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Lysosome-Oriented, Dual Stages pH-Responsive Polymeric Micelles for β-Lapachone Delivery

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Synthesis of prodrug (I), (V) and (VI)

The three prodrugs were synthesized using the same method. For example, prodrug (IV) was prepared in the following procedure. β-lap (100 mg, 0.41 mmol), acetic hydrazine (49.7mg, 0.45 mmol) (or equivalent mole of 5-amino-1-pentanol, or 4-amino-2-methyl-phenol) and 100 μL HOAc were mixed with 5 mL anhydrous methanol and kept refluxing in a single neck flask for 24 hrs. After removing the solvent under vacuum, the residues were redissolved in 30 mL of EtOAc, followed by rinse with saturated $NaHCO_3$ aqueous solution 3 times, and saturated NaCl aqueous solution for another 3 times. 53.5 mg of prodrug (IV) was obtained by silicone gel chromatography with eluent of Hexane/ EtOAc= 4:1.

(Z)-N'-(2,2-dimethyl-5-oxo-3,4-dihydro-2H-benzo[h]chromen-6(5H)-ylidene)acetohydrazide (I), yield, 80.2%, **MS:** 299.1 $[M+H]^+$, **^{1}HNMR** (500MHz, $CDCl_3$) δ: 8.121 (1H, s), 7.861-7.864 (1H, d, J=7.5), 7.491-7.427(2H, m), 2.560-2.534 (2H, t, J=6.5), 2.468 (3H, s), 1.849-1.823(2H, t, J=6.5), 1.661(1H, s), 1.437 (6H, s). **^{13}CNMR** (125MHz, $CDCl_3$) δ: 181.5(1C), 175.2(1C), 161.2(1C), 131.8(1C), 129.9(1C), 128.6(1C), 126.9(1C), 123.3(3C), 111.4(1C), 78.5(1C), 31.6 (1C), 26.8(2C), 19.9(1C), 16.0(1C).

(E)-6-(5-hydroxypentylimino)-2,2-dimethyl-3,4-dihydro-2H-benzo[h]chromen-5(6H)-one (V), yield 52%, **MS:** 326.2 $[M+H]^+$, **^{1}HNMR** (500MHz, $CDCl_3$) δ: 8.331-8.314 (1H, d, J=7.0), 8.278-8.261 (1H, d, J=7.0), 7.582-7.552 (1H, t, J=7.5), 7.469-7.438 (1H, t, J=7.5), 3.728-3.703 (2H, t, J=6.5Hz), 3.063-2.991 (4H, m), 2.063-2.004 (2H, m), 1.966-1.940 (2H, t, J=6.5), 1.771-1.716(2H, m), 1.455(6H, s). **^{13}CNMR**(125MHz,$CDCl_3$) δ: 164.5 (1C), 147.8 (1C), 147.4 (1C), 126.9 (1C), 125.2 (1C), 124.5 (2C), 123.9 (1C), 122.8 (1C), 121.8 (1C), 102.0 (1C), 75.3 (1C), 62.3 (1C), 32.4 (1C), 32.0 (1C), 26.9 (2C), 26.1 (1C), 23.4(1C), 17.6 (1C).

3,3,11-trimethyl-2,3,13,13a-tetrahydrobenzo[a]pyrano[2,3-c]phenol-xazin-12(1H)-one (VI). Yield, 43%, **MS:** 346.1 $[M+H]^+$, **^{1}HNMR** (500MHz,$CDCl_3$) δ: 8.821-8.805 (1H, d, J=8.0Hz), 8.251-8.235 (1H, d, J=8.0Hz), 7.726-7.696(1H, t, J=7.5Hz), 7.592-7.562(1H, t, J=7.5Hz), 6.771(1H, s), 6.364-6.360(1H, d, J=2Hz), 3.017-2.991(2H, t, J=6.5Hz), 2.580(3H, s), 2.029-2.002(2H, t, J=6.5Hz), 1.516(6H, s). **^{13}CNMR**(125MHz,$CDCl_3$) δ: 185.8 (1C), 155.5 (1C), 151.2 (1C), 143.5 (1C), 142.9 (1C), 142.4 (1C), 132.1 (1C), 130.4 (1C), 128.8 (1C), 126.4(1C), 123.8 (1C), 123.2 (1C), 122.4 (1C), 122.3 (1C), 105.1 (1C), 104.8(1C), 78.3(1C), 31.8(1C), 27.0(2C), 16.7(2C).

Synthesis of (II) and (III) [1]

β-lap (94.2 mg, 0.389 mmol), 1,1,1-Trimethanolethane 2-(Hydroxymethyl)-2-methyl-1,3-propanediol(140 mg, 1.17 mmol) and p-toluenesulfonic acid (10.3 mg, 0.054 mmol) was added into 15 mL anhydrous benzene in a single neck flask, decorated with a Dean-Stark trap. the reaction was kept refluxing for 48 hrs. After removing benzene under vacuum, the residues were redissolve in 30 mL EtOAc, followed by rinse with saturated $NaHCO_3$ aqueous solution for 3 times, and saturated NaCl aqueous solution 3 times. 25.7 mg of prodrug (II) and 15.3 mg of prodrug (III) were obtained by silicone gel chromatography with eluent Hexane/ EtOAc=6:1.

(2'r, 5'r)-5'-(hydroxymethyl)-2,2,5'-trimethyl-3,4-dihydrospiro[benzo[h]chromene-6,2'-[1,3]dio xan] -5(2H)-one (II) yield 35%, **MS:** 345.1$[M+H]^+$, **^{1}HNMR** (500MHz,$CDCl_3$) δ: 7.821-7.803 (1H, dd, J=1.0), 7.743-7.726(1H, dd, J=1.0), 7.461-7.428(1H, ddd, J=1.5),7.401-

7.368(1H, ddd, J=1.5), 4.611-4.588 (2H, d, J=11.5), 4.010(2H, s), 3.786-3.763(2H, d, J=11.5), 2.458-2.431(2H, t, J=6.5), 1.807-1.781(2H, t, J=6.5), 1.457 (3H, s), 1.409 (6H, s). **^{13}CNMR** (125MHz, $CDCl_3$) δ: 194.1 (1C), 160.7 (1C), 139.0 (1C), 130.5 (1C), 129.5 (1C), 128.4 (1C), 127.4 (1C), 123.7 (1C), 107.0 (1C), 89.7 (1C), 78.3 (1C), 67.7 (1C), 66.4 (2C), 35.0 (1C), 32.0 (1C), 27.0 (2C), 17.5(1C), 15.9 (1C).

(2's, 5's)-5'-(hydroxymethyl)-2,2,5'-trimethyl-3,4-dihydrospiro[benzo[h]chromene-6,2'-[1,3]dio xan]-5(2H)-one (III) yield= 25%, **MS:** 345.1 $[M+H]^+$, **^{1}HNMR** (500MHz,$CDCl_3$) δ: 7.912-7.894(1H, dd, J=1.5), 7.754-7.736 (1H, dd, J=1.5), 7.486-7.453(1H, ddd, J=1.5), 7.395-7.377(1H, ddd, J=1.5), 4.769- 4.747 (2H, d, J=11.0), 3.632-3.609 (2H, d, J=11.5), 3.480 (2H, s), 2.453-2.426 (2H, t, J=7.0), 1.794-1.768(2H, t, J=6.5), 1.445(3H, s), 1.396(6H, s).**^{13}CNMR**(125MHz,$CDCl_3$) δ:194.3(1C), 160.7 (1C), 139.0(1C), 130.5(1C), 129.4(1C), 128.1(1C), 127.3(1C), 123.4 (1C), 106.9 (1C), 89.5(1C), 79.3 (1C), 68.7(1C), 67.5 (2C), 35.1(1C), 31.9 (1C), 26.9(2C), 19.8(1C), 15.9(1C).

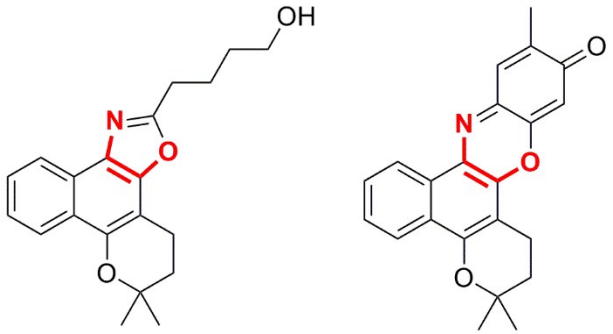
Characterization of compound (IV)

(E)-1-(4-(2,2-dimethyl-5-oxo-3,4-dihydro-2H-benzo[h]chromen-6(5H)-ylideneamino)phenyl)-1H-pyrrole-2,5-dione (VI), yield, 72.6 %, **MS:** 413.1 $[M+H]^+$, **^{1}HNMR** (500MHz,$CDCl_3$) δ: 8.221-8.207 (1H, d, J=7.0), 7.846-7.826 (1H, d, J=10.0), 7.559-7.481 (2H,m), 7.340-7.322 (2H, d, J=9), 6.829-6.804 (4H,m), 2.432-2.406 (2H, t, J=6.5), 1.810-1.784 (2H, t, J=6.5), 1.444 (6H, s). **^{13}CNMR**(125MHz,$CDCl_3$) δ: 177.9 (1C), 170.2 (2C), 162.0 (1C), 153.0 (1C), 151.1 (1C), 134.3 (2C), 132.7 (1C), 131.6 (1C), 130.5 (2C), 130.3 (1C), 127.3 (1C), 126.6 (2C), 126.1 (1C), 123.7 (1C), 116.8 (2C), 112.4 (1C), 78.8 (1C), 32.0 (1C), 27.0 (2C), 16.3 (1C).

The absolute structure conformation of prodrug (II) and Prodrug (III).

The chemical shift on ^{1}HNMR of prodrug (II) and Prodrug (III) were different at several protons, they were in different spatial conformation. The chemical shifts and the coupling effect of the proton on methyl group or $C\underline{H}_2$-OH on the 1, 3-dioxane and α proton on the benzyl group were examined. The ^{1}HNMR spectra indicate that proton chemical shifts of $C\underline{H}_2$-OH ranges from 3.97 to 3.47 in prodrug (II) and Prodrug (III), while the chemical shif ts of HO-CH_2-C-$C\underline{H}_3$ on 1,3-dioxane were 0.84 to 1.41, this indicate the two group in different electrons environment. The stretch direction of the HO-CH_2- group and the -$C\underline{H}_3$ group on the chiral carbon atom lead their protons in different electron environment. The absolute conformation of prodrug (II) and Prodrug (III) were confirmed by one dimensional nuclear Overhauser effect (1D- NOE). The positive NOE effects were observed not only among the protons of –$C\underline{H}_2$-OH group to its adjacent –CH_3 groups and one –CH_2-O- groups of the 1, 3-dioxane structure in prodrug (II), but also between $C\underline{H}_2$-OH and α-proton of the aromatic ring, this indicate that the $C\underline{H}_2$-OH group stretch toward the benzyl direction, there is a shorter spatial length between them (**Figure S15)**. However, The positive NOE effect only observed between the protons of –$C\underline{H}_2$-OH group and its adjacent –CH_3 groups and the two –CH_2-O- groups of 1, 3-dioxane structure in prodrug (III), indicating the CH_2-OH group stretch opposite to the benzyl direction **(Figure S16)**.

Figure S1: Chemical structure of β-lap derivatives (V) and (VI)



(V) **(VI)**

Figure S2: ^{1}HNMR of （I）

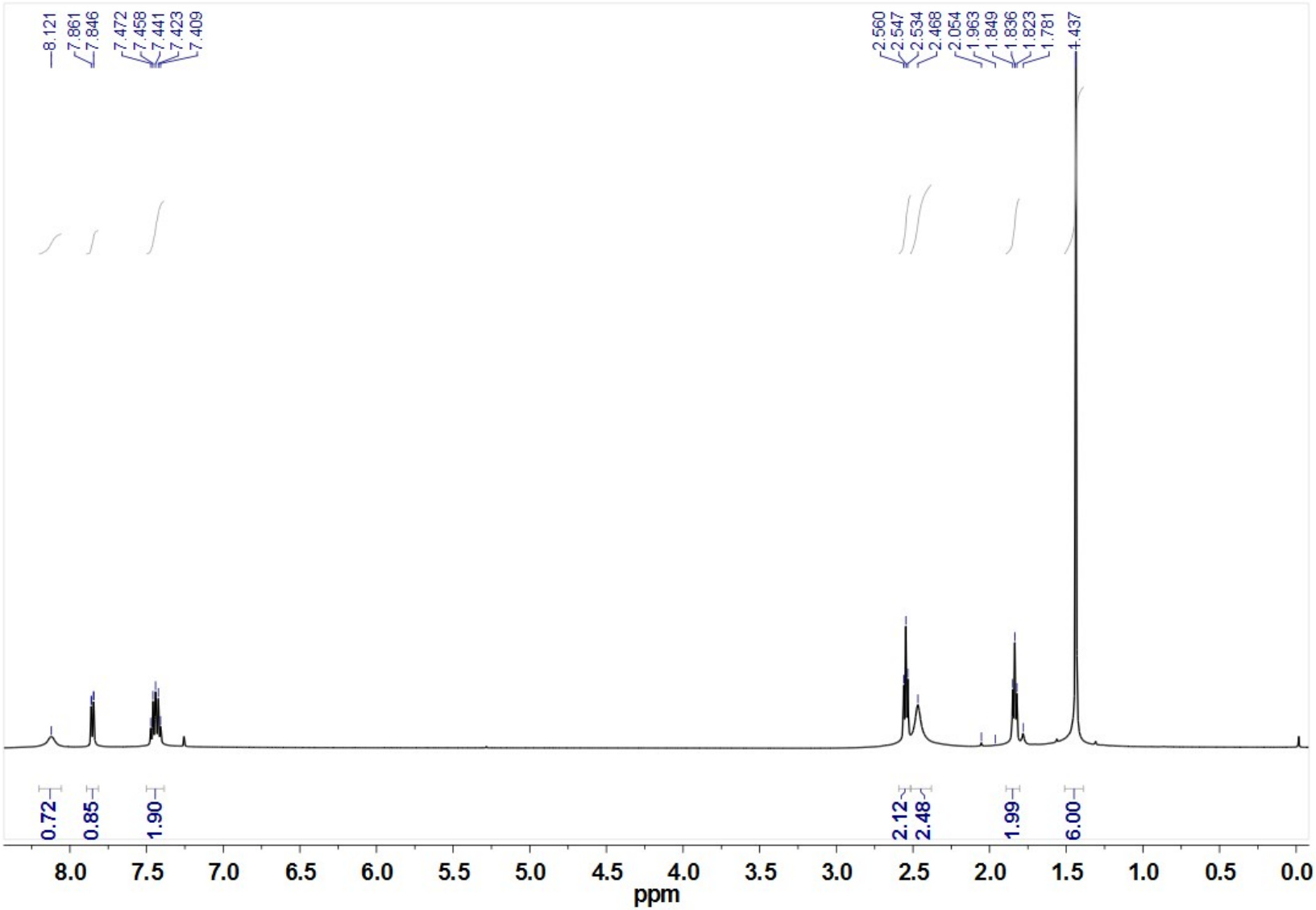


Figure S3: ^{13}CNMR of （I）

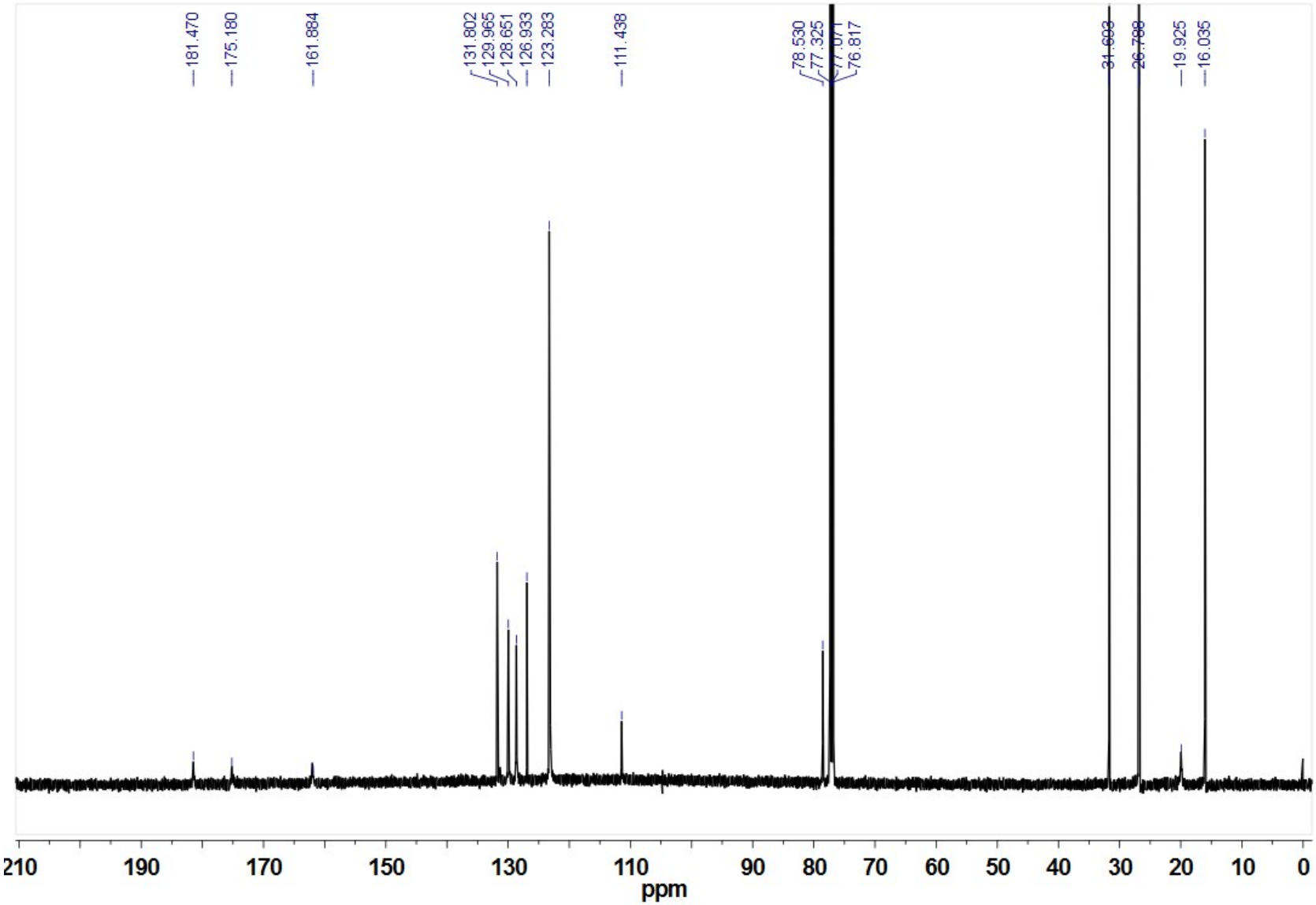


Figure S4: ^{1}HNMR of （II）

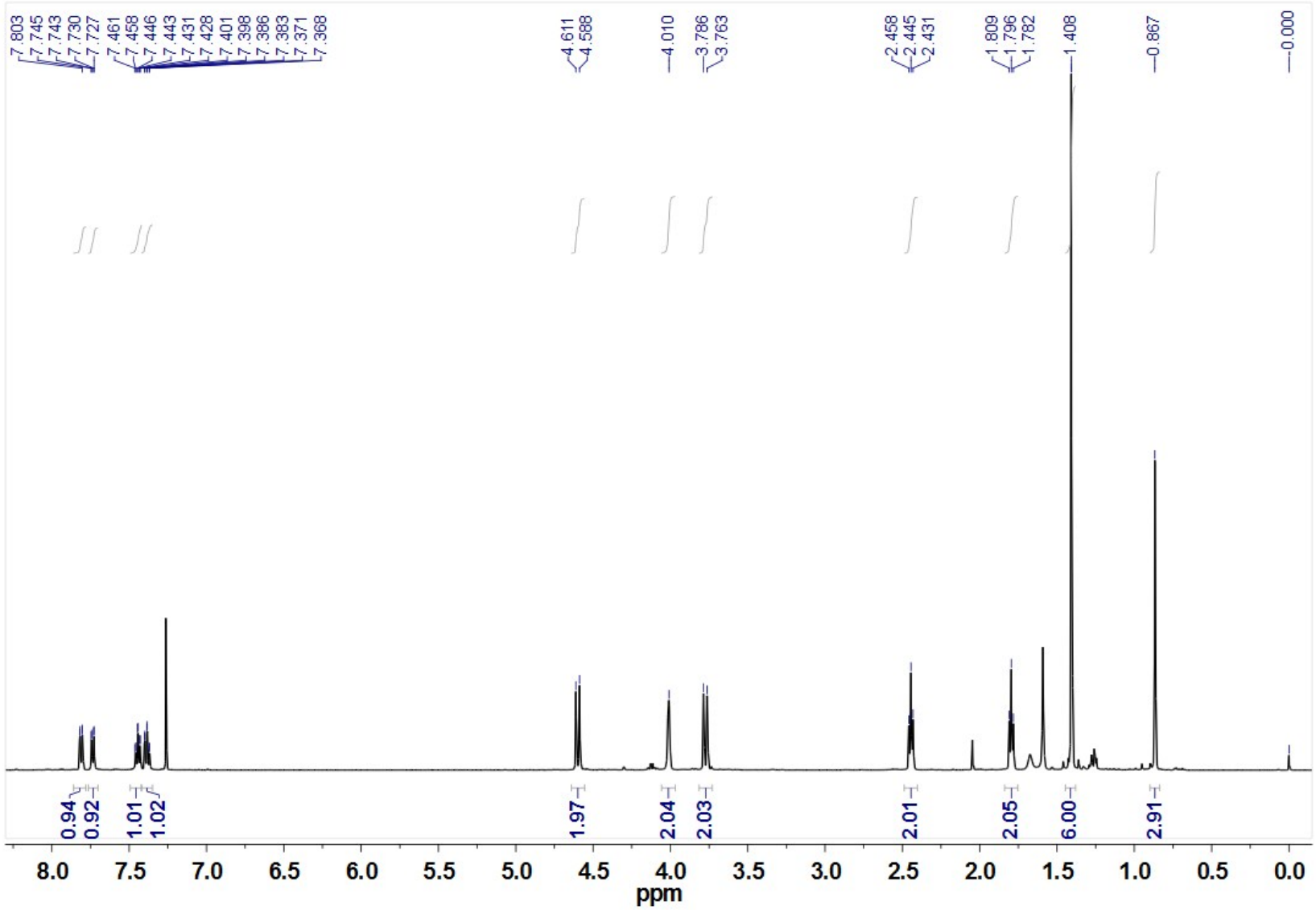


Figure S5: ^{13}CNMR of （II）

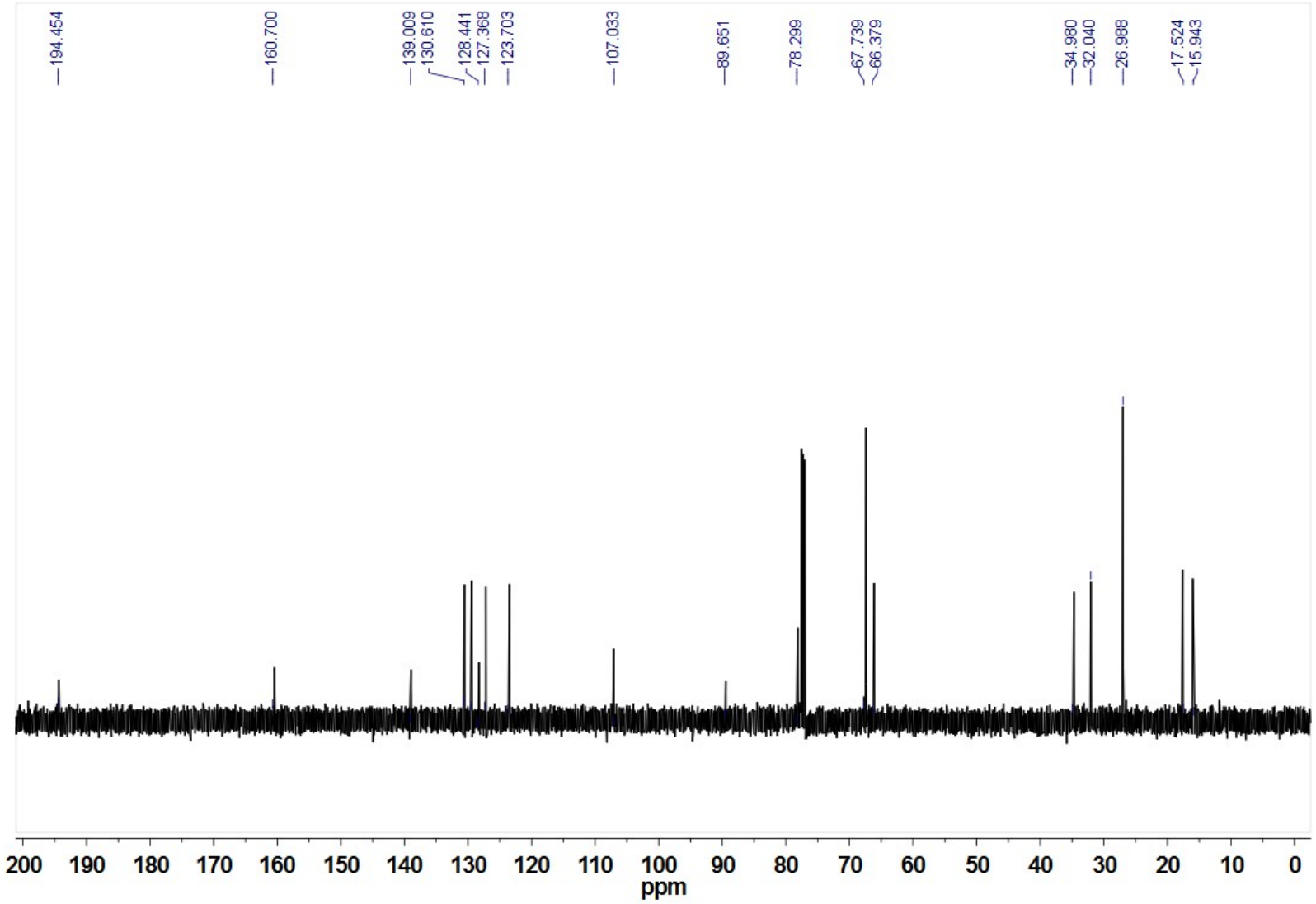


Figure S6: ^{1}HNMR of （III）

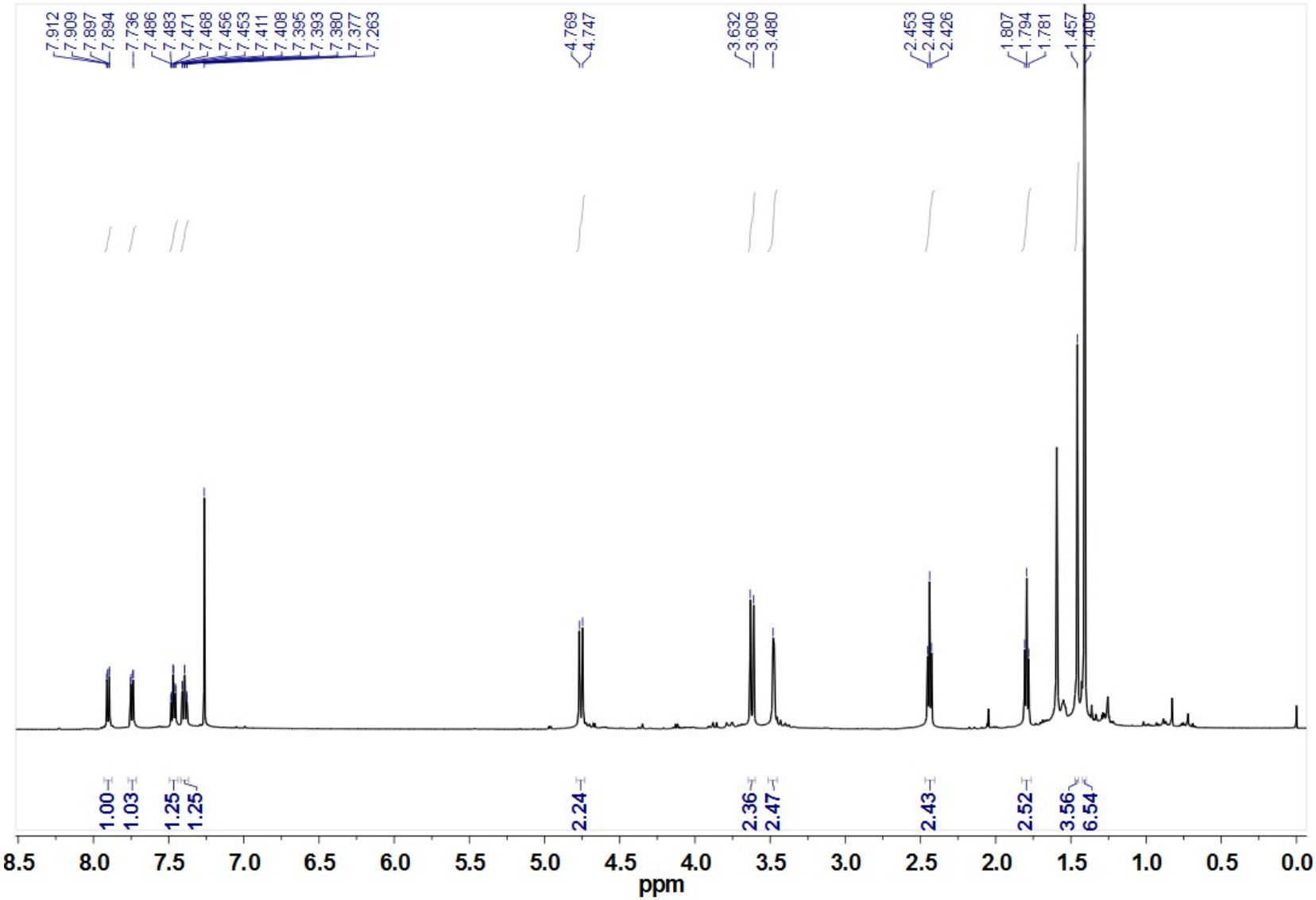


Figure S7: ^{13}CNMR of （III）

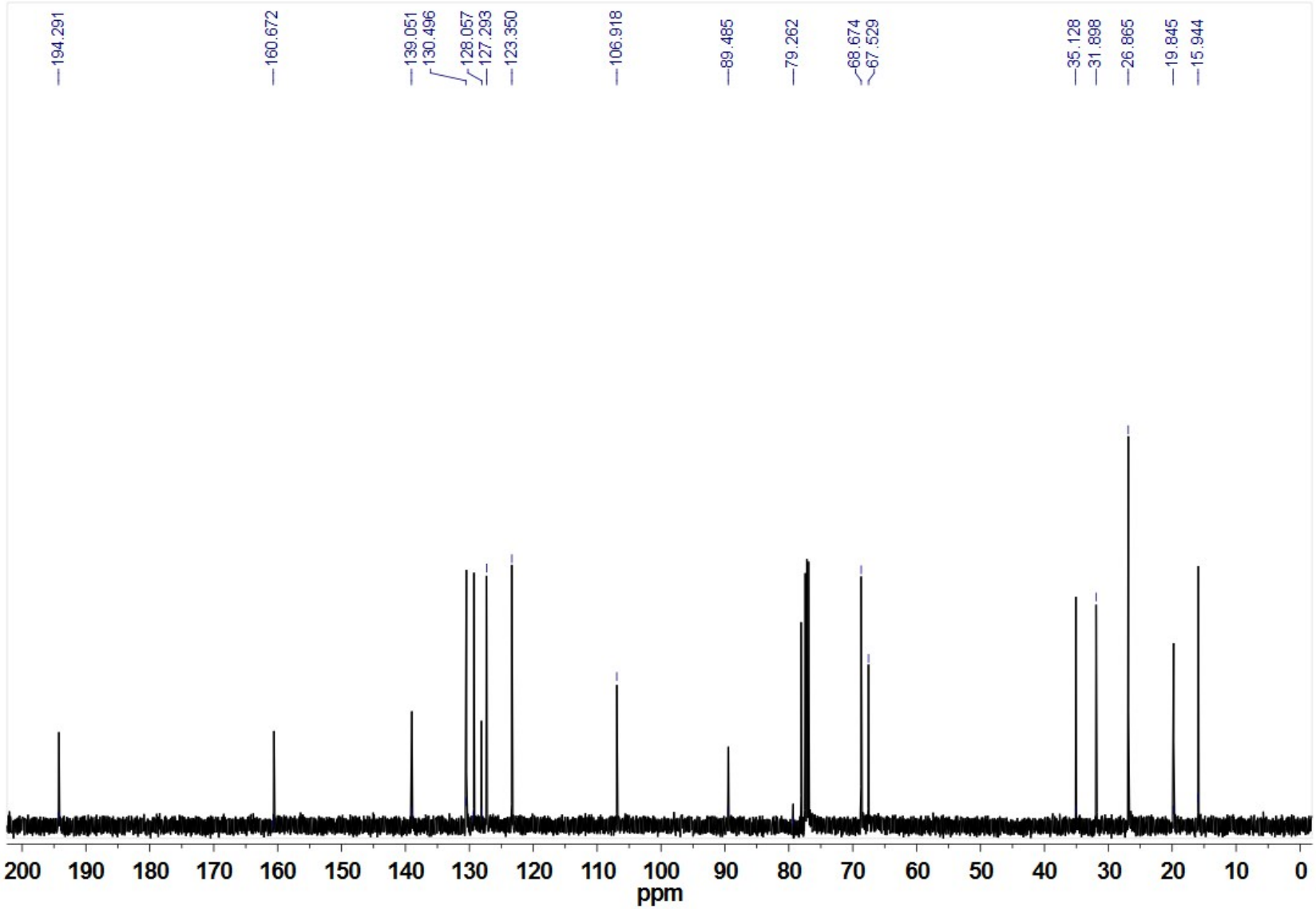


Figure S8: ^{1}HNMR of （IV）

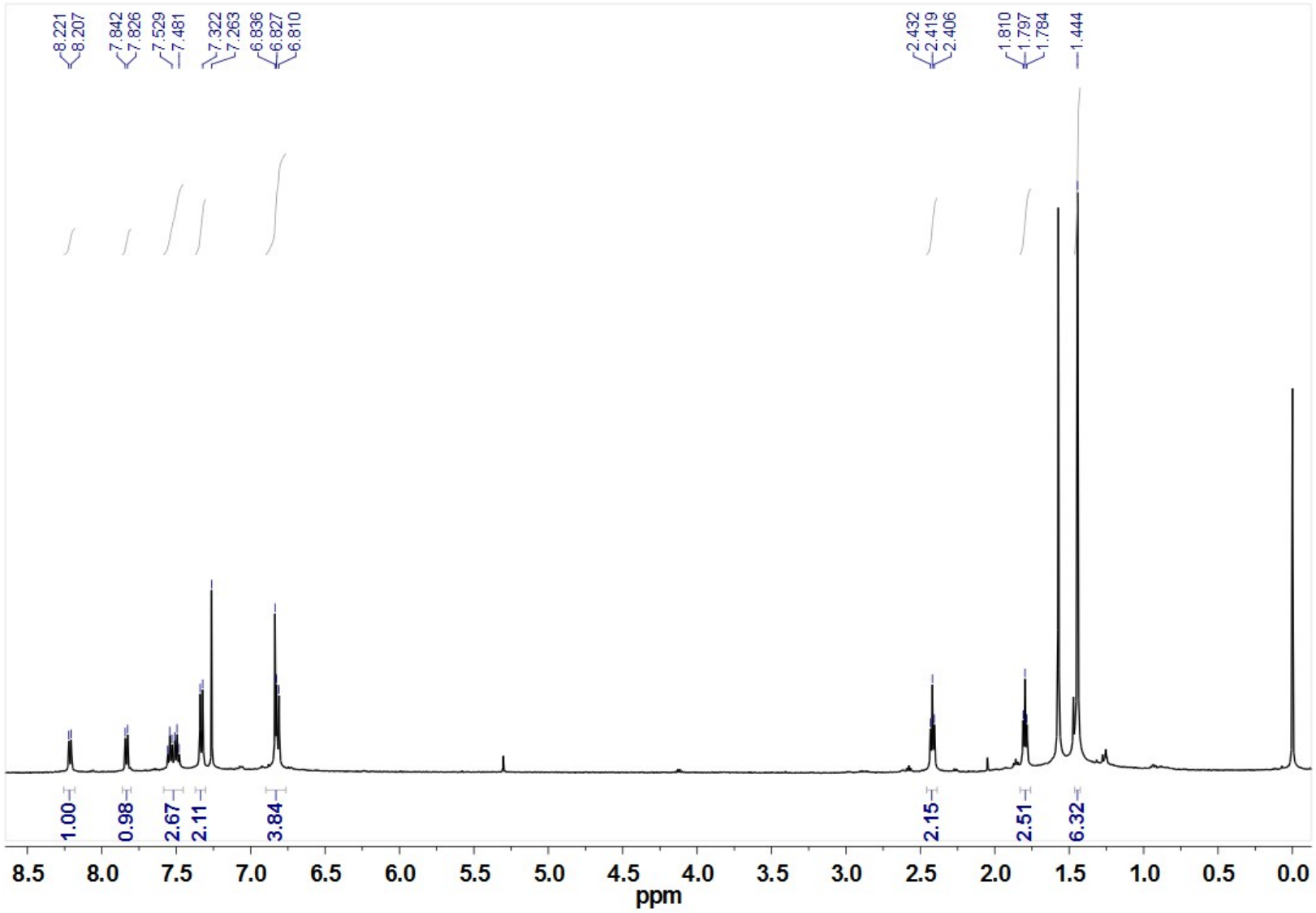


Figure S9: ^{13}CNMR of （IV）

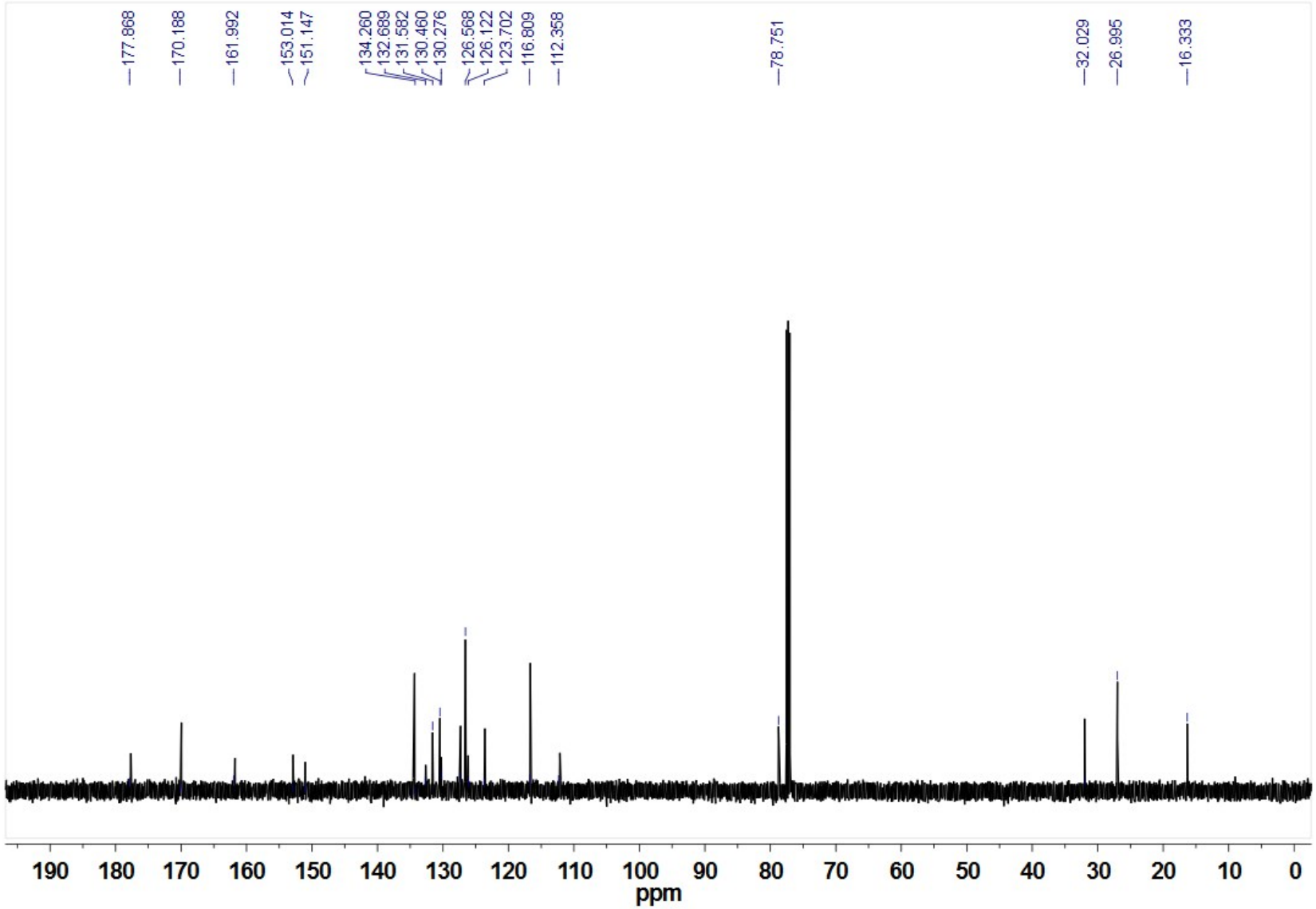


Figure S10: ^{1}HNMR of （V）

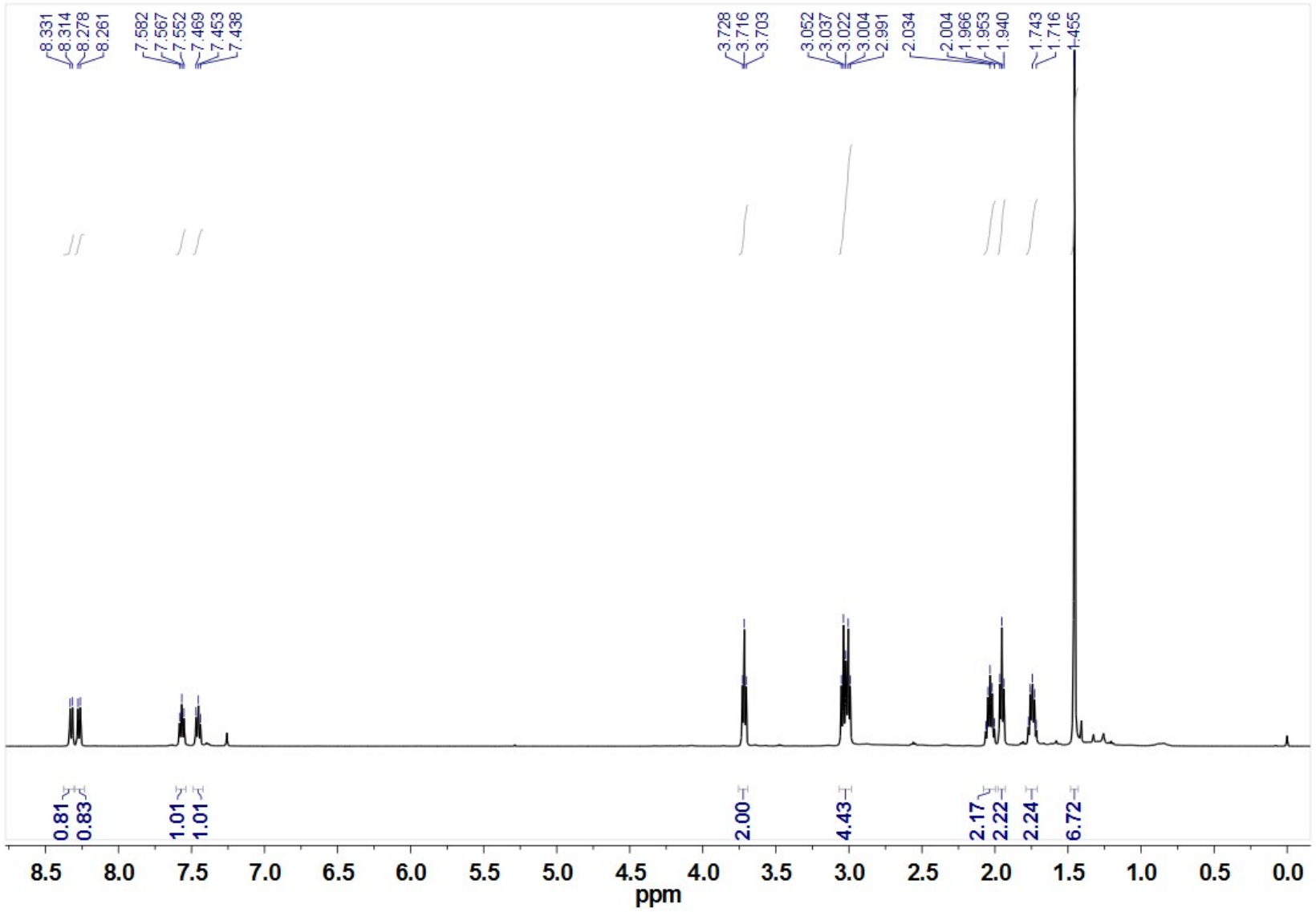


Figure S11: ^{13}CNMR of （V）

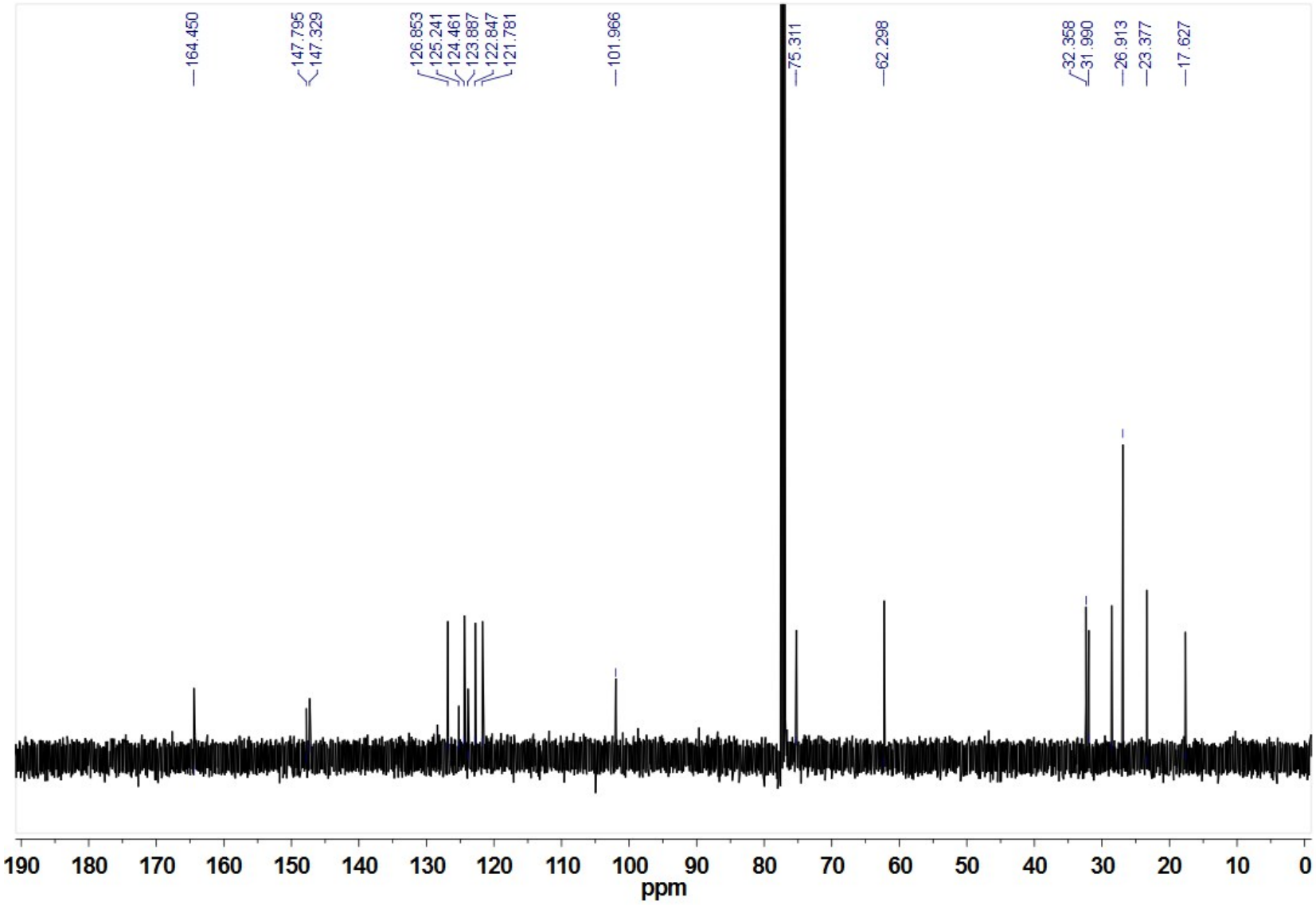


Figure S12: ^{1}HNMR of （VI）

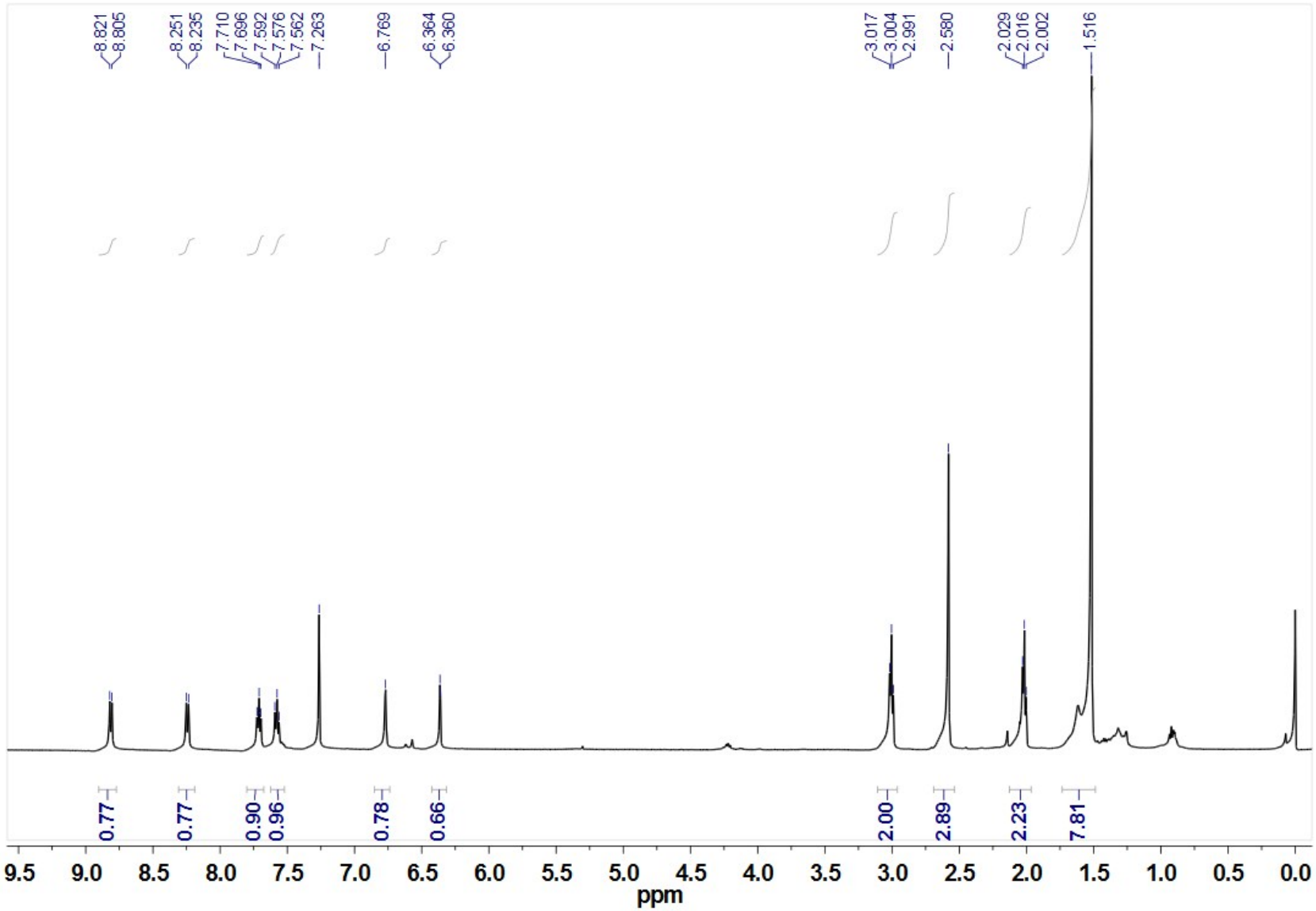


Figure S13: ^{13}CNMR of （VI）

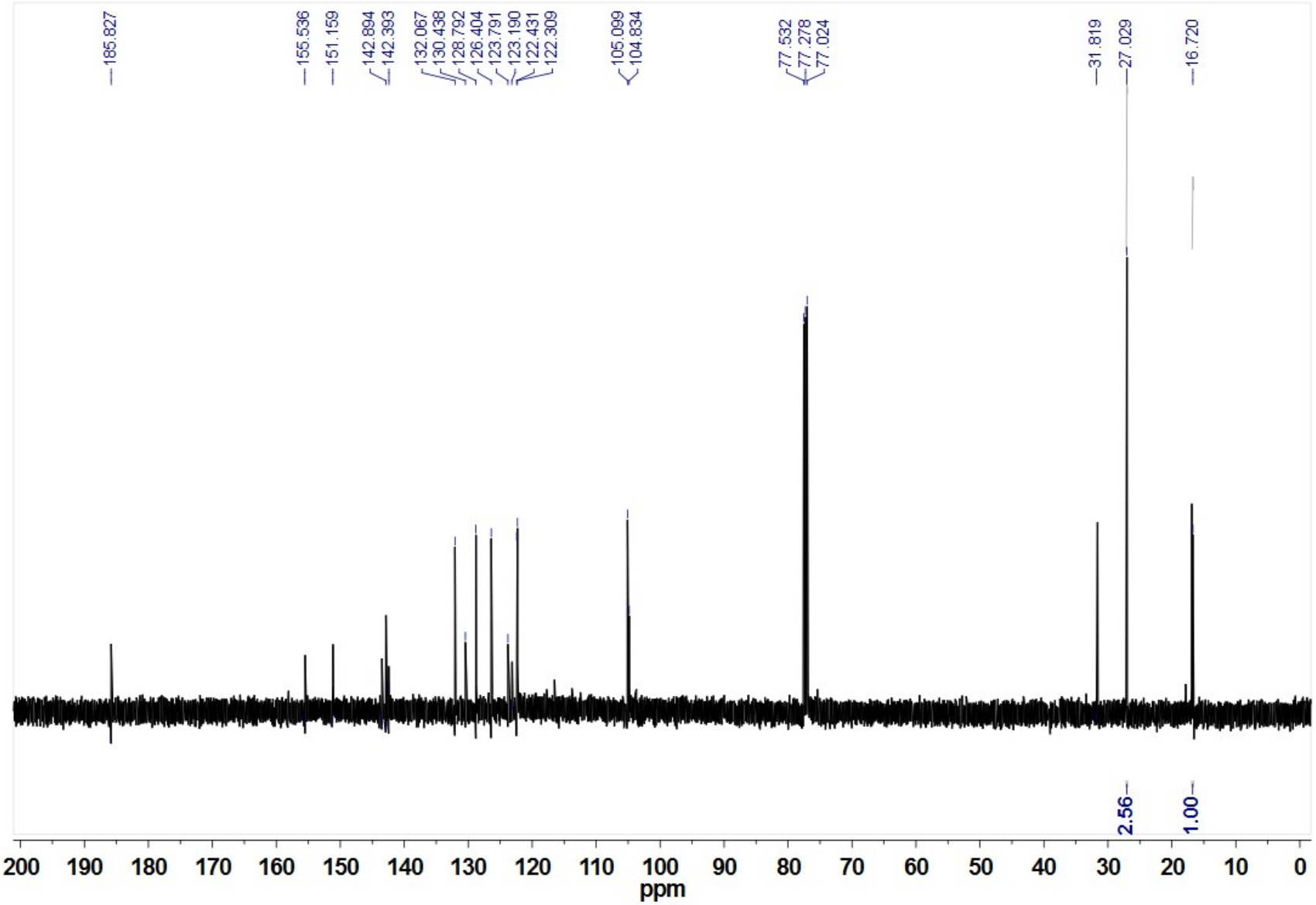


Figure S14: Ketal prodrug hydrolysis profile at pH 1.0 at different time point 0, 2, 6, 12 and 24 hrs: (a) prodrug (II); (b) prodrug (III). β-lap standard UV-Vis spectrum was overlaid for comparisons.

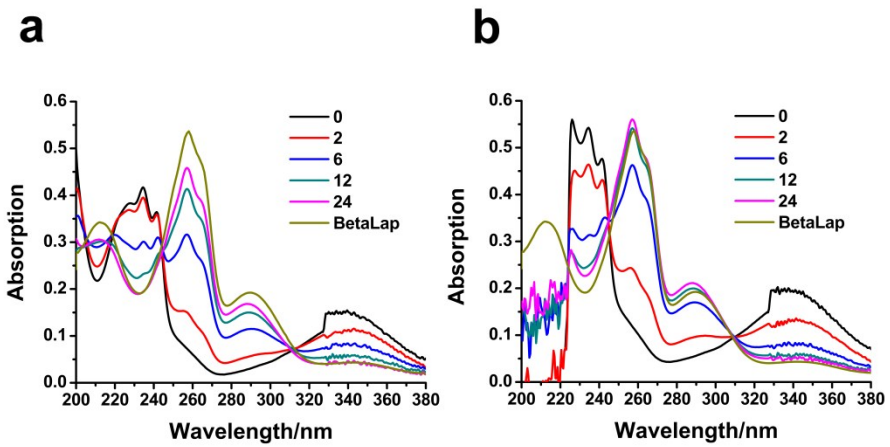


Figure S15: 1D-NOE of prodrug (II)

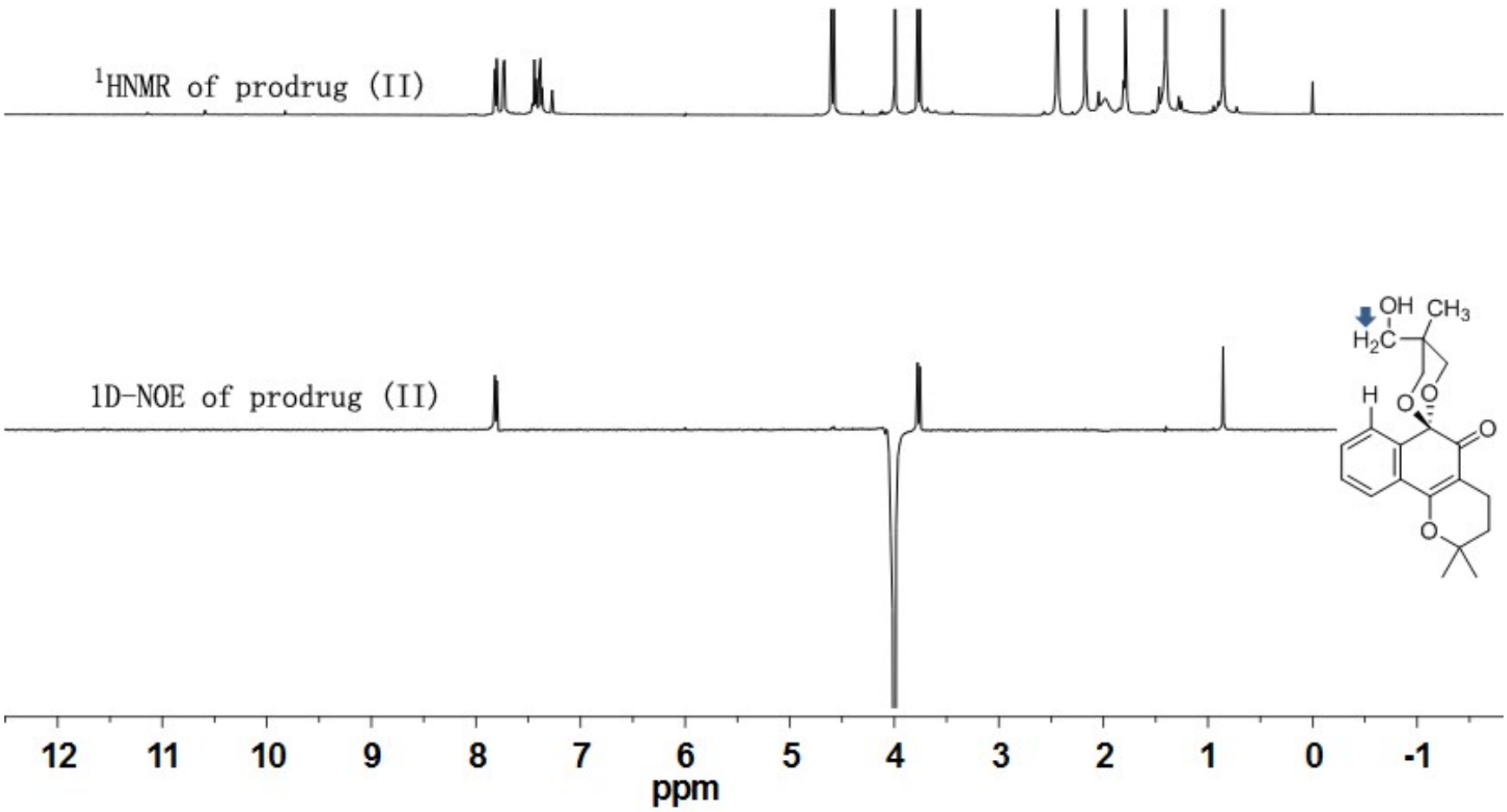


Figure S16: 1D-NOE of prodrug (III)

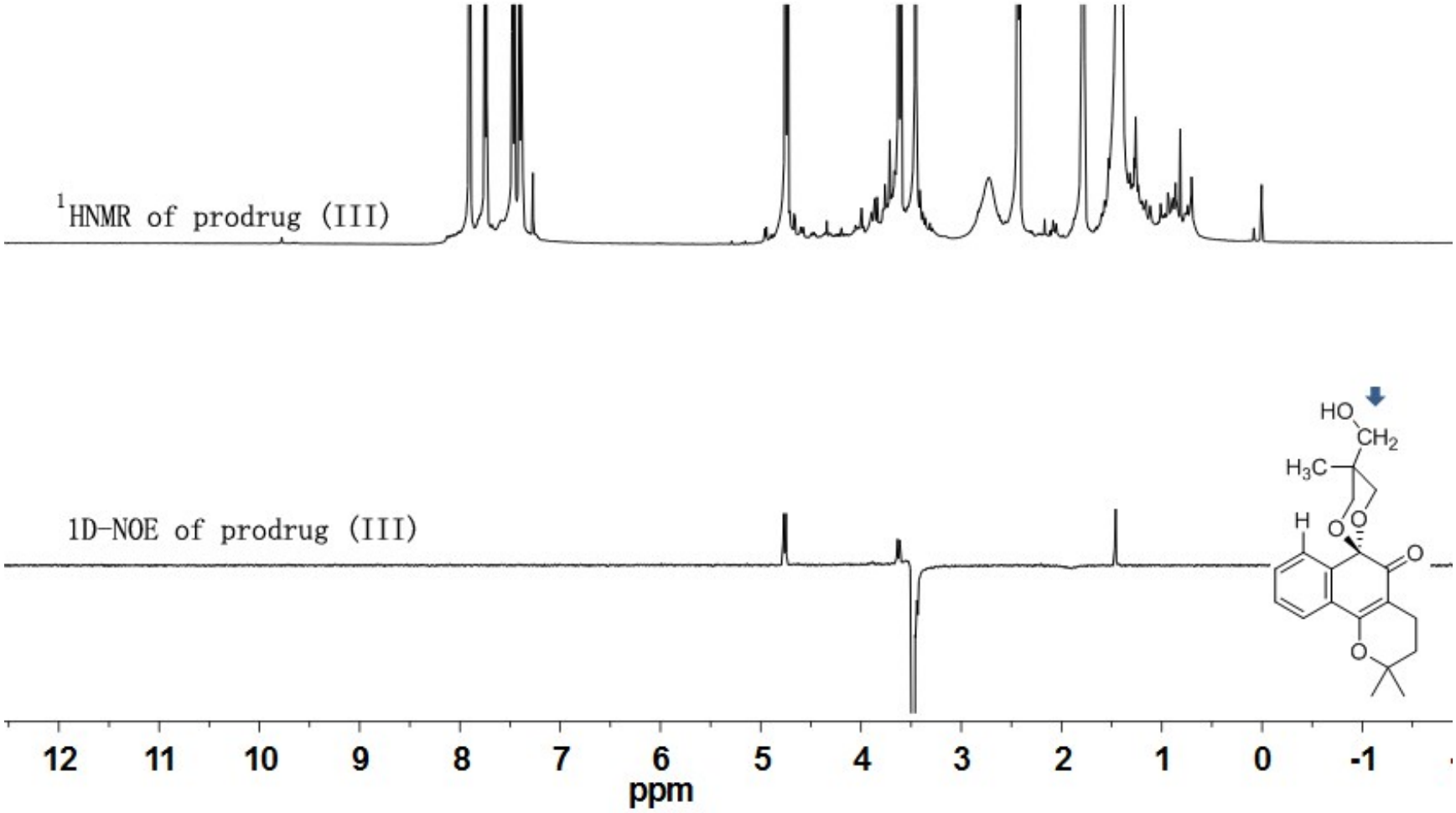
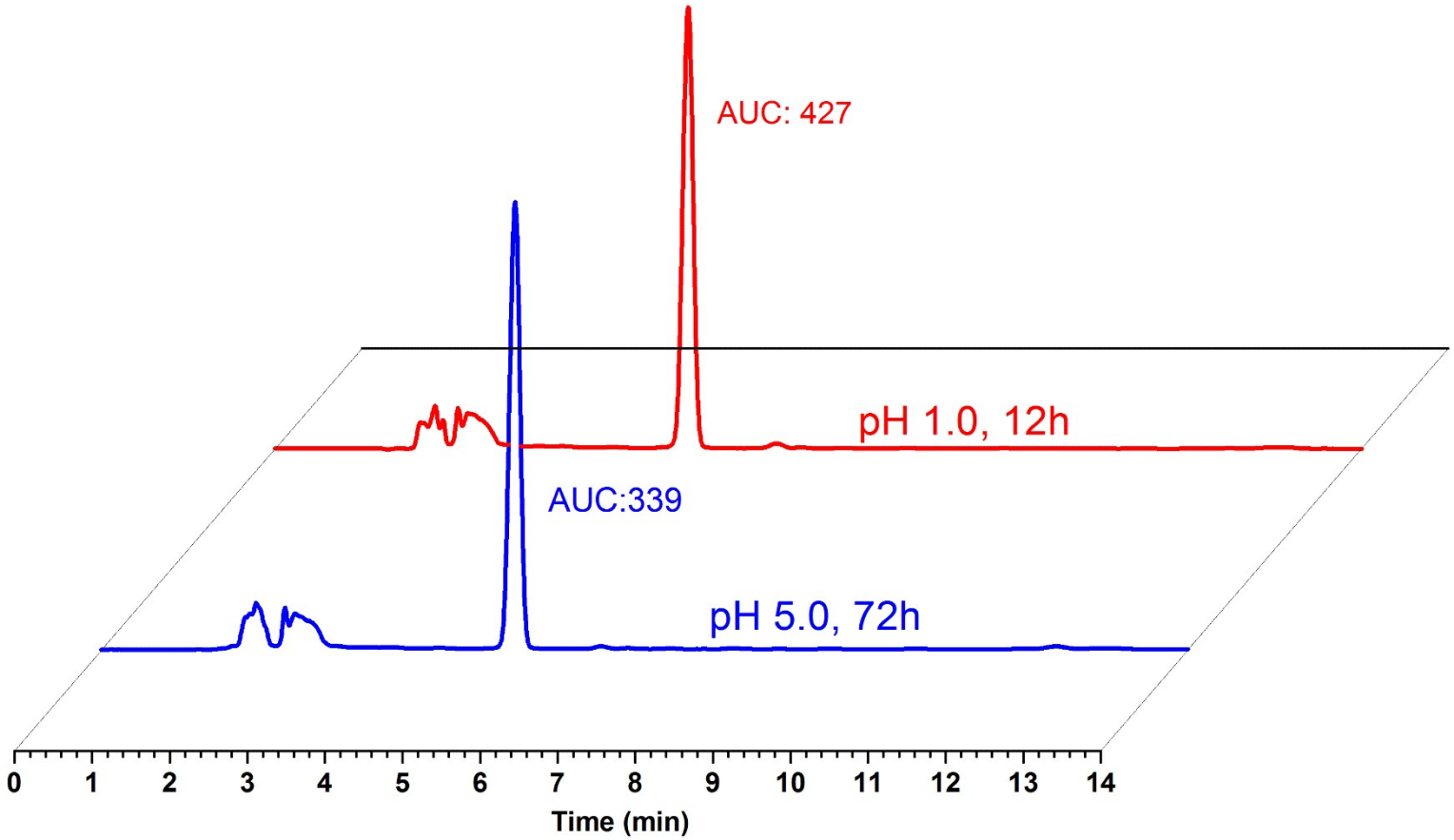


Figure S17: HPLC trace of polymer mPEG-*b*-P(DPA$_{45}$-*r*-PDSM(IV)$_6$) hydrolysis at pH 1.0 for 12 h and pH5.0 for 72 h(after 72h in pH 5.0 about 79% (339/427=0.79) β-Lap released from polymer micelles).



[1] A.P. Griset, J. Walpole, R. Liu, A. Gaffey, Y.L. Colson, M.W. Grinstaff, Expansile nanoparticles: synthesis, characterization, and in vivo efficacy of an acid-responsive polymeric drug delivery system, J. Am. Chem. Soc., 131 (2009) 2469-2471.