Synthesis of Branched Monodisperse Oligoethylene Glycols and ^{19}F MRI-Traceable Biomaterials through Reductive Dimerization of Azides

Jing Zhang,[†] Yuan Yuan,[†] Yu Li,[‡] Hao Yang,[†] Huaibin Zhang,[†] Shizhen Chen,[‡] Xin Zhou,[‡] Zhigang Yang*,[†] and Zhong-Xing Jiang*,[†]

[†]Hubei Province Engineering and Technology Research Center for Fluorinated Pharmaceuticals and School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, China.

[‡]State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, National Center for Magnetic Resonance in Wuhan, Wuhan Institute of Physics and Mathematics, Innovative Academy of Precision Measurement Science and Technology, Chinese Academy of Sciences, Wuhan, Wuhan 430071, China.

Table of contents

1. General information S2

2. *n*-octanol/water partition coefficients (logP) measurement S2

3. Solvent-dependent ^{19}F NMR of **40** and **41** S2

4. Dynamic light scattering of **41** S3

5. *In vitro* ^{19}F MRI experiments of **40** and **41** S3

6. Cytotoxicity assay of **40** and **41** S3

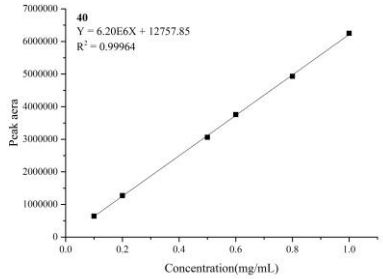
7. Copies of ^{1}H/^{13}C/^{19}F NMR spectra and mass spectra (HRMS) of compounds. S4

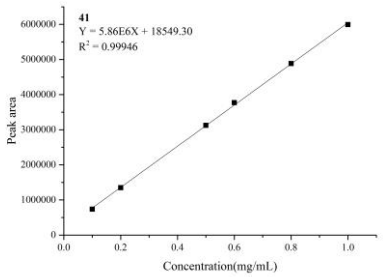
1. General information

^{1}H, ^{19}F and ^{13}C NMR spectra were recorded on a Bruker 400 MHz. Chemical shifts are in ppm and coupling constants (*J*) are in Hertz (Hz). ^{1}H NMR spectra were referenced to tetramethylsilane (d, 0.00 ppm) using $CDCl_3$ as solvent, ^{13}C NMR spectra were referenced to solvent carbons (77.16 ppm for $CDCl_3$). ^{19}F NMR spectra were referenced to 2% perfluorobenzene (s, -164.90 ppm) in $CDCl_3$. The splitting patterns for ^{1}H NMR spectra are denoted as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), b (broad) and combinations thereof. MS spectra were recorded on an Asilent LCMS-1100 spectrometer. MALDI-TOF mass spectra were recorded on a MALDI-TOF/TOF 5800 (AB SCIEX) spectrometer using the reflector mode for positive ions with *α*-cyano-4-hydroxylcinnamic acid as matrix.

2. n-Octanol/Water partition coefficients (logP) measurement

The logP values of compounds **40**-**41** were measured following shake-flask method. Briefly, the product was dissolved in distilled *n*-octanol saturated with water. Then 1 mL of this solution was mixed with an equal volume of water saturated with distilled *n*-octanol and mixed on a vortex device. After shaking the mixture overnight, water phase was separated by centrifugation. Equal-volume samples of the shaken water phase and the starting solution were subsequently taken and analyzed by HPLC. The peak area was measured at λ = 254 nm, and compared with calibration curve to obtain the concentration of the peptide. LogP values were determined from: $Lg[(C_s-C_w)/C_w]$, where C_s and C_w are the concentrations of the starting water solution and the water phase of the compound, respectively.





3. Solvent-dependent ^{19}F NMR of 40 and 41

Solvent-dependent ^{19}F NMR spectra were referenced to 10% sodium trifluomethanesulfonate (s, -79.61 ppm) in D_2O at 25 °C.

4. Dynamic light scattering of 41

Solution of **41** in H_2O at 0.07 mM was used for DLS analysis. The particle size was measured at an angle of 90° in a 10 mm diameter cell at the room temperature with a Dynamic Light Scattering (DLS) Analyzer (Malvern ZetasizerNano 3690). Eleven scans were run for each measurement and the measurement was repeated 3 times. The particle size and polydispersity index (PDI) were calculated by Malvern software.

5. In vitro ^{19}F MRI experiments of 40 and 41

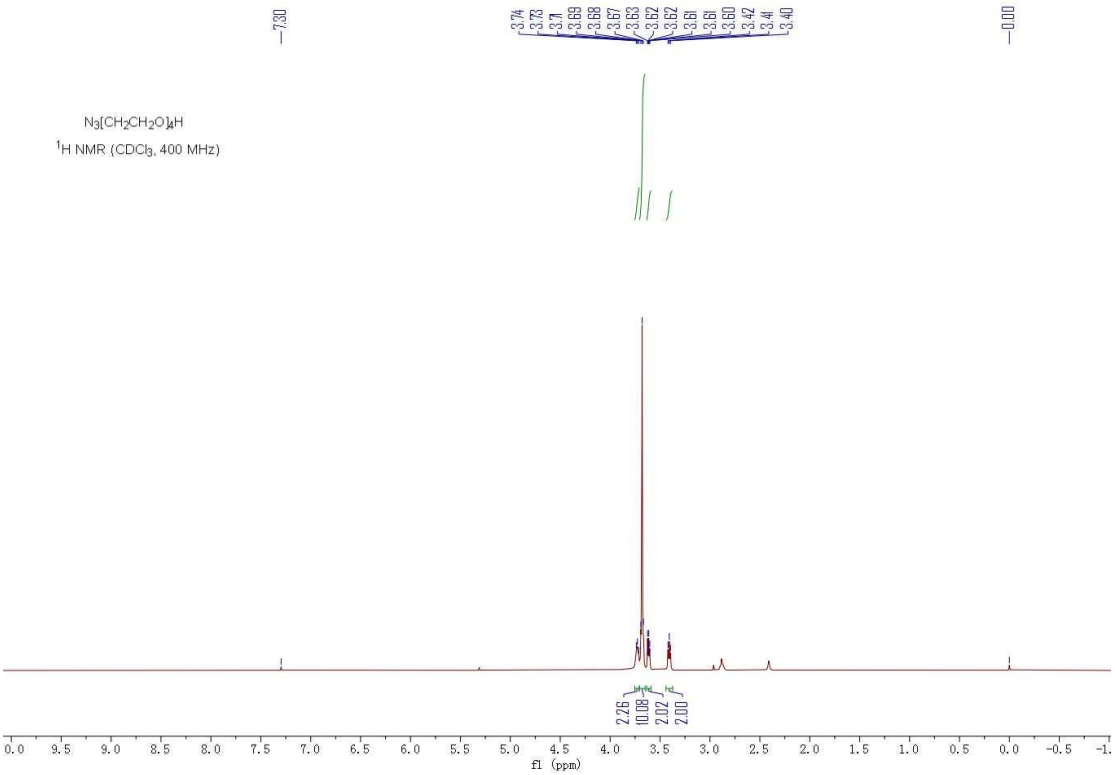
All magnetic resonance imaging (MRI) experiments were performed on a 400 MHz Bruker BioSpec MRI system. The temperature of the magnet room was maintained at 25 °C during the entire MRI experiment. The ^{19}F in vitro images were acquired using a gradient-echo (GRE) pulse sequence, method = RARE, matrix size = 32 × 32, SI = 20 mm, FOV = 3.0 cm, TR = 2500 ms, TE = 2.8 ms, scan time = 160 s.

6. Cytotoxicity assay of 40 and 41

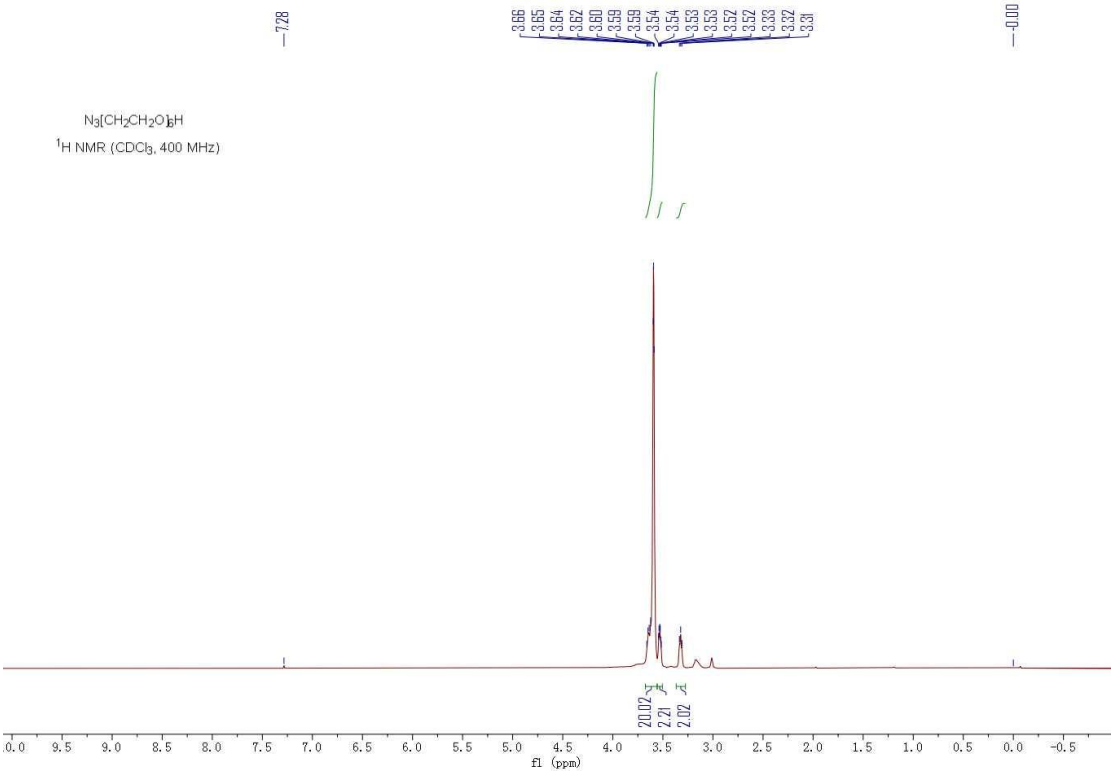
HepG2 cells were cultured in DMEM medium containing 10% FBS and 1% streptomycin double antibody. L929 cells were cultured in alpha-MEM medium containing 10% FBS and 1% streptomycin double antibody. All cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2 and the growth medium was replaced with fresh media every 24 h.
The cell viability assay of compounds **40** and **41** were investigated in L929 cell lines and HepG2 cell lines *in vitro* by MTT assay. L929 cells HepG2 cells were seeded into a 96-well plate for several hours. Subsequently, a gradient concentration of the compounds ranging from 62 μg/mL to 1000 μg/mL were added in a series of wells. Every concentration was set with five wells at least. The wells with 100 μL culture medium alone were used as negative control and wells containing cells alone were used as positive control. After incubation for 24 h, the medium was replaced with 100 μL MTT (1.0 mg/mL) solution and incubated for 4 h. Then the medium was replaced with 200 μL DMSO and the absorbance value was measured at 490 nm using a microplate reader (Bio Tek Instruments, USA).

7. Copies of $^{1}H/^{13}C/^{19}F$ NMR, MS and mass spectra (HRMS) of compounds

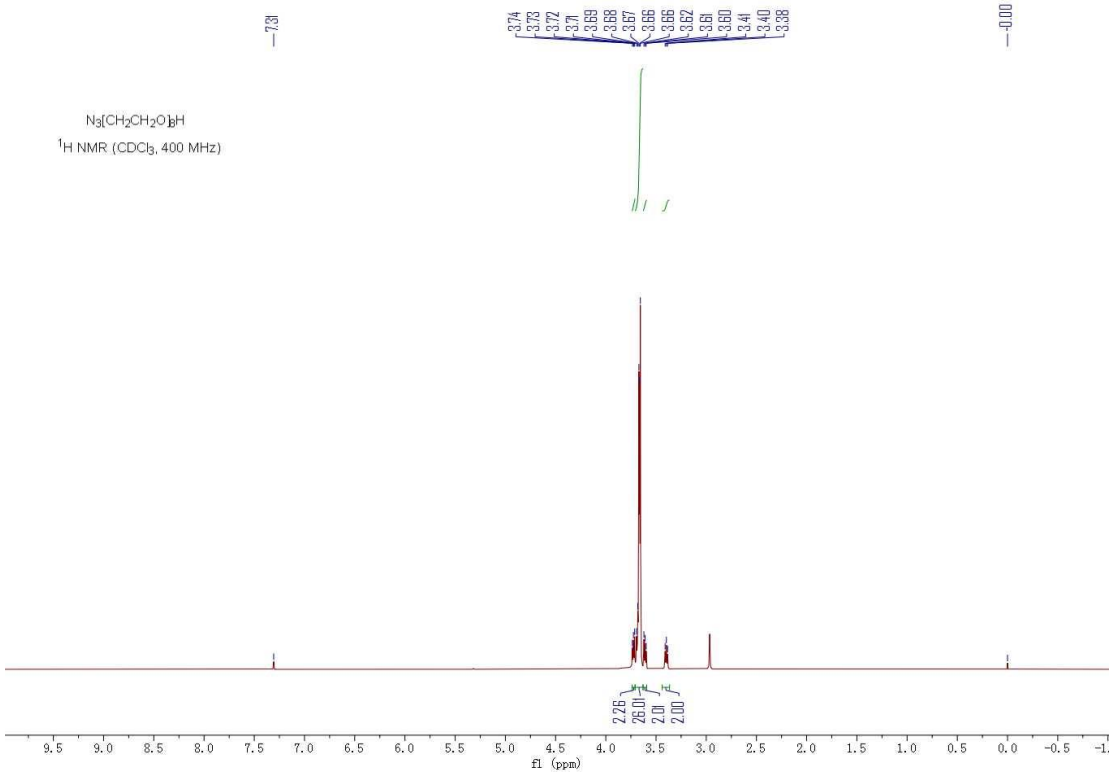
^{1}H NMR of compound **2**



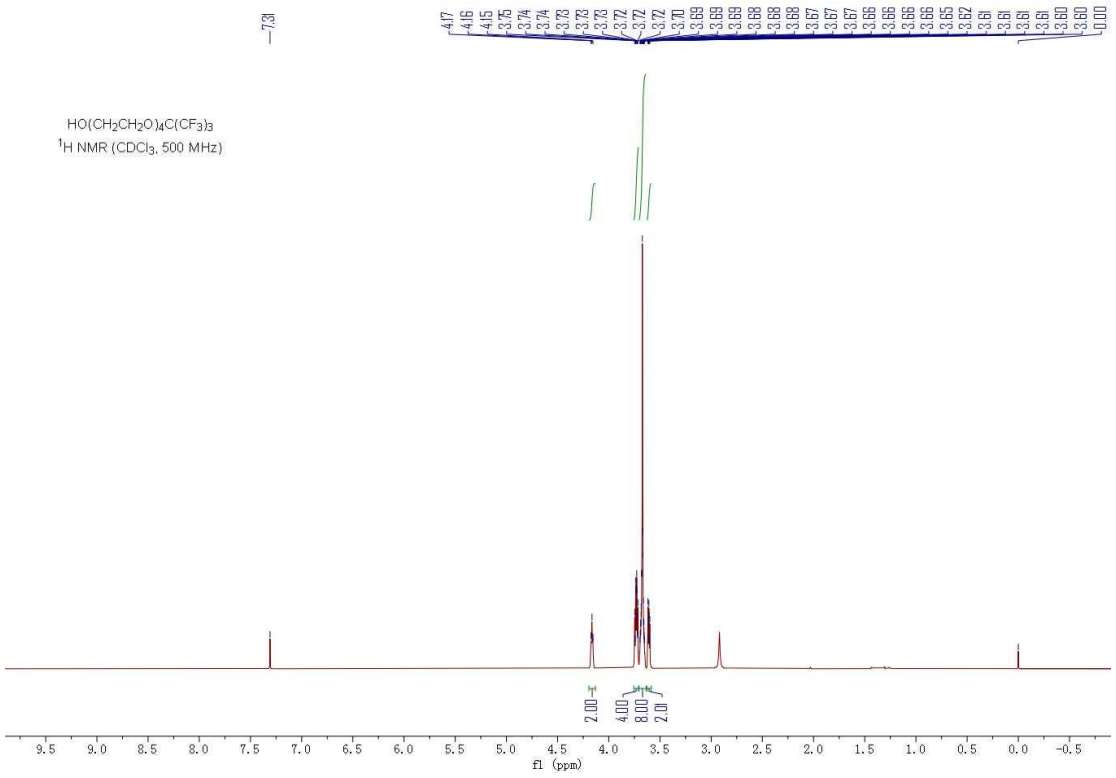
^{1}H NMR of compound **3**



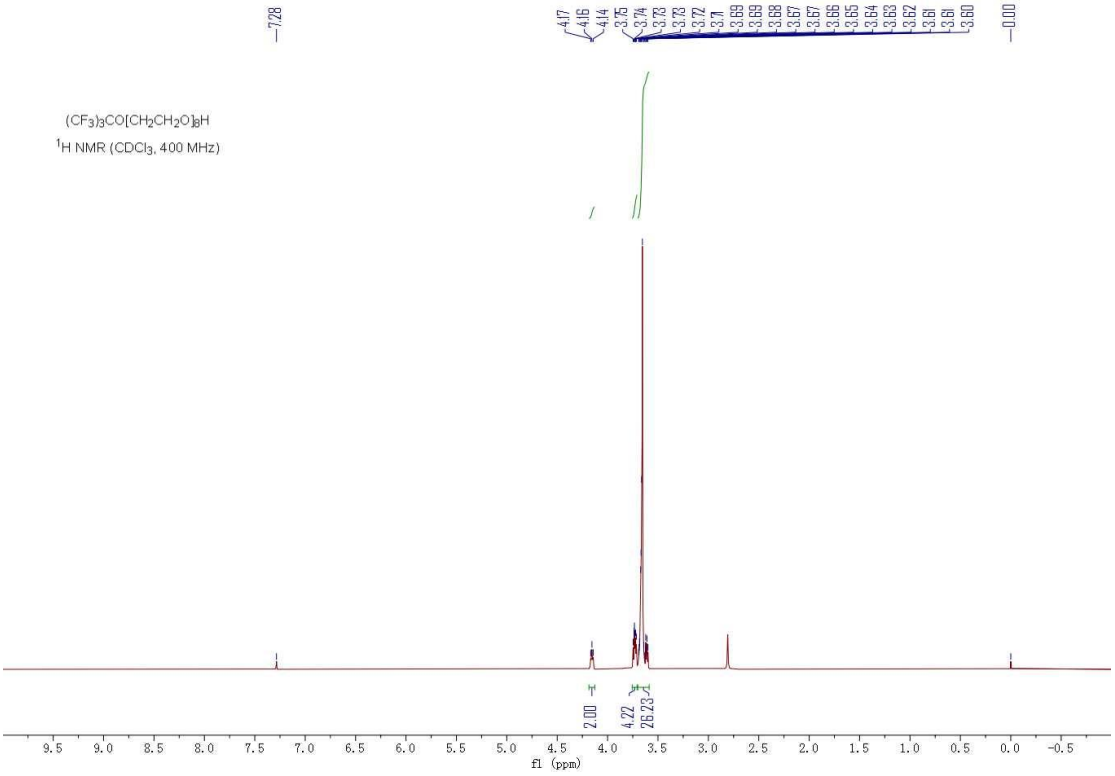
^{1}H NMR of compound **4**



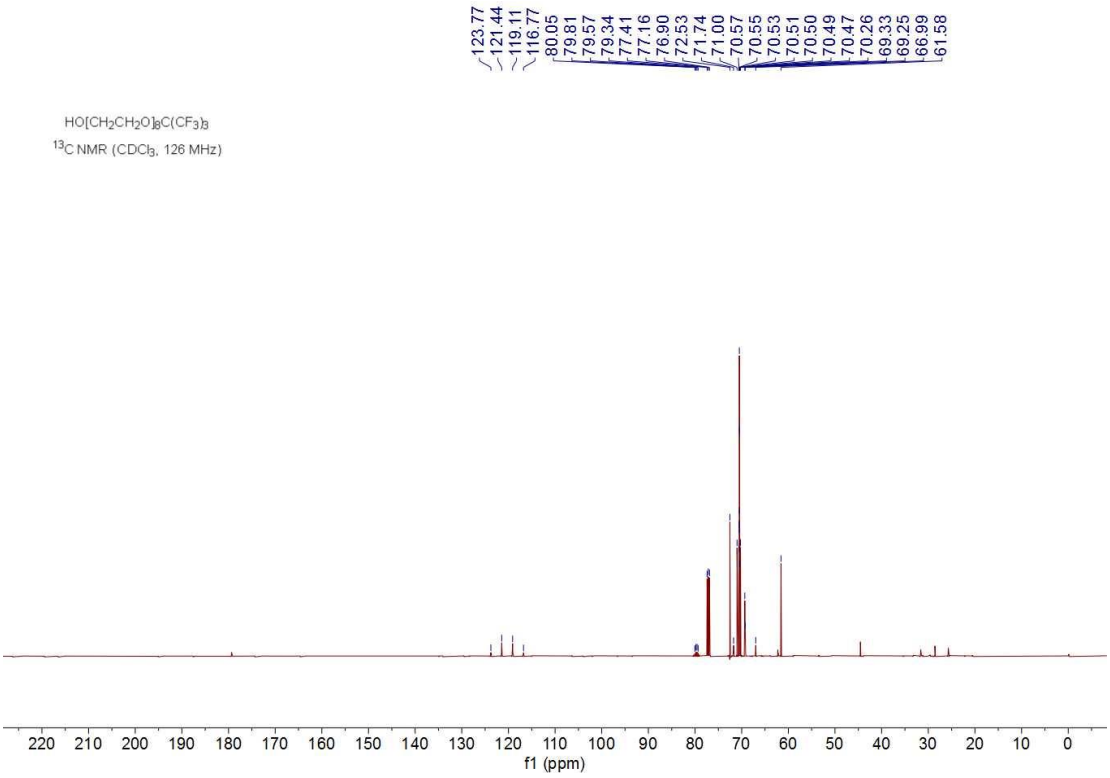
^{1}H NMR of compound **5**



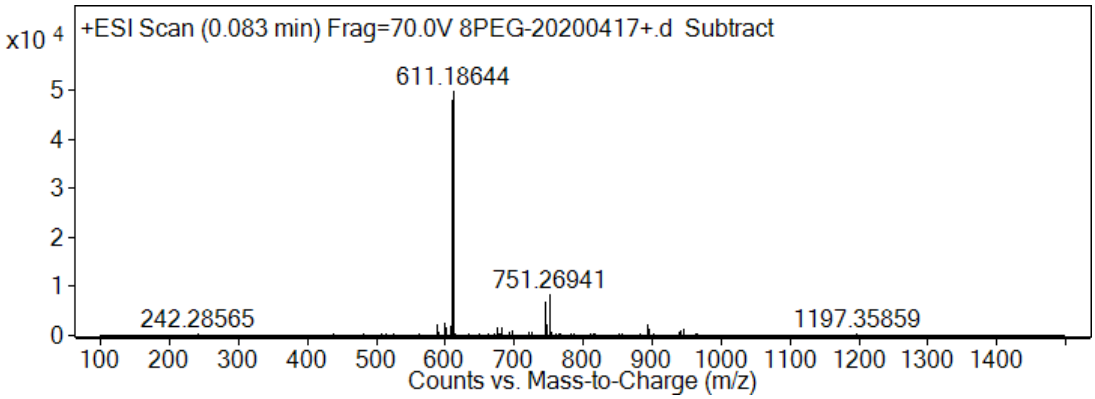
^{1}H NMR of compound **6**



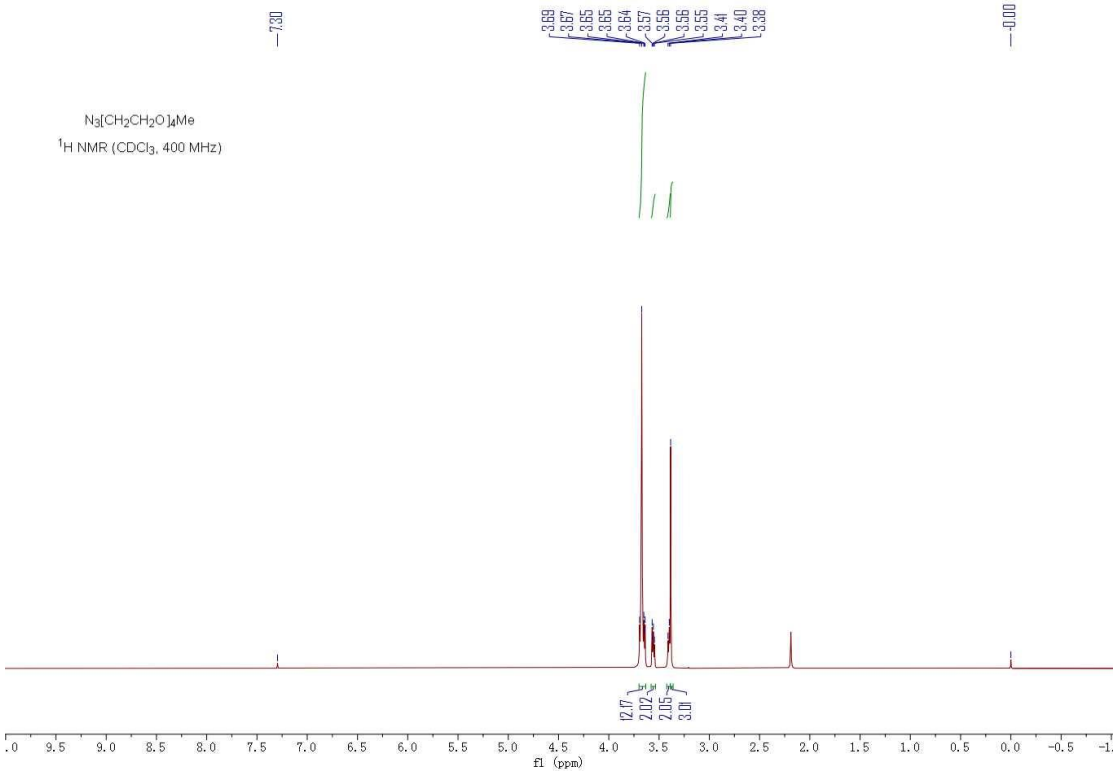
^{13}C NMR of compound **6**



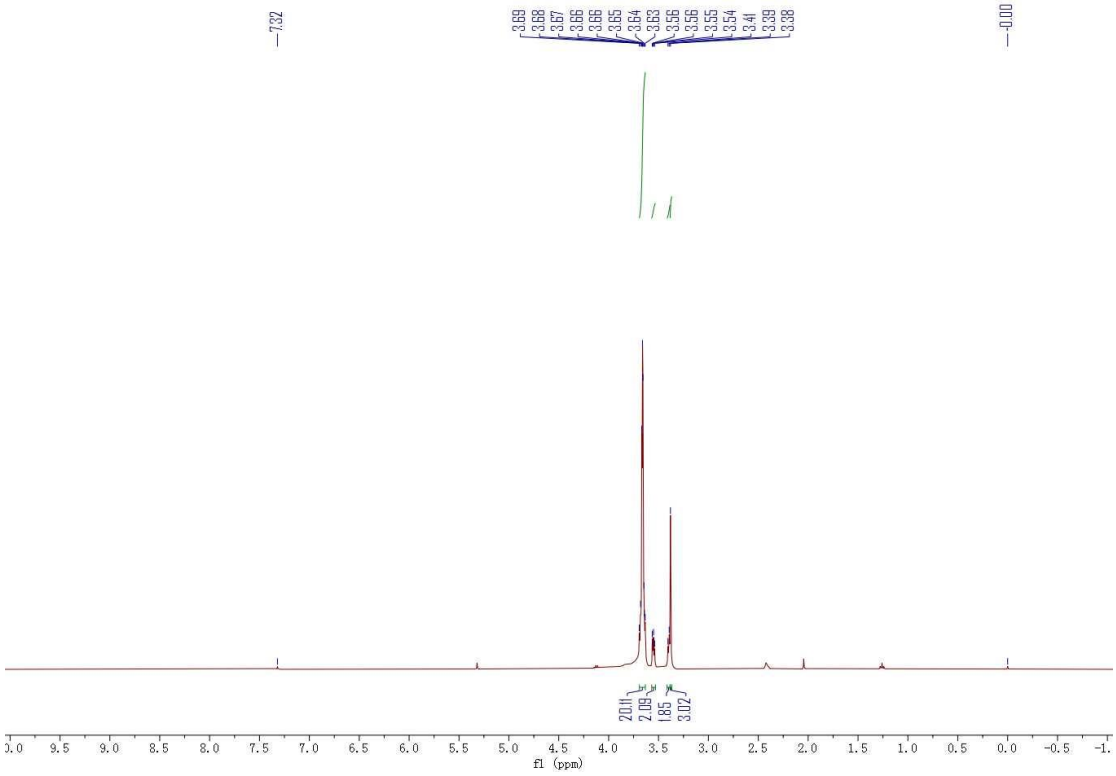
HRMS of compound **6**



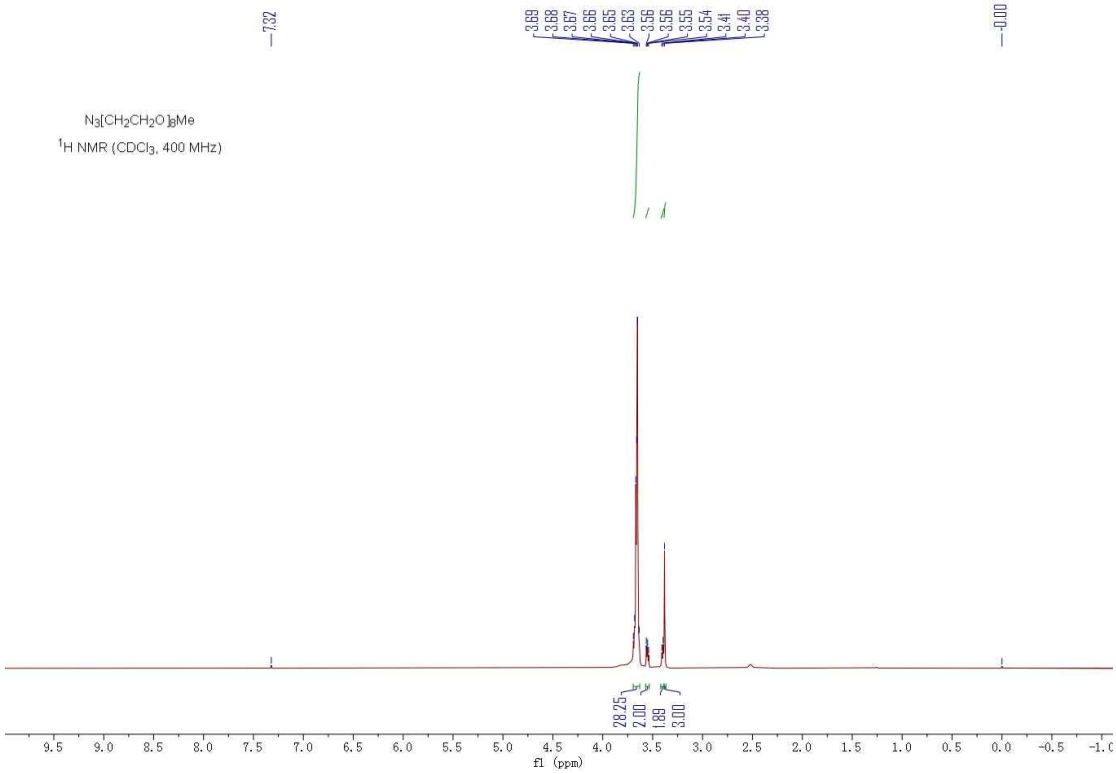
^{1}H NMR of compound **7**



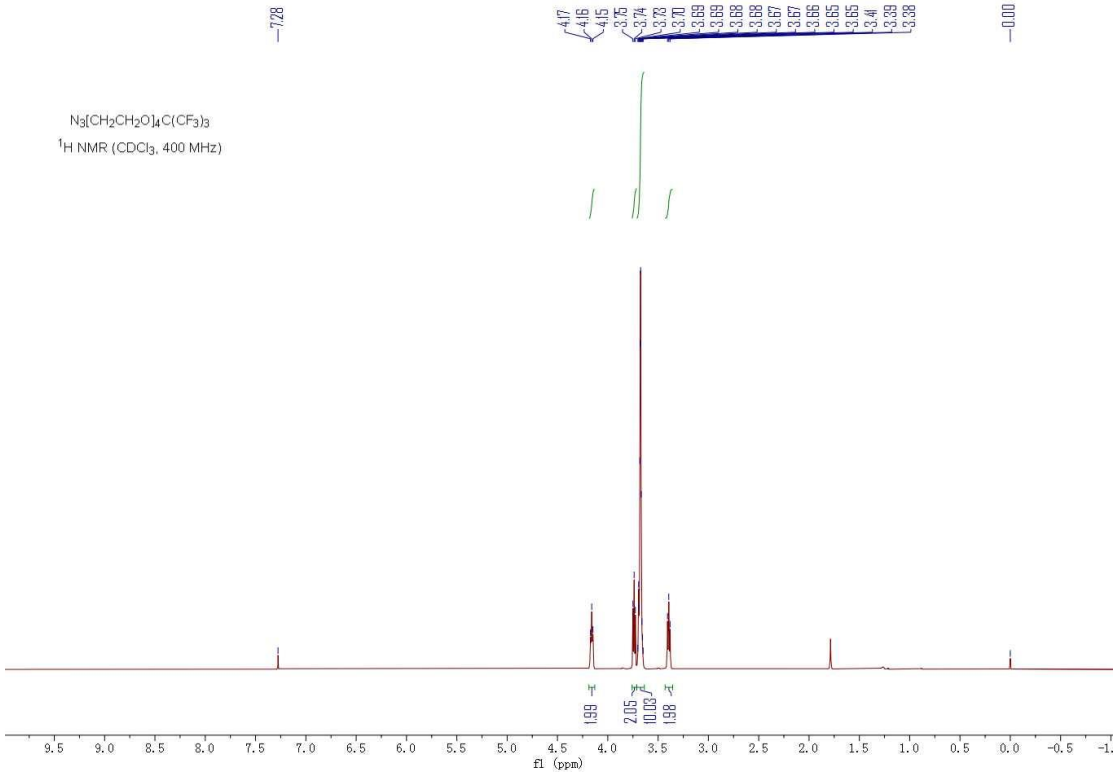
^{1}H NMR of compound **8**



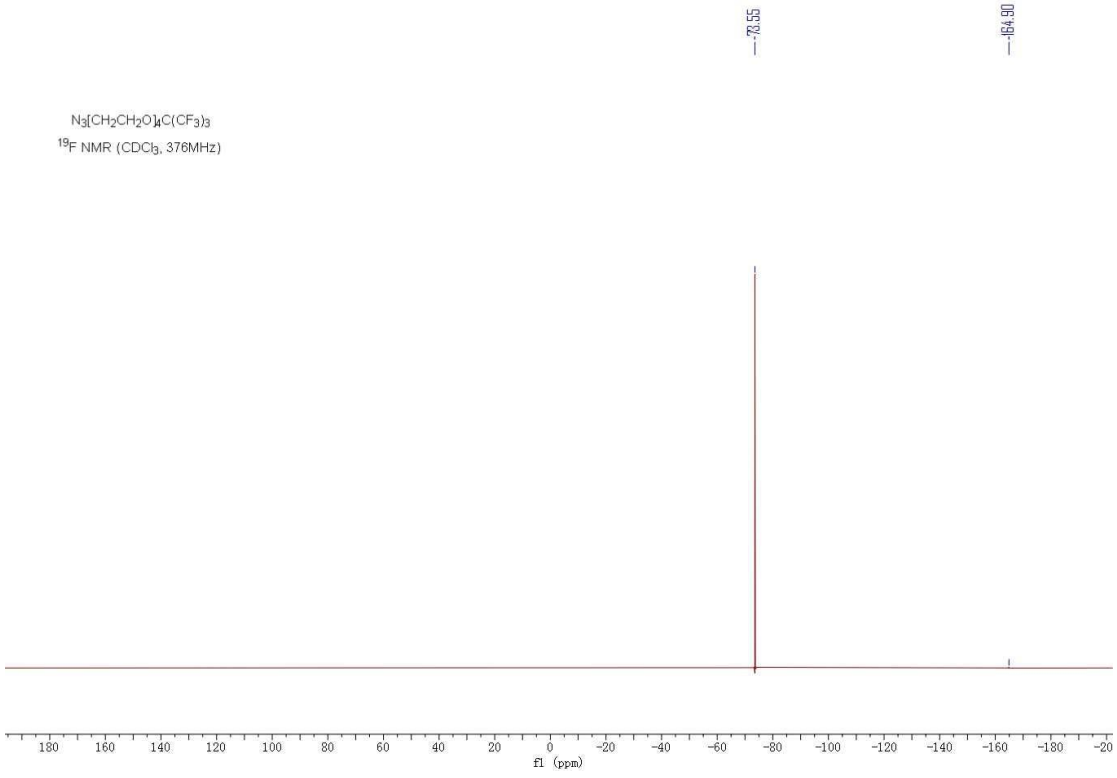
^{1}H NMR of compound **9**



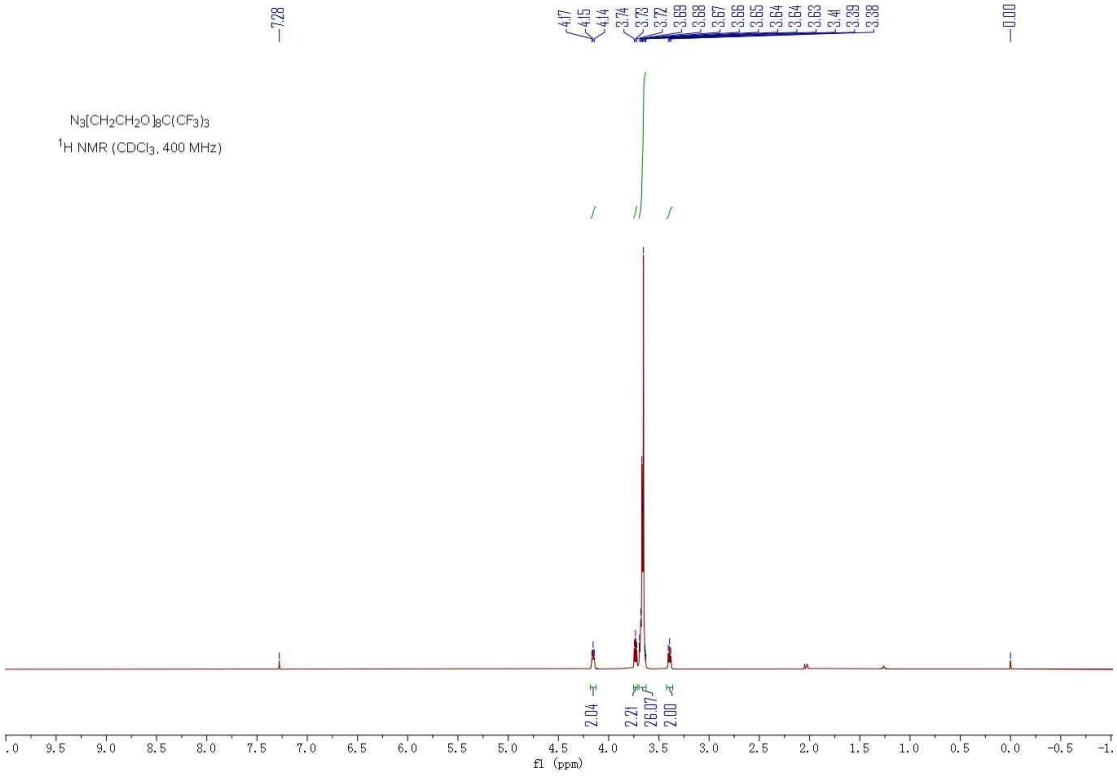
^{1}H NMR of compound **10**



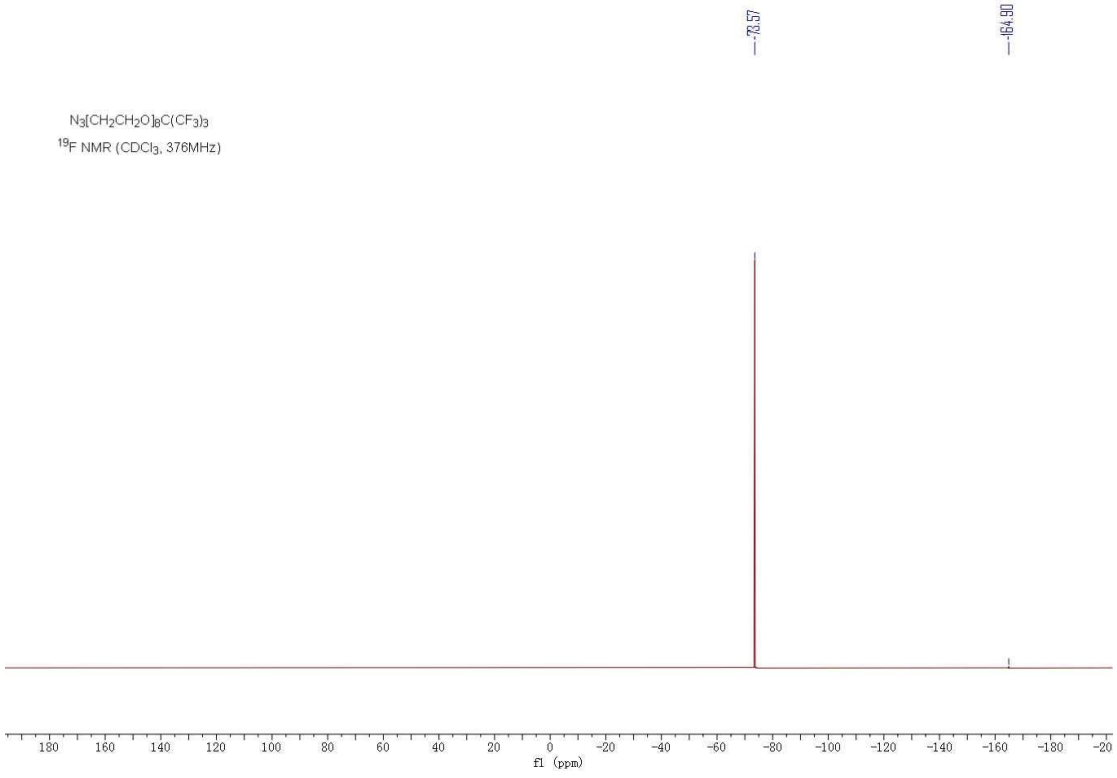
^{19}F NMR of compound **10**



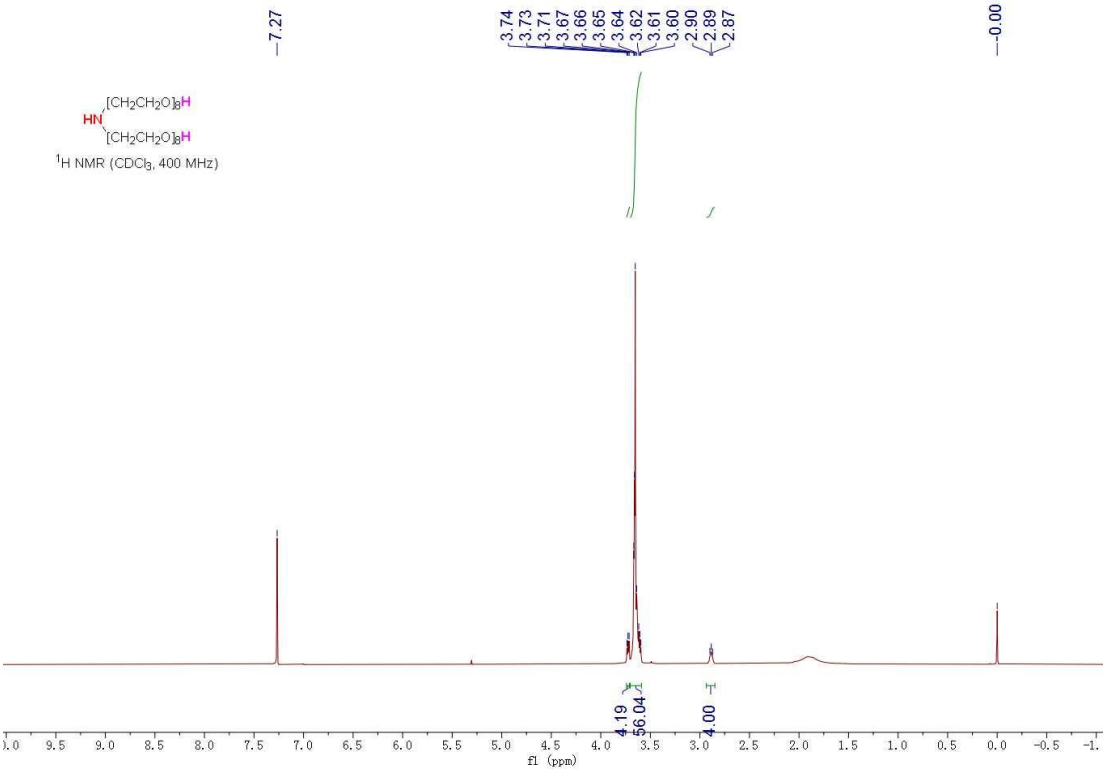
^{1}H NMR of compound **11**



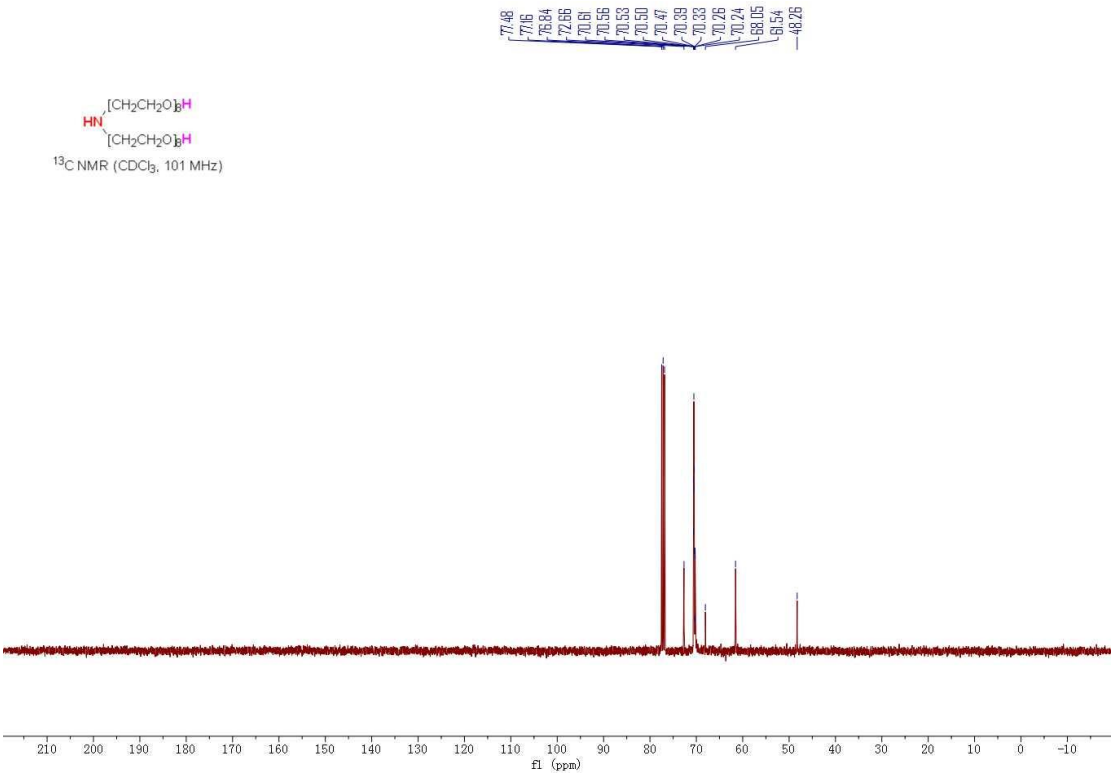
^{19}F NMR of compound **11**



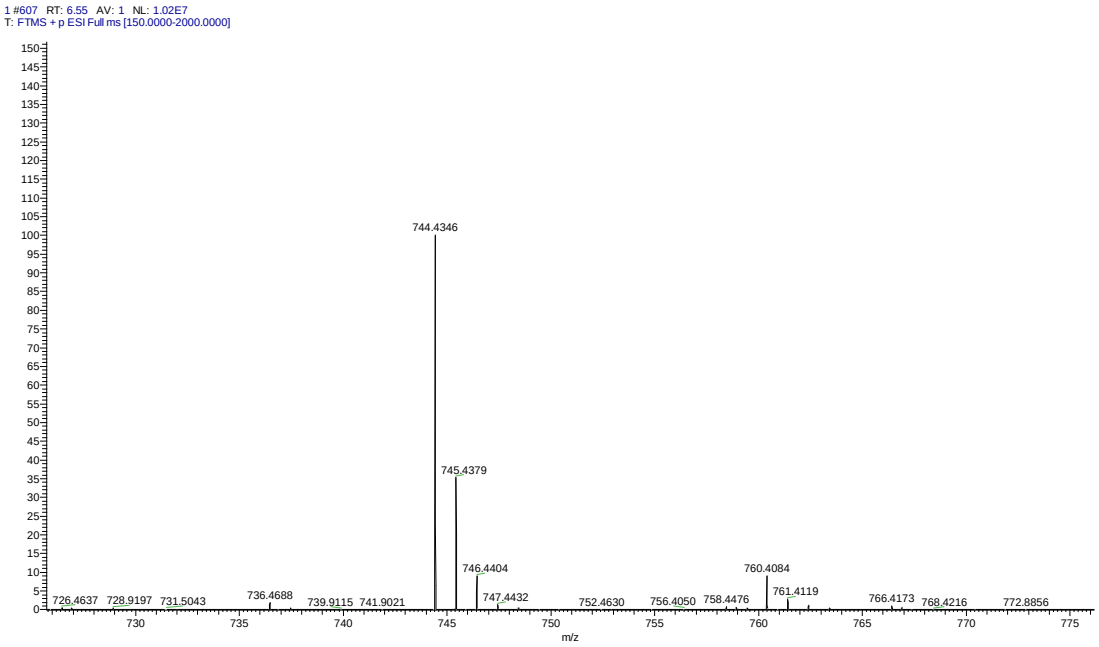
^{1}H NMR of compound **12**



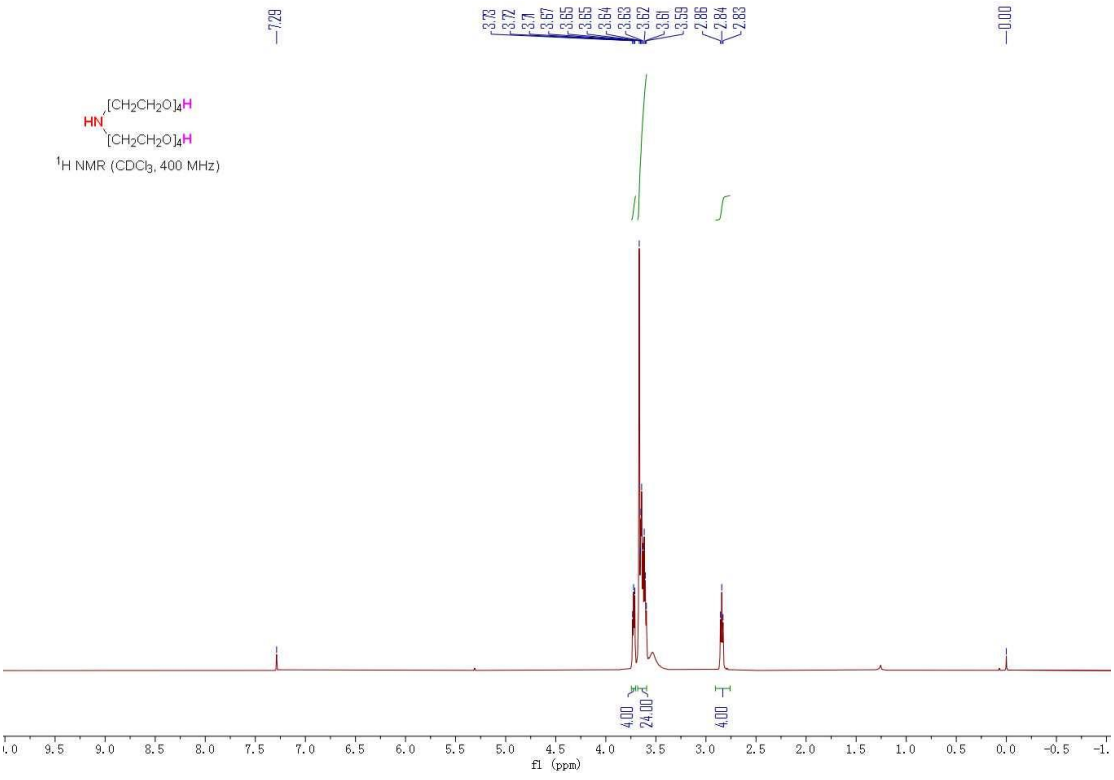
^{13}C NMR of compound **12**



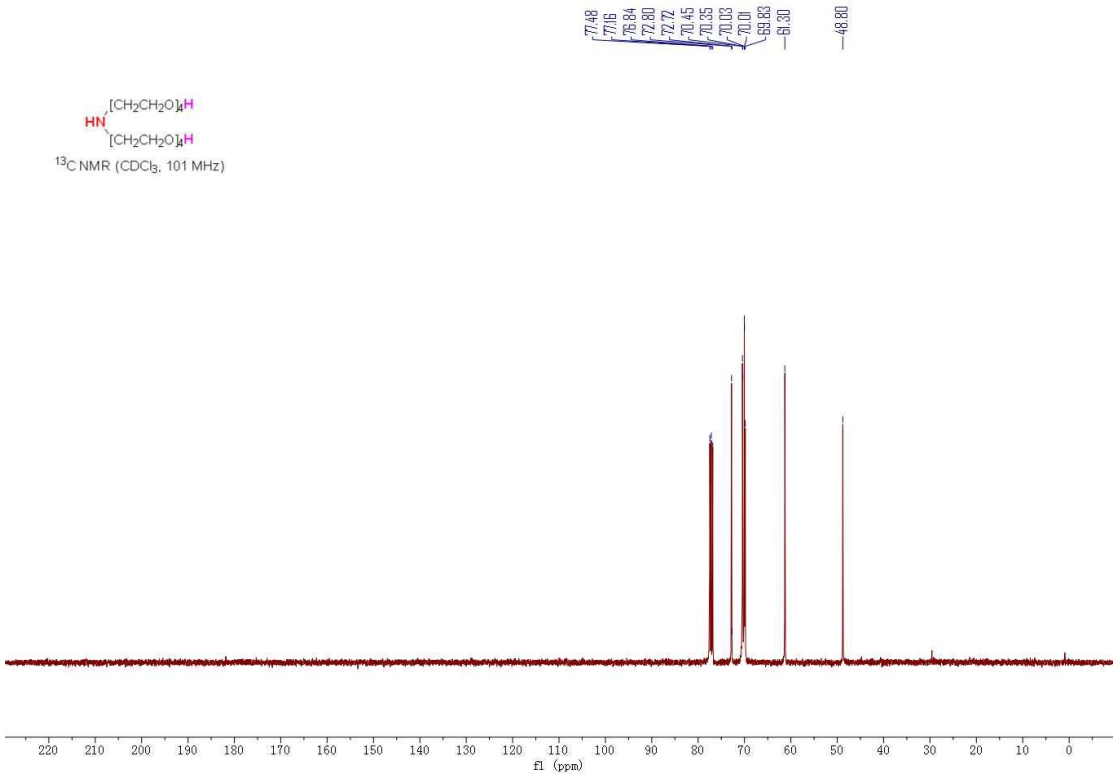
HRMS of compound **12**



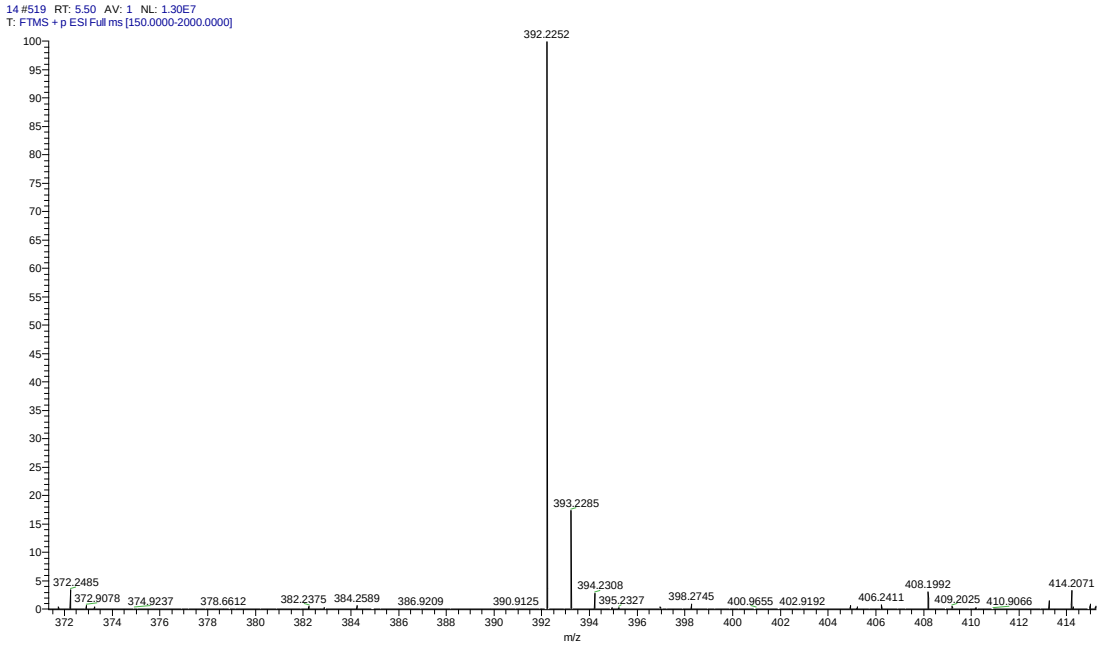
^{1}H NMR of compound **13**



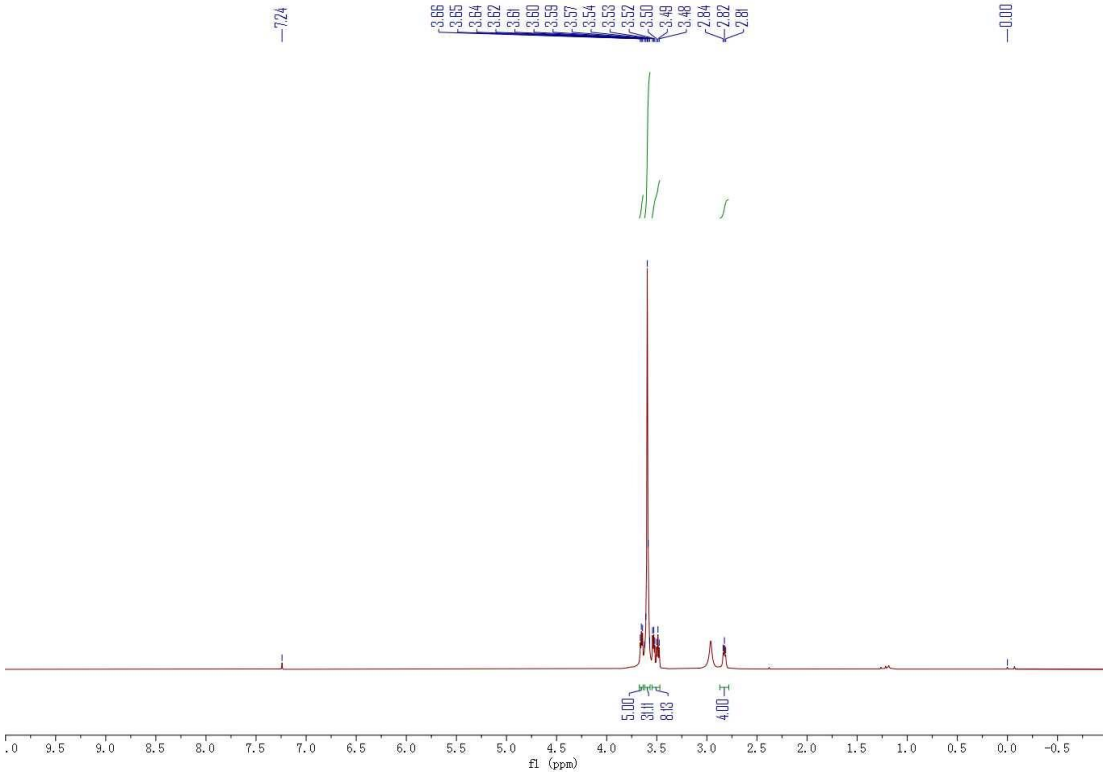
^{13}C NMR of compound **13**



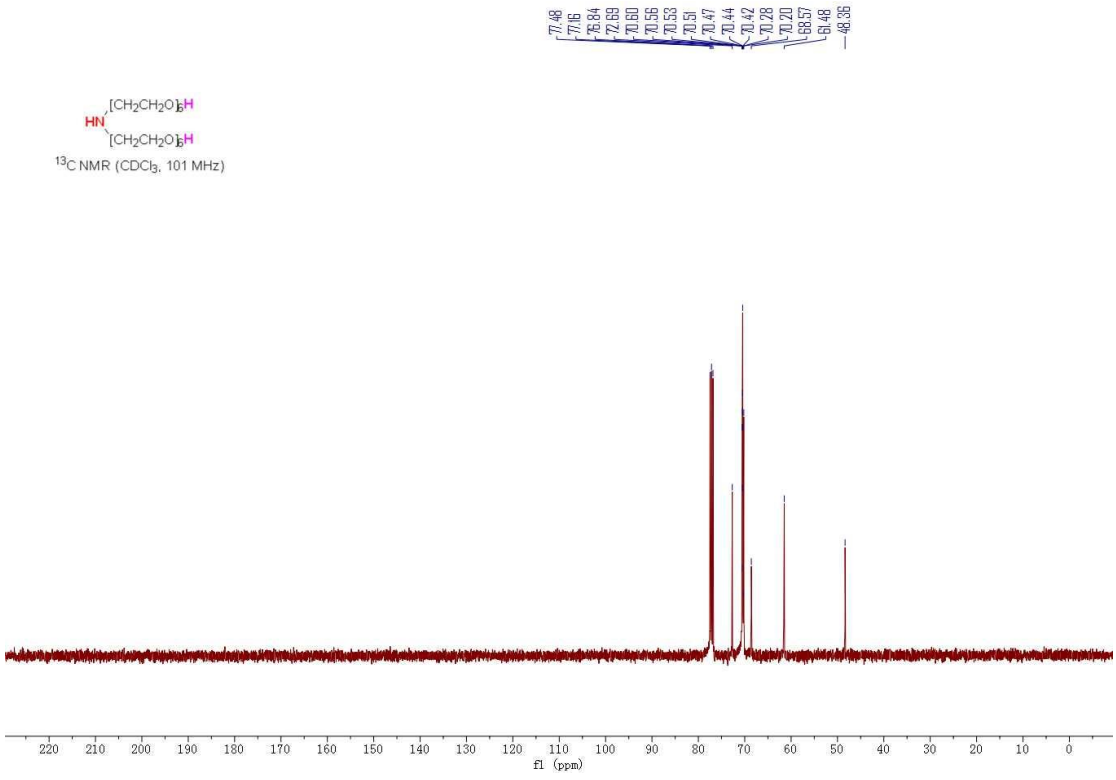
HRMS of compound **13**



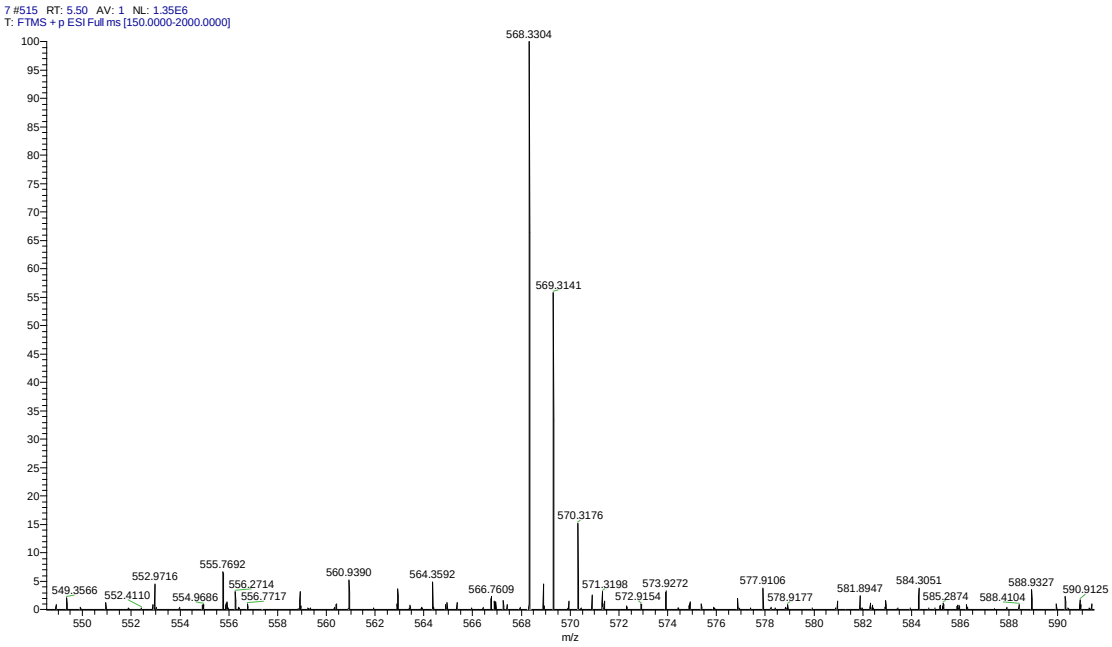
^{1}H NMR of compound **14**



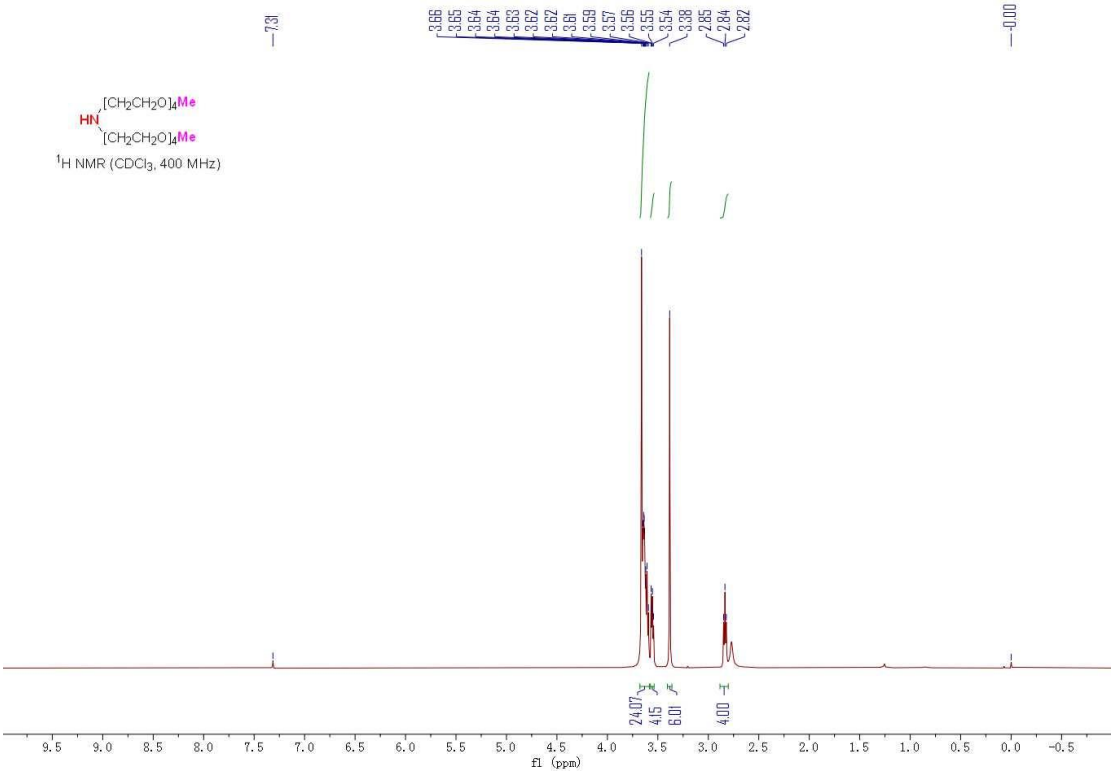
^{13}C NMR of compound **14**



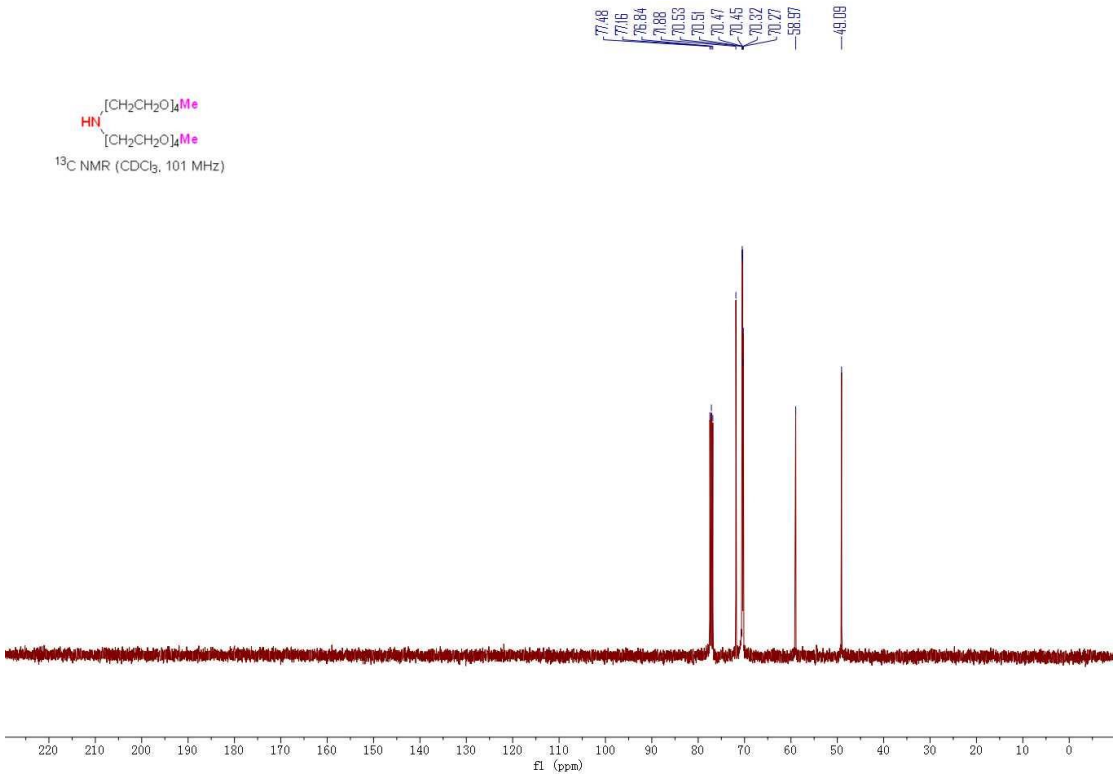
HRMS of compound **14**



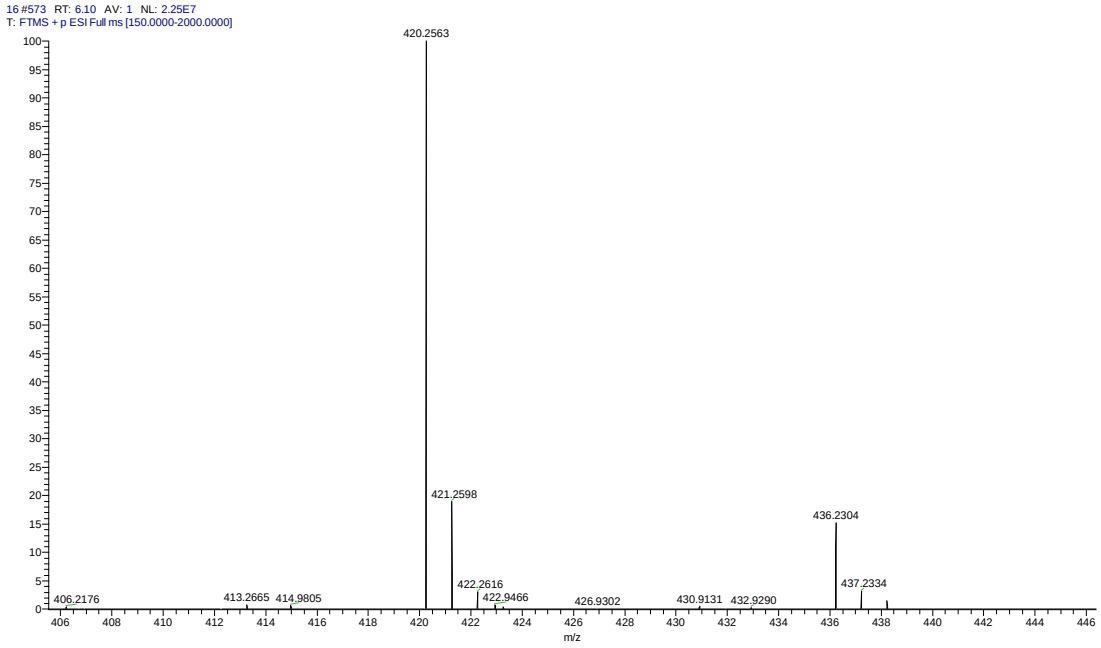
^{1}H NMR of compound **15**



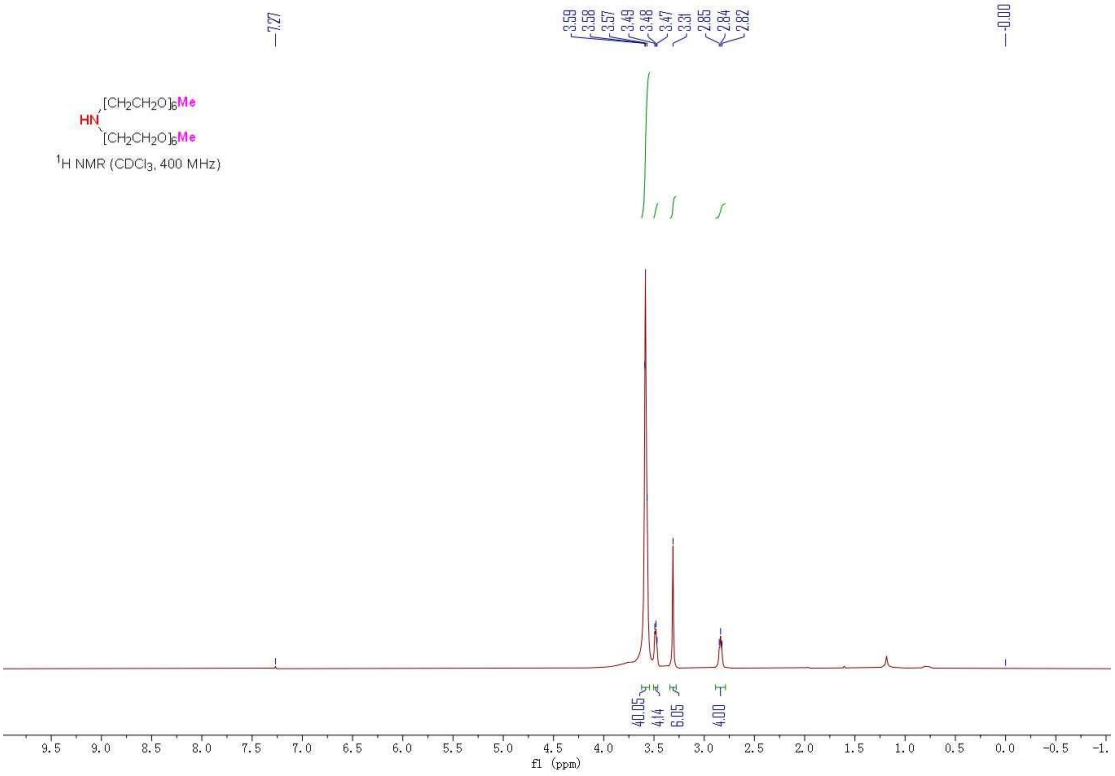
^{13}C NMR of compound **15**



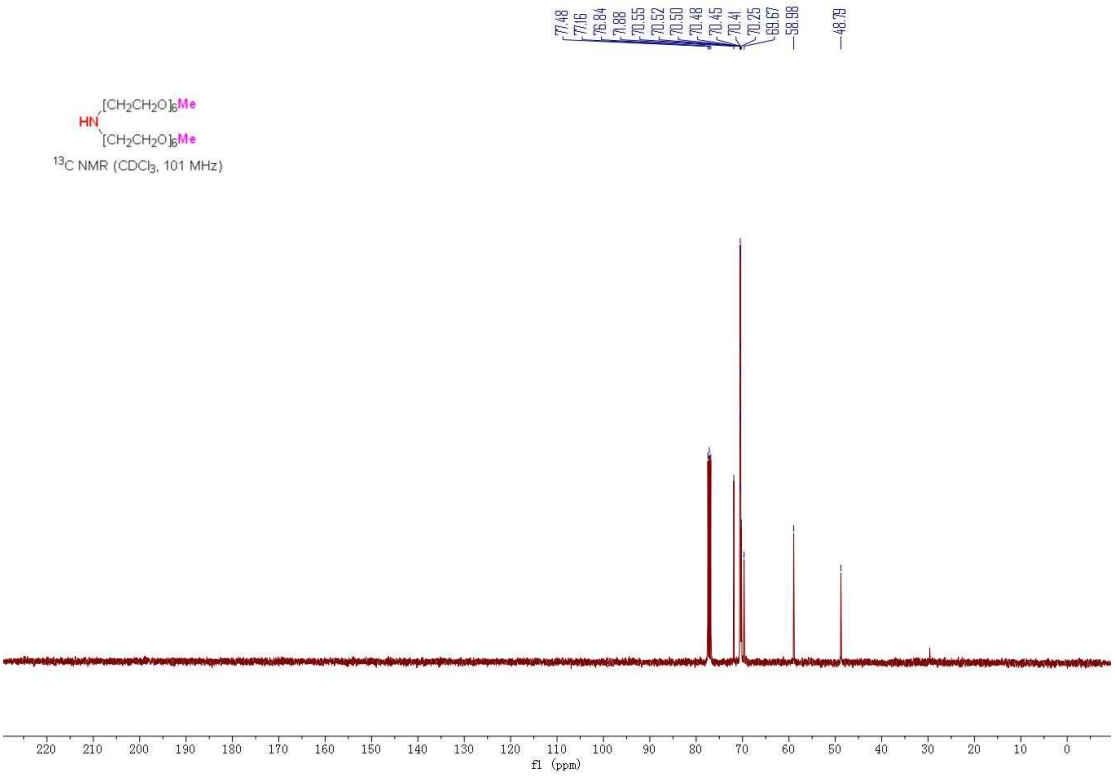
HRMS of compound **15**



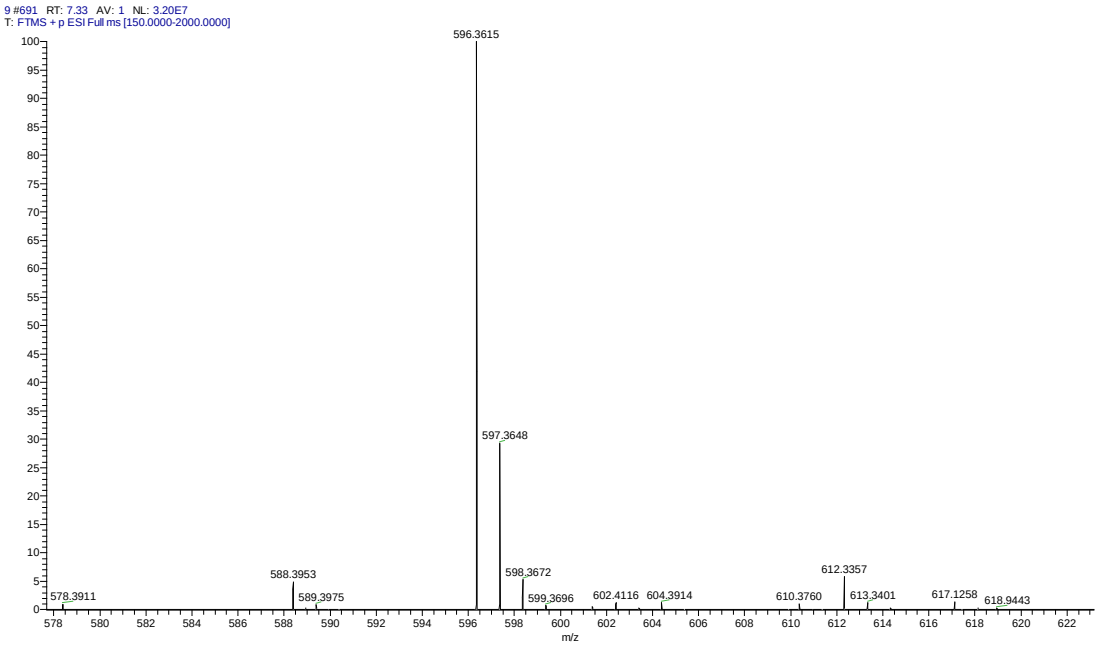
^{1}H NMR of compound **16**



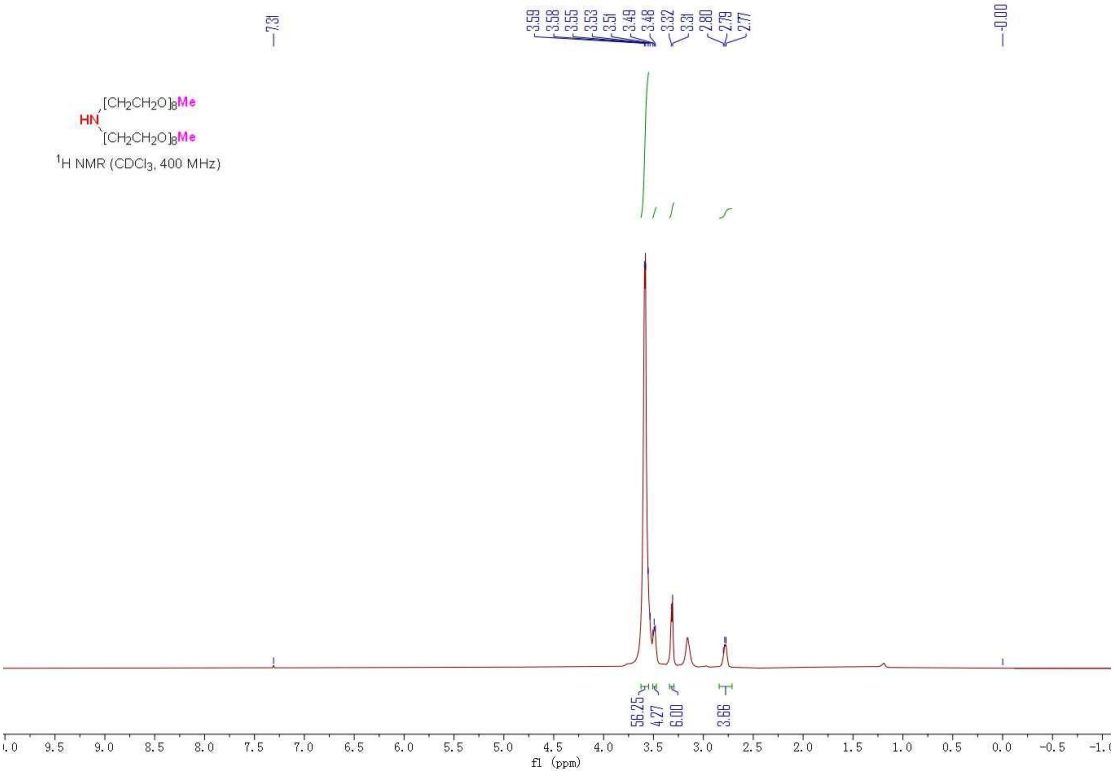
^{13}C NMR of compound **16**



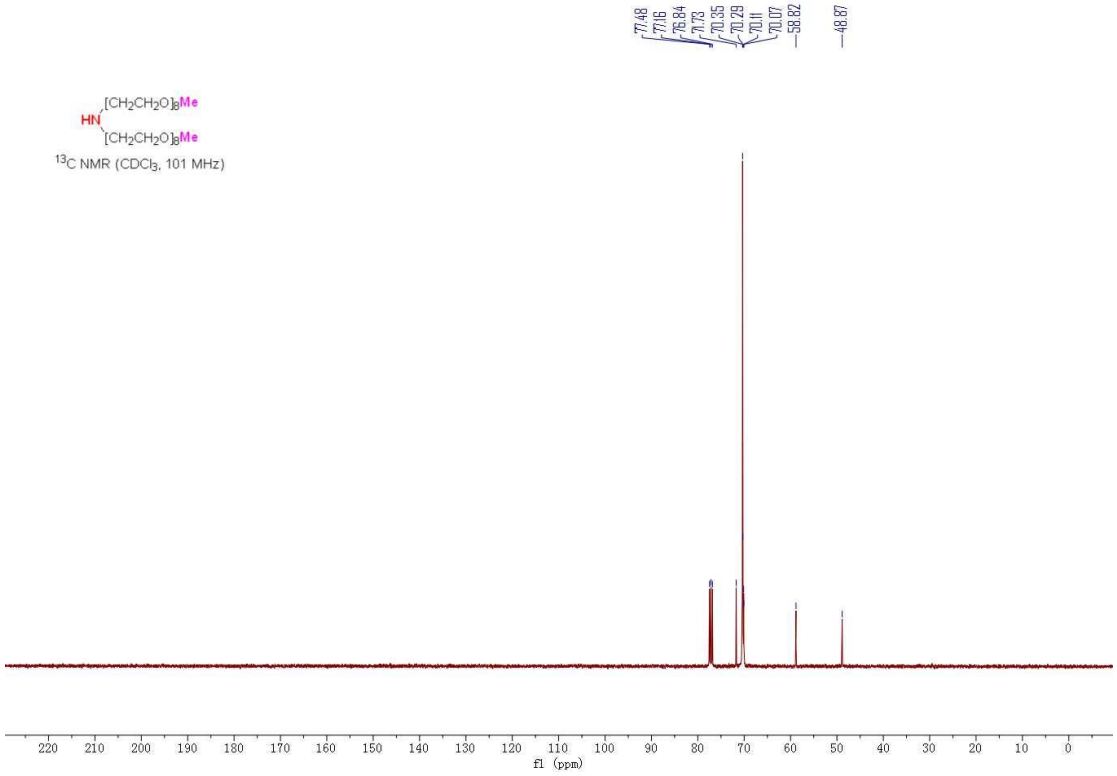
HRMS of compound **16**



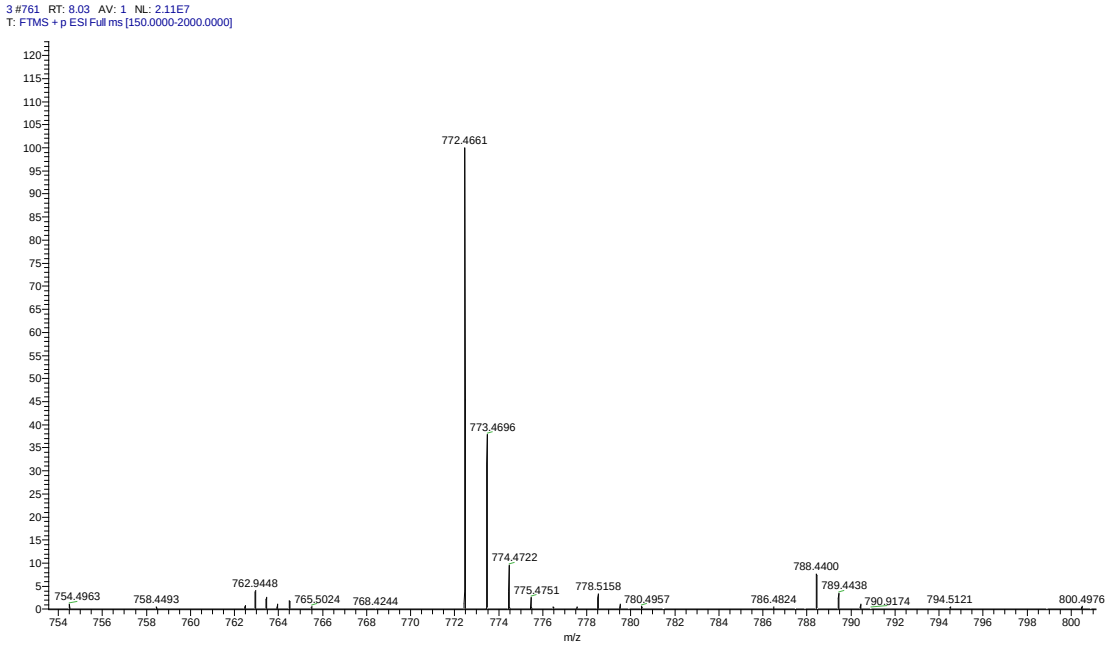
^{1}H NMR of compound **17**



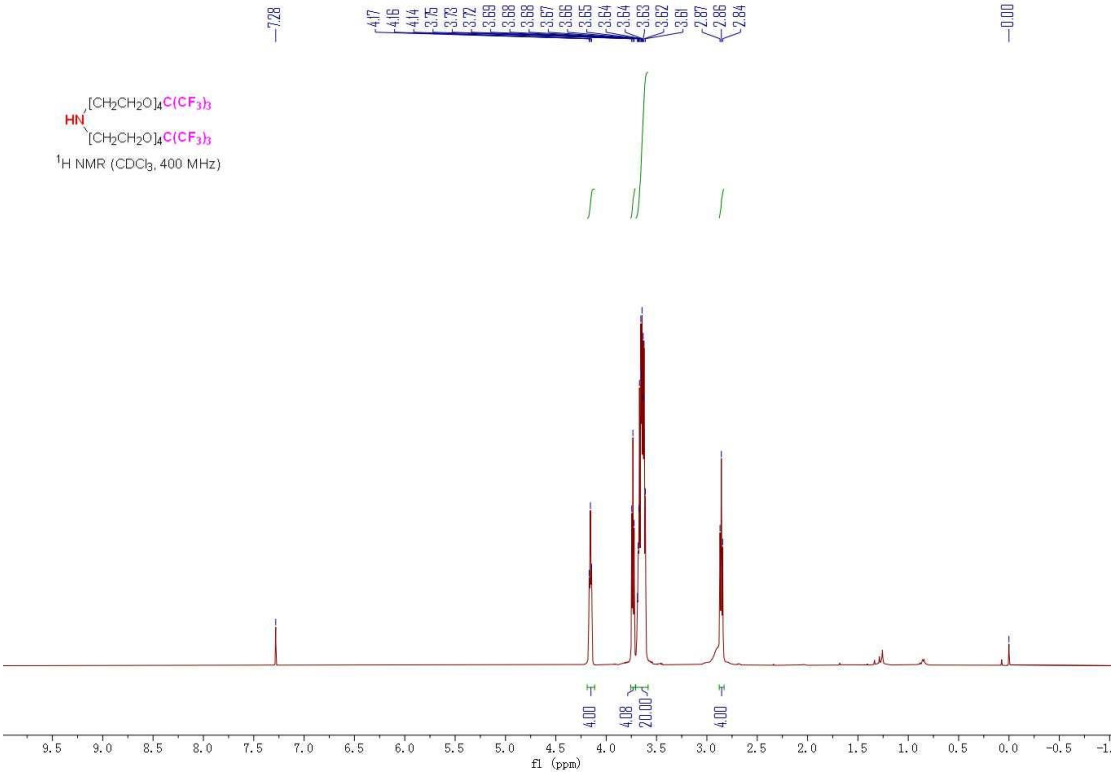
^{13}C NMR of compound **17**



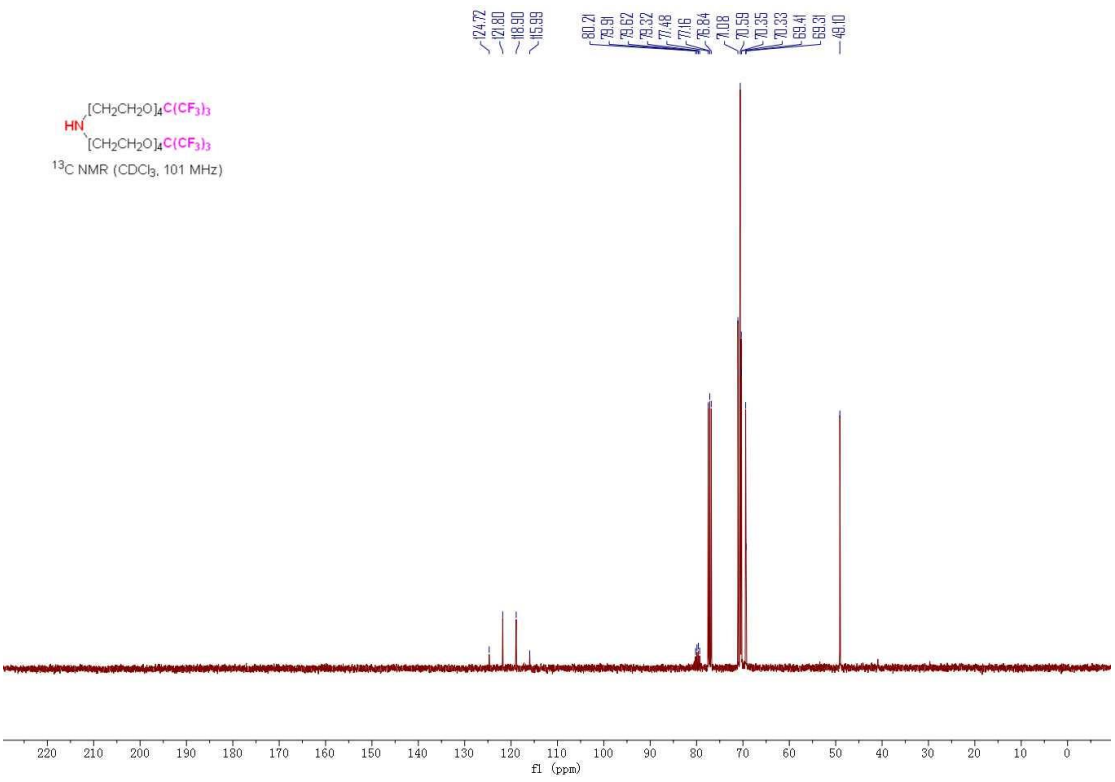
HRMS of compound **17**



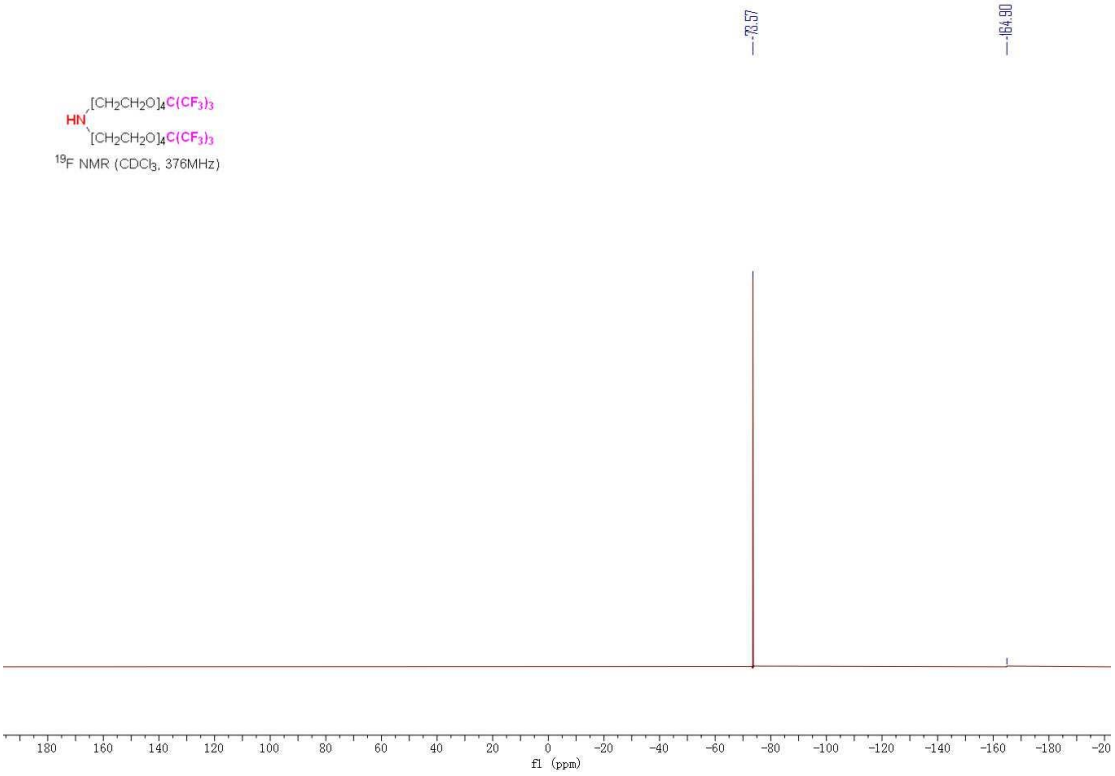
^{1}H NMR of compound **18**



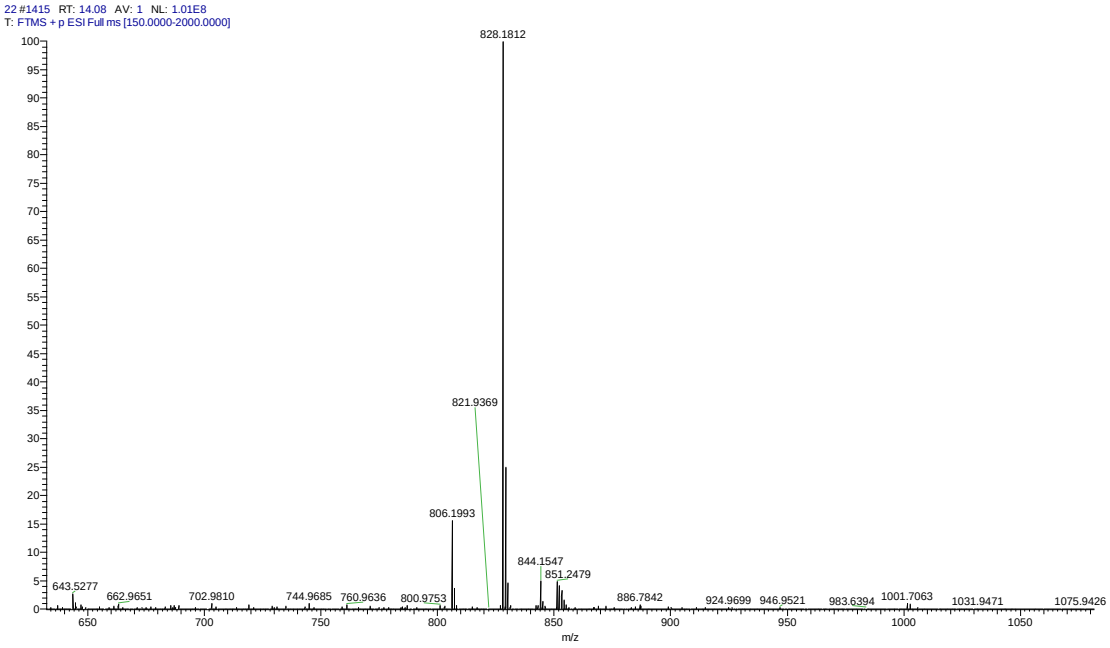
^{13}C NMR of compound **18**



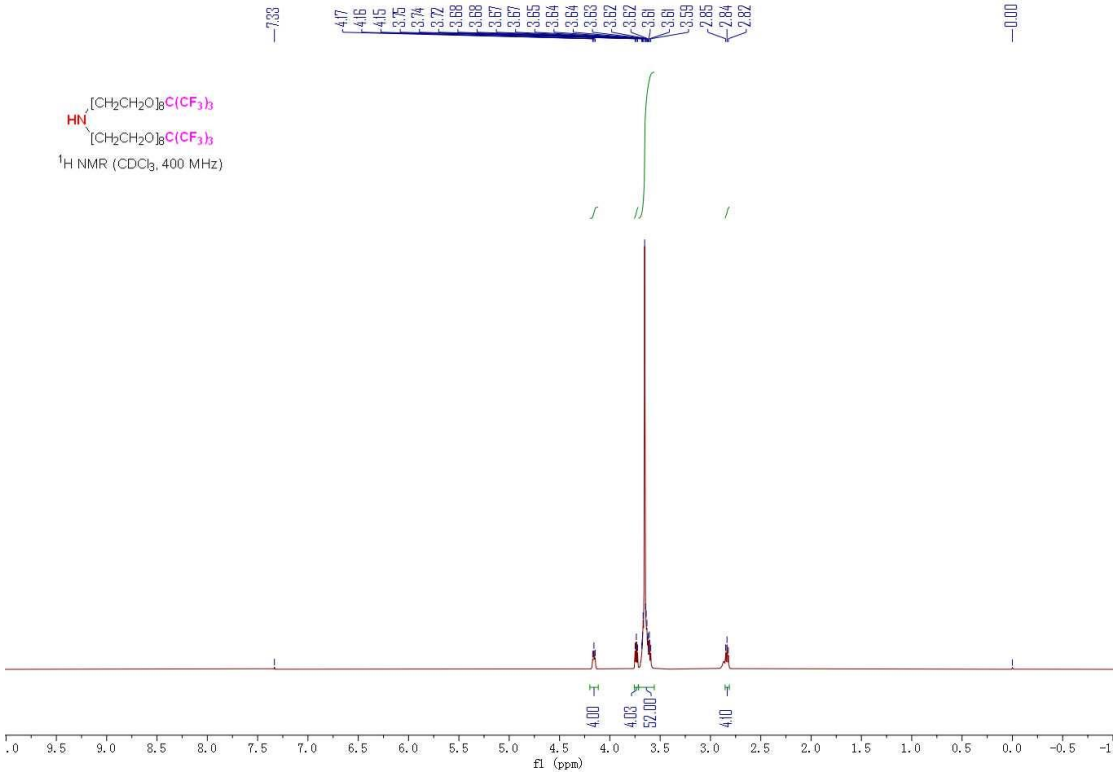
^{19}F NMR of compound **18**



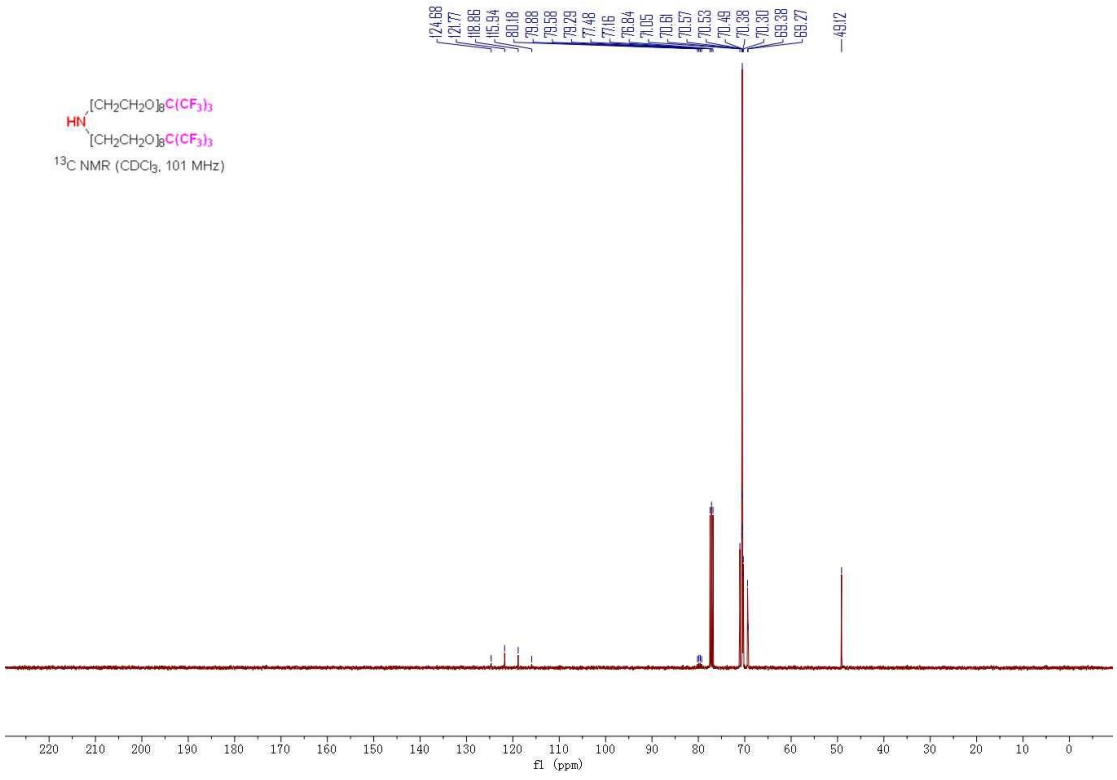
HRMS of compound **18**



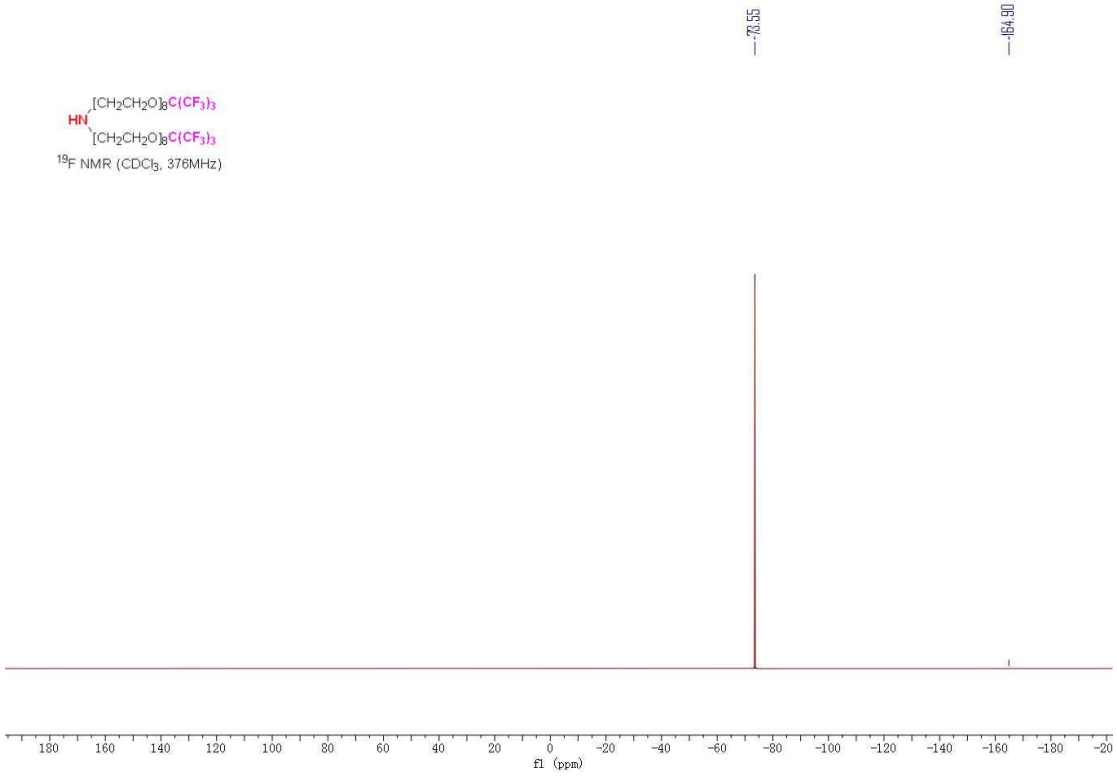
^{1}H NMR of compound **19**



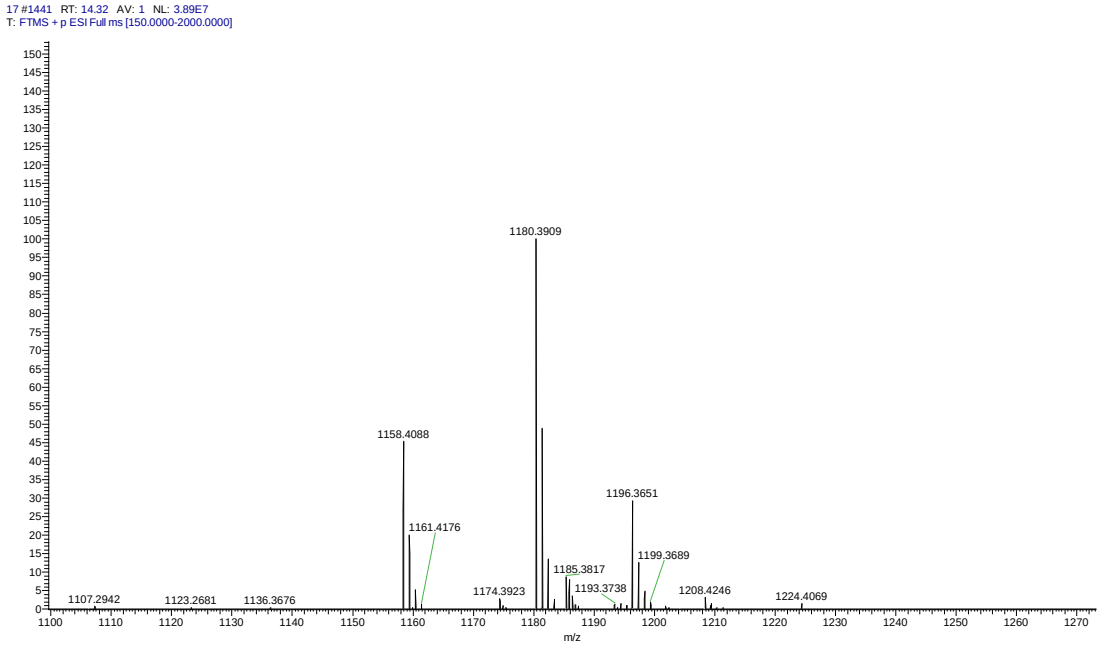
^{13}C NMR of compound **19**



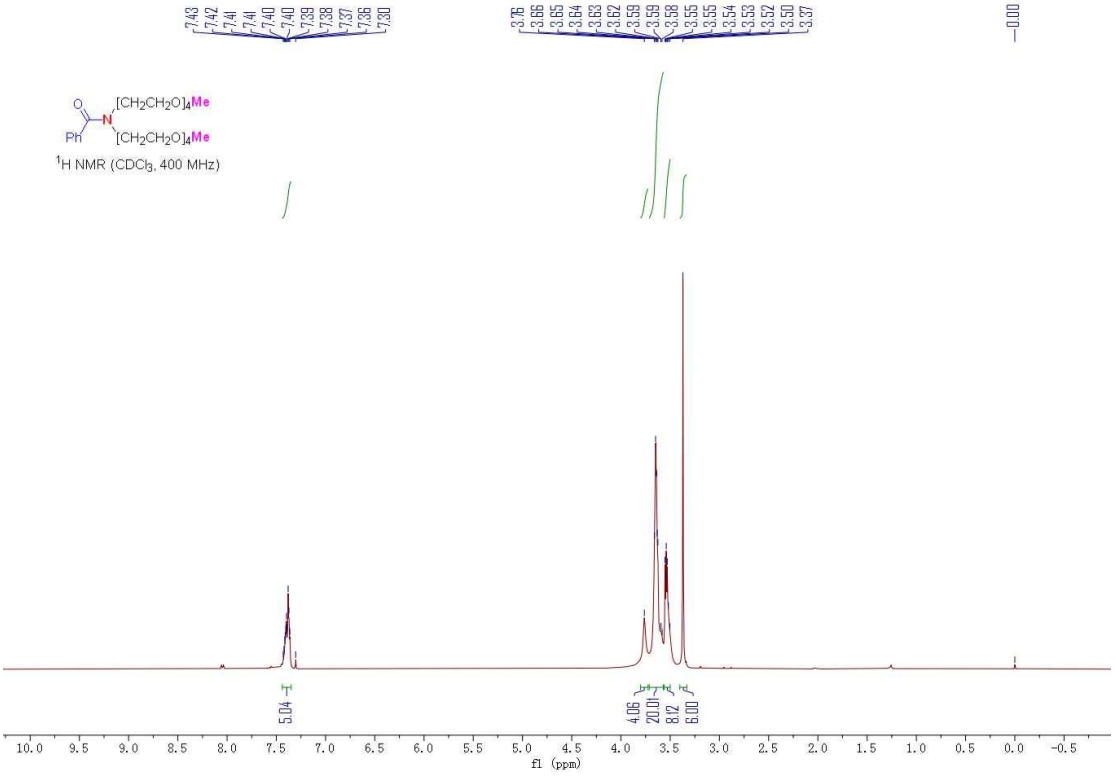
^{19}F NMR of compound **19**



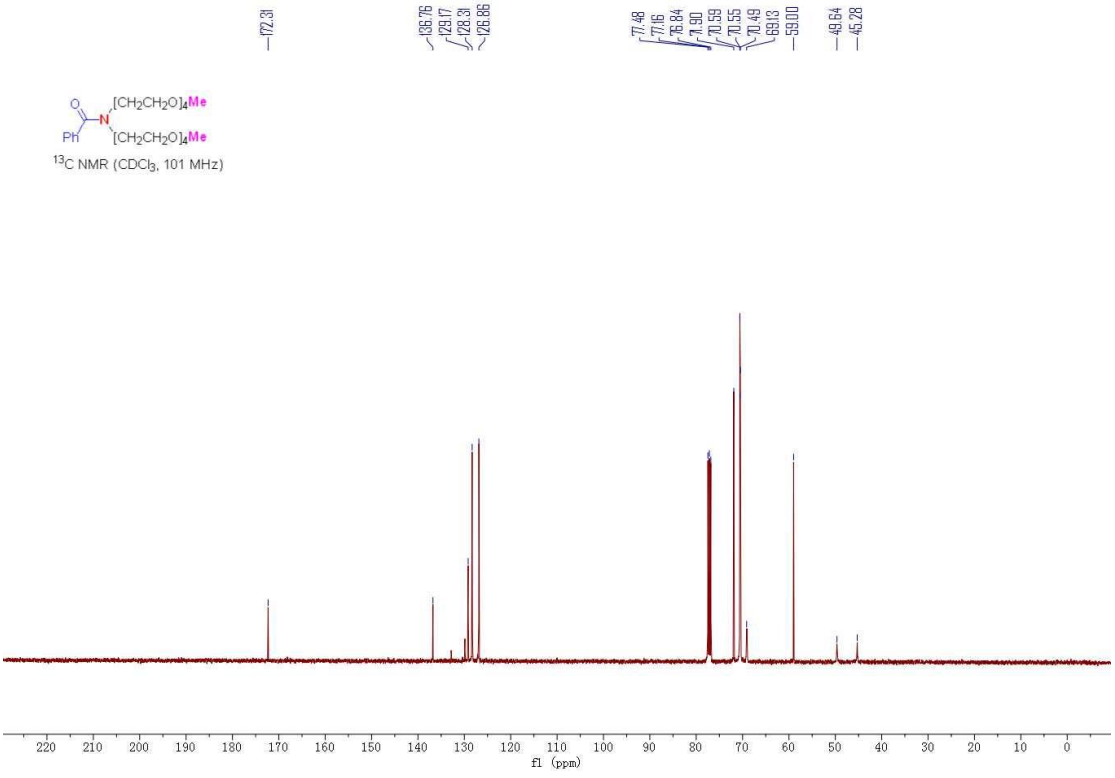
HRMS of compound **19**



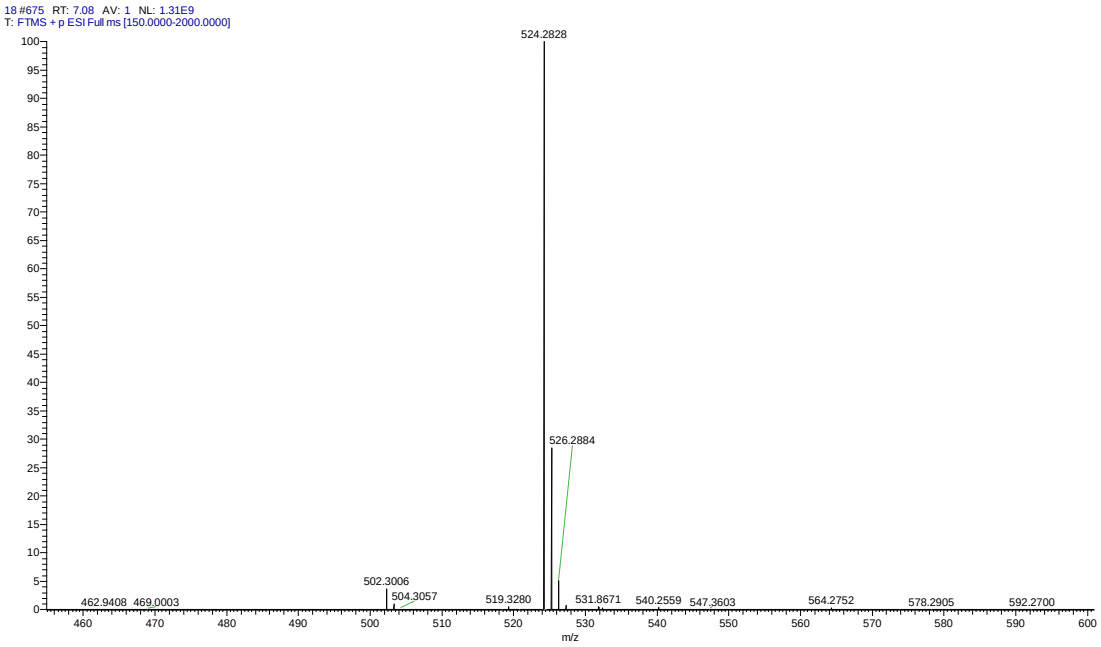
^{1}H NMR of compound **20**



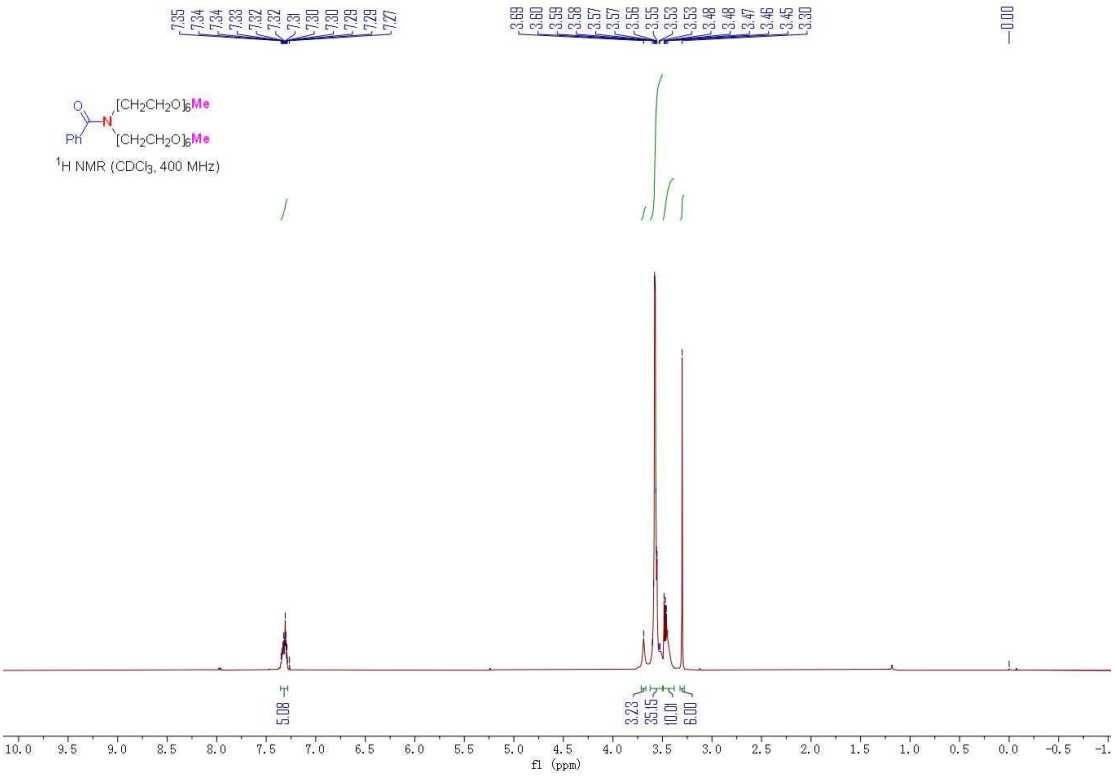
^{13}C NMR of compound **20**



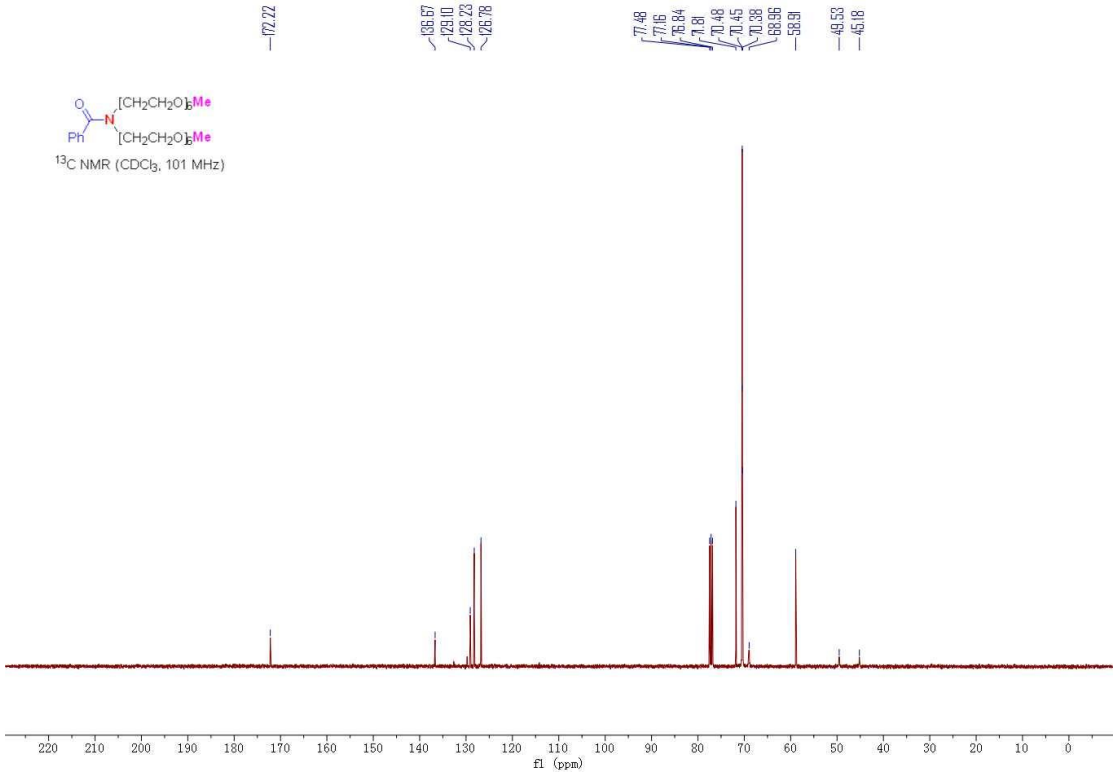
HRMS of compound **20**



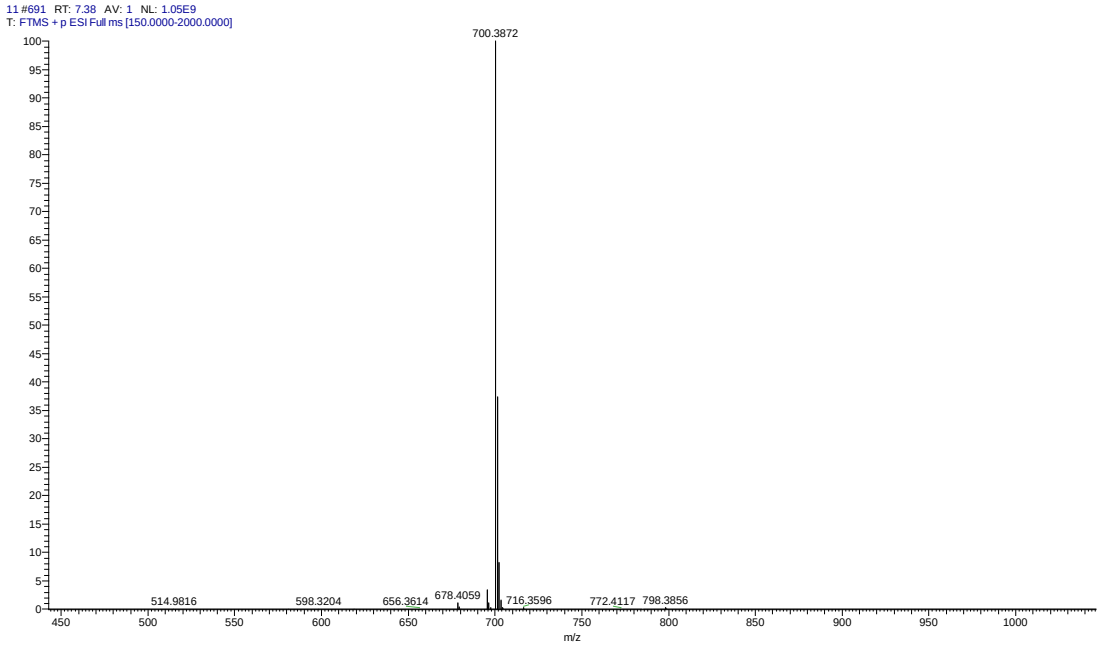
^{1}H NMR of compound **21**



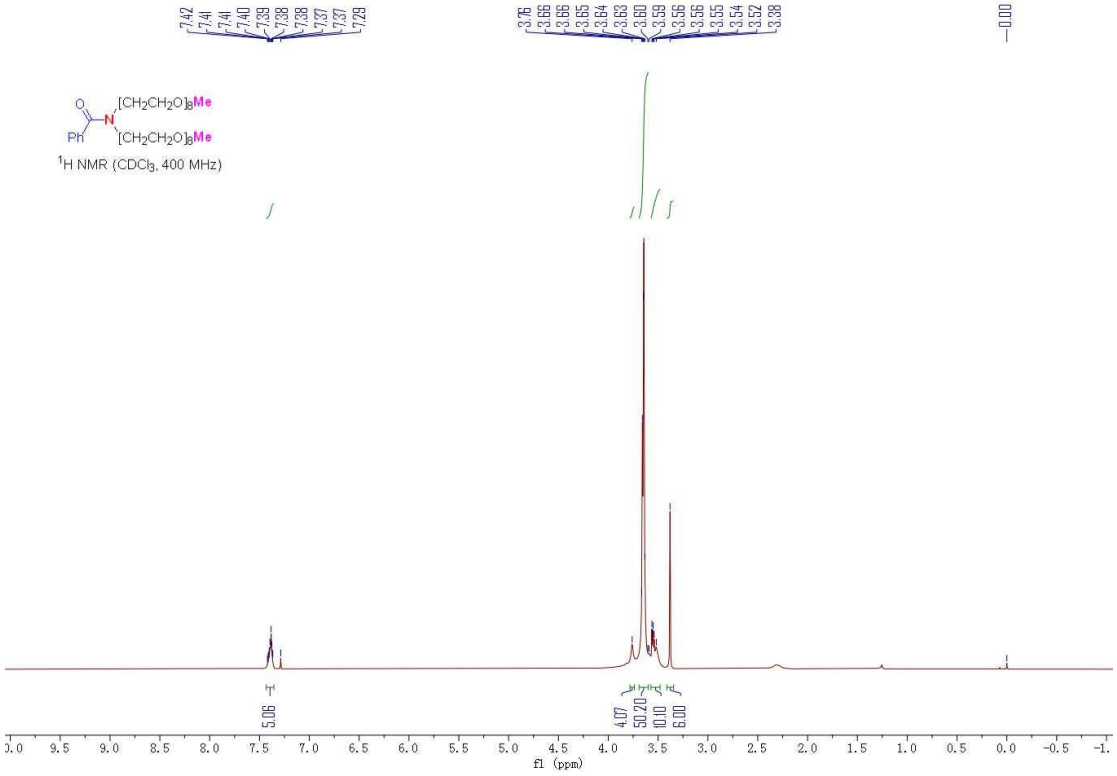
^{13}C NMR of compound **21**



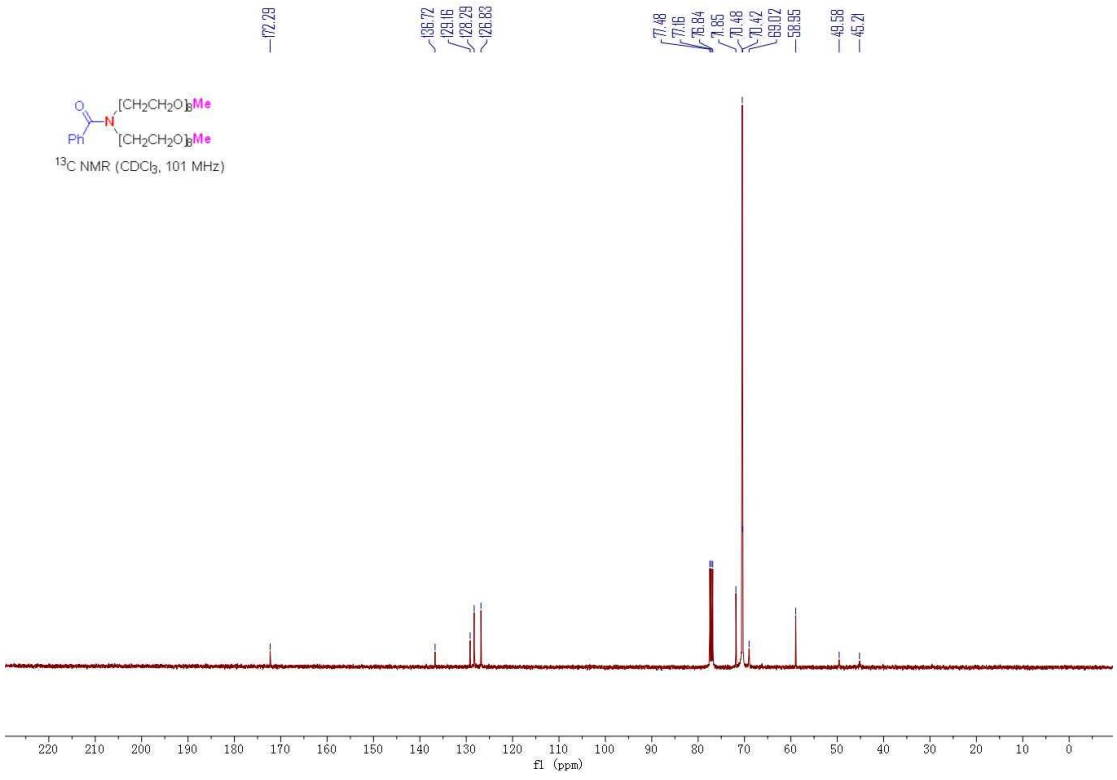
HRMS of compound **21**



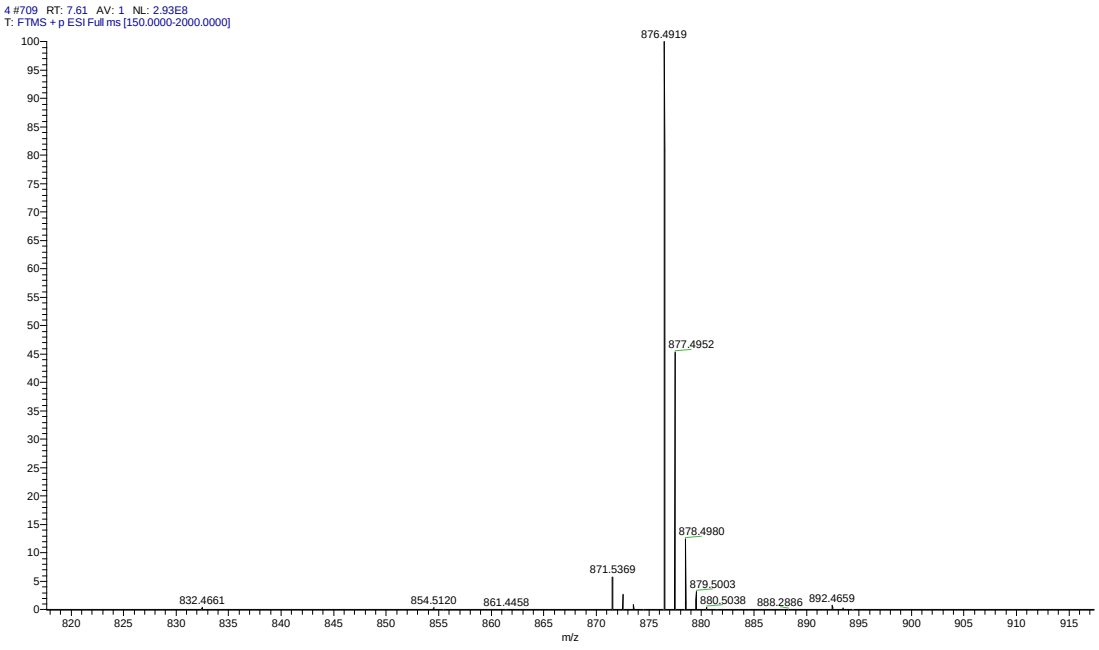
^{1}H NMR of compound **22**



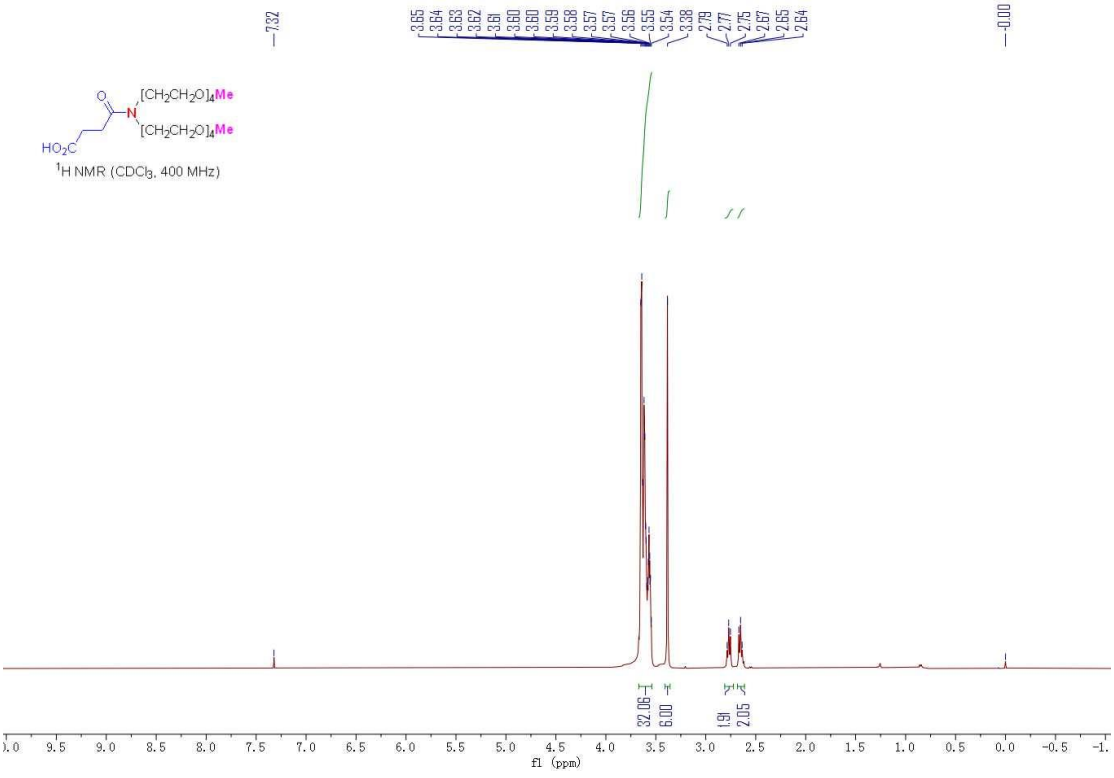
^{13}C NMR of compound **22**



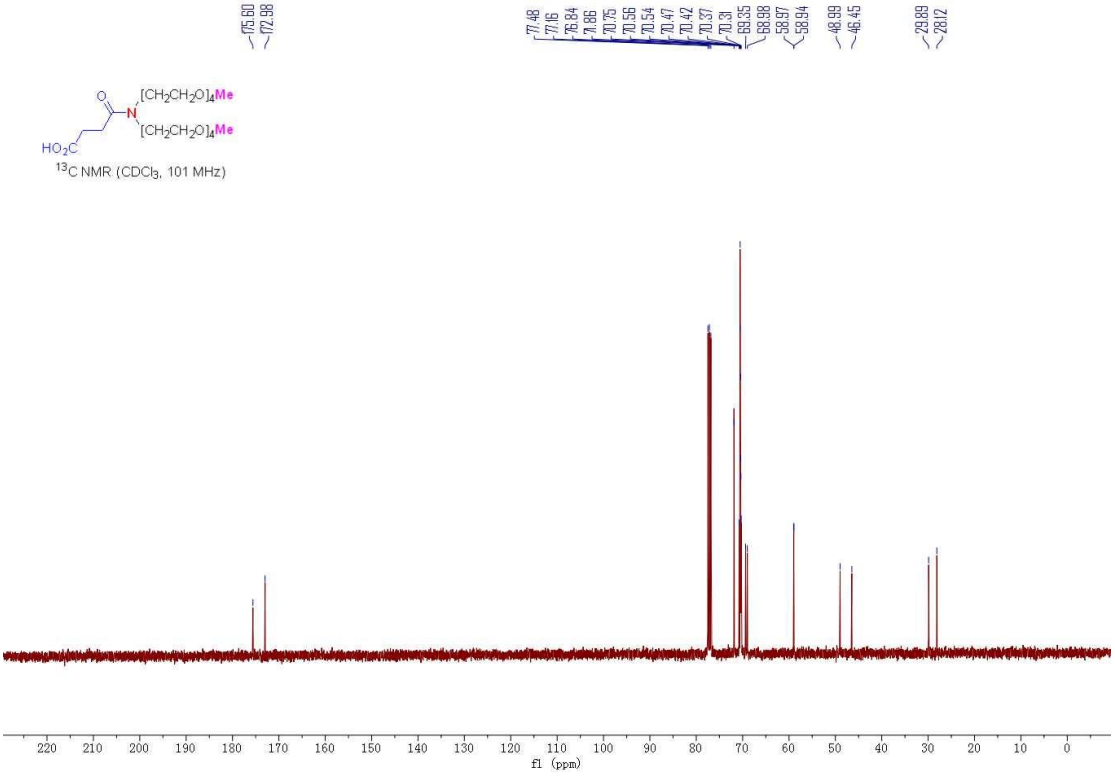
HRMS of compound **22**



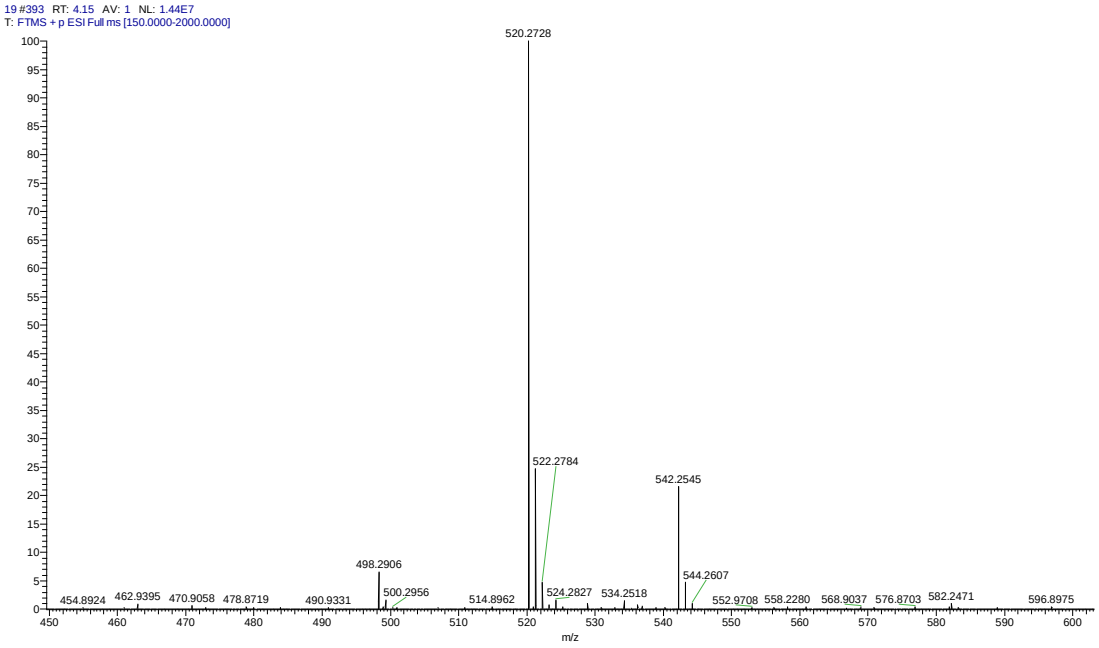
^{1}H NMR of compound **23**



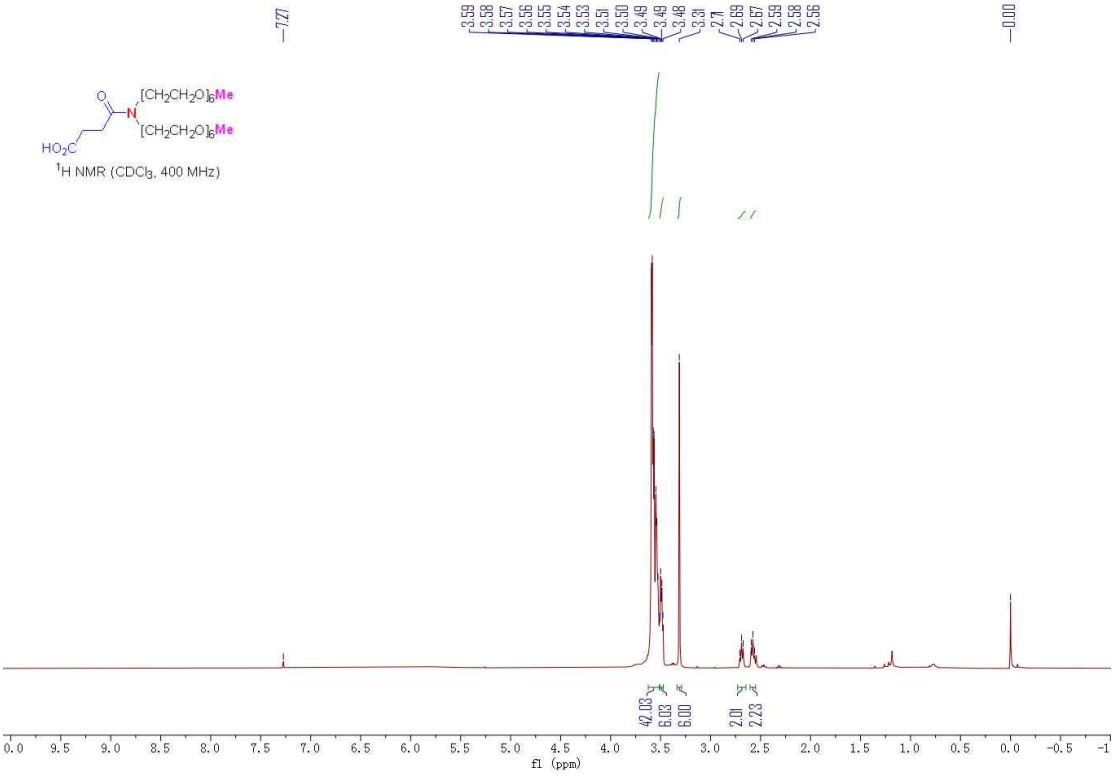
^{13}C NMR of compound **23**



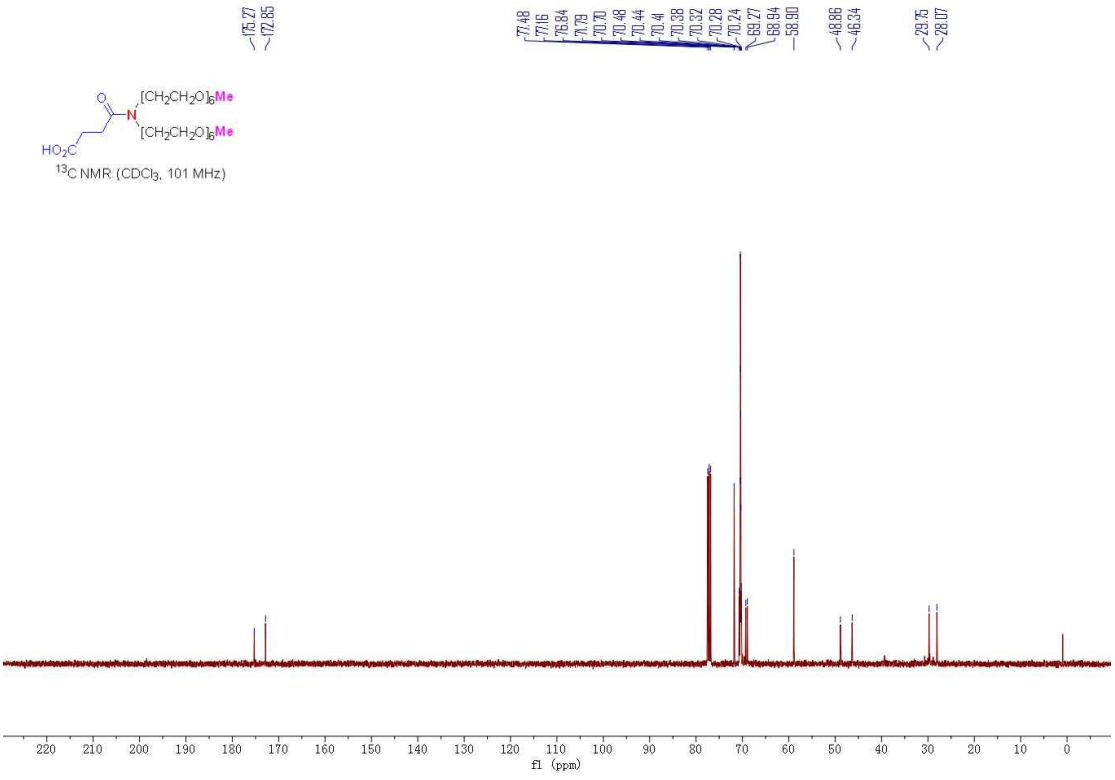
HRMS of compound **23**



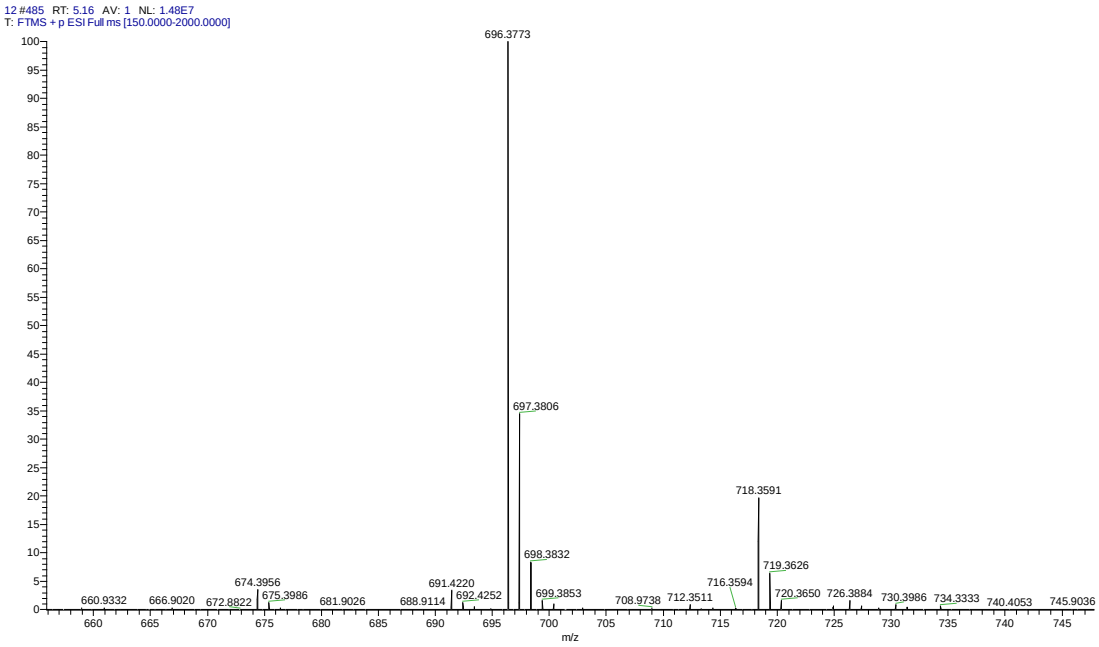
^{1}H NMR of compound **24**



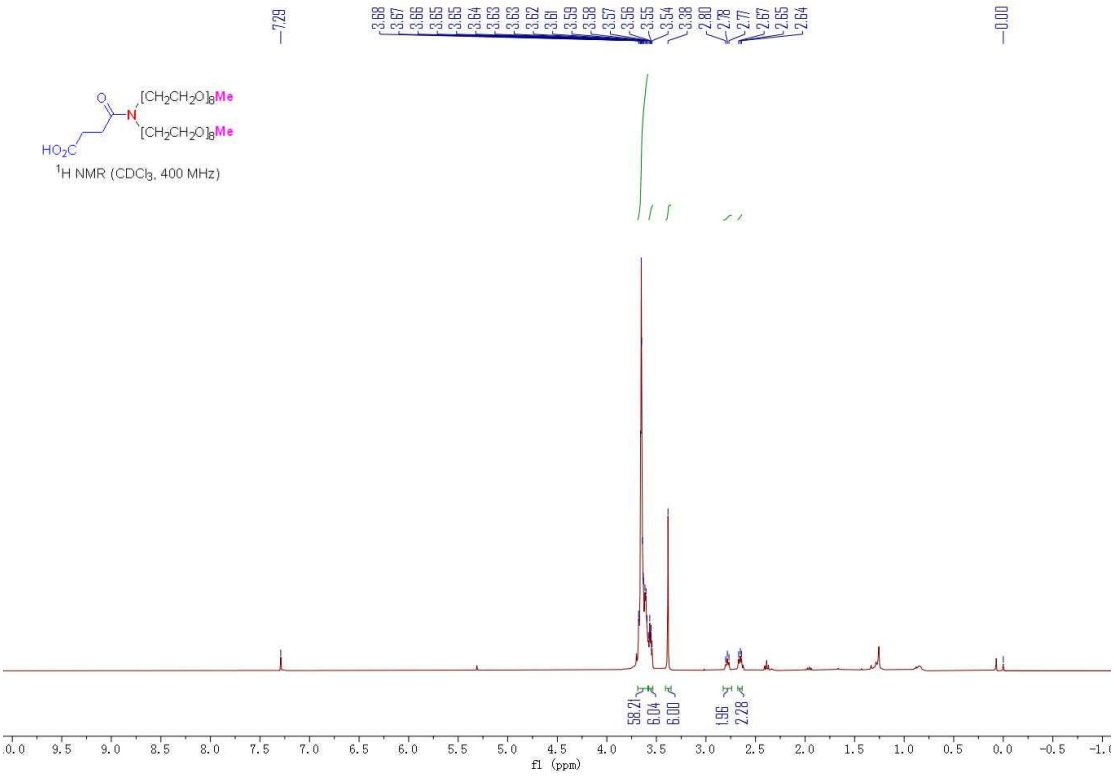
^{13}C NMR of compound **24**



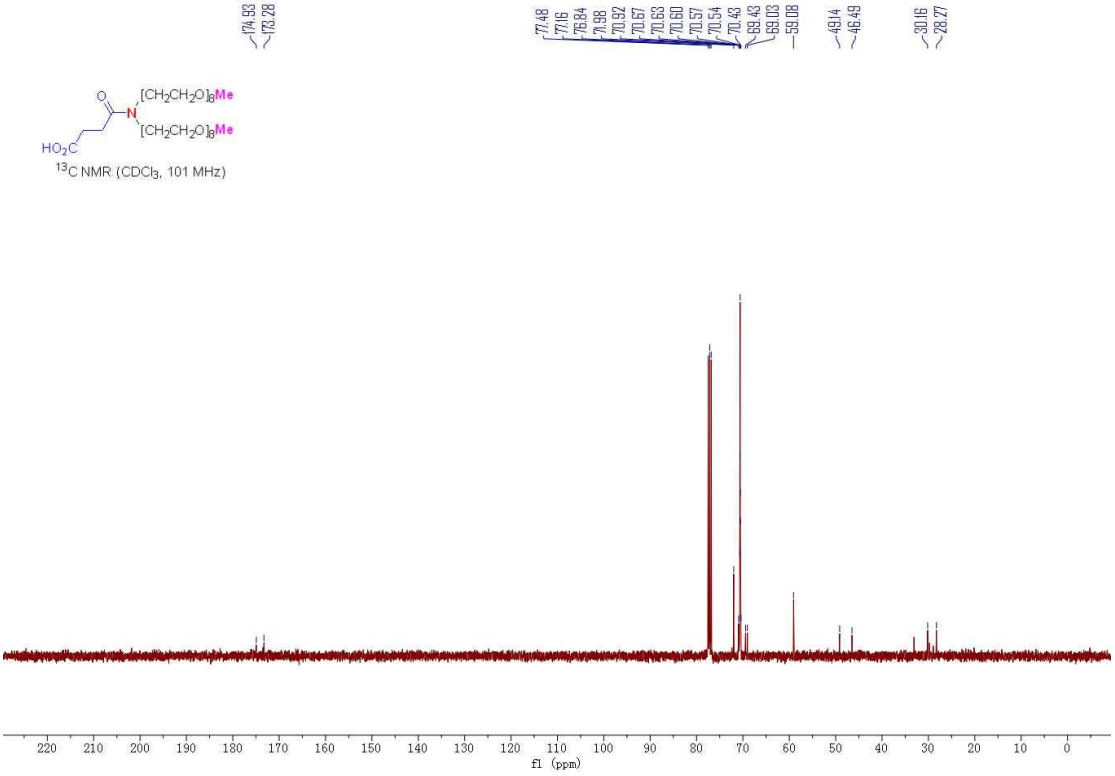
HRMS of compound **24**



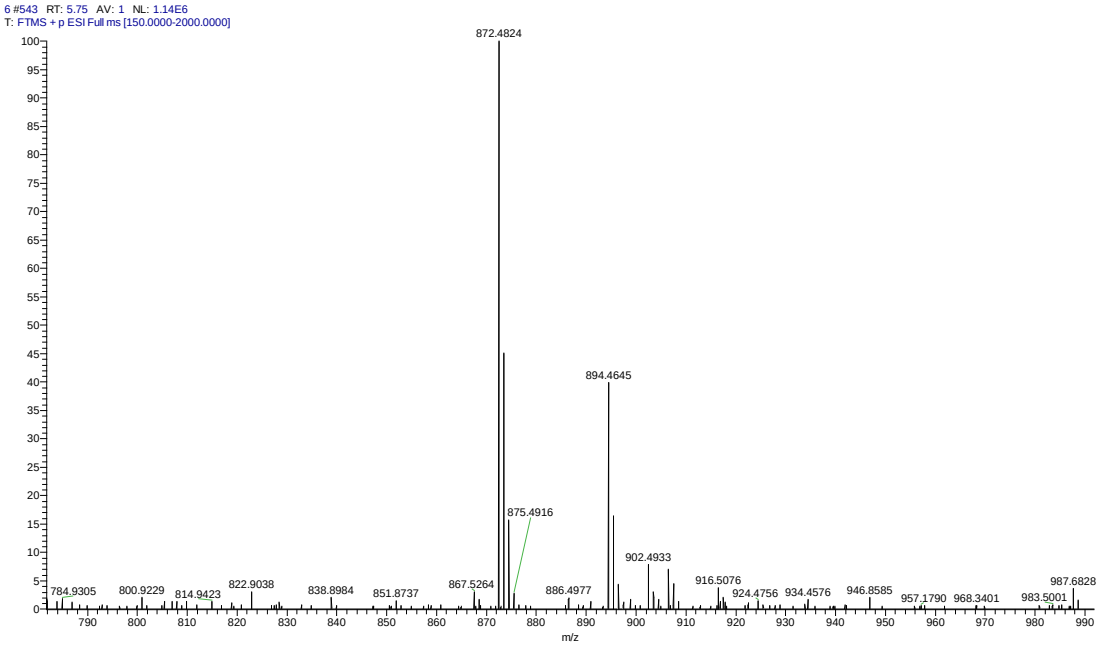
^{1}H NMR of compound **25**



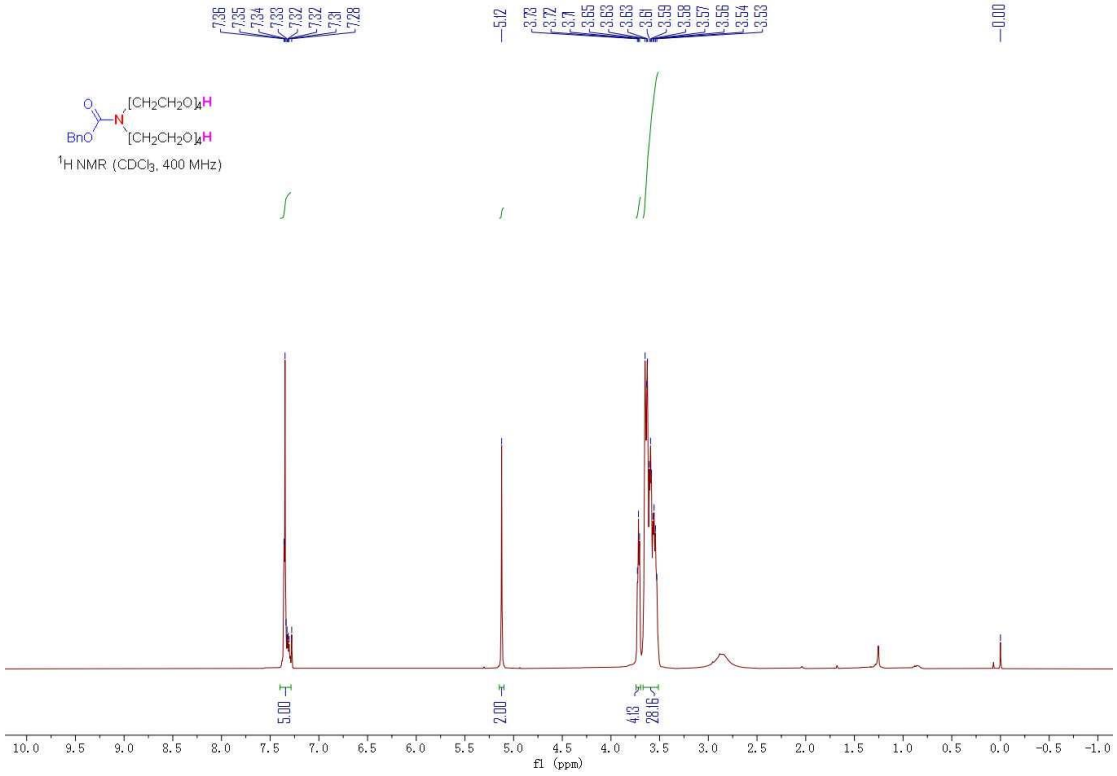
^{13}C NMR of compound **25**



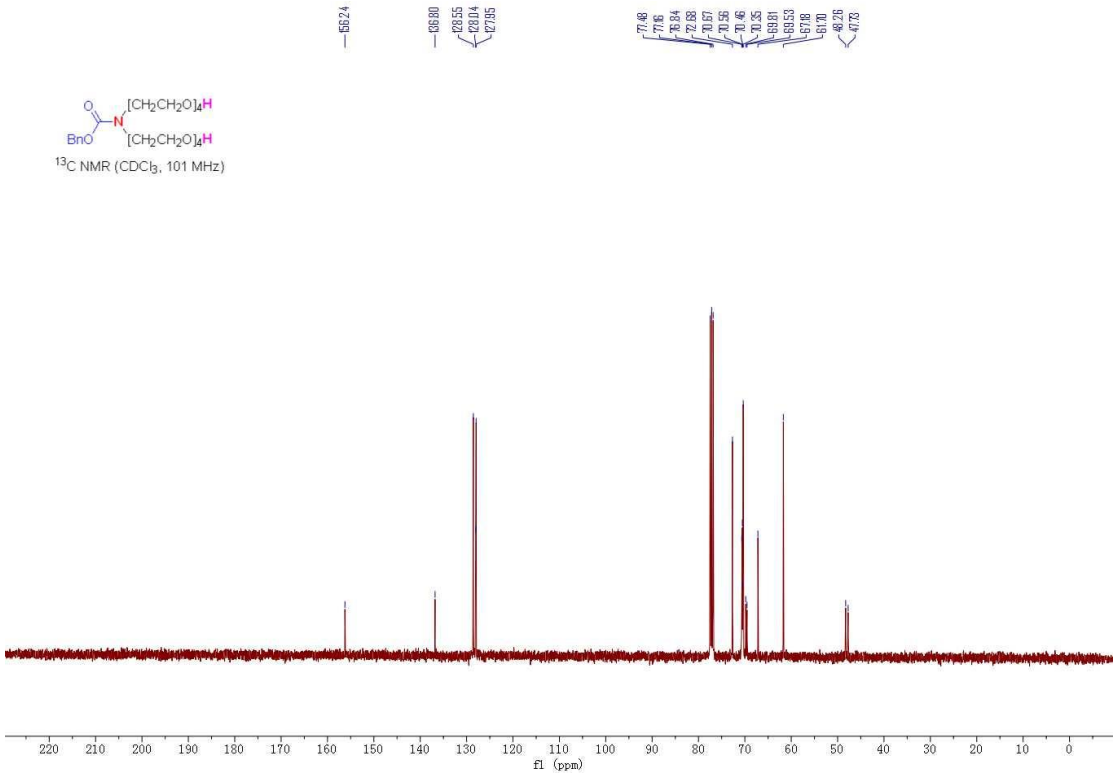
HRMS of compound **25**



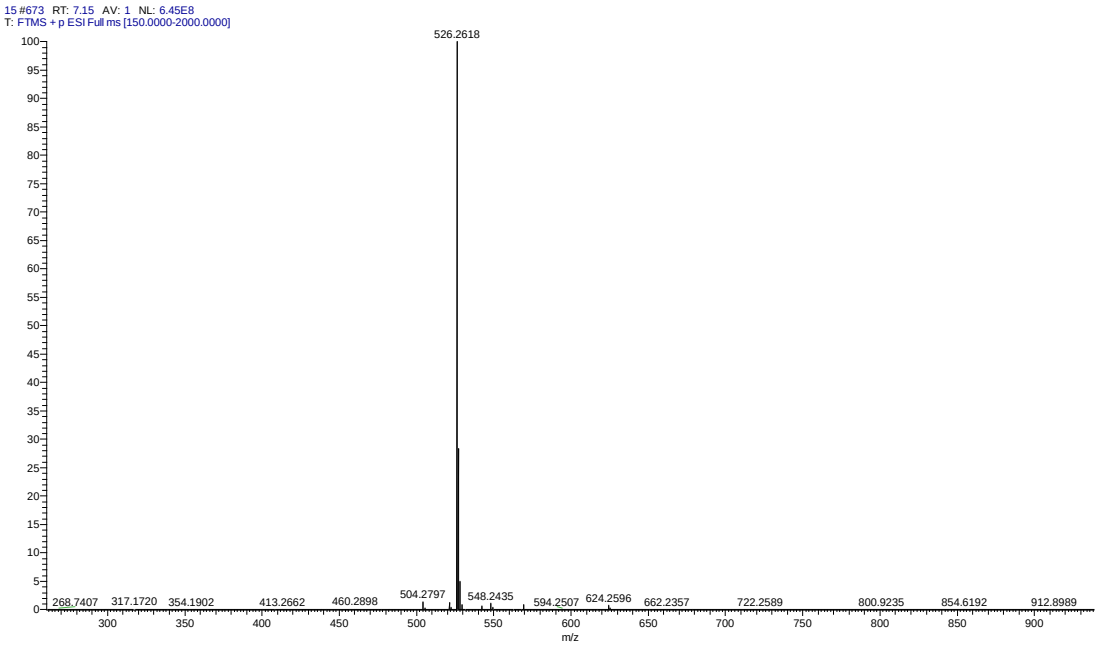
^{1}H NMR of compound **26**



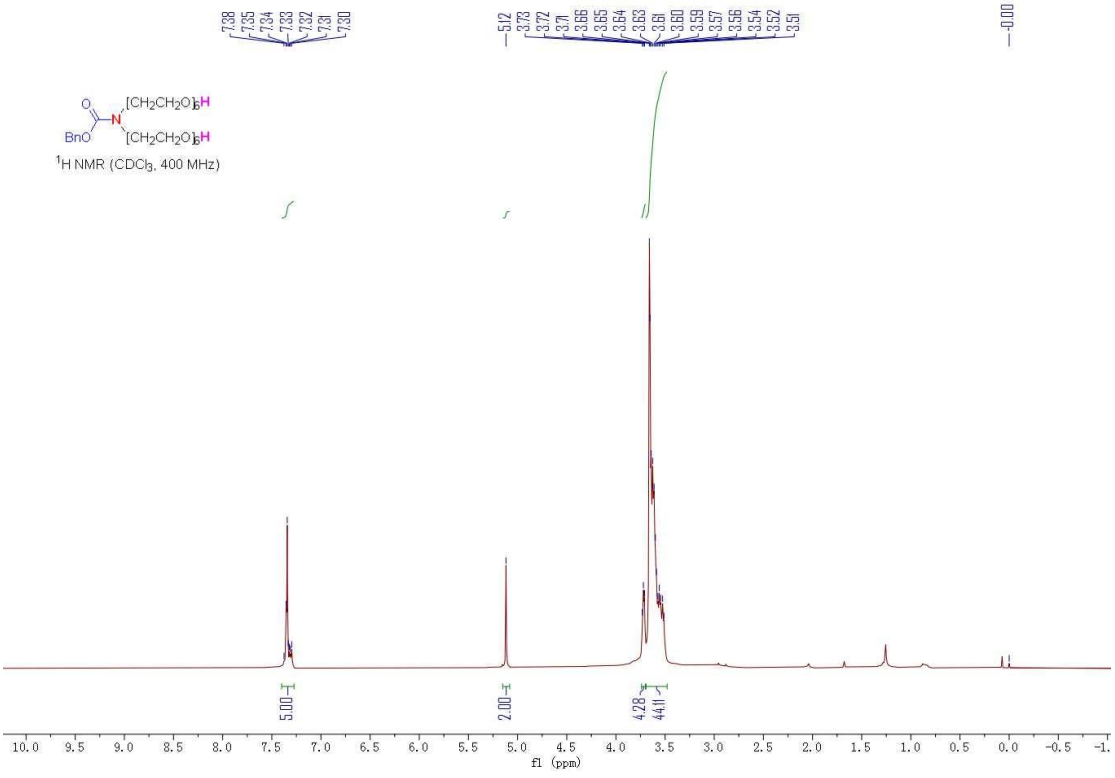
^{13}C NMR of compound **26**



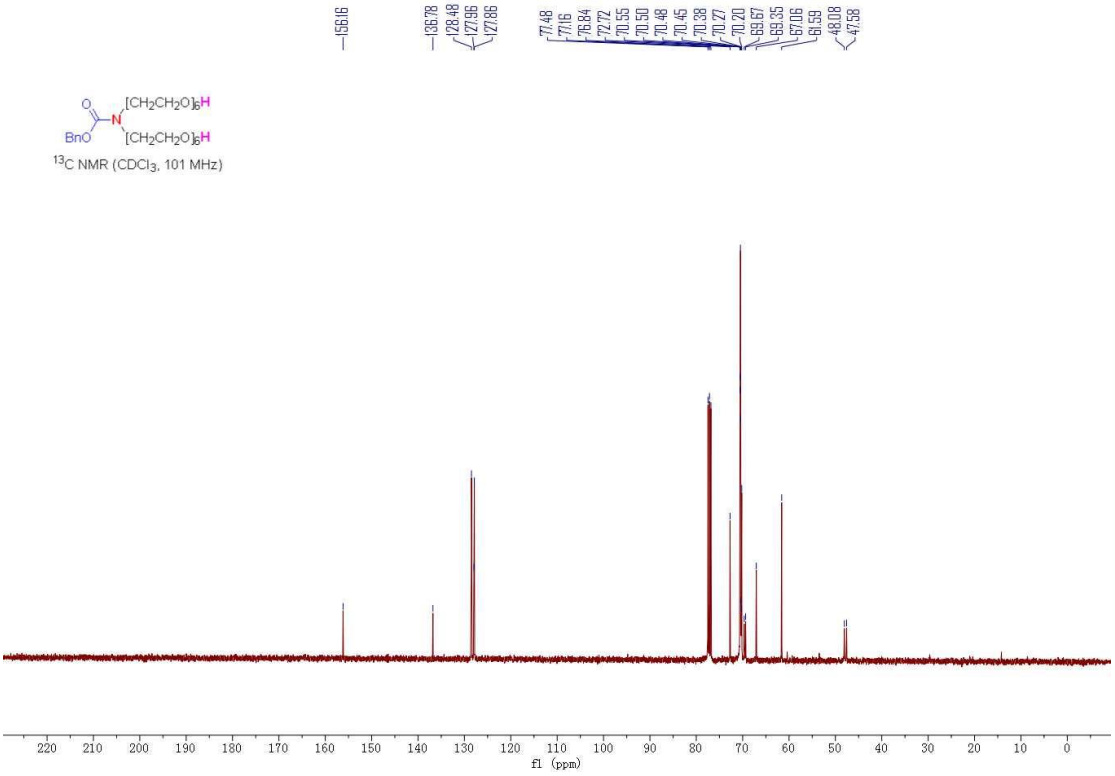
HRMS of compound **26**



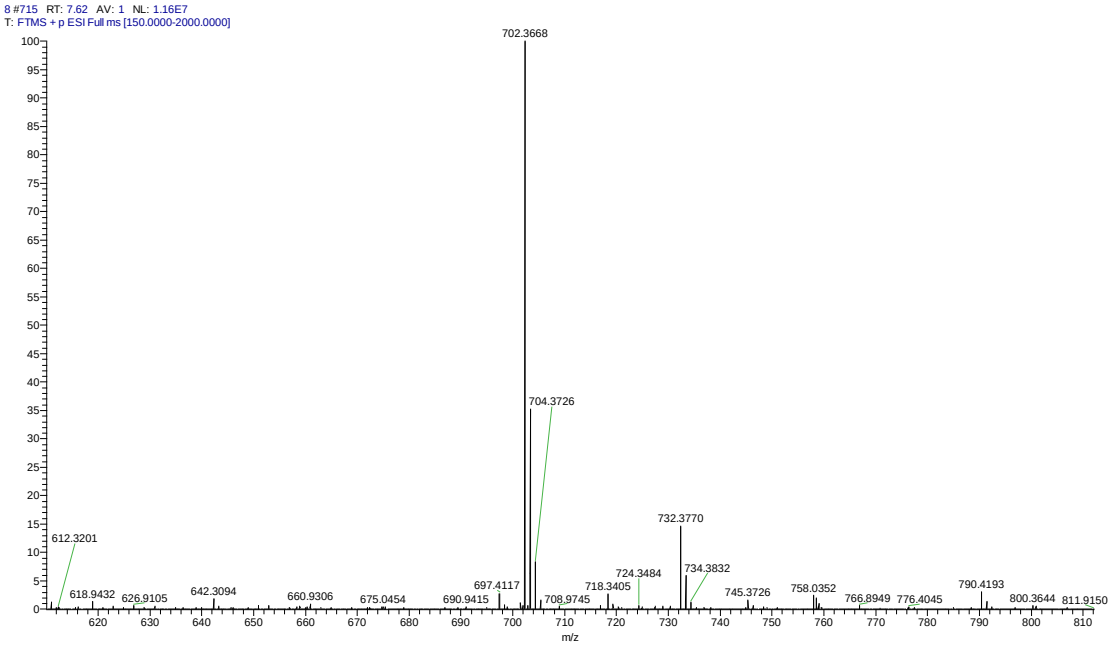
^{1}H NMR of compound **27**



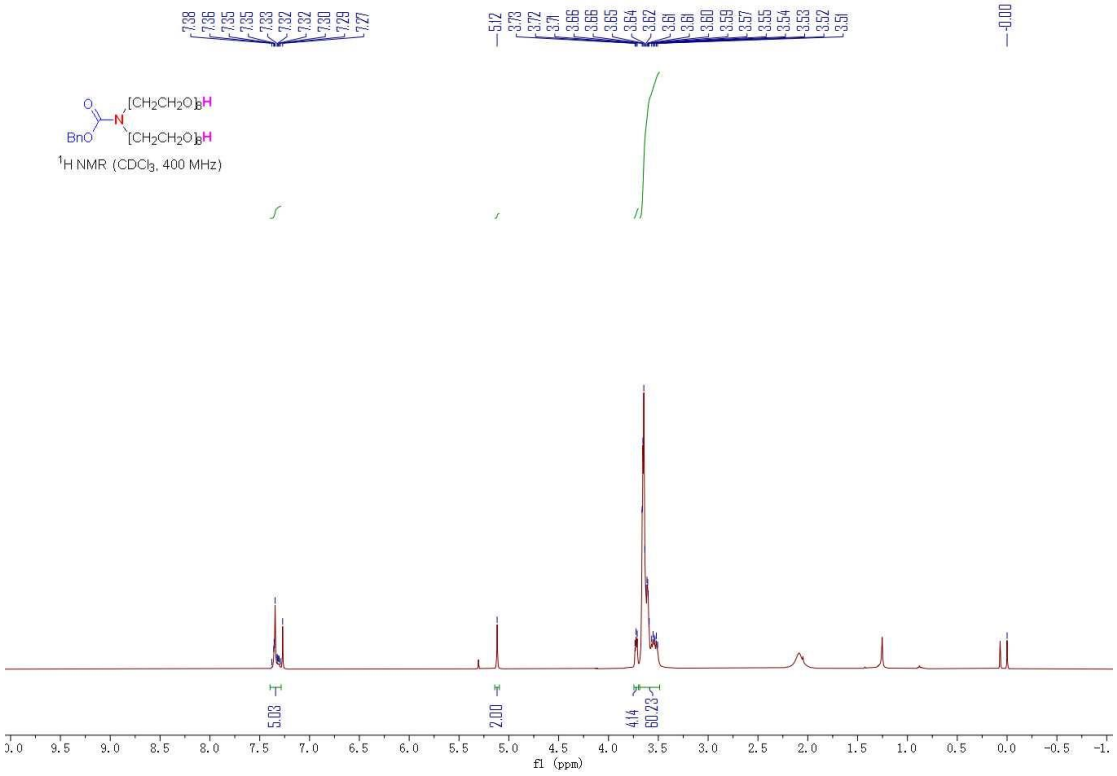
^{13}C NMR of compound **27**



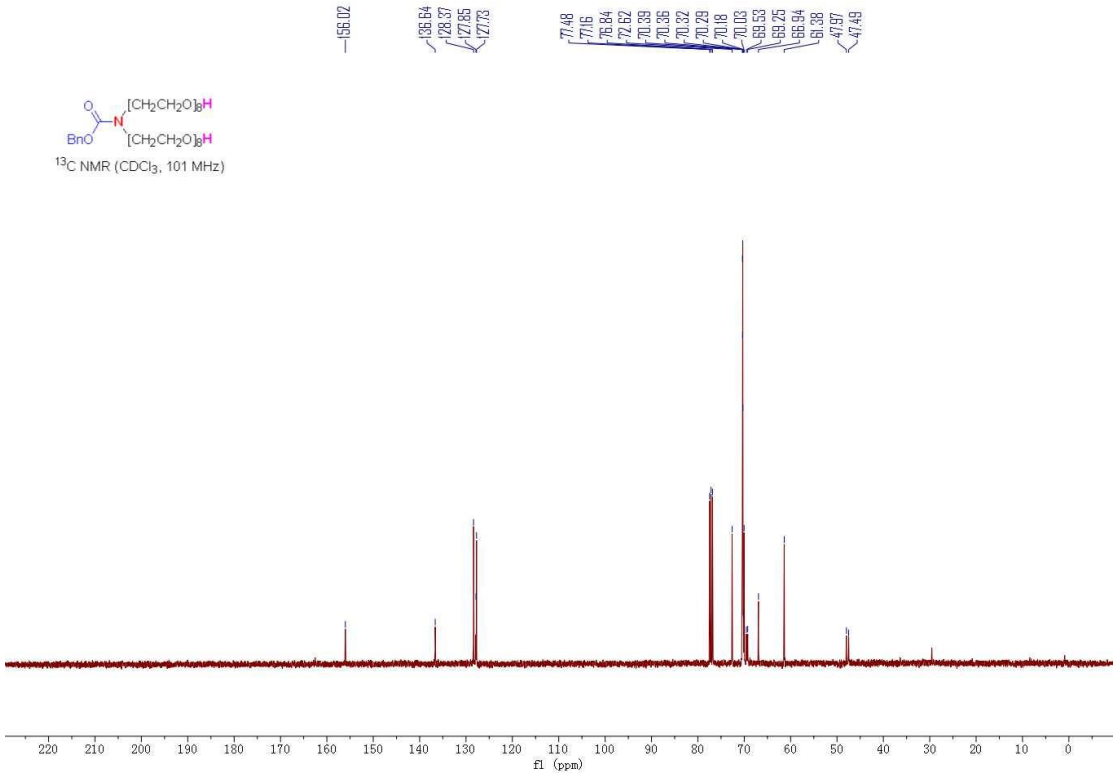
HRMS of compound **27**



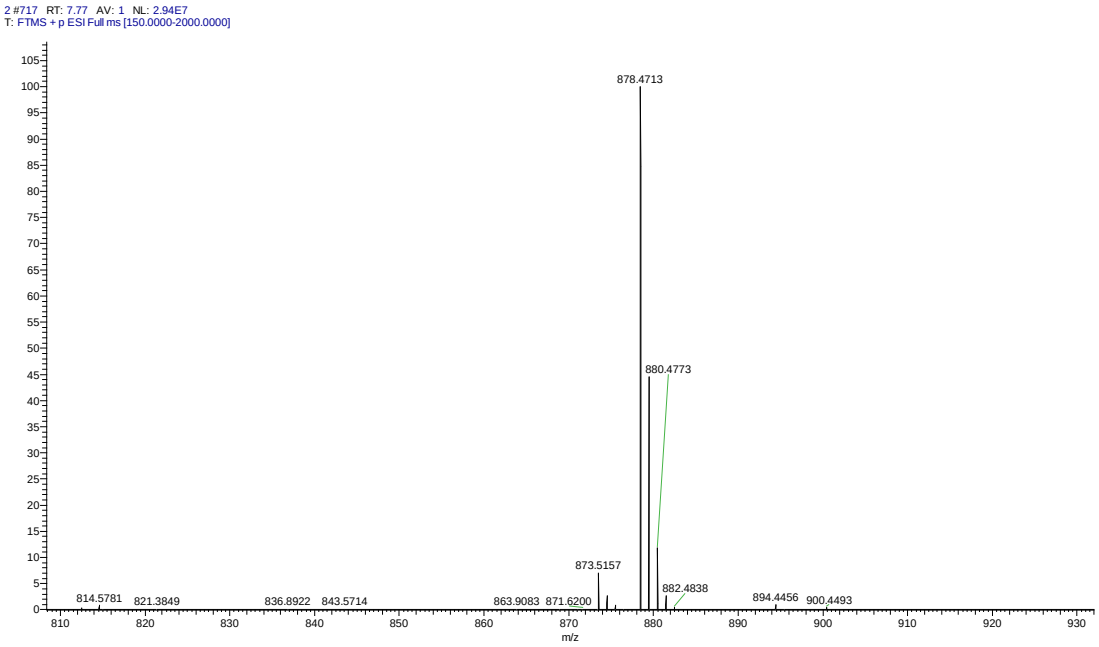
^{1}H NMR of compound **28**



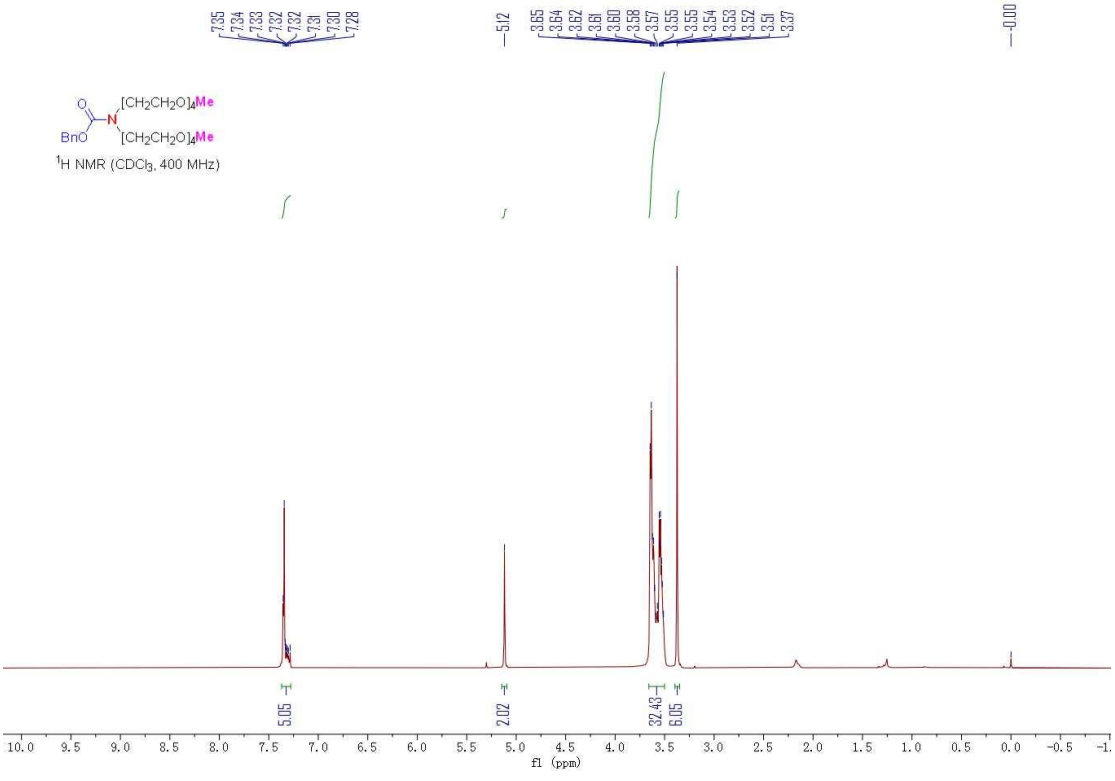
^{13}C NMR of compound **28**



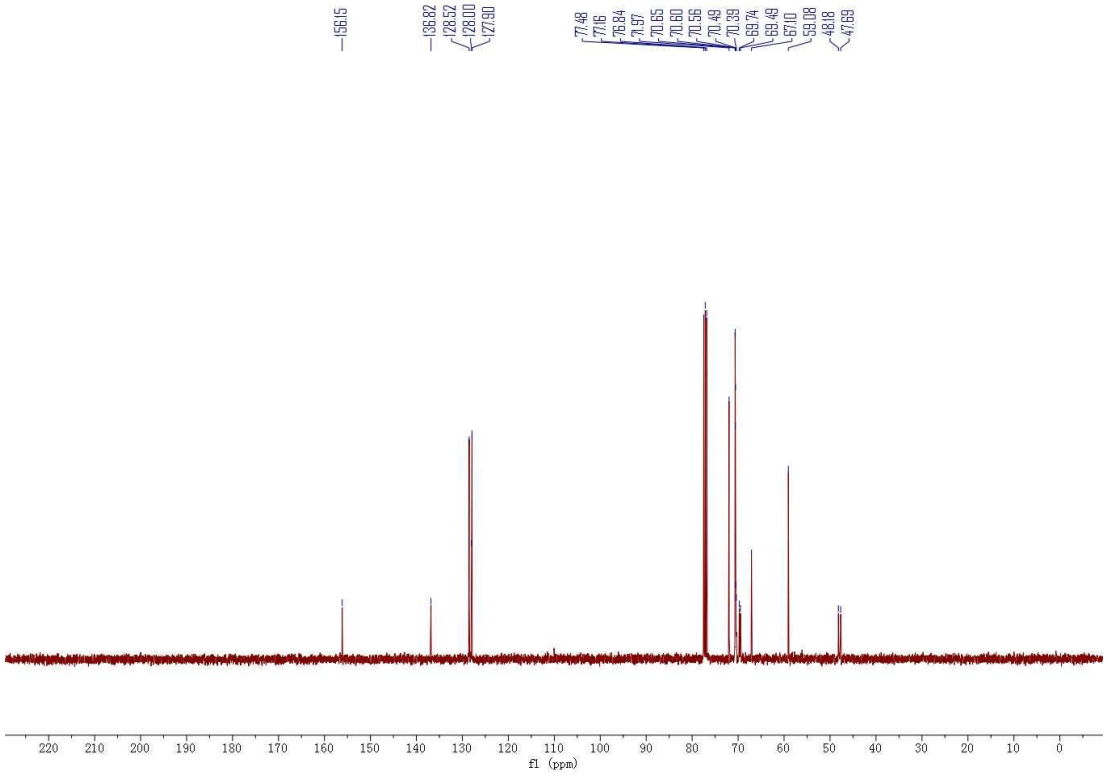
HRMS of compound **28**



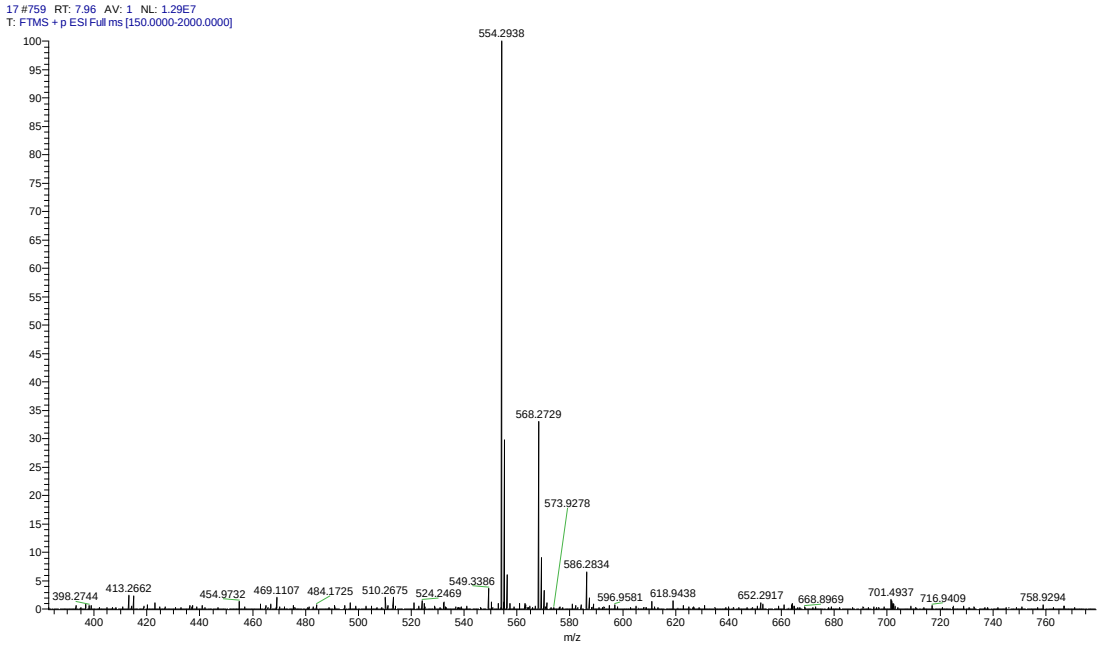
^{1}H NMR of compound **29**



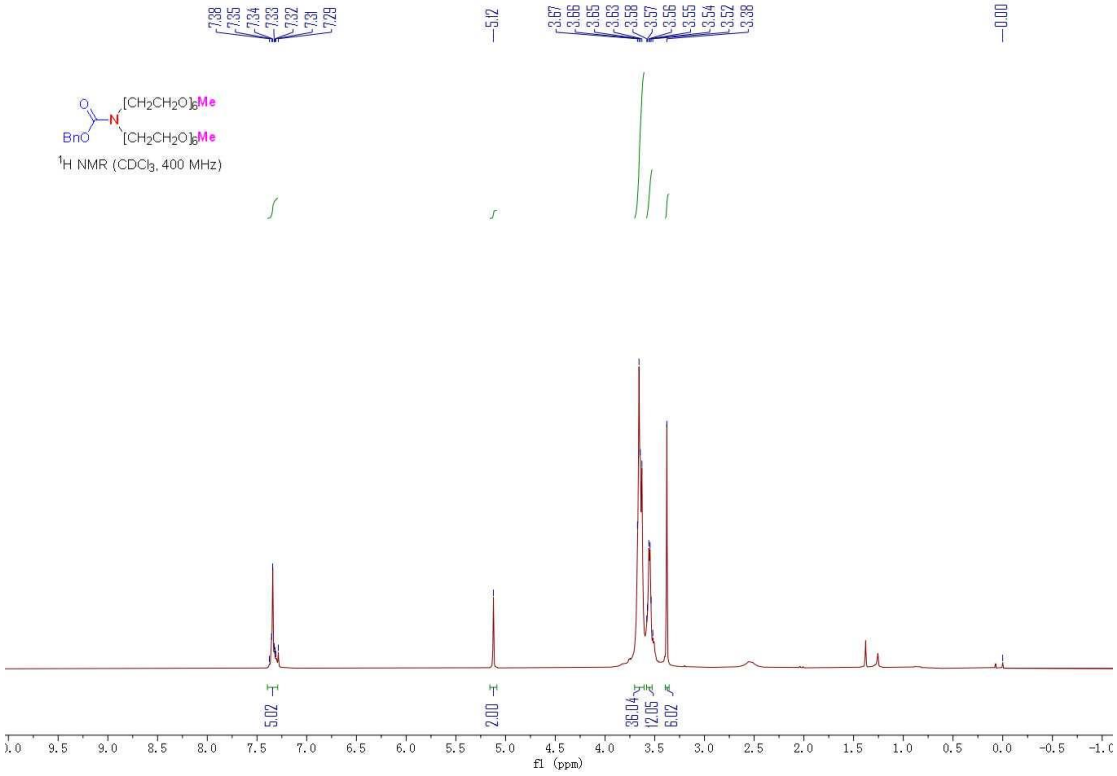
^{13}C NMR of compound **29**



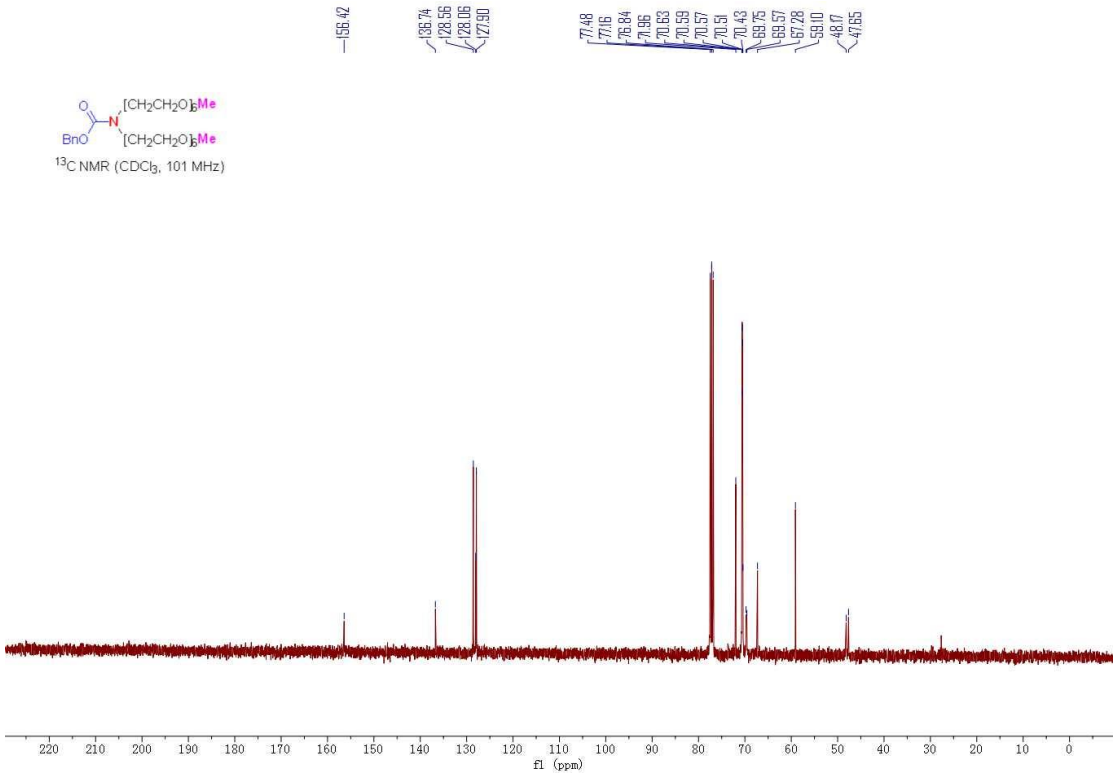
HRMS of compound **29**



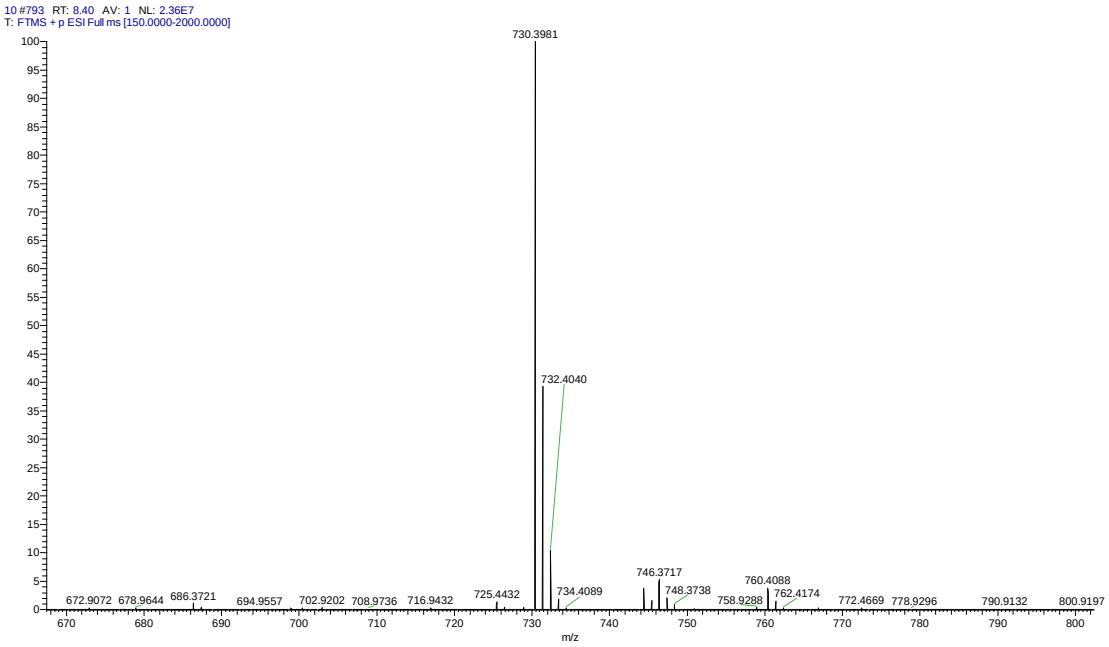
^{1}H NMR of compound **30**



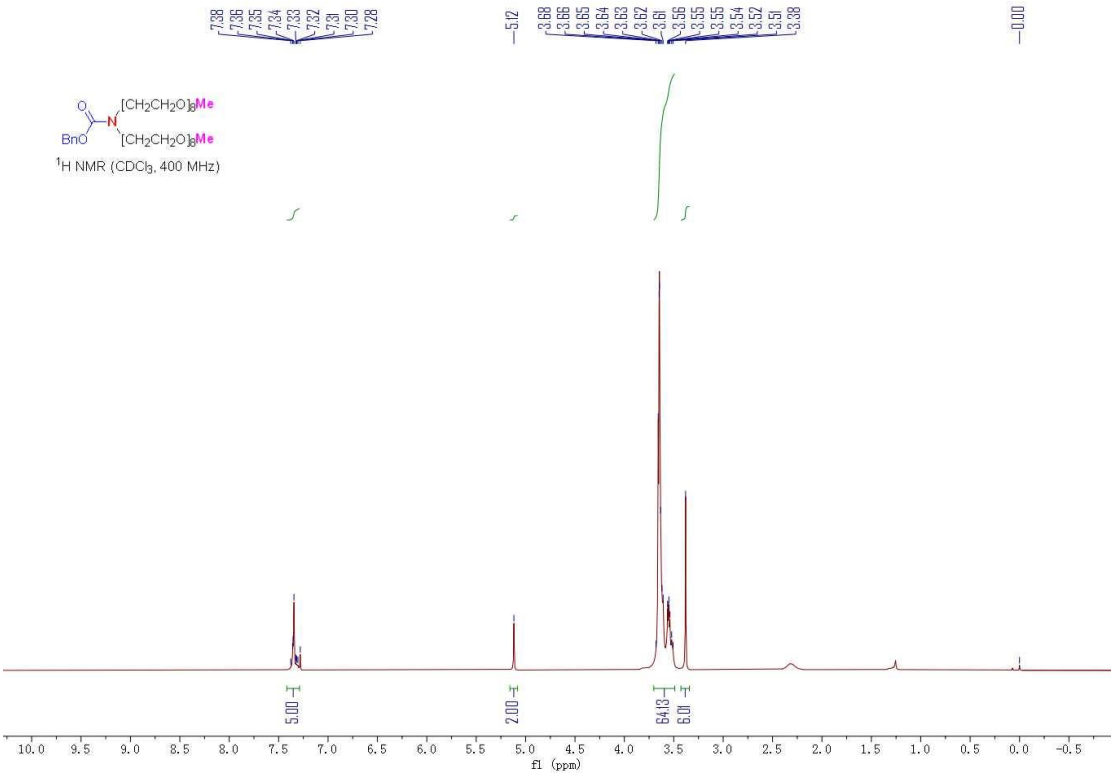
^{13}C NMR of compound **30**



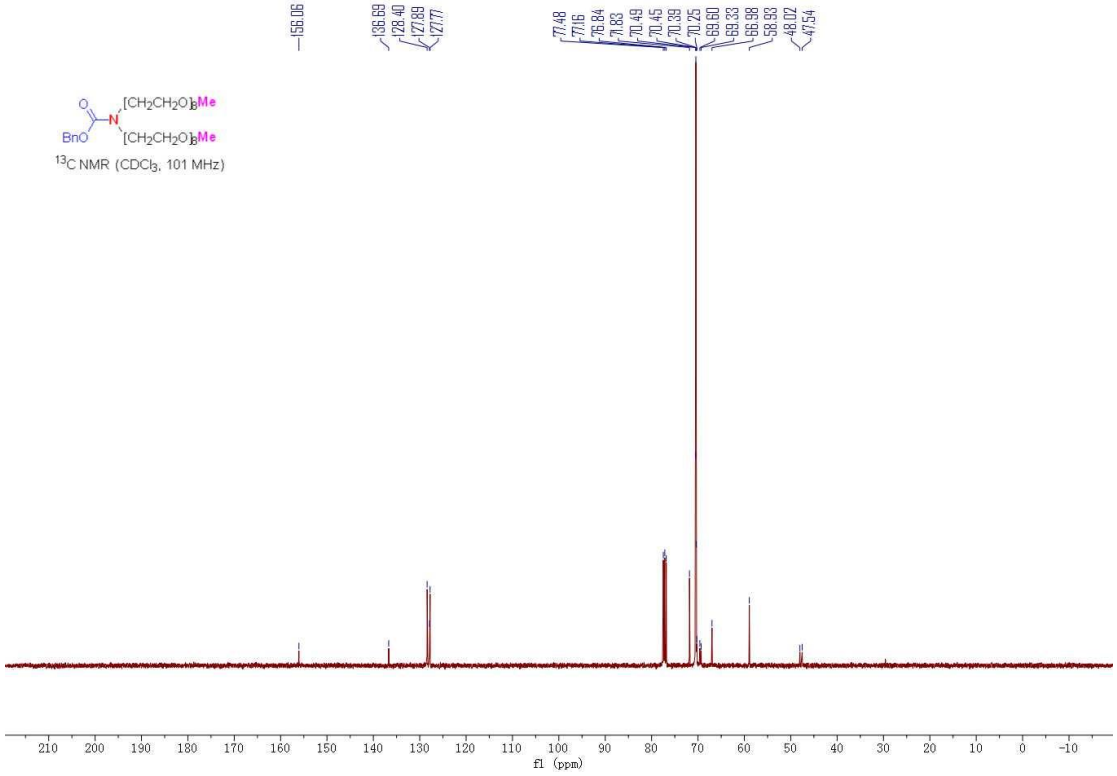
HRMS of compound **30**



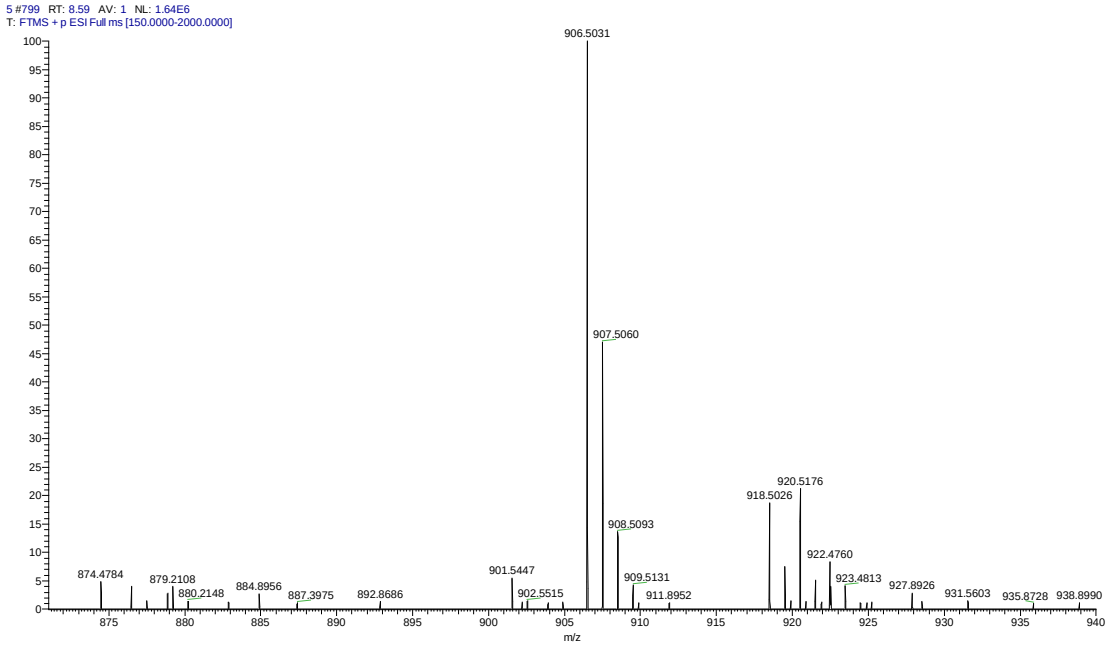
^{1}H NMR of compound **31**



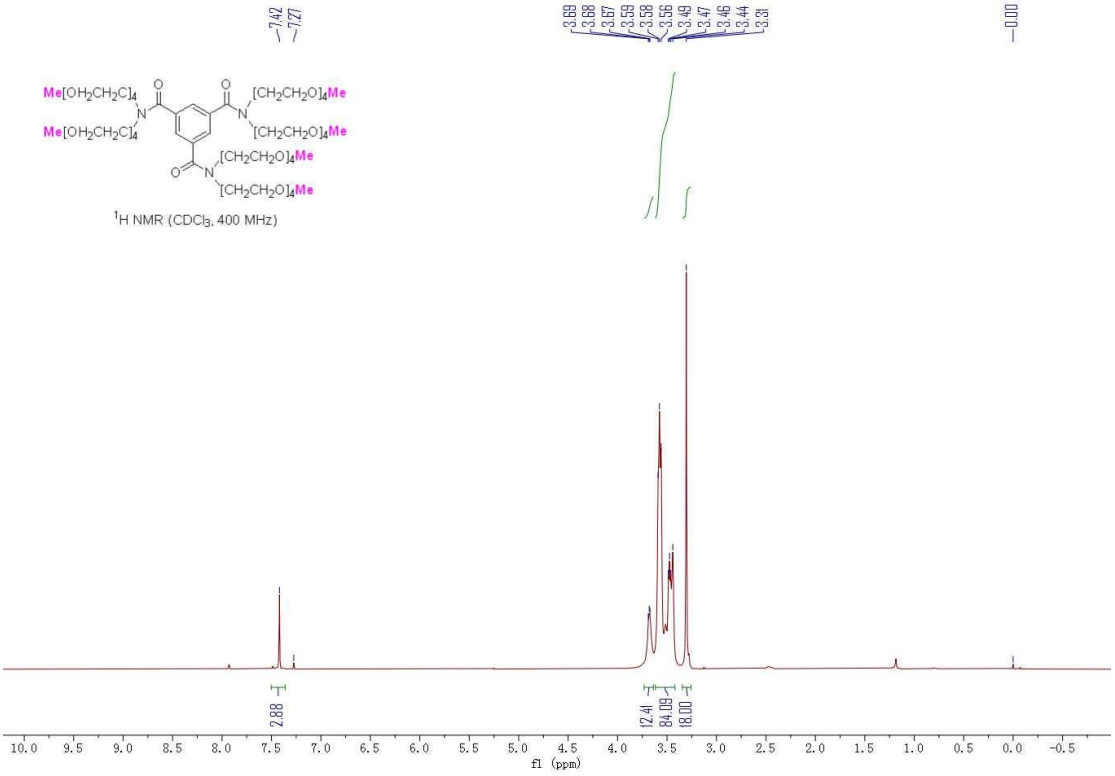
^{13}C NMR of compound **31**



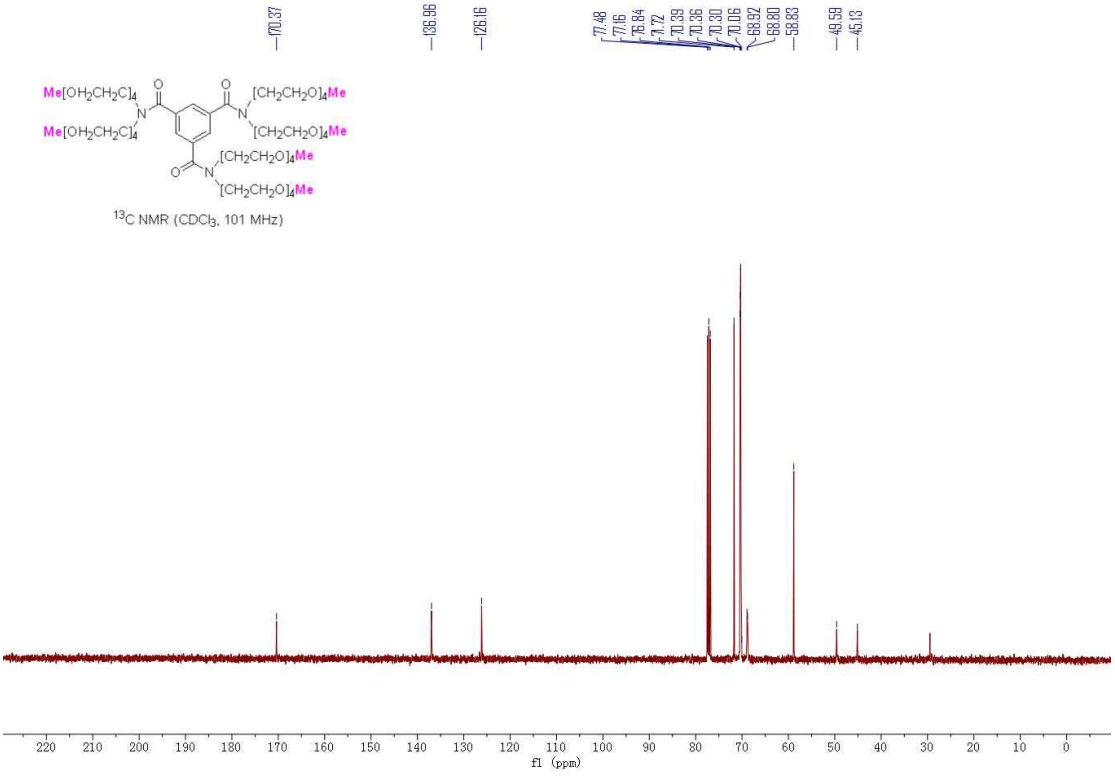
HRMS of compound **31**



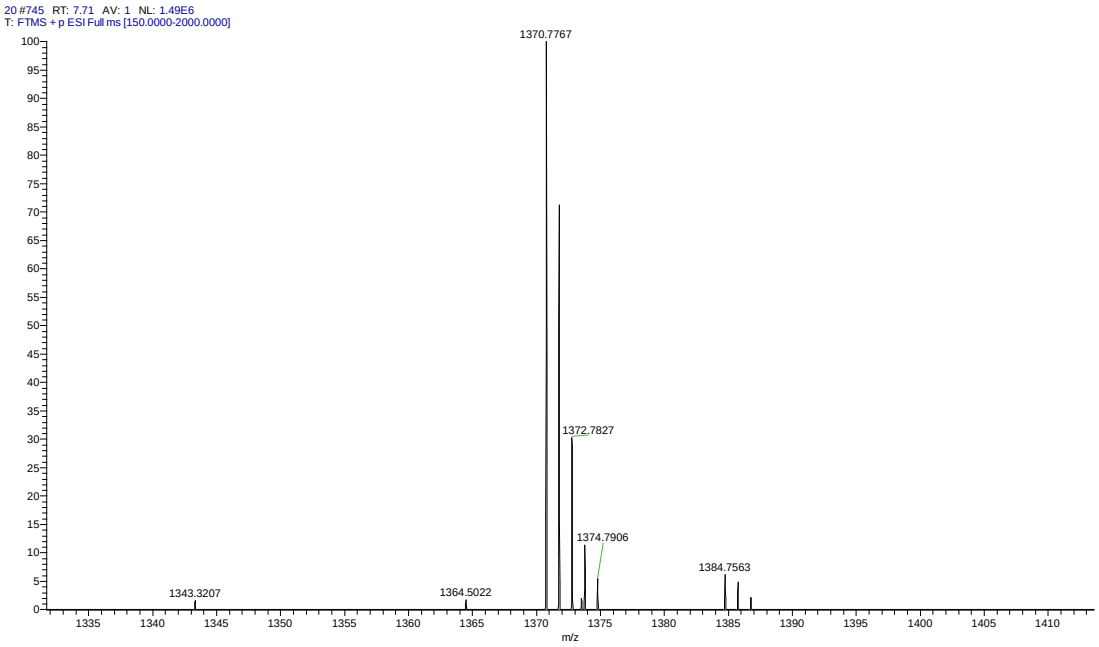
^{1}H NMR of compound **32**



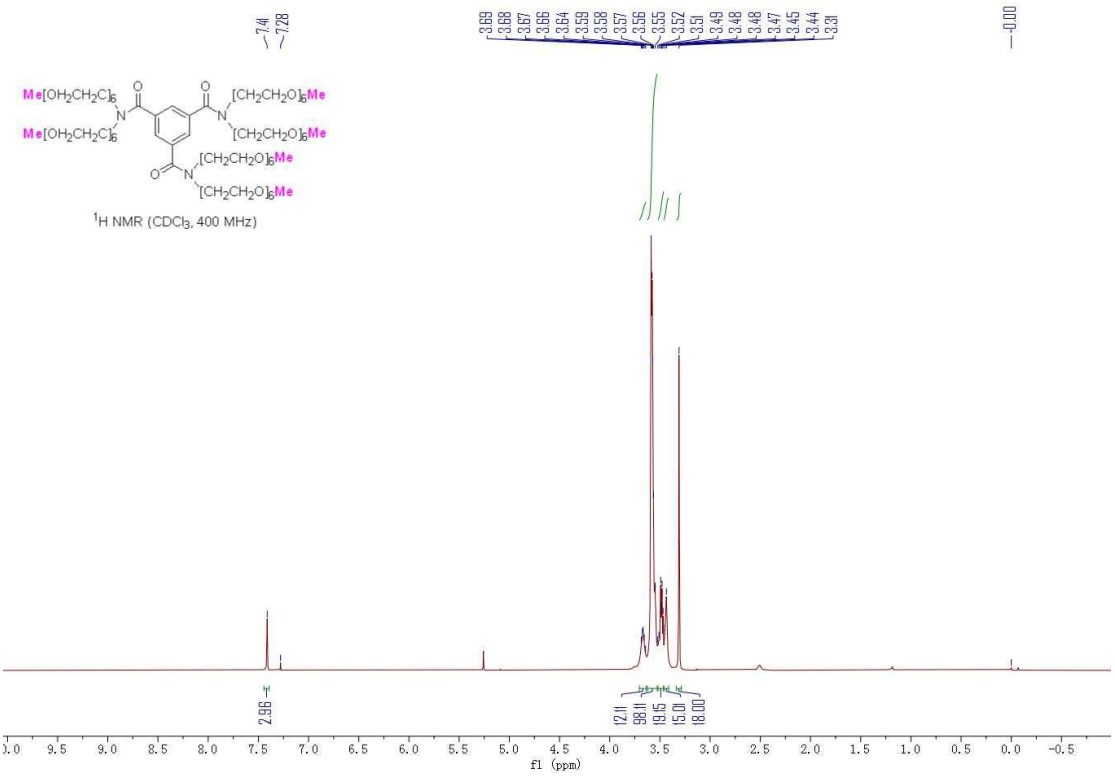
^{13}C NMR of compound **32**



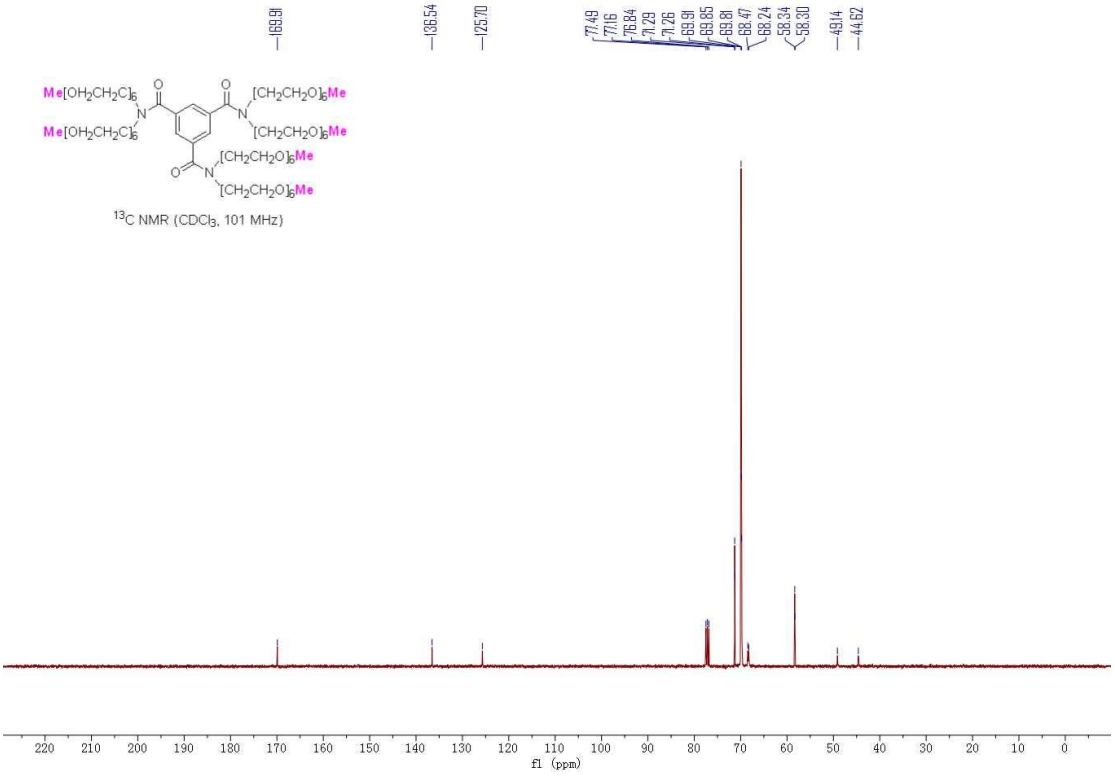
HRMS of compound **32**



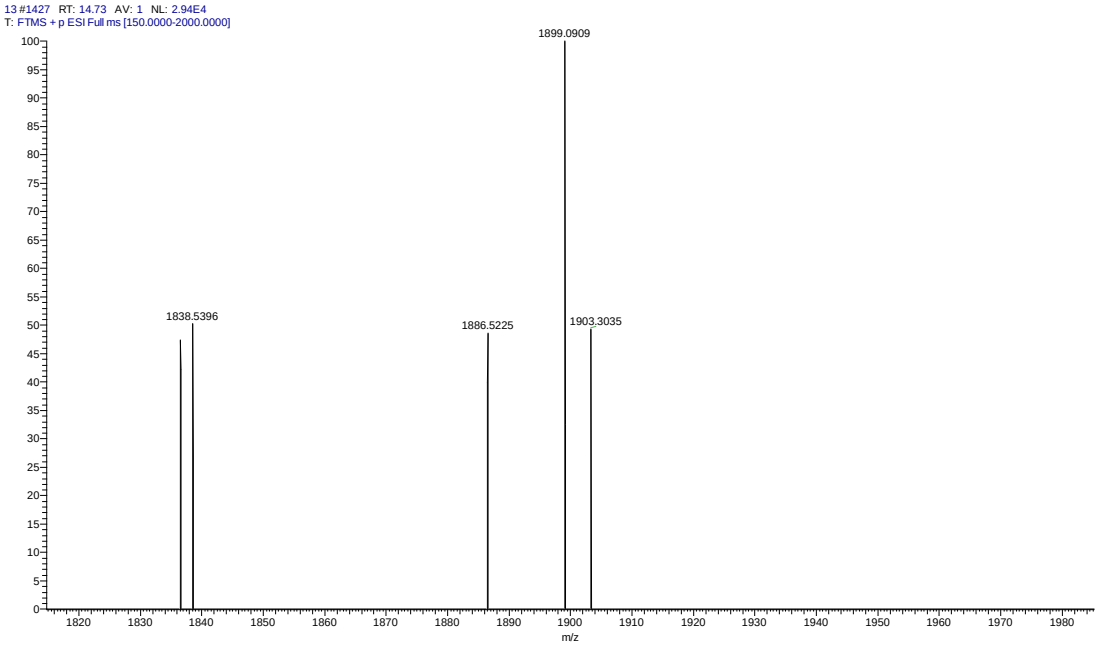
^{1}H NMR of compound **33**



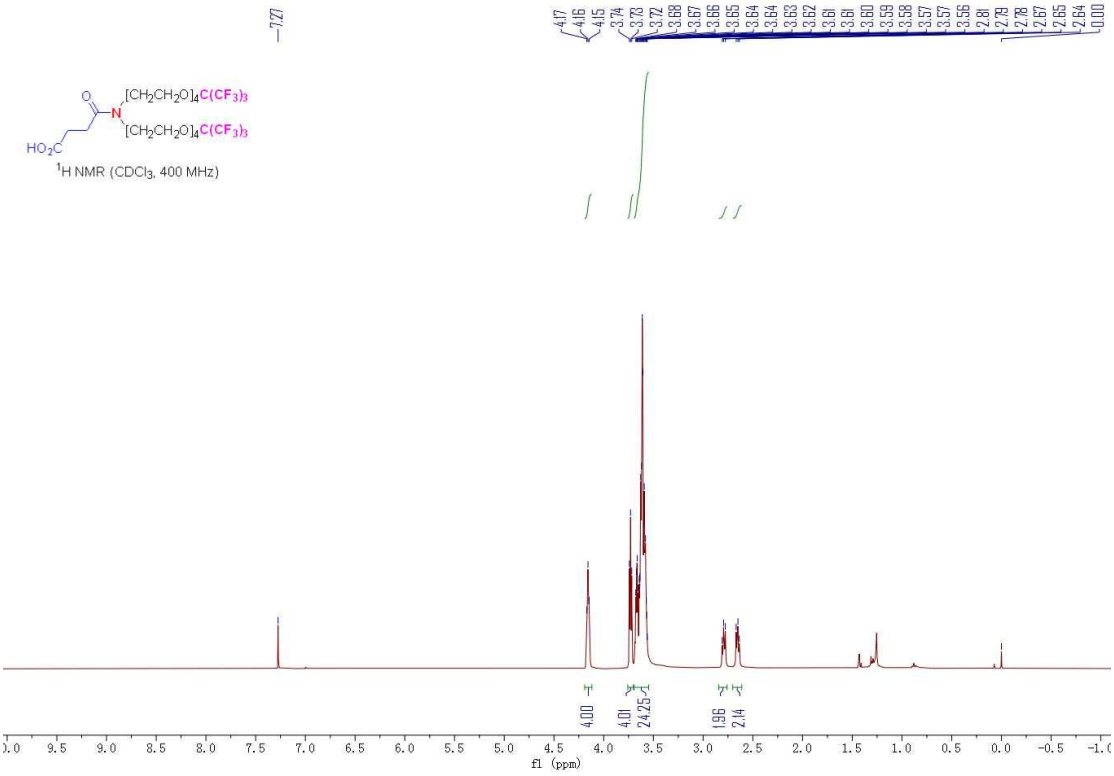
^{13}C NMR of compound **33**



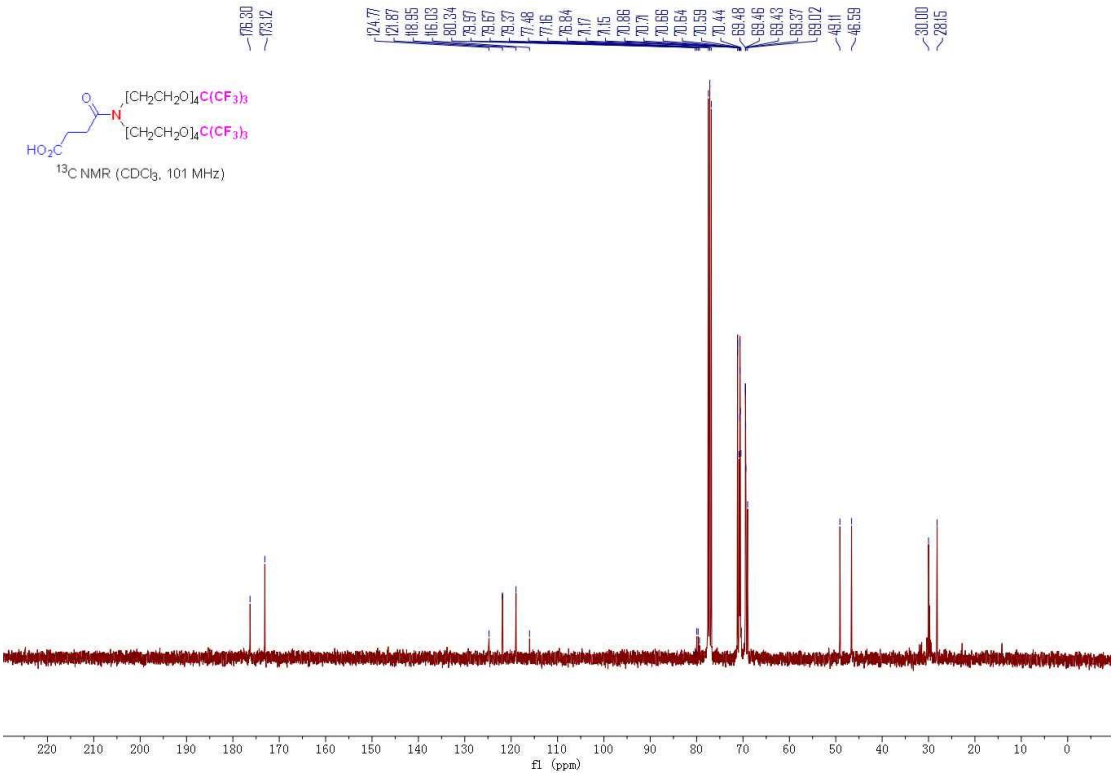
HRMS of compound **33**



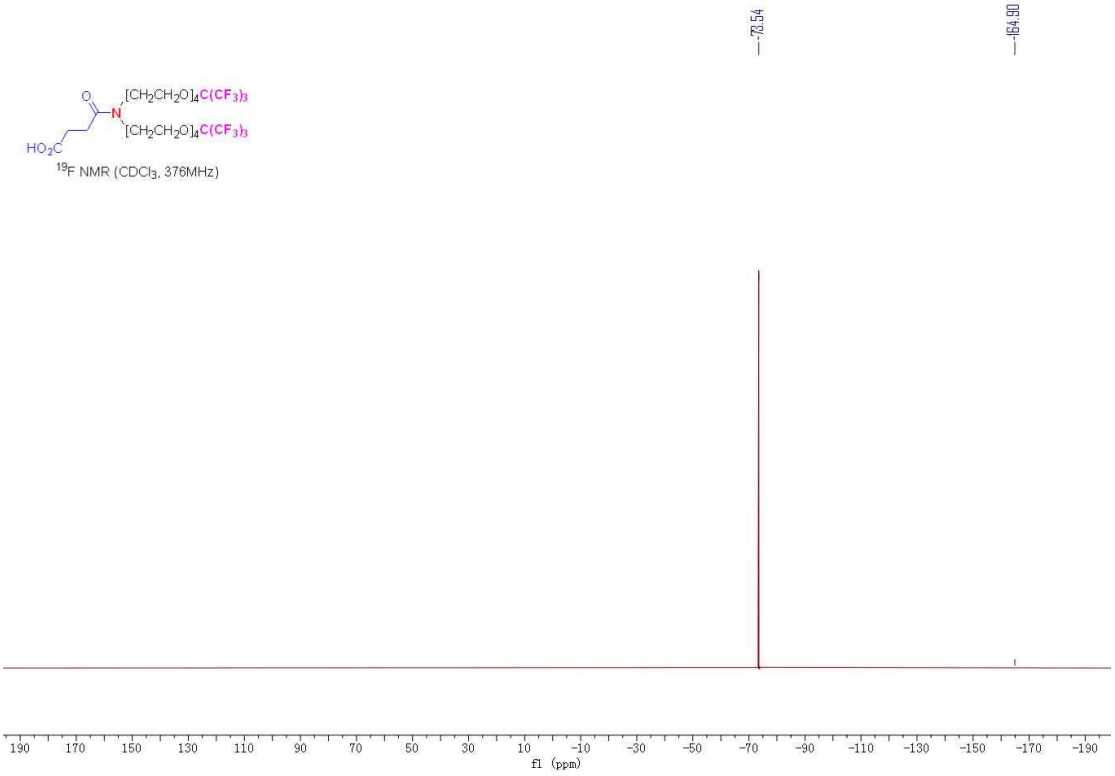
^{1}H NMR of compound **34**



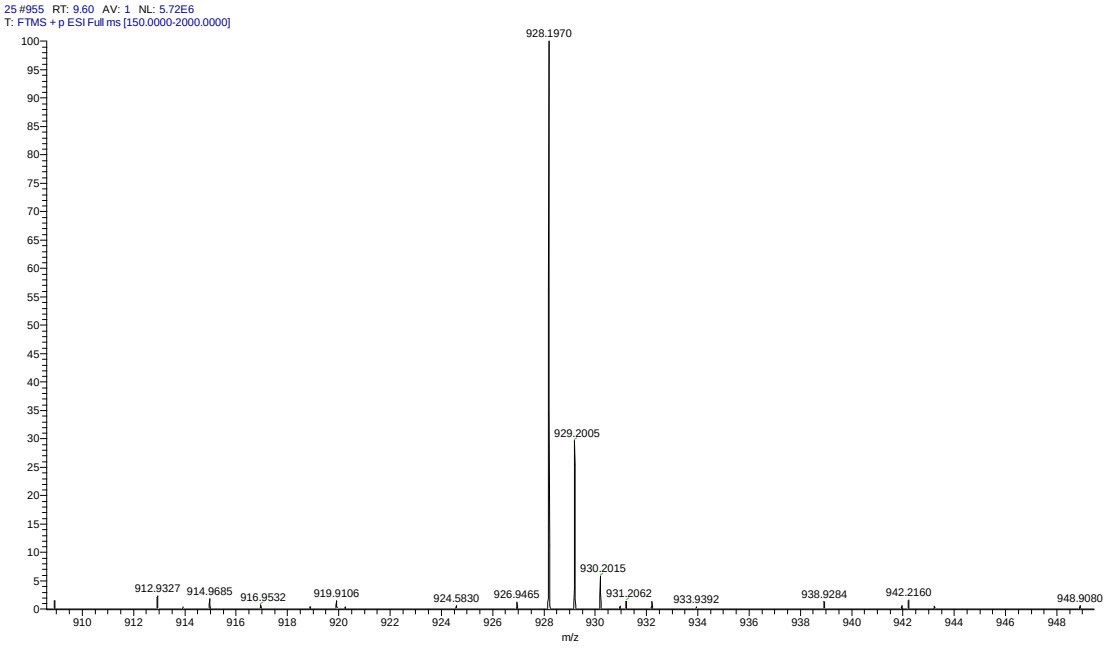
^{13}C NMR of compound **34**



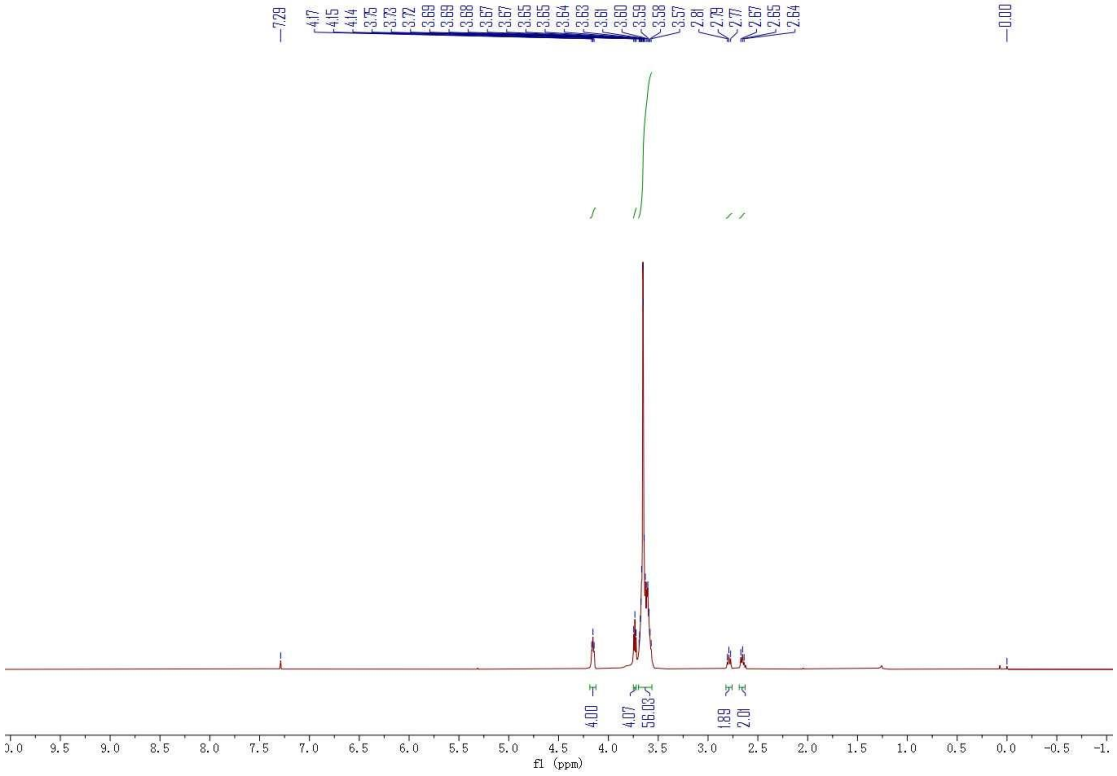
^{19}F NMR of compound **34**



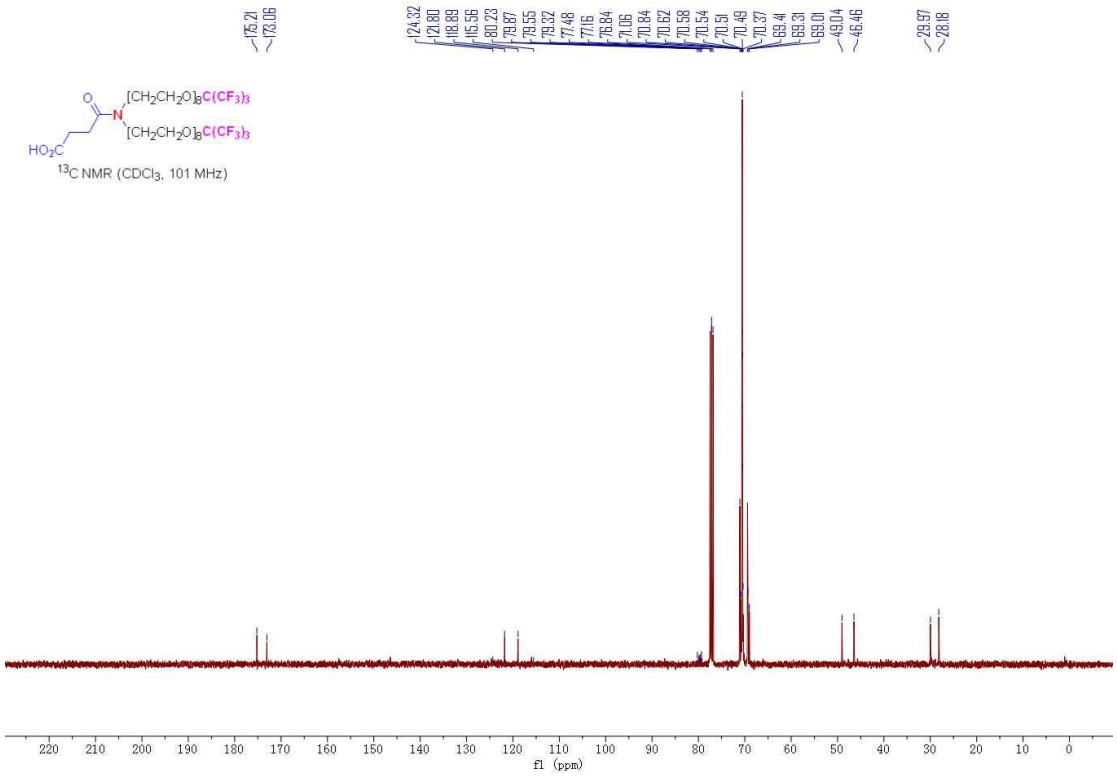
HRMS of compound **34**



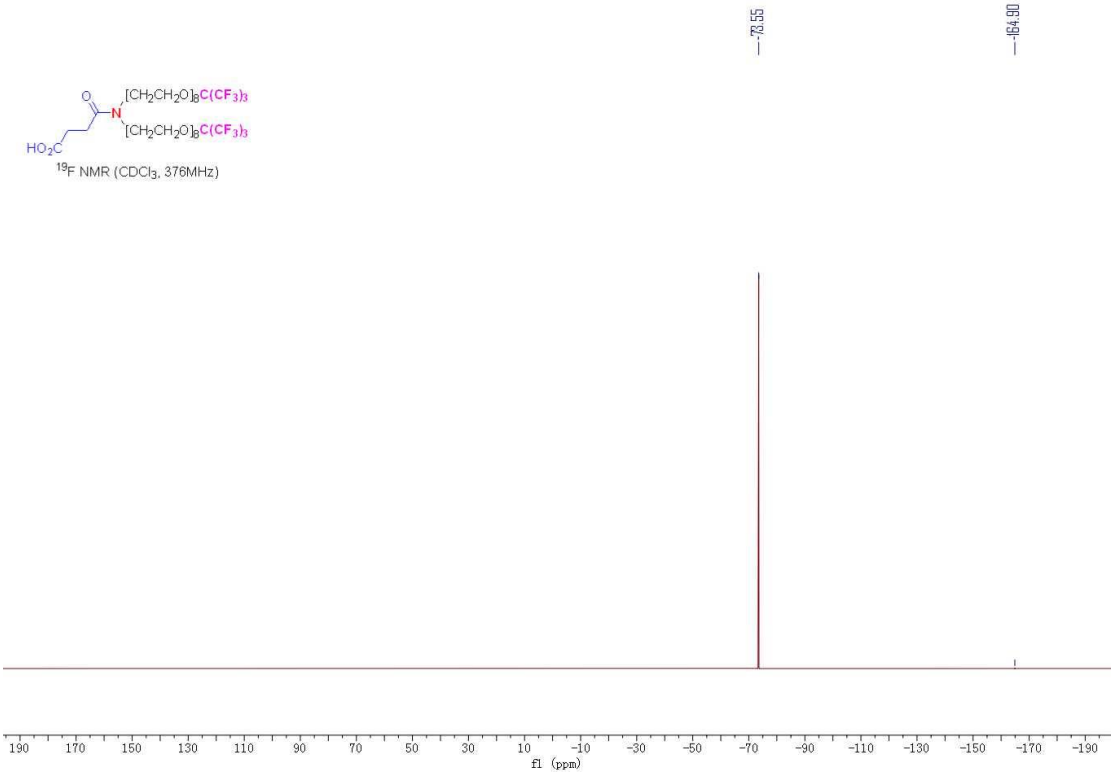
^{1}H NMR of compound **35**



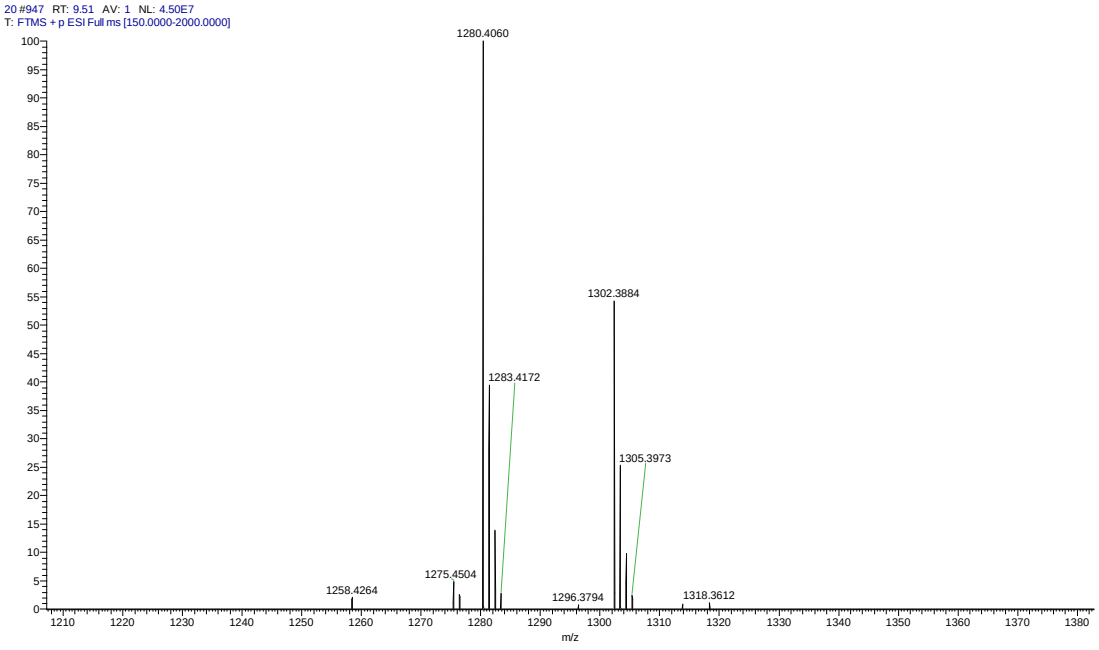
^{13}C NMR of compound **35**



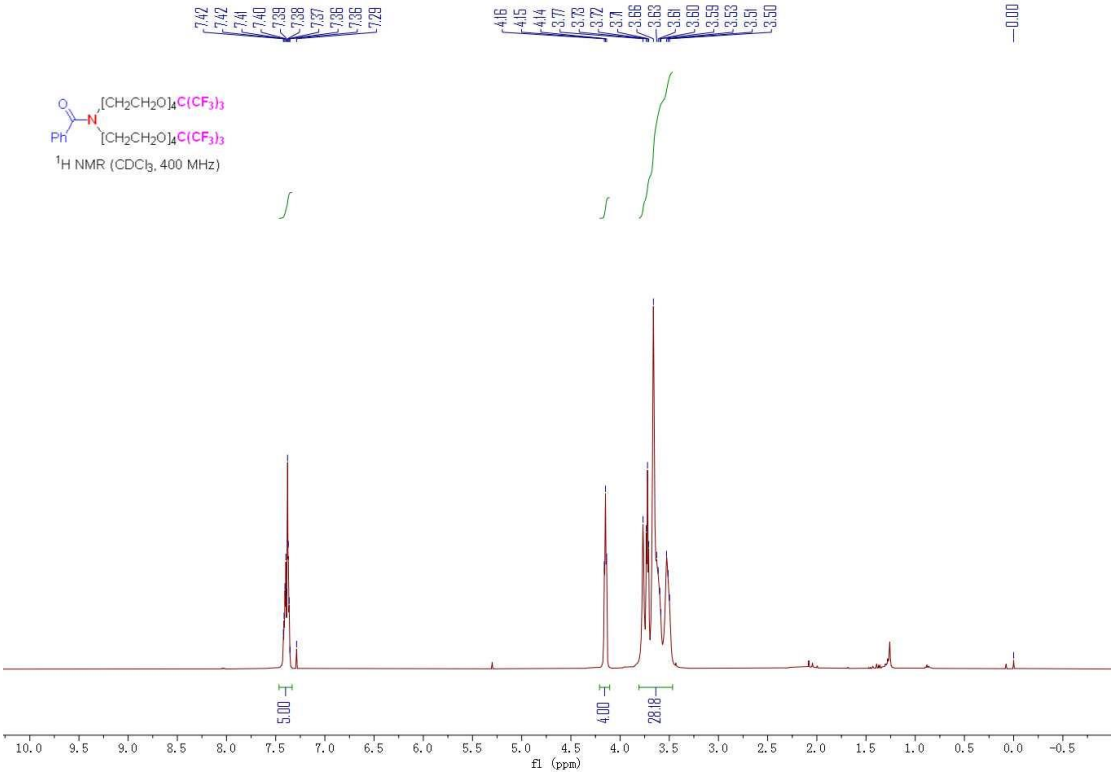
^{19}F NMR of compound **35**



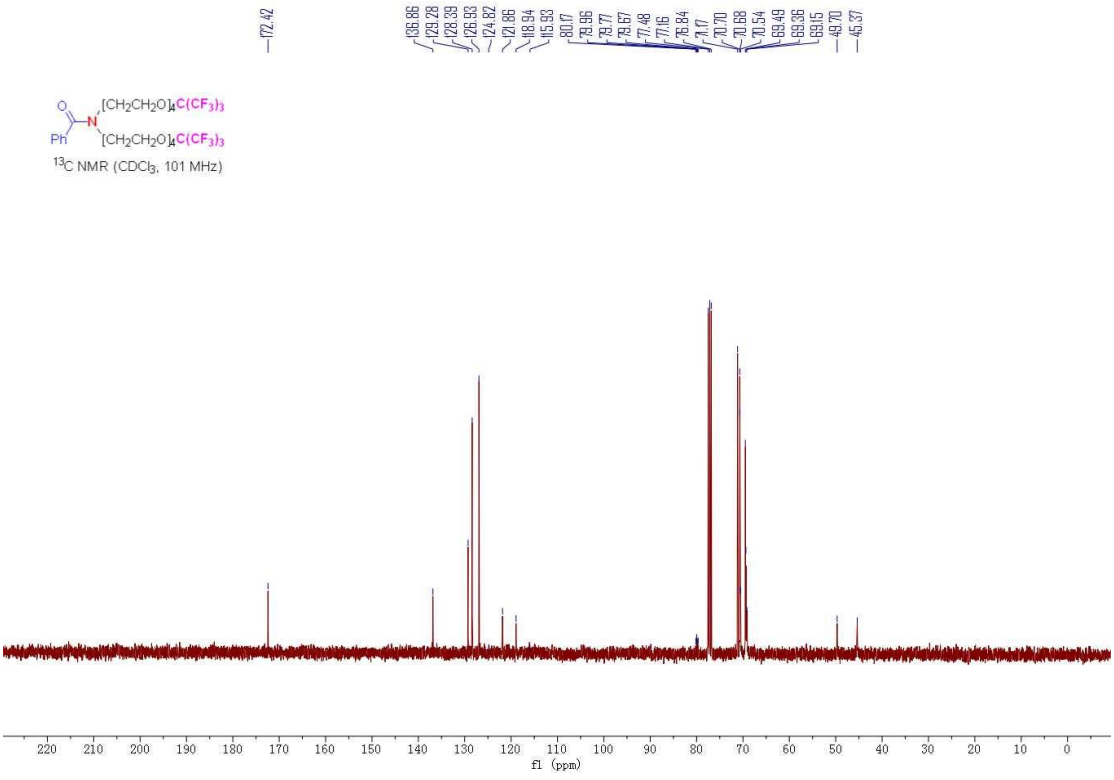
HRMS of compound **35**



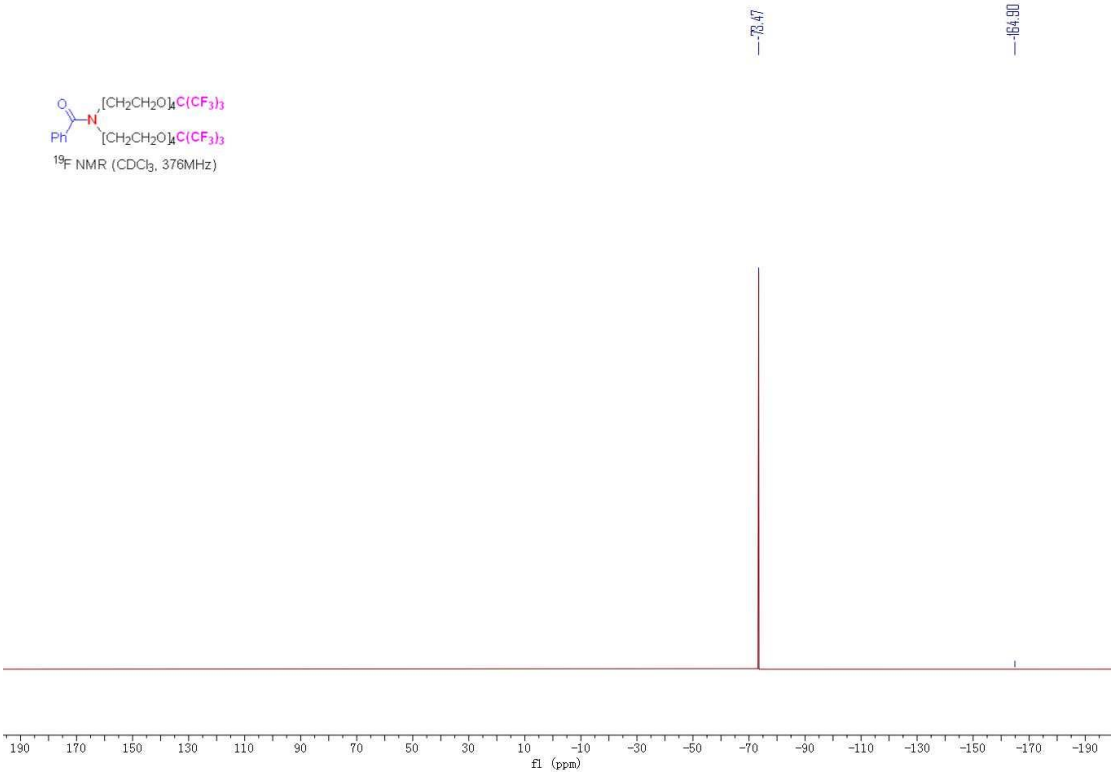
^{1}H NMR of compound **36**



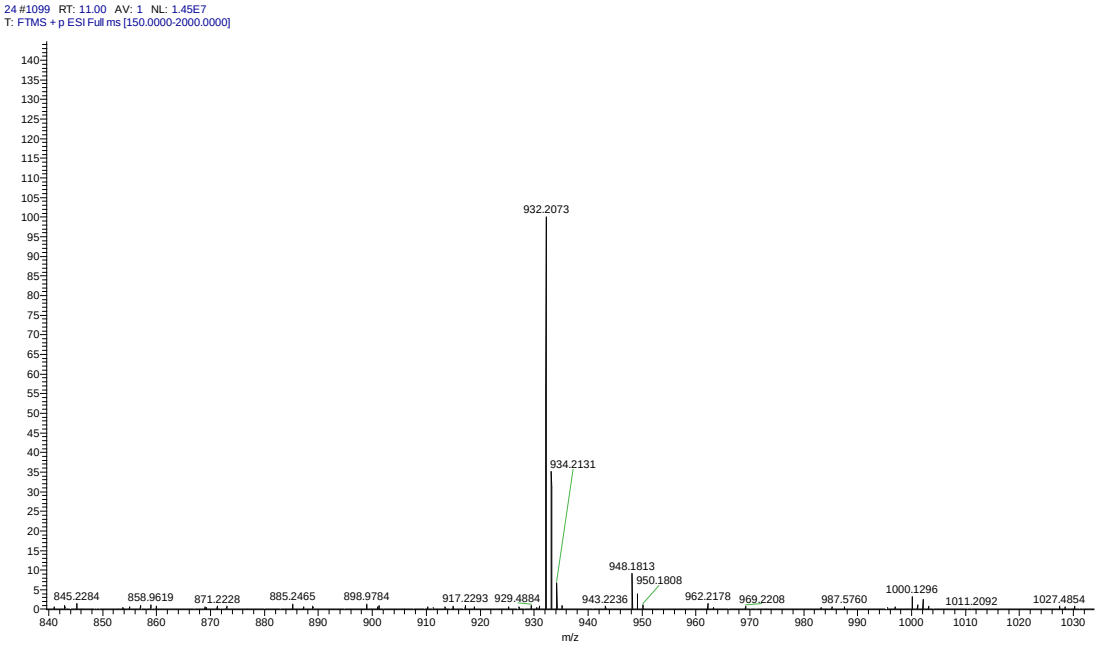
^{13}C NMR of compound **36**



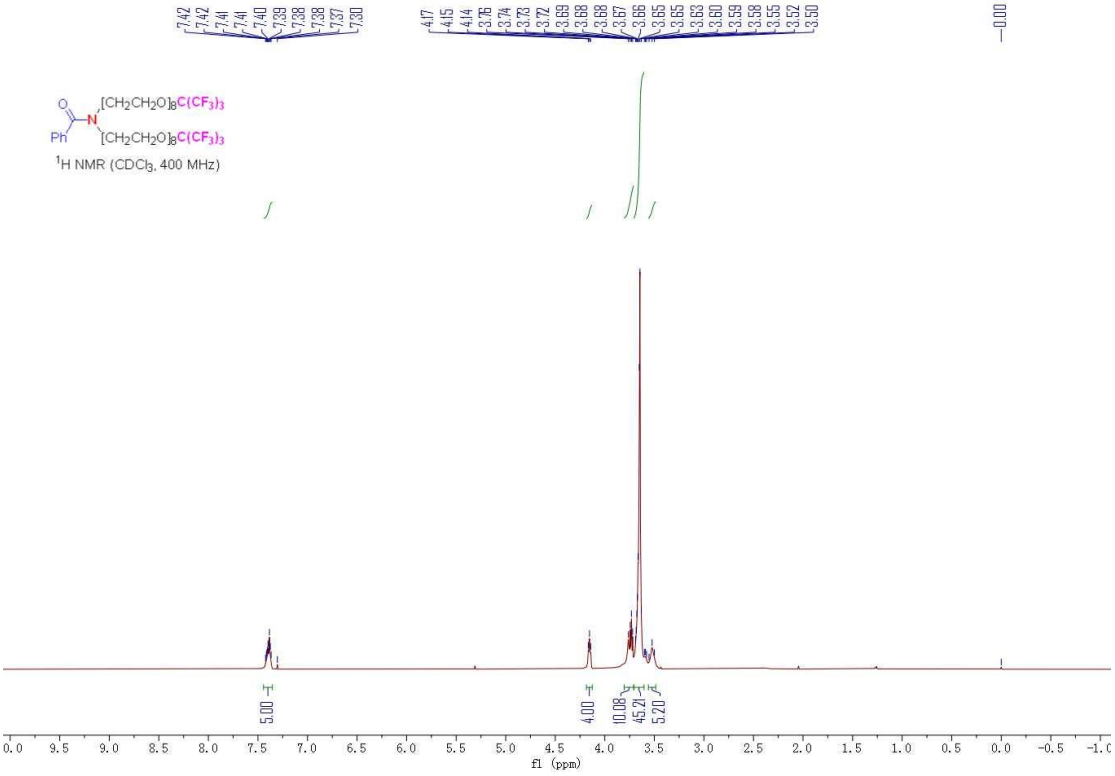
^{19}F NMR of compound **36**



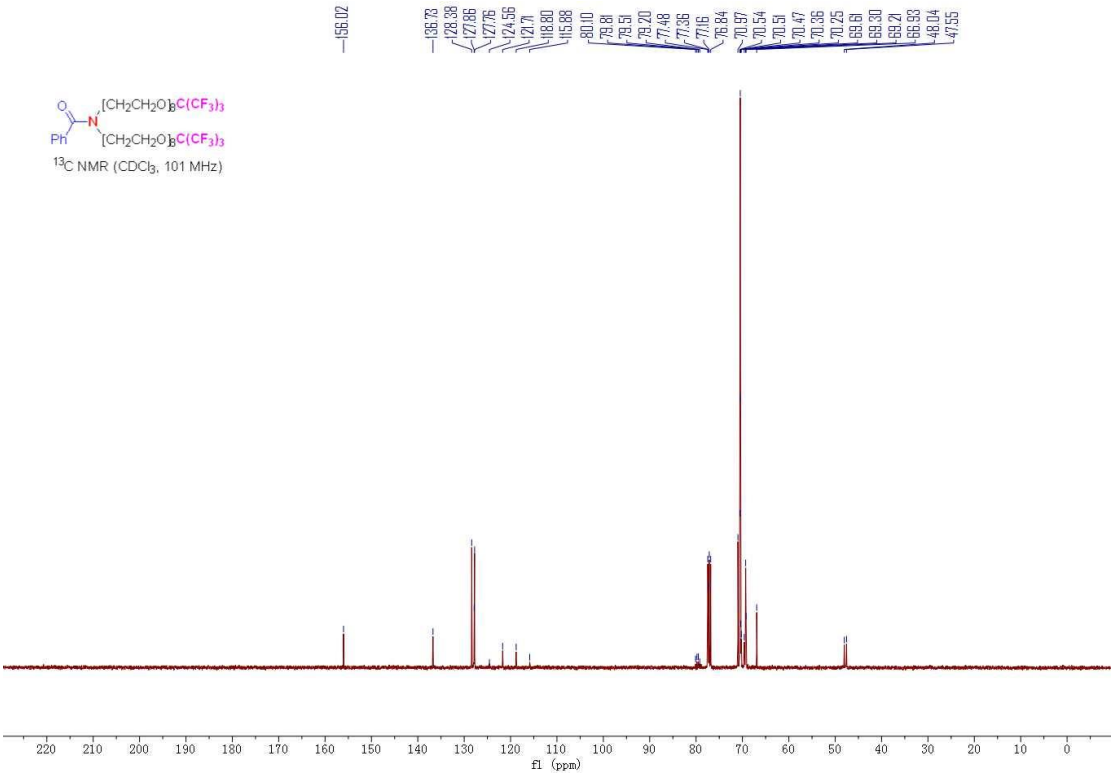
HRMS of compound **36**



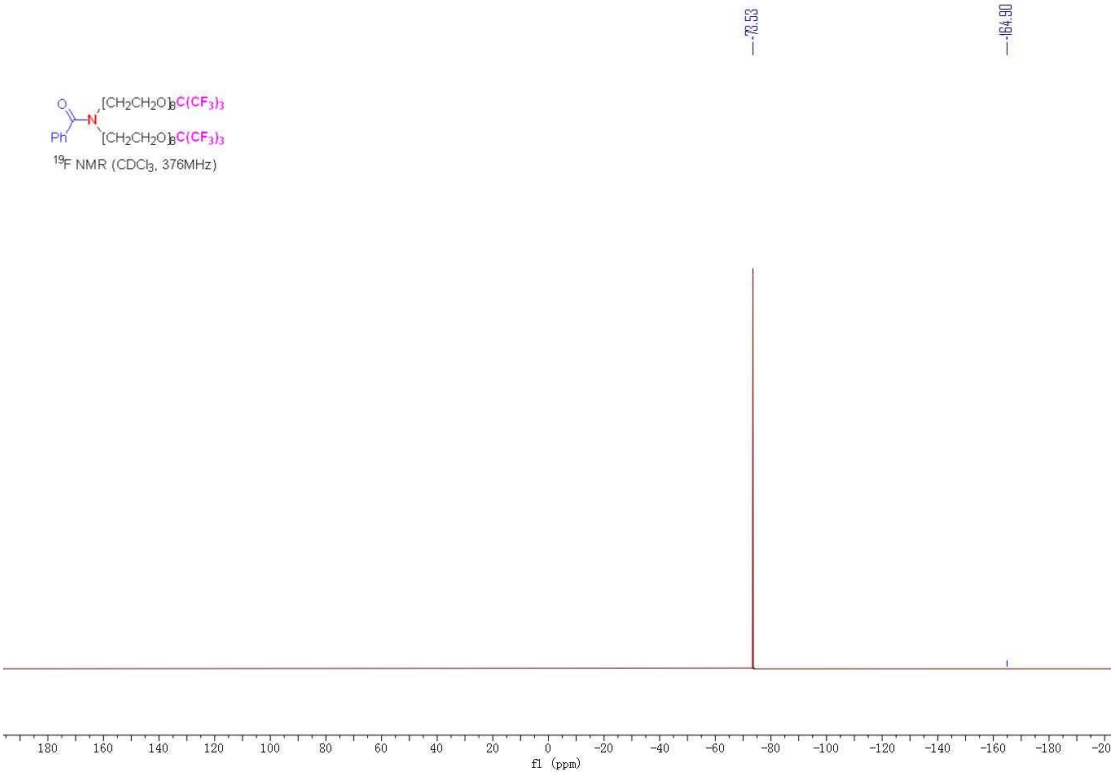
^{1}H NMR of compound **37**



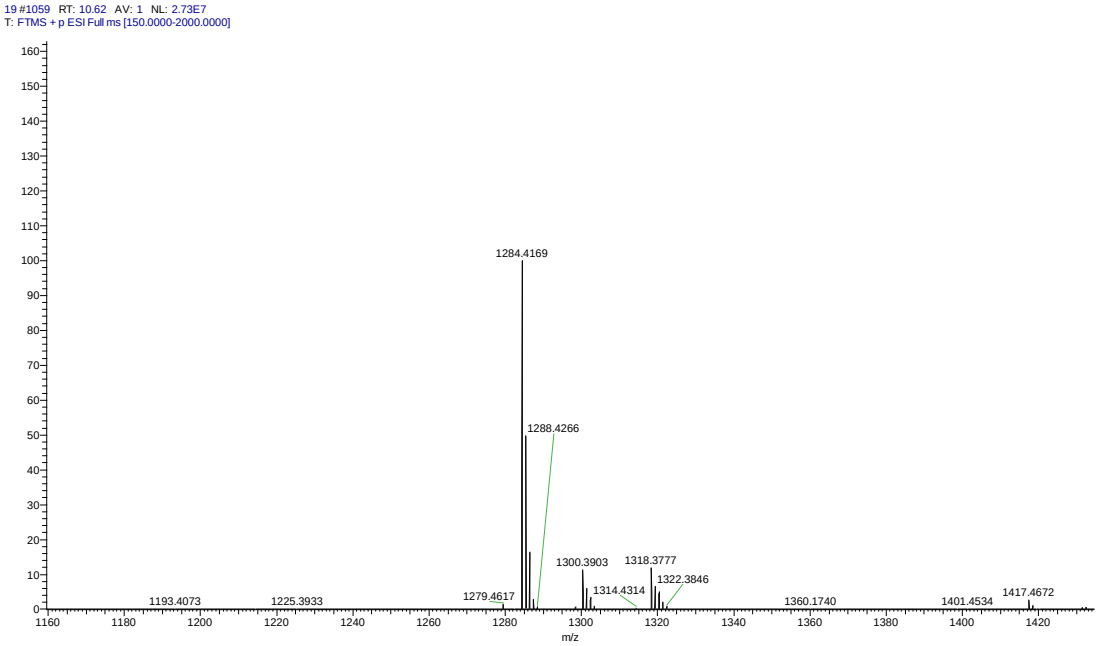
^{13}C NMR of compound **37**



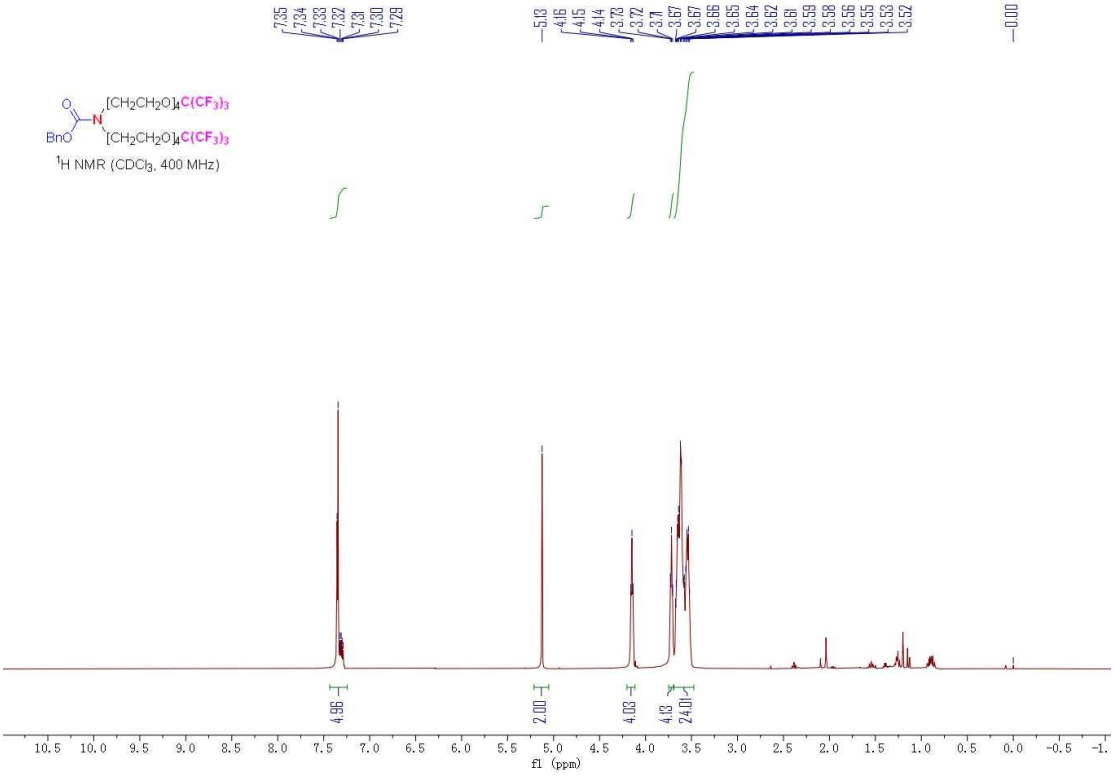
^{19}F NMR of compound **37**



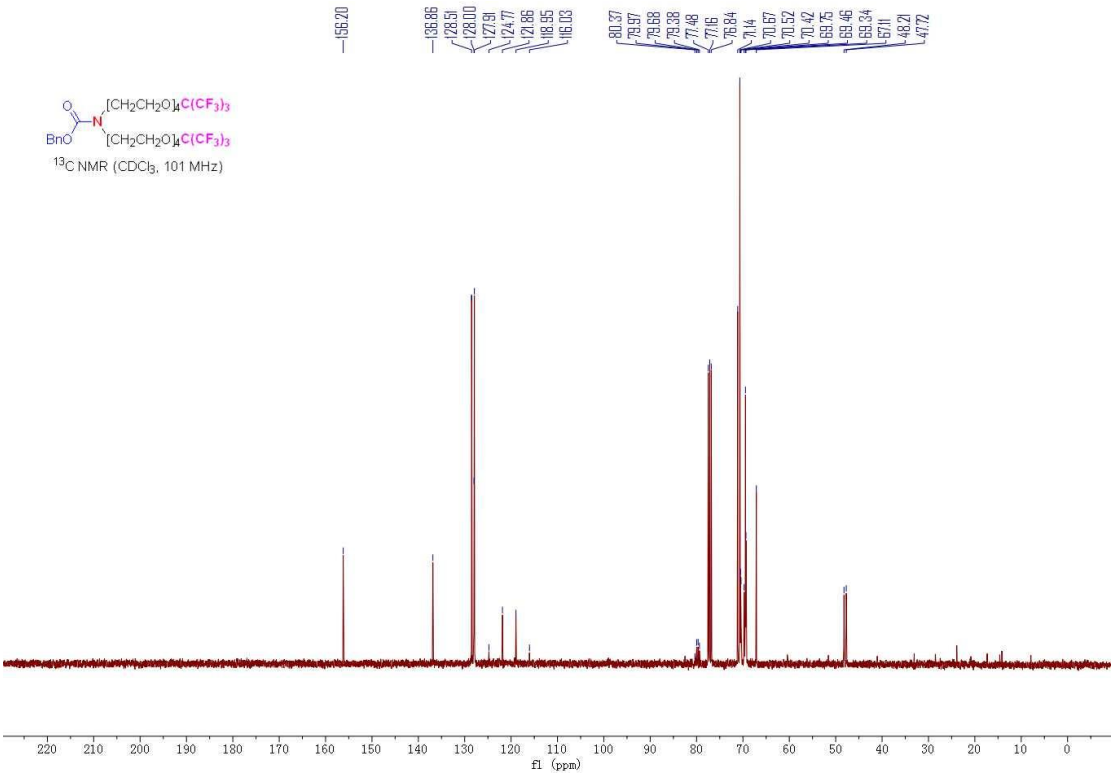
HRMS of compound **37**



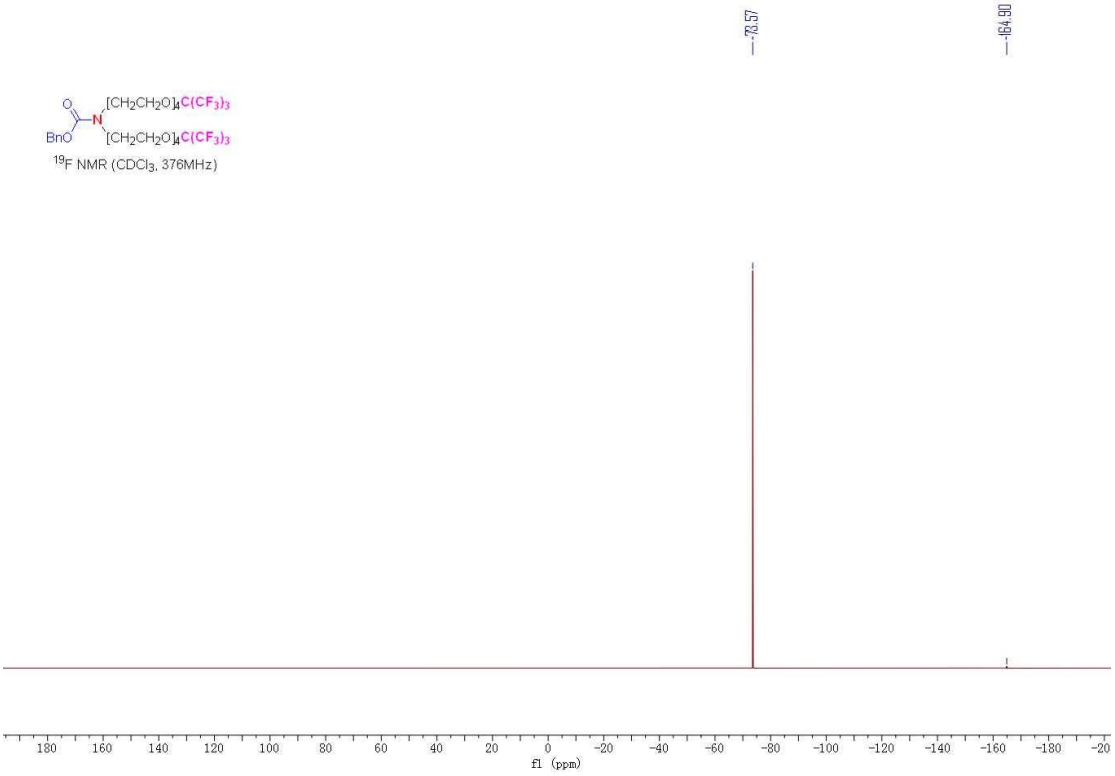
^{1}H NMR of compound **38**



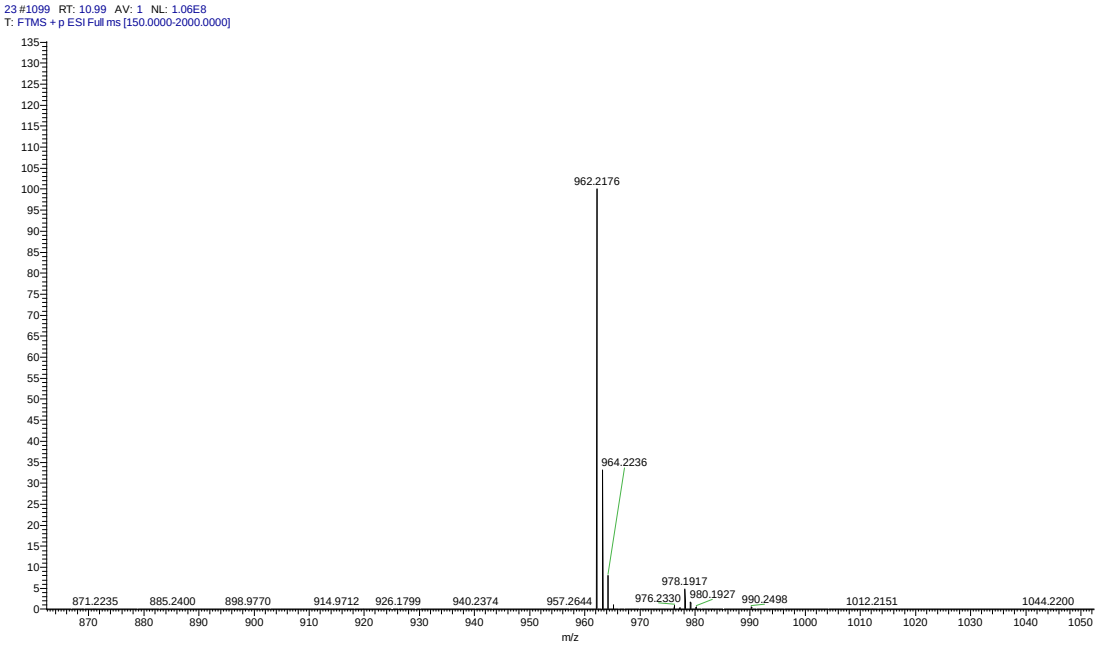
^{13}C NMR of compound **38**



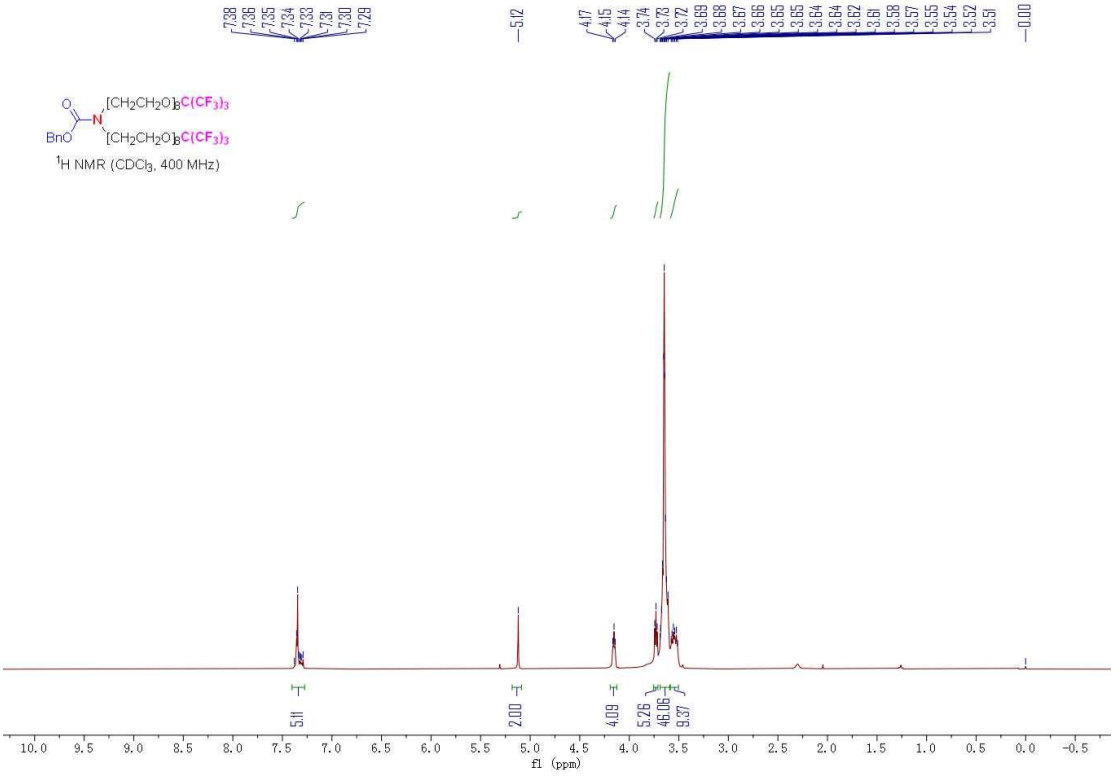
^{19}F NMR of compound **38**



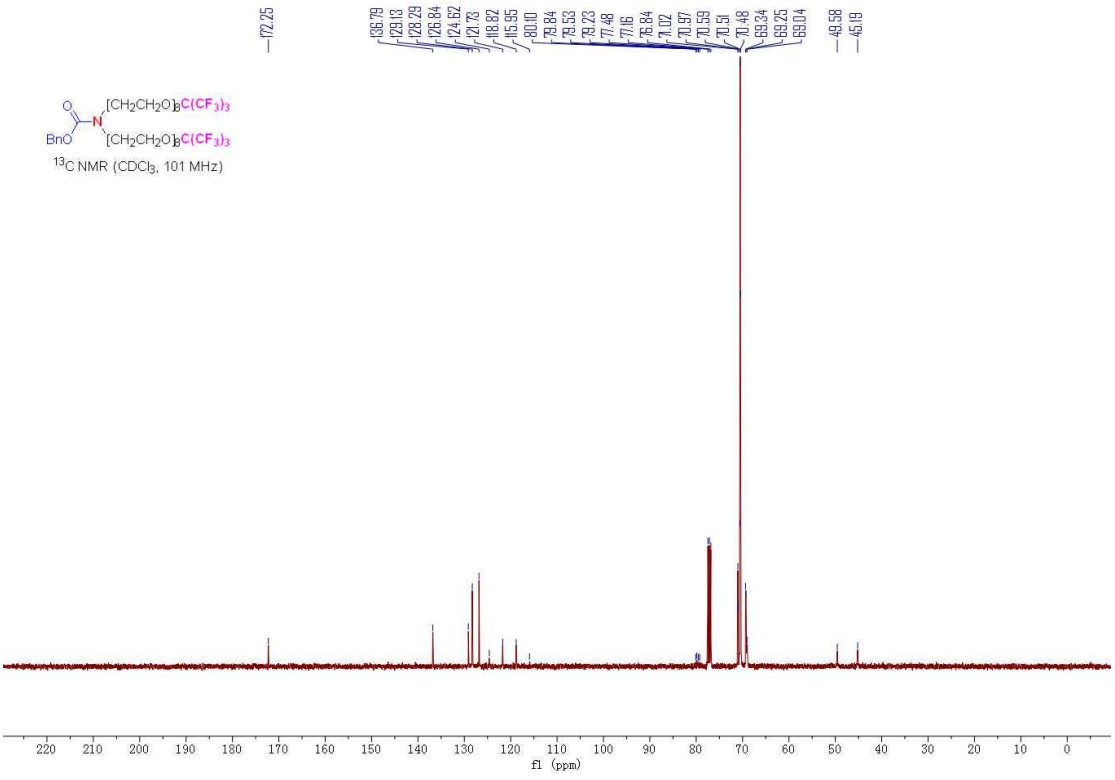
HRMS of compound **38**



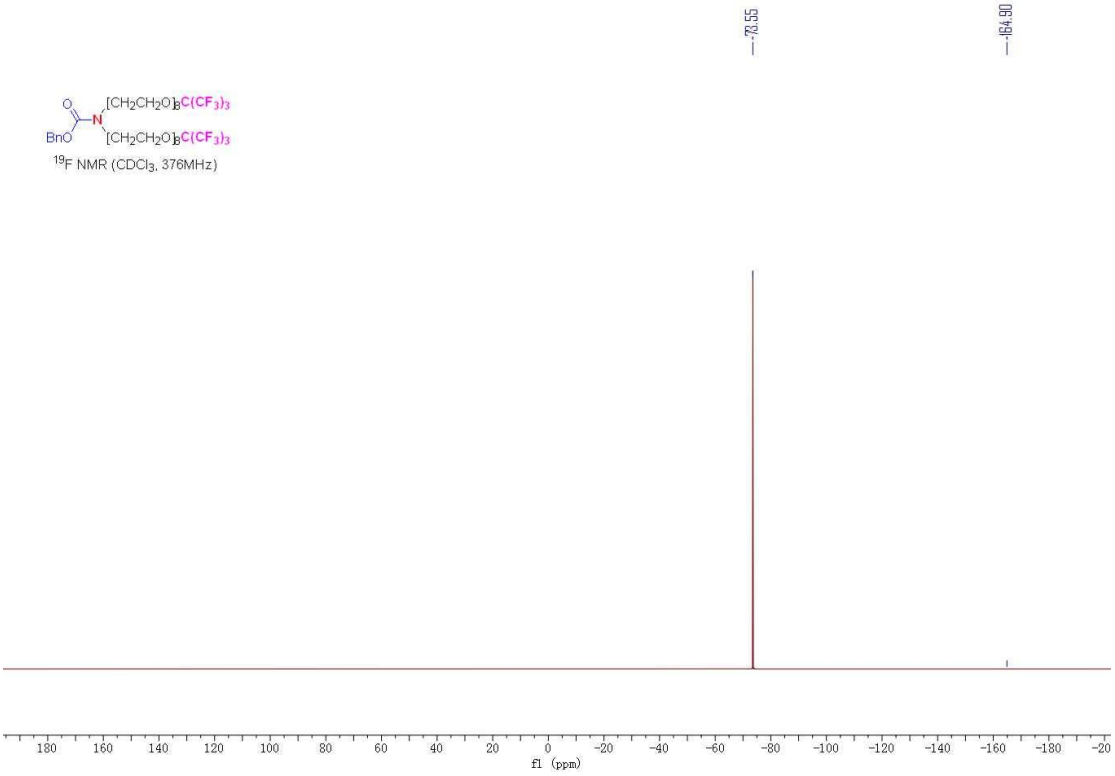
^{1}H NMR of compound **39**



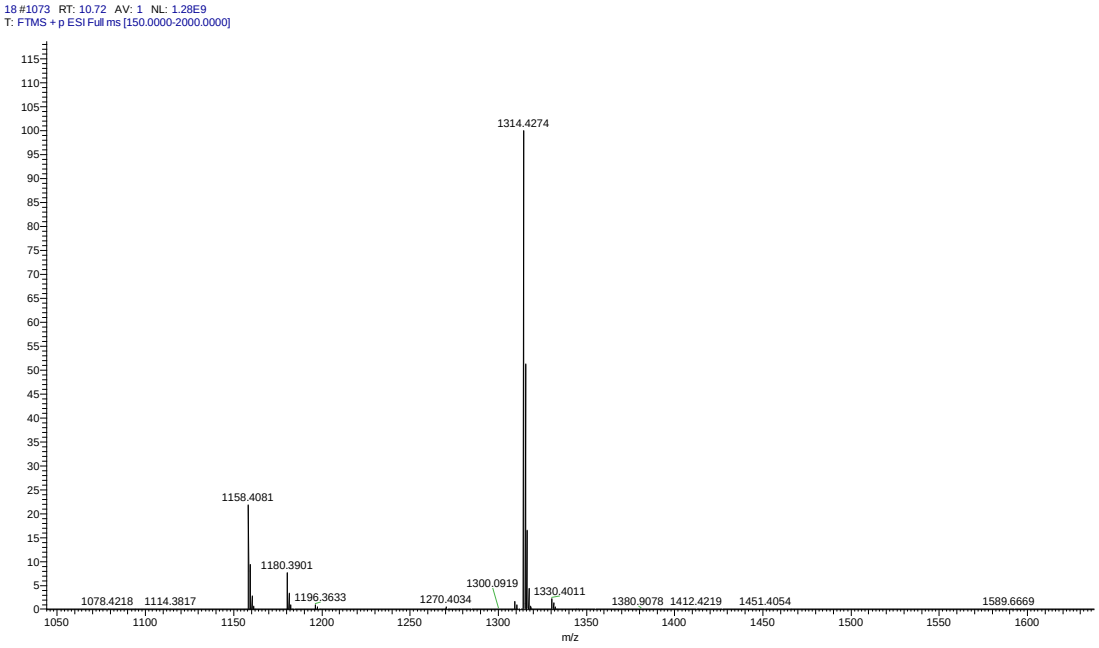
^{13}C NMR of compound **39**



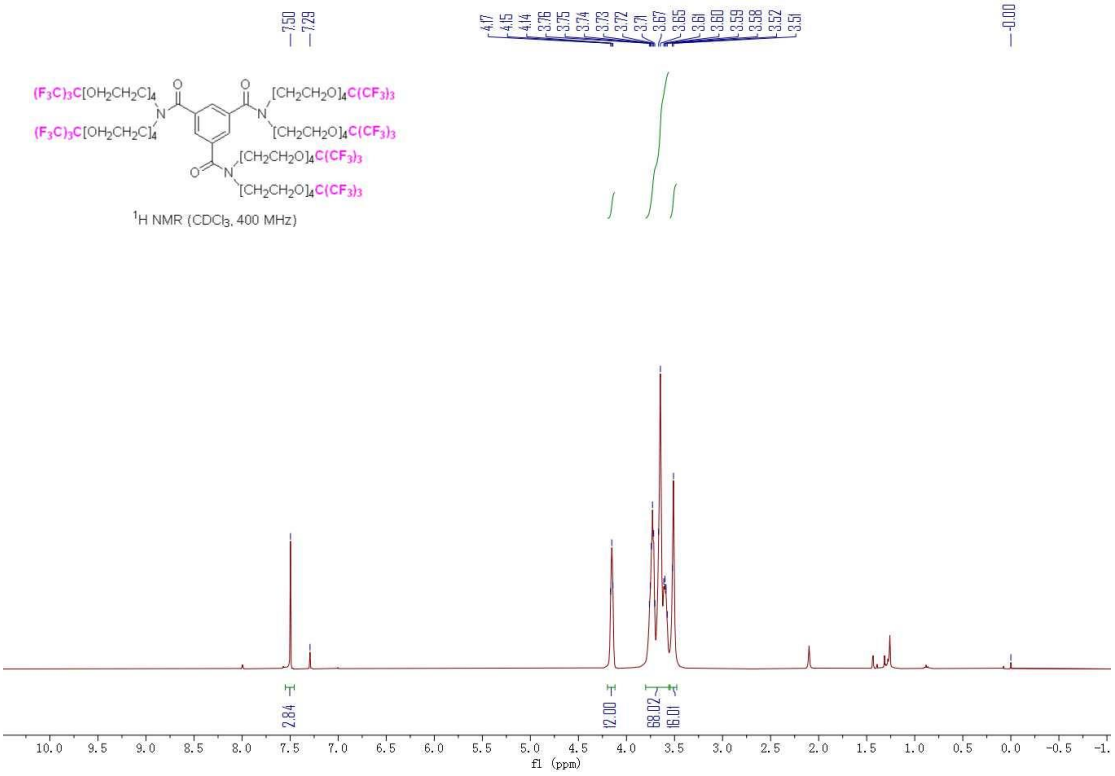
^{19}F NMR of compound **39**



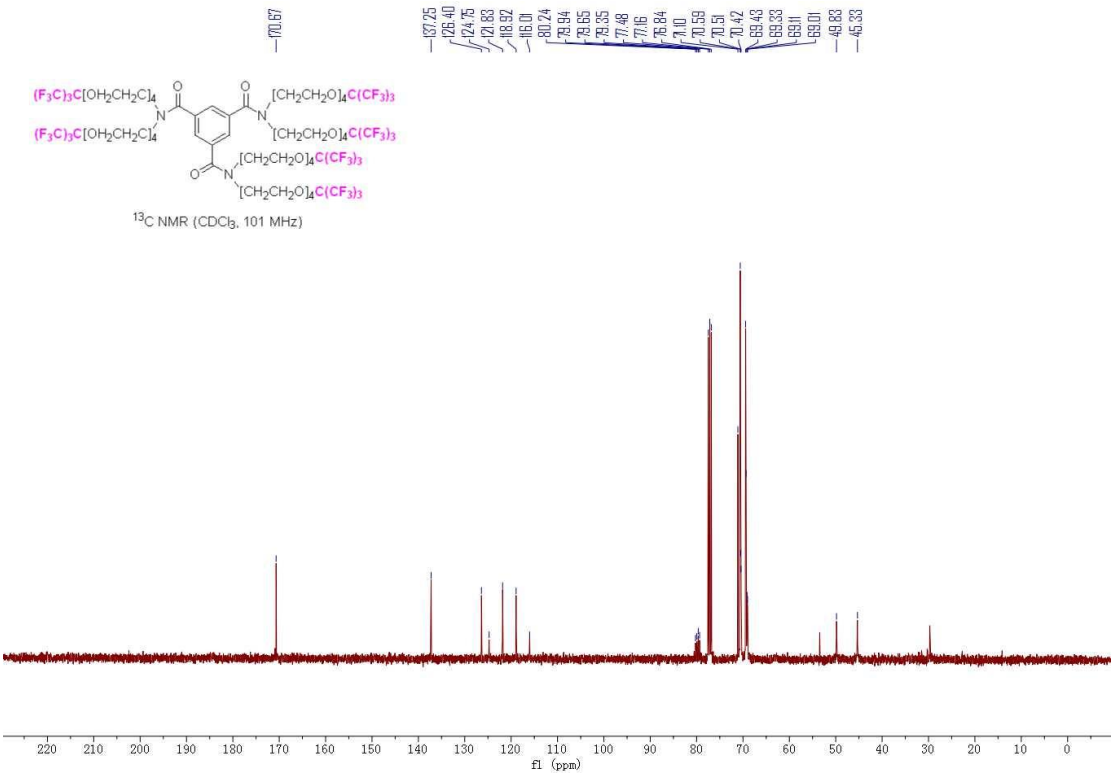
HRMS of compound **39**



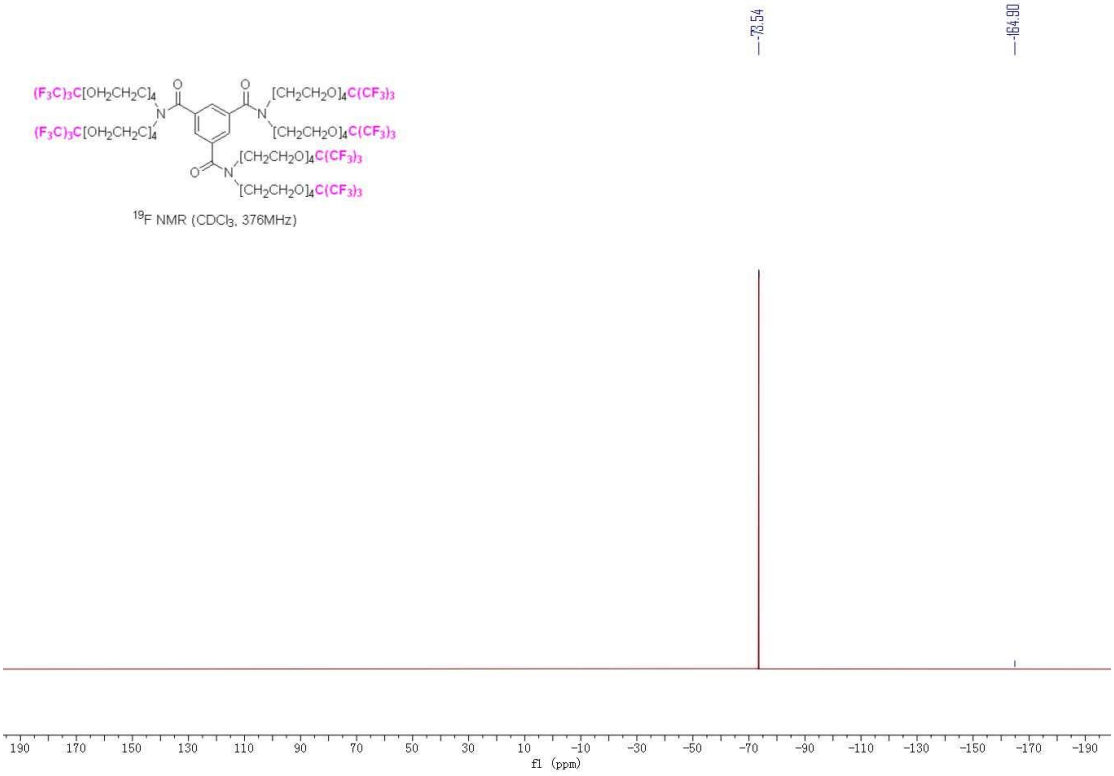
^{1}H NMR of compound **40**



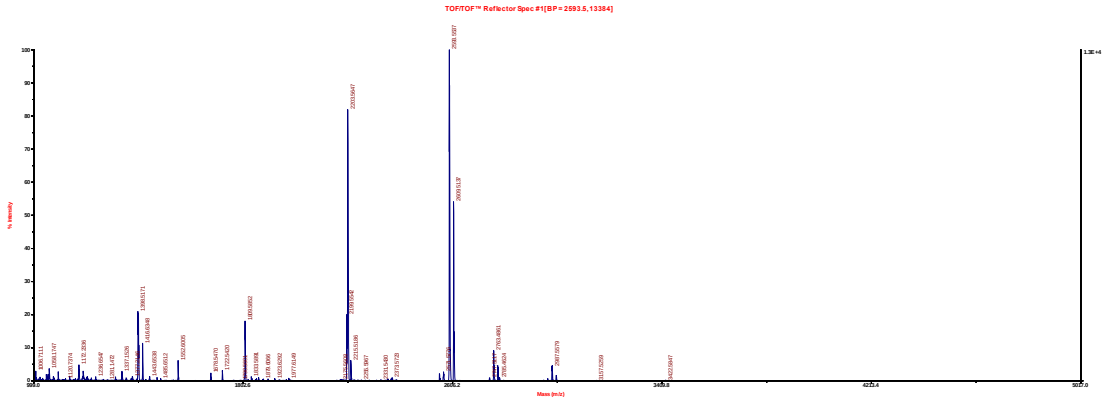
^{13}C NMR of compound **40**



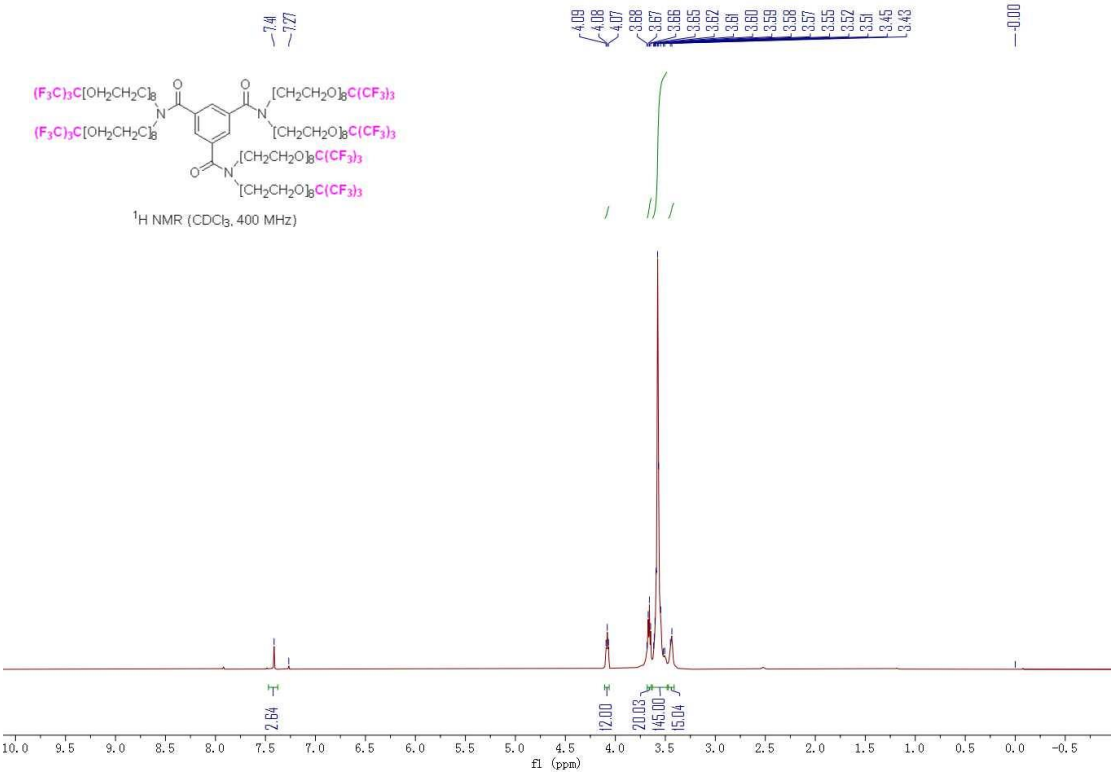
^{19}F NMR of compound **40**



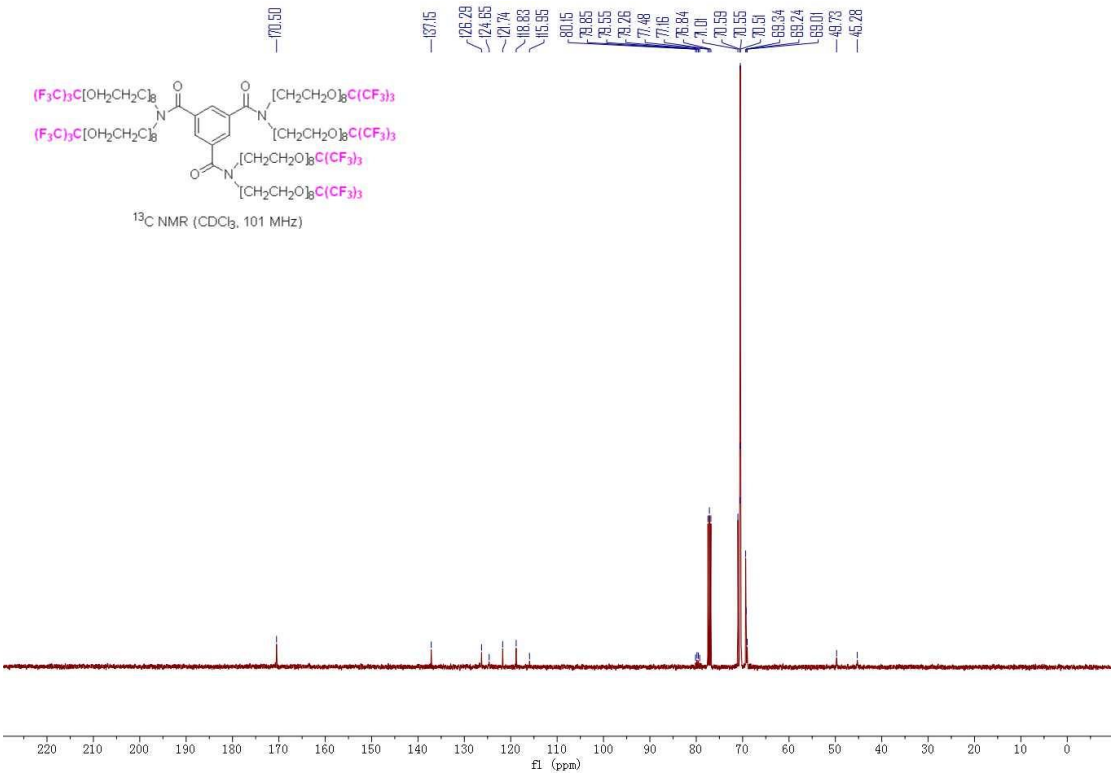
HRMS of compound **40**



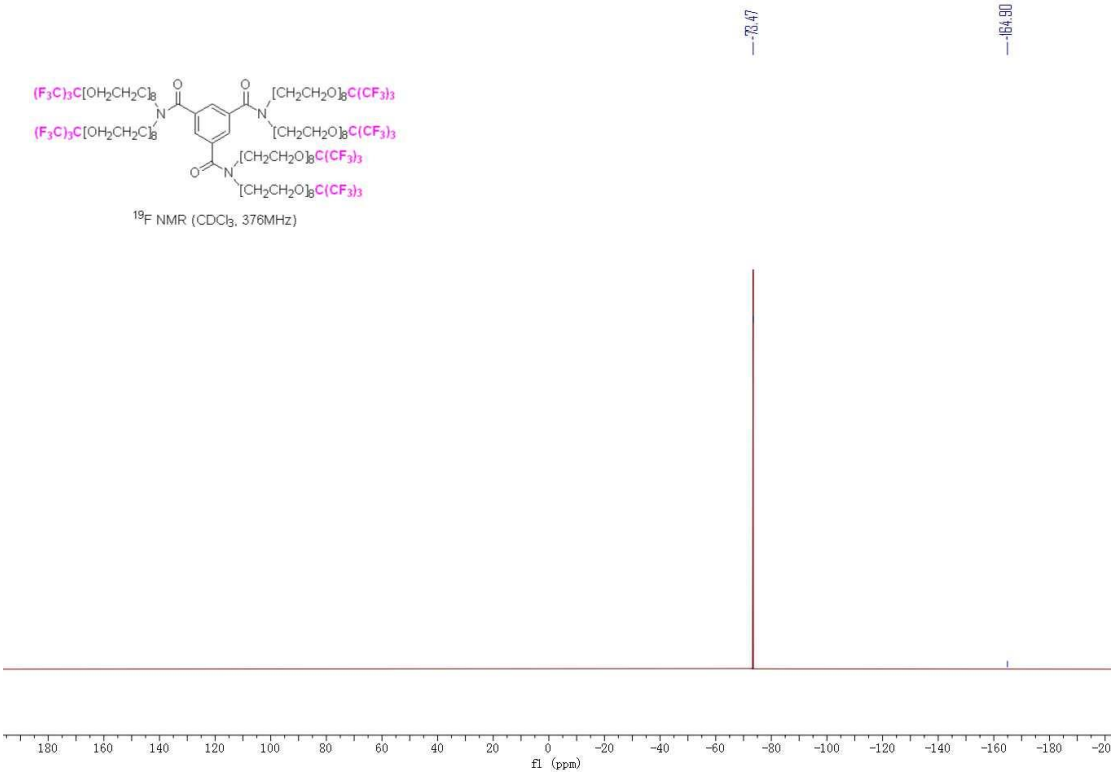
^{1}H NMR of compound **41**



^{13}C NMR of compound **41**



^{19}F NMR of compound **41**



HRMS of compound **41**