Exploring α7-Nicotinic Receptor Ligand Diversity by Scaffold Enumeration from the Chemical Universe Database GDB

Supporting Information

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Virtual Screening

Table S1. Composition of diamine scaffolds generated from GDB-11.[a)]

Atoms	Cycles				Amines		Rotatable Bonds					
	0	1	2	>2	1°	2°	0	1	2	>2	SUM	%
<9	25	72	55	9	110	51	74	41	27	19	161	0.07
9	85	349	484	239	689	468	594	282	152	129	1157	0.5
10	369	1832	3582	3135	4954	3964	4663	2261	1075	919	8918	3.82
11	1545	9275	23530	32778	34969	32159	34155	18359	8103	6511	67128	28.7
12	4784	26774	58266	66384	129911	26297	56852	46709	26627	26020	156208	66.9
SUM	6808	38302	85917	102545	170633	62939	96338	67652	35984	33598	233572	100

[a)] General formula $C_xH_yN_2$, $1 \leq x \leq 10$, excluding aziridines. Distribution of the other non-tertiary N atom according to size of diamines, 1° = primary amines. 2° = secondary amines.

Docking studies. The protonation state of all the compounds was set at pH=7.4, and the compounds were expanded to 3D structures using CORINA for Autodock or LigPrep for Glide. AutoDockTools (ADT3) and Glide were applied to prepare and parameterize the receptor protein and ligands in batch mode. All structures were then docked into the target receptor (pdb code: 1UW6.pdb). The most favorable docking conformation and its estimated binding energy value was taken to be the final docking result for that input structure, considering each diastereoisomer independently.

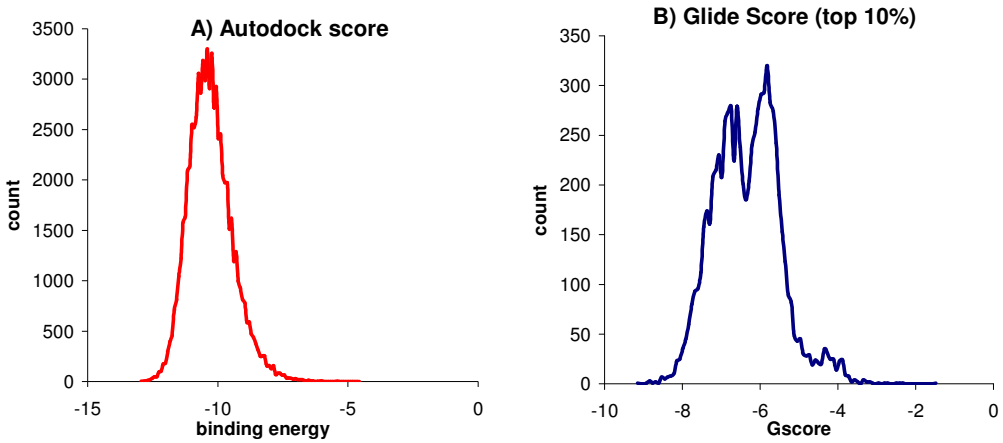


Figure S1. Docking data. **A.** Distribution of docking scores for the best binding stereoisomers of each of the 72,745 SMILES scored by Autodock. **B.** Docking scores for Glide. For Glide the program automatically rejected the weakly docking compounds and only stored data for the top 10% of the SMILES.

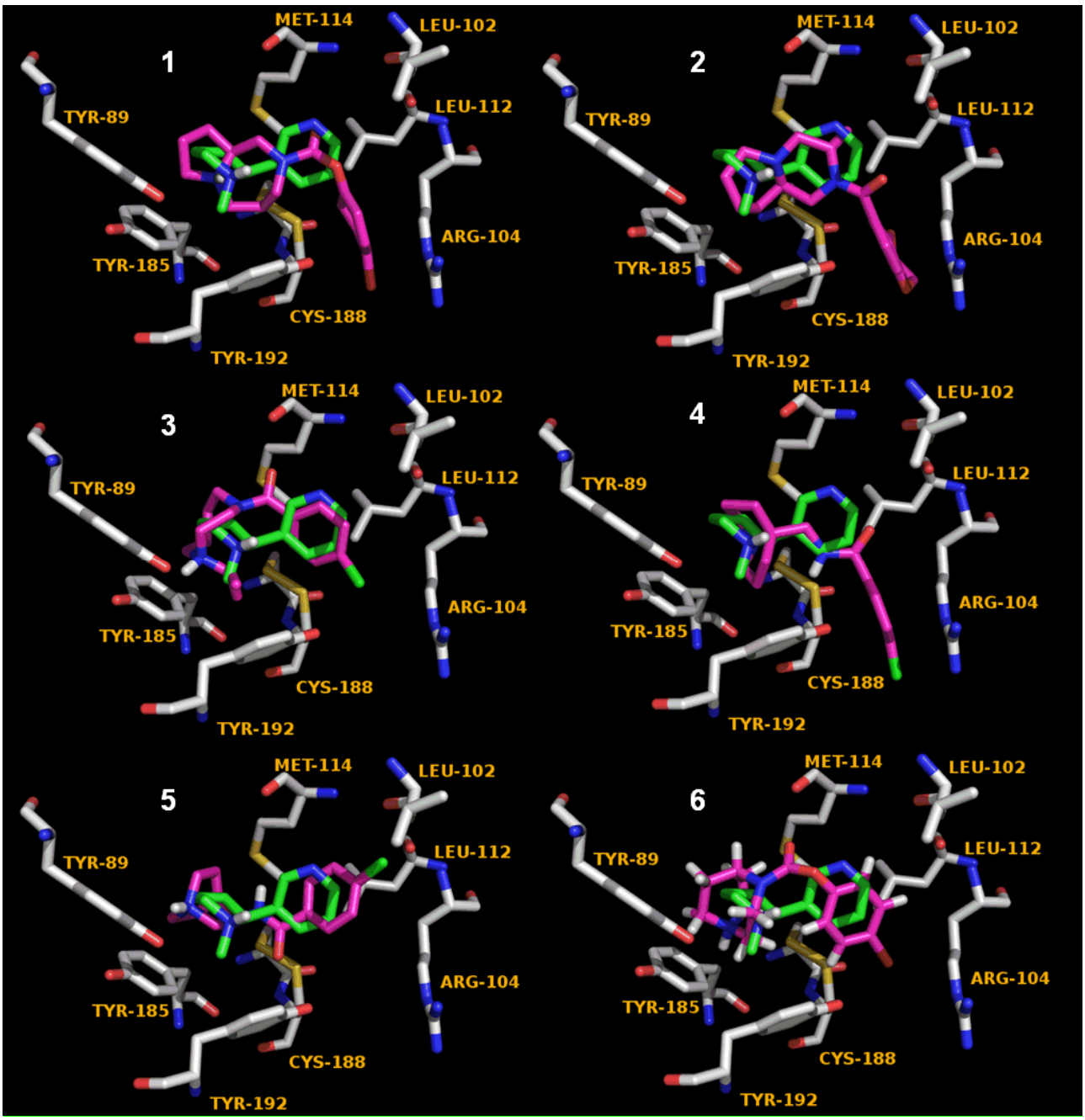


Figure S1C. Docking poses of ligands **1-4** and the references **5** and **6** docked in the nicotinic binding site of 1UW6.pdb. Green: reference crystallographic nicotine ligand. Magenta: ligand in its most favourable docked pose as estimated by Autodock (**1** and **2**) or Glide (**4 - 6**).

Electrophysiology

All experiments were carried out at the human α7 nAChR receptors expressed in *Xenopus* oocytes using the method of cDNA expression. *Xenopus* oocytes were prepared and injected using standard procedures. Acetylcholine-evoked currents wer recorded using the standard two electrode voltage-clamp configuration (TVEC).

Briefly, ovaries were harvested from *Xenopus Laevis* females that have been deeply anesthetized and pithed according to the animal rights rule from the Geneva canton. A small piece of ovary was isolated for immediate preparation while the remaining part was placed at 4 ^{o}C in a sterile Barth solution containing in mM NaCl 88, KCl 1, $NaHCO_3$ 2.4, HEPES 10, $MgSO_4$.7H2O 0.82, $Ca(NO_3)_2.4H_2O$ 0.33, $CaCl_2.6H_2O$ 0.41, at pH 7.4, and supplemented with 20 µg/ml of kanamycine, 100 unit/ml penicillin and 100 µg/ml streptomycin. On the second day following dissociation, oocytes were injected with 2 ng of cDNA per oocyte containing the genes encoding for the human α7 nAChR using an automated injector.[1] All recordings were performed at 18 ^{o}C and cells superfused with OR2 medium containing in mM: NaCl 82.5, KCl 2.5, HEPES 5, $CaCl_2.2H_2O$ 1.8, pH 7.4. Cells were held at –80 mV. Data will be filtered at 10 Hz, captured at 100 Hz and analyzed using proprietary data acquisition and analysis software running under Matlab (Mathworks Inc.).

Concentration inhibition curves were fitted using the empirical Hill equation in the form:

$$y = 1 / 1+(x/IC_{50})^{nH}$$

where: y = the fraction of evoked current, x = the antagonist concentration, IC_{50} = concentration for 50% inhibition and, *nH* = the apparent co-operativity.

Concentration activation curves were fitted using the empirical Hill equation:

$$y = 1 / 1 + (EC_{50}/x)nH$$

where: y = the fraction of ACh-evoked current, x = the agonist concentration, EC_{50} = the concentration causing 50 % activation and nH the apparent cooperativity.

[1] Hogg RC, Bandelier F, Benoit A, Dosch R, Bertrand D. An automated system for intracellular and intranuclear injection. J Neurosci Methods 2008;169:65-75.

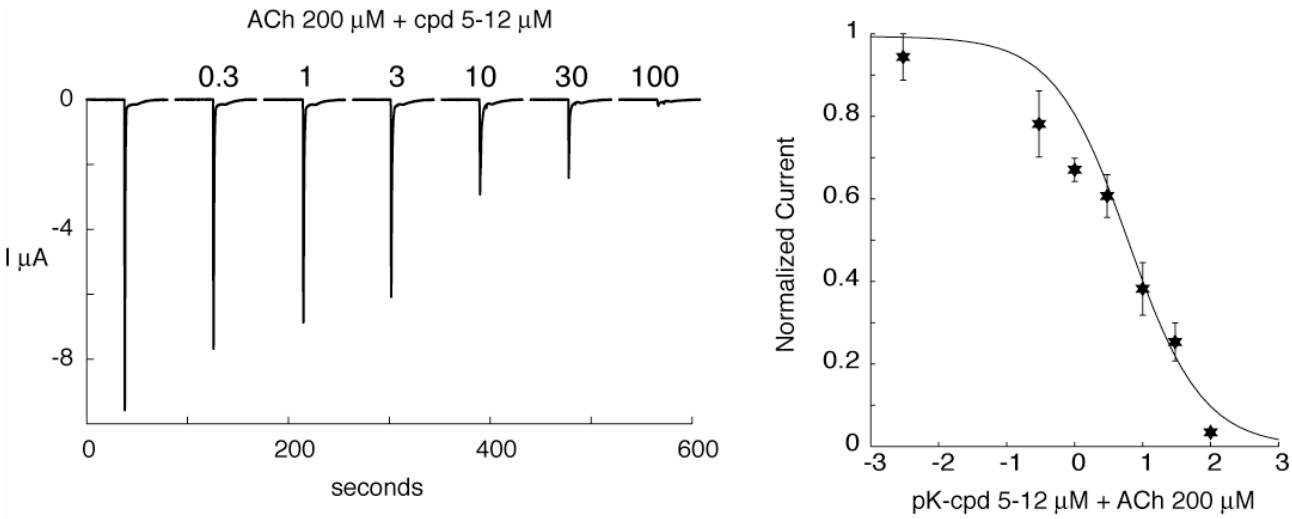


Figure S2. Compound **2** (5-12) inhibits in a dose dependent manner the ACh-evoked current.. Typical effects of compound 2 are shown in the inset. Plot of the normalized peak current as a function of the logarithm of the compound concentration yielded a typical concentration inhibition curve (n=5). The continuous line is the best fit with the Hill equation, yielding an IC_{50} of 6 µM and a Hill coefficient of 0.8.

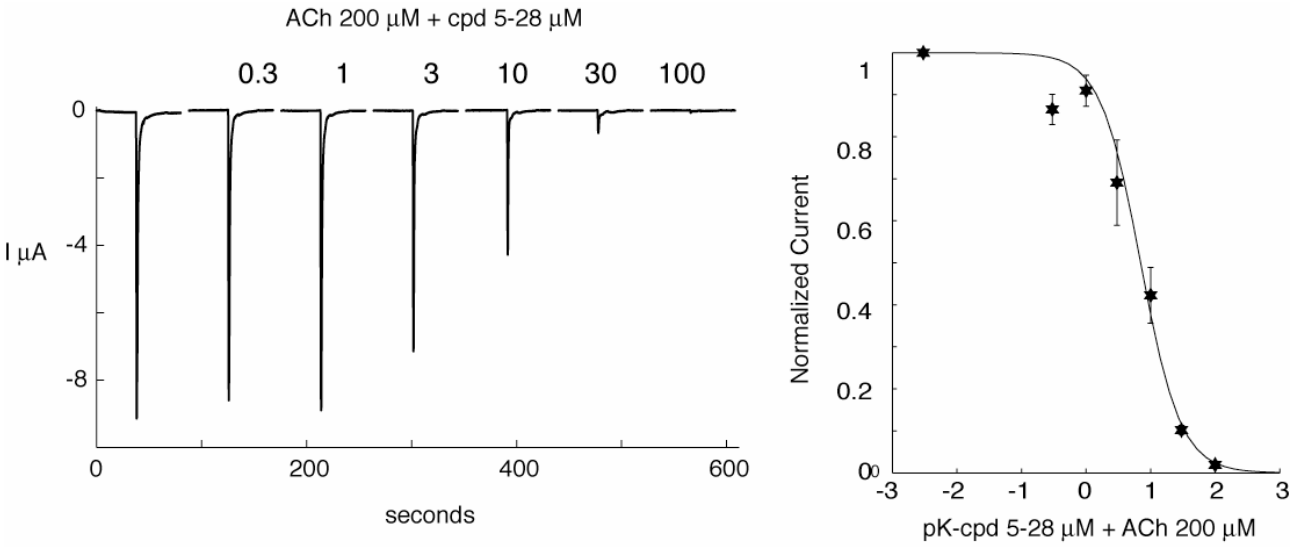


Figure S3. Determination of IC_{50} of compound **3** (5-28). Data were obtained as in Figure S2 and were fitted with an IC_{50} of 7 µM and a Hill coefficient of 1.4 (n=5).

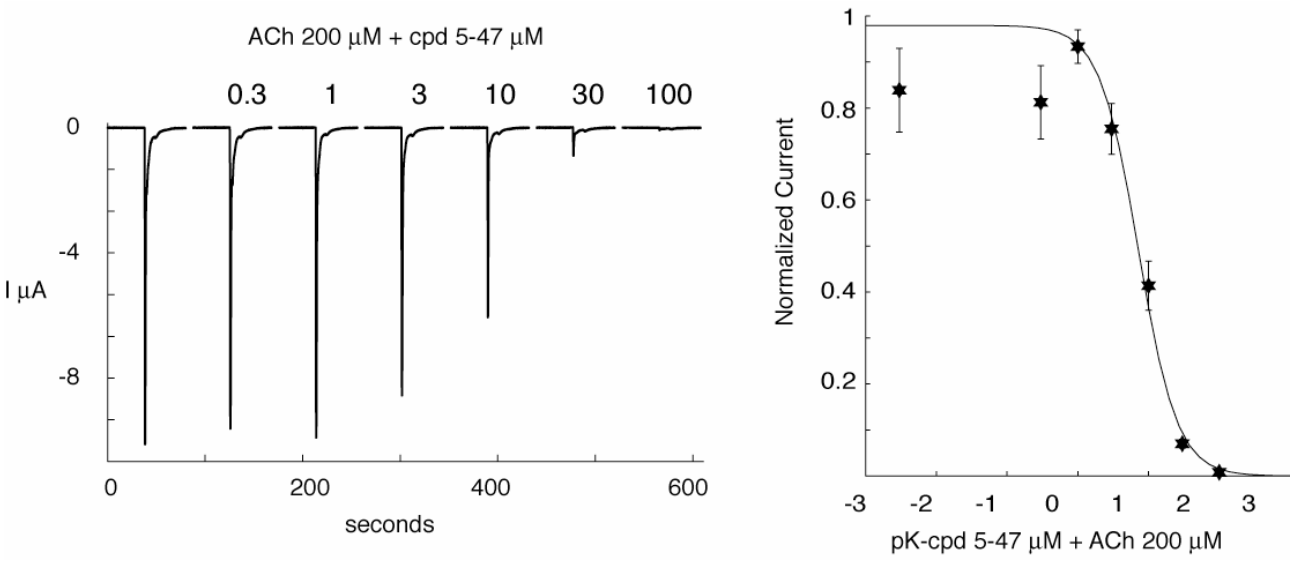
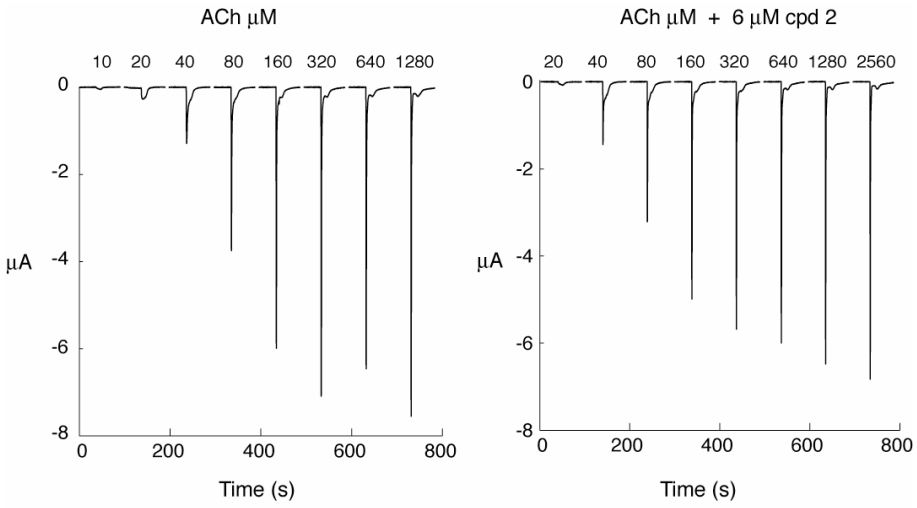
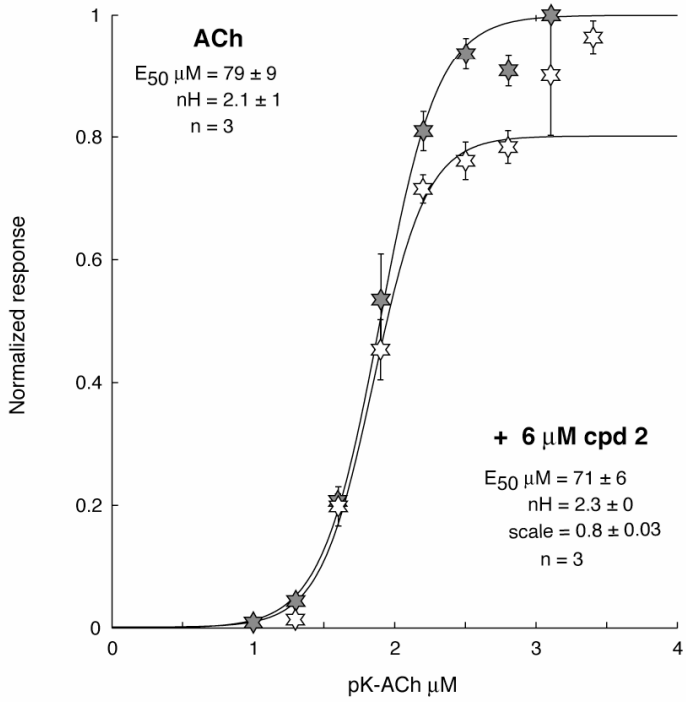


Figure S4. Determination of IC_{50} of compound **4** (5-47) Data were obtained as in Figure S2 and were fitted with an IC_{50} of 7.2 µM and a Hill coefficient of 1.58 (n = 5).


ACh μM
10 20 40 80 160 320 640 1280
0
-2
-4
-6
-8
μA
0 200 400 600 800
Time (s)
ACh μM + 6 μM cpd 2
20 40 80 160 320 640 1280 2560
0
-2
-4
-6
-8
μA
0 200 400 600 800
Time (s)


ACh
E50 μM = 79 ± 9
nH = 2.1 ± 1
n = 3
+ 6 μM cpd 2
E50 μM = 71 ± 6
nH = 2.3 ± 0
scale = 0.8 ± 0.03
n = 3
Normalized response
0
0.2
0.4
0.6
0.8
1
0 1 2 3 4
pK-ACh μM

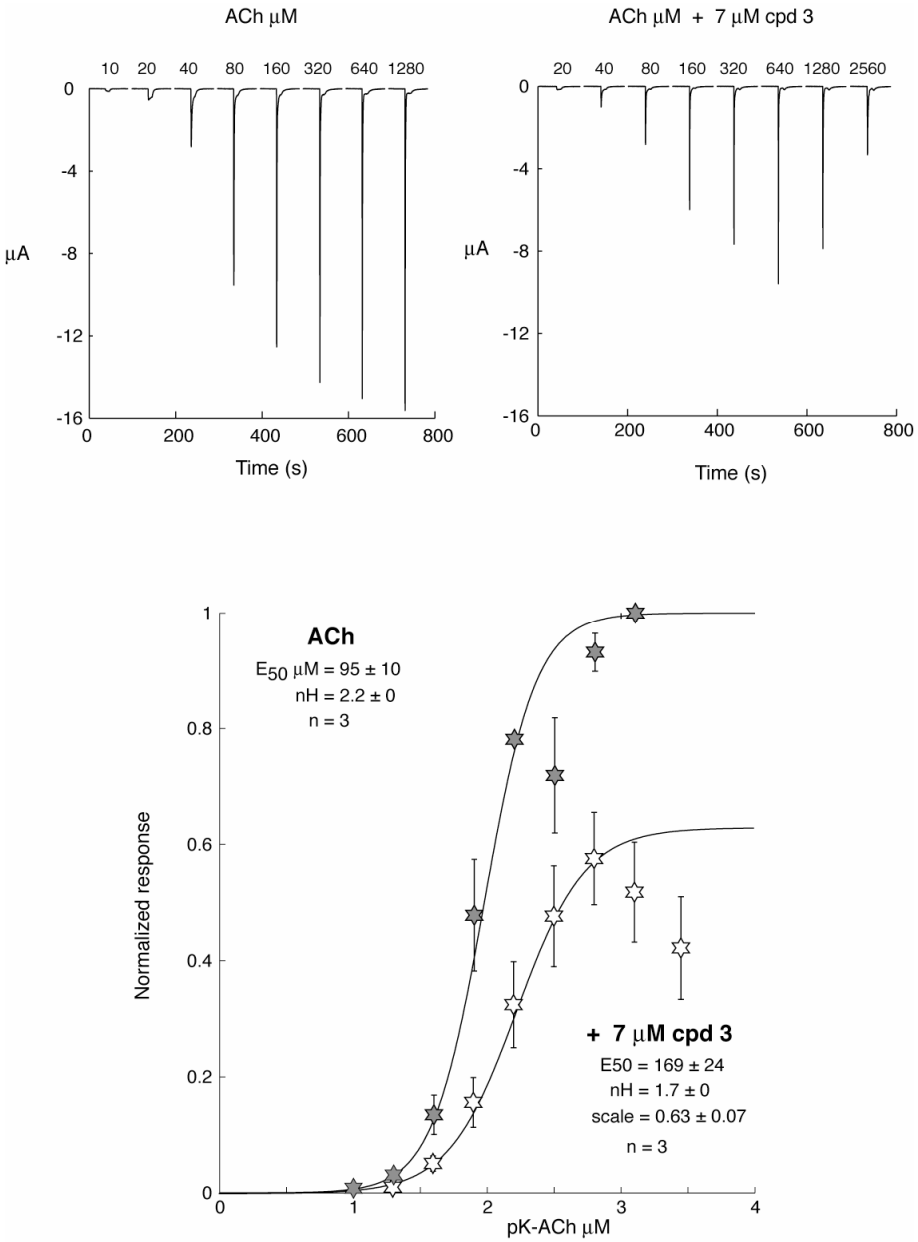


Figure S5. . Effects of compounds 2 (5-12) and 3 (5-28) on the ACh concentration activation curve. To examine in more details the effects of compounds the ACh concentration activation curve was determined in absence or presence of a fixed concentration of the compound. Typical currents evoked by a series of ACh concentrations are shown in the left panel. ACh pulses were applied at 2 minutes interval to allow full recovery between pulses. Plot of the peak inward current as a function of the logarithm of the ACh concentration yielded typical concentration activation curves that were readily fitted with the empirical Hill equation. EC50's and nH coefficients for the best fit (continuous curves) are indicated on the graph.

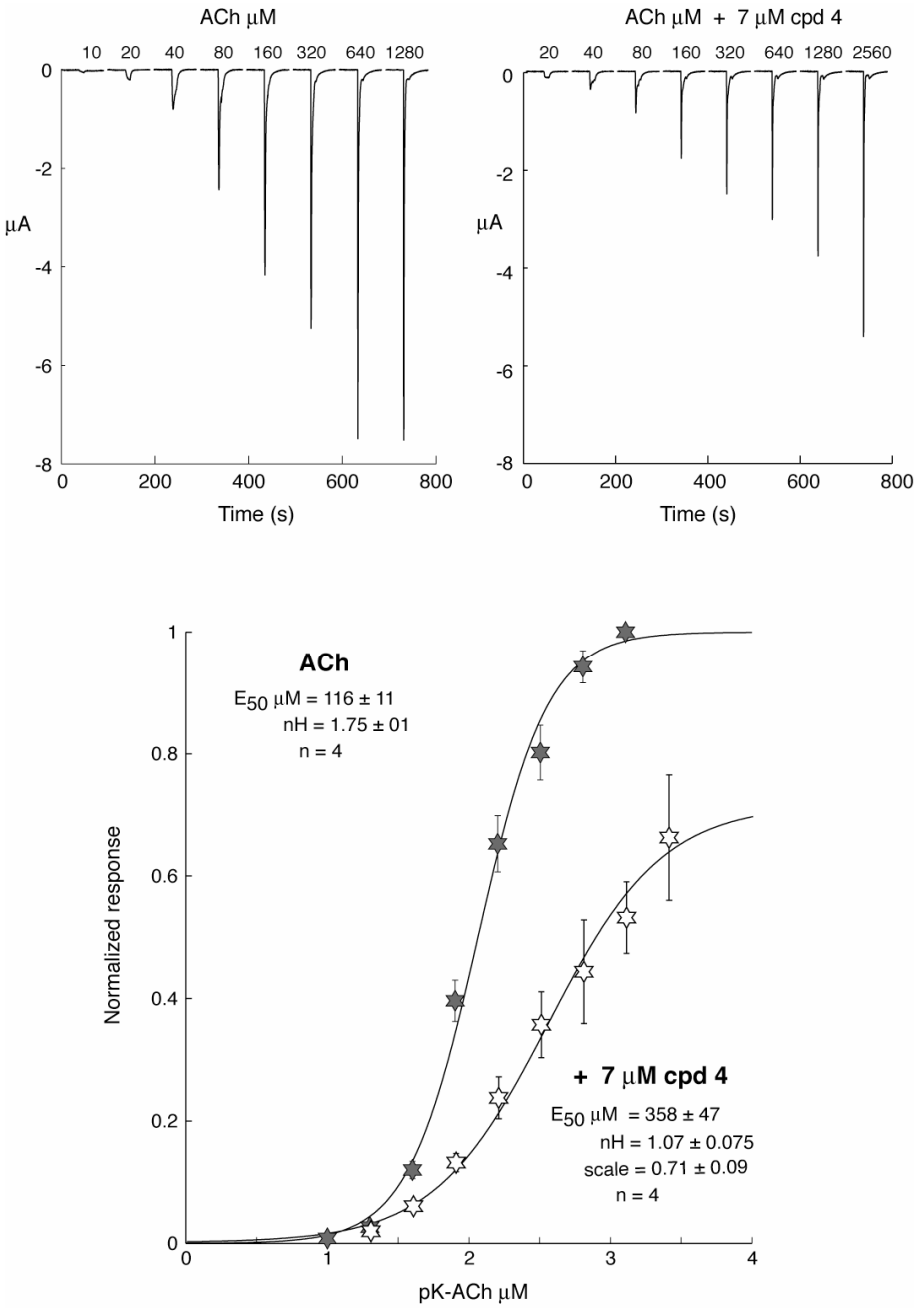
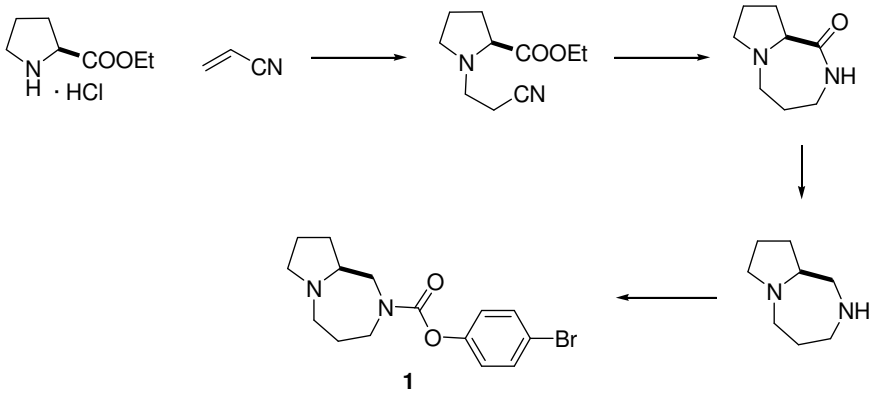


Figure S6. Effects of compound 4 on the ACh concentration activation curve. To examine in more details the effects of compounds the ACh concentration activation curve was determined in absence or presence of a fixed concentration of the compound. Typical currents evoked by a series of ACh concentrations are shown in the left panel. ACh pulses were applied at 2 minutes interval to allow full recovery between pulses. Plot of the peak inward current as a function of the logarithm of the ACh concentration yielded typical concentration activation curves that were readily fitted with the empirical Hill equation. EC50 and nH coefficients for the best fit (continuous curves) are indicated on the graph. Note that exposure to compound 4 caused a non-surmontable inhibition indicative of a non-competitive blockade of the receptor.

Synthesis

General. All reagents were purchased from commercial sources without further purification. All solvents used in reactions were distilled from technical quality. Dry solvents were obtained directly from a solvent drying system. Chromatographic purifications (flash) were performed with Silica Gel 60 from Fluka (0.04-0.063 nm; (230-400) mesh ASTM). Preparative RP-HPLC was performed with a Waters Delta Prep 4000 system with a Waters Prepak Cartridge (500 g) as column and Waters 486 Tunable Absorbance Detector. Analytical RP-HPLC was performed on Waters 600E systems with a Waters Atlantis (4.6 mm × 100 mm, dC_{18}, 5 lm) column, UV detection with Waters 996 photodiode array detector. Eluents for all systems were: A: water with 0.1% TFA, C: Acetonitrile/water (90/10) with 0.1% TFA and D: Acetonitrile/water (40/60) with 0.1% TFA. NMR spectra were acquired on a Bruker 300 MHz or a Bruker 400 MHz instrument. ^{1}H and ^{13}C chemical shifts are quoted relative to solvent signals.[2] Carbons multiplicities have been assigned either by Attached Proton Test (APT) or Distorsionless Enhancement Polarization Transfer (DEPT) experiments. Mass spectra were obtained by electron spray ionization (ES-MS) on a Micromass Autospec Q (Waters / Micromass) instrument in the positive mode.

Synthesis of ligand 1



1

(*S*)-ethyl 1-(2-cyanoethyl)pyrrolidine-2-carboxylate.[3] Following the procedure described by Guryn et al[3], L-proline ethyl ester hydrochloride (7.8 g, 43 mmol) and acrylonitrile (13 mL, 197.8 mmol) were dissolved in 15 mL of water. When the mixture was heated up to 50 ºC, a solution of KOH (2.41 g, 43 mmol) in 35 mL of water was added. The reaction was stirred at 50 ºC for 1.5 hours and then 1 more hour at 70 ºC. After cooling down to room temperature, the mixture was extracted with Et_2O, the organic fractions dried (Na_2SO_4), filtered and evaporated under reduced pressure to obtain the compound as a colorless oil (4.92 g, 58% yield). **^{1}H NMR** ($CDCl_3$, 300 MHz)

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[2] Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. NMR chemical shifts of common laboratory solvents as trace impurities. *J. Org. Chem.*, **1997**, *62*, 7512-7515

[3] Guryn, R. Synthesis of 1,5-diazabicyclo[5.3.0]decane and 1,5-diazabicyclo[5.4.0]undecane. *Pol. J. Chem*. **1985**, *59*, 1243-1246

δ 4.16 (q, J = 7 Hz, 2H), 3.31-3.24 (m, 1H), 3.20-3.11 (m, 1H), 3.05 (td, J_1 = 15 Hz, J_2 = 12 Hz, 1H), 2.84-2.74 (m, 1H), 2.52 (t, J = 7 Hz, 3H), 2.19-2.02 (m, 1H), 2.00-1.76 (m, 3H), 1.26 (t, J = 7 Hz, 3H).

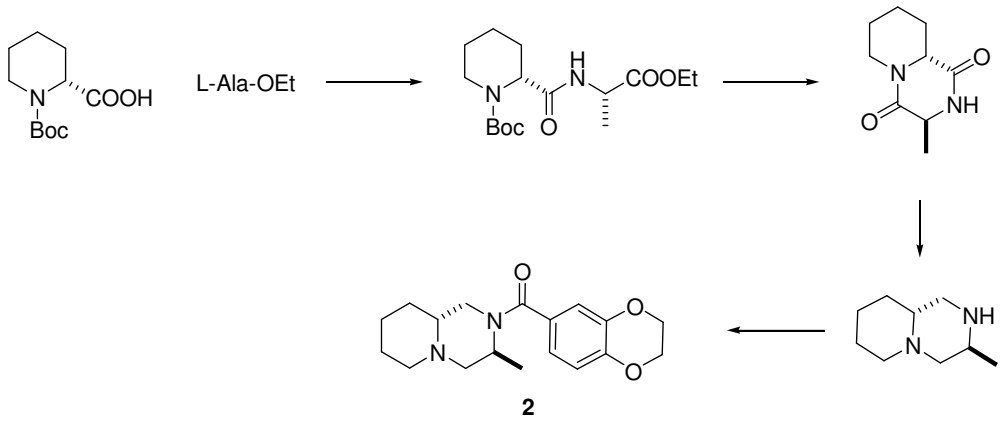
(*S*)-octahydropyrrolo[1,2-*a*][1,4]diazepin-1-one.[3] Following the procedure described by Guryn et al[3], (*S*)-ethyl 1-(2-cyanoethyl)pyrrolidine-2-carboxylate (4.92 g, 25.1 mmol) was dissolved in MeOH (150 mL). To this solution was added ~ 5 g of Nickel Raney (50% in water), trying to remove as much water as possible by decantation. The reaction was placed with H_2 at 48 bar for 6 hours at 80°C (until the reaction stops consuming H_2). The mixture was filtered over Celite and the cake was washed with MeOH. The organic fraction was evaporated, and the obtained solid was recristallized with acetone to obtain the pure product as a white solid (2.21 g, 57% yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 6.40 (br s, 1H), 3.39-3.03 (m, 5H), 2.67-2.51 (m, 1H), 2.50-2.30 (m, 2H), 1.94-1.62 (m, 5H).

(*S*)-octahydro-1H-pyrrolo[1,2-*a*][1,4]diazepine.[3] In a 2-necked round-bottom flask equipped with a condenser was prepared a suspension of $LiAlH_4$ (0.765 g, 20.16 mmol) in dry THF (20 mL) under Ar. That suspension was cooled down to 0 ºC followed by the addition dropwise of a solution of the previously synthesized lactame (1.55 g, 10.08 mmol) in dry THF (20 mL). When the H_2 release stopped, the mixture was heat up to reflux for 14 hours. The reaction was quenched by adding dropwise a saturated solution of Rochelle's salt in water (11.4 g of salt in 6 mL of water). This mixture was vigorously stirred for 30 minutes, filtered over Celite and the cake washed with THF. The organic solvent was then evaporated under reduced pressure and the residue was distilled under reduced pressure (15 mbar, 110 ºC). The desired amine was obtained as a colorless oil (0.516g, 36% yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 3.07-2.86 (m, 5H), 2.59-2.28 (m, 4H), 2.16 (br s, 1H), 1.97-1.58 (m, 5H), 1.45-1.29 (m, 1H).

(*S*)-4-bromophenyl hexahydro-1*H*-pyrrolo[1,2-*a*][1,4]diazepine-2(3*H*)-carboxylate, TFA salt (1). 4-bromophenyl chloroformate (105 µL, 0.74 mmol), EDC hydrochloride (0.178 g, 0.93 mmol), HOBt hydrate (0.126 g, 0.93 mmol), *N*-methyl morpholine (203 µL, 1.85 mmol) and (*S*)-octahydro-1*H*-pyrrolo[1,2-*a*][1,4]diazepine (0.104 g, 0.74 mmol) were dissolved in this order in CH_2Cl_2 (1 mL). The reaction was stirred for 14 hours at room temperature under N_2. Once the reaction was completed, it was extracted with saturated $NaHCO_3$ (x3). The combined organic fractions were dried (Na_2SO_4), filtered and evaporated under reduced pressure. The crude was purified using a preparative RP-HPLC and only fractions with a purity >95% were lyophilized. The desired compound was obtained as a colorless oil (26 mg, 0.059mmol, 8% yield). **^{1}H NMR** (D_2O, 400 MHz) mixture of rotamers δ 7.66 (d, J = 9 Hz, 2H), 7.14 (d, J = 7 Hz, 2H), 4.40-3.56 (m, 7H), 3.47-

3.20 (m, 2H), 2.54-1.85 (m, 6H); **^{13}C NMR** (D_2O, 75 MHz) mixture of 4 rotamers δ 155.8 (C), 155.6 (C), 155.3 (C), 155.0 (C), 149.8 (C), 149.7 (C), 149.6 (C), 132.6 (CH), 123.7 (CH), 118.8 (C), 118.7 (C), 118.7 (C), 67.0 (CH), 66.9 (CH), 66.2 (CH), 65.5 (CH), 57.2 (CH_2), 57.1 (CH_2), 56.9 (CH_2), 56.8 (CH_2), 53.7 (CH_2), 53.6 (CH_2), 51.4 (CH_2), 51.3 (CH_2), 47.3 (CH_2), 47.0 (CH_2), 46.9 (CH_2), 46.8 (CH_2), 46.2 (CH_2), 43.9 (CH_2), 43.7 (CH_2), 28.1 (CH_2), 28.0 (CH_2), 27.7 (CH_2), 27.6 (CH_2), 26.2 (CH_2), 26.0 (CH_2), 25.2 (CH_2), 25.0 (CH_2), 23.3 (CH_2), 21.7 (CH_2), 21.6 (CH_2); **MS** (ESI$^+$) *m/z* 339.2 ([M+1, Br^{79}]$^+$), 341.2 ([M+1, Br^{81}]$^+$); **HRMS** (ESI$^+$) calcd. for $C_{15}H_{20}Br^{79}N_2O_2$ 339.0708 ([M+1]$^+$]) found 339.0698; **analytical RP-HPLC** isocratic A/D (50:50) 10 min, flow 1.0 mL/min, λ = 254 nm, t_R = 5.2 min.

Synthesis of ligand 2



(3*S*,9*aR*)-hexahydro-3-methyl-6*H*-pyrido[1,2-*a*]pyrazine-1,4-dione.[4] Procedure adapted from patent WO 2005/058327. To a solution of (*R*)-(+)-*N*-Boc-2-piperidinecarboxylic acid (2.0 g, 8.72 mmol) in CH_2Cl_2 (30 mL) was added EDC hydrochloride (2.01 g, 10.4 mmol), HOBt hydrate (1.22 g, 8.98 mmol), triethylamine (2.43 mL, 21.8 mmol) and L-alanine ethyl ester hydrochloride (1.34 g, 8.72 mmol). The reaction was stirred for 15 hours. Once the reaction was finished, it was extracted with HCl 0.5M and brine. The aqueous phase was then basified to pH = 8 with Na_2CO_3 and extracted with CH_2Cl_2. All the organic phases from the acid and basic extractions were combined, dried (Na_2SO_4) and filtered. The organic solvent was evaporated under reduced pressure. TFA (8 mL) was added to the solid residue, the mixture was stirred for 1 hour and, afterwards, the excess of TFA was evaporated under reduced pressure. The residue was then dissolved in MeOH (75 mL) and triethylamine (8 mL) was added to the solution. This mixture was heated up to reflux for 4 hours under N_2. The organic solvents were evaporated to dryness and a white solid was obtained. This solid was purified by recrystallization (48% hexane, 48% ether, 4% ethanol) to give the compound (0.624 g, 34% yield, ~87% purity by NMR, the impurity is triethylamine hydrochloride). **^{1}H NMR**

[4] Reyniers, F. S.; Borremans, F.; Anteunis, M. Solution Conformation of Cyclic Dipeptides of Pipecolic and Thiapipecolic Acid Combined with Glycine and Sarcosine. *Bull. Soc. Chim. Belg*., **1985**, *94*, 413-420

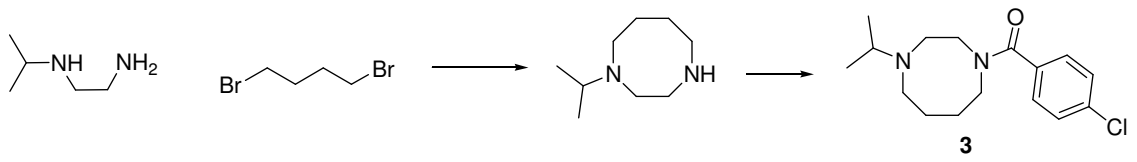
($CDCl_3$, 300 MHz) δ 4.66 (dm, J = 13 Hz, 1H), 4.08 (q, J = 7 Hz, 1H), 3.88-3.78 (m, 1H), 2.49 (td, J_1= 13 Hz, J_2= 3 Hz, 1H), 2.35-2.24 (m, 1H), 2.03-1.93 (m, 1H), 1.76-1.65 (m, 1H), 1.65-1.35 (m, 6H).

(3*S*,9*aR*)-octahydro-3-methyl-1*H*-pyrido[1,2-*a*]pyrazine.[5] Procedure adapted from patent WO 2005/058327. In a 2-necked round-bottom flask with reflux was prepared a suspension of $LiAlH_4$ (0.311 g, 8.19 mmol) in dry THF (10 mL) under Ar. That suspension was cooled down to 0 ºC followed by the addition dropwise of the diketopiperazine (0.498 g of 87% purity, 0.447 g real weight, 2.45 mmol) dissolved in 10 mL of dry THF. When the H_2 release stopped, the mixture was heat up to reflux for 14 hours. The reaction was quenched by adding dropwise a saturated solution of Rochelle's salt in water. This mixture was stirred for 3 hours, filtered over Celite and the cake washed with THF. The organic solvent was then evaporated under reduced pressure. The residue was extracted with CH_2Cl_2/brine, the organic fractions dried (Na_2SO_4), filtered and evaporated under reduced pressure. The oily residue was distilled under reduced pressure (2 mbar, 100 ºC) to yield the compound as a colorless oil (0.084g, 22% yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 3.00-2.87 (m, 1H), 2.86-2.66 (m, 3H), 2.55 (ddd, J_1 = 12 Hz, J_2 = 10 Hz, J_3 = 0.7 Hz, 1H), 2.06-1.94 (m, 1H), 1.81-1.45 (m, 7H), 1.36-1.08 (m, 2H), 1.00 (dd, J_1 = 6 Hz, J_2 = 0.7 Hz, 3H).

((3*S*,9*aR*)-hexahydro-3-methyl-1*H*-pyrido[1,2-*a*]pyrazin-2(6*H*)-yl)(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)methanone, TFA salt (2). 1,4-benzodioxane-6-carboxylic acid (0.023 g, 0.13 mmol), EDC hydrochloride (0.031 g, 0.16 mmol), HOBt hydrate (0.022 g, 0.16 mmol), *N*-methyl morpholine (36 µL, 0.32 mmol) and (3*S*,9*aR*)-octahydro-3-methyl-1*H*-pyrido[1,2-*a*]pyrazine (0.104 g, 0.13 mmol) were dissolved in this order in CH_2Cl_2 (0.5 mL). The reaction was stirred for 14 hours at room temperature under N_2. Once the reaction was completed, it was extracted with saturated $NaHCO_3$ (x3). The organic fractions were dried (Na_2SO_4), filtered and evaporated under reduced pressure. The crude was purified using a preparative RP-HPLC and only fractions with a purity >95% were lyophilized. The desired compound was obtained as a colorless oil (22 mg, 0.053 mmol, 41% yield). **^{1}H NMR** (D_2O, 400 MHz at 353K) δ 7.63-7.45 (m, 3H), 5.29 (br s, 1H), 4.89 (s, 2H), 4.88 (s, 2H), 4.63-4.38 (m, 2H), 4.29-3.80 (m, 4H), 3.69-3.55 (m, 1H), 2.59-1.93 (m, 9H); **^{13}C NMR** (D_2O, 100 MHz at 353K) δ 120.9 (CH), 118.3 (CH), 116.4 (CH), 65.4 (CH_2), 65.2 (CH_2), 57.4 (CH), 54.0 (CH_2), 47.8 (CH_2), 46.2 (br CH), 42.7 (br CH_2), 21.7 (CH_2), 21.3 (CH_2), 17.4 (CH_2), 15.3 (CH_3); **MS** (ESI^+) *m/z* 317.4 ($[M+1]^+$); **HRMS** (ESI^+) calcd. for $C_{18}H_{25}N_2O_3$ 317.1865 ($[M+1]^+$) found 317.1860; **analytical RP-HPLC** 100A → A/D (30:70) 20 min, flow 1.4 mL/min, λ = 254 nm, t_R = 13.2 min

[5] Bradser, C. K.; Telang, S. A. 2-azaquinolizinium oxides. *J. Org. Chem*., **1966**, *31*, 941-943

Synthesis of ligand 3

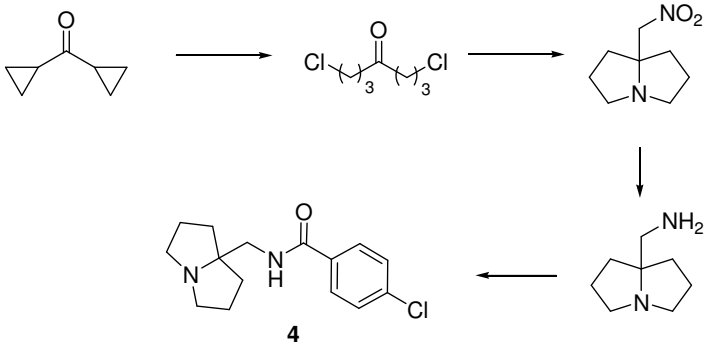


1-isopropyl-1,4-diazocane.[6] *N*-isopropylethylenediamine (2.5 mL, 19.57 mmol), 1,4-dibromobutane (2.32 mL, 19.57 mmol) and Na_2CO_3 (3.1 g, 29.35 mmol) were mixed in *o*-xylene (100 mL). The reaction was purged under N_2 and heated up to 130 ºC for 18 hours. When the reaction was finished, xylene was distilled off and the residue was extracted with AcOEt/HCl 1M. The aqueous phase whan then basified with NaOH in pellets to pH = 9 and extracted with AcOEt. This organic phase was dried (Na_2SO_4), filtered and the solvent evaporated under reduced pressure to give the desired amine as a yellow oil (1.28 g, 42% yield), which was used without further purification for the next step. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 2.78 (sep, J = 6 Hz, 1H), 2.74-2.67 (m, 2H), 2.62-2.56 (m, 2H), 2.52-2.46 (m, 4H), 1.79-1.70 (br m, 5H), 1.06 (d, J = 6 Hz, 6H).

(4-chlorophenyl)(4-isopropyl-1,4-diazocan-1-yl)methanone (3). 4-chlorobenzoic acid (0.023 g, 0.13 mmol), EDC hydrochloride (0.031 g, 0.16 mmol), HOBt (0.022 g, 0.16 mmol), *N*-methyl morpholine (36 µL, 0.32 mmol) and 1-isopropyl-1,4-diazocane (0.020 g, 0.13 mmol) were dissolved in this order in CH_2Cl_2 (0.5 mL). The reaction was stirred for 14 hours at room temperature under N_2. Once the reaction was completed, it was extracted with saturated $NaHCO_3$ (x3). The organic fractions were dried (Na_2SO_4), filtered and evaporated under reduced pressure. The final compound slowly hydrolizes in 0.1% TFA (HPLC conditions). For that reason, purification was done by column chromatography (neutral alumina, CH_2Cl_2/MeOH 0.5%) to give **3** as a yellow oil (20 mg, 0.068 mmol, 53% yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.33-7.26 (m, 2H), 7.25-7.18 (m, 2H), 3.86 (br s, 1H), 3.40 (br s, 2H), 2.83-2.00 (br m, 6H), 1.70 (br s, 4H), 1.10 (br s, 6H); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 170.5 (C), 135.8 (C), 135.0 (C), 128.6 (CH), 127.6 (CH), 54.8 (br CH_2), 54.1 (CH), 50.1 (br CH_2), 39.8 (br CH_2), 23.4 (CH_2), 20.9 (CH_3); **MS** (ESI^+) *m/z* 295.4 ([M+1, Cl^{35}]$^+$), 297.2 ([M+1, Cl^{37}]$^+$); **HRMS** (ESI^+) calcd. for $C_{16}H_{24}Cl^{35}N_2O$ 295.1572 ([M+1]$^+$]) found 295.1566.

[6] Majchrzak, M.; Kotelko, A.; Guryn, R. Pochodne oktahydrodwuazocyny-1,5 i oktahydrodwuazocyny-1,4 o spodziewanym dzialaniu farmakologicznym: I: Syntheza N-alkilowych pochodnych oktahydrodwuazocyny-1,5 i oktahydrodwuazocyny-1,4. *Acta Polon. Pharm*., **1975**, *32*, 145-148

Synthesis of ligand 4



5-nitromethyl-1-azabicyclo[3.3.0]octane.[7] Procedure adapted from Suzuki[8] and Oka[7]. Neat dicyclopropyl ketone (3 mL, 26.6 mmol) was cooled down to 0 ºC followed by bubbling HCl gas through the ketone for 30 min. The HCl flow was stopped, the reaction was allowed to warm up to room temperature and it was stirred for 3 hours. The procedure was repeated again once more and, once finished, pure 1,7-dichloro-heptan-4-one was obtained in quantitative yield (4.87 g). This ketone (3.05 g, 16.6 mmol) was mixed together with nitromethane (3.4 mL, 62.7 mmol) and the solution cooled down to 0 ºC. Then, NH_3 gas was bubbled through the solution for 30 minutes. The NH_3 flow was stopped, the reaction warm up to room temperature for 2 hours. A white precipitate appeared. The described procedure was repeated 2 more times and finally the reaction stirred for 14 more hours at room temperature. The mixture was extracted with CH_2Cl_2/NaOH 0.1M and the organic phases dried (Na_2SO_4), filtered and the solvent evaporated to dryness. The crude was purified by column chromatography (silica gel, CH_2Cl_2/MeOH 3%) to yield the desired compound as a colorless solid (1.7 g, 60% yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 4.25 (s, 2H), 3.09-2.99 (m, 2H), 2.65-2.54 (m, 2H), 2.19-2.07 (m, 2H), 1.95-1.57 (m, 6H).

5-aminomethyl-1-azabicyclo[3.3.0]octane.[7] Procedure adapted from Oka et al.[7] To a solution of 5-nitromethyl-1-azabicyclo[3.3.0]octane (1.7 g, 9.99 mmol) and NaOH (0.352 g, 8.80 mmol) in EtOH (8 mL), ~0.600 g of Nickel Raney (50% in water) were added, trying to remove as much water as possible by decantation. The mixture was placed under H_2 (1 atm) at room temperature and stirred for 15 hours. After filtration over Celite, the filtrate was evaporated under reduced pressure. This residue (containing the diamine and NaOH) was used without further purification due to instability of the molecule and the difficulties to purify it. The ^{1}H NMR matches with the one described in the literature.[7]

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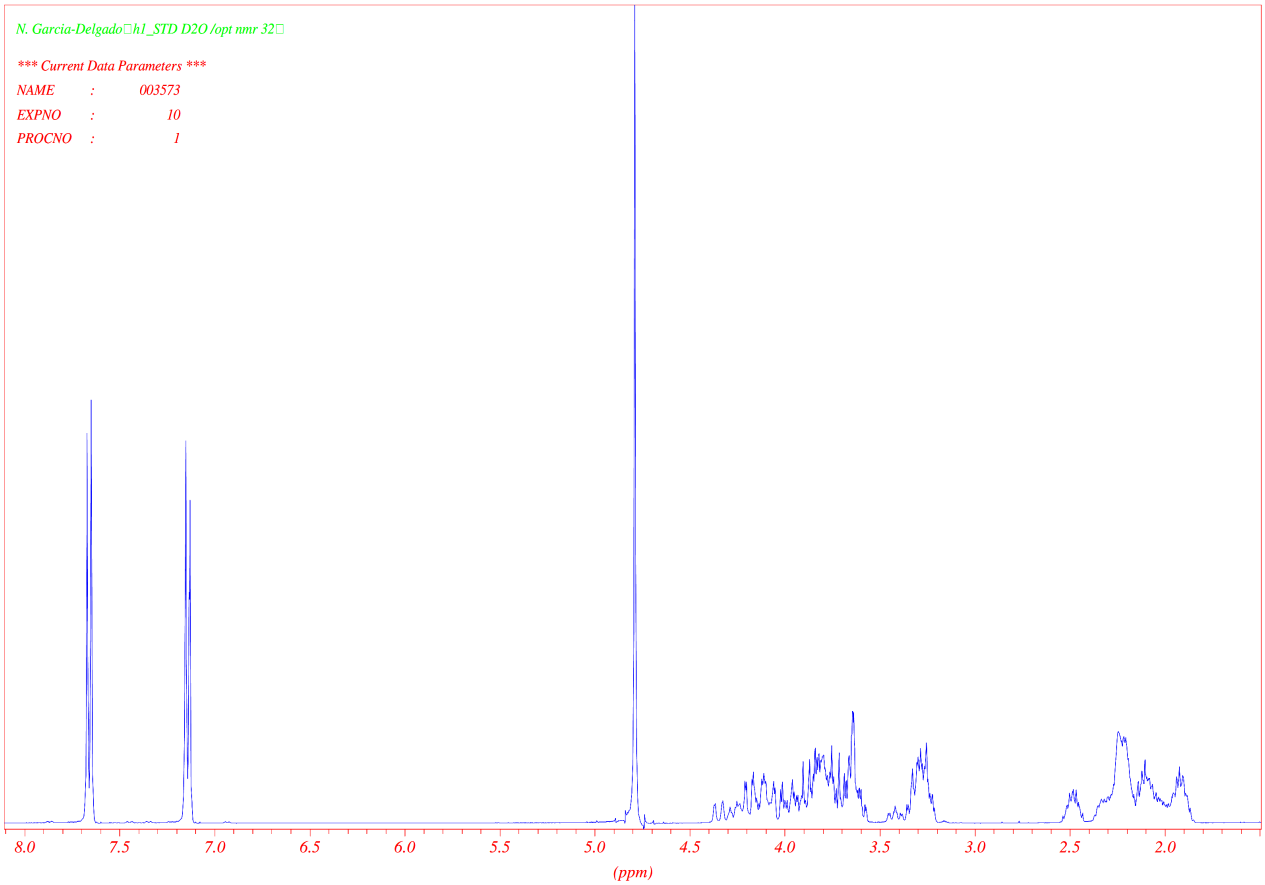
[7] Oka, M.; Matsumoto, Y.; Hirooka, K.; Suzuki, T. Synthesis of 1-azabicyclo[3.3.0]octane derivatives and their effects as piracetam-like nootropics. *Chem. Pharm. Bull.*, **2000**, *48*, 1121-1124

[8] Suzuki, T.; Oka, M.; Maeda, K.; Furusawa, K.; Mitani, T.; Kataoka, T. *N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-2-nitroalanine, a potent muscarinic agonist. *Chem Pharm. Bull.*, **1997**, *45*, 1218-1220

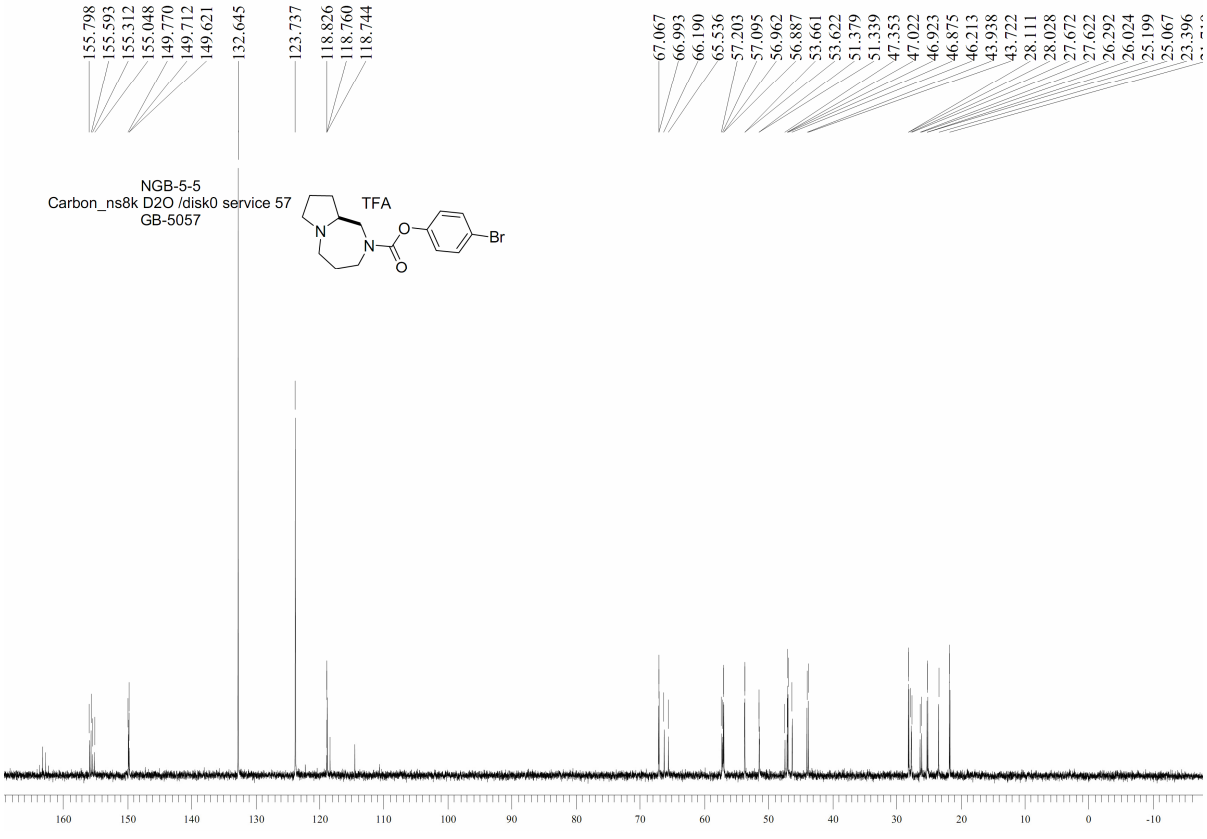
4-chloro-*N*-((hexahydro-1*H*-pyrrolizin-7*a*-yl)methyl)benzamide (4). 4-chlorobenzoic acid (0.056 g, 0.36 mmol), EDC hydrochloride (0.085 g, 0.45 mmol), HOBt (0.060 g, 0.45 mmol), *N*-methyl morpholine (98 μL, 0.89 mmol) and 5-aminomethyl-1-azabicyclo[3.3.0]octane (0.050 g, 0.36 mmol) were dissolved in this order in CH_2Cl_2 (1 mL). The reaction was stirred for 14 hours at room temperature under N_2. Once the reaction was completed, it was extracted with saturated $NaHCO_3$ (x3). The organic fractions were dried (Na_2SO_4), filtered and evaporated under reduced pressure. Purification by column chromatography (neutral alumina, CH_2Cl_2/MeOH 2%) gave **4** as a yellow solid (40 mg, 0.143 mmol, 40% yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 8.12 (br s, 1H), 7.91 (d, J = 8 Hz, 2H), 7.40 (d, J = 8 Hz, 2H), 3.63 (d, J = 6 Hz, 2H), 3.44-3.27 (m, 2H), 2.89-2.73 (m, 2H), 2.11-1.70 (m, 8H); **^{13}C NMR** ($CDCl_3$, 75 MHz) δ 166.6 (C), 137.7 (C), 132.0 (C), 128.8 (CH), 128.7 (CH), 78.0 (br C), 55.4 (CH_2), 45.6 (CH_2), 35.7 (CH_2), 24.7 (CH_2); **MS** (ESI^+) *m/z* 279.2 ($[M+1, Cl^{35}]^+$), 2281.2 ($[M+1, Cl^{37}]^+$); **HRMS** (ESI^+) calcd. for $C_{15}H_{20}Cl^{35}N_2O$ 279.1264 ($[M+1]^+$) found 279.1260; **Mp** (ºC) 158-163.

NMR spectra

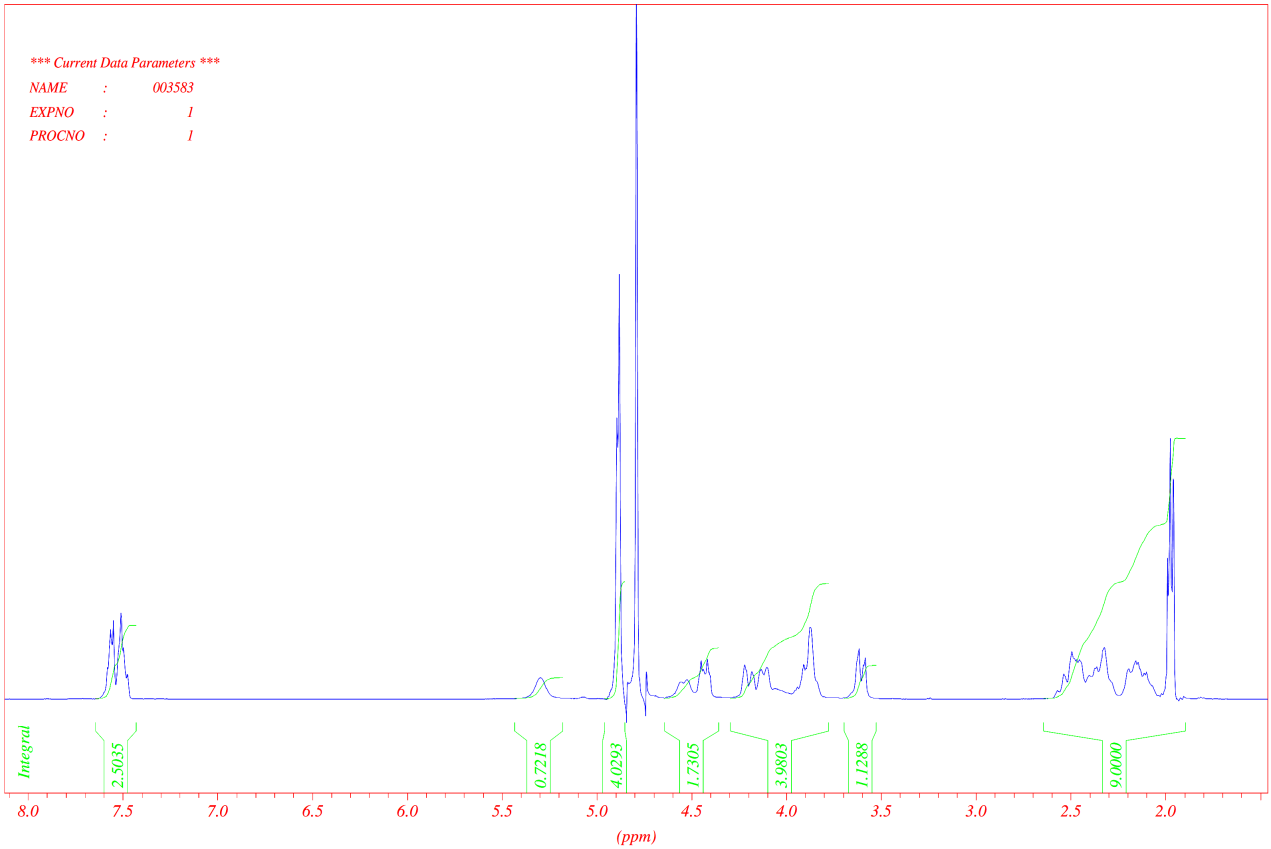
^{1}H-NMR spectrum of **1** TFA salt (D_2O, 400 MHz)



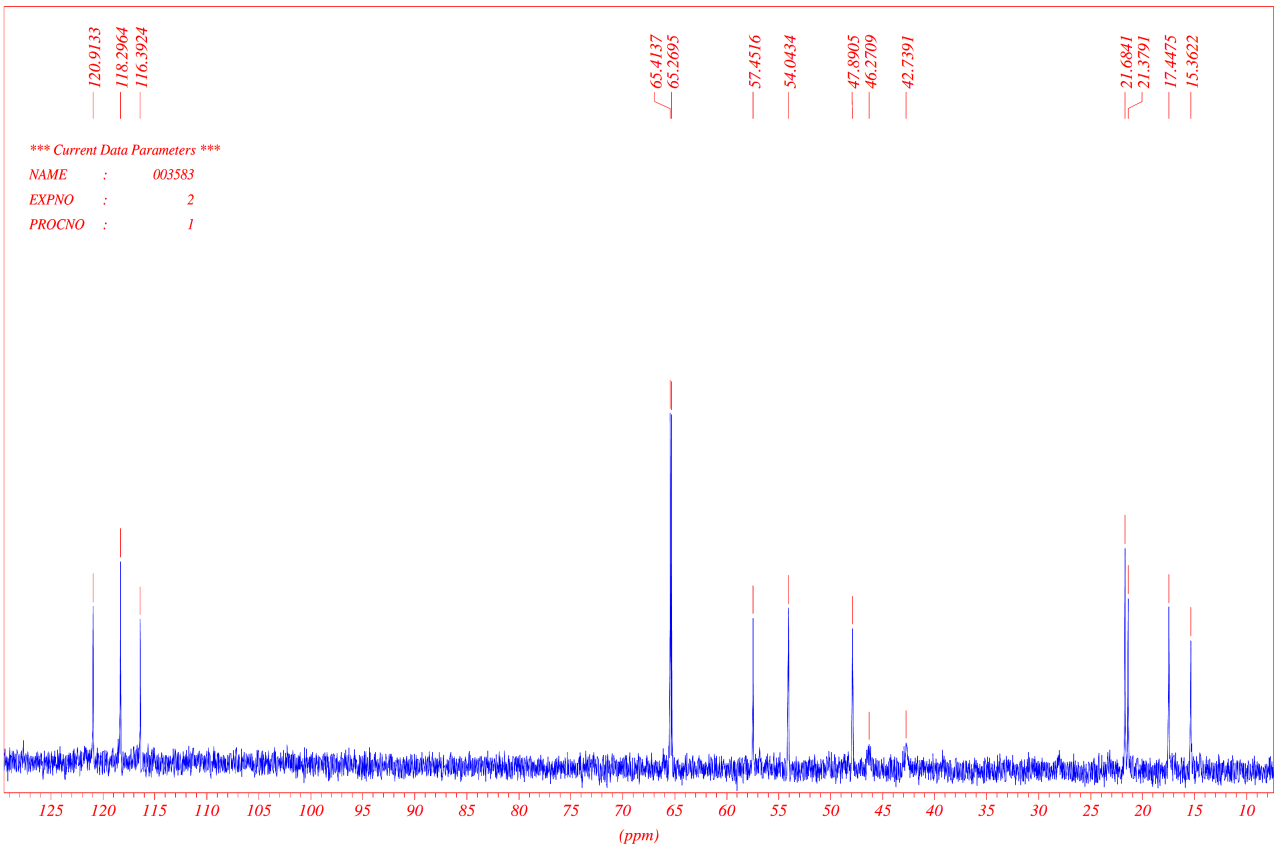
^{13}C-NMR spectrum of **1** TFA salt (D_2O, 75 MHz)



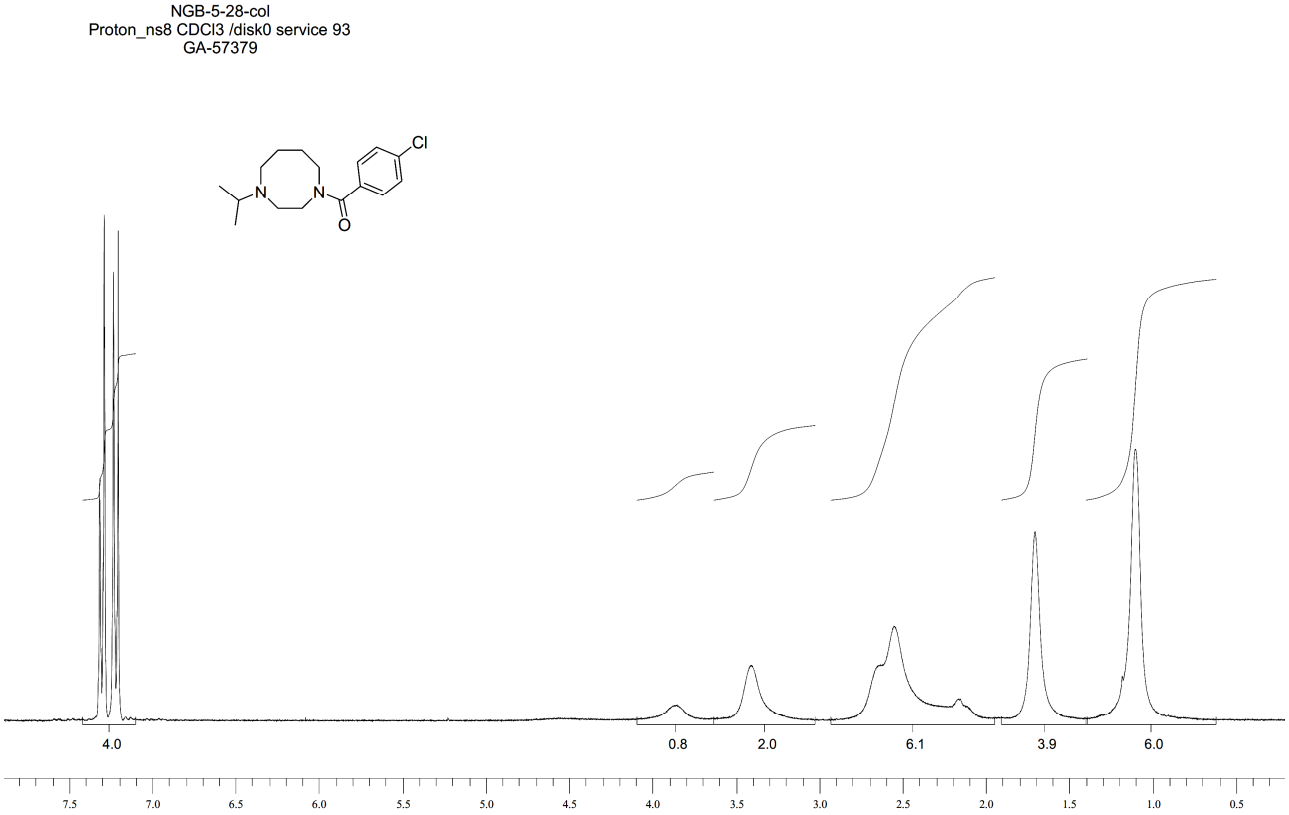
^{1}H-NMR spectrum of **2** TFA salt (D_2O, 400 MHz)



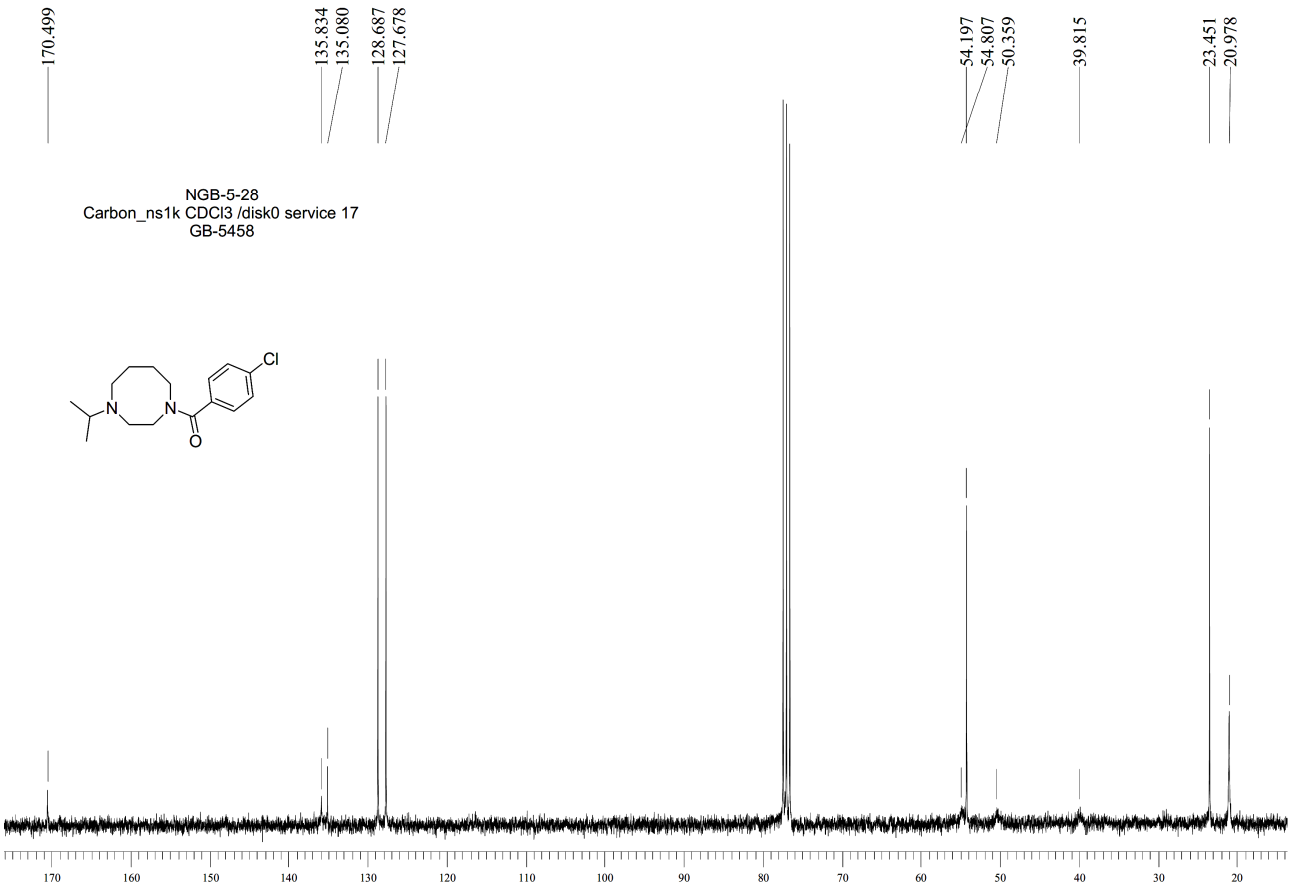
^{13}C-NMR spectrum of **2** TFA salt (D_2O, 100 MHz)



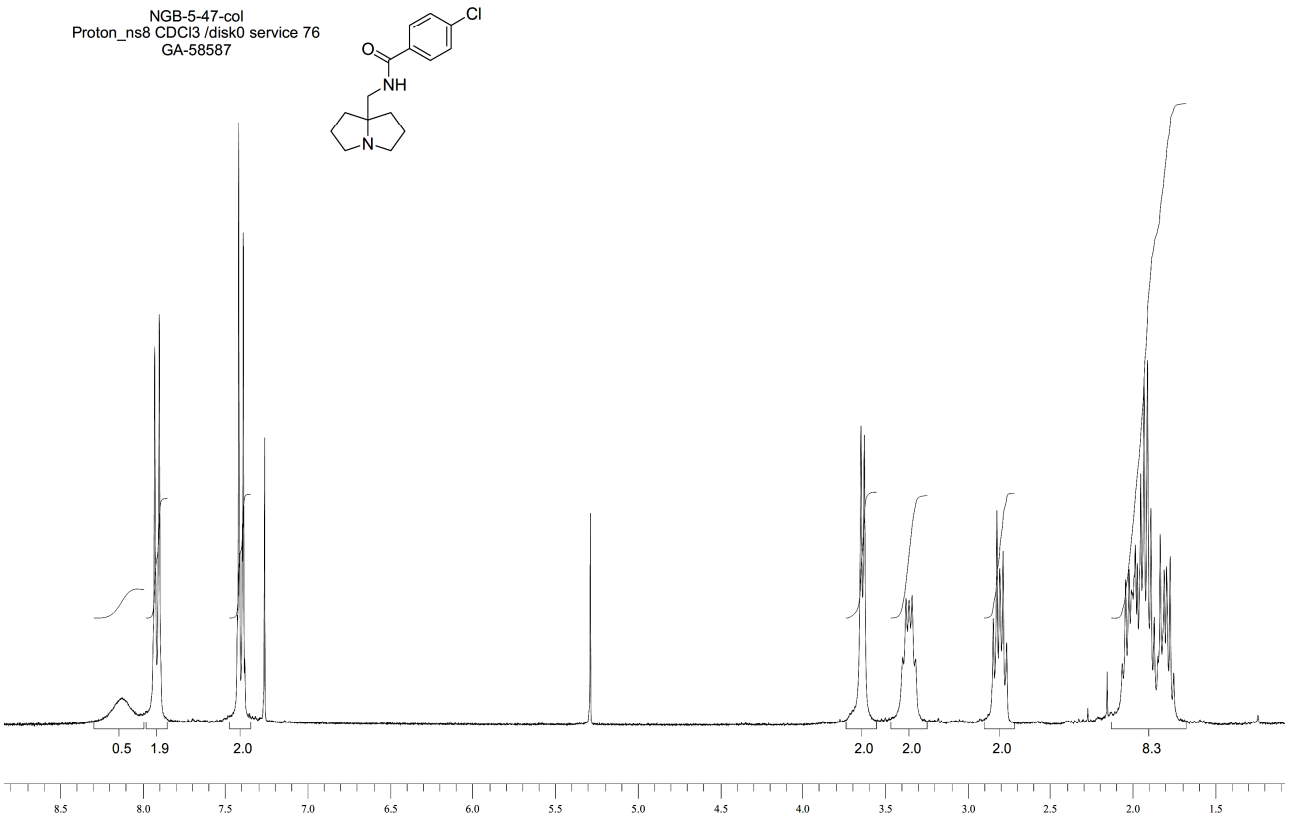
^{1}H-NMR spectrum of **3** ($CDCl_3$, 300 MHz)



^{13}C-NMR spectrum of **3** ($CDCl_3$, 75 MHz)



^{1}H-NMR spectrum of **4** ($CDCl_3$, 300 MHz)



^{13}C-NMR spectrum of **4** ($CDCl_3$, 75 MHz)