Supporting Information

Design, Synthesis, Biological Application of Novel Photoaffinity Probes of Dihydropyridine Derivative, BAY R3401

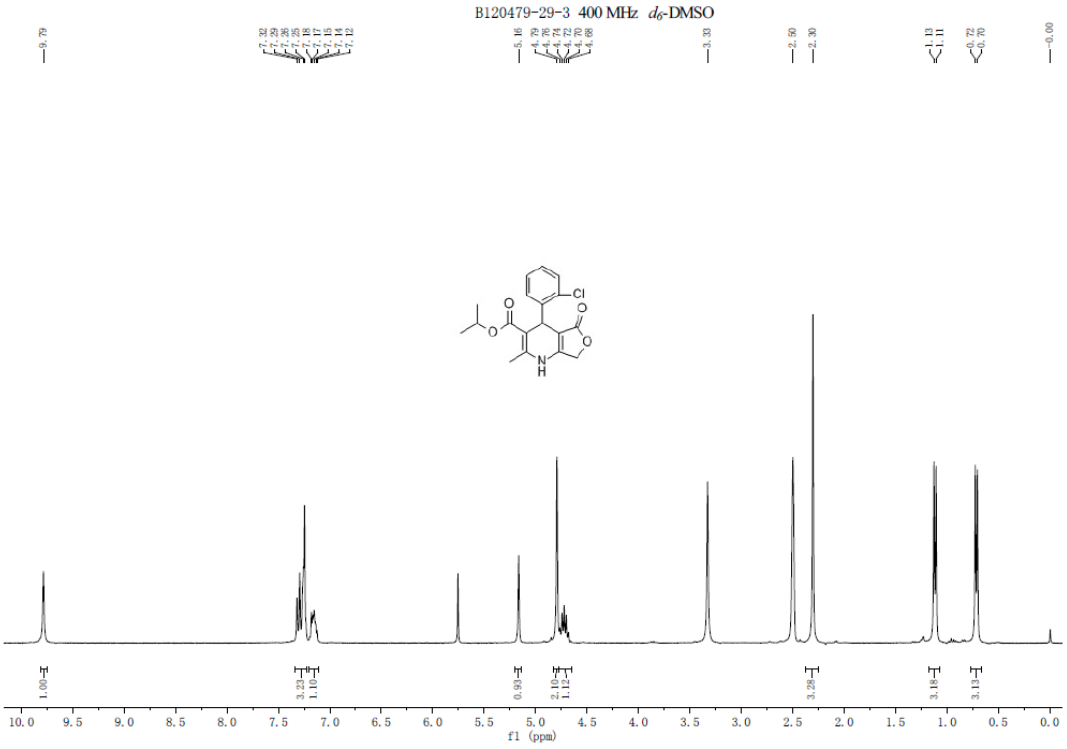
Liying Zhang [1,*], Zhiwei Yan [1], Youde Wang [1], Chengjun Song [2], Guangxin Miao [1].

Contents

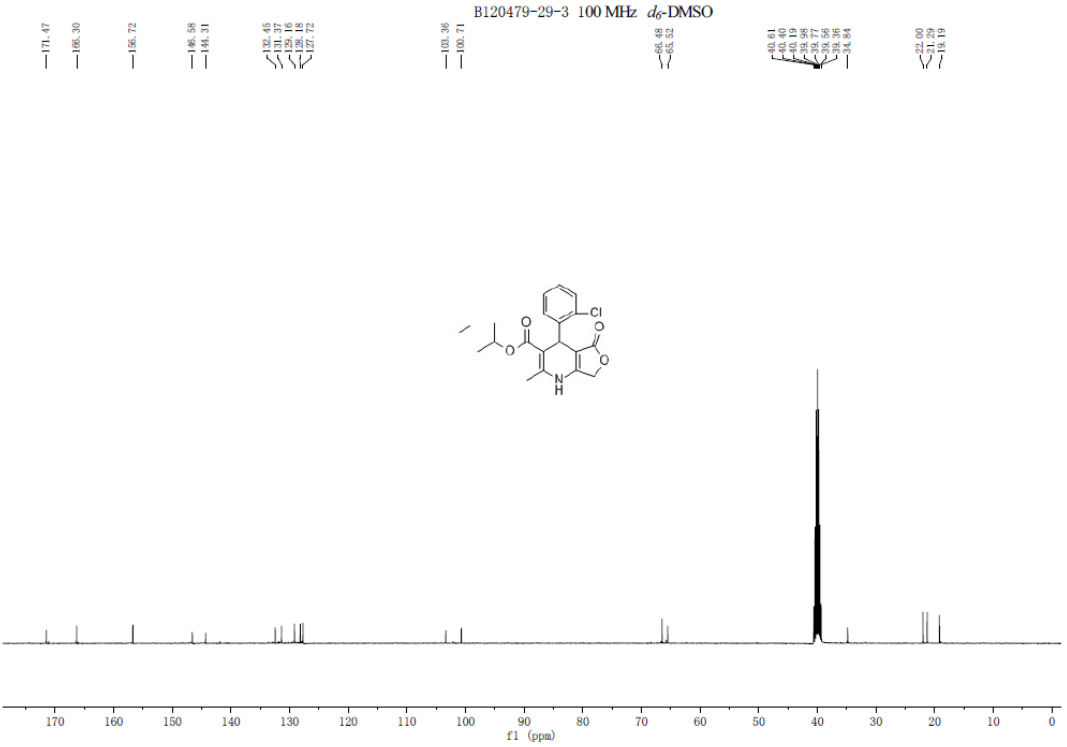
Coomassie brilliant blue (CBB) poststaining and destaining protocol.

The soluble proteomes prepared from HepG2 cells was diluted to 2.0 mg/mL with 50 mM Tris–HCl buffer (pH 7.4). The labeling reation was initiated by incubating proteomes with the probe (dissolved in DMSO) at 4 °C for 8 h, and then exposed to UV 365 nm (220v, 6W, 365 nm) at a distance of 3 cm. The reaction mixture was centrifuged at 48000 g for 10 min, then the supernatant was removed and the precipitate was resuspended in lysis buffer (urea 480 mg/L, chaps 40 mg/L, Tris-base 4.8 mg/L, DTT 10 mg/L, Ampholate 50 ml/L, and bromophenol blue 0.002%) at 4 °C for 1 h and centrifuged at 18000 g for 2 h. The supernatant was dialyzed against 50 mM Tris–HCl buffer (pH 7.4) and then subjected to SDS-PAGE electrophoresis. The samples were analyzed on Coomassie blue staining, based on the published method.[1] 2.5 g of Coomassie blue R250 was dissolved in 1000 mL of 50% (v/v) methanol, 10% (v/v) acetic acid and 40% (v/v) water with stirring as needed. The solution was filtered to remove any insoluble material. The final concentration of Coomassie blue R250 was 0.25% (w/v). After electrophoresis, the apparatus was disassembled and the gel was immersed into CBB solution. The gel was stained at room temperature overnight with gentle agitation. The Coomassie stain was removed by aspiration after staining. The gel was then immersed into the destaining solution composed of 50% (v/v) methanol, 10% (v/v) acetic acid and 40% (v/v) water which allowed the gel to destain with gentle agitation. The destaining step was repeated several times with removal of destaining solution at each change by aspiration. Destaining was continued until the protein bands were seen clearly without any background staining of the gel.

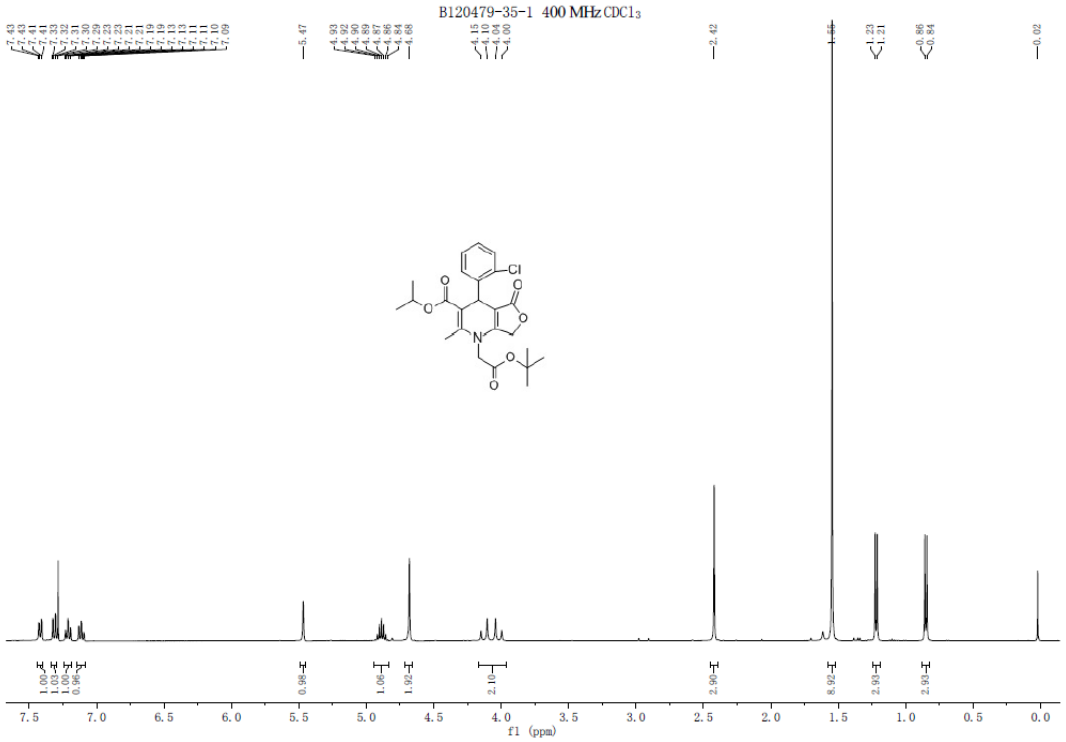
^{1}H NMR spectra of compound **10**



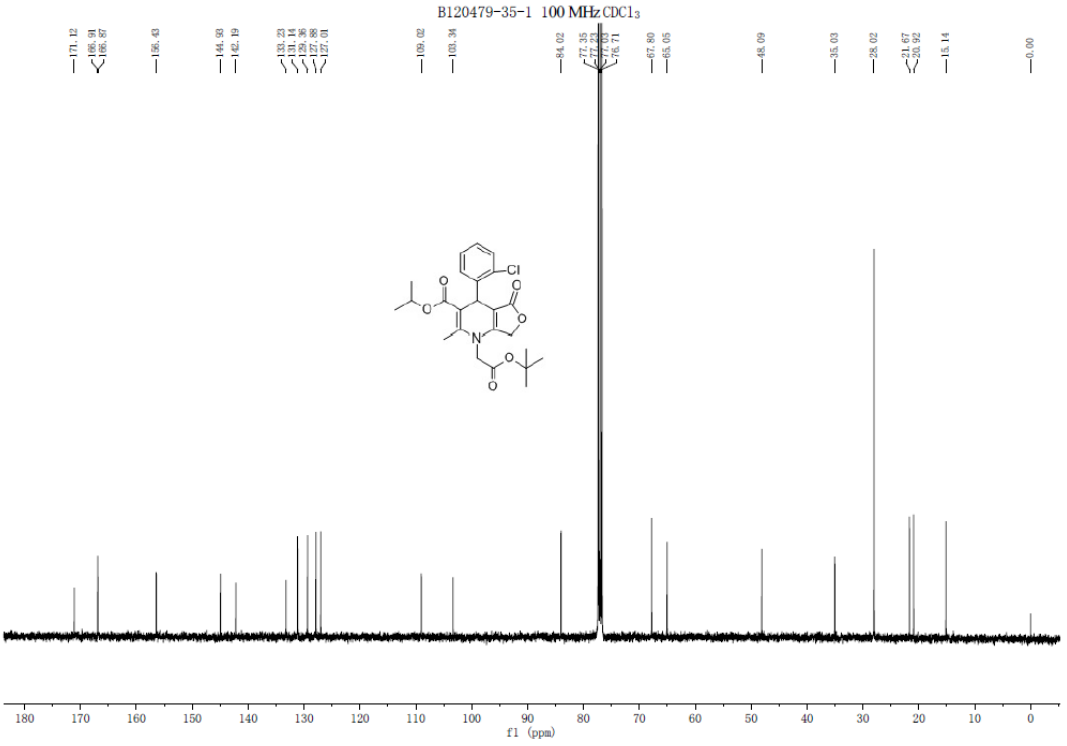
^{13}C NMR spectra of compound **10**



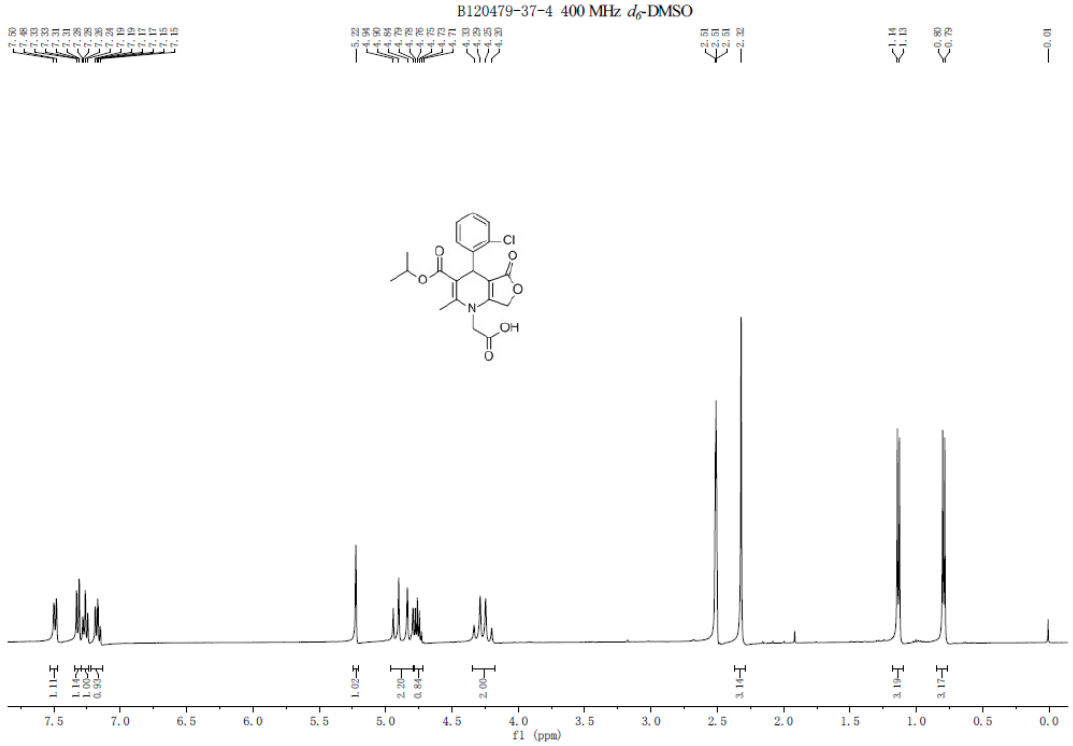
^{1}H NMR spectra of compound **11**



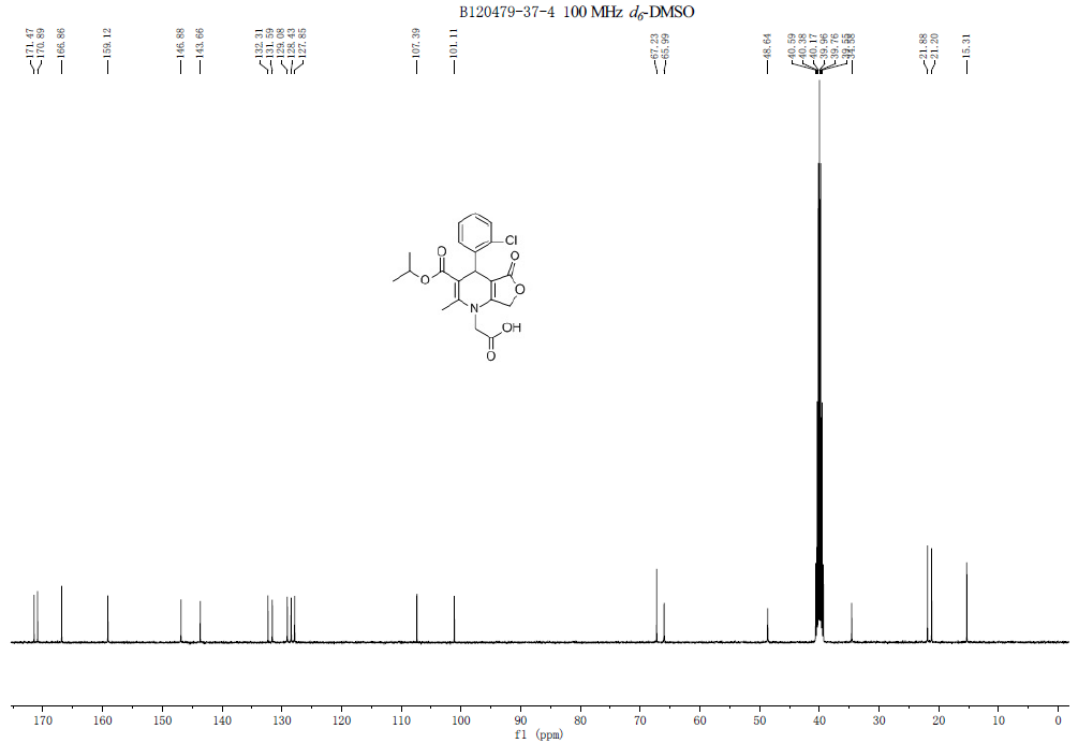
^{13}C NMR spectra of compound **11**



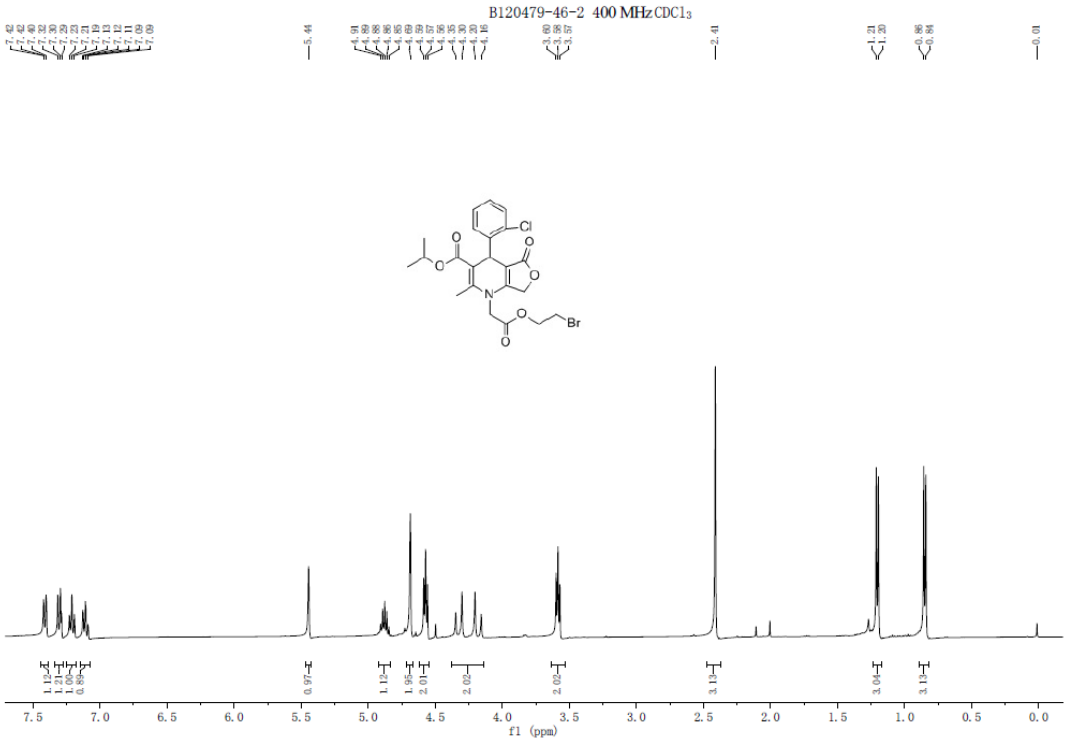
^{1}H NMR spectra of compound **12**



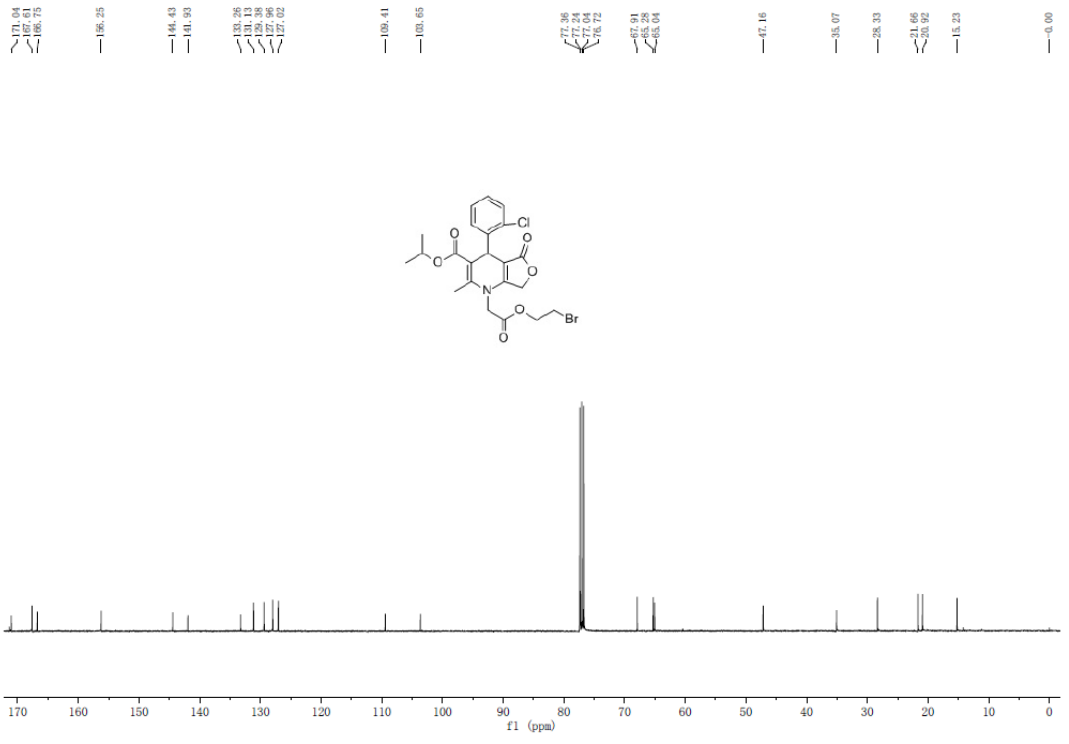
^{13}C NMR spectra of compound **12**



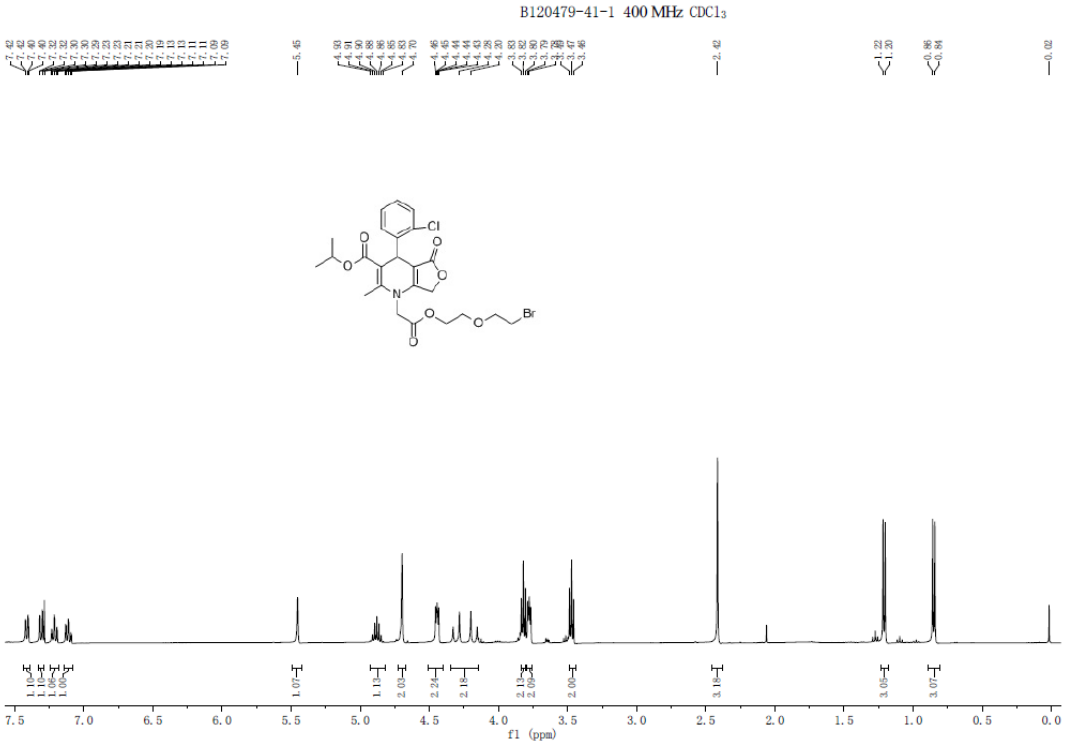
^{1}H NMR spectra of compound **13a**



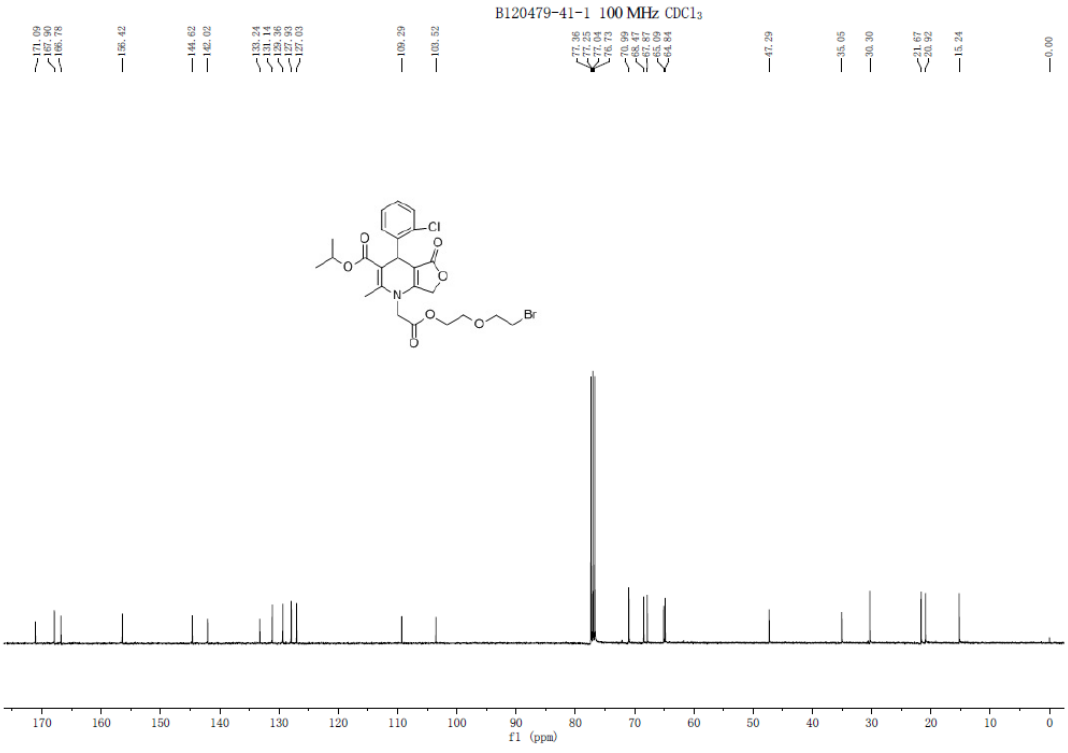
^{13}C NMR spectra of compound **13a**



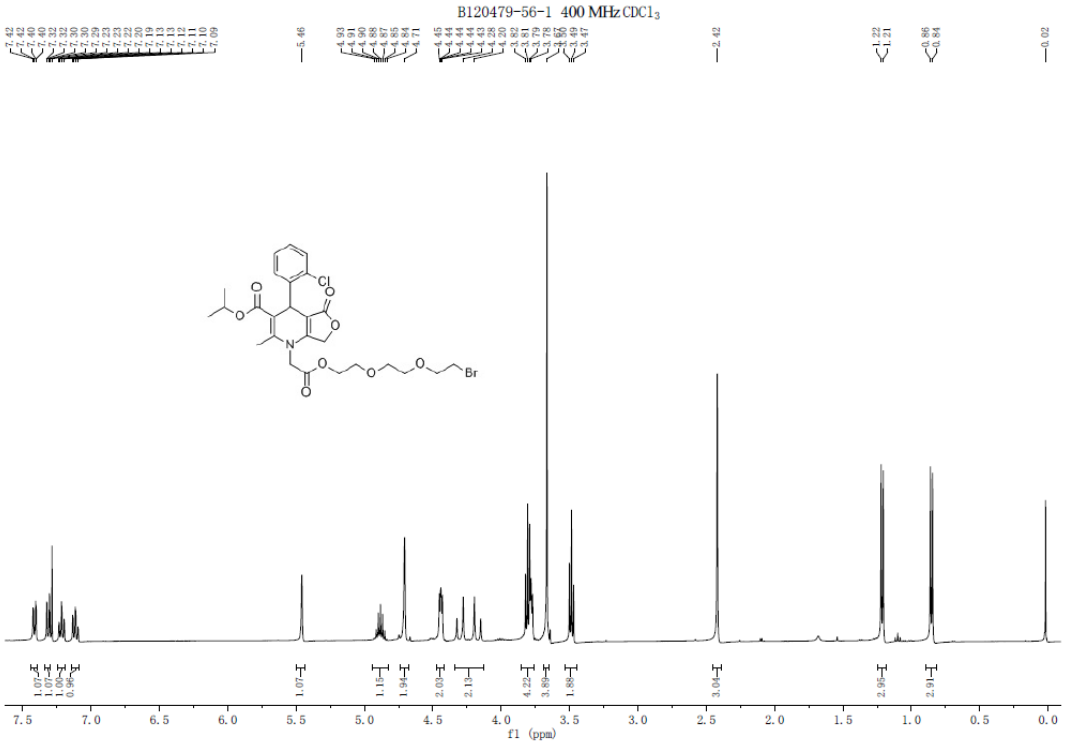
^{1}H NMR spectra of compound **13b**



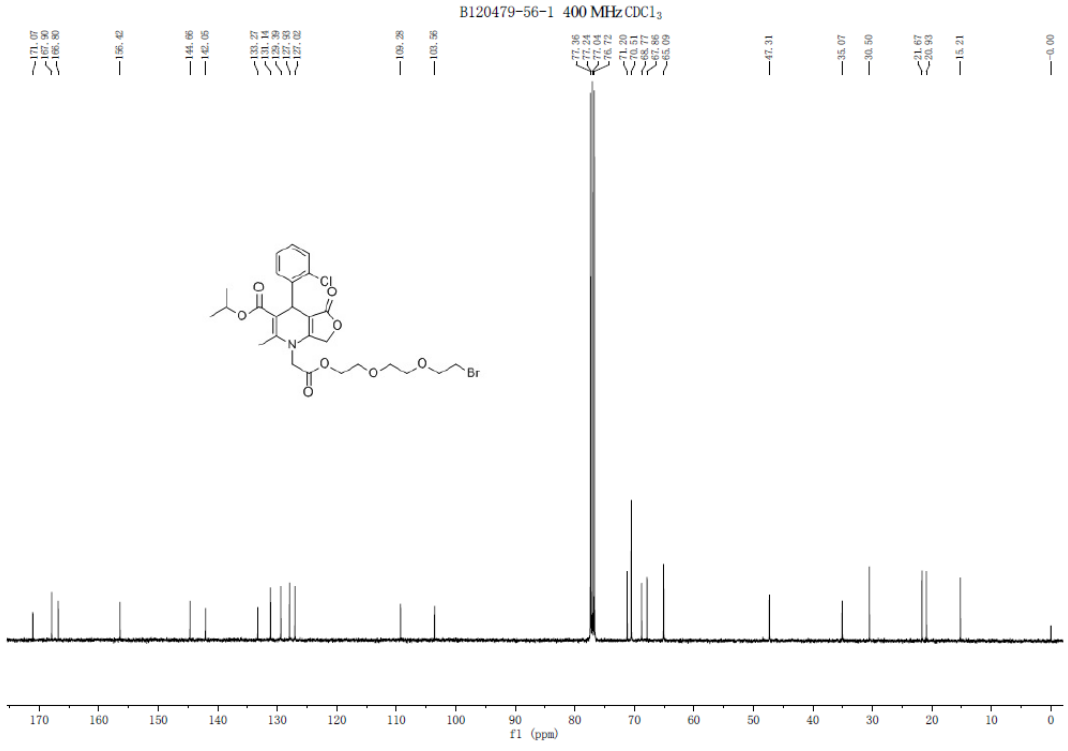
^{13}C NMR spectra of compound **13b**



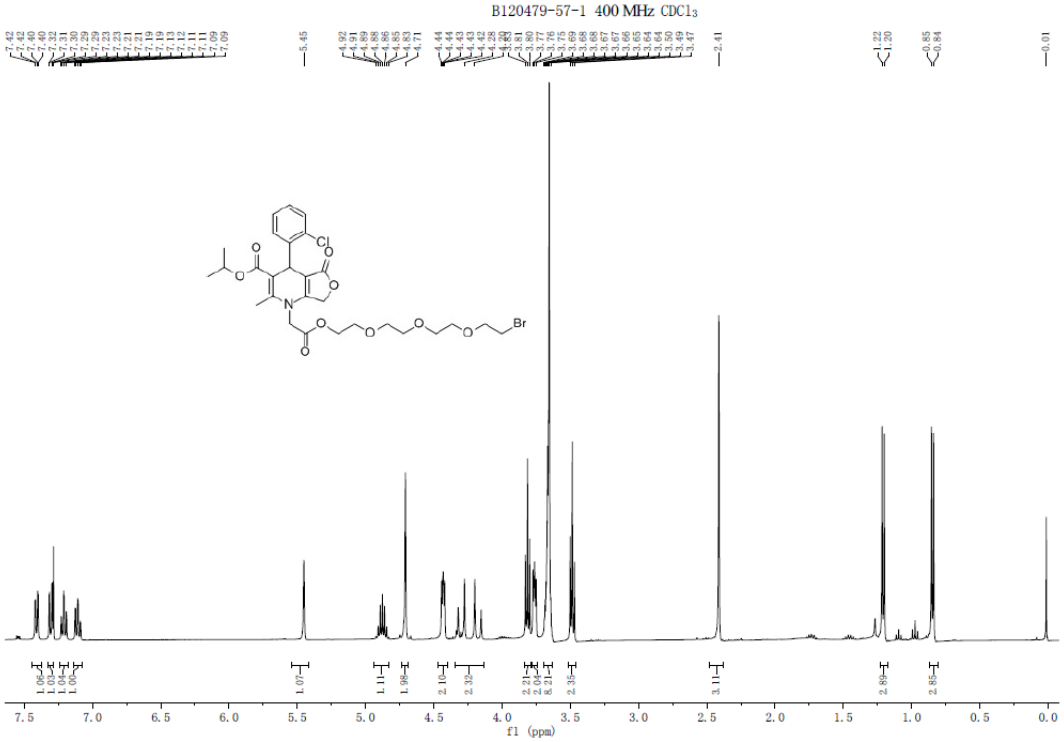
^{1}H NMR spectra of compound **13c**



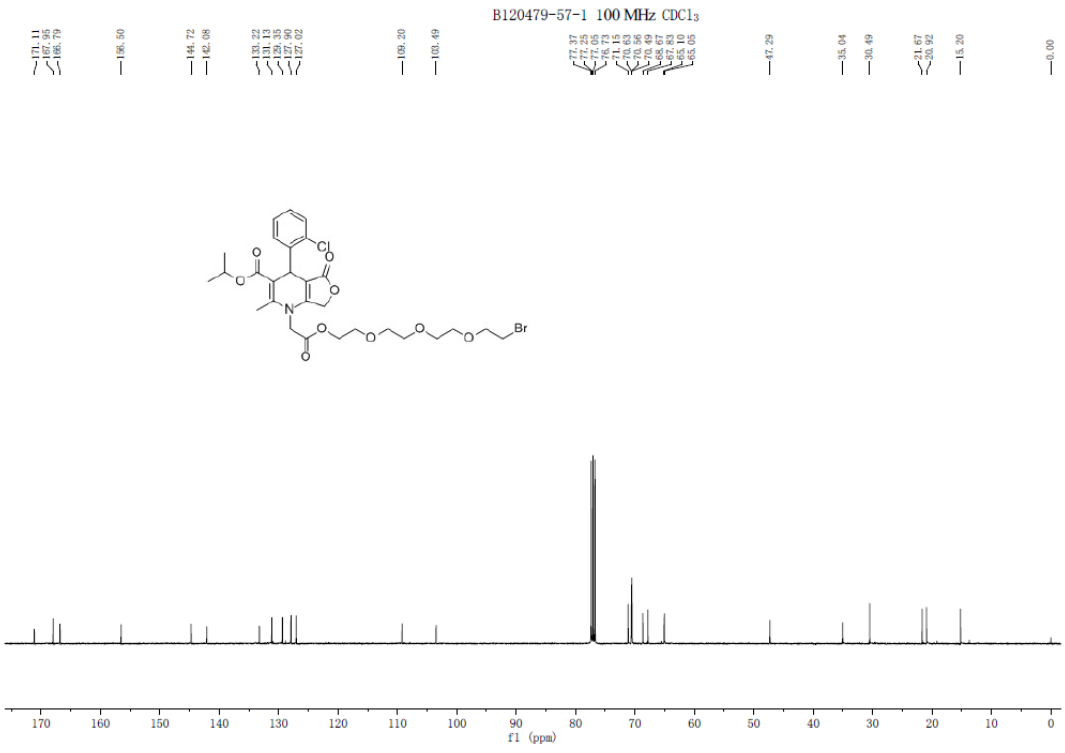
^{13}C NMR spectra of compound **13c**



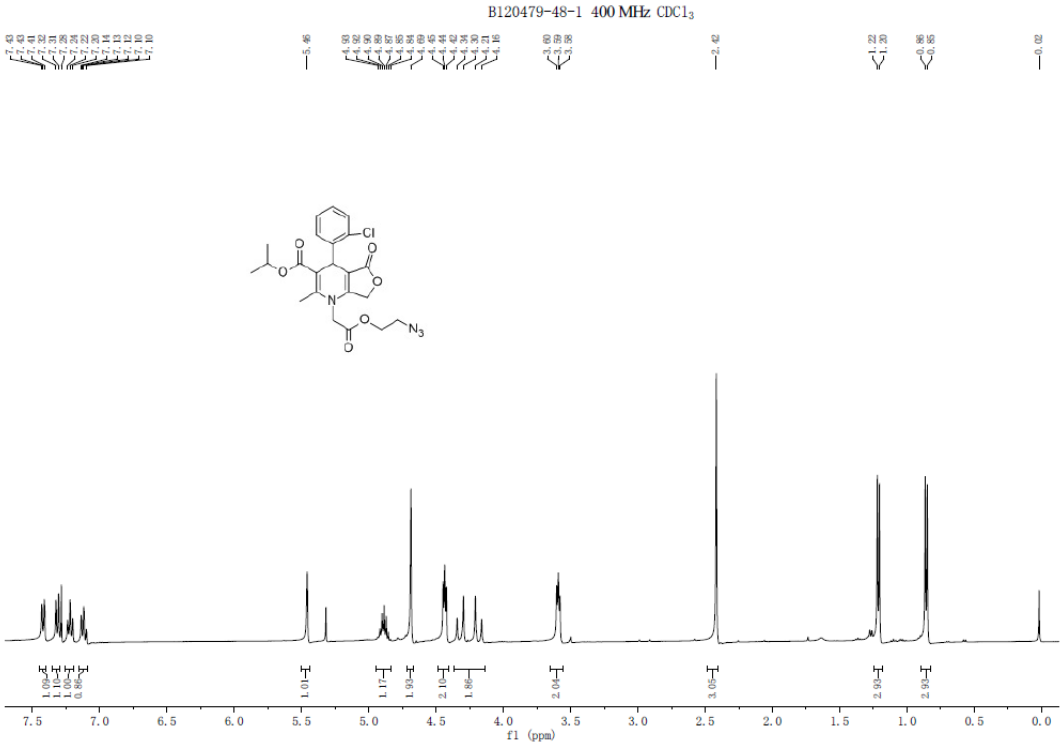
^{1}H NMR spectra of compound **13d**



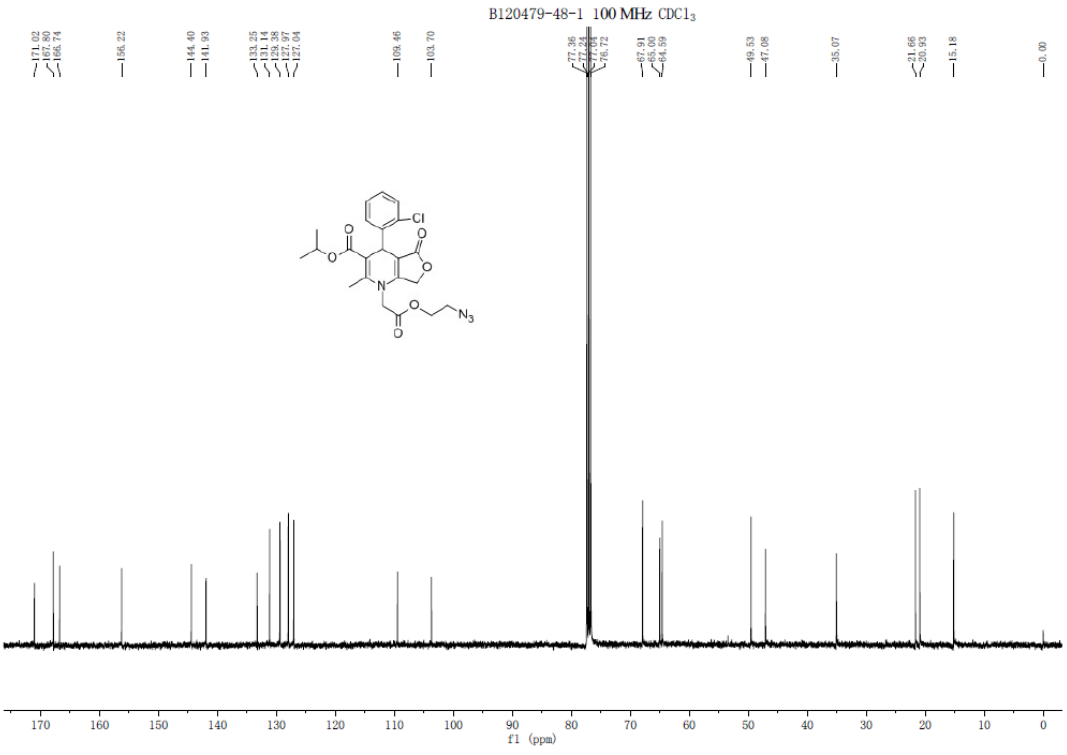
^{13}C NMR spectra of compound **13d**



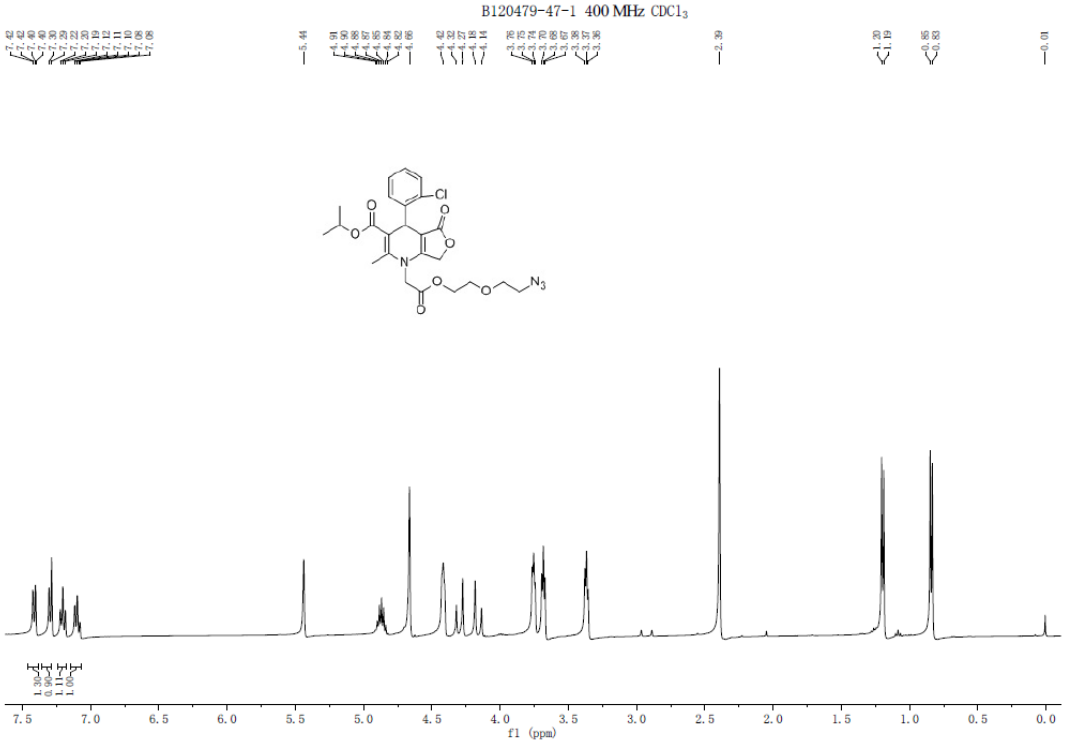
^{1}H NMR spectra of compound **6a**



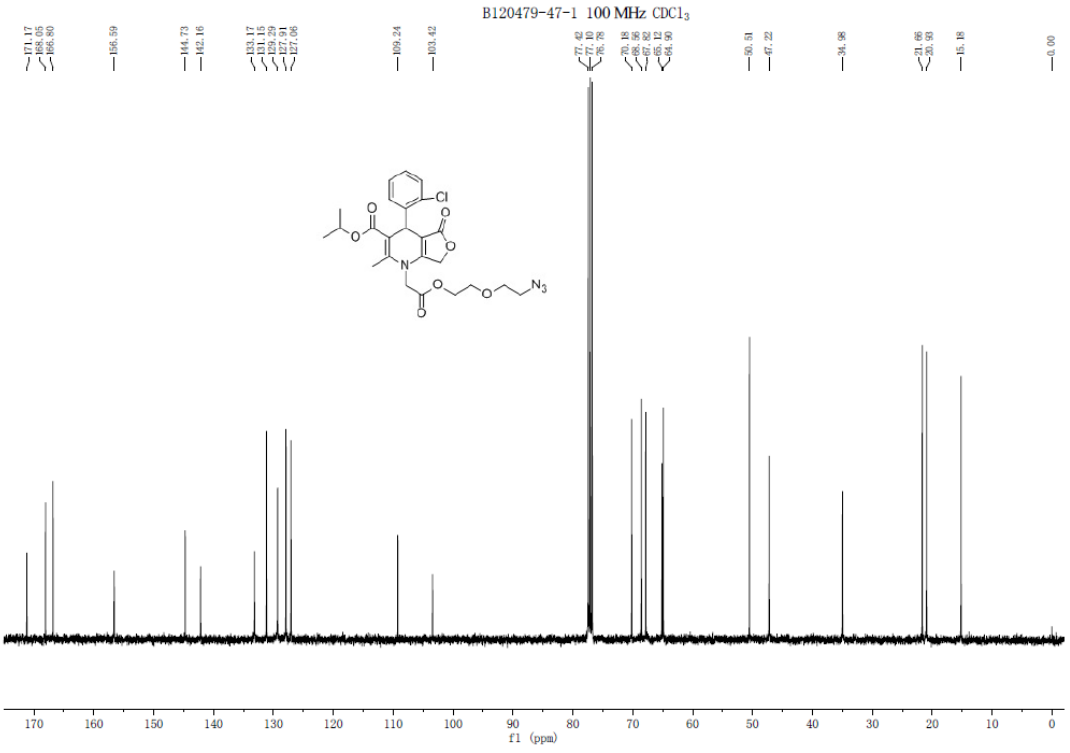
^{13}C NMR spectra of compound **6a**



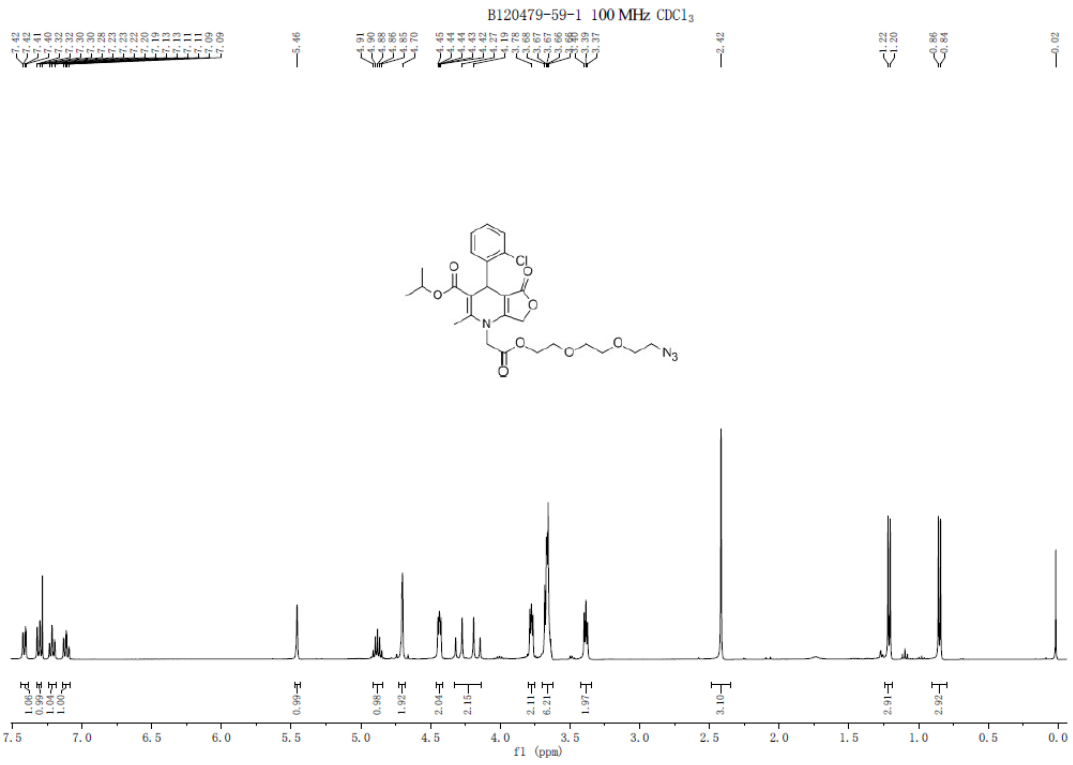
^{1}H NMR spectra of compound **6b**



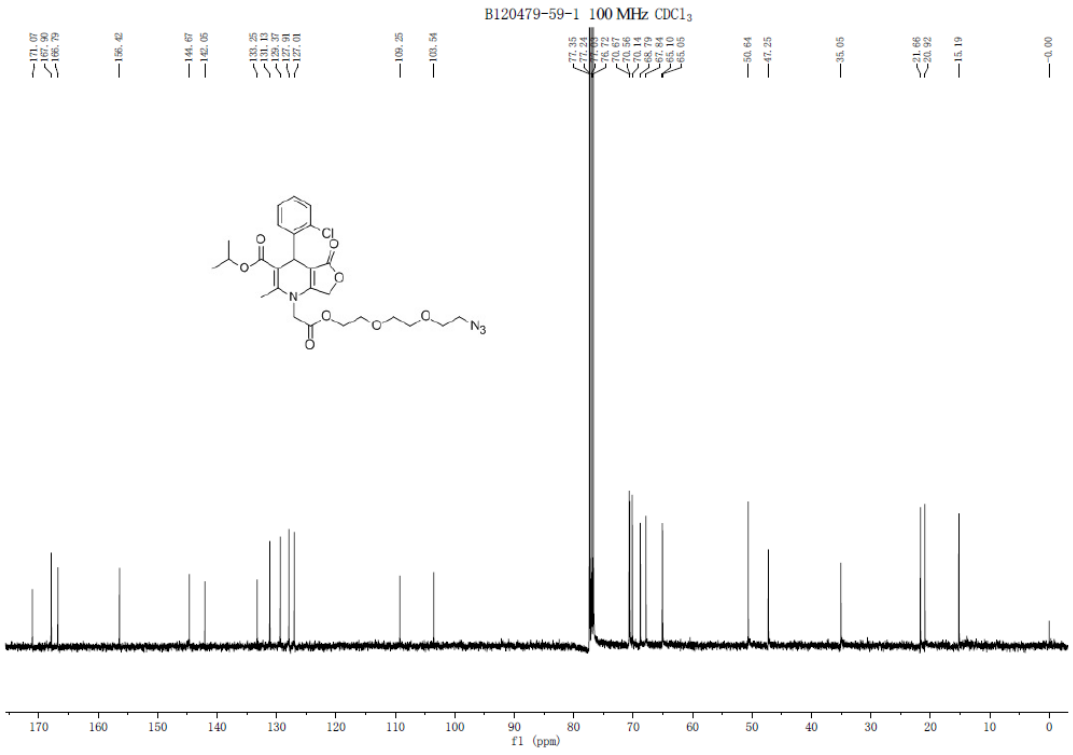
^{13}C NMR spectra of compound **6b**



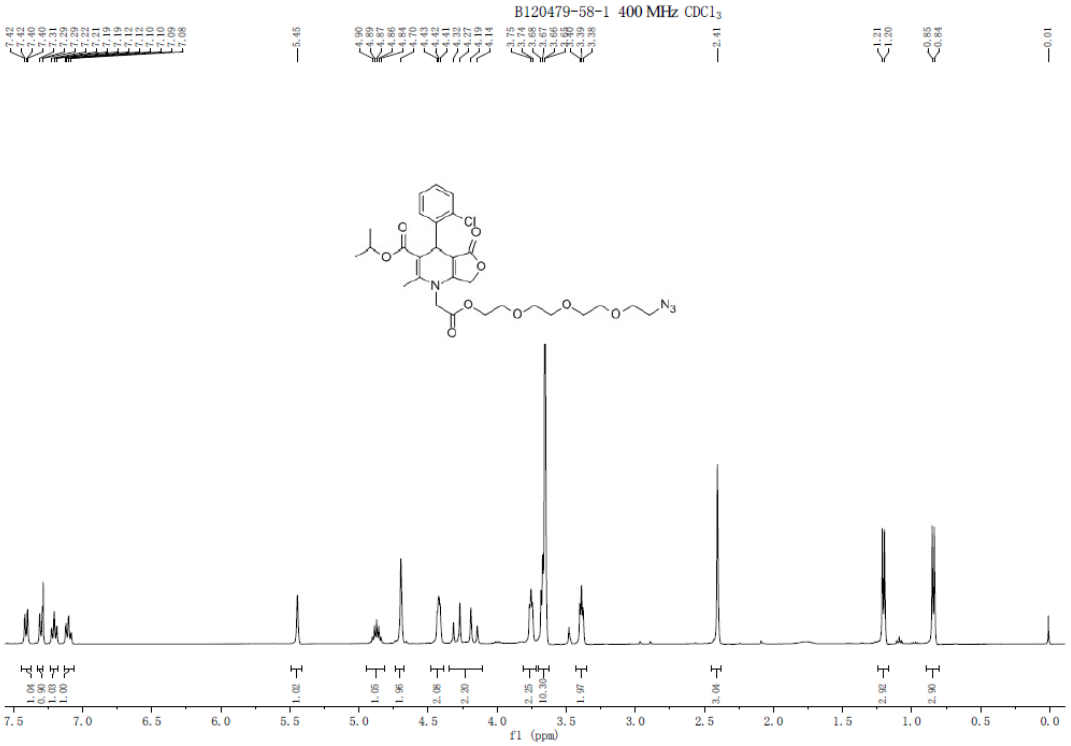
^{1}H NMR spectra of compound **6c**



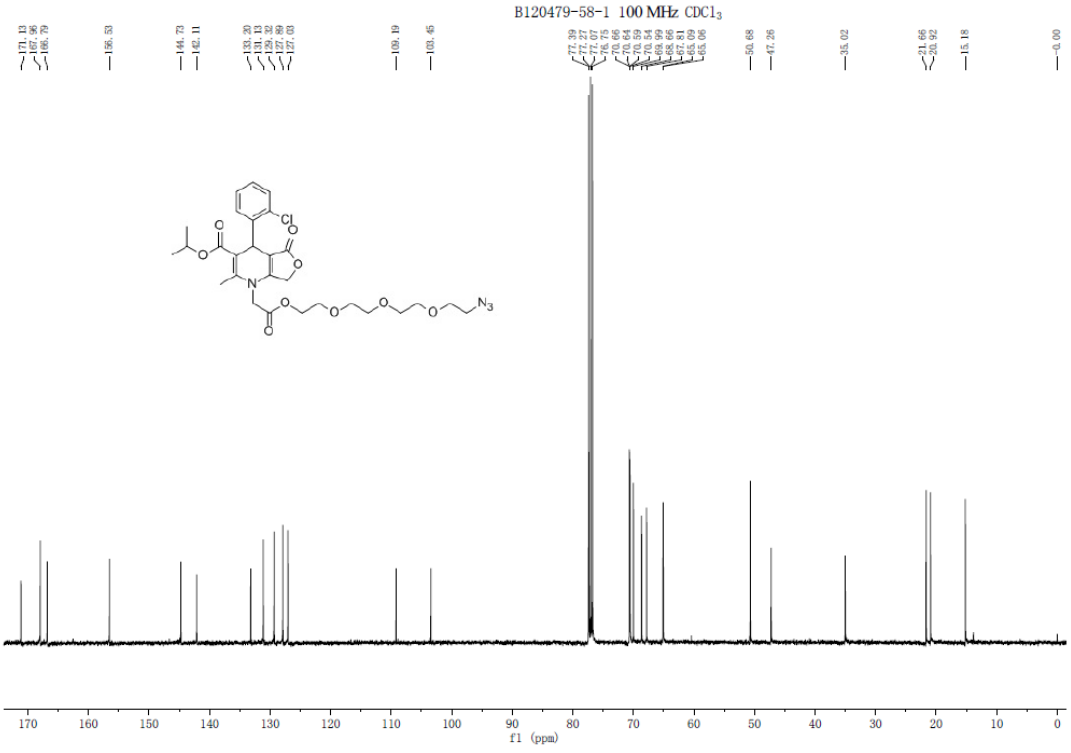
^{13}C NMR spectra of compound **6c**



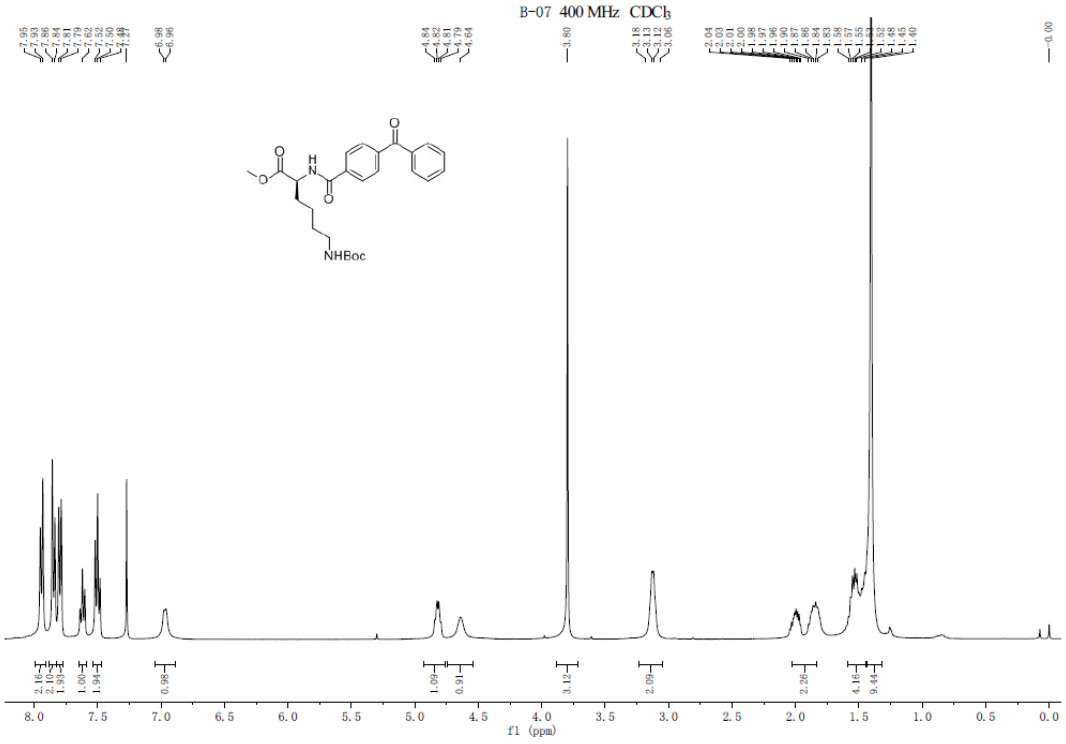
^{1}H NMR spectra of compound **6d**



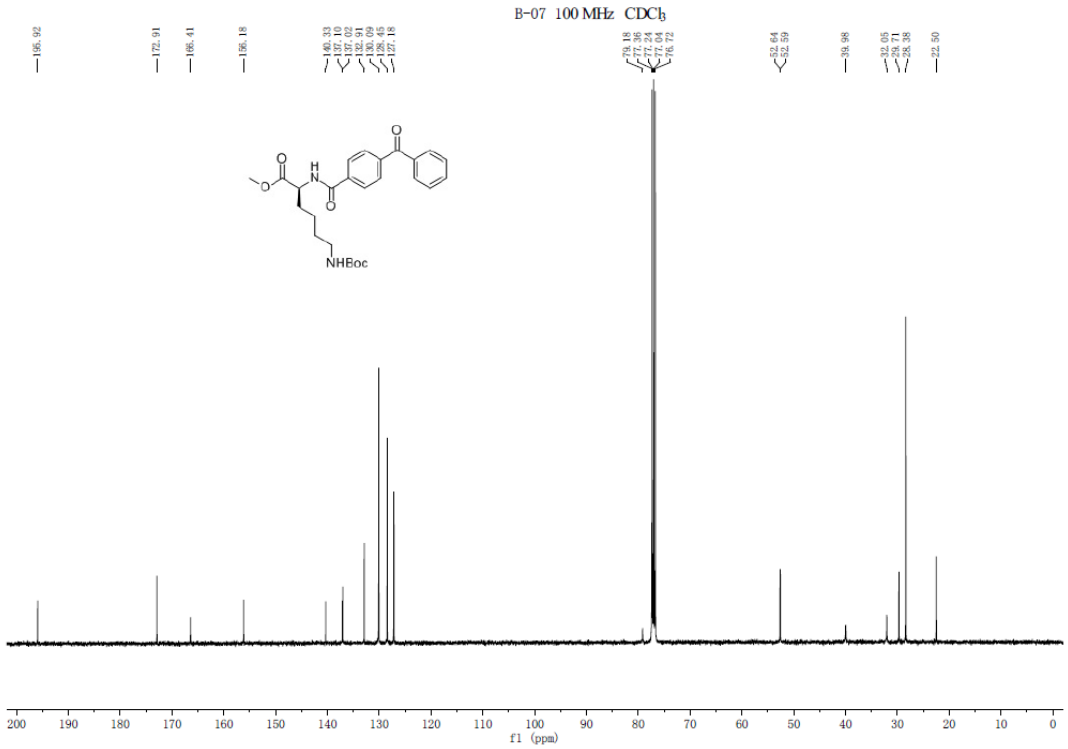
^{13}C NMR spectra of compound **6d**



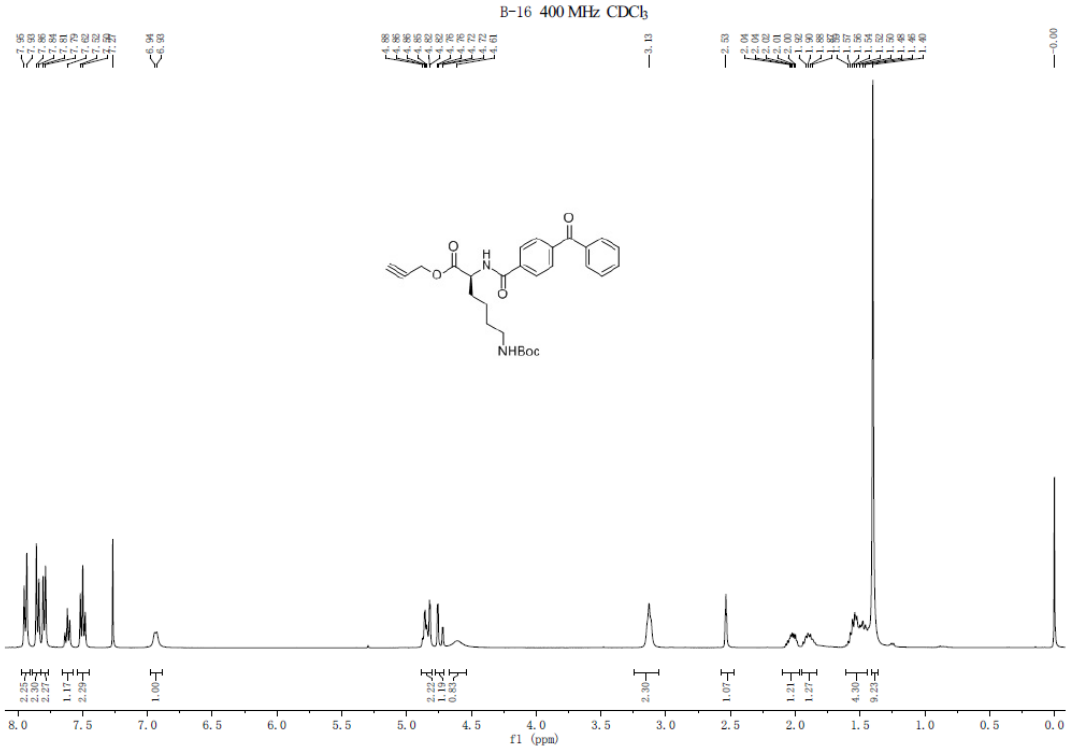
^{1}H NMR spectra of compound **16**



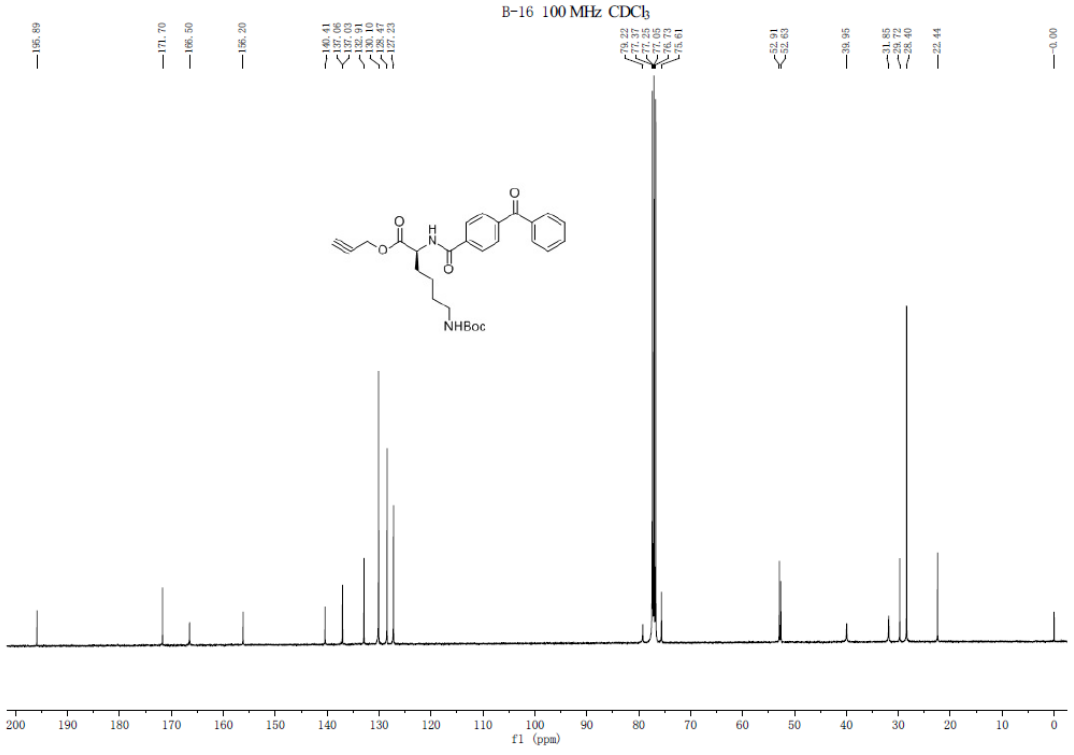
^{13}C NMR spectra of compound **16**



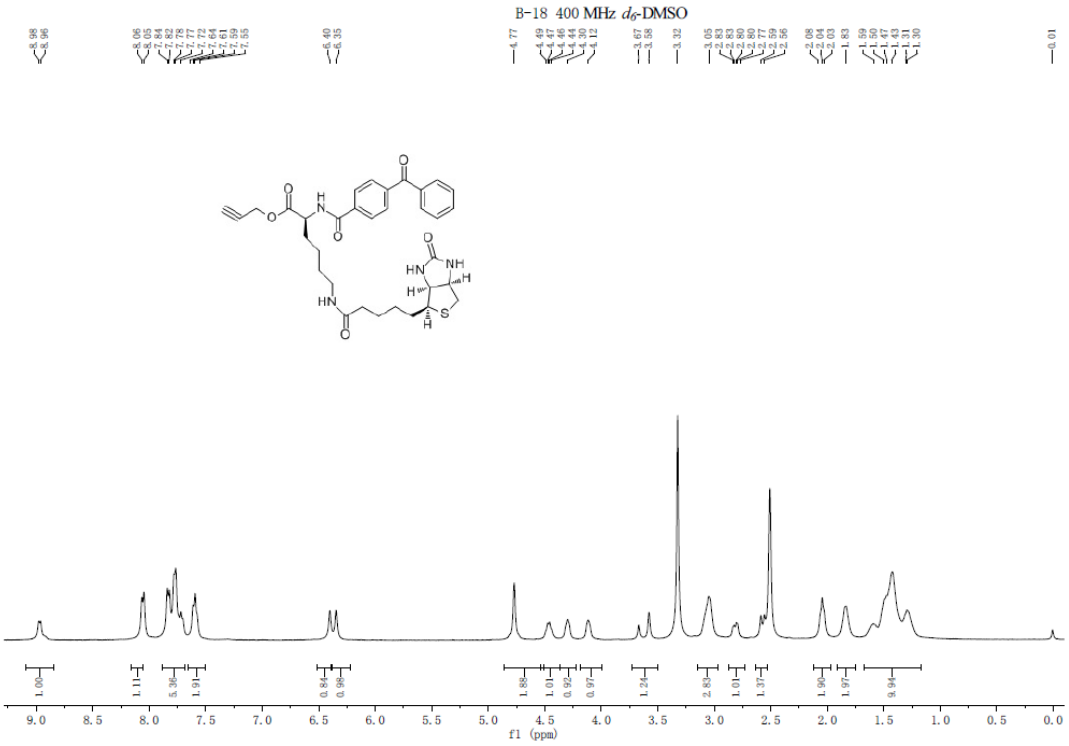
^{1}H NMR spectra of compound **18**



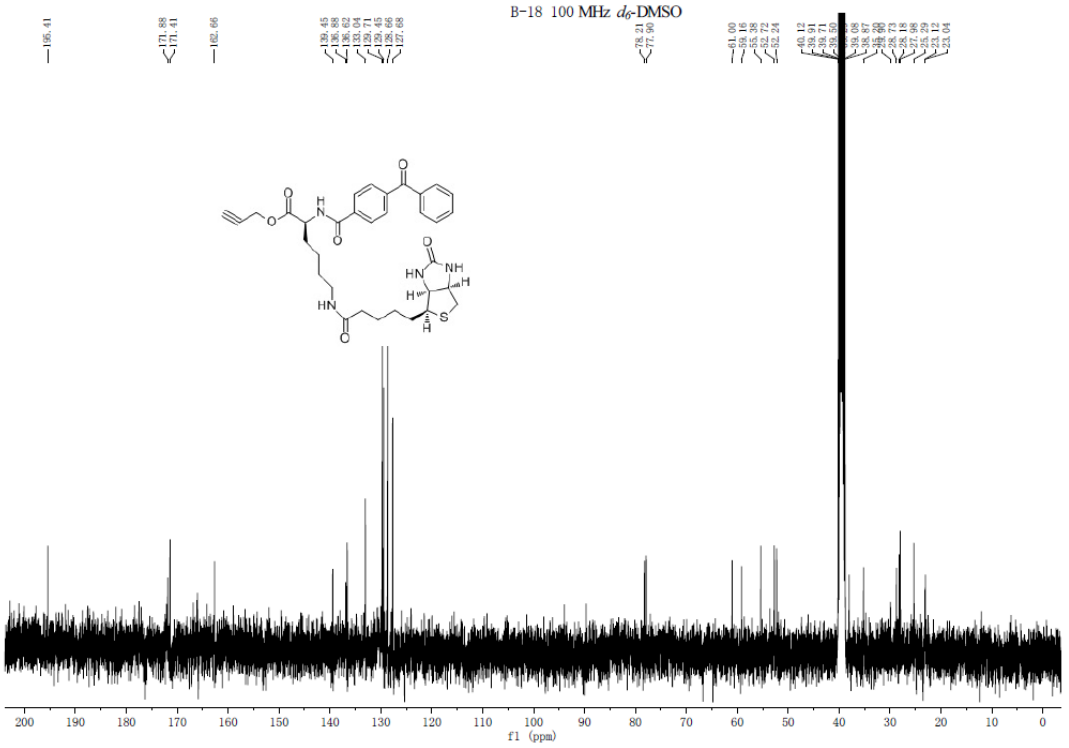
^{13}C NMR spectra of compound **18**



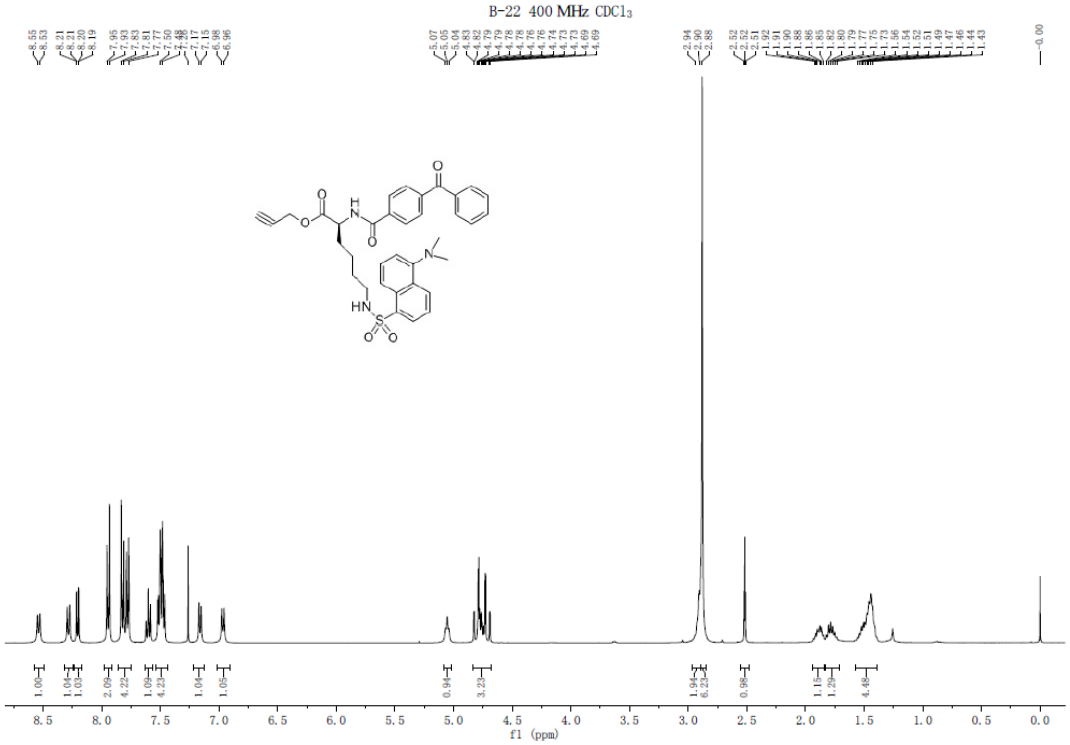
^{1}H NMR spectra of compound**20**



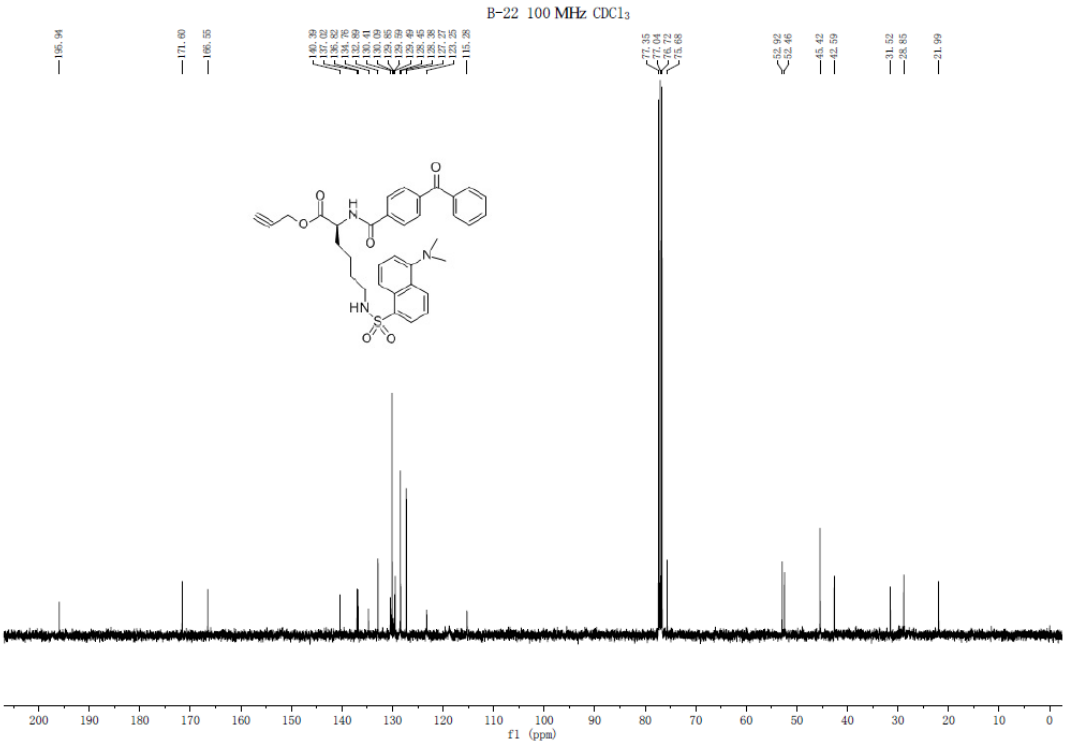
^{13}C NMR spectra of compound**20**



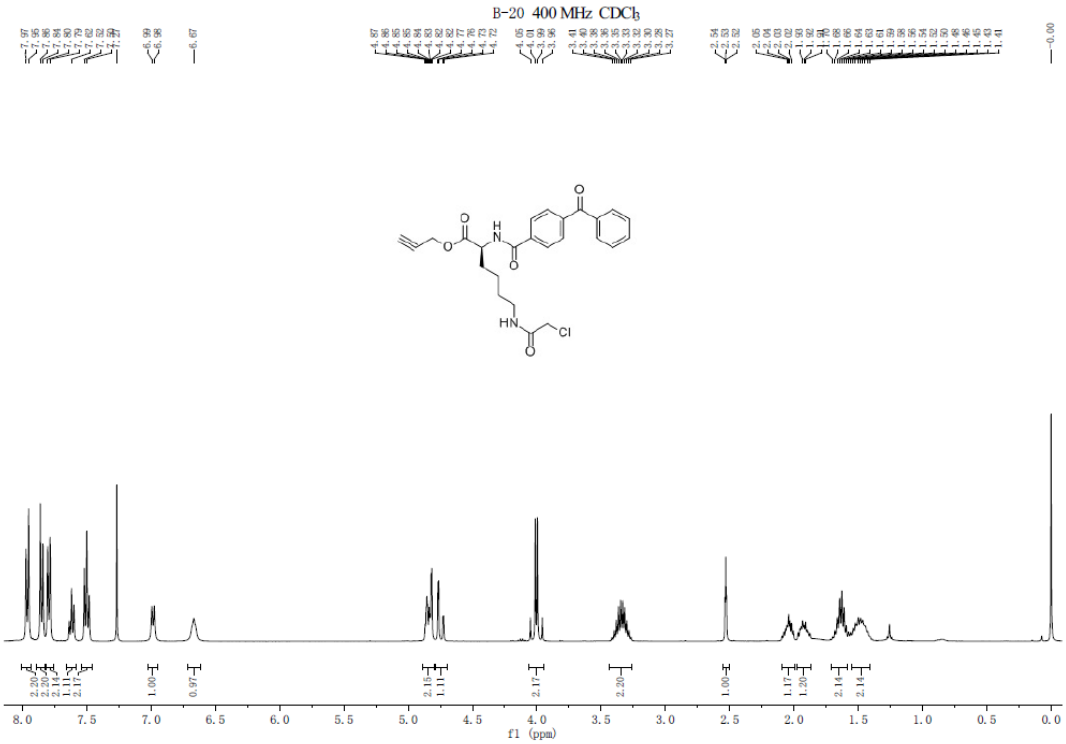
^{1}H NMR spectra of compound **21**



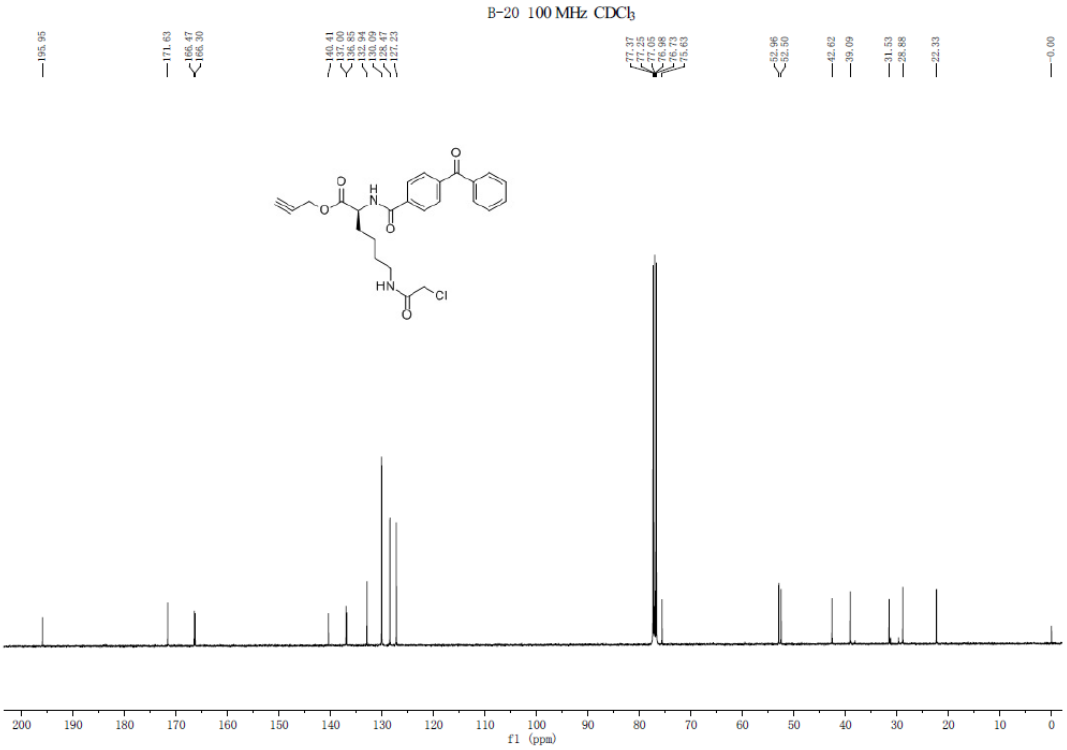
^{13}C NMR spectra of compound **21**



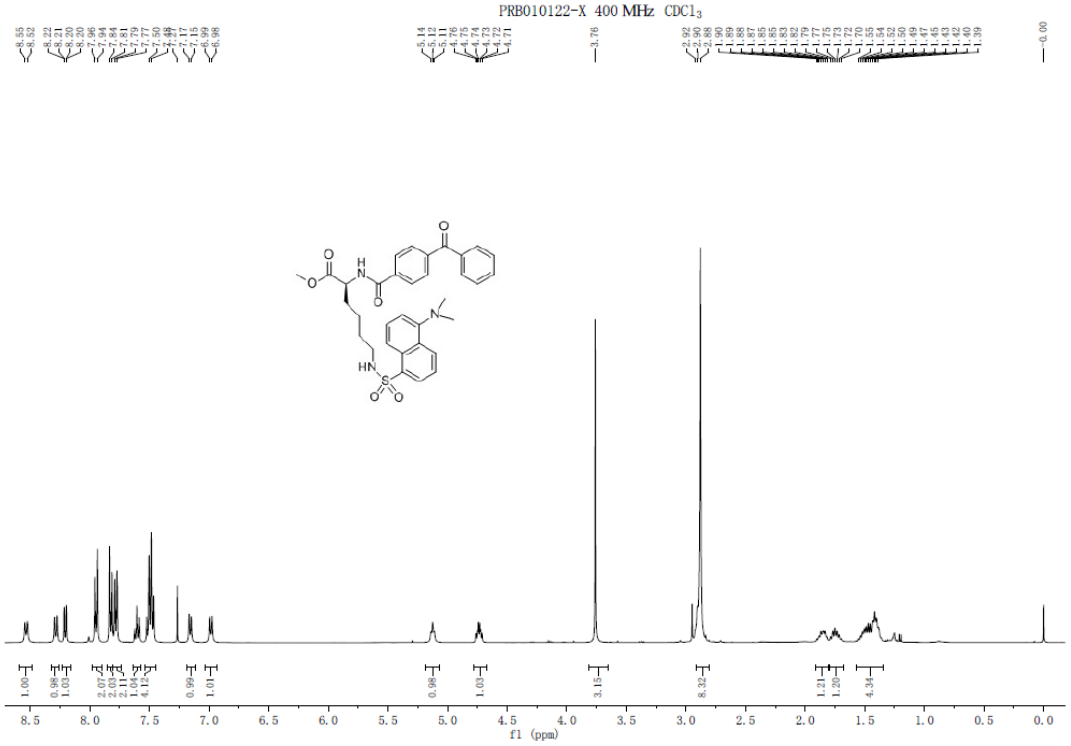
^{1}H NMR spectra of compound**22**



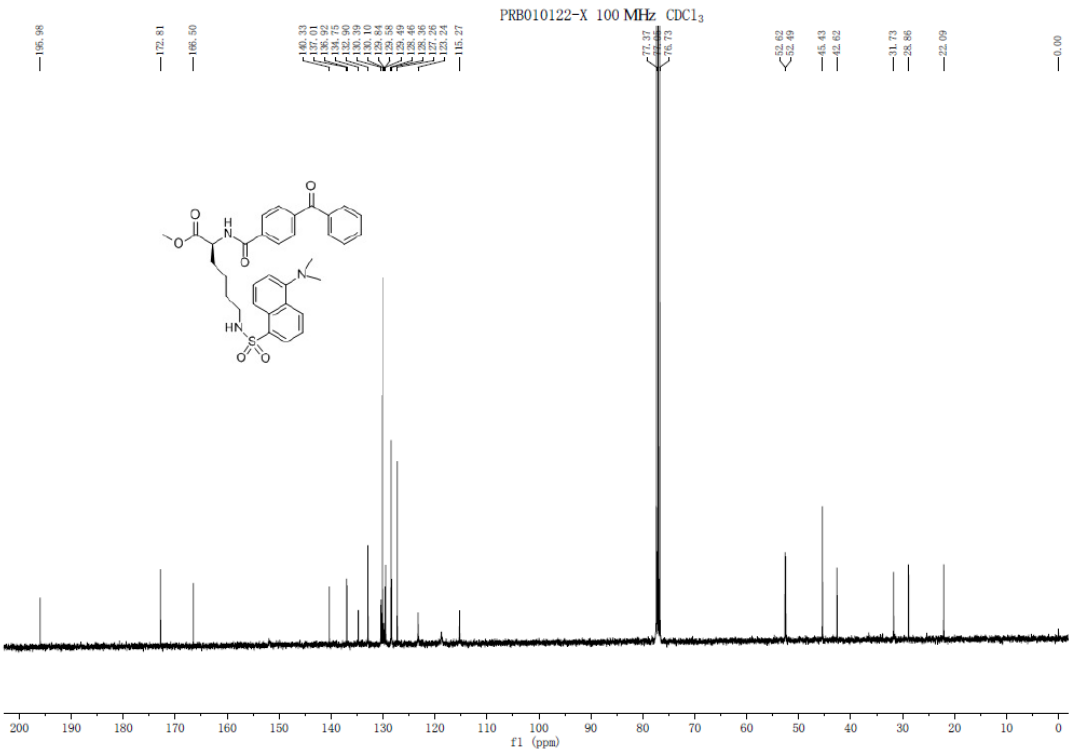
^{13}C NMR spectra of compound**22**



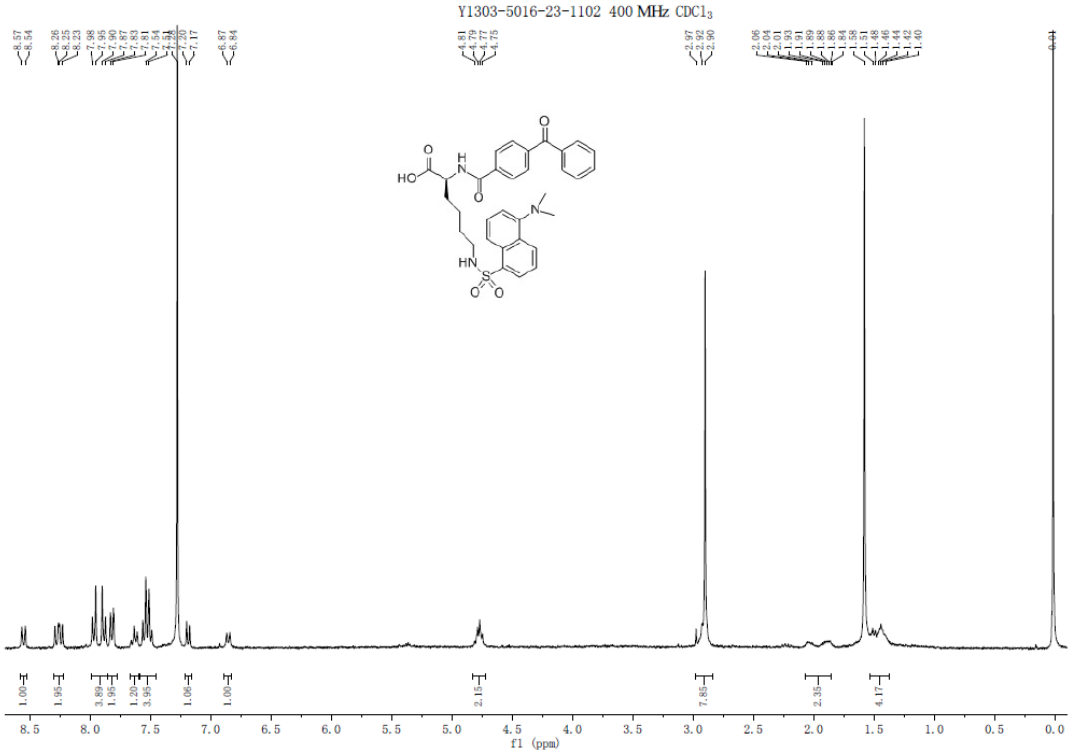
^{1}H NMR spectra of compound **24**



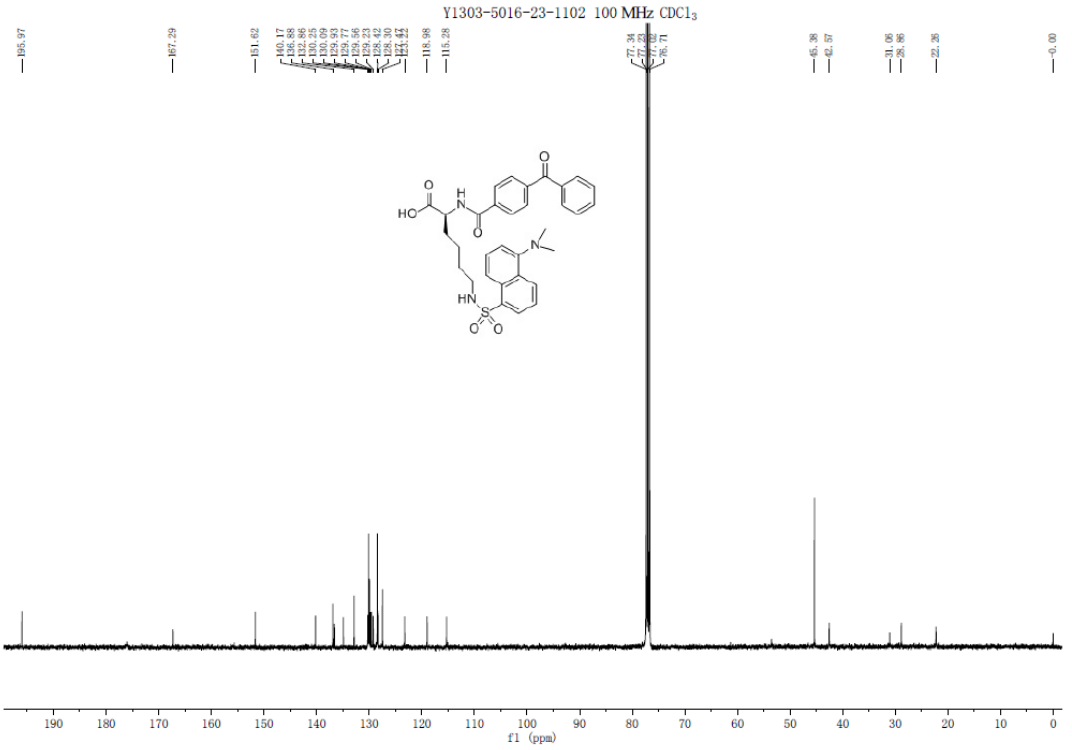
^{13}C NMR spectra of compound **24**



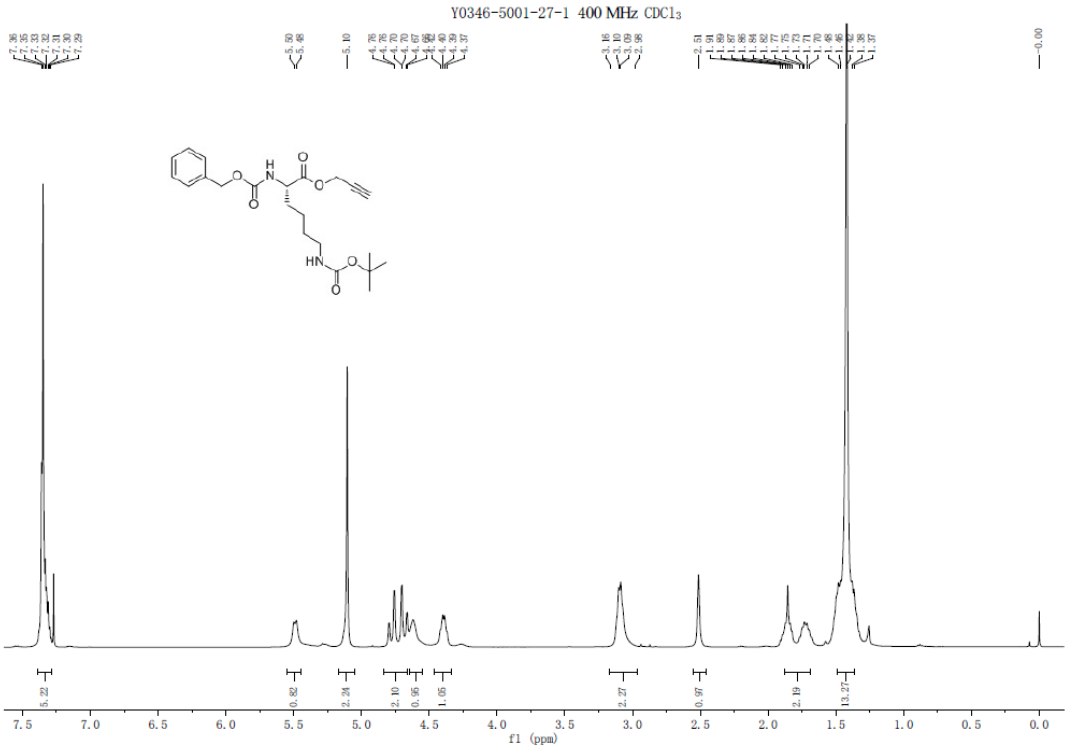
^{1}H NMR spectra of compound **25**



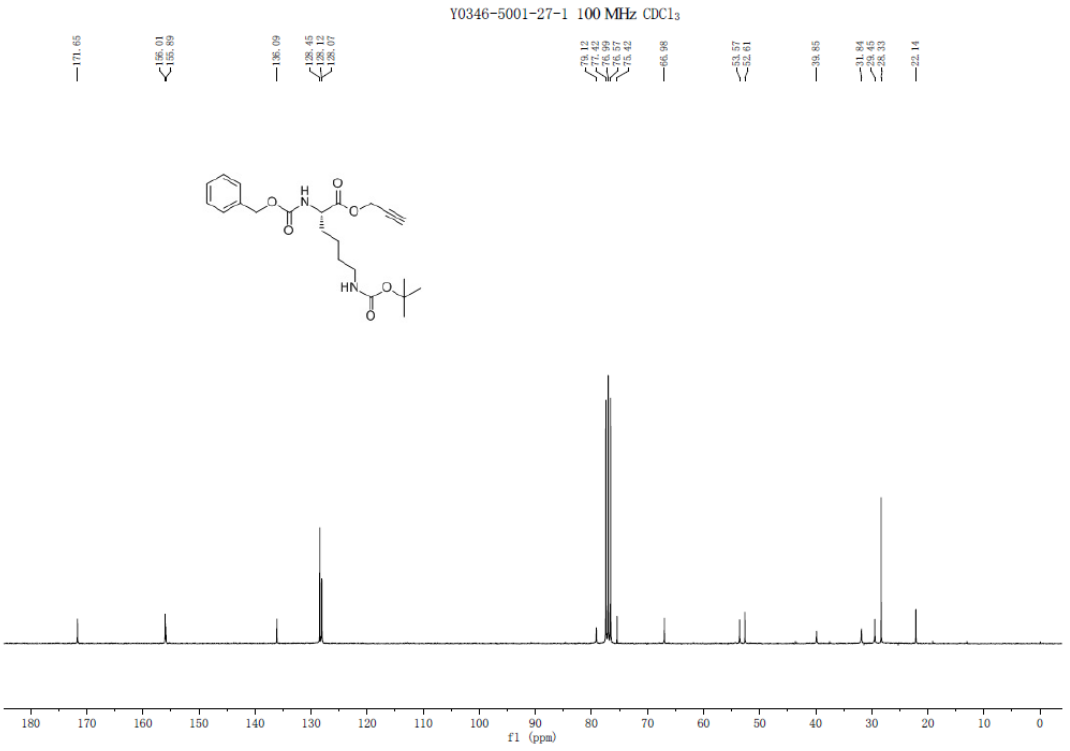
^{13}C NMR spectra of compound **25**



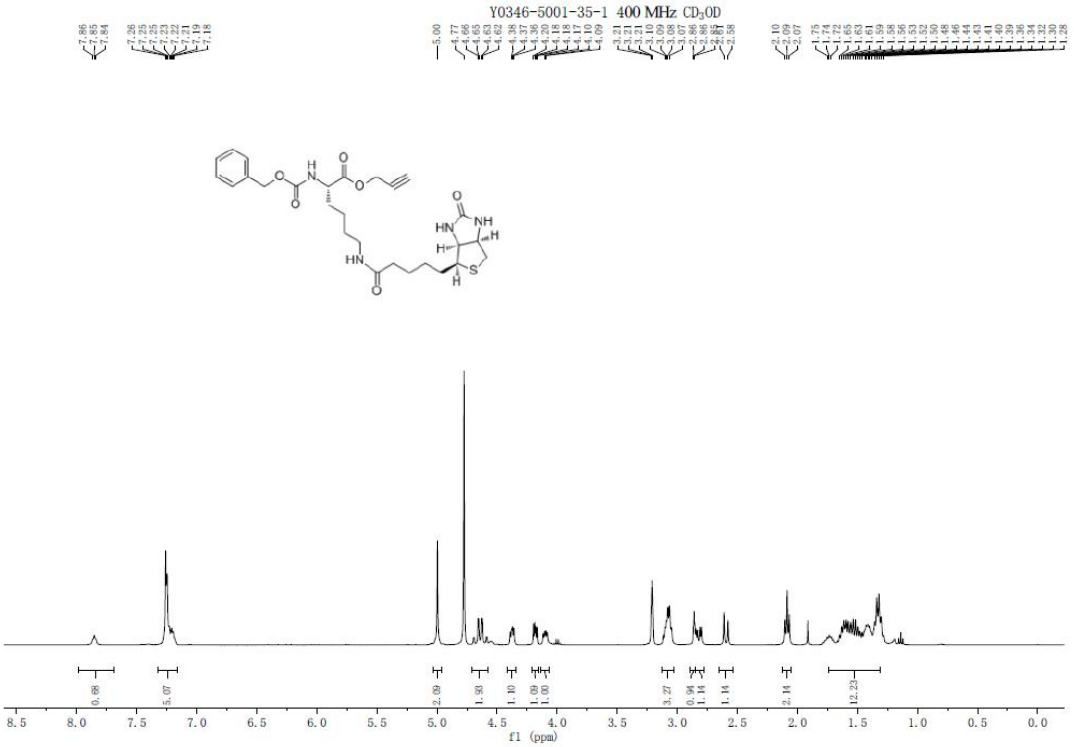
^{1}H NMR spectra of compound **27**



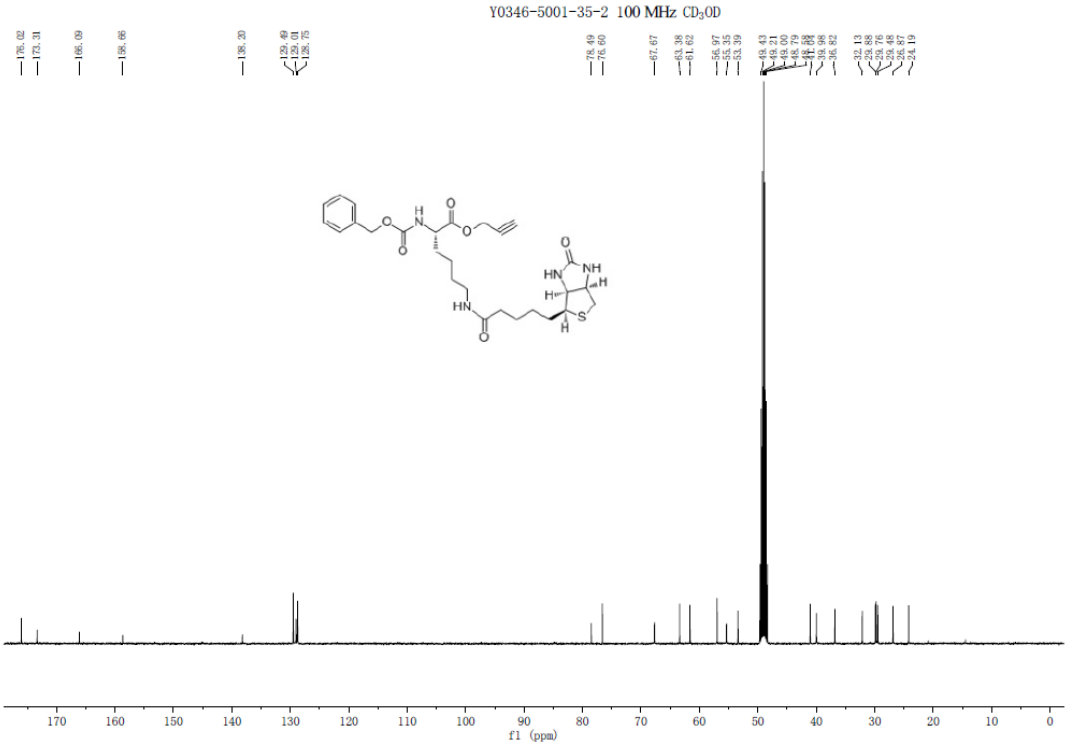
^{13}C NMR spectra of compound **27**



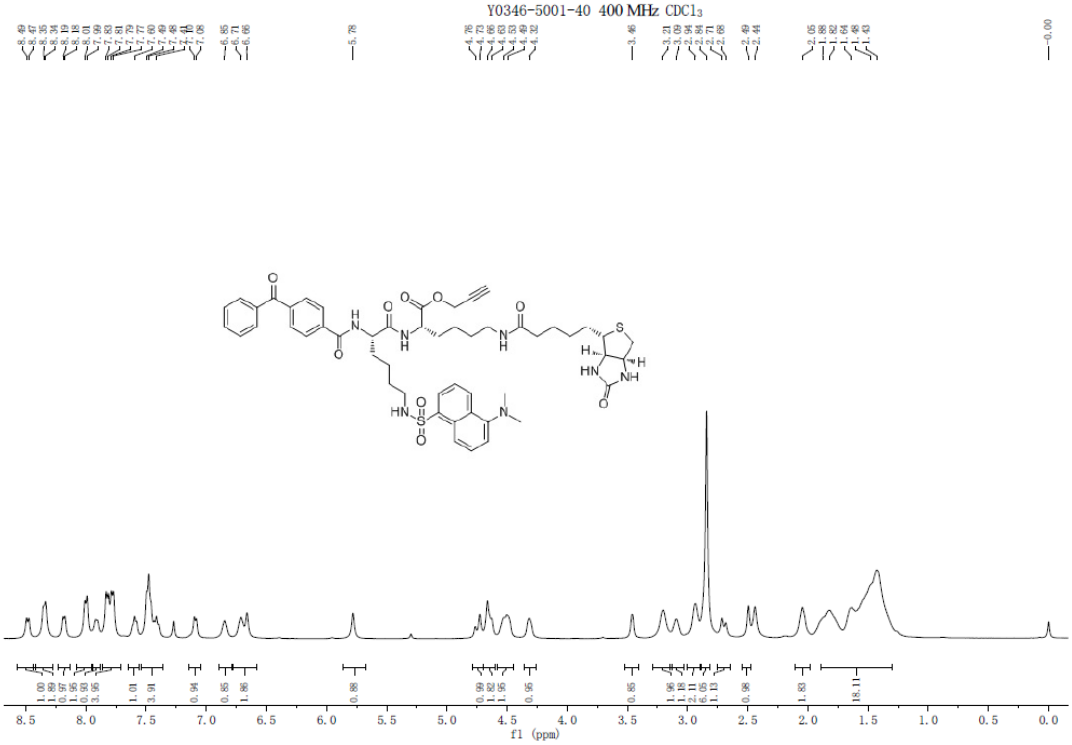
^{1}H NMR spectra of compound **28**



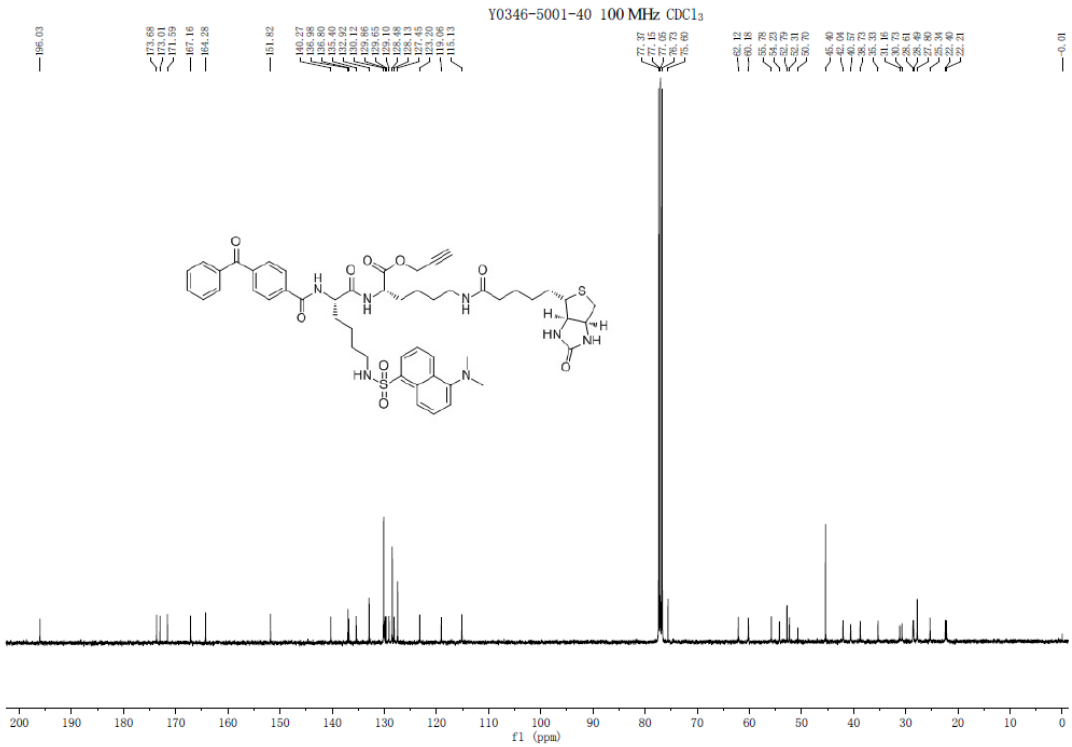
^{13}C NMR spectra of compound **28**



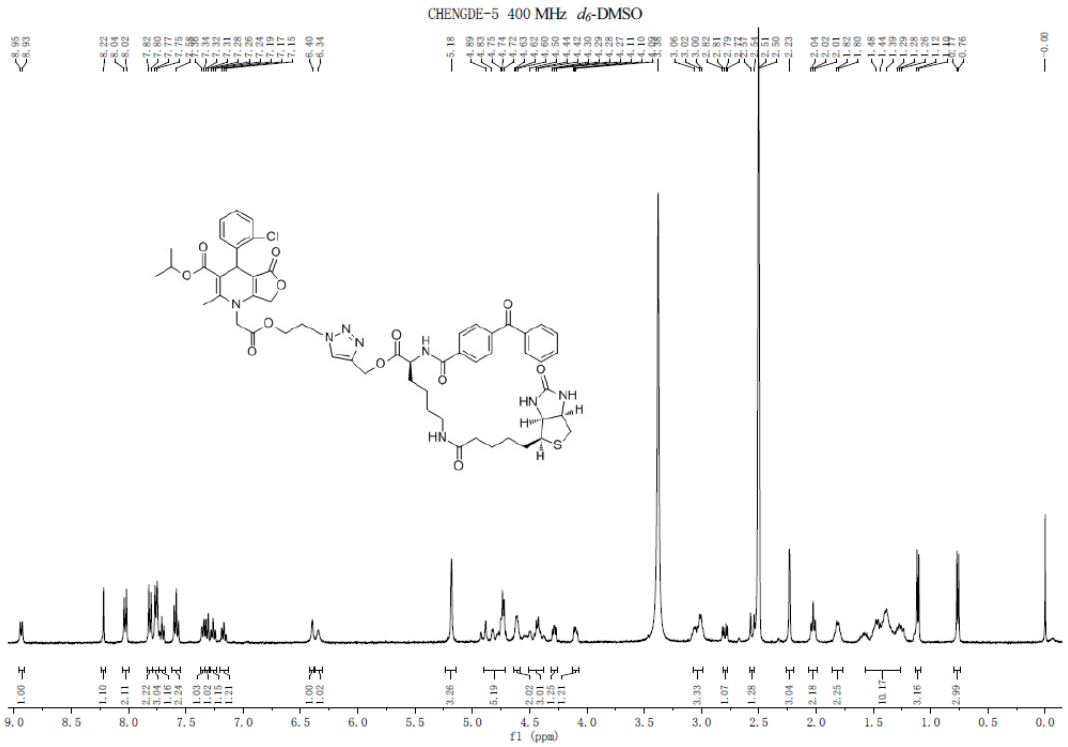
^{1}H NMR spectra of compound **30**



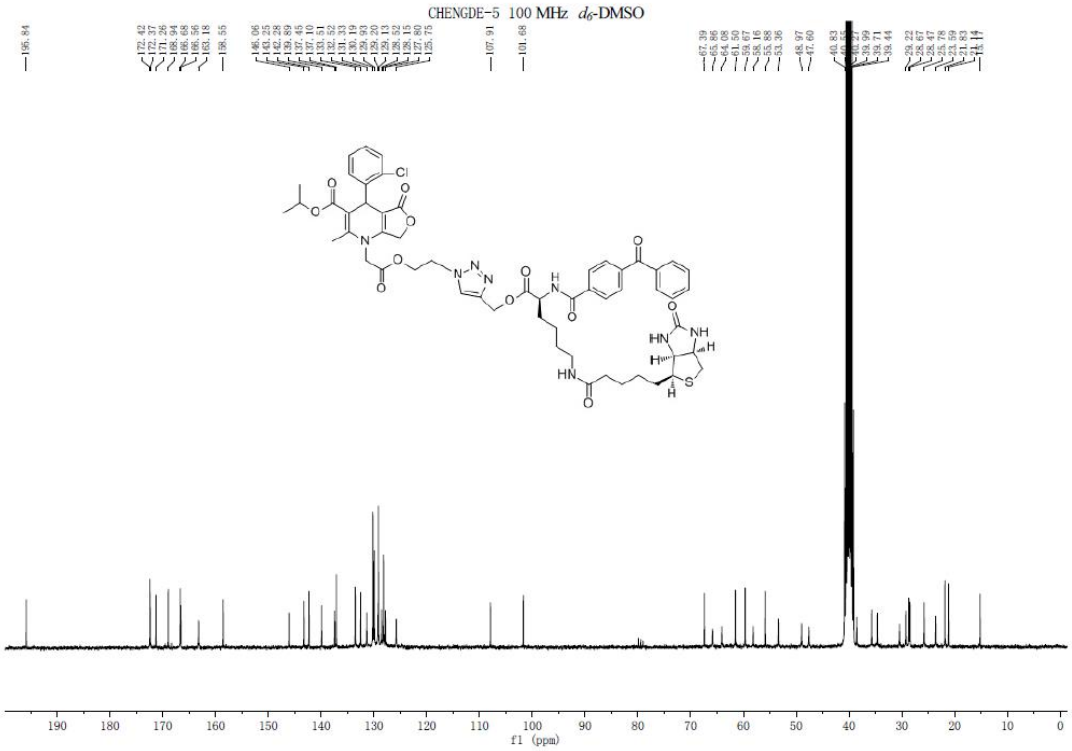
^{13}C NMR spectra of compound **30**



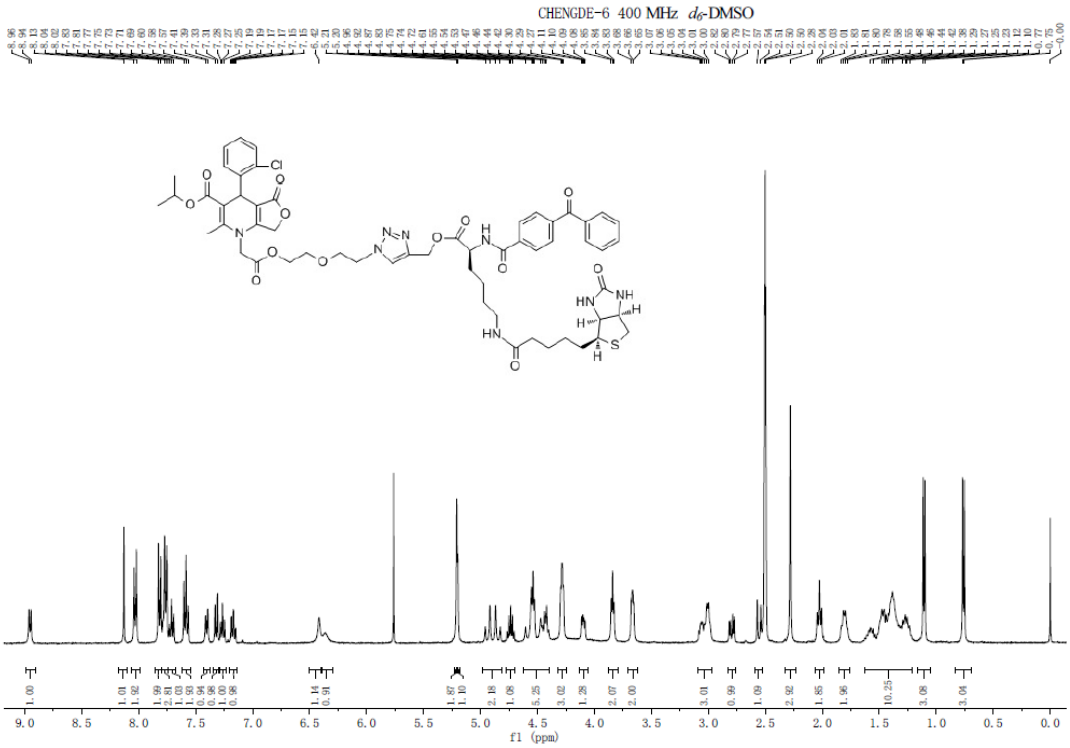
^{1}H NMR spectra of compound **2a**



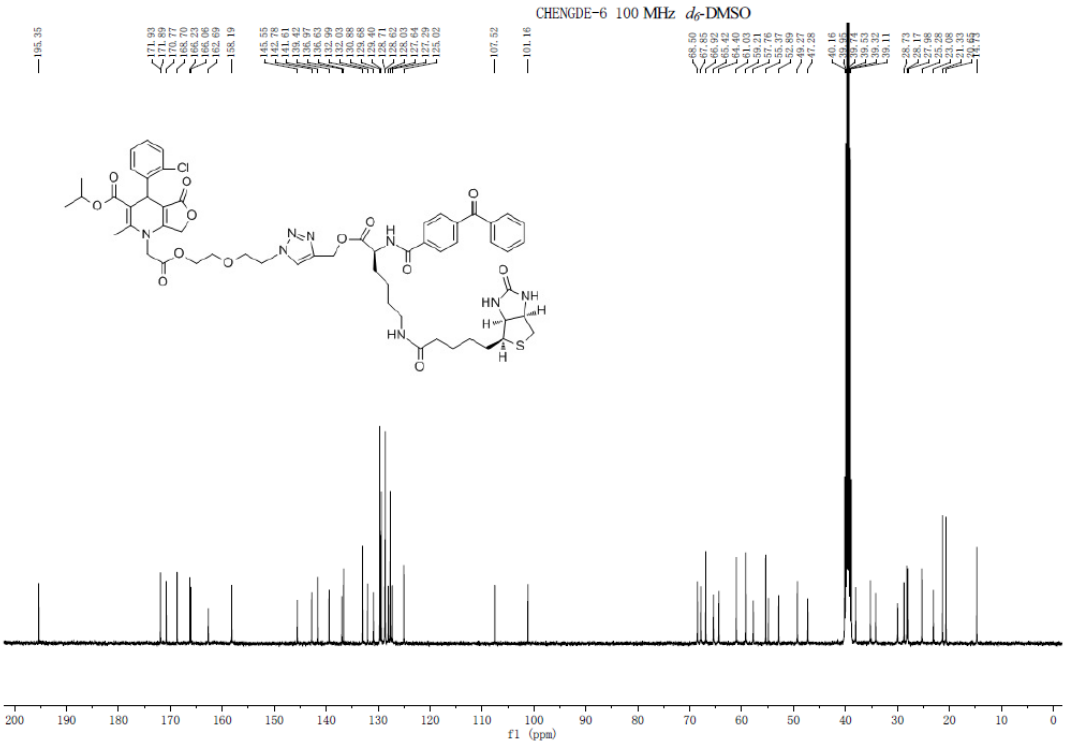
^{13}C NMR spectra of compound **2a**



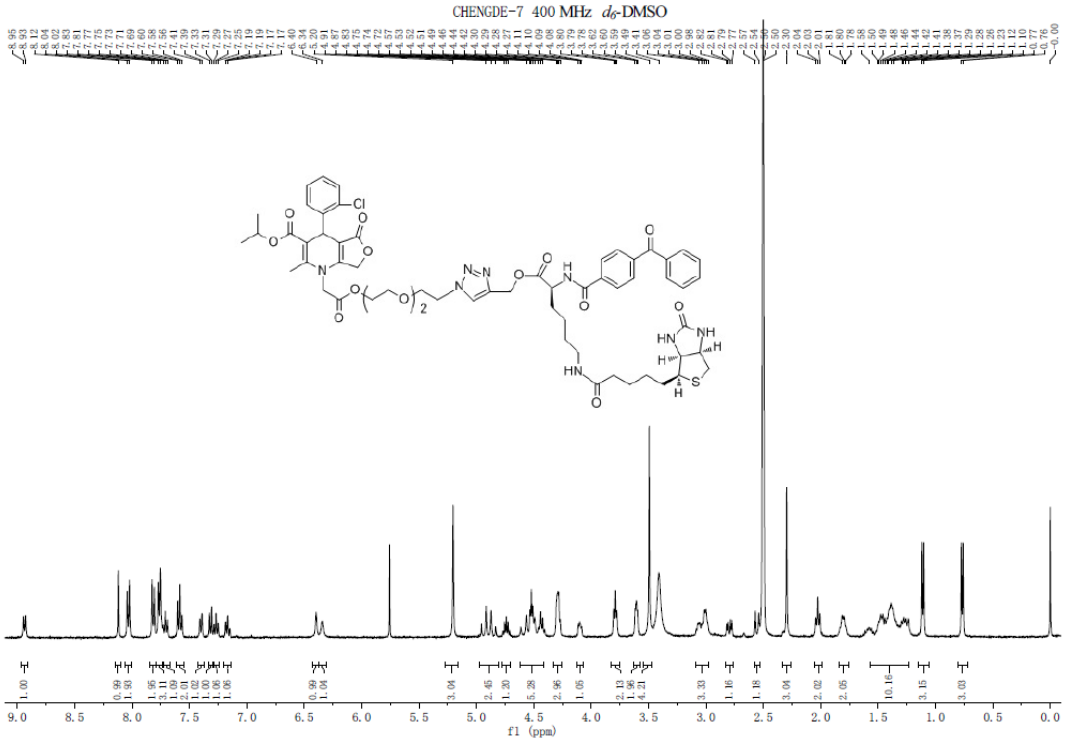
^{1}H NMR spectra of compound **2b**



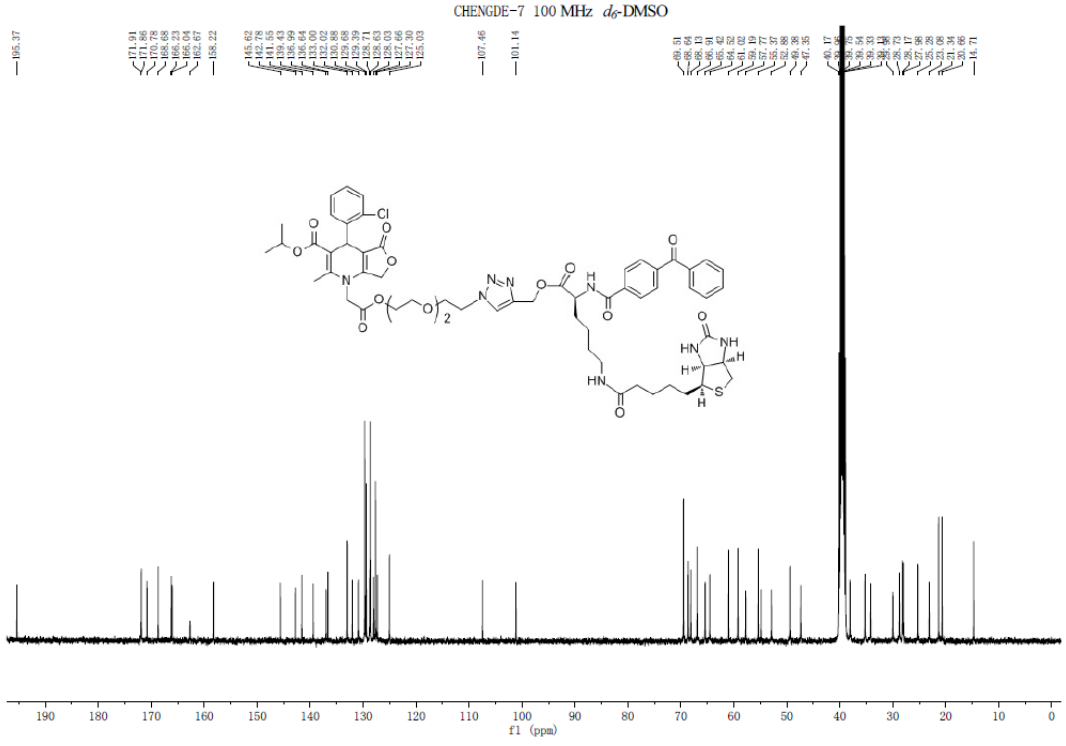
^{13}C NMR spectra of compound **2b**



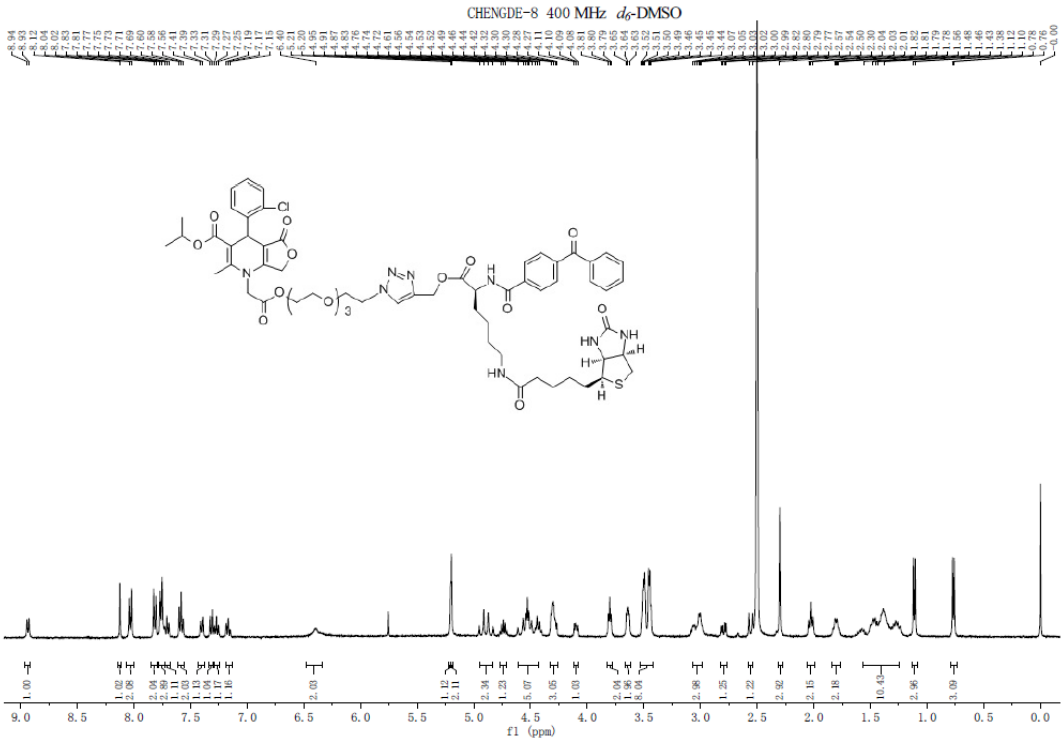
^{1}H NMR spectra of compound **2c**



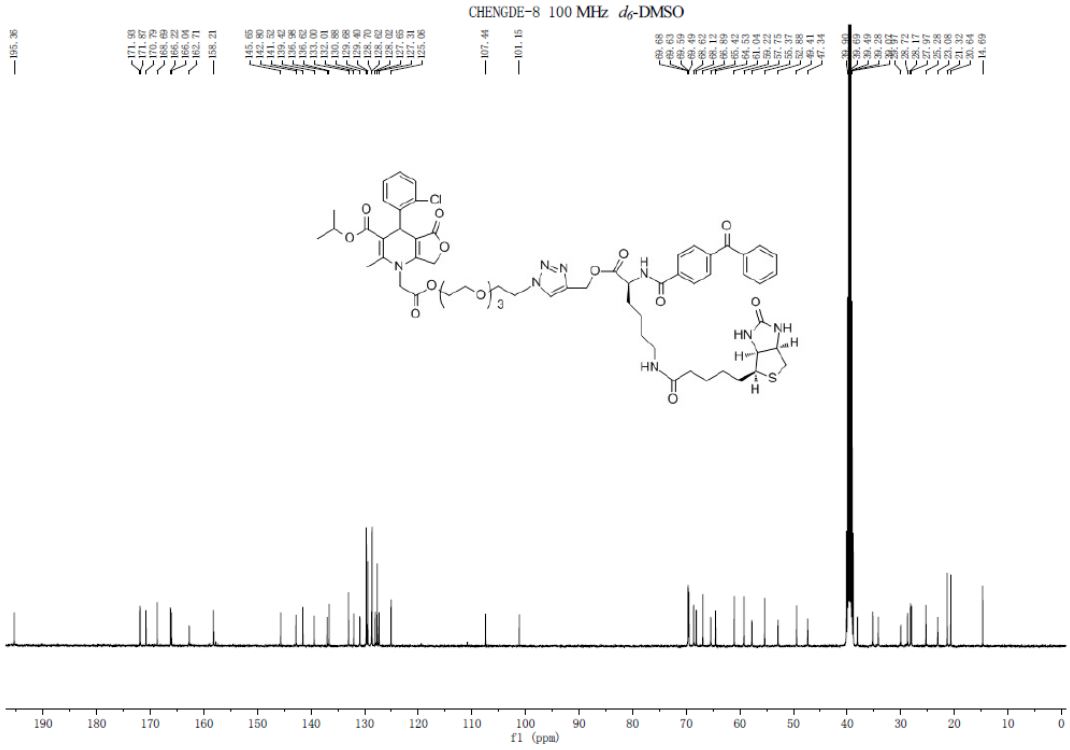
^{13}C NMR spectra of compound **2c**



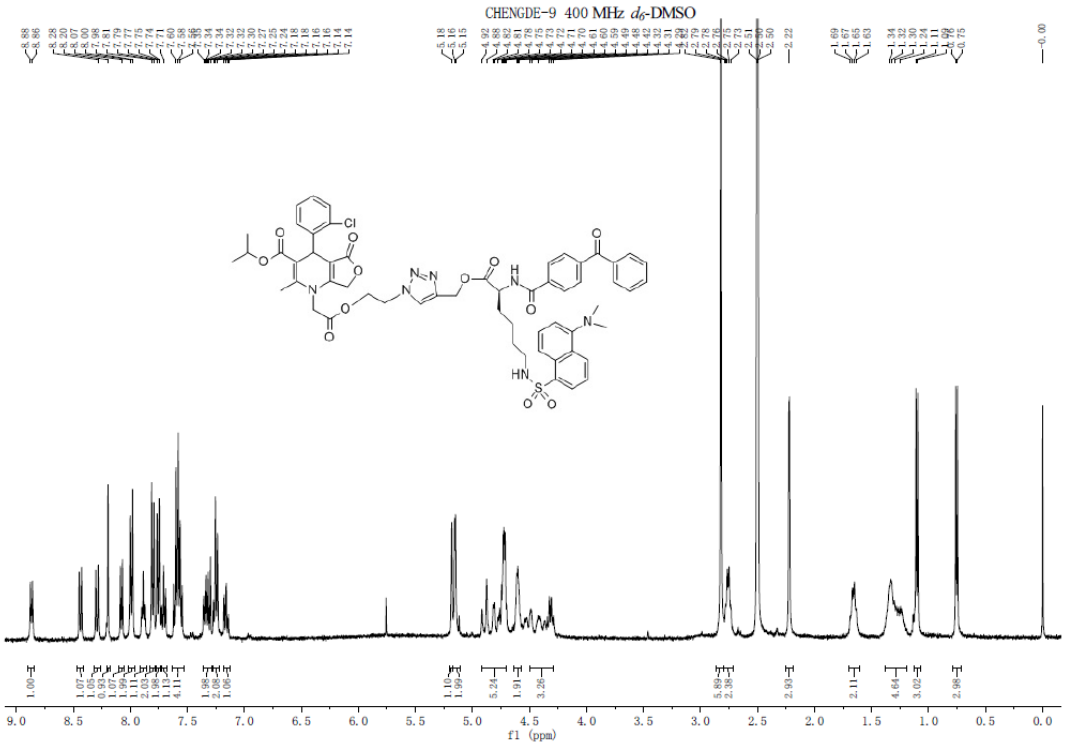
^{1}H NMR spectra of compound **2d**



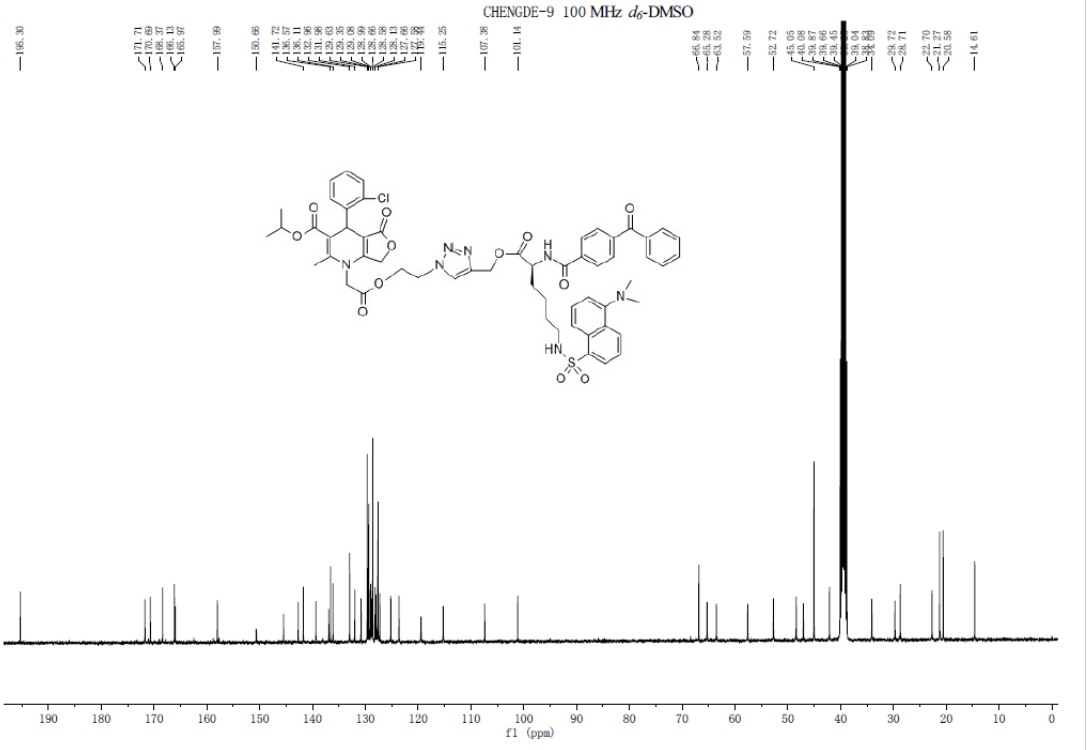
^{13}C NMR spectra of compound **2d**



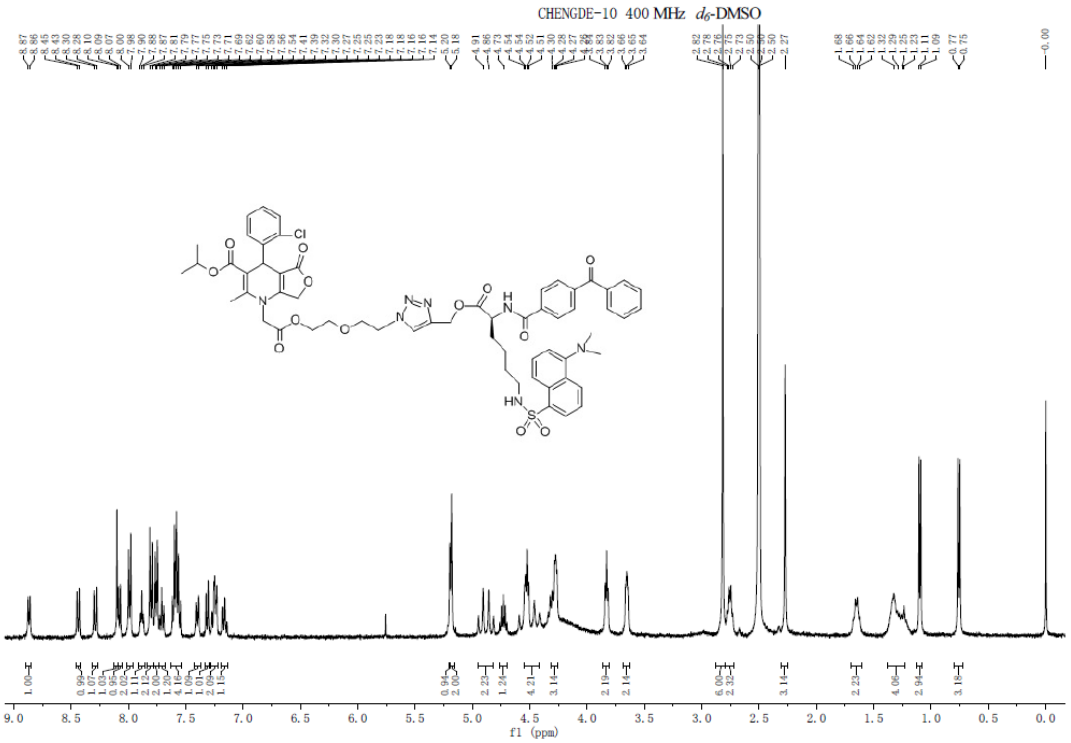
^{1}H NMR spectra of compound **3a**



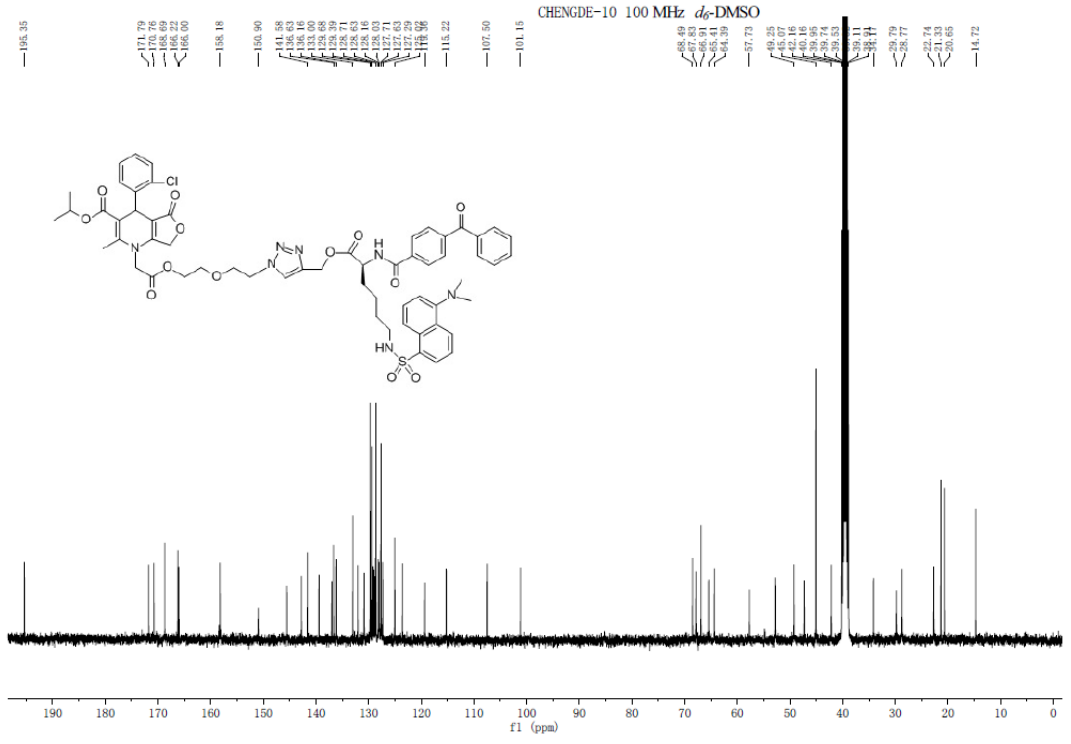
^{13}C NMR spectra of compound **3a**



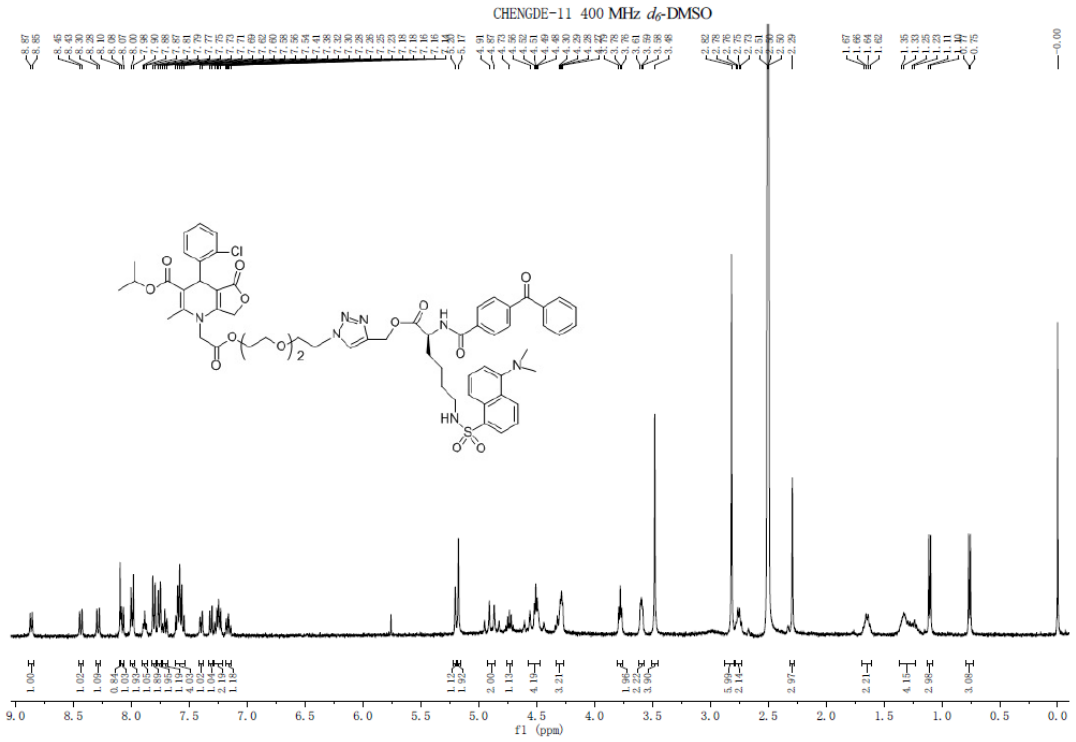
^{1}H NMR spectra of compound **3b**



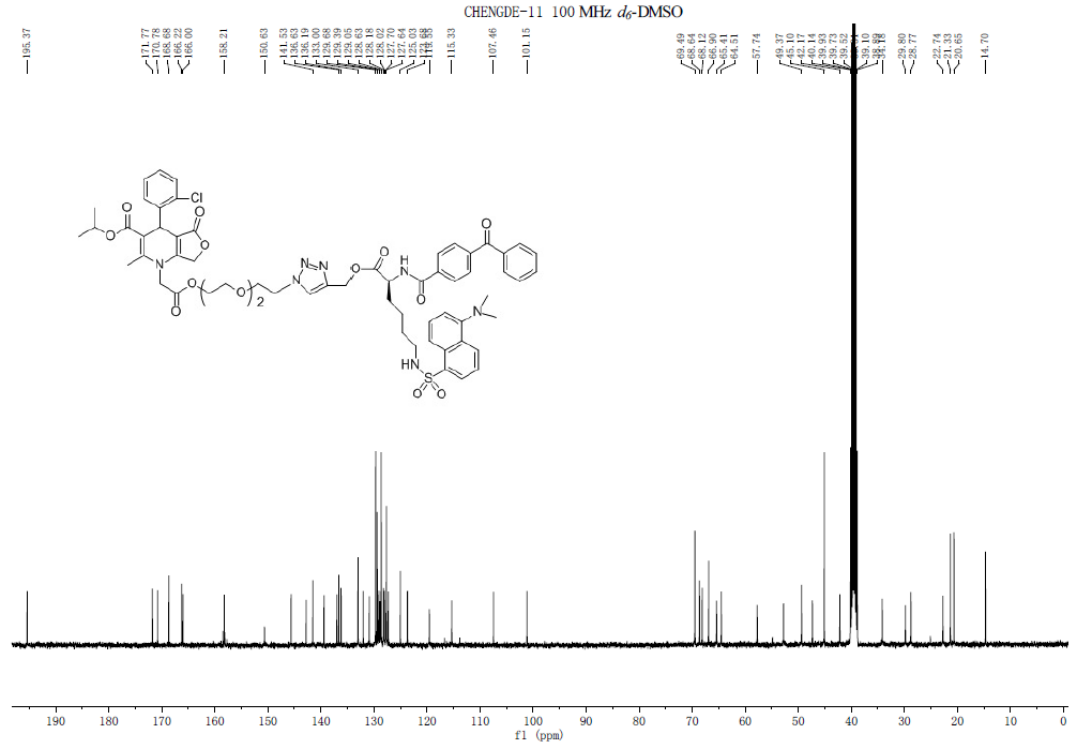
^{13}C NMR spectra of compound **3b**



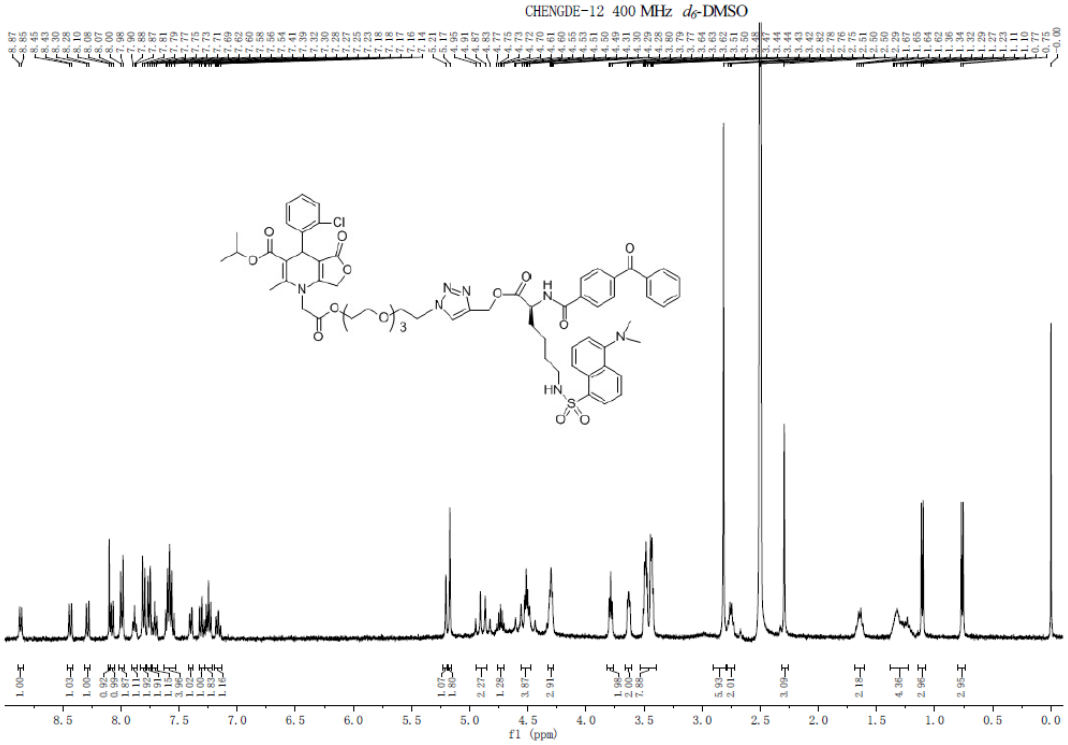
^{1}H NMR spectra of compound **3c**



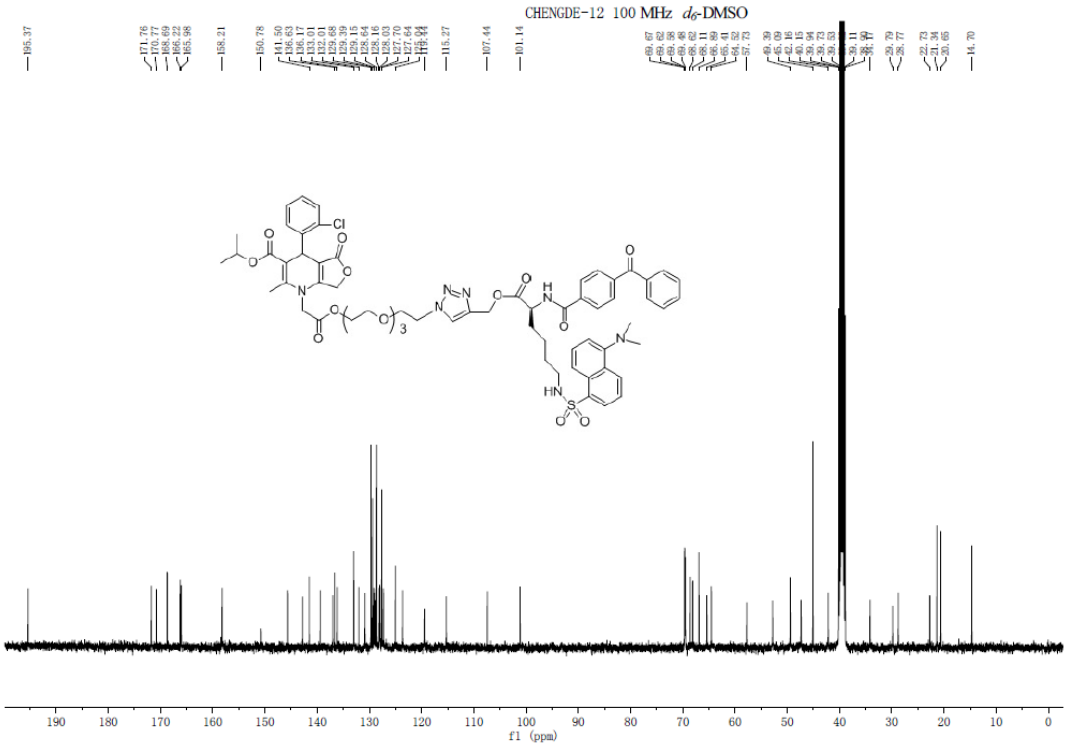
^{13}C NMR spectra of compound **3c**



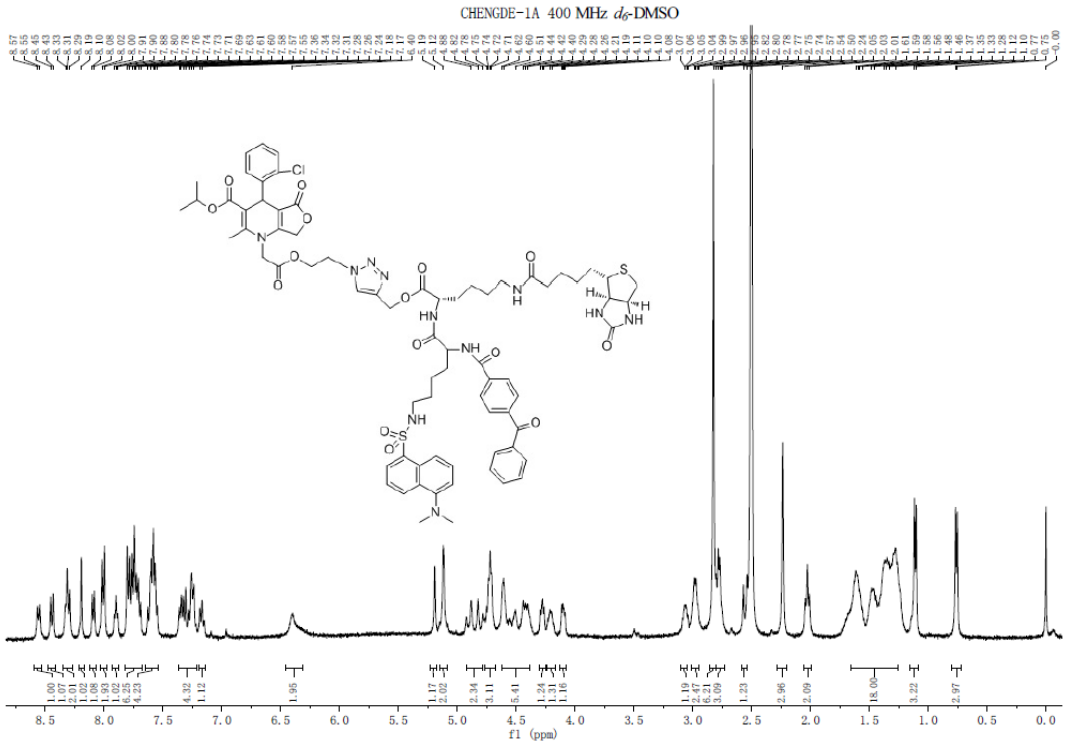
^{1}H NMR spectra of compound **3d**



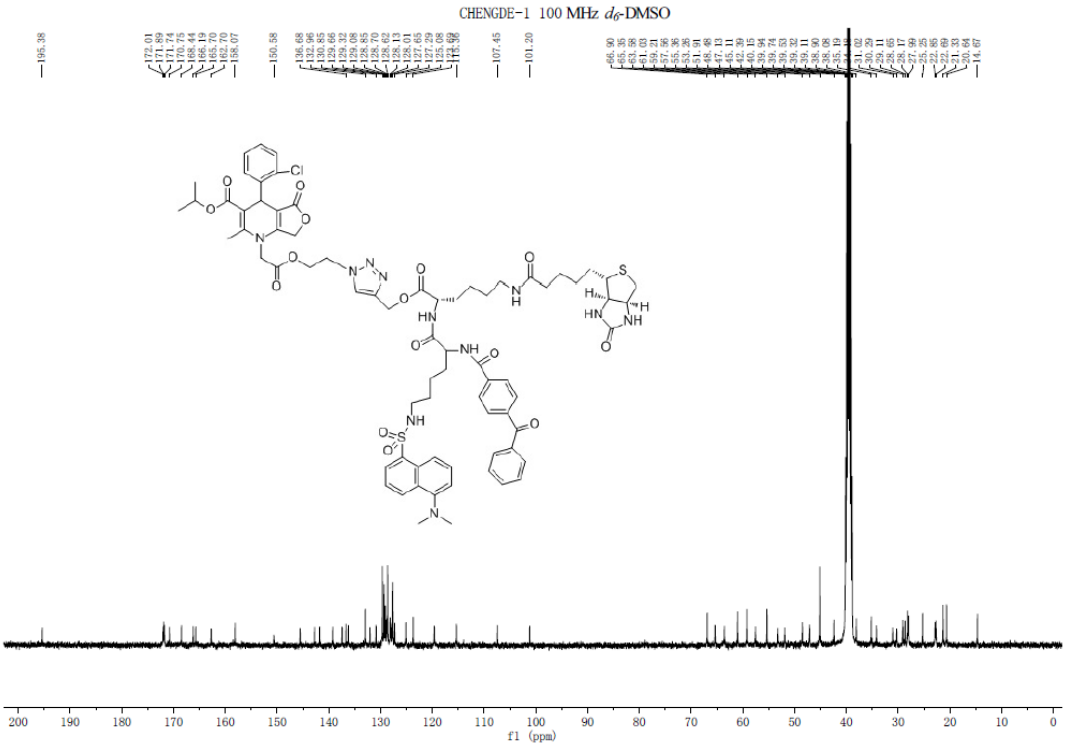
^{13}C NMR spectra of compound **3d**



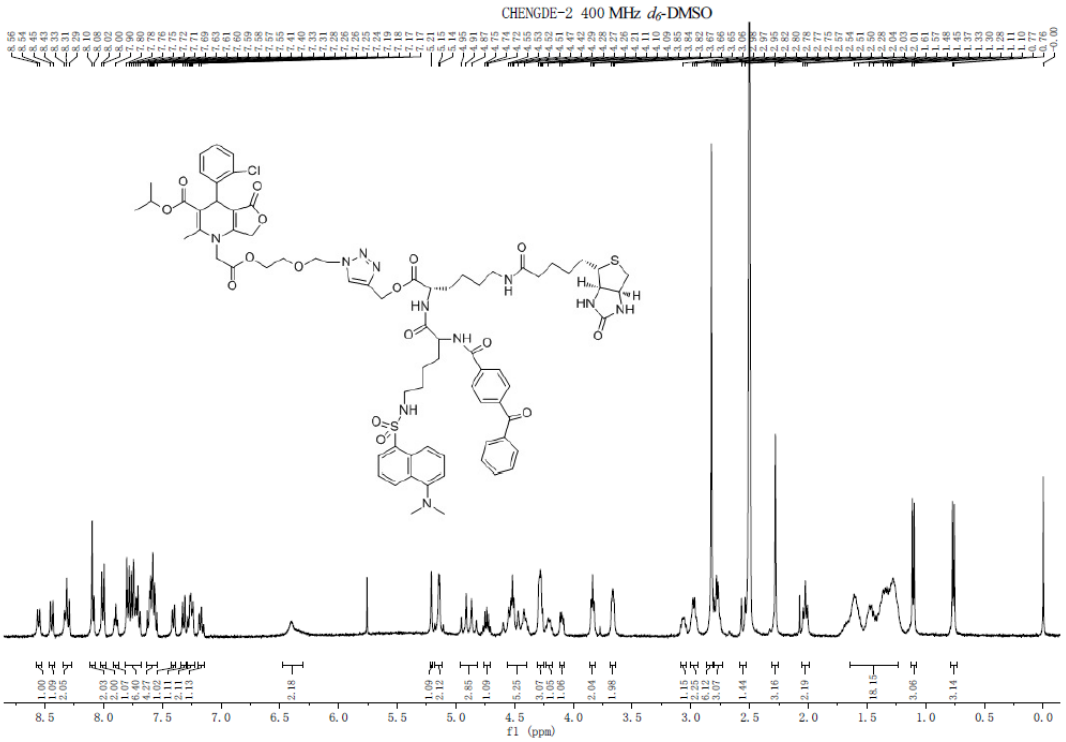
^{1}H NMR spectra of compound **4a**



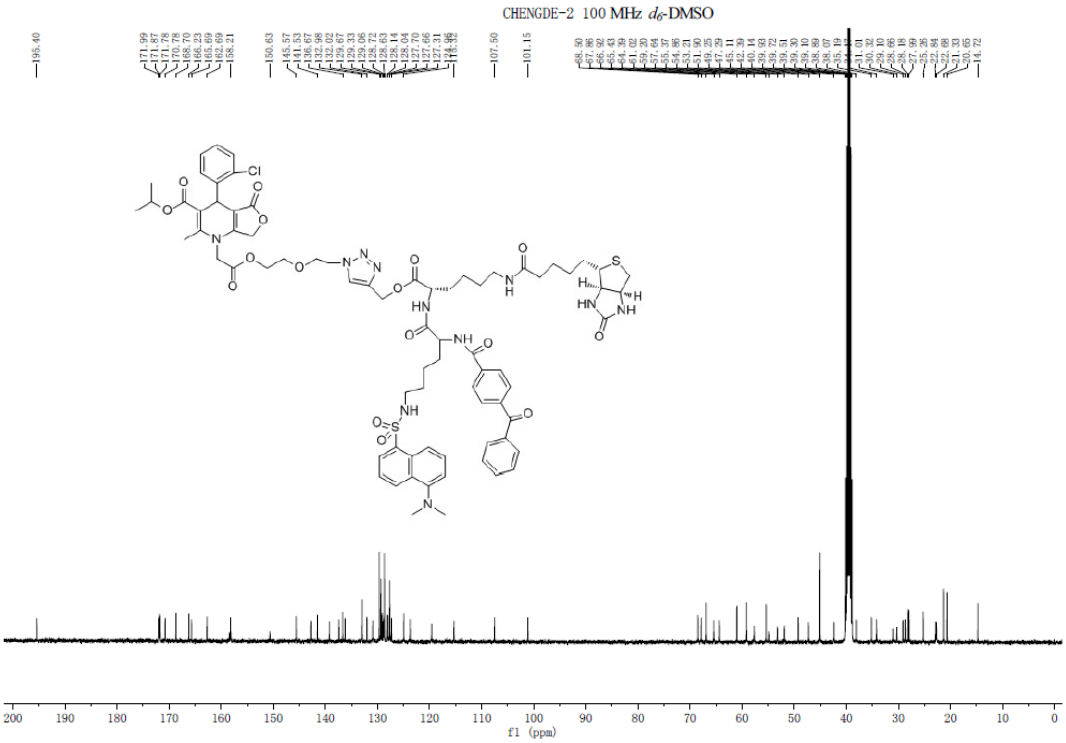
^{13}C NMR spectra of compound **4a**



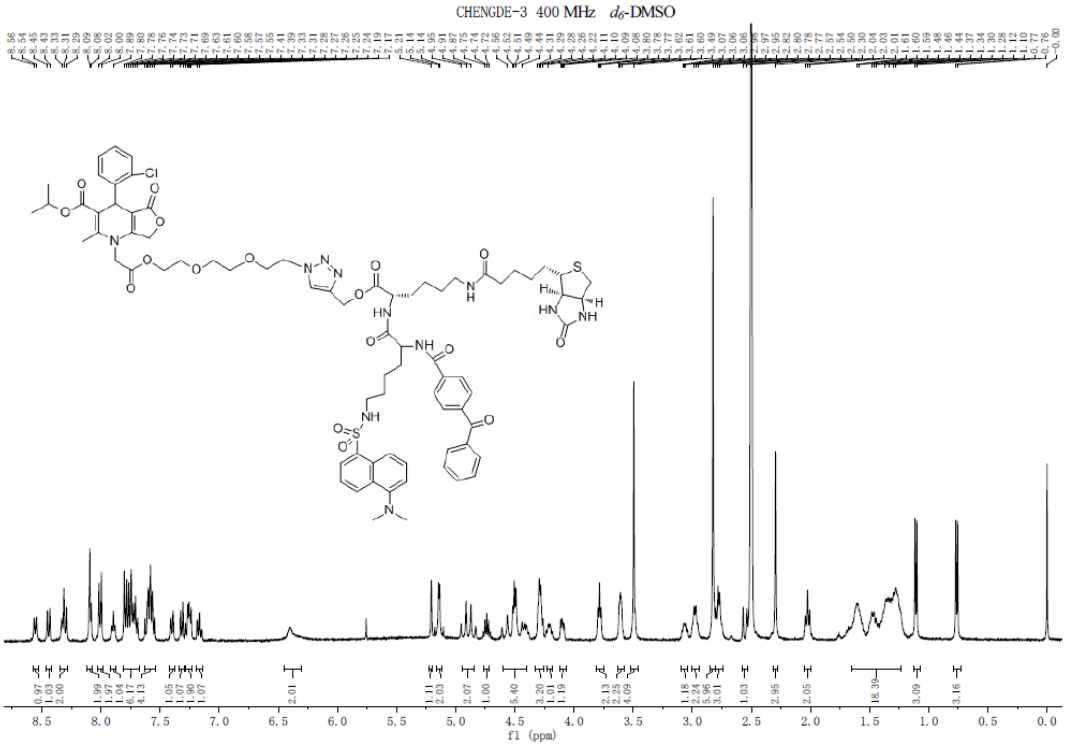
^{1}H NMR spectra of compound **4b**



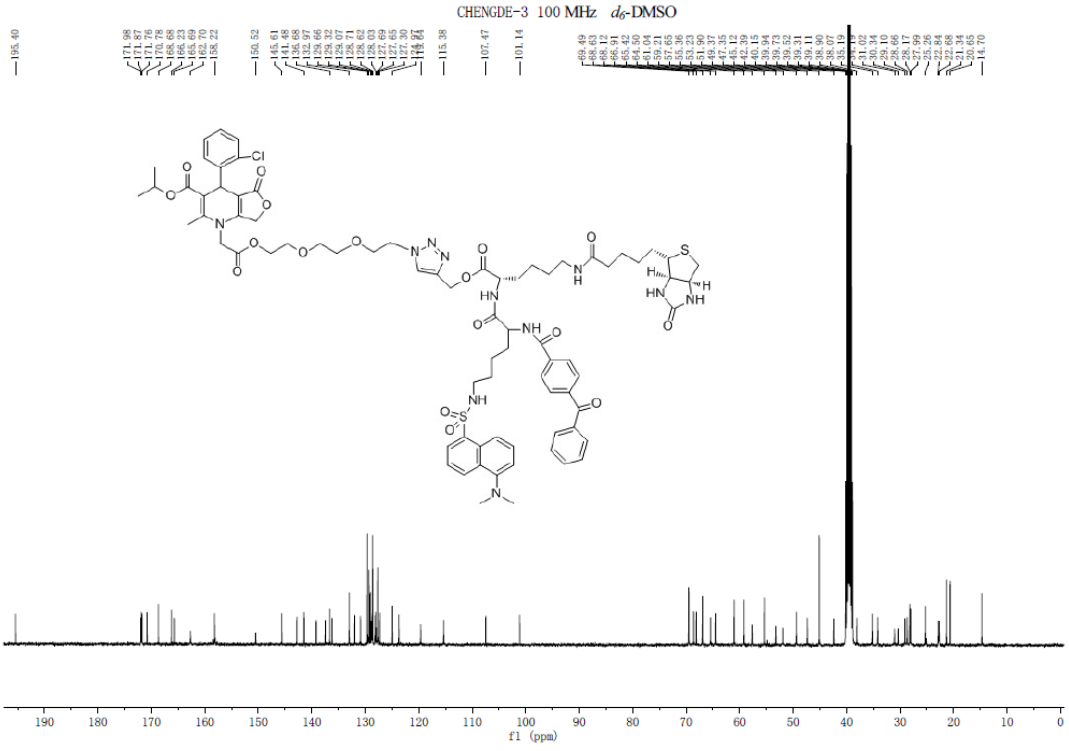
^{13}C NMR spectra of compound **4b**



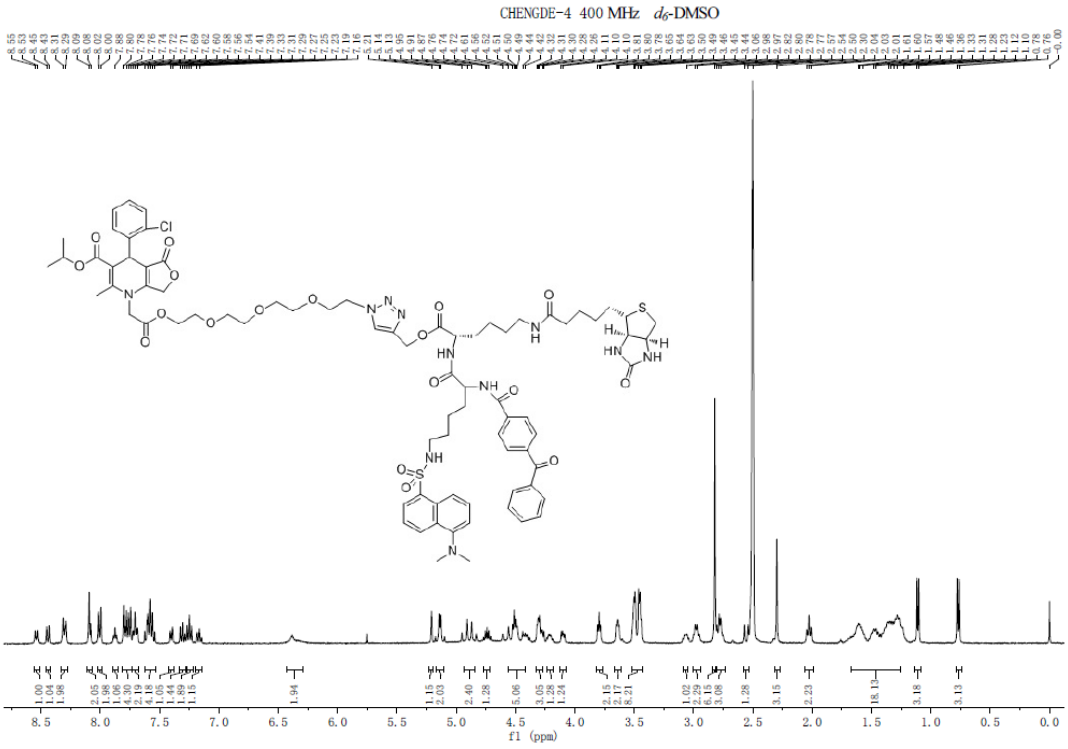
^{1}H NMR spectra of compound **4c**



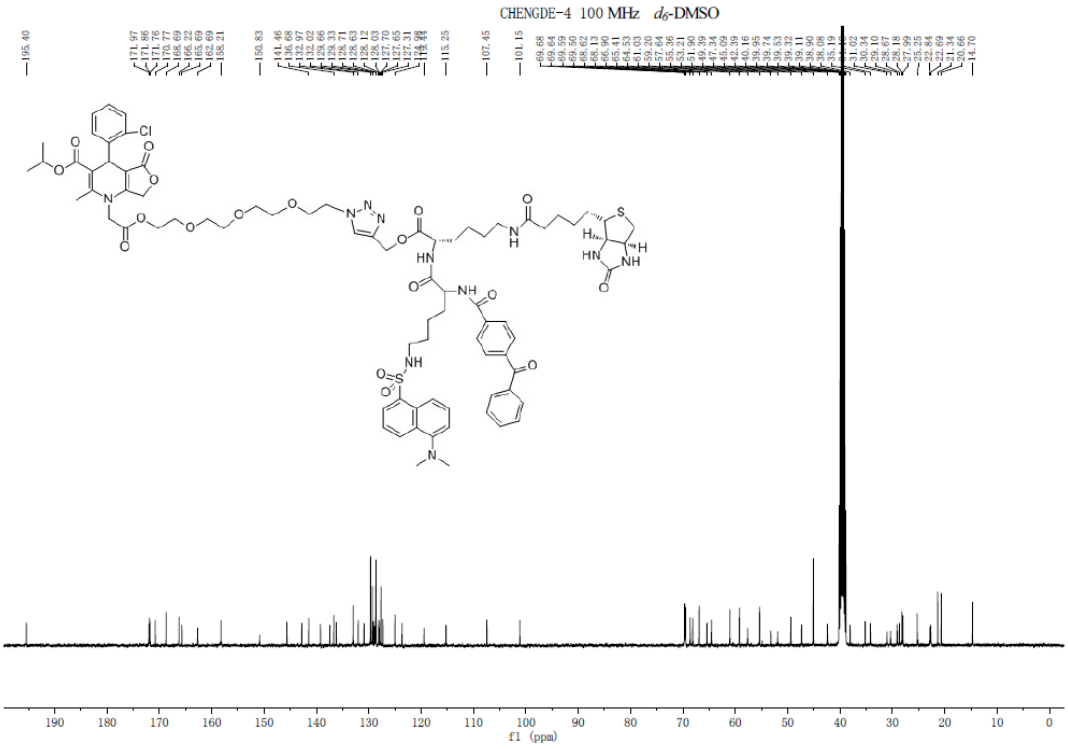
^{13}C NMR spectra of compound **4c**



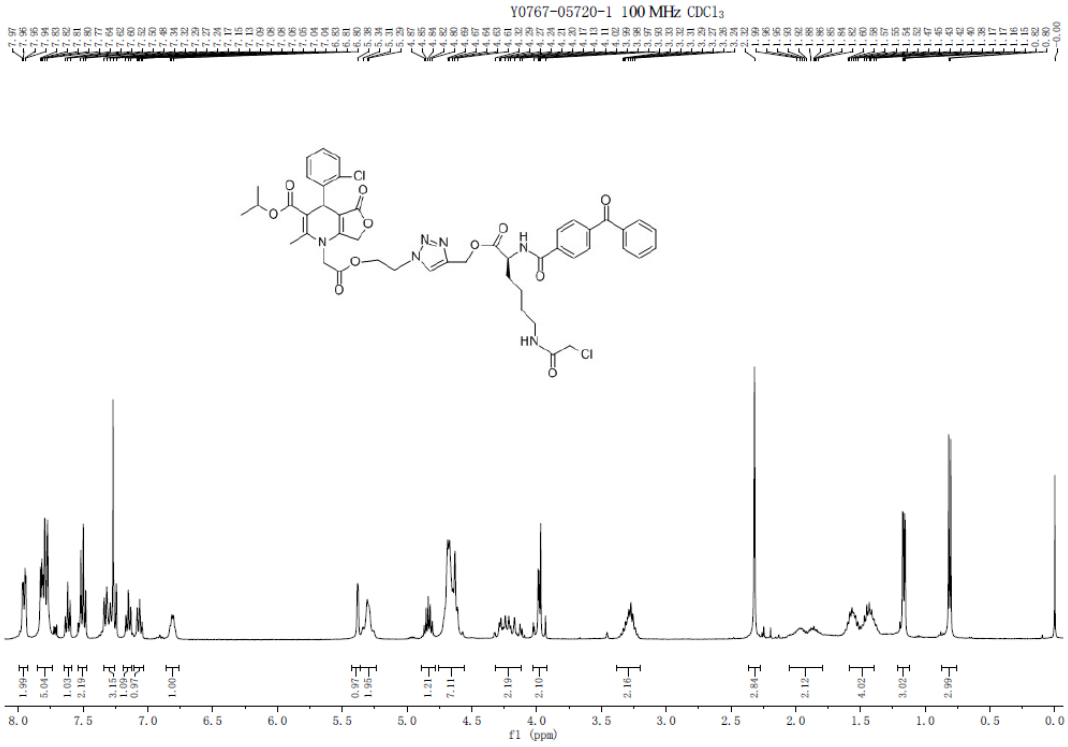
^{1}H NMR spectra of compound **4d**



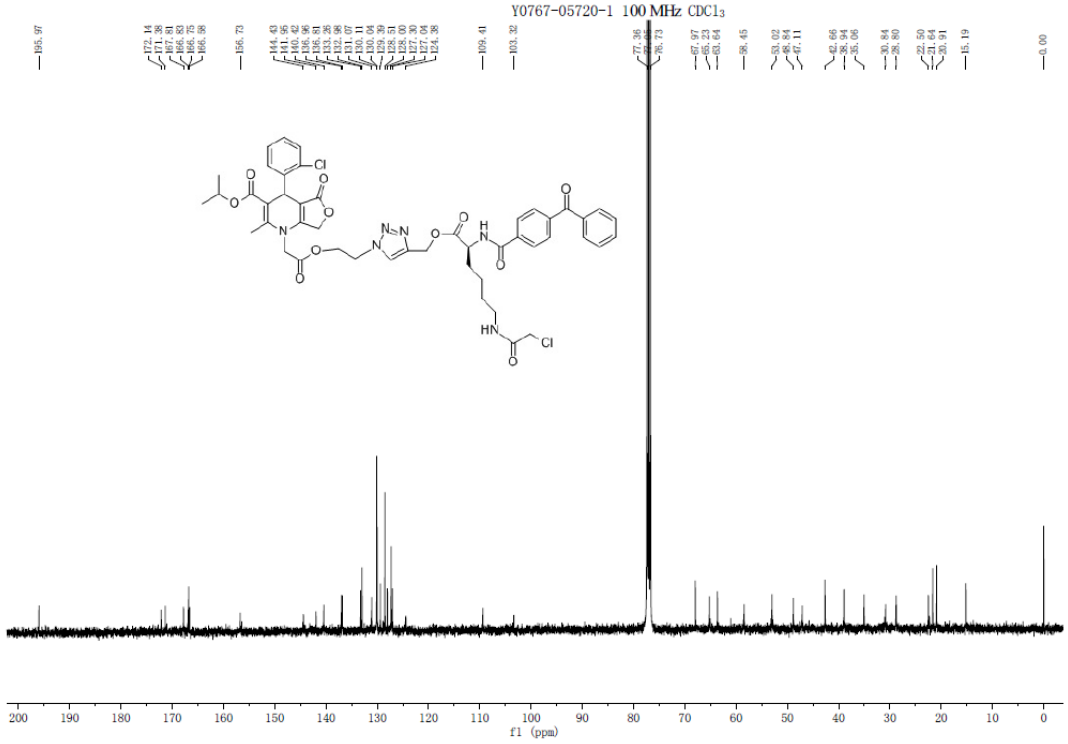
^{13}C NMR spectra of compound **4d**



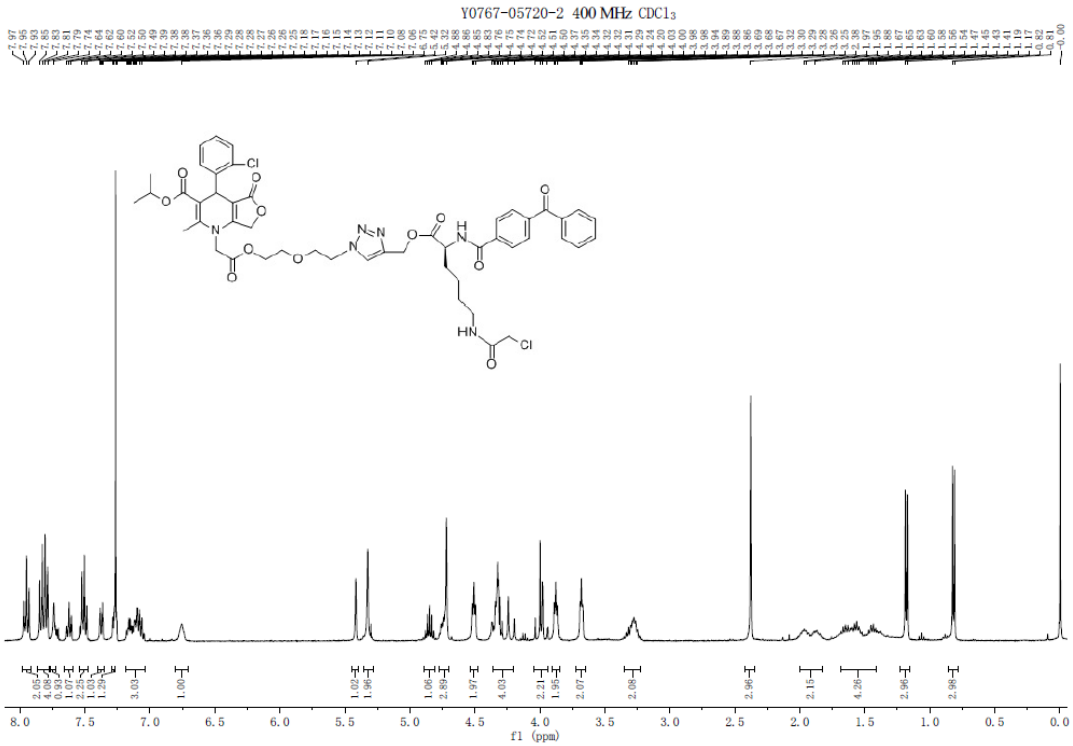
^{1}H NMR spectra of compound **31a**



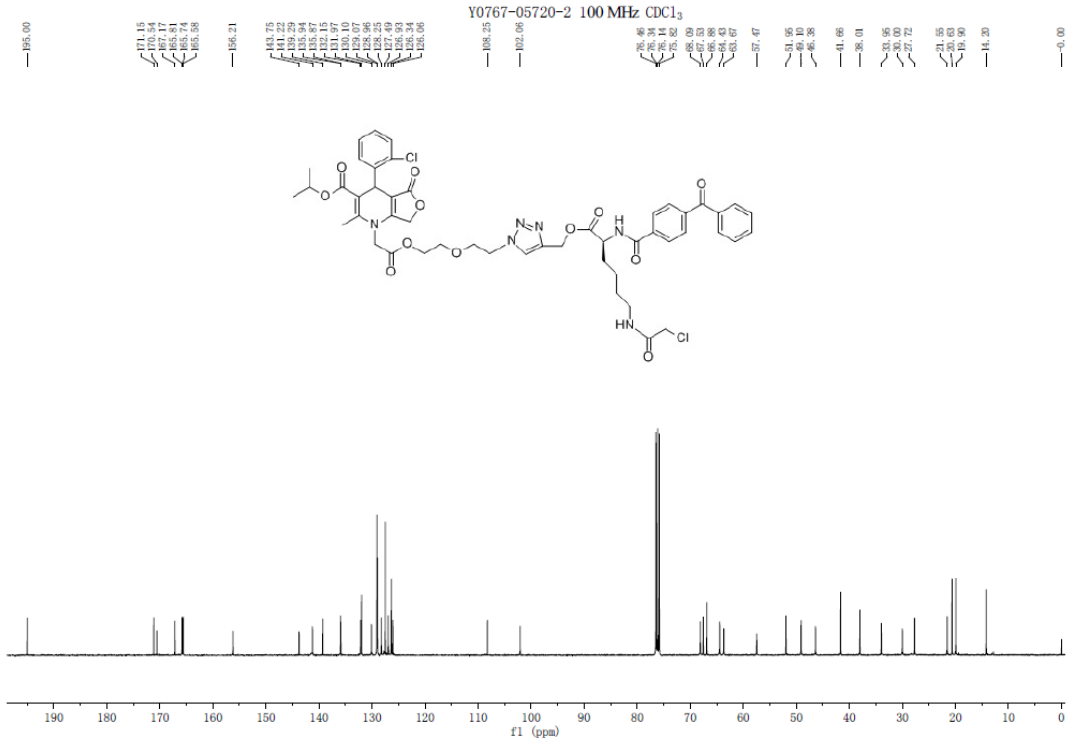
^{13}C NMR spectra of compound **31a**



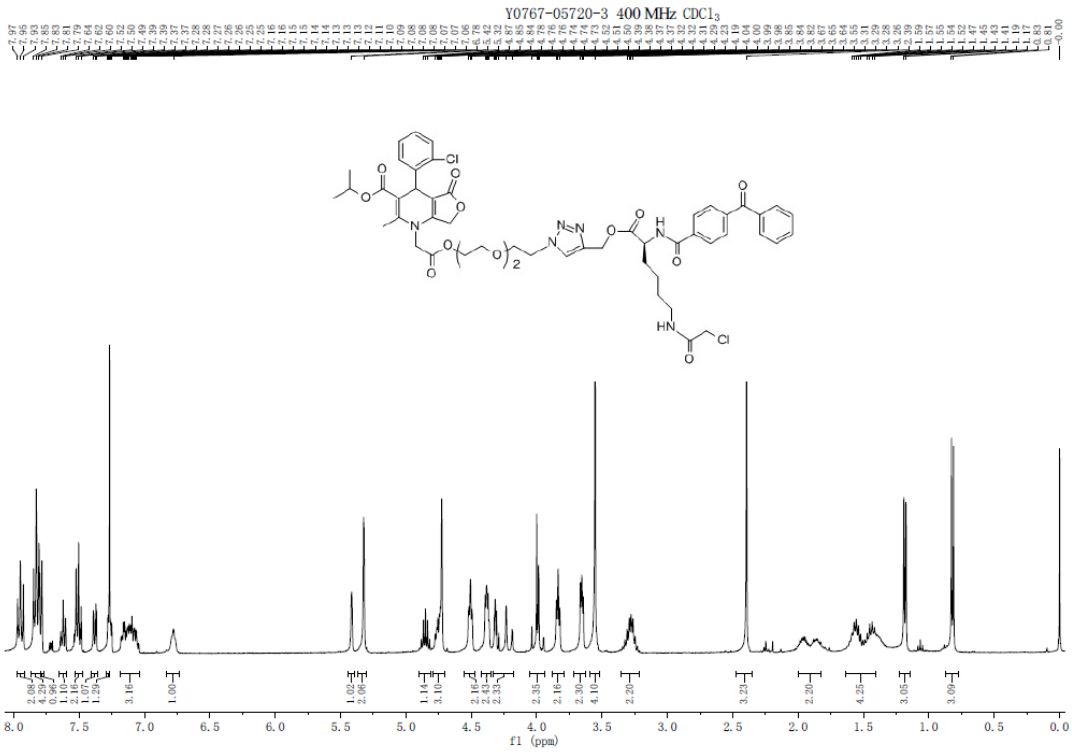
^{1}H NMR spectra of compound **31b**



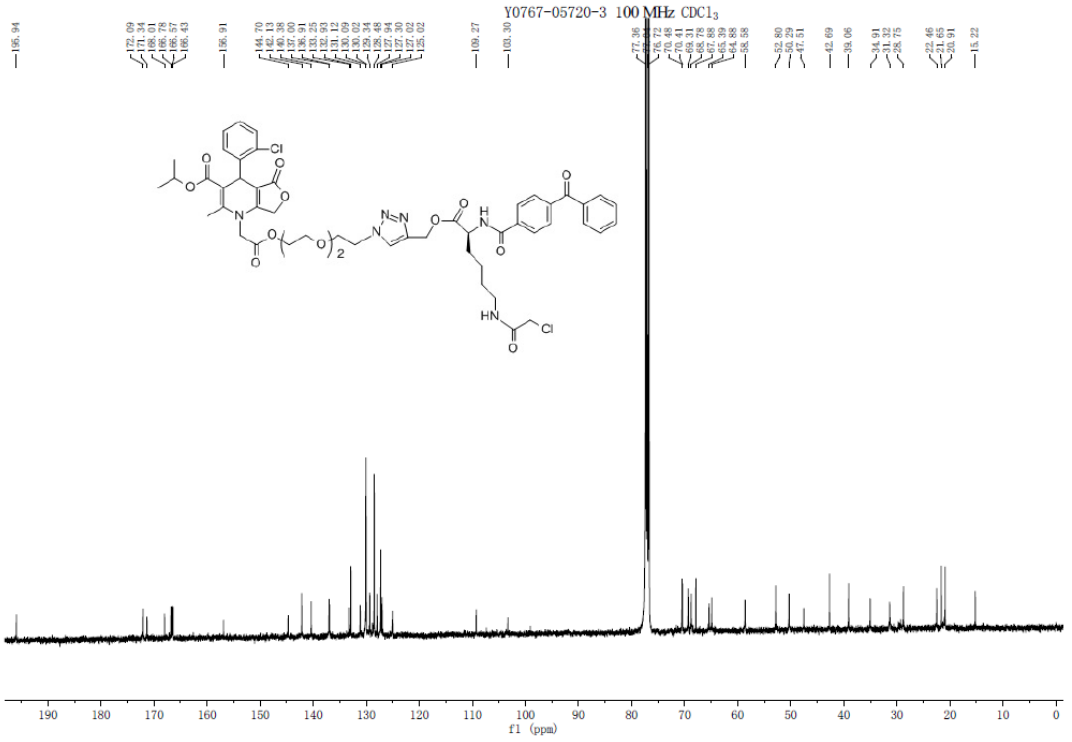
^{13}C NMR spectra of compound **31b**



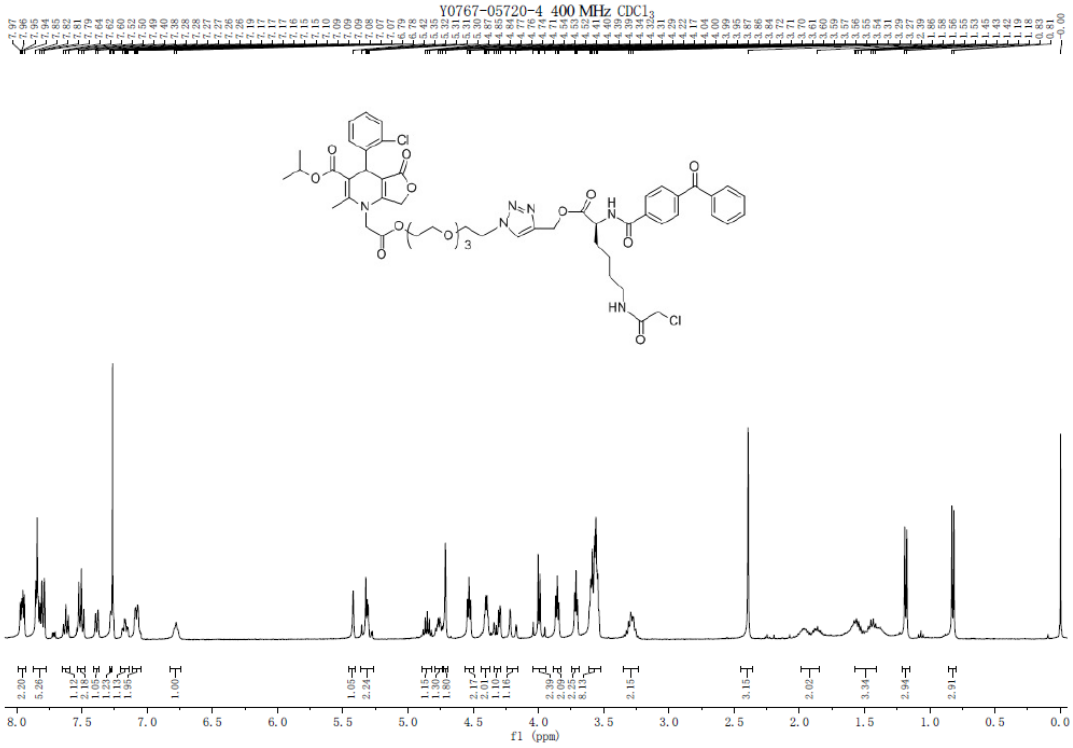
^{1}H NMR spectra of compound **31c**



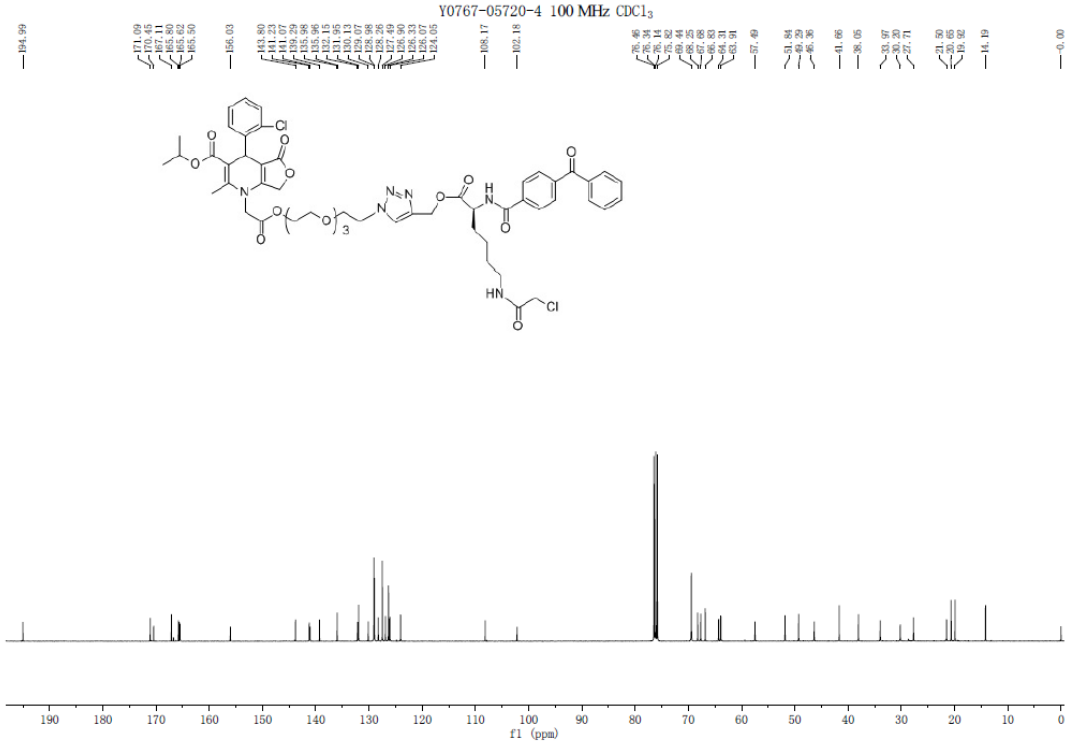
^{13}C NMR spectra of compound **31c**



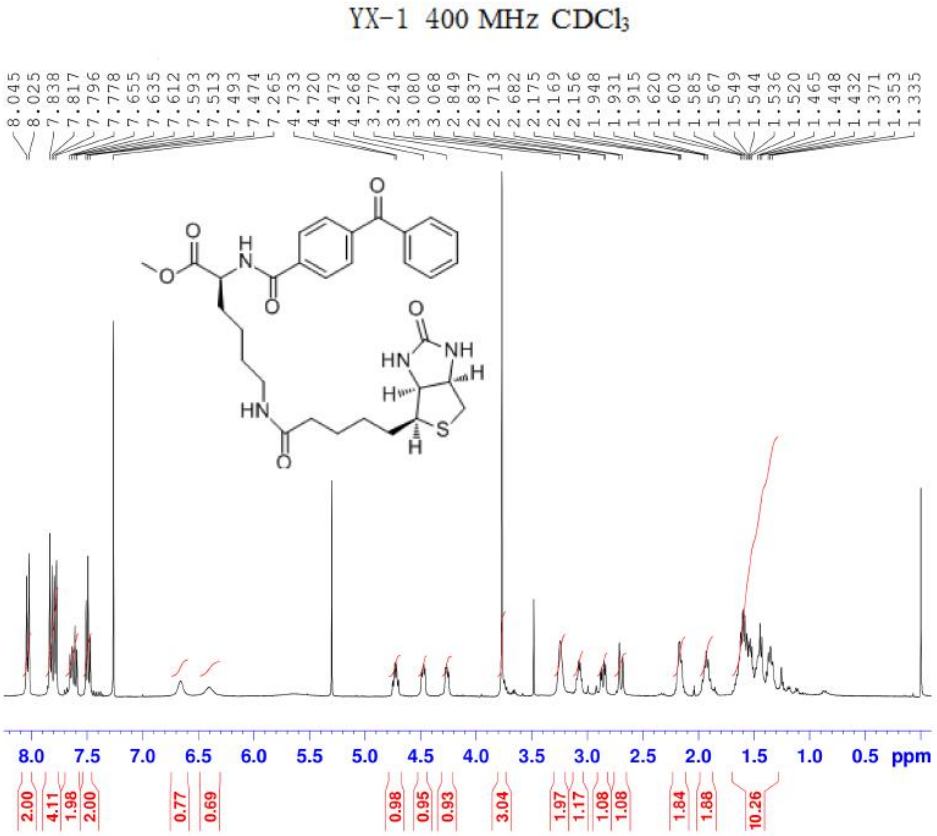
^{1}H NMR spectra of compound **31d**



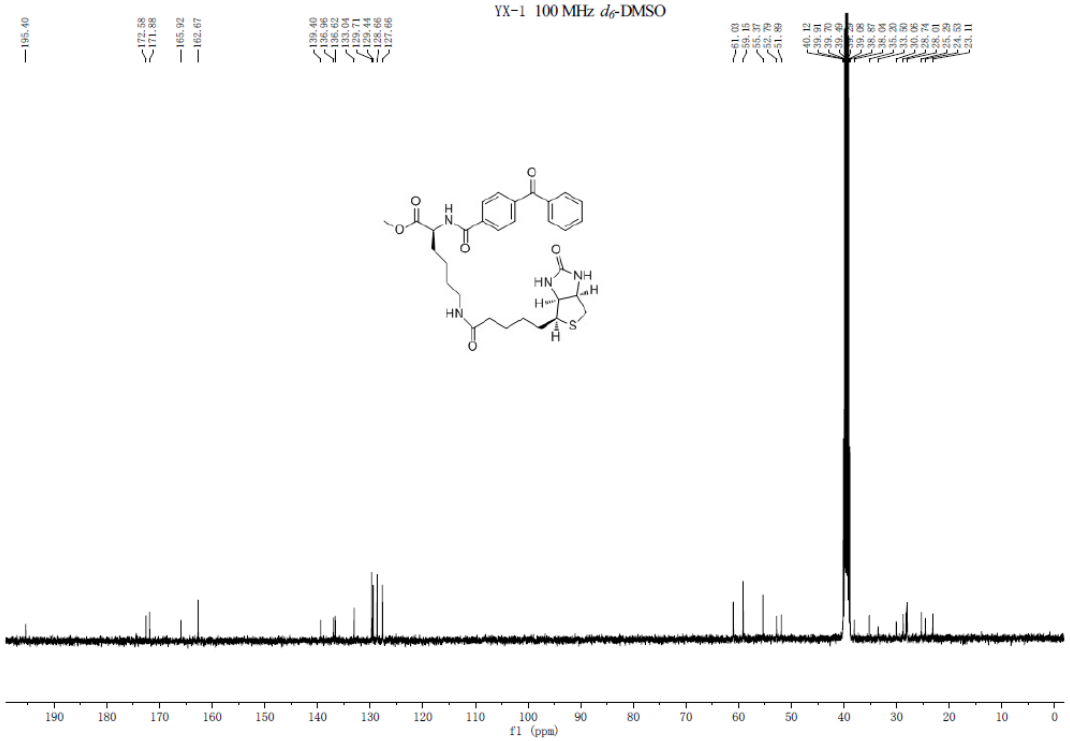
^{13}C NMR spectra of compound **31d**



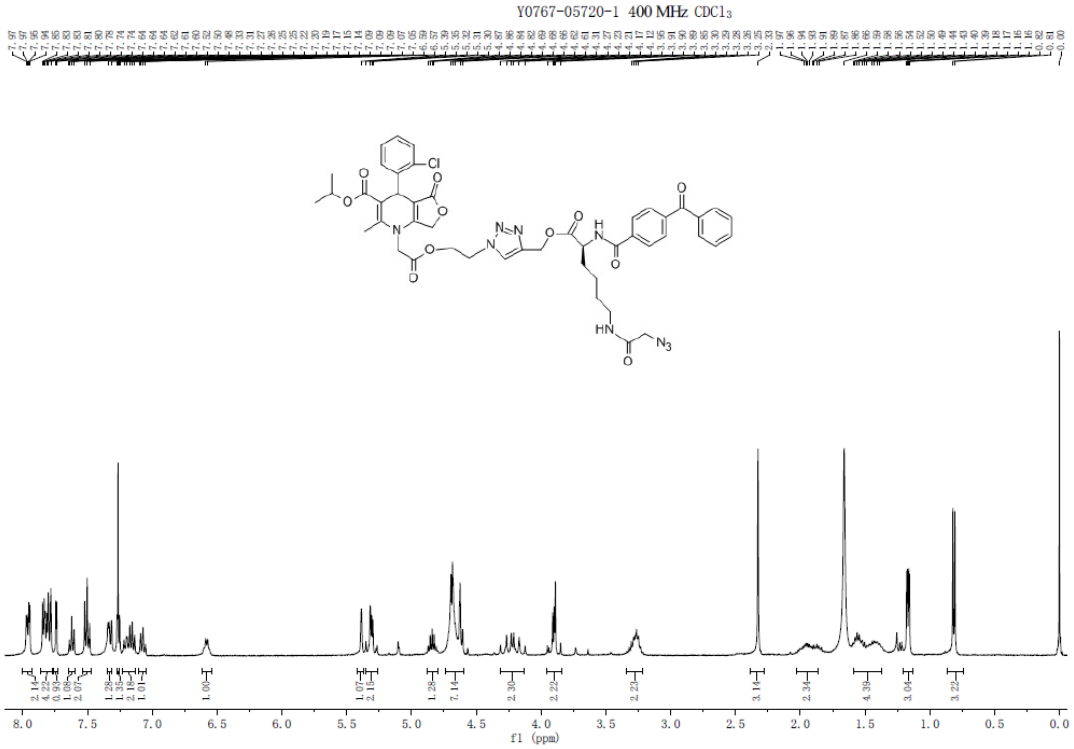
^{1}H NMR spectra of compound **32**



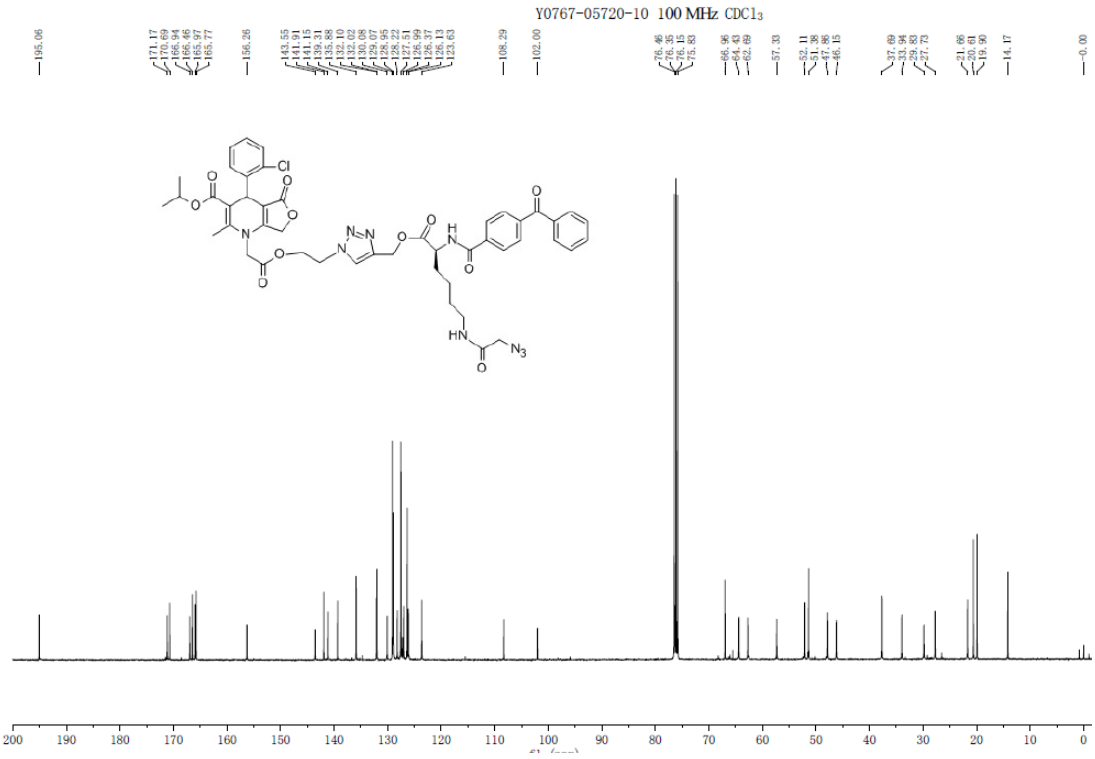
^{13}C NMR spectra of compound **32**



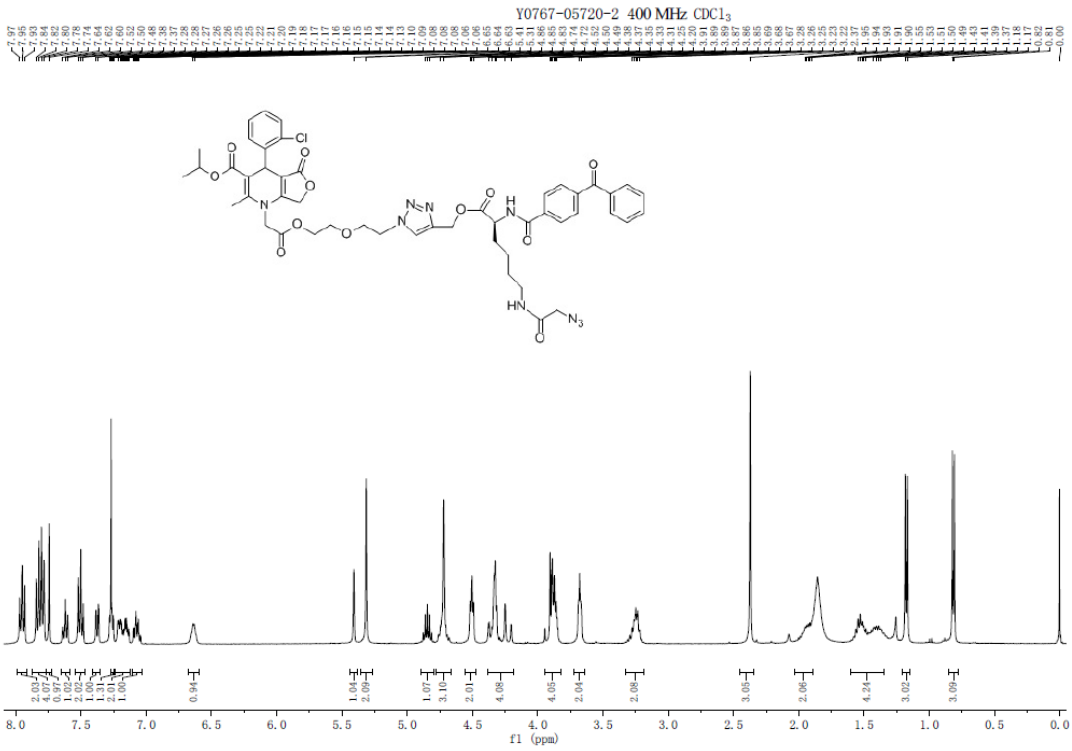
^{1}H NMR spectra of compound **5a**



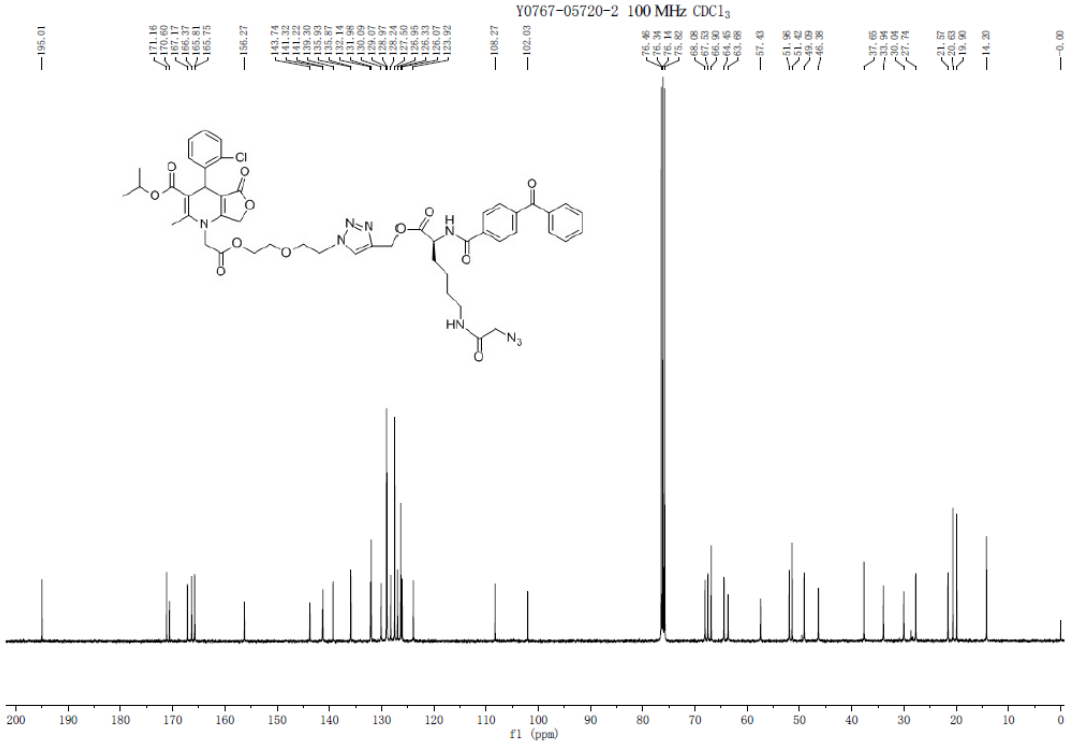
^{13}C NMR spectra of compound **5a**



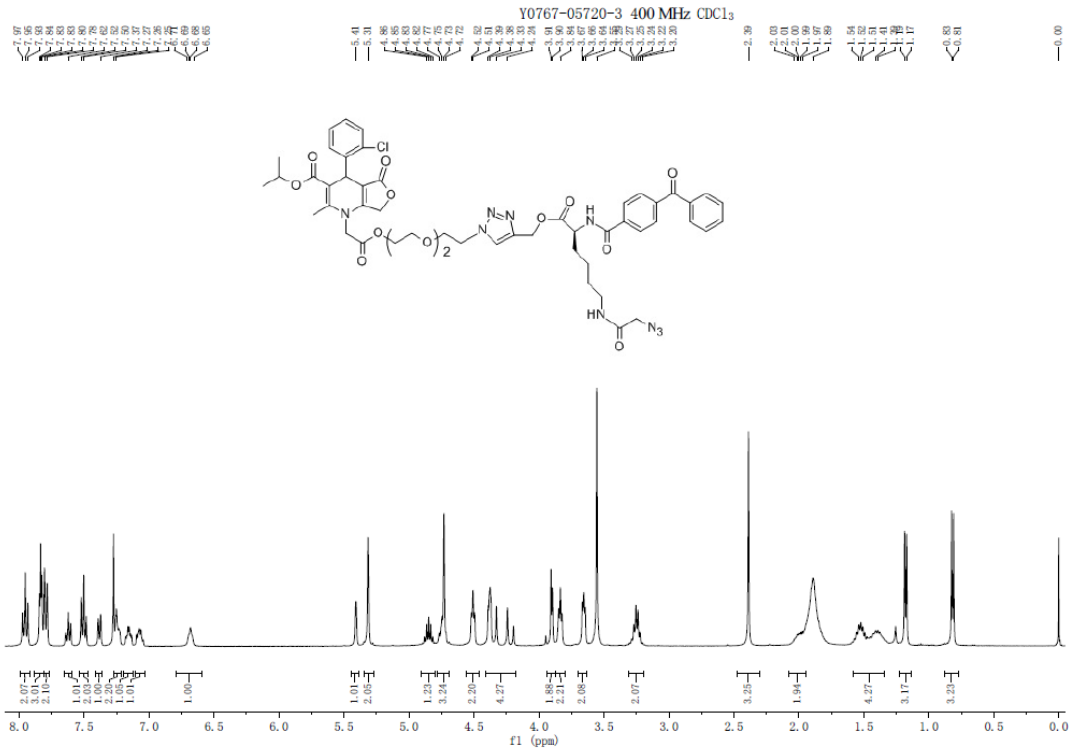
^{1}H NMR spectra of compound **5b**



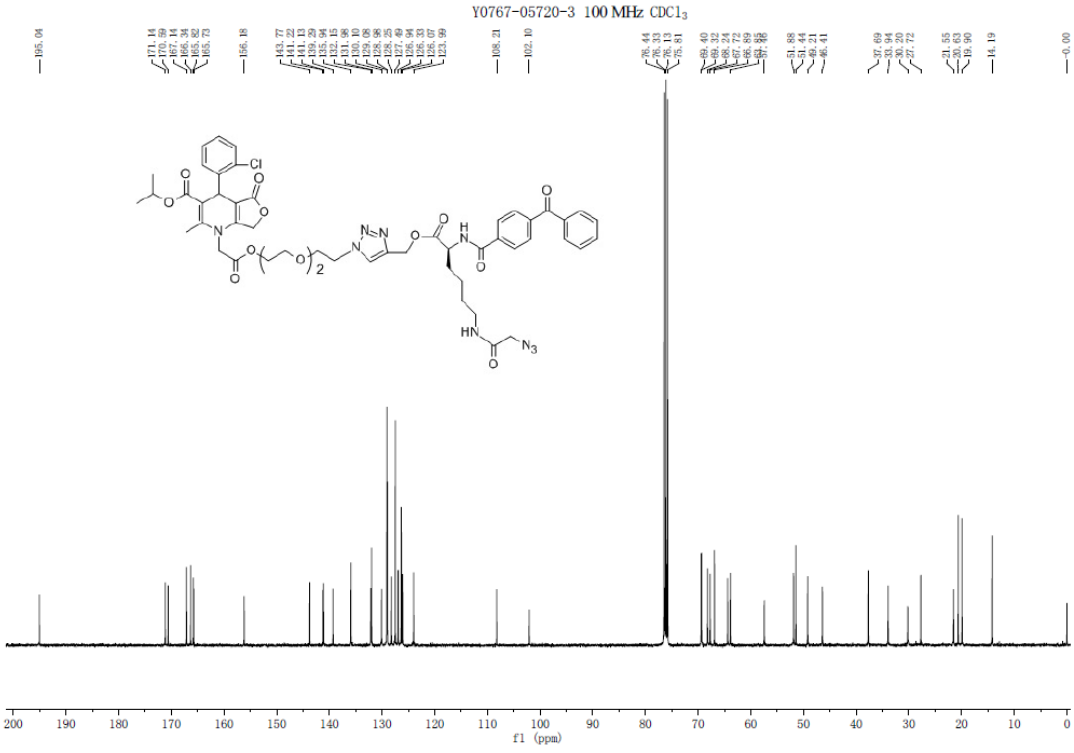
^{13}C NMR spectra of compound **5b**



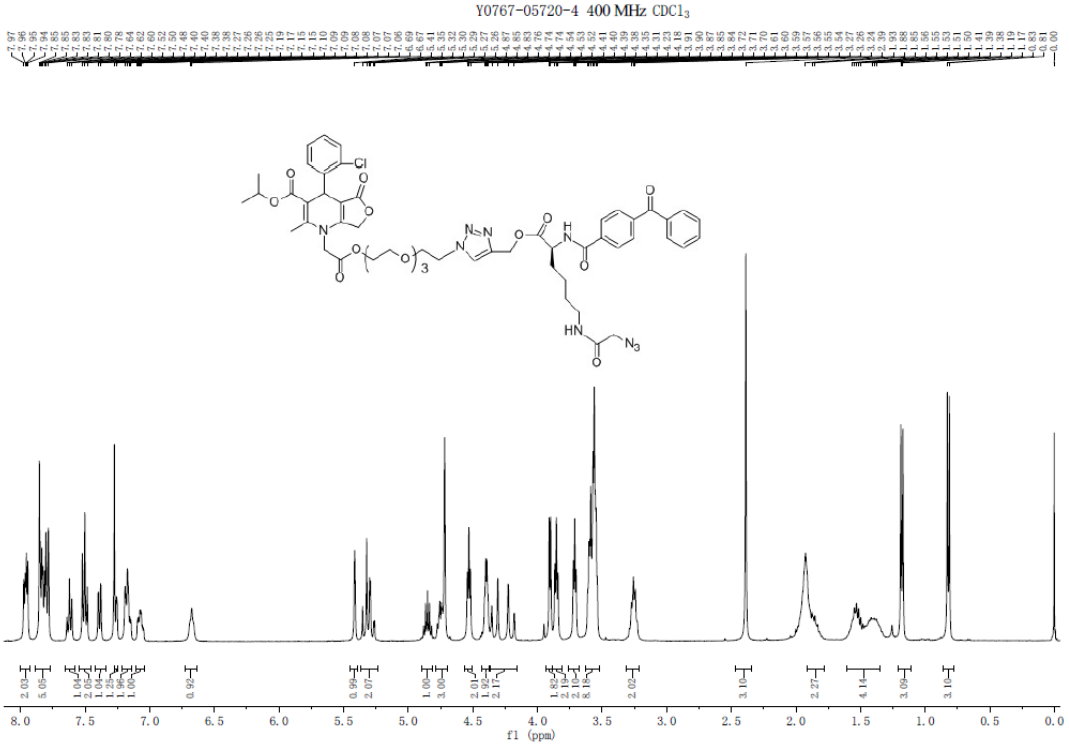
^{1}H NMR spectra of compound **5c**



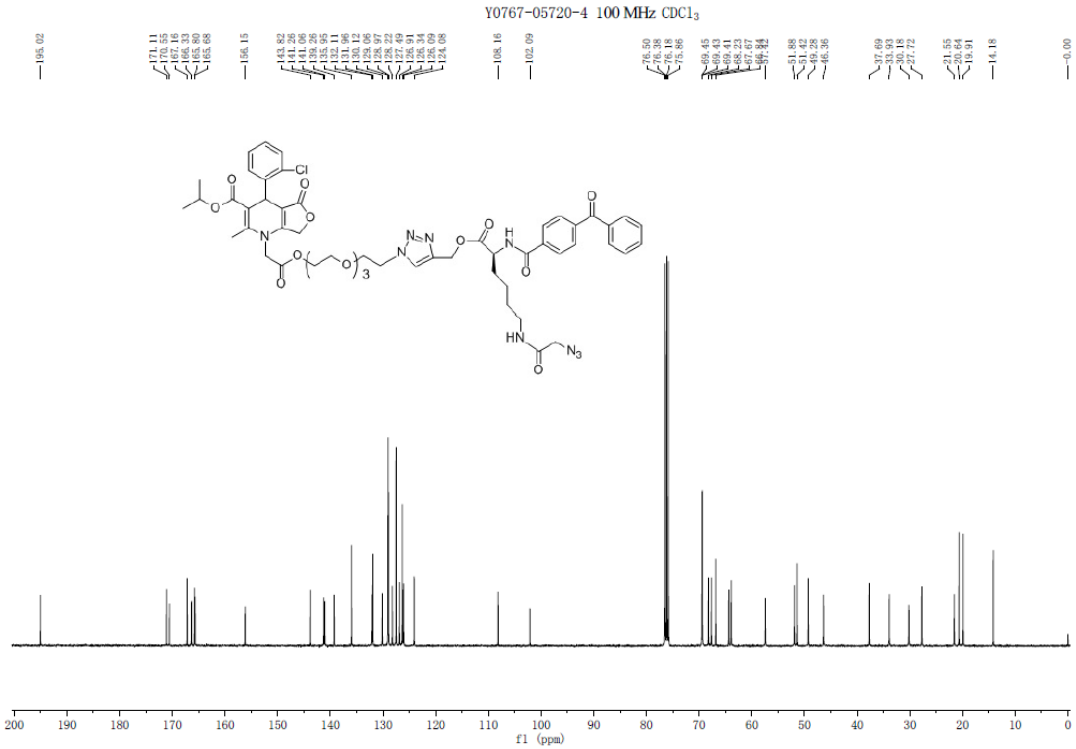
^{13}C NMR spectra of compound **5c**



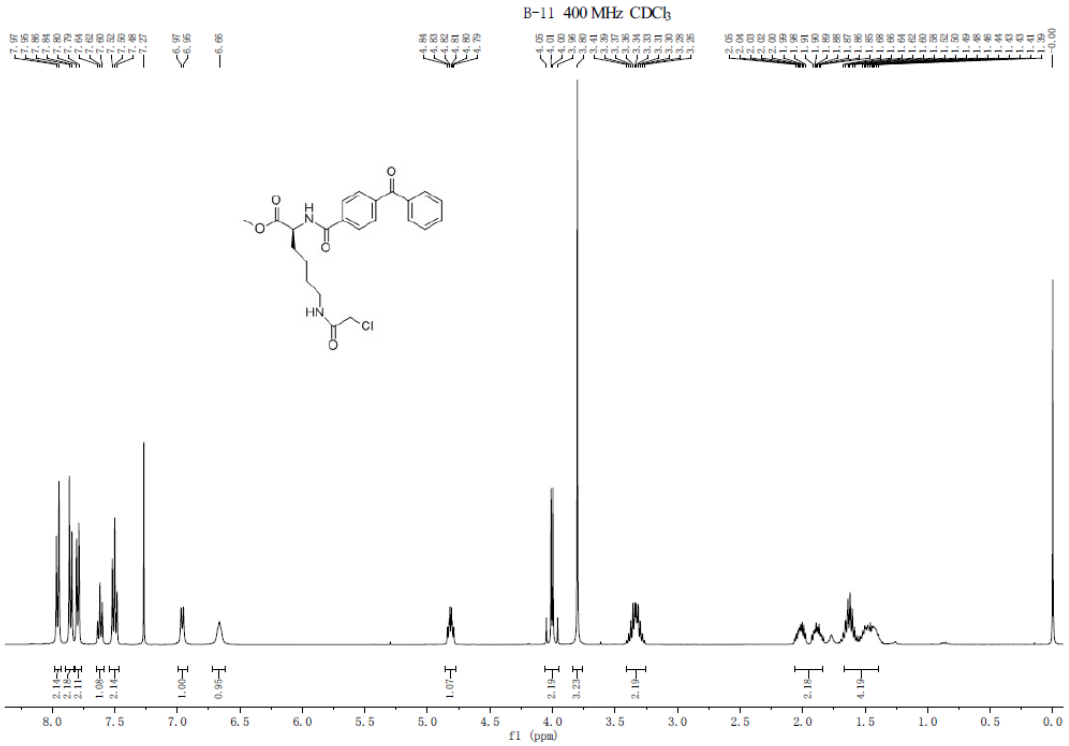
^{1}H NMR spectra of compound **5d**



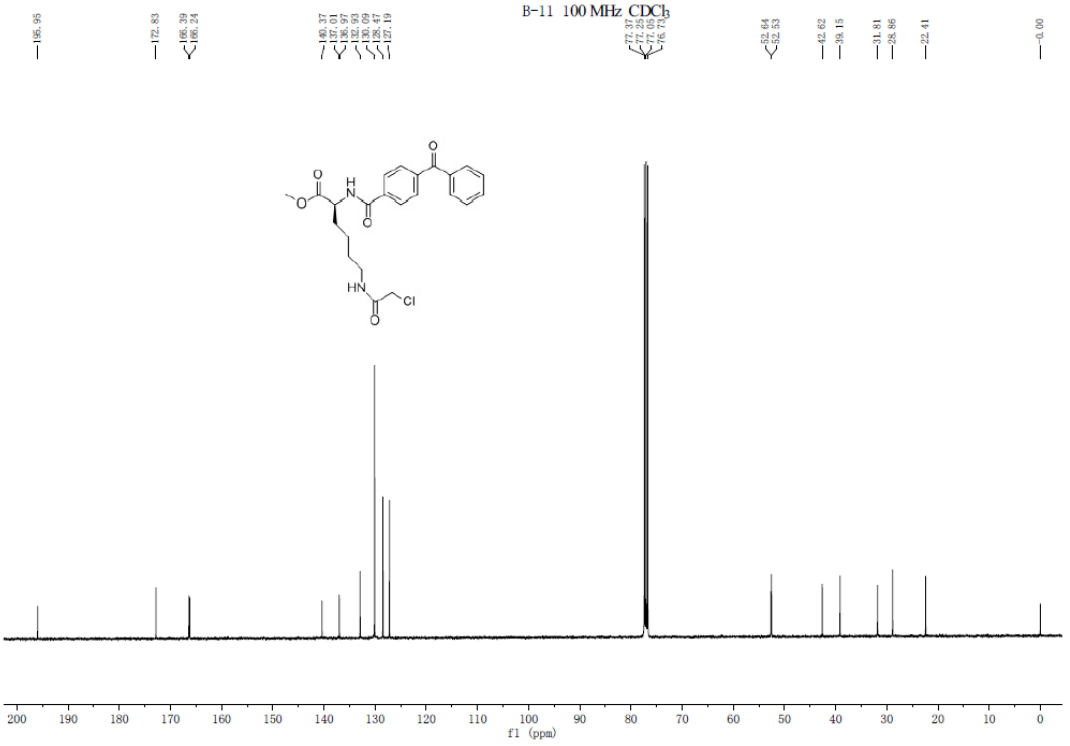
^{13}C NMR spectra of compound **5d**



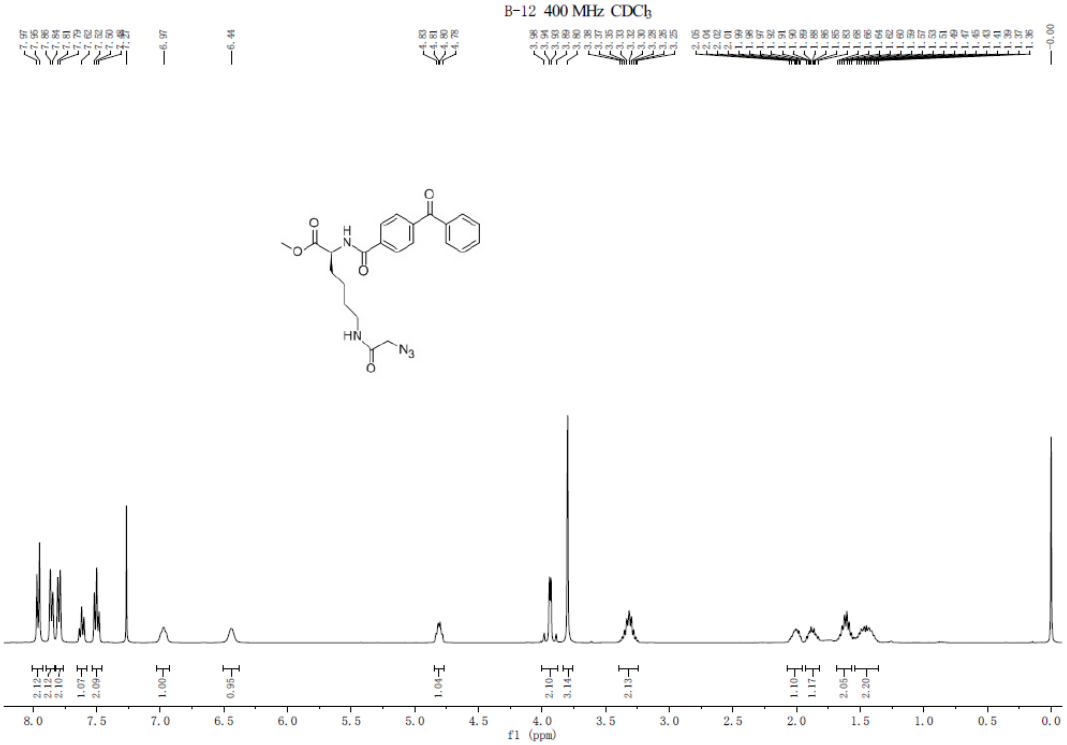
^{1}H NMR spectra of compound **33**



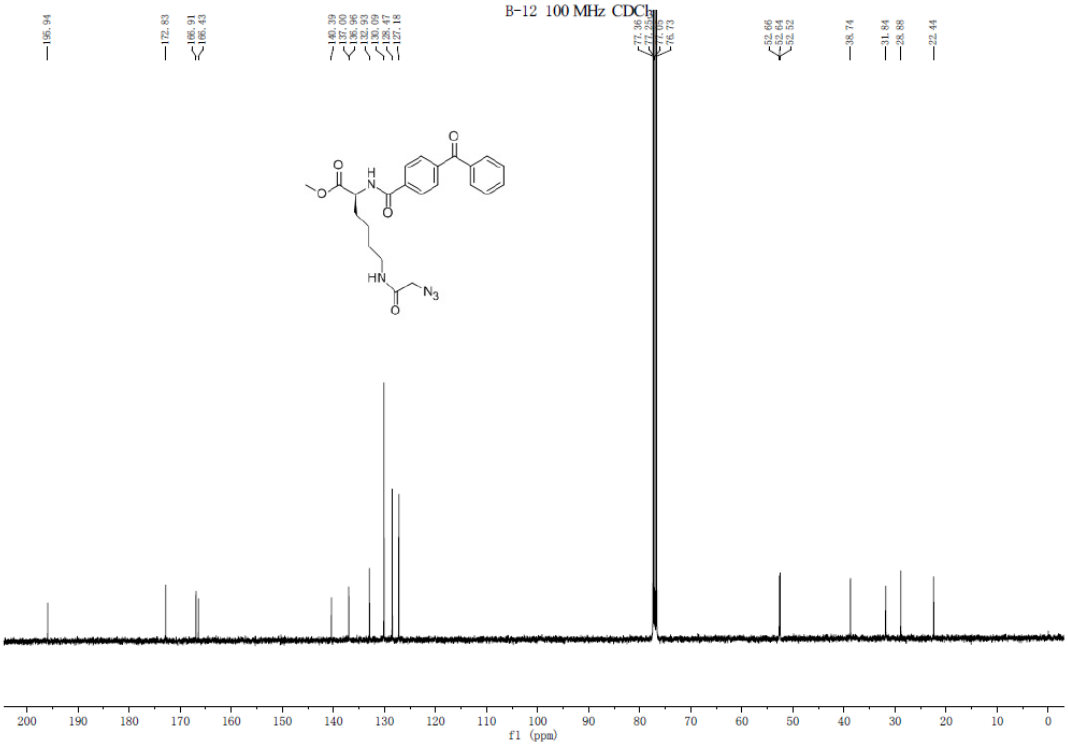
^{13}C NMR spectra of compound **33**



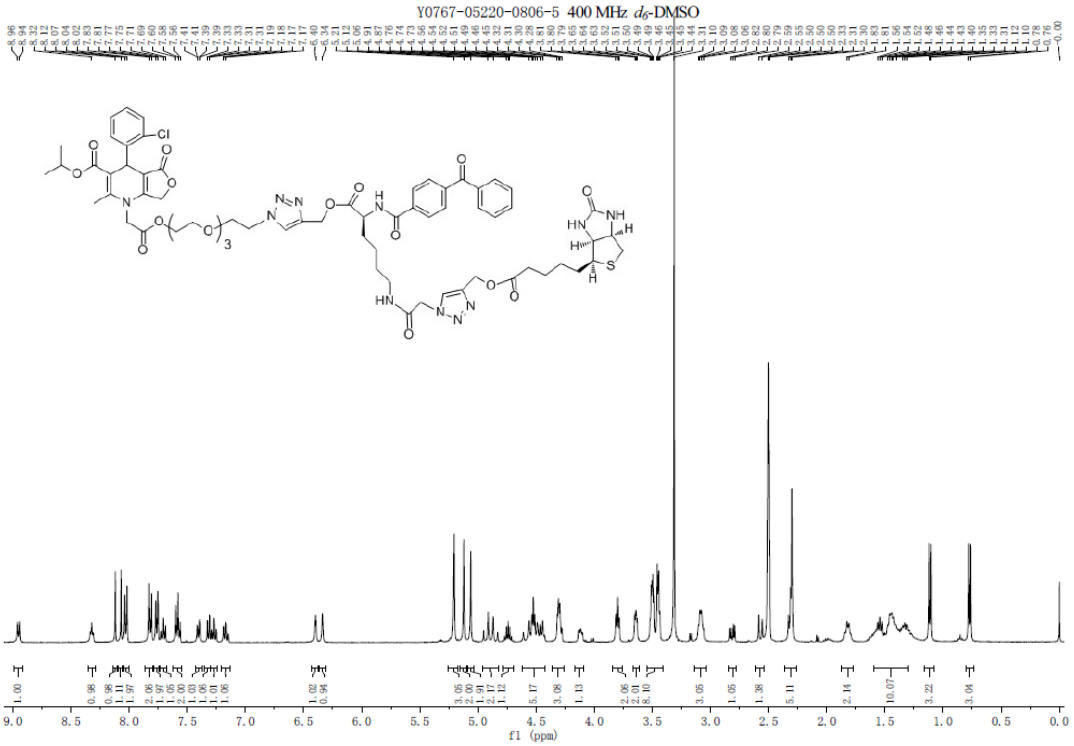
^{1}H NMR spectra of compound **34**



^{13}C NMR spectra of compound **34**



^{1}H NMR spectra of compound **36**



^{13}C NMR spectra of compound **36**

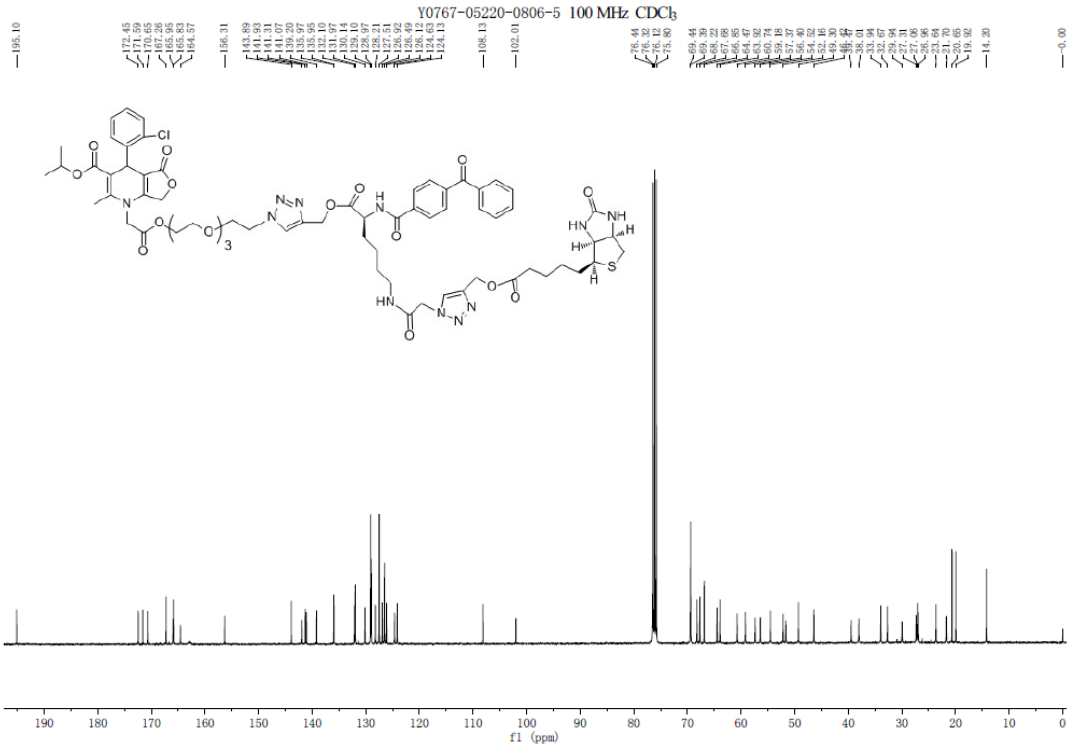
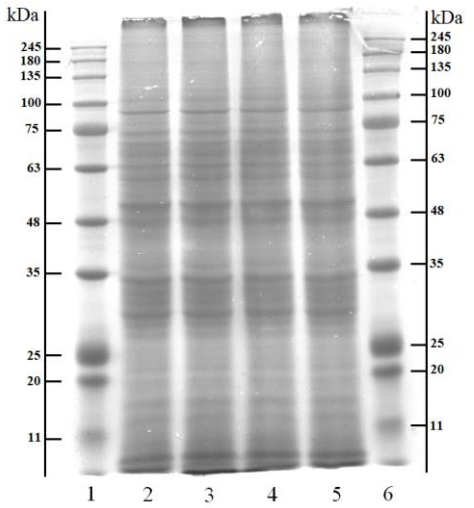


Figure 1. Image of the SDS-PAGE gel stained with Coomassie R-250.



Photoaffinity labeling of the soluble proteomes prepared from HepG2 cells followed SDS-PAGE electrophoresis separated and stained with Coomassie blue (CB) as described in the Experimental procedures. Samples were prepared by incubating 2.0 mg/mL proteomes at different conditions: (Lane 1 and 6) Marker; **(**Lane 2) With 10 μM probe **2d** and exposed to UV light for 30 min; (Lane 3) With 10 μM probe **2d** and **BAY R3401** then exposed to UV light for 30 min; (Lane 4) With 10 μM control compound **32** and exposed to UV light for 30 min; (Lane 5) With 0 μM probe **2d** and exposed to UV light for 30 min.

References

Yu, Y.; Qin, A.; Feng, C.; Lu, P.; Ng, K.M.; Luo, K.Q.; Tang, B.Z. An amine-reactive tetraphenylethylene derivative for protein detection in SDS-PAGE. *Analyst.* **137**, 5592-5596. (2012).