Electronic Supplementary Material (ESI) for Dalton Transactions.

Supplementary Information

Synthesis and antiproliferative activity of benzimidazole-based, trinuclear neutral cyclometallated and cationic, *N^N*-chelated ruthenium(II) complexes

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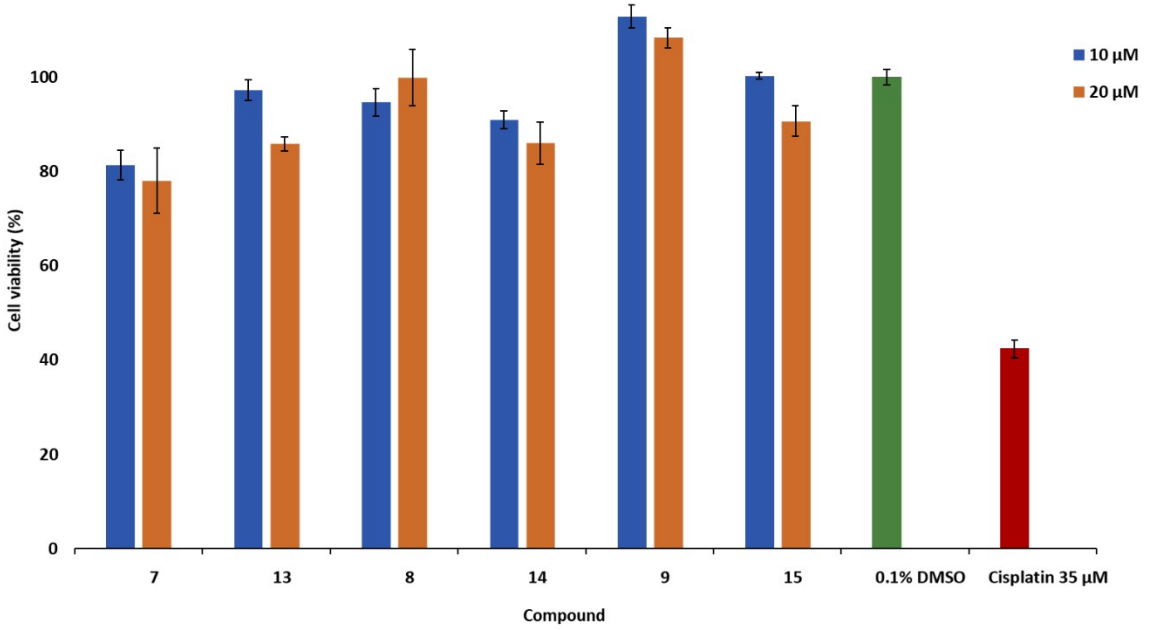

Figure S1: The *in vitro* activity of the 2-phenyl ligands (**7** - **9**) and corresponding cyclometallated Ru(II) complexes (**13** - **15**) against the MCF-7 breast cancer cell line at 10 µM and 20 µM. Vehicle (0.1 % DMSO) treated cells, and cells treated with 35 µM cisplatin (the IC_{50} of cisplatin in MCF-7 cells treated for 48 hours [44]) were included as vehicle and positive controls, respectively.

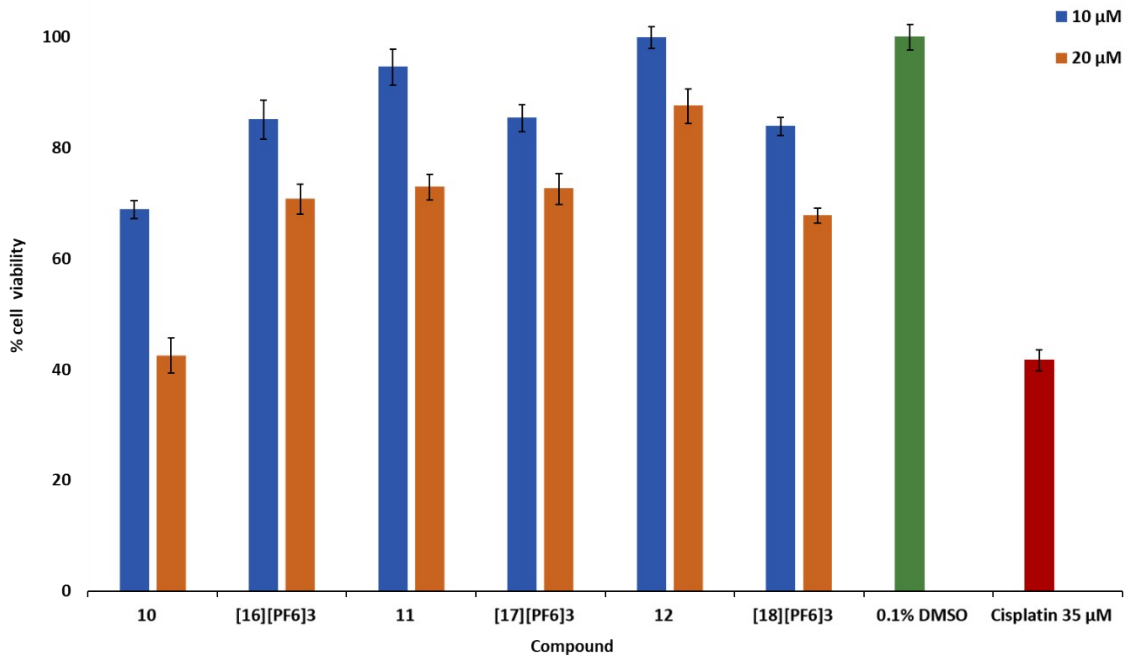

Figure S2: The *in vitro* activity of the 2-pyridyl ligands (**10 – 12**) and corresponding *N^N*-Ru(II) cationic complexes (**[16][PF_6]$_3$** - **[18][PF_6]$_3$**) against the MCF-7 breast cancer cell line at 10 µM and 20 µM. Vehicle (0.1 % DMSO) treated cells, and cells treated with 35 µM cisplatin (the IC_{50} of cisplatin in MCF-7 cells treated for 48 hours [44]) were included as vehicle and positive controls, respectively

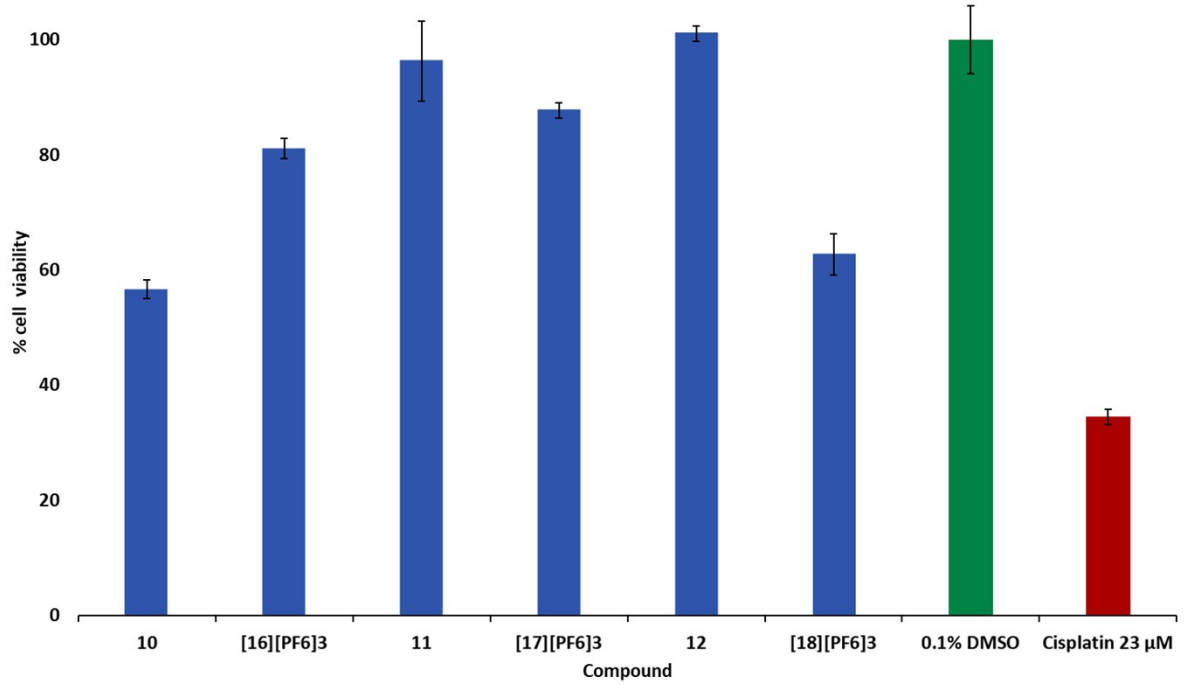

Figure S3: The *in vitro* activity of the 2-pyridyl ligands (**10 – 12**) and corresponding N^N-Ru(II) cationic complexes (**[16][PF_6]$_3$** - **[18][PF_6]$_3$**) against the MDA-MB-231 breast cancer cell line at 20 µM. Vehicle (0.1 % DMSO) treated cells, and cells treated with 23 µM cisplatin (the IC_{50} of cisplatin in MDA-MB-231 cells treated for 48 hours[46]) were included as vehicle and positive controls, respectively.

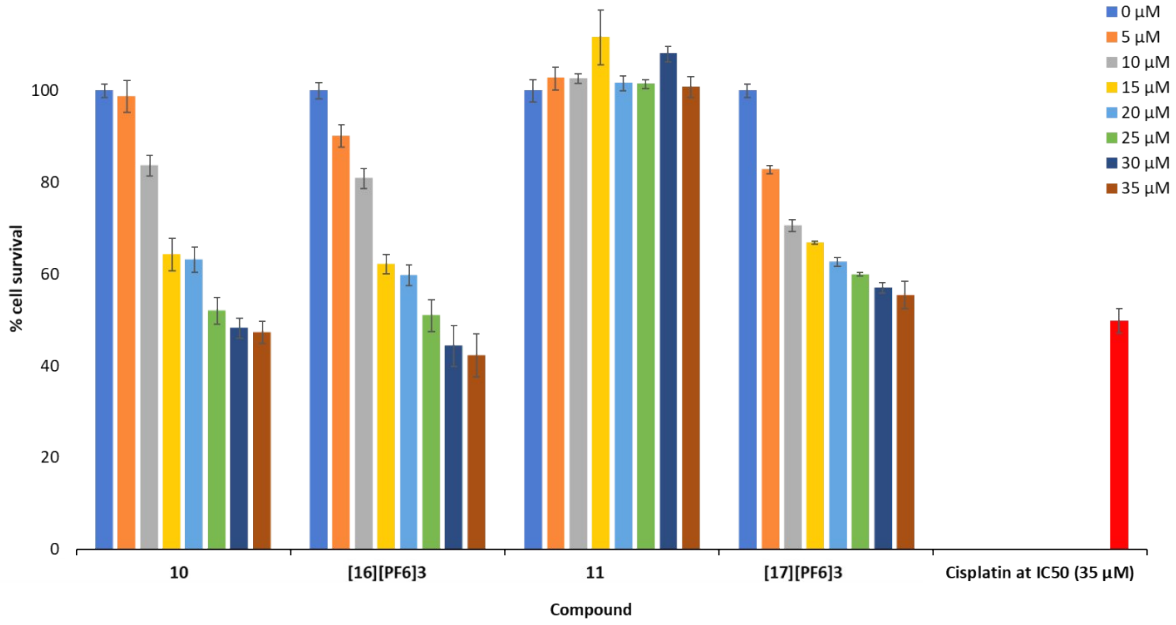

Figure S4: Treatment of MCF-7 cells with increasing concentrations of **10**, **[16]PF_6]$_3$**, **11**, **[17]PF_6]$_3$** and cisplatin at IC_{50} for 48 hours

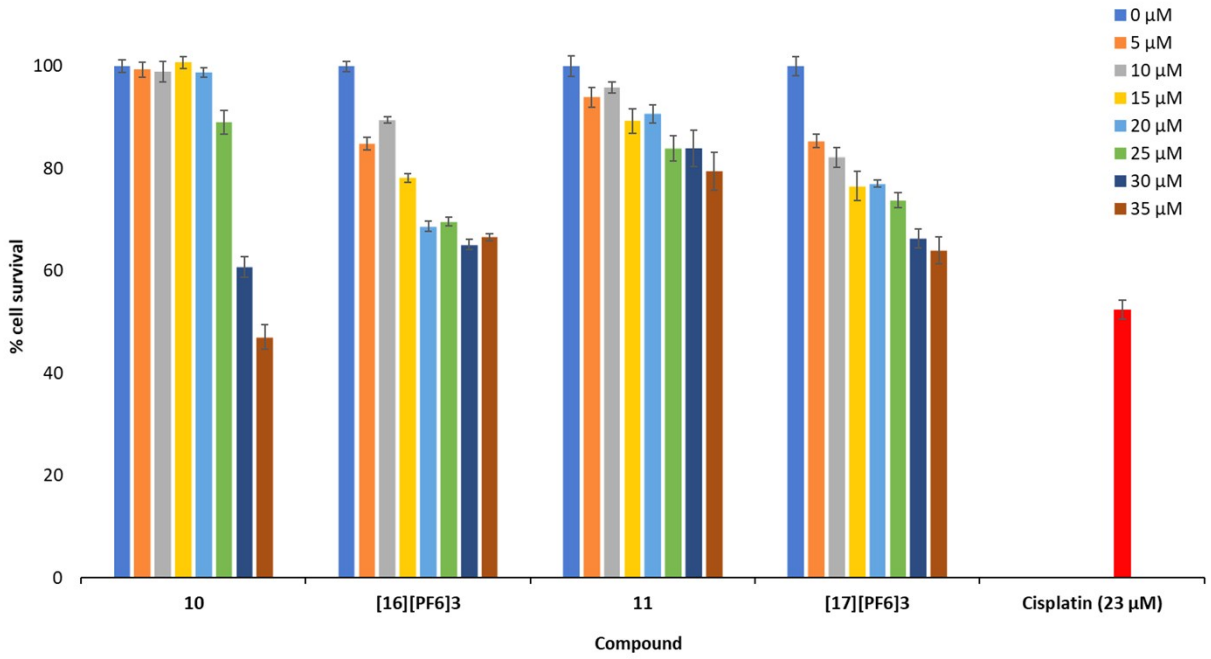

Figure S5: Treatment of MDA-MB-231 cells with increasing concentrations of **10**, **[16]$PF_6]_3$**, **11**, **[17]$PF_6]_3$** and cisplatin at IC_{50} for 48 hours

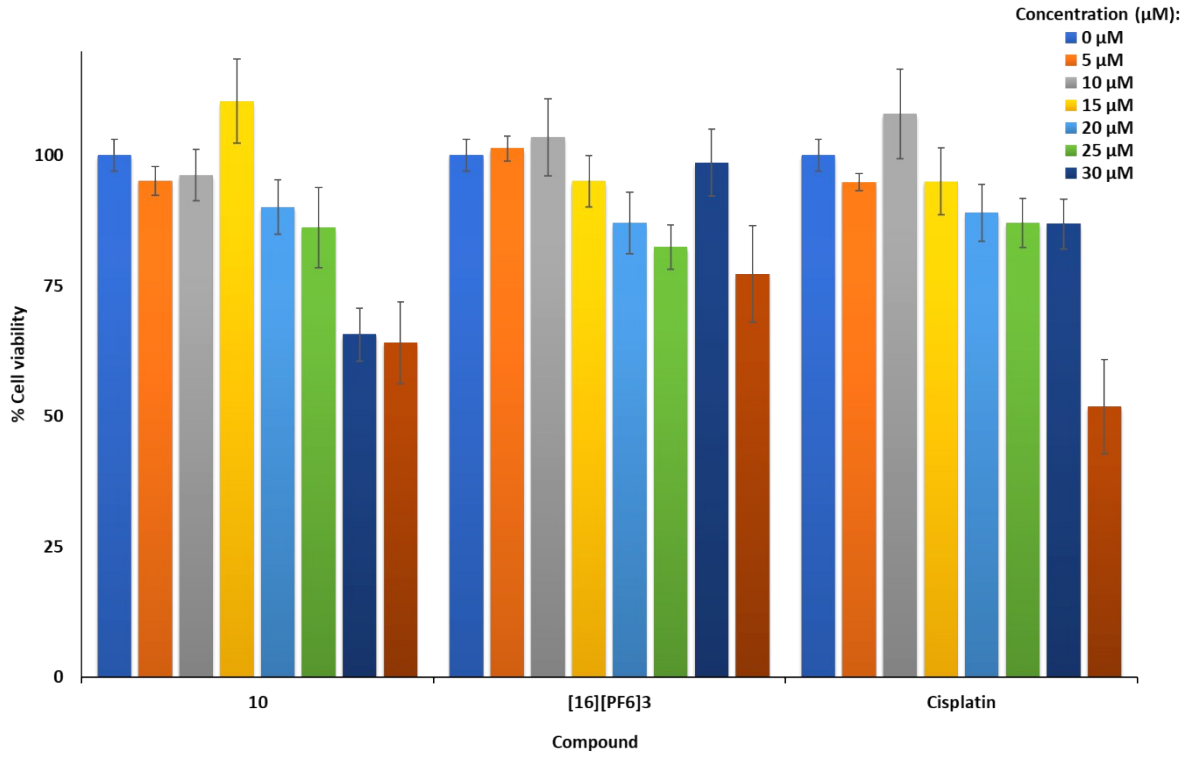

Figure S6: Treatment of non-tumorigenic MCF-12A cells with increasing concentrations of **10**, **[16]$PF_6]_3$** and cisplatin for 48 hours

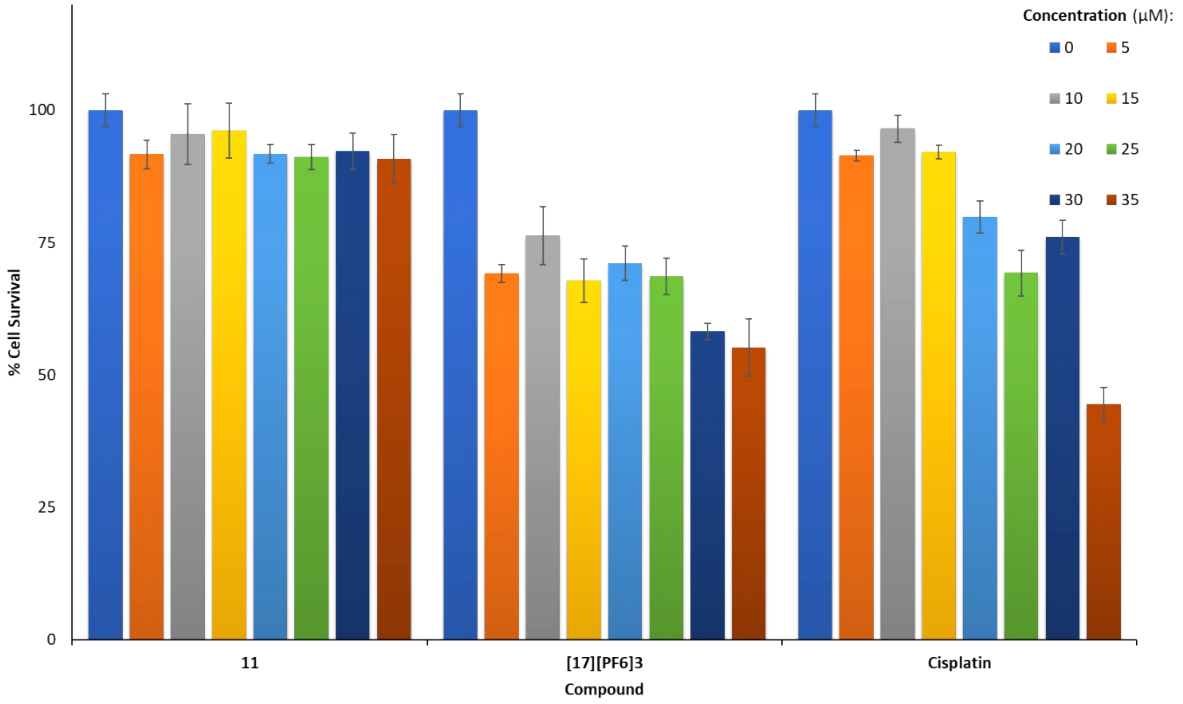

Figure S7: Treatment of non-tumorigenic MCF-12A cells with increasing concentrations of **11**, **[17]PF_6]$_3$** and cisplatin for 48 hours

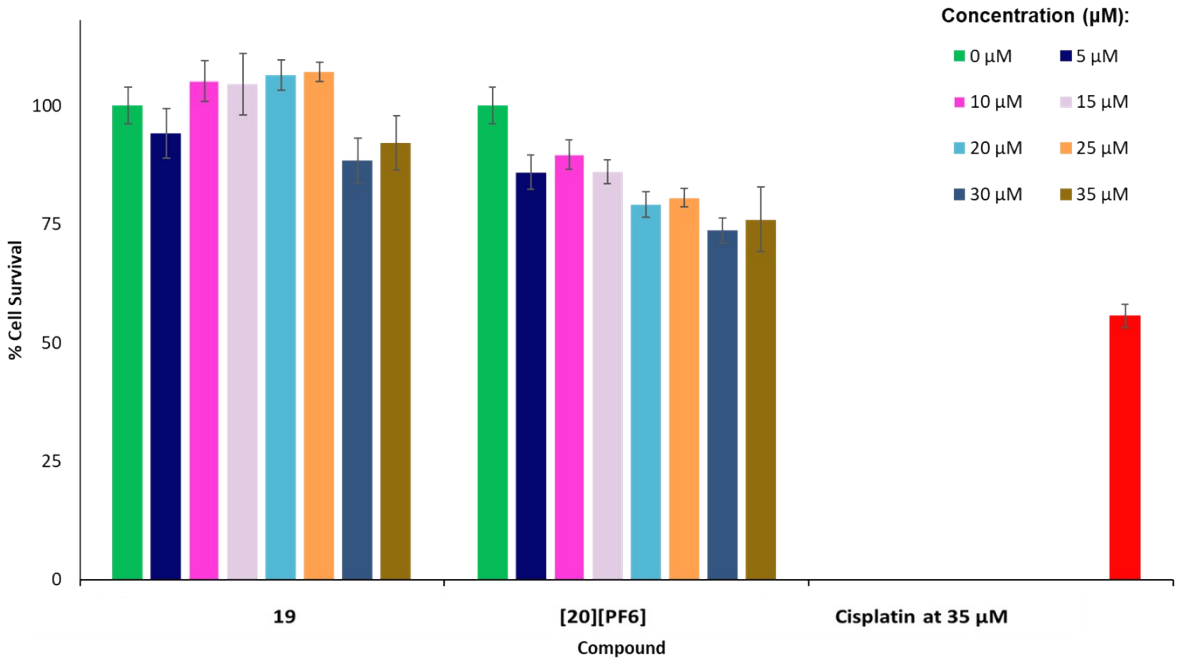

Figure S8:Treatment of MCF-7 breast cancer cells with increasing concentrations of **19** and **[20]PF6]** and cisplatin at IC_{50} of 48 hours

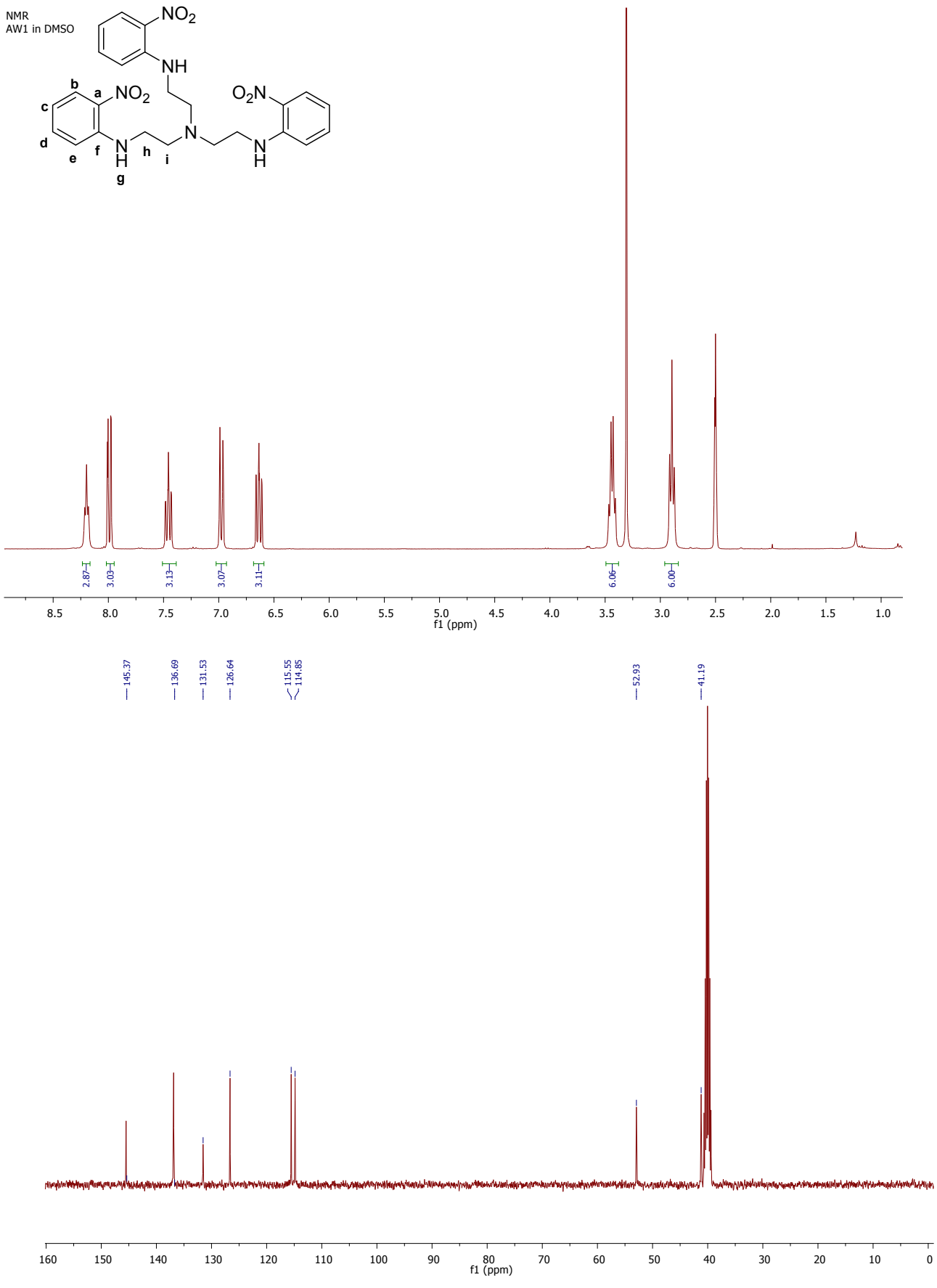

Figure S9: NMR Spectra of **1** in DMSO(d_6).

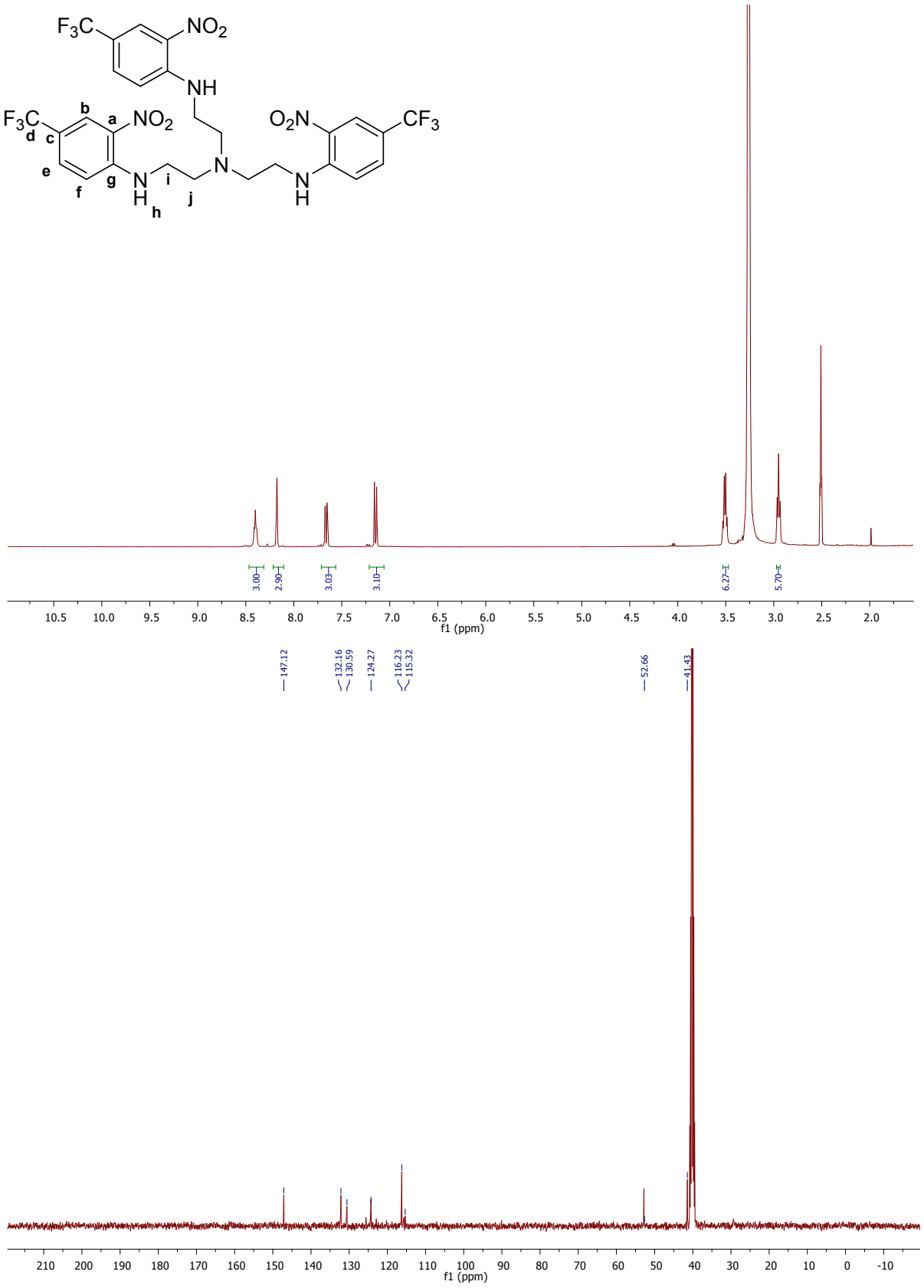

Figure S10: NMR Spectra of **2** in DMSO(d_6).

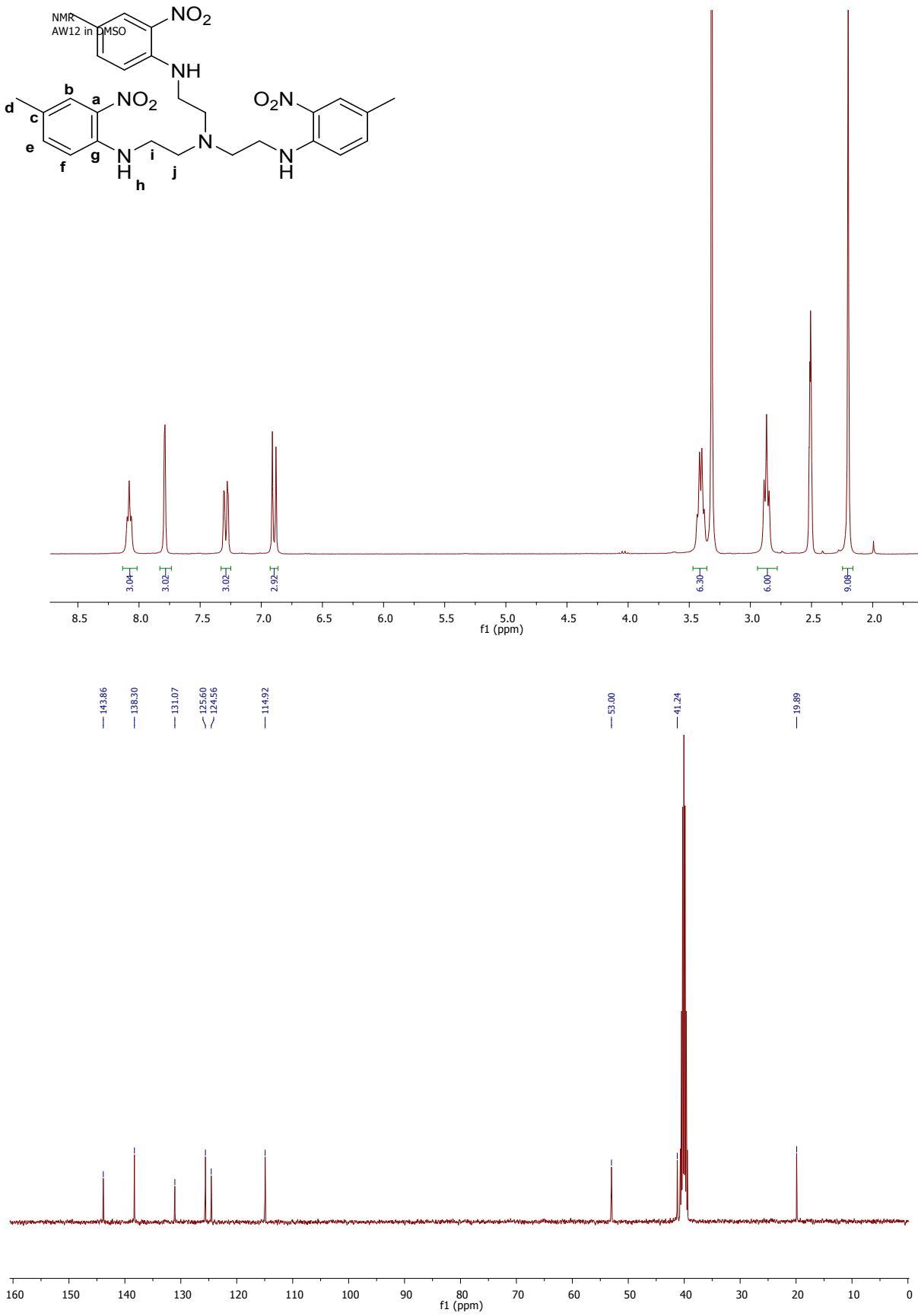

Figure S11: NMR Spectra of **3** in DMSO(d_6).

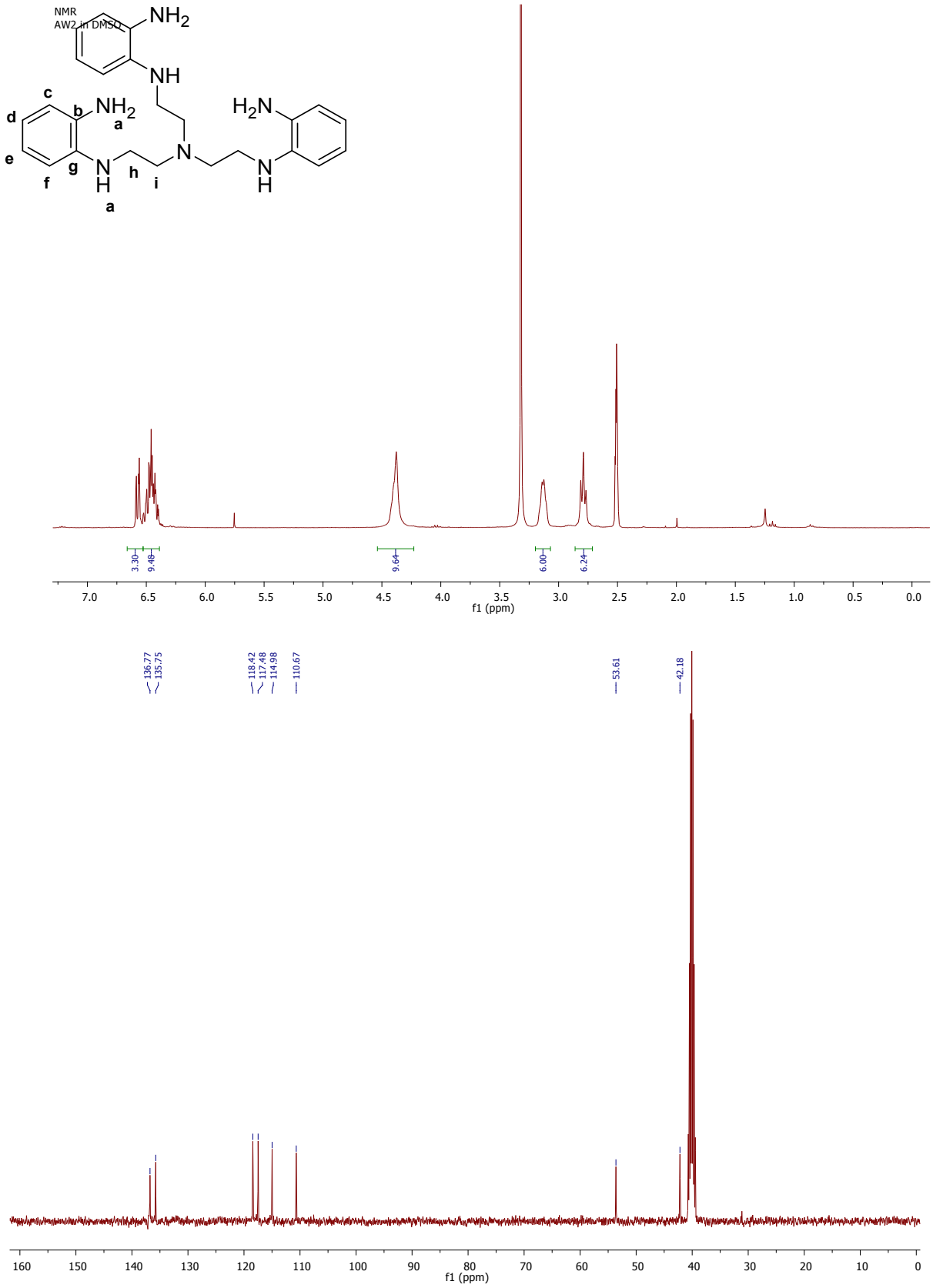

Figure S12: NMR Spectra of **4** in DMSO(d_6).

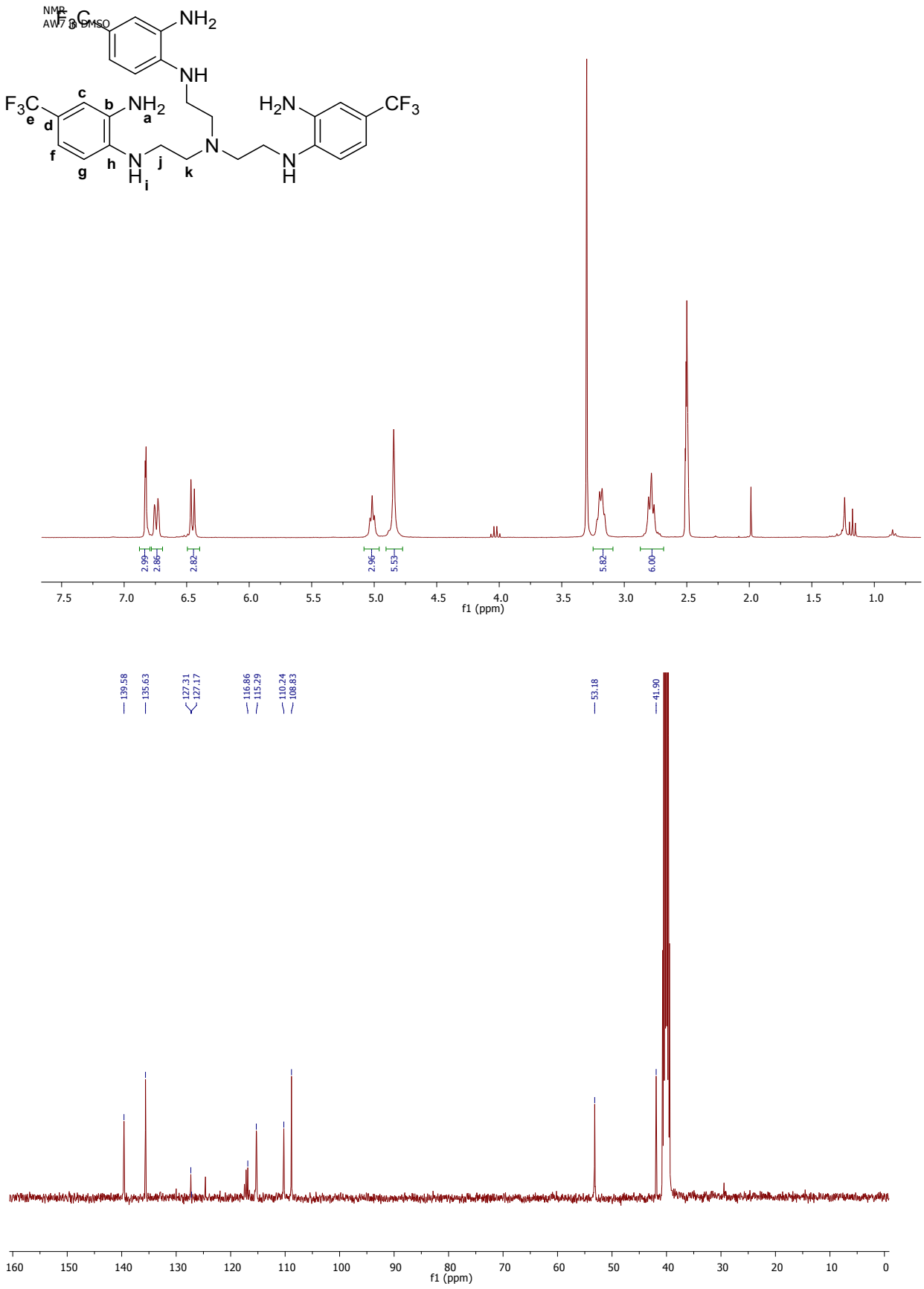

Figure S13: NMR Spectra of **5** in DMSO(d_6).

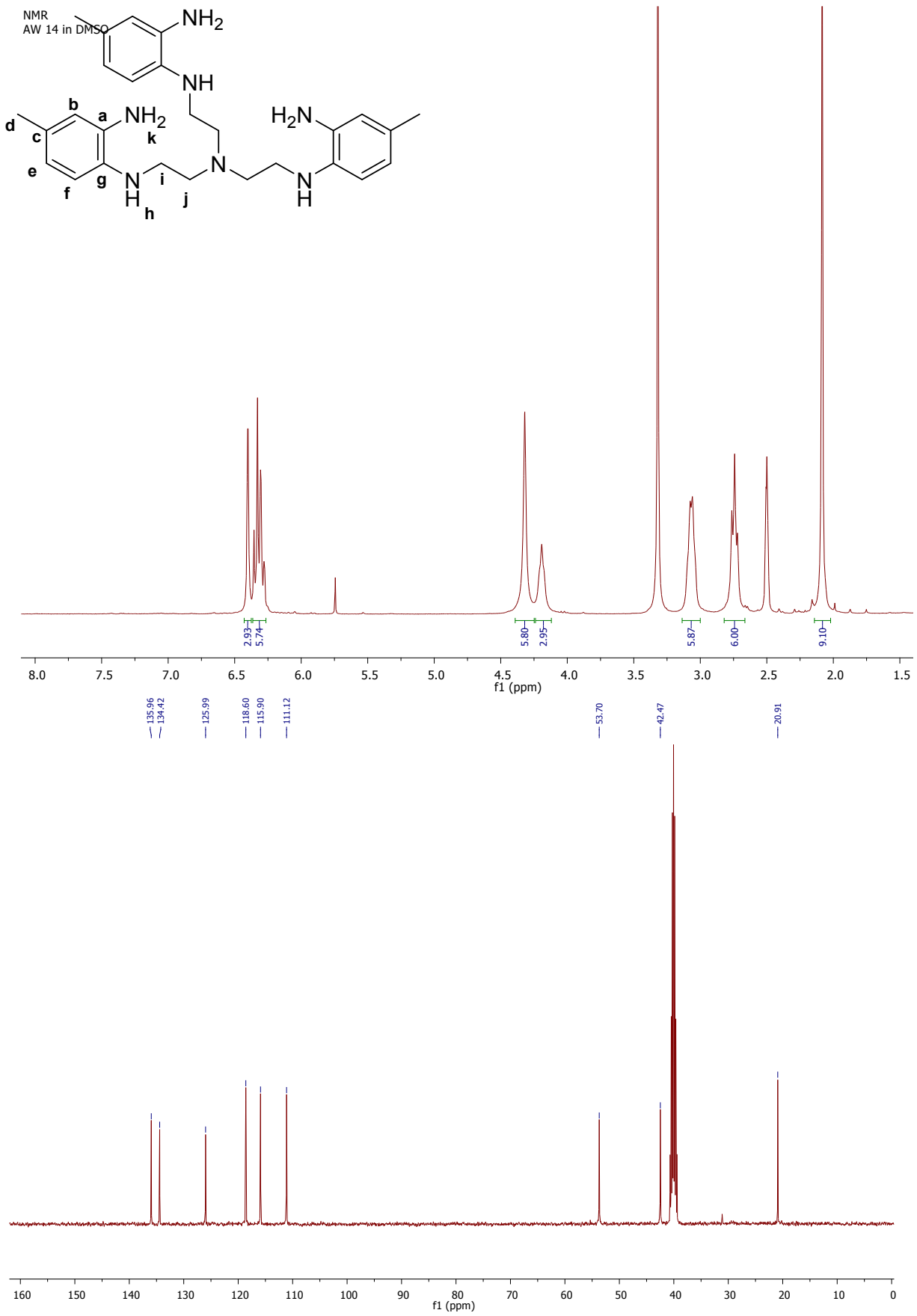

Figure S14: NMR Spectra of **6** in DMSO(d_6).

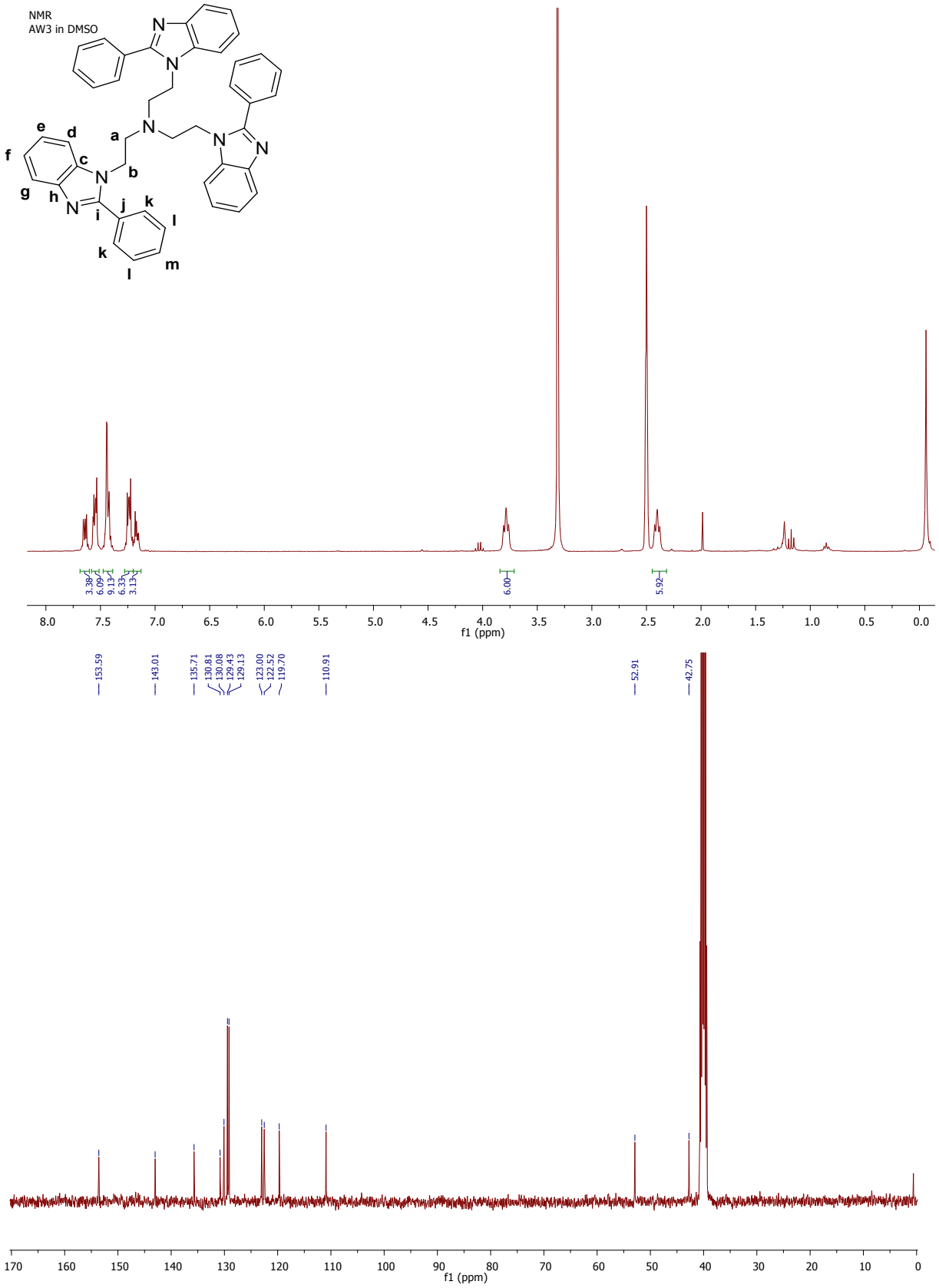

Figure S15: NMR Spectra of **7** in DMSO(d_6).

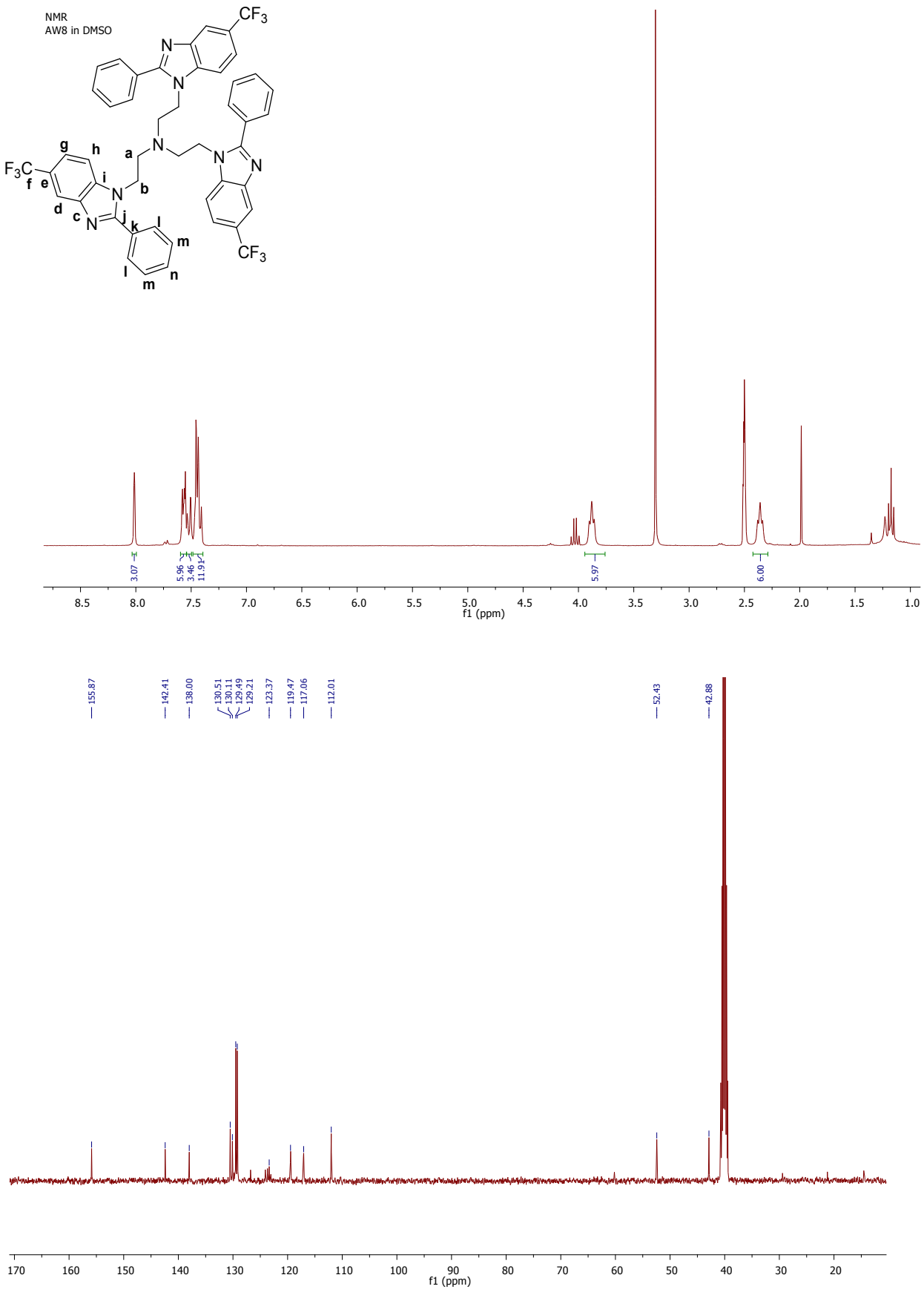

Figure S16: NMR Spectra of **8** in DMSO(d_6).

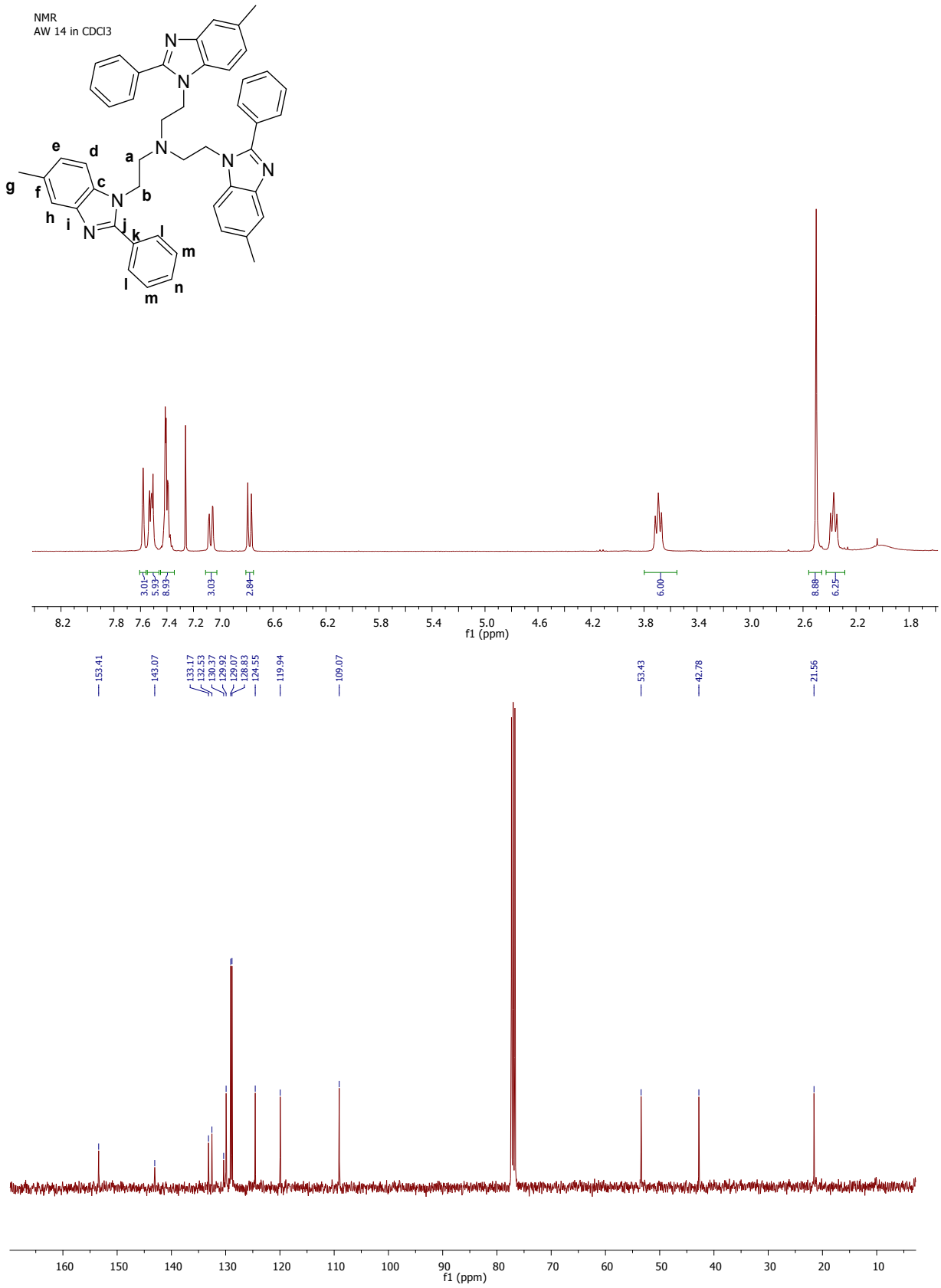

Figure S17: NMR Spectra of **9** in CDCl3.

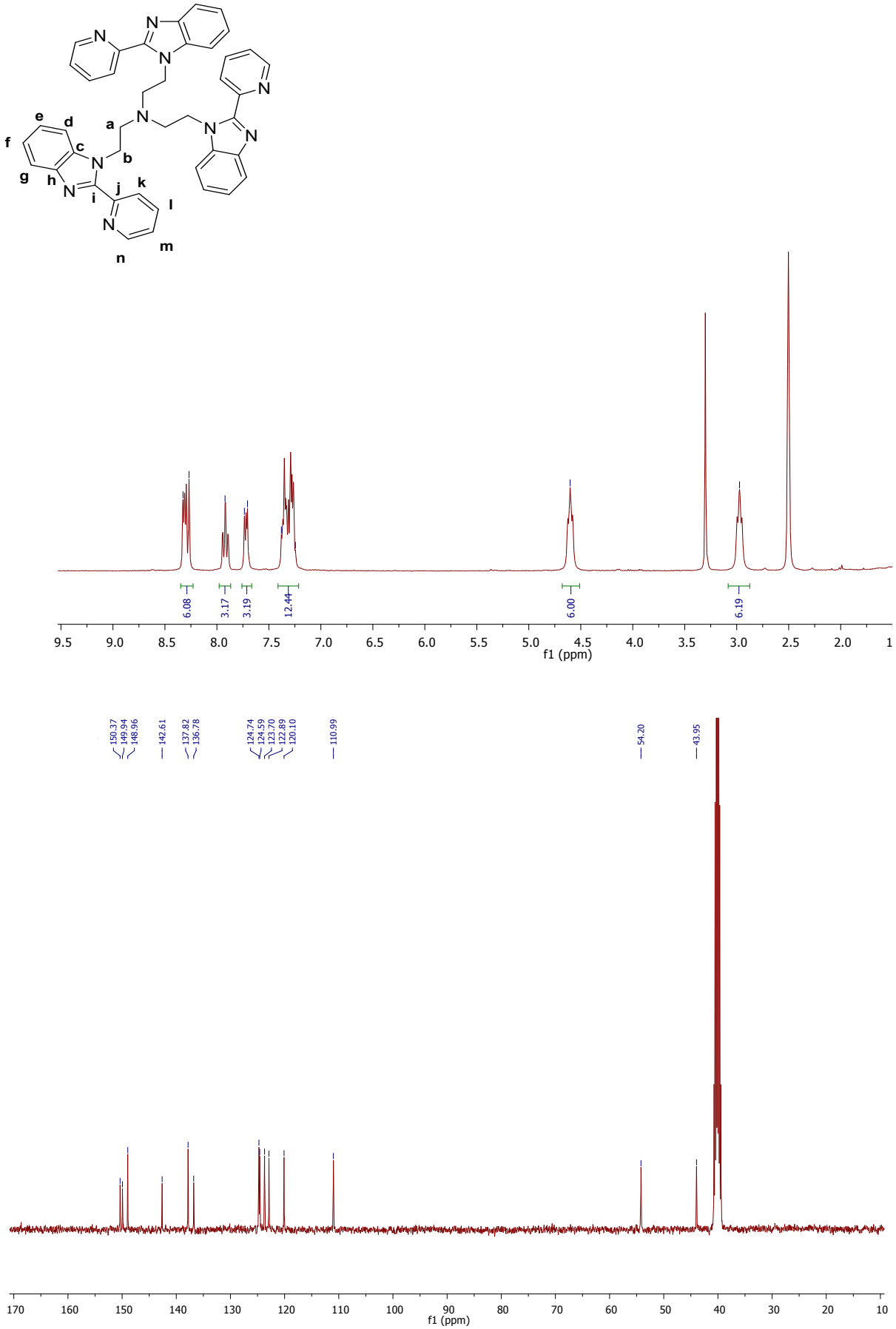

Figure S18: NMR Spectra of **10** in DMSO(d_6).

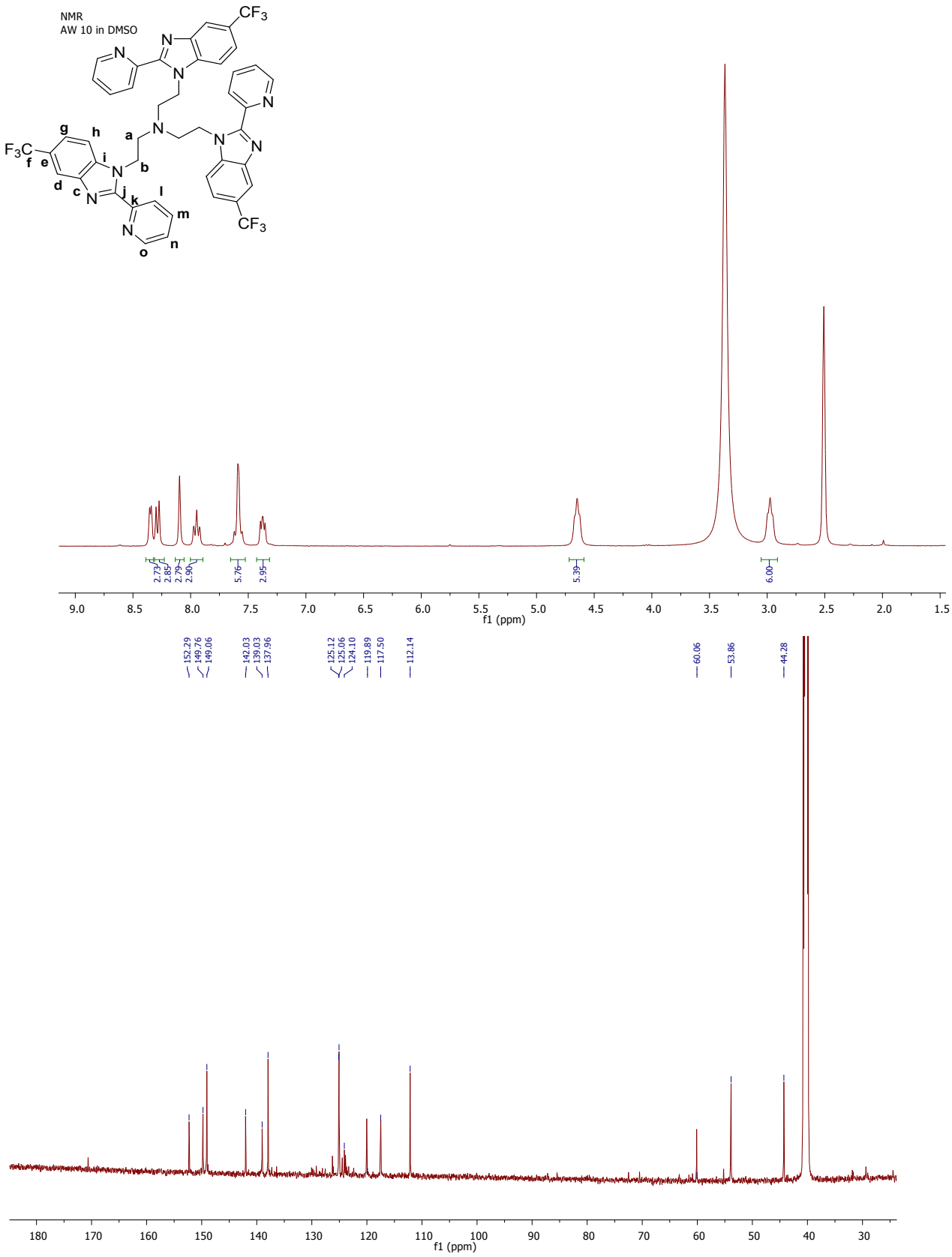

Figure S19: NMR Spectra of **11** in DMSO(d_6).

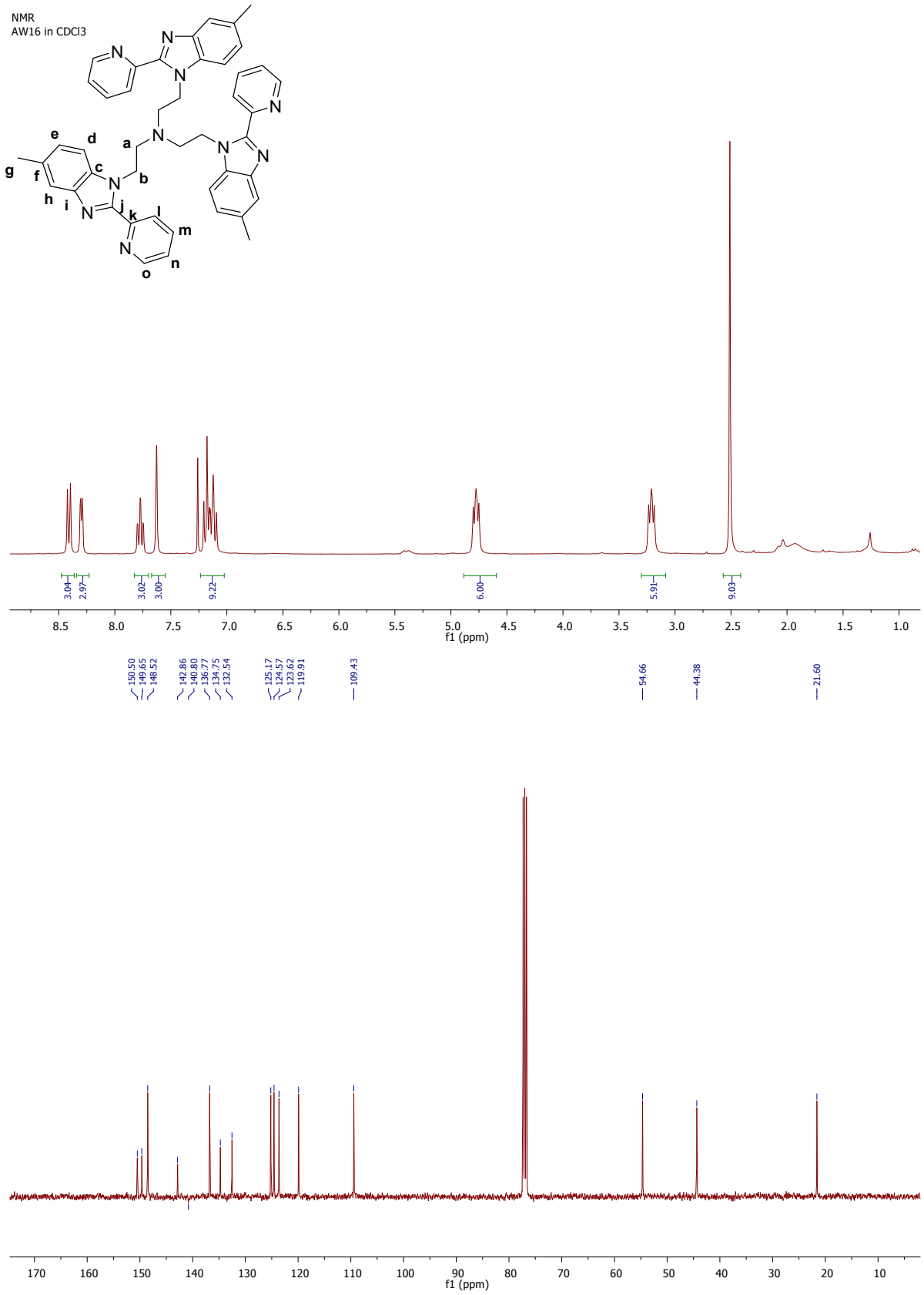

Figure S20: NMR Spectra of **12** in $CDCl_3$.

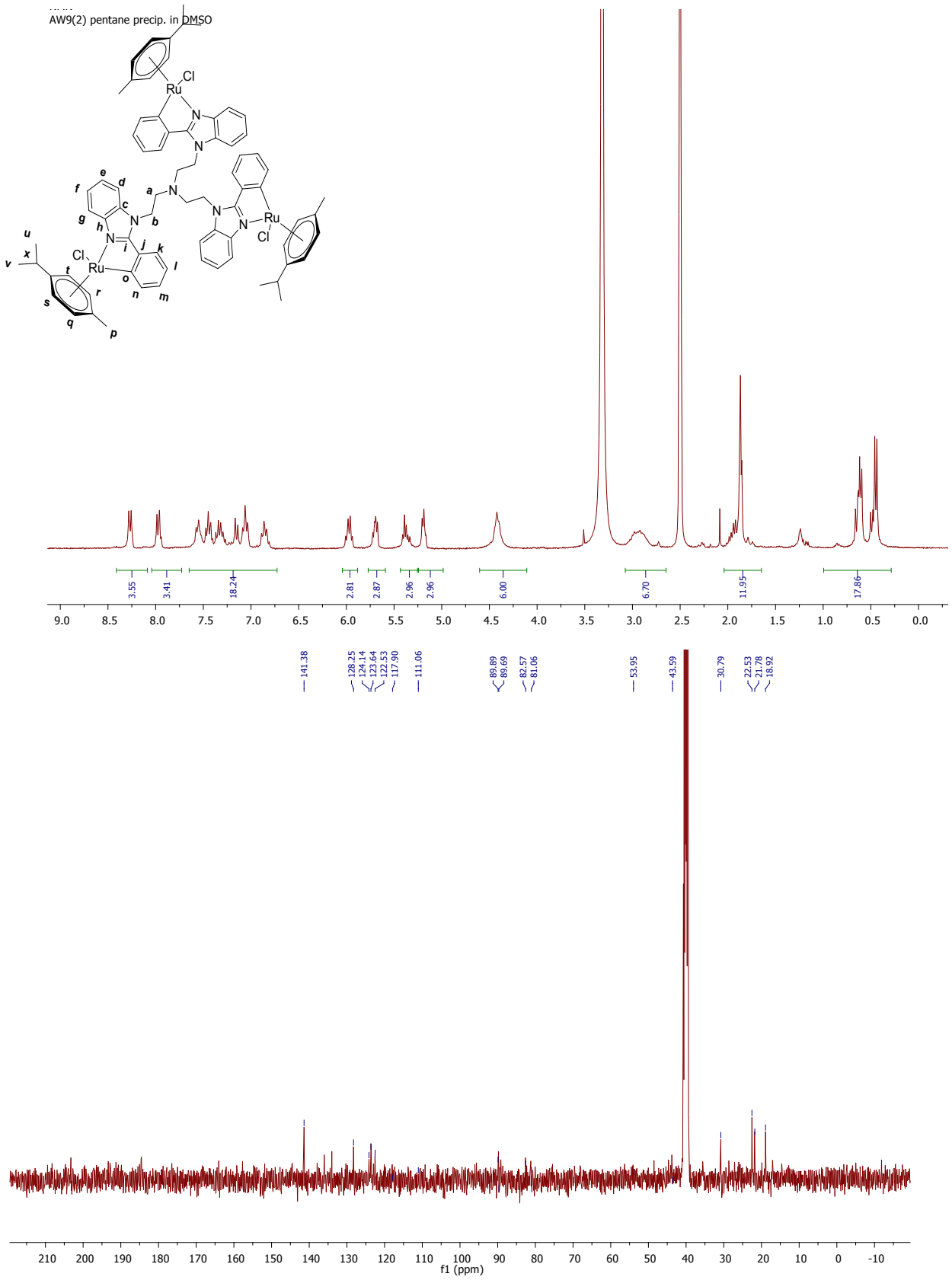

Figure S21: NMR Spectra of **13** in DMSO(d_6).

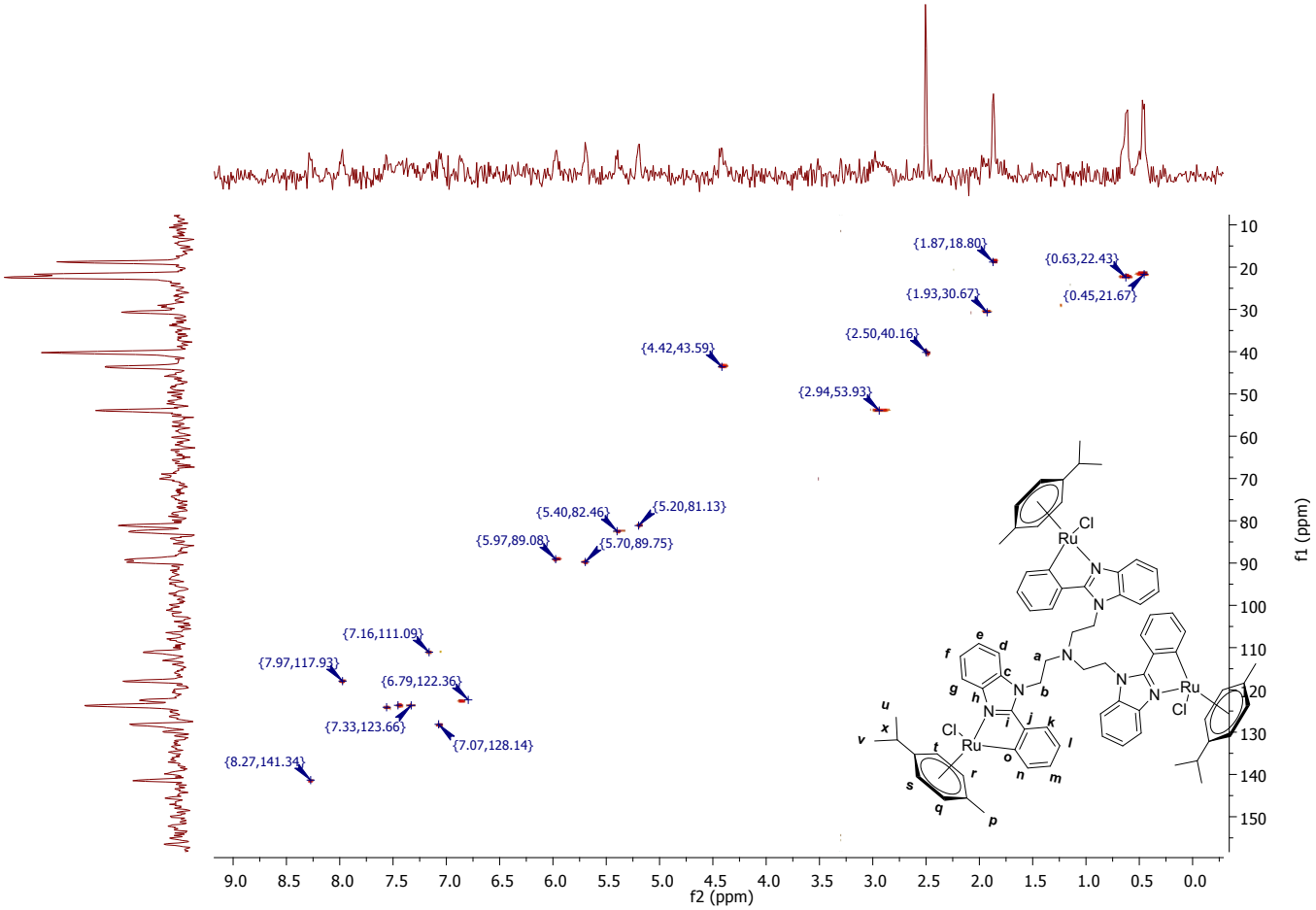

Figure S22: HSQC of **13** in DMSO(d_6).

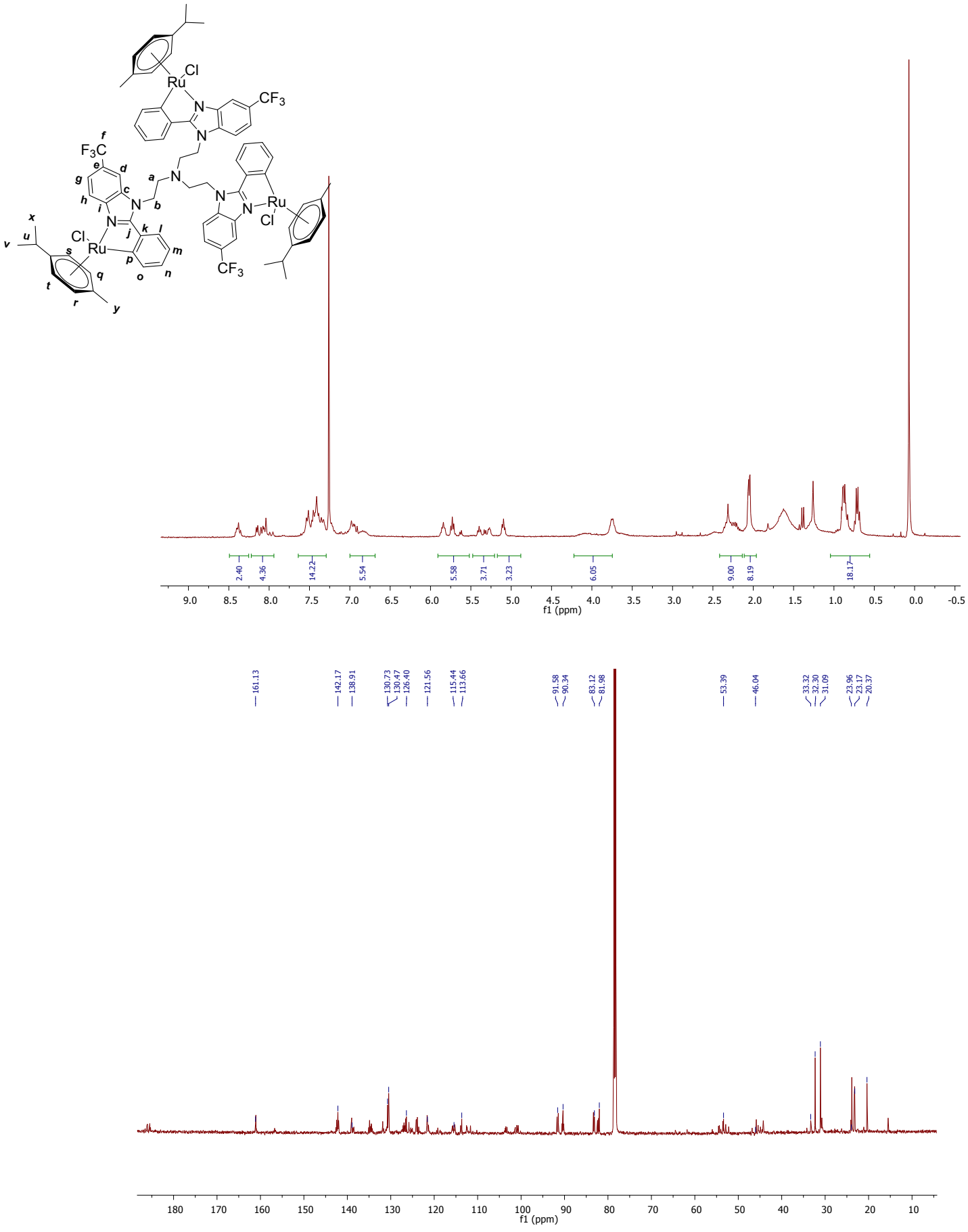

Figure S23: NMR Spectra of **14** in DMSO(d_6).

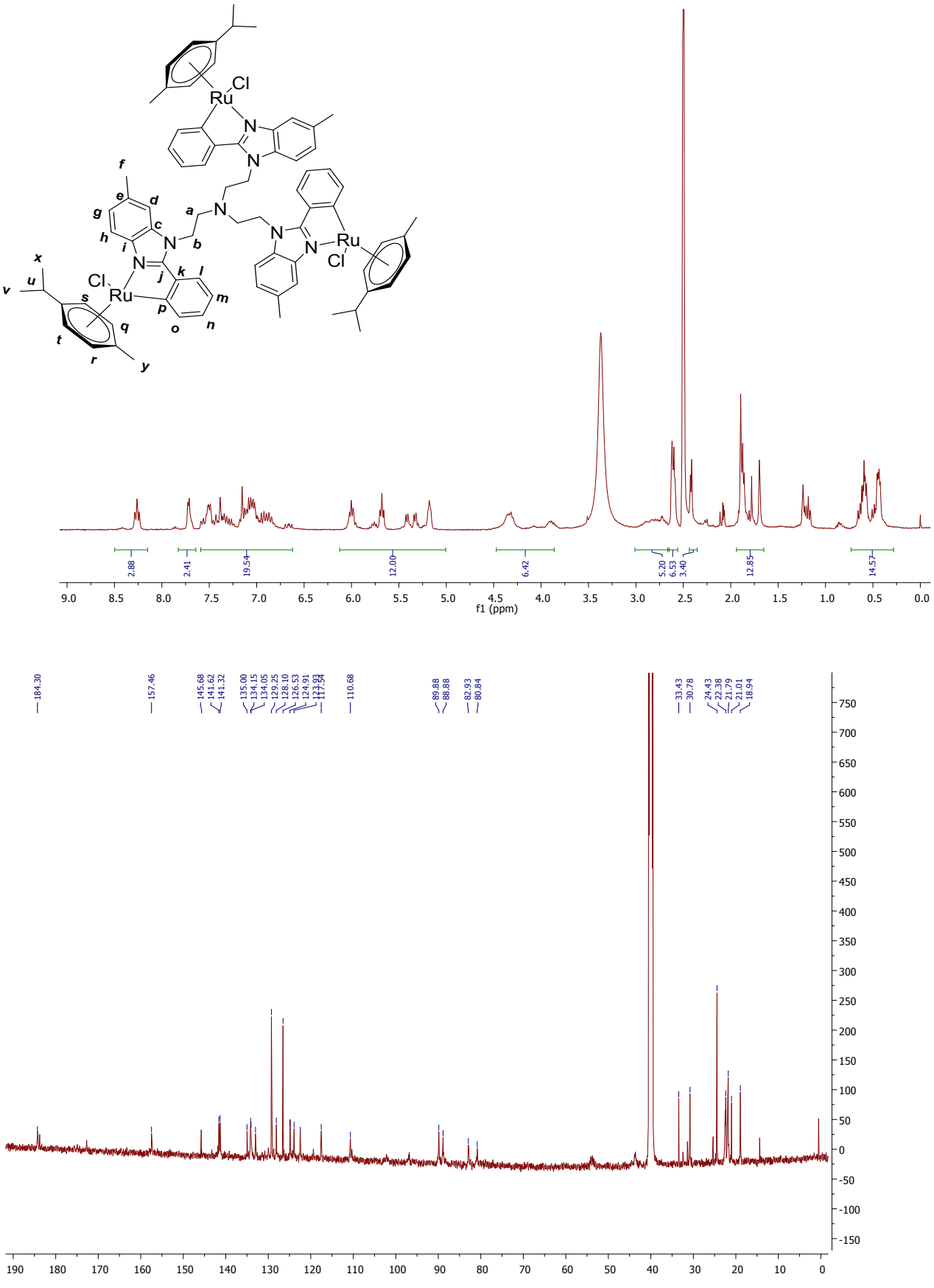

Figure S24: NMR Spectra of **15** in DMSO(d_6).

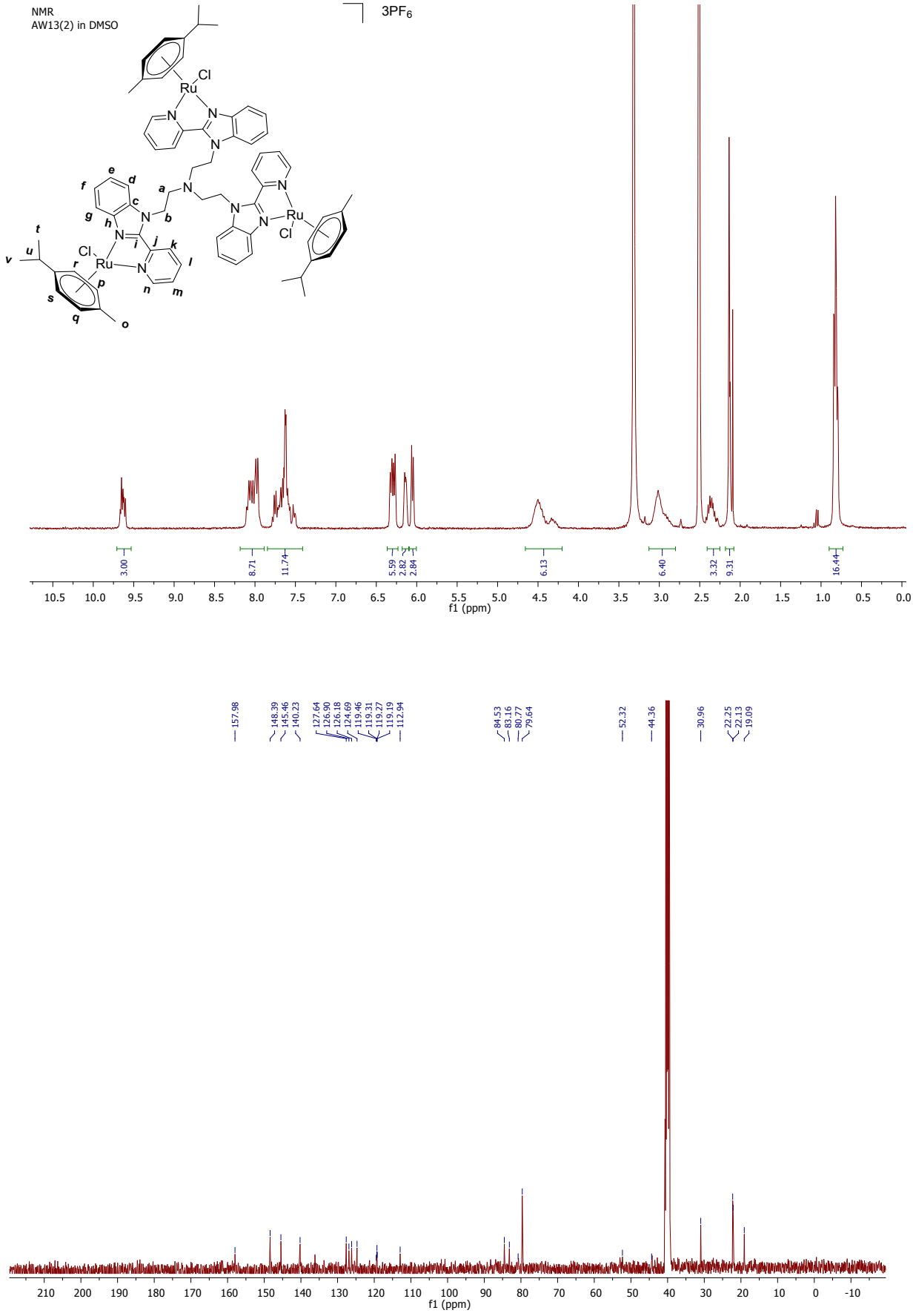

Figure S25: NMR Spectra of **[16][PF$_6$]$_3$** in DMSO(d$_6$).

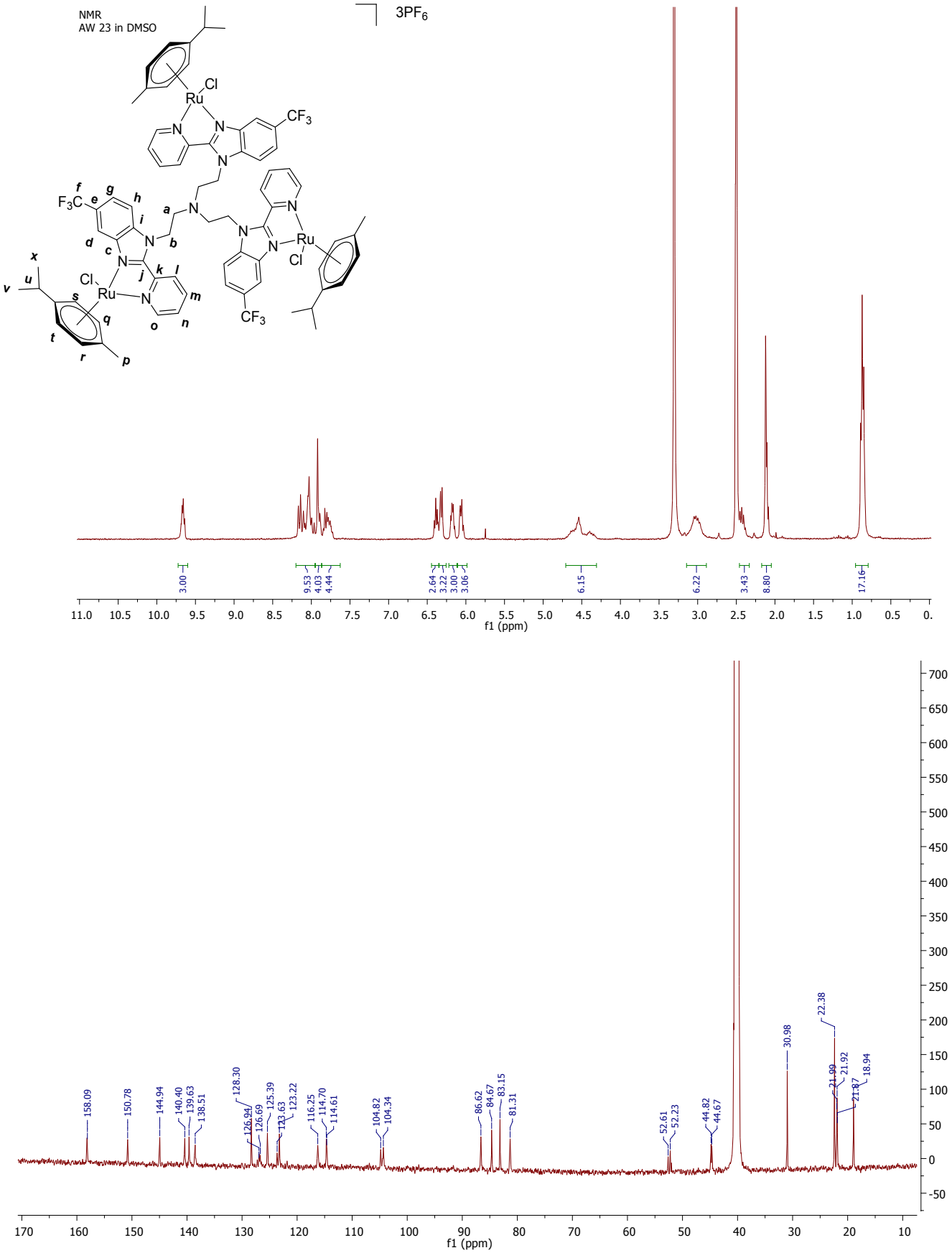

Figure S26: NMR Spectra of **[17][PF$_6$]$_3$** in DMSO(d$_6$).

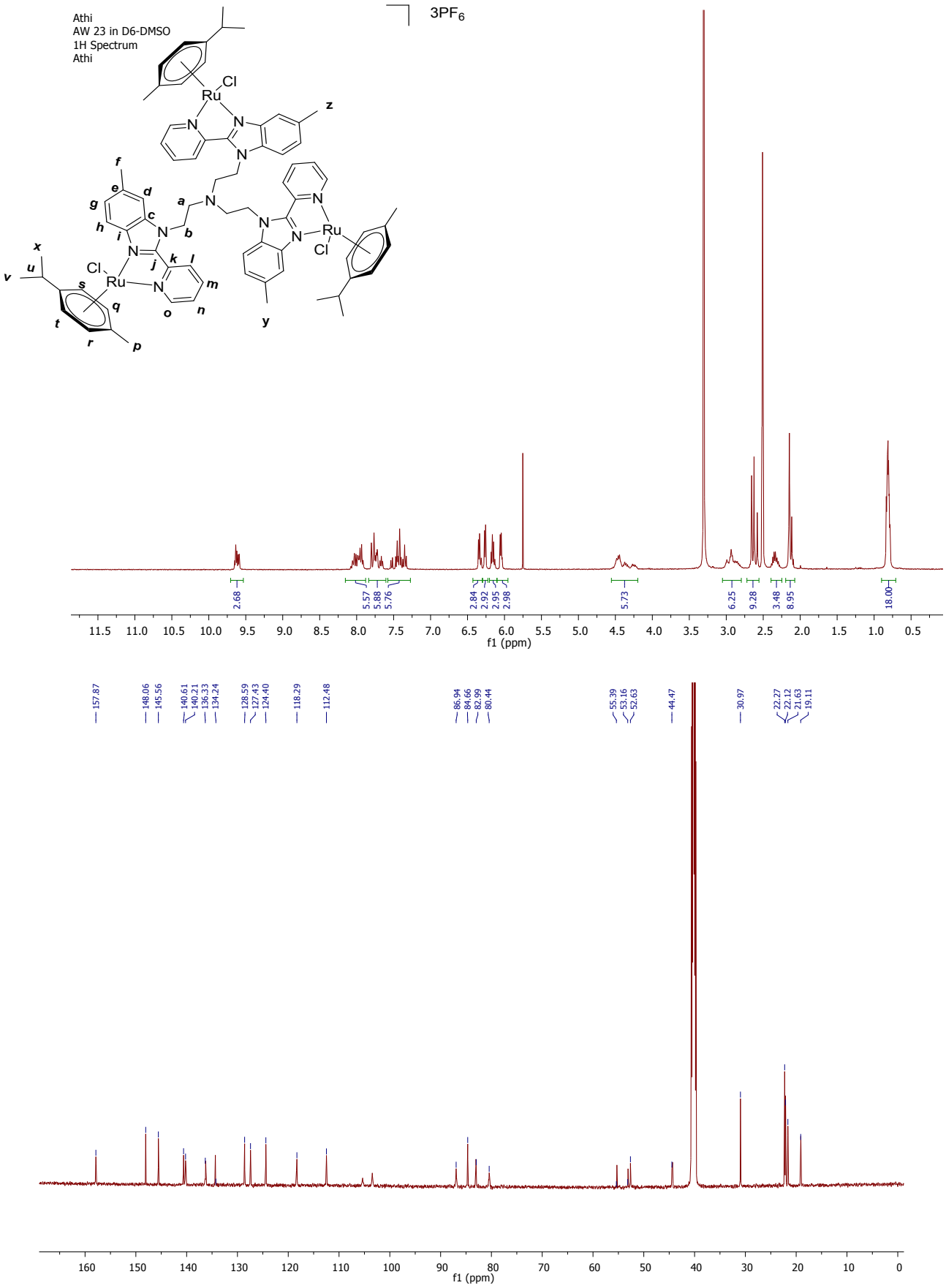

Figure S27: NMR Spectra of **[18][PF$_6$]$_3$** in DMSO(d$_6$).

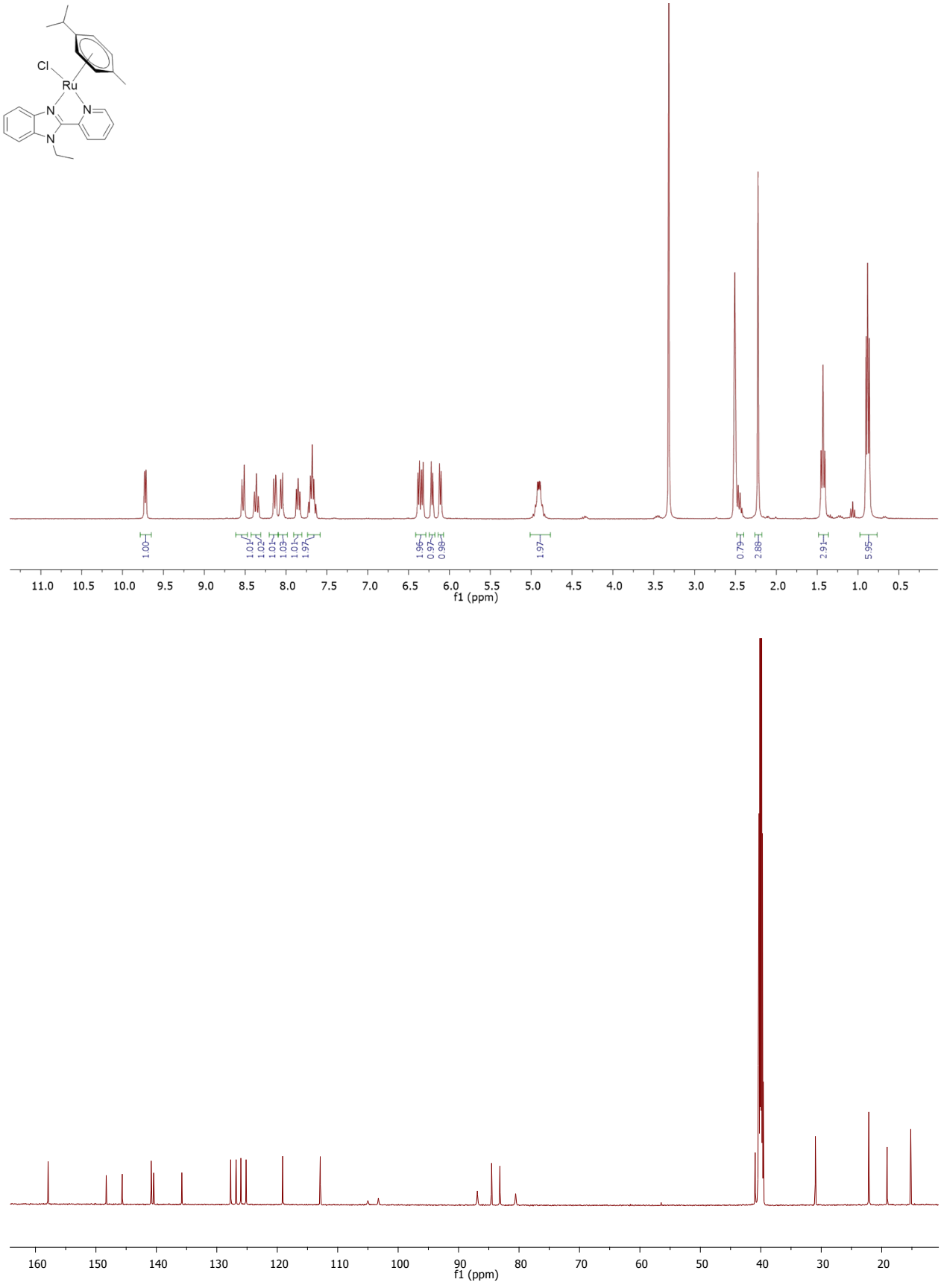

Figure S28: NMR spectra of **[20][PF_6]** in DMSO(d_6).

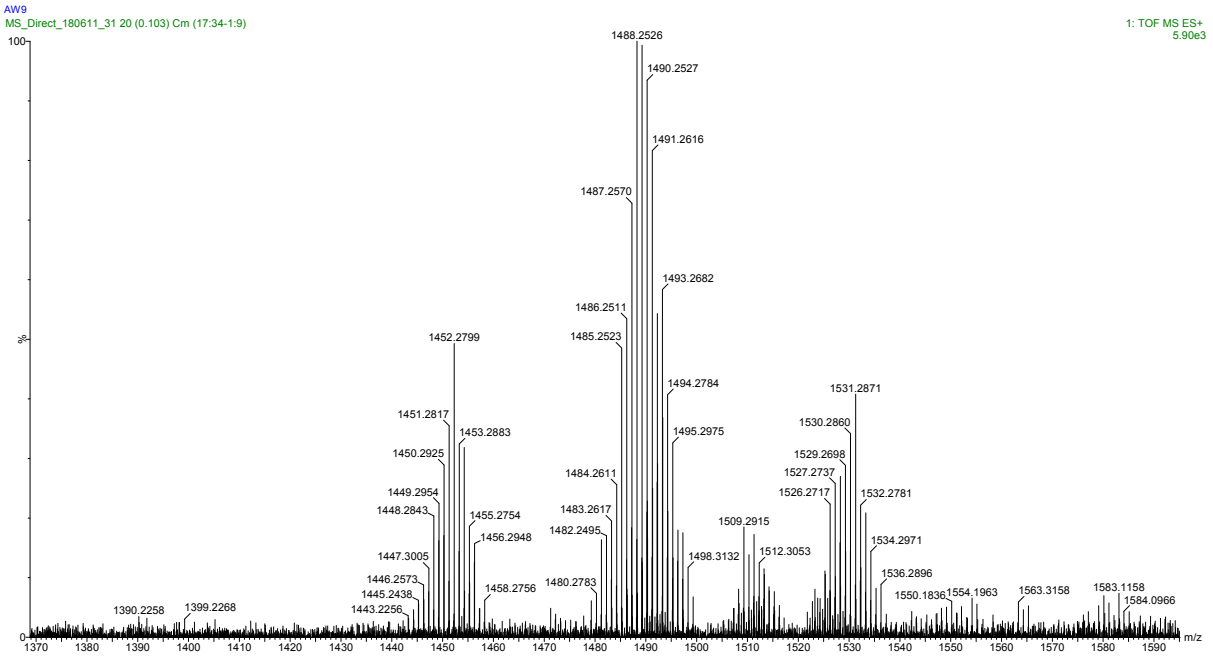

Figure S29: The HR-ESI Mass Spectrum of **13** recorded in the positive-ion mode (+ve).

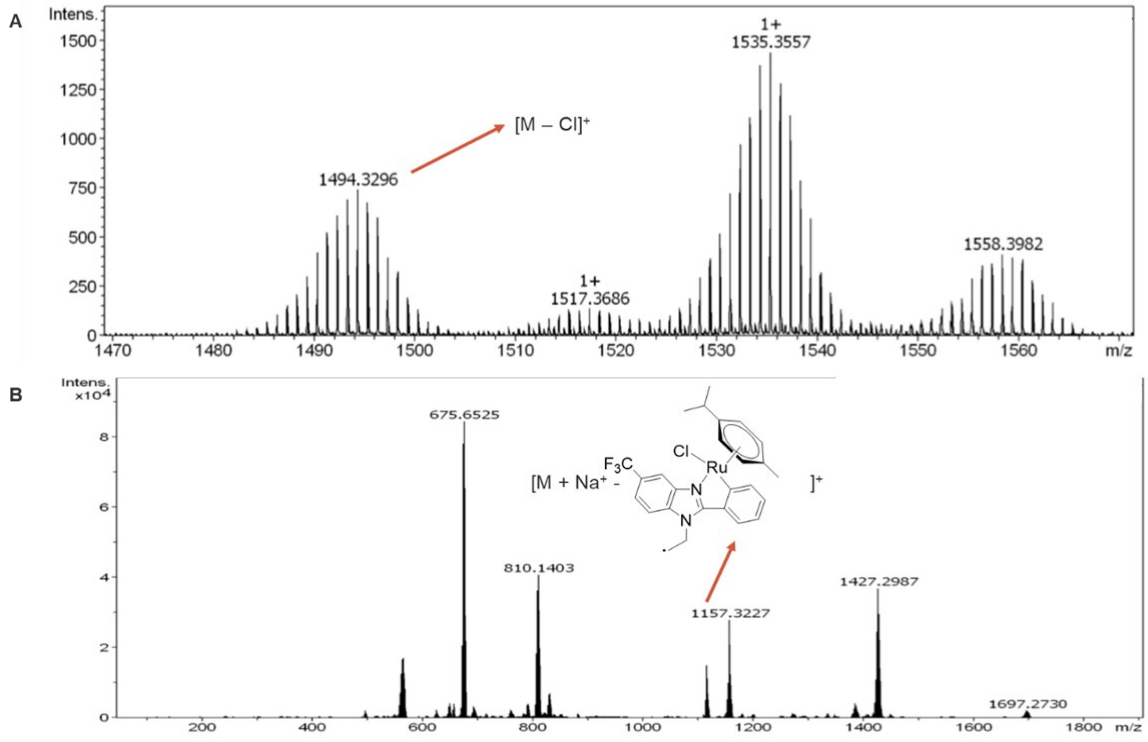

Figure S30: The HR-ESI Mass Spectra of **15** (A) and **14** (B) recorded in the positive-ion mode (+ve), with assigned characteristic fragments observed

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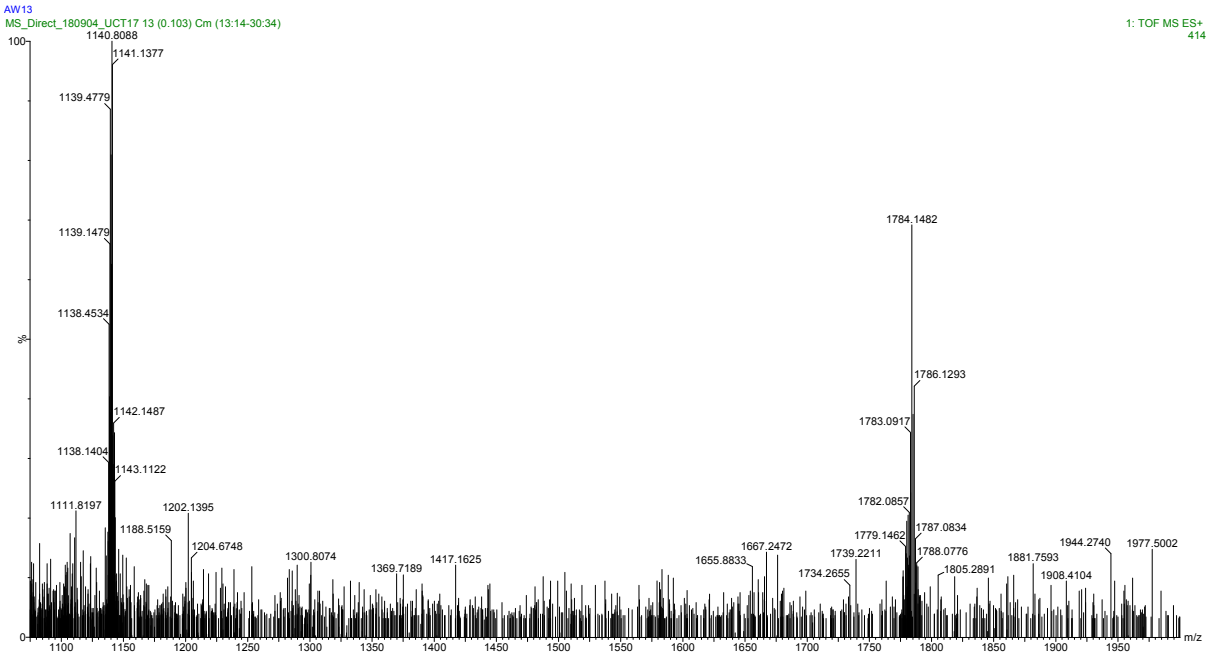

Figure S31: The HR-ESI Mass Spectrum of **[16][PF_6]$_3$** recorded in the positive-ion mode (+ve).

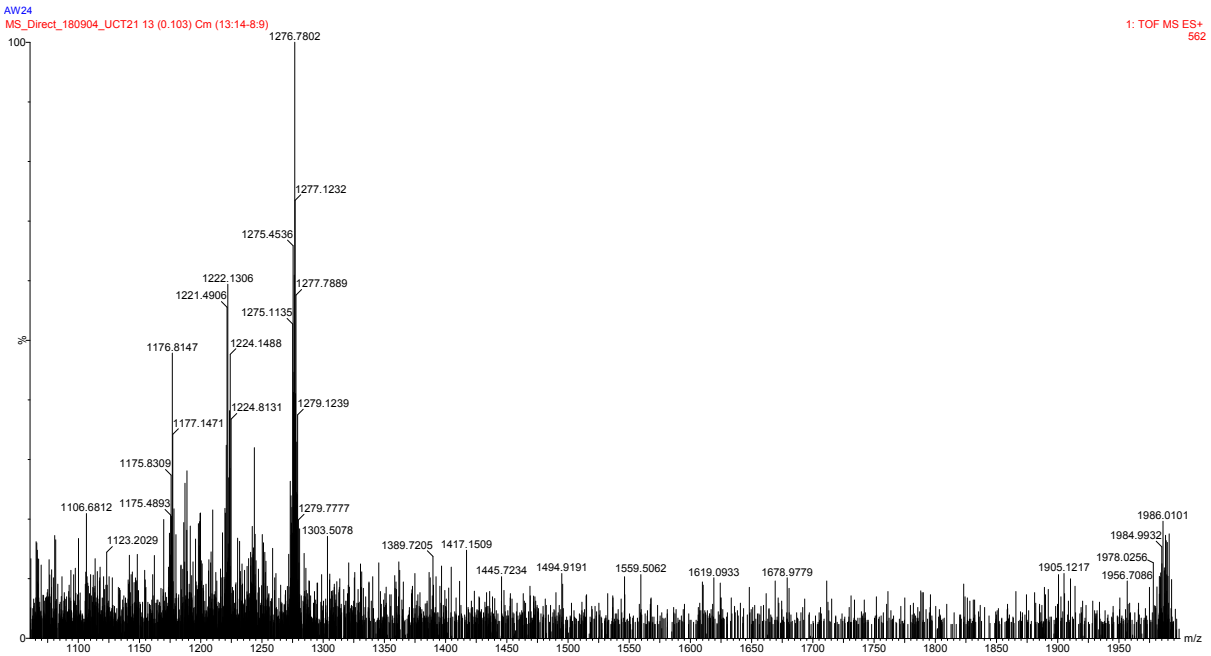

Figure S32: The HR-ESI Mass Spectrum of **[17][PF_6]$_3$** recorded in the positive-ion mode (+ve).

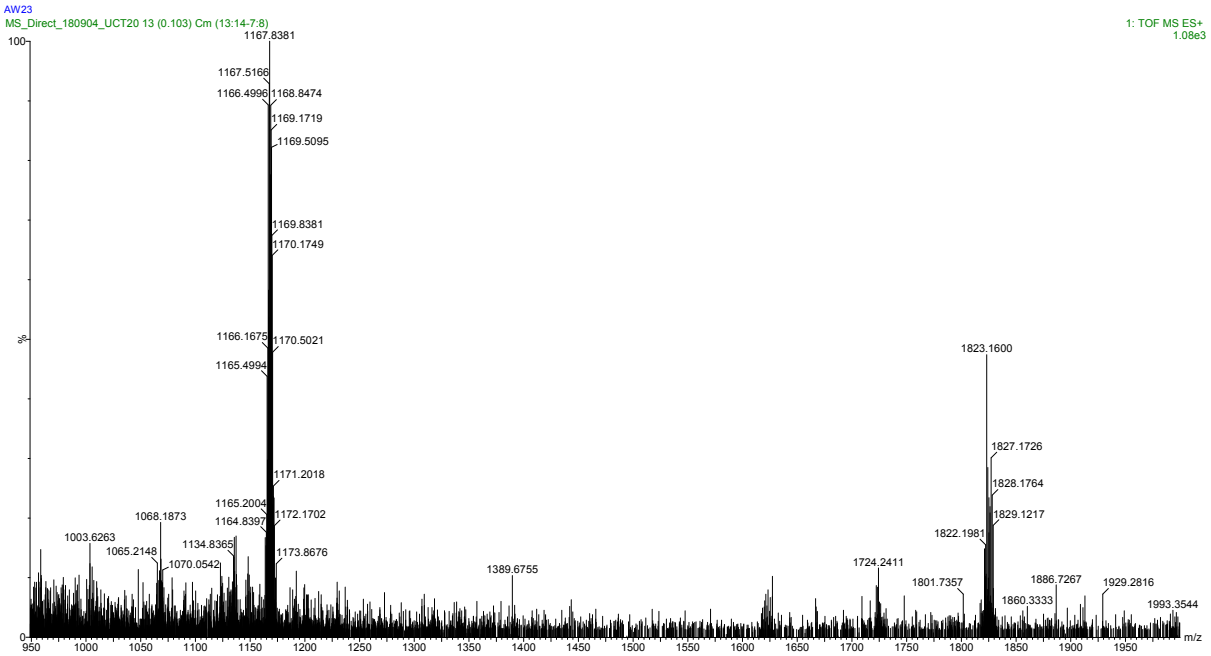

Figure S33: The HR-ESI Mass Spectrum of **[18][PF_6]$_3$** recorded in the positive-ion mode (+ve).

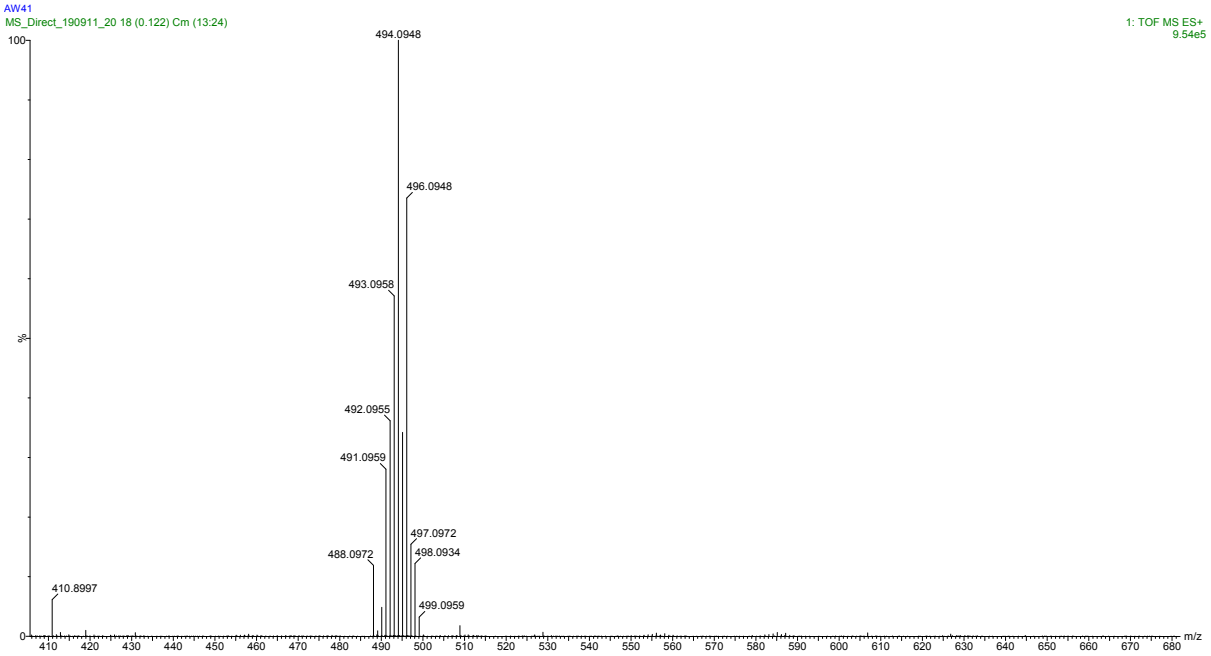

Figure S34: The HR-ESI Mass Spectrum of **[20][PF_6]** recorded in the positive-ion mode (+ve).

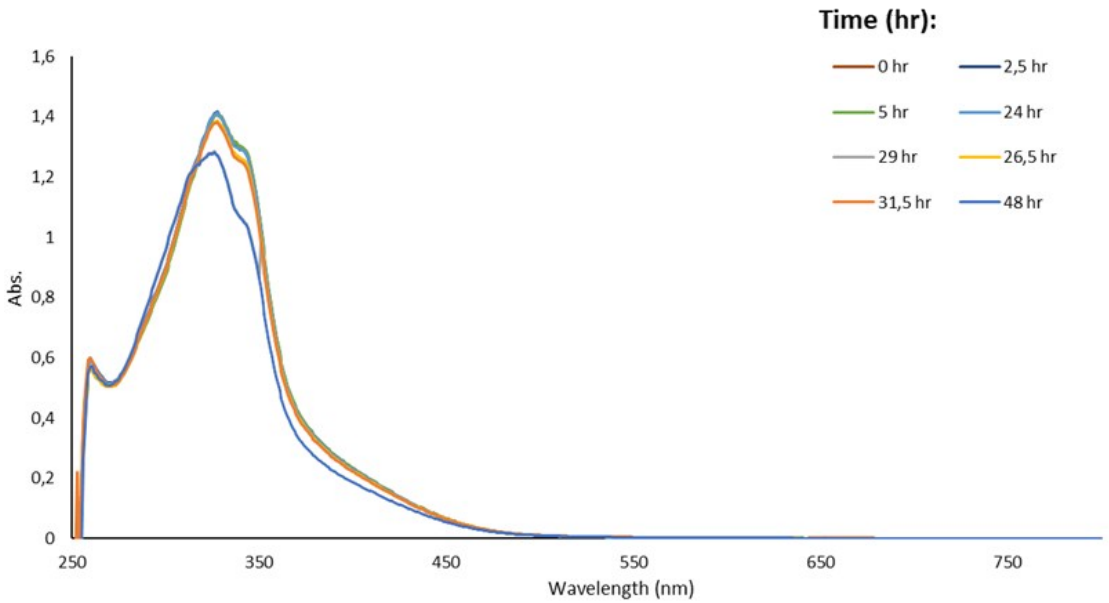

Figure S35: The UV/ Vis spectra of **[16][PF_6]$_3$** in DMSO recorded over 48 hours at 37 °C.

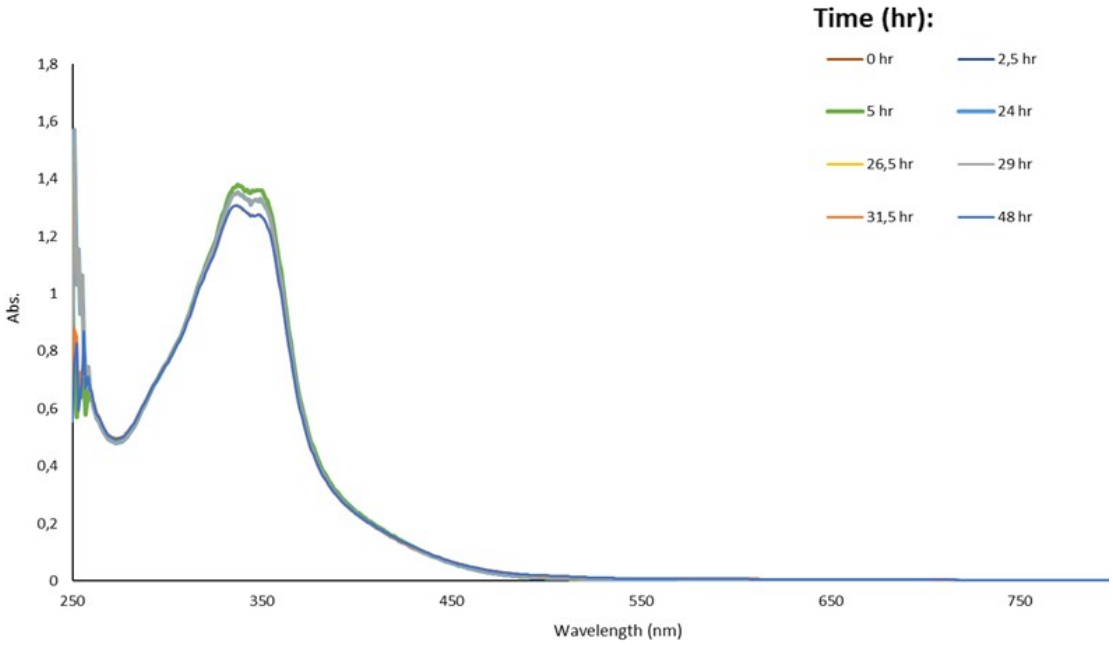

Figure S36: The UV/ Vis spectra of **[17][PF_6]$_3$** in DMSO recorded over 48 hours at 37 °C

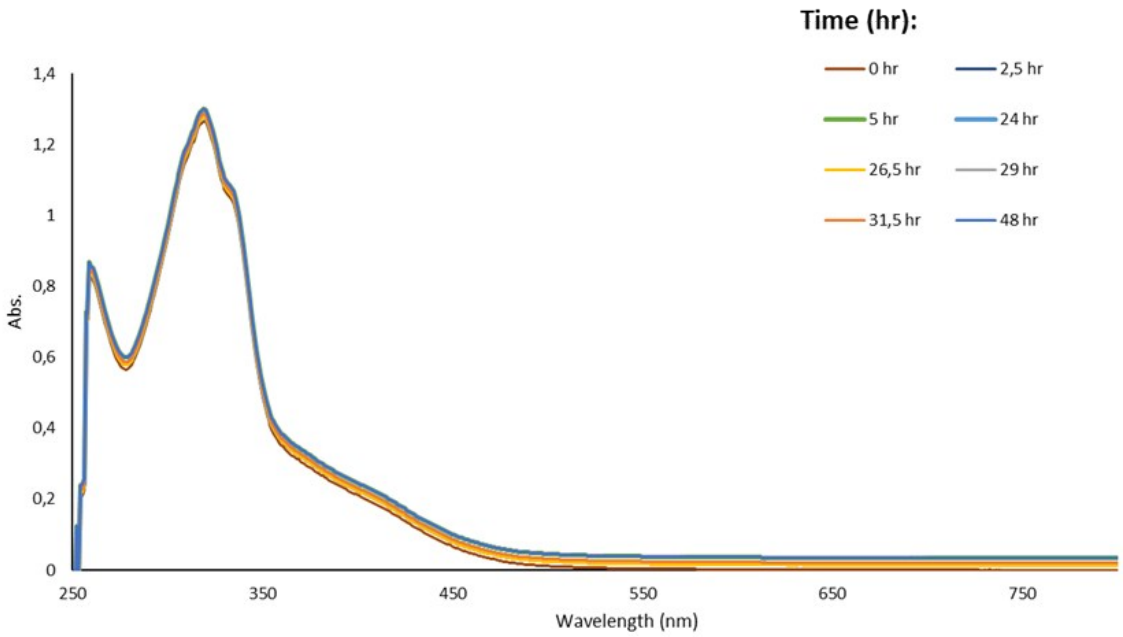

Figure S37: The UV/ Vis spectra of **[18][PF_6]$_3$** in DMSO recorded over 48 hours at 37 °C

Synthesis of the tris-benzimidazole ligands and their precursors

The first step towards the synthesis of the tris-benzimidazole ligands is a nucleophilic aromatic substitution (S_NAr) reaction, in which an excess of a commercially available substituted *ortho*-halogenated nitrobenzene is reacted with tris(2-aminoethyl)amine. The core prerequisite of a S_NAr reaction is that a strong electron-withdrawing group is present in either the *ortho*- or *para*- positions, relative to the potential leaving group, which serves to stabilize the Meisenheimer complex formed as the reaction proceeds. The desired compounds were isolated and purified *via* column chromatography to yield either bright yellow solids (**1** and **2**) or a dark orange solid (**3**) in moderate yields (35 – 48%) and are soluble in several polar organic solvents including ethanol, methanol, acetone and dimethylsulfoxide. The ^{1}H NMR spectra of the tris-nitrobenzene compounds **1** – **3** reveals the presence of a triplet at 8.1 – 8.5 ppm corresponding to the secondary amine protons which supports complete substitution of all three arms of the tris-core. Furthermore, the splitting pattern observed in the aromatic regions of the spectra (6.5 – 8.0 ppm) is consistent with a splitting pattern associated with a 1,2,4-trisubstituted aromatic ring with three electronically discrete functional groups. The only difference is compound **1,** as **1** consists of a 1,2-disubstituted aromatic ring.

The synthesis of the tris-1,2-benzenediamines from the tris-nitrobenzenes (**1** – **3**) involves the reduction of the nitro- functionalities to primary amine functionalities. This was achieved by using zinc and ammonium chloride as reducing agents under mild conditions. This reduction proved to be highly efficient affording the tris-1,2-diamines in excellent yields (75 – 97%).

The comparison of each ^{1}H NMR spectrum of the tris-1,2-diamines (**4** – **6**) with the corresponding tris-nitrobenzene (**1** – **3**) results in two main observations. Firstly, the signal between 4.3 – 3.8 ppm in each spectrum which corresponds to the protons of the primary amine functionalities is the first indicator of successful reduction of the nitro- functionalities to primary amine groups. Secondly, the aromatic signals of **4 - 6** converge and some coalesce relative to the aromatic signals of the corresponding nitro-benzenes (**1** – **3**), attesting to the similar electronic effects of the primary and secondary amine groups on the aromatic ring which corroborates successful reduction. Further analysis of the tris-1,2-diamines (**4** – **6**) using infrared (IR) spectrometry revealed two absorption bands between 3299 and 3376 cm^{-1}. These correspond to the symmetrical and asymmetrical stretches of the N-H bonds of the primary amine moieties and are thus indicative of the successful reduction of the nitro- groups to primary aryl amine groups.

Finally, the synthesis of the tris-benzimidazole ligands, from the tris-1,2-benzenediamines (**4** – **6**) entailed a cyclisation-condensation reaction with either benzaldehyde or 2-pyridinecarboxaldehyde in the presence of a catalytic amount of trifluoroacetic acid and magnesium sulfate.