Supporting Information

for

Optical Control of Dopamine Receptors Using a Photoswitchable Tethered Inverse Agonist

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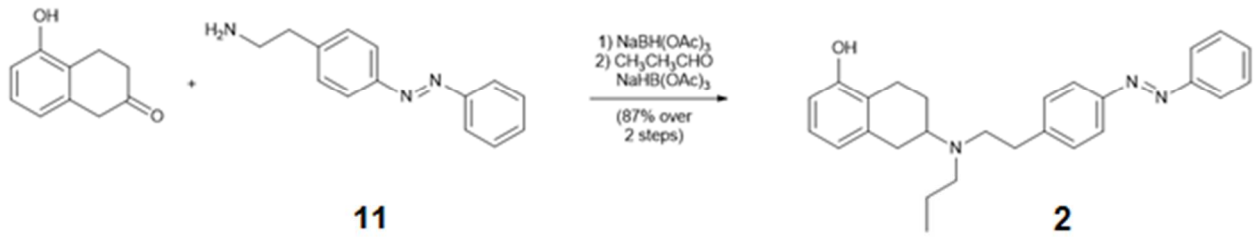
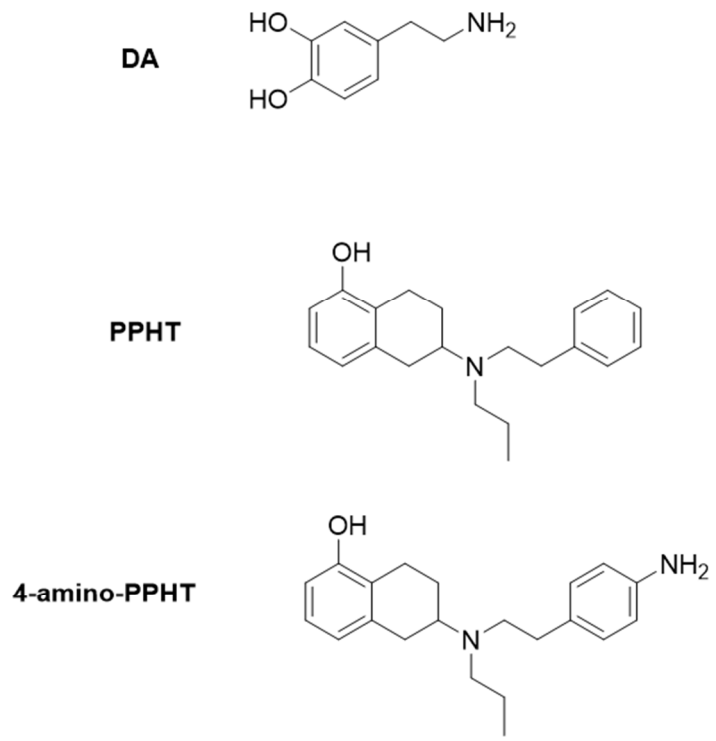
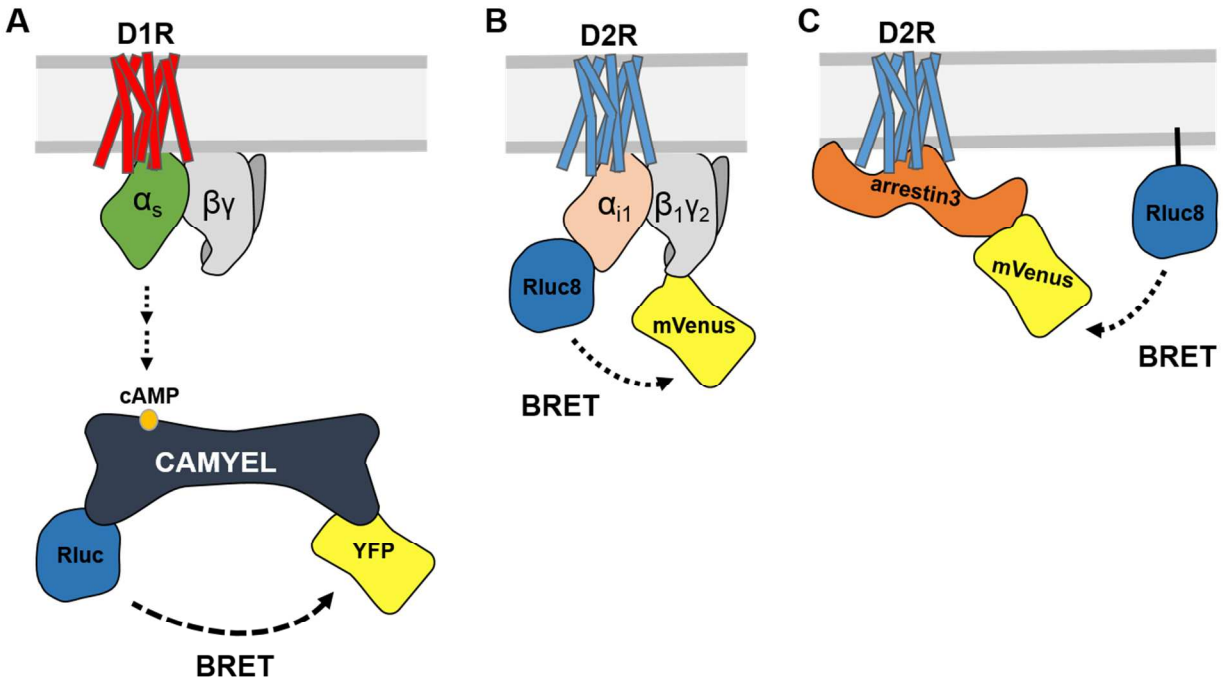


11 2

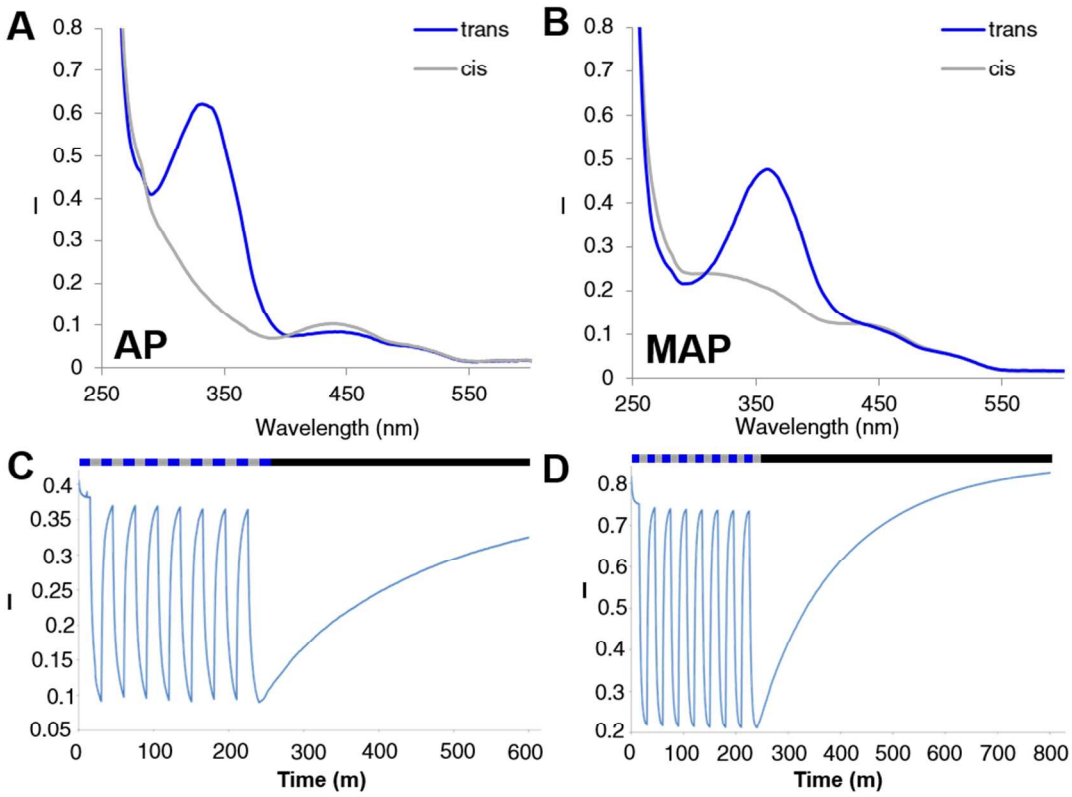
Supplementary Scheme 1. Synthesis of azobenzene-PPHT (AP).



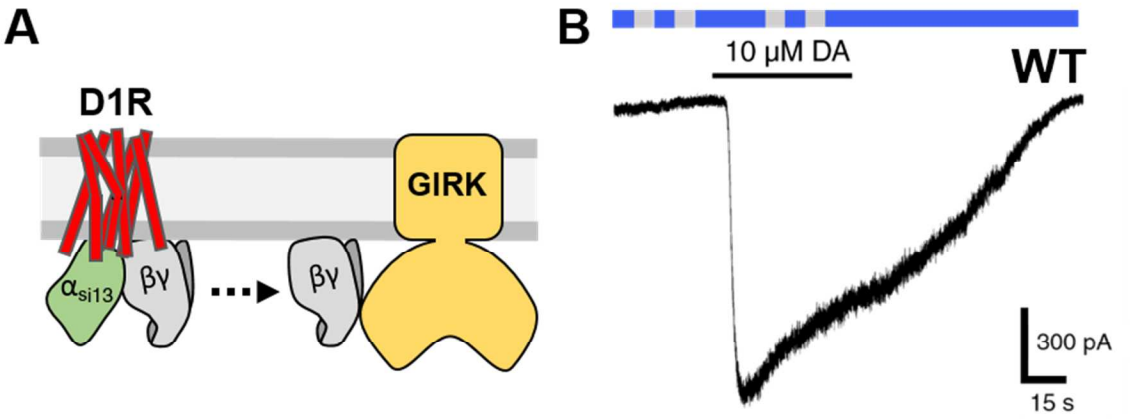
Supplementary Figure 1. Structures of dopamine (DA), PPHT and 4-amino-PPHT.



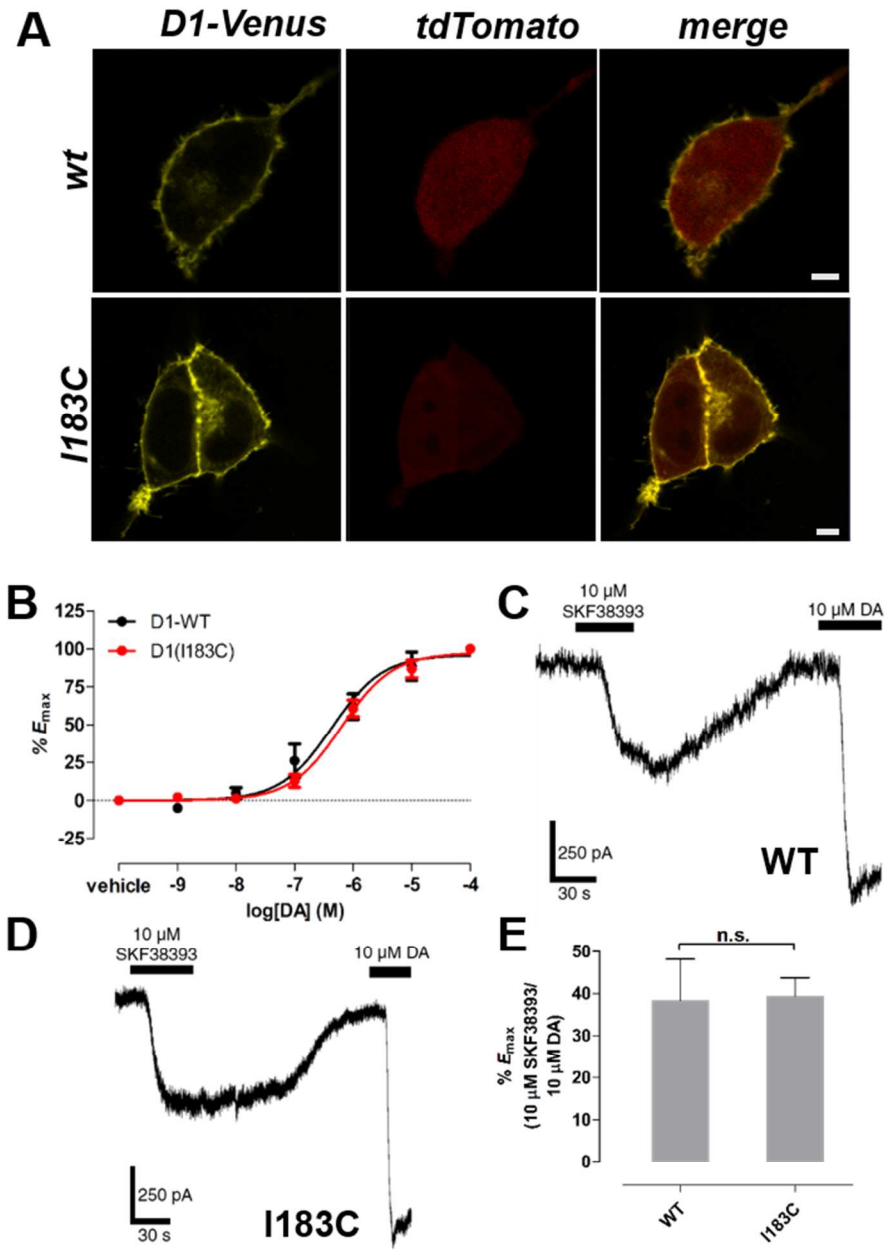
Supplementary Figure 2. Schematic representation of the bioluminescence resonance energy transfer (BRET)-based assays for measuring DAR function. (A) A D1R-mediated cAMP accumulation assay using the Epac-containing CAMYEL sensor. (B) A D2R-mediated G protein activation that measures conformational changes and/or the dissociation of G_{i1} in response to receptor activation. (C) A D2R-mediated arrestin recruitment that measures receptor-mediated arrestin3 translocation to the plasma membrane.



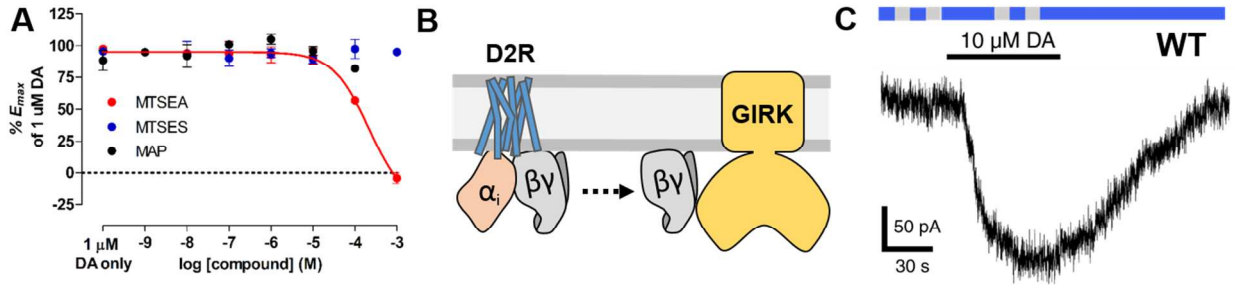
Supplementary Figure 3. Photochemical characterization of AP and MAP. (A) UV/Vis-spectra of the *cis*- and *trans*-isomers of AP (left) and MAP (right). (B) Switching of AP (left) and MAP (right) over several cycles of illumination with 360 and 460 nm light (grey and blue bars, respectively), as well the relaxation of their *cis*- to *trans*-isomers in the dark (black bars).



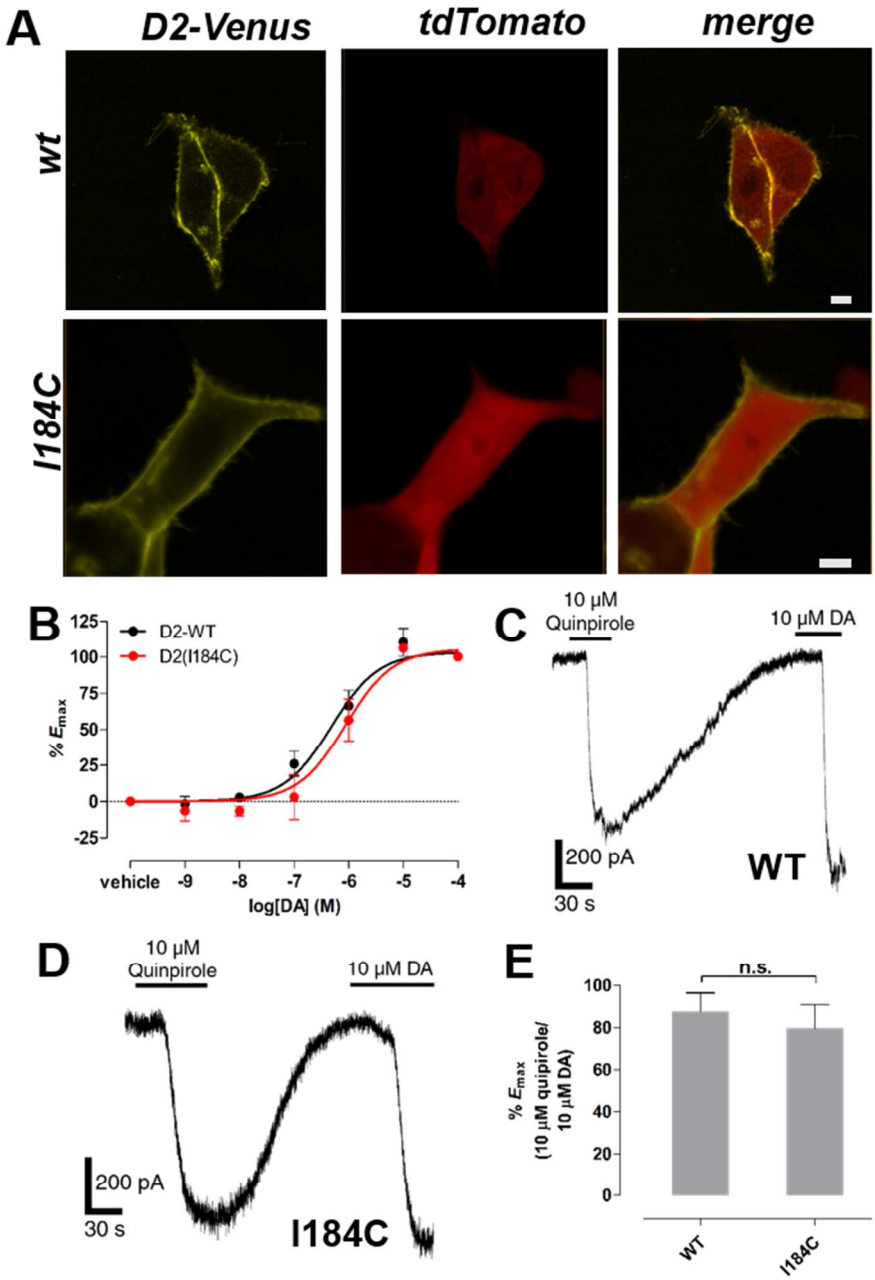
Supplementary Figure 4. MAP has no effect on wildtype D1R. (A) Schematic of the D1R-mediated GIRK activation assay. As a G_s-coupled receptor, D1R cannot activate GIRK channels. However, co-transfection of a chimeric G protein of $G\alpha_{i1}$ and $G\alpha_s$ ($G\alpha_{si13}$) allows D1R to activate GIRK. (B) After labeling and washout, MAP does not photoswitch wildtype D1R in response to 360 nm and 460 nm light in the D1R-mediated GIRK activation assay.



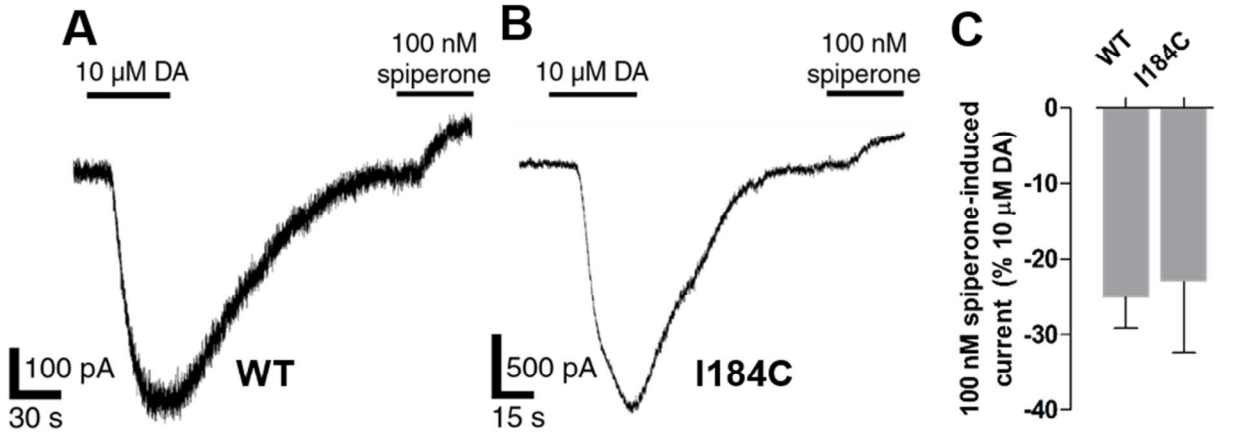
Supplementary Figure 5. Characterization of D1R(I183C). (A) D1R(I183C) expresses at the cell surface of HEK293T cells similar to wildtype D1R. D1Rs were tagged with mVenus at their C-termini. tdTomato was used as cytosolic marker. Bar= 5 µm. (B) DA activates wildtype D1R and D1R(I183C) with similar potency. Error bars represent S.E.M for n=5-7 cells per concentration of DA. (C) The selective D1-like receptor agonist SKF38393 partially activates wildtype D1R in the GIRK activation assay. (D) SKF38393 partially activates D1R(I183C) in the GIRK activation assay. (E) 10 µM SKF38393 similarly activates wildtype D1R and D1R(I183C) relative to 10 µM DA (n.s.= not significant, two-tailed, unpaired t-test). Error bars represent S.E.M for n=3-6 cells per receptor.



Supplementary Figure 6. MAP has no effect on wildtype D2R. (A) MTSEA but not MTSES or MAP react covalently with C118 of D2R, impairing receptor activation by DA in the D2R-mediated G protein activation assay. Error bars represent S.E.M. (B) Schematic of the D2R-mediated GIRK activation assay. (C) After labeling and washout, MAP does not photoswitch wildtype D2R in response to 360 nm and 460 nm light in the D2R-mediated GIRK activation assay.

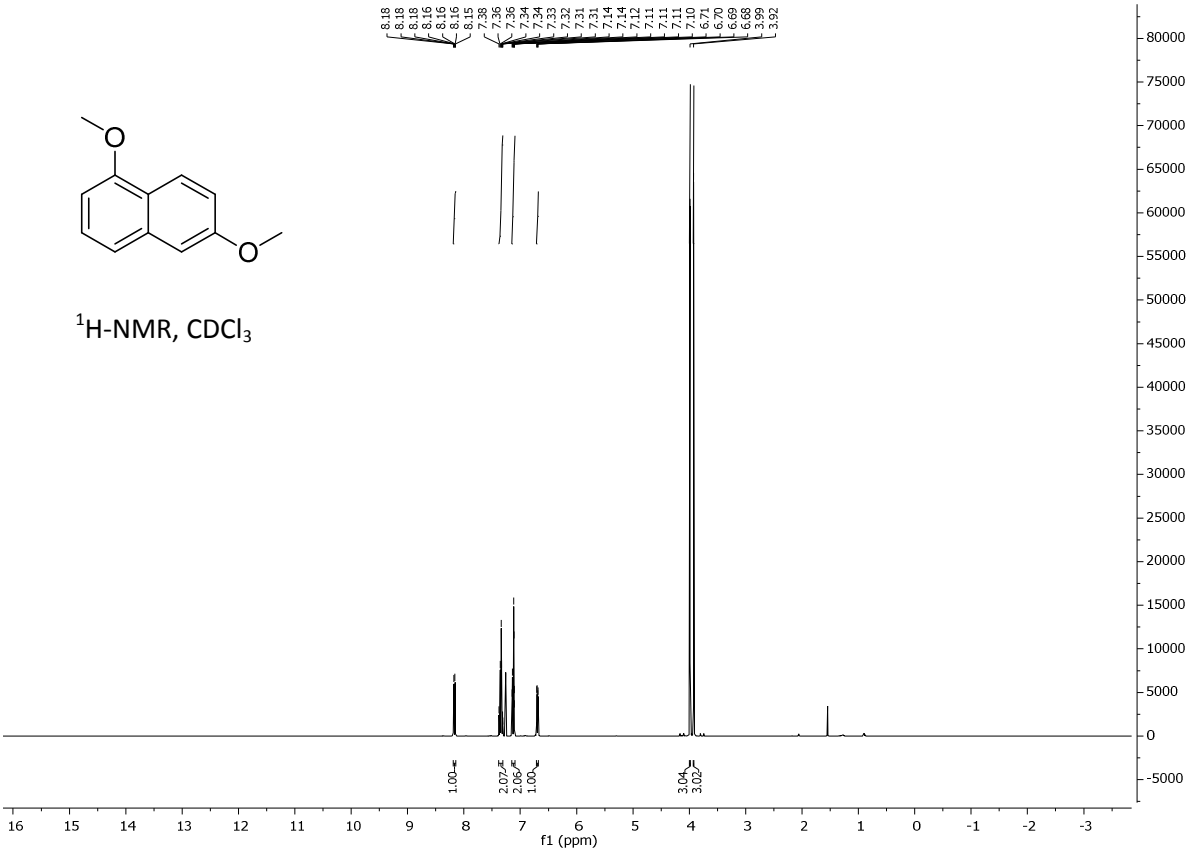


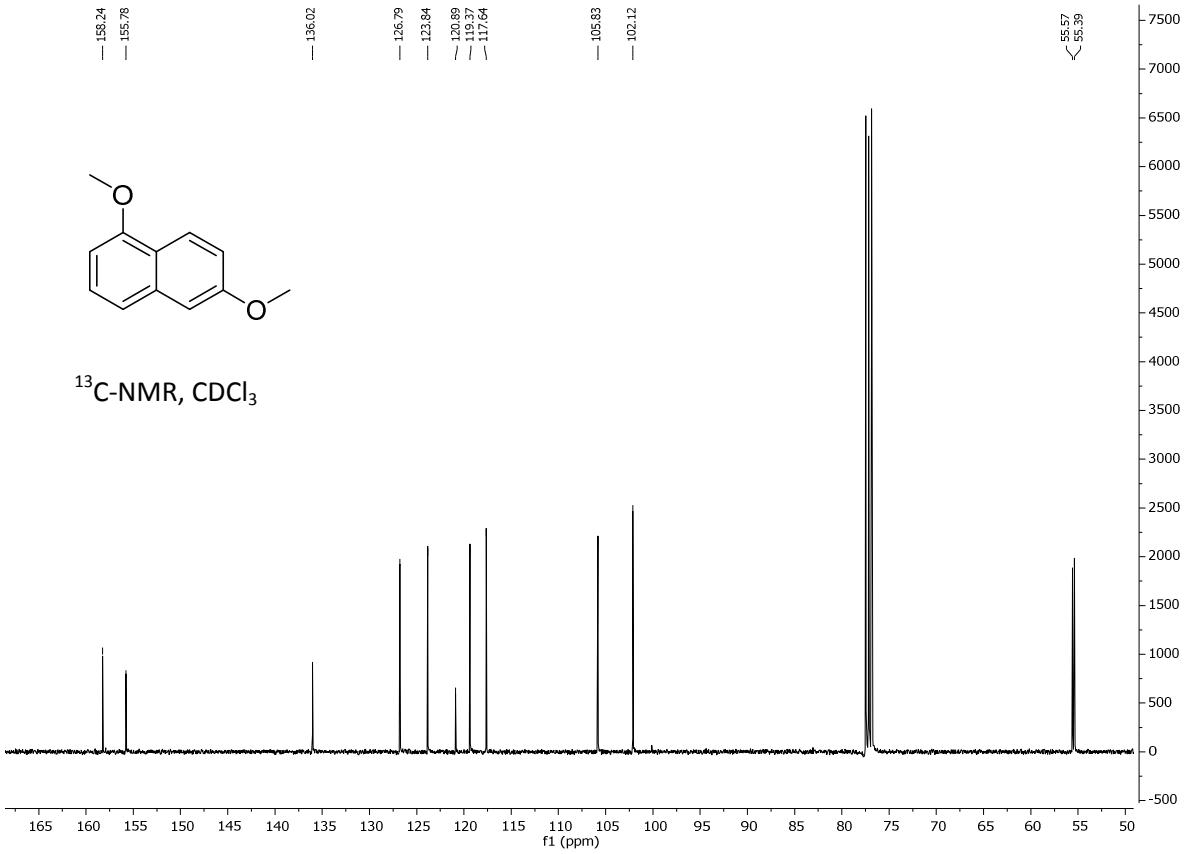
Supplementary Figure 7. Characterization of D2R(I184C). (A) D2R(I184C) expresses at the cell surface of HEK293T cells similar to wildtype D2R. D2Rs were tagged with mVenus at their C-termini. tdTomato was used as cytosolic marker. Bar= 5 µm. (B) DA activates wildtype D2R and D2R(I184C) with similar potency. Error bars represent S.E.M for n=3-8 cells per concentration of DA. (C) The selective D2-like receptor agonist quinpirole activates wildtype D2R in the GIRK activation assay. (D) Quinpirole activates D2R(I184C) in the GIRK activation assay. (E) 10 µM quinpirole similarly activates wildtype D2R and D2R(I184C) relative to 10 µM DA (n.s.= not significant, two-tailed, unpaired t-test). Error bars represent S.E.M for n=3-5 cells per receptor.

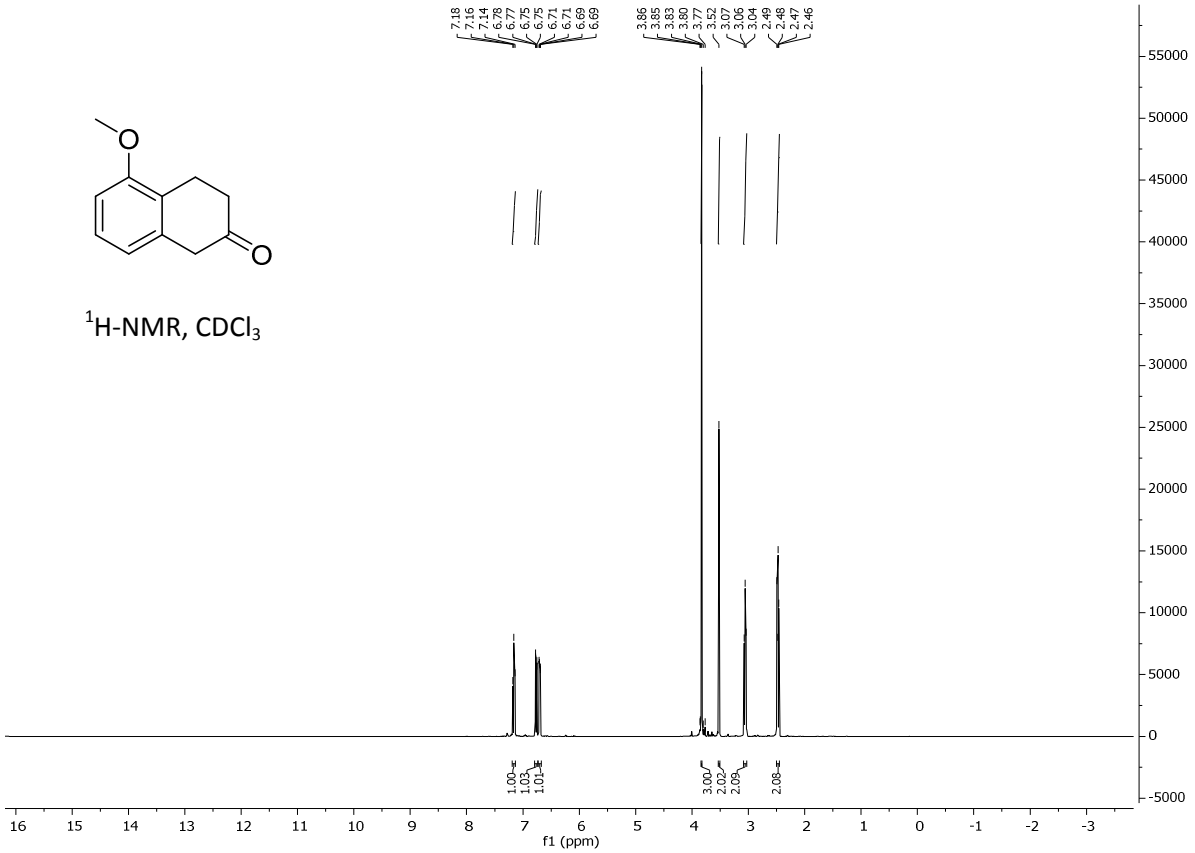


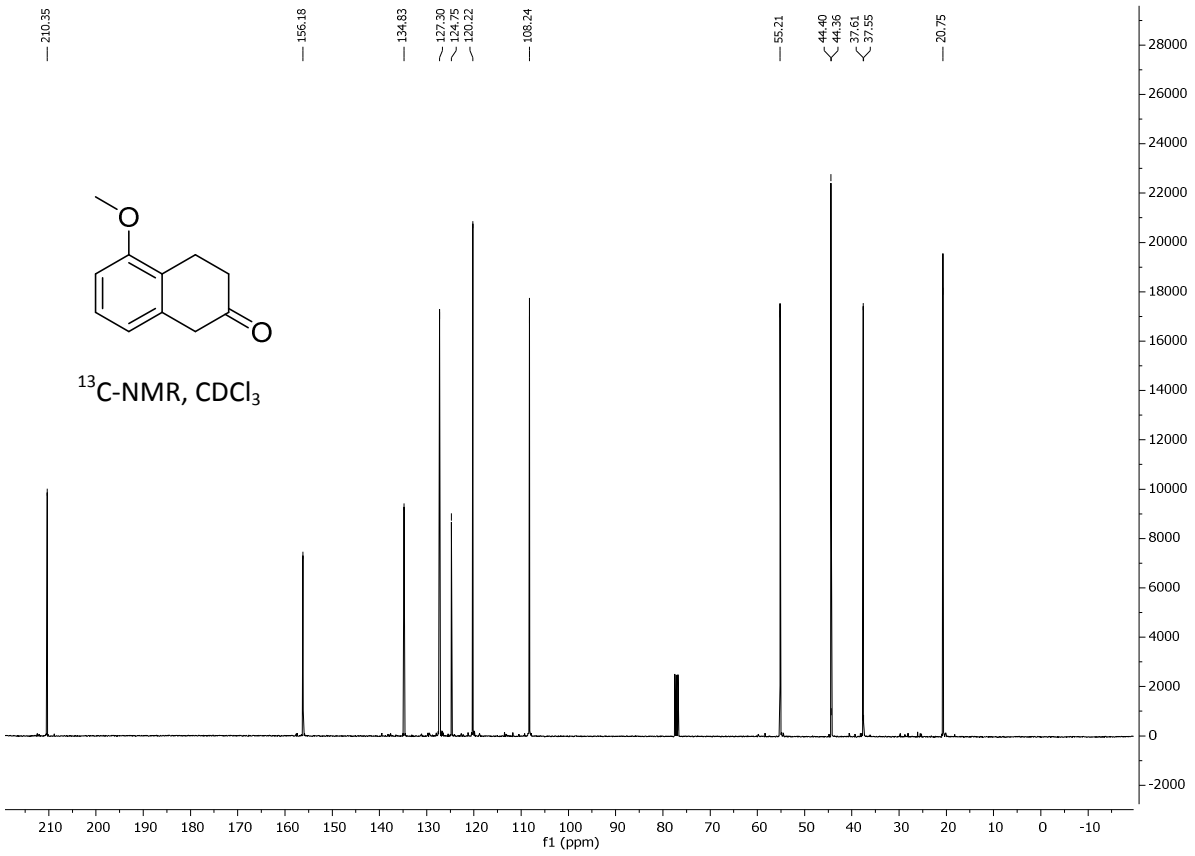
Supplementary Figure 8. D2R(I184C) is constitutively active in the GIRK-activation assay. (A) Basal inward current is reduced in response to spiperone in cells expressing wildtype D2R. (B) Basal inward current is reduced in response to spiperone in cells expressing D2R(I184C). (C) Summary of the effects of spiperone on basal inward current in cells expressing D2R wildtype and I184C. Error bars represent S.E.M for n=3-4 cells per receptor (not significant, one-way ANOVA).

Analytical Spectra




158.24
155.78
136.02
126.79
123.84
120.89
119.37
117.64
105.83
102.12
55.57
55.39
O
O
13C-NMR, CDCl3
f1 (ppm)


7.18
7.16
7.14
6.78
6.77
6.75
6.75
6.71
6.69
6.69
3.86
3.85
3.83
3.80
3.77
3.52
3.07
3.06
3.04
2.49
2.48
2.47
2.46
O
O
1H-NMR, CDCl3
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
1.00
1.03
1.01
3.00
2.02
2.09
2.08
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)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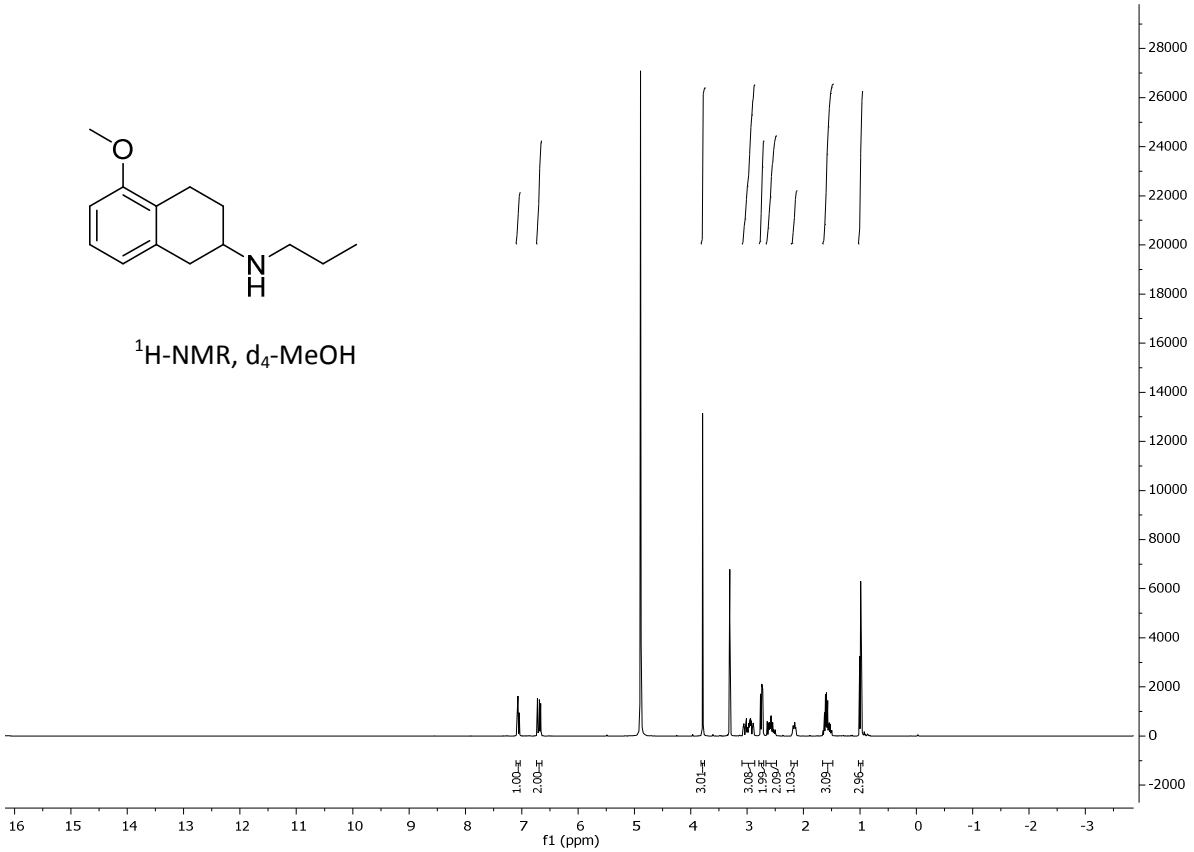
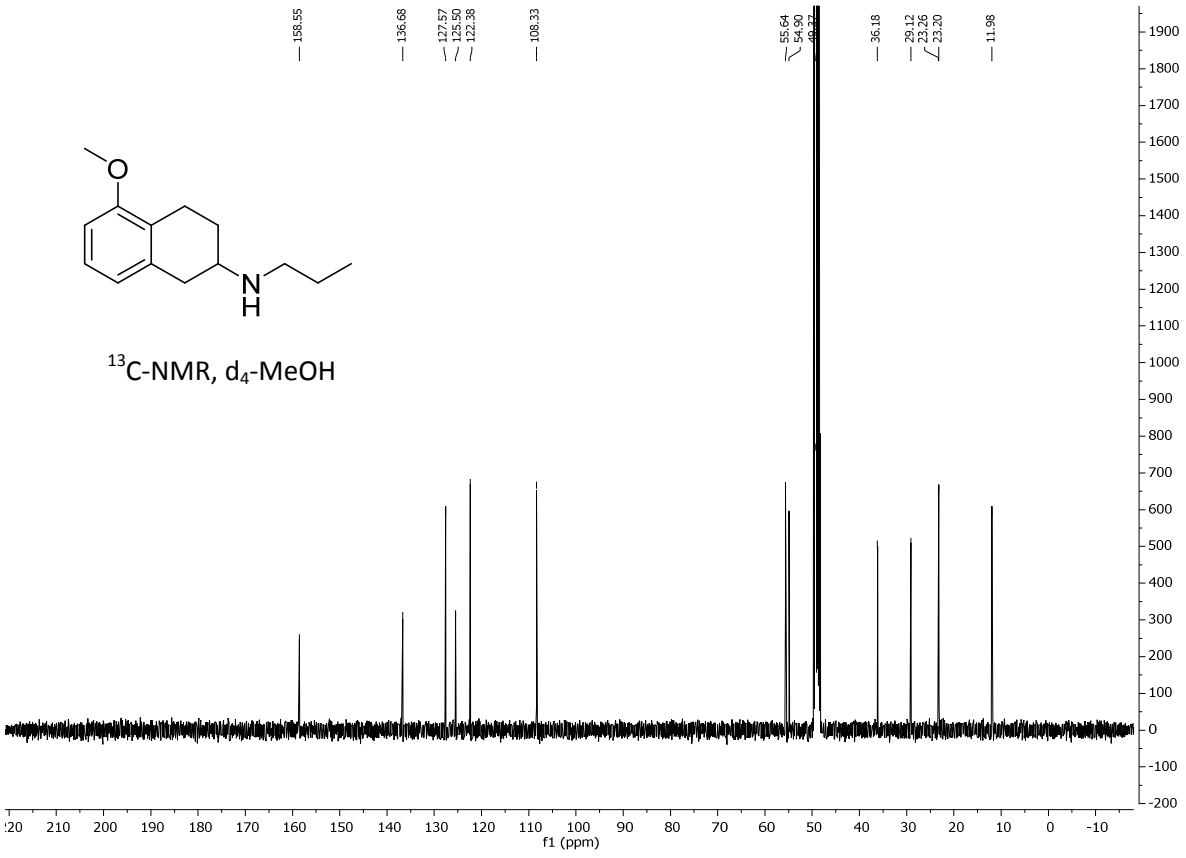
156.18
134.83
127.30
124.75
120.22
108.24
55.21
44.40
44.36
37.61
37.55
20.75
O
O
^{13}C-NMR, $CDCl_3$
f1 (ppm)


O
N
H
1H-NMR, d4-MeOH
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
1.00
2.00
3.01
3.08
1.99
2.09
1.03
3.09
2.96
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)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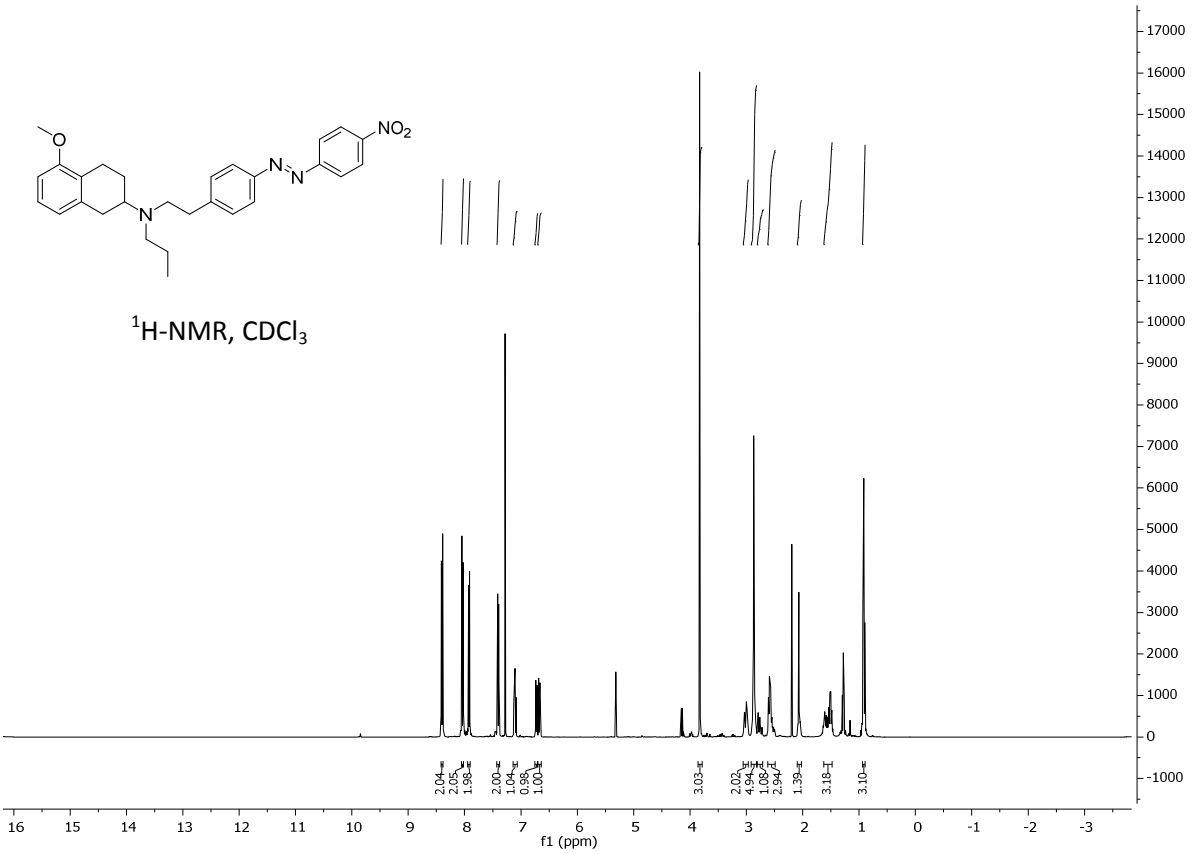
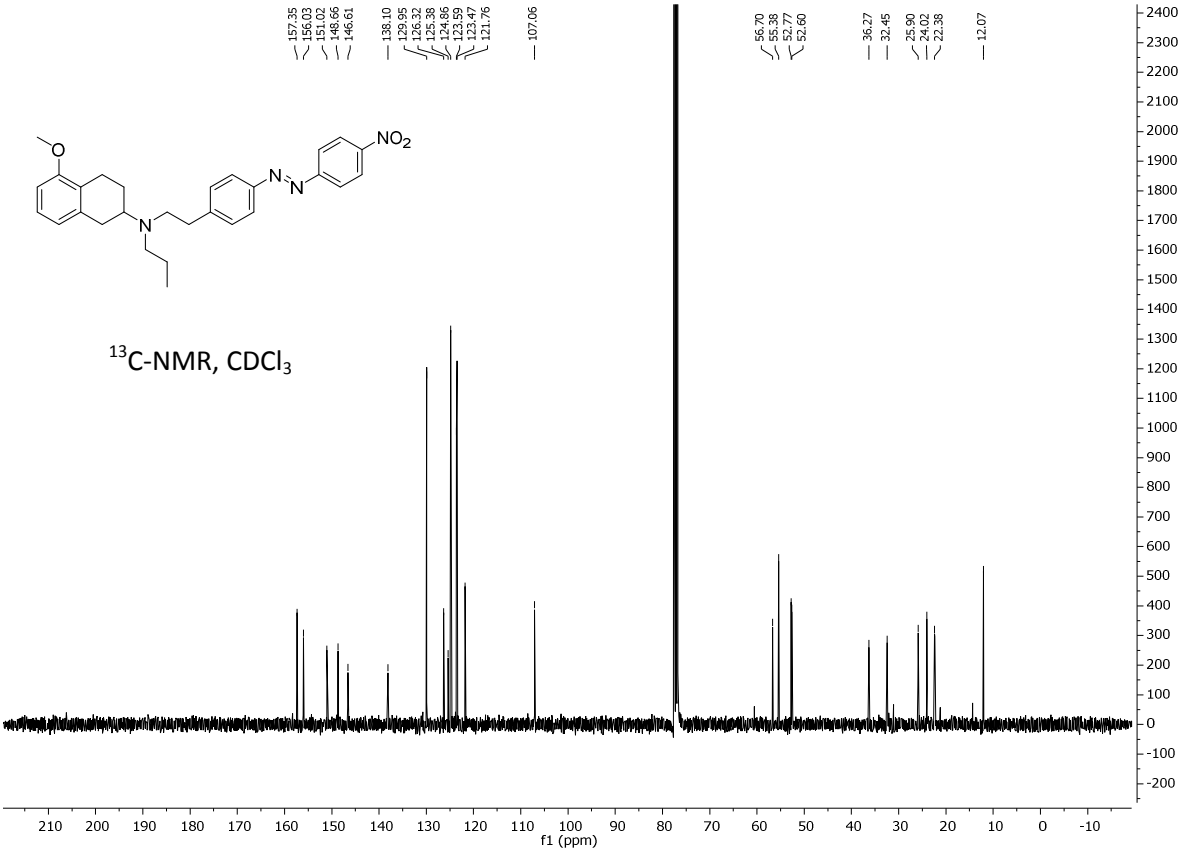
136.68
127.57
125.50
122.38
108.33
55.64
54.90
49.32
36.16
29.12
23.26
23.20
11.98
O
N
H
13C-NMR, d4-MeOH
f1 (ppm)


^{1}H-NMR, $CDCl_3$
f1 (ppm)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156.03
151.02
148.66
146.61
138.10
129.95
126.32
125.38
124.86
123.59
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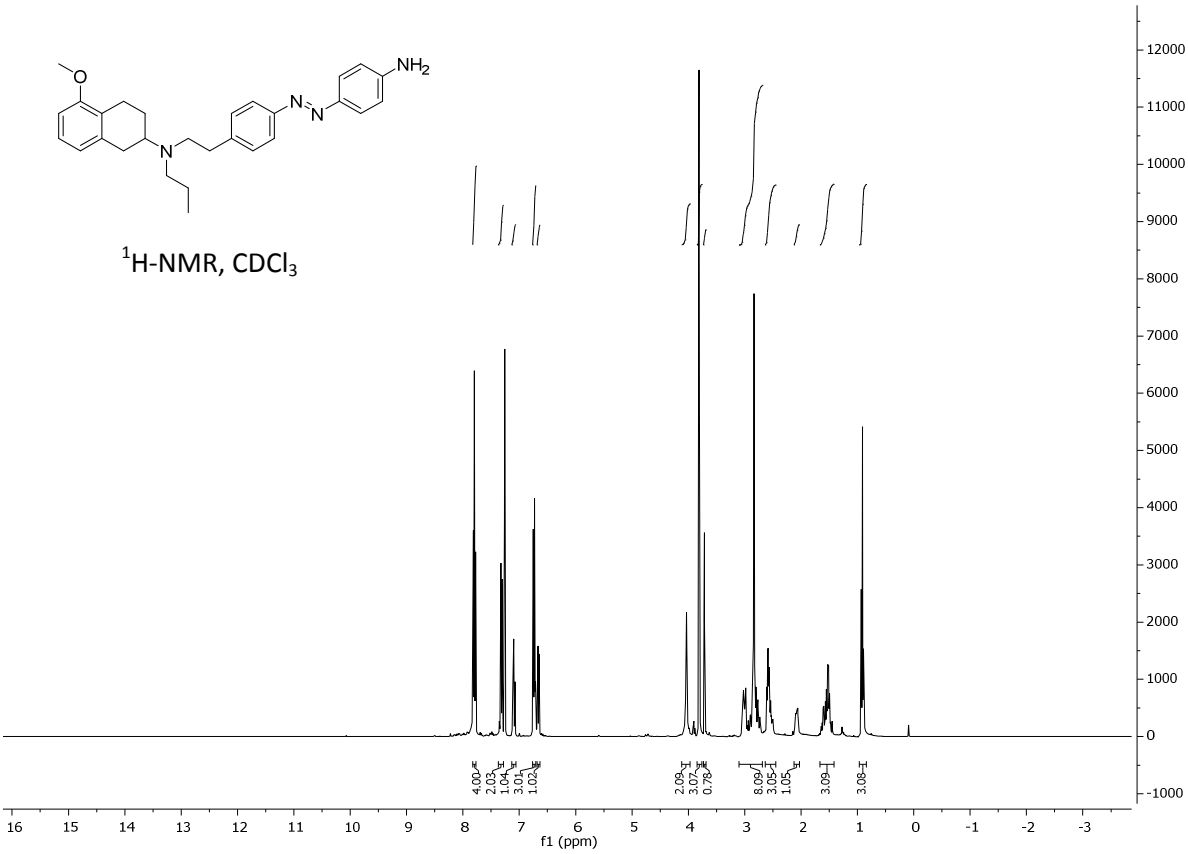
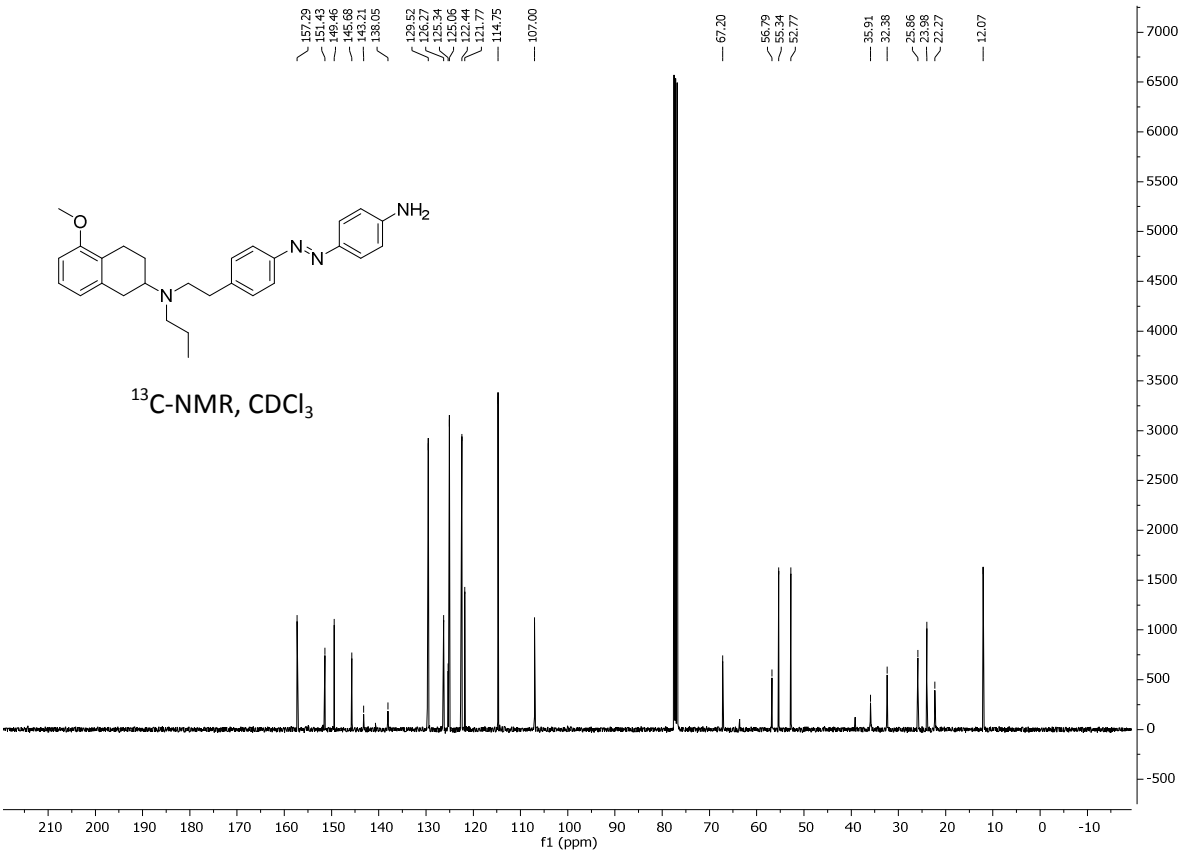
121.76
107.06
56.70
55.38
52.77
52.60
36.27
32.45
25.90
24.02
22.38
12.07
O
N
N
N
NO2
13C-NMR, CDCl3
f1 (ppm)


NH2
O
N
N
N
1H-NMR, CDCl3
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
4.00
2.03
1.04
3.01
1.02
2.09
3.07
0.78
8.09
3.05
1.05
3.09
3.08
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)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145.68
143.21
138.05
129.52
126.27
125.34
125.06
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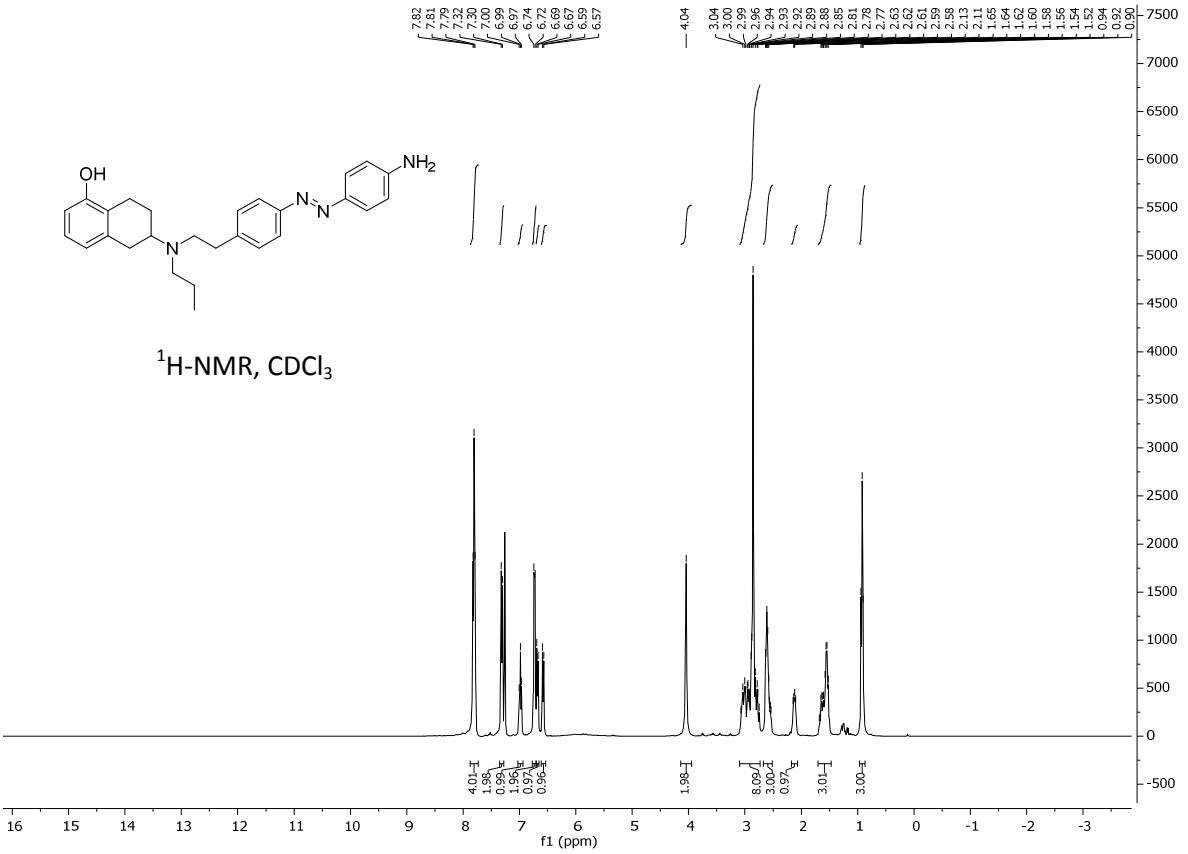
121.77
114.75
107.00
67.20
56.79
55.34
52.77
35.91
32.38
25.86
23.98
22.27
12.07
O
N
N
N
NH2
13C-NMR, CDCl3
f1 (ppm)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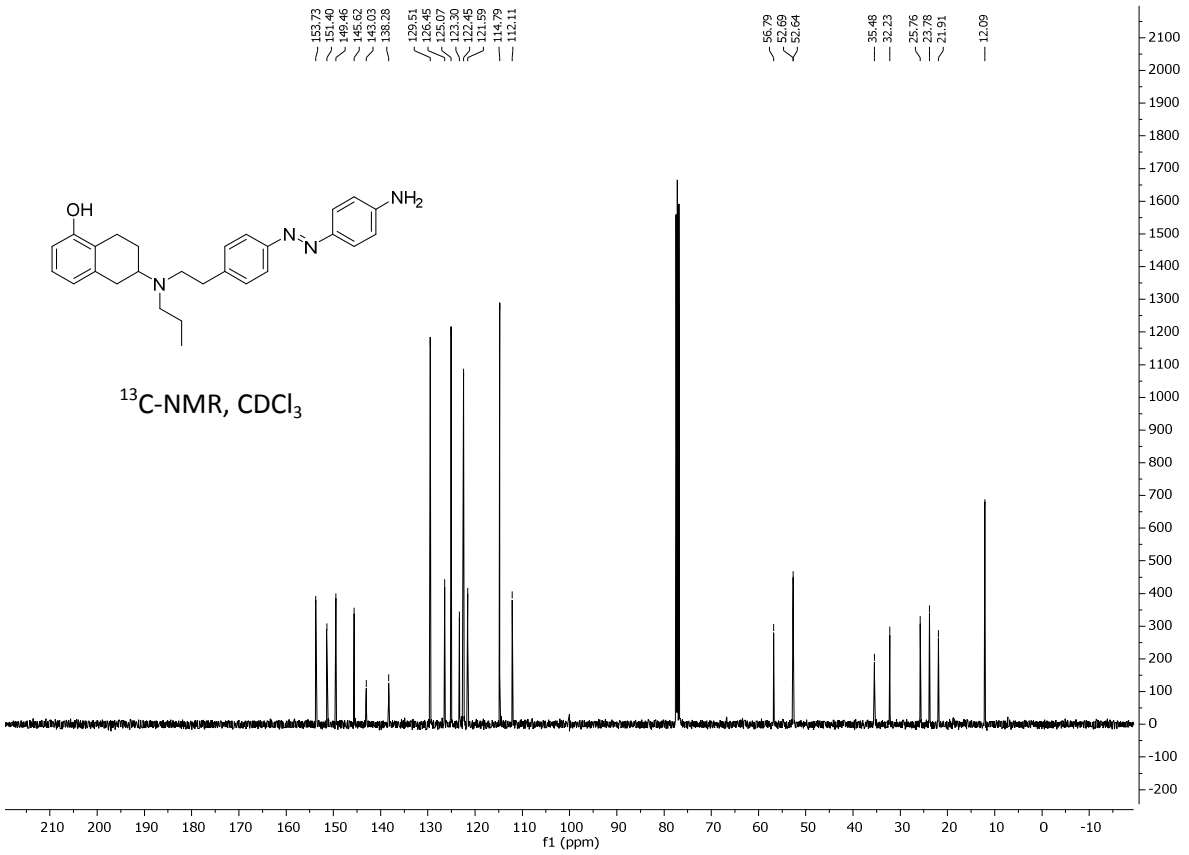
NH2
N
N
N
1H-NMR, CDCl3
f1 (ppm)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123.30
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114.79
112.11
56.79
52.69
52.64
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12.09
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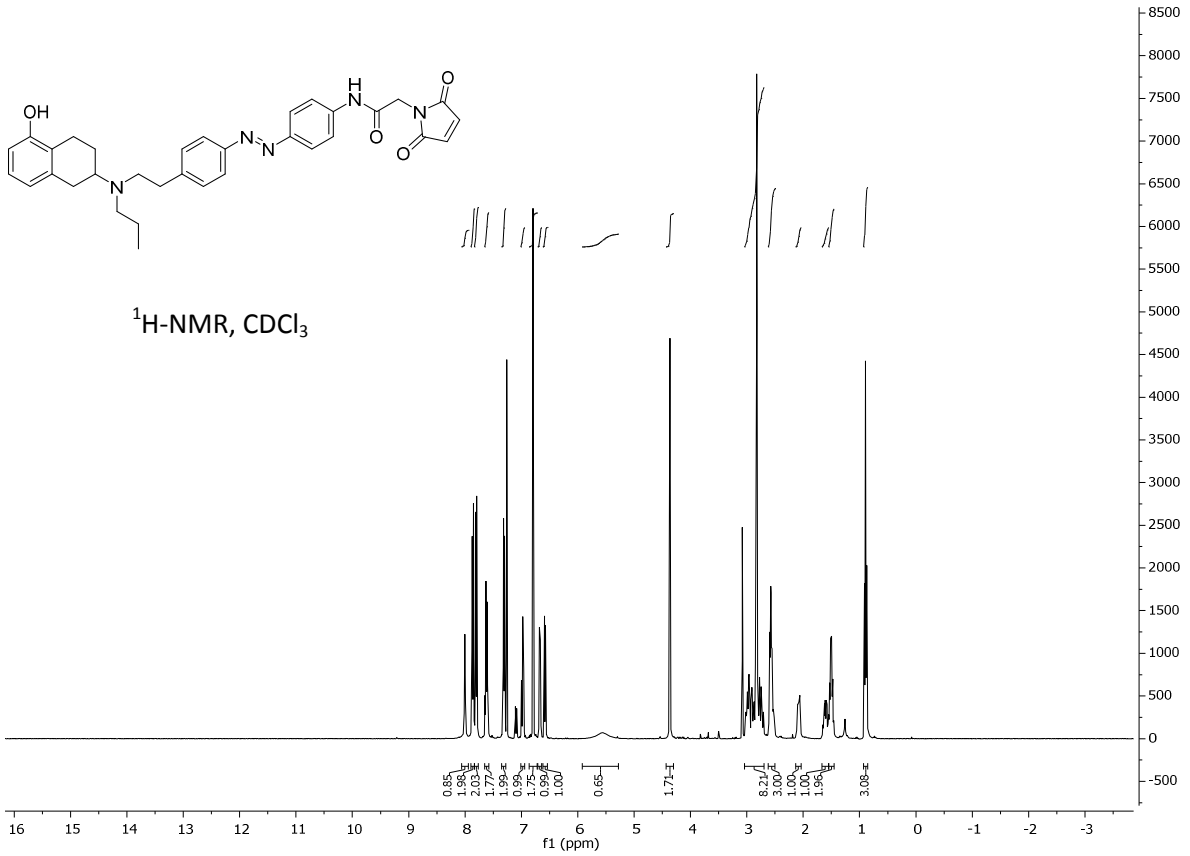
NH2
N
N
N
13C-NMR, CDCl3
f1 (ppm)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N
N
H
N
O
O
O
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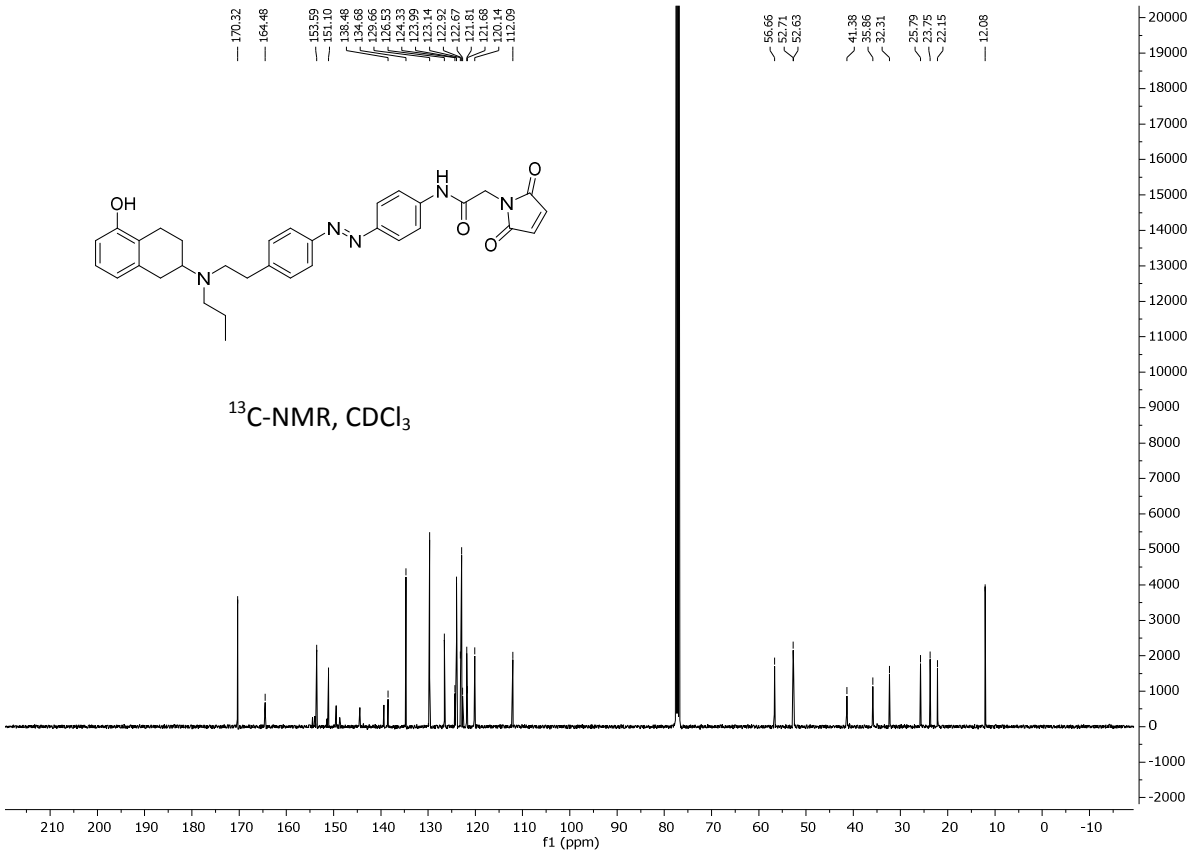
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
0.85
1.98
2.03
1.77
1.99
0.99
1.75
0.99
1.00
0.65
1.71
8.21
3.00
1.00
1.05
1.95
3.08
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
f1 (ppm)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121.68
120.14
112.09
56.66
52.71
52.63
41.38
35.86
32.31
25.79
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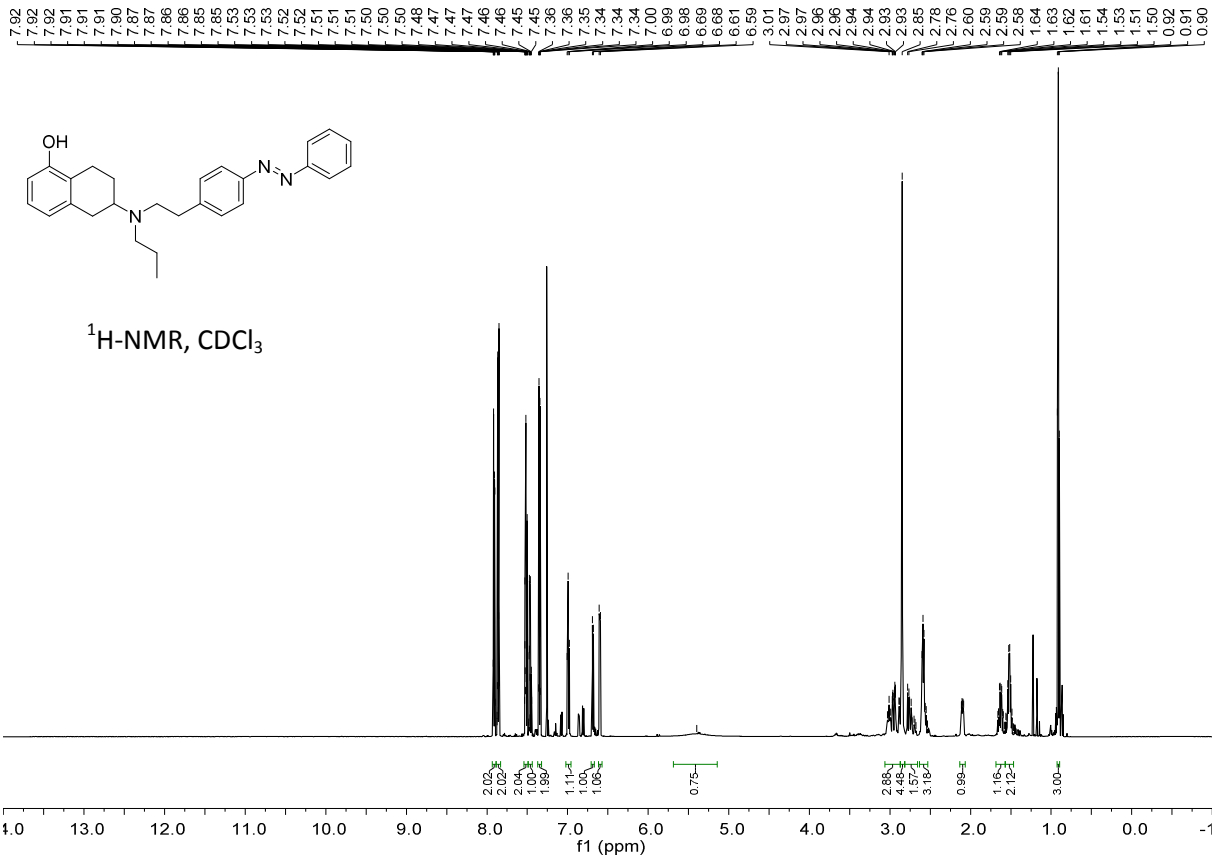
O
O
O
N
13C-NMR, CDCl3
f1 (ppm)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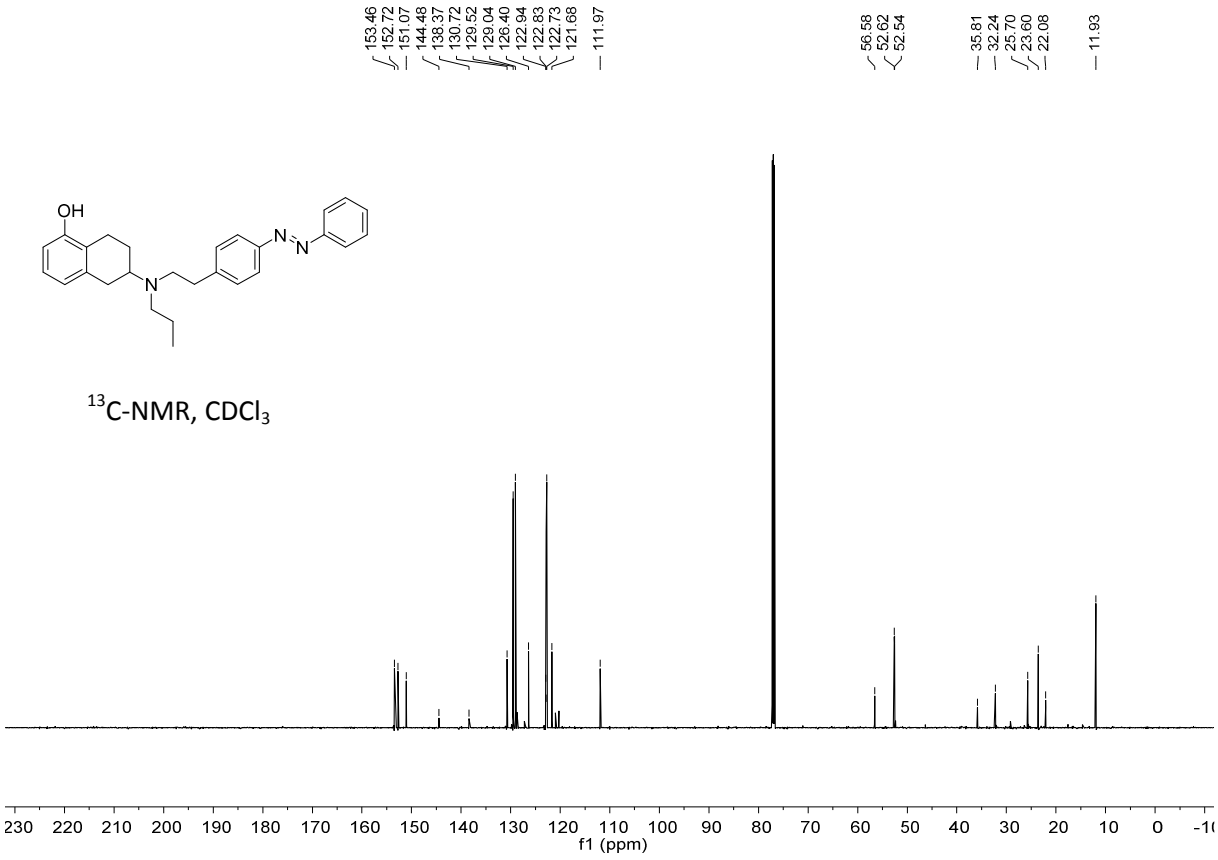
f1 (ppm)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111.97
56.58
52.62
52.54
35.81
32.24
25.70
23.60
22.08
11.93
OH
N
N
N
13C-NMR, CDCl3
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)