

## Supporting Information:

Chemo-Enzymatic Synthesis of New 2,4-Syn-Functionalized (*S*)-Glutamate Analogues and Structure-Activity Relationship Studies at Ionotropic Glutamate Receptors and Excitatory Amino Acid Transporters

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### **Table of Content:**

Elemental analysis data of <b>2b-p</b> , <b>6a-j</b> and <b>10</b>	page S2
<sup>1</sup> H and <sup>13</sup> C NMR spectra of <b>2b-p</b>	page S3-S15
HPLC analyses of <b>2e</b> , <b>2i</b> and <b>2o</b>	page S18-S19
X-Ray crystal data and refinement statistics	page S20
Figure S1	page S21

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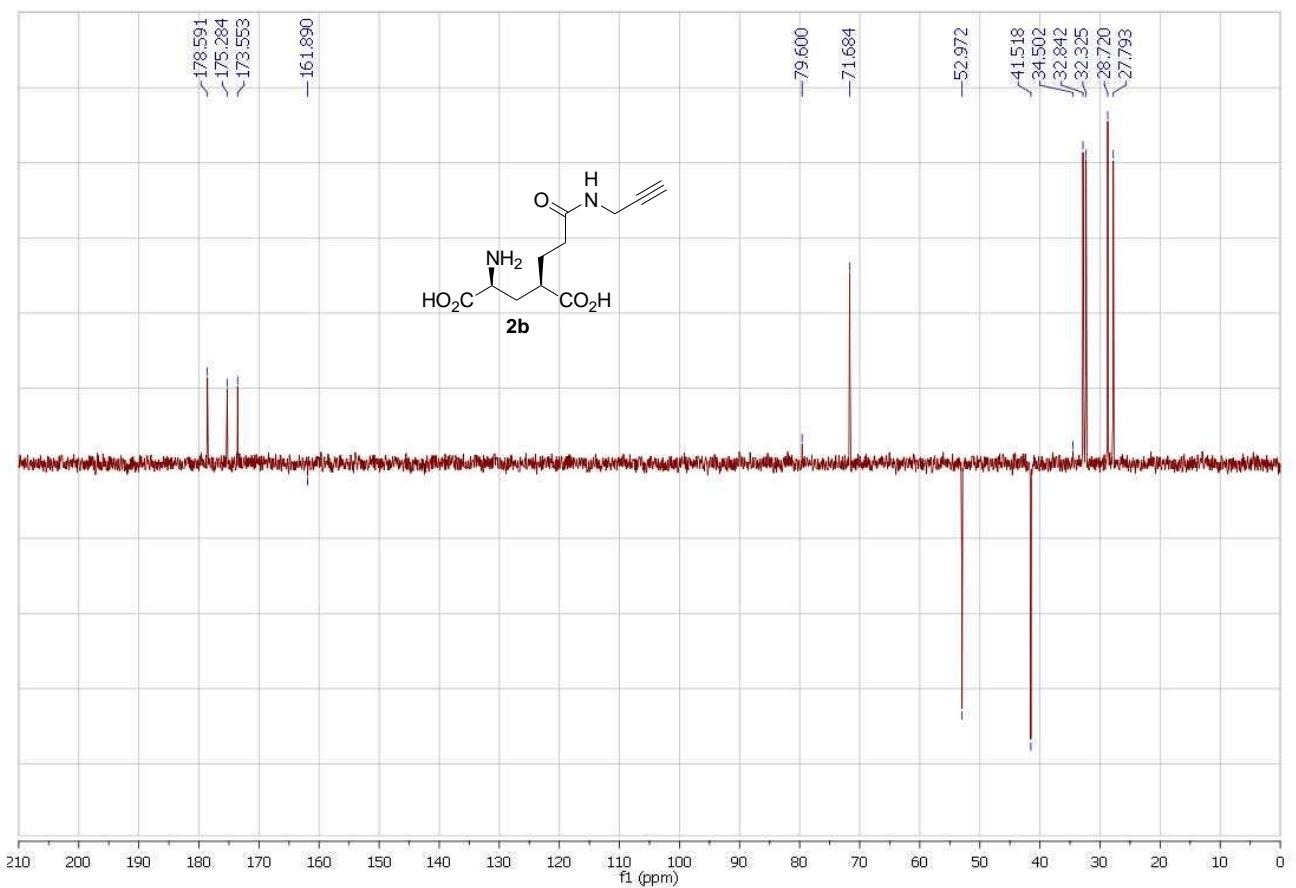
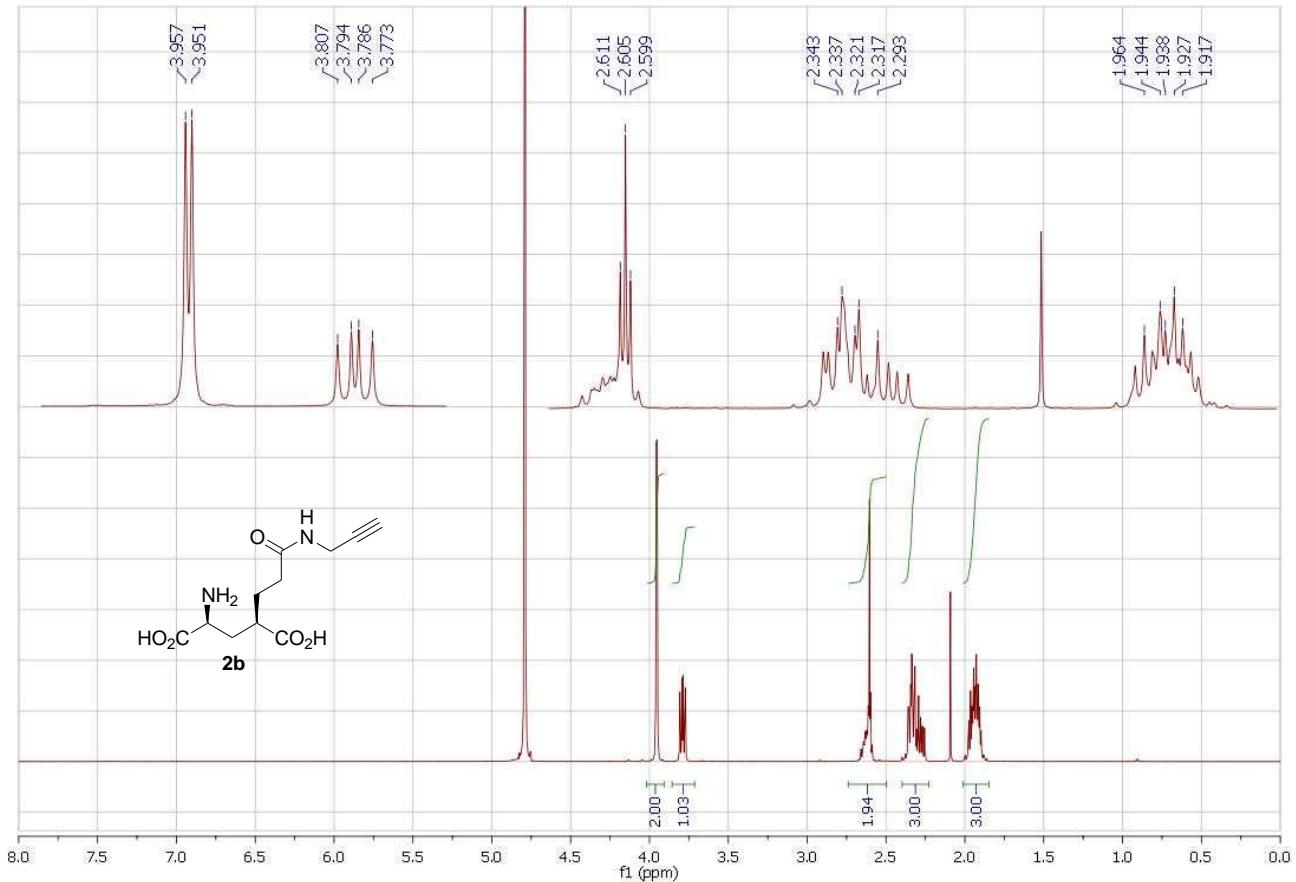
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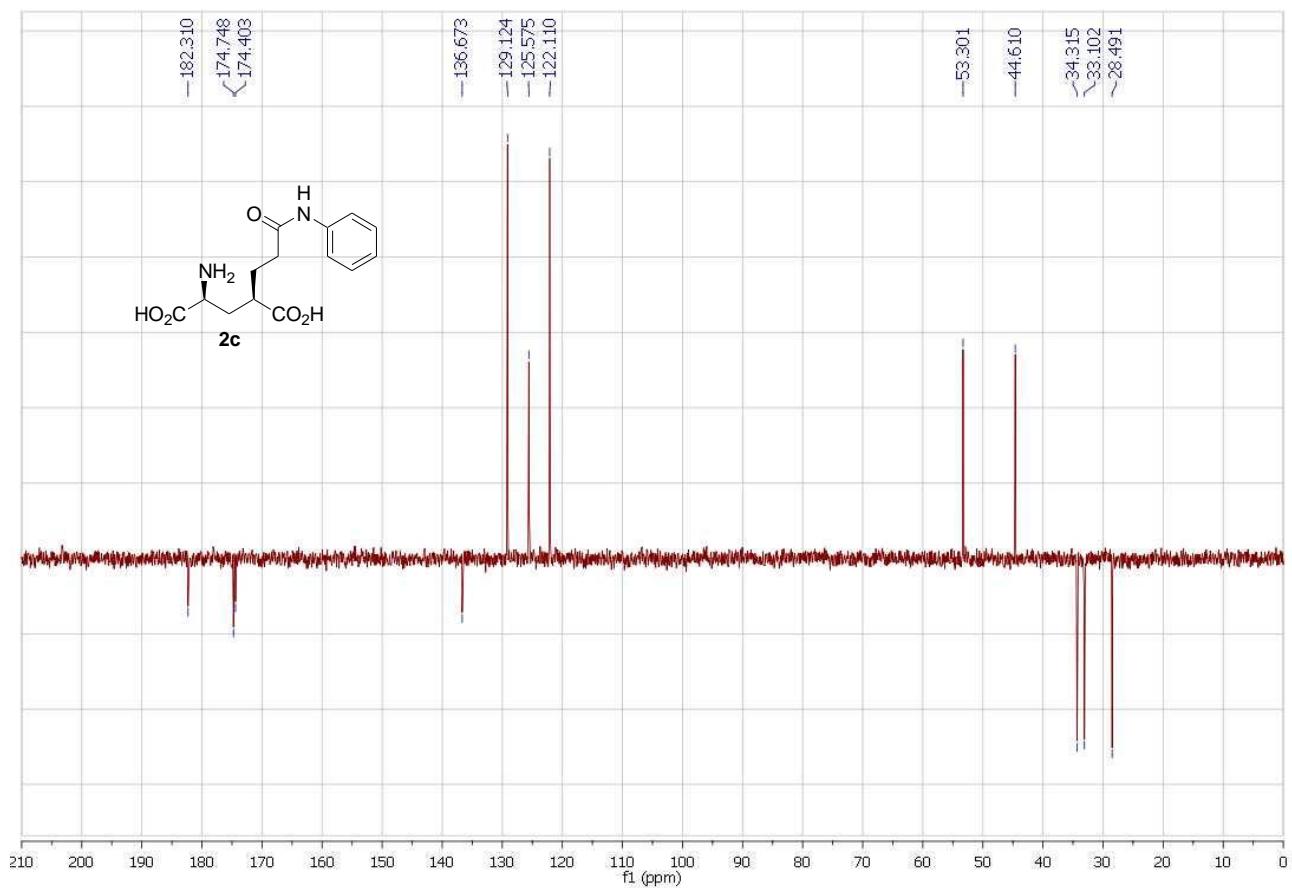
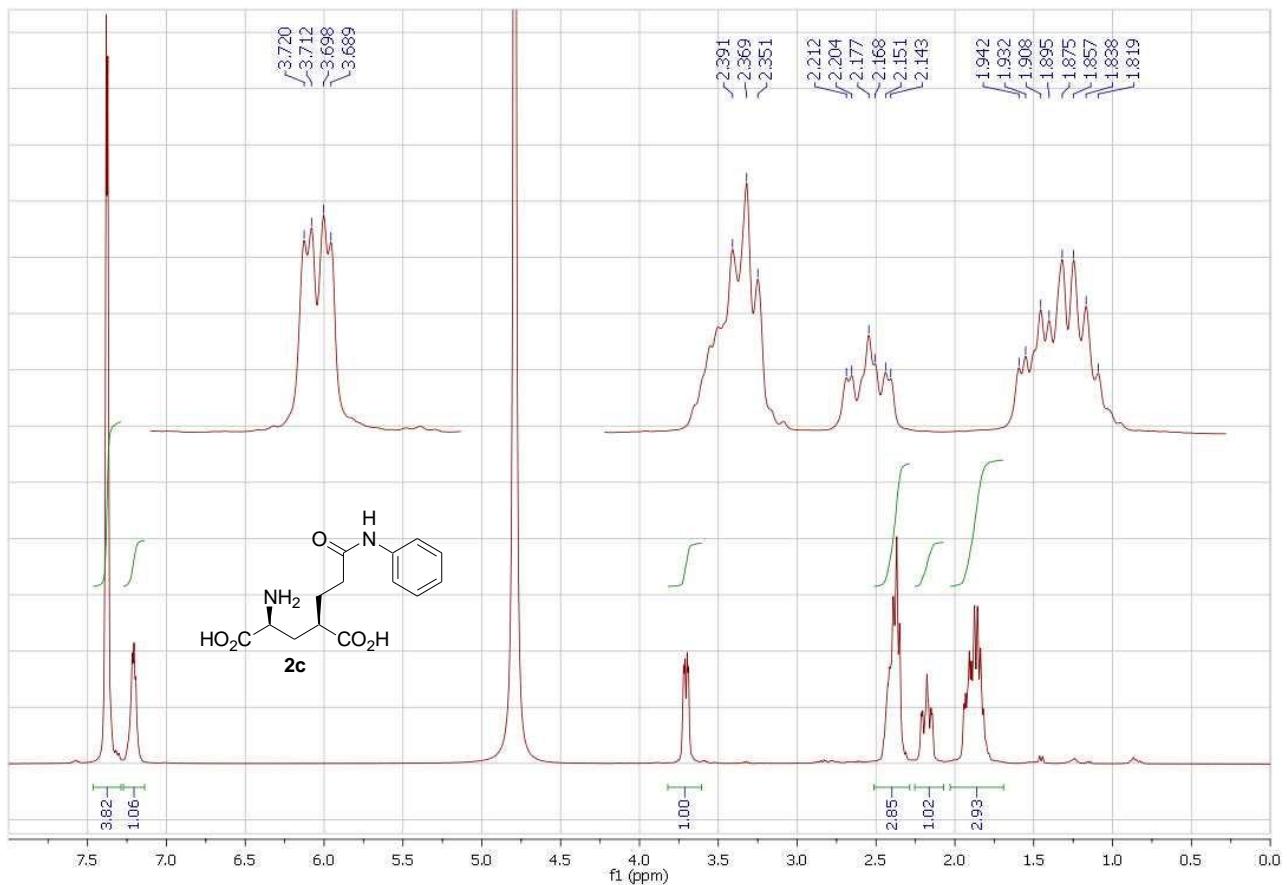
### **Corresponding Authors**

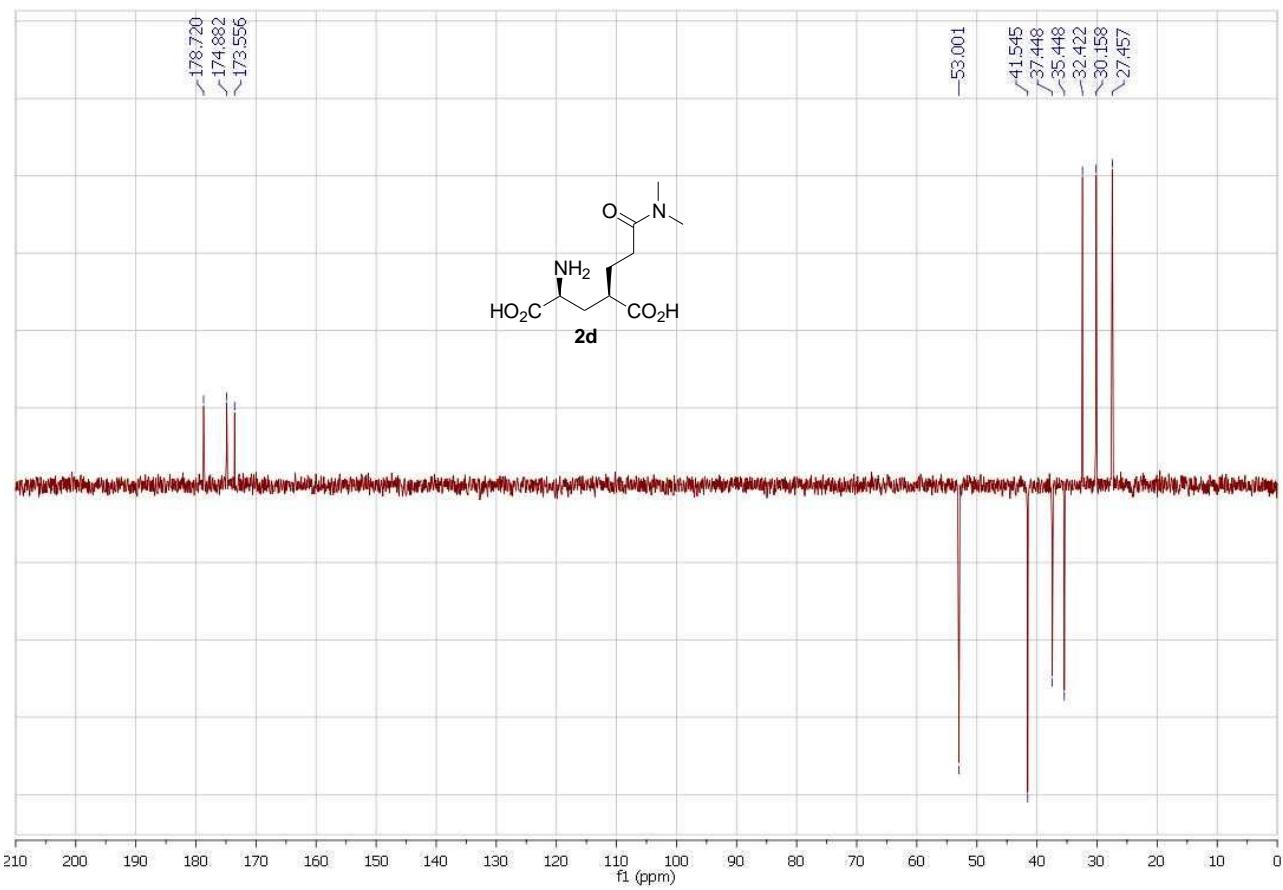
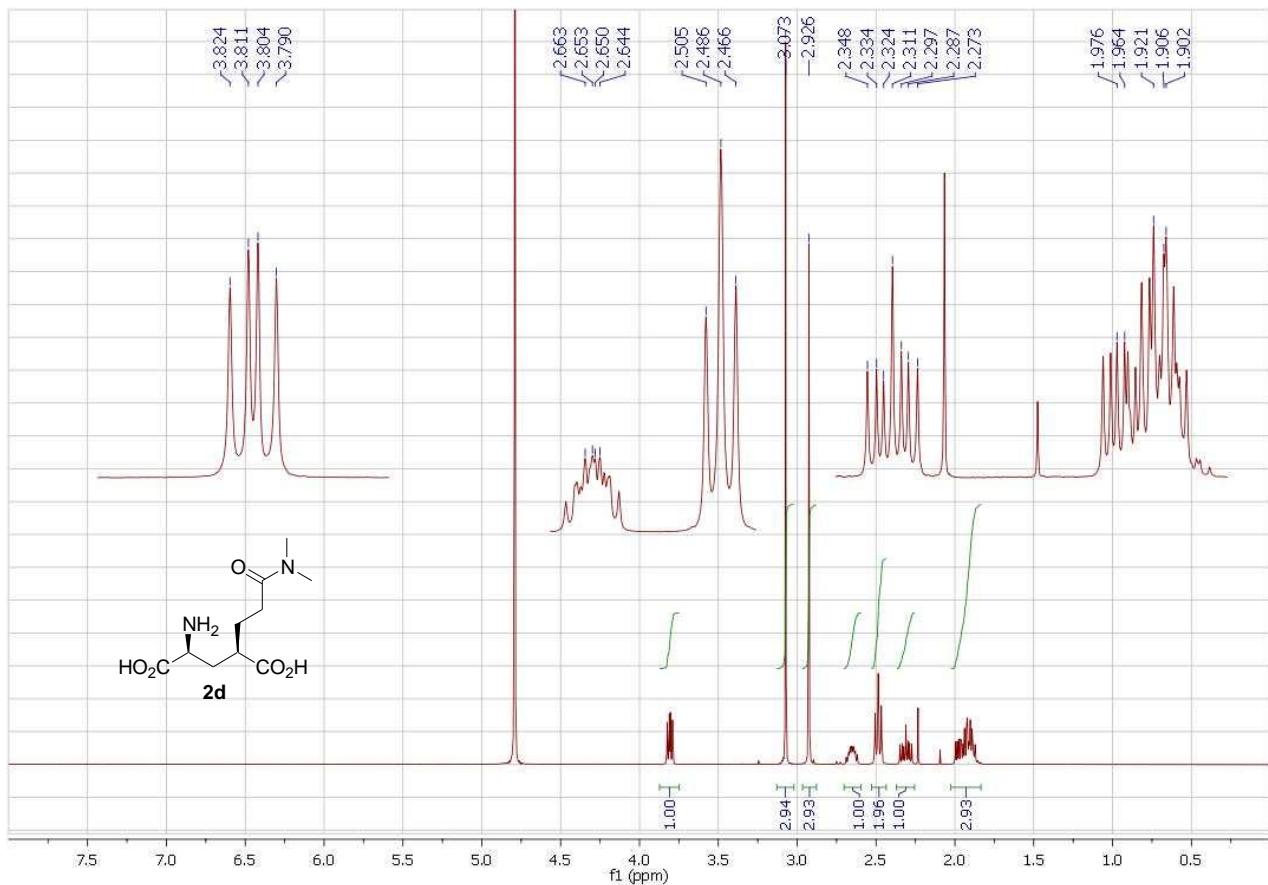
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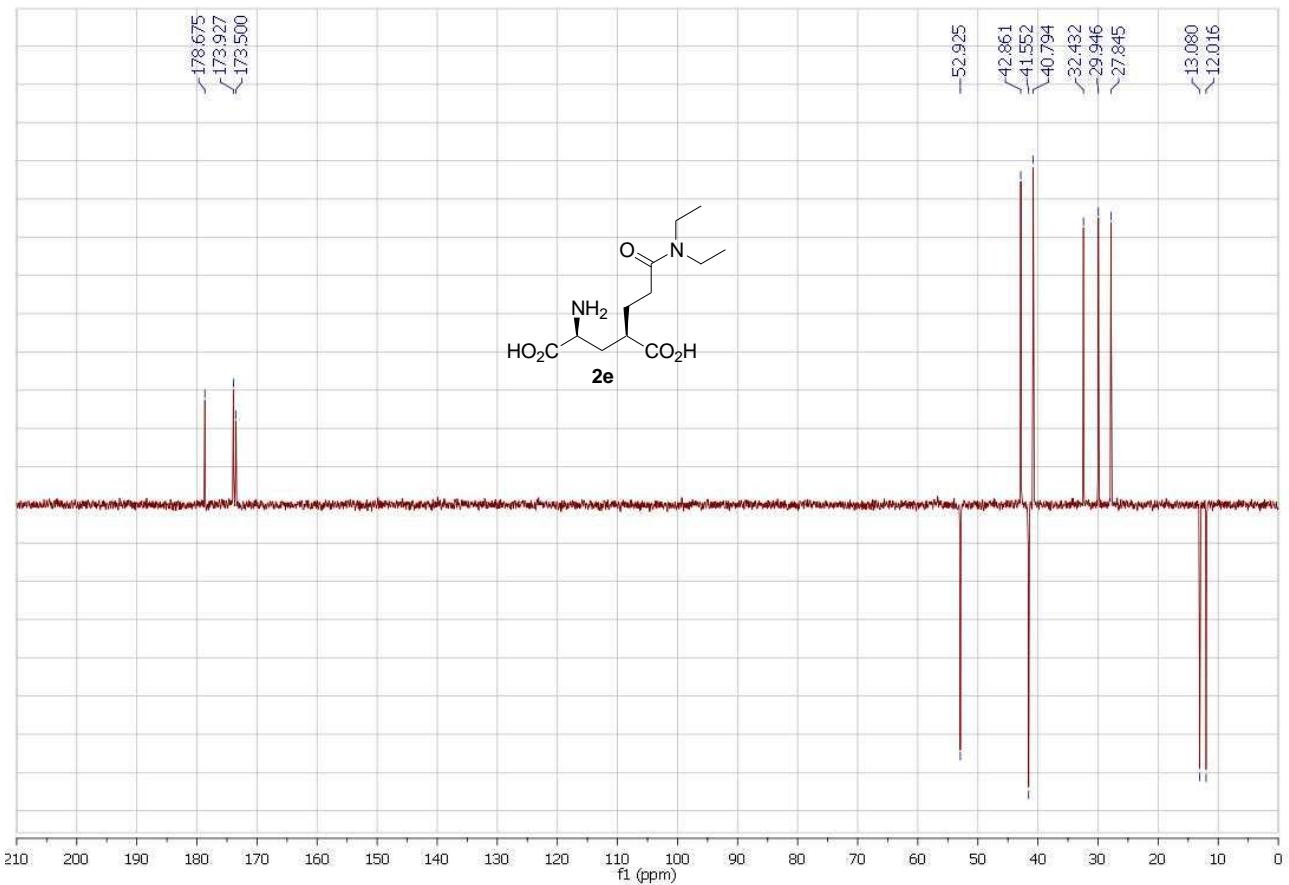
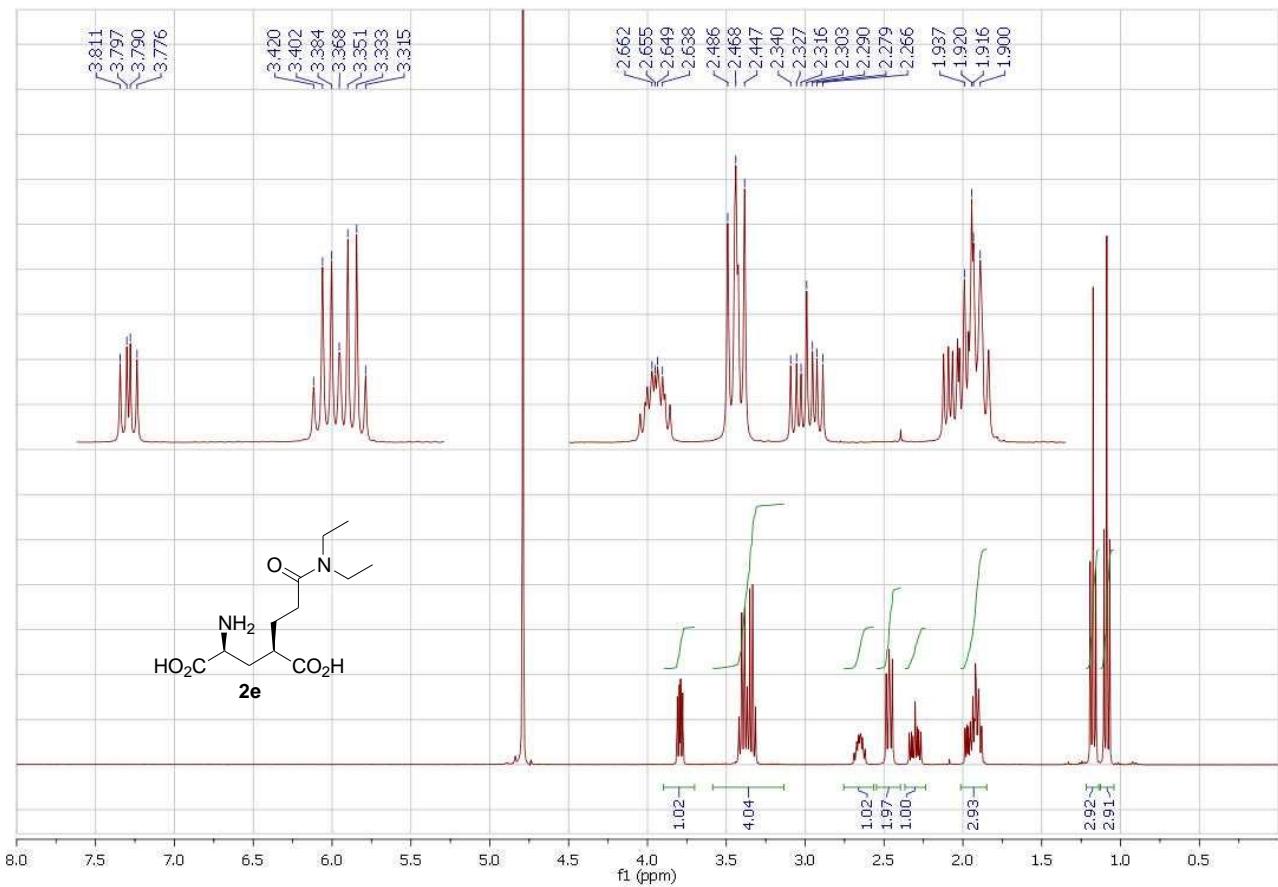
**Elemental analyses for 2b-p, 6a-j and 10**

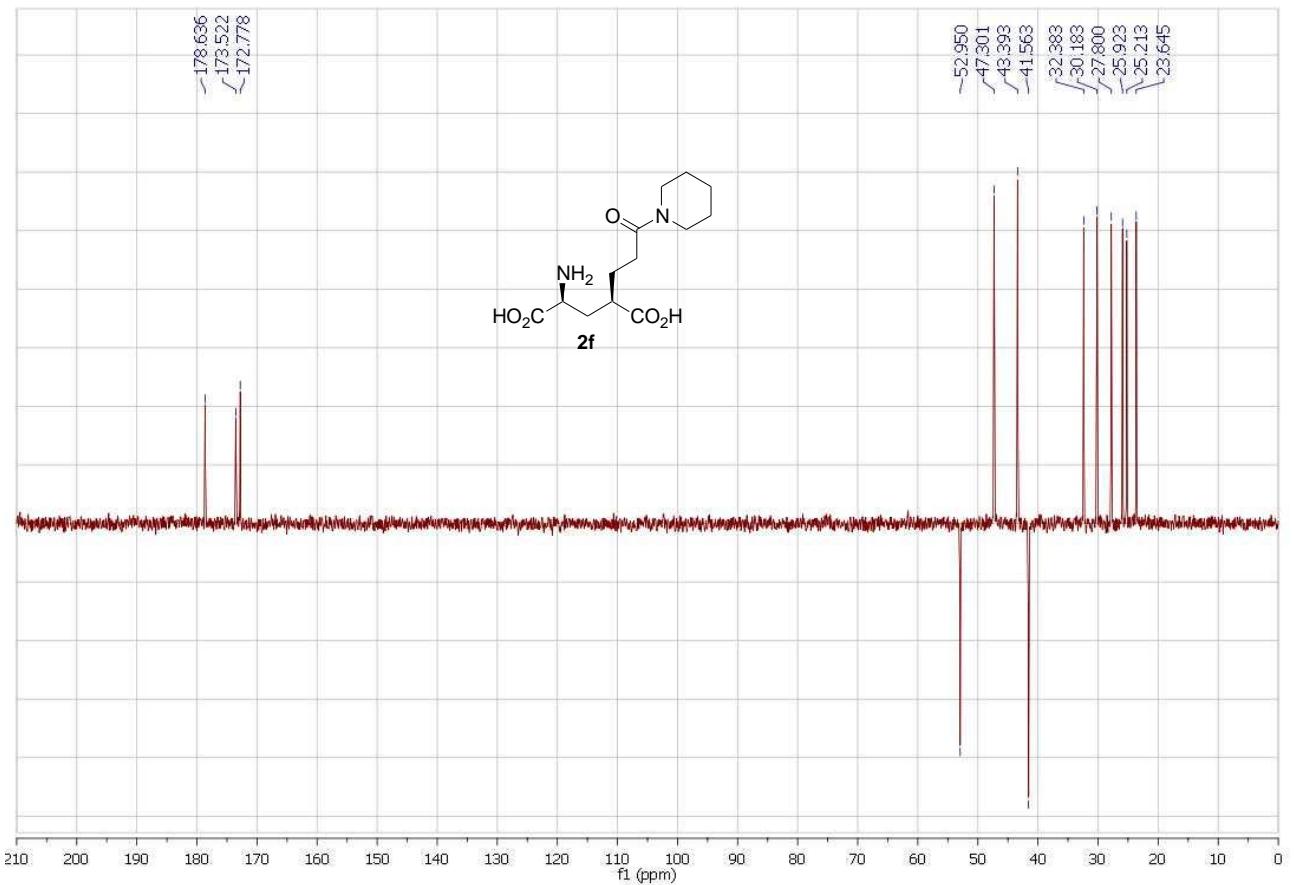
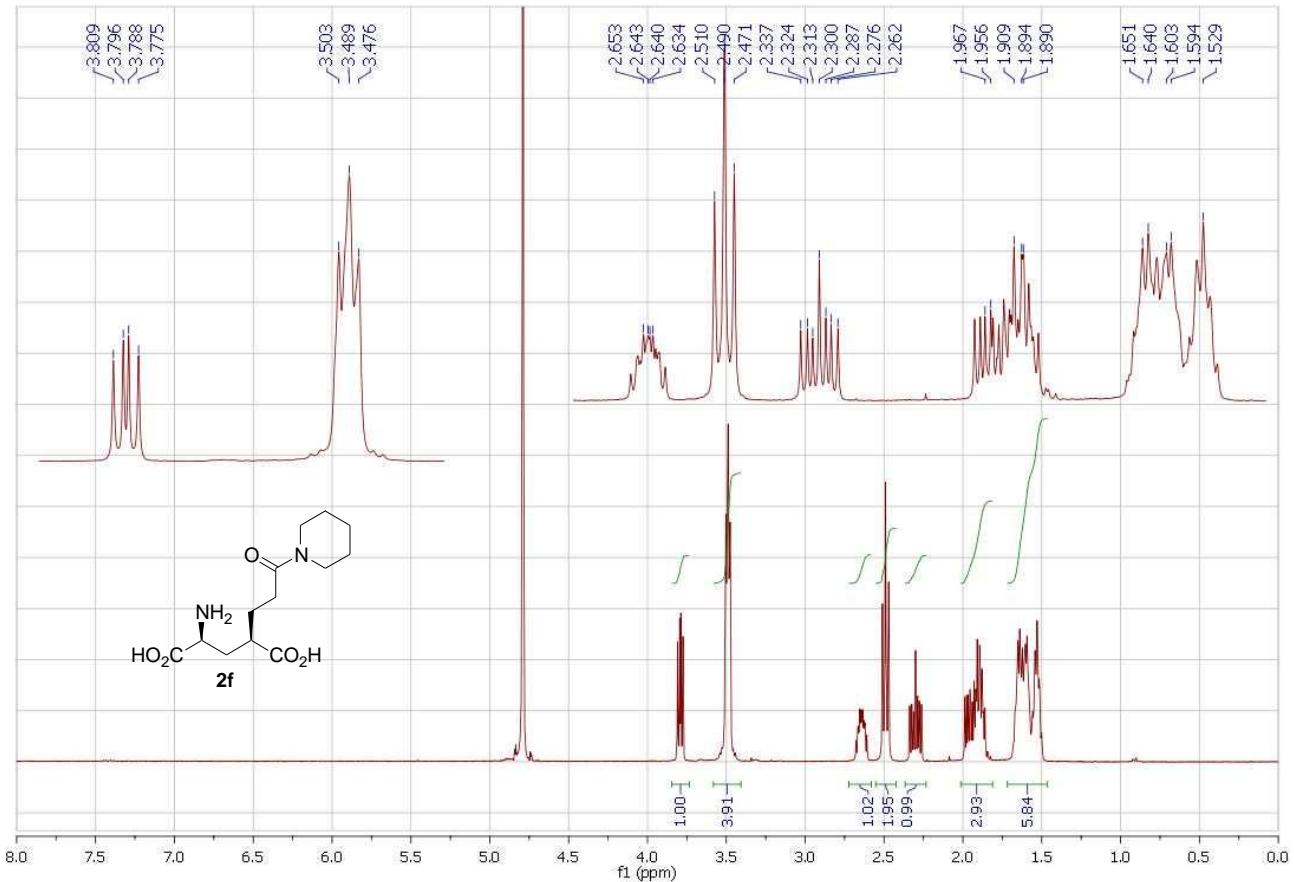
		Calcd			Found		
		C	H	N	C	H	N
<b>2b</b>	$\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_5 \cdot 0.5 \text{ H}_2\text{O}$	49.81	6.46	10.56	49.80	6.43	10.26
<b>2c</b>	$\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_5$	57.14	6.16	9.52	57.19	6.16	9.18
<b>2d</b>	$\text{C}_{10}\text{H}_{18}\text{N}_2\text{O}_5 \cdot \text{H}_2\text{O}$	45.45	7.63	10.60	45.30	7.51	10.64
<b>2e</b>	$\text{C}_{12}\text{H}_{22}\text{N}_2\text{O}_5 \cdot 0.5 \text{ H}_2\text{O}$	50.87	8.18	9.88	50.98	7.75	9.92
<b>2f</b>	$\text{C}_{13}\text{H}_{22}\text{N}_2\text{O}_5$	54.53	7.74	9.78	54.37	7.71	9.61
<b>2g</b>	$\text{C}_{12}\text{H}_{22}\text{N}_2\text{O}_6 \cdot \text{H}_2\text{O}$	46.74	7.84	9.09	46.70	7.70	9.12
<b>2h</b>	$\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_6 \cdot 0.25 \text{ H}_2\text{O}$	54.79	6.28	8.52	54.90	6.25	8.60
<b>2i</b>	$\text{C}_9\text{H}_{16}\text{N}_2\text{O}_6 \cdot 0.75 \text{ NH}_3 \cdot \text{H}_2\text{O}$	38.74	7.31	13.80	38.81	6.87	14.17
<b>2j</b>	$\text{C}_{14}\text{H}_{19}\text{N}_3\text{O}_5 \cdot 1.5 \text{ H}_2\text{O}$	49.99	6.59	12.49	50.21	6.21	12.24
<b>2k</b>	$\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_6 \cdot 0.25 \text{ H}_2\text{O}$	54.79	6.28	8.52	54.87	6.19	8.49
<b>2l</b>	$\text{C}_8\text{H}_{12}\text{N}_2\text{O}_4$	47.99	6.04	13.99	47.69	6.04	13.78
<b>2m</b>	$\text{C}_8\text{H}_{14}\text{N}_2\text{O}_6 \cdot 0.5 \text{ H}_2\text{O}$	39.51	6.22	11.52	39.31	6.01	11.41
<b>2n</b>	$\text{C}_7\text{H}_{14}\text{N}_2\text{O}_4 \cdot 0.75 \text{ H}_2\text{O}$	41.27	7.67	13.75	41.08	7.42	13.39
<b>2o</b>	$\text{C}_8\text{H}_{16}\text{N}_2\text{O}_4 \cdot 1.5 \text{ H}_2\text{O}$	41.55	8.28	12.11	41.62	7.65	12.03
<b>2p</b>	$\text{C}_8\text{H}_{13}\text{NO}_4 \cdot 0.5 \text{ H}_2\text{O}$	48.97	7.19	7.13	49.23	7.00	7.29
<b>6a</b>	$\text{C}_{10}\text{H}_{15}\text{NO}_6$	48.98	6.16	5.71	49.12	6.22	5.54
<b>6b</b>	$\text{C}_{13}\text{H}_{17}\text{NO}_6$	55.12	6.05	4.94	54.95	6.14	5.08
<b>6c</b>	$\text{C}_{16}\text{H}_{19}\text{NO}_6$	59.81	5.96	4.36	59.41	6.19	4.28
<b>6d</b>	$\text{C}_{12}\text{H}_{19}\text{NO}_6 \cdot 0.25 \text{ H}_2\text{O}$	51.88	7.08	5.04	51.53	7.01	4.87
<b>6e</b>	$\text{C}_{14}\text{H}_{23}\text{NO}_6 \cdot 0.25 \text{ H}_2\text{O}$	54.98	7.74	4.58	55.26	7.80	4.64
<b>6f</b>	$\text{C}_{15}\text{H}_{23}\text{NO}_6$	57.49	7.40	4.47	57.50	7.36	4.59
<b>6g</b>	$\text{C}_{14}\text{H}_{23}\text{NO}_7 \cdot 0.25 \text{ H}_2\text{O}$	52.25	7.36	4.35	52.29	7.42	4.42
<b>6h</b>	$\text{C}_{17}\text{H}_{21}\text{NO}_7 \cdot 0.5 \text{ H}_2\text{O}$	56.66	6.15	3.89	56.68	6.14	4.03
<b>6i</b>	$\text{C}_{11}\text{H}_{17}\text{NO}_7 \cdot 0.25 \text{ H}_2\text{O}$	47.23	6.30	5.01	47.53	6.18	5.07
<b>6j</b>	$\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_6 \cdot 0.25 \text{ H}_2\text{O}$	56.38	6.06	8.22	56.35	6.03	8.11
<b>10</b>	$\text{C}_{10}\text{H}_{13}\text{NO}_5$	52.86	5.77	6.16	52.89	5.77	6.39

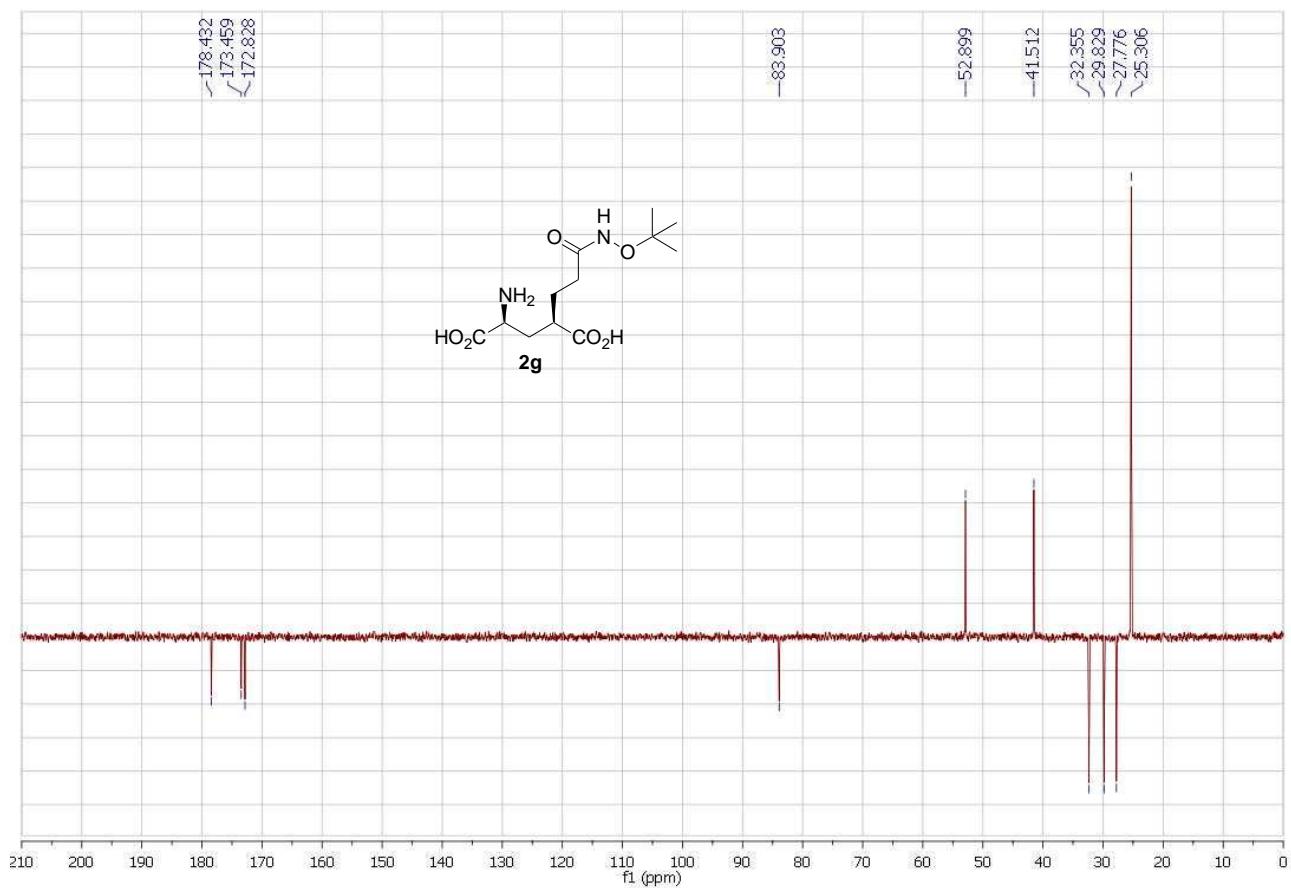
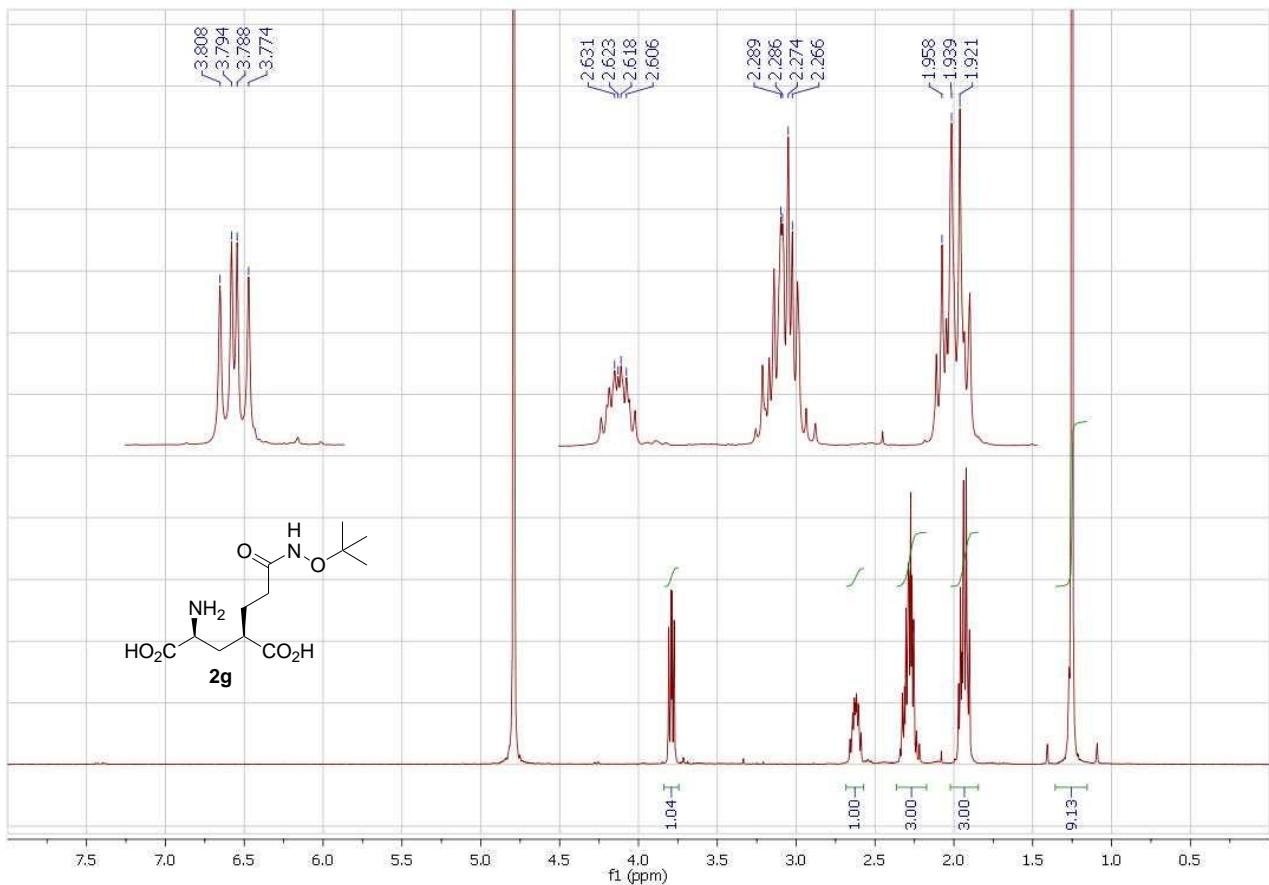


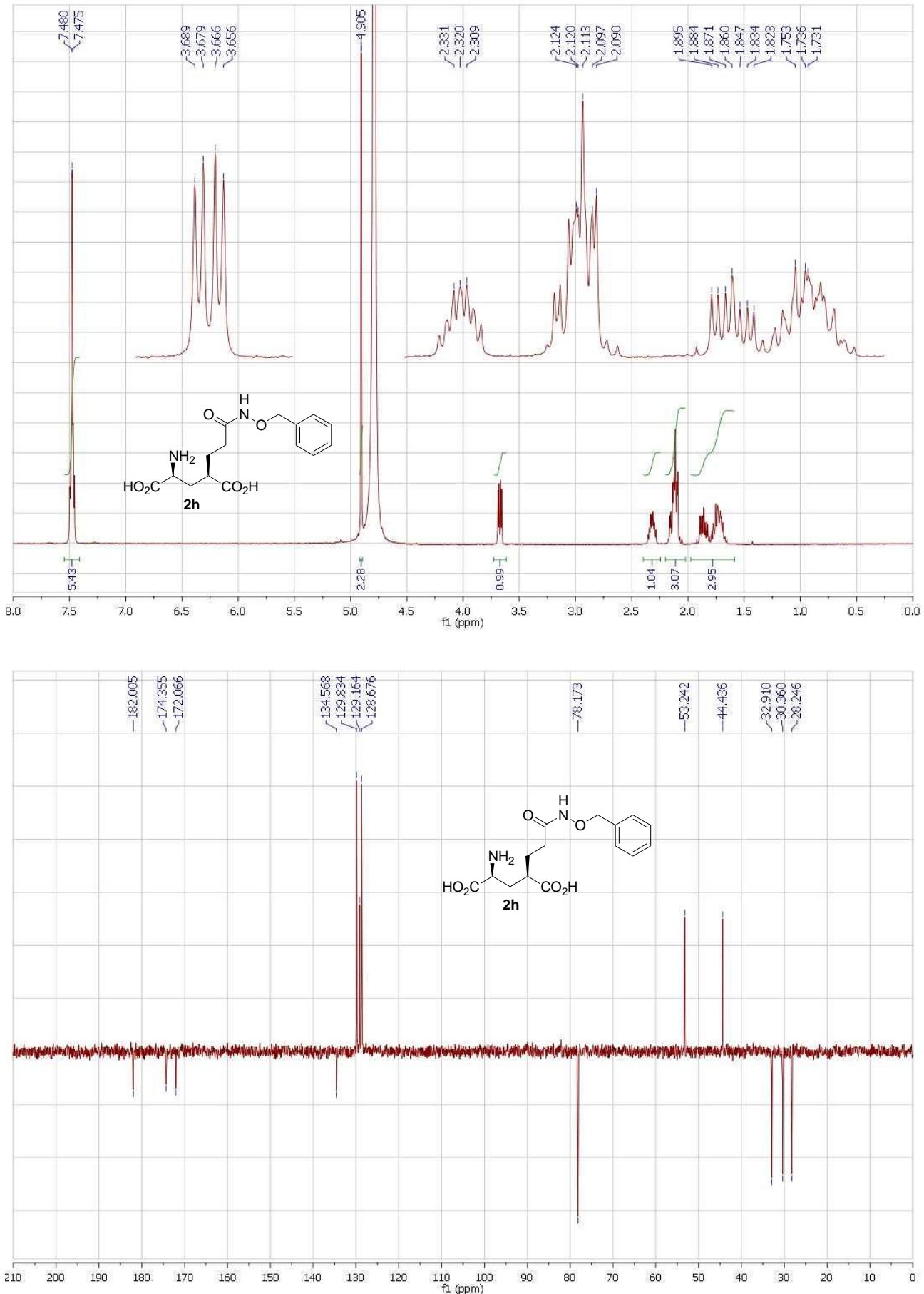


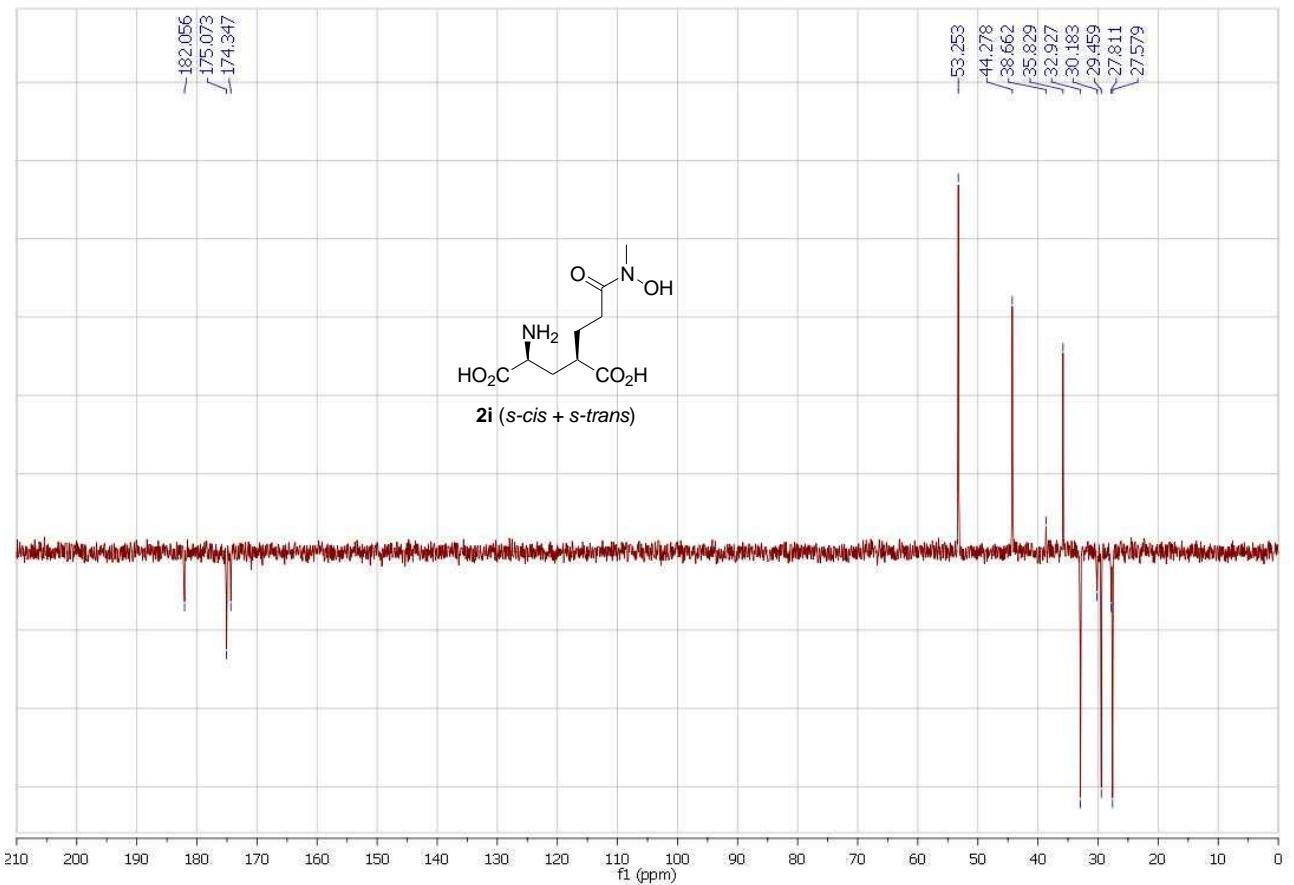
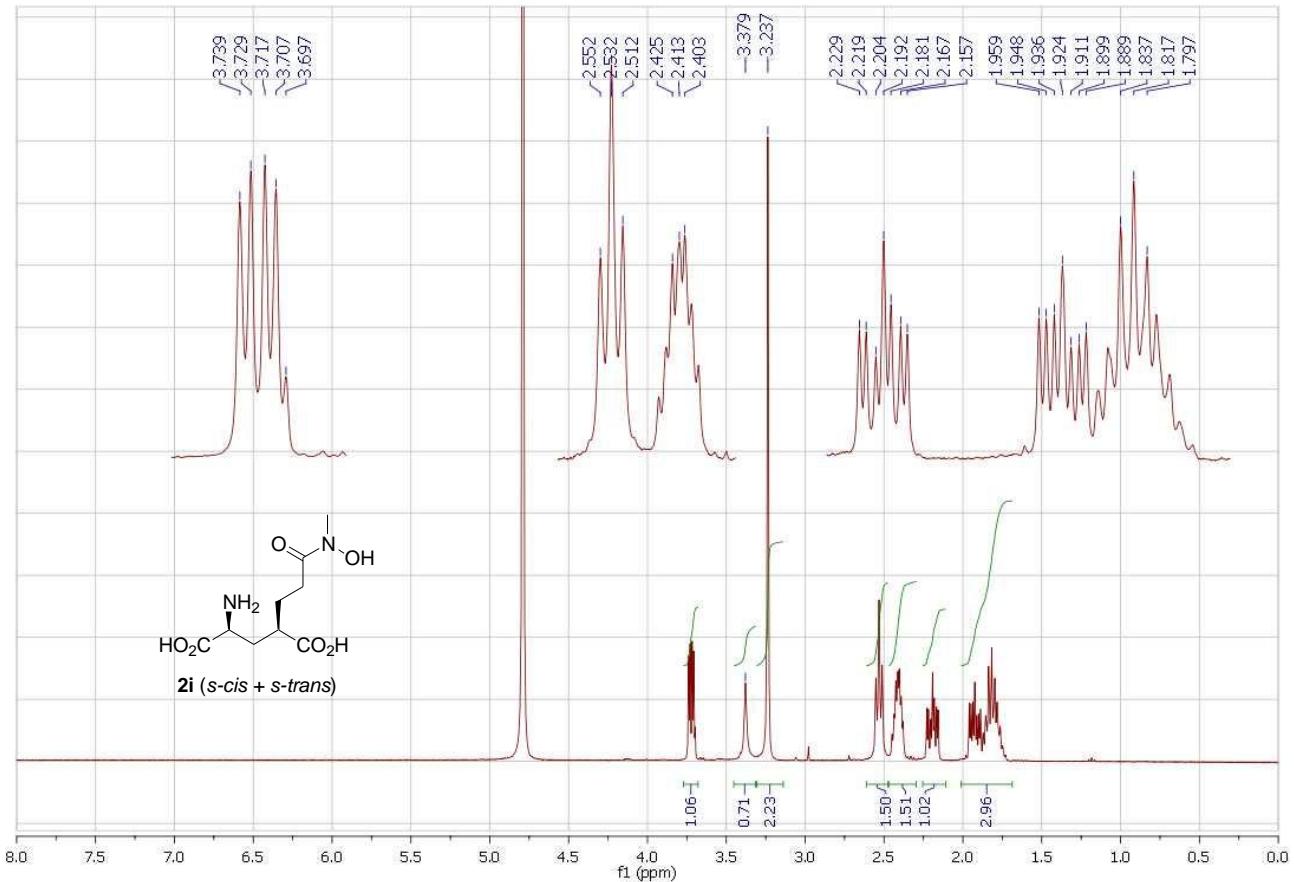


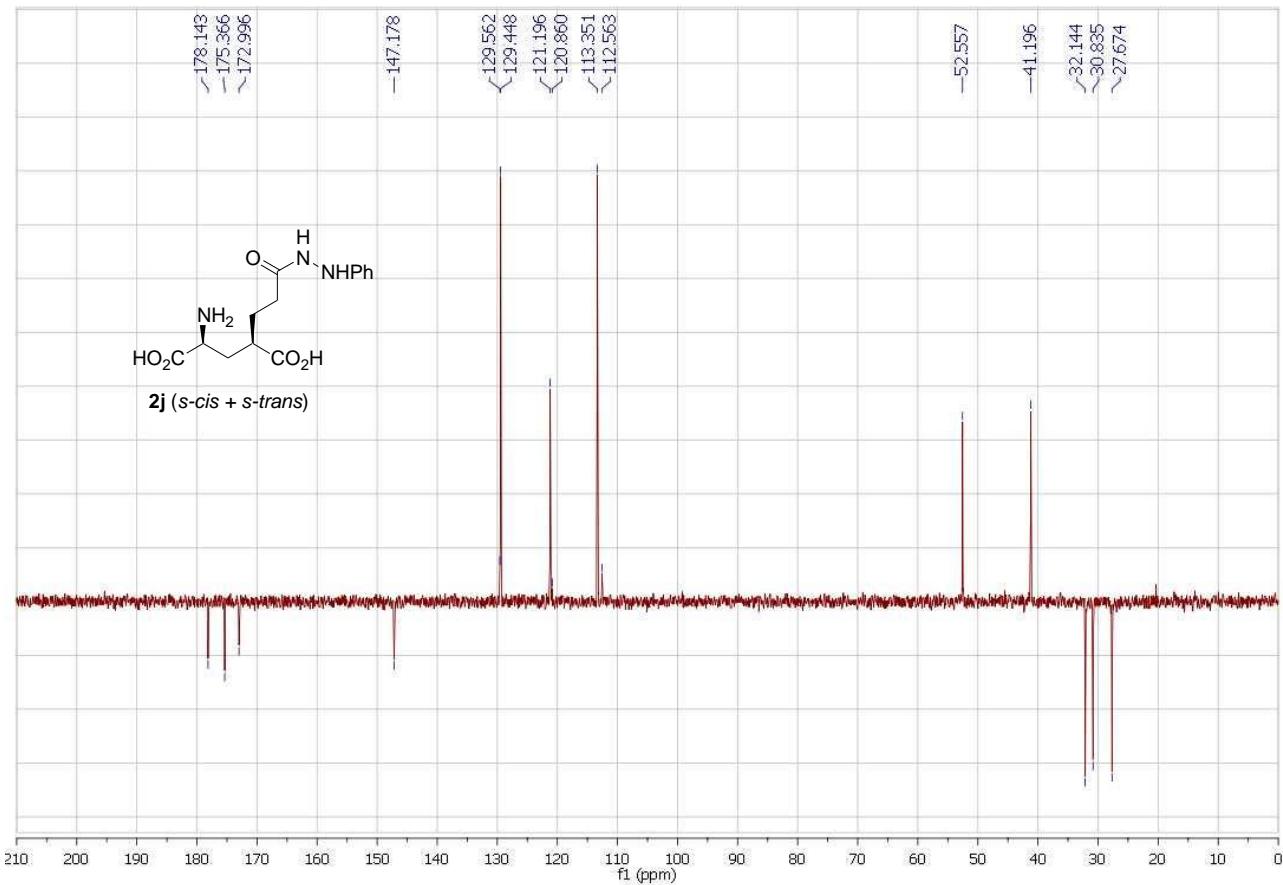
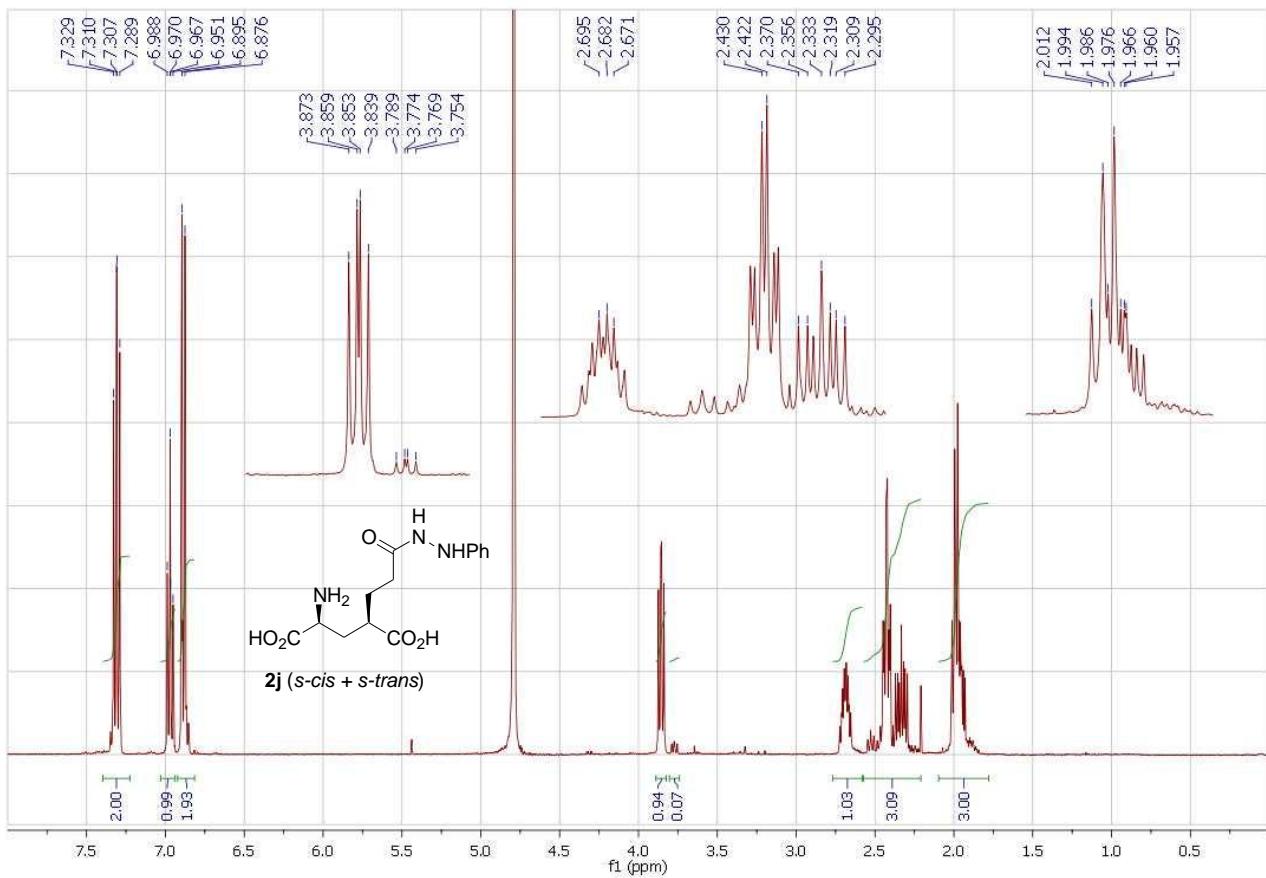


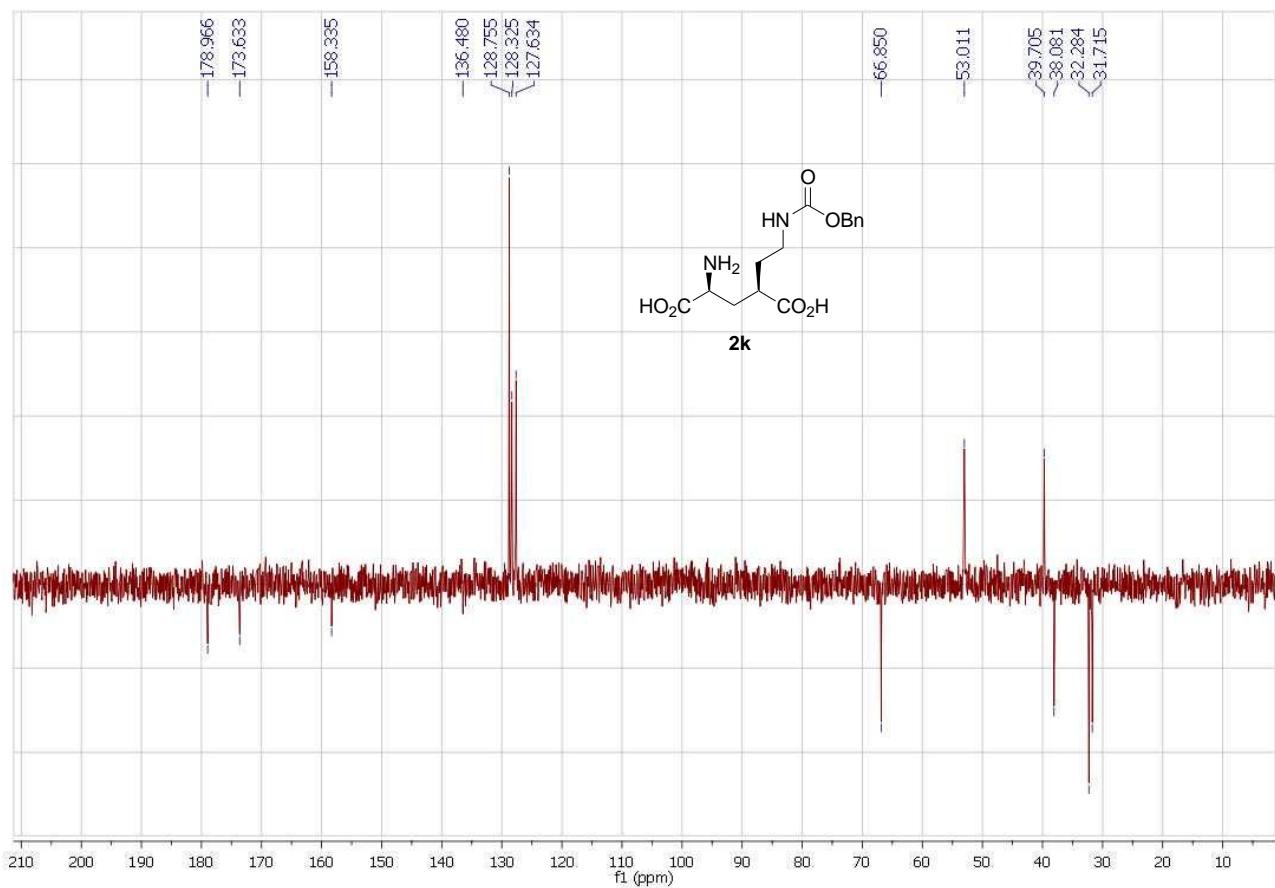
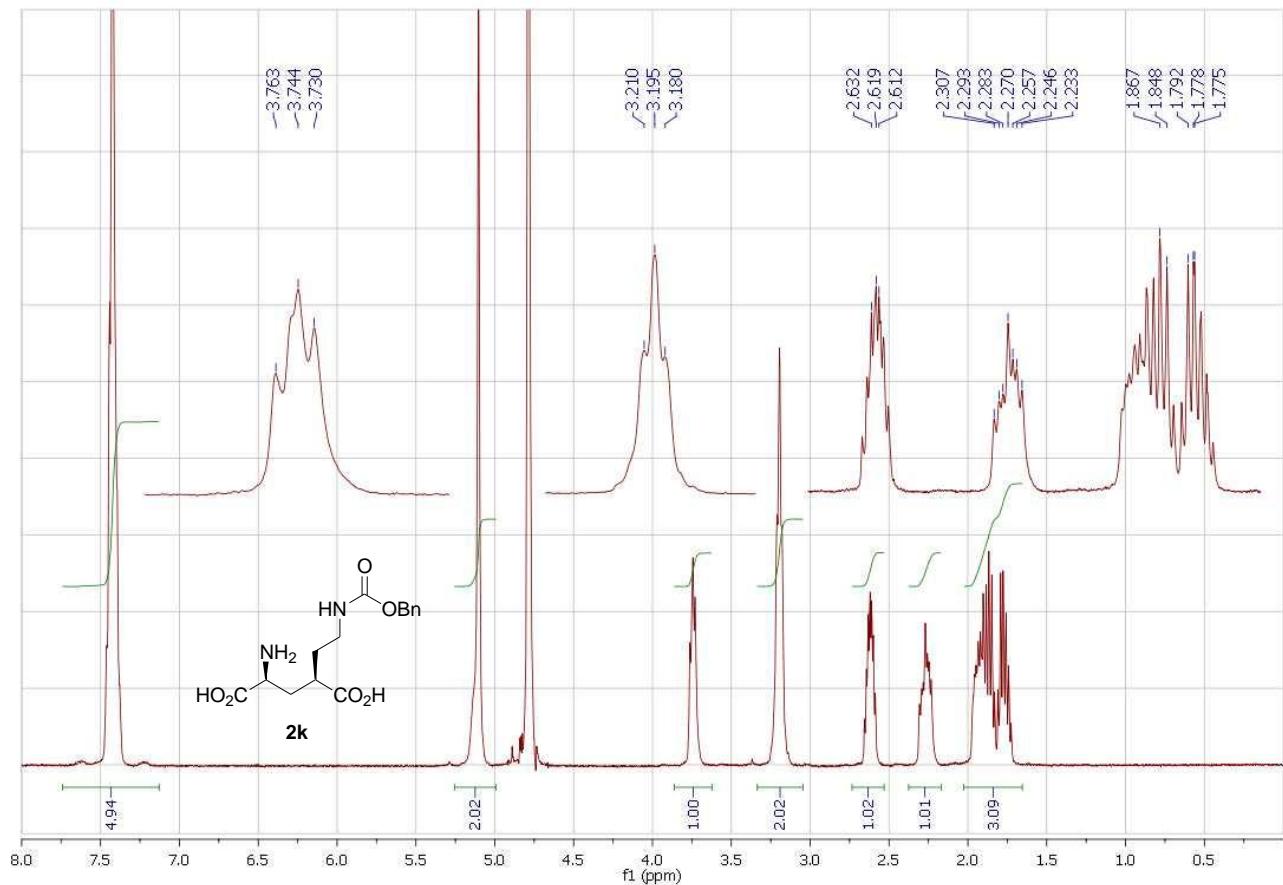


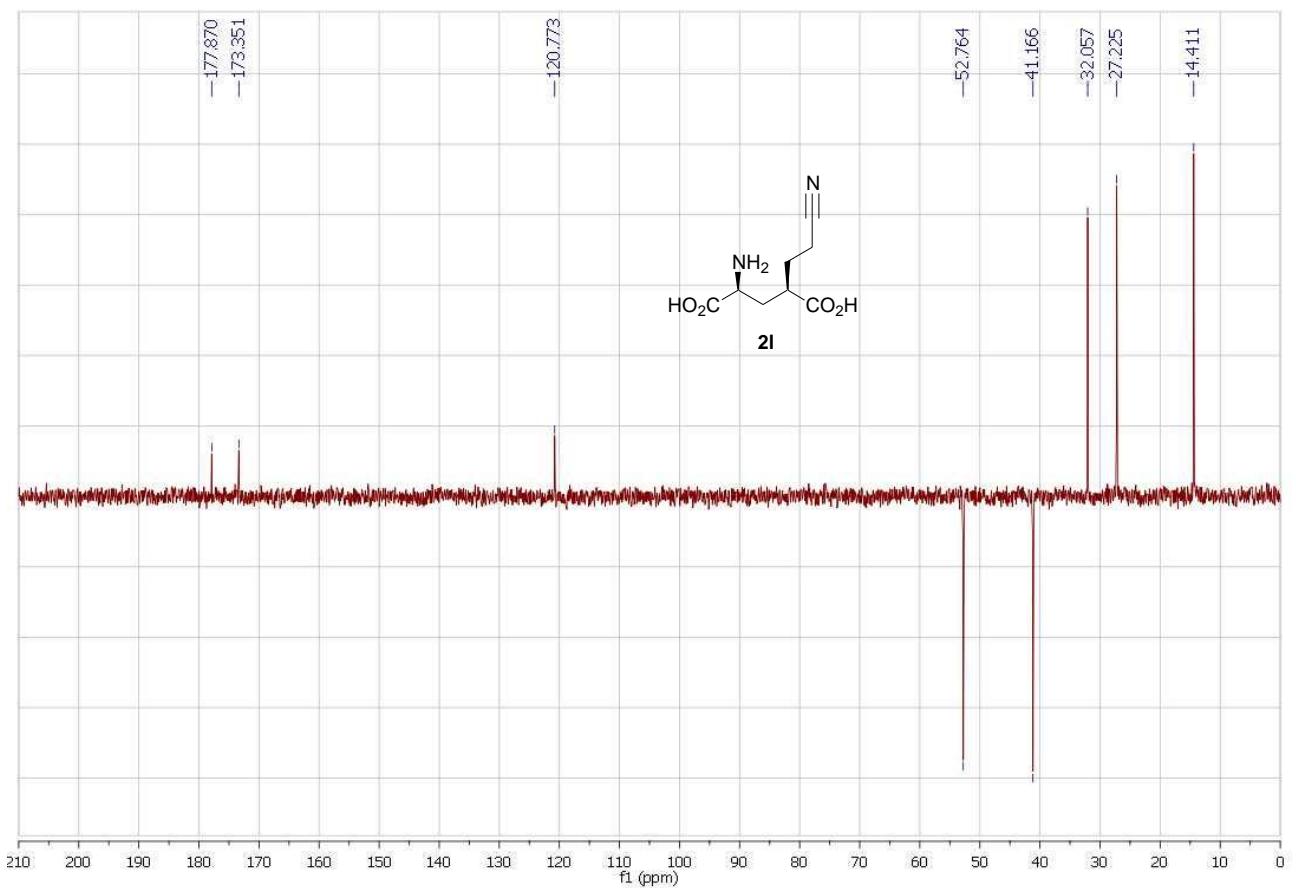
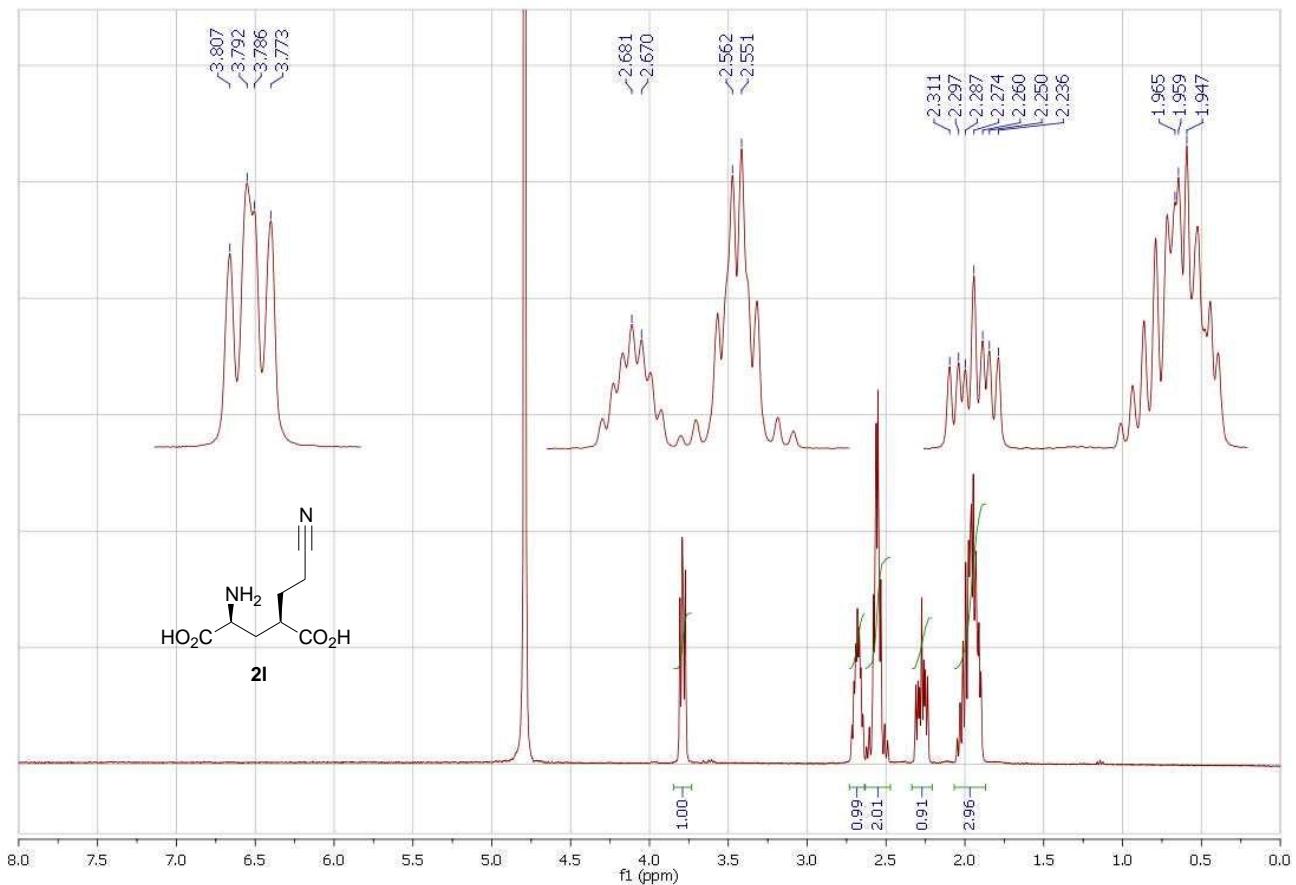


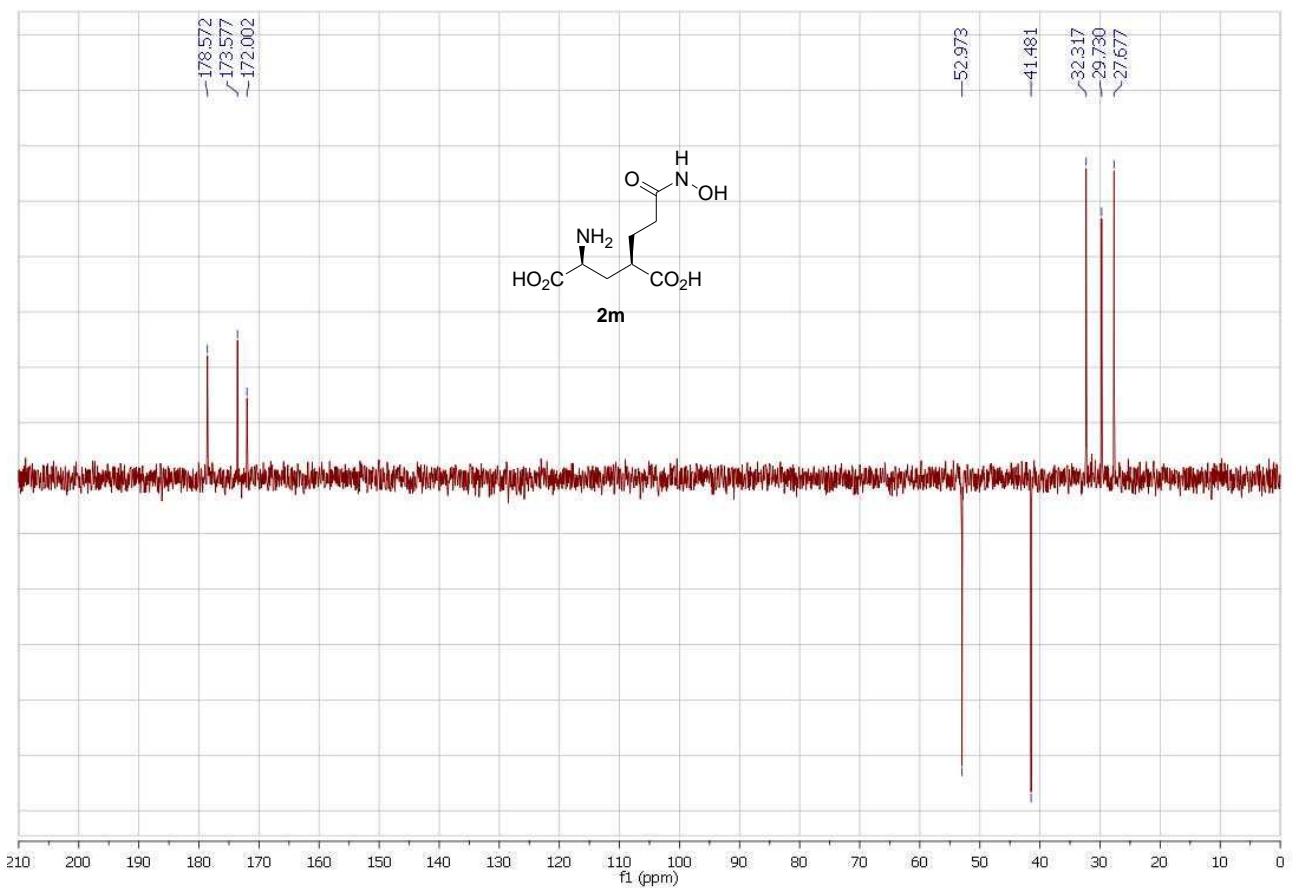
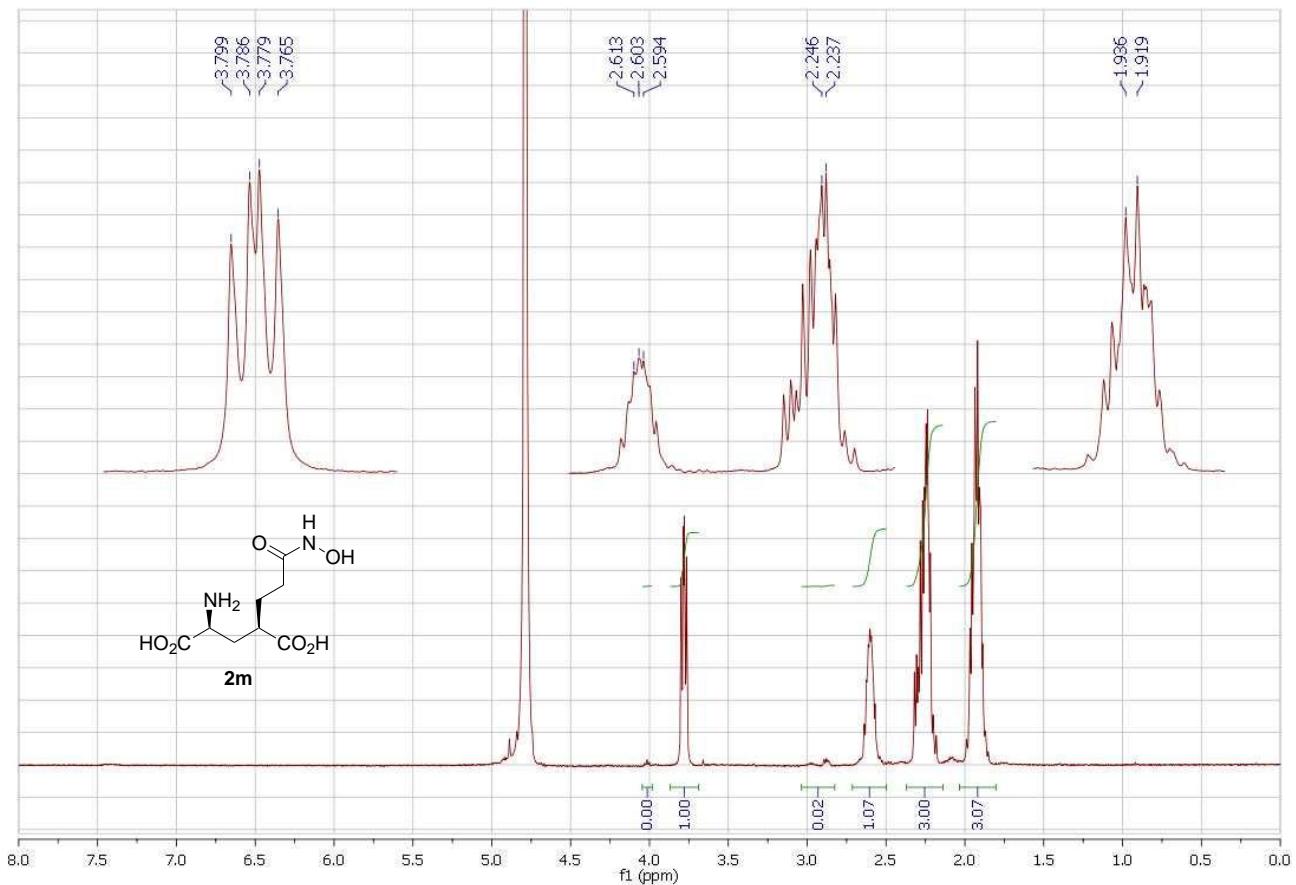


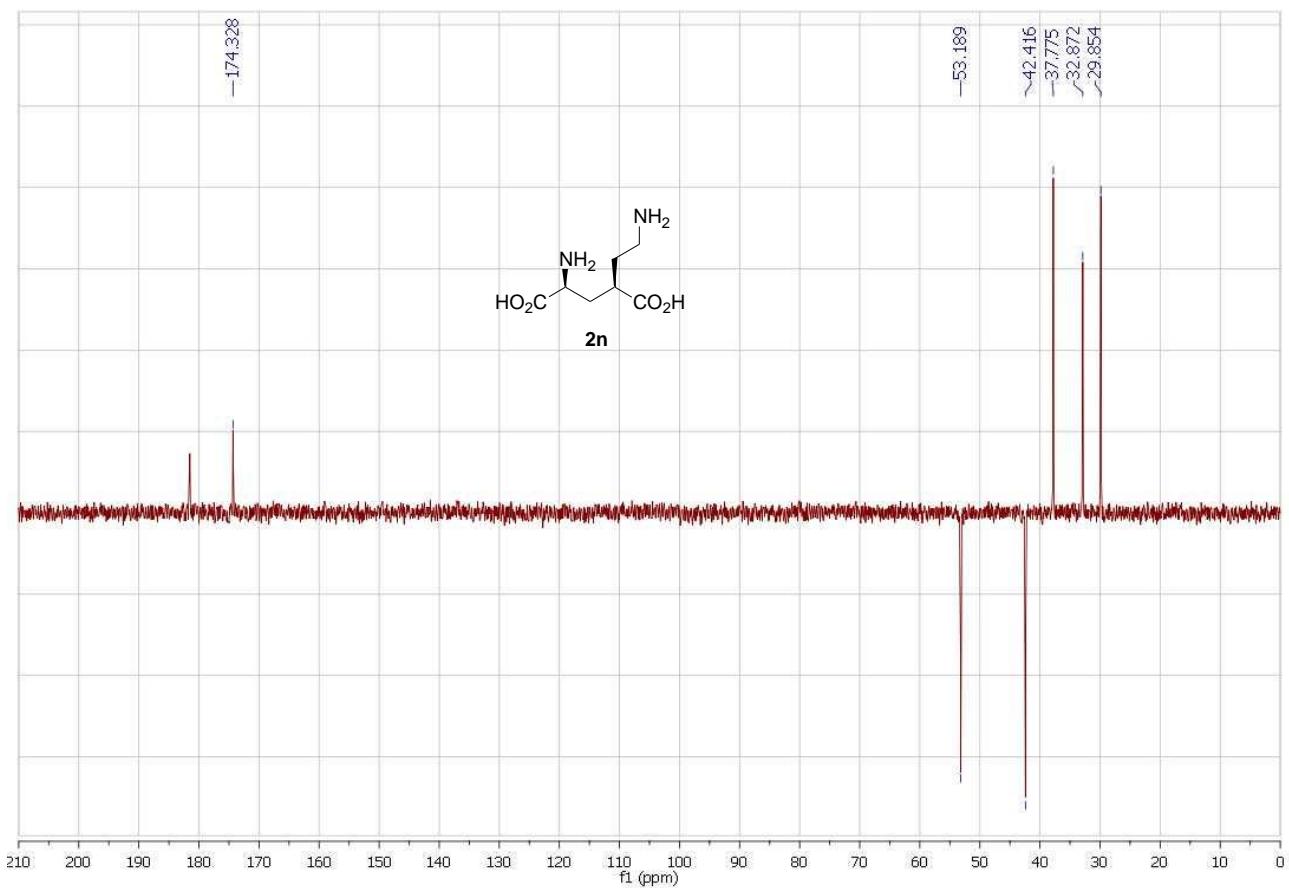
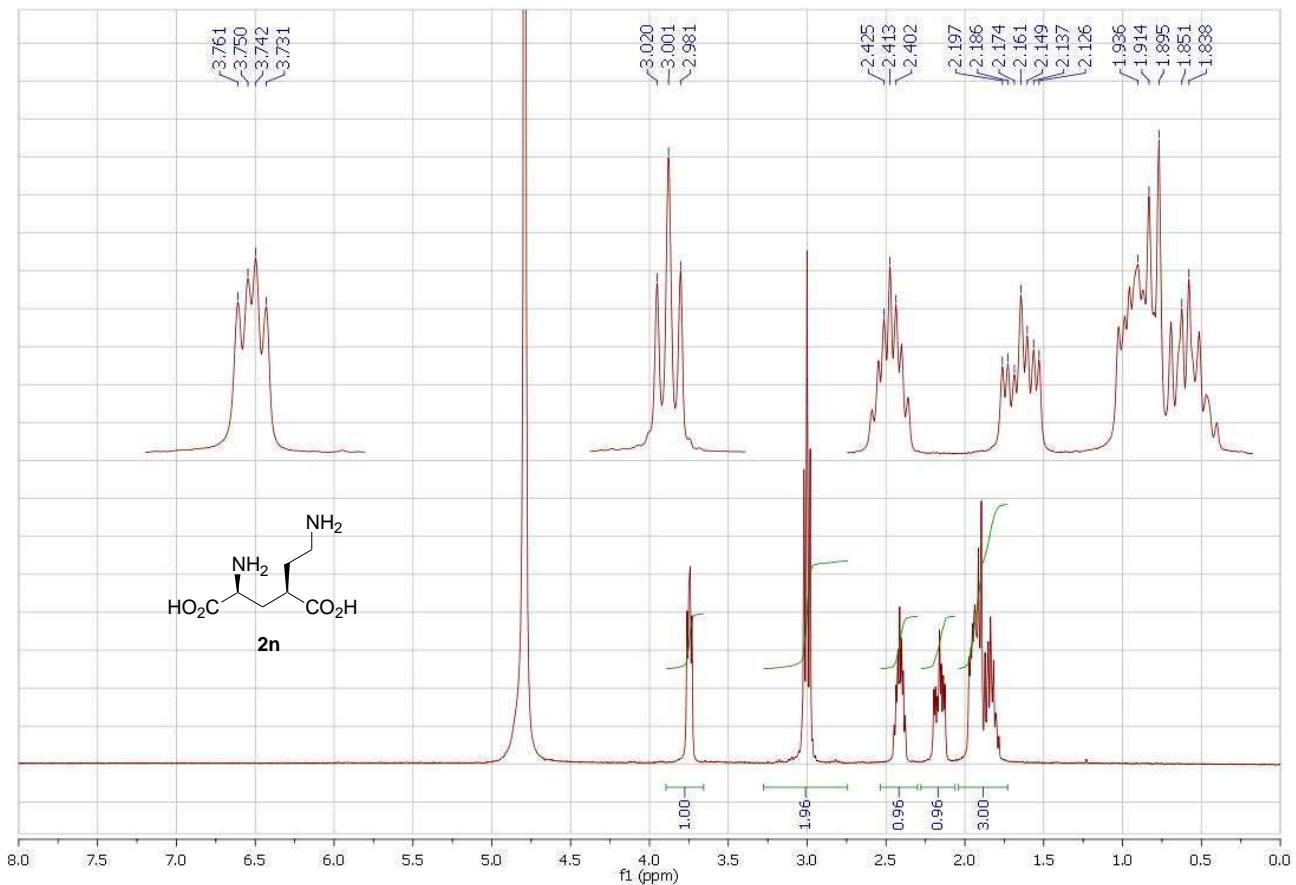


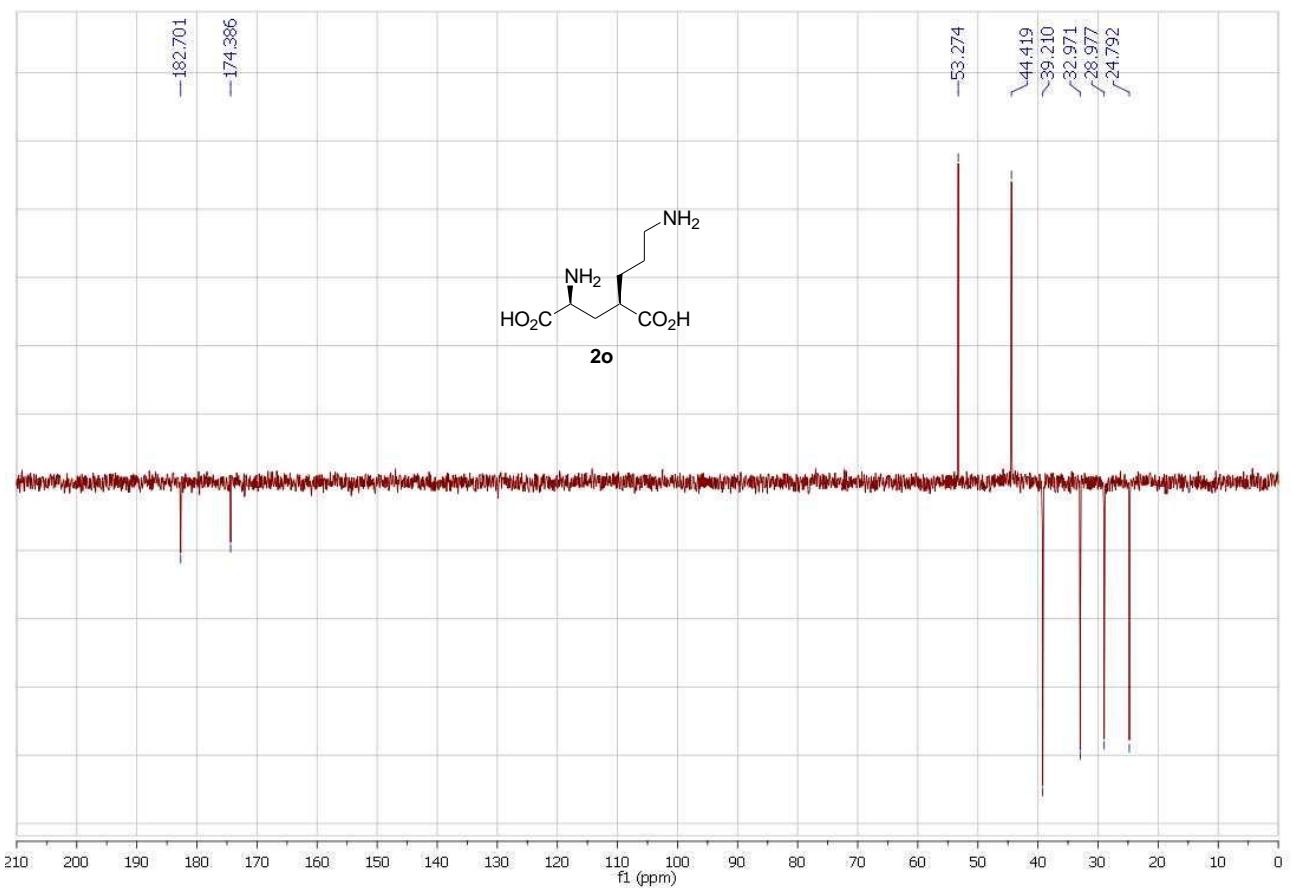
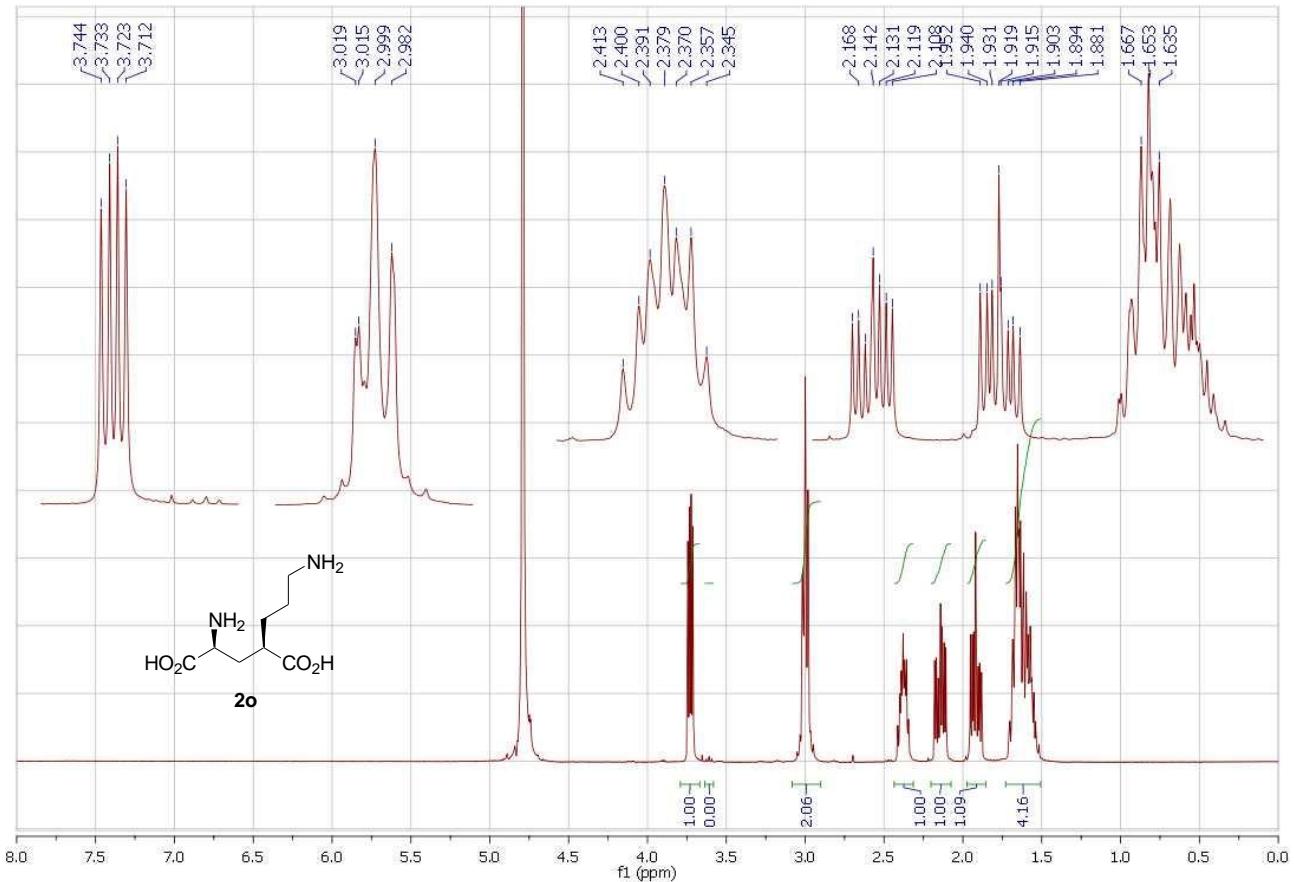


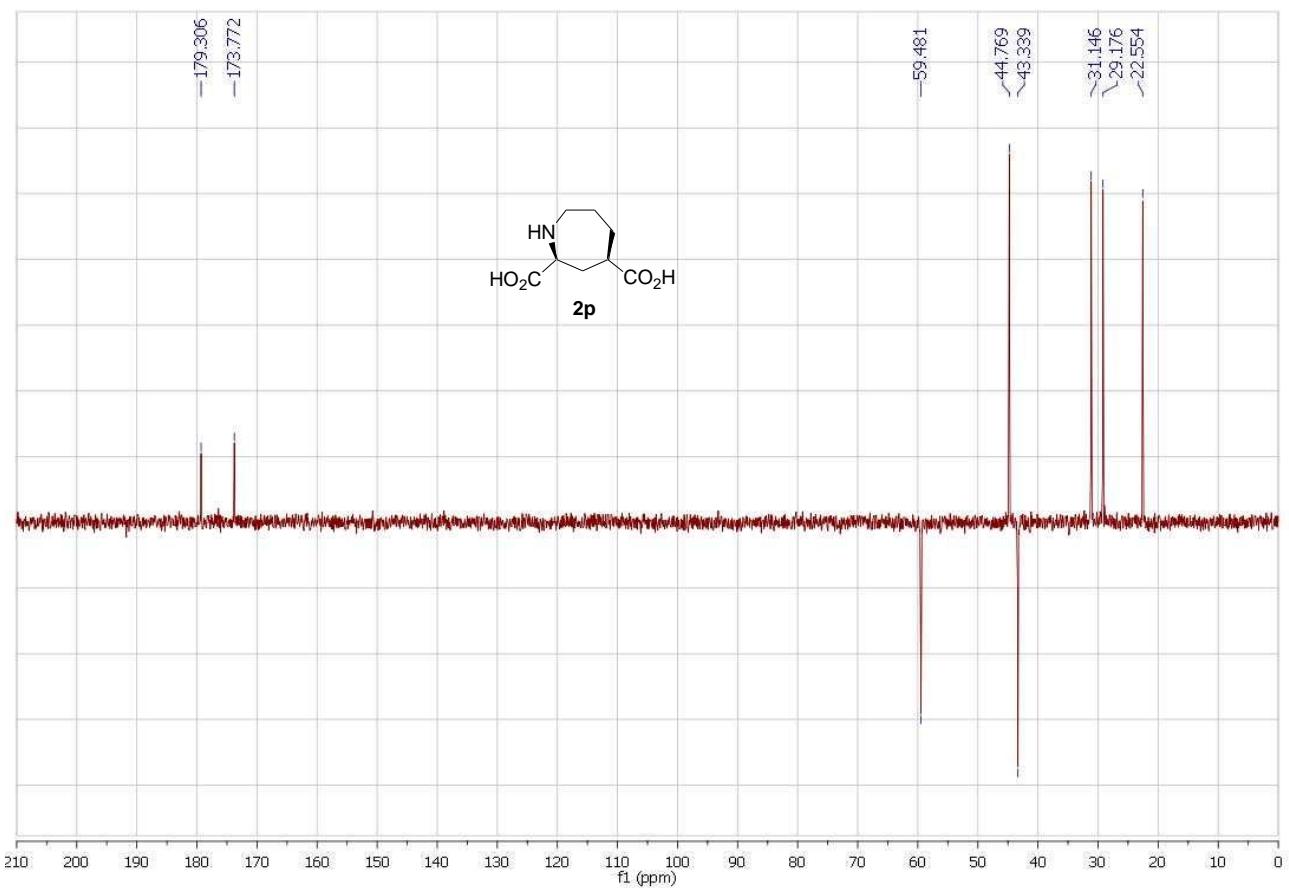
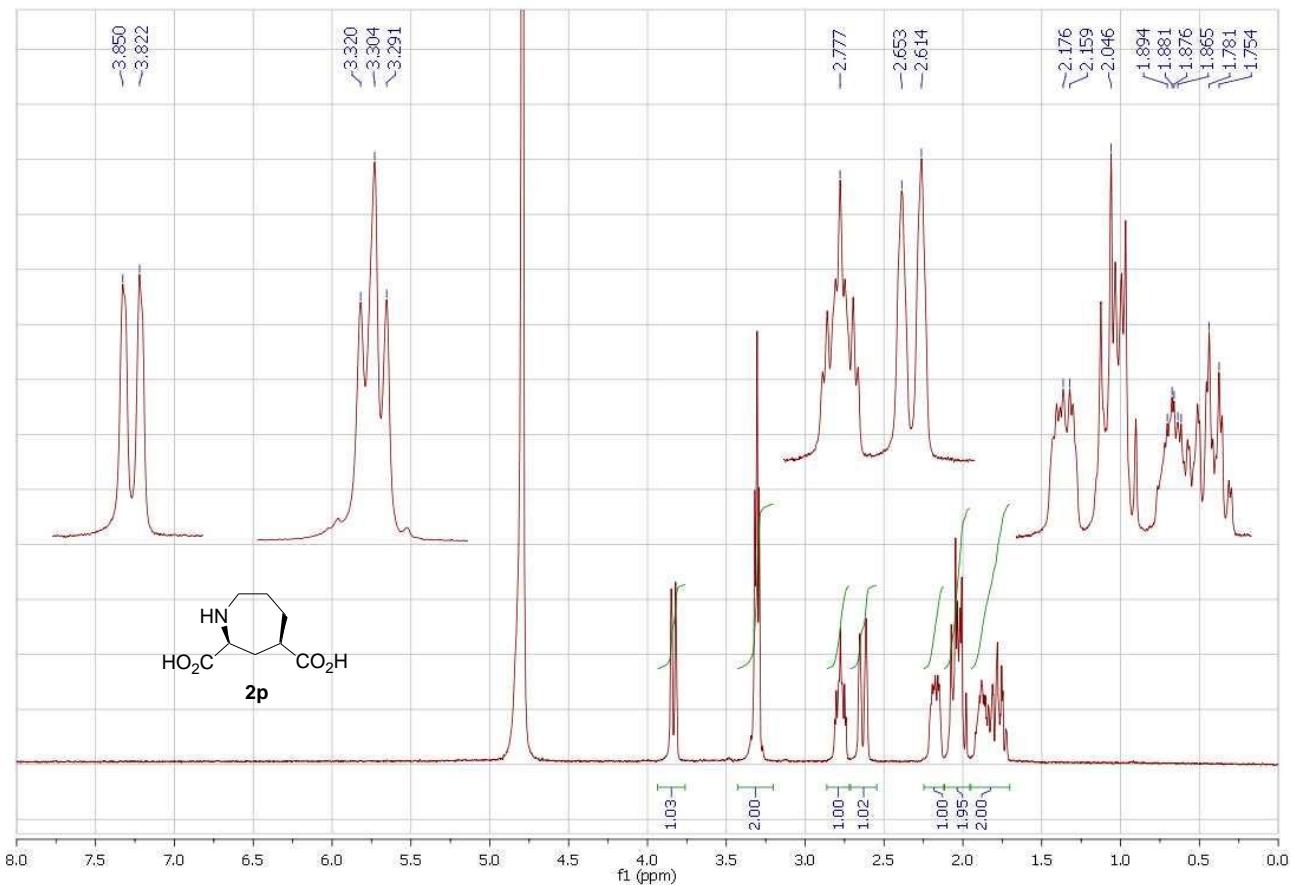






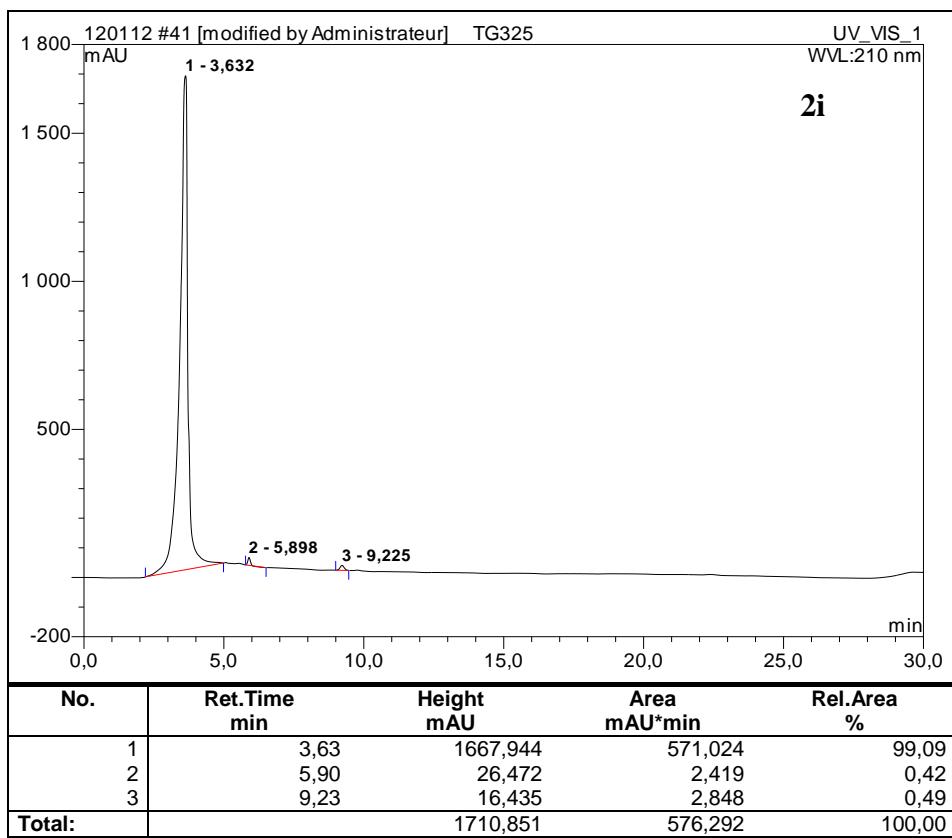
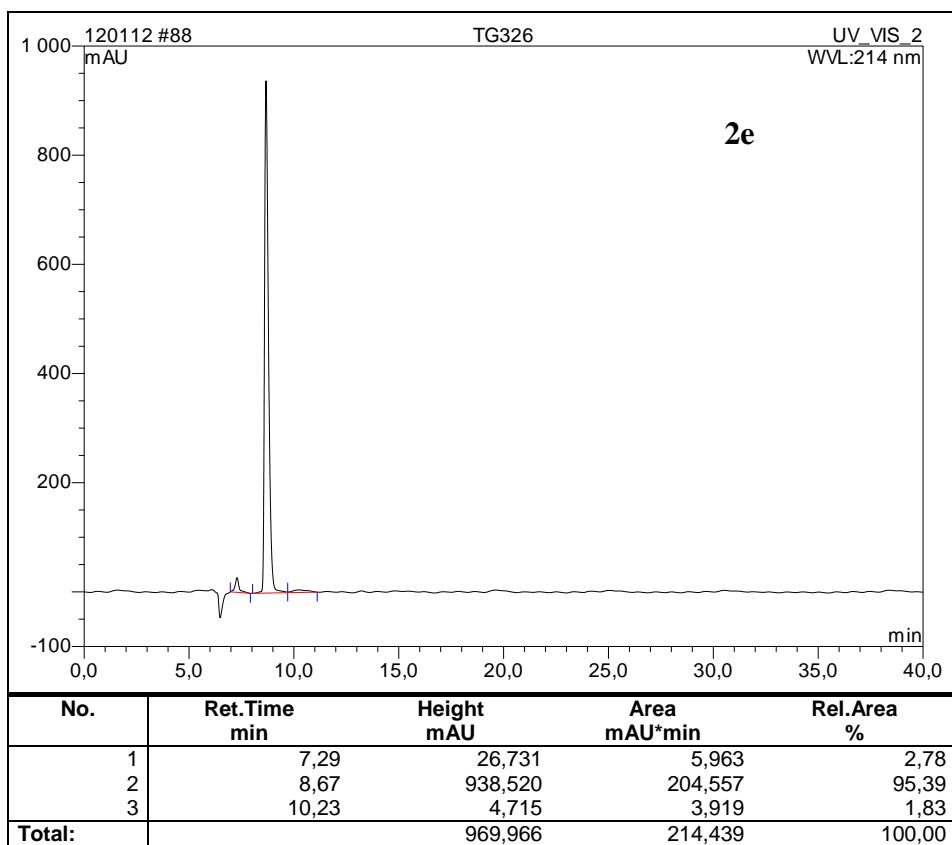


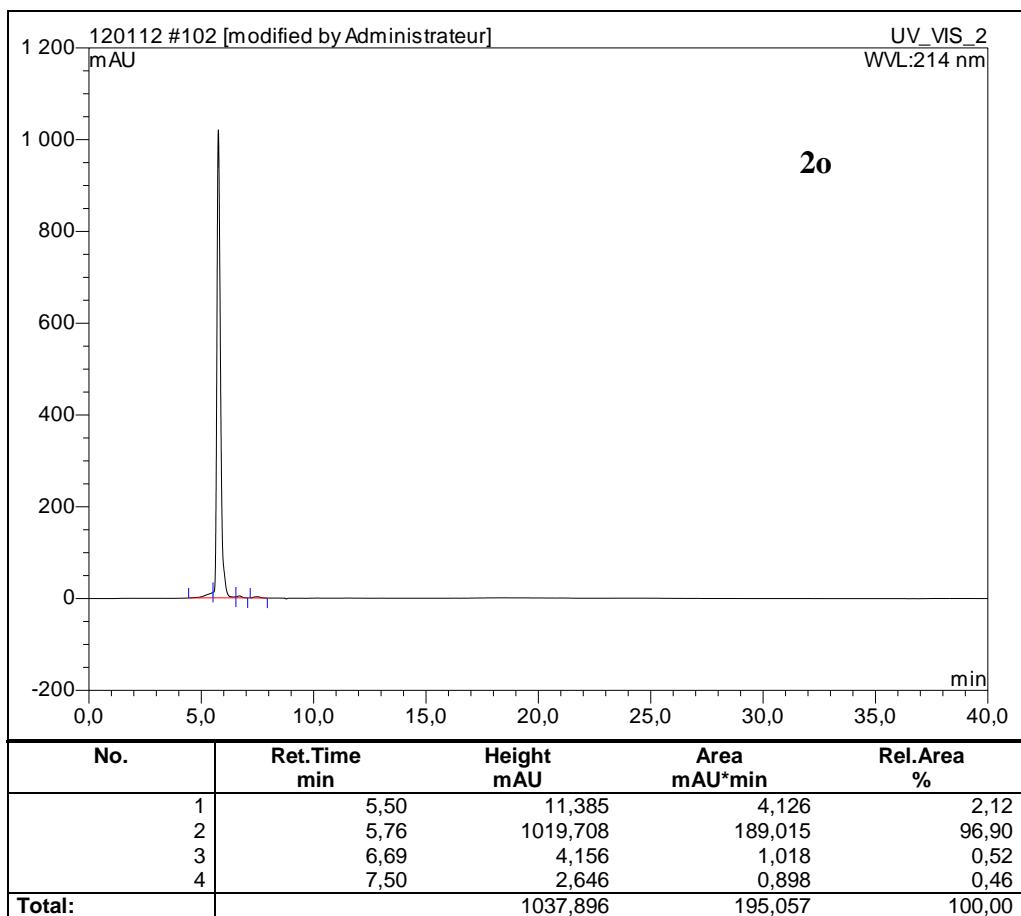




### HPLC analysis of **2e**, **2i** and **2o**:

C18 column (Uptisphere ODB, 5 $\mu$ m, 250x4.6 mm); flow rate 0.5 (**2o**, **2e**) or 0.8(**2i**) mL.min<sup>-1</sup>; UV detection (210-214 nm); 30°C; Eluent: H<sub>2</sub>O / CH<sub>3</sub>CN 95:5 (**2i**, **2o**), 80:20 (**2e**).





**Table S1.** Data collection and refinement statistics of GluA2 LBD and GluK3 LBD, both crystallized with **2i**.

Data	GluA2-LBD:2i	GluK3-LBD:2i
Beamline	I911-3	I911-3
Space group	$P2_12_12$	$P4_122$
Unit cell dimensions		
a (Å)	59.61	68.18
b (Å)	95.64	68.18
c (Å)	48.55	126.52
Molecules (a.u.) <sup>a</sup>	1	1
Resolution (Å)	50.64 - 1.24 (1.31-1.24)	68.18 - 2.65 (2.79 - 2.65)
Unique reflections	78559 (11146)	9229 (1277)
Average redundancy	6.1 (5.3)	4.6 (4.6)
Completeness (%)	99.0 (97.7)	98.7 (98.7)
R <sub>merge</sub> (%) <sup>c</sup>	7.0 (30.9)	10.8 (39.0)
I/σ(I)	6.1 (2.0)	5.6 (1.9)
<b>Refinement</b>		
Amino acid residues	263	253
Ligand molecules	1	1
Sulfate/phosphate	3/-	-/1
Lithium/potassium/chloride	1/-/-	-/2/1
Water/glycerol	340/4	51/-
R <sub>work</sub> (%) <sup>d</sup> /R <sub>free</sub> (%) <sup>e</sup>	13.7/15.5	19.8/26.7
<i>Average B-values (Å<sup>2</sup>) for:</i>		
Amino acid residues/ligand	11/12	50/44
Sulfate/phosphate	48/-	-/120
Lithium/potassium/chloride	7/-/-	-/57/50
Water/glycerol	24/28	33/-
R.M.S. deviation bond length(Å)/angles (degrees)	0.008/1.3	0.017/1.2
Residues in allowed regions of Ramachandran plot (%) <sup>f</sup>	100.0	100.0

<sup>a</sup> a.u.: asymmetric unit.<sup>b</sup> Numbers in parentheses are for the outermost bin. $R_{merge} = \sum_h \sum_i |I_i(h) - \langle I(h) \rangle| / \sum_h \sum_i |I_i(h)|$ , where  $I_i(h)$  is the  $i^{\text{th}}$  measurement.<sup>d</sup>  $R_{\text{work}} = \sum_{hkl} (|F_{o,hkl}| - |F_{c,hkl}|) / |F_{o,hkl}|$ , where  $|F_{o,hkl}|$  and  $|F_{c,hkl}|$  are the observed and calculated structure factor amplitudes.<sup>e</sup>  $R_{\text{free}}$  is equivalent to  $R_{\text{work}}$ , but calculated with 5% reflections omitted from the refinement process.<sup>f</sup> The Ramachandran plots were calculated using Procheck [Laskowski *et al.*, 1993].

**Figure S1.** Zoom on the ligand-binding site of GluA2-LBD with **2i** and  $2F_o-F_c$  omit map at  $1\sigma$  carved around the ligand, Glu423 and five water molecules. The compound **2i** (thick black line) was built in a conformation in which the hydroxamic acid moiety points towards Tyr471 and Pro499. However, alternative conformations of **2i** with the hydroxamic acid moiety pointing towards Ser673 and Glu423 might also be present, as positive  $F_o-F_c$  electron density (green, contoured at  $3\sigma$ ) is seen extending from the C3' atom of **2i**. Thus water molecules W1 and W2 might be displaced by alternative conformations. The presence of alternative conformations is also indicated by negative  $F_o-F_c$  density (red, contoured at  $-3\sigma$ ) at the hydroxamic acid moiety. This negative density disappears if the occupancy of atoms comprising the hydroxamic acid moiety is set to 0.8. Additional positive and negative densities are seen in the vicinity of the hydroxamic acid moiety and Glu423, which might be caused by a high mobility of Glu423.

