**Supporting Information for**

**Original article**

# Thio-ProTide strategy: a novel $H_2S$ donor–drug conjugate (DDC) alleviates hepatic injury via innate lysosomal targeting

**Haowen Jin, Jie Ma, Bixin Xu, Sitao Xu, Tianyu Hu, Xin Jin, Jiankun Wang, Guangji Wang*, Le Zhen***

*Key Laboratory of Drug Metabolism and Pharmacokinetics, Research Unit of PK–PD Based Bioactive Components and Pharmacodynamic Target Discovery of Natural Medicine of Chinese Academy of Medical Sciences, China Pharmaceutical University, Nanjing 210009, China*

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*Corresponding authors.

E-mail addresses: Le Zhen (i_m_zhenle@163.com), Guangji Wang (guangjiwang@hotmail.com).

**Running title:** ProTide-based H2S donor–drug conjugate

## Contents

## General Information

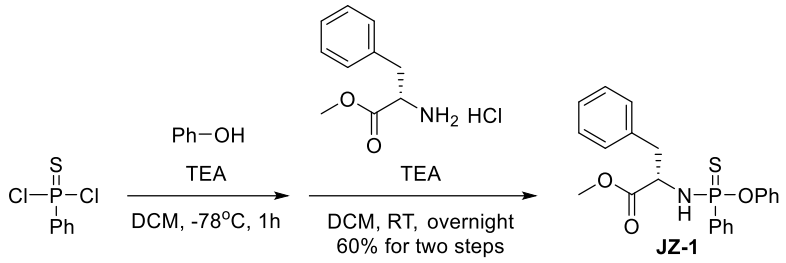
Starting materials, solvents, and reagents were received from commercial sources (Adamas-*beta*, Bide Pharmatech, Aladdin, Energy Chemical and Sigma–Aldrich) and unless otherwise noted and were used without purification.

Analytical thin-layer chromatography (TLC) was carried out on Merck 60 F254 pre-coated silica gel plate (0.2 mm thickness). Visualization was accomplished with UV light (254 nm) or staining with phosphomolybdic acid followed by heating. Flash column chromatography was performed over silica gel (200–300 mesh). $^{1}$H NMR and $^{13}$C NMR spectra were recorded at ambient temperature using Bruker 300M or 500M spectrometers, chemical shifts (in ppm) were referenced to $CDCl_3$ ($\delta$ = 7.26 ppm for $^{1}$H NMR and $\delta$ = 77.2 ppm for $^{13}$C NMR), DMSO-$d_6$ ($\delta$ = 2.50 ppm for $^{1}$H NMR and $\delta$ = 39.5 ppm for $^{13}$C NMR), methanol-$d_4$ ($\delta$ = 3.31 ppm for $^{1}$H NMR and $\delta$ = 49.0 ppm for $^{13}$C NMR), as internal standards. Deuterated solvents (Cambridge Isotope Laboratories and Adamas-*beta*) were used for NMR spectroscopic analyses. Data for $^{1}$H NMR are recorded as following abbreviations: multiplicity (s = singlet, d = doublet, t = triplet, q = quarter, m = multiplet), coupling constant (*J*, Hz). High resolution mass spectrometry (HRMS) data were acquired using the electrospray ionization time-of-flight (ESI-TOF) method. The intermediate generation and prodrug stability were measured by a LC–MS/MS system containing a Shimadzu HPLC system (Kyoto, Japan) coupled with a SCIEX API 4000 triple-quadrupole mass spectrometer (Foster City, CA, USA) equipped with an electrospray ionization (ESI) interface in positive or negative ionization mode. Analyst software version 1.5.1 (SCIEX) was used for data acquisition and analysis. Excitation and emission measurements were recorded with a 96-well plate reader (BioTek SYNERGY-H1 multi-mode reader, Winooski, VT, USA). Fluorescence imaging were acquired using Olympus FV3000 laser scanning confocal microscope (Olympus, Tokyo, Japan) and BioTek Lionheart FX automated microscope (BioTek, VT, USA).

# 1. Synthesis of donors, probes and relevant compounds

## 1.1. Synthesis of JZ-1–JZ-11

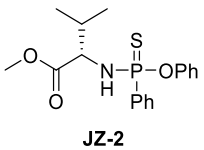
*Methyl (phenoxy(phenyl)phosphorothioyl)-L-phenylalaninate* (**JZ-1**)




**Scheme S1.** Synthesis of **JZ-1**

Phenylphosphonothioic dichloride (422 mg, 2 mmol) was dissolved in anhydrous dichloromethane (DCM) (5 mL). To this solution was added phenol (188 mg, 2 mmol) and trimethylamine (TEA) (304 μL, 2.2 mmol) in anhydrous DCM (2 mL) under an Ar atmosphere at –78 °C. The reaction was stirred at –78 °C for 1 h. A solution of methyl L-phenylalaninate hydrochloride (430 mg, 2 mmol) and TEA (608 μL, 4.4 mmol) in anhydrous DCM (5 mL) was added, the reaction was stirred at room temperature overnight. The mixture was diluted with DCM (10 mL), washed with water (30 mL), dried over $Na_2SO_4$, and concentrated under vacuum. The crude material was purified by flash chromatography [petroleum ether (PE)/ethyl acetate (EA) = 10:1] to afford the product **JZ-1** as a colorless oil (496 mg, 60.3%). **JZ-1** was a mixture of two inseparable diastereomers. $^1$H NMR (300 MHz, $CDCl_3$) 8.03 – 7.82 (m, 2H), 7.60 – 7.40 (m, 3H), 7.36 – 7.20 (m, 6H), 7.15 (d, *J* = 7.2 Hz, 2H), 7.04 (s, 2H), 4.54 – 4.36 (m, 1H), 3.90 – 3.69 (m, 1H), 3.58 (d, *J* = 17.7 Hz, 3H), 3.06 – 2.87 (m, 2H); $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 172.8 (dd), 150.8 (dd), 135.7, 134.9 (d), 133.8 (d), 132.1 (d, ), 132.0 (d), 131.0 (d), 130.8, 129.5 (d), 129.4 (d), 128.7, 128.6, 128.5, 128.4, 127.1 (d), 124.9 (d), 121.6 (d), 121.5 (d), 56.1 (d), 52.1 (d), 40.7 (dd); $^{31}$P NMR (202 MHz, $CDCl_3$) $\delta$ 74.76, 74.32; HRMS (ESI) for $C_{22}H_{23}NO_3PS$ $[M+H]^+$ calcd. for 412.1131, found 412.1126.

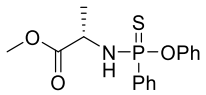
*Methyl (phenoxy(phenyl)phosphorothioyl)-L-valinate* (**JZ-2**)




**JZ-2** was prepared from L-valine methyl ester hydrochloride (334 mg, 2 mmol) and phenylphosphonothioic dichloride (422 mg, 2 mmol) following similar synthetic procedures described

for **JZ-1**, the crude material was purified by flash chromatography (PE/EA = 20:1) to afford the product **JZ-2** as a colorless oil (387 mg, 53.2%). **JZ-2** was a mixture of two inseparable diastereomers. $^{1}$H NMR (300 MHz, $CDCl_3$) 8.09–7.97 (m, 2H), 7.61–7.46 (m, 3H), 7.36–7.27 (m, 2H), 7.26–7.10 (m, 3H), 4.11–3.86 (m, 1H), 3.86–3.73 (m, 1H), 3.62 (d, *J* = 13.5 Hz, 3H), 2.08–1.89 (m, 1H), 0.89 (m, 3H), 0.83 (m, 3H); $^{13}$C NMR (126 MHz, Chloroform-*d*) $\delta$ 173.3 (dd), 151.0 (dd), 135.0 (d), 133.9 (d), 132.2 (d), 132.1 (d), 131.1 (dd), 129.4, 128.6 (d), 128.5 (d), 124.8, 121.5, 121.4, 60.2 (d), 52.0 (d), 32.5 (dd), 18.8 (d), 17.9 (d); $^{31}$P NMR (202 MHz, $CDCl_3$) $\delta$ 75.95; HRMS (ESI) for $C_{18}H_{22}NO_3PS$ $[M+H]^+$ calcd. for 364.1131, found 364.1125.

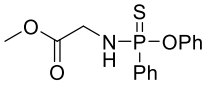
*Methyl (phenoxy(phenyl)phosphorothioyl)-L-alaninate* (**JZ-3**)




**JZ-3**

**JZ-3** was prepared from L-alanine methyl ester hydrochloride (278 mg, 2 mmol) and phenylphosphonothioic dichloride (422 mg, 2 mmol) following similar synthetic procedures described for **JZ-1**, the crude material was purified by flash chromatography (PE/EA = 20:1) to afford the product **JZ-3** as a colorless oil (278 mg, 41.4%). **JZ-3** was a mixture of two inseparable diastereomers. $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.13–7.89 (m, 2H), 7.66–7.45 (m, 3H), 7.42–7.03 (m, 5H), 4.27–4.11 (m, 1H), 3.98–3.83 (m, 1H), 3.66 (d, *J* = 13.9 Hz, 3H), 1.34 (m, 3H); $^{13}$C NMR (126 MHz, Chloroform-*d*) $\delta$ 174.1 (dd), 150.7 (d), 135.0 (d), 133.9 (d), 132.1 (dd), 131.0 (d), 130.9 (d), 129.4 (d), 128.7 (d), 128.6 (d), 124.92, 121.7 (d), 121.6 (d), 52.4 (d), 50.7 (d), 21.2 (d); $^{31}$P NMR (202 MHz, $CDCl_3$) $\delta$ 74.34, 73.83; HRMS (ESI) for $C_{16}H_{19}NO_3PS$ $[M+H]^+$ calcd. for 336.0818, found 336.0814.

*Methyl (phenoxy(phenyl)phosphorothioyl)glycinate* **(JZ-4)**

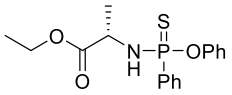



**JZ-4**

**JZ-4** was prepared from glycine methyl ester hydrochloride (250 mg, 2 mmol) and phenylphosphonothioic dichloride (422 mg, 2 mmol) following similar synthetic procedures described for **JZ-1** , the crude material was purified by flash chromatography (PE/EA = 10:1) to afford the product **JZ-4** as a colorless oil (290 mg, 45.1%). **JZ-4** was a mixture of two enantiomers. $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.09–7.97 (m, 2H), 7.61–7.49 (m, 3H), 7.35 (m, 2H), 7.26–7.13 (m, 3H), 4.01–

3.88 (m, 2H), 3.73 (s, 3H); $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 171.0, 170.9, 150.7, 150.6, 134.5, 133.3, 132.3, 132.2, 131.0, 130.9, 129.5, 128.8, 128.6, 125.0, 121.6, 121.6, 121.6, 52.4, 43.5; $^{31}$P NMR (202 MHz, $CDCl_3$) $\delta$ 75.30; HRMS (ESI) for $C_{15}H_{17}NO_3PS$ $[M+H]^+$ calcd. for 322.0661, found 322.0662.

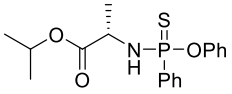
*Ethyl (phenoxy(phenyl)phosphorothioyl)-L-alaninate* (**JZ-5**)



**JZ-5**

**JZ-5** was prepared from L-alanine ethyl ester hydrochloride (459 mg, 3 mmol) and phenyl phosphonic dichloride (633 mg, 3 mmol) following similar synthetic procedures described for **JZ-1**, the crude material was purified by flash chromatography (PE/EA = 50:1) to afford the product **JZ-5** as a colorless oil (320 mg, 30.6%). **JZ-5** was a mixture of two inseparable diastereomers. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.11–7.93 (m, 2H), 7.63–7.44 (m, 3H), 7.38–7.29 (m, 2H), 7.30–7.12 (m, 3H), 4.25–3.90 (m, 3H), 3.99 (br s, 1H), 1.33 (dd, $J$ = 25.7, 7.0 Hz, 3H), 1.29–1.16 (m, 3H).; $^{13}$C NMR (101 MHz, chloroform-*d*) $\delta$ 173.6 (d), 150.7 (d), 135.1 (d), 132.1 (dd), 130.9 (dd), 129.4 (d), 128.6 (dd), 124.9, 121.6 (dd), 61.5 (d), 50.7 (d), 21.2 (dd), 14.1; $^{31}$P NMR (121 MHz, $CDCl_3$) $\delta$ 74.24, 73.65; HRMS (ESI) for $C_{17}H_{21}NO_3P$ $[M+H]^+$ calcd. for 350.0974, found 350.0974.

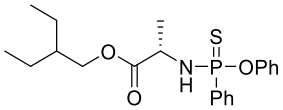
*Isopropyl (phenoxy(phenyl)phosphorothioyl)-L-alaninate* (**JZ-6**)



**JZ-6**

**JZ-6** was prepared from L-alanine isopropyl ester hydrochloride (334 mg, 2 mmol) and phenylphosphonothioic dichloride (422 mg, 2 mmol) following similar synthetic procedures described for **JZ-1**, the crude material was purified by flash chromatography (PE/EA = 20:1) to afford the product **JZ-6** as a colorless oil (301 mg, 41.4%). **JZ-6** was a mixture of two inseparable diastereomers. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.12–7.86 (m, 2H), 7.64–7.40 (m, 3H), 7.38–7.07 (m, 5H), 5.08–4.83 (m, 1H), 4.24–4.04 (m, 1H), 4.03–3.87 (m, 1H), 1.32 (m, 3H), 1.25–1.15 (m, 6H); $^{13}$C NMR (126 MHz, chloroform-*d*) $\delta$ 173.2 (dd), 150.8 (dd), 135.1 (d), 134.0 (d), 132.1 (d), 132.0 (d), 130.9 (dd), 129.4 (dd), 128.6 (d), 128.5 (d), 124.9, 121.7 (d), 121.6 (d), 69.2 (d), 50.9 (d), 21.7 (d), 21.6 (d), 21.3 (dd); $^{31}$P NMR (202 MHz, DMSO-$d_6$) $\delta$ 75.99, 74.13; HRMS (ESI) for $C_{18}H_{23}NO_3PS$ $[M+H]^+$ calcd. for 364.1131, found 364.1127.

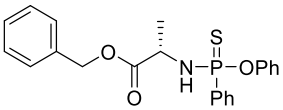
*2-Ethylbutyl (phenoxy(phenyl)phosphorothioyl)-L-alaninate* (**JZ-7**)



**JZ-7**

**JZ-7** was prepared from L-alanine 2-ethylbutyl ester hydrochloride (627 mg, 3 mmol) and phenyl phosphonic dichloride (633 mg, 3 mmol) following similar synthetic procedures described for **JZ-1**, the crude material was purified by flash chromatography (PE/EA = 50:1) to afford the product **JZ-7** as a colorless oil (250 mg, 20.6%). **JZ-7** was a mixture of two inseparable diastereomers. $^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.09–7.97 (m, 2H), 7.61–7.49 (m, 3H), 7.35 (t, $J$ = 7.7 Hz, 2H), 7.27–7.15 (m, 3H), 4.29–4.14 (m, 1H), 4.09–3.93 (m, 3H), 1.56–1.46 (m, 1H), 1.43–1.27 (m, 7H), 0.95–0.84 (m, 6H); $^{13}$C NMR (101 MHz, chloroform-*d*) $\delta$ 173.9 (dd), 150.7 (d), 135.1, 133.6, 132.1 (dd), 130.9 (dd), 129.4 (d), 128.6 (dd), 124.9, 121.6 (dd), 67.5 (d), 50.7 (d), 40.2, 23.2 (d), 21.4, 11.0; $^{31}$P NMR (162 MHz, $CDCl_3$) $\delta$ 74.39, 73.73; HRMS (ESI) for $C_{21}H_{29}O_3NP$ $[M+H]^+$ calcd. for 406.1600, found 406.1601.

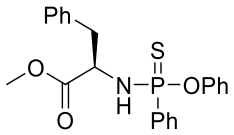
*Benzyl (phenoxy(phenyl)phosphorothioyl)-L-alaninate* (**JZ-8**)



**JZ-8**

**JZ-8** was prepared from L-alanine benzyl ester hydrochloride (654 mg, 3 mmol) and phenyl phosphonic dichloride (633 mg, 3 mmol) following similar synthetic procedures described for **JZ-1**, the crude material was purified by flash chromatography (PE/EA = 50:1) to afford the product **JZ-8** as a colorless oil (479 mg, 38.9%). **JZ-8** was a mixture of two inseparable diastereomers. $^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.08–7.97 (m, 2H), 7.60–7.53 (m, 1H), 7.53–7.47 (m, 2H), 7.39 (t, $J$ = 6.4 Hz, 3H), 7.35–7.29 (m, 4H), 7.25–7.15 (m, 3H), 5.11 (d, $J$ = 23.7 Hz, 2H), 4.32–4.19 (m, 3H), 4.02–3.91 (m, 1H), 1.43–1.30 (m, 3H); $^{13}$C NMR (101 MHz, chloroform-*d*) $\delta$ 173.6 (dd), 150.7 (d), 135.2 (d), 135.0 (d), 133.6 (d), 132.1 (dd), 130.9 (dd), 129.7, 129.4 (d), 128.6 (dd), 128.2 (d), 124.9, 121.6 (dd), 120.7, 115.3, 67.2 (d), 50.7 (d), 21.2 (dd); $^{31}$P NMR (121 MHz, $CDCl_3$) $\delta$ 74.34, 73.76; HRMS (ESI) for $C_{22}H_{23}NO_3P$ $[M+H]^+$ calcd. for 412.1131, found 412.1131.

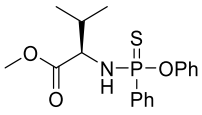
*Methyl (phenoxy(phenyl)phosphorothioyl)-D-phenylalaninate* (**JZ-9**)



**JZ-9**

**JZ-9** was prepared from D-phenylalaninate methyl ester hydrochloride (645 mg, 3 mmol) and phenylphosphonothioic dichloride (633 mg, 3 mmol) following similar synthetic procedures described for **JZ-1**, the crude material was purified by flash chromatography (PE/EA = 50:1) to afford the product **JZ-9** as a colorless oil (432 mg, 35.0%). **JZ-9** was a mixture of two inseparable diastereomers. $^{1}$H NMR (400 MHz, chloroform-*d*) $\delta$ 8.01–7.85 (m, 2H), 7.61–7.43 (m, 3H), 7.37–7.27 (m, 2H), 7.30–7.21 (m, 3H), 7.22–7.01 (m, 5H), 4.54–4.36 (m, 1H), 3.88–3.71 (m, 1H), 3.59 (d, *J* = 24.9 Hz, 3H), 3.09–2.88 (m, 2H); $^{13}$C NMR (101 MHz, chloroform-*d*) $\delta$ 172.8 (dd), 150.8 (dd), 135.7, 134.9 (d), 133.5 (d), 132.1 (dd), 130.9 (dd), 129.4 (dd), 128.5 (dd), 127.1, 124.9, 121.5 (dd), 56.0 (d), 52.1 (d), 40.6 (dd); $^{31}$P NMR (121 MHz, $CDCl_3$) $\delta$ 74.66, 74.26; HRMS (ESI) for $C_{22}H_{23}NO_3PS$ $[M+H]^+$ calcd. for 412.1131, found 412.1130.

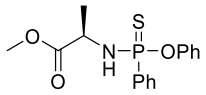
*Methyl (phenoxy(phenyl)phosphorothioyl)-D-valinate* (**JZ-10**)



**JZ-10**

**JZ-10** was prepared from D-valine methyl ester hydrochloride (501 mg, 3 mmol) and phenylphosphonothioic dichloride (633 mg, 3 mmol) following similar synthetic procedures described for **JZ-1**, the crude material was purified by flash chromatography (PE/EA = 50:1) to afford the product **JZ-10** as a colorless oil (350 mg, 32.1%). **JZ-10** was a mixture of two inseparable diastereomers. $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.09–7.97 (m, 2H), 7.62–7.46 (m, 3H), 7.36–7.28 (m, 2H), 7.25–7.12 (m, 3H), 4.11–3.88 (m, 1H), 3.88–3.75 (m, 1H), 3.62 (d, *J* = 14.1 Hz, 3H), 2.06–1.90 (m, 1H), 0.90 (dd, *J* = 6.8, 4.0 Hz, 3H), 0.83 (dd, *J* = 6.9, 4.3 Hz, 3H); $^{13}$C NMR (101 MHz, chloroform-*d*) $\delta$ 173.3 (dd), 150.9 (dd), 135.0 (d), 133.6 (d), 132.1 (dd), 131.0 (dd), 129.7, 129.3, 128.5 (dd), 124.8, 121.5 (d), 60.1 (d), 52.0 (d), 32.5 (dd), 18.8 (d), 17.9 (d); $^{31}$P NMR (121 MHz, $CDCl_3$) $\delta$ 75.90; HRMS (ESI) for $C_{18}H_{23}NO_3PS$ $[M+H]^+$ calcd. for 364.1131, found 364.1131.

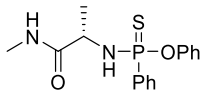
*Methyl (phenoxy(phenyl)phosphorothioyl)-D-alaninate* (**JZ-11**)



**JZ-11**

**JZ-11** was prepared from D-alanine methyl ester hydrochloride (278 mg, 2 mmol) and phenylphosphonothioic dichloride (422 mg, 2 mmol) following similar synthetic procedures described for **JZ-1** , the crude material was purified by flash chromatography (PE/EA = 20:1) to afford the product **JZ-11** as a colorless oil (170 mg, 25.3%). **JZ-11** was a mixture of two inseparable diastereomers. $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.13–7.91 (m, 2H), 7.61–7.47 (m, 3H), 7.38–7.28 (m, 2H), 7.27–7.07 (m, 3H), 4.30–4.12 (m, 1H), 4.04–3.86 (m, 1H), 3.66 (d, $J$ = 14.1 Hz, 3H), 1.34 (m, 3H); $^{13}$C NMR (126 MHz, chloroform-*d*) $\delta$ 170.94 (d), 150.7 (d), 134.5, 133.3, 132.2 (d), 131.0, 130.9, 129.5, 128.8, 128.6, 125.0, 121.6, 121.6, 52.5, 43.5; $^{31}$P NMR (202 MHz, $CDCl_3$) $\delta$ 74.34, 73.83; HRMS (ESI) for $C_{16}H_{19}NO_3PS$ $[M+H]^+$ calcd. for 336.0818, found 336.0814.

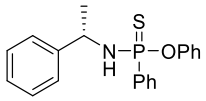
*O-phenyl N-((S)-1-(methylamino)-1-oxopropan-2-yl)-P-phenylphosphonamidothioate* (**JZ-12**)



**JZ-12**

**JZ-12** was prepared from (*S*)-2-amino-*N*-methylpropanamide hydrochloride (416 mg, 3 mmol) and phenyl phosphonic dichloride (633 mg, 3 mmol) following similar synthetic procedures described for **JZ-1**, the crude material was purified by flash chromatography (PE/EA = 10:1) to afford the product **JZ-12** as a colorless oil (321 mg, 32.1%). **JZ-12** was a mixture of two inseparable diastereomers. $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.06–7.93 (m, 2H), 7.61–7.45 (m, 3H), 7.32 (t, $J$ = 7.7 Hz, 2H), 7.24–7.13 (m, 3H), 6.26 (d, $J$ = 45.6 Hz, 1H), 4.17–3.90 (m, 2H), 2.73–2.57 (m, 3H), 1.39–1.22 (m, 3H);$^{13}$C NMR (101 MHz, chloroform-*d*) $\delta$ 173.3 (d), 150.5, 134.7 (d), 132.4, 130.8 (dd), 129.5, 128.7 (dd), 125.1 (d), 121.7 (d), 121.4 (d, $J$ = 5.1 Hz), 51.7, 26.2 (d), 20.7 (d); $^{31}$P NMR (162 MHz, $CDCl_3$) $\delta$ 75.55, 75.13; HRMS (ESI) for $C_{16}H_{20}N_2O_2PS$ $[M+H]^+$ calcd. for 335.0978, found 335.0971.

*O-phenyl P-phenyl-N-((S)-1-phenylethyl) phosphonamidothioate* (**JZ-13**)

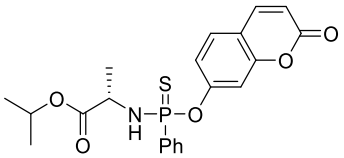


**JZ-13**

**JZ-13** was prepared from *(S)*-(–)-alpha-methylbenzylamine (363 mg, 3 mmol) and phenyl phosphonic dichloride (633 mg, 3 mmol) following similar synthetic procedures described for **JZ-1**, the crude

material was purified by flash chromatography (PE/EA = 5:1) to afford the product **JZ-13** as a colorless oil (361 mg, 34.1%). **JZ-13** was a mixture of two inseparable diastereomers. $^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.07–7.89 (m, 2H), 7.62–7.37 (m, 3H), 7.35–7.30 (m, 2H), 7.29–7.21 (m, 4H), 7.21–7.10 (m, 3H), 7.02–6.97 (m, 1H), 4.71–4.36 (m, 1H), 1.53–1.40 (m, 3H);$^{13}$C NMR (101 MHz, chloroform-*d*) $\delta$ 150.9 (dd), 144.2 (dd), 135.0 (d), 131.9 (dd), 131.0 (dd), 129.3 (d), 128.6 (d), 128.5, 128.4 (d), 127.2 (d), 126.1 (d, 124.7 (d), 121.4 (t), 52.4 (dd), 25.0 (dd); $^{31}$P NMR (162 MHz, $CDCl_3$) $\delta$ 73.00, 72.87; HRMS (ESI) for $C_{20}H_{21}NOPS$ $[M+H]^+$ calcd. for 354.1076, found 354.1076.

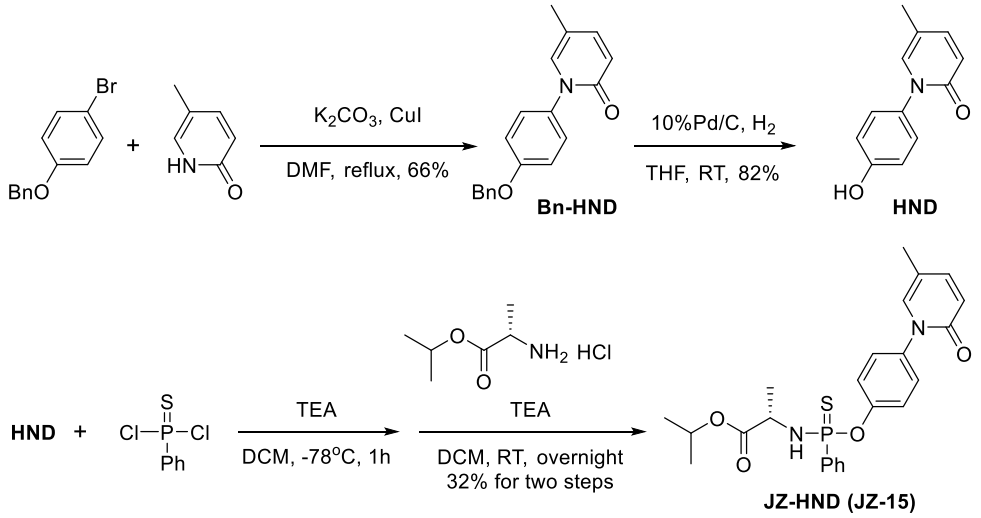
*Isopropyl (((2-oxo-2H-chromen-7-yl)oxy)(phenyl)phosphorothioyl)-L-alaninate* (**JZ-14**)



**JZ-14**

**JZ-14** was prepared from L-alanine isopropyl ester hydrochloride (334 mg, 2 mmol), phenylphosphonothioic dichloride (422 mg, 2 mmol) and 7-hydroxycoumarin (324mg, 2 mmol) following similar synthetic procedures described for **JZ-1**, the crude material was purified by flash chromatography (PE/EA = 20:1) to afford the product **JZ-14** as a colorless oil (280 mg, 32.4%). **JZ-14** was a mixture of two inseparable diastereomers. $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.09–7.88 (m, 2H), 7.67 (d, $J$ = 9.5 Hz, 1H), 7.63–7.47 (m, 3H), 7.43 (d, $J$ = 8.9 Hz, 1H), 7.18 (m, 2H), 6.36 (d, $J$ = 9.5 Hz, 1H), 5.04–4.88 (m, 1H), 4.21–3.99 (m, 2H), 1.36 (m, 3H), 1.24–1.16 (m, 6H); $^{13}$C NMR (126 MHz, chloroform-*d*) $\delta$ 173.0 (dd), 160.4 (d), 154.7 (d), 153.6 (d), 142.9, 134.3 (d), 133.1 (d), 132.5 (d), 132.5 (d), 130.9 (dd), 128.8 (dd), 128.5 (d), 118.6 (dd), 115.9, 115.5, 110.1 (dd), 69.4 (d), 50.8 (d), 21.7 (d), 21.6 (d), 21.2 (d); $^{31}$P NMR (202 MHz, DMSO-$d_6$) $\delta$ 76.99, 75.43; HRMS (ESI) for $C_{21}H_{23}NO_5PS$ $[M+H]^+$ calcd. for 432.1029, found 432.1028.

*Isopropyl ((4-(5-methyl-2-oxopyridin-1(2H)-yl)phenoxy)(phenyl)phosphorothioyl)-L-alaninate* (**JZ-HND**/**JZ-15**)




**Scheme S2.** Synthesis of **HND** and **JZ-HND**

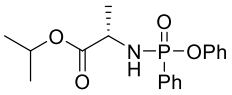
1-(Benzyloxy)-4-bromobenzene (1578 mg, 6.0 mmol) and 5-methylpyridin-2-ol (545 mg, 5.0 mmol) were dissolved in anhydrous DMF (10 mL), to this solution was added anhydrous $K_2CO_3$ (1380 mg, 10 mmol) and CuI (190 mg, 0.10 mmol) under an Ar atmosphere. The reaction was stirred at 160 °C for 8 h. the reaction was diluted with 50 mL saturated $NaHCO_3$ aqueous solution then extracted by EA (100 mL×3), the organic phase was dried over $Na_2SO_4$, filtered and concentrated under vacuum. The crude product was purified by flash chromatography (PE/EA = 1:1) to afford the product **Bn-HND** as a white solid (958 mg, 65.8%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.57–7.26 (m, 9H), 7.15–7.07 (m, 2H), 6.40 (d, $J$ = 9.2 Hz, 1H), 5.17 (s, 2H), 2.04 (s, 3H).

**Bn-HND** (873 mg, 3 mmol) and 10% Pd/C (87.3 mg) were dissolved in THF, the reaction was stirred at room temperature overnight under $H_2$ atmosphere. The reaciton was filtered with celite, and the reaction solution was concentrated under vacuum. The crude product was washed with EA to obtain **HND** as a white solid (4.92 g, 81.5%). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.38 (dd, $J$ = 9.3, 2.5 Hz, 1H), 7.21–7.14 (m, 1H), 7.05–6.96 (m, 2H), 6.73–6.62 (m, 3H), 2.14 (s, 3H).

Phenylphosphonothioic dichloride (422 mg, 2 mmol) was dissolved in anhydrous DCM (5 mL). To this solution was added **HND** (402 mg, 2 mmol) and TEA (304 µL, 2.2 mmol) in anhydrous DCM (2 mL) under an Ar atmosphere at –78 °C. The reaction was stirred at –78 °C for 1 h. L-Alanine isopropyl ester hydrochloride (334 mg, 2 mmol) and TEA (608 µL, 4.4 mmol) in anhydrous DCM (5 mL) was added subsequently. The reaction was stirred overnight at room temperature. The mixture was diluted with DCM (10 mL), washed with water (30 mL), dried over $Na_2SO_4$, and concentrated

under vacuum. The crude material was purified by flash chromatography (DCM /MeOH = 50:1) to afford the product **JZ-HND** as a yellow solid (300 mg, 32%). **JZ-HND** was a mixture of two inseparable diastereomers. $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.08–7.95 (m, 2H), 7.61–7.47 (m, 3H), 7.35–7.27 (m, 4H), 7.26 (s, 1H), 7.12 (s, 1H), 6.64 (d, $J$ = 9.3 Hz, 1H), 5.00 (m, 1H), 4.23–4.06 (m, 1H), 4.00 (t, $J$ = 9.3 Hz, 1H), 2.10 (s, 3H), 1.30 (d, $J$ = 7.0 Hz, 3H), 1.25–1.16 (m, 6H); $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 173.17 (dd), 160.94, 150.15 (dd), 143.57, 137.91 (d), 136.61, 135.93, 134.77, 132.40, 131.14 (d), 131.04 (d), 128.89 (t), 128.23, 122.73 (d), 122.31 (d), 120.68, 114.56, 68.46, 51.12 (d), 21.92 (d), 21.89 (d), 20.17 (dd), 16.80; $^{31}$P NMR (202 MHz, DMSO) $\delta$ 76.56, 74.59; HRMS (ESI) for $C_{24}H_{27}N_2NaO_4PS$ $[M+Na]^+$ calcd. for 493.1321, found 493.1327.

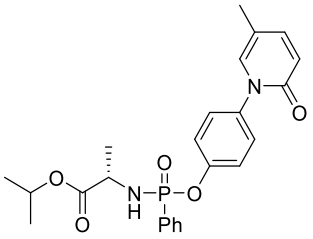
*Isopropyl (phenoxy(phenyl)phosphoryl)-L-alaninate* (**JZ-16**)




**JZ-16**

**JZ-16** was prepared from L-alanine isopropyl ester hydrochloride (501 mg, 3 mmol) and phenyl phosphonic dichloride (585 mg, 3 mmol) following similar synthetic procedures described for **JZ-1**, the crude material was purified by flash chromatography (PE/EA = 20:1) to afford the product **JZ-16** as a colorless oil (460 mg, 44.1%). **JZ-16** was a mixture of two inseparable diastereomers. $^{1}$H NMR (300 MHz, Methanol-$d_4$) $\delta$ 8.03–7.86 (m, 2H), 7.74–7.47 (m, 4H), 7.40–7.14 (m, 4H), 5.39–5.22 (m, 1H), 4.05–3.89 (m, 1H), 1.35–1.25 (m, 3H), 1.25–1.07 (m, 6H); $^{13}$C NMR (126 MHz, methanol-$d_4$) $\delta$ 173.3 (d), 150.6, 132.3 (d), 132.2 (d), 131.3 (d), 129.3, 128.3 (d), 128.1, 124.6 (d), 120.8 (d), 120.5 (d), 68.6, 49.7, 20.5 (d), 19.4 (d), 19.2 (d); $^{31}$P NMR (202 MHz, Methanol-$d_4$) $\delta$ 21.02, 20.30; HRMS (ESI) for $C_{18}H_{22}NNaO_4P$ $[M+Na]^+$ calcd. for 370.1179, found 370.1176.

*Isopropyl ((4-(5-methyl-2-oxopyridin-1(2H)-yl)phenoxy)(phenyl)phosphoryl)-L-alaninate* (**JZ-17**)

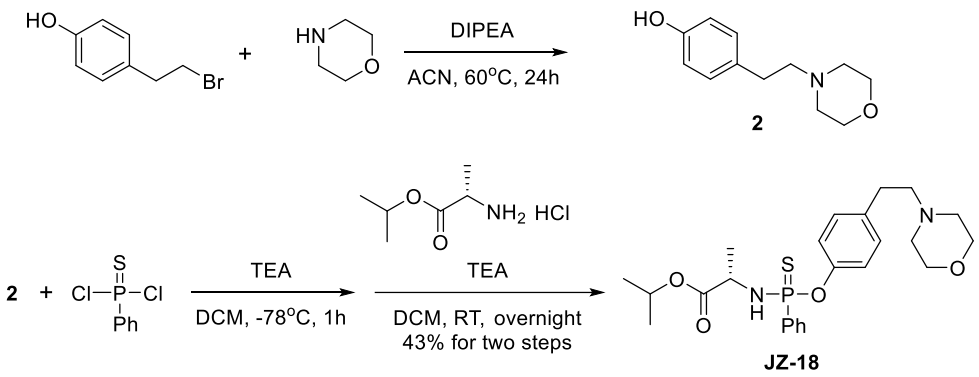



**JZ-17**

**JZ-17** was prepared from L-alanine isopropyl ester hydrochloride (1307 mg, 6.19 mmol) and phenyl phosphonic dichloride (1208 mg, 6.19 mmol) following similar synthetic procedures described for **JZ-**

**HND**, the crude material was purified by flash chromatography (PE/EA = 1:1) to afford the product **JZ-17** as a colorless oil (1270 mg, 45%). **JZ-17** was a mixture of two inseparable diastereomers. $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.00–7.86 (m, 2H), 7.63–7.44 (m, 3H), 7.40–7.30 (m, 4H), 7.26 (d, $J$ = 2.4 Hz, 1H), 7.07 (s, 1H), 6.62 (d, $J$ = 9.2 Hz, 1H), 5.07–4.93 (m, 1H), 4.14–4.00 (m, 1H), 3.85–3.68 (m, 1H), 2.11 (s, 3H), 1.37 (dd, $J$ = 7.0, 2.2 Hz, 3H), 1.21 (dd, $J$ = 6.3, 2.9 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 173.18 (dd), 161.68, 150.48 (dd), 142.75, 137.43 (d), 135.29, 132.59 (d), 131.67 (t), 130.67, 129.27, 128.68 (dd), 127.89 (d), 121.38, 121.34 (dd), 115.16, 69.24 (d), 49.69 (d), 21.69 (d), 21.63, 21.42 (dd), 17.04; $^{31}$P NMR (162 MHz, DMSO) $\delta$ 15.01, 14.44; MS (ESI) for $C_{24}H_{27}N_2NaO_5P$ $[M+Na]^+$ calcd. for 477.1550, found 477.1503.

*Isopropyl ((4-(2-morpholinoethyl)phenoxy)(phenyl)phosphorothioyl)-L-alaninate* (**JZ-18**)



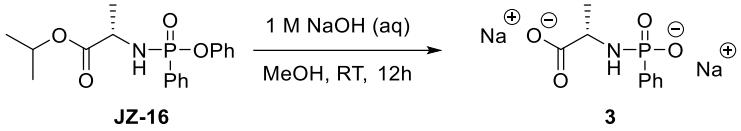

**Scheme S3.** Synthesis of **JZ-18**

4-Hydroxyphenethyl bromide (1000 mg, 5 mmol) was dissolved in acetonitrile (ACN) (20 mL), to this solution was added morpholine (435 mg, 5 mmol) and *N*,*N*-diisopropylethylamine (DIPEA) (870 µL, 5 mmol). This reaction was stirred at 60 °C for 24 h, and then part of the ACN was removed. The residue was cooled to room temperature, the precipitate was filtrated and washed with water, which dried in a vacuum affording compound **2** as a white solid (770 mg, 74.3%). $^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.12–7.08 (m, 2H), 6.81–6.75 (m, 2H), 3.79 (t, $J$ = 4.7 Hz, 4H), 2.81–2.75 (m, 2H), 2.63–2.59 (m, 2H), 2.58–2.52 (m, 4H). The NMR data agreed with those in a literature report[1].

**JZ-18** was prepared from L-alanine isopropyl ester hydrochloride (395 mg, 2.37 mmol) and **2** (490 mg, 2.37 mmol) following similar synthetic procedures described for **JZ-1**, the crude material was purified by flash chromatography (PE/EA = 20:1) to afford the product **JZ-18** as a colorless oil (486 mg, 43.0%). **JZ-18** was a mixture of two inseparable diastereomers. $^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.09–7.97 (m, 2H), 7.63–7.48 (m, 3H), 7.21–7.11 (m, 4H), 5.07–4.93 (m, 1H), 4.21–4.05 (m, 1H), 4.00–3.92 (m, 1H), 3.80 (s, 4H), 2.91–2.78 (m, 2H), 2.75–2.45 (m, 6H), 1.33 (m, 3H), 1.26–1.17 (m, 6H); $^{13}$C NMR (126 MHz, chloroform-*d*) $\delta$ 173.2 (dd, J = 17.2, 6.9 Hz), 149.0 (d), 136.7, 135.2 (d),

134.0 (d), 132.1 (d), 132.0 (d), 130.9 (dd), 129.6 (d), 128.6 (d), 128.5 (d), 121.6 (d), 121.5 (d), 69.2, 69.1, 67.0, 60.7, 53.7, 50.9, 50.8, 32.6, 21.7 (d), 21.6 (d), 21.3 (dd); $^{31}$P NMR (162 MHz, $CDCl_3$) $\delta$ 74.32, 73.69; HRMS (ESI) for $C_{24}H_{34}N_2O_4PS$ $[M+H]^+$ calcd. for 477.1972, found 477.1956.

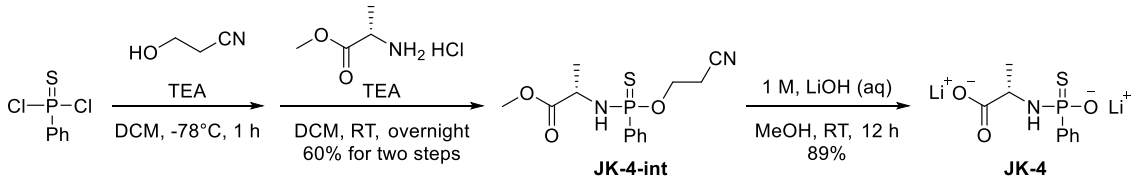
### 1.2 Synthesis of compound 3




**Scheme S4.** Synthesis of compound **3**

**JZ-16** (300 mg, 0.86 mmol) was dissolved in methanol (3 mL). To this mixture was added 1 mol/L NaOH solution (3 mL), the resulting mixture was stirred at room temperature for 12 h and then concentrated to dryness. The resulting solid was suspended in anhydrous ACN and filtered off. The filtrate was concentrated. The final product **3** was obtained as white solid (128 mg, 65.0%). $^1$H NMR (300 MHz, MeOH-$d_4$) $\delta$ 7.69–7.53 (m, 2H), 7.21 (s, 3H), 3.20–3.06 (m, 1H), 0.95 (d, $J$ = 6.7 Hz, 3H); $^{13}$C NMR (126 MHz, $D_2O$) $\delta$ 185.01–182.39 (m), 137.3, 136.1, 130.6 (d), 130.3, 129.9, 128.2 (d), 52.3, 21.9 (d); $^{31}$P NMR (202 MHz, $D_2O$) $\delta$ 16.52; MS (ESI) for $C_9H_{11}NO_4P[M–H]^–$ calcd. for 228.0431, found 228.0415.

### 1.3 Synthesis of JK-4



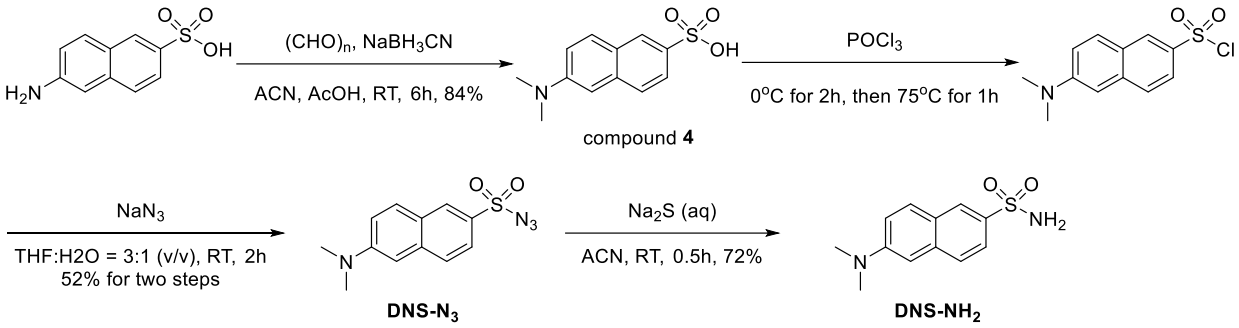

**Scheme S5.** Synthesis of **JK-4**

**JK-4** was prepared following the reported procedure[2]. Phenylphosphonothioic dichloride (633 mg, 3 mmol) was dissolved in anhydrous dichloromethane (DCM) (5 mL). To this solution was added 3-hydroxypropionitrile (213 mg, 3 mmol) and trimethylamine (TEA) (417 µL, 3 mmol) in anhydrous DCM (2 mL) under an Ar atmosphere at –78 °C. The reaction was stirred at –78 °C for 1 h. A solution of L-alanine methyl ester hydrochloride (420 mg, 3 mmol) and TEA (834 µL, 6 mmol) in anhydrous DCM (5 mL) was added, the reaction was stirred at room temperature overnight. The mixture was diluted with DCM (10 mL), washed with water (30 mL), dried over $Na_2SO_4$, and concentrated under vacuum. The crude material was purified by flash chromatography [petroleum ether (PE)/ethyl acetate

(EA) = 30:1] to afford the product **JZ-4-int** as a colorless oil (562 mg, 60.2%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.97–7.86 (m, 2H), 7.61–7.47 (m, 3H), 4.43–4.22 (m, 2H), 4.19–3.91 (m, 1H), 3.85–3.67 (m, 1H), 3.73 (d, $J$ = 34.4 Hz, 3H), 2.88–2.78 (m, 2H), 1.42 (d, $J$ = 7.2 Hz, 1H), 1.35 (d, $J$ = 7.1 Hz, 2H).

**JK-4-int** (100 mg, 0.32 mmol) was dissolved in methanol (3 mL). To this mixture was added 1 mol/L LiOH solution (3 mL), the resulting mixture was stirred at room temperature for 12 h and then concentrated to dryness. The resulting solid was suspended in anhydrous MeOH and filtered off. The filtrate was concentrated. The crude product was washed with ethyl ether to obtain product **JK-4** as white solid (70 mg, 89.1%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.79–7.66 (m, 2H), 7.29–7.13 (m, 3H), 3.20–3.09 (m, 1H), 1.11–1.04 (m, 1H), 0.90 (d, $J$ = 6.9 Hz, 2H); $^{31}$P NMR (121 MHz, MeOD) $\delta$ 55.46.

### 1.4 Synthesis of DNS-$N_3$ and DNS-$NH_2$




**Scheme S6.** Synthesis of **DNS-$N_3$** and **DNS-$NH_2$**

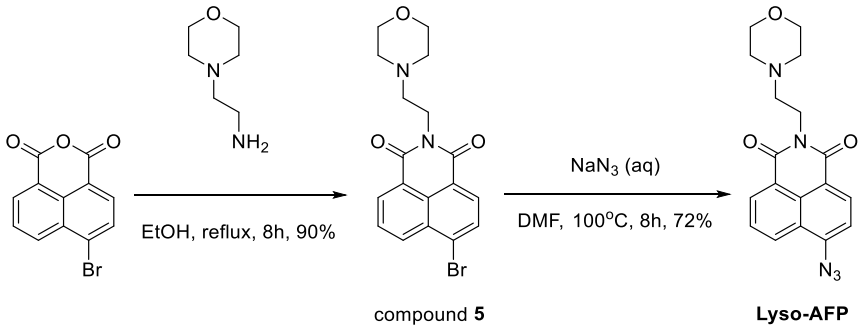
**DNS-$N_3$** was prepared following the reported procedure[3]. 6-Aminonaphthalene-2-sulphonic acid (220 mg, 1 mmol) was dissolved in ACN (3 mL), paraformaldehyde (200 mg, 6 mmol) was added to this solution. After the solution was stirred at room temperature for 10 min, $NaBH_4CN$ (200 mg, 3 mmol) was slowly added. Then, AcOH (1 mL) was added in 4 portions during 4h. The reaction was further stirred at room temperature for 2 h. Then the solution was filtered. The resulting precipitate was washed with water and MeOH and then dried to obtain the compound **4** as a light yellow solid (210 mg, 83.7%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.99 (s, 1H), 7.85 (d, $J$ = 9.1 Hz, 1H), 7.71 – 7.55 (m, 2H), 7.33 (d, $J$ = 9.1 Hz, 1H), 7.16 (s, 1H), 3.07 (s, 6H). The NMR data agreed with those in a literature report[3].

Compound **4** (130 mg, 0.518 mmol) was dissolved in 2 mL $POCl_3$ in ice bath under an Ar atmosphere. The reaction mixture was stirred at 0 °C for 2 h, then heated to 75 °C and stirred for 1 h. The reaction mixture was poured into ice water (10 mL) and the product was extracted with EA (10 mL). The organic phase was dried over $Na_2SO_4$, filtered and concentrated under vacuum. The resulting crude product was dissolved in tetrahydrofuran (THF) (3 mL) and added into a stirred solution of $NaN_3$ (290 mg, 4.5 mmol) in a mixture of THF/$H_2O$ (3 mL, v/v = 1:1). The reaction mixture was stirred at

room temperature for 2 h. After the organic solvent was evaporated, a yellow solid was obtained and purified by flash chromatography purification (PE/EA = 20: 1) to give compound **DNS-N$_3$** as a light yellow solid (74 mg, 51.9% in two steps). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.33 (s, 1H), 7.82 (d, $J$ = 9.1 Hz, 1H), 7.73 (s, 2H), 7.27–7.21 (m, 1H), 6.90 (d, $J$ = 2.6 Hz, 1H), 3.14 (s, 6H); MS (ESI) for $C_{12}H_{13}N_4O_2S$ $[M+H]^+$ calcd. for 277.0754, found 277.0755. The NMR data agreed with those in a literature report[3].

**DNS-N$_3$** (100 mg, 0.36 mmol) was dissolved in ACN (10 mL), to this solution was added aqueous $Na_2S$ solution (260 mg, 3.33 mmol) (2 mL). The reaction was stirred at room temperature for 30 min and the solvent was concentrated under vacuum. The crude product was purified by flash chromatography purification (DCM/MeOH = 50: 1) to give compound **DNS-NH$_2$** (65 mg, 72.2%)**.** $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.18 (s, 1H), 7.95–7.87 (m, 1H), 7.77 (d, $J$ = 7.5 Hz, 1H), 7.69 (s, 1H), 7.35–7.27 (m, 1H), 7.26–7.18 (m, 2H), 6.98 (s, 1H), 3.05 (s, 6H). The NMR data agreed with those in a literature report[3].

### 1.5 Synthesis of Lyso-AFP




**Scheme S7.** Synthesis of **Lyso-AFP**

**Lyso-AFP** was prepared following the reported procedure[3], 4-bromo-1,8-naphthalic anhydride (5 g, 18 mmol) and 4-(2-aminoethyl)-morpholine (5 g, 36 mmol) were dissolved in ethanol (100 mL), and the solution was refluxed for 8 h. After cooling to room temperature, the yellow precipitate was filtered, collected and vacuum dried at room temperature to obtain compound **5** as a yellow solid (6.3 g, 89.9%). A solution of $NaN_3$ (195 mg, 3 mmol) in water (3 mL) was added into the solution of compound **5** (1169 mg, 3 mmol) in *N*,*N*-dimethylformamide (DMF) (20 mL). The reaction mixture was stirred at 100 °C for 8 h. Then the reaction solution was poured into ice water. The yellow precipitate was filtrated and further purified by flash chromatography (DCM/MeOH = 100:1) to afford the probe **Lyso-AFP** as a bright yellow solid (757 mg, 71.8%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.63 (d, $J$ = 6.5 Hz, 1H), 8.59 (s, 1H), 8.45 (d, $J$ = 7.7 Hz, 1H), 7.77–7.72 (m, 1H), 7.47 (d, $J$ = 7.9 Hz, 1H), 4.35 (d, $J$ = 7.5 Hz,

2H), 3.78–3.65 (m, 4H), 2.83–2.71 (m, 2H), 2.71–2.50 (m, 4H), MS (ESI) for $C_{18}H_{18}N_5O_3$ $[M+H]^+$ calcd. for 352.1404, found 352.1422. The NMR data agreed with those in a literature report[4].

## 2. Validation of $H_2S$ release

### 2.1 Calibration curves for $H_2S$ determination

The stock solution of probe **DNS-N$_3$** in ACN (1 mmol/L) and $Na_2S$ in purified water (1 mmol/L) was freshly prepared. The stock solution of probe (10 μL) and 0, 1, 2, 3, 6, 8, 10 μmol/L of $Na_2S$ solution (10 μL) were added sequentially to 980 μL of TBS buffer (10 mmol/L, pH 7.4, 37 °C). The resulting solution was vortexed for 5 min, 100 μL of reaction solution was transferred into a black 96-well plate containing 100 μL of ACN. The fluorescence was measured in a plate reader (BioTek SYNERGY-H1 multi-mode reader, Winooski, VT) ($E_x$ = 325 nm, $E_m$ = 450 nm). The calibration curve was obtained by fitting the linear regression to a set of fluorescence intensities against the indicated concentrations of $Na_2S$ (see **Fig. S1**). The above assays were repeated in triplicate and recorded as the mean ± SD from three experiments.

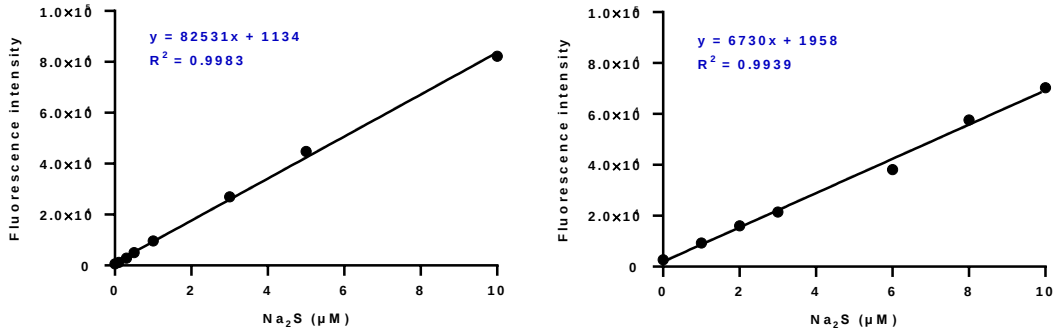



**Figure S1.** Representative calibration curves of $H_2S$ using probe **DNS-N$_3$** (10 μmol/L).

### 2.2 Enzyme-catalyzed $H_2S$ release

The stock solution of probe **DNS-N$_3$** in ACN (1 mmol/L) and stock solution of donor **JZ**s in MeOH (500 μmol/L) were freshly prepared. The stock solution of *carboxypeptidase Y* (CPY, C3888, Sigma–Aldrich) was prepared in deionized water (6 μg/mL) and the stock solution of recombinant human *cathepsin A* (CTSA, Cl11, Novoprotein, Suzhou, China) was prepared in deionized water (25 μg/mL). CTSA was activated with the method similar to that from a previous report[5]. 10 μL of stock solution

of the donor **JZ**s and 10 μL of stock solution of probe were added sequentially to a mixture containing 970 μL of TBS buffer (10 mmol/L, pH = 7.4) and 10 μL of stock solution of enzyme. (The final concentration were 60 ng/mL for CPY and 250 ng/mL for CTSA). After incubation of the reaction solution for indicated time (0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10 h) at 37 °C, 100 μL of reaction solution was transferred into black 96-well plate containing 100 μL of ACN ($n$=3). The fluorescence intensity was measured ($E_x$ = 325 nm, $E_m$ = 450 nm). $H_2S$ concentration was calculated based on the above mentioned calibration curves (Fig. S1). In the control group, the procedure was the same as described previously, only the donor solution was replaced with blank methanol. All experimental results were obtained by subtracting the background of the control group from the fluorescence values of the experimental group. The above assays were repeated in triplicate and reported as the mean ± SD of three experiments. The results are shown in **Figs. 2A** and **8B**.

### 2.3 $H_2S$ released within the tissue homogenates

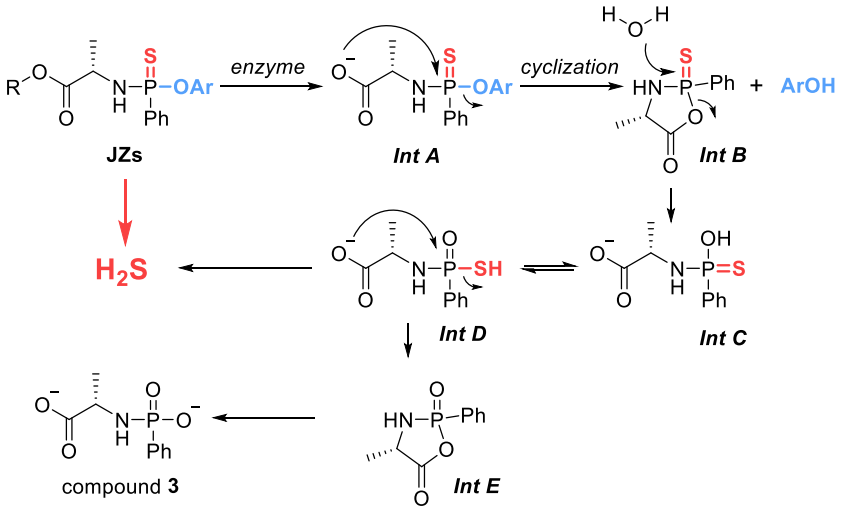
The *in vitro* $H_2S$ release capacity of donor **JZs** was investigated using homogenized liver and homogenized intestine. Liver homogenates (0.1 g/mL) were prepared from liver of SD rats in PBS buffer (10 mmol/L, pH = 7.4). Preparation of intestine homogenates (0.1 g/mL) from intestine of SD rats in PBS buffer (10 mmol/L, pH = 7.4). The stock solution of donors was prepared in MeOH (10 mmol/L) and the stock solution of probe were prepared in ACN (500 μmol/L). 25 μL of donor stock solution was added to 975 μL of liver homogenates or intestine homogenates at 37 °C. After incubation for the indicated time (at 1, 10, 20, 30, 40 min), 25 μL of reaction solution was mixed with 25 μL of probe solution (1 mmol/L) and vortexed for 5 min. The resulting mixed solution was diluted 100 times with ACN and added to a black 96-well plate. The fluorescence intensity was measured ($E_x$ = 325 nm, $E_m$ = 450 nm) using a plate reader (BioTek SYNERGY-H1 multi-mode reader, Winooski, VT, USA). In the control group, the procedure was the same as described previously, only the donor solution was replaced with blank methanol. All experimental results were obtained by subtracting the background of the control group from the fluorescence values of the experimental group. The above assays were repeated in triplicate and reported as the mean ± SD of three experiments. The Bicinchoninic Acid (BCA) protein assay kit (P0012S, Beyotime, China) was used for the quantification of total protein in homogenates. The results are shown in **Fig. 3**.

## 3. Validation of the pathway of the $H_2S$ donor activation

### 3.1 Proposed mechanism for $H_2S$ release

The donors is cleaved by CPY or CTSA to give metabolic intermediate A (**Int A**). The resulting carboxylic acid anion attacks the phosphorus, releasing the phenol moiety. The obtained **Int B** is similar as a mixed anhydride which is readily hydrolyzed via circle opening. Then **Int C** undergoes a tautomeric transformation. The resulting important intermediate **Int D** undergoes spontaneous cyclization, like **Int A** to **Int B**, yielding $H_2S$ and **Int E**. Finally, **Int E** is converted to **3** via hydrolysis, and the released $H_2S$ can be trapped by the probe **DNS-N$_3$** to give **DNS-NH$_2$** (Scheme S8).

Based on the above mechanism, donor **JZ-7** was used to characterize the kinetics of the phenolic group leaving inferred with the aid of tracing the fluorescence signal produced by 7-hydroxycoumarin (Section 3.2). The donor **JZ-6**, compound **3** and **DNS-NH$_2$** are used to describe the kinetic characteristics of donor degradation and $H_2S$ formation in the following LC–MS/MS assay (Section 3.3).




**Scheme S8**. Proposed mechanism for $H_2S$ release.

### 3.2 Measurement of 7-hydroxycoumarin released from JZ-14

The stock solution of **JZ-14** in MeOH (500 μmol/L) was freshly prepared, **CPY** stock solution was prepared in deionized water with different concentration (0.6, 6, 60 μg/mL). 10 μL of **JZ-14** stock solution was added to a mixture containing 980 μL of TBS buffer (10 mmol/L, pH = 7.4) and 10 μL of different concentration of **CPY** stock solution (final concentration = 6, 60, 600 ng/mL). At 1, 2, 3, 4, 5, 10, 30, 60, 90, 180, 240, 300, 360 min, 20 μL of reaction solution was added to a black 96-well plate containing 180 μL ACN per well (*n*=3). In the control group, the procedure was the same as

above mentioned, but without the addition of CPY solution. In the control group, the procedure was the same as described previously, only the donor solution was replaced with blank methanol. All experimental results were obtained by subtracting the background of the control group from the fluorescence values of the experimental group. The above assays were repeated in triplicate and reported as the mean ± SD of three experiments. The results are shown in **Fig. S2A**.

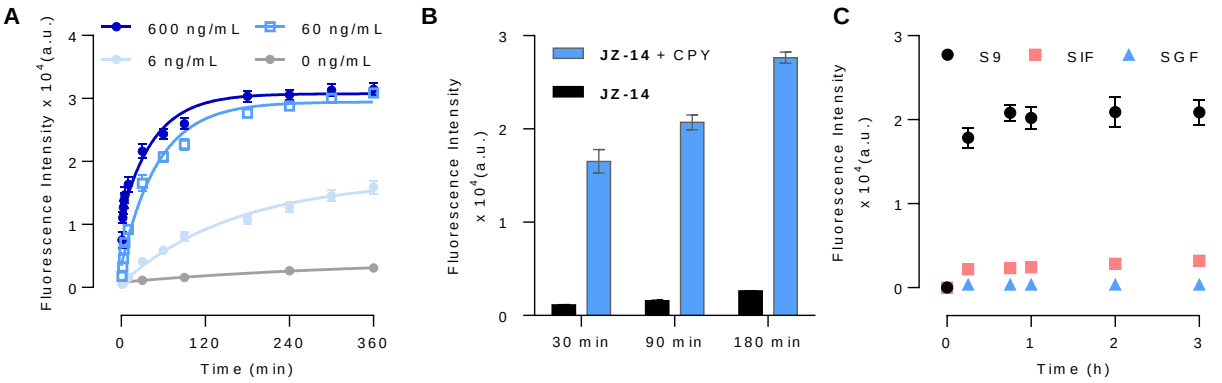



**Figure S2.** (A) 7-hydroxycoumarin (7-HC) release from **JZ-14**. Reaction conditions: **JZ-14** (5 μmol/L), CPY (6, 60, 600 ng/mL) in TBS (with 1% menthol, pH 7.4, 37 °C), followed by fluorescence intensity measurement ($\lambda_{ex}/\lambda_{em}$ = 350/455 nm). $k$ = 0.0189 $min^{-1}$, $t_{1/2}$ = 36.7 min (CPY = 60 ng/mL). (B) Stability of the donor **JZ-14** under different conditions.

The addition of CPY (60 ng/mL) to TBS buffer was able to increase the fluorescence intensity by 10 to 15-fold, suggesting that **JZ-7** is relatively stable in the buffer and its activation is CPY dependent (Fig. S2B). Stability in different biological matrices is an important factor for the druggability of prodrugs. **JZ-7** has desirable stability in both simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), while it can release 7-hydroxycoumarin in rat liver S9 (Fig. S2C), indirectly responding to intrahepatic specific activation of the donor **JZs**.

### 3.3 $H_2S$ donor activation monitored by LC–MS/MS

(1) HPLC condition

The study was carried out on a Shimadzu LC-20A HPLC system (Kyoto Japan). HPLC autosampler temperature: 4 °C. Injection volume: 5 μL. The chromatographic separation was performed on Waters XSelect HSS T3 column (3.5 μm, 4.6 mm×150 mm) with a flow rate of 0.6 mL/min at 40 °C. A gradient elution program was conducted with mobile phase A (0.1% formic acid for positive ionization mode or 0.01% ammonia for negative ionization mode) and mobile phase B (methanol) shown in the following **Tables S1 and S2**.

(2) MS condition

The compounds were analyzed by a LC–MS/MS system containing a Shimadzu HPLC system (Kyoto, Japan) coupled with a SCIEX API 4000 triple-quadrupole mass spectrometer (Foster City, CA, USA) equipped with an electrospray ionization (ESI) interface in negative ionization mode or positive ionization mode. Analyst software version 1.5.1 (SCIEX) was used for data acquisition and analysis. MRM parameters: Selected Multiple Reaction Monitoring (MRM) was conducted for the quantification analysis in the mass analyzers. The declustering potential (DP) and collision energy (CE) were optimized as listed in above **Table S3**.

Ion source parameters: The pressure of collision gas, curtain gas, ion source gas 1 and ion source gas 2 were 12, 30, 65 and 70 psi, respectively. The ion source temperature was maintained at 550 °C. The optimized ion spray voltage was –4500 V and dwell time of 100 ms for the analytes and IS applied.

**Table S1.** Gradient elution program in positive ionization mode.

| Time (min) | Flow rate (mL/min) | Phase B (%) |
|---|---|---|
| 0.00 | 0.6 | 10 |
| 2.00 | 0.6 | 10 |
| 3.00 | 0.6 | 60 |
| 6.00 | 0.6 | 60 |
| 7.00 | 0.6 | 95 |
| 13.00 | 0.6 | 95 |
| 13.50 | 0.6 | 10 |
| 16.00 | 0.6 | 10 |
| 16.01 | 0.6 | Stop |

**Table S2.** Gradient elution program in negative ionization mode.

| Time (min) | Flow rate (mL/min) | Phase B (%) |
|---|---|---|
| 0.01 | 0.6 | 5 |
| 1.00 | 0.6 | 5 |
| 2.00 | 0.6 | 60 |
| 4.00 | 0.6 | 60 |
| 5.00 | 0.6 | 95 |
| 7.00 | 0.6 | 95 |
| 7.50 | 0.6 | 5 |
| 10.50 | 0.6 | 5 |
| 10.51 | 0.6 | Stop |

**Table S3.** MRM parameters for LC–MS/MS

| Prodrug | ESI mode | MRM transitions (m/z) | Declustering Potential (V) | Collision Energy (eV) |
|---|---|---|---|---|
| **JZ-3** | – | 334.0/93.0 | –73 | –36 |
| **JZ-6** | – | 362.0/93.1 | –28 | –39 |
| **JZ-18** | – | 475.3/206.1 | –95 | –35 |
| **3** | – | 228.0/88.1 | –20 | –28 |
| **JK-4** | – | 243.9/209.8[a] | –80 | –33 |
| | | 241.9/181.9[b] | –35 | –25 |
| **4-MU**[a] | – | 174.8/133.0 | –42 | –36 |
| **JZ-HND** | + | 471.3/202.3 | 110 | 22 |
| **HND** | + | 201.8/64.9 | 90 | 45 |
| **DNS-$NH_2$** | + | 251.0/235.9 | 60 | 33 |
| **DNS-$N_3$** | + | 277.3/185.0 | 20 | 18 |
| **4-MU**[c] | + | 177.0/103.0 | 18 | 36 |

[a] Easily changeable Q1/Q3.
[b] For quantification.
[c] 4-Methylumbelliferone (4-MU), internal standard (IS).

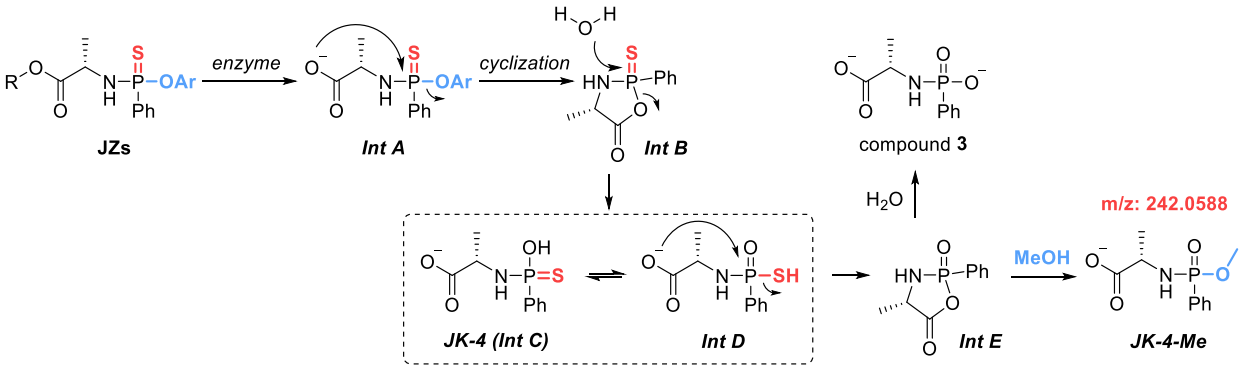
(3) Calibration curves

Calibration curves for each sample or standard were obtained as plots of relative intensities (ratios of analytes and IS peak areas) by linear regression using a weighting factor of the reciprocal of the relative concentration ($1/x^2$) versus square of concentration. See **Table S4**.

**Table S4.** Linearity of representative calibration curves.

| Compound | Mean linear relationship | Regression coefficient (*r*) |
|---|---|---|
| **JZ-3** | $y = 0.00225\,x + 0.0759$ | 0.9925 |
| **JZ-6** | $y = 0.00133\,x - 0.0156$ | 0.9960 |
| **JZ-18** | $y = 0.00249\,x + 0.028$ | 0.9940 |
| **3** | $y = 0.0000264\,x - 0.00537$ | 0.9964 |
| **JK-4** | $y = 0.211\,x + 0.00104$ | 0.9916 |
| **JZ-HND** | $y = 1.38\,x + 0.0221$ | 0.9906 |
| **HND** | $y = 0.863\,x + 0.0189$ | 0.9951 |
| **DNS-$NH_2$** | $y = 0.00196\,x + 0.00984$ | 0.9928 |
| **DNS-$N_3$** | $y = 0.00391\,x - 0.0468$ | 0.9958 |

(4) **JK-4** detection




**Scheme S9**. Generation and transformation of **JK-4** as an intermediate.

The results of our experiments demonstrated that **JK-4** (**Int-C**) exhibited a high degree of instability. Nevertheless, we discovered that it could be derivatized with methanol for the purpose of quantification. The rationale and practicality of this method were verified. As documented in the literature[2], **JK-4** can be rapidly hydrolyzed in aqueous solution, resulting in the production of compound **3**. Similarly, the use of methanol for the extraction of samples after enzymatic reactions results in the rapid alcoholization of **JK-4**, producing **JK-4-Me** (Scheme S9).

Following the synthesis of **JK-4**, the conversion of **JK-4** (5 mg) to **JK-4-Me** in MeOD (0.6 mL) was observed in real time using NMR spectroscopy (Fig. S3). Subsequently, the methanol extraction of the sample in a buffer system was simulated, demonstrating that **JK-4** can be derivatized by methanol to form **JK-4-Me** during the LC–MS/MS experiment (Fig. S4). This process was found to be rapid and met the current preliminary quantitative-quantitative needs. **JK-4**: $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.79–7.66 (m, 2H), 7.29–7.13 (m, 3H), 3.20–3.09 (m, 1H), 1.11–1.04 (m, 1H), 0.90 (d, $J$ = 6.9 Hz, 2H); **$^{31}$P NMR (121 MHz, MeOD) $\delta$ 55.46. JK-4-Me**: $^{1}$H NMR (300 MHz, Methanol-$d_4$) δ 7.80 – 7.70 (m, 2H), 7.54 – 7.39 (m, 3H), 3.63 – 3.53 (m, 1H), 3.54 – 3.40 (m, 1H), 3.30 (s, 3H), 1.26 (d, $J$ = 7.0 Hz, 3H).**$^{31}$P NMR (121 MHz, MeOD) $\delta$ 25.16, 25.04.** $C_{10}H_{13}NO_4PS$ $[M–H]^-$ calcd. for 242.0588, found 242.0586.

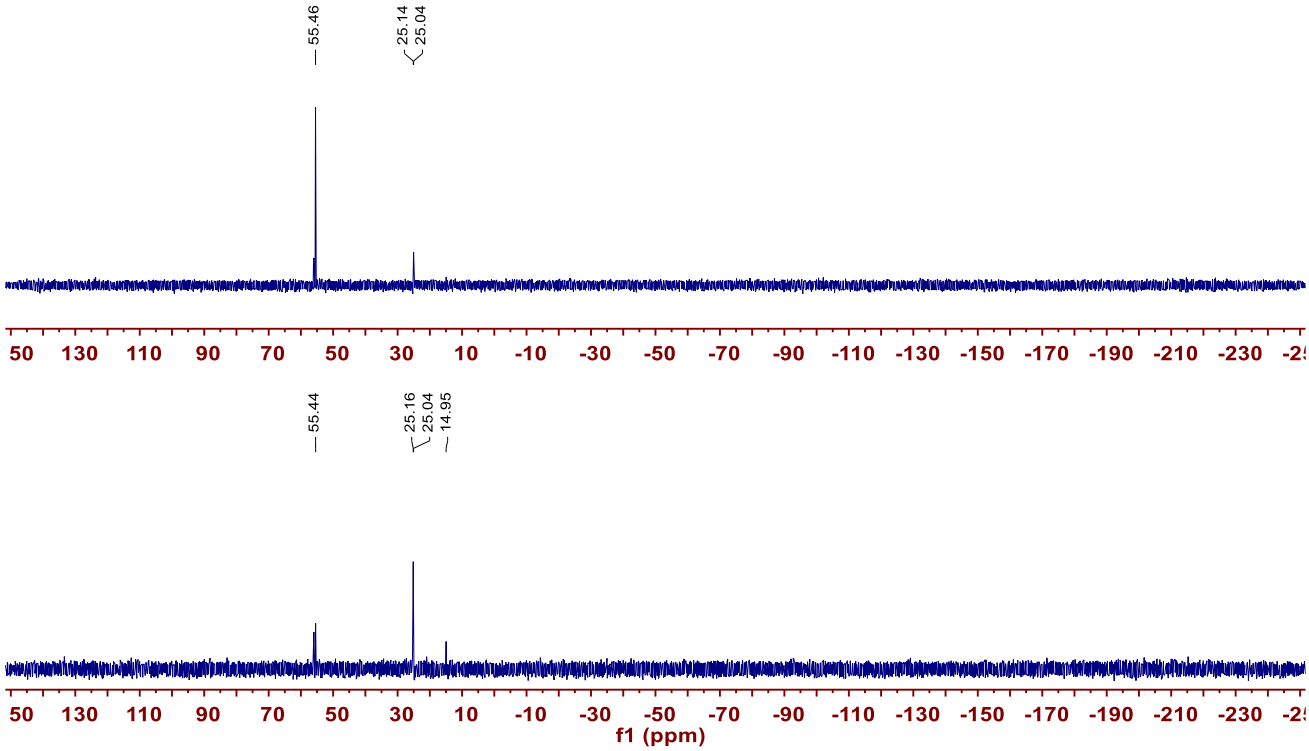



**Figure S3.** Conversion of **JK-4** to **JK-4-Me** by $^{31}$P NMR.

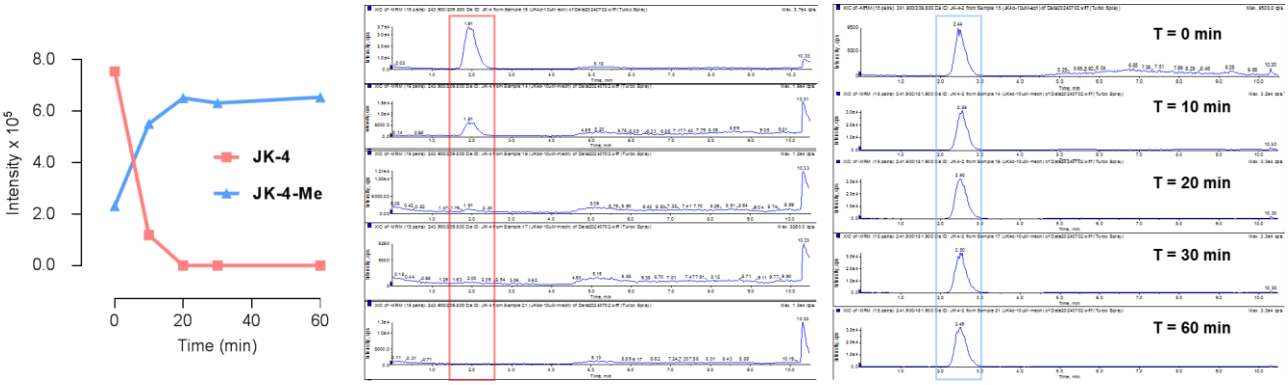



**Figure S4.** Conversion of **JK-4** to **JK-4-Me** by LC–MS$^2$. LC–MS/MS chromatogram of **JK-4** (red box) and **JK-4-Me** (blue box). **JK-4**: $t_R$= 1.91 min, **JK-4-Me**: $t_R$= 2.50 min.

(5) Reaction solution

CPY/CTSA assay: The stock solution of probe (**DNS-N$_3$**) in ACN (1 mmol/L) and each donor solution in MeOH (500 μmol/L) was freshly prepared. The stock solution of **JK-4** in DMSO (20 mmol/L) was freshly prepared. **CTSA** (12.5 μg/mL) or **CPY** (6 μg/mL) stock solution was prepared in deionized water. CTSA was activated according to the previous reported method[5]. 20 μL of donor solution (final concentration = 5 μmol/L) and 20 μL of probe stock solution (final concentration = 10 μmol/L) were added into 1940 μL of TBS buffer (10 mmol/L, pH = 7.4) with 20 μL of CPY stock solution (60 ng/mL) or 10 μL of CTSA stock solution (125 ng/mL) at 37 °C. At 0, 0.5, 1, 2, 4, 6, 8, 12, 24 h, 50 μL reaction solution was added into 200 μL MeOH containing 200 ng/mL of 4-MU (IS). The mixture was centrifuged twice at 18,000 rpm at 4 °C for 5 min to remove invisible impurities, and 80 μL of supernatant was used as sample for LC–MS/MS. The concentration was calculated based on calibration curve. This assay was repeated in triplicate and recorded as the mean ± SD ($n$=3).

Tissue homogenates: The *in vitro* degradation of JZ-6 was investigated in intestine and liver homogenates. Preparation of liver homogenates (0.1 g/mL) from liver of SD rats in PBS buffer (10 mmol/L, pH = 7.4). Preparation of intestine homogenates (0.1 g/mL) from intestine of SD rats in PBS buffer (10 mmol/L, pH = 7.4). JZ-6 stock solution (500 μmol/L) in MeOH was prepared. 20 μL of JZ-6 stock solution (final concentration = 5 μmol/L) was added into 1980 μL of different tissue homogenates at 37 °C. At 1, 5, 10, 20, 40, 60 min, 50 μL reaction solution was added into 200 μL ACN containing 200 ng/mL of 4-MU (IS). The mixture was centrifuged twice at 18,000 rpm at 4 °C for 5 min to remove invisible impurities, and 80 μL of supernatant was used as sample for LC–MS/MS. The concentration was calculated based on calibration curve. This assay was repeated in triplicate and

recorded as the mean ± SD from three experiments. The Bicinchoninic Acid (BCA) protein assay kit (P0012S, Beyotime, China) was used for the quantification of total protein in homogenates.

TBS buffer: The stock solution of each donor solution in MeOH (500 μmol/L) was freshly prepared. 20 μL of donor solution (final concentration = 5 μmol/L) added into 1980 μL of TBS buffer (10 mmol/L, pH = 7.4) 37 °C. At 1, 5, 10, 20, 40, 60, 120, 240, 480, 1440 min, 50 μL reaction solution was added into 200 μL ACN containing 200 ng/mL of 4-MU (IS). The mixture was centrifuged twice at 18,000 rpm at 4 °C for 5 min to remove invisible impurities, and 80 μL of supernatant was used as sample for LC–MS/MS. The concentration was calculated based on calibration curve. This assay was repeated in triplicate and recorded as the mean ± SD (*n*=3).

(6) Supplementary results

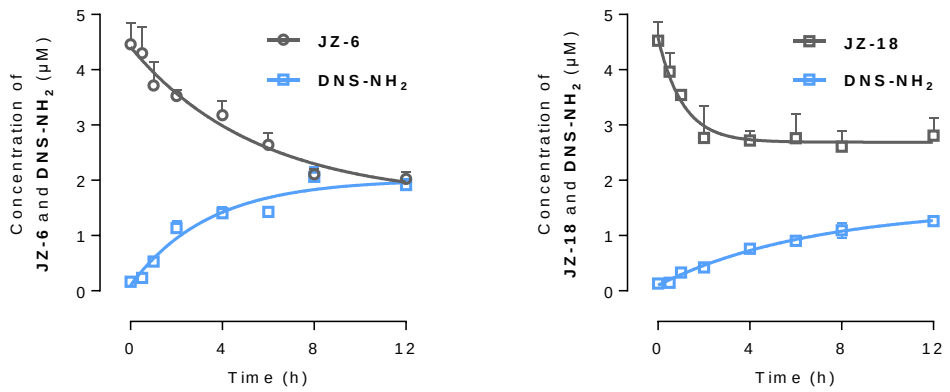



**Figure S5.** CTSA-catalyzed $H_2S$ release of **JZ-6** and **JZ-18**. Generation of **DNS-NH2** after donors (5 μmol/L) activation by CTSA (250 ng/mL) in TBS (with 1% menthol, pH 7.4, 37 °C), followed by LC–MS/MS quantification. Degradation of **JZ-6**: $k$ = 0.1816 $h^{-1}$, $t_{1/2}$ = 3.8 h, generation of **DNS-NH2**: $k$ = 0.2929 $h^{-1}$, $t_{1/2}$ = 2.4 h; degradation of **JZ-18**: $k$ = 0.9296 $h^{-1}$, $t_{1/2}$ = 0.75 h, generation of **DNS-NH2**: $k$ = 0.1471 $h^{-1}$, $t_{1/2}$ = 4.7 h. Data represent the average ± SD ($n$ = 3). The half-life is calculated from the equation, $t_{1/2}$= 0.693/$k$, where $k$ (the rate constant) is obtained by curve fitting with a single-exponential function.

The study describes the kinetic changes of **DNS-NH2** generation and the degradation of **JZ-6** (5 μmol/L) by CTSA (250 ng/mL) in the reaction system using LC–MS/MS. The results showed that **JZ-6** was gradually degraded in the reaction system. Along with the degradation, **DNS-NH2** gradually accumulated. **JZ-18** exhibited a distinct kinetic profile with rapid degradation followed by a plateau phase (Fig. S5).

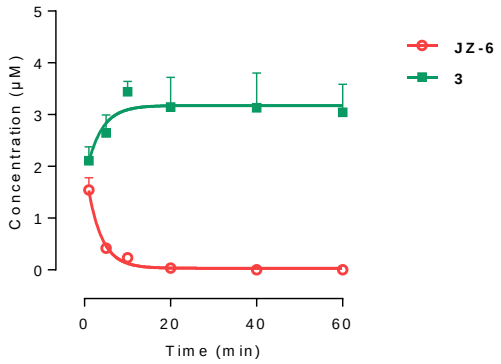



**Figure S6**. Degradation of donor **JZ-6** and generation of compound **3** within liver homogenates.

The donor **JZ-6** was rapidly degraded within the homogenate and minimized at 20 min (Fig. S6). At the same time, we detected the generation of compound **3**, a key metabolic intermediate, suggesting that the donor is also likely to complete activation within the homogenate as expected. More notably, compound **3** accumulated to its highest concentration at 20 min, a peak time that coincided with the peak time of $H_2S$ in the liver homogenate.

The results of LC–MS/MS assay are shown in **Figs. 2C**, **4**, **S4**–**S6**. The representative LC–MS/MS chromatograms of **JZ-6**, **3** and **DNS-NH2** was shown in **Fig. S7** (CPY-catalyzed reaction) and **Fig. S8** (CTSA-catalyzed reaction). The representative LC–MS/MS chromatogram of **JZ-HND**, **3** and **HND** was shown in **Fig. S9** (CPY-catalyzed reaction). The LC–MS/MS chromatograms of **JZ-18**, **3** and **DNS-NH2** are shown in **Fig. S10** (CTSA-catalyzed reaction). The LC–MS/MS chromatogram of **JZ-6** degradation in liver homogenates is shown in **Fig. S11**.

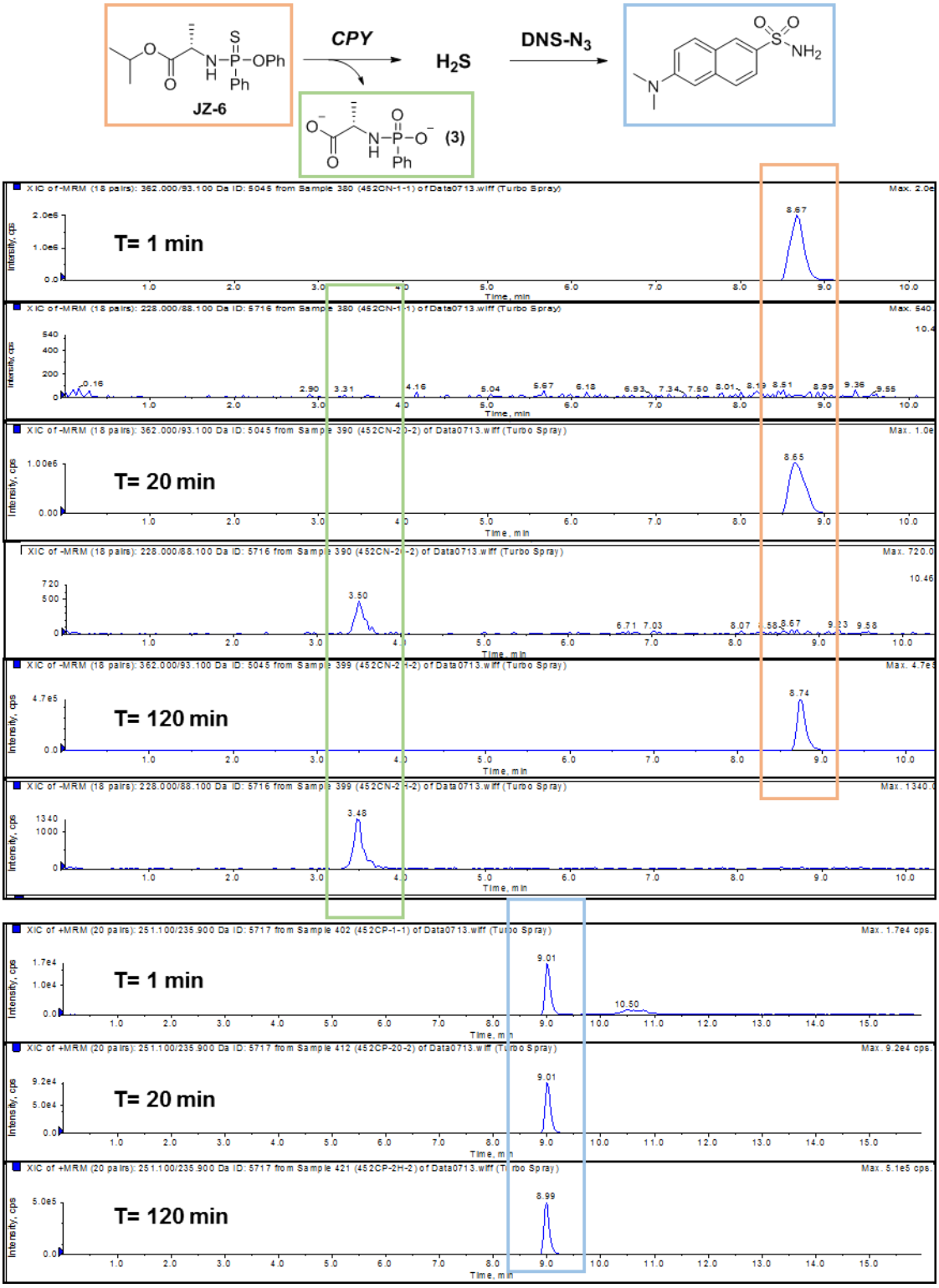



**Figure S7**. LC–MS/MS chromatogram of **JZ-6** (orange box), **3** (green box) and **DNS-NH2** (blue box) during CPY (60 ng/mL)-mediated degradation of the donor. **JZ-6**: $t_R$= 8.65 min, **3**: $t_R$= 3.50 min, **DNS-NH2**: $t_R$= 9.00 min.

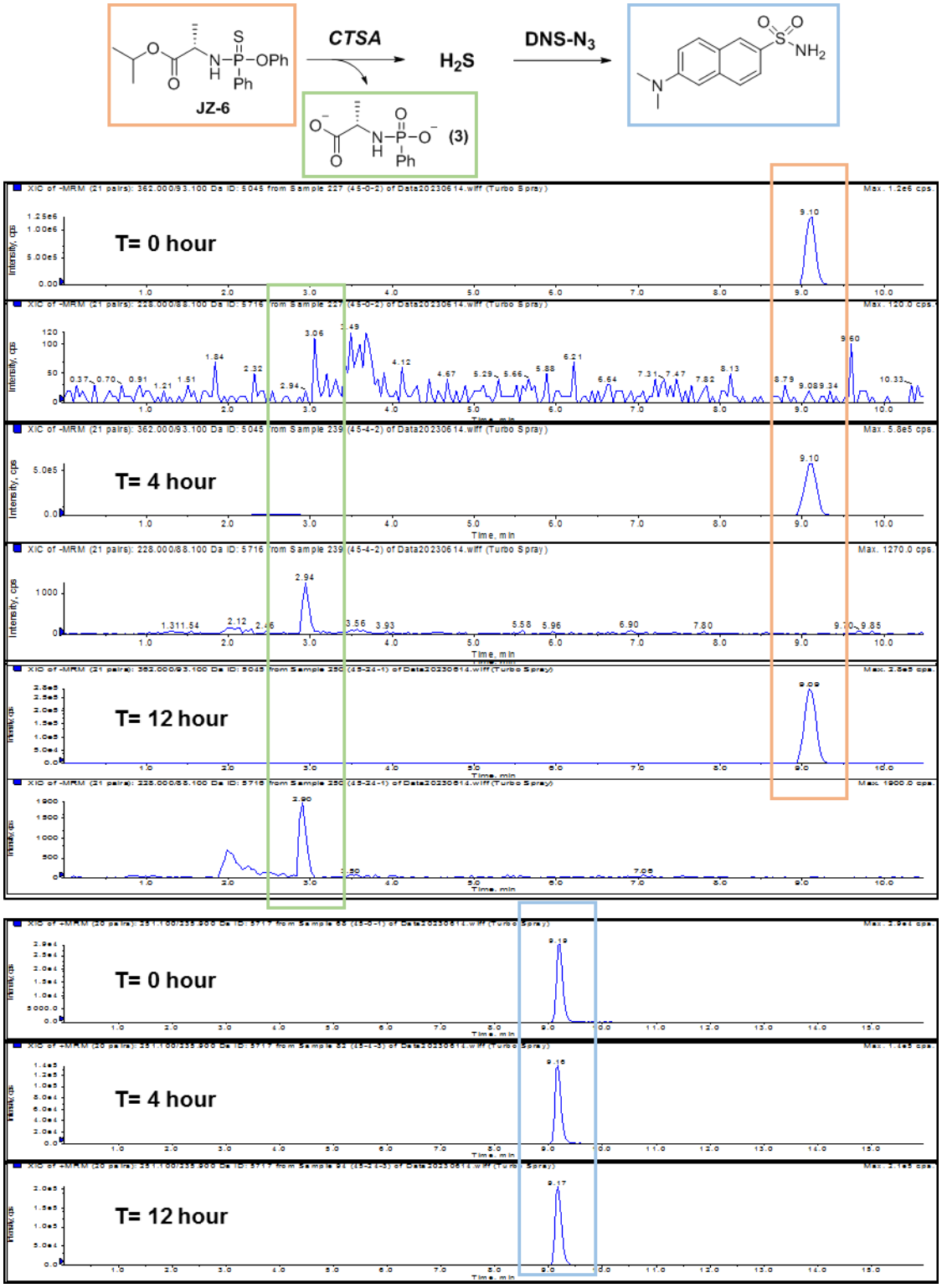



**Figure S8**. LC–MS/MS chromatogram of **JZ-6** (orange box), **3** (green box) and **DNS-NH2** (blue box) during CTSA (125 ng/mL)-mediated degradation of the donor. **JZ-6**: $t_R$= 8.65 min, **3**: $t_R$= 3.00 min, **DNS-NH2**: $t_R$= 9.17 min.

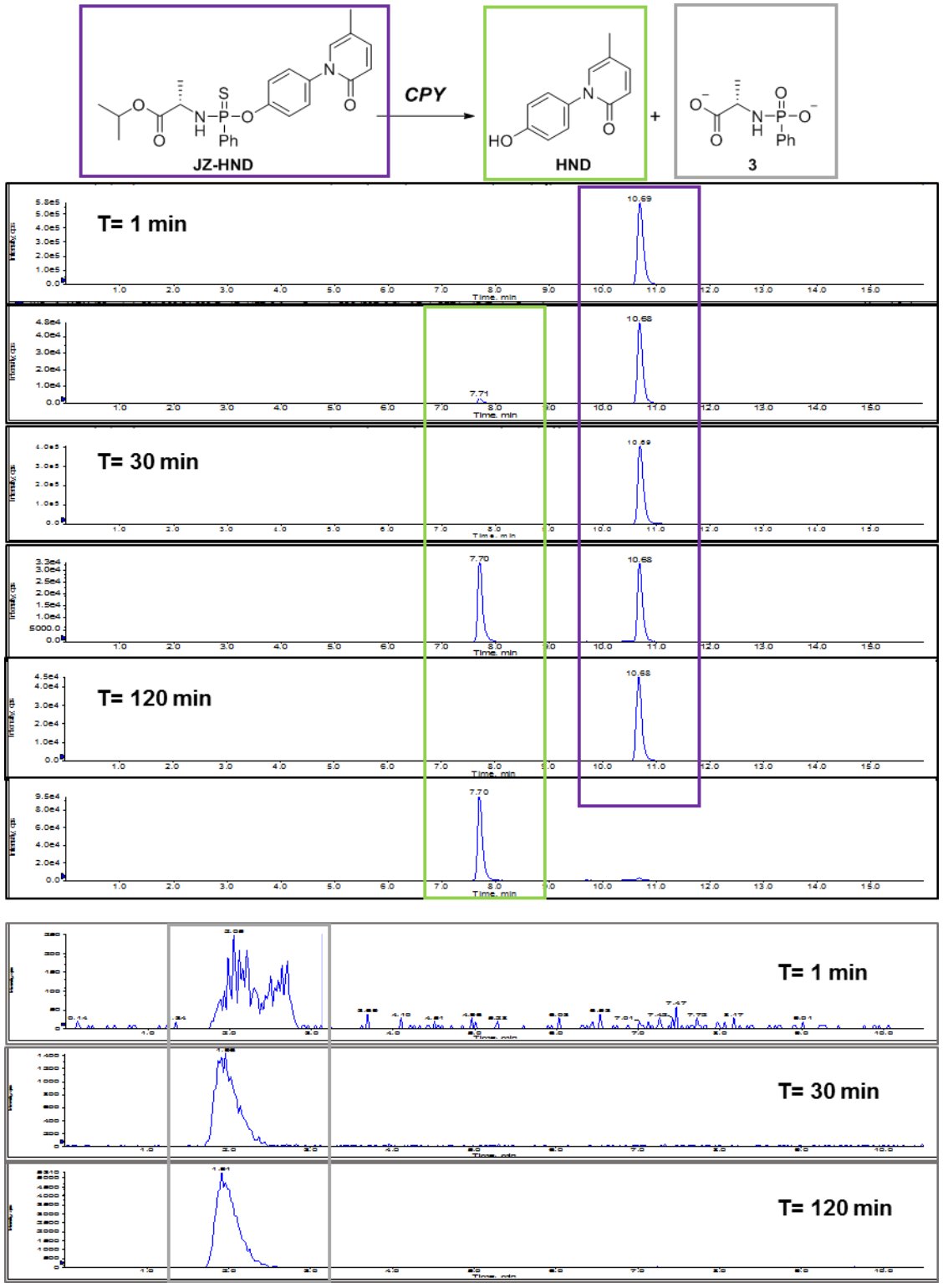



**Figure S9**. LC–MS/MS chromatogram of **JZ-HND** (purple box), **HND** (green box) and **3** (gray box) during CPY (60 ng/mL)-mediated degradation of the donor. **JZ-HND**: $t_R$= 10.68 min, **HND**: $t_R$= 7.70 min, **3**: $t_R$= 1.95 min.

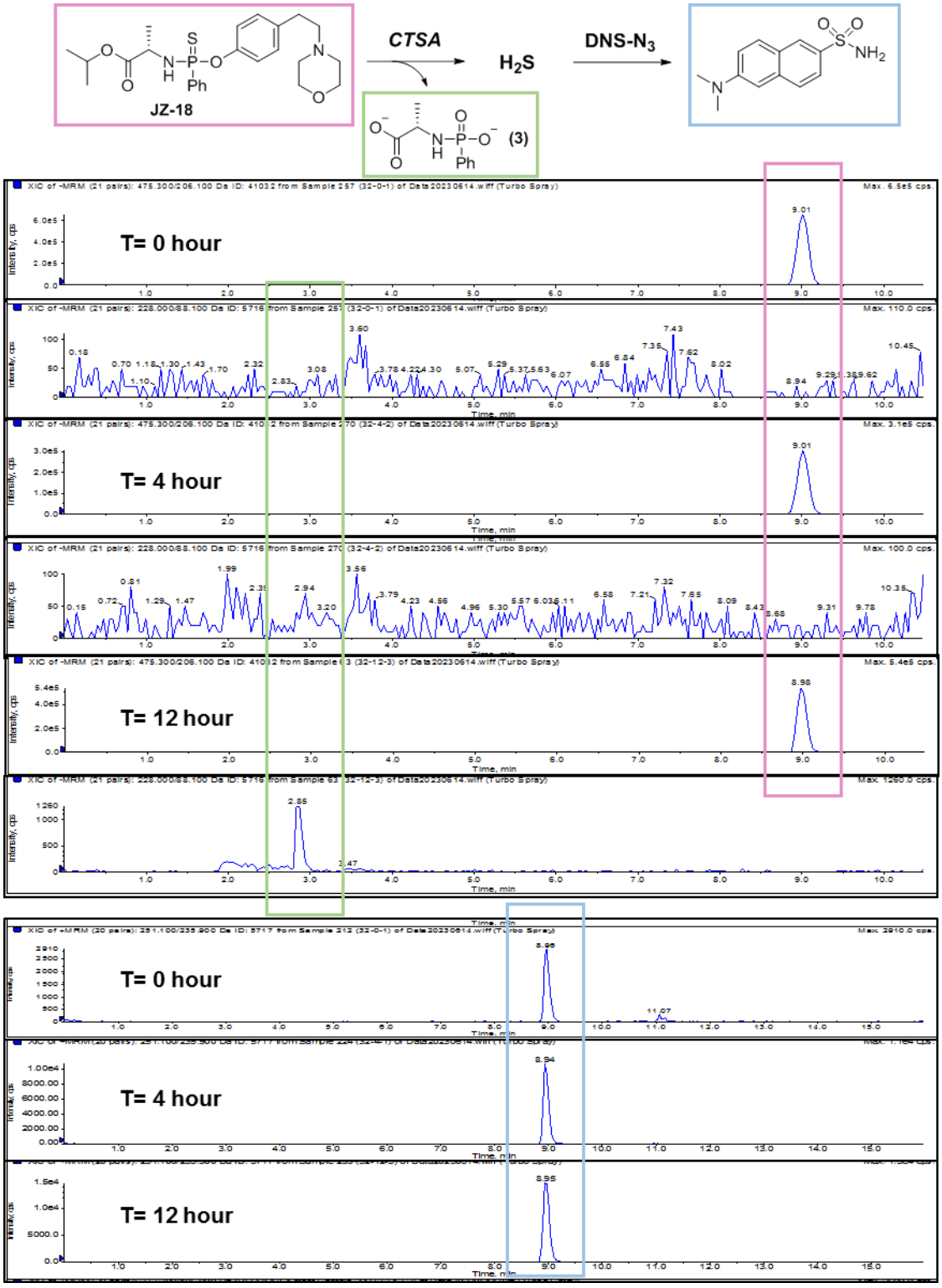



**Figure S10**. LC–MS/MS chromatogram of **JZ-18** (pink box), **3** (green box) and **DNS-NH2** (blue box) during CTSA (125 ng/mL)-mediated degradation of the donor. **JZ-18**: $t_R$= 8.99 min, **3**: $t_R$= 2.85 min, **DNS-NH2**: $t_R$= 9.00 min.

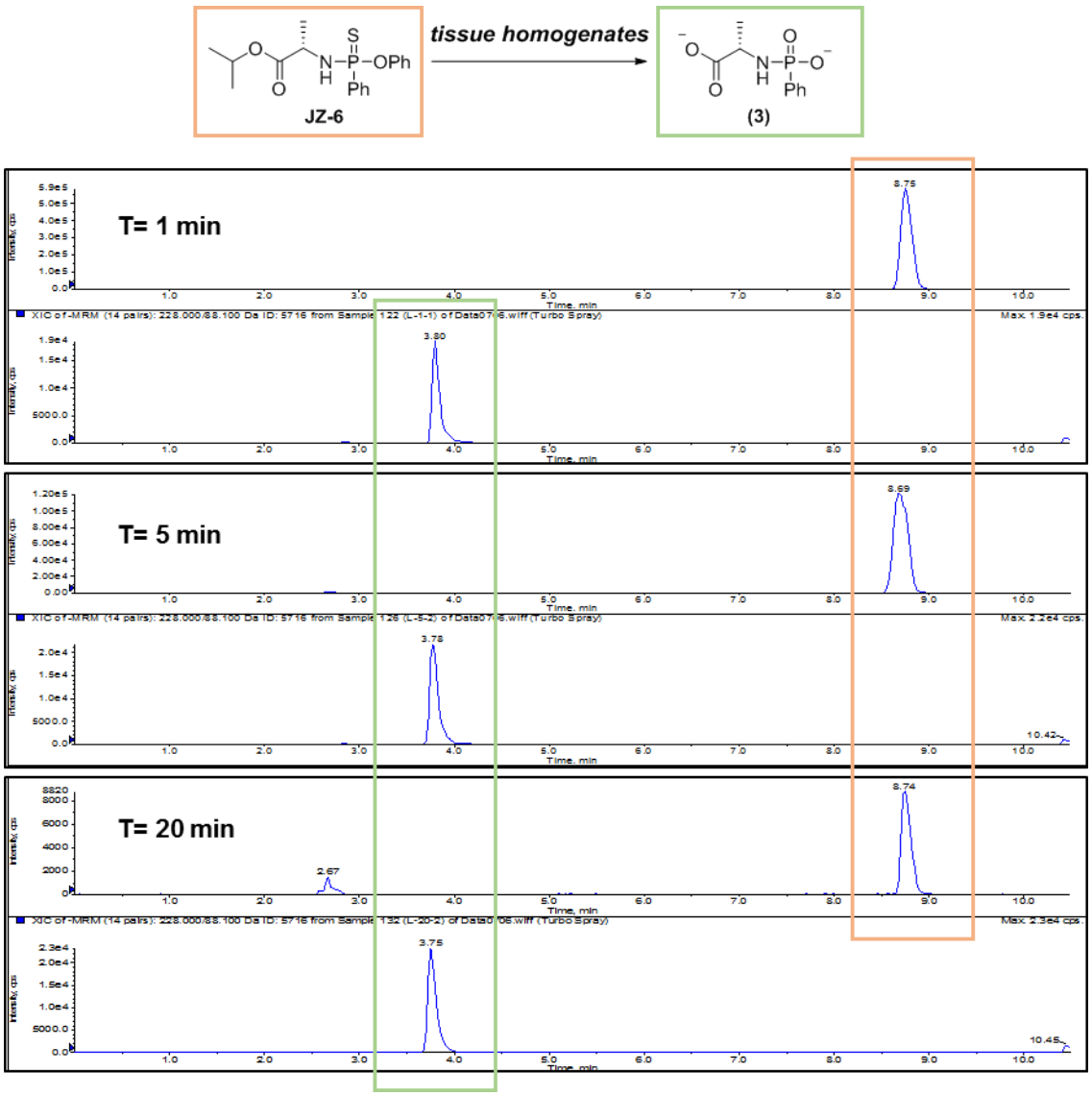



**Figure S11**. LC–MS/MS chromatogram of **JZ-6** (orange box) and **3** (green box) during liver homogenate (0.1 g/mL)-mediated degradation of the donor. **JZ-6**: $t_R$ = 8.65 min, **3**: $t_R$ = 3.50 min.

## 4. Cellular assays

### 4.1 Cell culture

The immortalized human hepatic stellate cell line LX-2, HeLa cell lines and macrophage cell line RAW 264.7 was acquired from the Cell Bank of Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). Hepatocarcinoma cell line HepG2 were provided by KeyGEN BioTECH (Nanjing, China). Cells were maintained in complete high-glucose Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Genetimes Technology Inc., Shanghai, China) and 1% penicillin–streptomycin (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) in an incubator containing 95% humidified air and 5% $CO_2$ at 37 °C. In LX-2 cell assay, a recombinant human TGF-$\beta$1 (5 ng/mL; Solarbio, Beijing, China) was added with compounds to the cell culture for 24 h for detection of fibrosis factors.

### 4.2 Cell viability

The Cell Counting Kit-8 (CCK-8) (US Everbright® Inc., Suzhou, China) was applied to measure the cell viability of LX-2 and HepG2 cells (Fig. S12). Cells were inoculated into 96-well plates at a density of $5 \times 10^3$ cells/mL and allowed to adhere overnight. Cells were treated with either DMSO (0.1%, control) or donors at different concentrations (as indicated in the figures) for 24 h at 37 °C. 100 μL of CCK-8 solution was added to each well and cells were incubated for 2–4 h at 37 °C. Absorbance was measured at 450 nm using a plate reader (Synergy H1 Hybrid Multi-Mode Reader, BioTek Instruments, Inc., Winooski, Vermont, USA). The results were expressed as the percentage of cell viability (%) with respect to the control (medium-treated cells). This assay was repeated in five independent experiments and recorded as the mean ± SD.

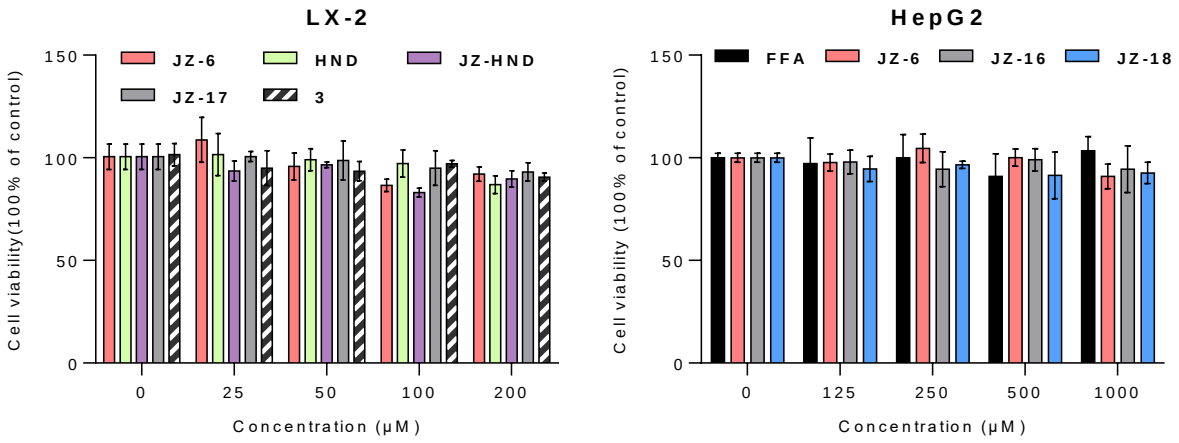



**Figure S12**. Effect of compounds on viability of LX-2 cells (left panel) and HepG2 cells (right panel).

### 4.3 Subcellular fractionation

Lysosomal fractionation was performed as described previously in our group with minor modifications[6,7]. Briefly, the cells were scraped, collected by centrifugation (600 × *g*, 5 min, 4 °C), and washed twice with ice-cold PBS. Break the cells with Dounce homogenizer and centrifuge the sample at 1000 × *g* for 10 min. The resulting supernatant was transferred and centrifuged at 20,000 × *g* for 20 min to afford the crude lysosomal fraction (CLF). CLF was re-suspended in a OptiPrep™ density gradient medium solution. The solution was seperated by density gradient centrifugation (100,000 × *g* for 1 h), and the top band was collected as the lysosome fractions.

### 4.4 Cellular pharmacokinetics

Cellular pharmacokinetics was performed as described previously in our group with minor modifications[6,8]. HepG2 cells were seeded in 6-well cell culture plates ($2 \times 10^5$ cells/well) and grown to 90% confluence. The cells were treated with **JZ-HND** or **HND** at 37 °C for the designated time. Then, the medium was removed. The cells were washed with ice-cold PBS three times and lysed in cold 70% methanol solution. The mixture was vortexed for 5 min for more complete analyte abstract and protein precipitation, and then centrifuged at 18,000 rpm for 5 min. An aliquot (100 μL) of the supernatant was transferred to a new tube, diluted with acetonitrile solution containing IS, and recentrifuged at 18,000 rpm before LC–MS/MS analysis (Section 3.3, Tables S1–S3). Cellular accumulations were calibrated by cellular protein content, which was determined using a BCA Protein Assay Kit (Beyotime, China). All experiments were conducted in triplicate.

Lysosomal uptake of compound **3** was determined in the presence or absence of inhibitors. The cells were treated with **JZ-6** (25 μmol/L)/BNPP (20 μmol/L), **JZ-6** (25 μmol/L)/talaprevir (5 μmol/L), **JZ-6** (25 μmol/L) or **JZ-18** (25 μmol/L) for 6 h. Then lysosomes were seperated as mentioned above (Section 4.3). The fractions were washed with ice-cold PBS and lysed in cold 70% methanol solution. The mixture was vortexed for 5 min and centrifuged at 18,000 rpm for 5 min. The aliquots were used to determine drug concentrations by LC–MS/MS (Section 3.3, Tables S1–S3). Lysosomal accumulations were calibrated by protein content, which was determined using a BCA Protein Assay Kit (Beyotime, China). All experiments were conducted in triplicate.

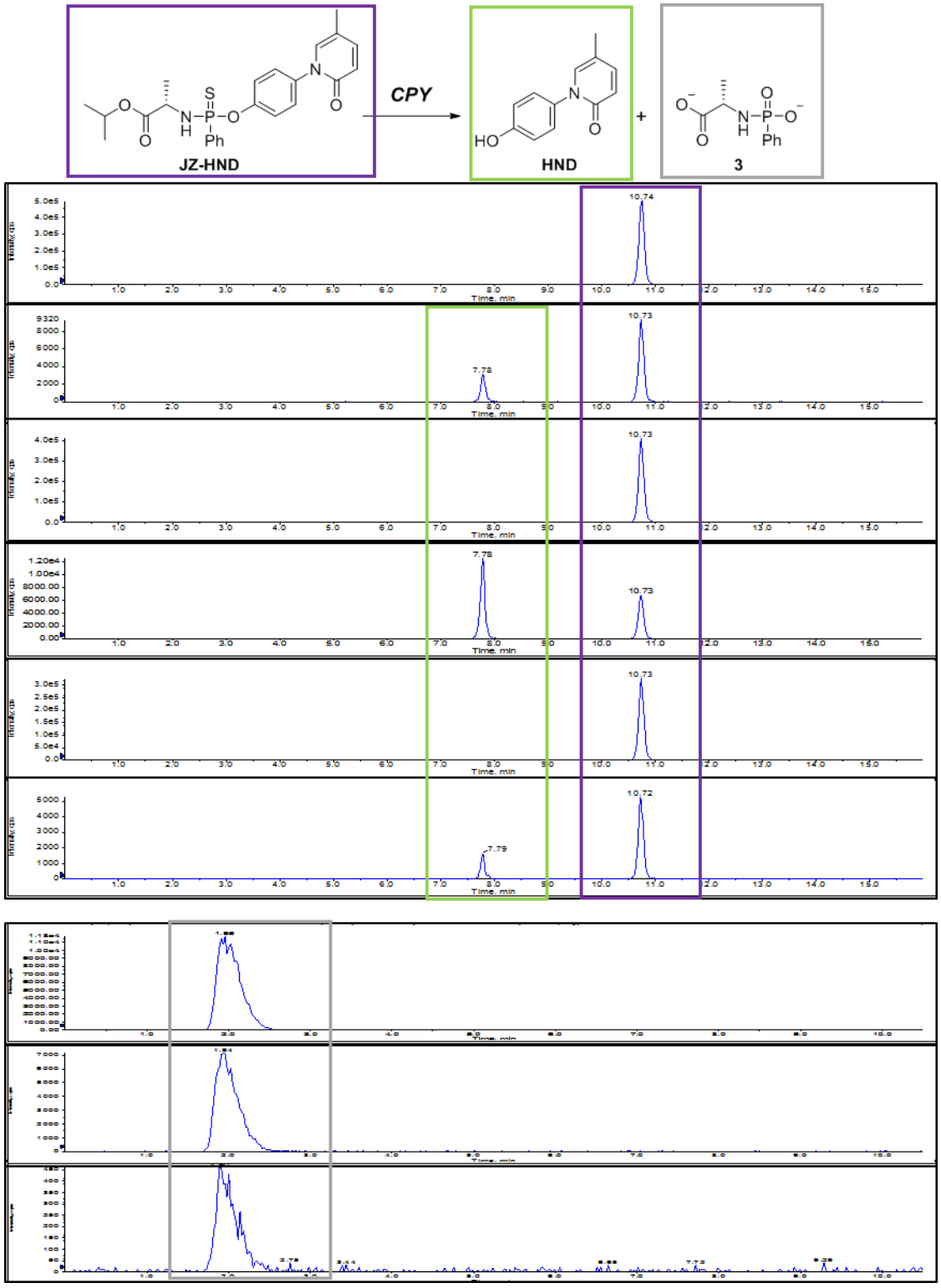



**Figure S13**. Intracellular pharmacokinetics of **JZ-HND** (purple box), **HND** (green box) and **3** (gray box) in HepG2 cells. **JZ-HND**: $t_R$= 10.73 min, **HND**: $t_R$= 7.78 min, **3**: $t_R$= 1.95 min.

### 4.5 Cell imaging

#### 4.5.1 Confocal microscopic images of $H_2S$ release in cellular lysosome

HeLa cells were inoculated into 12-well plates and cultured overnight. Cells were co-incubated with 100 μmol/L of the donors at 37 °C for 3 h or NaHS (100 μmol/L) for 0.5 h, and washed with PBS buffer to remove extracellular donors. Cells were then co-incubated with the $H_2S$-responsive fluorescent probe Lyso-AFP (10 μmol/L) dissolved in DMEM (with 0.1% pluronic F-127) and commercially available LysoTracker (50 nmol/L) dissolved in DMEM at 37 °C in the dark for 0.5 h and washed with PBS buffer. Cells were then co-incubated with Hoechst 33342 (1:1000 dilution) dissolved in DMEM for 10 min and washed with PBS buffer. Intracellular fluorescence in cells was monitored using a confocal laser scanning microscope (OLYMPUS FV300). $E_x/E_m$: 346 nm/460 nm for Hoechst 33342. $E_x/E_m$: 488 nm/535 nm for Lyso-AFP. $E_x/E_m$: 577 nm/590 nm for LysoTracker.

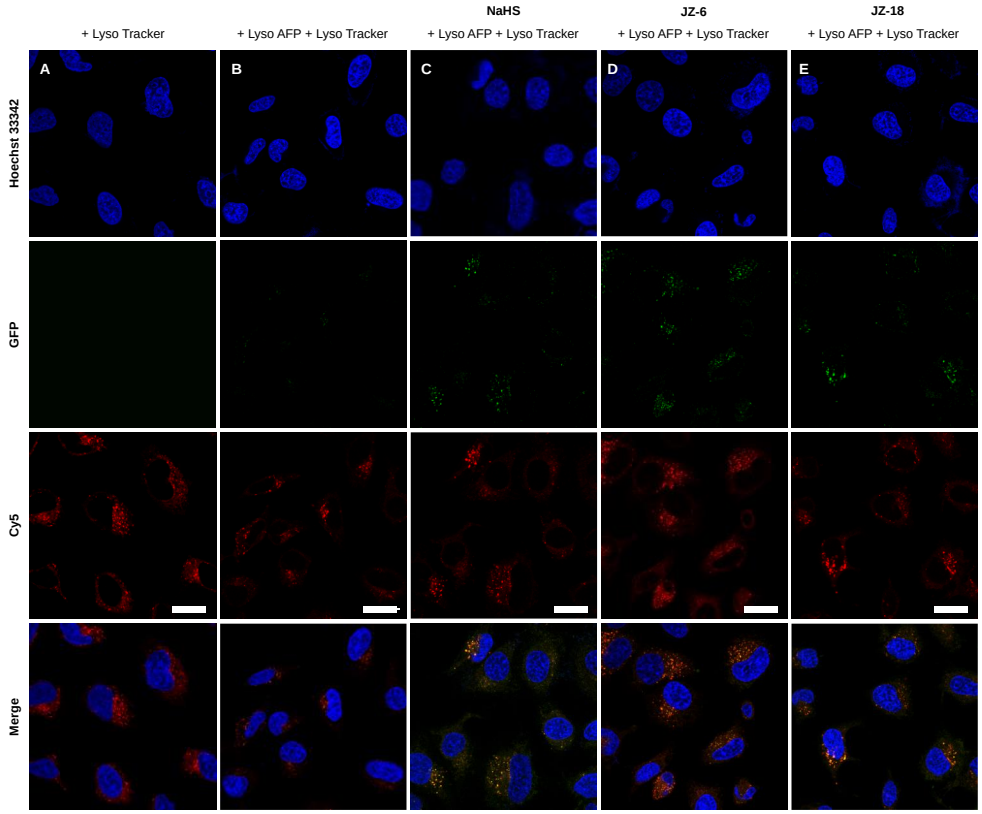



**Figure S14.** Confocal images of lysosome-localized $H_2S$ delivery in Hela cells. Cells were incubated with LysoTracker (50 nmol/L), Hoechst 33342 (1:1000 dilution) and (A) vehicle. (B) Lyso-AFP (10 μmol/L). (C) Lyso-AFP (10 μmol/L) and NaHS (100 μmol/L, positive control). (D) Lyso-AFP (10 μmol/L) and **JZ-6** (100 μmol/L). (E) Lyso-AFP (10 μmol/L) and **JZ-18** (100 μmol/L). Cells were incubated with NaHS for 0.5 h or donors for 3 h, and then with probes, followed by imaging. $E_x/E_m$: 346 nm/460 nm for Hoechst 33342. $E_x/E_m$: 488 nm/535 nm for Lyso-AFP; $E_x/E_m$: 577 nm/590 nm for LysoTracker. Bar scale: 10 μm.

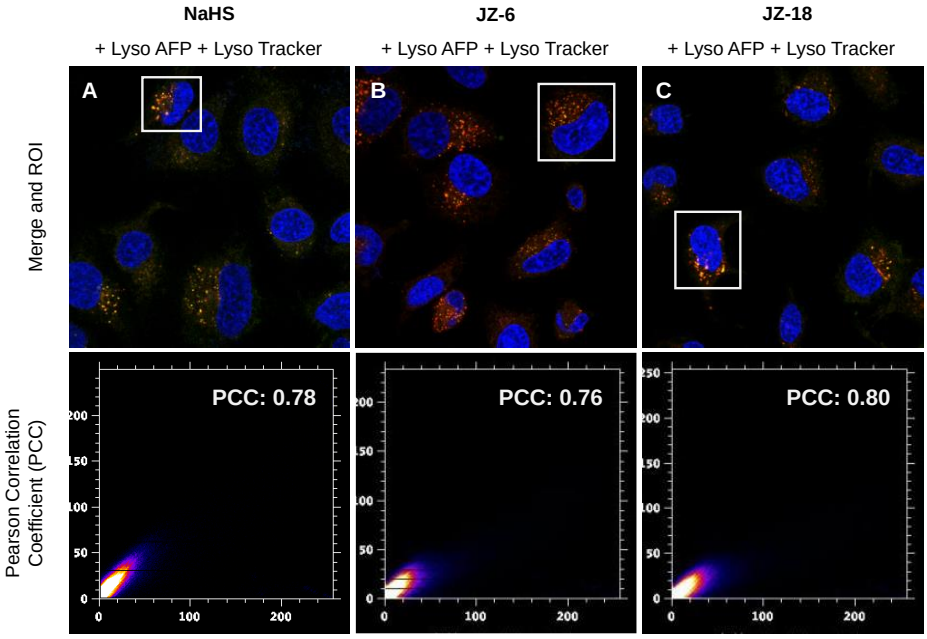



**Figure S15.** Intensity profile of regions of interest (ROI) and intensity correlation plot of Lyso-AFP and Lyso-Tracker. For Pearson Correlation Coefficient (PCC) calculation, *X* axis represent LysoTracker intensity and *Y* axis represent Lyso-AFP. PCC: 0.78 (A), 0.76(B), 0.80(C).

### 4.5.2 Acridine orange (AO) staining

HepG2 cells were seeded in a laser confocal dish and pretreated with **JZ-6** (50 μmol/L) with or without RSL3 (20 μmol/L) for 1 h. Then, the cells were incubated with AO (5 μg/mL, MCE HY-101879) for 15 min at room temperature in the dark. After washing, the cells were observed by a confocal laser scanning microscope OLYMPUS FV300 (Olympus, Tokyo, Japan). AO: $E_x$ = 488 nm, $E_m1$ = 530 nm (Green), $E_m2$ = 640 nm (Red).

### 4.5.3 Reactive oxygen species (ROS) detection

ROS was detected using a ROS Assay Kit (R253; Dojindo Laboratories, Kumamoto, Japan). LX-2 or HepG2 cells were incubated in a 12-well plate at a density of $1 \times 10^5$ cells/well and cultured overnight. In LX-2 cells, cells were co-treated with compounds (100 μmol/L) and TGF-$\beta$1 (10 ng/mL) for 24 h. In HepG2 cells, cells were pretreated with compounds (100 μmol/L) for 8 h before FFA solution (1 mmol/L) treatment for 24 h. The cells were then incubated with 10 μmol/L of DCFH-DA for 30 min and Hoechst 33342 (1:1000 diluted) for 10 min at 37 °C. The images were obtained with a fluorescence microscope (BioTek Lionheart FX, USA) using DIC for brightfield imaging and GFP filter cube for fluorescence imaging (**Figs. 5B** and **7A**). DCFH-DA: $E_x$ = 488 nm, $E_m$= 517 nm, Hoechst (33342): $E_x$

= 346 nm, $E_m$ = 460 nm. The fluorescence intensity was analyzed from a series of images using Image J Software.

### 4.6 Gene expression analysis (qPCR assay)

Total RNA from LX-2 cells or liver tissue was extracted using Trizol (Invitrogen), and 2 μg of total RNA was used for reverse transcription using the One Step TB Green™ PrimeScript™ RT-PCR Kit II (Takara, Kusatsu, Japan). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using a SYBR Green Supermix kit (Takara). Reactions were performed in triplicate for each sample. Relative expression was normalized to the expression levels of beta-actin. The following primer sequences were used.

**Table S5** Primer sequences used in qPCR assay.

| Gene | | Primer sequence (5′–3′) |
|---|---|---|
| Human | | |
| *ACTA2* | Forward | CTATGAGGGCTATGCCTTGCC |
| | Reverse | GCTCAGCAGTAGTAACGAAGGA |
| *COL1A1* | Forward | GTGCGATGACGTGATCTGTGA |
| | Reverse | CGGTGGTTTCTTGGTCGGT |
| *ACTB* | Forward | GCGTGACATTAAGGAGAAG |
| | Reverse | GAAGGAAGGCTGGAAGAG |
| Mouse | | |
| *Tgfb1* | Forward | CCACCTGCAAGACCATCGAC |
| | Reverse | CTGGCGAGCCTTAGTTTGGAC |
| *Col1a1* | Forward | TAGGCCATTGTGTATGCAGC |
| | Reverse | ACATGTTCAGCTTTGTGGACC |
| *Col3a1* | Forward | CTGTAACATGGAAACTGGGGAAA |
| | Reverse | CCATAGCTGAACTGAAAACCACC |
| *Acta2* | Forward | CCCAGACATCAGGGAGTAATGG |
| | Reverse | TCTATCGGATACTTCAGCGTCA |
| *Gpx1* | Forward | CCACCGTGTATGCCTTCTCC |
| | Reverse | AGAGAGACGCGACATTCTCAAT |
| *Sod1* | Forward | AACCAGTTGTGTTGTCAGGAC |
| | Reverse | CCACCATGTTTCTTAGAGTGAGG |
| *Sod2* | Forward | CAGACCTGCCTTACGACTATGG |
| | Reverse | CTCGGTGGCGTTGAGATTGTT |
| *Cat* | Forward | GGAGGCGGGAACCCAATAG |
| | Reverse | GTGTGCCATCTCGTCAGTGAA |
| *Nfkb1* | Forward | ATGGCAGACGATGATCCCTAC |
| | Reverse | CGGAATCGAAATCCCCTCTGTT |
| *Nfkbia* | Forward | TGAAGGACGAGGAGTACGAGC |
| | Reverse | TGCAGGAACGAGTCTCCGT |
| *Il1b* | Forward | ATAACCTGCTGGTGTGTGACGTT |
| | Reverse | AAGGCCACAGGTATTTTGTCGTT |
| *Tnfa* | Forward | CTGAGGTCAATCTGCCCAAGTAC |
| | Reverse | CTTCACAGAGCAATGACTCCAAAG |
| *Actb* | Forward | ATGGAGGGGAATACAGCCC |
| | Reverse | TTCTTTGCAGCTCCTTCGTT |

### 4.7 Free fatty acids (FFA) preparation

The stock solution of FFA (5 mmol/L) was prepared as follows. Stock solutions of sodium oleate (OA) (100 mmol/L) and sodium palmitate (PA) (100 mmol/L) were prepared with deionized water heated to 80 °C. Simultaneously, a 5% (*w*/*v*) fatty acid-free bovine serum albumin (FAF-BSA, YeaSen, China) solution was prepared in DMEM at 37 °C in a water bath. To prepare a 5 mmol/L FFA stock solution, 0.2 mL of 100 mmol/L PA and 0.4 mL of 100 mmol/L OA were added to 3.8 mL of 5% FAF-BSA solution and 7.6 mL of 5% FAF-BSA solution, respectively, which were then heated at 37 °C for 5 min in a shaking water bath. PA/FAF-BSA solution and OA/FAF-BSA solution were mixed and sterile filtered using a 0.22 μm pore size filter, affording the FFA solution (5 mmol/L). The solution can be stored at 4 °C for up to 2 weeks.

### 4.8 Intracellular triglyceride (TG) measurement

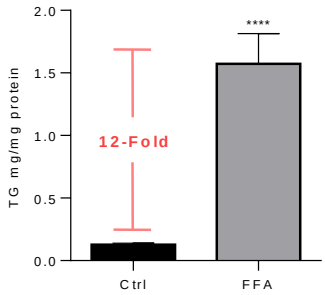



**Figure S16.** Intracellular triglyceride (TG) measurement of Hela cells after FFA treatment (1 mmol/L). HepG2 or HeLa cells were inoculated into 6-well plates at a density of $7\times10^5$ cells/well and cultured overnight. The cells were co-incubated with FFA (1 mmol/L) for 24 h. Intracellular TG content was measured according to the manufacture's recommended protocol using the TG Content Assay Kit (Cat# BC0625; Solarbio, Beijing, China). This assay was repeated in three independent experiments and recorded as the mean ± SD. Intracellular triglyceride content increased significantly after FFA modeling and the results are shown in Fig. S16.

### 4.9 Malondialdehyde (MDA) measurement

HepG2 cells were inoculated into 6-well plates at a density of $7\times10^5$ cells/well and cultured overnight. The cells were co-incubated with FFA (1 mmol/L) or plus **JZ-6** (20, 100, 500 μmol/L), **JZ-16** (500 μmol/L), NaHS (500 μmol/L) for 24 h. For lysosomal MDA measurement, the cells were pretreated with RSL3 (1 μmol/L) for 2 h and then treated with culture medium or **JZ-6** (100 μmol/L) for 24 h.

The cell lysates or pelleted crude lysosomal fractions (lysed in 50 mmol/L Tris HCl (pH 8), 150 mmol/L NaCl, 1 mmol/L EGTA, 1% NP-40, 0.1% SDS, 3% glycerol) were collected. MDA was then measured according to the manufacture's recommended protocol using the Lipid Peroxidation (MDA) Assay Kit (Sigma–Aldrich). The fluorescence intensity at excitation/emission wavelengths of 532/553 nm was measured on a microplate reader. The MDA concentration was normalized with cell counts. This assay was repeated in three independent experiments and recorded as the mean ± SD.

### 4.10 Ferroptosis rescue experiments

In the ferroptosis rescue experiments, HepG2 cells were inoculated into 96-well plates at a density of $5\times10^3$ cells/well and cultured overnight. The cells were pretreated with RSL-3 (4 μmol/L) for 2 h and then treated with culture medium or **JZ-6** (20 μmol/L), **JZ-16** (20 μmol/L), and Fer-1 (1 μmol/L) for 24 h. 100 μL of CCK-8 solution was added to each well and the cells were incubated for a further 2–4 h at 37 °C. The absorbance was measured at 450 nm using a microplate reader (Synergy H1 Hybrid Multi-Mode Reader, BioTek Instruments, Inc., Winooski, Vermont, USA). The results were expressed as the percentage of cell viability (%) with respect to the control (medium-treated cells). This assay was repeated in three independent experiments and recorded as the mean ± SD.

### 4.11 Enzyme-linked immunosorbent assay (ELISA)

The RAW 264.7 cells were inoculated into 12-well plates at a density of $2 \times 10^5$ cells/well and cultured overnight. The cells were pretreated with **JZ-6** (20, 100, and 500 μmol/L), **JZ-HND** (20, 100, and 500 μmol/L) or NaHS (20, 100, and 500 μmol/L) for 1 h before treatment with lipopolysaccharide (LPS, 1 μg/mL). Thereafter, the cell culture supernatant was collected. The concentrations of interleukin-1 beta (IL-1$\beta$) and interleukin-6 (IL-6) in the cell culture supernatant were quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Dakewe Biotech, China). This assay was repeated in three independent experiments and recorded as the mean ± SD.

## 5. Animal experiments

### 5.1 Animals

All animal protocols were approved by the Institutional Animal Care and Use Committee of China Pharmaceutical University. Male BALB/c mice (Six-week-old) were purchased from Beijing Vital River Animal Technology Co., Ltd. Mice were housed in pathogen-free conditions in a temperature-controlled environment at 22–24 °C with a 12-h/12-h light/dark cycle.

### 5.2 $CCl_4$-induced liver injury

The mice were randomly divided into 4 groups ($n = 6$ in each group) as follows: control group, $CCl_4$-treated model group, **JZ-HND** group (1 mmol/kg), and **HND** group (1 mmol/kg). $CCl_4$-treated mice were injected intraperitoneally with 20% $CCl_4$ (10 mL/kg of body weight) diluted in soybean oil three times per week for 4 weeks. **JZ-HND** (1 mmol/kg) or **HND** (1 mmol/kg) were orally administered once a day for 4 weeks.

### 5.3 Histopathology

The liver tissues were fixed in 4% paraformaldehyde solution. The haematoxylin & eosin (HE) and Masson trichrome staining was performed on paraffin-embedded liver sections according to standard procedures. Histological images of section tissues were captured with a light microscope (NIKON Eclipse ci, Nikon Instruments, Inc., Tokyo, Japan) for morphological analysis and for visualizing collagen expression.

### 5.4 Serum assays

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and superoxide dismutase (SOD) levels were measured using commercial kits according to the manufacturer's instructions (BC555 for ALT, BC1565 for AST, and S104195 for SOD, Solarbio, Beijing, China).

### 5.5 Glutathione peroxidase (GPX) activity

Hepatic tissue glutathione peroxidase (GPX) activity were measured using commercial kits according to the manufacturer's instructions (BC1195, Solarbio, Beijing, China).

### 5.6 Quantitative PCR analysis

qPCR assays were performed as mentioned above (Section 4.6). The primer sequences used are listed in **Table S5**.

### 5.7 Animal pharmacokinetics

The BALB/c mice were fasted for 12 h without water before the animal pharmacokinetics experiment. The mice were randomly divided into 3 groups ($n$ = 3 in each group), each group were intragastric administration with **JZ-HND** (1 mmol/kg, 0.5% CMC-Na).

Blood samples (approximately 60 μL) were collected from retro-orbital plexus under light isoflurane anesthesia such that the samples were obtained at eight time points post dose: 5, 15, 30 min and 1, 2, 4, 8, 12, and 24 h. Blood samples were collected at each time point into labeled microcentrifuge tubes containing sodium heparin as anticoagulant. Plasma was collected after centrifugation at 8000 rpm × 5 min. stored below −40 °C until LC−MS/MS analysis.

Liver samples were collected at 0.5, 4, 8 h post dose. Liver samples were homogenized using PBS buffer containing 70% MeOH in a ratio of 10:1 buffer to liver, this portion of sample was used to analyses the metabolism of **JZ-HND** in liver. Liver sample were homogenized using HPE-IAM solution (20 mmol/L, in MeOH) in a ratio of 10:1 buffer to liver, this portion of sample was used to analyses the $H_2S$ and sulfur compound levels and the resulting homogenates were stored below −40 °C until LC−MS/MS analysis.

**Table S6.** Pharmacokinetic parameters of JZ-HND and HND in mice plasma after oral administration of JZ-HND at single dose of 1.0 mmol/kg ($n$=3). Data are presented as mean ± SD.

| PK parameter | **JZ-HND** | HND |
|---|---|---|
| | Mean ± SD | Mean ± SD |
| $AUC_{0-24\ h}$ (h·μmol/L) | 33.74 ± 4.59 | 52.92 ± 4.81 |
| Cl/F (L/h/kg) | 29.95 ± 3.87 | 19.01 ± 1.82 |
| $C_{max}$ (μmol/L) | 13.33 ± 0.74 | 23.40 ± 7.82 |
| $t_{1/2}$ (h) | 2.16 ± 0.23 | 1.96 ± 0.38 |
| MRT (h) | 2.11 ± 0.28 | 2.28 ± 0.12 |
| $T_{max}$ (h) | 0.83 ± 0.29 | 0.5 |
| $V/F$ (L/kg) | 92.30 ± 17.01 | 54.62 ± 15.12 |

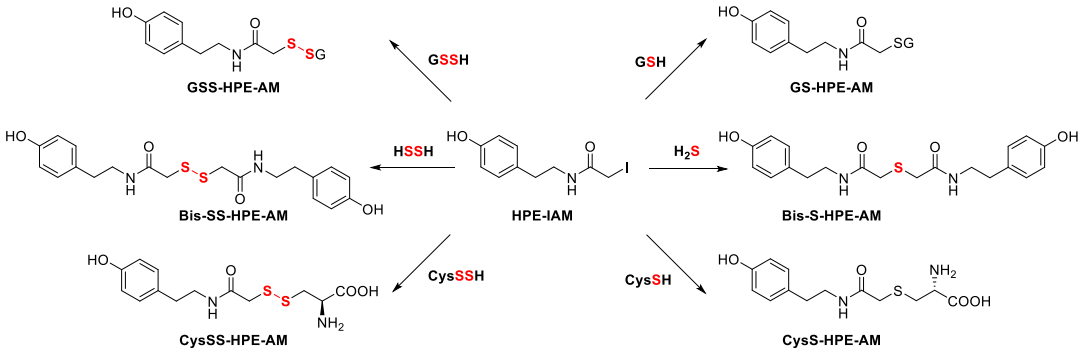
**Table S7.** Pharmacokinetic parameters of JZ-HND and HND in mice livers after oral administration of JZ-HND at single dose of 1.0 mmol/kg ($n$=3). Data are presented as mean ± SD.

| PK parameter | **JZ-HND** | HND |
|---|---|---|
| | Mean ± SD | Mean ± SD |
| $C_{max}$ (μmol/L) | 15.28 ± 8.59 | 3.09 ± 1.60 |

### 5.8 LC−MS/MS assay for the detection of $H_2S$ in mouse liver

(1) Sulfur-containing derivatives.

To ensure accurate quantitation of $H_2S$ production and endogenously formed persulfides in the mouse liver following the administration of **JZ-HND**, HPE-IAM was selected as the trapping agent for. The sulfur-containing derivatives generated after *S*-alkylation can indirectly reflect the amount of $H_2S$ and endogenous sulfur species, as well as their changes[9].




**Scheme S10.** Sulfur-containing derivatives.

Sulfur-containing derivatives were synthesized according methods reported by our group[9]. Analytical data **GS-HPE-AM**, **GSS-HPE-AM**, **Cys-S-HPE-AM** and **CysSS-HPE-AM** agree with literature values[9].

**Bis-S-HPE-AM**: $^1$H NMR (300 MHz, DMSO) $\delta$ 9.21 (s, 2H), 7.00 (d, $J$ = 8.4 Hz, 4H), 6.68 (d, $J$ = 8.5 Hz, 4H), 3.27–3.14 (m, 8H), 2.60 (t, $J$ = 6.2 Hz, 4H).

**Bis-SS-HPE-AM**: $^1$H NMR (300 MHz, DMSO) $\delta$ 9.19 (s, 2H), 7.00 (d, $J$ = 8.5 Hz, 4H), 6.67 (d, $J$ = 8.5 Hz, 4H), 3.45 (s, 4H), 3.24 (dd, $J$ = 14.5, 6.1 Hz, 4H), 2.60 (t, $J$ = 7.5 Hz, 4H).

(2) Analytical method

HPLC conditions: The $H_2S$ generation in liver were measured by a LC−MS/MS system containing a Shimadzu HPLC system (Kyoto, Japan) coupled with a SCIEX API 4000 triple quadrupole mass spectrometer (Foster City, CA, USA) equipped with an electrospray ionization (ESI) interface in positive or negative ionization mode. A gradient elution program was conducted with mobile phase A (0.1% formic acid and 2 mmol/L ammonium formate in water) and mobile phase B (methanol) shown in the following Table S7.

MS conditions: Ion source parameters: The pressure of curtain gas, collision gas, ion source gas 1 and ion source gas 2 were 12, 30, 60 and 70 psi, respectively. The ion source temperature was maintained at 550 °C. The optimized ion spray voltage was 4500 V and dwell time of 100 ms for the analytes and IS applied (Table S9).

**Table S8.** Gradient elution program.

| Time (min) | Flow rate (mL/min) | Phase B (%) |
|---|---|---|
| 0.01 | 1 | 10 |
| 1.00 | 1 | 10 |
| 1.50 | 1 | 70 |
| 3.00 | 1 | 70 |
| 3.10 | 1 | 10 |
| 6.01 | 1 | Stop |

**Table S9.** MRM parameters for LC−MS/MS.

| Prodrug | ESI mode | MRM transitions (*m*/*z*) | Declustering Potential (V) | Collision Energy (eV) |
|---|---|---|---|---|
| **CysS-HPE-AM** | + | 299.1/121.1 | 70 | 37 |
| **CysSS-HPE-AM** | + | 331.0/121.0 | 70 | 40 |
| **GS-HPE-AM** | + | 485.0/121.0 | 100 | 63 |
| **GSS-HPE-AM** | + | 517.2/338.2 | 87 | 23 |
| **Bis-S-HPE-AM** | + | 389.1/121.2 | 90 | 43 |
| **Bis-SS-HPE-AM** | + | 421.0/212.2 | 89 | 25 |
| **4-MU**[a] | + | 177.0/103.0 | 18 | 36 |

[a]4-Methylumbelliferone (4-MU), internal standard (IS).

(3) Calibration curves

Calibration curves for each sample or standard were obtained as plots of relative intensities (ratios of analytes and IS peak areas) by linear regression using a weighting factor of the reciprocal of the relative concentration ($1/x^2$) versus square of concentration. See **Table S10**.

**Table S10.** Linearity of representative calibration curves.

| Compound | Mean linear relationship | Regression coefficient (*r*) |
|---|---|---|
| **CysS-HPE-AM** | $y = 0.273\ x + 0.0113$ | 0.9905 |
| **CysSS-HPE-AM** | $y = 0.234\ x + 0.00482$ | 0.9938 |
| **GS-HPE-AM** | $y = 0.244\ x + 0.00456$ | 0.9926 |
| **GSS-HPE-AM** | $y = 0.636\ x + 0.135$ | 0.9914 |
| **Bis-S-HPE-AM** | $y = 6.29\ x + 0.0328$ | 0.9955 |
| **Bis-SS-HPE-AM** | $y = 23.3\ x + 0.167$ | 0.9966 |

## 6. Statistical analysis

GraphPad Prism 9.0 software (GraphPad Software) was used for statistical analysis. One-way ANOVA was used to test statistical significance between groups as appropriate; nonlinear regression was used to analyze drug disappearance curve; linear regression was used to analyze calibration curves. Pearson correlation coefficient analysis and intracellular fluorescence was used to evaluate the correlation by Image J. Data were expressed as mean ± SD where applicable.

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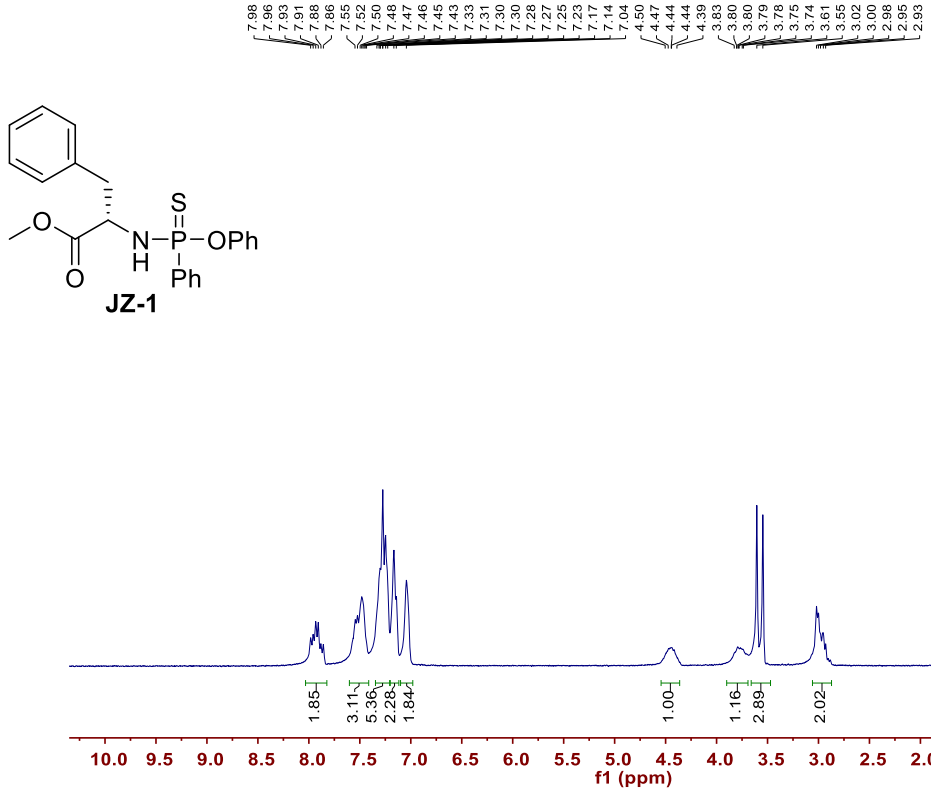
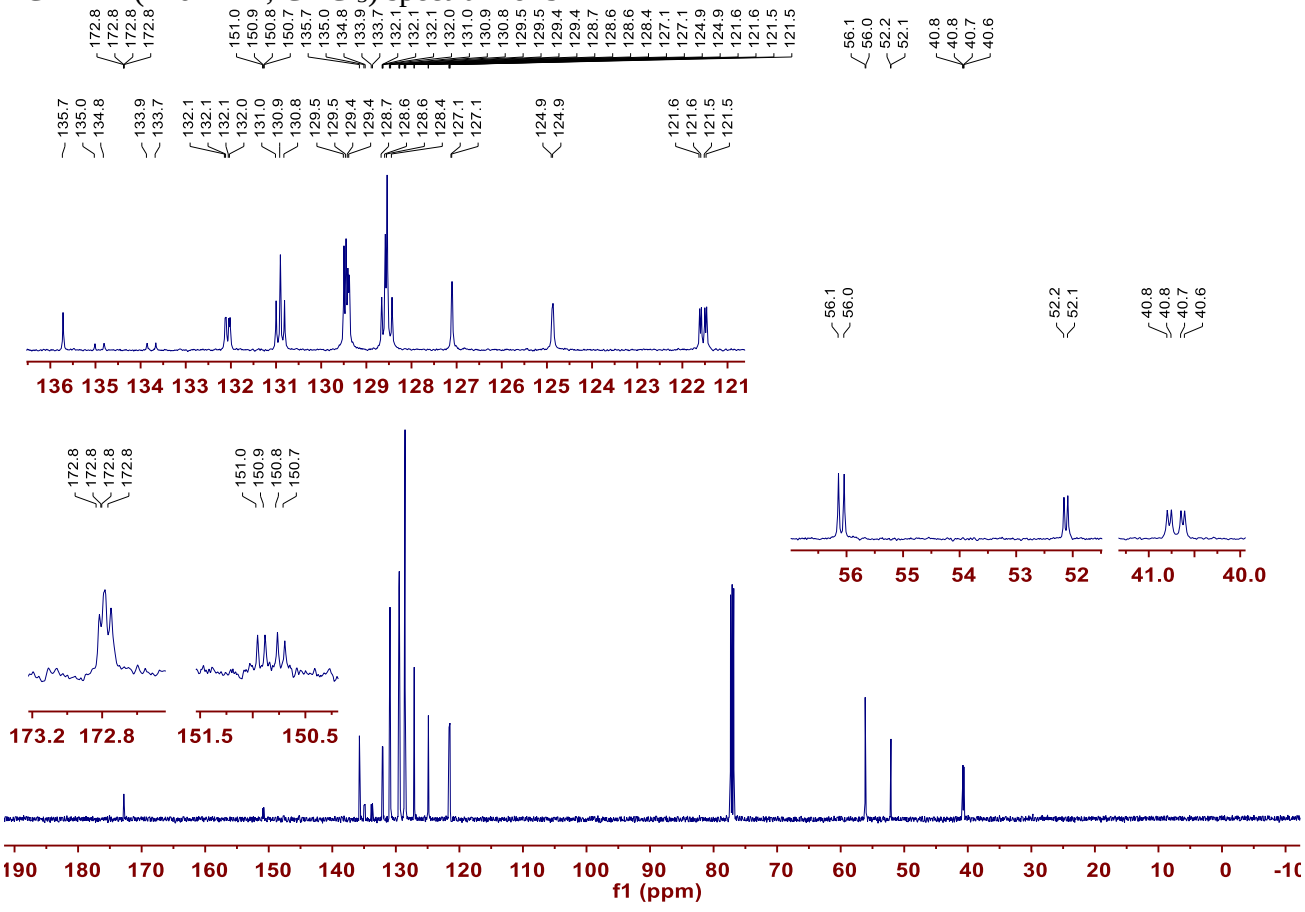
## NMR Spectra

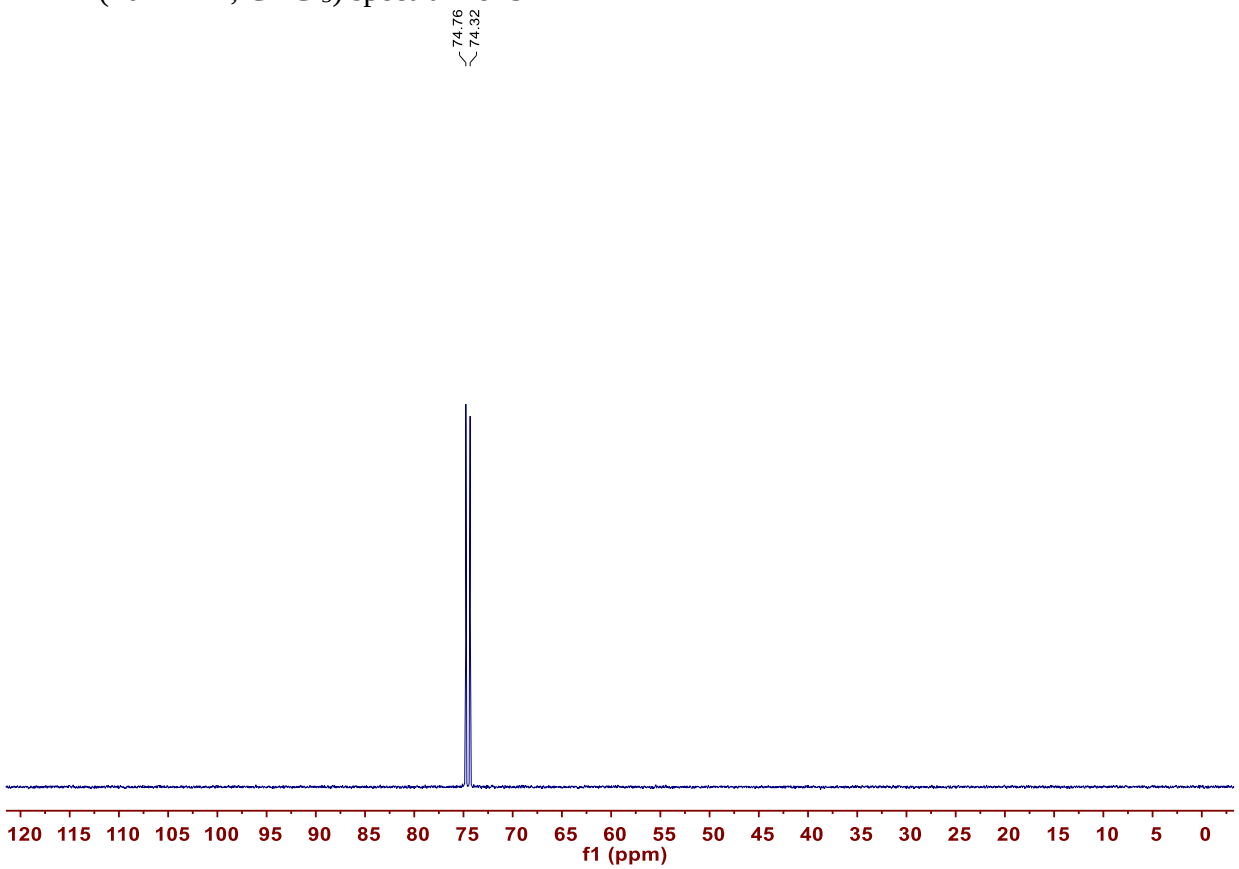
$^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of **JZ-1**



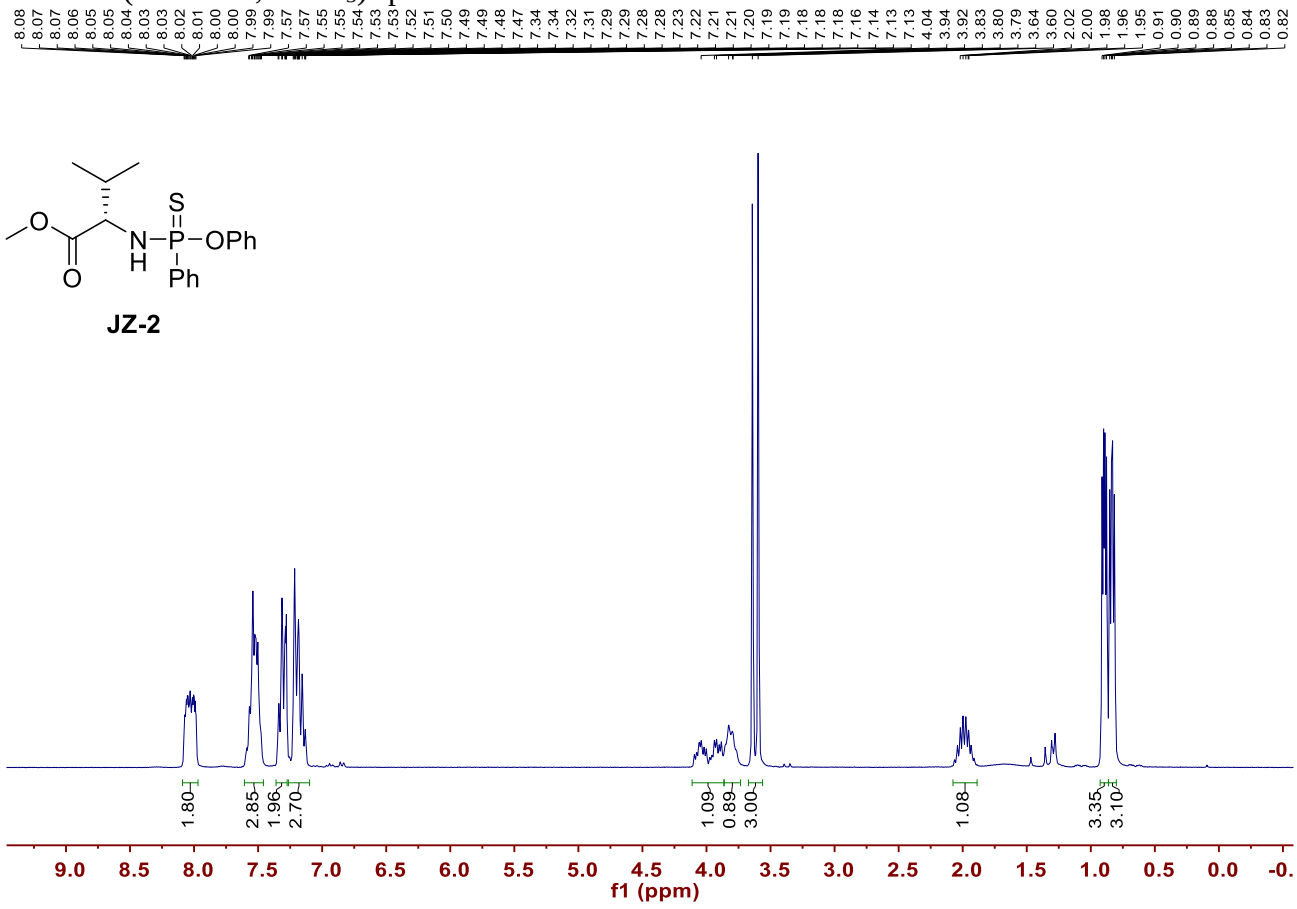

$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-1**



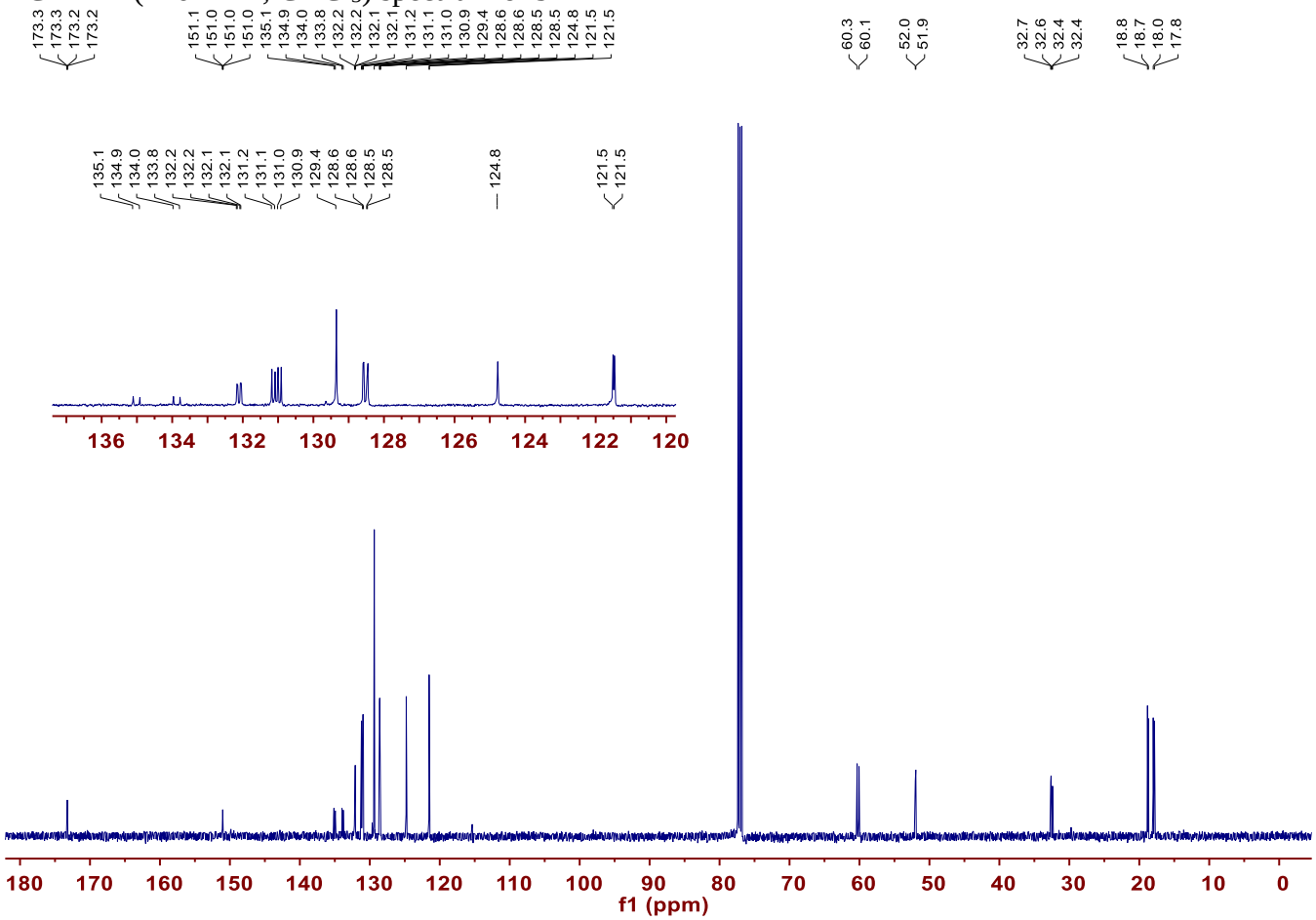
$^{31}$P NMR (202 MHz, $CDCl_3$) spectrum of **JZ-1**



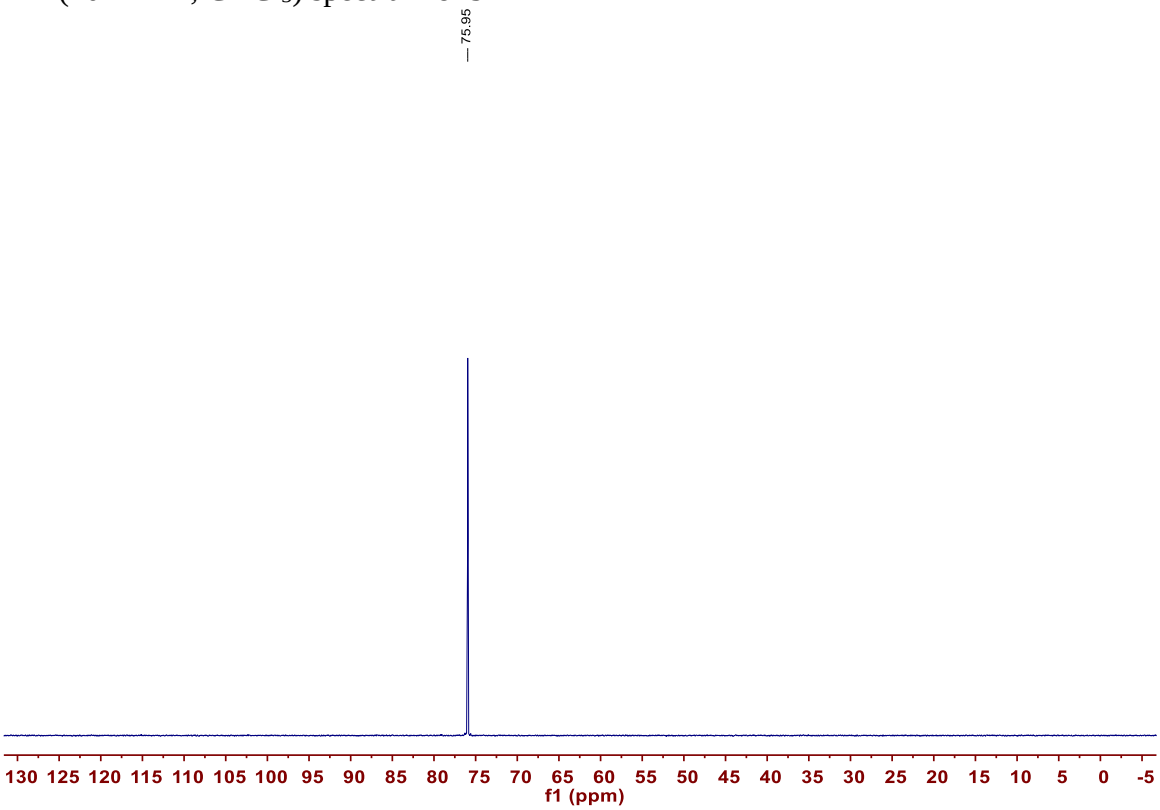

$^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of **JZ-2**



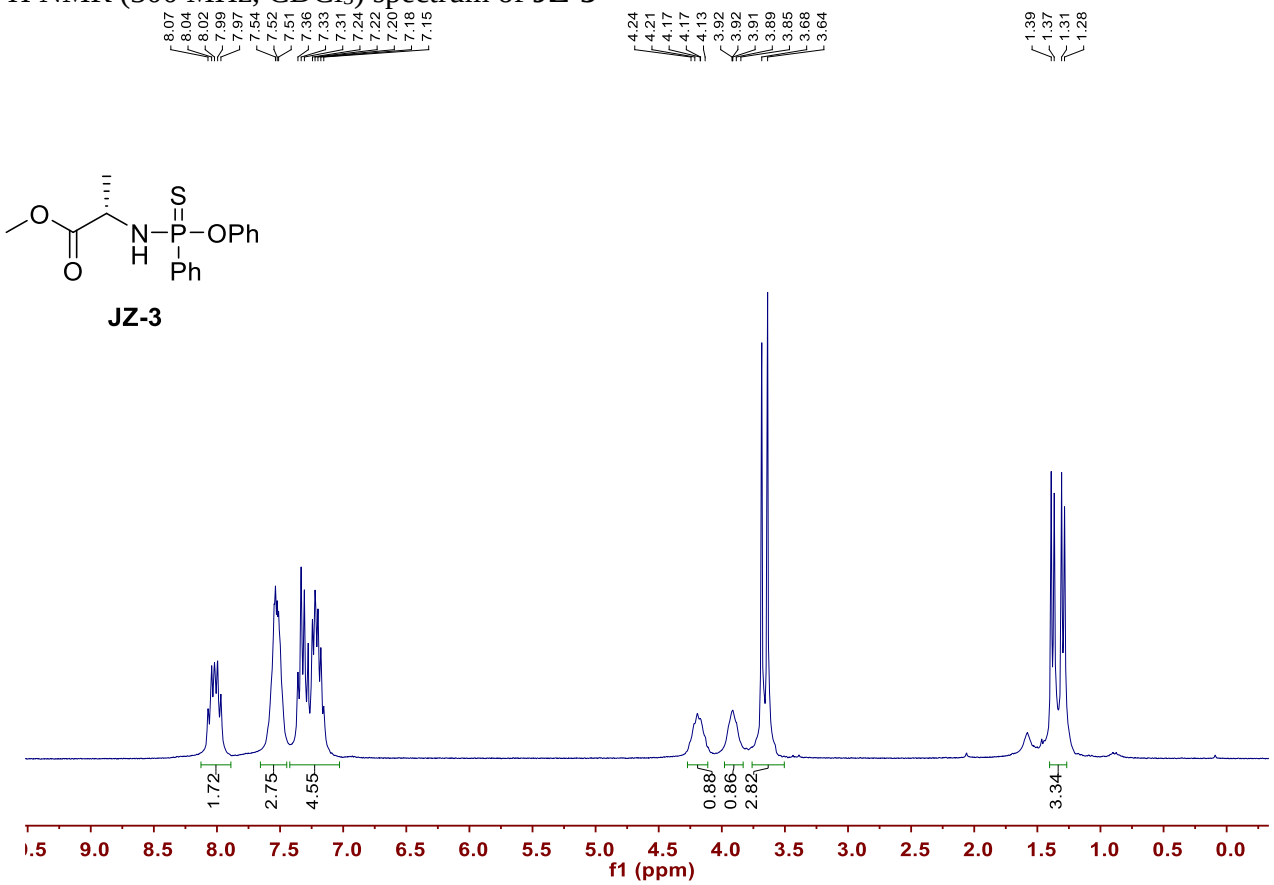
$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-2**



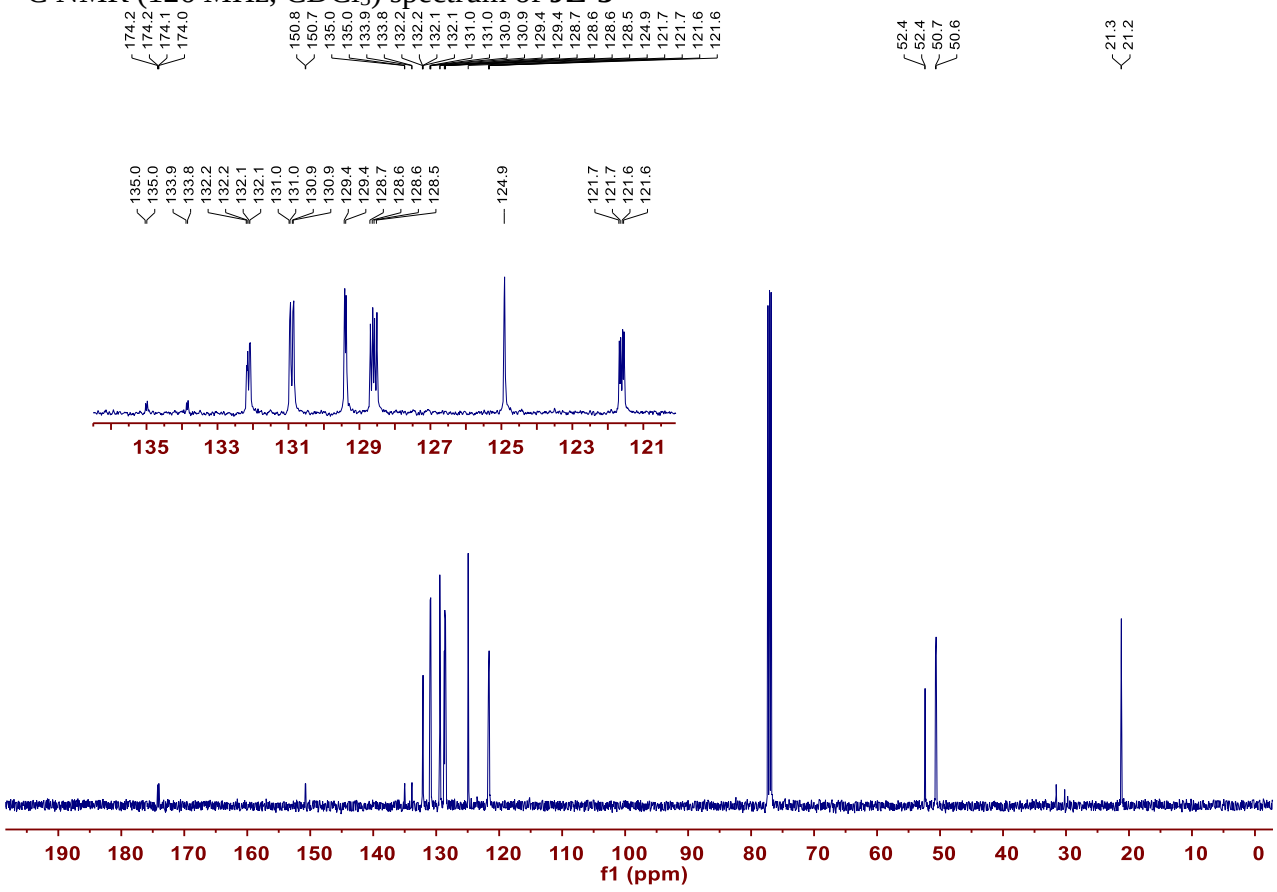

$^{31}$P NMR (202 MHz, $CDCl_3$) spectrum of **JZ-2**



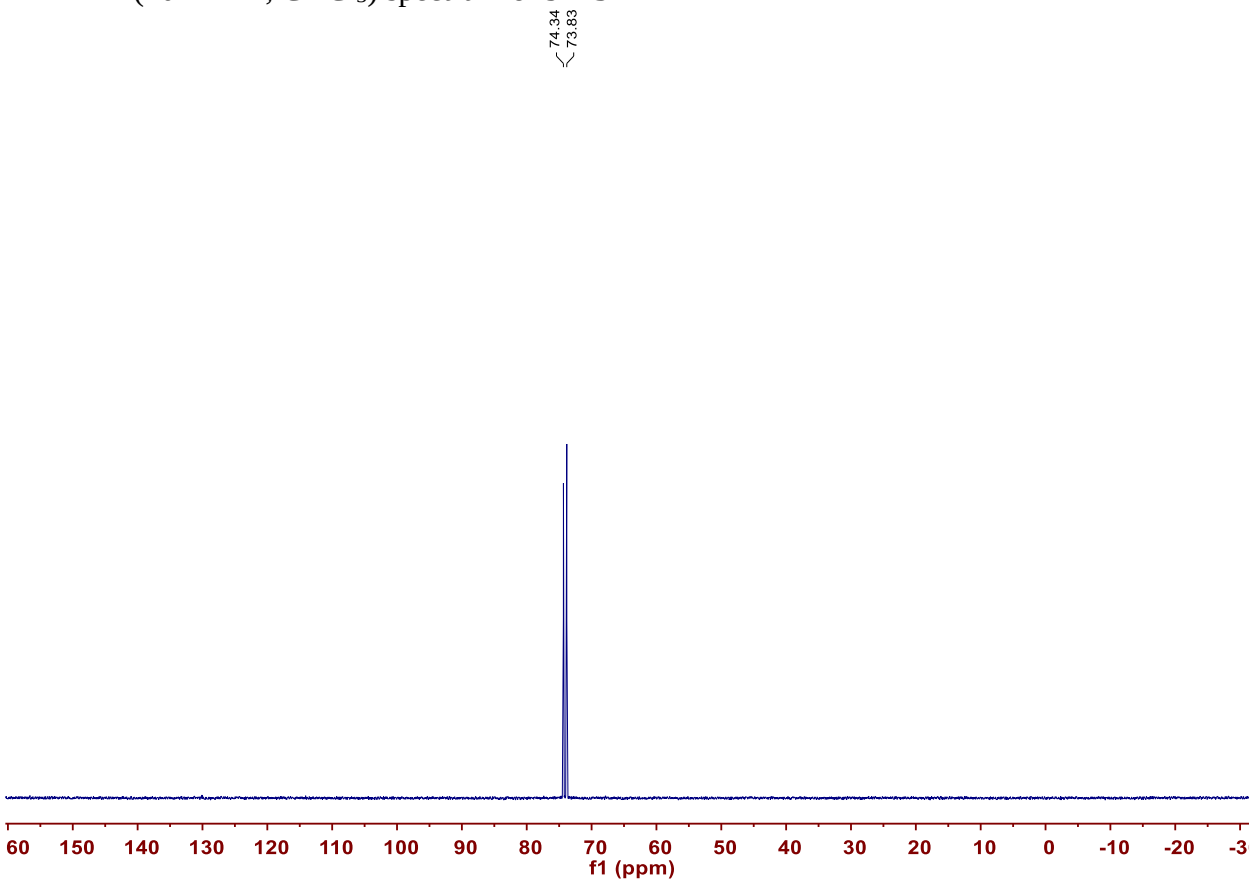
$^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of **JZ-3**



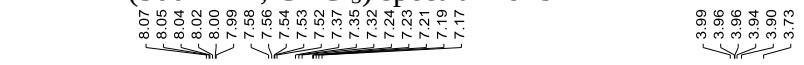

$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-3**

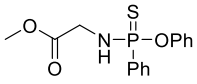


$^{31}P$ NMR (202 MHz, $CDCl_3$) spectrum of **JZ-3**

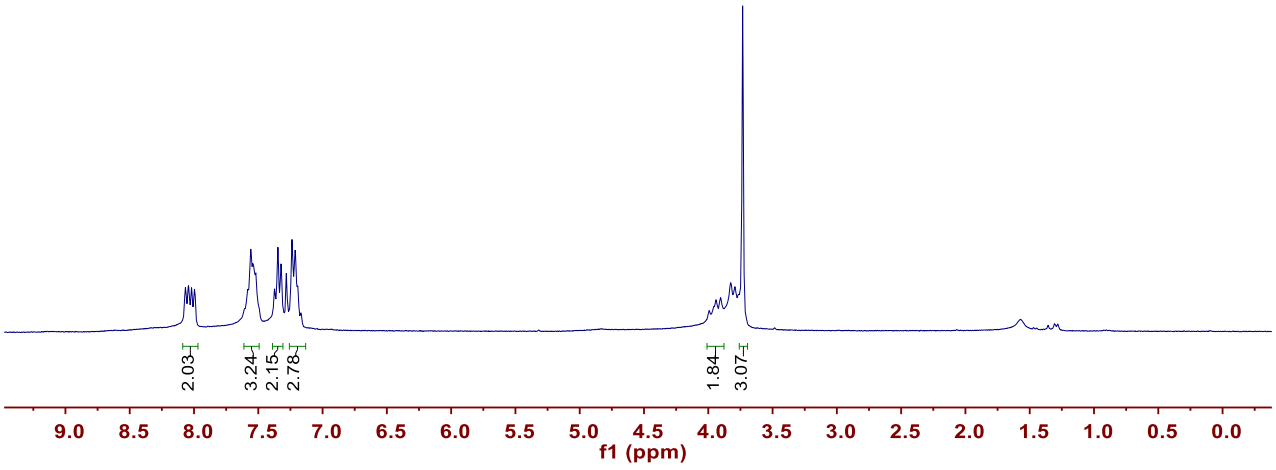



$^{1}H$ NMR (300 MHz, $CDCl_3$) spectrum of **JZ-4**

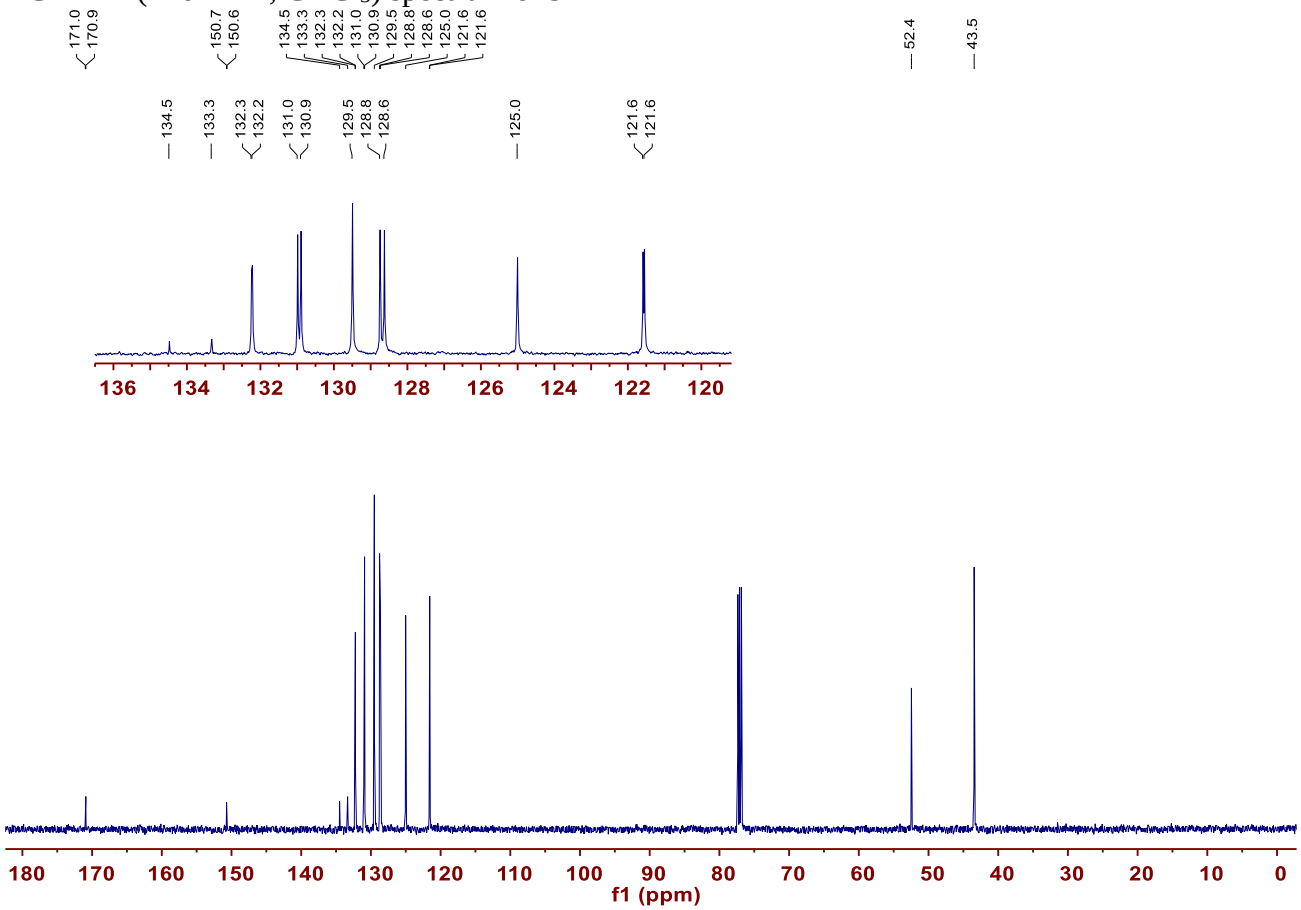


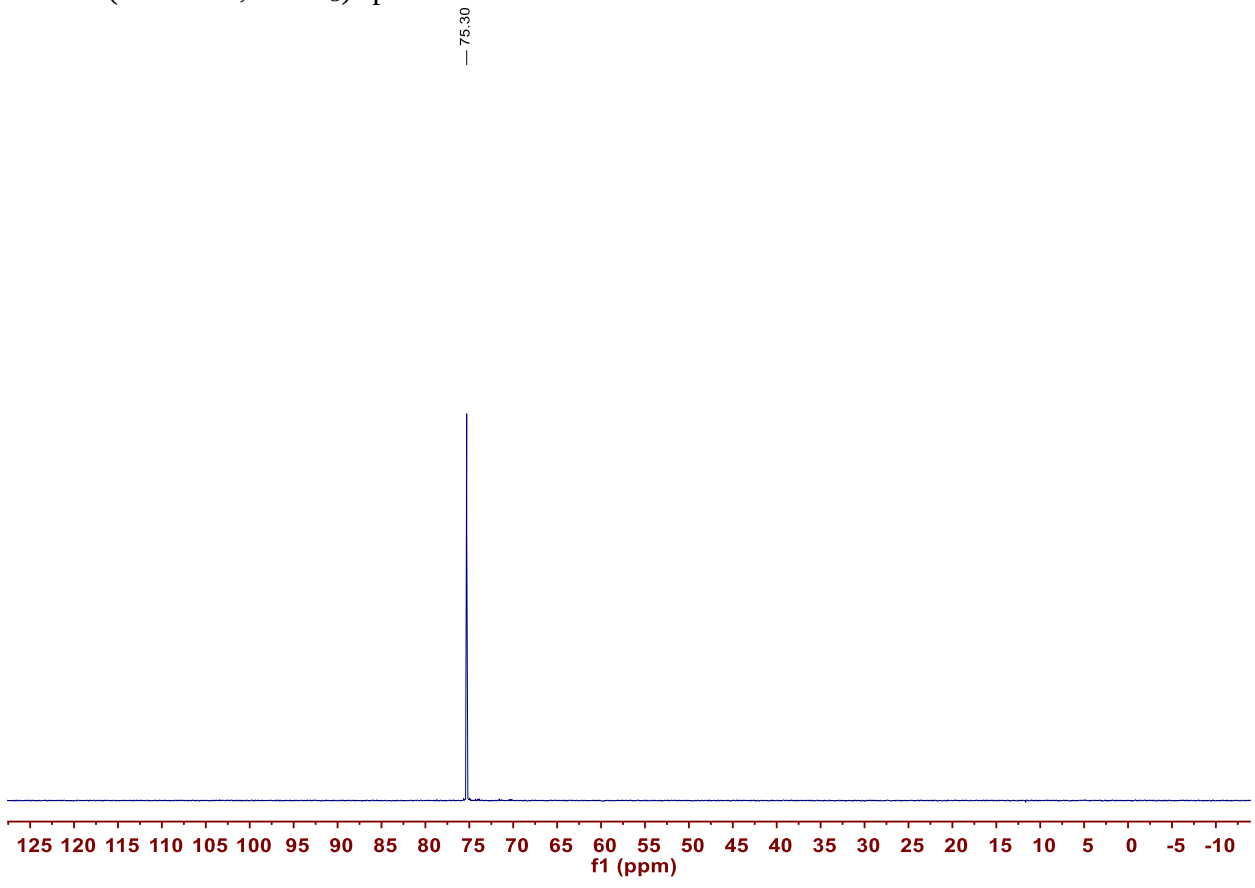

**JZ-4**



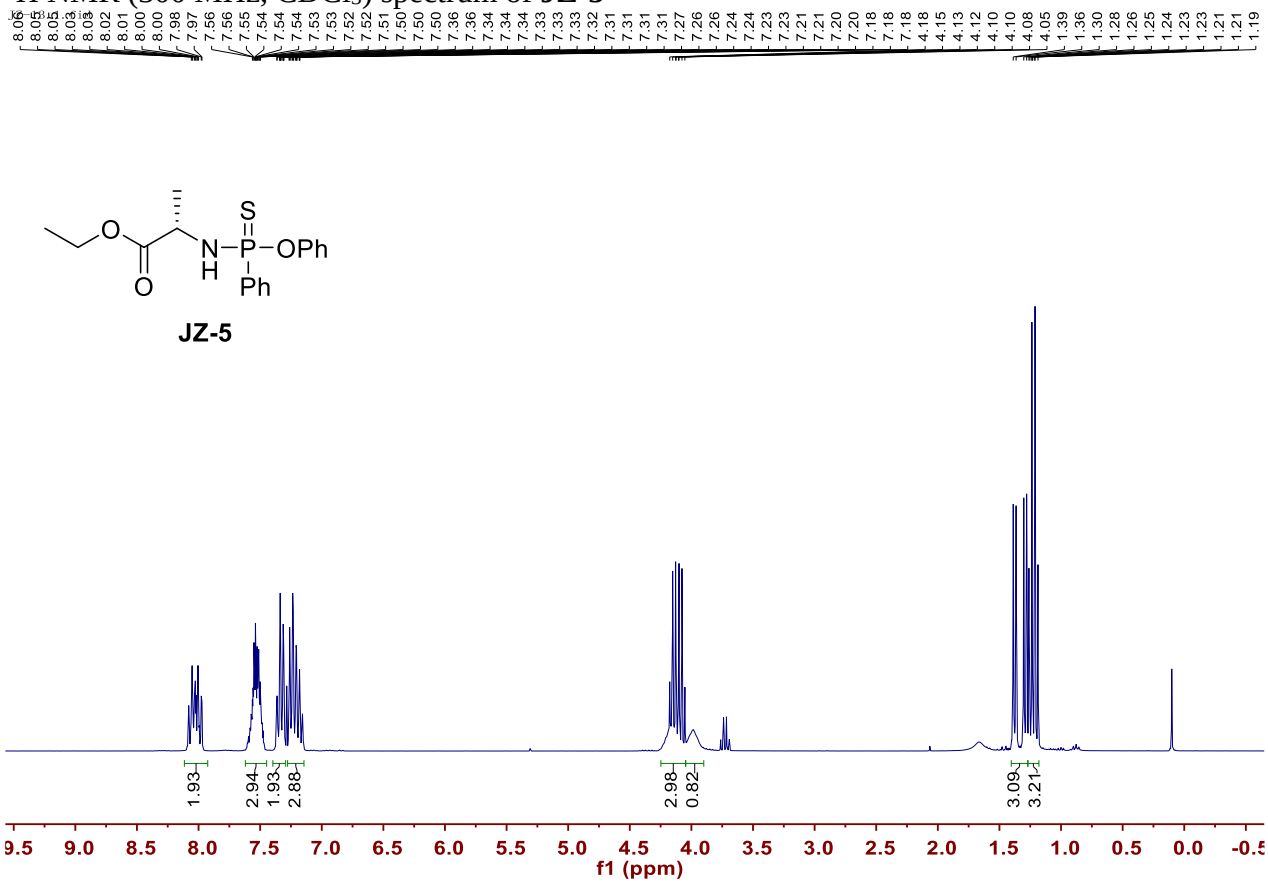
$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-4**



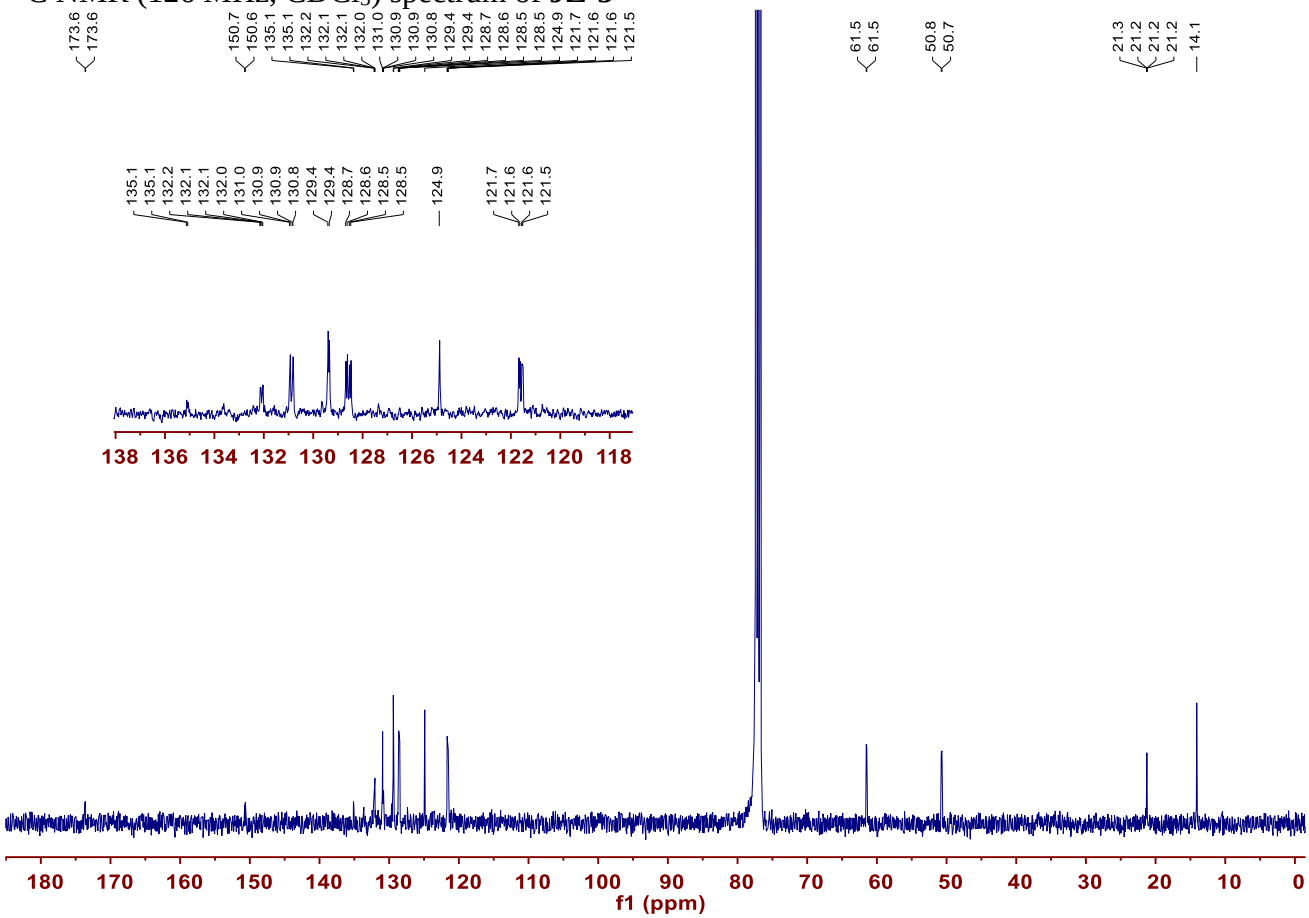

$^{31}$P NMR (202 MHz, $CDCl_3$) spectrum of **JZ-4**



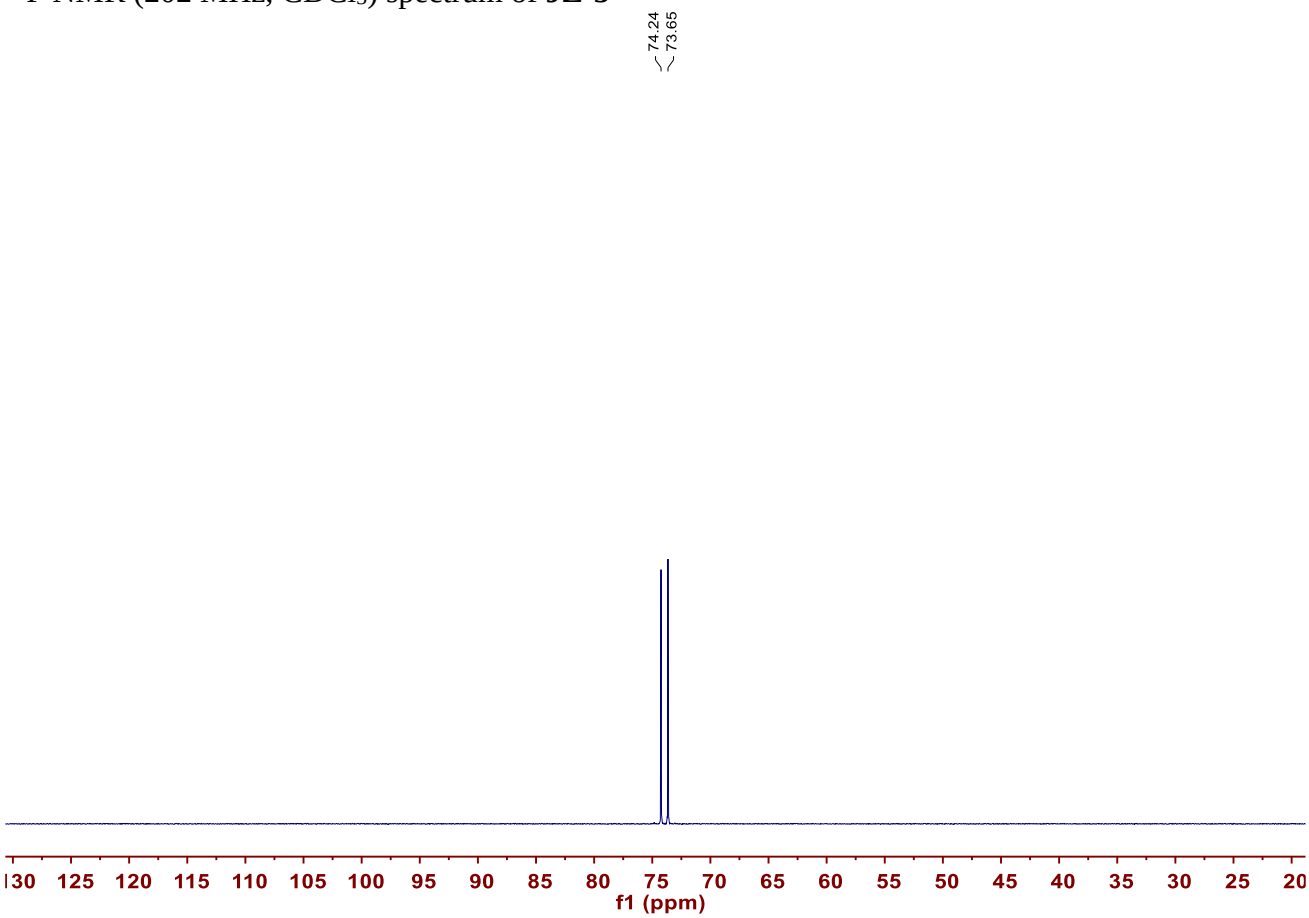
$^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of **JZ-5**



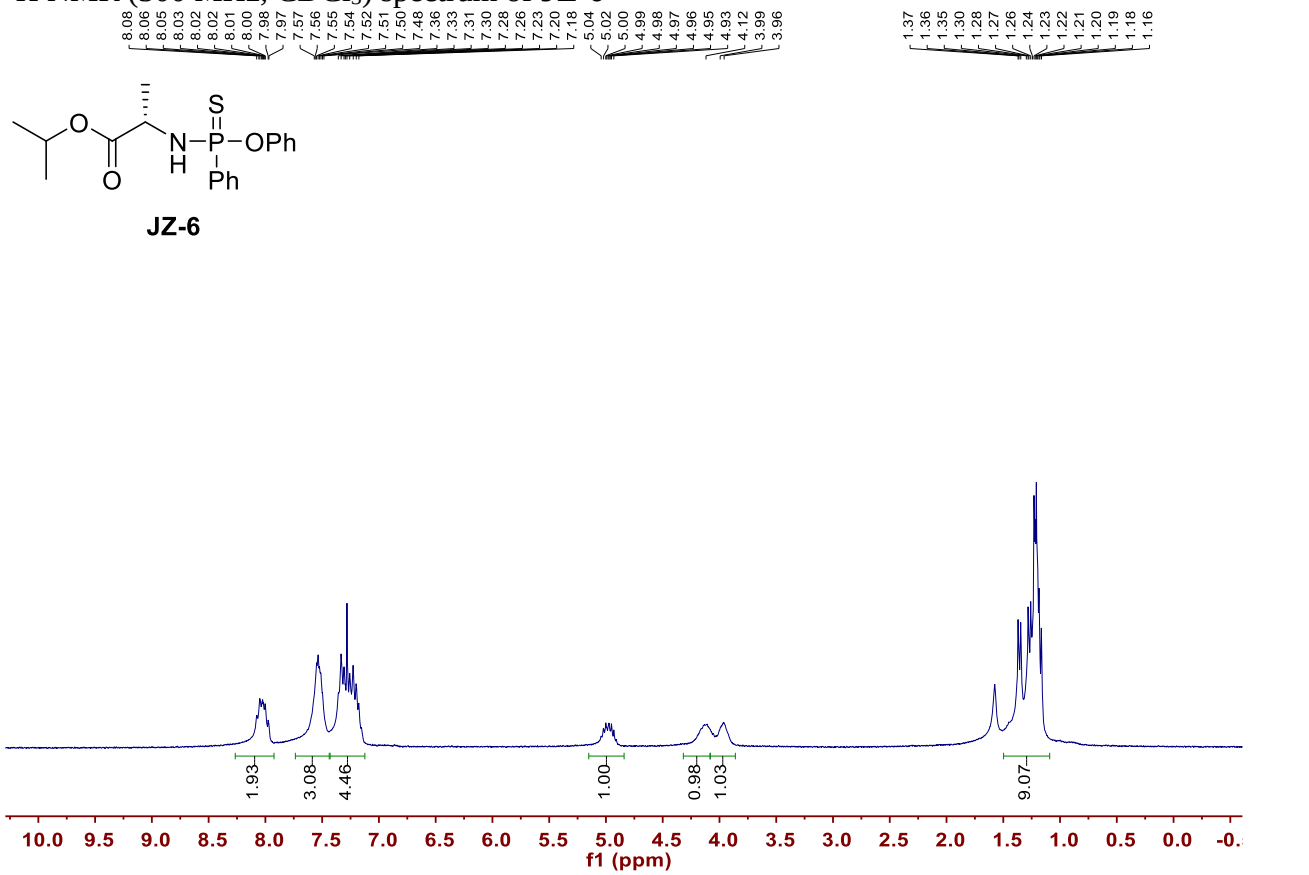

$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-5**



$^{31}$P NMR (202 MHz, $CDCl_3$) spectrum of **JZ-5**



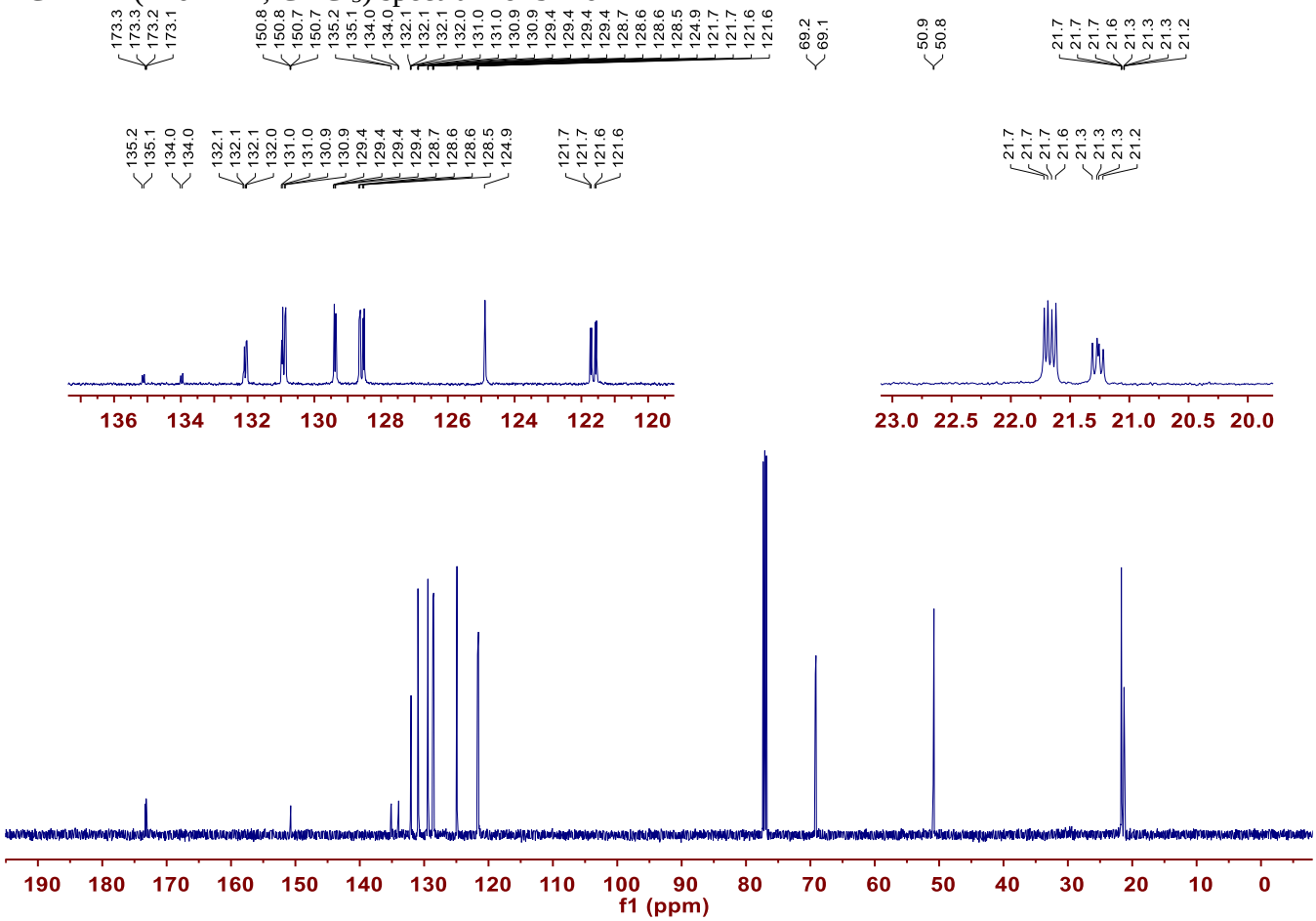

$^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of **JZ-6**



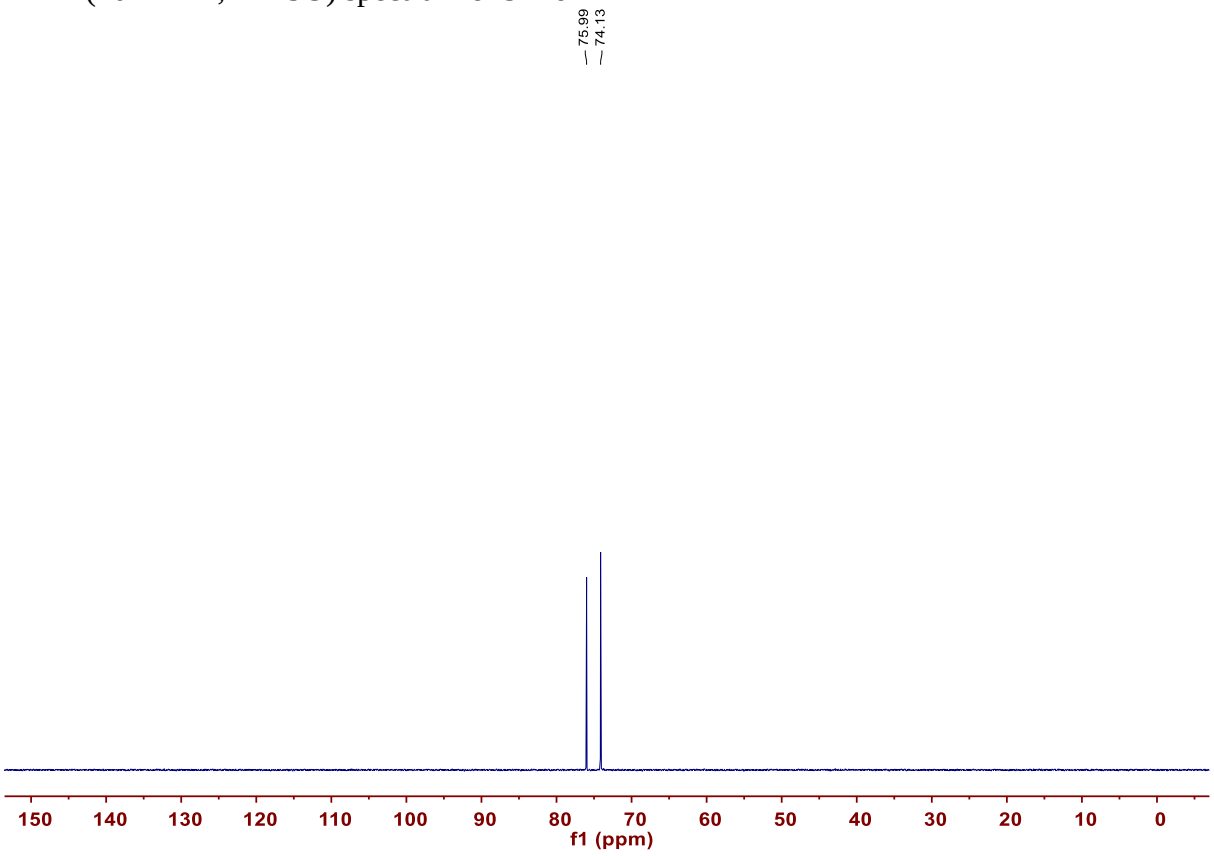



**JZ-6**

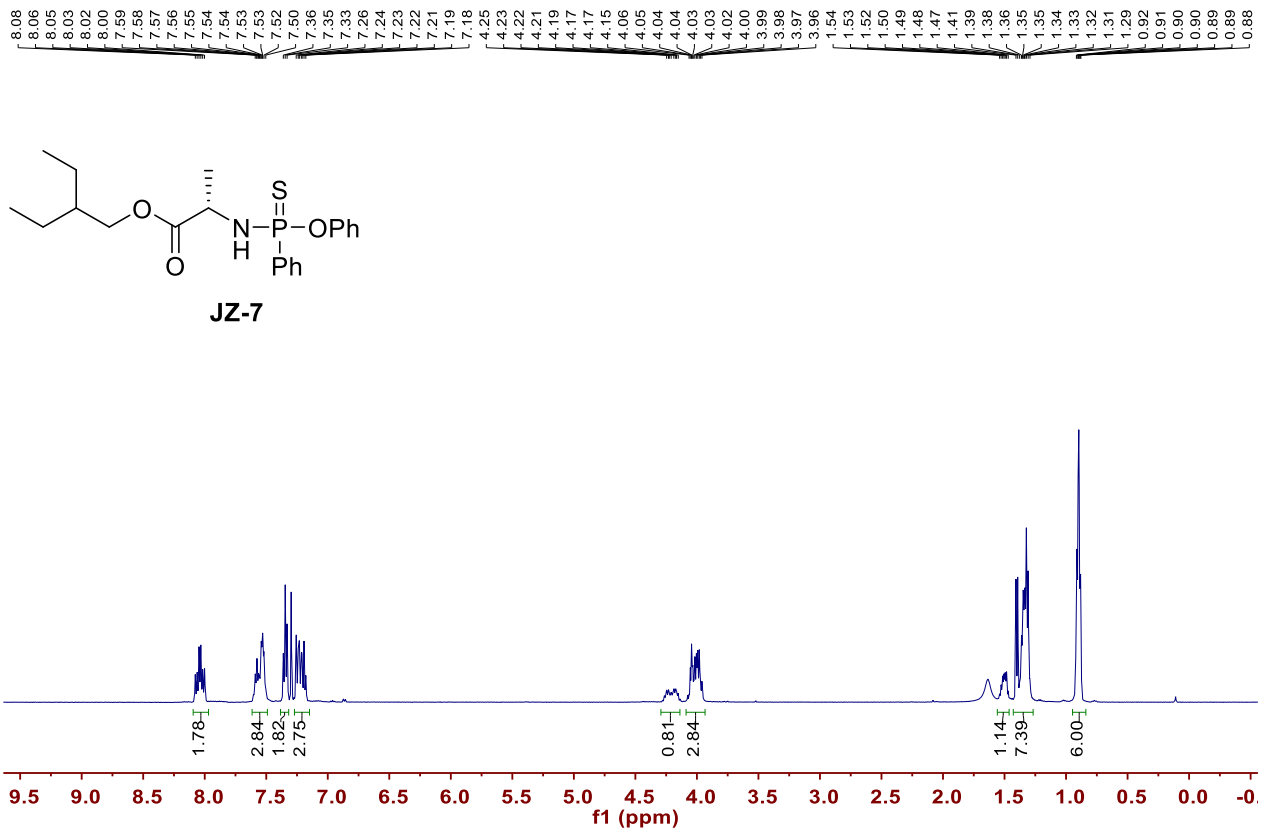
$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-6**



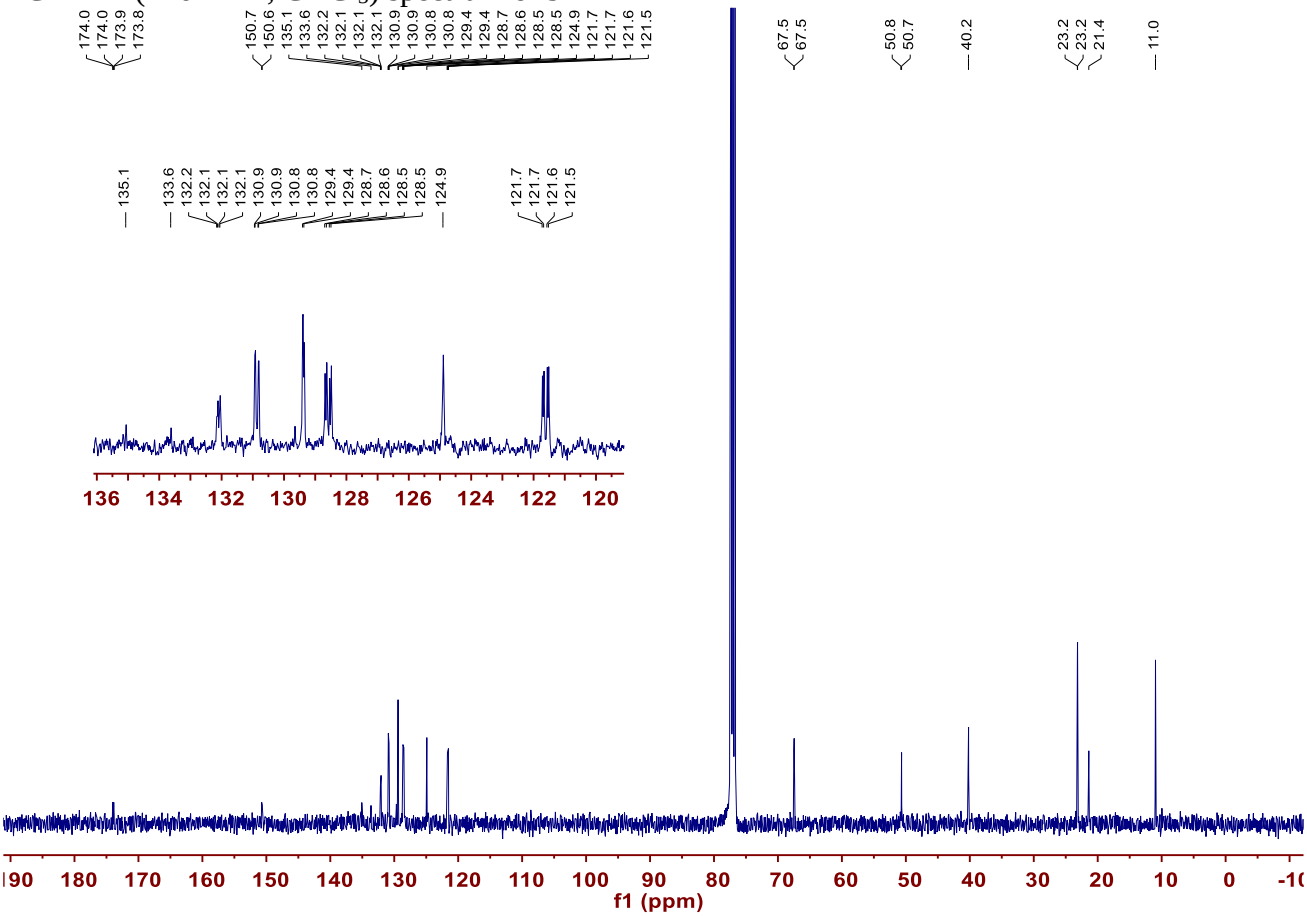

$^{31}$P NMR (202 MHz, DMSO) spectrum of **JZ-6**



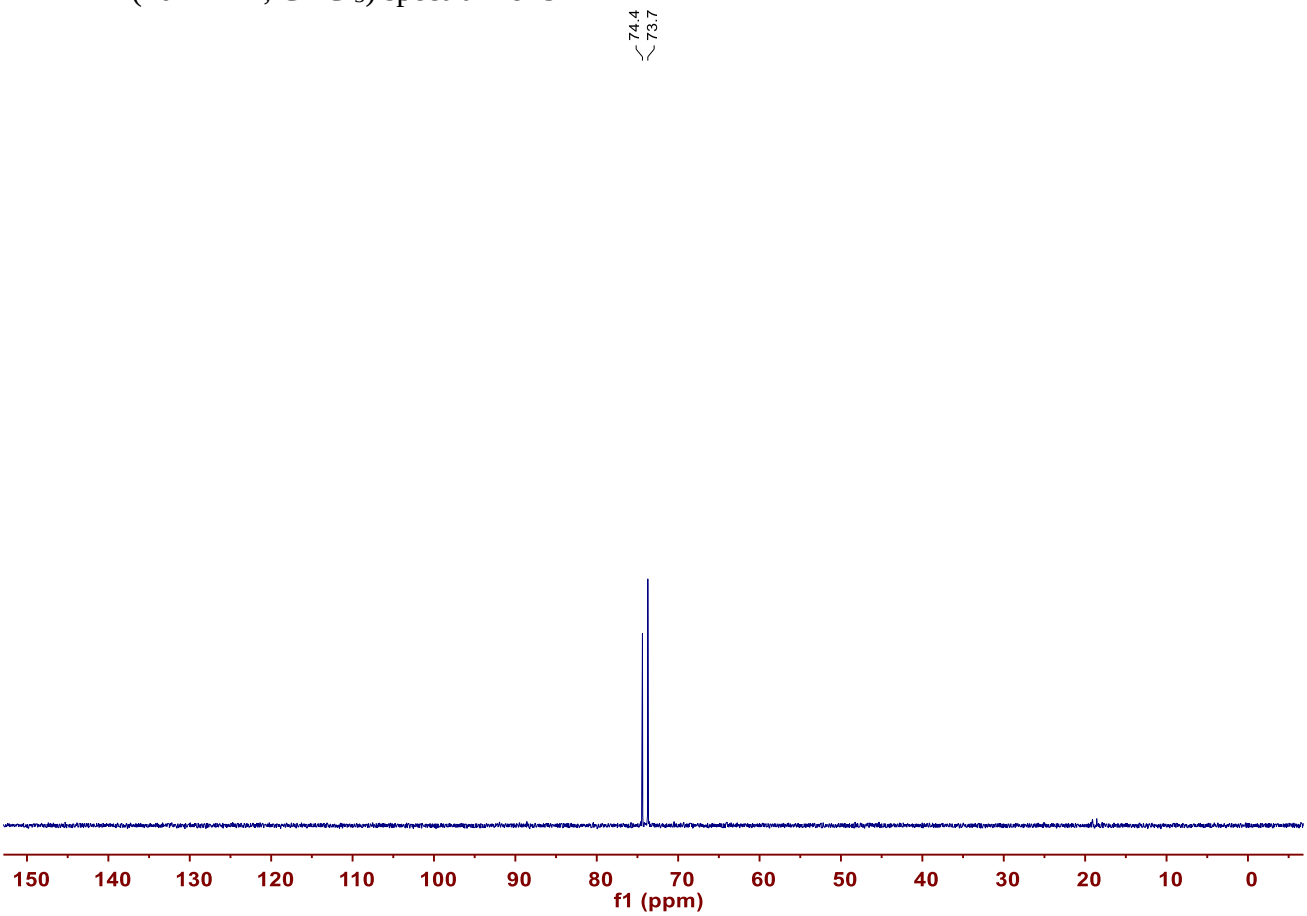
$^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of **JZ-7**



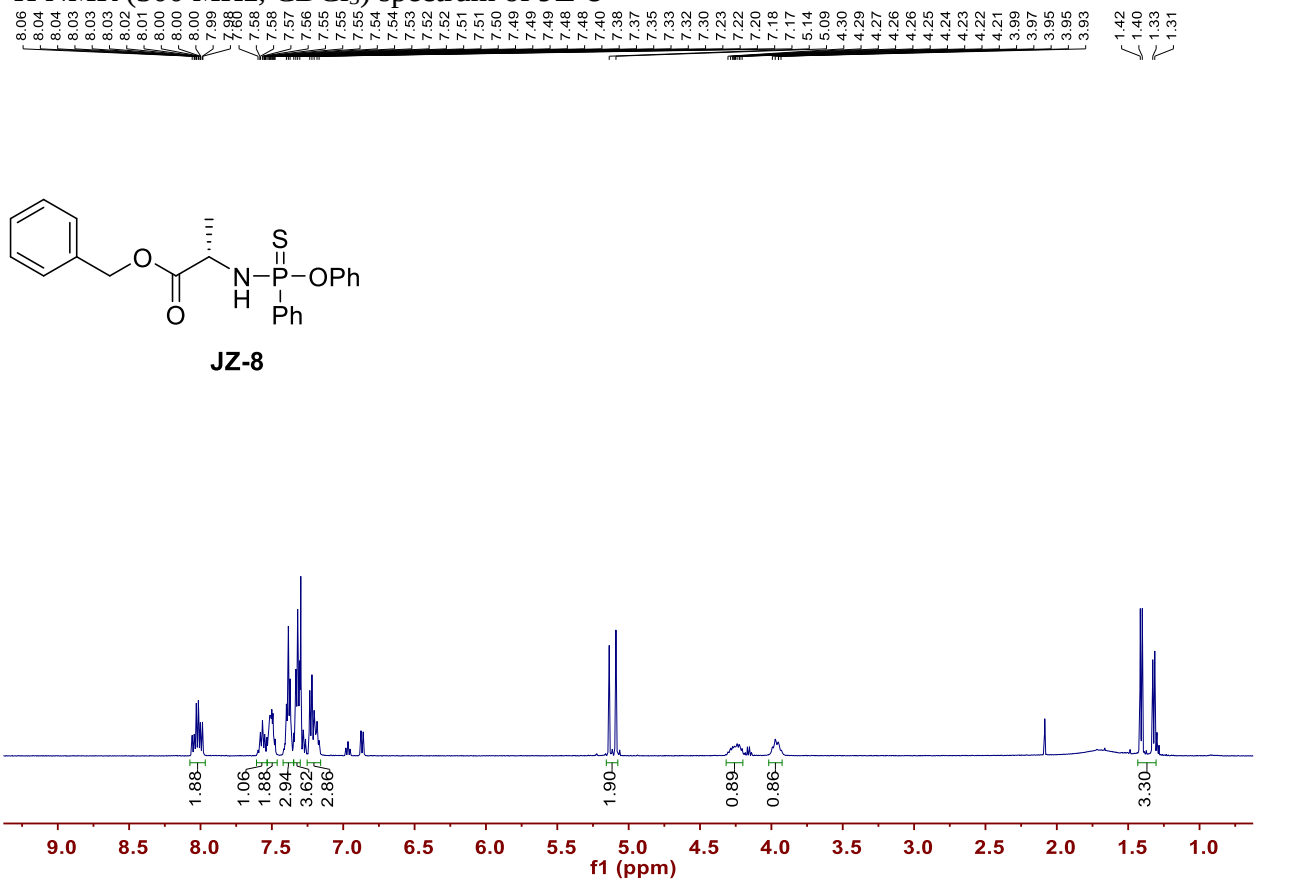

$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-7**



$^{31}P$ NMR (202 MHz, $CDCl_3$) spectrum of **JZ-7**




$^{1}H$ NMR (300 MHz, $CDCl_3$) spectrum of **JZ-8**

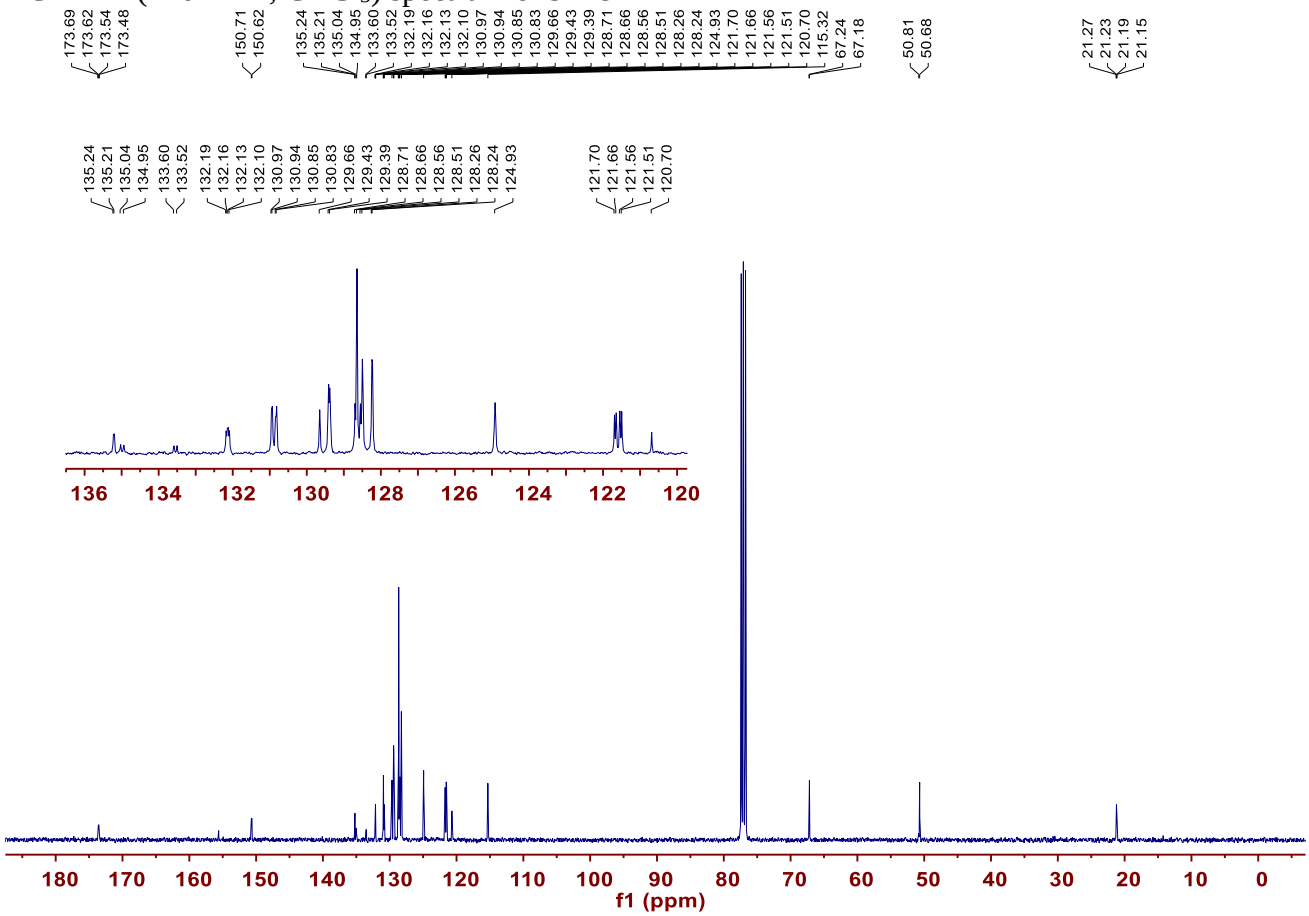




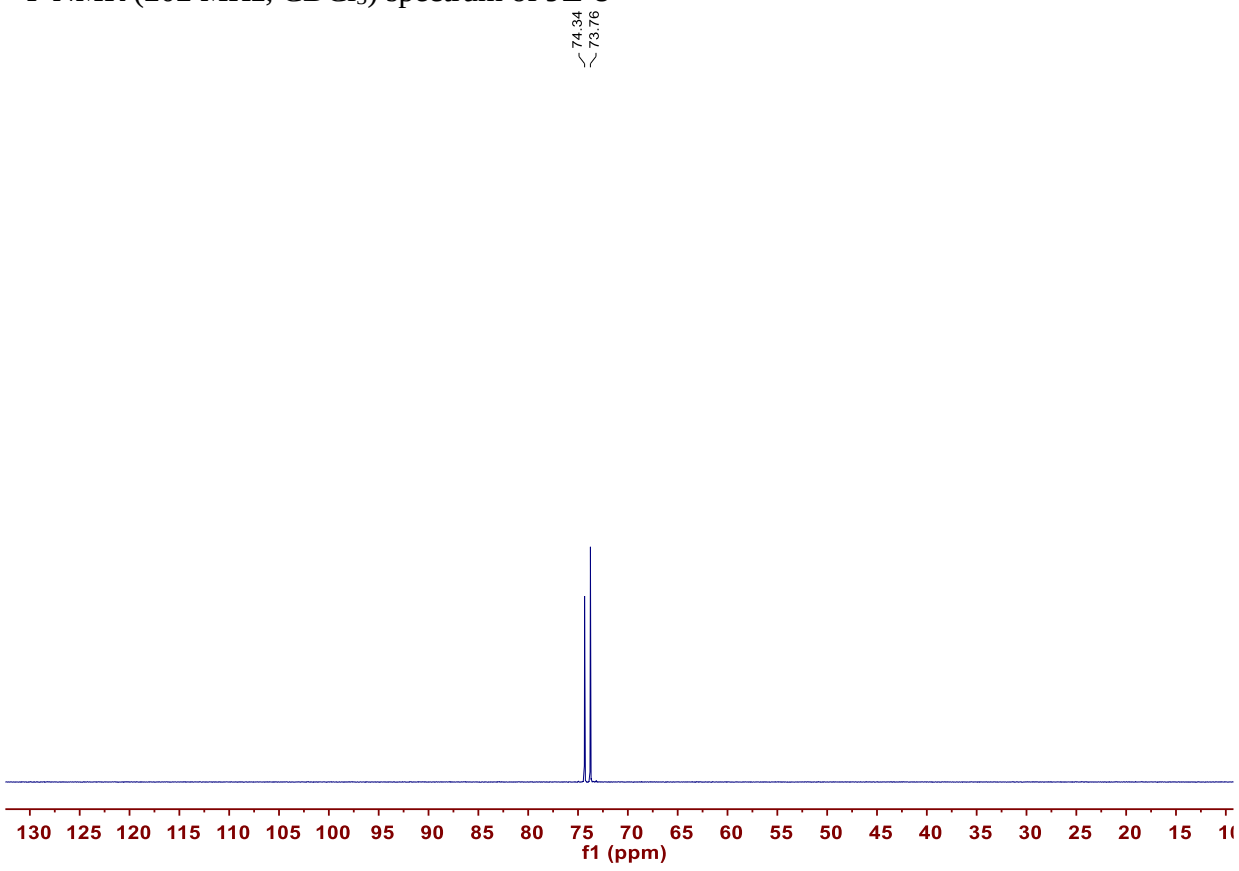


**JZ-8**

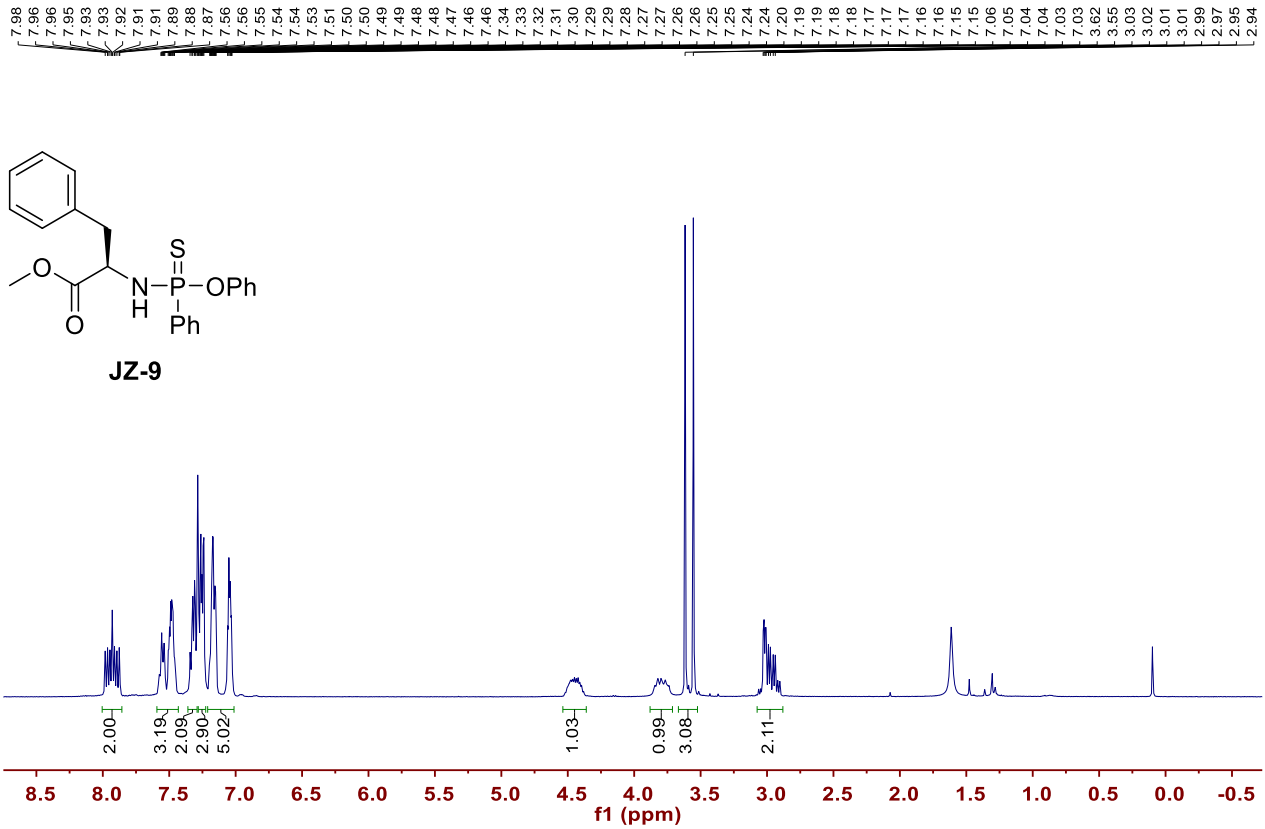
$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-8**



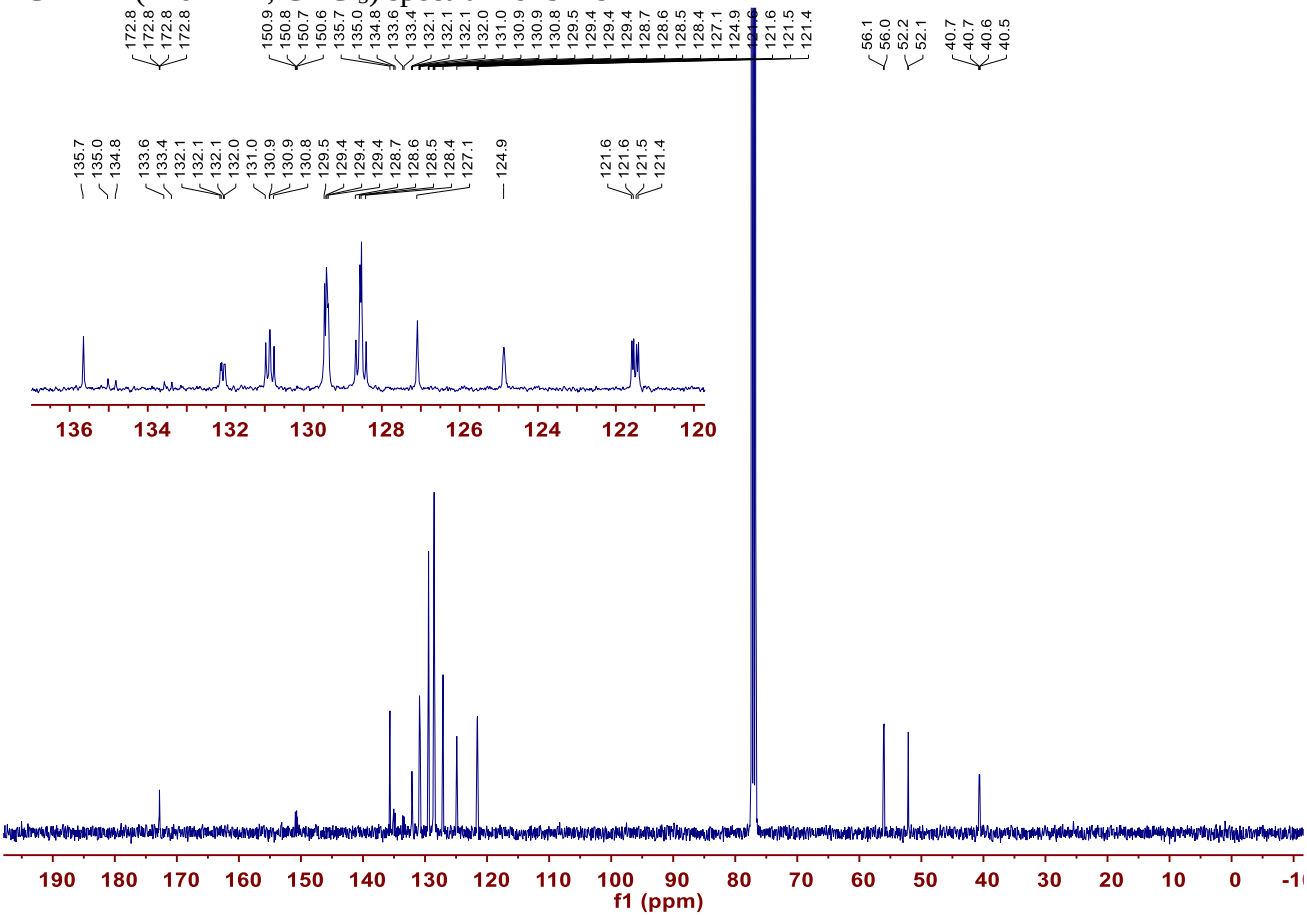

$^{31}$P NMR (202 MHz, $CDCl_3$) spectrum of **JZ-8**



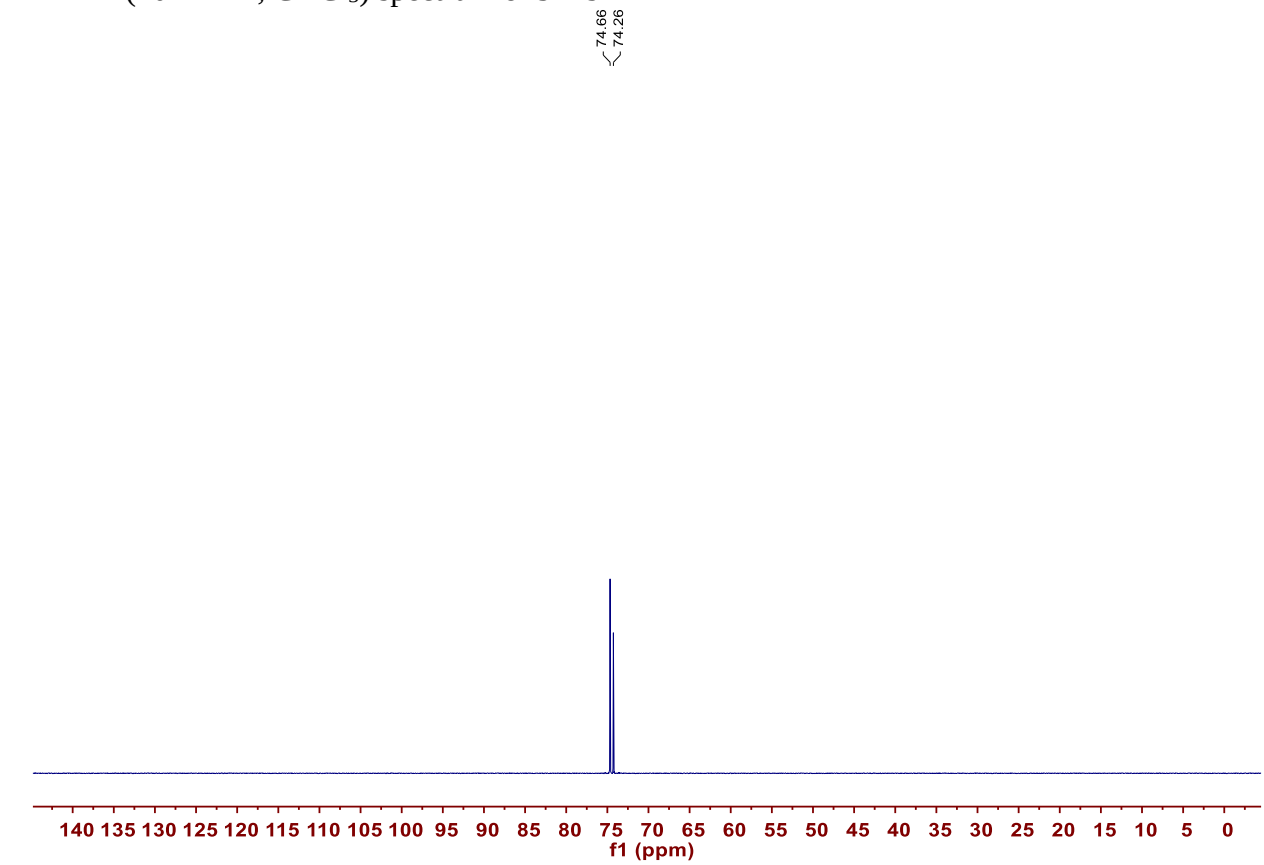
$^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of **JZ-9**



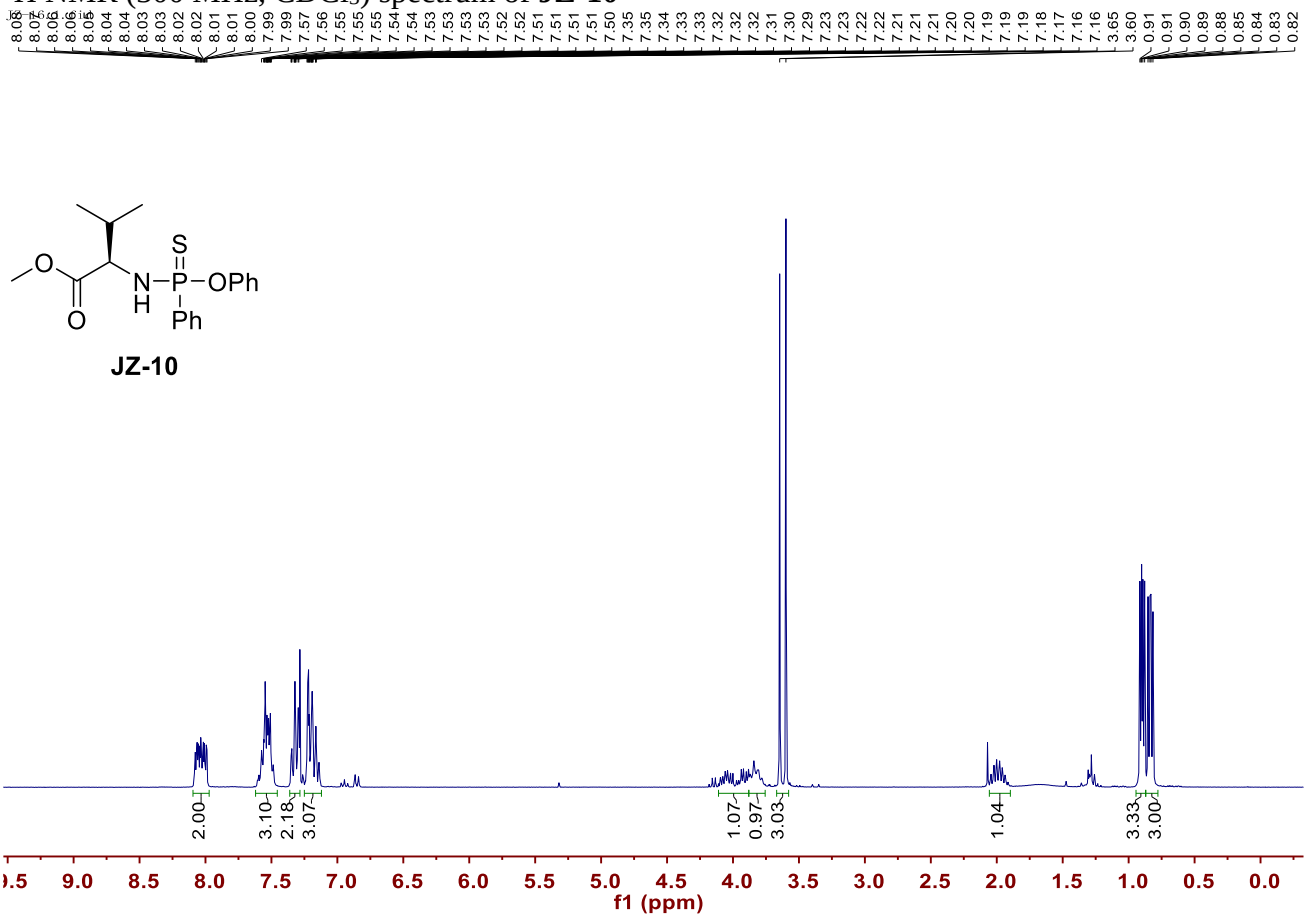

$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-9**



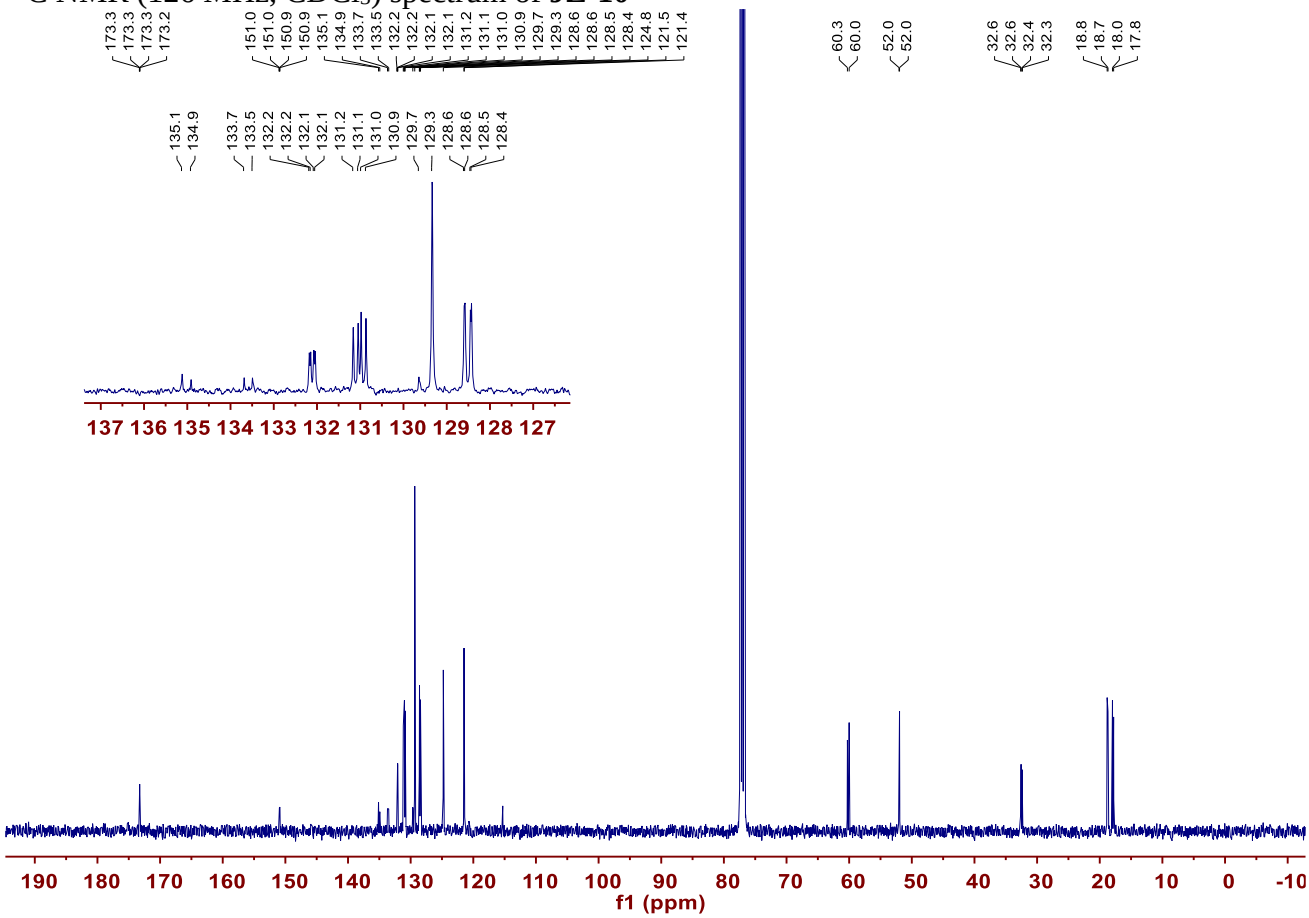
$^{31}P$ NMR (202 MHz, $CDCl_3$) spectrum of **JZ-9**



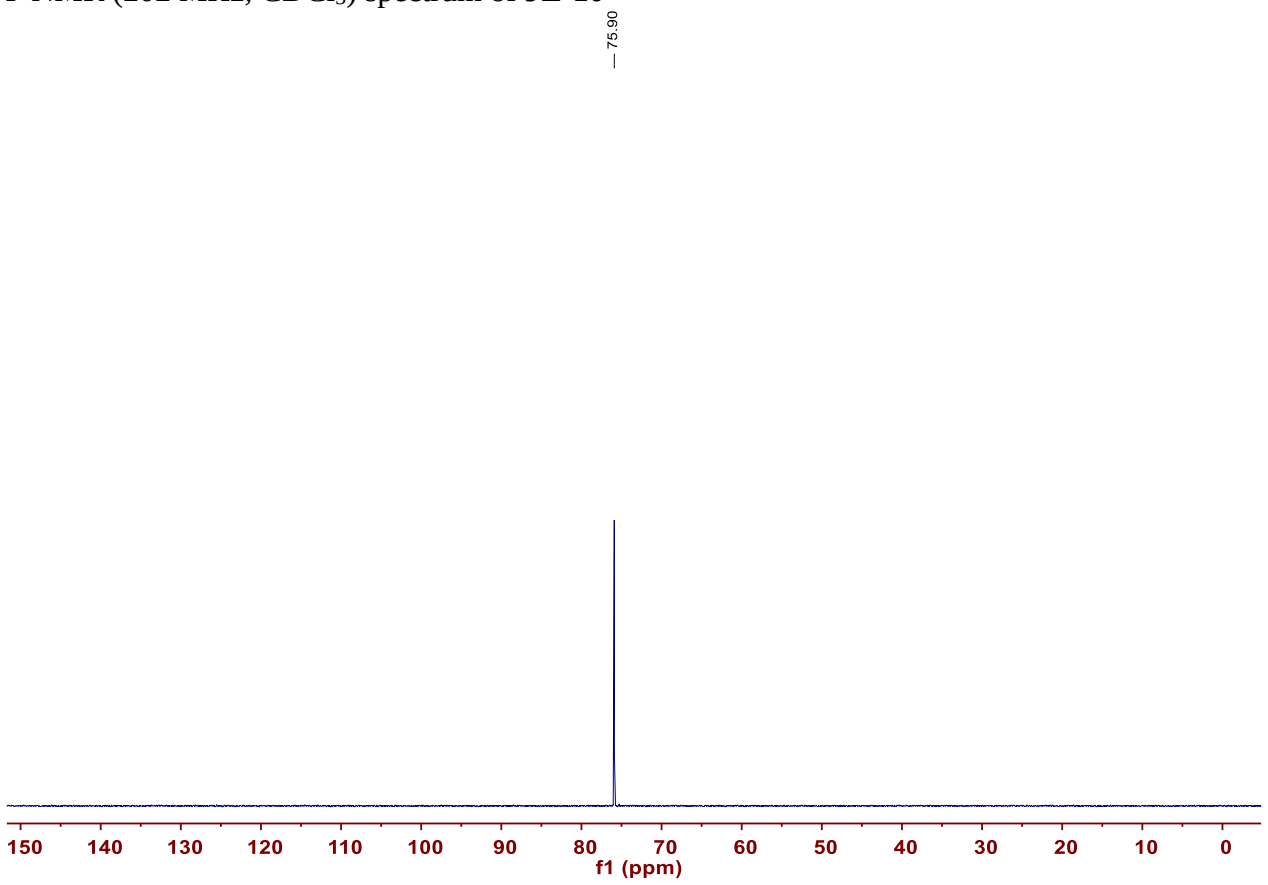

$^{1}H$ NMR (300 MHz, $CDCl_3$) spectrum of **JZ-10**



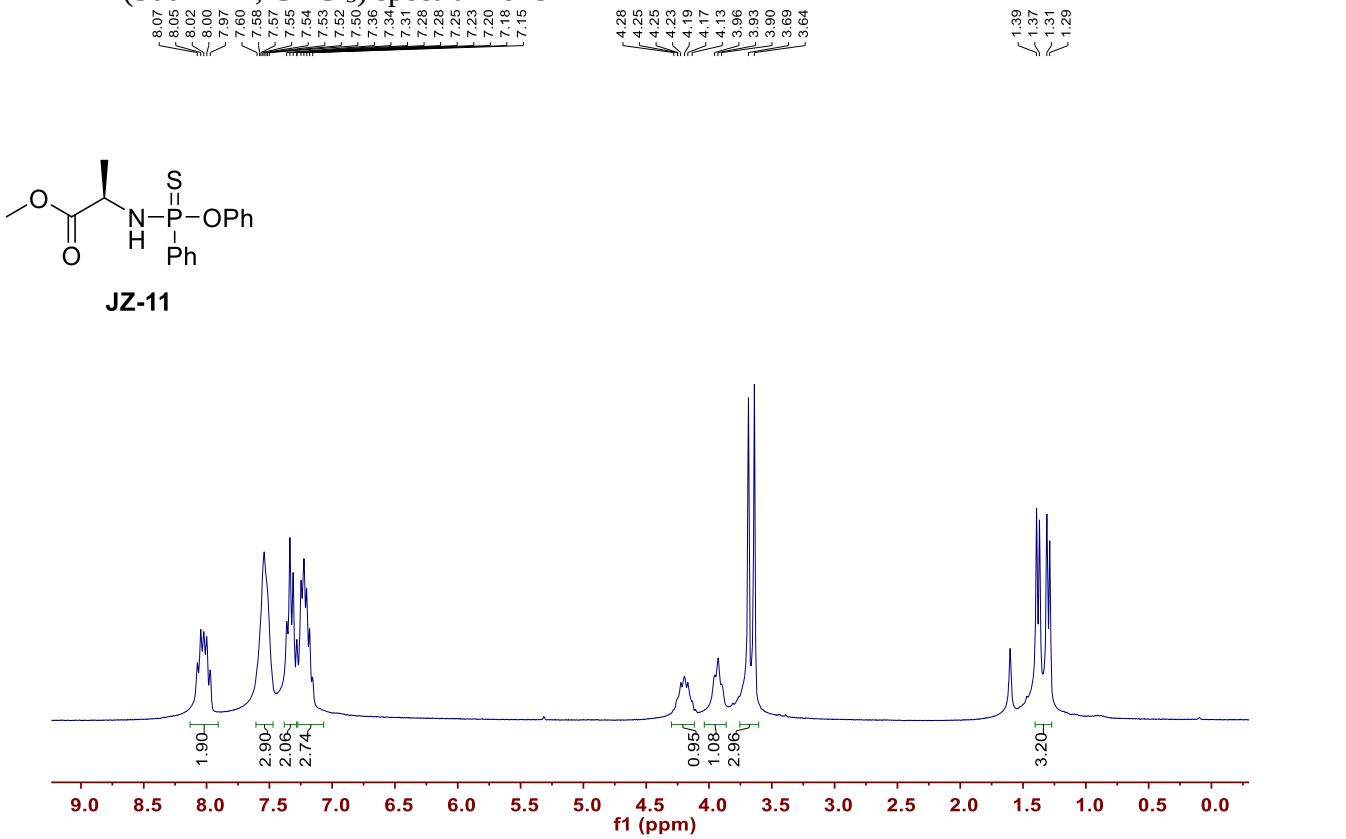
$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-10**



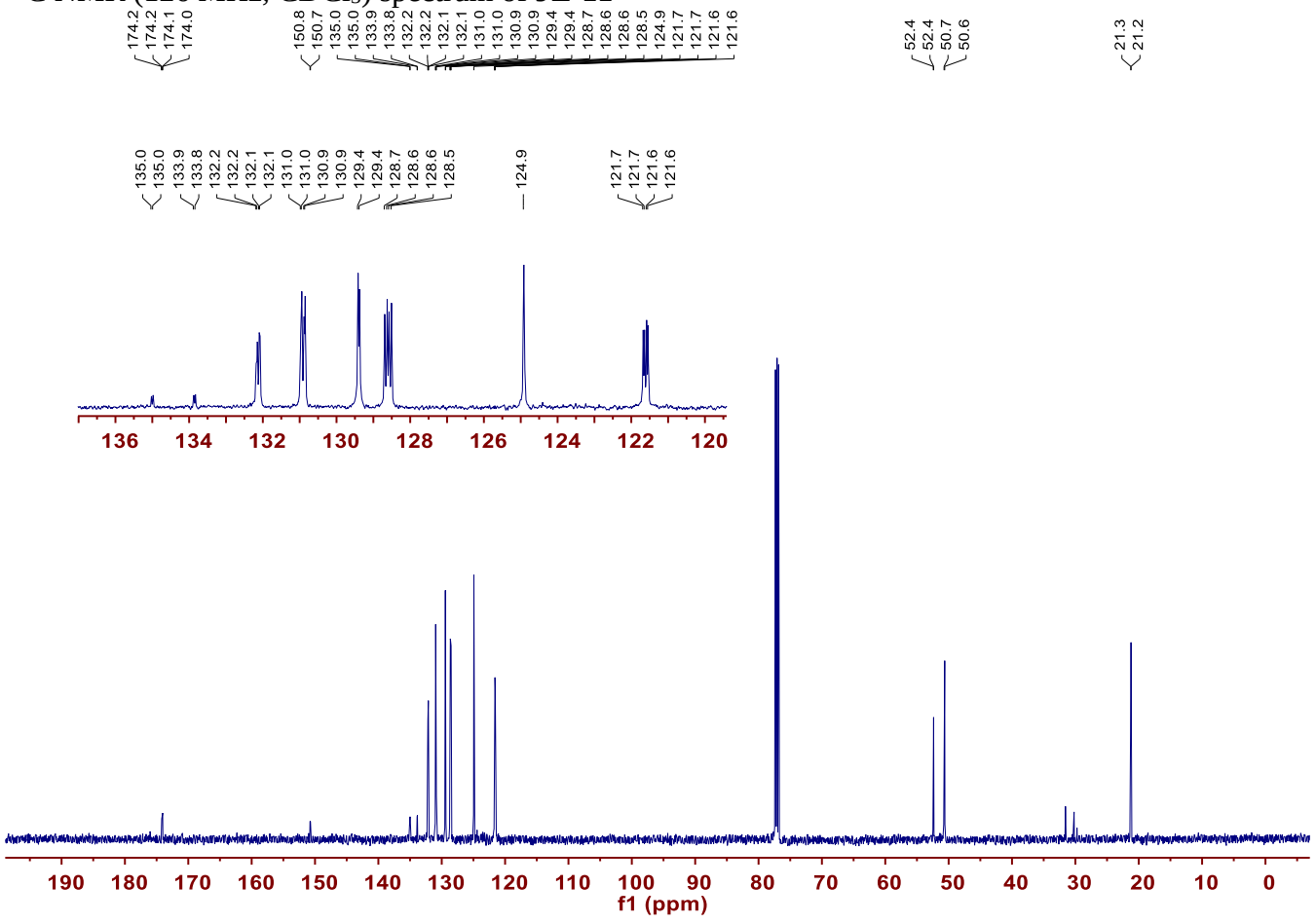

$^{31}$P NMR (202 MHz, $CDCl_3$) spectrum of **JZ-10**



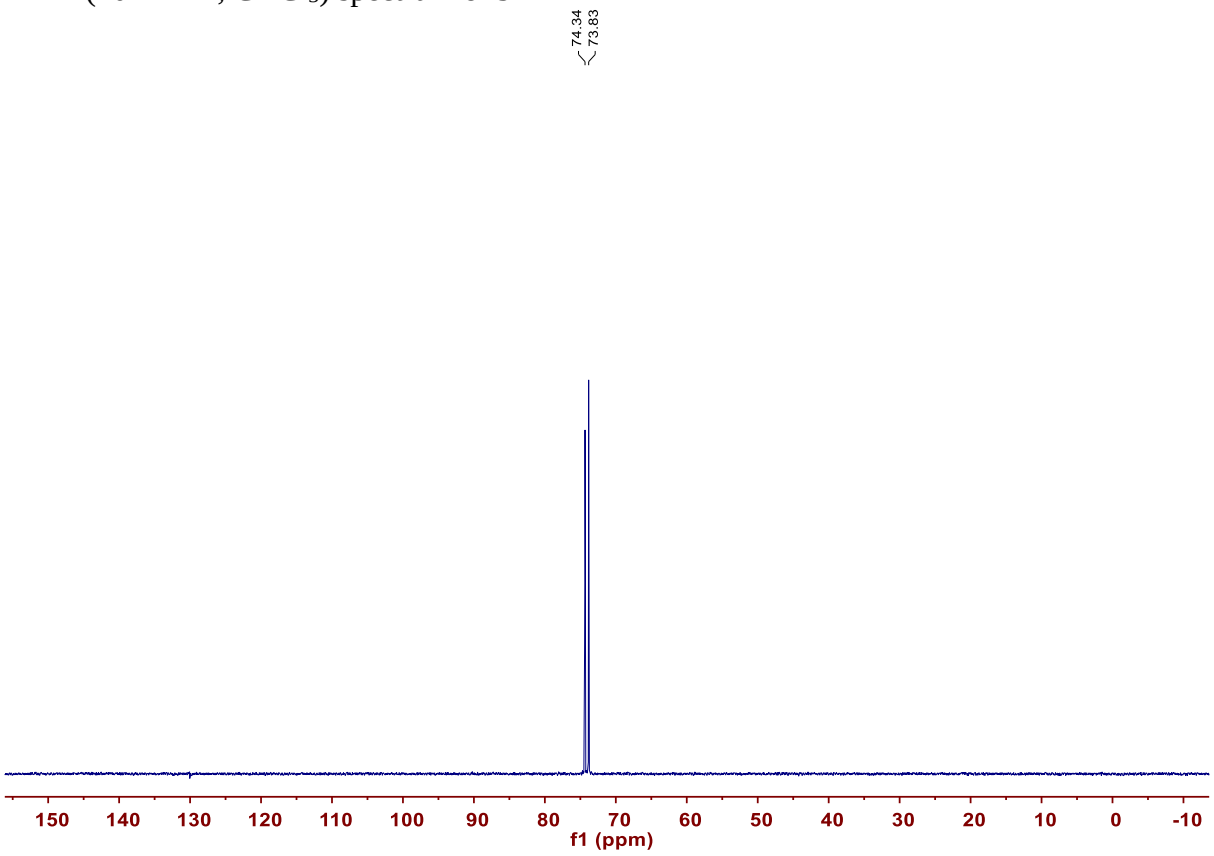
$^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of **JZ-11**



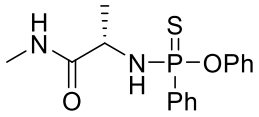

$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-11**



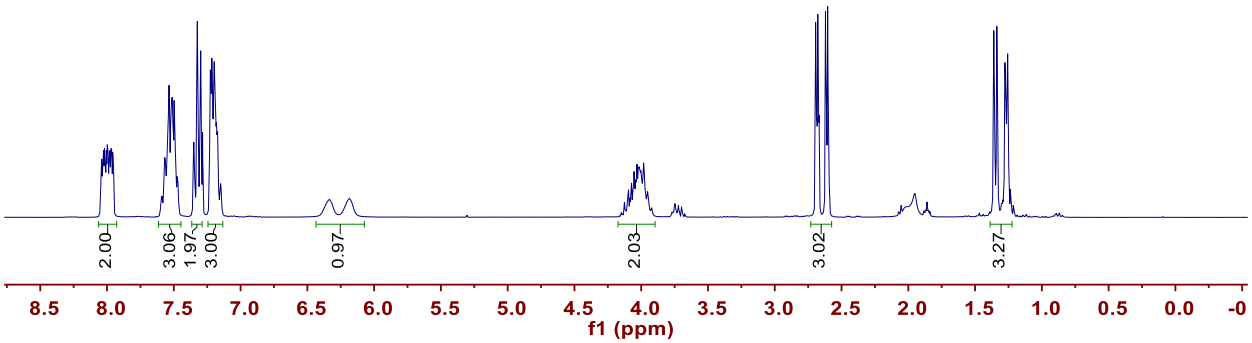
$^{31}$P NMR (202 MHz, $CDCl_3$) spectrum of **JZ-11**



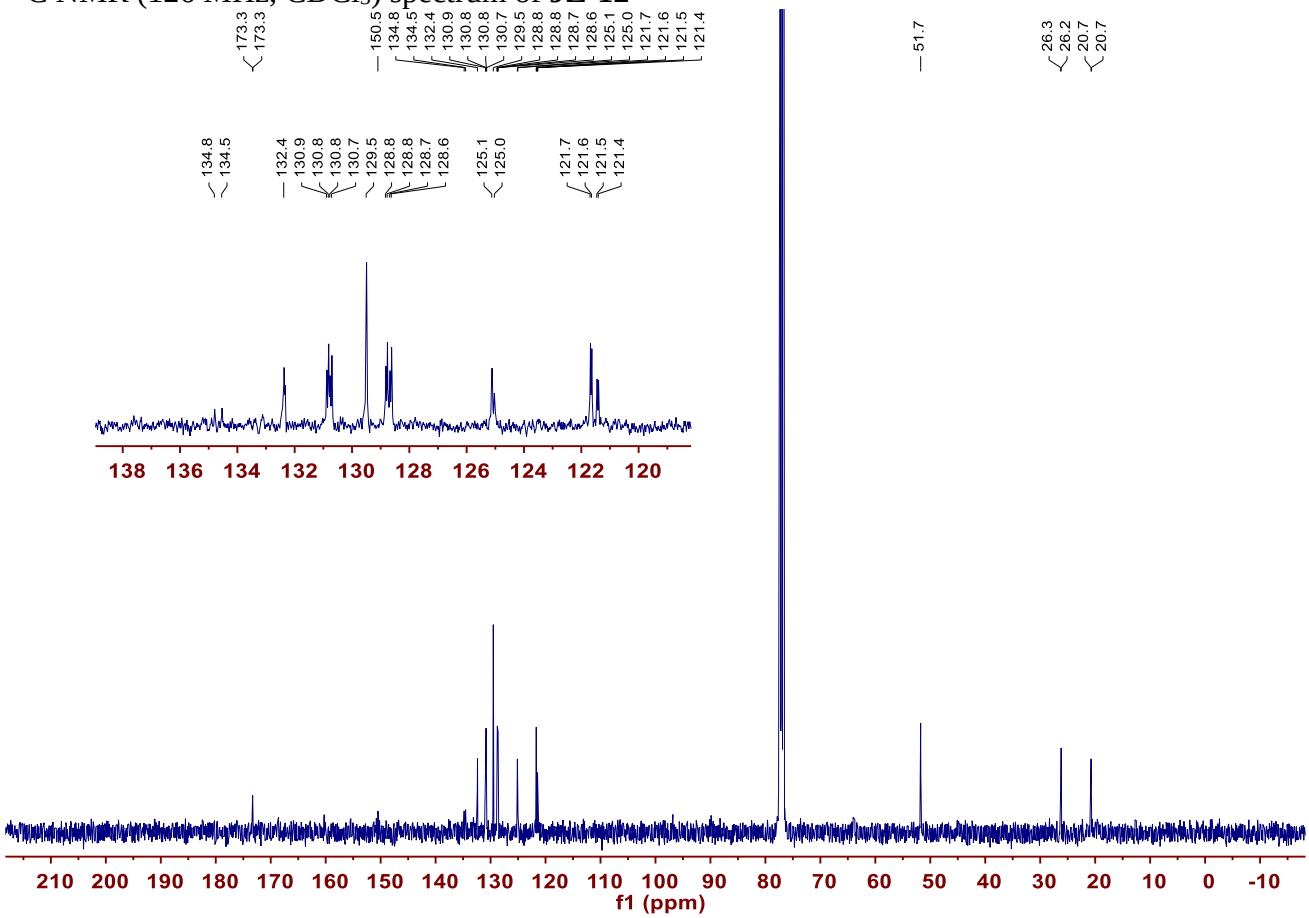

$^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of **JZ-12**



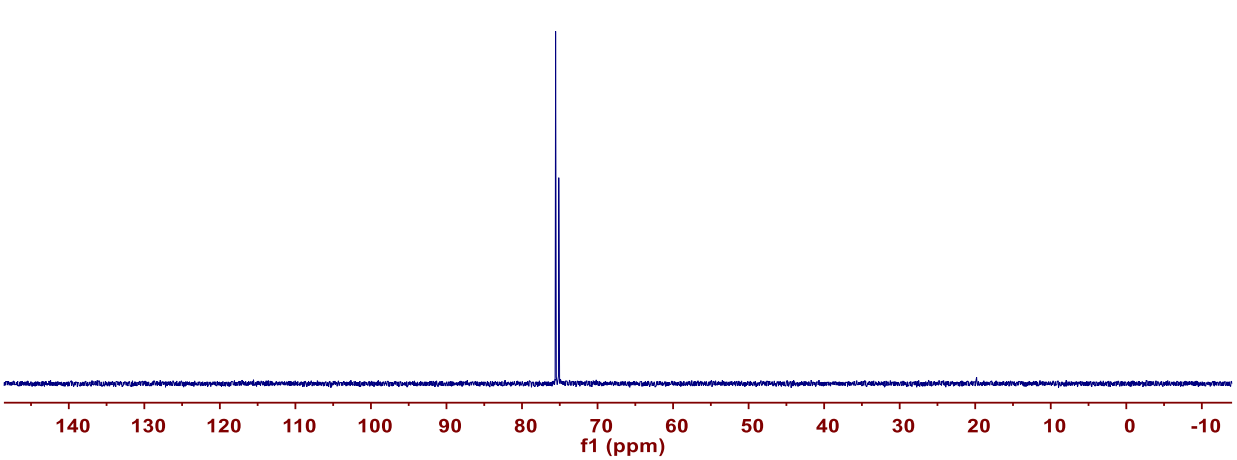
**JZ-12**



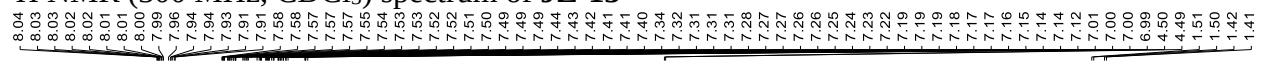
$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-12**

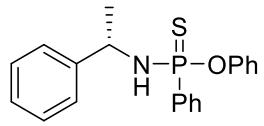



$^{31}$P NMR (202 MHz, $CDCl_3$) spectrum of **JZ-12**

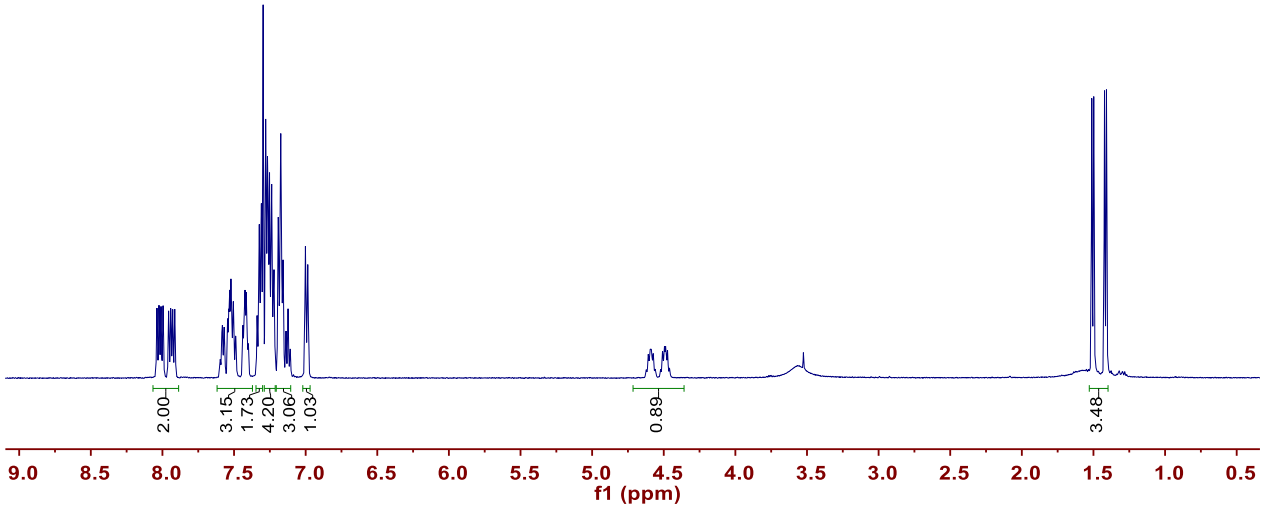


$^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of **JZ-13**

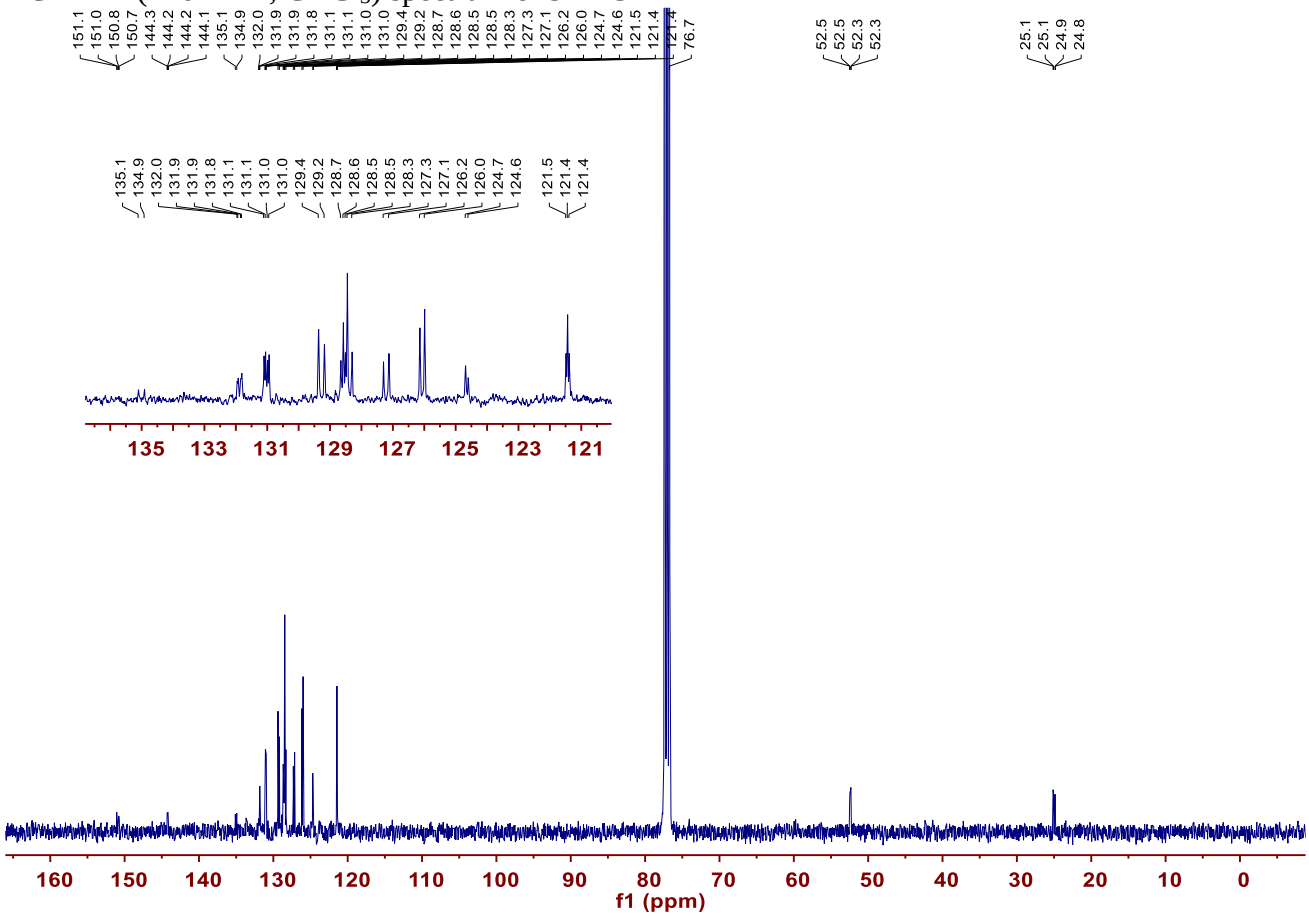




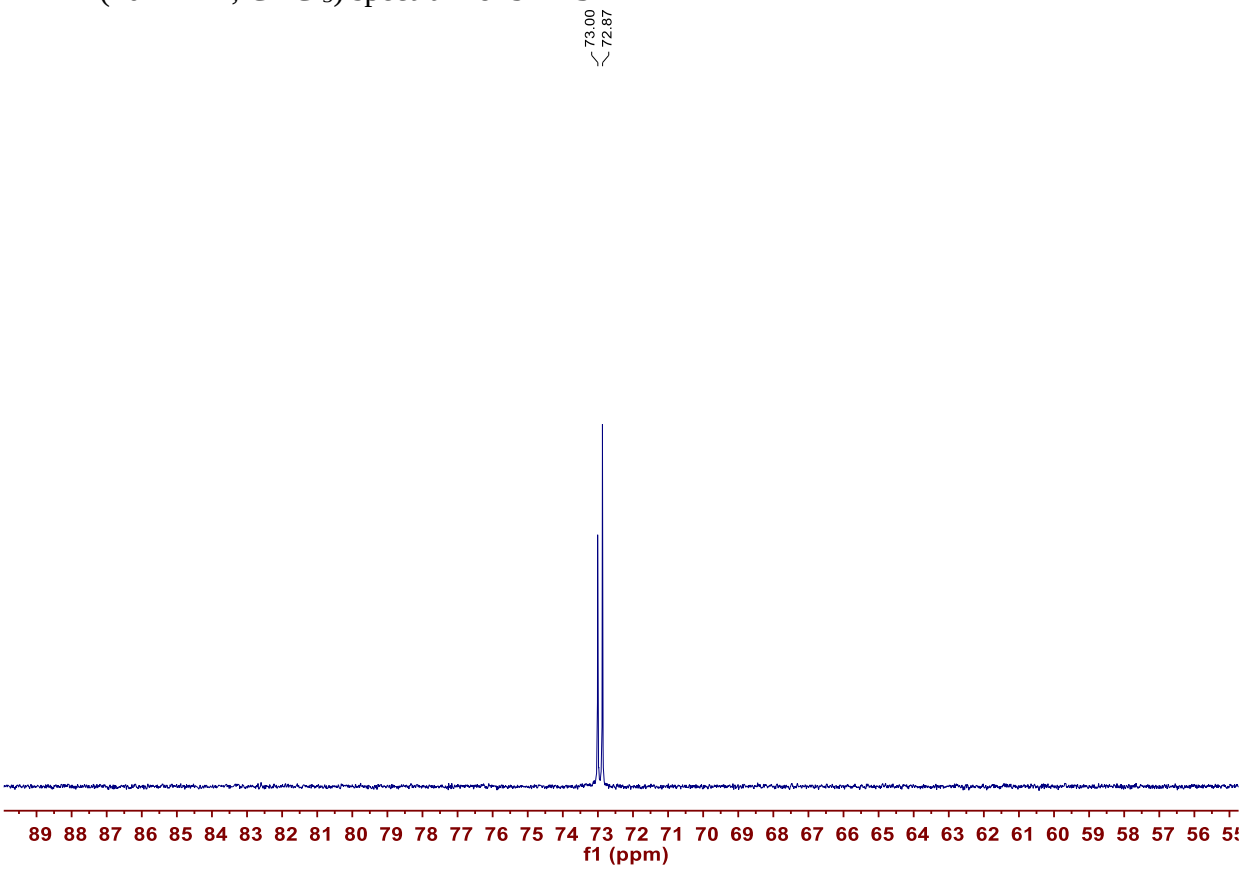

**JZ-13**



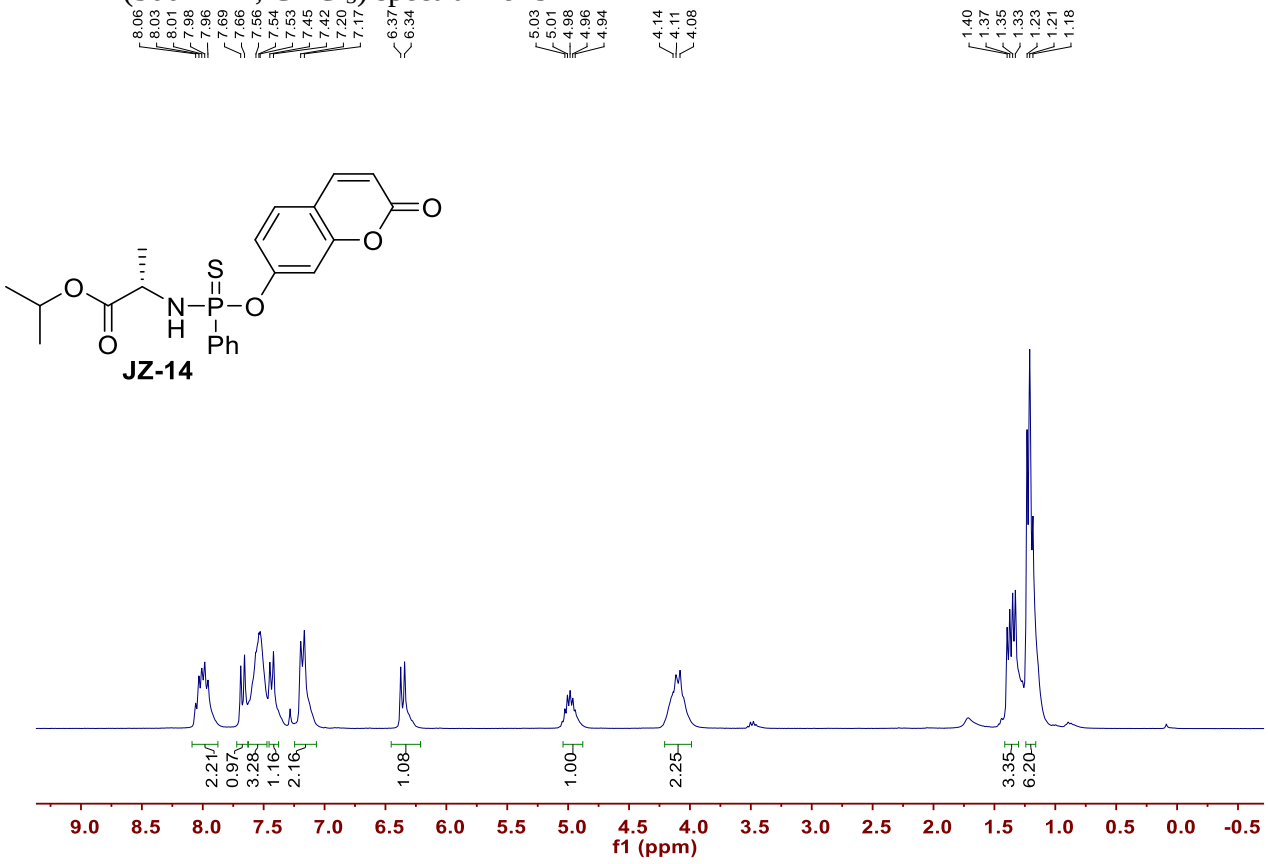

$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-13**



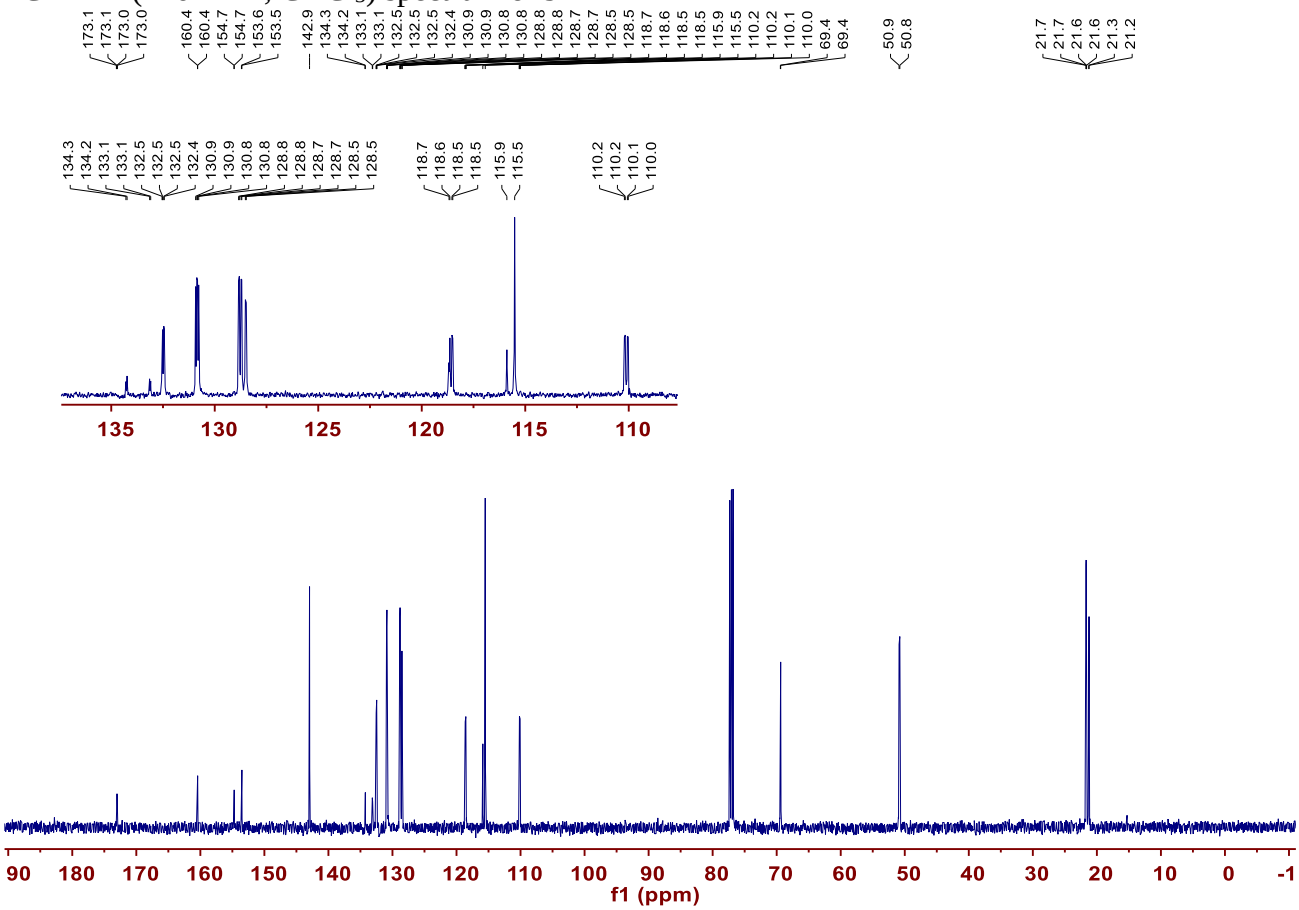
$^{31}$P NMR (202 MHz, $CDCl_3$) spectrum of **JZ-13**



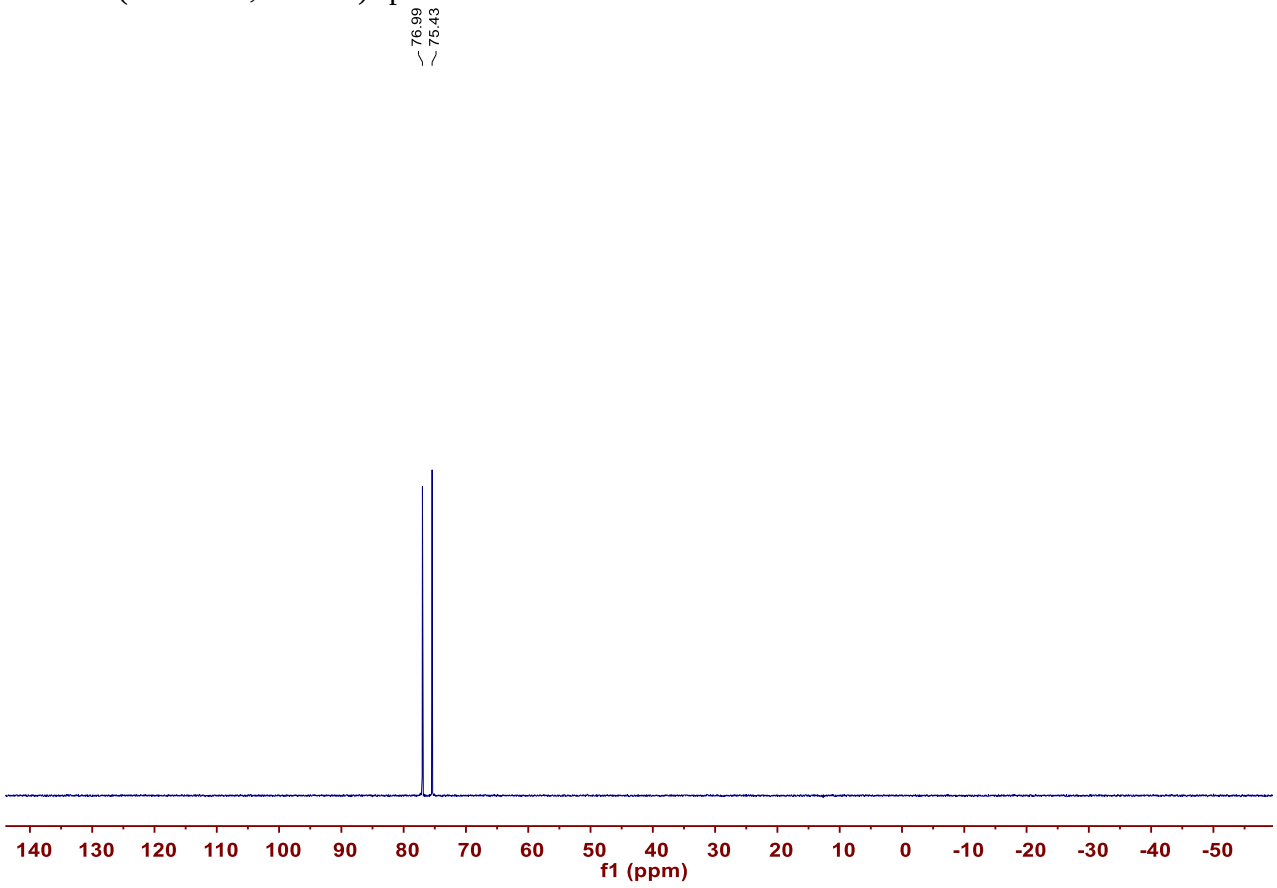

$^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of **JZ-14**



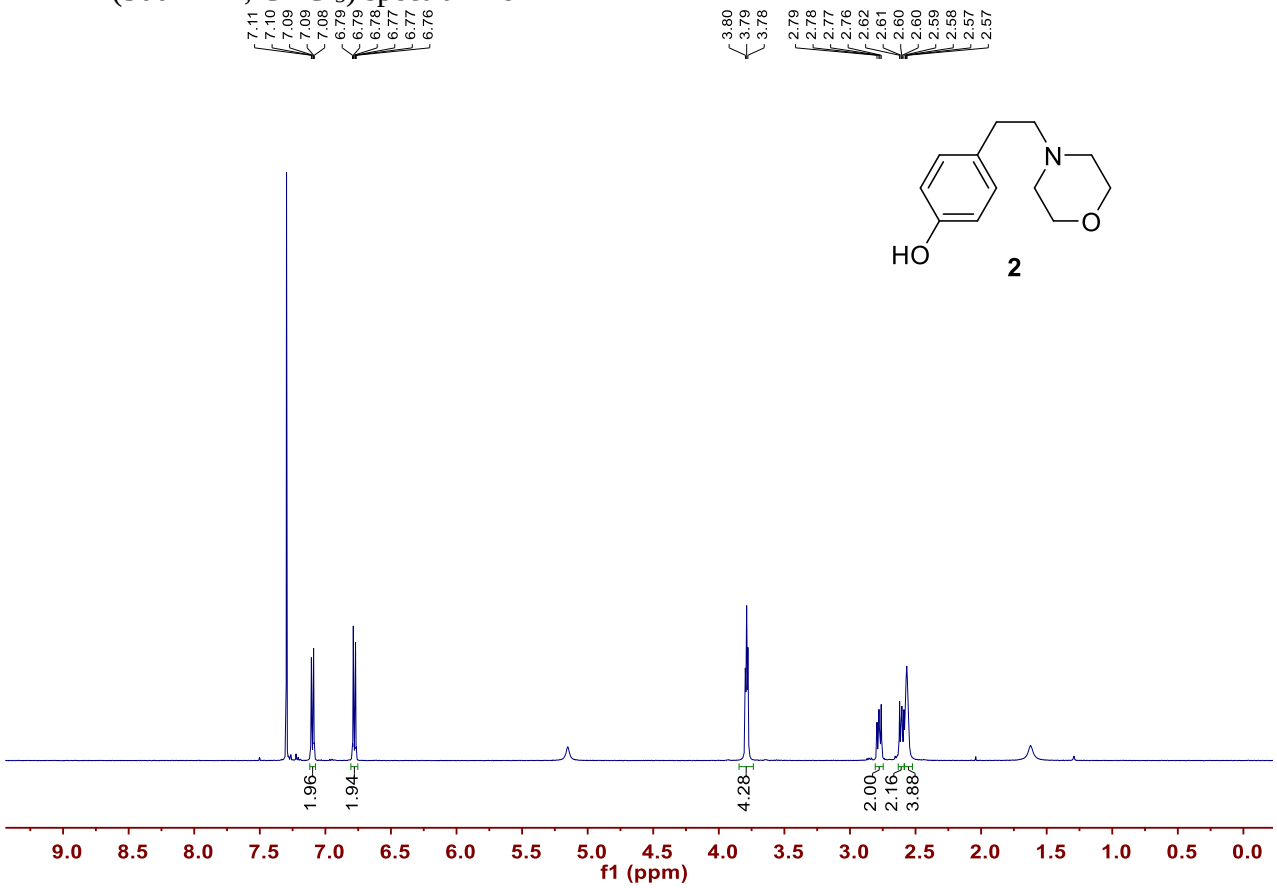
$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-14**



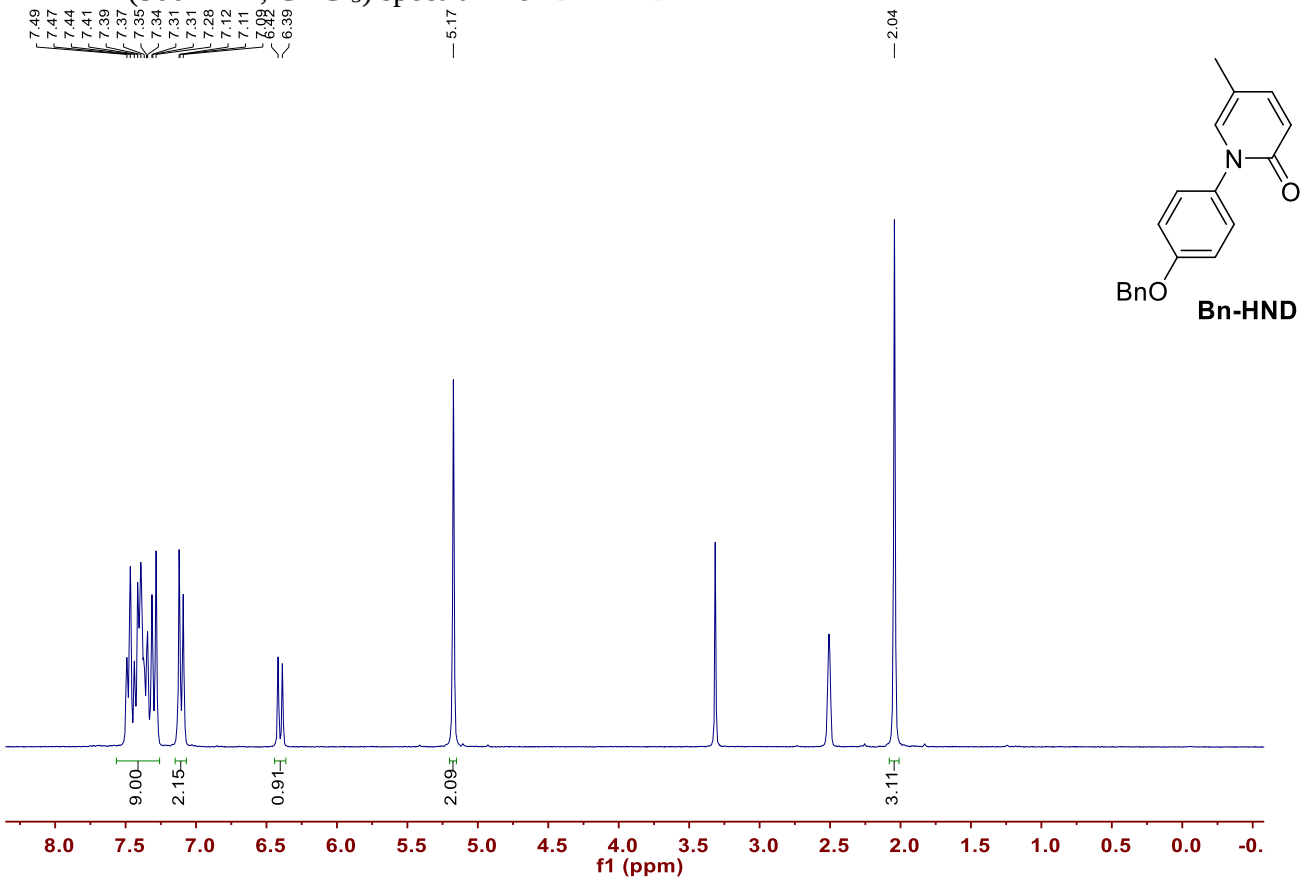

$^{31}$P NMR (202 MHz, DMSO) spectrum of **JZ-14**



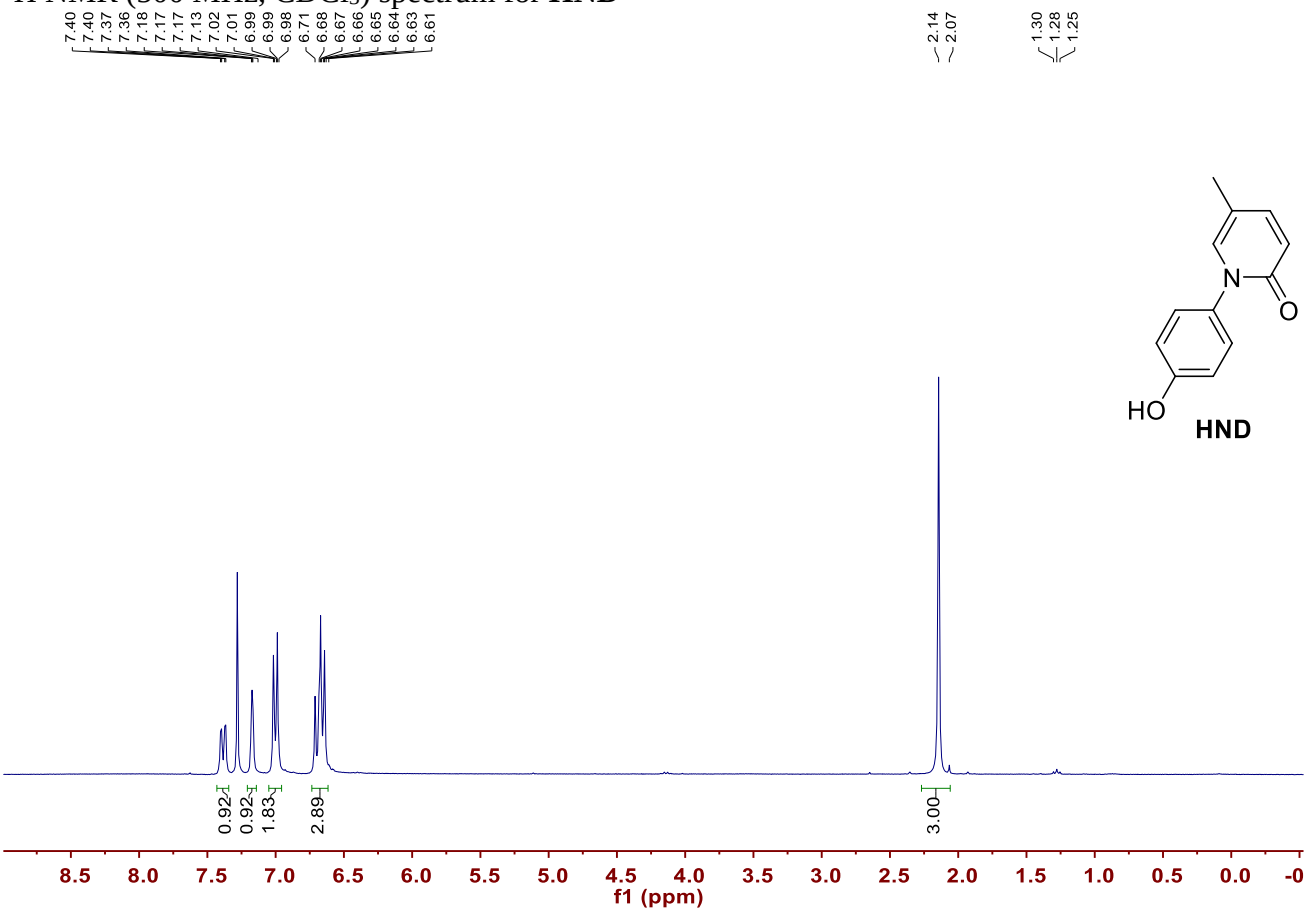
$^1$H NMR (500 MHz, $CDCl_3$) spectrum for **2**



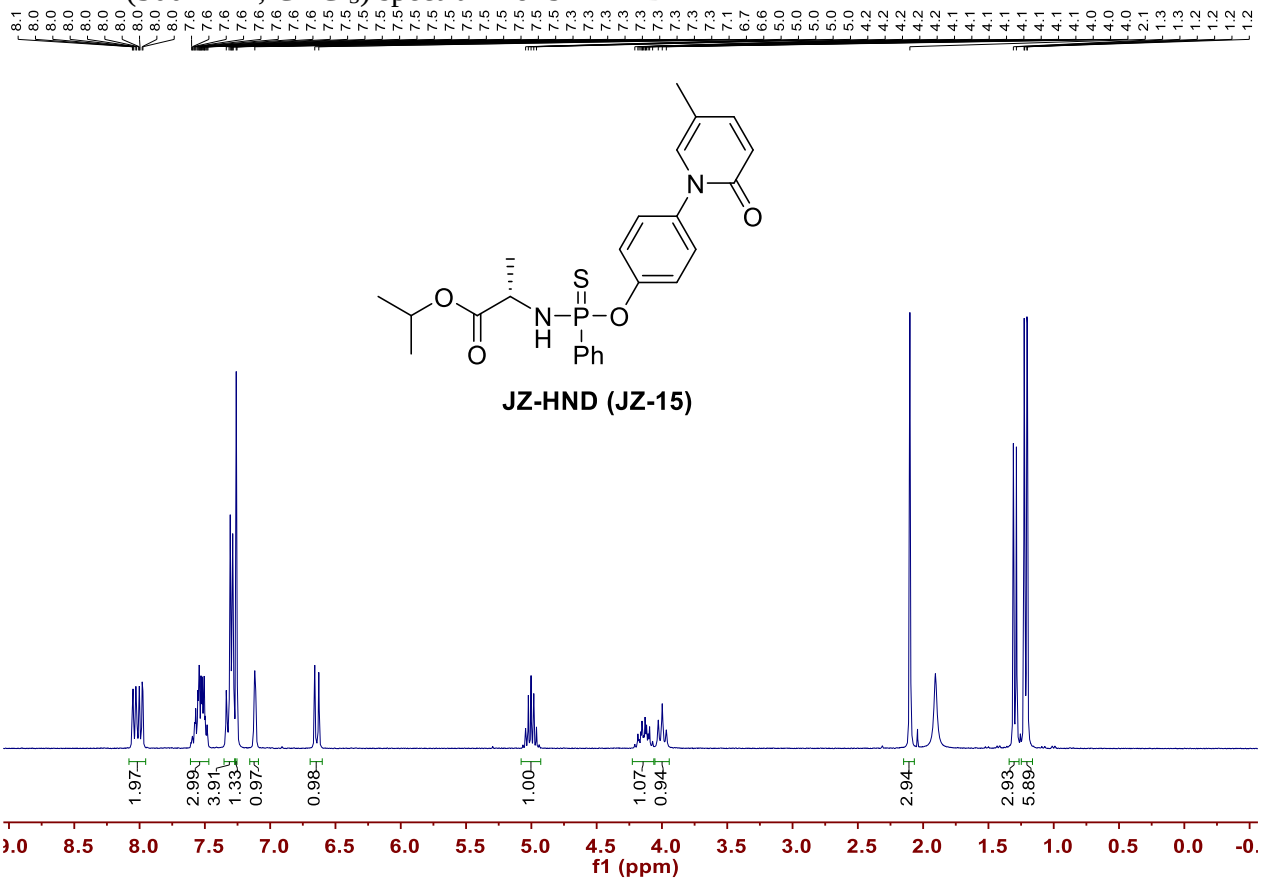

$^1$H NMR (500 MHz, $CDCl_3$) spectrum for **Bn-HND**



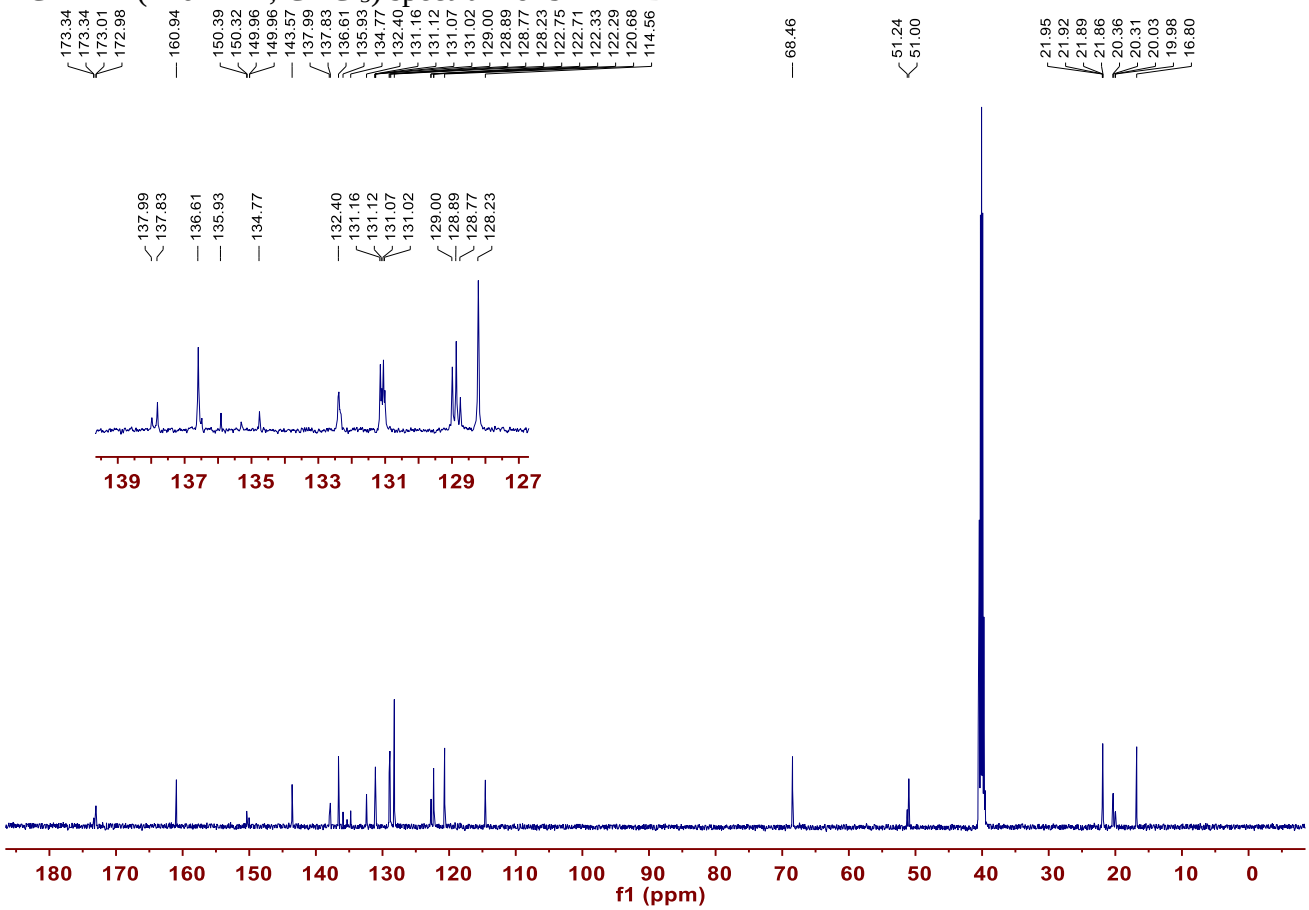
$^1$H NMR (500 MHz, $CDCl_3$) spectrum for **HND**



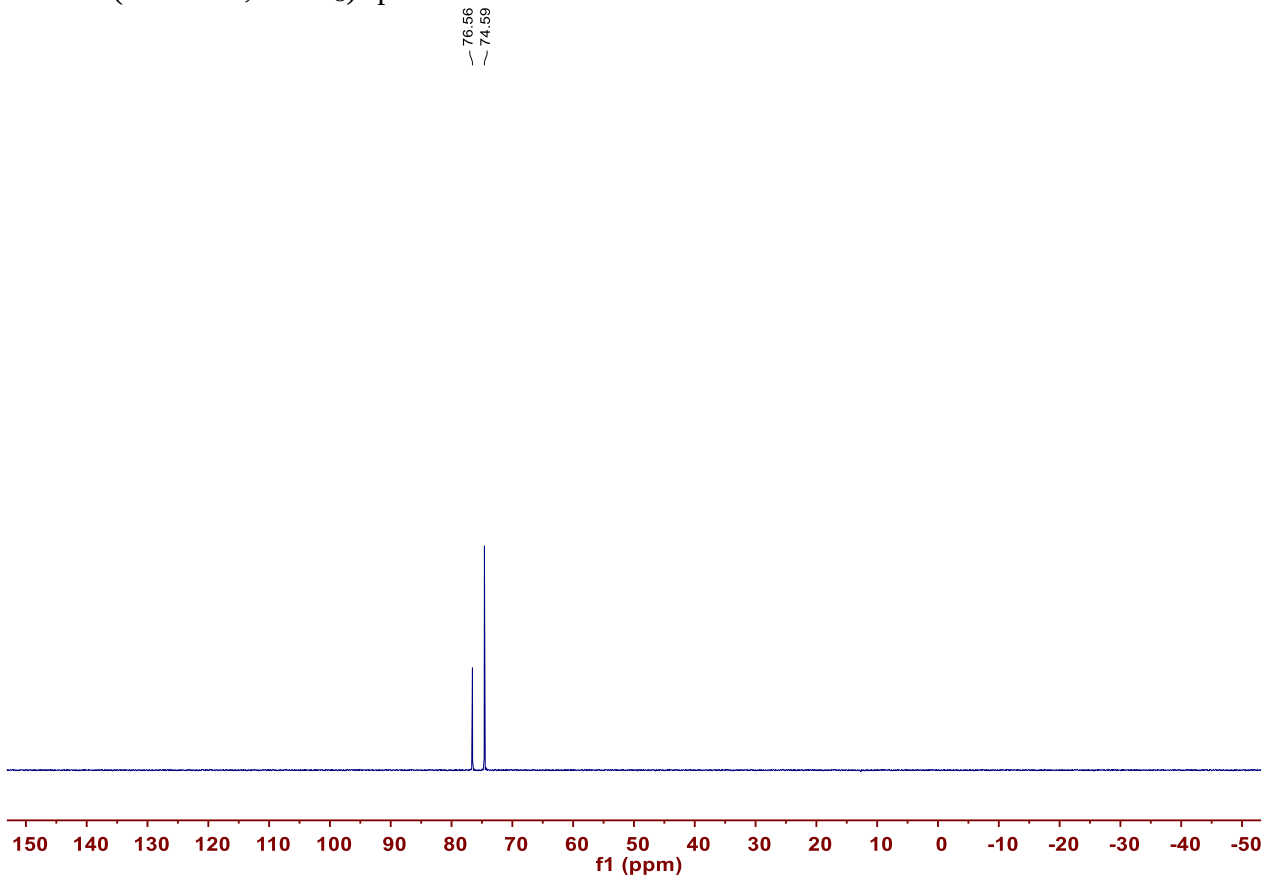

$^1$H NMR (500 MHz, $CDCl_3$) spectrum for **JZ-HND**



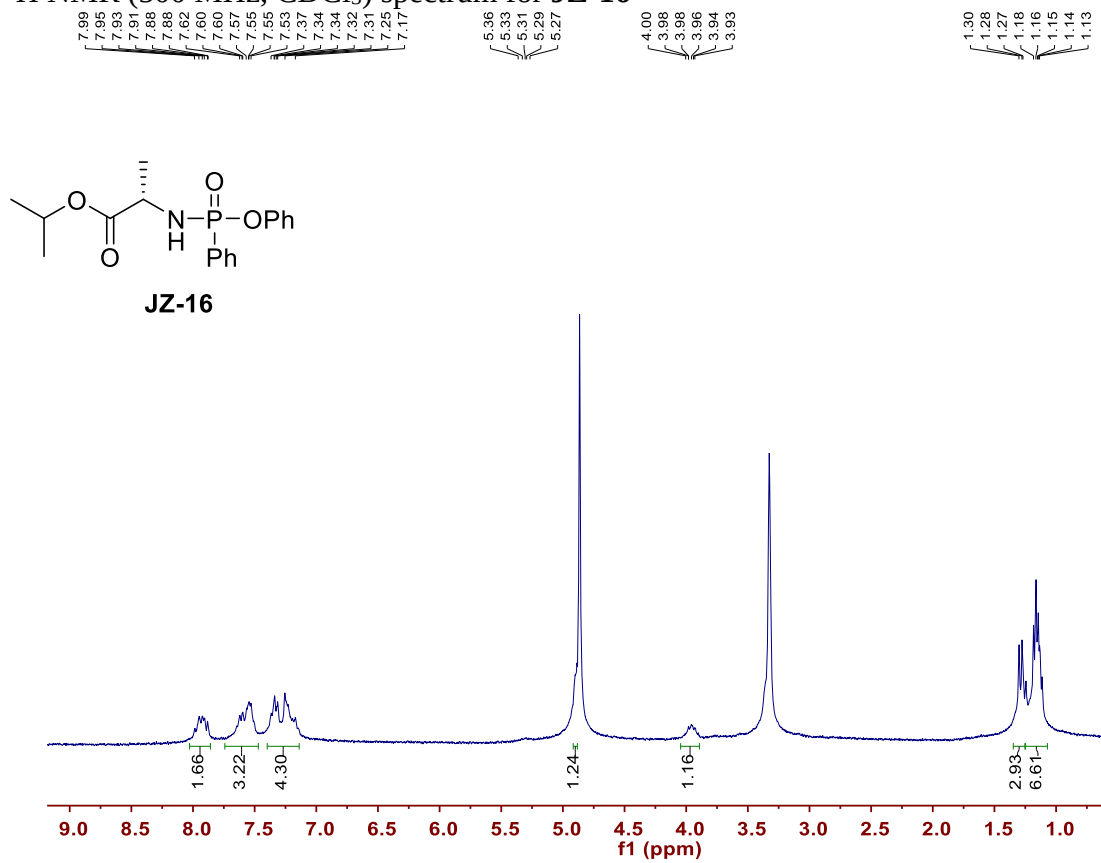
$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-HND**



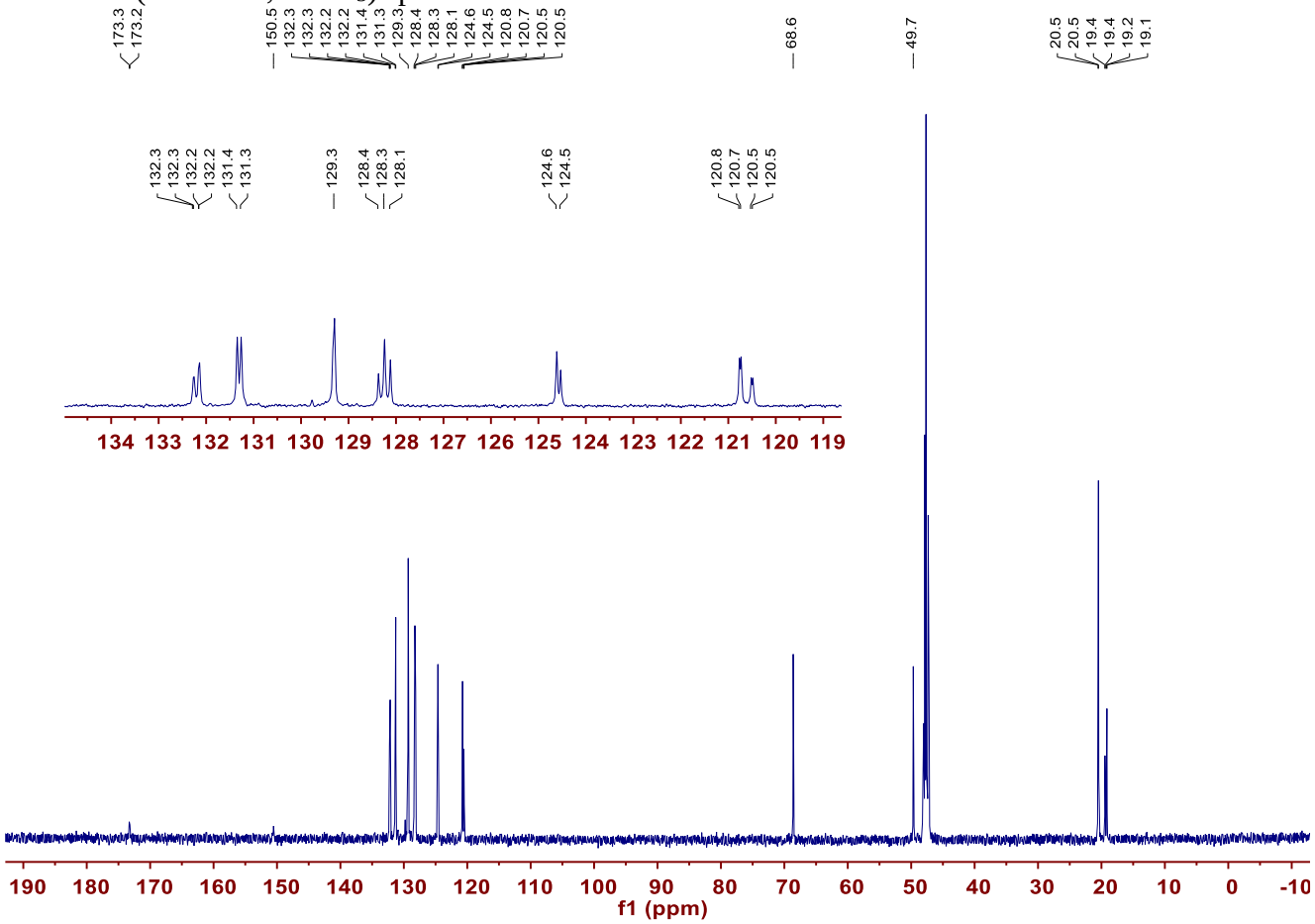

$^{31}$P NMR (162 MHz, $CDCl_3$) spectrum of **JZ-HND**



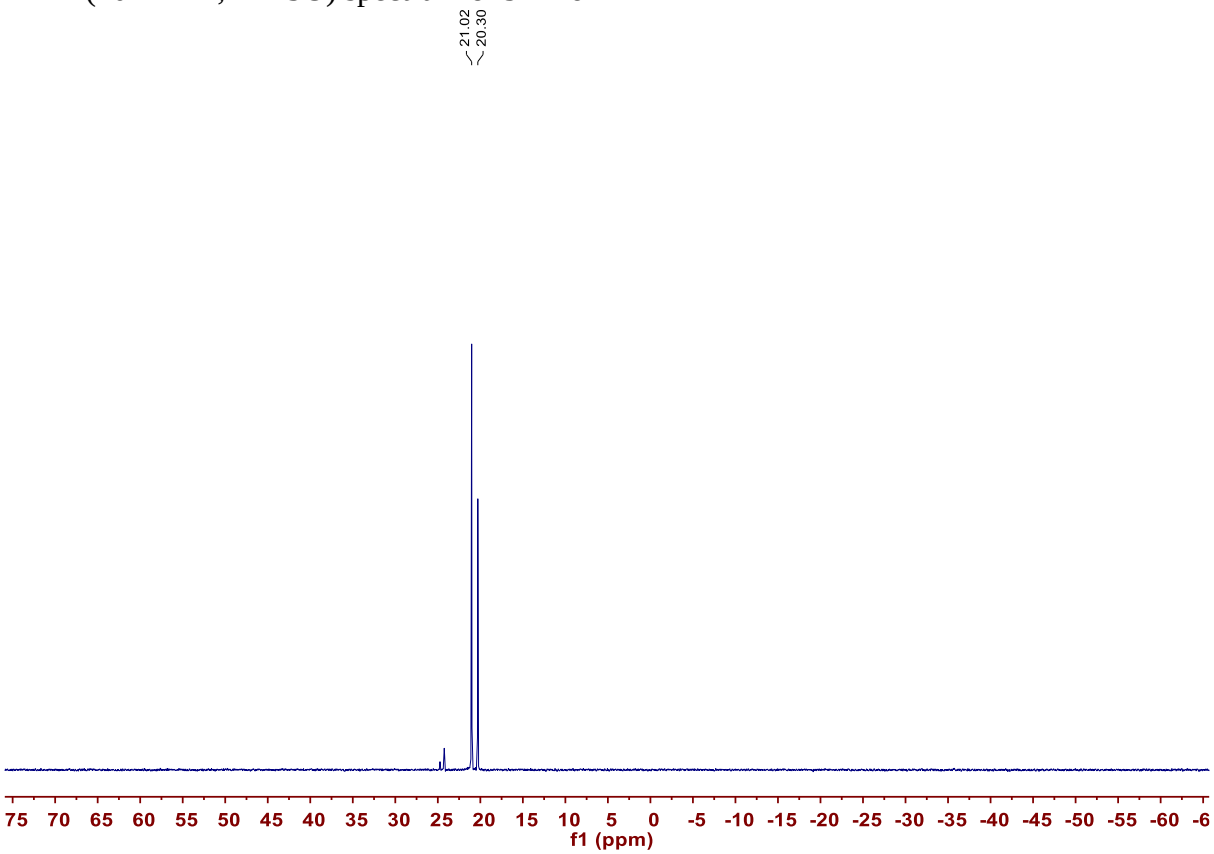
$^{1}$H NMR (500 MHz, $CDCl_3$) spectrum for **JZ-16**



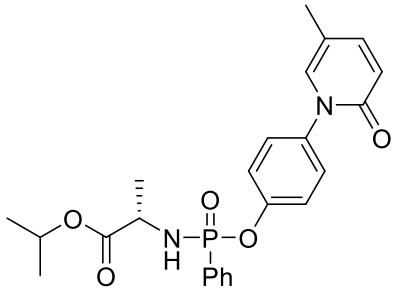

$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-16**



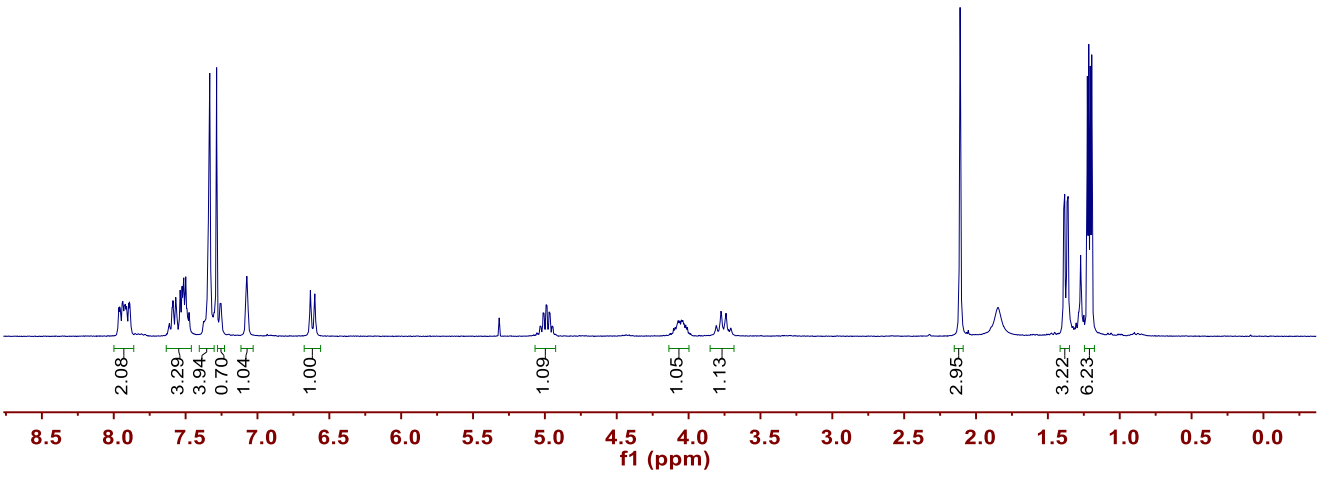
$^{31}$P NMR (202 MHz, DMSO) spectrum of **JZ-16**



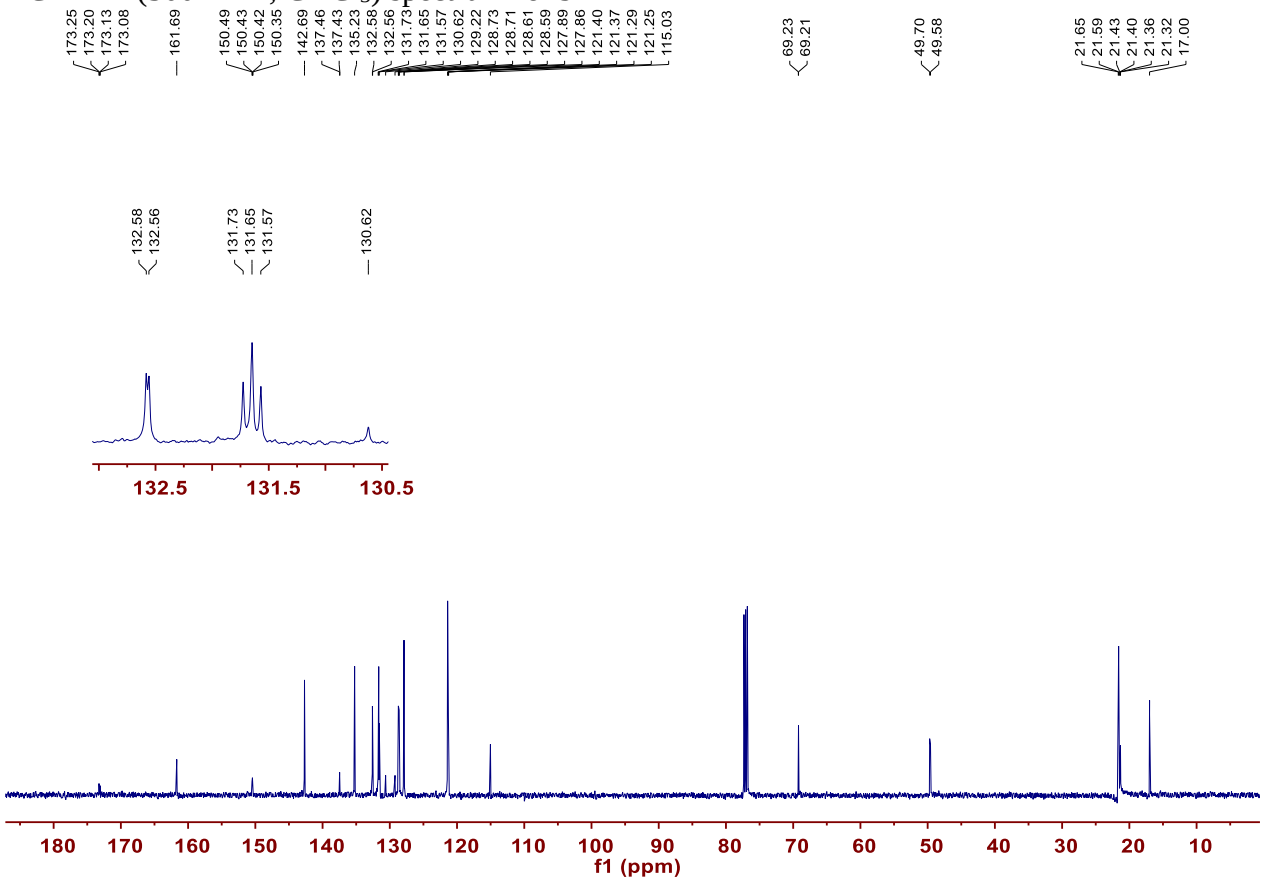

$^{1}$H NMR (500 MHz, $CDCl_3$) spectrum for **JZ-17**



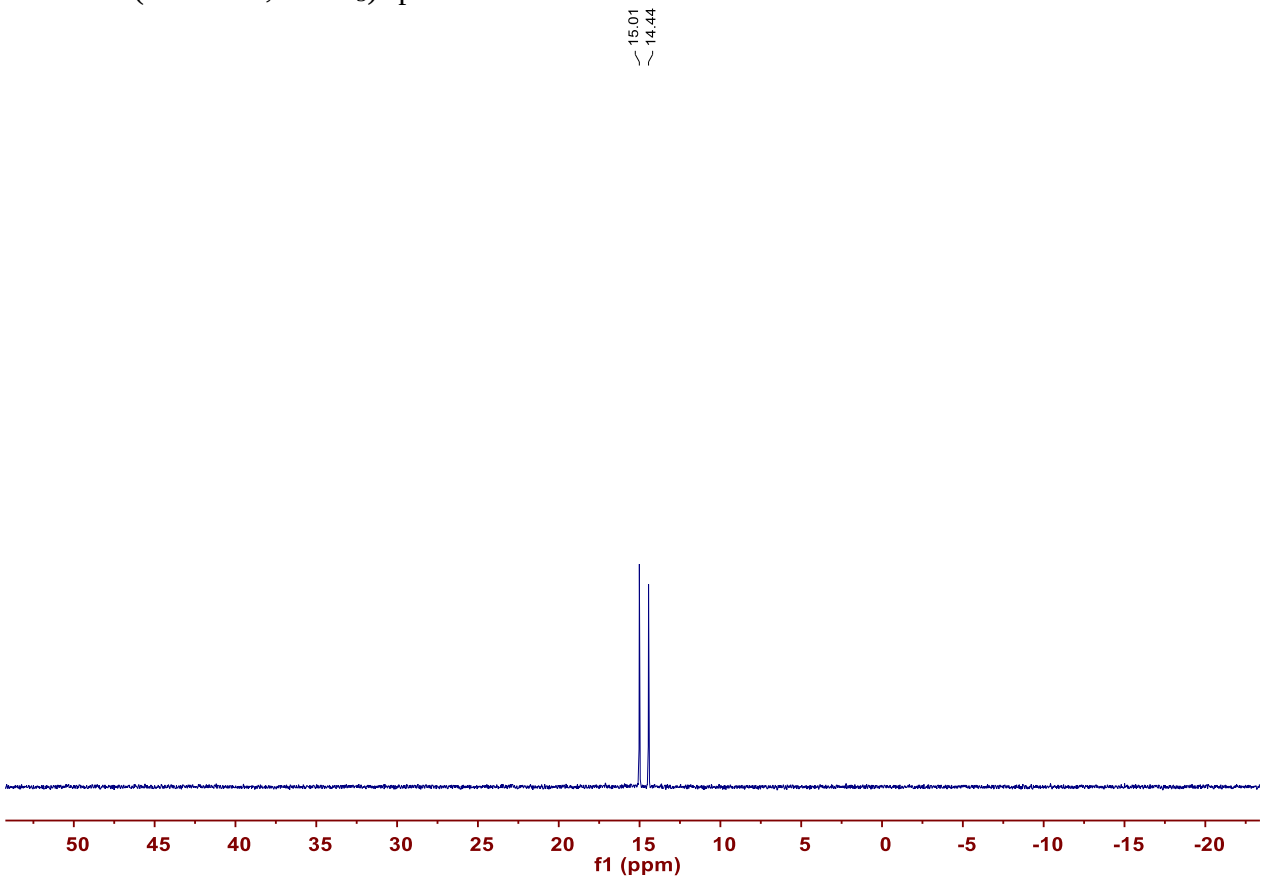

**JZ-17**



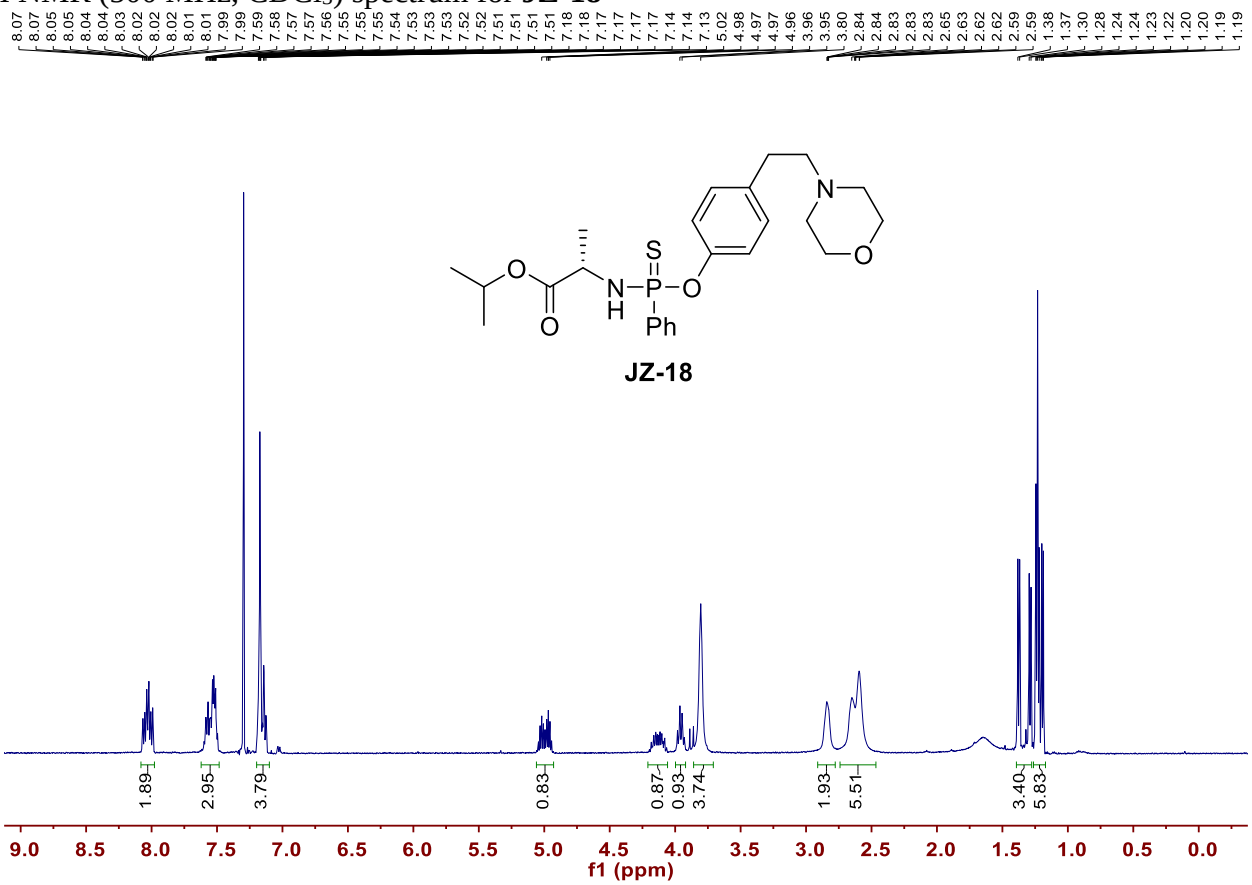
$^{13}$C NMR (500 MHz, $CDCl_3$) spectrum for **JZ-17**



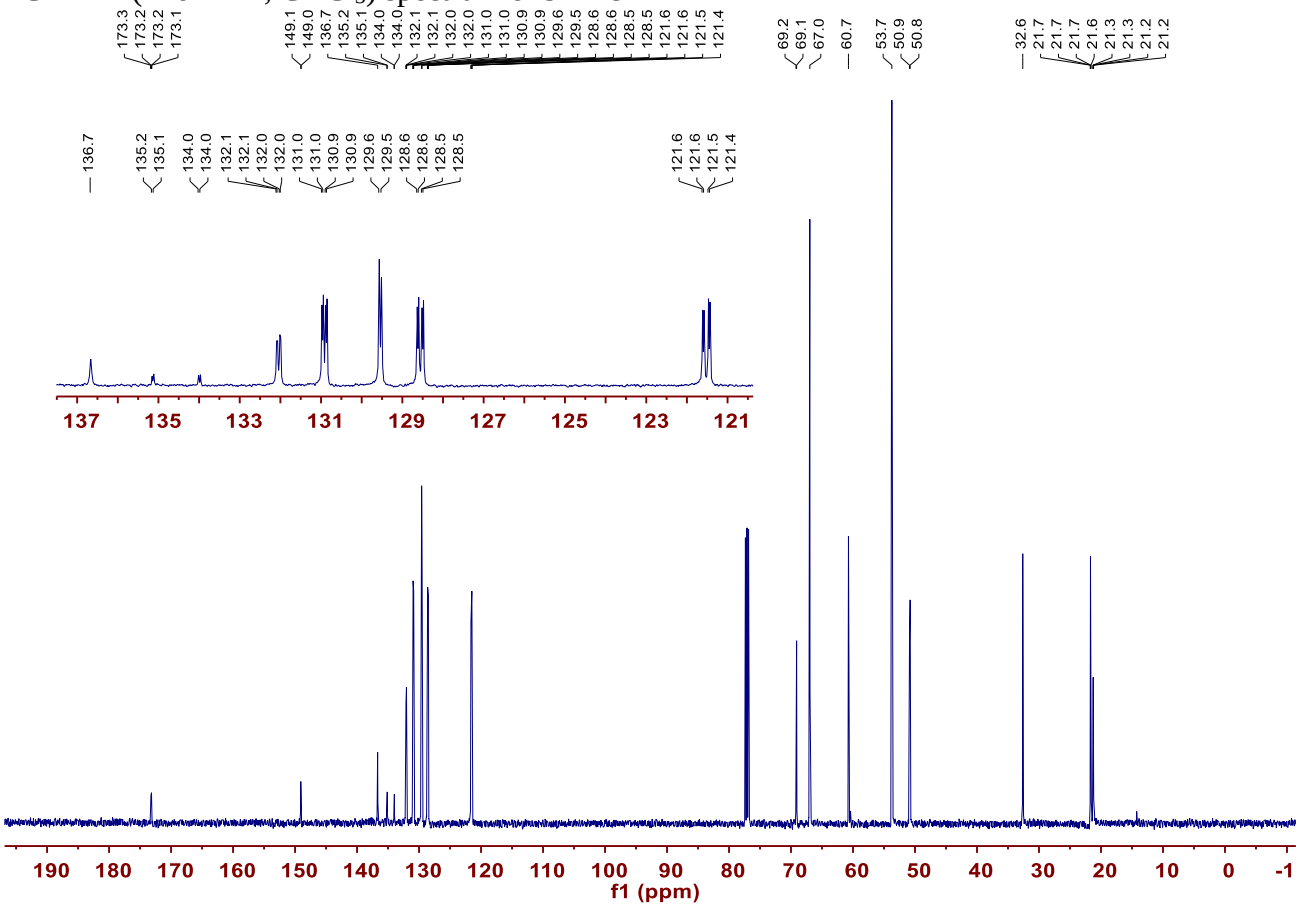

$^{31}$P NMR (162 MHz, $CDCl_3$) spectrum of **JZ-17**



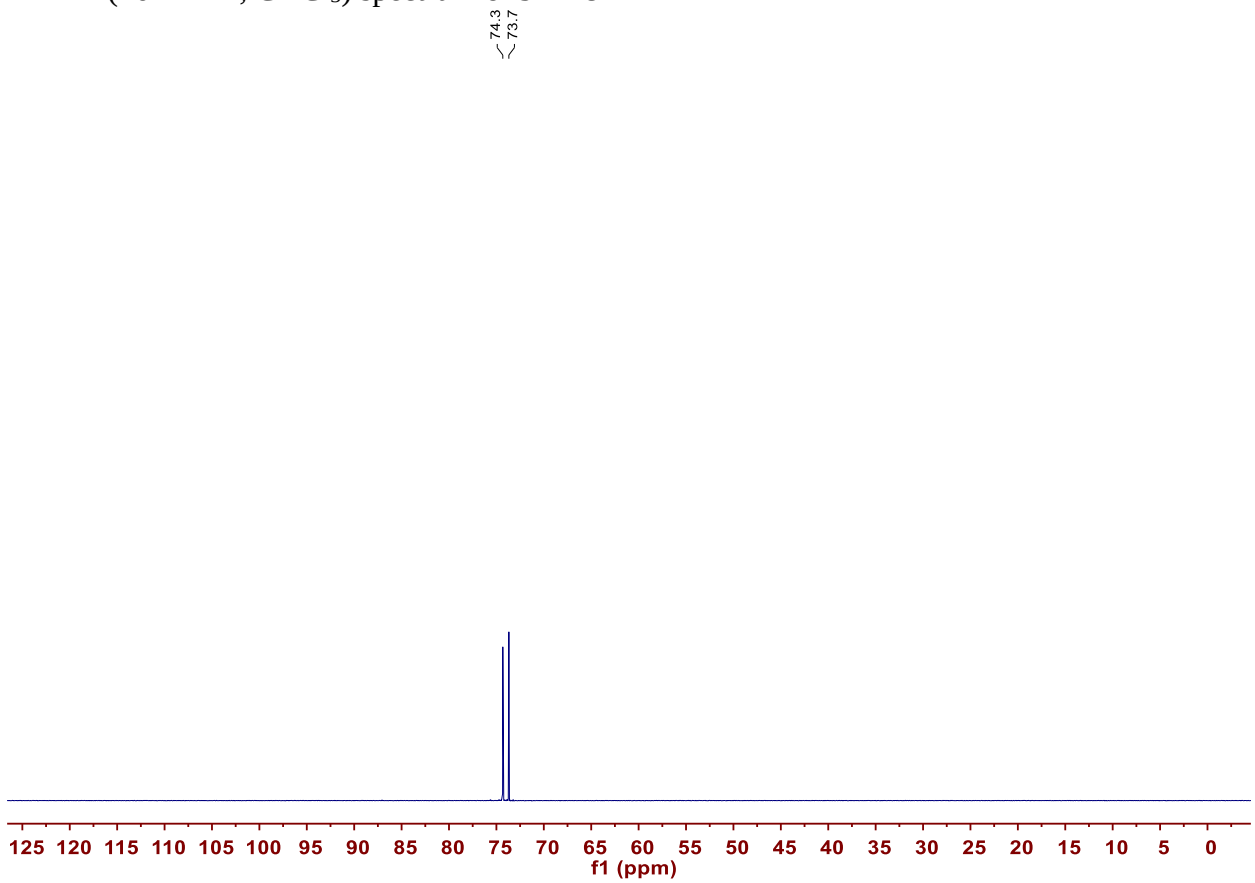
$^{1}$H NMR (500 MHz, $CDCl_3$) spectrum for **JZ-18**



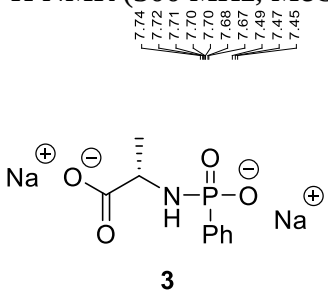

$^{13}$C NMR (126 MHz, $CDCl_3$) spectrum of **JZ-18**



$^{31}$P NMR (162 MHz, $CDCl_3$) spectrum of **JZ-18**

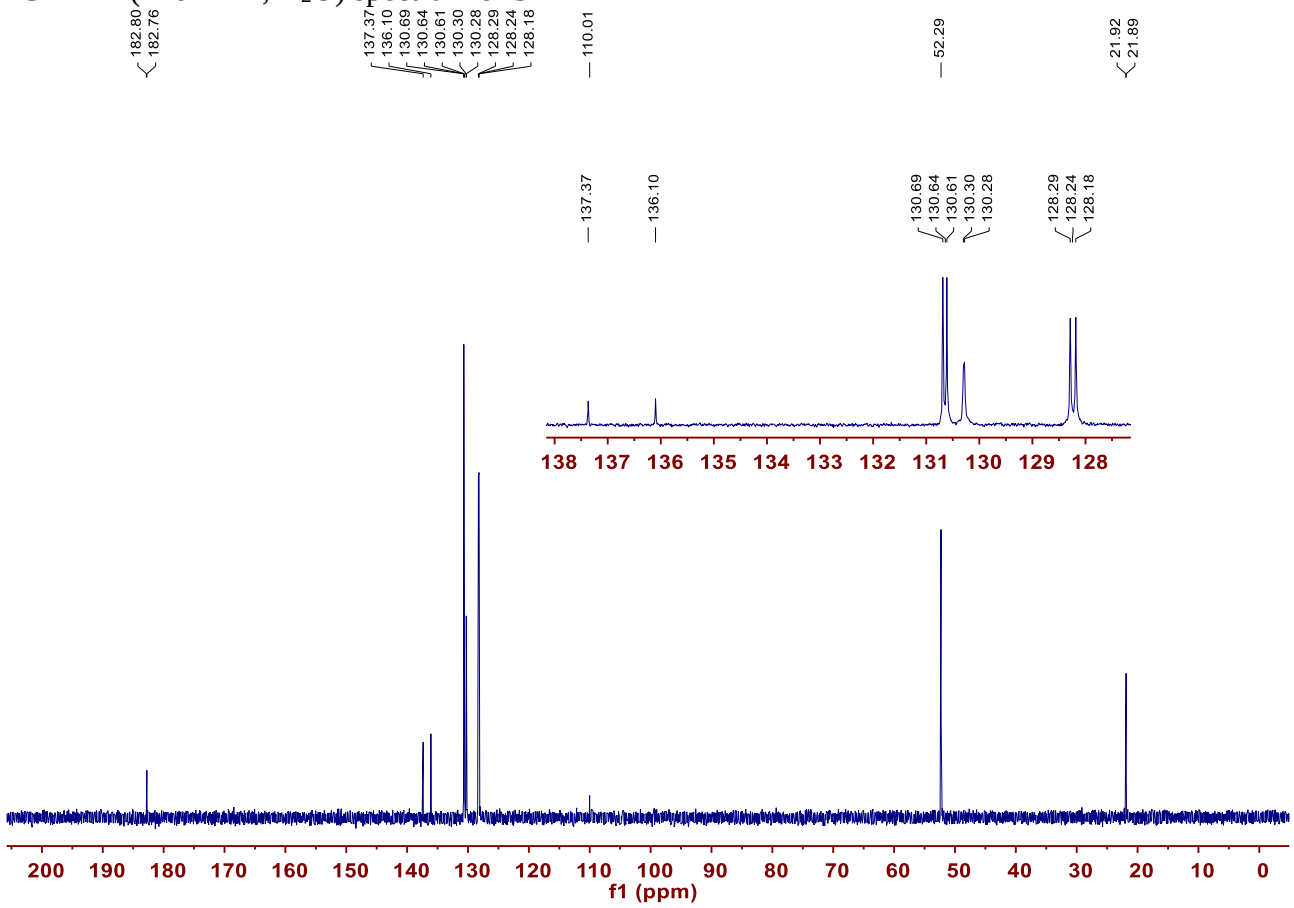



$^{1}$H NMR (300 MHz, MeOH-$d_4$) spectrum of **3**

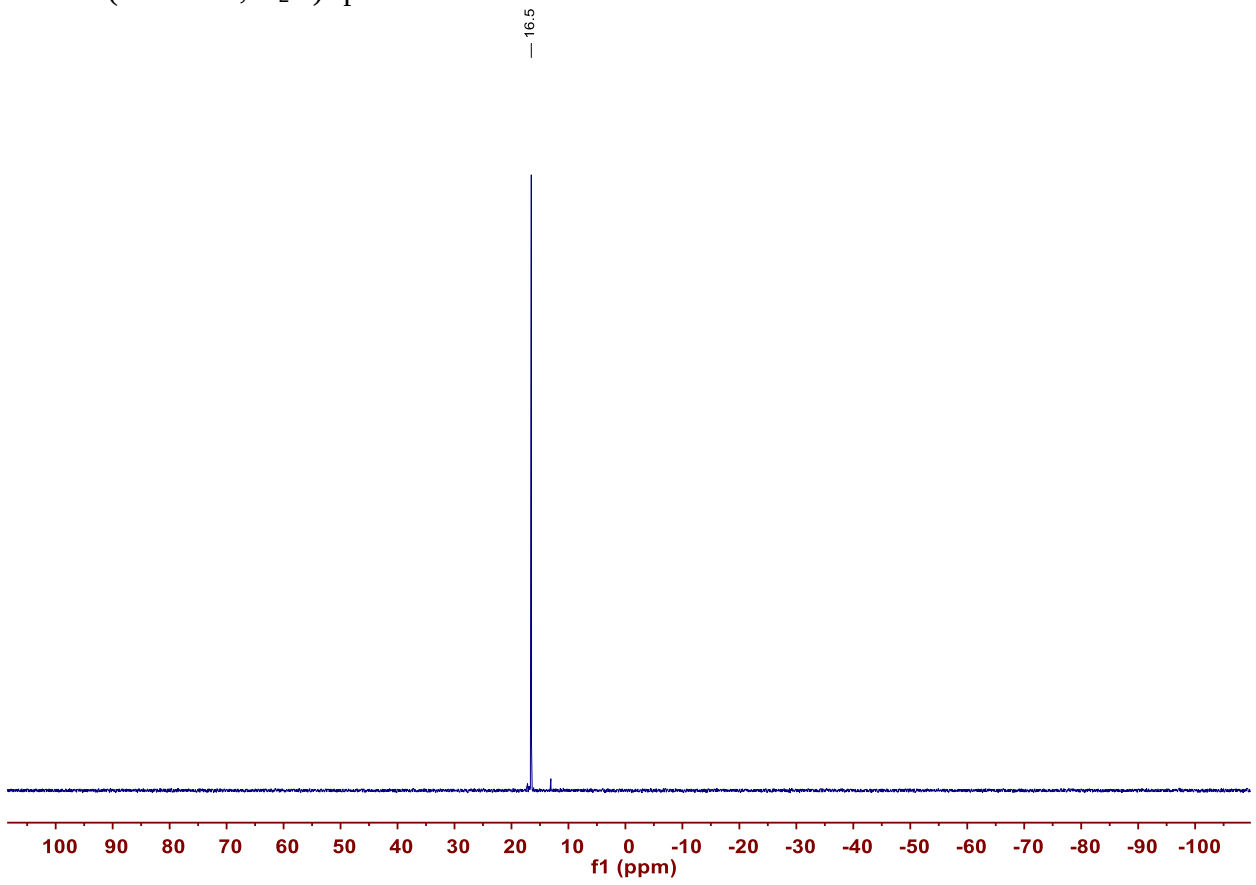



**3**

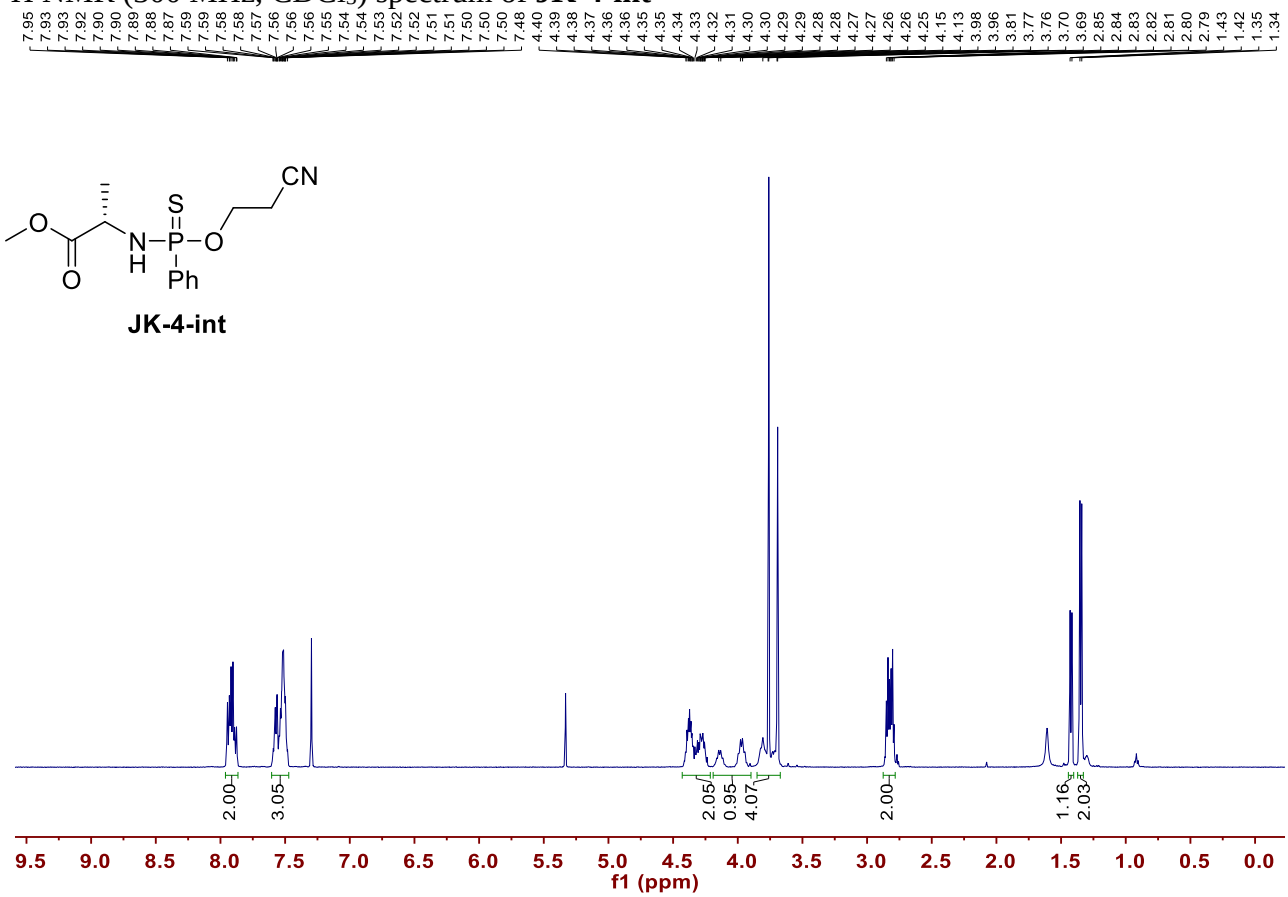
$^{13}$C NMR (126 MHz, $D_2O$) spectrum of **3**



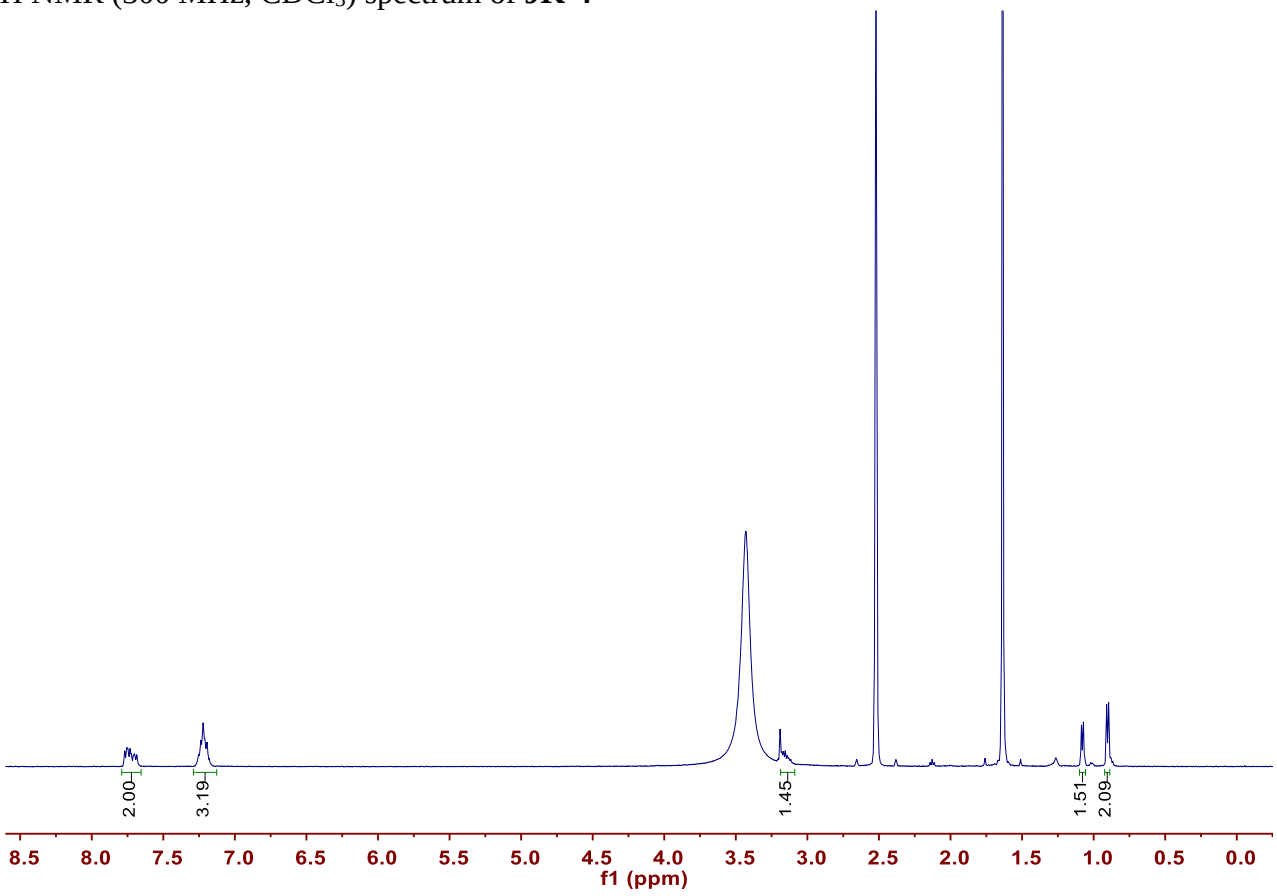

$^{31}$P NMR (202 MHz, $D_2O$) spectrum of **3**



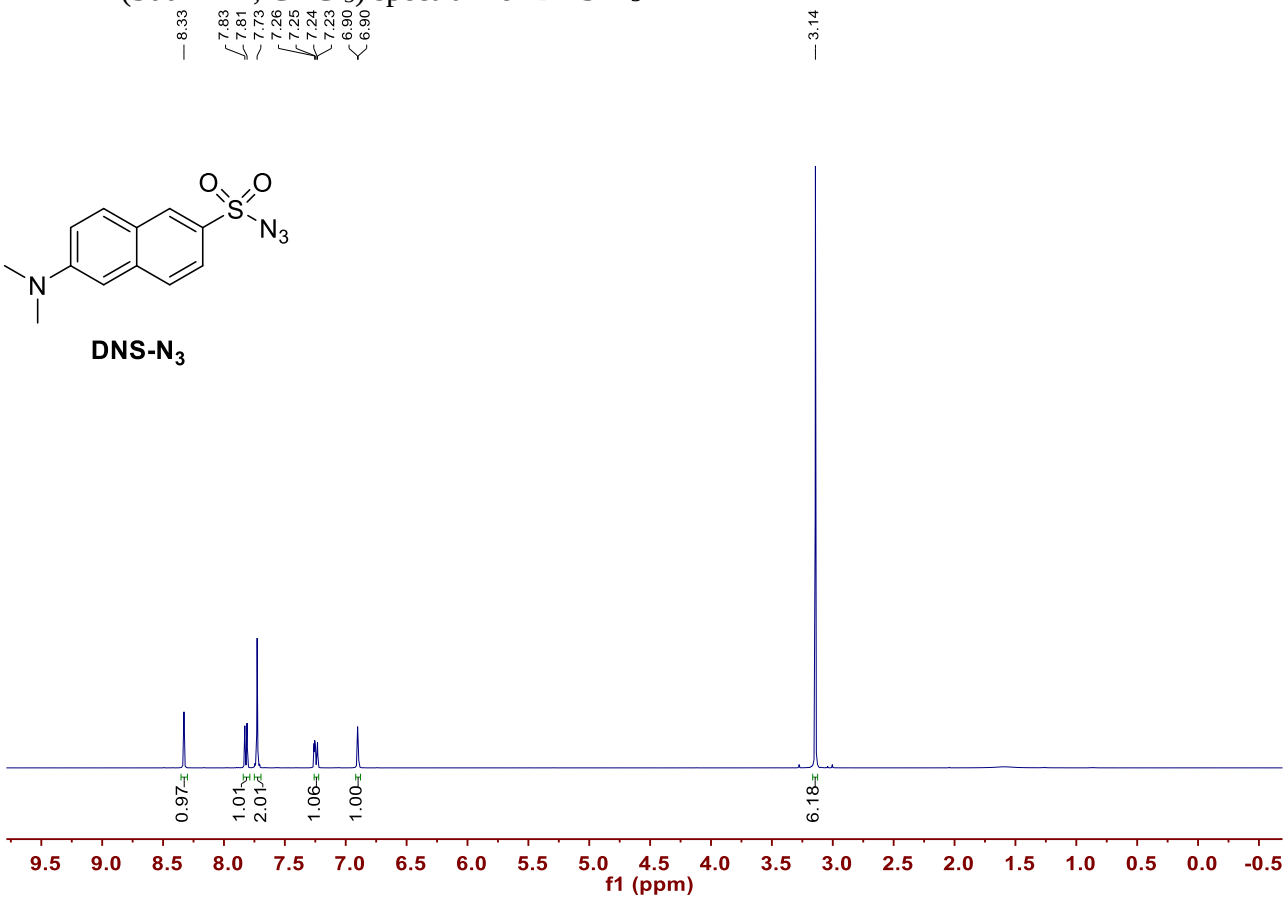
$^1$H NMR (300 MHz, $CDCl_3$) spectrum of **JK-4-int**




$^1$H NMR (300 MHz, $CDCl_3$) spectrum of **JK-4**

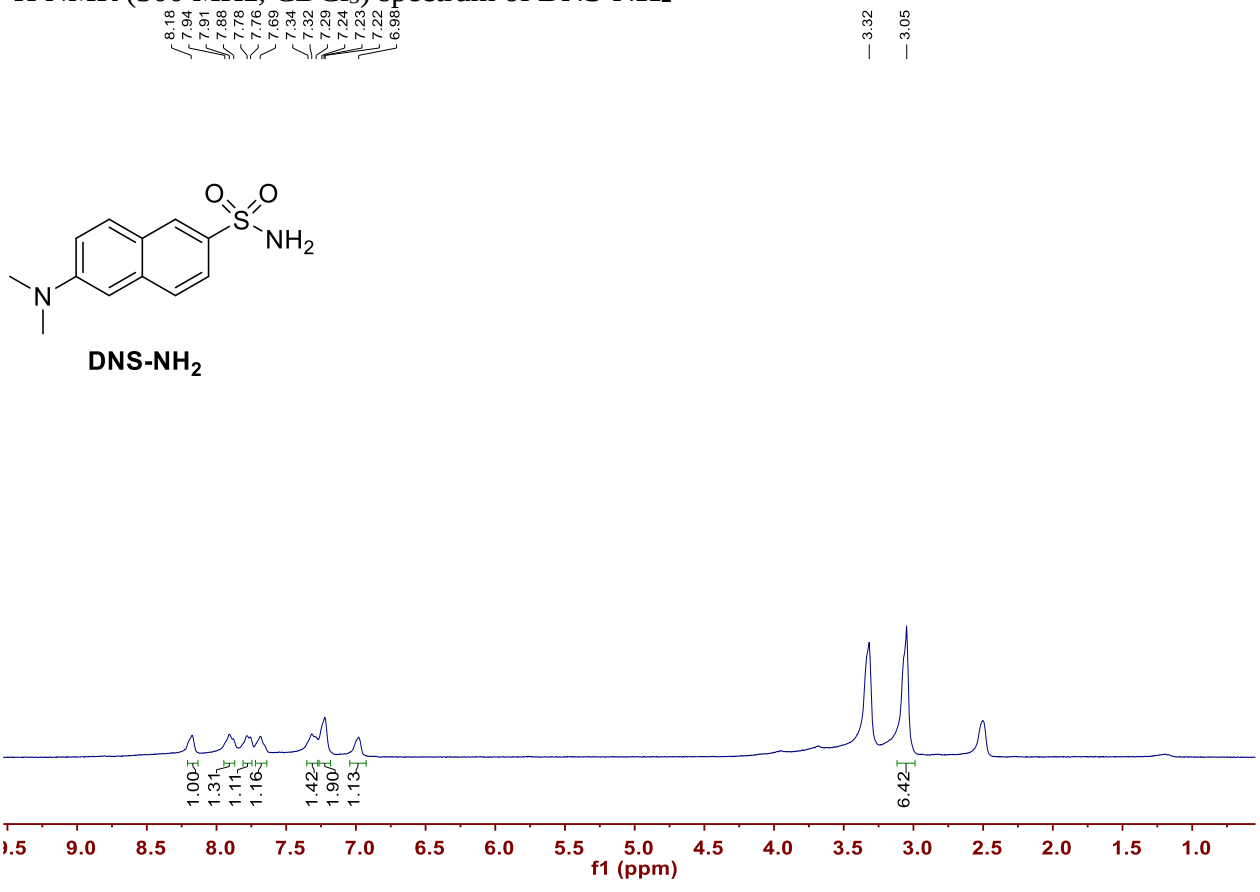


$^1$H NMR (300 MHz, $CDCl_3$) spectrum of **DNS-$N_3$**



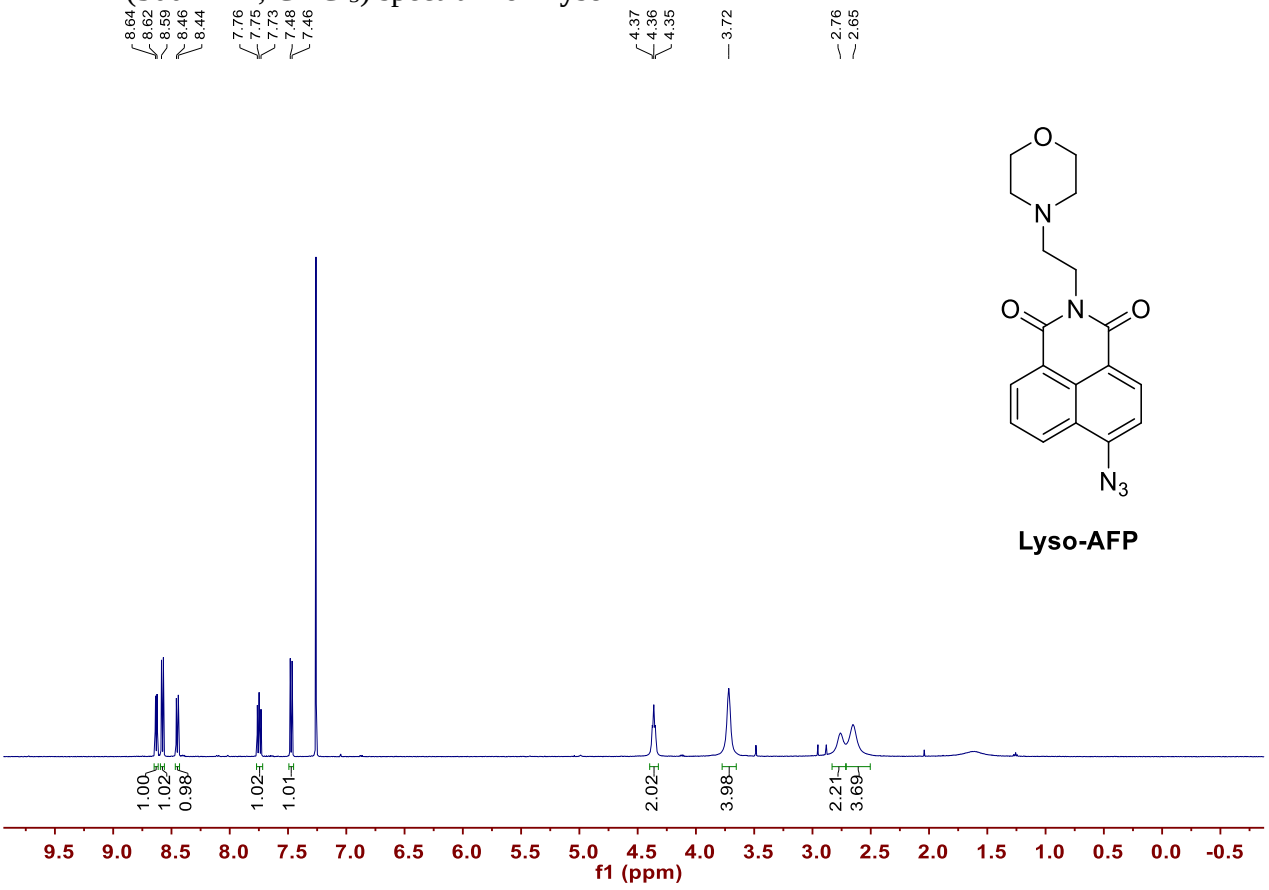

**DNS-$N_3$**

$^1$H NMR (300 MHz, $CDCl_3$) spectrum of **DNS-$NH_2$**

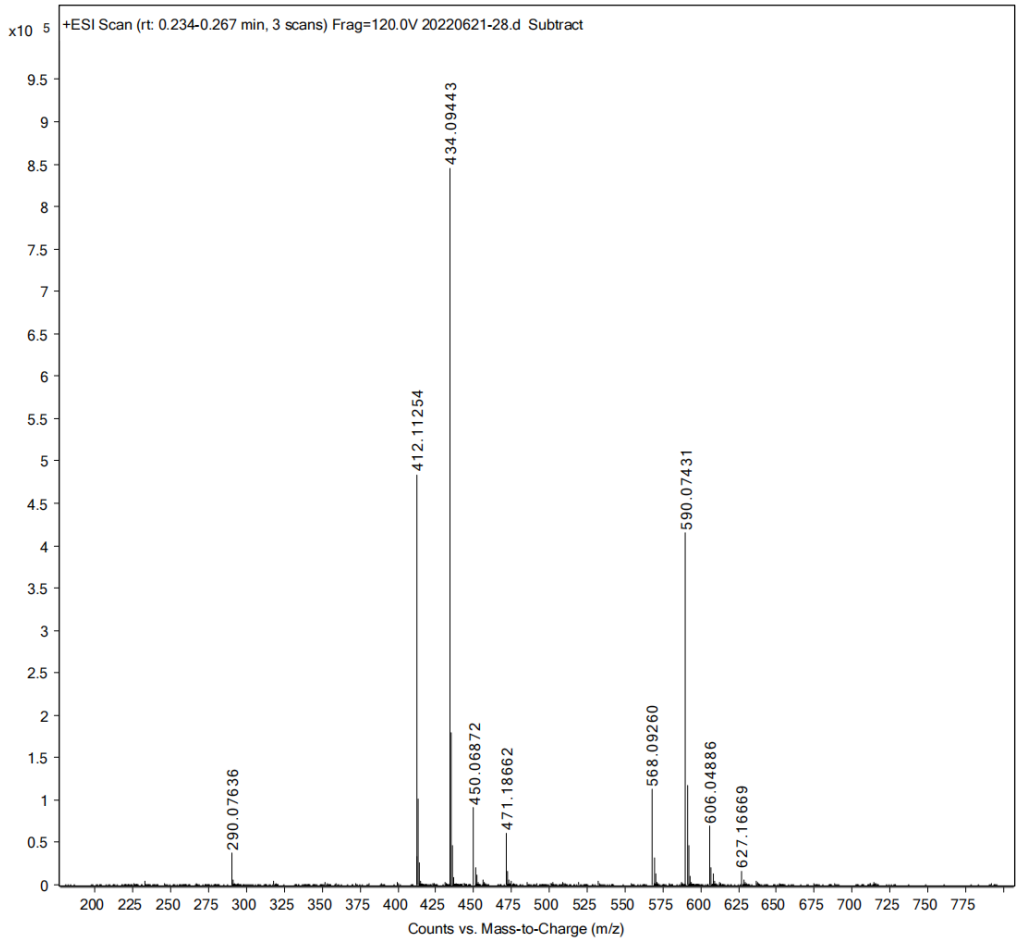



**DNS-$NH_2$**

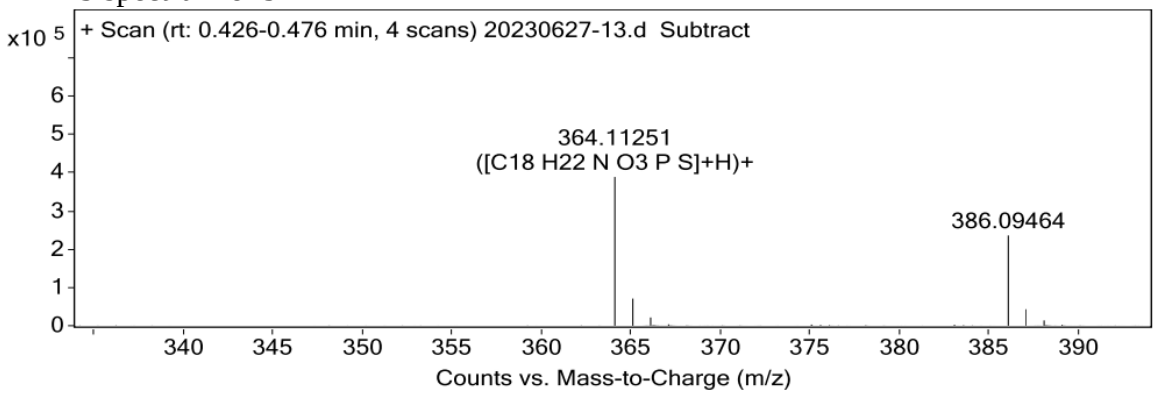
$^{1}$H NMR (300 MHz, $CDCl_3$) spectrum of **Lyso-AFP**



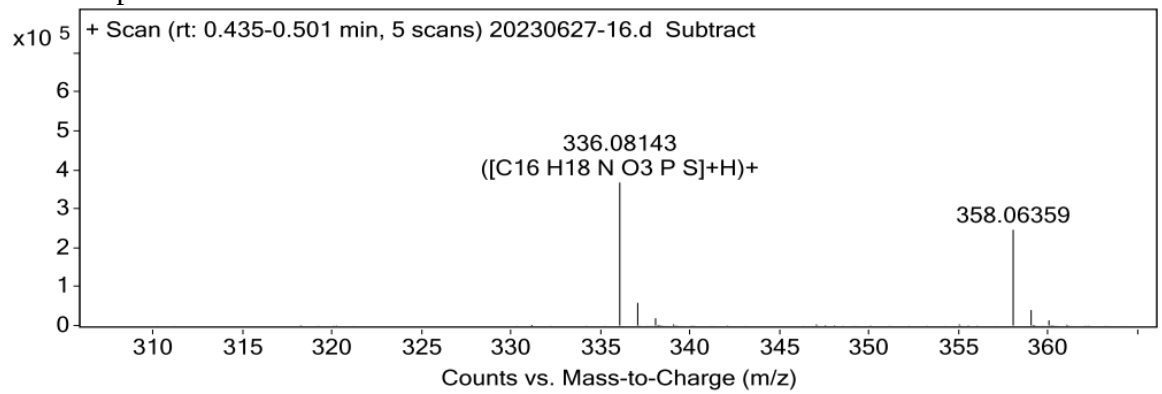
HRMS spectrum of **JZ-1**



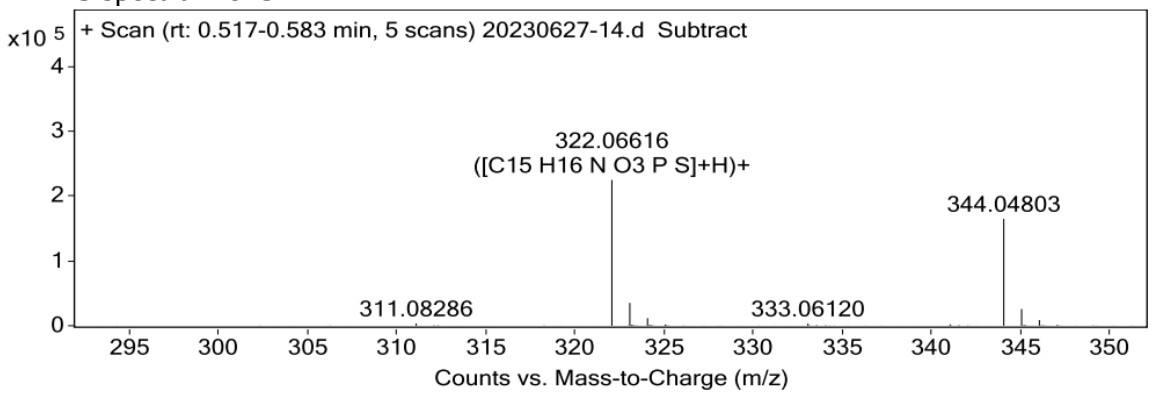

HRMS spectrum of **JZ-2**



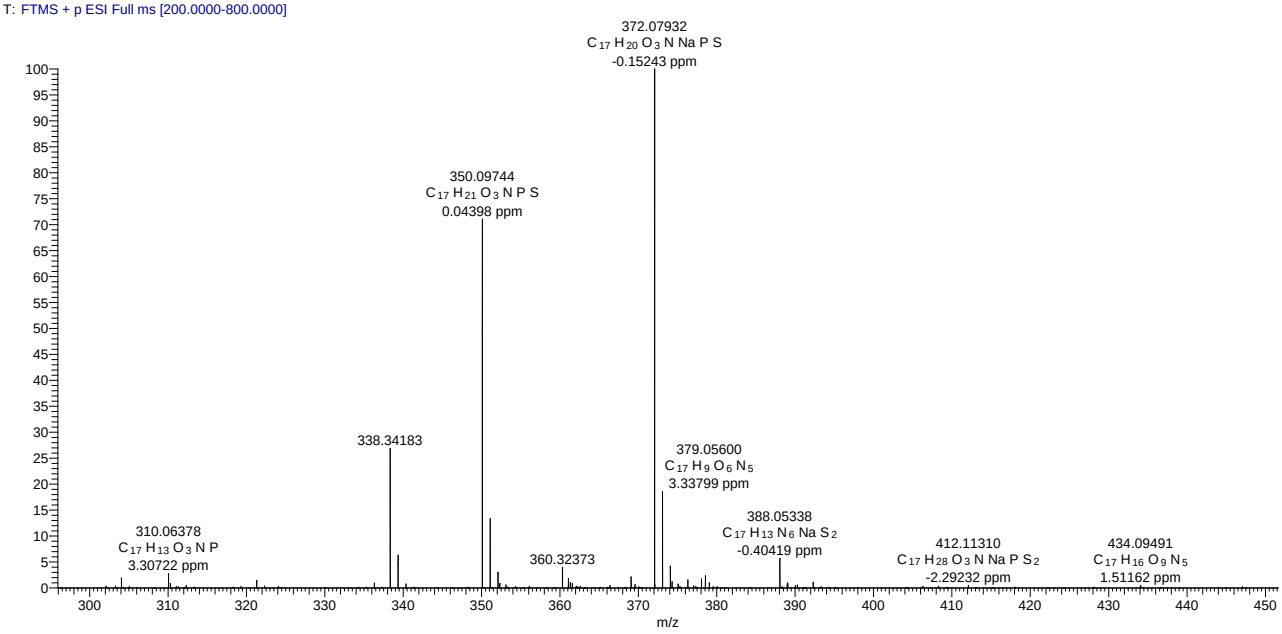

HRMS spectrum of **JZ-3**



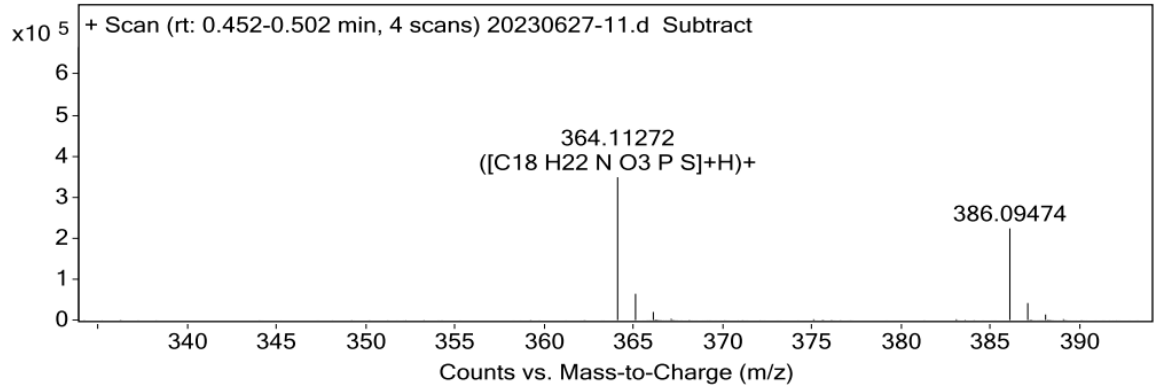
HRMS spectrum of **JZ-4**




HRMS spectrum of **JZ-5**

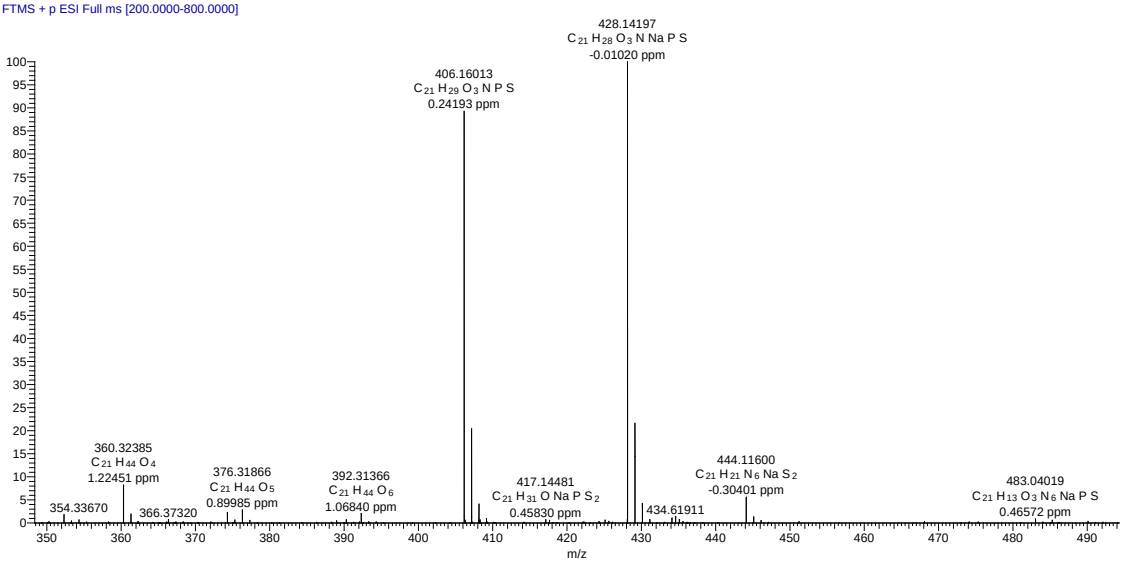



HRMS spectrum of **JZ-6**



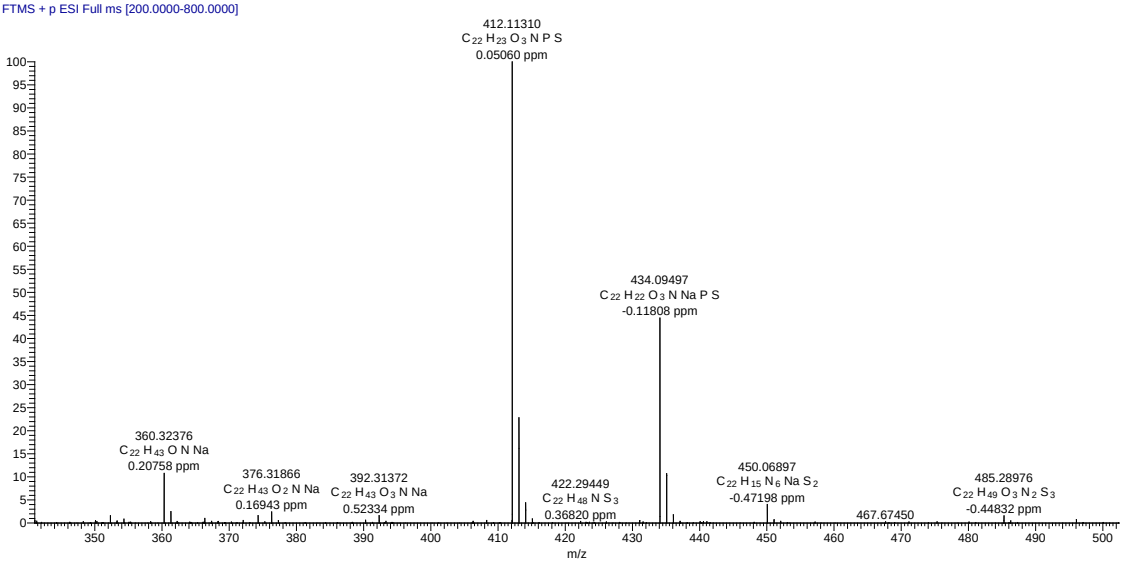
# HRMS spectrum of **JZ-7**

JZ-12 #9 RT: 0.06 AV: 1 NL: 3.35E9
T: FTMS + p ESI Full ms [200.0000-800.0000]



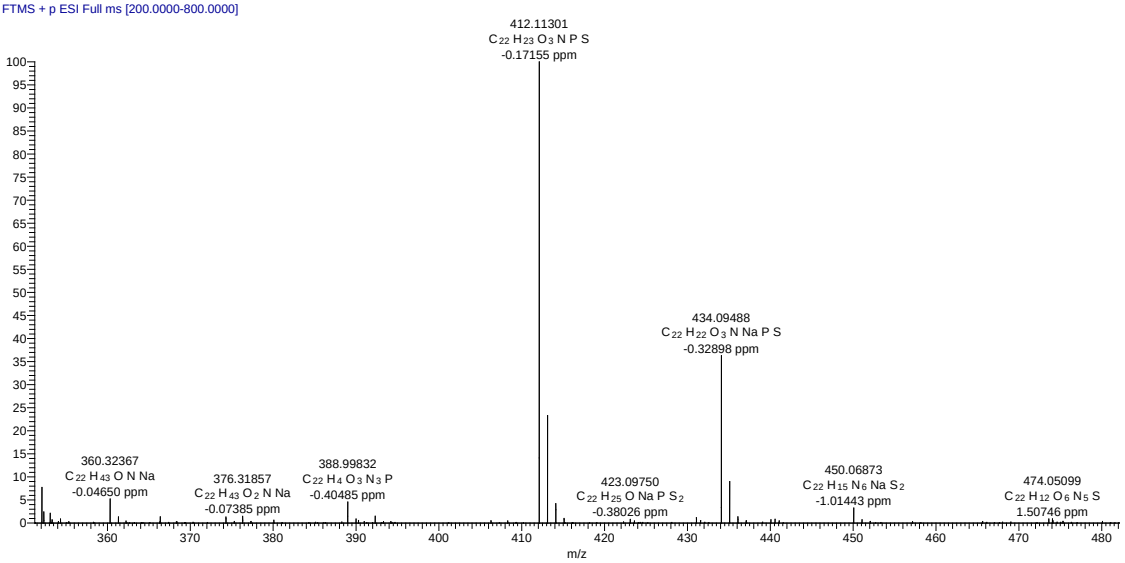

# HRMS spectrum of **JZ-8**

JZ-14 #11 RT: 0.08 AV: 1 NL: 3.01E9
T: FTMS + p ESI Full ms [200.0000-800.0000]

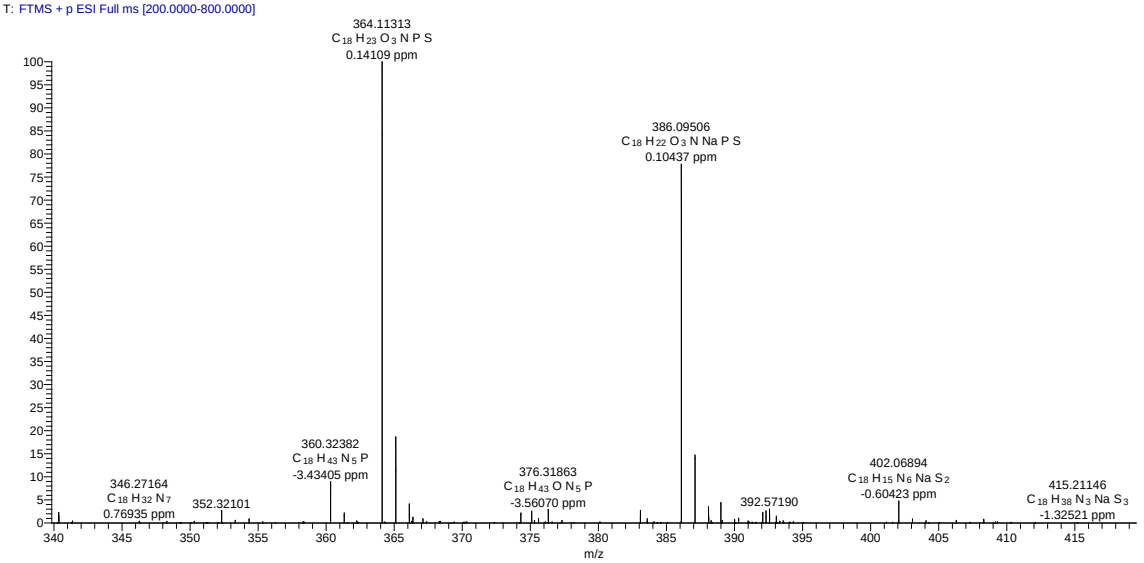



# HRMS spectrum of **JZ-9**

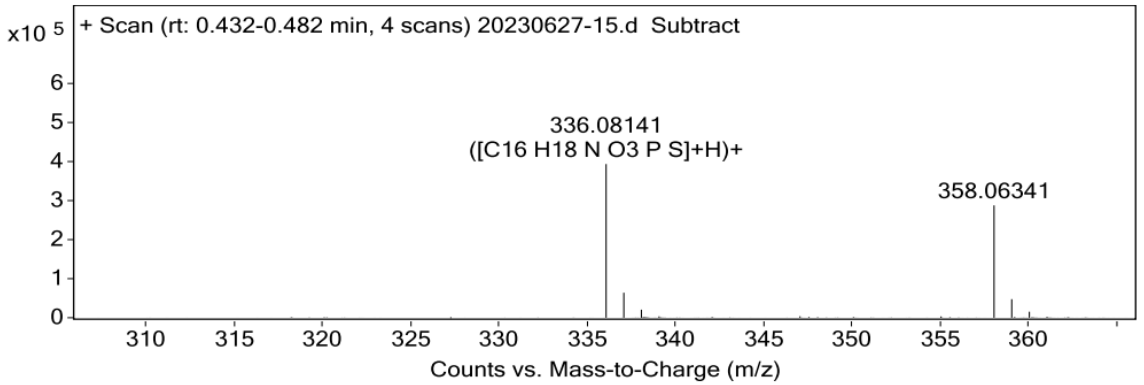
JZ-15 #11 RT: 0.08 AV: 1 NL: 2.12E9
T: FTMS + p ESI Full ms [200.0000-800.0000]



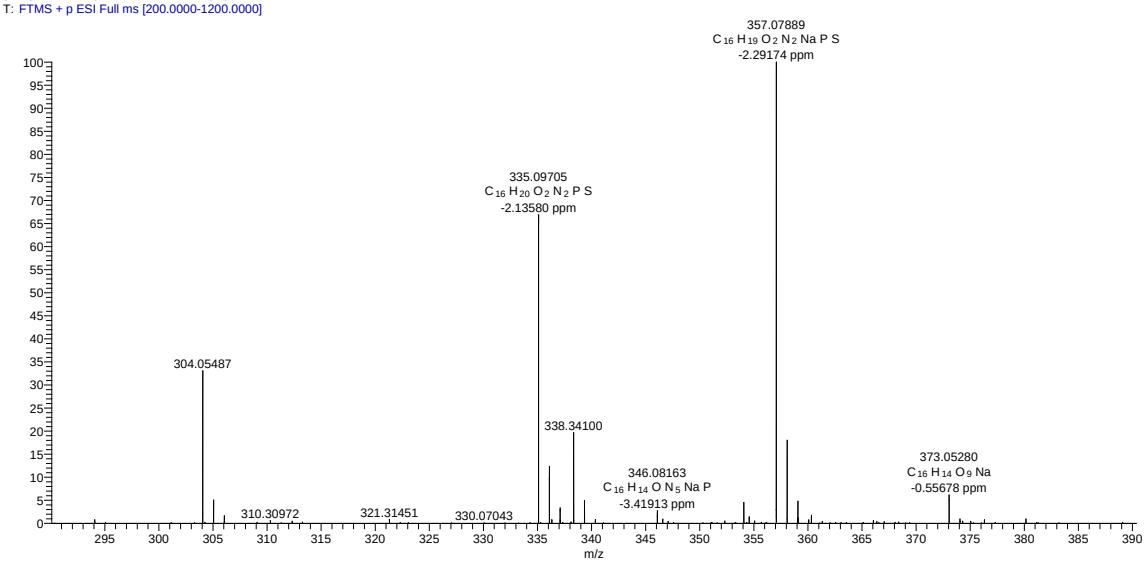
## HRMS spectrum of **JZ-10**



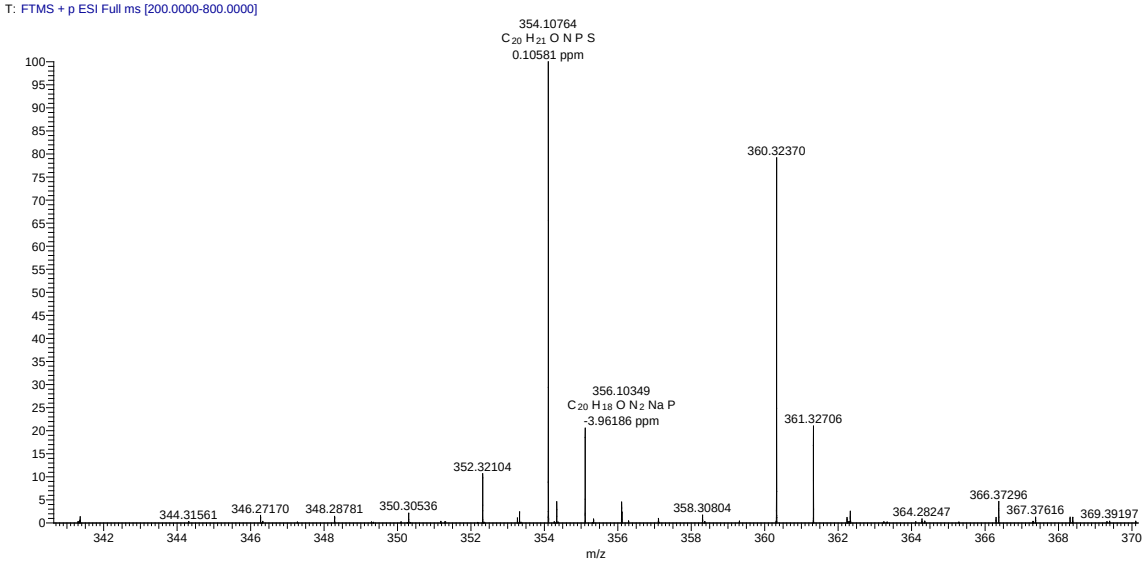

## HRMS spectrum of **JZ-11**



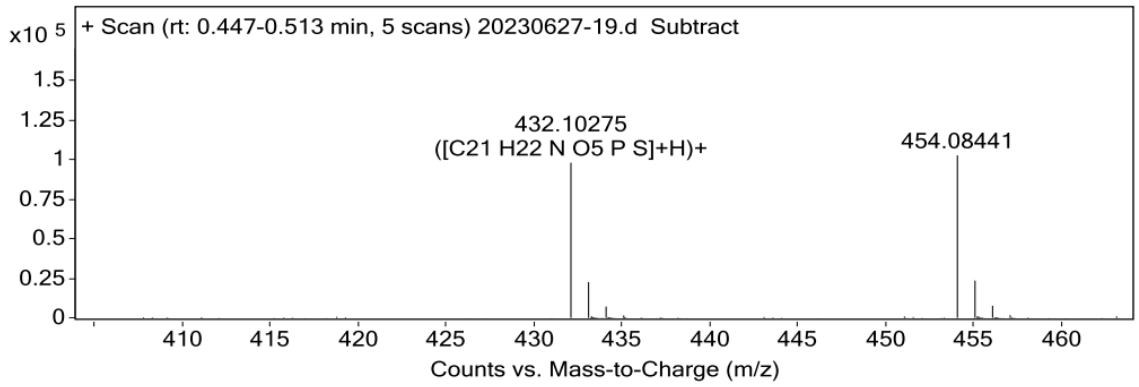

## HRMS spectrum of **JZ-12**



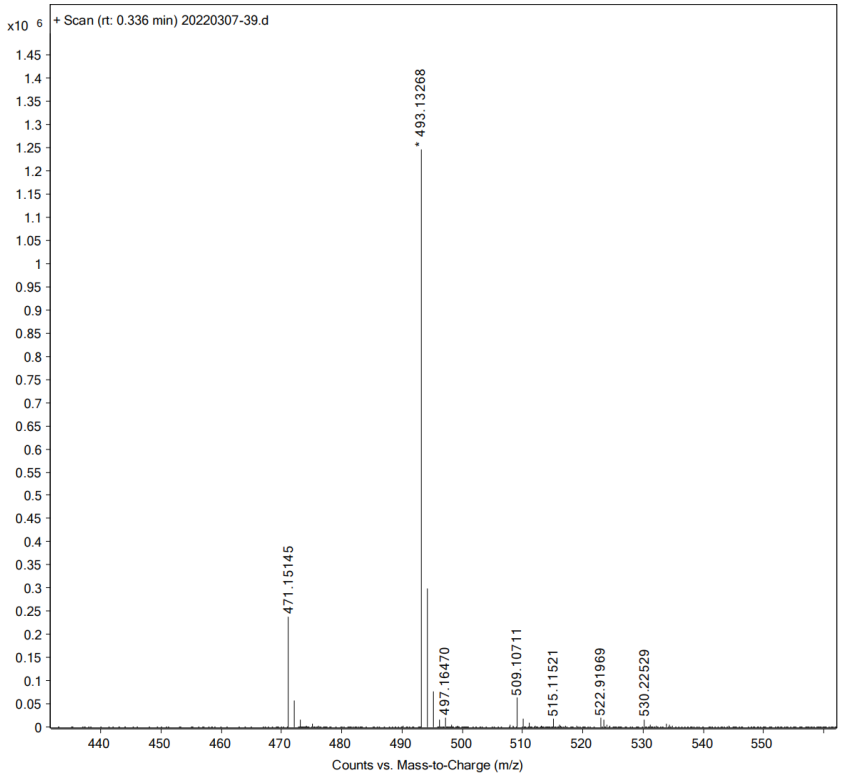
HRMS spectrum of **JZ-13**



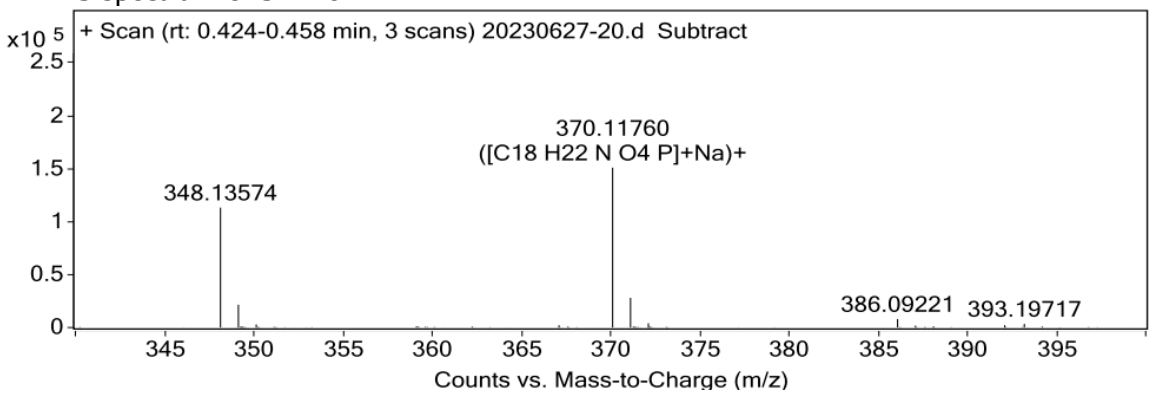

HRMS spectrum of **JZ-14**



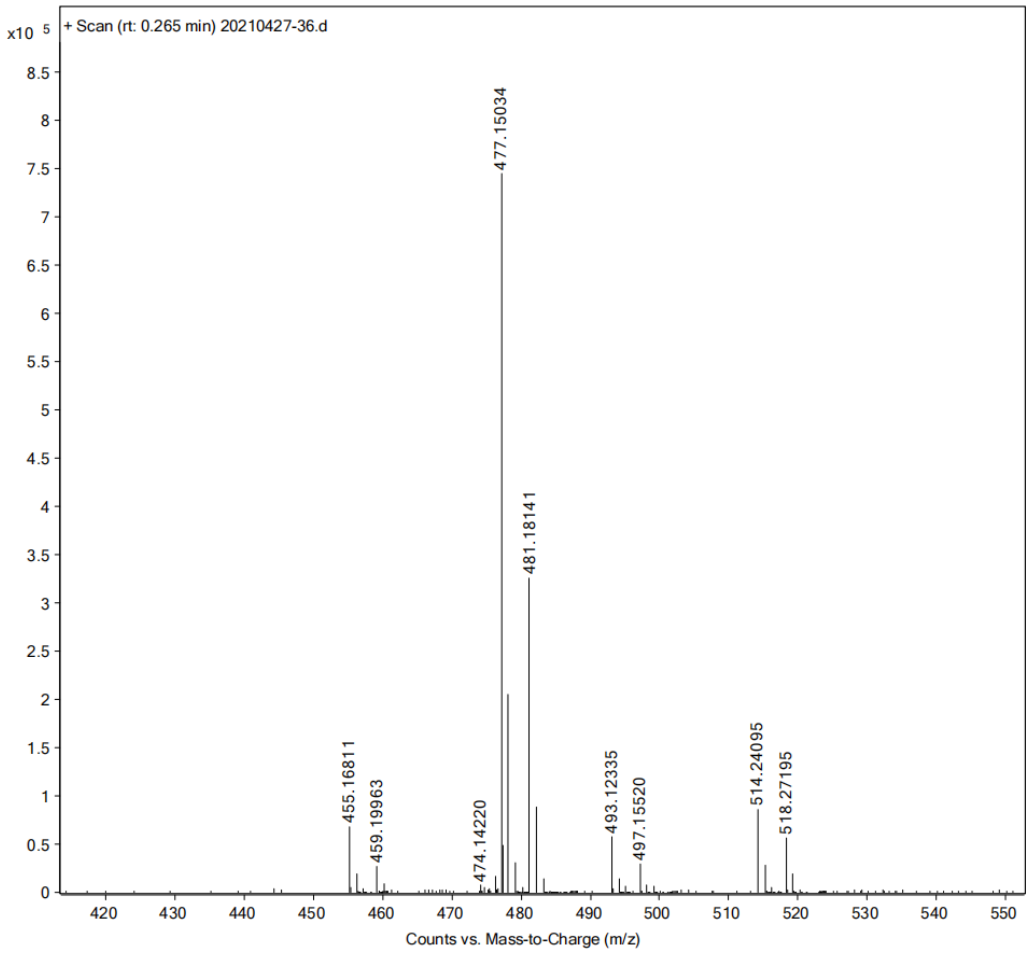

HRMS spectrum of **JZ-HND（JZ-15）**



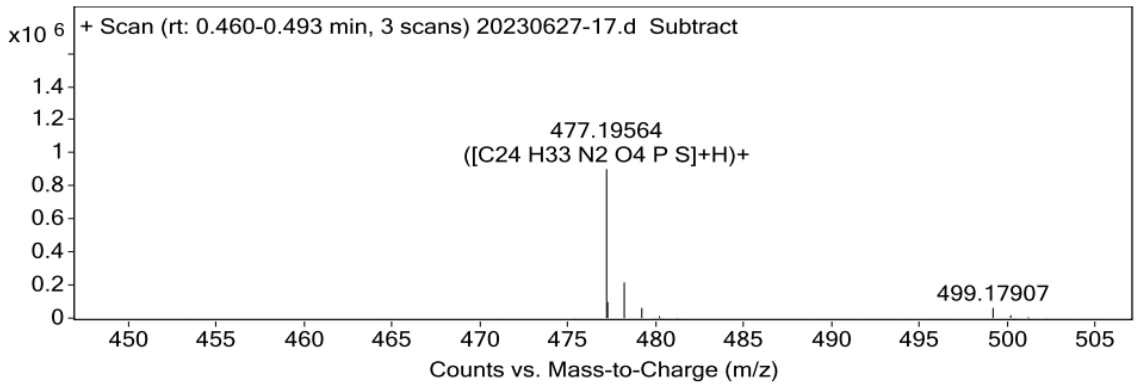
HRMS spectrum of **JZ-16**



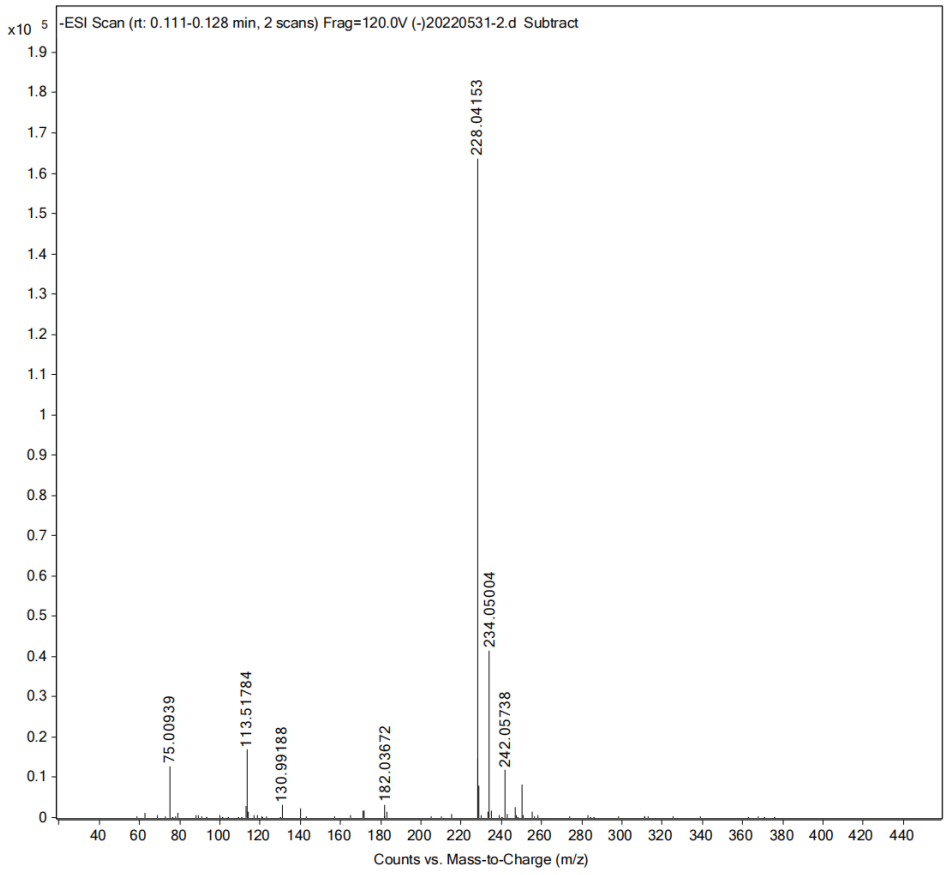

HRMS spectrum of **JZ-17**



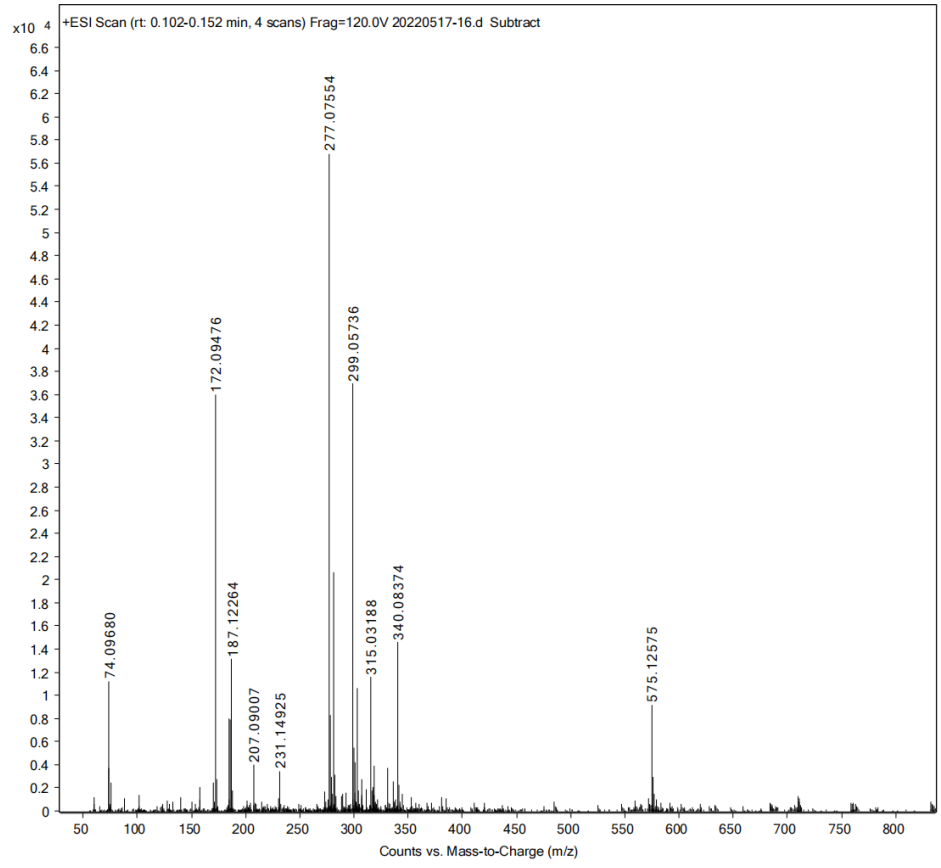

HRMS spectrum of **JZ-18**



HRMS spectrum for **3**




HRMS spectrum for **DNS-$N_3$**



HRMS spectrum for **Lyso-AFP**