SUPPORTING INFORMATION

Amine-containing Donepezil Analogues as Potent Acetylcholinesterase Inhibitors with Increased Polarity

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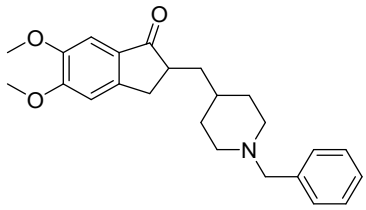
1. Materials

All commercially available starting materials, reagents, and solvents were used as supplied unless otherwise stated. Reported yields are isolated yields. Proton (^{1}H) and carbon (^{13}C) NMR were collected on Bruker NMR spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts (δ) are reported in parts-per-million (ppm) relative to the residual undeuterated solvent. High-resolution mass spectra were obtained using electron spray ionization (ESI).

Inhibitors 2c,[1] 2f, 2g, and 2k[2] were previously synthesised, but not tested for their AChE inhibition. 2a,b[3] and 6c[4] were synthesised by Sugimoto et al. during the development of donepezil and tested for their inhibition against AChE.

2. Analytical Data of Inhibitors

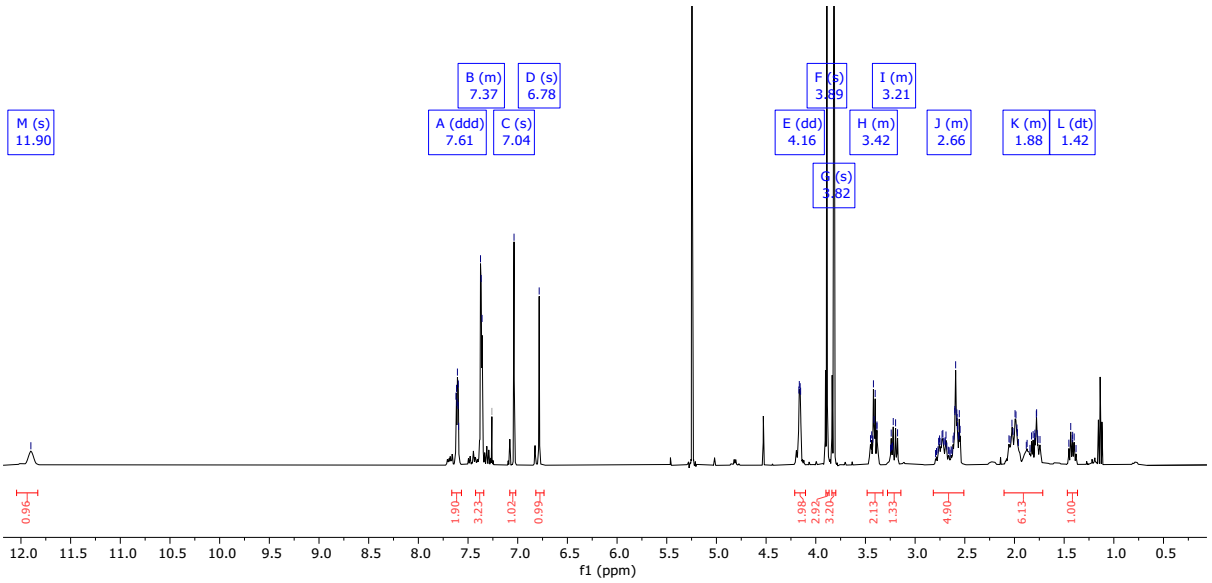
Donepezil (2a)



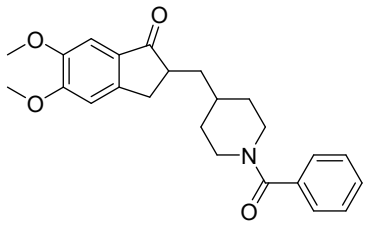
2-((1-benzylpiperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one

Obtained through the reaction of **1** with benzyl bromide, yellow oil, (63 %)

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.90 (s, 1H), 7.61 (ddd, J =4.8, 3.4, 1.7 Hz, 2H), 7.43 – 7.27 (m, 3H), 7.04 (s, 1H), 6.78 (s, 1H), 4.16 (dd, J =5.1, 1.8 Hz, 2H), 3.89 (s, 3H), 3.82 (s, 3H), 3.53 – 3.32 (m, 2H), 3.28 – 3.09 (m, 1H), 2.90 – 2.40 (m, 5H), 2.10 – 1.70 (m, 6H), 1.42 (dt, J =13.6, 7.5 Hz, 1H).



Donepezil-amide (2b)

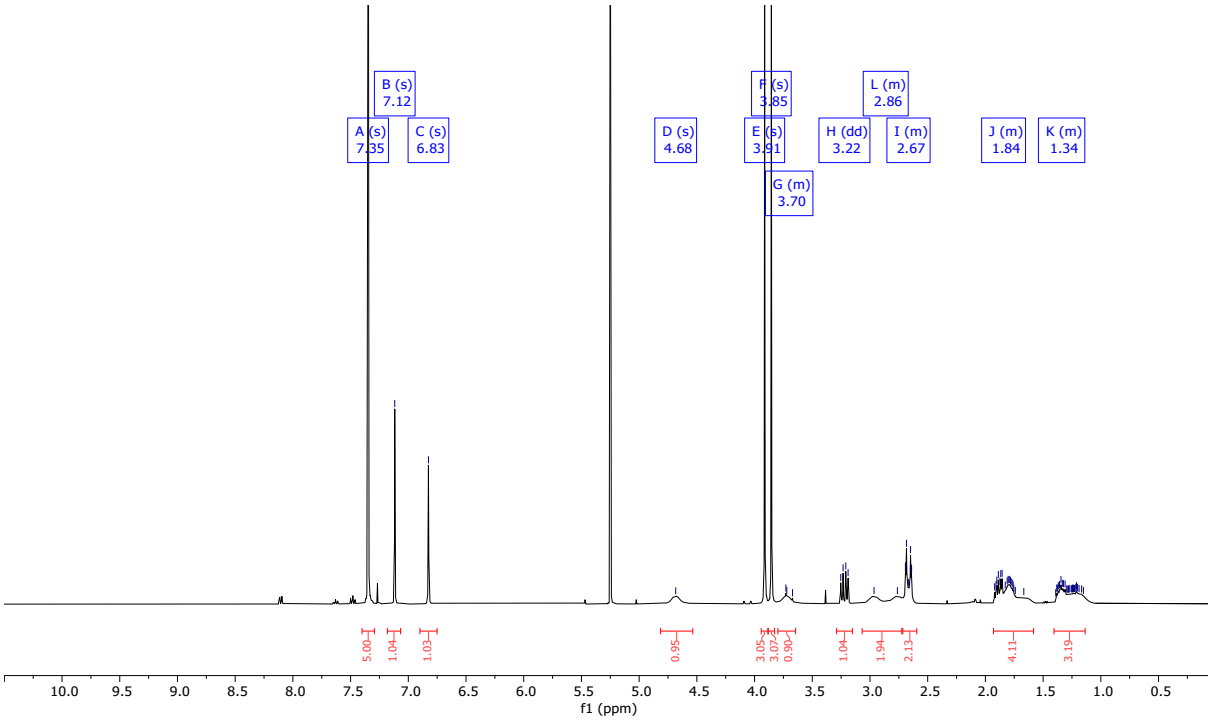


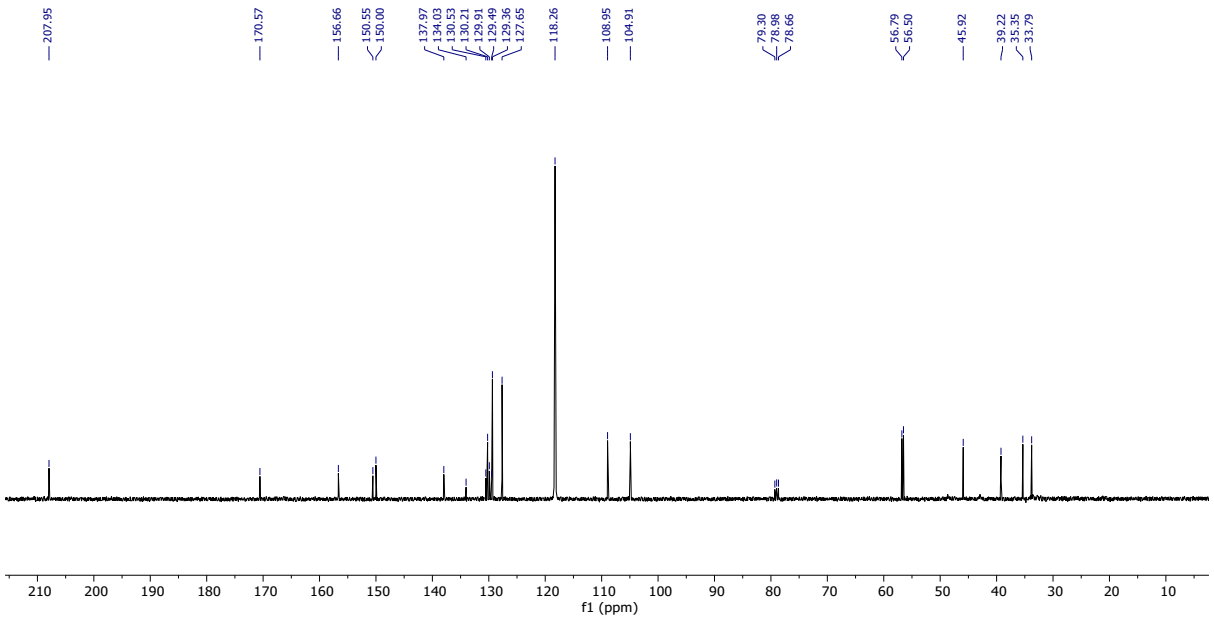
2-((1-benzoylpiperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one

Obtained through the reaction of **1** with benzoyl chloride, yellow oil, (98 %)

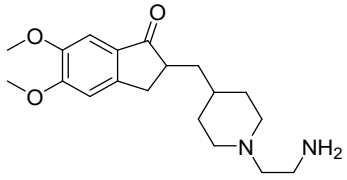
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (s, 5H), 7.12 (s, 1H), 6.83 (s, 1H), 4.68 (s, 1H), 3.91 (s, 3H), 3.85 (s, 3H), 3.79 – 3.59 (m, 1H), 3.22 (dd, *J* = 17.4, 8.0 Hz, 1H), 3.04 – 2.72 (m, 3H), 2.71 – 2.61 (m, 2H), 1.97 – 1.55 (m, 4H), 1.43 – 0.96 (m, 3H).

^{13}C NMR (101 MHz, CD_3CN**)** *δ* 207.95, 170.57, 156.66, 150.55, 150.00, 137.97, 134.03, 130.53, 130.21, 129.91, 129.49, 129.36, 127.65, 118.26, 108.95, 104.91, 79.30, 78.98, 78.66, 56.79, 56.50, 45.92, 39.22, 35.35, 33.79.




207.95
170.57
156.66
150.55
150.00
137.97
134.03
130.53
130.21
129.91
129.49
129.36
127.65
118.26
108.95
104.91
79.30
78.98
78.66
56.79
56.50
45.92
39.22
35.35
33.79
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

Donepezil-$(CH_2)_2$-NH_2 · 2 HCl (2c)



2-((1-(2-aminoethyl)piperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one

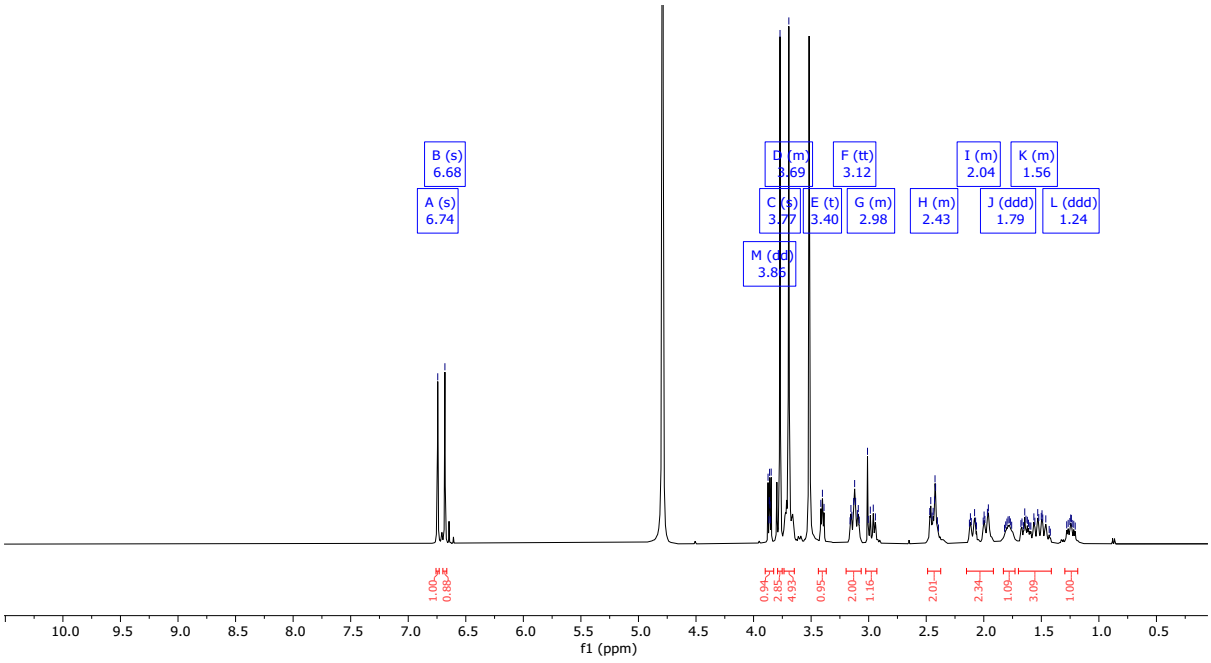
Obtained through the reaction of **1** with *N*-Boc-2-chloroethylamine and subsequent boc-deprotection, colourless solid (71 %).

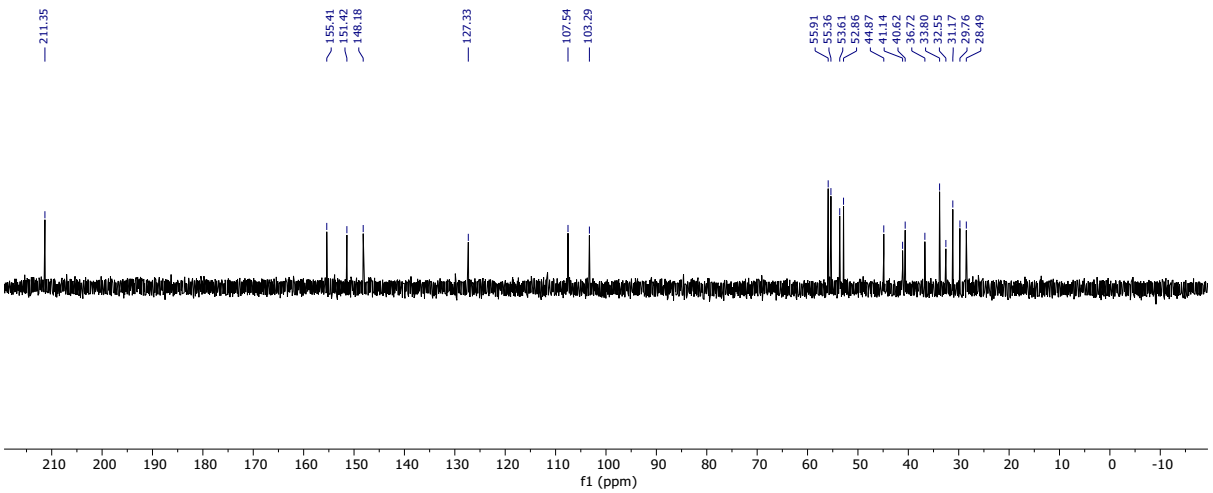
^{1}H NMR (400 MHz, D_2O) δ 6.74 (s, 1H), 6.68 (s, 1H), 3.86 (dd, 5.4 Hz, 1H), 3.77 (s, 3H), 3.73 – 3.67 (m, 5H), 3.40 (t, *J* = 5.5 Hz, 1H), 3.12 (tt, *J* = 12.5, 3.1 Hz, 2H), 3.05 – 2.89 (m, 1H), 2.53 – 2.32 (m, 2H), 2.16 – 1.95 (m, 2H), 1.79 (ddd, *J* = 12.7, 8.3, 4.3 Hz, 1H), 1.70 – 1.41 (m, 3H), 1.27 (ddd, *J* = 13.4, 11.2, 4.9 Hz, 1H).

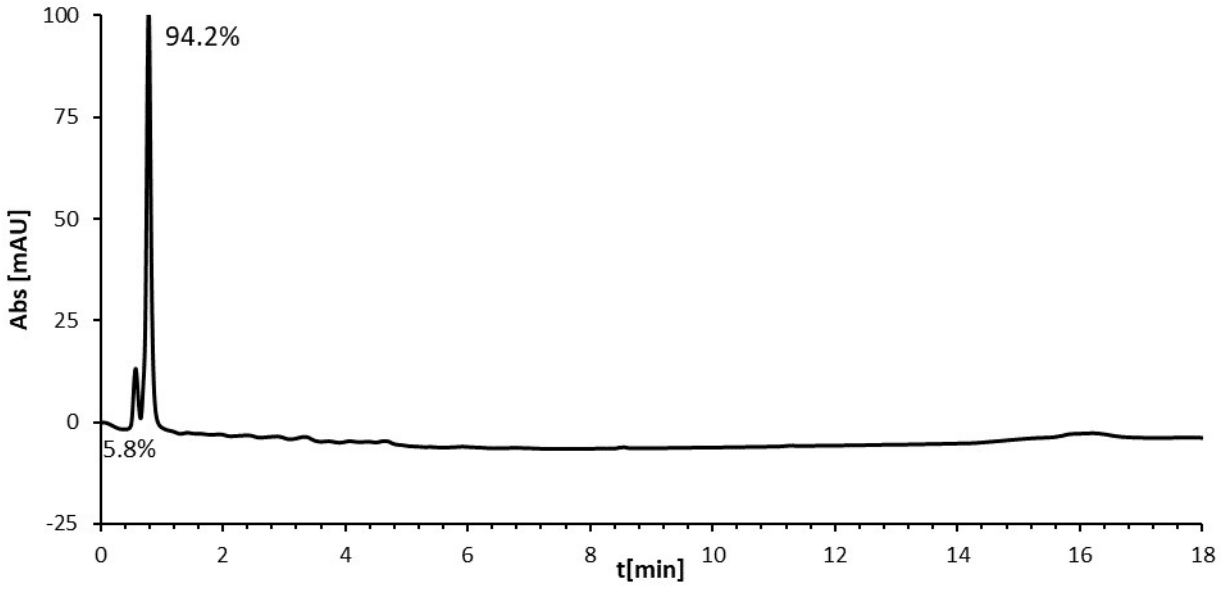
^{13}C NMR (101 MHz, D2O) δ 211.35, 155.41, 151.42, 148.18, 127.33, 107.54, 103.29, 55.91, 55.36, 53.61, 52.86, 44.87, 41.14, 40.62, 36.72, 33.80, 32.55, 31.17, 29.76, 28.49.

ESI [M+H]: 333.2173 found: 333.2171

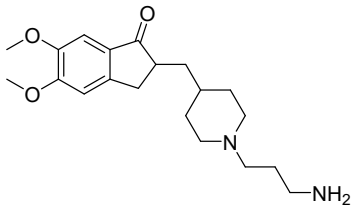
Purity: 94.2%




211.35
155.41
151.42
148.18
127.33
107.54
103.29
55.91
55.36
53.61
52.86
44.87
41.14
40.62
36.72
33.80
32.55
31.17
29.76
28.49
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


100
75
50
25
0
-25
Abs [mAU]
94.2%
5.8%
0 2 4 6 8 10 12 14 16 18
t[min]

Donepezil-$(CH_2)_3$-NH_2 · 2 HCl (2d)

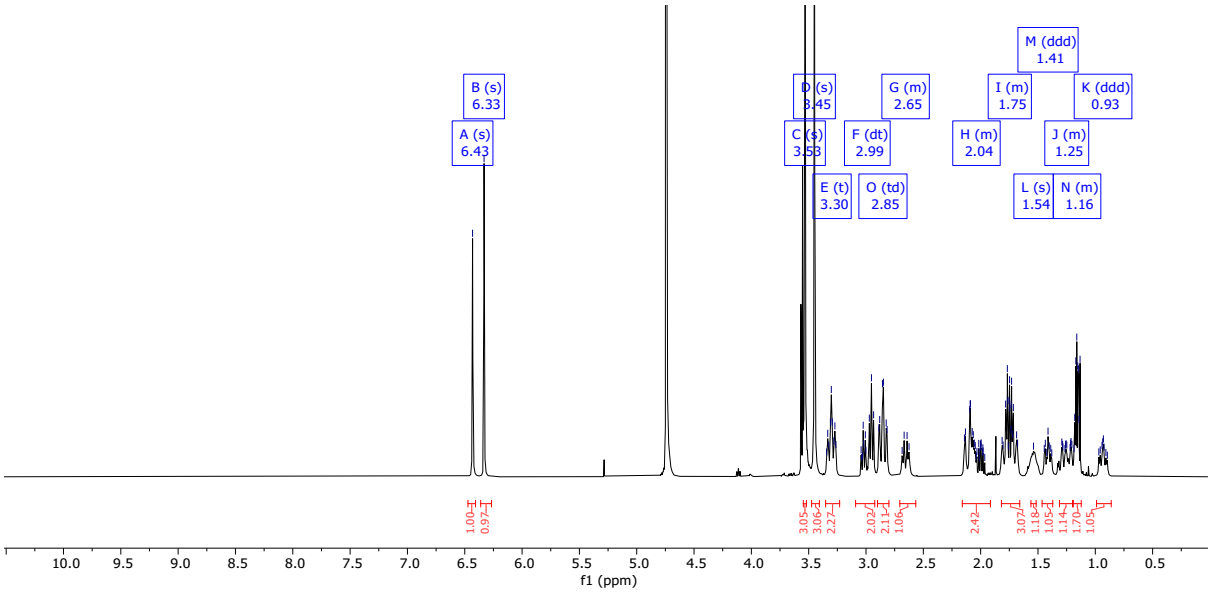


2-((1-(3-aminopropyl)piperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one

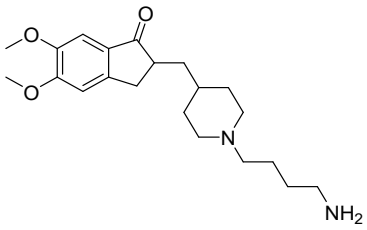
Obtained through the reaction of **1** with *N*-Boc-3-chloropropylamine and subsequent boc-deprotection, colourless solid (83%)

^{1}H NMR (400 MHz, D_2O) δ 6.43 (s, 1H), 6.33 (s, 1H), 3.53 (s, 3H), 3.45 (s, 3H), 3.30 (t, *J* = 6.4 Hz, 2H), 2.99 (dt, *J* = 28.8, 7.4 Hz, 2H), 2.85 (td, *J* = 12.9, 3.1 Hz, 2H), 2.71 – 2.57 (m, 1H), 2.16 – 1.91 (m, 2H), 1.82 – 1.66 (m, 3H), 1.54 (s, 1H), 1.41 (ddd, *J* = 13.1, 9.0, 3.7 Hz, 1H), 1.31 – 1.20 (m, 1H), 1.19 – 1.12 (m, 2H), 0.93 (ddd, *J* = 13.7, 11.2, 4.9 Hz, 1H).

ESI [M+H]: 347.2260 found: 347.2270



Donepezil-$(CH_2)_4$ -NH_2 · 2 HCl (2e)



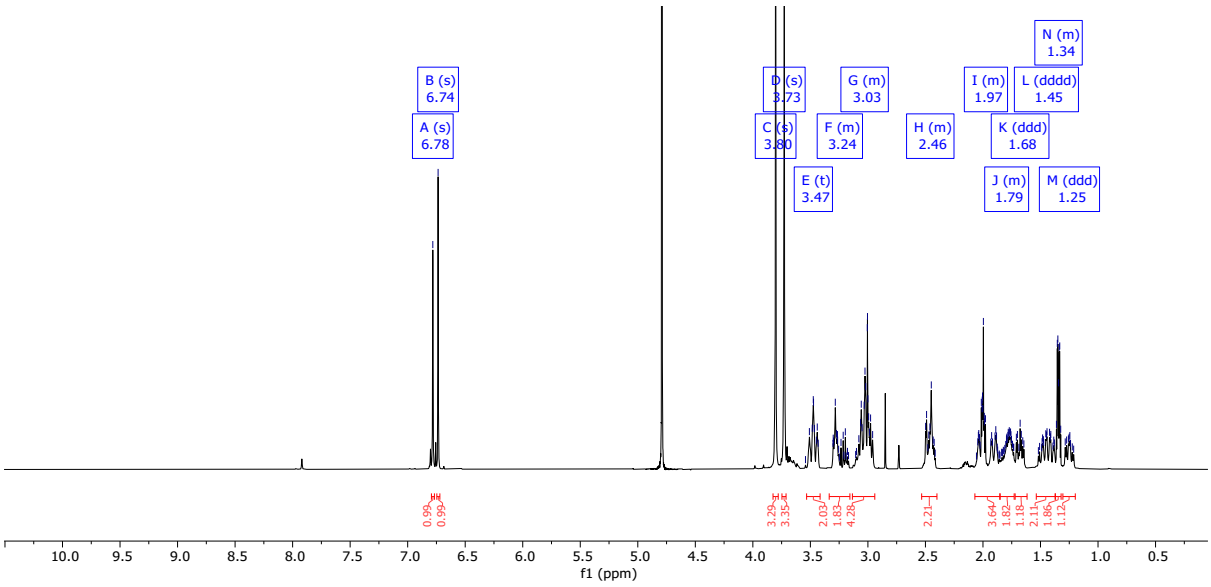
2-((1-(4-aminobutyl)piperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one

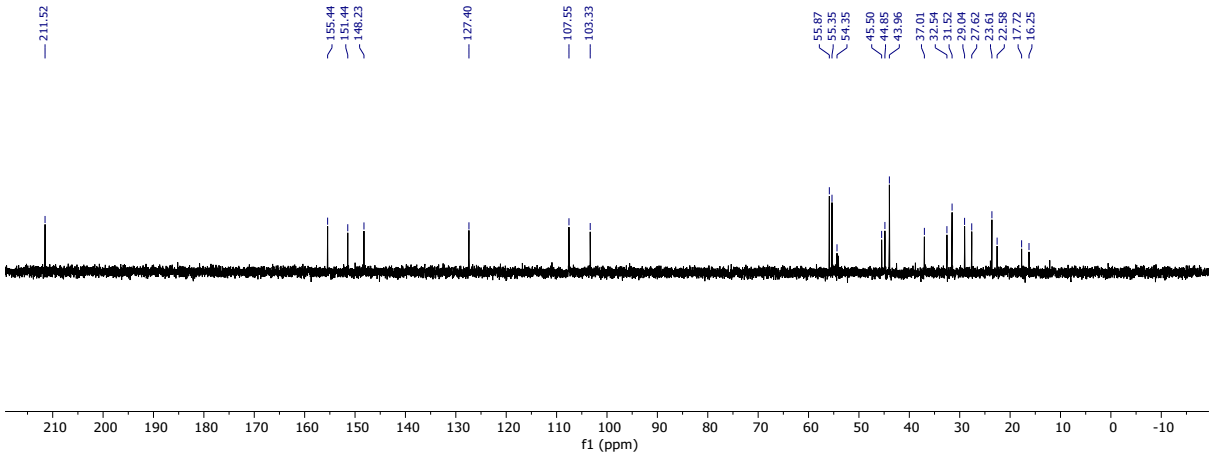
Obtained through the reaction of **1** with *N*-Boc-4-chlorobutylamine and subsequent boc-deprotection, colourless solid (76 %)

^{1}H NMR (400 MHz, D_2O) δ 6.78 (s, 1H), 6.74 (s, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 3.47 (t, 2H), 3.36 – 3.17 (m, 2H), 3.12 – 2.94 (m, 4H), 2.55 – 2.38 (m, 2H), 2.08 – 1.87 (m, 4H), 1.87 – 1.72 (m, 2H), 1.68 (ddd, *J* = 12.9, 8.8, 3.8 Hz, 1H), 1.45 (dddd, *J* = 27.3, 13.7, 8.2, 3.1 Hz, 2H), 1.37 – 1.32 (m, 2H), 1.25 (ddd, *J* = 13.3, 11.0, 4.9 Hz, 1H).

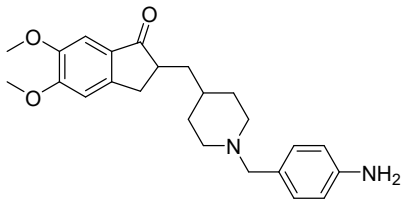
^{13}C NMR (101 MHz, D_2O) δ 211.52, 155.44, 151.44, 148.23, 127.40, 107.55, 103.33, 55.87, 55.35, 54.35, 45.50, 44.85, 43.96, 37.01, 32.54, 31.52, 29.04, 27.62, 23.61, 22.58, 17.72, 16.25.

ESI [M+H]: 361.2413 found: 361.2412




211.52
155.44
151.44
148.23
127.40
107.55
103.33
55.87
55.35
54.35
45.50
44.85
43.96
37.01
32.54
31.52
29.04
27.62
23.61
22.58
17.72
16.25
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

_p_-Amino-donepezil · 2 HCl (2f)



2-((1-(4-aminobenzyl)piperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one

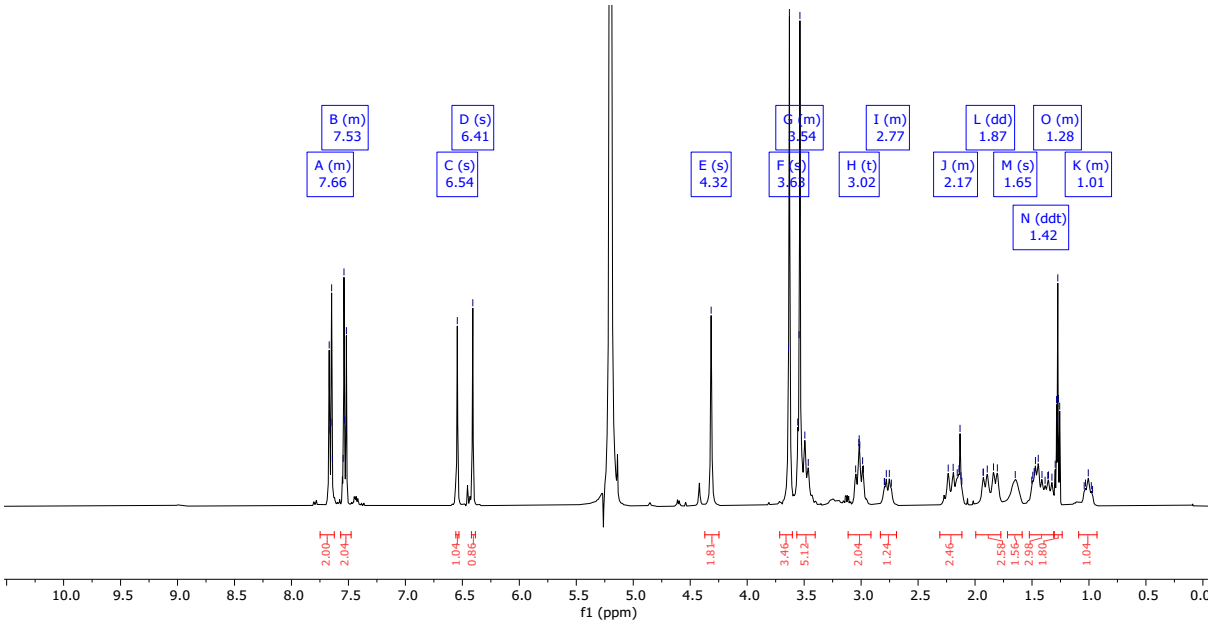
Obtained through the reaction of **1** with *N*-Boc-4-aminobenzyl chloride and subsequent boc-deprotection, yellow solid (87 %)

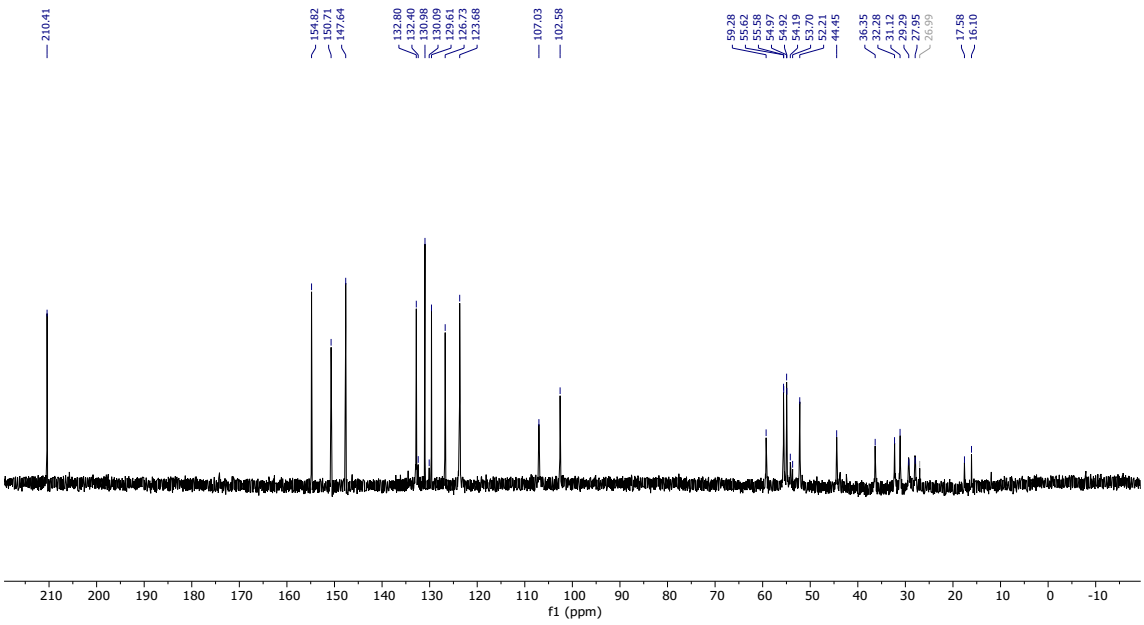
^{1}H NMR (400 MHz, D_2O) δ 7.75 – 7.62 (m, 2H), 7.57 – 7.48 (m, 2H), 6.54 (s, 1H), 6.41 (s, 1H), 4.32 (s, 2H), 3.63 (s, 3H), 3.56 – 3.40 (m, 5H), 3.02 (t, 2H), 2.83 – 2.69 (m, 1H), 2.31 – 2.12 (m, 2H), 1.87 (dd, J = 35.6, 13.8 Hz, 2H), 1.65 (s, 2H), 1.42 (ddt, J = 34.9, 24.0, 10.3 Hz, 3H), 1.31 – 1.24 (m, 2H), 1.09 – 0.93 (m, 1H).

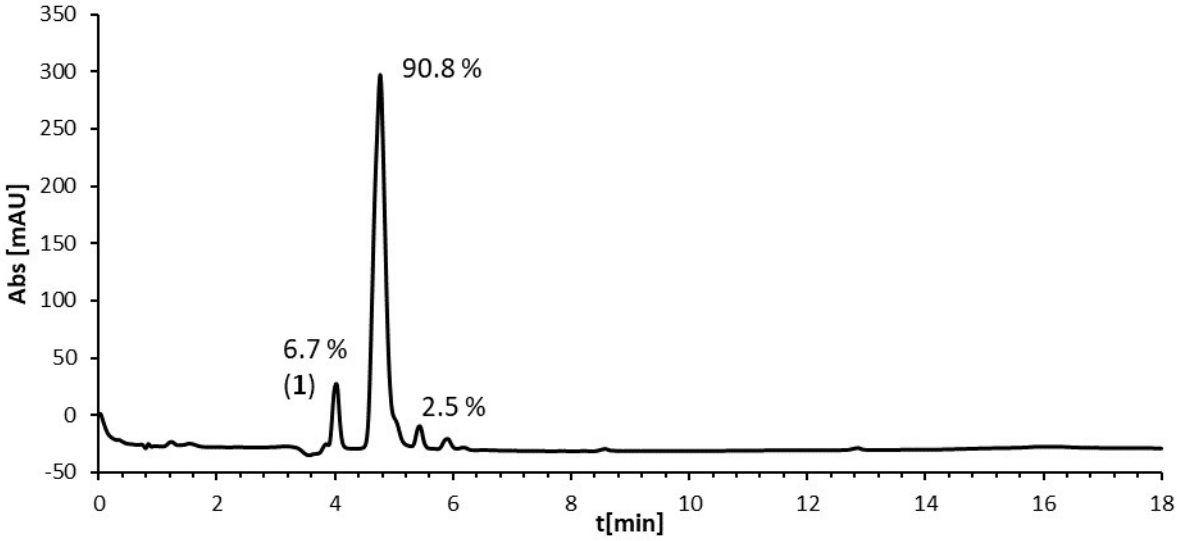
^{13}C NMR (101 MHz, D_2O) δ 210.41, 154.82, 150.71, 147.64, 132.80, 132.40, 130.98, 129.61, 126.73, 123.68, 107.03, 102.58, 59.28, 55.62, 55.58, 54.97, 54.92, 54.19, 53.70, 52.21, 44.45, 36.35, 32.28, 31.12, 29.29, 27.95, 26.99, 17.58, 16.10.

ESI [M+2H]: 396.2326 found: 396.2329

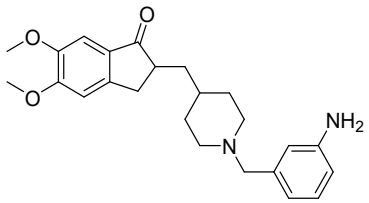
Purity: 90.8%




210.41
154.82
150.71
147.64
132.80
132.40
130.98
130.09
129.61
126.73
123.68
107.03
102.58
59.28
55.62
55.58
54.97
54.92
54.19
53.70
52.21
44.45
36.35
32.28
31.12
29.29
27.95
26.99
17.58
16.10
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


350
300
250
200
150
100
50
0
-50
Abs [mAU]
90.8 %
6.7 %
(1)
2.5 %
0 2 4 6 8 10 12 14 16 18
t[min]

***m*-Amino-donepezil · 2 HCl (2g)**



2-((1-(3-aminobenzyl)piperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one

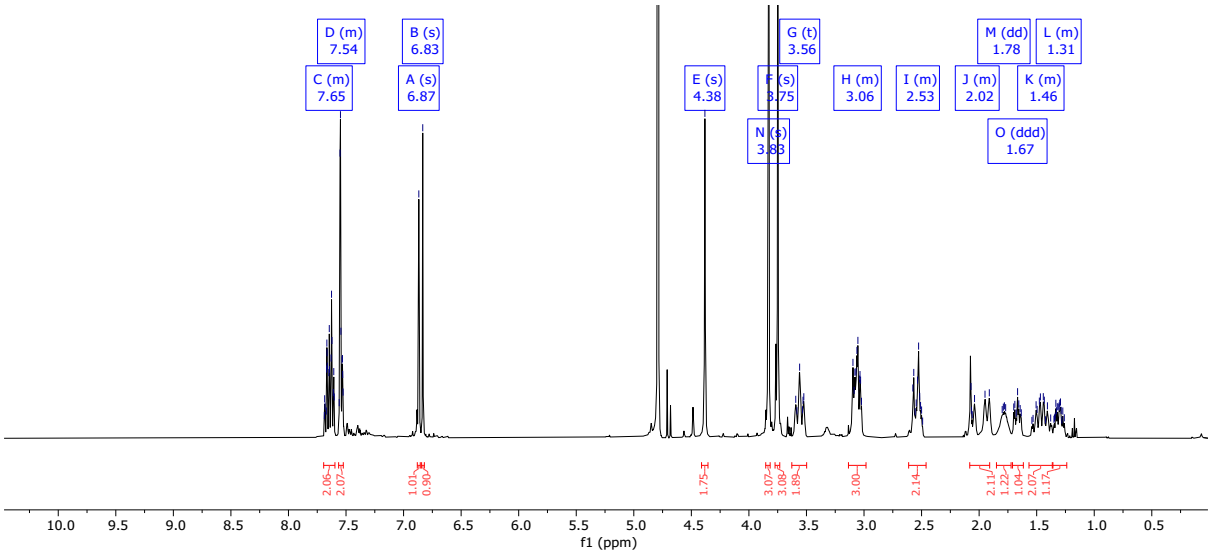
Obtained through the reaction of **1** with *N*-Boc-3-aminobenzyl chloride and subsequent boc-deprotection, yellow solid (92 %)

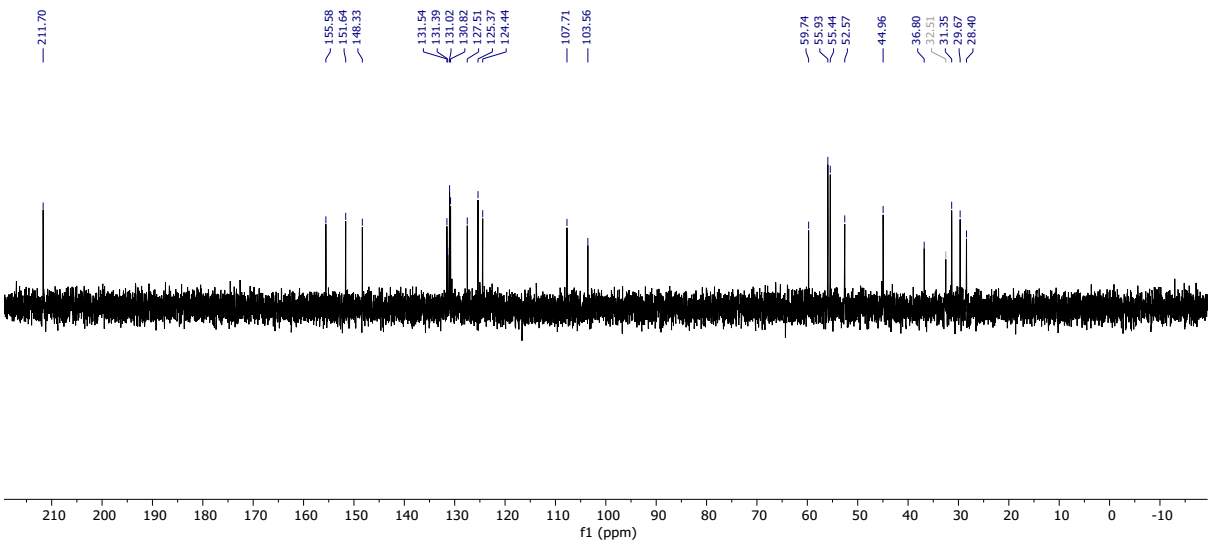
^{1}H NMR (400 MHz, D_2O) δ 7.69 – 7.58 (m, 2H), 7.57 – 7.52 (m, 2H), 6.87 (s, 1H), 6.83 (s, 1H), 4.38 (s, 2H), 3.83 (s, 3H), 3.75 (s, 3H), 3.56 (t, *J* = 14.0 Hz, 2H), 3.15 – 2.99 (m, 3H), 2.63 – 2.45 (m, 2H), 2.09 – 1.89 (m, 2H), 1.78 (dd, *J* = 7.9, 4.2 Hz, 1H), 1.67 (ddd, *J* = 12.9, 8.6, 3.8 Hz, 1H), 1.56 – 1.37 (m, 2H), 1.36 – 1.24 (m, 1H).

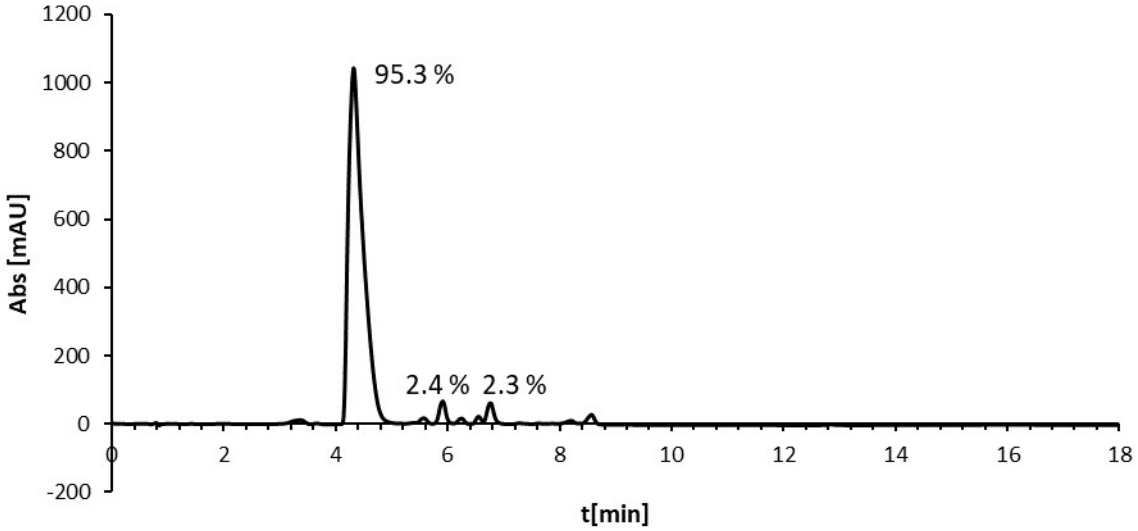
^{13}C NMR (101 MHz, D_2O) *δ* 211.31, 155.44, 151.39, 148.23, 132.11, 131.10, 130.90, 130.60, 127.85, 127.37, 125.79, 124.84, 107.58, 103.34, 62.97, 59.72, 55.94, 55.40, 52.62, 44.93, 36.77, 32.57, 31.42, 29.67, 28.41.

ESI [M+H]: 395.2330 found: 395.2330

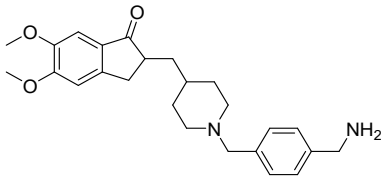
Purity: 95.3 %




211.70
155.58
151.64
148.33
131.54
131.39
131.02
130.82
127.51
125.37
124.44
107.71
103.56
59.74
55.93
55.44
52.57
44.96
36.80
32.51
31.35
29.67
28.40
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


95.3 %
2.4 %
2.3 %
Abs [mAU]
t[min]
1200
1000
800
600
400
200
0
-200
0 2 4 6 8 10 12 14 16 18

***p*-Aminomethyl donepezil · 2 HCl (2h)**



2-((1-(4-(aminomethyl)benzyl)piperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one

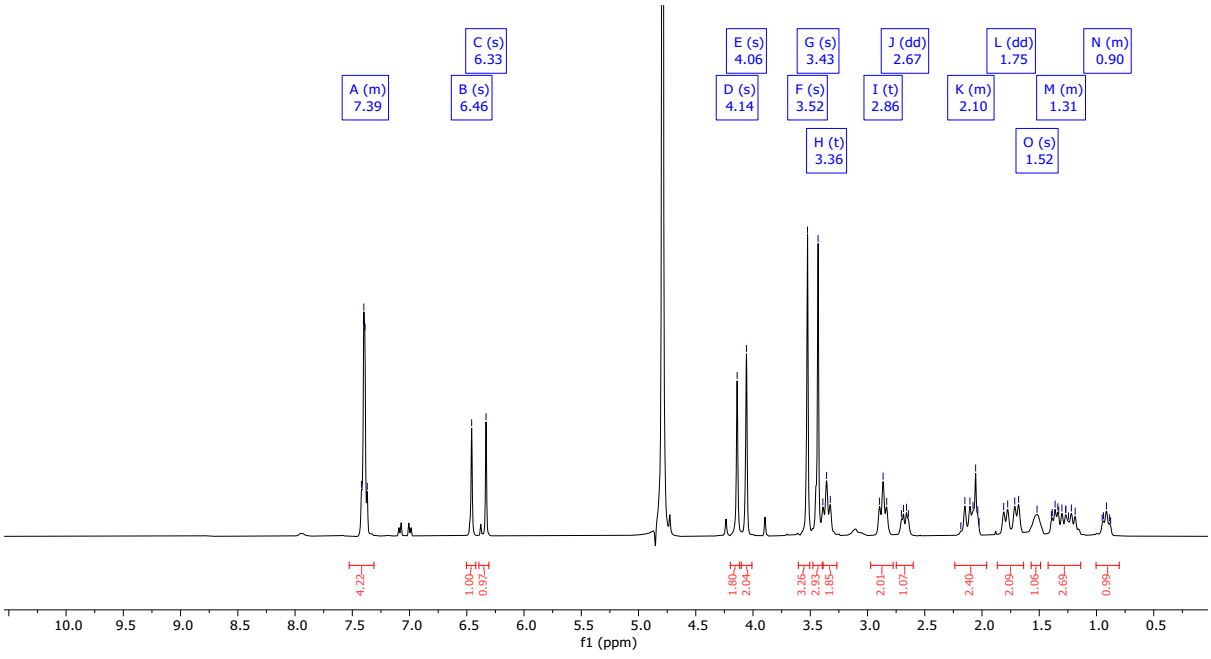
Obtained through the reaction of **1** with 4-cyanobenzyl chloride and subsequent reduction with hydrogen, colorless solid (83 %)

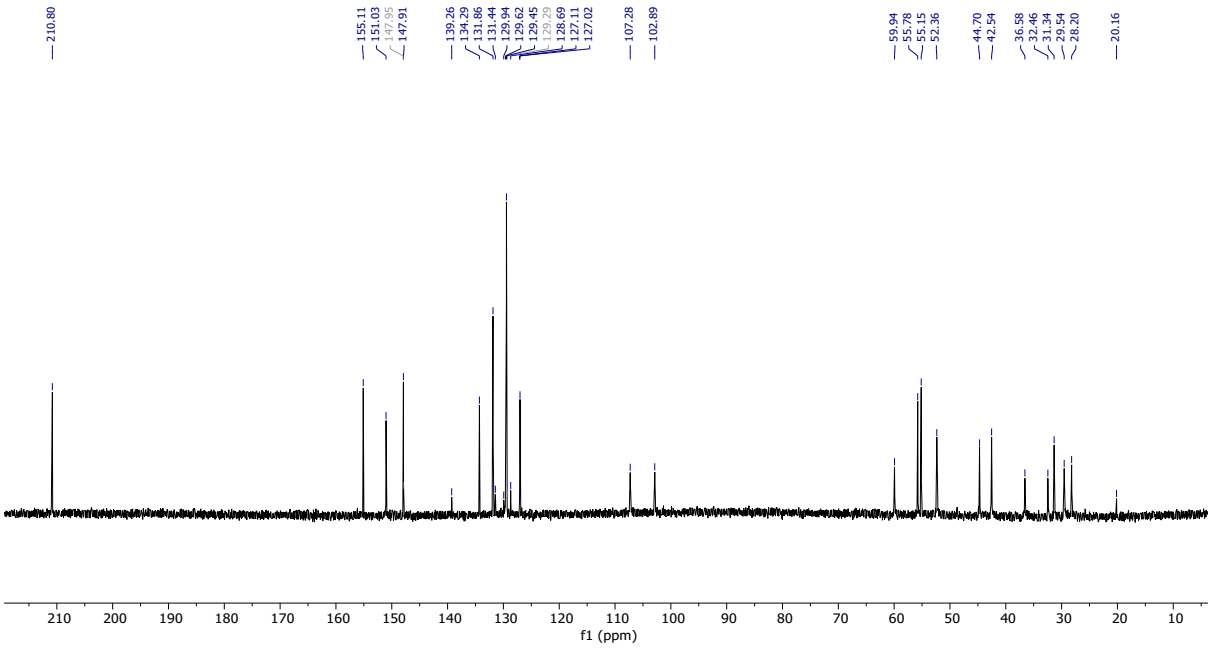
^{1}H NMR (400 MHz, D_2O) δ 7.53 – 7.31 (m, 4H), 6.46 (s, 1H), 6.33 (s, 1H), 4.14 (s, 2H), 4.06 (s, 2H), 3.52 (s, 3H), 3.43 (s, 3H), 3.36 (t, J =13.0 Hz, 2H), 2.86 (t, J =12.7 Hz, 2H), 2.67 (dd, J =17.4, 6.9 Hz, 1H), 2.24 – 1.96 (m, 2H), 1.75 (dd, J =38.5, 13.8 Hz, 2H), 1.52 (s, 1H), 1.42 – 1.14 (m, 3H), 1.00 – 0.80 (m, 1H).

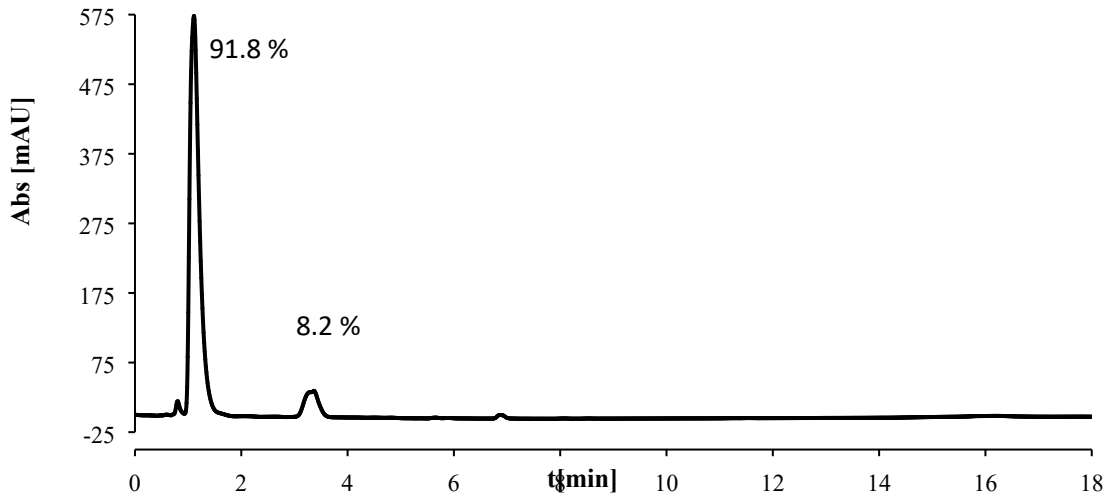
^{13}C NMR (101 MHz, D_2O) δ 211.47, 155.44, 151.48, 148.21, 139.34, 133.56, 131.92, 131.60, 130.45, 130.06, 129.71, 129.60, 129.27, 129.06, 127.37, 125.63, 107.59, 103.35, 60.17, 55.89, 55.35, 52.48, 44.89, 43.00, 42.68, 36.75, 32.52, 31.38, 29.65, 28.35, 20.30.

ESI [M+H]: 409.2481 found: 409.2486

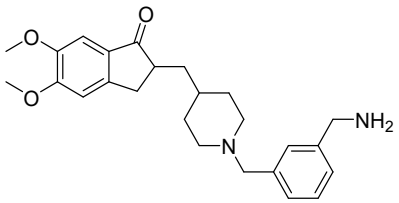
Purity: 91.8%




210.80
155.11
151.03
147.95
147.91
139.26
134.29
131.86
131.44
129.94
129.62
129.45
129.29
128.69
127.11
127.02
107.28
102.89
59.94
55.78
55.15
52.36
44.70
42.54
36.58
32.46
31.34
29.54
28.20
20.16
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


575
475
375
275
175
75
-25
Abs [mAU]
91.8 %
8.2 %
0 2 4 6 8 10 12 14 16 18
t[min]

_m_-Aminomethyl donepezil · 2 HCl (2i)



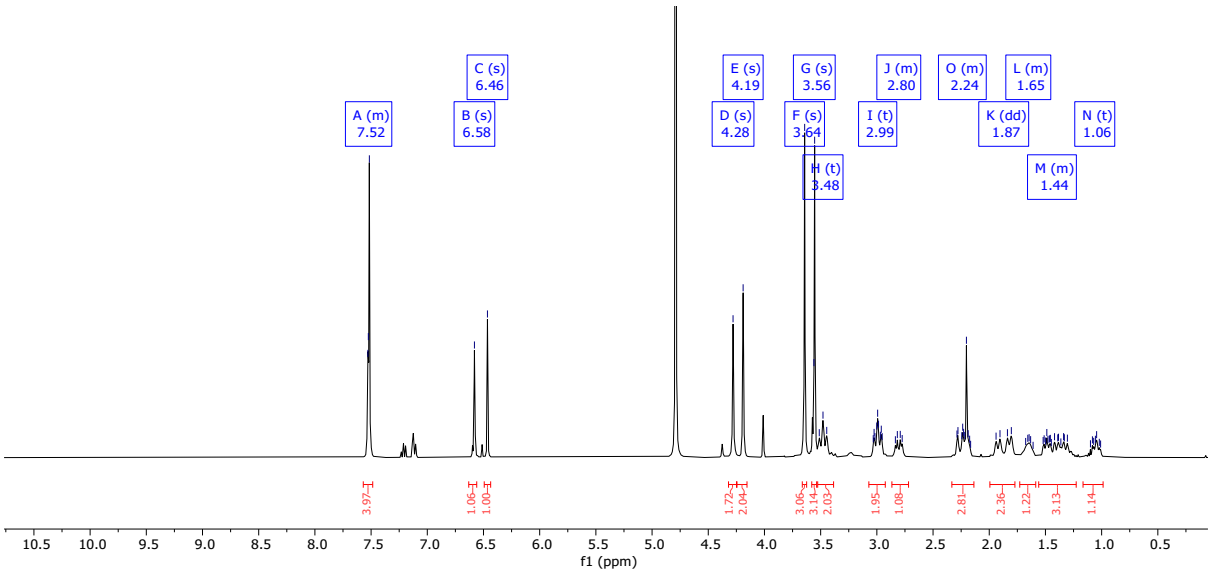
2-((1-(3-(aminomethyl)benzyl)piperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one

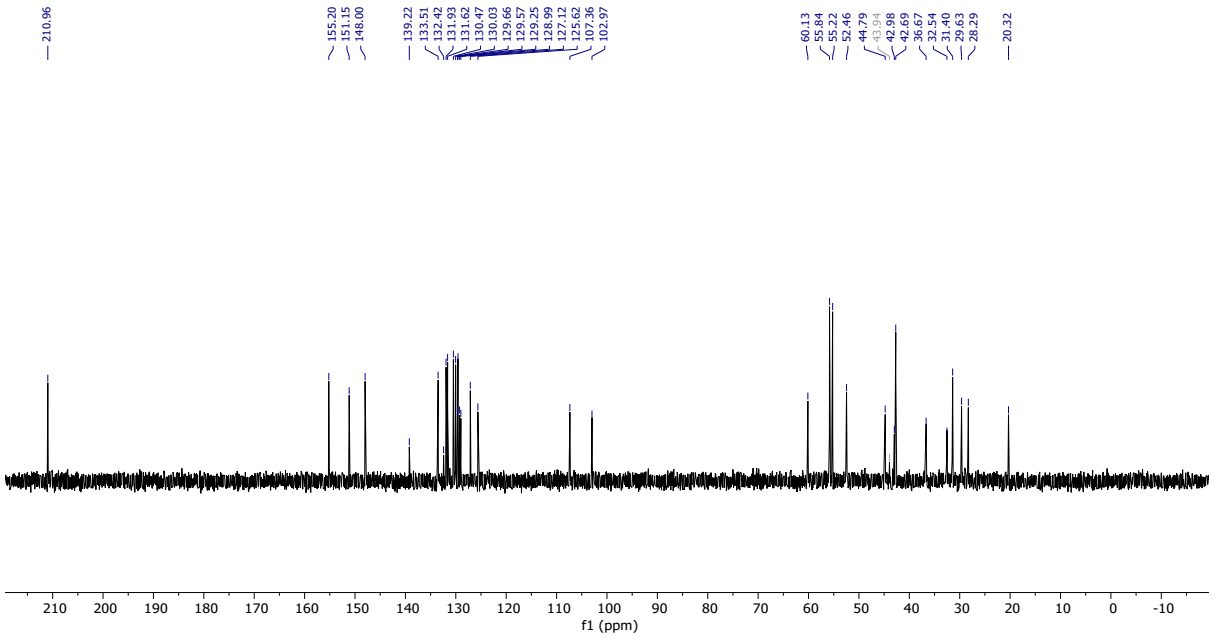
Obtained through the reaction of **1** with 3-cyanobenzyl chloride and subsequent reduction with hydrogen, colorless solid (78 %)

^{1}H NMR (400 MHz, D_2O) δ 7.58 – 7.47 (m, 4H), 6.58 (s, 1H), 6.46 (s, 1H), 4.28 (s, 2H), 4.19 (s, 2H), 3.64 (s, 3H), 3.56 (s, 3H), 3.48 (t, *J* = 13.3 Hz, 2H), 2.99 (t, *J* = 3.1 Hz, 2H), 2.86 – 2.71 (m, 1H), 2.37 – 2.14 (m, 3H), 1.87 (dd, *J* = 40.4, 14.0 Hz, 2H), 1.72 – 1.61 (m, 1H), 1.54 – 1.26 (m, 3H), 1.06 (t, 1H).

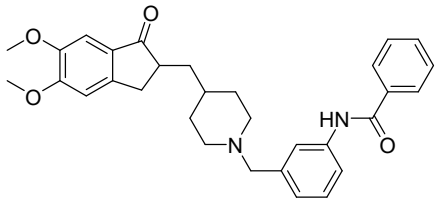
^{13}C NMR (101 MHz, D_2O) *δ* 210.96, 155.20, 151.15, 148.00, 139.22, 133.51, 132.42, 131.93, 131.62, 130.47, 130.03, 129.66, 129.57, 129.25, 128.99, 127.12, 125.62, 107.36, 102.97, 60.13, 55.84, 55.22, 52.46, 44.79, 43.94, 42.98, 42.69, 36.67, 32.54, 31.40, 29.63, 28.29, 20.32.

ESI [M+H]: 409.248 found: 409.249




210.96
155.20
151.15
148.00
139.22
133.51
132.42
131.93
131.62
130.47
130.03
129.66
129.57
129.25
128.99
127.12
125.62
107.36
102.97
60.13
55.84
55.22
52.46
44.79
42.98
42.69
36.67
32.54
31.40
29.63
28.29
20.32
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

_m_-Benzamide donepezil · HCl (2j)



N-(3-((4-((5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl)methyl)phenyl) benzamide

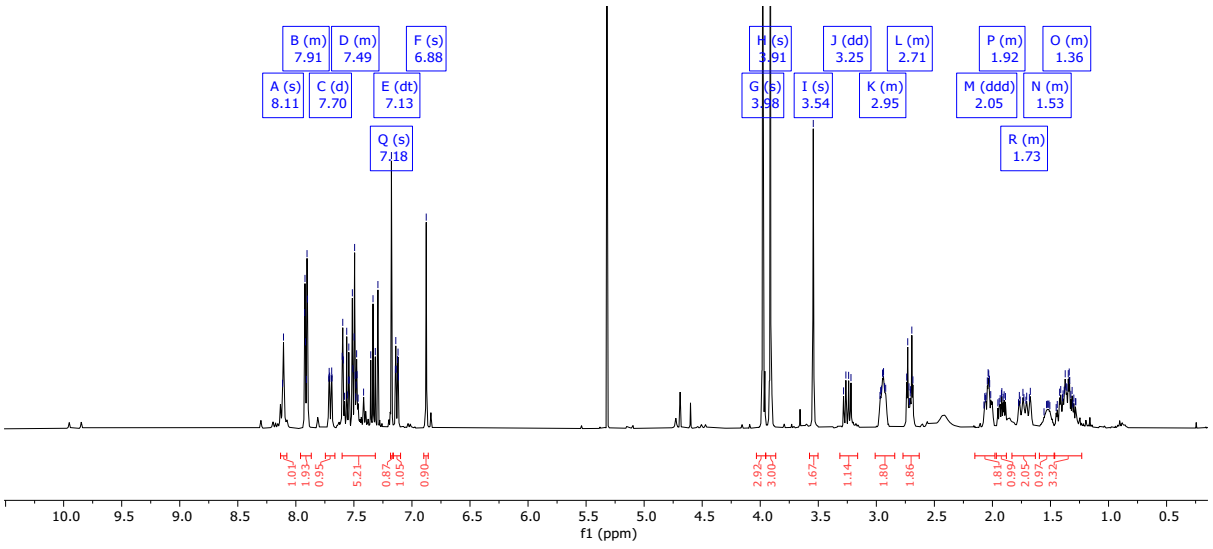
m-Amino donepezil (**2g**) and triethylamine (6 eq.) were dissolved in chloroform and benzoyl chloride (2 eq.) was added dropwise. The reaction mixture was stirred overnight at room temperature, before 0.1 M NaOH was added to hydrolyse unreacted acid chloride. The organic phase was washed 2x with 0.1 M NaOH, 0.1 M HCl and 3x with brine. Removal of the solvent under reduced pressure yielded **2j** as a colourless solid (78 %).

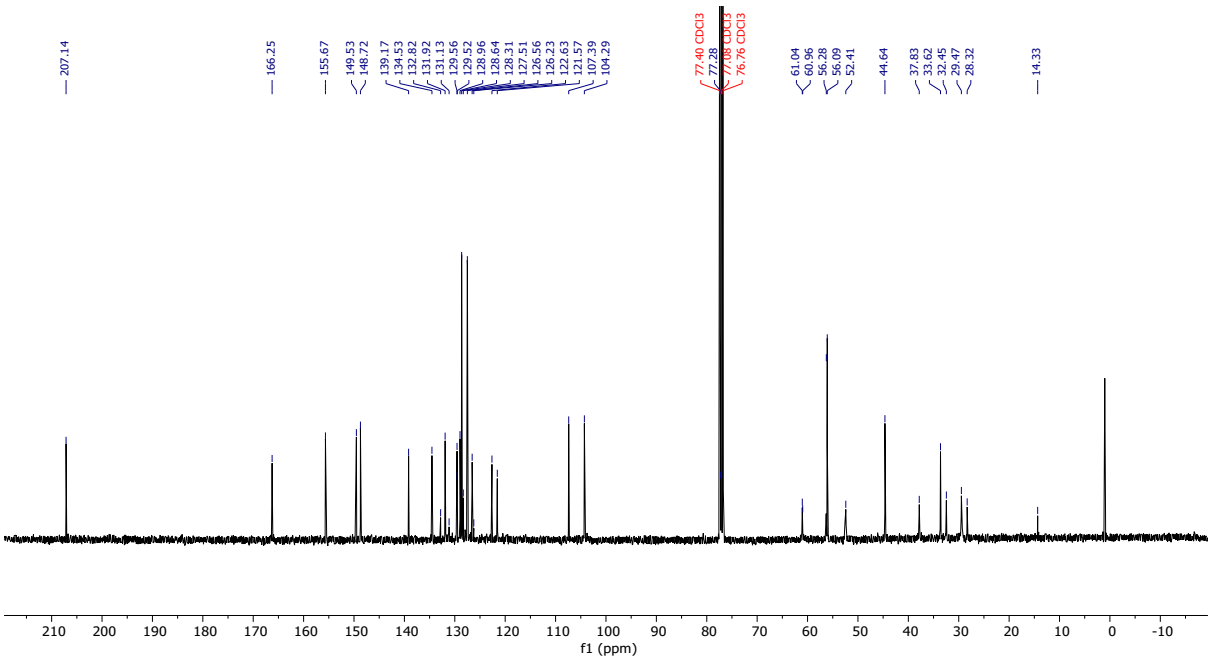
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.96 – 7.88 (m, 2H), 7.70 (d, *J* = 9.4 Hz, 1H), 7.65 – 7.26 (m, 5H), 7.18 (s, 1H), 7.13 (dt, *J* = 7.5, 1.3 Hz, 1H), 6.88 (s, 1H), 3.98 (s, 3H), 3.91 (s, 3H), 3.54 (s, 2H), 3.25 (dd, *J* = 17.5, 8.0 Hz, 1H), 3.03 – 2.87 (m, 2H), 2.79 – 2.59 (m, 2H), 2.05 (ddd, *J* = 11.6, 4.8, 2.7 Hz, 1H), 1.95 – 1.81 (m, 1H), 1.80 – 1.63 (m, 2H), 1.59 – 1.49 (m, 1H), 1.46 – 1.18 (m, 3H).

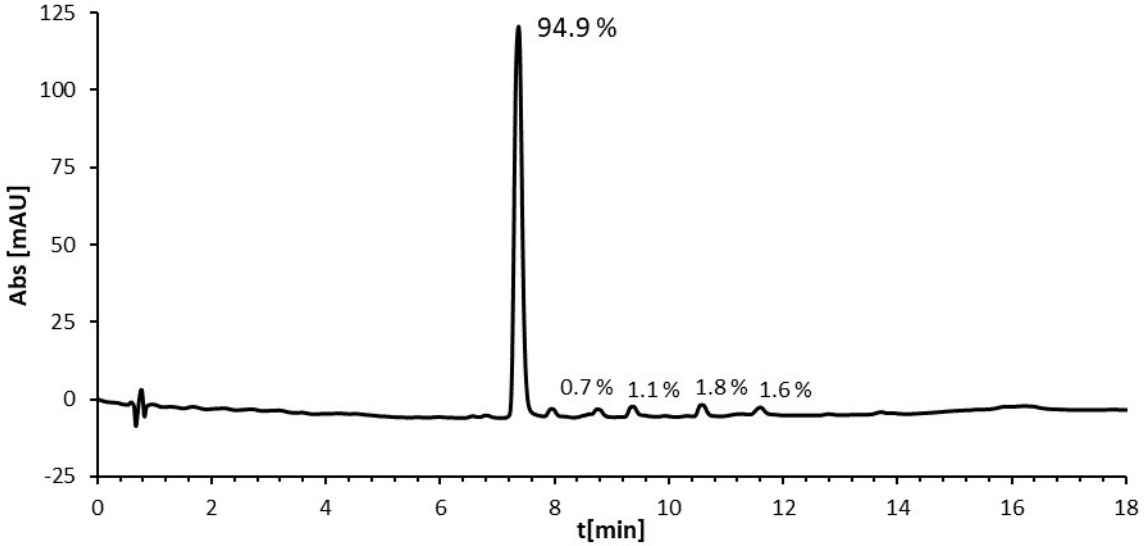
^{13}C NMR (101 MHz, $CDCl_3$) δ 207.14, 166.25, 155.67, 149.53, 148.72, 139.17, 134.53, 132.82, 131.92, 131.13, 129.56, 129.52, 128.96, 128.64, 128.31, 127.51, 126.56, 126.23, 122.63, 121.57, 107.39, 104.29, 77.40, 77.28, 77.08, 76.76, 61.04, 60.96, 56.28, 56.09, 52.41, 44.64, 37.83, 33.62, 32.45, 29.47, 28.32, 14.33.

ESI [M+H]: 499.2591 found: 499.2591

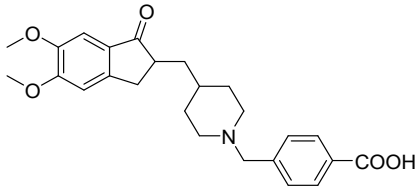
Purity: 94.9%




207.14
166.25
155.67
149.53
148.72
139.17
134.53
132.82
131.92
131.13
129.56
129.52
128.96
128.64
128.31
127.51
126.56
126.23
122.63
121.57
107.39
104.29
77.40 CDCl3
77.28
77.08 CDCl3
76.76 CDCl3
61.04
60.96
56.28
56.09
52.41
44.64
37.83
33.62
32.45
29.47
28.32
14.33
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


125
100
75
50
25
0
-25
Abs [mAU]
94.9 %
0.7 %
1.1 %
1.8 %
1.6 %
0 2 4 6 8 10 12 14 16 18
t[min]

_p_-Carboxy donepezil · $N(Et)_3$ (2k)



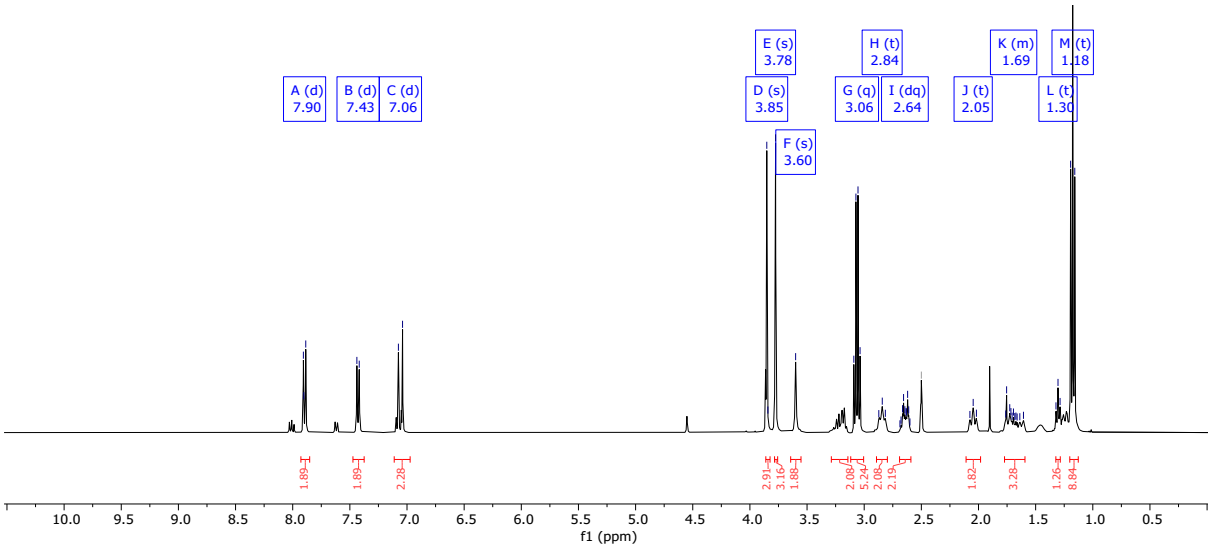
4-((4-((5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidin-1-yl)methyl) benzoic acid

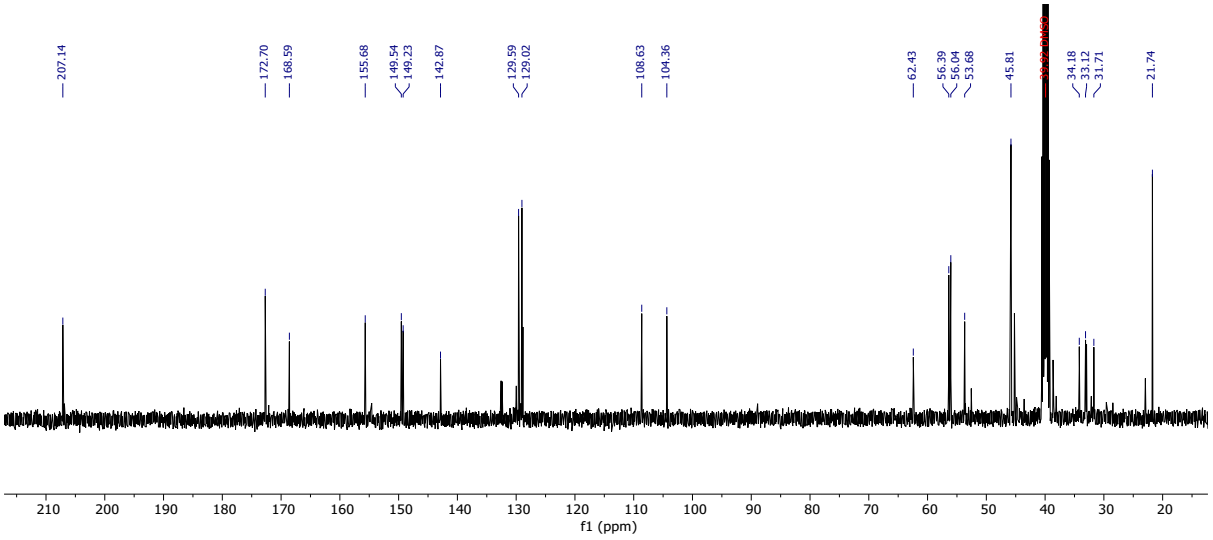
Obtained through the reaction of **1** with 4-(bromomethyl)benzoic acid and subsequent extraction with 0.1 M NH_3, yellow solid (54 %)

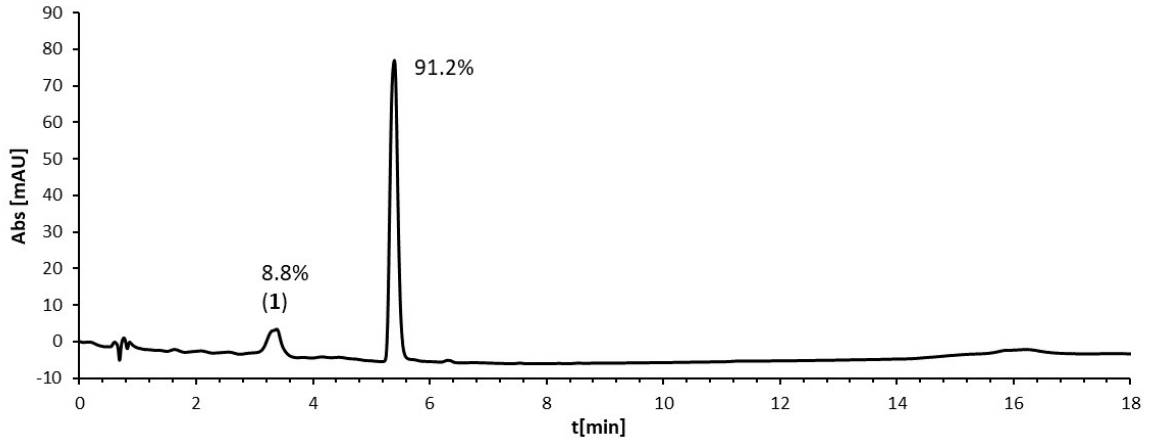
^{1}H NMR (400 MHz, DMSO) δ 7.90 (d, J = 8.3 Hz, 2H), 7.43 (d, J = 8.3 Hz, 2H), 7.06 (d, J = 14.5 Hz, 2H), 3.85 (s, 3H), 3.78 (s, 3H), 3.60 (s, 2H), 3.27 – 3.15 (m, 2H), 3.06 (q, J = 7.3 Hz, 6H), 2.84 (t, J = 10.8 Hz, 2H), 2.64 (dq, J = 13.5, 4.5 Hz, 2H), 2.05 (t, J = 11.4 Hz, 2H), 1.79 – 1.59 (m, 3H), 1.30 (t, J = 7.2 Hz, 1H), 1.18 (t, J = 7.3 Hz, 9H).

^{13}C NMR (101 MHz, DMSO) δ 207.14, 172.70, 168.59, 155.68, 149.54, 149.23, 142.87, 129.59, 129.02, 108.63, 104.36, 62.43, 56.39, 56.04, 53.68, 45.81, 39.92, 34.18, 33.12, 31.71, 21.74.

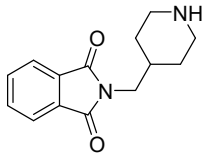
Purity: 91.2%




207.14
172.70
168.59
155.68
149.54
149.23
142.87
129.59
129.02
108.63
104.36
62.43
56.39
56.04
53.68
45.81
34.18
33.12
31.71
21.74
f1 (ppm)


91.2%
8.8%
(1)
Abs [mAU]
t[min]

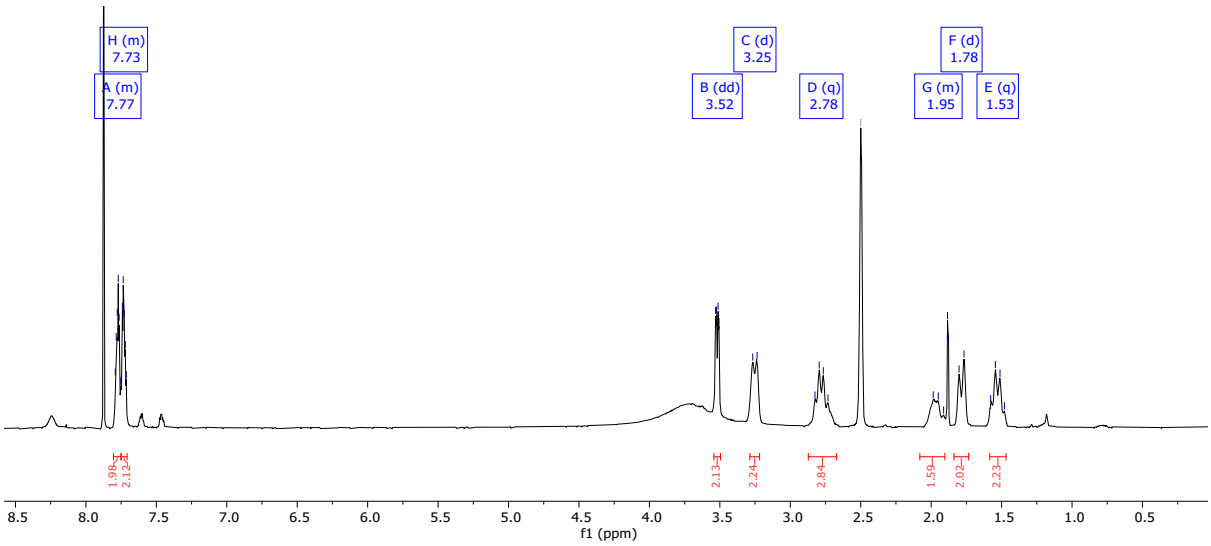
Piperidine 4-methyl phthalimide · HCl (5a)



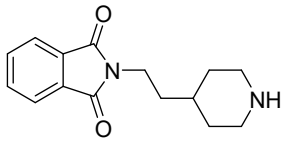
2-(piperidin-4-ylmethyl)isoindoline-1,3-dione

A solution of 150 mg of phthalic anhydride (1.01 mmol) and 4-(aminomethyl)piperidine dissolved in 0.5 ml of acetonitrile was slowly heated to 150° C while stirring. Upon evaporation of the solvent and the reaction mixture reaching the melting point of phthalic anhydride, the obtained orange liquid is kept at 150° C for another hour. After cooling to room temperature, the residue was dissolved in 3 ml of methanol and the solvent was subsequently removed under reduced pressure to obtain the product as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 – 7.75 (m, 2H), 7.75 – 7.71 (m, 2H), 3.52 (dd, J = 7.0, 2.7 Hz, 2H), 3.25 (d, J = 12.2 Hz, 2H), 2.78 (q, J = 12.6 Hz, 3H), 2.08 – 1.90 (m, 2H), 1.78 (d, J = 14.0 Hz, 2H), 1.53 (q, J = 13.2 Hz, 2H).



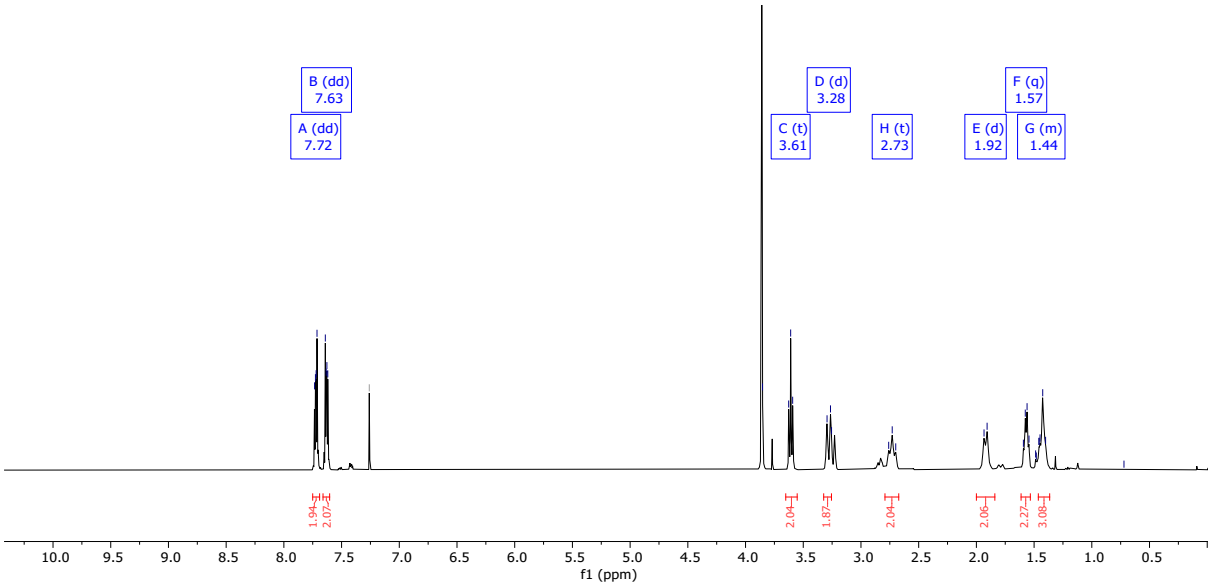
Piperidine 4-ethyl phthalimide · HCl (5b)



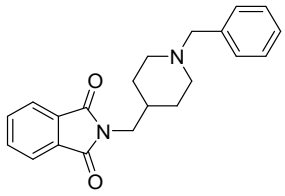
2-(2-(piperidin-4-yl)ethyl)isoindoline-1,3-dione

According to the protocol outlined in 5a using 4-(aminoethyl)piperidine

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.63 (dd, *J* = 5.5, 3.0 Hz, 2H), 3.61 (t, *J* = 6.9 Hz, 2H), 3.28 (d, *J* = 12.0 Hz, 2H), 2.73 (t, *J* = 12.2 Hz, 2H), 1.92 (d, *J* = 10.6 Hz, 2H), 1.57 (q, *J* = 6.6 Hz, 2H), 1.46 – 1.36 (m, 3H).



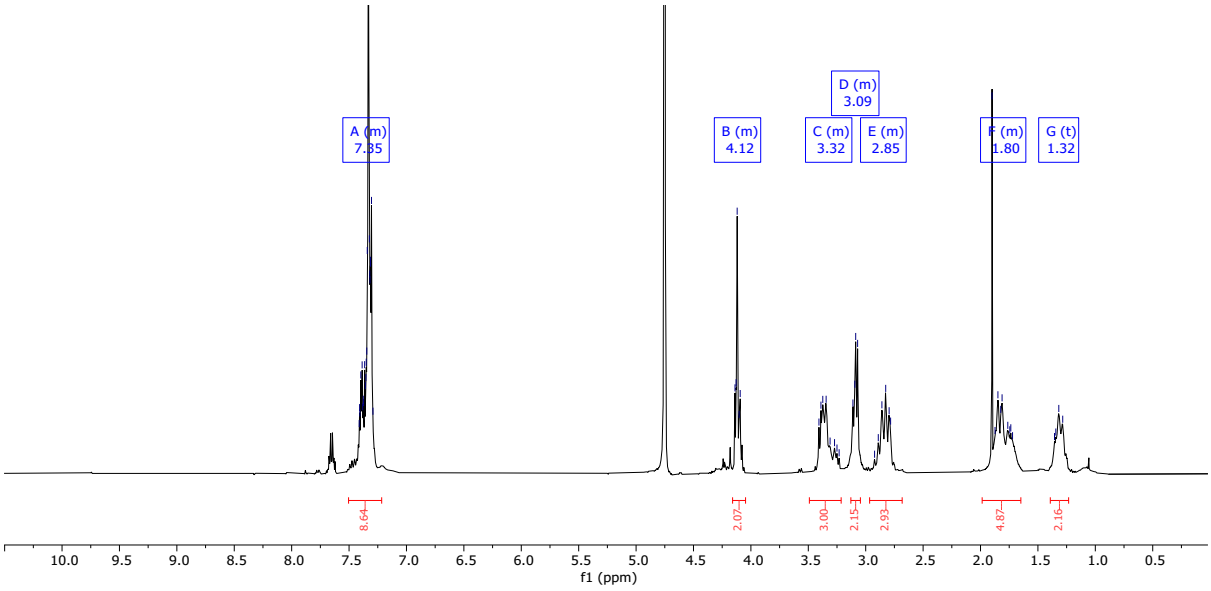
Benzyl-piperidine 4-methyl phthalimide · HCl (6a)



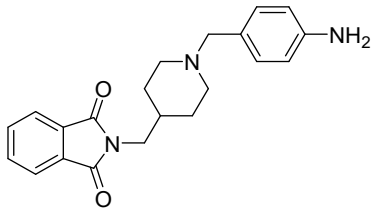
2-((1-benzylpiperidin-4-yl)methyl)isoindoline-1,3-dione

Obtained through the reaction of **5a** with benzyl bromide, colorless solid (64 %)

^{1}H NMR (400 MHz, D_2O) δ 7.50 – 7.22 (m, 9H), 4.19 – 4.05 (m, 2H), 3.54 – 3.23 (m, 3H), 3.14 – 2.99 (m, 2H), 2.85 (m, 3H), 1.98 – 1.64 (m, 5H), 1.32 (t, J = 14.3 Hz, 2H)



4-Amino benzyl-piperidine 4-methyl phthalimide · 2 HCl (6b)

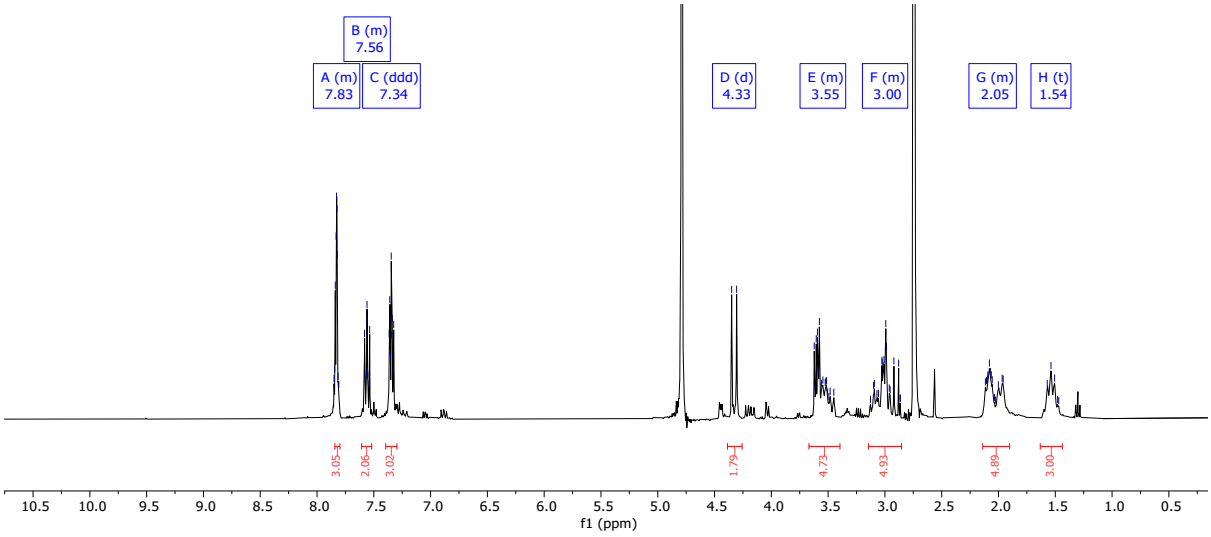


2-((1-(4-aminobenzyl)piperidin-4-yl)methyl)isoindoline-1,3-dione

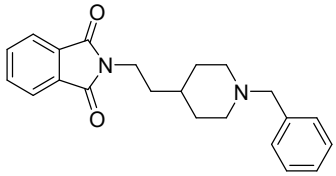
Obtained through the reaction of **5a** with *N*-Boc-4-aminobenzyl chloride and subsequent boc-deprotection, orange solid (54 %)

^{1}H NMR (400 MHz, D_2O) δ 7.94 – 7.77 (m, 3H), 7.61 – 7.52 (m, 2H), 7.34 (ddd, *J* = 8.6, 4.8, 2.2 Hz, 2H), 4.33 (d, *J* = 17.6 Hz, 2H), 3.71 – 3.38 (m, 5H), 3.21 – 2.84 (m, 5H), 2.19 – 1.93 (m, 5H), 1.54 (t, *J* = 13.0 Hz, 3H).

ESI [M+H]: 350.1863, found: 350.1863



Benzyl-piperidine 4-ethyl phthalimide · HCl (6c)



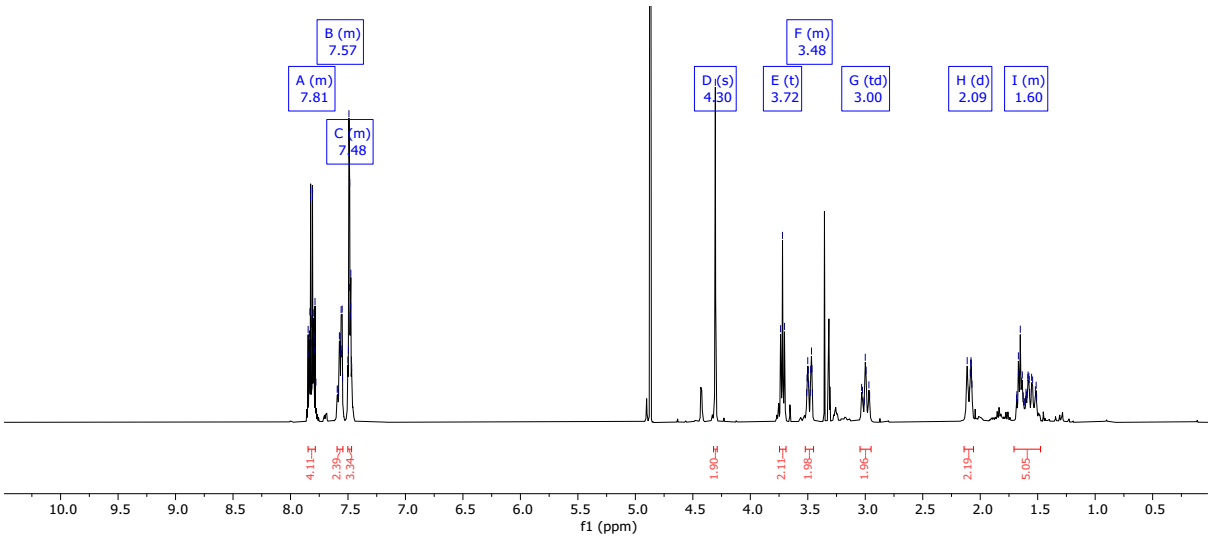
2-(2-(1-benzylpiperidin-4-yl)ethyl)isoindoline-1,3-dione

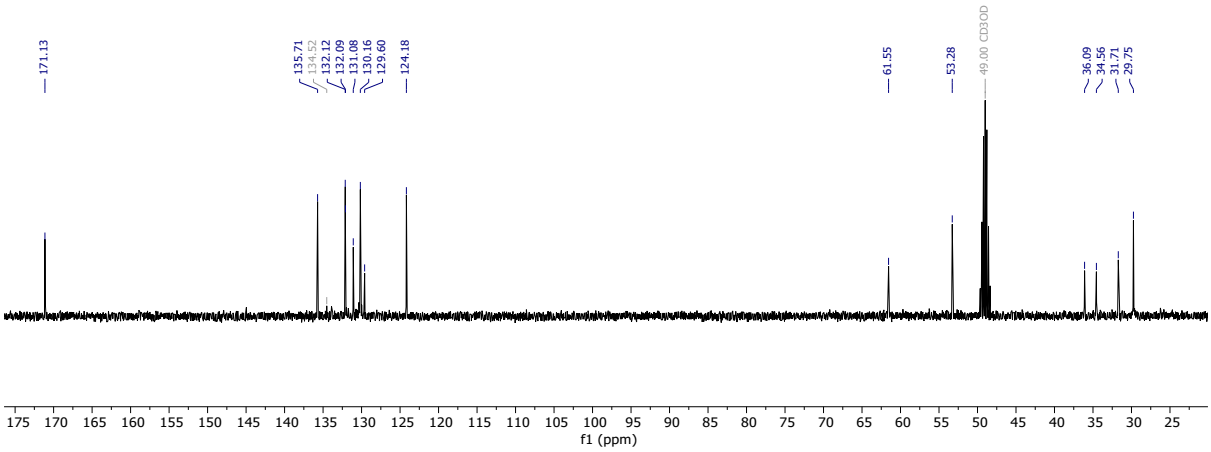
Obtained through the reaction of **5b** with benzyl bromide, colorless solid (86 %)

^{1}H NMR (400 MHz, MeOD) δ 7.94 – 7.75 (m, 4H), 7.64 – 7.54 (m, 2H), 7.51 – 7.45 (m, 3H), 4.30 (s, 2H), 3.72 (t, *J* = 6.8 Hz, 2H), 3.55 – 3.46 (m, 2H), 3.00 (td, *J* = 12.6, 2.8 Hz, 2H), 2.09 (d, *J* = 13.5 Hz, 2H), 1.73 – 1.47 (m, 5H).

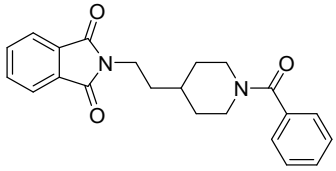
^{13}C NMR (101 MHz, MeOD) *δ* 171.13, 135.71, 134.52, 132.12, 132.09, 131.08, 130.16, 129.60, 124.18, 61.55, 53.28, 36.09, 34.56, 31.71, 29.75.

ESI [M+H]: 349.1911, found: 349.1911





Benzoyl-piperidine 4-ethyl phthalimide (6d)



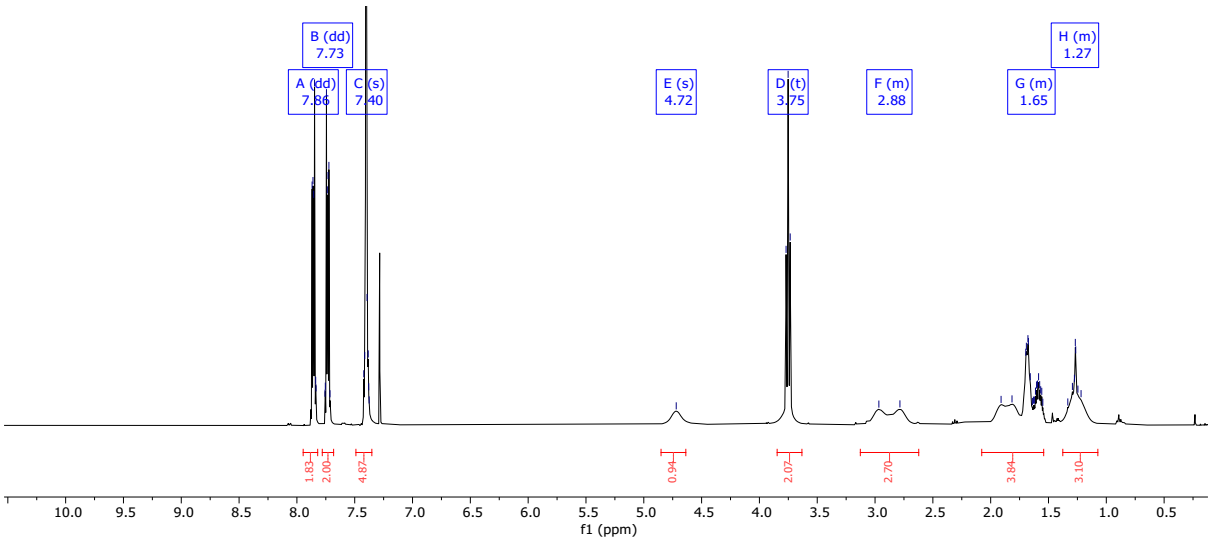
2-(2-(1-benzoylpiperidin-4-yl)ethyl)isoindoline-1,3-dione

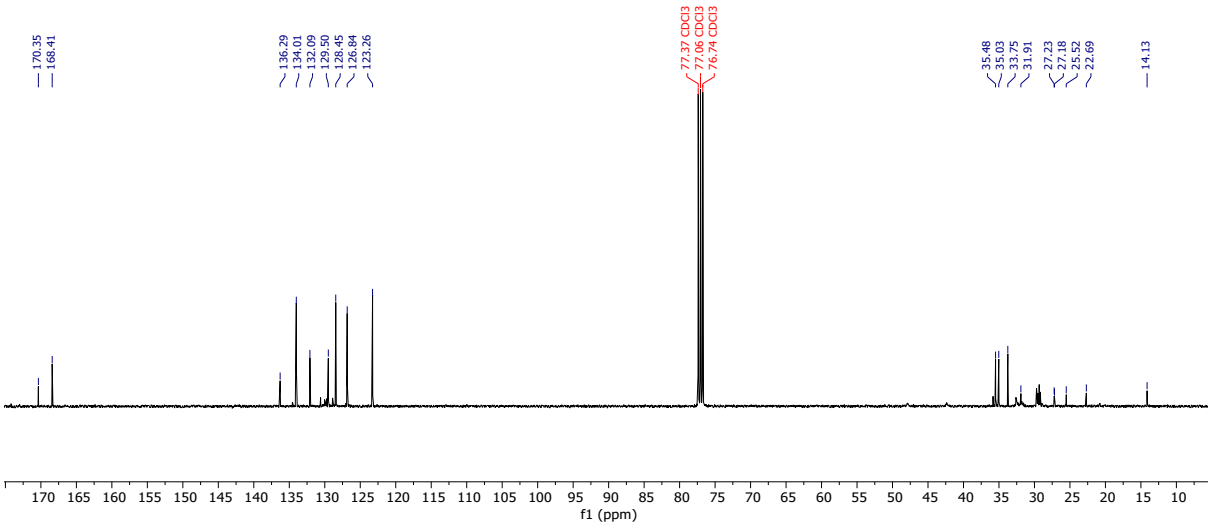
Obtained through the reaction of **5b** with benzoyl chloride, yellow solid (85 %)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.73 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.40 (s, 5H), 4.72 (s, 1H), 3.75 (t, *J* = 7.1 Hz, 2H), 3.13 – 2.62 (m, 3H), 2.08 – 1.54 (m, 4H), 1.38 – 1.07 (m, 3H).

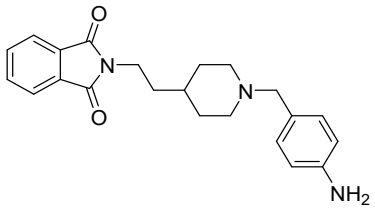
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.35, 168.41, 136.29, 134.01, 132.09, 129.50, 128.45, 126.84, 123.26, 77.37, 77.06, 76.74, 35.48, 35.03, 33.75, 31.91, 27.23, 27.18, 25.52, 22.69, 14.13.

ESI [M+Na]: 385.1523, found: 385.1523





Phth-Et-Bn-4-amine · 2 HCl (6e)



2-(2-(1-(4-aminobenzyl)piperidin-4-yl)ethyl)isoindoline-1,3-dione

Obtained through the reaction of **5b** with *N*-Boc-4-aminobenzyl chloride and subsequent boc-deprotection, yellow solid (86 %)

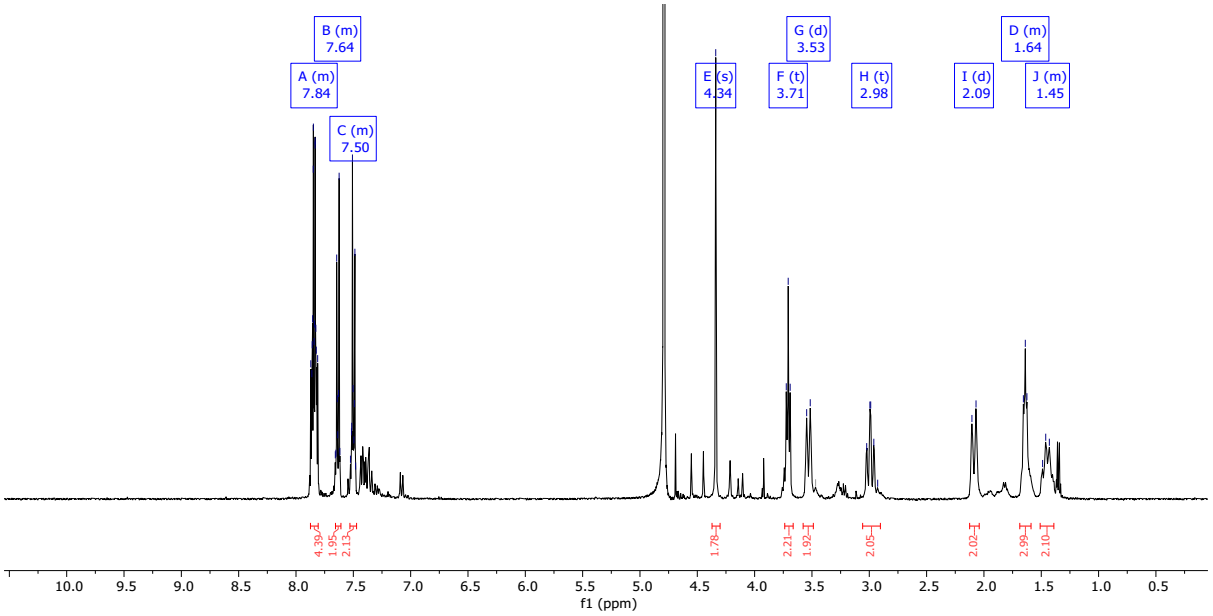
^{1}H NMR (400 MHz, D_2O) δ 7.87 – 7.80 (m, 4H), 7.66 – 7.61 (m, 2H), 7.53 – 7.47 (m, 2H), 4.34 (s, 2H), 3.71 (t, *J* = 6.8 Hz, 2H), 3.53 (d, *J* = 12.5 Hz, 2H), 2.98 (t, *J* = 13.2 Hz, 2H), 2.09 (d, *J* = 14.3 Hz, 2H), 1.68 – 1.60 (m, 3H), 1.48 – 1.41 (m, 2H)

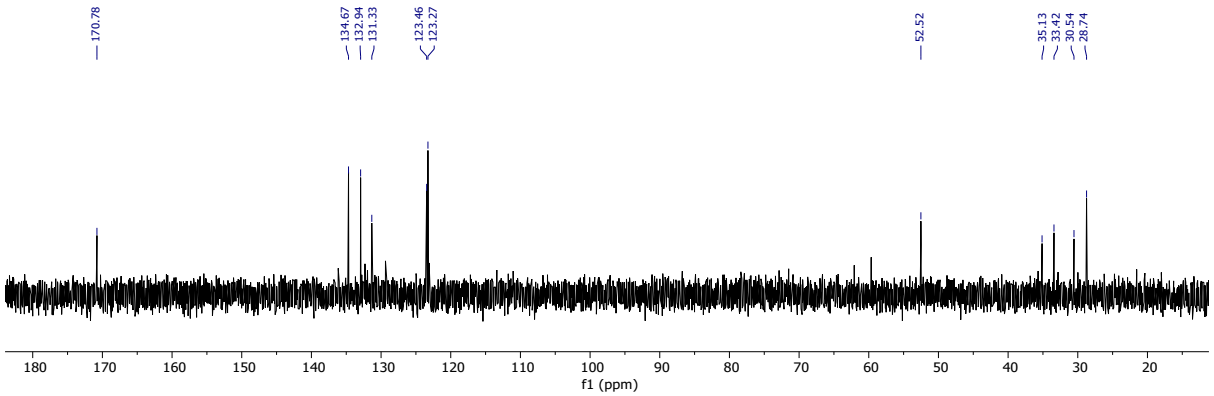
^{13}C NMR (101 MHz, D_2O) δ 170.78, 134.67, 132.94, 131.33, 123.46, 123.27, 52.52, 35.13, 33.42, 30.54, 28.74.

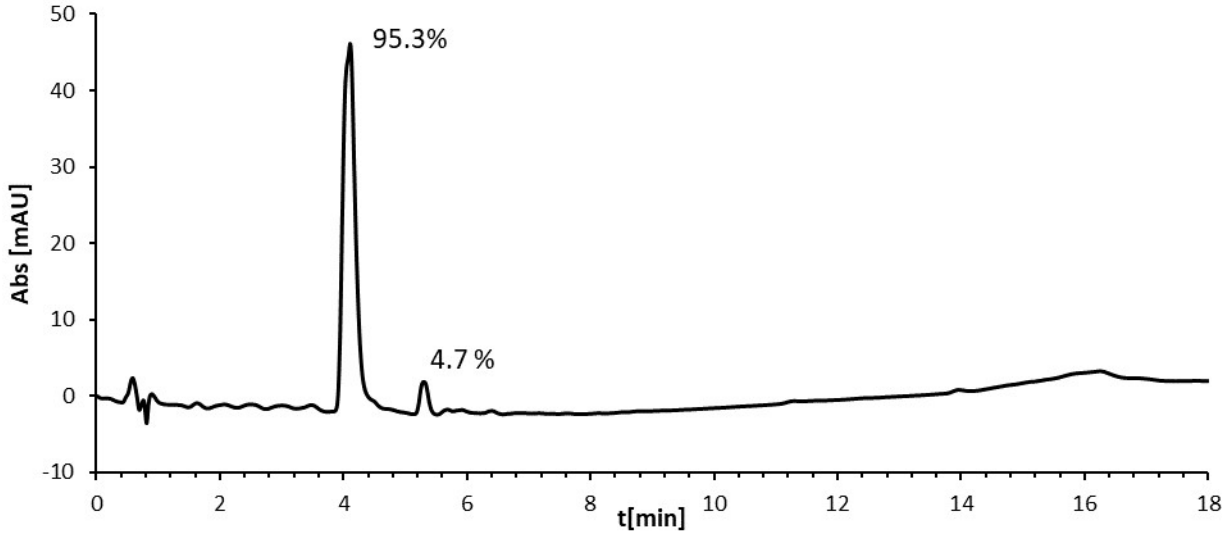
ESI [M+H]: 364.2020, found: 364.2020

Obtained through the reaction of **5b** with *N*-Boc-4-aminobenzyl chloride and subsequent boc-deprotection, yellow solid (86 %)

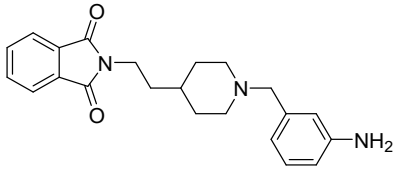
Purity: 95.3%




170.78
134.67
132.94
131.33
123.46
123.27
52.52
35.13
33.42
30.54
28.74
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


95.3%
4.7 %
Abs [mAU]
t[min]
50
40
30
20
10
0
-10
0
2
4
6
8
10
12
14
16
18

Phth-Et-Bn-3-amine · 2 HCl (6f)



2-(2-(1-(3-aminobenzyl)piperidin-4-yl)ethyl)isoindoline-1,3-dione

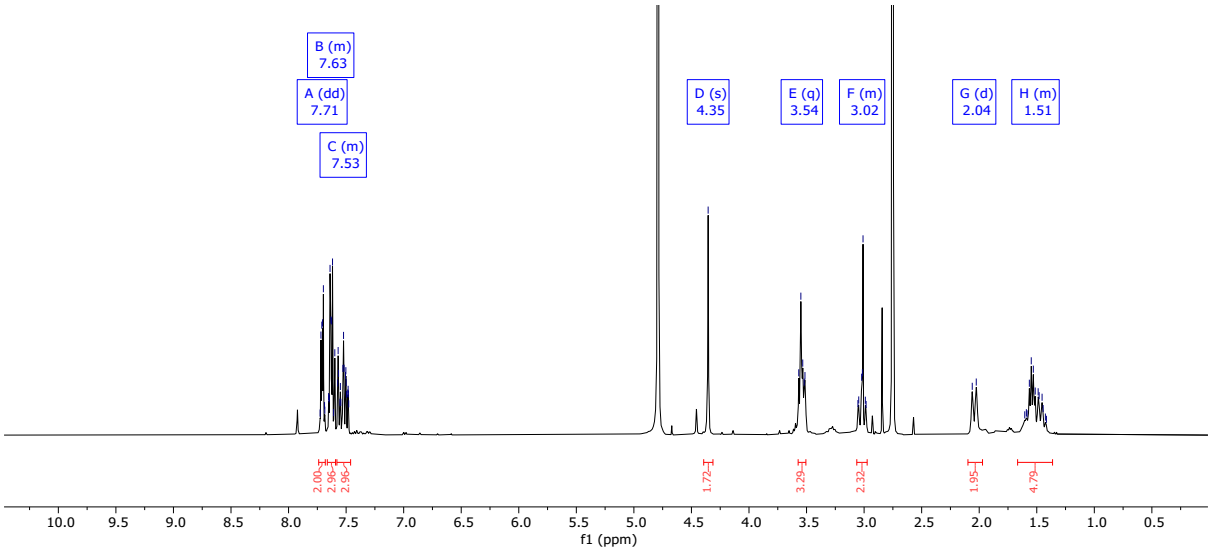
Obtained through the reaction of **5b** with *N*-Boc-3-aminobenzyl chloride and subsequent boc-deprotection, orange solid (74 %)

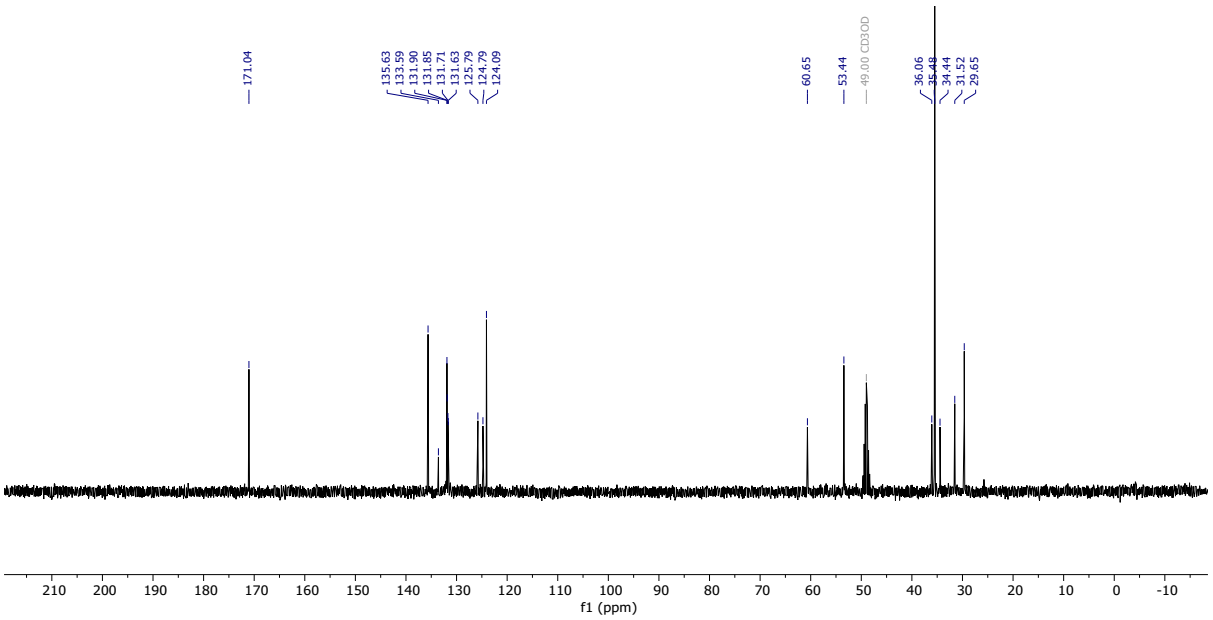
^{1}H NMR (400 MHz, D_2O) δ 7.71 (dd, J = 5.5, 3.0 Hz, 2H), 7.66 – 7.61 (m, 3H), 7.61 – 7.46 (m, 3H), 4.35 (s, 2H), 3.54 (q, J =7.6 Hz, 3H), 3.09 – 2.96 (m, 2H), 2.04 (d, J =14.0 Hz, 2H), 1.70 – 1.35 (m, 5H).

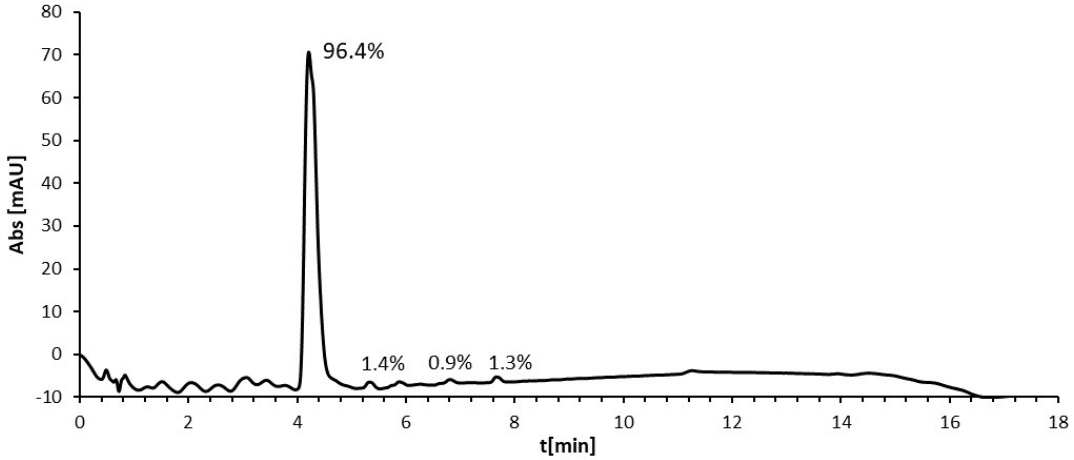
^{13}C NMR (101 MHz, D_2O) δ 171.04, 135.63, 133.59, 131.90, 131.85, 131.63, 125.79, 124.79, 124.09, 60.65, 53.44, 36.06, 35.48, 34.44, 31.52, 29.65.

ESI [M+H]: 364.2020, found: 364.2020

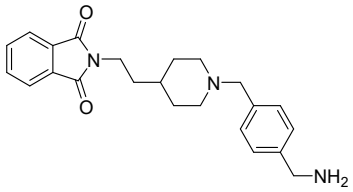
Purity: 96.4%




171.04
135.63
133.59
131.90
131.85
131.71
131.63
125.79
124.79
124.09
60.65
53.44
49.00 CD3OD
36.06
34.48
34.44
31.52
29.65
f1 (ppm)


96.4%
1.4%
0.9%
1.3%
Abs [mAU]
t[min]

Phth-Et-Bn-4-methylamine · 2 HCl (6g)



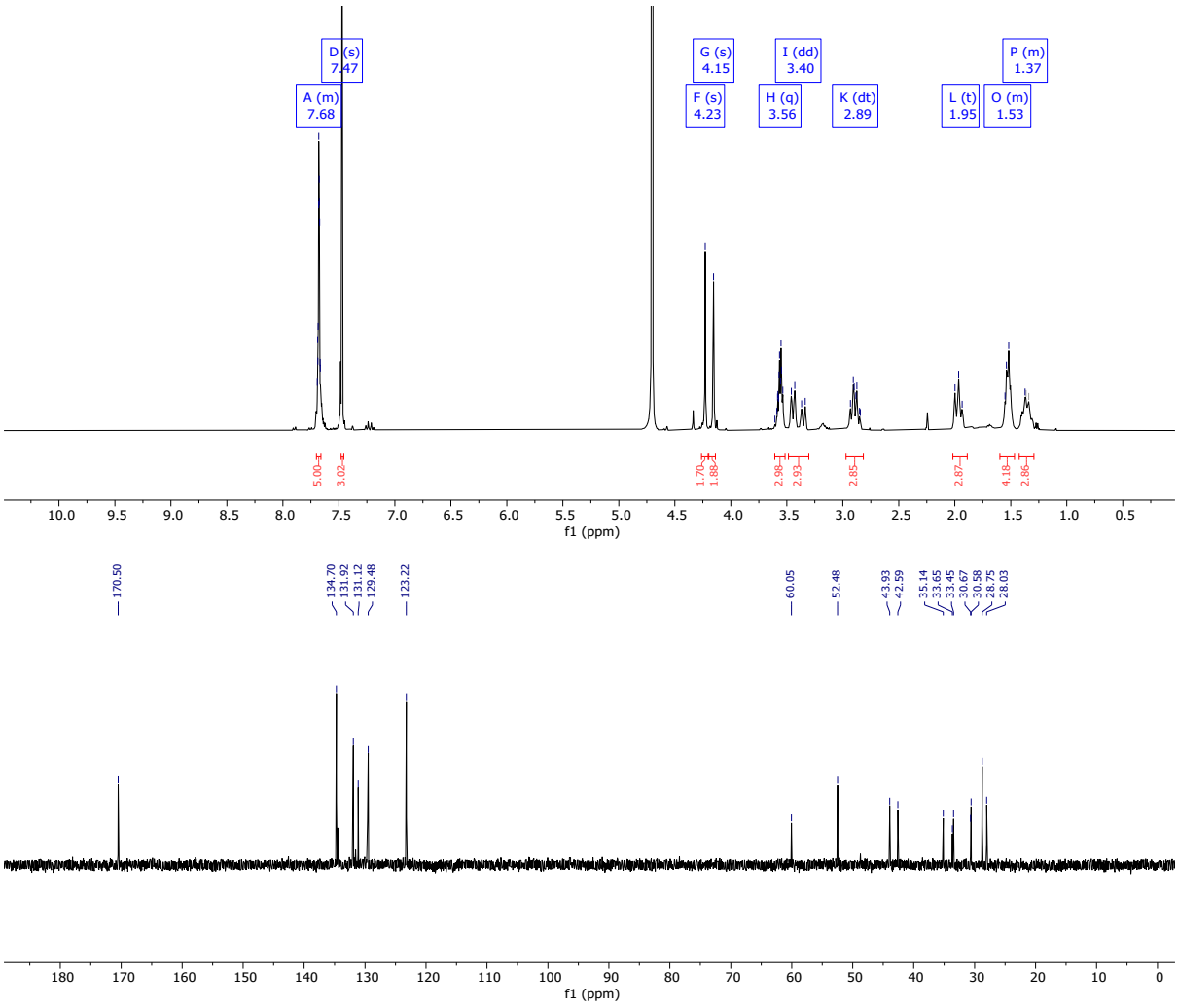
2-(2-(1-(4-(aminomethyl)benzyl)piperidin-4-yl)ethyl)isoindoline-1,3-dione

Obtained through the reaction of **5b** with 4-cyanobenzyl chloride and subsequent reduction with hydrogen, yellow solid (43 %)

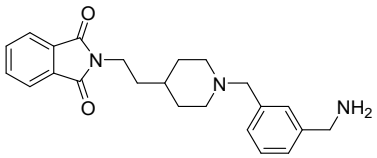
^{1}H NMR (400 MHz, D_2O) δ 7.70 – 7.67 (m, 5H), 7.47 (s, 3H), 4.23 (s, 2H), 4.15 (s, 2H), 3.56 (q, *J* = 2.9 Hz, 3H), 3.40 (dd, *J* = 36.9, 12.6 Hz, 3H), 2.89 (dt, *J* = 15.9, 11.3 Hz, 3H), 1.95 (t, *J* = 12.5 Hz, 3H), 1.57 – 1.48 (m, 4H), 1.39 – 1.35 (m, 3H).

^{13}C NMR (101 MHz, D_2O) δ 170.50, 134.70, 131.92, 131.12, 129.48, 123.22, 60.05, 52.48, 43.93, 42.59, 35.14, 33.65, 33.45, 30.67, 30.58, 28.75, 28.03.

ESI [M+H]: 378.2176, found 378.2176



Phth-Et-Bn-3-methylamine · 2 HCl (6h)

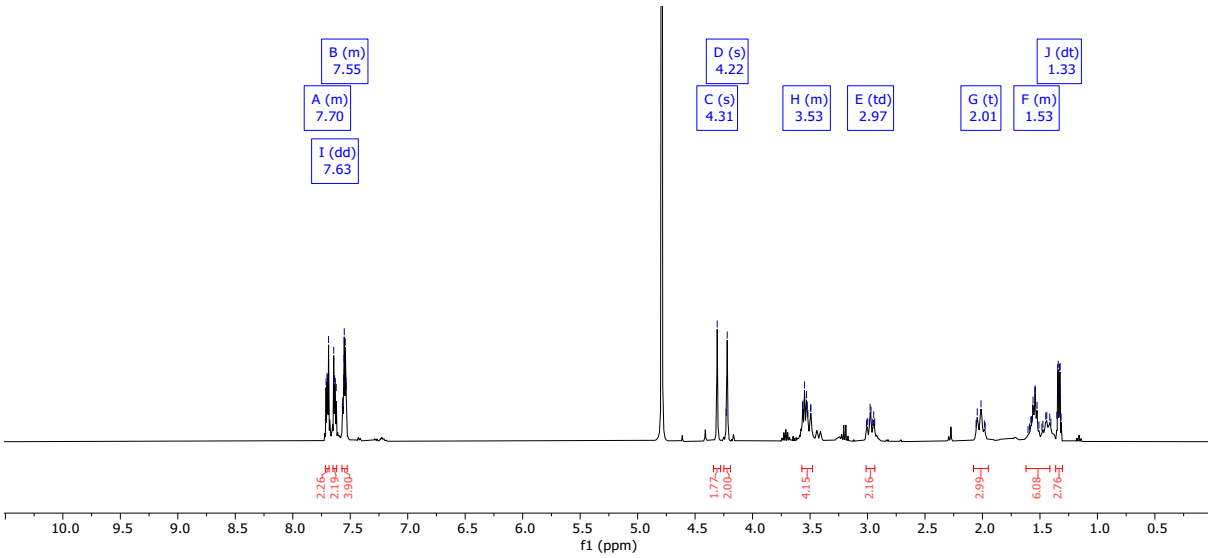


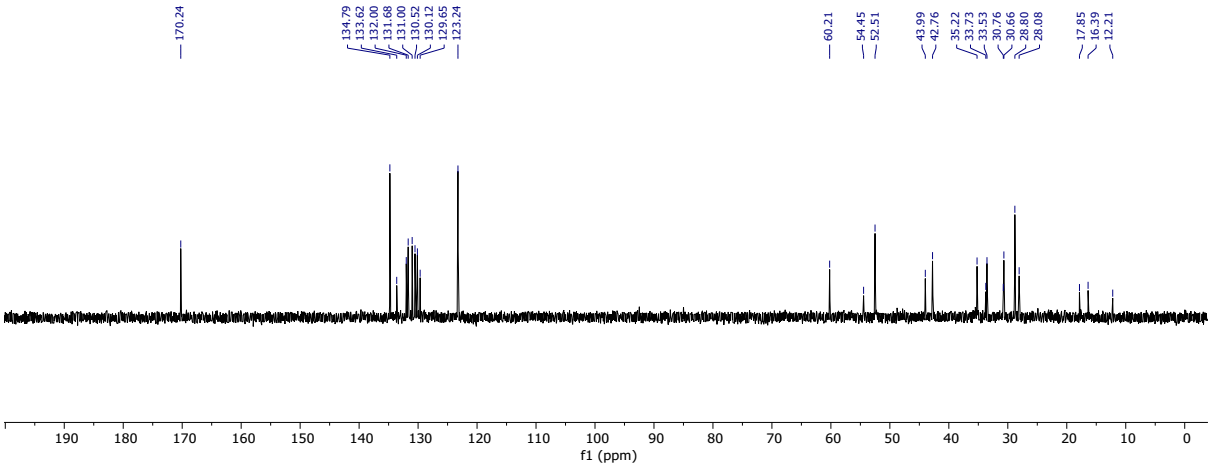
2-(2-(1-(3-(aminomethyl)benzyl)piperidin-4-yl)ethyl)isoindoline-1,3-dione

Obtained through the reaction of **5b** with 3-cyanobenzyl chloride and subsequent reduction with hydrogen, yellow solid (64 %)

^{1}H NMR (400 MHz, D_2O) δ 7.72 – 7.68 (m, 2H), 7.63 (dd, *J* = 5.6, 3.1 Hz, 2H), 7.57 – 7.53 (m, 4H), 4.31 (s, 2H), 4.22 (s, 2H), 3.57 – 3.48 (m, 4H), 2.97 (td, *J* = 12.7, 3.0 Hz, 2H), 2.01 (t, *J* = 12.4 Hz, 3H), 1.62 – 1.41 (m, 6H), 1.33 (dt, *J* = 7.5, 2.6 Hz, 3H).

ESI [M+H]: 378.2175 found, 378.2175





3. Determination of Acetylcholinesterase Activity

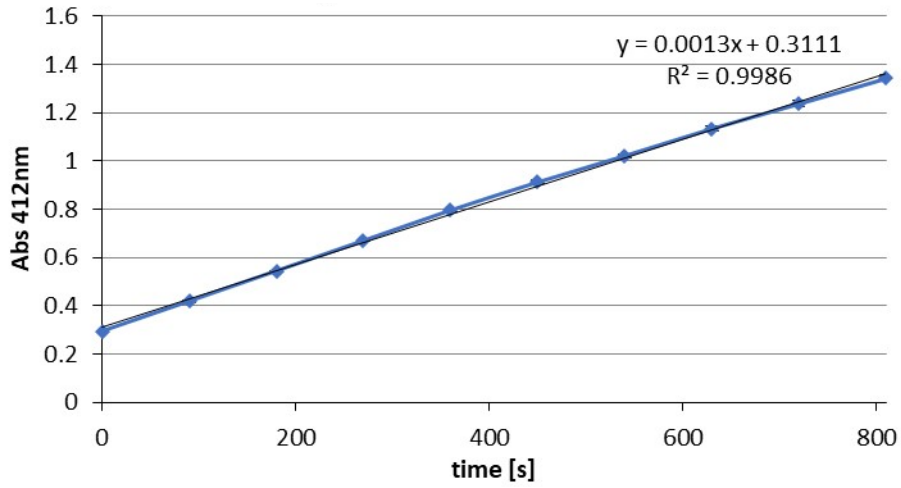


Figure S1: AChE assay in the absence of any inhibitor showing the linearity of the system.

4. Molecular Docking Data

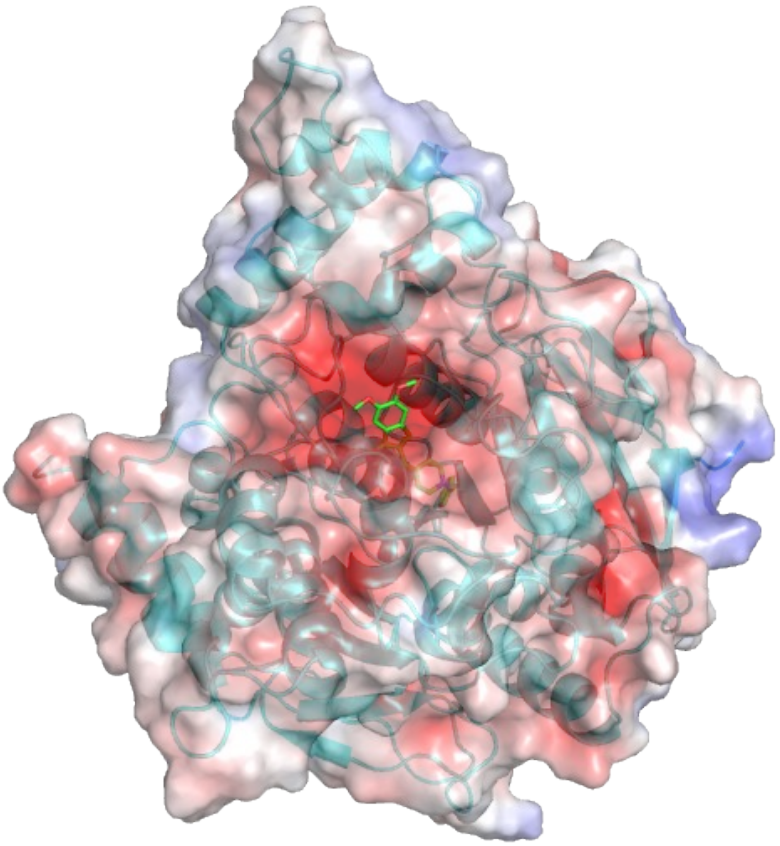


Figure S2: AChE surface coloured by electrostatic potential, red: negative, blue: positive. PDB-ID 6O4W, donepezil depicted with green carbon atoms. The electrostatic potential was calculated using the Adaptive Poisson-Boltzmann Solver (APBS) [5] plugin within PyMOL

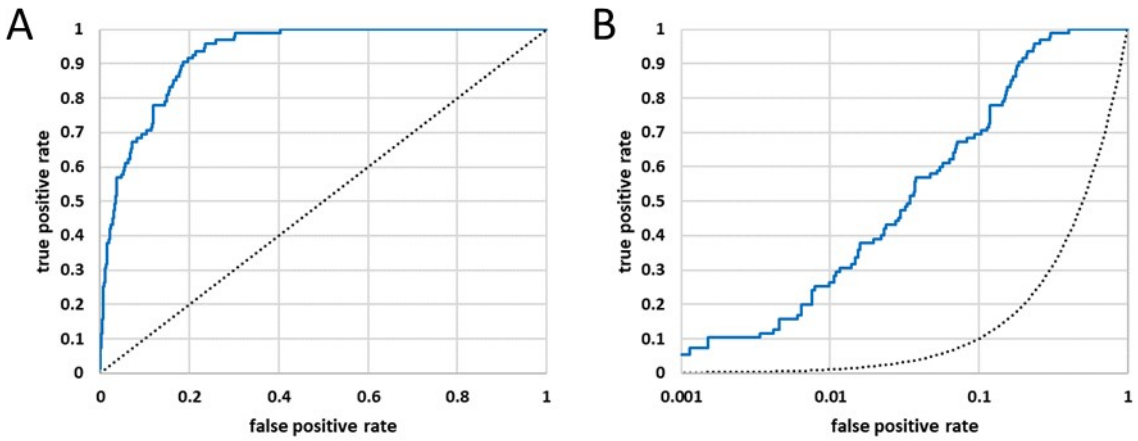


Figure S3: ROC binder-decoy discrimination analysis for 95 binders and 2649 decoys. A) The ROC curve is depicted as a blue line (ROC-AUC = 0.93). Generally, possible AUC-values are between 0 and

1 with 1 describing a perfect discrimination and 0.5 a random distribution as indicated by the dotted line. B) Semilogarithmic ROC to evaluate early enrichment is depicted by the blue line (adjusted ROC $logAUC_{0.1\ 100\%} = 0.37$). Possible adjusted ROC-$logAUC_{0.1\text{-}100\%}$ values are between -14.5 and 0.85 for perfect discrimination. Random distribution is indicated by a dotted line (ROC-$logAUC_{0.1\text{-}100\%} = 0$). The obtained results indicate very strong discrimination of binders from decoys by the scoring function.

Table S1: Physicochemical properties, docking scores and inhibition results. Physicochemical properties were predicted with MOE. Docking was conducted with HYBRID. For racemic ligands only the value for the better scoring enantiomer is shown.

compound	H-bond acceptors	H-bond donors	h_logP	SlogP	logP(o/w)	h_logD	TPSA [Å]	rotatable bonds	pK_a	pK_b	docking score [kcal/mol]	pIC_{50}	IC_{50} [nM]
acotiamide	9	3	2.7	3.0	2.0	0.7	113.0	10	7.5	4.9	-13.3	5.5	3000[6]
1	4	1	2.0	2.4	2.1	-0.9	47.6	6	-	4.0	-12.5	5.0	9450
2a (donepezil)	4	0	3.8	4.6	4.1	1.6	38.8	8	-	4.7	-18.8	8.0	11.0
2b	5	0	3.9	4.0	3.6	3.9	55.8	7	-	-	-17.5	4.7	17,900
2c	5	2	1.8	2.1	1.4	-1.3	64.8	8	-	3.9	-14.2	5.0	11,200
2d	5	2	2.1	2.5	1.9	-2.8	64.8	9	-	3.8	-15.7	5.0	10,800
2e	5	2	2.3	2.9	2.3	-3.2	64.8	10	-	3.7	-16.9	4.8	14,600
2f	5	2	3.1	4.2	3.5	0.8	64.8	8	-	4.4	-18.0	7.0	99.1
2g	5	2	3.2	4.2	3.5	0.8	64.8	8	-	4.5	-17.9	7.3	47.3
2h	5	2	3.4	4.4	3.4	-1.1	64.8	9	-	4.3	-18.6	5.2	5800
2i	5	2	3.4	4.4	3.4	-1.1	64.8	9	-	4.4	-15.6	6.0	1030
2j	6	1	5.1	5.9	5.1	3.0	67.9	10	-	4.7	-8.8	4.6	22,800
2k	6	1	3.3	4.3	3.8	0.9	76.1	9	4.6	4.8	-16.8	4.1	72,200
6a	4	0	3.8	3.5	3.6	1.8	40.6	6	-	4.9	-11.8	4.6	22,800
6b	5	2	3.2	3.0	2.9	0.9	66.6	6	-	4.6	-10.7	4.6	25,200
6c	4	0	4.1	3.9	4.0	1.9	40.6	7	-	4.7	-16.9	7.9	13.2
6d	5	0	4.1	3.2	3.4	4.1	57.7	6	-	-	-18.6	4.2	63,000
6e	5	2	3.4	3.4	3.3	1.0	66.6	7	-	4.4	-17.2	6.5	327
6f	5	2	3.4	3.4	3.4	1.1	66.6	7	-	4.5	-17.1	6.8	150
6g	5	2	3.6	3.6	3.3	-0.9	66.6	8	-	4.4	-19.1	5.8	1535
6h	5	2	3.6	3.6	3.3	-0.8	66.6	8	-	4.4	-18.6	6.5	299.0

5. References

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