New Dual Inhibitors of Tyrosyl-DNA Phosphodiesterase 1 and 2 Based on Deoxycholic Acid: Design, Synthesis, Cytotoxicity, and Molecular Modeling

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NMR ^{1}H and **^{13}C** of DCA derivatives (**2, 3a-e, 4d-f**)

Table S1. The binding affinities as predicted by the scoring functions used to the catalytic TDP1 binding pocket and their measured IC_{50} values.

Figure S1. The correlation plot of measured IC_{50} values against their CS counterparts.

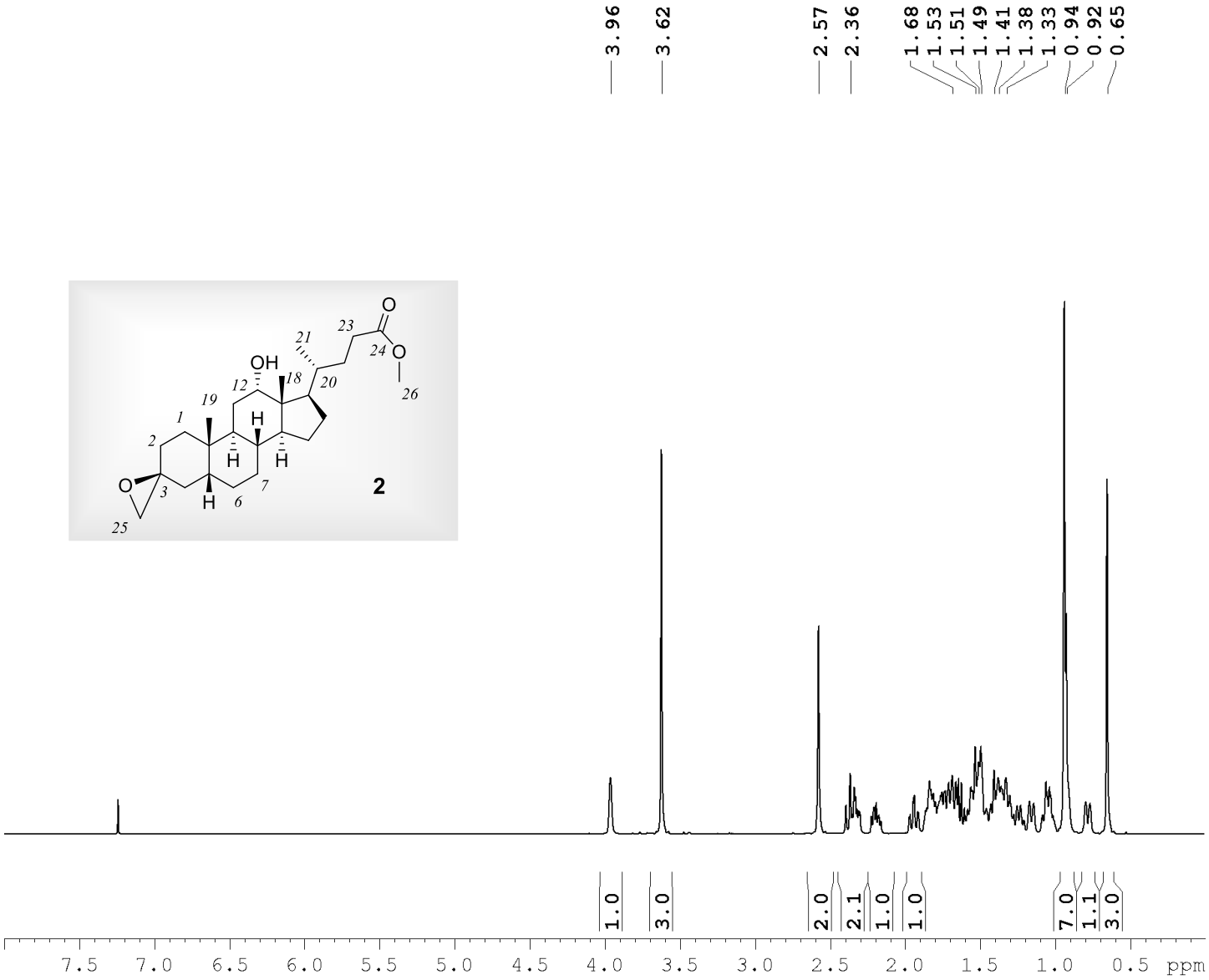
Table S2. The binding affinities as predicted by the scoring functions used to the catalytic TDP2 binding pocket and their measured IC_{50} values.

Table S3. The molecular descriptors and their corresponding Known Drug Indexes 2a and 2b ($KDI_{2a/2b}$).

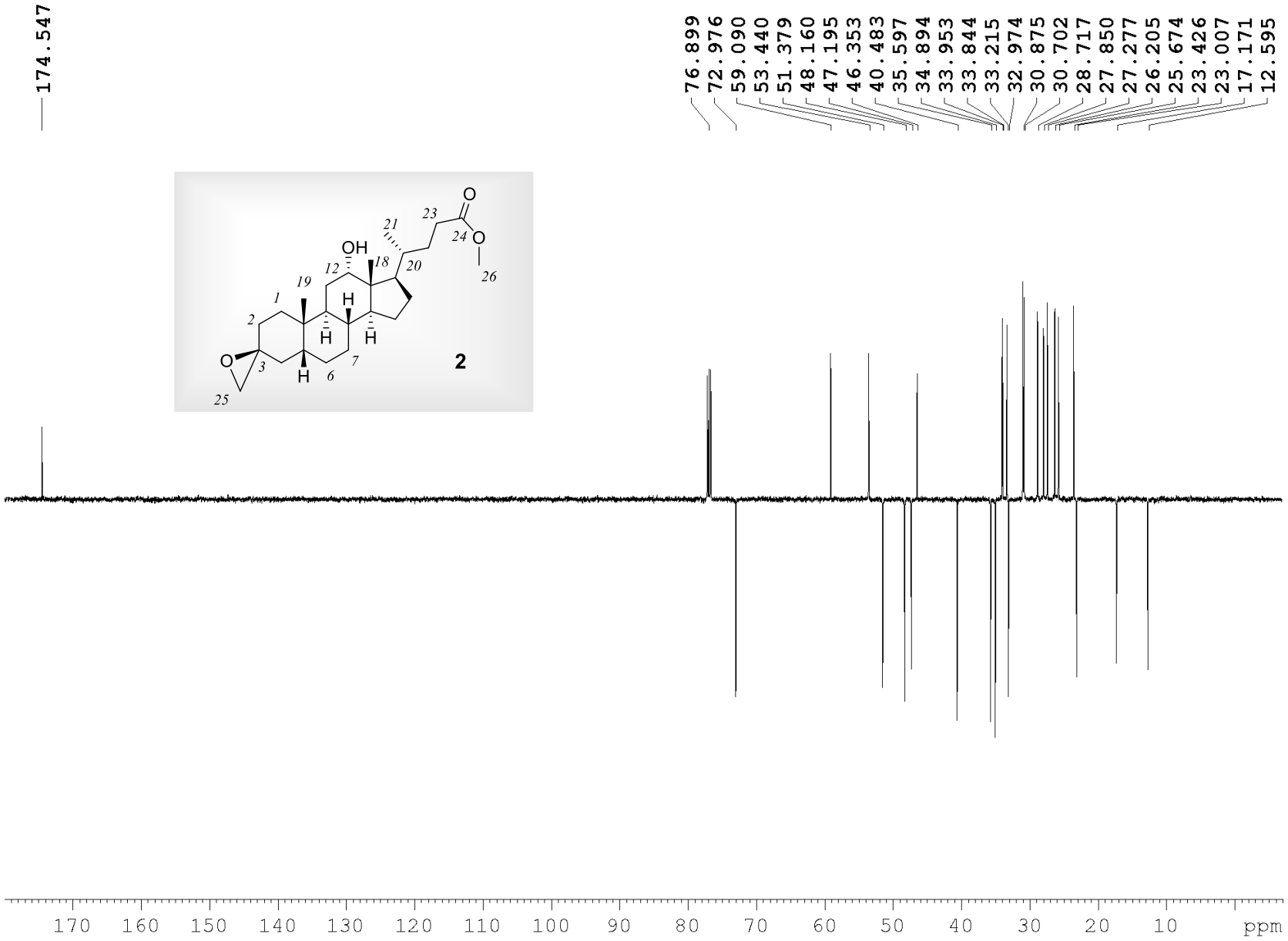
Table S4. Definition of lead-like, drug-like and Known Drug Space (KDS) in terms of molecular descriptors. The values given are the maxima for each descriptor for the volumes of chemical space used.

HPLC analyses for compound **3a-e, 4d-f** and 3α,12α-*bis*-methoxy deoxycholic *para*-bromoanilide (compound **A**) and 3α-benzyloxy deoxycholic tryptamide (compound **B**)

Spectrum of compound **2,** ^{1}H NMR, 500MHz, $CDCl_3$



Spectrum of compound **2,** ^{13}C NMR, 125MHz, $CDCl_3$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23.426
23.007
17.171
12.595
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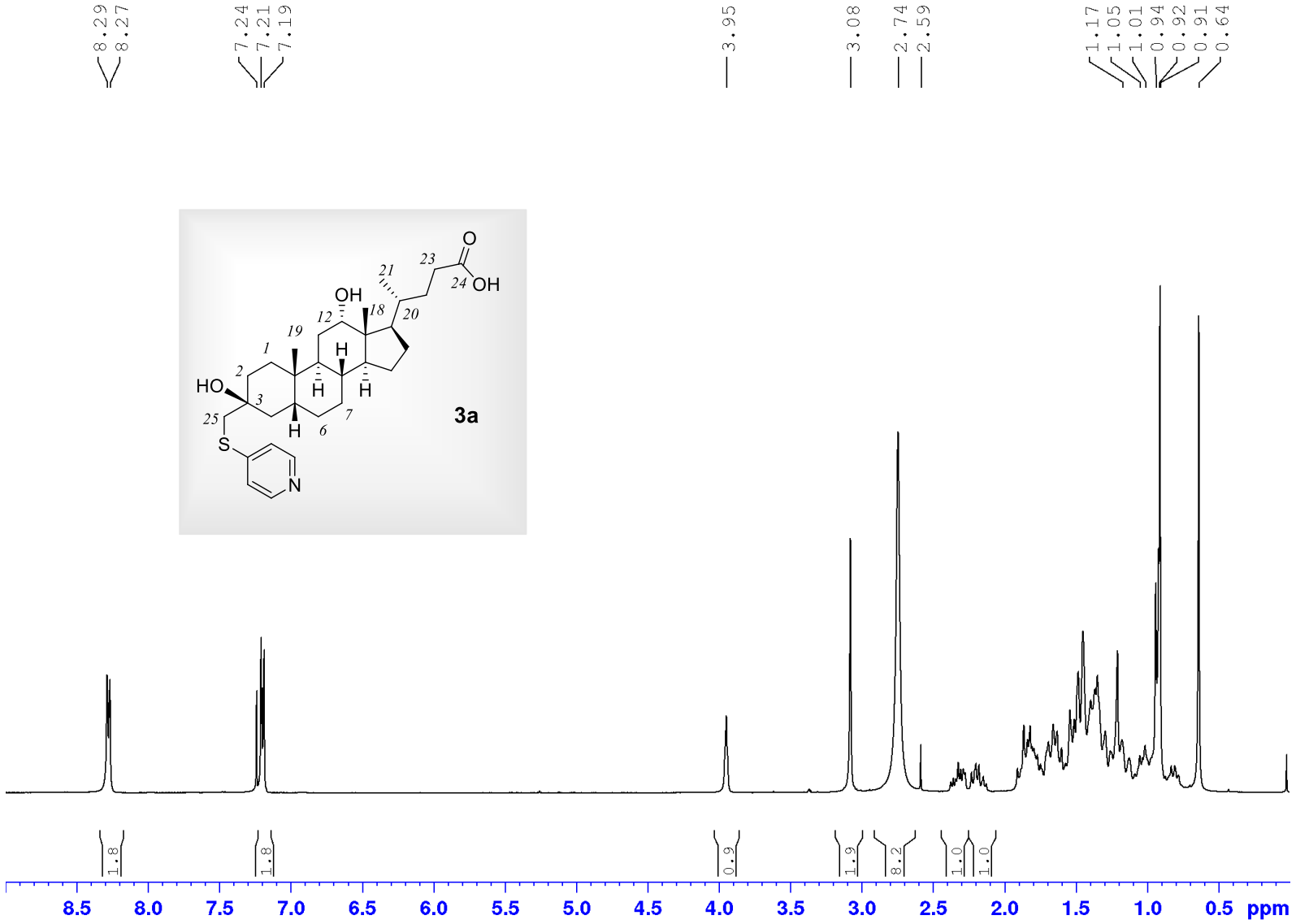
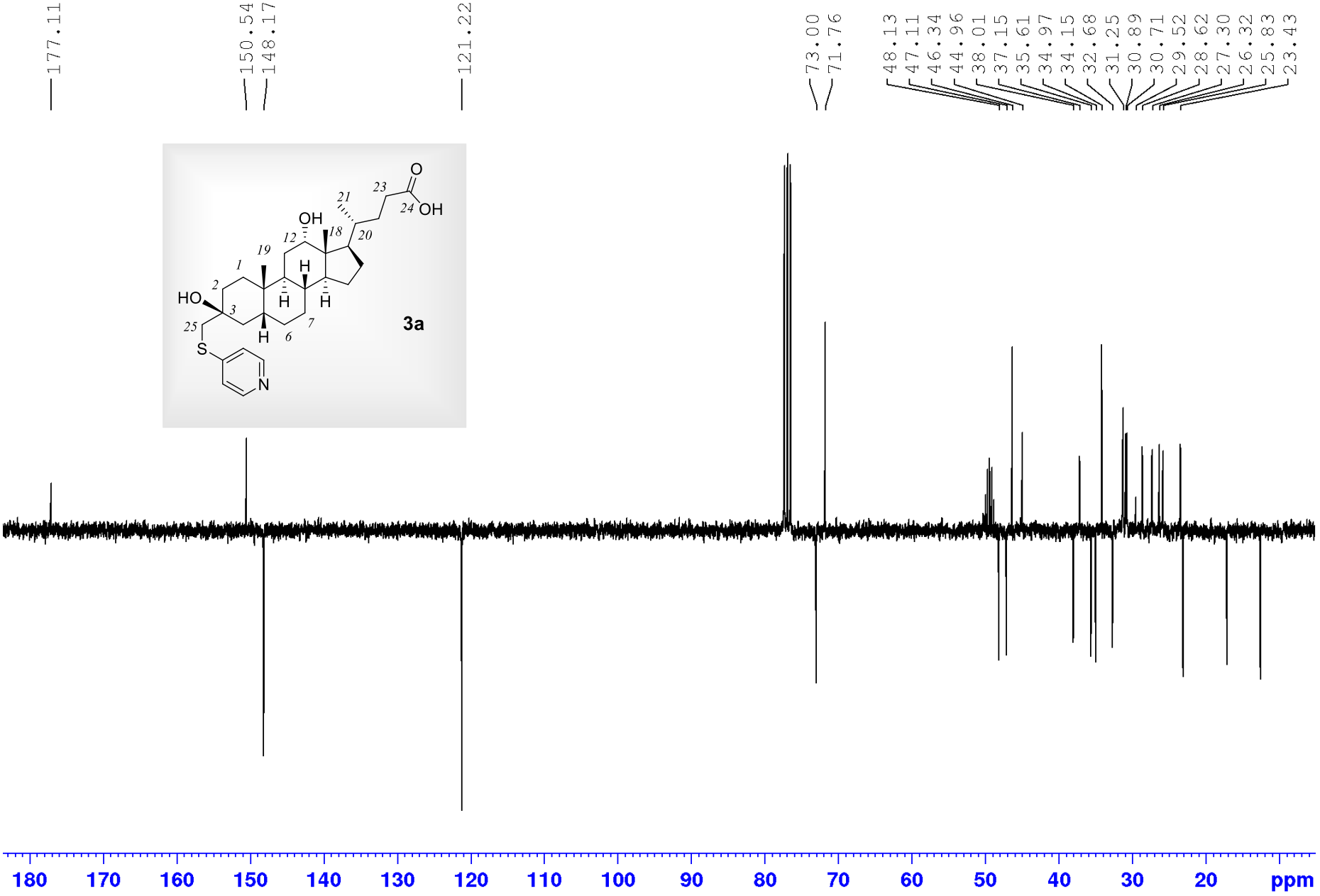
2

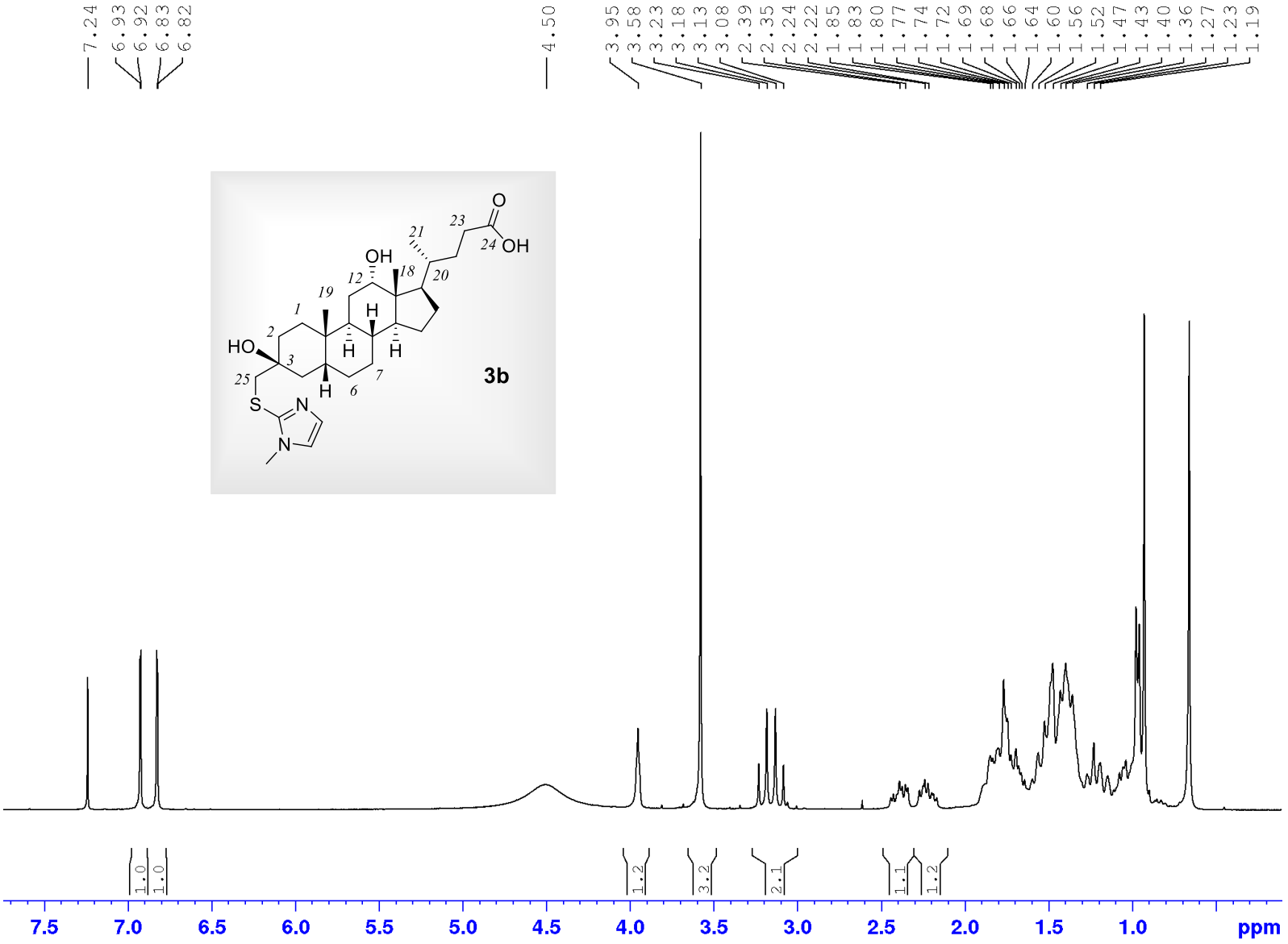
Spectrum of compound **3a,** ^{1}H NMR, 300MHz, $CDCl_3$+CD_3OD



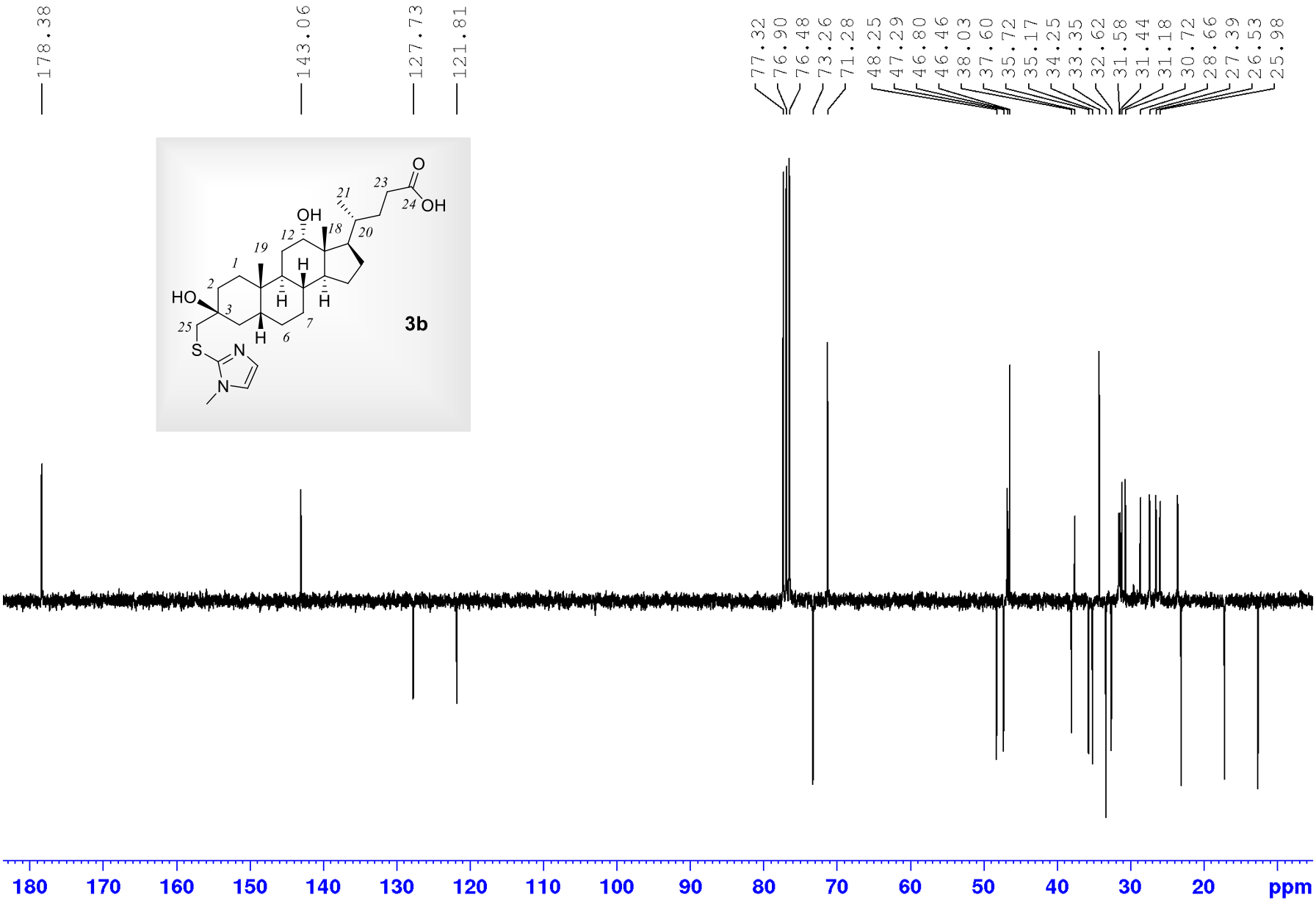
Spectrum of compound **3a,** ^{13}C NMR, 75MHz, $CDCl_3$+CD_3OD



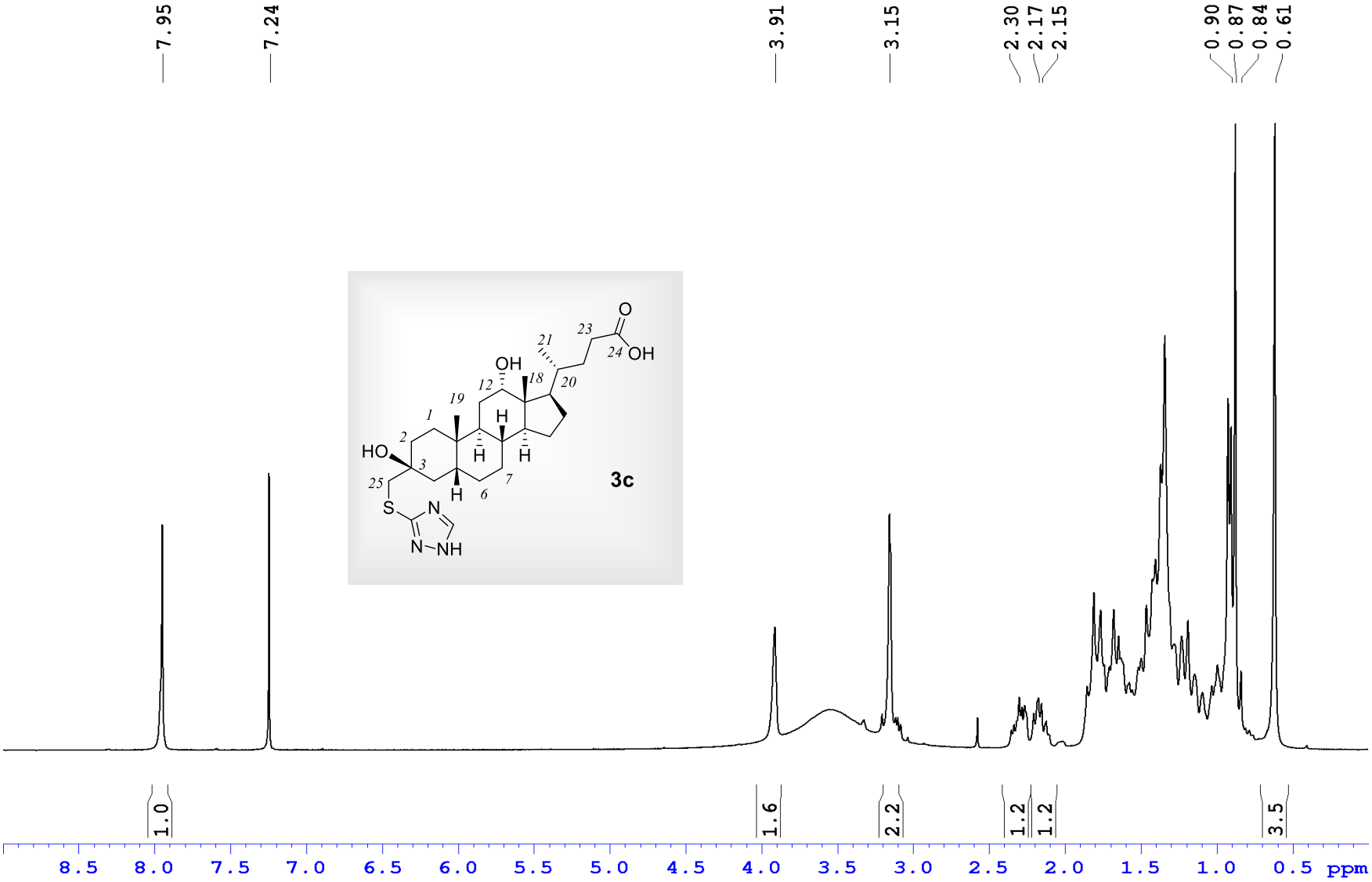
Spectrum of compound **3b,** ^{1}H NMR, 300MHz, $CDCl_3$



Spectrum of compound **3b,** ^{13}C NMR, 75MHz, $CDCl_3$+CD_3OD

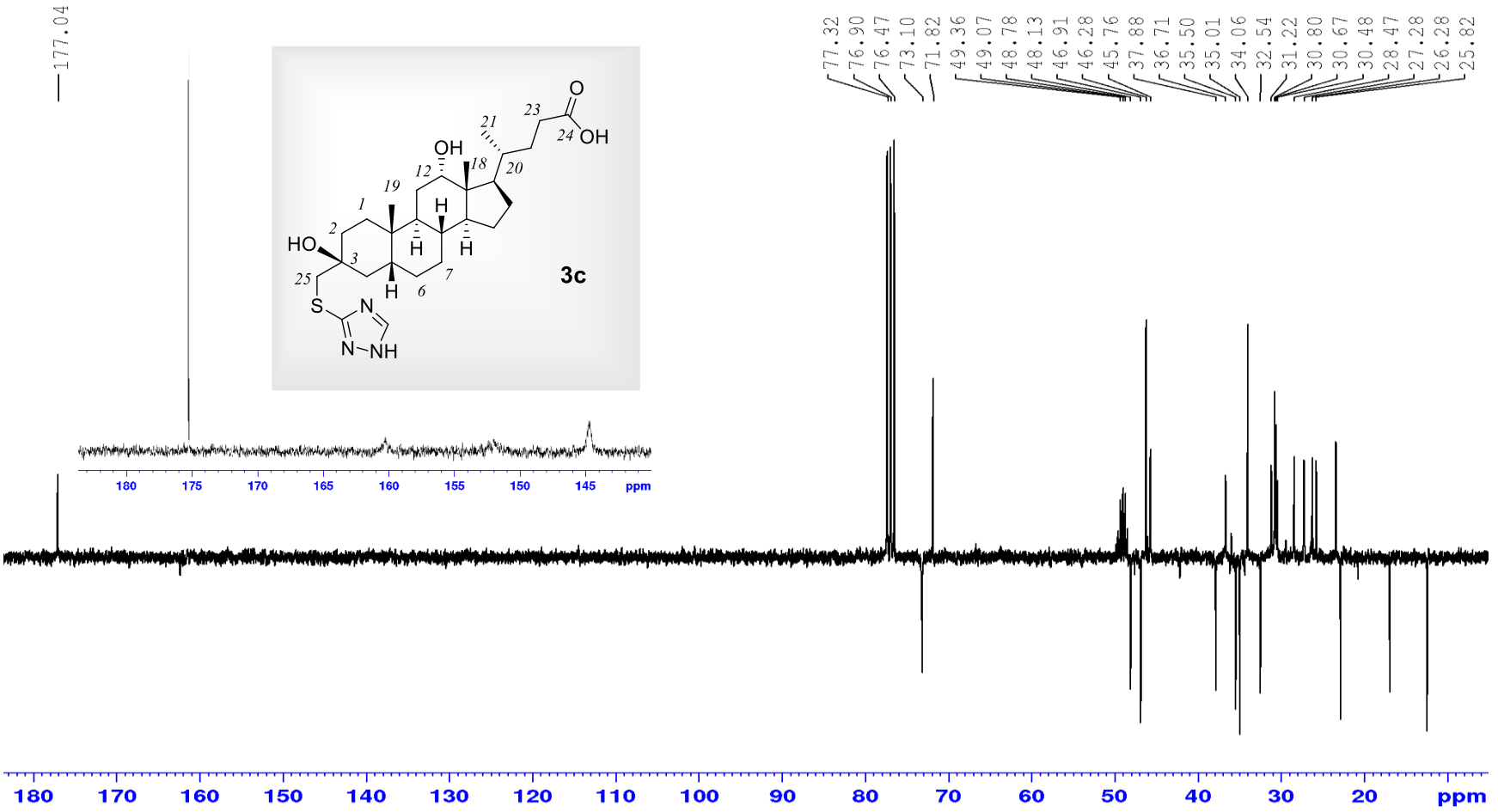


Spectrum of compound **3c,** ^{1}H NMR, 300 MHz, $CDCl_3$+CD_3OD

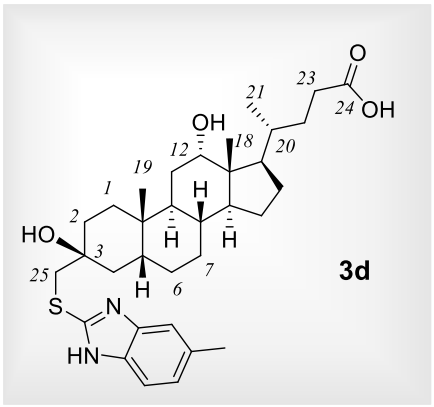


3c

Spectrum of compound **3c,** ^{13}C NMR, 75 MHz, $CDCl_3$+CD_3OD

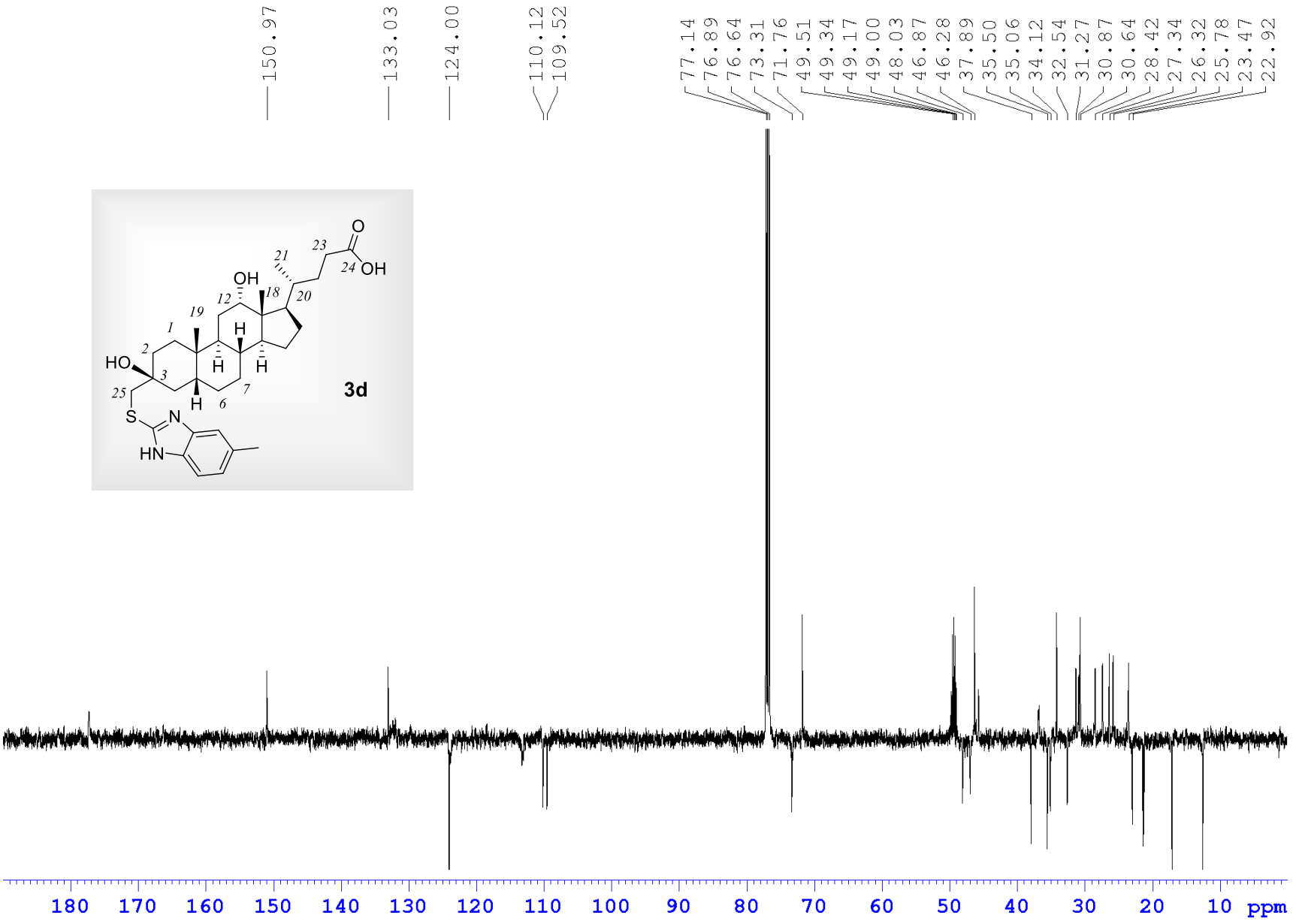


Spectrum of compound **3d,** ^{1}H NMR, 500 MHz, $CDCl_3$+CD_3OD

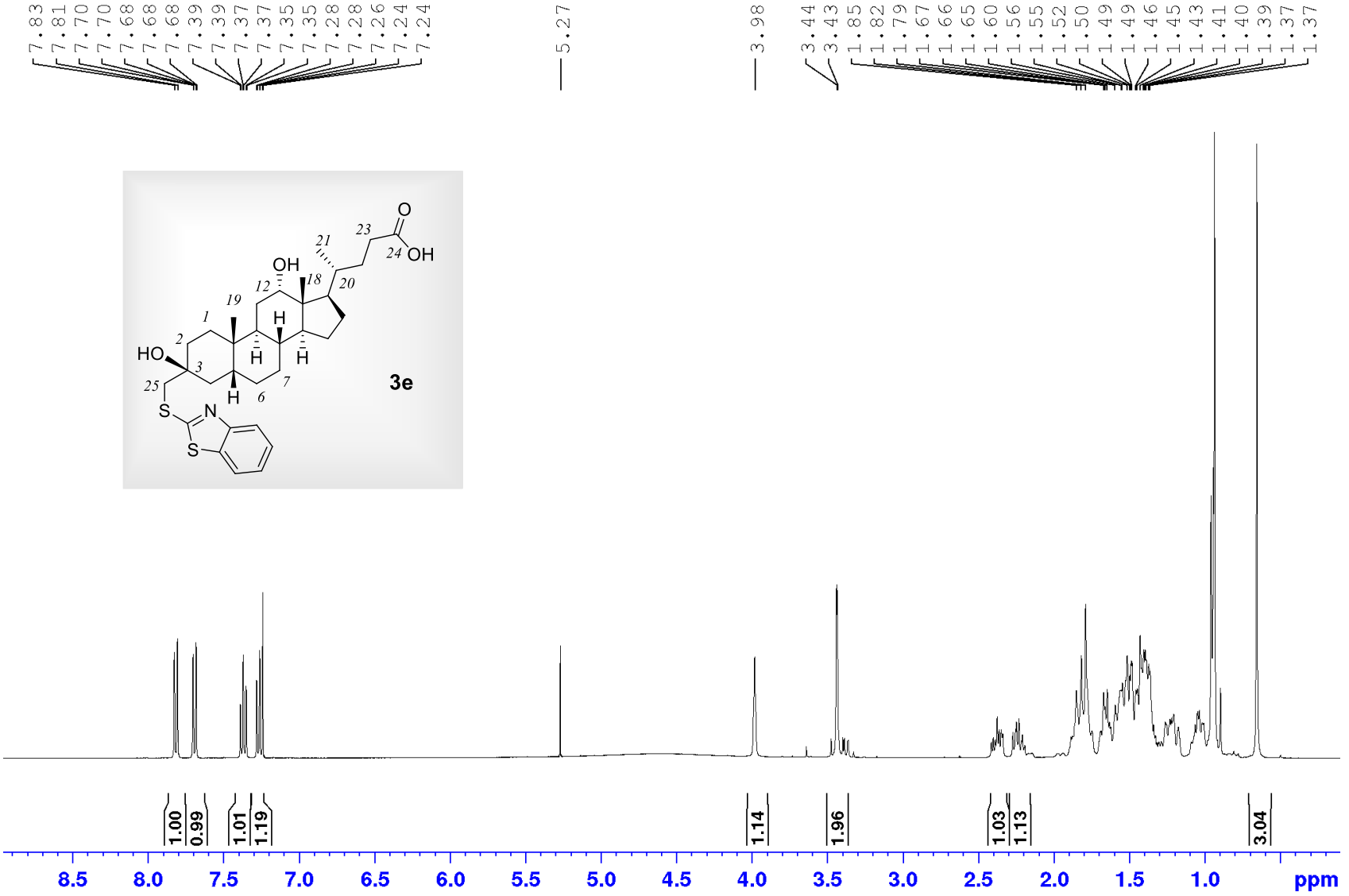


3d

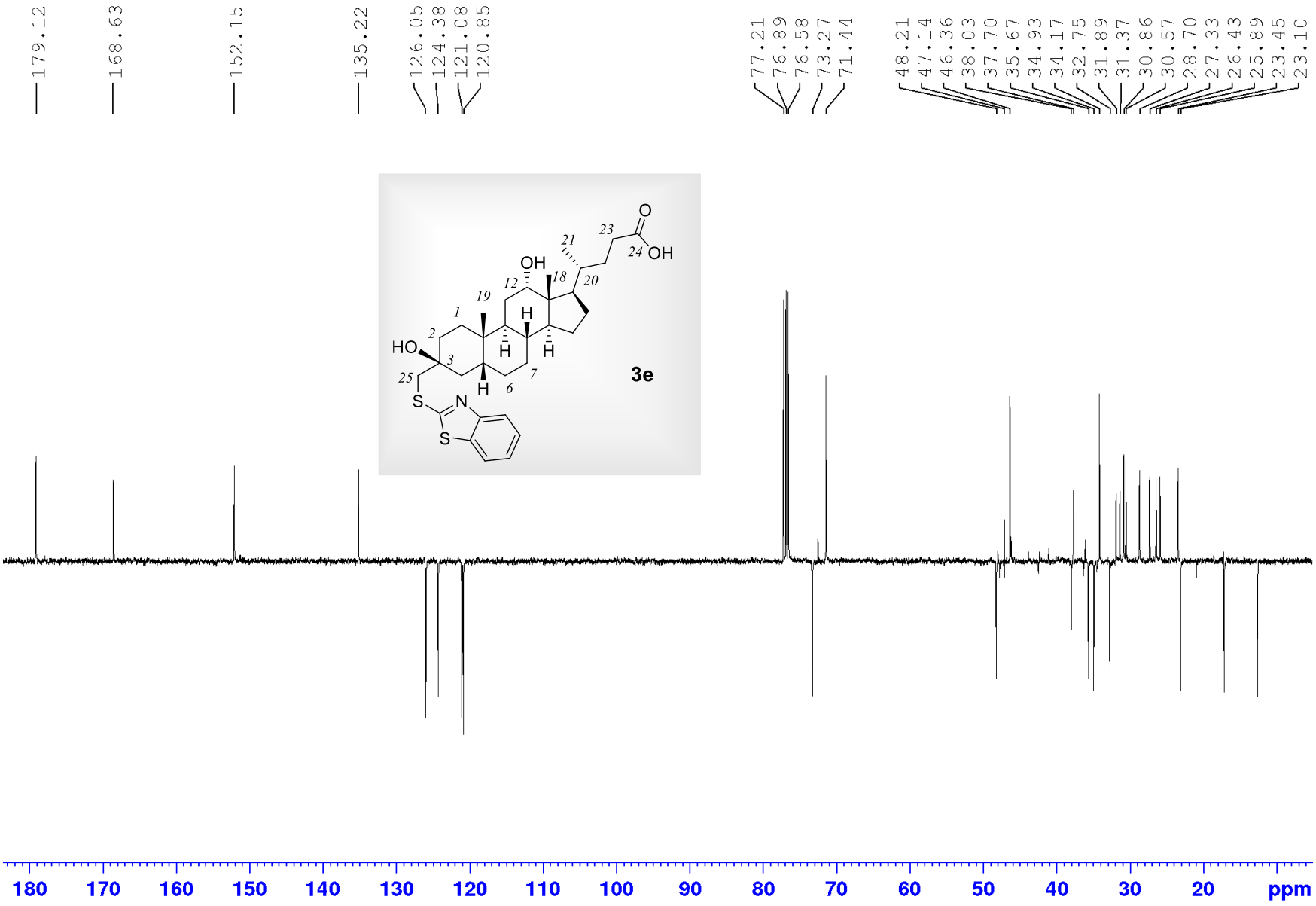
Spectrum of compound **3d,** ^{13}C NMR, 125 MHz, $CDCl_3$+CD_3OD



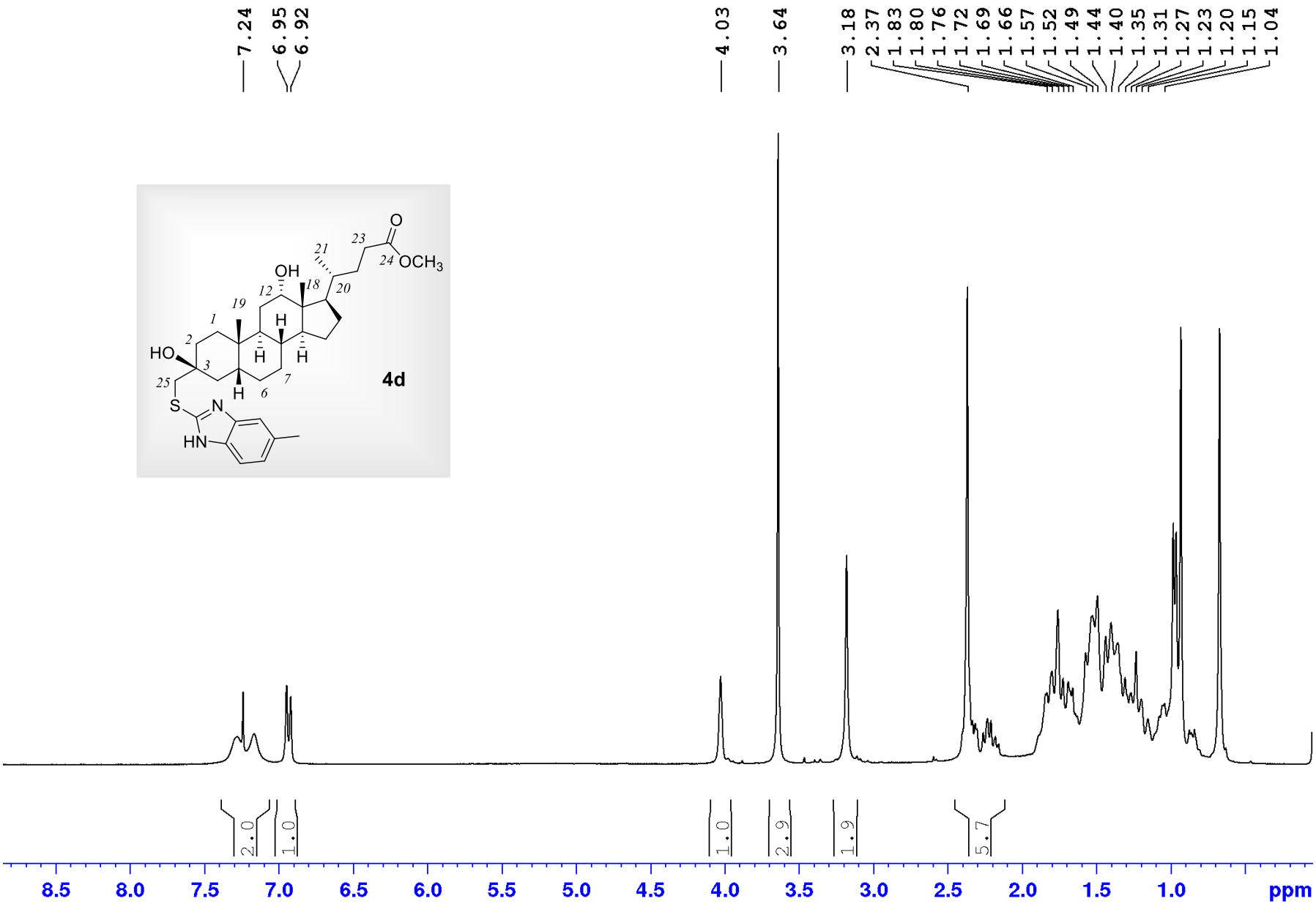
Spectrum of compound **3e,** ^{1}H NMR, 400 MHz, $CDCl_3$



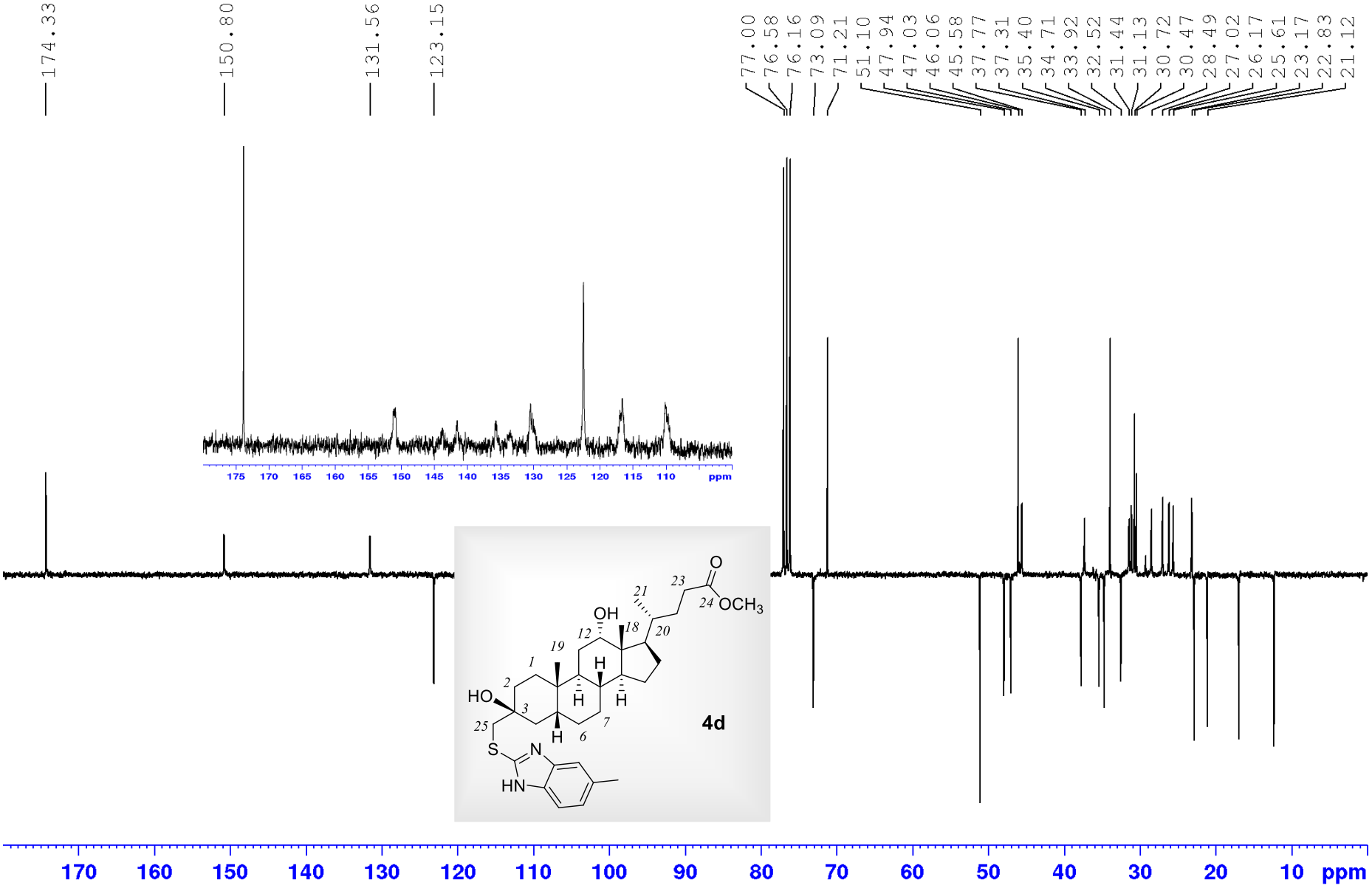
Spectrum of compound **3e,** ^{13}C NMR, 100 MHz, $CDCl_3$



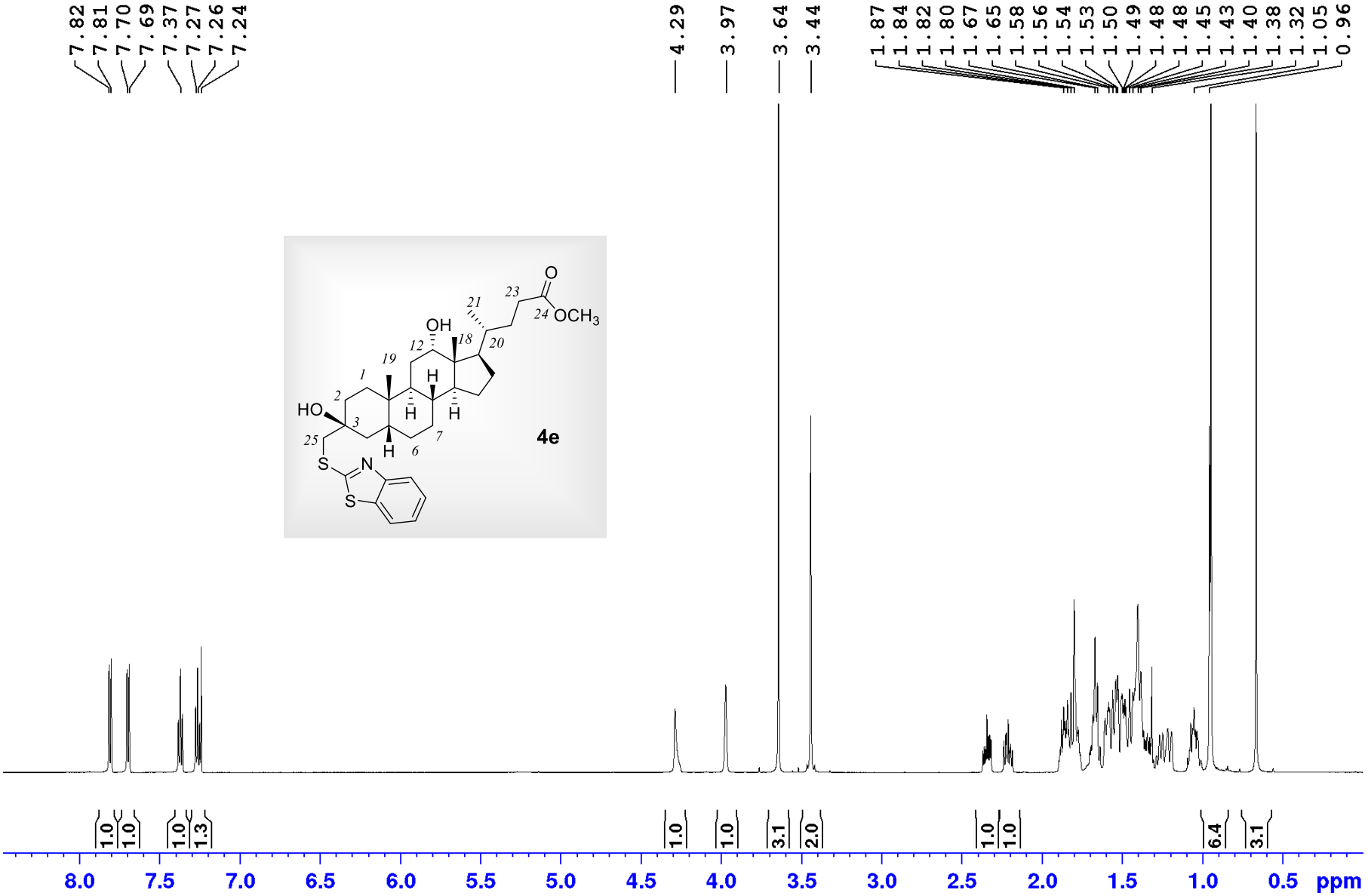
Spectrum of compound **4d,** ^{1}H NMR, 300 MHz, $CDCl_3$



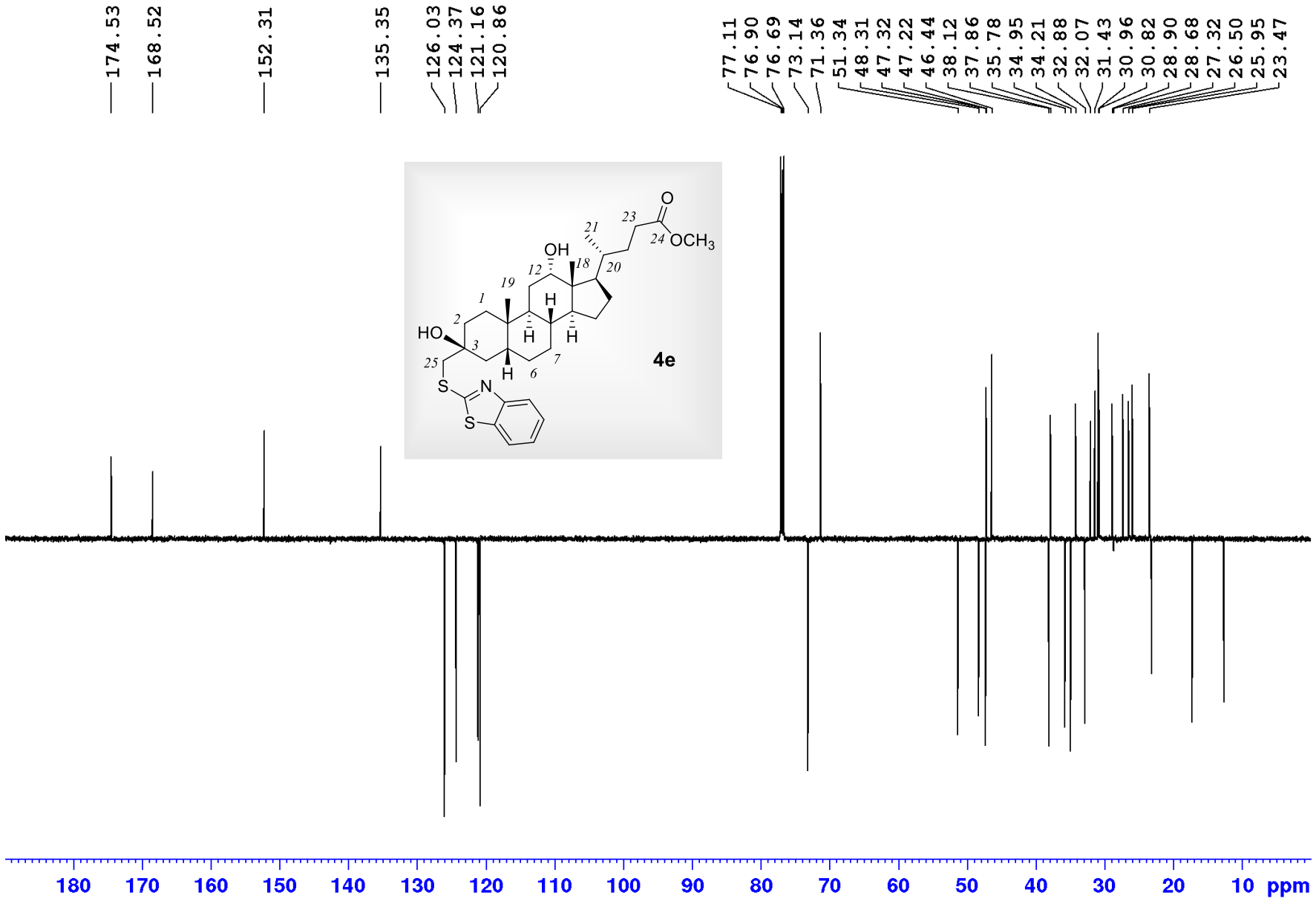
Spectrum of compound **4d,** ^{13}C NMR, 75 MHz, $CDCl_3$



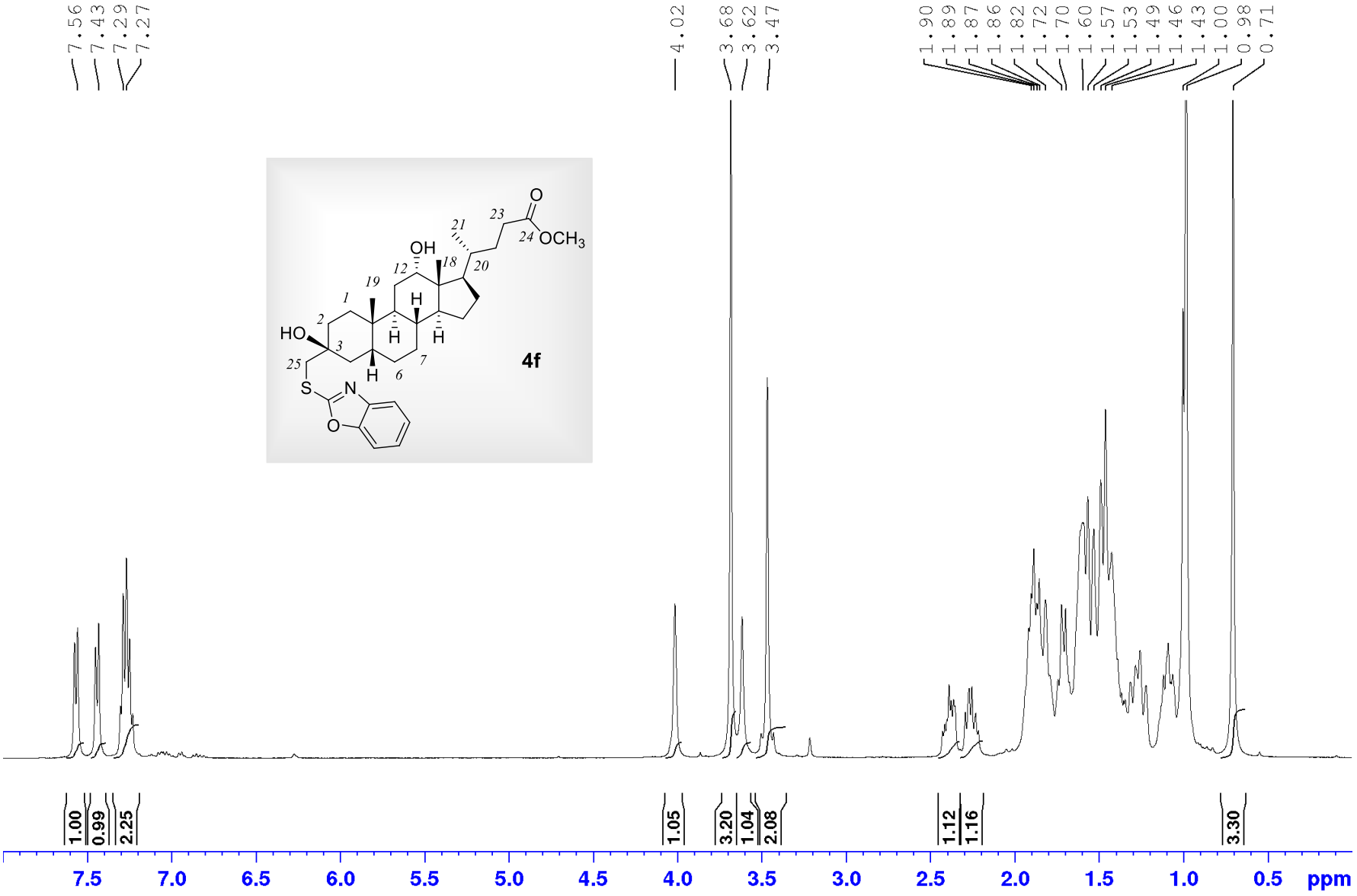
Spectrum of compound **4e,** ^{1}H NMR, 600 MHz, $CDCl_3$

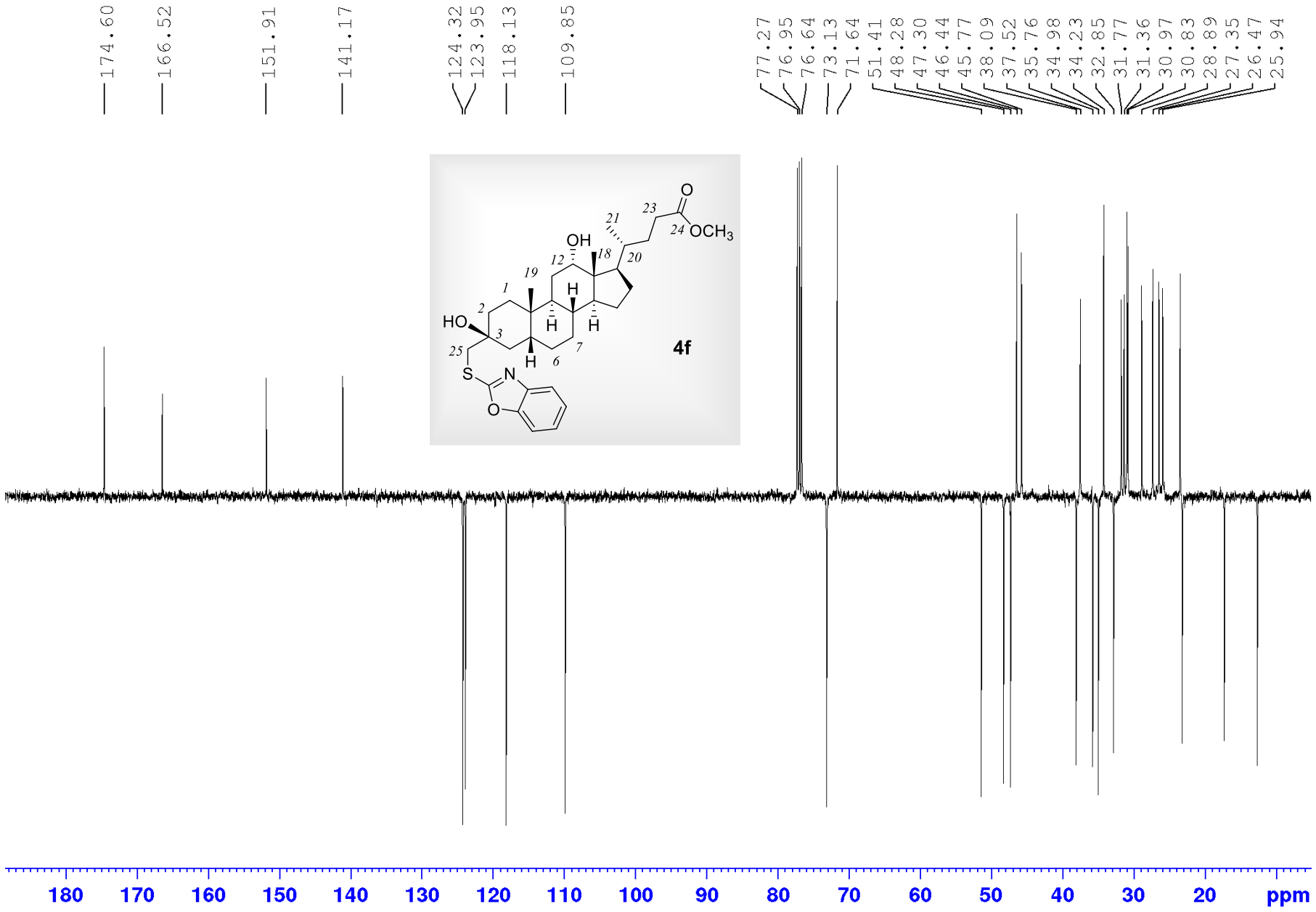


Spectrum of compound **4e,** ^{13}C NMR, 125 MHz, $CDCl_3$



Spectrum of compound **4f,** ^{1}H NMR, 400 MHz, $CDCl_3$

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S
N
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130
120
110
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50
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30
20
ppm

Table S1. The binding affinities as predicted by the scoring functions used to the catalytic TDP1 binding pocket and their measured IC_{50} values.

Ligand	ASP	ChemPLP	CS	GS	IC_{50}, μM
3a	45.0	55.8	22.9	48.5	22 ± 2
3b	45.7	60.4	20.3	48.8	30 ± 7
3c	47.6	50.5	16.2	54.1	32 ± 4
3d	43.3	63.5	24.4	58.1	25 ± 4
3e	47.2	62.4	22.2	52.3	14 ± 2
4d	44.0	63.8	27.6	57.3	0.94 ± 0.04
4e	45.1	60.5	24.4	52.5	0.63 ± 0.03
4f	43.6	59.4	25.1	53.3	1.8 ± 0.5

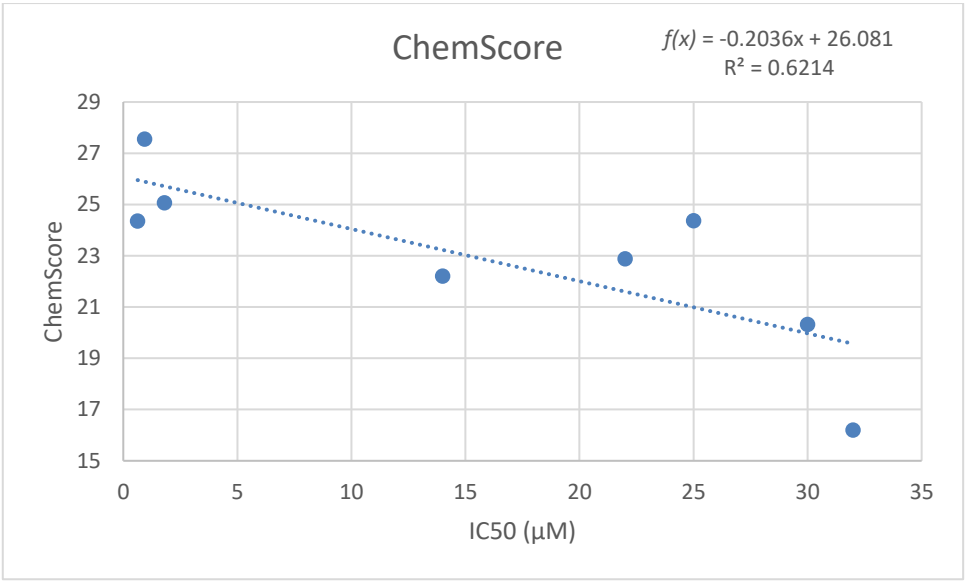


Figure S1. The correlation plot of measured IC_{50} values against their CS counterparts.

Table S2. The binding affinities as predicted by the scoring functions used to the catalytic TDP2 binding pocket and their measured IC_{50} values.

Ligand	**ASP**	**ChemPLP**	**CS**	**GS**	**IC_{50}, µM**
3a	36.0	54.7	21.7	50.1	N/A
3b	38.5	56.8	18.5	43.5	N/A
3c	33.8	52.6	14.4	49.3	N/A
3d	38.1	64.8	23.3	56.2	250
3e	39.0	62.4	21.3	53.9	76
4d	38.9	65.1	26.0	57.1	218
4e	39.6	62.6	24.0	55.1	N/A
4f	39.9	61.3	23.8	55.0	N/A

Table S3. The molecular descriptors and their corresponding Known Drug Indexes 2a and 2b ($KDI_{2a/2b}$).

	RB	MW	HD	HA	Log P	PSA	KDI_{2A}	KDI_{2B}
3a	9	515.8	3	6.45	5.4	92.6	4.12	0.07
3b	9	518.8	3	5.95	5.6	96.4	4.07	0.06
3c	9	505.7	4	7.45	4.2	125.6	3.74	0.05
3d	9	568.8	4	5.95	6.5	108.1	3.24	0.01
3e	9	571.8	3	5.95	6.6	93.4	3.65	0.02
4d	9	582.8	3	5.95	7.0	94.1	3.55	0.01
4e	9	585.9	2	5.95	7.1	79.4	3.75	0.01
4f	9	569.8	2	6.45	6.7	88.7	3.82	0.02
TDP1 - R^2	X	0.686	0.602	0.171	0.685	0.550	0.025	0.435
TDP2 - R^2	X	0.021	0.412	X	0.036	0.452	0.658	0.980

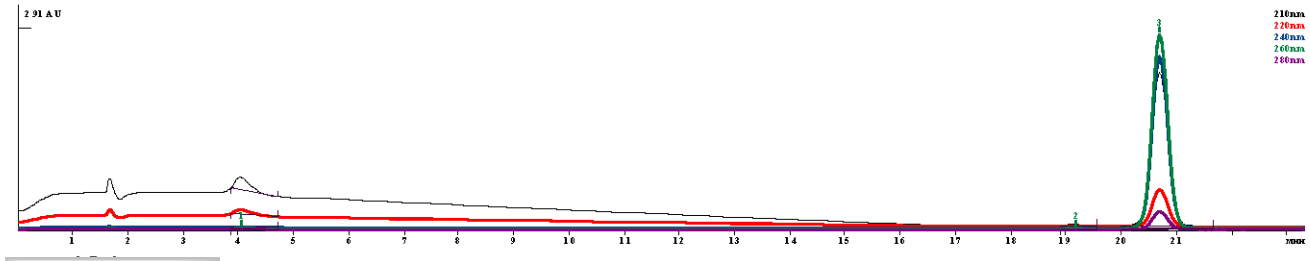
Table S4. Definition of lead-like, drug-like and Known Drug Space (KDS) in terms of molecular descriptors. The values given are the maxima for each descriptor for the volumes of chemical space used.

	Lead-like Space	**Drug-like Space**	**Known Drug Space**
Molecular weight (g mol^{-1})	300	500	800
Lipophilicity (Log P)	3	5	6.5
Hydrogen bond donors (HD)	3	5	7
Hydrogen bond acceptors (HA)	3	10	15
Polar surface area ($Å^2$) (PSA)	60	140	180
Rotatable bonds (RB)	3	10	17

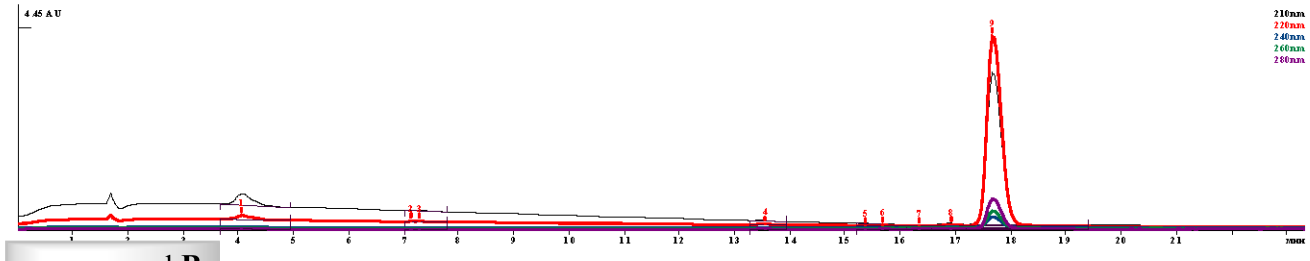
HPLC analyses for compound **3a-e, 4d-f** and 3α,12α-*bis*-methoxy deoxycholic *para*-bromoanilide (compound **A**) and 3α-benzyloxy deoxycholic tryptamide (compound **B**) HPLC analyses were carried out on a MilichromA-02, using a ProntoSIL 120-5-C18 AQ column (BISCHOFF, 2.0 × 75 mm column, grain size 5.0 μm). The mobile phase was Millipore purified water with 0.1% trifluoroacetic acid (eluent **A**) at a flow rate of 150 μL/min at 35°C with UV detection at 210, 220, 240, 260 and 280 nm. A typical run time was 25 min with a gradient of 30–100% acetonitrile (eluent **B**)

Eluent A+B, flow 500 μL/min								
Volume , μL	500	500	1000	1500	2000	2500	3000	3500
eluent A, %	100	70	50	30	10	10	0	0
eluent B, %	0	30	50	70	90	90	100	100

Compound	Log P (*in silico*)	HLPC, *time*
3a	5.4	9 min 53 s (1483 μL)
3b	5.6	9 min 47 s (1469 μL)
3d	6.5	10 min 46 s (1614 μL)
4d	7.0	12 min 20 s (1849 μL)
3c	4.2	12 min 20 s (1950 μL)
3e	6.6	14 min 45 s (2212 μL)
4f	6.7	16 min 28 s (2471 μL)
4e	7.1	17 min 08 s (2569 μL)
3α-benzyloxy deoxycholic tryptamide (compound **B**)	8.0	17 min 40 s (2649 μL)
3α,12α-*bis*-methoxy deoxycholic *para*-bromoanilide (compound **A**)	7.4	20 min 41 s (3102 μL)



compound **A**



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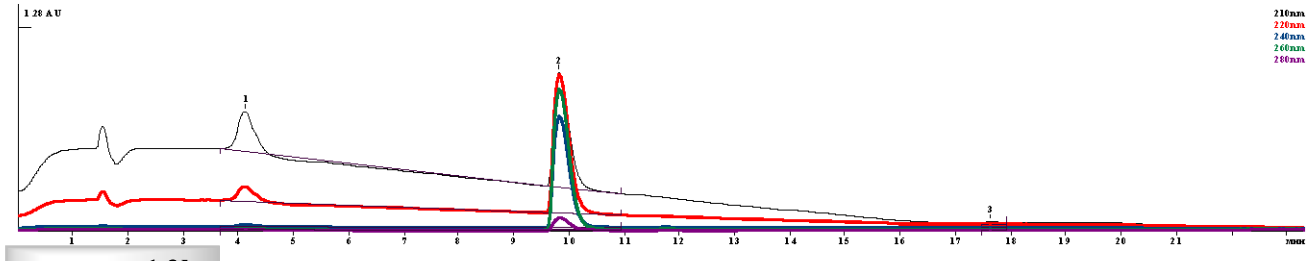
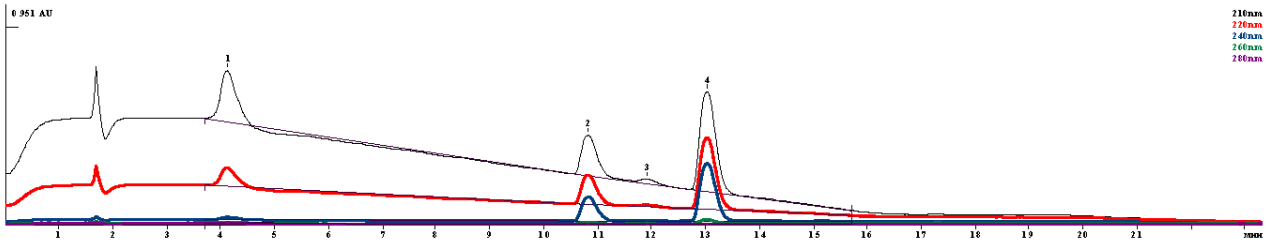
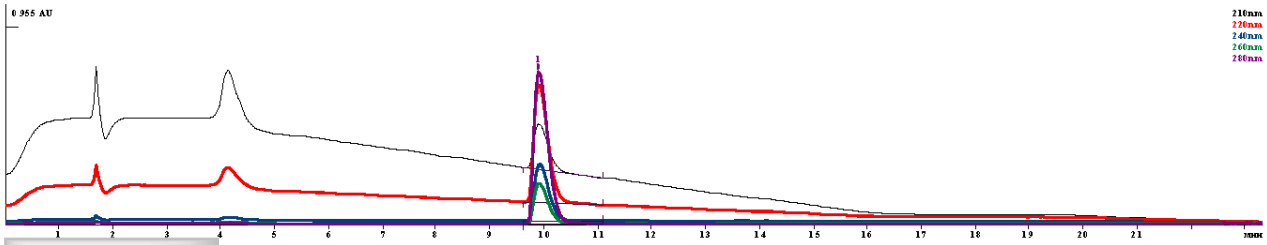


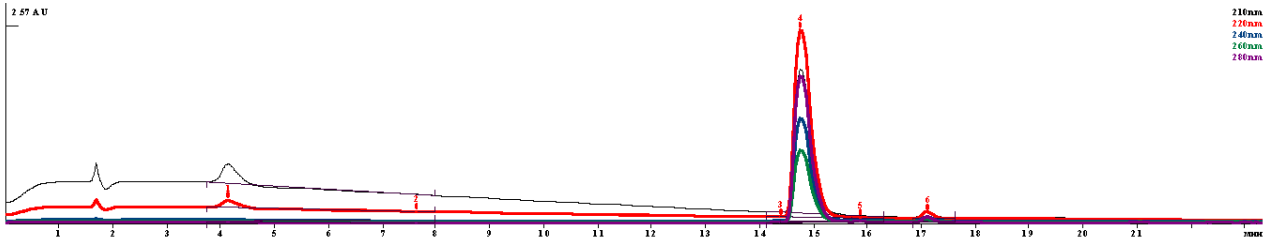
compound **3b**



compound **3c**



compound **3a**



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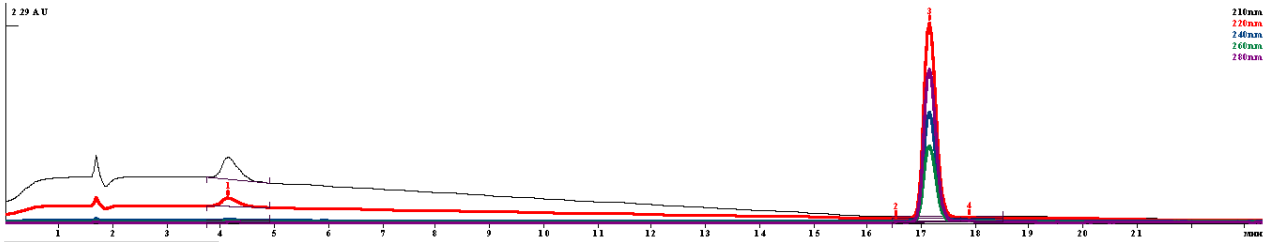
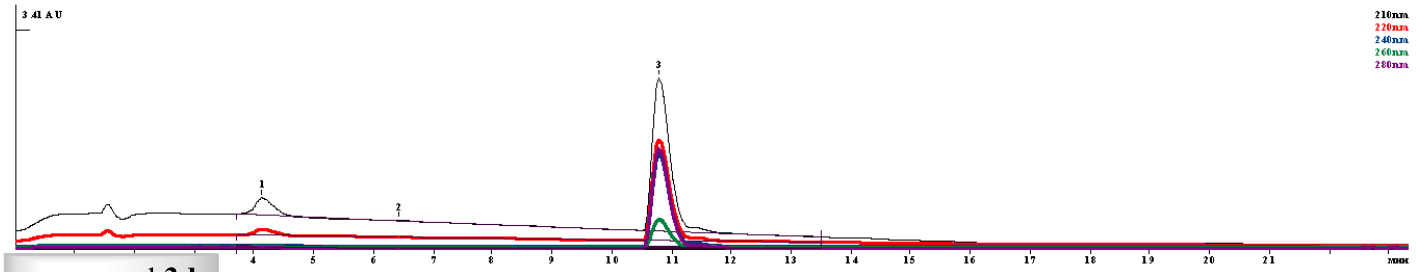
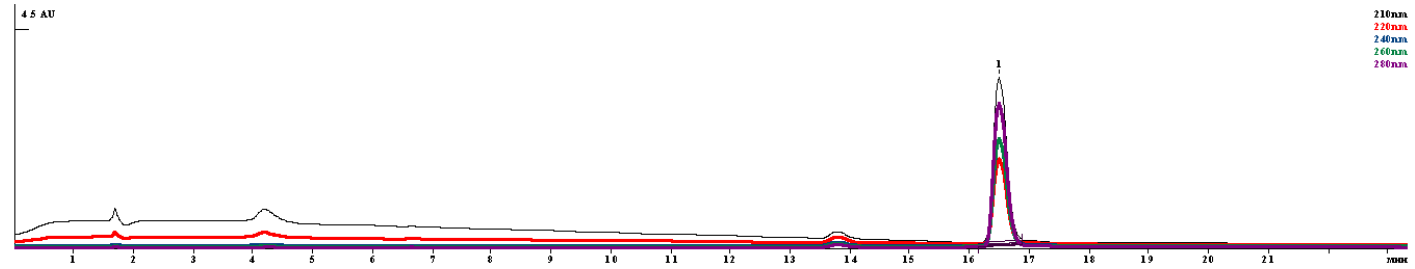


compound **4e**



compound **3d**



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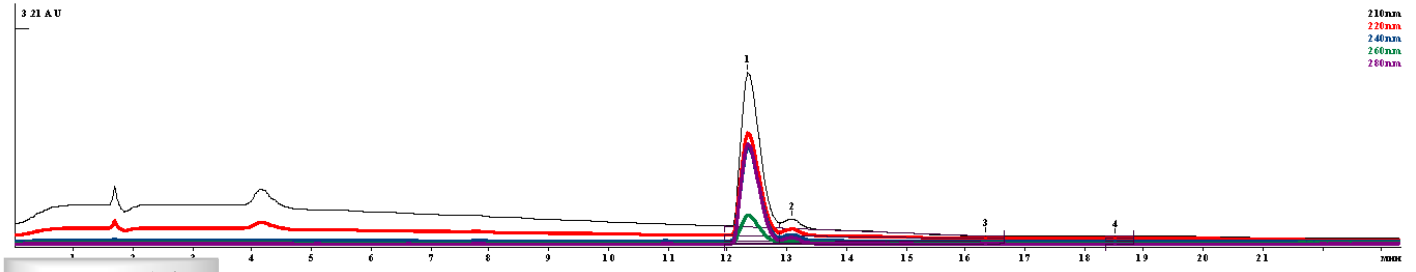

compound **4d**