Supplemental Data for

Structural and computational basis for potent inhibition of glutamate carboxypeptidase II by carbamate-based inhibitors

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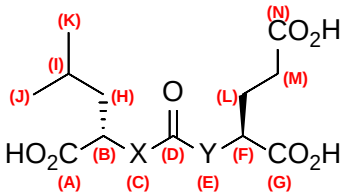
Table S1 Primary energy data: molecular energies in gas-phase, E(el), solvation energies, G(solv) and thermal corrections.

Compound ID	E(el) [a.u.]	G(solv) [kcal·mol^{-1}]		Thermal corrections [kJ·mol^{-1}]	Free energy [a.u.]		Free energy w/o thermal corrections [a.u.]	
	aug-cc-pVDZ/ dlpno-ccsd(t)	water	octanol		water	octanol	water	octanol
enzyme_geom								
ZJ-43	-1101.030638	-380.5771607	-332.192925	x	x	x	-1101.63713	-1101.56002
5	-1120.873229	-378.0424948	-330.589002	x	x	x	-1121.47568	-1121.40006
7	-1120.877541	-376.0584747	-329.46543	x	x	x	-1121.47683	-1121.40258
local_min								
ZJ-43	-1101.064086	-360.6436077	-318.508895	623.809	-1101.40121	-1101.33407	-1101.63881	-1101.57166
5	-1120.894983	-366.4478244	-321.317768	596.758	-1121.25166	-1121.17974	-1121.47895	-1121.40704
7	-1120.897908	-366.5066557	-322.604834	596.78	-1121.25467	-1121.18471	-1121.48197	-1121.41201
global_min								
ZJ-43	-1101.045102	-377.7762178	x	632.525	-1101.40621	x	x	x
5	-1120.871343	-385.9885252	x	594.17	-1121.26015	x	x	x
7	-1120.906017	-363.3795128	x	593.544	-1121.25903	x	x	x

Table S2 ^{1}H and ^{13}C NMR data of compounds **ZJ-43**, **5**, and **7**

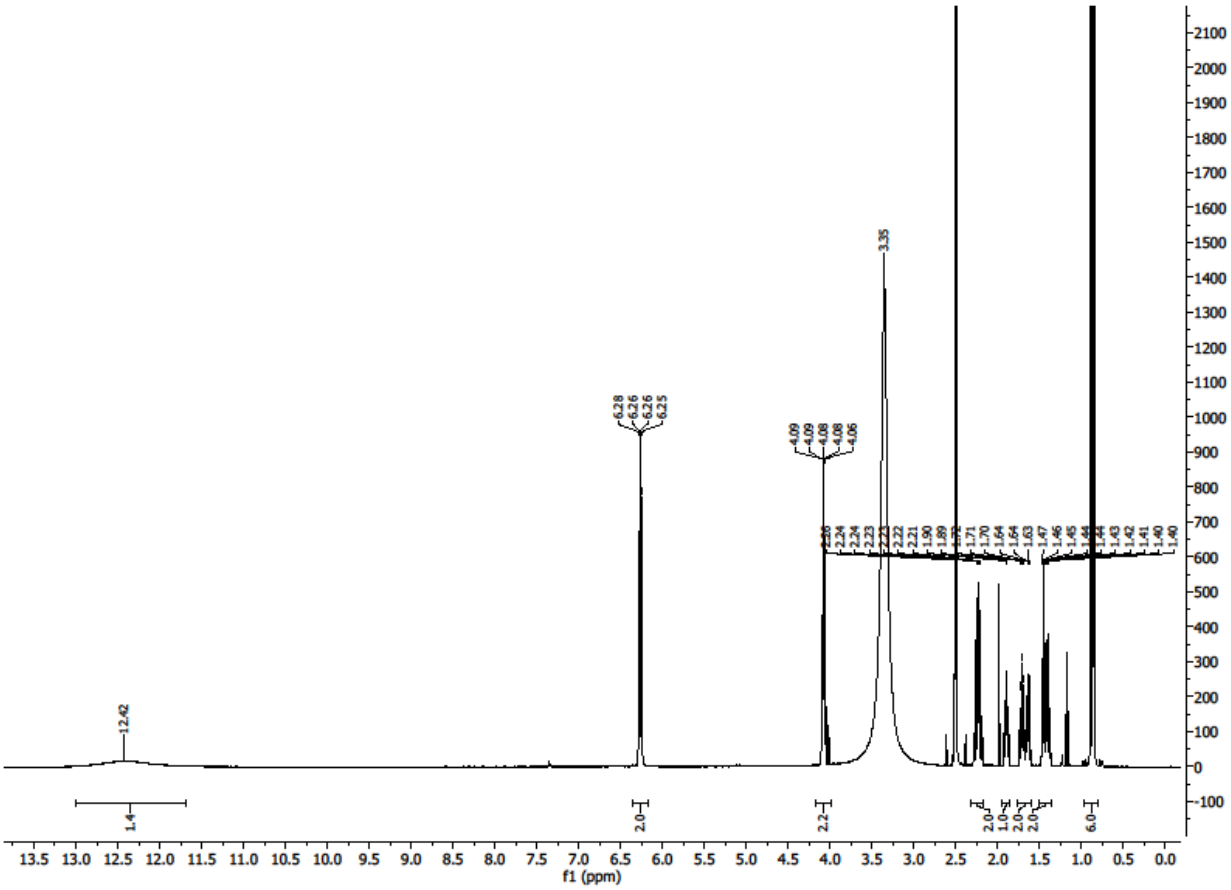


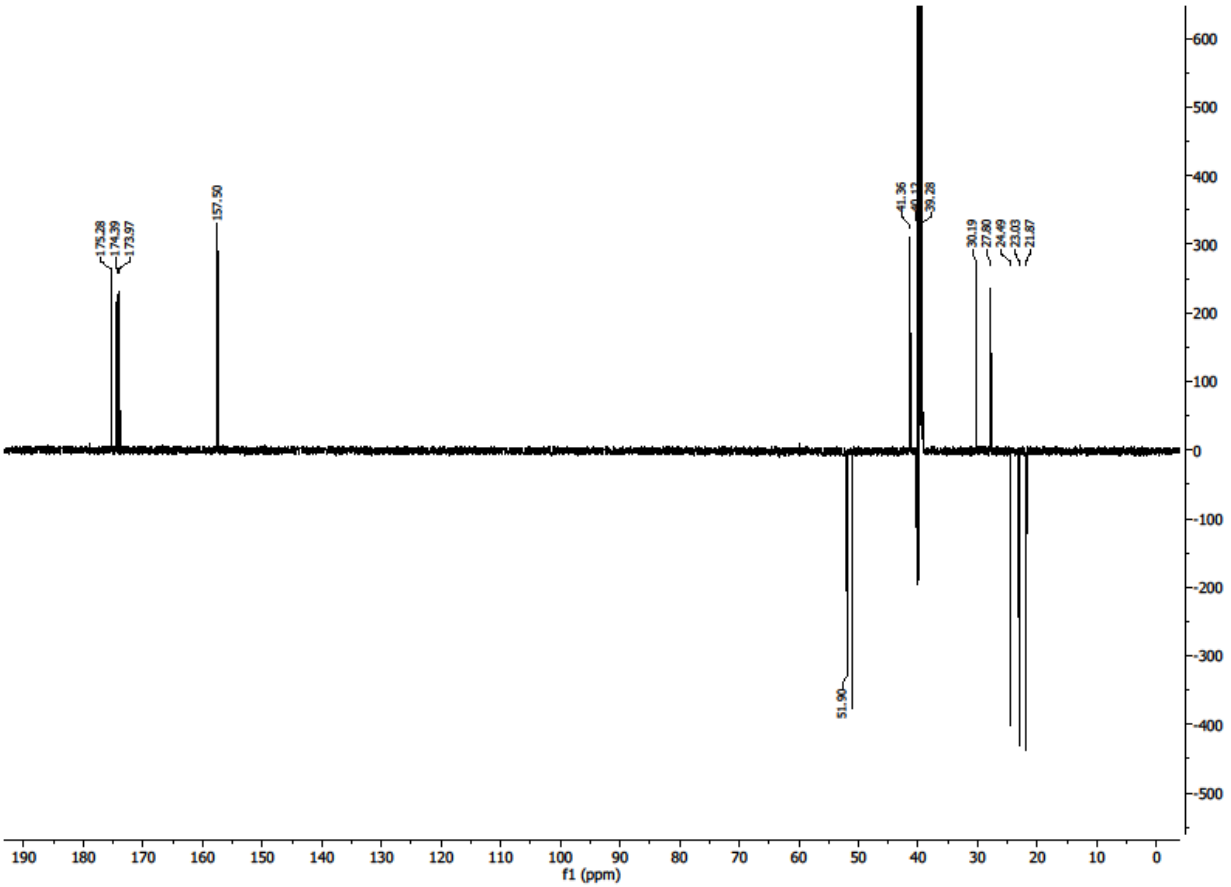
ZJ-43 (X = Y = NH)
5 (X = O, Y = NH)
7 (X = NH, Y = O)

	ZJ-43 X = NH, Y = NH	**5** X = O, Y = NH	**7** X = NH, Y = O
		^{1}H NMR (DMSO-*d*$_6$)	
A	12.42 br	12.58 br	12.56 br
B	4.075 ddd, *J*(B,C) = 8.3; *J*(B,H1) = 10.4; *J*(B,H2) = 4.6	4.73 dd, *J*(B,H1) = 10.4; *J*(B,H2) = 4.6)	3.93 ddd, *J*(B,C) = 8.2; *J*(B,H1) = 10.4; *J*(B,H2) = 4.6)
C	6.27 d, *J*(C,B) = 8.3	--	7.62 d, *J*(C,B) = 8.2
E	6.255 d, *J*(E,F) = 8.4	7.61 d, *J*(E,F) = 8.1	--
F	4.08 td, *J*(F,E) = 8.4; *J*(F,L1) = 5.3; *J*(F,L2) = 8.6	3.95 ddd, *J*(F,E) = 8.1; *J*(F,L1) = 5.0; *J*(F,L2) = 9.4	4.73 dd, *J*(F,L1) = 4.9; *J*(F,L2) = 8.2
G	12.42 br	12.58 br	12.56 br
H1	1.46 dd (H1,B = 8.3; H1H2 = 13.6; H1,I = 5.6)	1.64 ddd (H1,B = 9.8; H1H2 = 14.0; H1,I = 4.7)	1.53 ddd, (H1,B = 10.4; H1H2 = 13.6; H1,I = 5.0)
H2	1.40 ddd, *J*(H2,B) = 5.6; *J*(H2H1) = 13.6; *J*(H2,I) = 9.5	1.54 ddd, *J*(H2,B) = 4.1; *J*(H2H1) = 14.0; *J*(H2,I) = 8.8	1.45 ddd, *J*(H2,B) = 4.6; *J*(H2H1) = 13.6; *J*(H2,I) = 9.1
I	1.64 m, *J*(I,H1) = 5.2; *J*(I,H2) = 9.5; *J*(I,J) = 6.6; *J*(I,K) = 6.6	1.735 m, *J*(I,H1) = 4.7; *J*(I,H2) = 8.8; *J*(I,J) = 6.6; *J*(I,K) = 6.6	1.67 m, *J*(I,H1) = 5.0; *J*(I,H2) = 9.1; *J*(I,J) = 6.6; *J*(I,K) = 6.6
J	0.885 d, *J*(J,I) = 6.6	0.91 d, *J*(J,I) = 6.6	0.885 d, *J*(J,I) = 6.6
K	0.855 d, *J*(K,I) = 6.6	0.89 d, *J*(K,I) = 6.6	0.84 d, *J*(K,I) = 6.6
L1	1.90 dddd, *J*(L1,F) = 5.3; *J*(L1,L2) = 13.8; *J*(L1,M1) = 6.7; *J*(L1,M2) = 9.0	1.94 dddd, *J*(L1,F) = 5.0; *J*(L1,L2) = 13.9; *J*(L1,M1) = 7.1; *J*(L1,M2) = 8.4	2.00 m, *J*(L1,F) = 4.9; *J*(L1,L2) = 14.2; *J*(L1,M1) = 6.7; *J*(L1,M2) = 9.0
L2	1.71 dddd, *J*(L2,F) = 8.6; *J*(L2,L1) = 13.8; *J*(L2,M1) = 9.0; *J*(L2,M2) = 6.0	1.75 dddd, *J*(L2,F) = 9.4; *J*(L2,L1) = 13.9; *J*(L2,M1) = 7.1; *J*(L2,M2) = 8.4	1.875 m, *J*(L2,F) = 8.2; *J*(L2,L1) = 14.2; *J*(L2,M1) = 8.9; *J*(L2,M2) = 5.8
M1	2.26 ddd, *J*(M1,L1) = 6.7; *J*(M1,L2) = 9.0; *J*(M1,M2) = 16.5	2.31 ddd, *J*(M1,L1) = 7.1; *J*(M1,L2) = 7.8; *J*(M1,M2) = 16.8	2.37 m, *J*(M1,L1) = 6.7; *J*(M1,L2) = 8.9; *J*(M1,M2) = 16.8
M2	2.21 ddd, *J*(M2,L1) = 9.0; *J*(M2,L2) = 6.0; *J*(M2,M1) = 16.5	2.28 ddd, *J*(M2,L1) = 8.4; *J*(M2,L2) = 6.2; *J*(M2,M1) = 16.8	2.31 m, *J*(M2,L1) = 9.0; *J*(M2,L2) = 5.8; *J*(M2,M1) = 16.8
N	12.42 br	12.58 br	12.56 br
		^{13}C NMR (DMSO-*d*$_6$)	
A	175.28	172.64	174.44
B	51.06	70.66	52.32
D	157.50	155.92	155.73
F	51.90	53.24	71.12
G	174.39	173.64	171.99
H	41.36	39.70	39.94
I	24.49	24.33	24.42
J	23.03	23.04	23.08
K	21.87	21.66	21.39
L	27.79	26.42	26.54
M	30.19	30.27	29.49
N	173.97	173.97	173.72

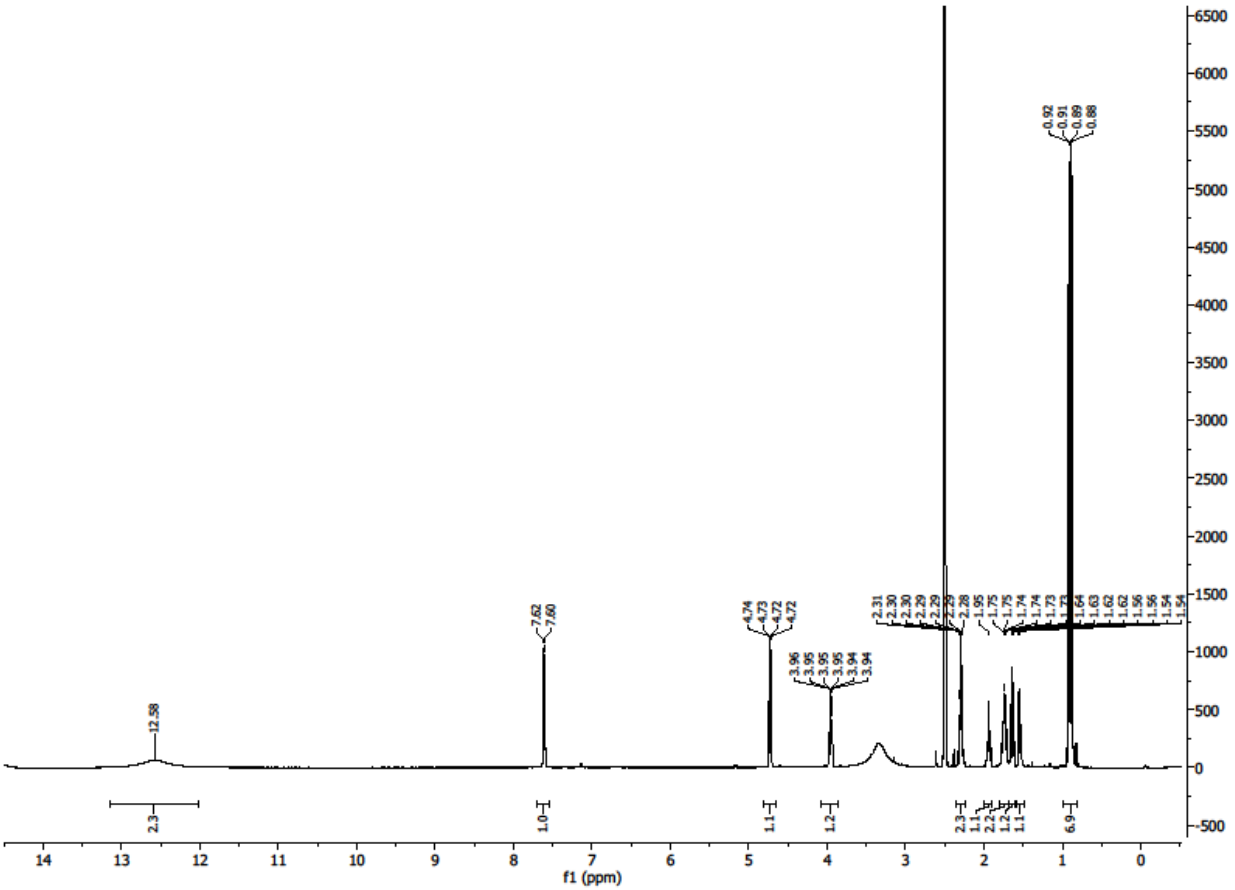
NMR spectra of **ZJ-43**, **5** and **7** were measured on Bruker AVANCE 600 instrument with a cryo-probe (^{1}H at 600 MHz and ^{13}C at 150.9 MHz frequency) in DMSO-*d*$_6$ at 25 °C (see below for ^{1}H and ^{13}C spectra). The 2D–H,H–COSY, 2D–H,H–ROESY, 2D–H,C–HSQC and 2D–H,C–HMBC spectra were used for the structural assignment of proton and carbon signals. Chemical shifts are referenced to the solvent signal using relations: δ_H(DMSO) = 2.50 ppm and δ_C (DMSO) = 39.7 ppm. Very similar values of proton vicinal couplings indicate also very similar conformation properties of three studied inhibitors in solution. General preferred *trans*- arrangement of the amide and carbamate bonds together with large values of ^{3}J(N,H) = 8.1–8.4 Hz suggest preferred extended conformation of the inhibitor backbone. The observed ^{3}J(Hα,Hβ) and ^{3}J(Hβ,Hγ) are typical for *trans*- and *gauche*- couplings of geminal protons and *trans*-orientation of carbon atoms in both side chains. The 2D-H,H-ROESY spectrum showed only *intra*-residual NOEs contacts. The absence of any *inter*-residual NOE contacts can be explained by preferred extended conformations of backbone and side-chains where average distance between side-chain protons of two residues is above NOE observation range (more than ca 3.5 Å) and some contribution of flexibility of the inhibitor molecules in solution.

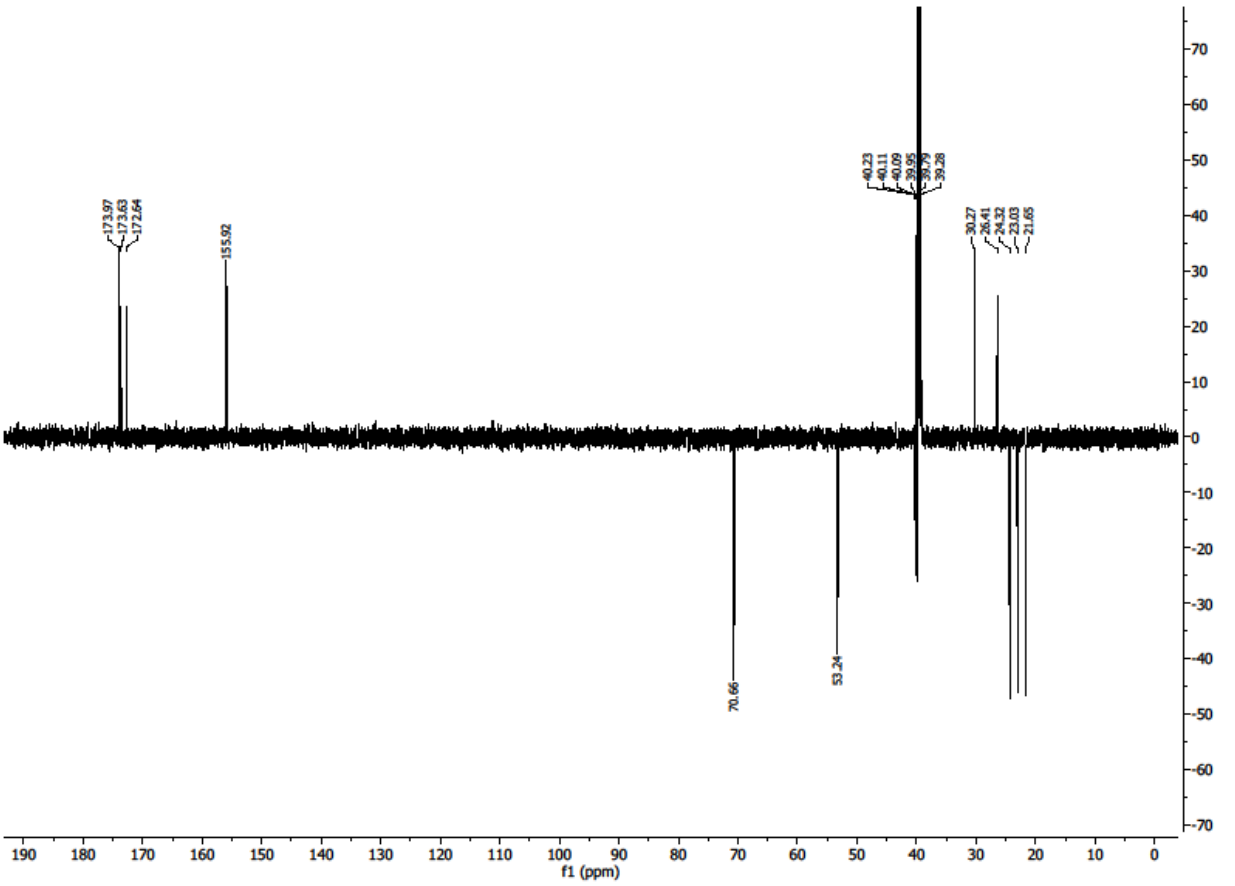
^{1}H and ^{13}C spectra of **ZJ-43**



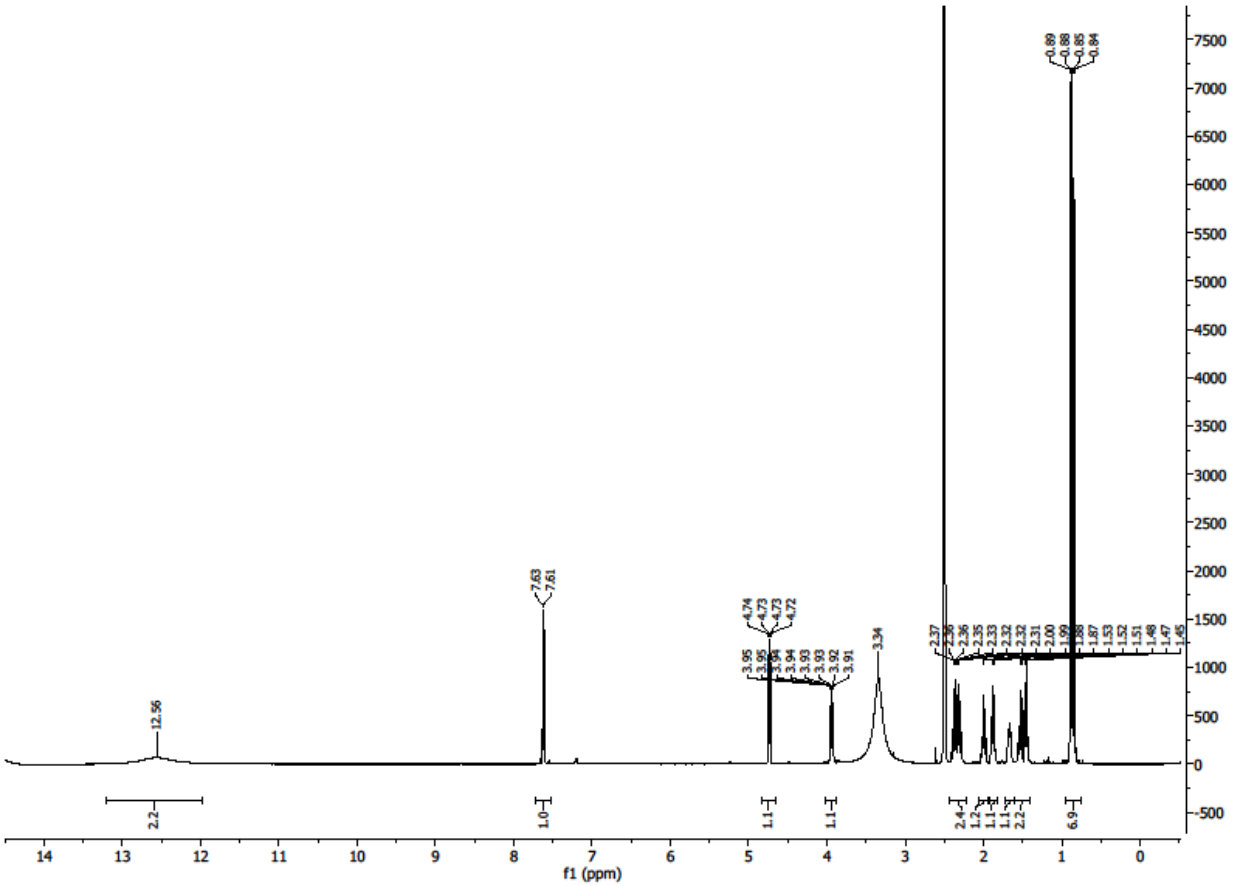


^{1}H and ^{13}C spectra of compound **5**





^{1}H and ^{13}C spectra of compound **7**



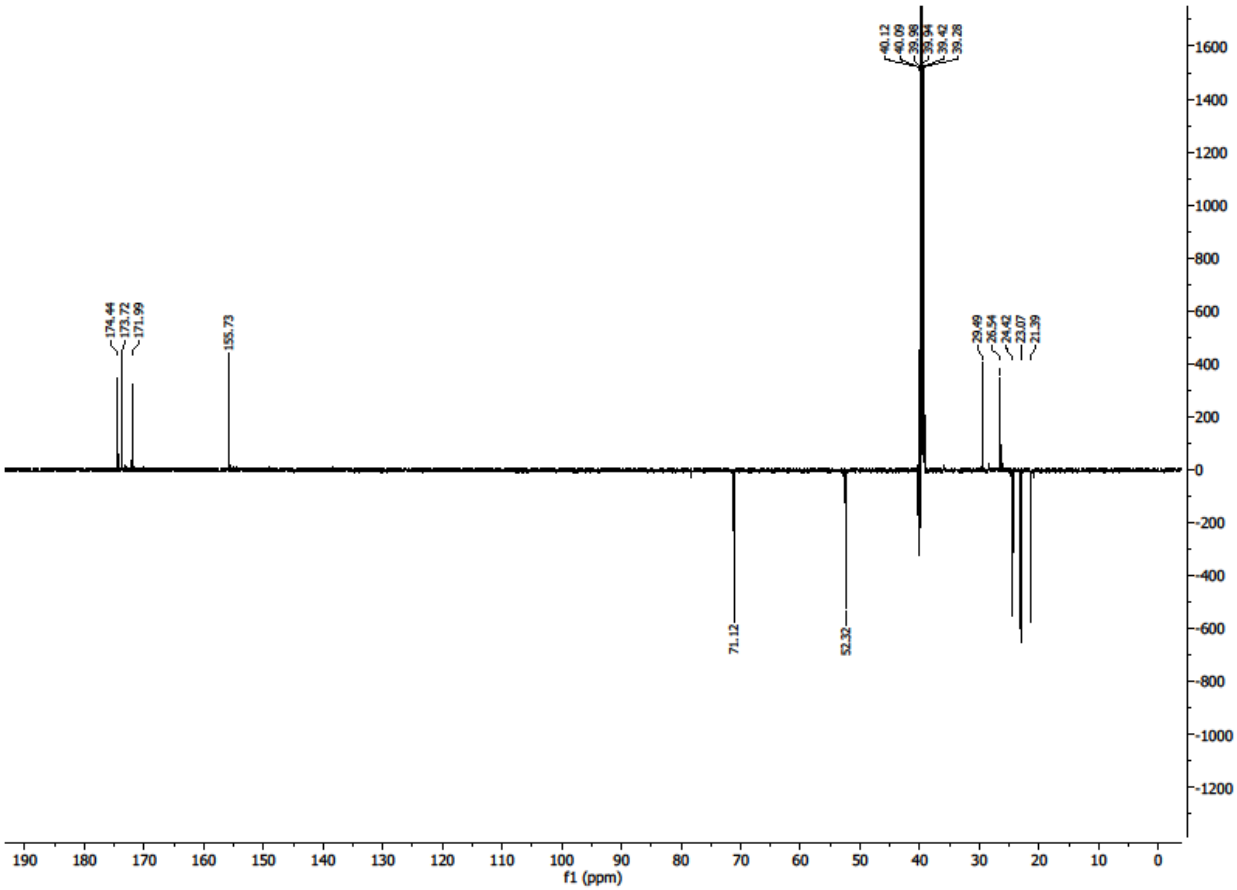
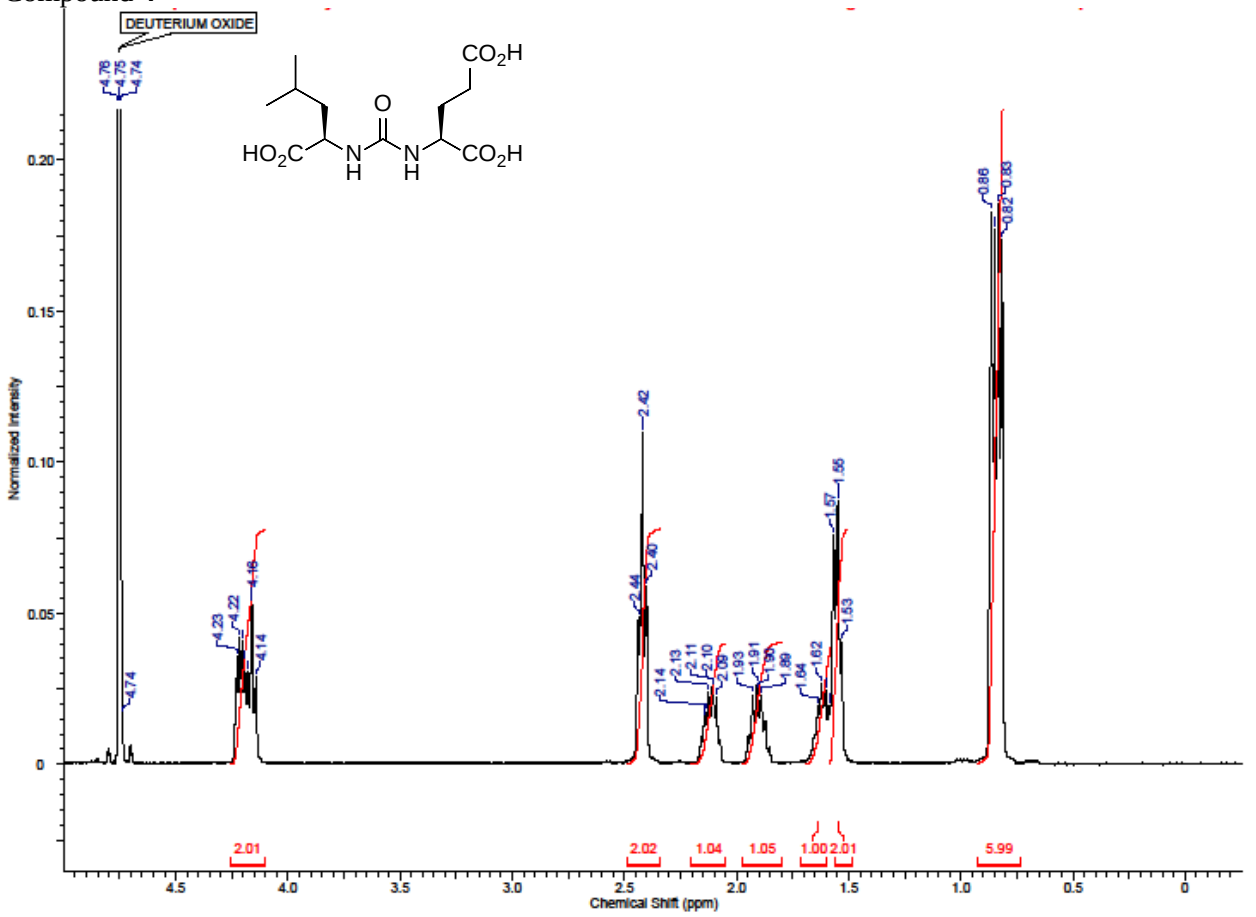
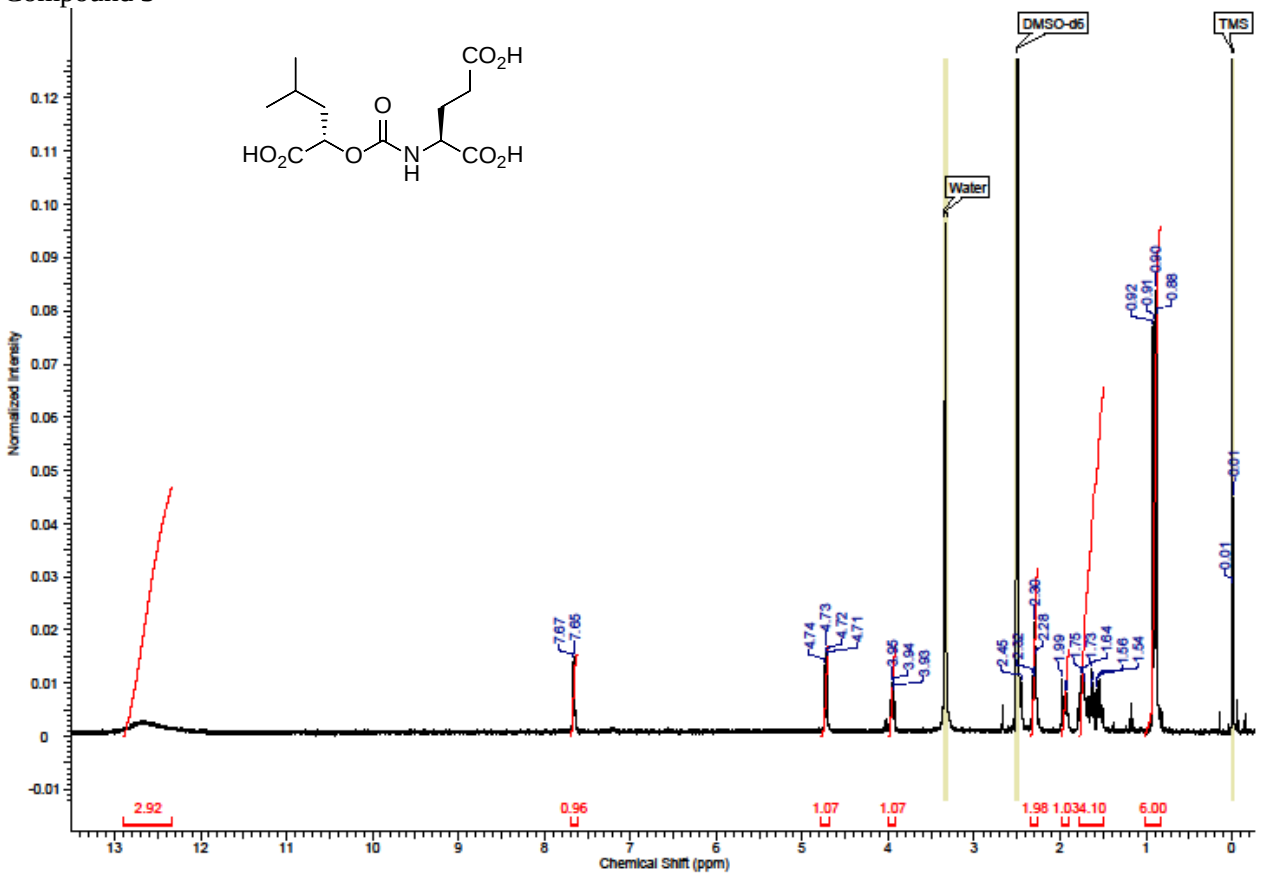


Table S3 ^{1}H spectra of test compounds **4-10**

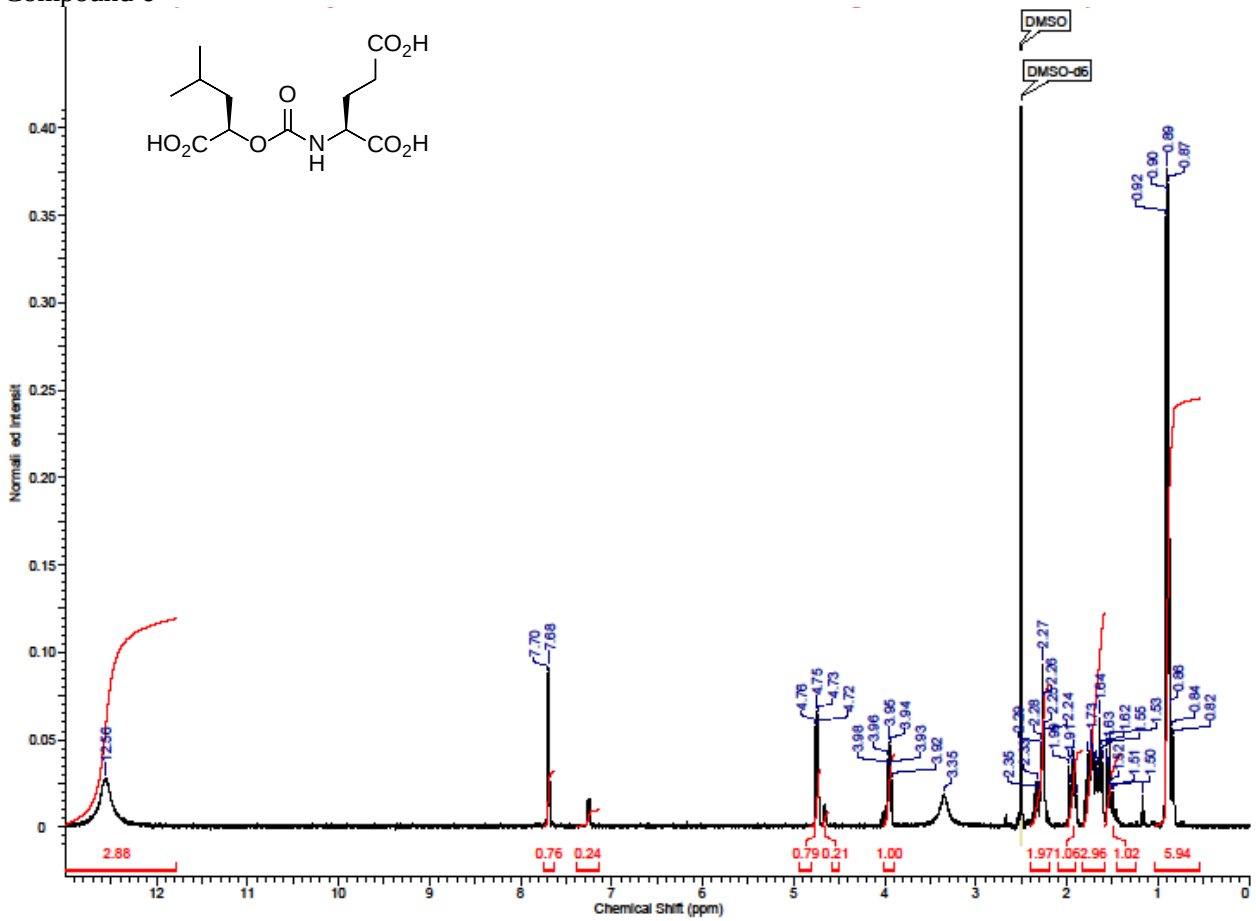
Compound **4**



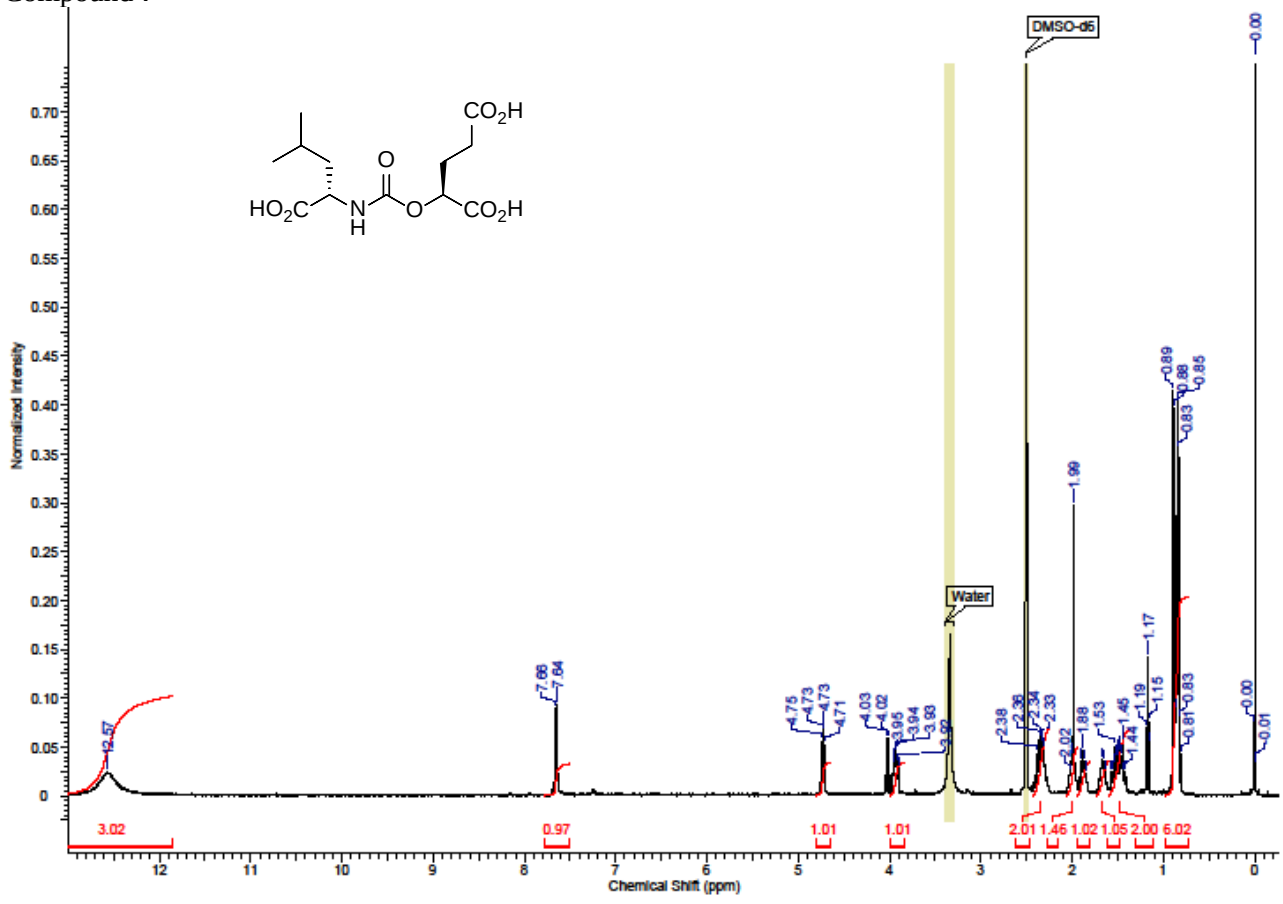
Compound **5**



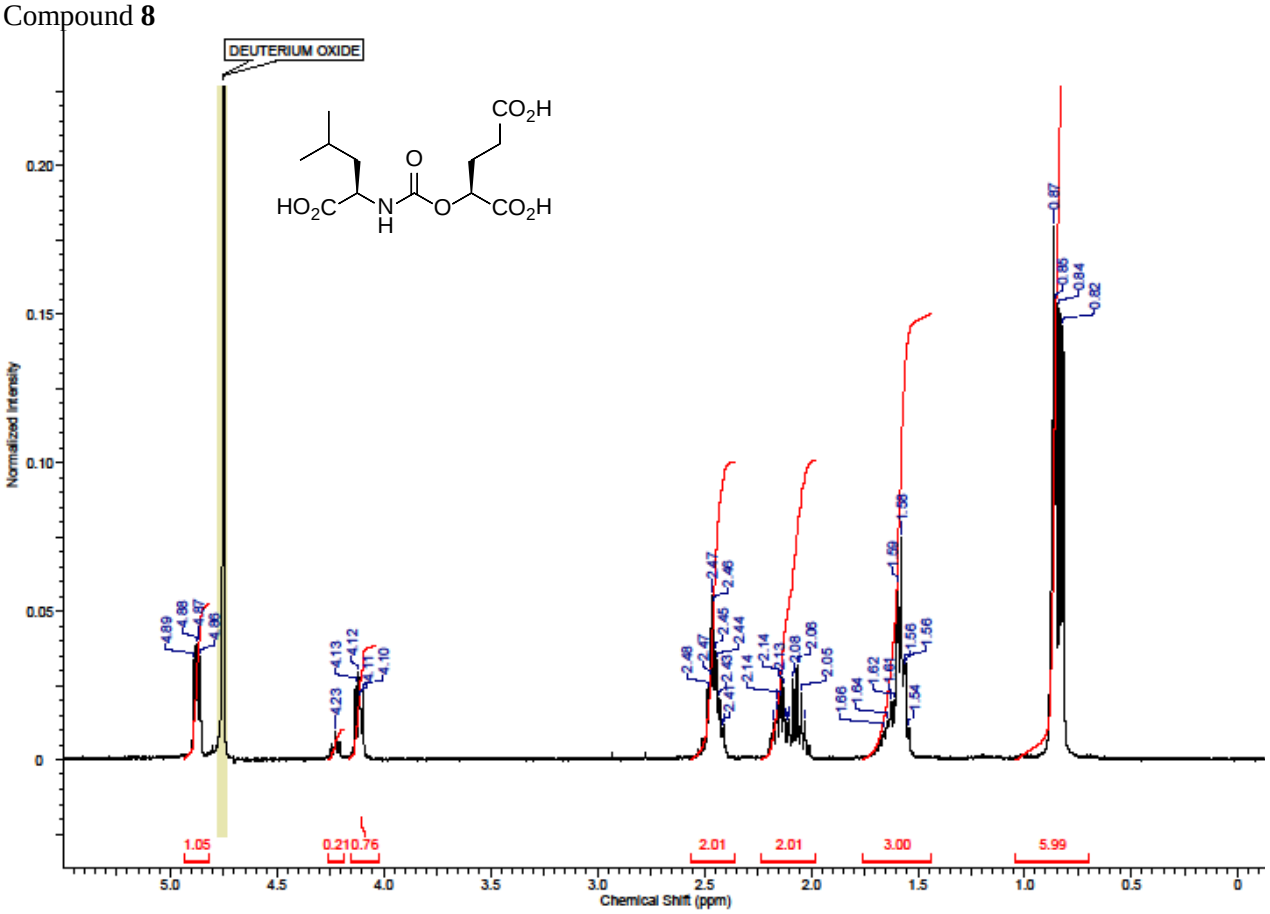
Compound **6**



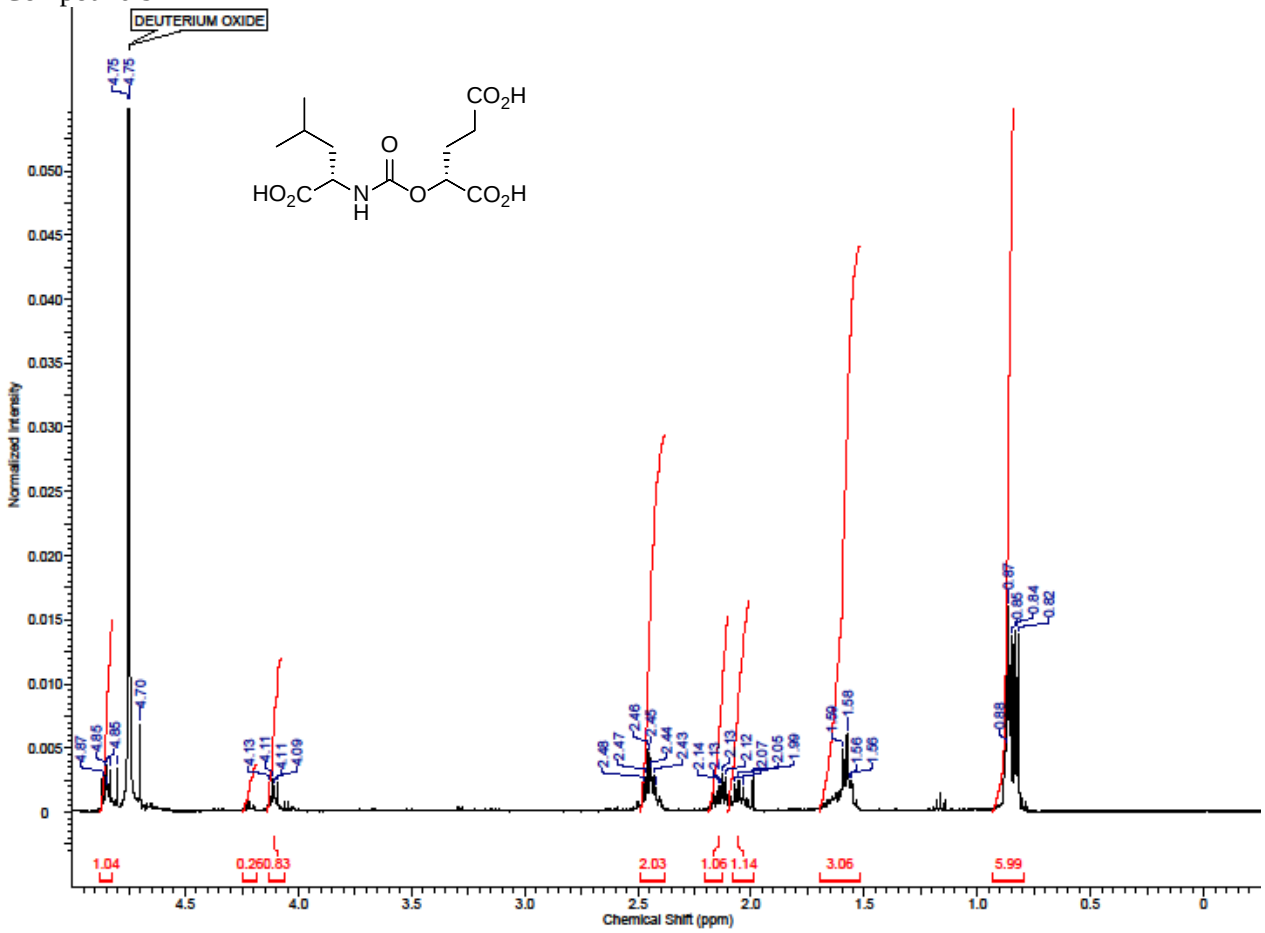
Compound **7**



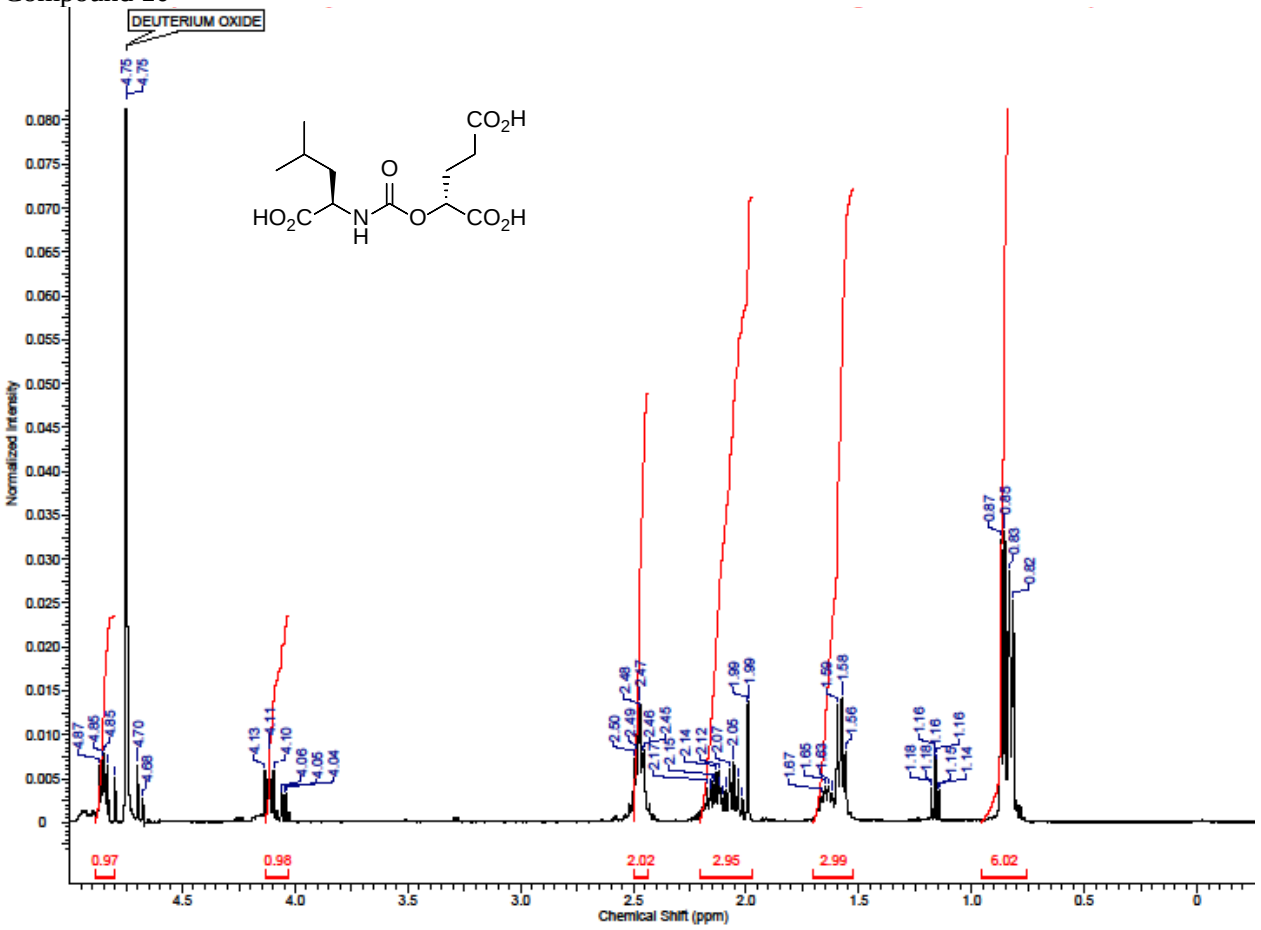
Compound **8**



Compound **9**



Compound **10**


DEUTERIUM OXIDE
Normalized Intensity
Chemical Shift (ppm)
0.97
0.98
2.02
2.95
2.99
6.02