

## **A Comprehensive Study of Novel Pregnenolone-Isoxazole Hybrids as Selective Inducers of Mitochondrial Apoptosis in Breast Cancer Cells**

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Dmitry Vasilenko,<sup>6</sup> Elena Chernoburova,<sup>1</sup> Elena Averina,<sup>6</sup> Igor Zavarzin,<sup>1</sup> and Yulia Volkova<sup>1\*</sup>

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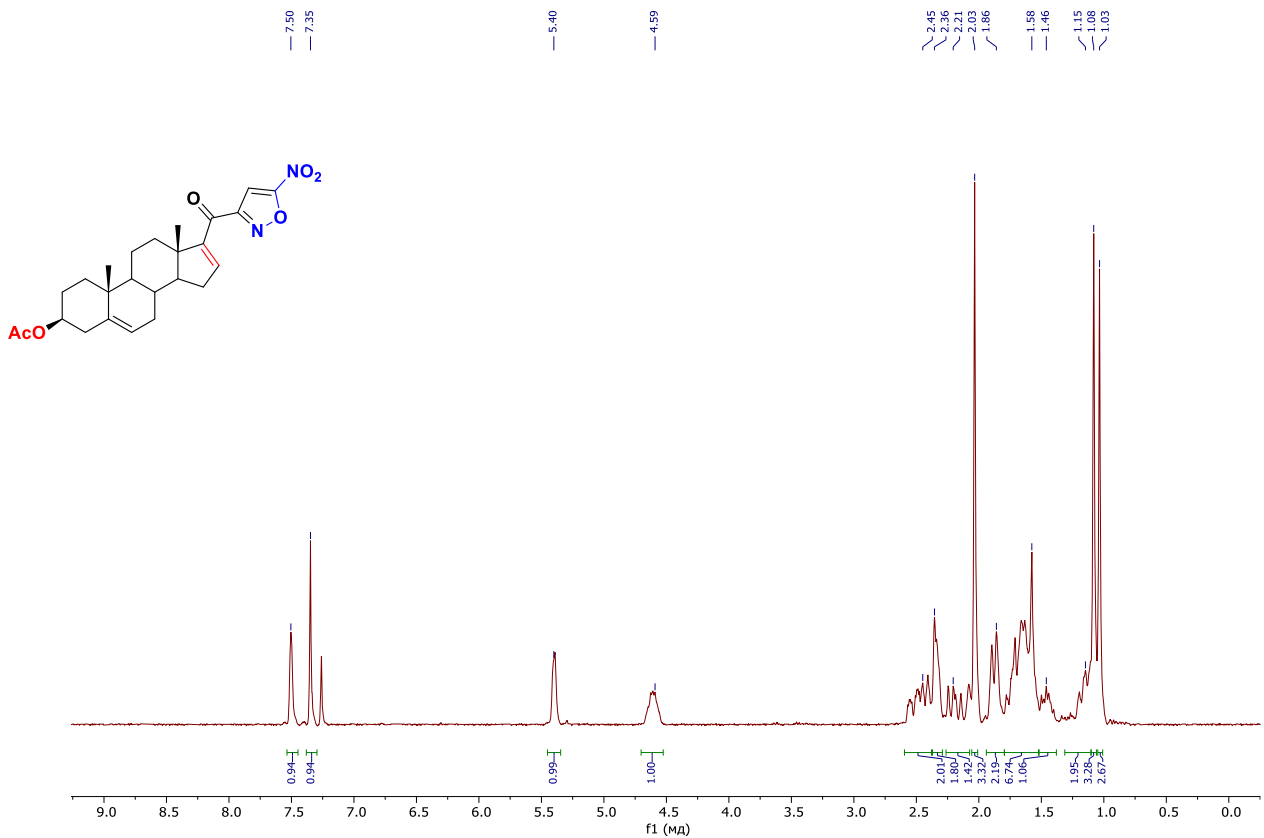
3 - Gause Institute of New Antibiotics, Bol'shaya Pirogovskaya ulitsa 11, Moscow 119021, Russia.

4 - Laboratory of Molecular Oncobiology, Institute of Gene Biology, Russian Academy of Sciences, ulitsa Vavilova 34/5, Moscow 119334, Russia.

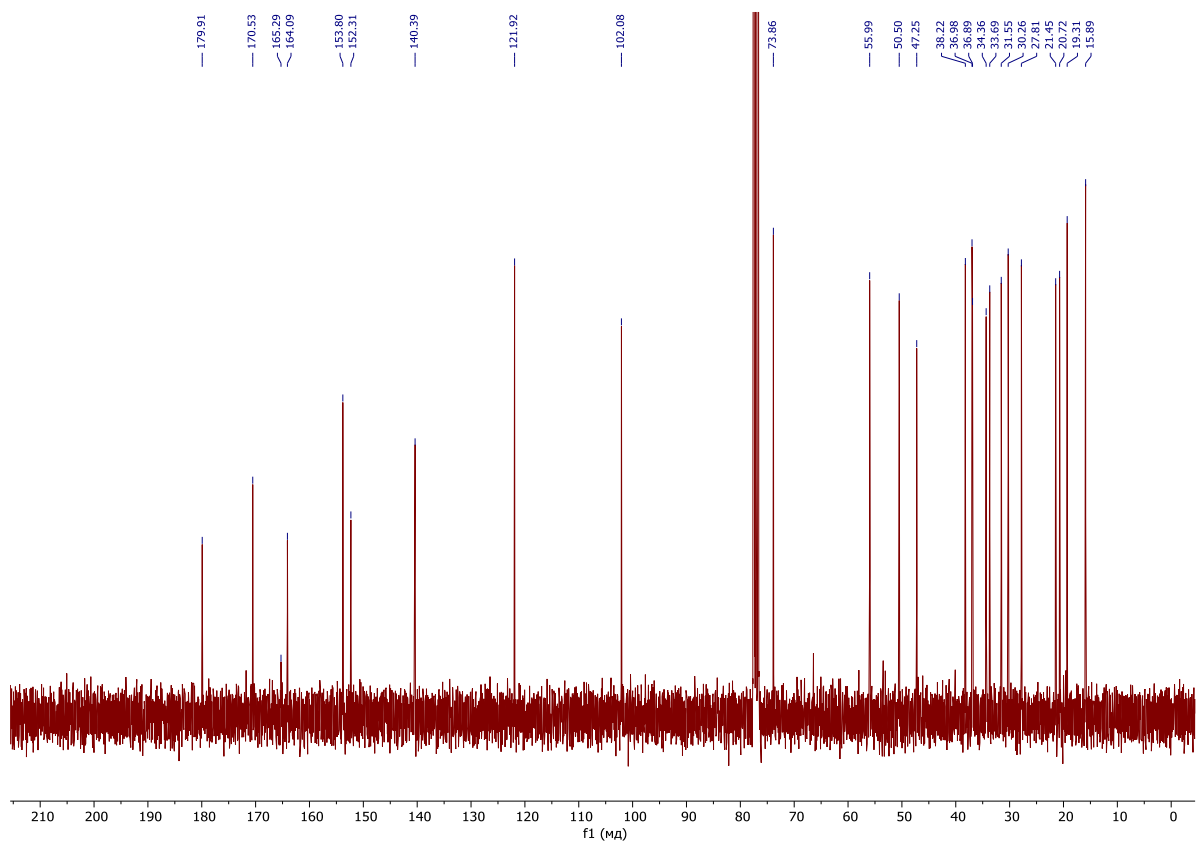
5 - Ufa Institute of Chemistry, Ufa Federal Research Center of the RAS, prospect Octyabrya 71, Ufa 450054, Russia.

6 - Department of Chemistry, Lomonosov Moscow State University, Leninskie Gory, 1-3, Moscow, 119991 Russia.

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<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 21-(5-nitroisoxazole)-pregna-5,16-dien-3 $\beta$ -ol-20-one (**2a**)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 21-(5-nitroisoxazole)-pregna-5,16-dien-3 $\beta$ -ol-20-one (**2a**)

**Analysis Info**

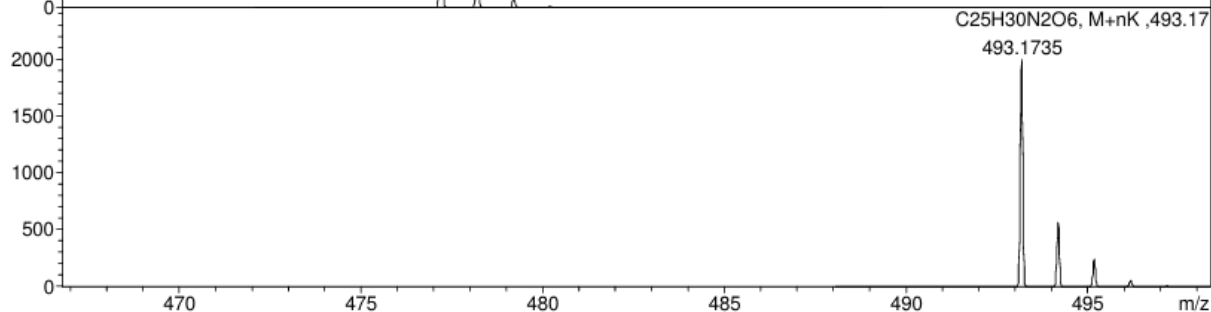
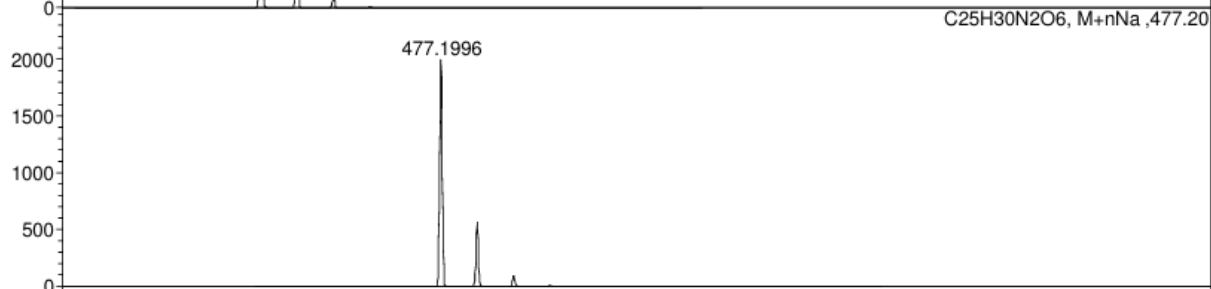
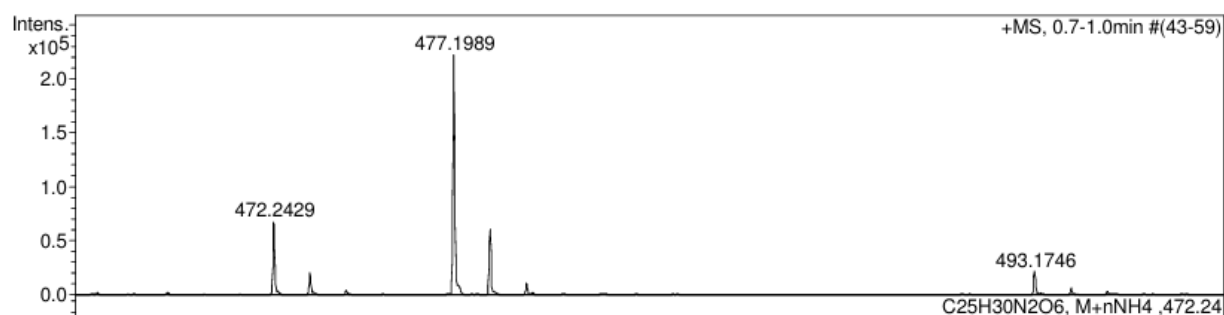
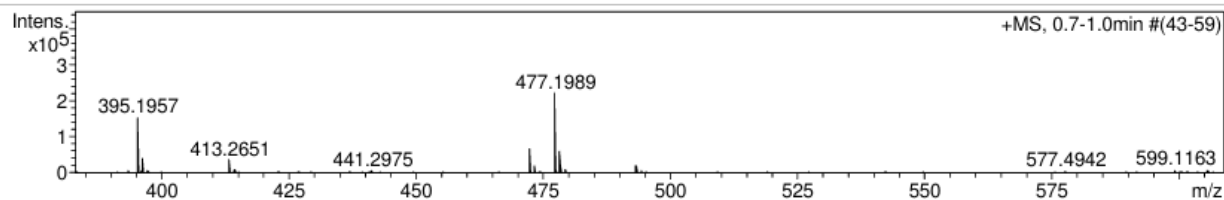
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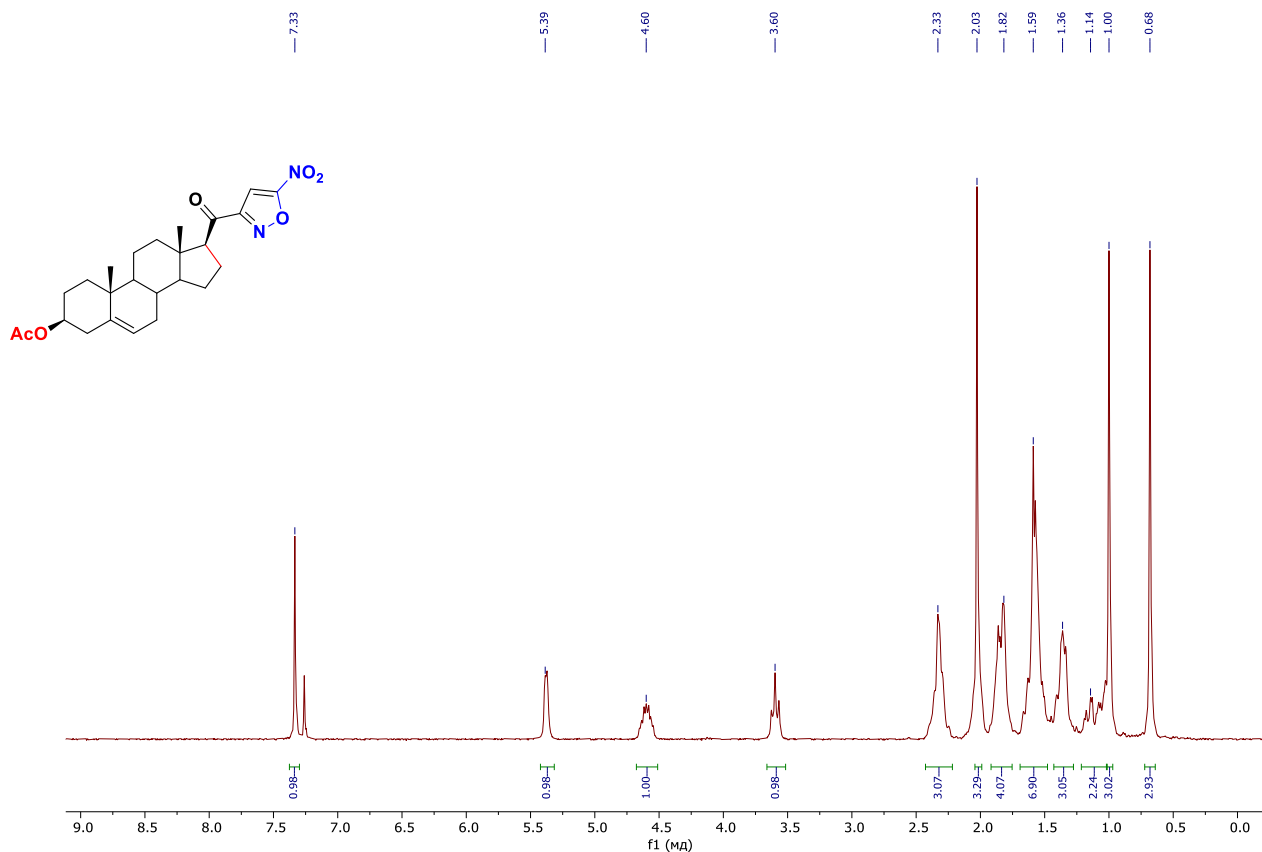
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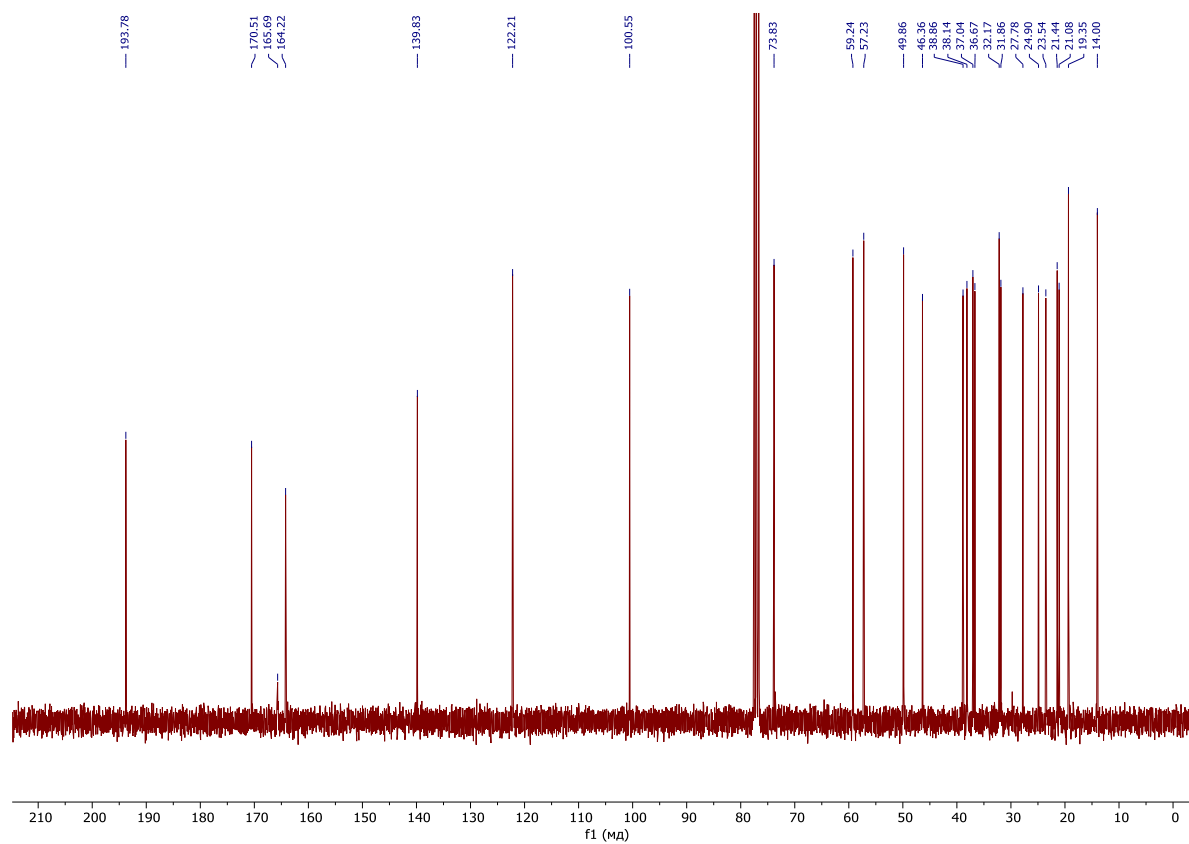
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HRMS (ESI/Q-TOF) of 3-acetate 21-(5-nitroisoxazole)-pregna-5,16-dien-3 $\beta$ -ol-20-one (**2a**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 21-(5-nitroisoxazole)-pregna-5-en-3β-ol-20-one (**2b**)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 21-(5-nitroisoxazole)-pregna-5-en-3β-ol-20-one (**2b**)

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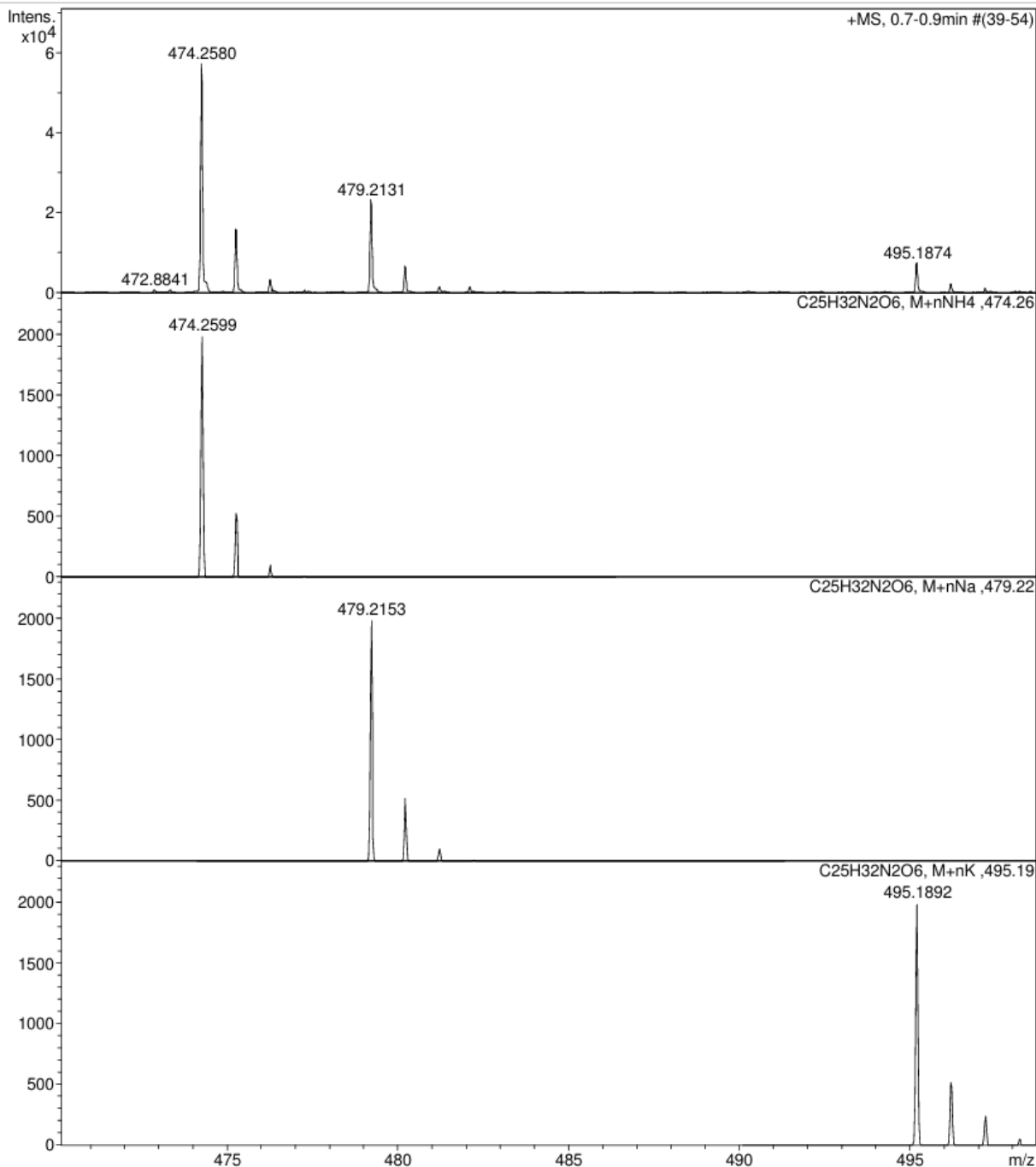
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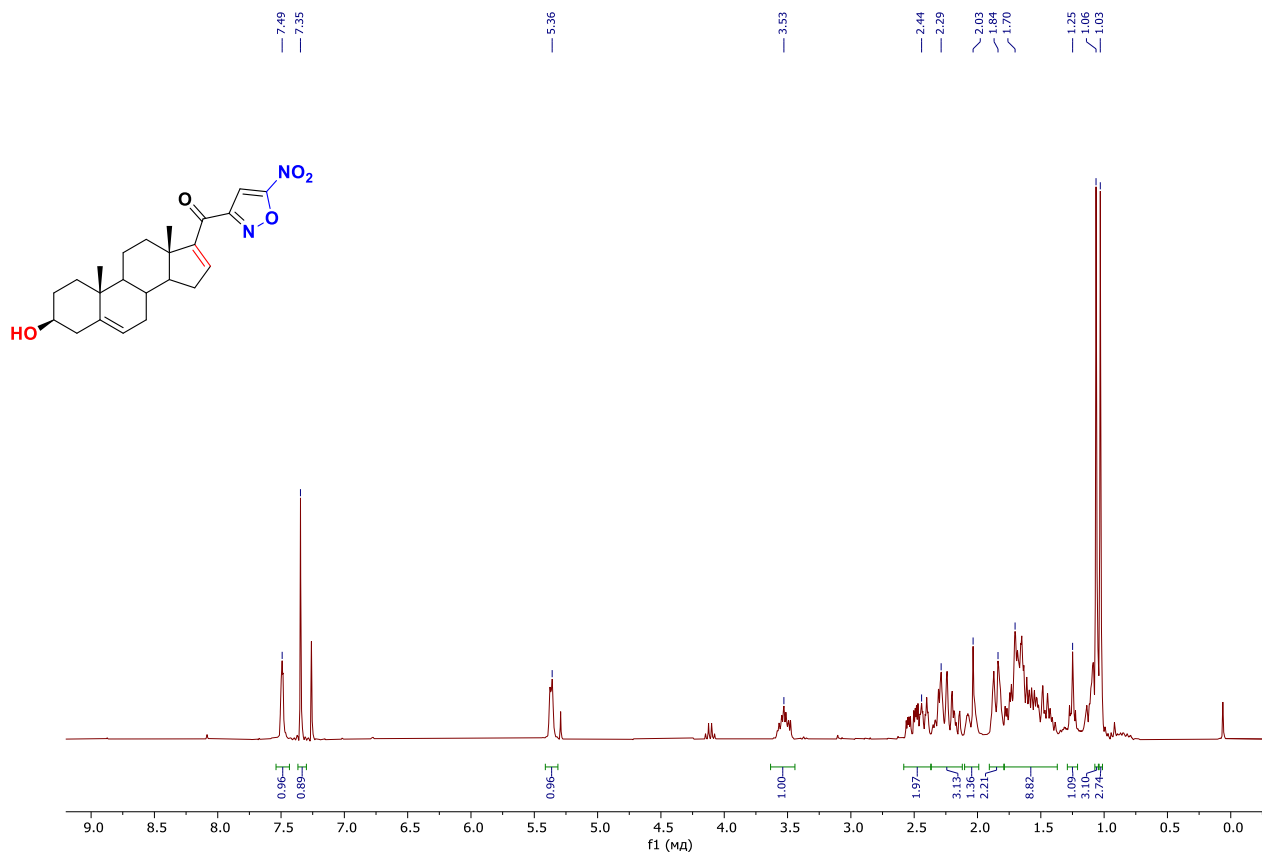
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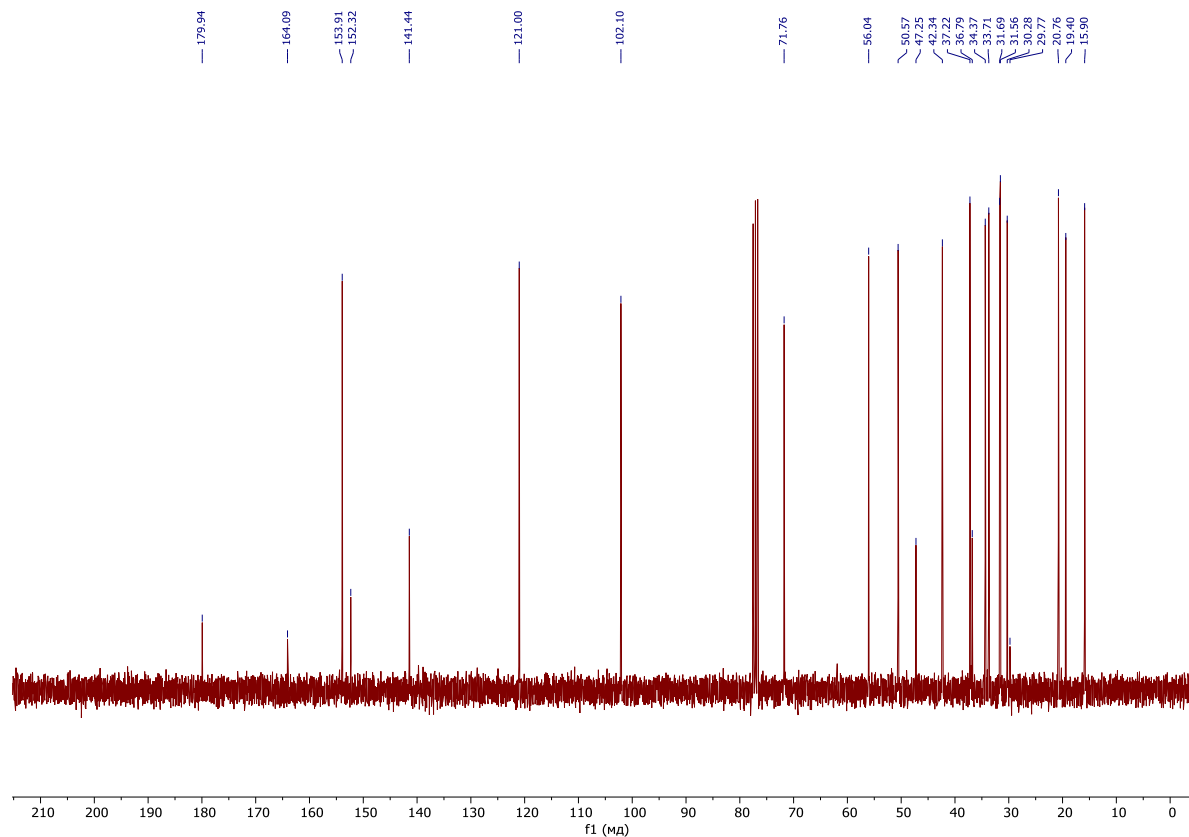
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HRMS (ESI/Q-TOF) of 3-acetate 21-(5-nitroisoxazole)-pregna-5-en-3 $\beta$ -ol-20-one (**2b**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 21-(5-nitroisoxazole)-pregna-5,16-dien-3β-ol-20-one (2c)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 21-(5-nitroisoxazole)-pregna-5,16-dien-3β-ol-20-one (2c)

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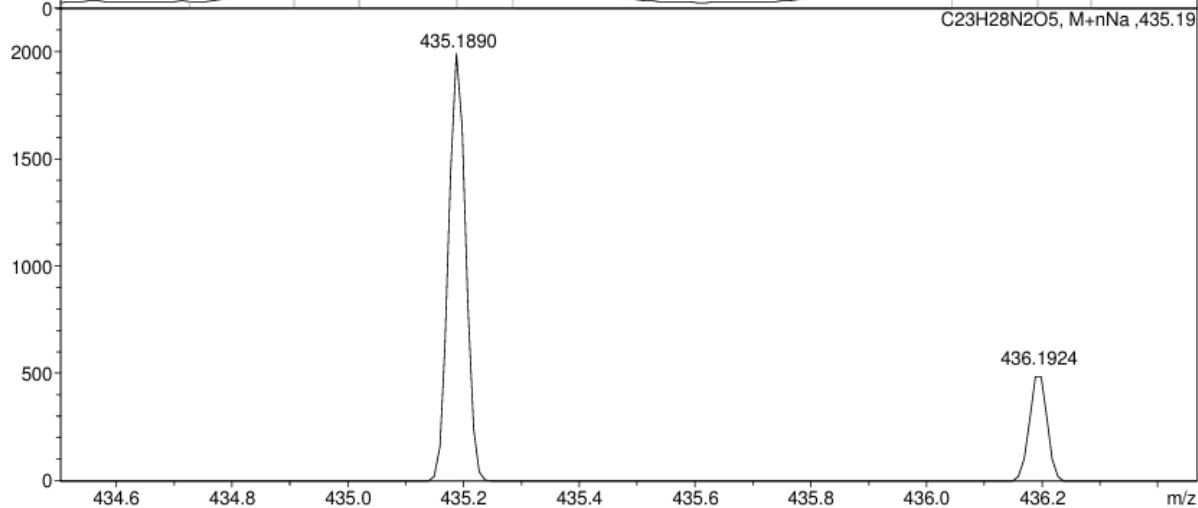
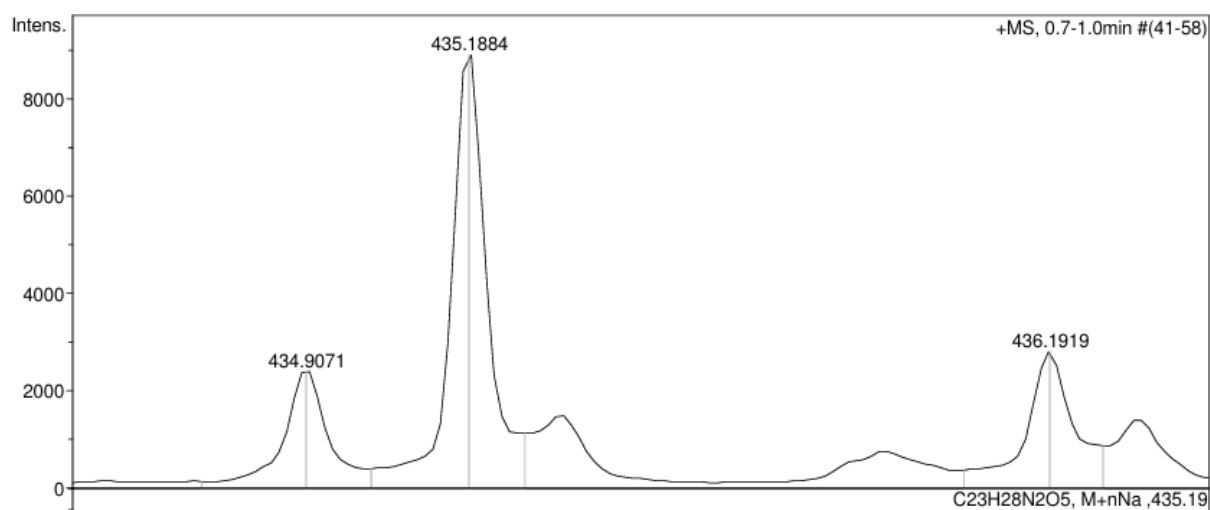
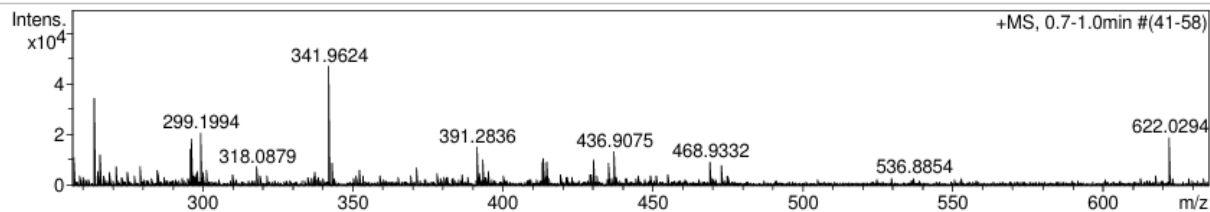
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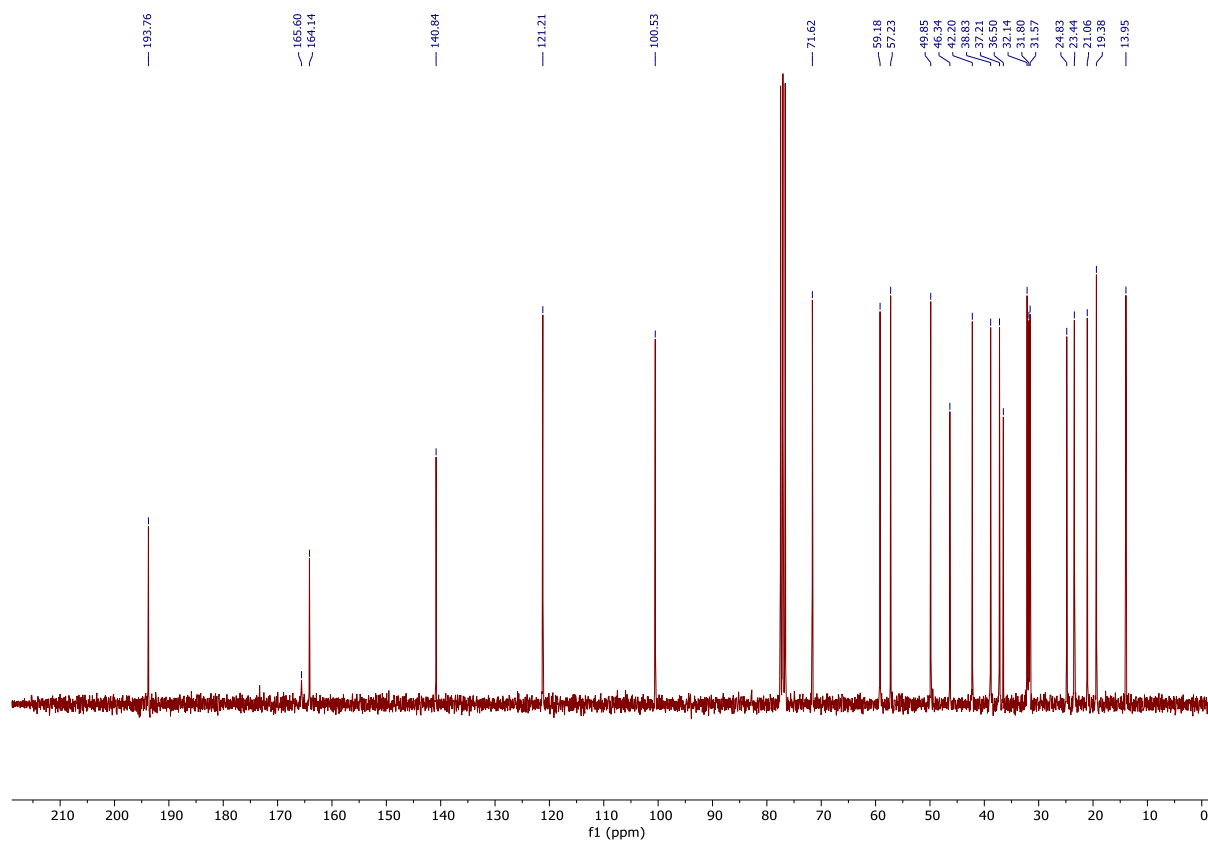
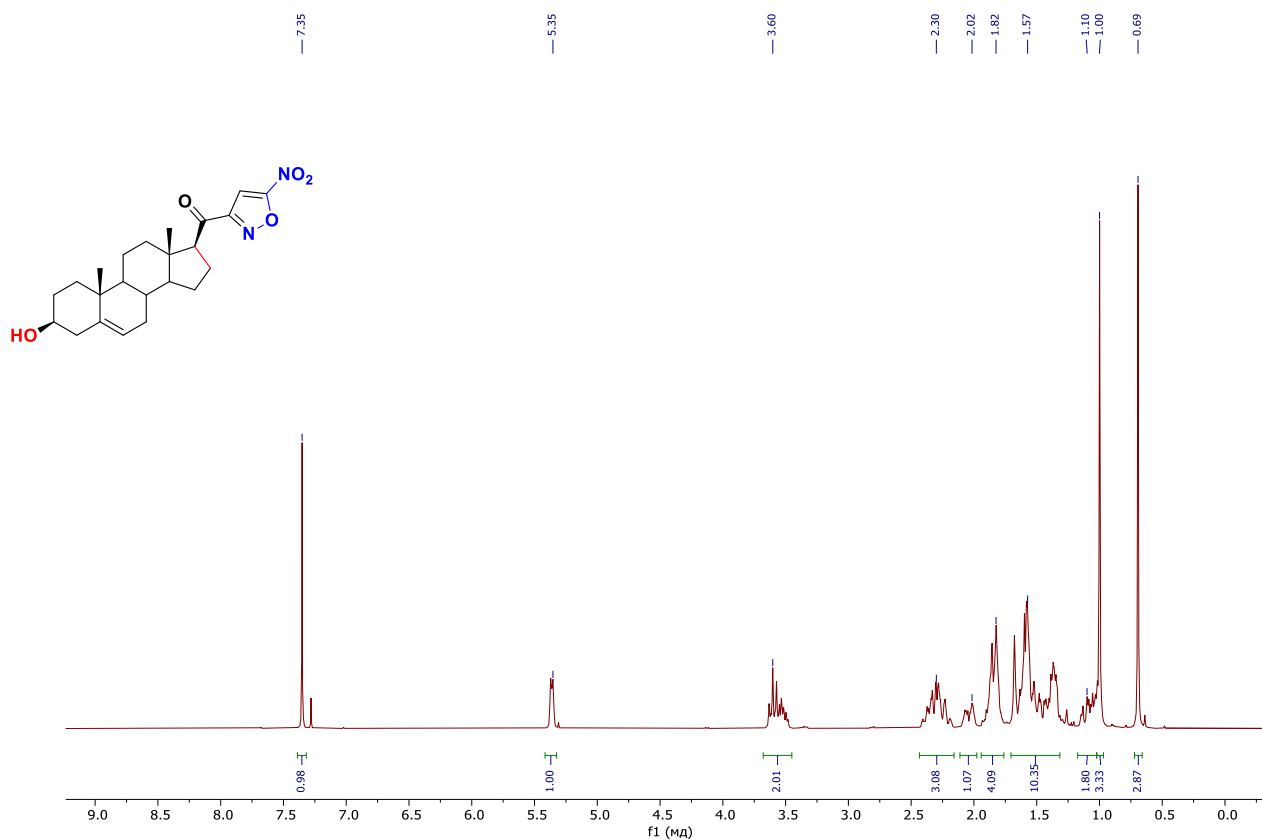
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HRMS (ESI/Q-TOF) of 21-(5-nitroisoxazole)-pregna-5,16-dien-3 $\beta$ -ol-20-one (**2c**)



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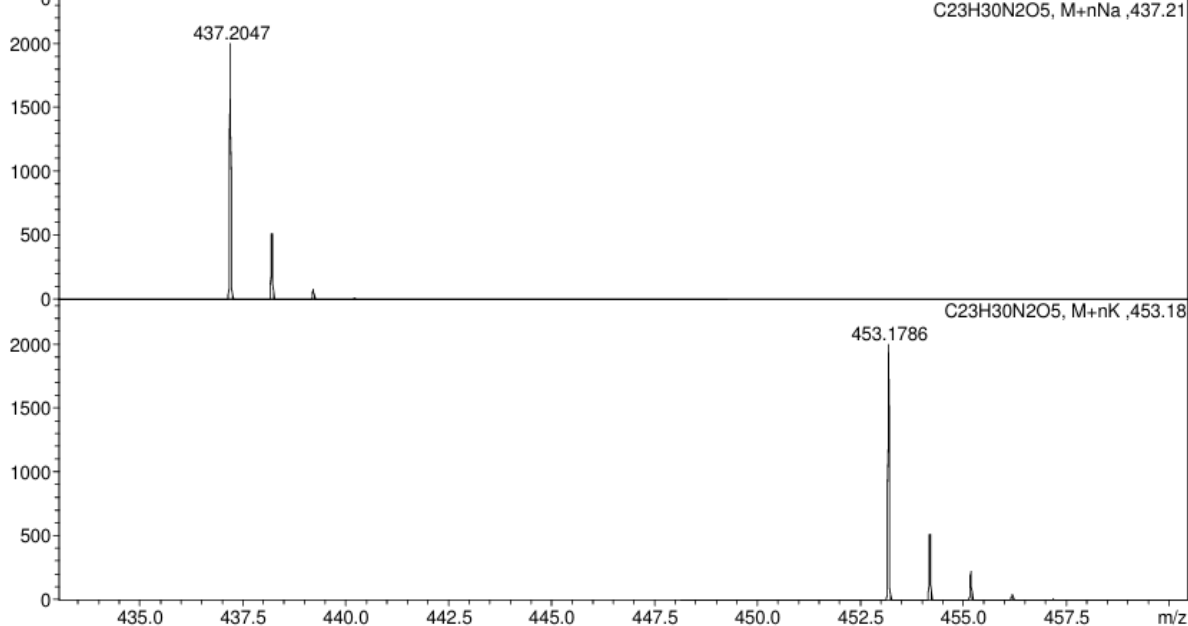
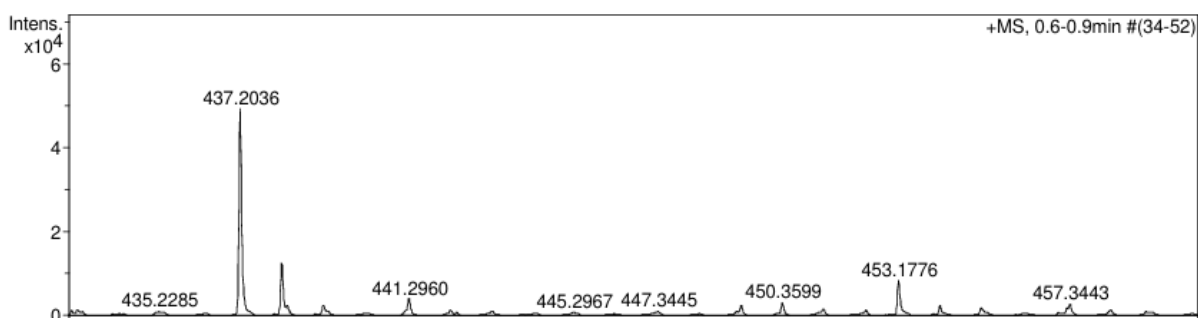
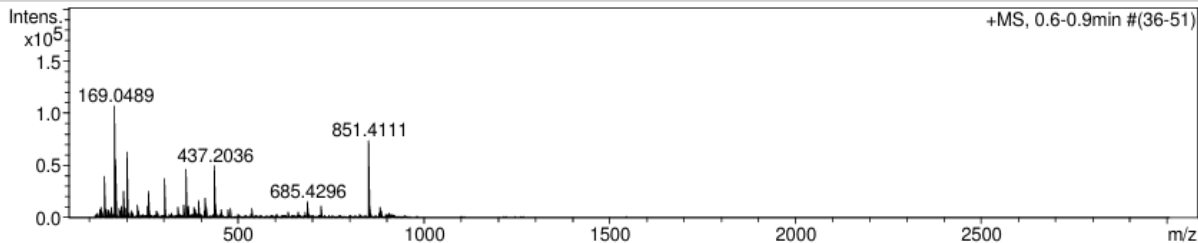
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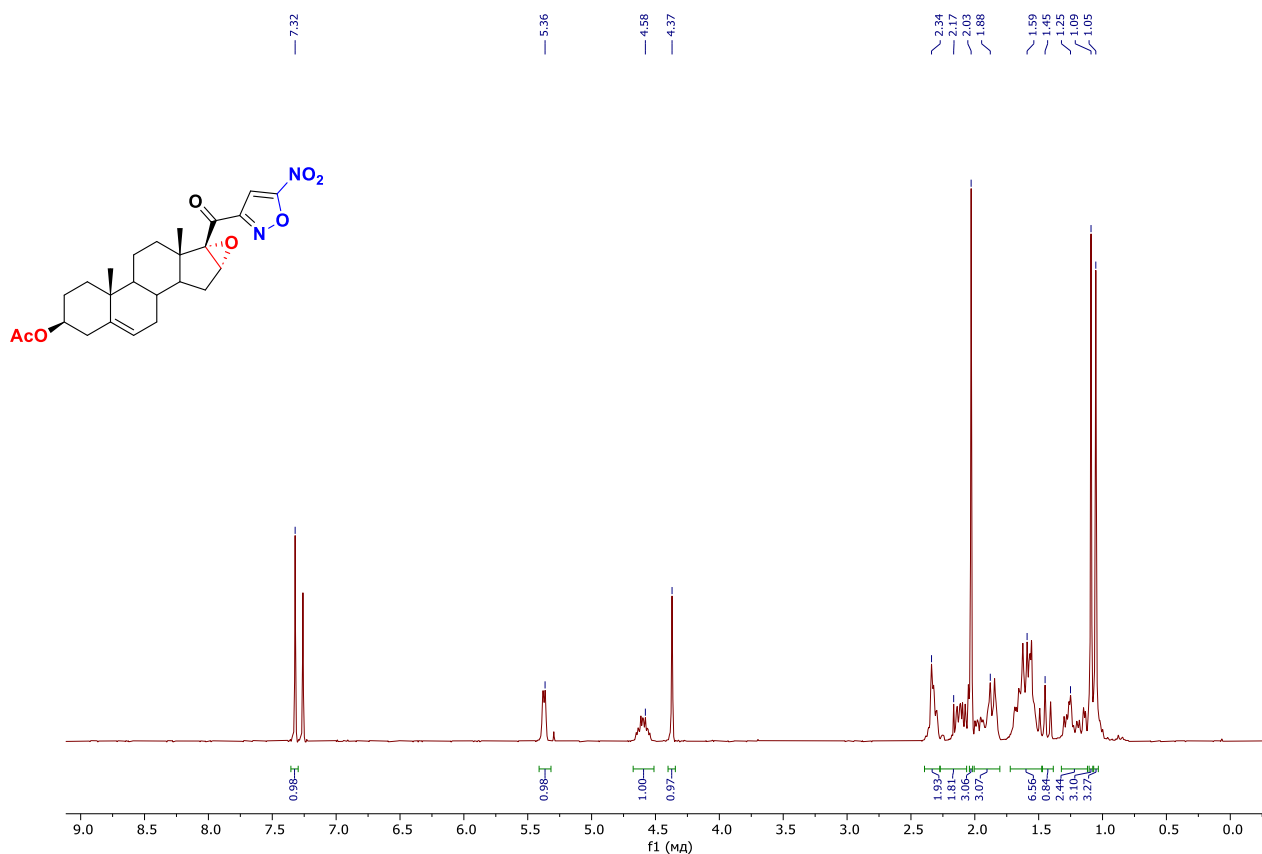
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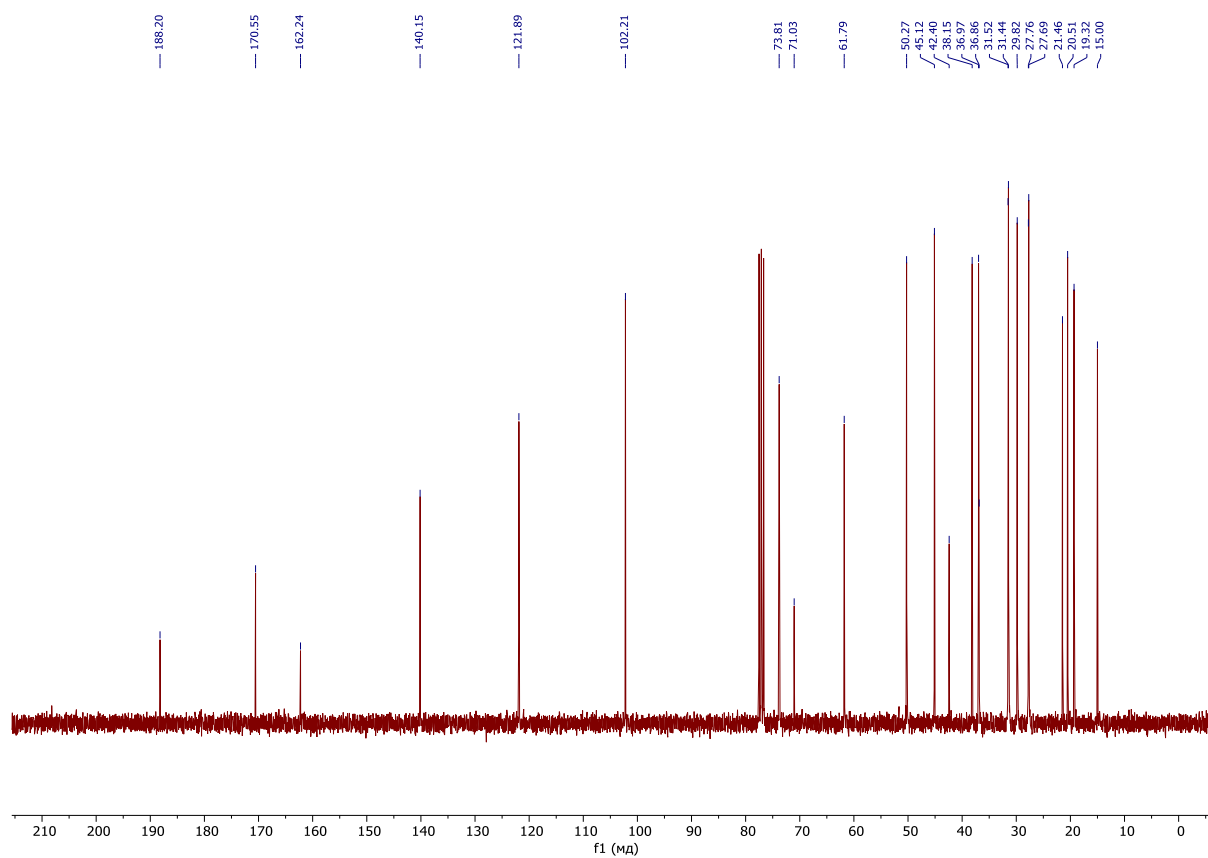
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HRMS (ESI/Q-TOF) of 21-(5-nitroisoxazole)-pregna-5-en-3 $\beta$ -ol-20-one (**2d**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 16 $\alpha$ ,17 $\alpha$ -epoxy-21-(5-nitroisoxazole)-pregna-5-en-3 $\beta$ -ol-20-one (2e)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 16 $\alpha$ ,17 $\alpha$ -epoxy-21-(5-nitroisoxazole)-pregna-5-en-3 $\beta$ -ol-20-one (2e)



**Analysis Info**

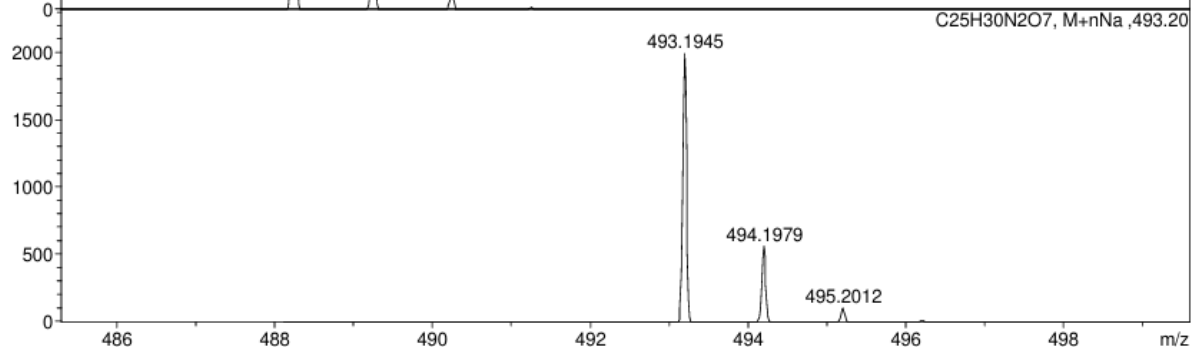
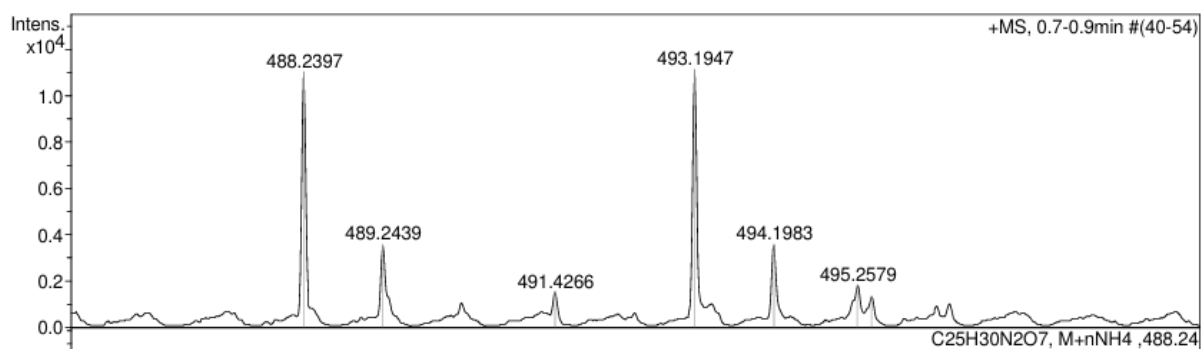
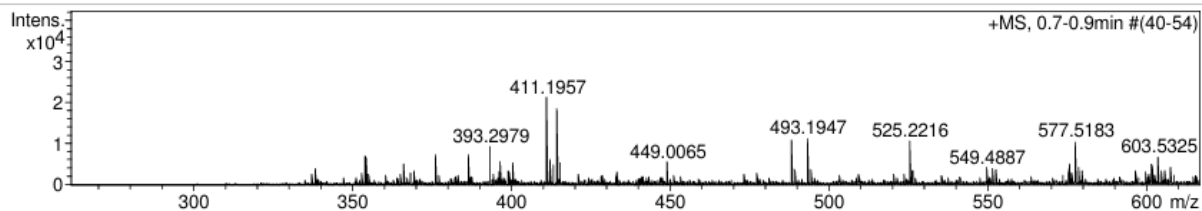
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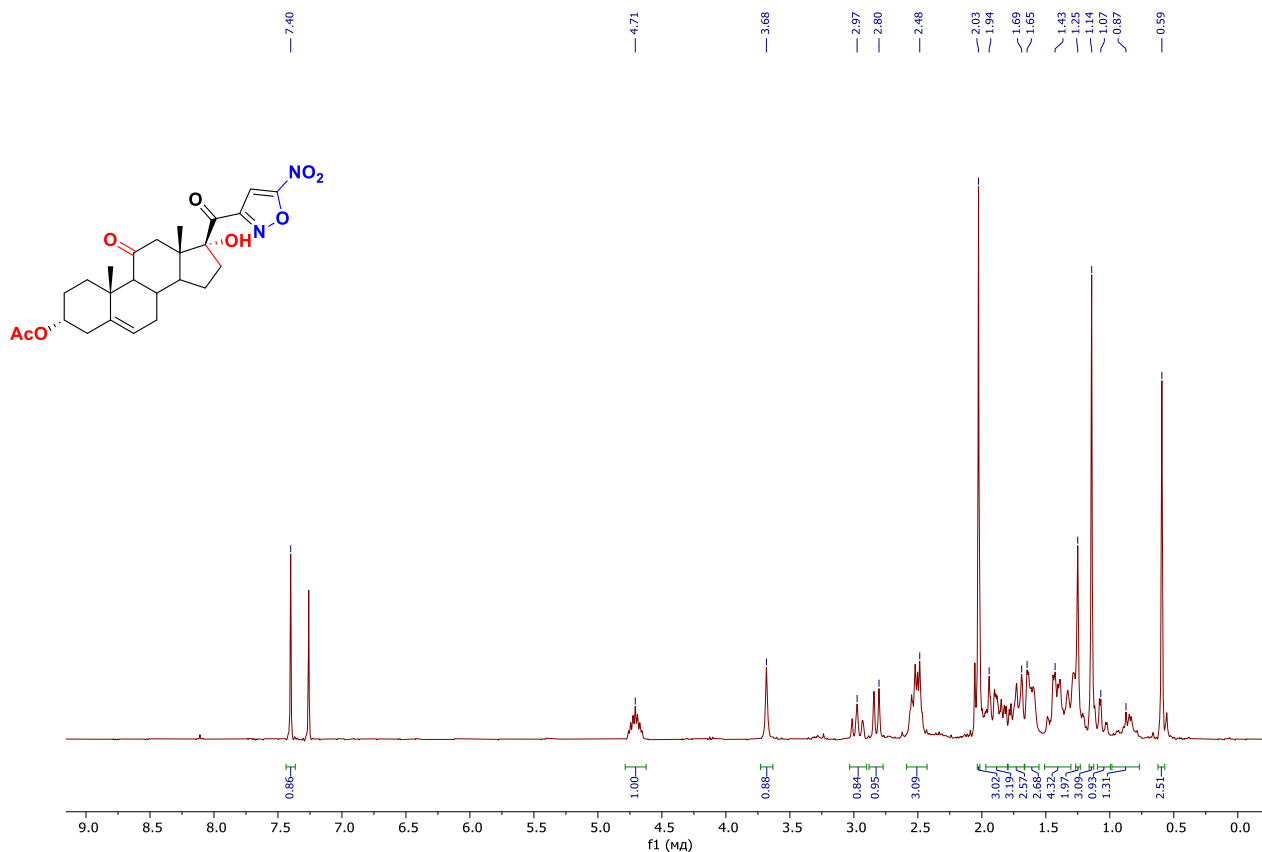
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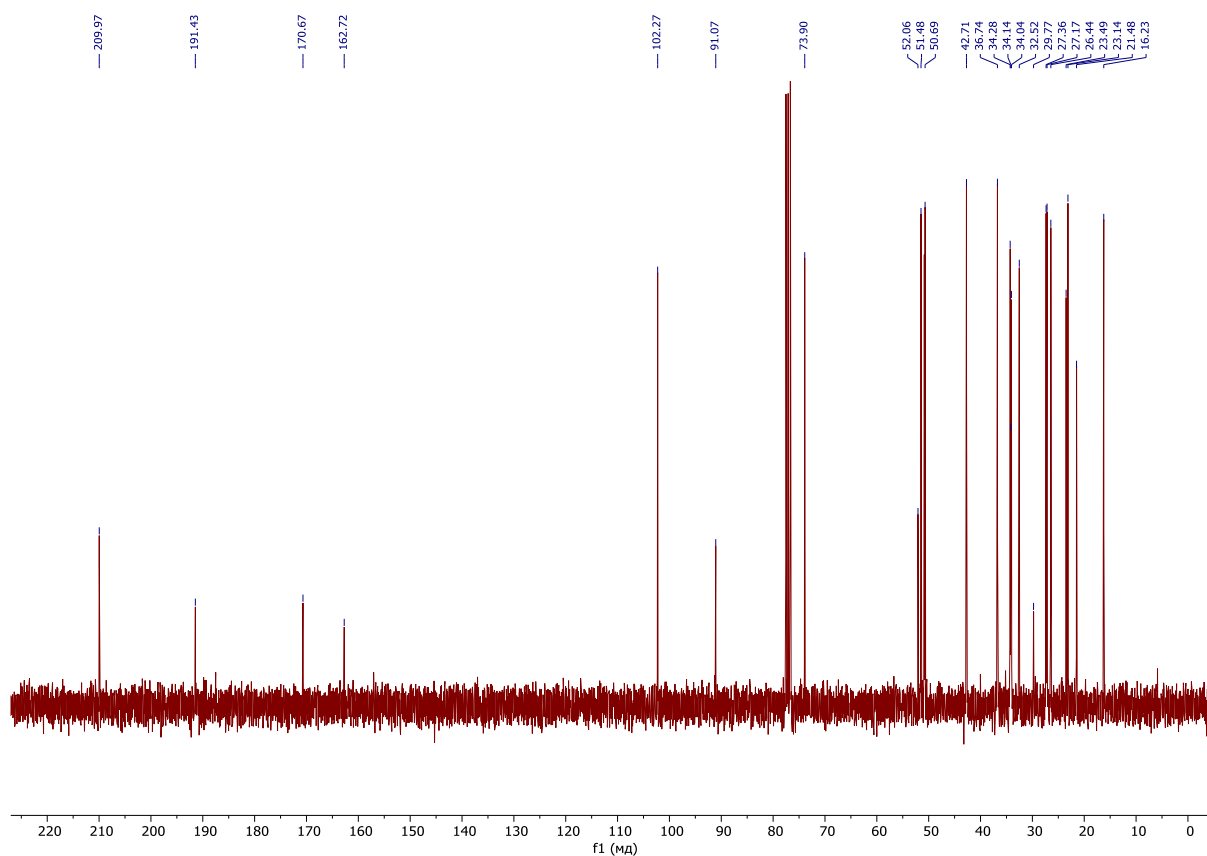
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HRMS (ESI/Q-TOF) of 3-acetate 16 $\alpha$ ,17 $\alpha$ -epoxy-21-(5-nitroisoxazole)-pregna-5-en-3 $\beta$ -ol-20-one (**2e**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate of 21-(5-nitroisoxazole)-pregna-3β-ol, 17α-hydroxy-11,20-dione, 5β (2f)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate of 21-(5-nitroisoxazole)-pregna-3β-ol, 17α-hydroxy-11,20-dione, 5β (2f)



**Analysis Info**

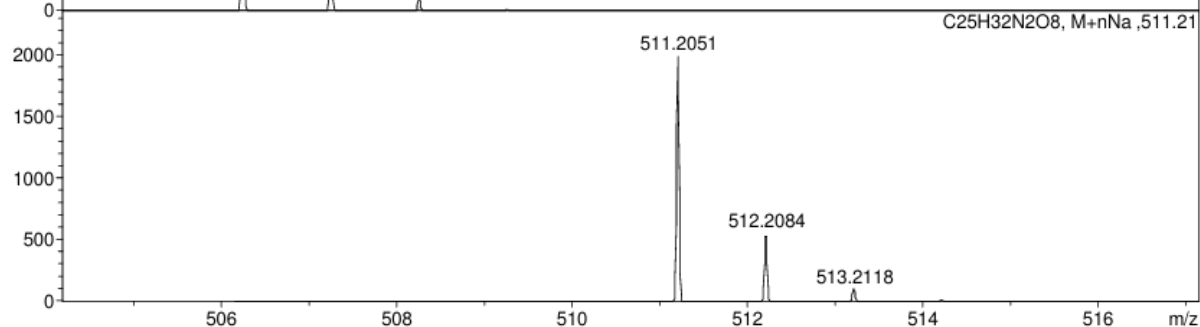
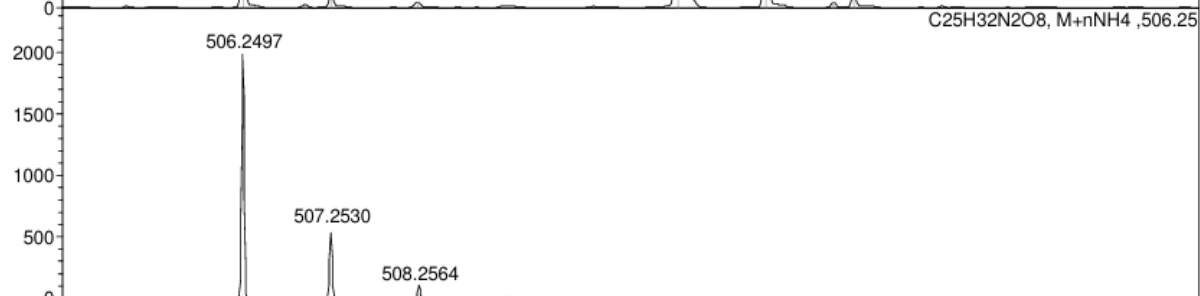
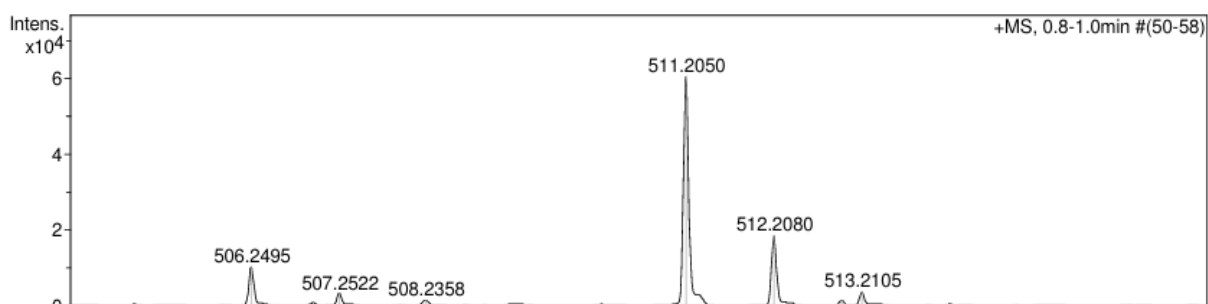
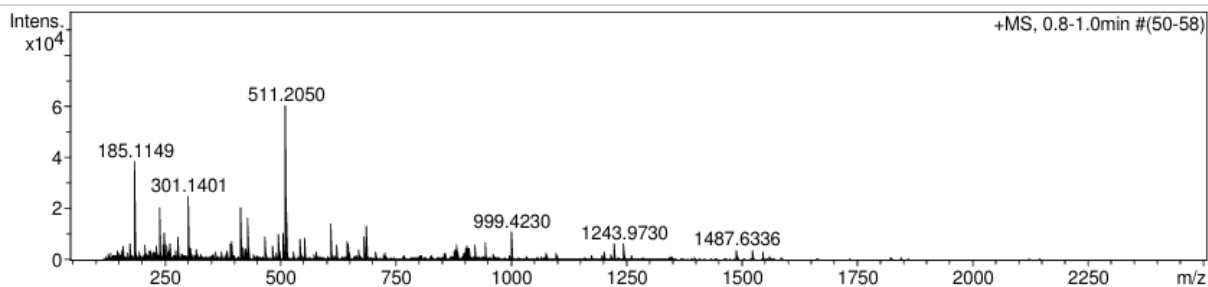
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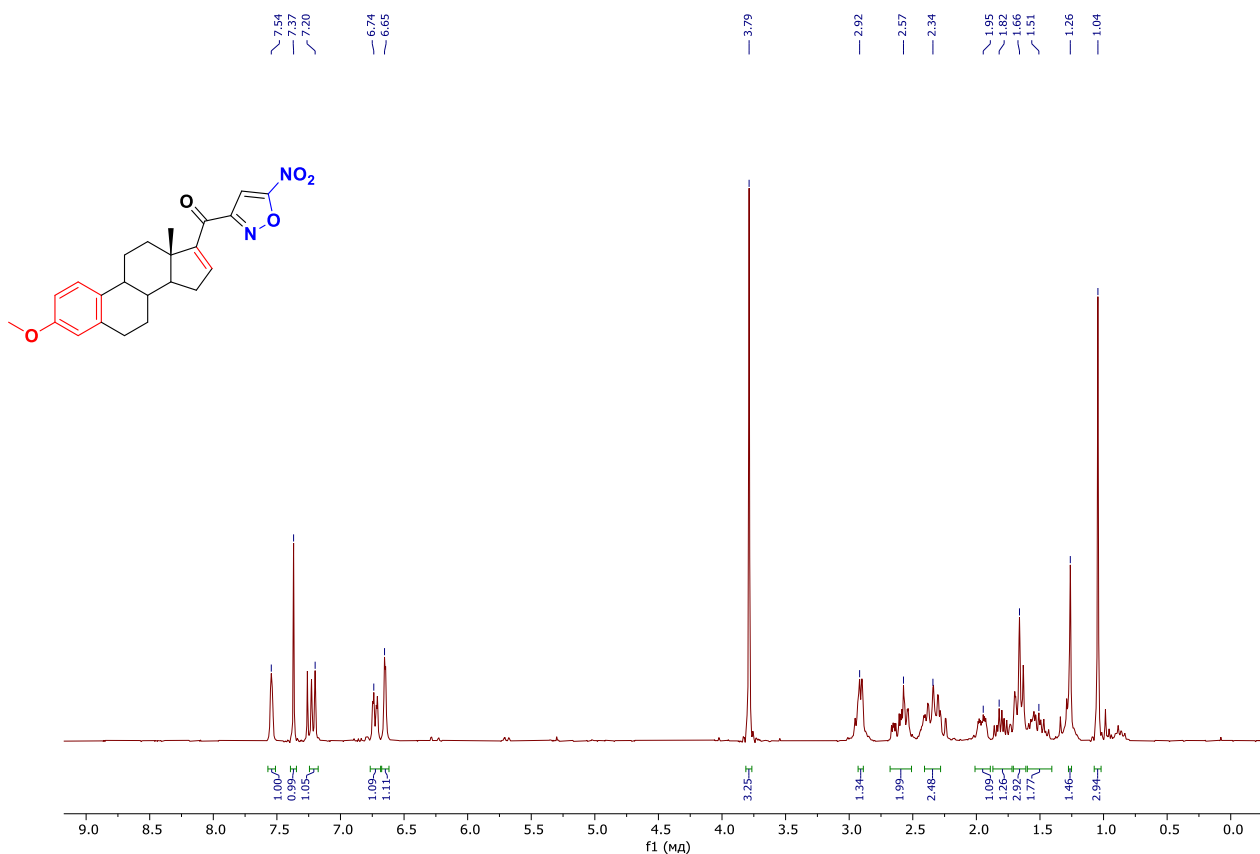
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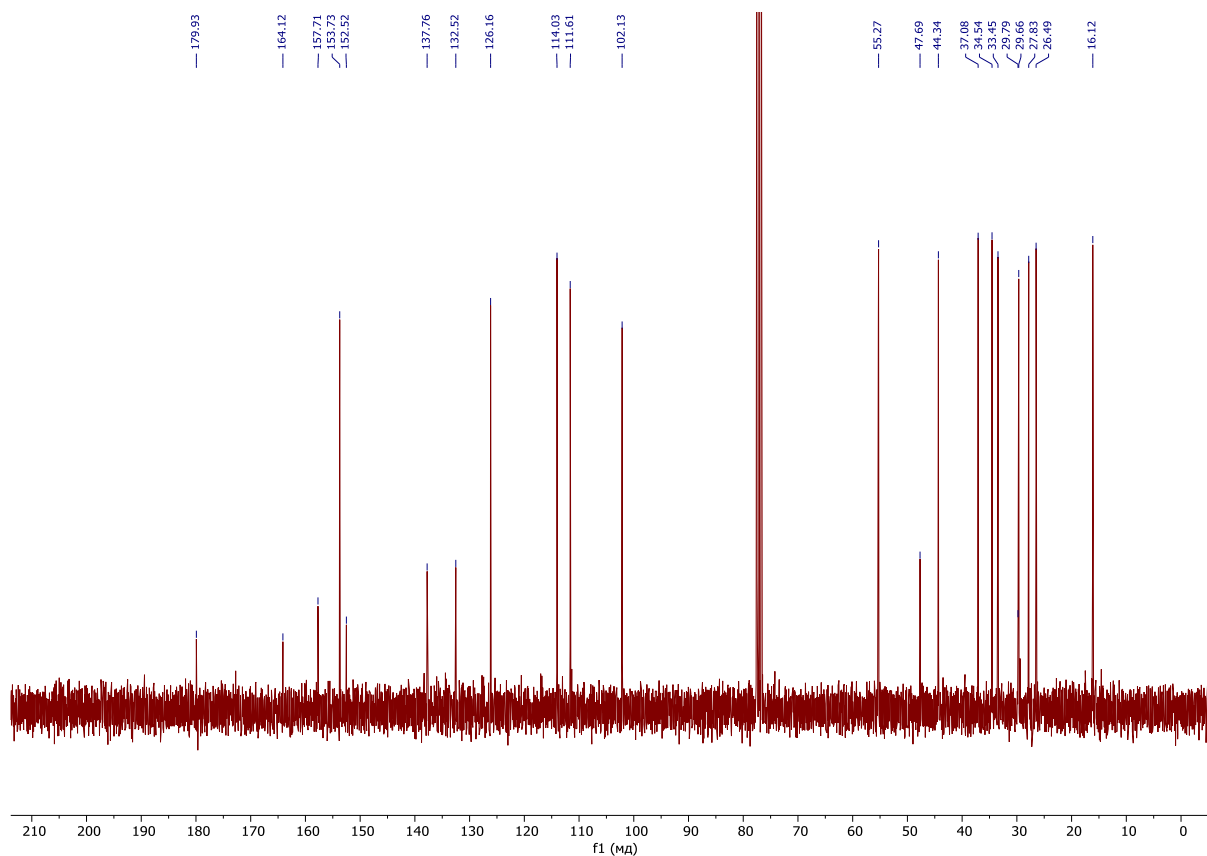
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HRMS (ESI/Q-TOF) of 3-acetate of 21-(5-nitroisoxazole)-pregna-3 $\beta$ -ol, 17 $\alpha$ -hydroxy-11,20-dione, 5 $\beta$  (**2f**)

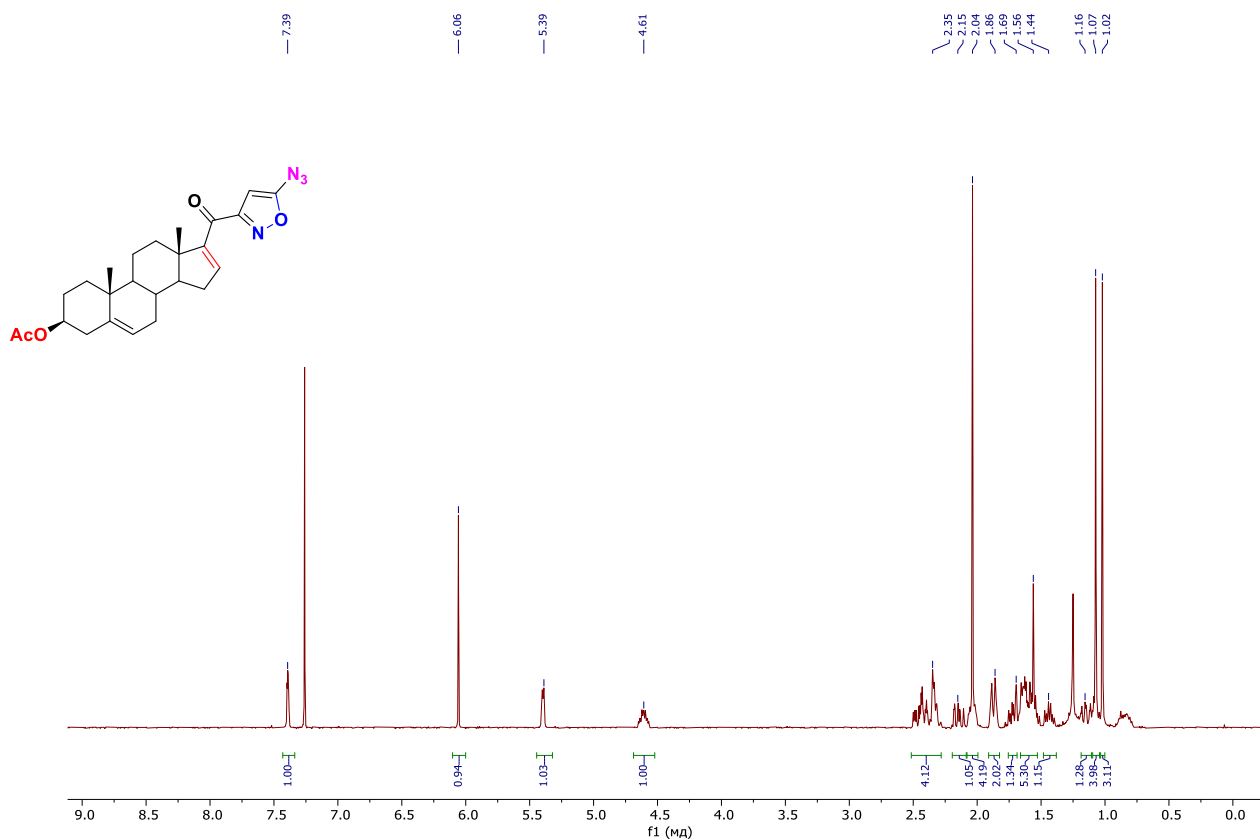


<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 21-(5-nitroisoxazole)-3-methoxy-19-nor-pregna-1,3,5(10),16,21-pentaen-20-one (2g)

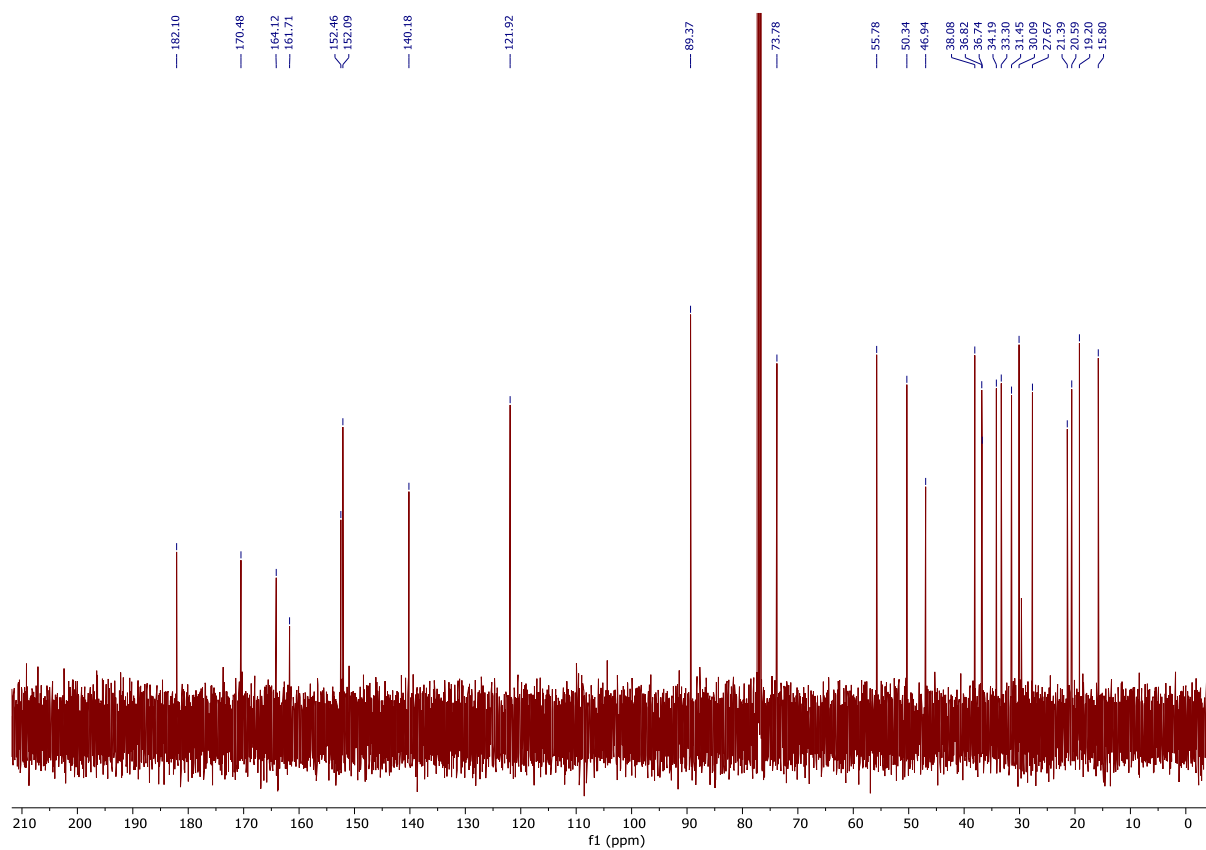


<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 21-(5-nitroisoxazole)-3-methoxy-19-nor-pregna-1,3,5(10),16,21-pentaen-20-one (2g)





$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) spectrum of 3-acetate 20-[3'-(5''-azide)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (3a)



$^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ ) spectrum of 3-acetate 20-[3'-(5''-azide)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (3a)

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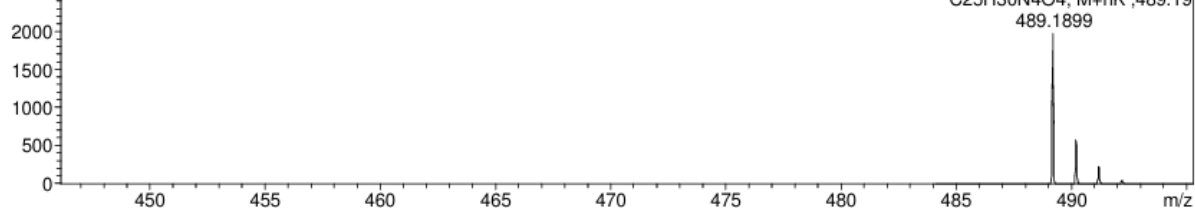
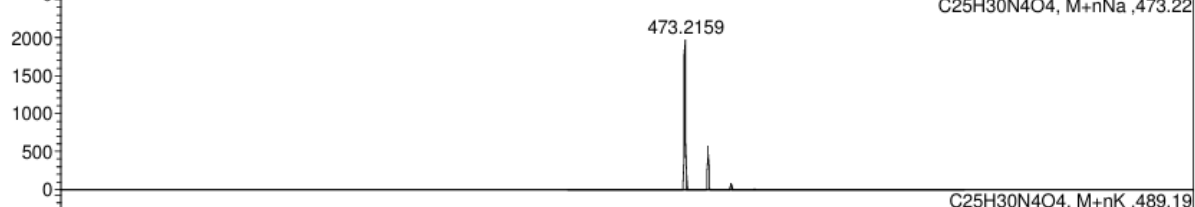
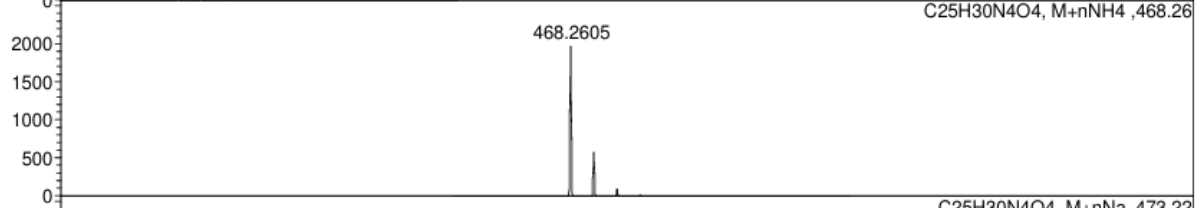
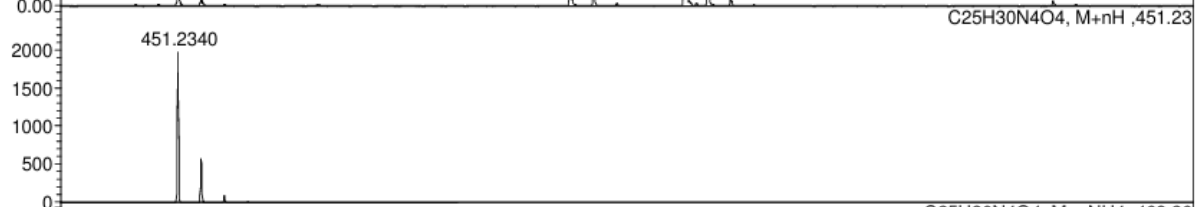
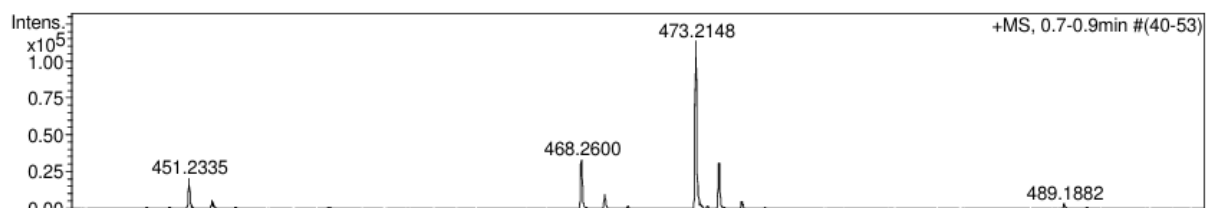
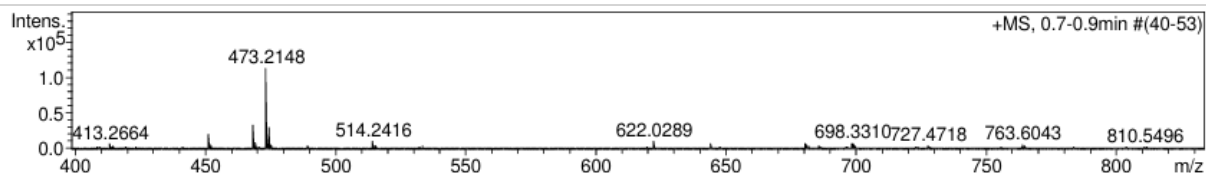
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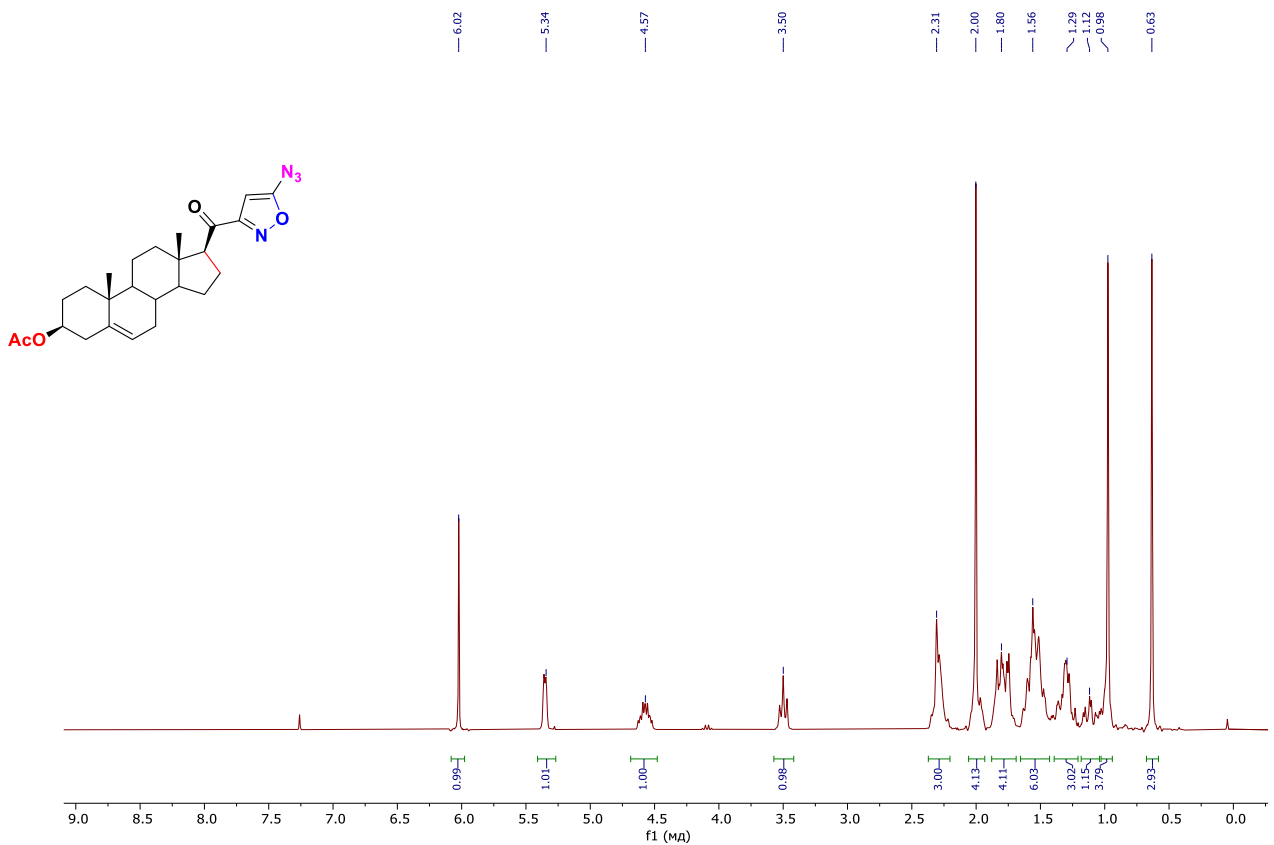
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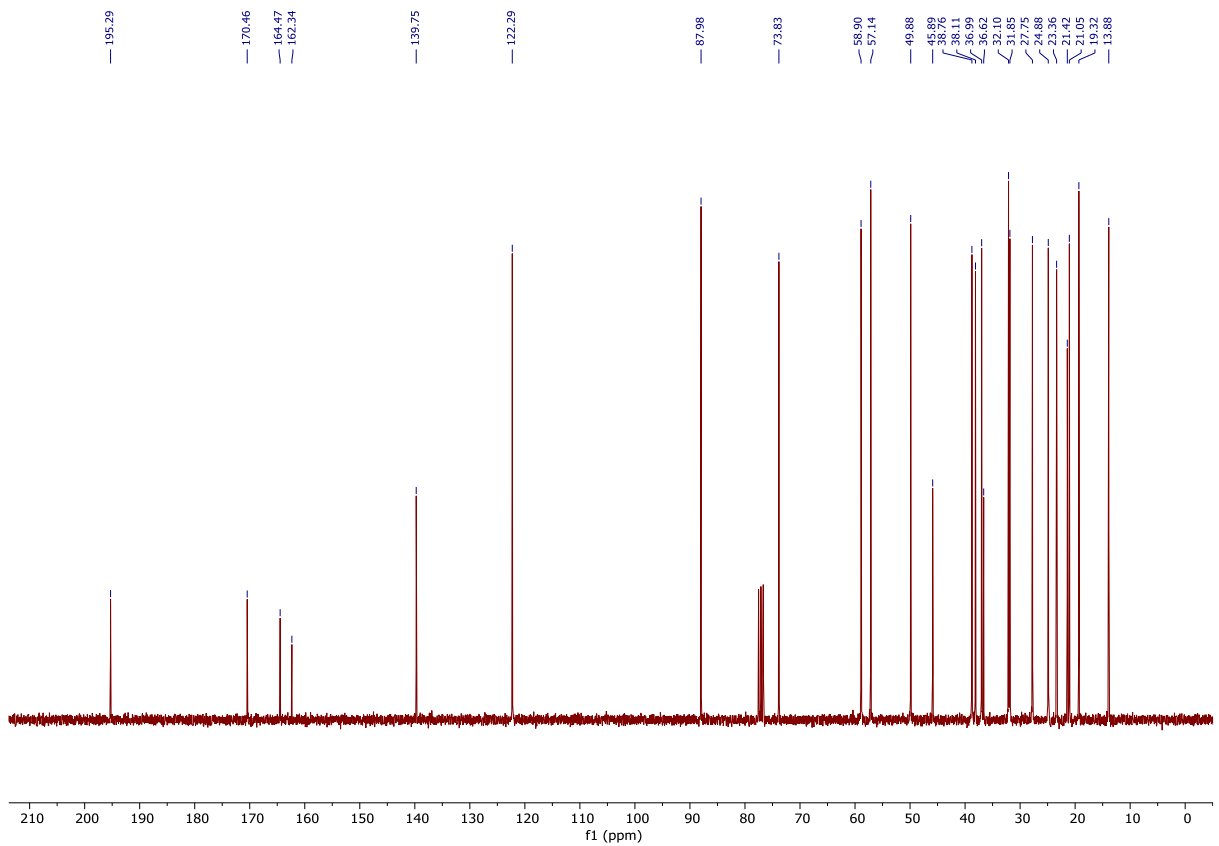
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HRMS (ESI/Q-TOF) of 3-acetate 20-[3`-(5``-azide)isoxazole]-pregn-5,16-dien-3β-ol-20-one (**3a**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 20-[3'-(5''-azide)isoxazole]-pregn-5-en-3 $\beta$ -ol-20-one (**3b**)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 20-[3'-(5''-azide)isoxazole]-pregn-5-en-3 $\beta$ -ol-20-one (**3b**)

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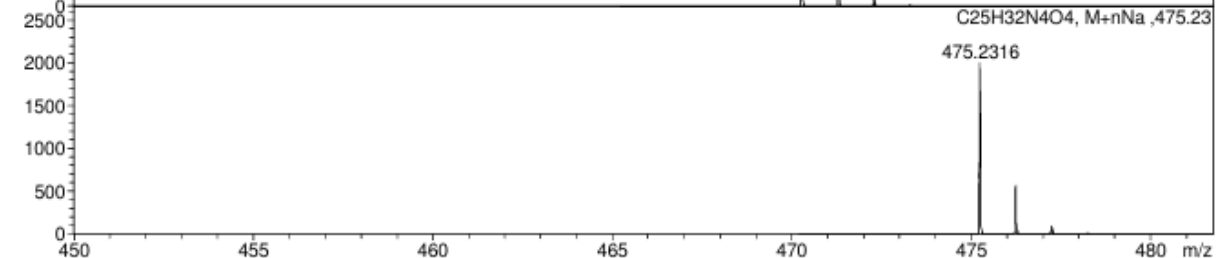
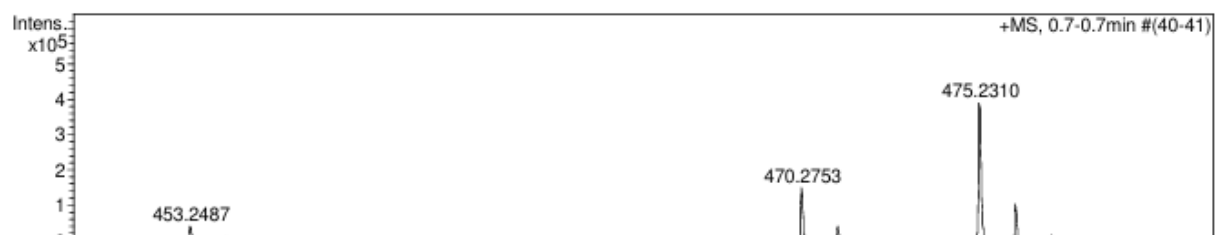
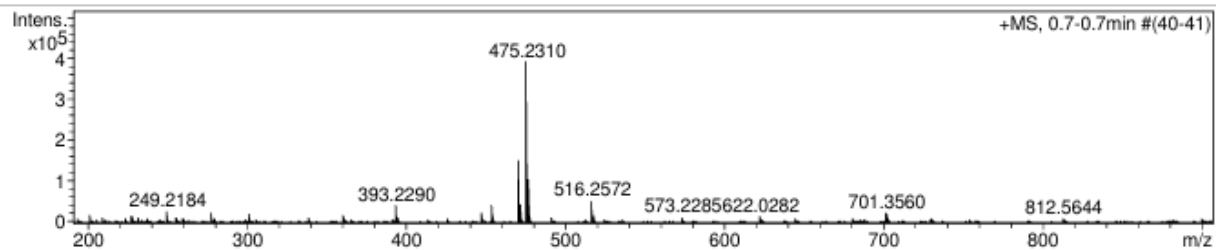
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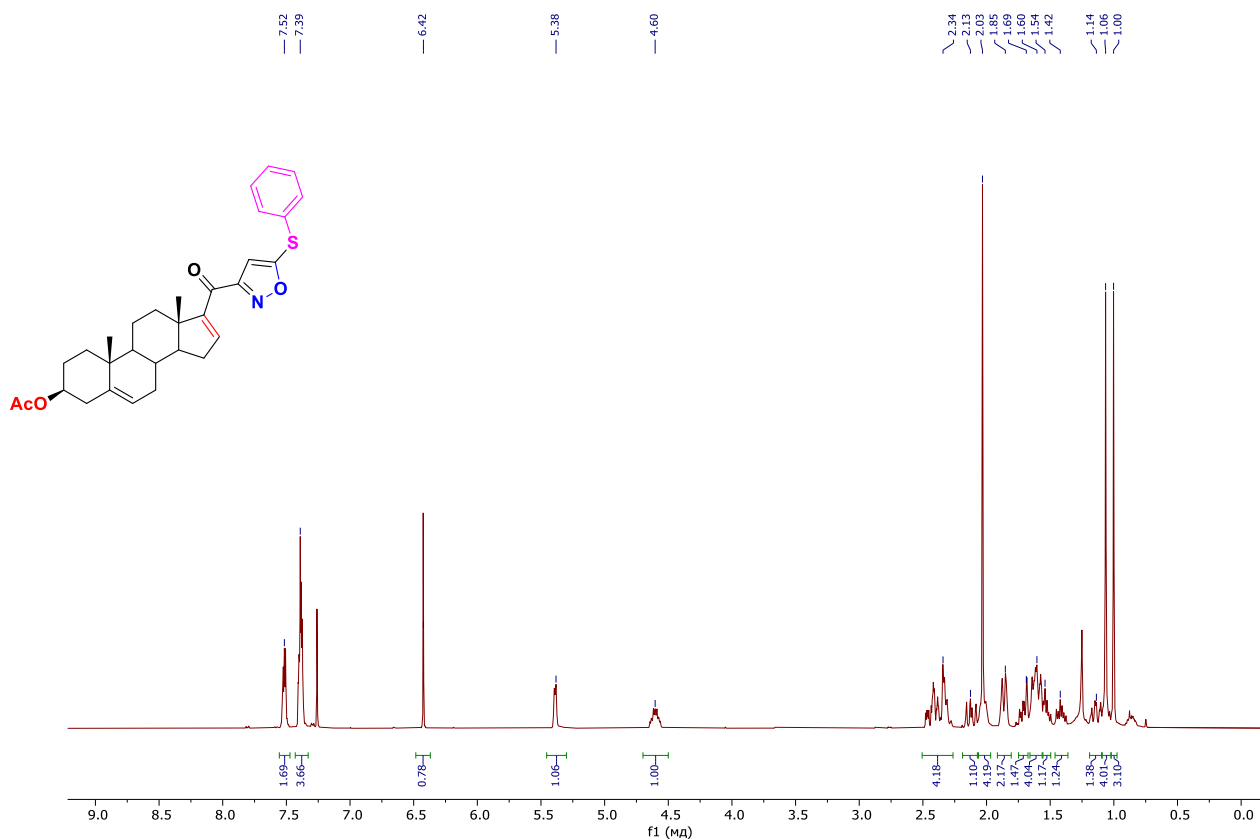
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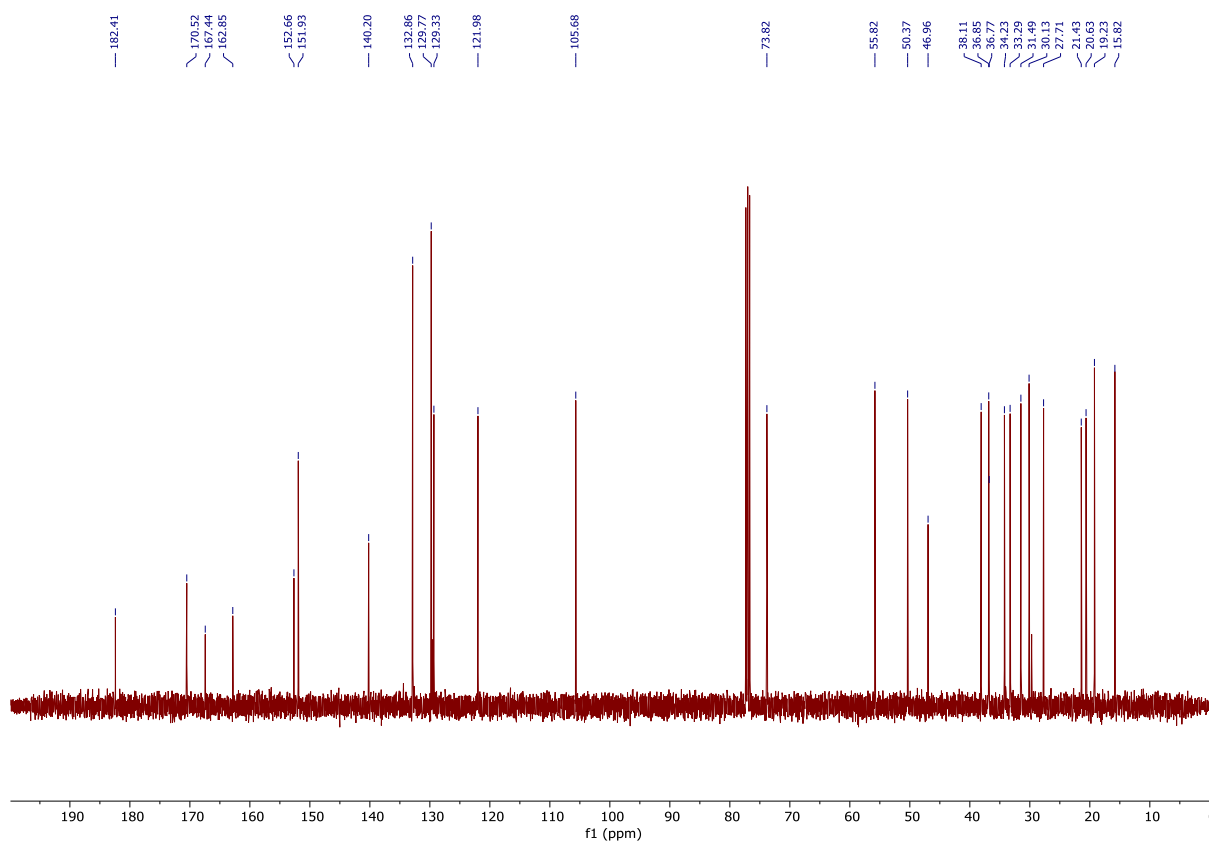
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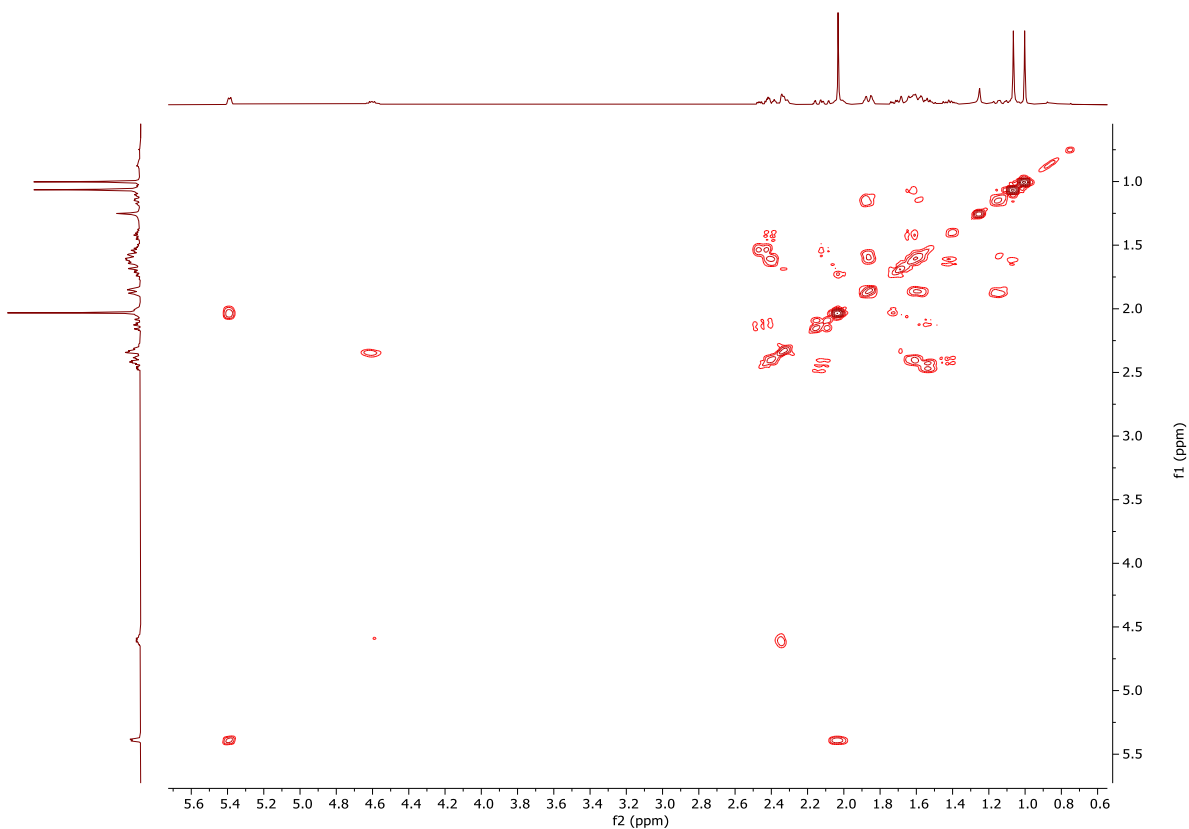
HRMS (ESI/Q-TOF) of 3-acetate 20-[3'-(5''-azide)isoxazole]-pregn-5-en-3 $\beta$ -ol-20-one (**3b**)



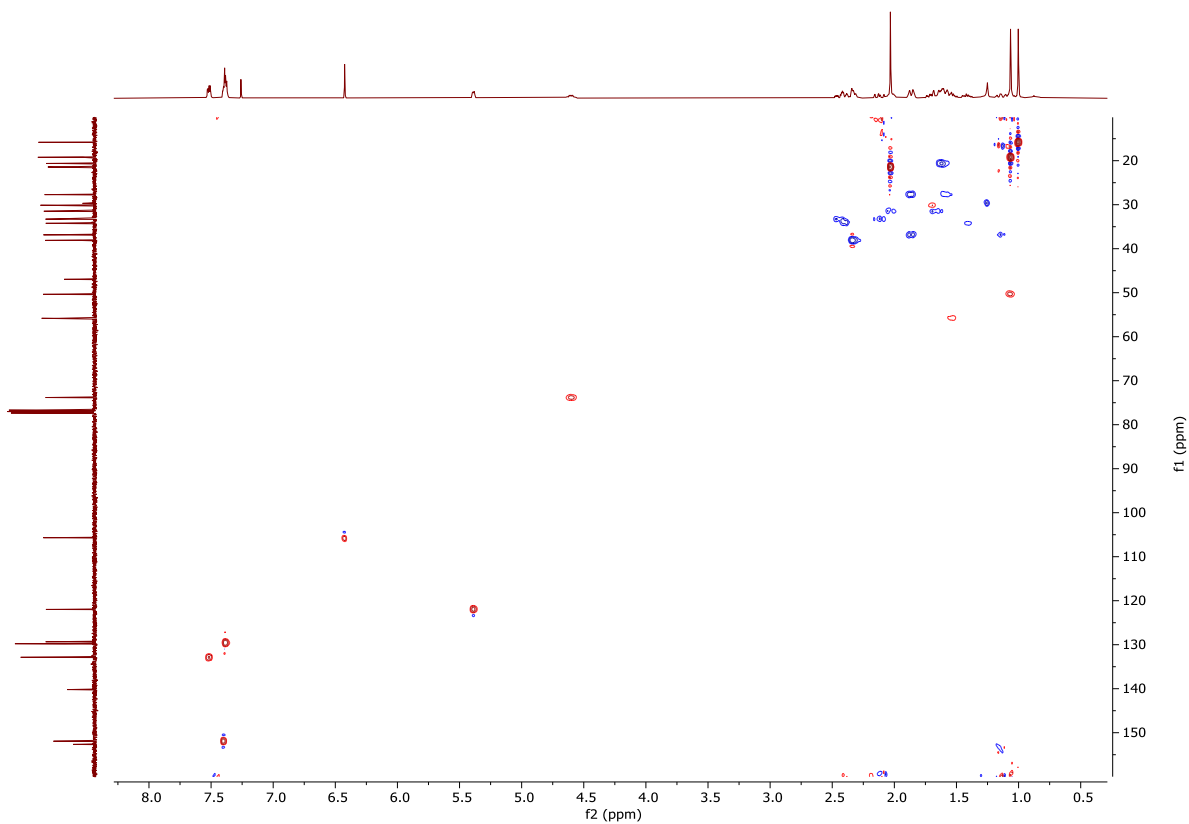
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) spectrum of 3-acetate 20-[3'-(5''-tiophenole)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (4a)



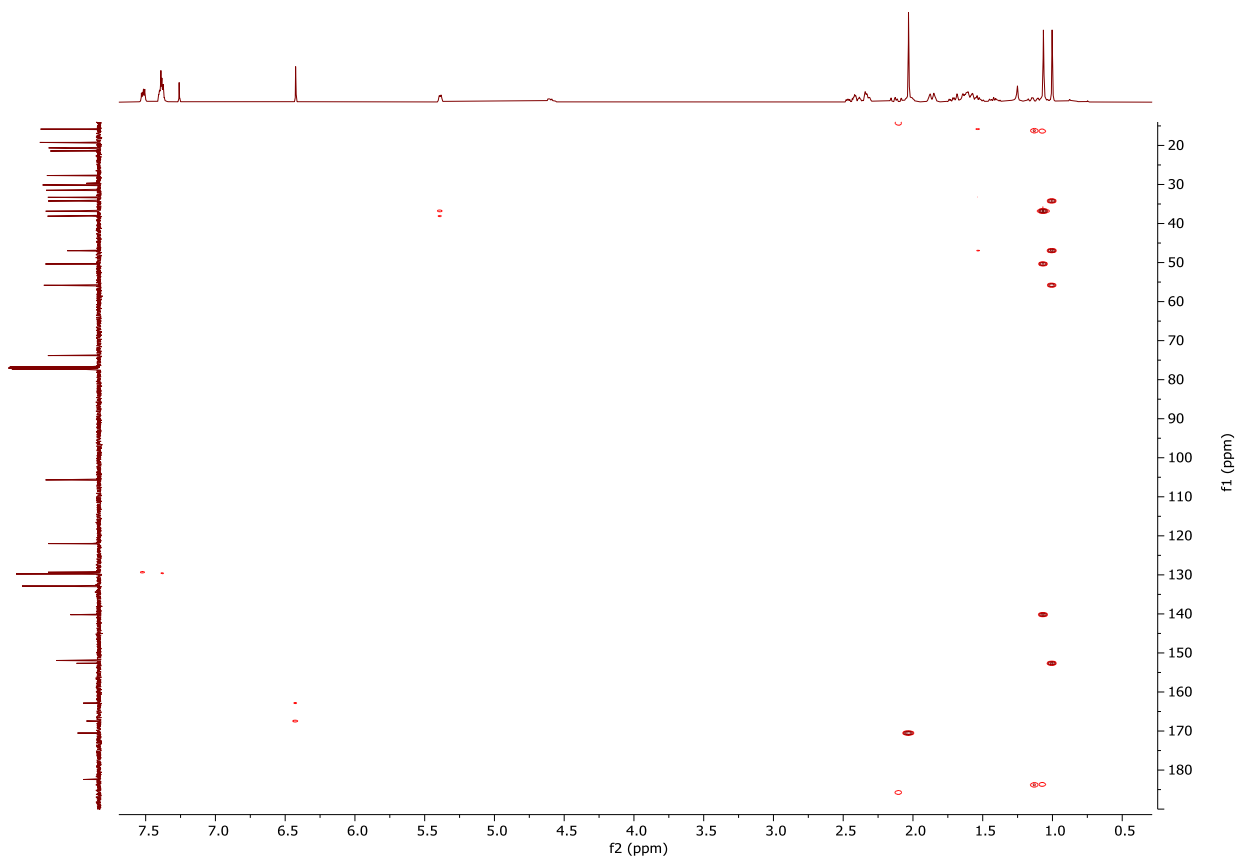
<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) spectrum of 3-acetate 20-[3'-(5''-tiophenole)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (4a)



COSY spectrum of 3-acetate 20-[3`-(5``-tiophenole)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (**4a**)



HSQC spectrum of 3-acetate 20-[3`-(5``-tiophenole)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (**4a**)



HMBC spectrum of 3-acetate 20-[3`-(5``-tiophenole)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (**4a**)

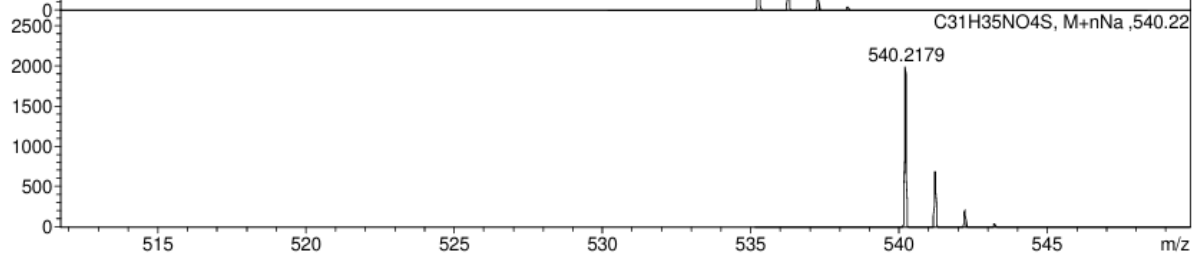
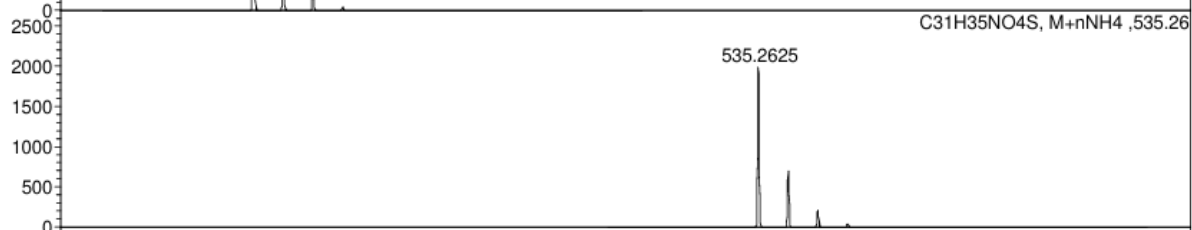
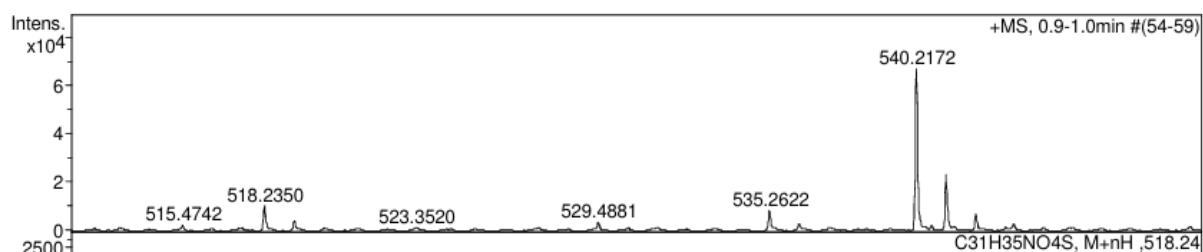
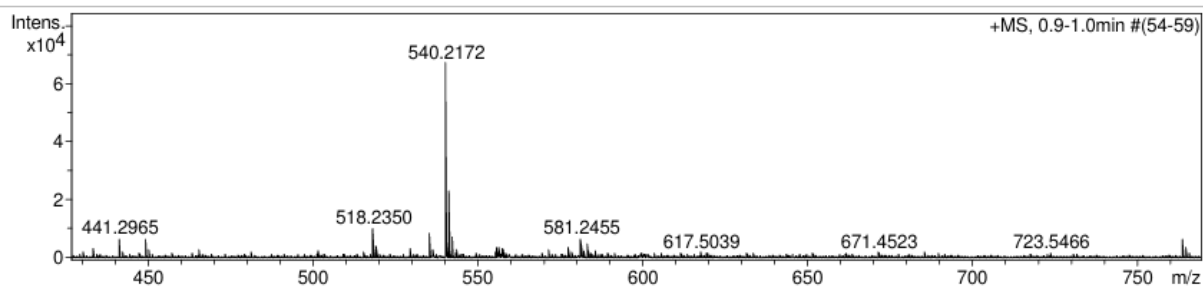
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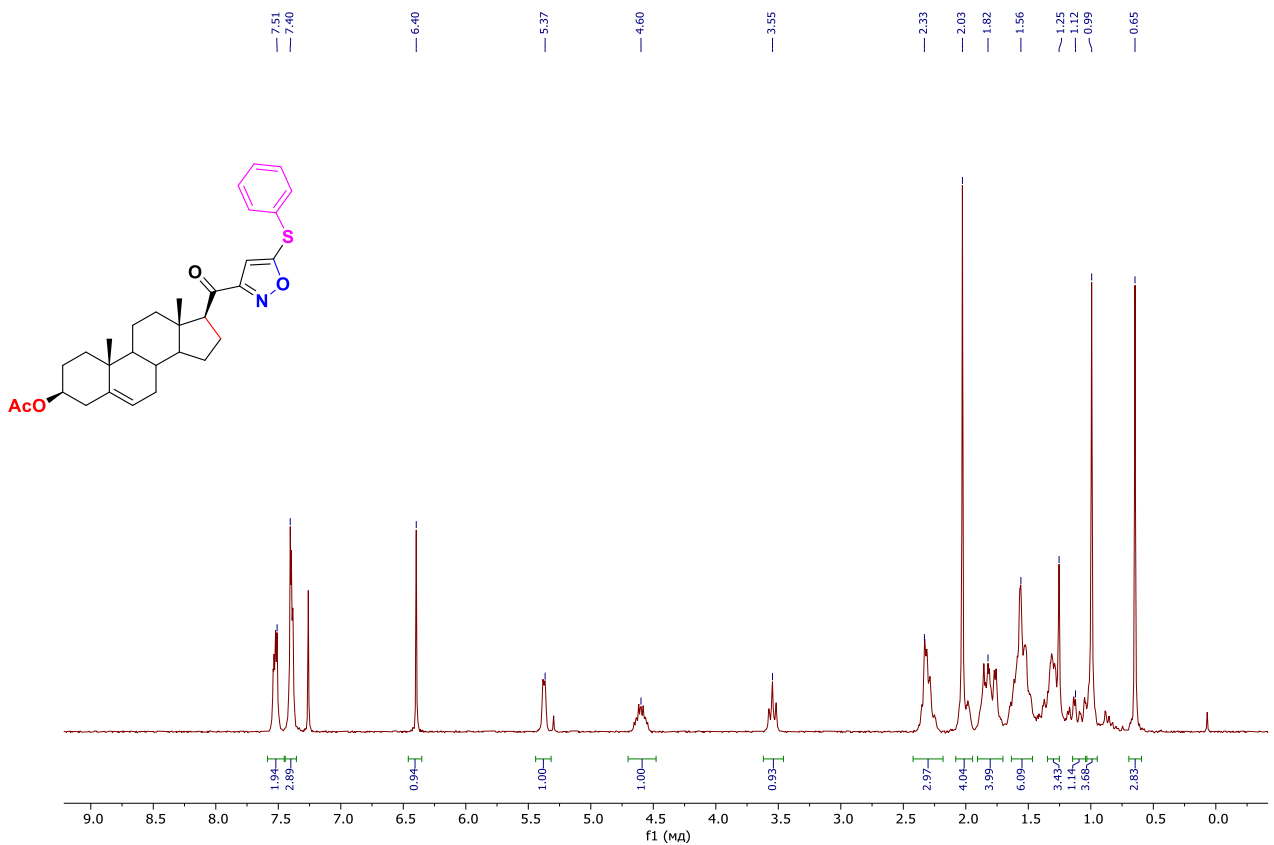
Acquisition Date 02.11.2023 12:54:51  
Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

**Acquisition Parameter**

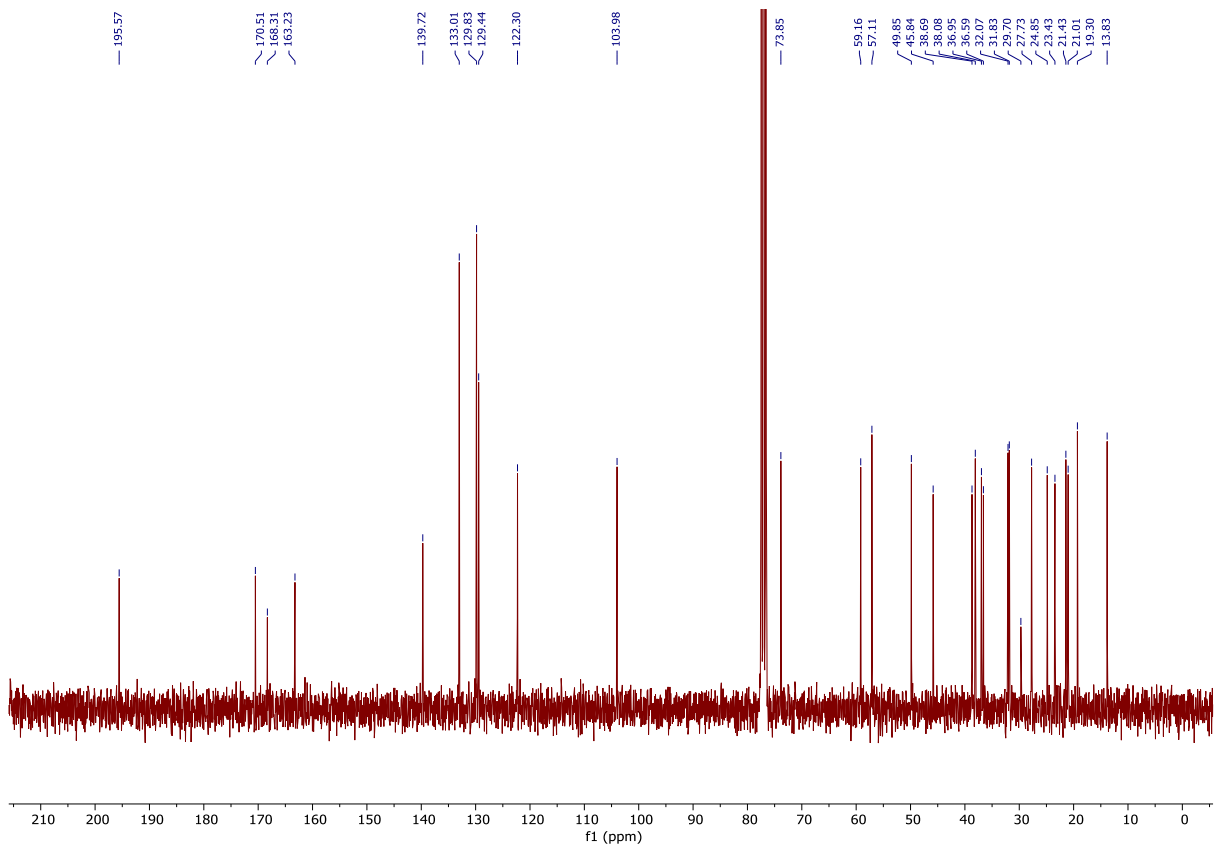
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-[3'-(5''-tiophenole)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (**4a**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 20-[3'-(5''-tiophenole)isoxazole]-pregn-5-en-3β-ol-20-one (4b)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 20-[3'-(5''-tiophenole)isoxazole]-pregn-5-en-3β-ol-20-one (4b)

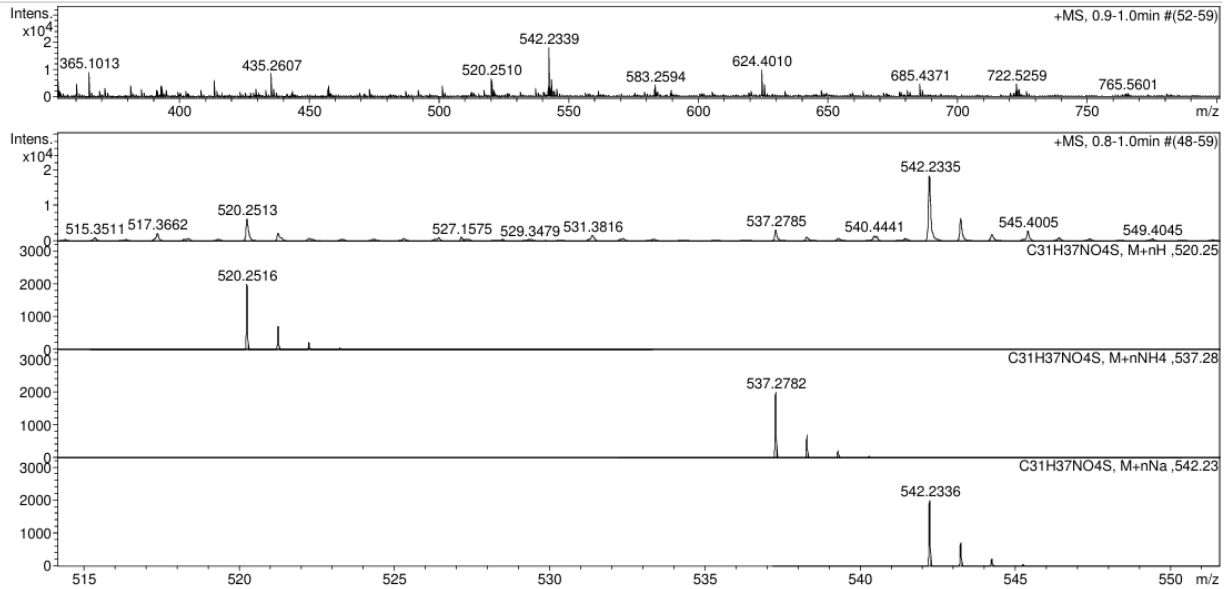
**Analysis Info**

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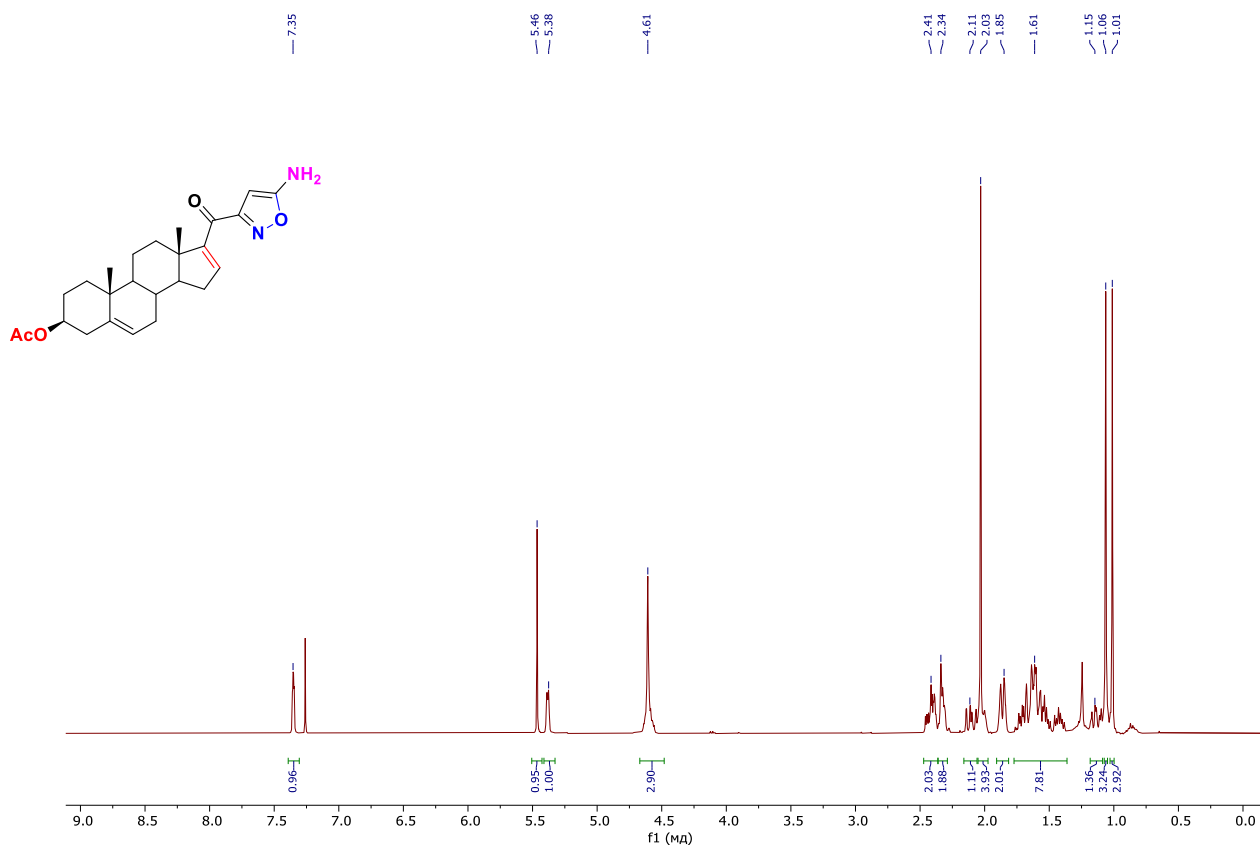
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Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

**Acquisition Parameter**

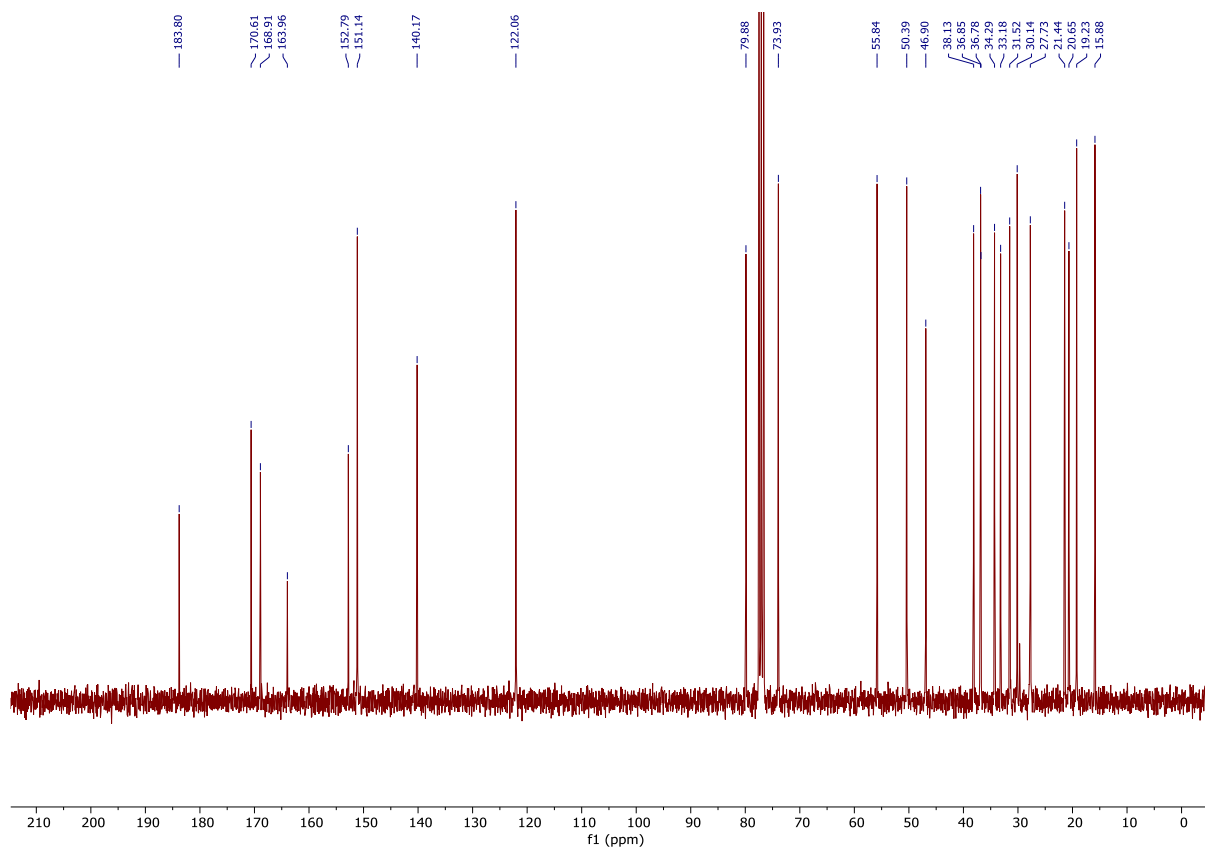
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-[3`-(5``-tiophenole)isoxazole]-pregn-5-en-3β-ol-20-one (**4b**)



$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz) spectrum of 3-acetate 20-[3'-(5''-amine)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (5a)



$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz) spectrum of 3-acetate 20-[3'-(5''-amine)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (5a)

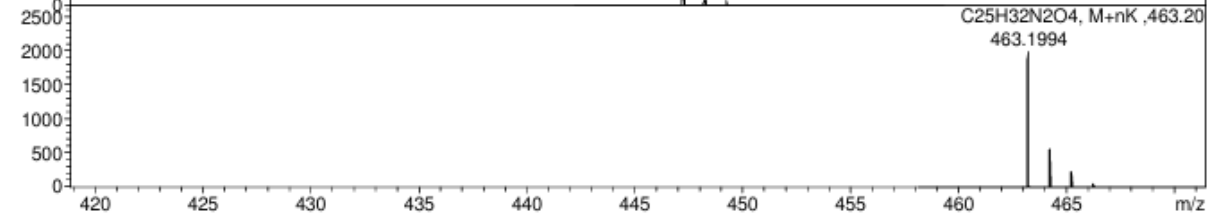
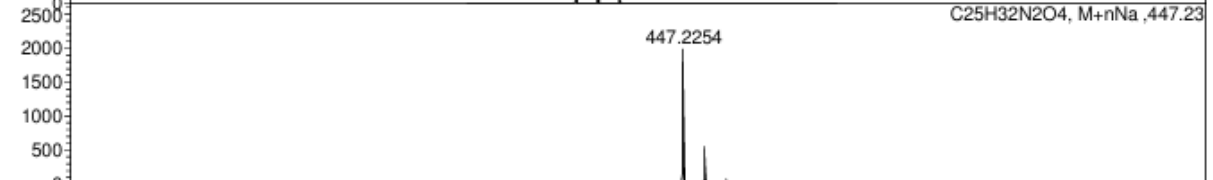
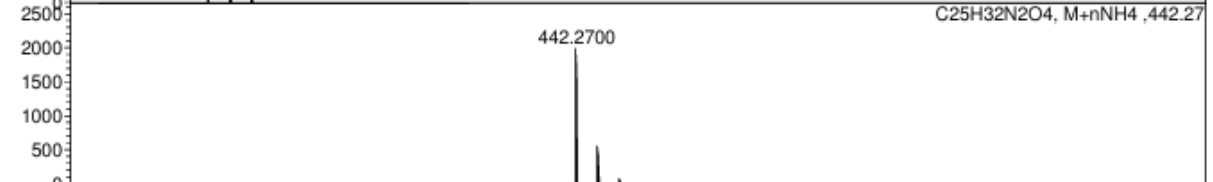
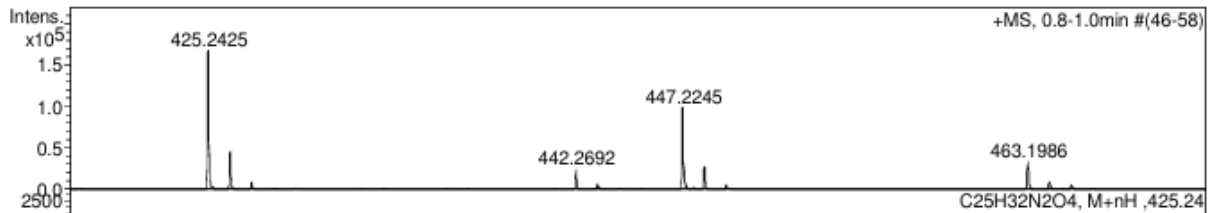
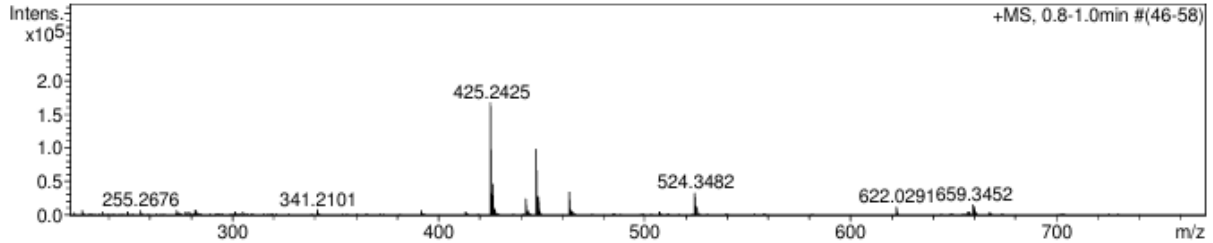
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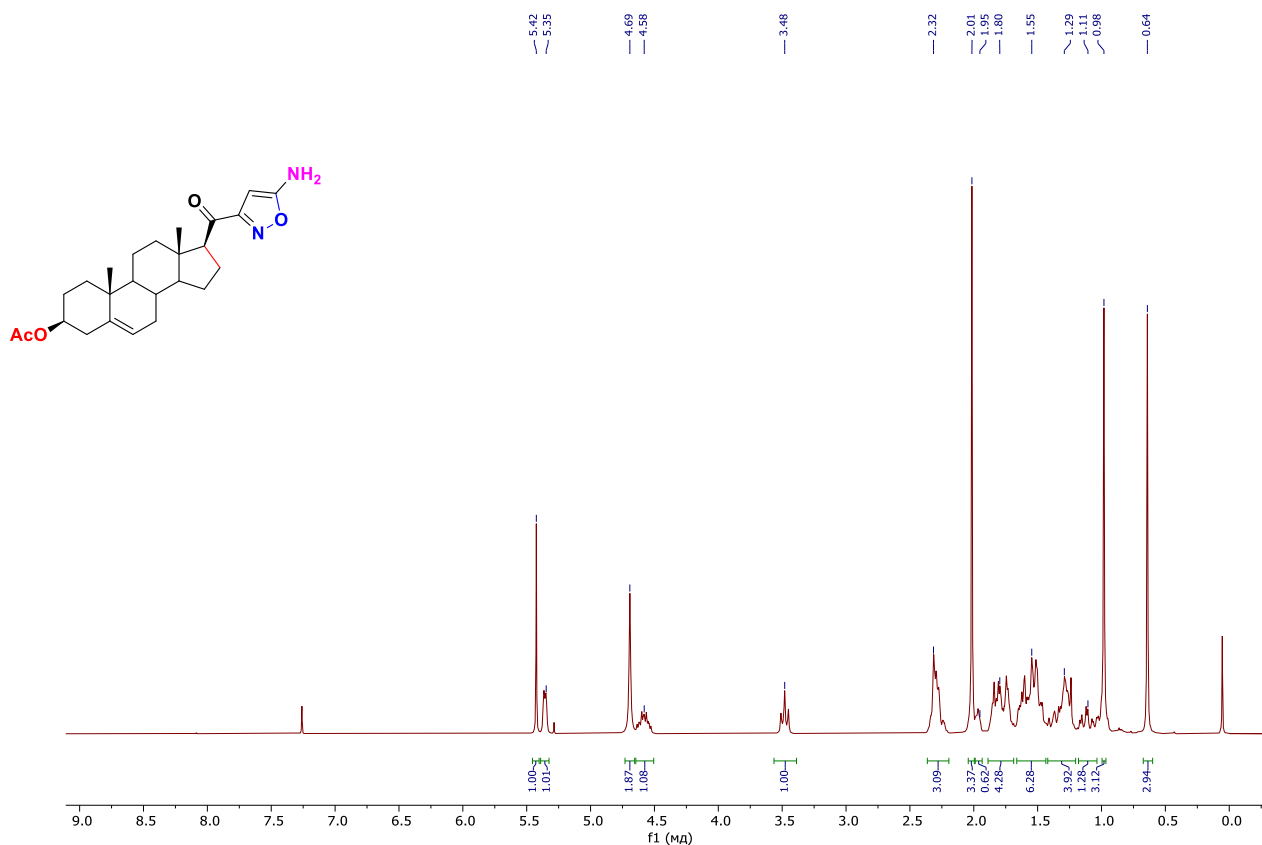
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Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

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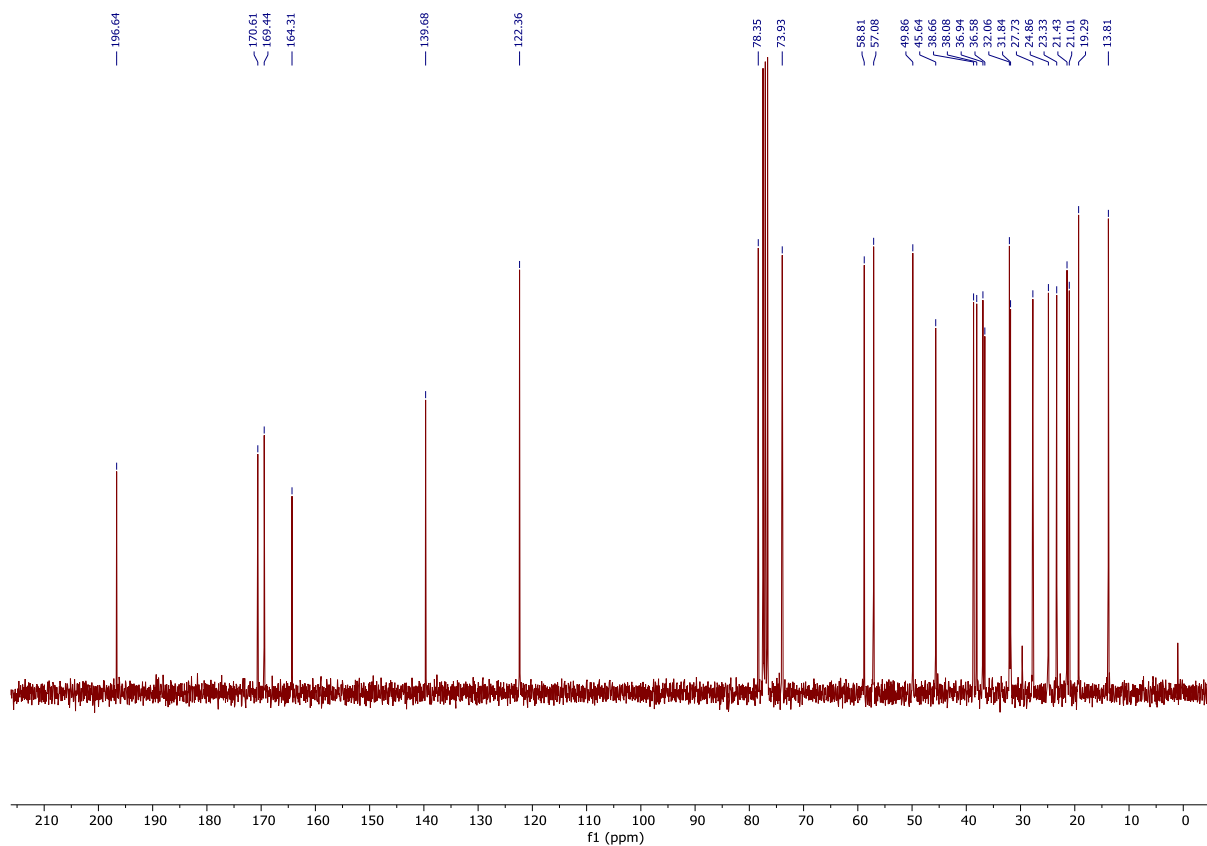
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-[3'-(5''-amine)isoxazole]-pregn-5,16-dien-3β-ol-20-one (5a)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 20-[3'-(5''-amine)isoxazole]-pregn-5-en-3β-ol-20-one (5b)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 20-[3'-(5''-amine)isoxazole]-pregn-5-en-3β-ol-20-one (5b)

**Analysis Info**

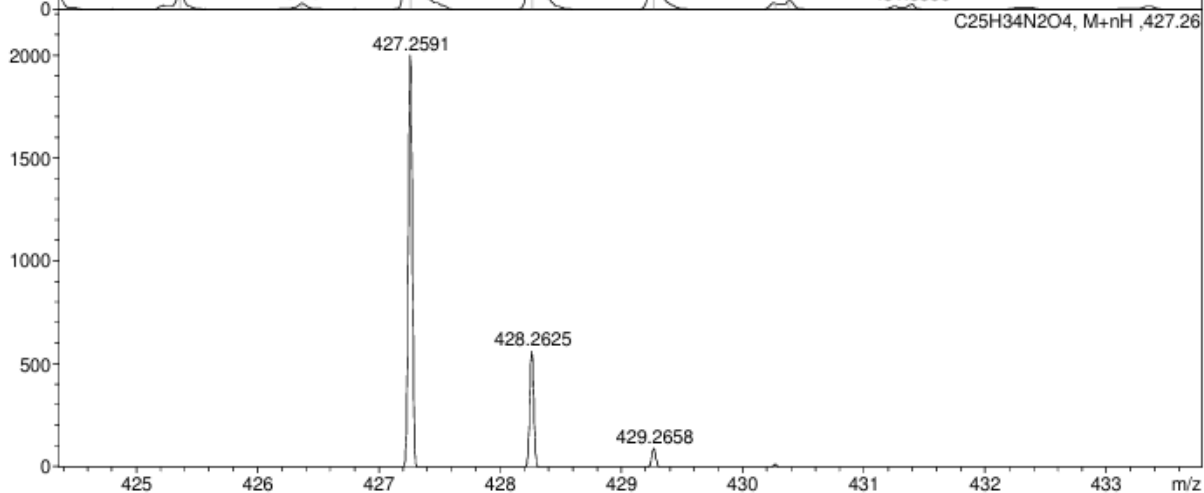
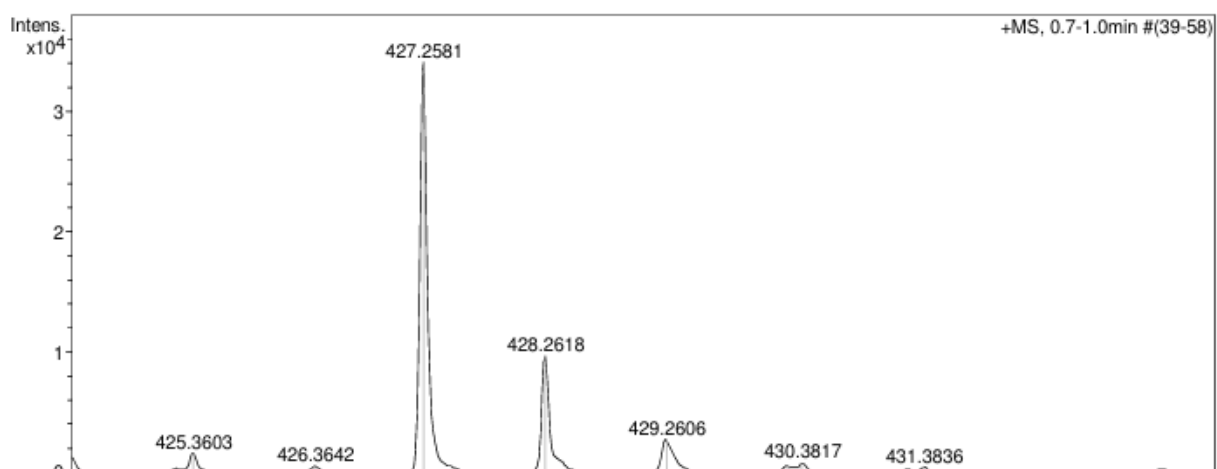
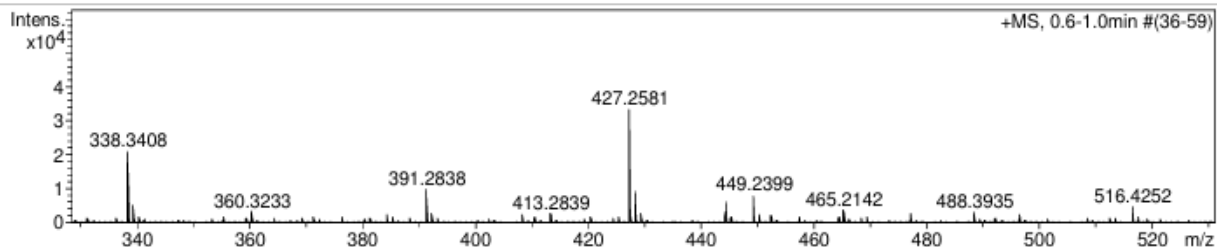
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Acquisition Date 28.02.2024 16:26:56

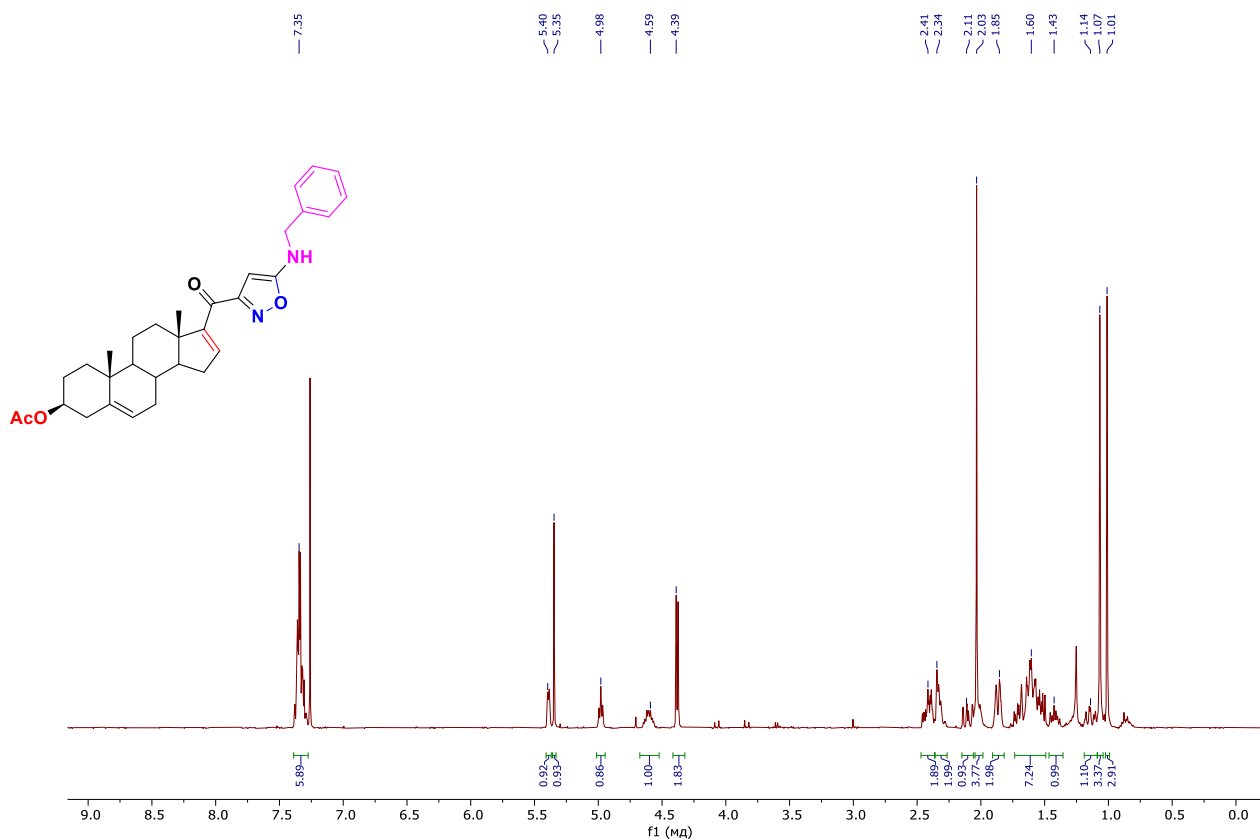
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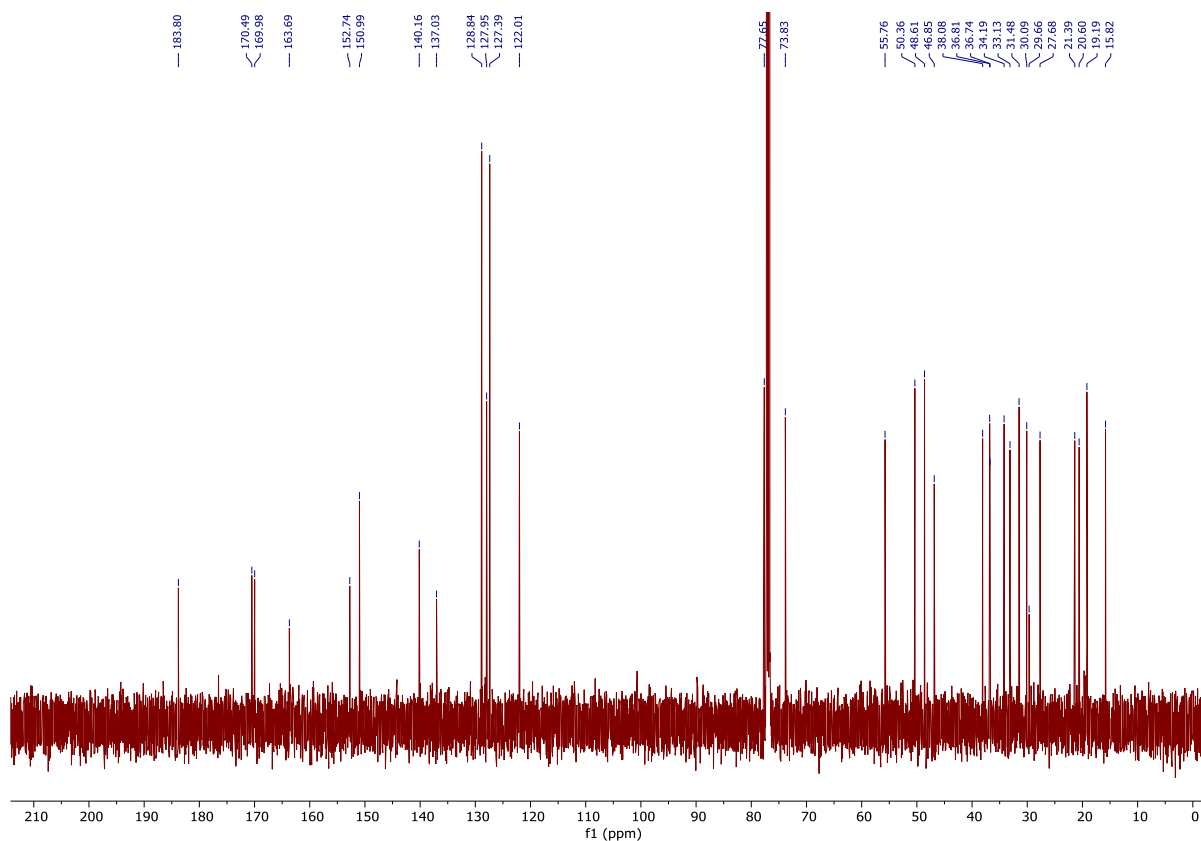
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-[3`-(5``-amine)isoxazole]-pregn-5-en-3β-ol-20-one (5b)



$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ) spectrum of 3-acetate 20-[3''-(5'-benzylamine)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (7a)



$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ) spectrum of 3-acetate 20-[3''-(5'-benzylamine)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (7a)

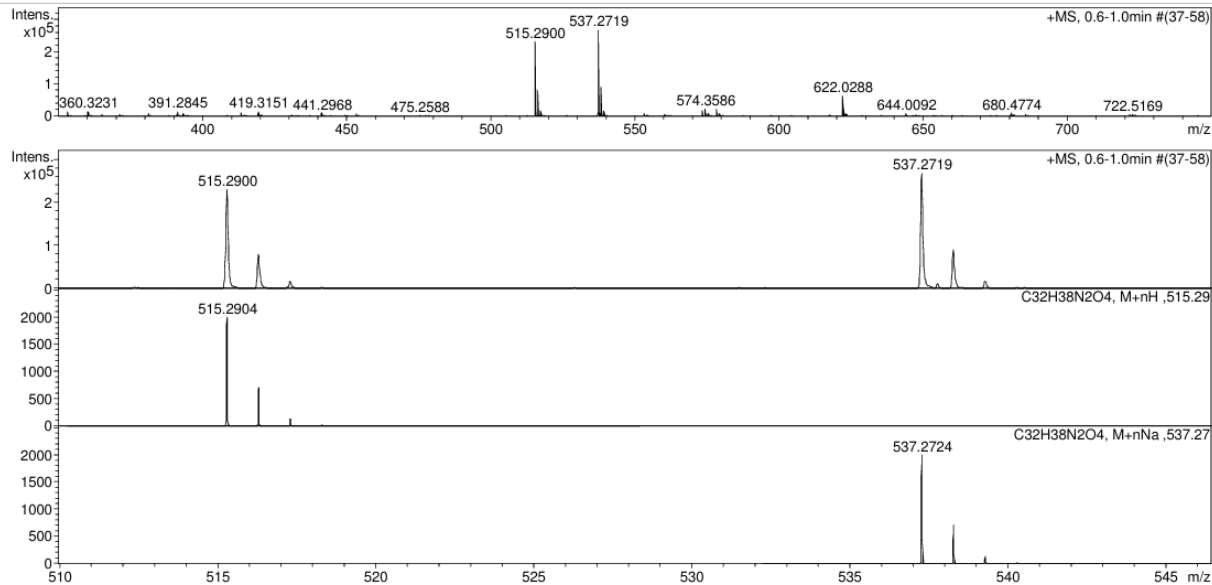
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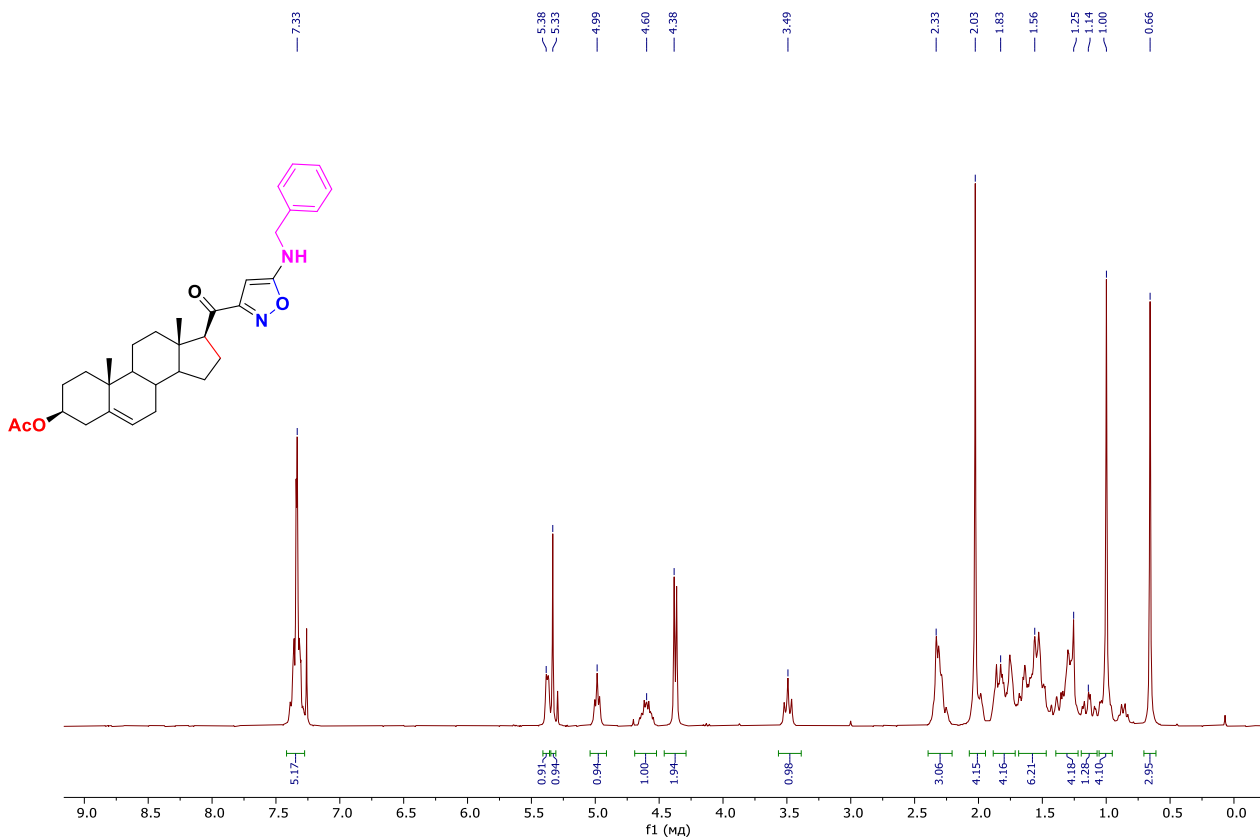
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Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

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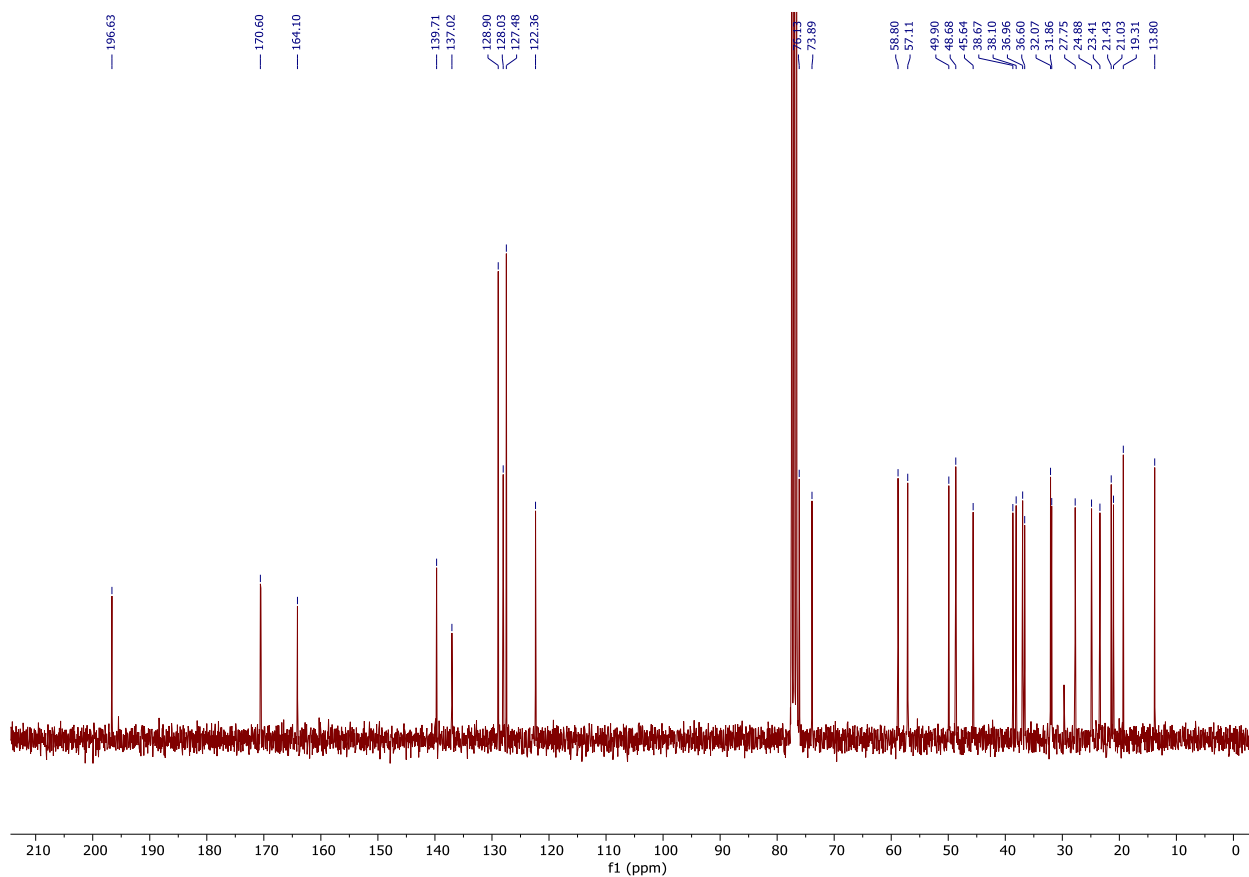
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-[3''-(5'-benzylamine)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (**7a**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 20-[3'-(5''-benzylamine)isoxazole]-pregna-5-en-3β-ol-20-one (7b)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 20-[3'-(5''-benzylamine)isoxazole]-pregna-5-en-3β-ol-20-one (7b)

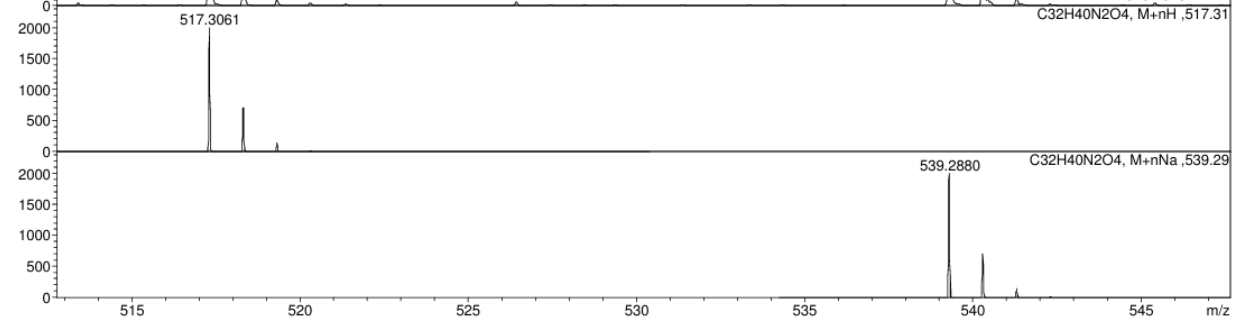
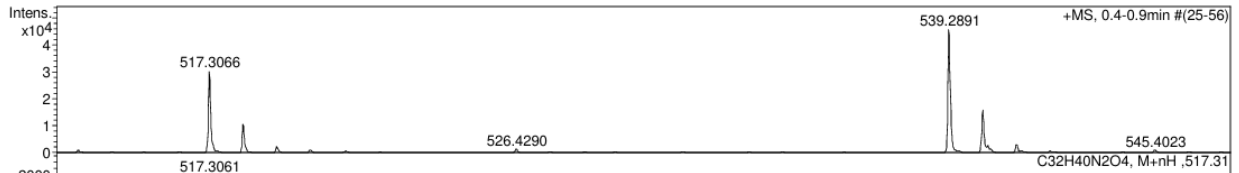
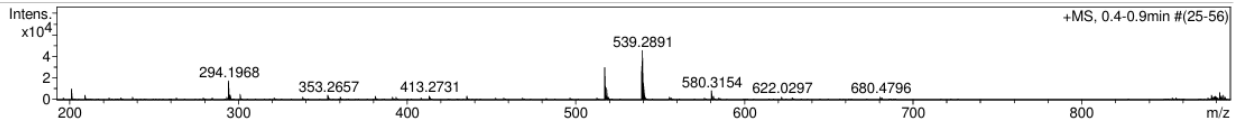
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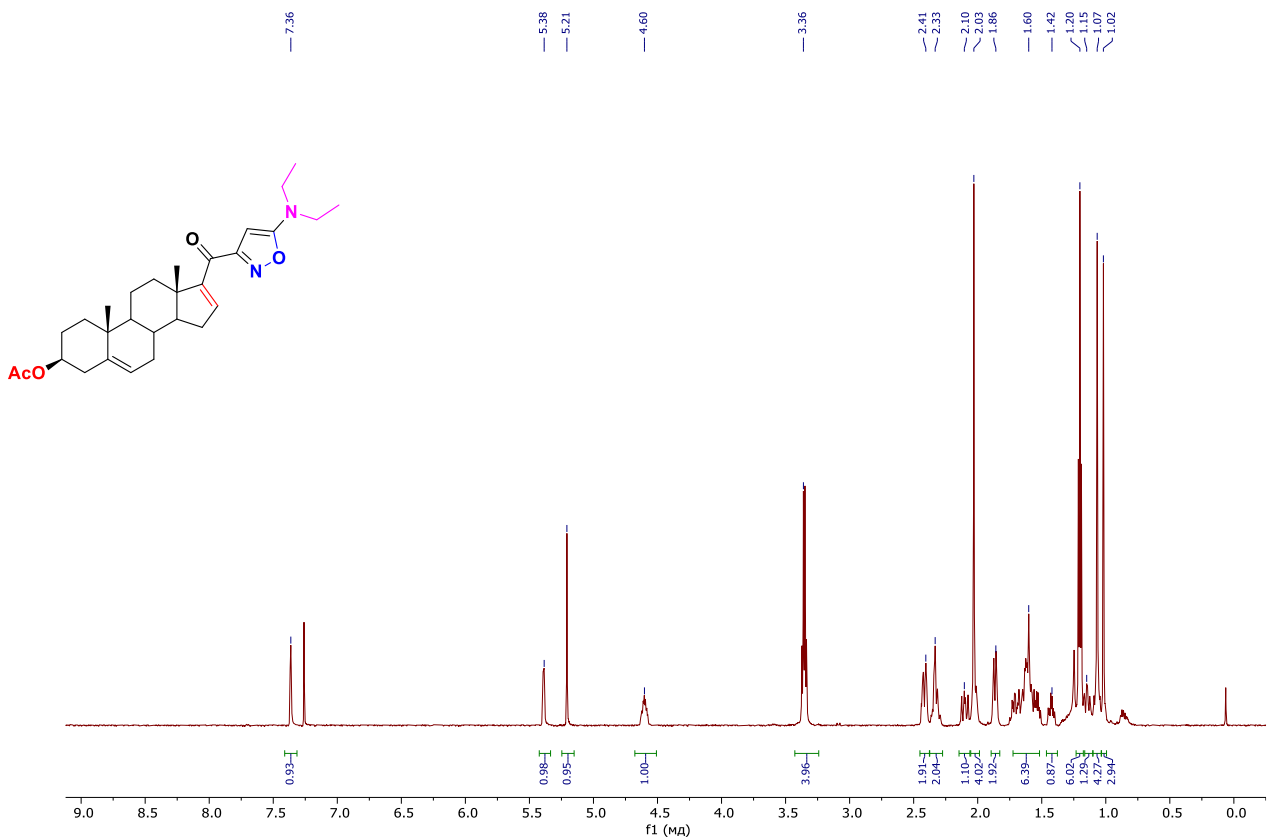
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Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

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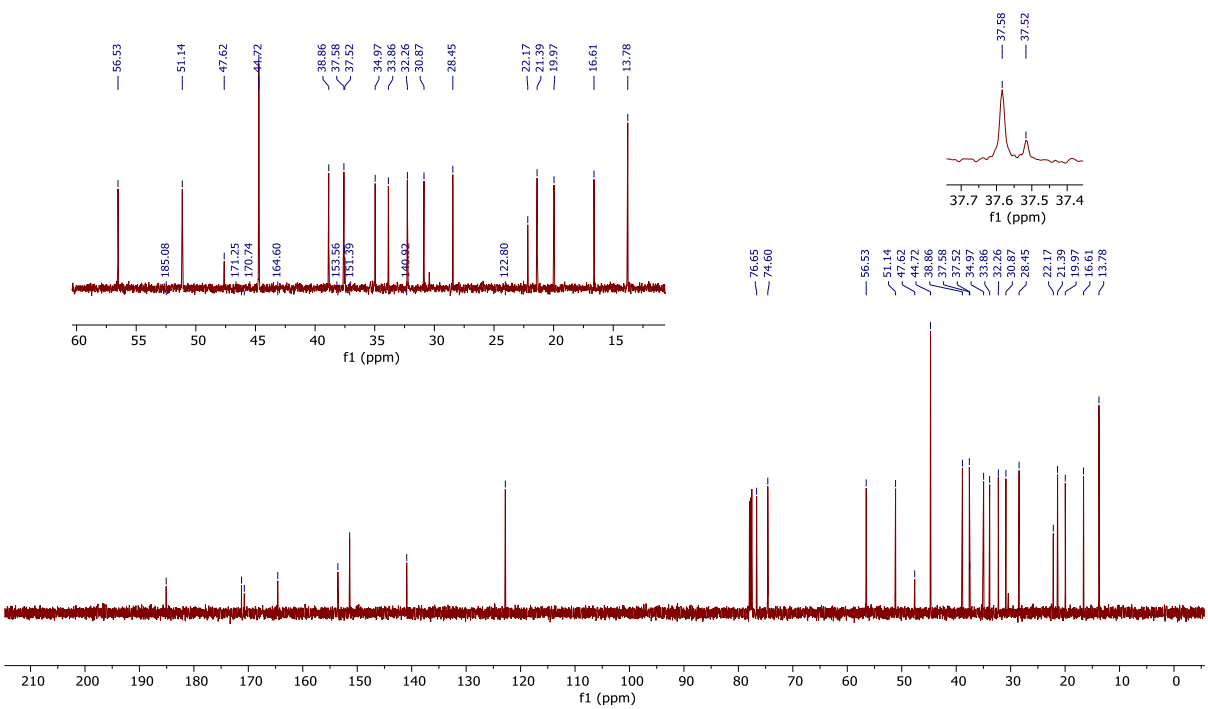
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-[3'-(5''-benzylamine)isoxazole]-pregna-5-en-3 $\beta$ -ol-20-one (**7b**)



<sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) spectrum of 3-acetate 20-[3'-(5''-diethylamine)isoxazole]-pregn-5,16-dien-3β-ol-20-one (7c)



<sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) spectrum of 3-acetate 20-[3'-(5''-diethylamine)isoxazole]-pregn-5,16-dien-3β-ol-20-one (7c)

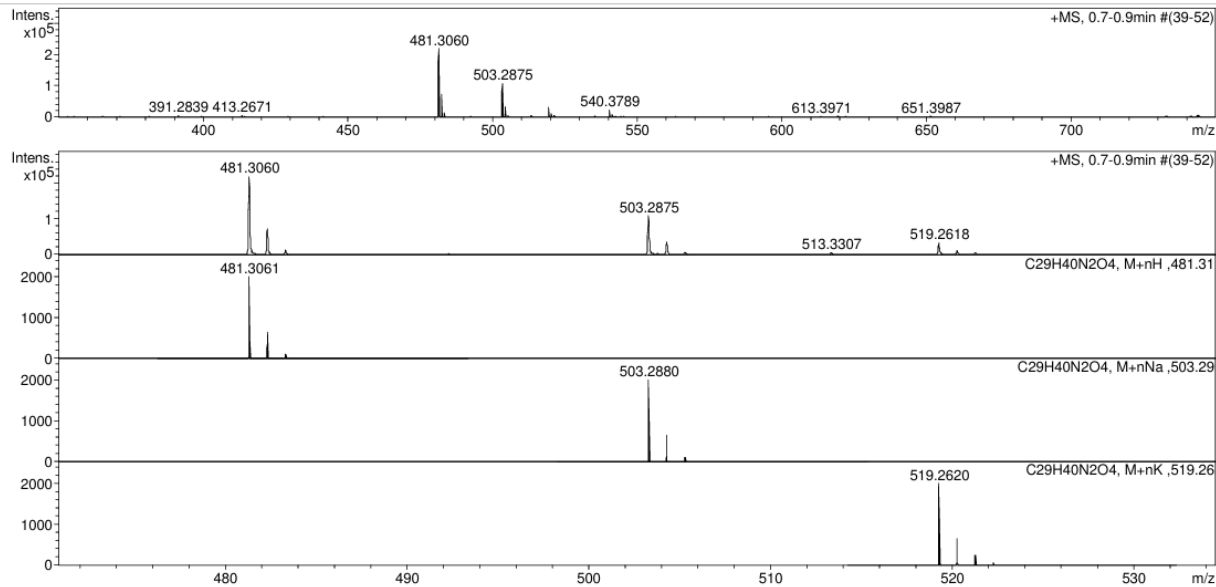
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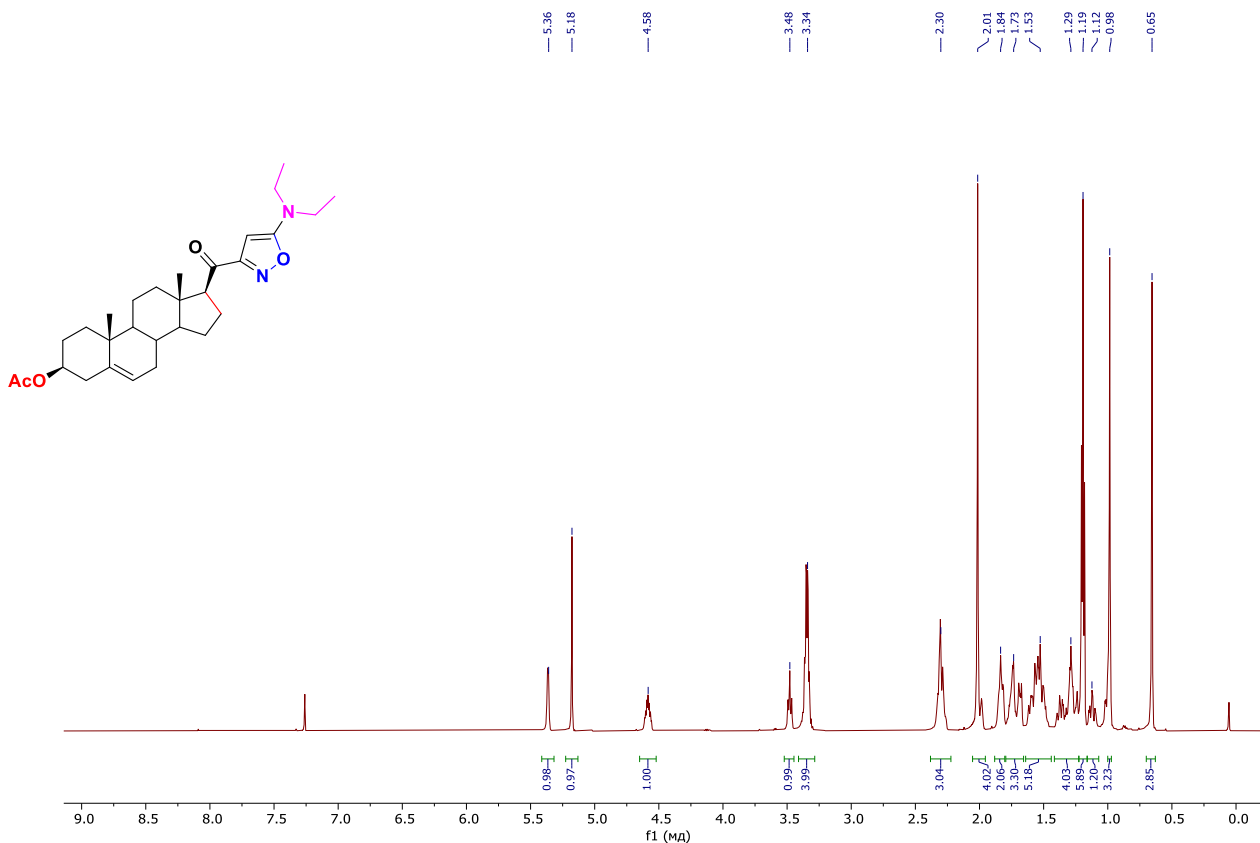
Acquisition Date 30.11.2023 15:48:47  
Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

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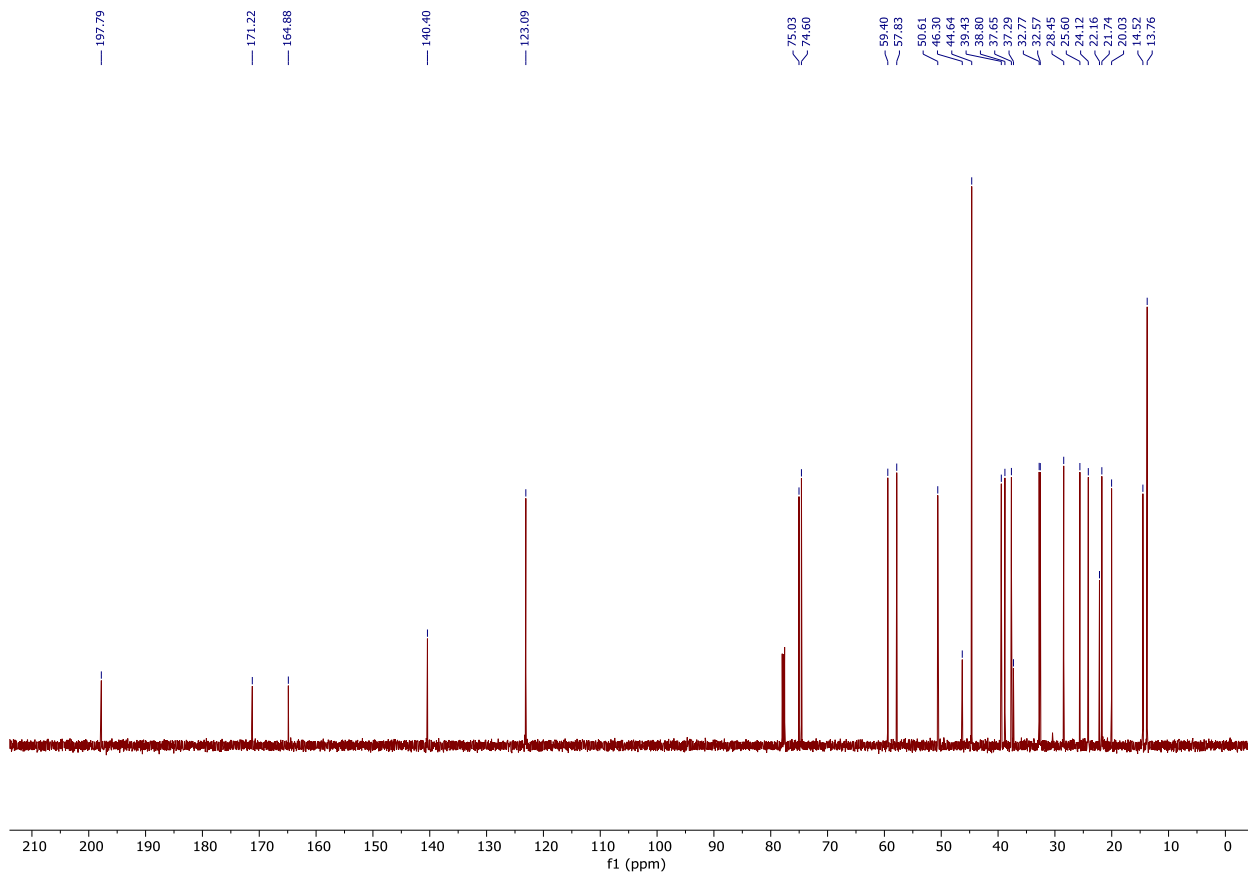
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-[3`-(5``-diethylamine)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (**7c**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz) spectrum of 3-acetate 20-(5'-diethylamineisoxazole)-pregna-5-en-3β-ol-20-one (7d)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 151 MHz) spectrum of 3-acetate 20-(5'-diethylamineisoxazole)-pregna-5-en-3β-ol-20-one (7d)

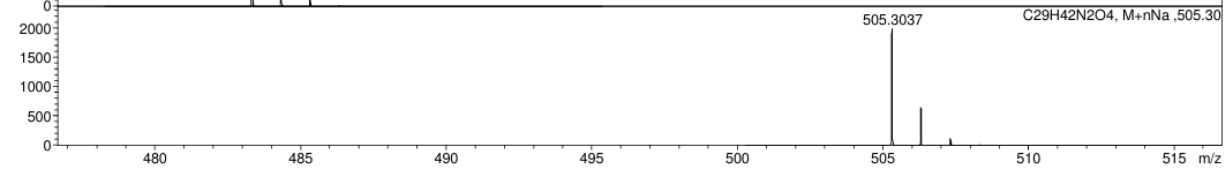
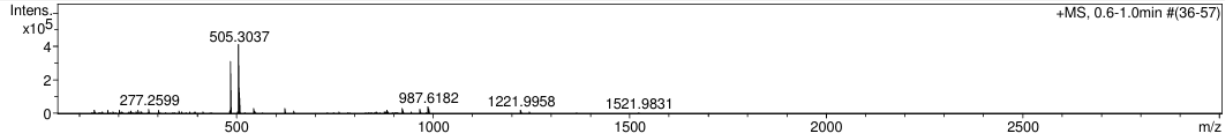
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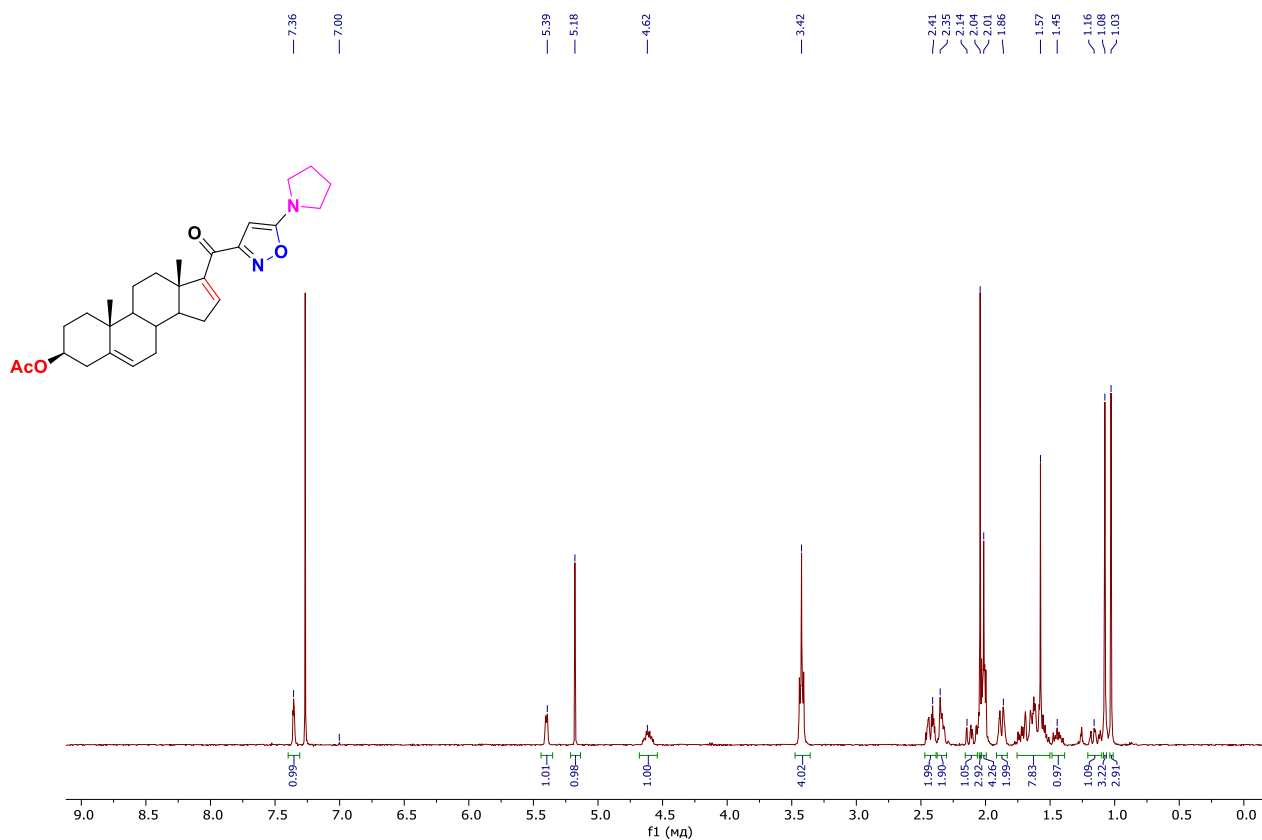
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Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

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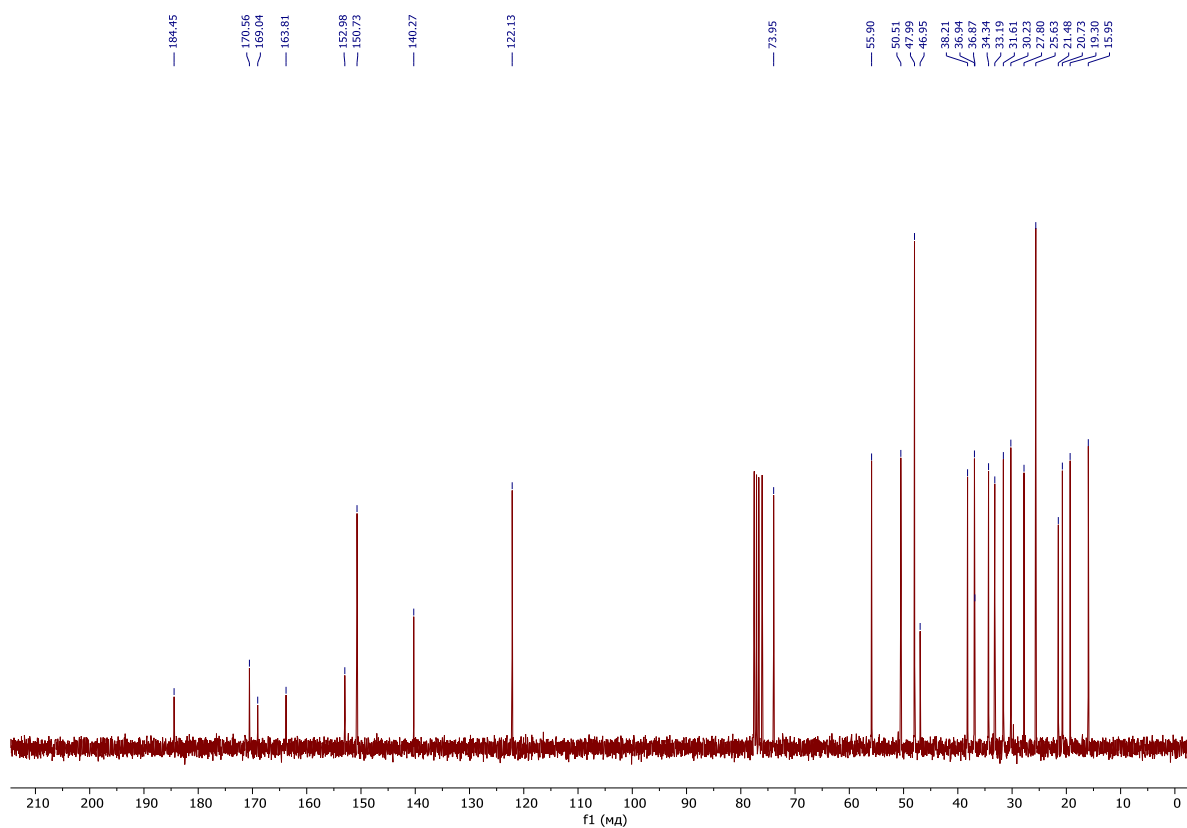
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-(5`-diethylamineisoxazole)-pregna-5-en-3β-ol-20-one (**7d**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 20-(5-pyrrolidineisoxazole)-pregna-5,16-diene-3β-ol-20-one (7e)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 20-(5-pyrrolidineisoxazole)-pregna-5,16-diene-3β-ol-20-one (7e)

**Analysis Info**

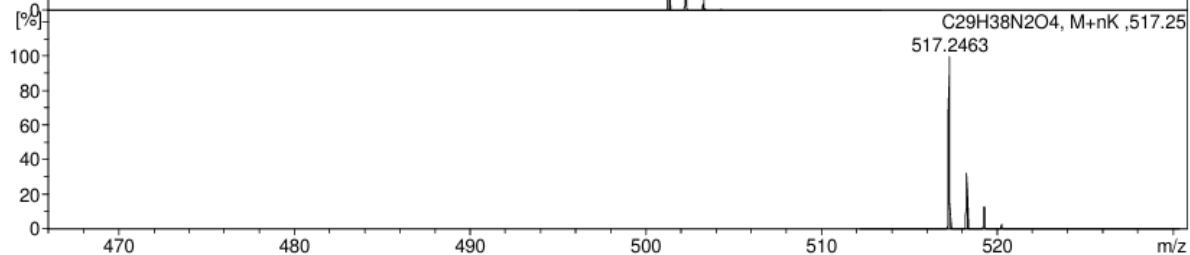
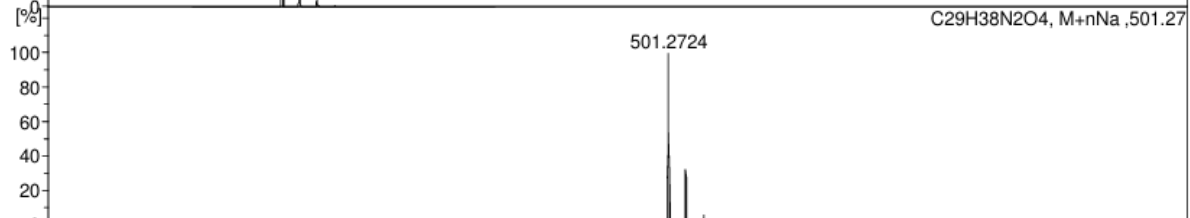
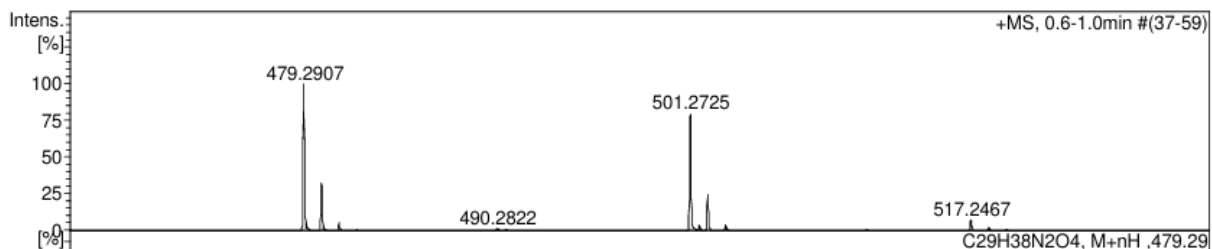
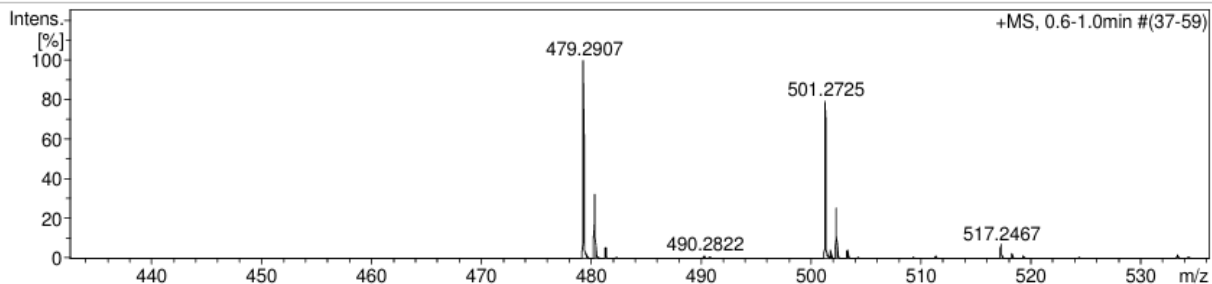
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Acquisition Date 02.11.2023 13:05:42

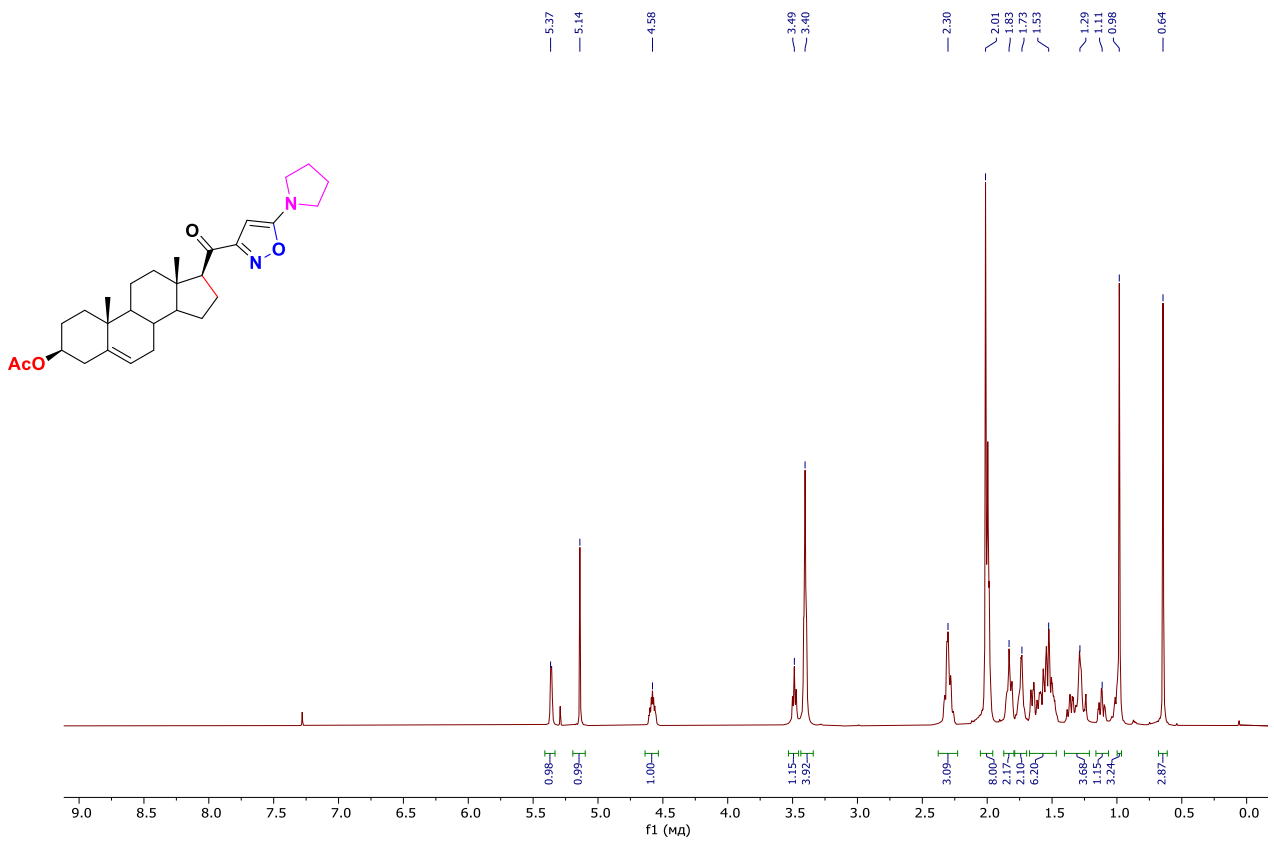
Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

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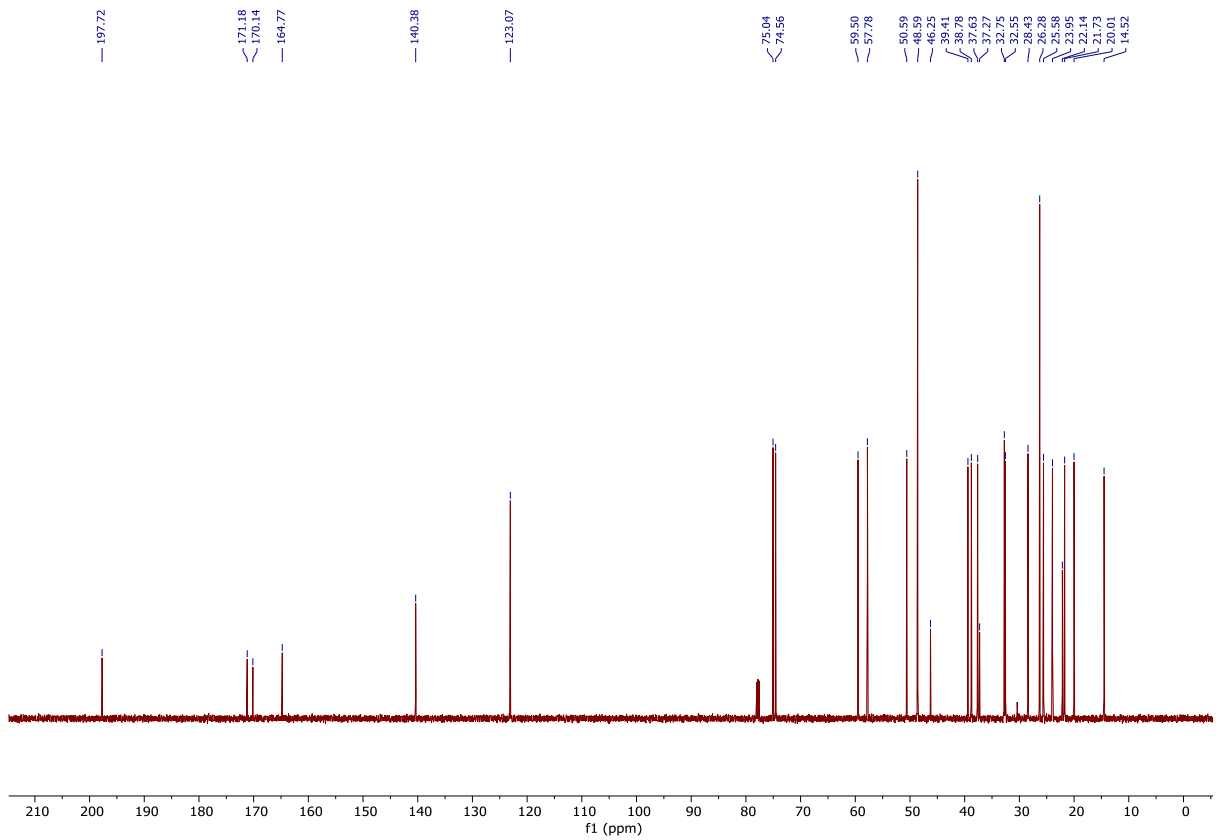
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-(5-pyrrolidineisoxazole)-pregna-5,16-diene-3 $\beta$ -ol-20-one (**7e**)



$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz) spectrum of 3-acetate 20-(5'-pirrolidineisoxazole)-pregna-5-en-3 $\beta$ -ol-20-one (7f)



$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 151 MHz) spectrum of 3-acetate 20-(5'-pirrolidineisoxazole)-pregna-5-en-3 $\beta$ -ol-20-one (7f)

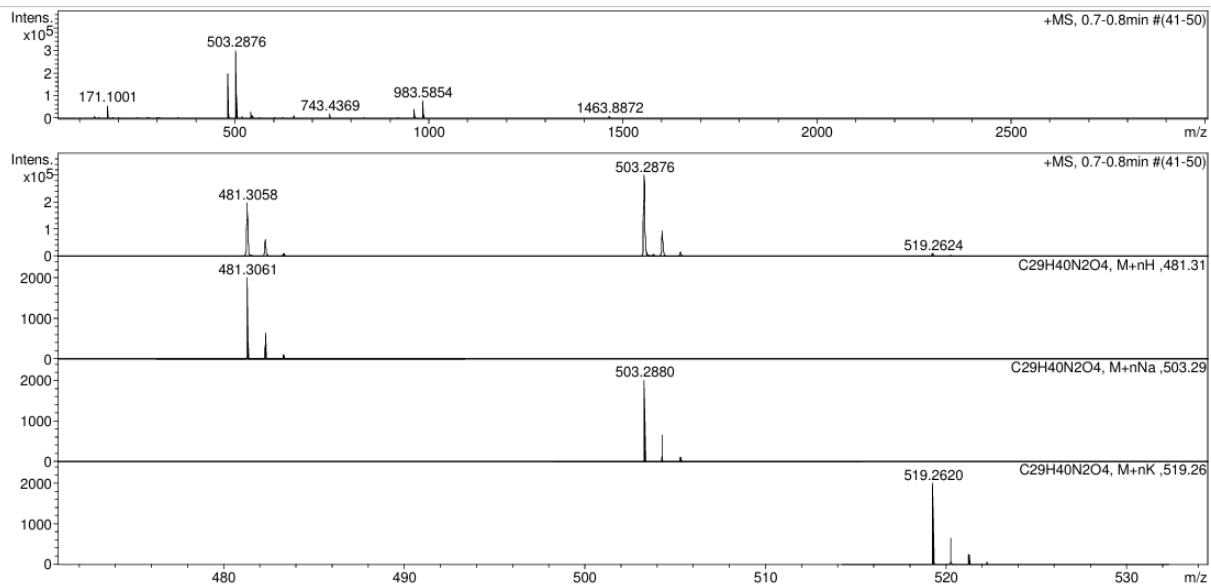
**Analysis Info**

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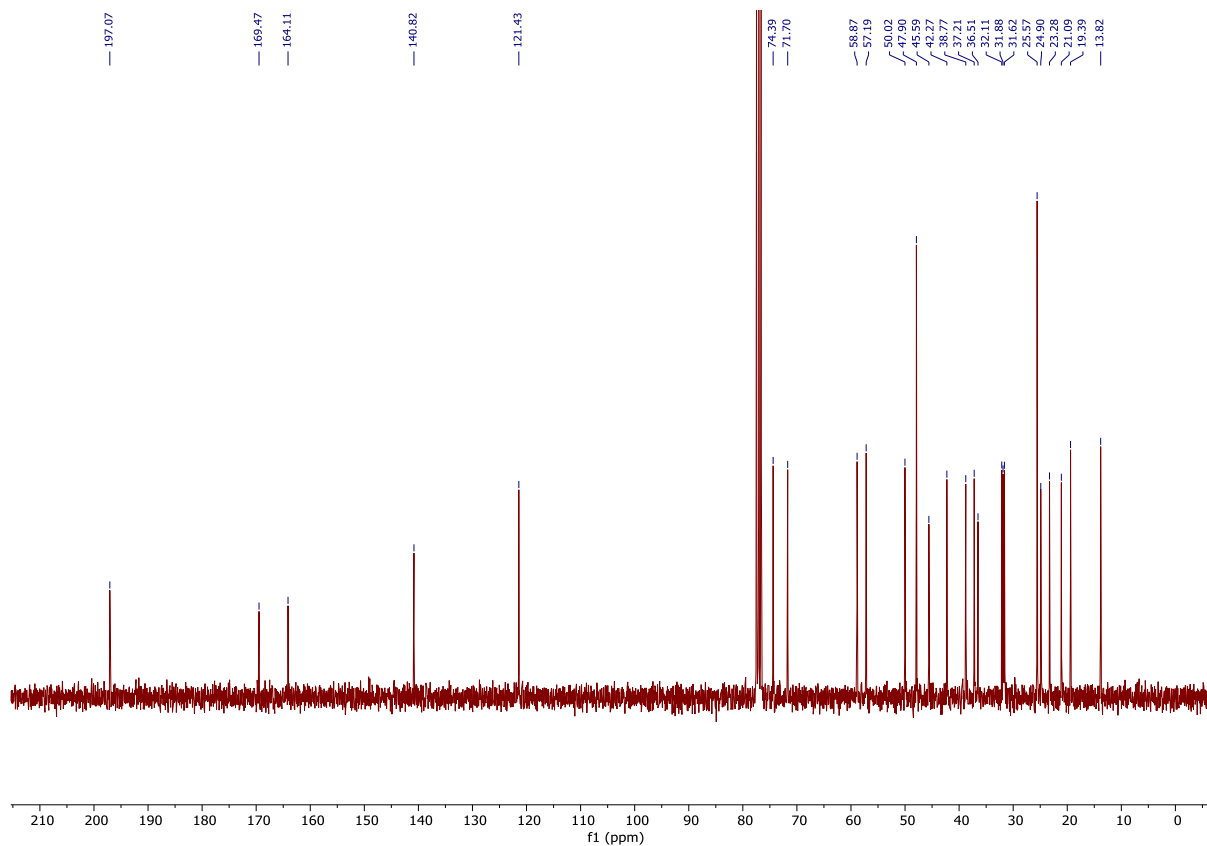
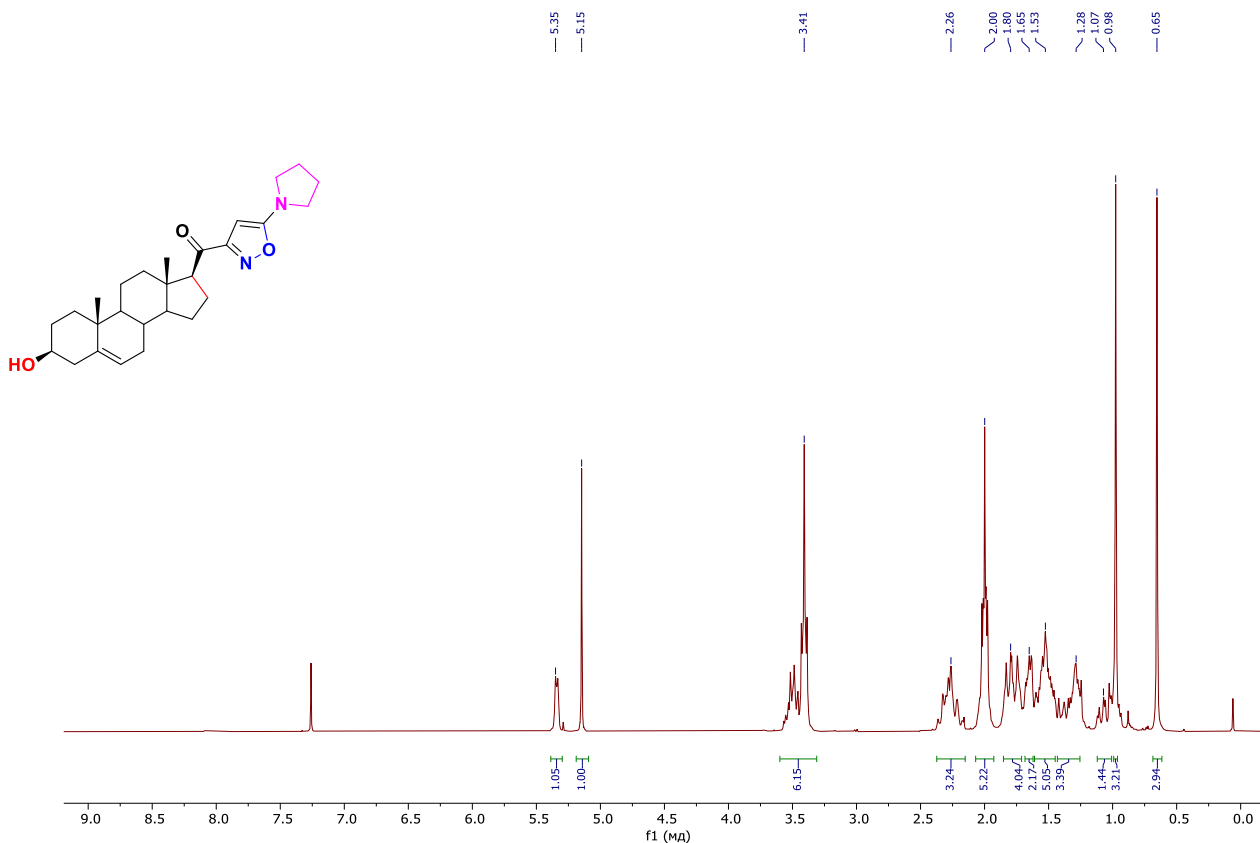
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Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

**Acquisition Parameter**

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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-(5'-pirrolidineisoxazole)-pregna-5-en-3 $\beta$ -ol-20-one (**7f**)



**Analysis Info**

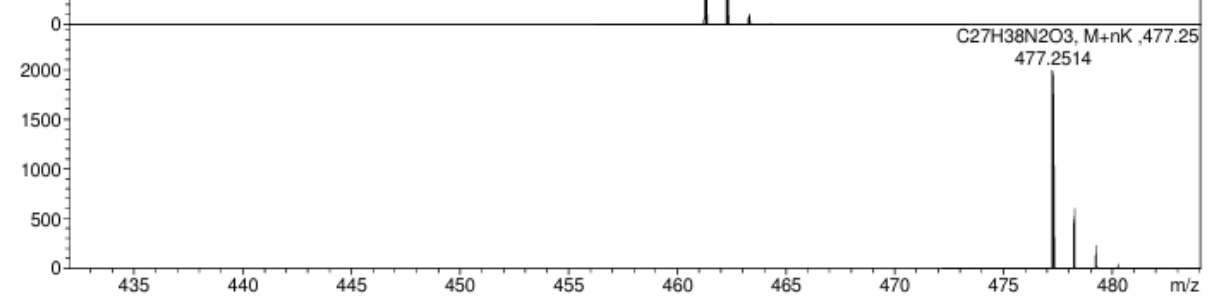
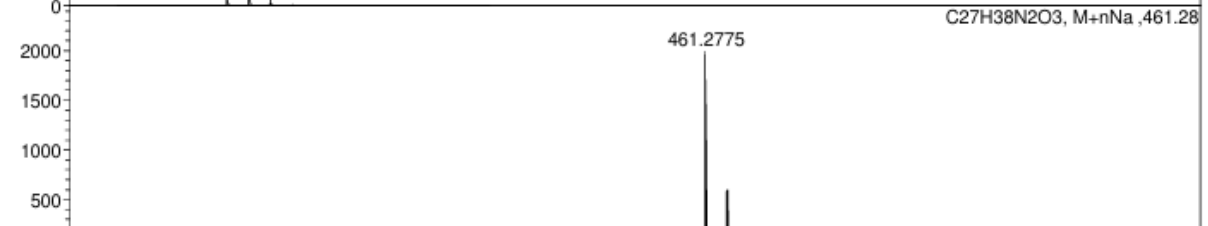
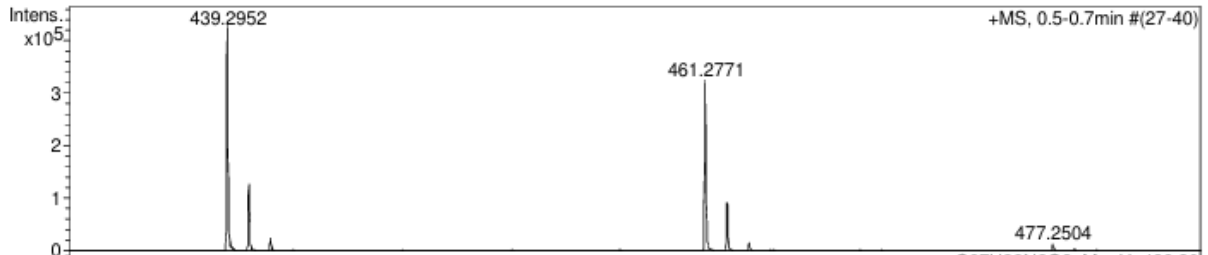
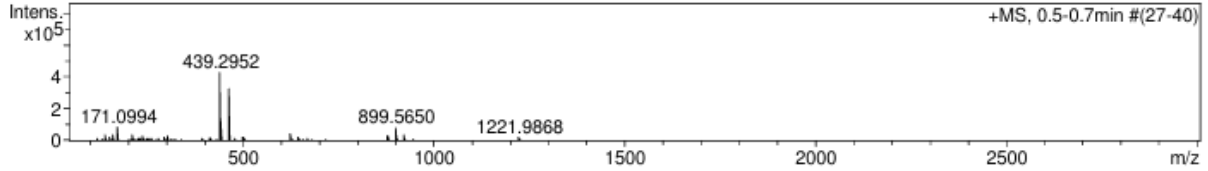
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Acquisition Date 20.03.2024 17:20:44

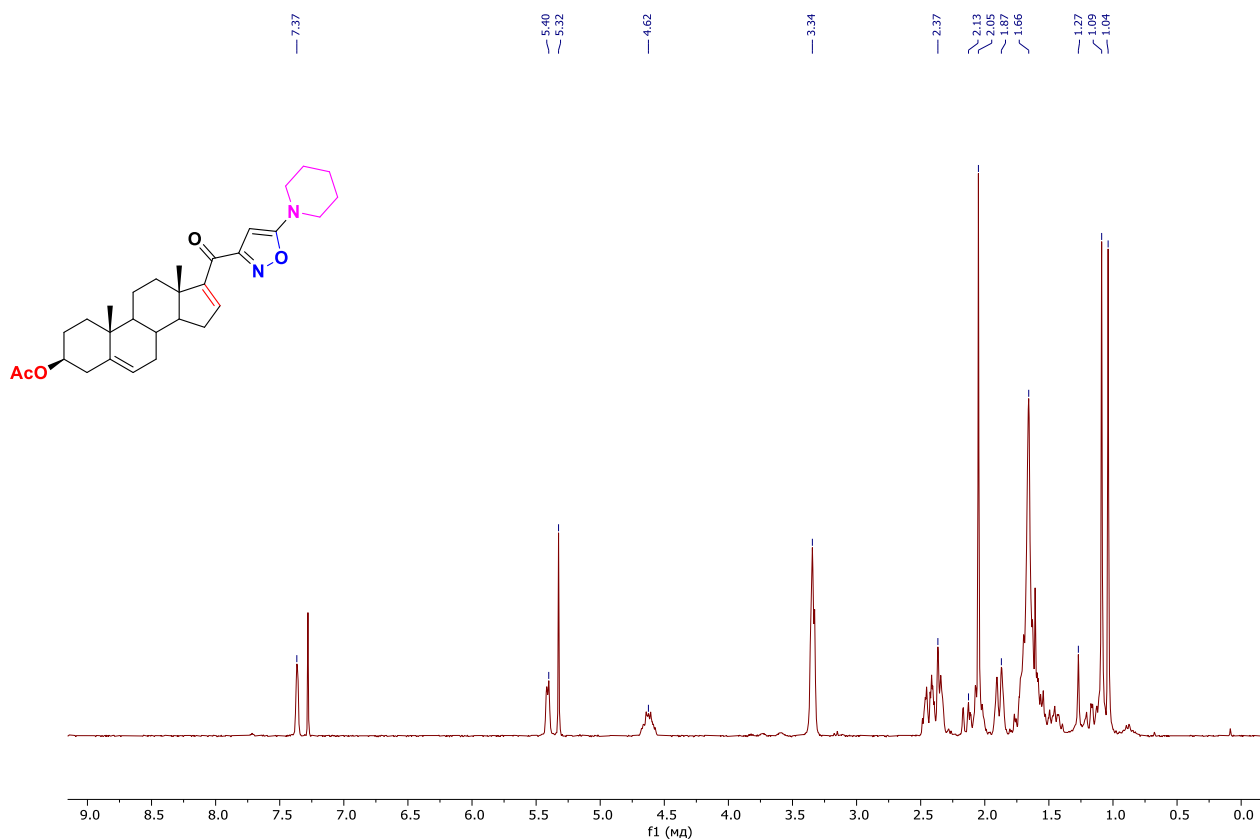
Operator BDAL@DE  
Instrument / Ser# microTOF 10248

**Acquisition Parameter**

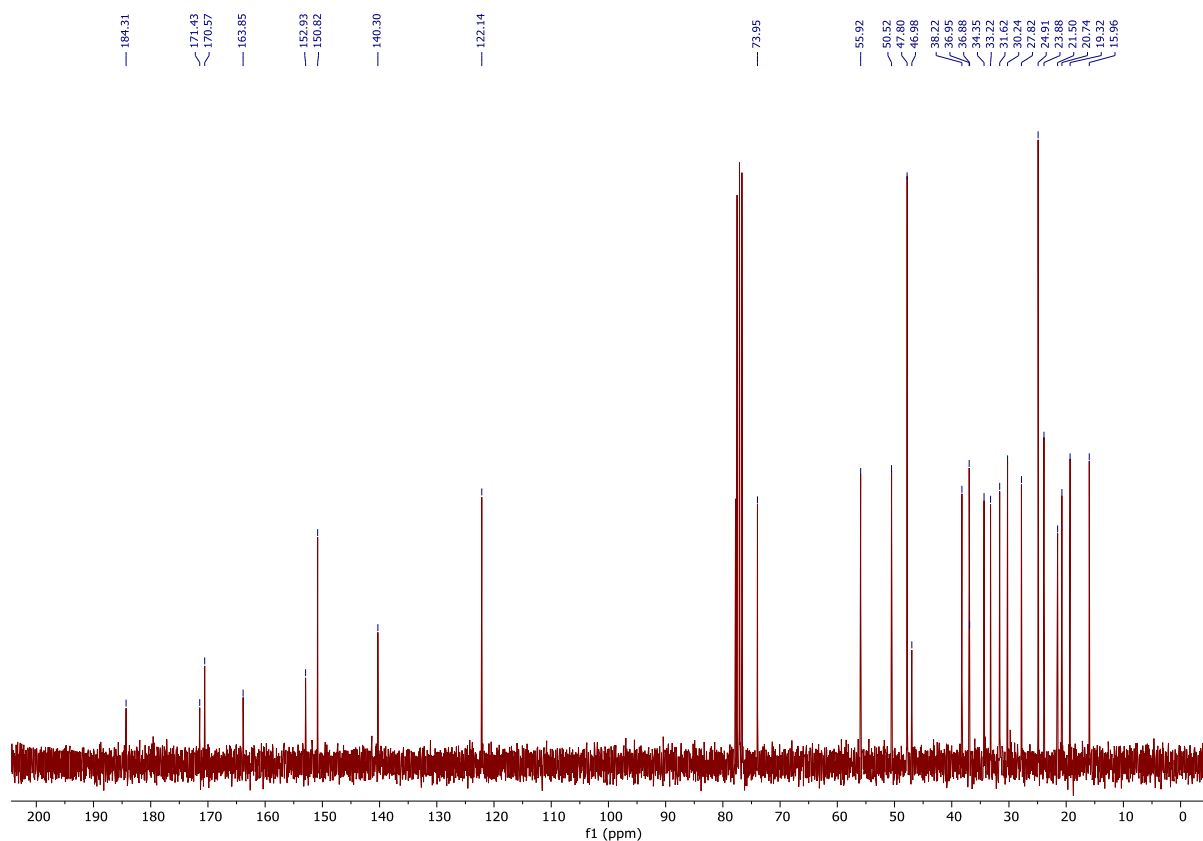
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HRMS (ESI/Q-TOF) of 20-[3`-(5``-pyrrolidine)isoxazole]-pregn-5-en-3β-ol-20-one (**7g**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 20-(5'-piperidine isoxazole)-pregna-5,16-diene-3β-ol-20-one (7h)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 20-(5'-piperidine isoxazole)-pregna-5,16-diene-3β-ol-20-one (7h)

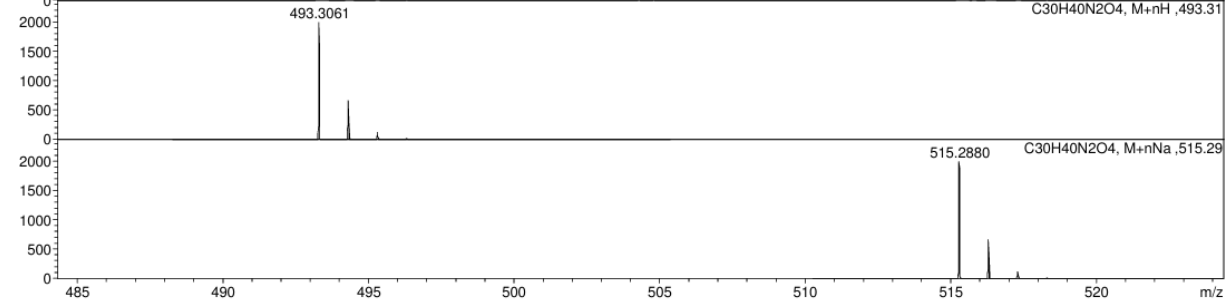
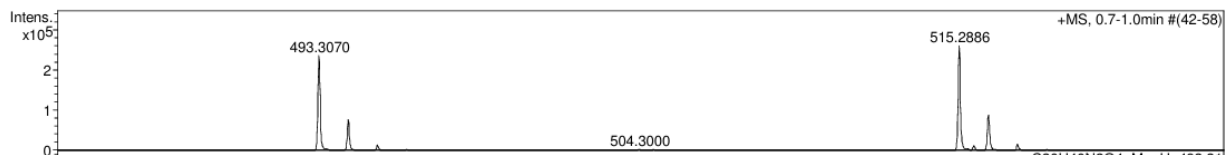
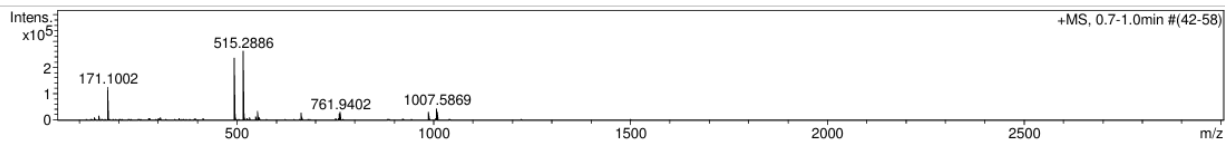
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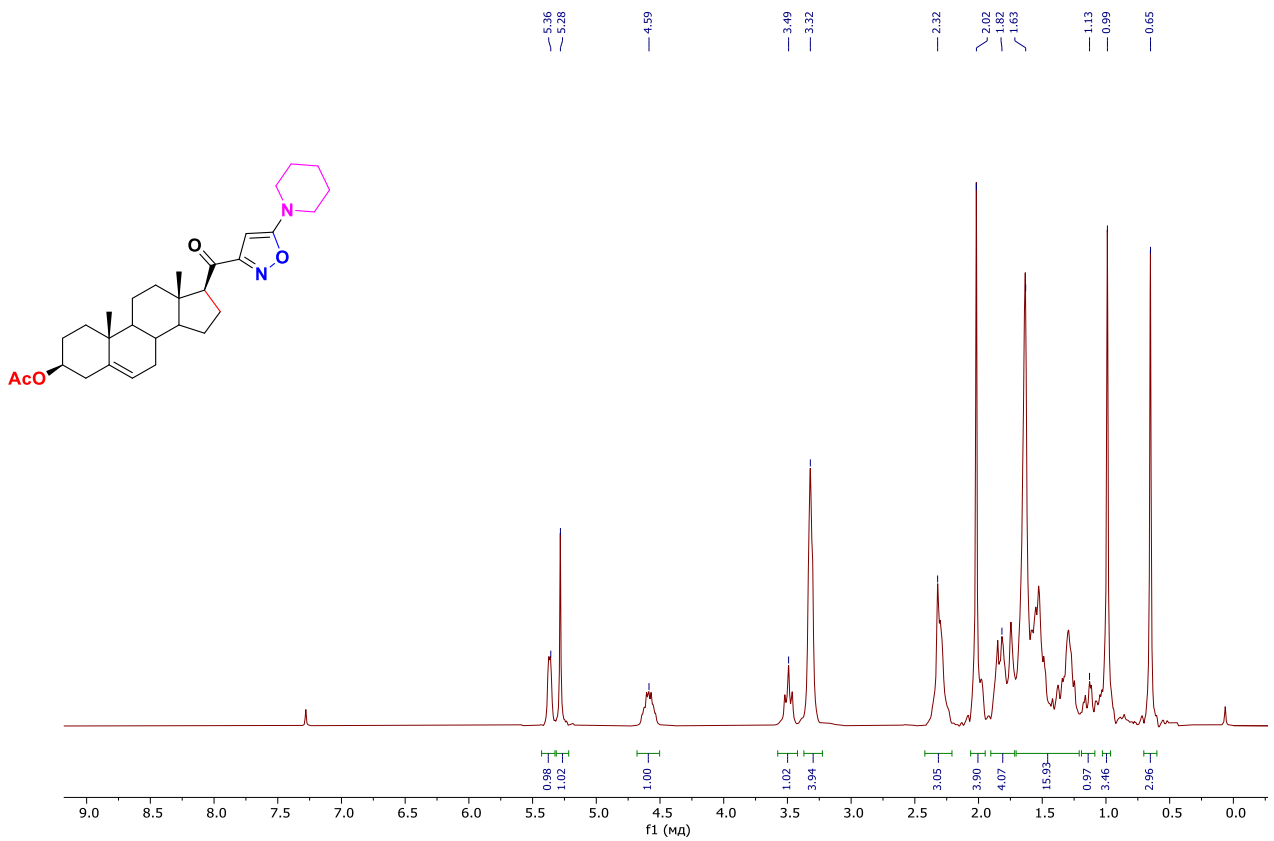
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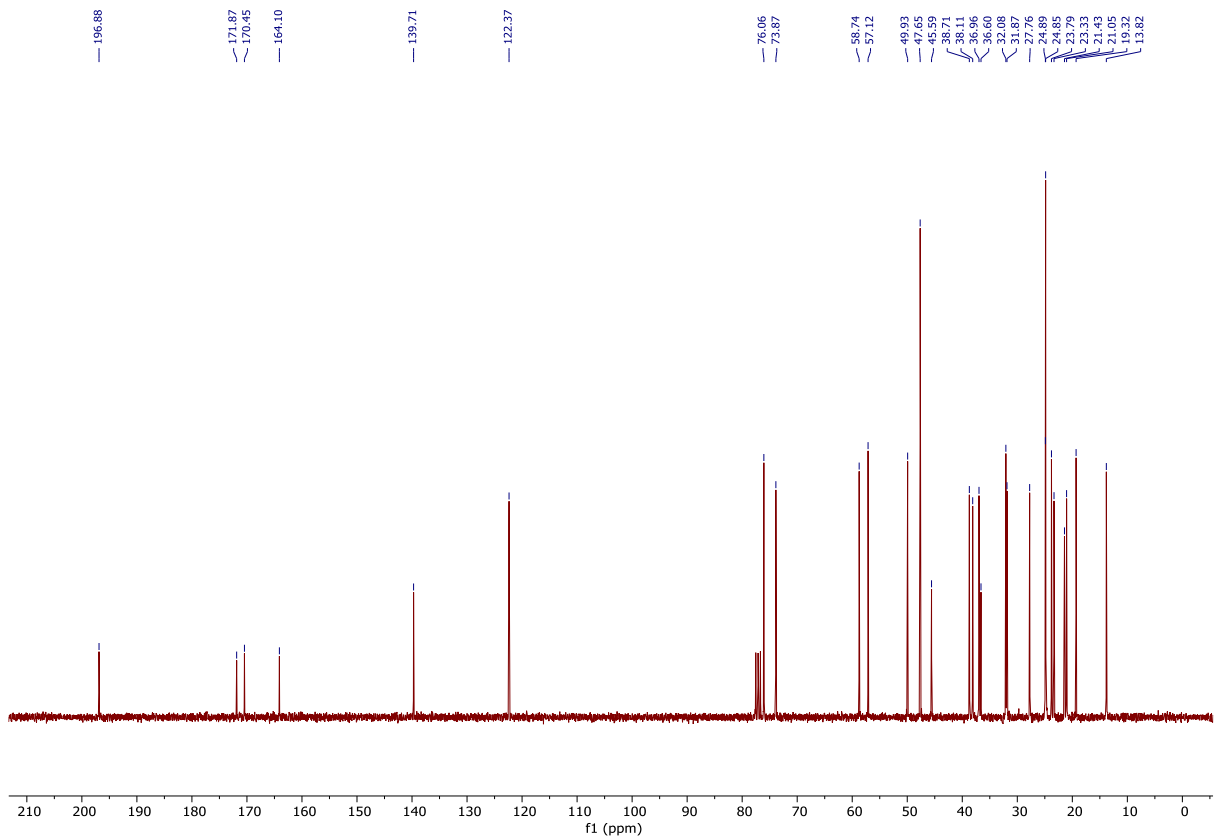
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-(5'-piperidine isoxazole)-pregna-5,16-diene-3 $\beta$ -ol-20-one (**7h**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 20-[5'-piperidine isoxazole]-pregna-5-en-3β-ol-20-one (7i)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 20-[5'-piperidine isoxazole]-pregna-5-en-3β-ol-20-one (7i)

**Analysis Info**

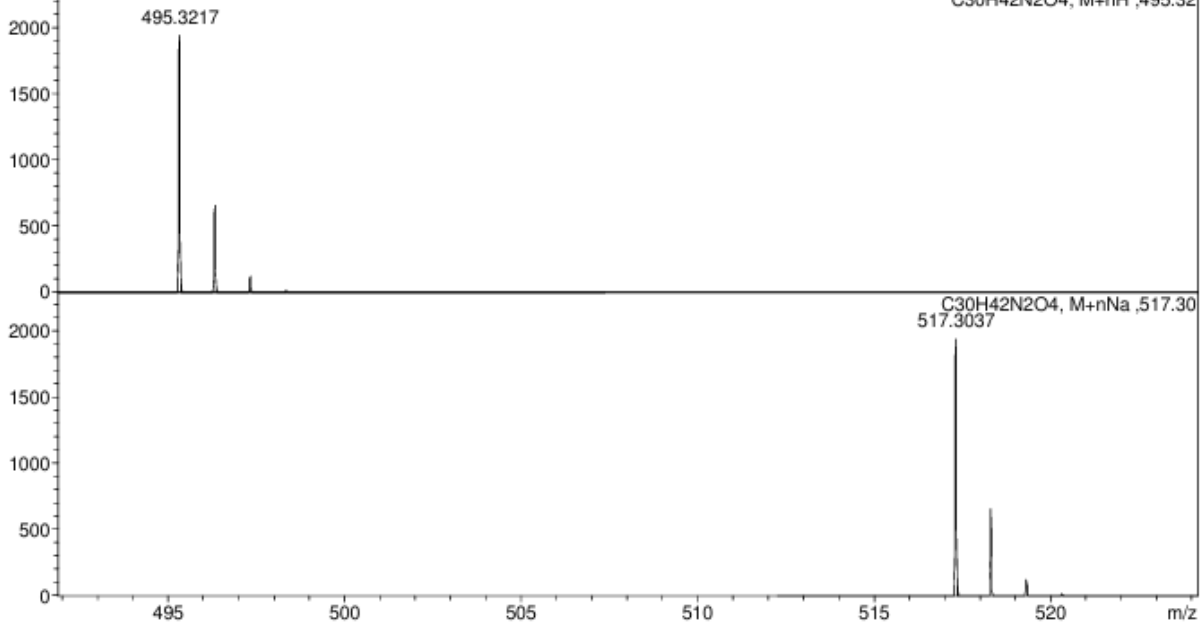
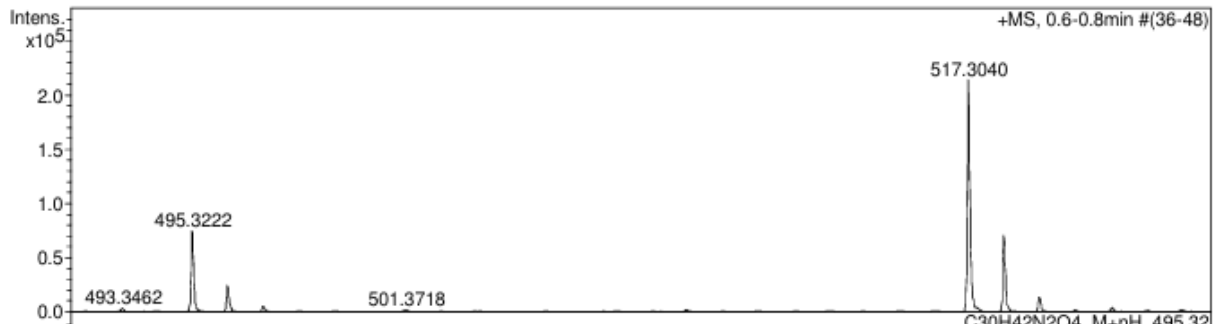
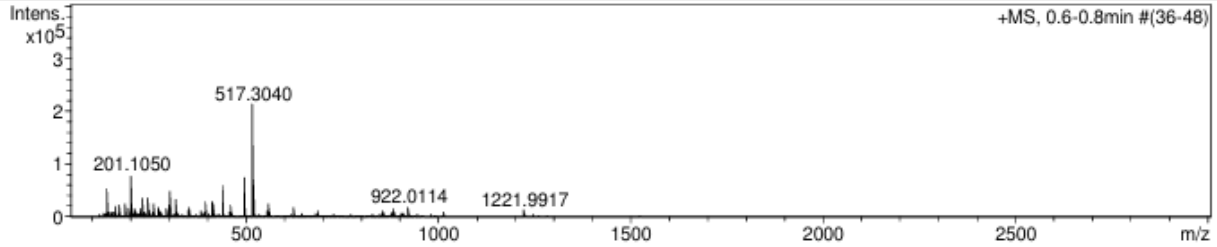
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Acquisition Date 14.12.2023 14:20:39

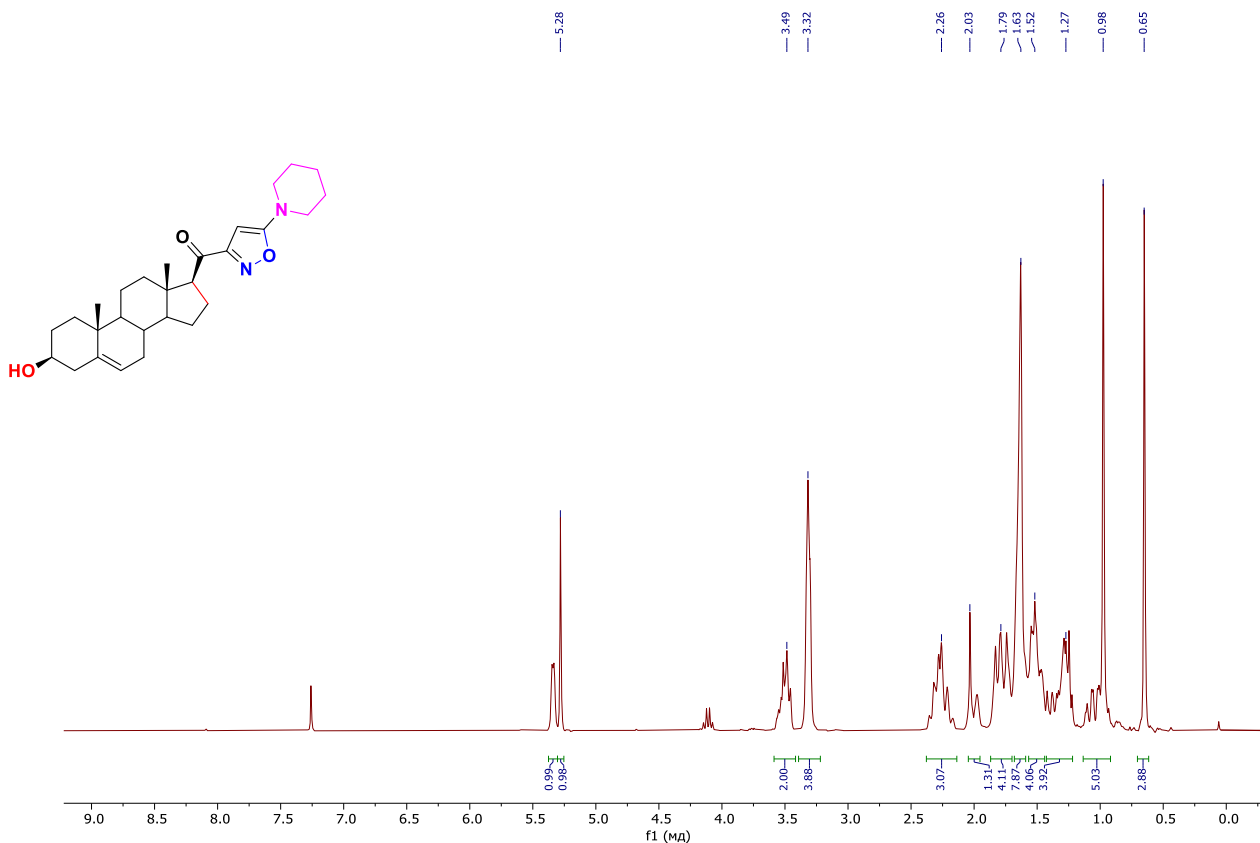
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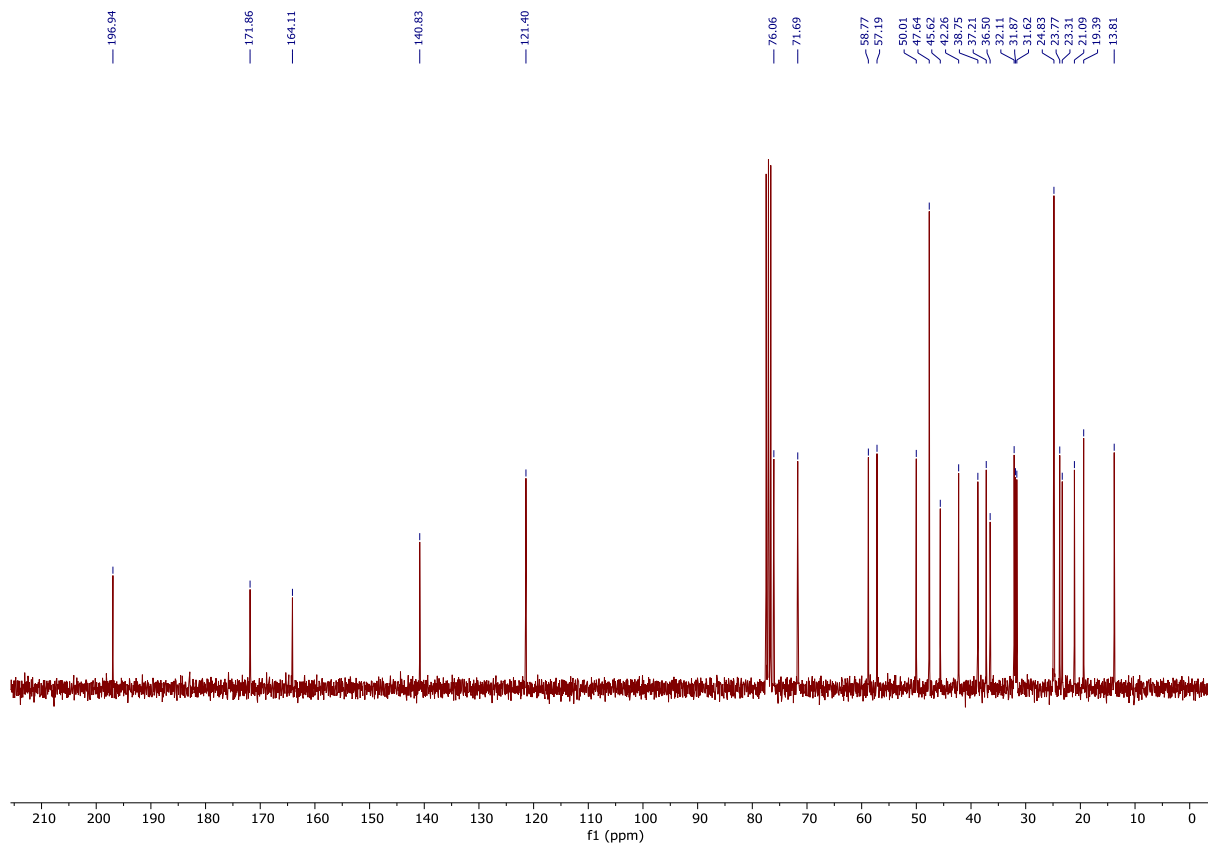
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-[5`-piperidine isoxazole]-pregna-5-en-3 $\beta$ -ol-20-one (7i)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 20-[3'-(5''-piperidine)isoxazole]-pregn-5-en-3β-ol-20-one (7j)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 20-[3'-(5''-piperidine)isoxazole]-pregn-5-en-3β-ol-20-one (7j)

**Analysis Info**

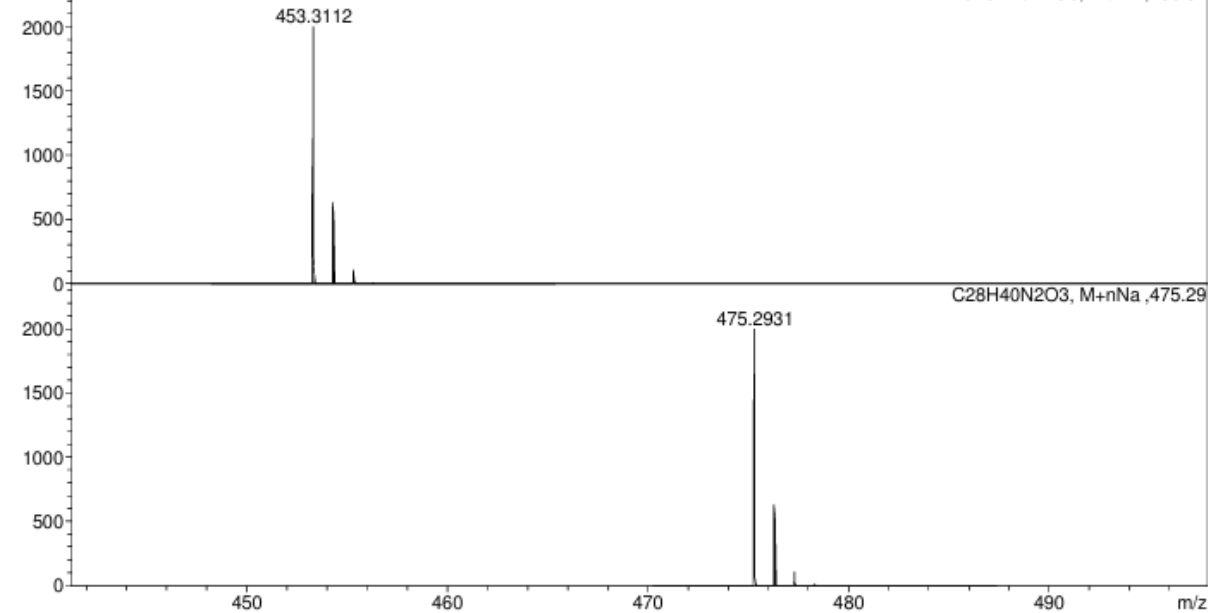
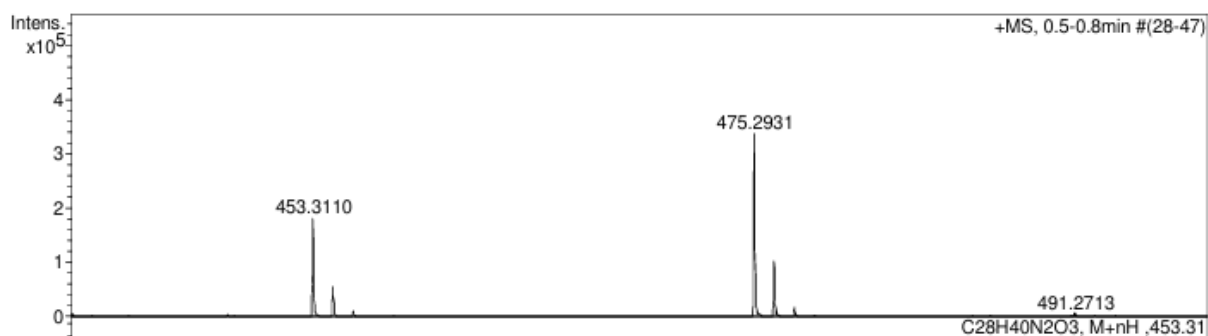
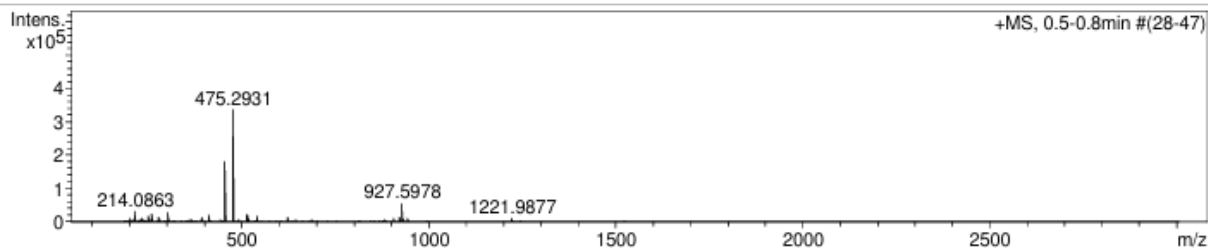
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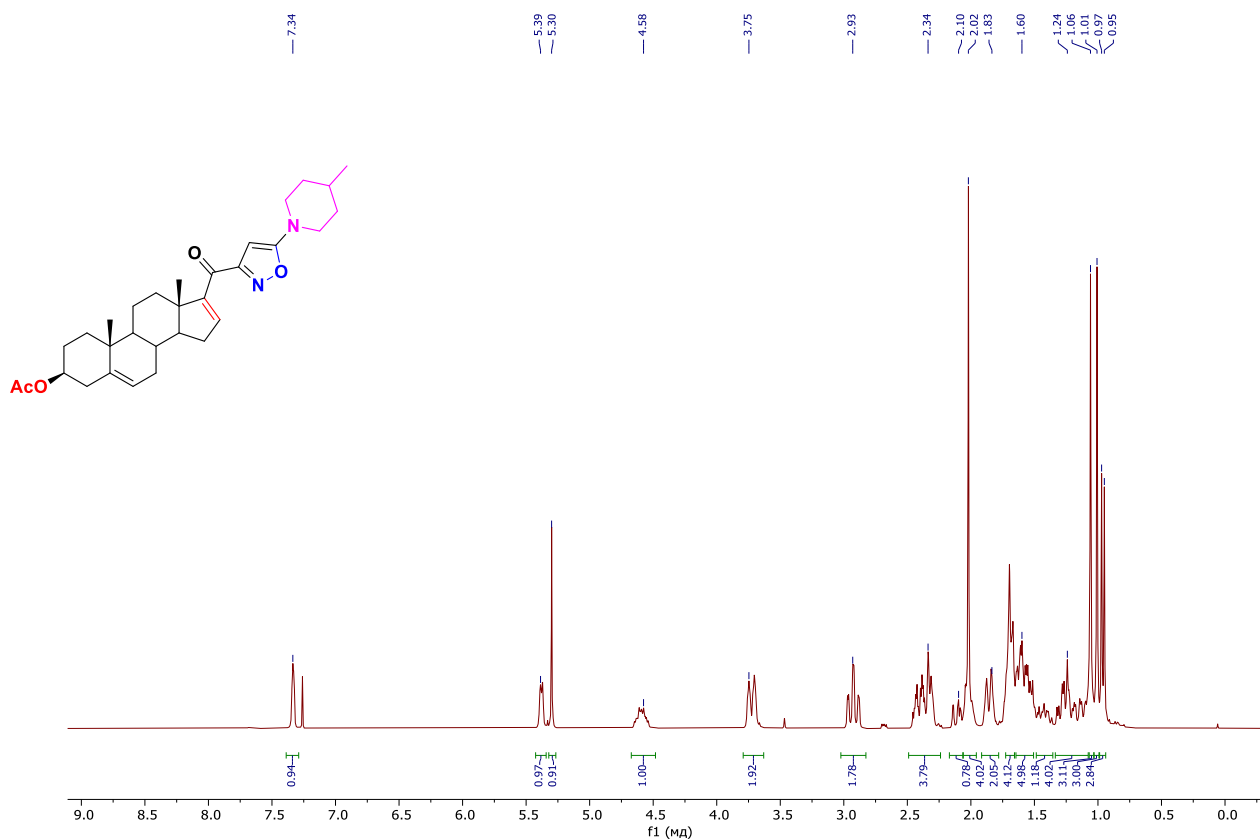
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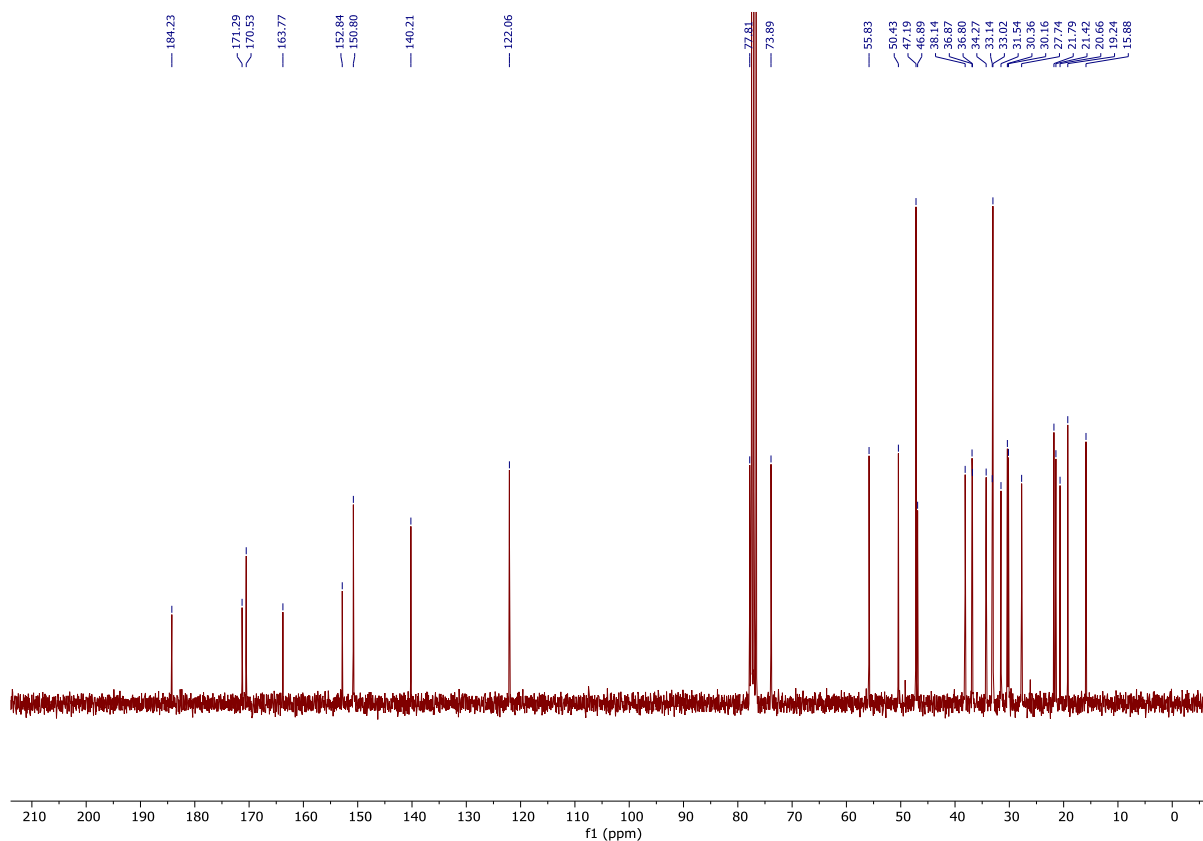
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HRMS (ESI/Q-TOF) of 20-[3'-(5''-piperidine)isoxazole]-pregn-5-en-3β-ol-20-one (7j)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 20-[5'-(4''-methylpiperidine)isoxazole]-pregna-5,16-diene-3β-ol-20-one (7k)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 20-[5'-(4''-methylpiperidine)isoxazole]-pregna-5,16-diene-3β-ol-20-one (7k)

**Analysis Info**

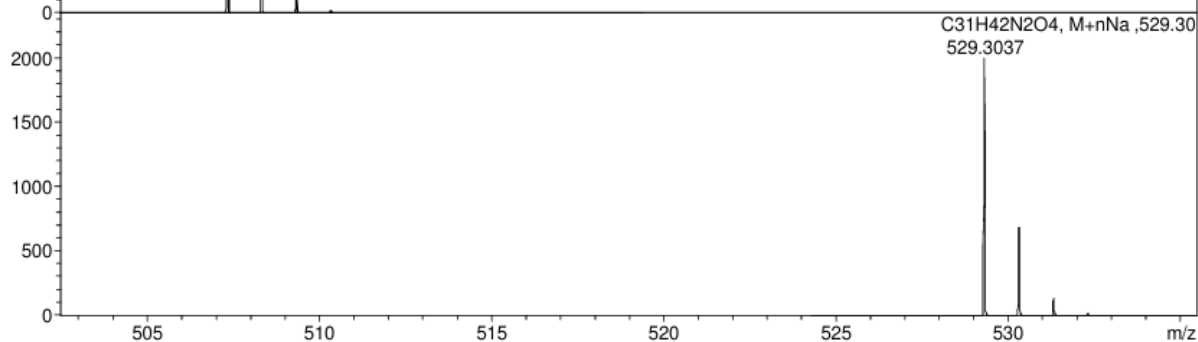
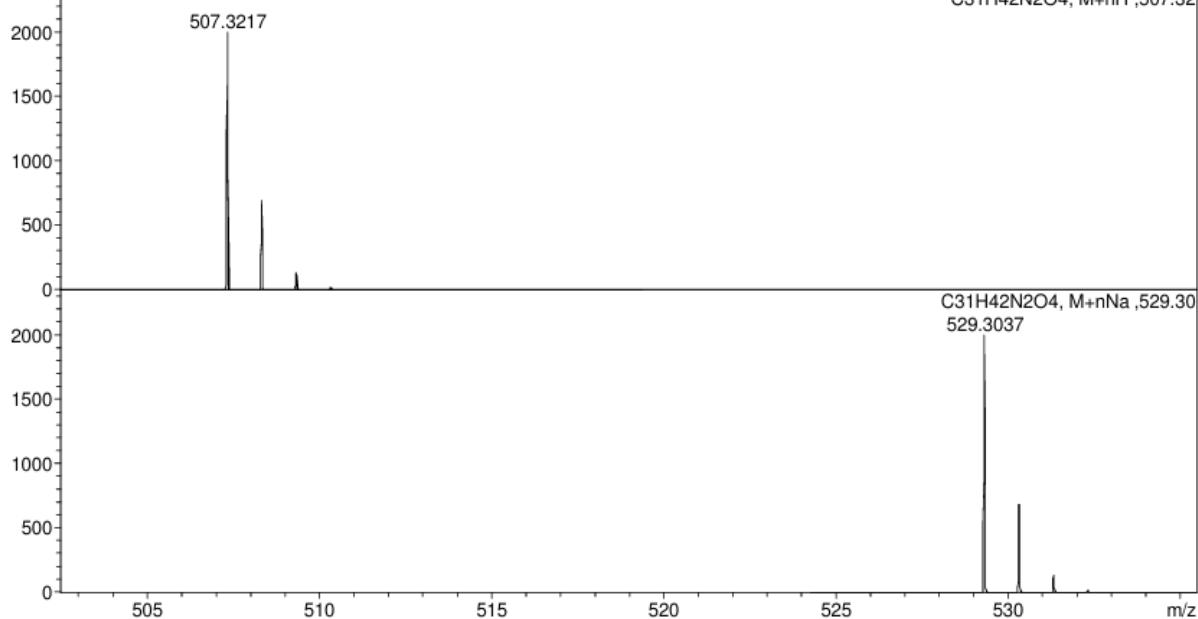
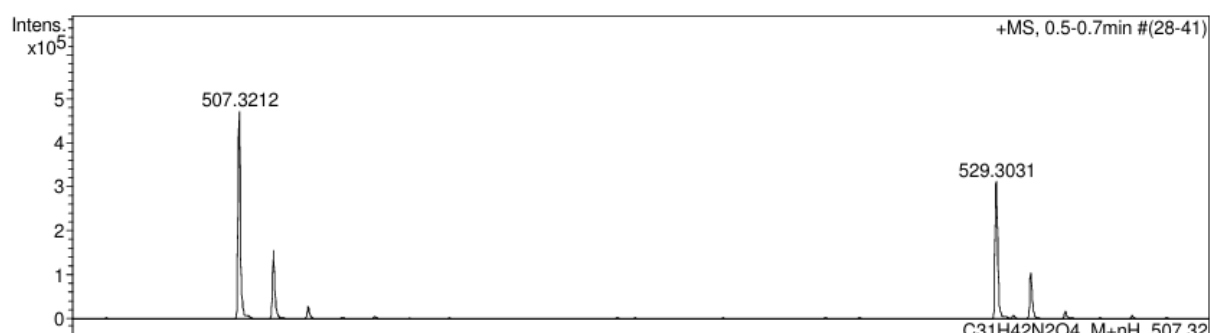
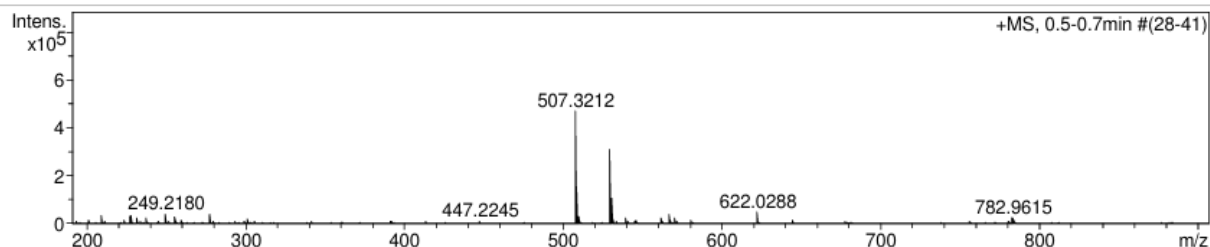
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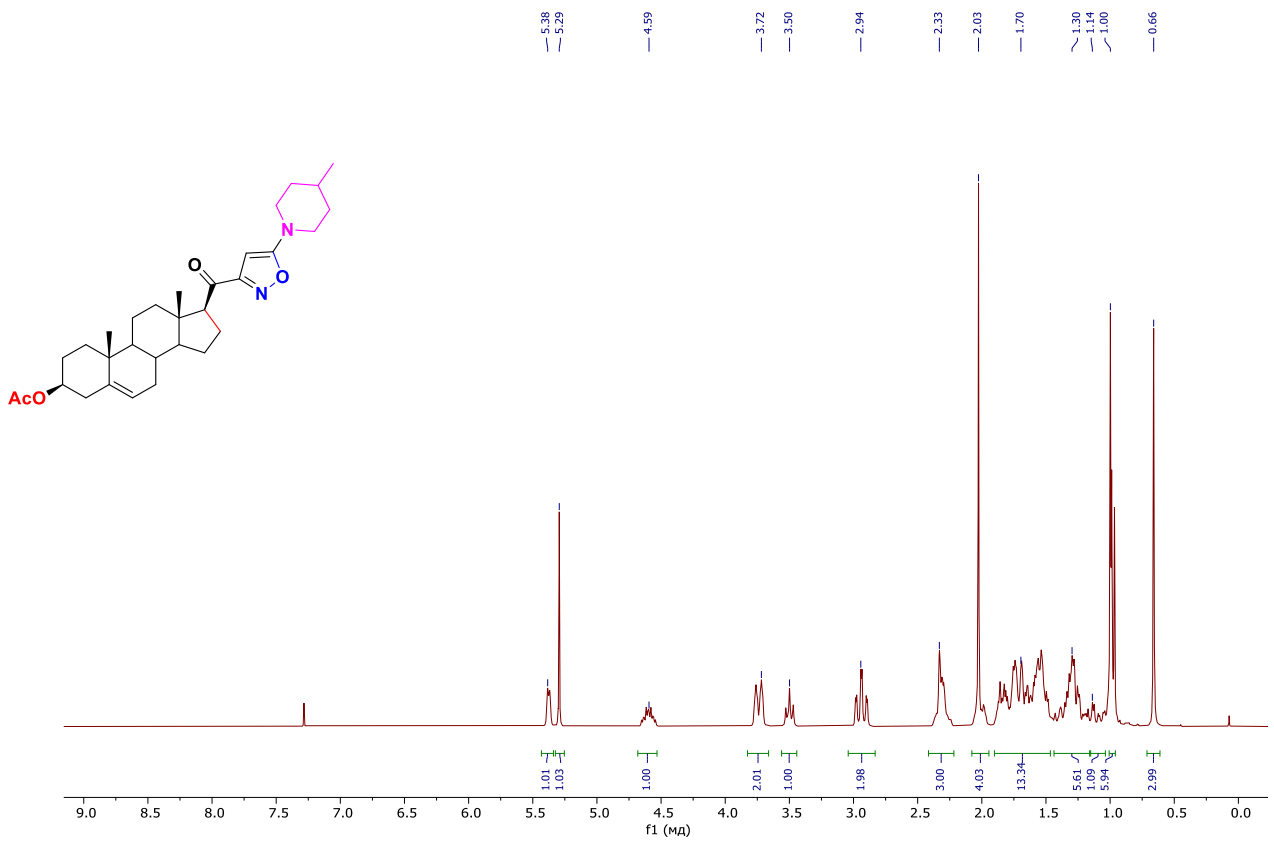
Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

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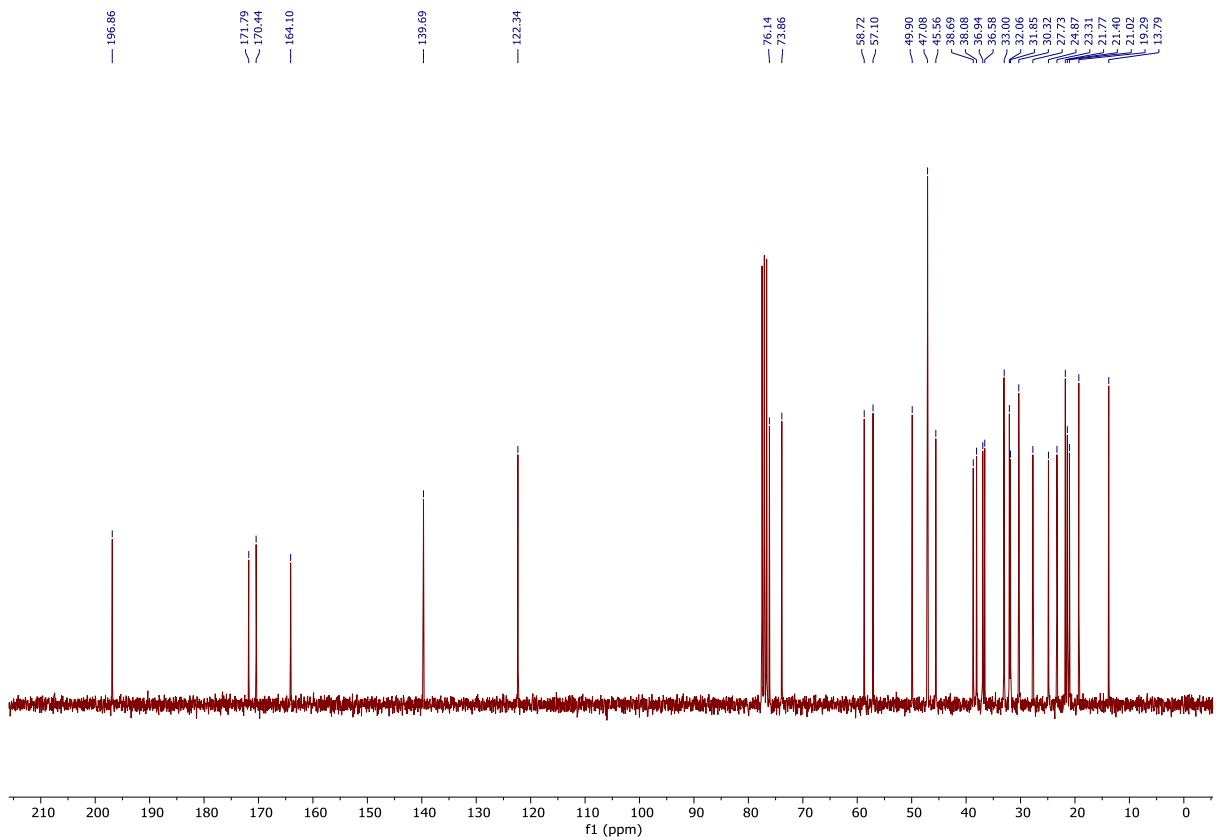
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-[5`-(4``-methylpiperidine)isoxazole]-pregna-5,16-diene-3β-ol-20-one (**7k**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 20-[5'-(4''-methylpiperidine)isoxazole]-pregna-5-en-3β-ol-20-one (71)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 20-[5'-(4''-methylpiperidine)isoxazole]-pregna-5-en-3β-ol-20-one (71)

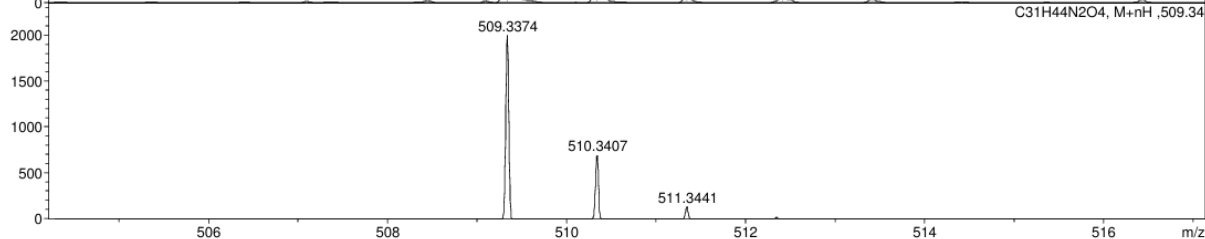
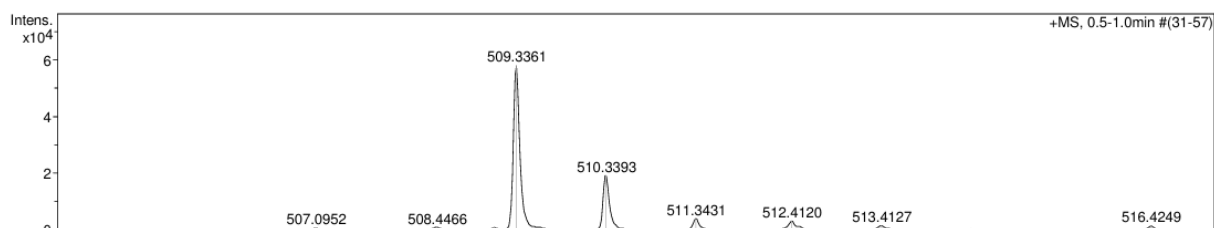
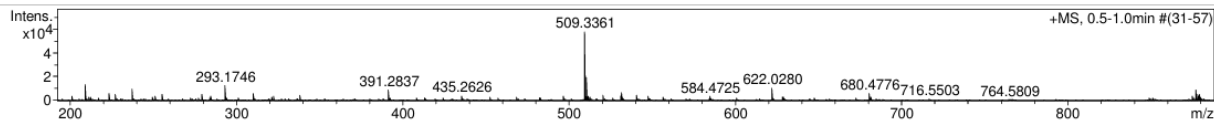
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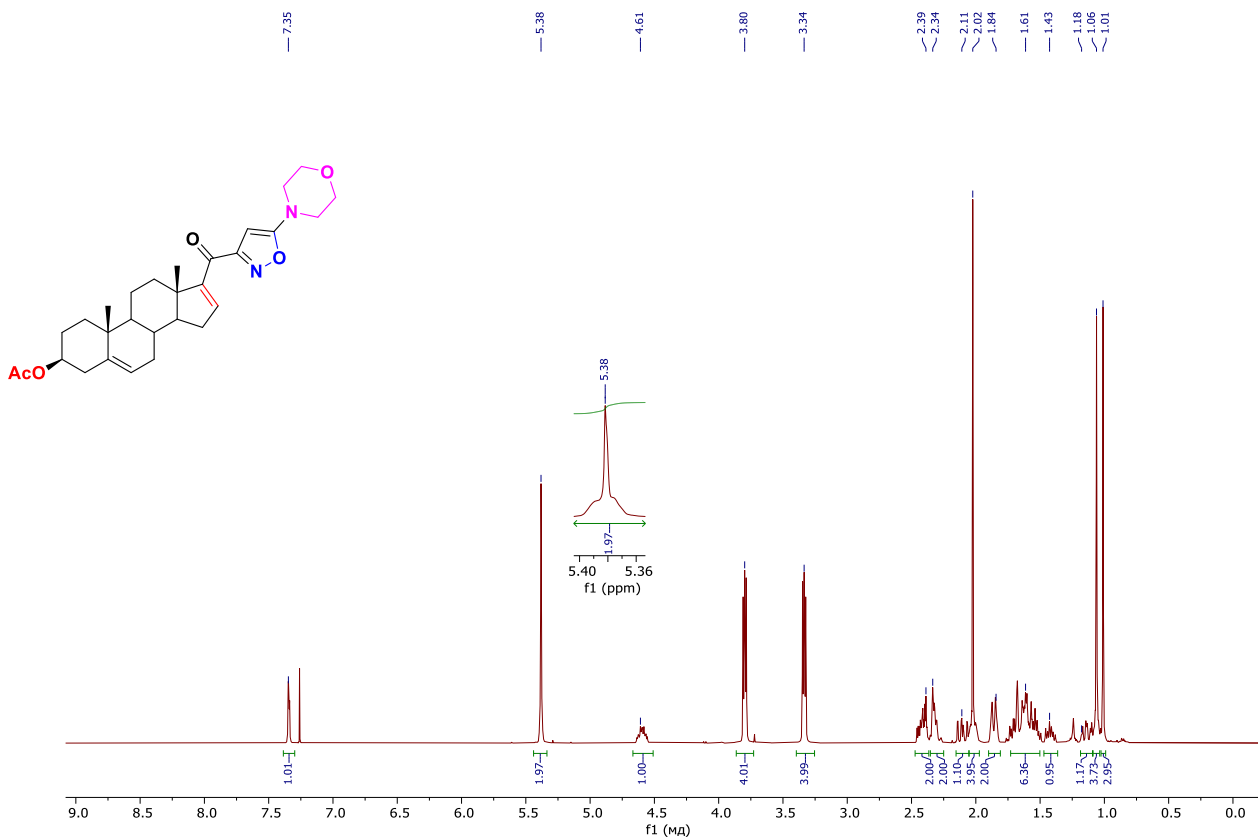
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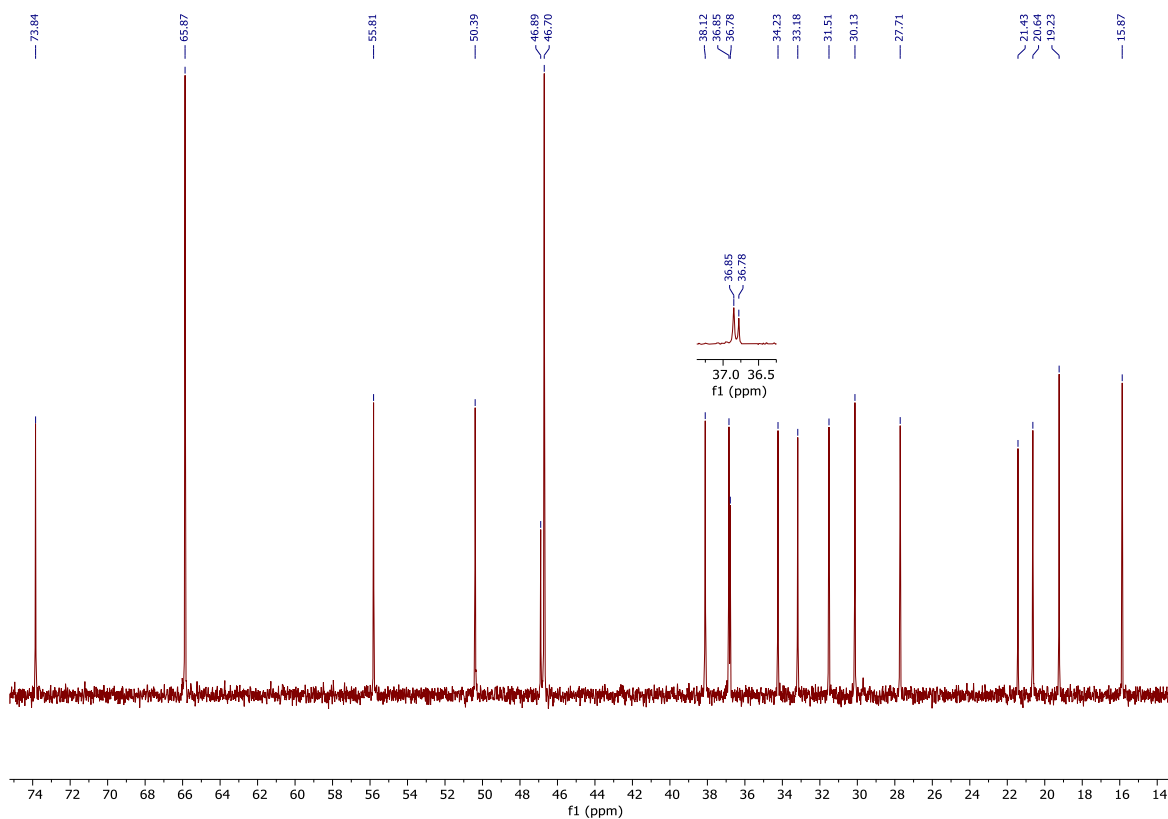
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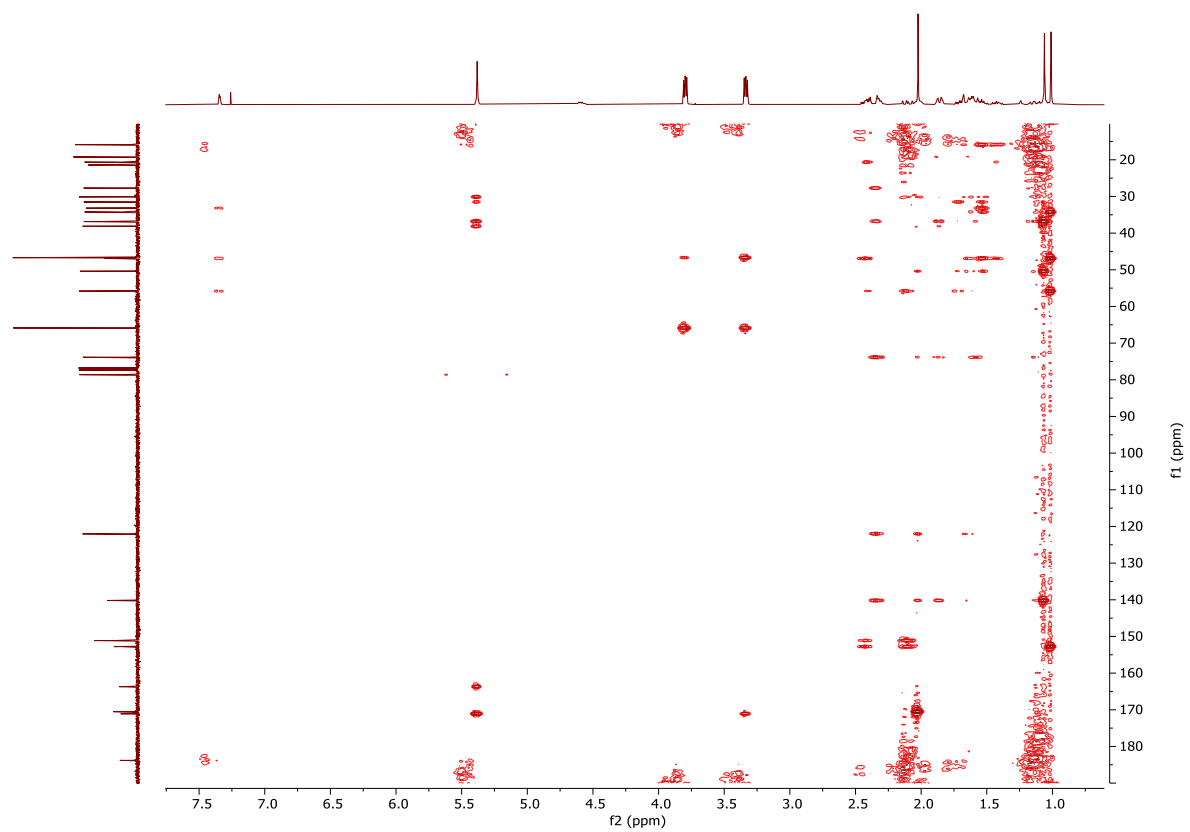
HRMS (ESI/Q-TOF) of 3-acetate 20-[5`-(4``-methylpiperidine)isoxazole]-pregna-5-en-3 $\beta$ -ol-20-one (**71**)



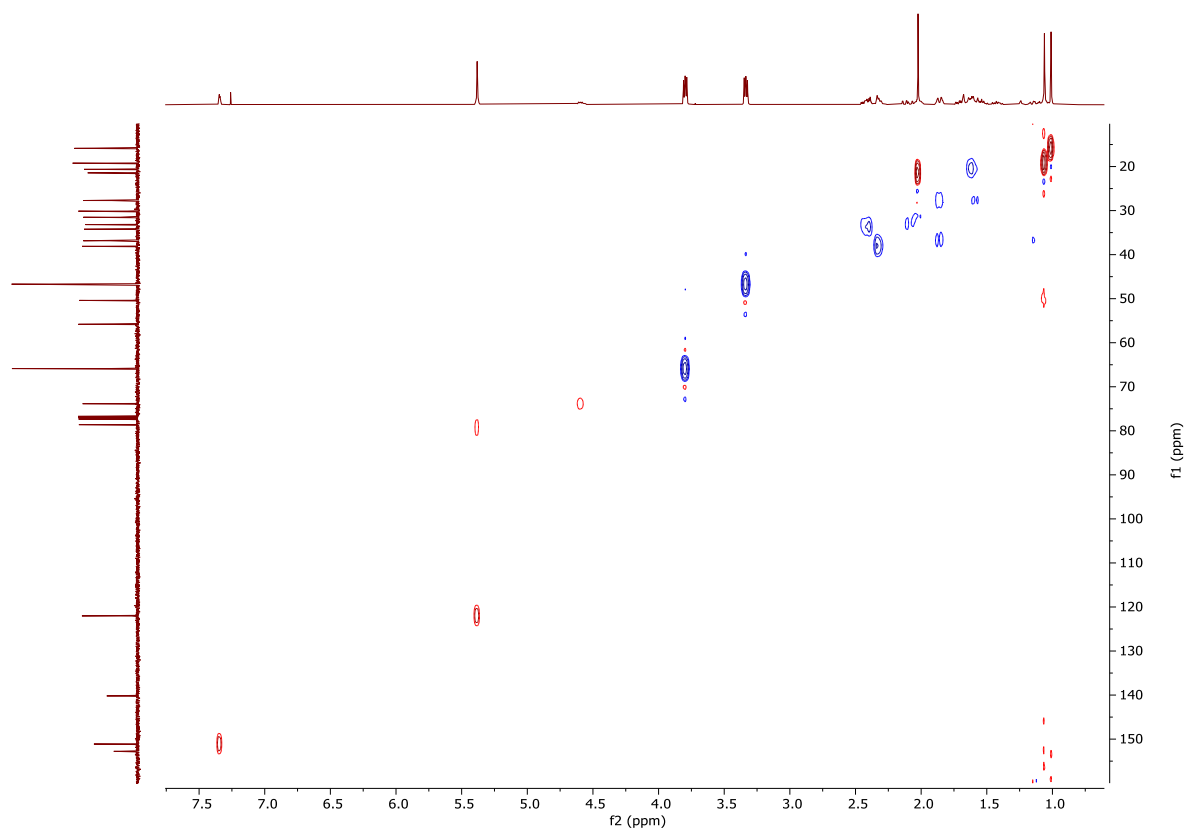
$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz) spectrum of 3-acetate 20-[3'-(5''-morpholine)isoxazole]-pregn-5,16-dien-3β-ol-20-one (**7m**)



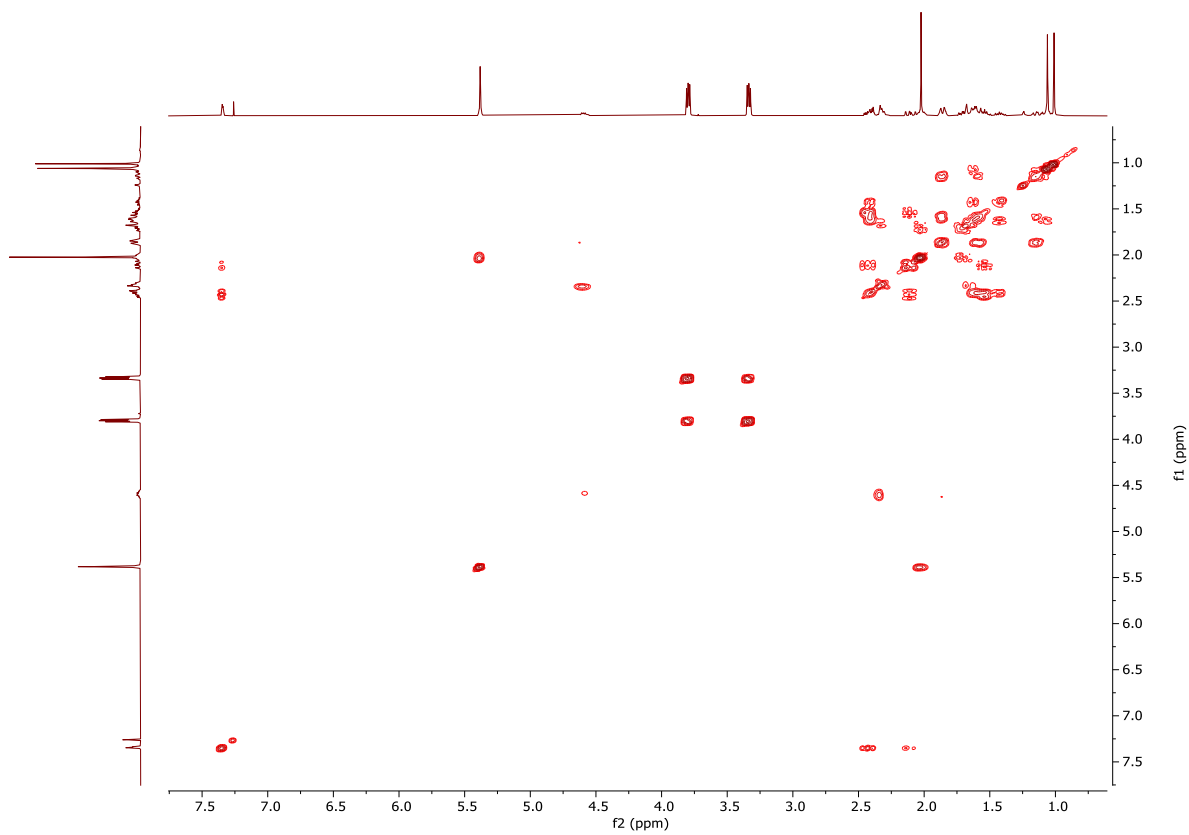
$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz) spectrum of 3-acetate 20-[3'-(5''-morpholine)isoxazole]-pregn-5,16-dien-3β-ol-20-one (**7m**)



HMBC spectrum of 3-acetate 20-[3`-(5``-morpholine)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (**7m**)



HSQC spectrum of 3-acetate 20-[3`-(5``-morpholine)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (**7m**)



COSY spectrum of 3-acetate 20-[3`-(5``-morpholine)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (**7m**)

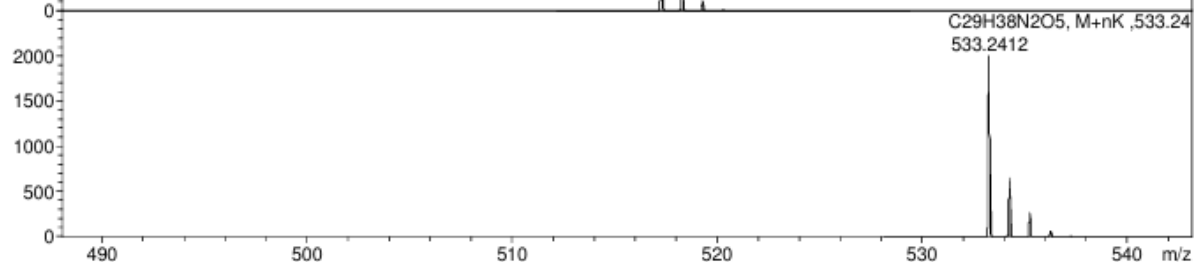
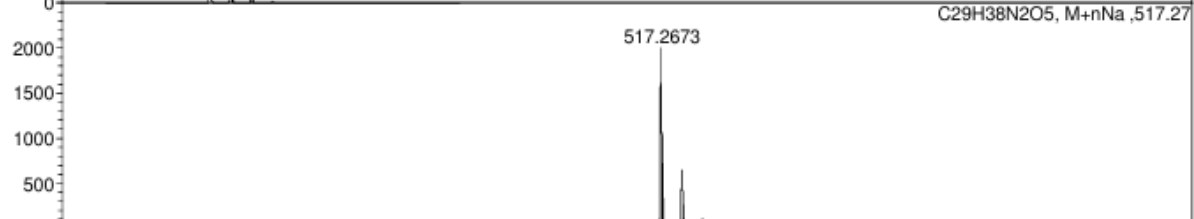
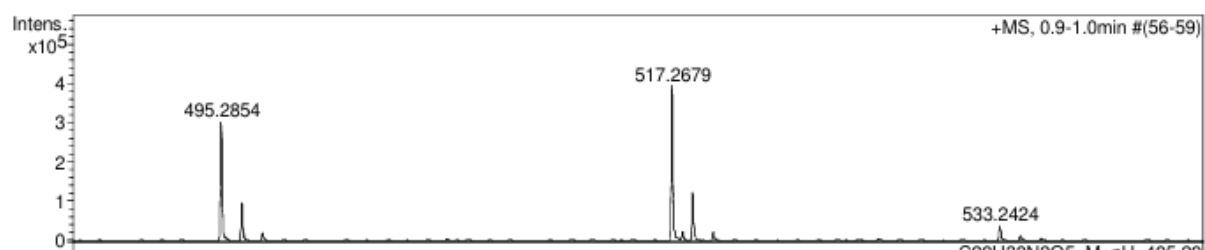
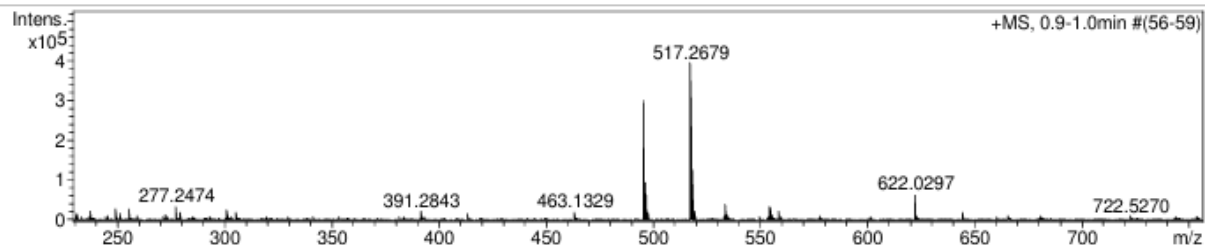
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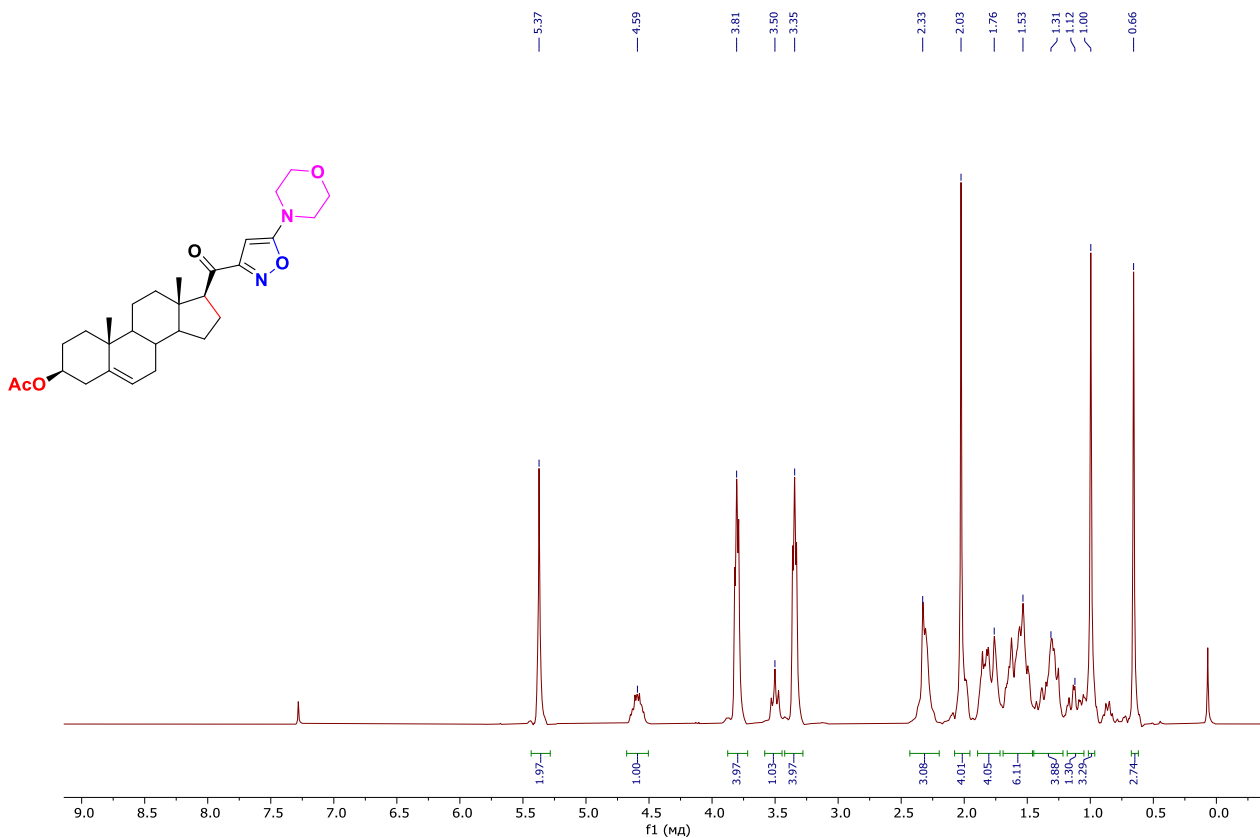
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Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

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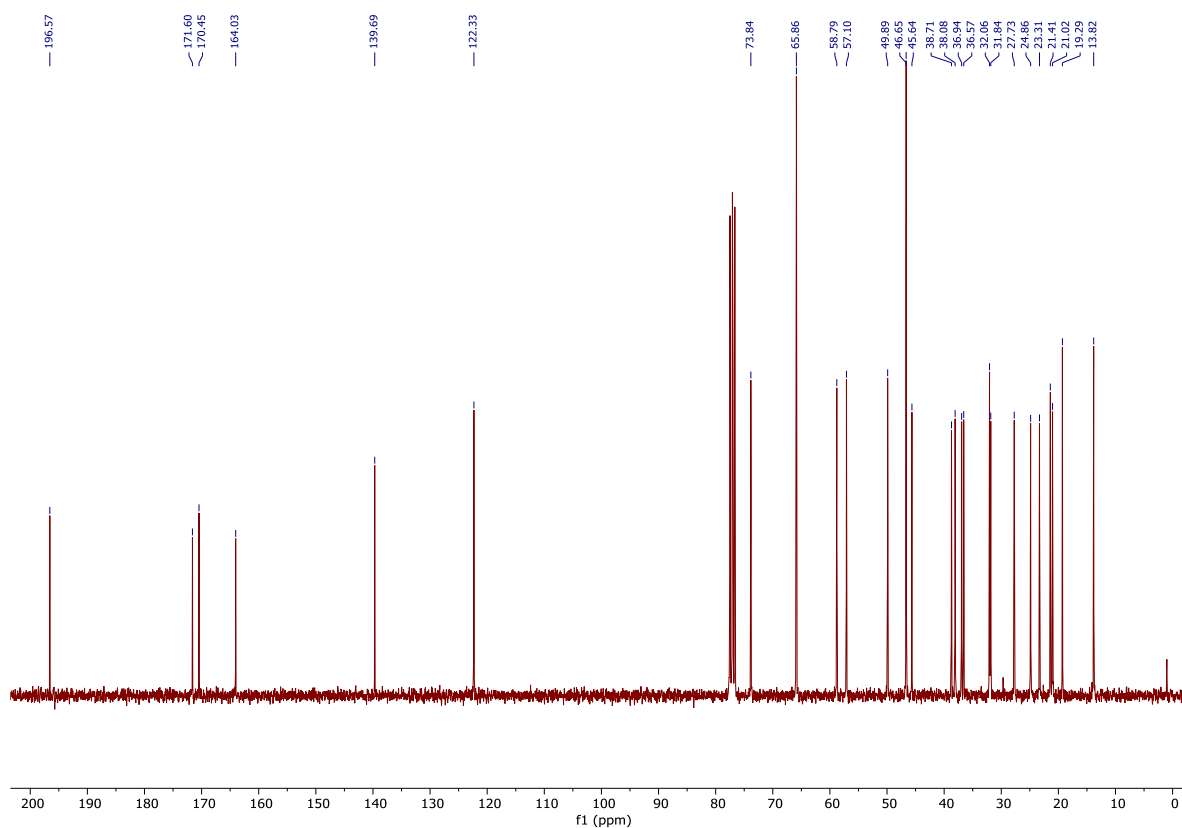
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-[3'-(5''-morpholine)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (**7m**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 20-(5'-morpholineisoxazole)-pregna-5-en-3β-ol-20-one (7n)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 20-(5'-morpholineisoxazole)-pregna-5-en-3β-ol-20-one (7n)

**Analysis Info**

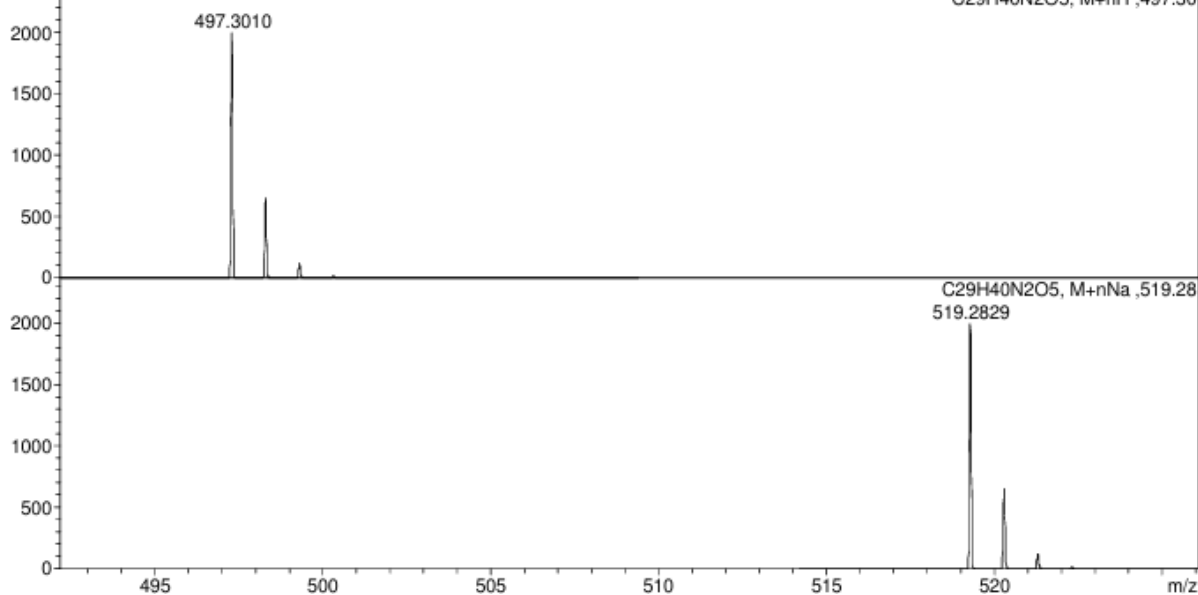
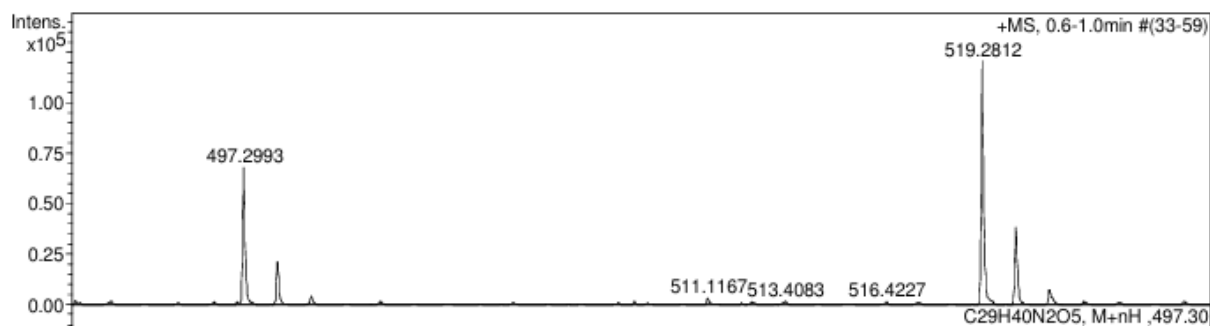
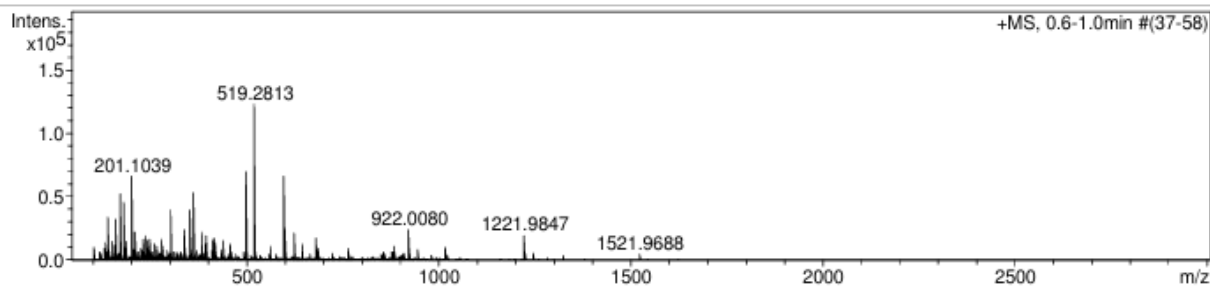
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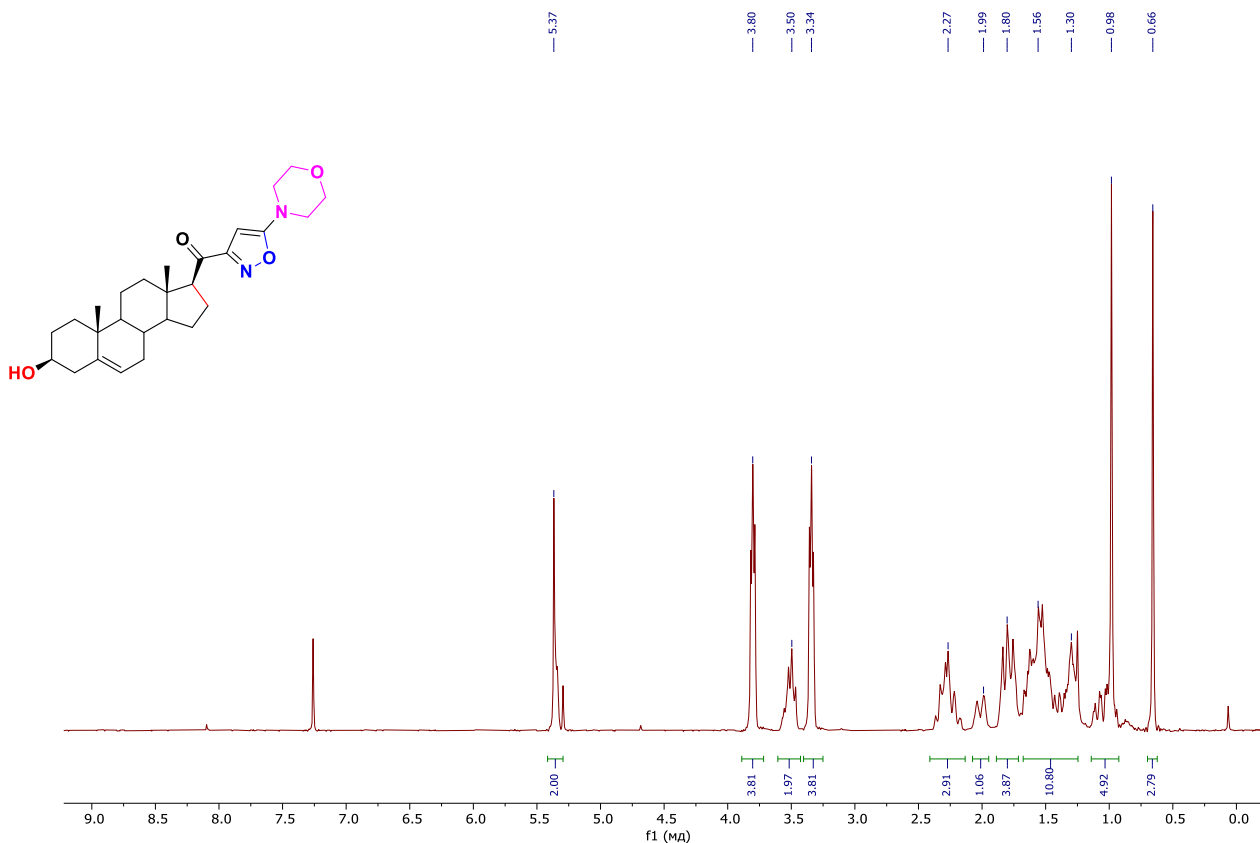
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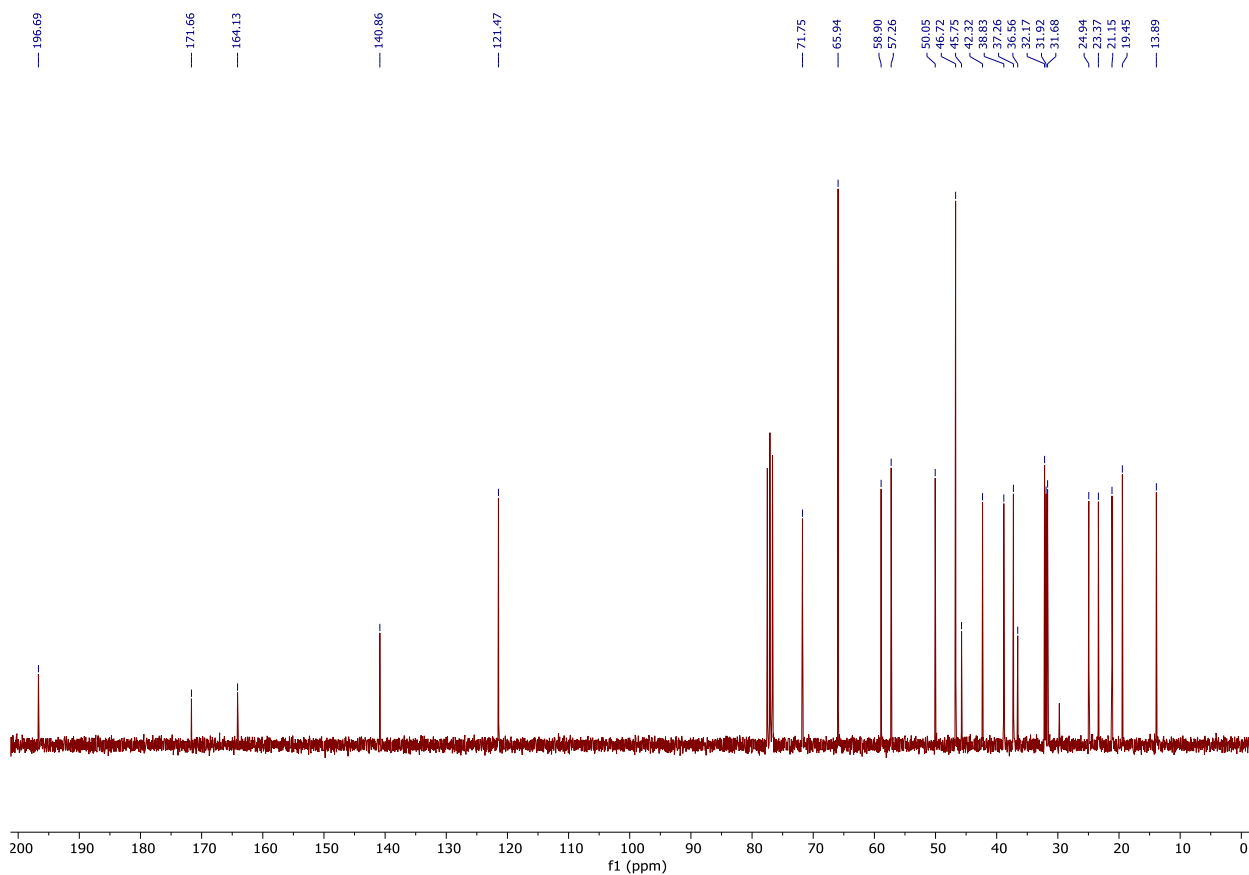
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HRMS (ESI/Q-TOF) of 3-acetate 20-(5'-moroholineisoxazole)-pregna-5-en-3 $\beta$ -ol-20-one (**7n**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 20-[3'-(5''-morpholine)isoxazole]-pregn-5-en-3β-ol-20-one (7o)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 20-[3'-(5''-morpholine)isoxazole]-pregn-5-en-3β-ol-20-one (7o)

**Analysis Info**

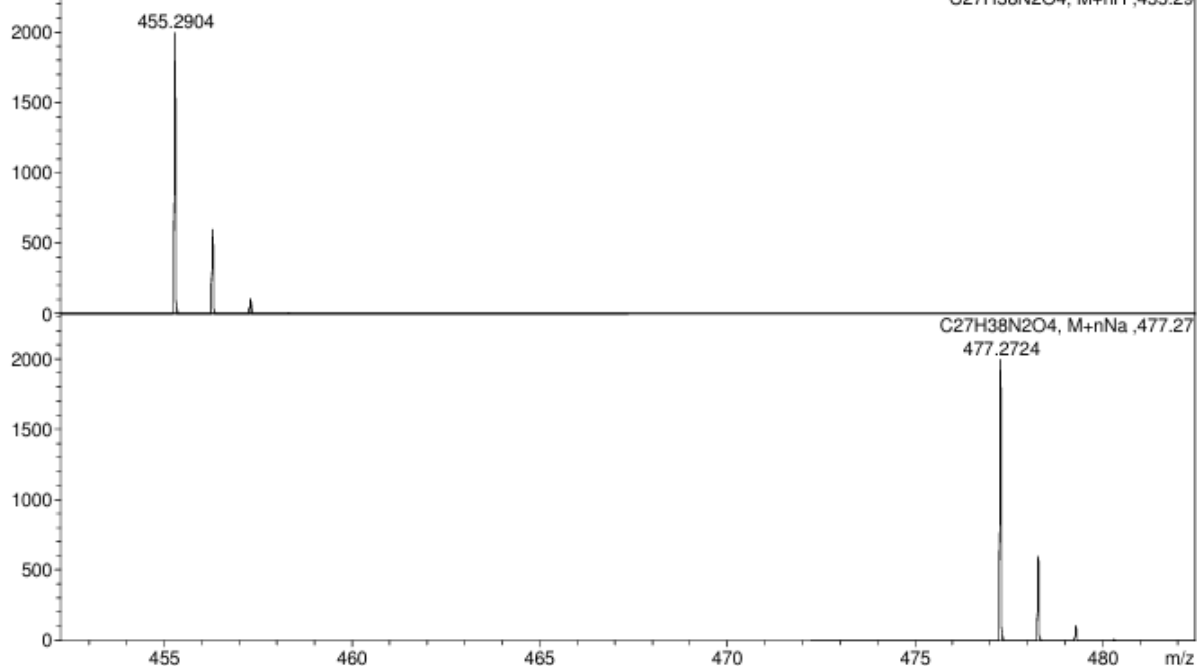
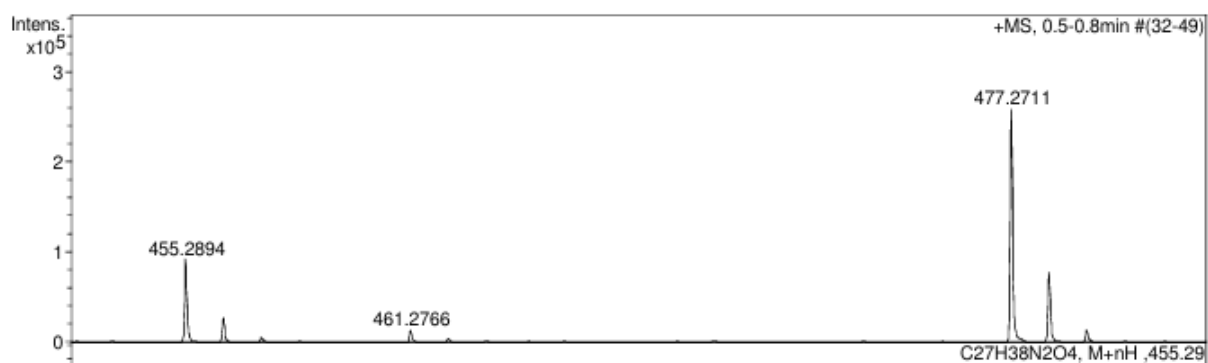
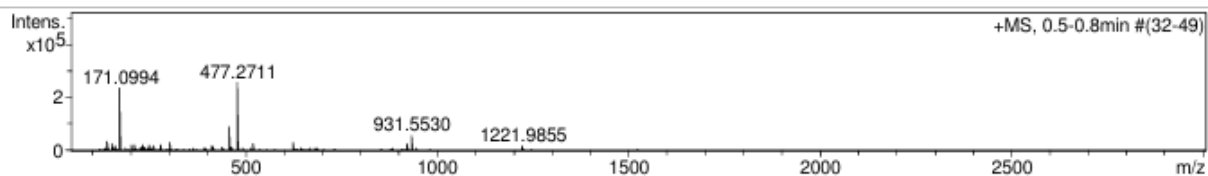
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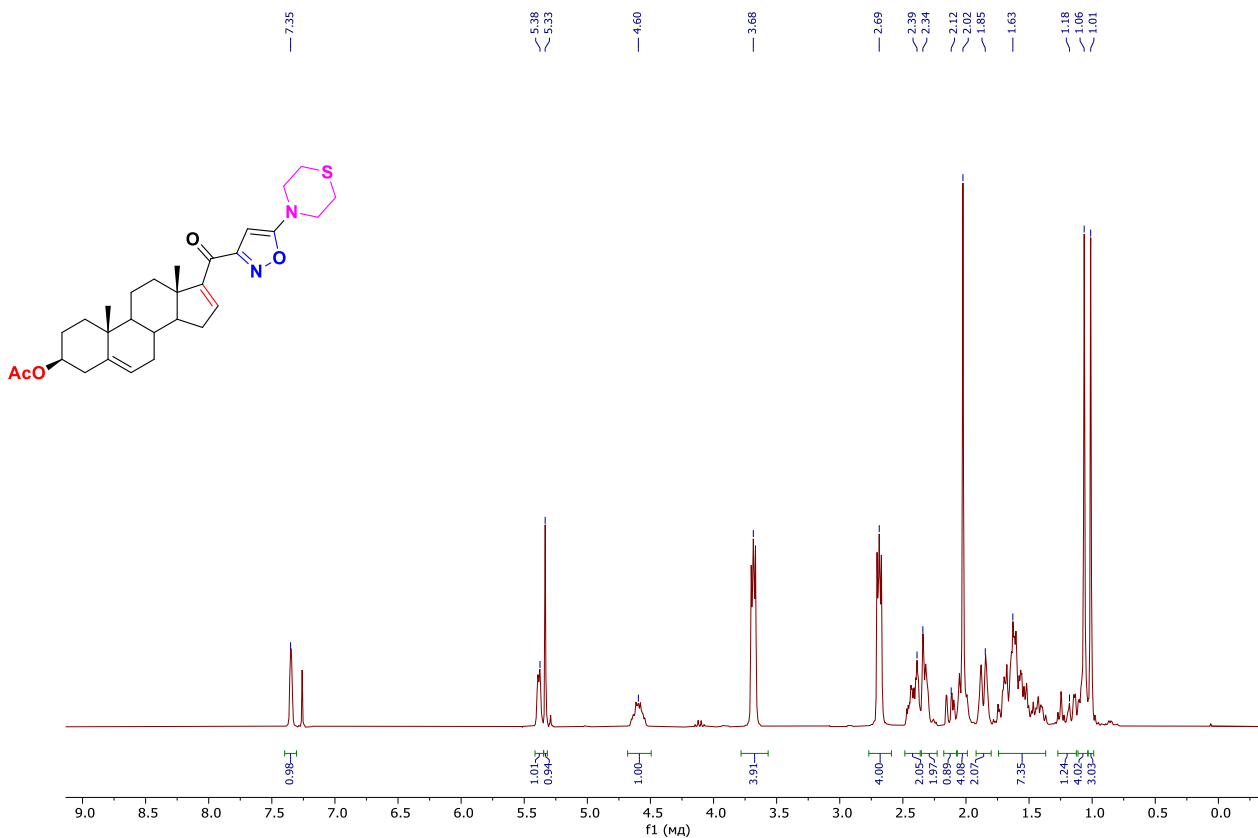
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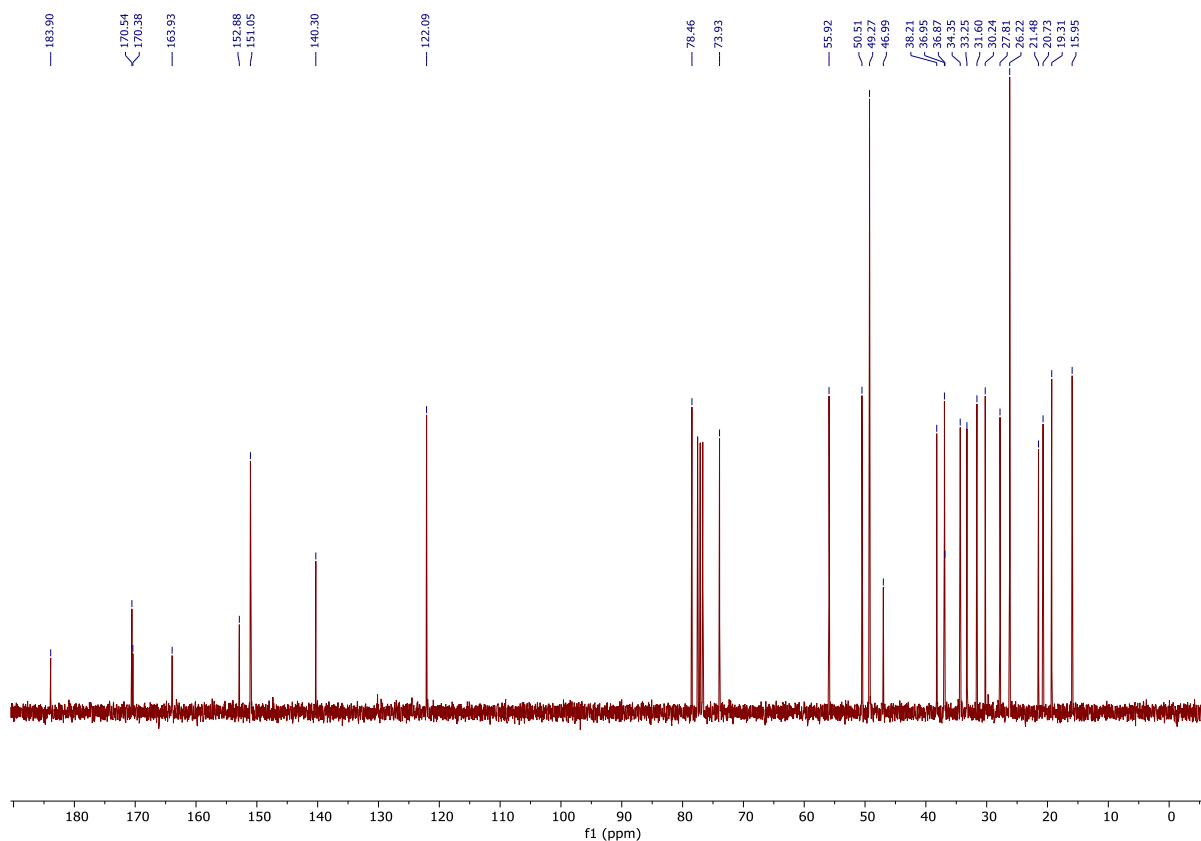
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 20-[3'-(5''-morpholine)isoxazole]-pregn-5-en-3 $\beta$ -ol-20-one (**7o**)



<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) spectrum of 3-acetate 20-[3'-(5''-tiomorfoline)isoxazole]-pregn-5,16-dien-3β-ol-20-one (7p)



<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) spectrum of 3-acetate 20-[3'-(5''-tiomorfoline)isoxazole]-pregn-5,16-dien-3β-ol-20-one (7p)

**Analysis Info**

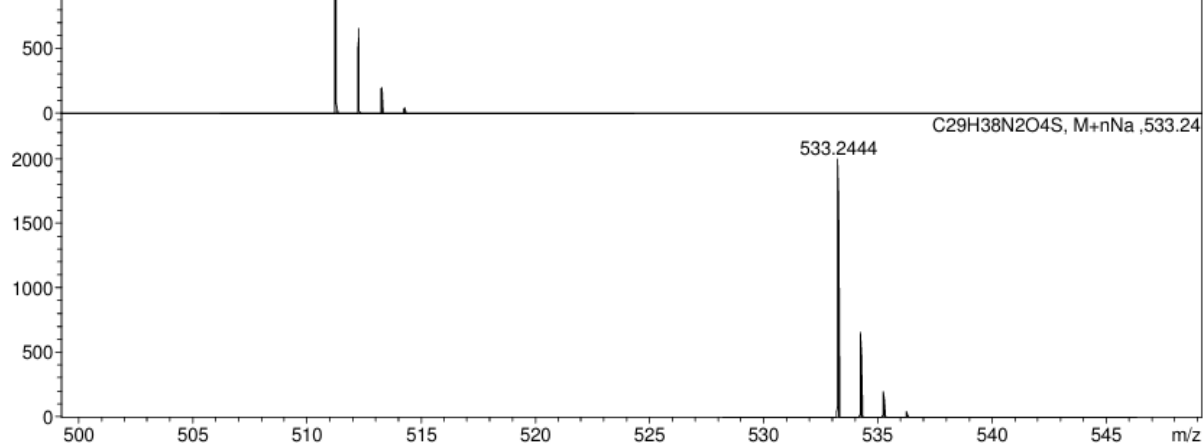
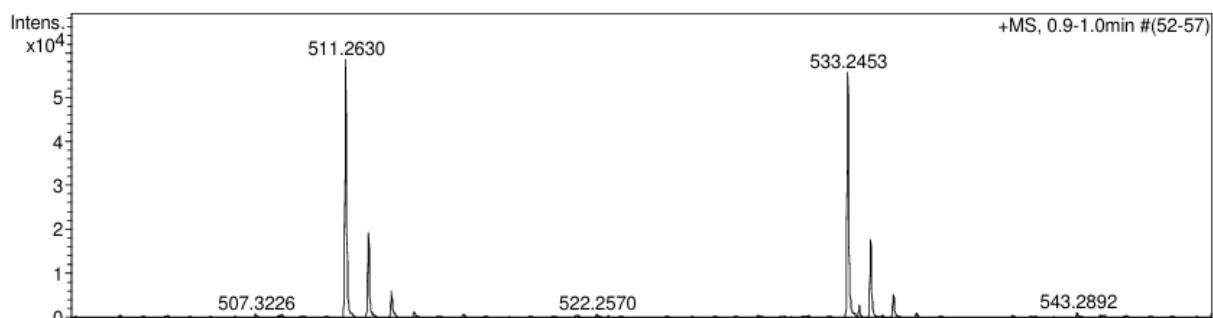
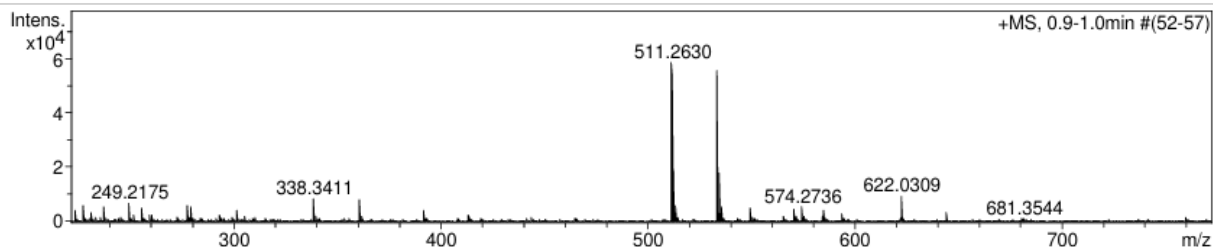
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Acquisition Date 08.02.2024 17:53:49

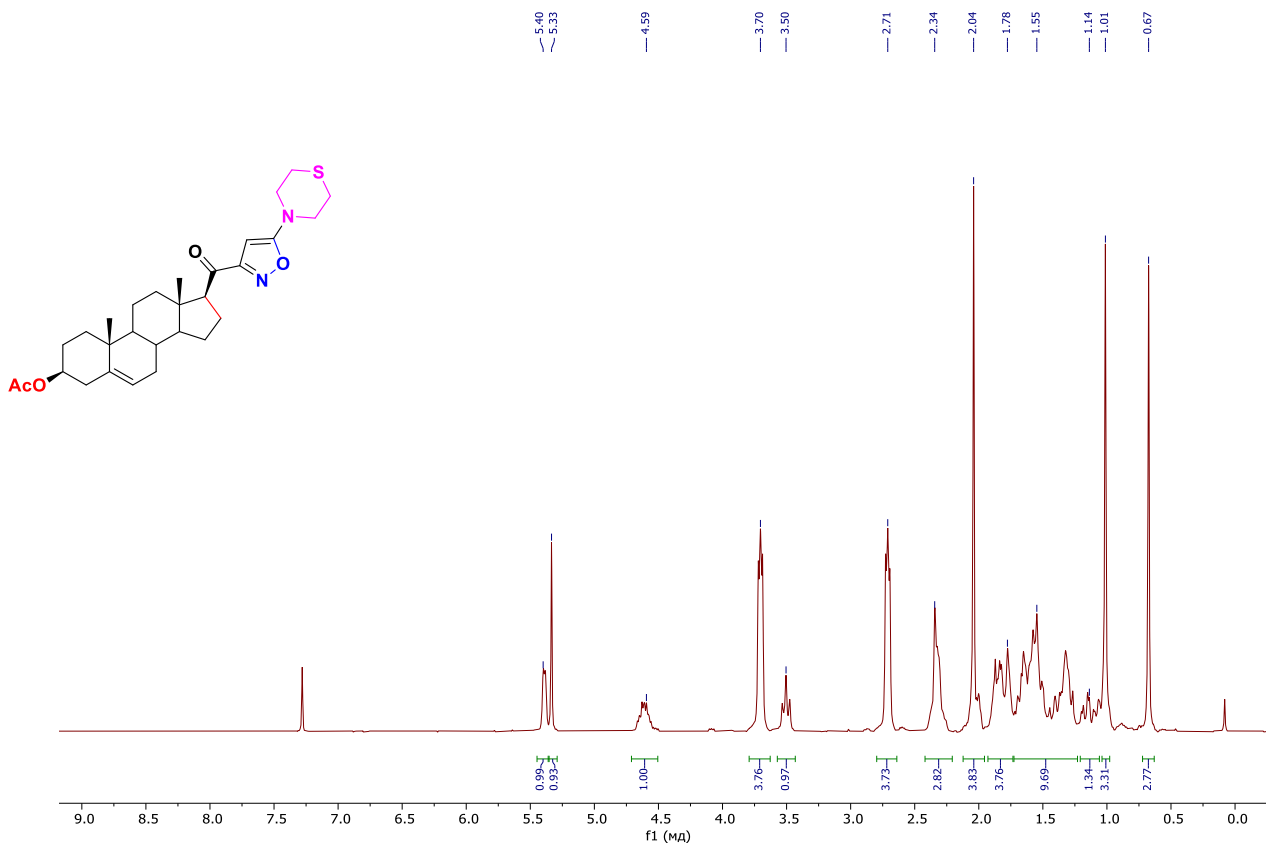
Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

**Acquisition Parameter**

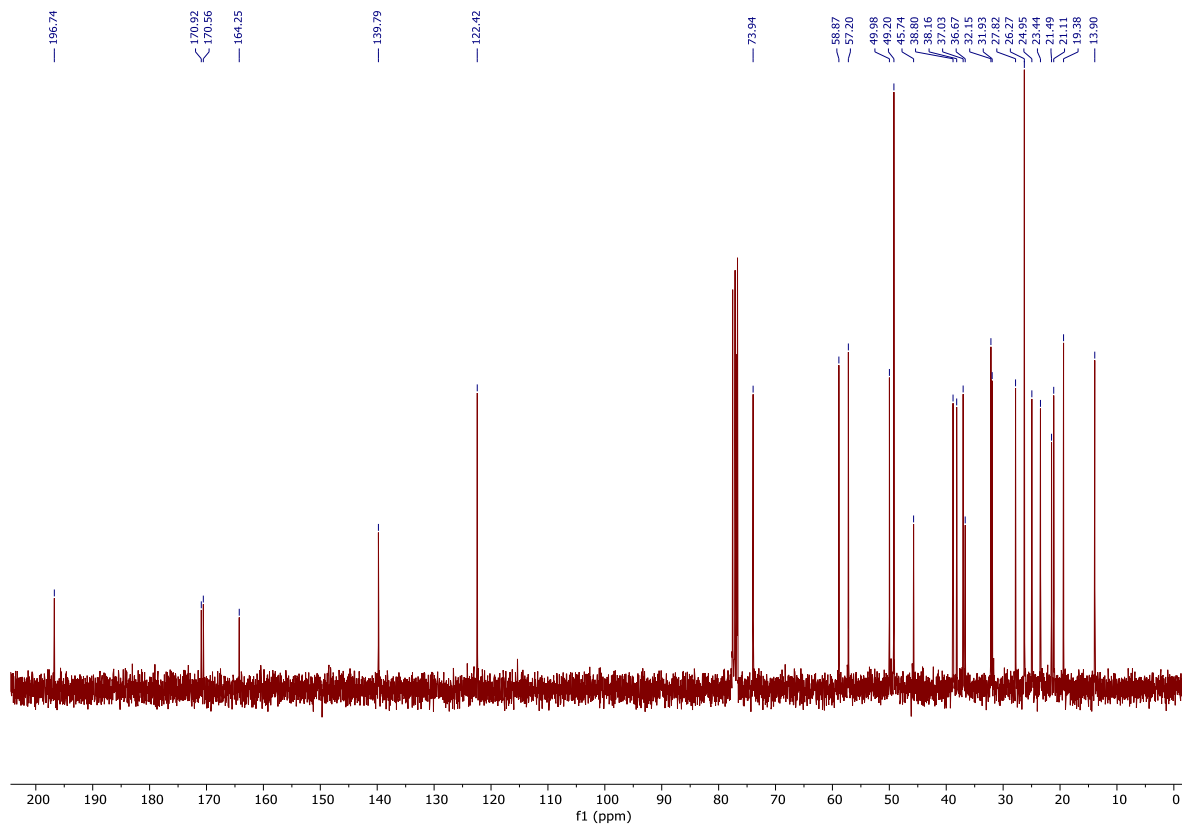
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-[3<sup>-</sup>-(5<sup>-</sup>-tiomorphone)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (**7p**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 20-(5'-tiomoroholineisoxazole)-pregna-5-en-3 $\beta$ -ol-20-one (**7q**)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 20-(5'-tiomoroholineisoxazole)-pregna-5-en-3 $\beta$ -ol-20-one (**7q**)

**Analysis Info**

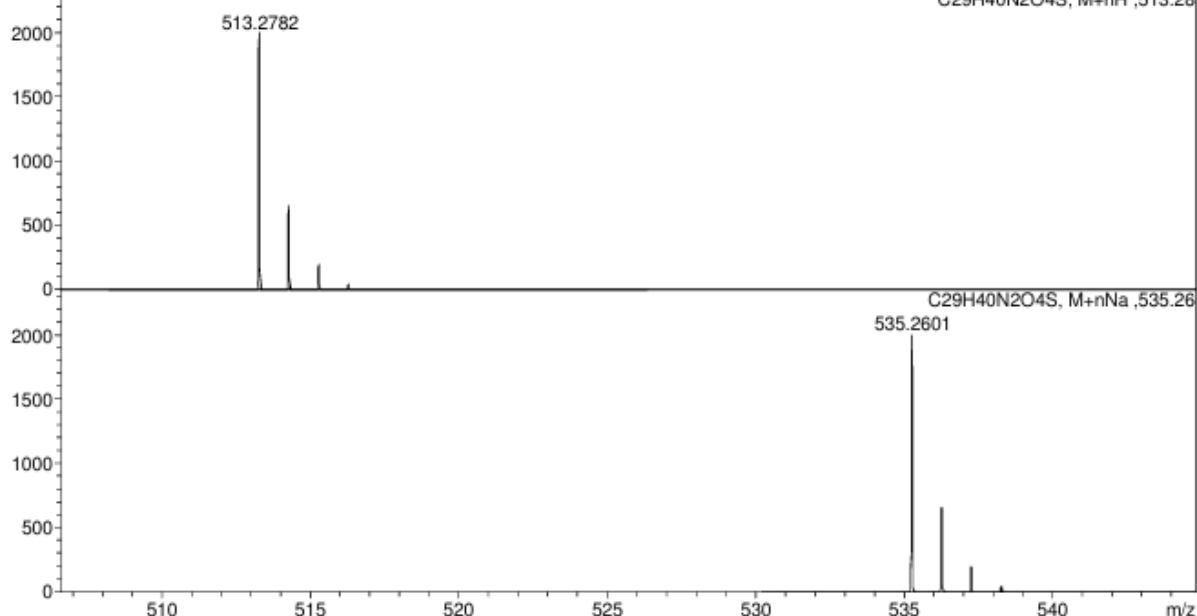
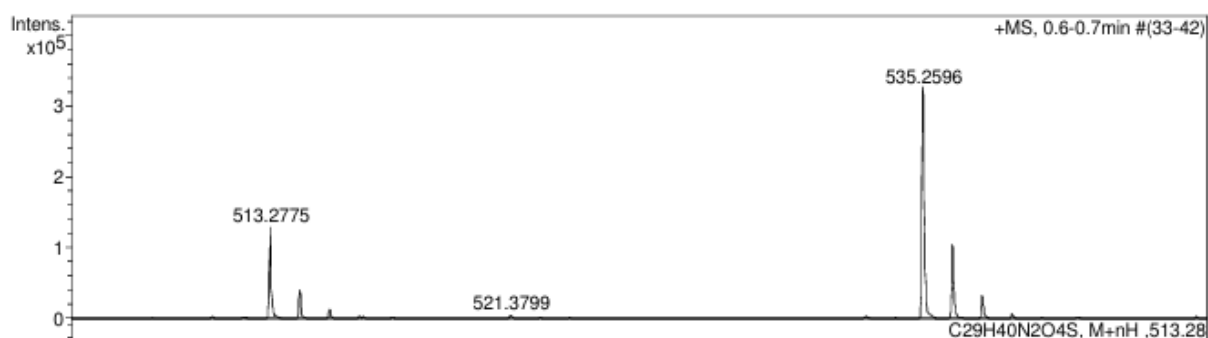
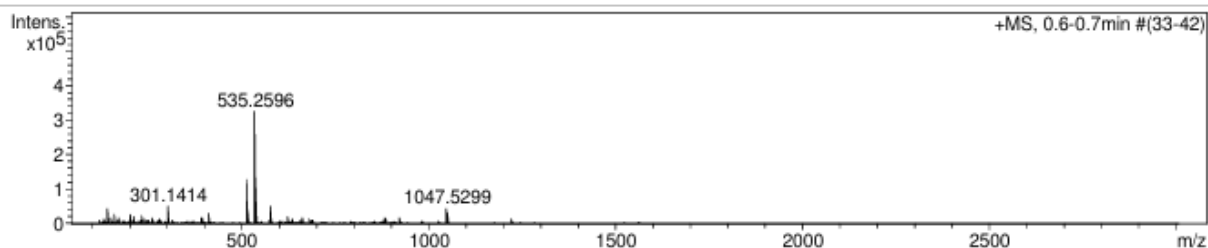
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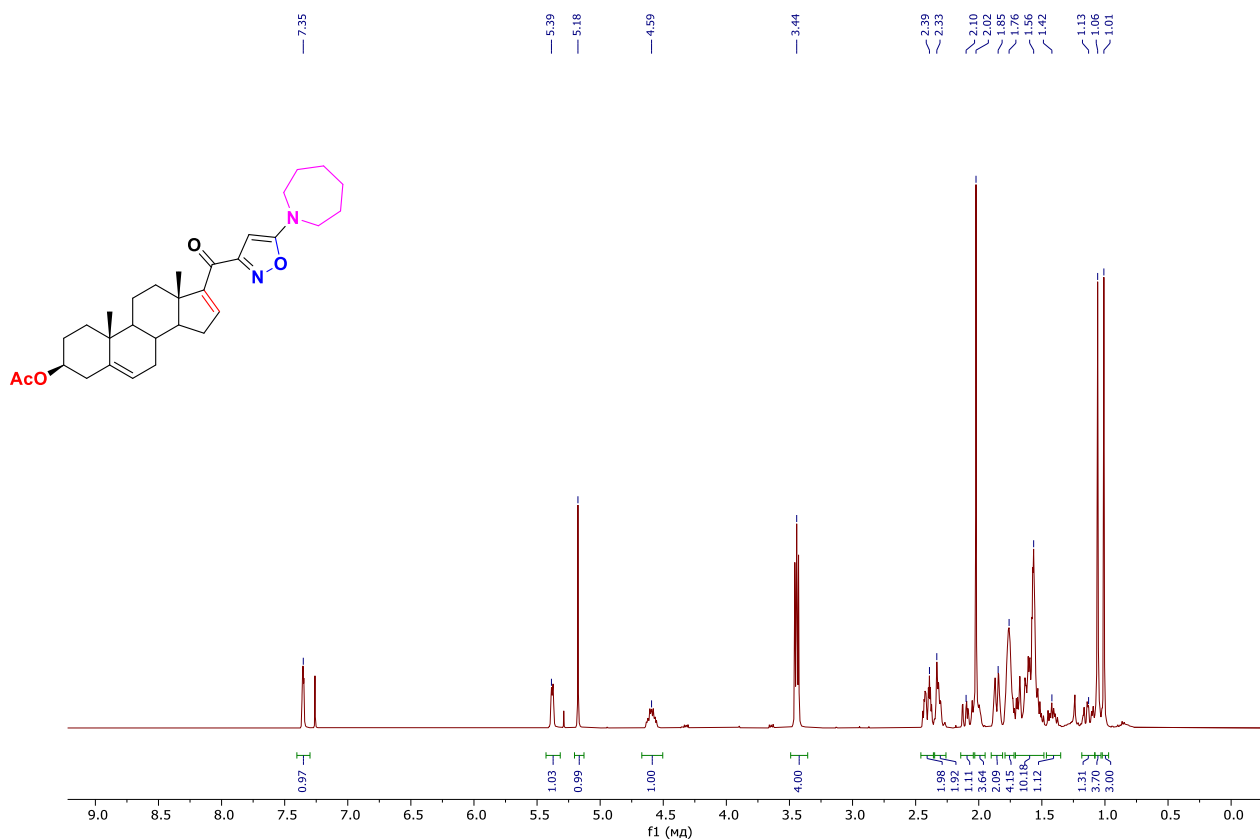
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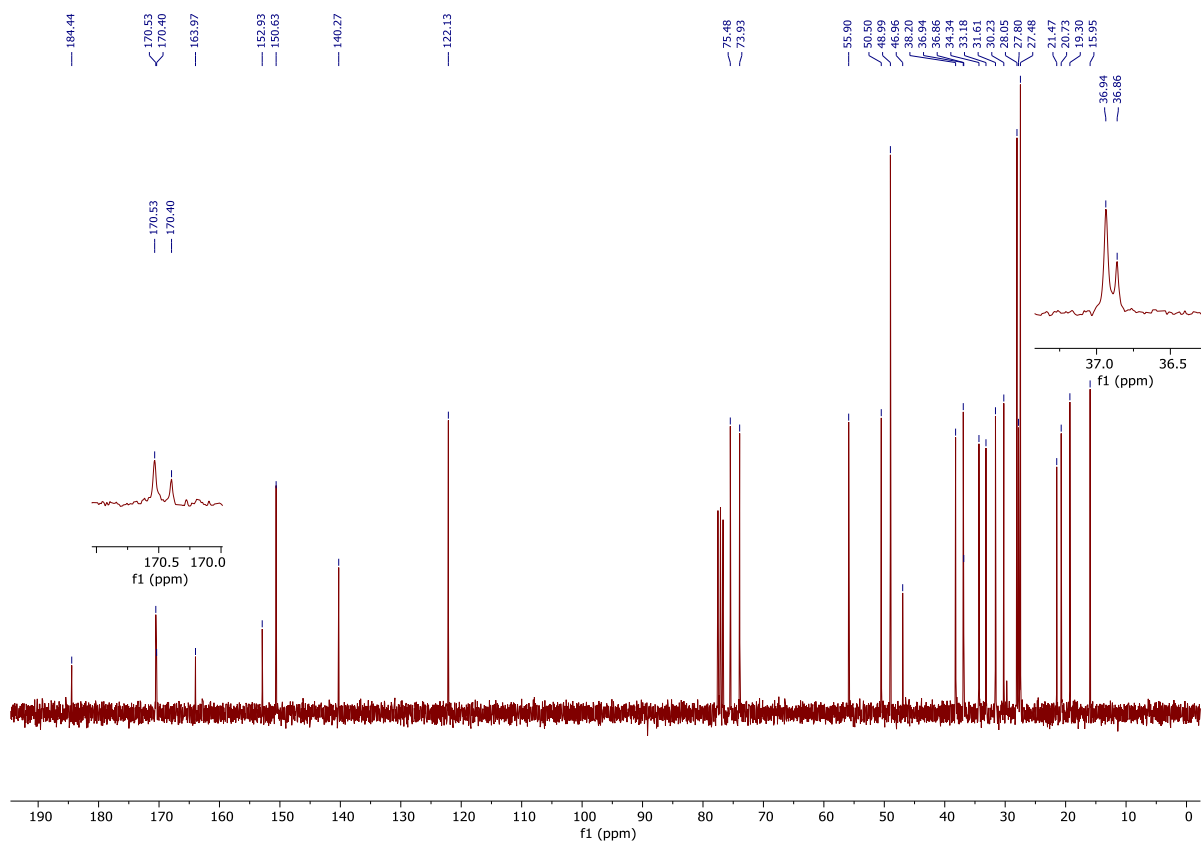
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-(5'-tiomorholineisoxazole)-pregna-5-en-3 $\beta$ -ol-20-one (**7q**)



<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) spectrum of 3-acetate 20-[3''-(5'-azepane)isoxazole]-pregn-5,16-dien-3β-ol-20-one (7r)



<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) spectrum of 3-acetate 20-[3''-(5'-azepane)isoxazole]-pregn-5,16-dien-3β-ol-20-one (7r)

**Analysis Info**

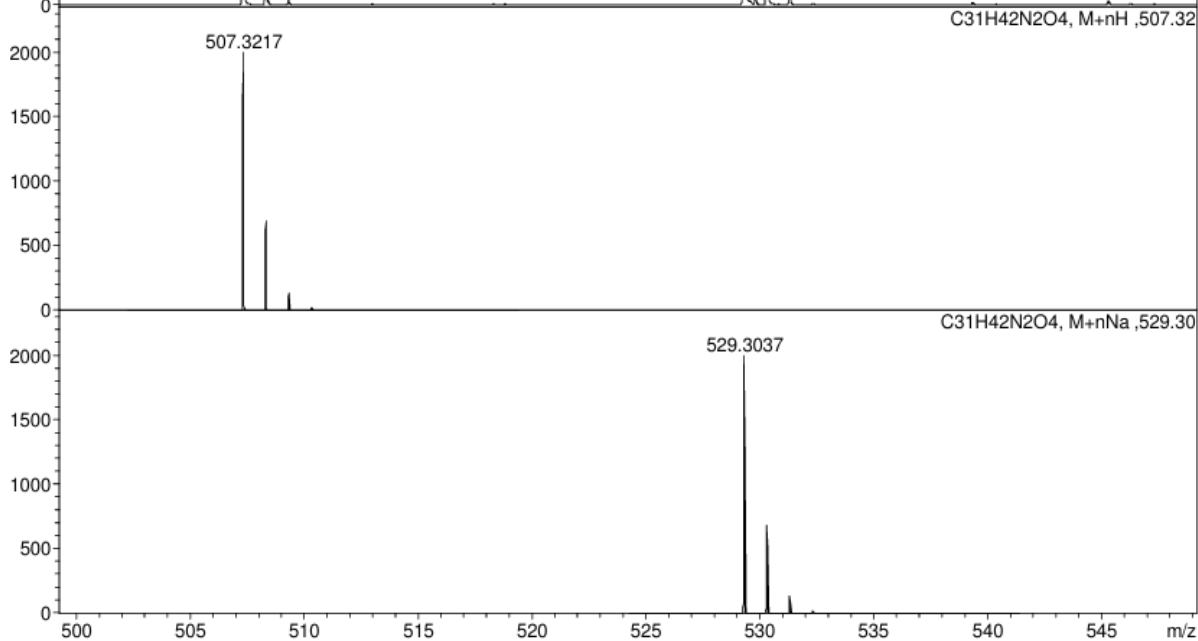
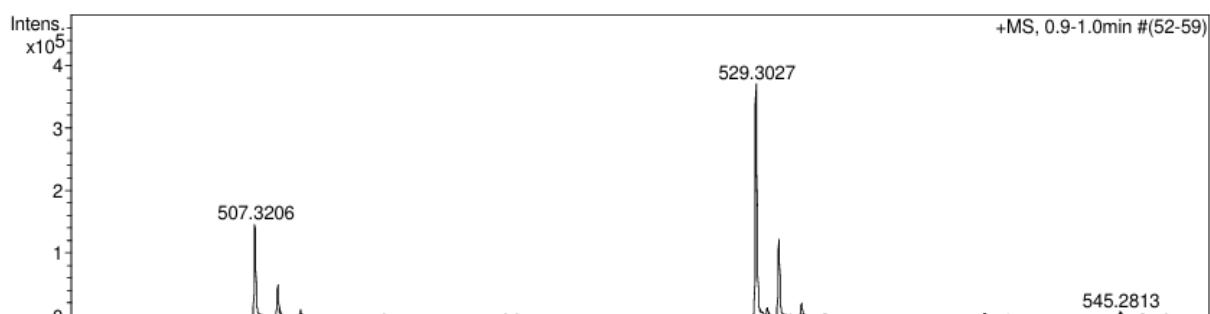
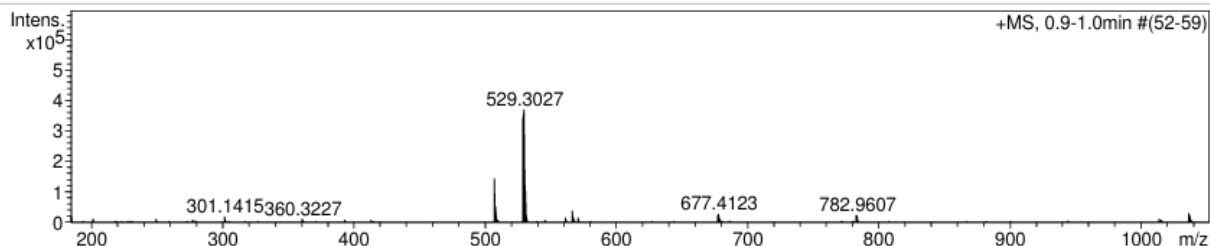
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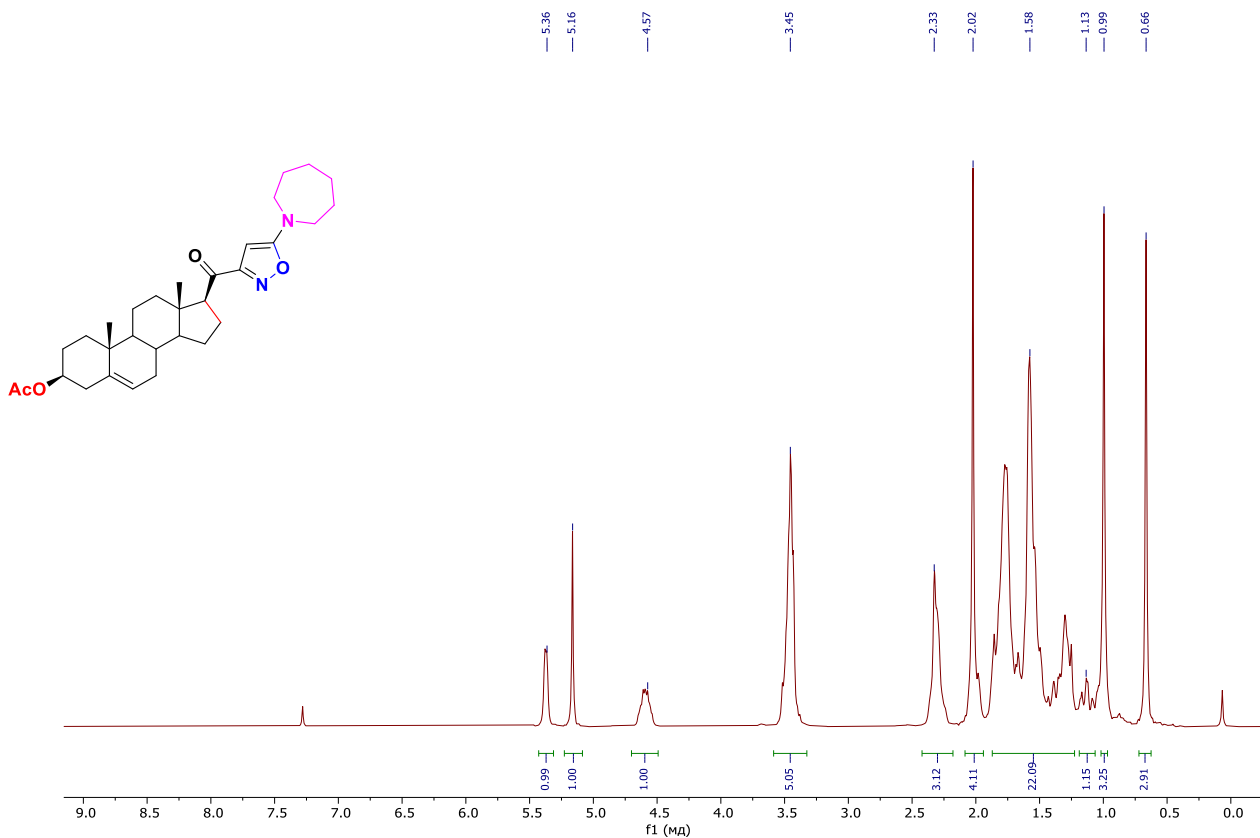
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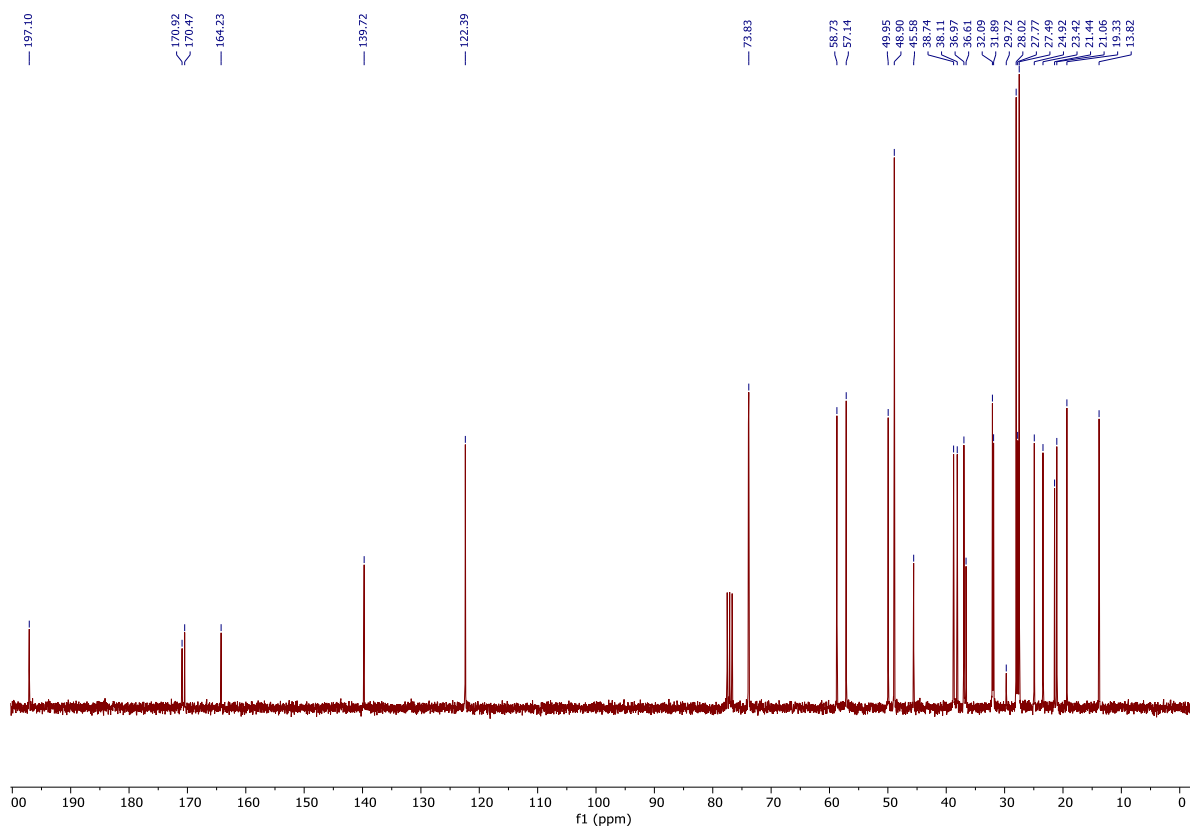
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-[3''-(5'-azepane)isoxazole]-pregn-5,16-dien-3 $\beta$ -ol-20-one (**7r**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 3-acetate 20-(5'-azepanisoazole)-pregna-5-en-3β-ol-20-one (7s)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 3-acetate 20-(5'-azepanisoazole)-pregna-5-en-3β-ol-20-one (7s)

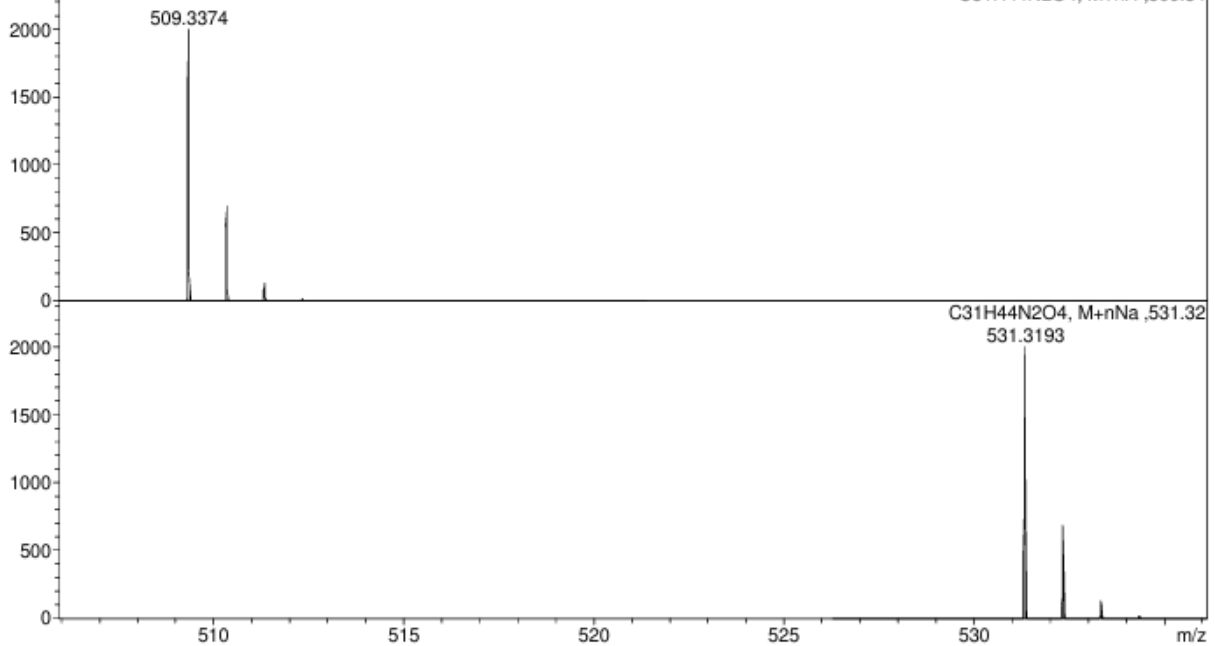
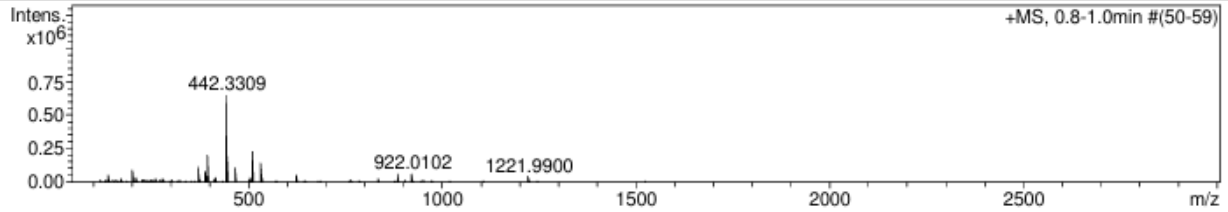
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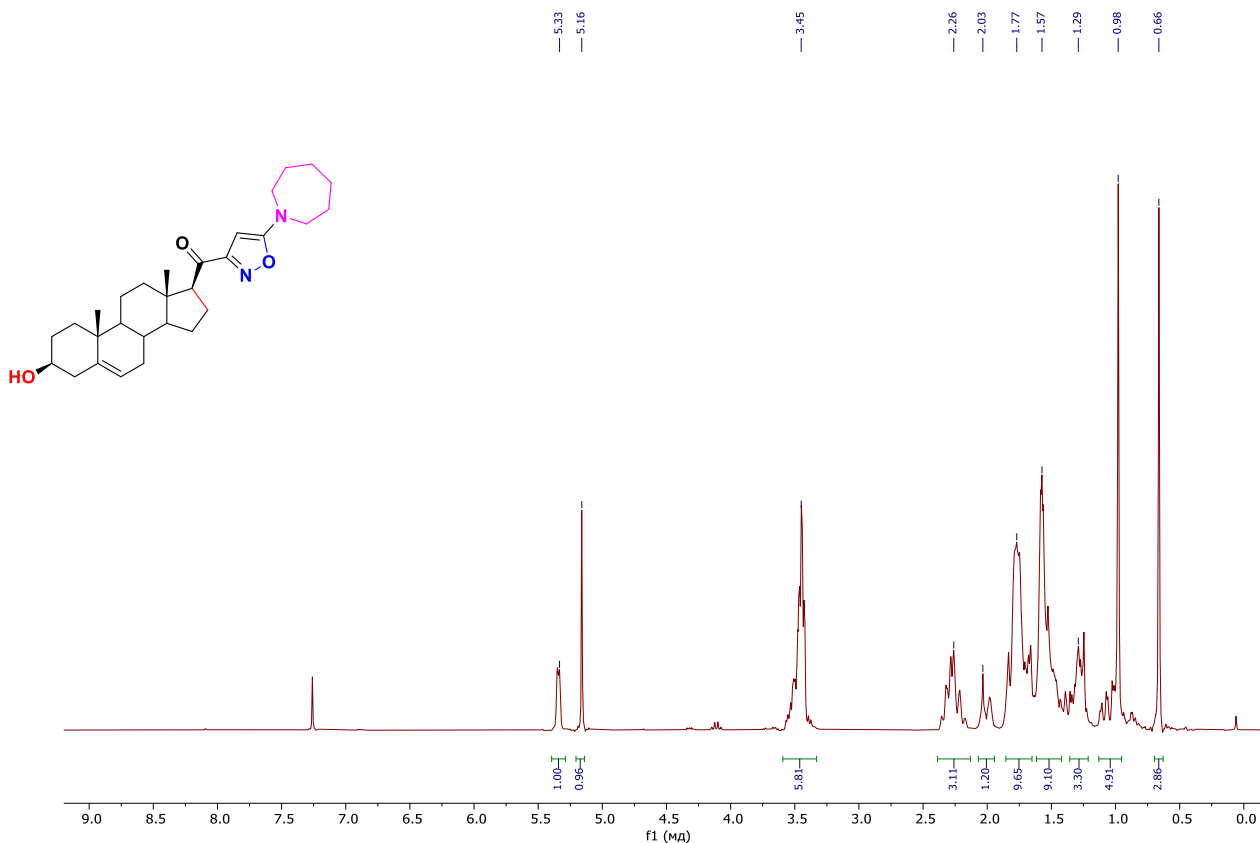
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Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

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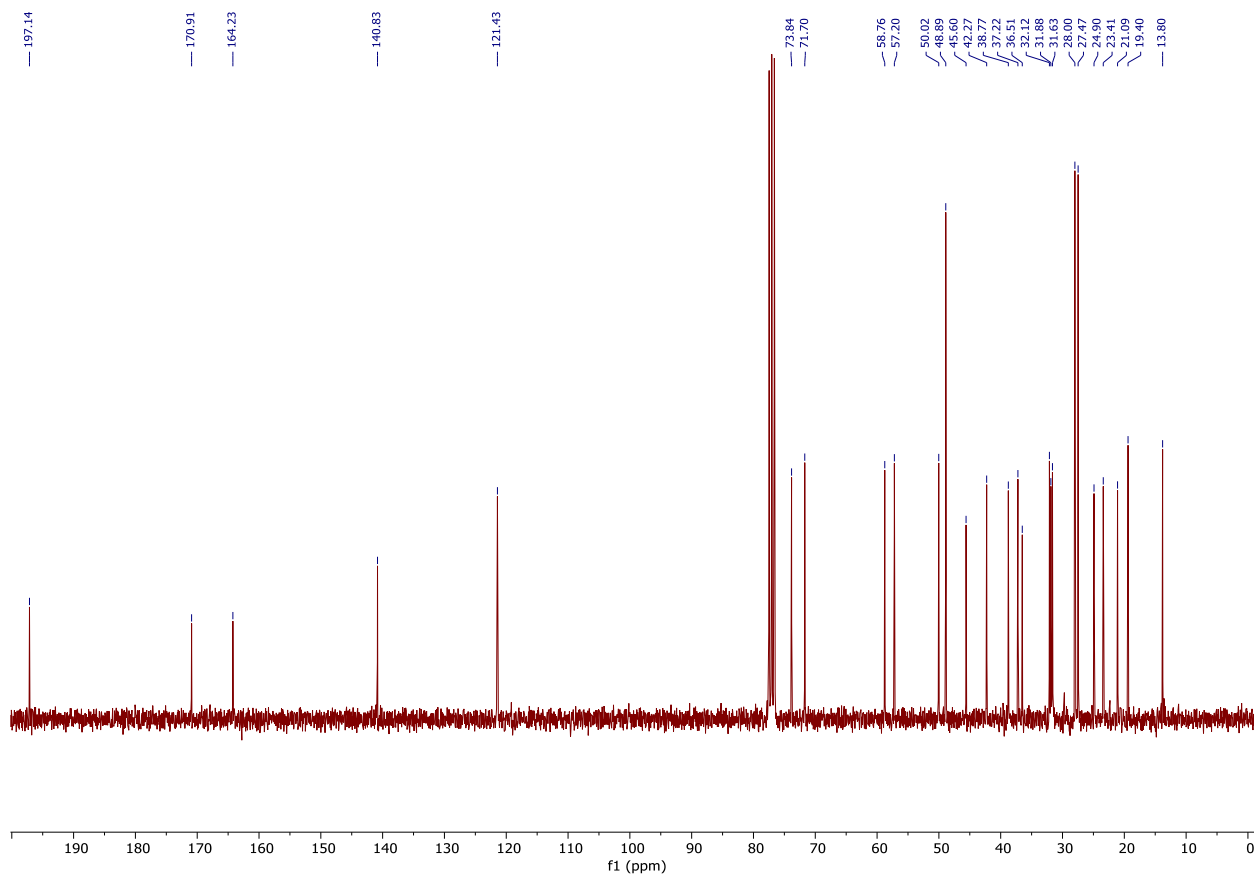
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



HRMS (ESI/Q-TOF) of 3-acetate 20-(5'-azepanisoazole)-pregna-5-en-3 $\beta$ -ol-20-one (**7s**)



<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) spectrum of 20-[3'-(5''-azepane)isoxazole]-pregn-5-en-3β-ol-20-one (7t)



<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) spectrum of 20-[3'-(5''-azepane)isoxazole]-pregn-5-en-3β-ol-20-one (7t)

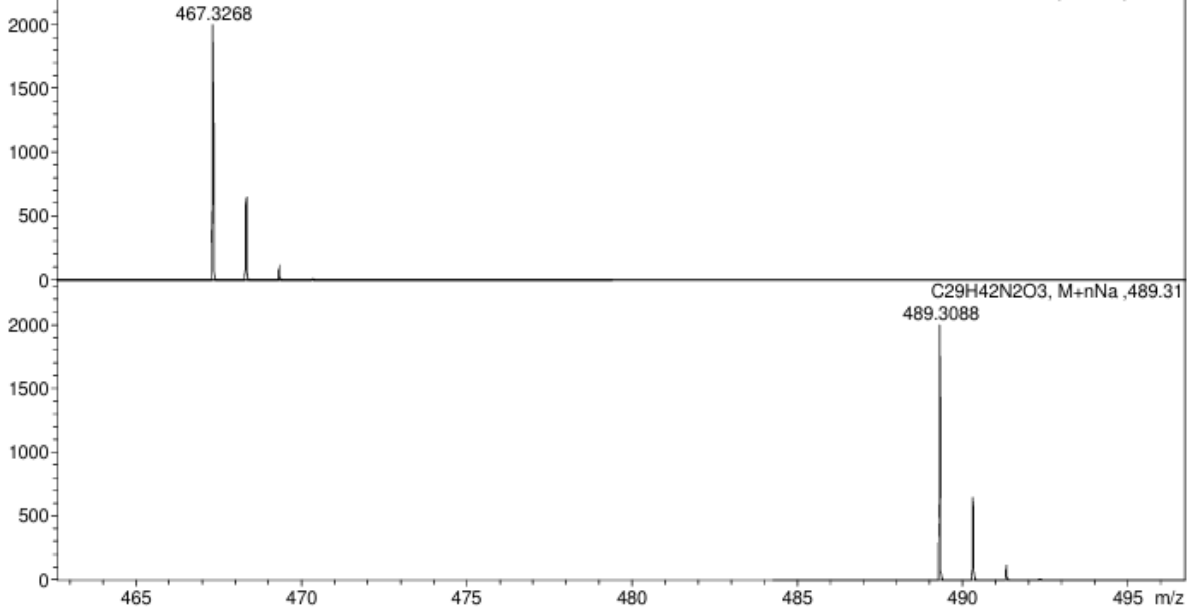
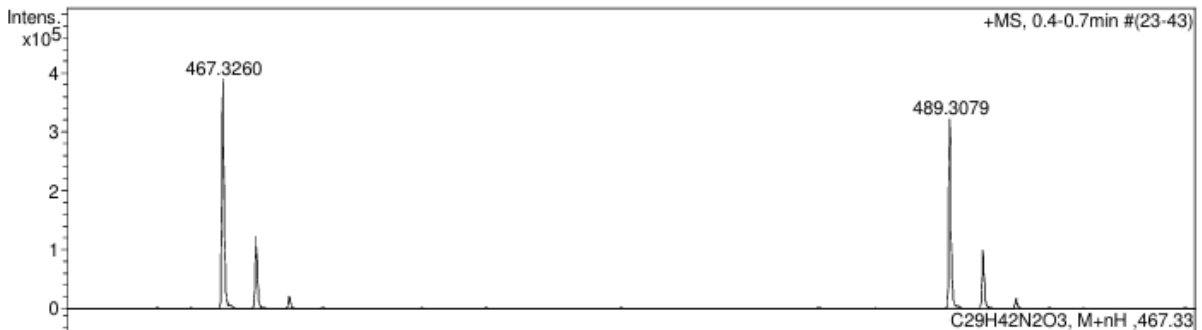
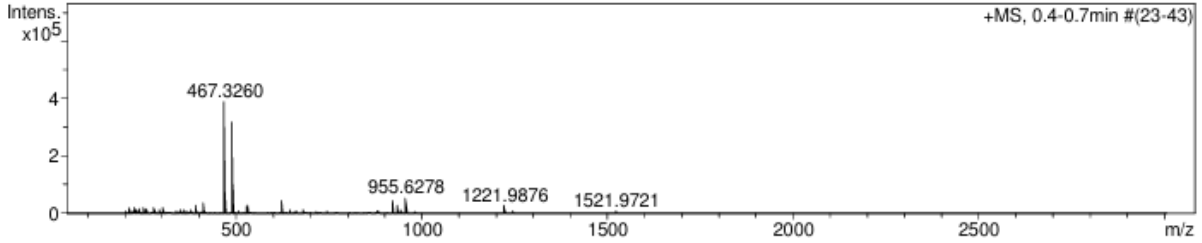
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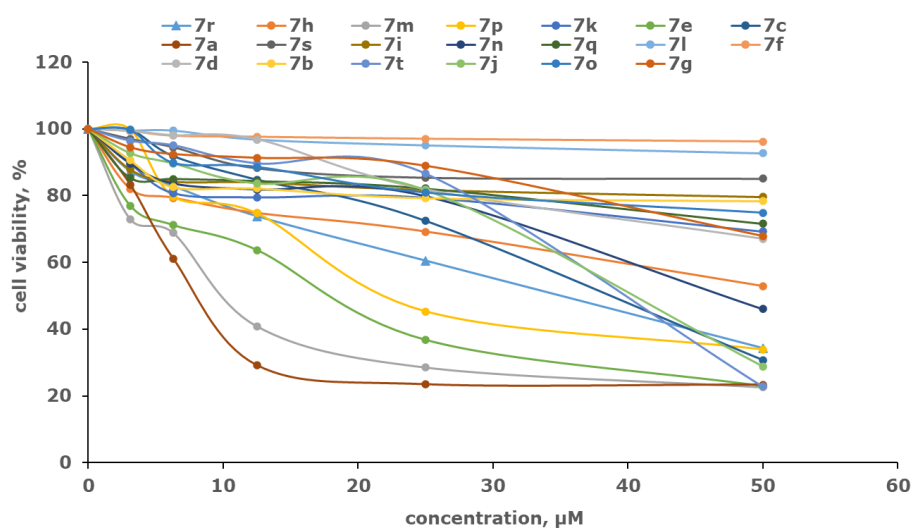
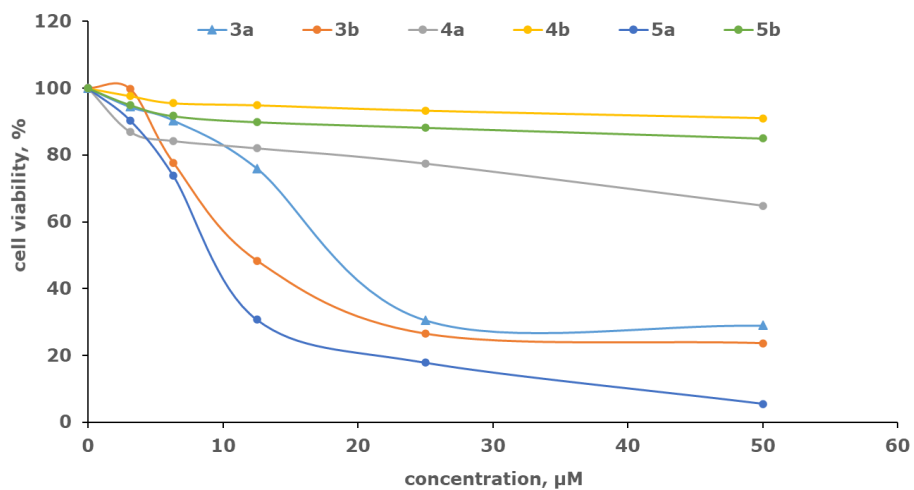
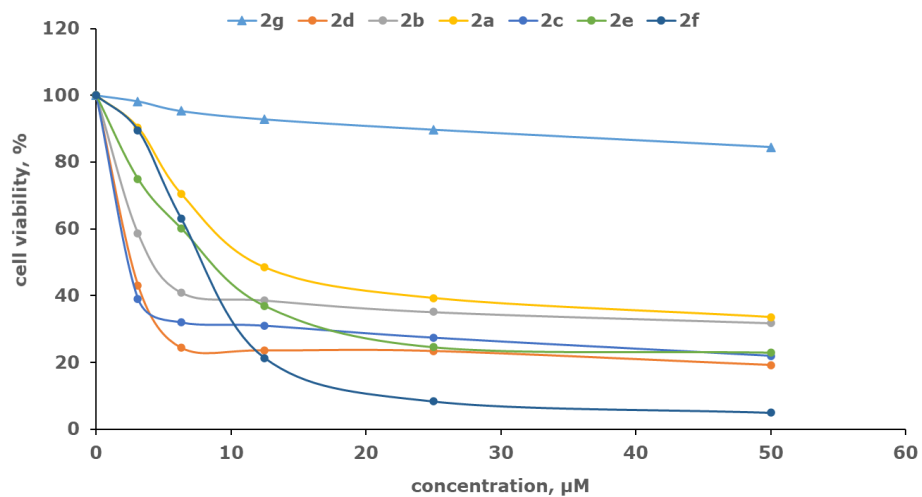
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Operator BDAL@DE  
Instrument / Ser# micrOTOF 10248

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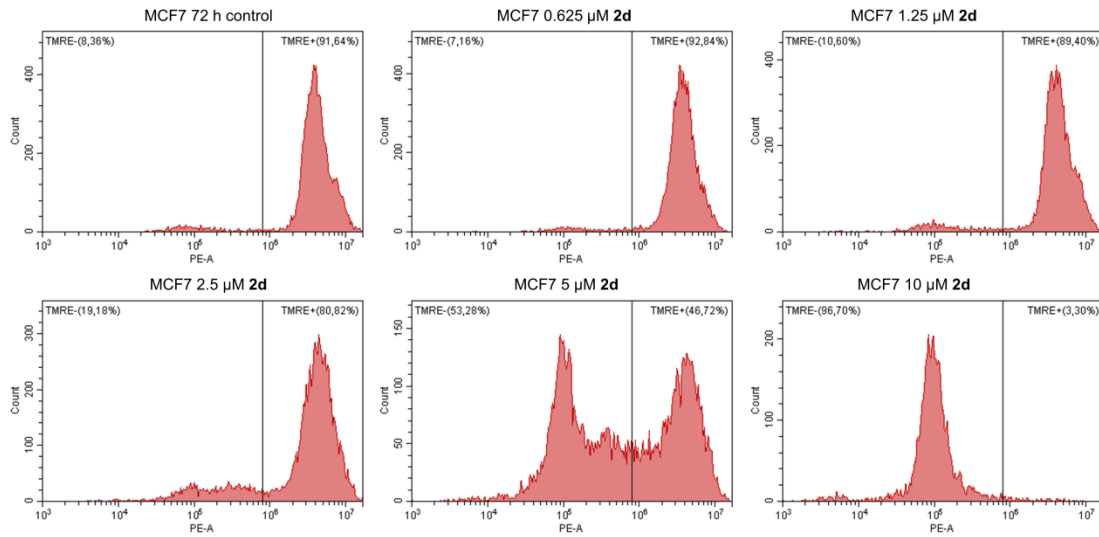
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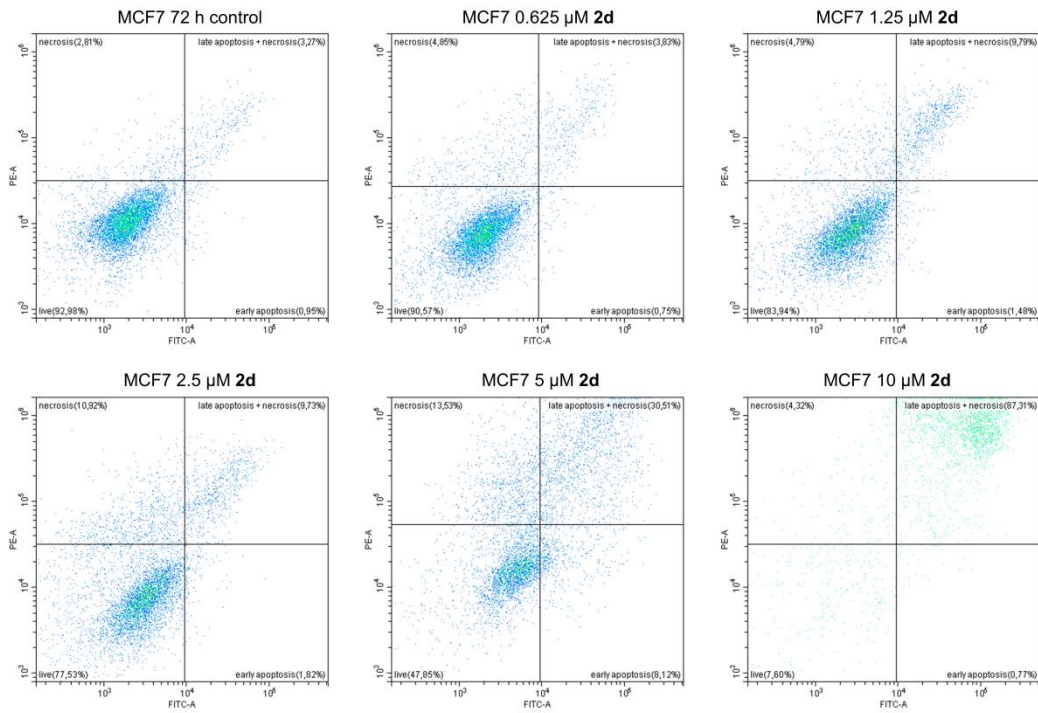
HRMS (ESI/Q-TOF) of 20-[3`-(5``-azepane)isoxazole]-pregn-5-en-3β-ol-20-one (7t)



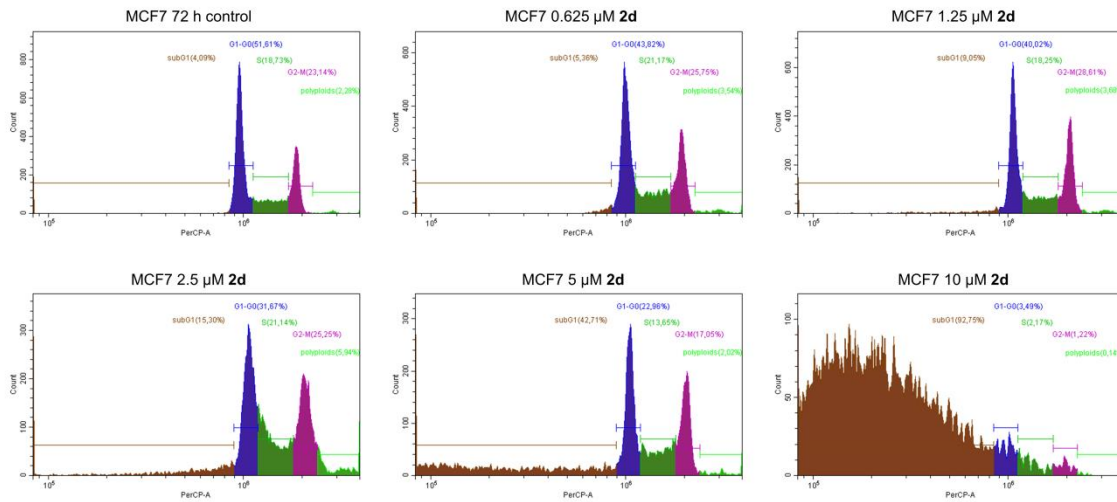
**Figure S1.** Antiproliferative activity of pregnenolone-isoxazole hybrids **2-5** and **7** against MCF7 breast cancer cells.



**Figure S2.** Representative flow cytometry histograms refer to Figure 4A.



**Figure S3.** Representative flow cytometry histograms refer to Figure 4B.



**Figure S4.** Representative flow cytometry histograms refer to Figure 4C.