Supporting Information

Engineering a GPCR – Ligand Pair that Simulates the Activation of D_{2L} by Dopamine

Nuska Tschammer, Miriam Dörfler, Harald Hübner, Peter Gmeiner*

Department of Chemistry and Pharmacy, Emil Fischer Center, Friedrich-Alexander-University, Schuhstr. 19, 91052 Erlangen, Germany.

Table 1. Receptor binding data for dopamine, the test compounds **3** and **5a-c** at the porcine dopamine D_1, serotonin 5-HT_{1A}, 5-HT_2 and adrenergic α_1 receptor as well as the human dopamine D_{2L}, D_{2S}, D_3 and $D_{4.4}$ receptors.

	K_i values (nM) ± SEM							
	[^{3}H] SCH 23390	[^{3}H]spiperone				[^{3}H] WAY 100635	[^{3}H] ketanserin	[^{3}H] prazosin
Compound	**pD_1**	**hD_{2L}**	**hD_{2S}**	**hD_3**	**h$D_{4.4}$**	**p5-HT_{1A}**	**p5-HT_2**	**pα_1**
3	13000 ± 500	370 ± 66	89 ± 16	44 ± 5.5	175 ± 17	200 ± 74	9800 ± 3400	1800 ± 0
5a	1100 ± 0	44 ± 3.8	43 ± 8.1	0.35 ± 0.041	7.2 ± 0.53	22 ± 0.50	570 ± 140	89 ± 8.0
5b	4300 ± 100	23 ± 3.3	8.2 ± 0.99	0.52 ± 0.048	0.51 ± 0.043	0.70 ± 0.11	360 ± 10	38 ± 1.5
(*R*)-5c	1800 ± 500	11 ± 1.6	5.3 ± 0.94	0.24 ± 0.027	1.2 ± 0.049	14 ± 5.3	2000 ± 700	62 ± 17
(*S*)-5c	1000 ± 80	40 ± 7.5	8.6 ± 2.8	0.47 ± 0.025	0.50 ± 0.026	5.8 ± 0	1400 ± 860	32 ± 0.50

Mean K_i values with SEM are derived from 2-10 experiments, each done in triplicate.

Figure 1. The mutant receptor D_{2L}F6.52W is not activated by the endogenous ligand dopamine but is fully functional under synthetic ligands. Mean curves with error bars representing the SEM are shown. (A) The incorporation of the [^{35}S]GTPγS was measured on membrane preparations of stably transfected CHO cells that expressed the $D_{2L}F390^{6.52}W$ receptor, treated with dopamine or quinpirole. No detectable [^{35}S]GTPγS incorporation was measured after dopamine treatment, whereas the EC_{50} value of quinpirole was 1500 nM. (B) The inhibitory effect of dopamine or quinpirole on cAMP accumulation was measured in stably transfected CHO cells that expressed the $D_{2L}F390^{6.52}W$ receptor after stimulation of the adenylyl cylase in the presence of 20μM forskolin. No detectable cAMP inhibition was measured in the presence of dopamine; the EC_{50} value of quinpirole was 500 nM. (C) The suppression of quinpirole mediated the [^{35}S]GTPγS incorporation was measured on membrane preparation of stably transfected CHO cells that expressed the $D_{2L}F390^{6.52}W$ receptor. After prestimulation of receptor with 0.1 mM quinpirole the suppression of this effect was determined by increasing concentrations of the antagonist spiperone with an EC_{50} value of 2.6 nM. (D) The suppression of the inhibition of cAMP accumulation was measured in the stably transfected CHO cells that expressed the $D_{2L}F390^{6.52}W$ receptor after prestimulation with 5 μM quinpirole and 20 μM forskolin with increasing concentrations of the antagonist spiperone with an EC_{50} value of 6.6 nM.

Figure1.

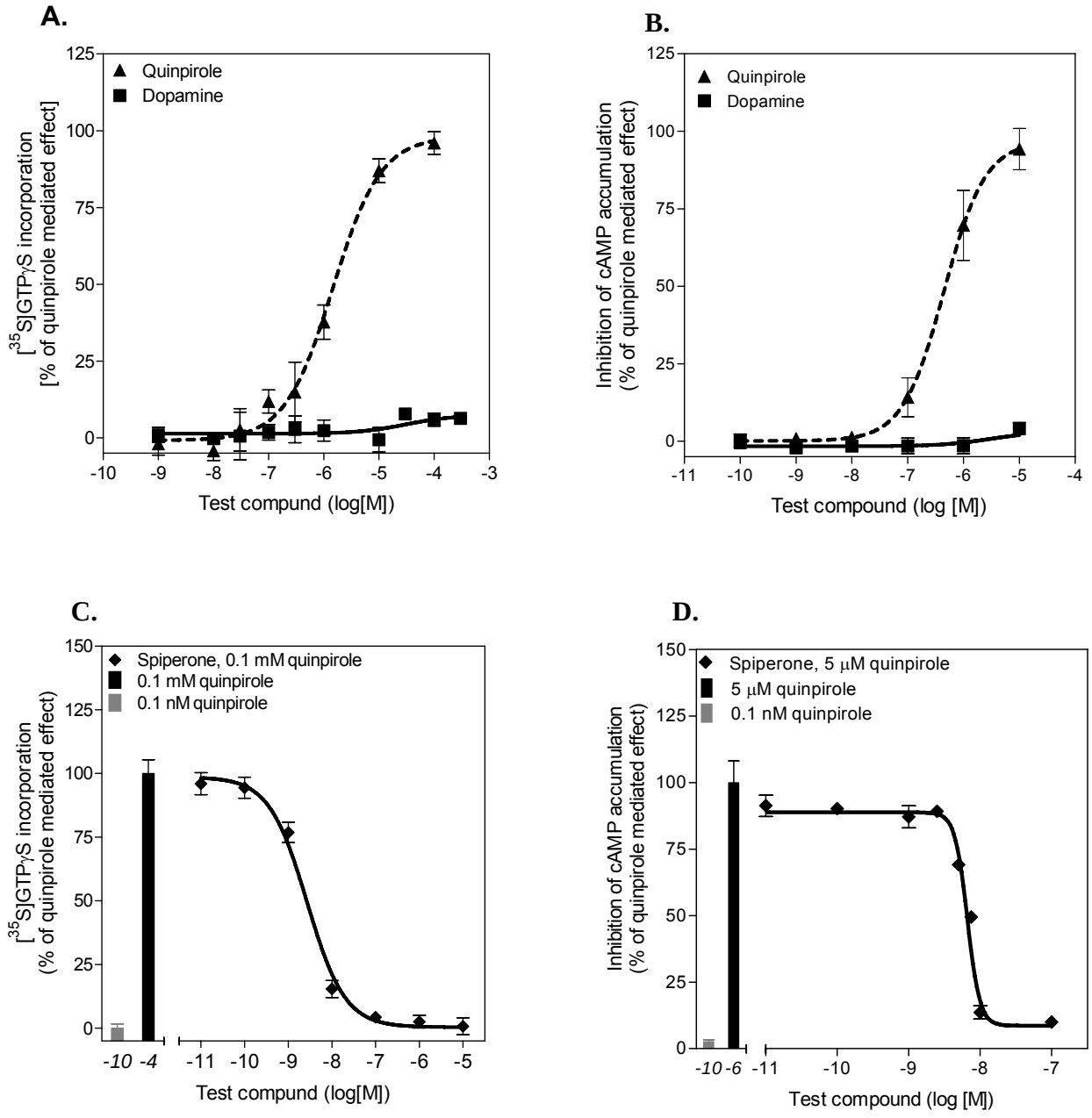
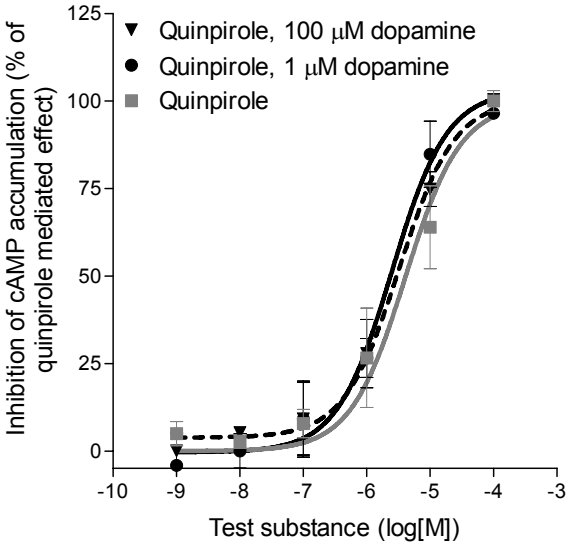

A.
Quinpirole
Dopamine
[35S]GTPγS incorporation
[% of quinpirole mediated effect]
Test compund (log[M])
B.
Quinpirole
Dopamine
Inhibition of cAMP accumulation
(% of quinpirole mediated effect)
Test compound (log [M])
C.
Spiperone, 0.1 mM quinpirole
0.1 mM quinpirole
0.1 nM quinpirole
[35S]GTPγS incorporation
(% of quinpirole mediated effect)
Test compund (log[M])
D.
Spiperone, 5 μM quinpirole
5 μM quinpirole
0.1 nM quinpirole
Inhibition of cAMP accumulation
(% of quinpirole mediated effect)
Test compound (log [M])

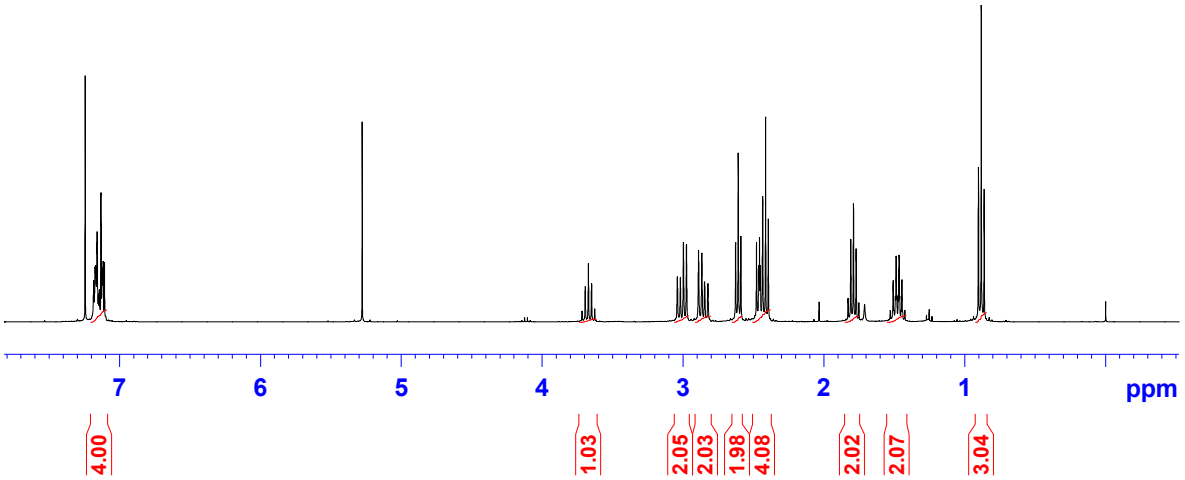
Figure 2: The potential antagonistic effect of dopamine on quinpirole stimulated inhibition of cAMP accumulation was measured in stably transfected CHO cells that expressed the $D_{2L}F390^{6.52}W$ receptor after stimulation of the adenylyl cylase in the presence of 20μM forskolin. Dopamine was not able to antagonize quinpirole stimulated inhibition of cAMP accumulation.



(*N*-Indan-2-yl-*N*-propyl)-4-aminobutyronitrile (4a)

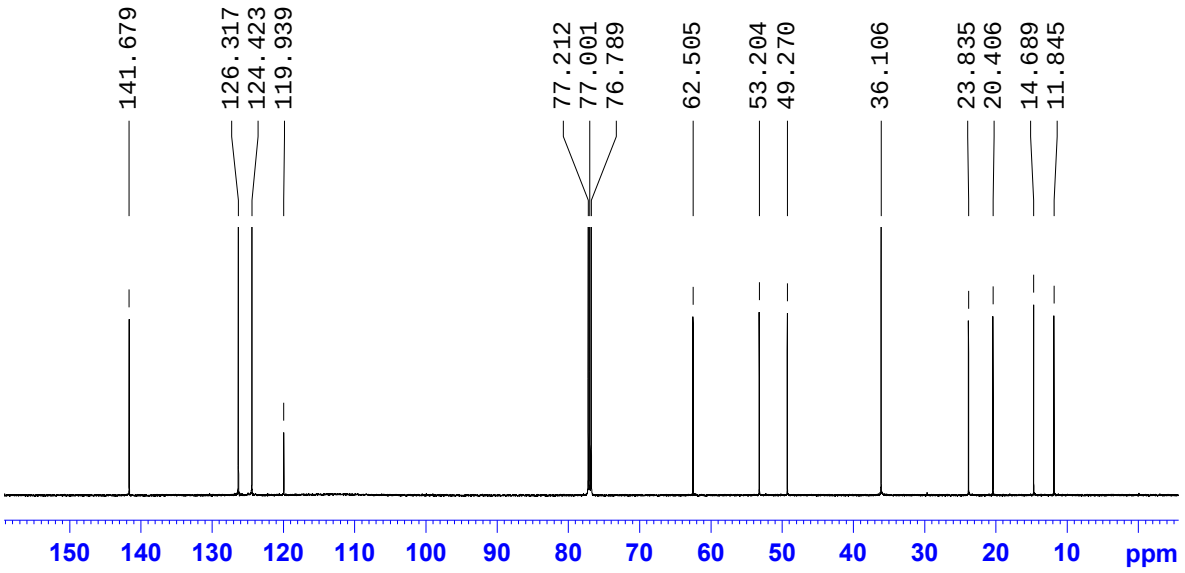
^{1}H-NMR:

^{1}H (360 MHz, $CDCl_3$):



^{13}C-NMR:

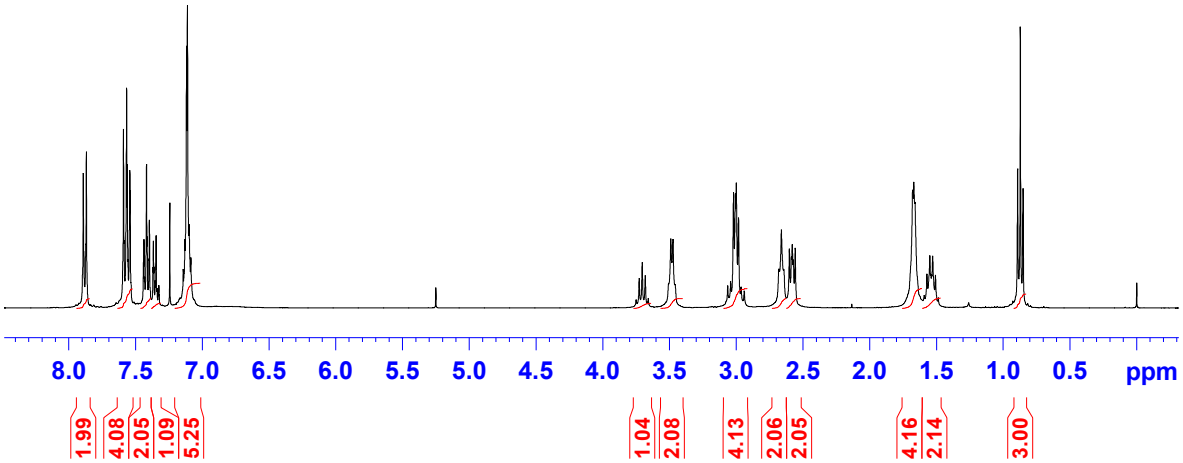
^{13}C (150 MHz, $CDCl_3$):



***N*-[(*N*'-Indan-2-yl-*N*'-propyl)-4-aminobutyl]-4-biphenyl carboxamide (5a)**

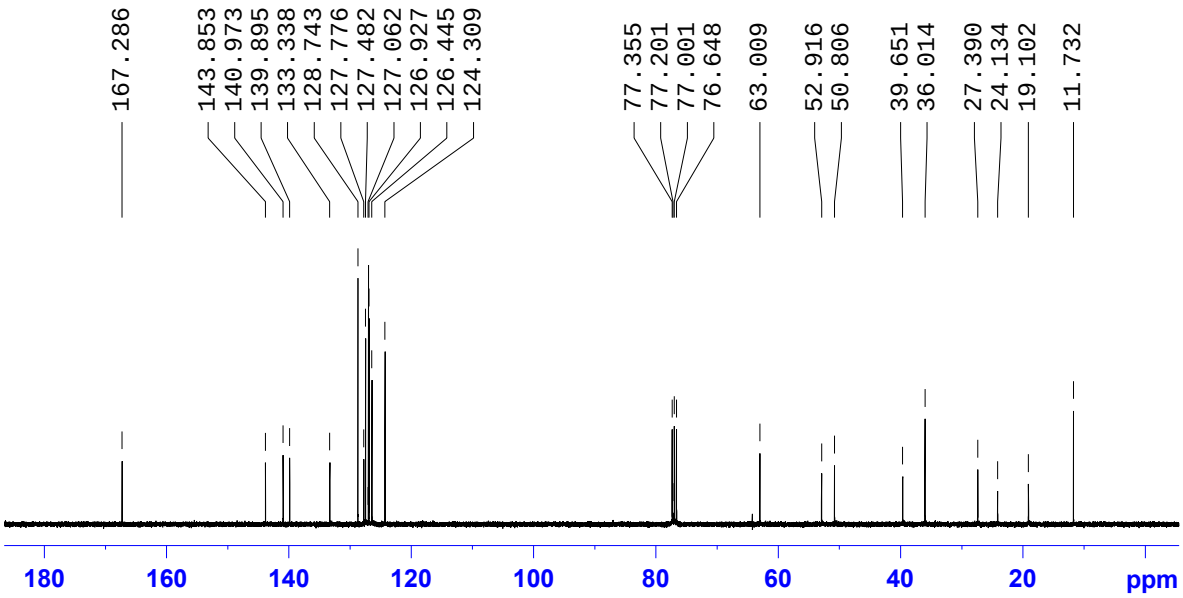
^{1}H-NMR:

^{1}H (360 MHz, $CDCl_3$):



^{13}C-NMR:

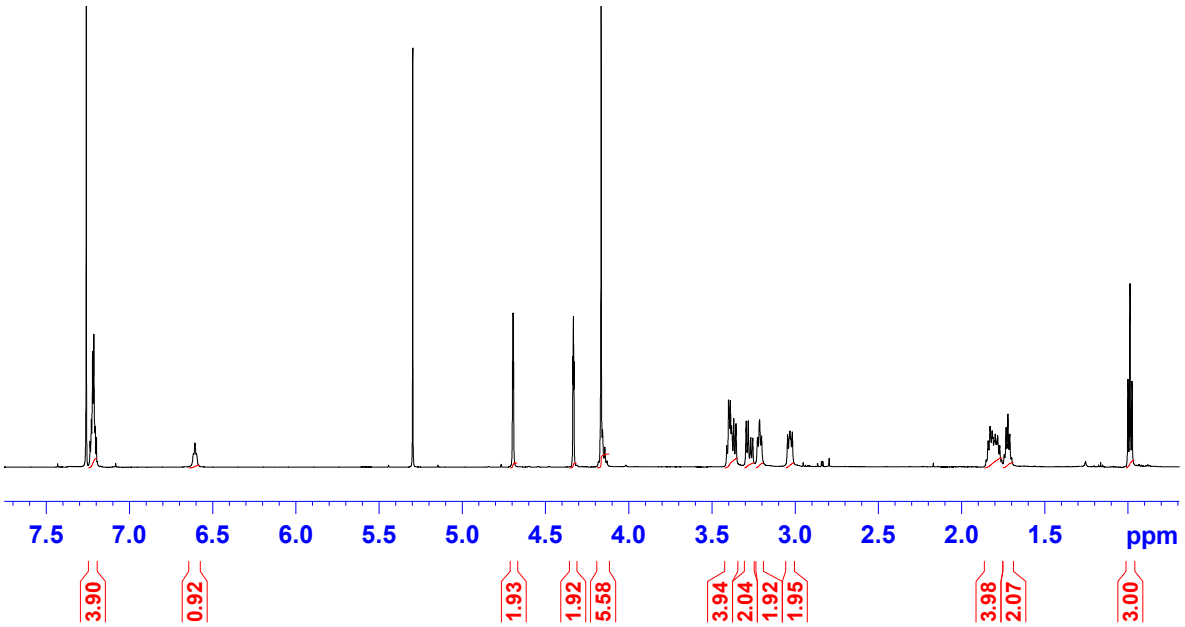
^{13}C (150 MHz, $CDCl_3$):



***N*-[(*N*'-Indan-2-yl-*N*'-propyl)-4-aminobutyl]ferrocenyl carboxamide (5b)**

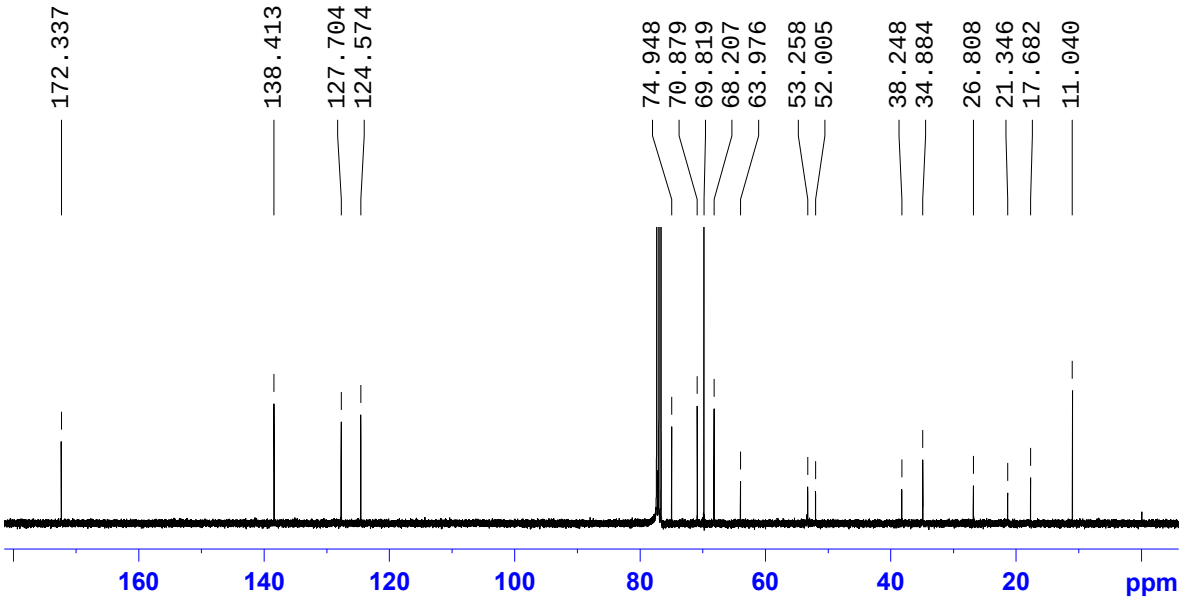
^{1}H-NMR:

^{1}H (600 MHz, $CDCl_3$):



^{13}C-NMR:

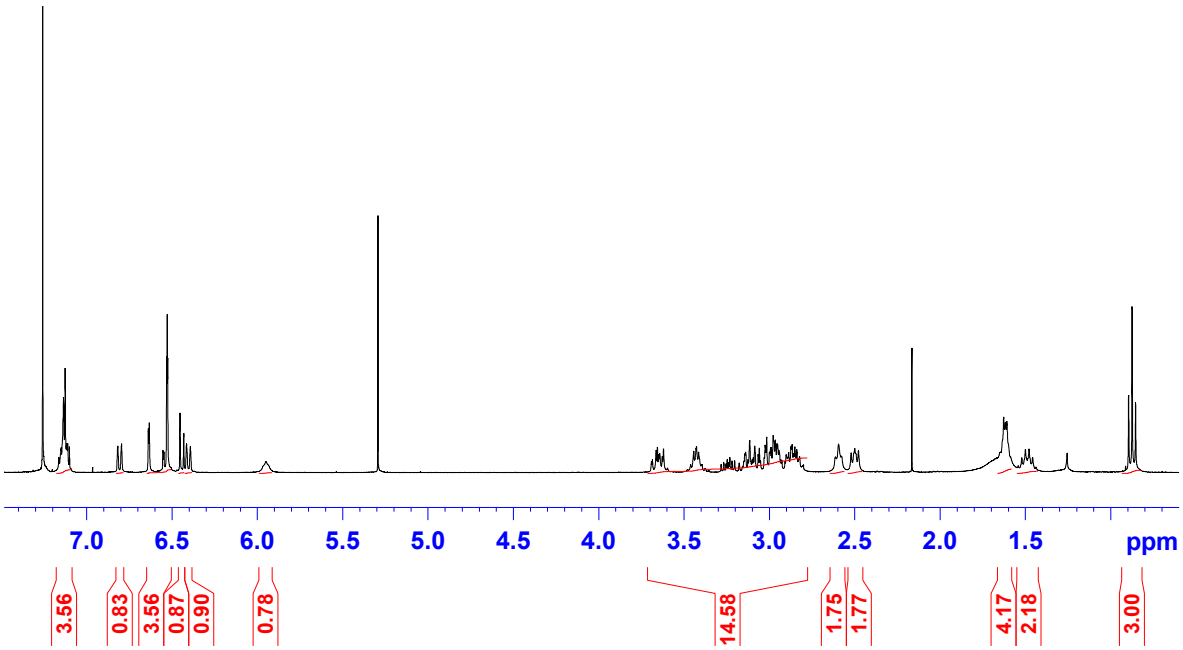
^{13}C (90 MHz, $CDCl_3$):



***N*-[(*N*'-Indan-2-yl-*N*'-propyl)-4-aminobutyl]-(*R*)-[2.2]paracyclophane carboxamide ((*R*)-5c)**

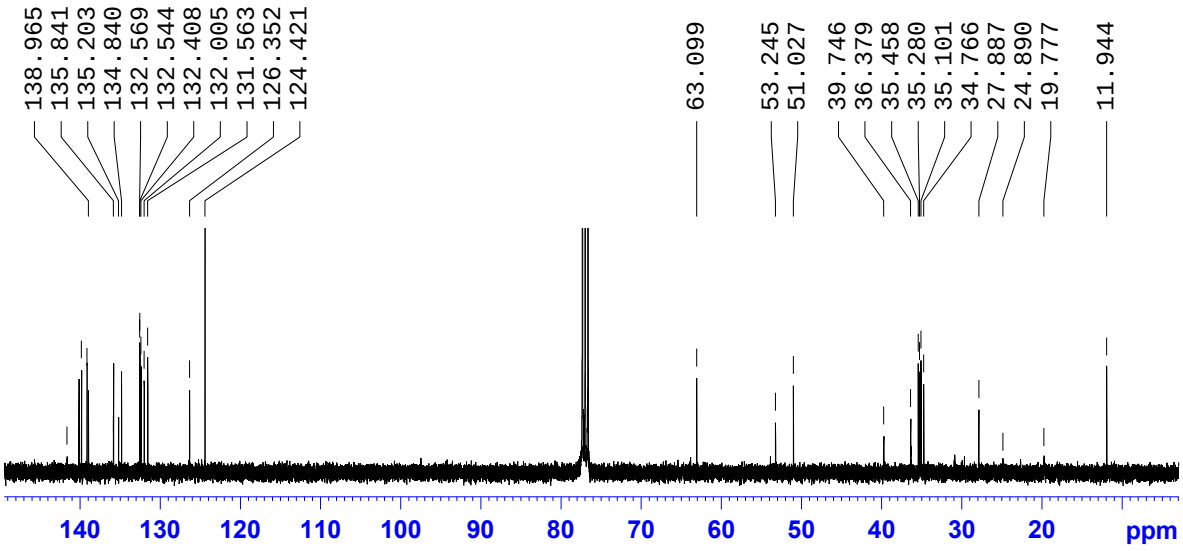
^{1}H-NMR:

^{1}H (360 MHz, $CDCl_3$):



^{13}C-NMR:

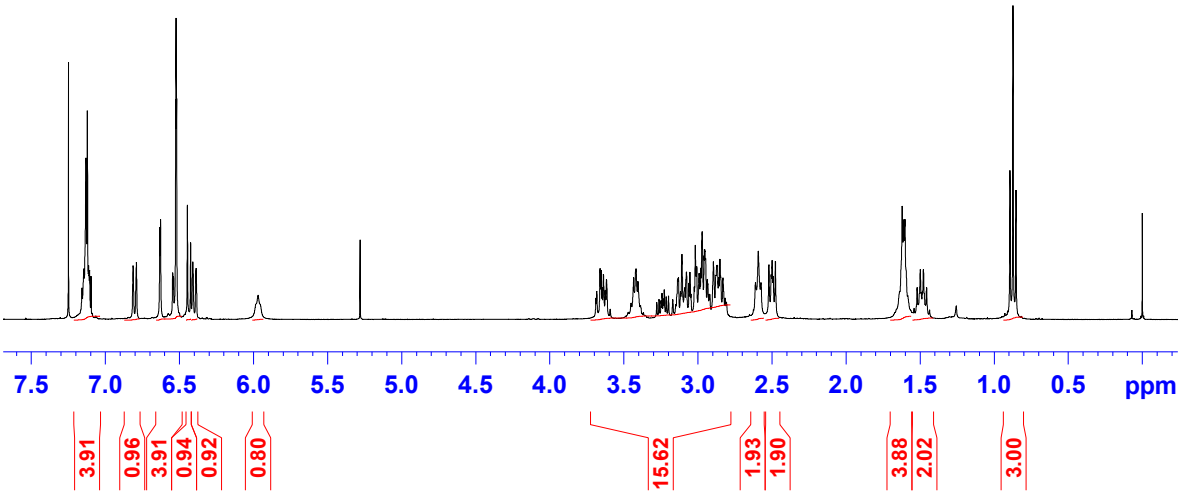
^{13}C (90 MHz, $CDCl_3$):



***N*-[(*N*'-Indan-2-yl-*N*'-propyl)-4-aminobutyl]-(*S*)-[2.2]paracyclophane carboxamide ((*S*)-5c)**

^{1}H-NMR:

^{1}H (360 MHz, $CDCl_3$):



^{13}C-NMR:

^{13}C (150 MHz, $CDCl_3$):