Chemical, computational and functional insights into the chemical stability of the Hedgehog pathway inhibitor GANT61

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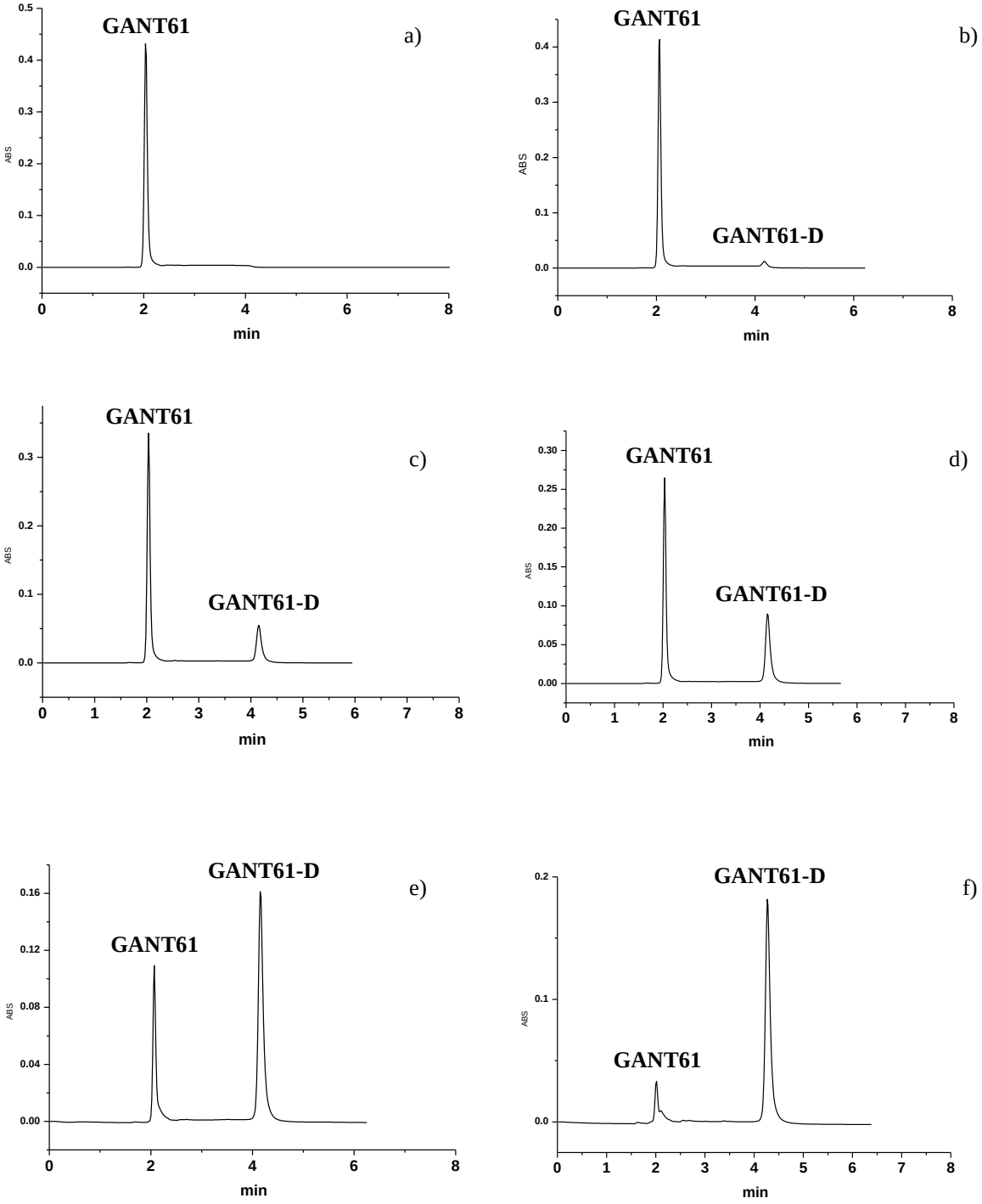


Figure S1. HPLC-UV chromatograms at different times confirm the disappearance of GANT61 and the formation of GANT61-D. **a)** GANT61 in CH_3CN; **b–f)** GANT61 in CH_3CN/100 mM NH_4OAc, pH=4.5; 95/5, v/v: b) t = 0 min; c) t = 15 min; d) t = 30 min; e) t = 75 min; f) t = 24 h.

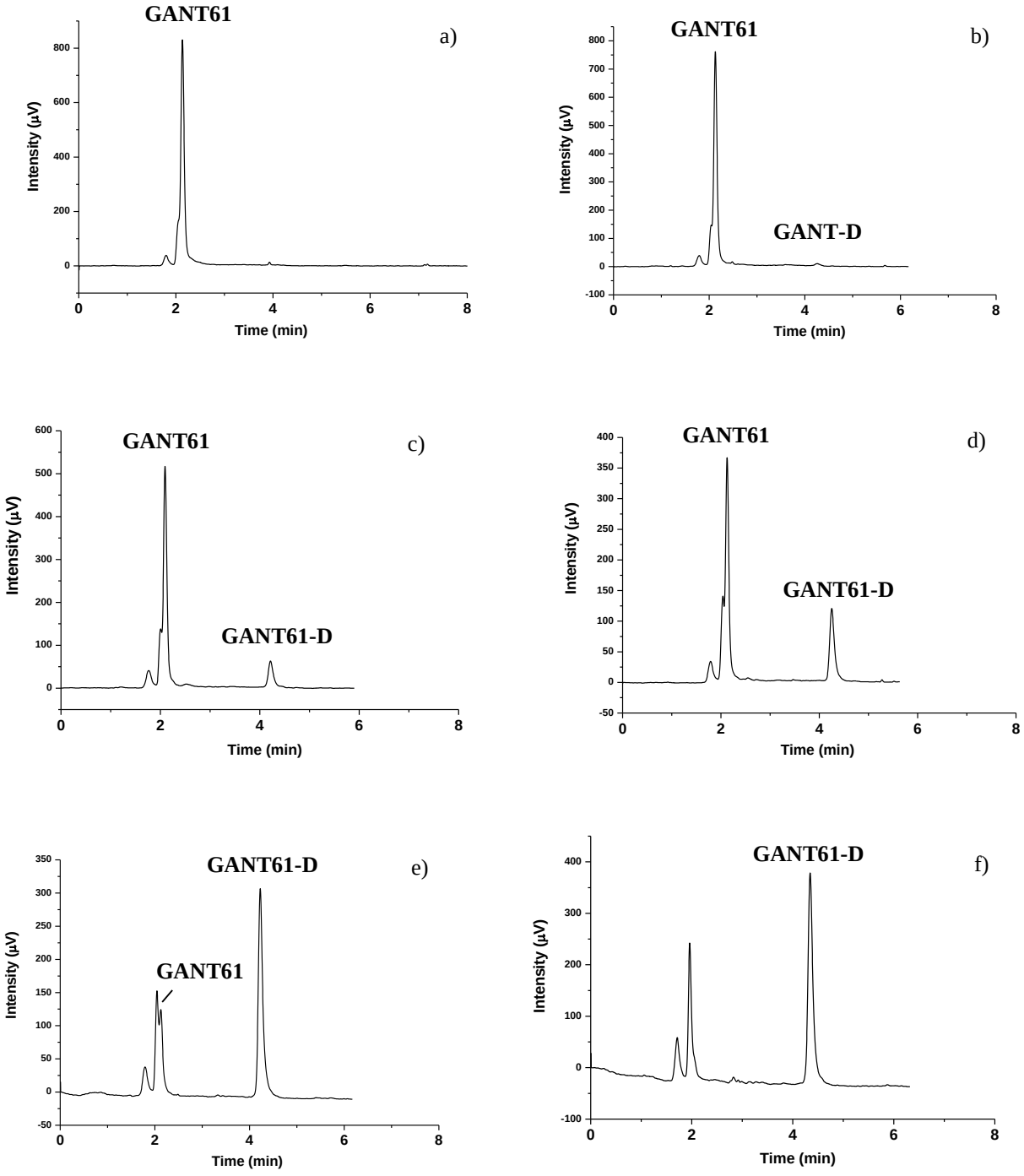


Figure S2. HPLC-ELSD chromatograms at different times confirm the disappearance of GANT61 and the formation of GANT61-D. **a)** GANT61 in CH_3CN; **b–f)** GANT61 in CH_3CN/100 mM NH_4OAc, pH=4.5; 95/5, v/v: b) t = 0 min; c) t = 15 min; d) t = 30 min; e) t = 75 min; f) t = 24 h.

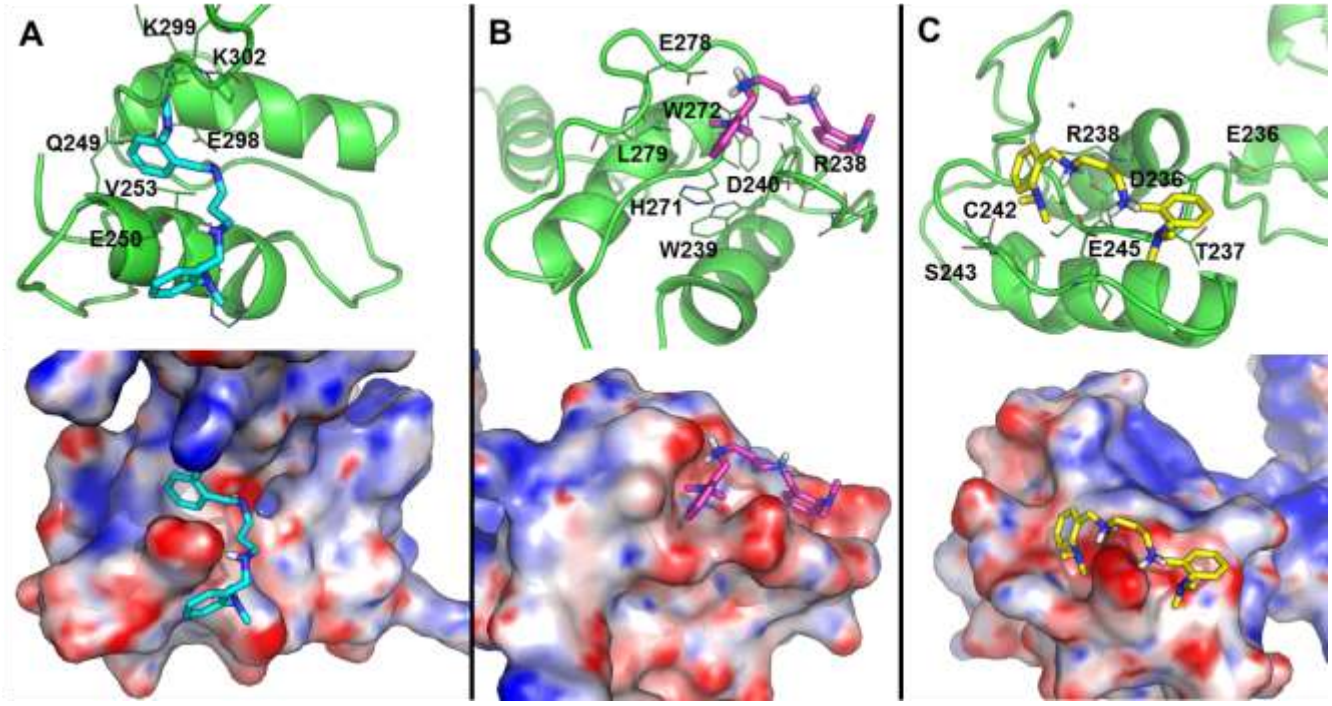
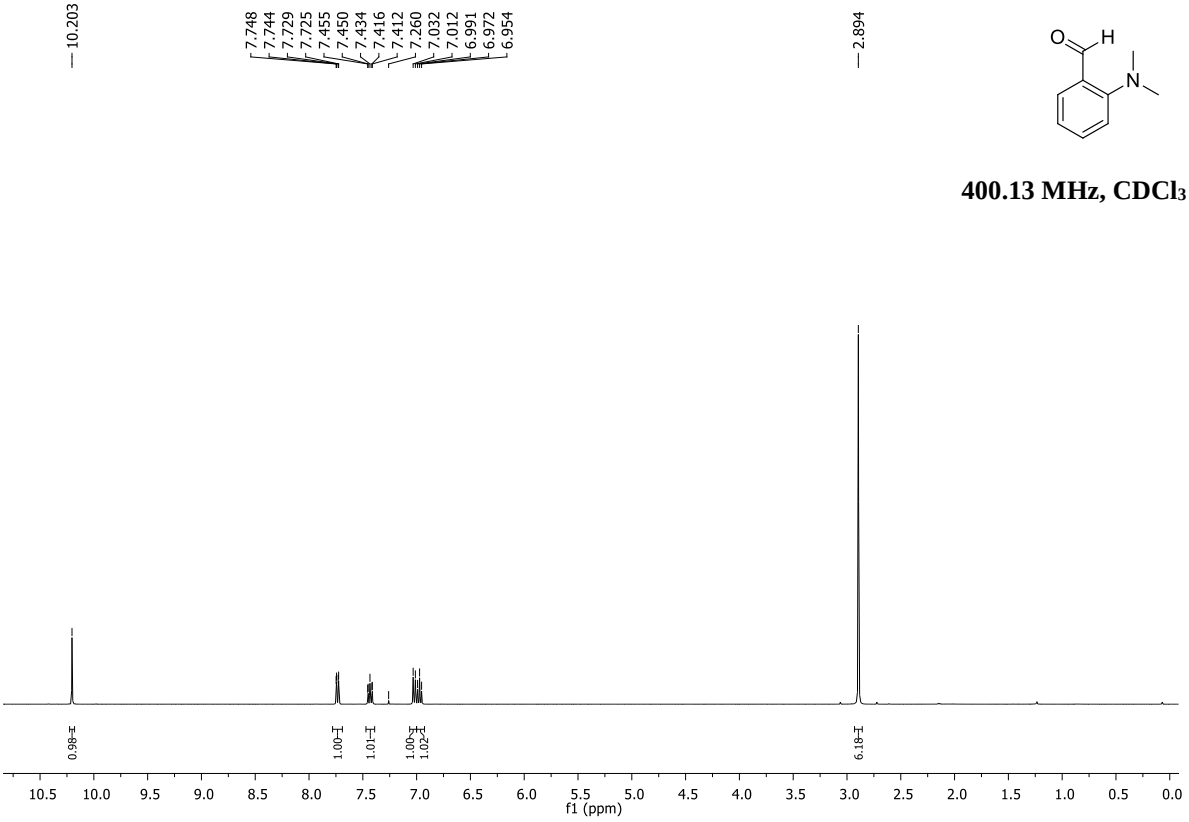
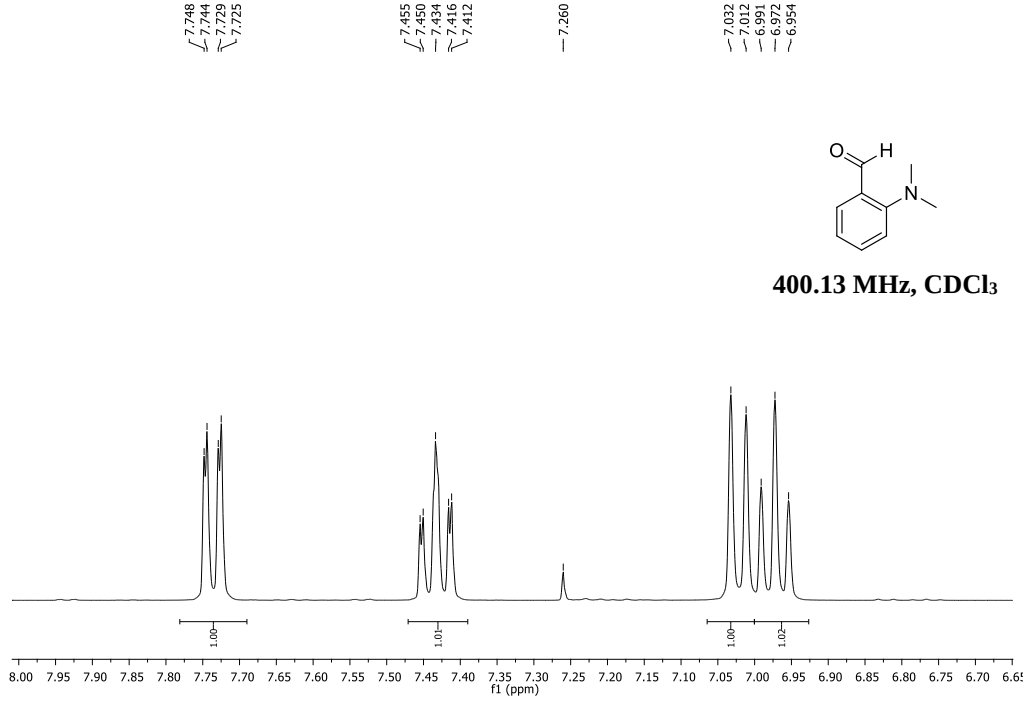


Figure S3. Magnification of GANT61-D binding modes to Gli1ZF. **A)** predicted binding mode of the neutral form of GANT61-D (cyan sticks); **B)** predicted binding mode of the mono-protonated form of GANT61-D (magenta sticks); **C)** predicted binding mode of the di-protonated form of GANT61-D (yellow sticks). Gli1ZF is showed as green cartoon in top panels and surface colored according with electrostatic potential in bottom panels. Residues within 4 Å from GANT61-D are shown as green sticks and are labeled.

^{1}H NMR of 2-(dimethylamino)benzaldehyde

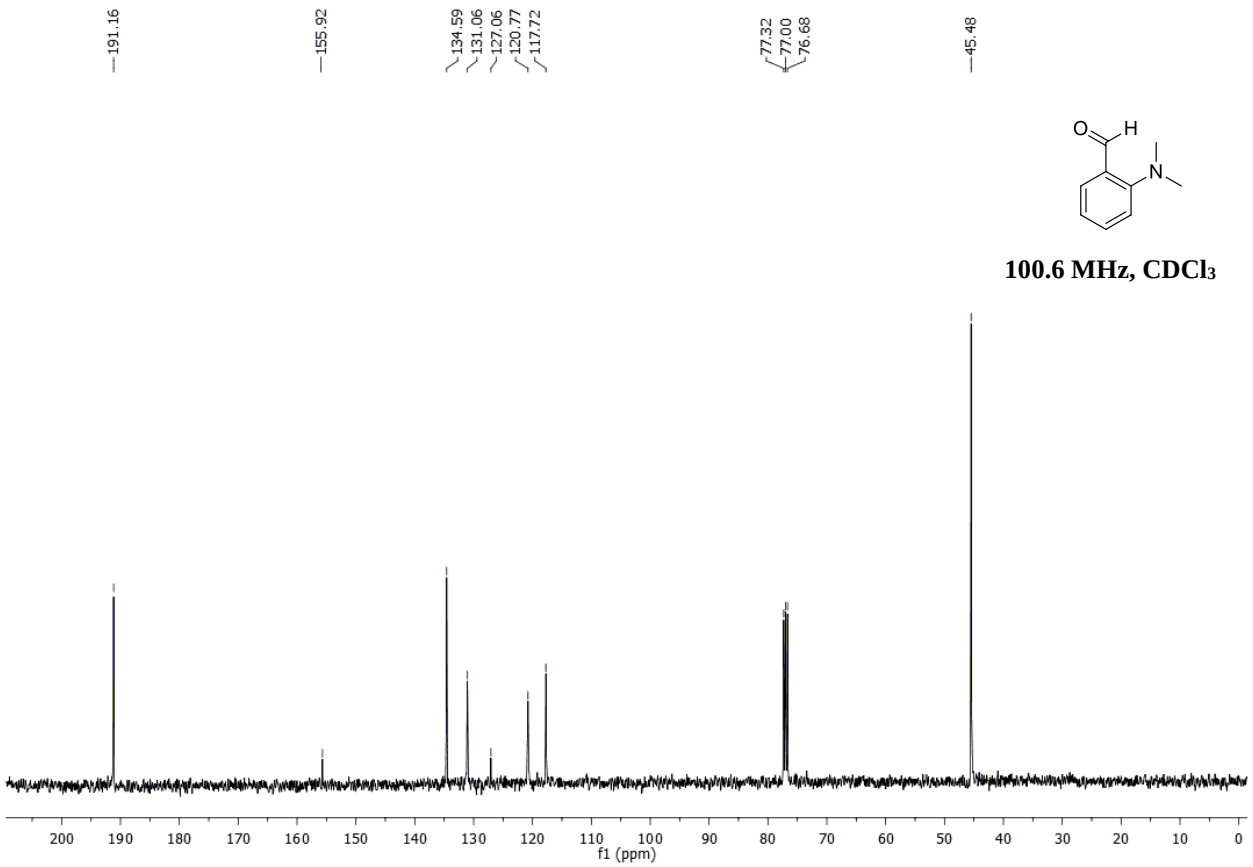


400.13 MHz, $CDCl_3$

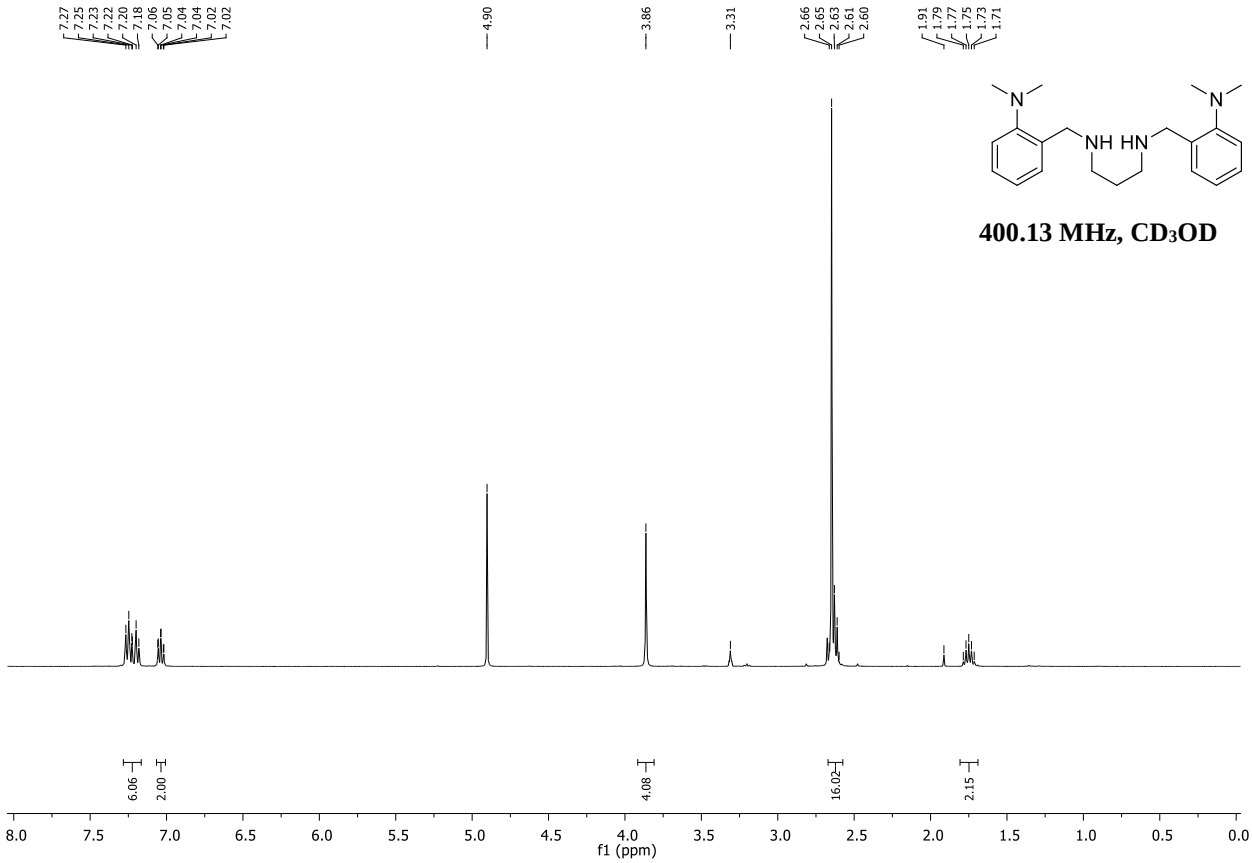


400.13 MHz, $CDCl_3$

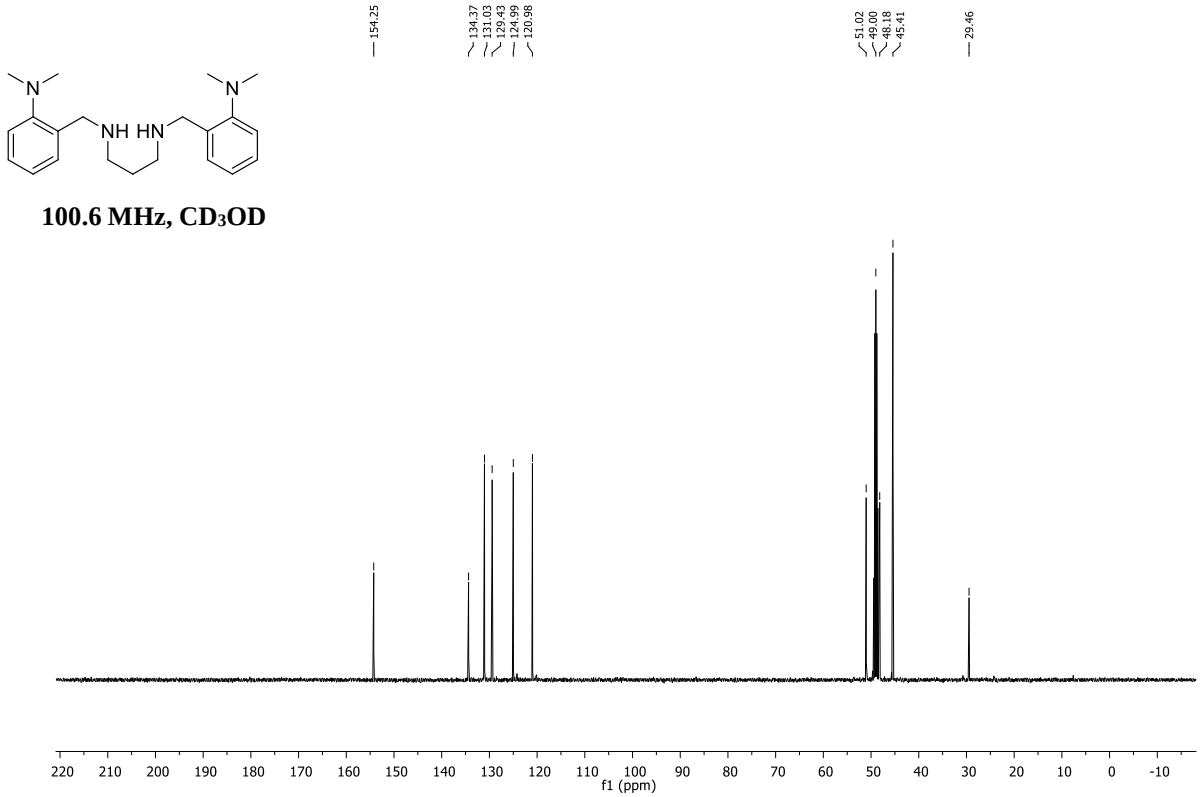
^{13}C NMR of 2-(dimethylamino)benzaldehyde



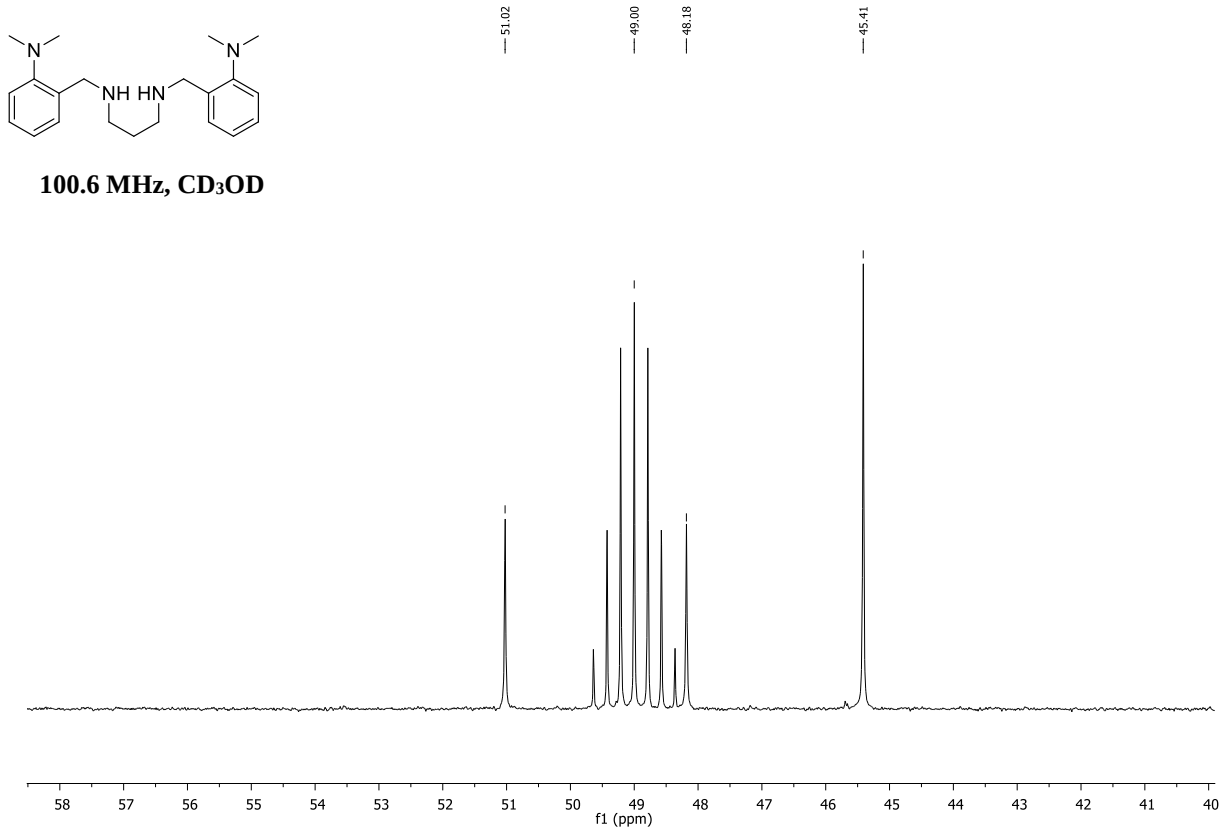
^{1}H NMR of GANT61-D



^{13}C NMR of GANT61-D

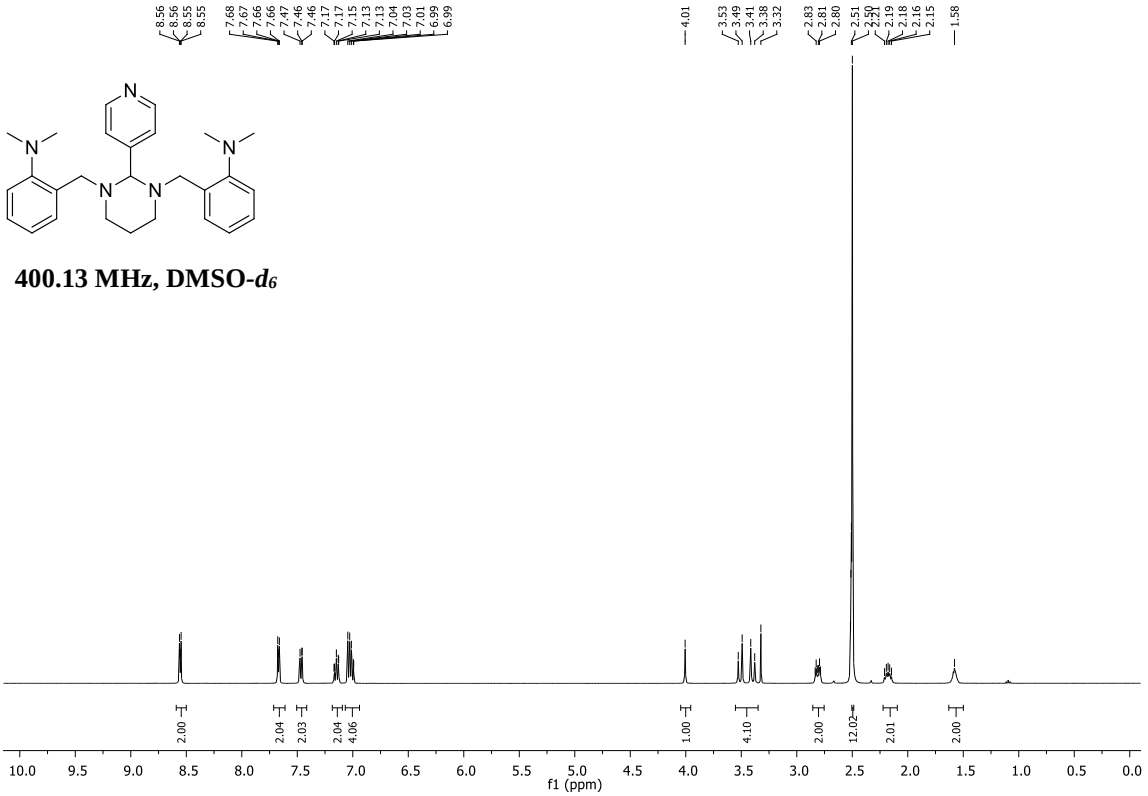


100.6 MHz, CD_3OD

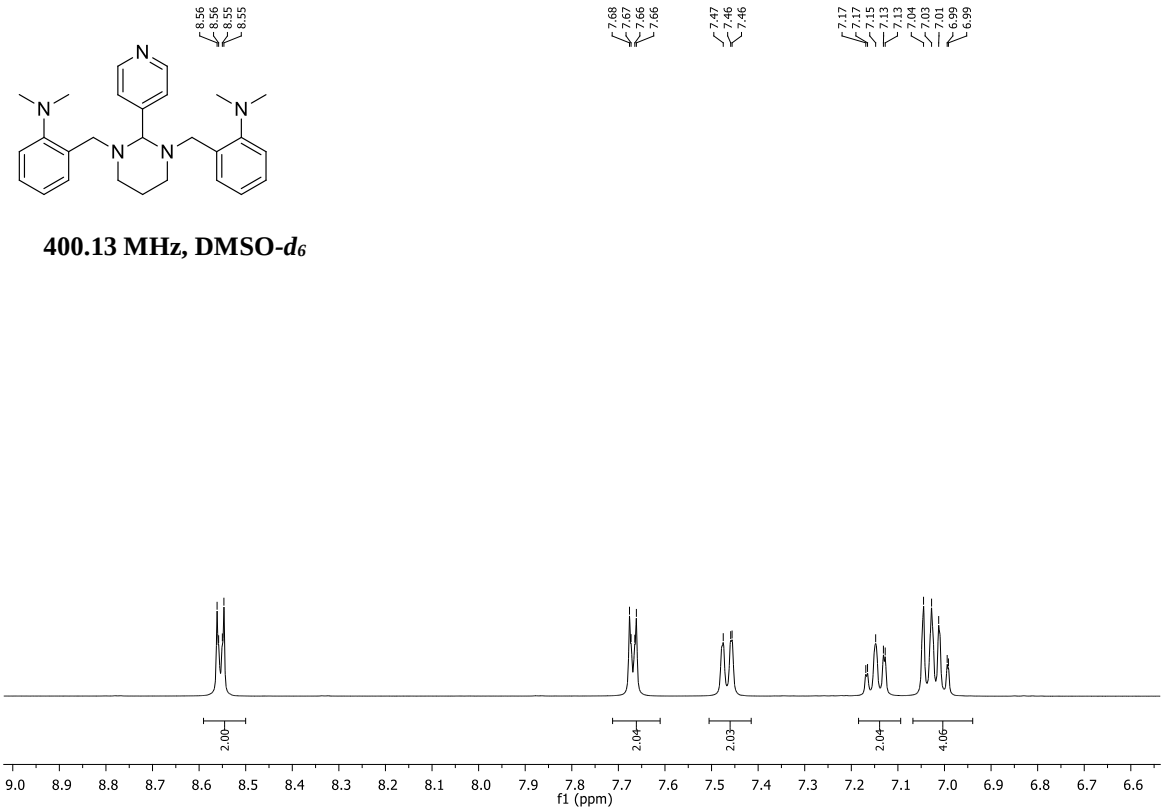


100.6 MHz, CD_3OD

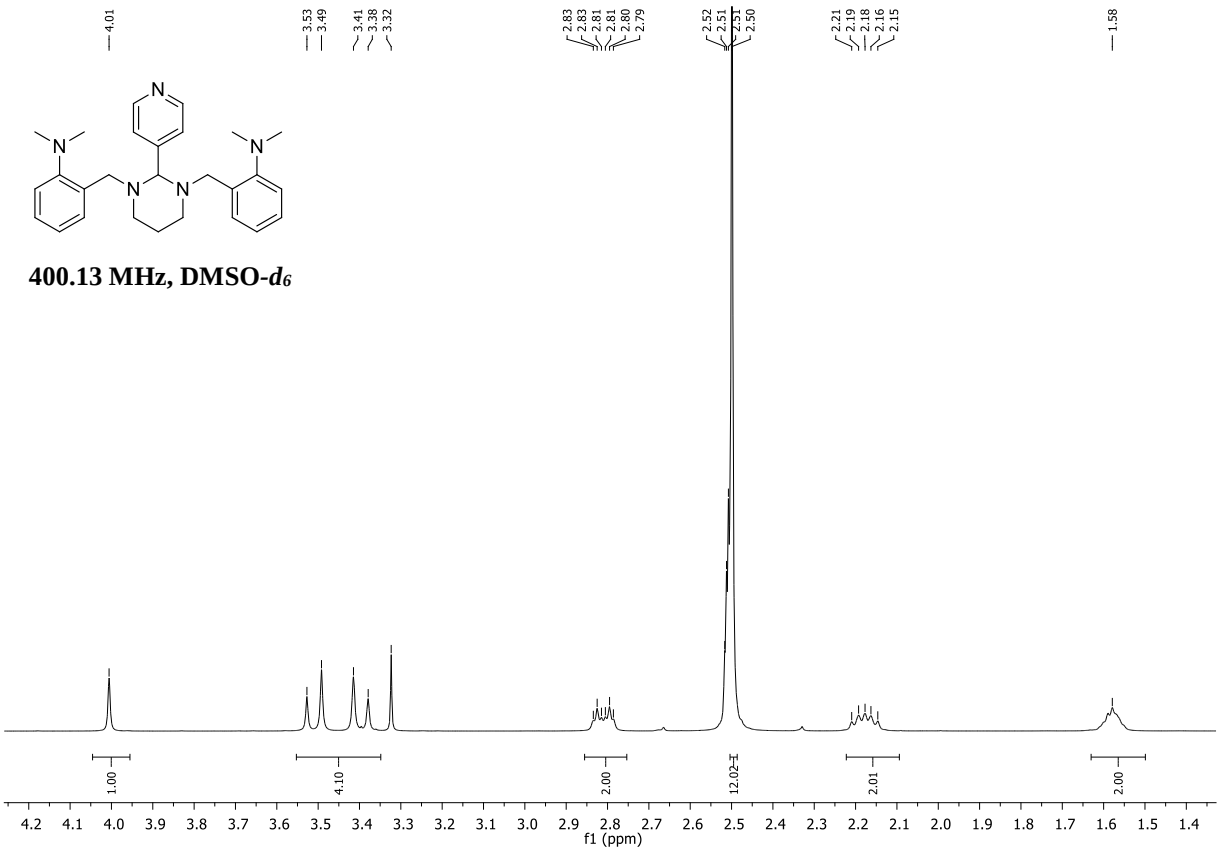
^{1}H NMR of GANT61



400.13 MHz, DMSO-*d*$_6$

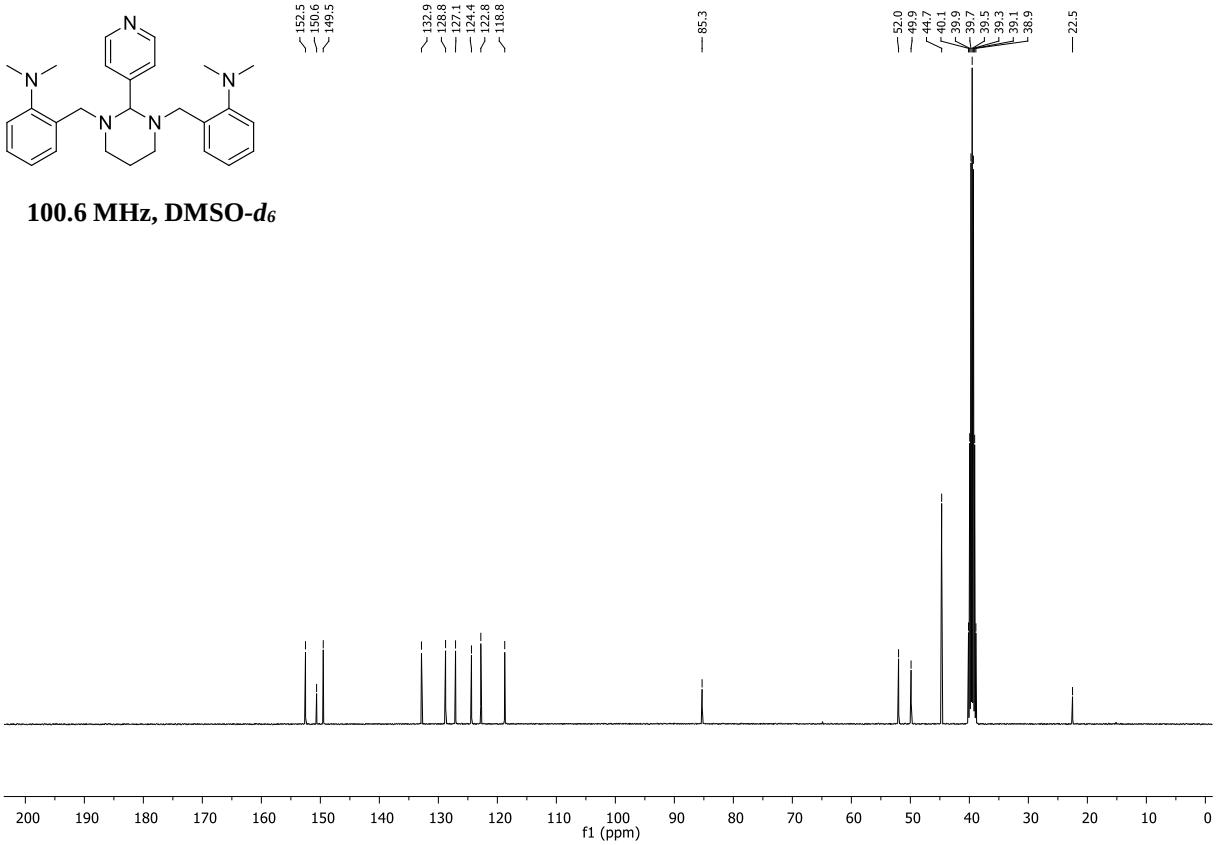


400.13 MHz, DMSO-*d*$_6$



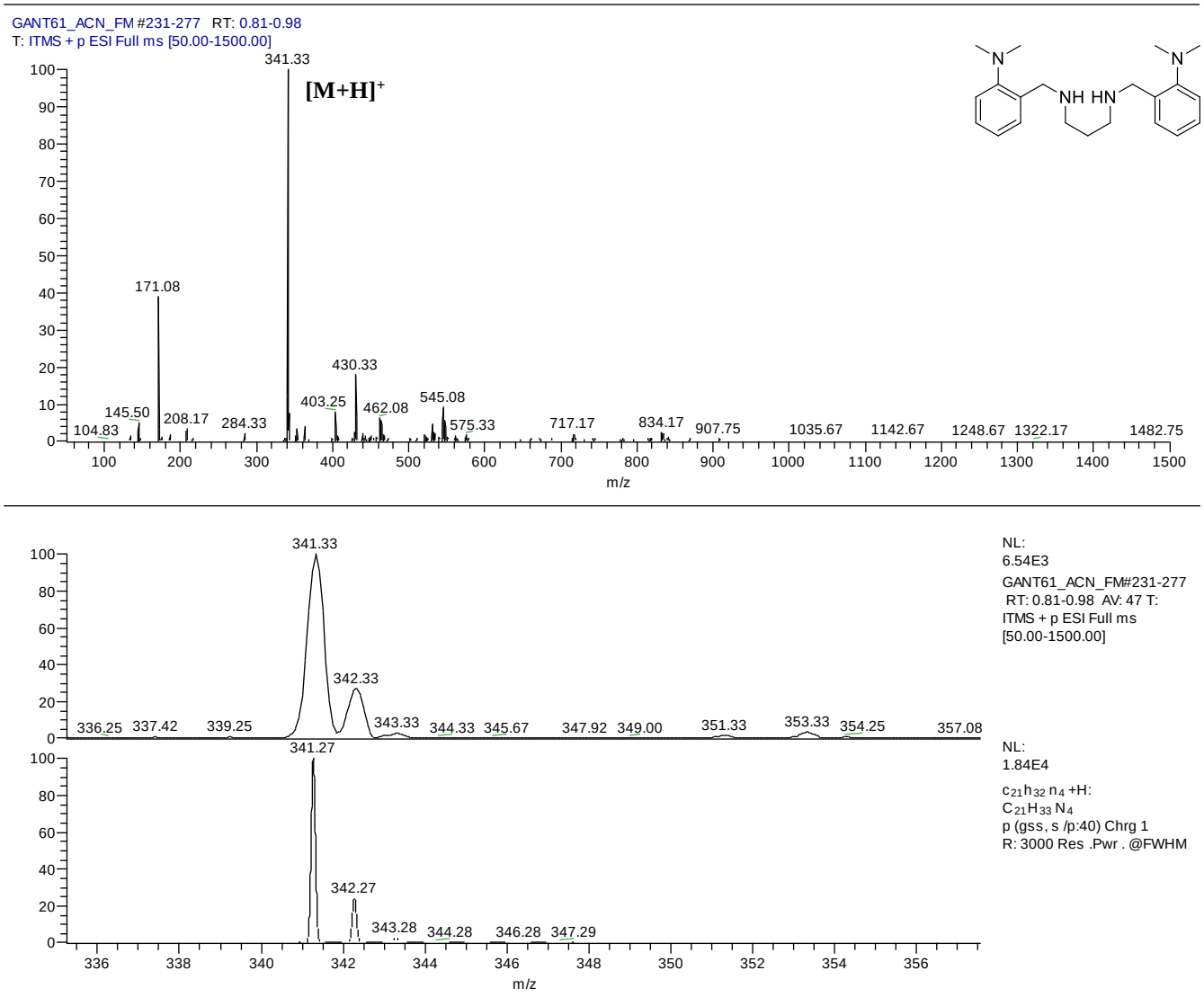
400.13 MHz, DMSO-*d*$_6$

^{13}C NMR of GANT61



100.6 MHz, DMSO-*d*$_6$

ESI MS of GANT61-D



ESI MS of GANT61