Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Multivalent manganese complexes decorated amphiphilic dextran micelles as sensitive MRI probes

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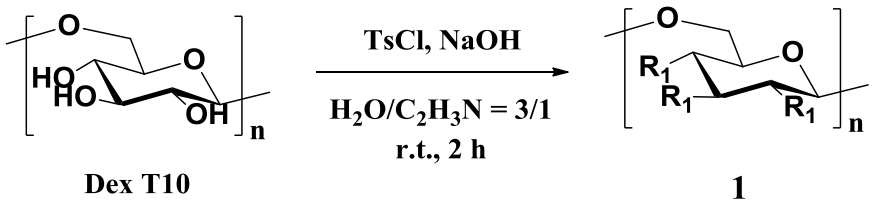
Experimental details:

1. Synthesis of amphiphilic dextran decorated with azide groups



Scheme S1: Synthesis of amphiphilic dextran grafting with azide groups

1.1 Synthesis of Dex-*g*-Ts (1)

8.1 g (50 mmol sugar units) Dextran T10 (Mw: 10 kDa) was completely dissolved in 50 mL water

and followed by drop wise adding 10 mL NaOH solution (5 M). The solution was then stirred at room temperature for 30 min. Paratoluensulfonyl chloride (TsCl, 9.5 g, 50 mmol, 1 eq) was dissolved in 20 mL acetonitrile and added drop wise into the dextran-NaOH solution. Then the solution was stirred at room temperature for 2 hours. The solid appeared in the solution after the reaction was collected and washed with ethanol for 5 times, and then the solvent was evaporated to afford a light yellow powder and dried in vacuum to obtain Dex-*g*-Ts. The partial sulfonation was confirmed by the peaks corresponding to Ts appeared on the ^{1}H NMR spectrum (DMSO-d_6, **Fig. S1**). The number of grafted Ts per 100 sugar units along dextran was about 43, calculated by comparing the integral areas between Ts (-CH_3) signal at 2.39 ppm and dextran ($H_{1,}$ $OH_{2,3,4}$) signal at 4.2-5.3 ppm in the ^{1}H NMR spectra.

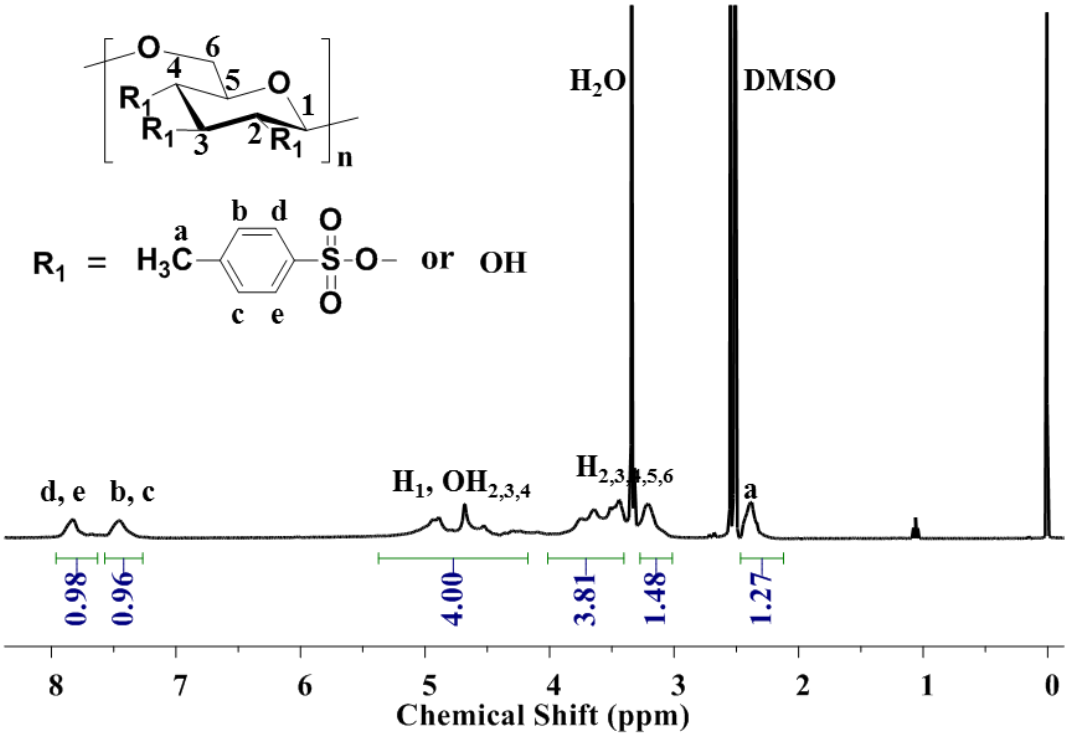


Figure S1. ^{1}H NMR spectrum of Dex-*g*-Ts (1) in DMSO

1.2 Synthesis of Dex-*g*-N_3 (2)

Dex-*g*-Ts (8.5 g, 30 mmol sugar units) was dissolved in 100 mL DMSO and stirred at room temperature for 30 min, followed by adding KI (996 mg, 6 mmol, 0.2 eq) and NaN_3 (3.9 g, 60 mmol, 2 eq). The reaction was then carried out at 70 ℃ for 72 hours. Most of the DMSO was distillated after the reaction and the mixture was dissolved in 50 mL DI water. The resulted solution was dialyzed 3 days against water (MWCO 6-8 kDa cutoff) and then lyophilized to obtain Dex-*g*-N_3. The number of grafted -N_3 per 100 sugar units along dextran was about 14, calculated by comparing the integral areas decrease between Ts (-CH_3) signal at 2.39 ppm and dextran (H_1, $OH_{2,3,4}$) signal at 4.2-5.3 ppm the ^{1}H NMR spectra (**Fig. S2**). And the result was further confirmed by fourier transform infrared spectrometer (FTIR), comparing Dex-*g*-Ts and Dex-*g*-N_3 (**Fig. S3**). The efficiency of modification of dextran with azide group by epichlorohydrin ring-open reaction in aqueous phase (**Fig. S4**) is much lower than that of replacement of sulfonation of dextran by

comparing the -N_3 peak appears on the FTIR.

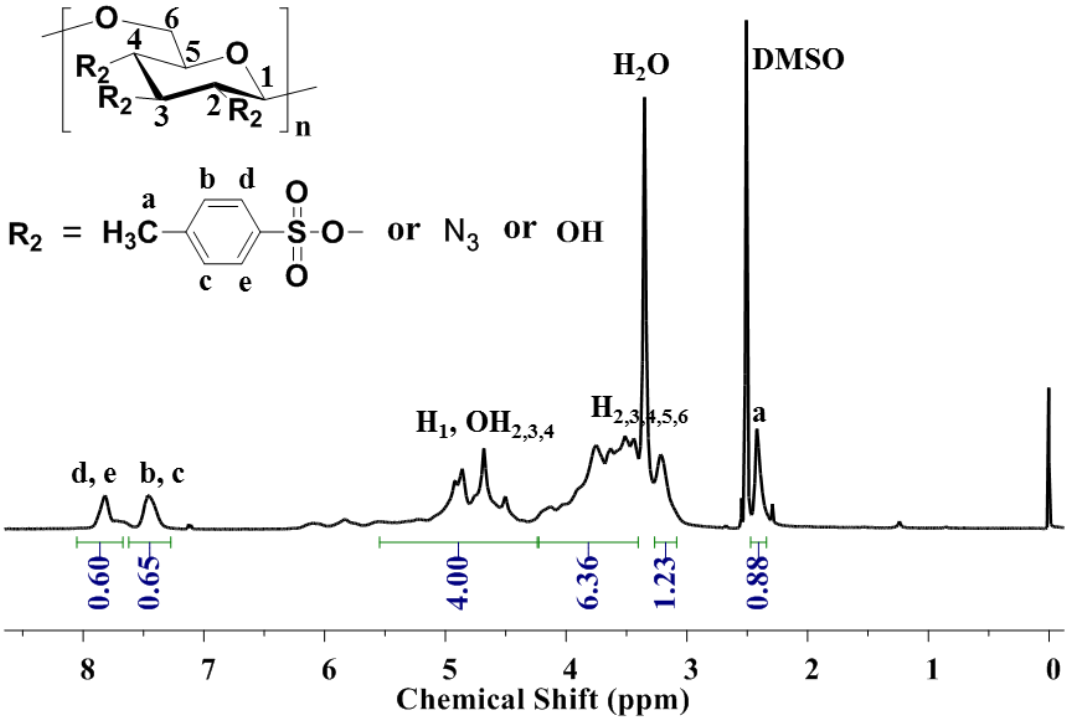


Figure S2. ^{1}H NMR spectrum of Dex-*g*-N_3 (2) in DMSO

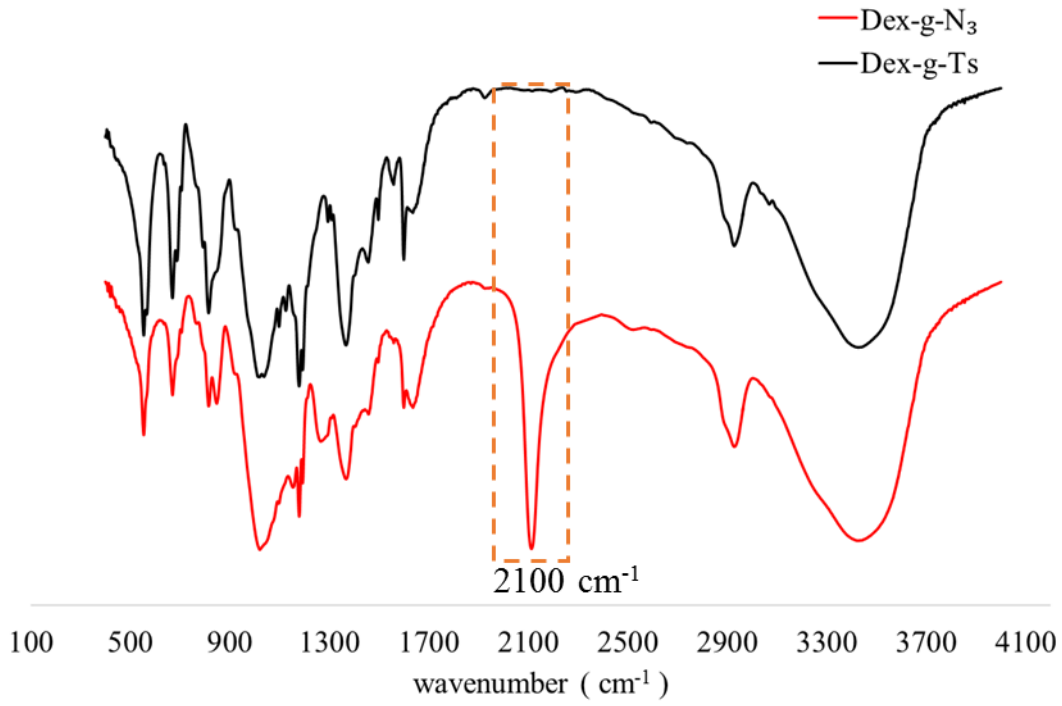


Figure S3. FT-IR spectrum of Dex-*g*-Ts (1) and Dex-*g*-N_3 (2)

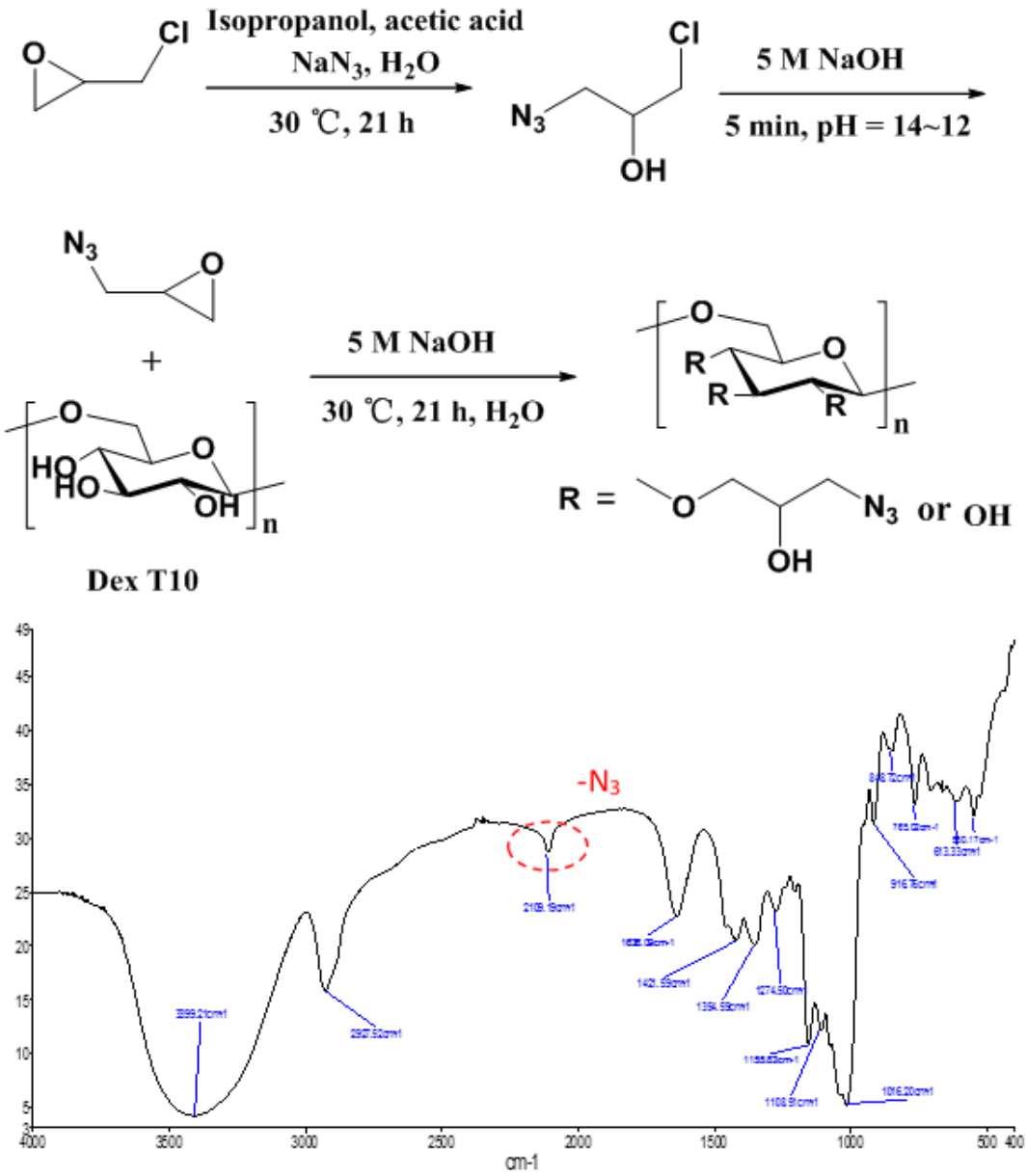


Figure S4. Modification of dextran with azide group by epichlorohydrin ring-open reaction: reaction route and FTIR characterization.

1.3 Synthesis of Dex-*g*-LA/N_3 (3)

Azide modified amphiphilic dextran Dex-*g*-LA/N_3 was synthesized by esterification reaction between the hydroxyl group (-OH) of dextran and the carboxyl group (-COOH) of lauric acid (LA) with the help of carbonyldiimidazole (CDI) (**Scheme S1**). Briefly, 4.0 g (20 mmol) anhydrous LA and 3.4 g (21 mmol, 5% molar excess with regard to the COOH groups) CDI were dissolved in 5 mL of anhydrous THF. The reaction was heated and refluxed under Ar for 3 h. Secondly, 4.6 g dry Dex-*g*-N_3 was dissolved in 35 mL anhydrous DMSO, after it was dissolved completely, the prepared solution of activated LA was injected into the reaction. The mixture was stirred for 24 hours at 70 ℃ under Ar atmosphere. The expected product was dialyzed 3 days against ethanol (MWCO 6-8 kDa cutoff) and then the solvent was evaporated and dried in vacuum to obtain Dex-*g*-LA/N_3. The

number of grafted LA per sugar units along dextran was about 0.29, calculated by comparing the integral areas between ^{1}H NMR spectrum (DMSO-d_6, **Fig. S5**): δ (ppm) 0.84 (-COOCH$_2$CH$_2$(CH$_2$)$_8$C**H**$_3$), 1.24 (-COOCH$_2$CH$_2$(C**H**$_2$)$_8$CH$_3$), 1.53 (-COOCH$_2$C**H**$_2$(CH$_2$)$_8$CH$_3$), 2.34 (-COOC**H**$_2$CH$_2$(CH$_2$)$_8$CH$_3$).

Linear control of LA on dextran backbone (from 0.017 to 0.37 LA per sugar unit) was carried out by adjusting the reaction time (**Fig. S6**). Dex-*g*-LA/N$_3$ nanomicelles can form and stably disperse in aqueous solution at the grafting ratio of LA from 0.09 to 0.29 LA per sugar unit, and corresponding CMC values were listed in Table S1.

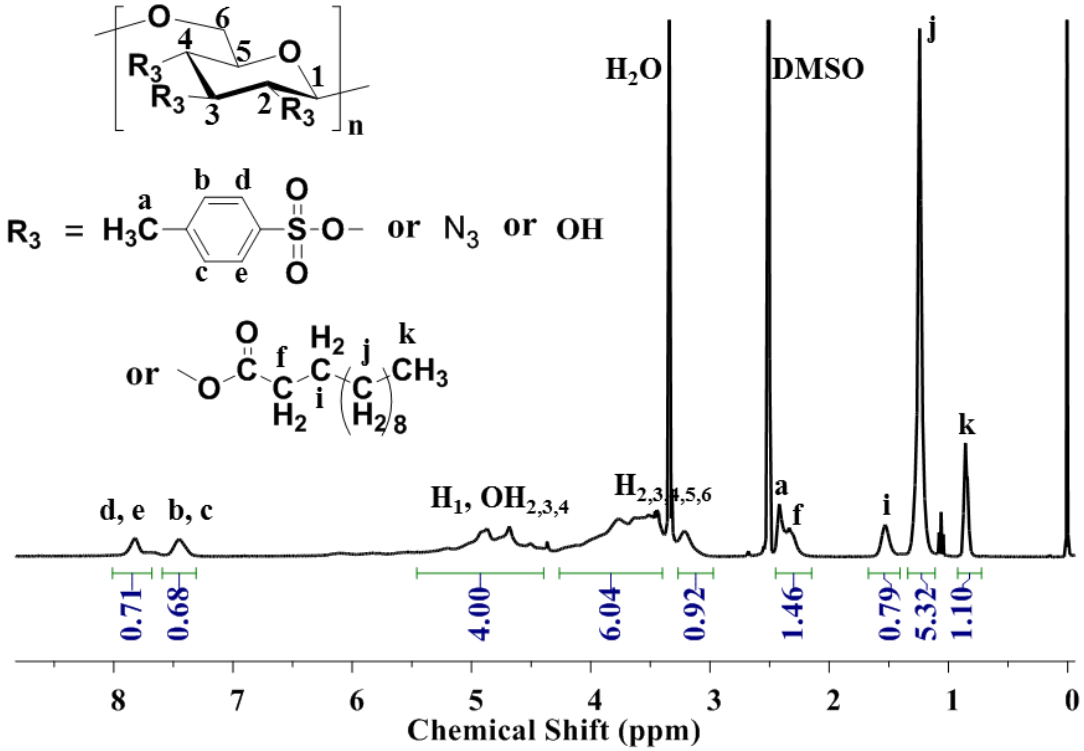
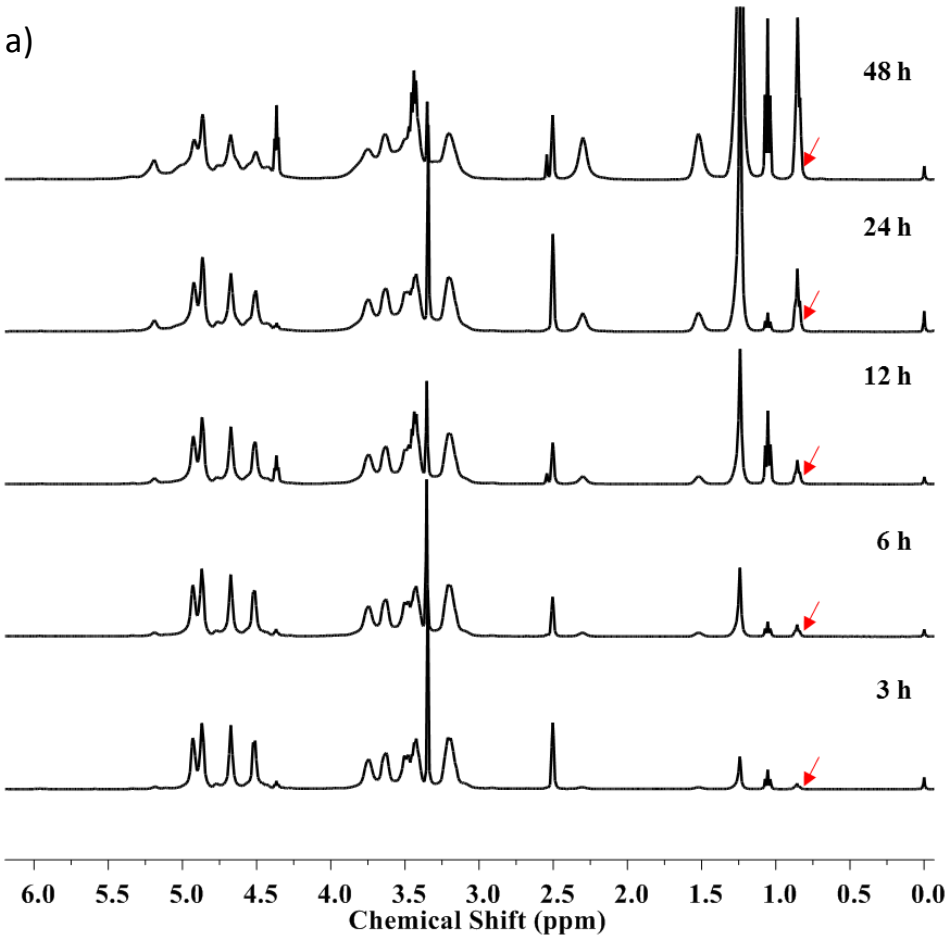


Figure S5. ^{1}H NMR spectrum of Dex-*g*-LA/Azide (3) in DMSO



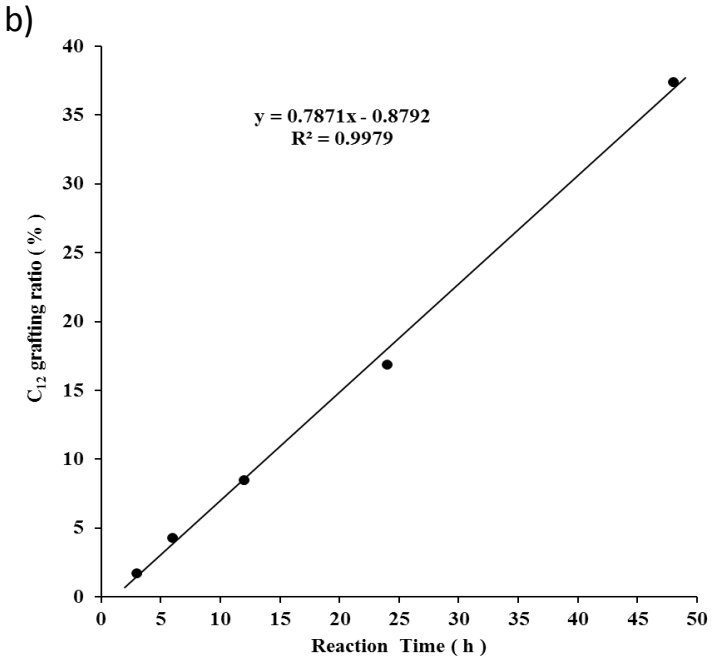


Figure S6. ^{1}H NMR spectrum of Dex-*g*-LA in DMSO at different reaction time (a); Linear control of LA on dextran backbone (b)

Table S1. CMC of Dex-*g*-LA/N_3 with different LA grafting ratio

LA grafting ratio (per sugar unit)	CMC (mg/L)
0.09	14.0
0.18	7.9
0.29	1.2

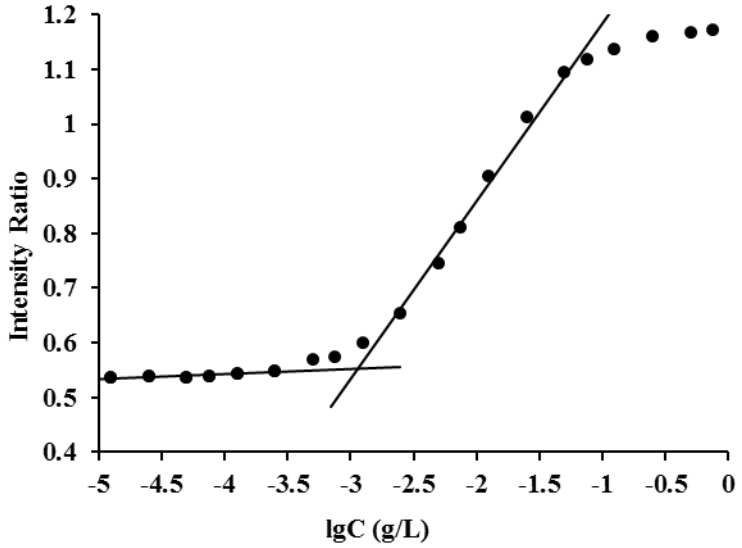


Figure S7. CMC of Dex-*g*-LA/N_3 with 0.29 LA per sugar unit

2. Synthesis of the ligand with alkynyl group (L)

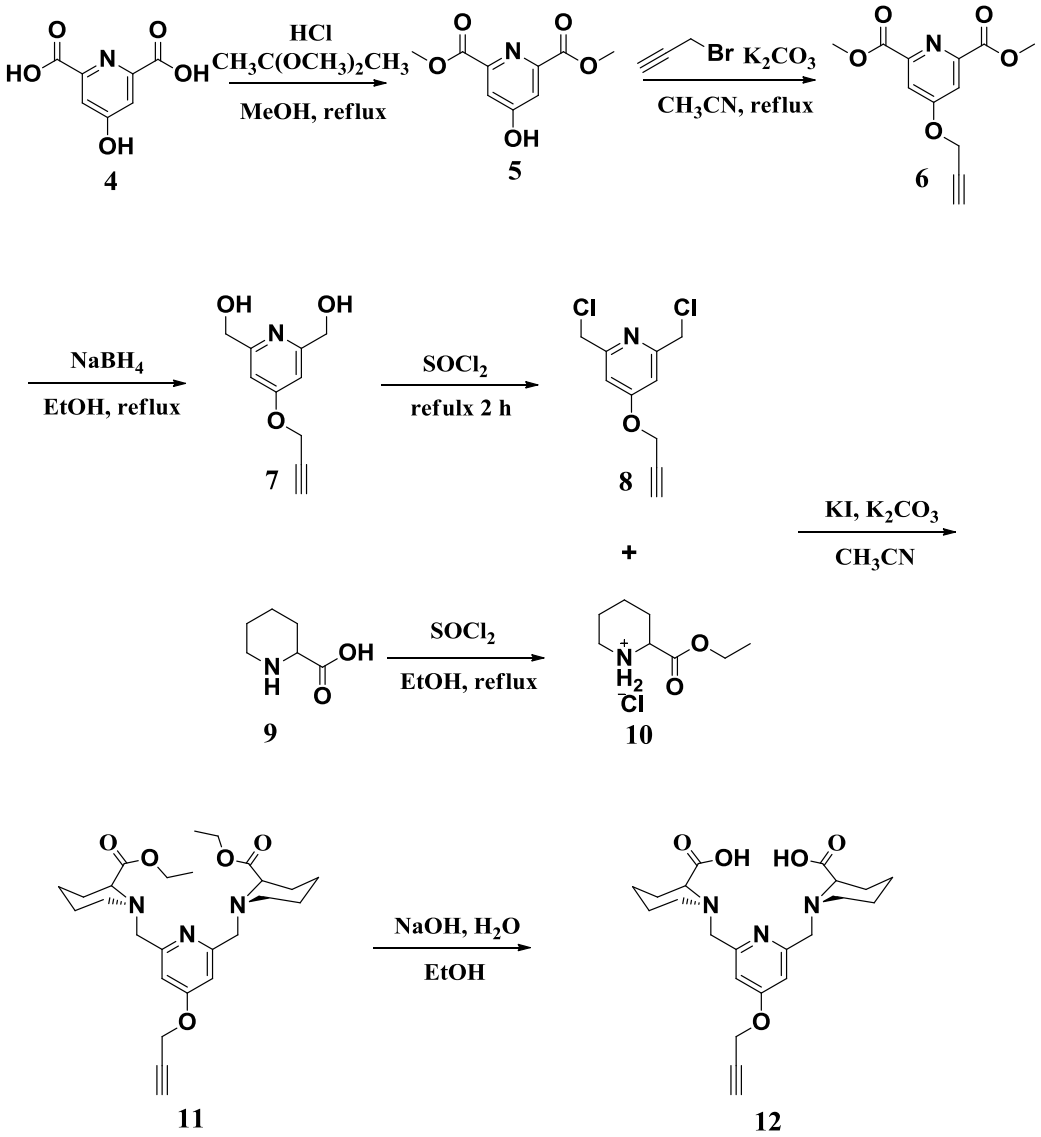
2.1 Synthesis of Dimethyl chelidamic ester hydrochloride (5)[1]

To a suspension of 10.0 g (54.6 mmol) chelidamic acid in 150 mL absolute MeOH was added 60 mL (75 mmol) 2, 2-dimethoxypropane and 7.5 mL (75 mmol) concentrated HCl. The mixture was refluxed for 4 h under a $CaCl_2$ drying tube. Following reflux, the temperature was allowed to go to room temperature, and the reaction was stirred overnight. After evaporation of the solvents and the addition of anhydrous ether (100 mL), the insoluble hydrochloride was filtered to give 11.9 g of crude product (88%) to next step reaction without further purified. ^{1}H NMR (400 MHz, DMSO) δ 7.65 (s, 2H), 3.89 (s, 6H).

2.2 Synthesis of dimethyl 4-(prop-2-yn-1-yloxy)pyridine-2,6-dicarboxylate (6)

Compound 5 (5.0 g, 20 mmol) was dissolved in 150 mL acetonitrile, K_2CO_3 (27.6 g, 200 mmol) was added, the solution was stirred for 30 min. Then, 3.4 mL (30 mmol) 3-bromo-1-propyne solution in toluene was added, heated to reflux for 12 h. Filtered the insoluble from solution, acetonitrile was evaporated off to obtain crude product. 50 mL chloroform added to dissolve the product, washed

three times with 20 mL water in a separatory funnel. Chloroform layer was dried with anhydrous magnesium sulfate. Filtered and evaporated off chloroform to get the product 4.5 g (90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 2H), 4.90 (d, *J* = 2.4 Hz, 2H), 4.05 (s, 6H), 2.65 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.58 (s), 164.99 (s), 149.86 (s), 114.87 (s), 77.64 (s), 77.23 (s), 56.40 (s), 53.35 (s). ESI-MS (m/z): 250.07 $(M+H)^+$, 272.05 $(M+Na)^+$, 288.03 $(M+K)^+$



Scheme S2: Synthesis route of the ligand with alkynyl group (L)

2.3 Synthesis of (4-(prop-2-yn-1-yloxy)pyridine-2,6-diyl)dimethanol (7)[2]

To a solution of compound 6 (4.5 g, 18 mmol) in 80 mL ethanol at 0 ℃ was added 2.7 g (72 mmol) $NaBH_4$ slowly. The reaction mixture was stirred at room temperature for 2 h and then heated to reflux for 5 h. The crude mixture was concentrated under vacuum and 100 mL saturated aqueous solution of potassium carbonate was added. The result mixture was stirred at 60 ° C for 2 h and then extracted with chloroform (100 mL, three times). The organic phase dried over sodium sulfate and concentrated. Compound 7 was obtained as a white solid; 2.8 g (80%). ^{1}H NMR (400 MHz, DMSO) δ 6.92 (s, 2H), 5.41 (s, 2H), 4.90 (d, J = 2.4 Hz, 2H), 4.48 (s, 4H), 3.66 (t, J = 2.4 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 165.21 (s), 163.53 (s), 105.08 (s), 79.37 (s), 79.01 (s), 64.40 (s), 55.75 (s). ESI-MS (m/z): 194.08 $(M+H)^+$, 216.06 $(M+Na)^+$

2.4 Synthesis of 2,6-bis(chloromethyl)-4-(prop-2-yn-1-yloxy)pyridine (8)[3]

Compound 7 (2.5 g, 13 mmol) was added slowly to 20 mL of $SOCl_2$ at 0 ℃. The reaction mixture was stirred at room temperature for 1 h and then heated to reflux for 2 h. The crude mixture was concentrated under vacuum and 20 mL of H_2O was added. The solution was filtrated and saturated aqueous solution of sodium bicarbonate was added in drops into the filtrate. The precipitate was isolated by filtration to afford compound 8; 2.4 g, (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 2H), 4.85 (d, *J* = 2.4 Hz, 2H), 4.73 (s, 4H), 2.66 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.49 (s), 157.20 (s), 109.51 (s), 77.59 (s), 77.23 (s), 56.34 (s), 44.87 (s). ESI-MS (m/z): 230.01 $(M+H)^+$

2.5 Synthesis of Ethyl piperidine-2-carboxylate (10)

To a solution of 5.0 g (39 mmol) DL-2-Piperidinecarboxylic acid in 150 mL ethanol at 0 ℃ was added 4.5 mL (58 mmol) $SOCl_2$ slowly. The result mixture was heated to reflux for 2 h and then concentrated under vacuum to give a crude oil. A 100 mL ethyl ether was added into the crude oil and stirred for several minutes, the suspension was filtrated to afford compound 10 as a white solid; 7.1 g, (93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.11 (s, 1H), 9.70 (s, 1H), 4.30 (d, *J* = 5.9 Hz, 2H), 3.94 (s, 1H), 3.69 (d, *J* = 33.7 Hz, 1H), 3.13 (s, 1H), 2.42 – 1.49 (m, 7H), 1.30 (t, J = 7.1 Hz, 3H). ESI-MS (m/z): 158.15 $(M+H)^+$

2.6 Synthesis of diethyl (1,1'-((4-(prop-2-yn-1-yloxy)pyridine-2,6-diyl)bis(methylene))bis(piperidine-2,1-diyl))diformate (11)

Compound 10 (5.8 g, 30 mmol) and anhydrous potassium carbonate (10 g, 100 mmol) was dissolved in 80 mL dry acetonitrile and Stirred at room temperature for 30 min. Then, Compound 8 (2.3 g, 10 mmol) and KI (166 mg, 1 mmol) were added in. The reaction mixture was refluxed until full conversion was observed by TLC. The crude product was purified by chromatography on silica gel (dichloromethane/ethyl acetate = 10/1) and compound 11 was obtained as a colorless oil; 3.8 g, (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (s, 2H), 4.78 (d, *J* = 2.4 Hz, 2H), 4.19 (q, 4H), 3.86 (d, *J* = 14.9 Hz, 2H), 3.59 (d, *J* = 14.9 Hz, 2H), 3.26 (m, 2H), 3.08 – 2.93 (m, 2H), 2.56 (t, *J* = 2.4 Hz, 1H), 2.28 (m, 2H), 1.96 – 1.34 (m, 12H), 1.27 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.78 (s), 165.02 (s), 160.49 (s), 107.31 (s), 77.68 (s), 76.05 (s), 64.28 (s), 61.86 (s), 60.31 (s), 55.42 (s), 50.40 (d, *J* = 3.7 Hz), 29.55 (s), 25.37 (s), 22.28 (s), 14.32 (s). ESI-MS (m/z): 472.28 $(M+H)^+$, 494.26 $(M+Na)^+$.

2.7 Synthesis of (1,1'-((4-(prop-2-yn-1-yloxy)pyridine-2,6-diyl)bis(methylene))bis(piperidine-2,1-diyl))diformic acid (L, 12)

Compound 11 (2.4 g，5 mmol) and NaOH (800 mg, 20 mmol) was stirred in ethanol (25 mL, 80% aq.) at room temperature until full conversion was observed by TLC. PH value of the result mixture was adjusted to 5.0 by adding HCl (1.0 M aq.), and the solvent was evaporated to afford a stringy solid. The residue was taken up in 50 mL chloroform and then dried over sodium sulfate and concentrated. Compound 12 was obtained as a white solid; 2.0 g, (95%). ^{1}H NMR (400 MHz, D_2O) δ 7.12 (d, *J* = 5.8 Hz, 2H), 3.92 (dd, *J* = 13.0, 4.7 Hz, 2H), 3.34 (t, *J* = 13.2 Hz, 2H), 2.81 (d, *J* =

10.6 Hz, 4H), 2.07 (t, J = 10.6 Hz, 2H), 1.99 – 1.84 (m, 2H), 1.84 – 1.19 (m, 12H). ESI-MS (m/z): 416.22 $(M+H)^+$

3. Cu (I) catalyzed azide alkyne cycloaddition

After we successfully obtained both amphiphilic dextran with azide groups (Dex-*g*-LA/N_3) and ligand with alkynyl group (L), copper(I)-catalyzed click chemistry reaction[4] was applied to connecting these two part together. Briefly, Dex-*g*-LA/N_3, CuBr (10 mol% per azide), Tris[(1-benzyl-1H-1,2,3-triazol-4-yl) methyl] amine (TBTA,10 mol% per azide group) and ligands were dissolved in water/DMSO 1:3 mixture and formed a cloudy solution. The reaction mixture was heated in an oil bath at 60 ℃ for 2 days. The copper ion-complexing ligand N,N,N',N'',N''-pentamethyldiethylenetriamine (5 eq to the copper ion) were dissolved in the reaction mixture and stirred for 24 hrs. The expected product was collected after the resulted solution was dialyzed 3 days against water (MWCO 10 kDa cutoff) and then lyophilized to obtain Dex-*g*-LA/L.

4. Preparation of Dex-*g*-LA/MnL nanomicelles

10 mg Dex-*g*-LA/L was dissolved in 1 mL THF, then the mixture was added into 10 mL MilliQ water under probe sonication, Dex-*g*-LA/L nanomicelles in water were obtained after evaporation of THF. Manganese chloride (5-10 times molar excess with regard to the L) was added into the micelles in water and stirred at room temperature overnight. The uncoordinated Mn (II) was removed by dialyzing the mixture for 3 days against water (MWCO 10 kDa cutoff).

5. T_1 relaxivity studies *in vitro*

T_1 relaxivity of Dex-*g*-LA/MnL nanomicelles and MnL were measured at 1.5 T on a clinical MR scanner (Siemens, Sonata) at room temperature as described before.[5] Concentration of Mn in Dex-*g*-LA/MnL nanomicelles or MnL solution was measured by elemental analyses using atomic absorption spectroscopy. Then, a series of aqueous solution samples with different Mn concentration (0.5, 0.4, 0.3, 0.25, 0.2, 0.15 and 0.1 mM) were prepared by dilution with water. The T_1-weighted images were acquired with a conventional spin echo acquisition with TR values ranging from 20 to 1000 ms (TE = 5.3 ms) at room temperature. Then, signal intensities collected were used to calculate T_1 times under different Mn concentration. Relaxivity values of r_1 were calculated through the curve fitting of $1/T_1$ time (s^{-1}) versus the manganese concentration (mM).

6. *In vivo* MRI studies

All studies involving animals were approved by the Animal Care and Use Committee of the Institute. MR imaging was carried out on a clinical 3.0 T scanner (Achieva, Philips). Sprague-Dawley (SD) Rats (180 - 220 g) were anaesthetized by pentobarbital sodium at the dose of 40 mg/kg body weight and placed within a 50 mm rat coil. After intravenous injection (via tail vein) of a total 0.5 mL solution of MnL or Dex-*g*-LA/MnL nanomicelles with the dosages of 0.1 Mn mmol/kg body weight, dynamic T_1-weighted images of chest and cervical region were obtained by a 3DCEMRA sequence (T1FFE, TE = 3 ms, TR = 7 ms, slice thickness = 0.9 mm, field of view = 100 mm × 100 mm, flip

angle 30 °). T_1-weighted MR images of hepar and kidney at before and 2 hours after administration of Dex-*g*-LA/MnL nanomicelles (0.1 Mn mmol/kg BW) were obtained with a T_1-weighted Fast Field Echo (T1TFE) imaging sequence (TE = 3 ms, TR = 10 ms, slice thickness = 2.0 mm, field of view = 65 mm × 65 mm, flip angle 15 °).

References

1. **R. Chênevert and M. Dickman, *The Journal of Organic Chemistry*, 1996, 61, 3332-3341.**
2. **K. Chifuku, T. Sawada, Y. Kuwahara and H. Shosenji, *Molecular Crystals and Liquid Crystals*, 2007, 470, 369-381.**
3. **U. Engelhardt, B. M. Rapko, E. N. Duesler, D. Frutos, R. T. Paine and P. H. Smith, *Polyhedron*, 1995, 14, 2361-2369.**
4. **T. R. Chan, R. Hilgraf, K. B. Sharpless and V. V. Fokin, *Organic Letters*, 2004, 6, 2853-2855.**
5. **H. Su, C. Wu, J. Zhu, T. Miao, D. Wang, C. Xia, X. Zhao, Q. Gong, B. Song and H. Ai, *Dalton Transactions*, 2012, 41, 14480-14483.**

Figures:

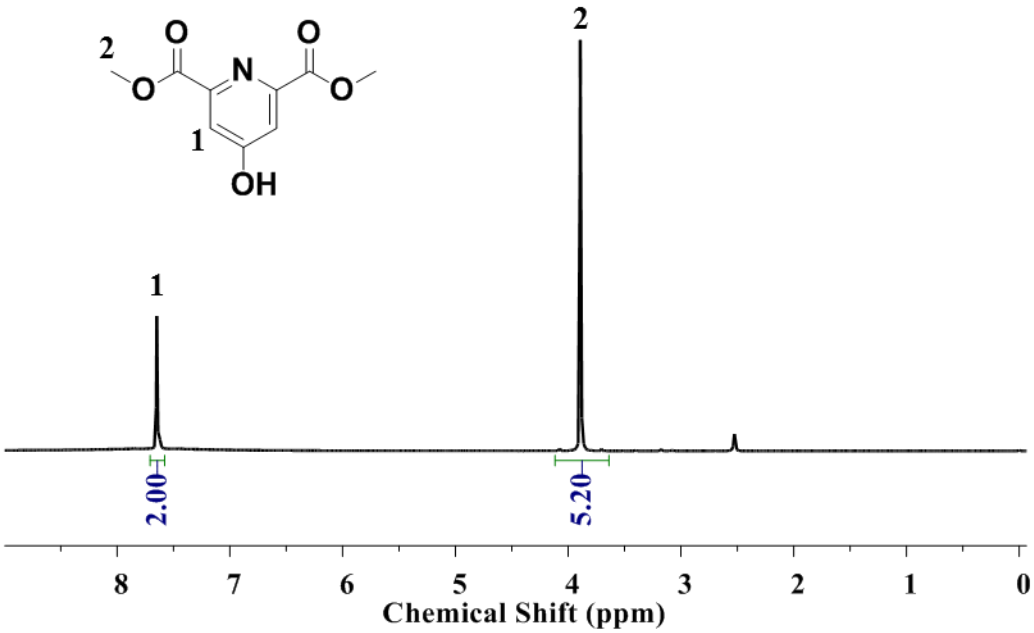


Figure S8. ^{1}H NMR spectrum of compound 5 in DMSO

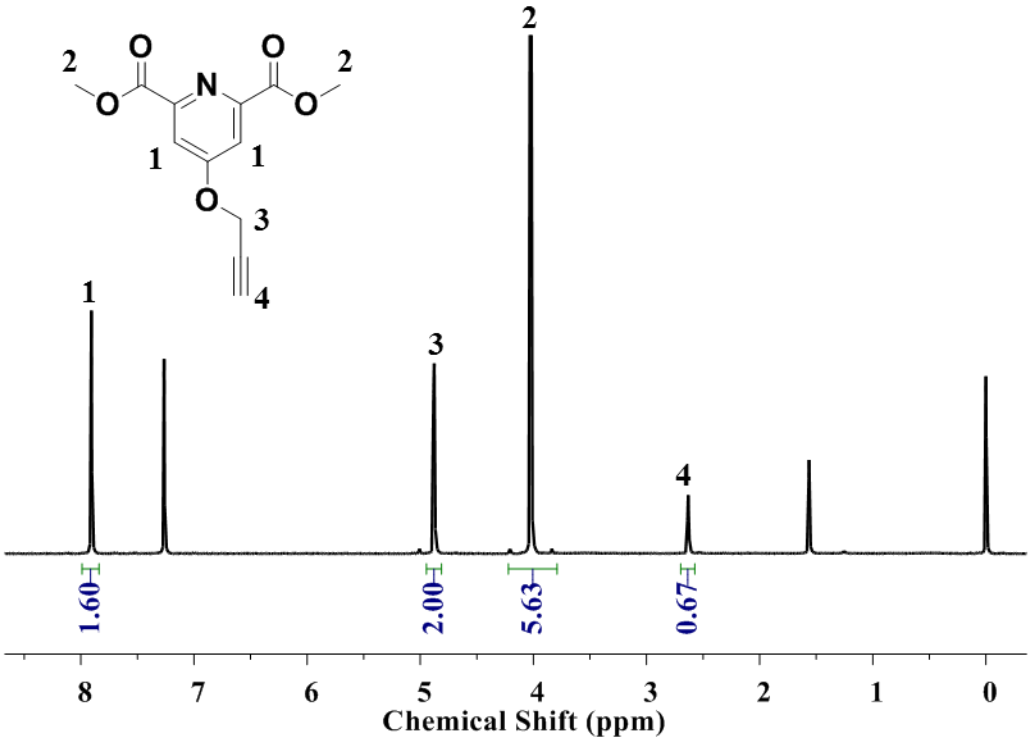


Figure S9. ^{1}H NMR spectrum of compound 6 in $CDCl_3$

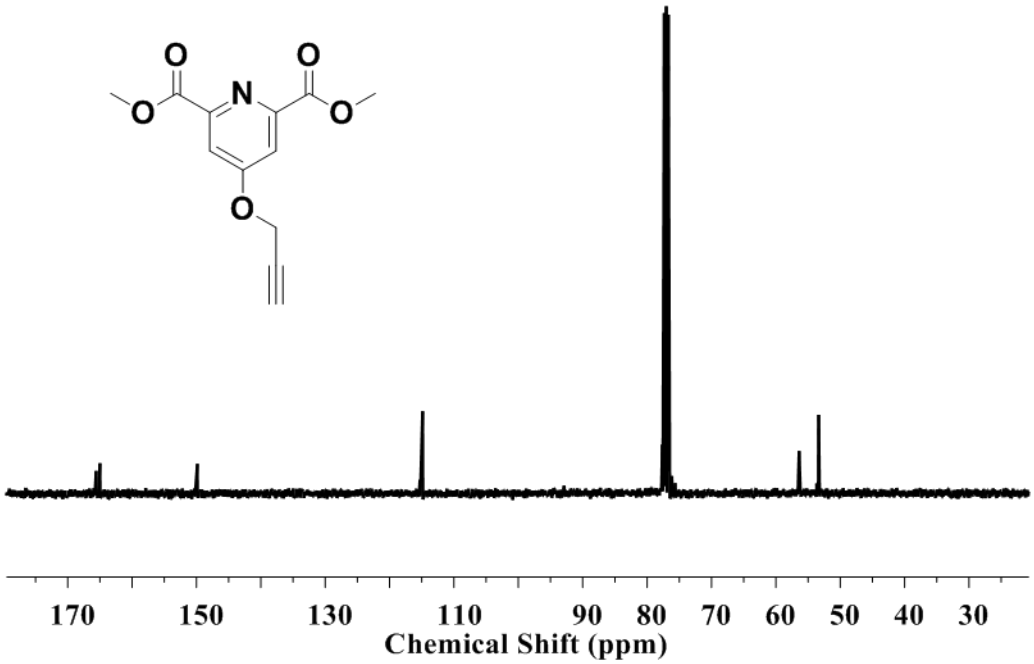


Figure S10. ^{13}C NMR spectrum of compound 6 in $CDCl_3$

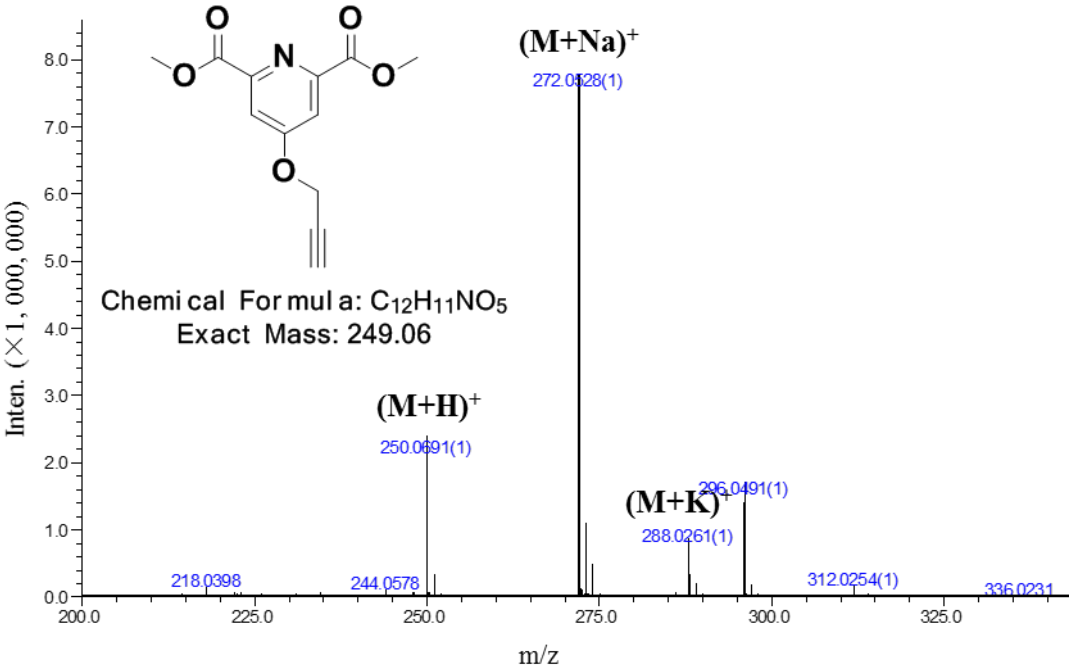


Figure S11. ESI-MS spectrum of compound 6

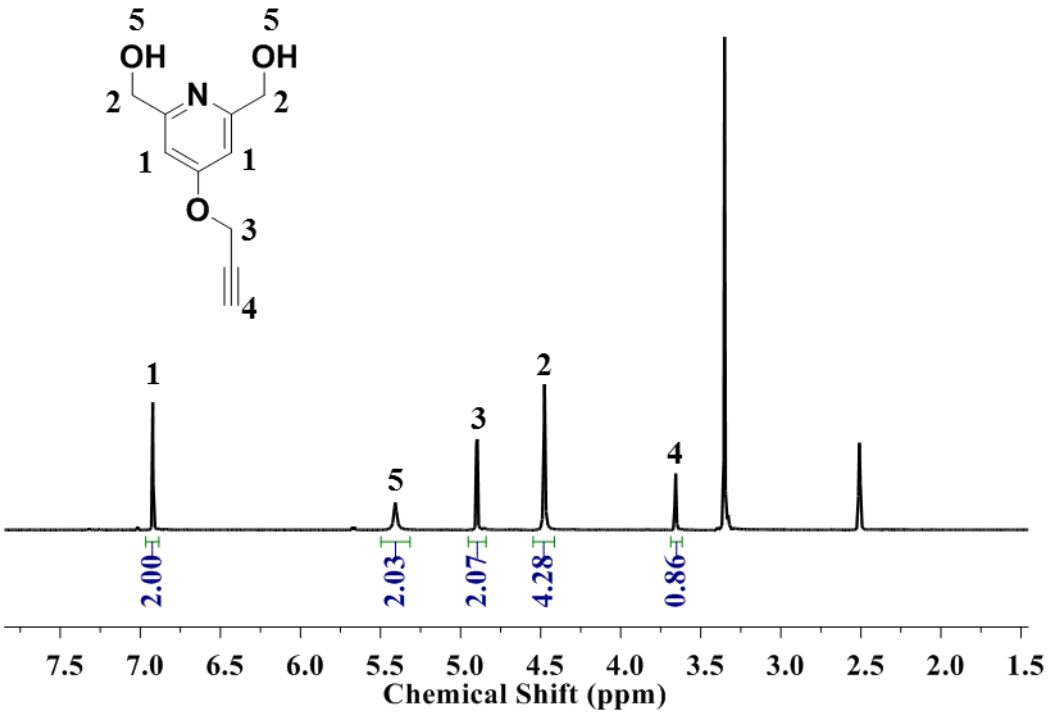


Figure S12. ^{1}H NMR spectrum of compound 7 in DMSO

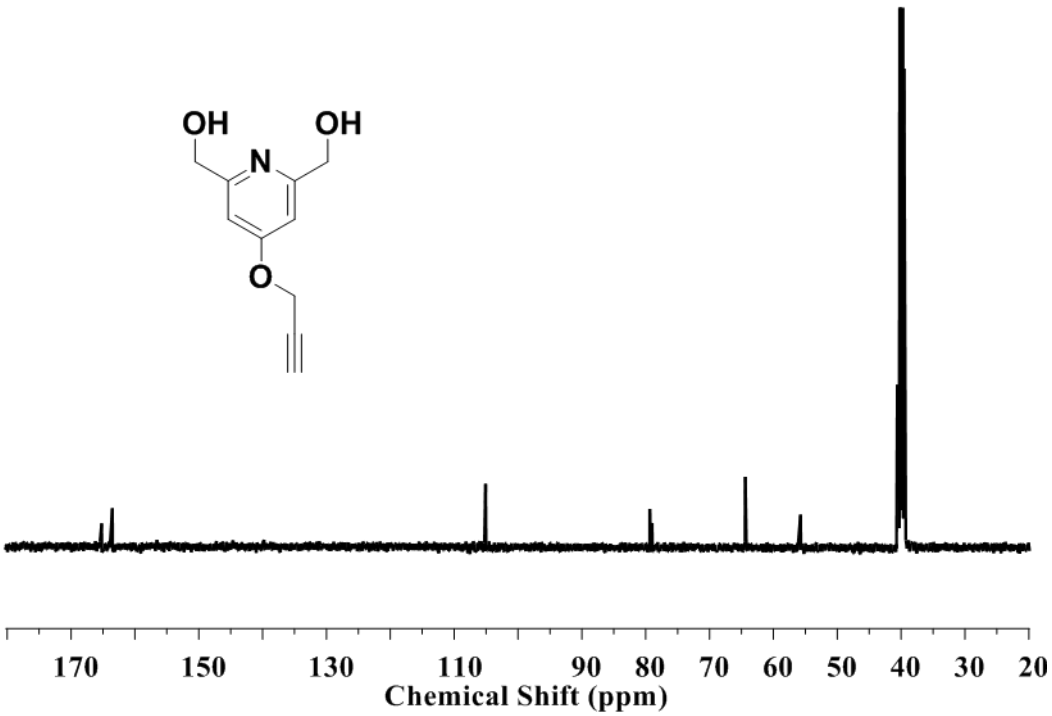


Figure S13. ^{13}C NMR spectrum of compound 7 in DMSO

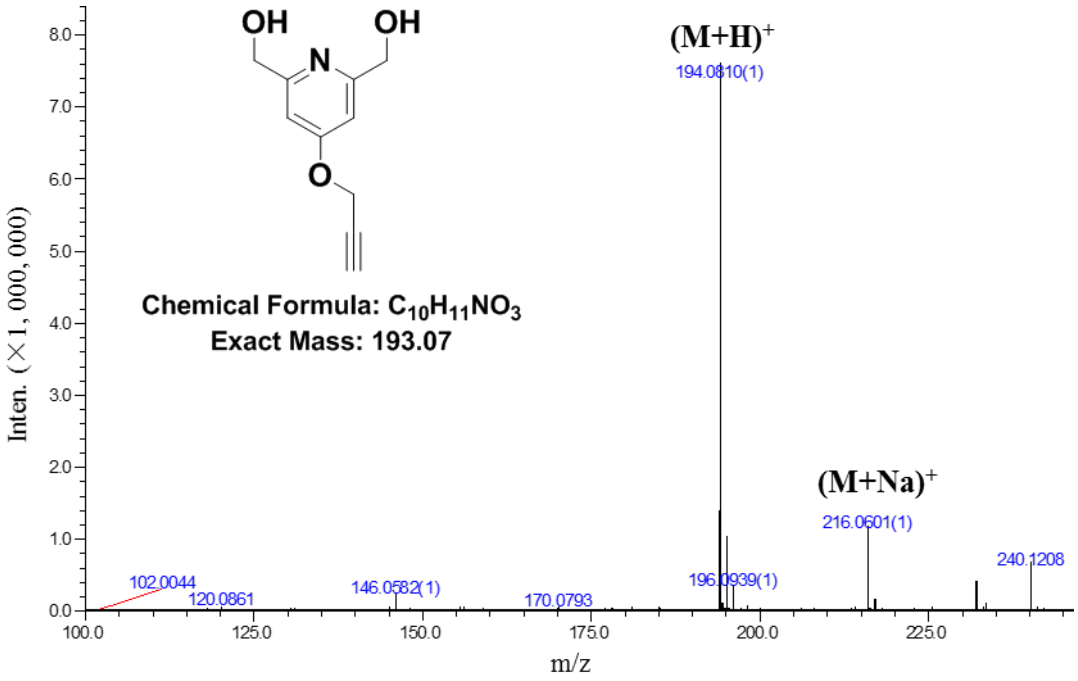


Figure S14. ESI-MS spectrum of compound 7

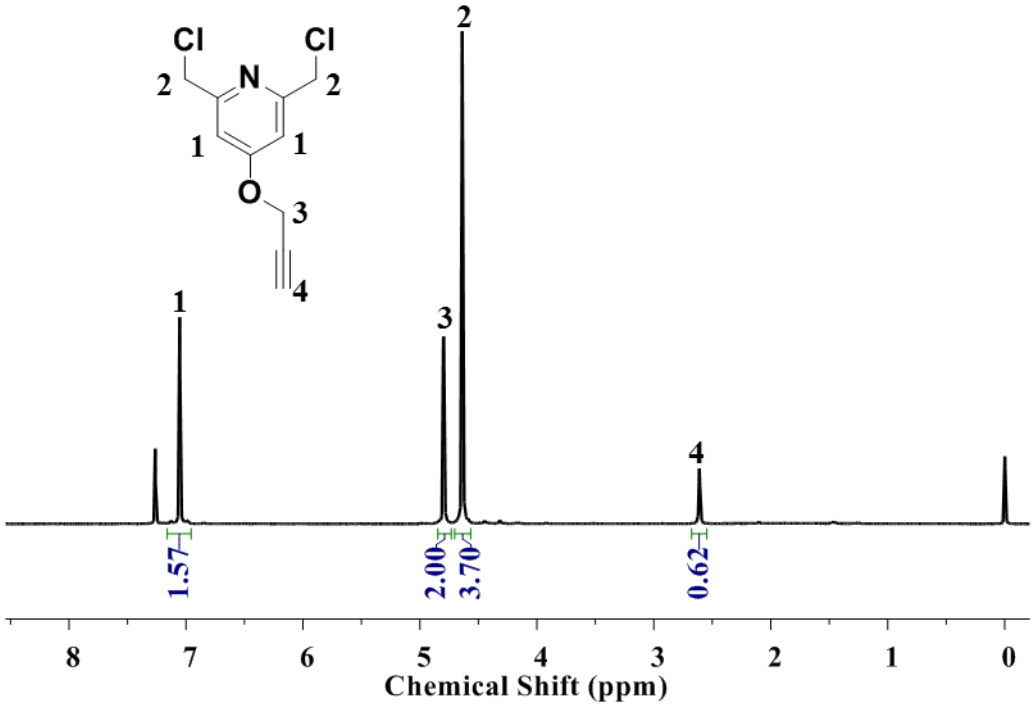


Figure S15. ^{1}H NMR spectrum of compound 8 in $CDCl_3$

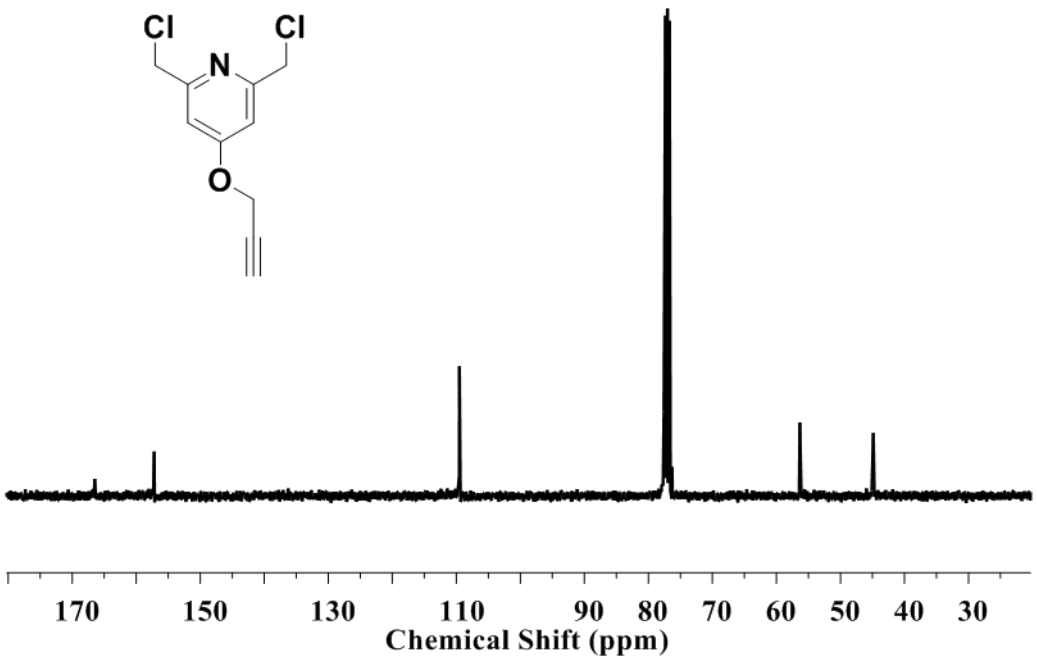


Figure S16. ^{13}C NMR spectrum of compound 8 in $CDCl_3$

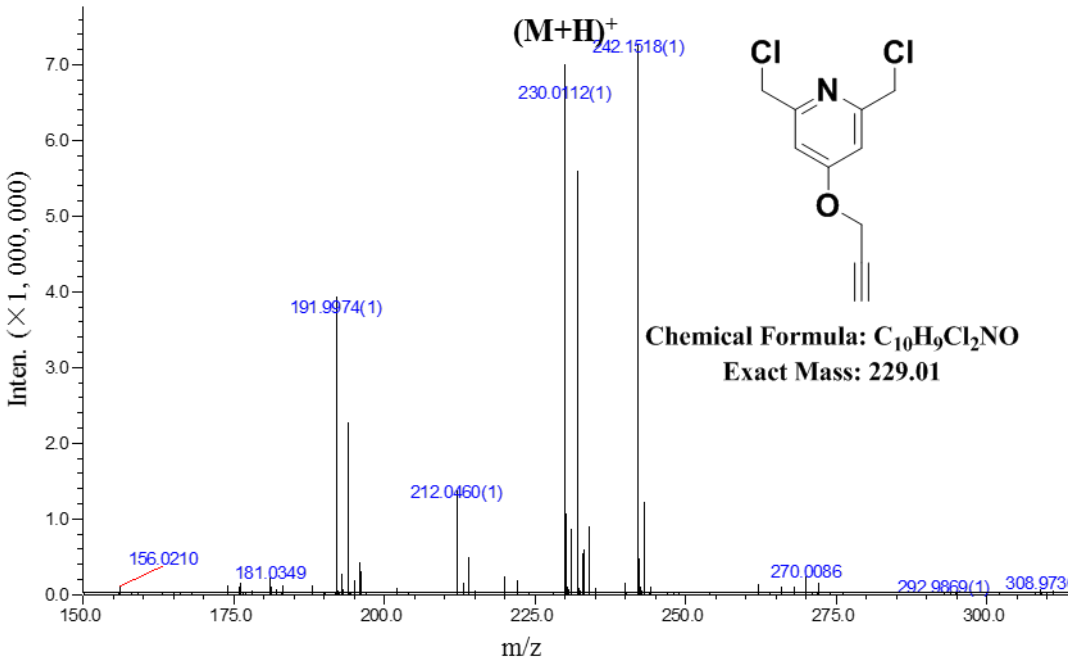


Figure S17. ESI-MS spectrum of compound 8

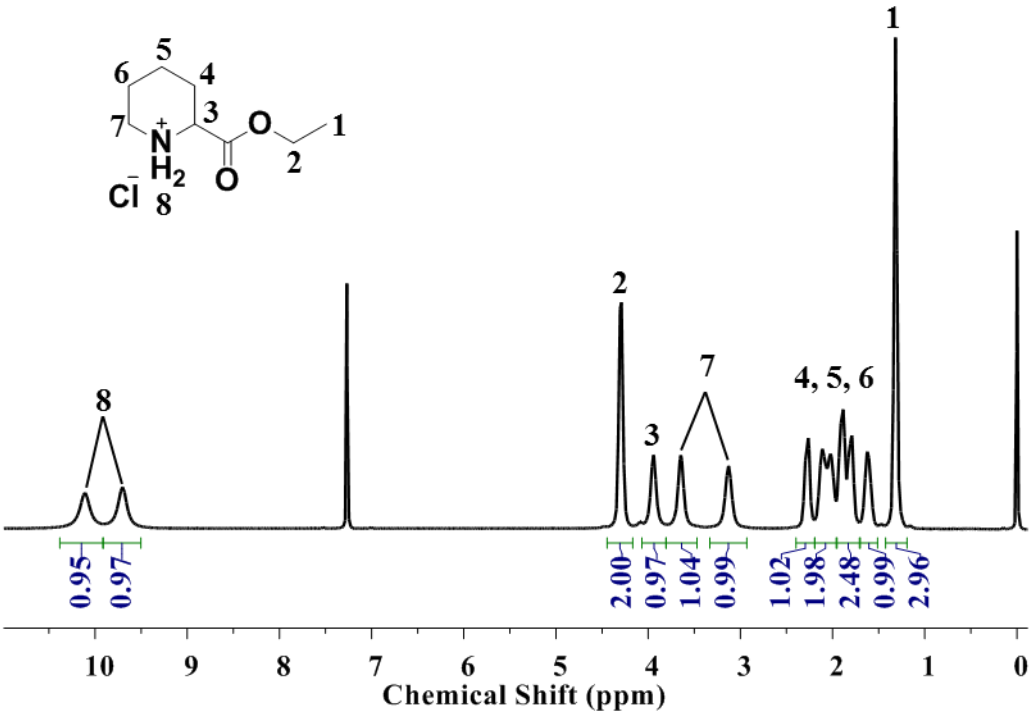


Figure S18. ^{1}H NMR spectrum of compound 10 in $CDCl_3$

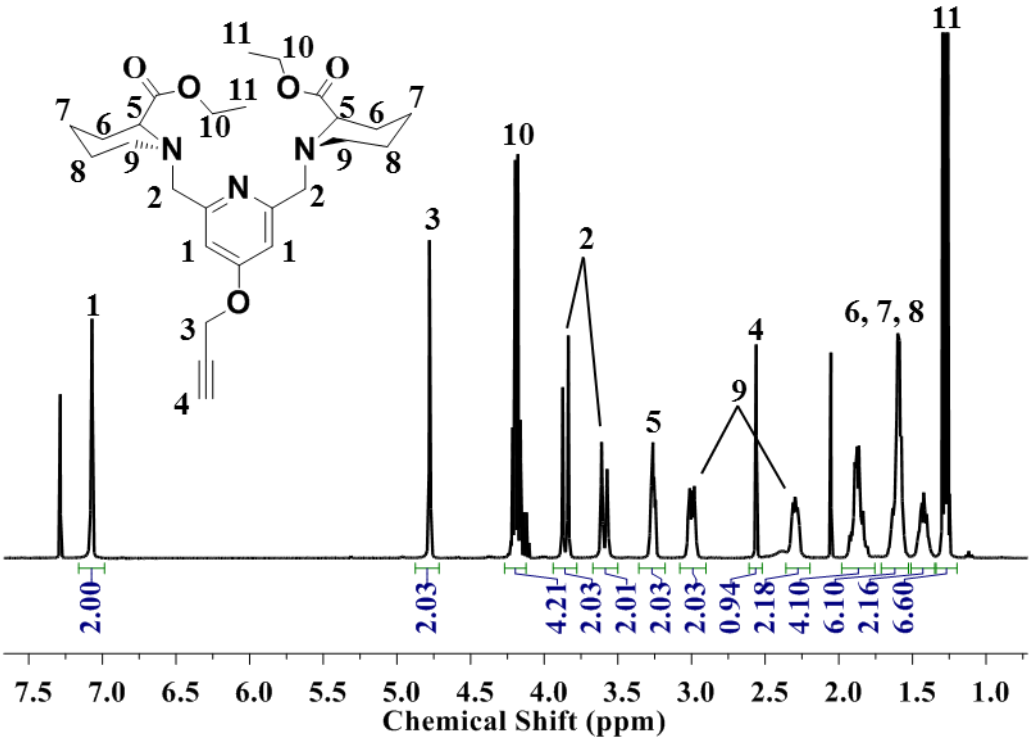


Figure S19. ^{1}H NMR spectrum of compound 11 in $CDCl_3$

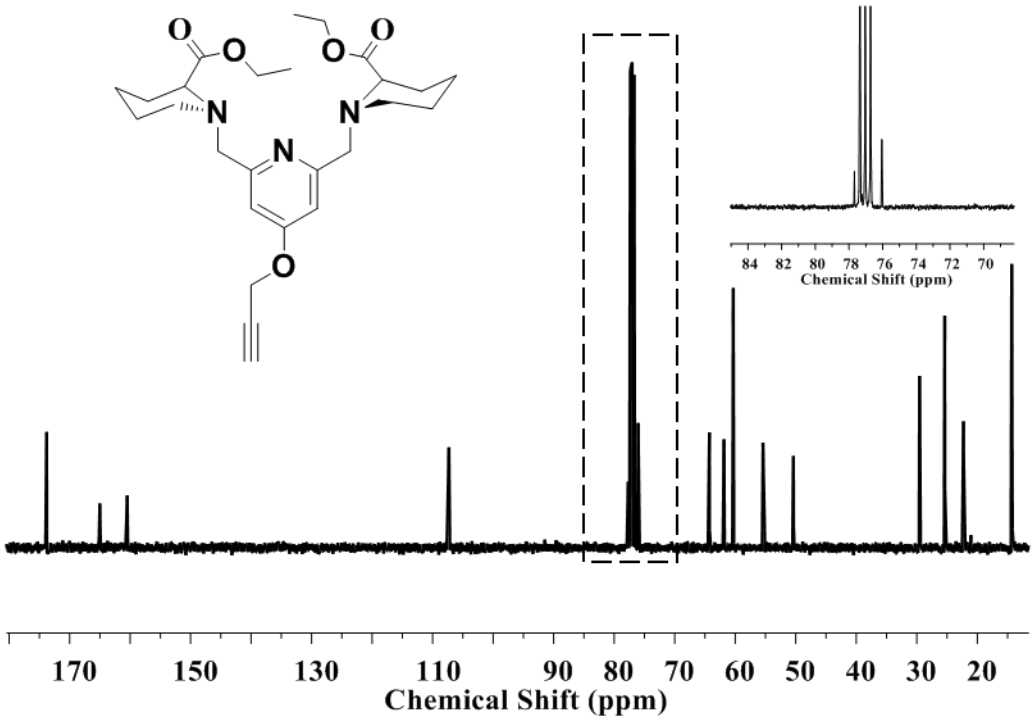


Figure S20. ^{13}C NMR spectrum of compound 11 in $CDCl_3$

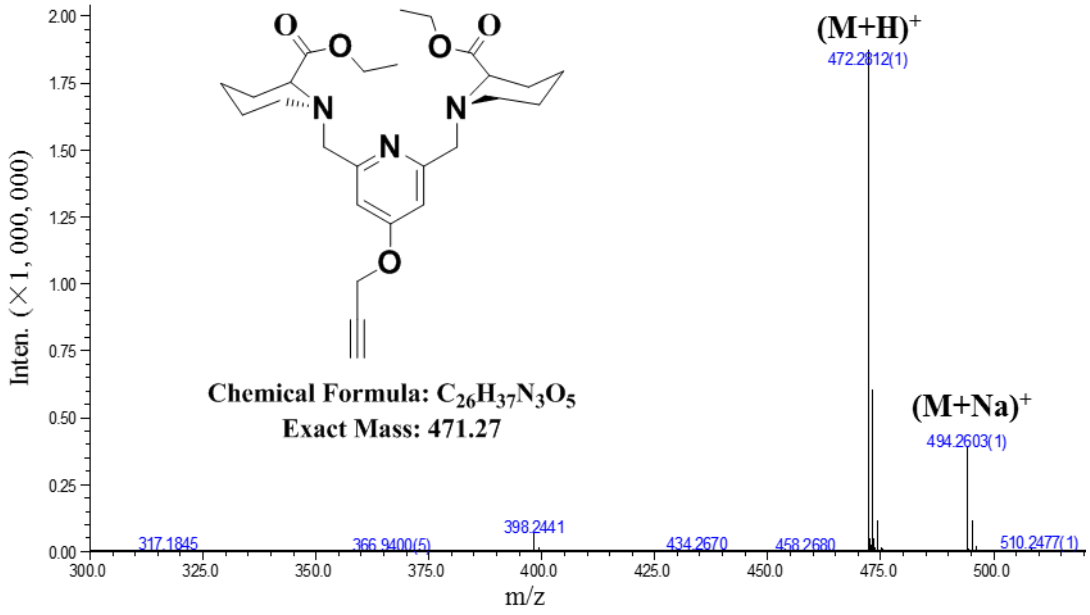


Figure S21. ESI-MS spectrum of compound 11

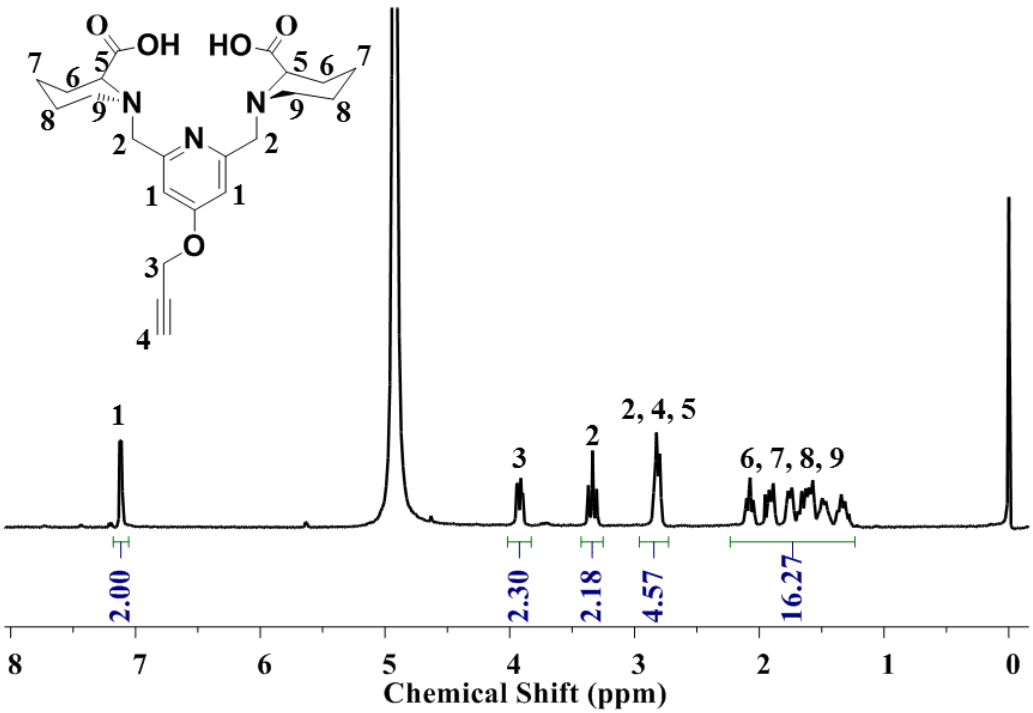


Figure S22. ^{1}H NMR spectrum of compound 12 in D_2O

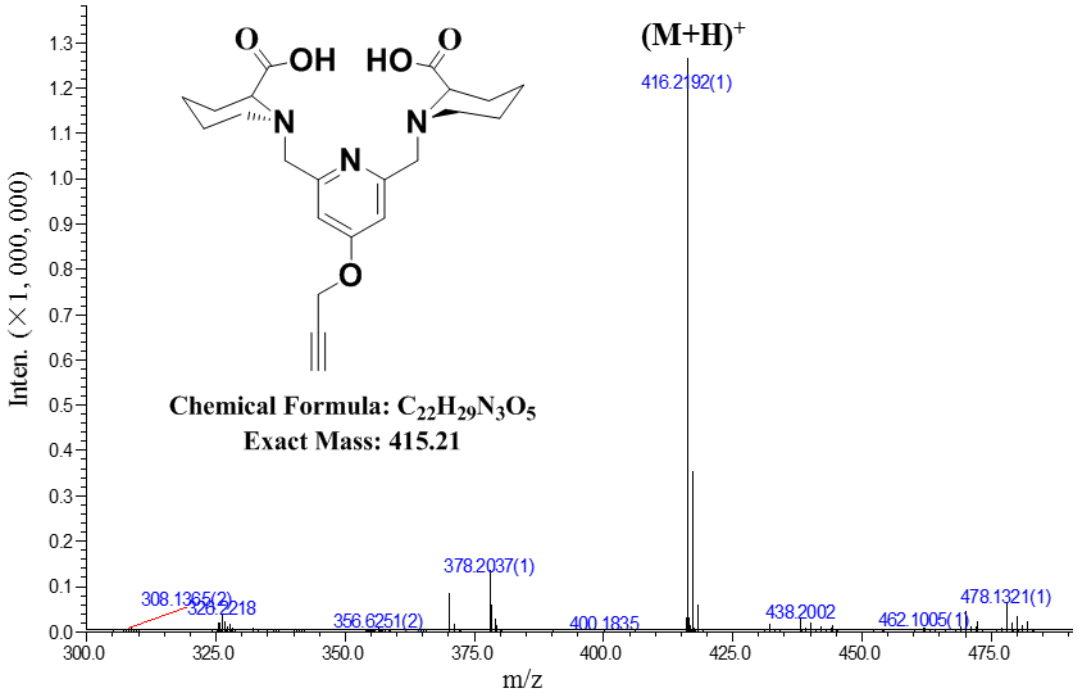


Figure S23. ESI-MS spectrum of compound 12