QSAR

The biological activity and toxicity of drug candidates can be assessed using computer calculations, without animal testing, significantly simplifying the drug development process. Quantitative structure-activity relationship (QSAR) is a widely used statistical tool for identifying the relationship between biological activity and molecular structure.[19] Computer modeling can also be used to predict the aqueous solubility and ability to cross the blood–brain barrier (BBB) for a particular molecule.

Potential pharmacological effects and their mechanisms of action can be predicted using the freely available PASS Online web service. The PASS Online algorithm is based on the description of structural formulas as a list of substructural atom-centric MNA (Multilevel Neighborhoods of Atoms) descriptors and a Bayesian algorithm[20]. The average prediction accuracy (AUC) calculated using the leave-one-out control method is 0.95. The PASS Online prediction output is a list of biological activities with probabilities of belonging to active compounds (Pa) and inactive compounds (Pi). Any activity prediction with Pa > Pi can be considered positive. Furthermore, a small Pa value may indicate that the compound may represent a new chemical class for the predicted biological activity. The biological activity prediction results for compounds **4 a–e** with a cutoff value of Pa > 0.5 are presented in Table 1.

Table 1. Predicted biological activity of the studied compounds using PASS Online with a threshold value of Pa > 0.5.

Compound	Pa	Pi	Activity
4 a	0.809	0.004	Antihypoxic
	0.740	0.012	Anti-ischemic, cerebral
	0.687	0.019	Treatment of acute neurological disorders
	0.599	0.022	Cytoprotective
	0.532	0.050	Anti-inflammatory
	0.590	0.002	Phospholipase A1 inhibitor
	0.517	0.027	Anticonvulsant
4 b	0.604	0.039	Anti-ischemic, cerebral
	0.585	0.040	Treatment of acute neurological disorders
	0.573	0.018	Anticonvulsant
	0.538	0.134	Treatment of phobias
	0.535	0.019	Antihypoxic

4 c-e	0.795 0.731 0.691 0.684 0.602 0.523 0.580 0.521	0.004 0.013 0.018 0.007 0.021 0.052 0.002 0.026	Antihypoxic Anti-ischemic, cerebral Treatment of acute neurological disorders Antianginal Cytoprotective Anti-inflammatory Phospholipase A1 inhibitor Anticonvulsant

The data presented in Table 1 indicate that all compounds likely possess antihypoxic and cerebral anti-ischemic activity and have potential for the treatment of acute neurological disorders, with **4 a** being the most promising candidate. All compounds except **4 b** also likely exhibit anticonvulsant, cytoprotective, and anti-inflammatory activity. Furthermore, the known molecular mechanism of action (phospholipase A1 inhibitor), associated with anti-inflammatory activity, is predicted with high probability. For the other predicted pharmacological effects listed above, the associated molecular mechanisms of action were not predicted with high probability. Compound **4 b** is predicted to be active in the treatment of phobias. Most of the compounds are also potential candidates for the treatment of neurodegenerative diseases and Alzheimer's disease, although these predictions have lower probabilities ($0.4 < Pa < 0.5$). Antispasmodic and vasodilatory activities, which may be related to antihypoxic and antiischemic properties, were predicted with the same probability. For compound **4 b**, these activities were predicted with lower probability ($0.3 < Pa < 0.4$). Prediction results for compounds **4 c-e** were similar, as PASS Online does not recognize differences in their structures, which have the same set of MNA descriptors.

To determine the safety of the tested compounds, cytotoxicity in normal cell lines and acute toxicity in rats were predicted LD_{50} using the CLC-Pred internet service[20,21]. The prediction results showed a weak probability of cytotoxicity for all studied compounds towards normal cell lines ($Pa < 0.2$). Acute toxicity for compounds **4 a–e**, predicted using the service GUSAR Acute Rat Toxicity[22,23] is given in Table 2.

Table 2. Results of LD_{50} prediction for rats using different administration methods using the service GUSAR Acute Rat Toxicity.

Compound	**Rats intraperitoneally LD_{50} (mg/kg), class**	**Rats intravenous LD_{50} (mg/kg), class**	**Rats oral LD_{50} (mg/kg), class**	**Rats subcutaneously LD_{50} (mg/kg), class**
4 a	241.8, 4	552.2, 5	1089, 4	451.4, 4
4 b	435.2, 4	212.2, 4	1239, 4	195.6, 4
4 c	358, 4	525.5, 5	1544, 4	397.4, 4
4 d	574.8, 5	497.9, 5	2760, 5	602.4, 4

4 e	597.5, 5	448.3, 5	3178, 5	897.9, 4

The prediction results in Table 2 show that all tested compounds are classified as slightly toxic (class 4) or non-toxic (class 5). Substances **4 a** and 4b are predicted to be more toxic than **4 c–e**. To predict the solubility of compounds **4 a–e** in water, as well as their ability to pass through the BBB, two Internet services were used: SwissADME and PreADME[24,25]. The forecast results are presented in Table 3.

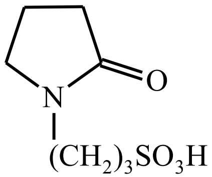
Table 3. Predicted aqueous solubility and BBB permeability for compounds **4 a–e** using services SwissADME and PreADME.

Compound	**SwissADME**		**PreADME**	
	Solubility in water	**BBB permeability**	**Solubility in water mg/L**	**BBB permeability (C.brain/C.blood)**
4 a	Well soluble	No	755774	0.133
4 b	Well soluble	No	41931	0.056
4 c	Well soluble	No	330540	0.175
4 d	Well soluble	No	143982	0.239
4 e	Well soluble	No	62495	0.339

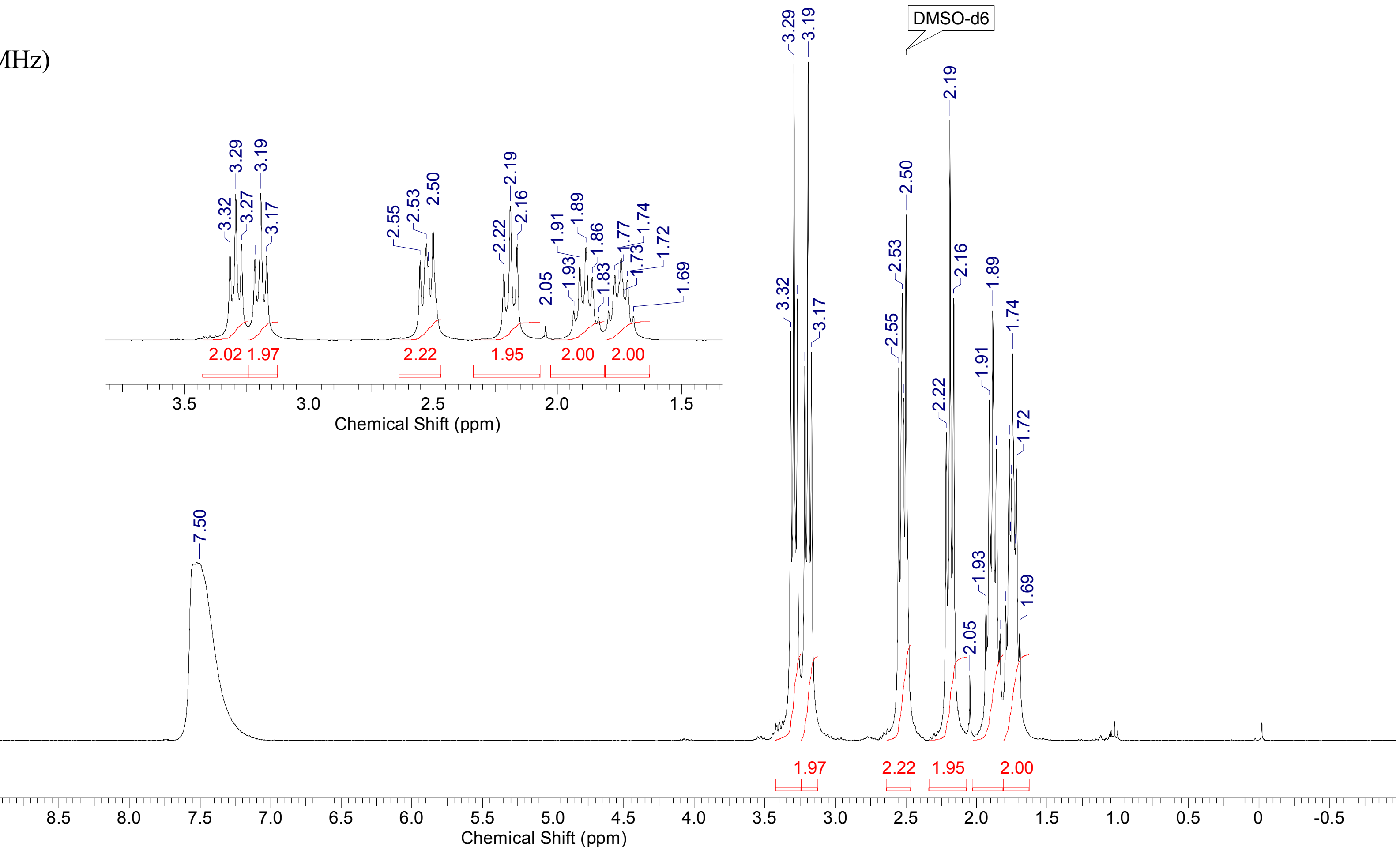
The data presented in Table 3 show good agreement between the water solubility and BBB penetration predictions for the two different services. All compounds are predicted to be highly water-soluble and poorly BBB-transporting. Compound **4 e** is less water-soluble and more readily crosses the BBB. Compound **4 b** is predicted to be the least water-soluble and the least likely to cross the BBB.

NMR spectra

[1]H NMR (DMSO-d6, 400 MHz)

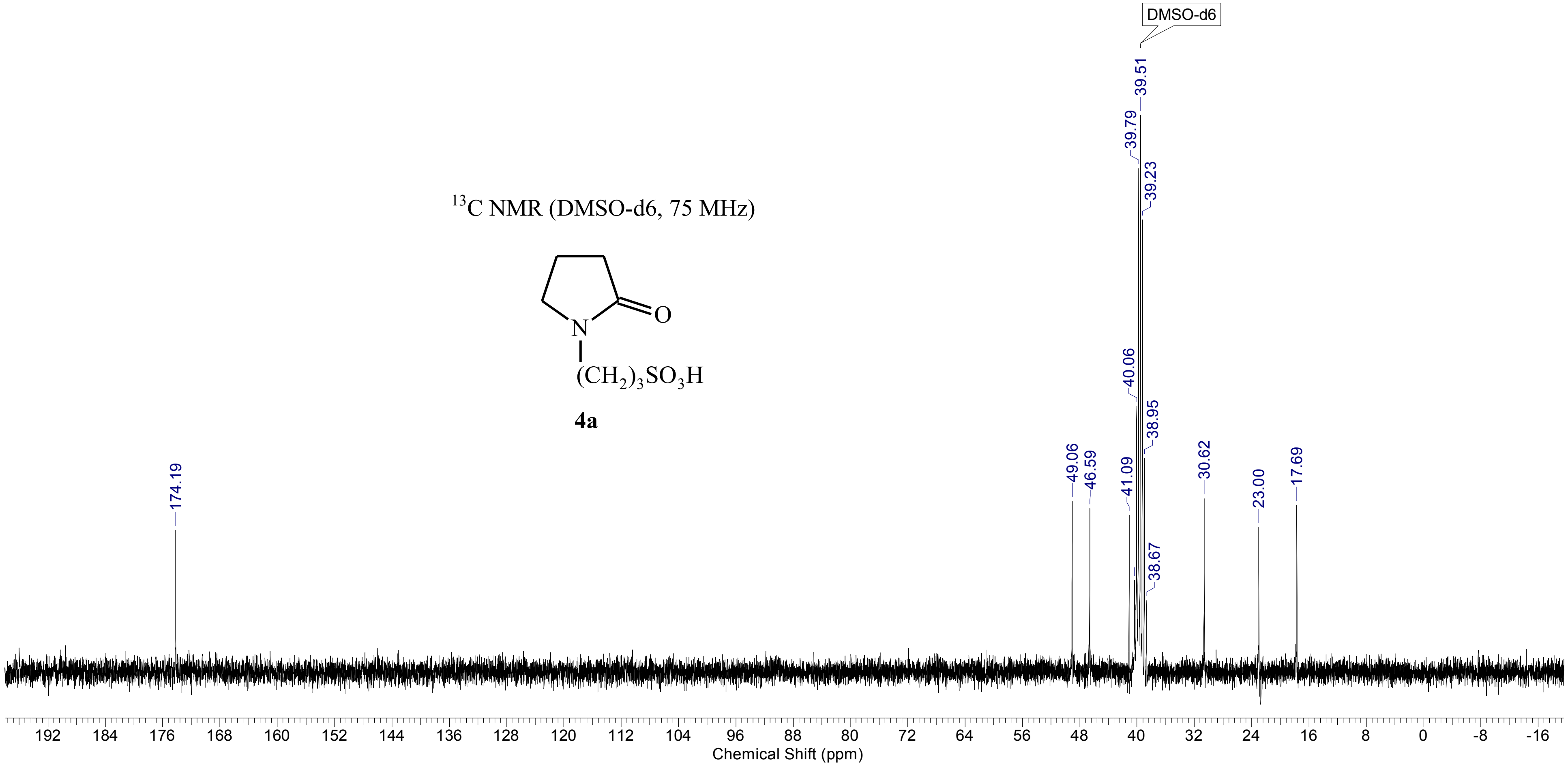


4a



[1]H NMR (DMSO-d6, 400 MHz, δ, ppm): 1.69-1.79 (m, 2H, $NCH_2\underline{CH_2}CH_2SO_3H$), 1.83-1.93 (m, 2H, $NCH_2\underline{CH_2}CH_2C(O)$), 2.19 (t, J_{HH} = 8.0, 2H, $\underline{CH_2}C(O)$), 2.53 (t, J_{HH} = 7.9, 2H, $\underline{CH_2}SO_3H$), 3.19 (t, J_{HH} =7.1, 2H, $N\underline{CH_2}CH_2CH_2SO_3H$), 3.29 (t, J_{HH} = 6.9, 2H, $N\underline{CH_2}CH_2CH_2C(O)$).

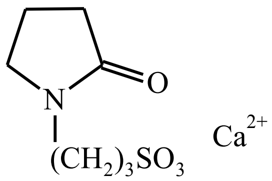
Note: the signal at 7.50 is DMSO water acidified with a proton of the group SO_3H



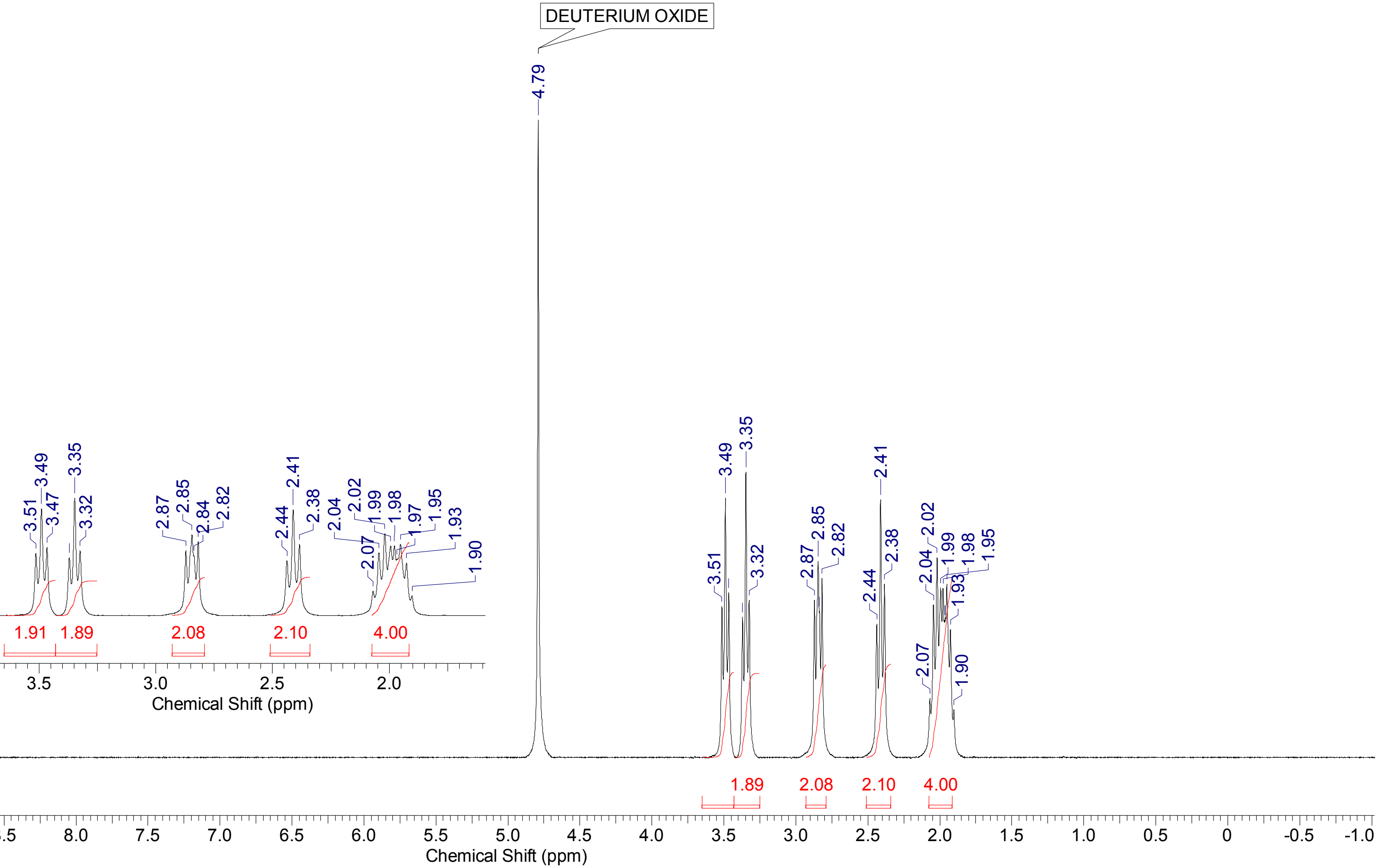
^{13}C NMR (DMSO-d6, 75 MHz, δ, ppm): 17.7 ($NCH_2\underline{CH_2}CH_2C(O)$), 23.0 ($NCH_2\underline{CH_2}CH_2SO_3H$), 30.6 ($NCH_2CH_2\underline{CH_2}C(O)$), 41.1

($NCH_2CH_2CH_2SO_3H$), 46.6 ($NCH_2CH_2CH_2C(O)$), 49.1 (CH_2SO_3H), 174.2 (C=O).

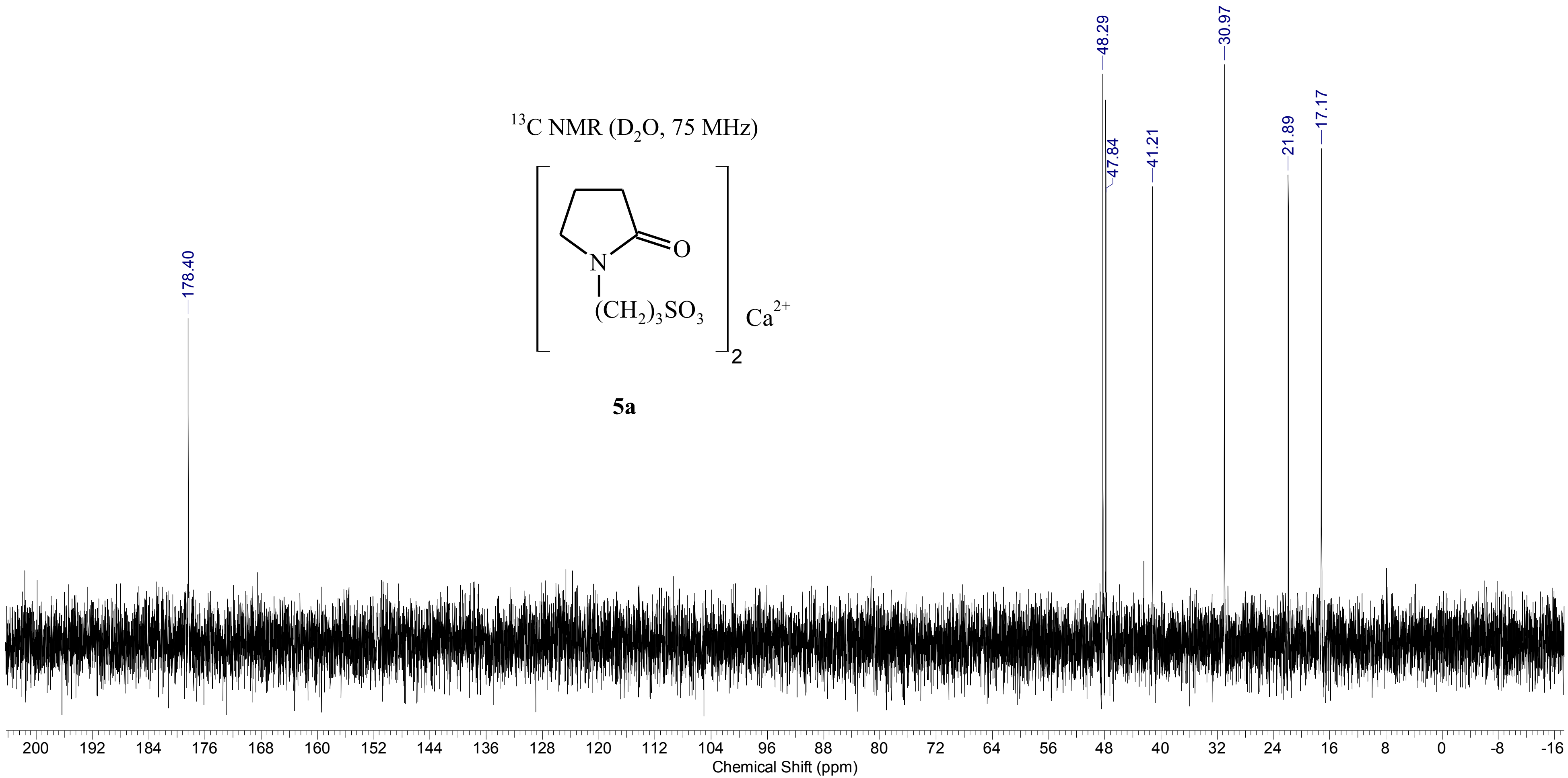
^{1}H NMR (D_2O, 400 MHz)



5a

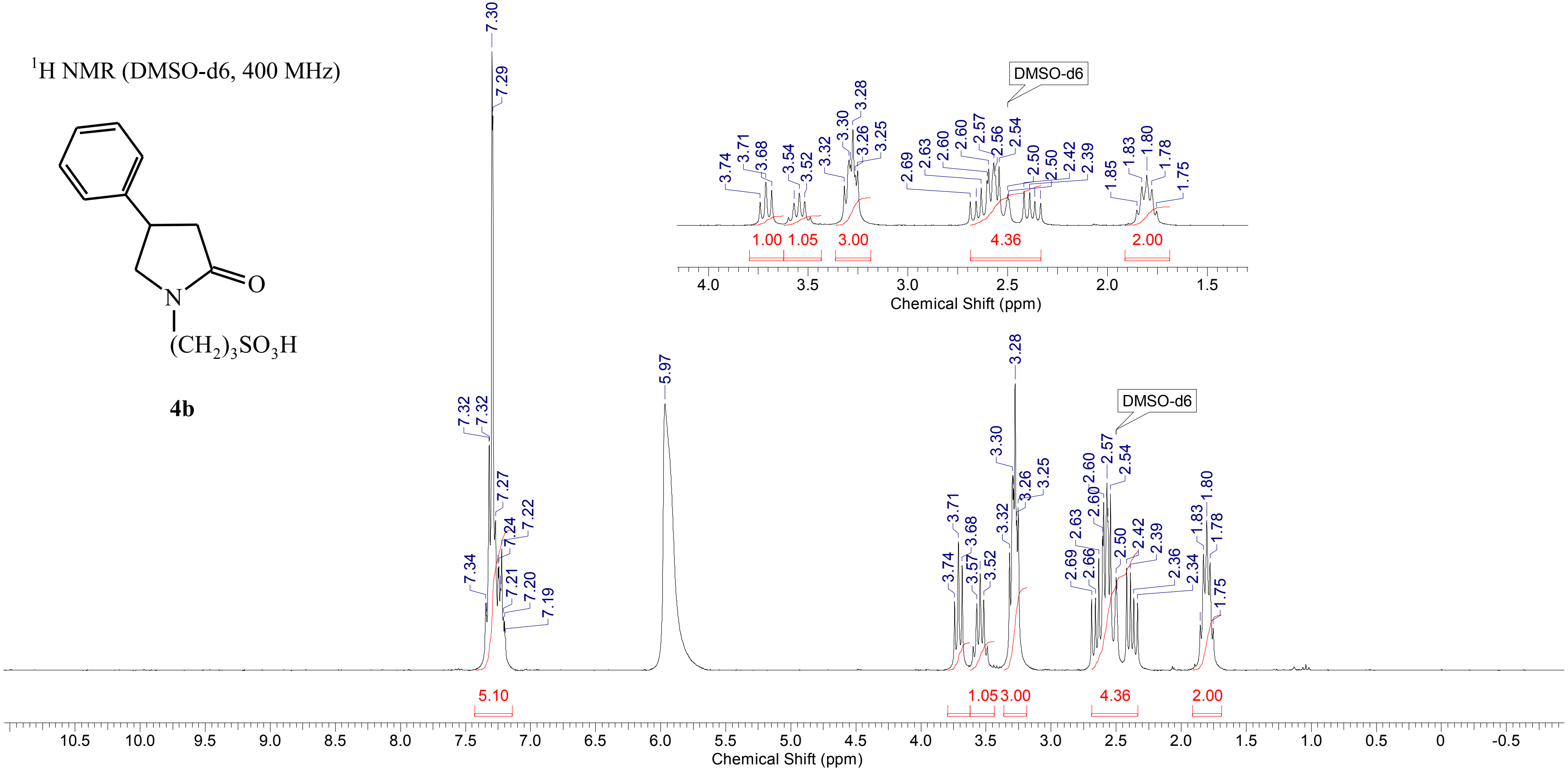


^{1}H NMR (D_2O, 400 MHz, δ, ppm): 1.90-2.07 (m, 4H, $NCH_2\underline{CH_2}CH_2SO_3H$ and $NCH_2\underline{CH_2}CH_2C(O)$), 2.41 (t, J_{HH} = 8.1, 2H, $\underline{CH_2}C(O)$), 2.85 (t, J_{HH} = 7.9, 2H, $\underline{CH_2}SO_3$), 3.35 (t, J_{HH} = 6.9, 2H, $N\underline{CH_2}CH_2CH_2SO_3$), 3.49 (t, J_{HH} = 7.1, 2H, $N\underline{CH_2}CH_2CH_2C(O)$).



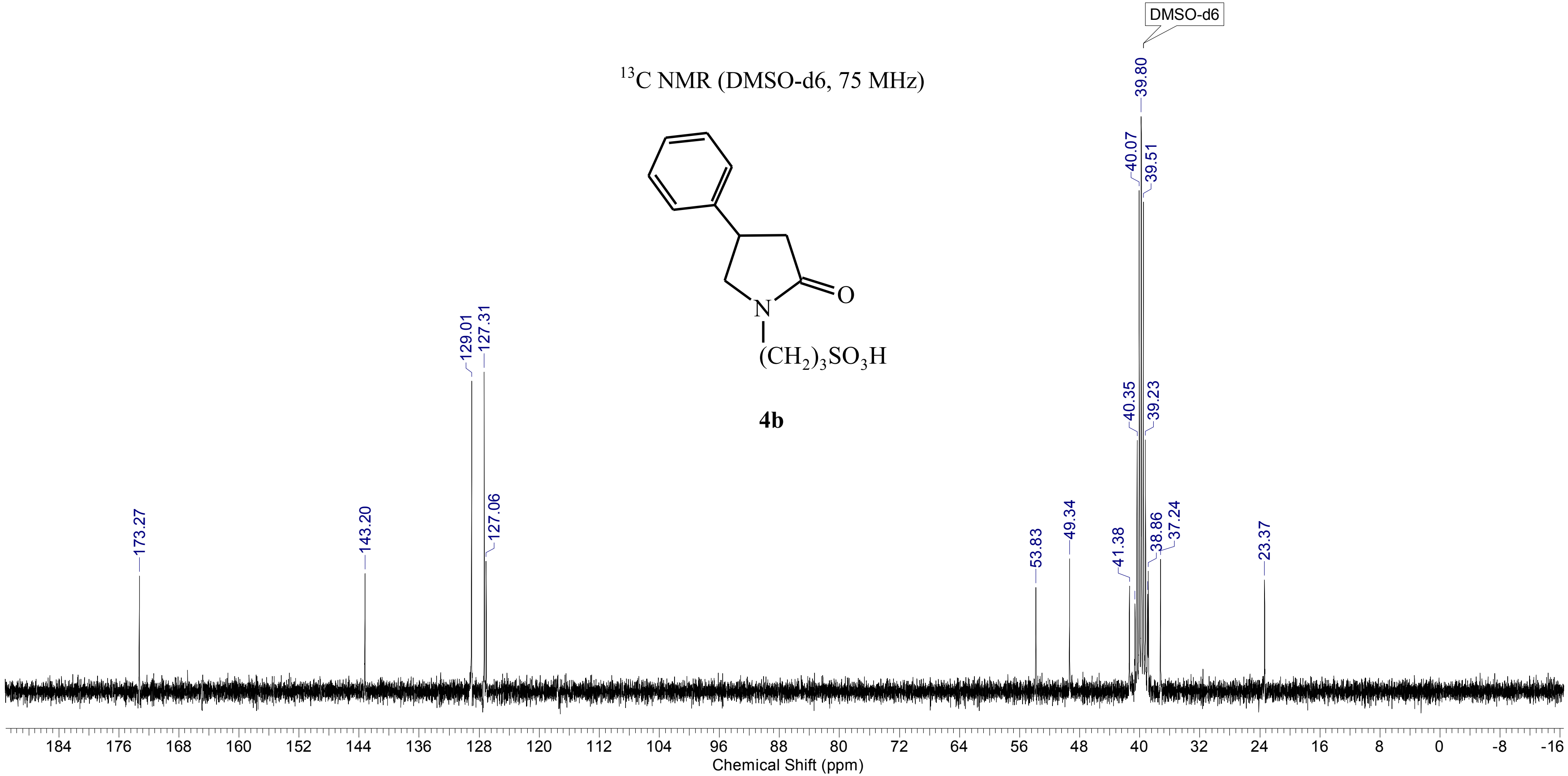
5a

^{13}C NMR (D_2O, 75 MHz, δ, ppm): 17.2 ($NCH_2\underline{CH_2}CH_2C(O)$), 21.9 ($NCH_2\underline{CH_2}CH_2SO_3H$), 30.9 ($NCH_2CH_2\underline{CH_2}C(O)$), 41.2 ($N\underline{CH_2}CH_2CH_2SO_3$), 47.8 ($N\underline{CH_2}CH_2CH_2C(O)$), 48.3 ($\underline{CH_2}SO_3$), 178.4 (C=O).

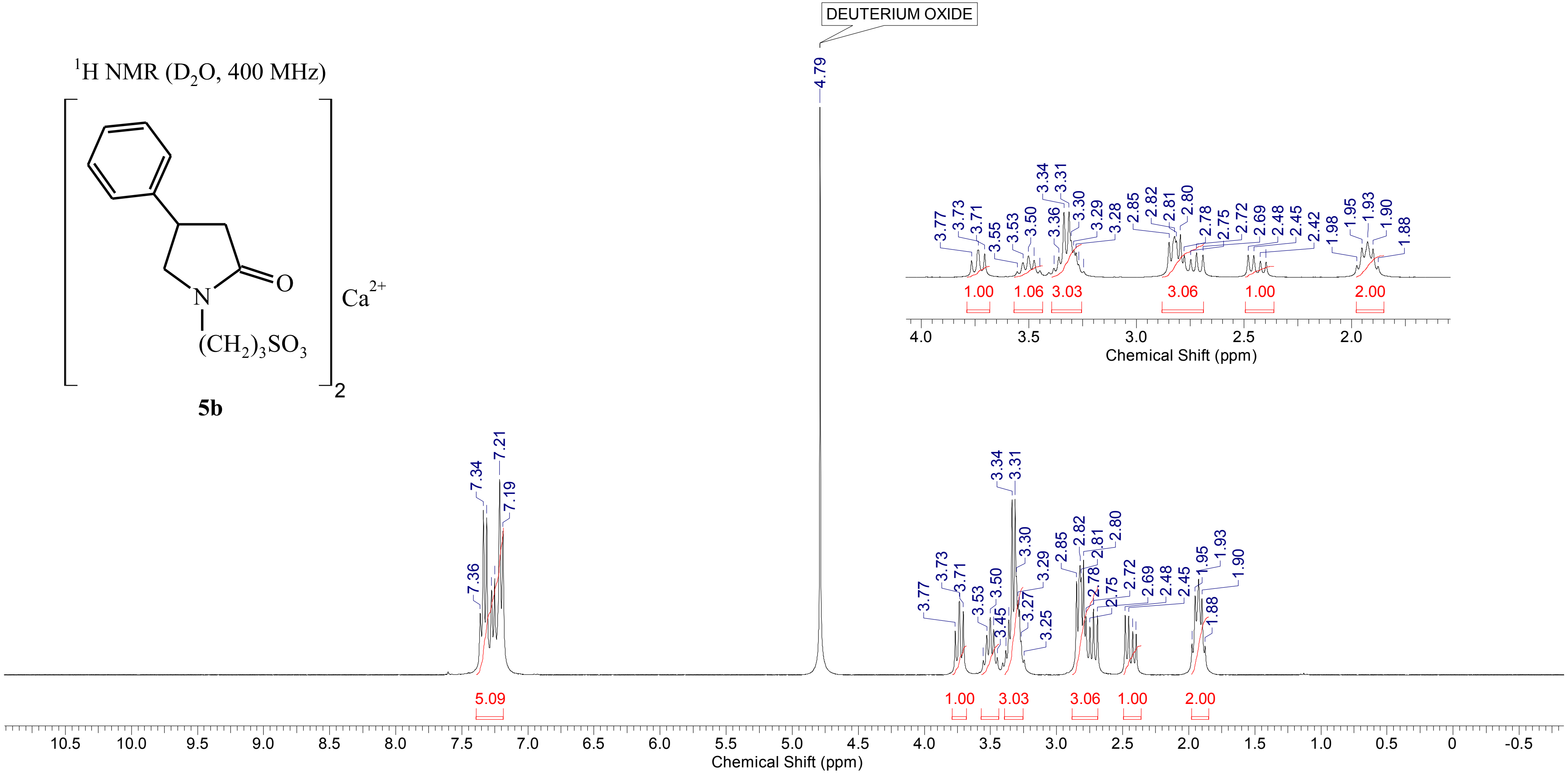


^{1}H NMR (DMSO-d$_6$, 400 MHz, δ, ppm): 1.75-1.85 (m, 2H, NCH$_2$<u>CH$_2$</u>CH$_2$SO$_3$H), 2.39-2.69 (m, 4H, <u>CH$_2$</u>SO$_3$ and <u>CH$_2$</u>C(O)), 3.25-3.32 (m, 3H, N<u>CH$_2$</u>CH$_2$CH$_2$SO$_3$H and <u>CH</u>Ph), 3.53 (t, J_{HH} =8.0, 1H, N<u>CH$_2$</u>CHPh), 3.71 (t, J_{HH} =8.7, 1H, N<u>CH$_2$</u>CHPh), 7.19-7.34 (m, 5H, Ph).

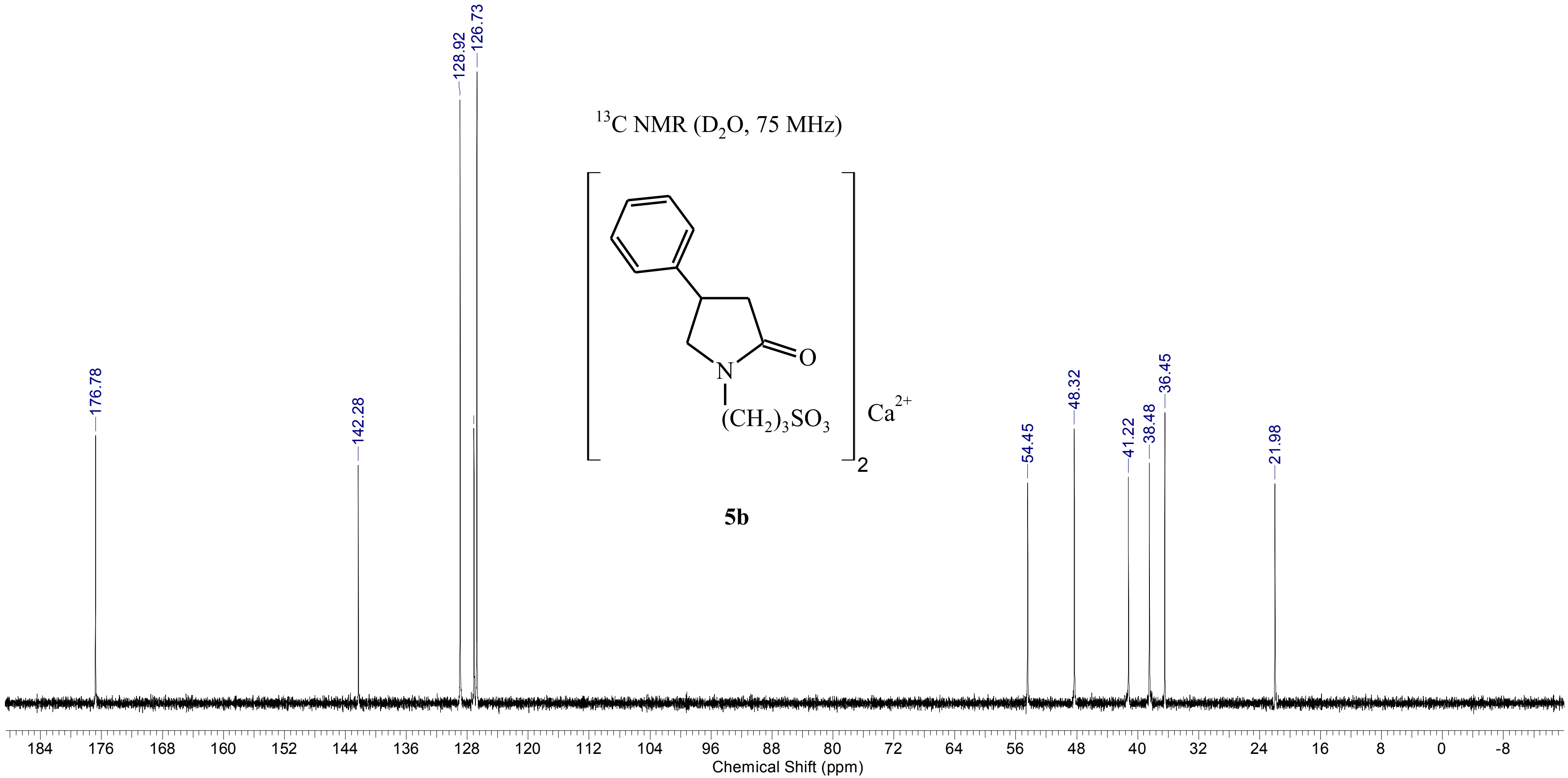
Note: signal 5.97 is DMSO water acidified with a proton of the group SO_3H



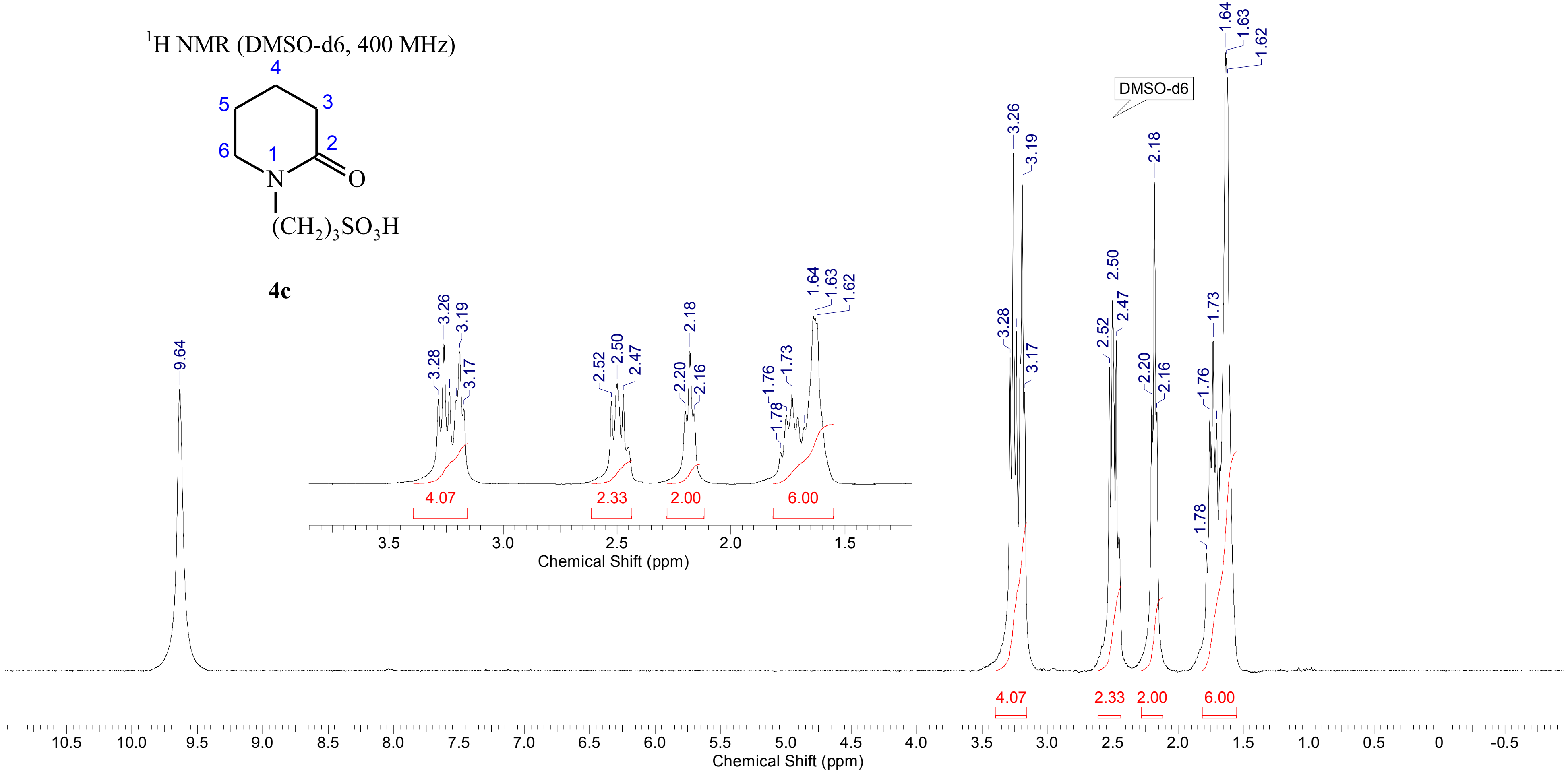
^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm): 23.4 (NCH$_2$<u>CH$_2$</u>CH$_2$SO$_3$H), 37.2 (<u>CH$_2$</u>C(O)), 38.9 (<u>CH</u>Ph), 41.4 (N<u>CH$_2$</u>CH$_2$CH$_2$SO$_3$H), 49.3 (<u>CH$_2$</u>SO$_3$), 53.8 (N<u>CH$_2$</u>CHPh), 127.0 (Ph), 127.3 (Ph), 129.0 (Ph), 143.2 (Ph), 173.3 (C=O).



^{1}H NMR (D_2O, 400 MHz, δ, ppm): 1.88-1.98 (q, J_{HH} =7.3, 2H, NCH$_2$<u>CH$_2$</u>CH$_2$SO$_3$), 2.42-2.48 (m, 1H, <u>CH$_2$</u>C(O)), 2.69-2.85 (m, 3H, <u>CH$_2$</u>SO$_3$ and <u>CH$_2$</u>C(O)), 3.28-3.36 (m, 3H, N<u>CH$_2$</u>CH$_2$CH$_2$SO$_3$H and <u>CH</u>Ph), 3.53 (t, J_{HH} =7.7, 1H, N<u>CH$_2$</u>CHPh), 3.73 (t, J_{HH} =9.0, 1H, N<u>CH$_2$</u>CHPh), 7.19-7.36 (m, 5H, Ph).

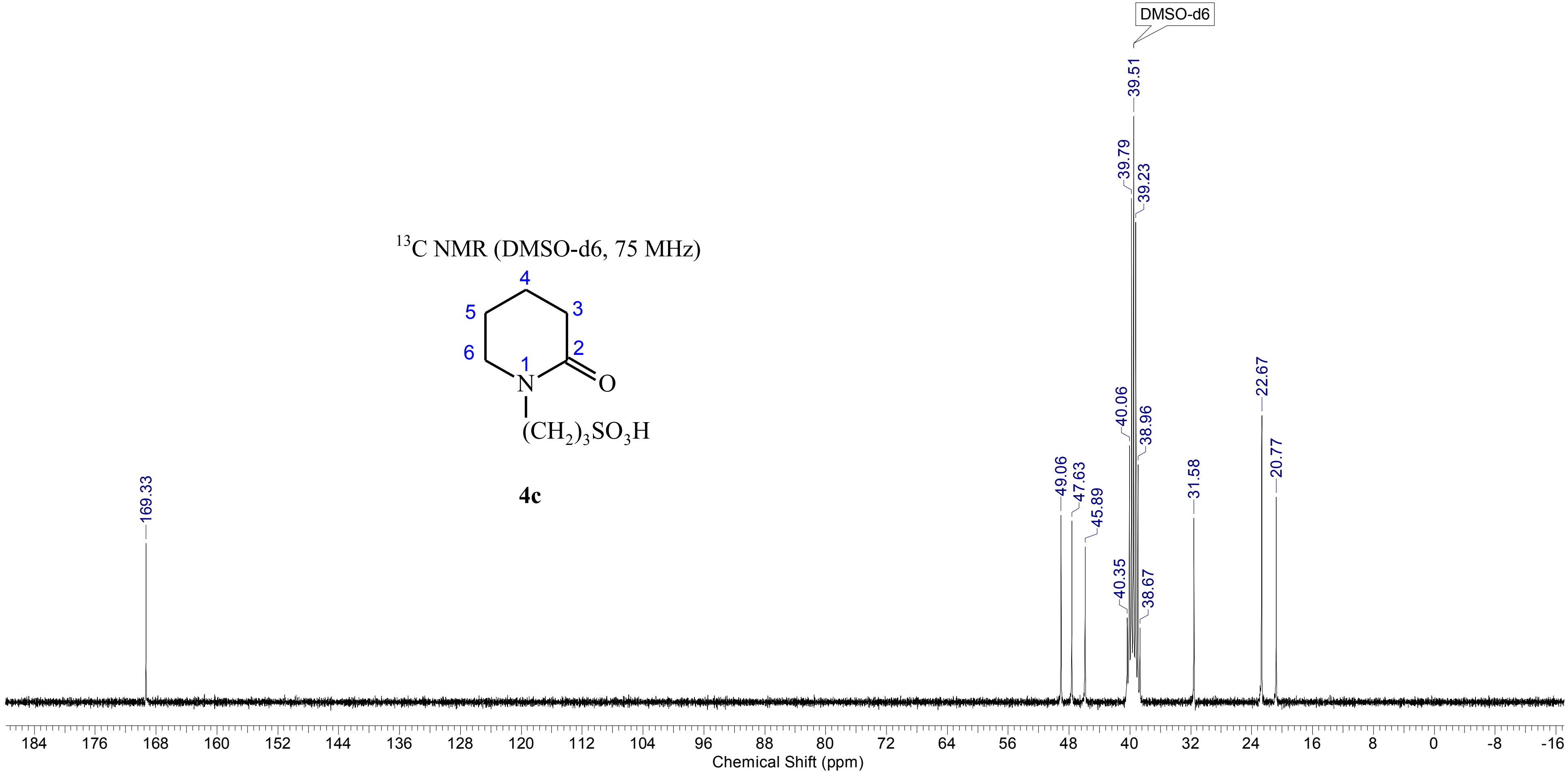


^{13}C NMR (D_2O, 75 MHz, δ, ppm): 21.9 ($NCH_2\underline{CH_2}CH_2SO_3$), 36.4 ($\underline{CH_2}C(O)$), 38.5 ($\underline{CH}Ph$), 41.2 ($N\underline{CH_2}CH_2CH_2SO_3$), 48.3 ($\underline{CH_2}SO_3$), 54.4 ($N\underline{CH_2}CHPh$), 126.7 (Ph), 127.1 (Ph), 128.9 (Ph), 142.3 (Ph), 176.8 (C=O).



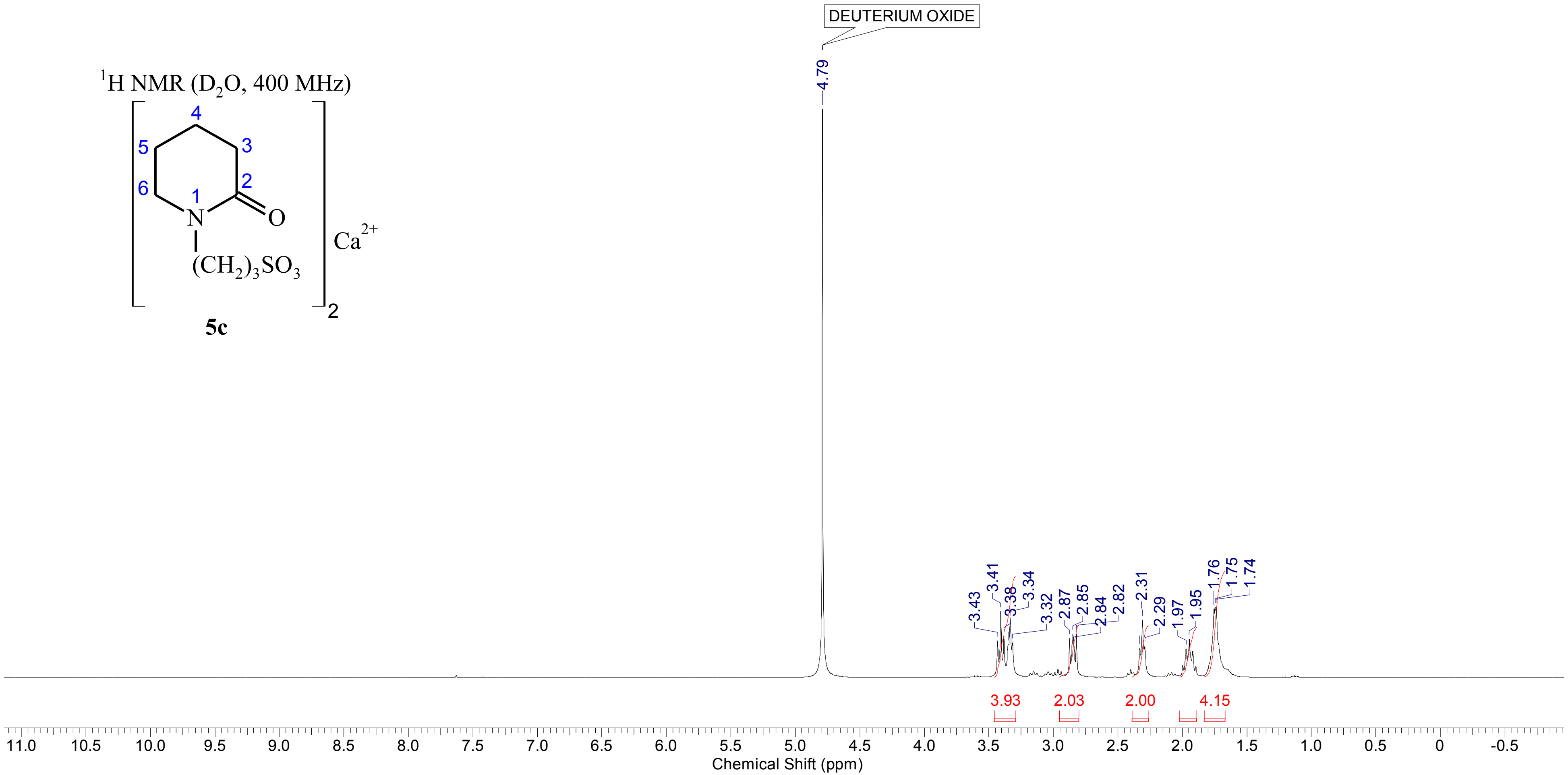
^{1}H NMR (DMSO-d$_6$, 400 MHz, δ, ppm): 1.62-1.78 (m, 6H, NCH$_2$<u>CH$_2$</u>CH$_2$SO$_3$H and C-4lactam and C-5lactam), 2.16-2.20 (m, 2H, <u>CH$_2$</u>C(O)), 2.47-2.52 (m, 2H, <u>CH$_2$</u>SO$_3$H), 3.17-3.28 (m, 4H, N<u>CH$_2$</u>CH$_2$CH$_2$SO$_3$H и C-6lactam).

Note: signal 9.64 is DMSO water acidified with a proton of the group SO$_3$H

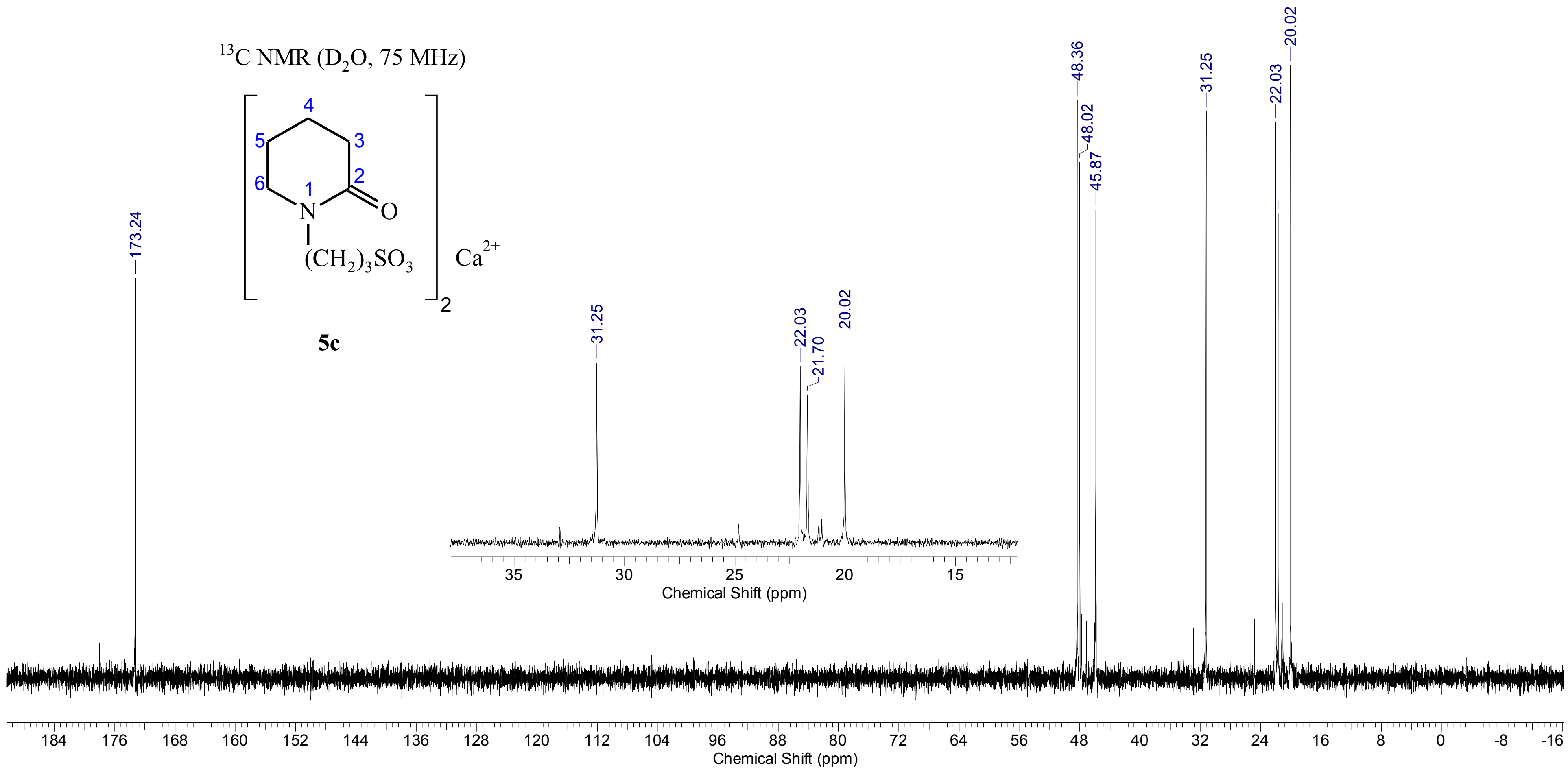


^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm): 20.8 ($NCH_2\underline{CH_2}CH_2SO_3H$), 22.7 (C-4lactam), 31.6 ($\underline{CH_2}C(O)$), 45.9 ($N\underline{CH_2}CH_2CH_2SO_3H$), 47.6 (C-6lactam), 49.1 ($\underline{CH_2}SO_3H$), 169.3 (C=O).

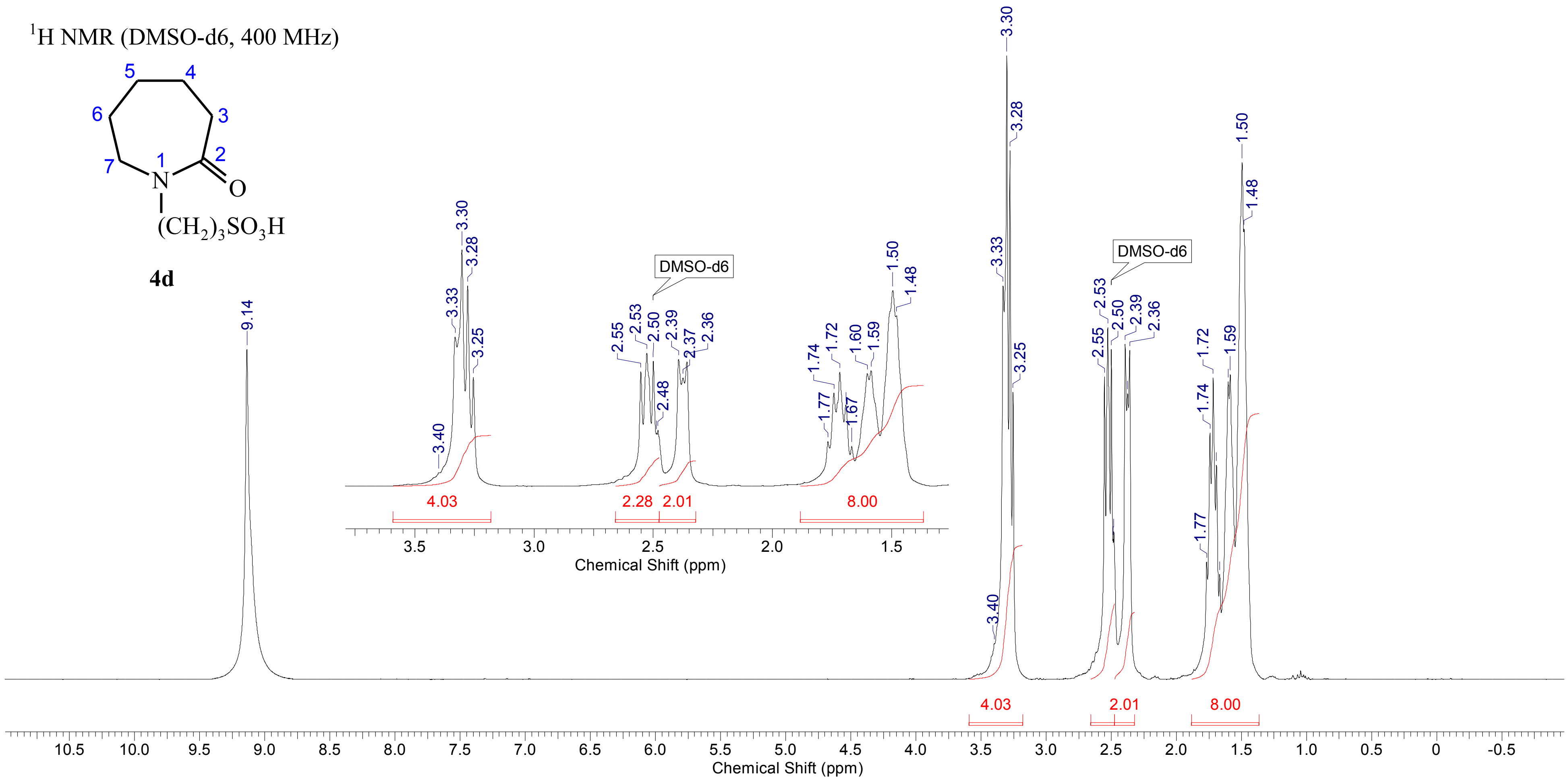
Note: the signal of one CH_2 group (C-5 lactam) is overlapped



^{1}H NMR (D_2O, 400 MHz, δ, ppm): 1.74-1.76 (m, 4H, C-4lactam and C-5lactam), 1.95-1.97 (m, 2H, $NCH_2CH_2CH_2SO_3$), 2.29-2.31 (m, 2H, $CH_2C(O)$), 2.82-2.87 (m, 2H, CH_2SO_3), 3.32-3.43 (m, 4H, $NCH_2CH_2CH_2SO_3$ and C-6lactam).

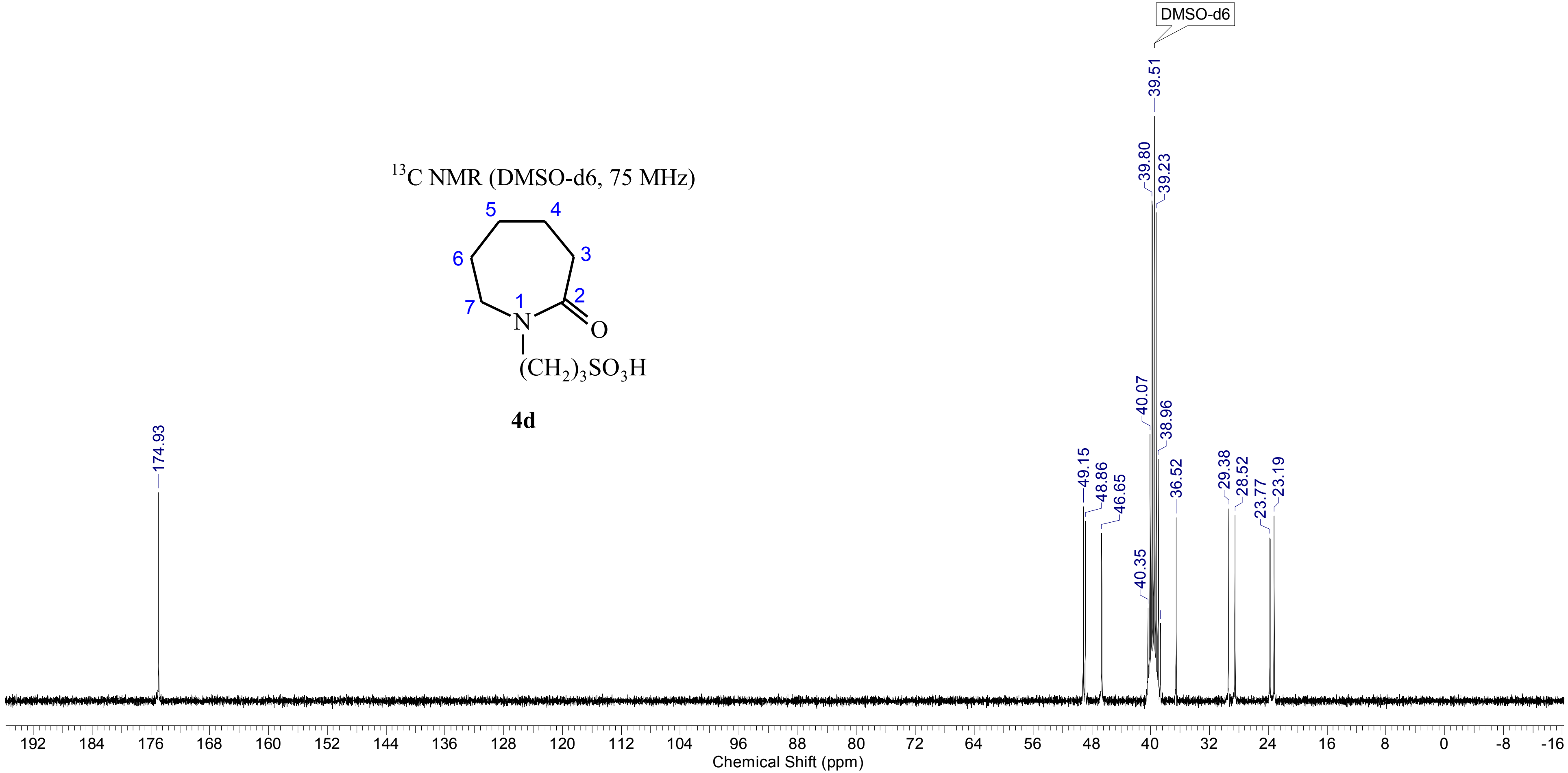


[13]C NMR (D_2O, 75 MHz, *δ*, ppm): 20.0 ($NCH_2\underline{CH_2}CH_2SO_3$), 21.7 (C-4lactam), 22.0 (C-5lactam), 31.2 ($\underline{CH_2}C(O)$), 45.9 ($N\underline{CH_2}CH_2CH_2SO_3$), 48.0 (C-6lactam), 48.4 ($\underline{CH_2}SO_3$), 173.2 (C=O).

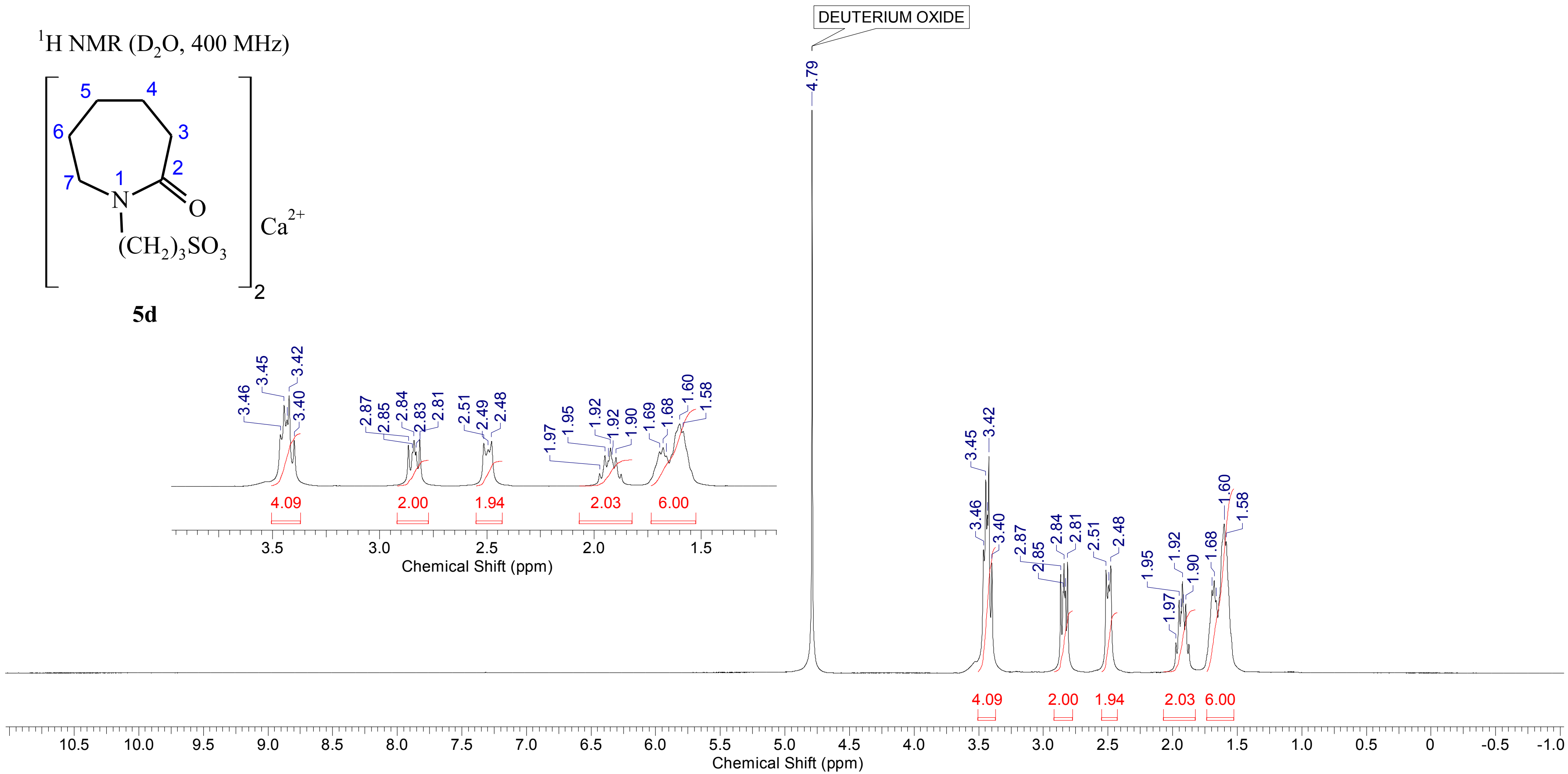


[1]H NMR (DMSO-d_6, 400 MHz, δ, ppm): 1.48-1.77 (m, 8H, $NCH_2\underline{CH_2}CH_2SO_3H$ and C-4лlactam and C-5lactam and C-6lactam), 2.36-2.39 (m, 2H, $\underline{CH_2}C(O)$), 2.48-2.55 (m, 2H, $\underline{CH_2}SO_3H$), 3.25-3.40 (m, 4H, $N\underline{CH_2}CH_2CH_2SO_3H$ and C-7lactam).

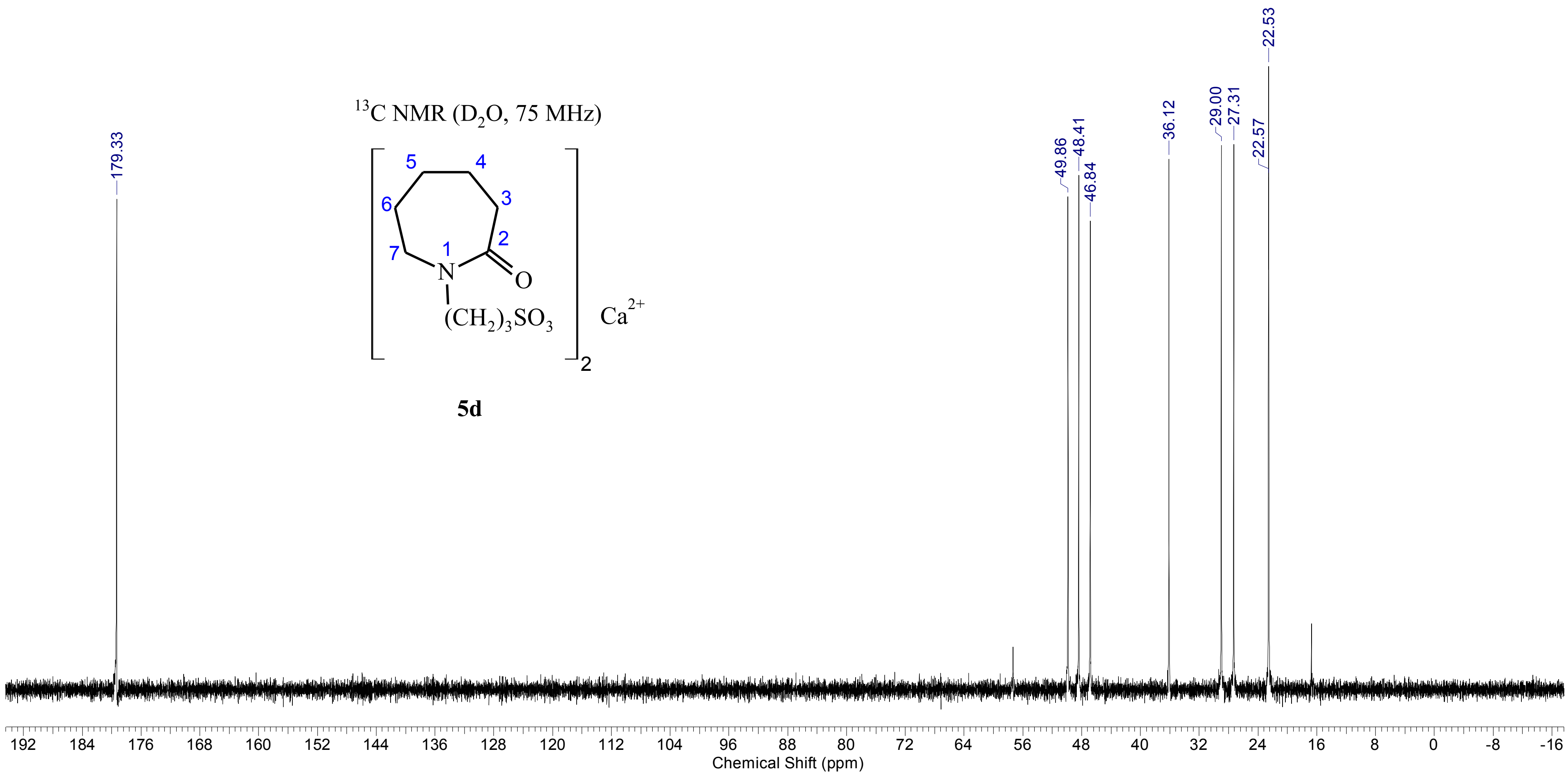
Note: signal 9.14 is DMSO water acidified with a proton of the group SO_3H



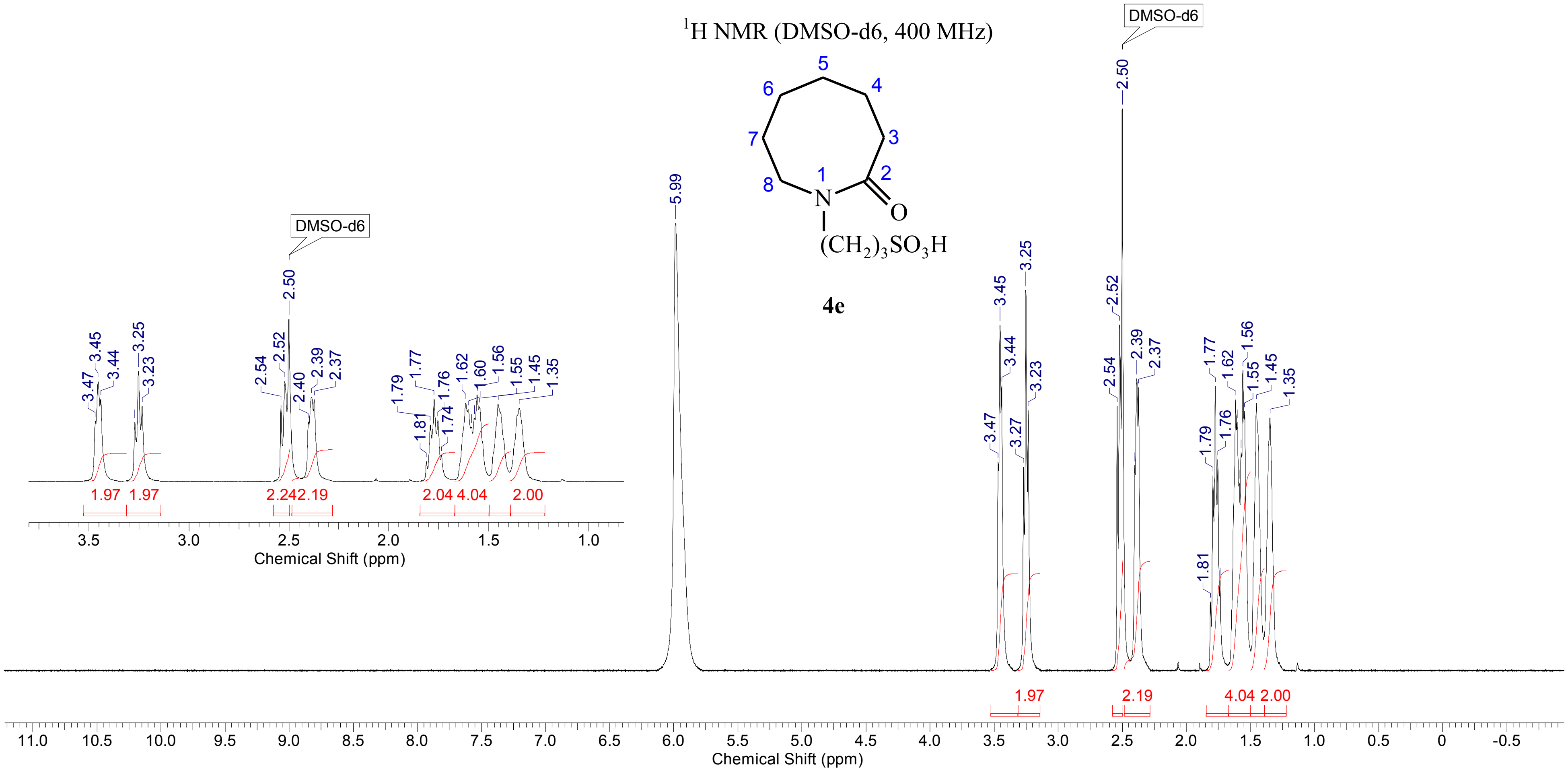
^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm): 23.2 (NCH$_2$<u>CH$_2$</u>CH$_2$SO$_3$), 23.8 (C-4lactam), 28.5 (C-5lactam), 29.4 (C-6lactam), 36.5 (<u>CH$_2$</u>C(O)), 46.6 (N<u>CH$_2$</u>CH$_2$CH$_2$SO$_3$H), 48.9 (C-7lactam), 49.1 (<u>CH$_2$</u>SO$_3$), 174.9 (C=O).



^{1}H NMR (D_2O, 400 MHz, δ, ppm): 1.58-1.69 (m, 6H, C-4lactam and C-5lactam and C-6lactam), 1.87-1.97 (m, 2H, $NCH_2\underline{CH_2}CH_2SO_3$), 2.48-2.51 (m, 2H, $\underline{CH_2}C(O)$), 2.81-2.87 (m, 2H, $\underline{CH_2}SO_3$), 3.40-3.46 (m, 4H, $N\underline{CH_2}CH_2CH_2SO_3$ and C-7lactam).

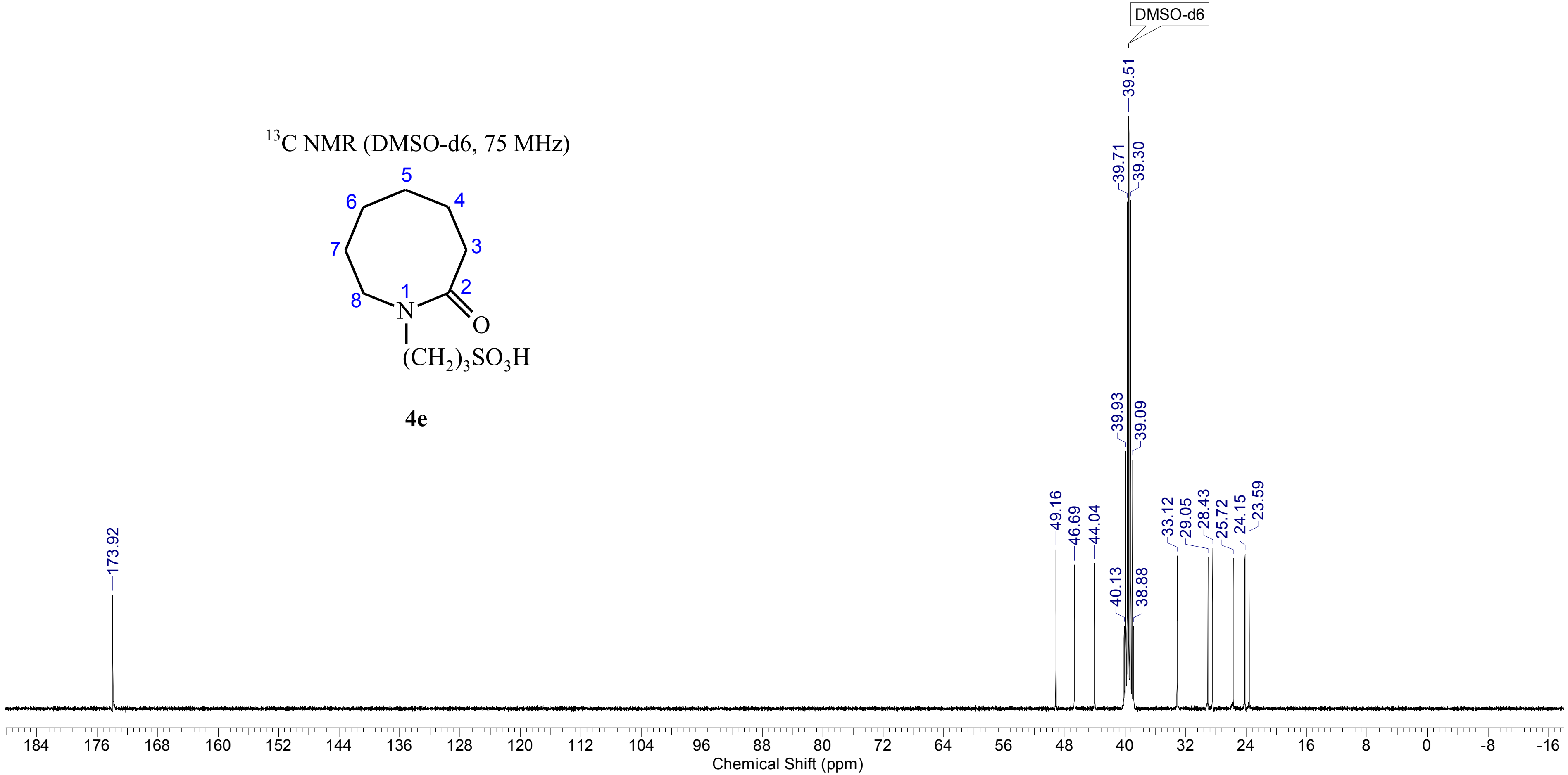


^{13}C NMR (D_2O, 75 MHz, δ, ppm): 22.5 ($NCH_2\underline{CH_2}CH_2SO_3$), 22.6 (C-4lactam), 27.3 (C-5lactam), 29.0 (C-6lactam), 36.1 ($\underline{CH_2}C(O)$), 46.8 ($N\underline{CH_2}CH_2CH_2SO_3$), 48.4 ($\underline{CH_2}SO_3$), 49.9 (C-7lactam), 179.3 (C=O).

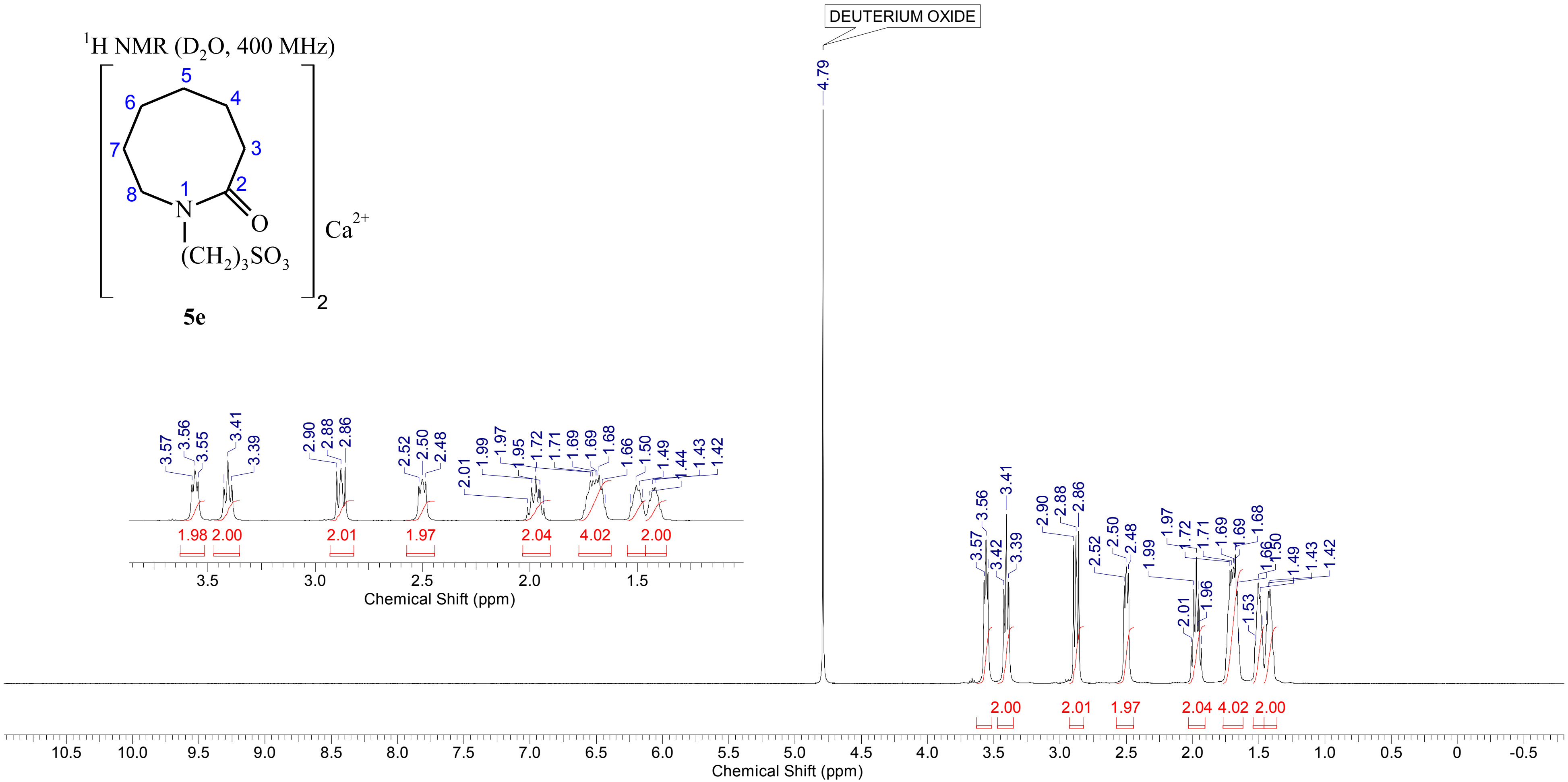


^{1}H NMR (DMSO-d$_6$, 400 MHz, δ, ppm): 1.35 (m, 2H, C-5lactam), 1.45 (m, 2H, C-4lactam), 1.55-1.62 (m, 4H, C-6lactam and C-7lactam), 1.74-1.81 (m, 2H, NCH$_2$<u>CH$_2$</u>CH$_2$SO$_3$), 2.37-2.40 (m, 2H, <u>CH$_2$</u>SO$_3$), 2.52-2.54 (m, 2H, <u>CH$_2$</u>C(O)), 3.23- 3.27 (m, 2H, N<u>CH$_2$</u>CH$_2$CH$_2$SO$_3$), 3.44-3.47 (m, 2H, C-8lactam).

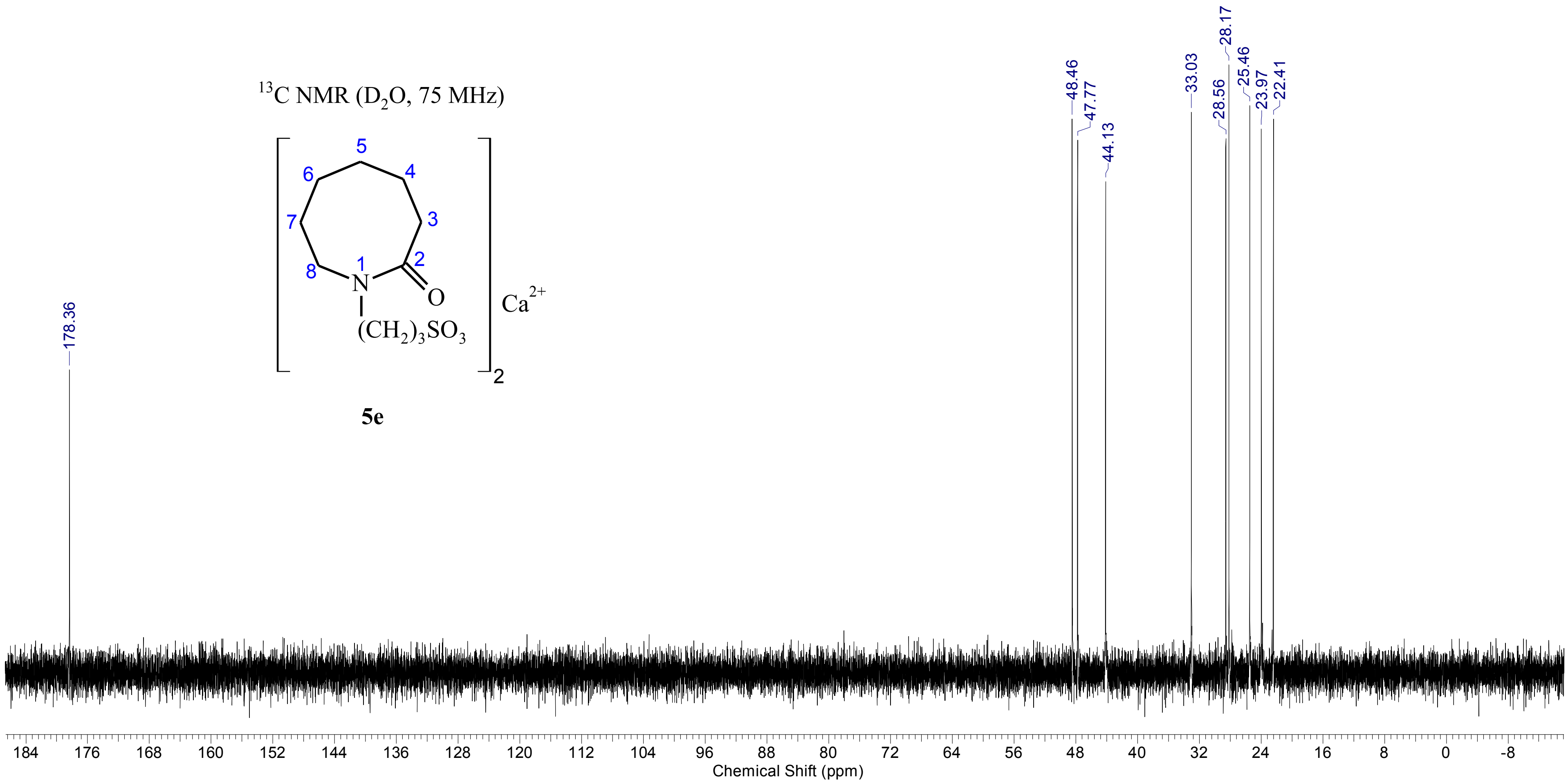
Note: signal 5.99 is DMSO water acidified with a proton of the group SO$_3$H



^{13}C NMR (DMSO-d$_6$, 75 MHz, δ, ppm): 23.6 (NCH$_2$$\underline{CH_2}CH_2SO_3$), 24.1 (C-4lactam), 25.7 (C-5lactam), 28.4 (C-6lactam), 29.0 (C-7lactam), 33.1 ($\underline{CH_2}$C(O)), 44.0 (N$\underline{CH_2}$CH$_2$CH$_2$SO$_3$), 46.7 (C-8lactam), 49.2 ($\underline{CH_2}$SO$_3$), 173.9 (C=O).



^{1}H NMR (D_2O, 400 MHz, δ, ppm): 1.42-1.44 (m, 2H, C-5lactam), 1.48-1.53 (m, 2H, C-4lactam), 1.65-1.72 (m, 4H, C-6lactam and C-7lactam), 1.93-2.01 (q, J_{HH} =7.7, 2H, $NCH_2\underline{CH_2}CH_2SO_3$), 2.48-2.52 (m, 2H, $\underline{CH_2}SO_3$), 2.86-2.90 (m, 2H, $\underline{CH_2}C(O)$), 3.41 (t, J_{HH} =7.1, 2H, $N\underline{CH_2}CH_2CH_2SO_3$), 3.56 (t, J_{HH} =5.7, 2H, C-8lactam).



^{13}C NMR (D_2O, 75 MHz, δ, ppm): 22.4 ($NCH_2\underline{CH_2}CH_2SO_3$), 23.9 (C-4lactam), 25.5 (C-5lactam), 28.2 (C-6lactam), 28.6 (C-7lactam), 33.0 ($\underline{CH_2}C(O)$), 44.1 ($N\underline{CH_2}CH_2CH_2SO_3$), 47.8 (C-8lactam), 48.5 ($\underline{CH_2}SO_3$), 178.4 (C=O).