Chiral recognition of flexible melatonin receptor ligands induced by conformational equilibria

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Supplementary Material

Figures S1-13. ^{1}H NMR spectra of compounds **6**, **7**, **8**, **9**, **10** and **12**, ^{13}C NMR spectra of compounds **6**, **7**, **9**, **10** and **12**, NOESY spectrum of compound **10** and COSY spectrum of compound **12**.

Figures S14-19. Chiral HPLC chromatograms of compounds **6**, **(*R*)-6**, **(*S*)-6**, **10**, **(*R*)-10** and **(*S*)-10**.

Figure S20. Free-energy surfaces calculated from the MD simulation of **(*S*)-10** in POPC membrane model and in TIP3P water molecules.

Protocol S1. Equilibration protocol for MD simulations of MT_2 receptor-ligand complexes.

Figure S21. Time-evolution of the free energy surface calculated from the MD simulation of **(*S*)-10** in chloroform.

Figure S1. ^{1}H NMR spectrum (200 MHz, $CDCl_3$) of compound **6**.

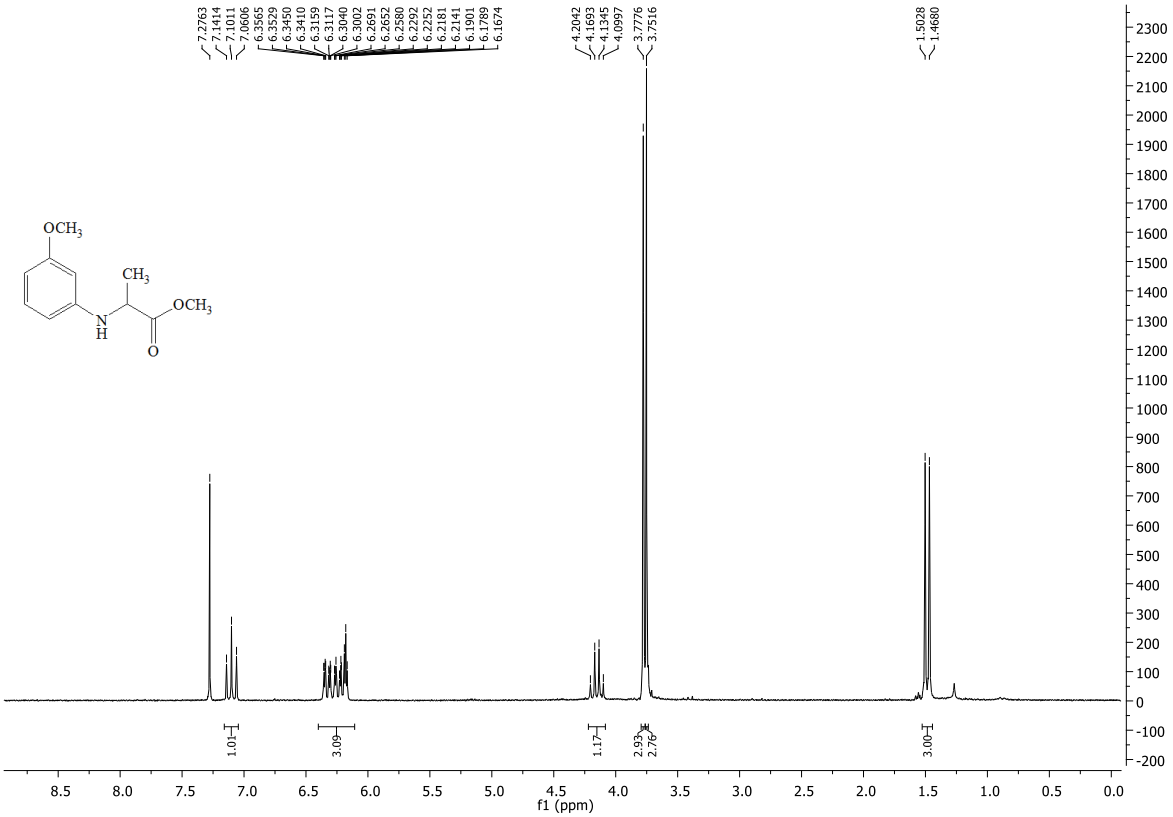


Figure S2. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **6**.

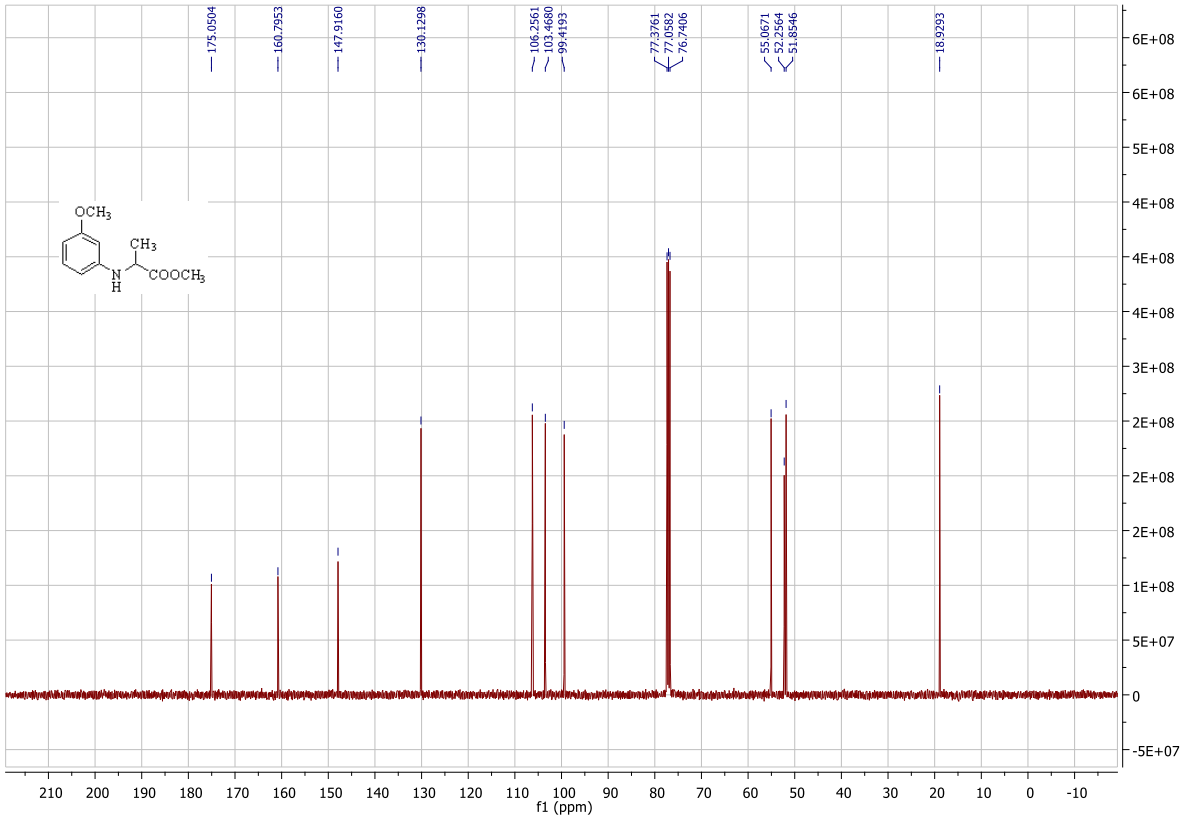


Figure S3. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **7**.

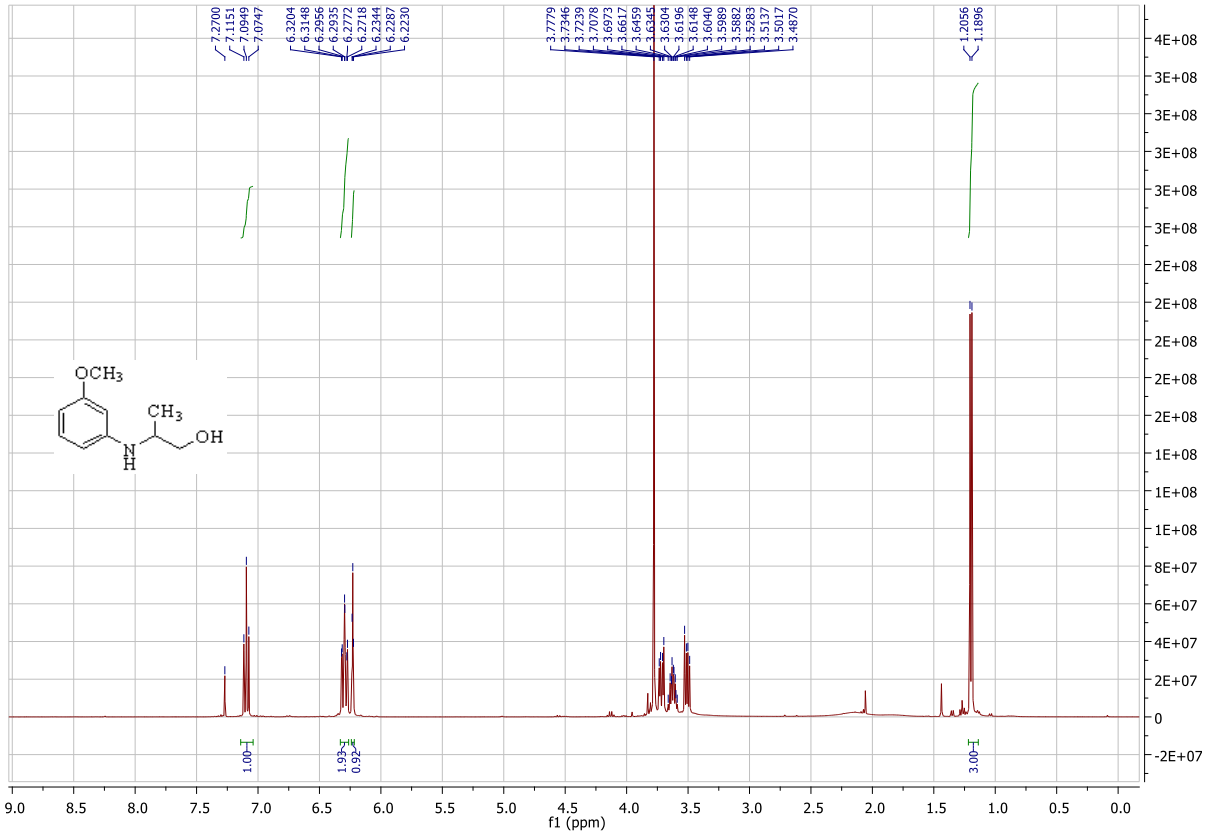


Figure S4. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **7**.

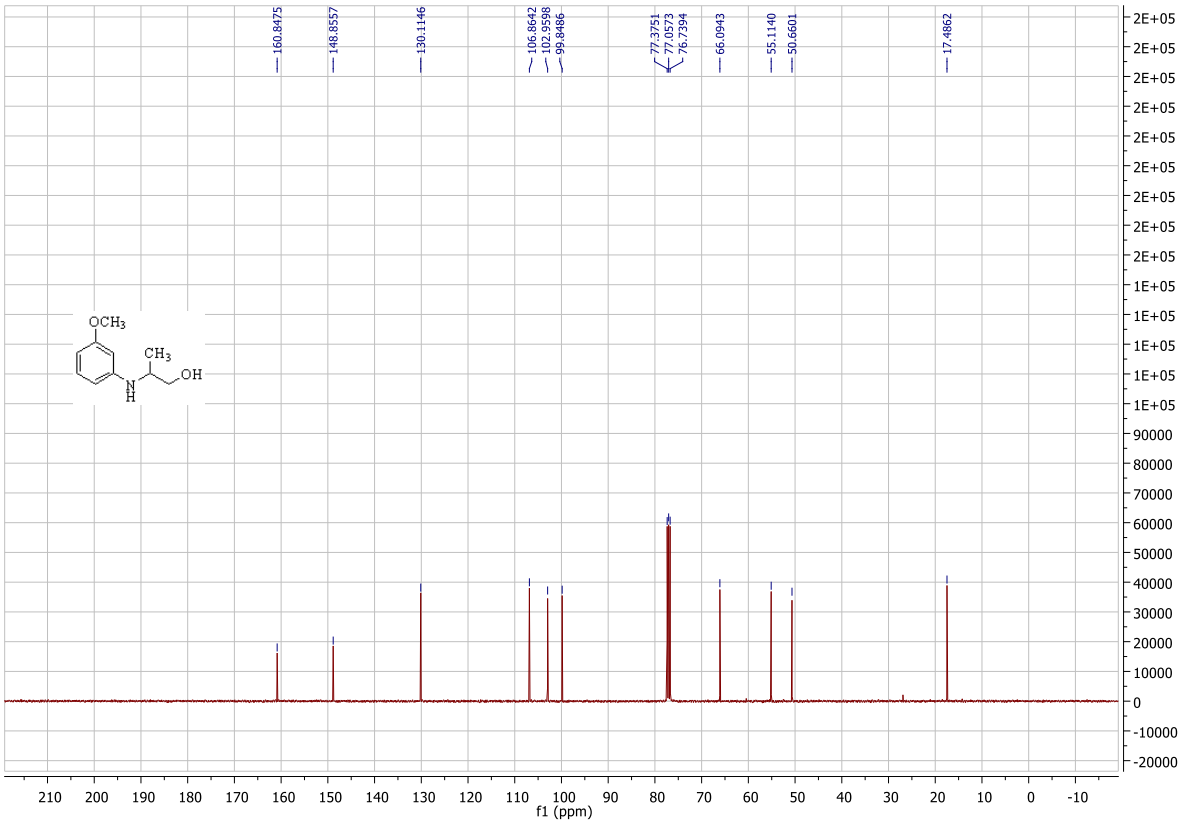


Figure S5. ^{1}H NMR spectrum (200 MHz, $CDCl_3$) of compound **8**.

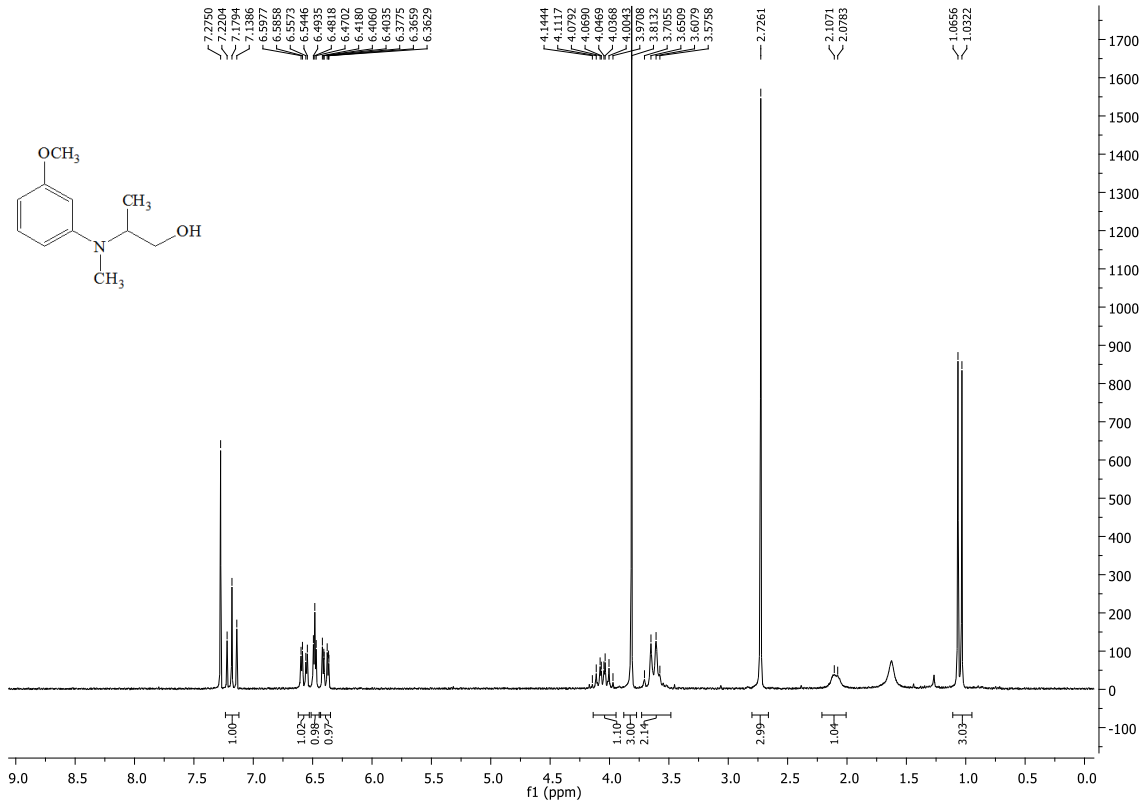


Figure S6. ^{1}H NMR spectrum (200 MHz, $CDCl_3$) of compound **9**.

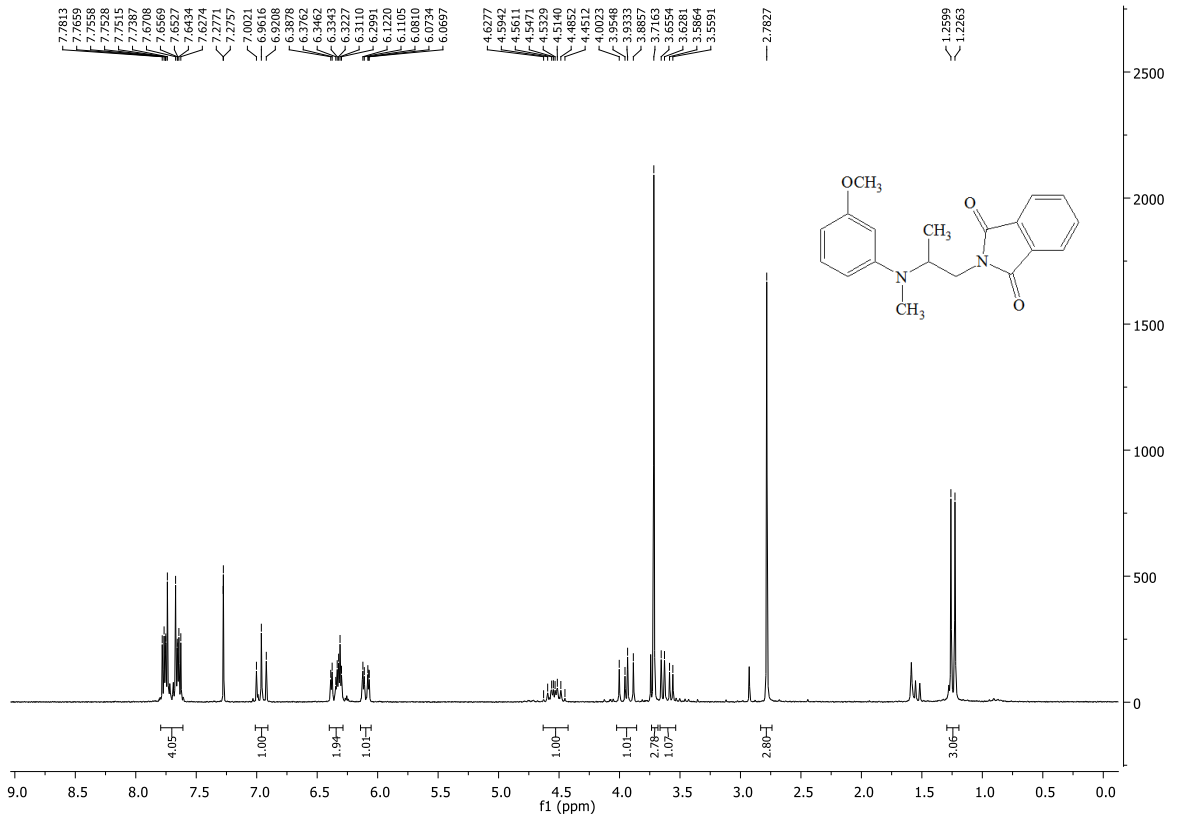


Figure S7. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **9**.

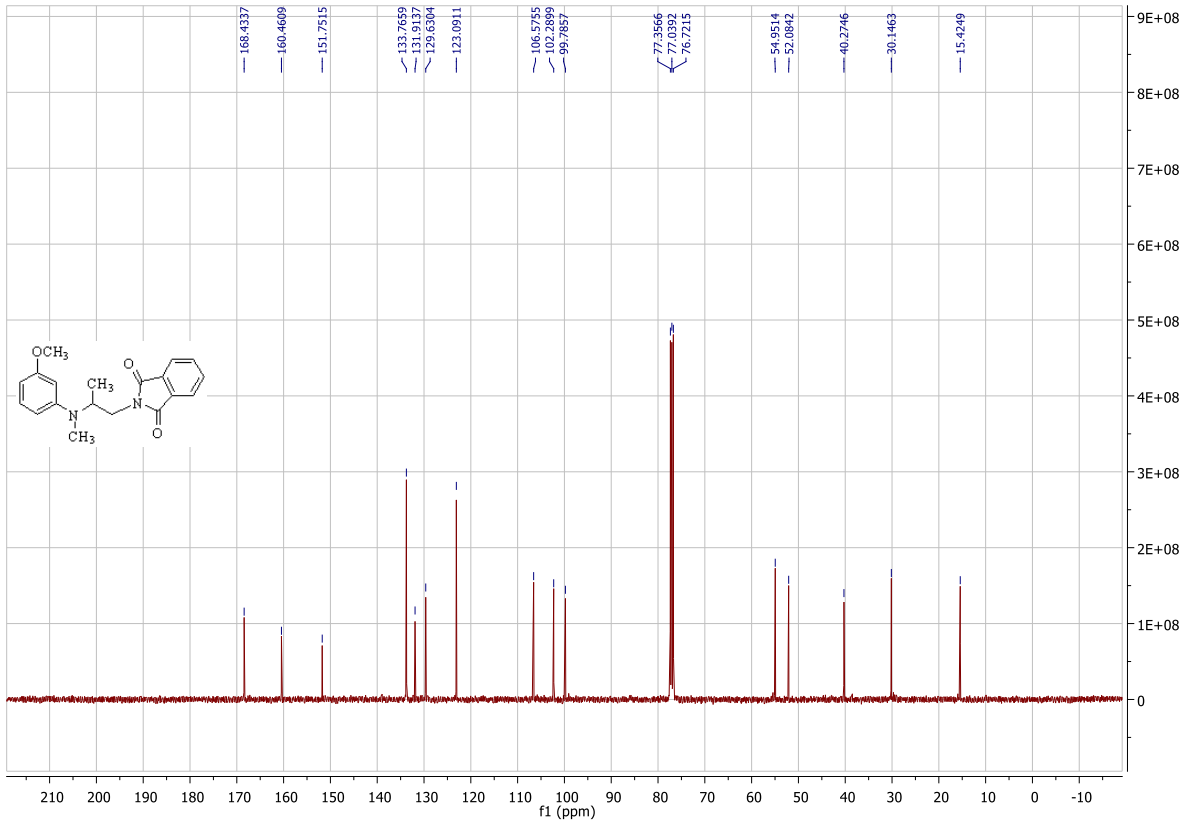


Figure S8. ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of compound **10**.

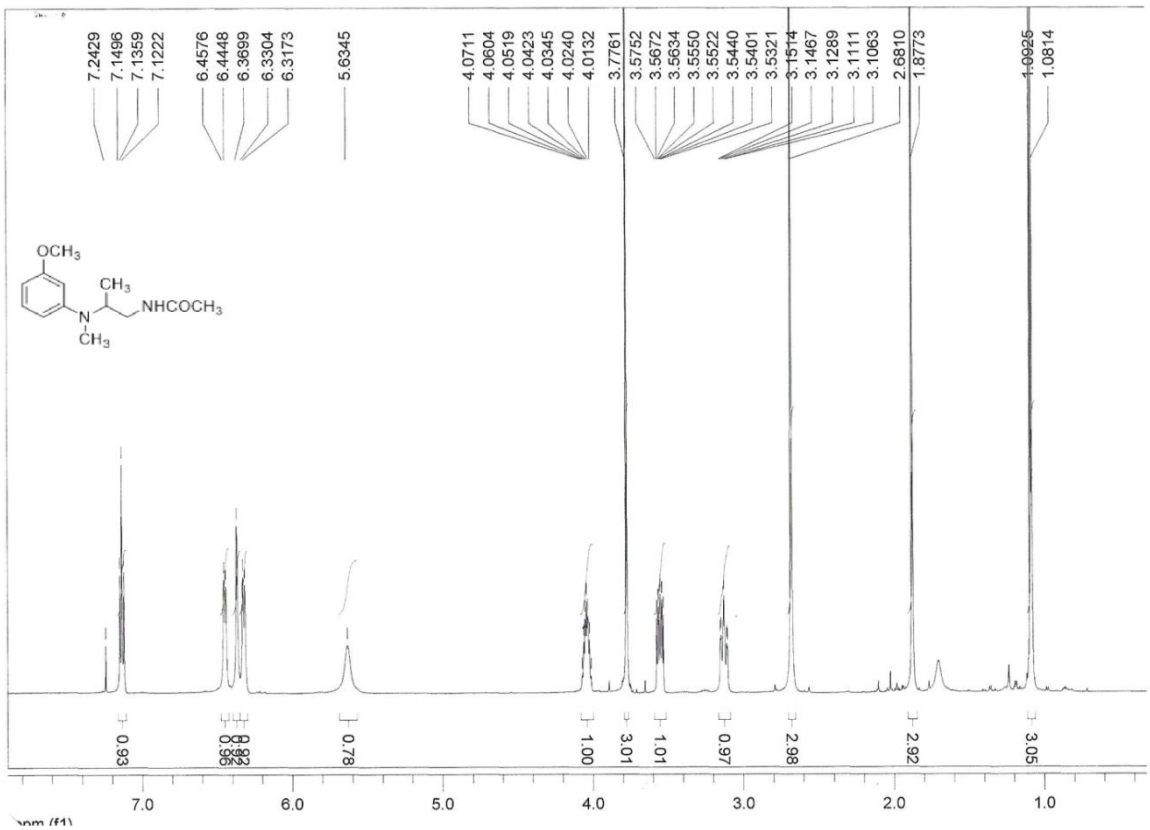


Figure S9. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **10**.

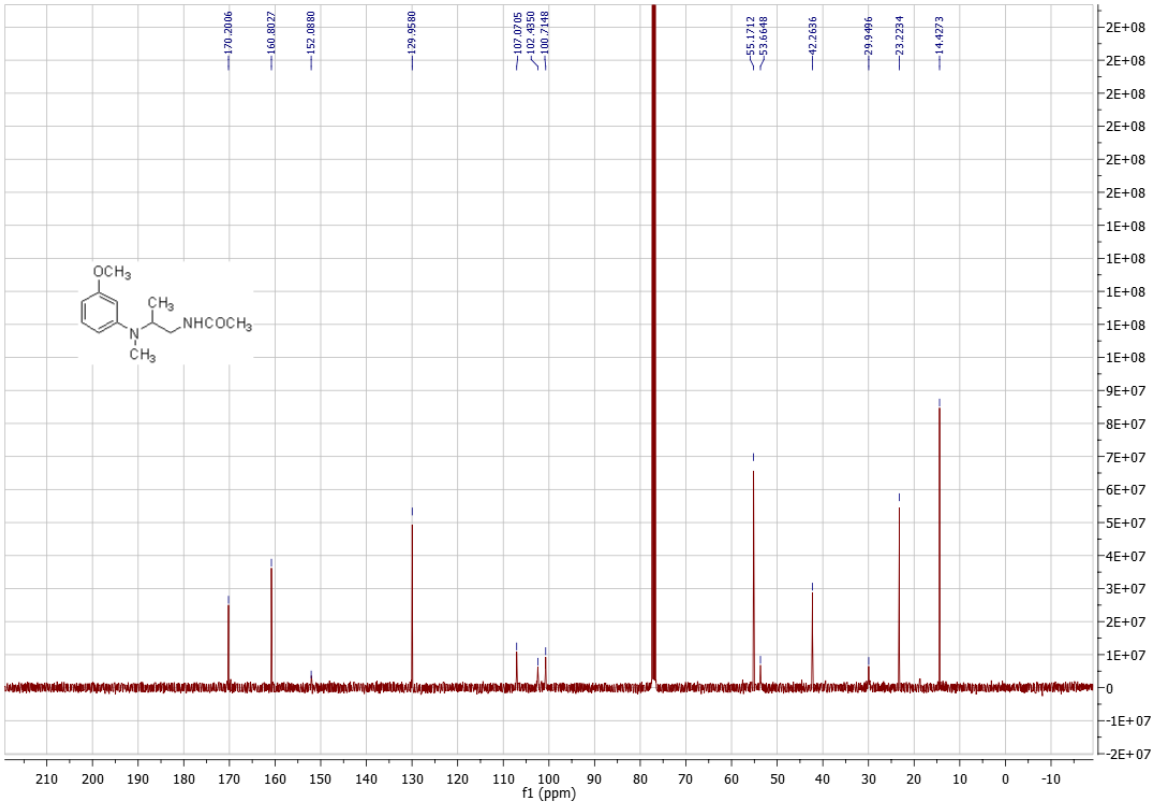
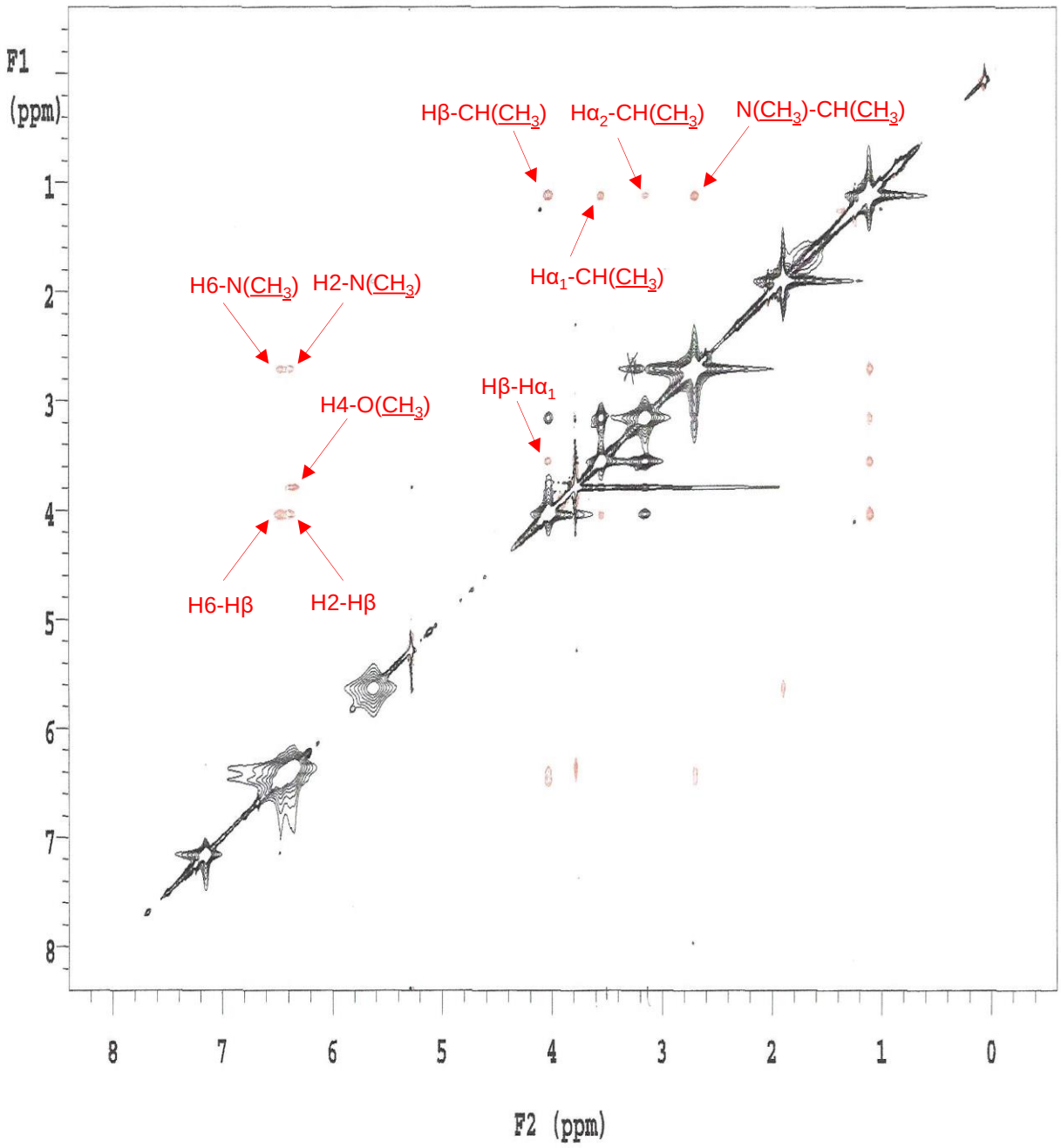


Figure S10. NOESY peaks (in red) of compound **10** (600 MHz, $CDCl_3$, 298 K). Proton assignment: (600 MHz, $CDCl_3$) δ 1.09 ($CH\underline{CH_3}$), 1.89 ($CO\underline{CH_3}$), 2.68 ($N\underline{CH_3}$), 3.13 ($H\alpha_2$), 3.55 ($H\alpha_1$), 3.77 ($O\underline{CH_3}$), 4.04 (Hβ), 5.63 ($N\underline{H}$), 6.32 (H4), 6.37 (H2), 6.45 (H6), 7.13 (H5).



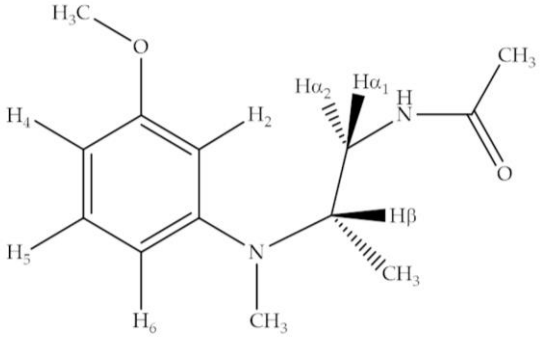


Figure S11. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **12**.

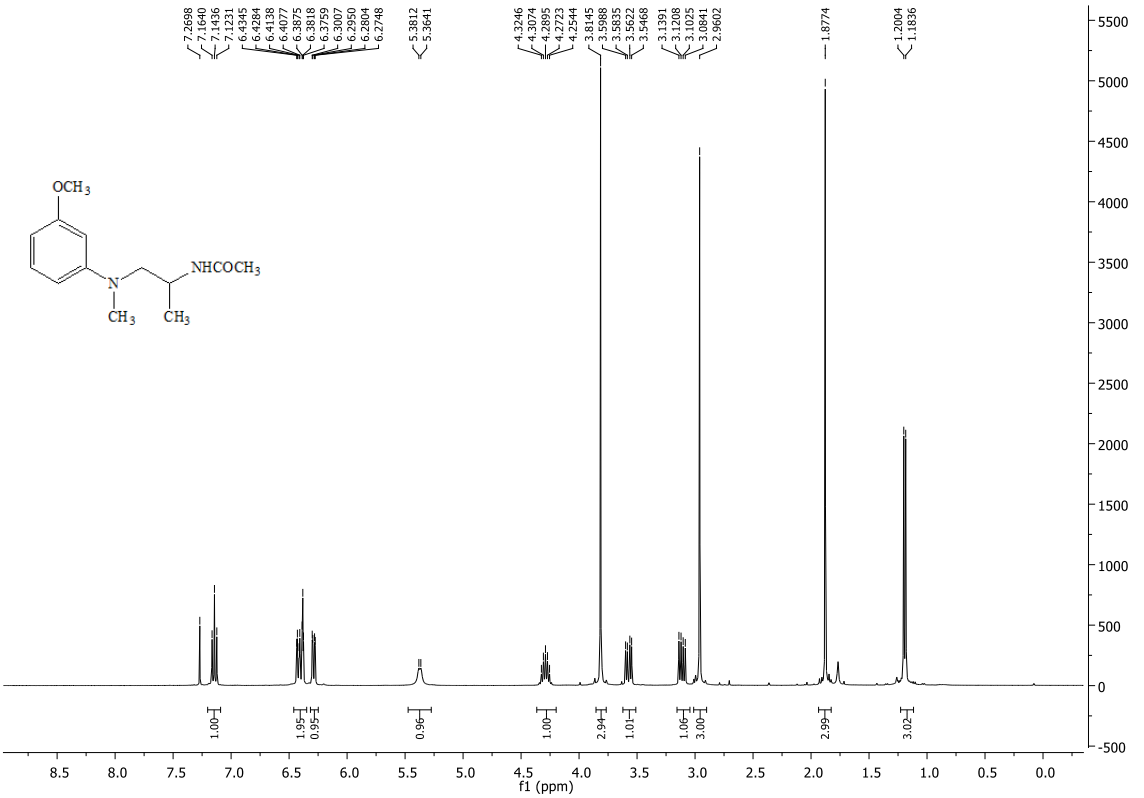


Figure S12. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **12**.

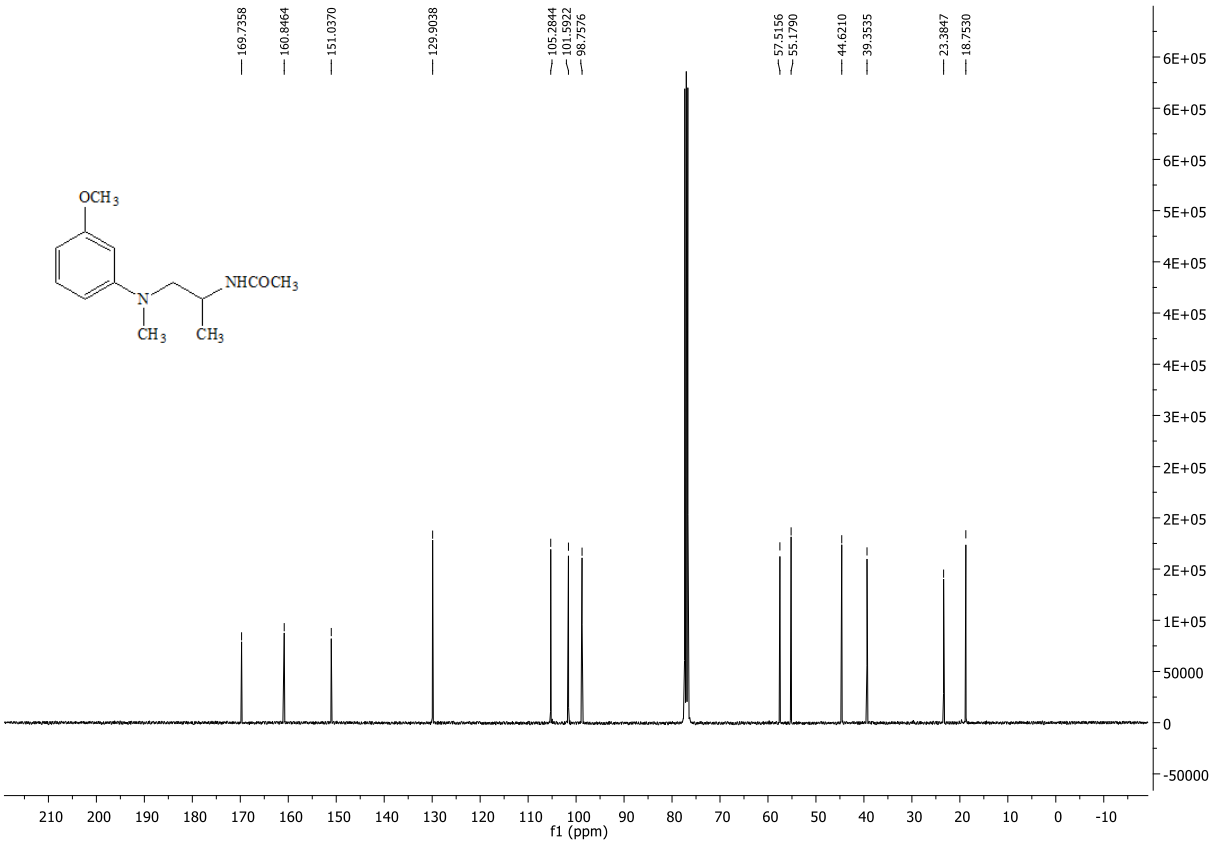
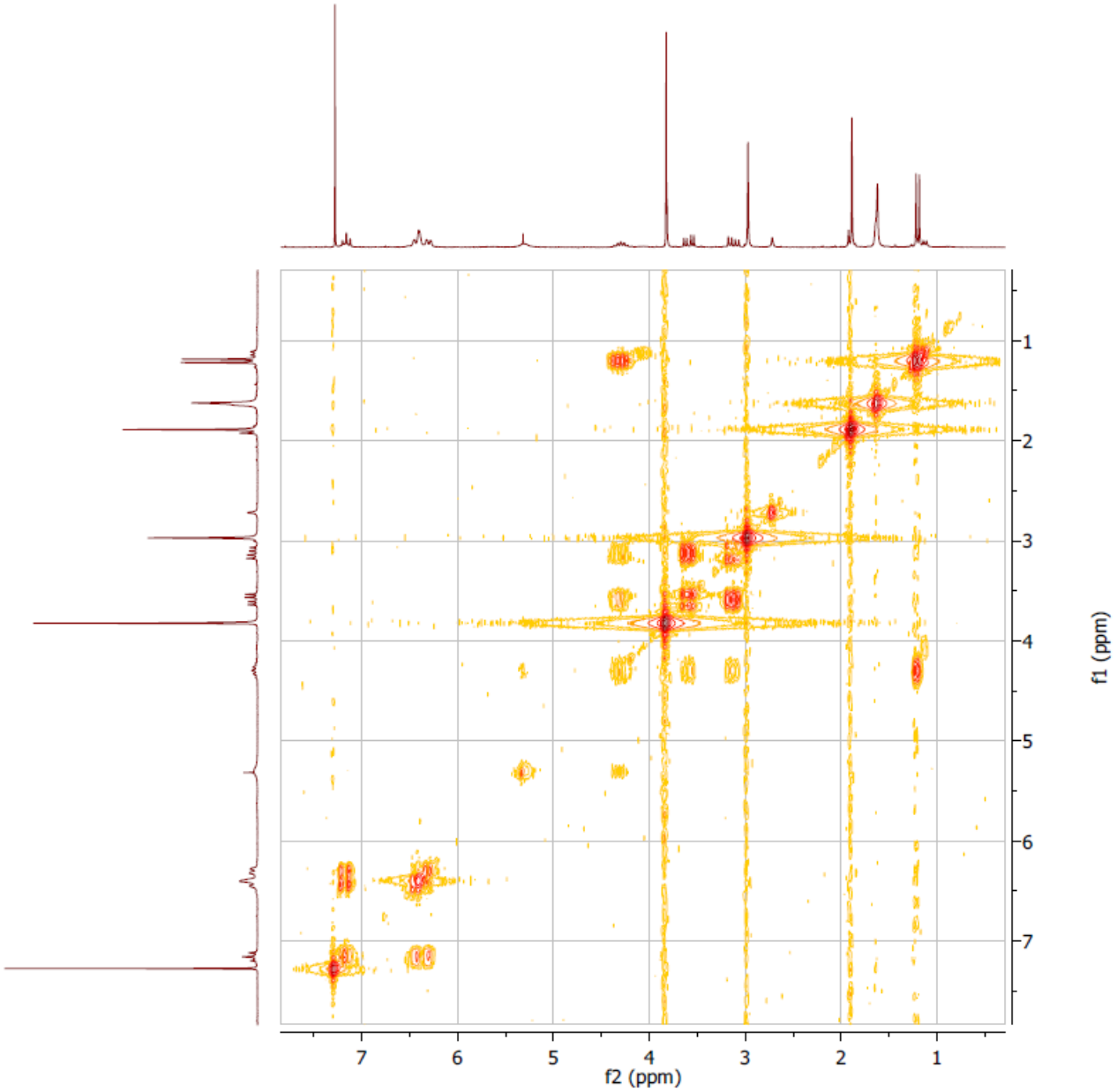


Figure S13. COSY spectrum (400 MHz, $CDCl_3$) of compound **12**.



The observed NH-CHα correlation and the absence of a coupling between CH_2 protons and amidic NH is in agreement with the proposed structure.

Figure S14. Chiral HPLC of compound **6**.

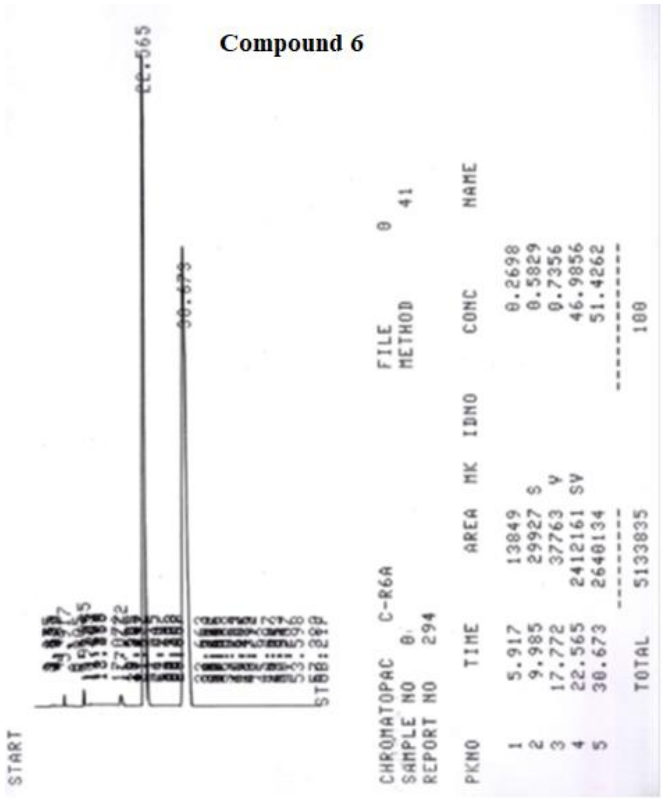


Figure S15. Chiral HPLC of compound **(*R*)-6**.

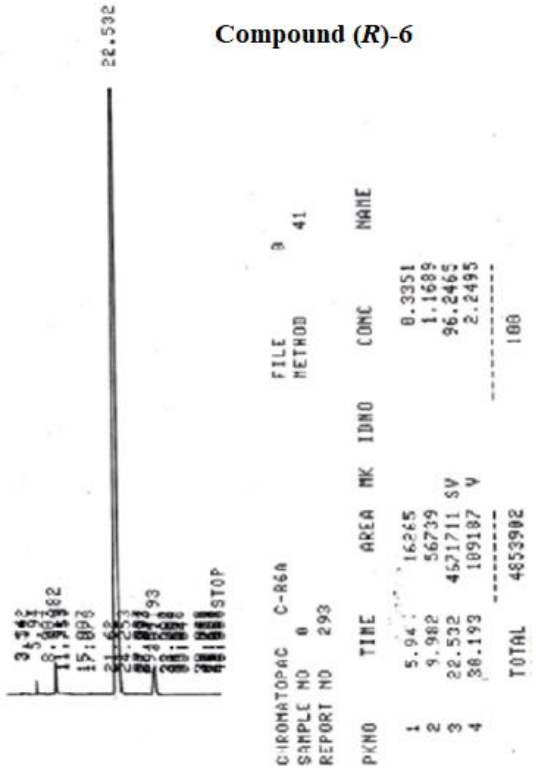


Figure S16. Chiral HPLC of compound **(*S*)-6**.

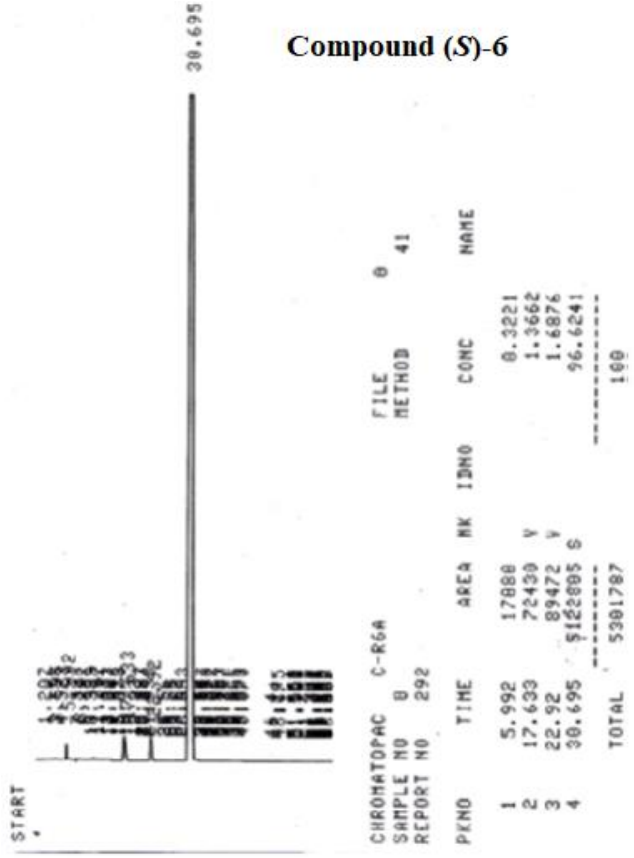


Figure S17. Chiral HPLC of compound **10**.

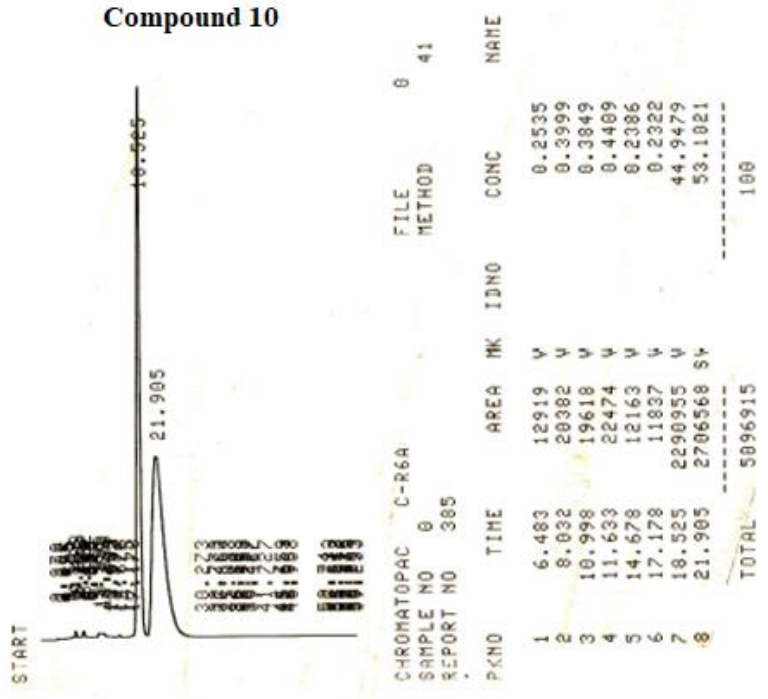


Figure S18. Chiral HPLC of compound **(*R*)-10**.

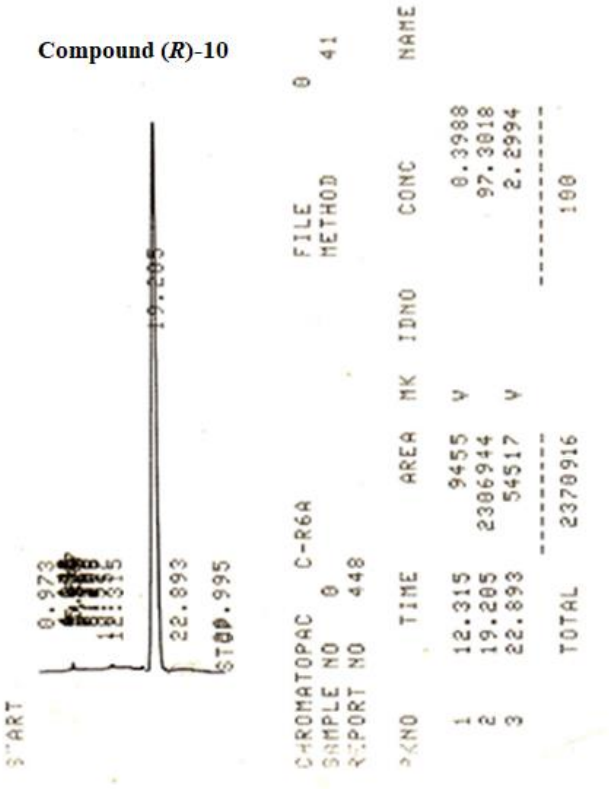


Figure S19. Chiral HPLC of compound **(*S*)-10**.

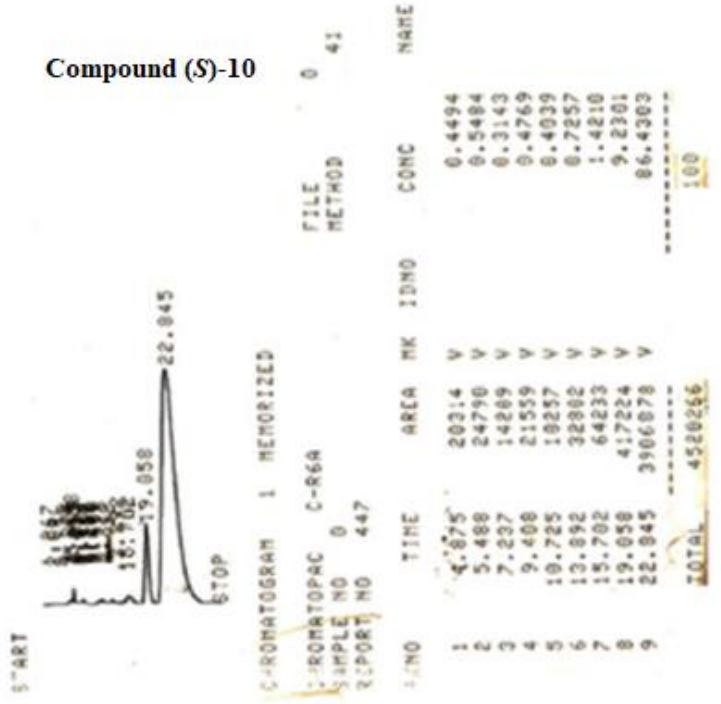
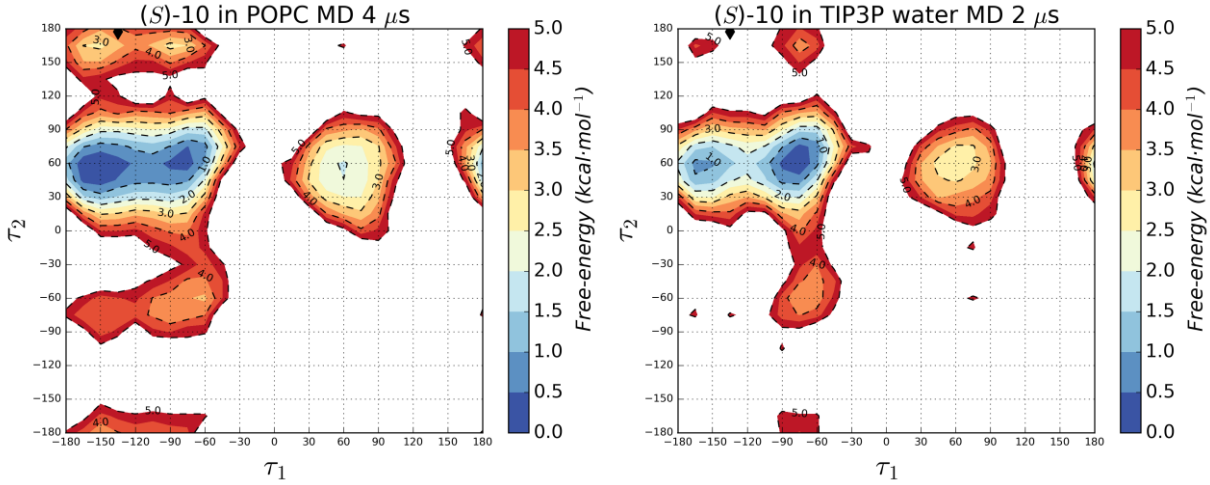


Figure S20. Free-energy surfaces calculated from the MD simulation of **(*S*)-10** in POPC (left) and in TIP3P water molecules (right). Dihedrals values assumed in the minimized docking pose are shown as a black diamond.



Protocol S1. Equilibration protocol for MD simulations of MT_2 receptor-ligand complexes

The equilibration protocol consists of a prolonged version of the default relaxation procedure implemented in Desmond 5.4 for protein-membrane systems.

1. 50 ps of Brownian dynamics in NVT ensemble with 1 ps timestep (3 ps for long-range electrostatics) for at 10 K with a force constant of 50 kcal·mol^{-1}·Å^{-2} on the solute heavy atoms;
2. 200 ps of Brownian dynamics in NPT ensemble with 1 ps timestep (3 ps for long-range electrostatics) at 100 K with a force constant of 20 kcal·mol^{-1}·Å^{-2} on the solute heavy atoms. A directional restraint on Z-axis was applied to membrane heavy atoms with a force constant of 5 kcal·mol^{-1}·Å^{-2};
3. 500 ps in NPγT ensemble with 1ps timestep (3 ps for long-range electrostatics) at 100 K with a force constant of 10 kcal·mol^{-1}·Å^{-2} on the solute heavy atoms. A directional restraint on Z-axis was applied to membrane heavy atoms with a force constant of 2 kcal·mol^{-1}·Å^{-2};
4. 1.5 ns of gradual heating in NPγT ensemble from 100 K to 300 K with timesteps used during production (2 ps and 6 ps for long-range electrostatics, see main text) and a force constant of 10 kcal·mol^{-1}·Å^{-2} on the solute heavy atoms. A directional restraint on Z-axis was applied to choline heads polar heteroatoms with a force constant of 2 kcal·mol^{-1}·Å^{-2};
5. 4.0 ns in NVT ensemble divided in steps with gradual release of restraints and timesteps as in production:
    - 1.0 ns with restraints on backbone and ligand heavy atoms of 5 kcal·mol^{-1}·Å^{-2};
    - 1.0 ns with restraints on alpha carbons and ligand heavy atoms of 5 kcal·mol^{-1}·Å^{-2};
    - 1.0 ns with restraints on alpha carbons of 2.5 kcal·mol^{-1}·Å^{-2} and ligand heavy atoms of 1 kcal·mol^{-1}·Å^{-2}. Henceforth, restraints on cap-termini backbone heavy atoms are kept as in the production phase (see main text);
    - 1.0 ns with further differentiated restraint on the alpha carbons (helices alpha carbons are restrained with a spring constant of 2.5 kcal·mol^{-1}·Å^{-2}, while for the other alpha carbons it is reduced to 0.1 kcal·mol^{-1}·Å^{-2}) and ligand heavy atoms restrained with a force constant of 0.1 kcal·mol^{-1}·Å^{-2};
6. 1.0 ns in NPγT ensemble with timesteps and restraints as in the production phase.

Figure S21. Time-evolution of the free energy surface calculated from the MD simulation of **(*S*)-10** in explicit chloroform.