Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Nitroxide-fluorophore double probes: a potential tool for studying membrane heterogeneity by ESR and fluorescence

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1. Absorption and emission spectra

1.1 Procedures

All absorption spectra were recorded with $5 \cdot 10^{-5}$ M solutions of probe in methanol. Fluorescent spectra were recorded with 10^{-7} M solutions of probe in methanol. Reduction of nitroxide of double probe: to the 10^{-4} M solution of the probe was added solution of ascorbic acid (20 equiv) in methanol, the mixture was allowed to stand for 20 min and than diluted to the final concentration with methanol.

Labelling of liposomes with probes: solvent was removed under reduced pressure from 100 µL of 10^{-4} M stock solution of the probe in methanol. Alternatively the nitroxide was first reduced as described above with solution of ascorbic acid in methanol and than the solvent was removed under reduced pressure. To the resuting film of probe 10 µL of 10^{-3} M solution of TRITON X-100 in PBS buffer (pH = 7.4) and 990 µL of PBS buffer were added. To the 40 µL of the obtained solution 13,3 µL of liposomes (lipid concentration was $3 \cdot 10^{-3}$ M, preparation is described below) were added and the mixture was diluted to the final volume of 4 mL with PBS buffer. Concentrations of components in the final mixture were 10^{-7} M for tested probe, 10^{-7} M for TRITON X-100 and $4 \cdot 10^{-5}$ M for lipids.

The liposomes were prepared by a reverse phase evaporation method originally described by Moscho et al. (Moscho, Orwar, Chiu, Modi, & Zare, 1996) and later modified by Kulin et al. (Kulin, Kishore, Helmerson, & Locascio, 2003). The lipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was dissolved in chloroform at a concentration of 30 mM. From this solution, 50 µL was added to a 50 mL round-bottom flask. Then, 3 mL of aqueous phase (10 mM Hepes buffer with 11 mM $MgCl_2$, pH = 7.4) was carefully added along the side of the flask wall using a pipet. The organic solvent, chloroform, was then removed in a rotary evaporator under reduced pressure. The final pressure was 40 mm of Hg at 40 °C while rotating at 40 rpm.

1.2 Spectra

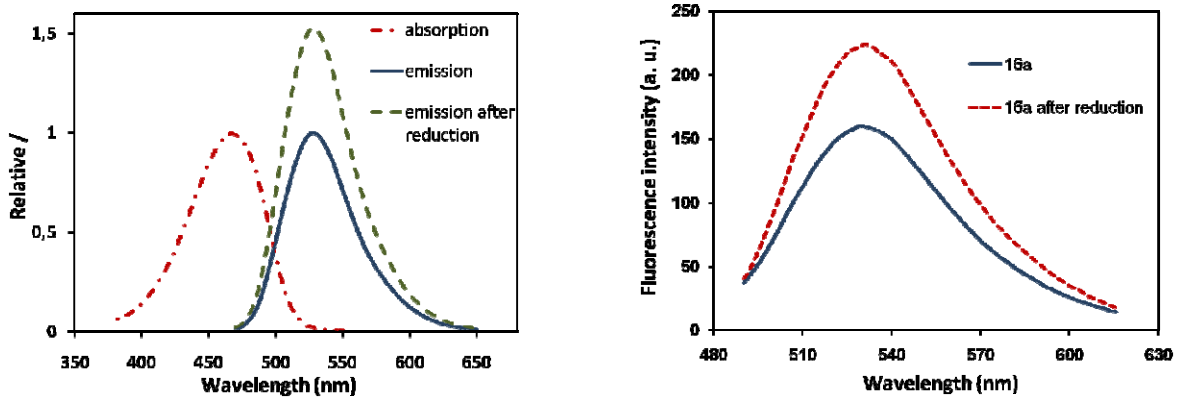

Figure S1. Left: Normalised absorption and fluorescence emission spectra shown together with fluorescence emission spectrum after reduction of the nitroxide of double probe **16a** in methanol (λ_{ex} = 450 nm). Right: Fluorescence emission spectra of double probe **16a** incorporated into the liposomes before and after reduction of the nitroxide (λ_{ex} = 470 nm).

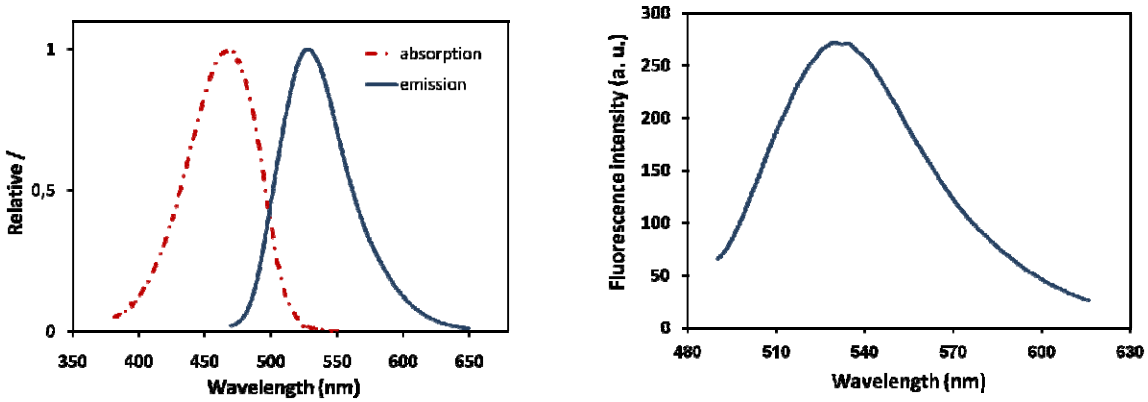

Figure S2. Left: Normalised absorption and fluorescence emission spectra of probe **16b** in methanol (λ_{ex} = 450 nm). Right: Fluorescence emission spectra of probe **16b** incorporated into the liposomes (λ_{ex} = 470 nm).

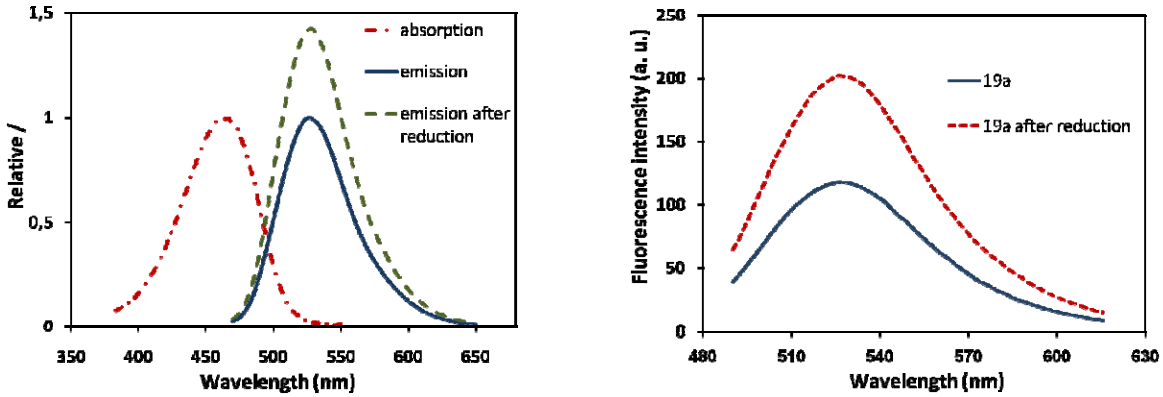

Figure S3. Left: Normalised absorption and fluorescence emission spectra shown together with fluorescence emission spectrum after reduction of the nitroxide of double probe **19a** in methanol (λ_{ex} = 450 nm). Right: Fluorescence emission spectra of double probe **19a** incorporated into the liposomes before and after reduction of the nitroxide (λ_{ex} = 470 nm).

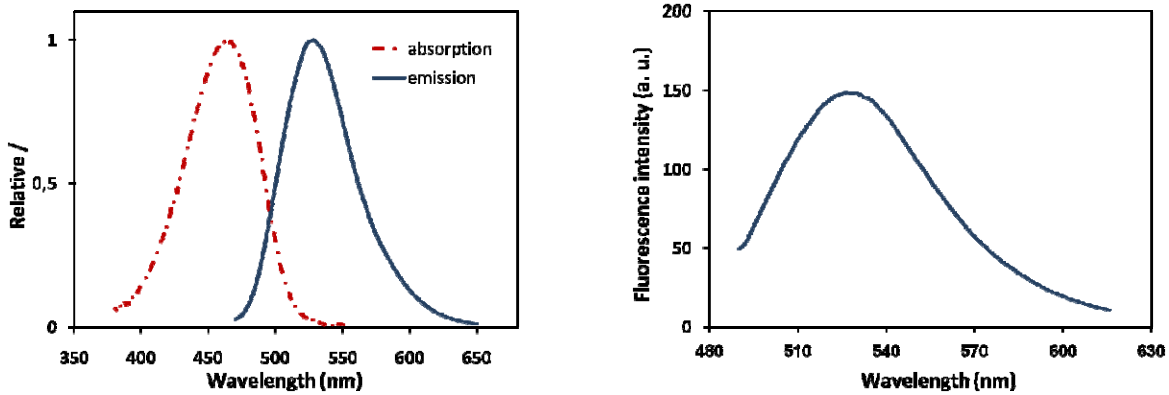

Figure S4. Left: Normalised absorption and fluorescence emission spectra of probe **19b** in methanol (λ_{ex} = 450 nm). Right: Fluorescence emission spectra of probe **19b** incorporated into the liposomes (λ_{ex} = 470 nm).

2. ESR spectra

2.1 Procedures

Liposomes were prepared from 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) (60%), cholesterol (40%) and PBS buffer (50 mg/mL) by lipid film method.

Labelling: a stock solution of probes in methanol (10^{-3} M) was added into a test tube. Ethanol was removed by a stream of nitrogen to obtain a film of componds **1**, **16a** and **19a** on the test tube wall. Liposomes were added to the film and the test tube was shaken for 30 min.

2.2 Spectra

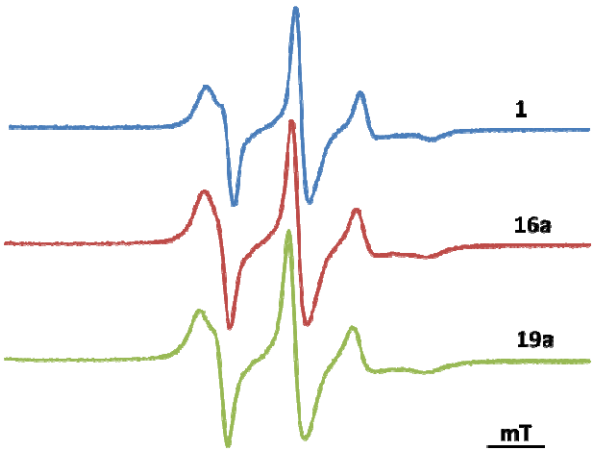

Figure S5. Normalised ESR spectra of liposomes labelled with **1**, **16a** and **19a**, spectra were recorded at 308 K.

3. ^{1}H and ^{13}C NMR spectra

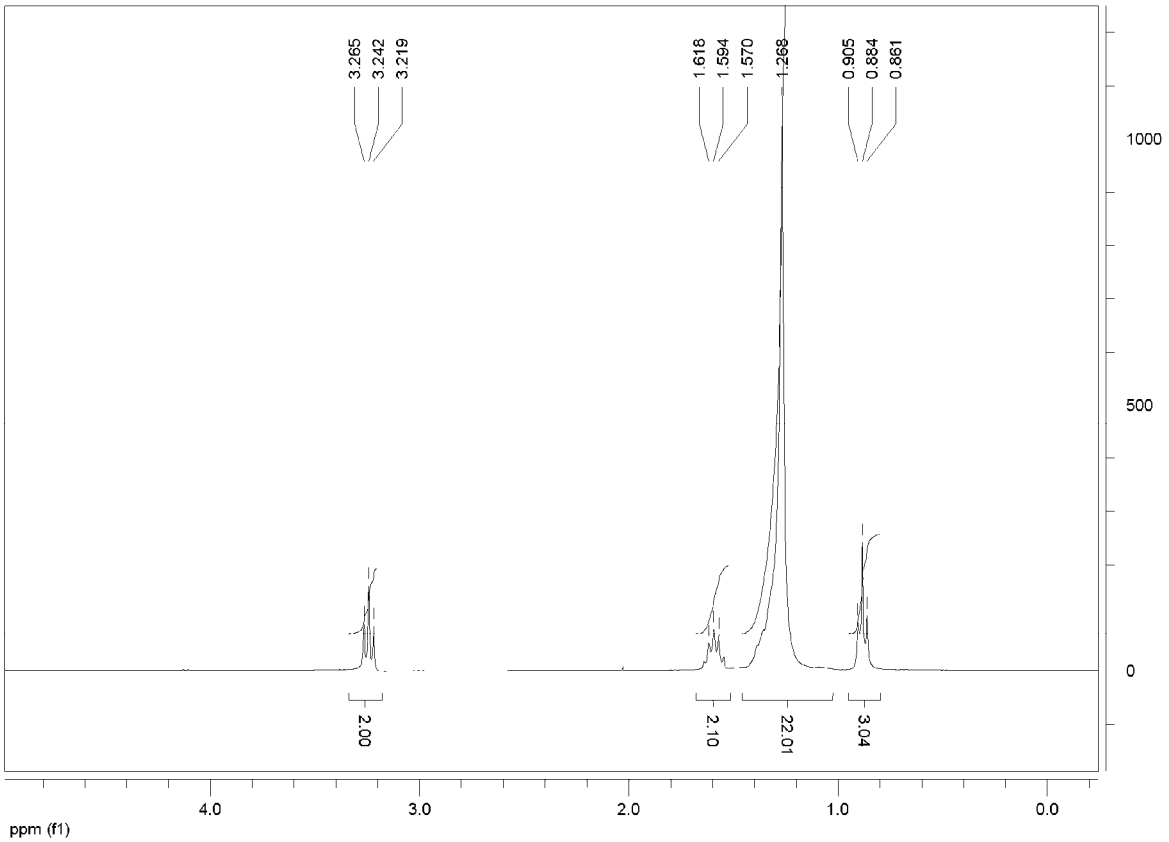

Figure S6. ^{1}H-NMR spectrum of compound **5** (300 MHz, $CDCl_3$).

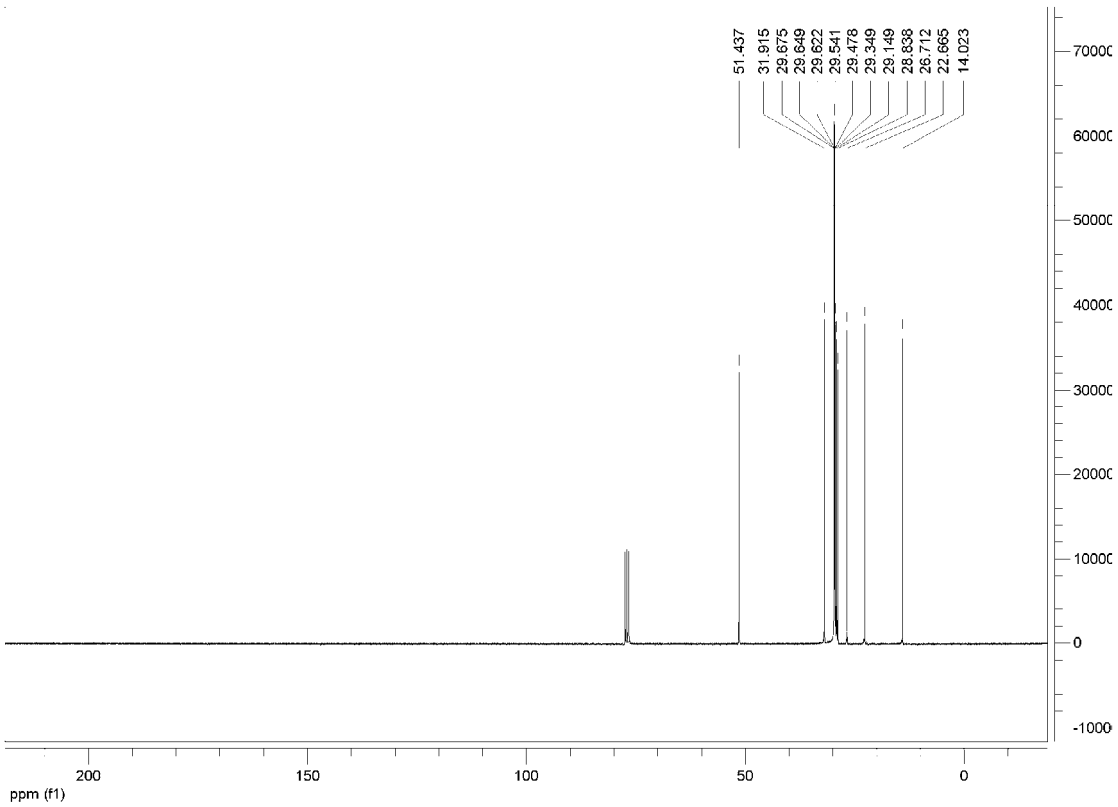

Figure S7. ^{13}C-NMR spectrum of compound **5** (75 MHz, $CDCl_3$).

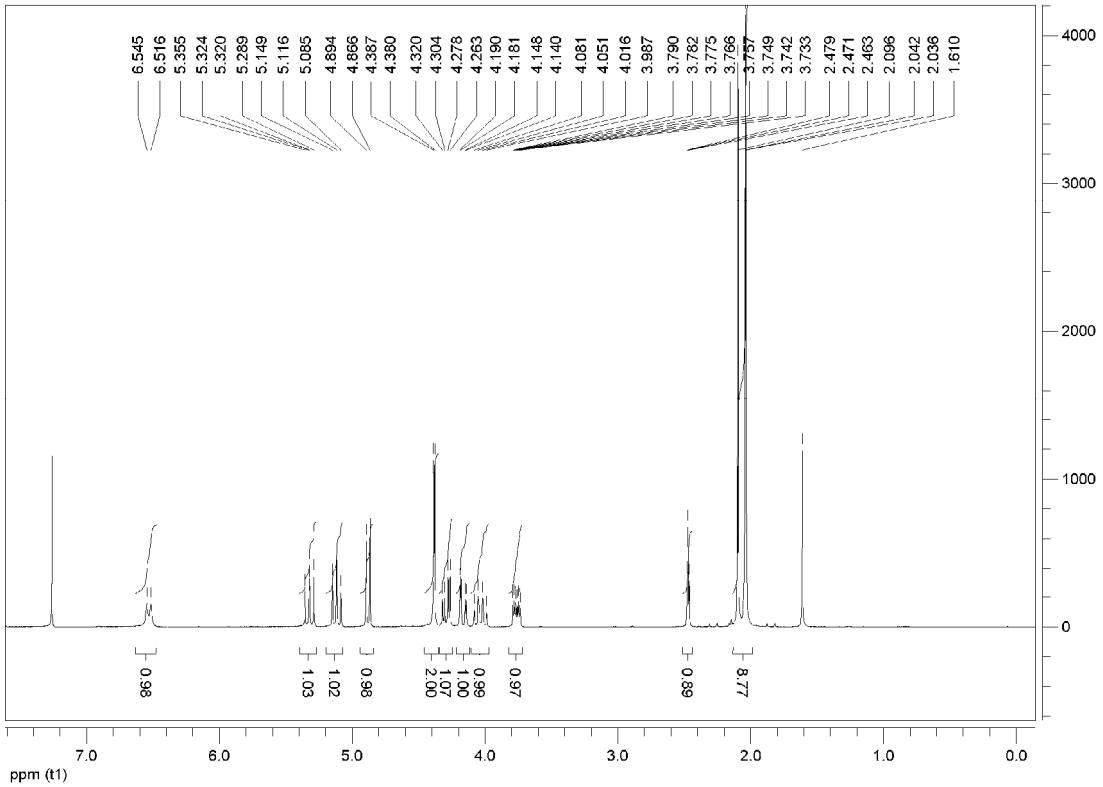

Figure S8. ^{1}H-NMR spectrum of compound **7** (300 MHz, $CDCl_3$).

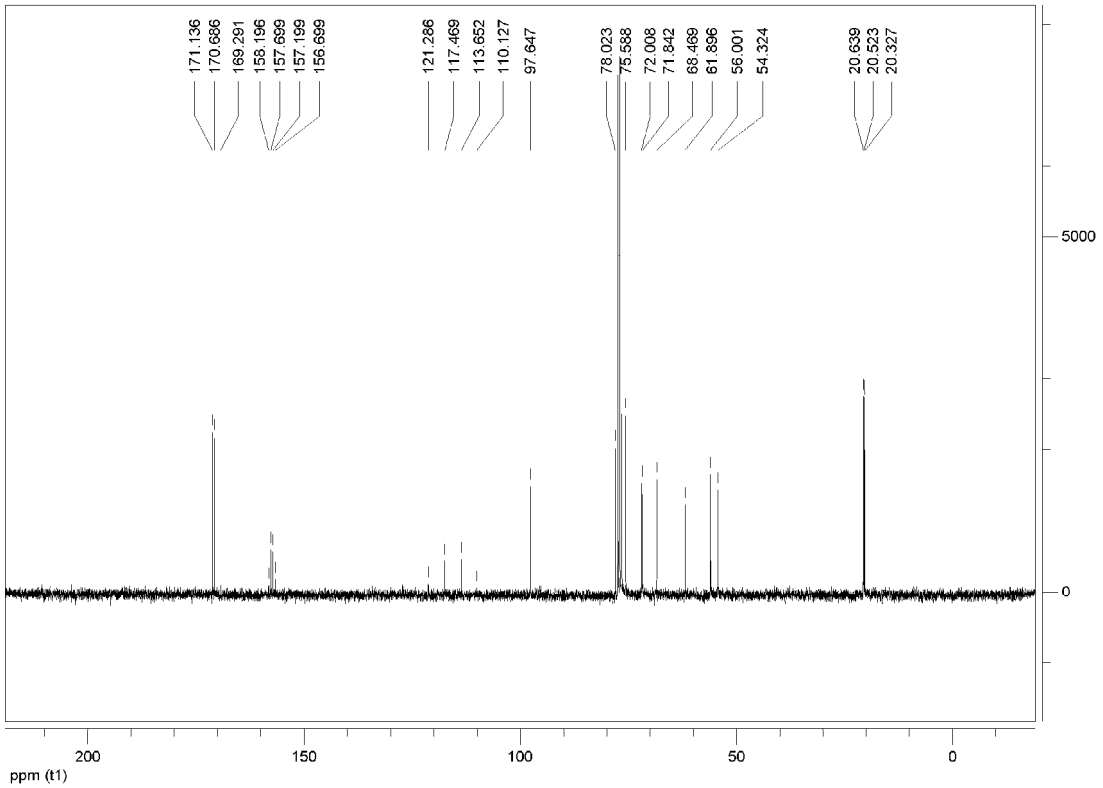

Figure S9. ^{13}C-NMR spectrum of compound **7** (75 MHz, $CDCl_3$).

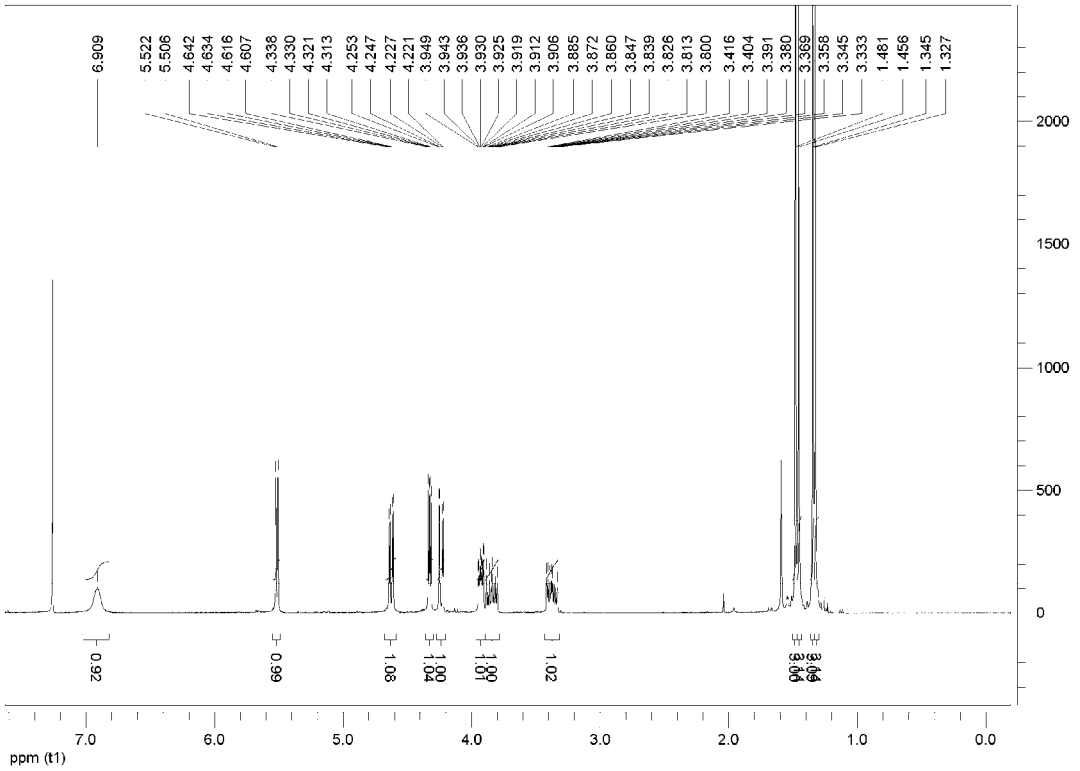

Figure S10. ^{1}H-NMR spectrum of compound **9** (300 MHz, $CDCl_3$).

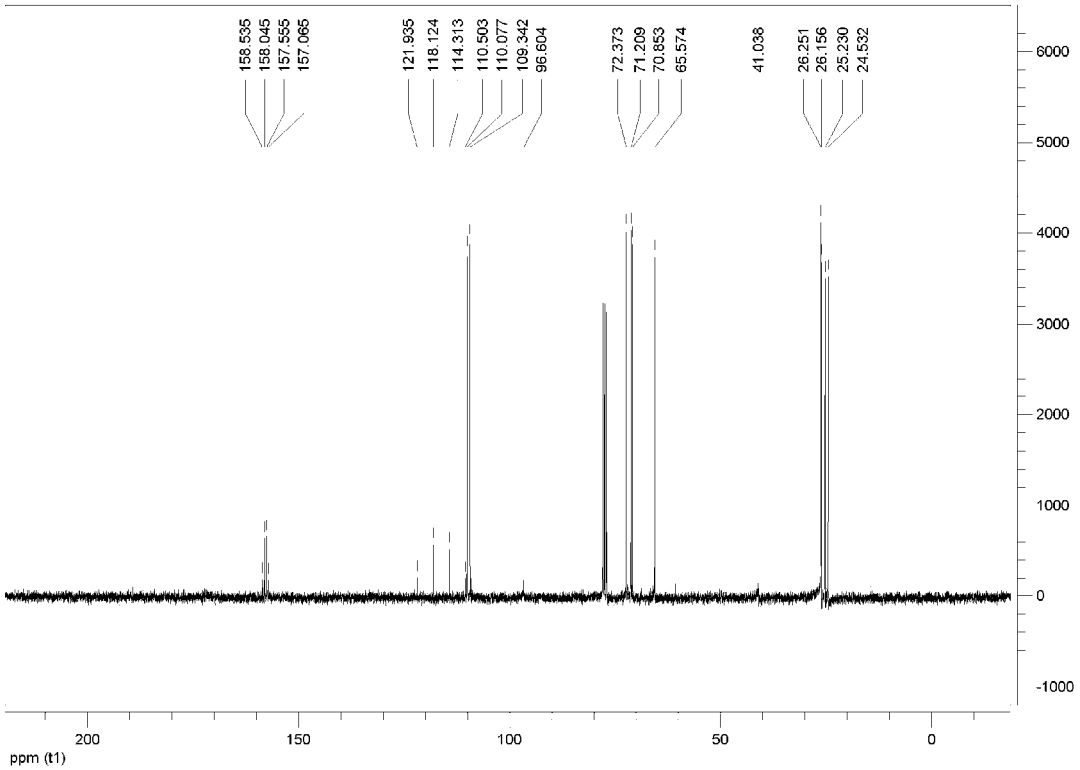

Figure S11. ^{13}C-NMR spectrum of compound **9** (75 MHz, $CDCl_3$).

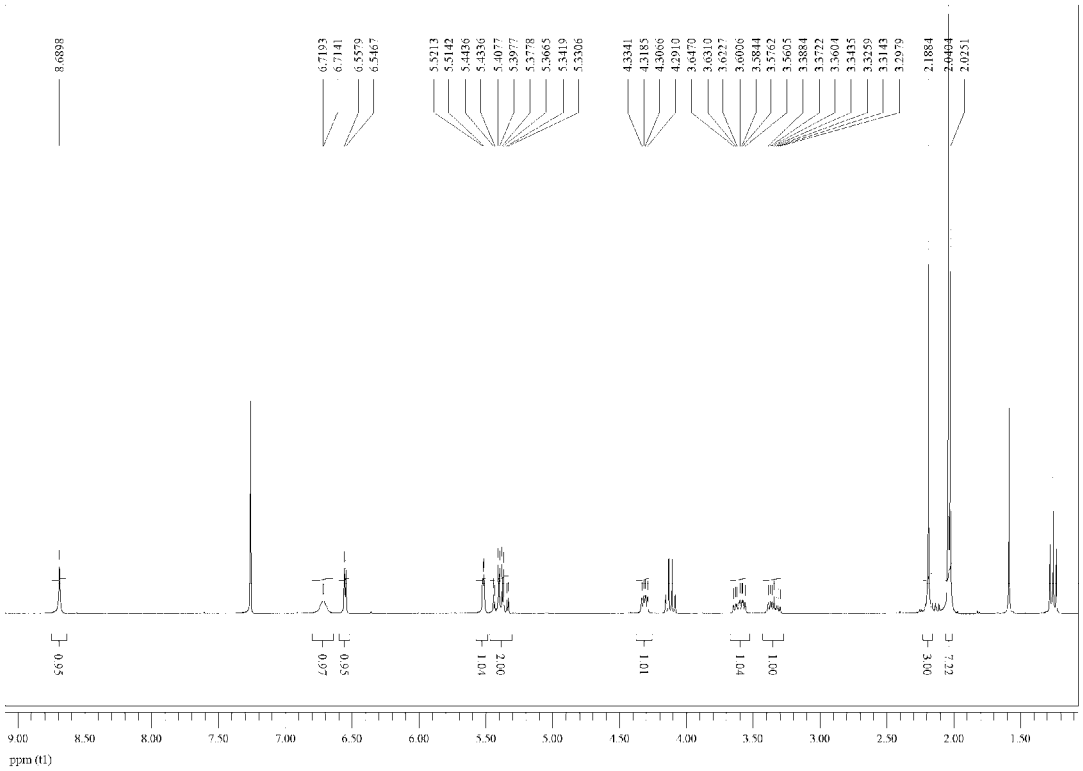

Figure S12. ^{1}H-NMR spectrum of compound **12** (300 MHz, $CDCl_3$).

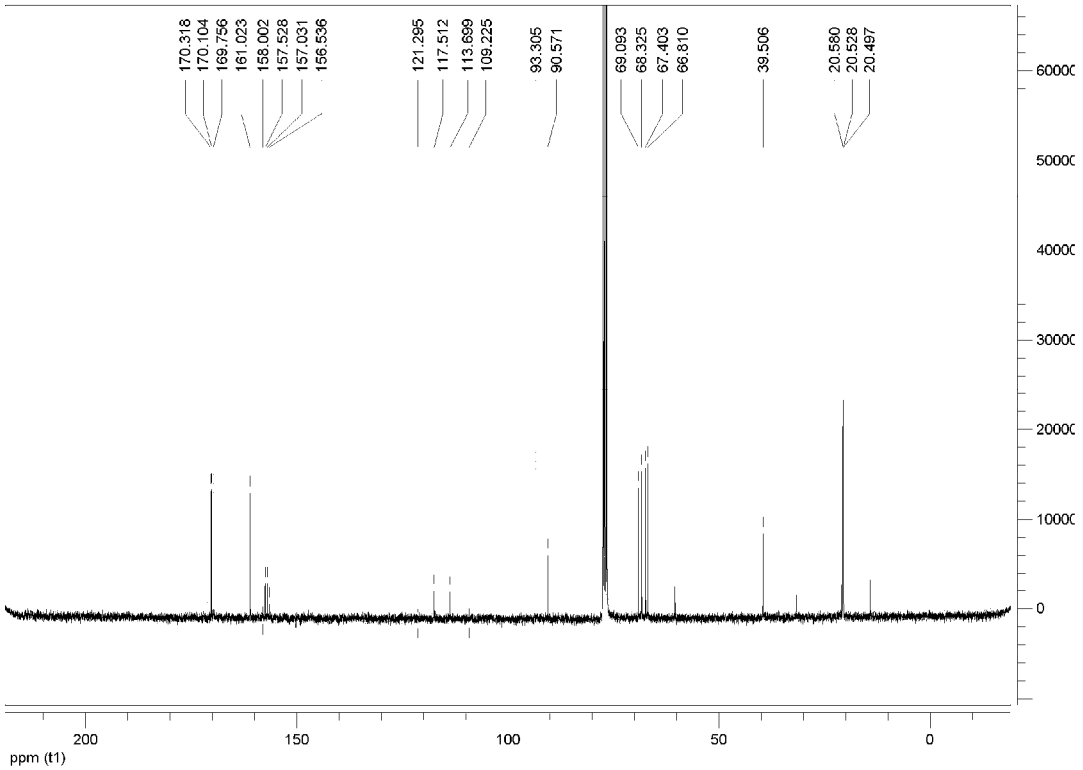

Figure S13. ^{13}C-NMR spectrum of compound **12** (75 MHz, $CDCl_3$).

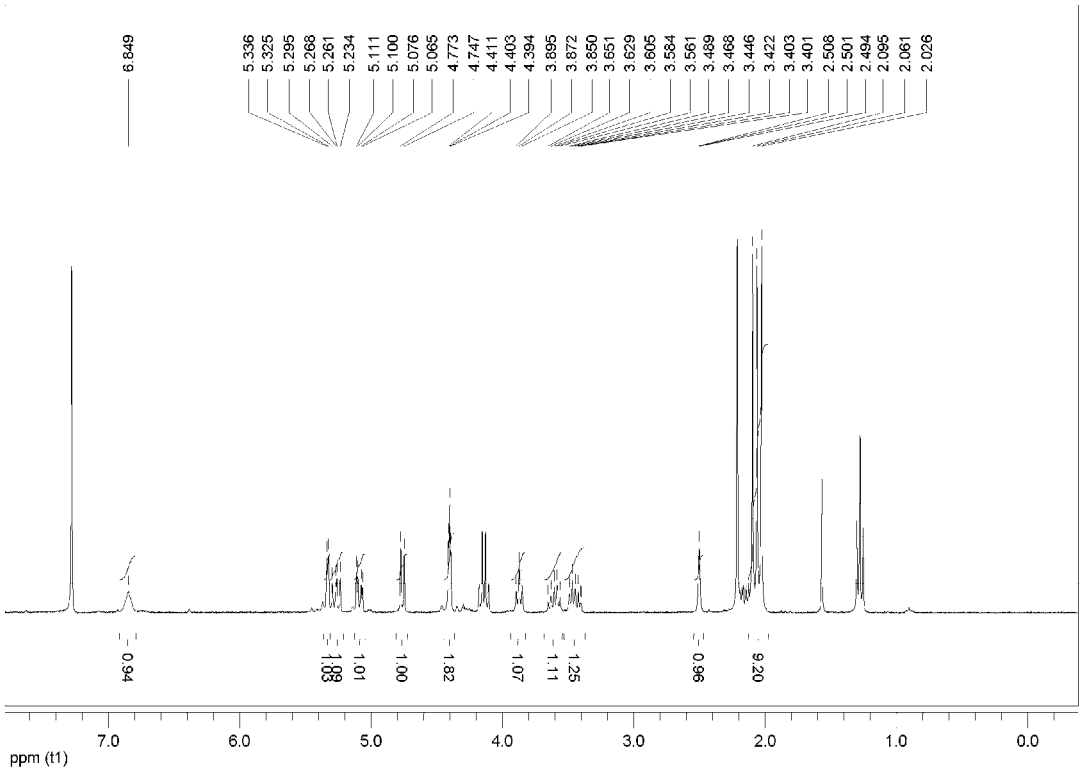

Figure S14. ^{1}H-NMR spectrum of compound **13** (300 MHz, $CDCl_3$).

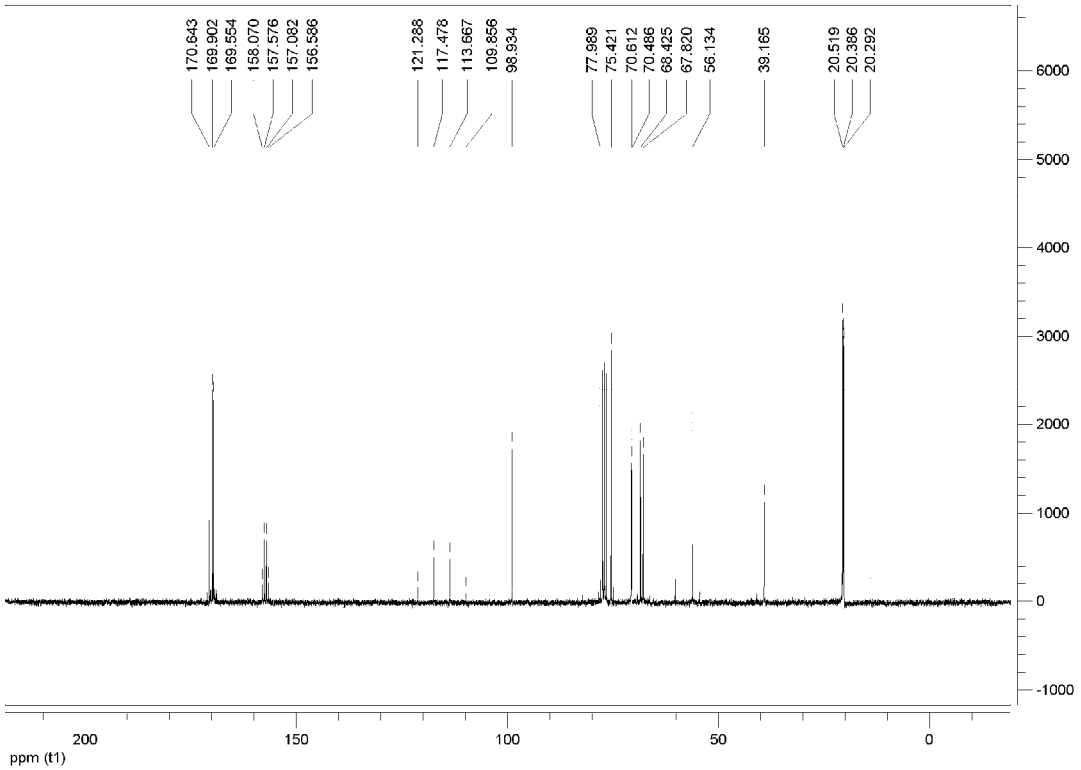

Figure S15. ^{13}C-NMR spectrum of compound **13** (75 MHz, $CDCl_3$).

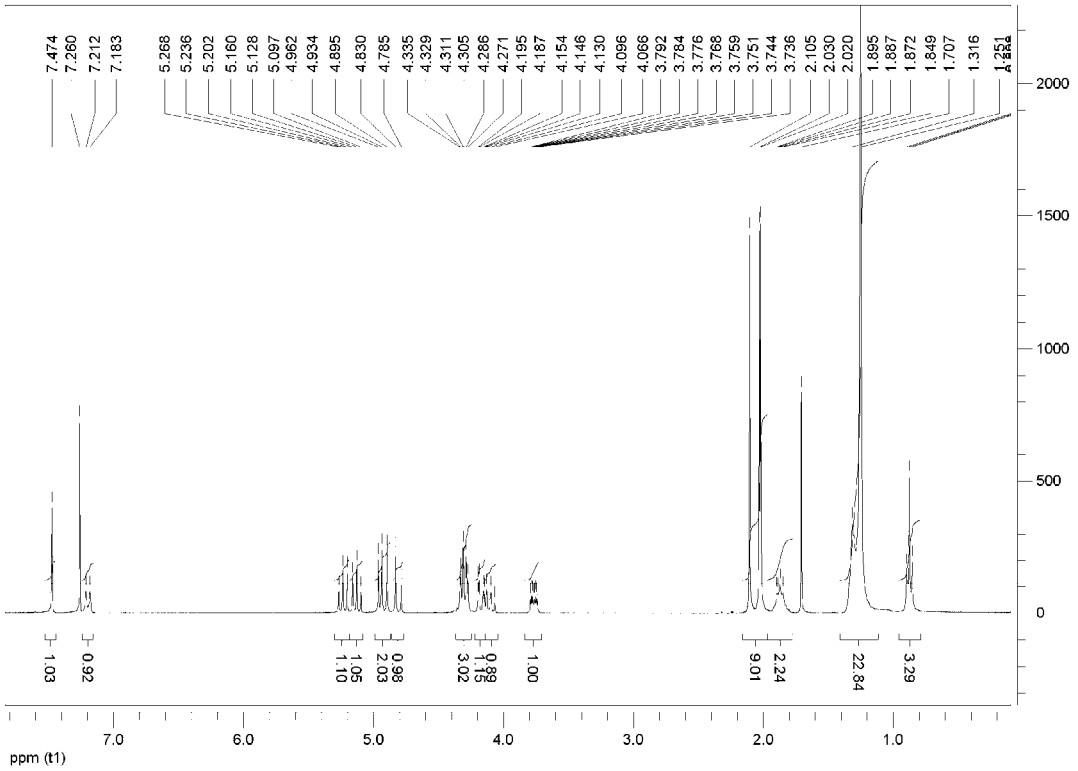

Figure S16. ^{1}H-NMR spectrum of compound **14b** (300 MHz, $CDCl_3$).

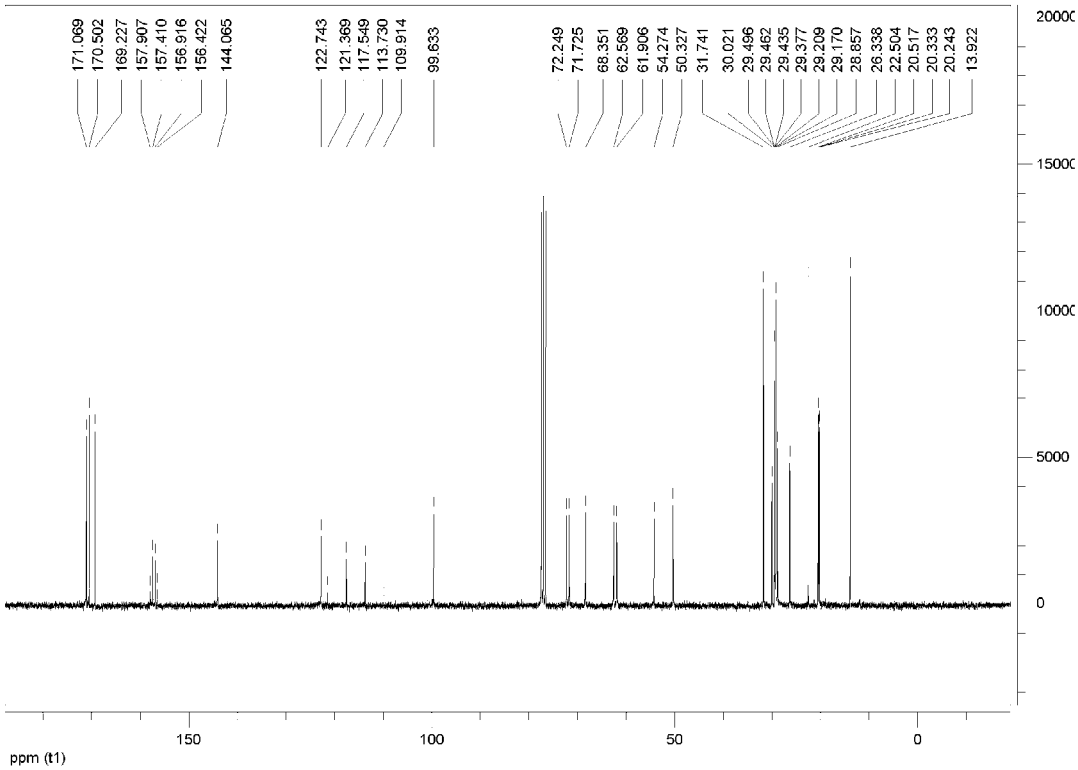

Figure S17. ^{13}C-NMR spectrum of compound **14b** (75 MHz, $CDCl_3$).

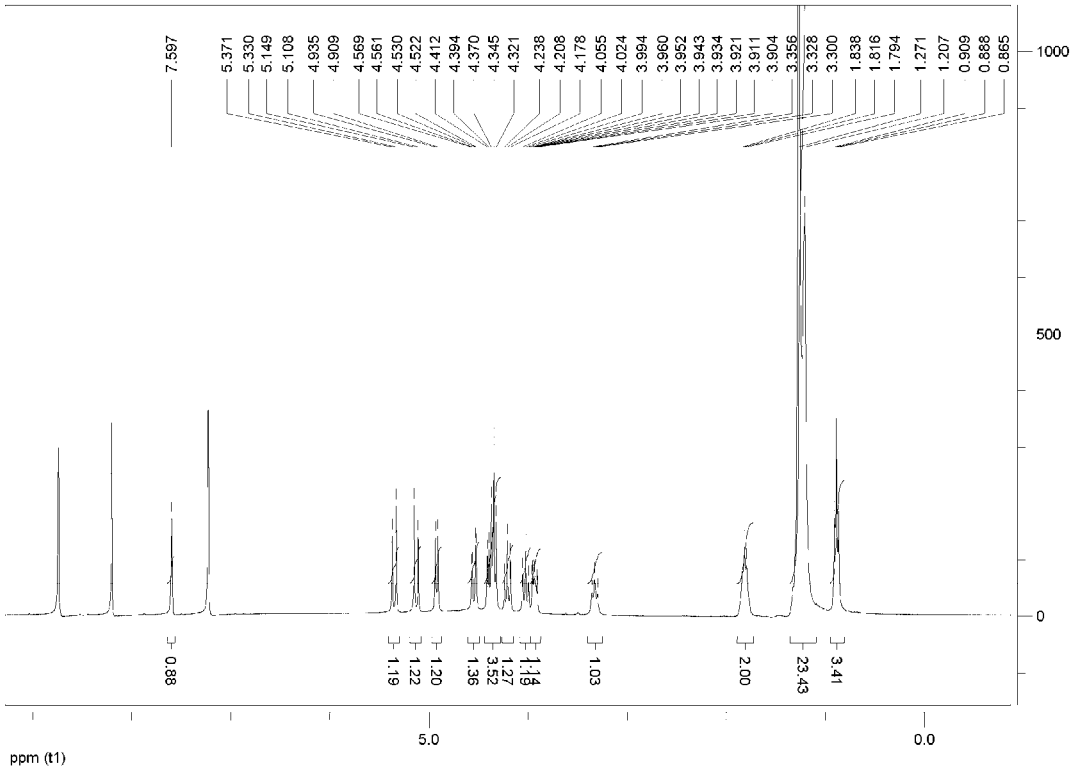

Figure S18. ^{1}H-NMR spectrum of compound **15b** (300 MHz, Pyr-d_5).

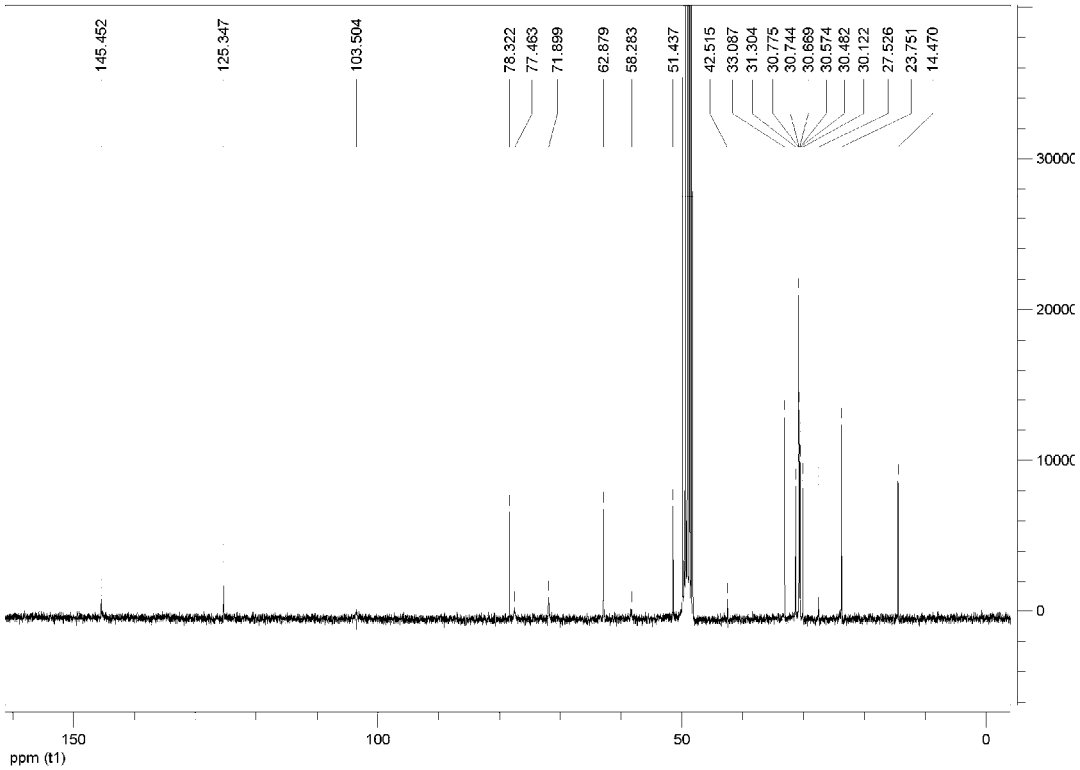

Figure S19. ^{13}C-NMR spectrum of compound **15b** (75 MHz, CD_3OD).

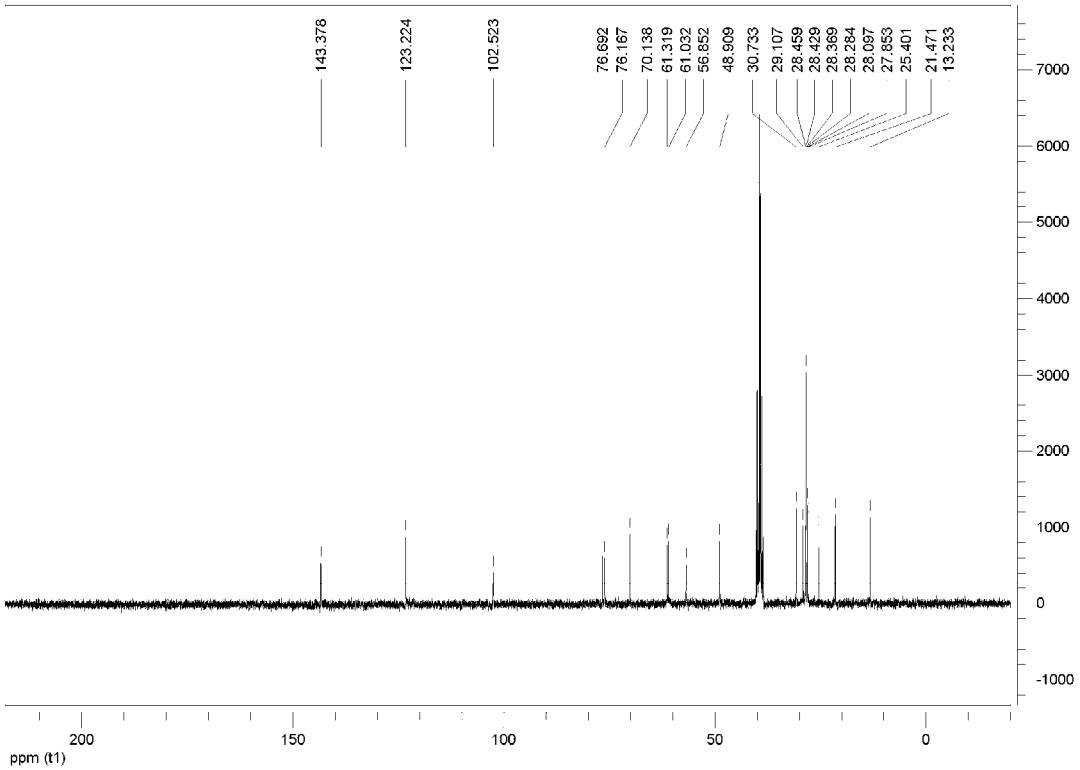

Figure S20. ^{1}H-NMR spectrum of compound **15b** (75 MHz, DMSO-d_6, recorded at 358K).

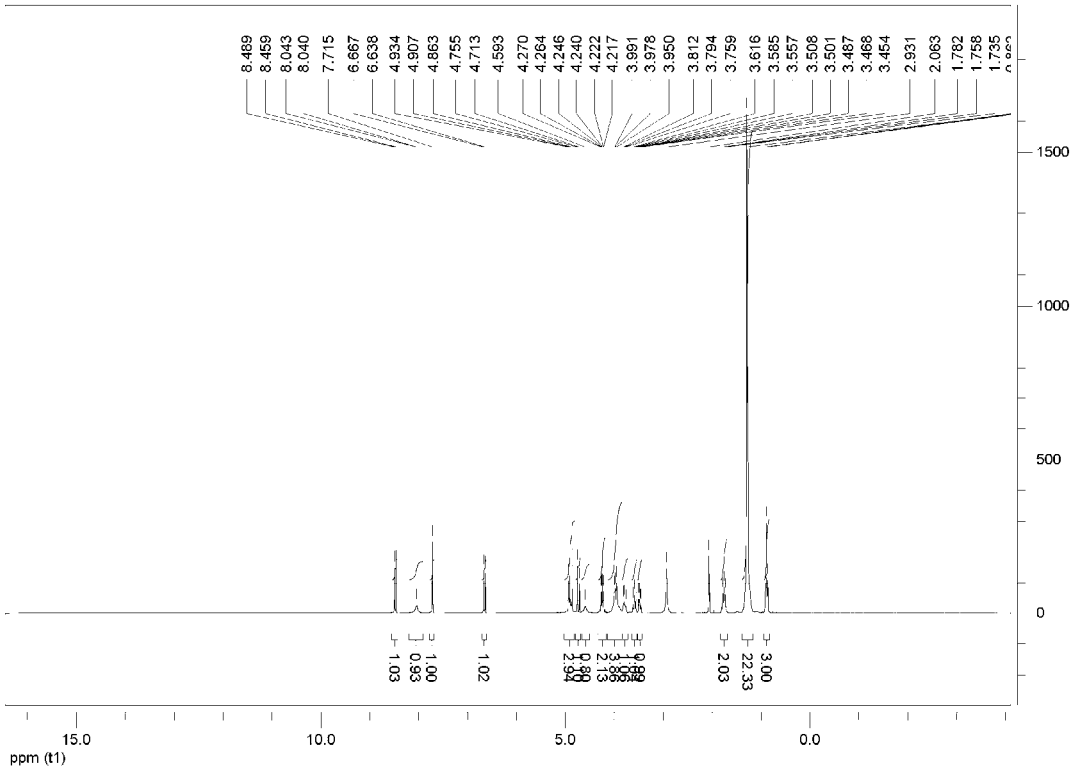

Figure S21. ^{1}H-NMR spectrum of compound **16b** (300 MHz, Acetone-d_6).

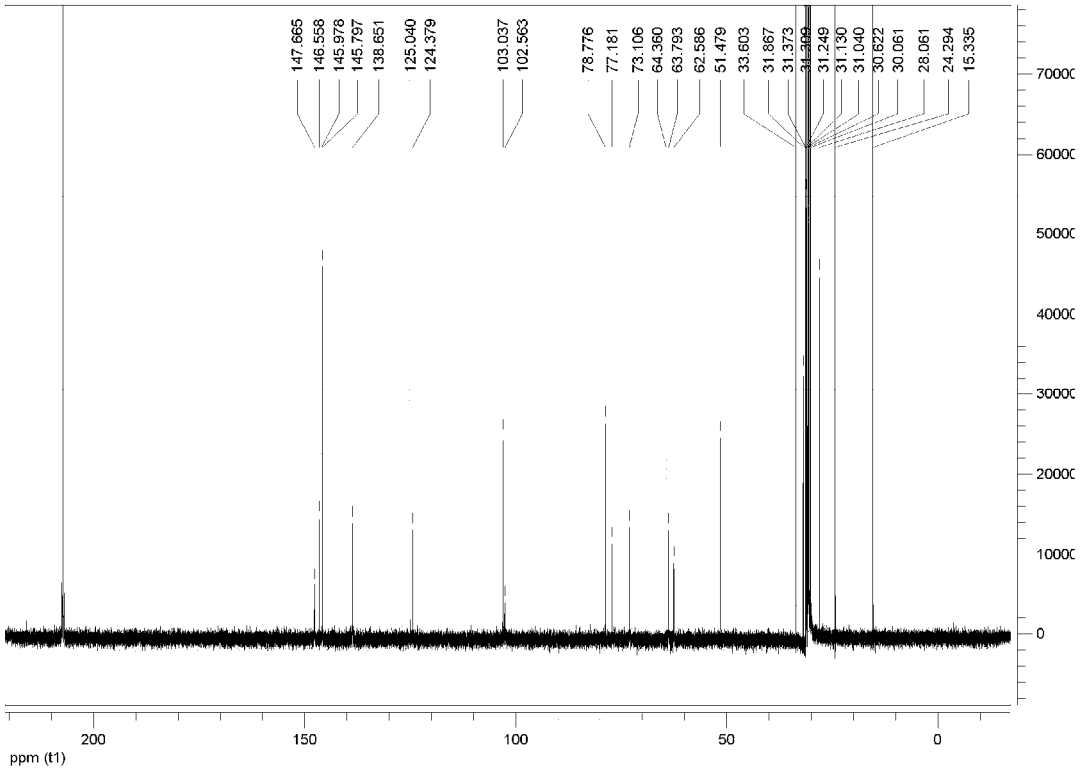

Figure S22. ^{13}C-NMR spectrum of compound **16b** (75 MHz, Acetone-d_6).

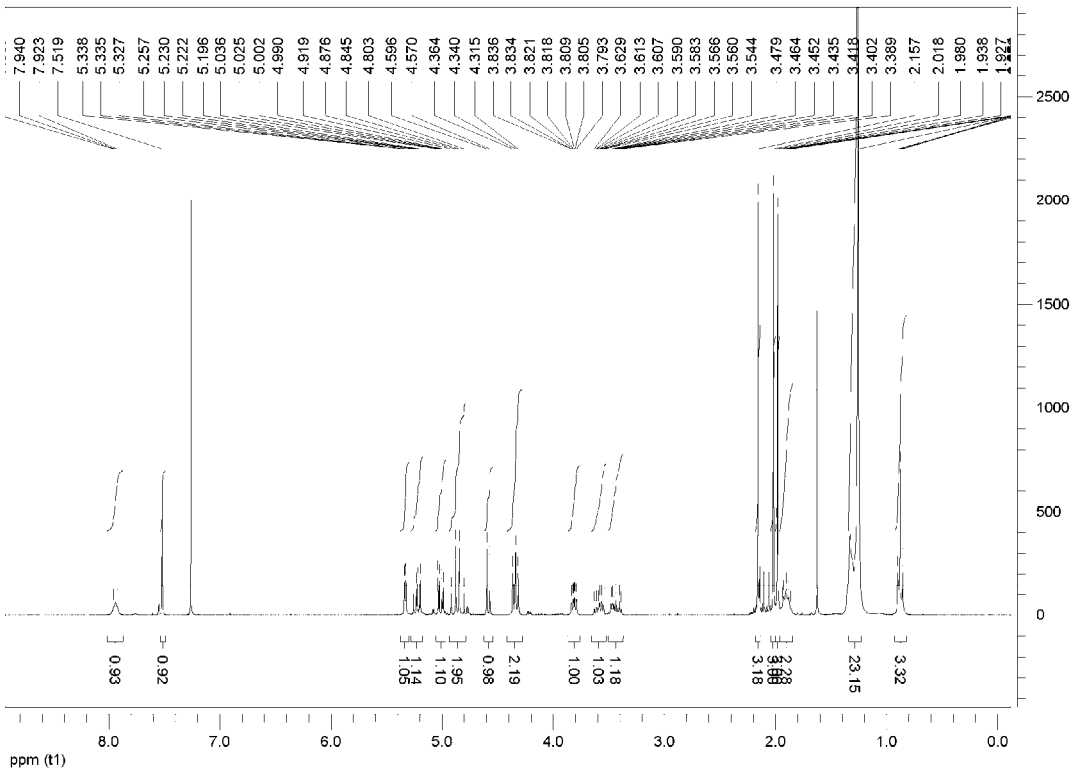

Figure S23. ^{1}H-NMR spectrum of compound **17b** (300 MHz, $CDCl_3$).

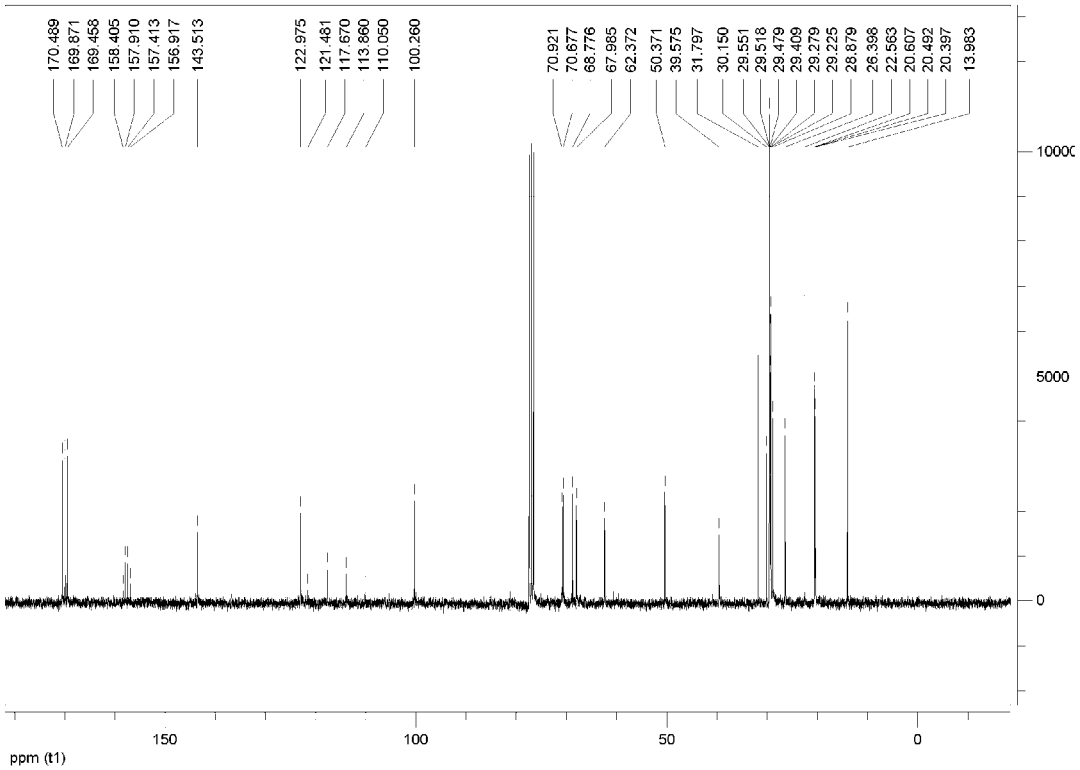

Figure S24. ^{13}C-NMR spectrum of compound **17b** (75 MHz, $CDCl_3$).

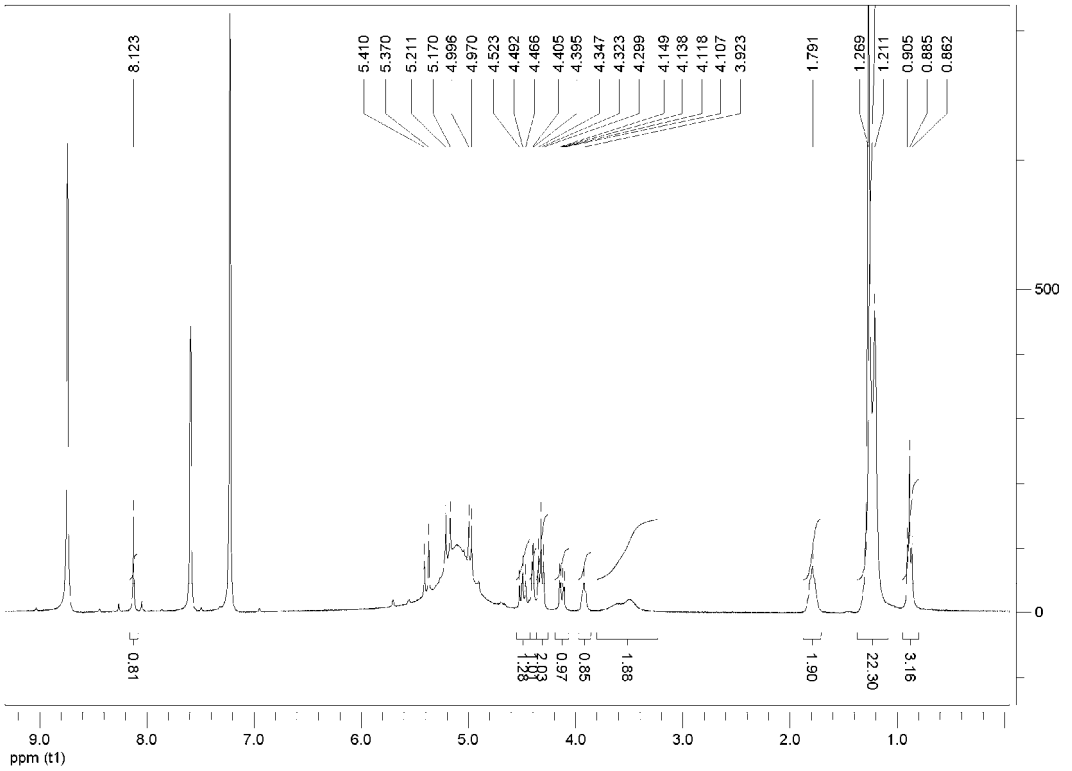

Figure S25. ^{1}H-NMR spectrum of compound **18b** (300 MHz, Pyr-d_5).

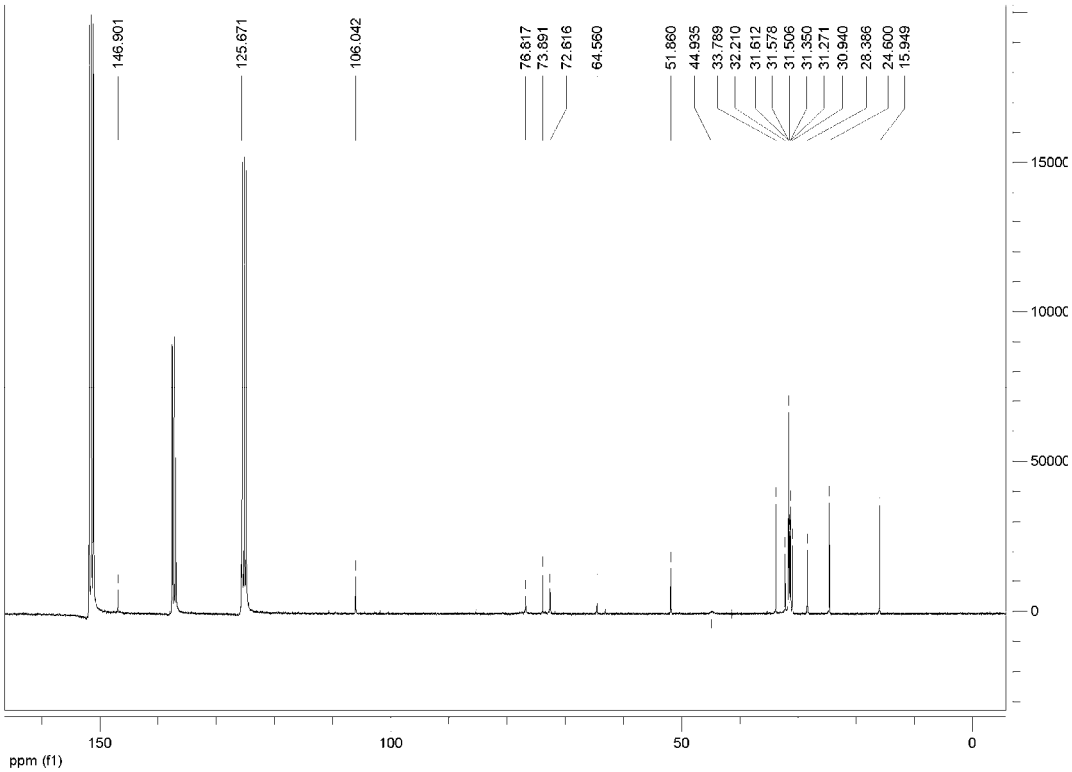

Figure S26. ^{13}C-NMR spectrum of compound **18b** (75 MHz, Pyr-d_5).

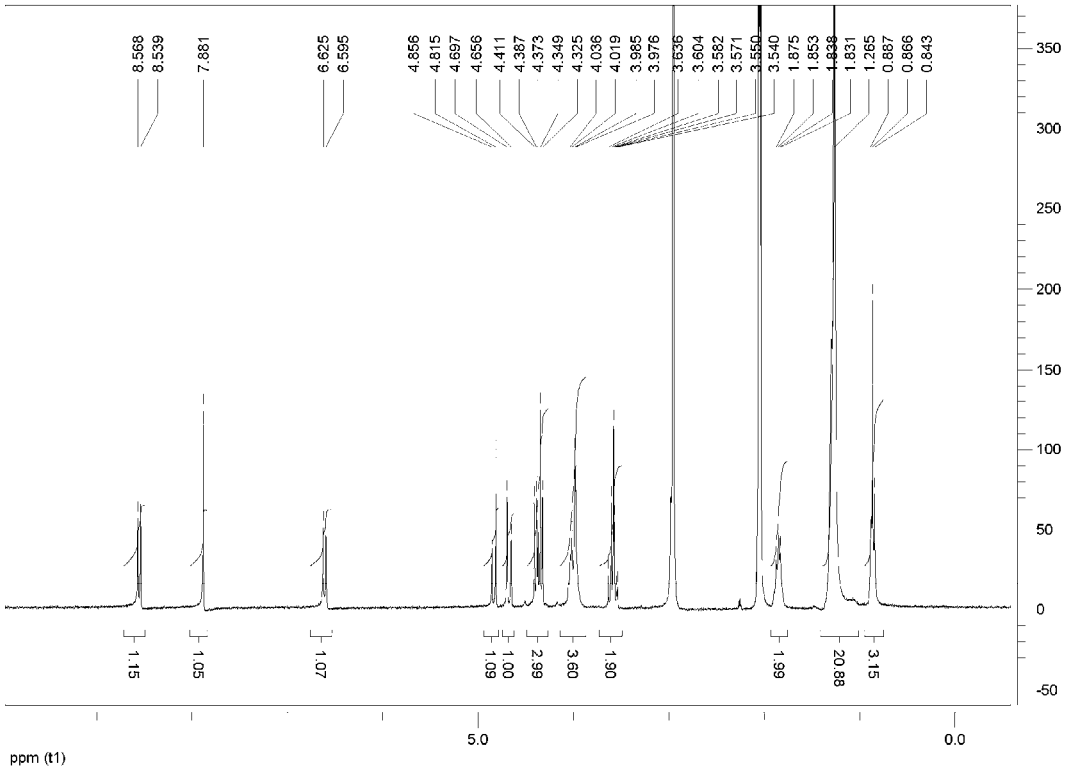

Figure S27. ^{1}H-NMR spectrum of compound **19b** (300 MHz, Acetone-d_6).

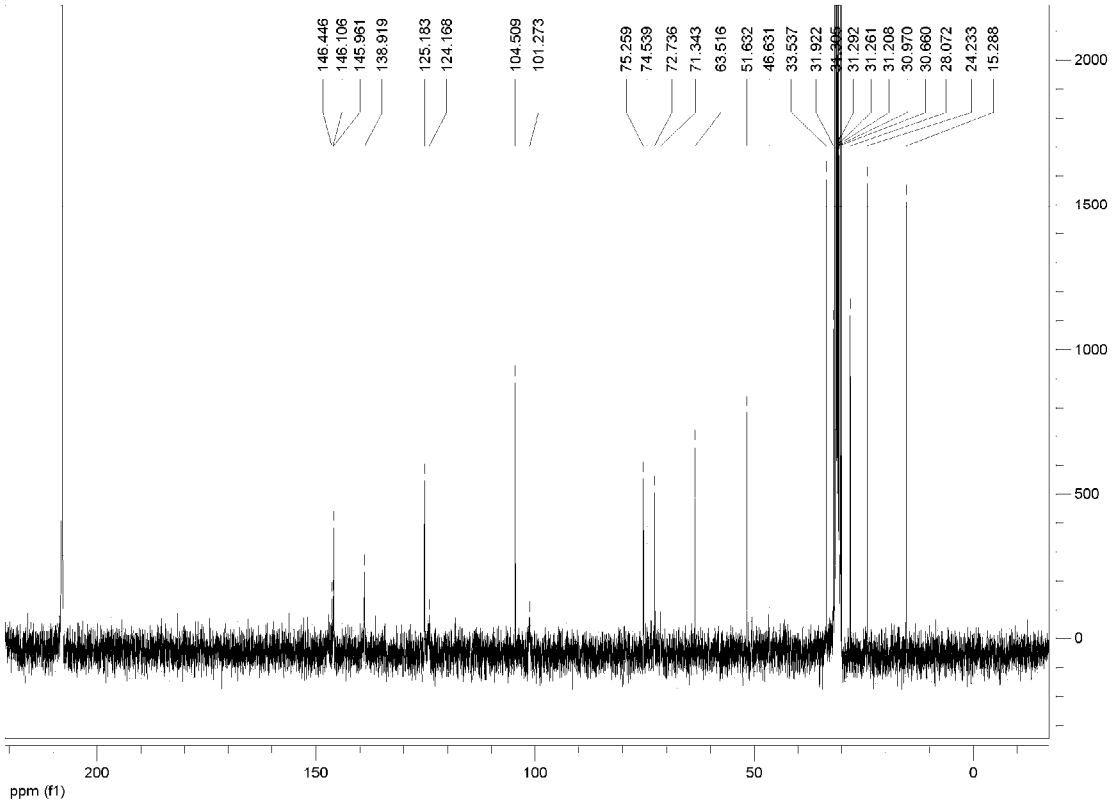

Figure S28. ^{13}C-NMR spectrum of compound **19b** (75 MHz, Acetone-d_6).

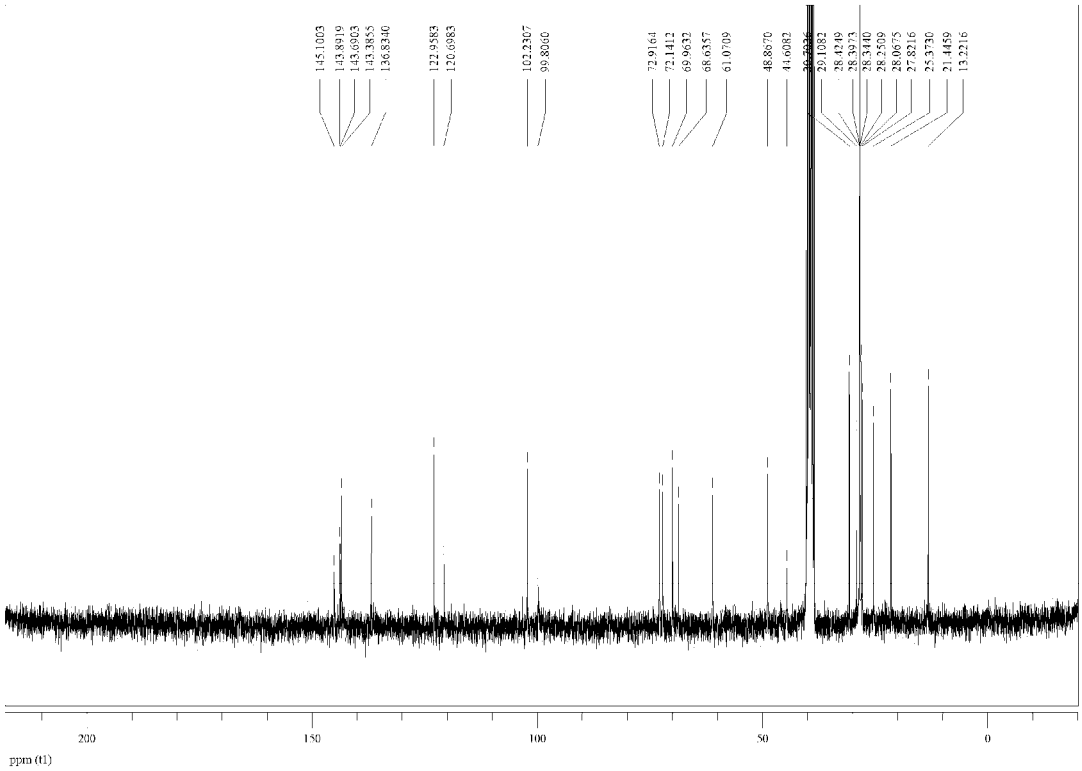

Figure S29. ^{13}C-NMR spectrum of compound **19b** (75 MHz, DMSO-d_6, recorded at 353K).