Supplementary Information (SI) for RSC Medicinal Chemistry.
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Supplementary information

Development of Potent and Selective Tetrahydro-β-Carboline-Based HDAC6 Inhibitors with Promising Activity Against Triple-Negative Breast Cancer

AUTHOR NAMES

Aya Fathy [a #], Amro Allam [a#], Ahmed K. ElHady [a,b#], Dalia S El-Gamil [c], Kai-Chun Lin [d], Yen-Hua Chang [d], Yu-Hsuan Lee [e], Sebastian Hilscher[f], Mike Schutkowski[f], Hany S. Ibrahim [f], Shun-Hua Chen[g], Chun-Hong Chen[h], Ashraf H. Abadi [a], Wolfgang Sippl [f], Po-Jen Chen[h], Yi-Sheng Cheng [d, e, i], and Mohammad Abdel-Halim[a*]*

AUTHOR ADDRESS

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo 11835, Egypt.

[b] School of Life & Medical Sciences, University of Hertfordshire hosted by Global Academic Foundation, New Administrative Capital, Cairo, Egypt

[c] Department of Chemistry, Faculty of Pharmacy, Ahram Canadian University, Cairo, 12451, Egypt

[d] Institute of Plant Biology, College of Life Science, National Taiwan University, Taipei 10617, Taiwan

[e] Department of Life Science, College of Life Science, National Taiwan University, Taipei 10617, Taiwan

[f] Institute of Pharmacy, Martin-Luther-University of Halle-Wittenberg, Halle (Saale), Germany

[g] School of Nursing, Fooyin University, Kaohsiung 831301, Taiwan

[h] Department of Medical Research, E-Da Hospital and Graduate Institute of Medicine, I-Shou University, Kaohsiung, 824410, Taiwan

[i] Genome and Systems Biology Degree Program, College of Life Science, National Taiwan University, Taipei 10617, Taiwan

[#]Both authors equally contributed to this work

*Correspondence: Mohammad Abdel-Halim, Faculty of Pharmacy and Biotechnology, Department of Pharmaceutical Chemistry, German University in Cairo, Cairo 11835, Egypt. Email: mohammad.abdel-

halim@guc.edu.eg and Po-Jen Chen, Department of Medical Research, E-Da Hospital and Graduate Institute of Medicine, I-Shou University, Kaohsiung, 824410, Taiwan. Email: ed113510@edah.org.tw

Content:

1) NCI anti-cancer screening experimental details
2) ^{1}H NMR and ^{13}C NMR Spectra of representative compounds

Experimental details for NCI anti-cancer screening

The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37° C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/mL gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µL of these different drug dilutions are added to the appropriate microtiter wells already containing 100 µL of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37°C, 5 % CO2, 95 % air, and 100 % relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 µL of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µL of 80 % TCA (final concentration, 16 % TCA). The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature.

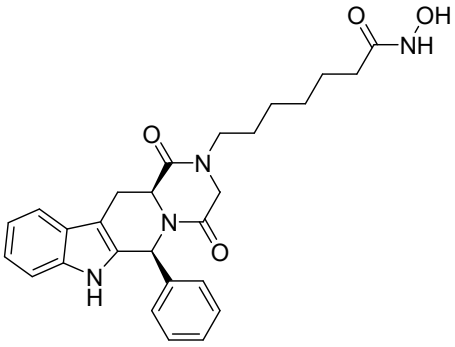
After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μL of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz

Half-maximal growth inhibitory concentration (GI50) is calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation.

^{1}H NMR and ^{13}C NMR Spectra of representative compounds



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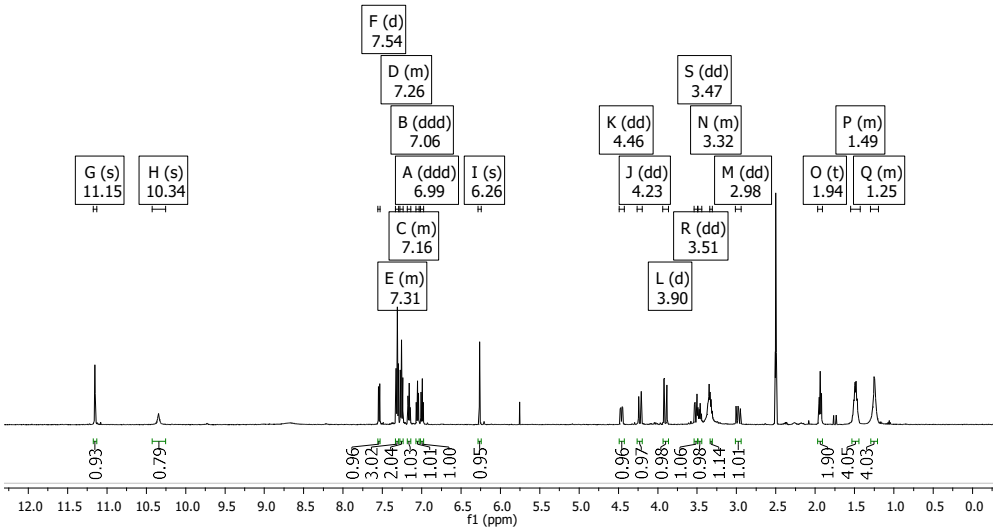

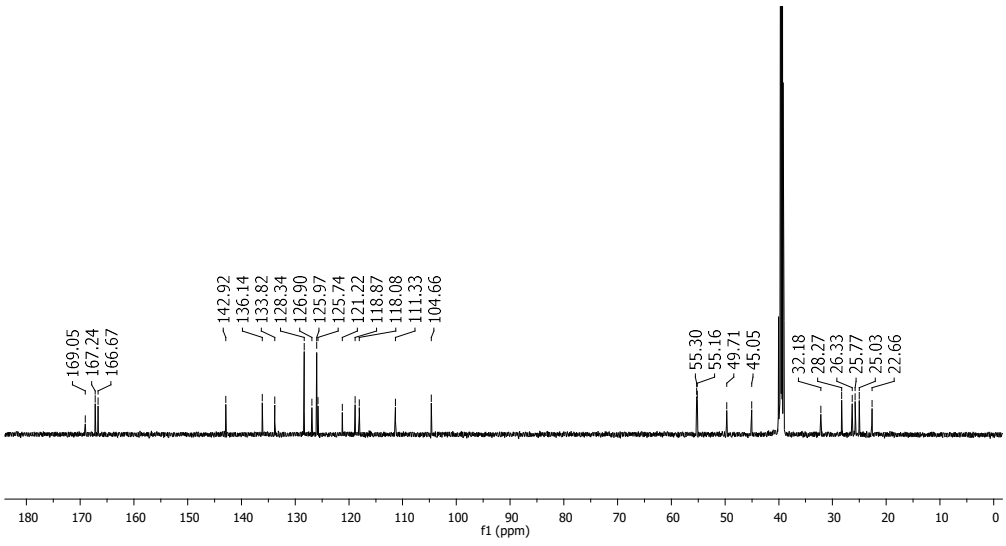

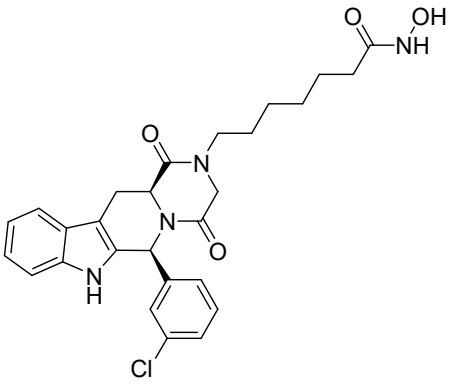
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N
H
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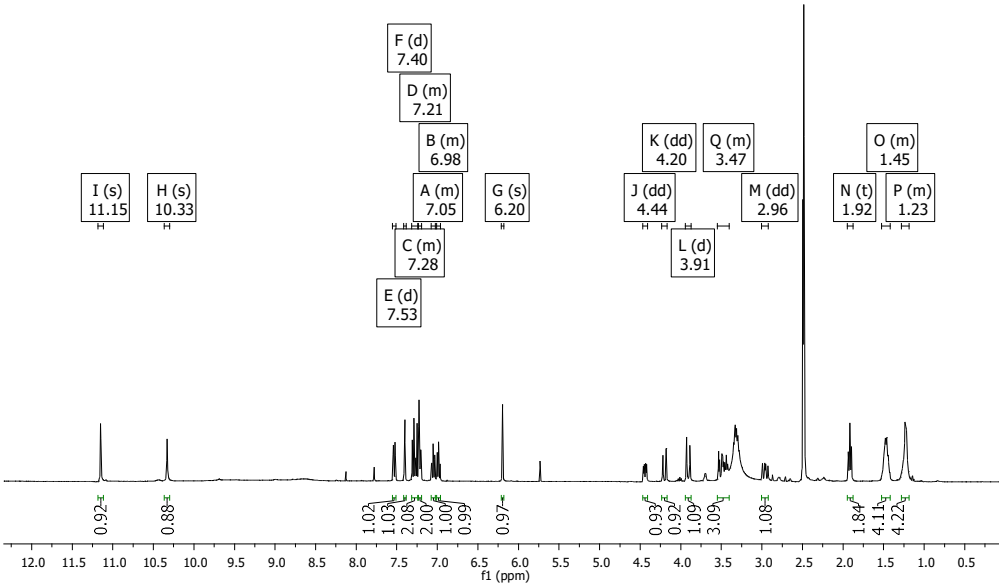
7c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I (s) 11.15
H (s) 10.33
K (dd) 4.20
Q (m) 3.47
J (dd) 4.44
M (dd) 2.96
L (d) 3.91
O (m) 1.45
N (t) 1.92
P (m) 1.23
0.92
0.88
1.02
1.03
2.08
2.00
1.00
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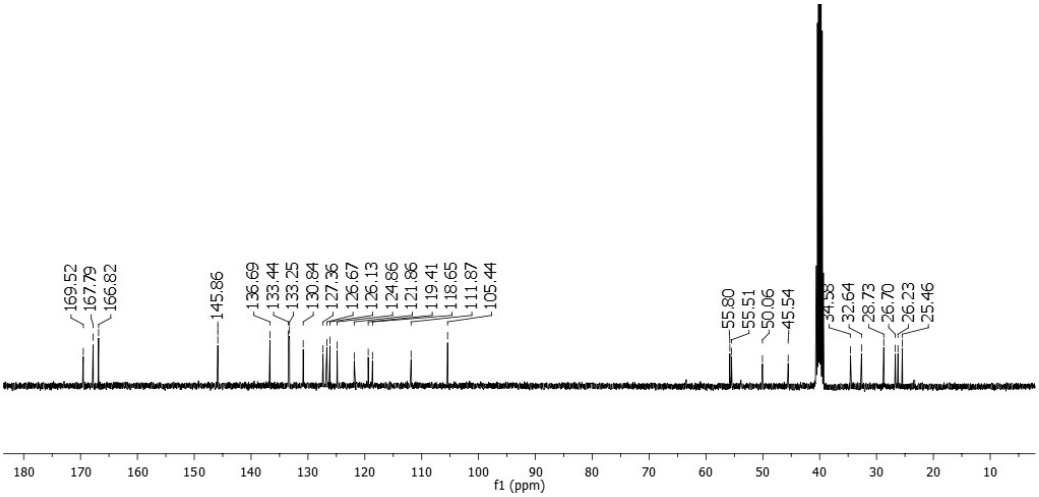
f1 (ppm)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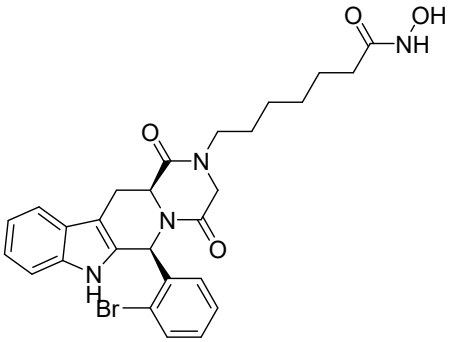
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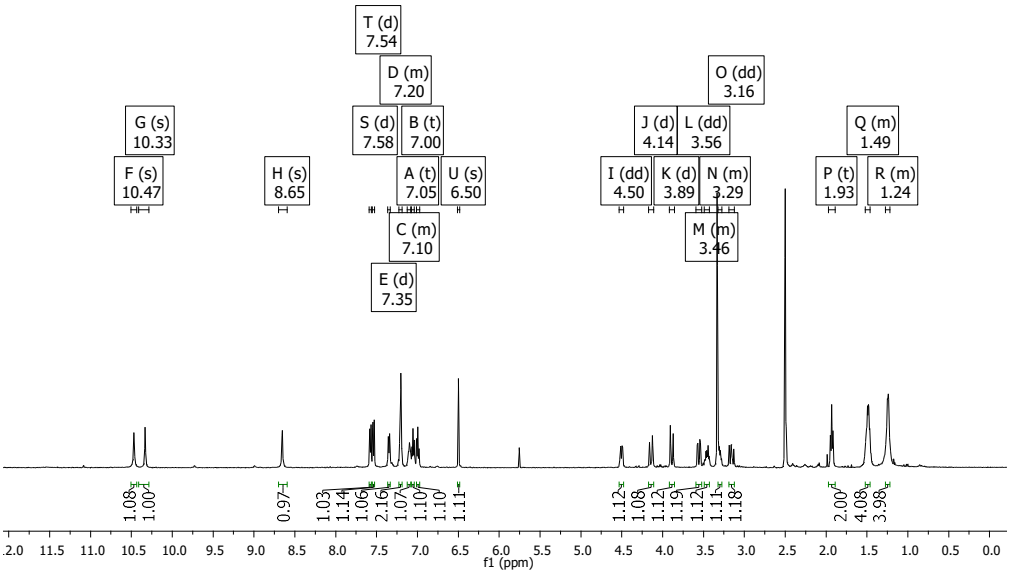
N
O
N
H
Br

8c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L (dd) 3.56
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F (s) 10.47
H (s) 8.65
A (t) 7.05
U (s) 6.50
I (dd) 4.50
K (d) 3.89
N (m) 3.29
P (t) 1.93
R (m) 1.24
C (m) 7.10
M (m) 3.46
E (d) 7.35
1.08
1.00
0.97
1.03
1.14
1.06
2.16
1.07
1.10
1.10
1.11
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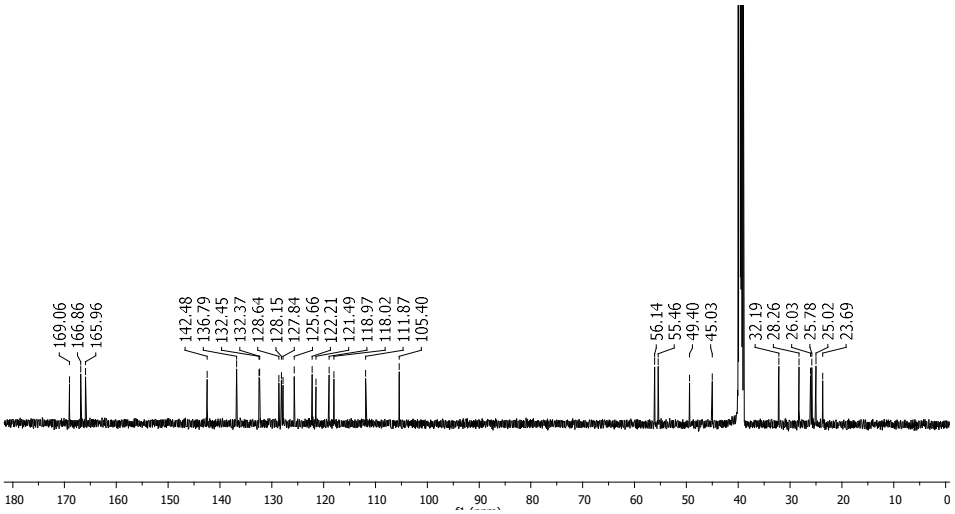
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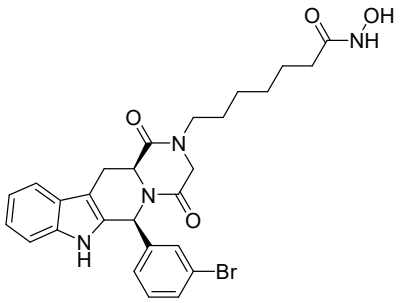
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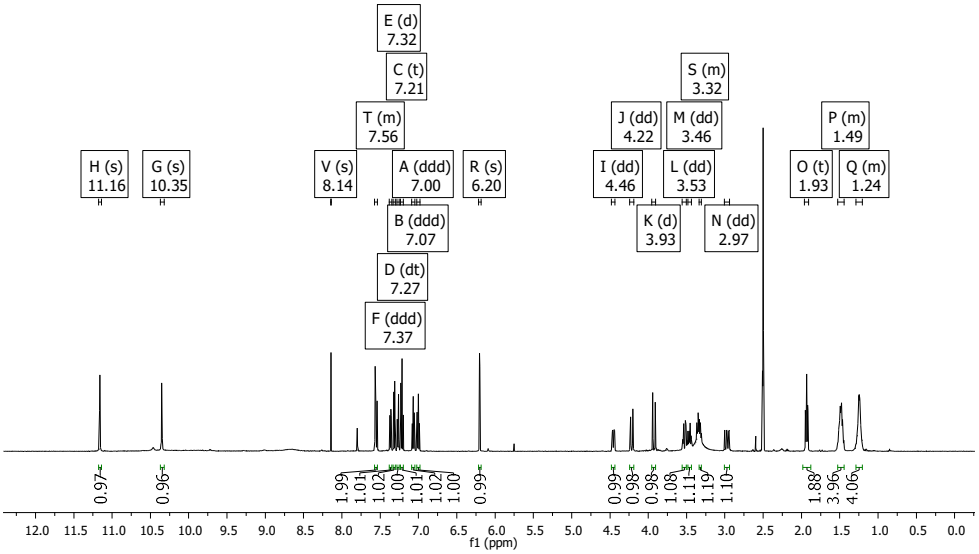
O
N
N
O
N
H
Br

9c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H (s) 11.16
G (s) 10.35
V (s) 8.14
A (ddd) 7.00
R (s) 6.20
I (dd) 4.46
L (dd) 3.53
O (t) 1.93
Q (m) 1.24
B (ddd) 7.07
K (d) 3.93
N (dd) 2.97
D (dt) 7.27
F (ddd) 7.37
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0.96
1.99
1.01
1.02
1.00
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1.00
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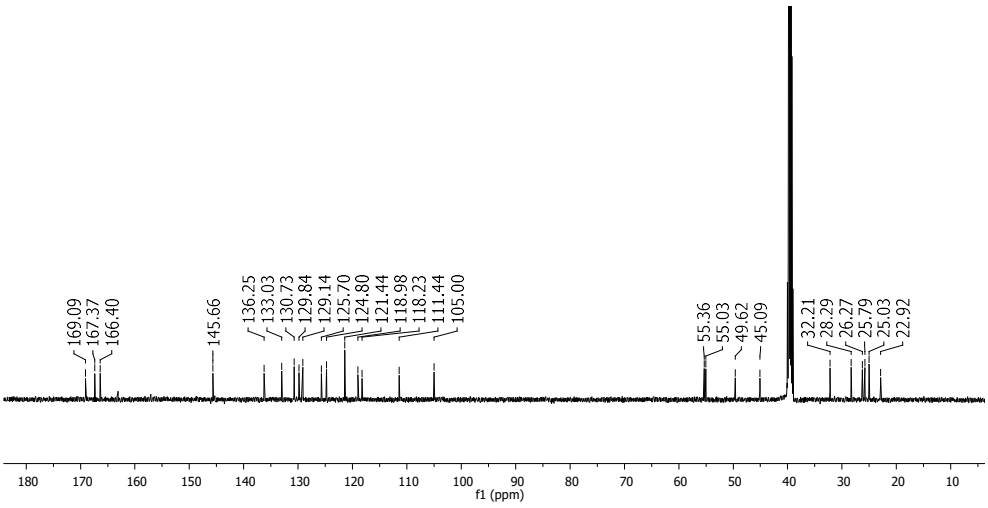
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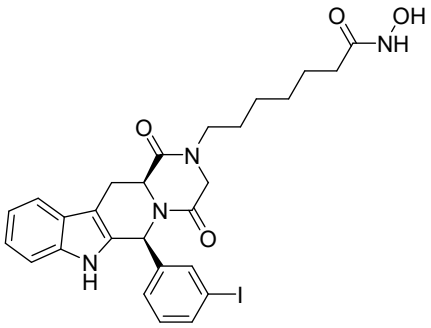
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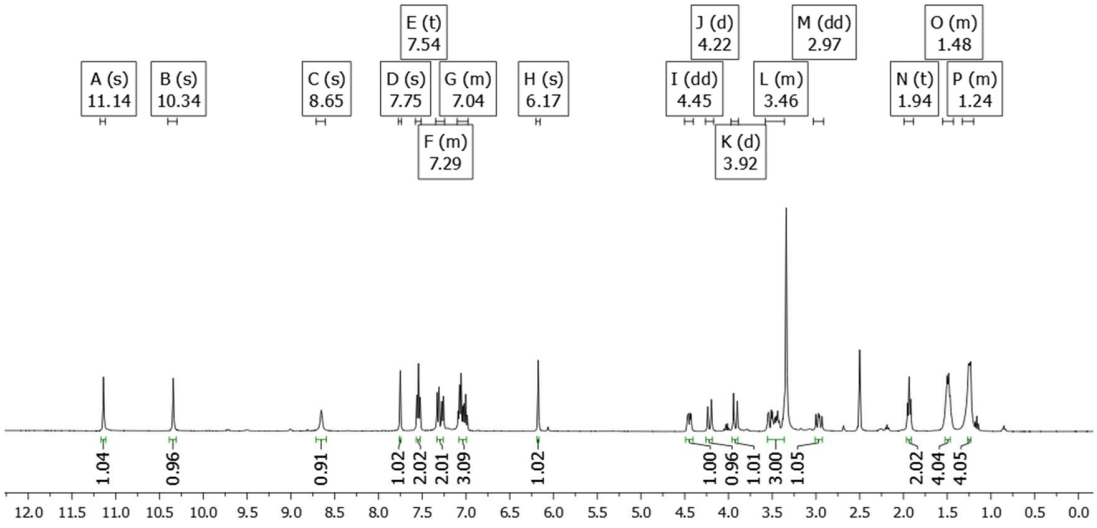
O
N
N
O
N
H
I

10c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G (m) 7.04
F (m) 7.29
H (s) 6.17
J (d) 4.22
I (dd) 4.45
K (d) 3.92
M (dd) 2.97
L (m) 3.46
O (m) 1.48
N (t) 1.94
P (m) 1.24
1.04
0.96
0.91
1.02
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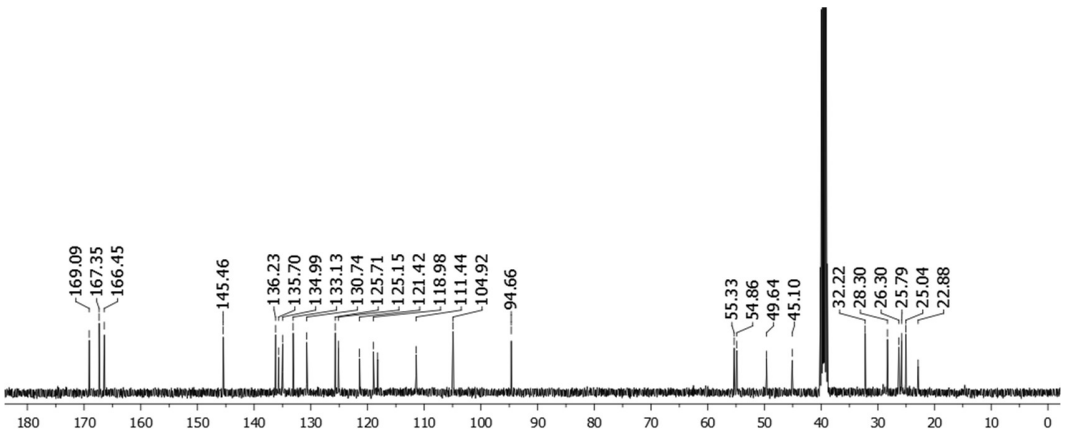
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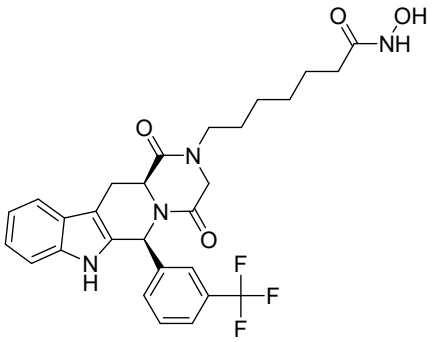

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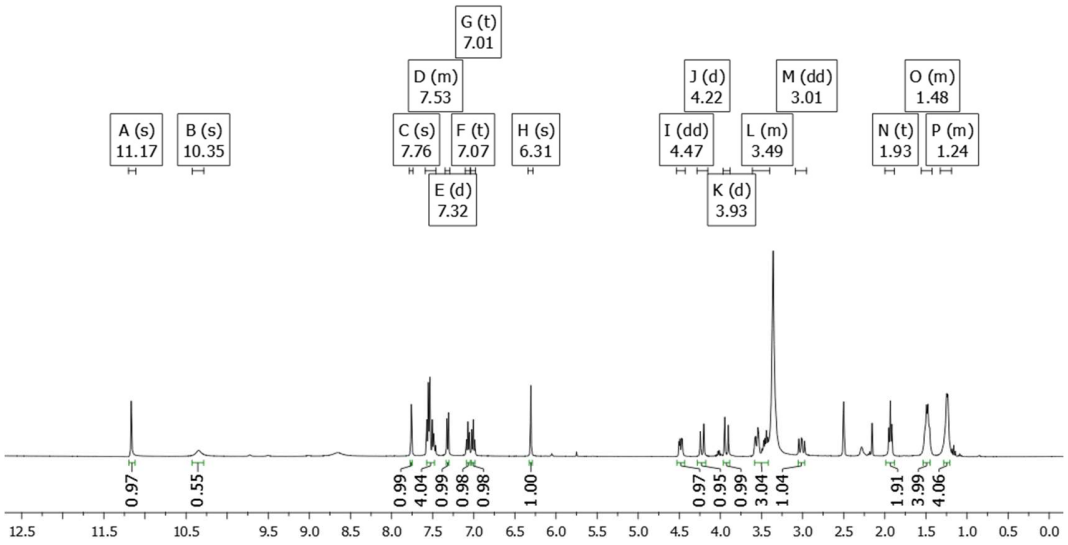
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C (s) 7.76
F (t) 7.07
H (s) 6.31
I (dd) 4.47
L (m) 3.49
N (t) 1.93
P (m) 1.24
E (d) 7.32
K (d) 3.93
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1.04
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3.99
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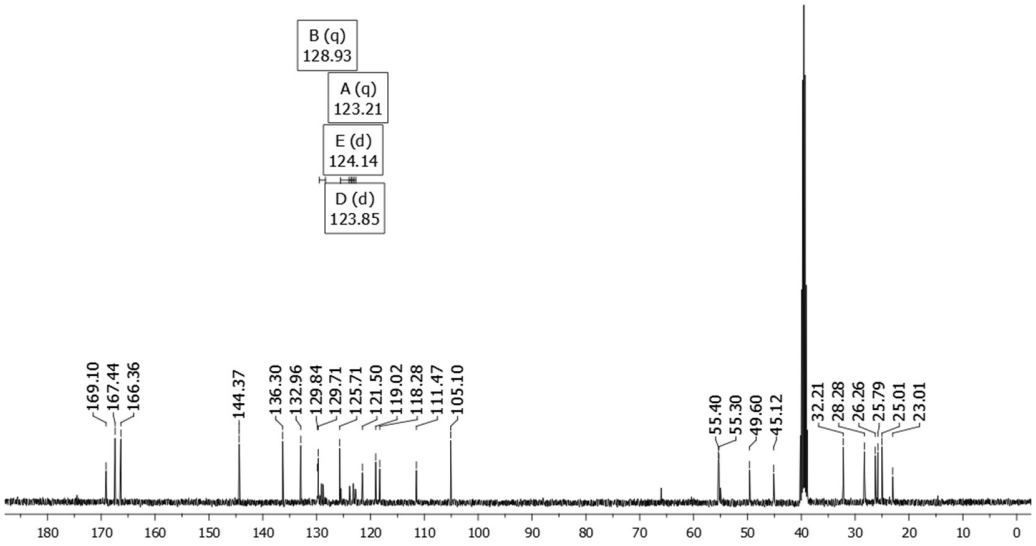
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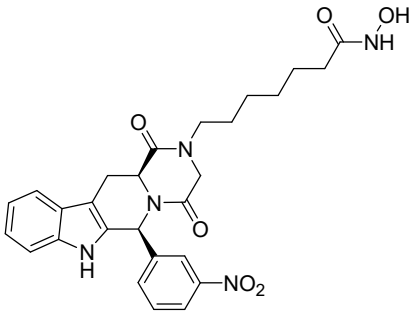
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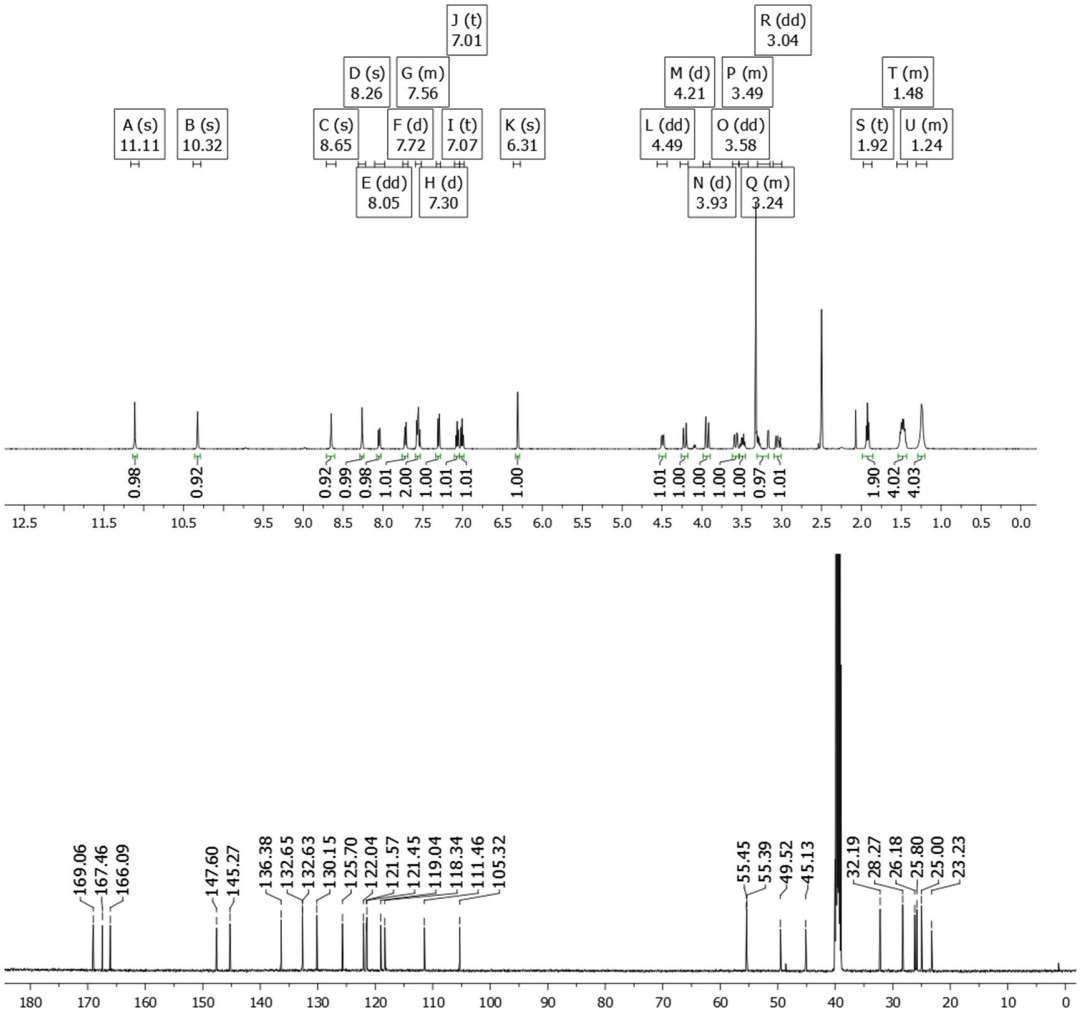
N
N
O
N
H
NO2

14c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H (d) 7.30
I (t) 7.07
J (t) 7.01
K (s) 6.31
L (dd) 4.49
M (d) 4.21
N (d) 3.93
O (dd) 3.58
P (m) 3.49
Q (m) 3.24
R (dd) 3.04
S (t) 1.92
T (m) 1.48
U (m) 1.24
169.06
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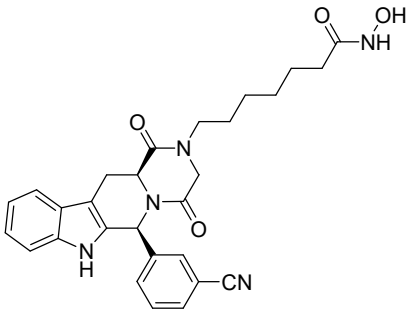
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N
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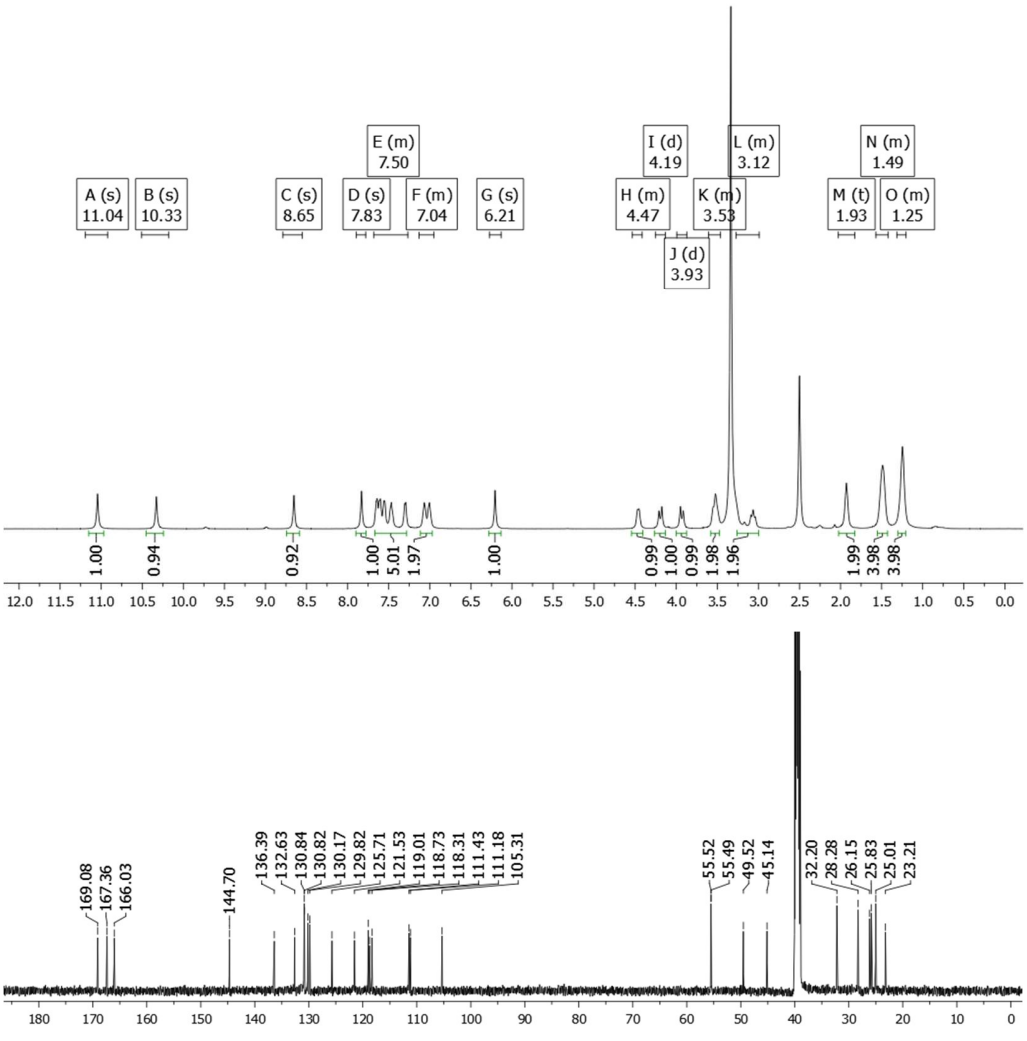
O
N
H
CN

16c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J (d) 3.93
K (m) 3.53
L (m) 3.12
M (t) 1.93
N (m) 1.49
O (m) 1.25
1.00 0.94 0.92 1.00 5.01 1.97 1.00 0.99 1.00 0.99 1.98 1.96 1.99 3.98 3.98
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
169.08
167.36
166.03
144.70
136.39
132.63
130.84
130.82
130.17
129.82
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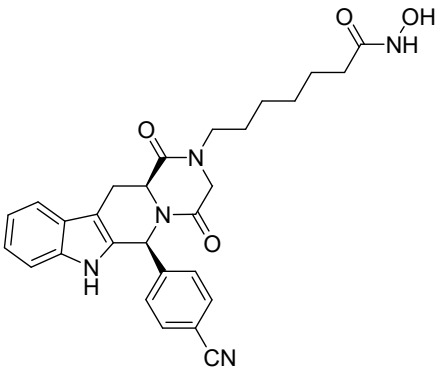
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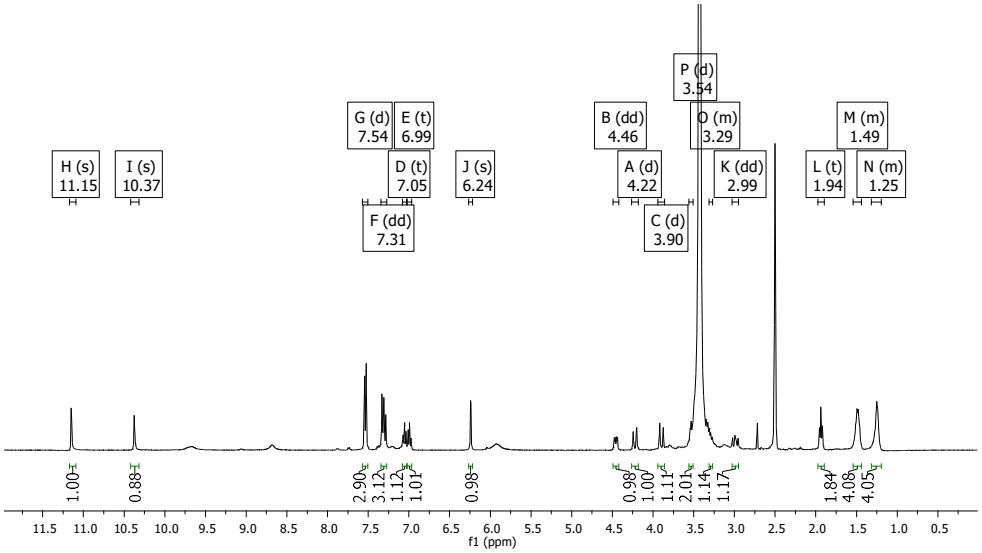
17c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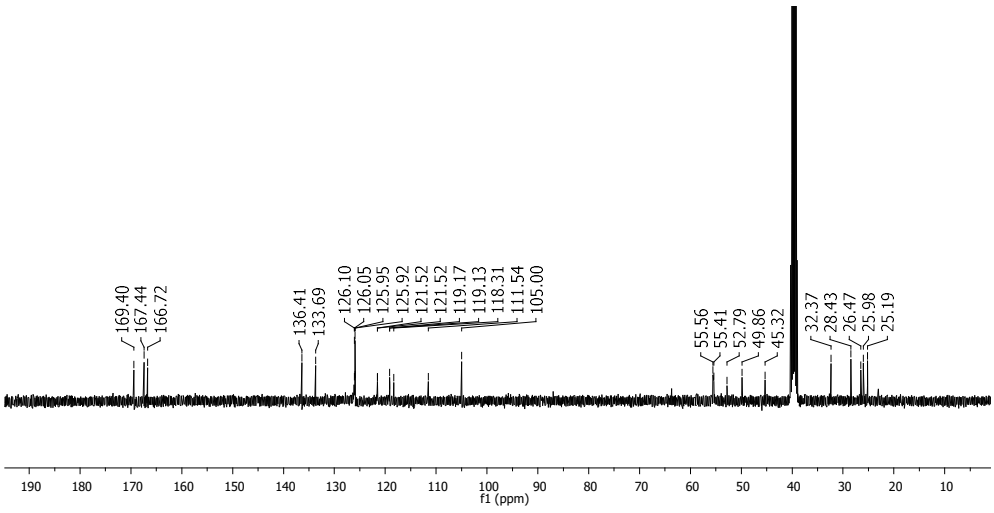
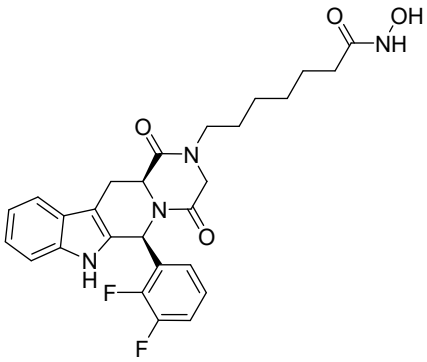
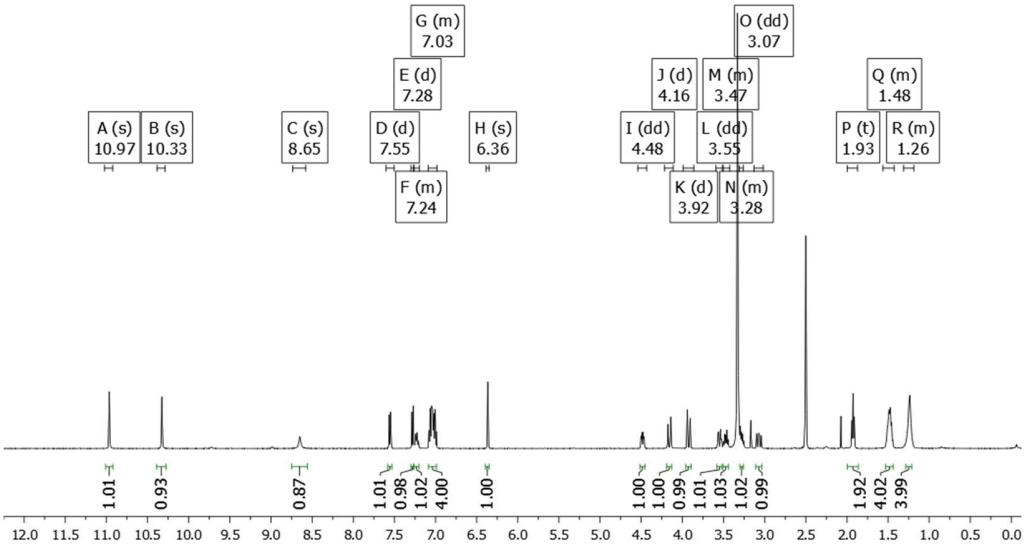
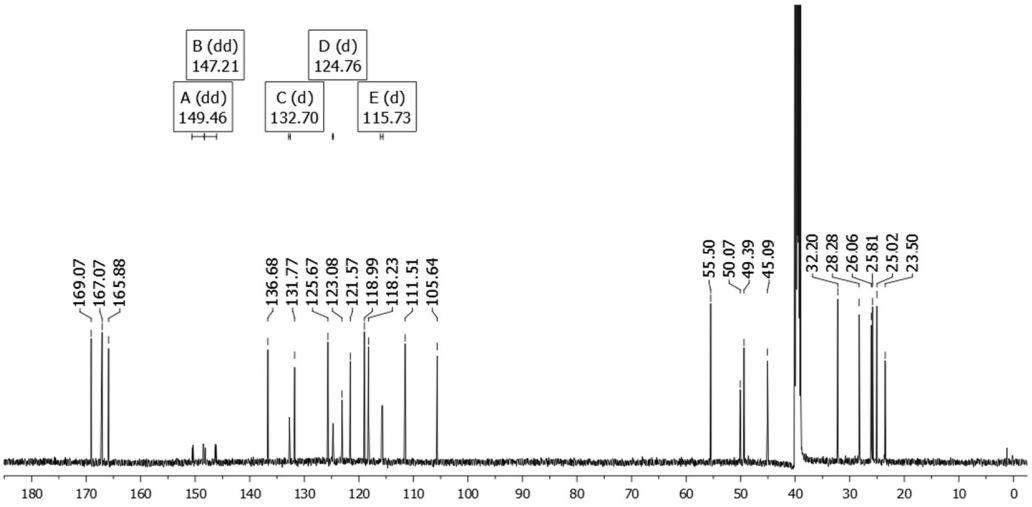
I (s) 10.37
G (d) 7.54
E (t) 6.99
D (t) 7.05
F (dd) 7.31
J (s) 6.24
B (dd) 4.46
A (d) 4.22
C (d) 3.90
P (d) 3.54
O (m) 3.29
K (dd) 2.99
M (m) 1.49
L (t) 1.94
N (m) 1.25
1.00
0.88
2.90
3.12
1.12
1.01
0.98
0.98
1.00
1.11
2.01
1.14
1.17
1.84
4.08
4.05
f1 (ppm)

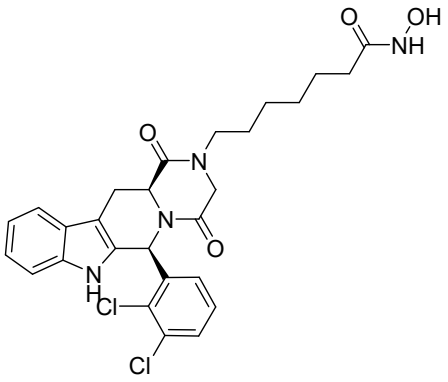

169.40
167.44
166.72
136.41
133.69
126.10
126.05
125.95
125.92
121.52
121.52
119.17
119.13
118.31
111.54
105.00
55.56
55.41
52.79
49.86
45.32
32.37
28.43
26.47
25.98
25.19
f1 (ppm)


O
OH
NH
O
N
N
O
N
H
F
F

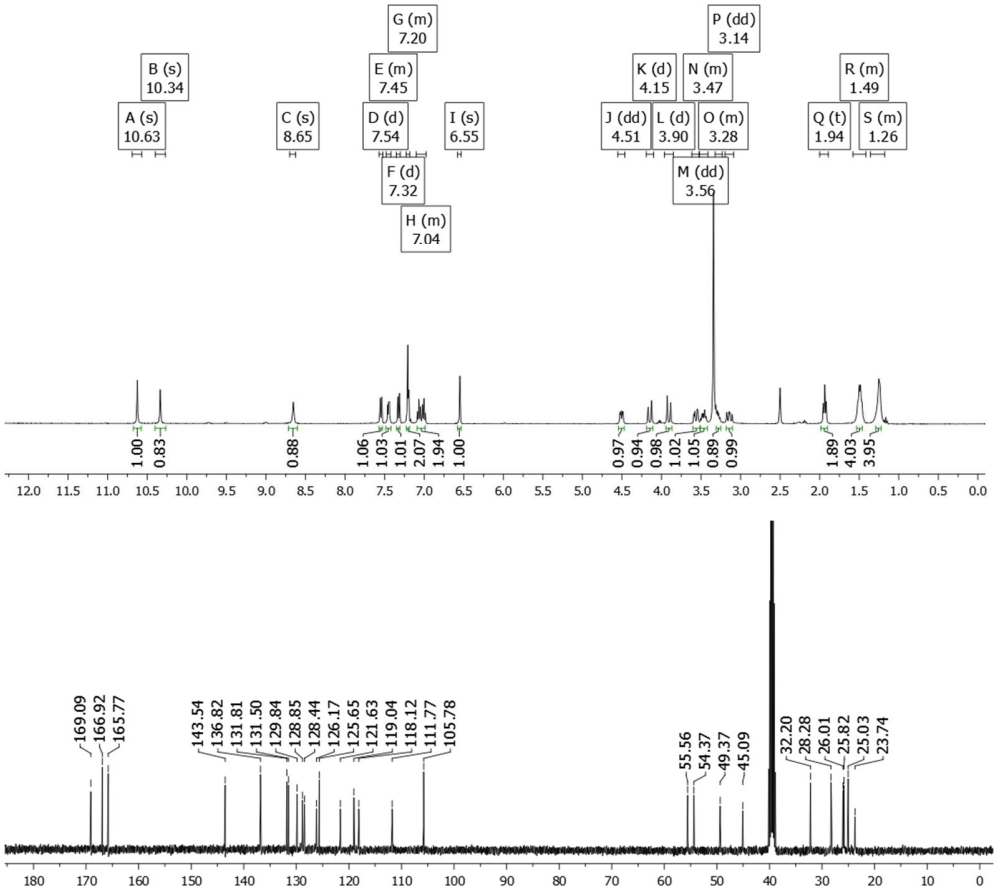
23c

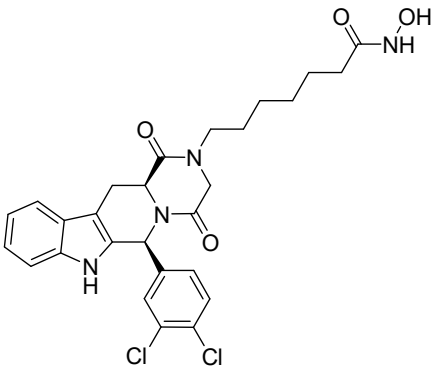

A (s) 10.97
B (s) 10.33
C (s) 8.65
D (d) 7.55
E (d) 7.28
F (m) 7.24
G (m) 7.03
H (s) 6.36
I (dd) 4.48
J (d) 4.16
K (d) 3.92
L (dd) 3.55
M (m) 3.47
N (m) 3.28
O (dd) 3.07
P (t) 1.93
Q (m) 1.48
R (m) 1.26
1.01
0.93
0.87
1.01
0.98
1.02
4.00
1.00
1.00
1.00
0.99
1.01
1.03
1.02
0.99
1.92
4.02
3.99
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0


A (dd) 149.46
B (dd) 147.21
C (d) 132.70
D (d) 124.76
E (d) 115.73
169.07
167.07
165.88
136.68
131.77
125.67
123.08
121.57
118.99
118.23
111.51
105.64
55.50
50.07
49.39
45.09
32.20
28.28
26.06
25.81
25.02
23.50
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

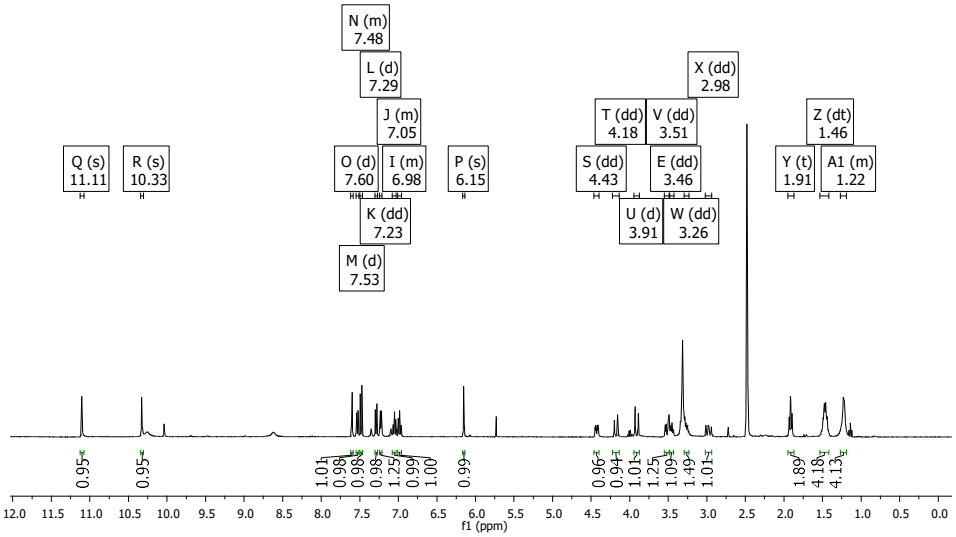

O
OH
NH
O
N
N
O
N
H
Cl
Cl

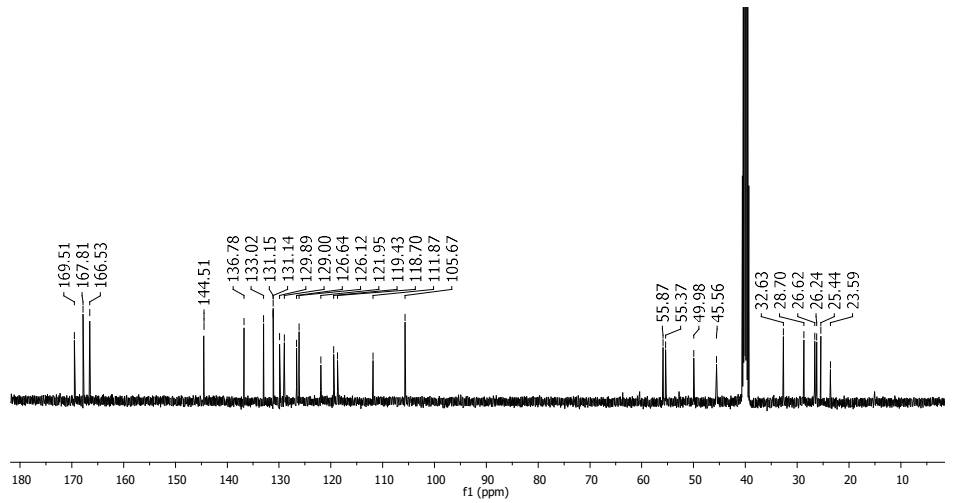
27c

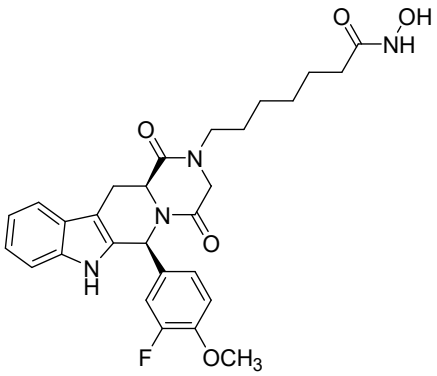

A (s) 10.63
B (s) 10.34
C (s) 8.65
D (d) 7.54
E (m) 7.45
F (d) 7.32
G (m) 7.20
H (m) 7.04
I (s) 6.55
J (dd) 4.51
K (d) 4.15
L (d) 3.90
M (dd) 3.56
N (m) 3.47
O (m) 3.28
P (dd) 3.14
Q (t) 1.94
R (m) 1.49
S (m) 1.26
1.00
0.83
0.88
1.06
1.03
1.01
2.07
1.94
1.00
0.97
0.94
0.98
1.02
1.05
0.89
0.99
1.89
4.03
3.95
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
169.09
166.92
165.77
143.54
136.82
131.81
131.50
129.84
128.85
128.44
126.17
125.65
121.63
119.04
118.12
111.77
105.78
55.56
54.37
49.37
45.09
32.20
28.28
26.01
25.82
25.03
23.74
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


O
OH
NH
O
N
N
O
N
H
Cl
Cl

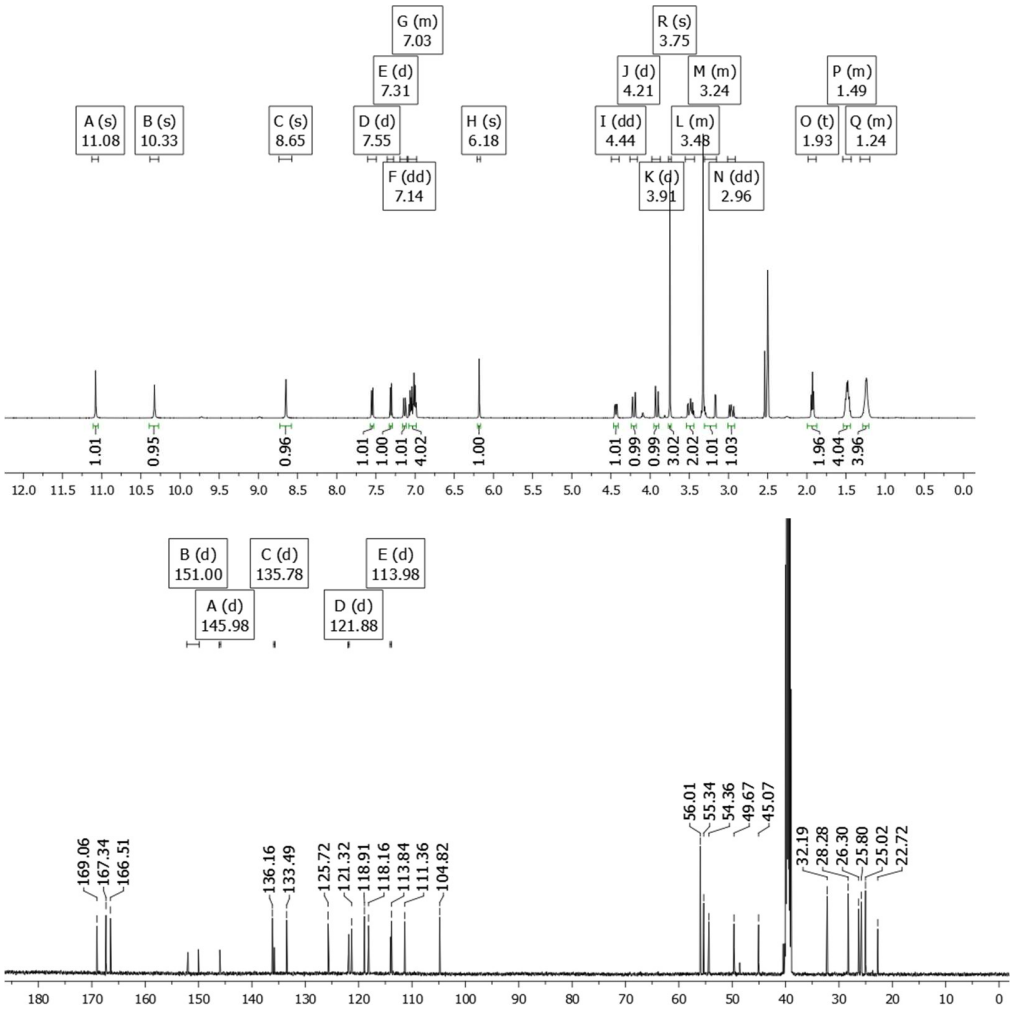
28c

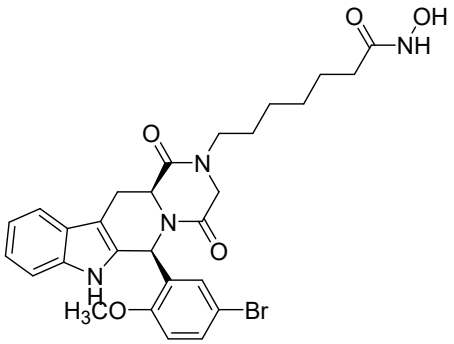

N (m) 7.48
L (d) 7.29
J (m) 7.05
X (dd) 2.98
T (dd) 4.18
V (dd) 3.51
Z (dt) 1.46
Q (s) 11.11
R (s) 10.33
O (d) 7.60
I (m) 6.98
P (s) 6.15
S (dd) 4.43
E (dd) 3.46
Y (t) 1.91
A1 (m) 1.22
K (dd) 7.23
U (d) 3.91
W (dd) 3.26
M (d) 7.53
0.95
0.95
1.01
0.98
0.98
0.98
1.25
0.99
1.00
0.99
0.96
0.94
1.01
1.25
1.08
1.49
1.01
1.89
4.18
4.13
f1 (ppm)


169.51
167.81
166.53
144.51
136.78
133.02
131.15
131.14
129.89
129.00
126.64
126.12
121.95
119.43
118.70
111.87
105.67
55.87
55.37
49.98
45.56
32.63
28.70
26.62
26.24
25.44
23.59
f1 (ppm)

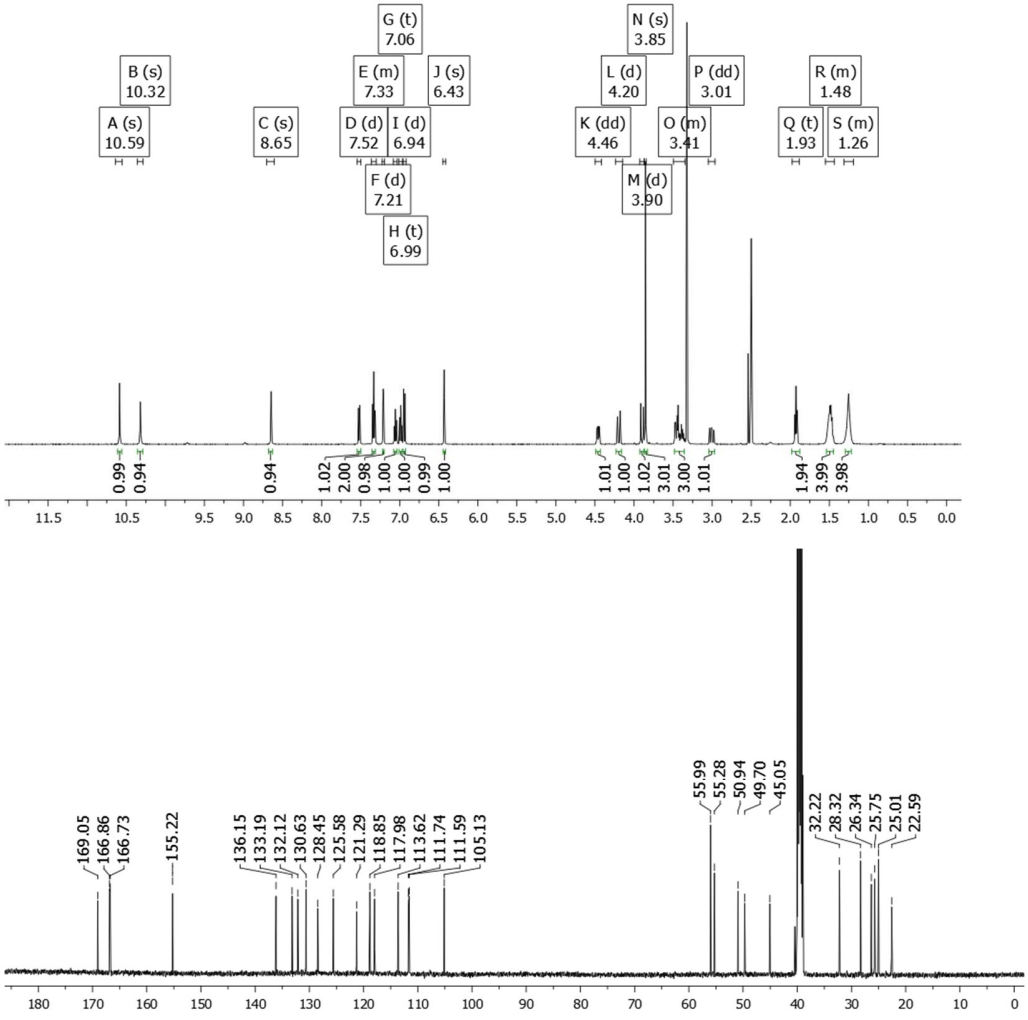

O
OH
NH
O
N
N
O
N
H
F
OCH3

33c


A (s) 11.08
B (s) 10.33
C (s) 8.65
D (d) 7.55
E (d) 7.31
F (dd) 7.14
G (m) 7.03
H (s) 6.18
I (dd) 4.44
J (d) 4.21
K (d) 3.91
L (m) 3.48
M (m) 3.24
N (dd) 2.96
O (t) 1.93
P (m) 1.49
Q (m) 1.24
R (s) 3.75
1.01 0.95 0.96 1.01 1.00 1.01 4.02 1.00 1.01 0.99 0.99 3.02 2.02 1.01 1.03 1.96 4.04 3.96
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
A (d) 145.98
B (d) 151.00
C (d) 135.78
D (d) 121.88
E (d) 113.98
169.06 167.34 166.51 136.16 133.49 125.72 121.32 118.91 118.16 113.84 111.36 104.82 56.01 55.34 54.36 49.67 45.07 32.19 28.28 26.30 25.80 25.02 22.72
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


O
OH
NH
O
N
N
O
N
H
H3CO
Br

34c


A (s) 10.59
B (s) 10.32
C (s) 8.65
D (d) 7.52
E (m) 7.33
F (d) 7.21
G (t) 7.06
H (t) 6.99
I (d) 6.94
J (s) 6.43
K (dd) 4.46
L (d) 4.20
M (d) 3.90
N (s) 3.85
O (m) 3.41
P (dd) 3.01
Q (t) 1.93
R (m) 1.48
S (m) 1.26
169.05
166.86
166.73
155.22
136.15
133.19
132.12
130.63
128.45
125.58
121.29
118.85
117.98
113.62
111.74
111.59
105.13
55.99
55.28
50.94
49.70
45.05
32.22
28.32
26.34
25.75
25.01
22.59