Electronic Supplementary Information

Synthesis, antiplasmodial and antileukemia activity of dihydroartemisinin-HDAC inhibitor hybrids as multi-target drugs

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1. Supplemental Tables

Table S1. Representative concentration-effect curves for key compounds.

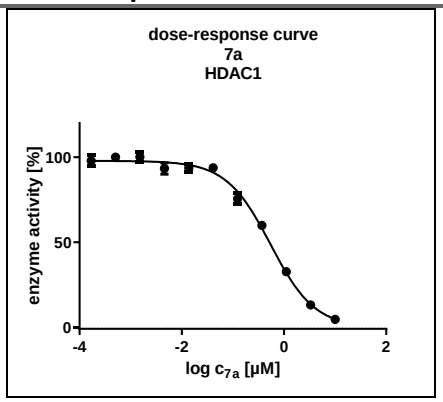
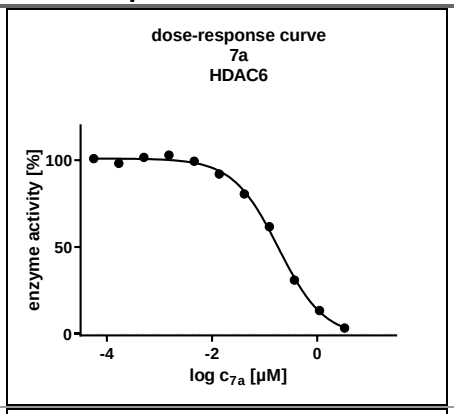
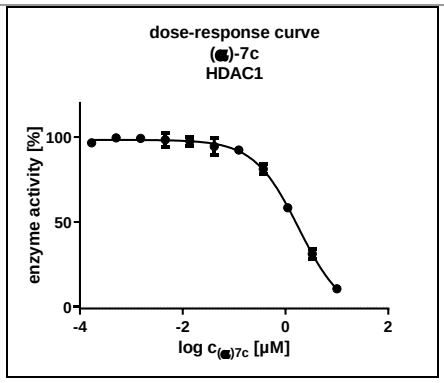
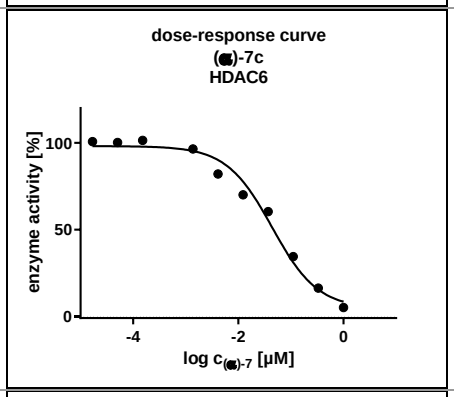
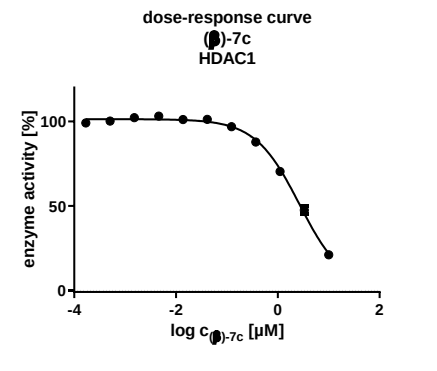
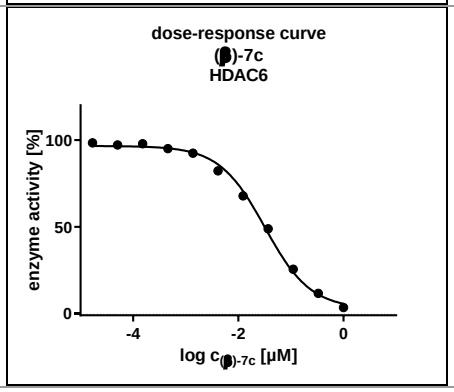
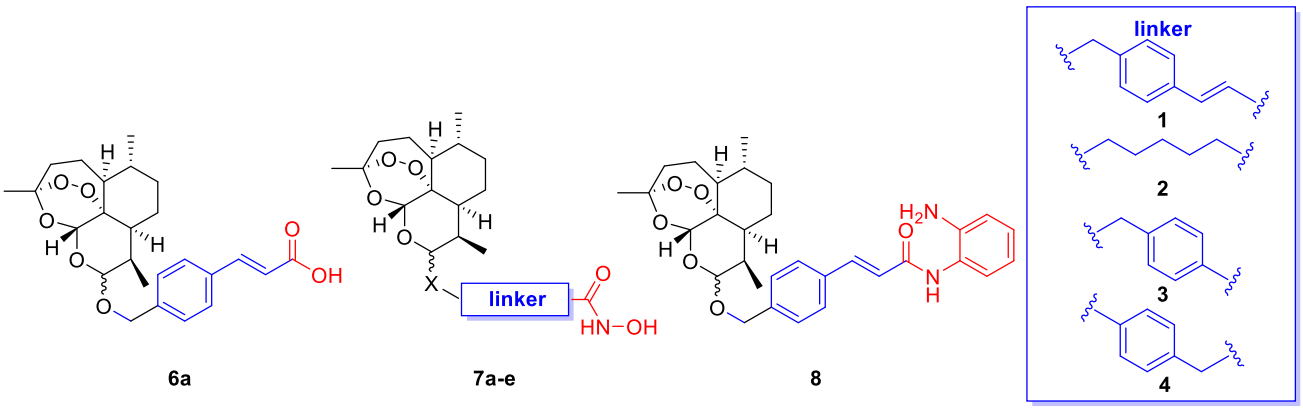
Compound	Dose-Response Curves HDAC1	Dose-Response Curves HDAC6
7a		
(α)-7c		
(β)-7c		

Table S2. Calculated LogP values, molecular weight, number of hydrogen bond donors and acceptors for the synthesized DHA-HDACi hybrids.



Compound	X	Linker	LogP[1]	Molecular Weight	H-Bond Donors[2]	H-Bond Acceptors[2]
6a	O	**1**	5.14	444.52	1	6
7a	O	**1**	4.65	459.54	2	7
7b	S	**2**	4.42	429.57	2	6
(α)-**7c**	S	**3**	5.03	449.56	2	6
(β)-**7c**	S	**3**	5.03	449.56	2	6
7d	O	**3**	4.31	433.50	2	7
(α)-**7e**	S	**4**	4.91	449.56	2	6
(β)-**7e**	S	**4**	4.91	449.56	2	6
8	O	**1**	5.58	534.65	2	7
DHA	-	-	3.15	284.35	1	5
Vorinostat	-	-	1.79	264,33	3	3

[1]Calculated by ChemDraw Professional v19.1
[2]Calculated by ChemOffice 19 MS Excel Plugin

Table S3. Selectivity indices range against healthy fibroblasts as compared to leukemia cells. Selectivity Index (SI) = (healthy fibroblast IC_{50})/(leukemia cell line IC_{50}). See Table 4 for fibroblast IC_{50} values.

Compound	K562	HL60	NALM6	HPB ALL	MOLM13
7a	21-42	5-10	42-87	5-10	29-60
(α)-7c	12-21	7-12	27-45	1.1-1.9	23-39
(β)-7c	36-43	6-8	59-71	1.2-1.4	39-47
Vorinostat	12-16	15-21	5-7	6-8	16-22

2. Docking of (α)-7c and (β)-7c

The crystal structure of human HDAC6 (PDB: 5EDU [1]) was obtained from the Protein Data Bank (PDB, www.rcsb.org). Chain A, corresponding to the Maltose-binding periplasmic protein and trichosatin A were deleted. All heteroatom records were removed, except for the metal ions (one zinc atom and two potassium atoms). Phenylhydroxamates with sterically demanding cap groups are known to bind via a Zn^{2+}-bound water molecule [1]. Considering this, a water molecule was introduced. Coordinates were identified from the aligned CD2-Domain of zHDAC6 (PDB: 6CGP [2]). The structure was optimized to the closest local energy minimum using RosettaRelax with coordinate constraints on the backbone and metal ion restraints [3]. Ligand input files (α)-**7c** and (β)-**7c** were created with ChemDraw. An initial 3D conformer with hydrogen atoms was constructed in Chem3D and energetically minimized using the MM2 force field, followed by the production of an ensemble of 1000 low-energy conformers with BCL:ConformerGenerator [4]. One conformer was placed in the binding pocket of HDAC6. A constraint file was constructed using the known distance of the zinc binding of trichostatin A [1]. Ligand docking was performed with RosettaLigand for an initial 5000 models. Those models were clustered according to their similarity in their binding mode. The depicted models are the best scoring models from the highest populated clusters [5–7].

Rosetta version 3.12 was used. The following commands were executed throughout the modeling process:

- Relax input starting structures:

```
rosetta/source/bin/relax.linuxgccrelease -s 5EDU_prepared.pdb rosetta/source/database/
-constrain_relax_to_start_coords -in:auto_setup_metals -nstruct 25 -out:prefix relaxed_
-ignore_unrecognized_res -overwrite -ignore_waters False
```

- Options for RosettaLigand:

```
-in:file:extra_res_fa ligand.params
-packing
        -ex1
        -ex2
        -no_optH false
        -flip_HNQ true
        -ignore_ligand_chi true


-parser
        -protocol dock.xml
```

-mistakes

-restore_pre_talaris_2013_behavior true

-constraints:cst_file constraint_5EDU_hydroxamicacid.cst

-out:path:all ../output/

- RosettaScripts protocol for executing RosettaLigand:

```
<ROSETTASCRIPTS>
        <SCOREFXNS>
            <ScoreFunction name="ligand_soft_rep" weights="ligand_soft_rep">
            </ScoreFunction>
            <ScoreFunction name="hard_rep" weights="ligand">
            </ScoreFunction>
        </SCOREFXNS>
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            <LigandArea name="inhibitor_final_sc" chain="X" cutoff="6.0"
            add_nbr_radius="true" all_atom_mode="false"/>
            <LigandArea name="inhibitor_final_bb" chain="X" cutoff="7.0"
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            <InterfaceBuilder name="side_chain_for_final" ligand_areas="inhibitor_final_sc"/>
            <InterfaceBuilder name="backbone"
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        <MOVEMAP_BUILDERS>
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            <MoveMapBuilder name="final"
            sc_interface="side_chain_for_final"bb_interface="backbone"minimize_water="false"\
            >
        </MOVEMAP_BUILDERS>
        <SCORINGGRIDS ligand_chain="X" width="15">
```

```
        <ClassicGrid grid_name="classic" weight="1.0"/>
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        <MOVERS>
            <StartFrom name="startFrom" chain="X">
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            </StartFrom>
            <ConstraintSetMover name="constraint" add_constraints="true"cst_file="./
            constraint_5EDU_hydroxamicacid.cst"/>
            <Transform name="transform" chain="X" box_size="7.0" move_distance="0.2"
            angle="20" cycles="500" repeats="1" temperature="5"/>
            <HighResDocker name="high_res_docker" cycles="6"
            repack_every_Nth="3"scorefxn="ligand_soft_rep"movemap_builder="docking"/>
            <FinalMinimizer name="final" scorefxn="hard_rep" movemap_builder="final"/>
            <ClearConstraintsMover name="clearconstraints"/>
            <InterfaceScoreCalculator name="add_scores" chains="X" scorefxn="hard_rep"/>
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        <PROTOCOLS>
            <Add mover_name="startFrom"/>
            <Add mover_name="constraint"/>
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            <Add mover_name="add_scores"/>
        </PROTOCOLS>
</ROSETTASCRIPTS>
```
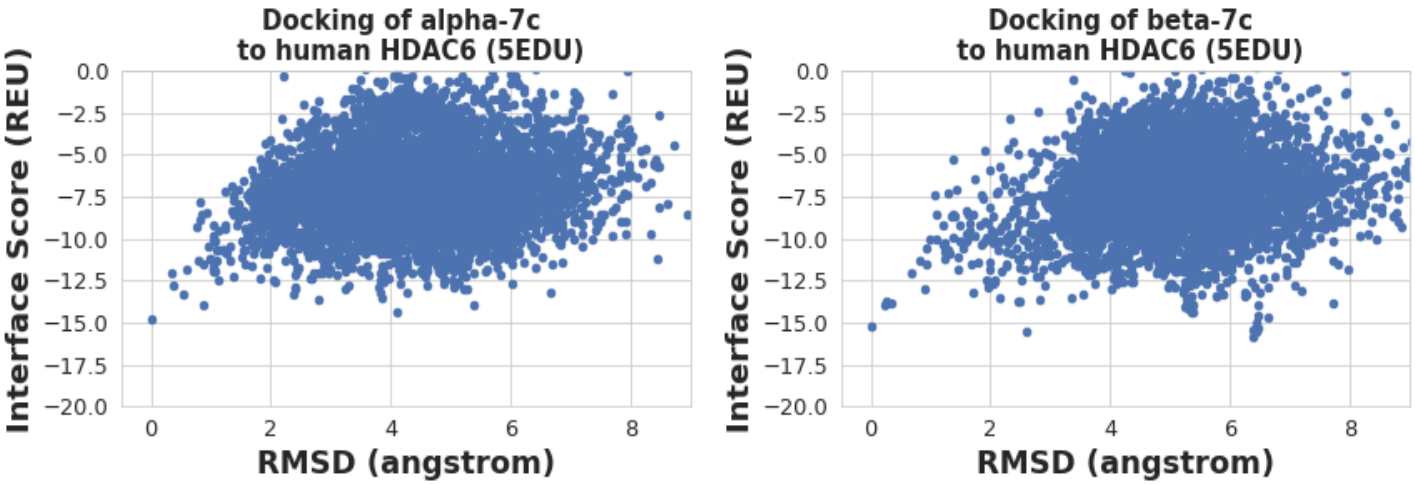

- Executing RosettaLigand:

```
cat ./listof_pdbs_5EDU | parallel -j 10 bash run_dock_parallel.sh
```

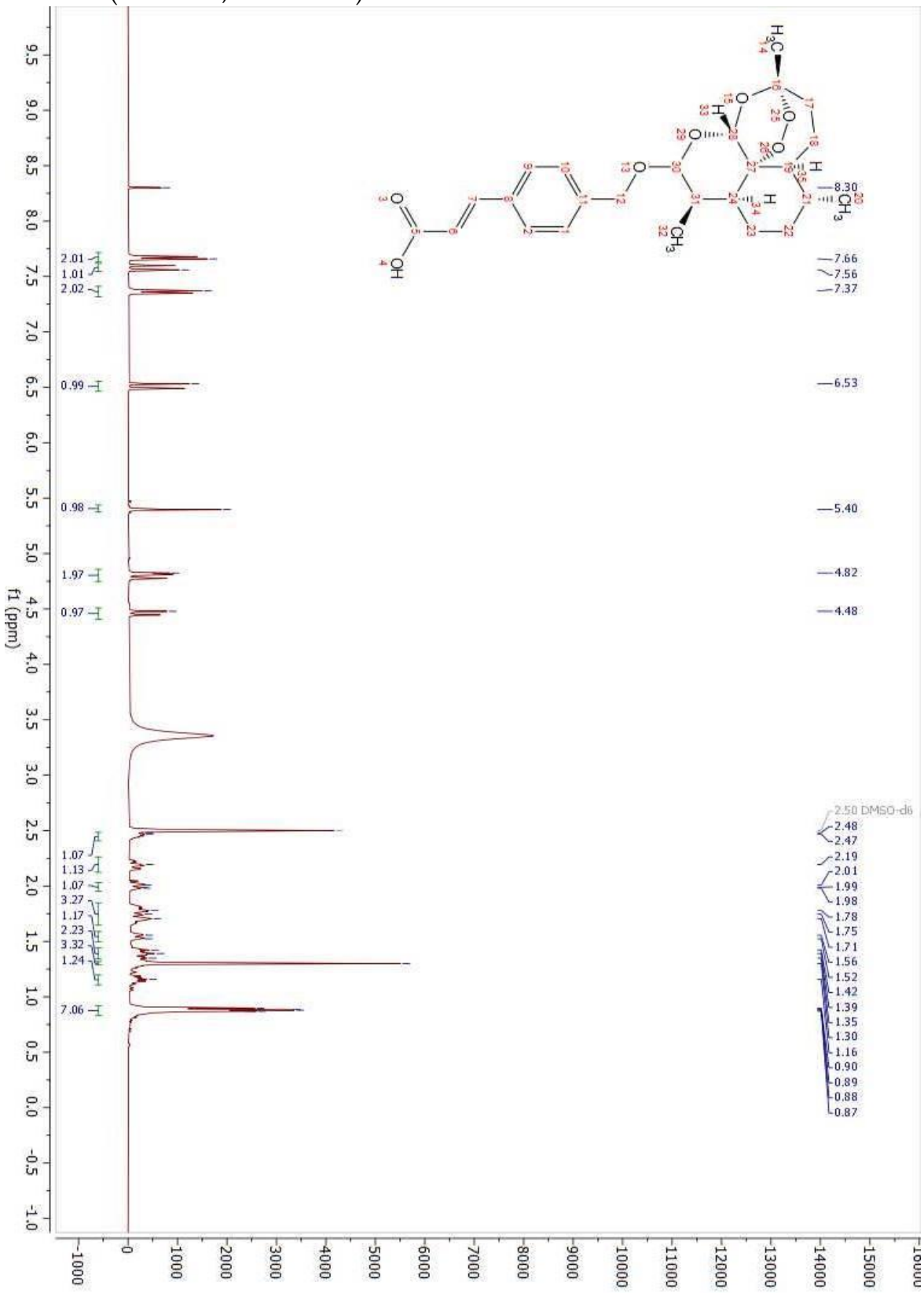
Ligand RMSD vs Interface Score plots of



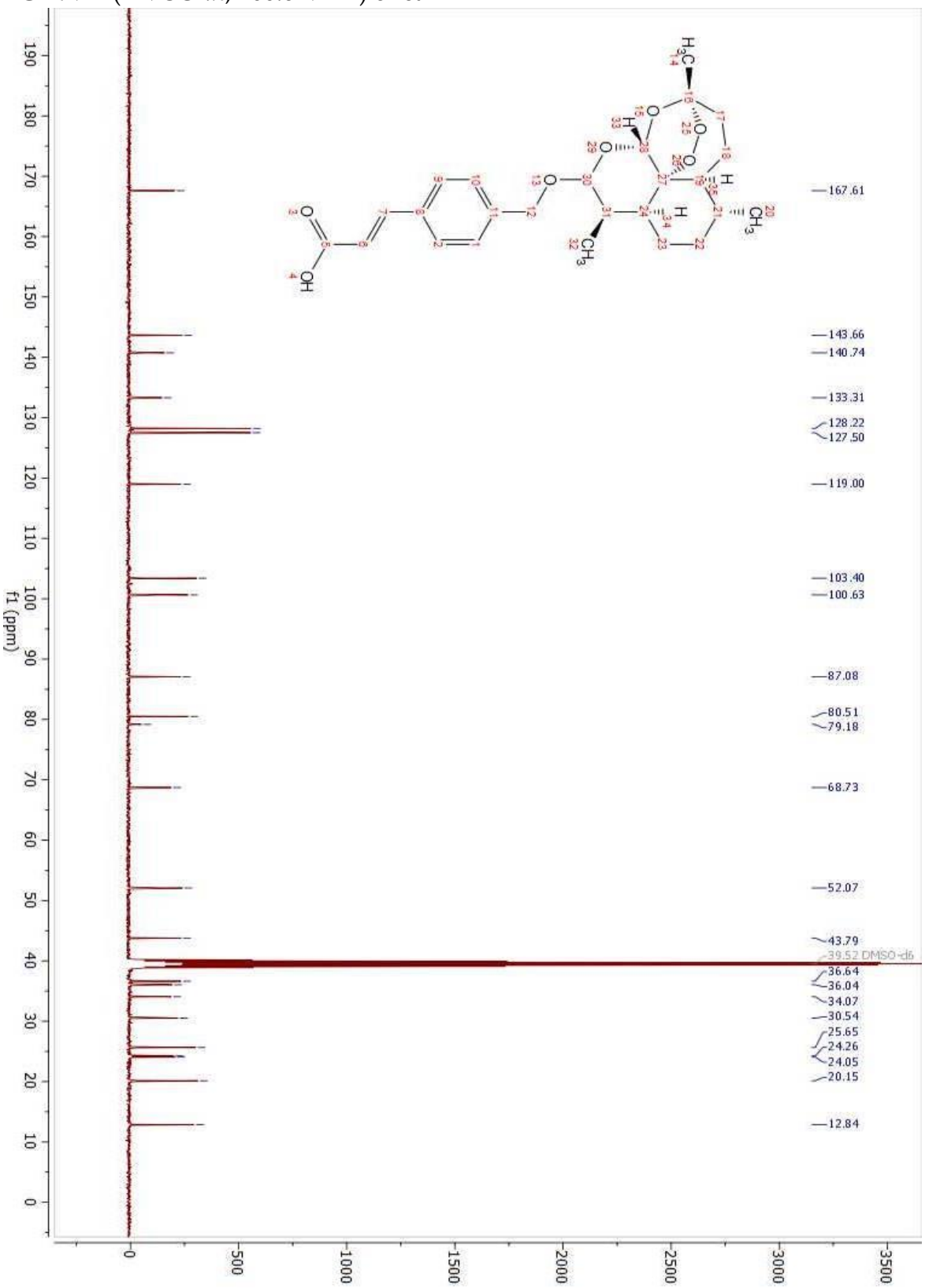
Ligand RMSD vs Interface Score plots for (α)-**7c** and (β)-**7c**. It was plotted against the models with the best scoring from the highest populated clusters.

3. ^{1}H and ^{13}C NMR spectra

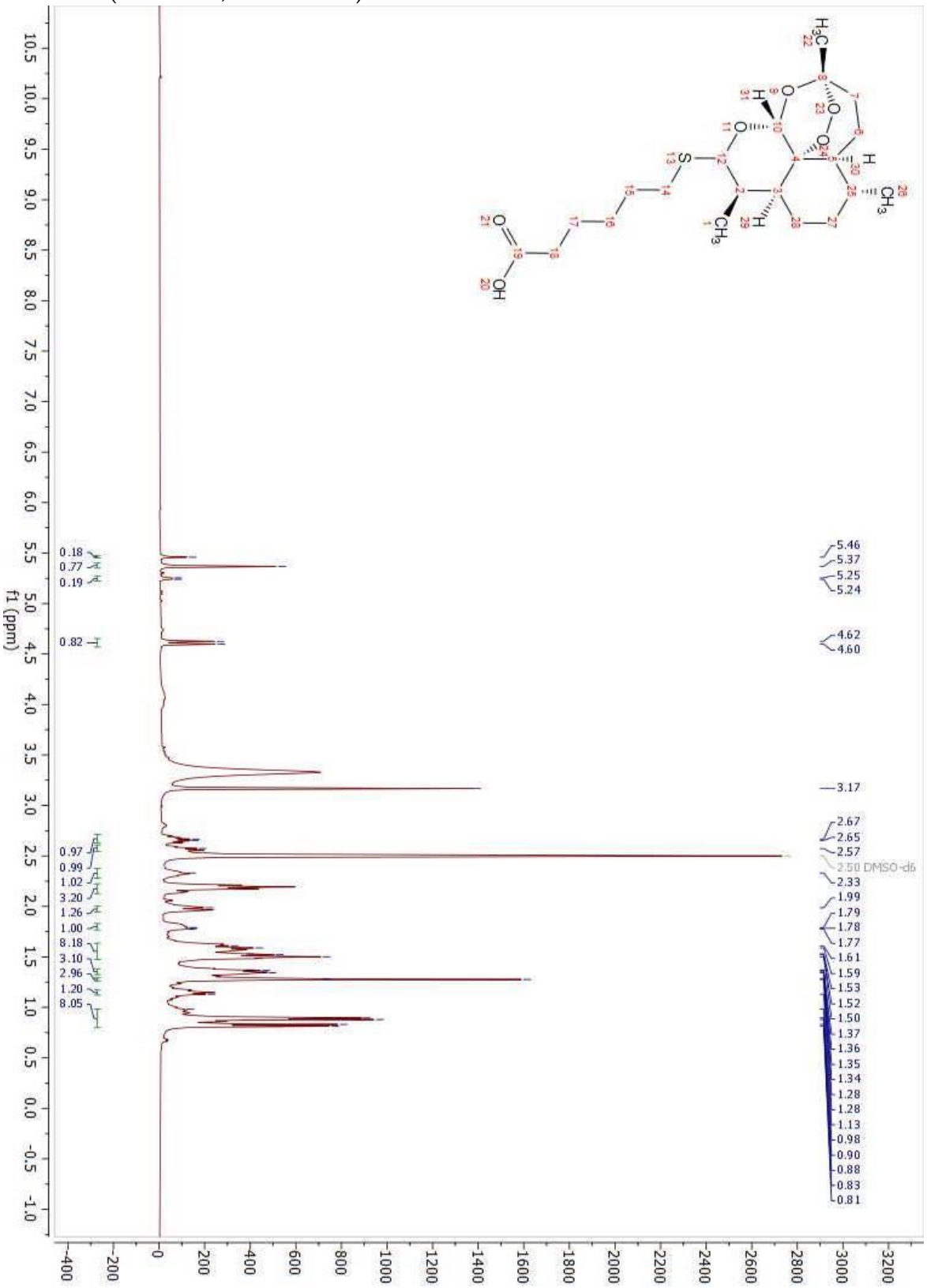
^{1}H-NMR (DMSO-d_6, 400.2 MHz) of **6a**



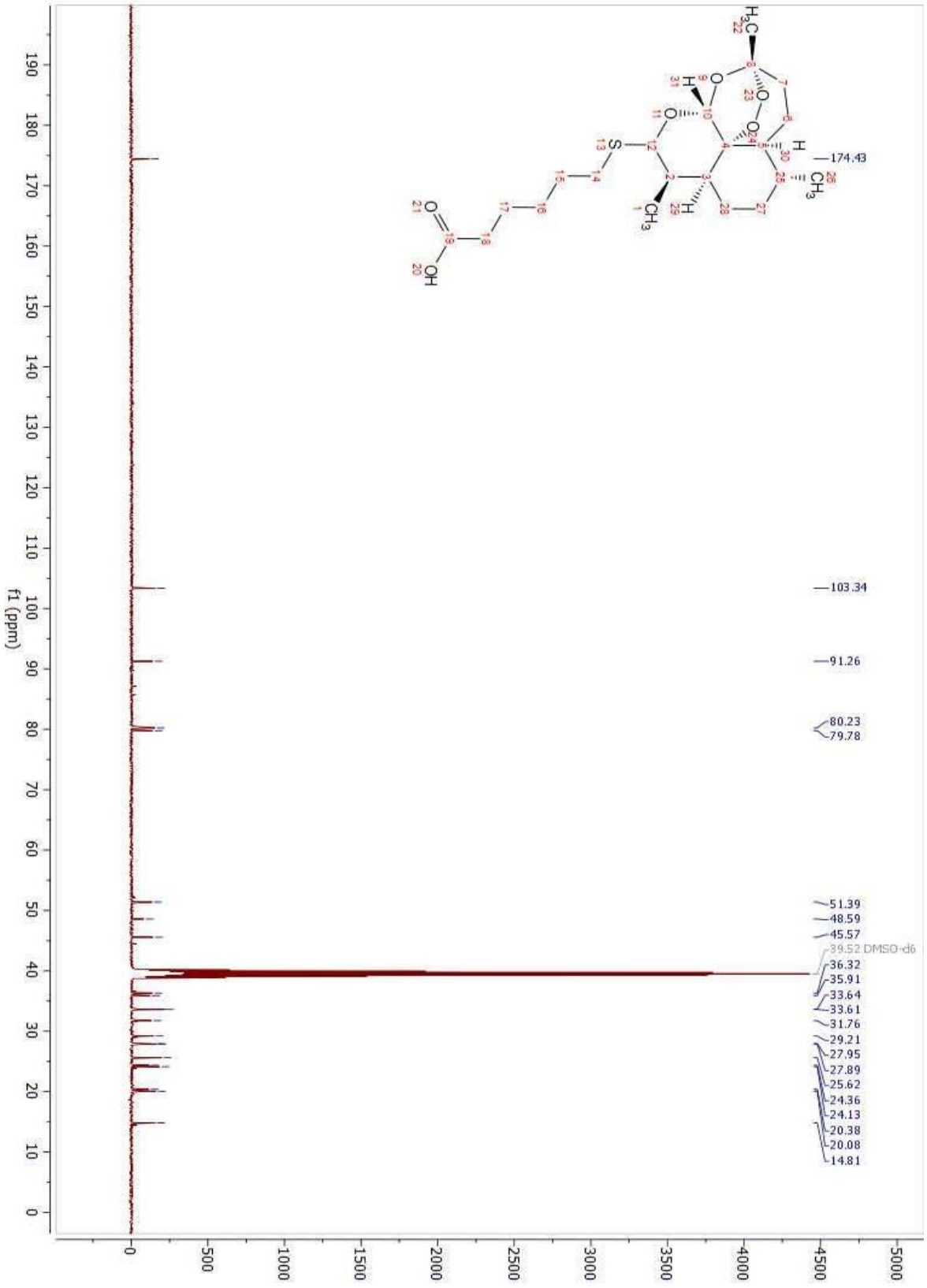
^{13}C-NMR (DMSO-d_6, 100.6 MHz) of **6a**



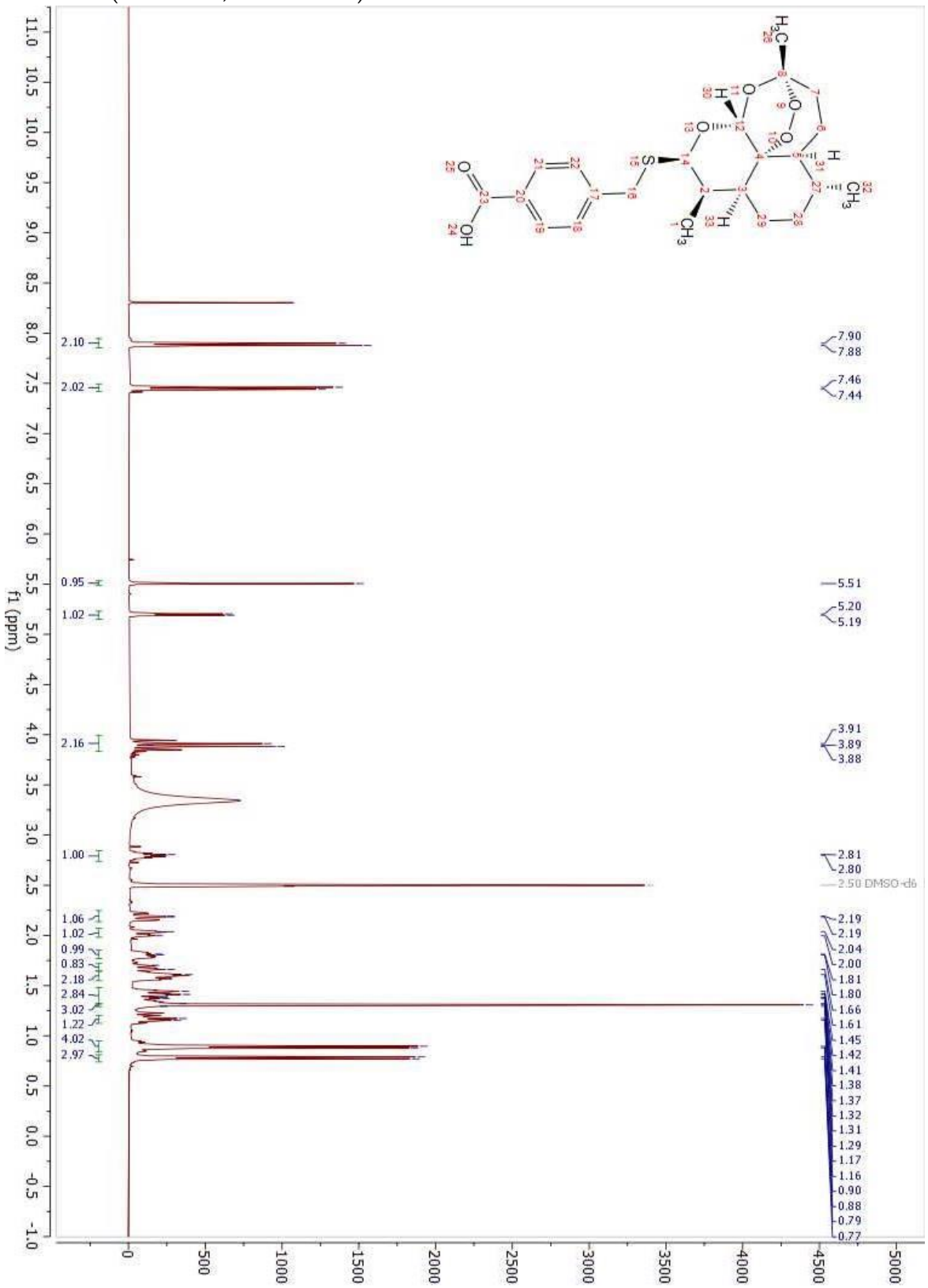
[1]H-NMR (DMSO-*d*$_6$, 400.2 MHz) of **6b**



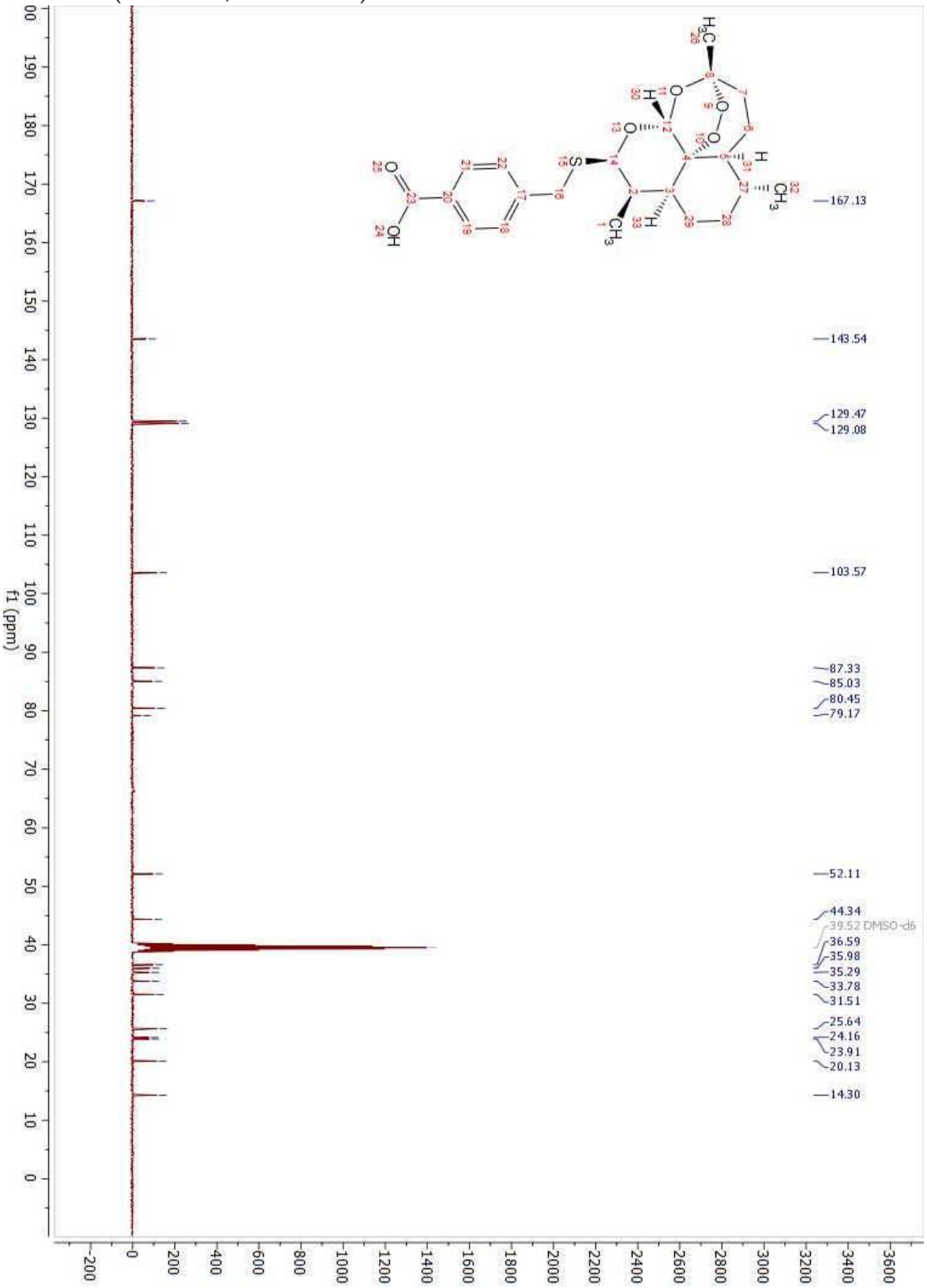
^{13}C-NMR (DMSO-d_6, 100.6 MHz) of **6b**



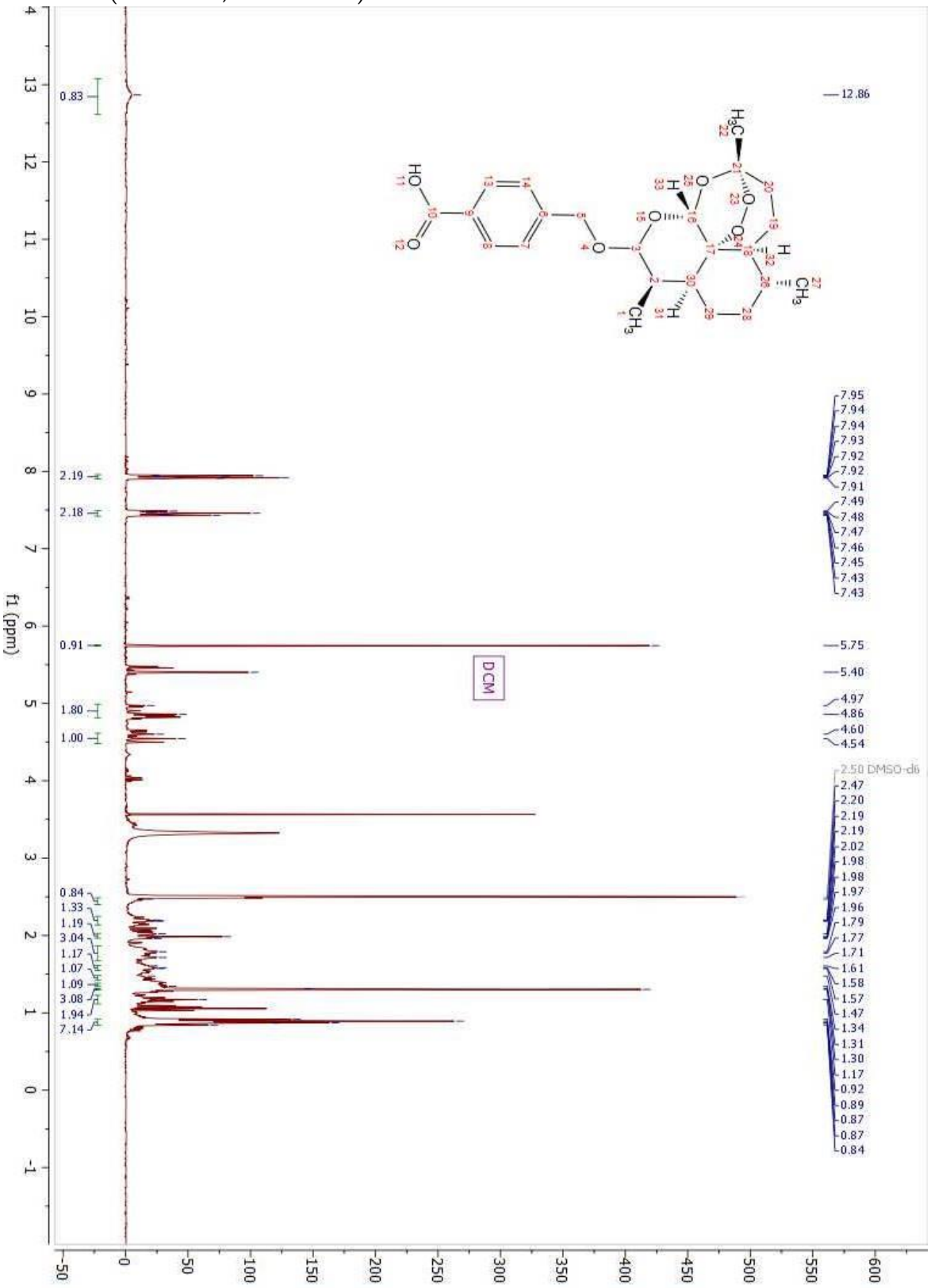
[1]H-NMR (DMSO-d_6, 400.2 MHz) of **6c**



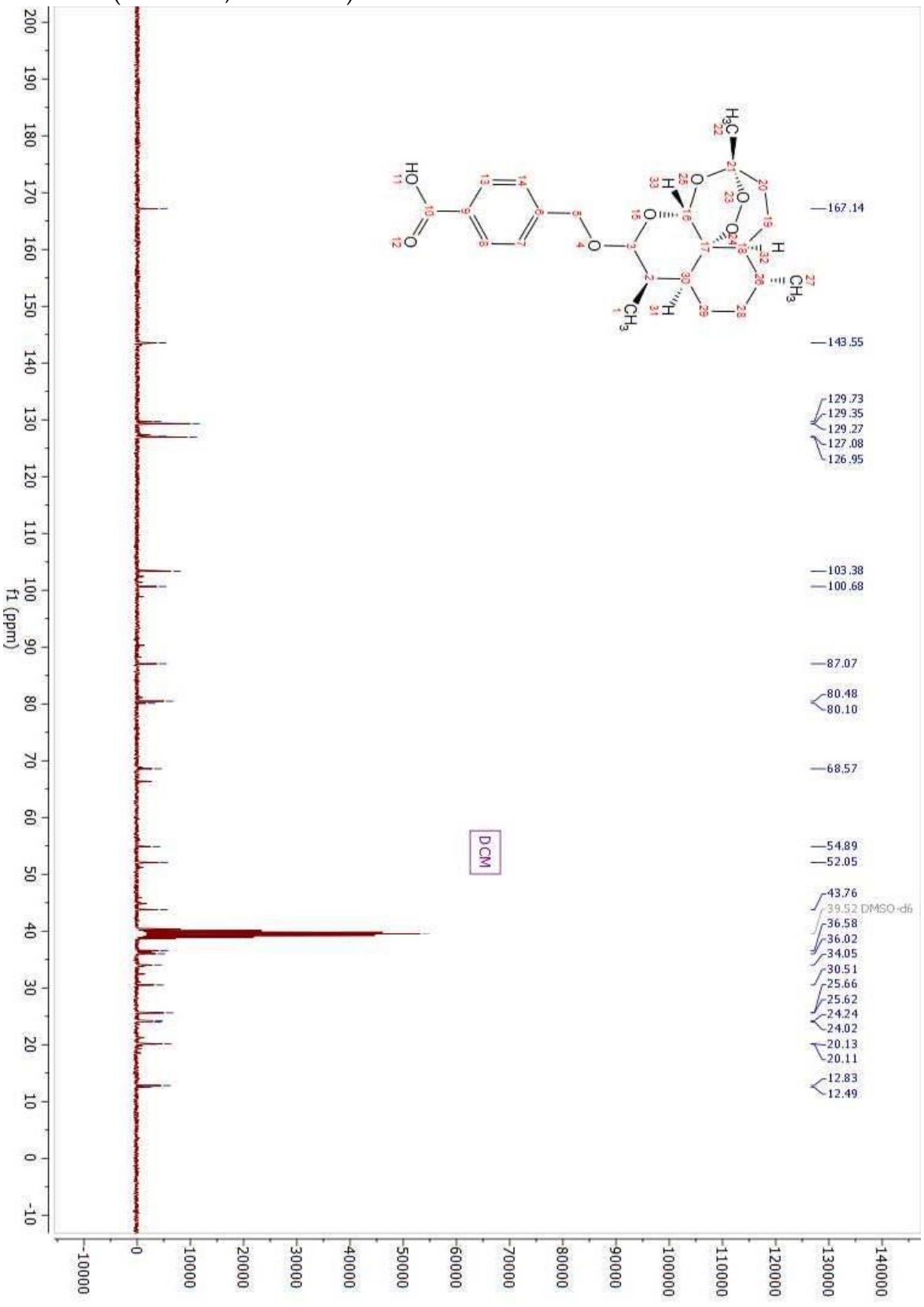
^{13}C-NMR (DMSO-d_6, 100.6 MHz) of **6c**



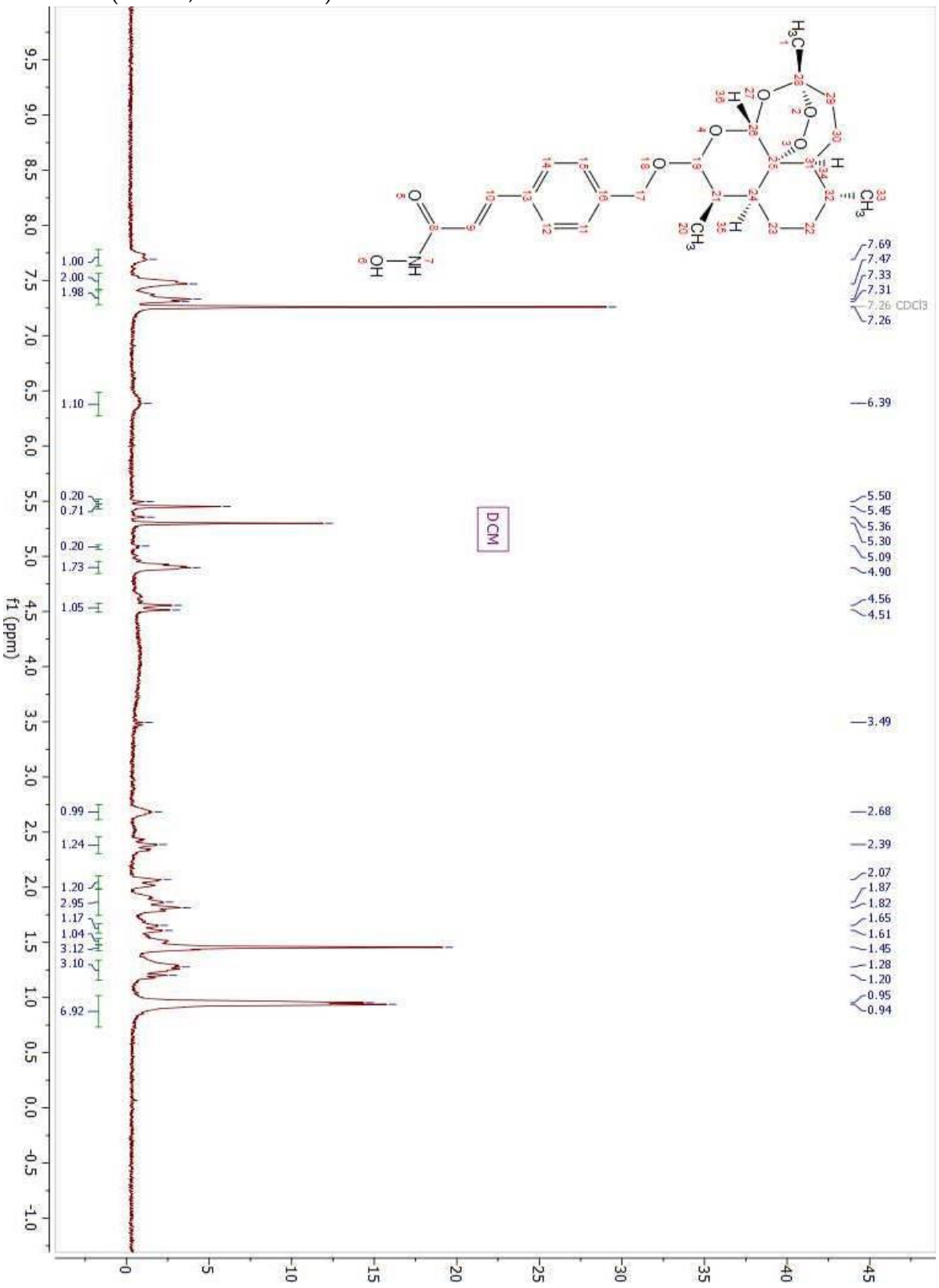
^{1}H-NMR (DMSO-d_6, 300.1 MHz) of **6d**



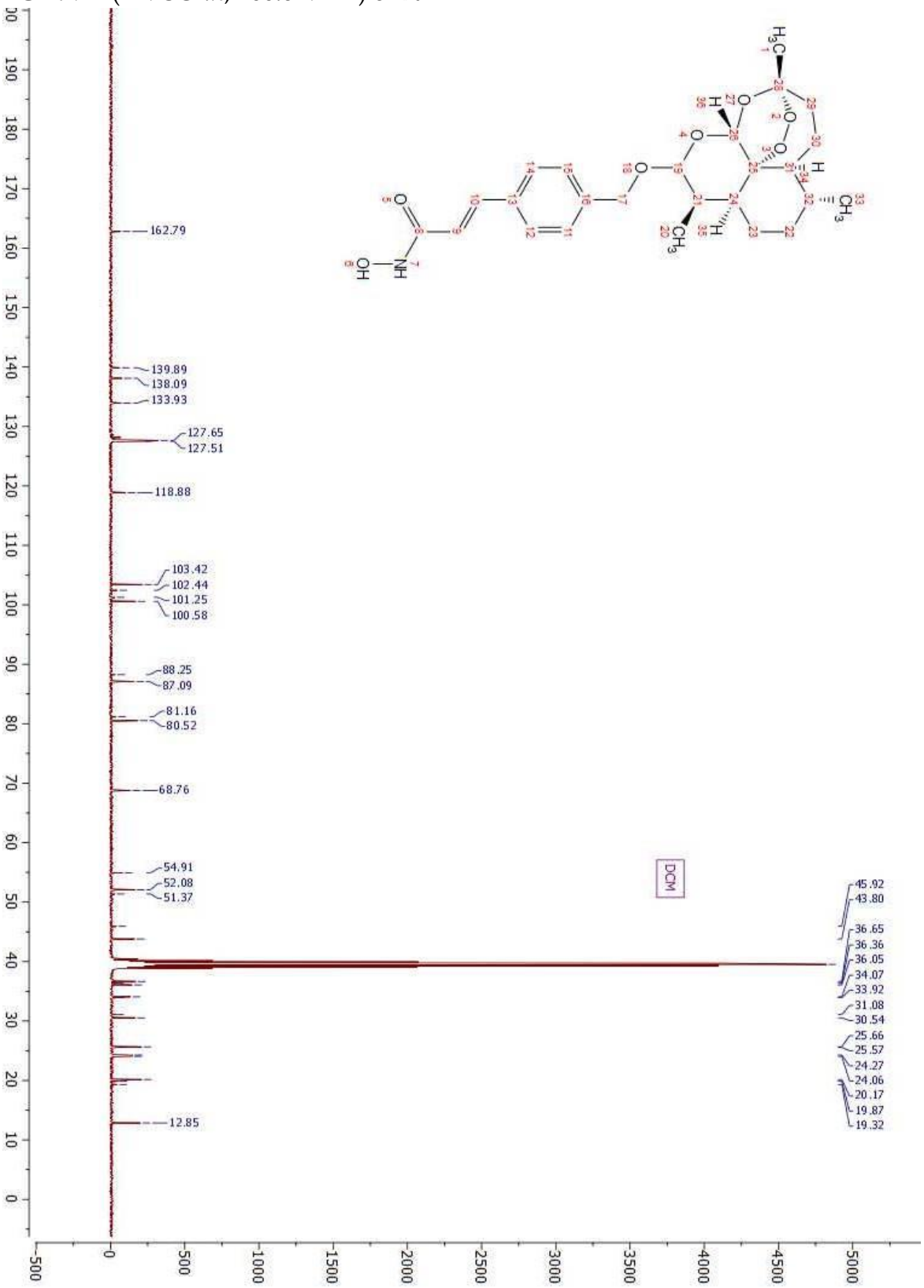
^{13}C-NMR (DMSO-d_6, 75.5 MHz) of **6d**



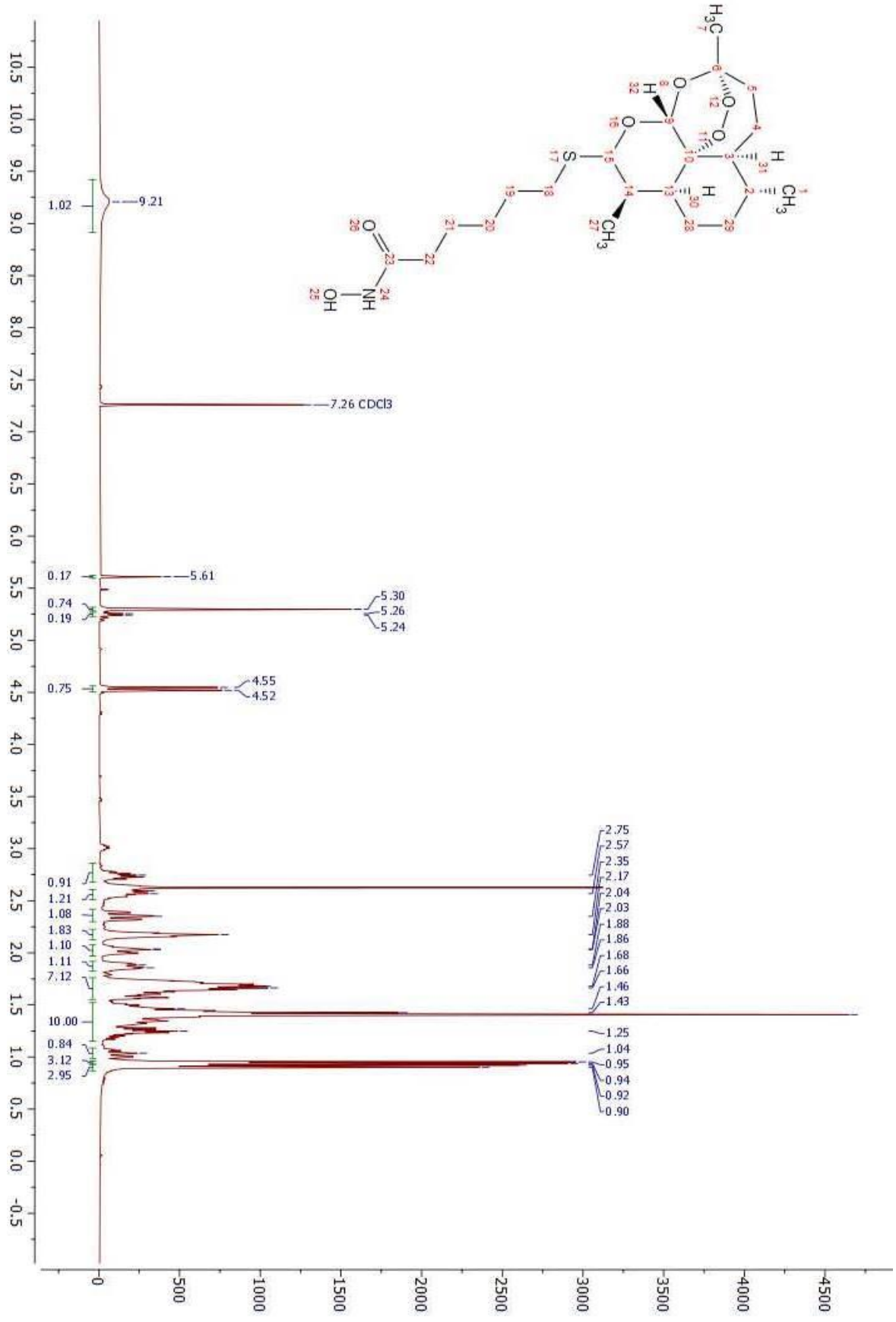
^{1}H-NMR ($CDCl_3$, 300.1 MHz) of **7a**



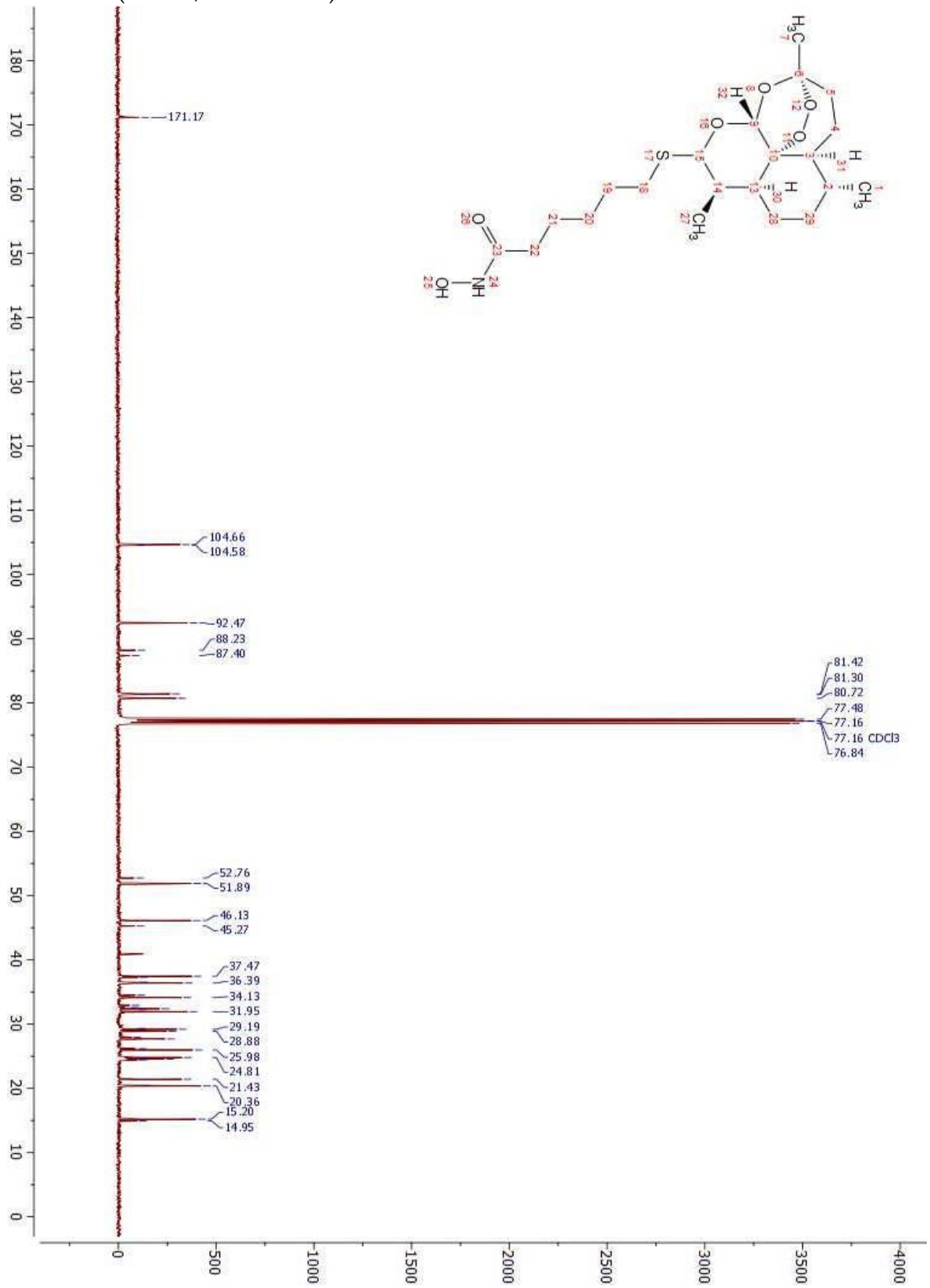
^{13}C-NMR (DMSO-d_6, 100.6 MHz) of **7a**



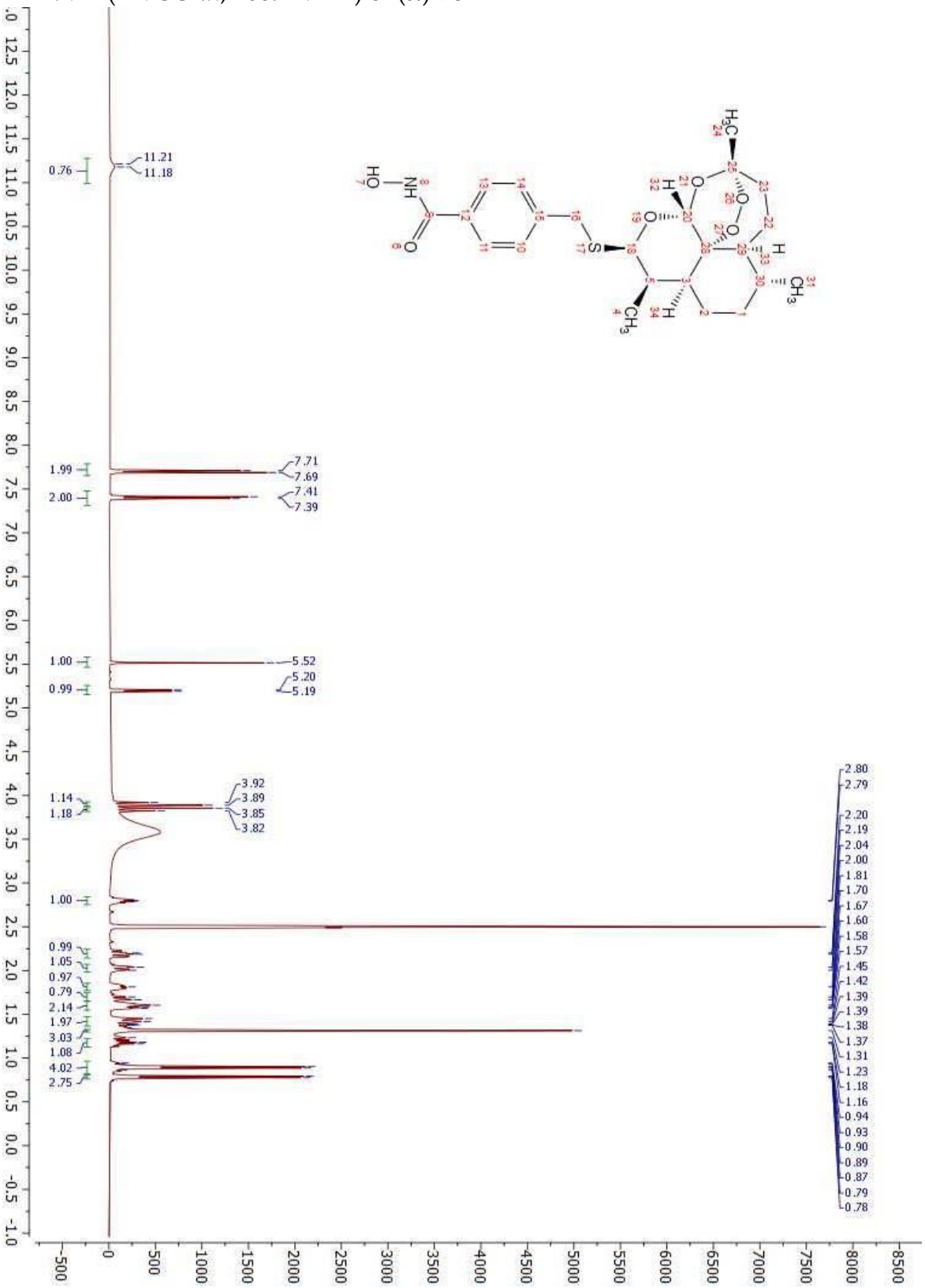
^{1}H-NMR ($CDCl_3$, 400.2 MHz) of **7b**



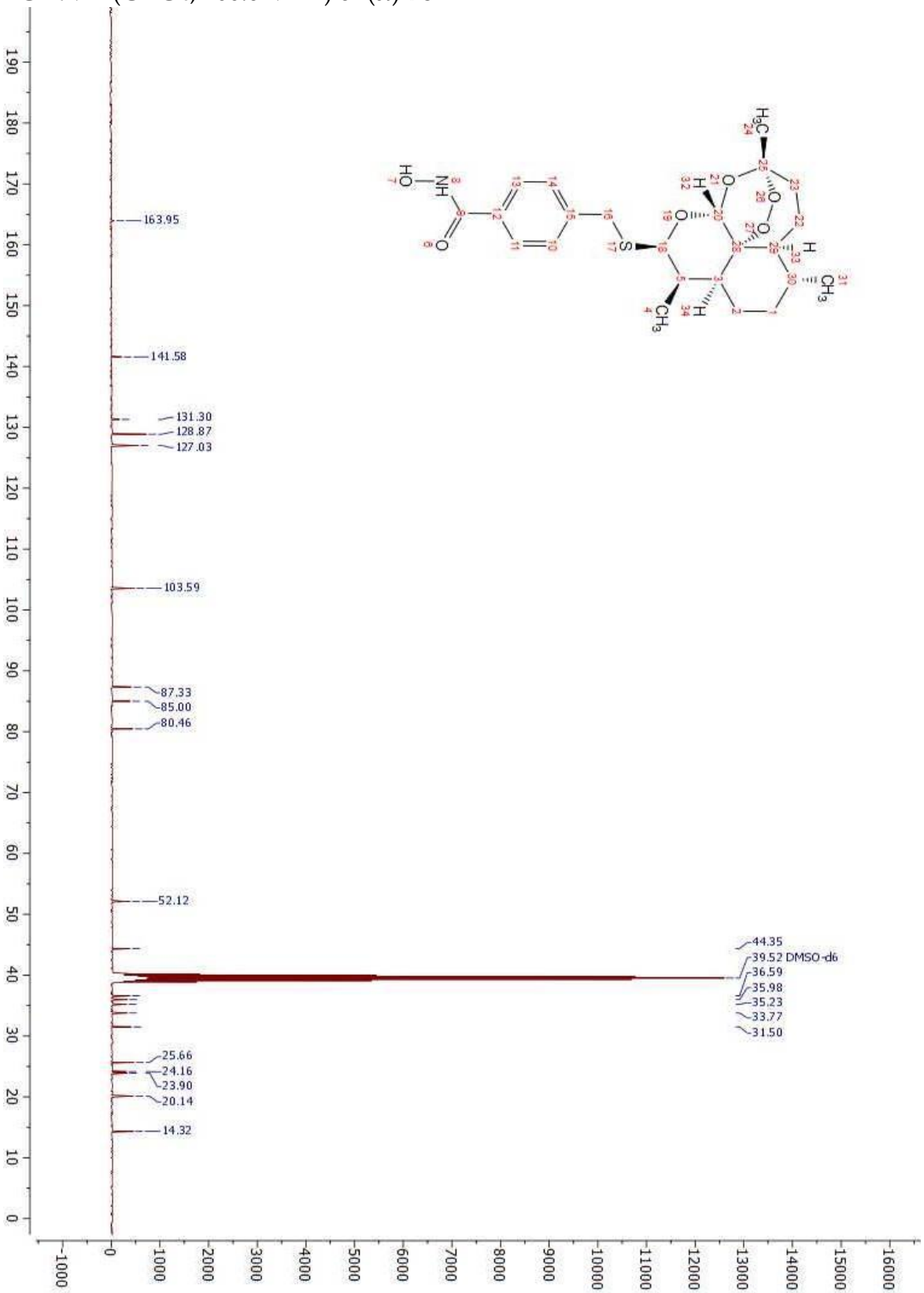
^{13}C-NMR ($CDCl_3$, 100.6 MHz) of **7b**



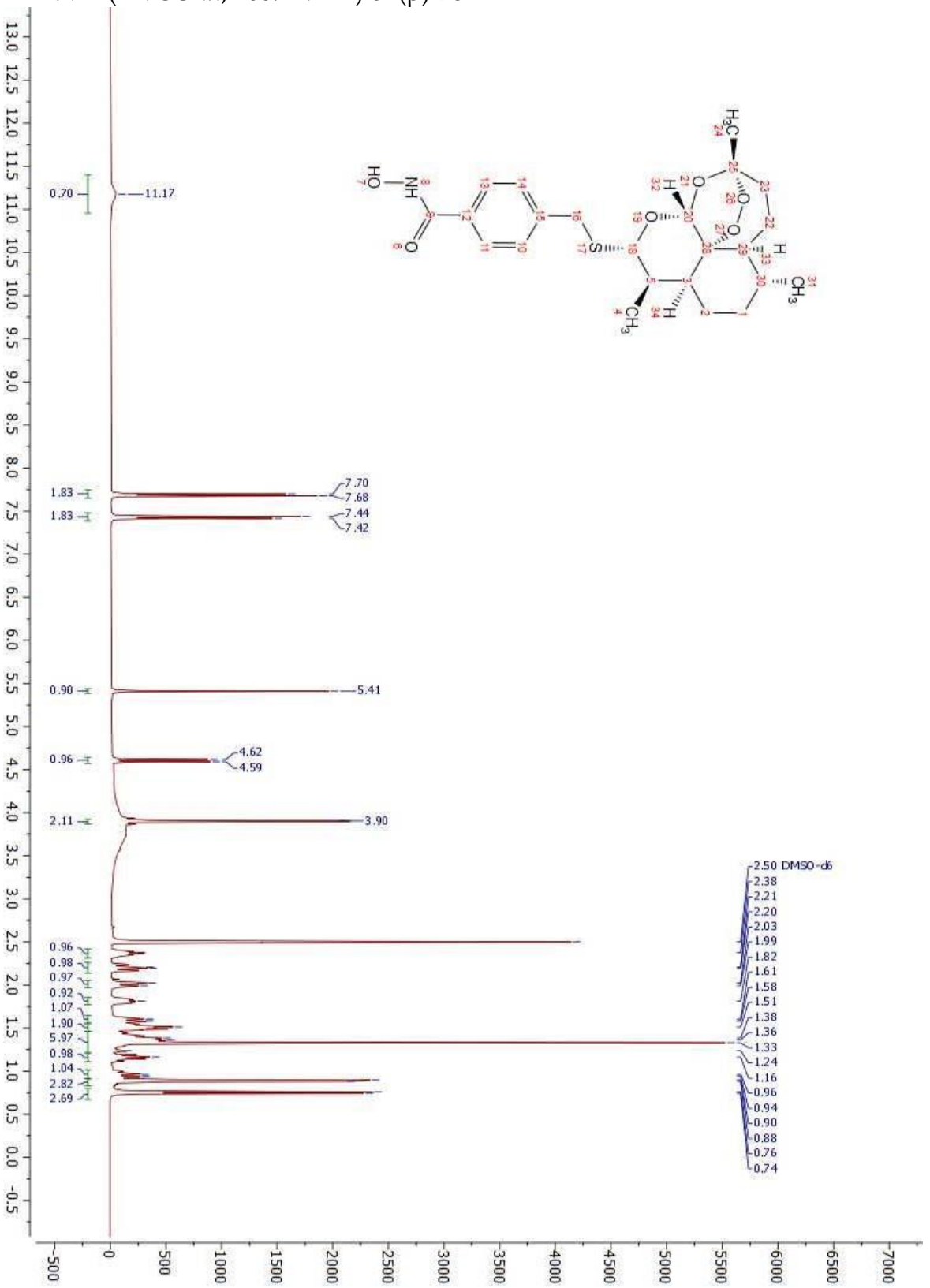
^{1}H-NMR (DMSO-d_6, 400.2 MHz) of (α)-**7c**



^{13}C-NMR ($CDCl_3$, 100.6 MHz) of (α)-**7c**



^{1}H-NMR (DMSO-d_6, 400.2 MHz) of (β)-**7c**



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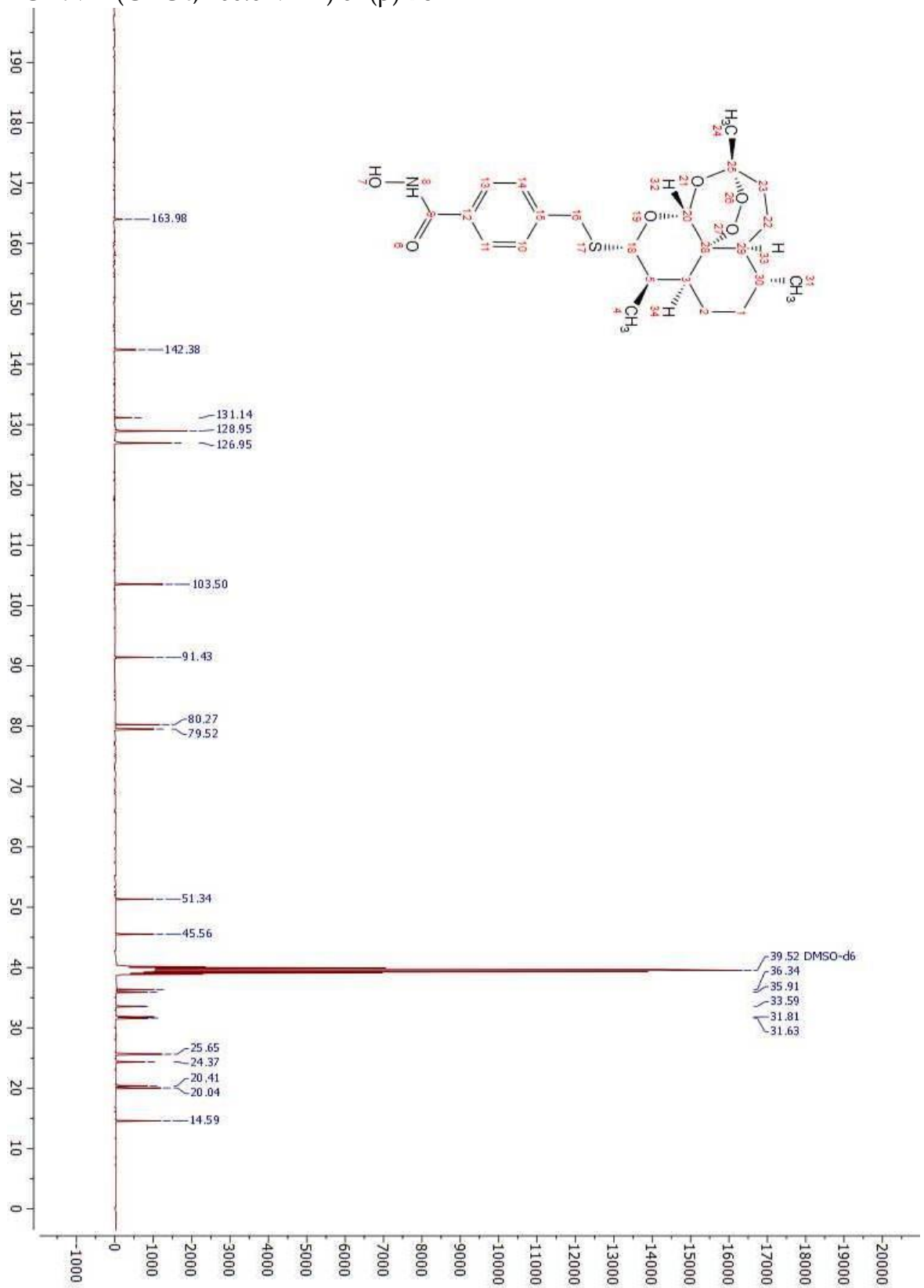
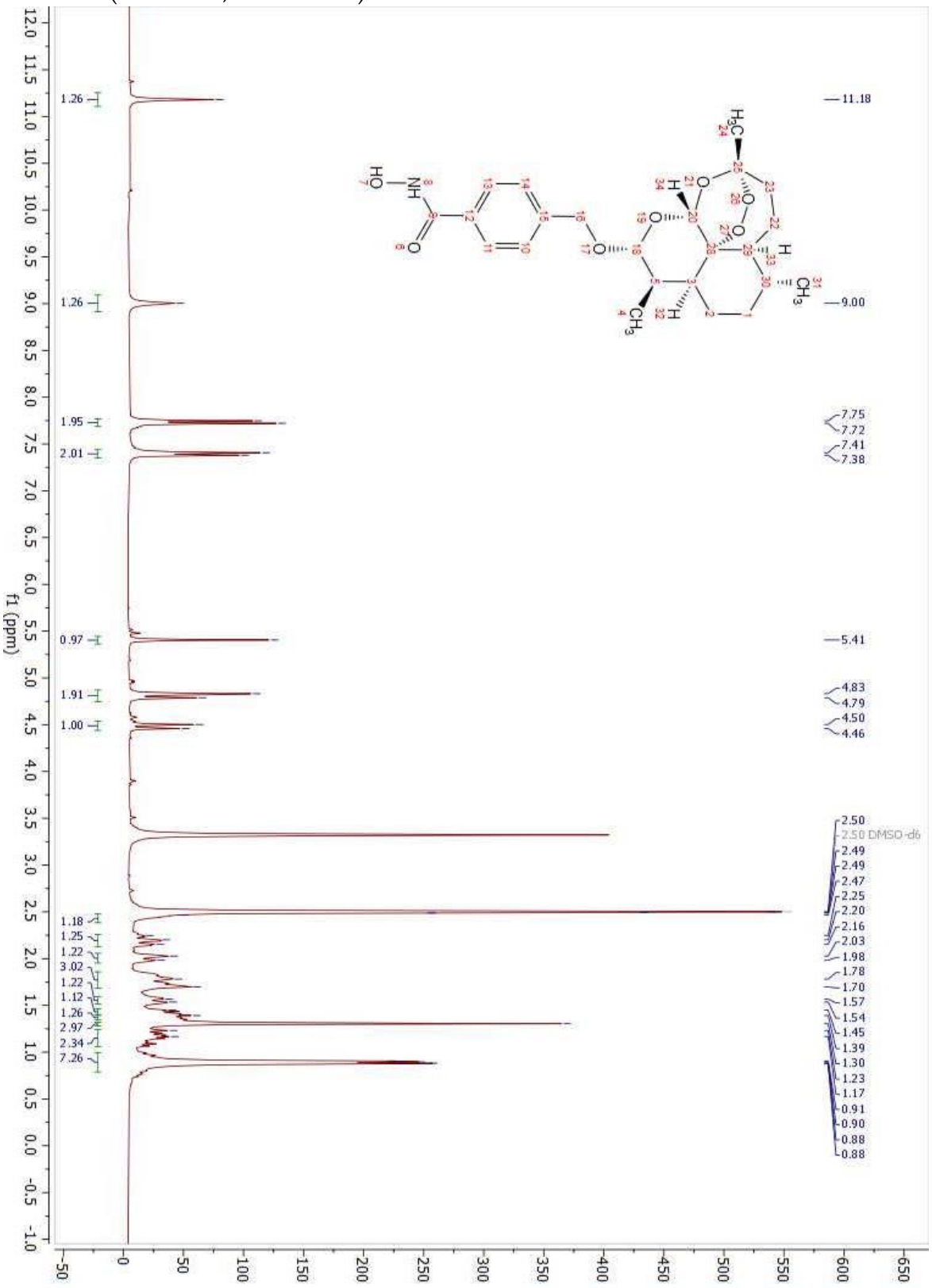
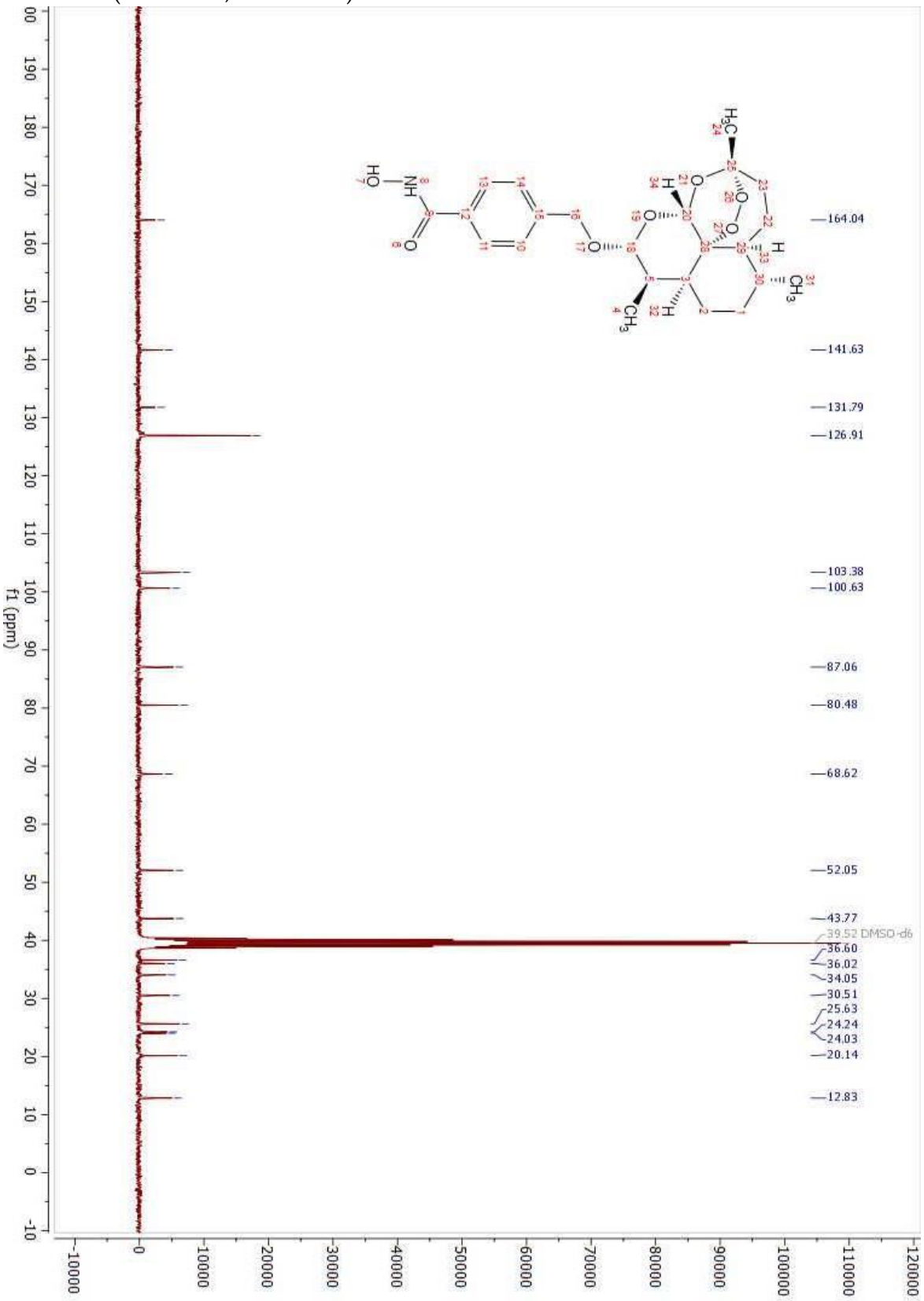


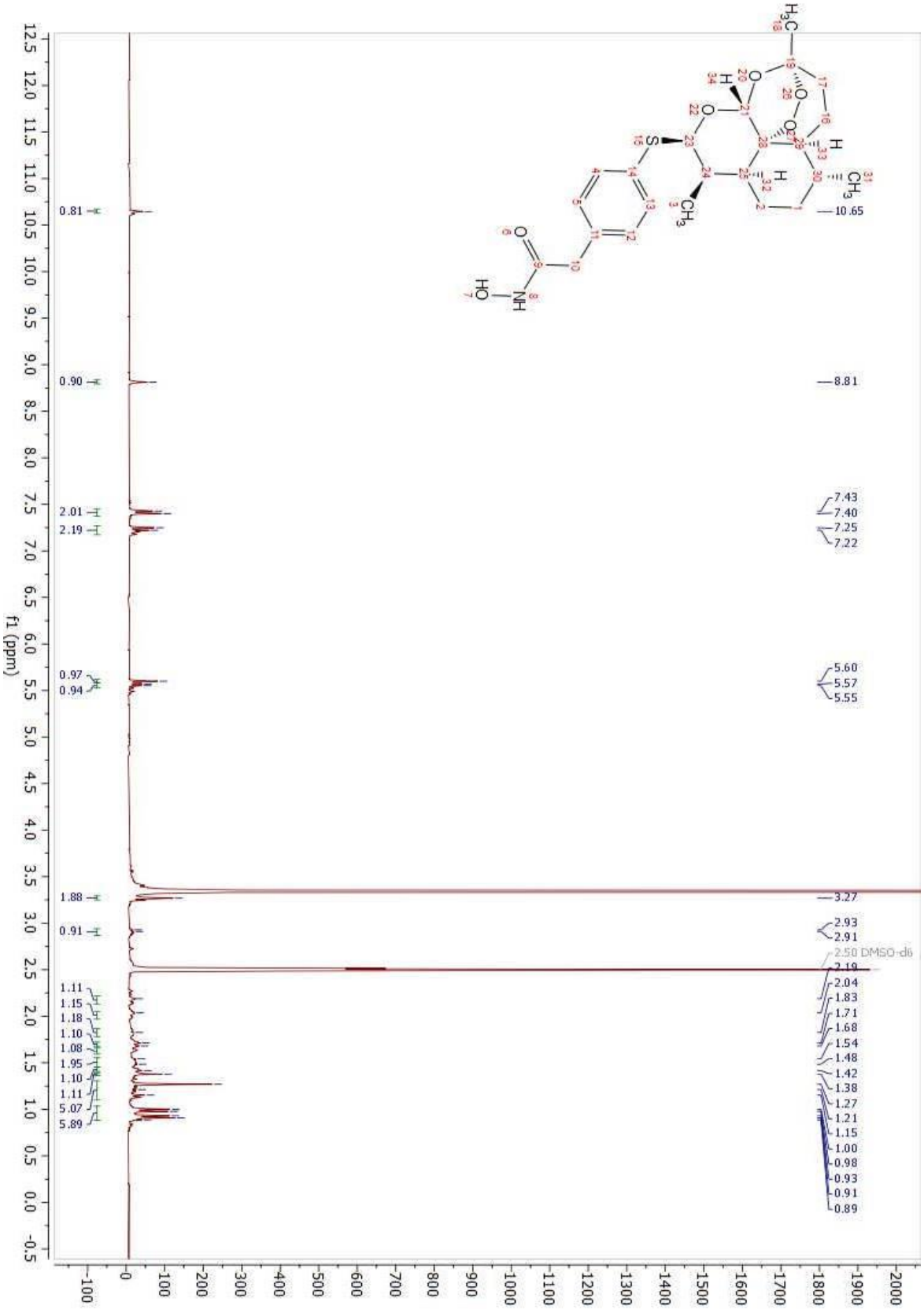
[1]H-NMR (DMSO-d_6, 300.8 MHz) of **7d**



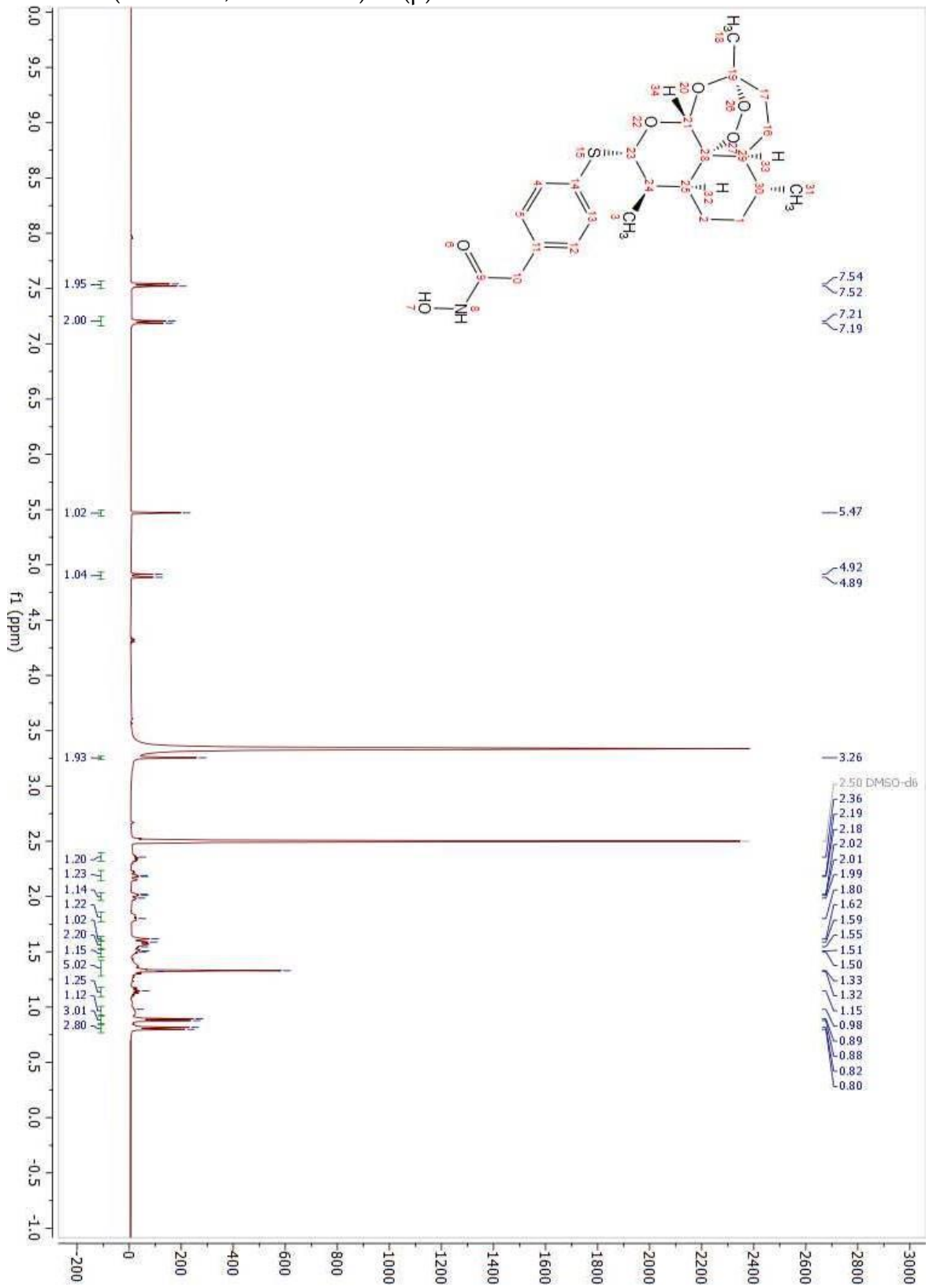
^{13}C-NMR (DMSO-d_6, 75.5 MHz) of **7d**



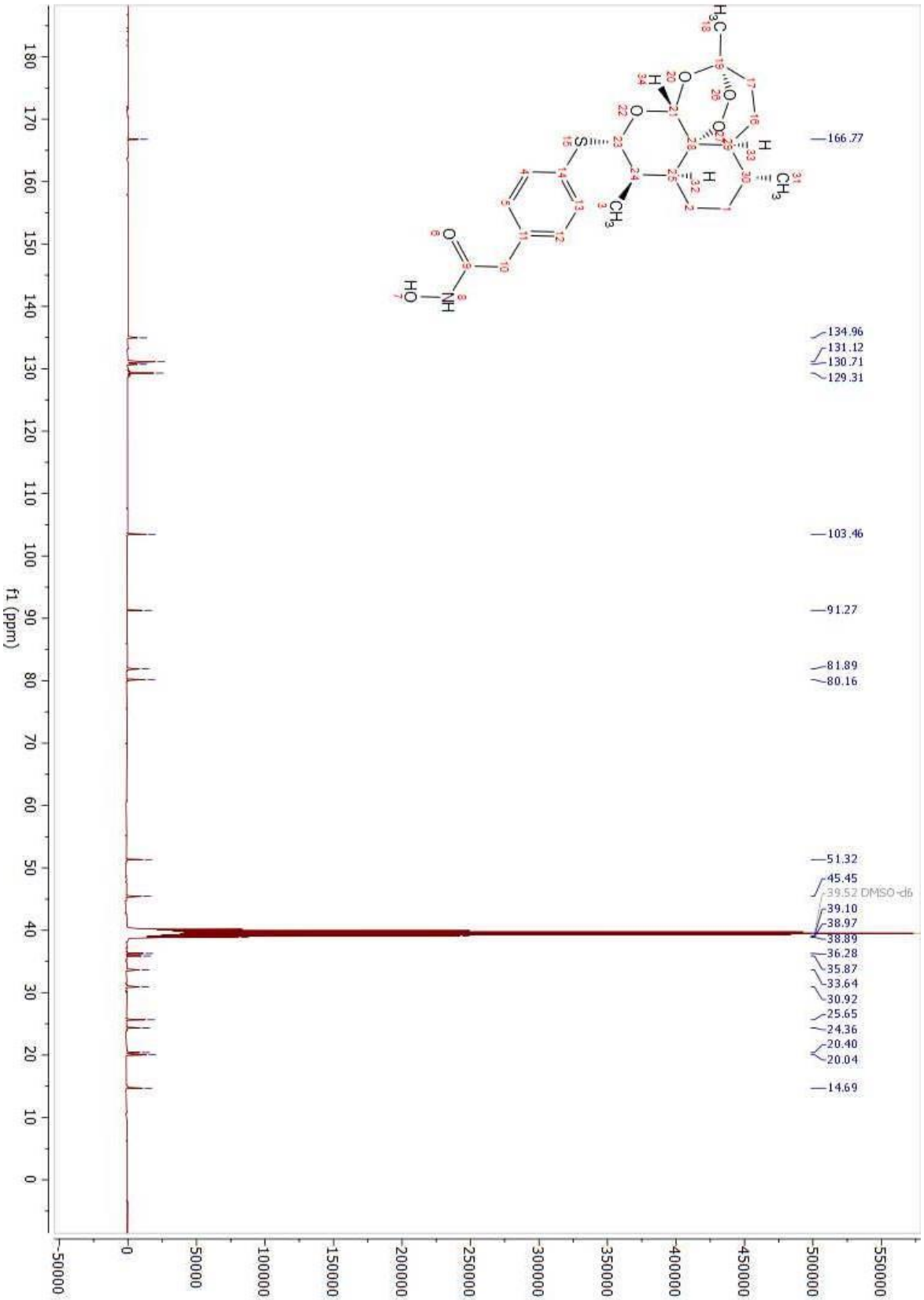
^{1}H-NMR (DMSO-d_6, 300.1 MHz) of (α)-**7e**



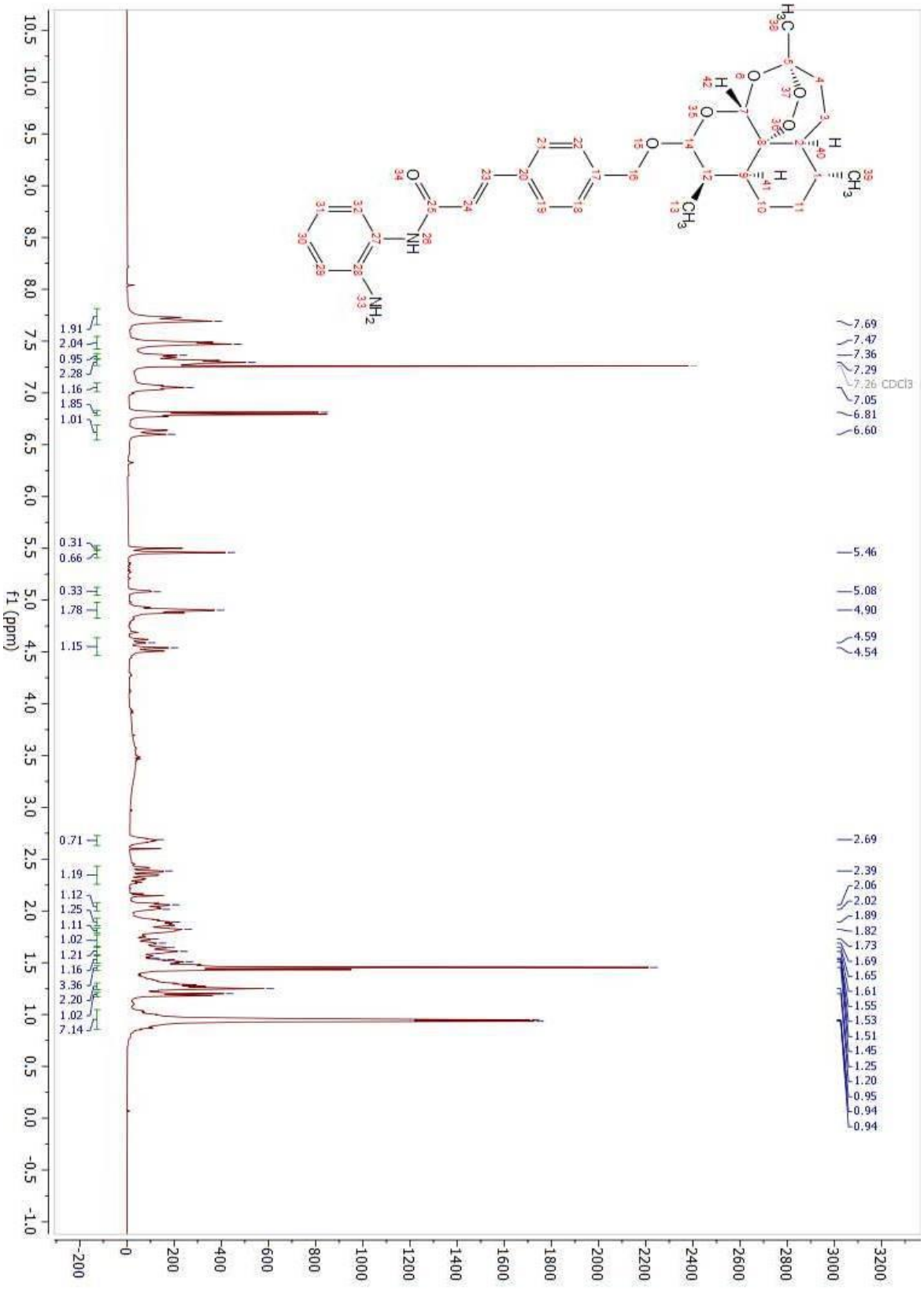
[1]H-NMR (DMSO-*d*$_6$, 400.0 MHz) of (β)-**7e**



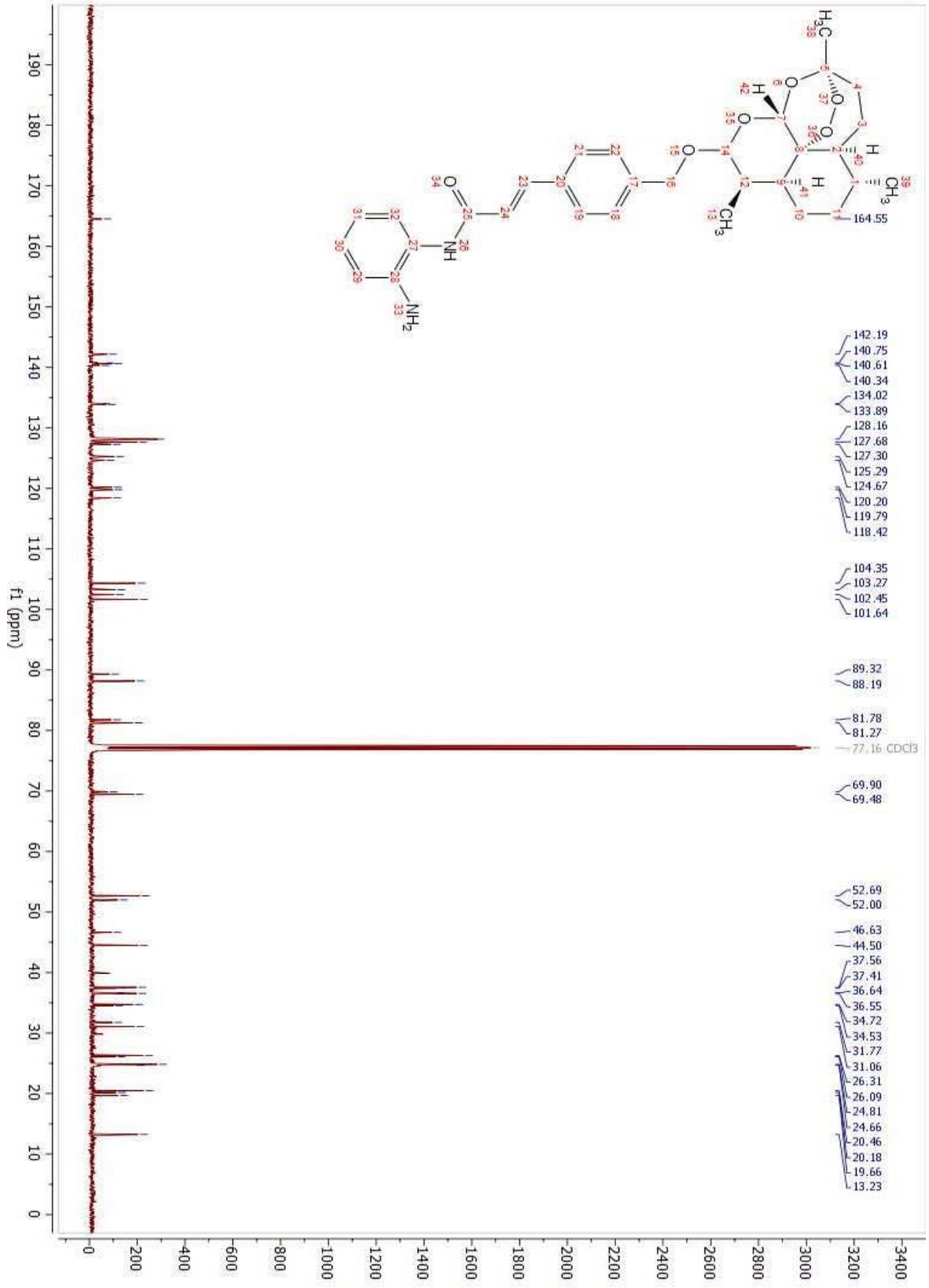
^{13}C-NMR (DMSO-d_6, 100.6 MHz) of (β)-**7e**



^{1}H-NMR ($CDCl_3$, 400.2 MHz) of **8**



^{13}C-NMR ($CDCl_3$, 100.6 MHz) of **8**



4. References

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2. Mackwitz, M.K.W.; Hamacher, A.; Osko, J.D.; Held, J.; Schöler, A.; Christianson, D.W.; Kassack, M.U.; Hansen, F.K. Multicomponent Synthesis and Binding Mode of Imidazo1,2- apyridine-Capped Selective HDAC6 Inhibitors. *Org. Lett.* **2018**, *20*, 3255–3258, doi:10.1021/acs.orglett.8b01118.
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6. Kaufmann, K.W.; Meiler, J. Using RosettaLigand for small molecule docking into comparative models. *PLoS One* **2012**, *7*, e50769, doi:10.1371/journal.pone.0050769.
7. DeLuca, S.; Khar, K.; Meiler, J. Fully Flexible Docking of Medium Sized Ligand Libraries with RosettaLigand. *PLoS One* **2015**, *10*, e0132508, doi:10.1371/journal.pone.0132508.