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Supporting Information

Fluorescent Tools for the Imaging of Dopamine D₂-Like Receptors

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1. Chemical purity and stability

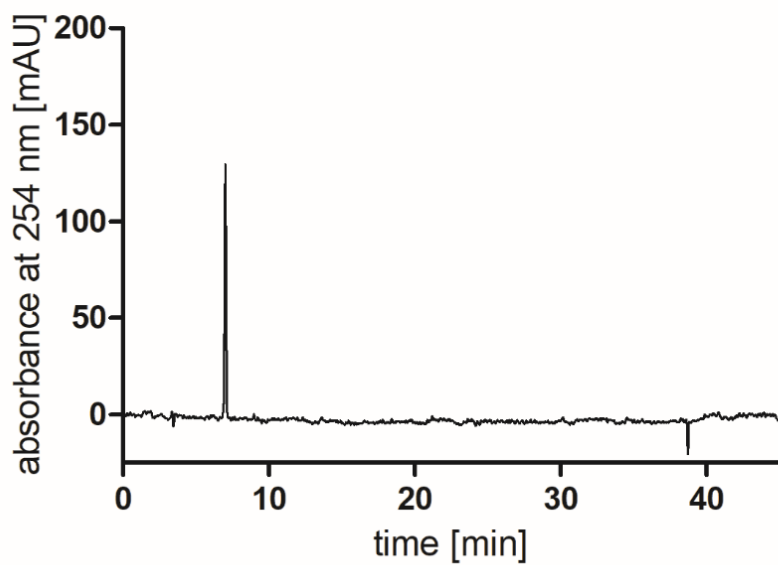


Figure S1. RP-HPLC analysis (purity control) of compound **16** (>99%, 254nm).

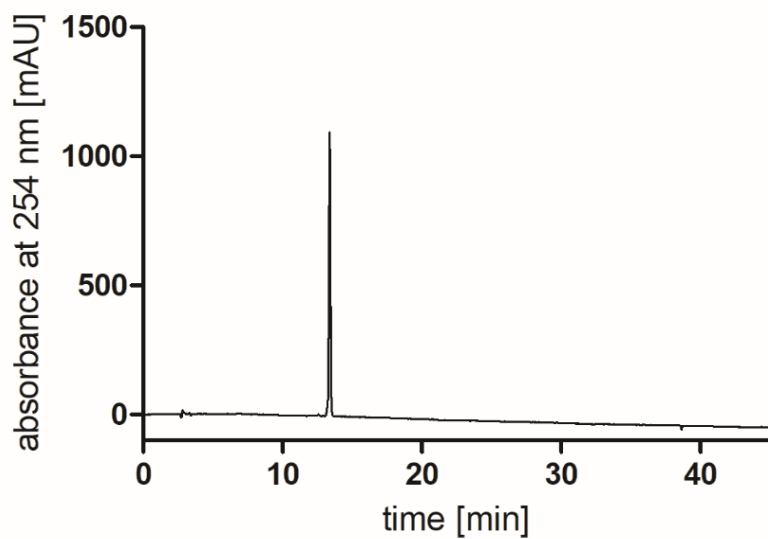


Figure S2. RP-HPLC analysis (purity control) of compound **17** (>99%, 254nm).

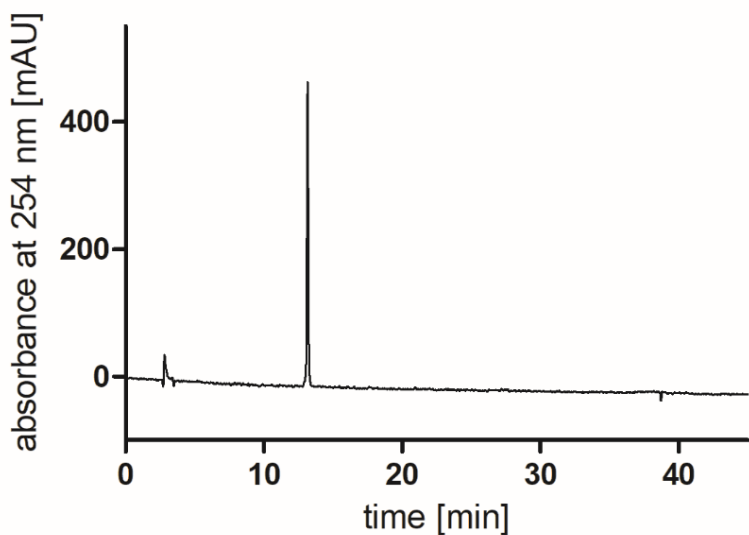


Figure S3. RP-HPLC analysis (purity control) of compound **20** (>99%, 254nm).

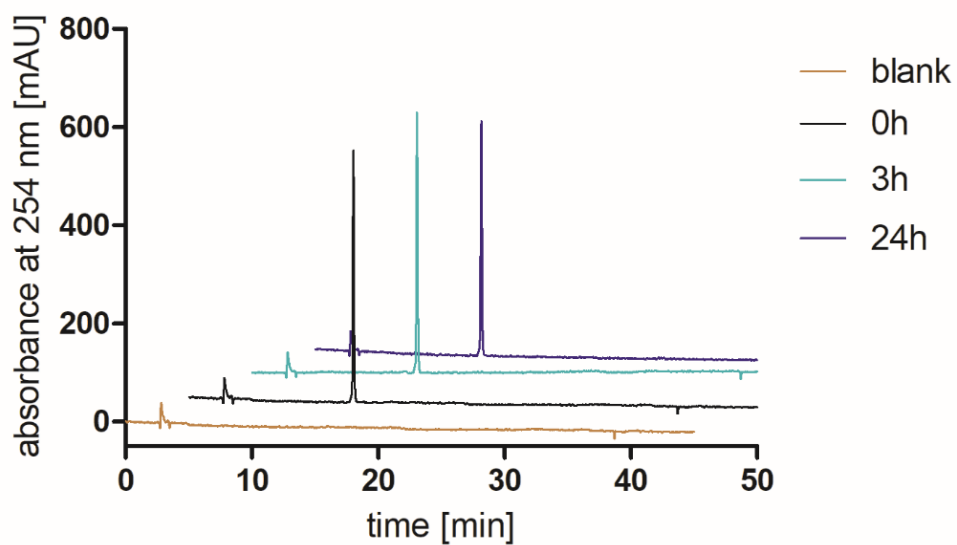


Figure S4. RP-HPLC analysis (stability control) of **20** after incubation in water/DMSO 1:1 at rt for up to 24 h. Compound **20** showed no decomposition.

2. Dopamine-induced G_{o1} activation

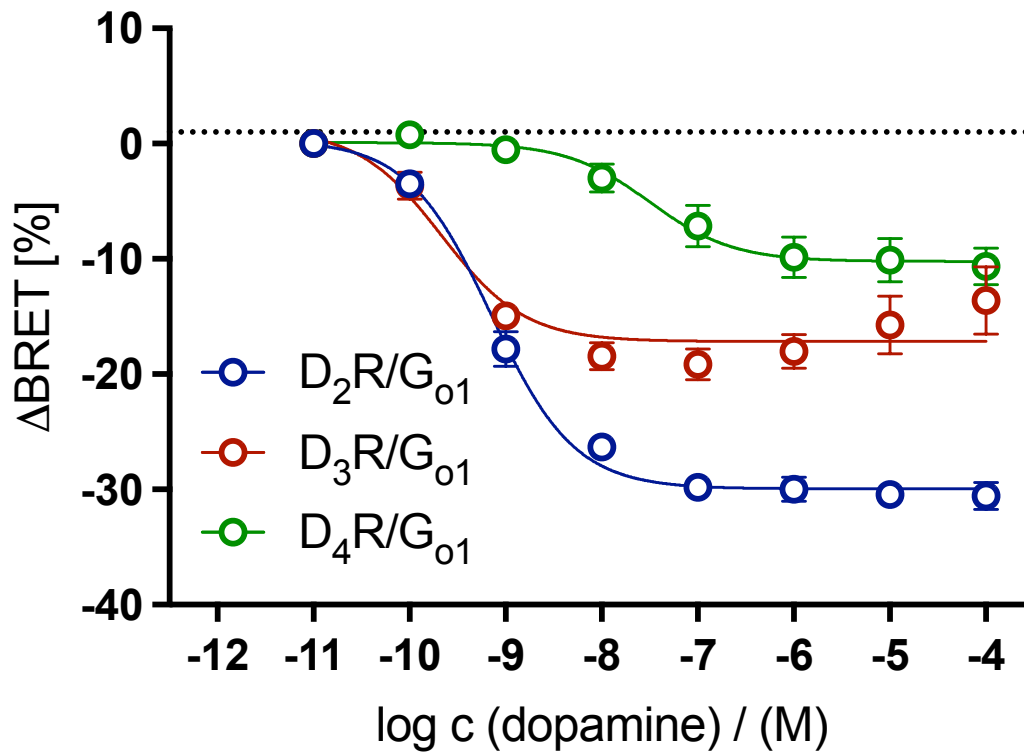


Figure S5. Concentration–response curves (CRCs) for G_{o1} activation of dopamine in HEK293A cells transiently expressing the G_{o1} BRET sensor along with the wild-type D_2R , D_3R or D_4R . Graphs represent the means of three independent experiments each performed in duplicate. Data were analyzed by nonlinear regression and were best fitted to sigmoidal concentration–response curves.

3. NMR spectra

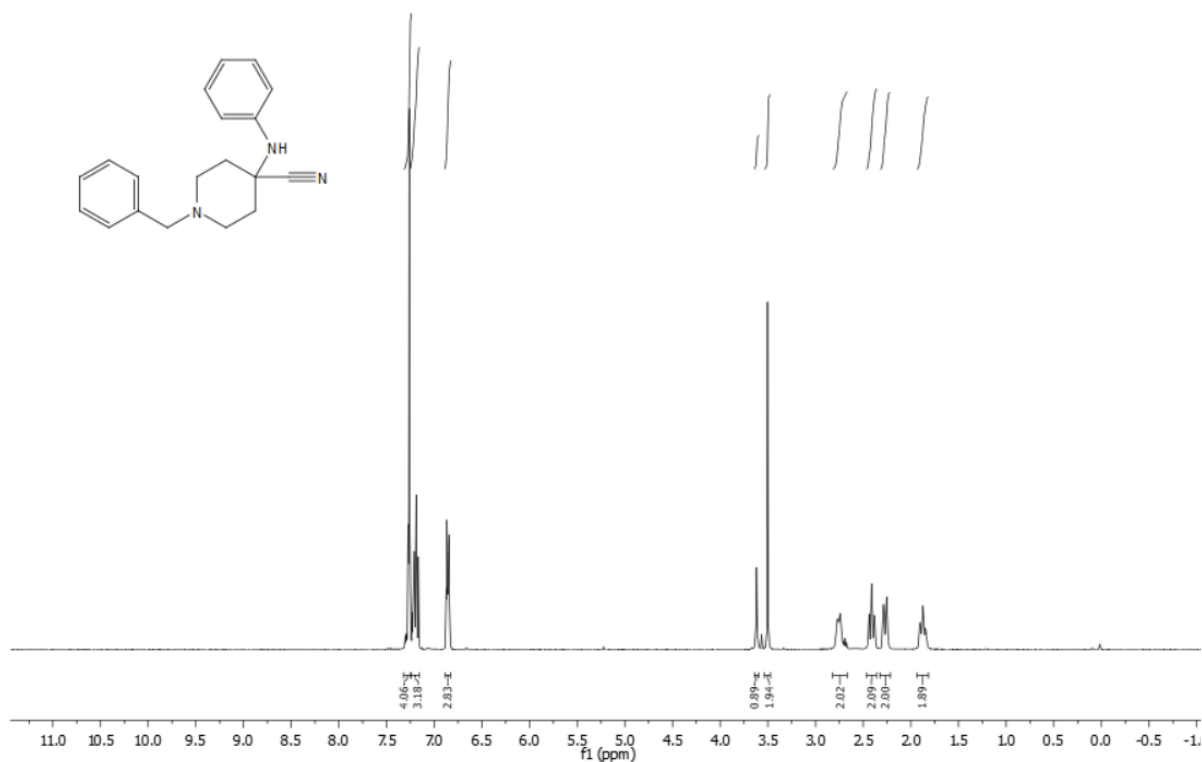


Figure S6. ¹H NMR spectrum (400 MHz, CDCl₃) of compound 2.

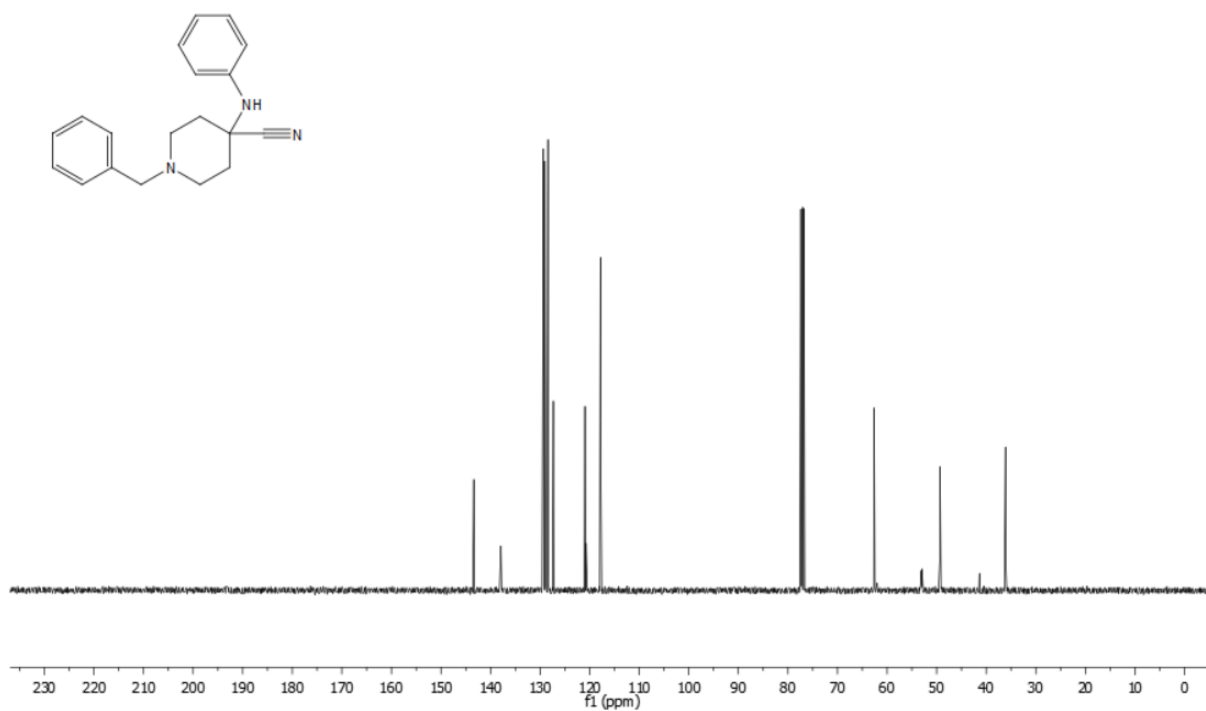


Figure S7. ¹³C NMR spectrum (101 MHz, CDCl₃) of compound 2.

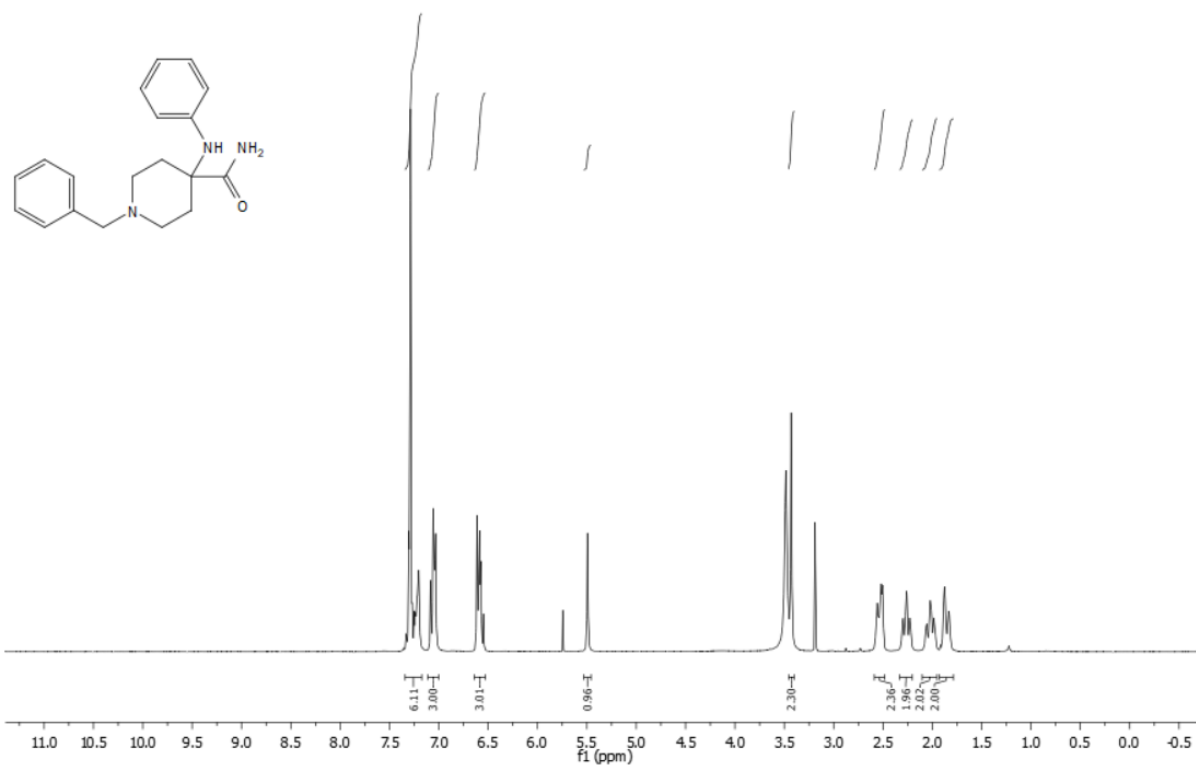


Figure S8. ¹H NMR spectrum (300 MHz, DMSO-d₆) of compound 3.

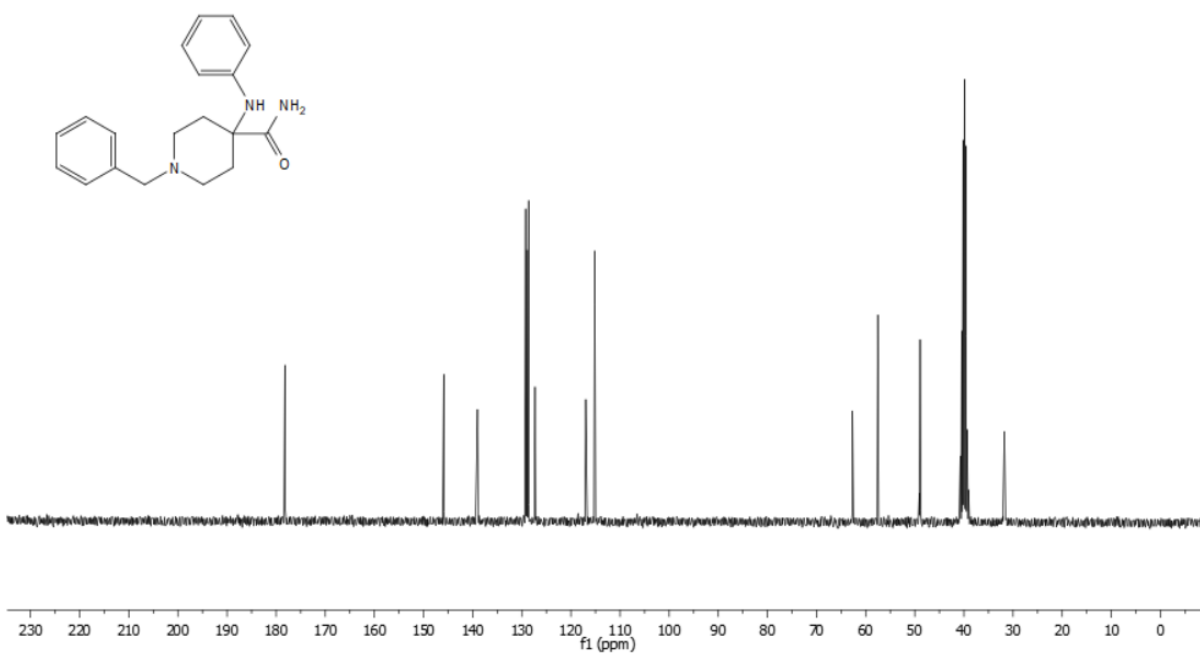


Figure S9. ¹³C NMR spectrum (101 MHz, DMSO-d₆) of compound 3.

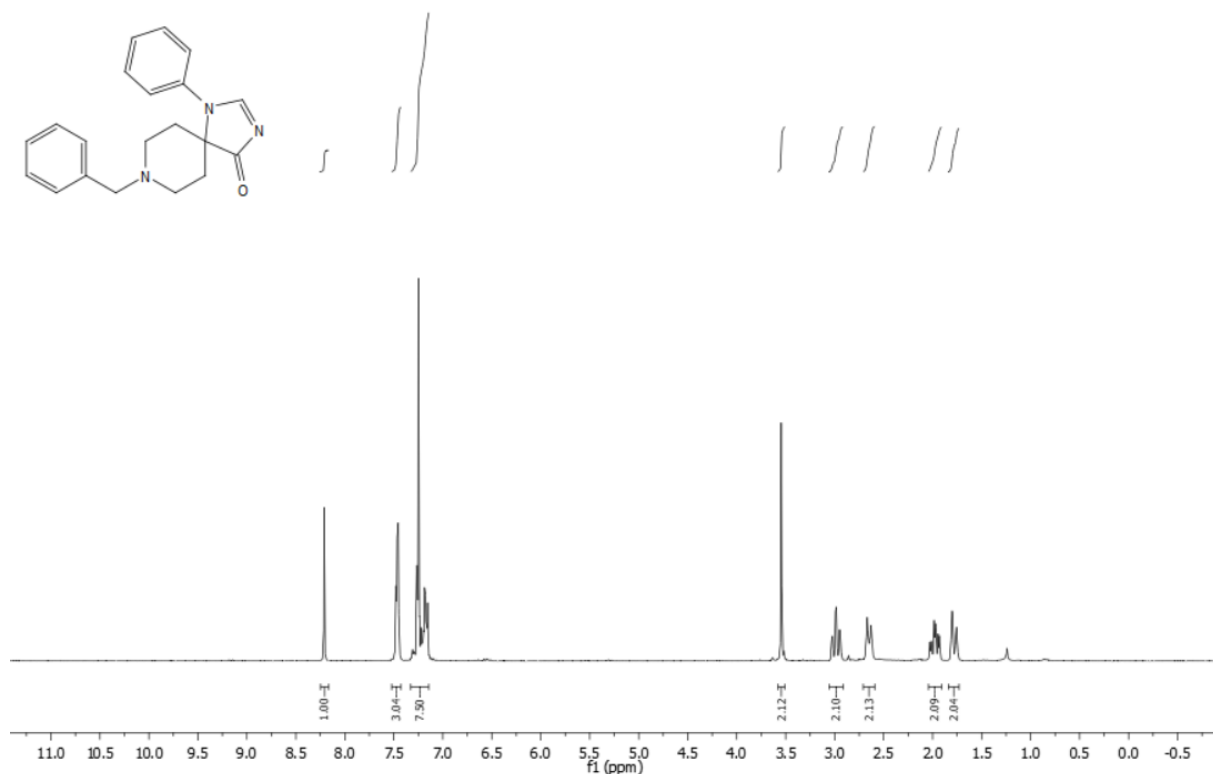


Figure S10. ¹H NMR spectrum (300 MHz, CDCl₃) of compound 4.

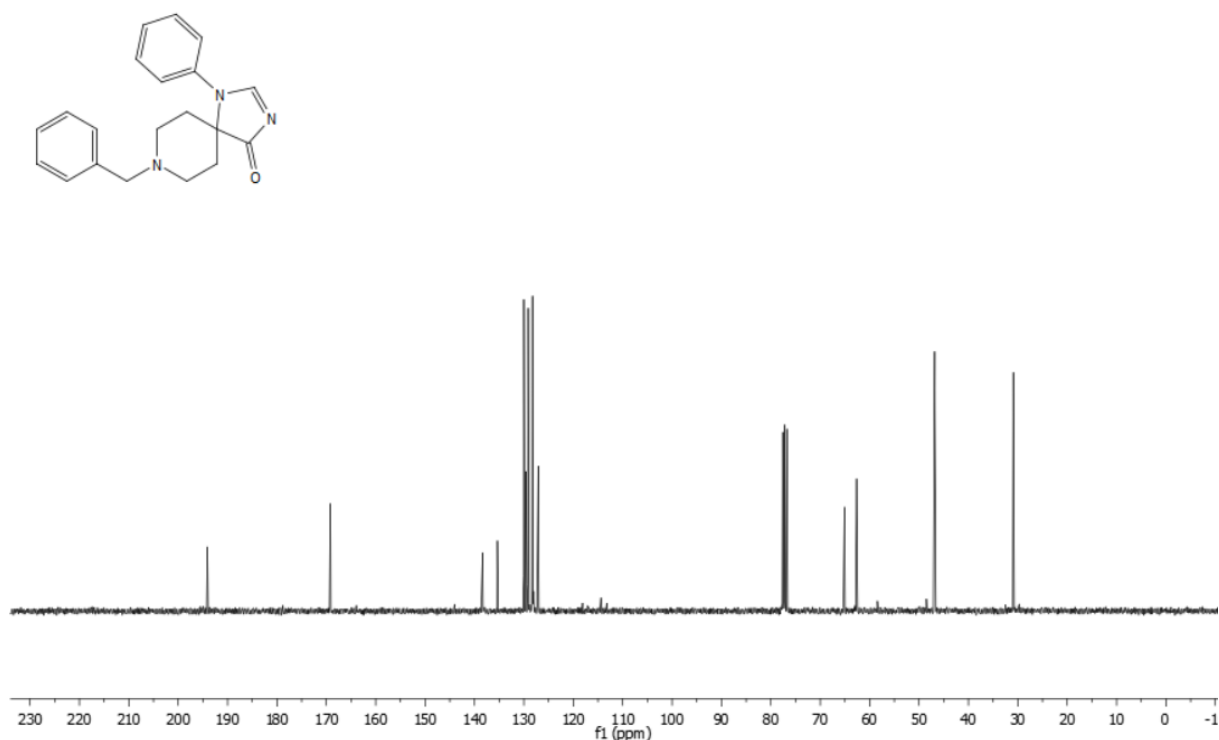


Figure S11. ¹³C NMR spectrum (75 MHz, CDCl₃) of compound 4.

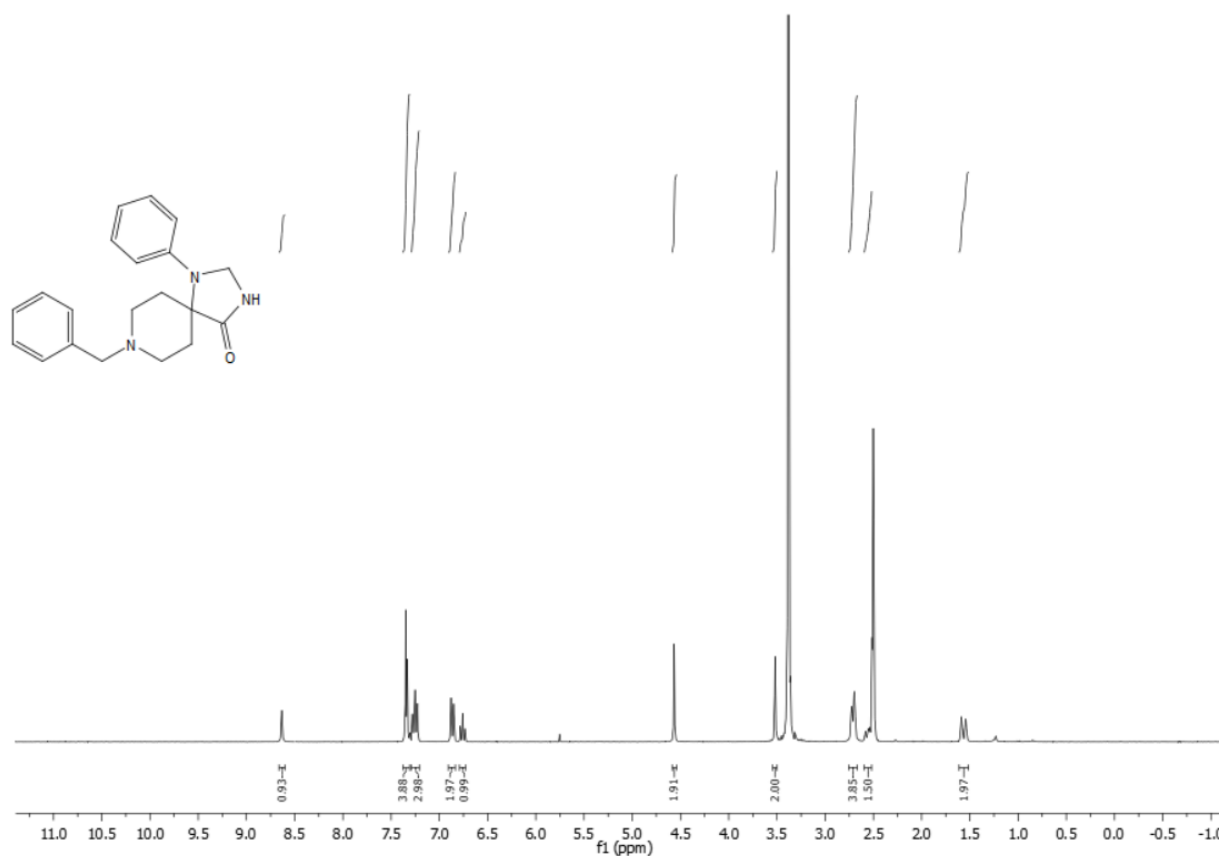


Figure S12. ^1H NMR spectrum (300 MHz, DMSO-d_6) of compound 5.

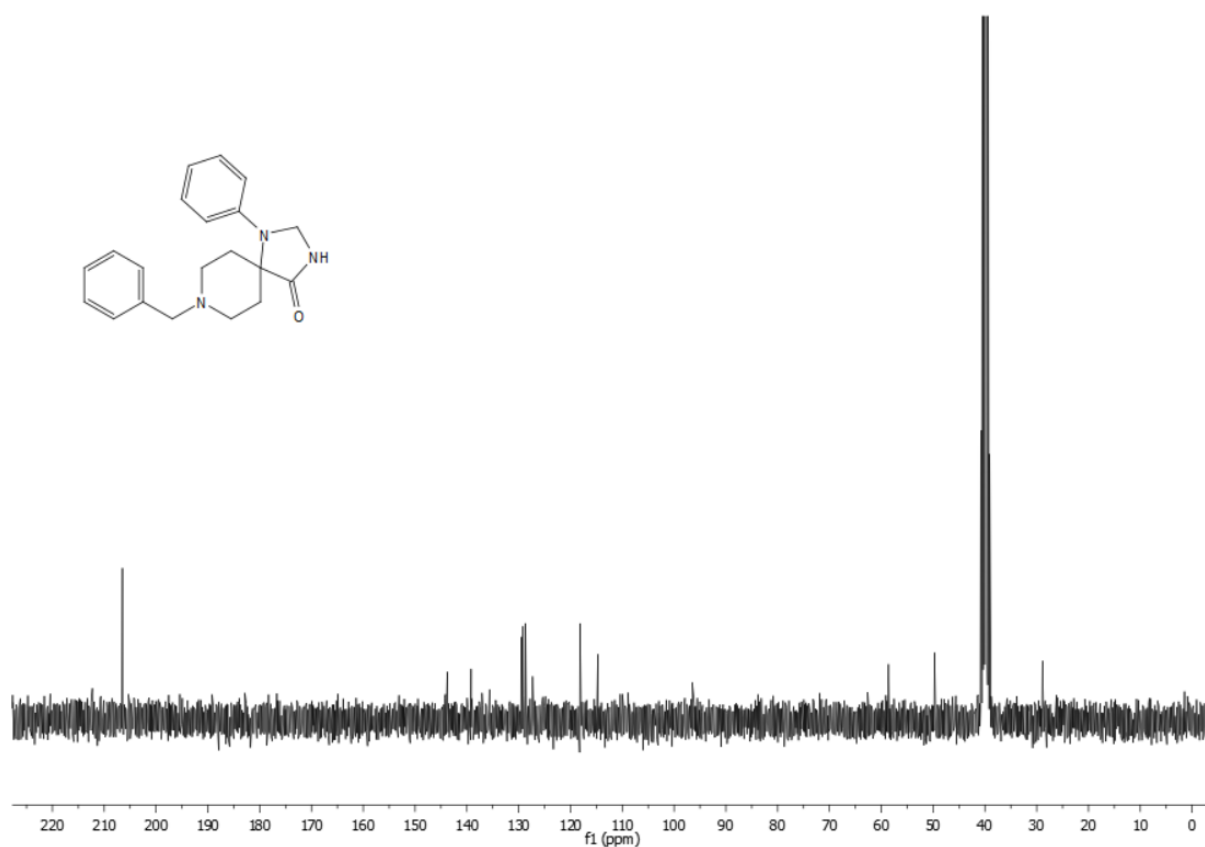


Figure S13. ^{13}C NMR spectrum (75 MHz, DMSO-d_6) of compound 5.

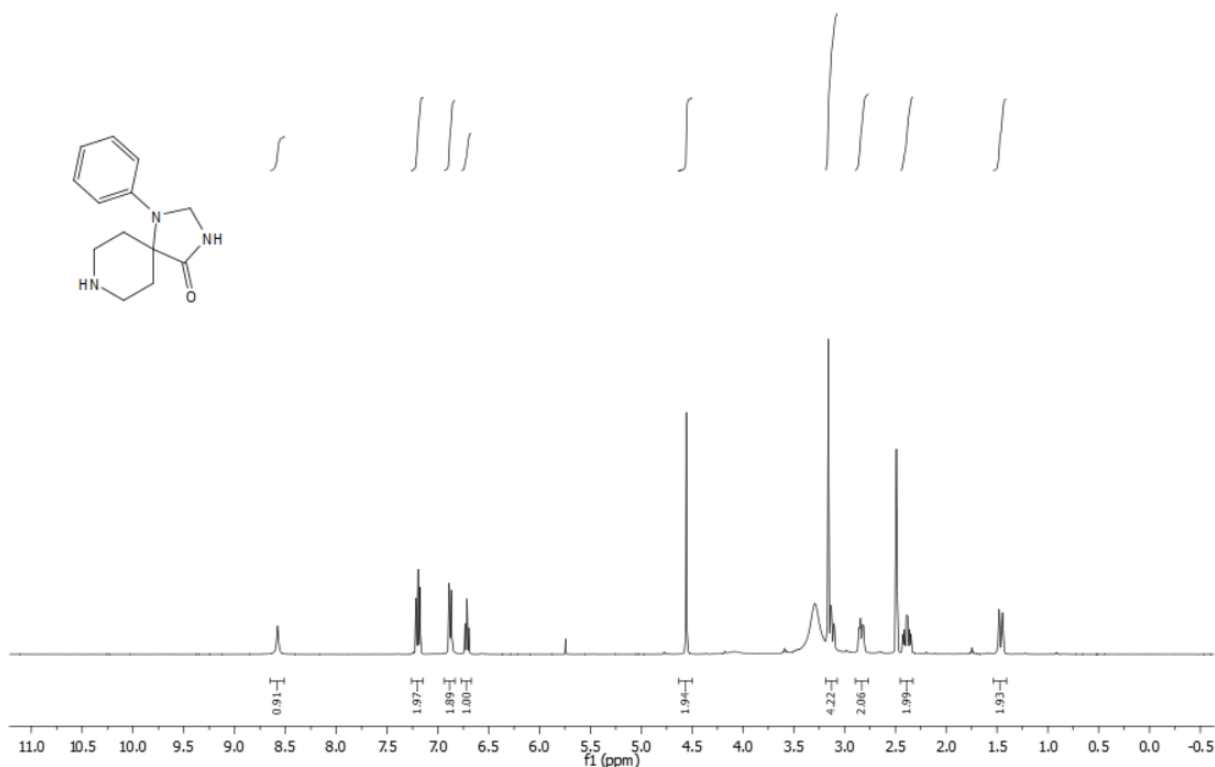


Figure S14. ¹H NMR spectrum (400 MHz, DMSO-d₆) of compound 6.

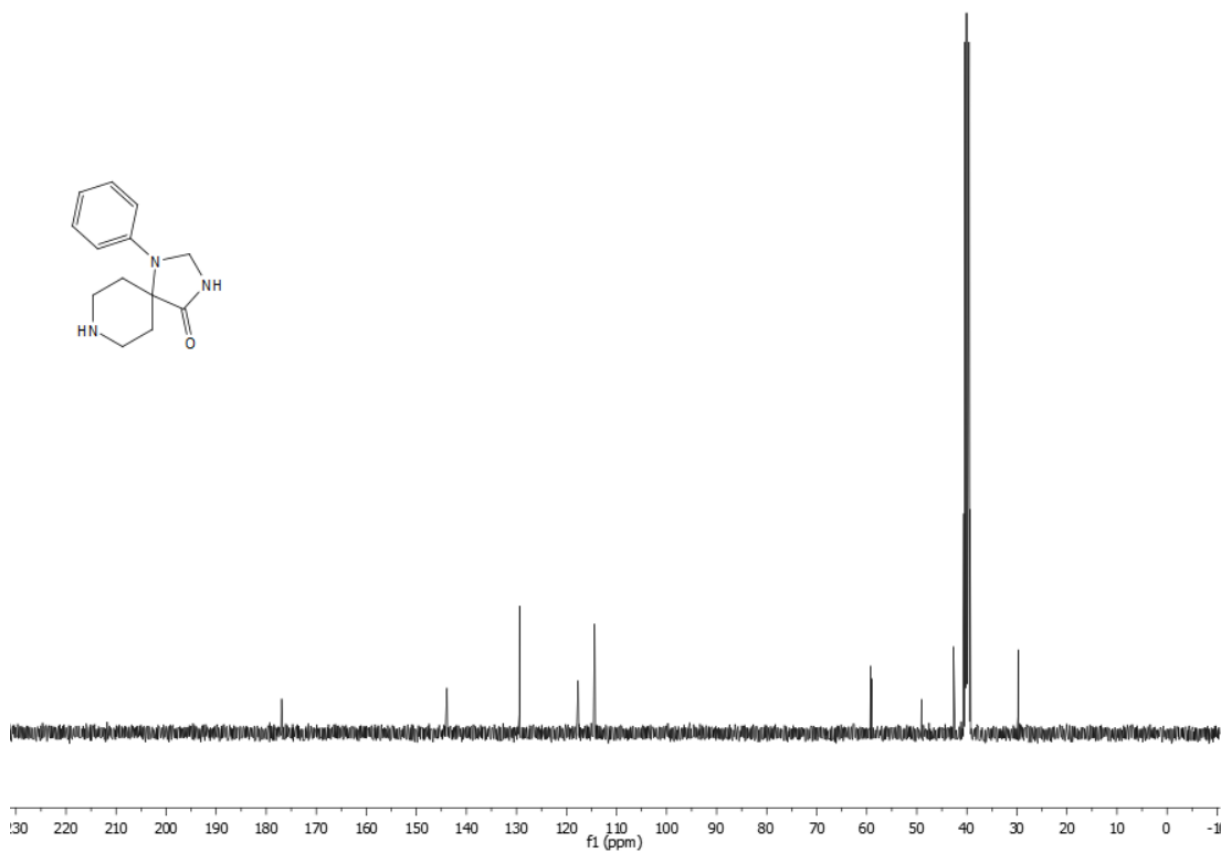
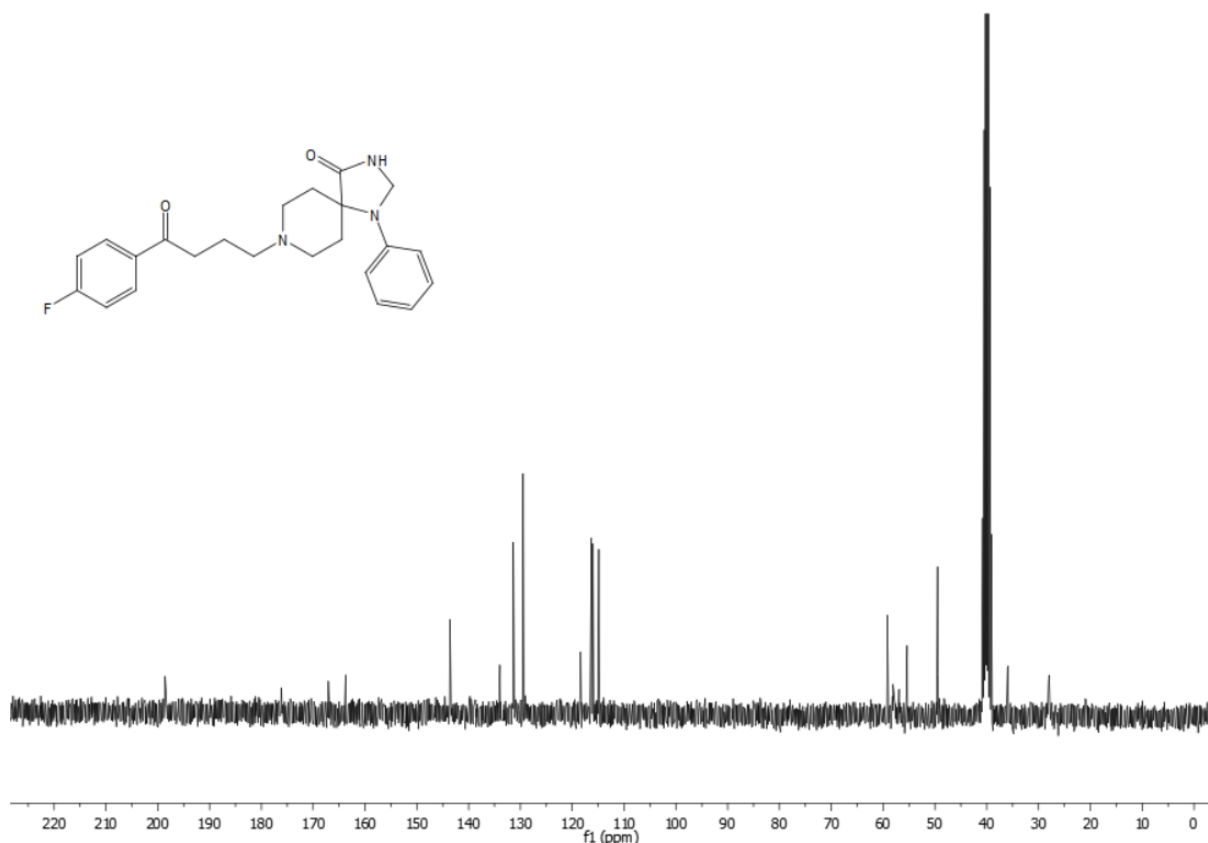
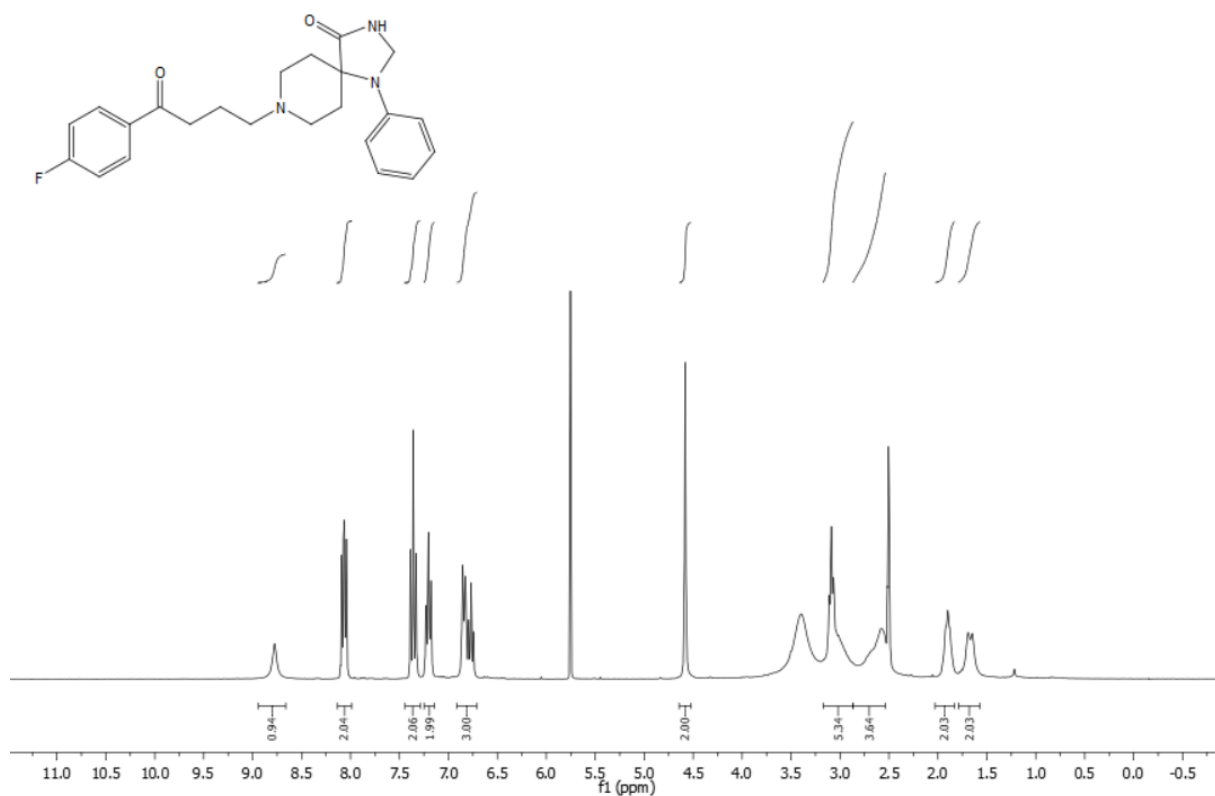


Figure S15. ¹³C NMR spectrum (101 MHz, DMSO-d₆) of compound 6.



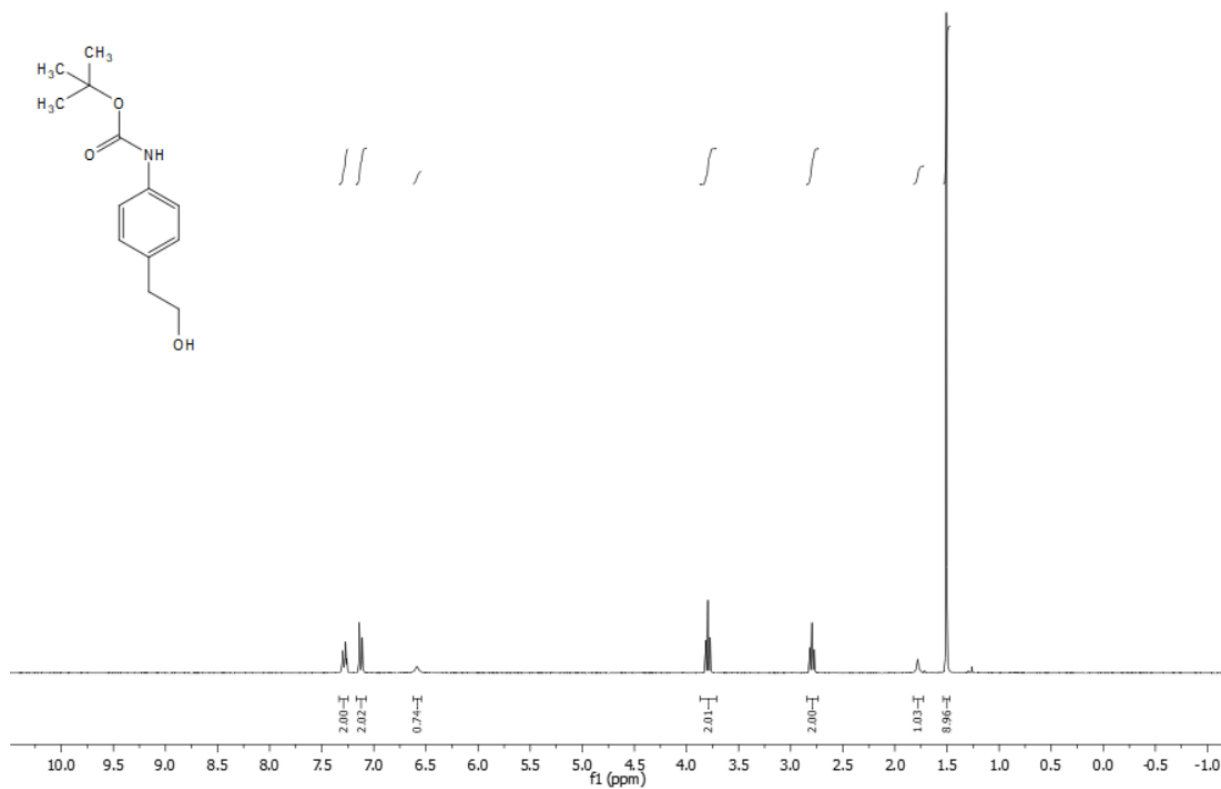


Figure S18. ^1H NMR spectrum (300 MHz, CDCl_3) of compound **8**.

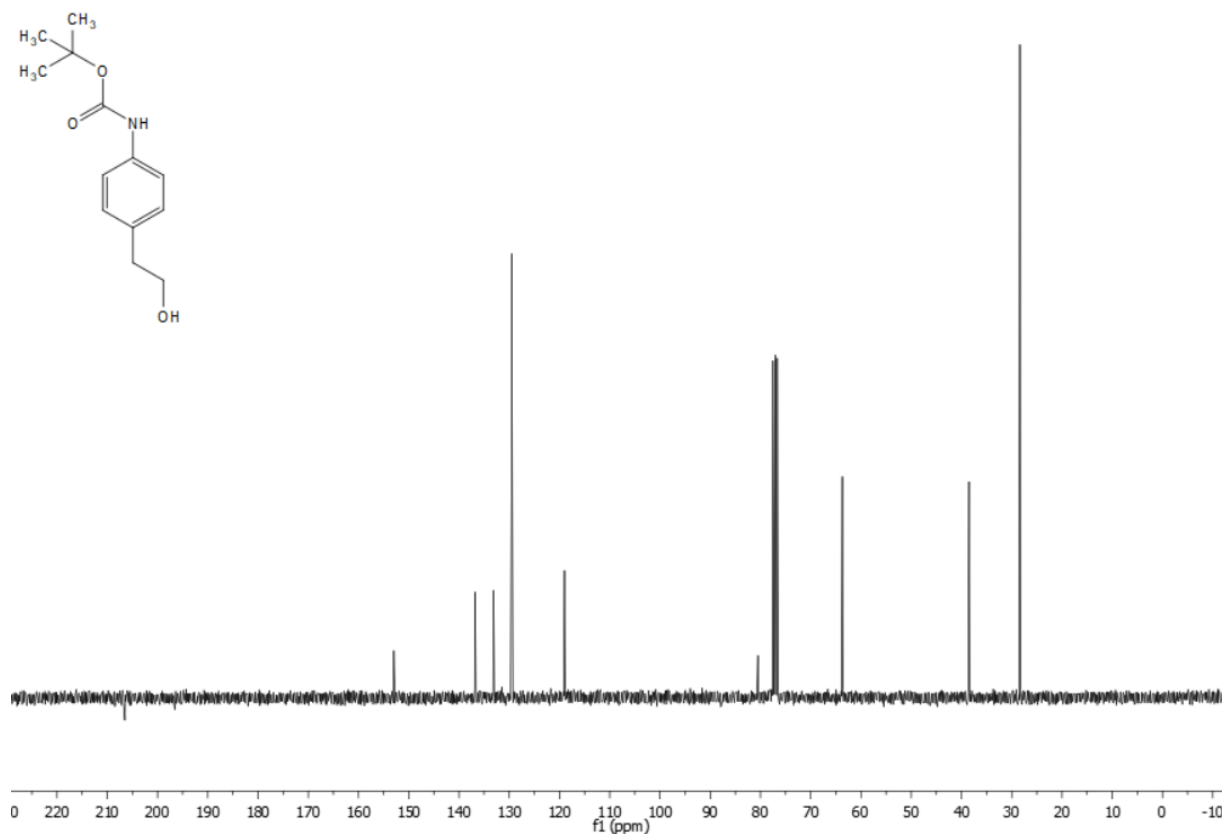


Figure S19. ^{13}C NMR spectrum (75 MHz, CDCl_3) of compound **8**.

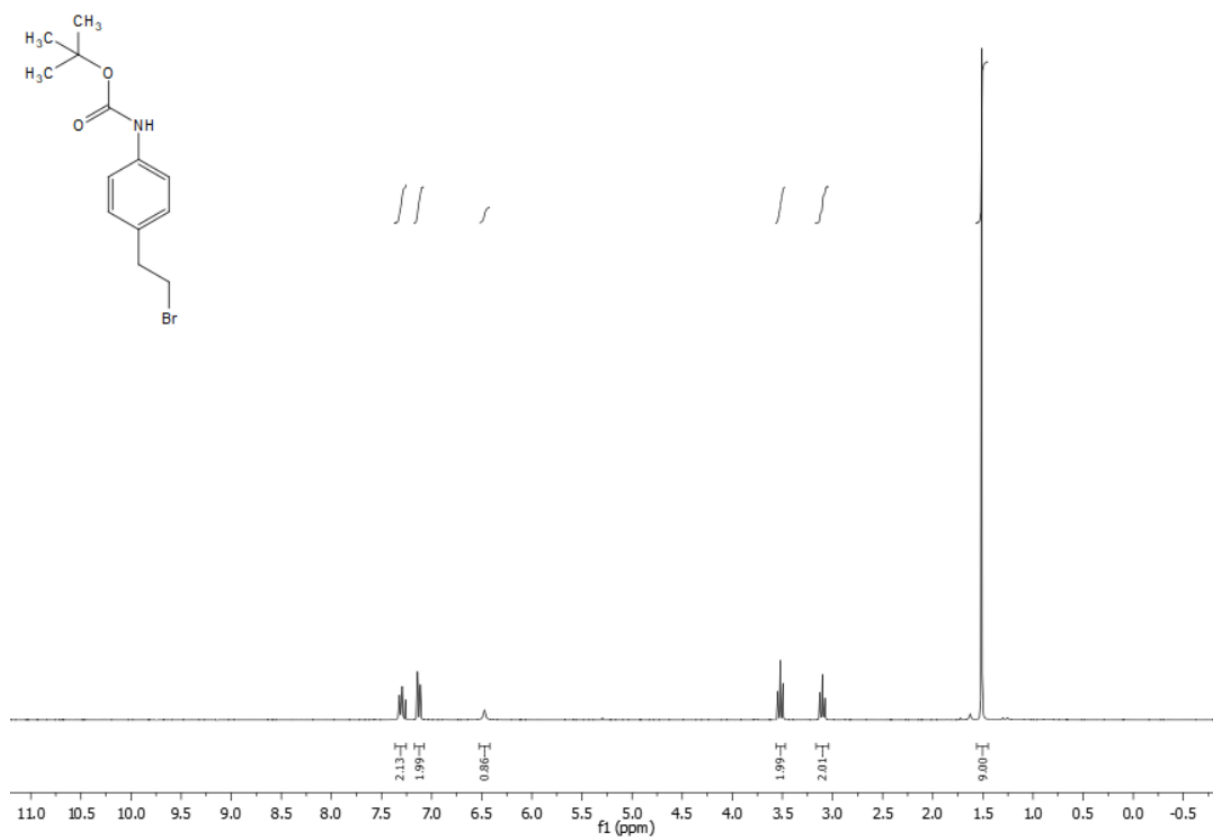


Figure S20. ¹H NMR spectrum (300 MHz, CDCl₃) of compound 9.

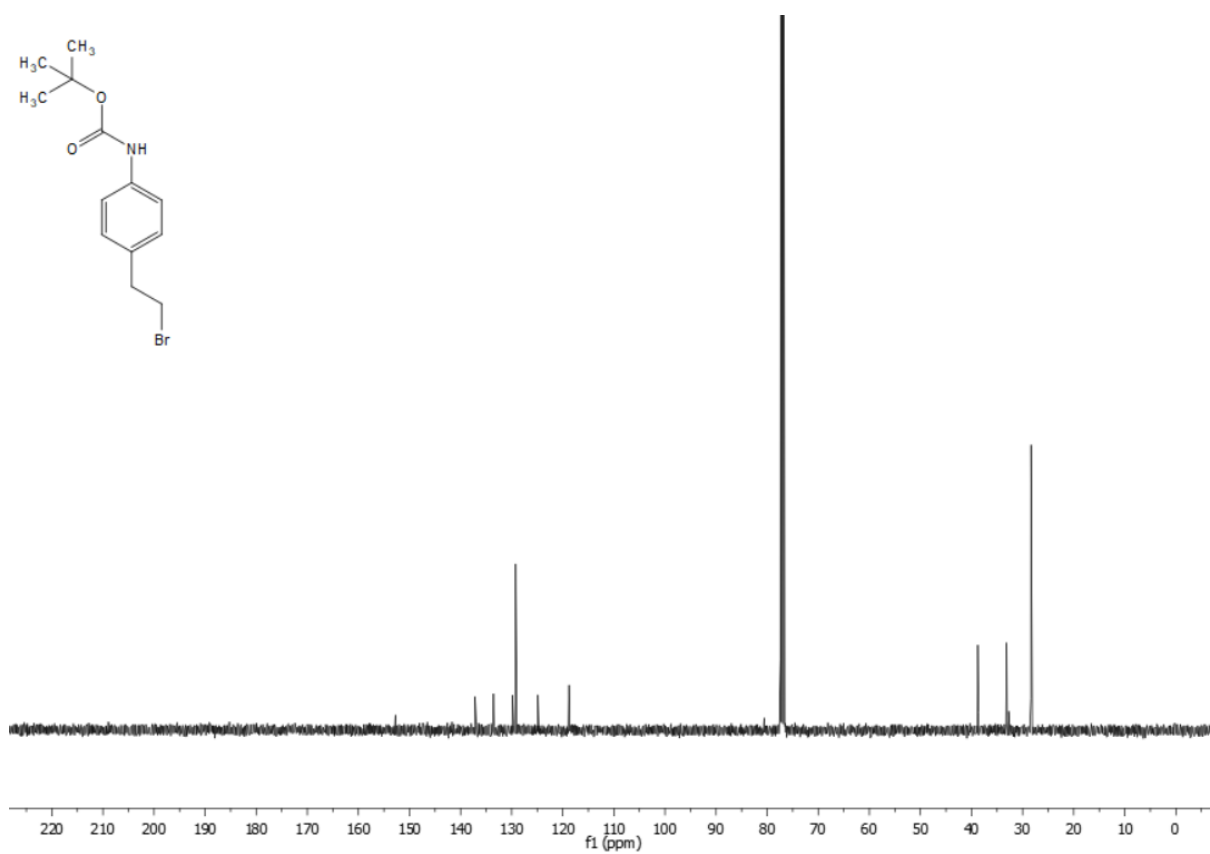


Figure S21. ¹³C NMR spectrum (75 MHz, CDCl₃) of compound 9.

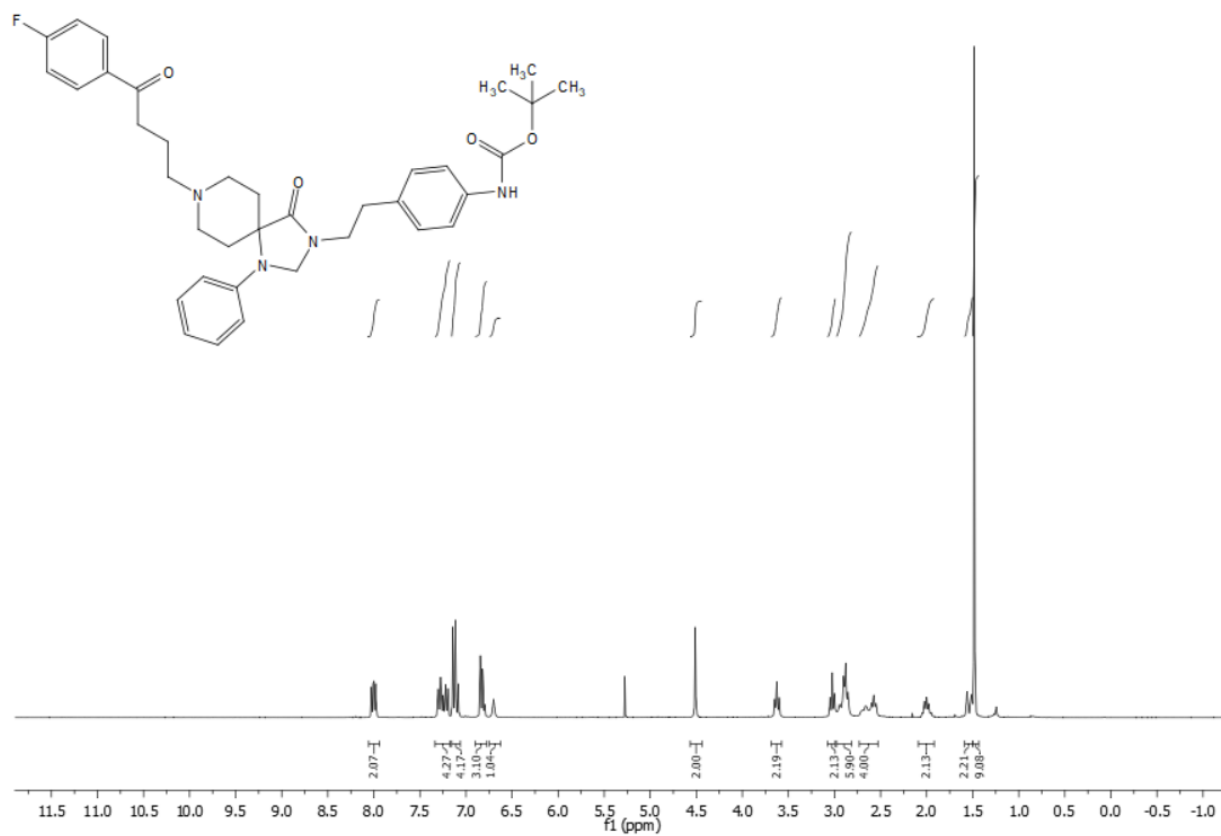


Figure S22. ¹H NMR spectrum (400 MHz, CDCl₃) of compound 10.

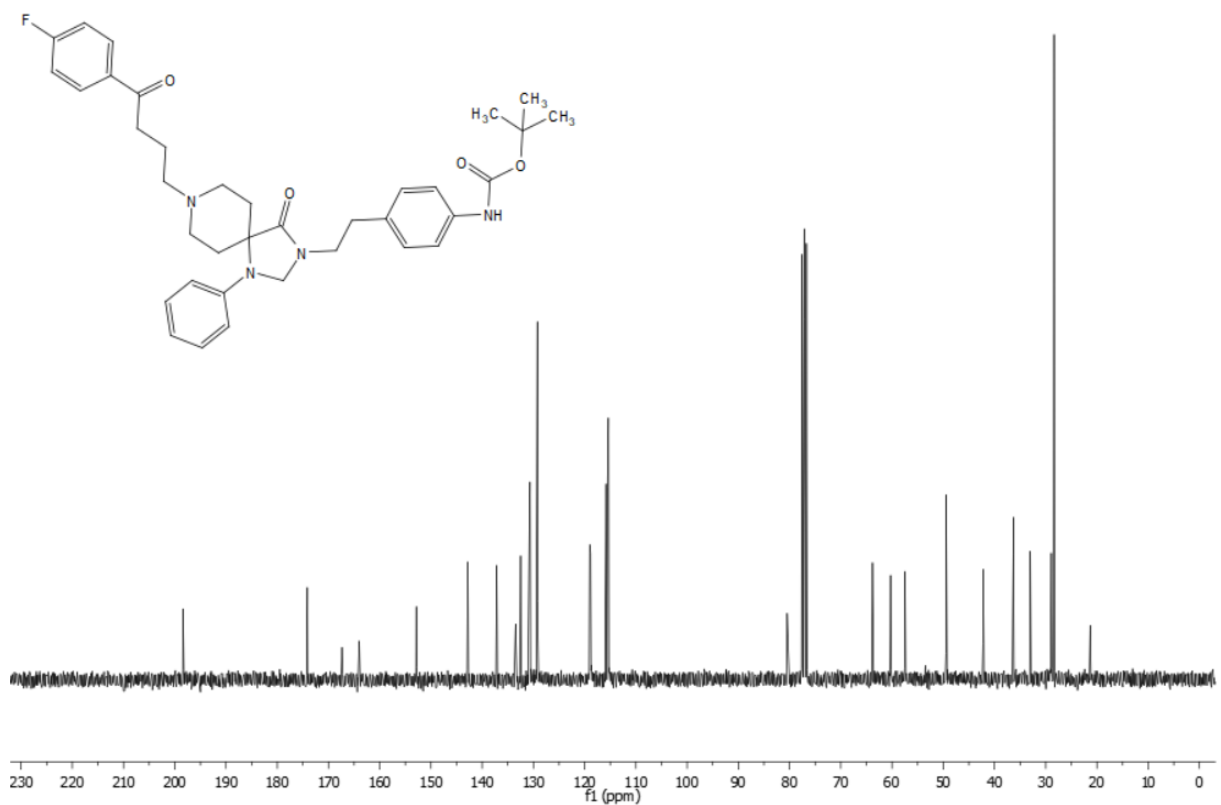


Figure S23. ¹³C NMR spectrum (75 MHz CDCl₃) of compound 10.

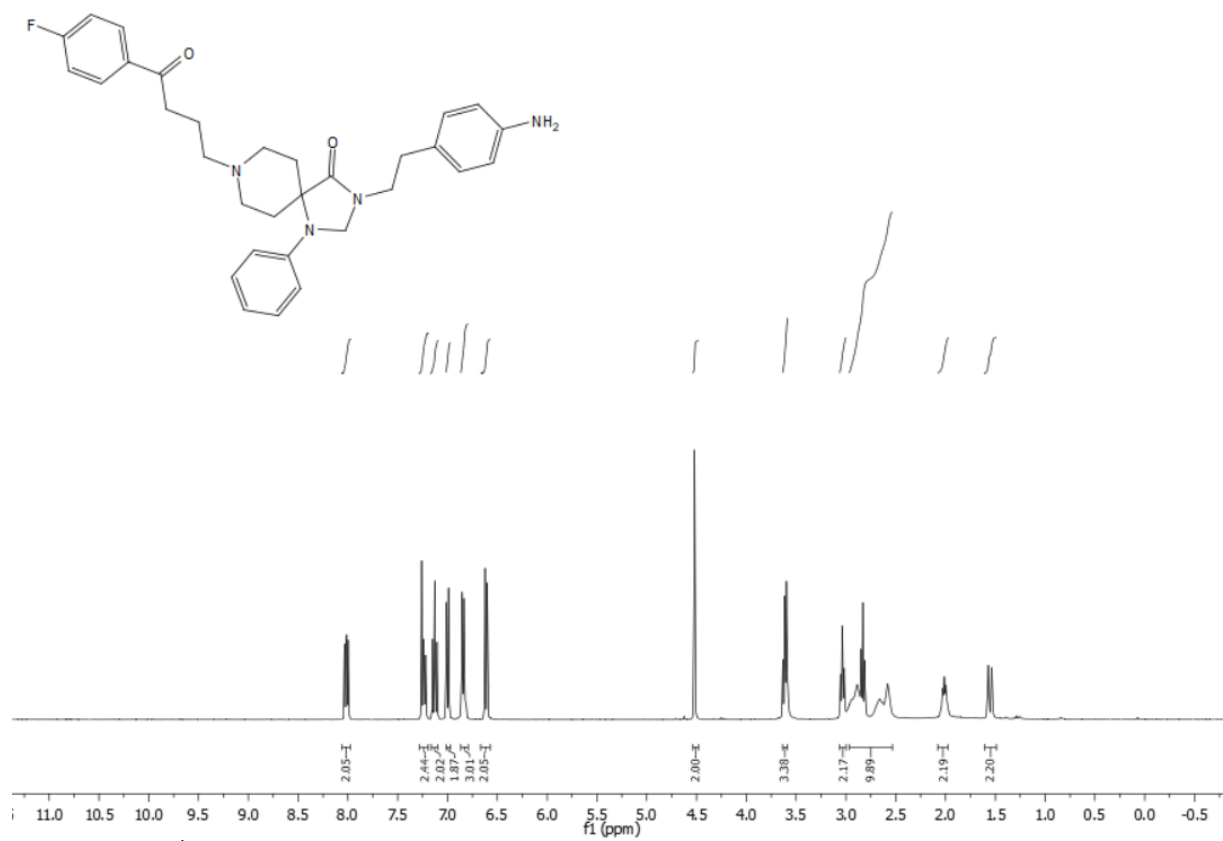


Figure S24. ¹H NMR spectrum (400 MHz, CDCl₃) of compound 11.

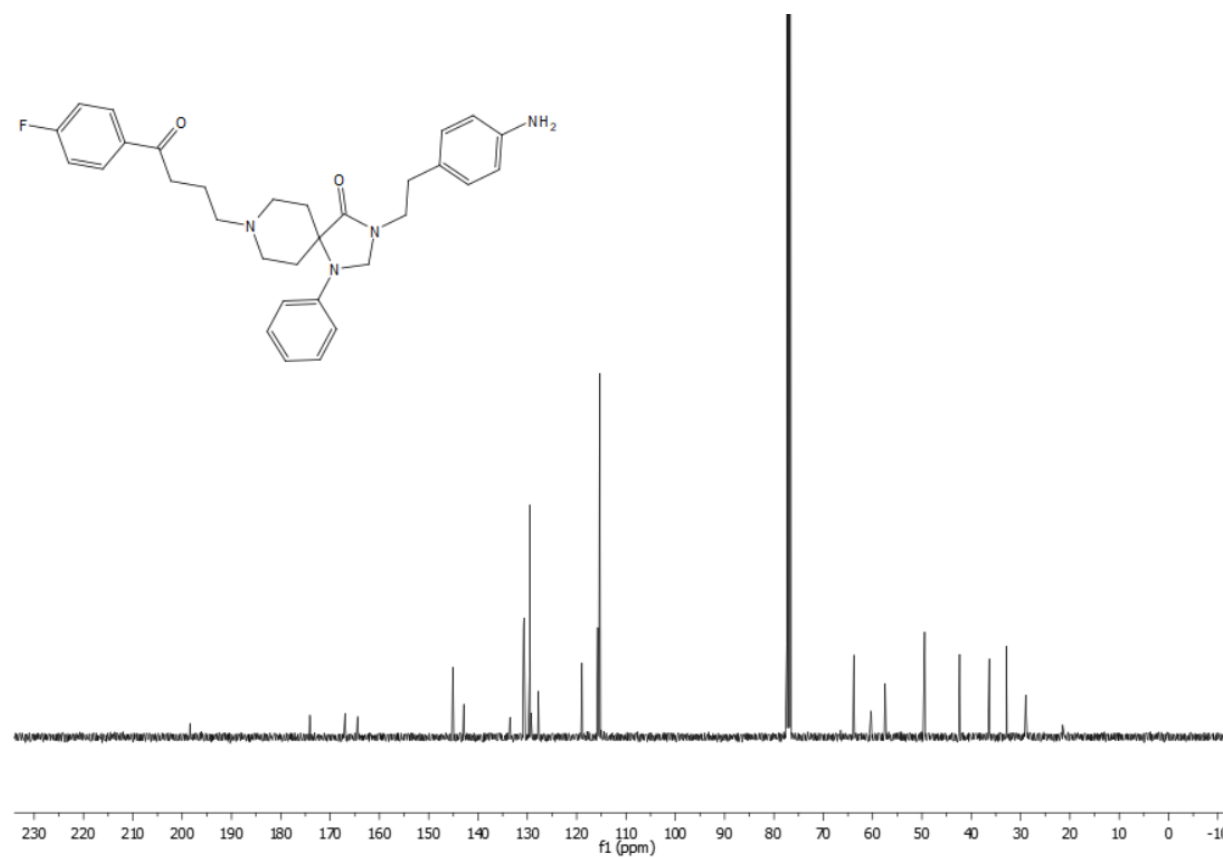


Figure S25. ¹³C NMR spectrum (101 MHz, CDCl₃) of compound 11.

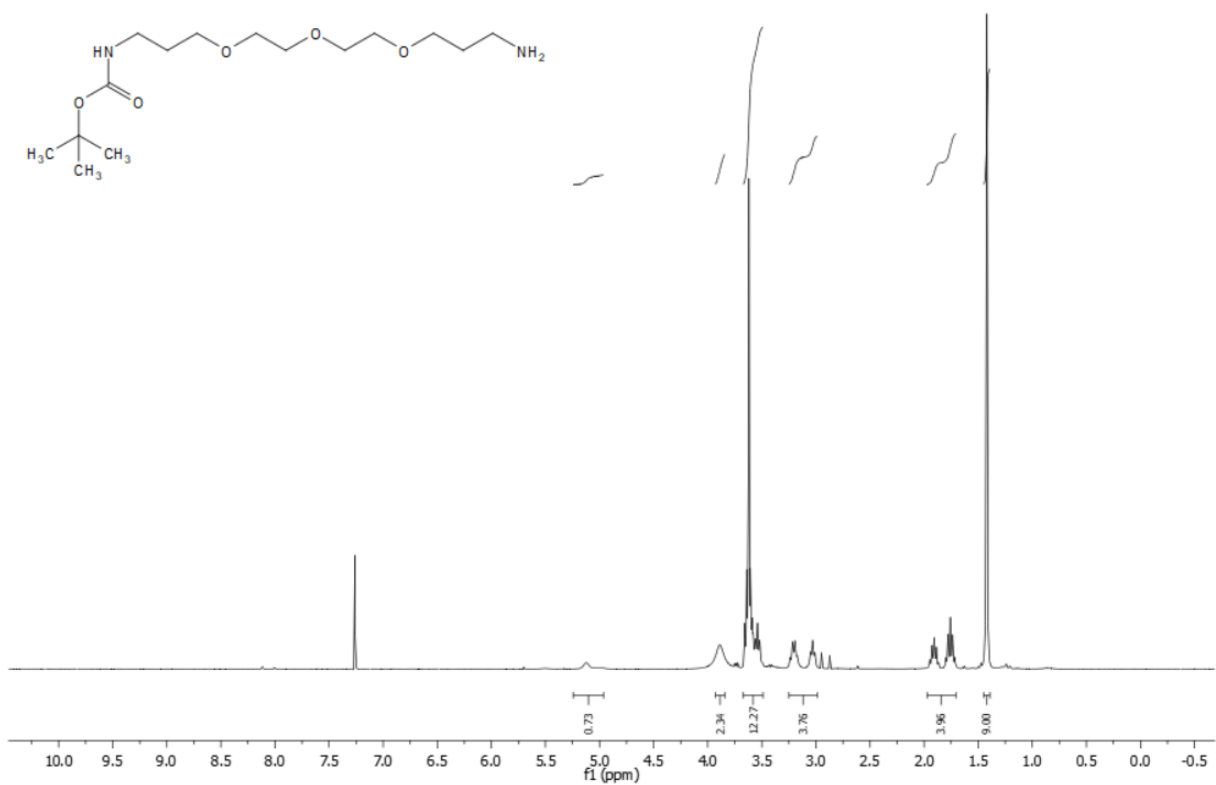


Figure S26. ¹H NMR spectrum (300 MHz, CDCl₃) of compound 12.

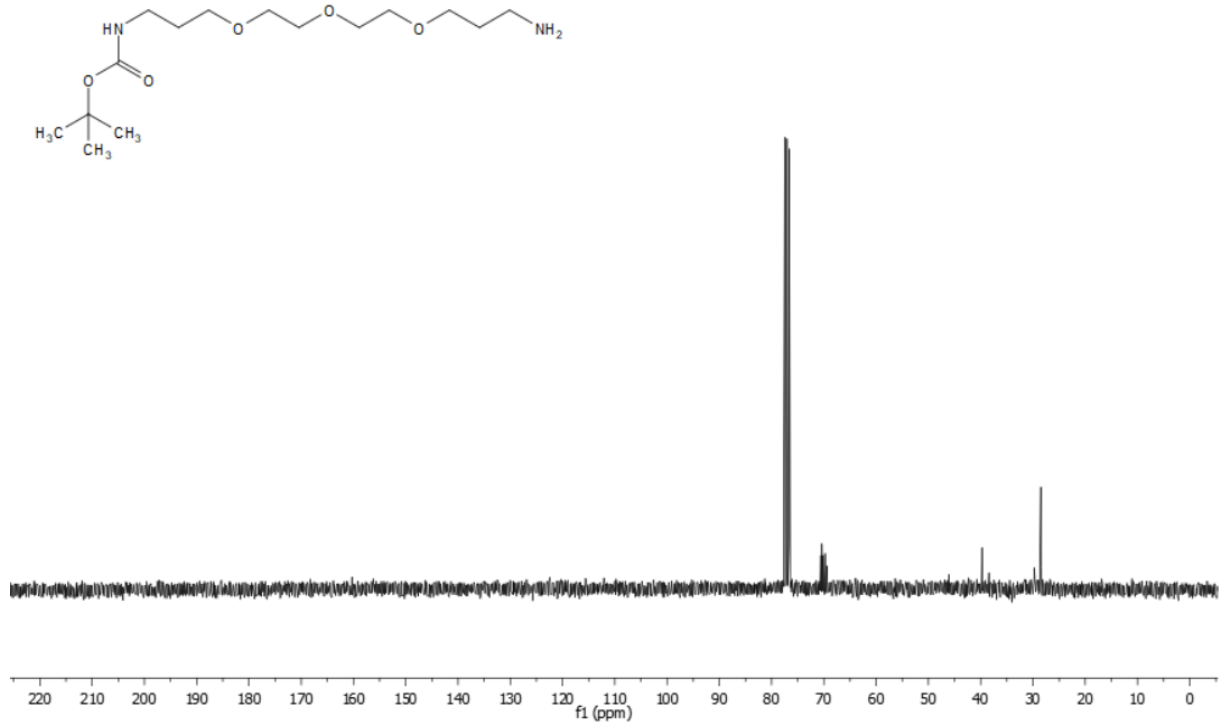


Figure S27. ¹³C NMR spectrum (75 MHz, CDCl₃) of compound 12.

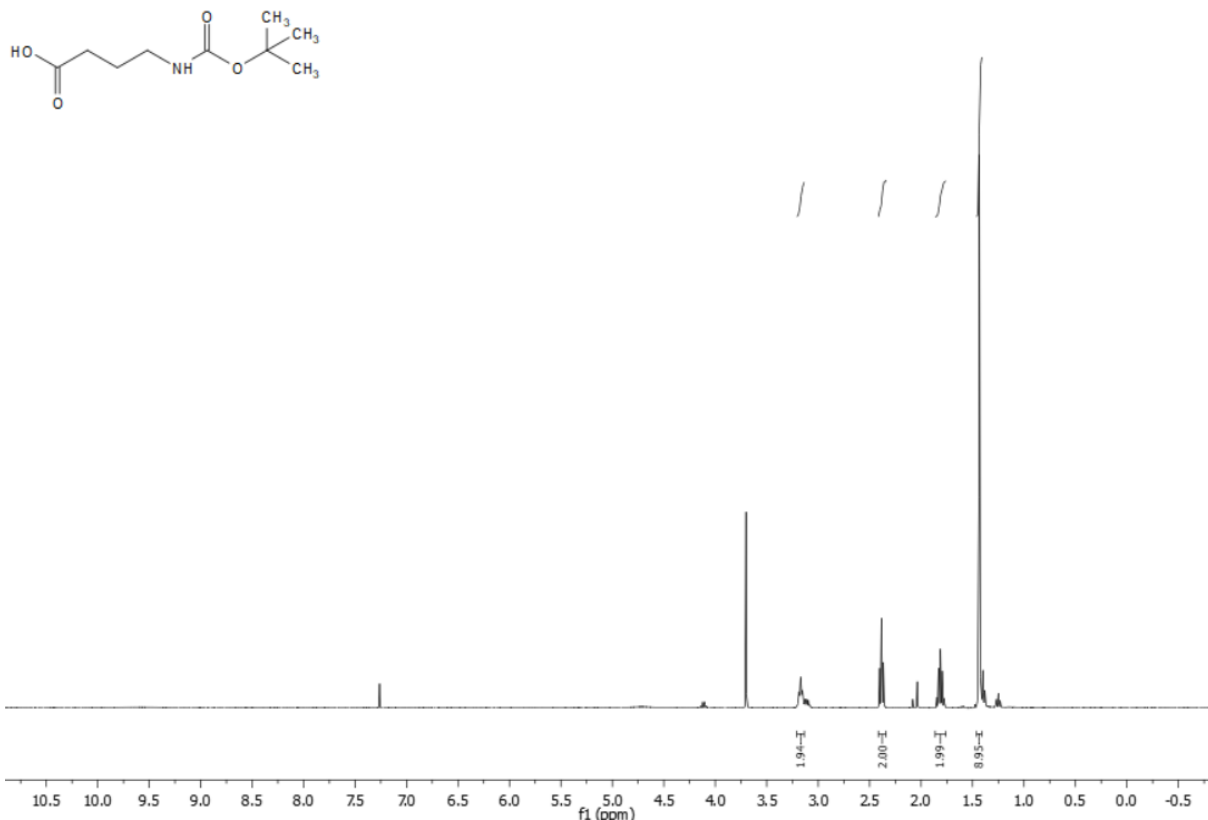


Figure S28. ¹H NMR spectrum (400 MHz, CDCl₃) of compound 13.

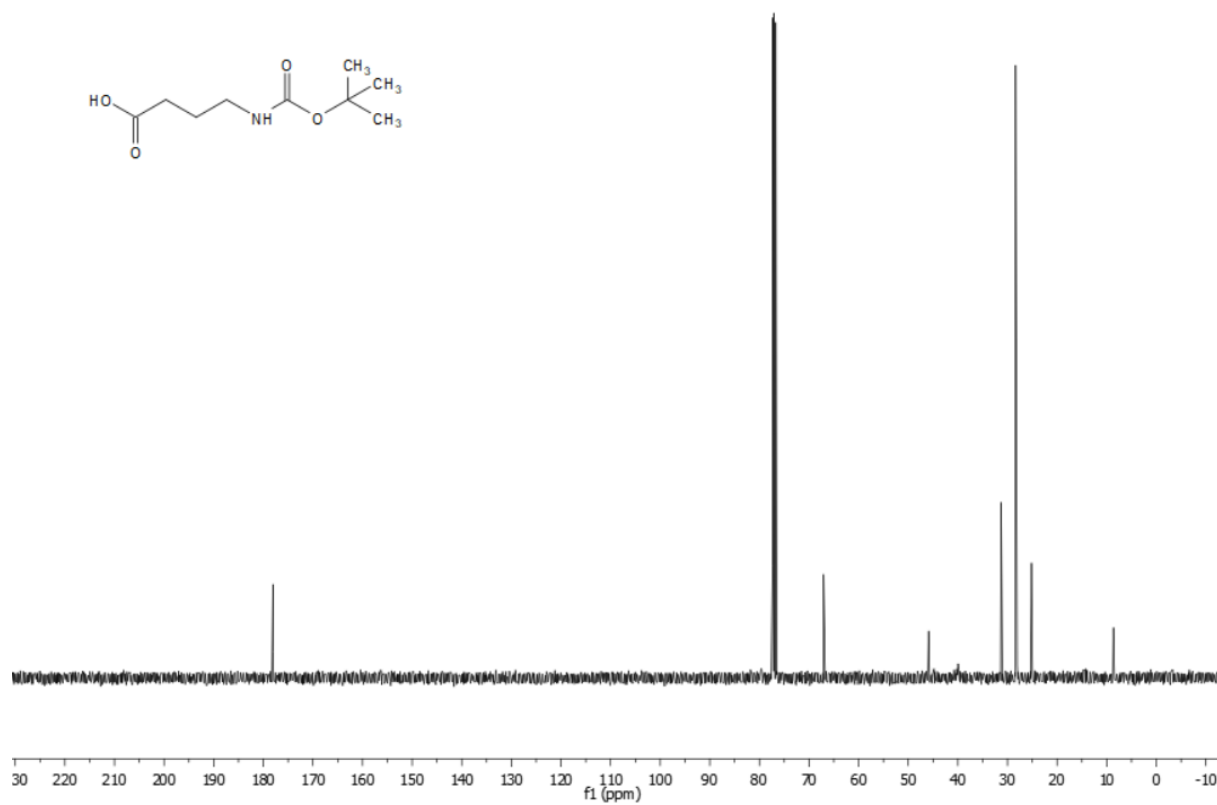
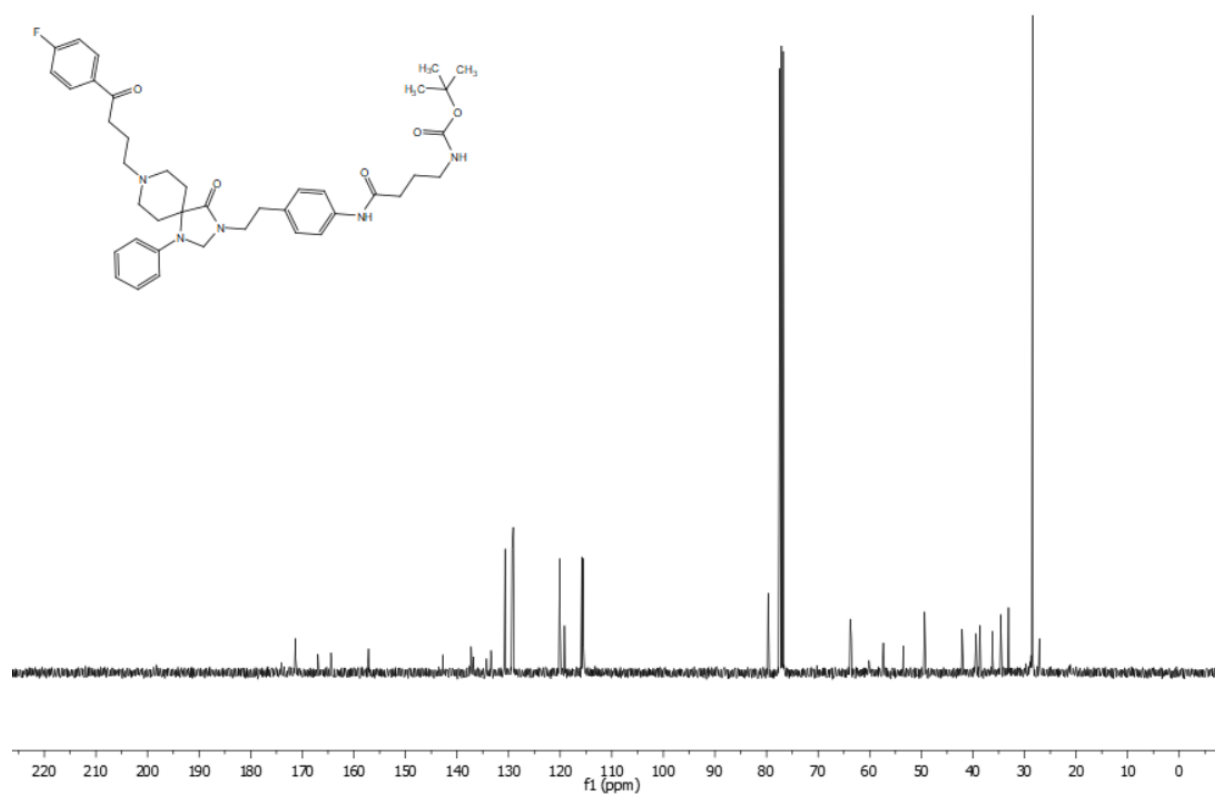
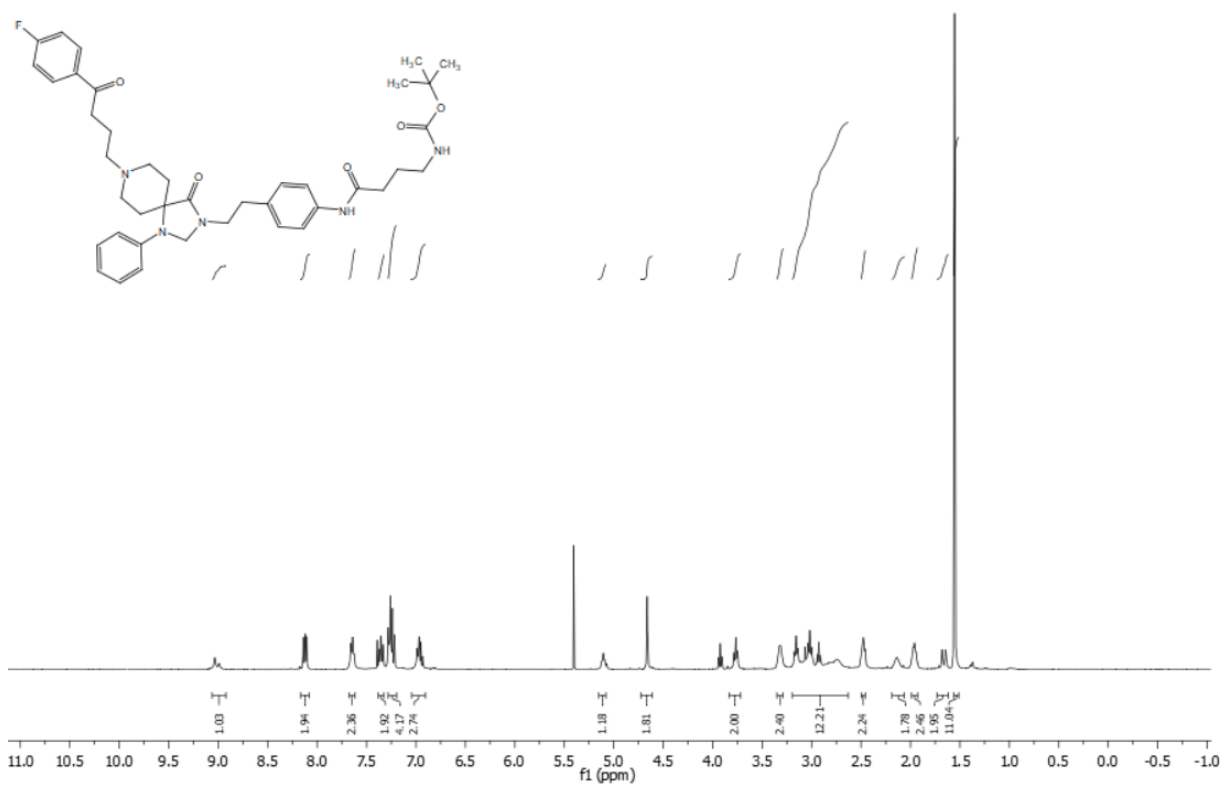


Figure S29. ¹³C NMR spectrum (101 MHz, CDCl₃) of compound 13.



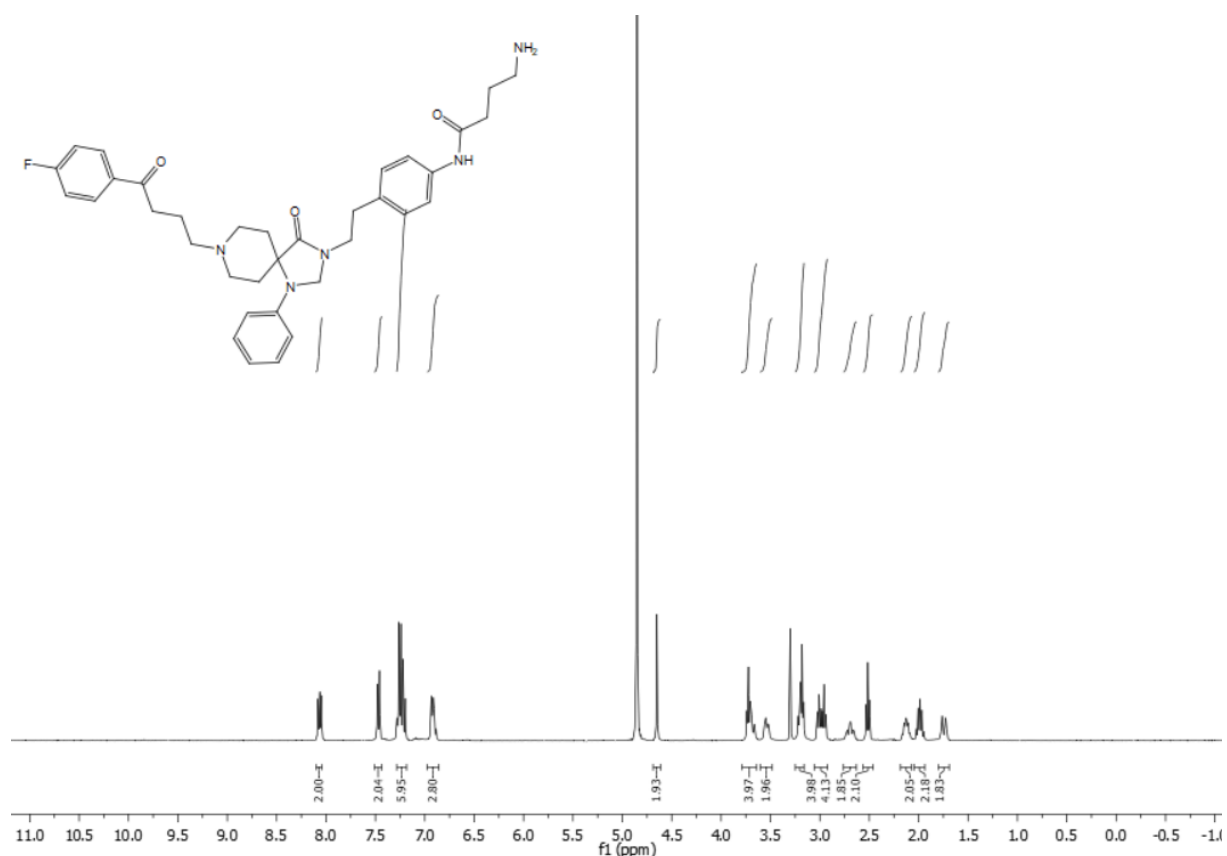


Figure S36. ^1H NMR spectrum (400 MHz, CD_3OD) of compound **19**.

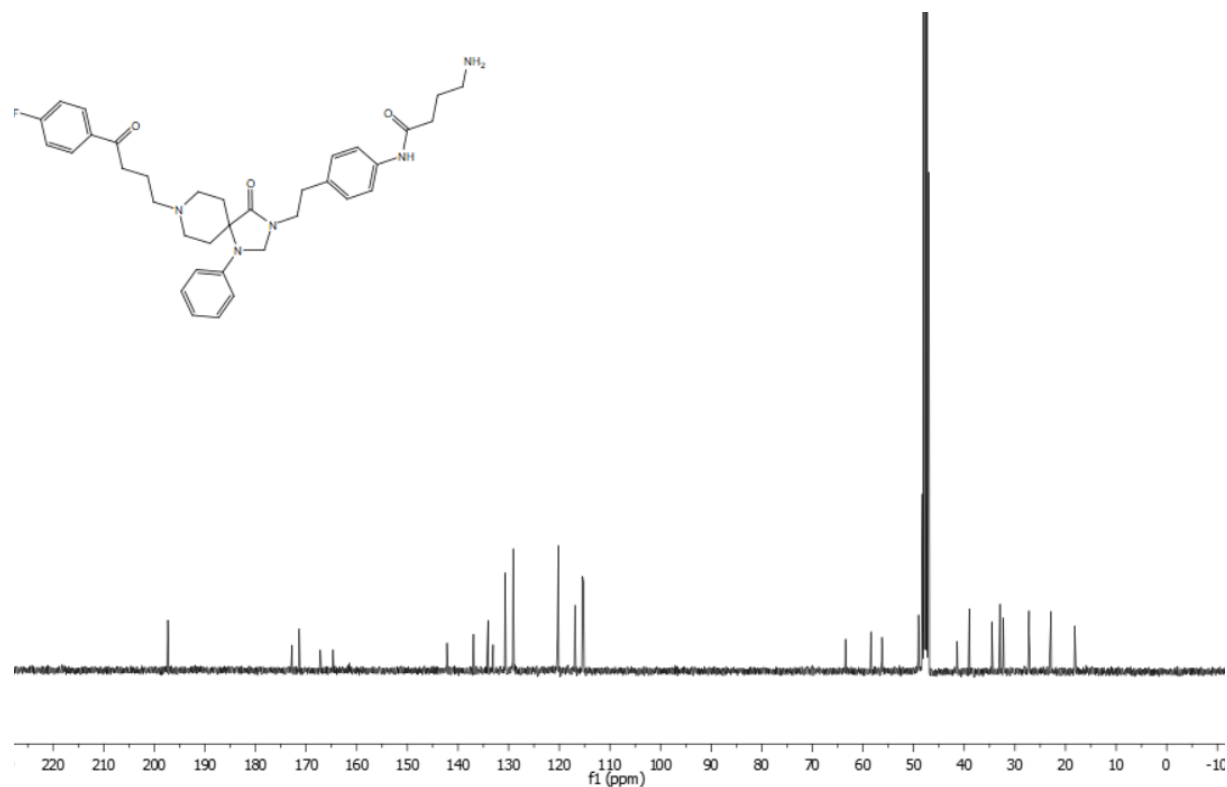


Figure S37. ^{13}C NMR spectrum (101 MHz, CD_3OD) of compound **19**.

5. Binding pose of spiperone bound to the D₂R

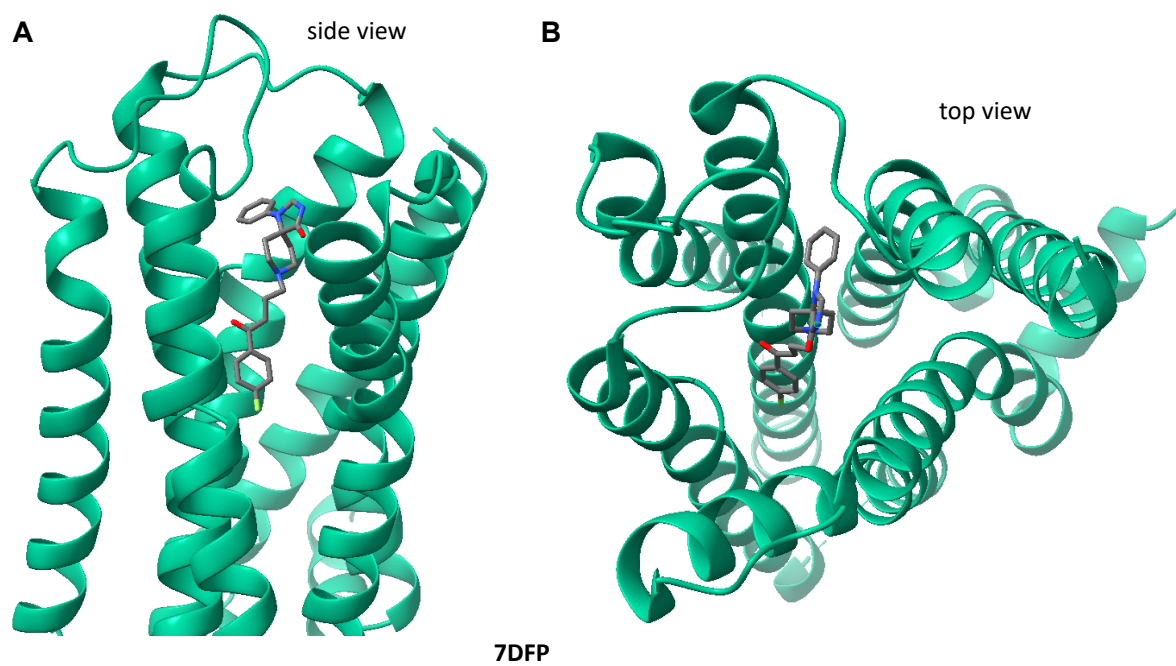


Figure S41. Binding pose of spiperone bound to the D₂R based on the cryo-EM structure 7DFP^[1] (side view, **A**; top view, **B**).

6. References

- [1] D. Im, A. Inoue, T. Fujiwara, T. Nakane, Y. Yamanaka, T. Uemura, C. Mori, Y. Shiimura, K. T. Kimura, H. Asada, N. Nomura, T. Tanaka, A. Yamashita, E. Nango, K. Tono, F. M. N. Kadji, J. Aoki, S. Iwata, T. Shimamura, *Nat Commun* **2020**, *11*, 6442.