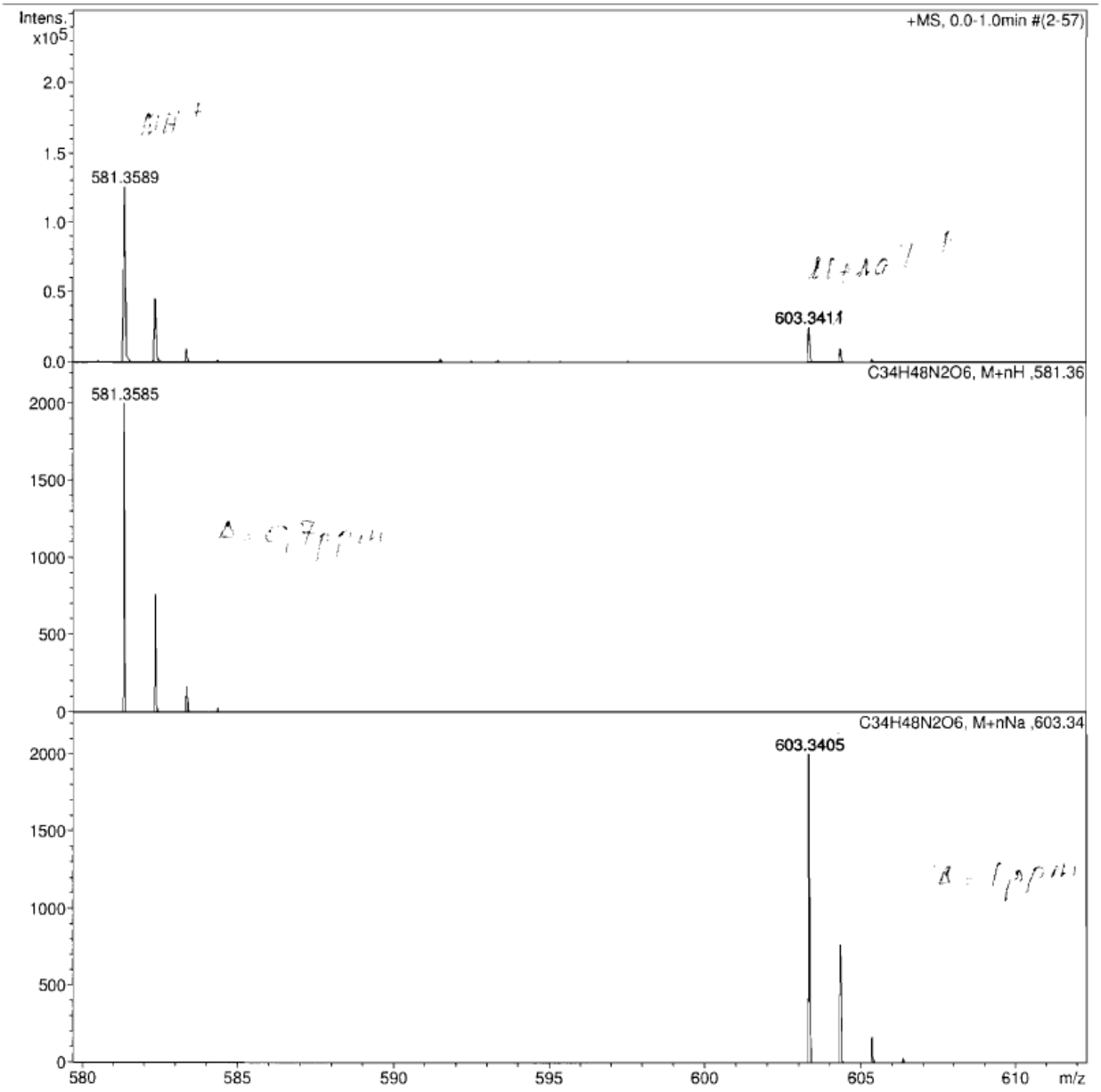

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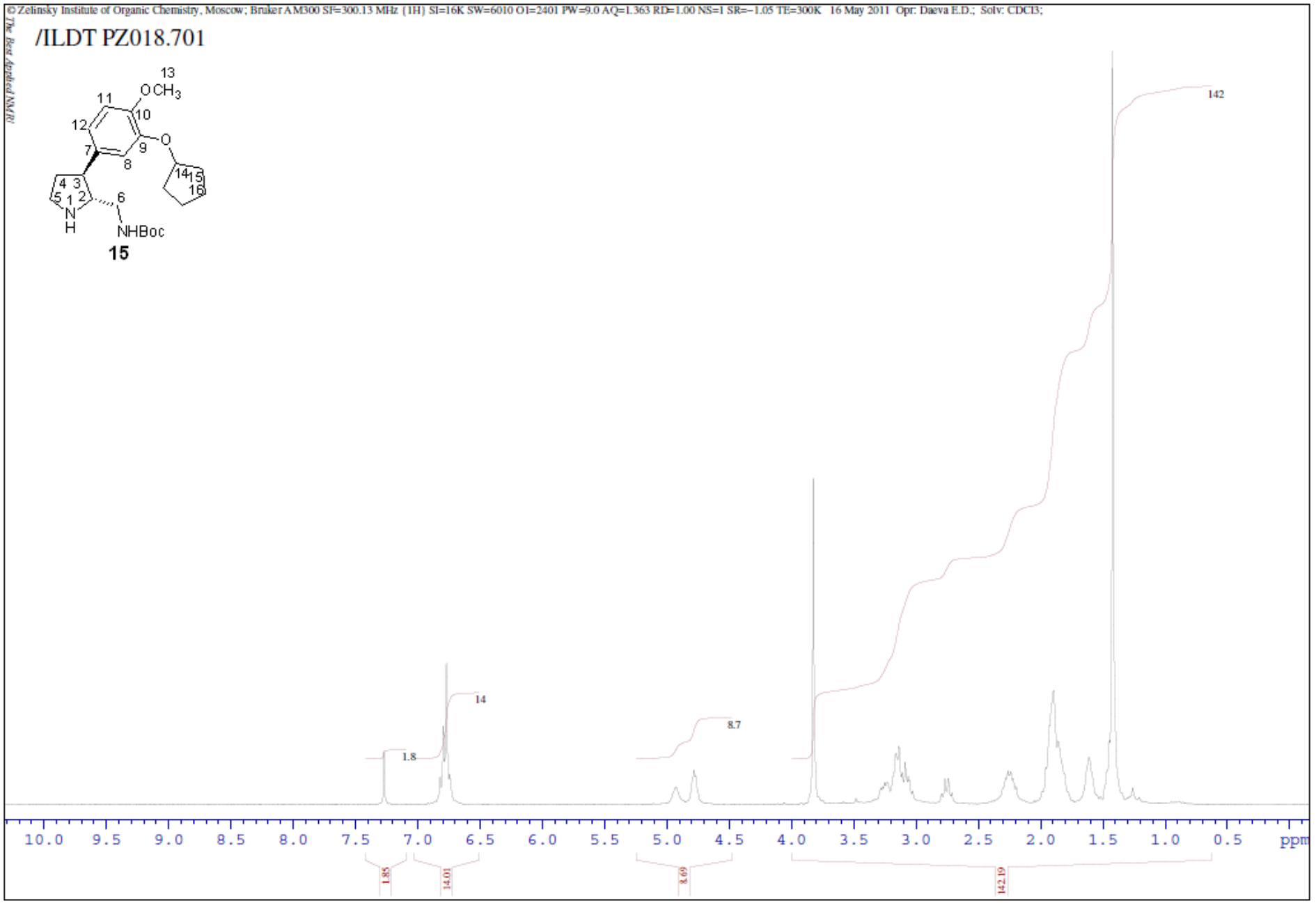

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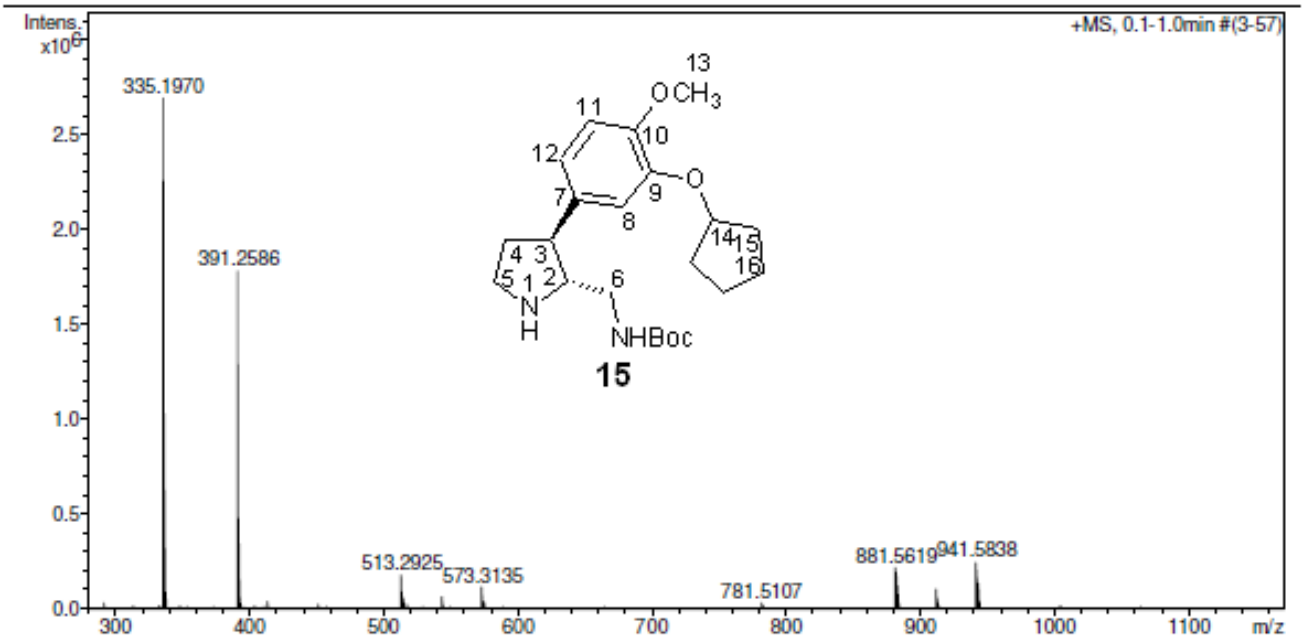

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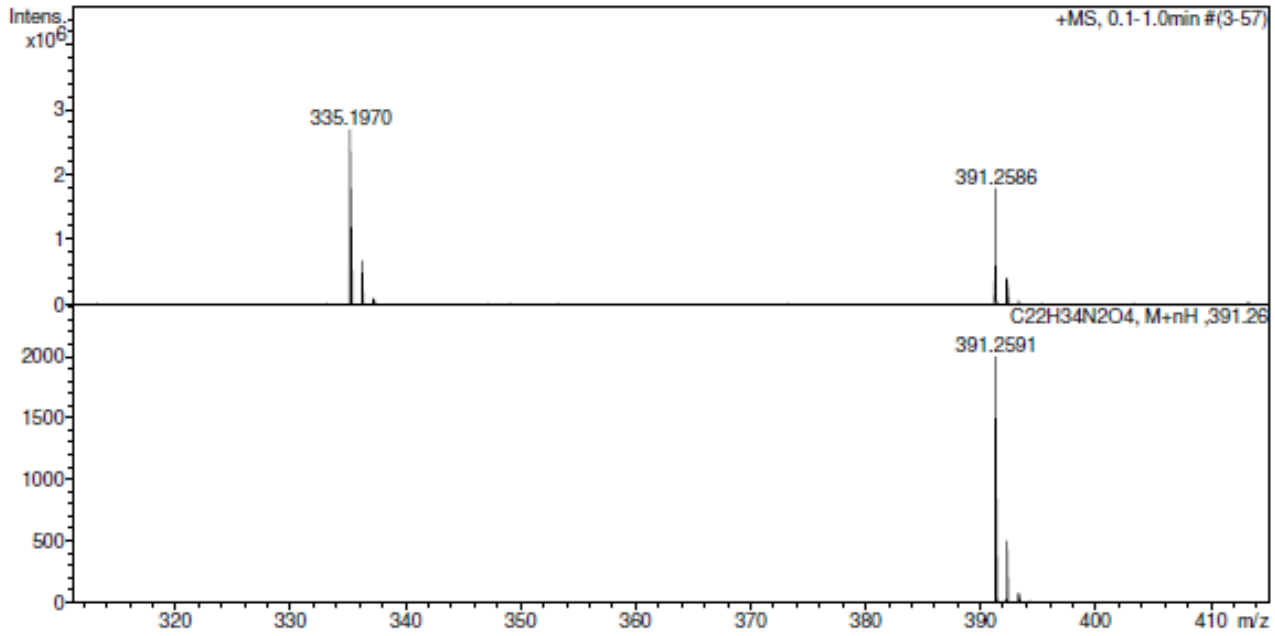


Evaluation of PDEIVB1 Inhibition Activity of CMPI 1 and CMPO 2 Enantiomers

Materials and methods.

PDE4B1 assay kit was used (BPS catalogue number 60342). A series of dilutions of the test compound (Table 1) were prepared with 10% DMSO in assay buffer and 5µl of the dilution was added to a 50µl reaction so that the final concentration of DMSO is 1% in all of reactions.

Table 1.

Compound I.D.	Stock Concentration	Dissolving Solvent	Test Range (µM)	Intermediate Dilution
(–)-(7*S*,7a*R*)-CMPI **1**	10mM	DMSO	0.001 - 10	10 % DMSO in PDE Assay Buffer
(+)-(7*R*,7a*S*)-CMPI **1**	10mM	DMSO	0.001 - 10	10 % DMSO in PDE Assay Buffer
(–)-(7*S*,7a*R*)-CMPO **2**	10mM	DMSO	0.001 - 10	10 % DMSO in PDE Assay Buffer
(+)-(7*R*,7a*S*)-CMPO **2**	10mM	DMSO	0.001 - 10	10 % DMSO in PDE Assay Buffer
rac-CMPO **2**	10mM	DMSO	0.001 - 10	10 % DMSO in PDE Assay Buffer

The enzymatic reactions were conducted at room temperature for 60 minutes in a 50µl mixture containing PDE assay buffer, 100nM FAM-cAMP, a PDE enzyme (Table 2) and the test compound (Table 1).

Table 2.

Assay	BPS Catalog Number	Enzyme Used (ng) / Reaction	Substrate
PDE4B1	60041	0.05	100 nM FAM-cAMP

After the enzymatic reaction, 100 µl of a binding solution (1:100 dilution of the binding agent with the binding agent diluent) was added to each reaction and the reaction was performed at room temperature for 30 minutes. Fluorescence intensity was measured at an excitation of 485 nm and an emission of 528 nm using a Tecan Infinite M1000 microplate reader.

Data analysis

PDE activity assays were performed in duplicate at each concentration. Fluorescence intensity is converted to fluorescence polarization using the Tecan Magellan6 software. The fluorescence

polarization data were analyzed using the computer software, Graphpad Prism. The fluorescence polarization (FP_t) in absence of the compound in each data set was defined as 100% activity. In the absence of PDE and the compound, the value of fluorescent polarization (FP_b) in each data set was defined as 0% activity. The percent activity in the presence of the compound was calculated according to the following equation: % activity = $(FP-FP_b)/(FP_t-FP_b)\times100\%$, where FP= the fluorescence polarization in the presence of the compound.

The values of % activity versus a series of compound concentrations were then plotted using non-linear regression analysis of Sigmoidal dose-response curve generated with the equation $Y=B+(T-B)/1+10^{((LogEC50-X)\times Hill\ \ Slope)}$, where Y=percent activity, B=minimum percent activity, T=maximum percent activity, X= logarithm of compound and Hill Slope=slope factor or Hill coefficient. The IC_{50} value was determined by the concentration causing a half-maximal percent activity.

Data for the effect of each compound on PDE4B1 activity is presented below.

Table 3. Data for the Effect of (–)-(7*S*,7a*R*)-CMPI **1** on PDE4B1 Activity

(–)-(7*S*,7a*R*)-CMPI **1** (Log [μM])	PDE Activity [(Fluorescent Polarization (mp)]		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	132	142	95.58	104.42
-3.0	126	130	90.06	93.38
-2.5	114	118	79.46	83.32
-2.0	86	91	54.69	59.54
-1.5	64	65	35.91	36.44
-1.0	39	44	13.93	17.93
-0.5	30	29	5.37	4.55
0.0	28	24	4.05	0.06
0.5	23	23	-0.93	-0.12
1.0	25	19	1.69	-4.12
Background	24	23	0.46	-0.46

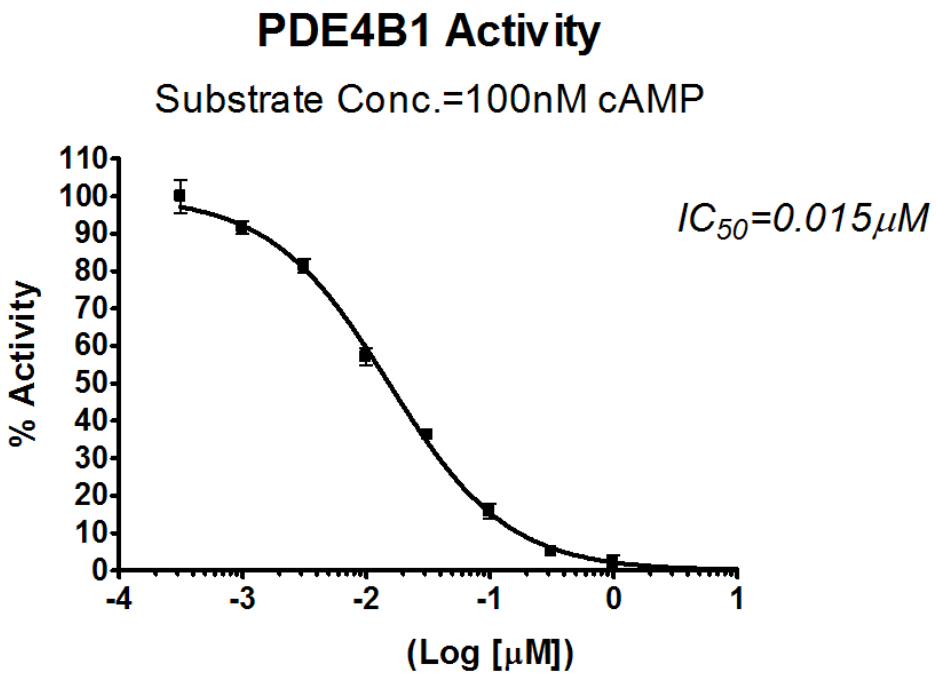


Table 4. Data for the Effect of (+)-(7*R*,7a*S*)-CMPI **1** on PDE4B1 Activity

(+)-(7*R*,7a*S*)-CMPI **1** (Log [μM])	PDE Activity [(Fluorescent Polarization (mp)]		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	138	130	103.22	96.78
-3.0	134	132	100.37	98.47
-2.5	130	131	96.59	97.60
-2.0	120	125	87.35	92.00
-1.5	116	113	83.77	81.43
-1.0	89	85	60.08	56.65
-0.5	56	55	31.12	30.46
0.0	41	36	17.48	13.77
0.5	29	29	7.37	7.36
1.0	28	34	6.45	11.52
Background	20	22	-0.78	0.78

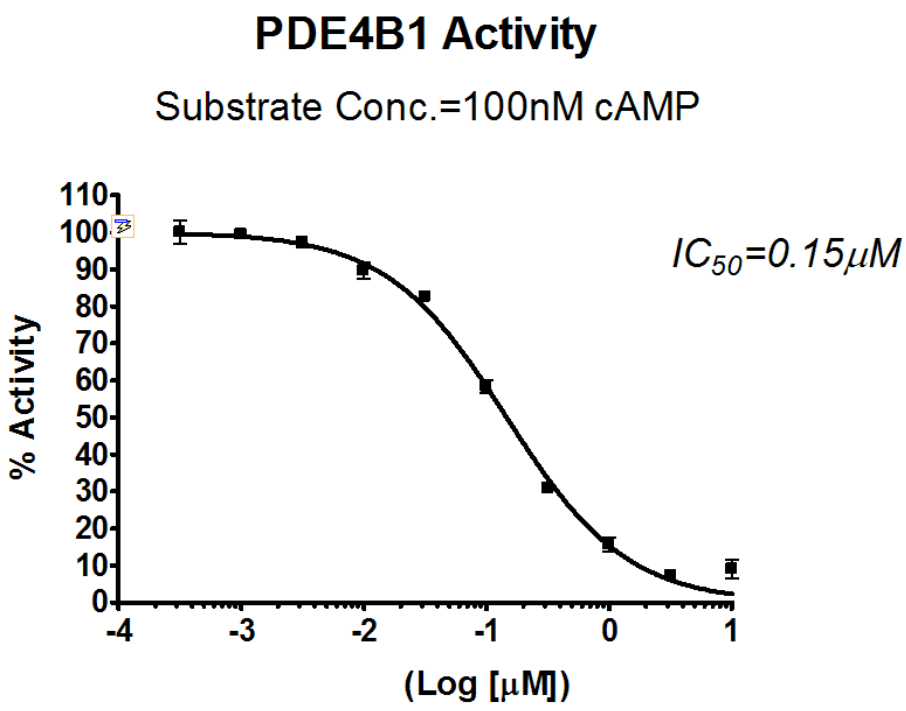


Table 5. Data for the Effect of (–)-(7*S*,7a*R*)-CMPO **2** on PDE4B1 Activity

(–)-(7*S*,7a*R*)-CMPO **2** (Log [μM])	PDE Activity [(Fluorescent Polarization (mp)]		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	124	128	98.18	101.82
-3.0	115	119	89.84	93.88
-2.5	108	121	83.15	95.85
-2.0	100	103	74.47	77.61
-1.5	76	79	51.53	53.91
-1.0	52	52	28.18	27.53
-0.5	40	34	15.88	9.80
0.0	28	24	3.97	0.47
0.5	25	31	1.39	6.91
1.0	25	27	1.56	3.65
Background	25	22	1.82	-1.82

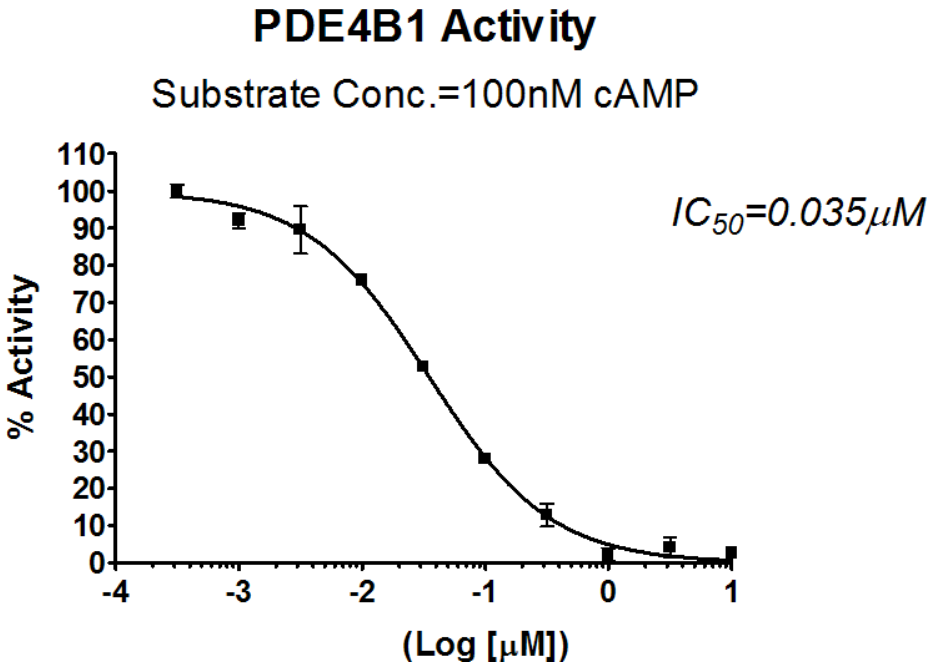


Table 6. Data for the Effect of (+)-(7*R*,7a*S*)-CMPO **2** on PDE4B1 Activity

(+)-(7*R*,7a*S*)-CMPO **2** (Log [μM])	PDE Activity [(Fluorescent Polarization (mp)]		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	118	122	98.06	101.94
-3.0	119	117	99.22	96.97
-2.5	115	113	94.81	92.34
-2.0	112	108	91.53	87.93
-1.5	97	98	76.61	77.99
-1.0	75	78	53.95	57.75
-0.5	57	58	36.35	37.38
0.0	40	47	18.99	26.13
0.5	26	29	4.76	7.32
1.0	25	25	3.72	3.19
Background	22	21	0.51	-0.51

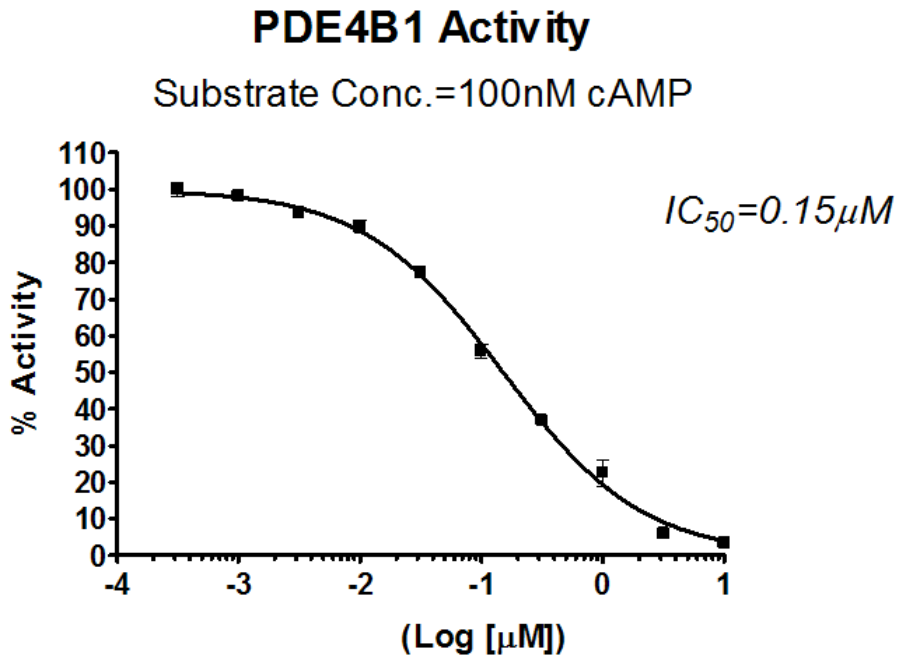
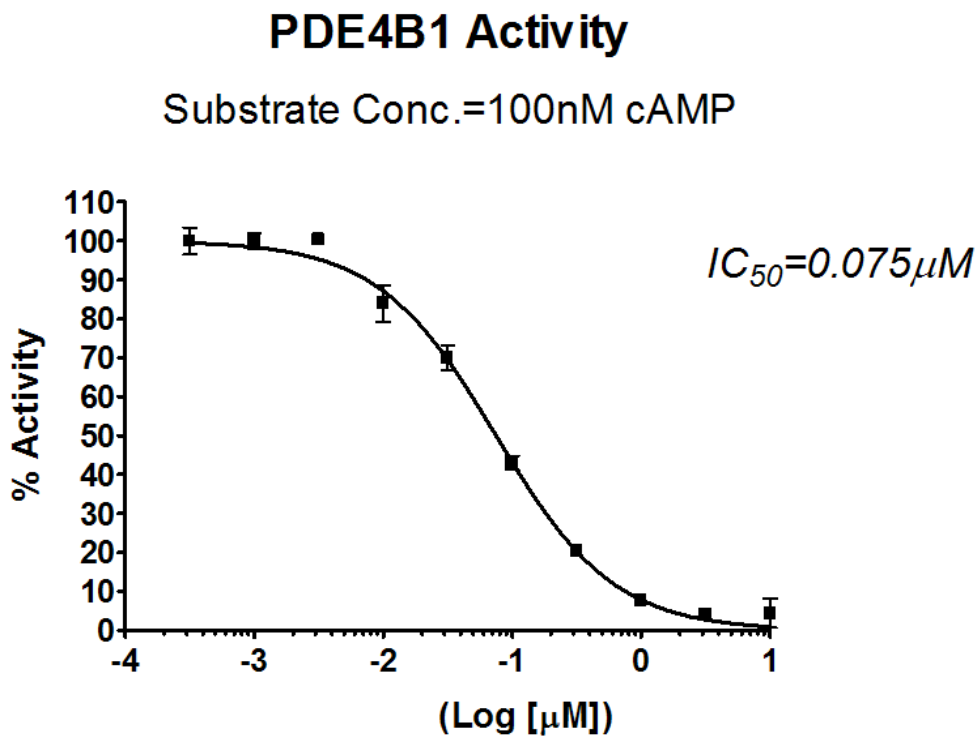


Table 7. Data for the Effect of *rac*-CMPO **2** on PDE4B1 Activity

rac-CMPO **2** (Log [μM])	PDE Activity [(Fluorescent Polarization (mp)]		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	128	136	96.60	103.40
-3.0	130	134	97.90	101.81
-2.5	133	132	100.83	99.79
-2.0	109	119	79.24	88.69
-1.5	95	102	66.96	73.04
-1.0	66	70	41.25	44.82
-0.5	41	44	19.13	21.52
0.0	28	28	7.28	7.76
0.5	23	25	3.12	5.10
1.0	20	29	0.66	8.14
Background	15	24	-4.18	4.18



Molecular docking of CMPI 1 and CMPO 2 enantiomers

Despite the structural diversity of the PDE IVB structures available to the moment (~35) and their co-crystallized ligands the binding site of the PDE IVB is rather rigid. For example, overlapping of the 3KKT and 1XLX results in the almost identical binding site except the C-capped terminus (see Figure 1) suggesting that the interactions of ligands with these binding sites will be very similar. Thus, only one PDE IVB structure (3KKT) was used to study binding of CMPI **1** and CMPO **2** enantiomers.

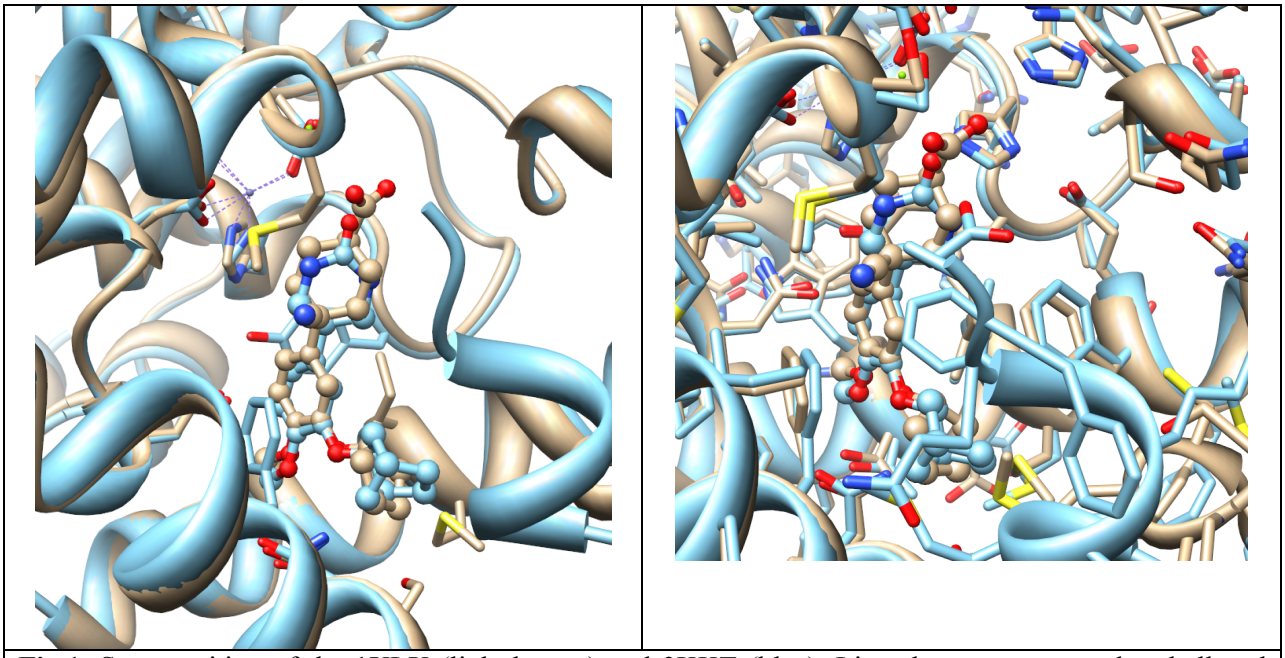


Fig.1. Superposition of the 1XLX (light-brown) and 3KKT (blue). Ligands are represented as ball and stick. Figure on the left – only some amino acids interacting with ligands are shown, on the right – all.

Protein structure was prepared according to classical AutoDock scenario: ligand and water molecules were removed, atoms of Zn^{2+} and Mg^{2+} were remained in the protein structure, nonpolar hydrogens, Gasteiger-Huckel charges and studied ligands CMPI **1** and CMPO **2** were added to protein. Spatial structures of the ligands were calculated with OpenBabel. Top scored poses according to predicted free energy of binding were selected for further discussion of protein-ligand interactions (Table 8). Visualization of interactions of molecules CMPI **1** and CMPO **2** with PDE IVB was done using Pymol v. 0.99.

Table 8. Predicted affinities for CMPI-**1** and CMPO-**2** enantiomers

Compound	Affinity, kcal/mol (AutoDock Vina)
(–)-(7*S*,7a*R*)-CMPI **1**	-10.2
(+)-(7*R*,7a*S*)-CMPI **1**	-10.5
(–)-(7*S*,7a*R*)-CMPO **2**	-10.5
(+)-(7*R*,7a*S*)-CMPO **2**	-10.4