Supplementary Material

Substitutional Diversity-Oriented Synthesis and In Vitro Anticancer Activity of Framework-Integrated Estradiol-Benzisoxazole Chimeras

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[2] Department of Biochemistry and Molecular Biology, Doctoral School of Biology, University of Szeged, Középfasor 52, H-6726 Szeged, Hungary
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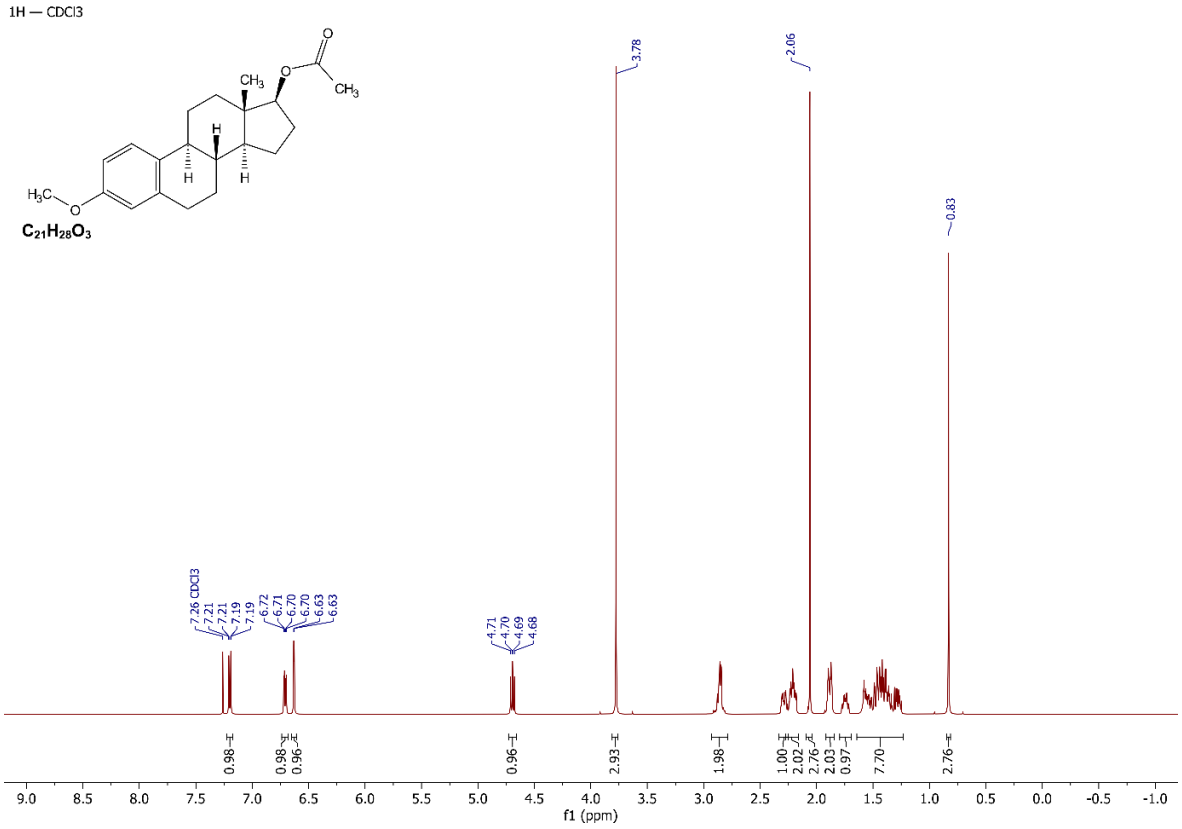
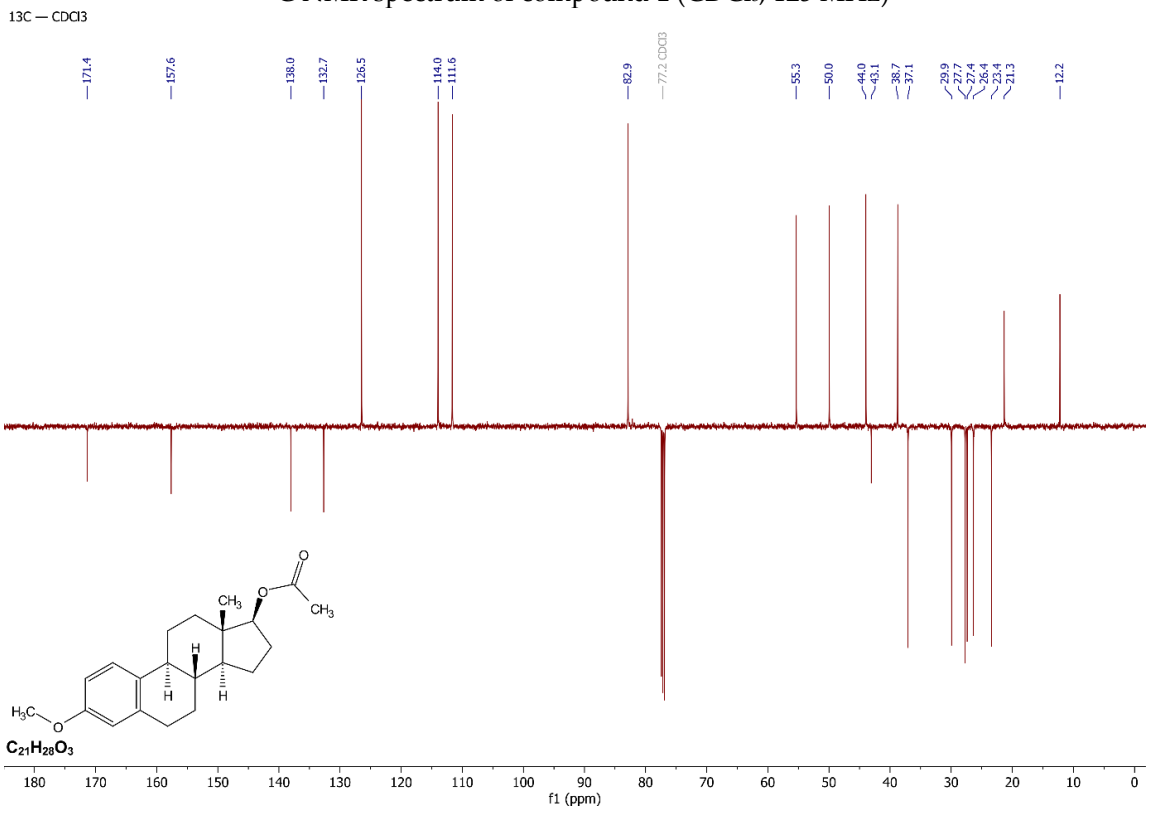
Dose-response curves *52-53*
List, sequence and appropriate concentrations of primers used in qRT-PCR *54*

^{1}H and ^{13}C NMR spectra of the synthesized compounds

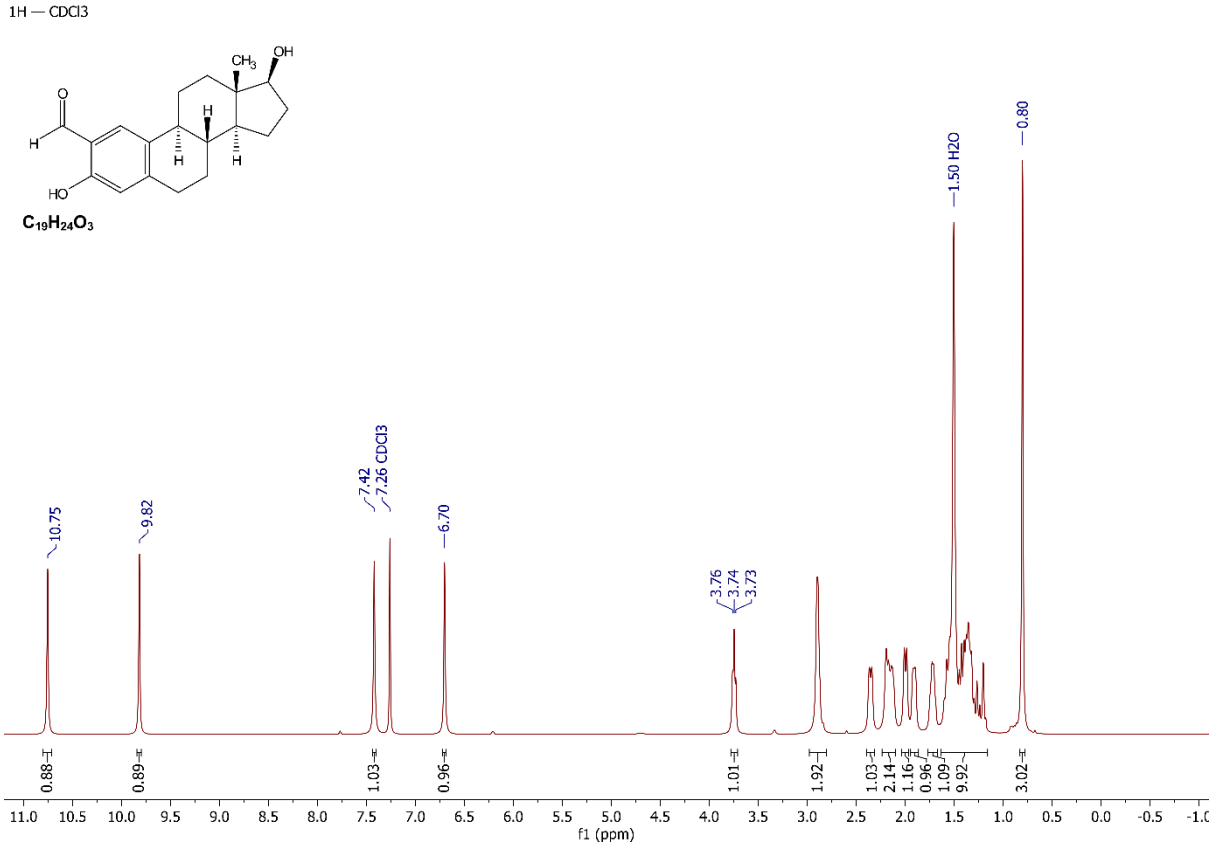
^{1}H NMR spectrum of compound **1** ($CDCl_3$, 500 MHz)



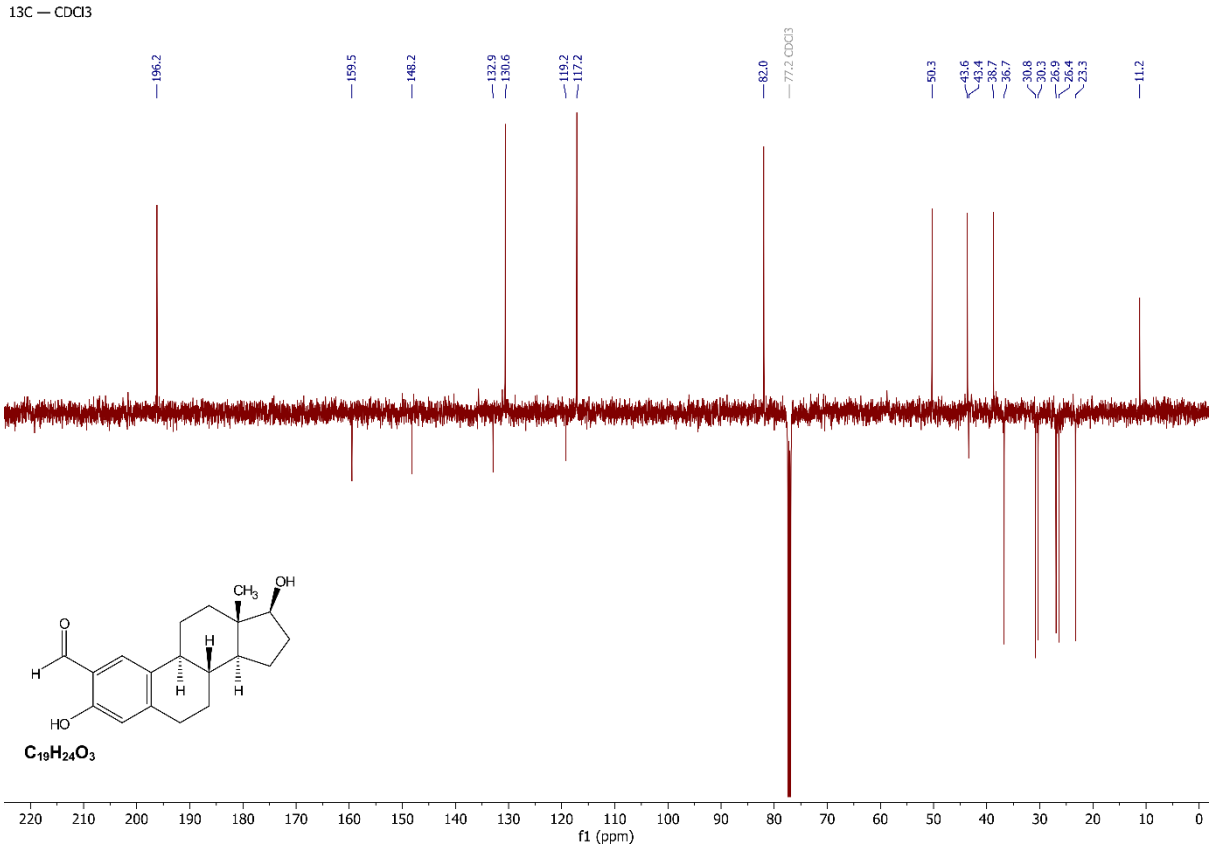
^{13}C NMR spectrum of compound **1** ($CDCl_3$, 125 MHz)



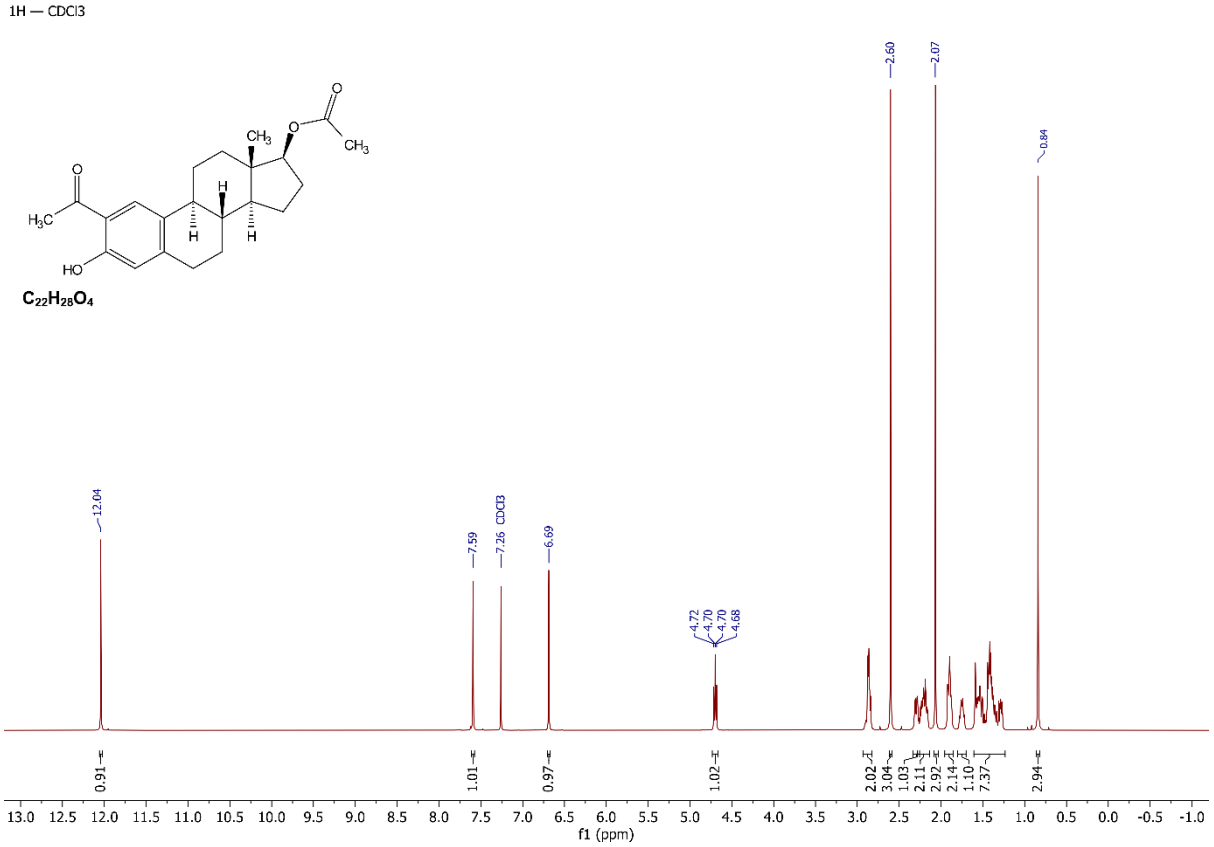
^{1}H NMR spectrum of compound **2a** ($CDCl_3$, 500 MHz)



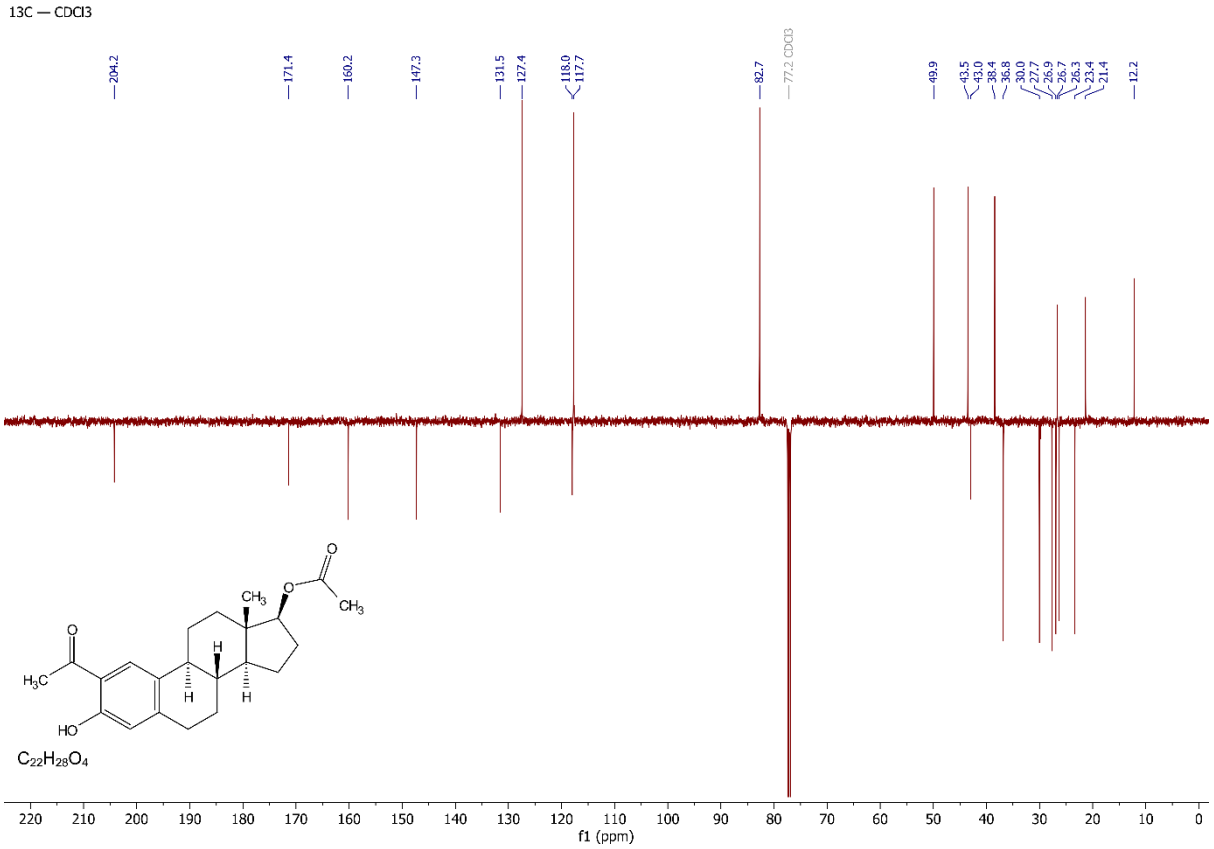
^{13}C NMR spectrum of compound **2a** ($CDCl_3$, 125 MHz)



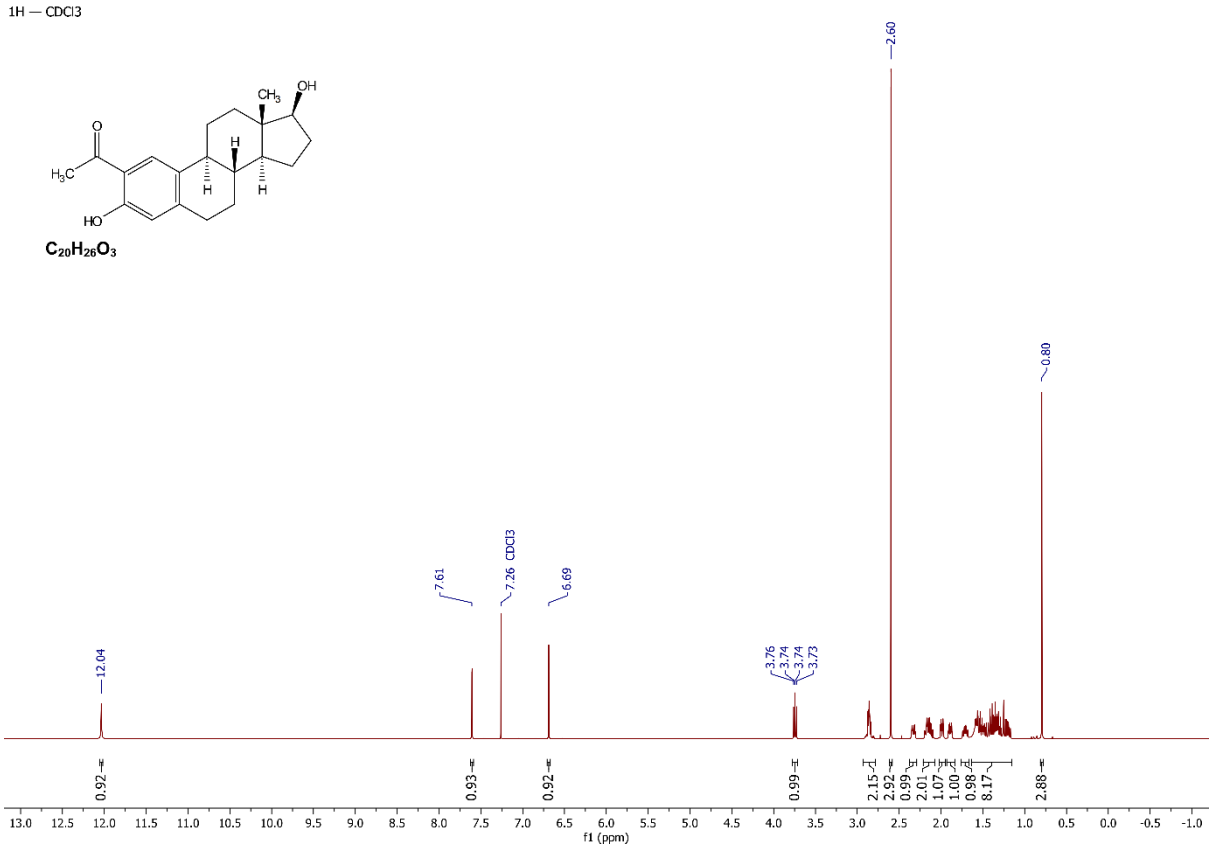
^{1}H NMR spectrum of compound **2b**-17Ac ($CDCl_3$, 500 MHz)



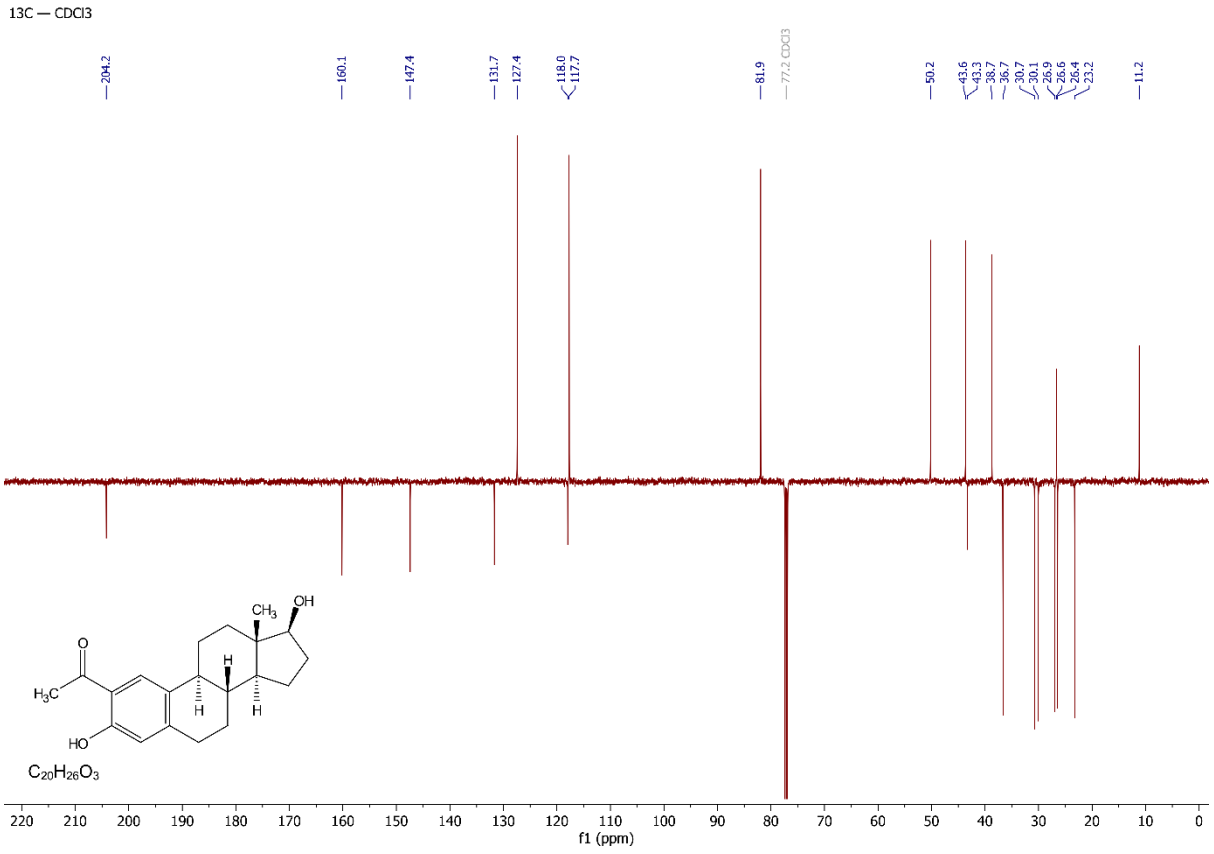
^{13}C NMR spectrum of compound **2b**-17Ac ($CDCl_3$, 125 MHz)



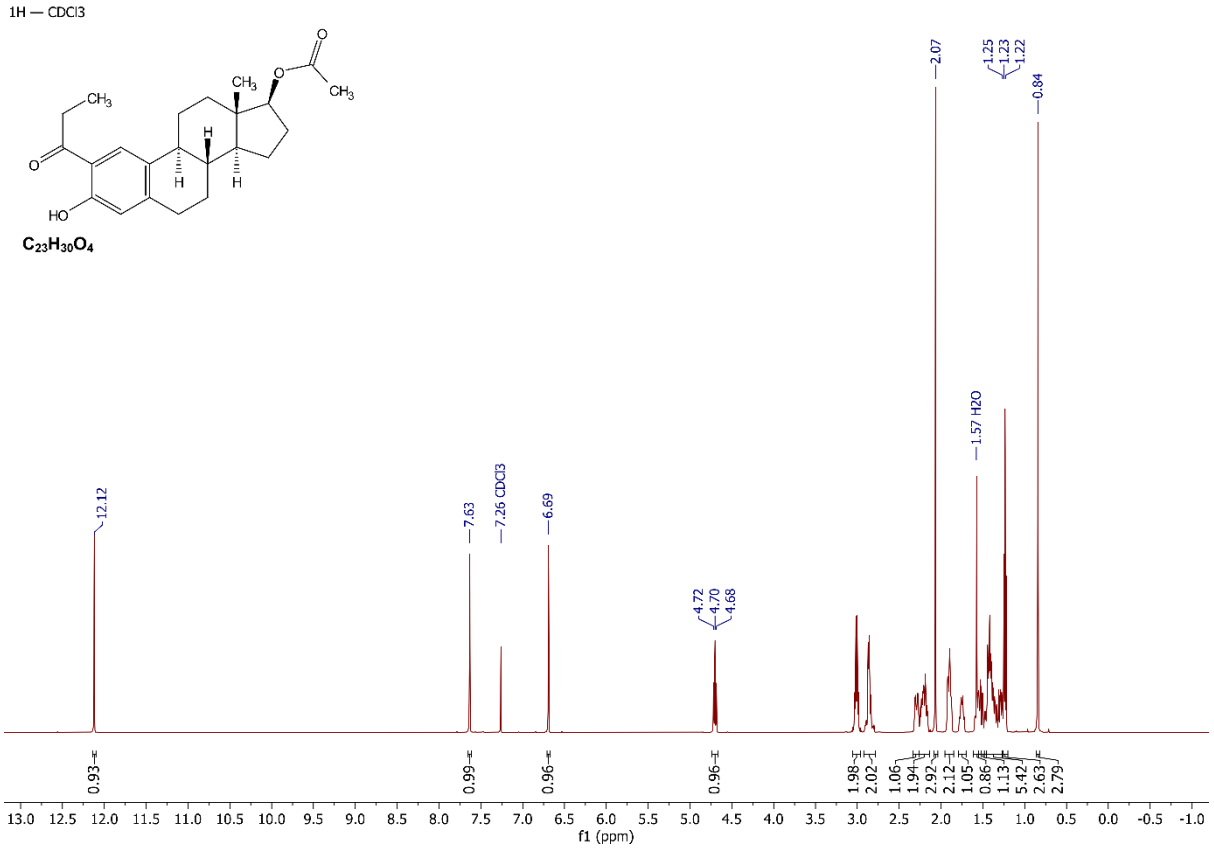
^{1}H NMR spectrum of compound **2b** ($CDCl_3$, 500 MHz)



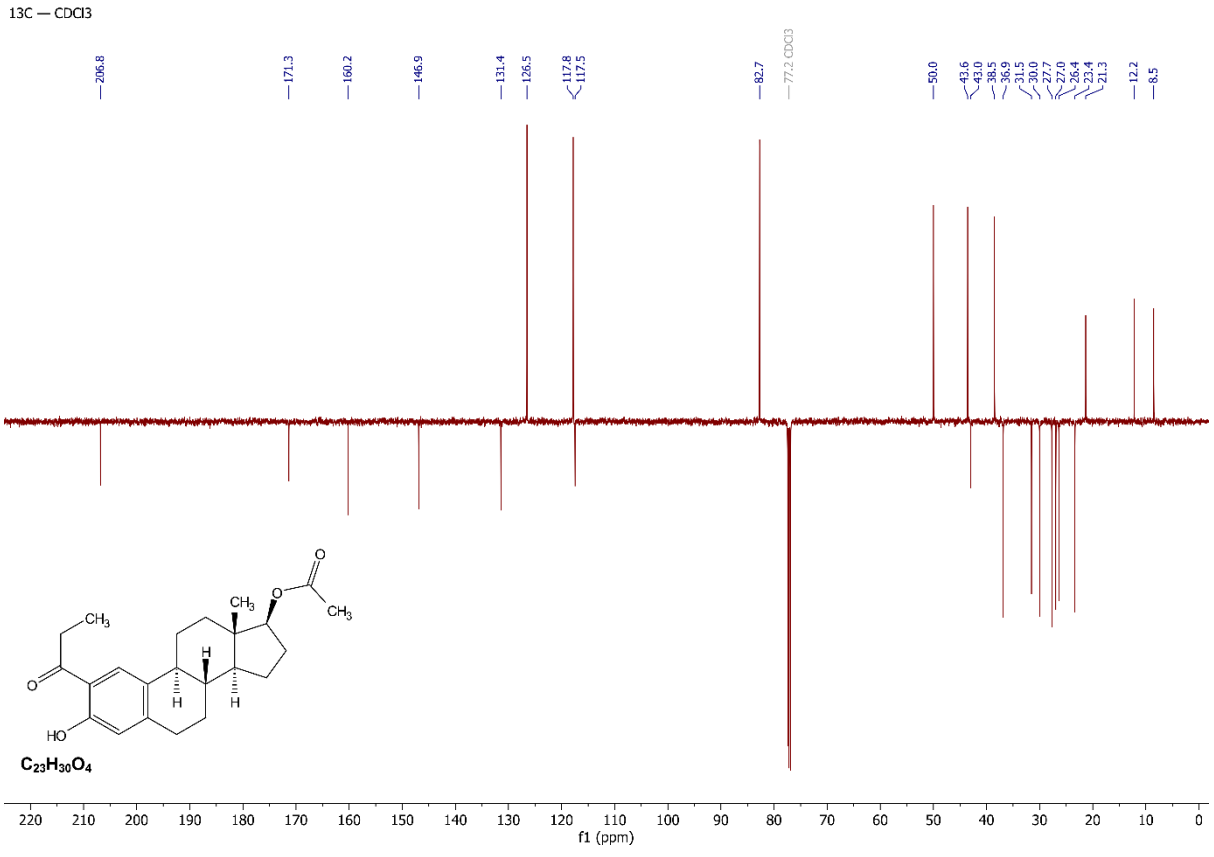
^{13}C NMR spectrum of compound **2b** ($CDCl_3$, 125 MHz)



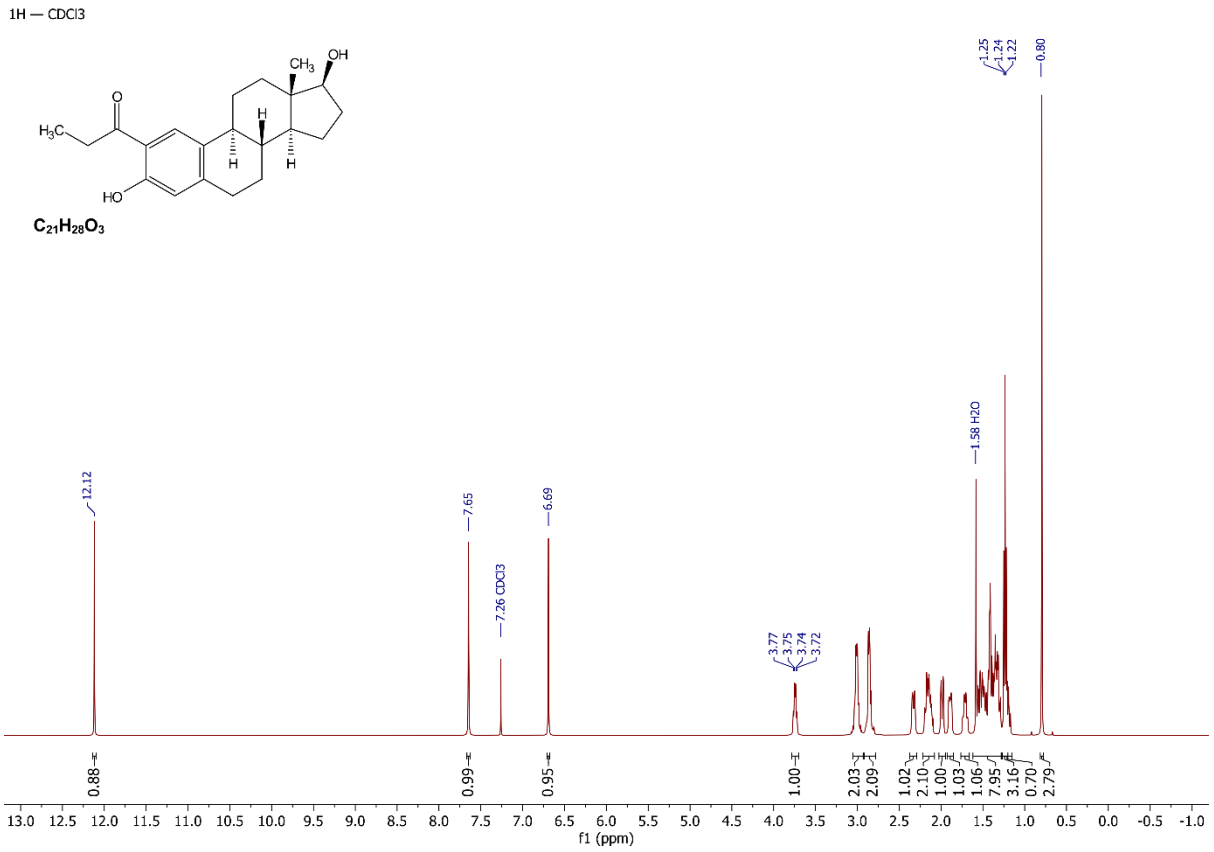
^{1}H NMR spectrum of compound **2c**-17Ac ($CDCl_3$, 500 MHz)



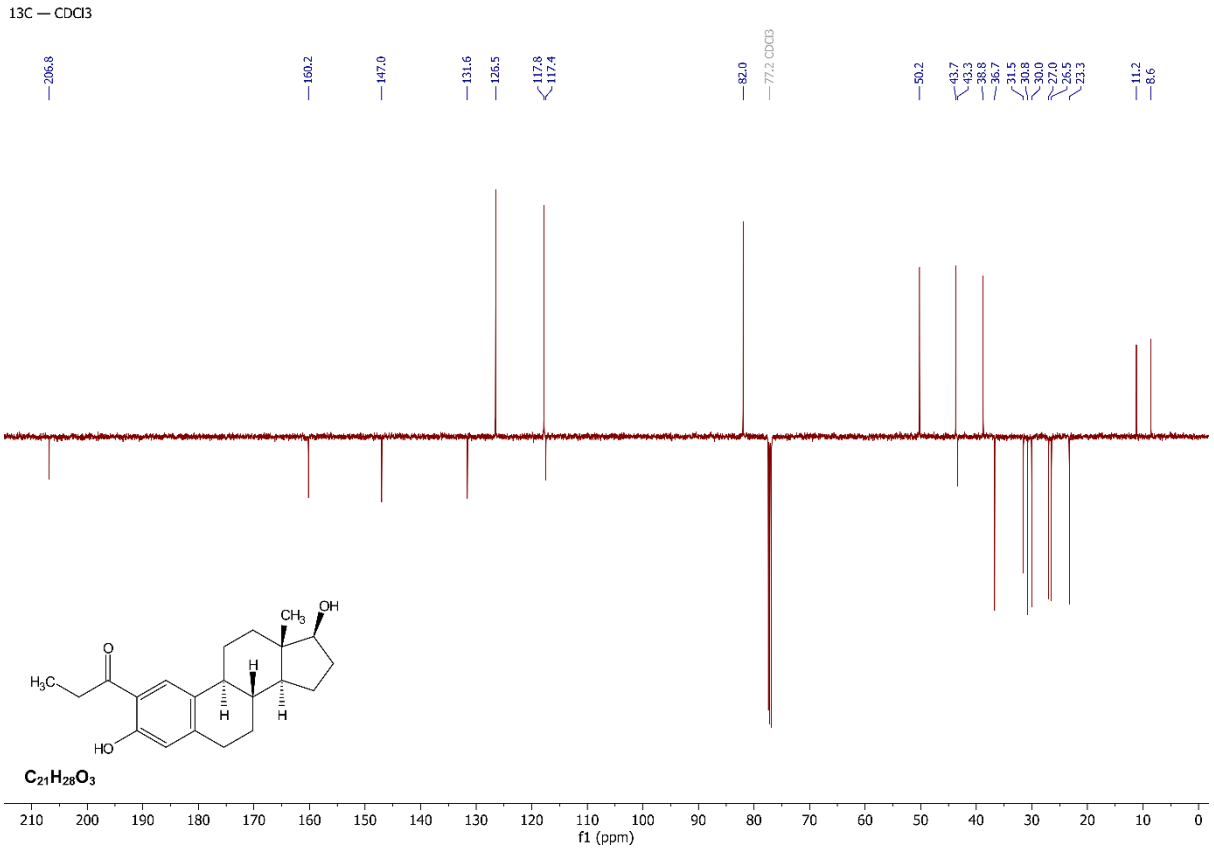
^{13}C NMR spectrum of compound **2c**-17Ac ($CDCl_3$, 125 MHz)



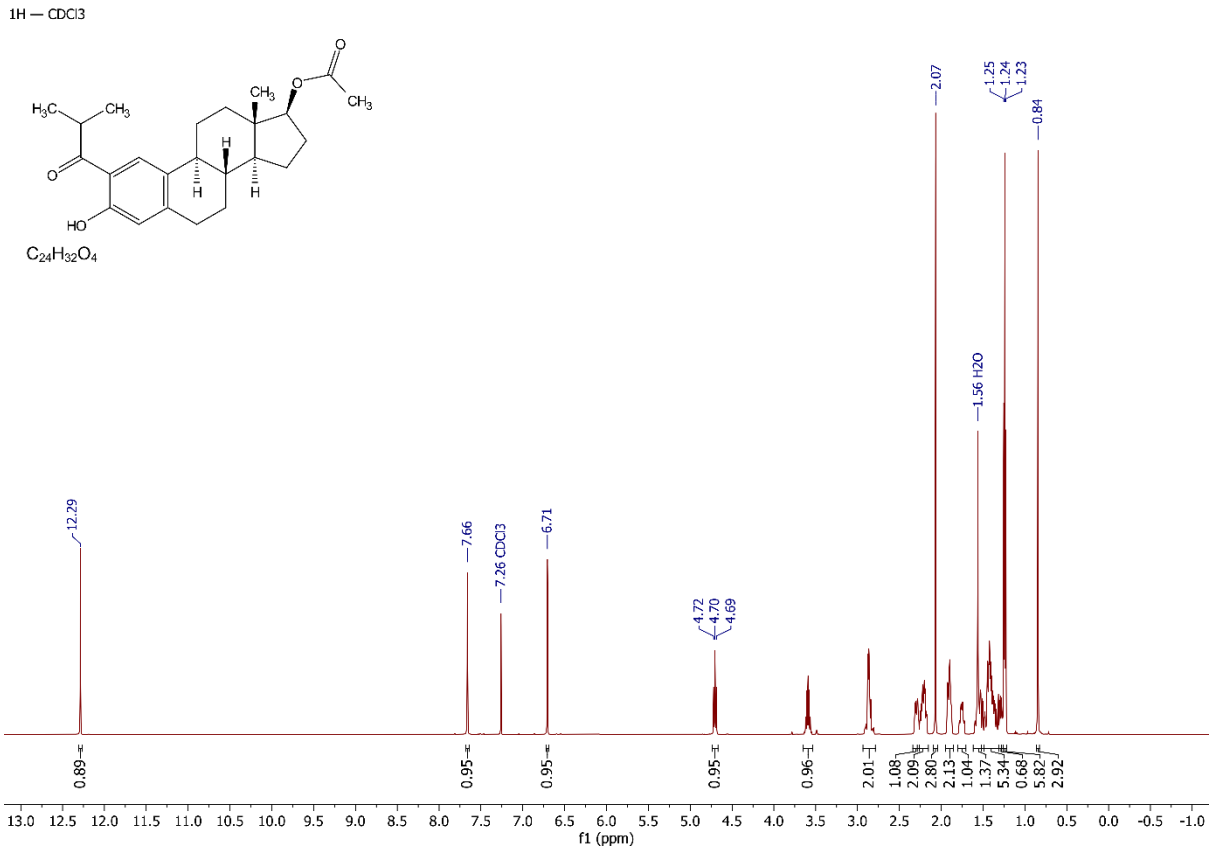
^{1}H NMR spectrum of compound **2c** ($CDCl_3$, 500 MHz)



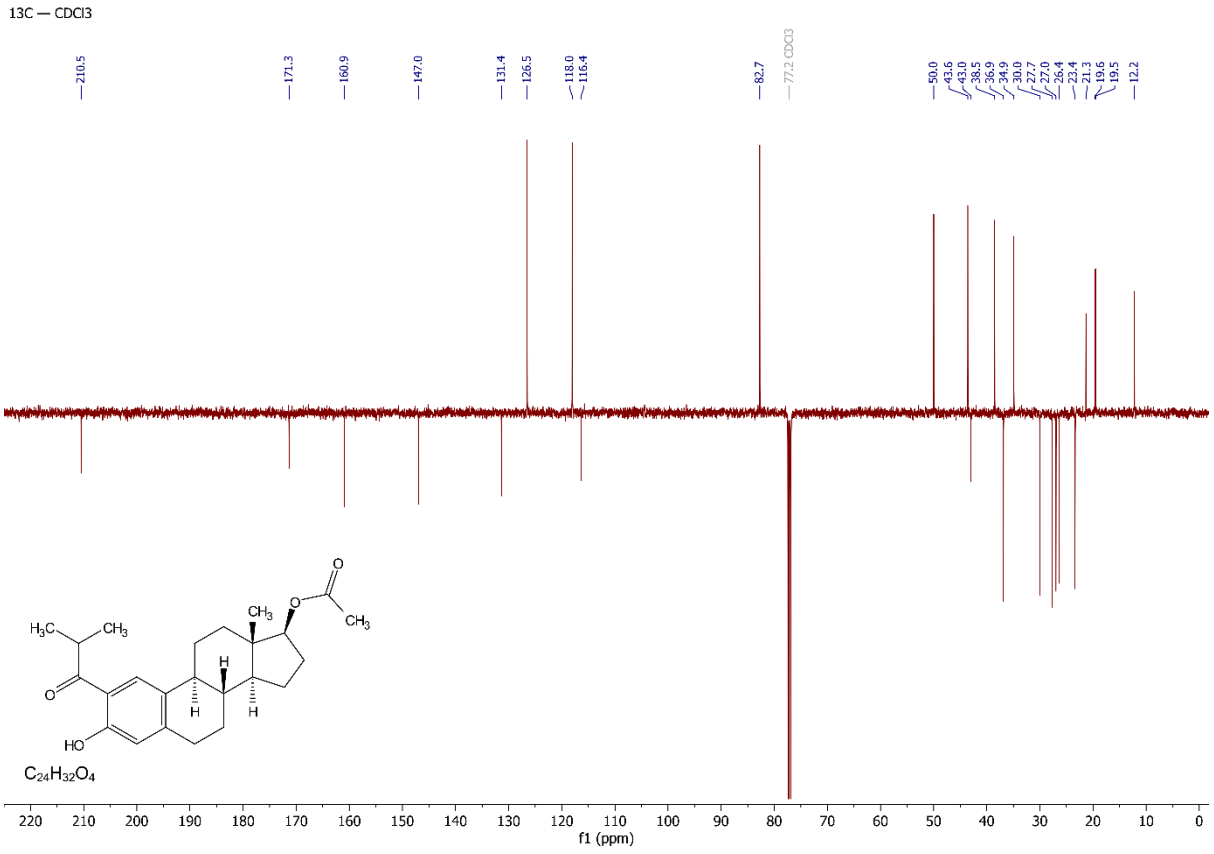
^{13}C NMR spectrum of compound **2c** ($CDCl_3$, 125 MHz)



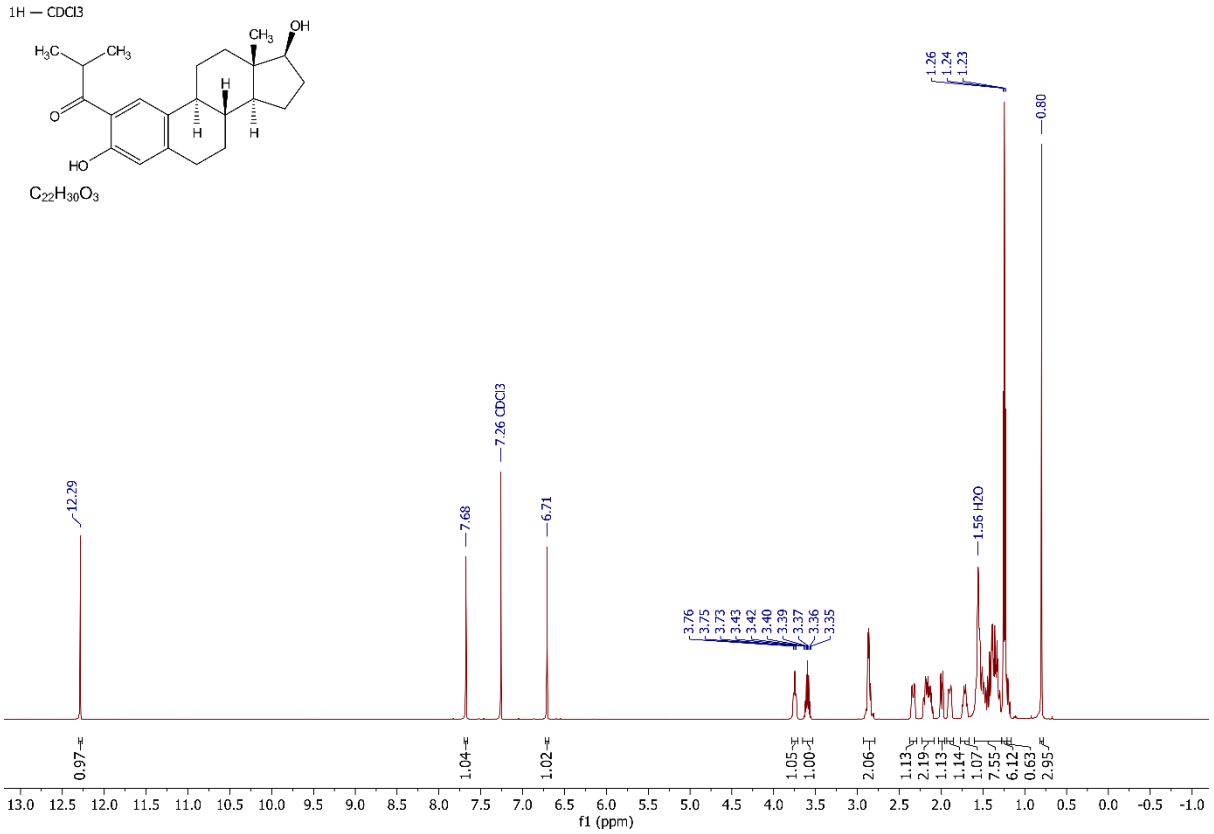
^{1}H NMR spectrum of compound **2d-**17Ac ($CDCl_3$, 500 MHz)



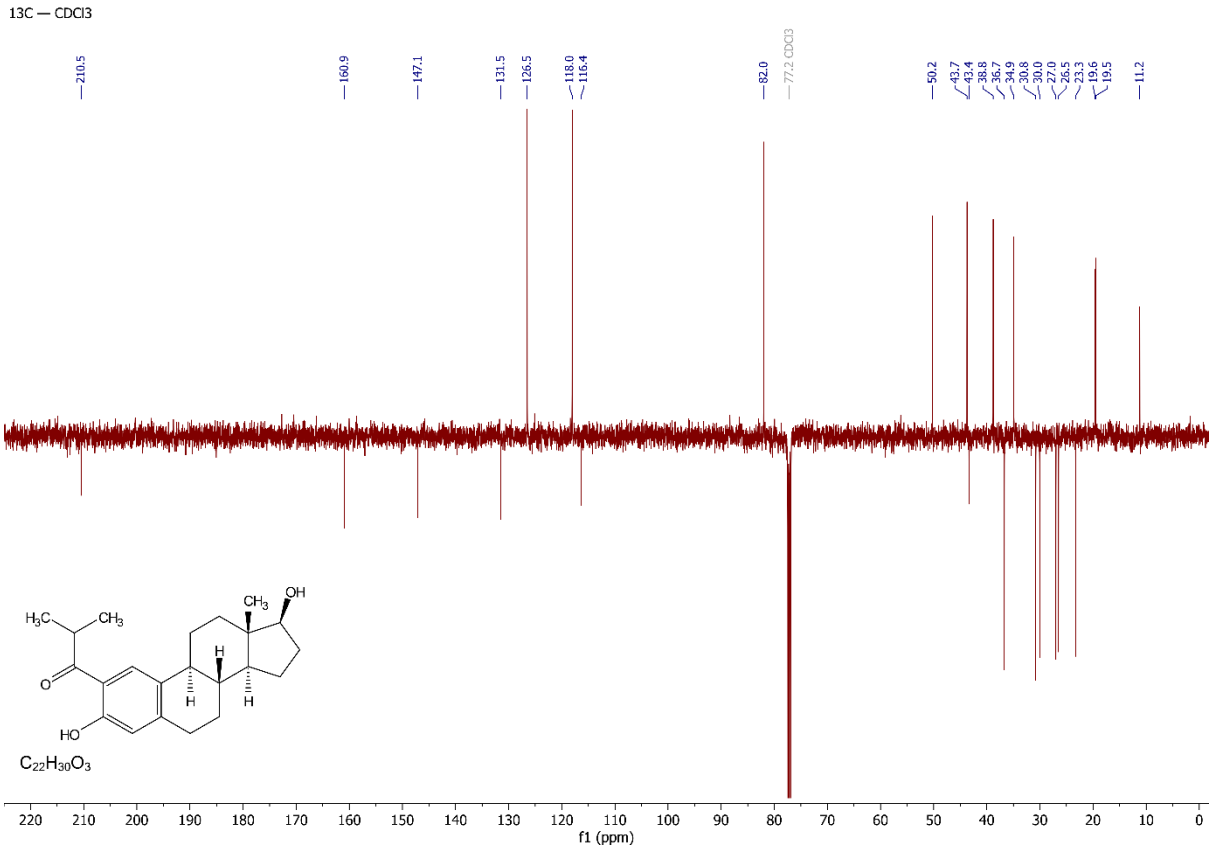
^{13}C NMR spectrum of compound **2d-**Ac ($CDCl_3$, 125 MHz)



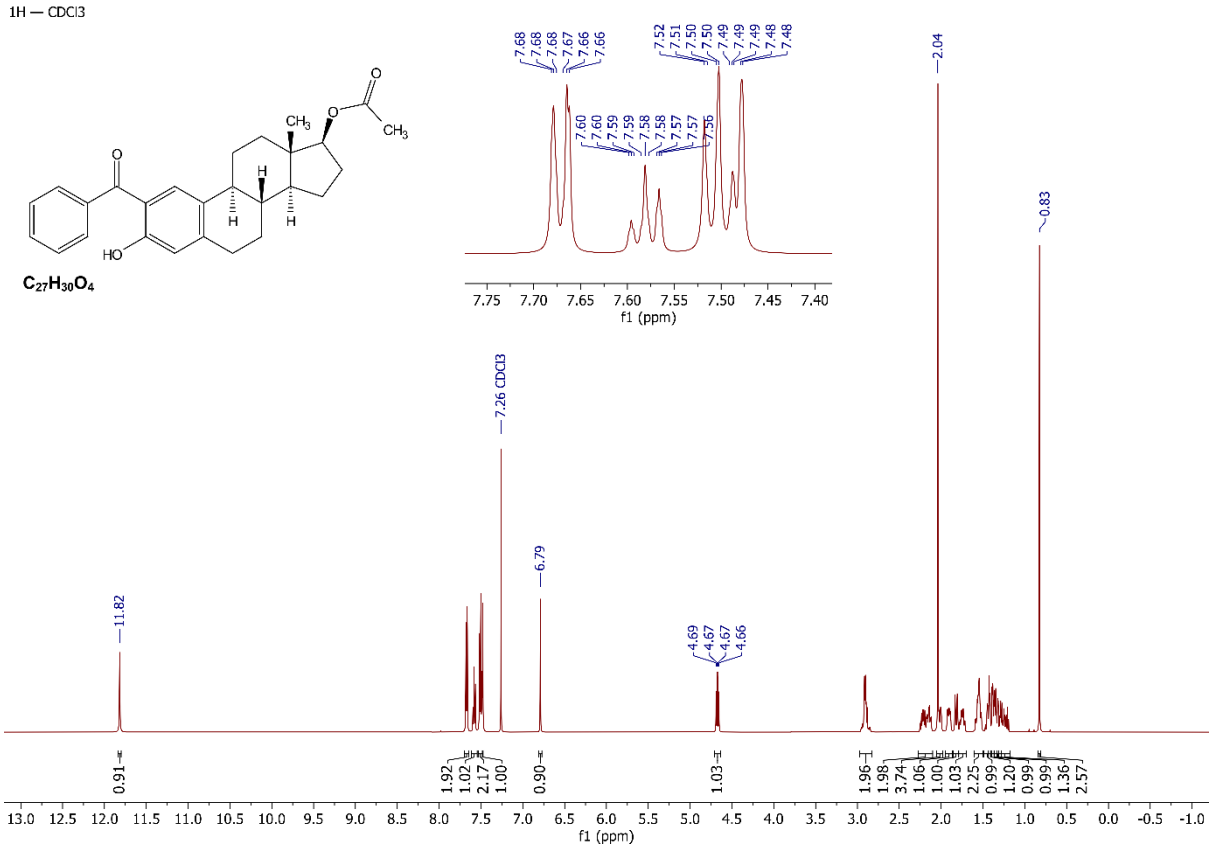
^{1}H NMR spectrum of compound **2d** ($CDCl_3$, 500 MHz)



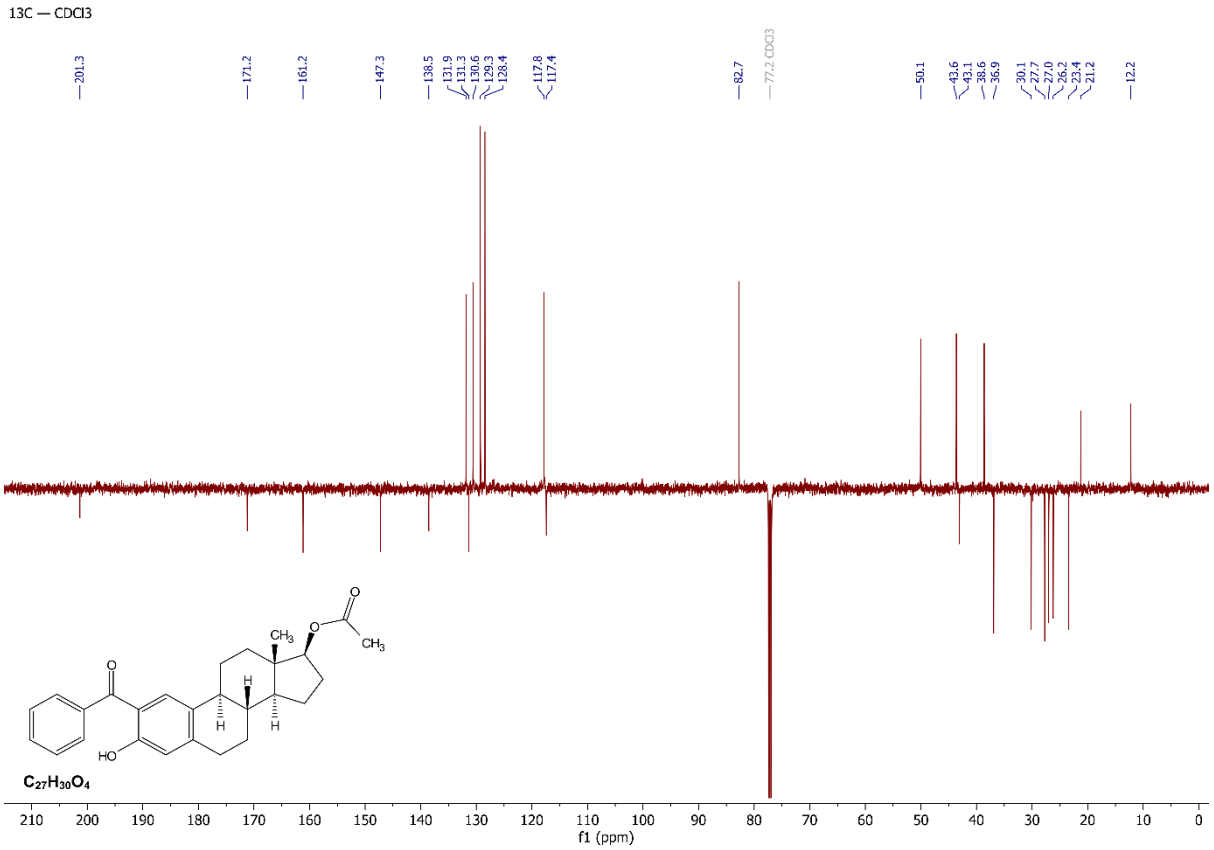
^{13}C NMR spectrum of compound **2d** ($CDCl_3$, 125 MHz)



^{1}H NMR spectrum of compound **2e**-17Ac ($CDCl_3$, 500 MHz)



^{13}C NMR spectrum of compound **2e**-17Ac ($CDCl_3$, 125 MHz)



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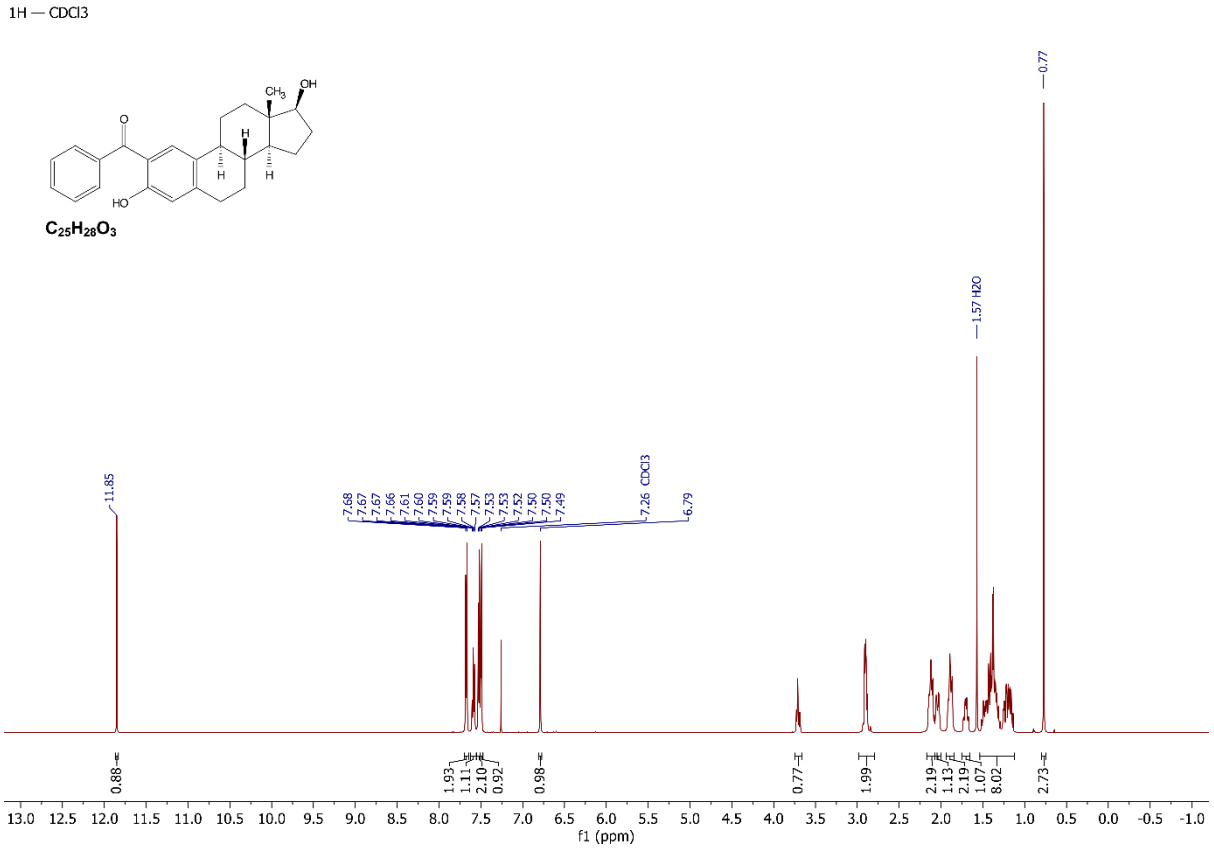
^{1}H NMR spectrum of compound **2e** ($CDCl_3$, 500 MHz)



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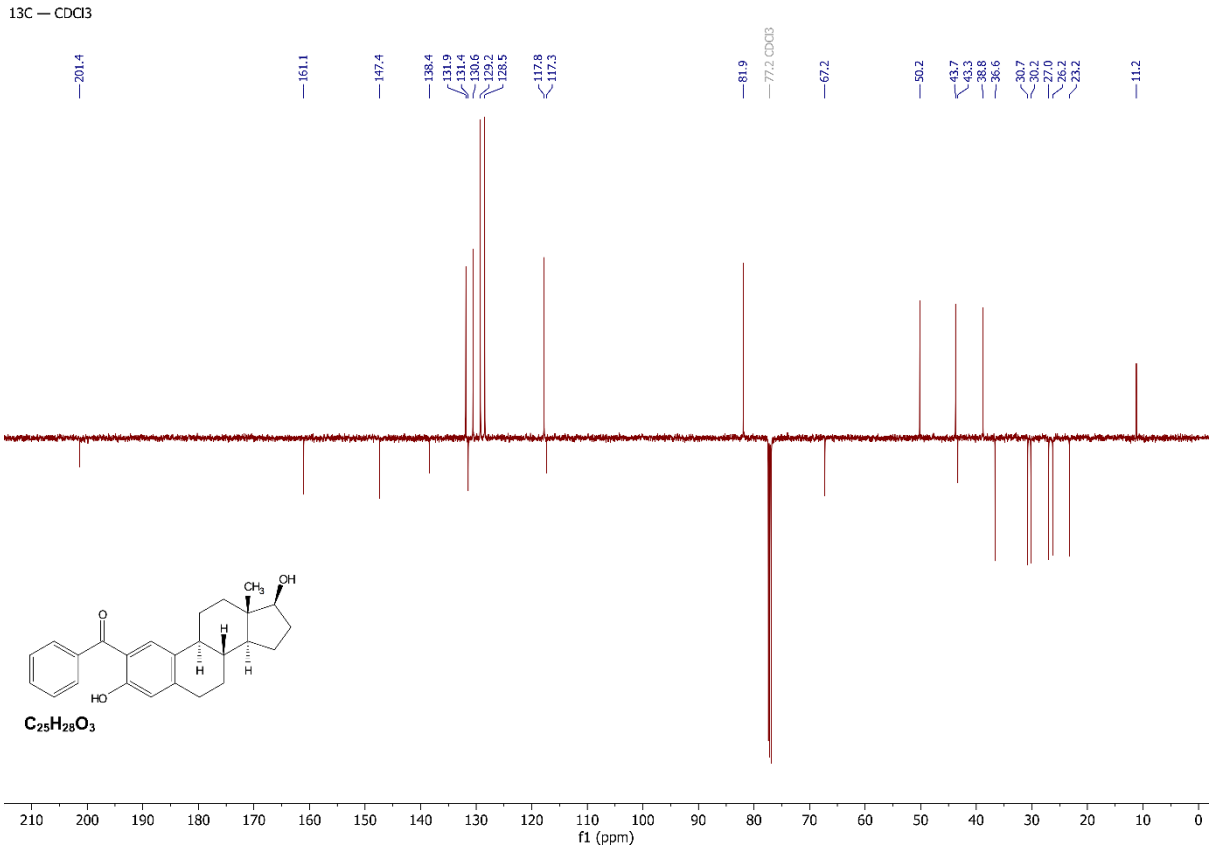
79

^{13}C NMR spectrum of compound **2e** ($CDCl_3$, 125 MHz)



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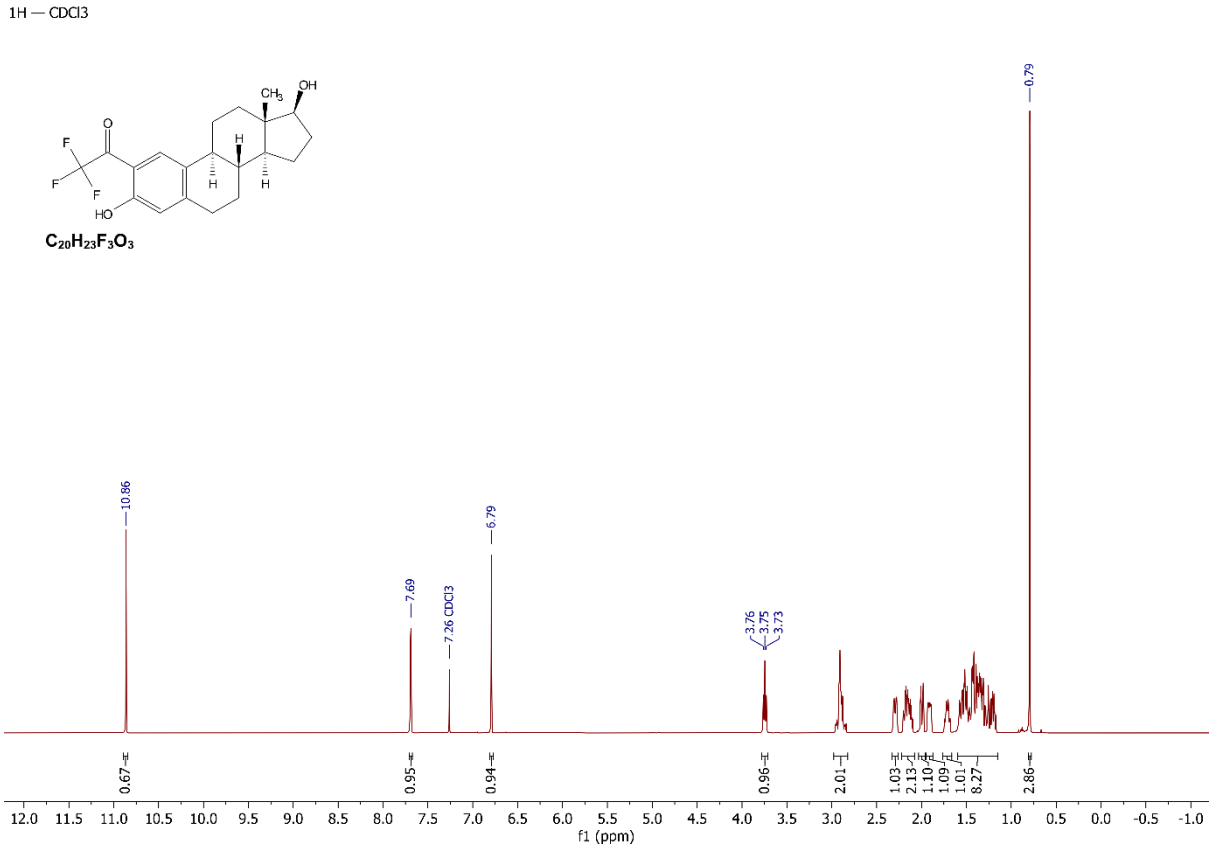
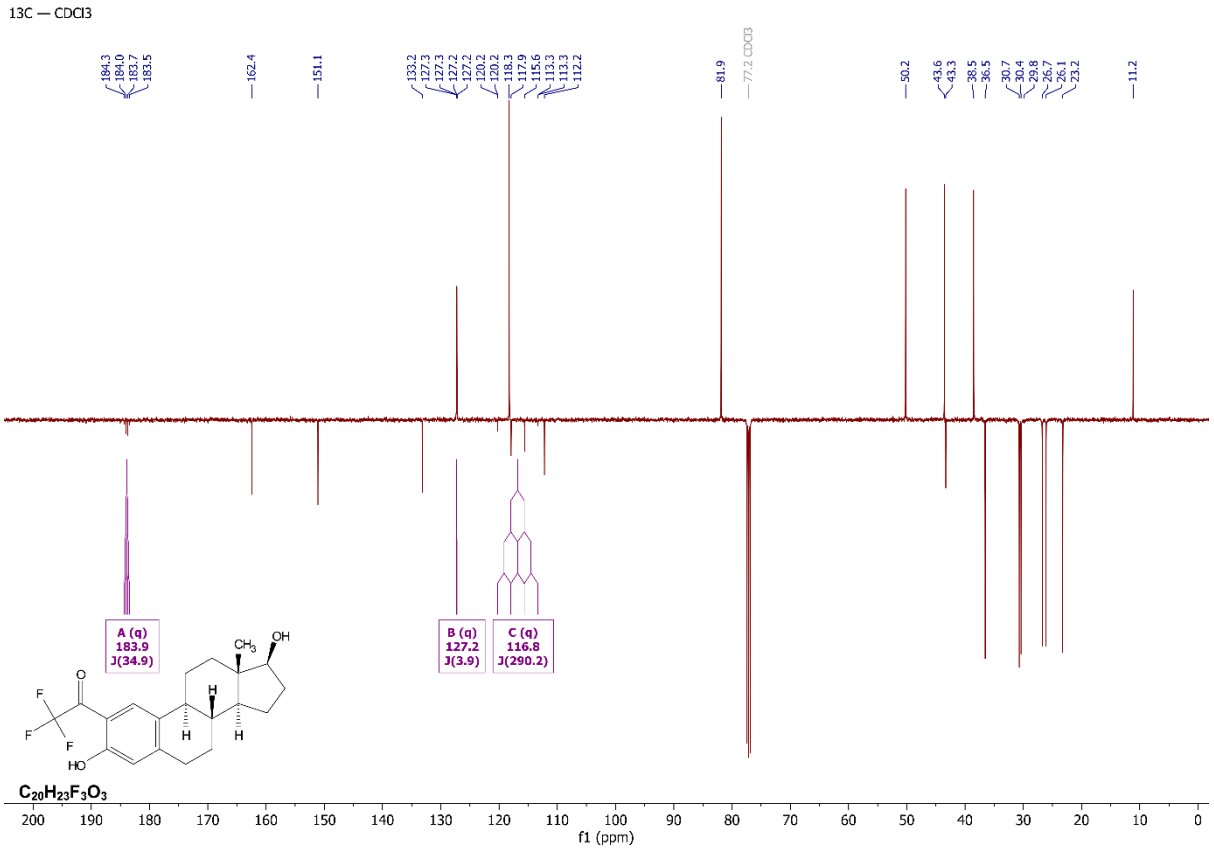
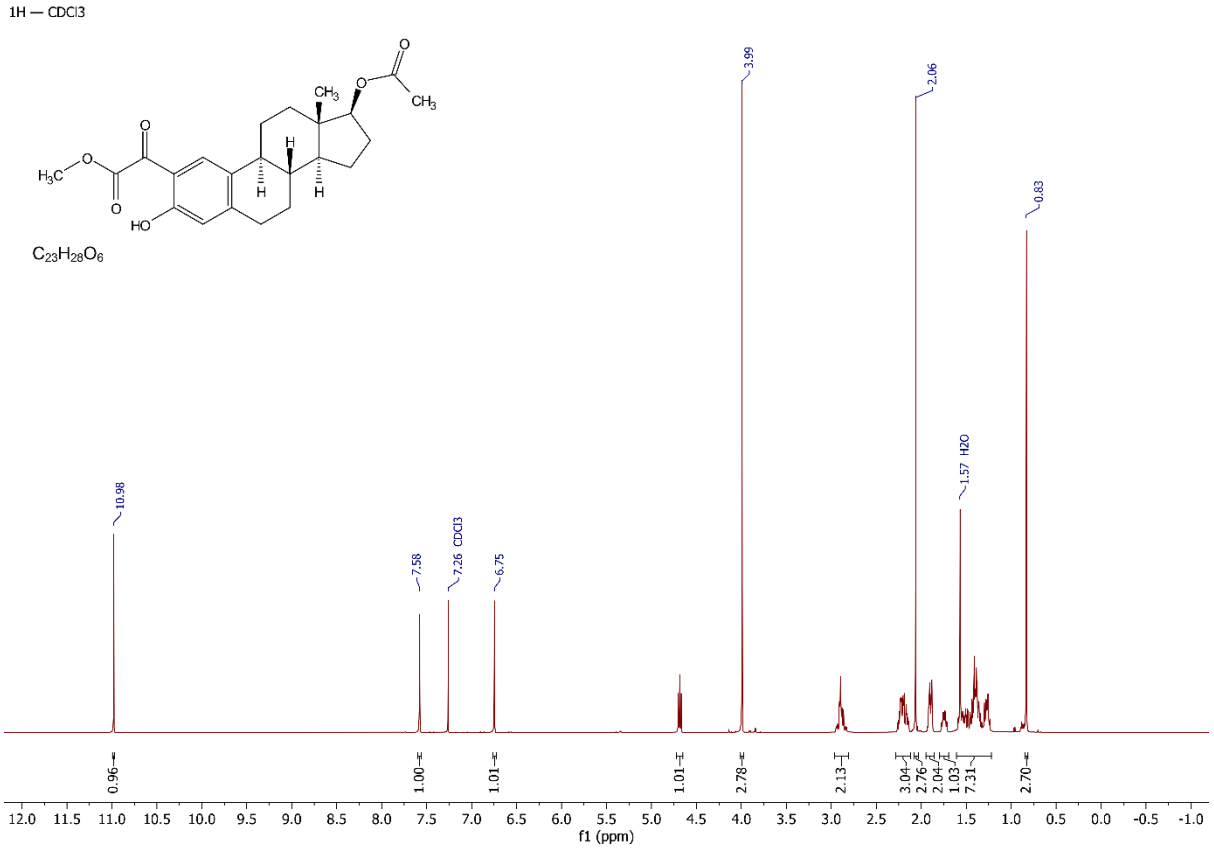
^{1}H NMR spectrum of compound **2f** ($CDCl_3$, 500 MHz)



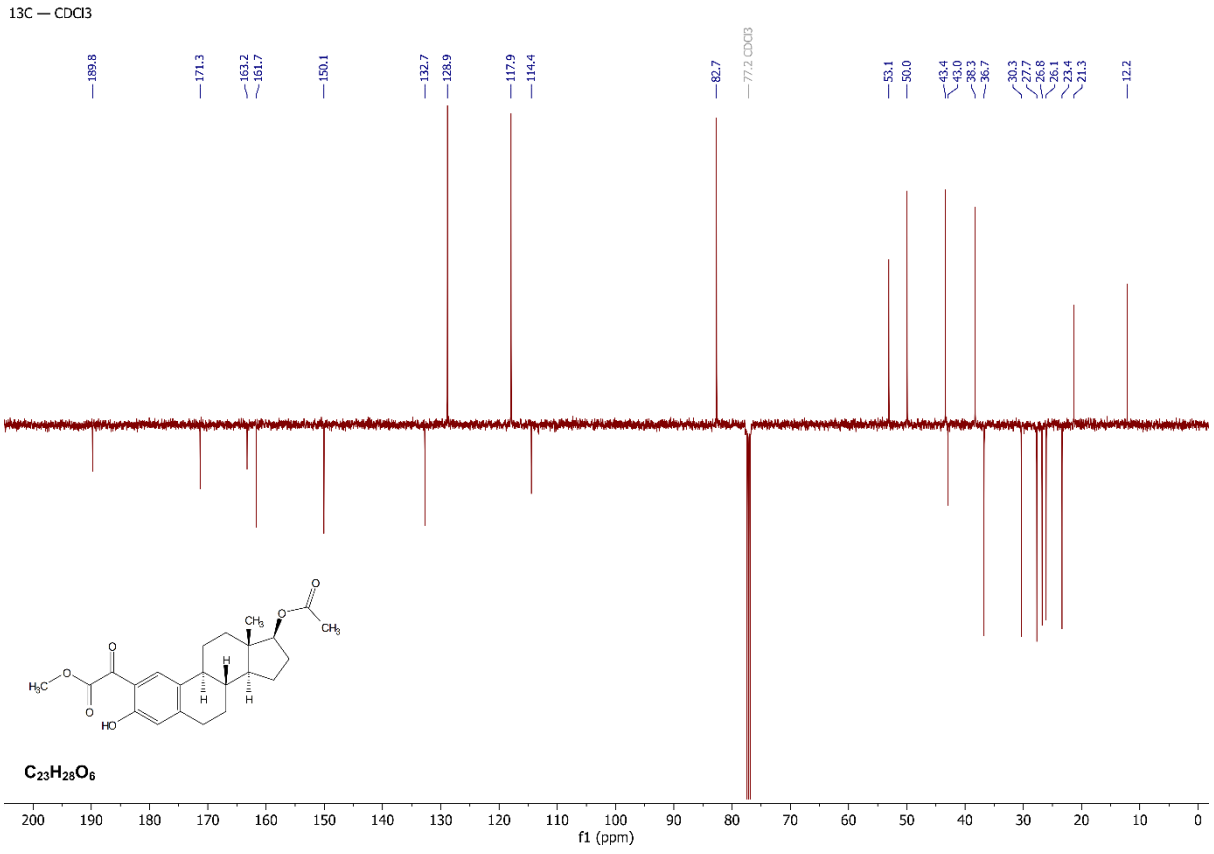
^{13}C NMR spectrum of compound **2f** ($CDCl_3$, 125 MHz)



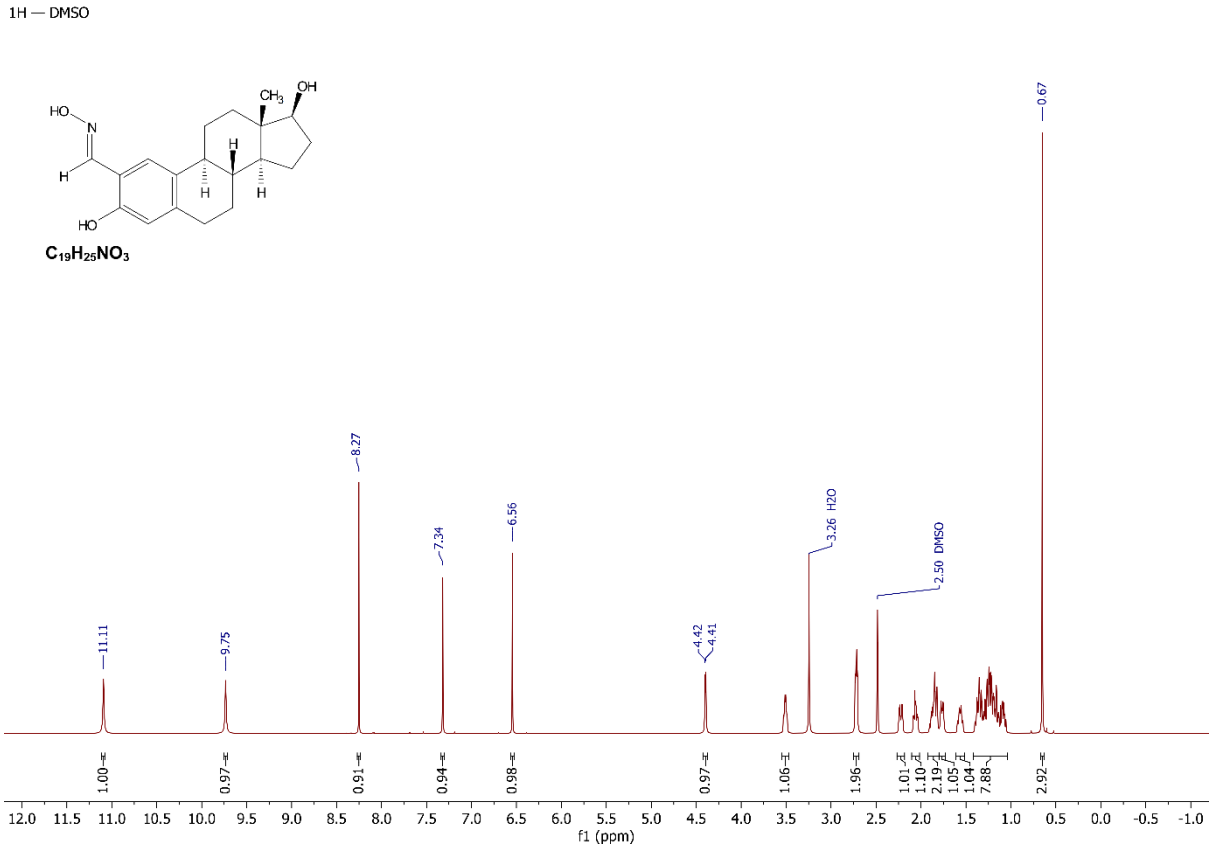
^{1}H NMR spectrum of compound **2g** ($CDCl_3$, 500 MHz)



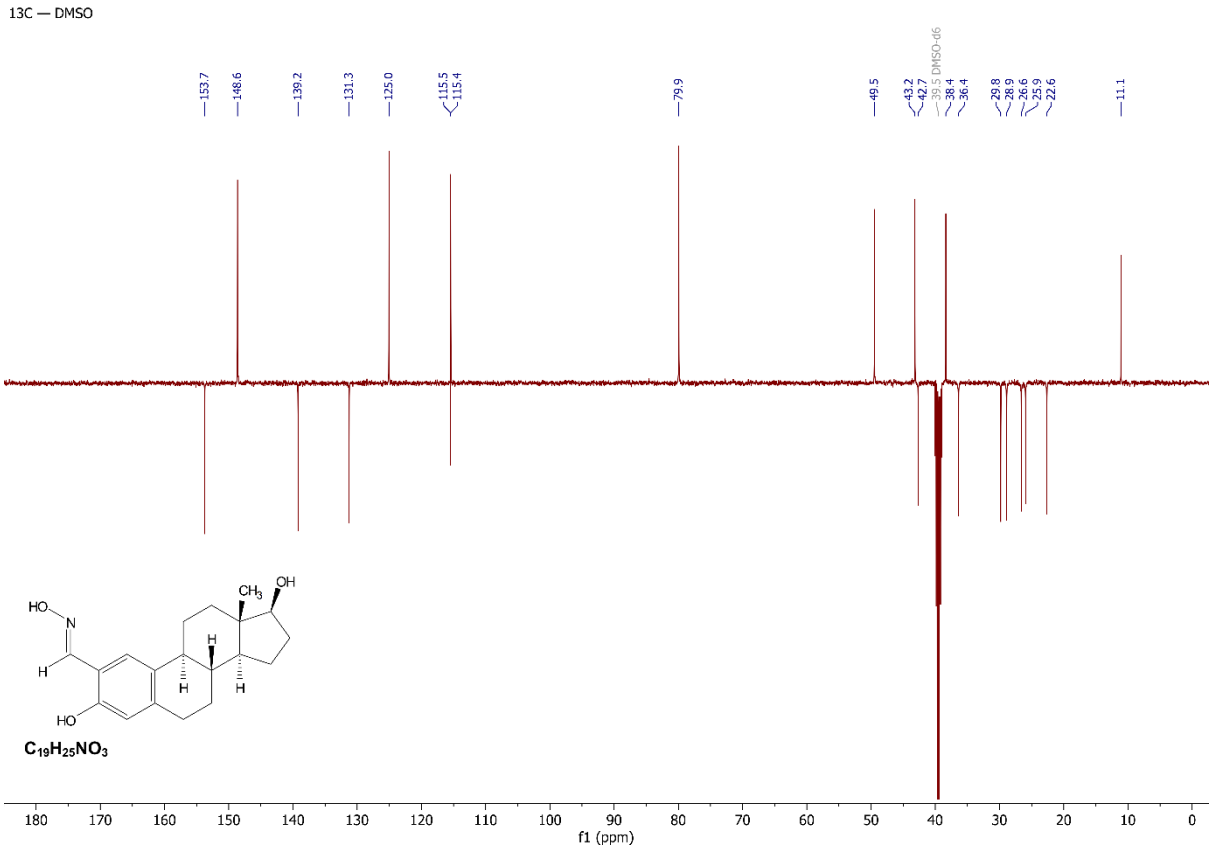
^{13}C NMR spectrum of compound **2g** ($CDCl_3$, 125 MHz)



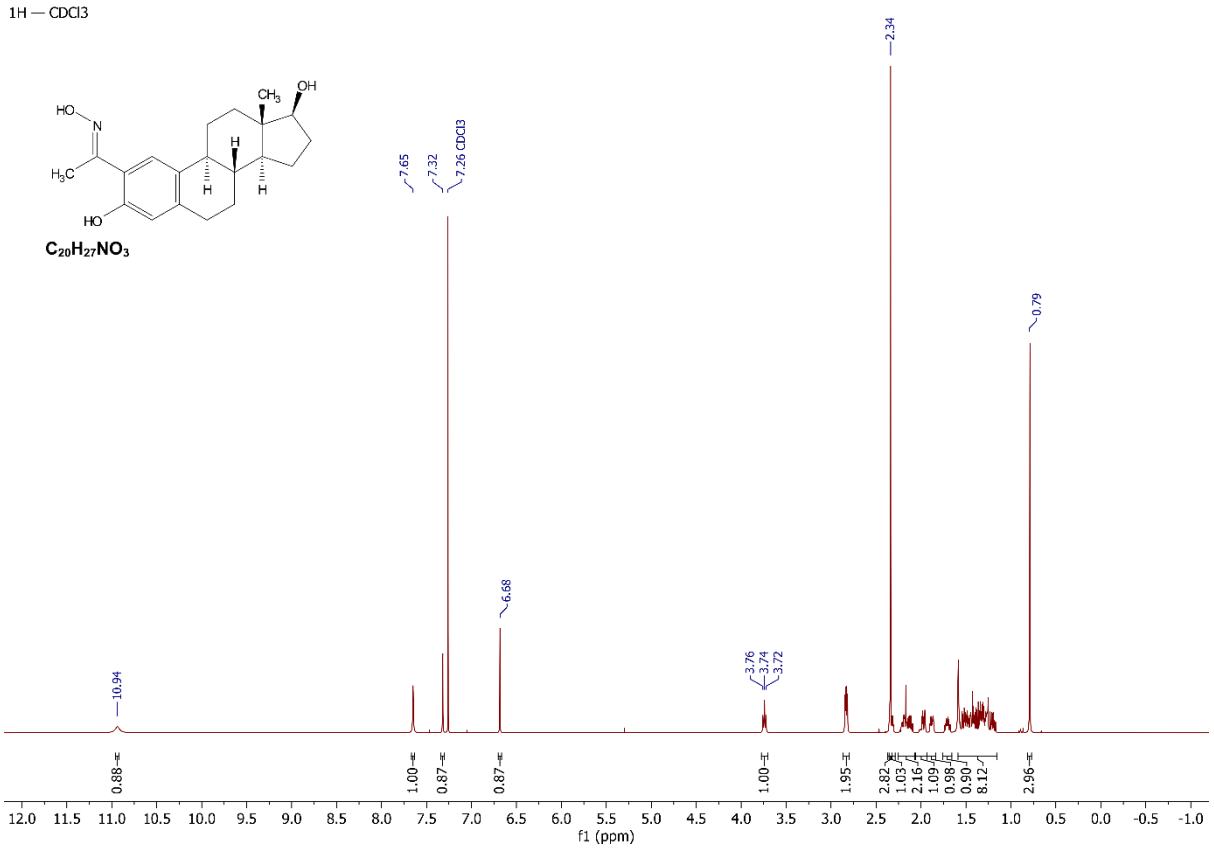
^{1}H NMR spectrum of compound **3a** (DMSO-d_6, 500 MHz)



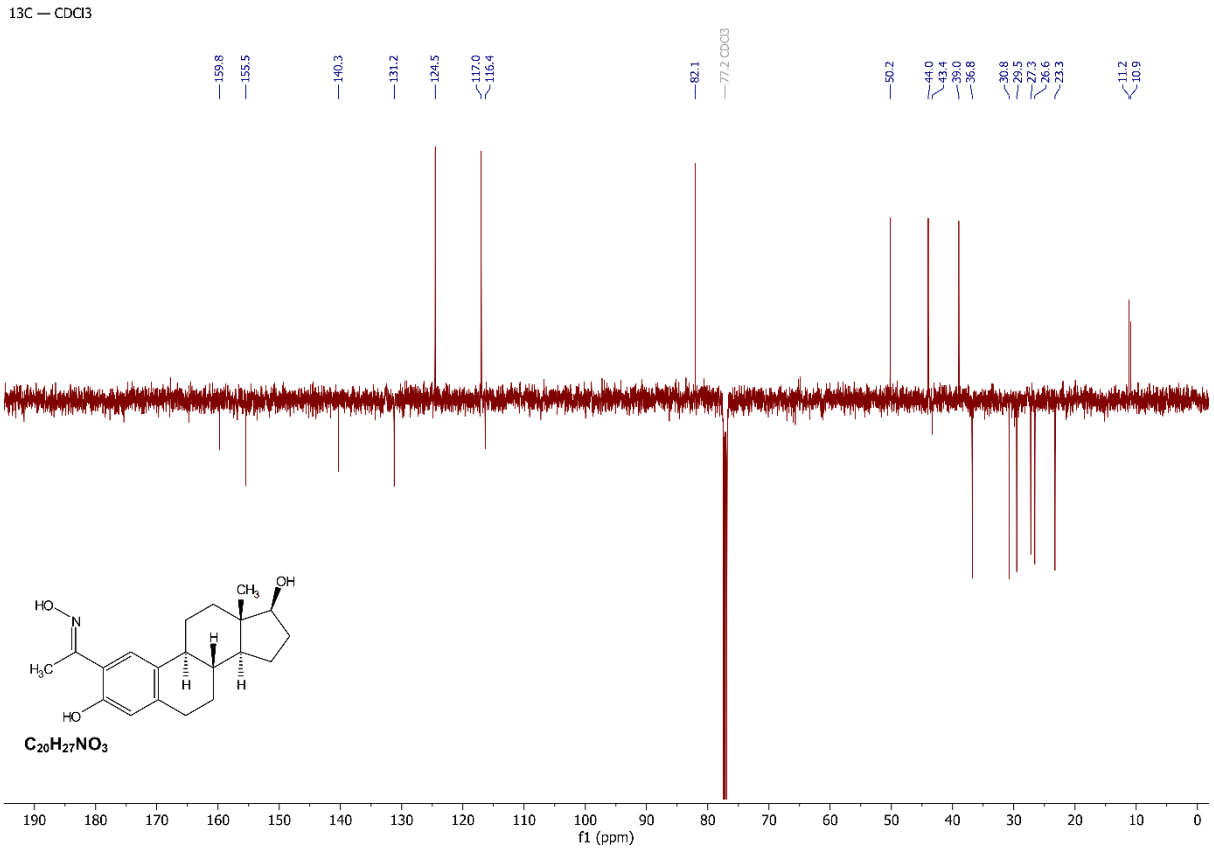
^{13}C NMR spectrum of compound **3a** (DMSO-d_6, 125 MHz)



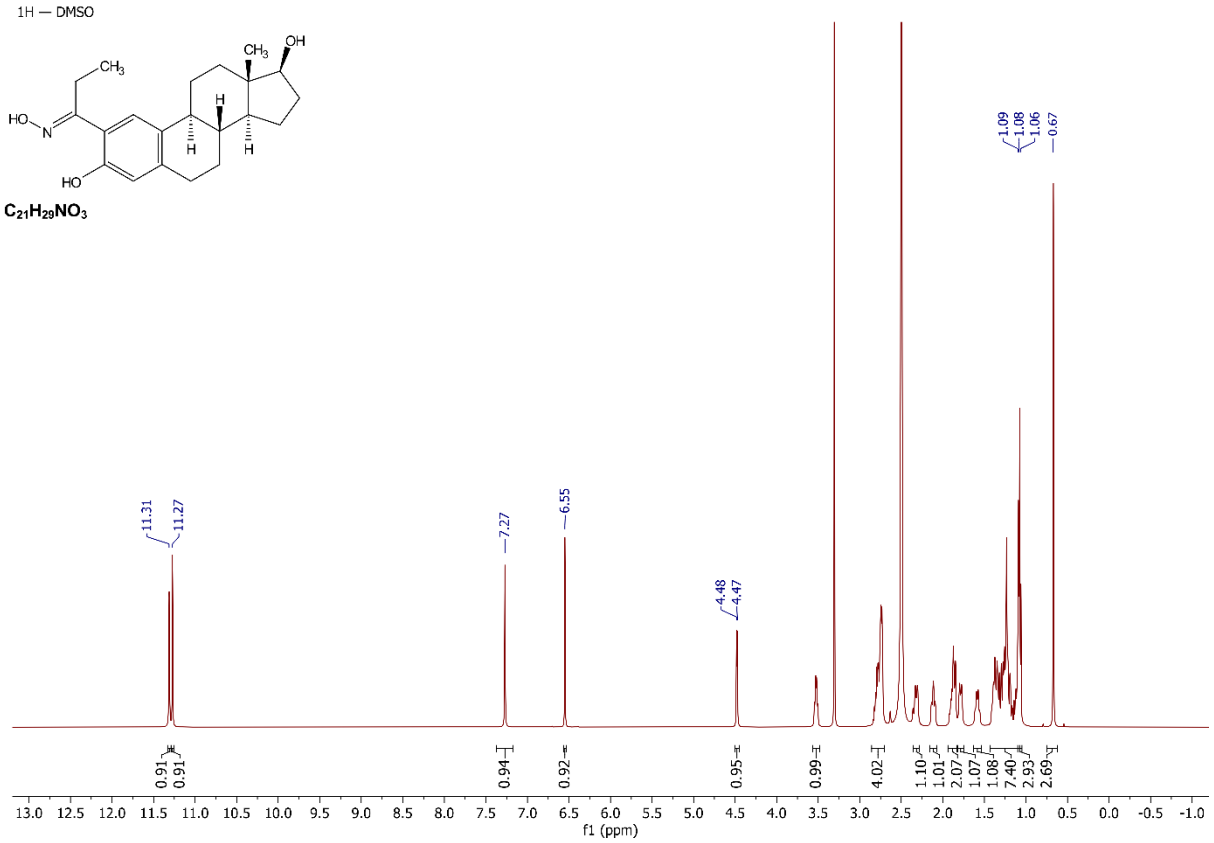
^{1}H NMR spectrum of compound **3b** ($CDCl_3$, 500 MHz)



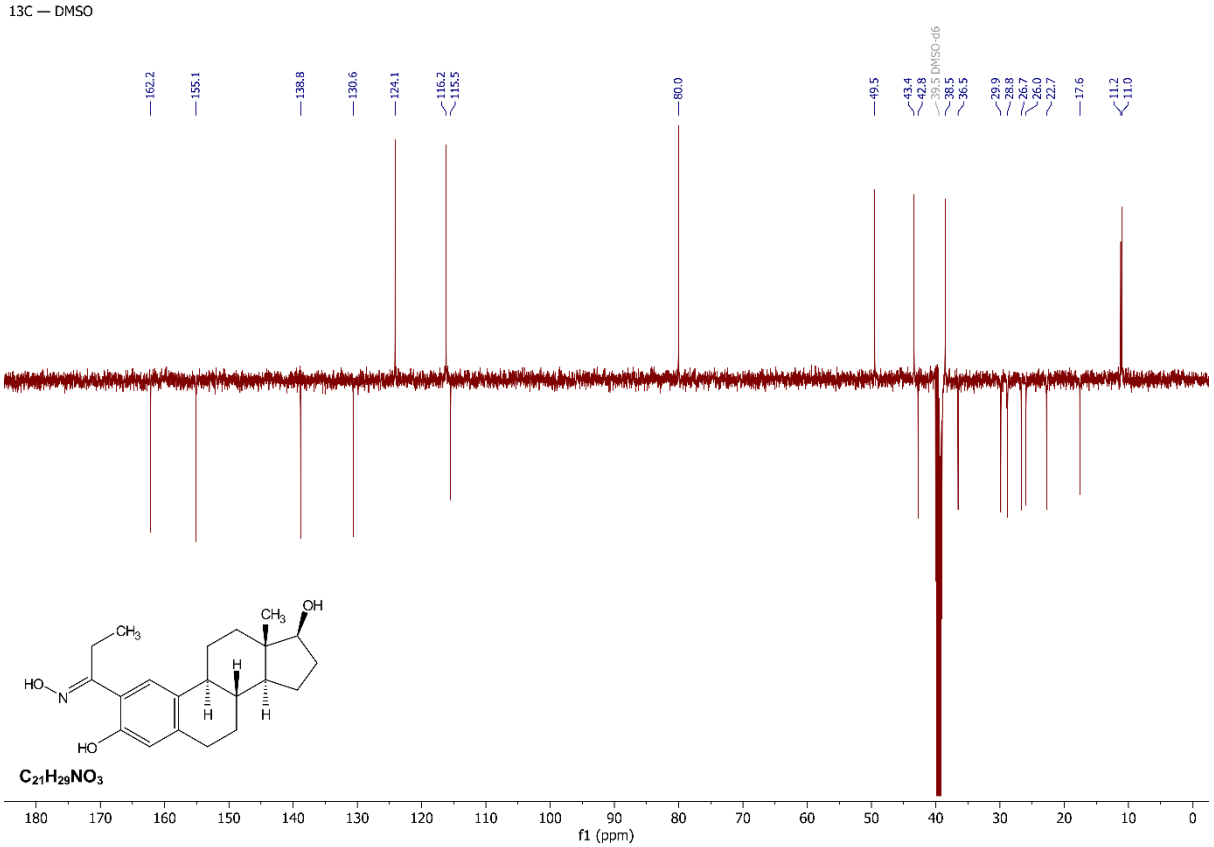
^{13}C NMR spectrum of compound **3b** ($CDCl_3$, 125 MHz)



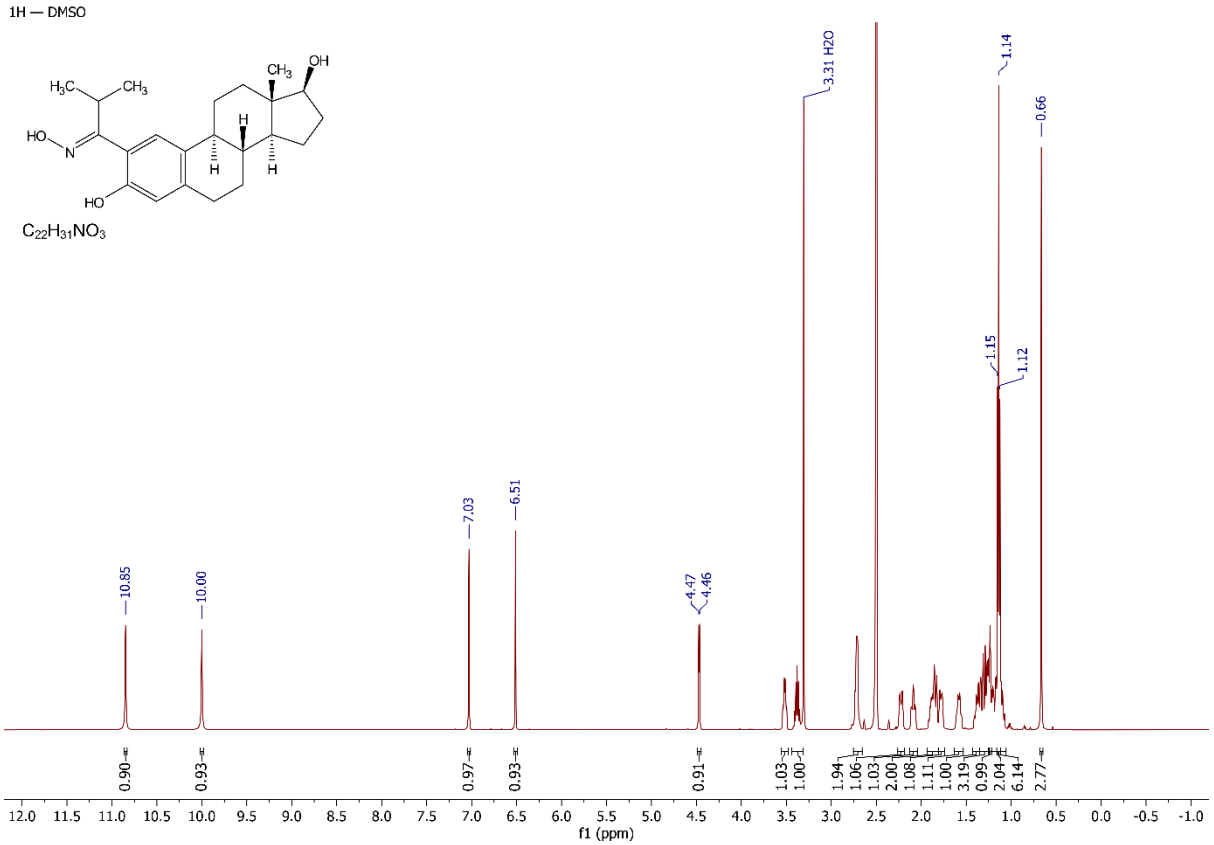
^{1}H NMR spectrum of compound **3c** (DMSO-d_6, 500 MHz)



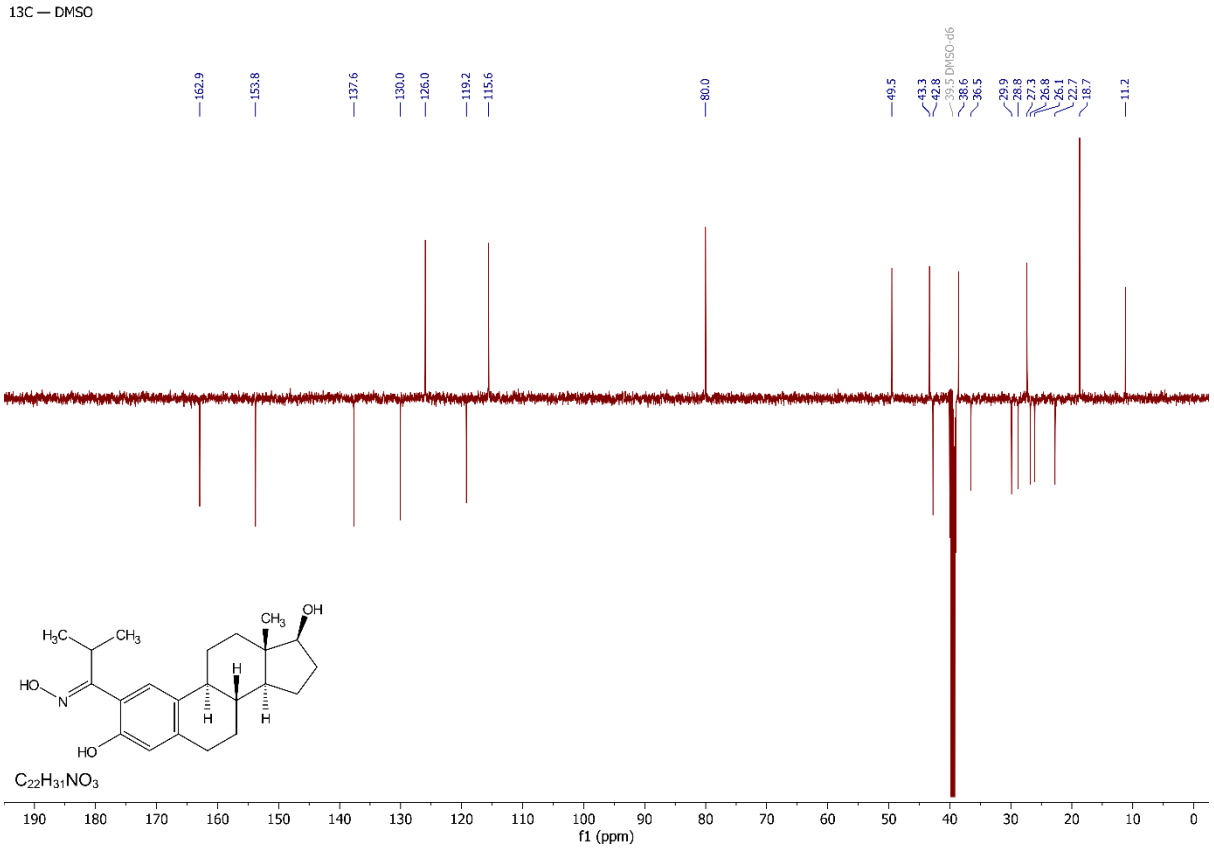
^{13}C NMR spectrum of compound **3c** (DMSO-d_6, 125 MHz)



^{1}H NMR spectrum of compound **3d-*E*** (DMSO-d_6, 500 MHz)



^{13}C NMR spectrum of compound **3d-*E*** (DMSO-d_6, 125 MHz)



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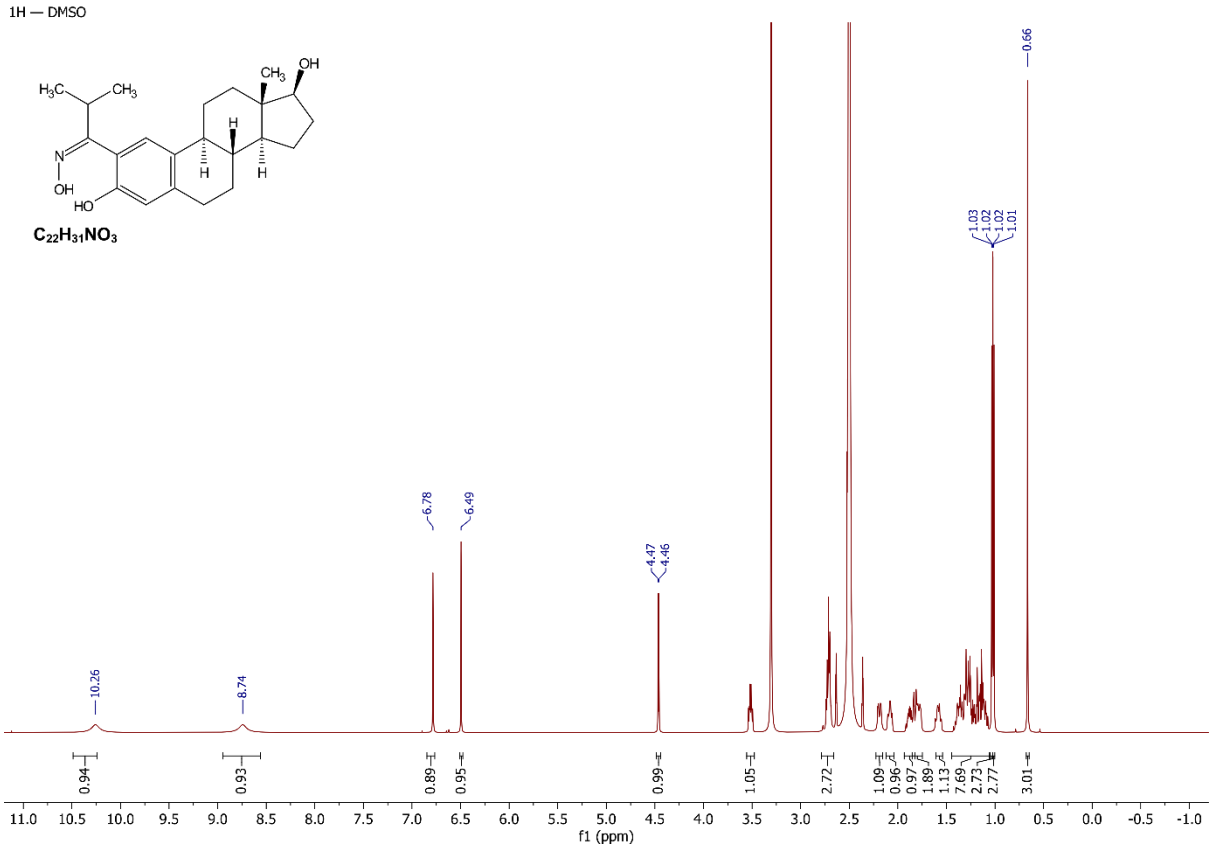
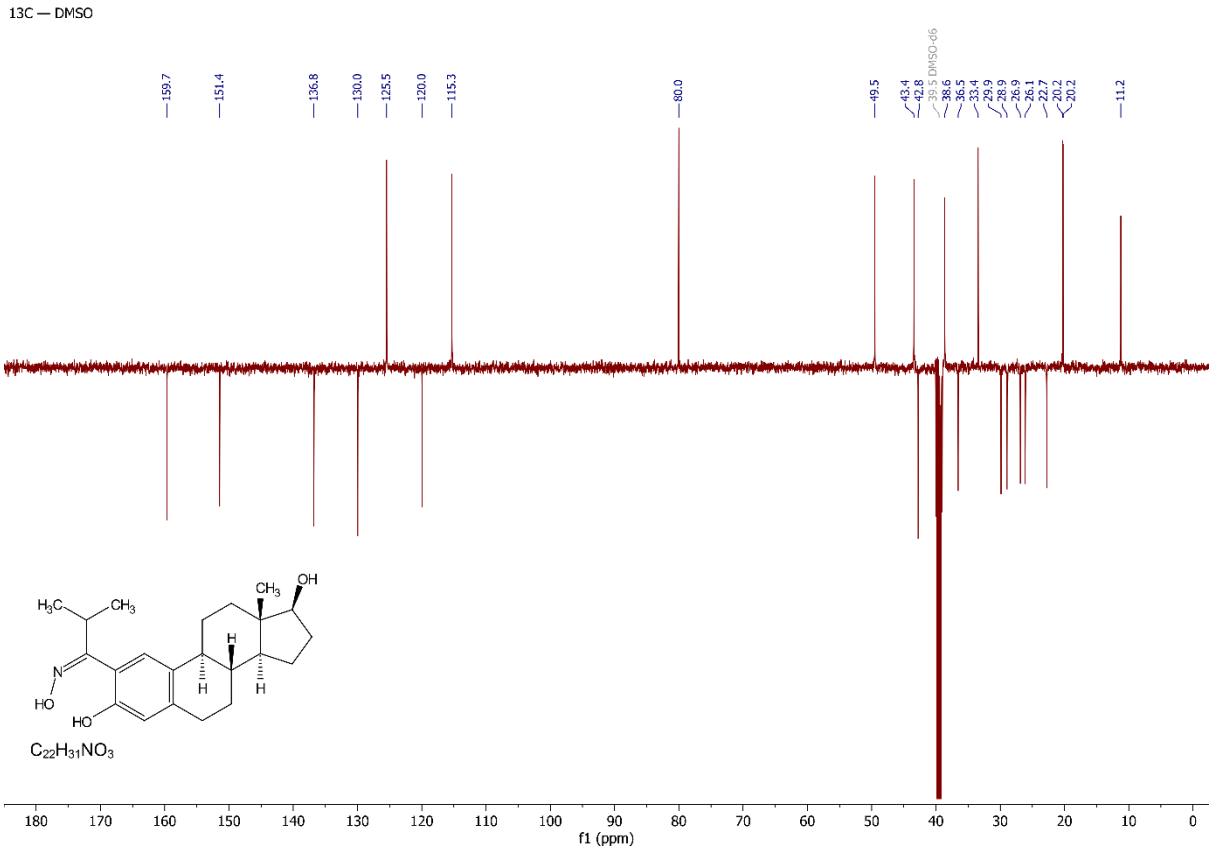
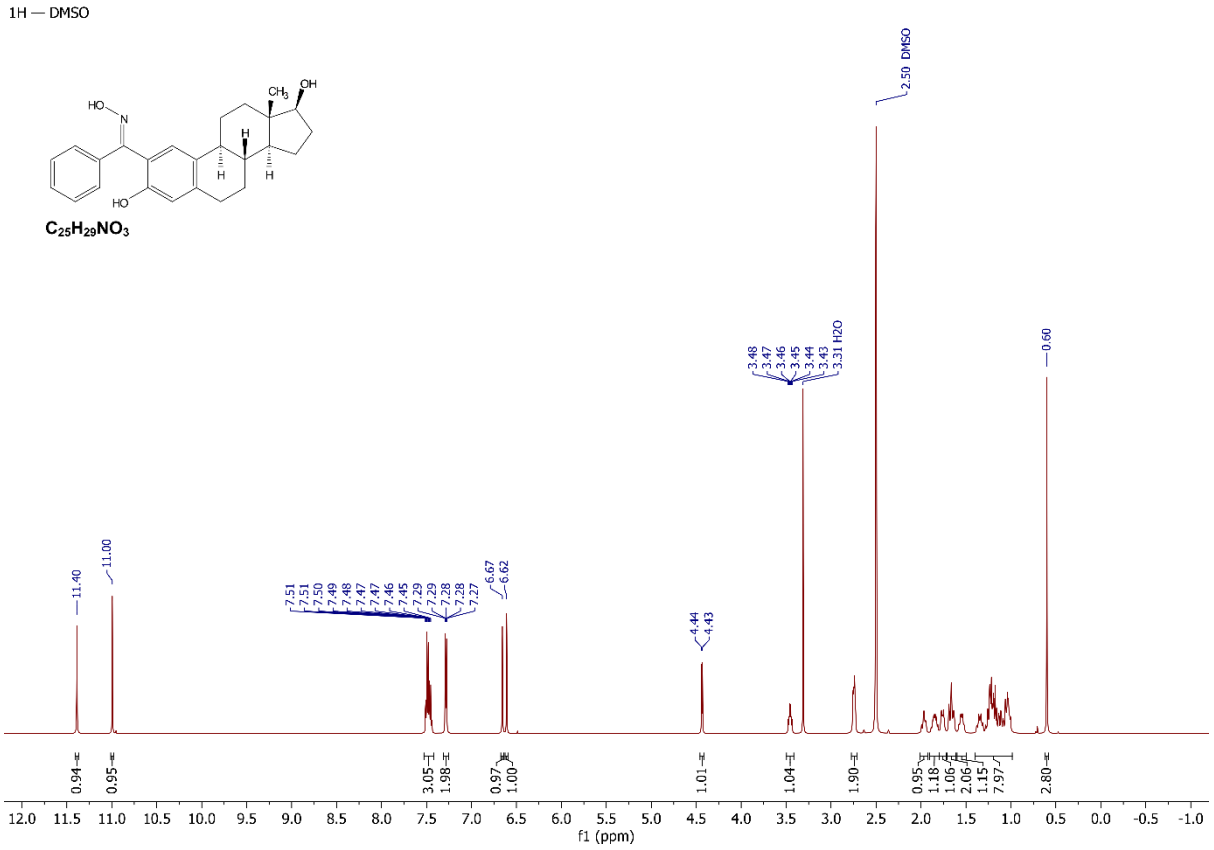


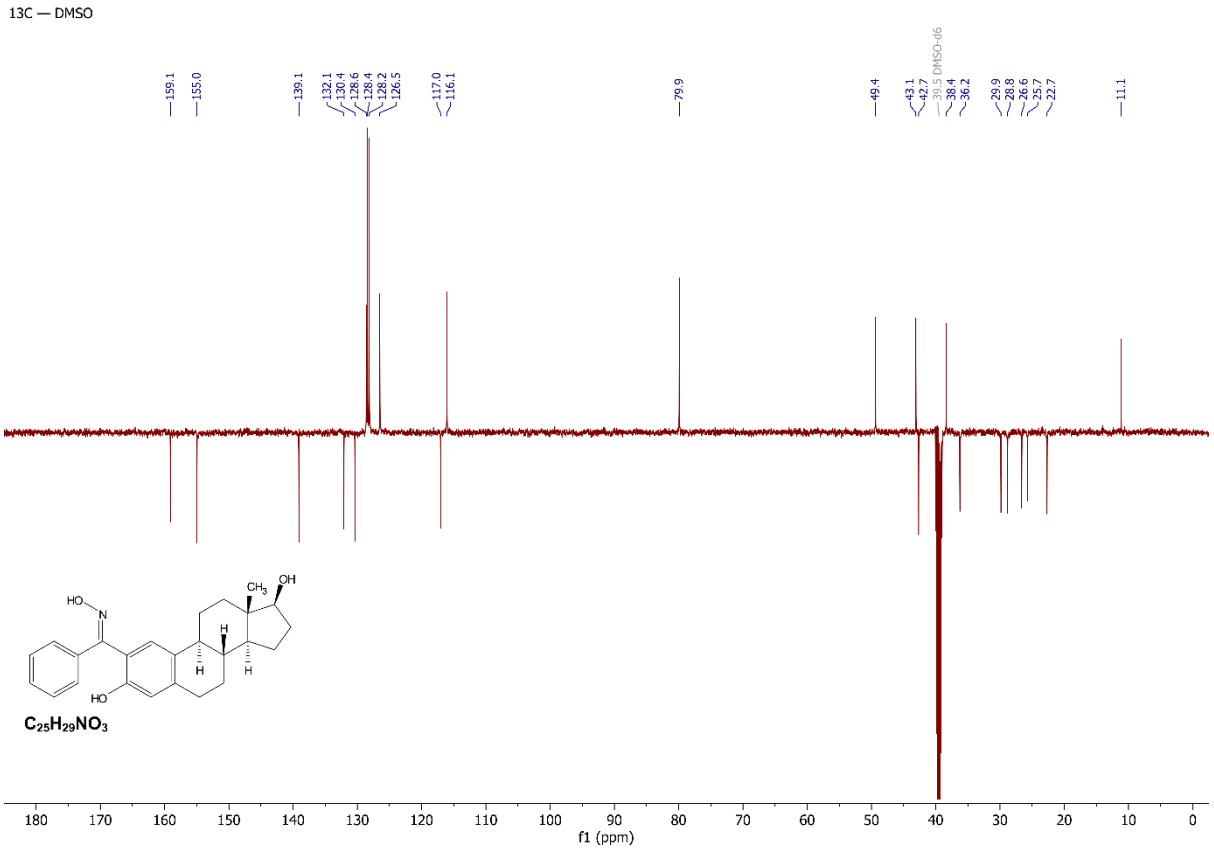
^{13}C NMR spectrum of compound **3d**-*Z* (DMSO-d_6, 125 MHz)



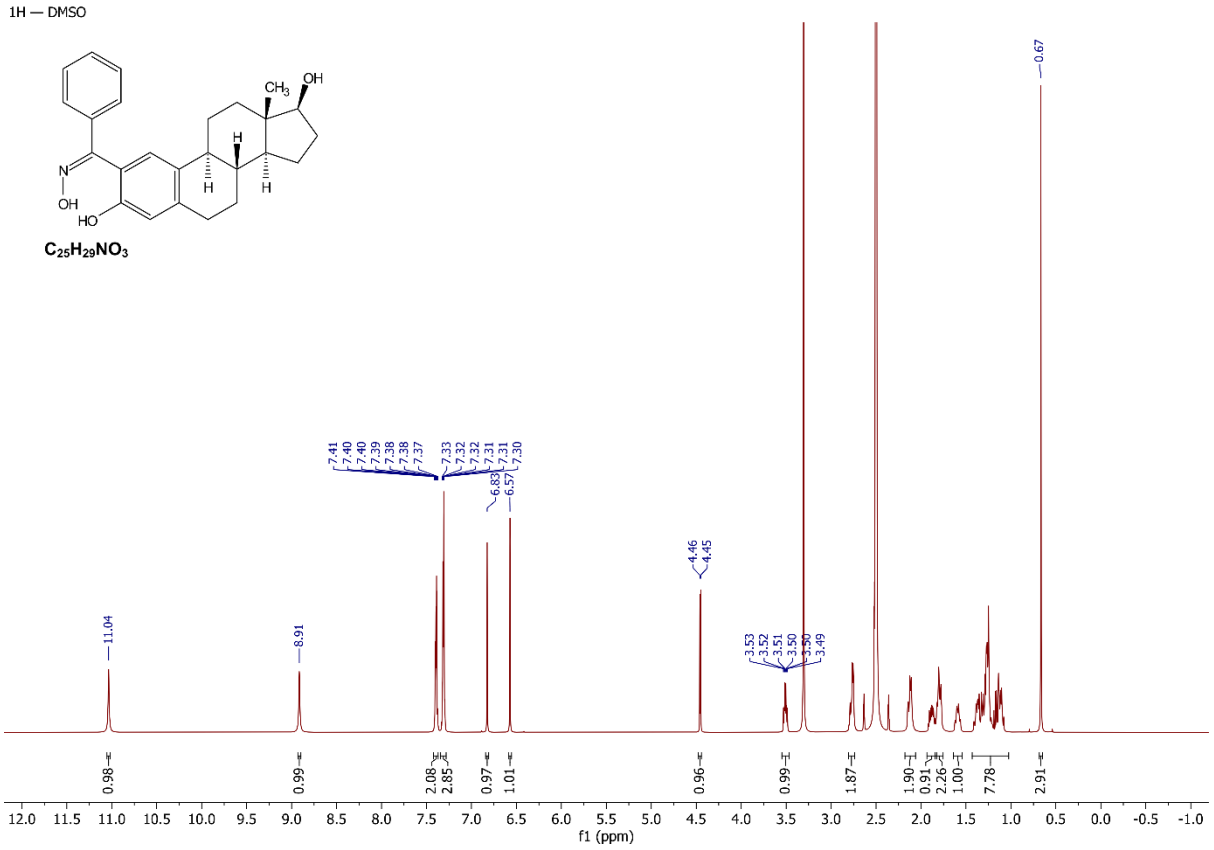
^{1}H NMR spectrum of compound **3e-***E* (DMSO-d_6, 500 MHz)



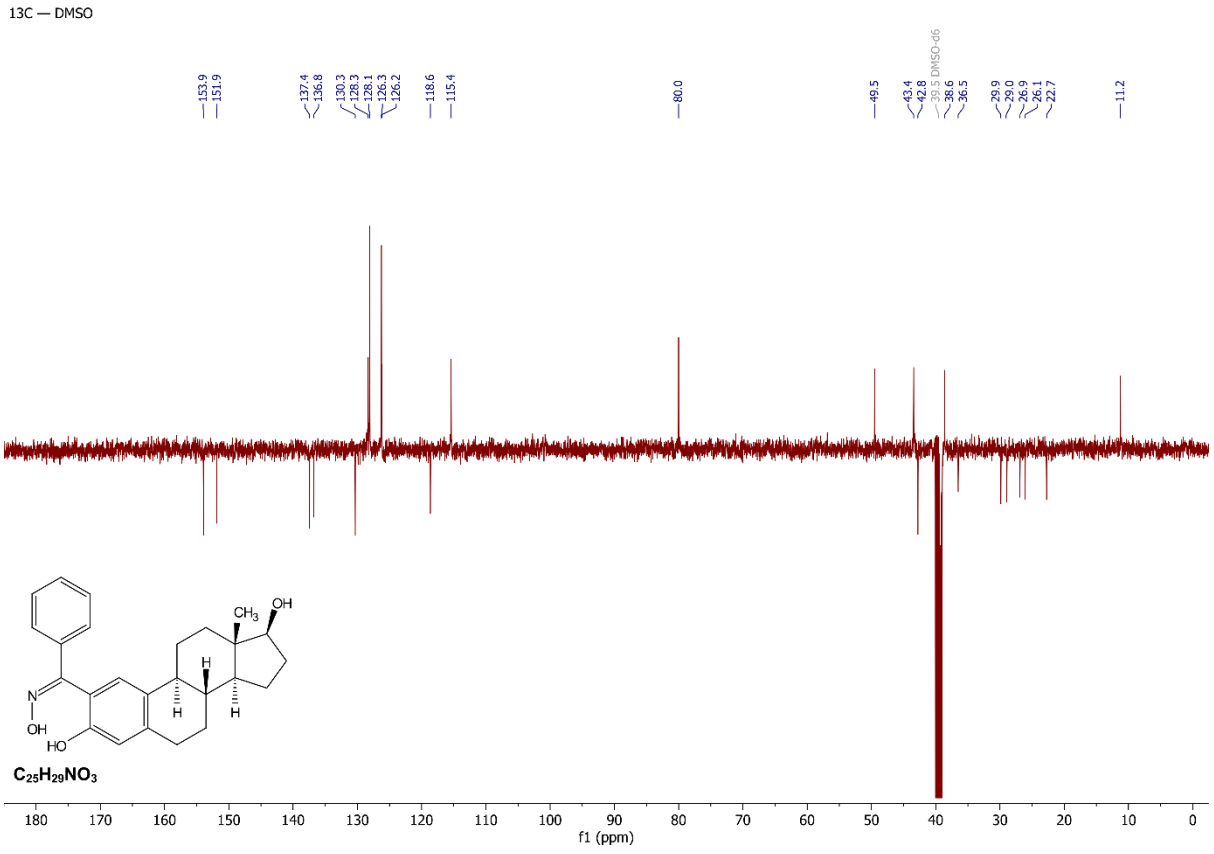
^{13}C NMR spectrum of compound **3e-***E* (DMSO-d_6, 125 MHz)



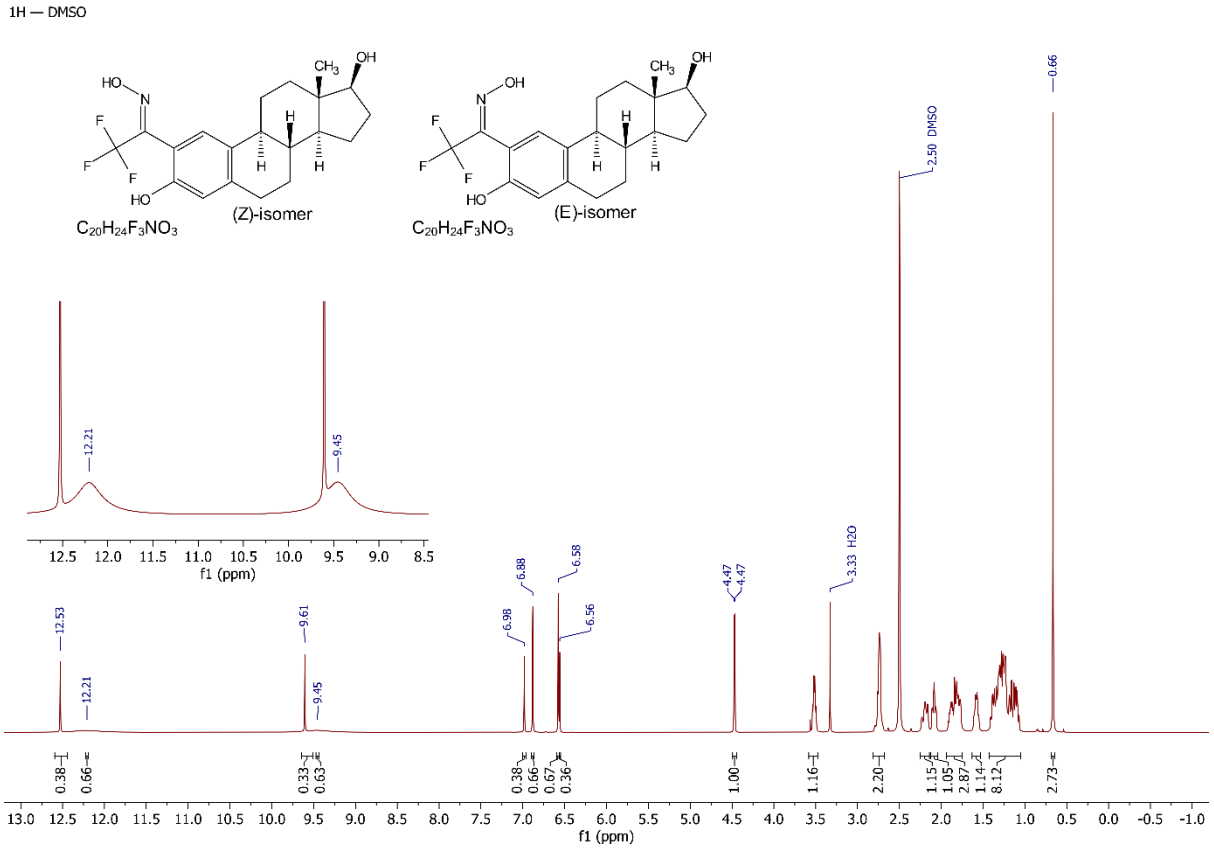
^{1}H NMR spectrum of compound **3e-***Z* (DMSO-d_6, 500 MHz)



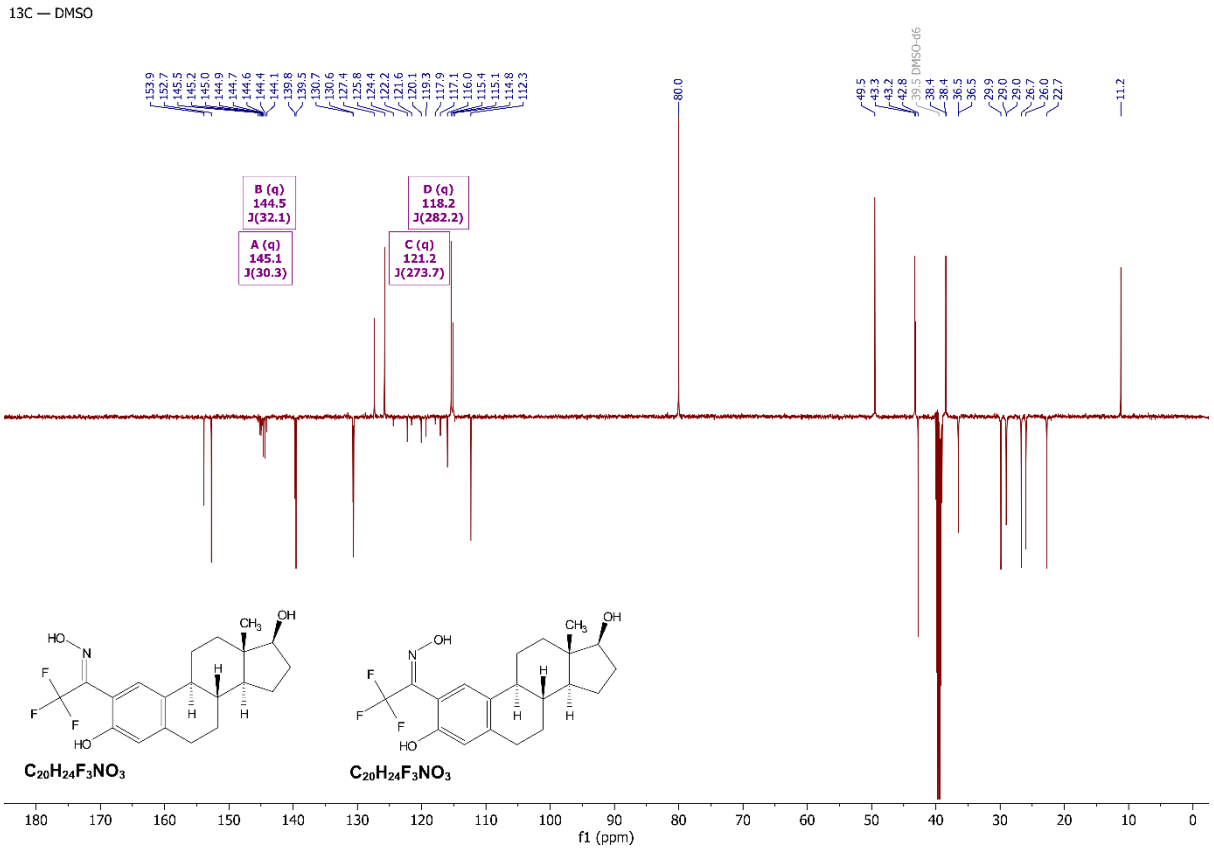
^{13}C NMR spectrum of compound **3e-***Z* (DMSO-d_6, 125 MHz)



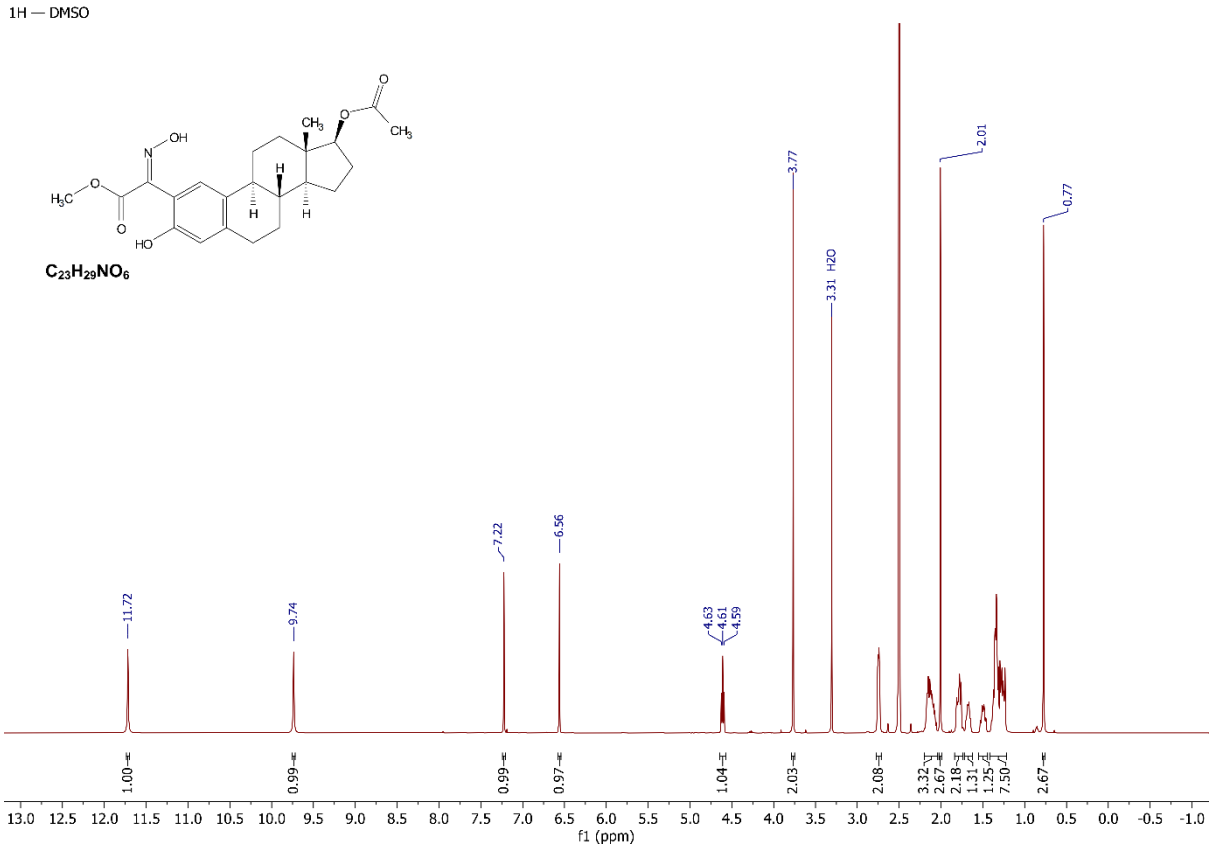
^{1}H NMR spectrum of compound **3f** (DMSO-*d*$_6$, 500 MHz)



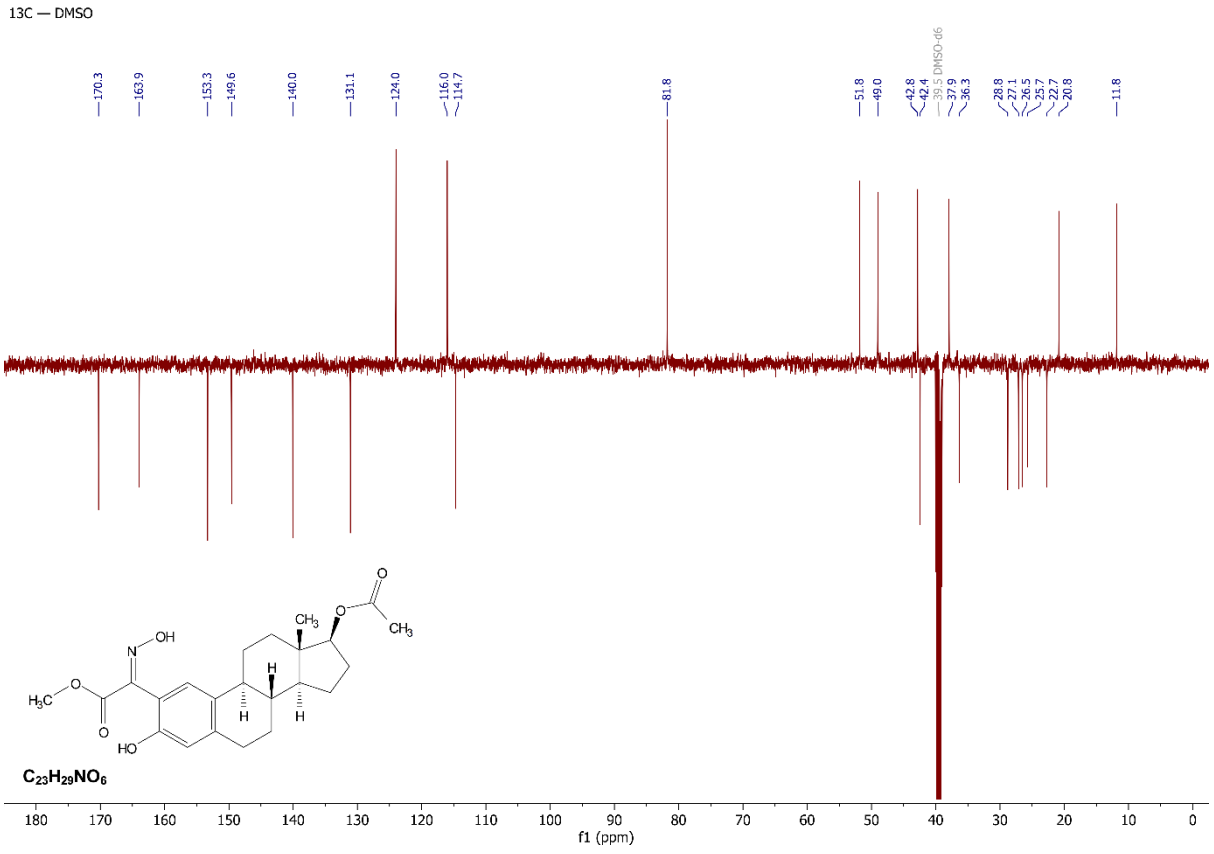
^{13}C NMR spectrum of compound **3f** (DMSO-*d*$_6$, 125 MHz)



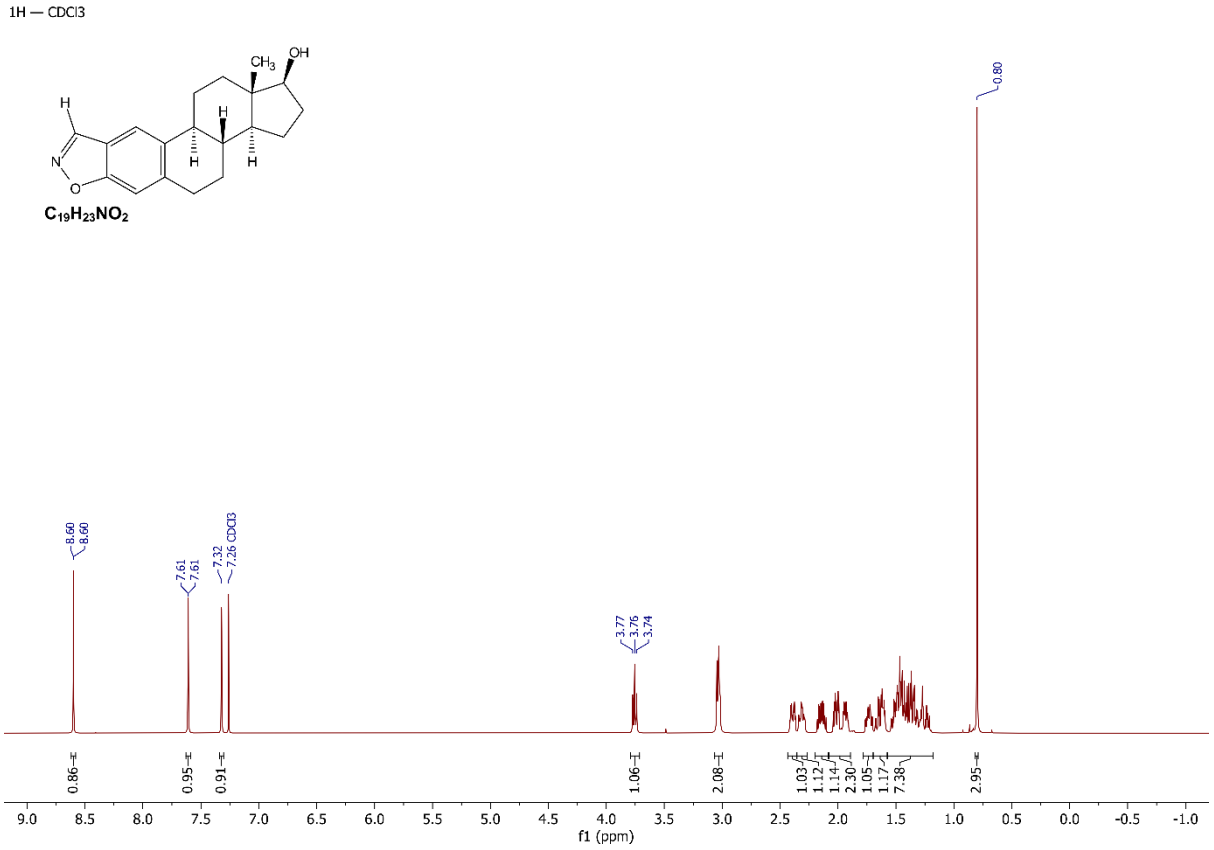
^{1}H NMR spectrum of compound **3g** (DMSO-*d*$_6$, 500 MHz)



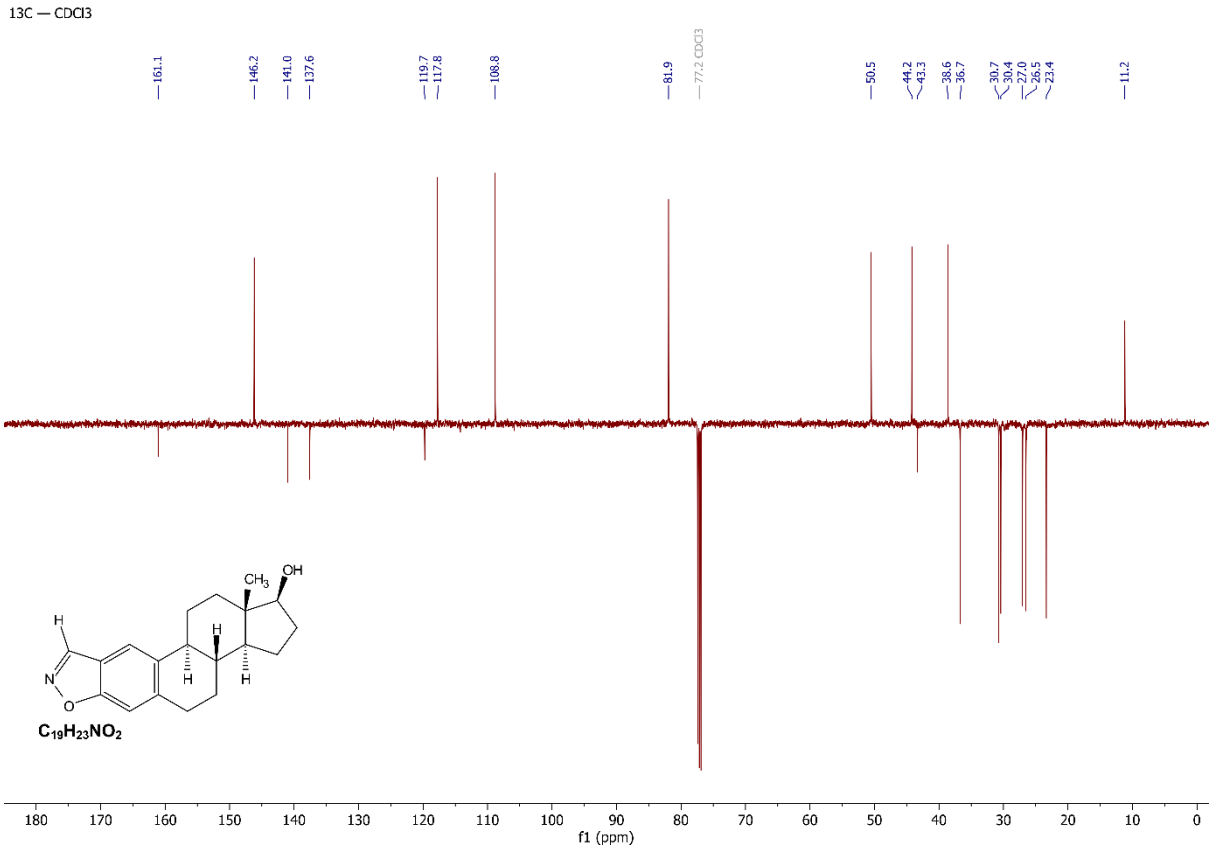
^{13}C NMR spectrum of compound **3g** (DMSO-*d*$_6$, 125 MHz)



^{1}H NMR spectrum of compound **4a** ($CDCl_3$, 500 MHz)



^{13}C NMR spectrum of compound **4a** ($CDCl_3$, 125 MHz)



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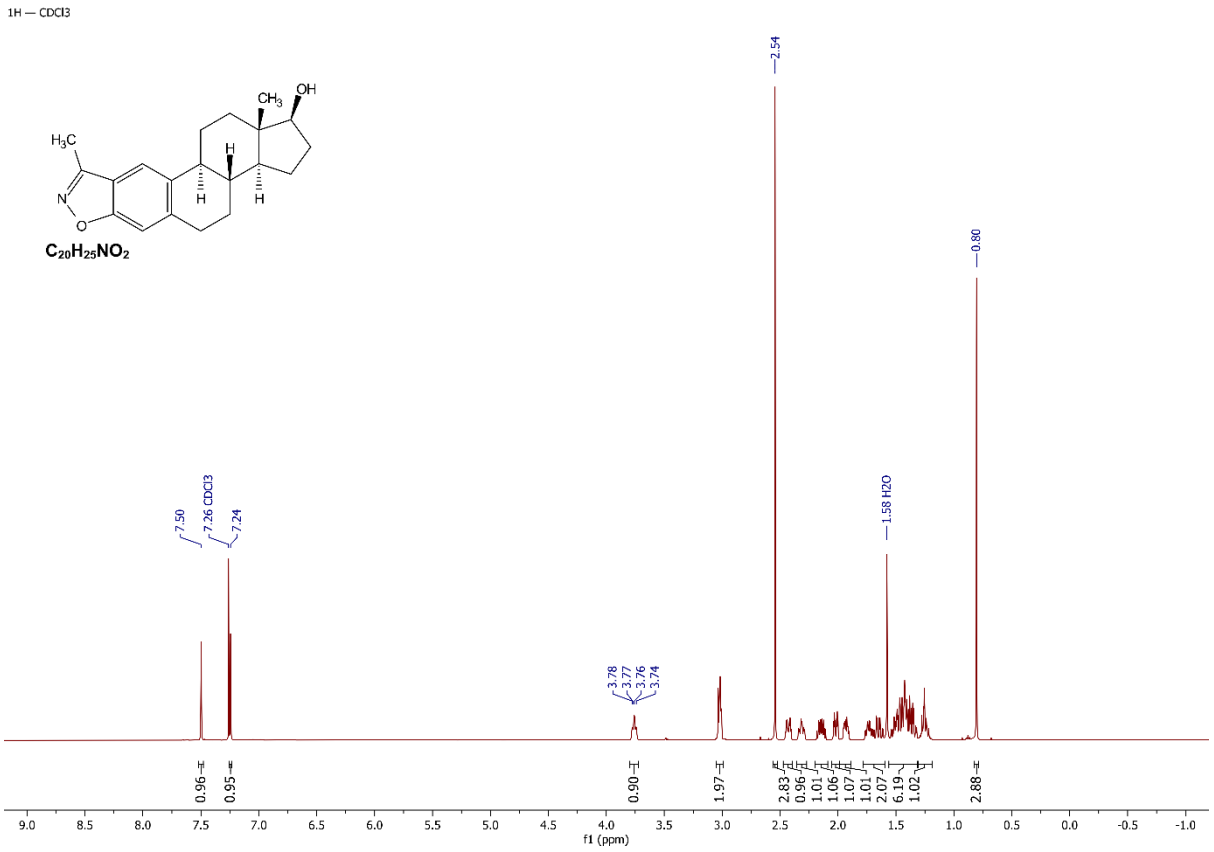
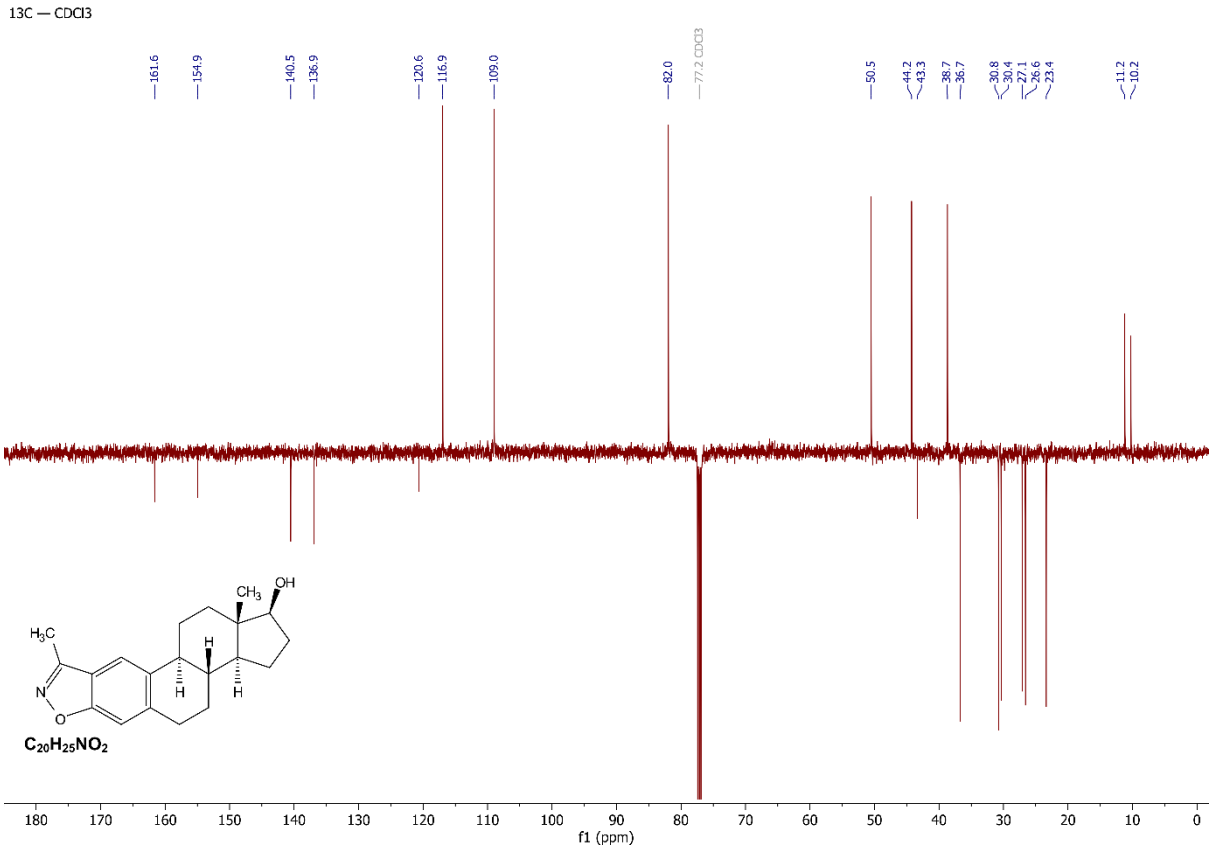
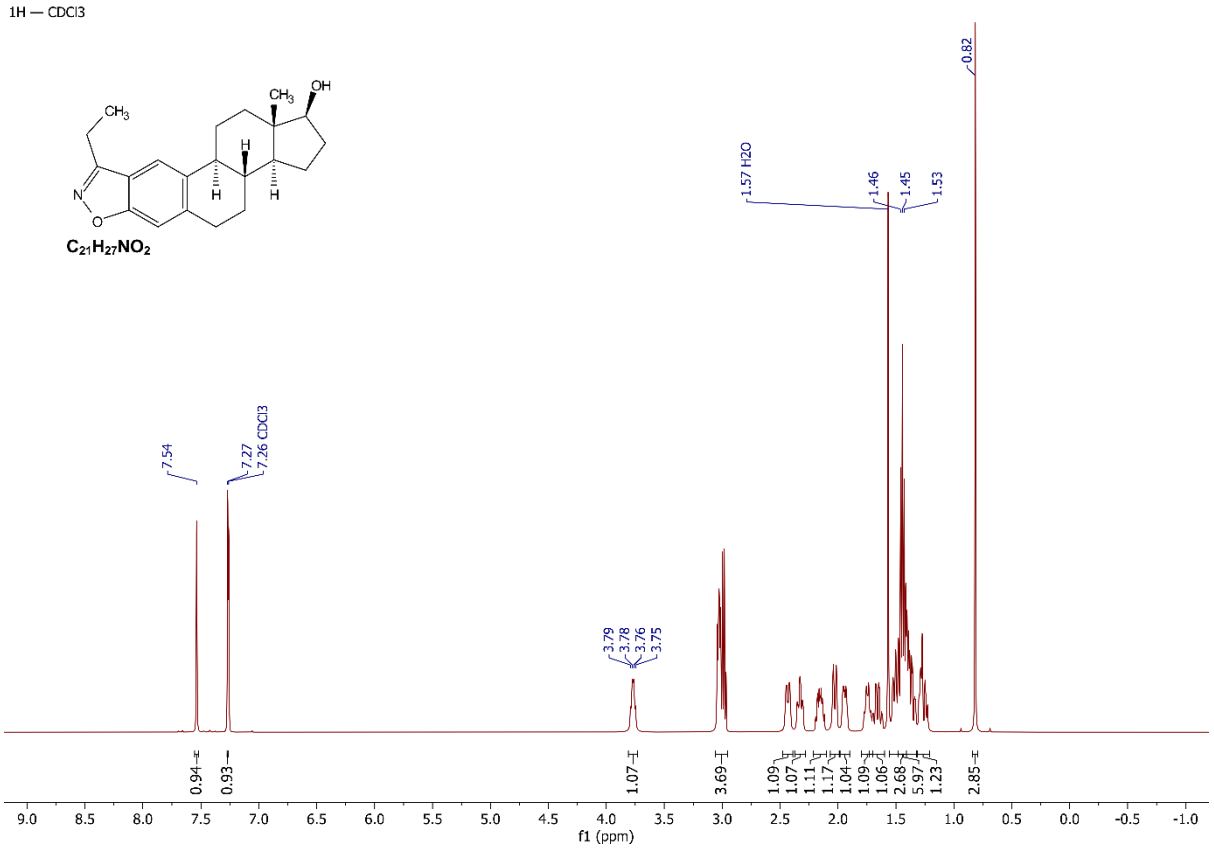


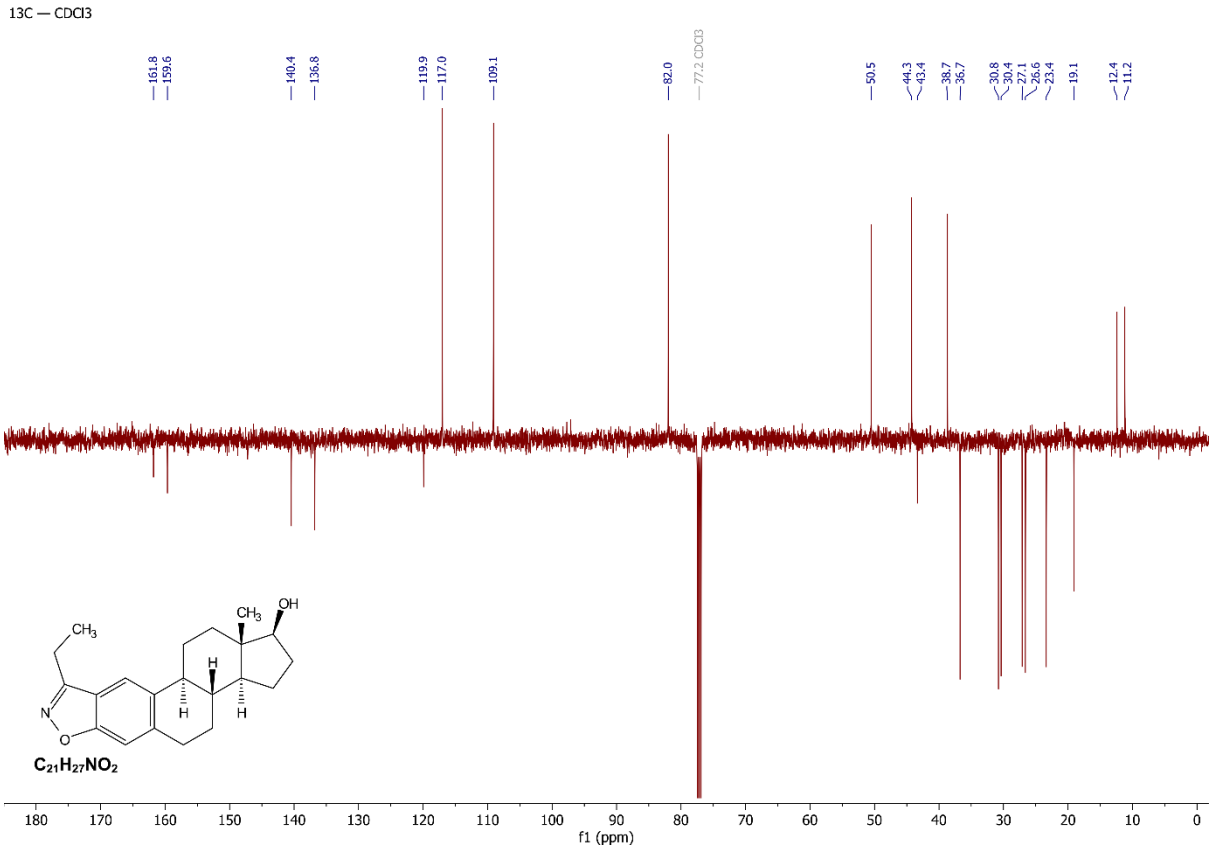
^{13}C NMR spectrum of compound **4b** ($CDCl_3$, 125 MHz)



^{1}H NMR spectrum of compound **4c** ($CDCl_3$, 500 MHz)



^{13}C NMR spectrum of compound **4c** ($CDCl_3$, 125 MHz)



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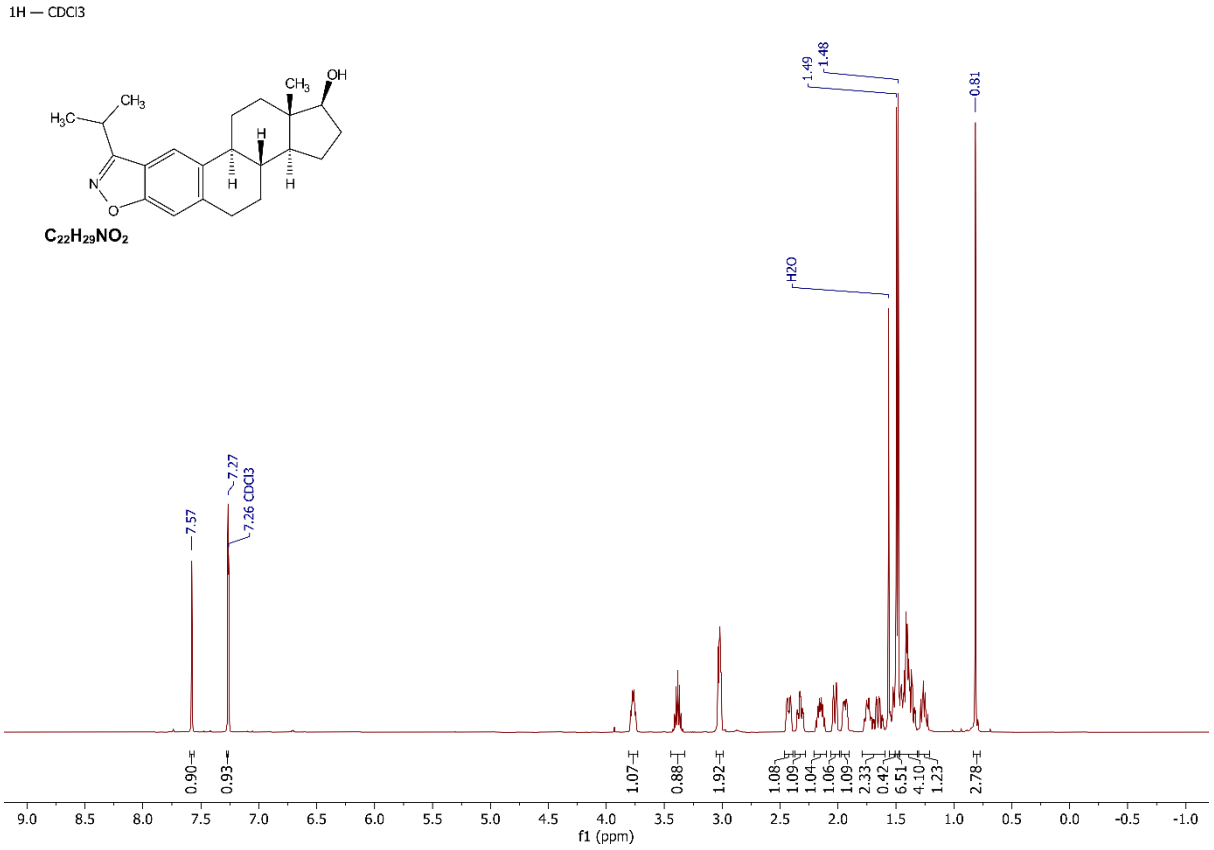
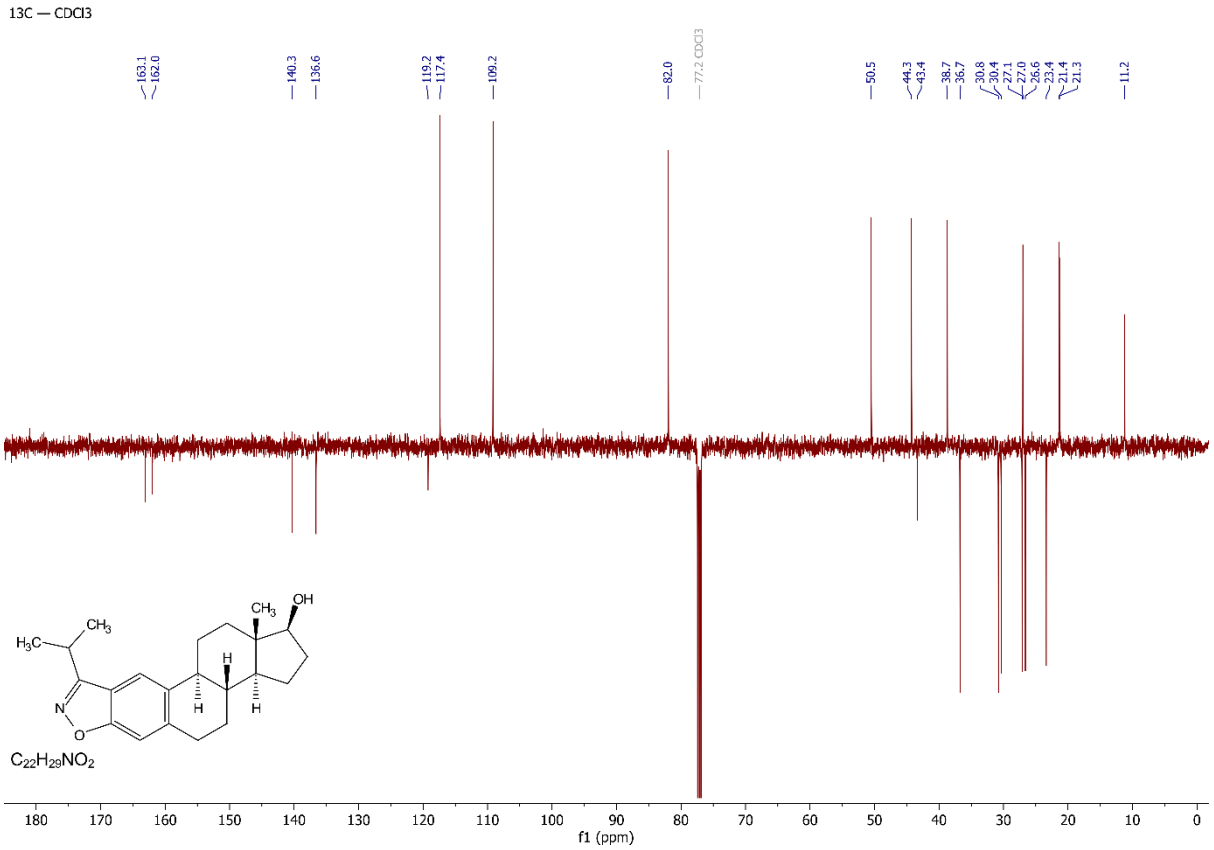
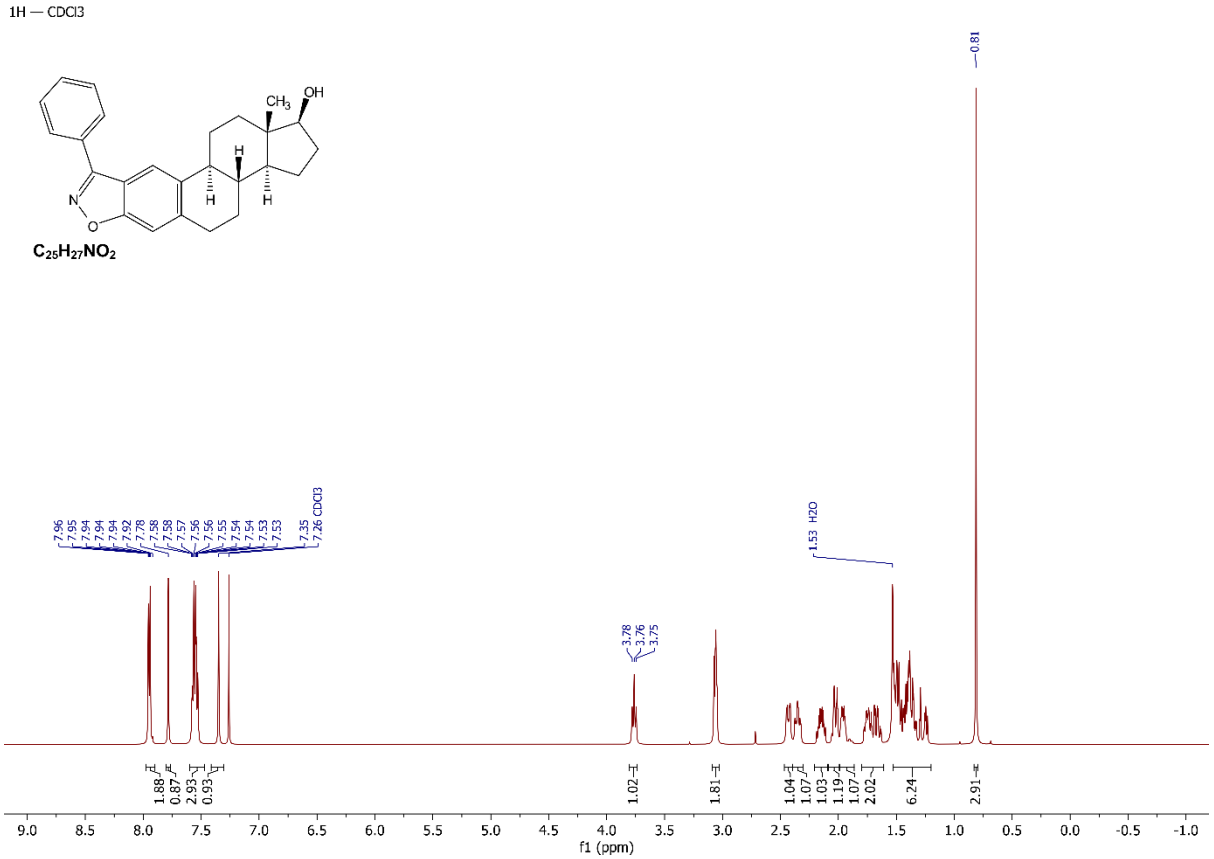


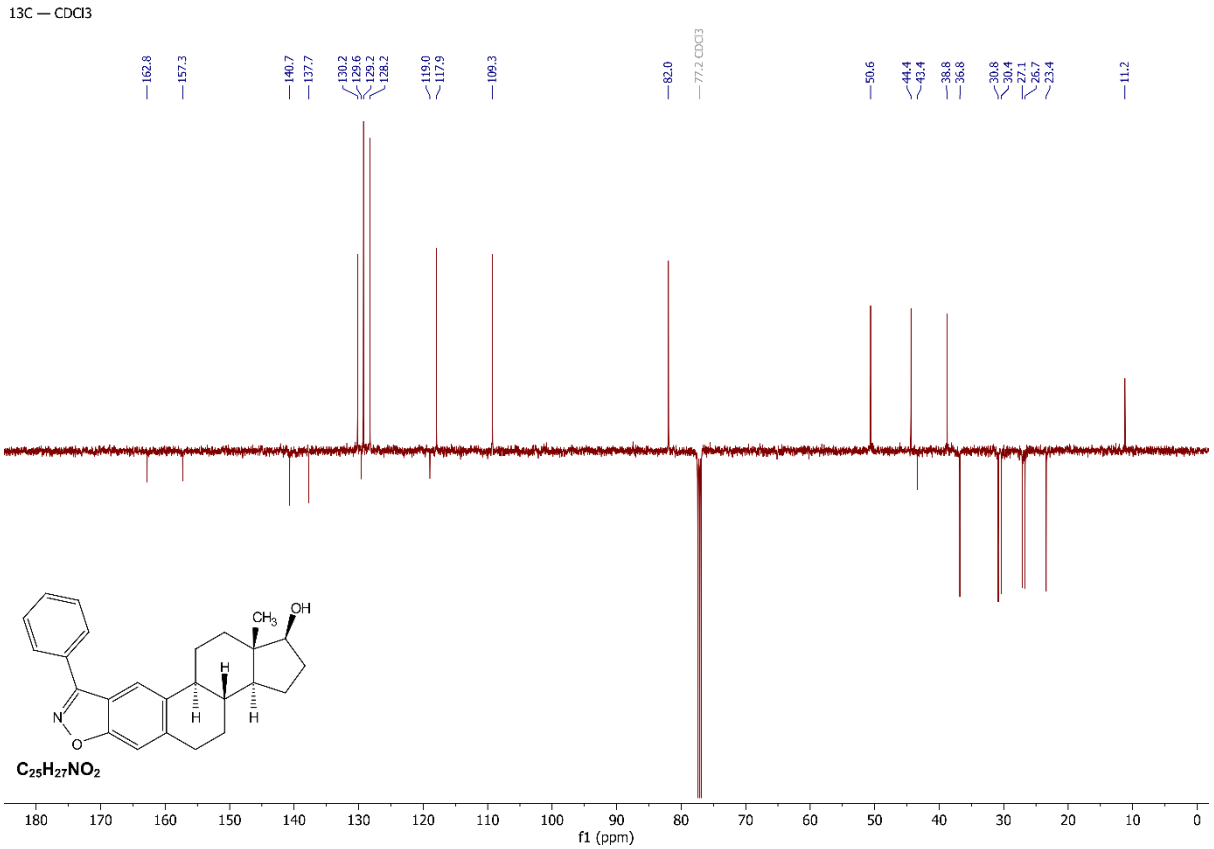
^{13}C NMR spectrum of compound **4d** ($CDCl_3$, 125 MHz)



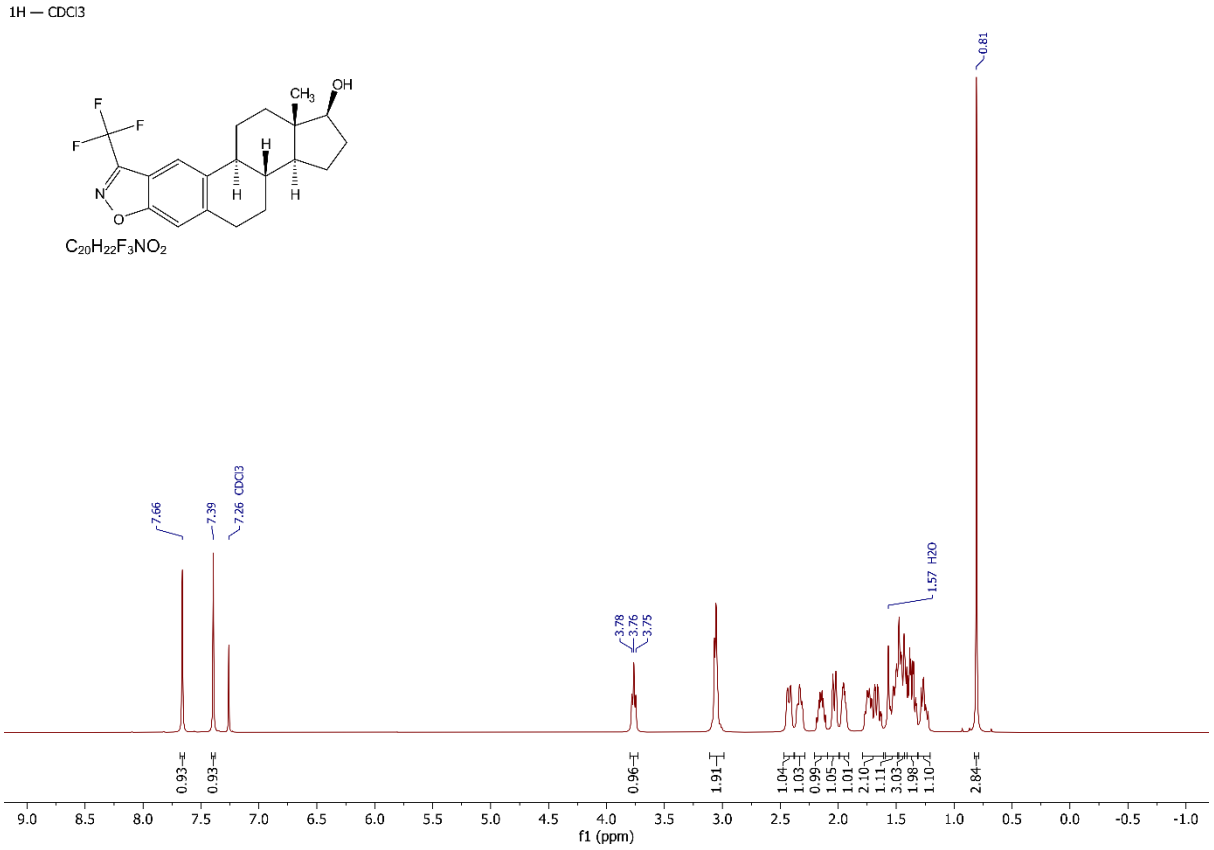
^{1}H NMR spectrum of compound **4e** ($CDCl_3$, 500 MHz)



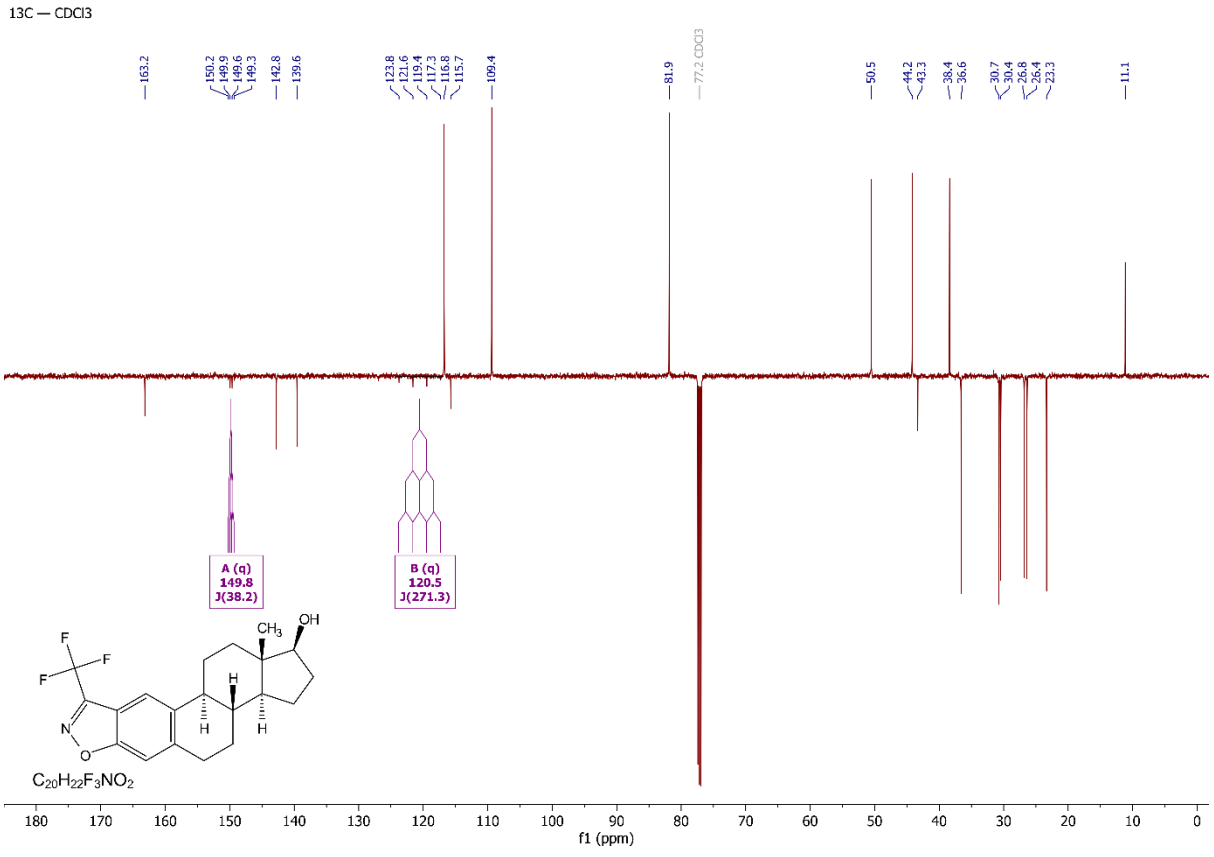
^{13}C NMR spectrum of compound **4e** ($CDCl_3$, 125 MHz)



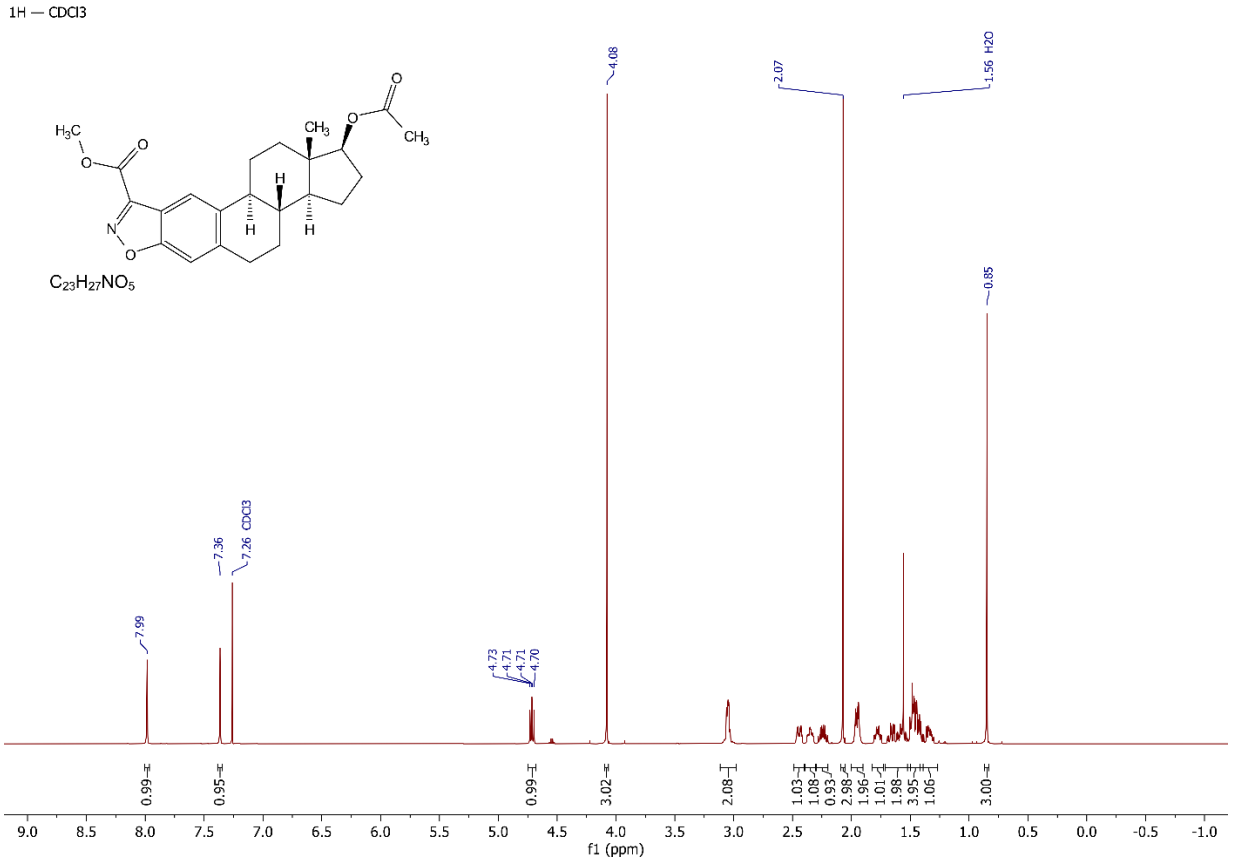
^{1}H NMR spectrum of compound **4f** ($CDCl_3$, 500 MHz)



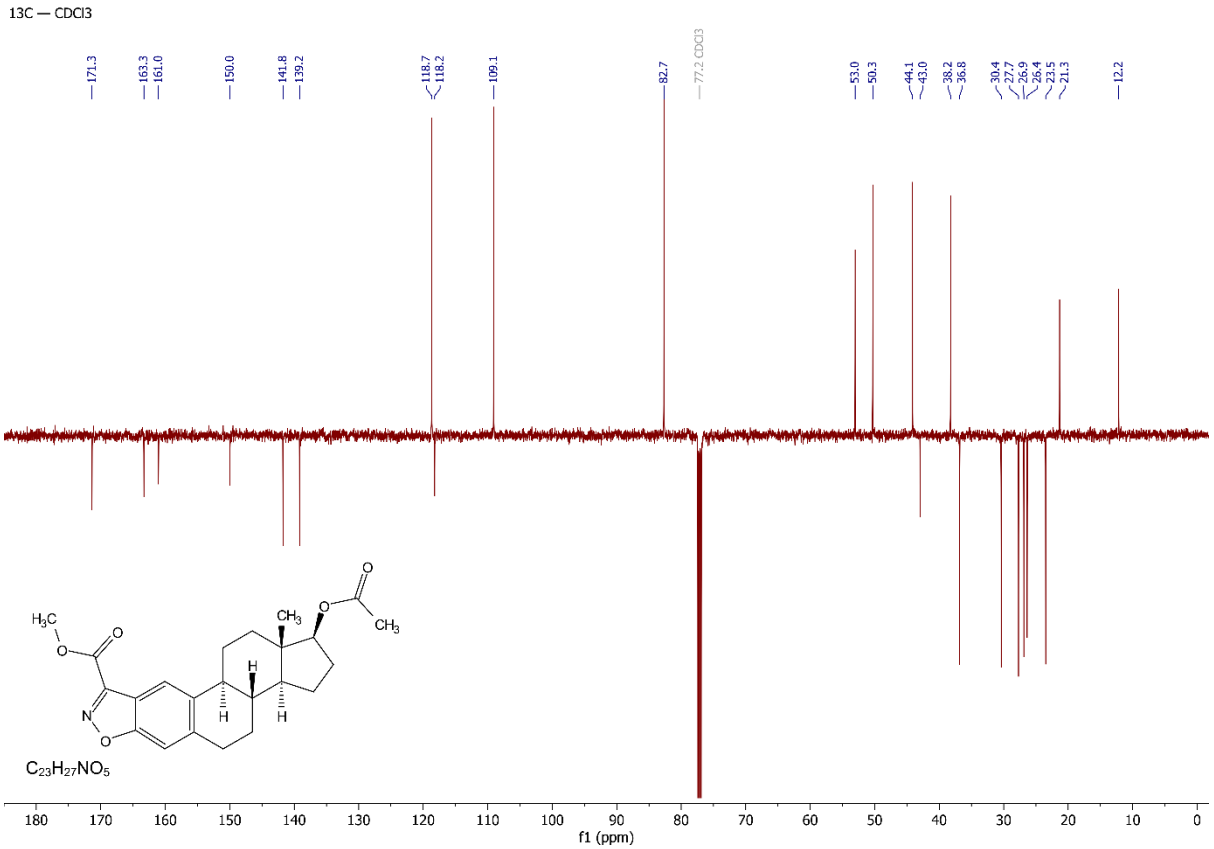
^{13}C NMR spectrum of compound **4f** ($CDCl_3$, 125 MHz)



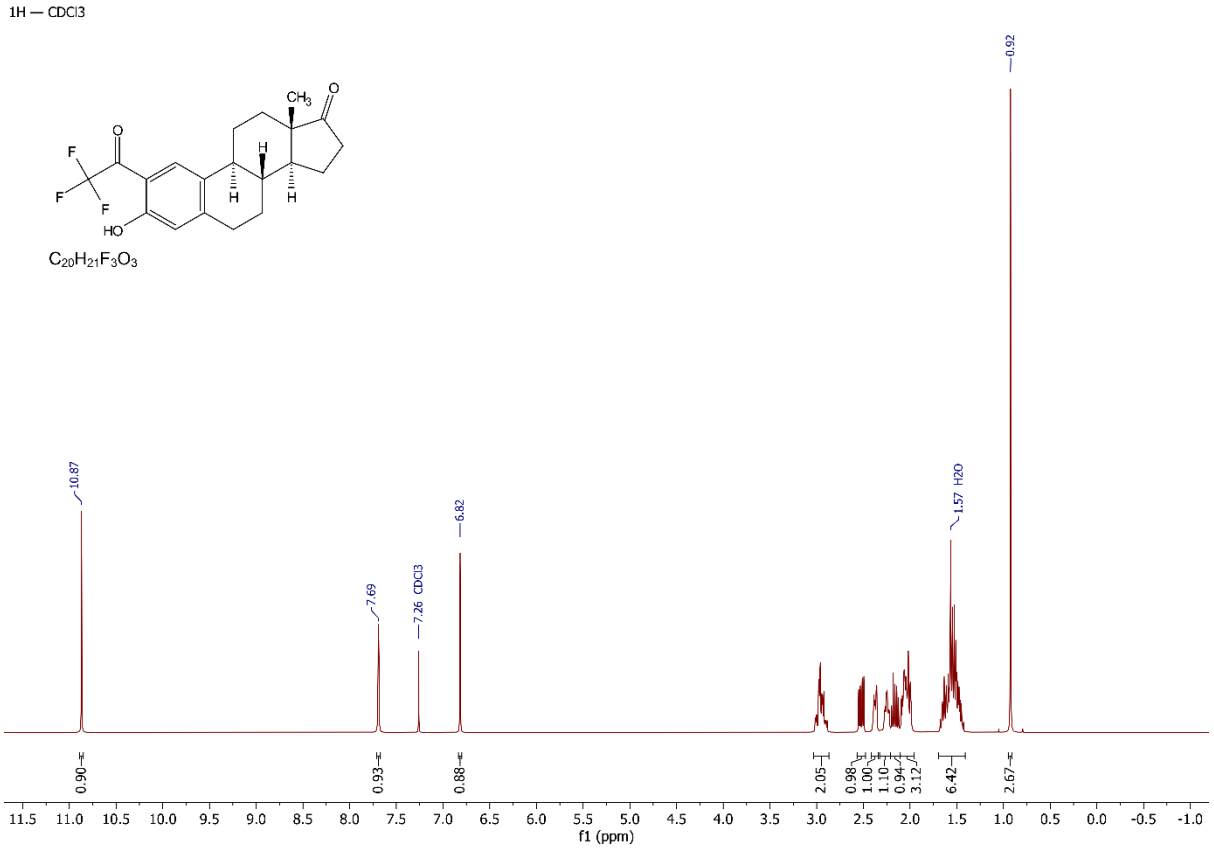
^{1}H NMR spectrum of compound **4g** ($CDCl_3$, 500 MHz)



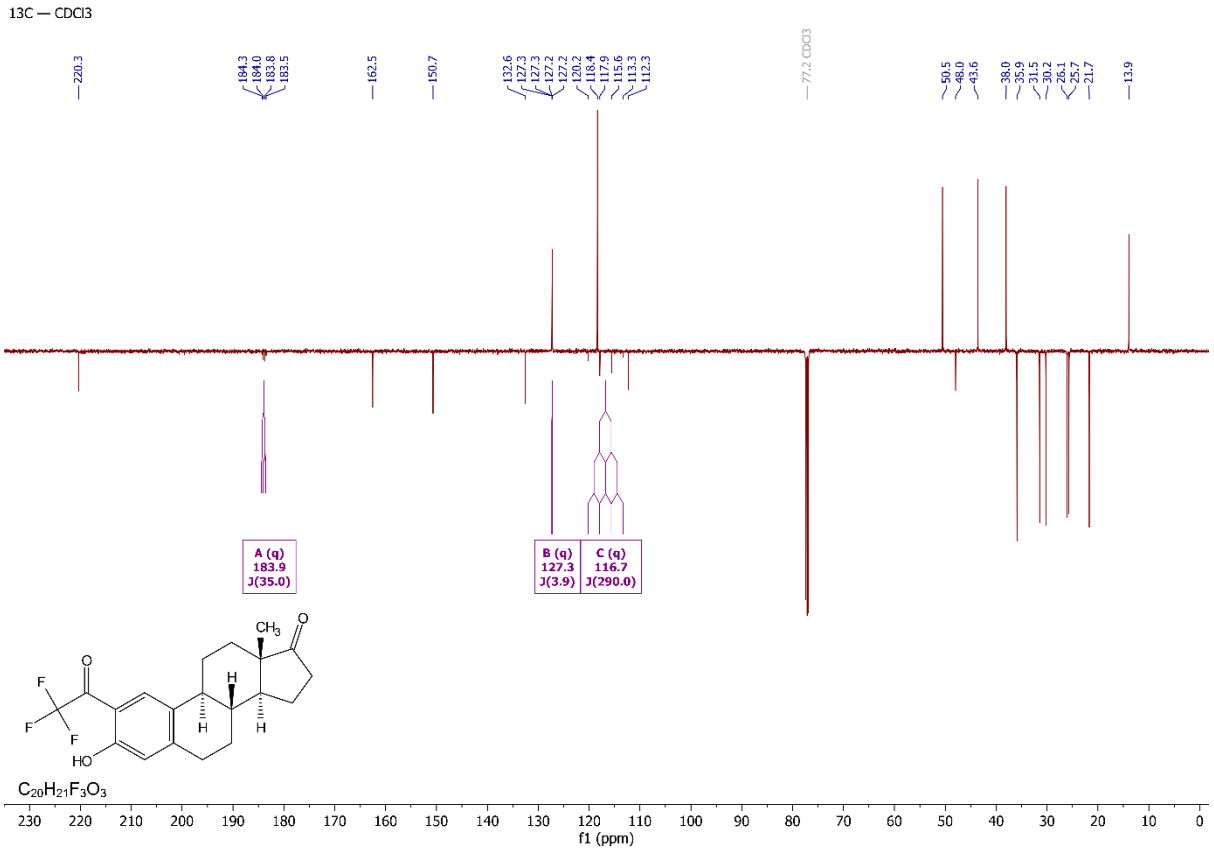
^{13}C NMR spectrum of compound **4g** ($CDCl_3$, 125 MHz)



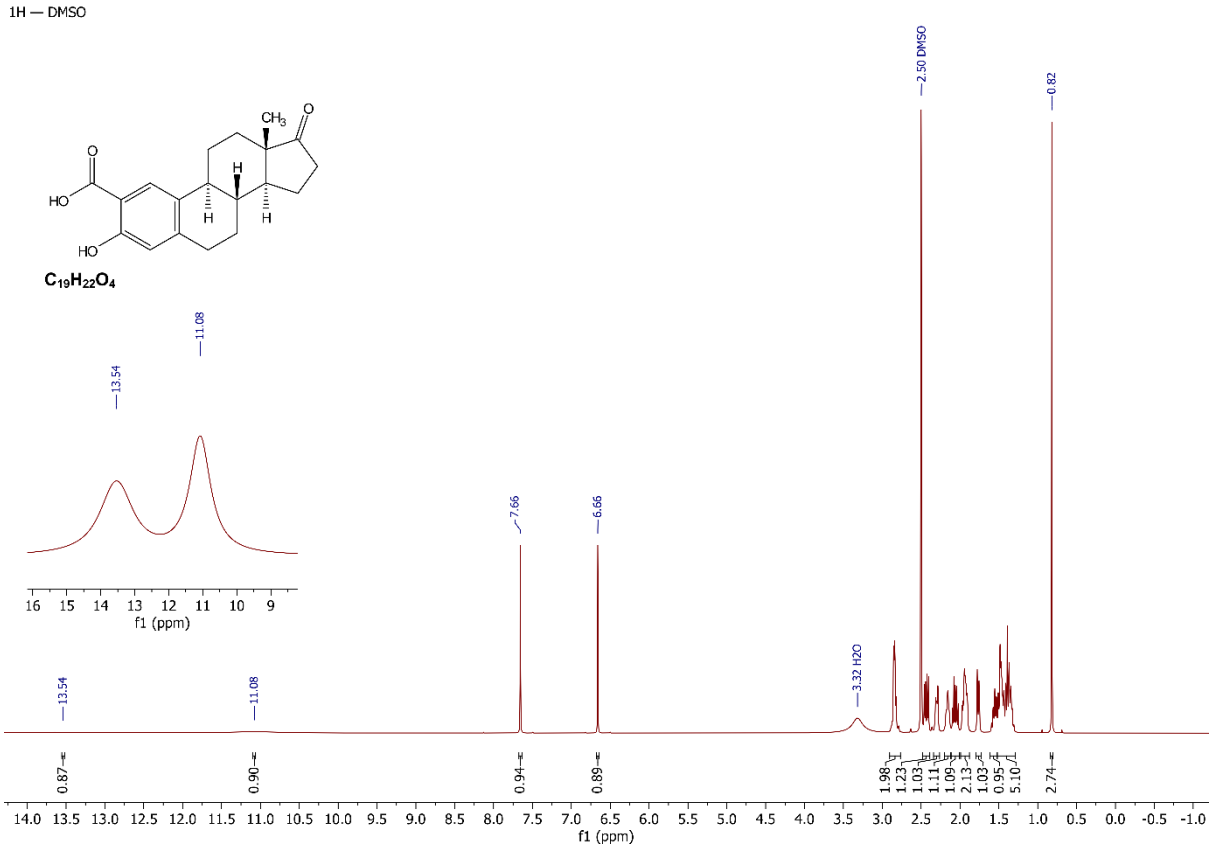
^{1}H NMR spectrum of compound **6a** ($CDCl_3$, 500 MHz)



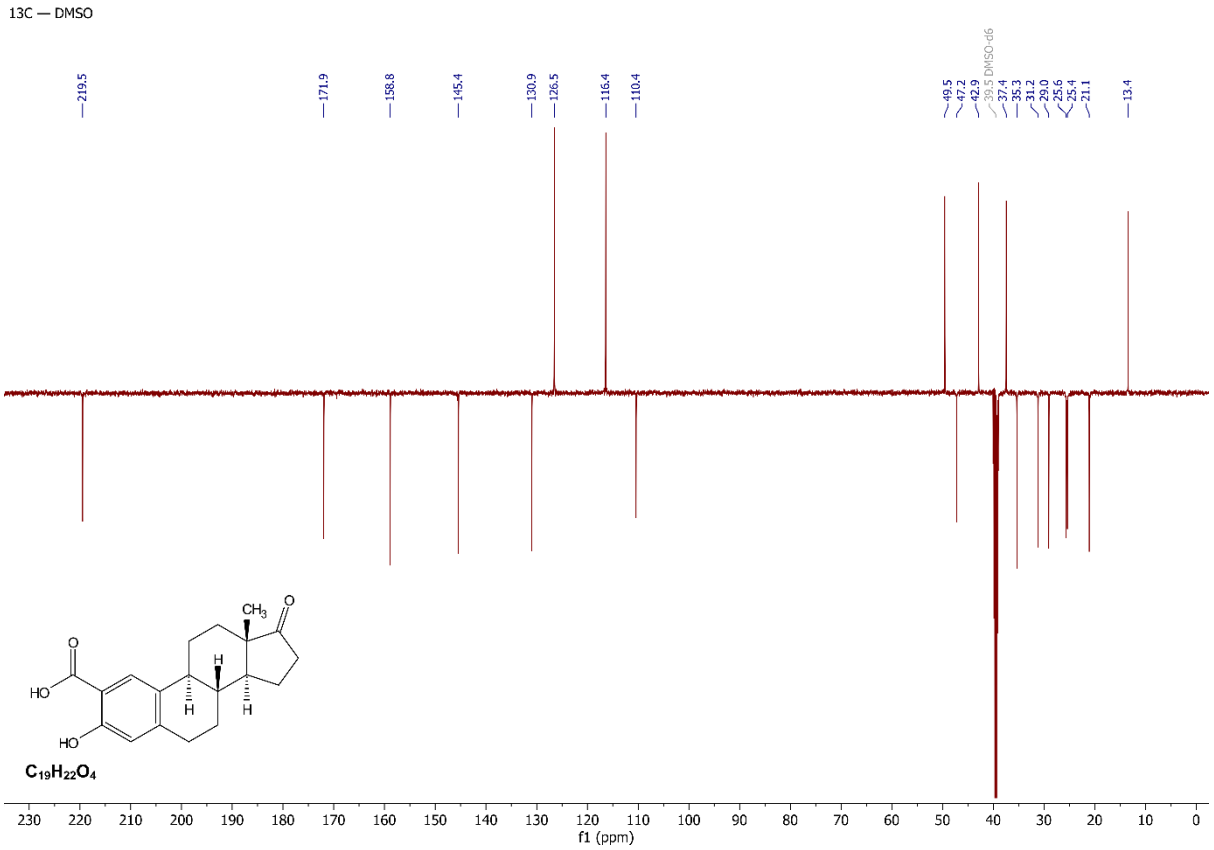
^{13}C NMR spectrum of compound **6a** ($CDCl_3$, 125 MHz)



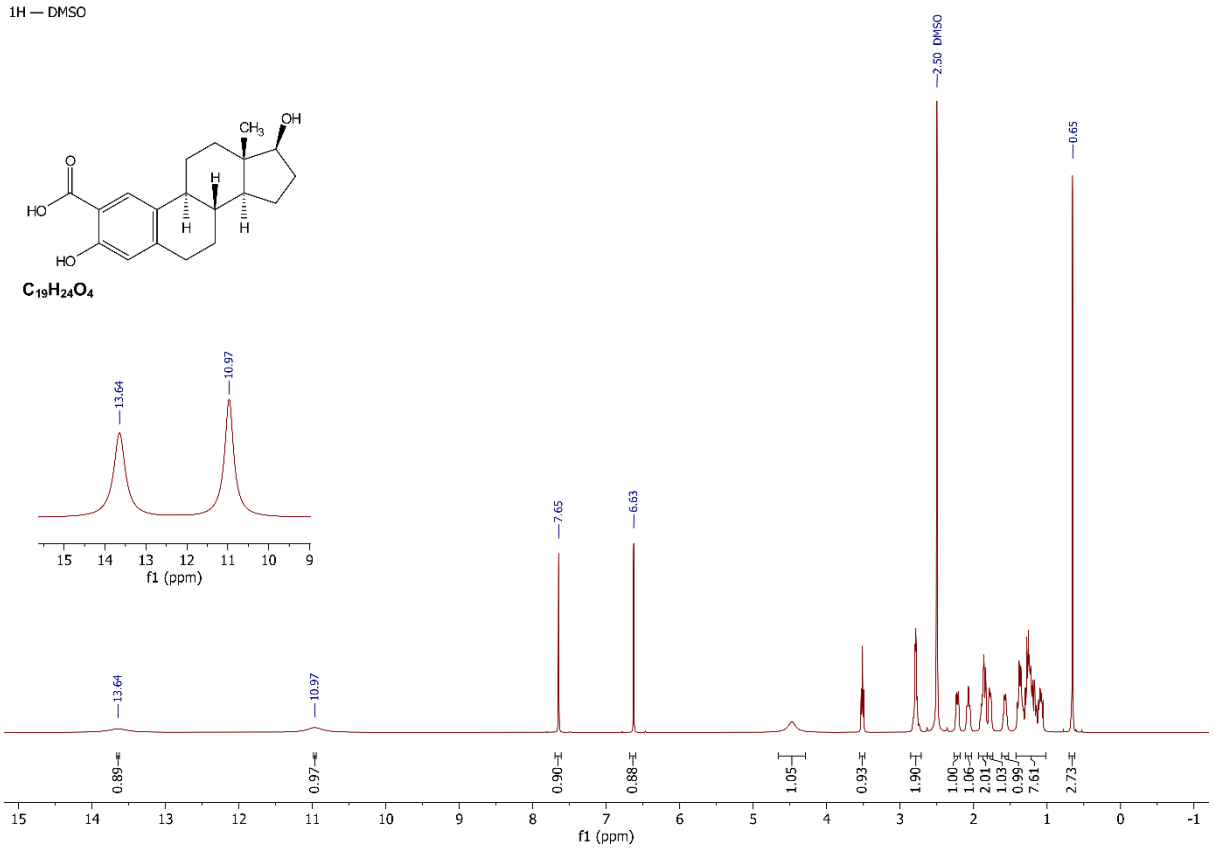
^{1}H NMR spectrum of compound **6b** (DMSO-d_6, 500 MHz)



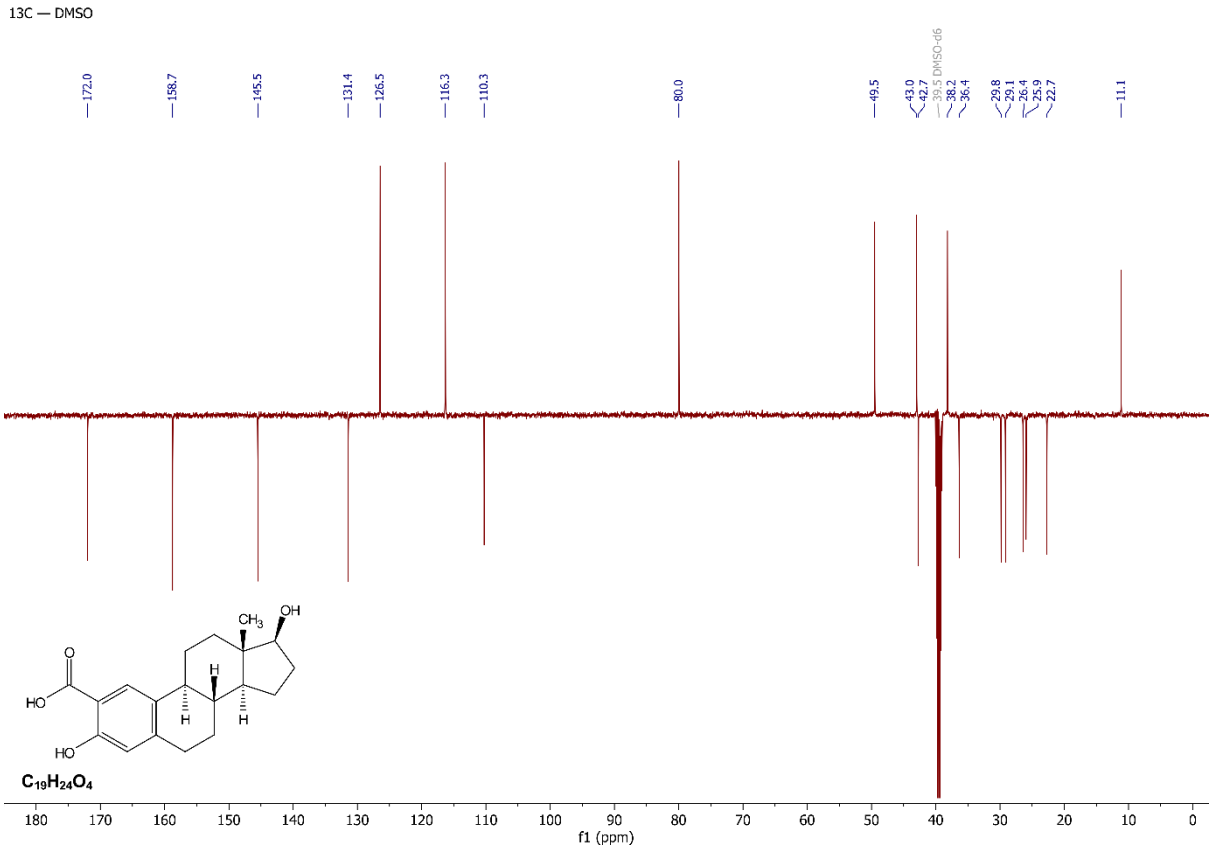
^{13}C NMR spectrum of compound **6b** (DMSO-d_6, 125 MHz)



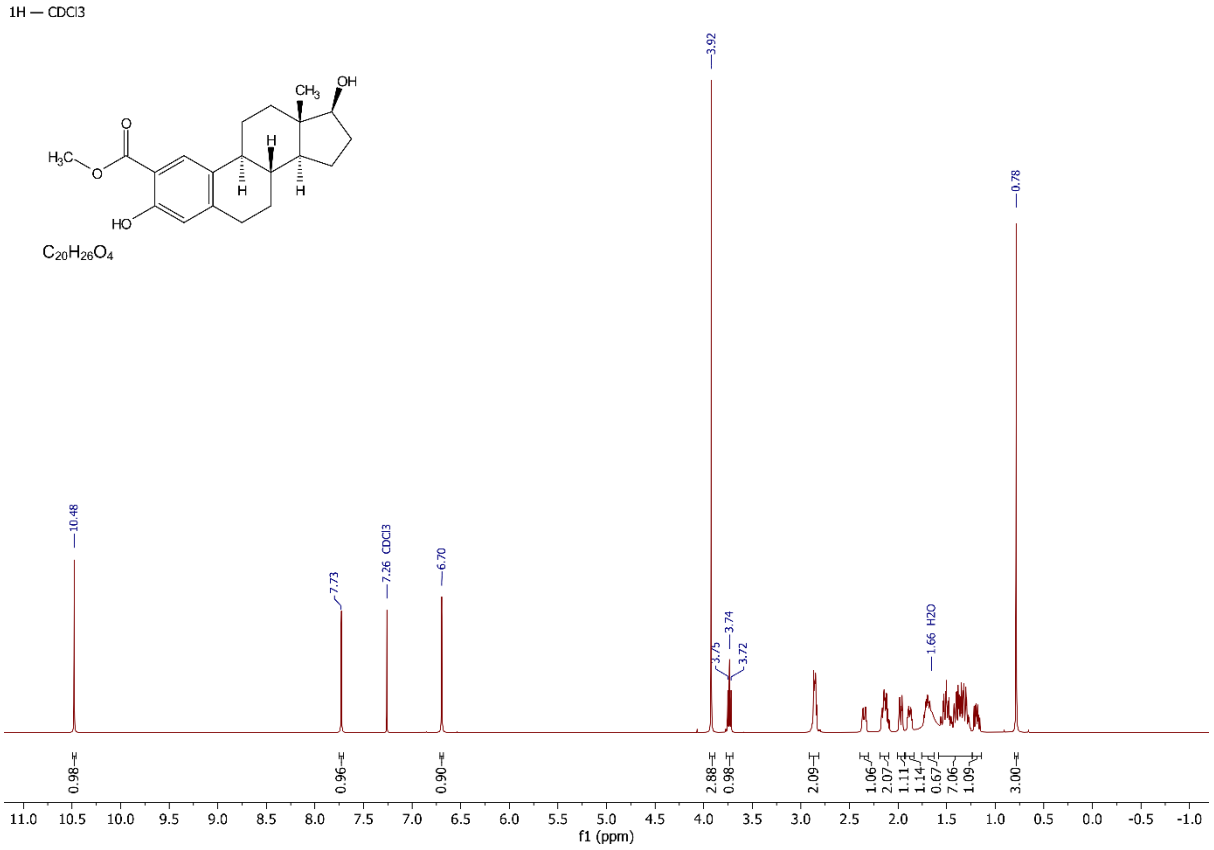
^{1}H NMR spectrum of compound **6c** (DMSO-d_6, 500 MHz)



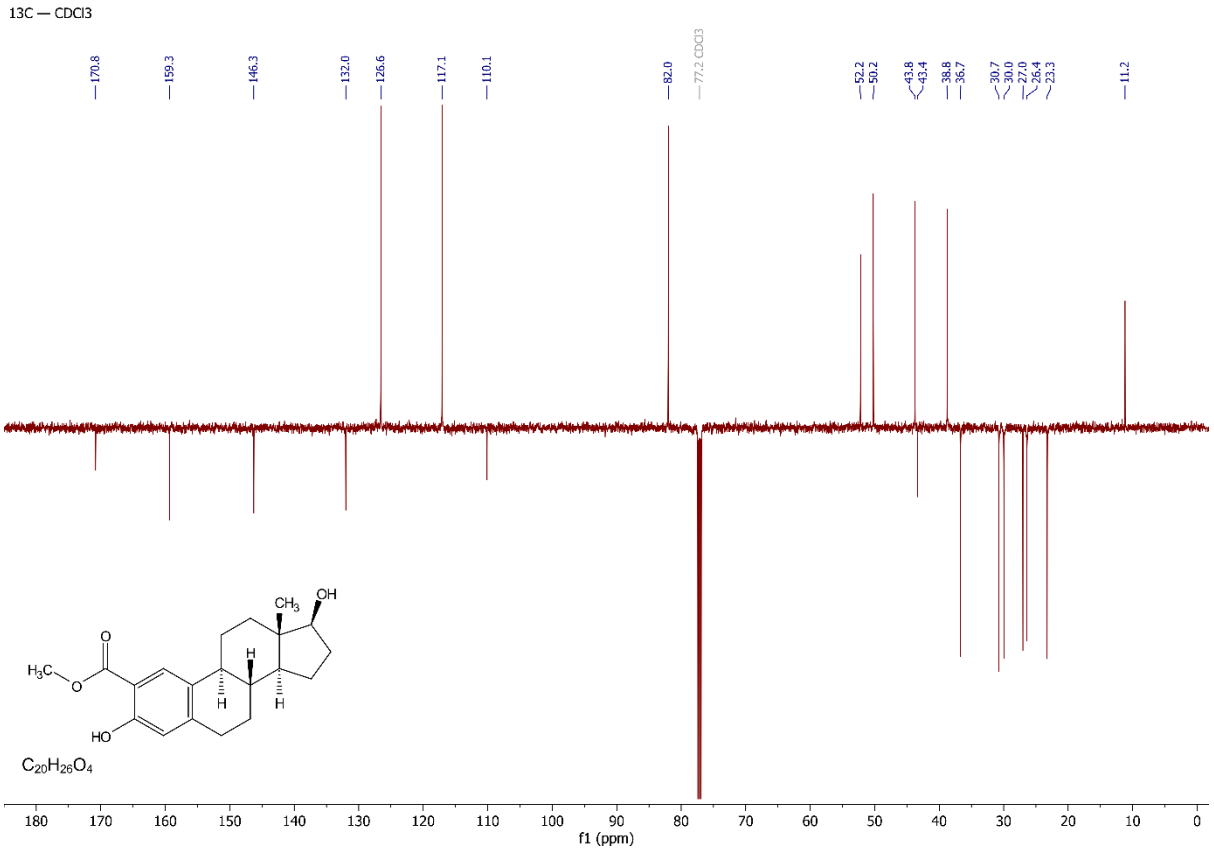
^{13}C NMR spectrum of compound **6c** (DMSO-d_6, 125 MHz)



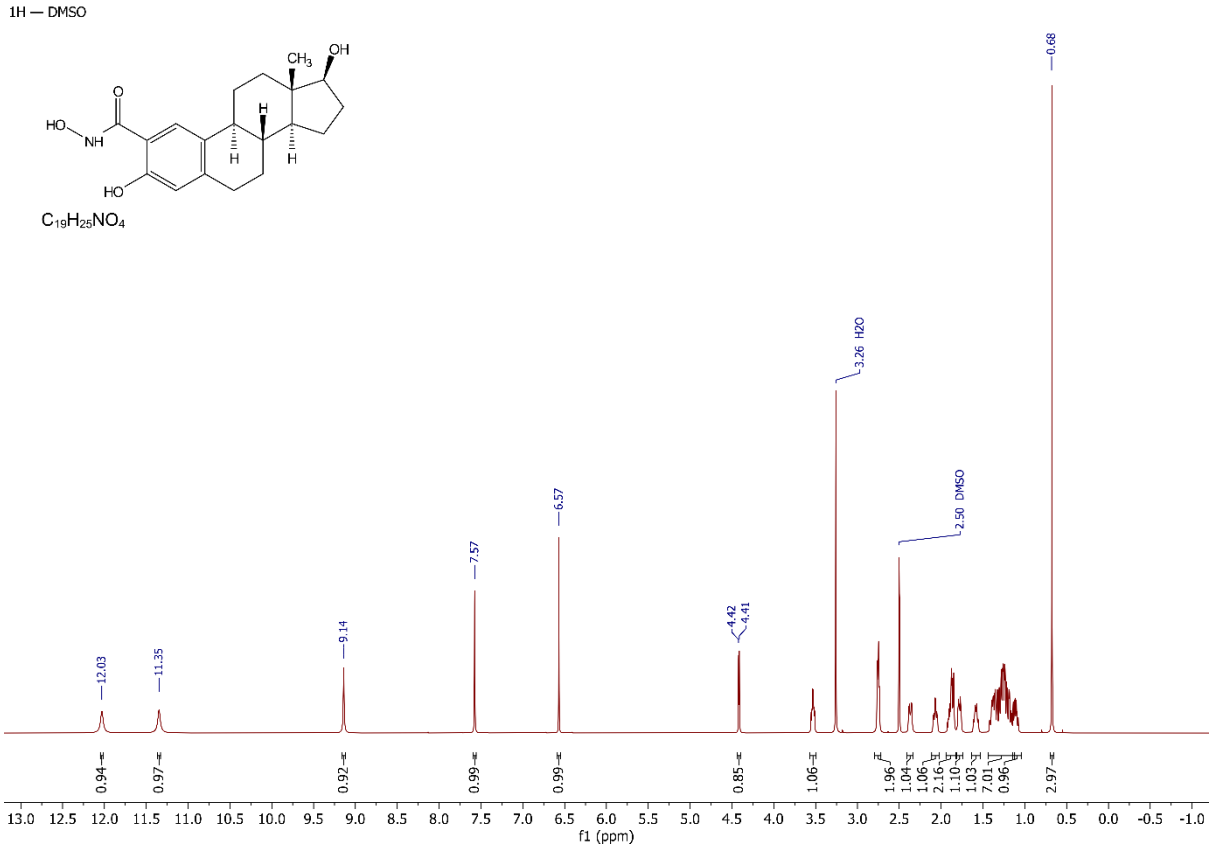
^{1}H NMR spectrum of compound **6d** ($CDCl_3$, 500 MHz)



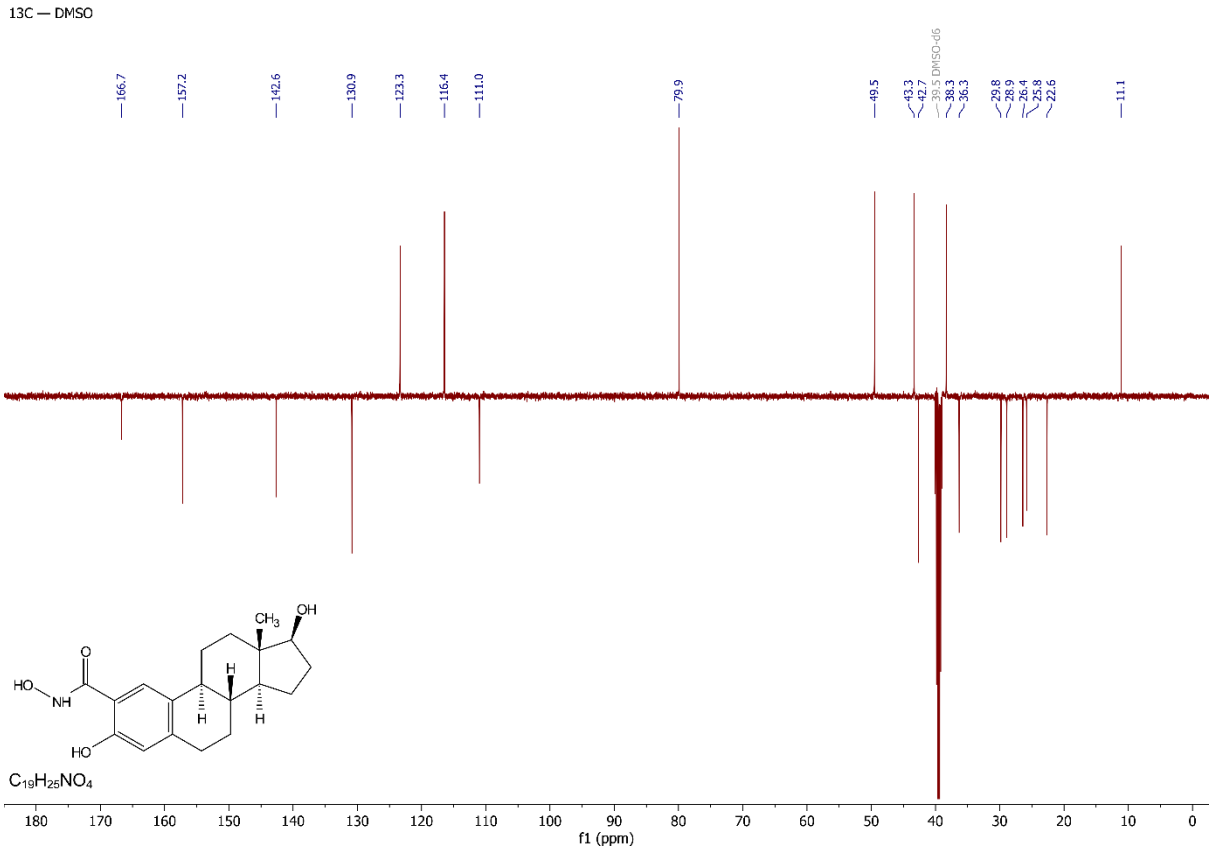
^{13}C NMR spectrum of compound **6d** ($CDCl_3$, 125 MHz)



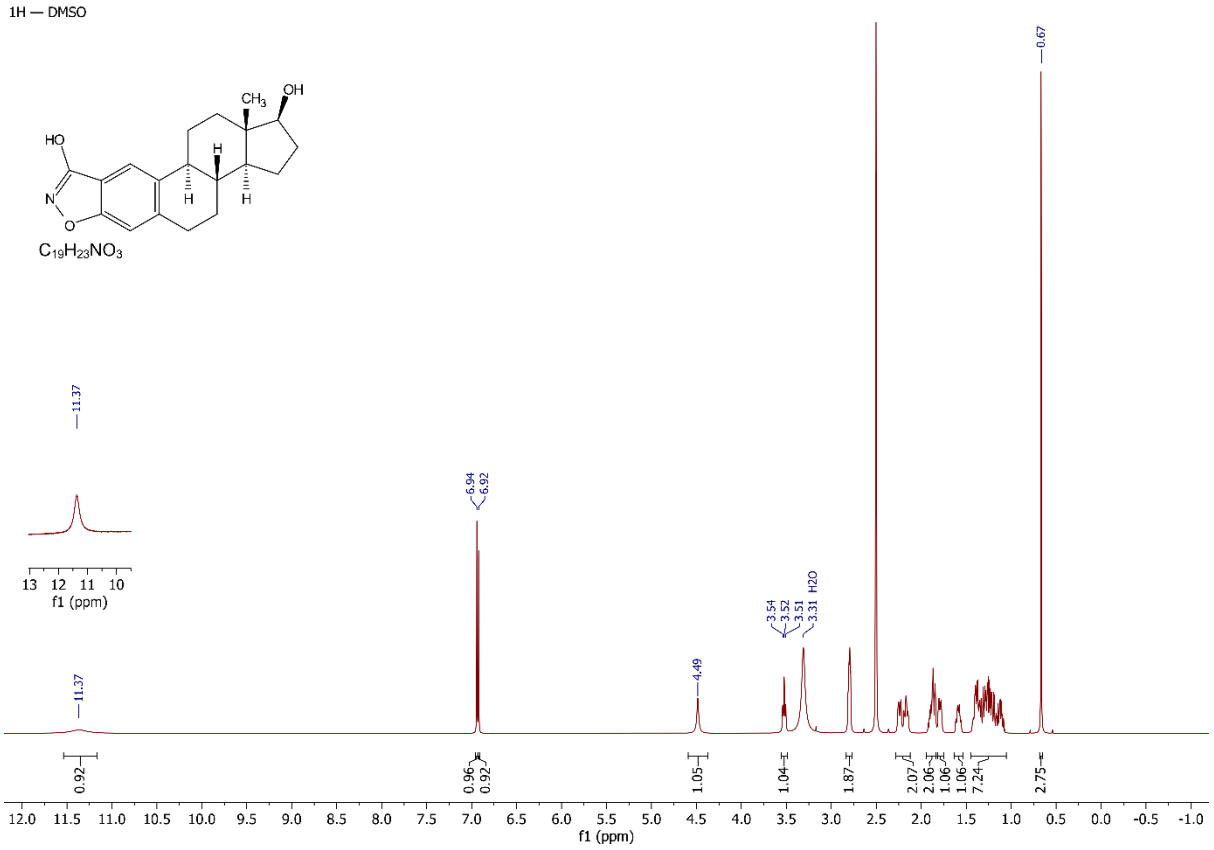
^{1}H NMR spectrum of compound **7** (DMSO-d_6, 500 MHz)



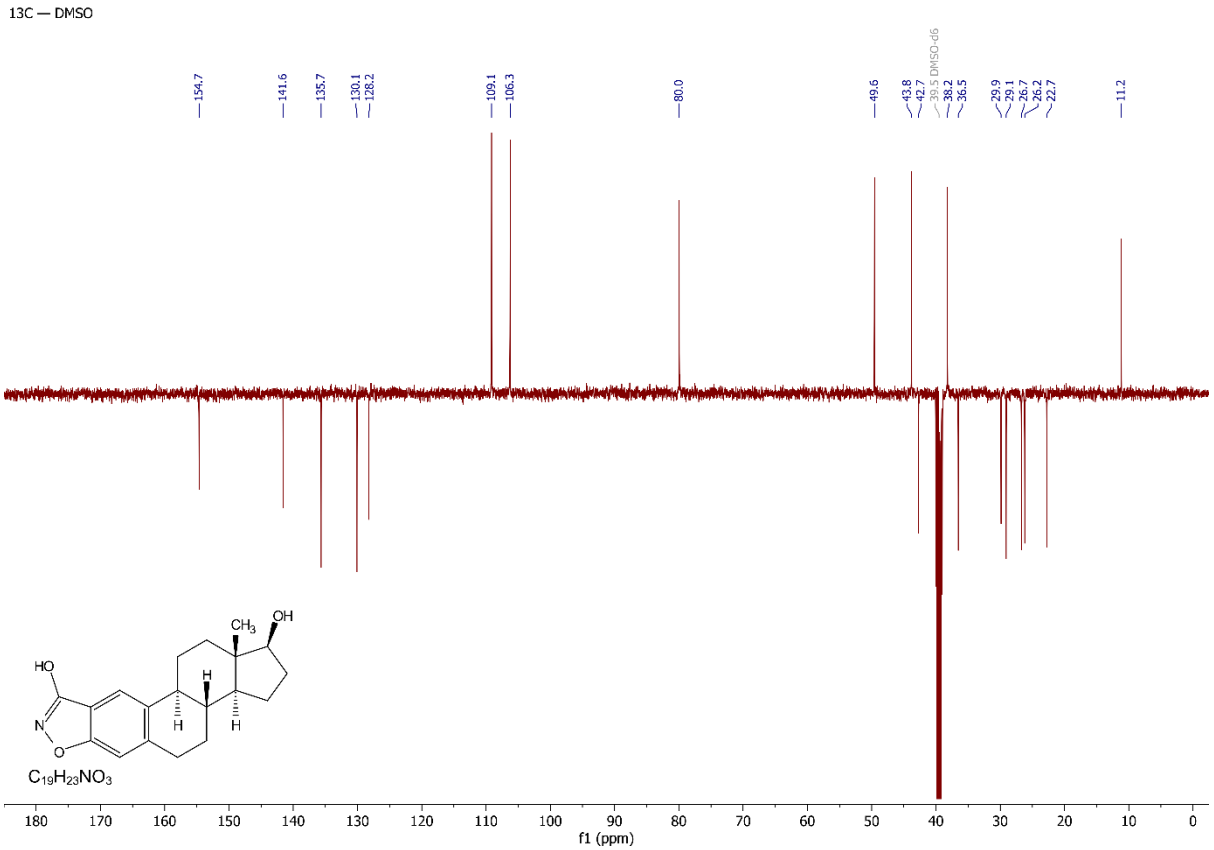
^{13}C NMR spectrum of compound **7** (DMSO-d_6, 125 MHz)



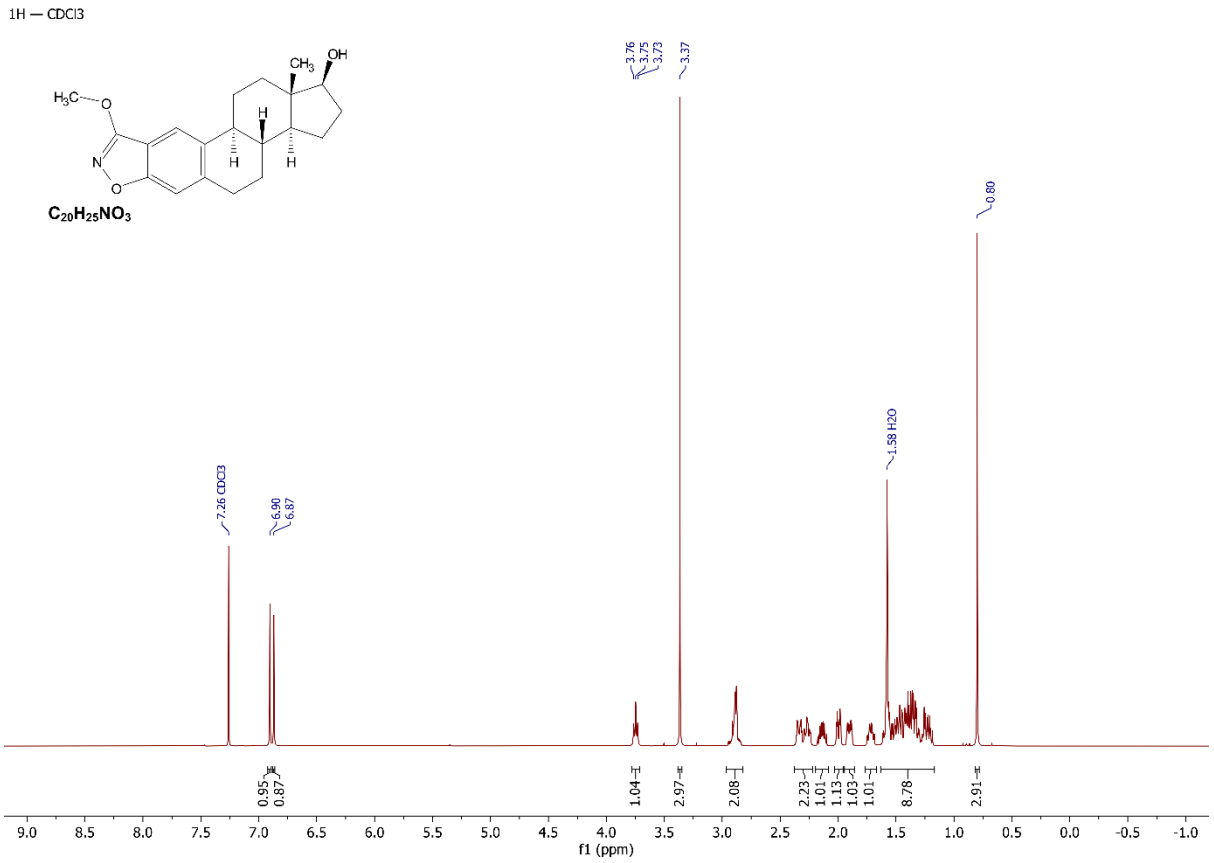
^{1}H NMR spectrum of compound **4h** (DMSO-d_6, 500 MHz)



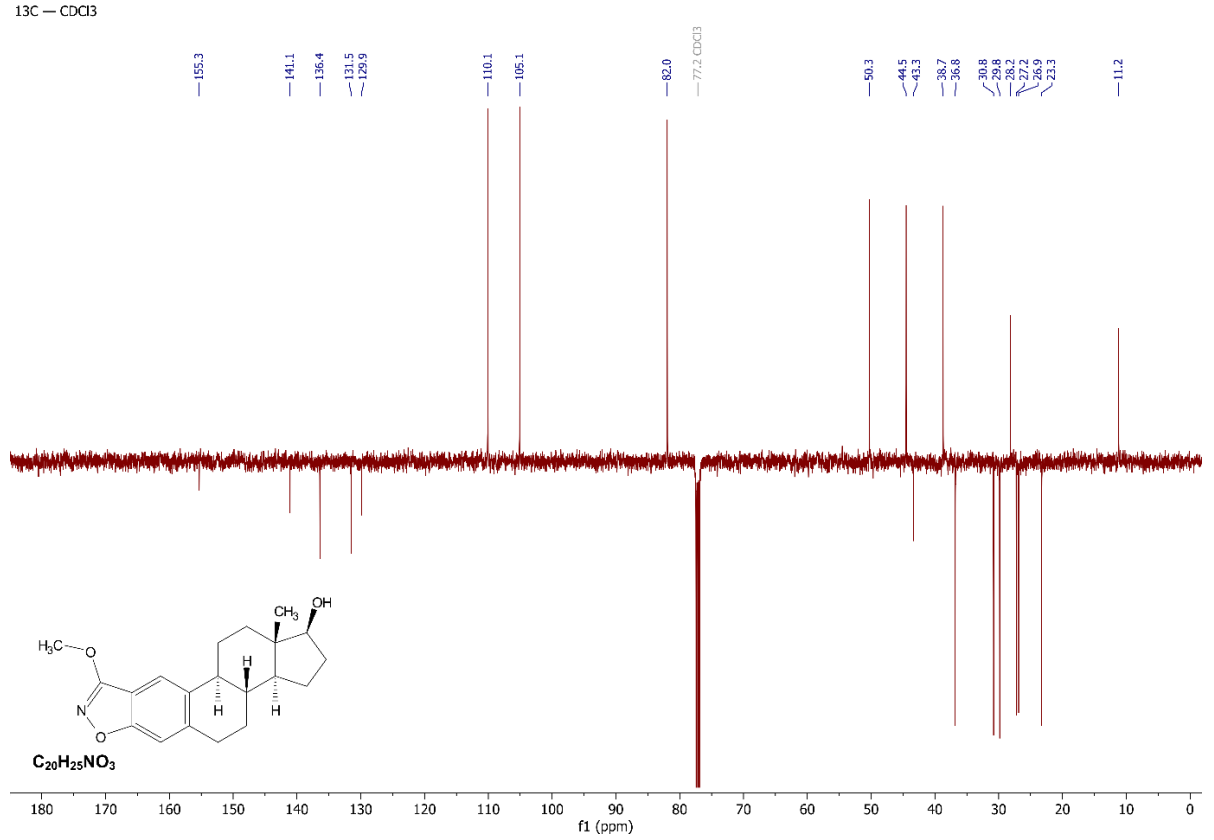
^{13}C NMR spectrum of compound **4h** (DMSO-d_6, 125 MHz)



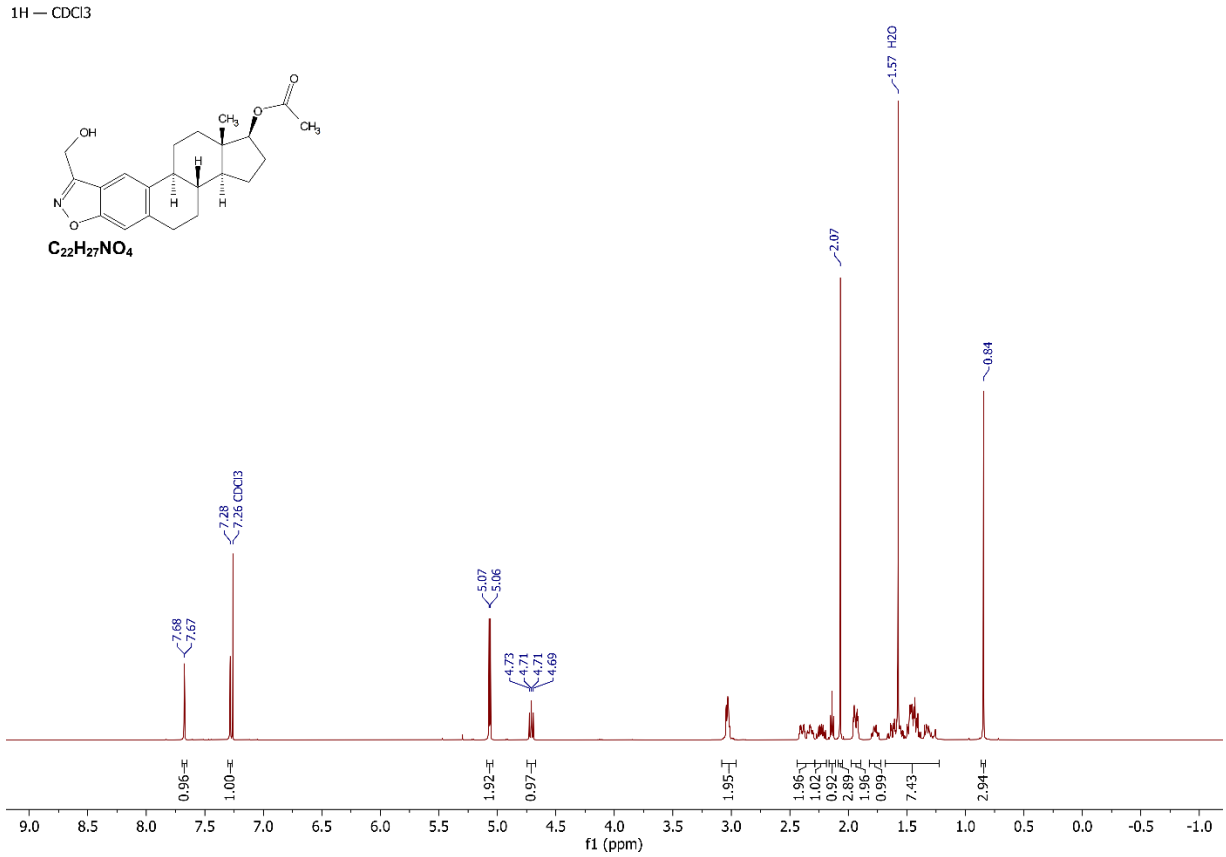
^{1}H NMR spectrum of compound **4i** ($CDCl_3$, 500 MHz)



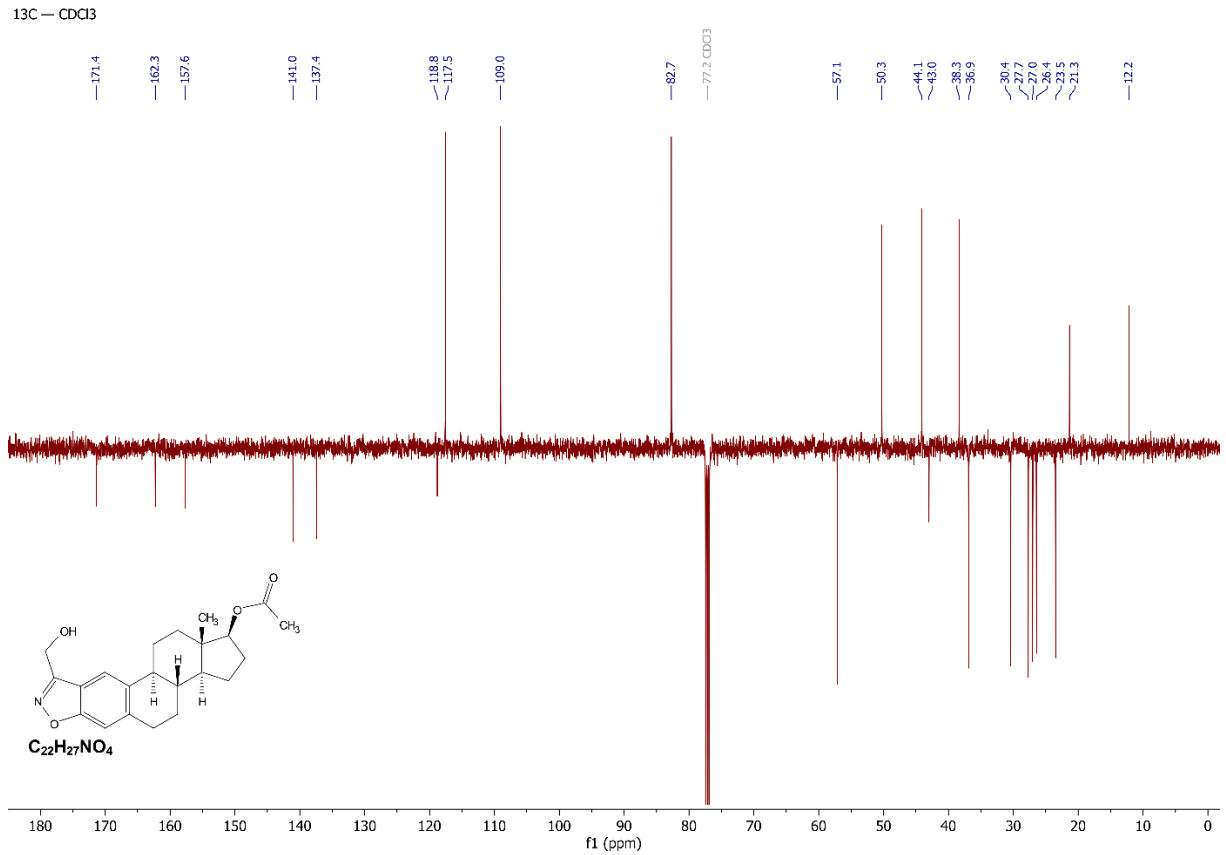
^{13}C NMR spectrum of compound **4i** ($CDCl_3$, 125 MHz)



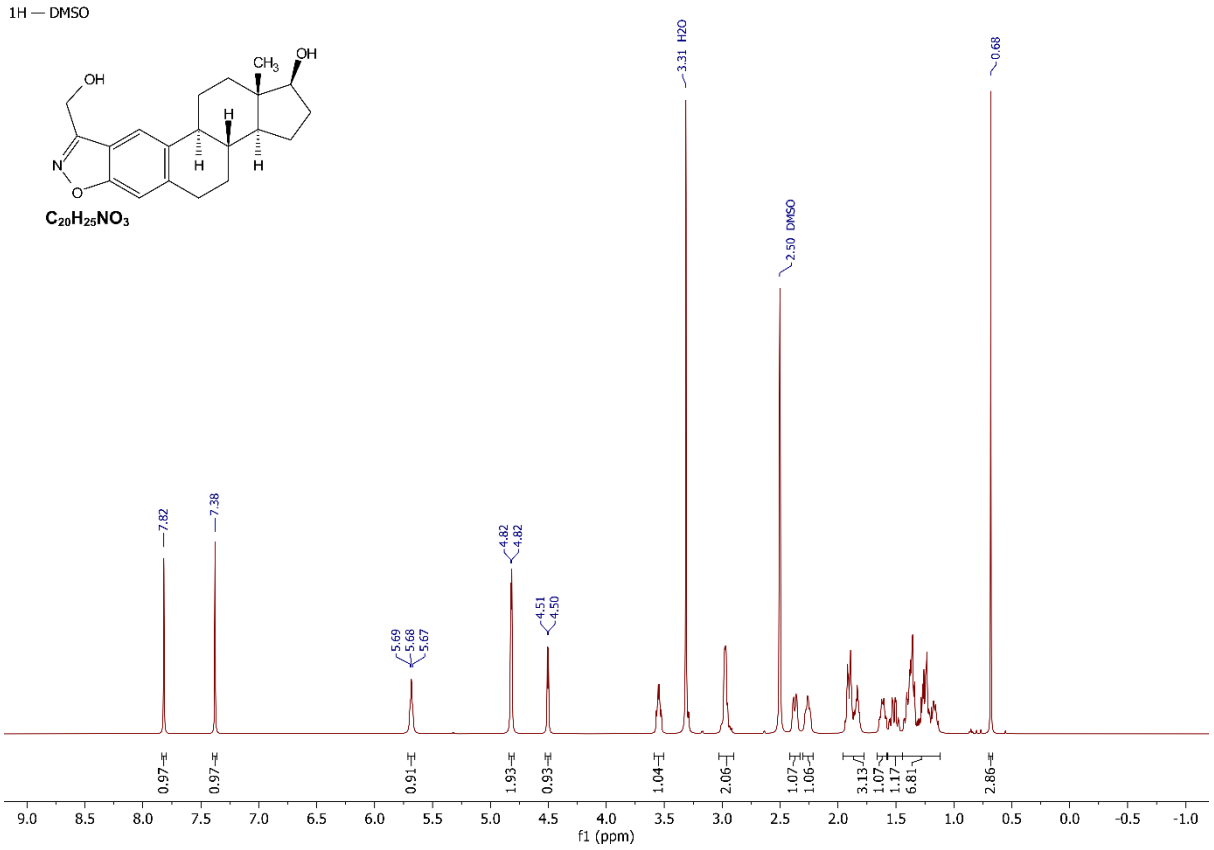
^{1}H NMR spectrum of compound **4j** ($CDCl_3$, 500 MHz)



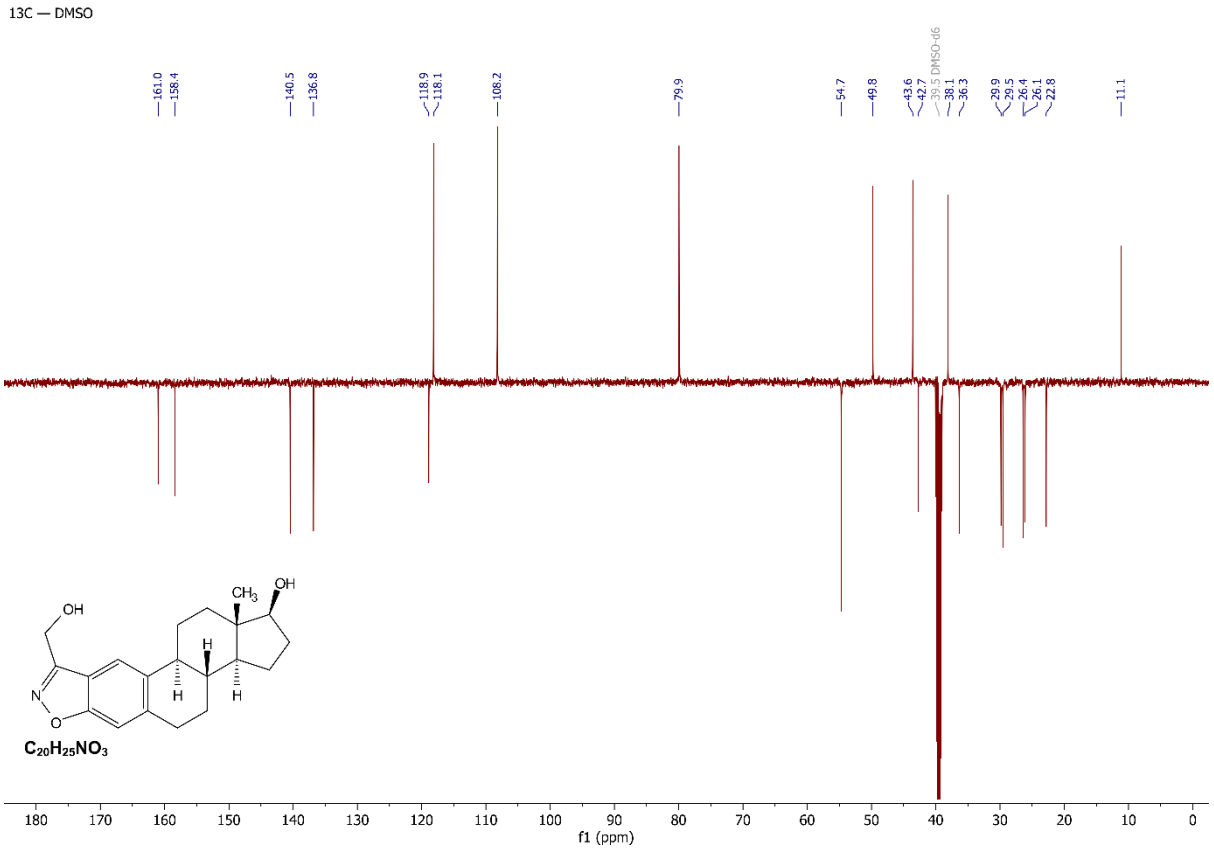
^{13}C NMR spectrum of compound **4j** ($CDCl_3$, 125 MHz)



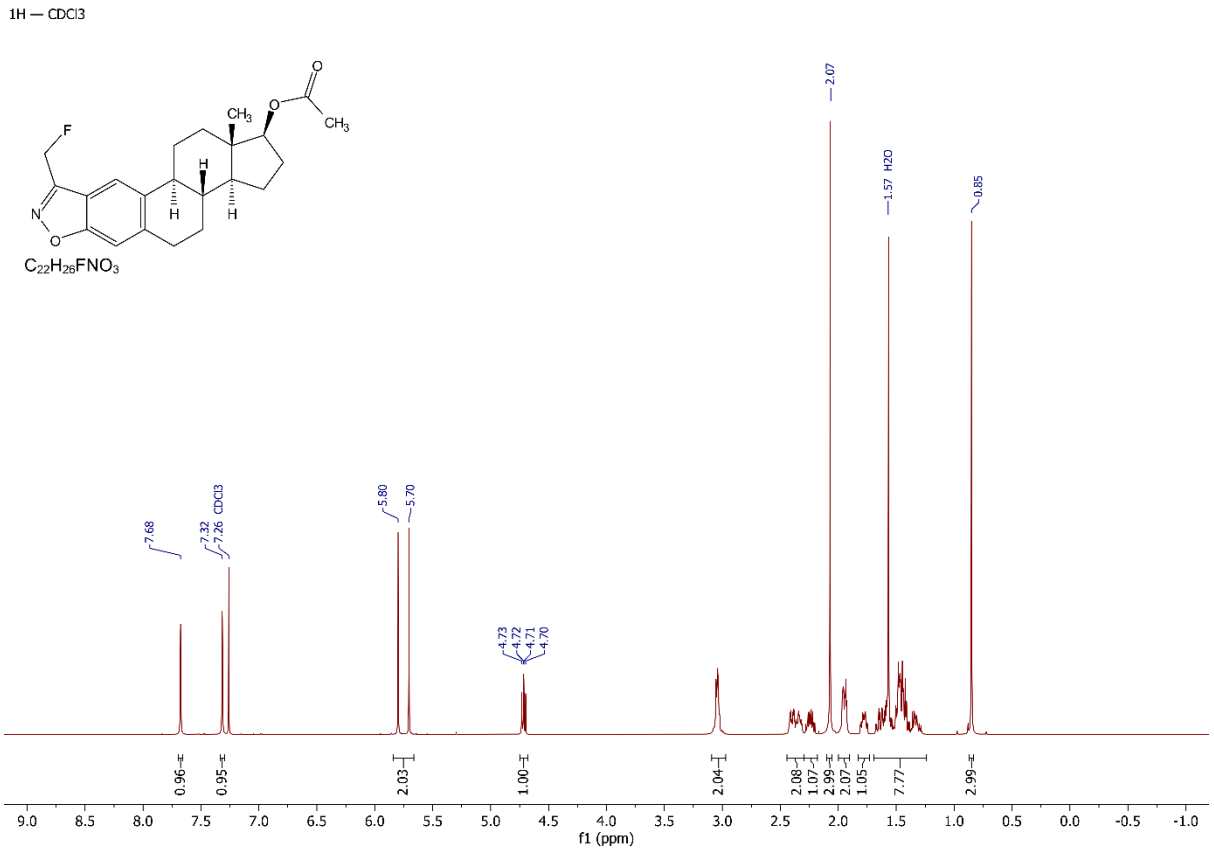
^{1}H NMR spectrum of compound **4k** (DMSO-d_6, 500 MHz)



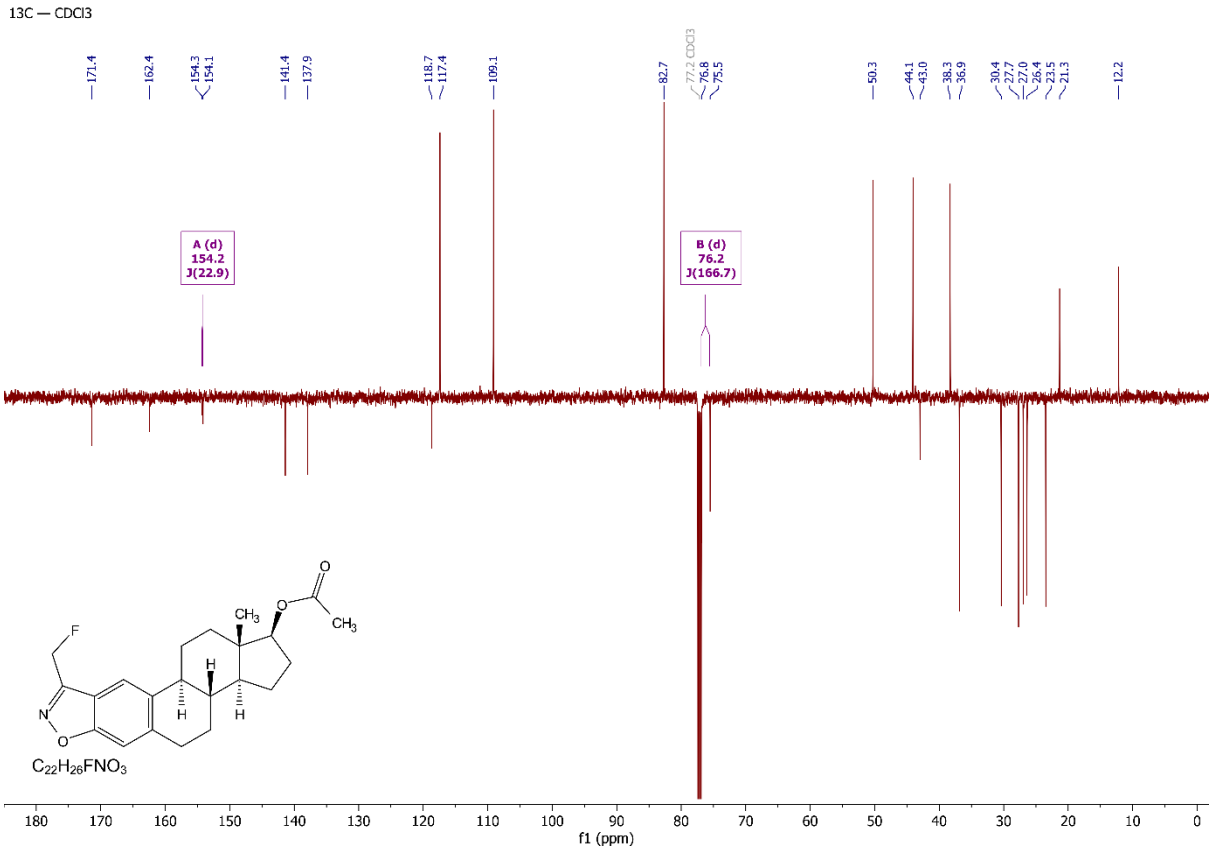
^{13}C NMR spectrum of compound **4k** (DMSO-d_6, 125 MHz)



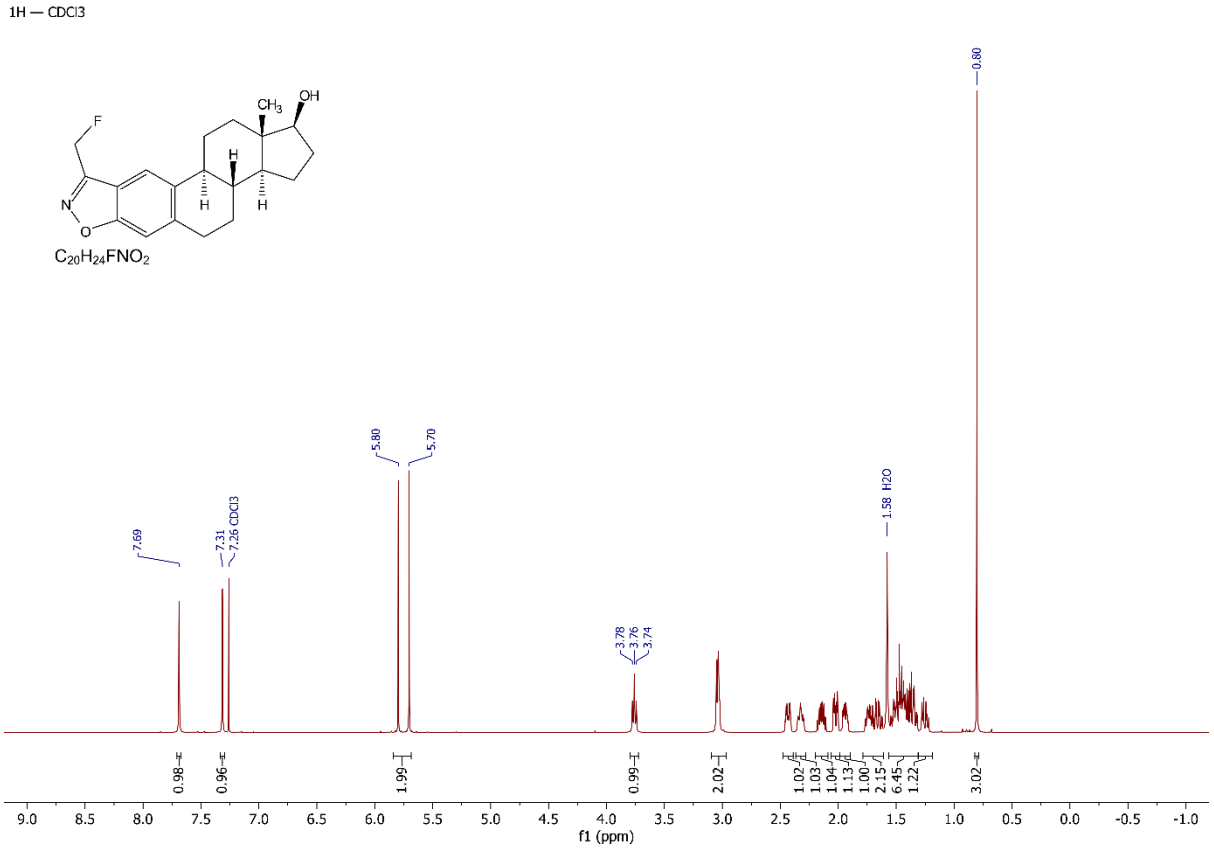
^{1}H NMR spectrum of compound **4l** ($CDCl_3$, 500 MHz)



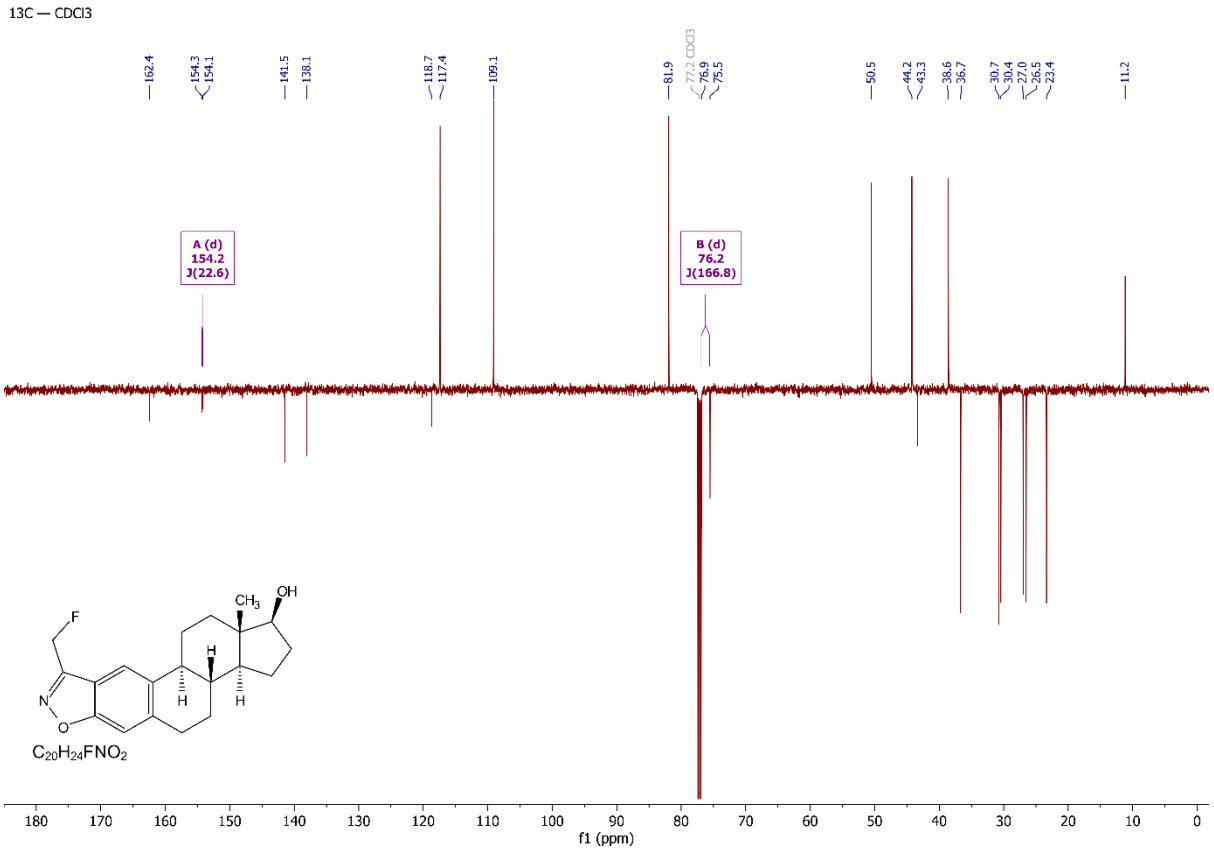
^{13}C NMR spectrum of compound **4l** ($CDCl_3$, 125 MHz)



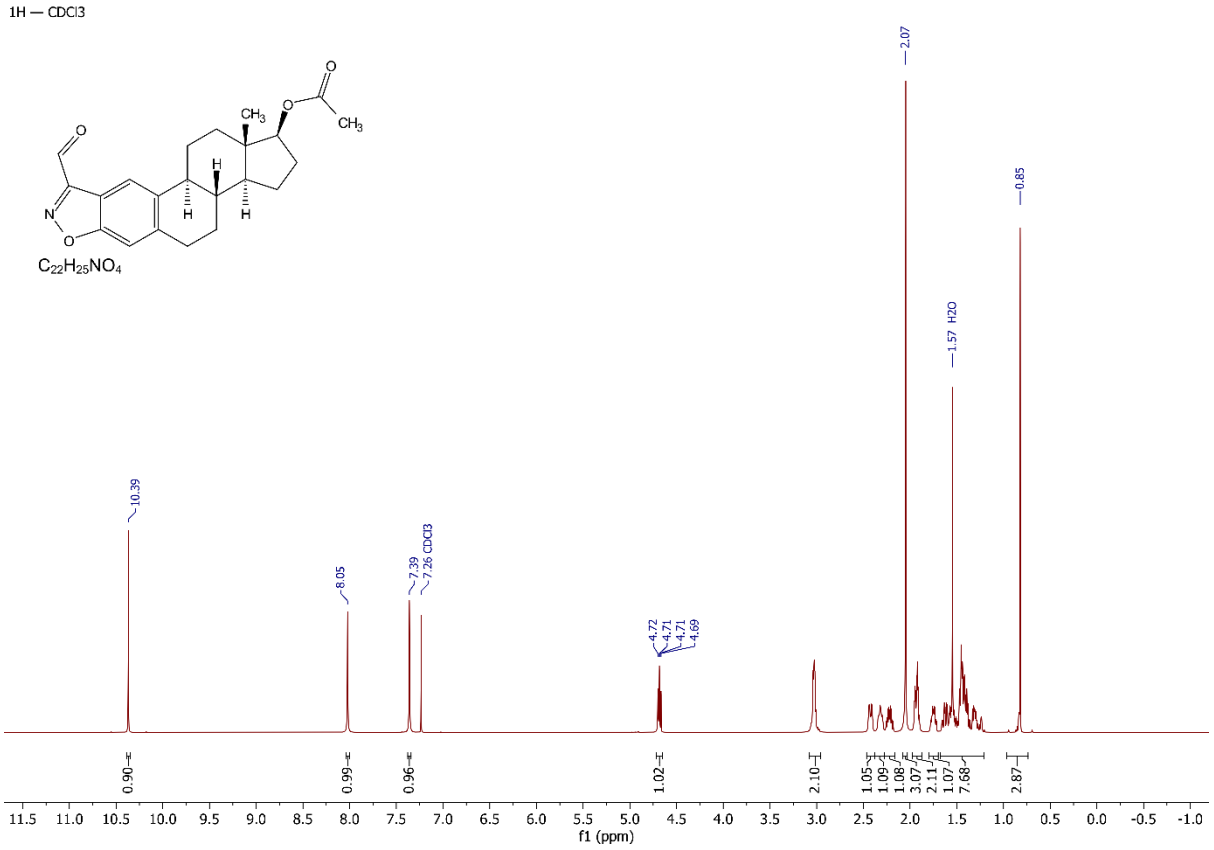
^{1}H NMR spectrum of compound **4m** ($CDCl_3$, 500 MHz)



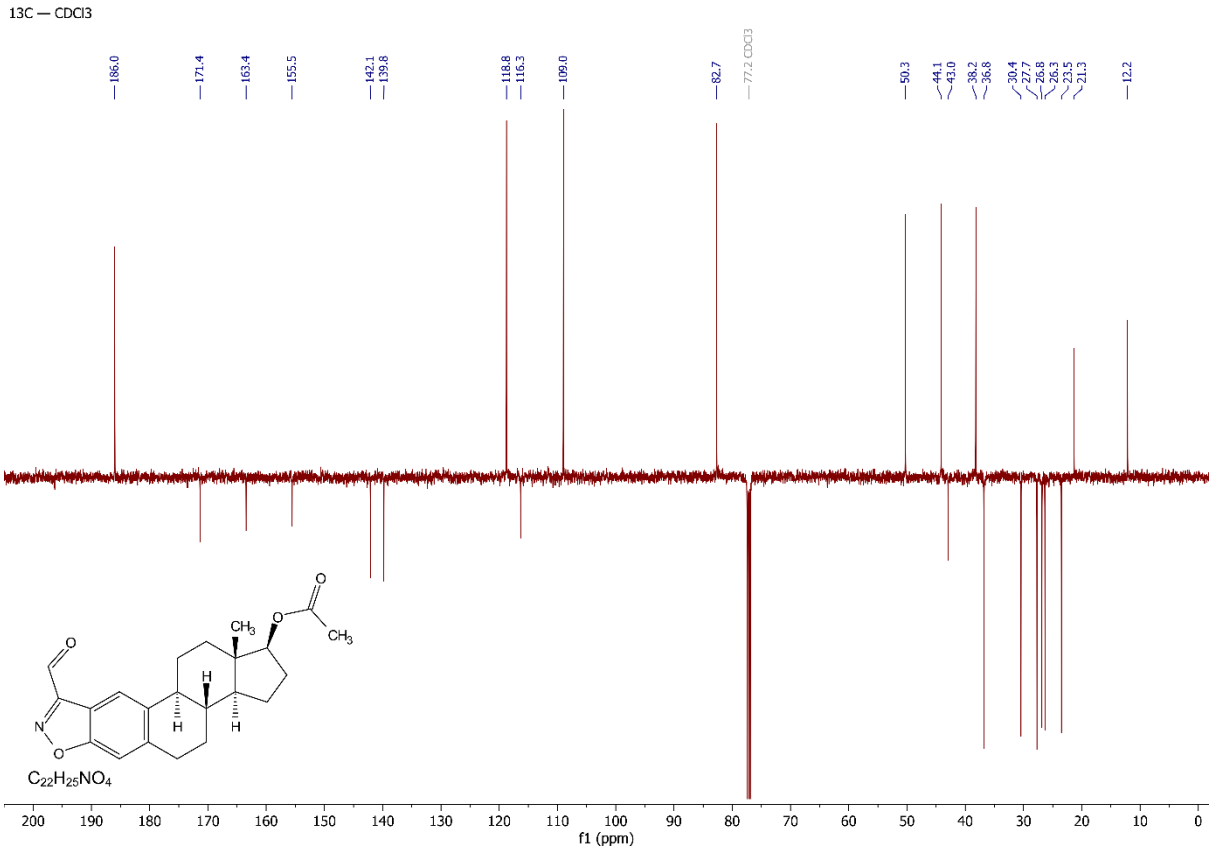
^{13}C NMR spectrum of compound **4m** ($CDCl_3$, 125 MHz)



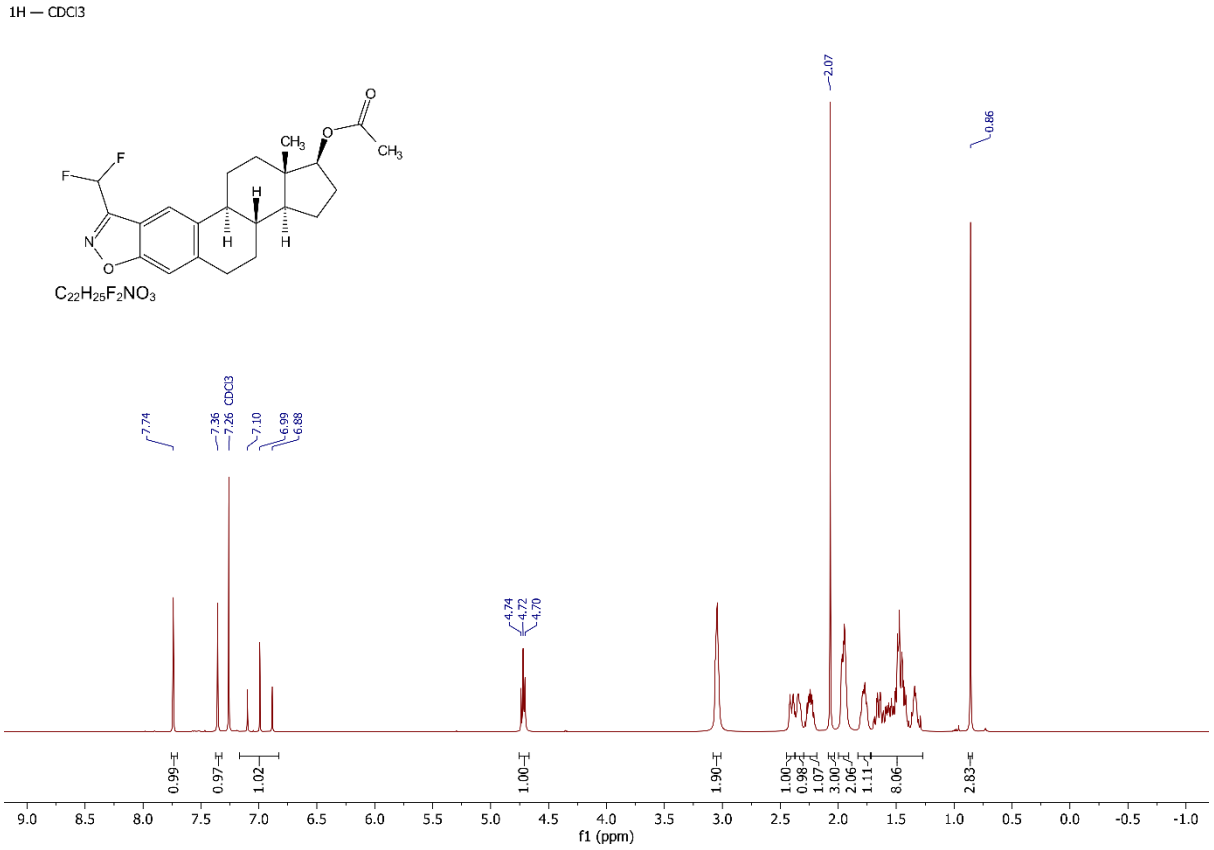
^{1}H NMR spectrum of compound **4n** ($CDCl_3$, 500 MHz)



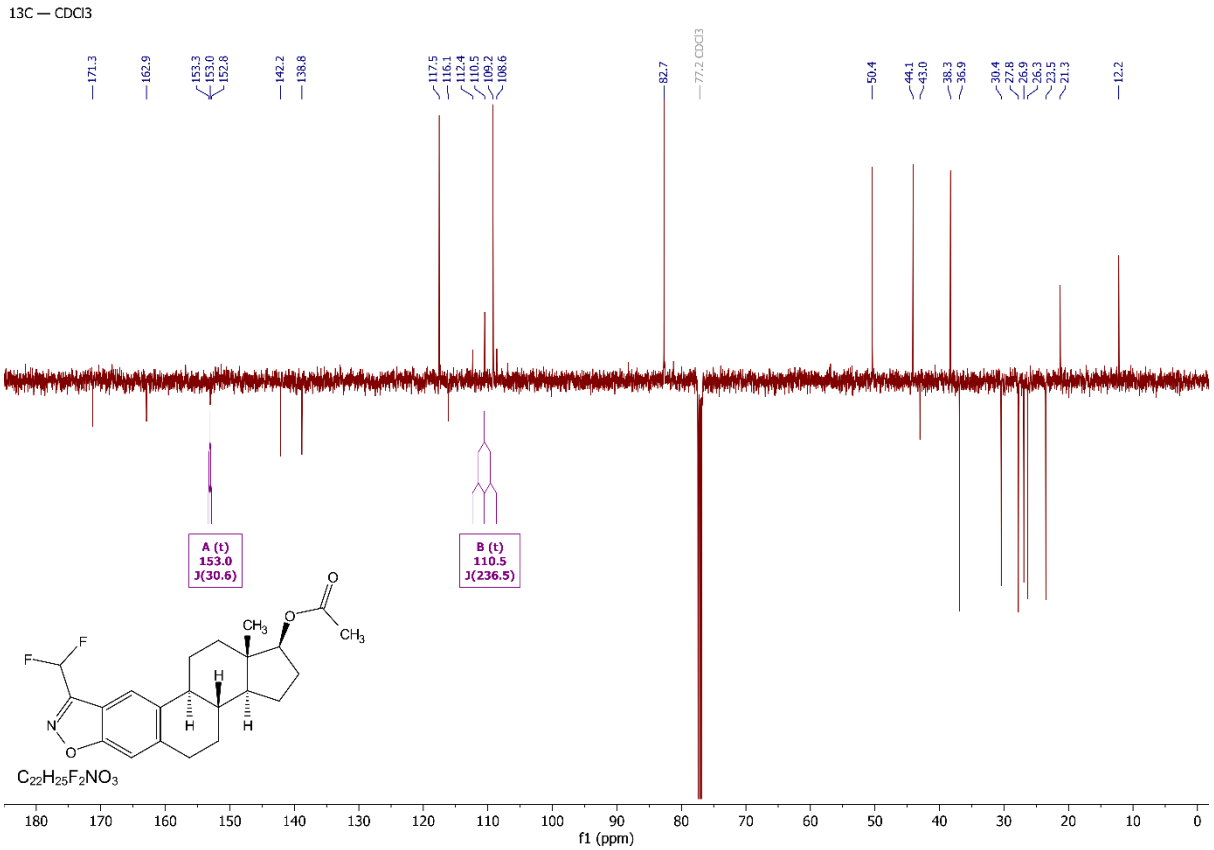
^{13}C NMR spectrum of compound **4n** ($CDCl_3$, 125 MHz)



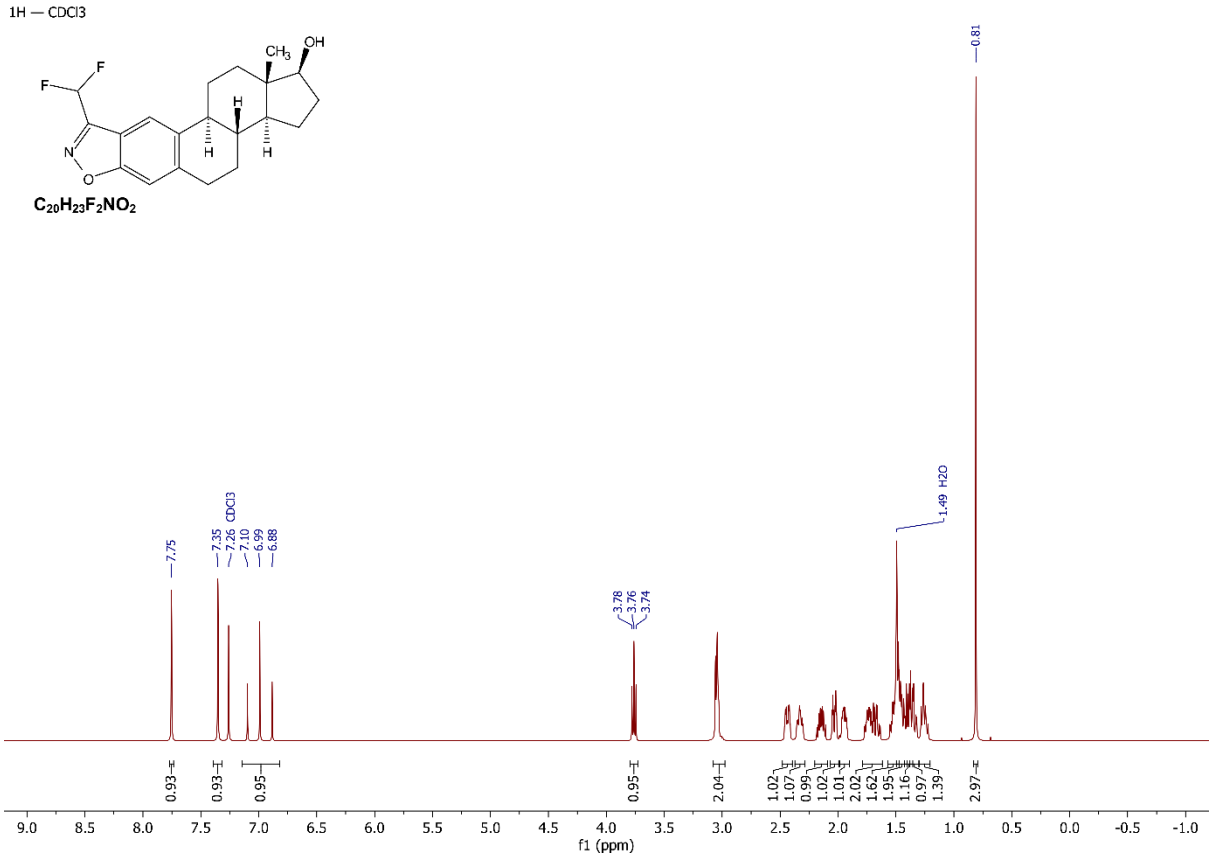
^{1}H NMR spectrum of compound **4o** ($CDCl_3$, 500 MHz)



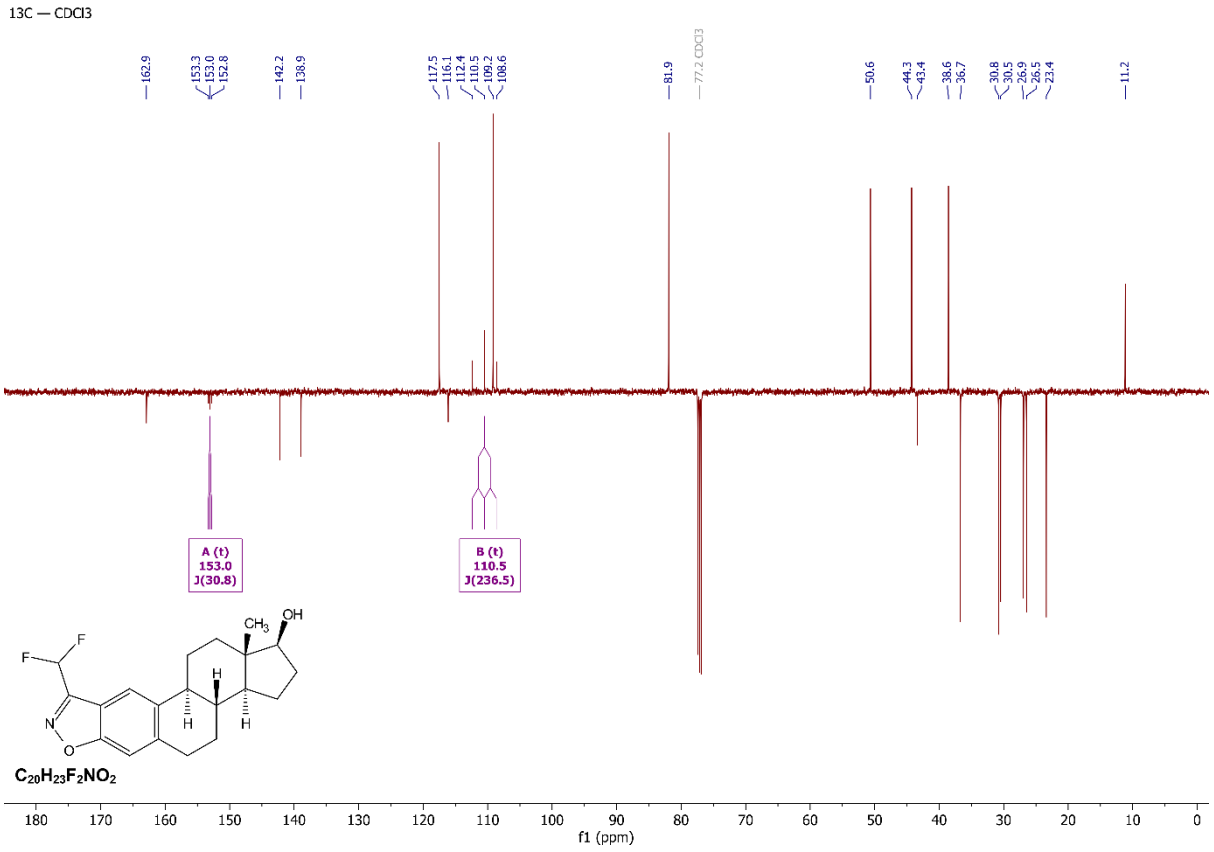
^{13}C NMR spectrum of compound **4o** ($CDCl_3$, 125 MHz)



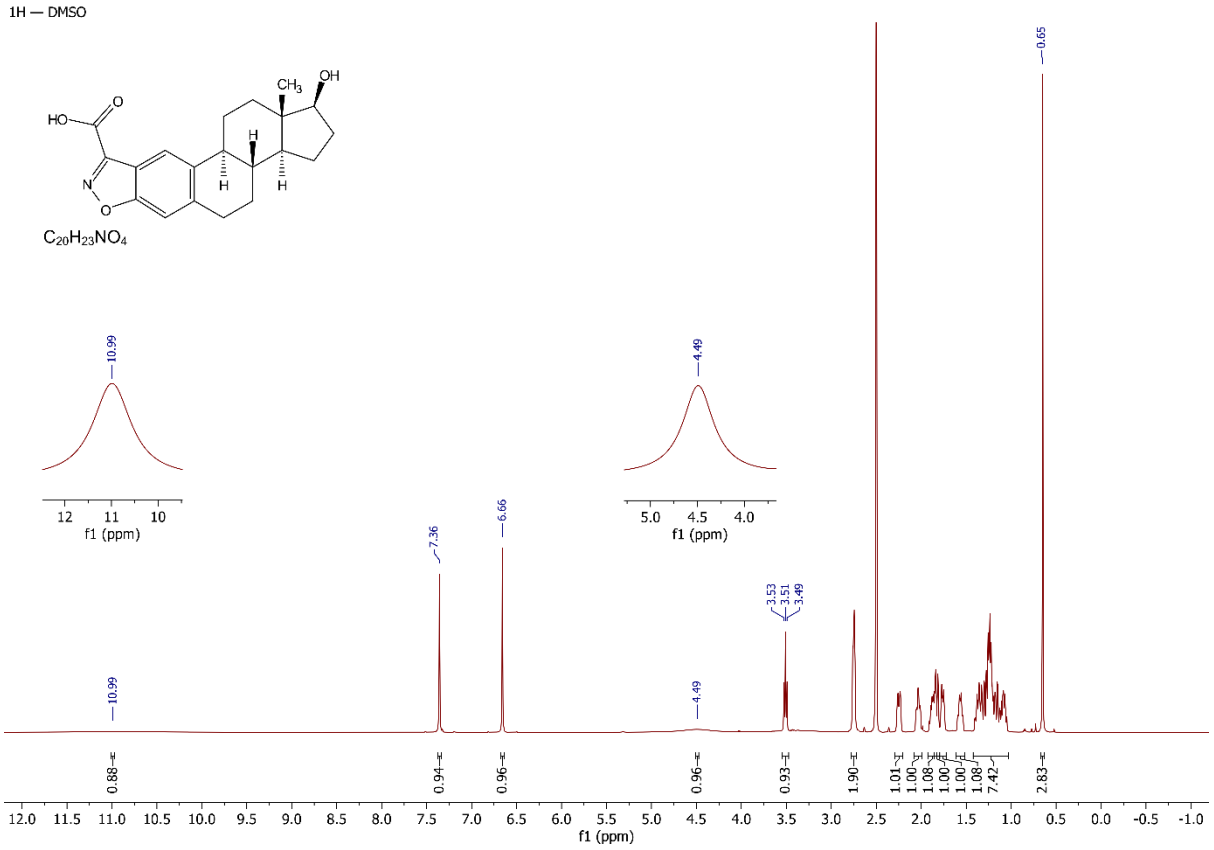
^{1}H NMR spectrum of compound **4p** ($CDCl_3$, 500 MHz)



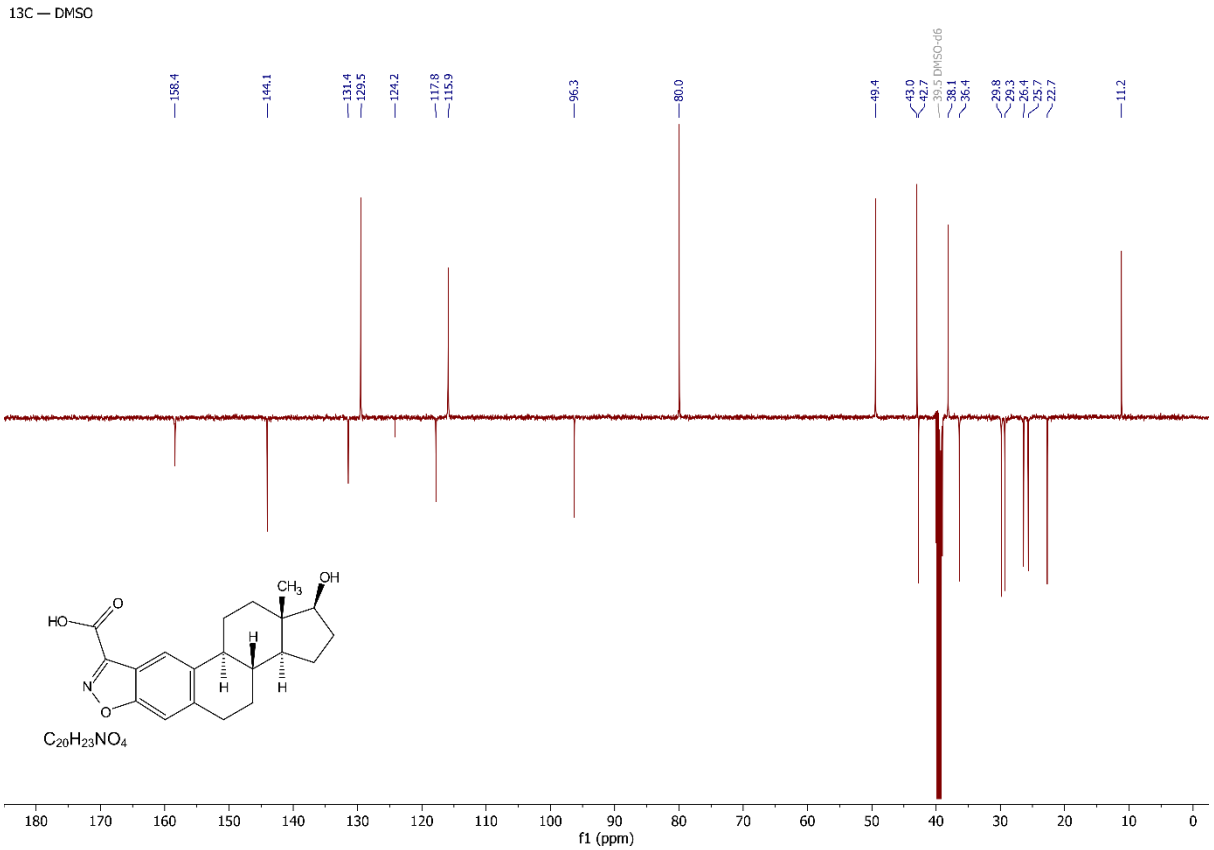
^{13}C NMR spectrum of compound **4p** ($CDCl_3$, 125 MHz)



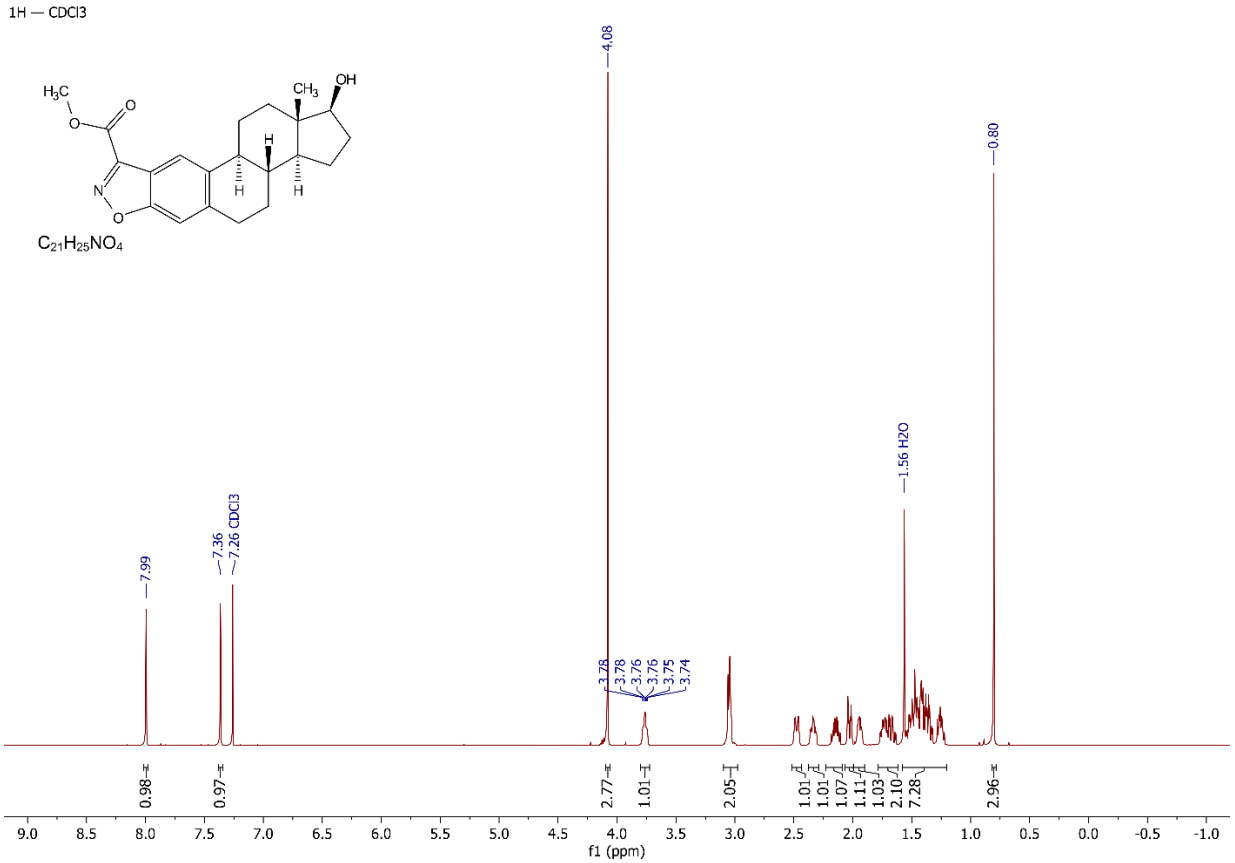
^{1}H NMR spectrum of compound **4q** (DMSO-d_6, 500 MHz)



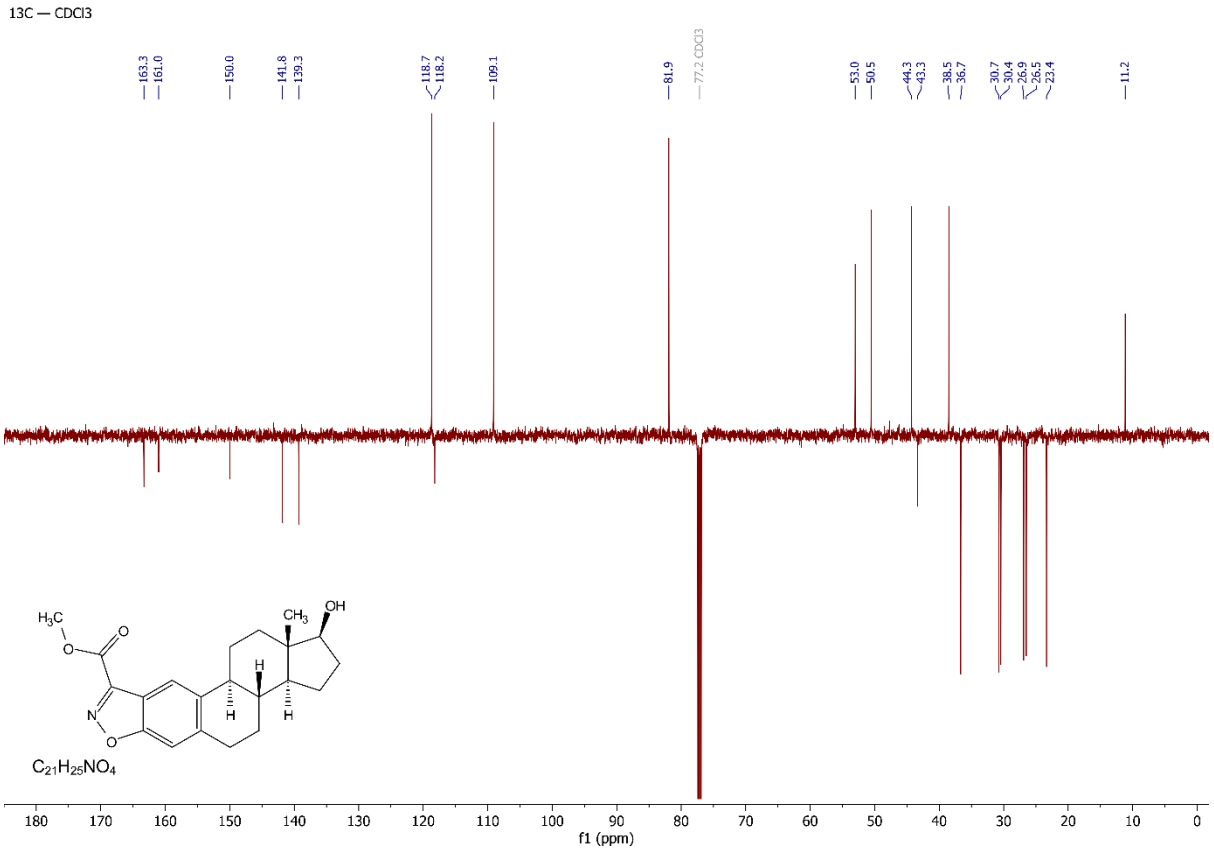
^{13}C NMR spectrum of compound **4q** (DMSO-d_6, 125 MHz)



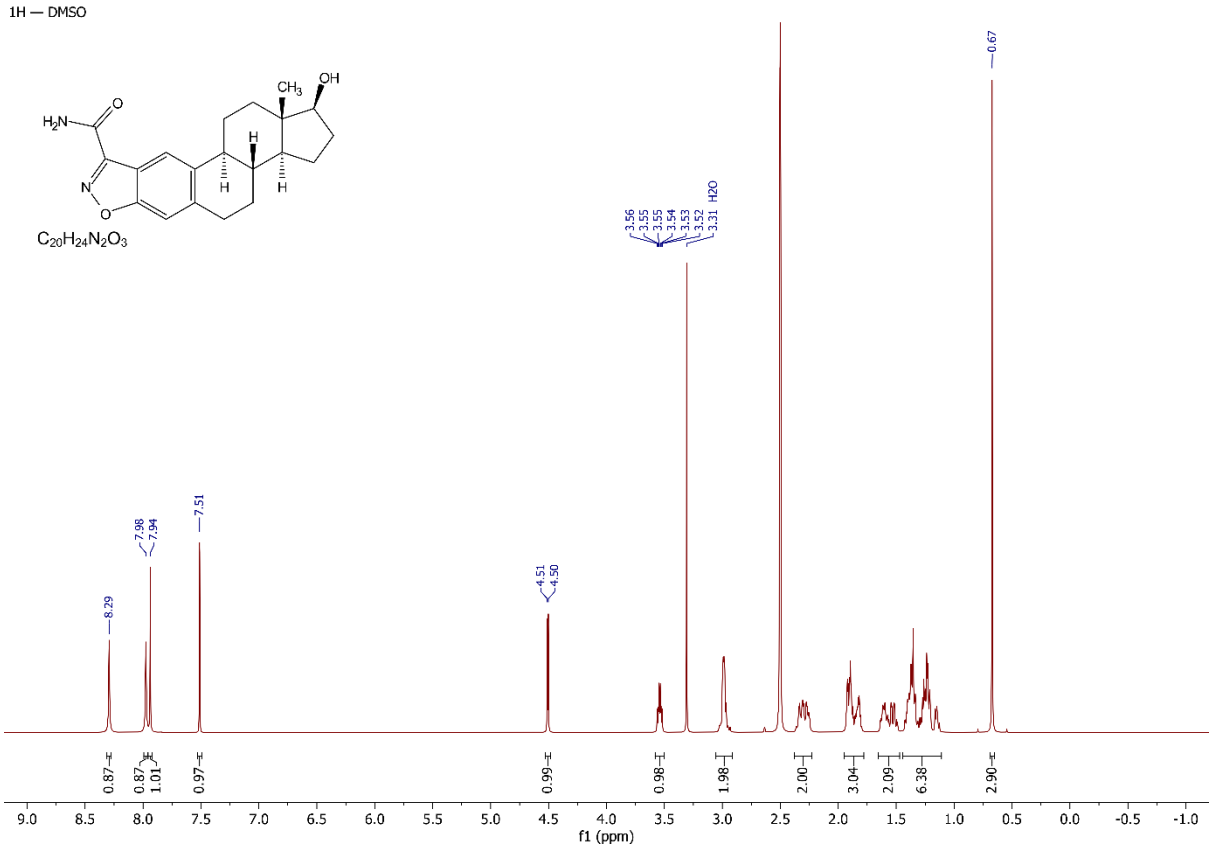
^{1}H NMR spectrum of compound **4r** ($CDCl_3$, 500 MHz)



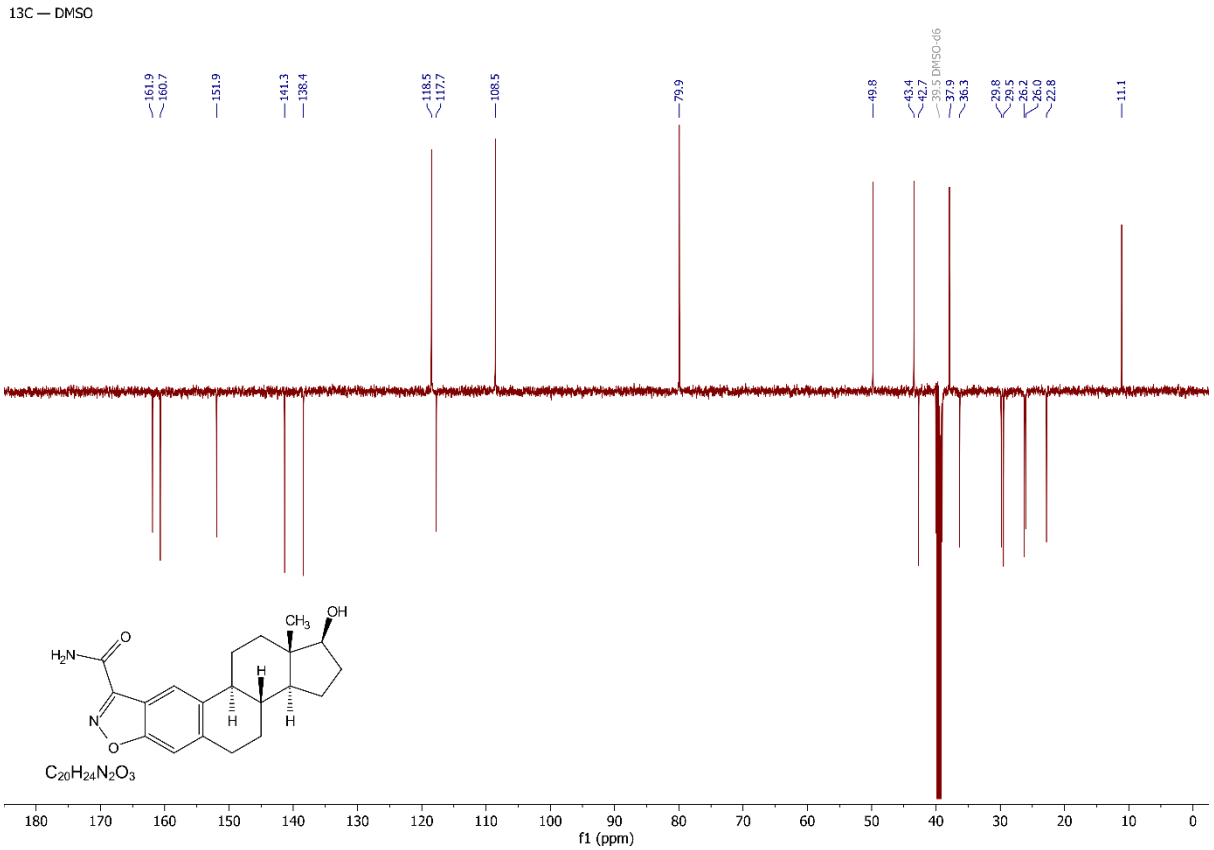
^{13}C NMR spectrum of compound **4r** ($CDCl_3$, 125 MHz)



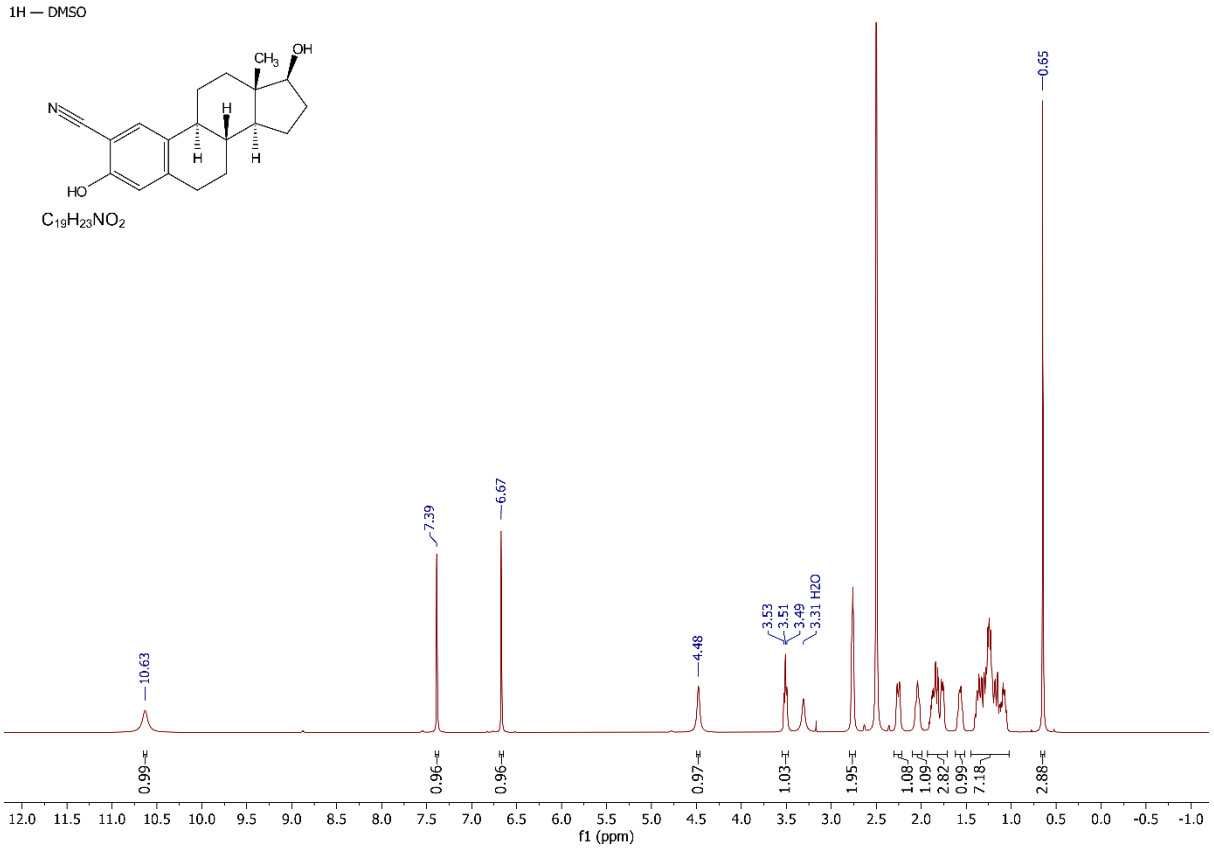
^{1}H NMR spectrum of compound **4s** (DMSO-d_6, 500 MHz)



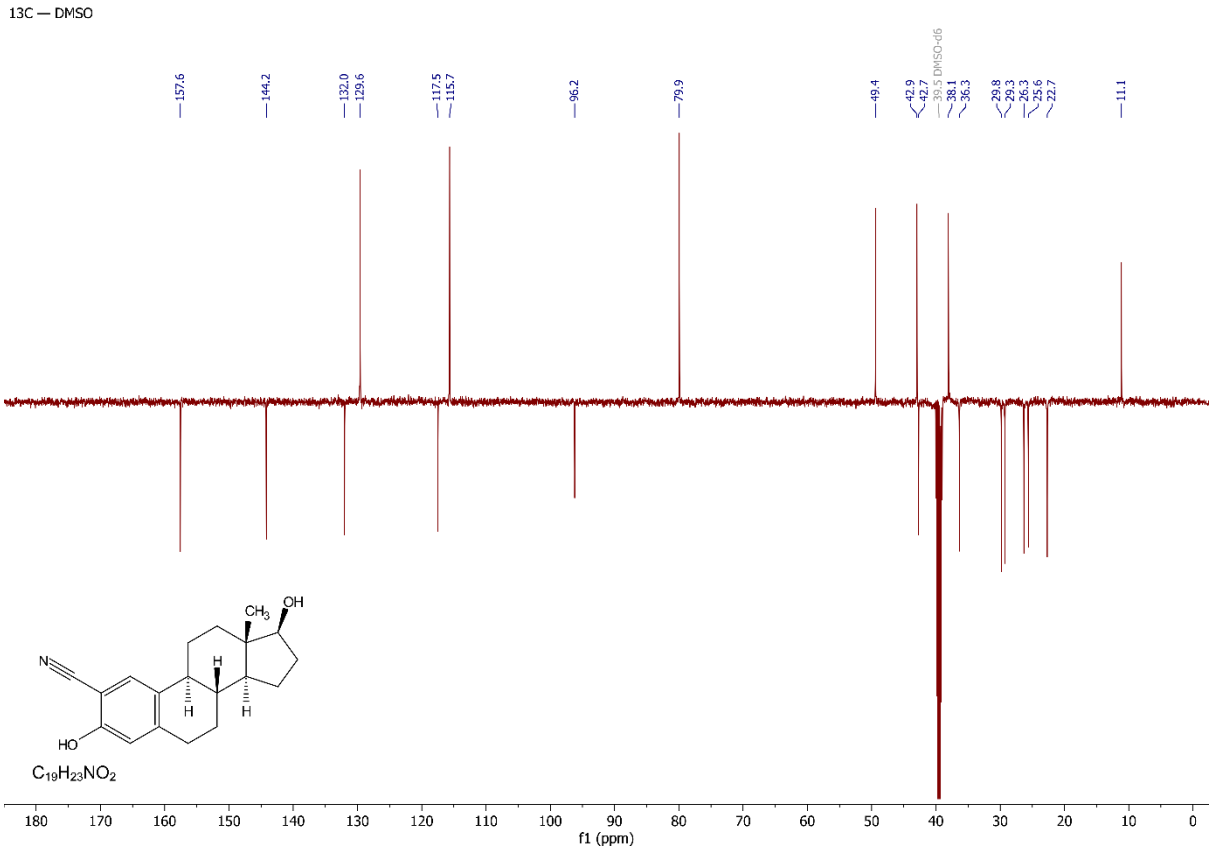
^{13}C NMR spectrum of compound **4s** (DMSO-d_6, 125 MHz)



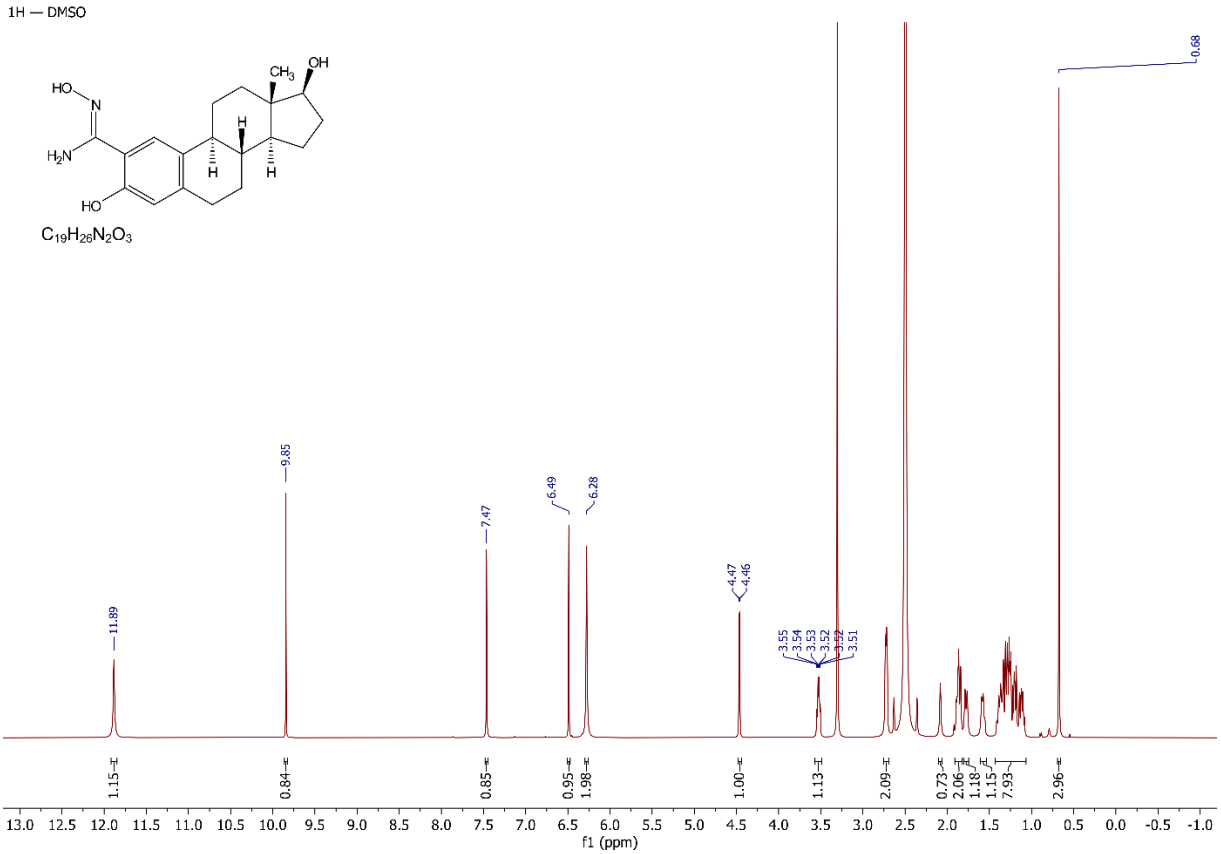
^{1}H NMR spectrum of compound **8** (DMSO-d_6, 500 MHz)



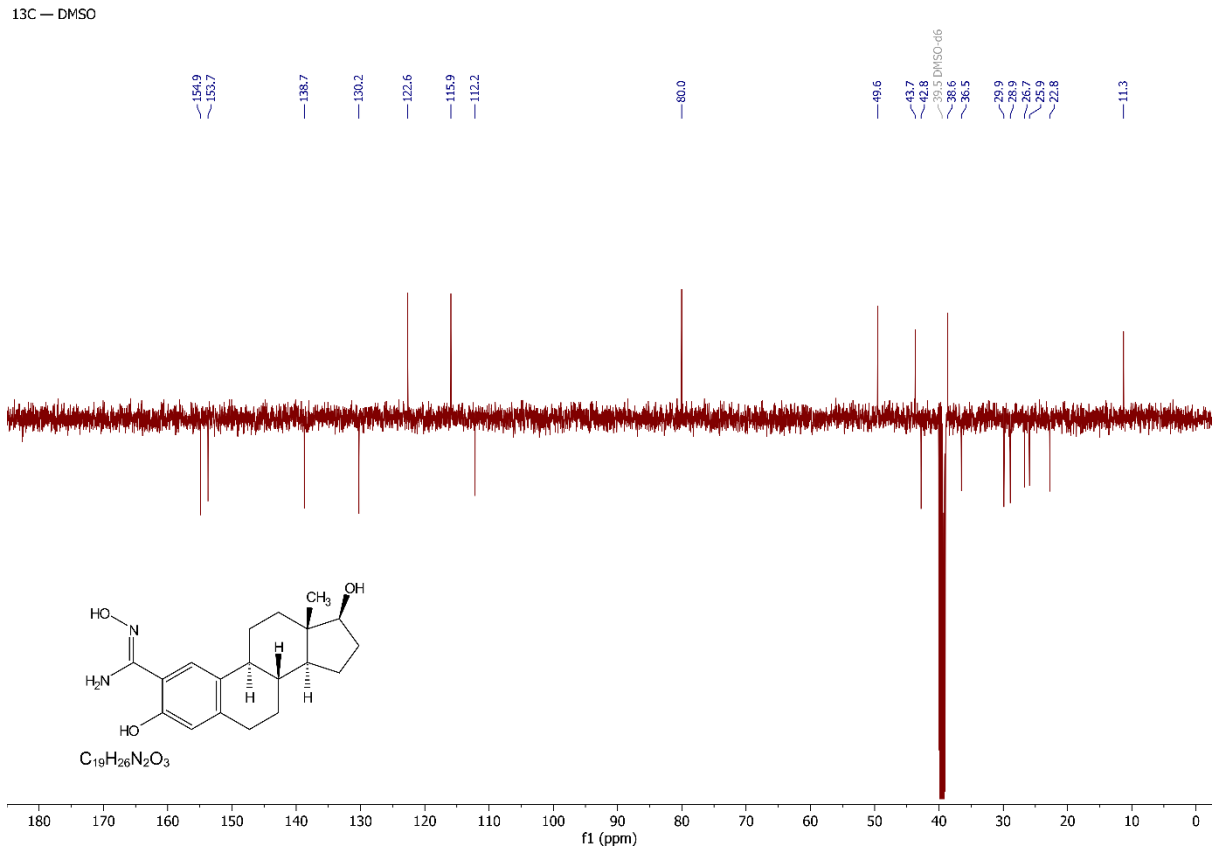
^{13}C NMR spectrum of compound **8** (DMSO-d_6, 125 MHz)



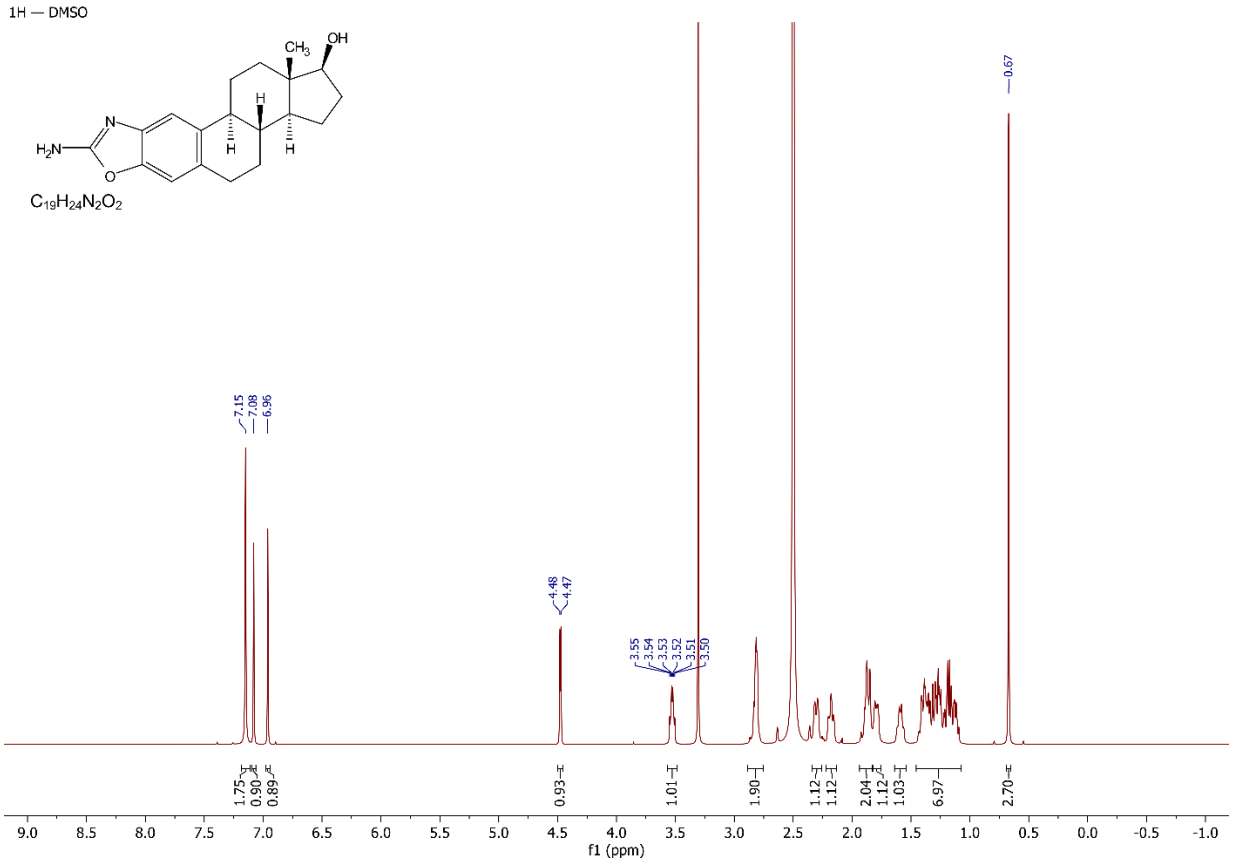
^{1}H NMR spectrum of compound **9** (DMSO-d_6, 500 MHz)



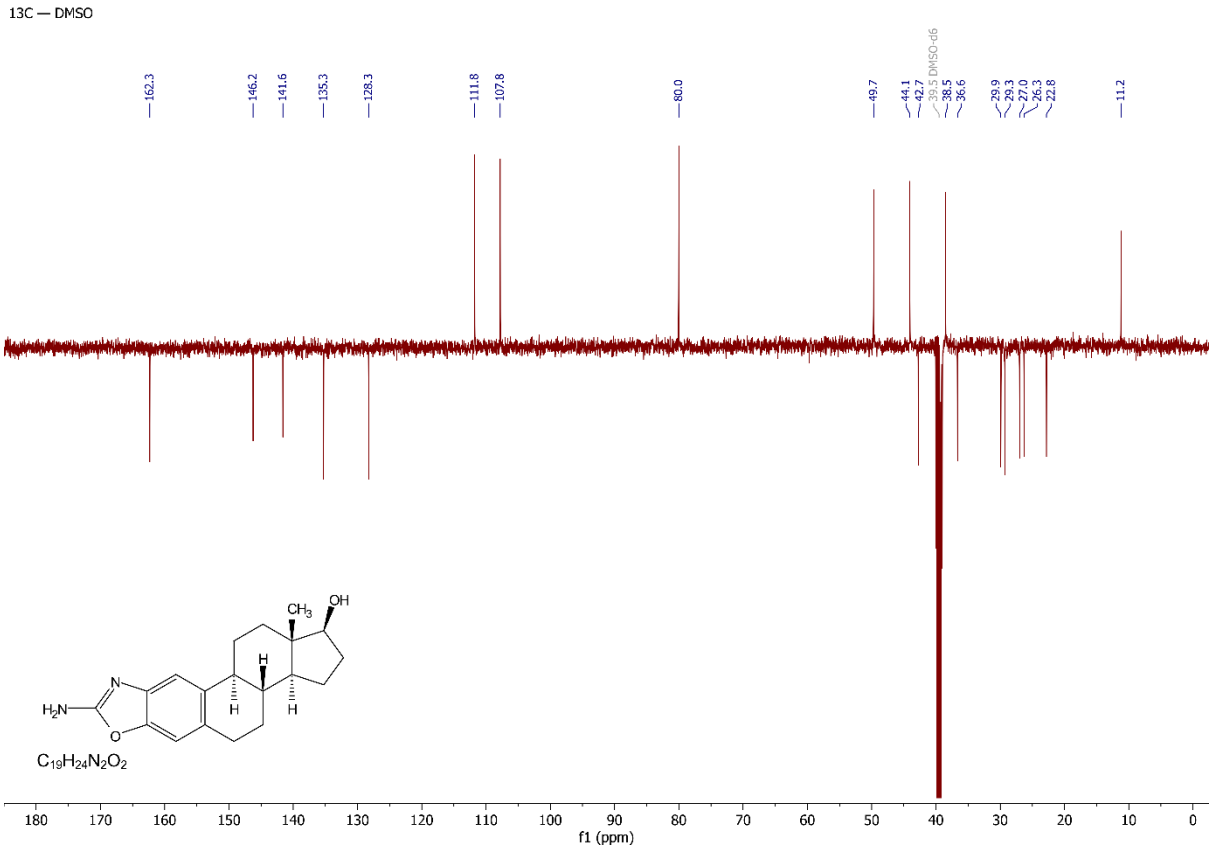
^{13}C NMR spectrum of compound **9** (DMSO-d_6, 125 MHz)



^{1}H NMR spectrum of compound **10** (DMSO-d_6, 500 MHz)



^{13}C NMR spectrum of compound **10** (DMSO-d_6, 125 MHz)



*Predicted pharmacokinetic parameters of the synthesized compounds**

R	HBD[1]	HBA[2]	Exact mass (Da)	LogP	TPSA[3] (Å²)	Rotatable Bonds
H	1	2	297.17	3.432	46.26	0
Me	1	2	311.19	3.564	46.26	0
Et	1	2	325.20	4.264	46.26	1
^{i}Pr	1	2	339.22	4.807	46.26	1
Ph	1	2	373.20	5.466	46.26	1
CH_2F	1	2	329.18	3.638	46.26	1
CHF_2	1	2	347.17	3.979	46.26	1
CF_3	1	2	365.16	4.696	46.26	1
COOH	2	4	341.16	3.476	83.56	1
COOMe	1	3	355.18	3.622	72.56	2
$CONH_2$	2	3	340.18	2.669	89.35	1
CH_2OH	2	3	327.18	2.747	66.49	1
OH	2	3	313.17	2.959	58.56	0
OMe	1	3	327.18	3.869	55.49	1
Ideal Values[4]	**<5**	**<10**	**<500**	**<5**	**<140**	**<10**

* Predicted with Chemaxon's Chemicalize software

[1] Hydrogen bond donor

[2] Hydrogen bond acceptor

[3] Topological polar surface area

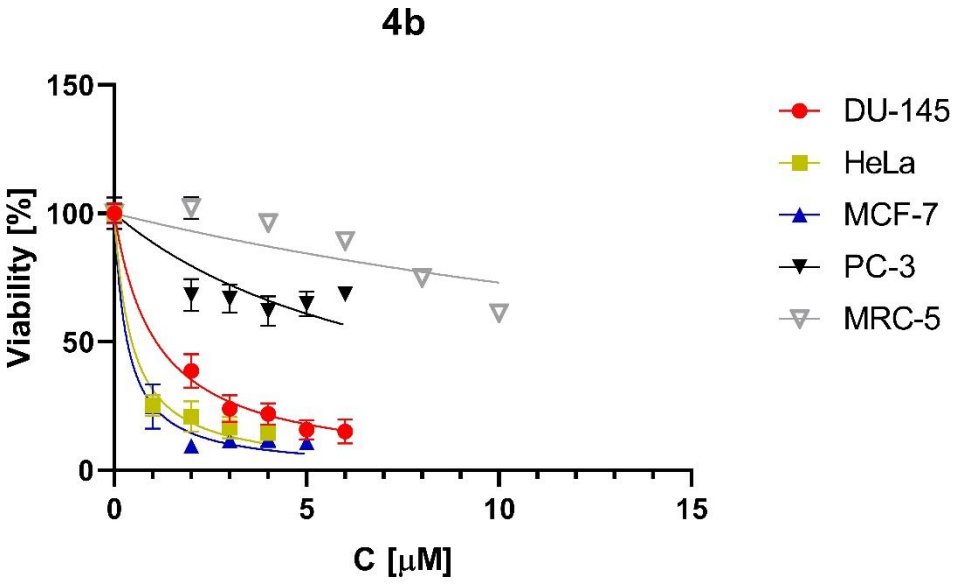
[4] Based on the Lipinski's and Veber's criteria

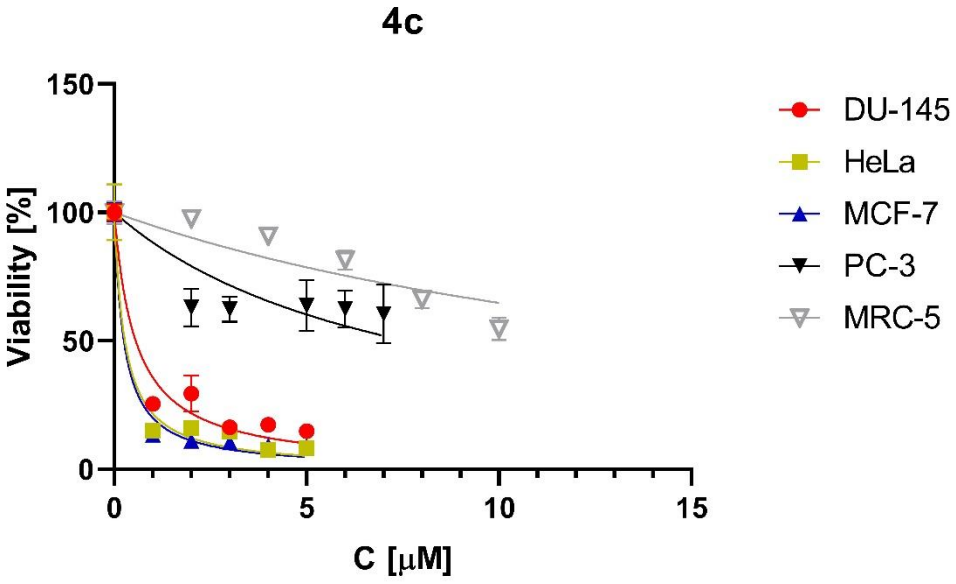
Mean values of primary growth inhibitory screen used for heat map construction

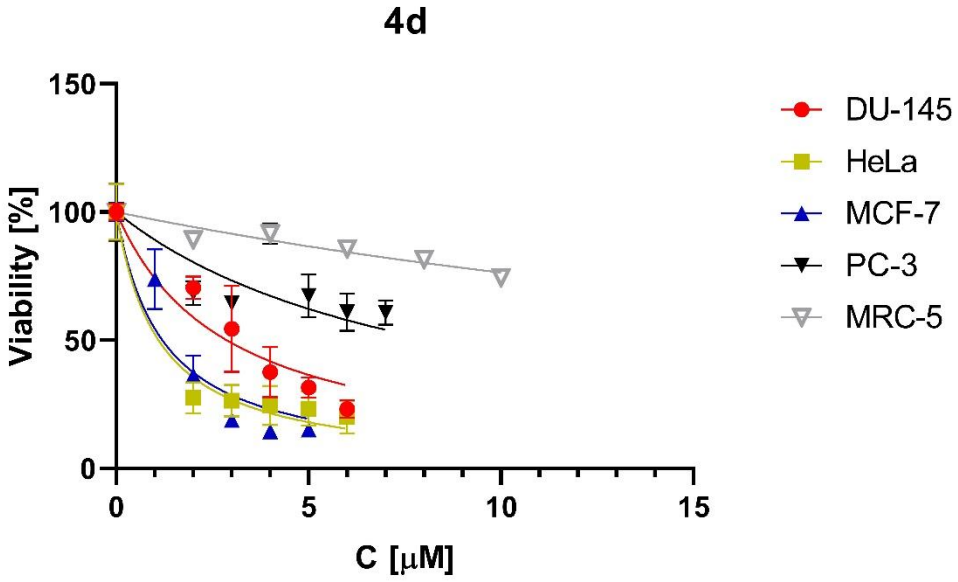
	MRC-5	**DU-145**	**PC3**	**HeLa**	**MCF-7**
2a	100±9.6	100±9.3	94±7.7	37.2±6.4	100±2.5
2b	100±6.3	99.6±8	85.1±8.4	96.4±4	100±6.9
2c	100±10.1	88.5±7.4	71.1±7.3	82.1±7.1	82.3±7
2d	100±10.1	76.9±11.5	73.9±7.9	100±3.7	88±7.9
2e	100±9.6	100±4.7	82.5±6.7	87.8±6.8	100±6.4
2f	100±10.4	98.6±11.6	79.4±7.8	100±9.5	100±4.8
4a	100±8.6	100±7.7	83.6±4.2	1.4±0.3	100±7.2
4b	90.1±9.2	14.7±4	30.2±5	10.7±5.1	6.5±3.9
4c	99.2±10	11.5±3.1	32.3±3.8	3.8±2.5	23.6±3.2
4d	98.3±11	41.1±9.5	51.6±9.6	17.2±9.5	39.4±2.6
4e	100±9.3	100±5.9	64.2±2.9	49.8±8.2	100±6.6
4f	100±8.5	100±7.6	85.7±8.2	100±3.2	100±9.2
4g	100±5.5	100±6.1	94.6±9.2	31±9.3	100±3.7
4h	100±10	40.8±7.4	85.4±5.3	0.4±0.4	100±4.6
4i	100±10	100±6.6	100±1.9	51.7±6.1	100±5.4
4j	100±8.8	75.9±3.4	87.3±7	100±4	98.2±7.6
4k	95.6±5.5	100±6.7	89.7±8	48.5±6.6	90.5±5.3
4l	91.5±6.7	73.9±3.2	76.1±2.4	100±7.1	97.2±4.5
4m	96.4±5.8	73.8±3.9	82.8±4.2	76.5±7.2	89.4±7.4
4n	100±5.6	83.6±5.6	88.4±4.4	93.5±7.1	100±9.4
4o	96.1±10	100±5	78.2±7.5	60.3±1.7	100±8.7
4p	99.2±8.6	78.3±8.9	77.4±8.5	91.3±5	100±6.9
4q	100±6.6	72.3±5.8	85.8±8.3	1.5±0.4	97.8±7.1
4r	100±10.2	74.4±8	74.9±5.3	2.6±1	100±3.9
4s	100±3.2	100±3.6	88.3±3.6	71.1±4.6	100±8.8
6c	100±4.7	90.1±6	94.3±9.4	92.7±2.2	100±10
6d	92.8±7.6	67.1±4.6	62.6±3	22±5.8	38.4±4.5
7	100±9.1	100±5.1	87.9±9.2	22.9±10.7	100±7.3
8	100±6.2	100±5.2	94.2±6.4	1.6±0.3	97.5±5.9
9	99.7±6.8	57.4±5.9	53.9±5.9	16.7±6.7	57.1±2.8
Control	100±9.1	100±5.2	100±2.6	100±6.3	99.5±3

Mean ± SD values of primary growth inhibitory screen (given as cell viability) used for heat map construction. The compounds were tested in 2.5 μM concentration for 72 h. Control represents viability of cells receiving no treatment.

Dose-response curves







Representative cell viability curves to determine growth inhibition and IC_{50} values following treatments with the selected compounds **4b**, **4c**, **4d** on different cell lines. On X-axis the applied concentrations in μM are represented. Based on the viability data, IC_{50} values were obtained, and represented in Table 1.

List, sequence and appropriate concentrations of primers used for qRT-PCR.

Gene	Forward-primer Reverse-primer	Final concentration [nM]
GAPDH	5'-TGCACCACCAACTGCTTAGC-3' 5'-GGCATGGACTGTGGTCATGAG-3'	200
BAX	5' TGCTTCAGGGTTTCATCCAG 3' 5' GGCGGCAATCATCCTCTG 3'	200
Casp-3	5' ACATGGCGTGTCATAAAATACC 3' 5' CACAAAGCGACTGGATGAAC 3'	200
p21	5'-CAGCAGAGGAAGACCATGTG-3' 5'-GGCGTTTGGAGTGGTAGAAA-3'	100
p53	5'-CCCTTCCCAGAAAACCTACC-3' 5'-CTCCGTCATGTGCTGTGACT-3'	200