# Supporting information for:
# Selective inhibitors of Human Neuraminidase-3

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**Protein expression**

Optimized Neu3 and Neu4 genes were synthesized and subcloned into vector pMAL-c2x in DNA2.0. The plasmids of MBP-Neu3 and MBP-Neu4 were transformed into expression cells, *E. coli* TB1. Protein was isolated by culturing *E. coli* TB1 cells containing plasmid pMBP-*Neu3* or *Neu4* in LB medium containing 0.2% glucose and ampicillin (100 μg/mL). The culture was grown at 30 °C with shaking until the $OD_{600}$ reached 0.3. The temperature was decreased to 20 °C, and fusion protein was induced by adding IPTG to a final concentration of 0.3 mM when $OD_{600}$ reached 0.5-0.6. Cells were harvested by centrifugation after 20 h of induction. The pellet was resuspended (50 mL per liter of medium) in resuspension buffer (20 mM MOPS, pH 7.2, 300 mM NaCl, 1mM EDTA, 10% glycerol and 0.05% triton X-100) and supplemented with a protease inhibitor tablet (Roche). The lysate was passed through a cell disruptor once at 20,000 psi and then immediately pelleted by centrifugation at 105,000 x g for 60 min at 4 °C. The supernatant was loaded onto an amylose column (New England Biolabs) equilibrated with 20 mM MOPS (10% glycerol and 300 mM NaCl, pH 7.2). MBP-fusion protein was eluted with running buffer containing 10% glycerol (v/v) and 10 mM maltose.

**Table S.1: HPLC Gradient elution used to check purity of inhibitors**

Purity of compounds for enzymatic assay was tested by HPLC using a C18 reverse phase column (XTerra RP C18, analytical, particle size: 3.5 μm, column dimensions: 4.8 × 150 mm). The gradient elution program was summarized below where solvent A was 0.1% trifluoroacetic acid in milliQ water and solvent B was 0.1% trifluoroacetic acid in acetonitrile. See Figure S.4 for HPLC traces.

| Time | Flow rate (mL/min) | A% | B% |
|---|---|---|---|
| **0** | 1 | 100 | 0 |
| **3** | 1 | 100 | 0 |
| **13** | 1 | 0 | 100 |
| **18** | 1 | 0 | 100 |

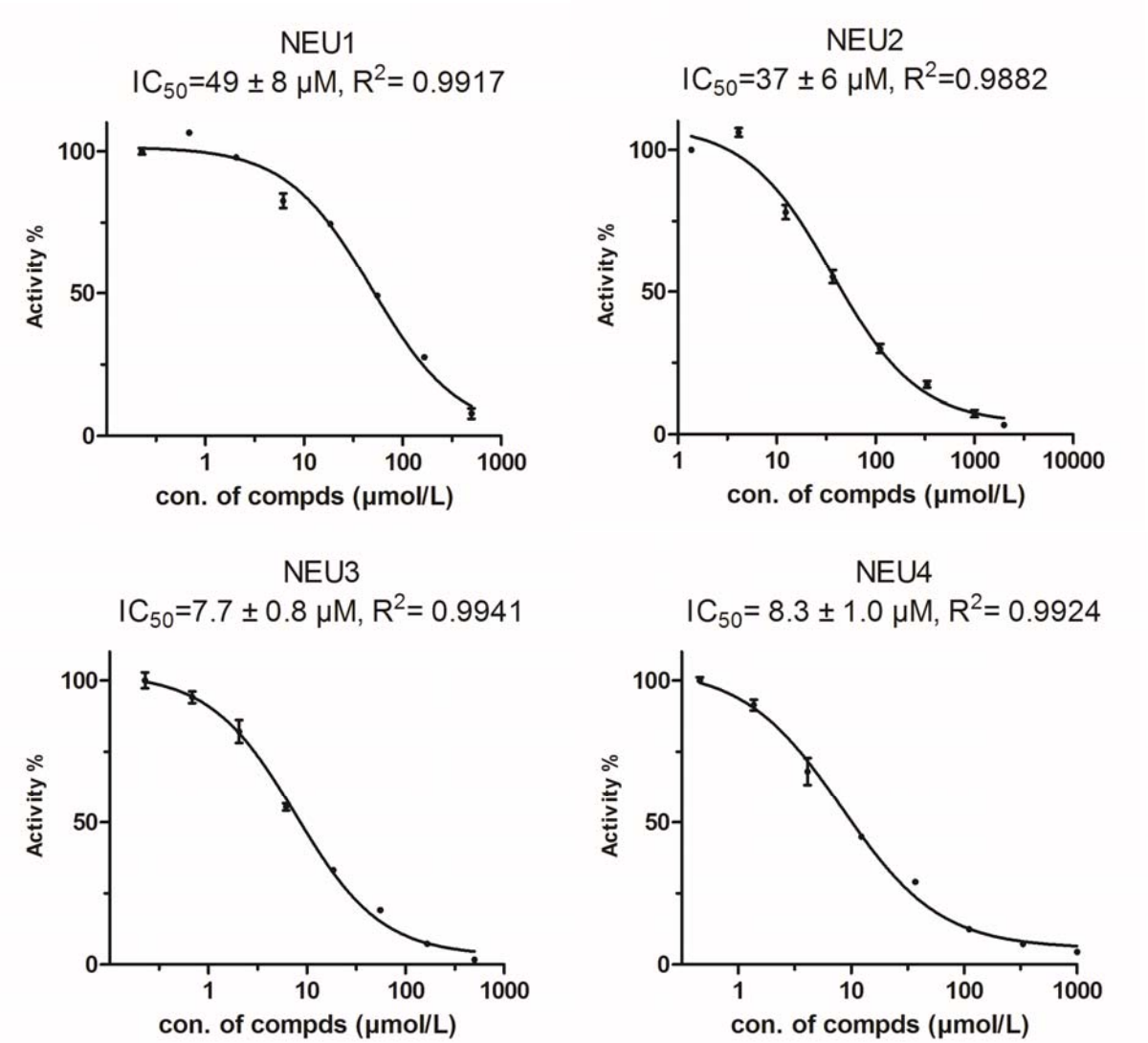
**Table S.2: Summary of HPLC and HRMS data for tested compounds**

| Compound | HPLC Trace | | HRMS | |
|---|---|---|---|---|
| | Retention Time min | Area % | Calculated m/z | Found m/z |
| **1(DANA)** | 1.657 | 98.70 | 290.0876 | 290.0879 |
| **2(C9-BA-DANA)** | 1.861 | 99.97% | 373.1616 | 373.1614 |
| **6(Zanamivir)** | 1.939 | 99.78 | 333.1405 | 333.1400 |
| **7a** | 11.085 | 97.69 | 460.1832 | 460.1834 |
| **7b** | 12.302 | 99.92 | 474.1625 | 474.1636 |
| **7c** | 4.433 | 97.35 | 432.1529 | 432.1513 |
| **7d** | 13.240 | 98.94 | 431.1567 | 431.1568 |
| **7e** | 12.674 | 98.74 | 447.1516 | 447.1527 |
| **7f** | 12.834 | 96.36 | 435.1316 | 435.1324 |
| **7g** | 13.886 | 99.11 | 485.1284 | 485.1282 |
| **7h** | 12.200 | 99.05 | 461.1309 | 461.1316 |
| **7i** | 14.041 | 99.97 | 493.1723 | 493.1729 |
| **7j** | 14.027 | 98.49 | 509.1672 | 509.1674 |
| **8a** | 12.307 | 97.04 | 502.1686 | 502.1683 |
| **8b** | 1.561 | 97.84 | 534.2101 | 534.2105 |
| **13** | 12.978 | 99.27 | 486.1373 | 486.1378 |
| **15** | 11.983 | 95.11 | 460.1468 | 460.1482 |
| **18** | 12.209 | 97.58 | 575.1738 | 575.1738 |
| **25a** | 3.100 | 99.05 | 345.1298 | 345.1302 |
| **25b** | 10.823 | 98.98 | 373.1611 | 373.1612 |
| **25c** | 1.805 | 98.52 | 357.1298 | 357.1305 |
| **25d** | 16.854 | 97.44 | 371.1454 | 371.1458 |

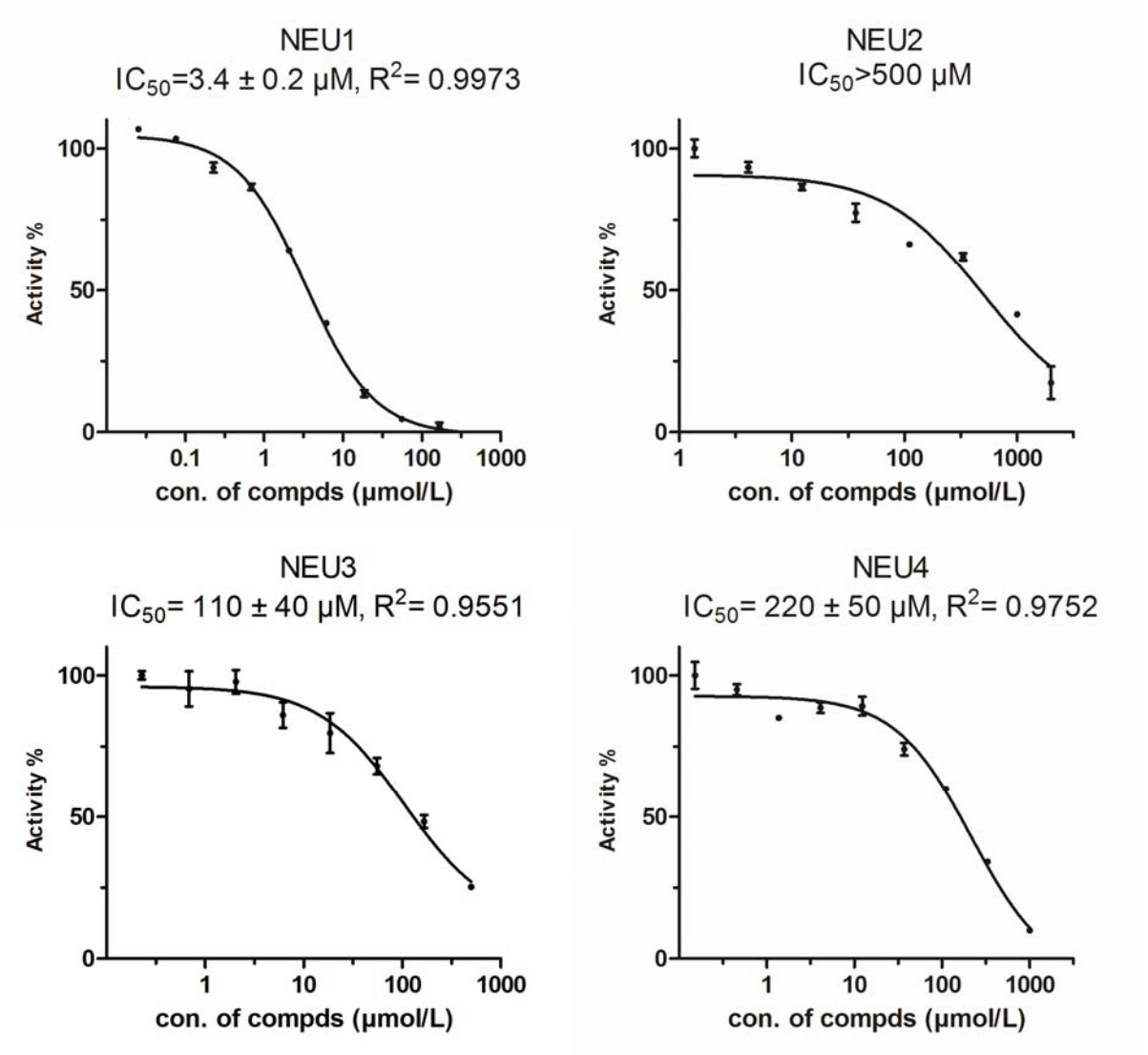
**Figure S.1: Inhibition of human neuraminidase isoforms by tested compounds**

**$IC_{50}$ curves using 4Mu-NANA as substrate**

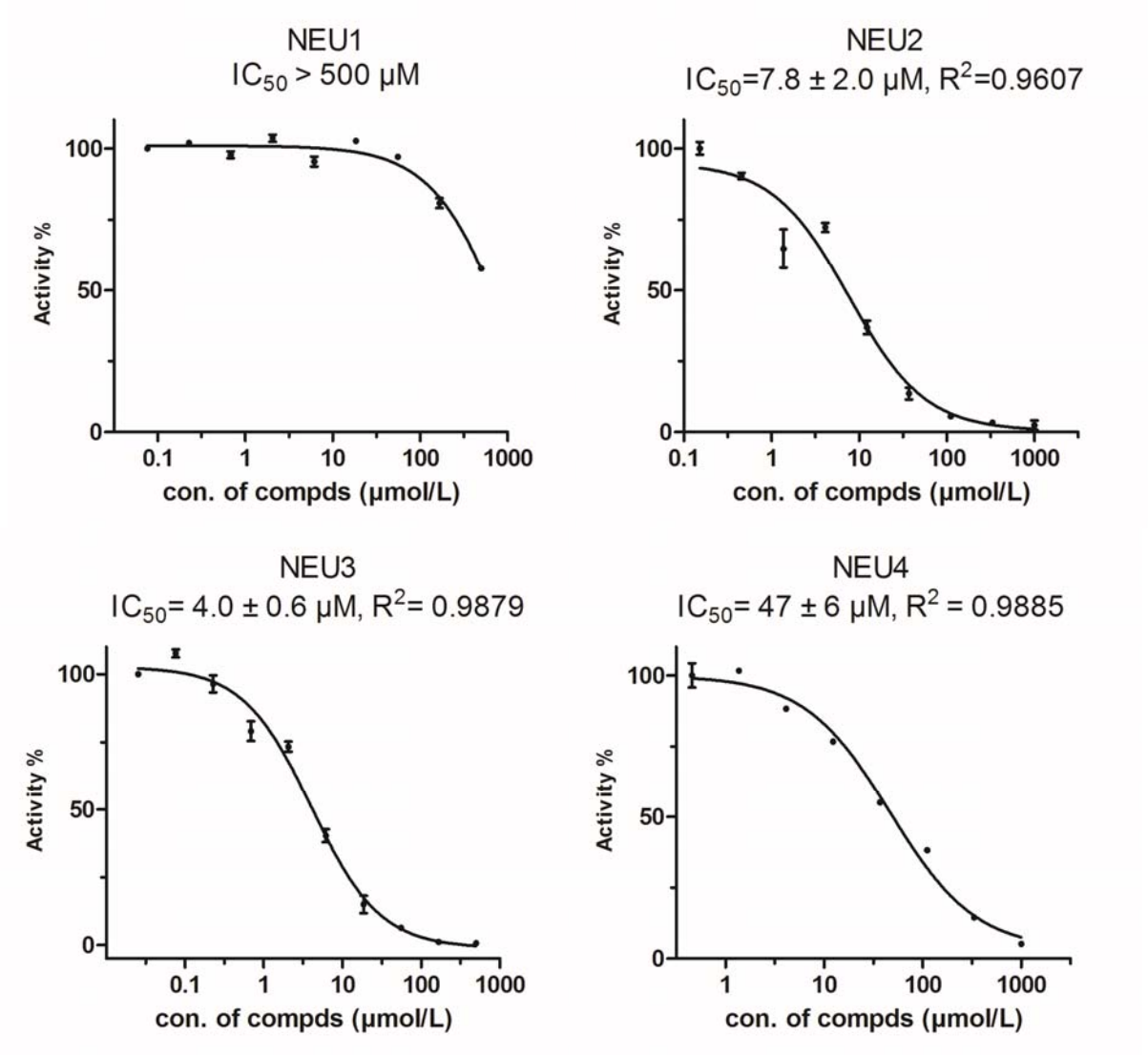
**1(DANA)**



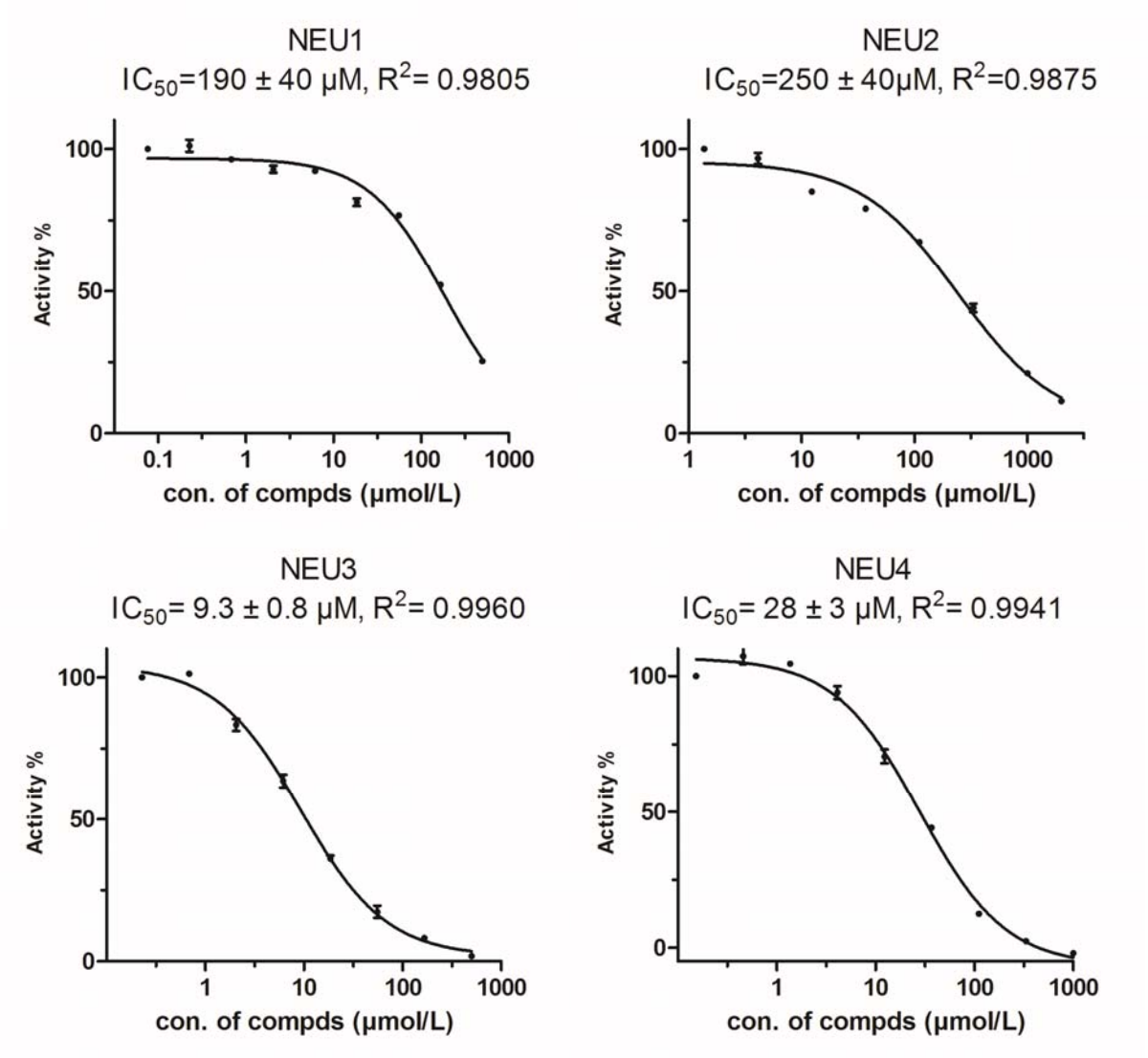
**2(C9-BA-DANA)**


NEU1
IC50=3.4 ± 0.2 μM, R2= 0.9973
Activity %
con. of compds (μmol/L)
NEU2
IC50>500 μM
Activity %
con. of compds (μmol/L)
NEU3
IC50= 110 ± 40 μM, R2= 0.9551
Activity %
con. of compds (μmol/L)
NEU4
IC50= 220 ± 50 μM, R2= 0.9752
Activity %
con. of compds (μmol/L)

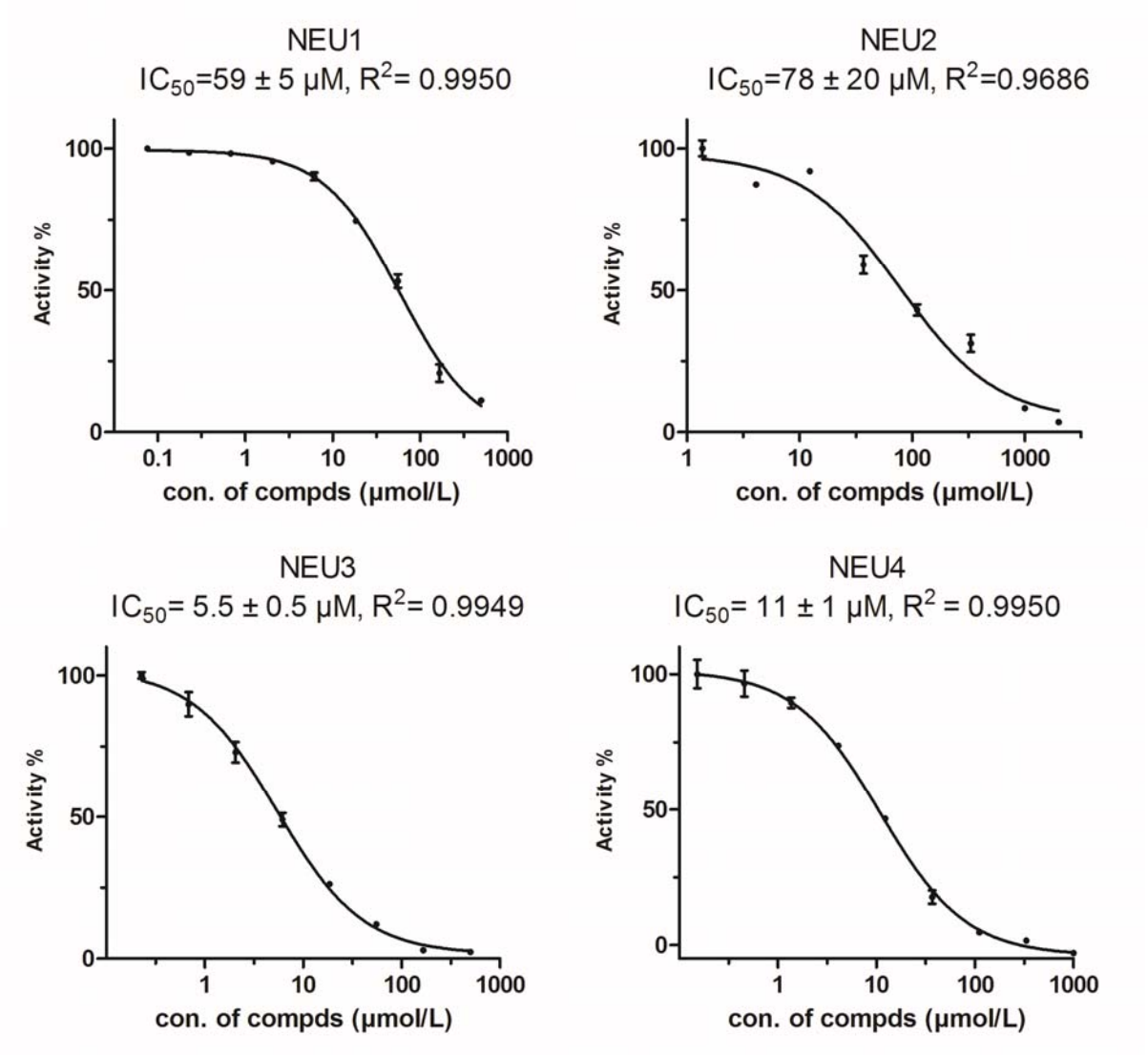
**6(Zanamivir)**


NEU1
IC50 > 500 μM
NEU2
IC50=7.8 ± 2.0 μM, R2=0.9607
NEU3
IC50= 4.0 ± 0.6 μM, R2= 0.9879
NEU4
IC50= 47 ± 6 μM, R2 = 0.9885
Activity %
con. of compds (μmol/L)

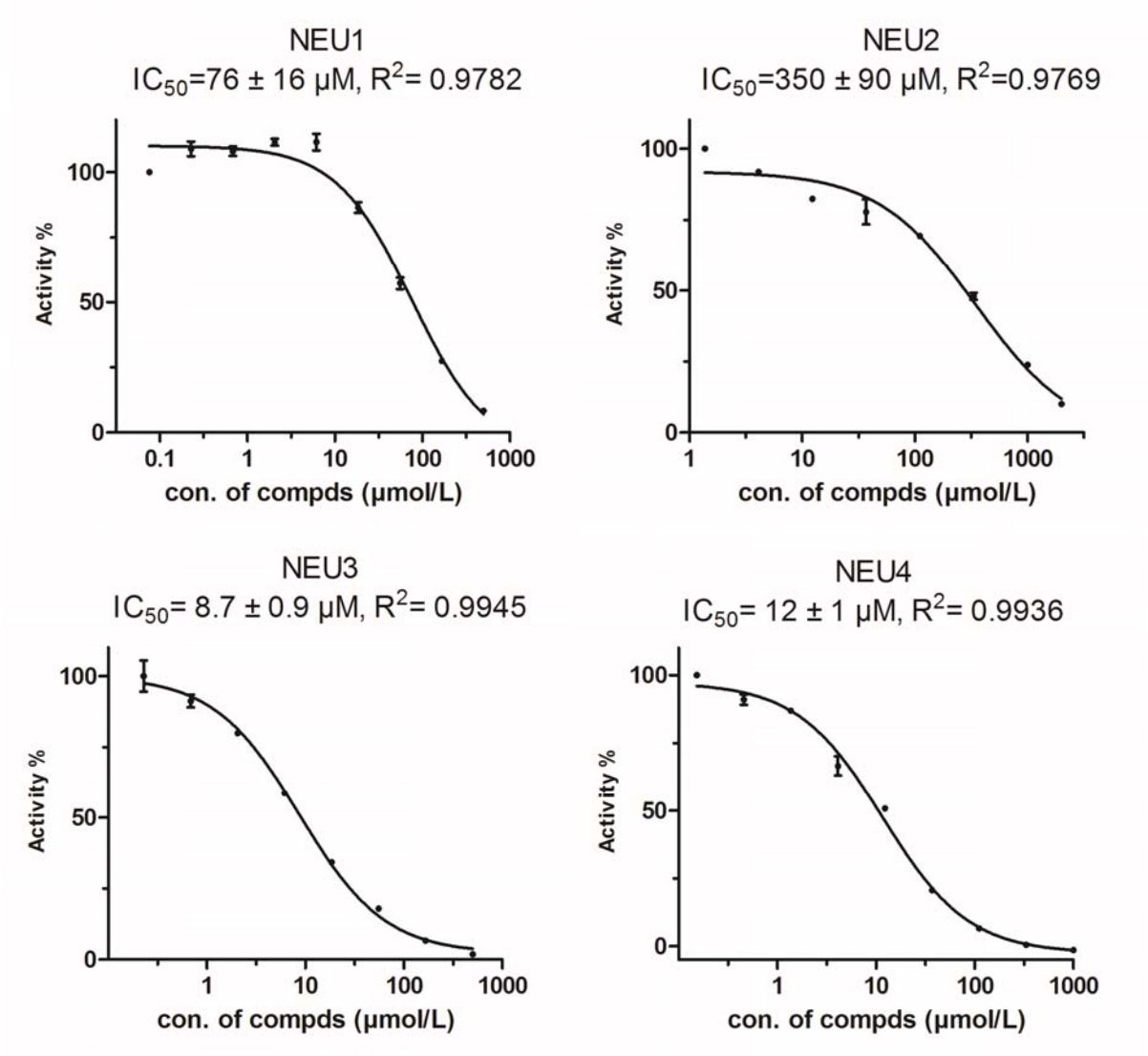
**7a**


NEU1
IC50=190 ± 40 μM, R2= 0.9805
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)
NEU2
IC50=250 ± 40μM, R2=0.9875
Activity %
100
50
0
1
10
100
1000
con. of compds (μmol/L)
NEU3
IC50= 9.3 ± 0.8 μM, R2= 0.9960
Activity %
100
50
0
1
10
100
1000
con. of compds (μmol/L)
NEU4
IC50= 28 ± 3 μM, R2= 0.9941
Activity %
100
50
0
1
10
100
1000
con. of compds (μmol/L)

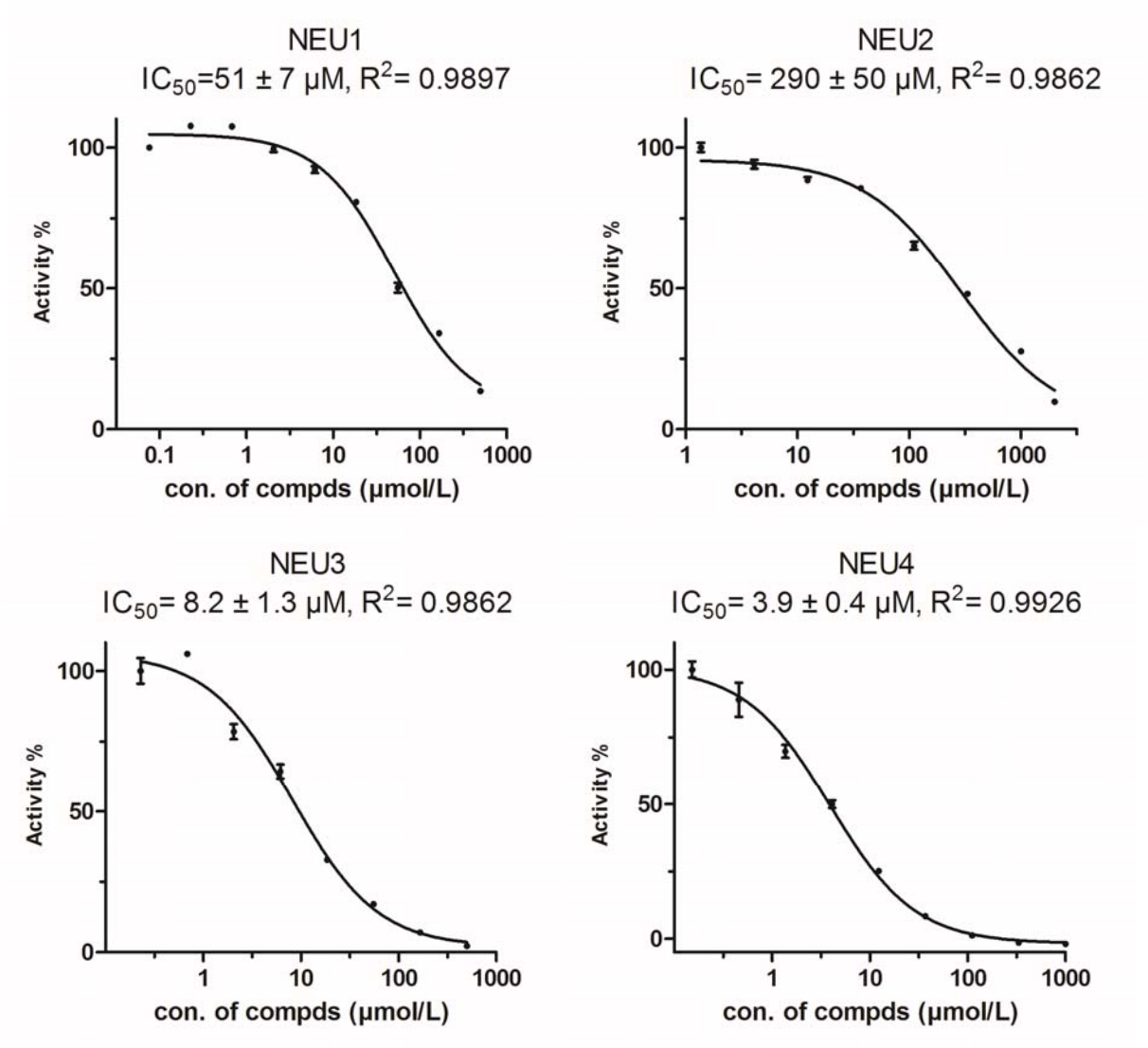
**7b**


NEU1
IC50=59 ± 5 μM, R2= 0.9950
NEU2
IC50=78 ± 20 μM, R2=0.9686
NEU3
IC50= 5.5 ± 0.5 μM, R2= 0.9949
NEU4
IC50= 11 ± 1 μM, R2 = 0.9950
Activity %
con. of compds (μmol/L)

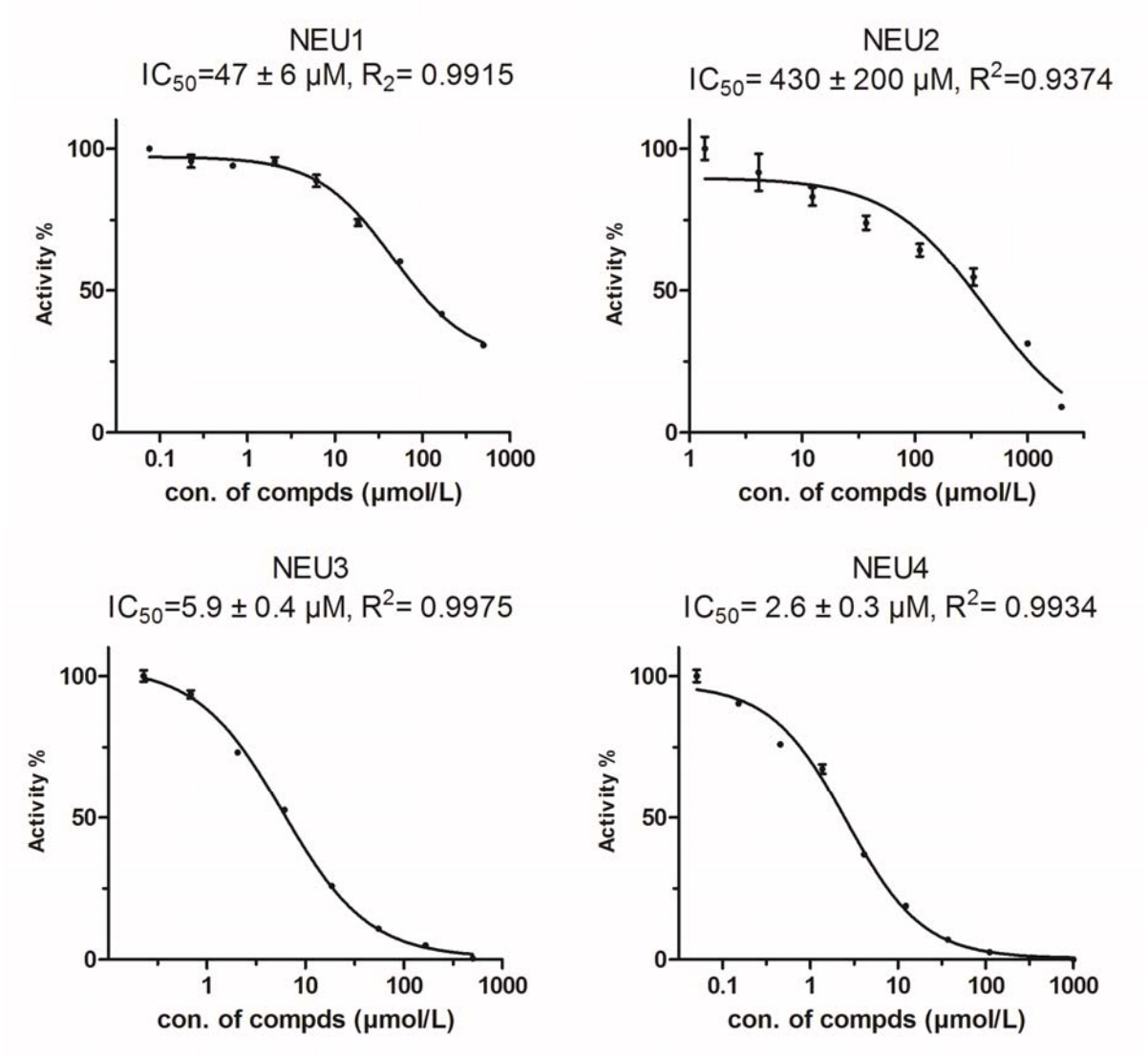
**7c**


NEU1
IC50=76 ± 16 μM, R2= 0.9782
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)
NEU2
IC50=350 ± 90 μM, R2=0.9769
Activity %
100
50
0
1
10
100
1000
con. of compds (μmol/L)
NEU3
IC50= 8.7 ± 0.9 μM, R2= 0.9945
Activity %
100
50
0
1
10
100
1000
con. of compds (μmol/L)
NEU4
IC50= 12 ± 1 μM, R2= 0.9936
Activity %
100
50
0
1
10
100
1000
con. of compds (μmol/L)

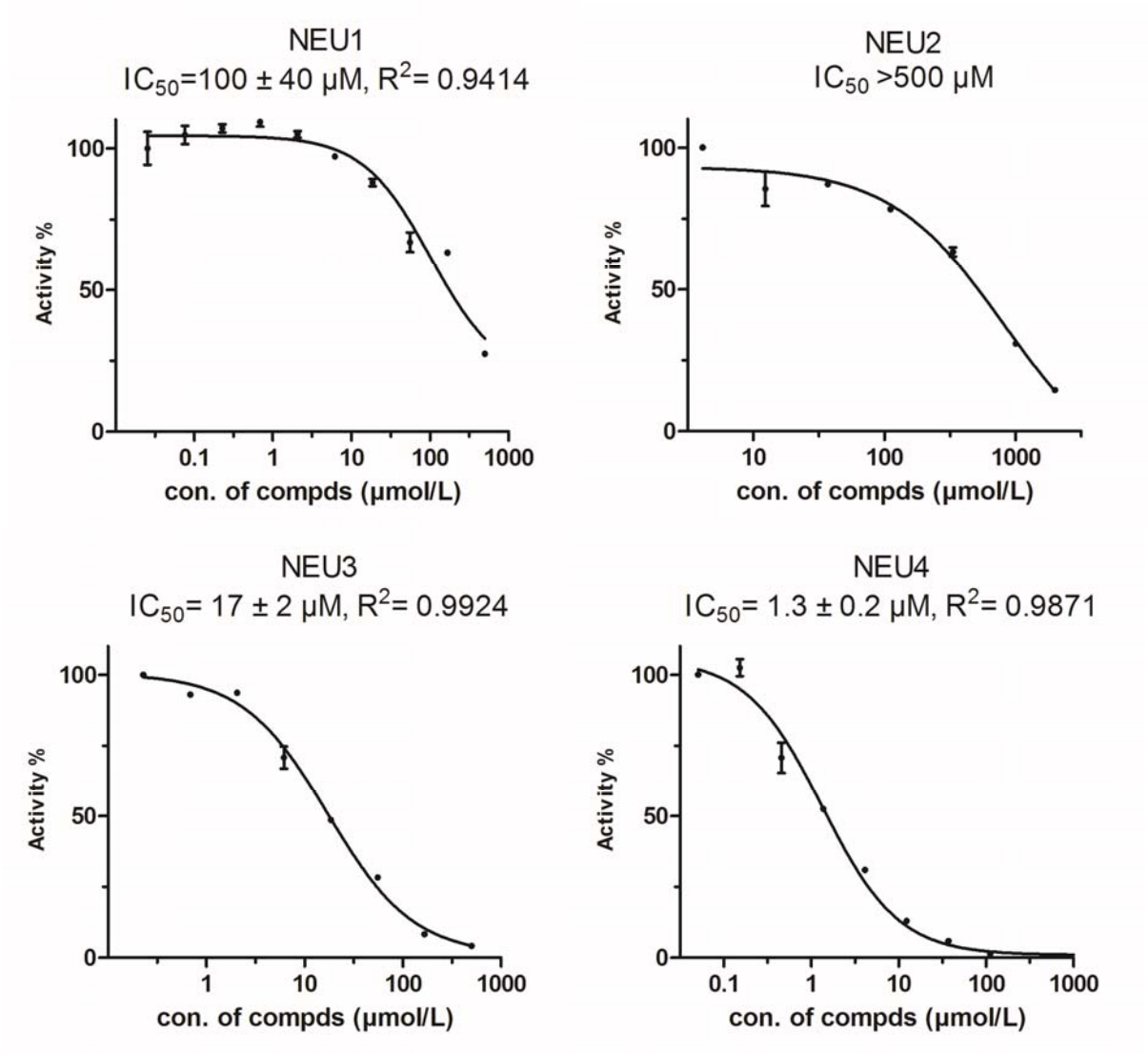
**7d**


NEU1
IC50=51 ± 7 μM, R2= 0.9897
NEU2
IC50= 290 ± 50 μM, R2= 0.9862
NEU3
IC50= 8.2 ± 1.3 μM, R2= 0.9862
NEU4
IC50= 3.9 ± 0.4 μM, R2= 0.9926
Activity %
con. of compds (μmol/L)

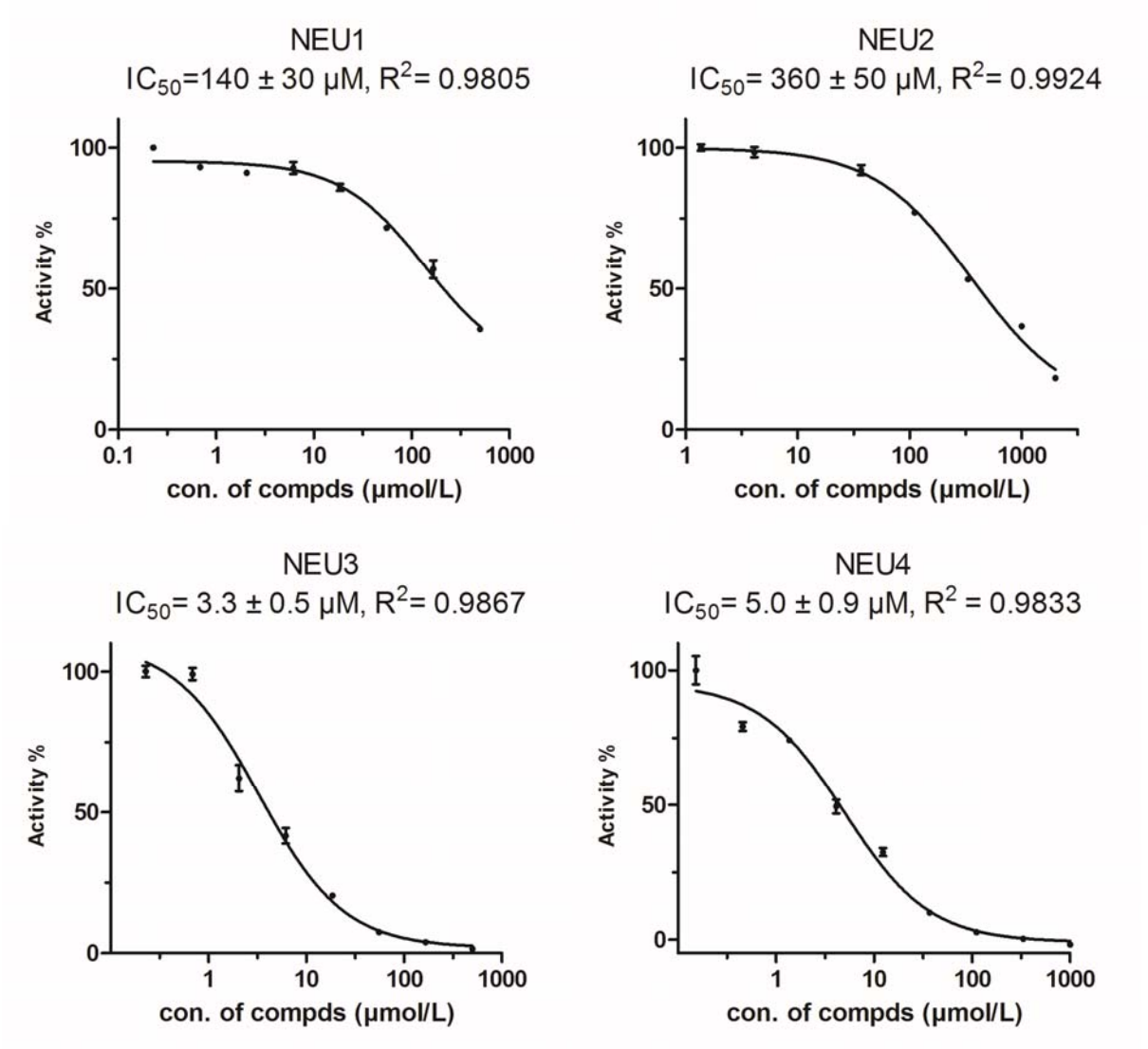
**7e**


NEU1
IC50=47 ± 6 μM, R2= 0.9915
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)
NEU2
IC50= 430 ± 200 μM, R2=0.9374
Activity %
100
50
0
1
10
100
1000
con. of compds (μmol/L)
NEU3
IC50=5.9 ± 0.4 μM, R2= 0.9975
Activity %
100
50
0
1
10
100
1000
con. of compds (μmol/L)
NEU4
IC50= 2.6 ± 0.3 μM, R2= 0.9934
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)

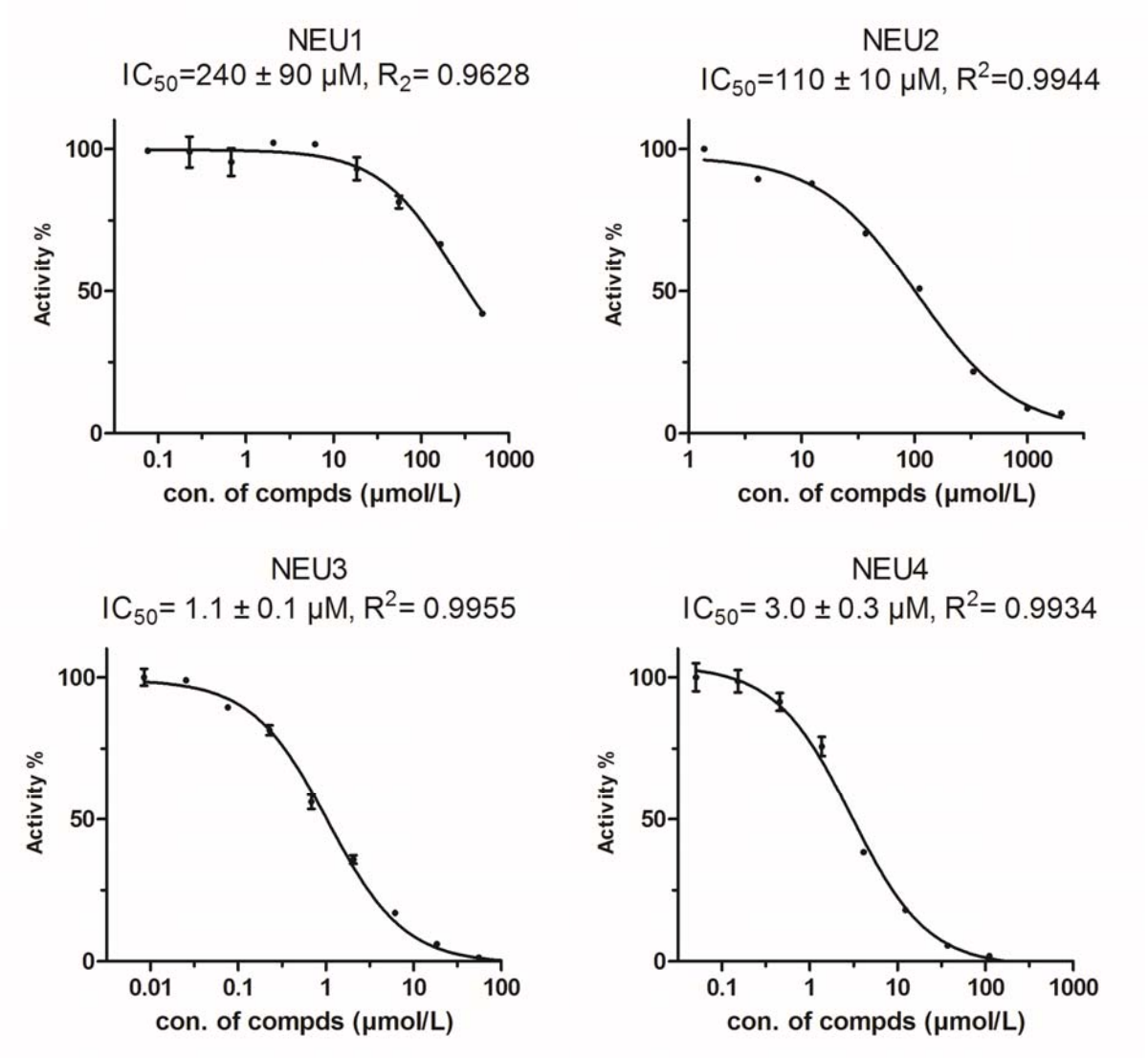
**7f**


NEU1
IC50=100 ± 40 μM, R2= 0.9414
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)
NEU2
IC50 >500 μM
Activity %
100
50
0
10
100
1000
con. of compds (μmol/L)
NEU3
IC50= 17 ± 2 μM, R2= 0.9924
Activity %
100
50
0
1
10
100
1000
con. of compds (μmol/L)
NEU4
IC50= 1.3 ± 0.2 μM, R2= 0.9871
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)

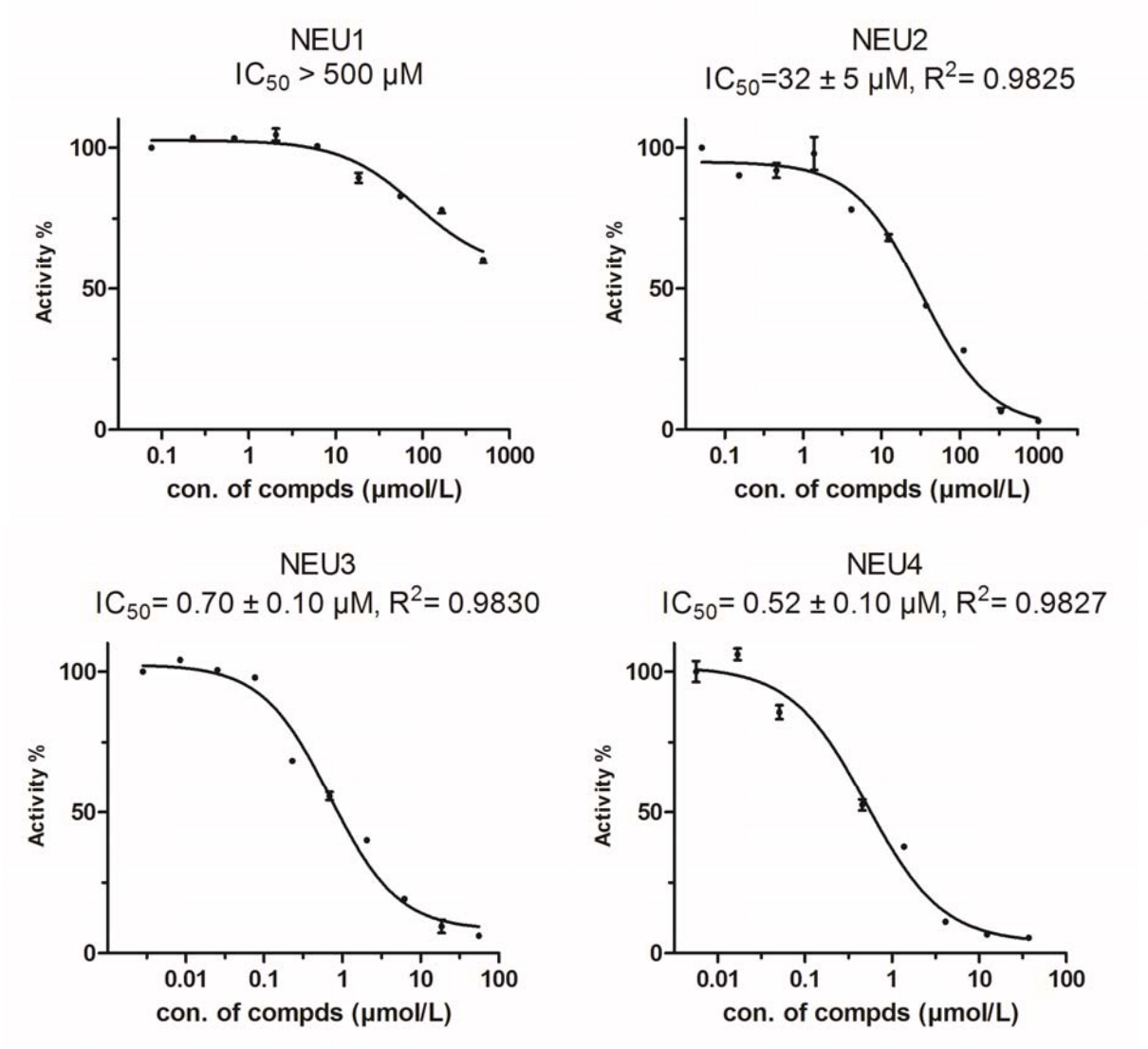
**7g**


NEU1
IC50=140 ± 30 μM, R2= 0.9805
NEU2
IC50= 360 ± 50 μM, R2= 0.9924
NEU3
IC50= 3.3 ± 0.5 μM, R2= 0.9867
NEU4
IC50= 5.0 ± 0.9 μM, R2 = 0.9833
Activity %
con. of compds (μmol/L)

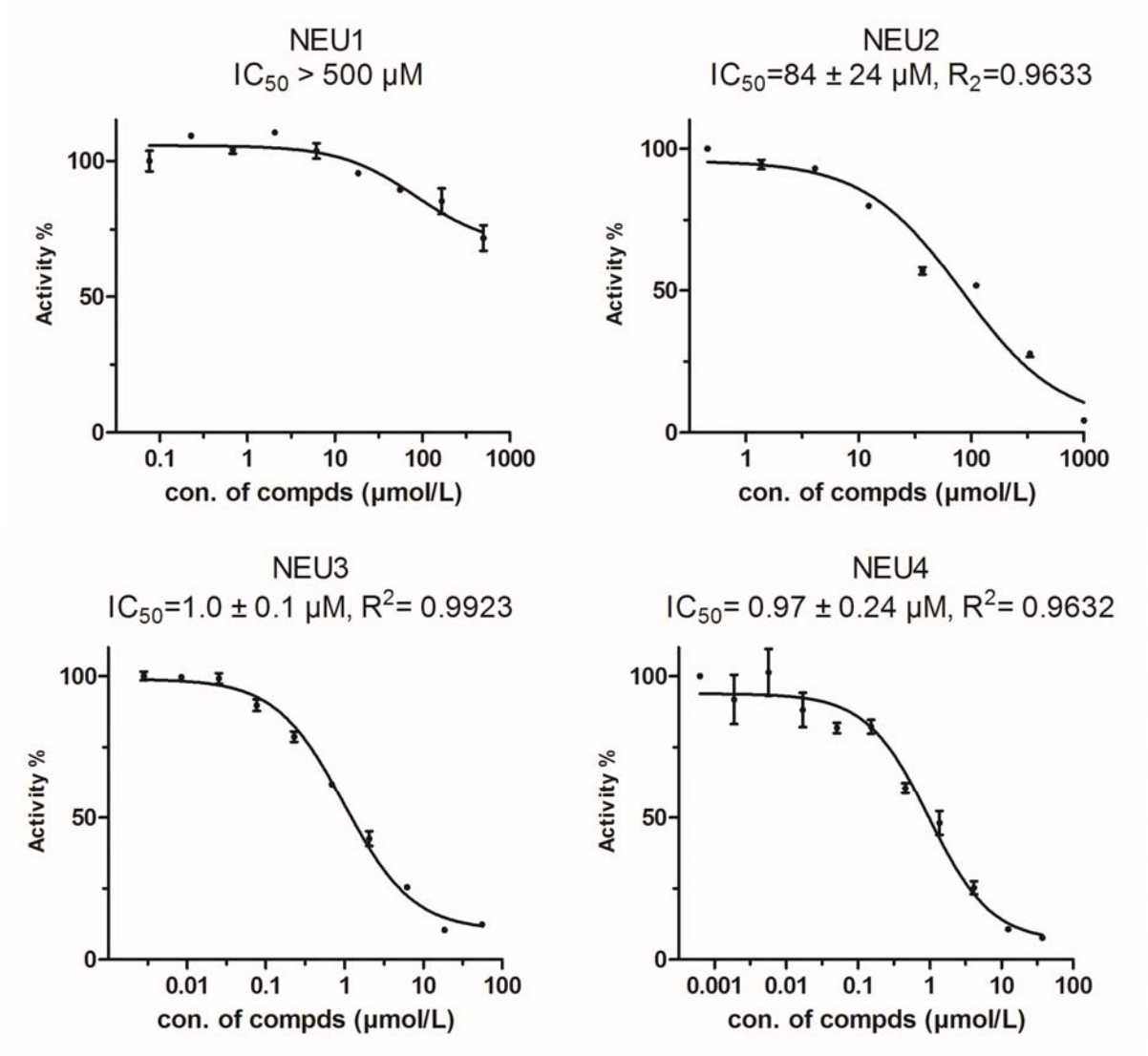
**7h**


NEU1
IC50=240 ± 90 μM, R2= 0.9628
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)
NEU2
IC50=110 ± 10 μM, R2=0.9944
Activity %
100
50
0
1
10
100
1000
con. of compds (μmol/L)
NEU3
IC50= 1.1 ± 0.1 μM, R2= 0.9955
Activity %
100
50
0
0.01
0.1
1
10
100
con. of compds (μmol/L)
NEU4
IC50= 3.0 ± 0.3 μM, R2= 0.9934
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)

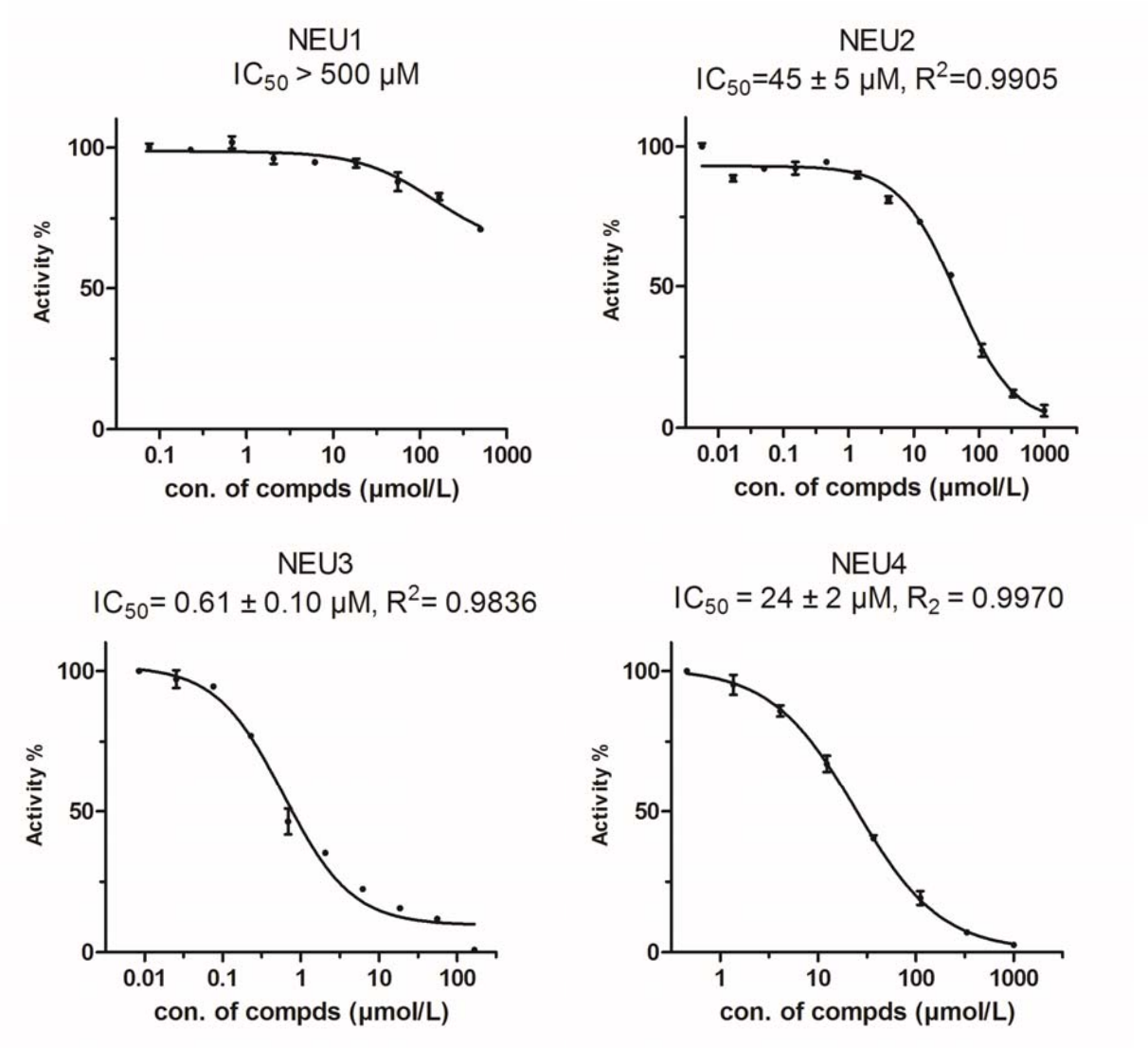
**7i**


NEU1
IC50 > 500 μM
NEU2
IC50=32 ± 5 μM, R2= 0.9825
NEU3
IC50= 0.70 ± 0.10 μM, R2= 0.9830
NEU4
IC50= 0.52 ± 0.10 μM, R2= 0.9827
Activity %
con. of compds (μmol/L)

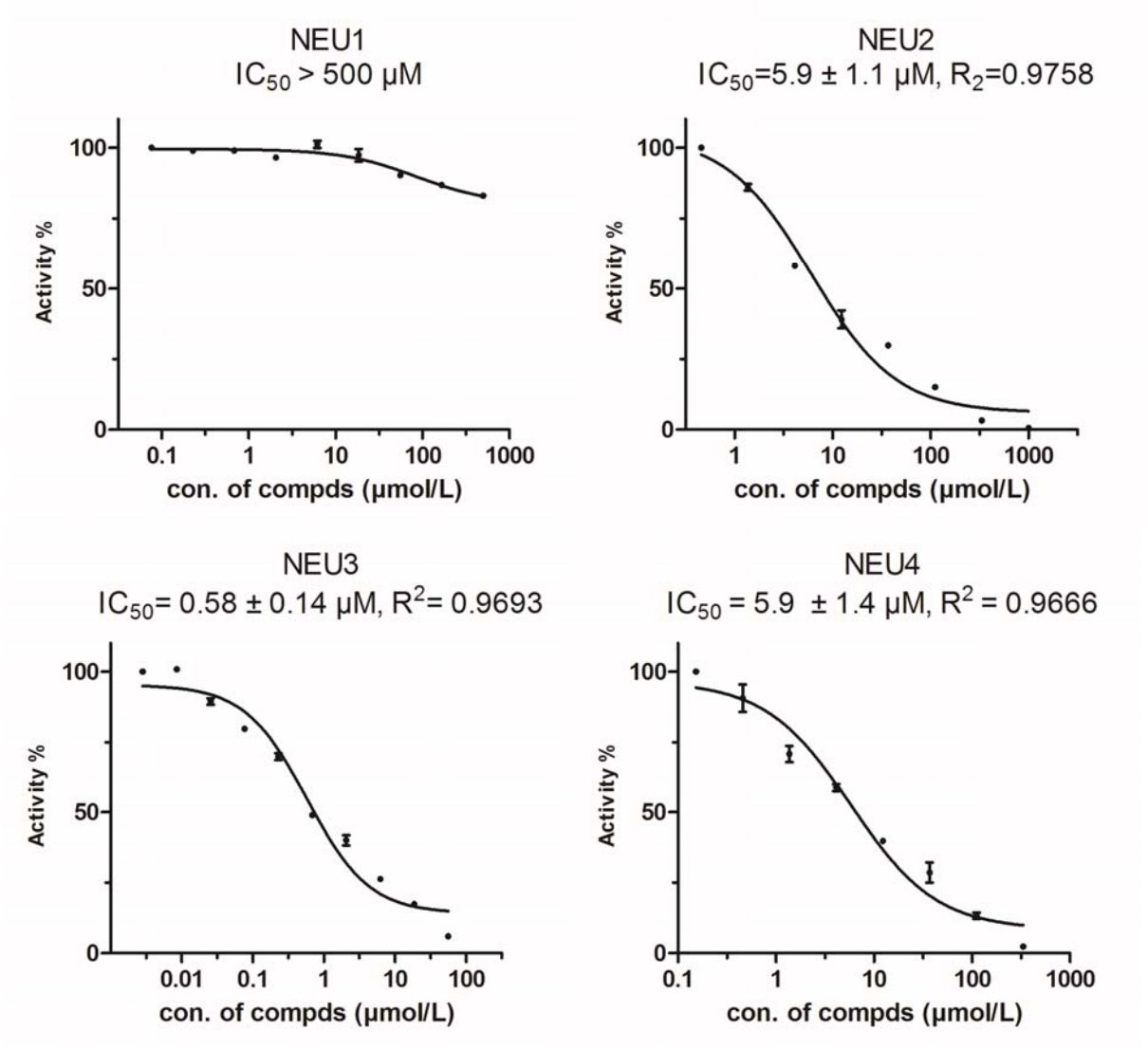
**7j**


NEU1
IC50 > 500 μM
Activity %
con. of compds (μmol/L)
NEU2
IC50=84 ± 24 μM, R2=0.9633
Activity %
con. of compds (μmol/L)
NEU3
IC50=1.0 ± 0.1 μM, R2= 0.9923
Activity %
con. of compds (μmol/L)
NEU4
IC50= 0.97 ± 0.24 μM, R2= 0.9632
Activity %
con. of compds (μmol/L)

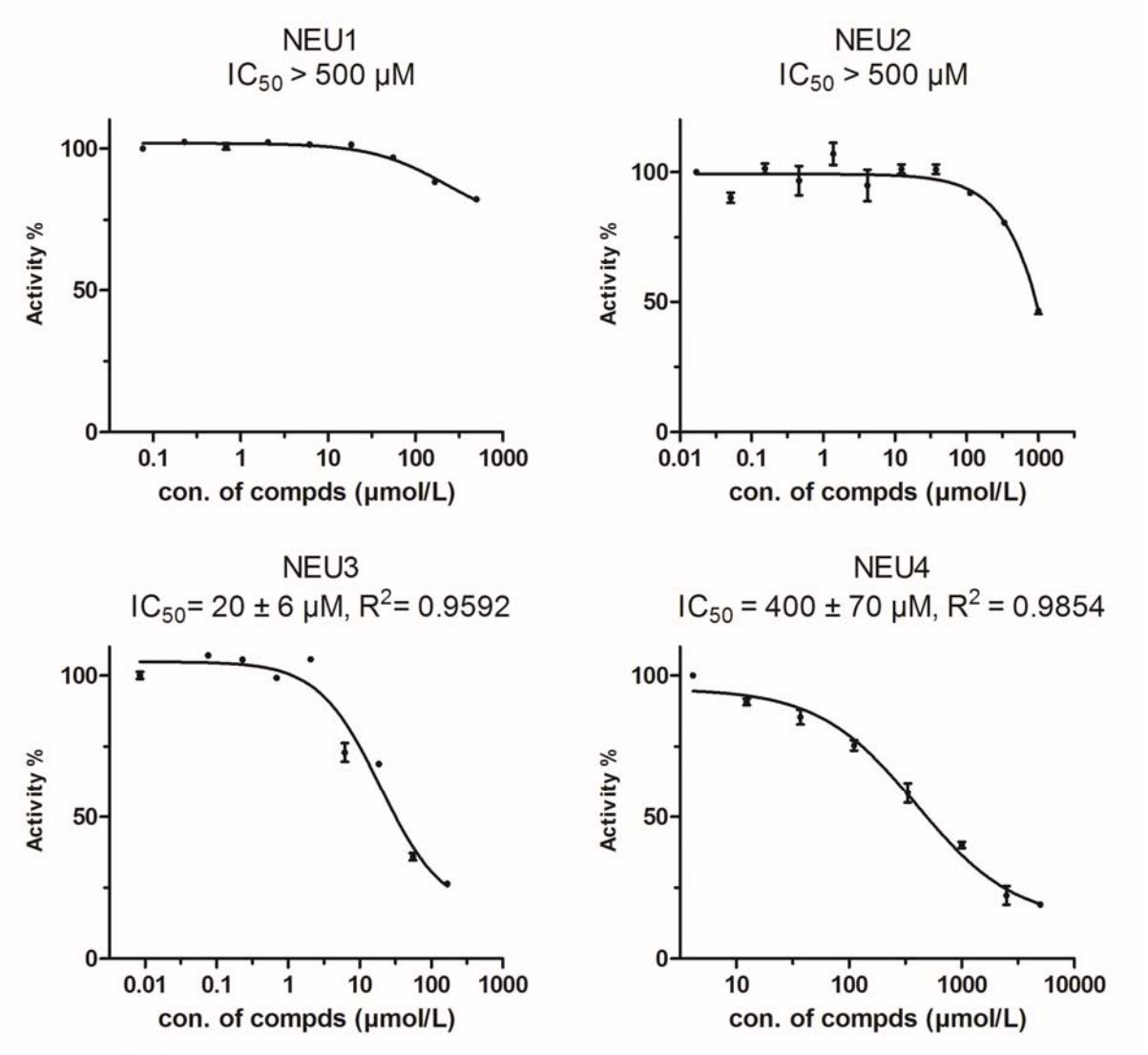
**8a**


NEU1
IC50 > 500 μM
NEU2
IC50=45 ± 5 μM, R2=0.9905
NEU3
IC50= 0.61 ± 0.10 μM, R2= 0.9836
NEU4
IC50 = 24 ± 2 μM, R2 = 0.9970
Activity %
con. of compds (μmol/L)

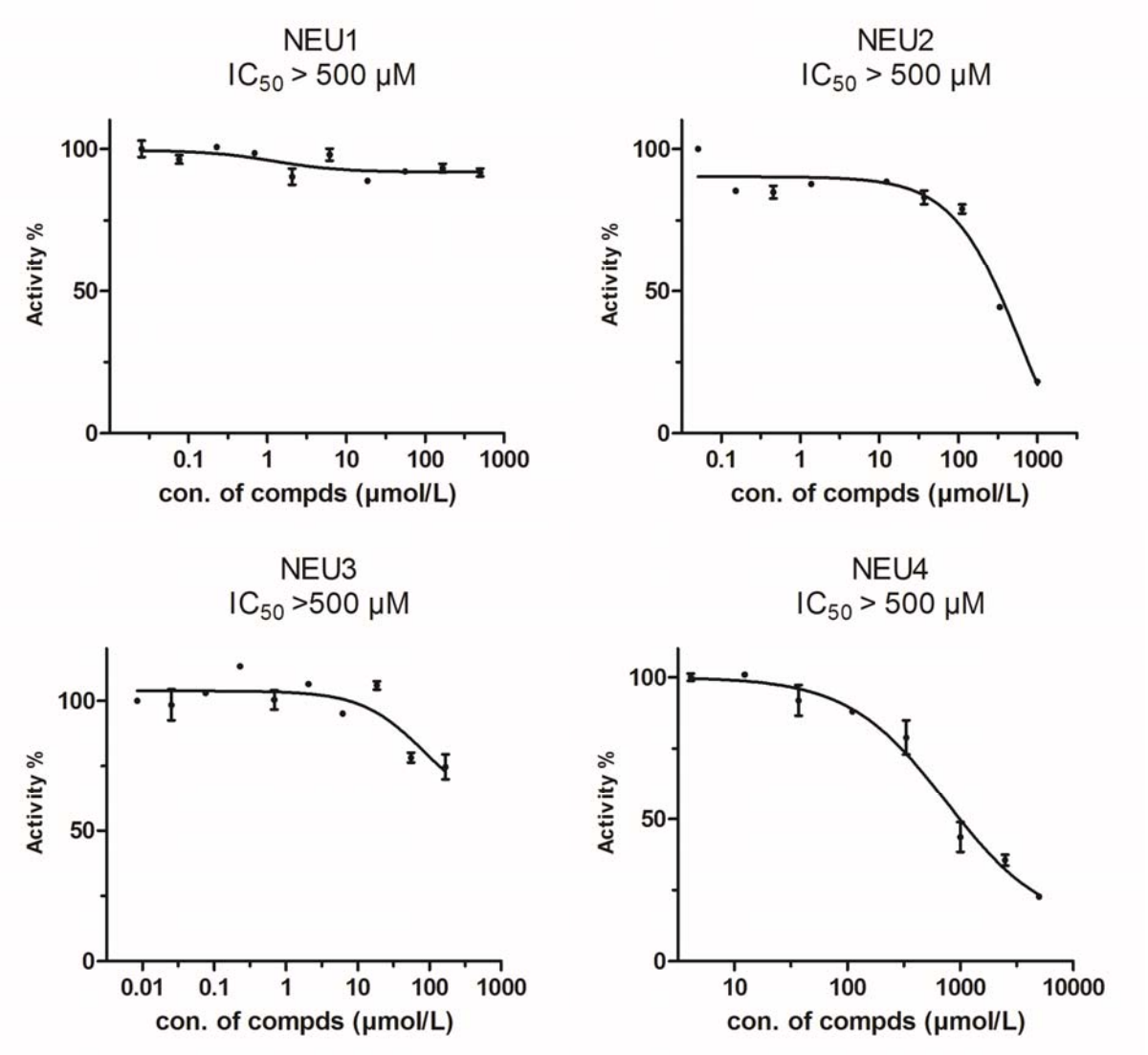
**8b**


NEU1
IC50 > 500 μM
NEU2
IC50=5.9 ± 1.1 μM, R2=0.9758
NEU3
IC50= 0.58 ± 0.14 μM, R2= 0.9693
NEU4
IC50 = 5.9 ± 1.4 μM, R2 = 0.9666
Activity %
con. of compds (μmol/L)

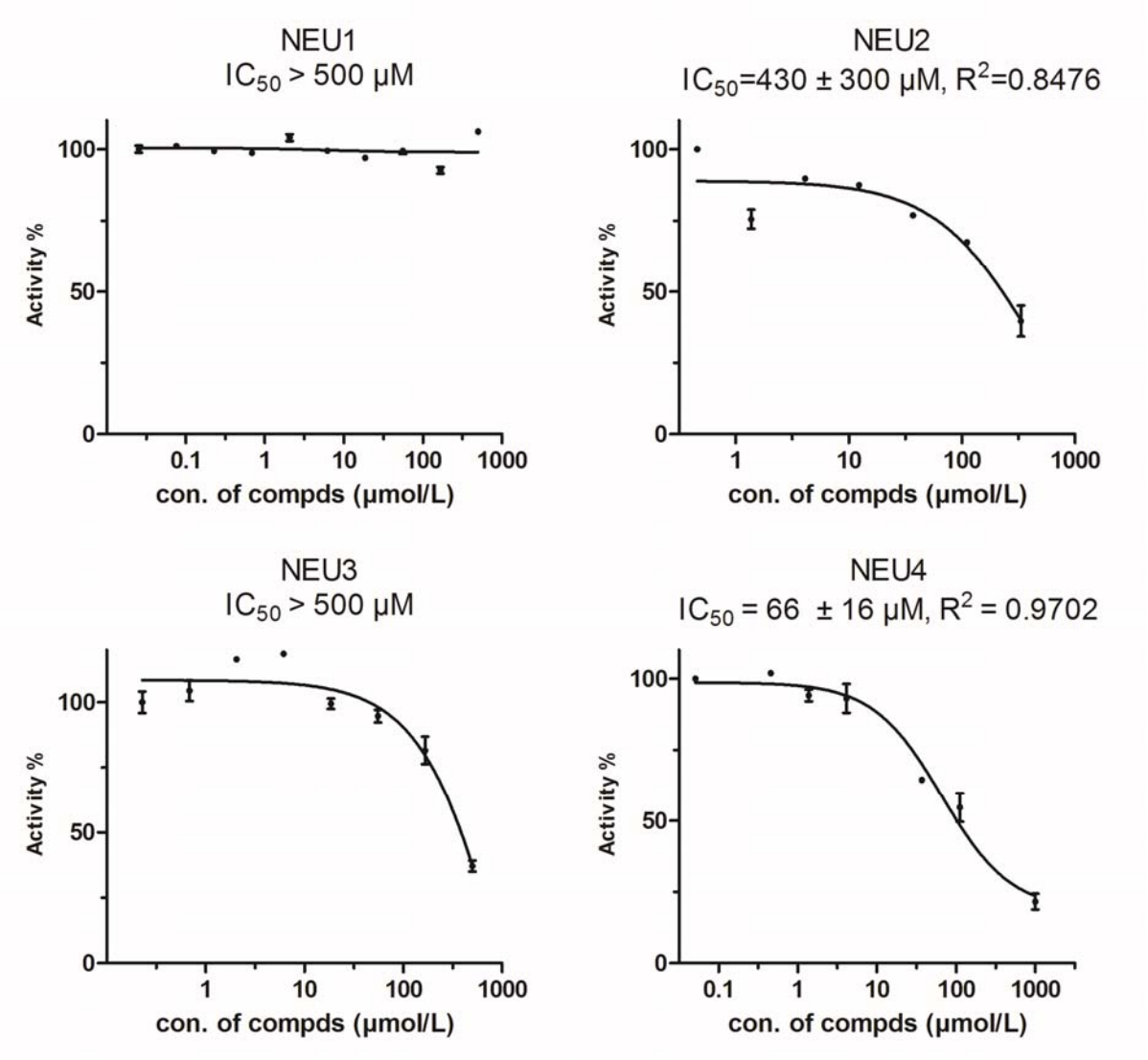
**13**


NEU1
IC50 > 500 μM
Activity %
con. of compds (μmol/L)
NEU2
IC50 > 500 μM
Activity %
con. of compds (μmol/L)
NEU3
IC50= 20 ± 6 μM, R2= 0.9592
Activity %
con. of compds (μmol/L)
NEU4
IC50 = 400 ± 70 μM, R2 = 0.9854
Activity %
con. of compds (μmol/L)

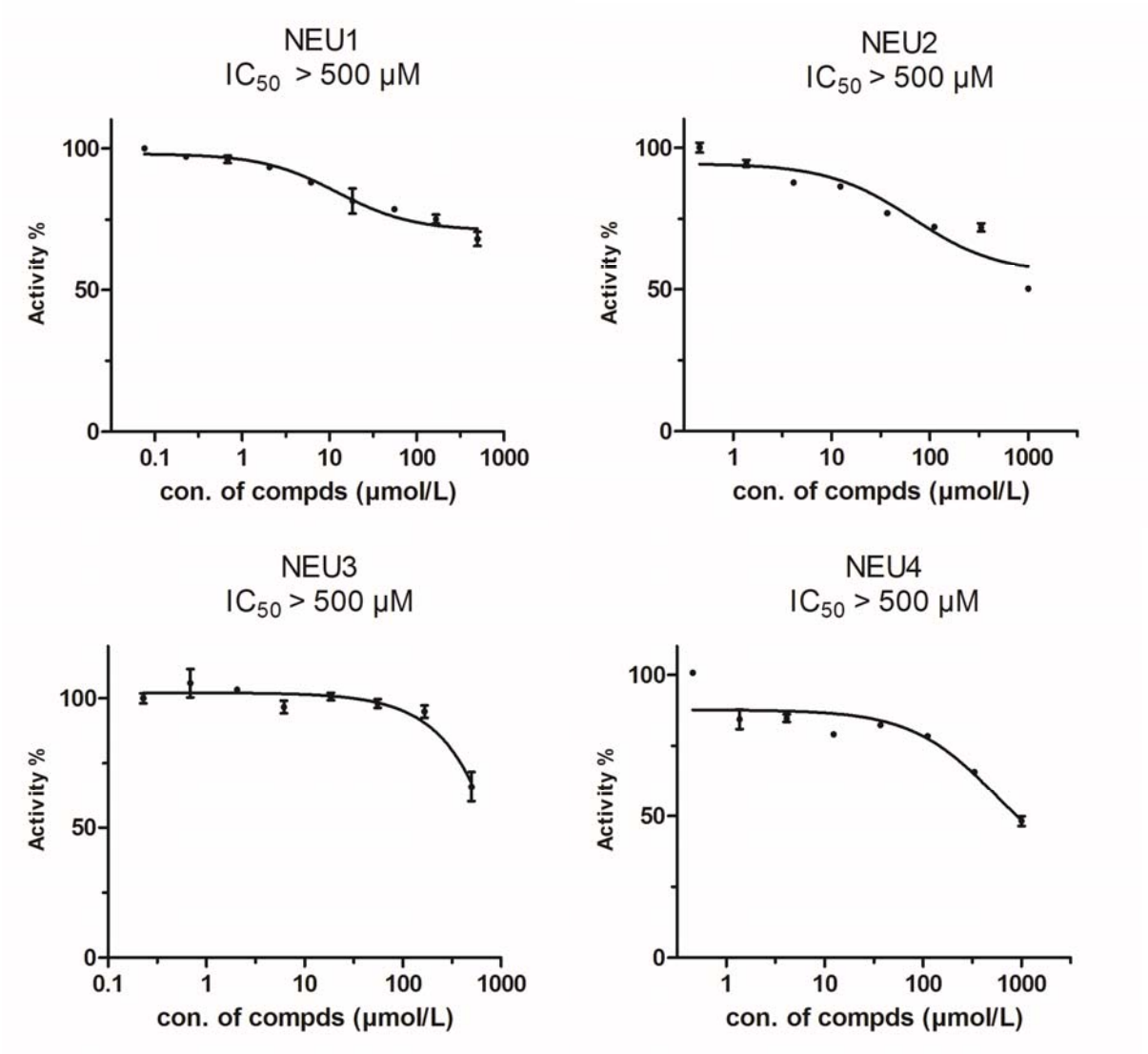
**15**


NEU1
IC50 > 500 μM
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)
NEU2
IC50 > 500 μM
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)
NEU3
IC50 >500 μM
Activity %
100
50
0
0.01
0.1
1
10
100
1000
con. of compds (μmol/L)
NEU4
IC50 > 500 μM
Activity %
100
50
0
10
100
1000
10000
con. of compds (μmol/L)

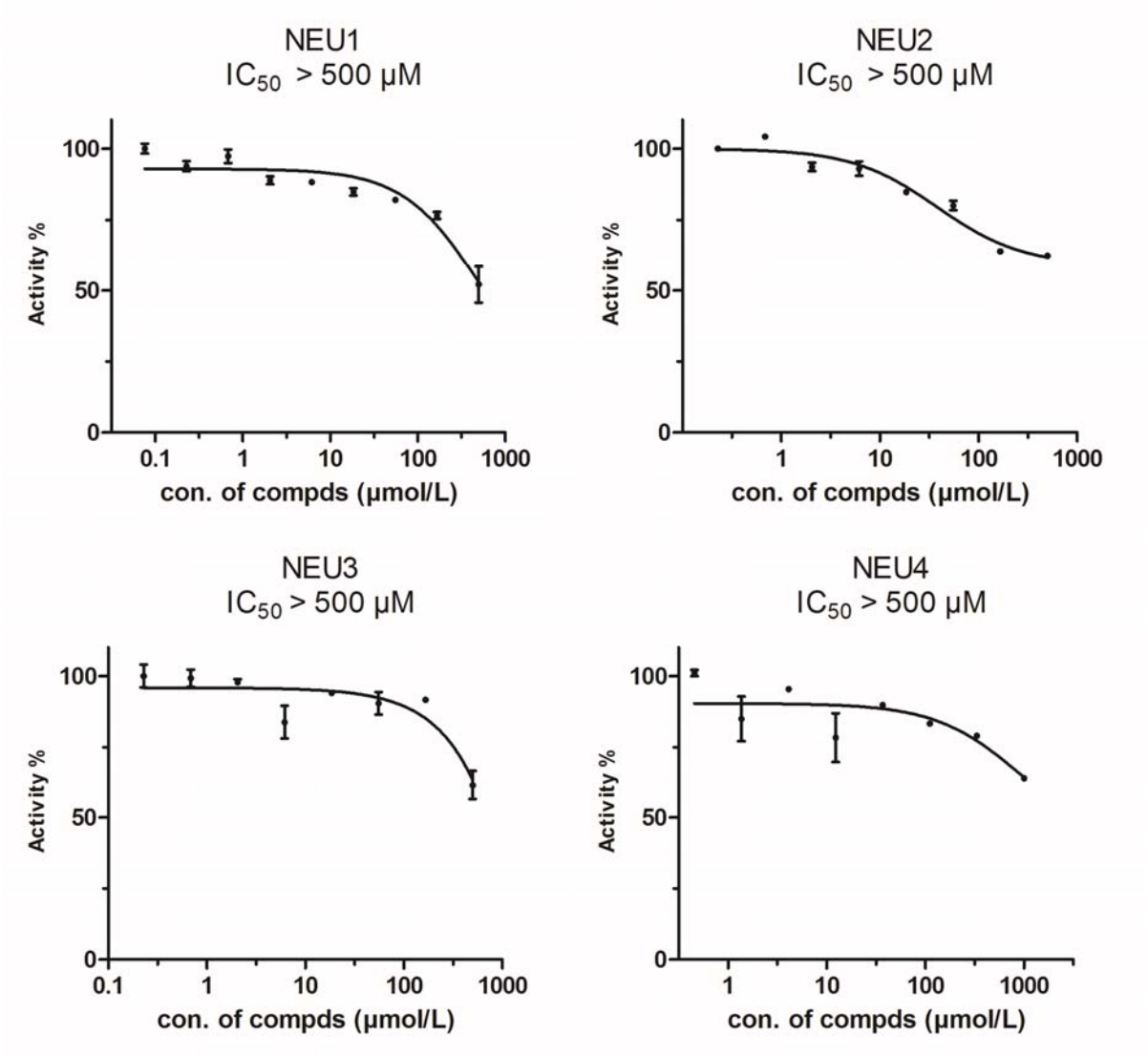
**18**


NEU1
IC50 > 500 μM
NEU2
IC50=430 ± 300 μM, R2=0.8476
NEU3
IC50 > 500 μM
NEU4
IC50 = 66 ± 16 μM, R2 = 0.9702
Activity %
con. of compds (μmol/L)

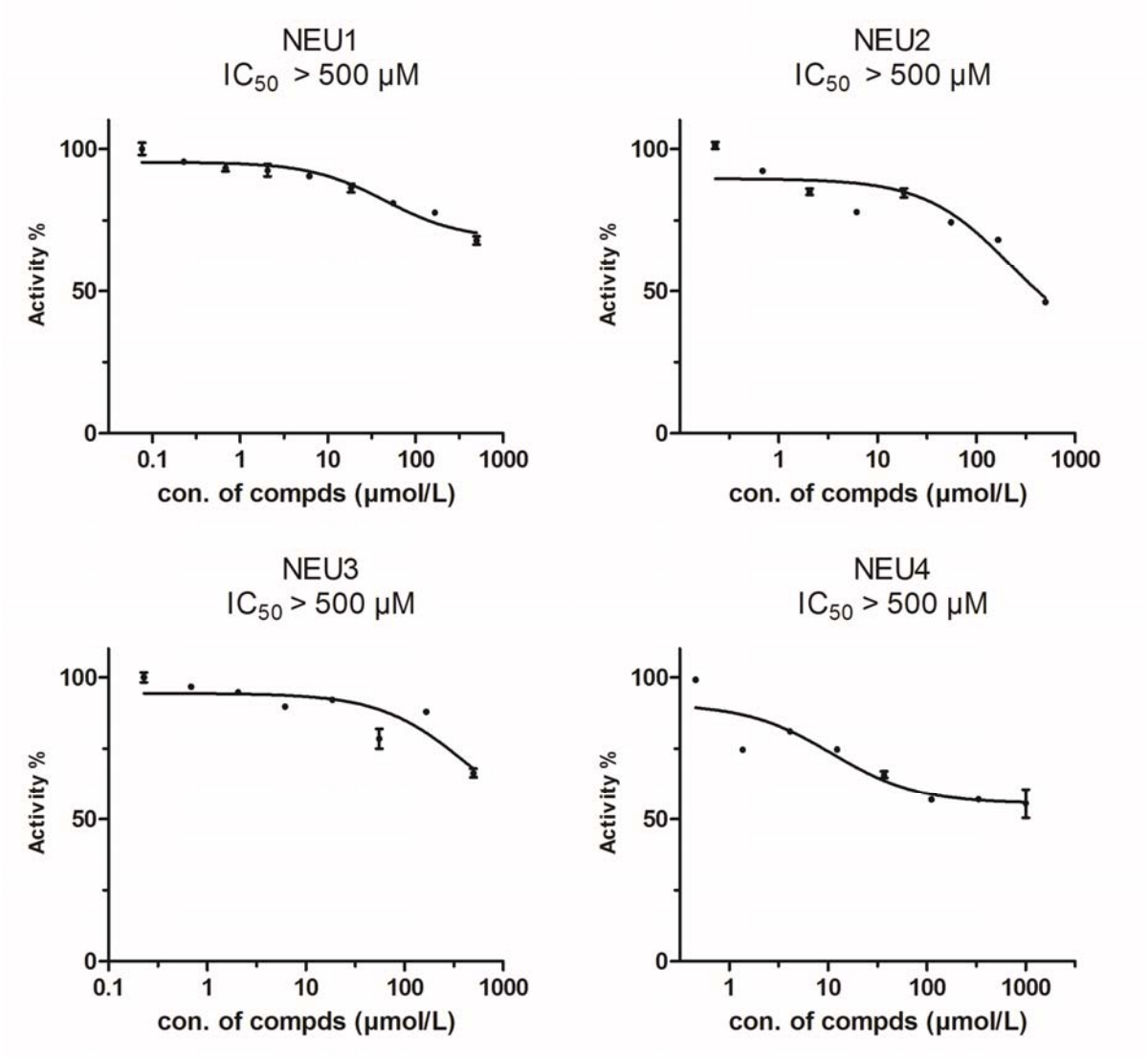
**25a**


NEU1
IC50 > 500 μM
NEU2
IC50 > 500 μM
NEU3
IC50 > 500 μM
NEU4
IC50 > 500 μM
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)

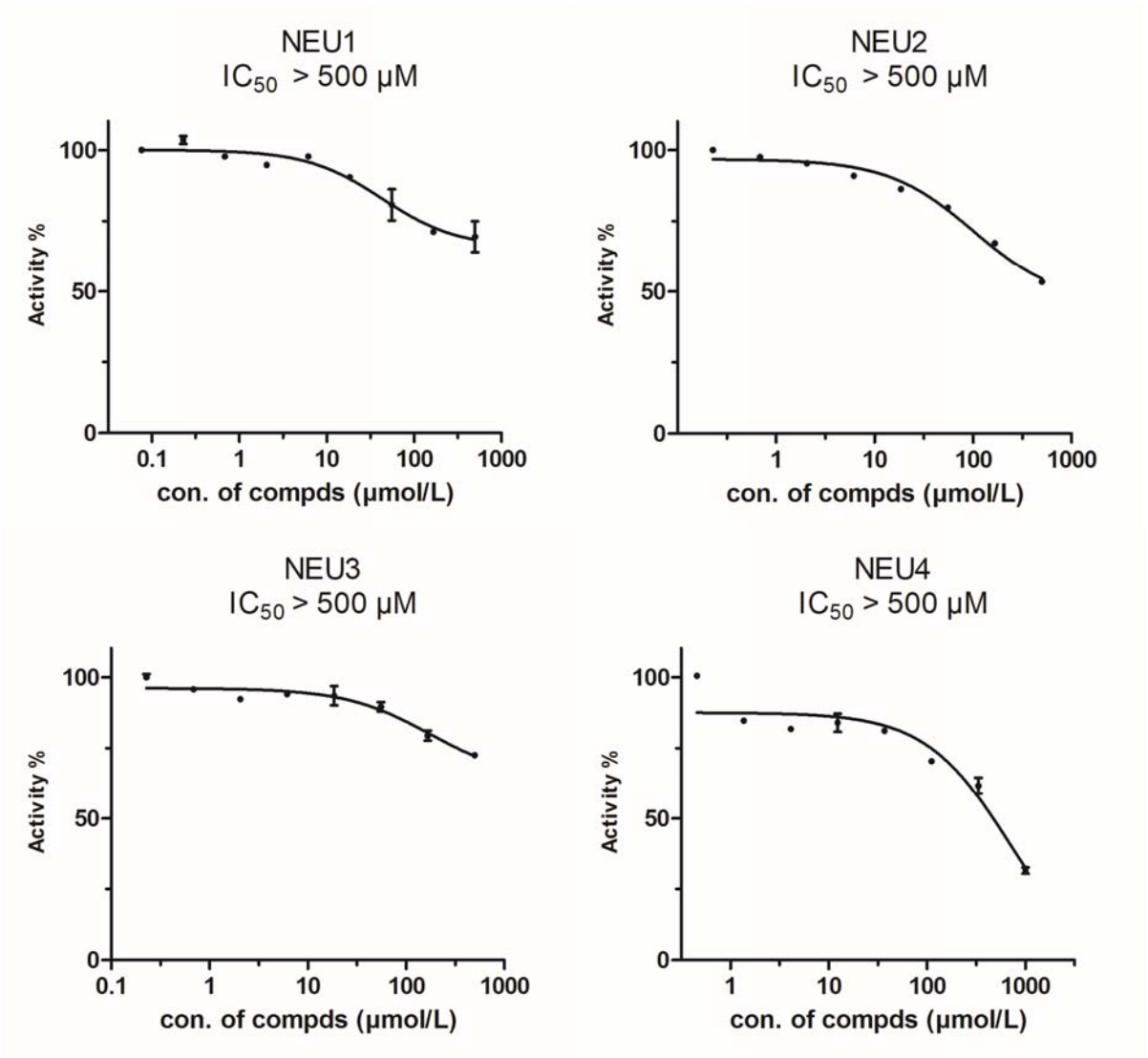
**25b**


NEU1
IC50 > 500 μM
NEU2
IC50 > 500 μM
NEU3
IC50 > 500 μM
NEU4
IC50 > 500 μM
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)

**25c**

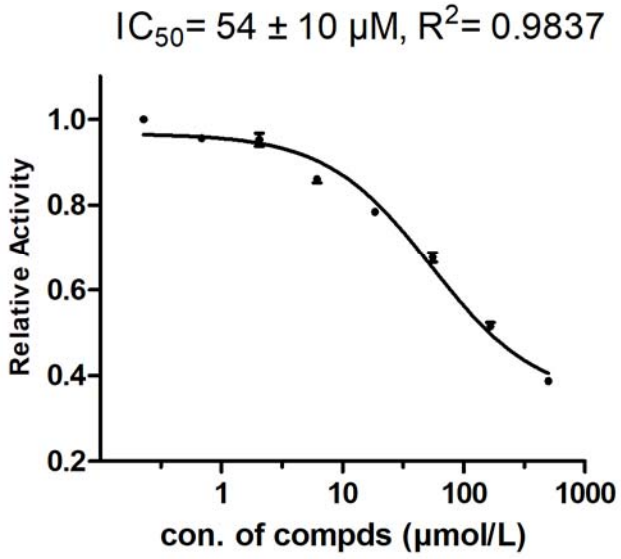

NEU1
IC50 > 500 μM
NEU2
IC50 > 500 μM
NEU3
IC50 > 500 μM
NEU4
IC50 > 500 μM
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)

**25d**

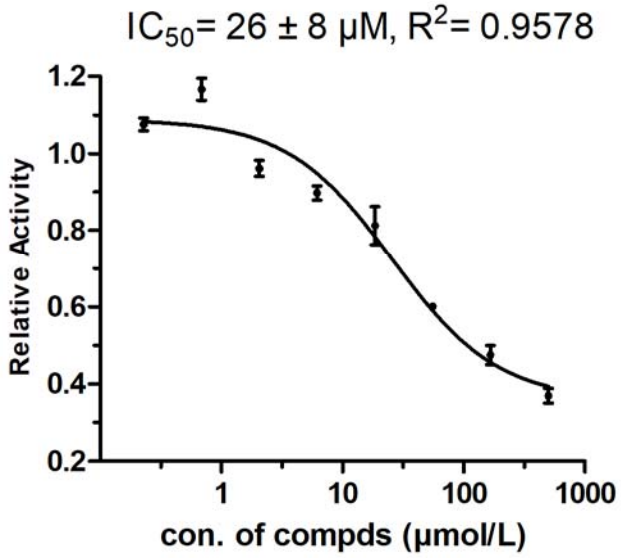

NEU1
IC50 > 500 μM
NEU2
IC50 > 500 μM
NEU3
IC50 > 500 μM
NEU4
IC50 > 500 μM
Activity %
100
50
0
0.1
1
10
100
1000
con. of compds (μmol/L)

**$IC_{50}$ curves using GM3 as substrate**

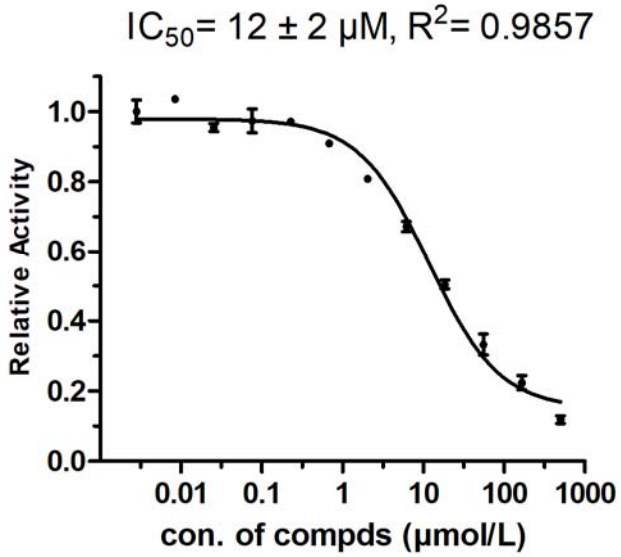
**DANA with NEU3**



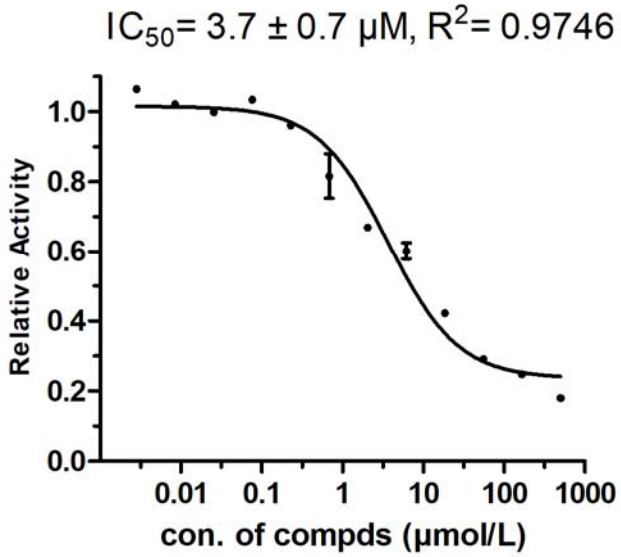

**DANA with NEU4**



**7i with NEU3**




**7i with NEU4**



**8b with NEU3**

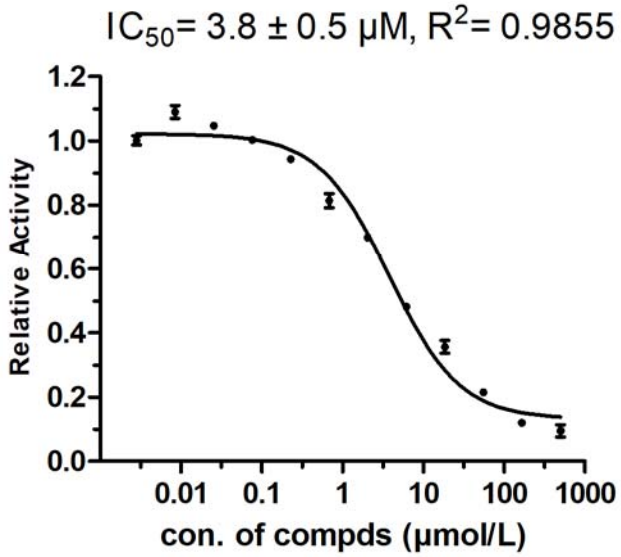
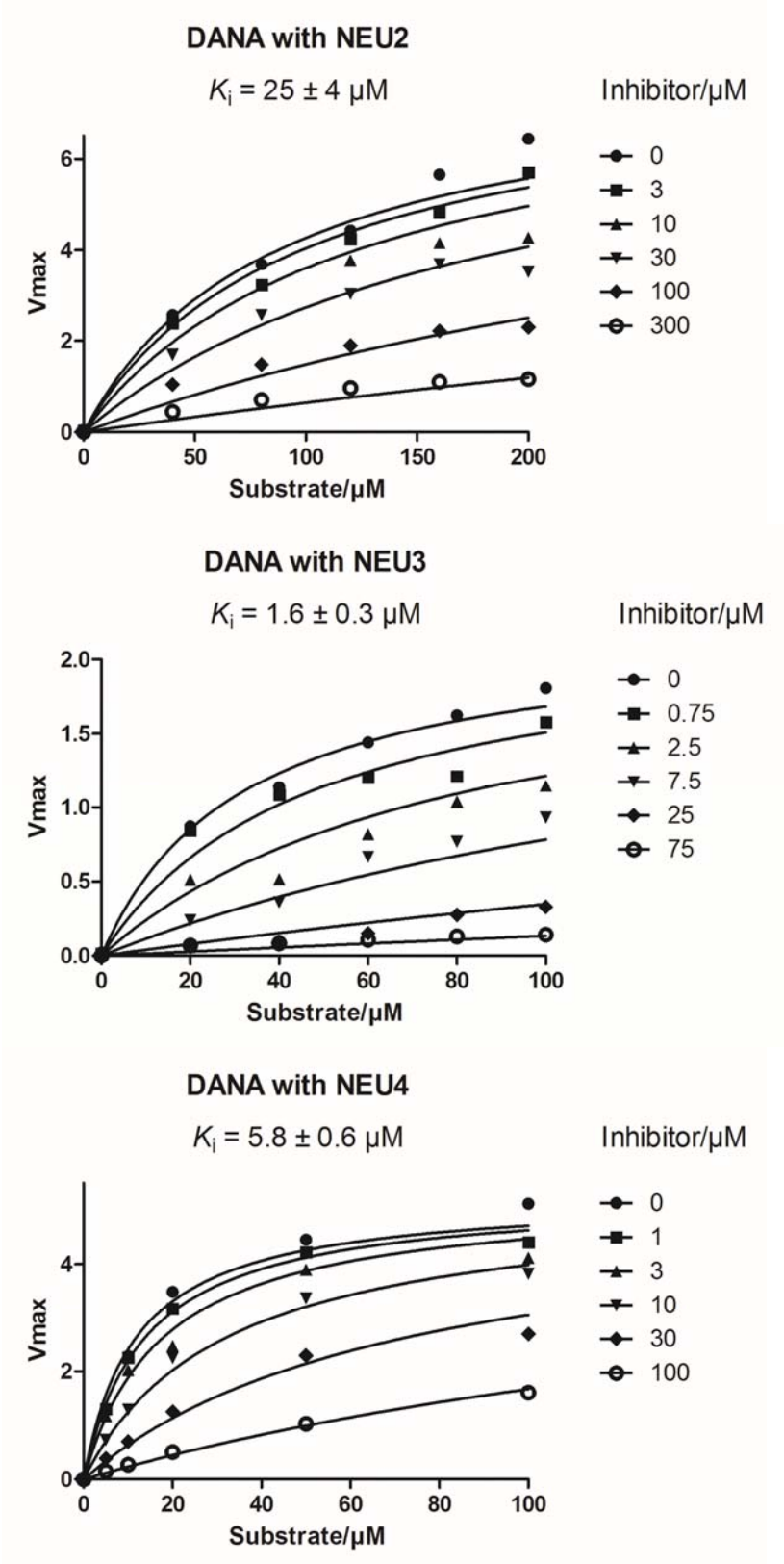
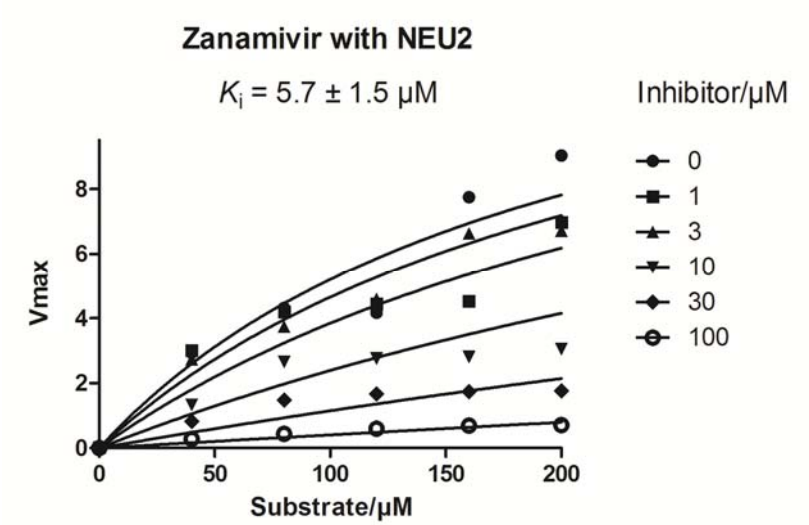
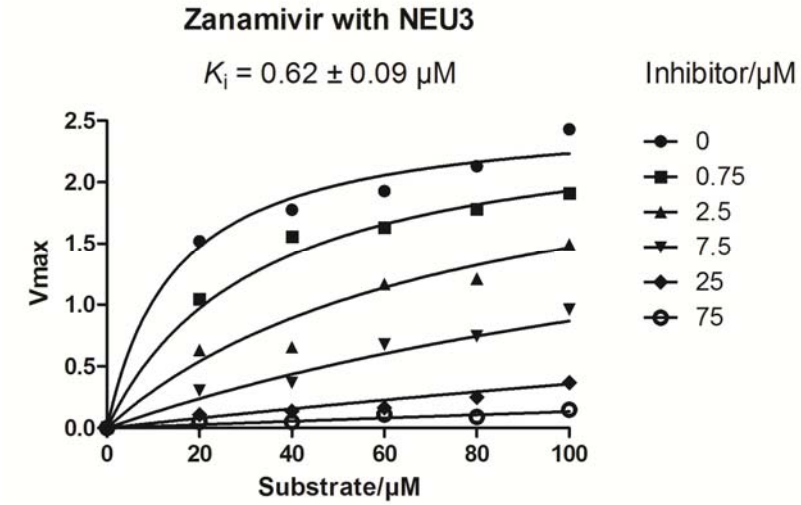
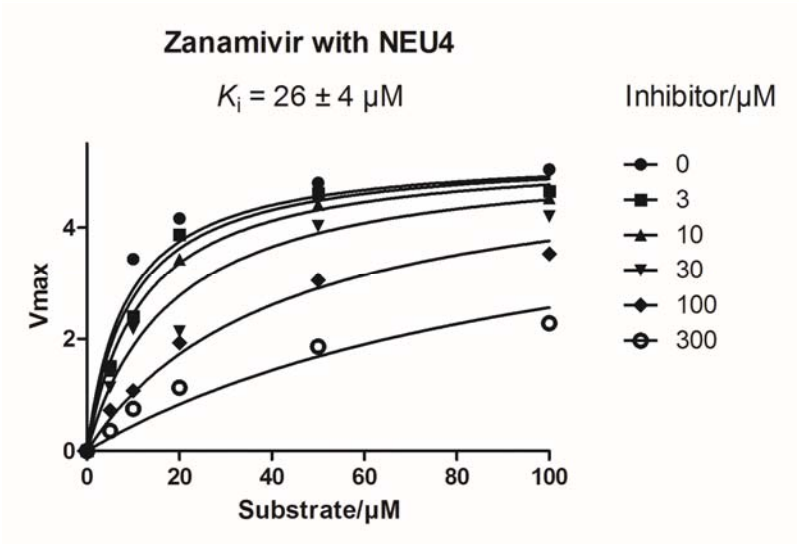

IC50= 3.8 ± 0.5 μM, R2= 0.9855
Relative Activity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.01
0.1
1
10
100
1000
con. of compds (μmol/L)

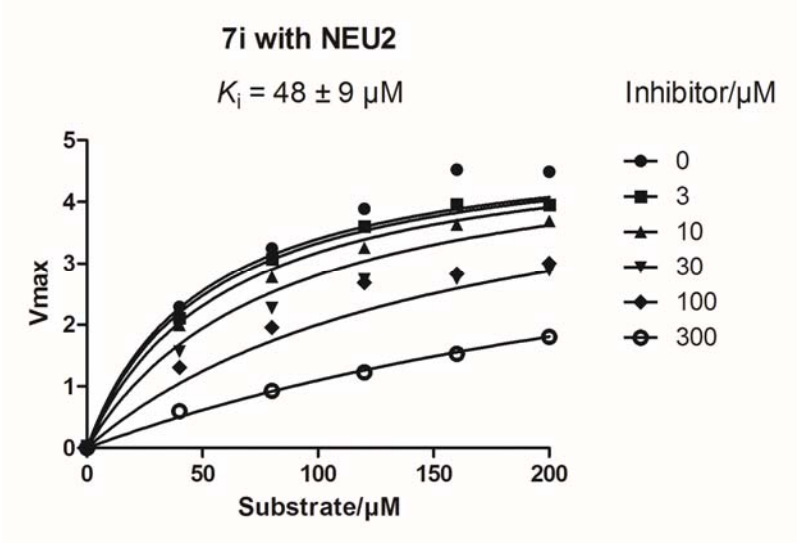
**Figure S.2: $K_i$ determinations**

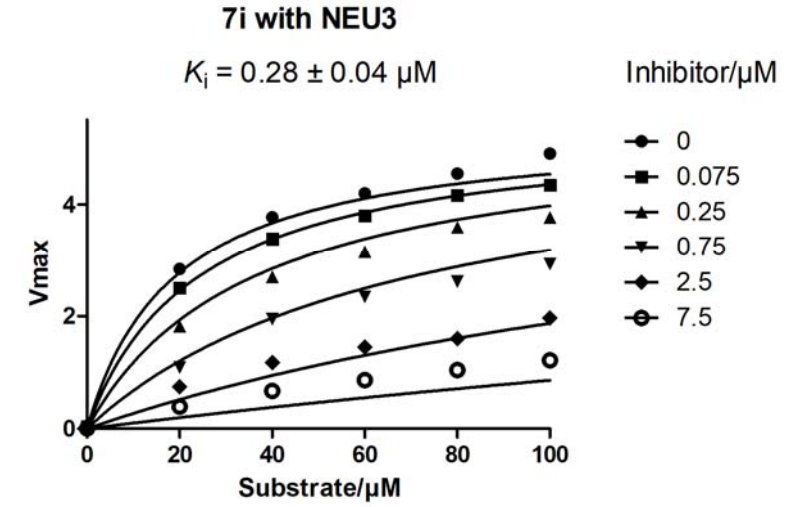


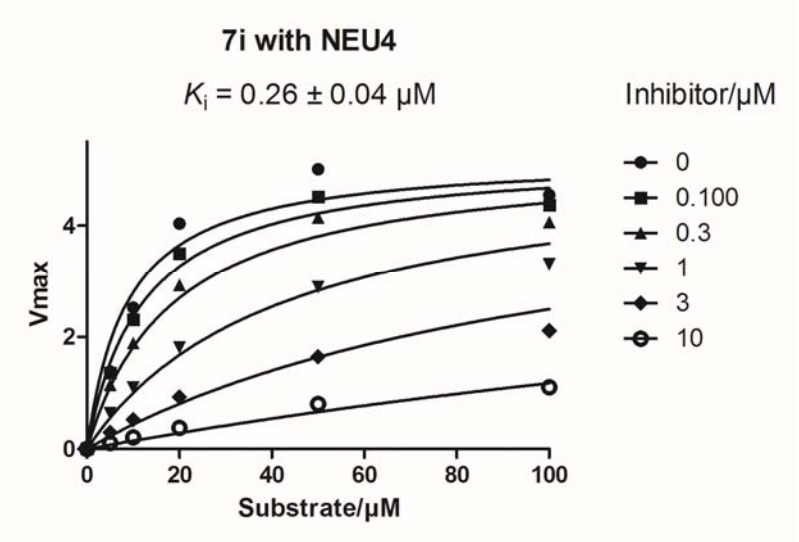

Zanamivir with NEU2
$K_i$ = 5.7 ± 1.5 μM
Inhibitor/μM
0
1
3
10
30
100
Vmax
Substrate/μM
0
2
4
6
8
0
50
100
150
200

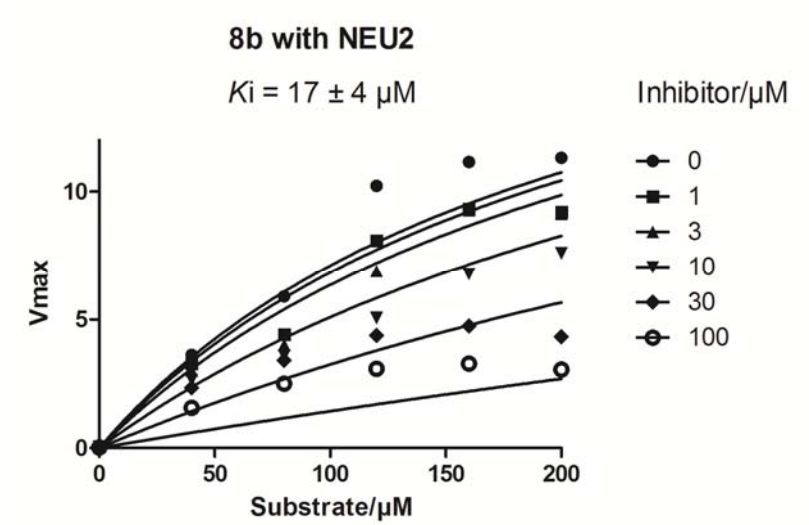

Zanamivir with NEU3
$K_i$ = 0.62 ± 0.09 μM
Inhibitor/μM
0
0.75
2.5
7.5
25
75
Vmax
Substrate/μM
0.0
0.5
1.0
1.5
2.0
2.5
0
20
40
60
80
100

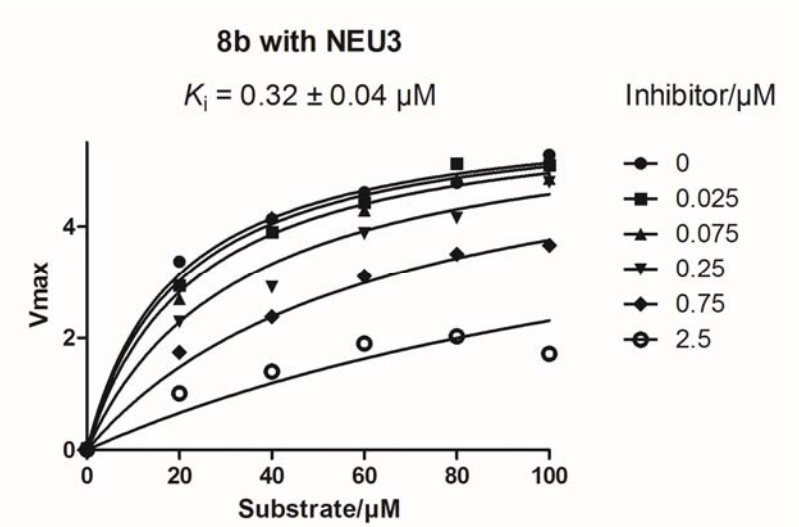

Zanamivir with NEU4
$K_i$ = 26 ± 4 μM
Inhibitor/μM
0
3
10
30
100
300
Vmax
Substrate/μM
0
2
4
0
20
40
60
80
100


7i with NEU2
$K_i$ = 48 ± 9 μM
Inhibitor/μM
0
3
10
30
100
300
Vmax
Substrate/μM


7i with NEU3
$K_i$ = 0.28 ± 0.04 μM
Inhibitor/μM
0
0.075
0.25
0.75
2.5
7.5
Vmax
Substrate/μM


7i with NEU4
$K_i$ = 0.26 ± 0.04 μM
Inhibitor/μM
0
0.100
0.3
1
3
10
Vmax
Substrate/μM


8b with NEU2
Ki = 17 ± 4 μM
Inhibitor/μM
0
1
3
10
30
100
Vmax
Substrate/μM


8b with NEU3
Ki = 0.32 ± 0.04 μM
Inhibitor/μM
0
0.025
0.075
0.25
0.75
2.5
Vmax
Substrate/μM

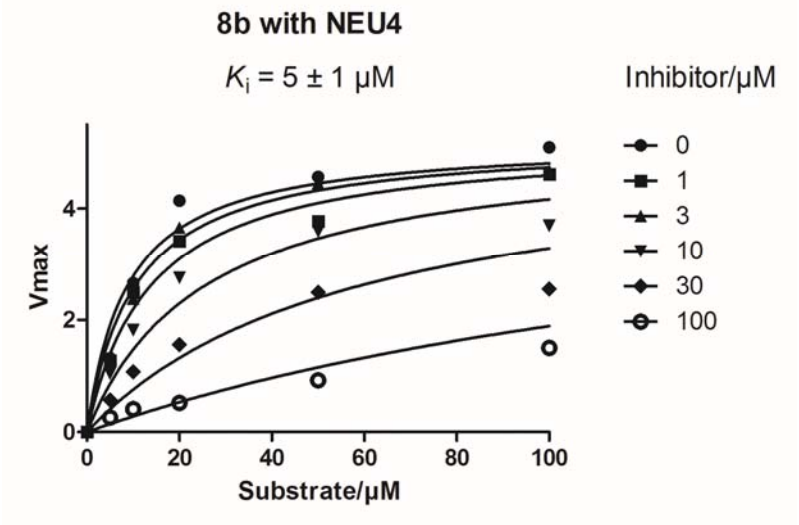
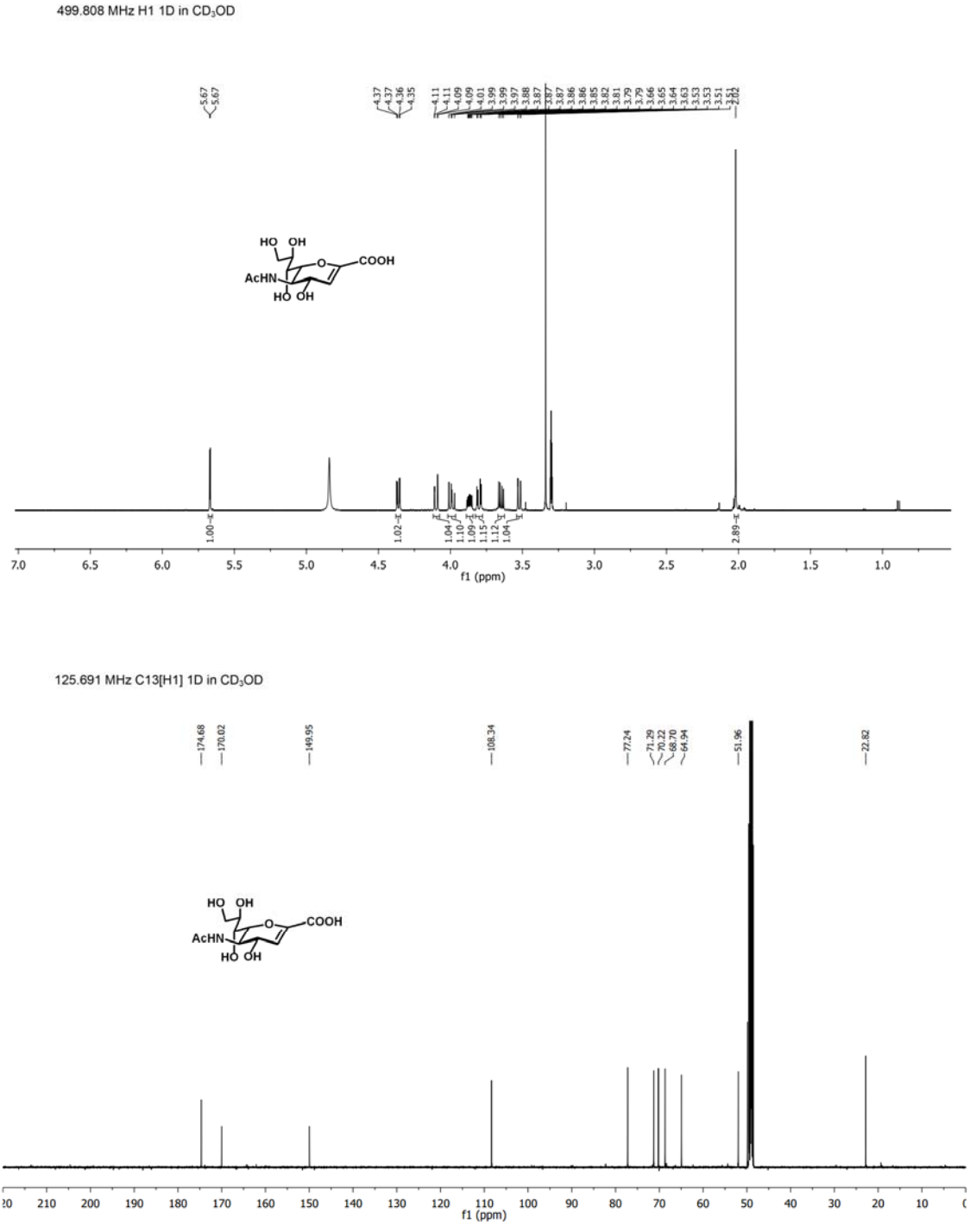

8b with NEU4
Ki = 5 ± 1 μM
Inhibitor/μM
0
1
3
10
30
100
Vmax
Substrate/μM

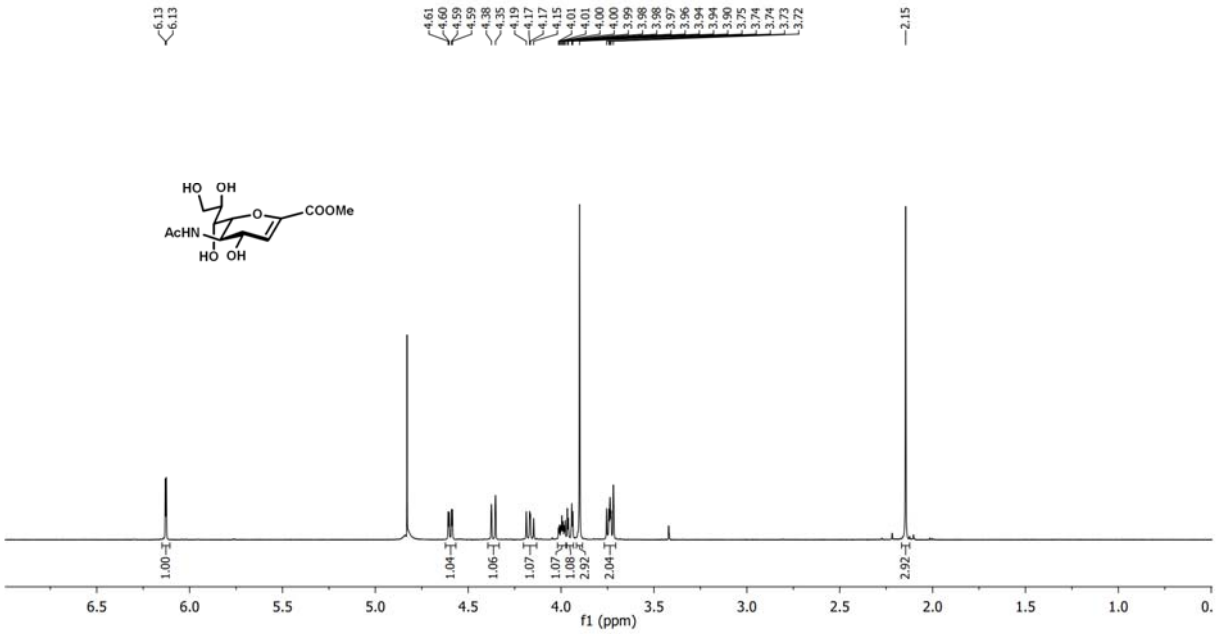
**Figure S.3: NMR ($^{1}H$ and $^{13}C$) spectra**

**1(DANA)**

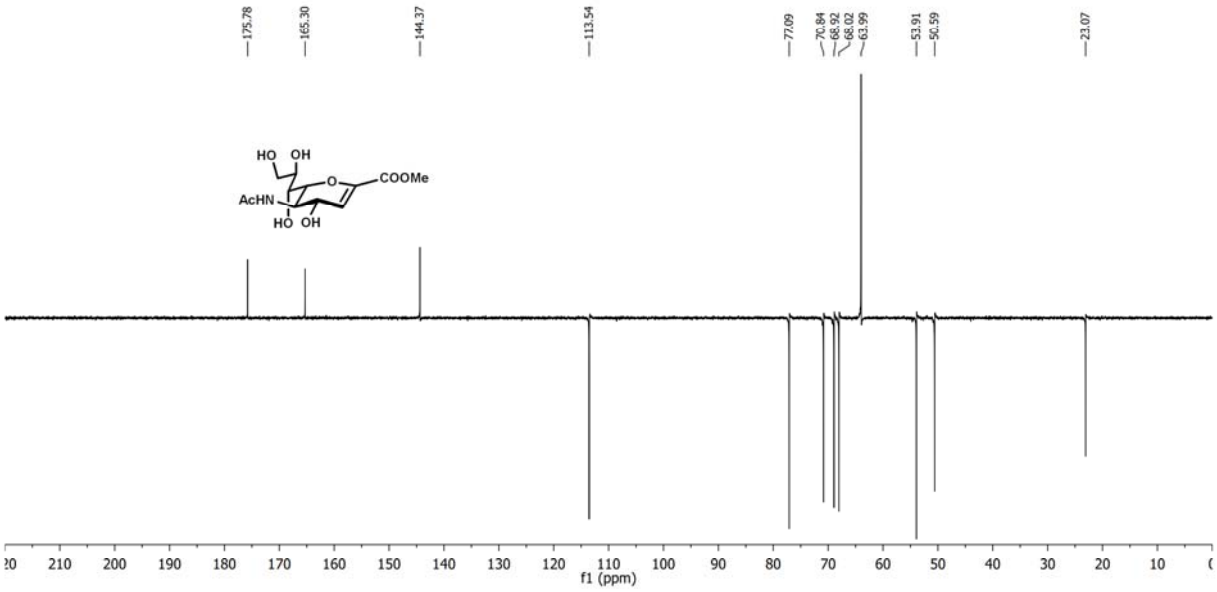


## 1-OMe (DANA-OMe)

499.808 MHz H1 1D in $D_2O$

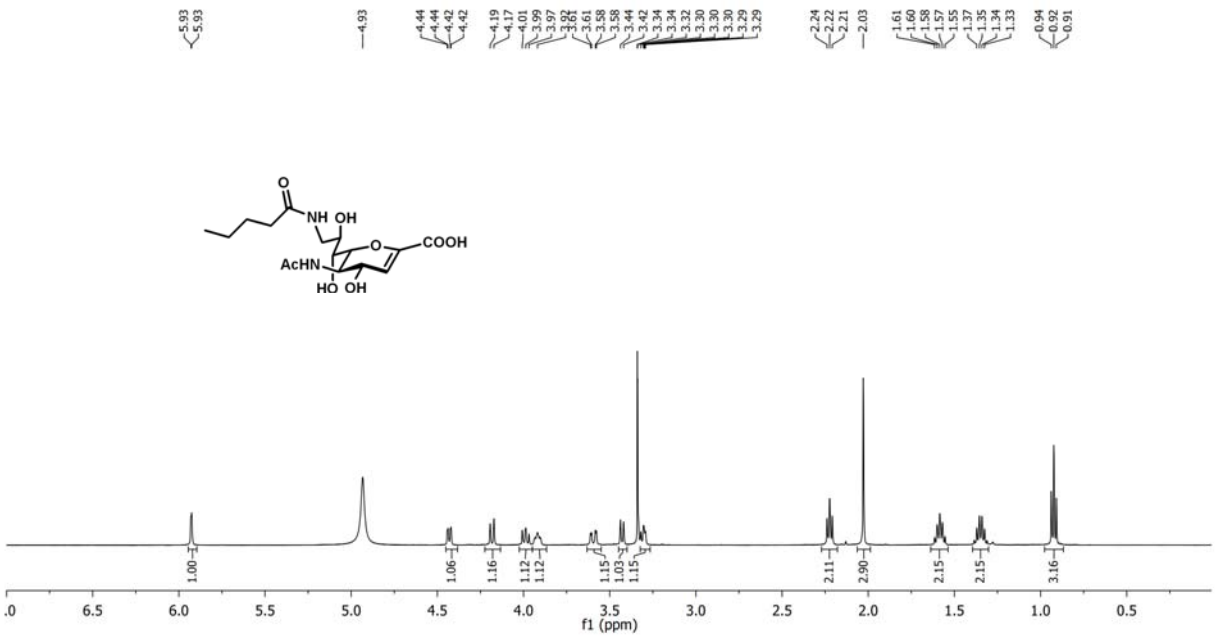



125.691 MHz C13[H1] DEPTq in $D_2O$

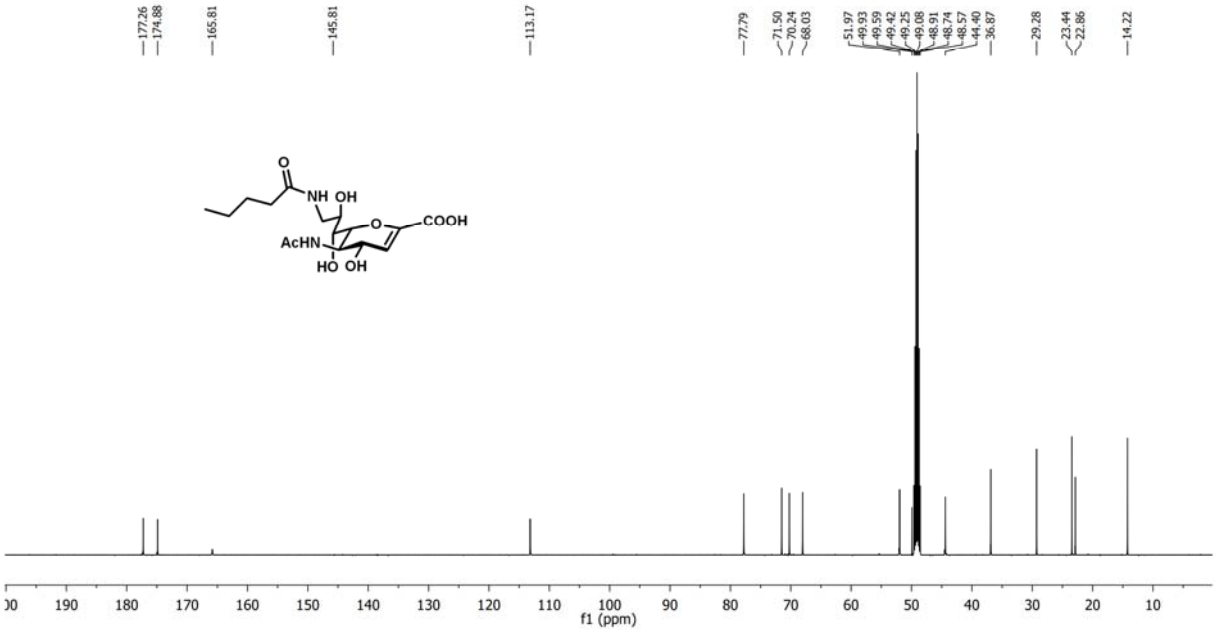


## 2(C9-BA-DANA)

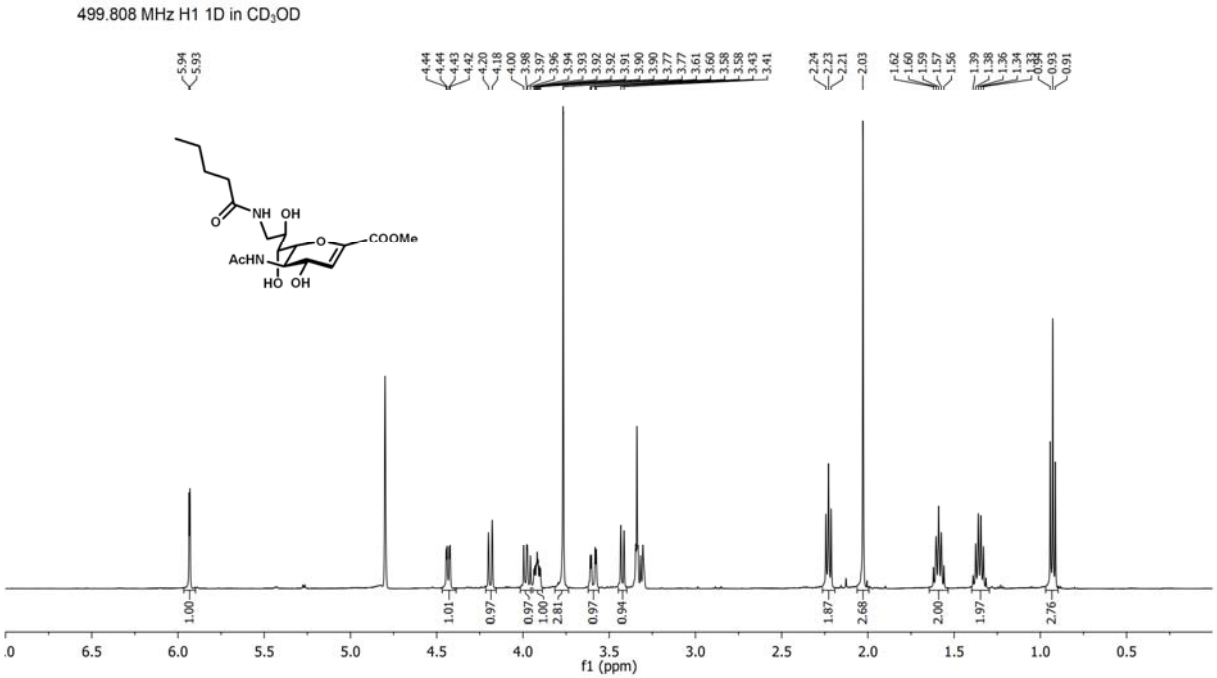
499.808 MHz H1 1D in $CD_3OD$

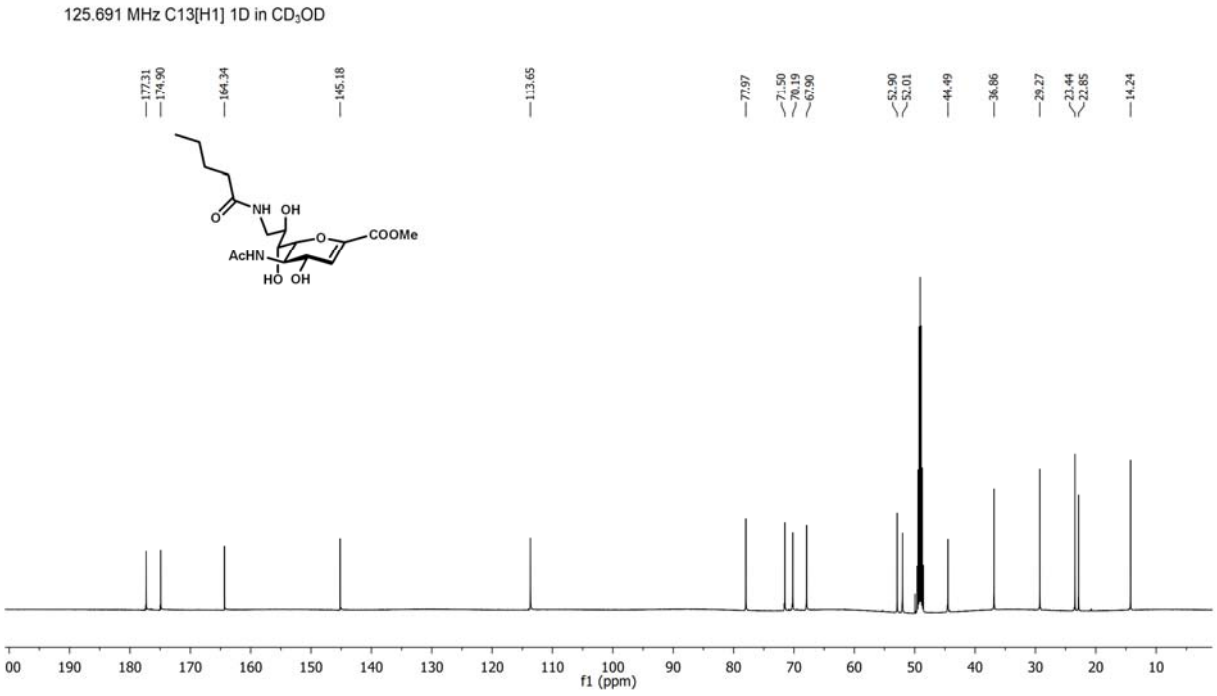



125.691 MHz C13[H1] 1D in $CD_3OD$

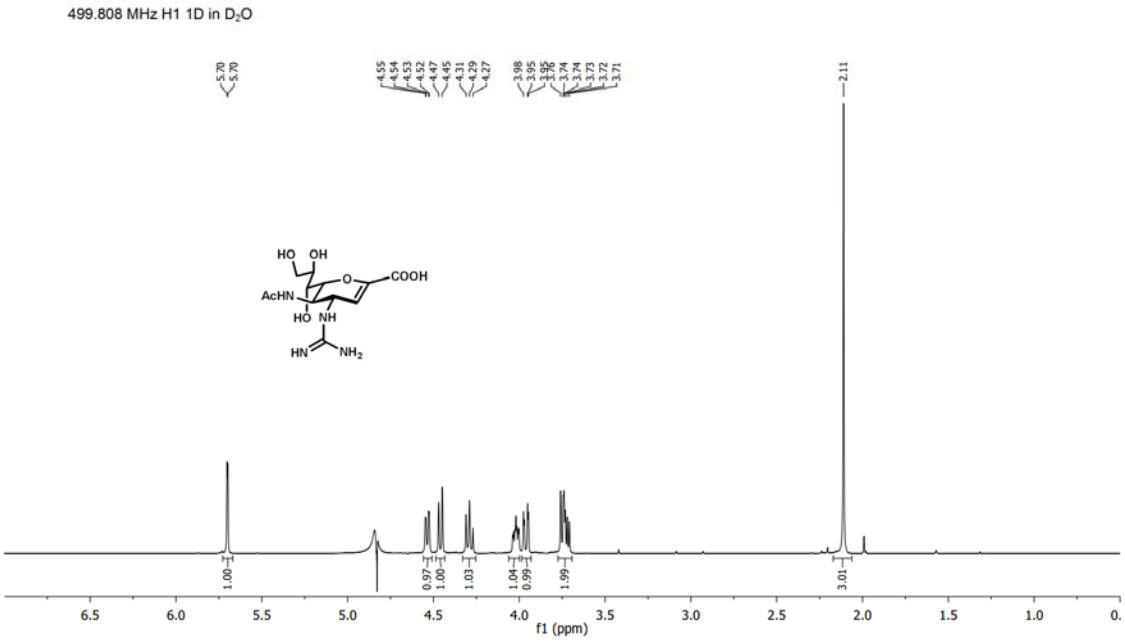


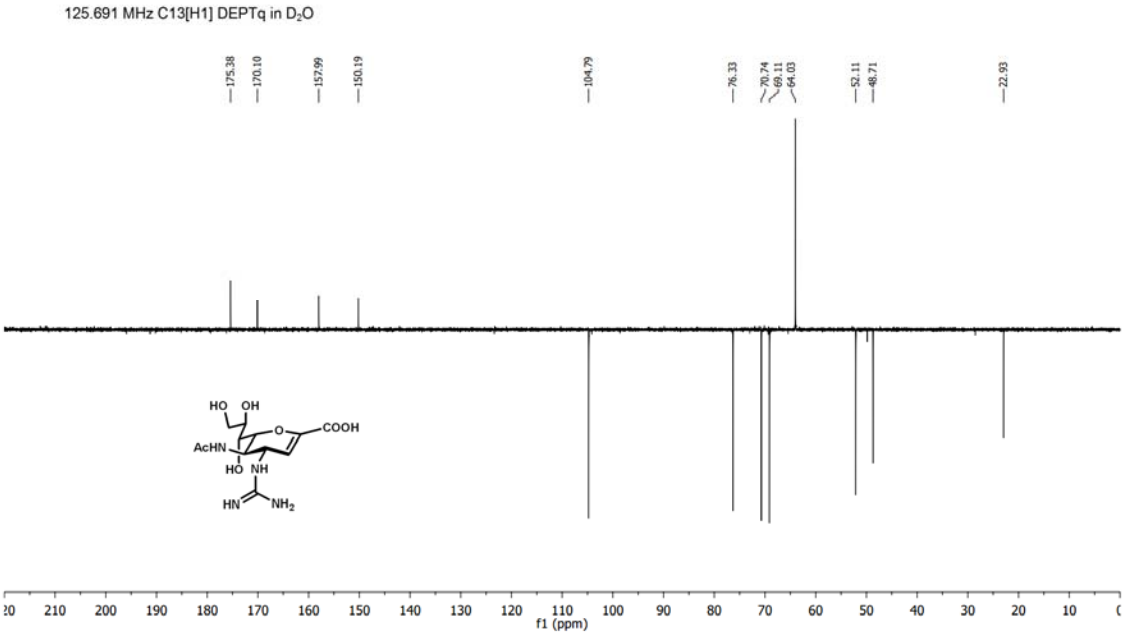
## 2-OMe (C9-BA-DANA-OMe)



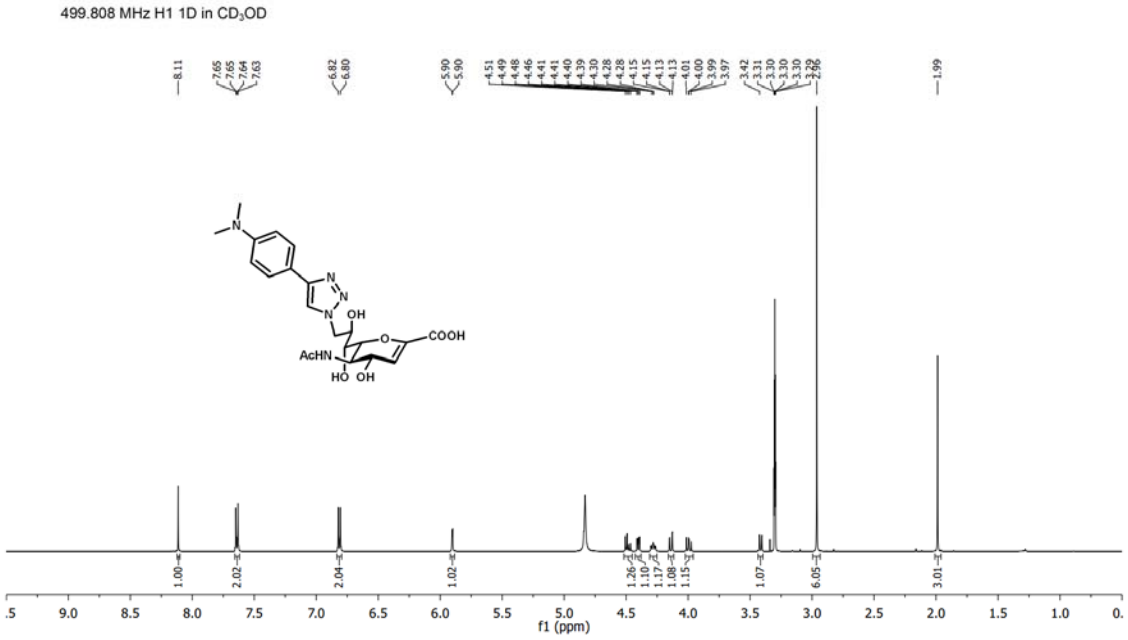



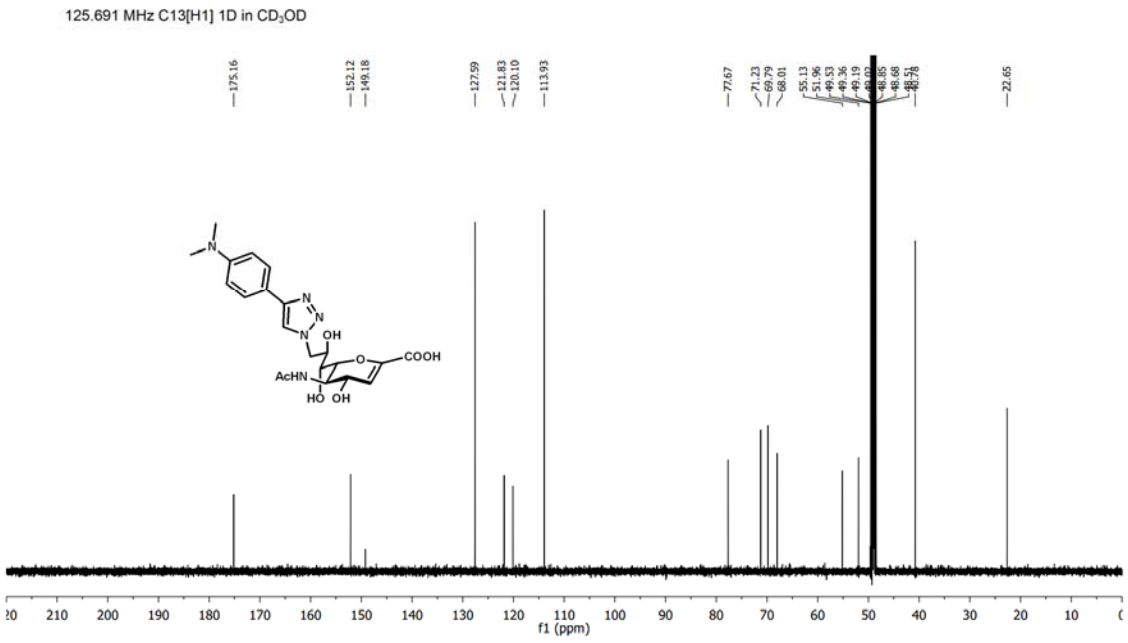
**6(Zanamivir)**


499.808 MHz H1 1D in D2O
5.70
5.70
4.55
4.54
4.53
4.52
4.47
4.45
4.31
4.29
4.27
3.98
3.95
3.95
3.76
3.74
3.74
3.73
3.72
3.71
2.11
HO
OH
COOH
AcHN
HO
NH
HN
NH2
1.00
0.97
1.00
1.03
1.04
0.99
1.99
3.01
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

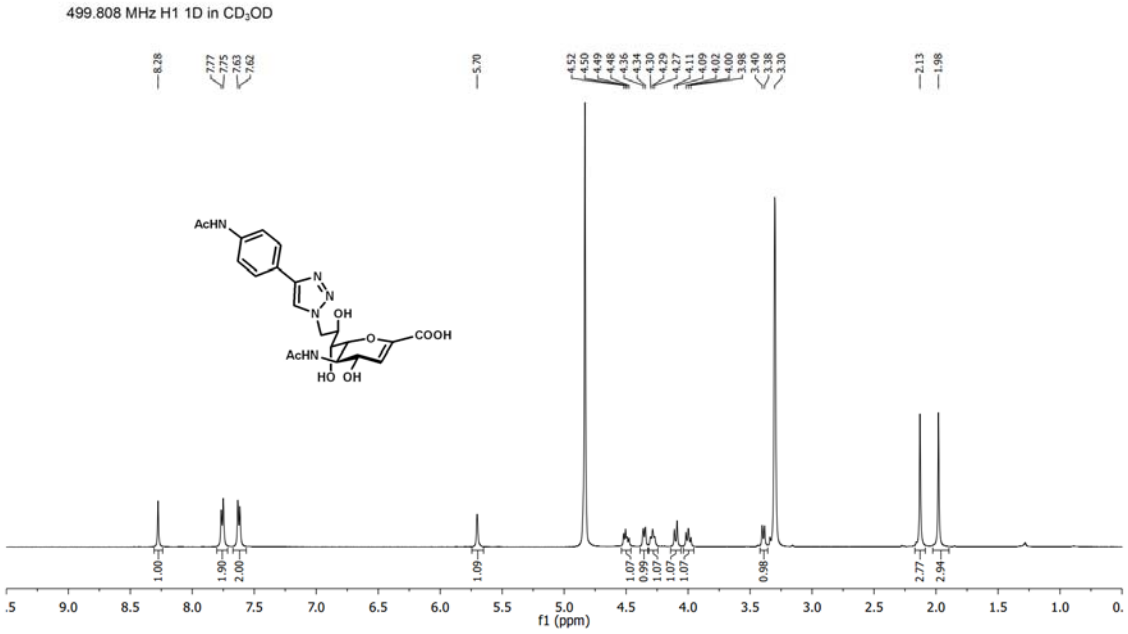

125.691 MHz C13[H1] DEPTq in D2O
175.38
170.10
157.99
150.19
104.79
76.33
70.74
69.11
64.03
52.11
48.71
22.93
HO
OH
COOH
AcHN
HO
NH
HN
NH2
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

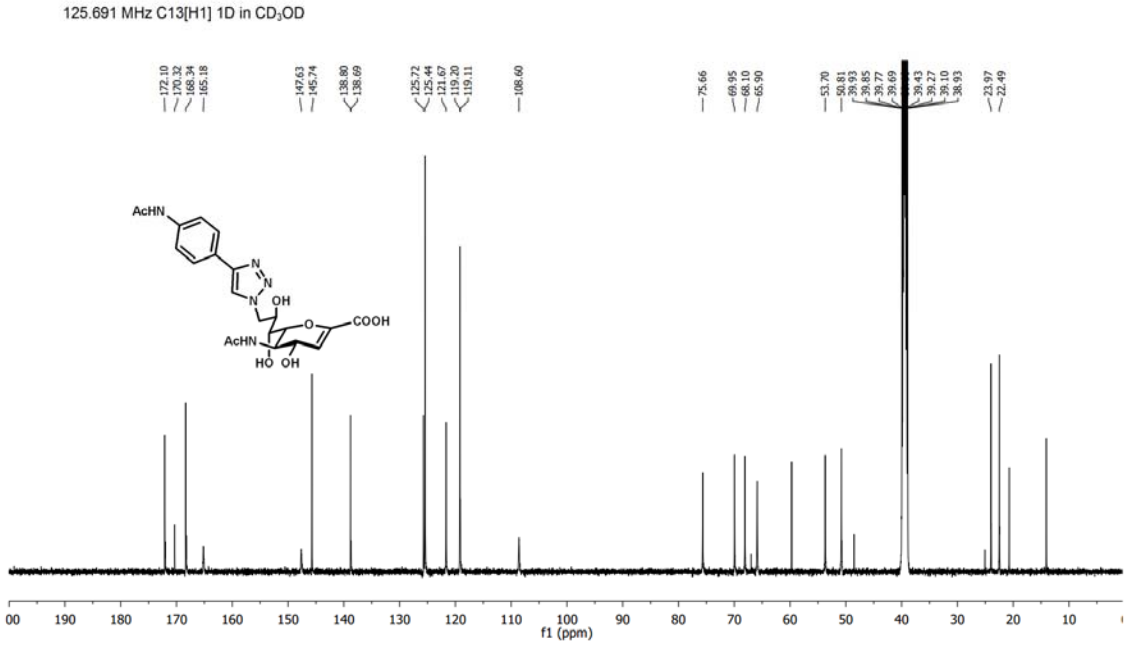
**7a**


499.808 MHz H1 1D in CD3OD
f1 (ppm)


125.691 MHz C13[H1] 1D in CD3OD
f1 (ppm)

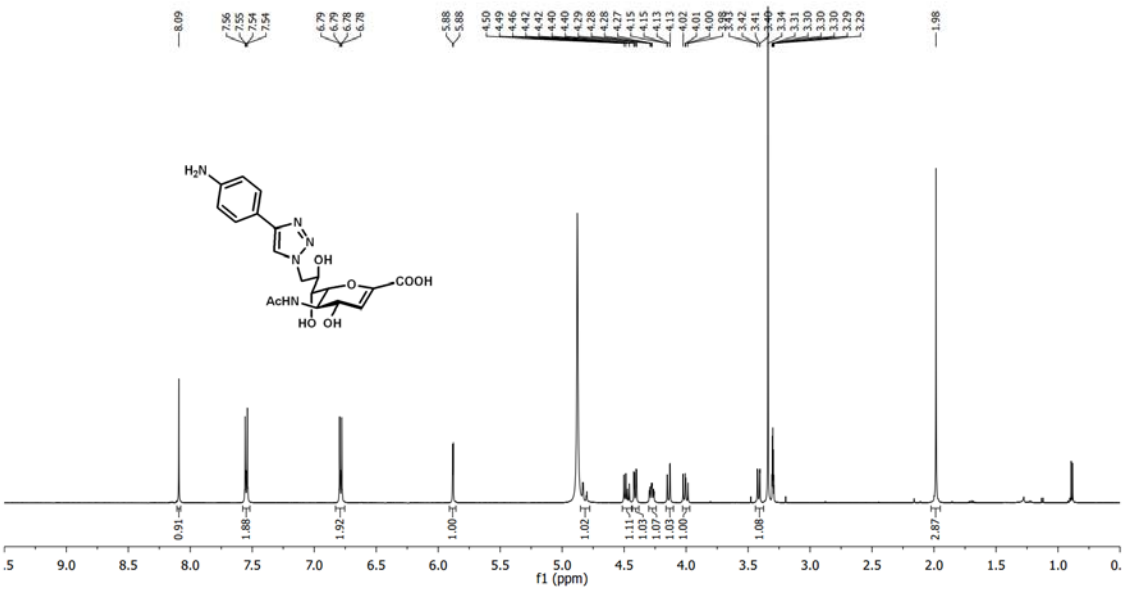
**7b**


499.808 MHz H1 1D in CD3OD
f1 (ppm)

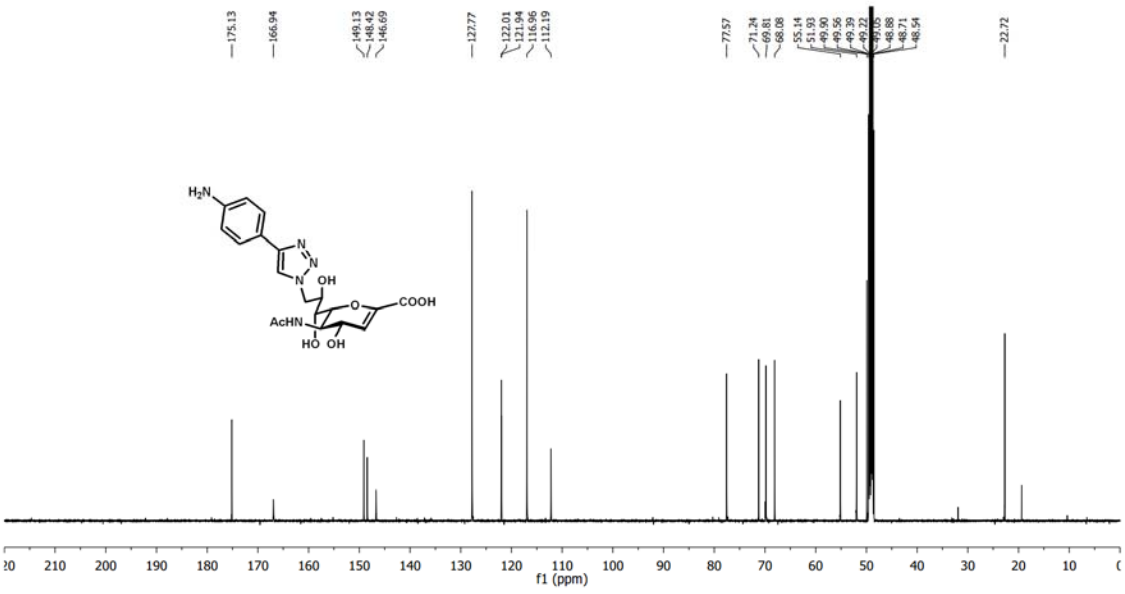

125.691 MHz C13[H1] 1D in CD3OD
f1 (ppm)

**7c**

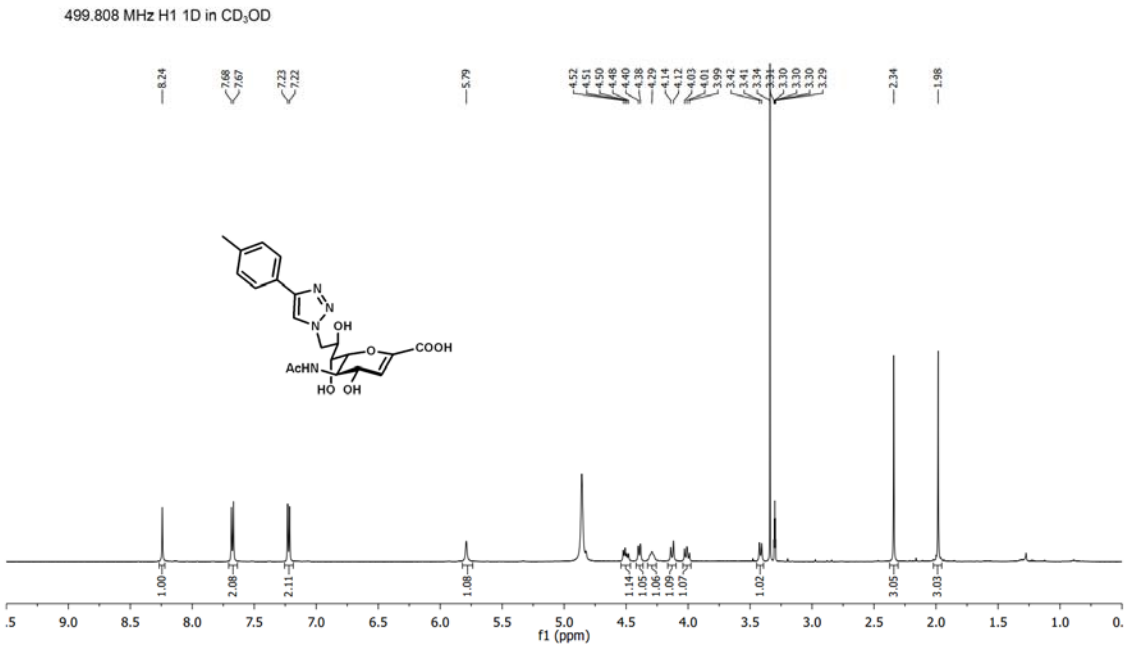
499.808 MHz H1 1D in $CD_3OD$

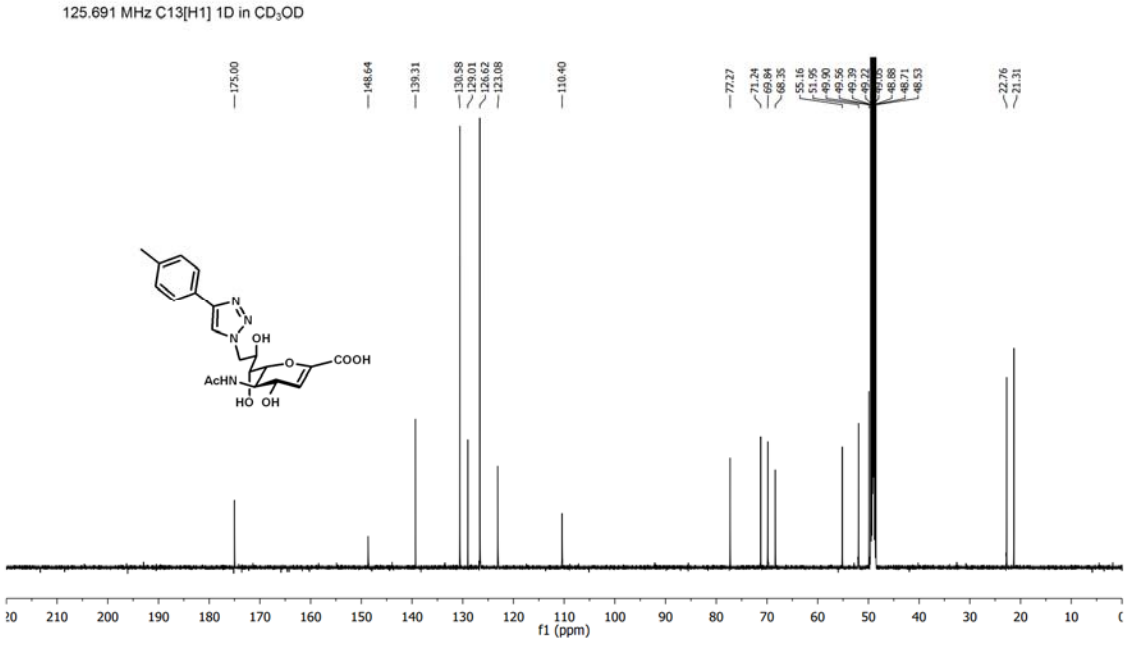



125.691 MHz C13[H1] 1D in $CD_3OD$



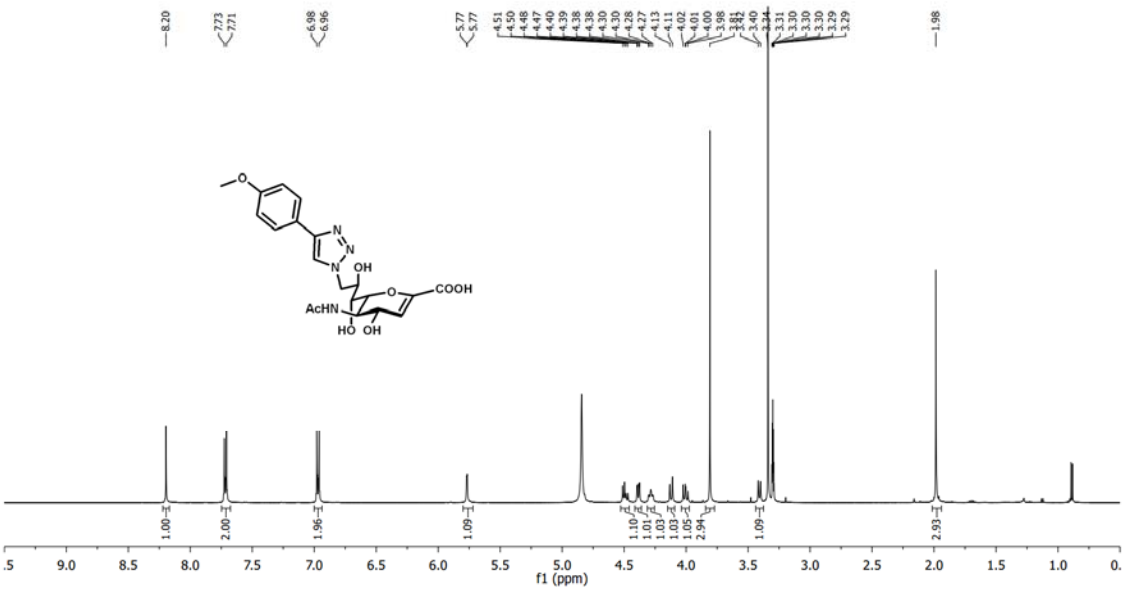
**7d**


499.808 MHz H1 1D in CD3OD
8.24
7.68
7.67
7.23
7.22
5.79
4.52
4.51
4.50
4.48
4.40
4.38
4.29
4.14
4.12
4.03
4.01
3.99
3.42
3.41
3.34
3.31
3.30
3.30
3.30
3.29
2.34
1.98
1.00
2.08
2.11
1.08
1.14
1.05
1.06
1.09
1.07
1.02
3.05
3.03
f1 (ppm)

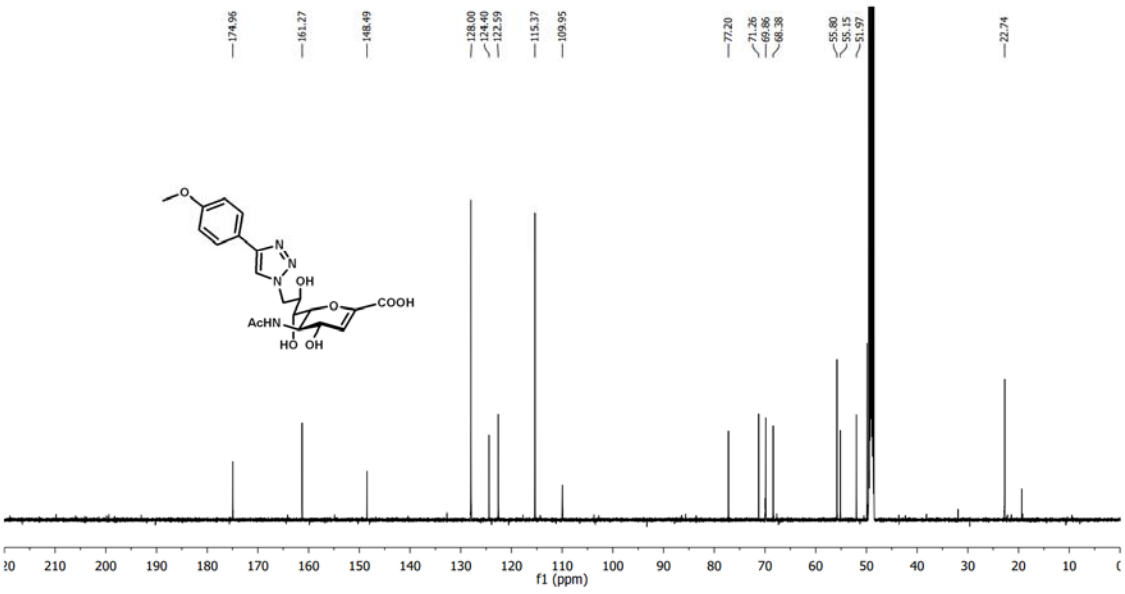

125.691 MHz C13[H1] 1D in CD3OD
175.00
148.64
139.31
130.58
129.01
126.62
123.08
110.40
77.27
71.24
69.84
68.35
55.16
51.95
49.90
49.56
49.39
49.22
49.05
48.88
48.71
48.53
22.76
21.31
f1 (ppm)

**7e**

499.808 MHz H1 1D in $CD_3OD$

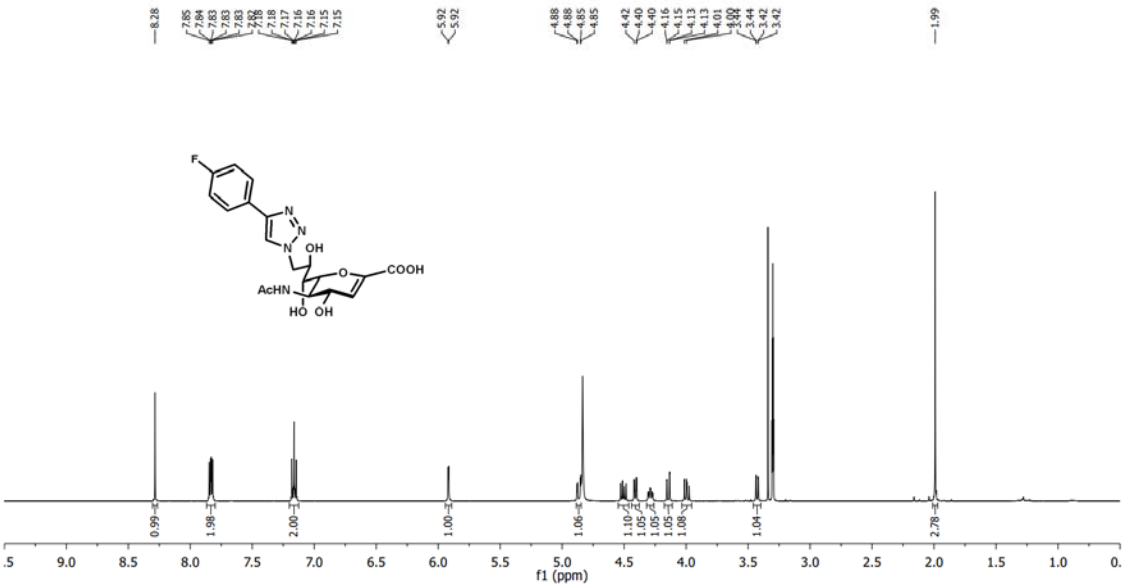



125.691 MHz C13[H1] 1D in $CD_3OD$

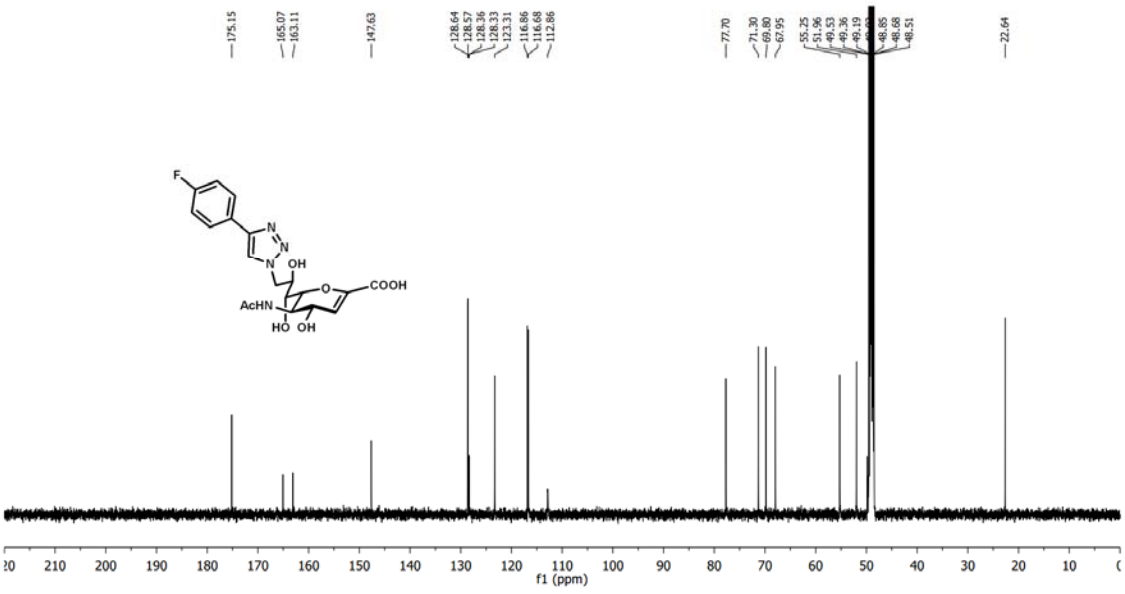


**7f**

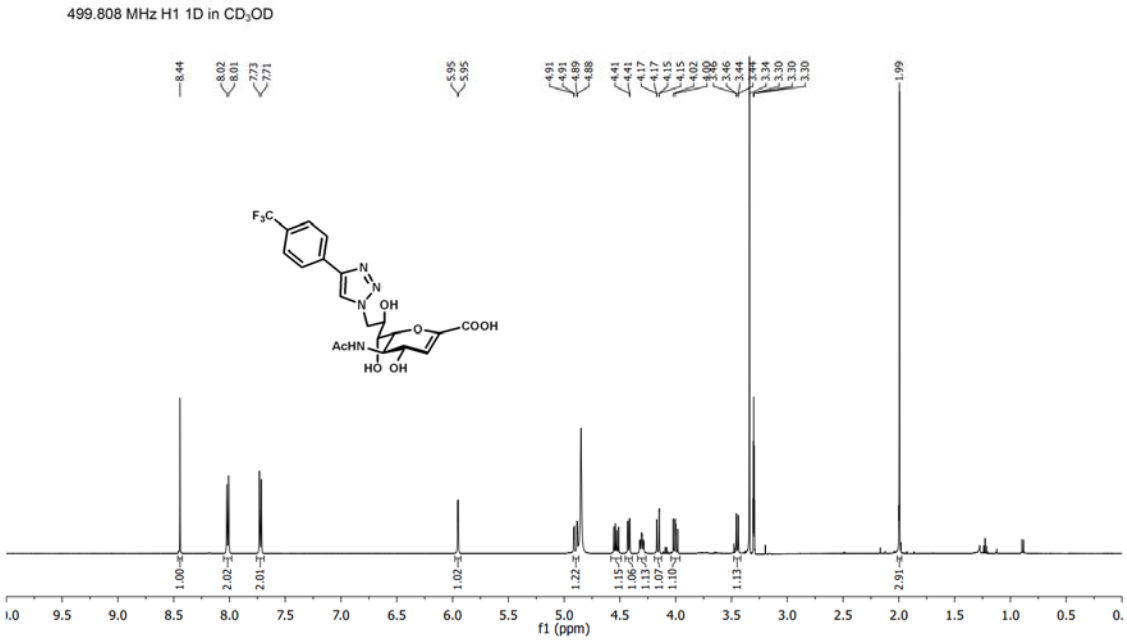
499.808 MHz H1 1D in $CD_3OD$

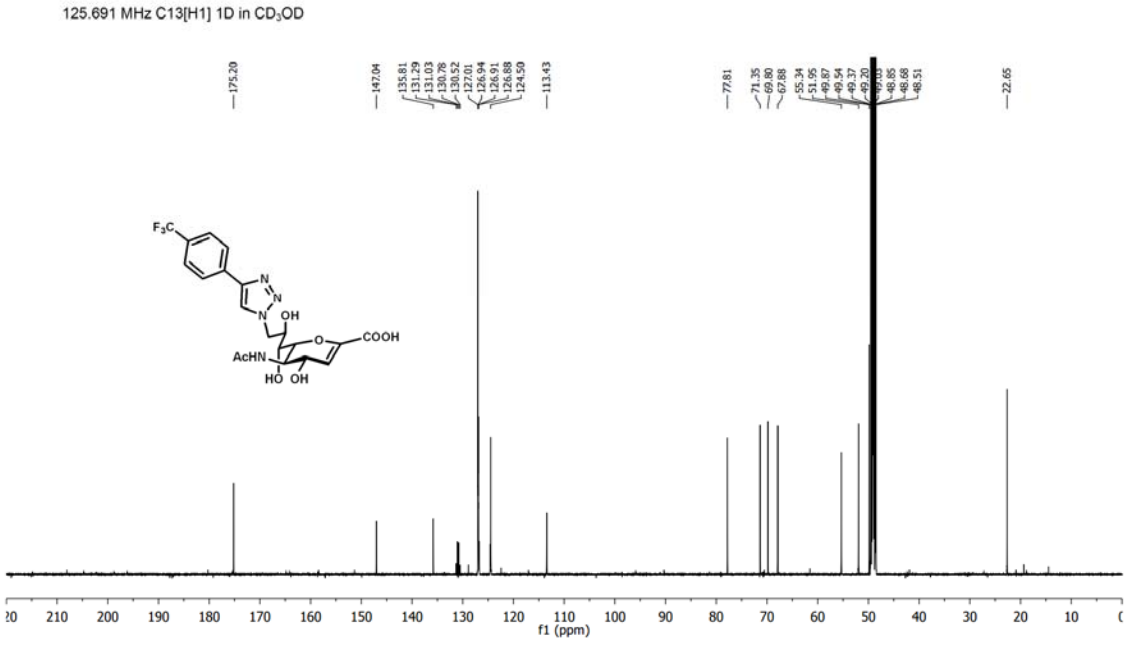



125.691 MHz C13[H1] 1D in $CD_3OD$

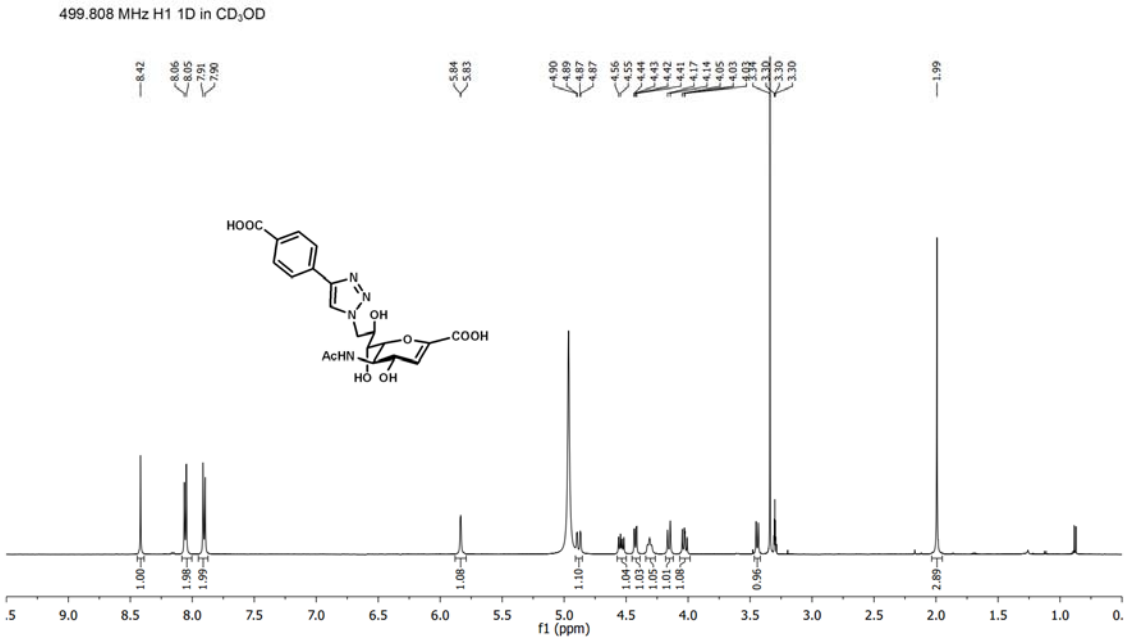


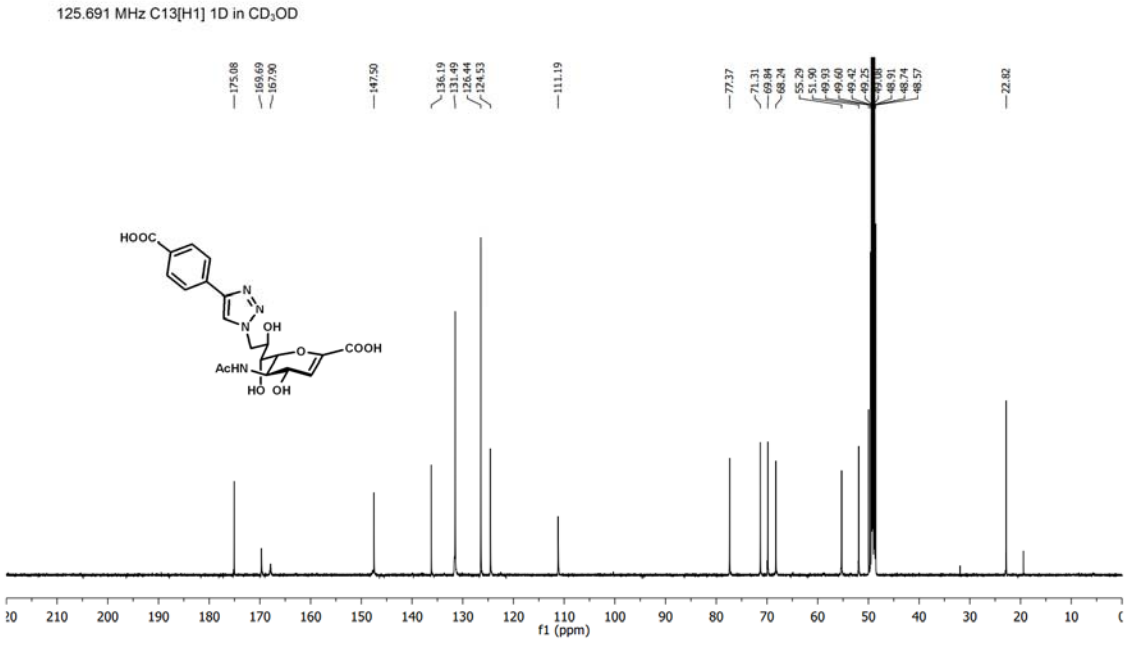
**7g**


499.808 MHz H1 1D in CD3OD
f1 (ppm)

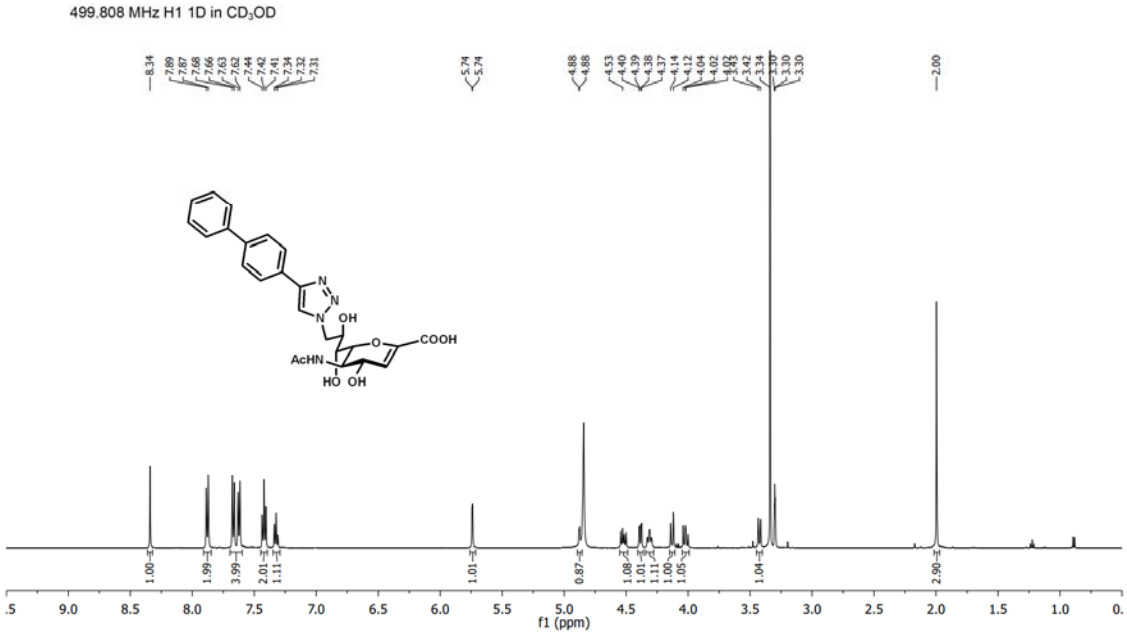

125.691 MHz C13[H1] 1D in CD3OD
f1 (ppm)

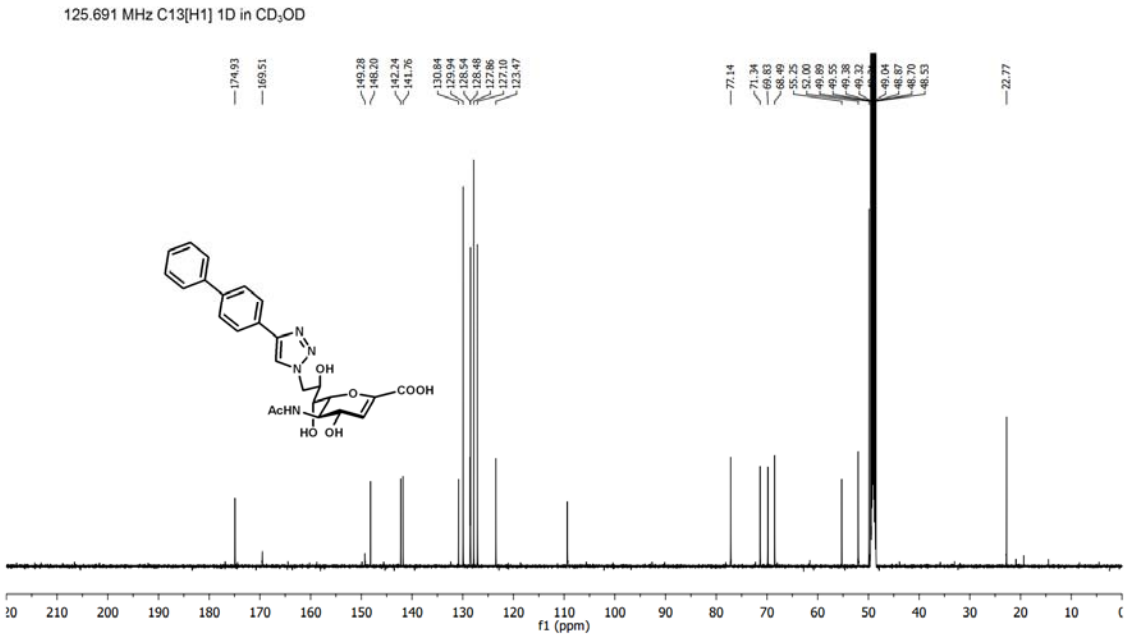
**7h**


499.808 MHz H1 1D in CD3OD
8.42
8.06
8.05
7.91
7.90
5.84
5.83
4.90
4.89
4.87
4.87
4.56
4.55
4.44
4.43
4.42
4.41
4.17
4.14
4.05
4.03
3.34
3.30
3.30
3.30
1.99
HOOC
N
N
N
OH
O
COOH
AcHN
HO
OH
1.00
1.98
1.99
1.08
1.10
1.04
1.03
1.05
1.01
1.08
0.96
2.89
f1 (ppm)

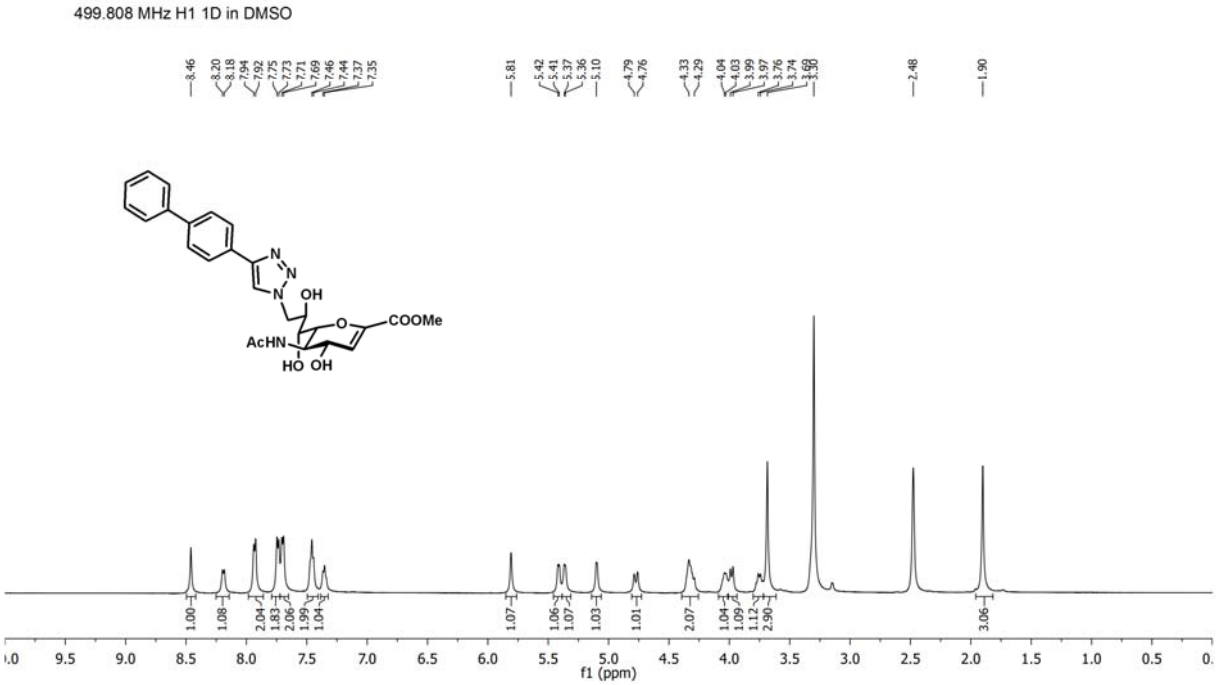

125.691 MHz C13[H1] 1D in CD3OD
175.08
169.69
167.90
147.50
136.19
131.49
126.44
124.53
111.19
77.37
71.31
69.84
68.24
55.29
51.90
49.93
49.60
49.42
49.25
49.08
48.91
48.74
48.57
22.82
HOOC
N
N
N
OH
O
COOH
AcHN
HO
OH
f1 (ppm)

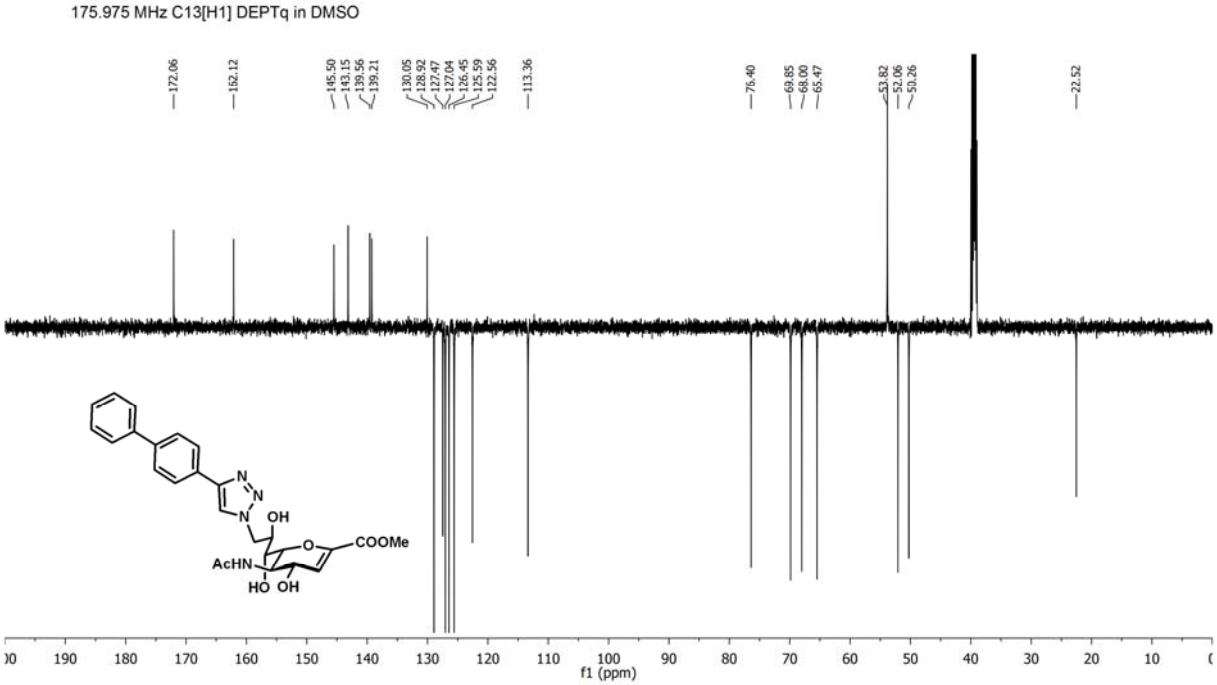
**7i**


499.808 MHz H1 1D in CD3OD
f1 (ppm)

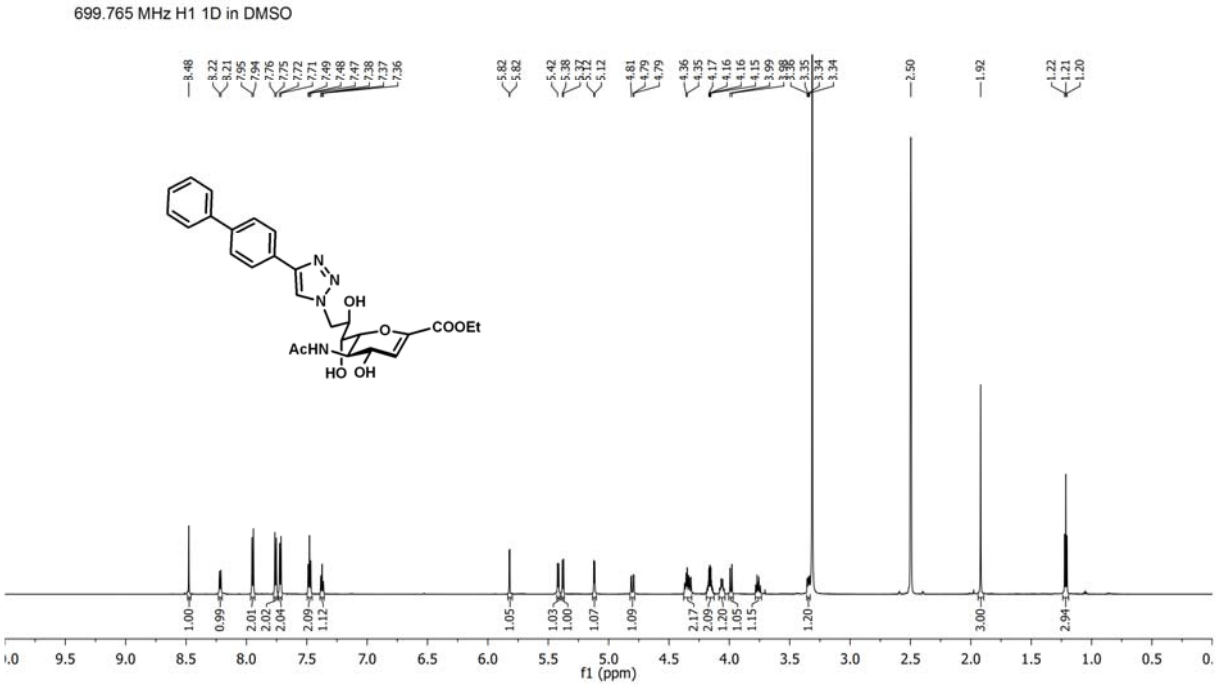

125.691 MHz C13[H1] 1D in CD3OD
f1 (ppm)

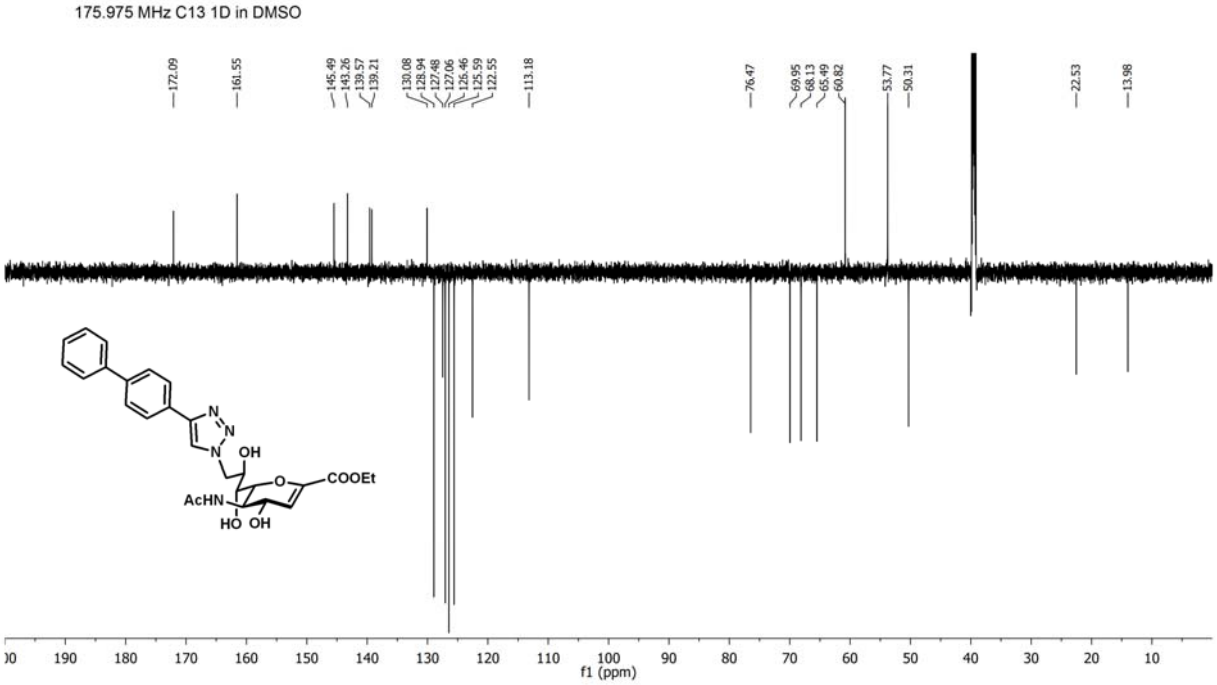
**7i-OMe**


499.808 MHz H1 1D in DMSO
8.46
8.20
8.18
7.94
7.92
7.75
7.73
7.71
7.69
7.46
7.44
7.37
7.35
5.81
5.42
5.41
5.37
5.36
5.10
4.79
4.76
4.33
4.29
4.04
4.03
3.99
3.97
3.76
3.74
3.69
3.50
2.48
1.90
OH
COOMe
AcHN
HO OH
1.00
1.08
2.04
1.83
2.06
1.99
1.04
1.07
1.06
1.07
1.03
1.01
2.07
1.04
1.09
1.12
2.90
3.06
f1 (ppm)

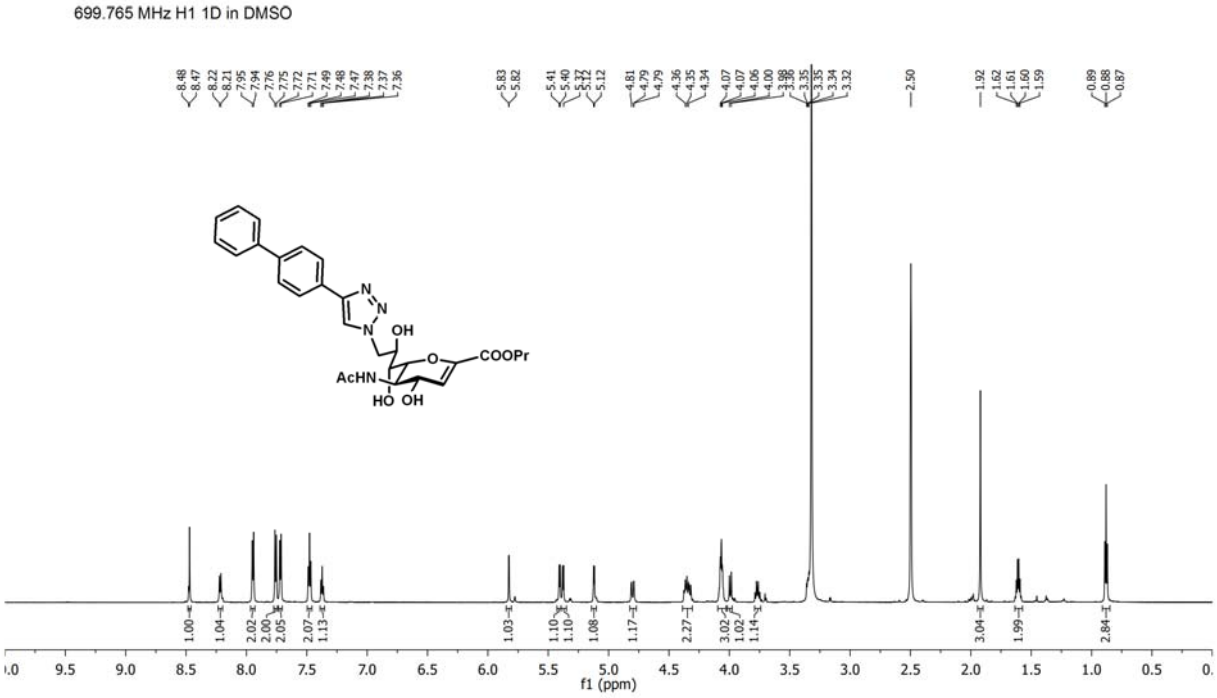

175.975 MHz C13[H1] DEPTq in DMSO
172.06
162.12
145.50
143.15
139.56
139.21
130.05
128.92
127.47
127.04
126.45
125.99
122.56
113.36
76.40
69.85
68.00
65.47
53.82
52.06
50.26
22.52
OH
COOMe
AcHN
HO OH
f1 (ppm)

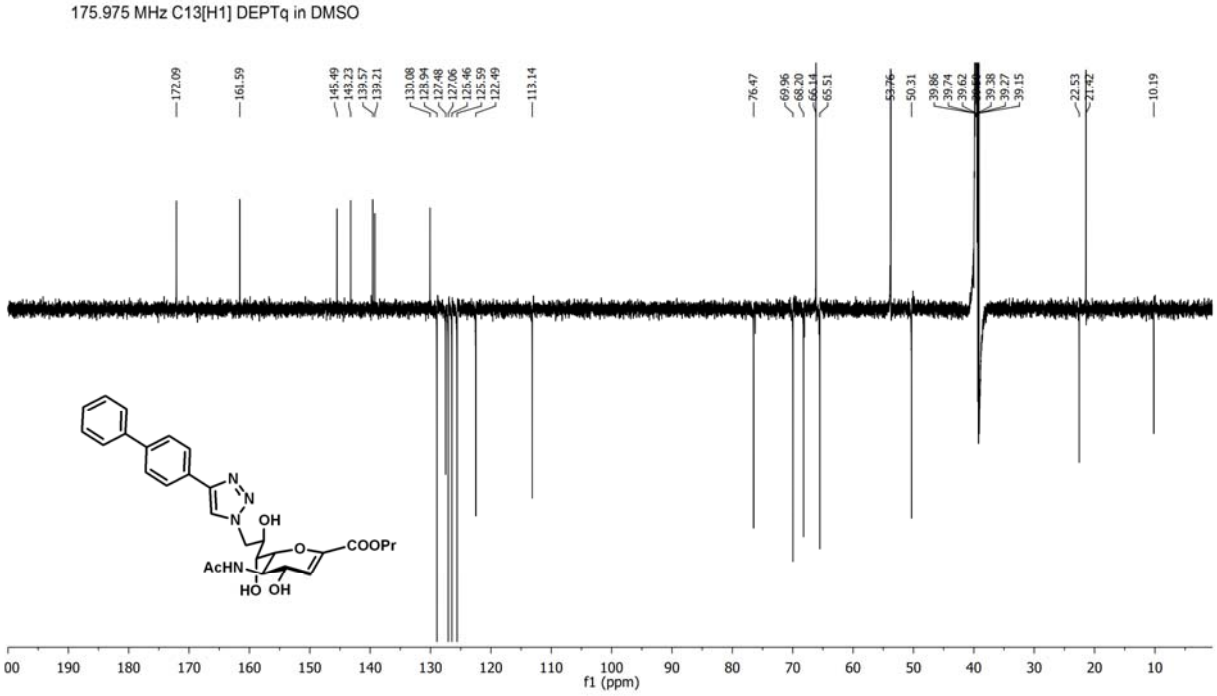
**7i-OEt**



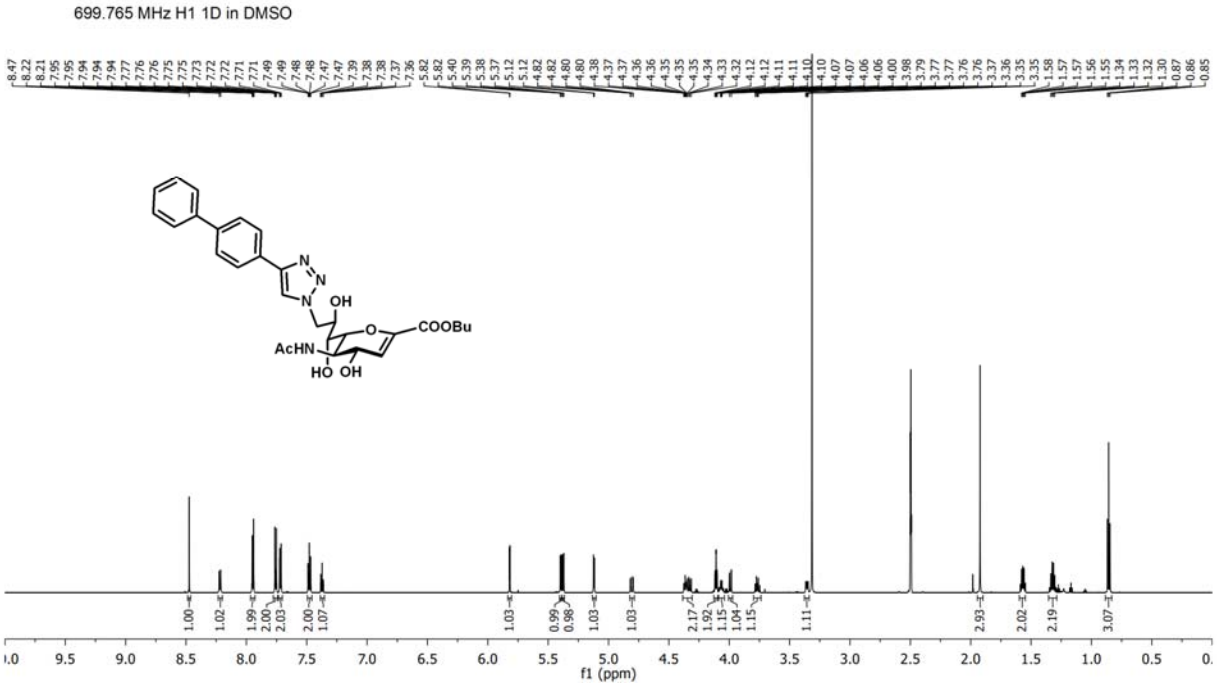



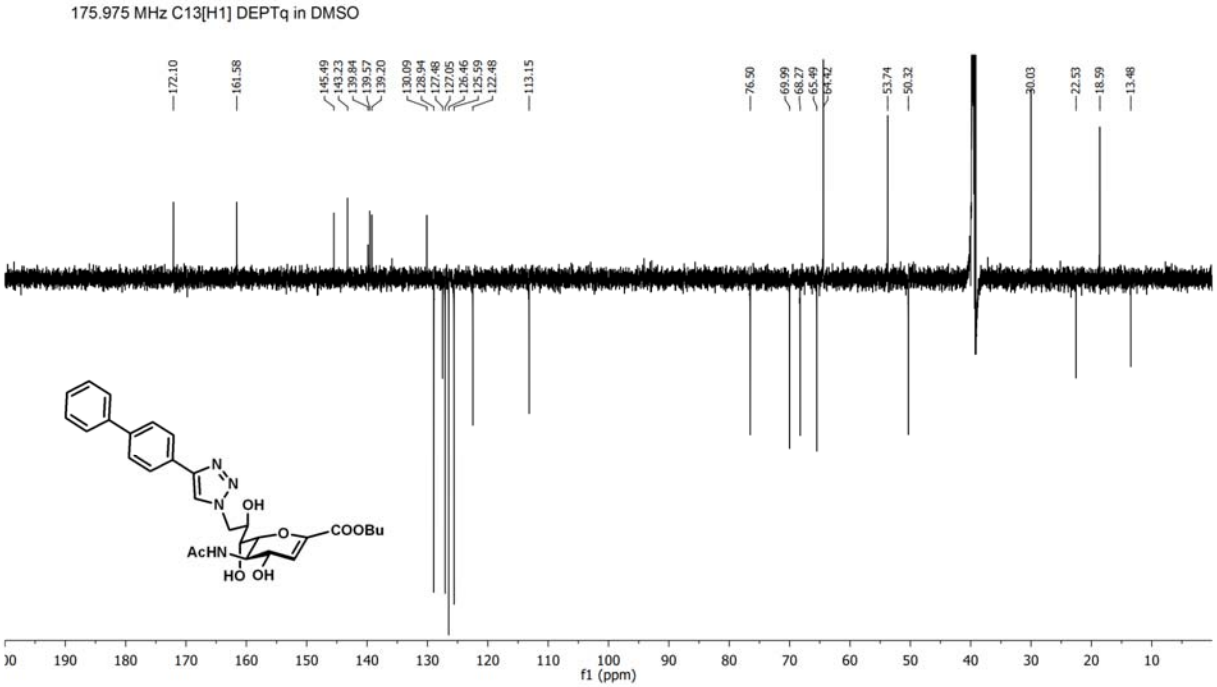
**7i-OPr**


699.765 MHz H1 1D in DMSO
f1 (ppm)


175.975 MHz C13[H1] DEPTq in DMSO
f1 (ppm)

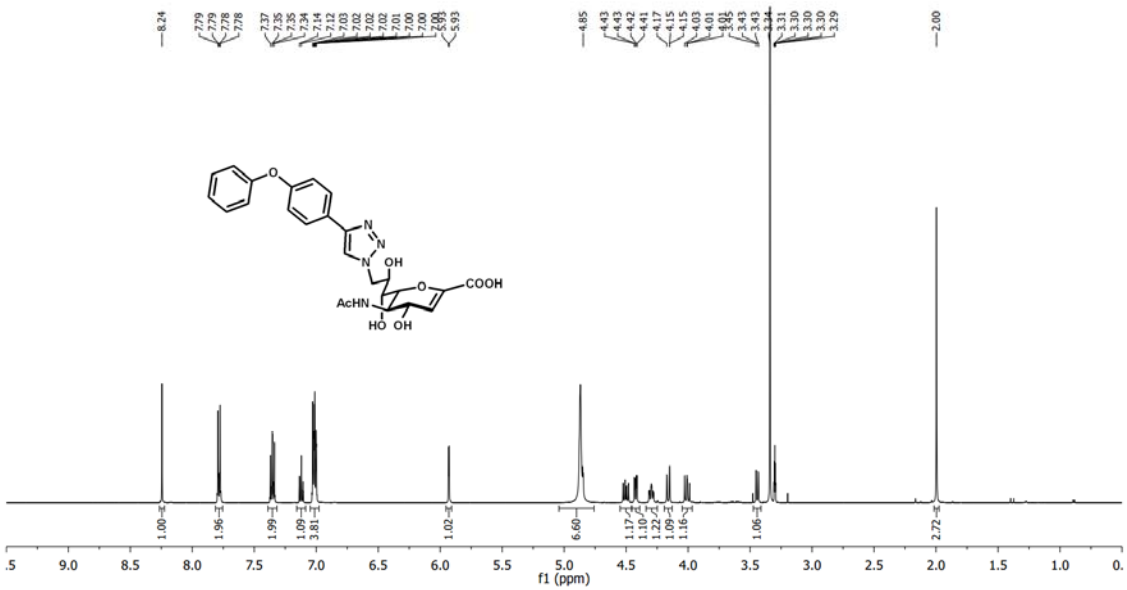
**7i-OBu**


699.765 MHz H1 1D in DMSO
f1 (ppm)

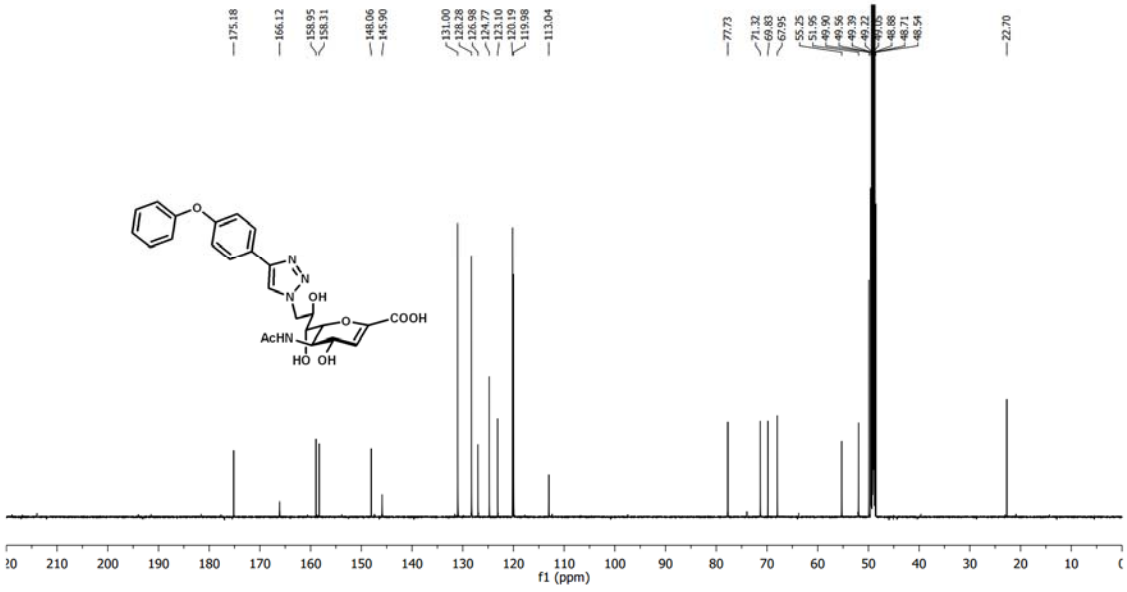

175.975 MHz C13[H1] DEPTq in DMSO
f1 (ppm)

**7j**

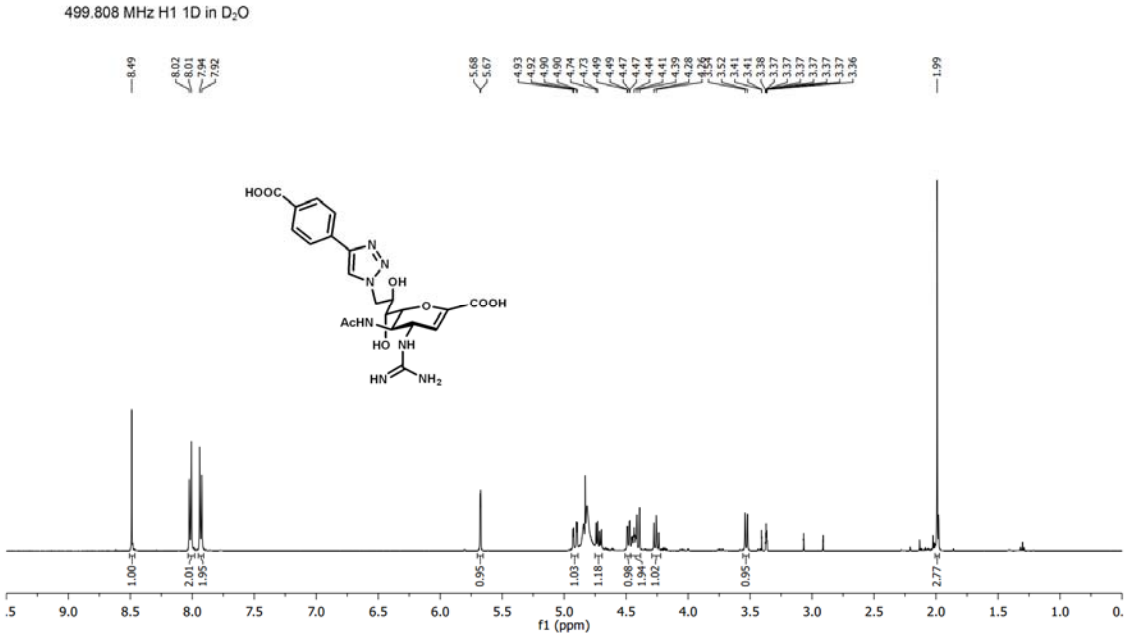
499.808 MHz H1 1D in $CD_3OD$

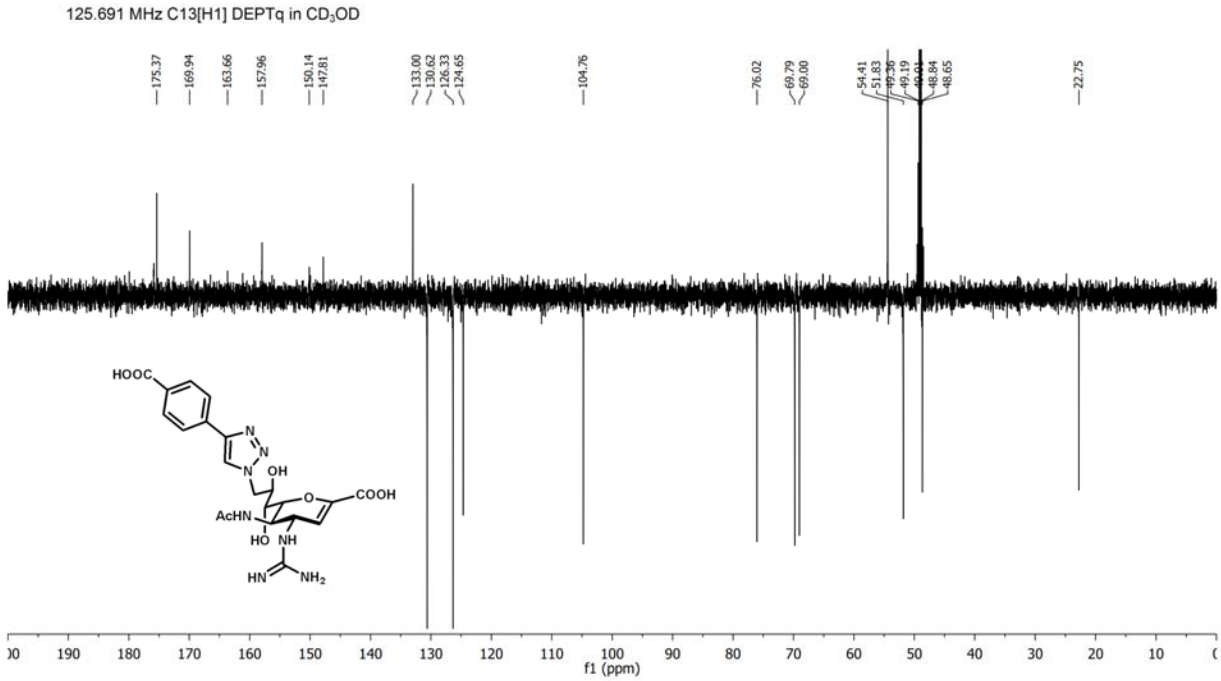



125.691 MHz C13[H1] 1D in $CD_3OD$

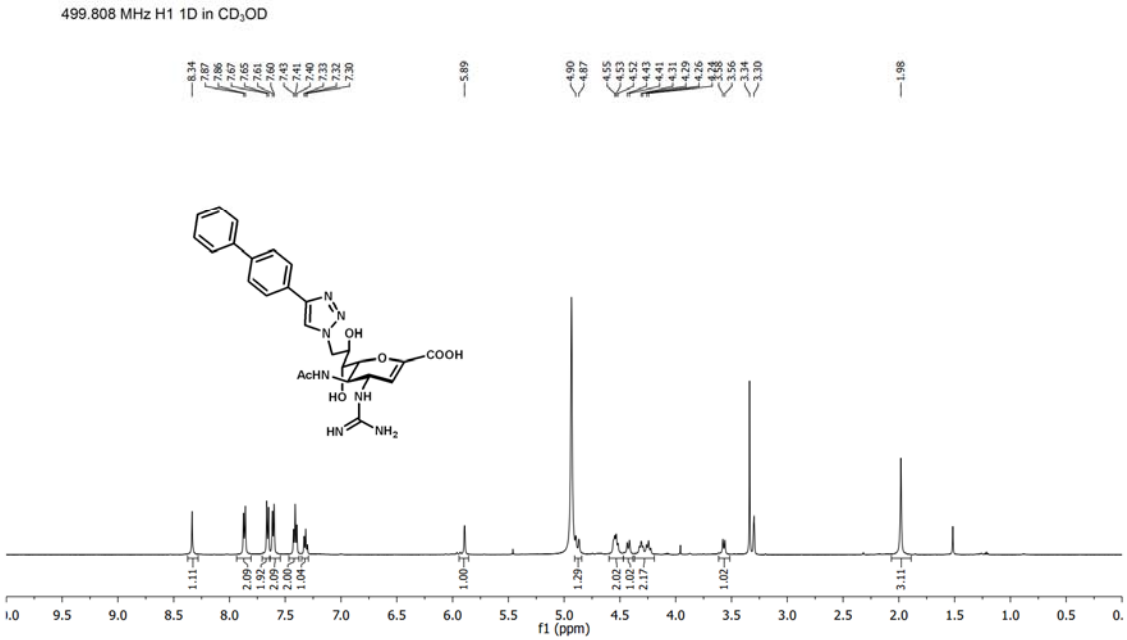


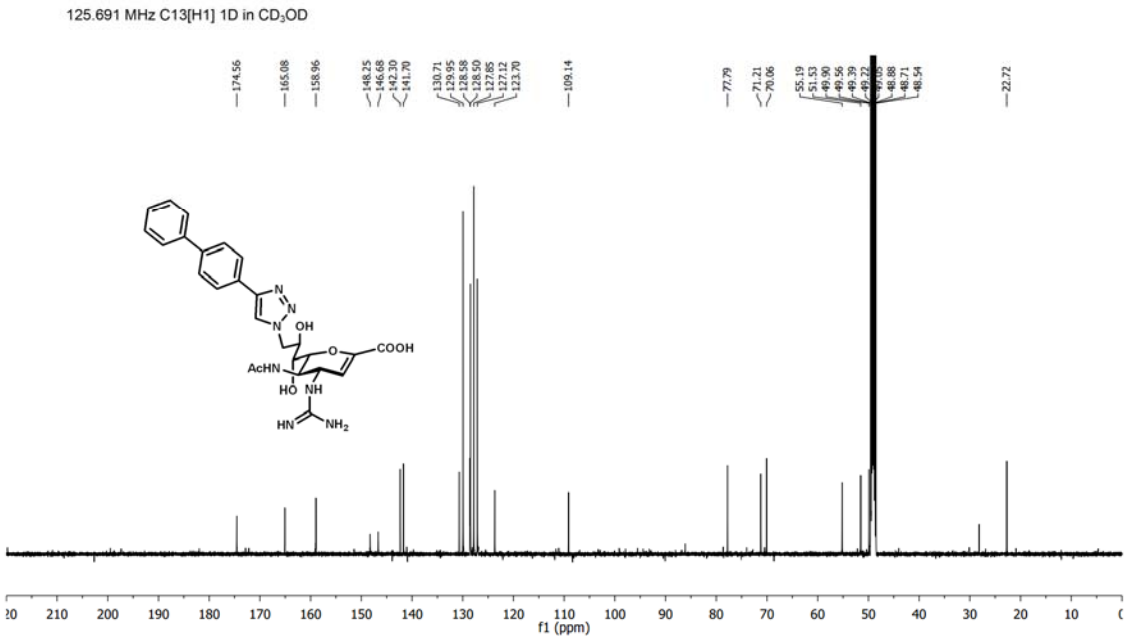
**8a**


499.808 MHz H1 1D in D2O
8.49
8.02
8.01
7.94
7.93
7.92
5.68
5.67
4.93
4.92
4.90
4.90
4.74
4.73
4.49
4.49
4.47
4.47
4.44
4.41
4.39
4.28
4.26
3.54
3.52
3.41
3.41
3.38
3.37
3.37
3.37
3.37
3.37
3.37
3.36
1.99
HOOC
COOH
AcHN
HO
NH
HN
NH2
OH
1.00
2.01
1.95
0.95
1.03
1.18
0.98
1.94
1.02
0.95
2.77
f1 (ppm)

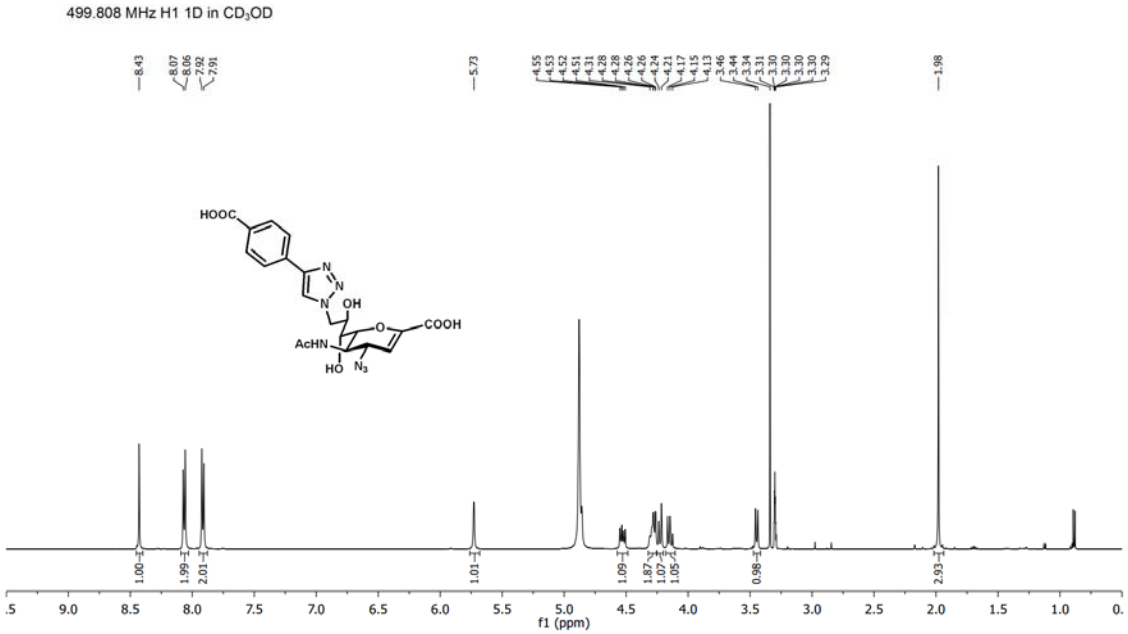

125.691 MHz C13[H1] DEPTq in CD3OD
175.37
169.94
163.66
157.96
150.14
147.81
133.00
130.62
126.33
124.65
104.76
76.02
69.79
69.00
54.41
51.83
49.36
49.19
49.01
48.84
48.65
22.75
HOOC
COOH
AcHN
HO
NH
HN
NH2
OH
f1 (ppm)

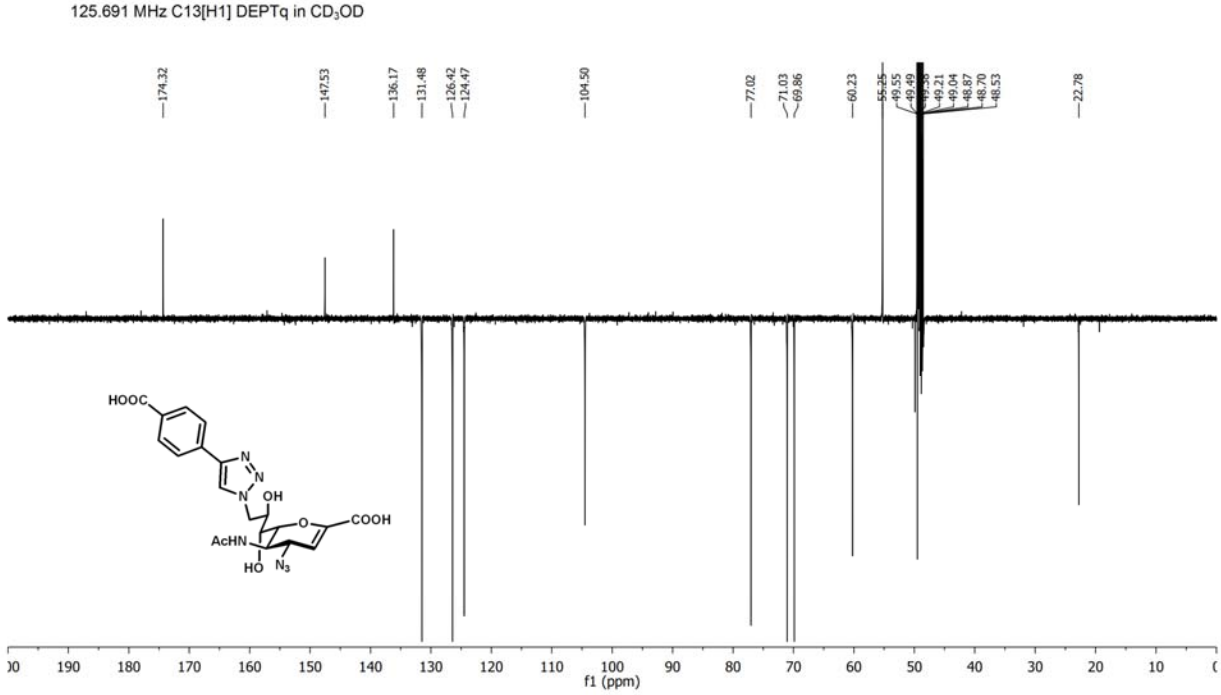
**8b**


499.808 MHz H1 1D in CD3OD
f1 (ppm)

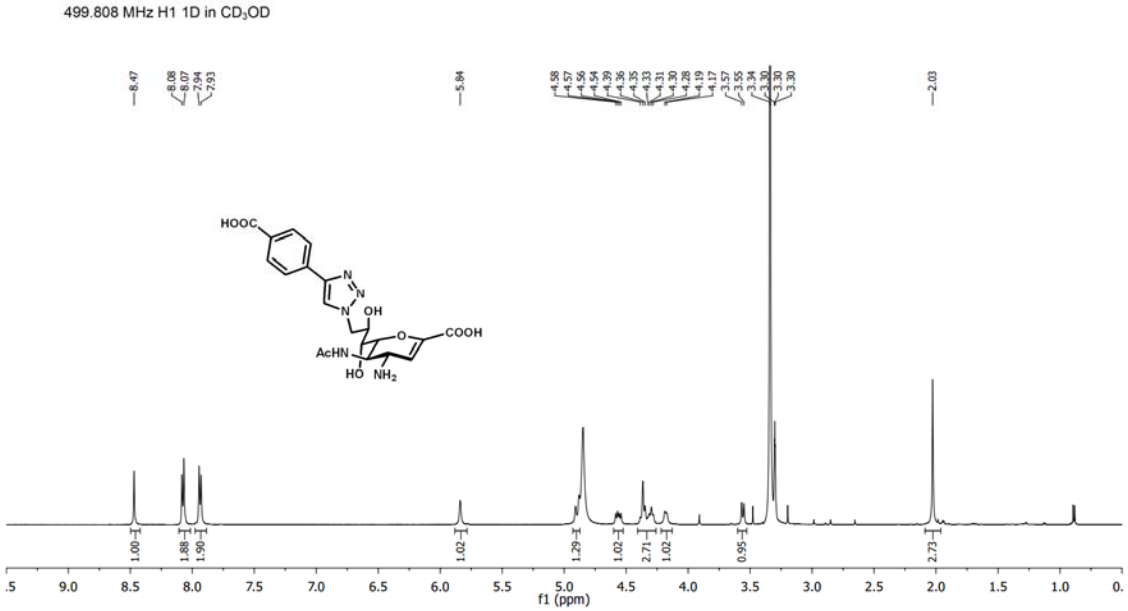

125.691 MHz C13[H1] 1D in CD3OD
f1 (ppm)

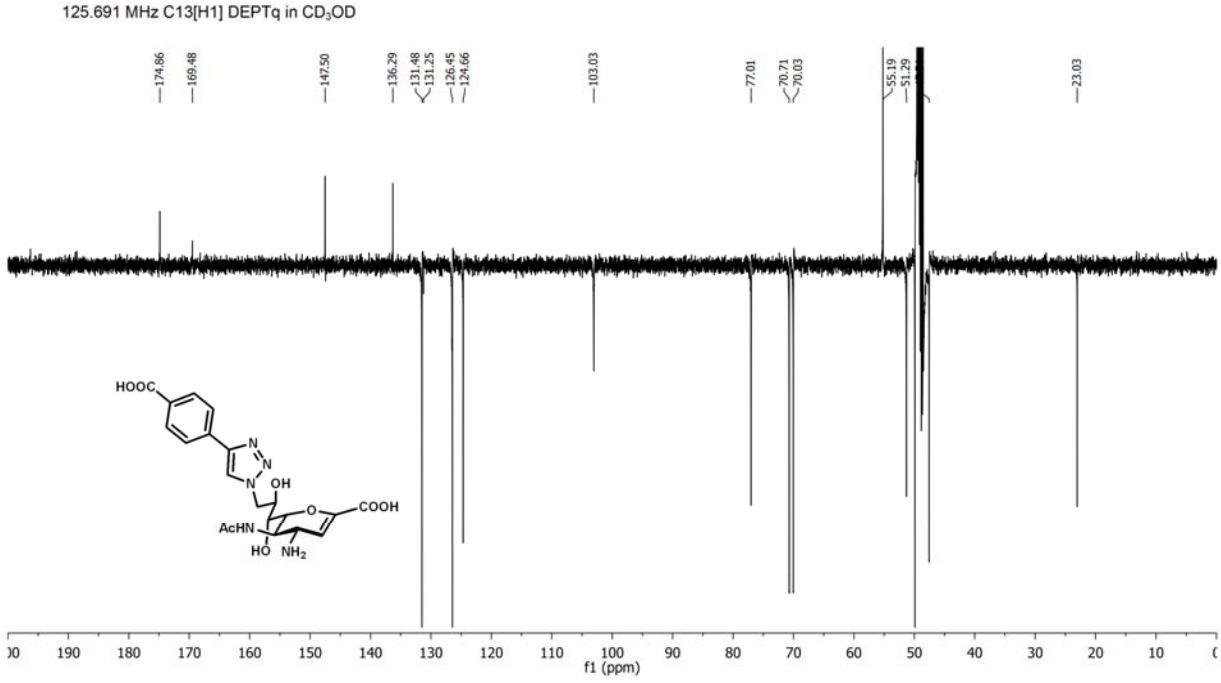
**13**


499.808 MHz H1 1D in CD3OD
8.43
8.07
8.06
7.92
7.91
5.73
4.55
4.53
4.52
4.51
4.31
4.28
4.28
4.26
4.26
4.24
4.21
4.17
4.15
4.13
3.46
3.44
3.34
3.31
3.30
3.30
3.30
3.30
3.29
1.98
HOOC
N
N
N
OH
O
COOH
AcHN
HO
N3
1.00
1.99
2.01
1.01
1.09
1.87
1.07
1.05
0.98
2.93
f1 (ppm)

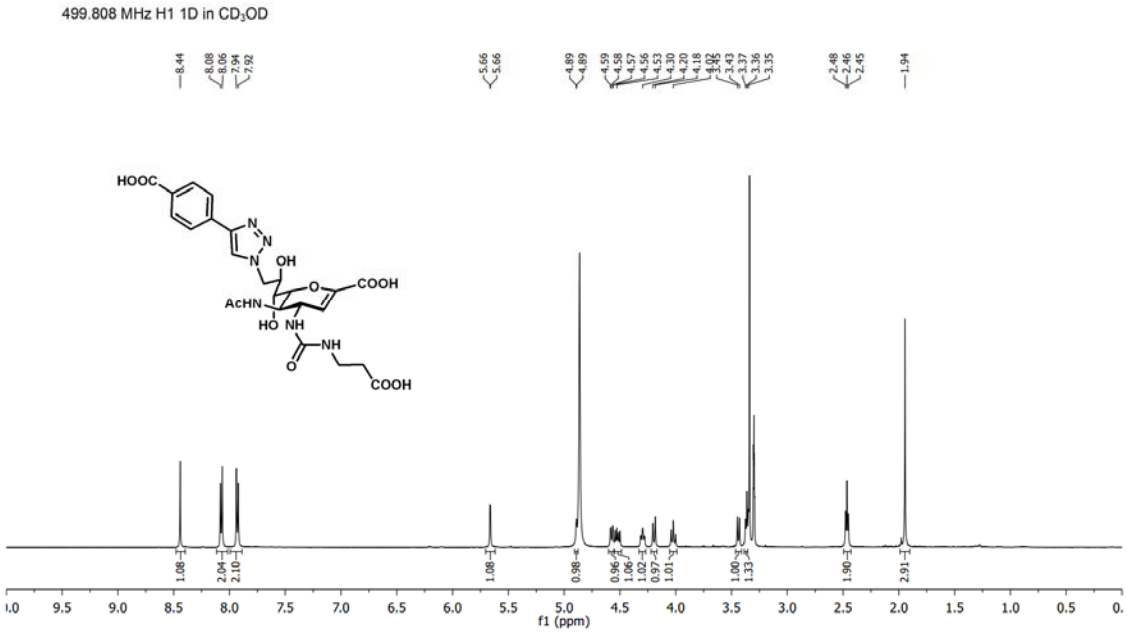

125.691 MHz C13[H1] DEPTq in CD3OD
174.32
147.53
136.17
131.48
126.42
124.47
104.50
77.02
71.03
69.86
60.23
55.25
49.55
49.49
49.38
49.21
49.04
48.87
48.70
48.53
22.78
HOOC
N
N
N
OH
O
COOH
AcHN
HO
N3
f1 (ppm)

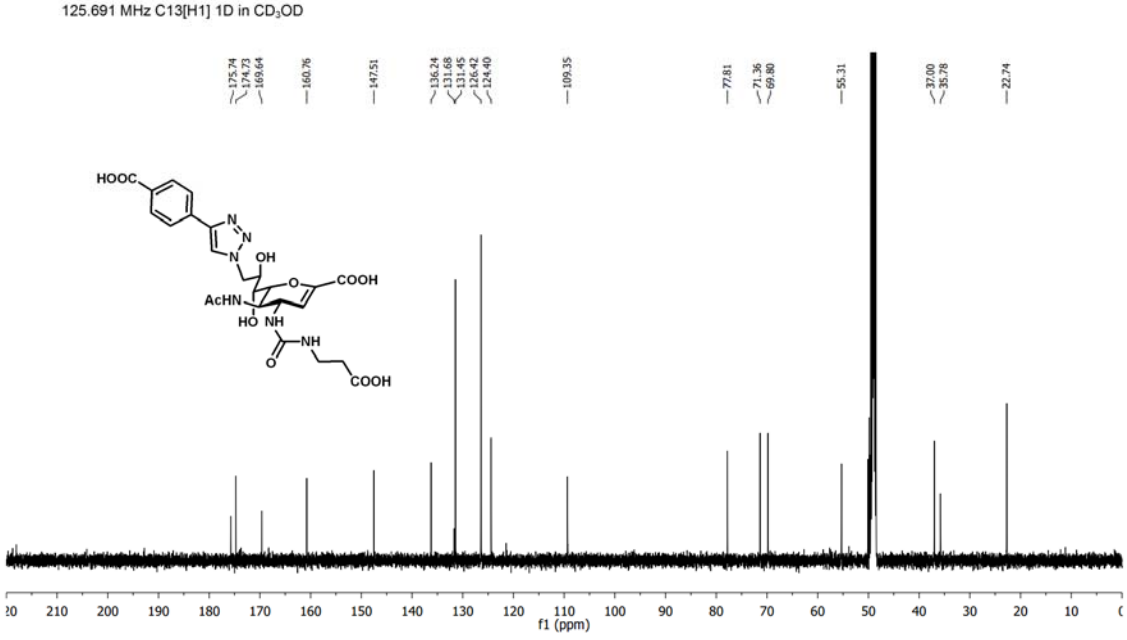
**15**


499.808 MHz H1 1D in CD3OD
8.47
8.08
8.07
7.94
7.93
5.84
4.58
4.57
4.56
4.54
4.39
4.36
4.35
4.33
4.31
4.30
4.28
4.19
4.17
3.57
3.55
3.34
3.30
3.30
3.30
2.03
HOOC
N
N
N
OH
O
COOH
AcHN
HO
NH2
1.00
1.88
1.90
1.02
1.29
1.02
2.71
1.02
0.95
2.73
f1 (ppm)

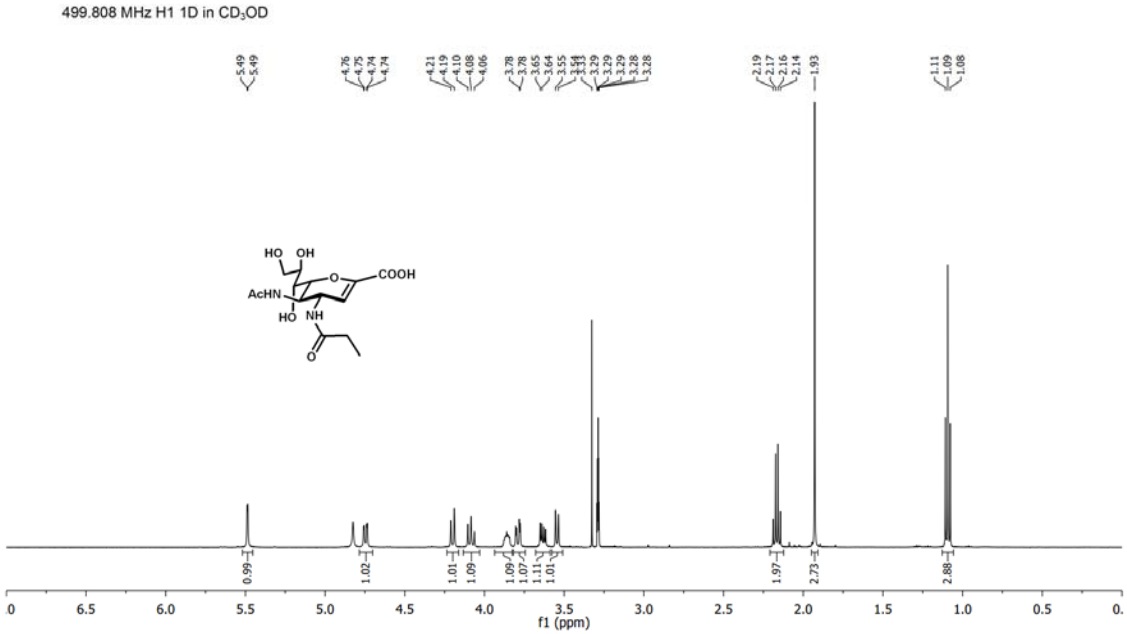

125.691 MHz C13[H1] DEPTq in CD3OD
174.86
169.48
147.50
136.29
131.48
131.25
126.45
124.66
103.03
77.01
70.71
70.03
55.19
51.29
23.03
HOOC
N
N
N
OH
O
COOH
AcHN
HO
NH2
f1 (ppm)

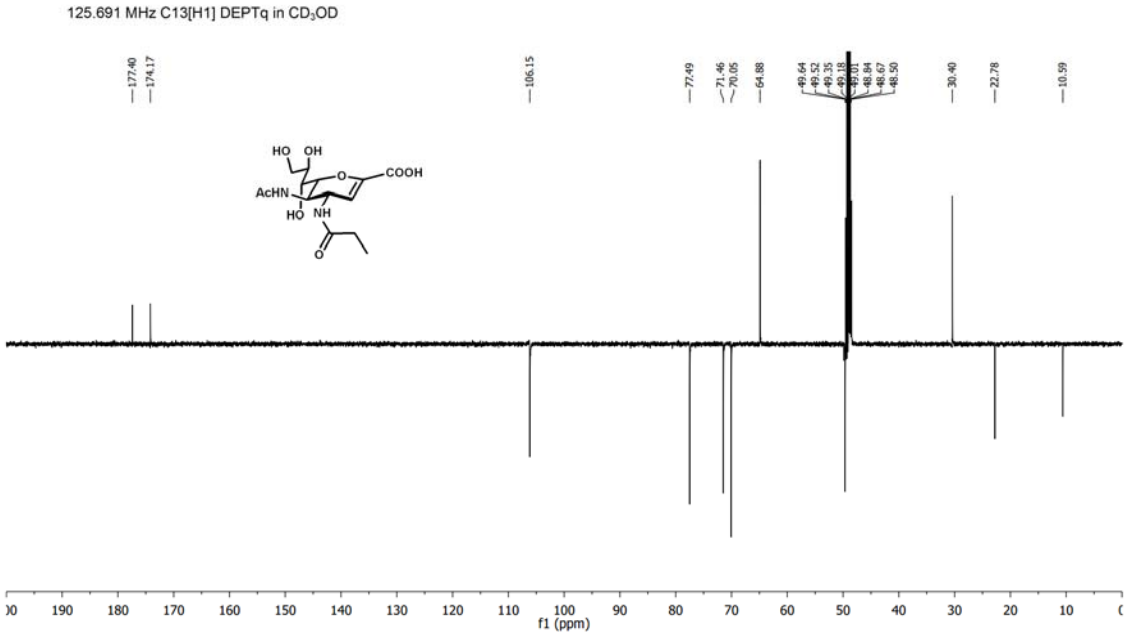
**18**


499.808 MHz H1 1D in CD3OD
8.44
8.08
8.06
7.94
7.92
5.66
5.66
4.89
4.89
4.59
4.58
4.57
4.56
4.53
4.30
4.20
4.18
3.43
3.37
3.36
3.35
2.48
2.46
2.45
1.94
HOOC
N
N
N
OH
O
COOH
AcHN
HO
NH
NH
O
COOH
1.08
2.04
2.10
1.08
0.98
0.96
1.06
1.02
0.97
1.01
1.00
1.33
1.90
2.91
f1 (ppm)

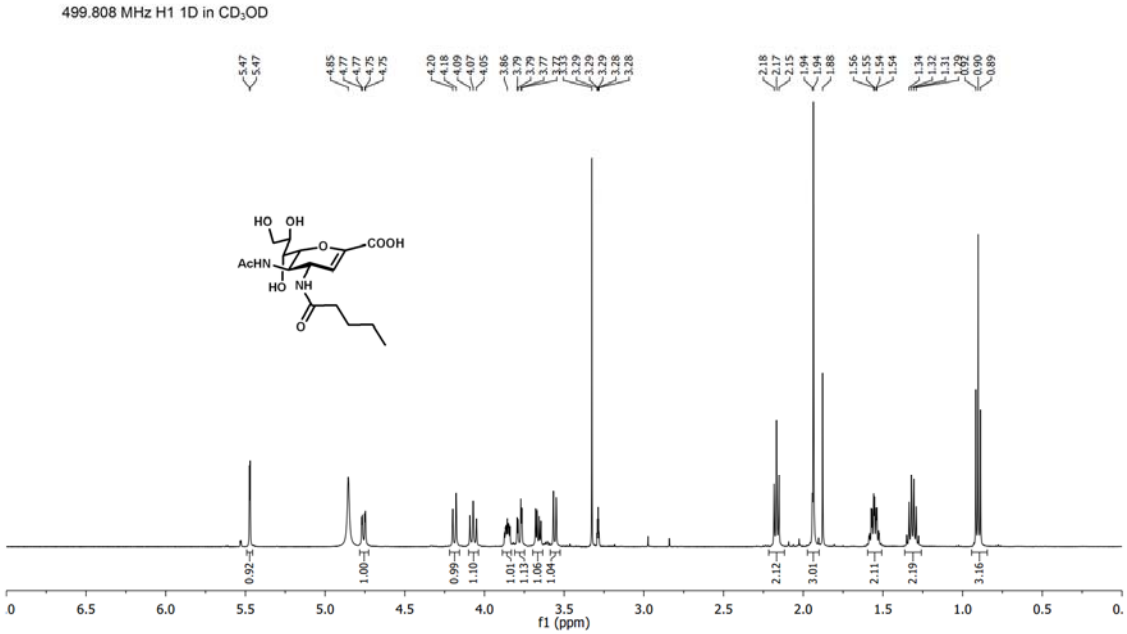

125.691 MHz C13[H1] 1D in CD3OD
175.74
174.73
169.64
160.76
147.51
136.24
131.68
131.45
126.42
124.40
109.35
77.81
71.36
69.80
55.31
37.00
35.78
22.74
HOOC
N
N
N
OH
O
COOH
AcHN
HO
NH
NH
O
COOH
f1 (ppm)

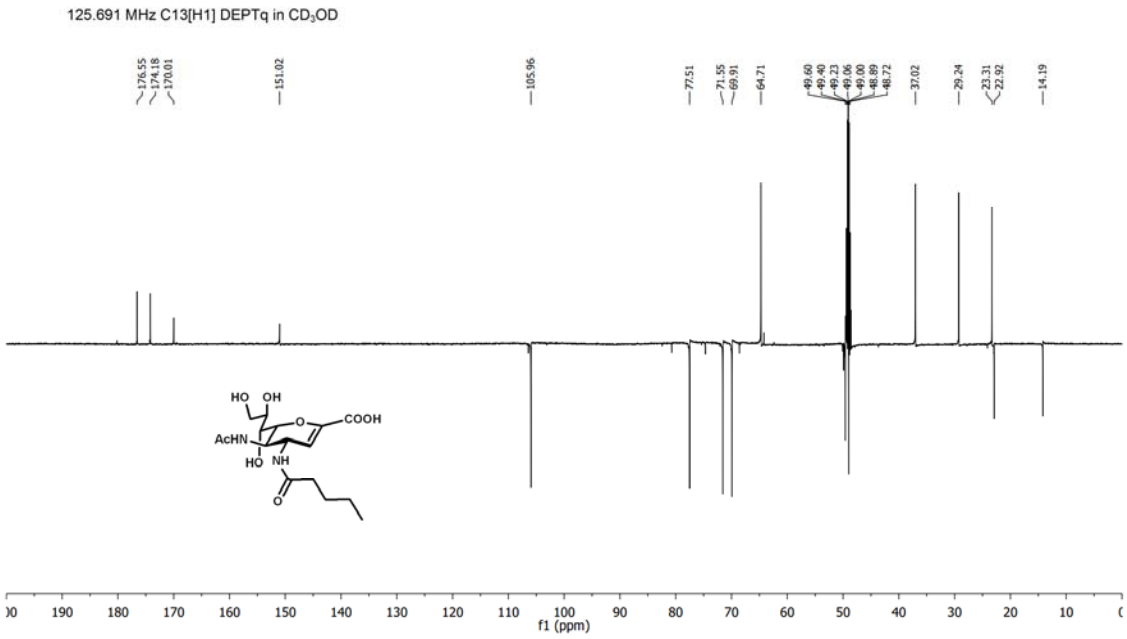
**25a**


499.808 MHz H1 1D in CD3OD
f1 (ppm)

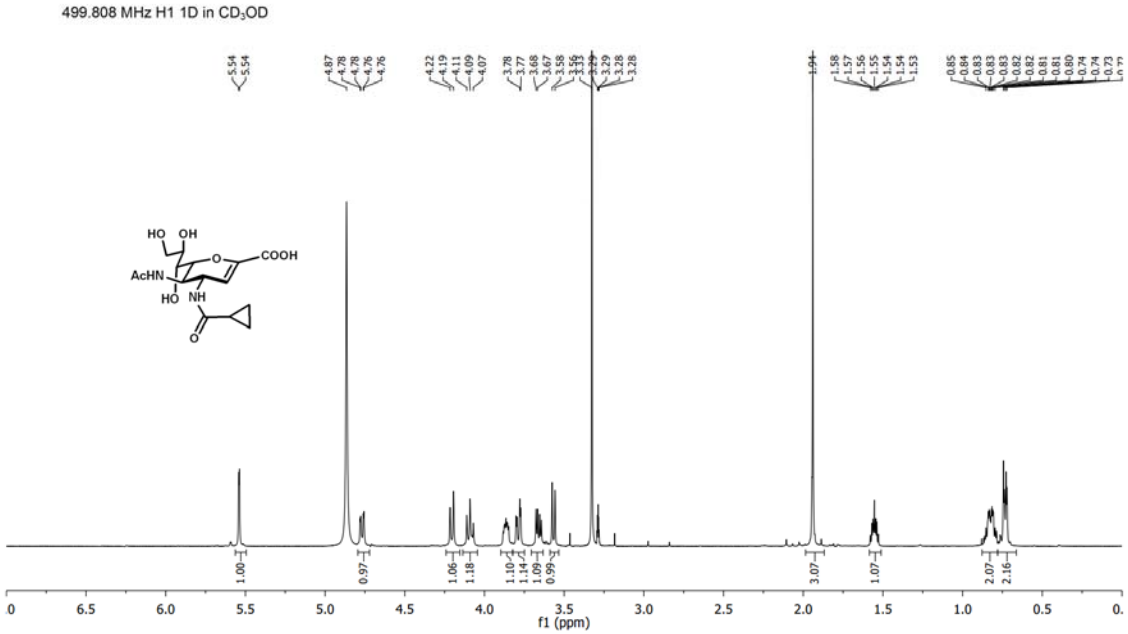

125.691 MHz C13[H1] DEPTq in CD3OD
f1 (ppm)

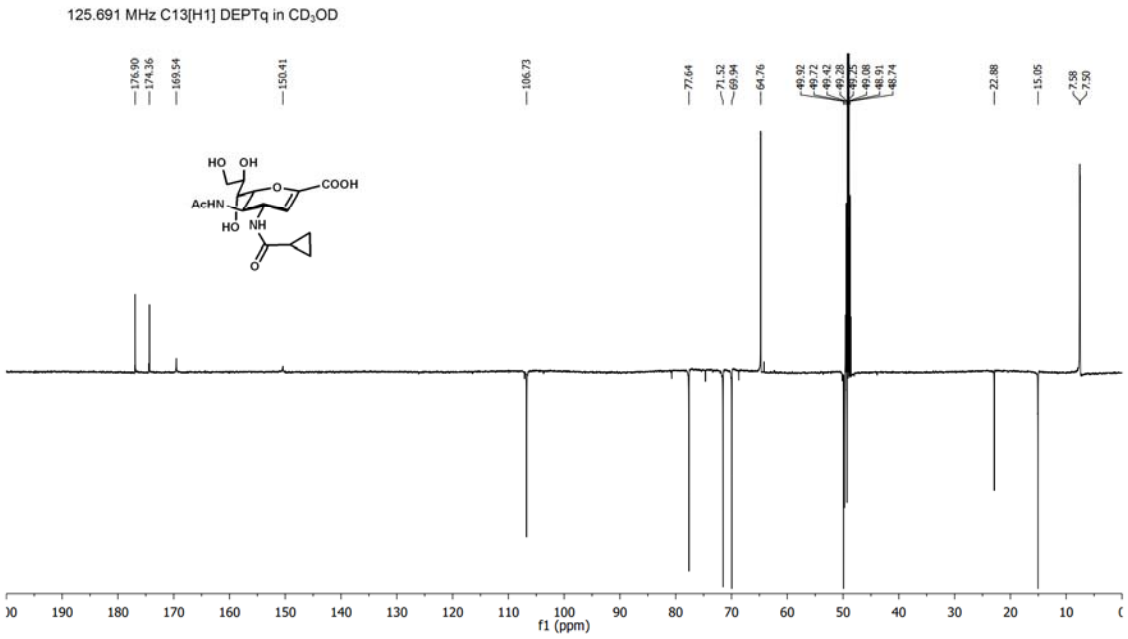
**25b**


499.808 MHz H1 1D in CD3OD
f1 (ppm)

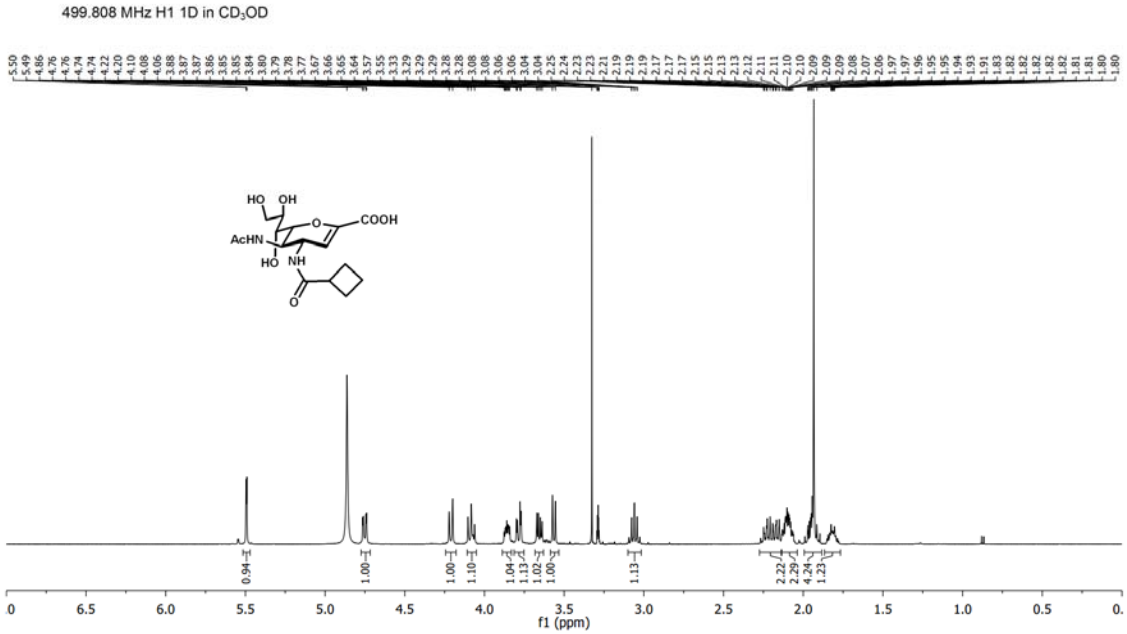

125.691 MHz C13[H1] DEPTq in CD3OD
f1 (ppm)

**25c**


499.808 MHz H1 1D in CD3OD
HO
OH
COOH
AcHN
HO
NH
O
f1 (ppm)


125.691 MHz C13[H1] DEPTq in CD3OD
176.90
174.36
169.54
150.41
106.73
77.64
71.52
69.94
64.76
49.92
49.72
49.42
49.28
49.25
49.08
48.91
48.74
22.88
15.05
7.58
7.50
HO
OH
COOH
AcHN
HO
NH
O
f1 (ppm)

**25d**


499.808 MHz H1 1D in CD3OD
HO
OH
COOH
AcHN
HO
NH
O
f1 (ppm)

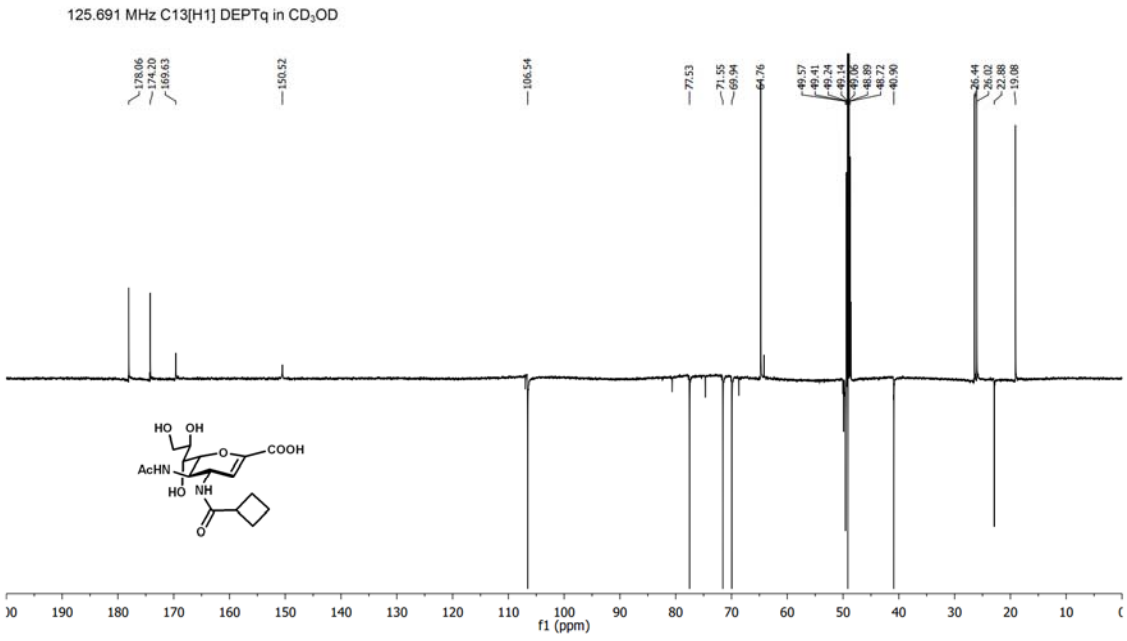
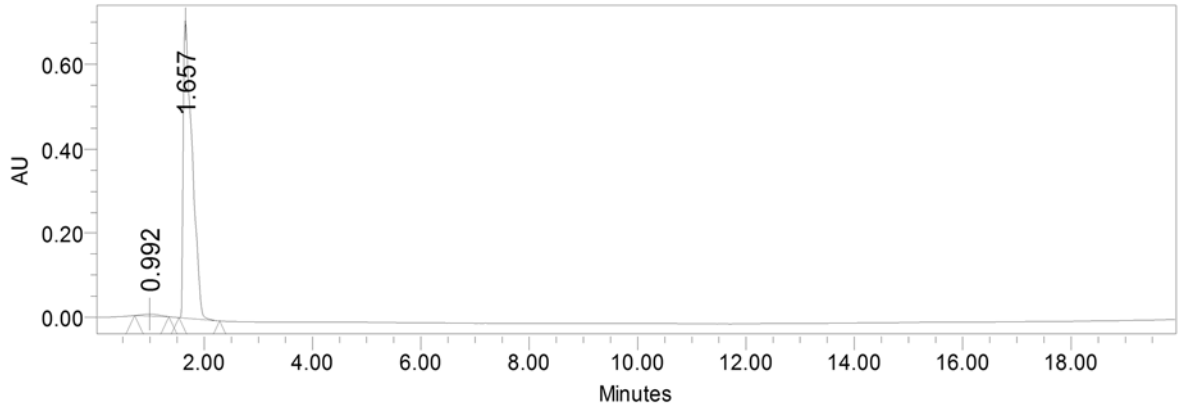

125.691 MHz C13[H1] DEPTq in CD3OD
178.06
174.20
169.63
150.52
106.54
77.53
71.55
69.94
64.76
49.57
49.41
49.24
49.14
49.06
48.89
48.72
40.90
26.44
26.02
22.88
19.08
HO
OH
COOH
AcHN
HO
NH
O
f1 (ppm)

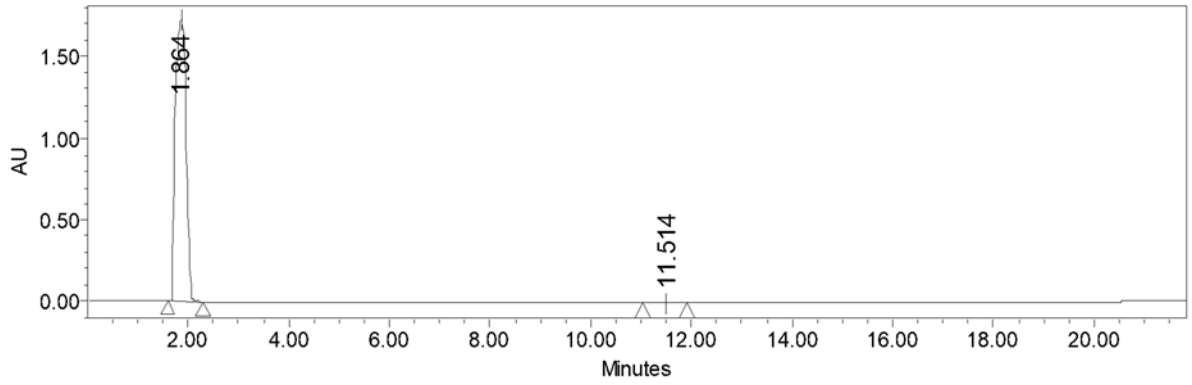
**Figure S.4: HPLC traces and LC-MS of esters**

**1(DANA)**



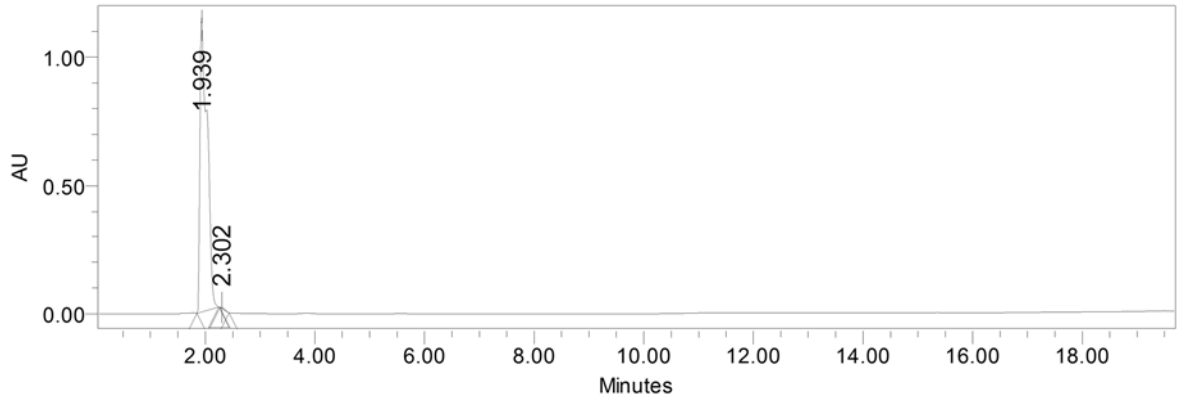

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 0.992 | 106518 | 1.30 |
| 2 | 1.657 | 8078848 | 98.70 |

**2(C9-BA-DANA)**



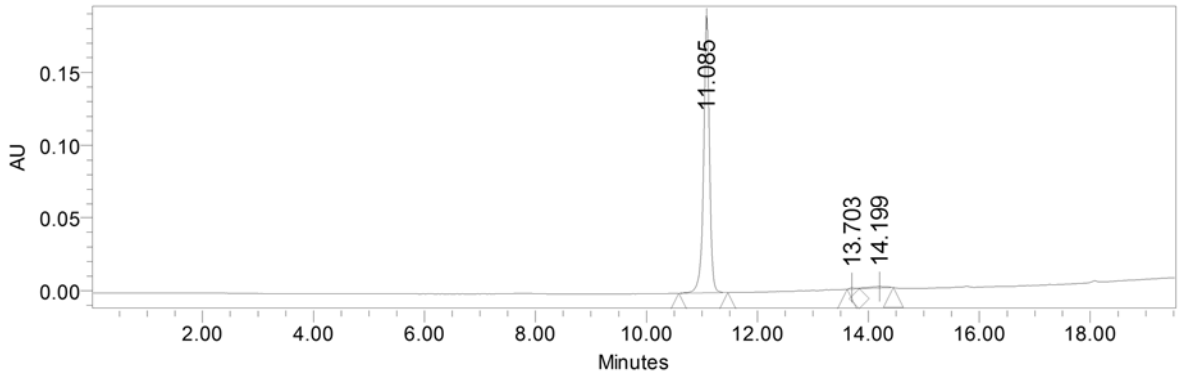

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 1.864 | 25156549 | 99.97 |
| 2 | 11.514 | 7667 | 0.03 |

**6(Zanamivir)**



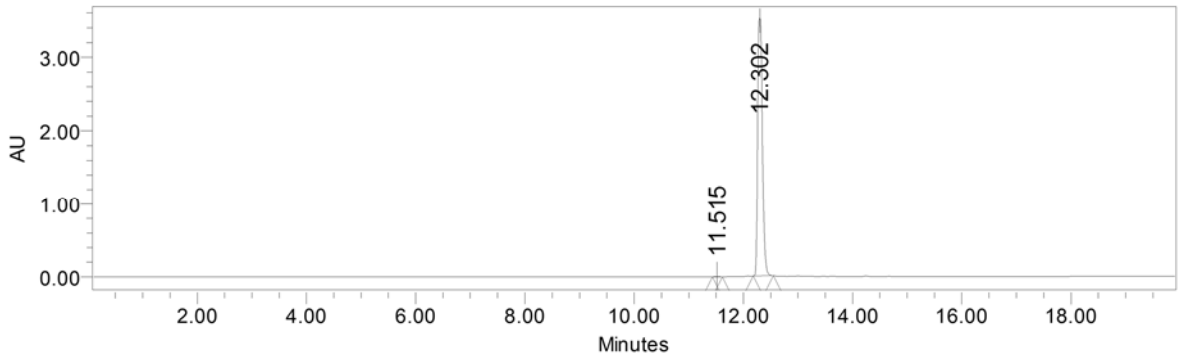

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 1.939 | 10488850 | 99.78 |
| 2 | 2.302 | 23638 | 0.22 |

**7a**



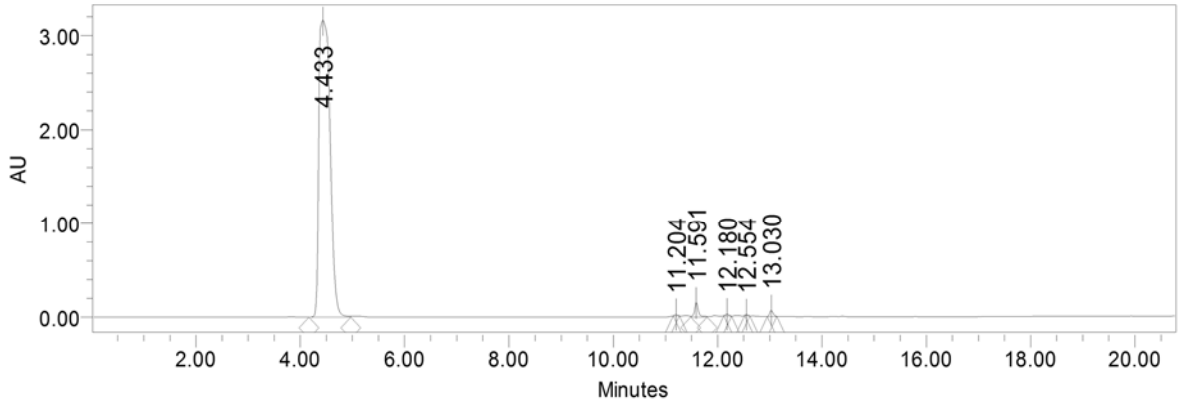

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 11.085 | 1402254 | 97.69 |
| 2 | 13.703 | 7549 | 0.53 |
| 3 | 14.199 | 25646 | 1.79 |

**7b**



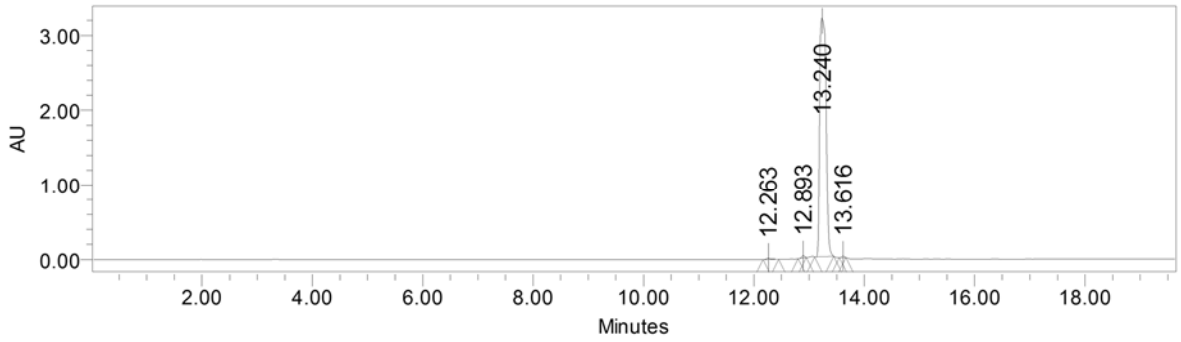

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 11.515 | 17119 | 0.08 |
| 2 | 12.302 | 21018453 | 99.92 |

**7c**



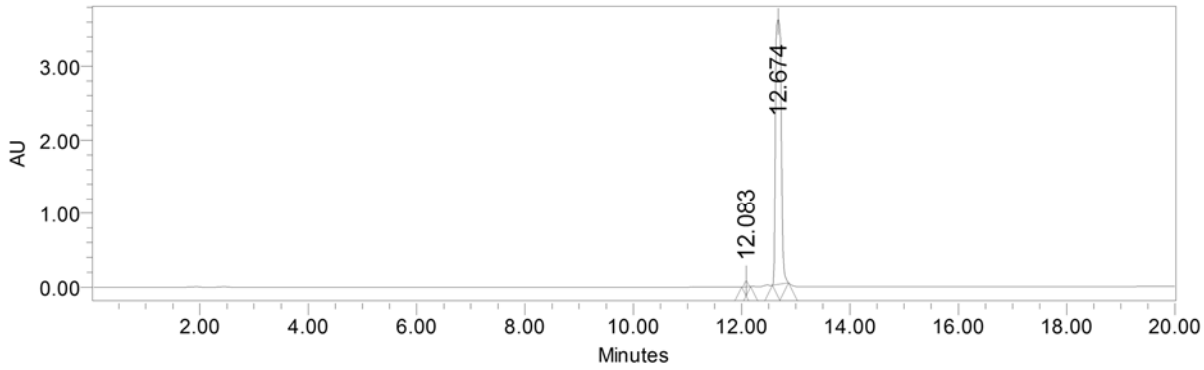

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 4.433 | 47355681 | 97.35 |
| 2 | 11.204 | 59740 | 0.12 |
| 3 | 11.591 | 798423 | 1.64 |
| 4 | 12.180 | 91828 | 0.19 |
| 5 | 12.554 | 43814 | 0.09 |
| 6 | 13.030 | 296047 | 0.61 |

**7d**



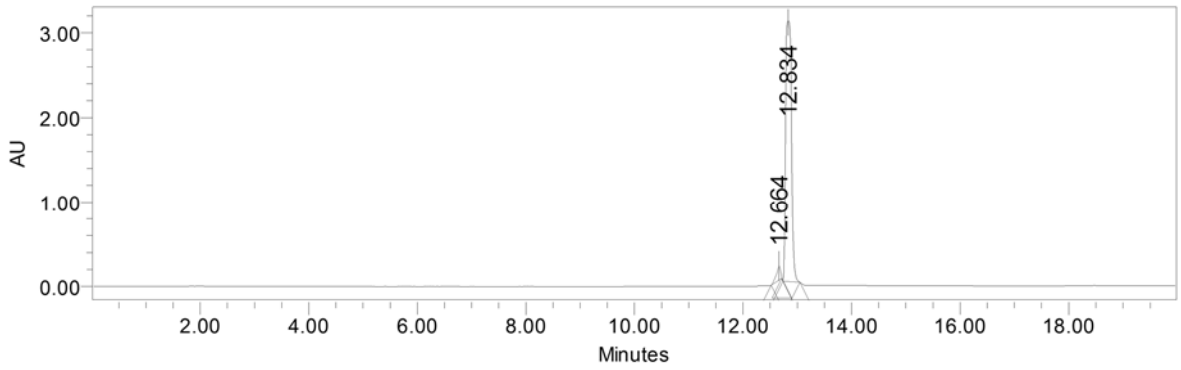

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 12.263 | 82134 | 0.32 |
| 2 | 12.893 | 119223 | 0.46 |
| 3 | 13.240 | 25686435 | 98.94 |
| 4 | 13.616 | 73491 | 0.28 |

**7e**



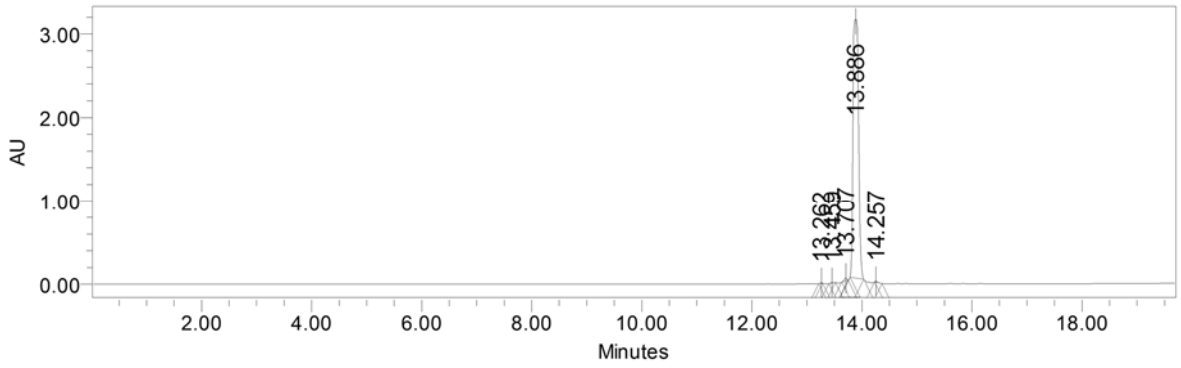

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 12.083 | 336719 | 1.26 |
| 2 | 12.674 | 26376013 | 98.74 |

**7f**



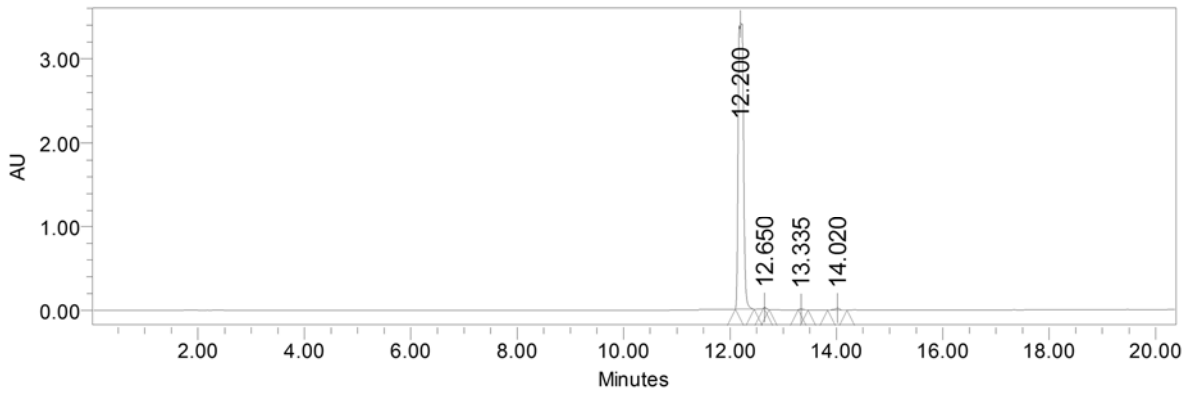

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 12.664 | 877386 | 3.64 |
| 2 | 12.834 | 23226938 | 96.36 |

**7g**



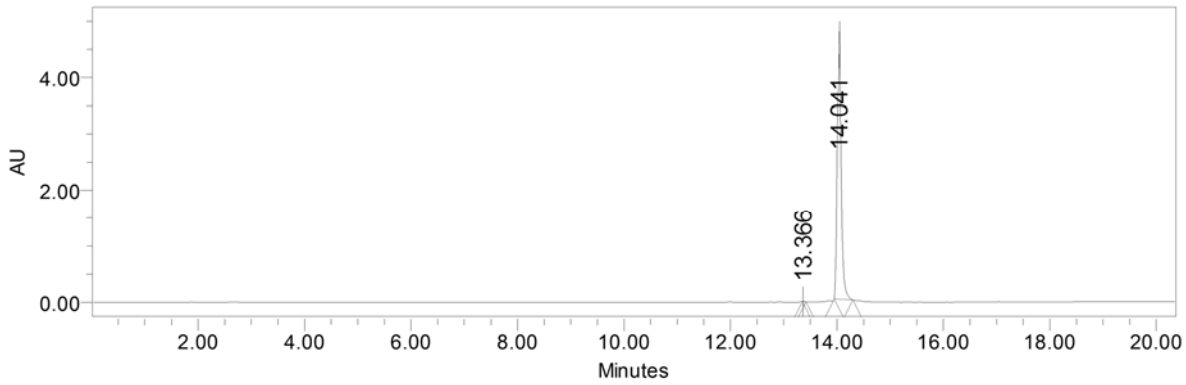

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 13.262 | 14442 | 0.07 |
| 2 | 13.459 | 30148 | 0.14 |
| 3 | 13.707 | 118468 | 0.55 |
| 4 | 13.886 | 21464337 | 99.11 |
| 5 | 14.257 | 29477 | 0.14 |

**7h**



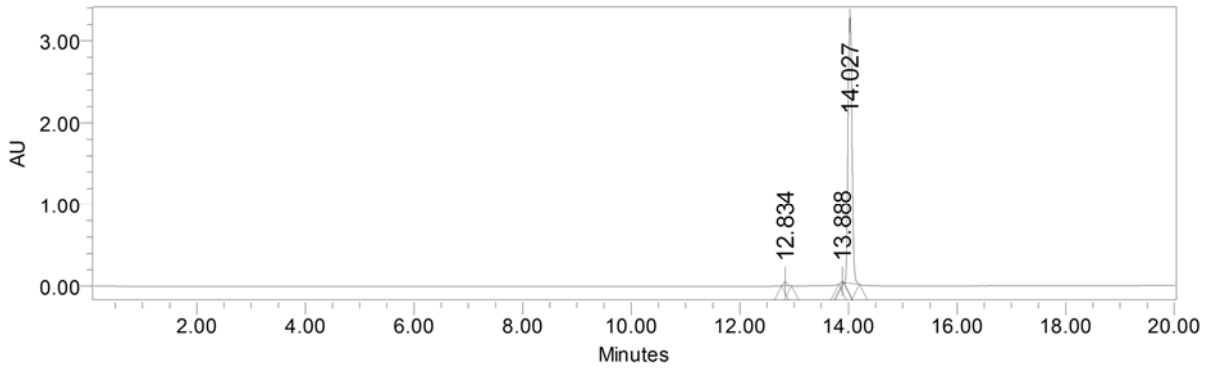

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 12.200 | 23946655 | 99.05 |
| 2 | 12.650 | 21704 | 0.09 |
| 3 | 13.335 | 59482 | 0.25 |
| 4 | 14.020 | 147588 | 0.61 |

**7i**



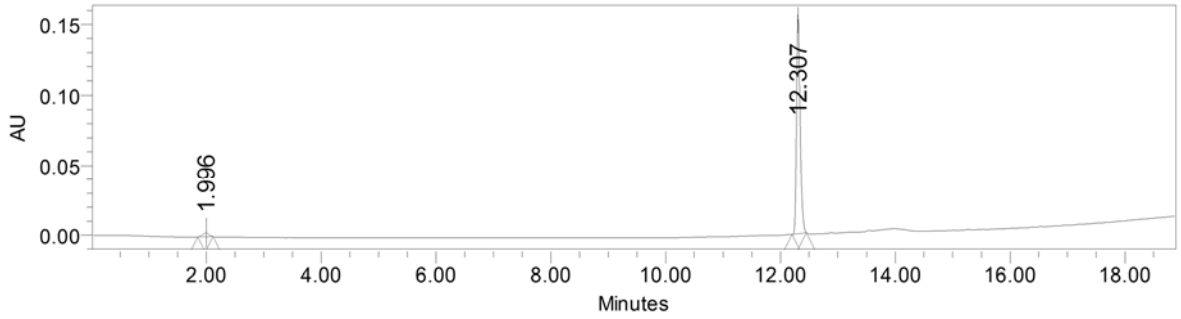

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 13.366 | 6371 | 0.03 |
| 2 | 14.041 | 23867337 | 99.97 |

**7j**



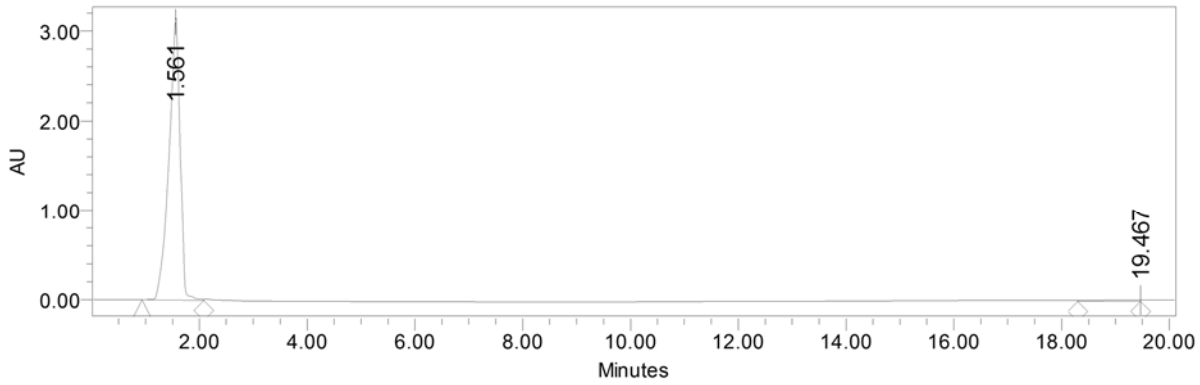

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 12.834 | 219939 | 1.31 |
| 2 | 13.888 | 32337 | 0.19 |
| 3 | 14.027 | 16503460 | 98.49 |

**8a**



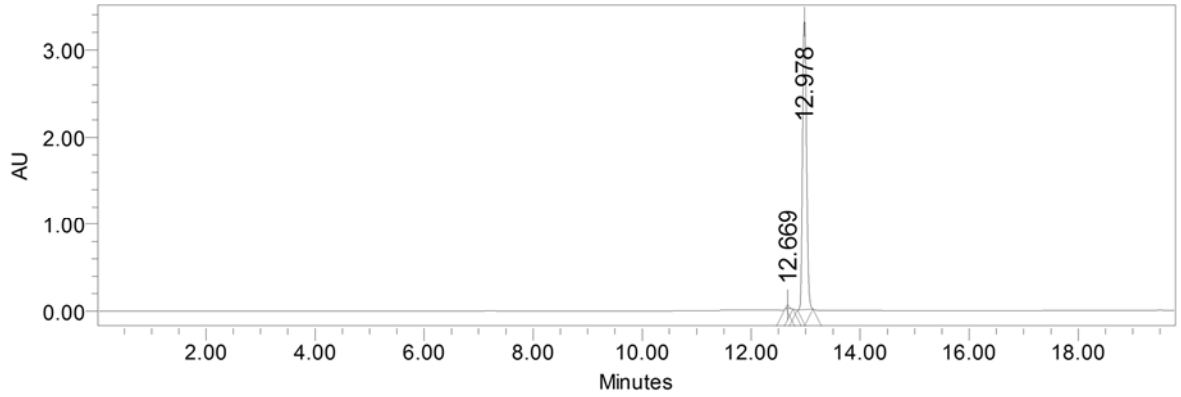

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 1.996 | 20928 | 2.96 |
| 2 | 12.307 | 686710 | 97.04 |

**8b**



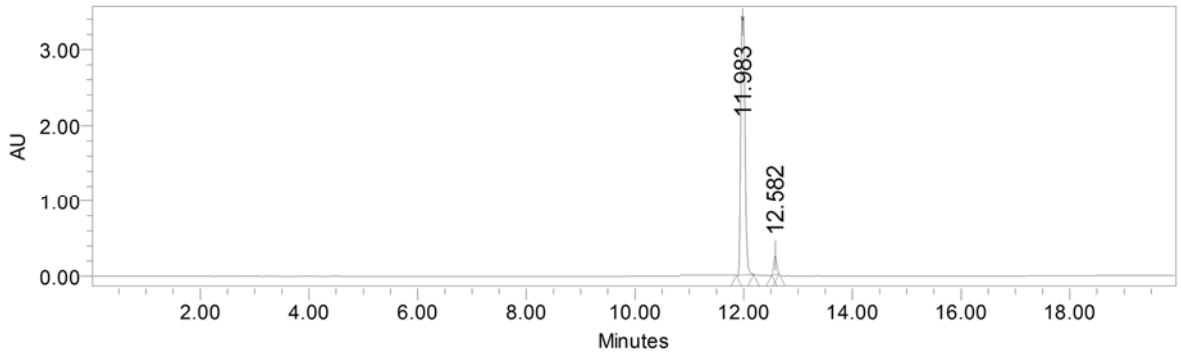

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 1.561 | 45407337 | 97.84 |
| 2 | 19.467 | 1016824 | 2.16 |

**13**



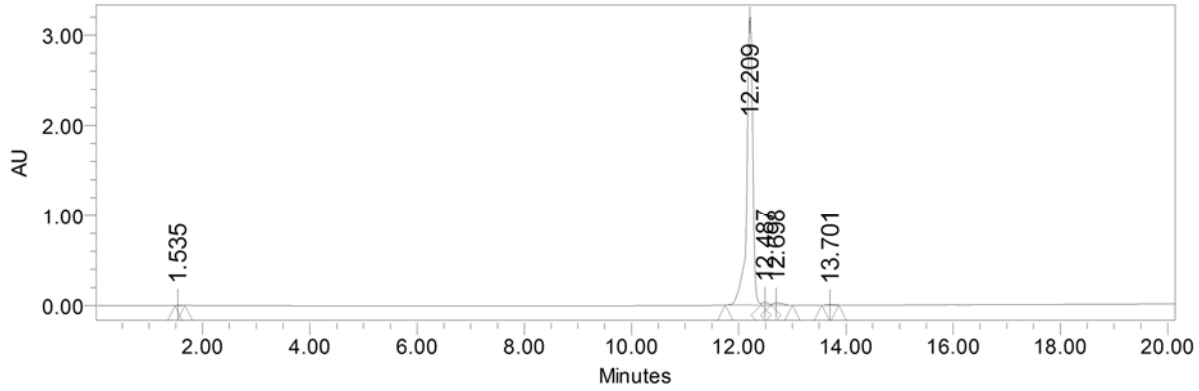

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 12.669 | 126606 | 0.73 |
| 2 | 12.978 | 17163838 | 99.27 |

**15**



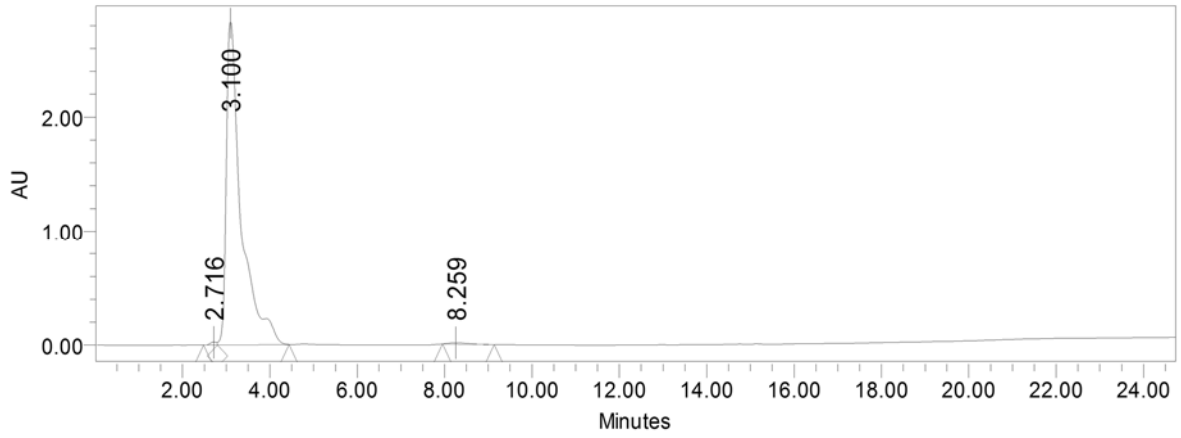

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 11.983 | 18920046 | 95.11 |
| 2 | 12.582 | 971880 | 4.89 |

**18**



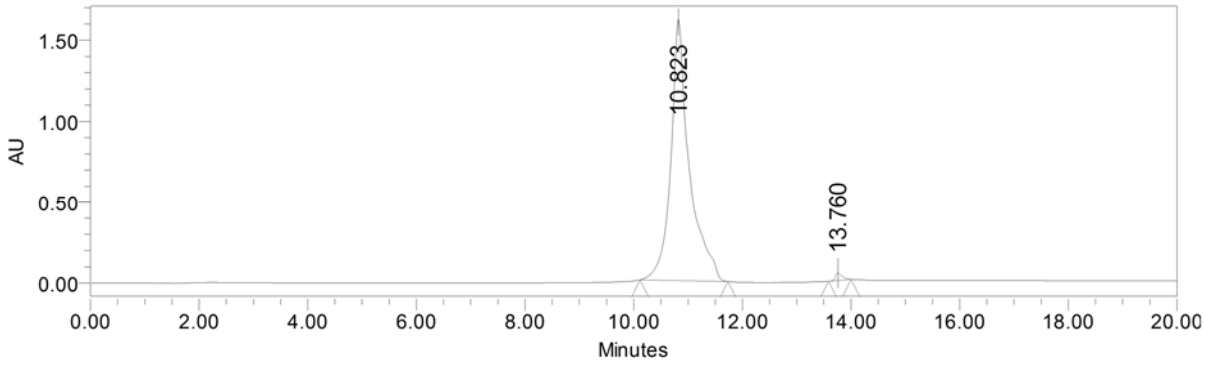

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 1.535 | 34220 | 0.13 |
| 2 | 12.209 | 24814748 | 97.58 |
| 3 | 12.487 | 232178 | 0.91 |
| 4 | 12.698 | 298943 | 1.18 |
| 5 | 13.701 | 49206 | 0.19 |

**25a**



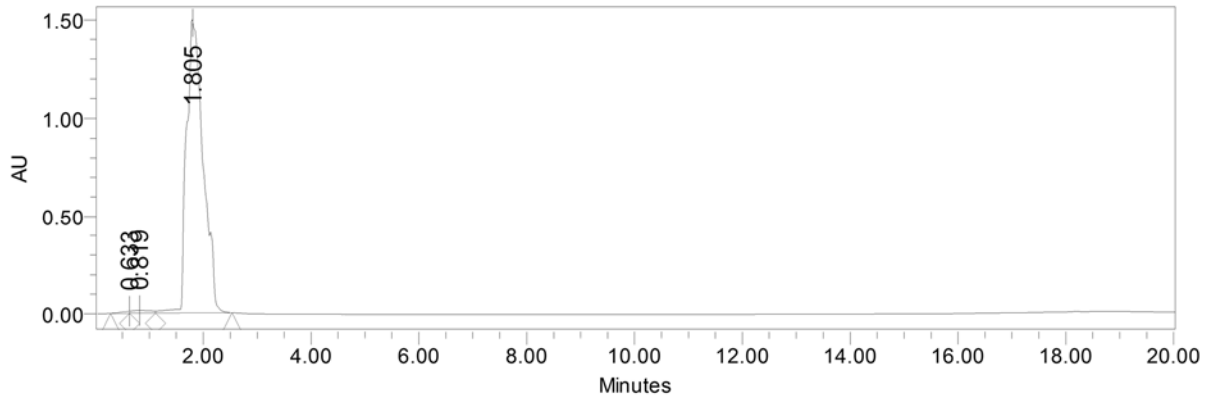

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 2.716 | 257020 | 0.38 |
| 2 | 3.100 | 66909481 | 99.05 |
| 3 | 8.259 | 384493 | 0.57 |

**25b**



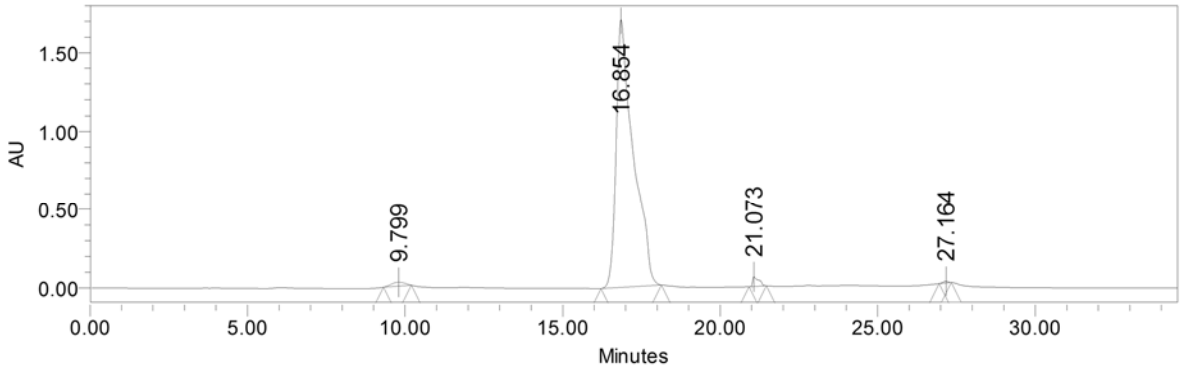

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 10.823 | 38232298 | 98.98 |
| 2 | 13.760 | 394274 | 1.02 |

**25c**




| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 0.633 | 110356 | 0.34 |
| 2 | 0.819 | 372916 | 1.14 |
| 3 | 1.805 | 32192143 | 98.52 |

**25d**



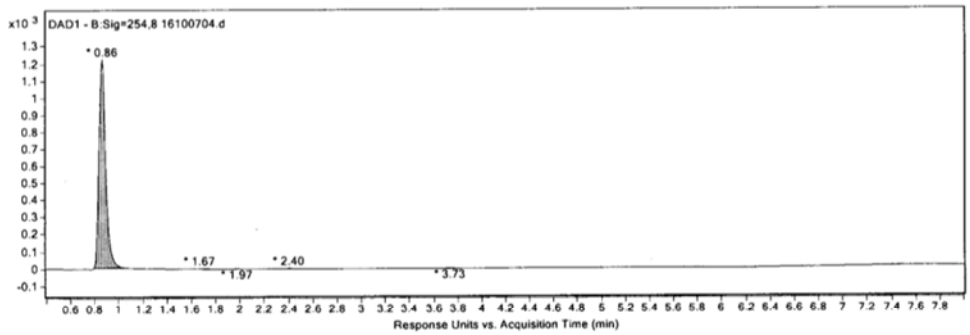

| | Retention Time | Area | % Area |
|---|---|---|---|
| 1 | 9.799 | 737757 | 1.11 |
| 2 | 16.854 | 64505616 | 97.44 |
| 3 | 21.073 | 841157 | 1.27 |
| 4 | 27.164 | 118339 | 0.18 |

**LC-MS analysis of esters.**

LC-MS Methods:
Solvent A: 0.1%FA in $H_2O$
Solvent B: 0.1%FA in ACN
Flow=0.5mL/min
First 0.3 mins: LC to waste
Kinetex Luna Evo C18 Polar with guard, 2.1x50mm, 1.6um, 100Å, temperature 40°C
Fragmentor: 150V
DAD: 254nm
Gradient:

| Time | %B |
|---|---|
| 0.00 | 2.00 |
| 0.50 | 2.00 |
| 5.00 | 95.0 |
| 8.00 | 95.0 |
| 9.0 | 2.00 |

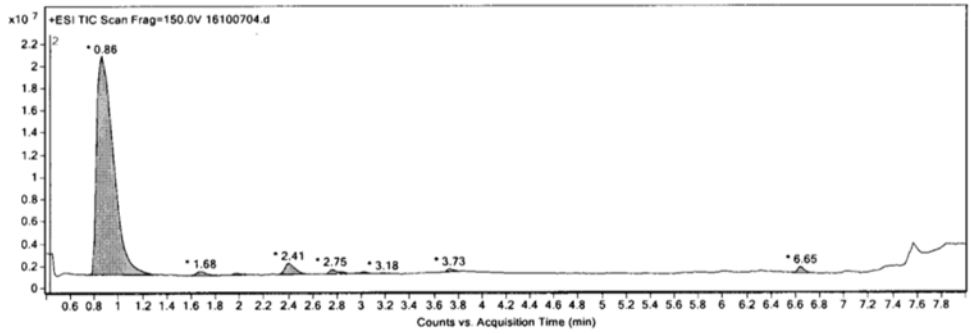
LC-MS results of **DANA-OMe**




**Integration Peak List**

| Peak | Start | RT | End | Height | Area | Area % |
|---|---|---|---|---|---|---|
| 1 | 0.79 | 0.86 | 1.04 | 1217.08 | 4634.97 | 100 |
| 2 | 1.61 | 1.67 | 1.76 | 0.85 | 3.74 | 0.08 |
| 3 | 1.88 | 1.97 | 2.17 | 1.41 | 6.5 | 0.14 |
| 4 | 2.28 | 2.4 | 2.59 | 2.28 | 11.28 | 0.24 |
| 5 | 3.59 | 3.73 | 3.81 | 1.27 | 5.76 | 0.12 |

**Fragmentor Voltage** 150 **Collision Energy** 0 **Ionization Mode** ESI




**Integration Peak List**

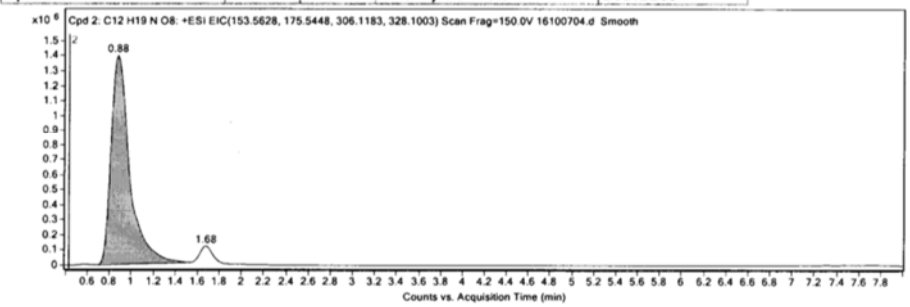
| Peak | Start | RT | End | Height | Area | Area % |
|---|---|---|---|---|---|---|
| 1 | 0.75 | 0.86 | 1.33 | 19651359 | 190908482 | 100 |
| 2 | 1.55 | 1.68 | 1.84 | 365139 | 2259383 | 1.18 |
| 3 | 1.9 | 1.97 | 2.12 | 159857 | 885389 | 0.46 |
| 4 | 2.31 | 2.41 | 2.58 | 981508 | 5647166 | 2.96 |
| 5 | 2.71 | 2.75 | 2.8 | 398485 | 1459311 | 0.76 |
| 6 | 2.82 | 2.83 | 2.91 | 177032 | 516037 | 0.27 |
| 7 | 2.93 | 3.02 | 3.1 | 161134 | 710349 | 0.37 |
| 8 | 3.13 | 3.18 | 3.29 | 50052 | 200265 | 0.1 |
| 9 | 3.67 | 3.73 | 3.84 | 256557 | 899922 | 0.47 |
| 10 | 6.57 | 6.65 | 6.78 | 549939 | 2092443 | 1.1 |

LC-MS results of **DANA-OMe** (continued)

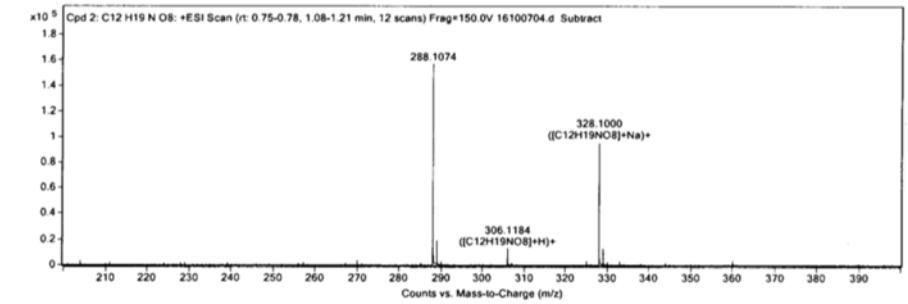
**Compound Table**

| Compound Label | RT | Mass | Abund | Formula | Tgt Mass | Diff (ppm) |
|---|---|---|---|---|---|---|
| Cpd 2: C12 H19 N O8 | 0.88 | 305.1109 | 96125 | C12 H19 N O8 | 305.1111 | -0.5 |
| Cpd 1: C12 H19 N O8 | 1.68 | 305.1111 | 45313 | C12 H19 N O8 | 305.1111 | 0.13 |

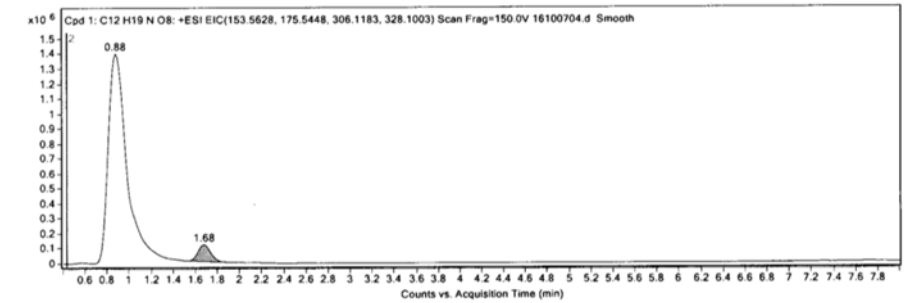
| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 2: C12 H19 N O8 | 328.1 | 0.88 | Find By Formula | 305.1109 |



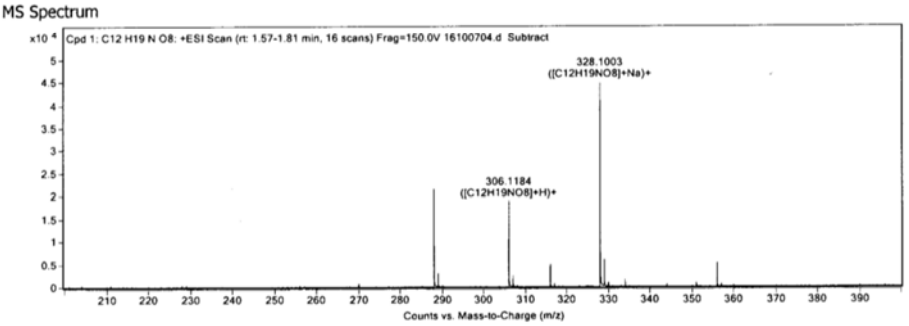

MS Spectrum



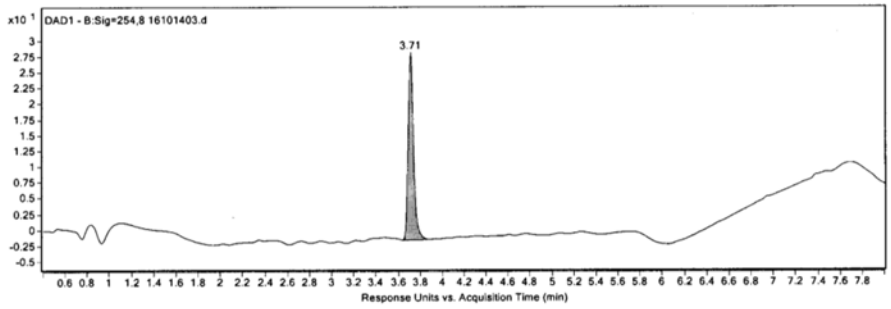

| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 1: C12 H19 N O8 | 328.1003 | 1.68 | Find By Formula | 305.1111 |




MS Spectrum



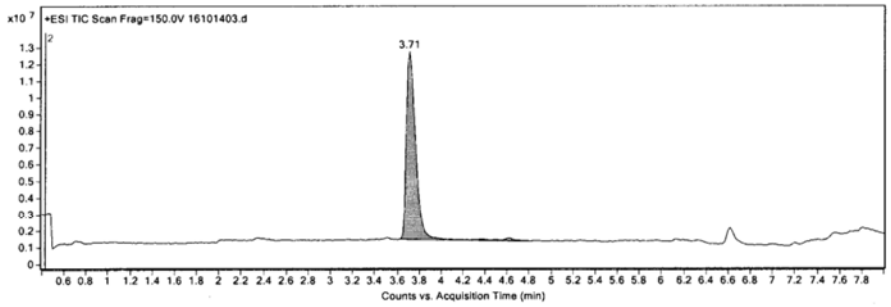
LC-MS results of **2-OMe**




**User Chromatogram Peak List**

| RT | Height | Height % | Area | Area % | Area Sum % | Width |
|---|---|---|---|---|---|---|
| 3.71 | 29.73 | 100 | 101.35 | 100 | 100 | 0.05 |

**Fragmentor Voltage** 150 **Collision Energy** 0 **Ionization Mode** ESI



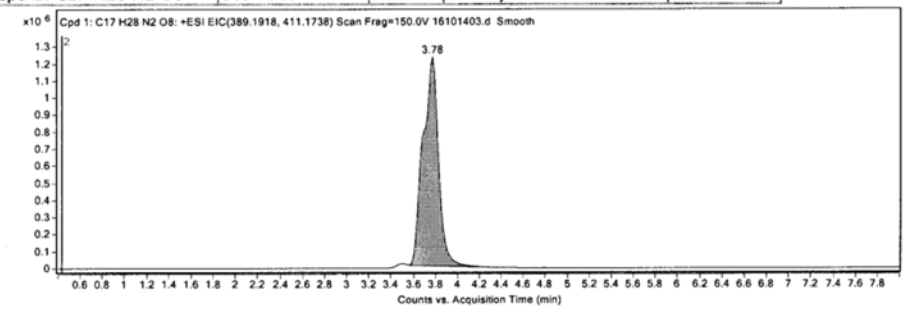

**User Chromatogram Peak List**

| RT | Height | Height % | Area | Area % | Area Sum % | Base Peak m/z | Width |
|---|---|---|---|---|---|---|---|
| 3.71 | 11274135 | 100 | 67797168 | 100 | 100 | 389.2024 | 1.18 |

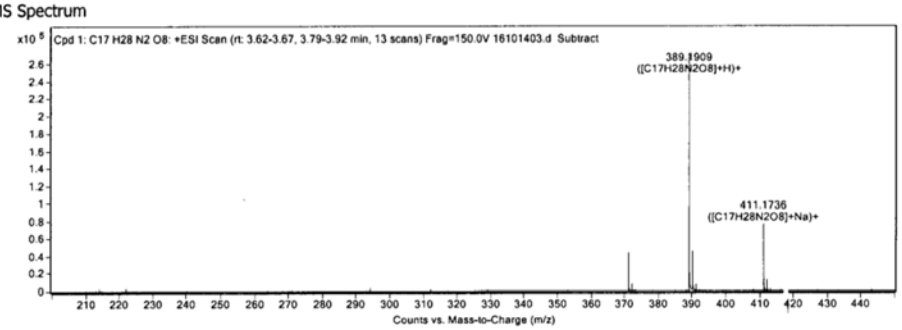
**Compound Table**

| Compound Label | RT | Mass | Abund | Formula | Tgt Mass | Diff (ppm) |
|---|---|---|---|---|---|---|
| Cpd 1: C17 H28 N2 O8 | 3.78 | 388.184 | 273668 | C17 H28 N2 O8 | 388.1846 | -1.42 |

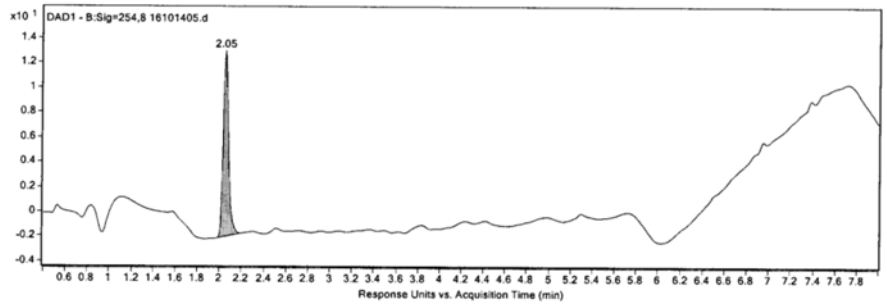
| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 1: C17 H28 N2 O8 | 389.1909 | 3.78 | Find By Formula | 388.184 |




MS Spectrum



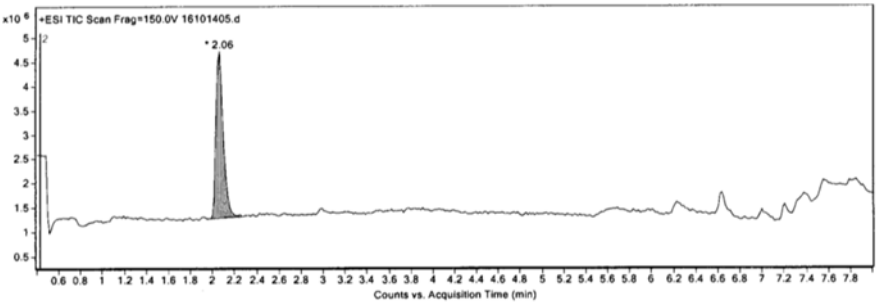
LC-MS results of **5-OMe**




**User Chromatogram Peak List**

| RT | Height | Height % | Area | Area % | Area Sum % | Width |
|---|---|---|---|---|---|---|
| 2.05 | 15.02 | 100 | 50.61 | 100 | 100 | 0.05 |

**Fragmentor Voltage** 150 **Collision Energy** 0 **Ionization Mode** ESI



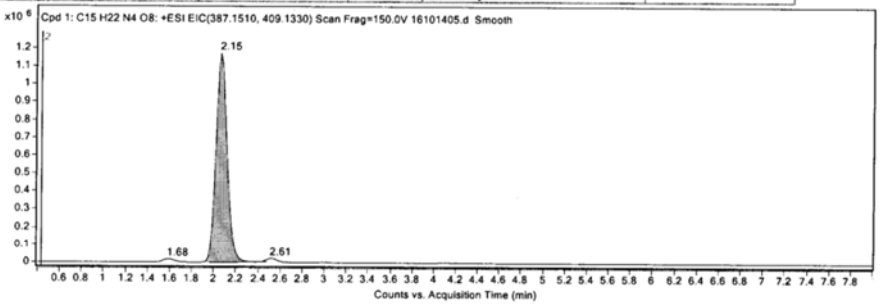

**User Chromatogram Peak List**

| RT | Height | Height % | Area | Area % | Area Sum % | Width |
|---|---|---|---|---|---|---|
| 2.06 | 3446556 | 100 | 15969380 | 100 | 100 | 0.3 |

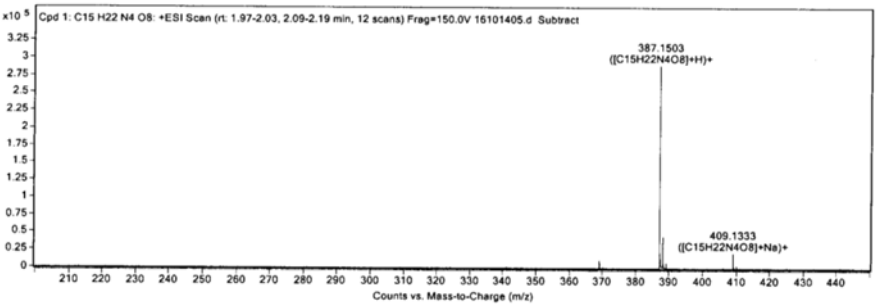
**Compound Table**

| Compound Label | RT | Mass | Abund | Formula | Tgt Mass | Diff (ppm) |
|---|---|---|---|---|---|---|
| Cpd 1: C15 H22 N4 O8 | 2.06 | 386.1432 | 21869 | C15 H22 N4 O8 | 386.1438 | -1.51 |

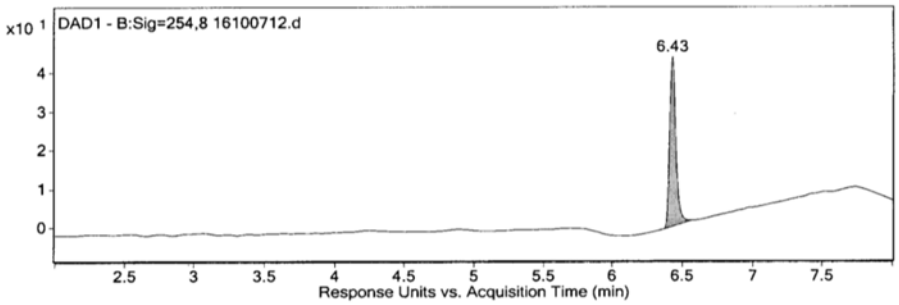
| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 1: C15 H22 N4 O8 | 409.1333 | 2.06 | Find By Formula | 386.1432 |




MS Spectrum



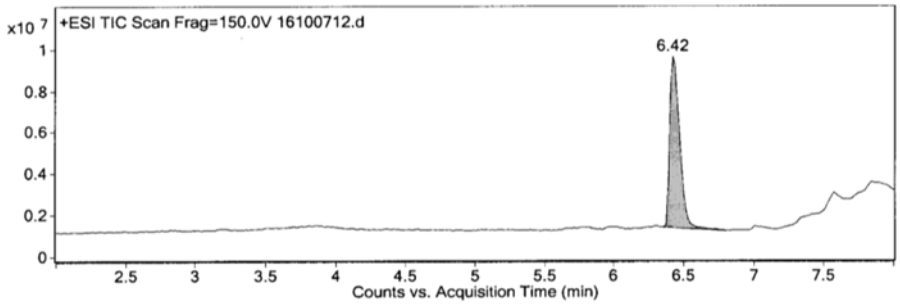
LC-MS results of **7i-OMe**




**Integration Peak List**

| Peak | Start | RT | End | Height | Area | Area % |
|---|---|---|---|---|---|---|
| 1 | 6.37 | 6.43 | 6.57 | 44.19 | 138.82 | 100 |

**Fragmentor Voltage** 150 **Collision Energy** 0 **Ionization Mode** ESI



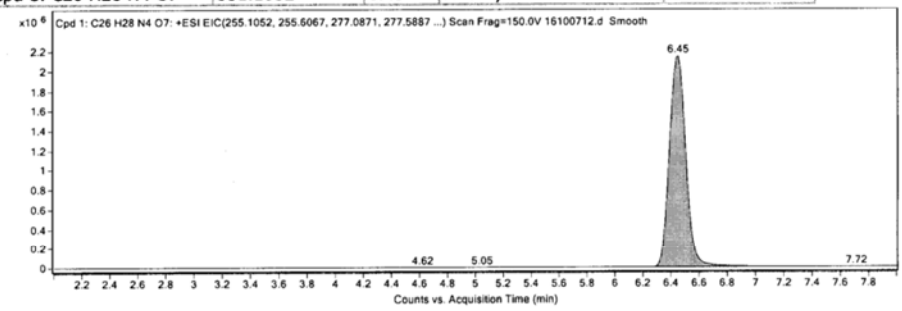

**Integration Peak List**

| Peak | Start | RT | End | Height | Area | Area % |
|---|---|---|---|---|---|---|
| 1 | 6.34 | 6.42 | 6.8 | 8251991 | 41362405 | 100 |

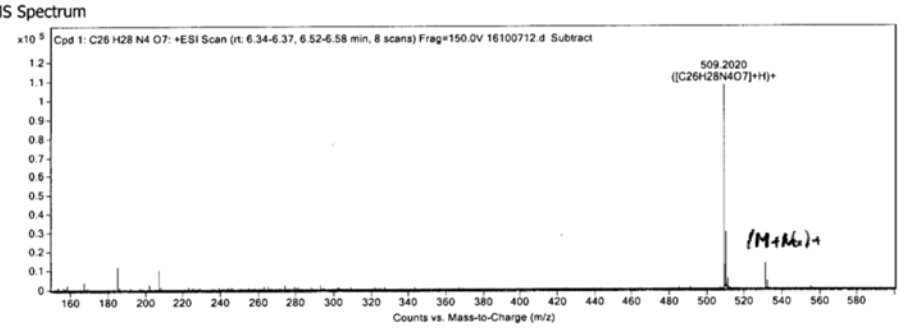
**Compound Table**

| Compound Label | RT | Mass | Abund | Formula | Tgt Mass | Diff (ppm) |
|---|---|---|---|---|---|---|
| Cpd 1: C26 H28 N4 O7 | 6.45 | 508.1952 | 13422 | C26 H28 N4 O7 | 508.1958 | -1.15 |

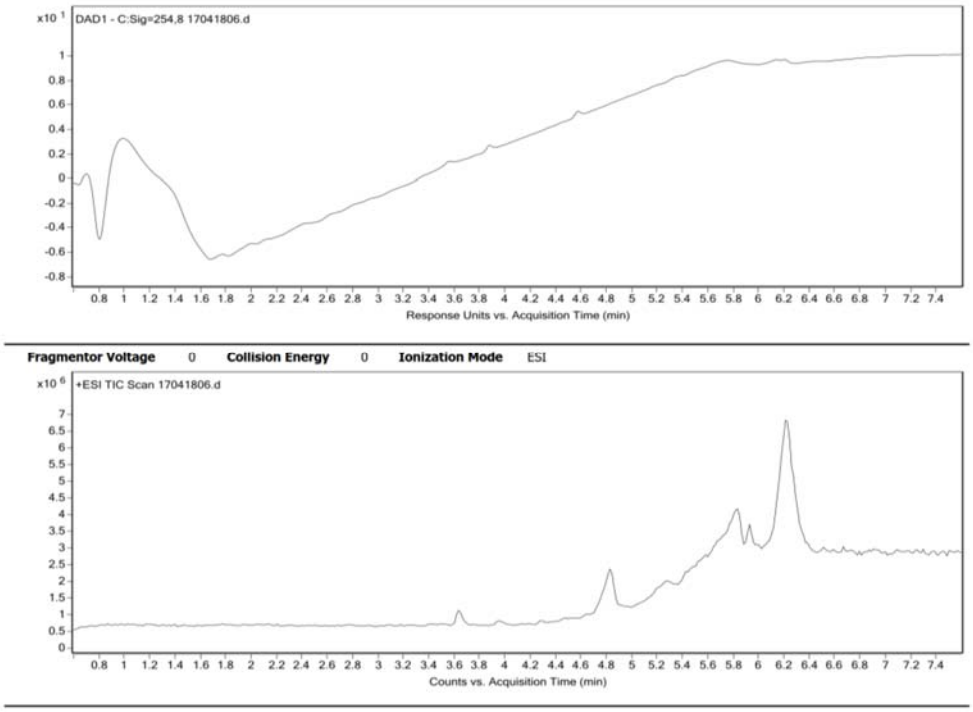
| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 1: C26 H28 N4 O7 | 531.1852 | 6.45 | Find By Formula | 508.1952 |



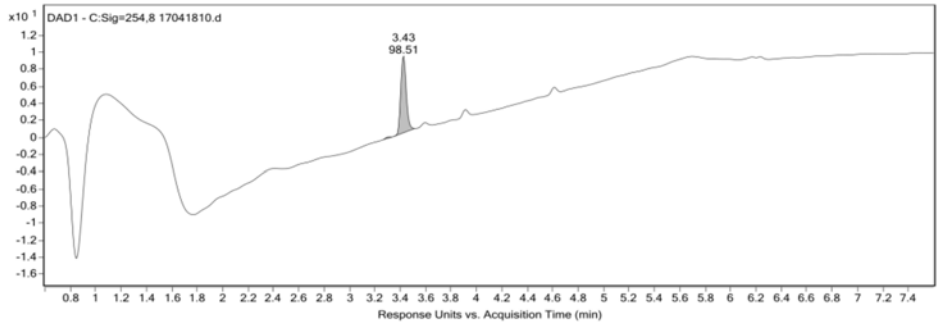

MS Spectrum



Solvent blank for **7i-OEt**, **7i-OPr**, **7i-OBu**



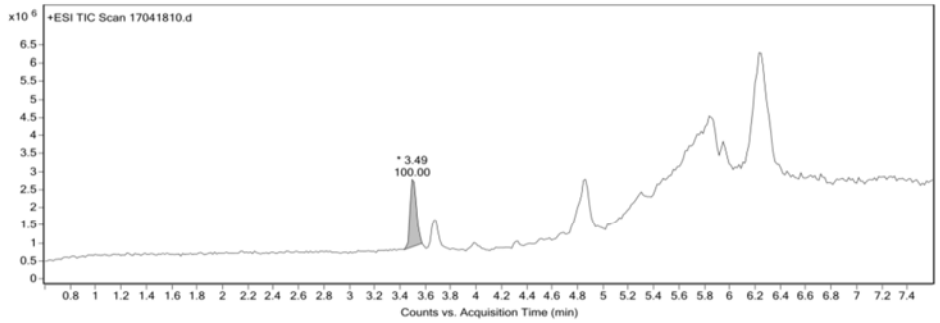
LC-MS results of **7i-OEt**




**User Chromatogram Peak List**

| RT | Height | Height % | Area | Area % | Area Sum % | Width |
|---|---|---|---|---|---|---|
| 3.34 | 0 | 0 | 0.41 | 1.51 | 1.49 | 0.07 |
| 3.43 | 8.98 | 100 | 27.37 | 100 | 98.51 | 0.17 |

**Fragmentor Voltage** 0 **Collision Energy** 0 **Ionization Mode** ESI



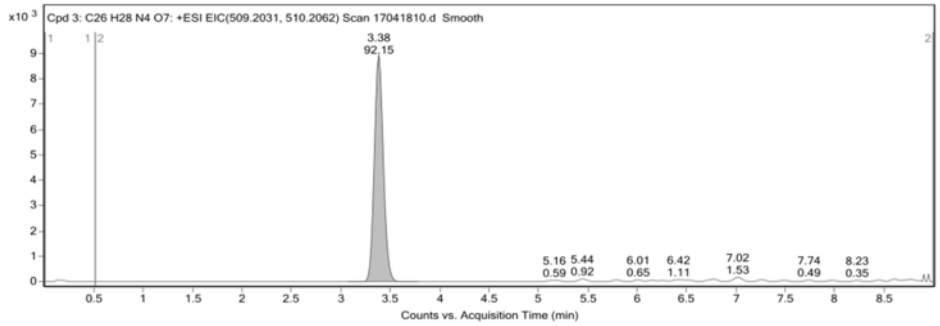

**User Chromatogram Peak List**

| RT | Height | Height % | Area | Area % | Area Sum % | Width |
|---|---|---|---|---|---|---|
| 3.49 | 1891988 | 100 | 6750054 | 100 | 100 | 0.14 |

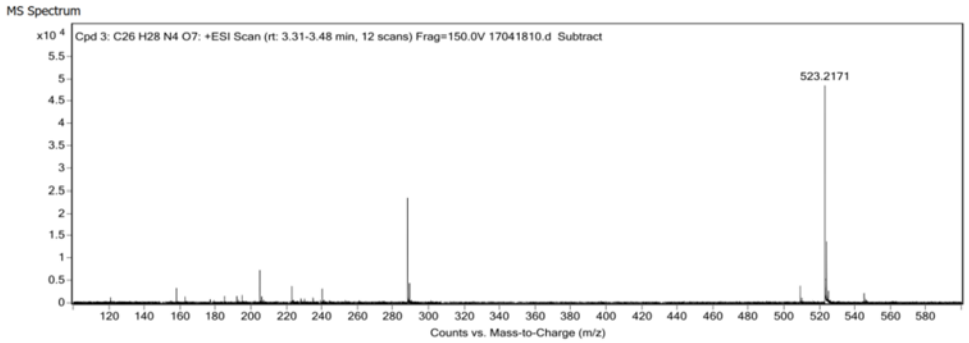
**Compound Table**

| Compound Label | RT | Mass | Abund | Formula | Tgt Mass | Diff (ppm) |
|---|---|---|---|---|---|---|
| Cpd 3: C26 H28 N4 O7 | 3.38 | 508.1959 | 3737 | C26 H28 N4 O7 | 508.1958 | 0.22 |
| Cpd 2: C27 H30 N4 O7 | 3.51 | 522.21 | 309038 | C27 H30 N4 O7 | 522.2114 | -2.79 |

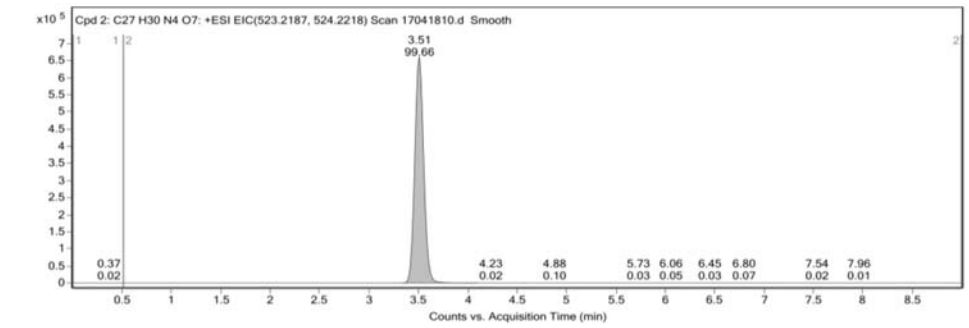
| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 3: C26 H28 N4 O7 | 509.2032 | 3.38 | Find By Formula | 508.1959 |

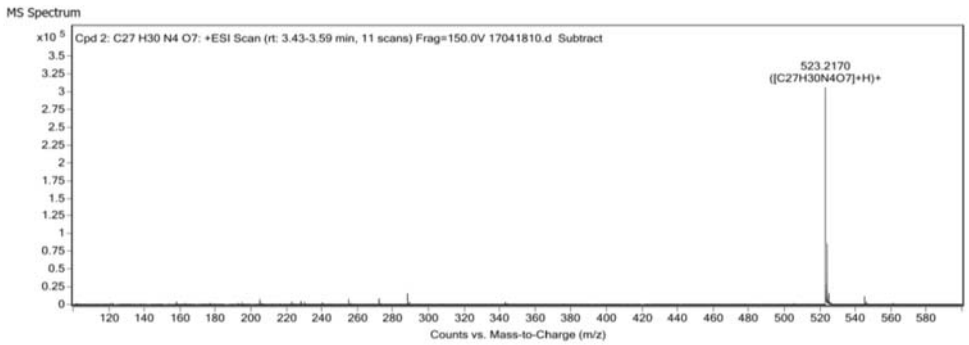


LC-MS results of **7i-OEt** (continued)

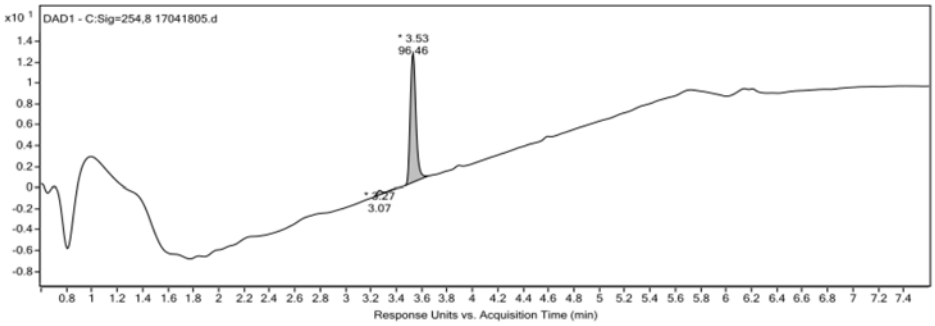



| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 2: C27 H30 N4 O7 | 523.217 | 3.51 | Find By Formula | 522.21 |



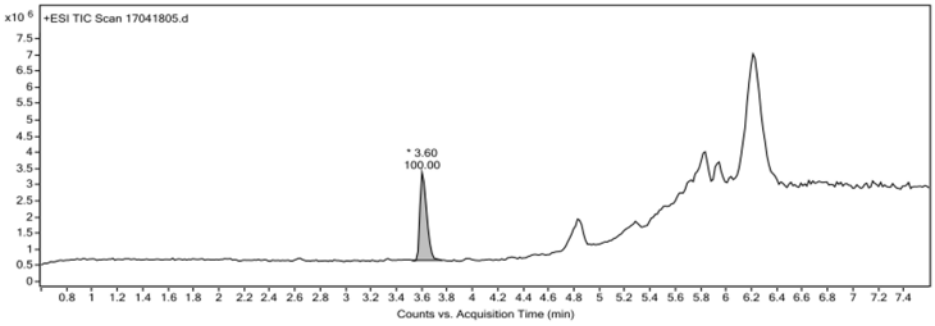
LC-MS results of **7i-OPr**




**User Chromatogram Peak List**

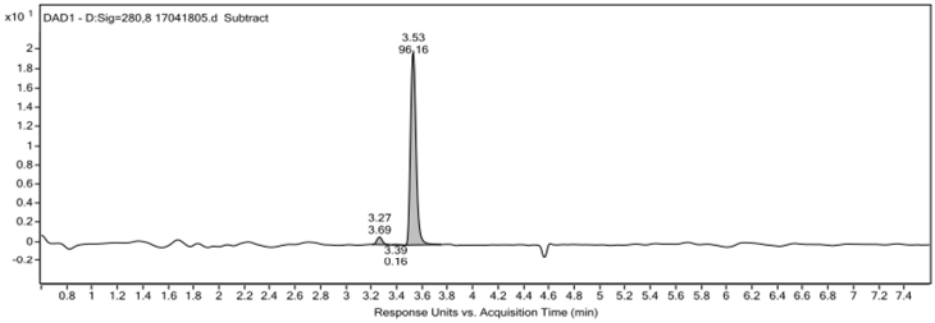
| RT | Height | Height % | Area | Area % | Area Sum % | Width |
|---|---|---|---|---|---|---|
| 3.27 | 0.41 | 3.36 | 1.22 | 3.18 | 3.07 | 0.11 |
| 3.41 | 0 | 0 | 0.19 | 0.49 | 0.47 | 0.08 |
| 3.53 | 12.33 | 100 | 38.4 | 100 | 96.46 | 0.18 |

**Fragmentor Voltage** 0 **Collision Energy** 0 **Ionization Mode** ESI




**User Chromatogram Peak List**

| RT | Height | Height % | Area | Area % | Area Sum % | Width |
|---|---|---|---|---|---|---|
| 3.6 | 2720616 | 100 | 10126901 | 100 | 100 | 0.24 |




**User Chromatogram Peak List**

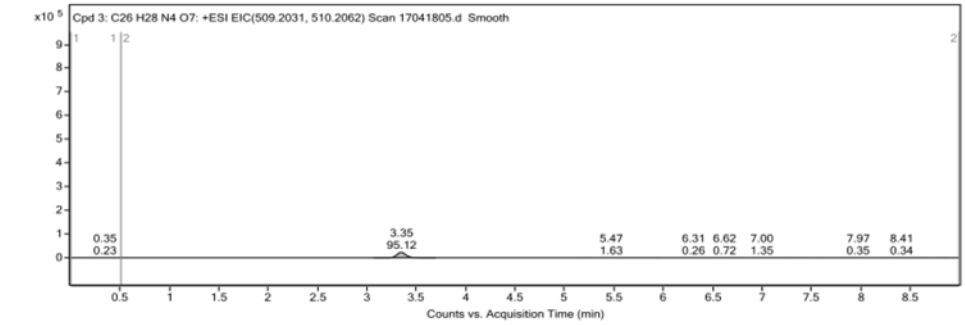
| RT | Height | Height % | Area | Area % | Area Sum % | Width |
|---|---|---|---|---|---|---|
| 3.27 | 0.79 | 3.94 | 2.36 | 3.83 | 3.69 | 0.15 |
| 3.39 | 0.03 | 0.17 | 0.1 | 0.16 | 0.16 | 0.11 |
| 3.53 | 20.02 | 100 | 61.59 | 100 | 96.16 | 0.29 |

**Compound Table**

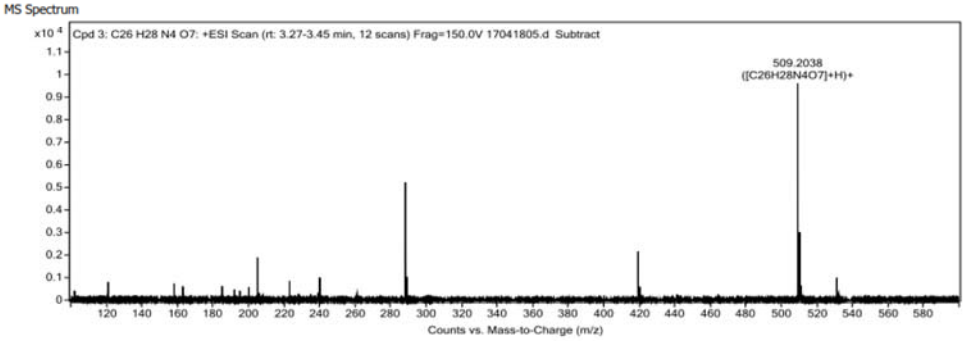
| Compound Label | RT | Mass | Abund | Formula | Tgt Mass | Diff (ppm) |
|---|---|---|---|---|---|---|
| Cpd 3: C26 H28 N4 O7 | 3.35 | 508.1964 | 9659 | C26 H28 N4 O7 | 508.1958 | 1.16 |
| Cpd 2: C27 H30 N4 O7 | 3.48 | 522.2111 | 234 | C27 H30 N4 O7 | 522.2114 | -0.63 |
| Cpd 1: C28 H32 N4 O7 | 3.62 | 536.2256 | 403346 | C28 H32 N4 O7 | 536.2271 | -2.86 |

| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 3: C26 H28 N4 O7 | 509.2038 | 3.35 | Find By Formula | 508.1964 |

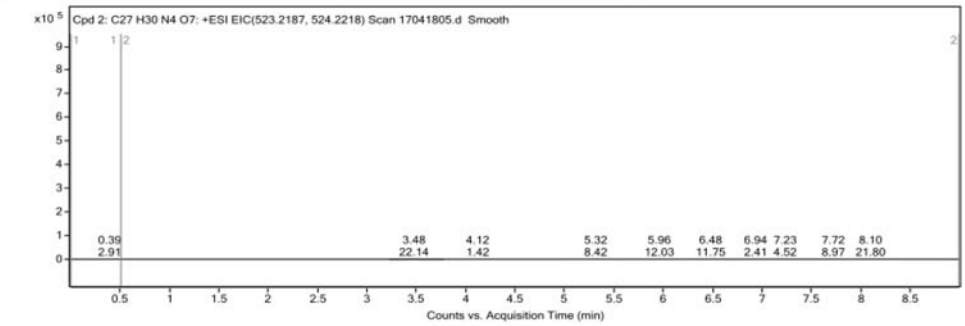
LC-MS results of **7i-OPr** (continued)



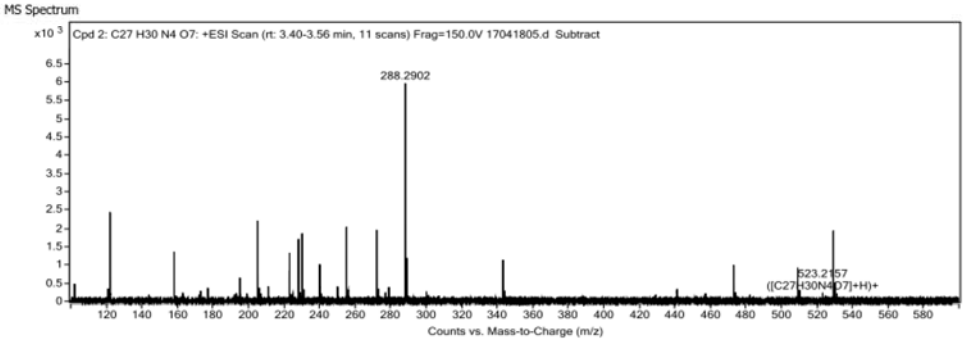

MS Spectrum




| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 2: C27 H30 N4 O7 | 523.2157 | 3.48 | Find By Formula | 522.2111 |



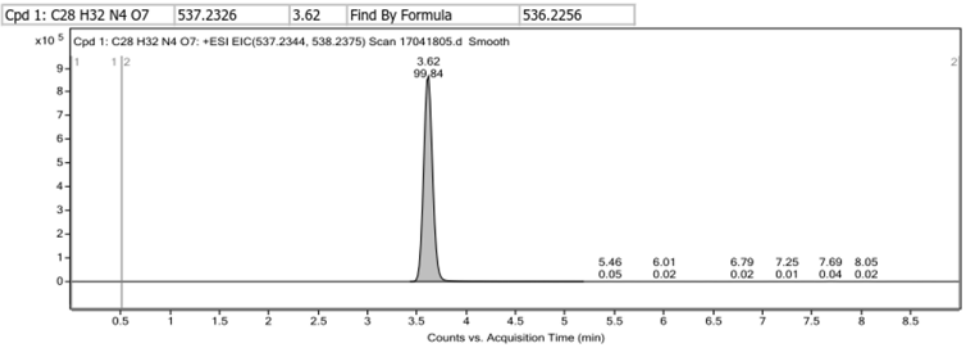

MS Spectrum



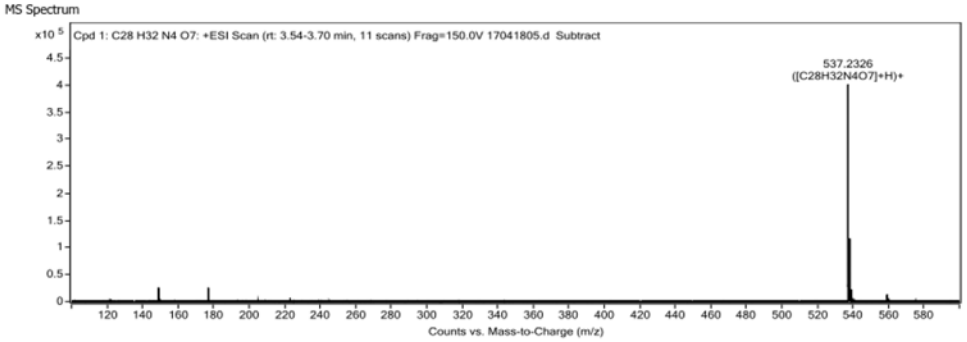

| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|

LC-MS results of **7i-OPr** (continued)

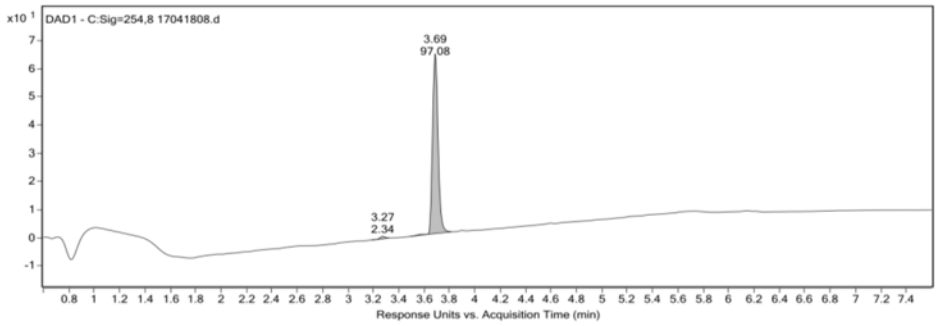
| Cpd 1: C28 H32 N4 O7 | 537.2326 | 3.62 | Find By Formula | 536.2256 |
|---|---|---|---|---|




MS Spectrum



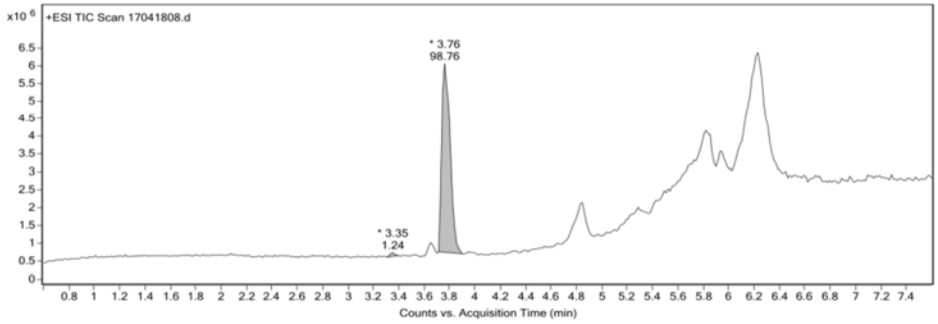
LC-MS results of **7i-OBu**




**User Chromatogram Peak List**

| RT | Height | Height % | Area | Area % | Area Sum % | Width |
|---|---|---|---|---|---|---|
| 3.27 | 0.84 | 1.32 | 4.67 | 2.41 | 2.34 | 0.15 |
| 3.59 | 0.19 | 0.3 | 1.16 | 0.6 | 0.58 | 0.14 |
| 3.69 | 63.87 | 100 | 193.51 | 100 | 97.08 | 0.2 |

**Fragmentor Voltage** 0 **Collision Energy** 0 **Ionization Mode** ESI




**User Chromatogram Peak List**

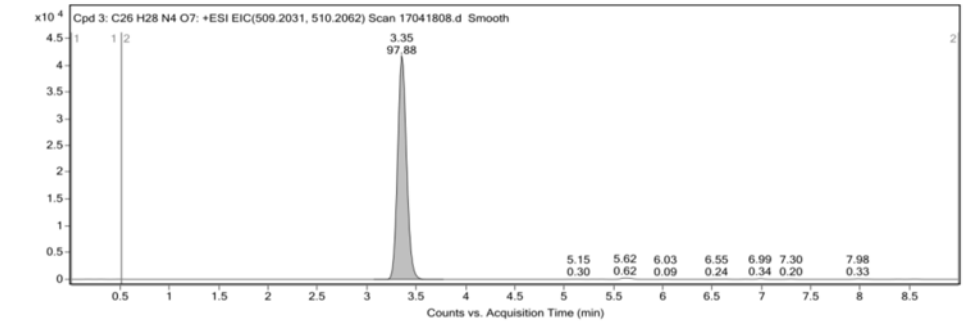
| RT | Height | Height % | Area | Area % | Area Sum % | Width |
|---|---|---|---|---|---|---|
| 3.35 | 107239 | 2.01 | 307419 | 1.25 | 1.24 | 0.09 |
| 3.76 | 5327845 | 100 | 24545715 | 100 | 98.76 | 0.19 |

LC-MS results of **7i-OBu** (continued)

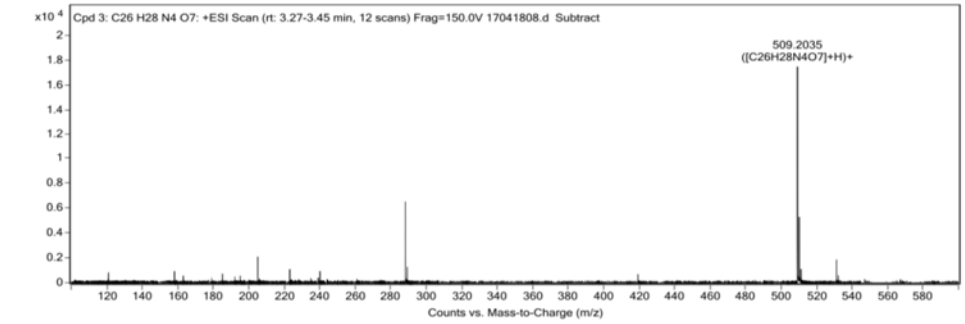
**Compound Table**

| Compound Label | RT | Mass | Abund | Formula | Tgt Mass | Diff (ppm) |
|---|---|---|---|---|---|---|
| Cpd 3: C26 H28 N4 O7 | 3.35 | 508.1962 | 17670 | C26 H28 N4 O7 | 508.1958 | 0.87 |
| Cpd 2: C27 H30 N4 O7 | 3.48 | 522.2118 | 432 | C27 H30 N4 O7 | 522.2114 | 0.73 |
| Cpd 1: C28 H32 N4 O7 | 3.62 | 536.2272 | 1508 | C28 H32 N4 O7 | 536.2271 | 0.1 |
| Cpd 4: C29 H34 N4 O7 | 3.78 | 550.2427 | 1018421 | C29 H34 N4 O7 | 550.2427 | -0.15 |

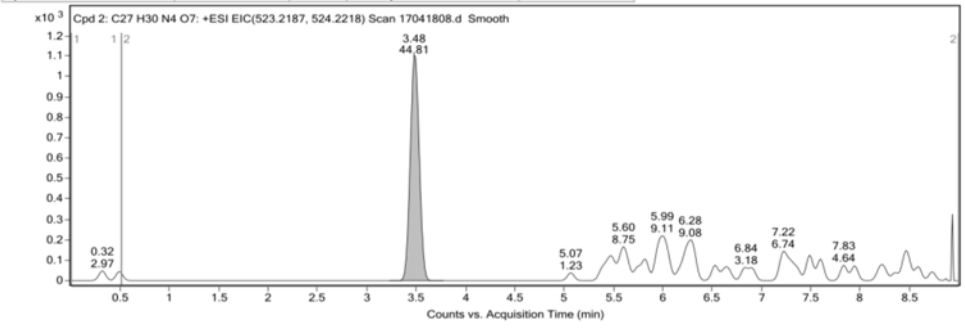
| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 3: C26 H28 N4 O7 | 509.2035 | 3.35 | Find By Formula | 508.1962 |



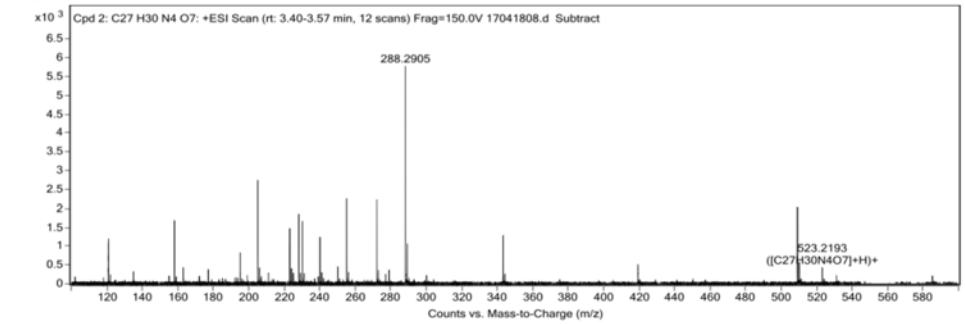

MS Spectrum




| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 2: C27 H30 N4 O7 | 523.2193 | 3.48 | Find By Formula | 522.2118 |

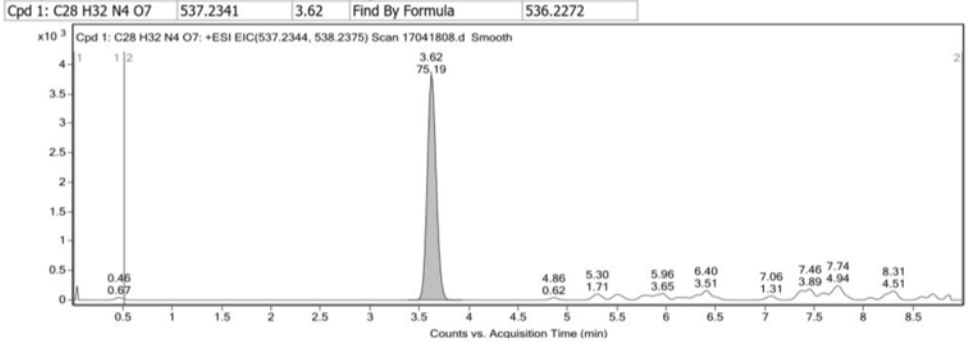



MS Spectrum

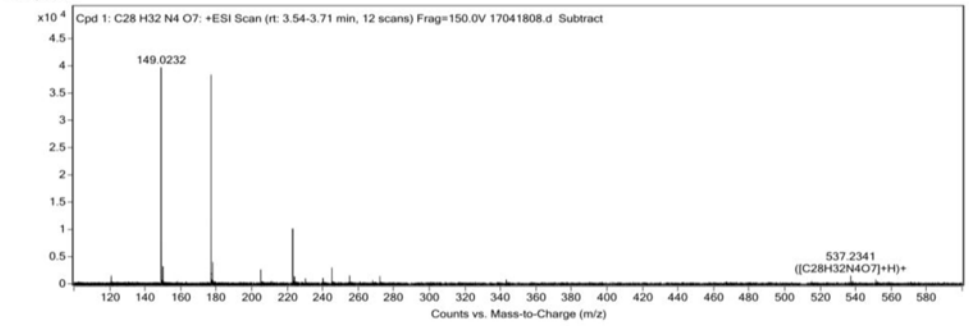


LC-MS results of **7i-OBu** (continued)

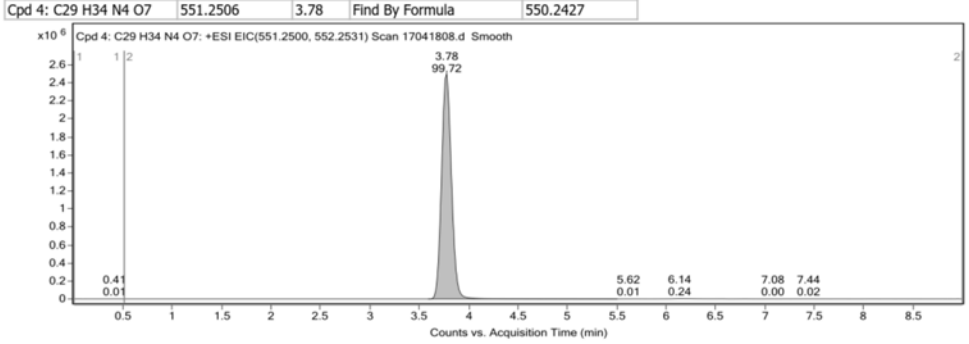
| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 1: C28 H32 N4 O7 | 537.2341 | 3.62 | Find By Formula | 536.2272 |



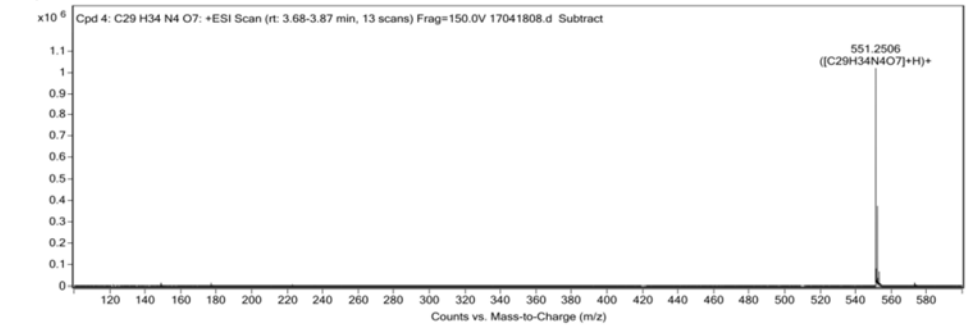

MS Spectrum



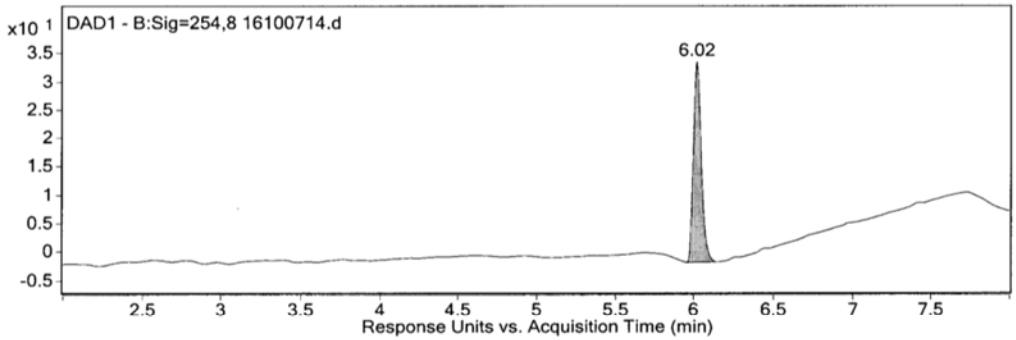

| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 4: C29 H34 N4 O7 | 551.2506 | 3.78 | Find By Formula | 550.2427 |




MS Spectrum



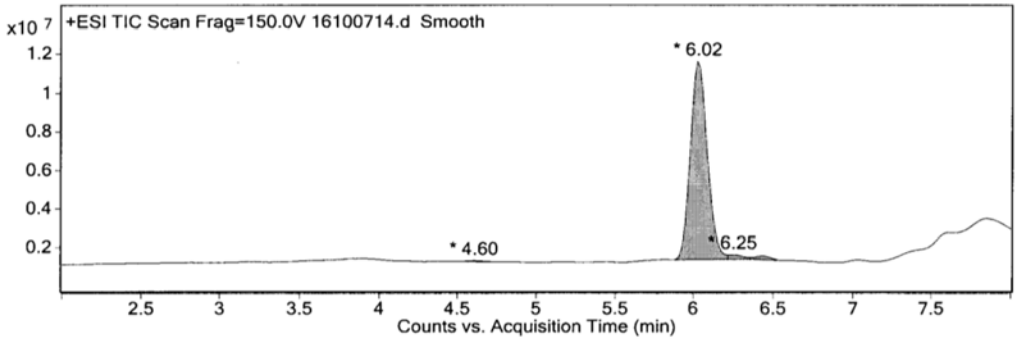
LC-MS results of **8b-OMe**




**Integration Peak List**

| Peak | Start | RT | End | Height | Area | Area % |
|---|---|---|---|---|---|---|
| 1 | 5.95 | 6.02 | 6.15 | 35.48 | 124.28 | 100 |

**Fragmentor Voltage** 150 **Collision Energy** 0 **Ionization Mode** ESI




**Integration Peak List**

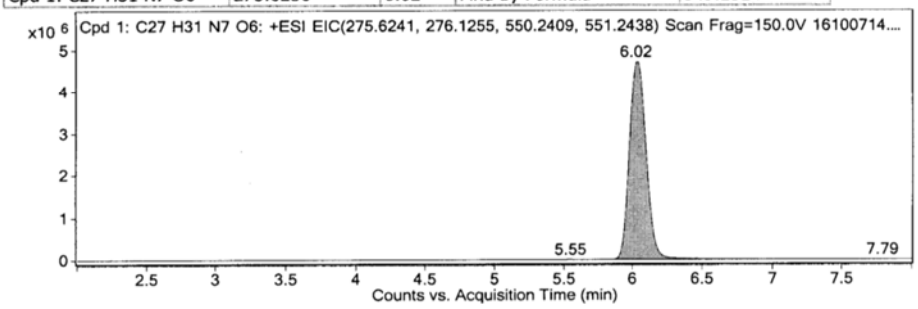
| Peak | Start | RT | End | Height | Area | Area % |
|---|---|---|---|---|---|---|
| 1 | 4.49 | 4.6 | 4.75 | 51600 | 313416 | 0.42 |
| 2 | 5.87 | 6.02 | 6.21 | 10197982 | 75480891 | 100 |
| 3 | 6.21 | 6.25 | 6.36 | 222235 | 1392216 | 1.84 |
| 4 | 6.36 | 6.44 | 6.56 | 192928 | 1236371 | 1.64 |

LC-MS results of **8b-OMe** (continued)

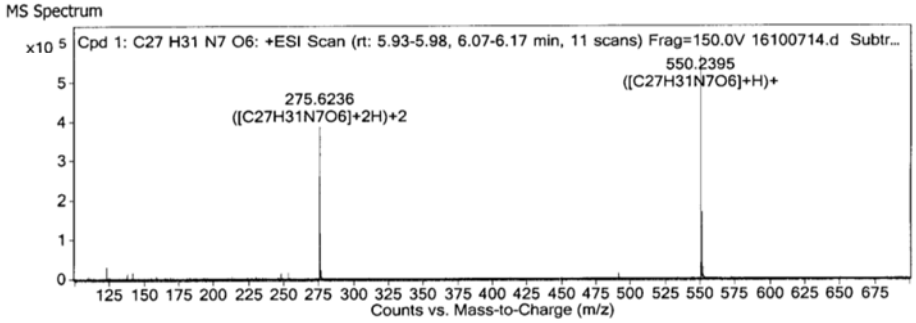
**Compound Table**

| Compound Label | RT | Mass | Abund | Formula | Tgt Mass | Diff (ppm) |
|---|---|---|---|---|---|---|
| Cpd 1: C27 H31 N7 O6 | 6.02 | 549.2324 | 386524 | C27 H31 N7 O6 | 549.2336 | -2.14 |

| Compound Label | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|
| Cpd 1: C27 H31 N7 O6 | 275.6236 | 6.02 | Find By Formula | 549.2324 |




MS Spectrum



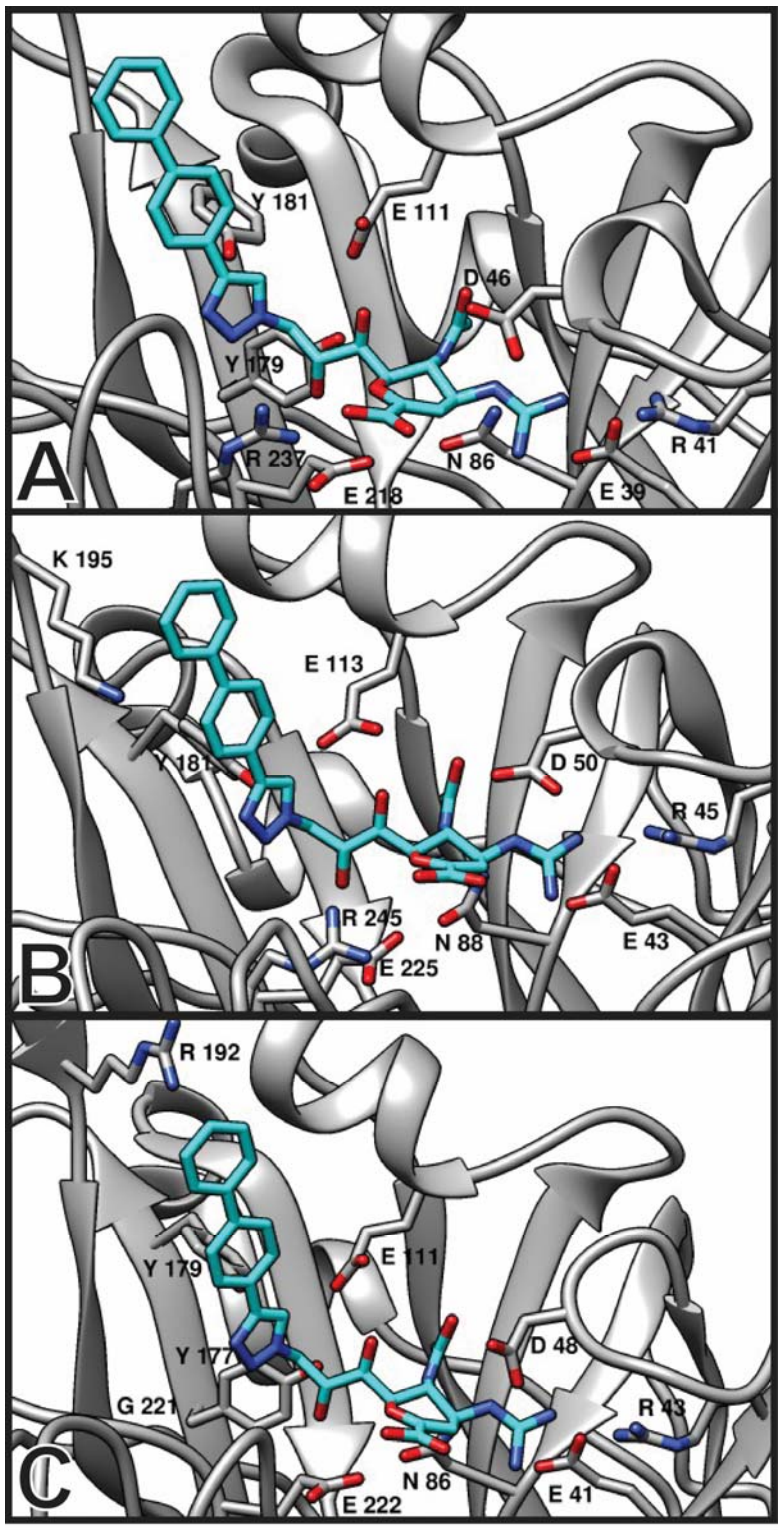



**Figure S.5: Starting structures for MD simulations of 8b with a) NEU2, b) NEU3, and c) NEU4.** The inhibitors are shown with cyan carbons, and key active site residues are labeled.

**Table S.3: Most populated H-bonds to glycerol side chain during MD simulations.**

| Cmpd | Neu2 H-bond | Neu2 Fraction Occupied | Neu3 H-bond | Neu3 Fraction Occupied | Neu4 H-bond | Neu4 Fraction Occupied |
|---|---|---|---|---|---|---|
| **8b** | *O7 ··· $H_2O$* [a] | *56 %* | *O7 ··· $H_2O$* [a] | *59 %* | *O7 ··· $H_2O$* [a] | *58 %* |
| | *N11 ··· $H_2O$* [a,c] | *35 %* | *N11 ··· $H_2O$* [a,c] | *29 %* | *N11 ··· $H_2O$* [a,c] | *19 %* |
| | **O7H ··· O(N5Ac)** [b] | **40 %** | O7H ··· E113 | 53 % | O7H ··· E111 | 70 % |
| | O8H ··· E218 | 53 % | O8H ··· E113 | 43 % | O8H ··· E222 | 82 % |
| | O7 | none | O7 | none | O7 ··· Y179 | < 1 % |
| | O8 ··· Y179 | 20 % | O8 ··· Y181 | 8 % | O8 ··· Y177 | < 1 % |
| | N11 ··· R237 [c] | 6 % | N10 ··· R245 [c] | 5 % | N10 ··· G221 [c,d] | 5 % |

[a] H-bonds to solvent are indicated in italics.
[b] Those in bold indicate intramolecular H-bonds.
[c] Note that O9H and O9 have been replaced with a triazole. The atom listed indicates the most populated H-bond to a triazole nitrogen. See **Error! Reference source not found.** for atom numbers.
[d] H-bond is to the backbone NH.

**Table S.4: Key H-bonds to C4 substituent during MD simulations.**

| Cmpd | Neu2 H-bond | Neu2 Fraction Occupied | Neu3 H-bond | Neu3 Fraction Occupied | Neu4 H-bond | Neu4 Fraction Occupied |
|---|---|---|---|---|---|---|
| **8b** | *Guanidine ··· $H_2O$* [a] | *46 %* | *Guanidine ··· $H_2O$* [a] | *41 %* | *Guanidine ··· $H_2O$* [a] | *32 %* |
| | Guanidine ··· E39 | 47 % | Guanidine ··· E43 | 92 % | Guanidine ··· E41 | 52 % |
| | Guanidine ··· D46 | 3 % | Guanidine ··· D50 | 27 % | Guanidine ··· D48 | 2 % |
| | Guanidine ··· N86 | 33 % | Guanidine ··· N88 | 50 % | Guanidine ··· N86 | 13 % |

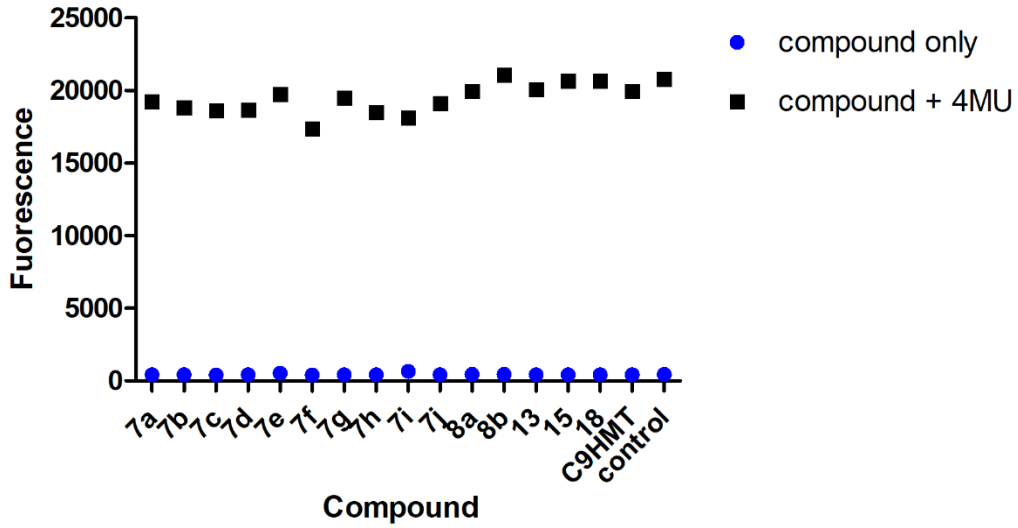
[a] H-bonds to solvent are indicated in italics.

**Table S.5: Most populated water bridges between the inhibitor 8b and enzyme during MD simulations.**

| Cmpd | Neu2 residues | Neu2 Fraction Occupied | Neu3 residues | Neu3 Fraction Occupied | Neu4 residues | Neu4 Fraction Occupied |
|---|---|---|---|---|---|---|
| **8b** | E111 *(glycerol)* | 65 % | D50 *(C4)* | 88 % | D48 *(C4)* | 64 % |
| | R237 *(glycerol)* | 34 % | R245 *(glycerol)* | 47 % | E111 *(glycerol)* | 55 % |
| | R41 *(C4)* | 23 % | E113 *(glycerol)* | 42 % | D48 & R83 *(C4/N5Ac)* | 32 % |
| | Q270 *(triazole)* | 10 % | H277 *(triazole)* | 17 % | R242 *(glycerol)* | 27 % |

[a] The closest region of the inhibitor is indicated in parenthesis.

**Test of the fluorescent properties of compounds screened.** A solution of each compound (2.5 µL; 4 mM), or of the sample combined with 4MU-NANA (2.5 µL; 125 µM), was diluted with water (15 µL) and glycine buffer (100 µL 0.2 M, pH 10.2) and transferred on 386-well plate. Fluorescence ($\lambda_{ex}$ = 365 nm; $\lambda_{em}$ = 445 nm) was detected by a plate reader (Molecular Devices, Sunnyvale CA). Compounds alone were not fluorescent, and no significant effects on the fluorescence of 4MU-NANA were observed.




**Test of the NEU activity of HEK293E cells.** The same amounts of Hek293E cells and a Lentivirus expressing Cathepsin A and Human neuraminidase 1(Cath-Neu1) with an N-terminal $His_6$-tag transduced Hek293E cells were seeded in 10 $cm^2$ plates individually. After the cells reached confluence, they were washed 3 times with 1 mL of PBS and harvested in 300 µL of PBS for each plate at the same density. The total harvested mixture was sonicated (10 seconds, 5 times at 60 Watt) on ice. Then lysis buffer (1 mM EDTA; 0.2 M NaCl; 20 mM MOPS; 0.1% Triton X-100. pH 7.2) was added, and left agitating for 1 hour on ice at 4°C. The neuraminidase activity of the crude cell lysate was determined with 50 µM 4MU-NANA.