Supporting Information

Bifunctional Pyridoxal Derivatives as Efficient Bioorthogonal Reagents for Biomacromolecule Modifications

Xianxian Mao, Wei Li, Shiyu Zhu, Juan Zou, Yuting Duan, Yuntao Wang, Jiayue Fei and Xiaojian Wang*

Institute of Advanced Synthesis, School of Chemistry and Molecular Engineering, Nanjing Tech University, Nanjing 211816, China

Table of Contents

Abbreviations

BSA……...……..…Albumin from bovine serum

CD_3OD…….……...……....Deuterated methanol

$CDCl_3$……..…….……....Deuterated chloroform

D_2O……...……...…….…….Deuterium oxide

DBU……....1,8-Diazabicyclo[5.4.0]undec-7-ene

DCM/CH_2Cl_2……Dichloromethane/Methylene chloride

DIPE……N,N-Diisopropylethylamine

DMF……..……...……..N, N-Dimethylformamide

DMP……..…….. 2,2-dimethoxypropane

DMSO……..……..……Dimethyl sulfoxide

DMSO-d_6…….……Dimethyl sulfoxide-d_6

DNase……Deoxyribonuclease

DPPA……Diphenylphosphoryl azide

ESI..... Electrospray ionization

Et_3N……..……..…..Triethylamine

EtOAc/EA…….....…….……..... Ethyl acetate

g……..……..gram

H……..…….....Proton

HBTU……..…1-Hydroxy Benzotriazole

H_2O…….Water

HOBT……1-Hydroxybenzotriazole

HP……...2-Hydrazinopyridine

HRMS…….…….....High resolution mass spectrometry

Hz……..…….....……..…Hertz

JEOL……..……..…….Japan Electron Optics Laboratory CO., LTD

M…….…….…...……...Molar

MeCN…… Acetonitrile

Me_2CO……Acetone

MeOH/CH_3OH..……..……..…Methanol

$MgCl_2$……..Magnesium chcoride

mg……..…milligram

min……..…….…Minute

mL……..Milliliter

mmol……..……..Millimoles

MnO_2…… …Manganese dioxide

N_2……...……...…….....Nitrogen

Na_2SO_4……...…….....…….…….....Sodium sulfate

NaF…… Sodium fluoride

NMR……Nuclear magnetic resonance

OD...…….Optical density

PBS…….Phosphate buffer saline

PE……....petroleum ether

PMSF……...…Phenylmethylsulfonyl fluoride

ppm……...…….……...…..parts per million

SDS-PAGE……Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

$SOCl_2$……Thionyl chloride

SPPS……. solid phase peptide synthesis

TFA…….....Trifluoroacetic acid

THF…….……...……Tetrahydrofuran

TIS……...Triisopropylsilane

TLC…...……Thin layer chromatography

Tris……..Tris(hydroxymethyl)aminoethane

$TsOH \cdot H_2O$……P-Toluenesulfonic acid monohydrate

UV……...……Ultraviolet

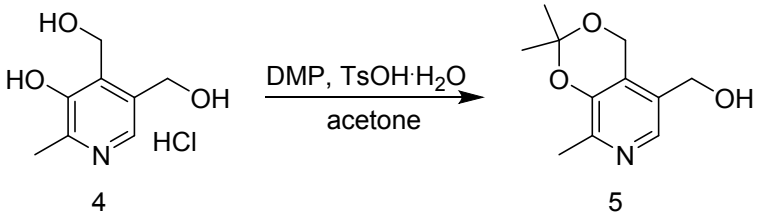
µL…….……..Micro litter

General materials and methods

All chemicals and solvents were purchased from commercial sources and used without further purification unless otherwise indicated. All reactions were performed under anhydrous conditions under an atmosphere of nitrogen. Reactions were monitored by TLC on HSGF254 silica gel plates. Detection was accomplished by examination under UV light (254 nm or 365 nm). Flash chromatography was performed on silica gel (100-200 mesh). ^{1}H NMR spectra were recorded in $CDCl_3$, DMSO-d_6, CD_3OD or D_2O on Bruker AVB-400 or JEOL ECZ400S spectrometer at 298K. TMS ($\delta\ (ppm)_H = 0.00$) was used as the internal reference. ^{13}C NMR spectra were recorded in either $CDCl_3$, DMSO-d_6, CD_3OD and D_2O at 100 MHz on Bruker AVB-400 or JEOL ECZ400S spectrometer, using the central resonances of $CDCl_3$ ($\delta\ (ppm)_C = 77.16$), DMSO-d_6 ($\delta\ (ppm)_C = 39.52$) or CD_3OD ($\delta\ (ppm)_C = 49.00$) as the internal references. Chemical shifts are reported in ppm and multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), and m (multiplet). Coupling constants, *J*, are reported in hertz (Hz). High-resolution mass spectra (HRMS) were obtained on a Waters ACQUITY UPLC/Xevo G2-XS Qtof system and are reported as m/z (relative intensity). Accurate masses are reported for the molecular ion (M^+) or a suitable fragmention. ESI-MS (/MS) spectra were recorded on a Thermo Liquid chromatography-mass spectrometry (Thermo Fisher Scientific, MSQ PLUS/U3000) equipped with a standard ESI ion source. Data acquisition and analysis were done with the Xcalibur (version 2.0, Thermo quest Finnigan) software package.

S1. Synthesis and characterization of small compounds

S1.1 Synthesis of (2,2,8-trimethyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methanol (5):



4 5

Figure S1. Synthesis of compound **5**

Compound **5** was prepared following the reported method.[1] To a stirred suspension of pyridoxine hydrochloride **4** (5.00 g, 24.3 mmol) and 2,2-dimethoxypropane (DMP, 50.1 mL, 408 mmol) in 75 mL of acetone was added p-toluenesulfonic acid monohydrate ($TsOH \cdot H_2O$, 18.5 g, 97.2 mmol), and this mixture was stirred for 20 h at RT. The dark brown solution was then neutralized with aqueous sodium bicarbonate, concentrated under reduced pressure, and extracted with DCM (3×100 mL). The organic layers were combined and dried over sodium sulfate. The crude product was purified via silica gel column chromatography (2:1, DCM/EA) to afford **5** (3.177 g, 63%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.73 (s, 1H), 4.92 (s, 2H), 4.52 (s, 2H), 2.34 (s, 3H), 1.54 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): *δ (ppm)* 147.33, 145.97, 138.25, 129.70, 125.98, 99.73, 59.78, 58.49, 24.66, 18.06. HRMS m/z Found: 210.1123, calculated: 210.1130 for $C_{11}H_{16}NO_3$ $[M+H]^+$.

S1.2 Synthesis of 5-(azidomethyl)-2,2,8-trimethyl-4H-[1,3]dioxino[4,5-c]pyridine (6)：

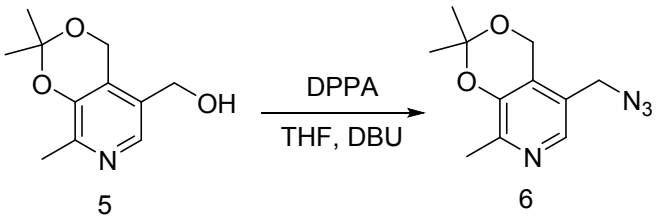


Figure S2. Synthesis of compound **6**

Diphenylphosphoryl azide (DPPA, 247 μL, 1.147 mmol) was added dropwise to a stirred solution of **5** (0.2 g, 0.956 mmol) in THF (5 mL) at 0 °C, and then DBU (171.4 μL, 1.147 mmol) was added. The reaction mixture was allowed to slowly warm up to room temperature and stirred for 4h. Upon completion, the mixture was diluted with DCM and washed with water and brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The crude product was purified via silica gel column chromatography (4:1-2:1, PE/EA) to afford **6** (0.216 g, 97%) as a light yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.96 (s, 1H), 4.86 (s, 2H), 4.23 (s, 2H), 2.43 (s, 3H), 1.58 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): *δ (ppm)* 149.12, 146.28, 139.81, 125.51, 123.95, 100.06, 58.42, 49.46, 24.76, 18.67. IR (KBr): ν = 2996, 2943, 2097, 1603, 1569, 860, 790, 665 cm^{-1}. HRMS m/z Found: 235.1188, calculated: 235.1195 for $C_{11}H_{15}N_4O_2$ $[M+H]^+$.

S1.3 Synthesis of 5-(azidomethyl)-4-(hydroxymethyl)-2-methylpyridin-3-ol (7)：

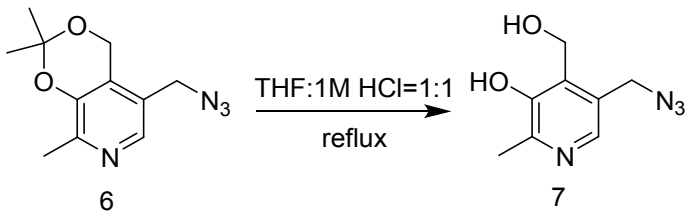


Figure S3. Synthesis of compound **7**

To a flask containing compound **6** (0.539 g, 2.302 mmol) was added a mixture of THF (4 mL) and 1 M HCl aqueous solution (4 mL) at room temperature. The mixture was refluxed under nitrogen for 4 h. After cooling to room temperature, the solvent was removed under vacuum. A light yellow residue was obtained, which was recrystallized with acetone to afford **7** (0.4244g, 95%) as a light yellow solid. ^{1}H NMR (400 MHz, CD_3OD): *δ (ppm)* 8.22 (s, 1H), 5.08 (s, 2H), 4.70 (s, 2H), 2.65 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): *δ (ppm)* 153.90, 142.99, 141.03, 132.54, 129.93 , 58.42, 48.26, 13.60. IR(KBr): ν = 3085, 2672, 2092, 1081, 1033, 863, 772, 659 cm^{-1}. HRMS m/z Found: 195.0876, calculated: 195.0882 for $C_8H_{11}N_4O_2$ $[M+H]^+$.

S1.4 Synthesis of 5-(azidomethyl)-3-hydroxy-2-methylisonicotinaldehyde (1)：

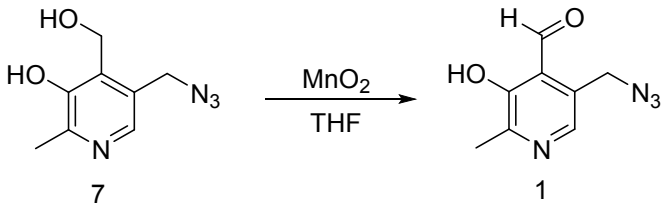


Figure S4. Synthesis of compound **1**

Manganese dioxide (MnO_2, 3.42 g, 39.37 mmol) was added to a stirred suspension of compound **7** (0.218 g, 1.12 mmol) in THF (15 mL) at room temperature. The reaction mixture was stirred for 6 h, monitored by TLC and then centrifuged. The supernatant was concentrated under reduced pressure and 15 mL of THF was added to the precipitate after centrifugation with stirring, and reacted for additional 6 h. The suspension was centrifuged and the supernatant was concentrated again under reduced pressure. The process was repeated for 2 more times and the crude product was combined and purified via silica gel column chromatography (3:1 EA/Me_2CO) to afford **1** (0.1171 g, 54%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): *δ (ppm)* 10.42 (s, 1H), 8.09 (s, 1H), 4.76 (s, 2H), 2.46 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): *δ (ppm)* 195.42, 153.91, 152.13, 139.95, 127.95, 123.24, 48.50, 19.60. IR (KBr): ν = 3419, 2927, 2860, 2127, 1672, 1093, 1214, 792, 765 cm^{-1}. HRMS m/z Found: 193.0720, calculated: 193.0726 for $C_8H_9N_4O_2$ $[M+H]^+$.

S1.5 Synthesis of 5-(chloromethyl)-2,2,8-trimethyl-4H-[1,3]dioxino[4,5-c]pyridine (8)：

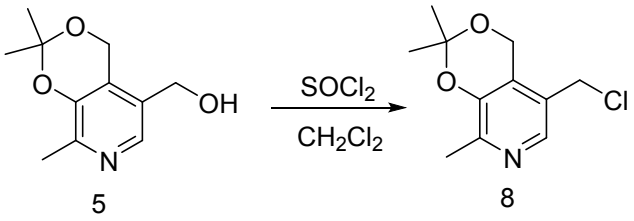


Figure S5. Synthesis of compound **8**

To a stirred solution of compound **5** (2.55 g, 12.2 mmol) in 51 mL of CH_2Cl_2, there was added dropwise with stirring a solution of 1.67 g (14.04 mmole) of thionyl chloride ($SOCl_2$) in 3.8 mL of CH_2Cl_2. After 2 h of reaction, the mixture was added dropwise into 50 mL of saturated sodium bicarbonate solution. The aqueous layer was extracted with DCM (3×30 mL) and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The crude product was purified via silica gel column chromatography (4:1-2:1, PE/EA) to afford **8** (2.525 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): *δ (ppm)* 7.89 (s, 1H), 4.94 (s, 2H), 4.58 (s, 2H), 2.39 (s, 3H), 1.55 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): *δ (ppm)* 148.99, 144.35, 134.61, 131.09, 130.79, 102.69, 58.37, 38.62, 24.72, 14.26. HRMS m/z Found: 228.0783, calculated: 228.0791 for $C_{11}H_{15}ClNO_2$ $[M+H]^+$.

S1.6 Synthesis of 2-(2,2,8-trimethyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)acetonitrile (9)：

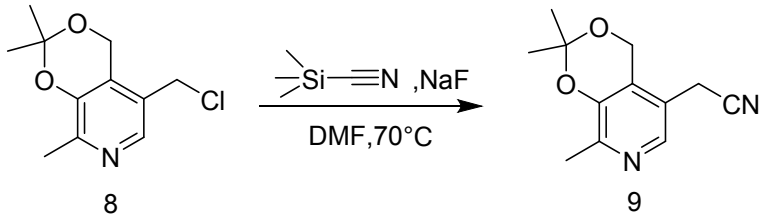


Figure S6. Synthesis of compound **9**

Sodium fluoride (NaF, 1.05 g, 24.93 mmol) was added to a stirred solution of compound **8** (2.84 g, 12.46 mmol) in DMF (30 mL). Trimethylsilyl cyanide (3.12 mL, 24.93 mmol) was added dropwise to the suspension. The reaction temperature was slowly warm to 70 °C and stirred overnight, after which TLC analysis indicated completion of the reaction. The reaction mixture was filtered and concentrated under reduced pressure, and the crude product was purified via silica gel column chromatography (3:1-1:1, PE/EA) to afford **9** (2.56 g, 94%)**.** ^{1}H NMR (400 MHz, $CDCl_3$): *δ (ppm)* 8.01 (s, 1H), 4.85 (s, 2H), 3.54 (s, 2H), 2.42 (s, 3H), 1.57 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): *δ (ppm)* 148.83, 146.19, 139.44, 124.83, 119.15, 116.17, 100.25, 58.22, 24.75, 18.60, 17.75. HRMS m/z Found: 219.1128, calculated: 219.1134 for $C_{12}H_{15}N_2O_2$ $[M+H]^+$.

S1.7 Synthesis of sodium 2-(5-sodiooxy**-4-(hydroxymethyl)-6-methylpyridin-3-yl)acetate (10)：**

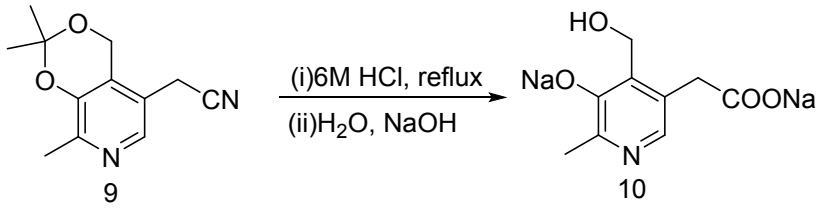


Figure S7. Synthesis of compound **10**

To a flask containing compound **9** (0.507 g, 2.32 mmol) was added 25 mL of 6 M HCl aqueous solution. The mixture was refluxed under nitrogen for 11 h. The solution was cooled, and the solvent was removed under vacuum. A light yellow residue was obtained, which was recrystallized with acetone to afford a light grey solid.

To a stirred solution of the light grey solid (53 mg, 0.284 mmol) in 3 mL H_2O was added 1 M NaOH to adjust the solution pH between 7 and 8. The solution was stirred at room temperature until TLC analysis indicated completion of the reaction. The reaction solution was lyophilized to afford a powder solid **10** (52 mg, sodium salt) for the next step of manganese dioxide oxidation. ^{1}H NMR (400 MHz, D_2O): *δ (ppm)* 7.36 (s, 1H), 4.61 (s, 2H), 3.49 (s, 2H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, D_2O): *δ (ppm)* 178.57, 160.13, 142.65, 140.28, 132.87, 126.91, 56.87, 38.70, 15.21. HRMS m/z Found: 220.0587, calculated: 220.0586 for $C_9H_{11}NNaO_4$ $[M+2H-Na]^+$.

S1.8 Synthesis of sodium 2-(4-formyl-5-hydroxy-6-methylpyridin-3-yl)acetic acid (2)：

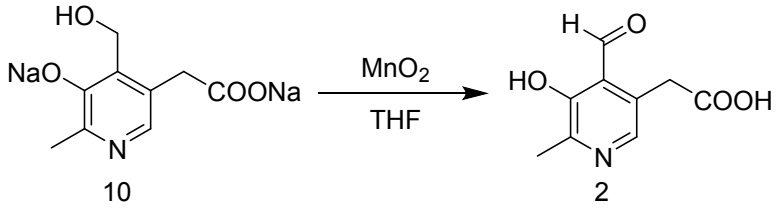


Figure S8. Synthesis of compound **2**

Manganese dioxide (MnO_2, 4.68 g, 53.86 mmol) was added to a stirred suspension of compound **10** (0.3033 g, 1.26 mmol) in THF (30 mL) at room temperature. The reaction mixture was stirred for 12 h and then centrifuged. The supernatant was concentrated under reduced pressure and 30 mL of THF was added to the precipitate after centrifugation with stirring, and then reacted for additional 12 h. The suspension was centrifuged and the supernatant was concentrated again under reduced pressure. The process was repeated for 2 more times and the crude product was combined and purified via silica gel column chromatography (3:1, EA/MeCN) to afford **2** (36.3 mg, 15%)**.** ^{1}H NMR (400 MHz, D_2O): *δ (ppm)* 10.30 (s, 1H), 7.15 (s, 1H), 3.52 (s, 2H), 2.28 (s, 3H). ^{13}C NMR (100 MHz, D_2O): *δ (ppm)* 196.30, 180.55, 167.73, 155.54, 132.25, 129.72, 125.76, 39.91, 18.82. (KBr): ν = 2928, 1704, 1674, 1614, 1066, 1009, 806, 703 cm^{-1}. HRMS m/z Found: 196.0607, calculated: 196.0610 for $C_9H_{10}NO_4$ $[M+H]^+$.

S1.9 Synthesis of 3-(4-formyl-5-hydroxy-6-methylpyridin-3-yl)propanoic acid (3)：

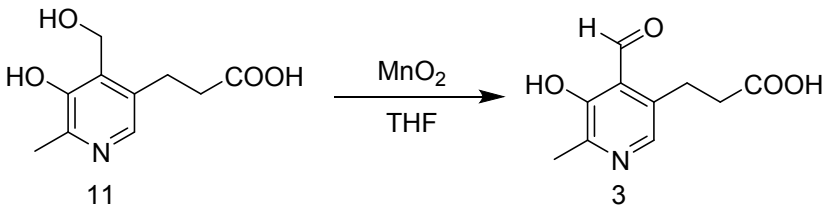


Figure S9. Synthesis of compound **3**

Compound **11** was prepared following the reported method.[2] Manganese dioxide (MnO_2, 5.738 g, 66.01 mmol) was added to a stirred suspension of compound **11** (0.4 g, 1.886 mmol) in THF (40 mL) at room temperature. The reaction mixture was stirred for 12 h and then centrifuged. The supernatant was concentrated under reduced pressure and 40 mL of THF was added to the resulting residue the precipitate after centrifugation with stirring, and then reacted for additional 12 h. The suspension was centrifuged and the supernatant was concentrated again under reduced pressure. The process was repeated for 2 more times and the crude product was combined and purified via silica gel column chromatography (3:1 EA/MeCN) to afford **3** (83.9 mg, 21%)**.** ^{1}H NMR (400 MHz, D_2O): *δ (ppm)* 10.35 (s, 1H), 7.24 (s, 1H), 2.95 (t, *J* = 7.2 Hz, 4H), 2.35 (t, *J* = 7.4 Hz, 4H), 2.27 (s, 3H). ^{13}C NMR (100 MHz, D_2O): *δ (ppm)* 196.75, 182.68, 167.97, 154.90, 134.75, 130.73, 125.27, 38.68, 27.16, 18.71. IR (KBr): ν = 3418, 2923, 2494, 1704, 1659, 1615, 1234, 1060, 699 cm^{-1}. HRMS m/z Found: 210.0765, calculated: 210.0766 for $C_9H_{10}NO_4$ $[M+H]^+$.

S1.10 Synthesis of 5-((2-((((5,6--Dihydro-11,12-didehydrodibenzo[a,e]cyclooctene -5-yl)oxy)carbonyl)amino)ethyl)amino)naphthalene-2-sulfonic acid (15)：

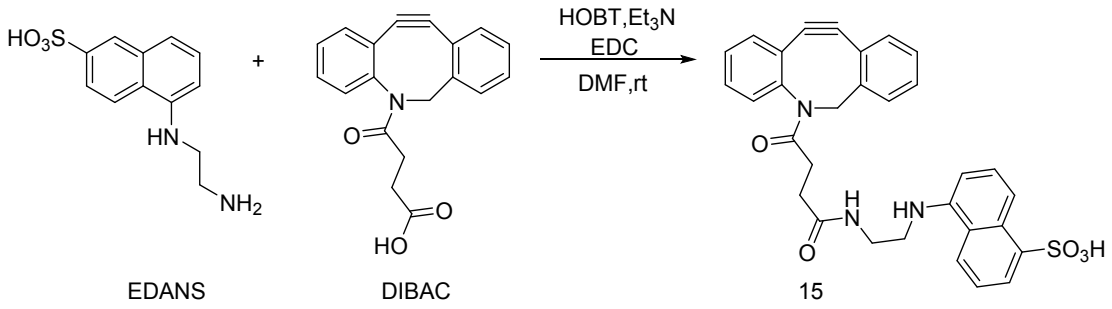


Figure S10. Synthesis of compound **15**

A solution of the DIBAC (50 mg, 0.2 mmol, was prepared following the reported method[3]) and HOBT （45 mg, 0.4 mmol）in DMF (5 mL) was added dropwise to a stirred solution of EDANS (54 mg, 0.24 mmol) in DMF (3 mL), then Et_3N (90 μL, 0.8 mmol) and EDC (65 mg, 0.4 mmol) was added. The rection mixture was stirred at room temperature for overnight until the reaction was complete (monitored by TLC), then the reaction mixture was evaporated under reduced pressure, and the crude product was purified via silica gel column chromatography (20:1-5:1, DCM/MeOH) to afford DIBAC-Flu **15** (88.5 mg, 80%). ^{1}H NMR (400 MHz, CD_3OD): *δ (ppm)* 8.21 (d, *J* = 8.6 Hz, 1H), 8.12 (dd, *J* = 16.0, 7.9 Hz, 2H), 7.58 – 7.54 (m, 1H), 7.45 – 7.36 (m, 6H), 7.25 – 7.15 (m, 3H), 6.67 (d, *J* = 7.6 Hz, 1H), 4.97 (d, *J* = 14.1 Hz, 2H), 3.62 (d, *J* = 13.9 Hz, 1H), 3.52 – 3.46 (m, 1H), 3.42 – 3.35 (m, 1H), 3.28 – 3.23 (m, 1H), 2.70 – 2.77 (m, 1H), 2.36 – 2.29 (m, 1H), 2.22 – 2.15 (m, 1H), 2.00 –1.93 (m, 1H). NMR (100 MHz, CD_3OD): *δ (ppm)* 176.33 , 174.58 , 153.18 , 150.01 , 142.57 , 134.02 , 132.13 , 131.18 , 130.59 , 130.29 , 129.77 , 129.42 , 129.10 ,128.97, 128.70 , 127.36 , 127.01 , 126.46 , 126.12 , 124.90 , 124.38 , 124.29 ,117.68, 116.16 , 109.32 , 101.97 ,57.29 ,45.89 , 40.02 , 32.60 , 32.09. HRMS m/z Found: 554.1755, calculated: 554.1750 for $C_{31}H_{28}N_3O_5S$ $[M+H]^+$.

S2. Computation Methods

All geometry optimizations were performed with Gaussian 16 software package,[4] at the M06-2X level and using the 6-311+G(2d,p) basis set in combination with the solvation model density (SMD) to mimic the water solvent effect and with RI approximation and Grimme's empirical dispersion correction D3.[5] The presence of energy minima of the ground states (zero imaginary frequencies) and saddle points for transition states (a single imaginary frequency) was checked by frequency calculations for the optimized geometries.

S3. Kinetic Study

To a UV-VIS cuvette, 3 mL of reactants in PBS 7.4 were added. The concentration of the reactants were as follows: for PL-N_3/HP, both were 50 μM, for PL-COOH 3/HP both were 50 μM, for PLP/HP both were 50 μM, for PL/HP, PL was 50 μM and HP was 1 mM, for 2-(azidomethyl)benzaldehyde/HP, 2-(azidomethyl)benzaldehyde was 50 μM and HP was 1 mM, and for 2-formyl benzoic acid /HP, 2-formyl benzoic acid was 50 μM and HP was 1 mM (Caution: HP is not stable in solution, and thus needs to be prepared fresh). For PL/HP, because the reaction is too slow, HP was used in large excess, and pseudo-first order reaction rate, k_{obs}, was obtained. The reaction was monitored by UV-VIS spectrometer at proper wavelengths, 358 nm for PL-N_3 /HP, 355 nm for PL-COOH-3/HP, 355 nm for PLP/HP, 350 nm for PL/HP, 350 nm for 2-(azidomethyl)benzaldehyde/HP, and 350 nm for 2-formyl benzoic acid/HP. Data collected was then fitted to the rate equation (1) for a reversible second-order reaction, except for PL/HP which was fitted to the rate equation (2) for a first-order reaction.[6]

$$c_p = c_0 - \frac{a_+(c_0-a_-)-a_-(c_0-a_+)e^{-k_1(a_+-a_-)t}}{(c_0-a_-)-(c_0-a_+)e^{-k_1(a_+-a_-)t}} \quad (1)$$

$$c_p = c_0\,(1-e^{-k_{obs}t}) \quad (2)$$

in which

$$a_+ = \frac{-k_{-1}+\sqrt{k^2_{-1}+4k_1k_{-1}x_0}}{2k_1}\,,\quad a_- = \frac{-k_{-1}-\sqrt{k^2_{-1}+4k_1k_{-1}x_0}}{2k_1}$$

$$k_{-1} = \frac{k_1}{K_{eq}}\,,\quad k_{obs} = k_1c_{HP}$$

and c_p is the concentration of the product, c_0 is the initial concentration of the aldehyde, c_{HP} is the concentration of HP, t is the reaction time, k_1 is rate constant of hydrazone formation, k_{-1} is rate constant of hydrazone hydrolysis, and K_{eq} is the equilibrium constant. The results of k_1, k_{-1}, and K_{eq} were reported as an average of three independent experiments.

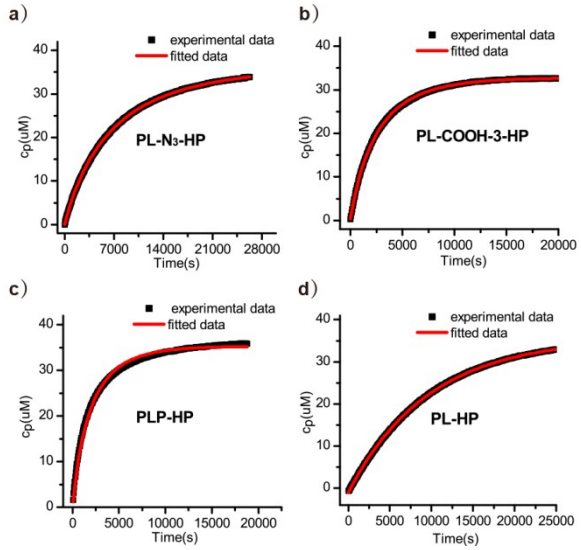


Figure S11. Formation of hydrazone over time. (a) 50 μM PL-N_3 and 50 μM HP, (b) 50 μM PL-COOH **3** and 50 μM HP, (c) 50 μM PLP and 50 μM HP, (d) 50 μM PL, and 1 mM HP. The black dots were experimental data which were collected every 60 s, and the red solid line was the data fifitted to the rate equation.

The yield of formation of hydrazone between PL-N_3 and HP (1:1) for 333 minutes is 65%. The yield of formation of hydrazone between PL-COOH **3** and HP (1:1) for 333 minutes is 65%.

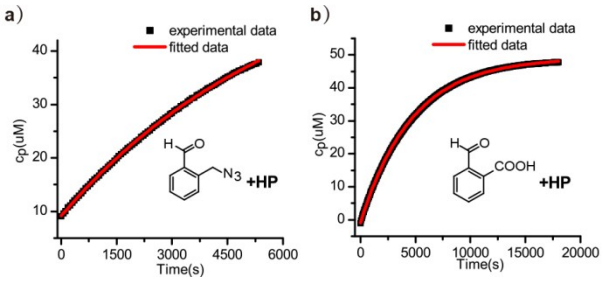


Figure S12. Formation of hydrazone over time. (a) 50 μM 2-(azidomethyl)benzaldehyde and 1 mM HP, (b) 50 μM 2-formyl benzoic acid and 1 mM HP. The black dots were experimental data which were collected every 60 s, and the red solid line was the data fifitted to the rate equation.

S4. Protein modification and Peptide synthesis

S4.1 Protein modification

S4.1.1 The modification of BSA with FTZ

Preparation of BSA-PL-FTZ conjugate. To a solution of BSA-FDIBO[7] (100 μL, 10 mg/mL in 1×PBS) was added 150 μL of the azido pyridoxal **1** (PL-N_3, 150 μL, 0.39 μmol, 2.6 mM in DMSO). The reaction mixture was left at room temperature for overnight to afford BSA-FDIBO-PL. Then the reaction mixture was washed with 1×PBS passing through an ultrafiltration tube (50 KD) to get rid of the excess PL-N_3. The resulting BSA-FDIBO-PL conjugate was examined by UV-VIS spectroscopy to quantity. A solution of **FTZ** (4 μL, 23.72 mM in DMSO) was added to the resulting BSA-FDIBO-PL solution (33 μL in 1×PBS). The reaction mixture was left at room temperature for 12 hours, then was washed with 1×PBS and 0.1 M Tris-HCl Buffer (pH 8.0) passing through an ultrafiltration tube (50 KD) to get rid of the excess FTZ. The resulting BSA-PL-FTZ conjugate was examined by UV-VIS spectroscopy and SDS-PAGE. The control experiment using just BSA or BSA-FDIBO with FTZ to yield BSA~FTZ or BSA-FDIBO~FTZ was analyzed with UV-VIS spectroscopy and SDS-PAGE under same conditions.

Preparation of BSA~PL~FTZ conjugate. To a solution of BSA (100 μL, 10 mg/mL in 1×PBS) was added 150 μL of the azido pyridoxal **1** (PL-N_3, 150 μL, 0.39 μmol, 2.6 mM in DMSO). The reaction mixture was left at room temperature for overnight to afford BSA~PL. Then the reaction mixture was washed with 1×PBS passing through an ultrafiltration tube (50 KD) to get rid of the excess PL-N_3. The resulting BSA~PL conjugate was examined by UV-VIS spectroscopy was quantity. A solution of **FTZ** (14 μL, 2.372 mM in DMSO) was added to the BSA~PL solution (20 μL in 1×PBS). The reaction mixture was left at room temperature for 12 hours, then was washed with 1×PBS and 0.1 M Tris-HCl Buffer (pH 8.0) passing through an ultrafiltration tube (50 KD) to get rid of the excess FTZ. The resulting BSA~PL~FTZ conjugate was examined by UV-VIS spectroscopy.

Preparation of BSA-PL-FTZ conjugate (Method 2). A solution of the azido pyridoxal **1** (PL-N_3, 1.5 μL, 0.075 μmol, 52 mM in DMSO) was incubated with a solution of **FTZ** (7.0 μL, 0.09 μmol, 12.78 mM in DMSO), then 1×PBS (21.5 μL) was added. The reaction mixture was left at room temperature for overnight to afford N_3-PL-FTZ **16**. Then to a solution of BSA-FDIBO (3.2 μL, 10 mg/mL in 1×PBS) and 1×PBS (23.8 μL) was added 3 μL of the N_3-PL-FTZ reaction solution. The reaction mixture was left at room temperature for 12 hours, and the resulting BSA-PL-FTZ solution was analyzed with SDS-PAGE without further purification.

Preparation of BSA~PL-FTZ. To a solution of BSA (3.2 μL, 10 mg/mL in 1×PBS) and 1×PBS (23.8 μL) was added 3 μL of the N_3-PL-FTZ reaction solution. The reaction mixture was left at room temperature for 12 hours, and the resulting BSA~PL-FTZ solution was analyzed with SDS-PAGE without further purification.

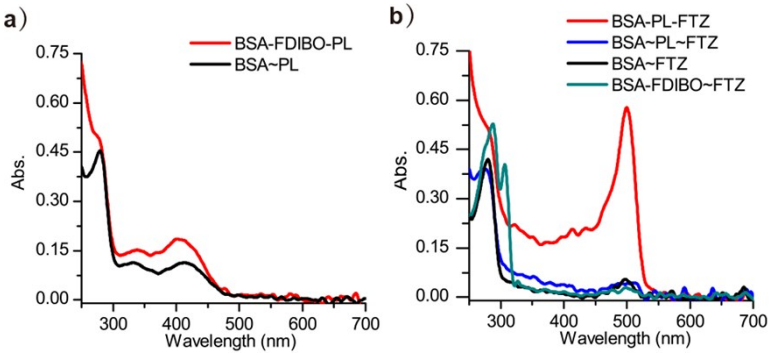


Figure S13. (a) UV-vis absorption spectra of BSA-FDIBO-PL (10.11 μM, red), BSA~PL (10.00 μM, black). (b) UV-vis absorption spectra of BSA- PL-FTZ (8.50 μM, red), BSA~PL~FTZ (8.49 μM, blue), BSA~FTZ (9.32μM, black) and BSA-FDIBO~FTZ (10.00 μM, green).

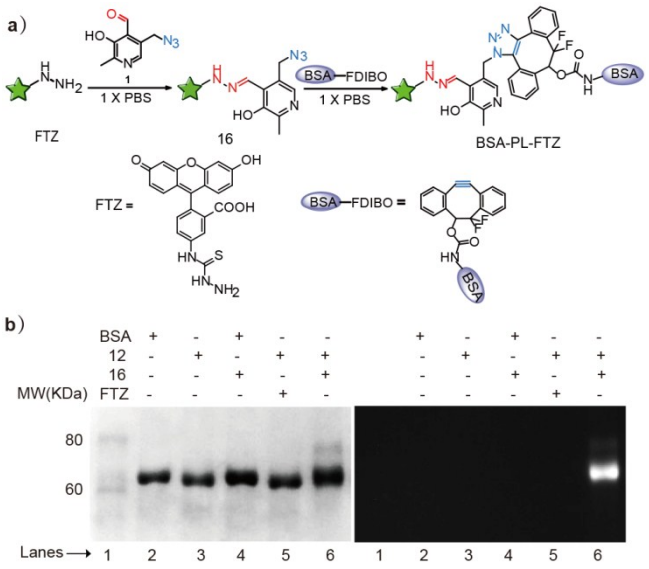


Figure S14. (a) Modification of BSA with FTZ (method 2). (b) SDS-PAGE visualized under UV light (right) and after Coomassie-blue stain (left). Well from left to right: protein ladder (each line: 80 kDa and 60. kDa, from top to bottom), BSA, BSA-FDIBO, BSA~FTZ, BSA-FDIBO~FTZ, and BSA-PL-FTZ from 2 to 6.

S4.1.2 The modification of eGFP with DIBAC-EDANS

Expression of Sortase A and eGFP and Sortase A–mediated hydrazinolysis of eGFP were performed following the reported method.[8] The plasmids of SrtA and eGFP were kindly provided by Prof. Lei Liu and Prof. Yiming Li (Tsinghua University).

Preparation of eGFP-PL-N_3 conjugate. The eGFP-$NHNH_2$ (20 μM) was incubated with azido pyridoxal **1** (PL-N_3, 400μM) in 1×PBS for 12 hours at room temperature. Then the reaction mixture was washed with 1×PBS passing through an ultrafiltration tube (10 KD) to get rid of the excess PL-N_3. The concentration of the resulting eGFP-PL-N_3 solution was quantitated by the absorption at 490 nm and then was stored in PBS 7.4 at 4 ˚C for next steps.

Preparation of eGFP-PL-EDANS conjugate. The eGFP-PL-N_3 (20 μM) was incubated with DIBAC-EDANS (200 μM) in 1×PBS for 12 hours at room temperature. Then the reaction mixture was washed with 1×PBS passing through an ultrafiltration tube (10 KD) to get rid of the excess DIBAC-EDANS. The resulting eGFP-PL-EDANS conjugate was examined by Fluorescence spectroscopy (λ_{ex} = 335 nm). The control experiment using just eGFP with DIBAC-EDANS to yield eGFP~EDANS was analyzed with Fluorescence spectroscopy under same conditions.

S4.1.3 The modification of BSA with eGFP

Preparation of BSA-PL-eGFP conjugate. The BSA-FDIBO (10 μM) was incubated with eGFP-PL-N_3 (160 μM) in 1×PBS for 12 hours at room temperature. Then the reaction mixture was washed with 1×PBS passing through an ultrafiltration tube (50 KD) to get rid of the excess eGFP-PL-N_3. The resulting BSA-PL-eGFP conjugate was examined by UV-VIS spectroscopy. The control experiment using just BSA-FDIBO and plain eGFP to yield BSA-FDIBO~eGFP was analyzed with UV-VIS spectroscopy under same conditions.

S4.2 FRET effect of the eGFP-PL-EDANS

The obtained eGFP-PL-EDANS conjugate (100 nM) was examined by Fluorescence spectroscopy upon excitation at 335 nm. The control experiment eGFP~EDANS, which was a mixture of 100 nM eGFP and 100 nM DIBAC-EDANS in 1× PBS was also examined by Fluorescence spectroscopy upon excitation at 335 nm. The unmodified eGFP was also examined by Fluorescence spectroscopy upon excitation at 335 nm and 490 nm respectively.

S4.3 Peptide synthesis

S4.3.1 Model peptides:

Model peptides Ac-RGDYKGGKG-NH_2 (peptiRGD) was synthesized through conventional Fmoc solid-phase chemistry on Rink Amide MBHA resin.

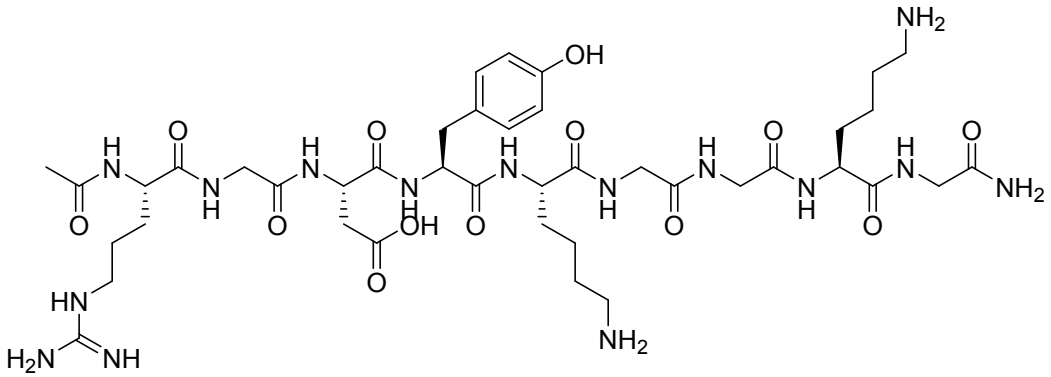


Figure S15. Structure of **peptiRGD**

S4.3.2 Modification of peptiRGD with PL-COOH 3 and Pyridoxal phosphate (PLP)

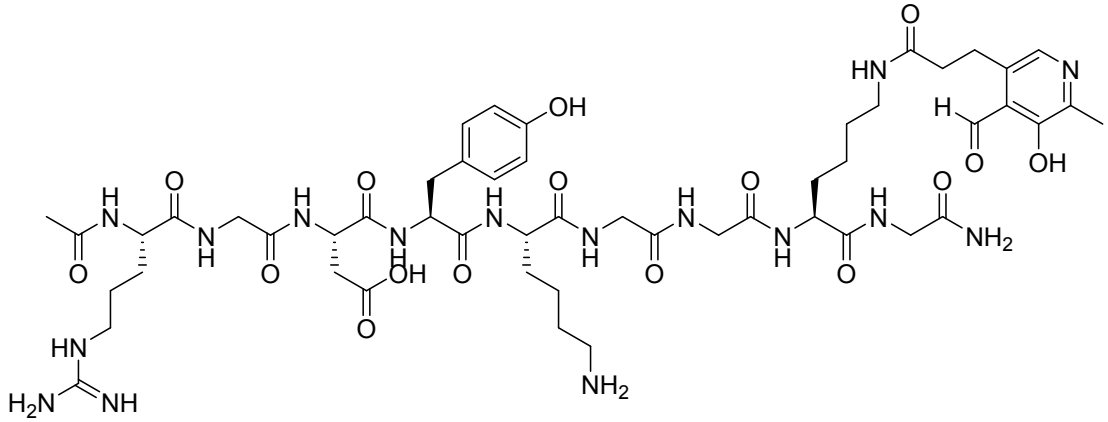


Figure S16. Structure of **RGDPL**

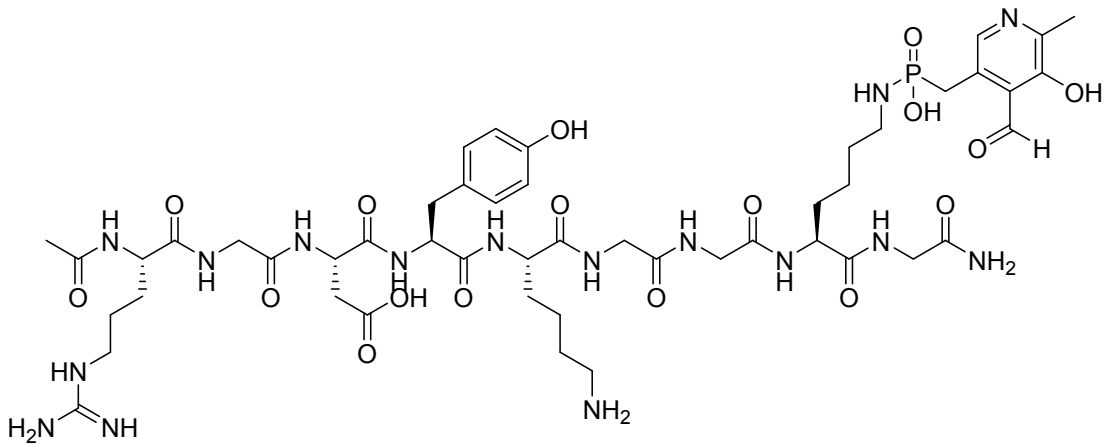


Figure S17. Structure of **RGDPLP**

Modified with PL-COOH 3: Fully protected peptidyl resin (10 mg) was placed in a reaction vessel and 3 mL of 1% TFA in DCM was added, which was then agitated gently for 10 min. The solution was drained and the resin was washed with DMF until the eluate was colorless. Then to the reaction vessel containing resin was added a mixture of PL-COOH **3** (3.867 mg, 5 eq) and HOBt (2.291 mg, 5 eq) in 100 μL of DMF, DIPEA (5.6 μL, 10 eq), and HBTU (6.318 mg, 4.9 eq) in 100 μL of DMF. After agitating gently for 2 h, the resin was washed with DMF (5 times) and DCM (5 times) to obtain PL-COOH **3** modified peptidyl resin. Then the modified resin was transferred to a 1.5 mL centrifuge tube, which was added 800 μL of FA/TIS/water (95:2.5:2.5 (v/v) and agitated gently for 5 h to attain deprotected peptidyl solution. After filtering the

resin, diethyl ether was added and the product: Ac-RGDYKGGK(PL)G-NH_2 (RGDPL) was obtained as precipitate, which was then examined by LC-MS.

Modified with Pyridoxal phosphate (PLP): Fully protected peptidyl resin (10 mg) was placed in a reaction vessel and 3 mL of 1% TFA in DCM was added, which was then agitated gently for 10 min. The solution was drained and the resin was washed with DMF until the eluate was colorless. Then to the reaction vessel containing resin was added a mixture of PLP (4.905 mg, 5 eq) and HOBt (2.291 mg, 5 eq) in 100 μL of DMF, DIPEA (5.6 μL, 10 eq), and EDC (3.475 mg, 4.9 eq) in 100 μL of DMF. After agitating gently for 2 h, the resin was washed with DMF (5 times) and DCM (5 times). Then the resin was transferred to a 1.5 mL centrifuge tube, which was added 800 uL of FA/TIS/water (95:2.5:2.5 (v/v) and agitated gently for 5 h to attain deprotected peptidyl solution. After filtering the resin, diethyl ether was added and the precipitate was examined by LC-MS.

Modification of RGDPL with 5-(hydrazinecarbothioamido)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid (FTZ) : The obtained RGDPL (1.7 μM) was incubated with FTZ (2.5 μM) in H_2O at pH 7.0 for 2 hours at room temperature and the reaction mixture was then analyzed by LC-MS without purification.

S4.3.3 LC-MS spectra

a. Ac-RGDYKGGKG-NH_2 (peptiRGD)

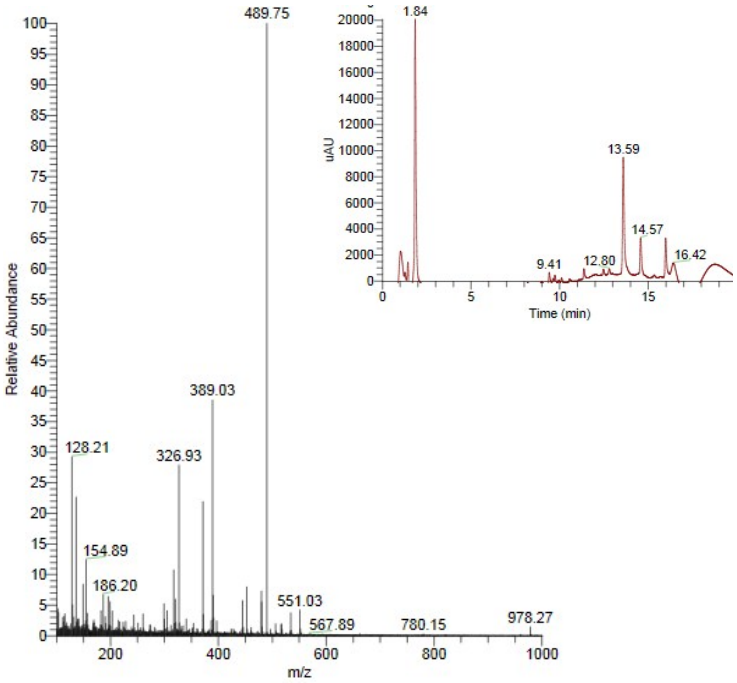


Figure S18. Mass analysis of **peptiRGD**

Product peak position: 1.84 min, ESI-MS: 489.75 ($[M+2H]^{2+}$) and 326.93 ($[M+3H]^{3+}$). Calculated mass for peptiRGD: 977.50.

b. Modification of peptiRGD with PL-COOH **3**

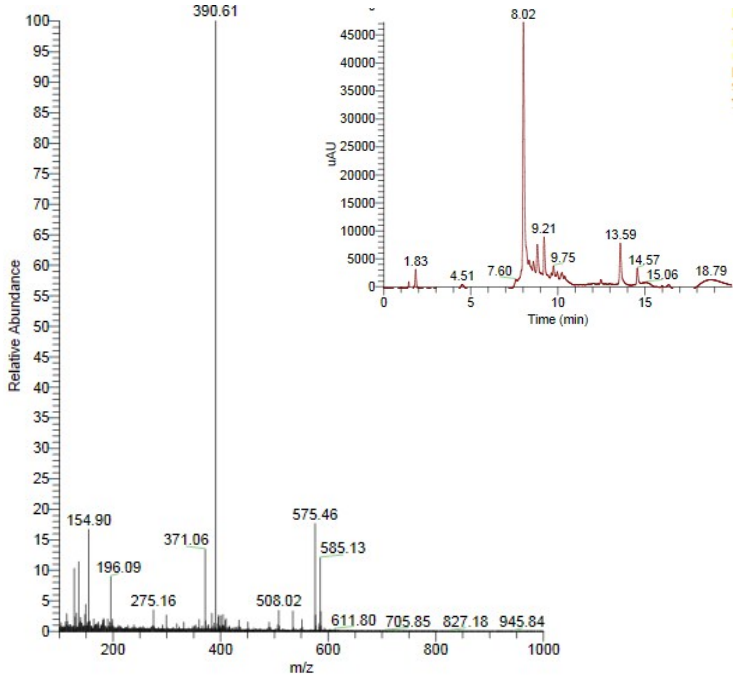


Figure S19. Mass analysis of modification of peptiRGD with PL-COOH **3**

Product peak position: 8.02 min, ESI-MS: 585.13 ($[M+2H]^{2+}$) and 390.61 ($[M+3H]^{3+}$). Calculated mass for RGDPL: 1168.56.

c. Modification of peptiRGD with PLP

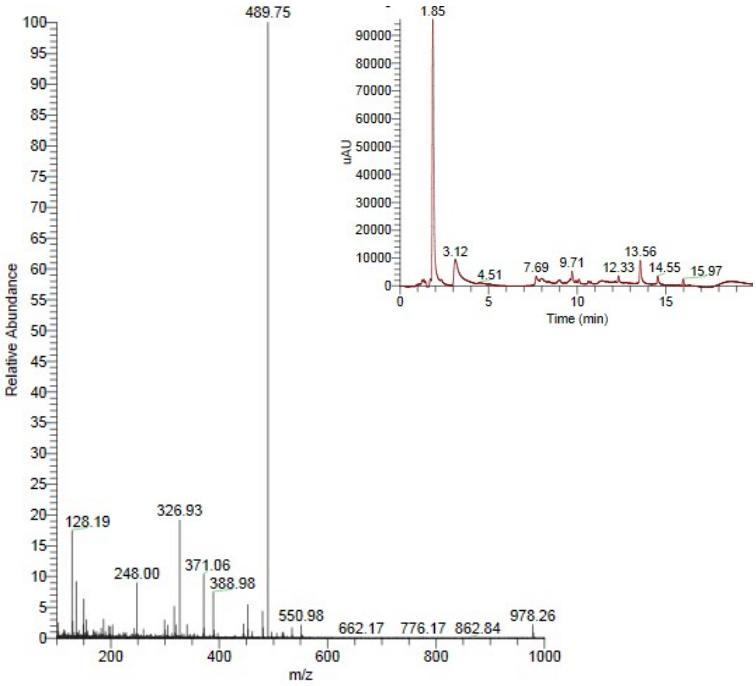


Figure S20. Mass analysis of modification of peptiRGD with PLP

Product peak position: 1.85 min, ESI-MS: 489.75 ($[M+2H]^{2+}$) and 326.93 ($[M+3H]^{3+}$), which was consistent with the mass of unmodified peptiRGD (977.50, calculated), but not the mass of expected product RGDPLP (1191.21, calculated).

d. Modification of RGDPL with FTZ

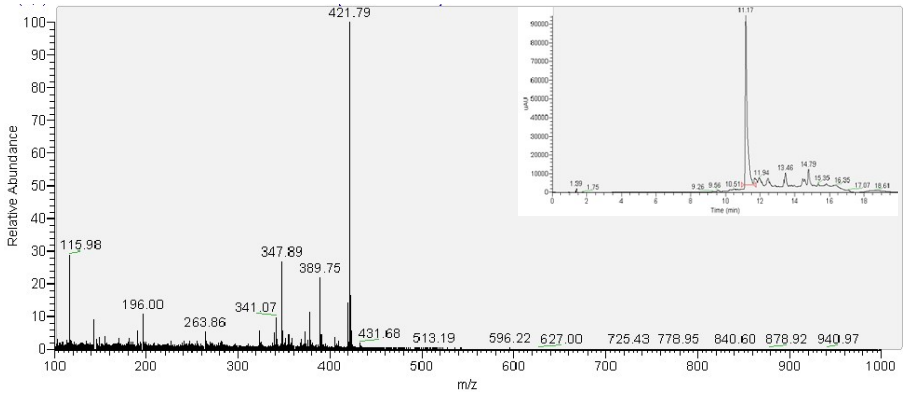


Figure S21. Mass analysis of FTZ

FTZ peak position: 11.17 min, ESI-MS: 421.79 ($[M+H]^{+}$). Calculated mass for FTZ: 421.07.

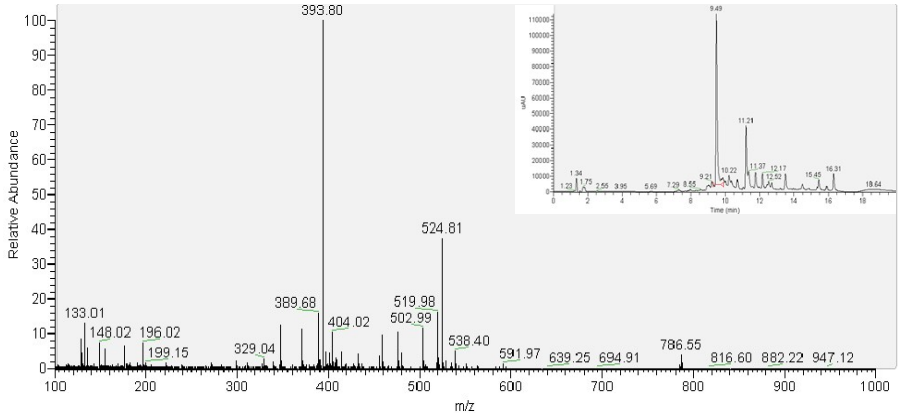


Figure S22. Mass analysis of modification of RGDPL with FTZ

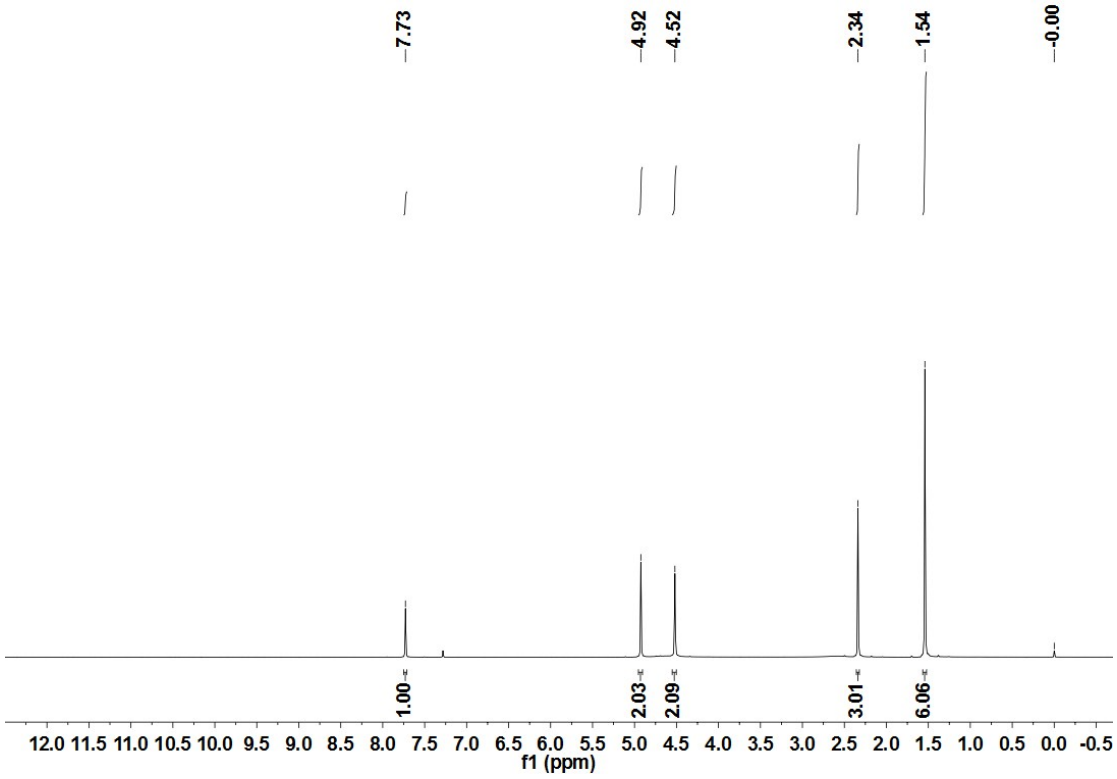
Product peak position: 9.49 min, ESI-MS: 786.65 ($[M+2H]^{2+}$) and 524.81 ($[M+3H]^{3+}$). Calculated mass for RGDPL-FTZ: 1571.63.

S5. General procedure for the SDS-PAGE analysis

The solution of sample (8.0 μL) was mixed with 5 X loading buffer (2.0 μL) in a 0.2 mL microcentrifuge tube. The samples were loaded onto a gel containing 5% stacking gel and 10% separating gel. The gel was run at 120 V for 15 min, and then at 180V for 40 min, with the Tris-glycine running buffer (25 mM Tris, 0.192 M glycine, and 0.1% (w/w) SDS, pH 8.3). After SDS-PAGE separation, the gel was washed with DI water and visualized with GenoSens 1850 for fluorescent signal. Then the gel was stained with Coomassie Brilliant Blue R250 and imaged for the protein staining signal.

S6. NMR Spectra

^{1}H NMR of 5



^{13}C NMR of 5

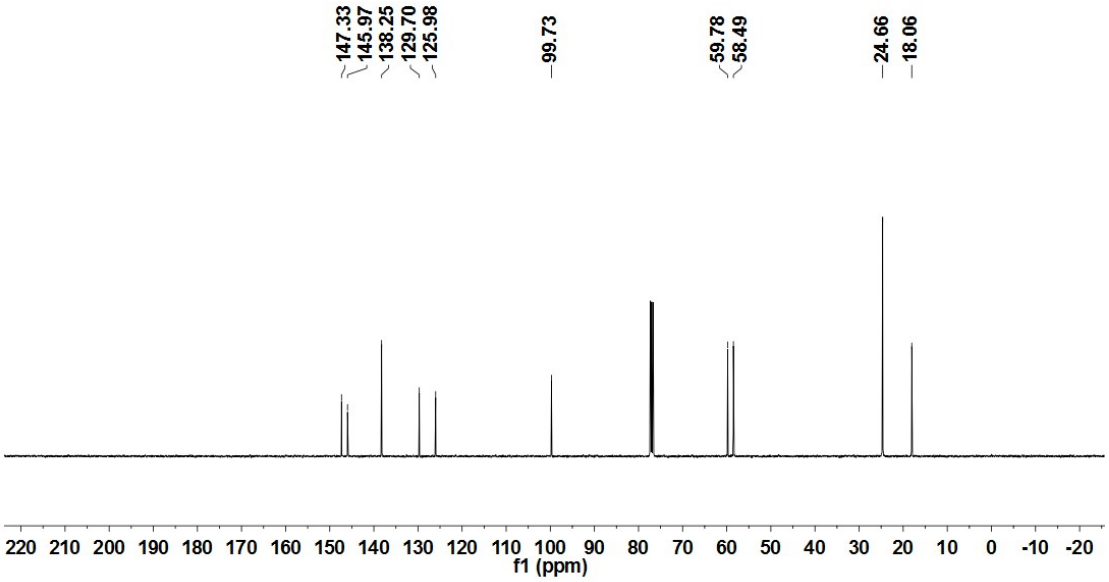
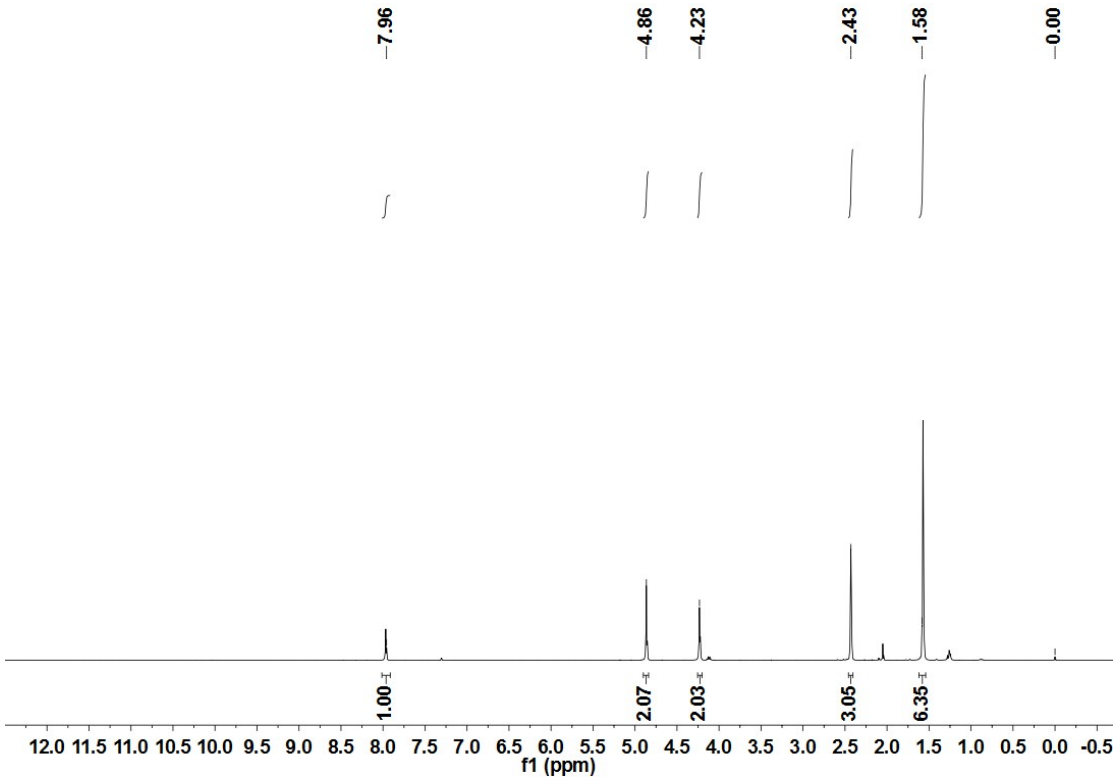


Figure S23. ^{1}H NMR and ^{13}C NMR spectra of **5** in $CDCl_3$.

^{1}H NMR of 6



^{13}C NMR of 6

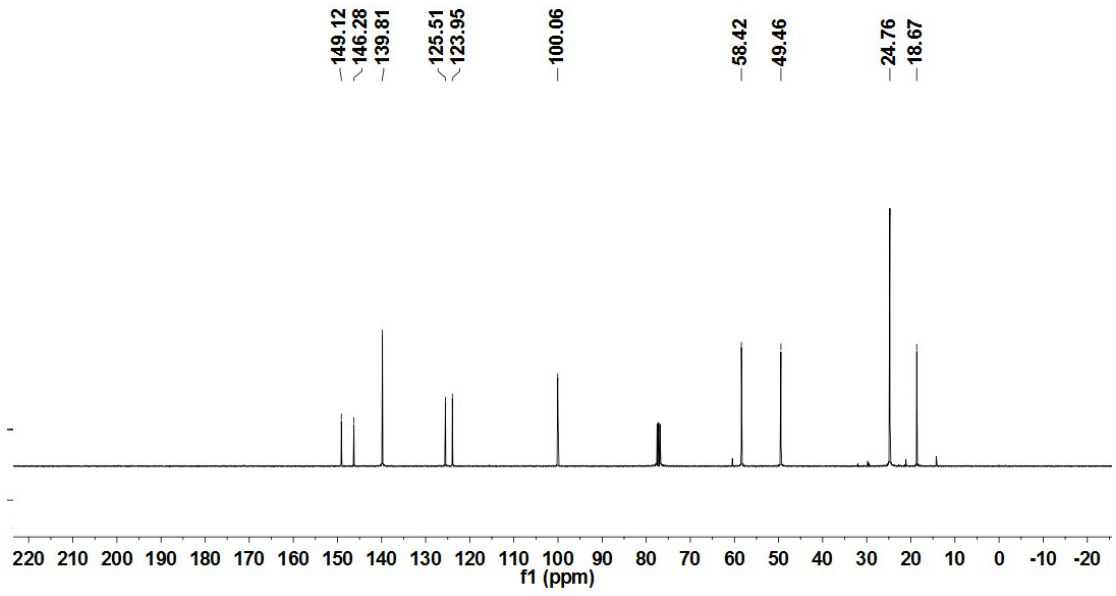
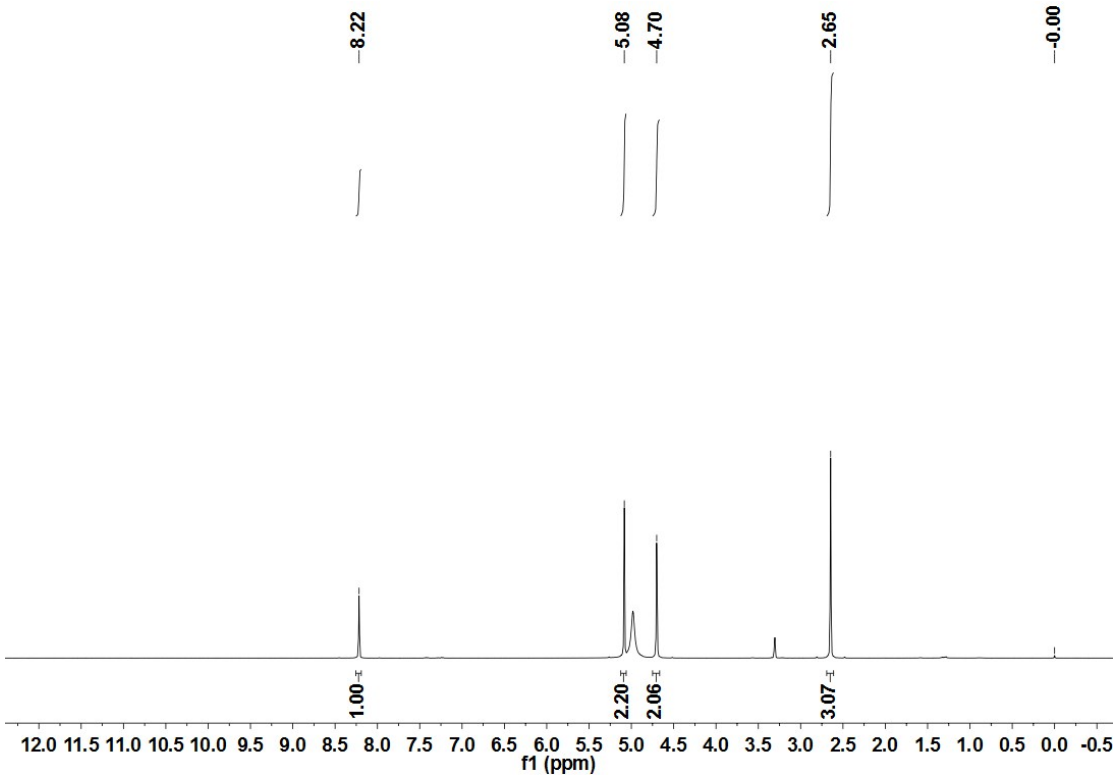


Figure S24. ^{1}H NMR and ^{13}C NMR spectra of **6** in $CDCl_3$.

^{1}H NMR of 7



^{13}C NMR of 7

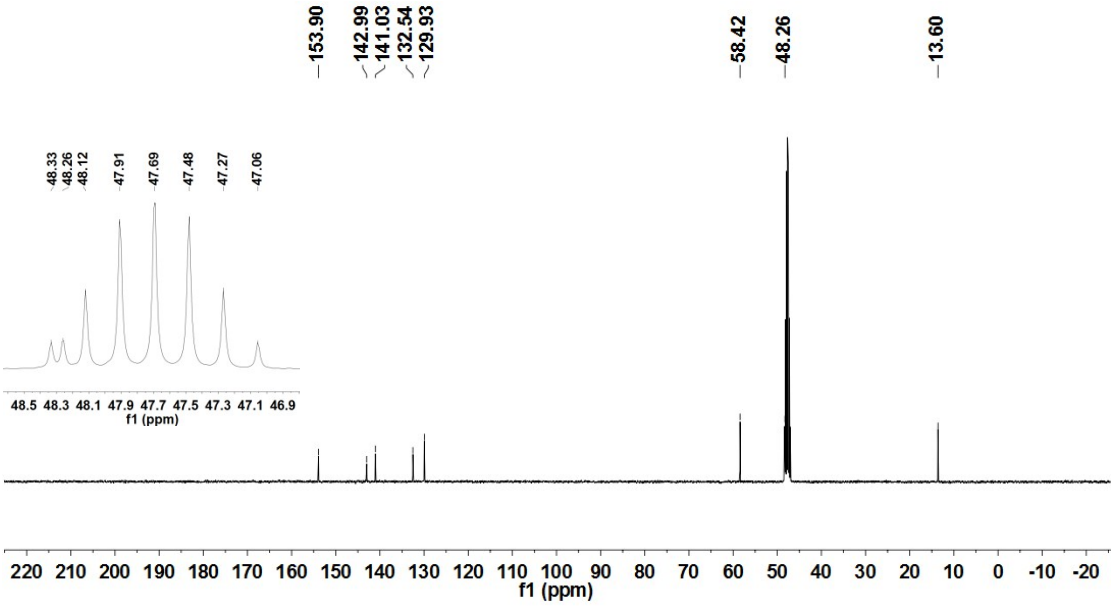
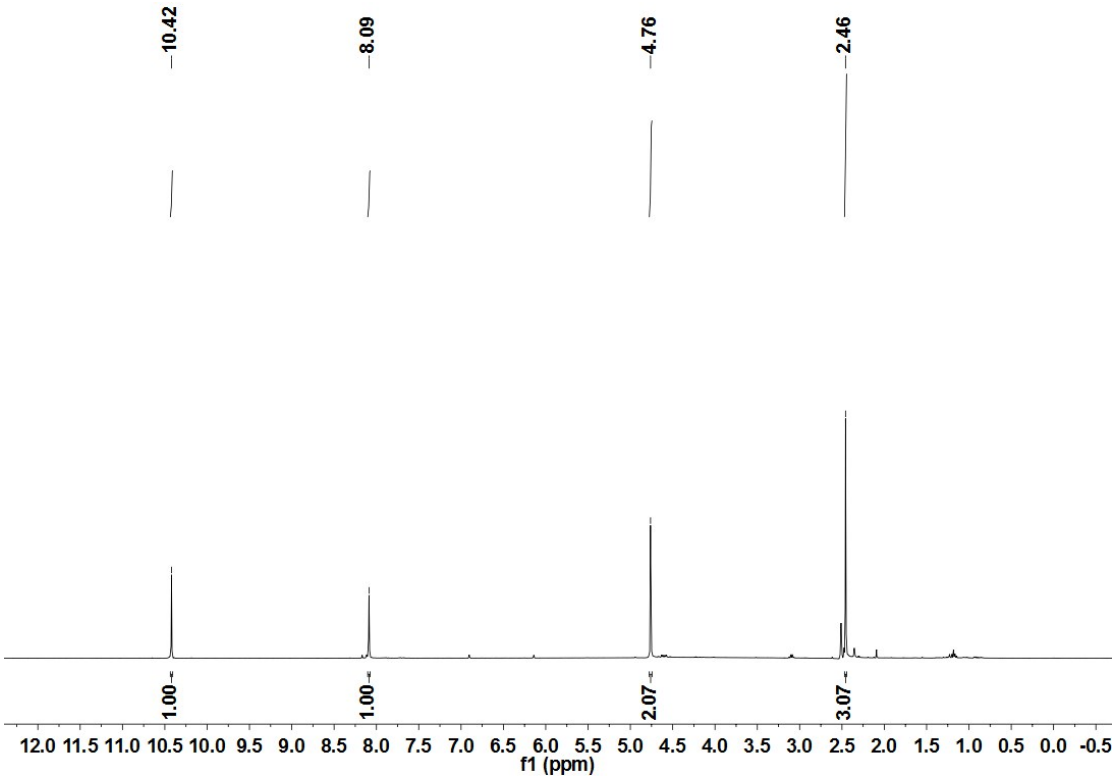


Figure S25. ^{1}H NMR and ^{13}C NMR spectra of **7** in D_2O.

^{1}H NMR of 1



^{13}C NMR of 1

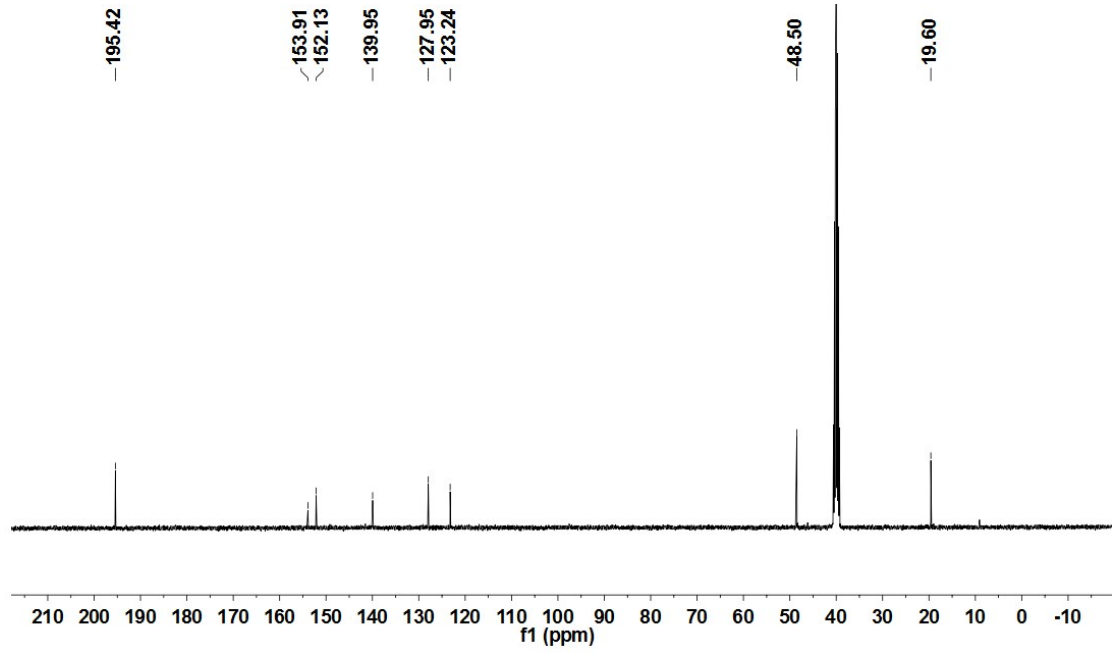
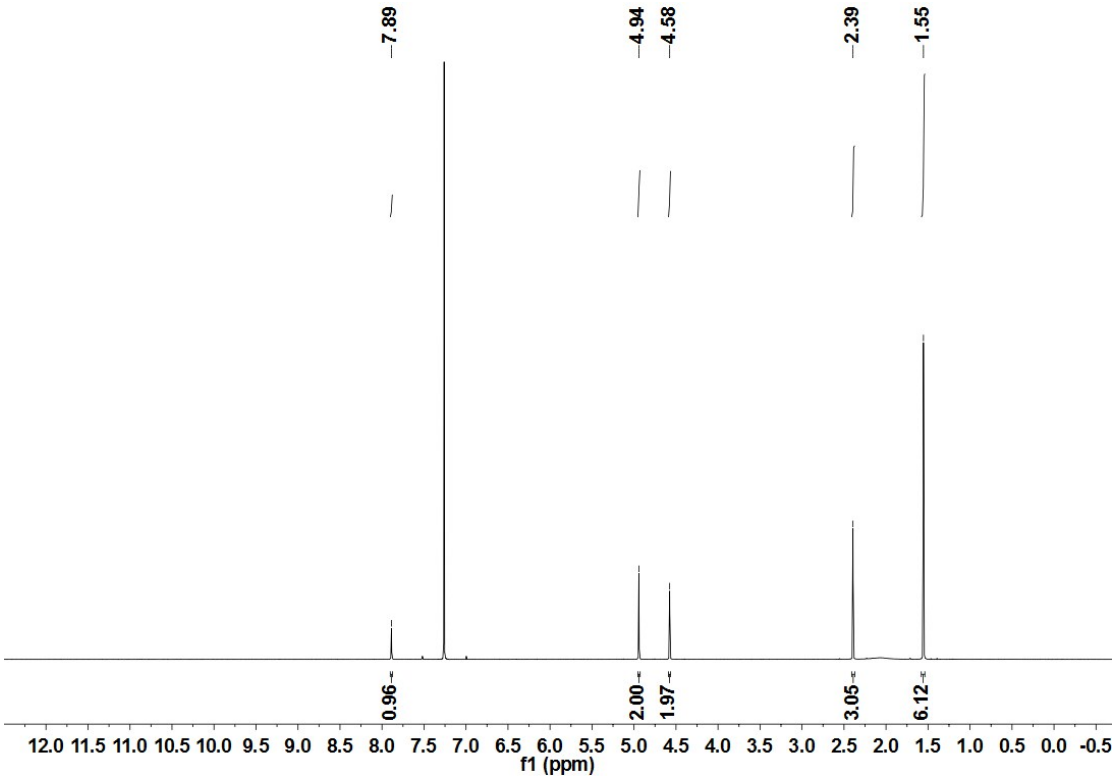


Figure S26. ^{1}H NMR and ^{13}C NMR spectra of **1** in DMSO-d_6.

^{1}H NMR of 8



^{13}C NMR of 8

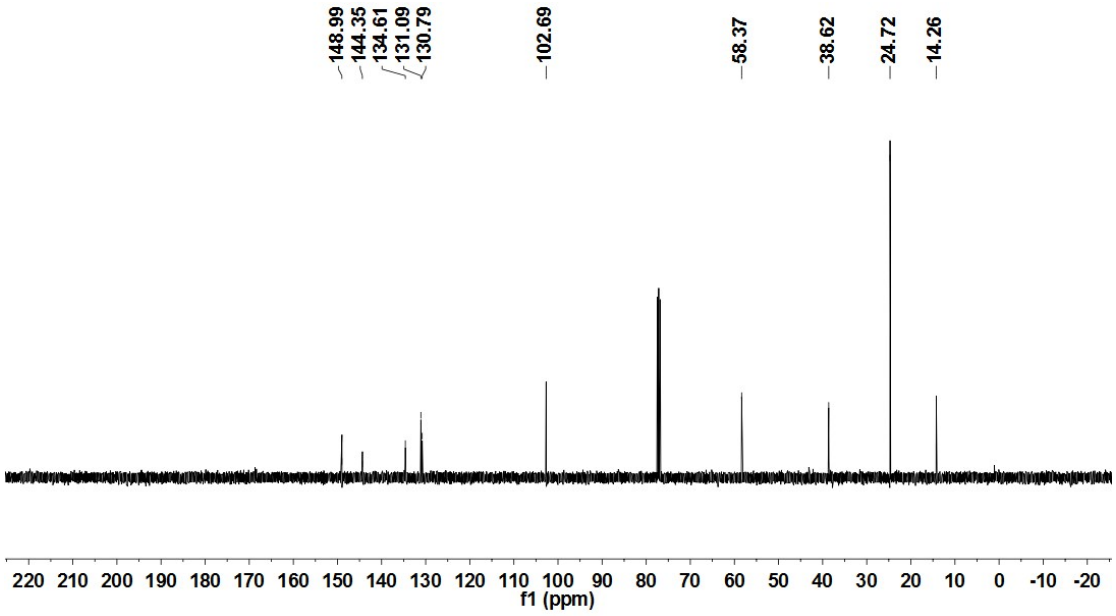
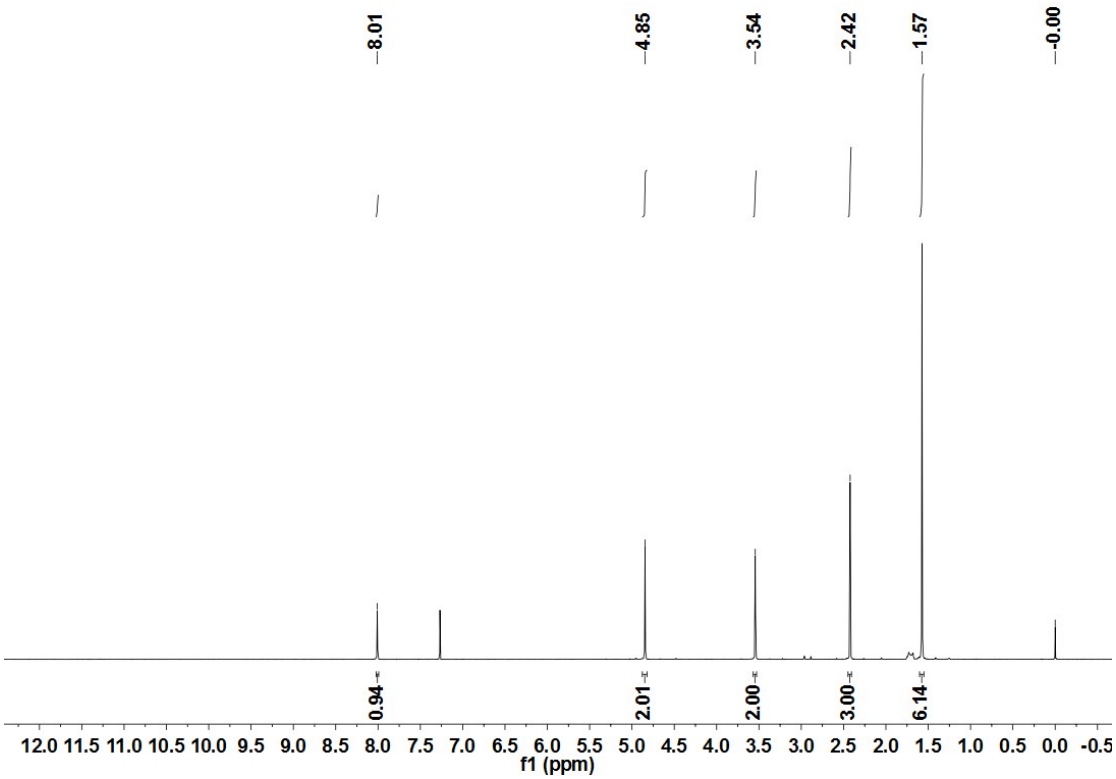


Figure S27. ^{1}H NMR and ^{13}C NMR spectra of **8** in $CDCl_3$.

^{1}H NMR of 9



^{13}C NMR of 9

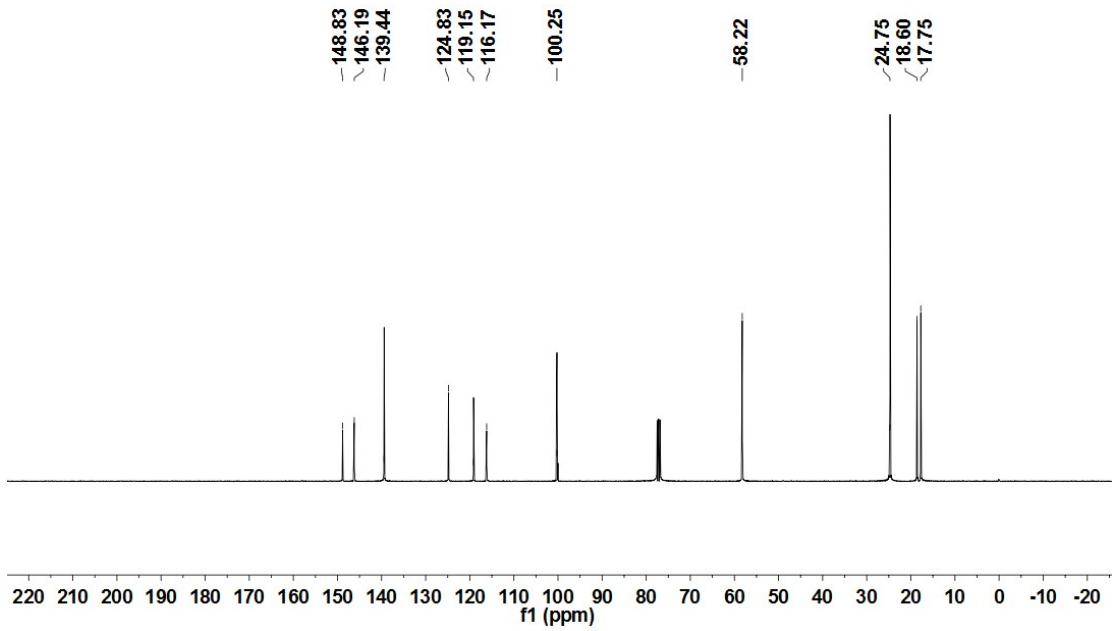
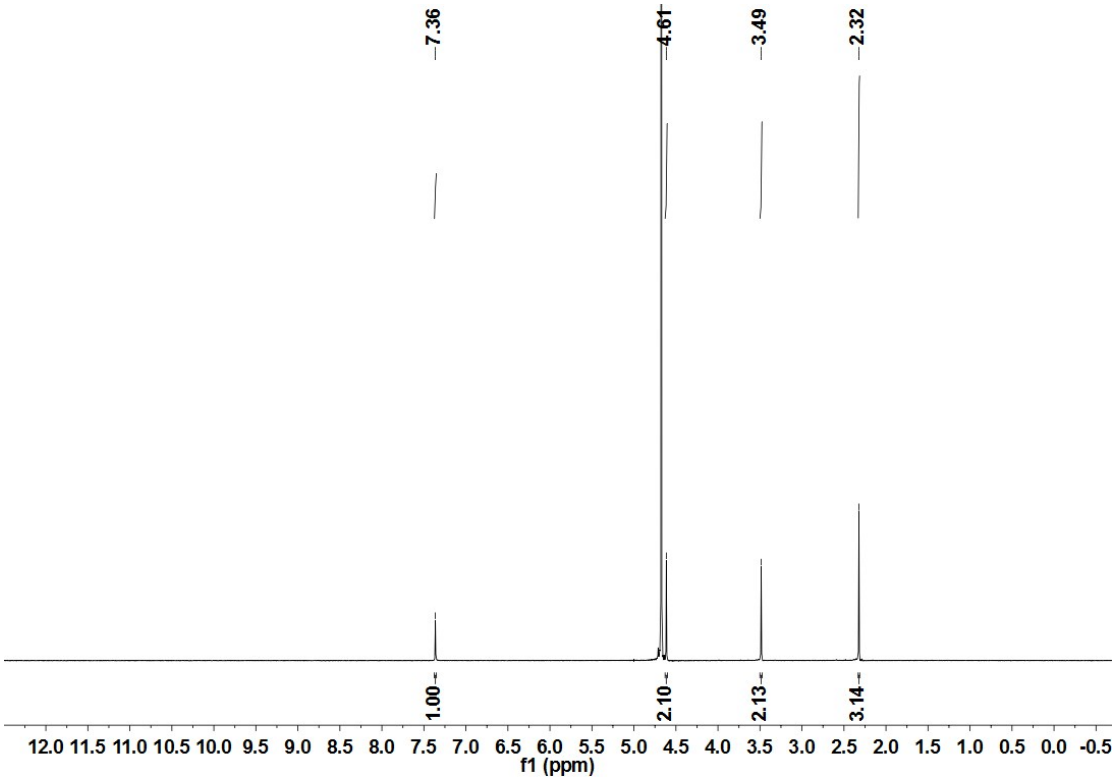


Figure S28. ^{1}H NMR and ^{13}C NMR spectra of **9** in $CDCl_3$.

^{1}H NMR of 10



^{13}C NMR of 10

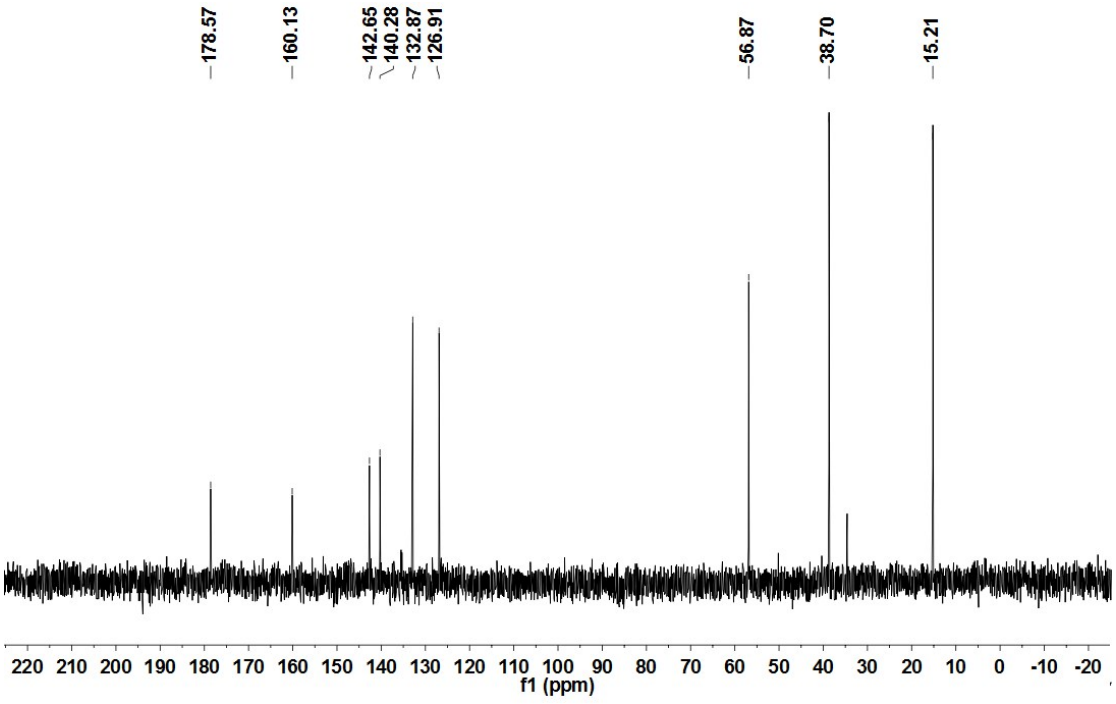
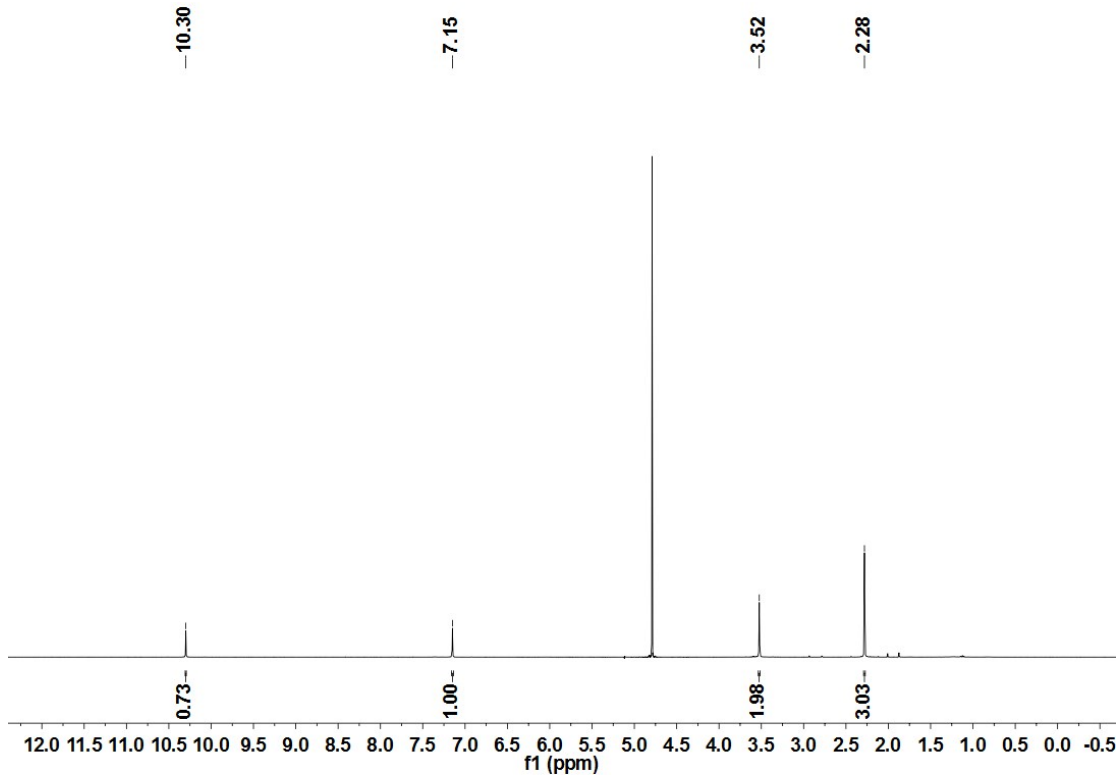


Figure S29. ^{1}H NMR and ^{13}C NMR spectra of **10** in D_2O.

^{1}H NMR of 2



^{13}C NMR of 2

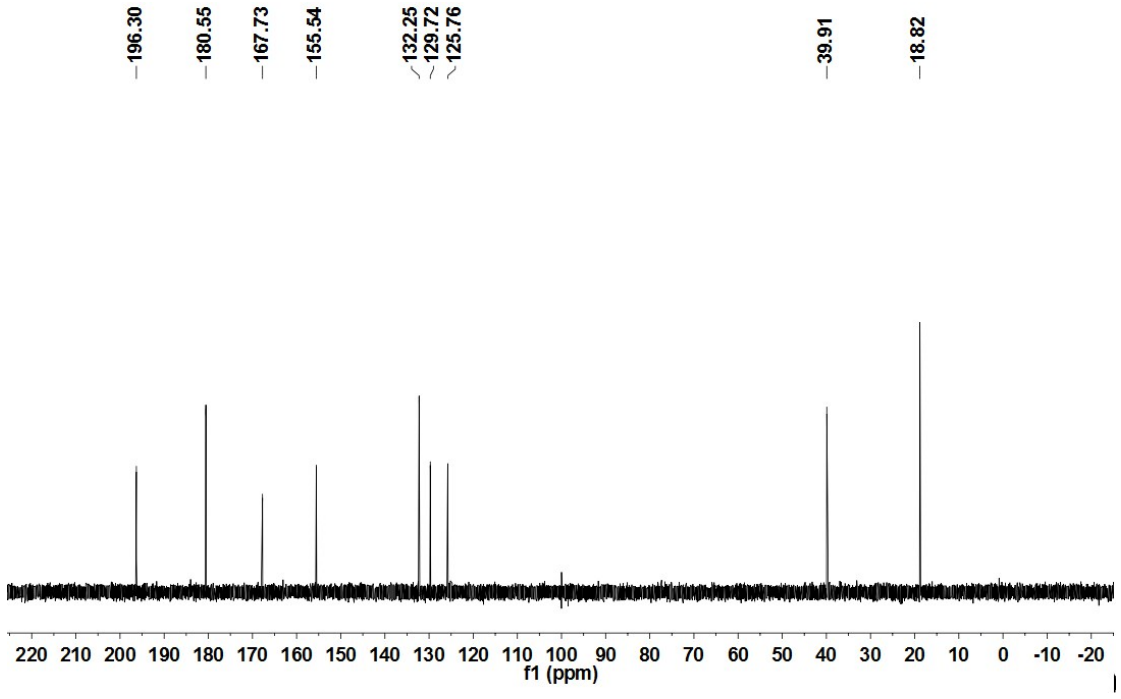
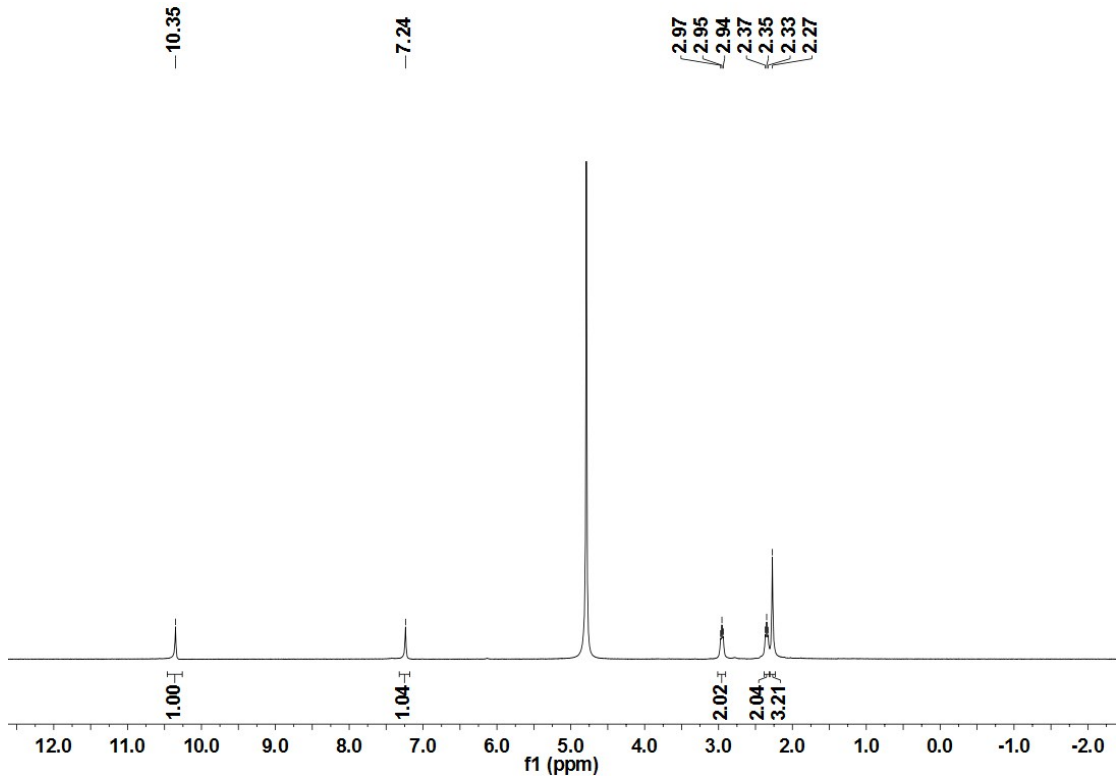


Figure S30. ^{1}H NMR and ^{13}C NMR spectra of **2** in D_2O.

^{1}H NMR of 3



^{13}C NMR of 3

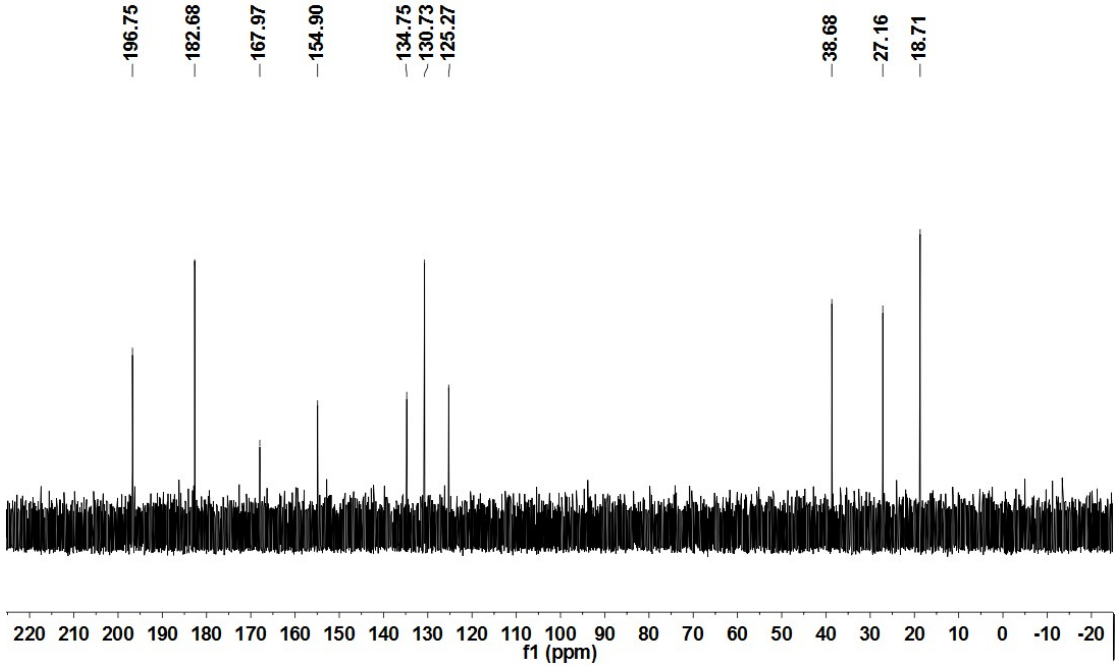
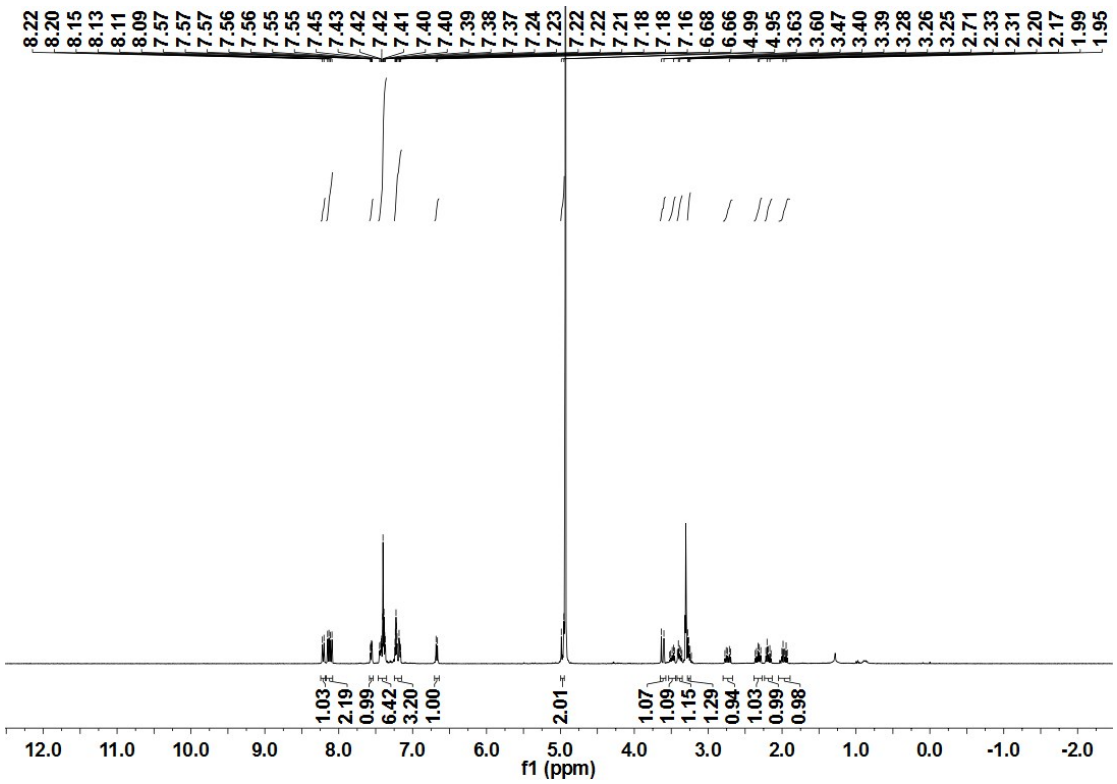


Figure S31. ^{1}H NMR and ^{13}C NMR spectra of **3** in D_2O.

^{1}H NMR of 15



^{13}C NMR of 15

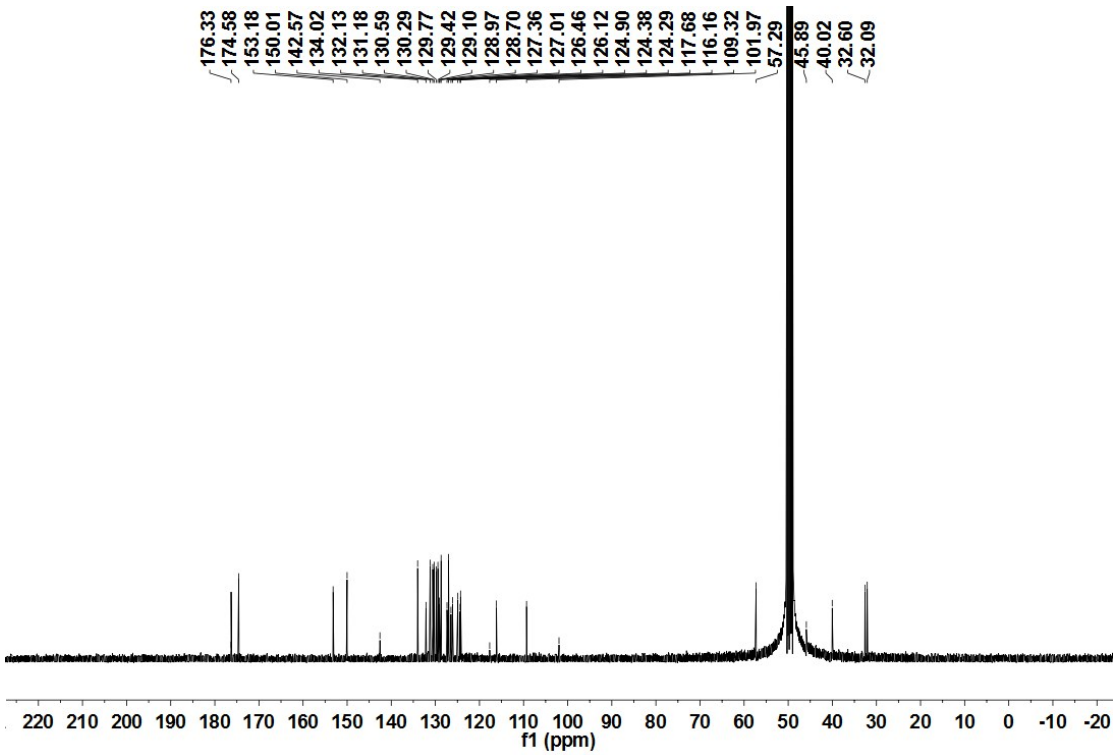


Figure S32. ^{1}H NMR and ^{13}C NMR spectra of **15** in CD_3OD.

S7. IR **Spectra**

IR of 6

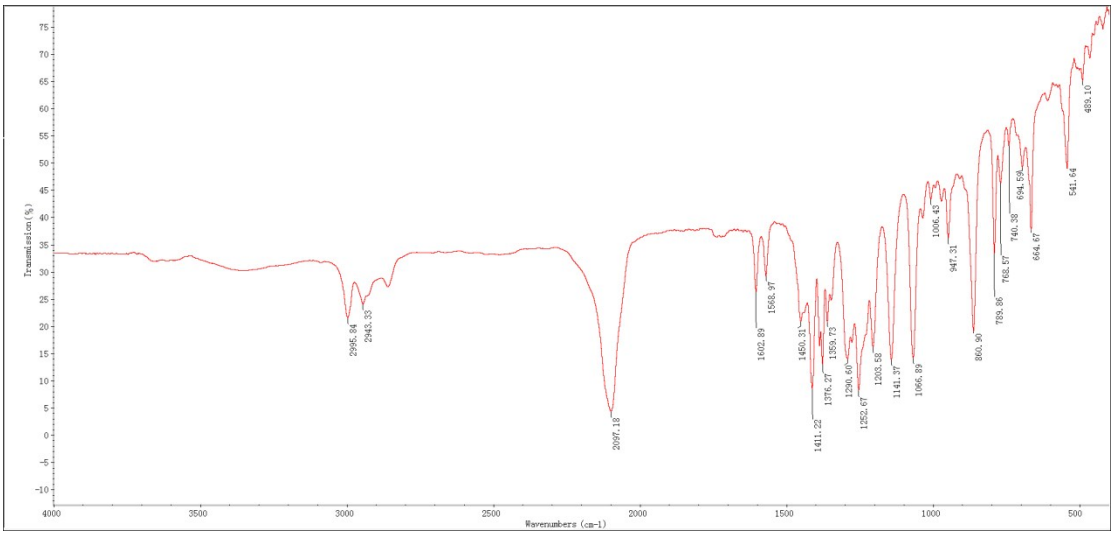


Figure S33. IR spectra of **6** on KBr.

IR of 7

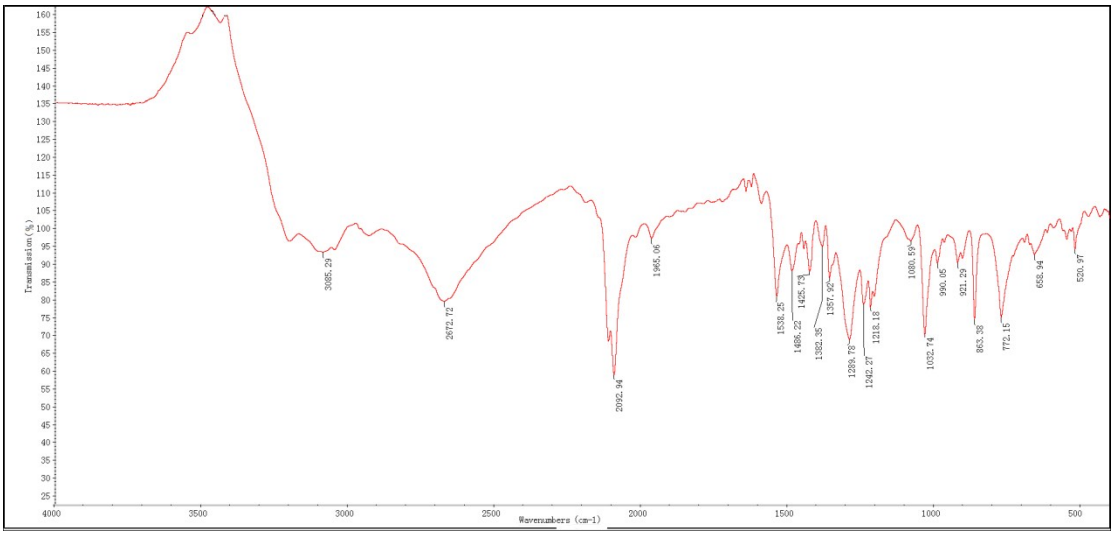


Figure S34. IR spectra of **7** on KBr.

IR of 1

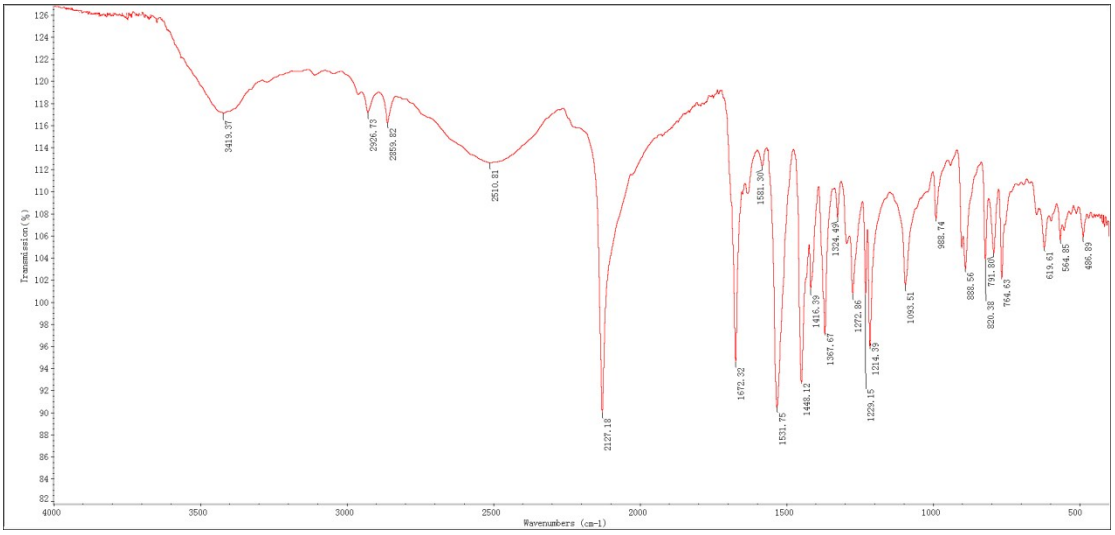


Figure S35. IR spectra of **1** on KBr.

IR of 2

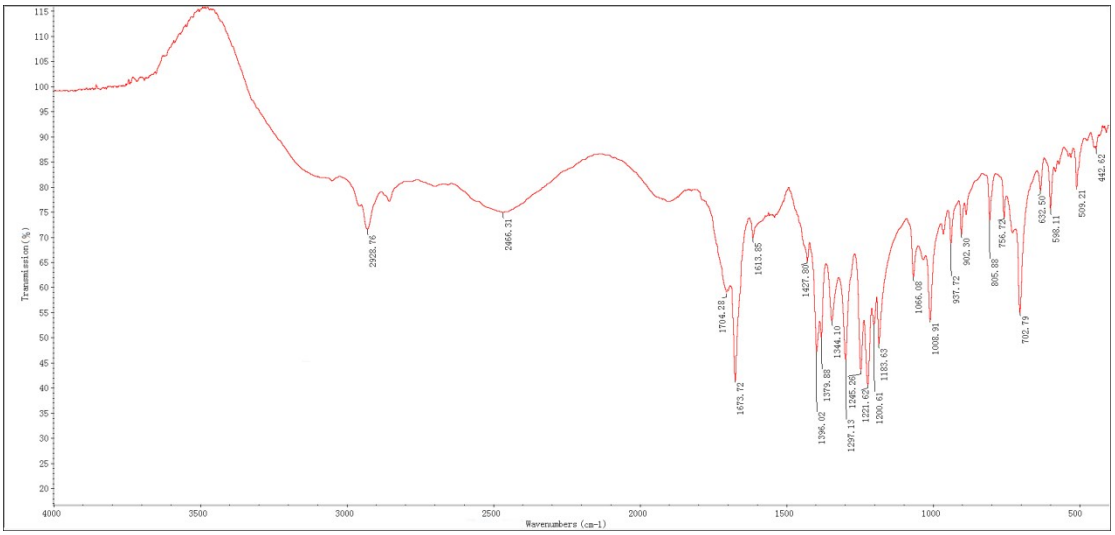


Figure S36. IR spectra of **2** on KBr.

IR of 3

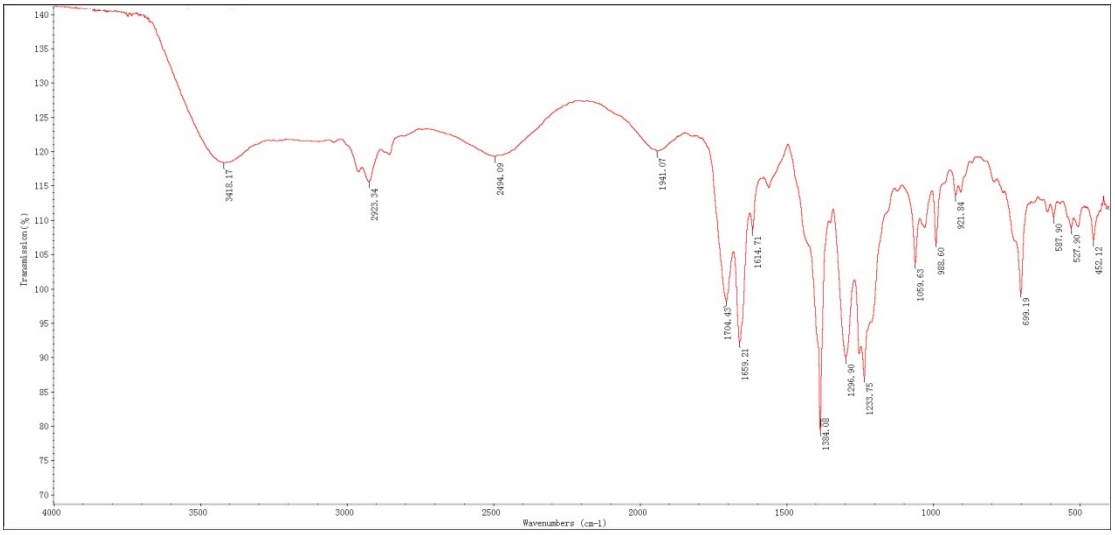


Figure S37. IR spectra of **3** on KBr.

S8. Cartesian coordinates of each compound at ground state

PLP + methyl amine

E	G
-1253.3744	-1253.185662

C	2.71878000	-1.87224800	-0.14913800
C	2.68897600	-0.48713200	0.07738900
C	1.47204900	0.17070400	0.20374400
C	0.30055600	-0.58915100	0.08596700
C	0.42237100	-1.94730300	-0.14103800
N	1.59950100	-2.57734500	-0.25536800
H	-0.46197100	-2.56204100	-0.23670000
C	1.37679700	1.66702400	0.39339000
H	0.47330300	1.90683400	0.96486900
N	1.36253200	2.33365600	-0.89840100
O	2.48573400	2.09450700	1.17948500
C	0.68510500	3.63179100	-0.82893300
H	-0.33586300	3.55066700	-0.43970600
O	3.89976300	0.14133300	0.14231000
H	2.41257100	3.04482400	1.33461200
C	4.03809700	-2.56766500	-0.28194800
H	4.61113000	-2.15900700	-1.11726400
H	4.64126200	-2.43051600	0.61823500
H	3.88055900	-3.63141900	-0.44655100
C	-1.05073300	0.07203800	0.20259200
H	-1.17691600	0.46103500	1.21971600
H	-1.10576900	0.92579900	-0.48360200
O	-2.07914700	-0.84929700	-0.09175000
P	-3.64158500	-0.28222800	0.04744400
O	-4.44874800	-1.50788600	-0.34386800
O	-3.79828200	0.13840900	1.50203200
O	-3.74251100	0.88164000	-0.92935400
H	1.24641300	4.30994700	-0.18500700
H	0.64838500	4.07372400	-1.82287500
H	0.85449200	1.74187800	-1.54968100
H	3.75550200	1.04950200	0.46570300

PL-N_3 + methyl amine

E	G
-774.875104	-774.685630

C	1.76960300	-1.94193600	-0.13536700
C	1.74310400	-0.55886600	0.09540700
C	0.53068600	0.11271100	0.18801200
C	-0.64427500	-0.63946400	0.06372100
C	-0.52667400	-2.00014000	-0.15575300
N	0.64622900	-2.63772600	-0.25933800
H	-1.41065000	-2.61425100	-0.25619500
C	0.49325300	1.61654700	0.35364800
H	-0.47348800	1.94285000	0.73757400
N	0.69238900	2.25300800	-0.92214000
O	1.48105700	1.94624700	1.34561700
C	0.65530500	3.71416200	-0.83970900
H	-0.29991000	4.02080200	-0.41092100
O	2.95441900	0.06535100	0.19602800
H	1.42600100	2.89114900	1.53782200
C	3.08527000	-2.64638200	-0.24877500
H	3.67735500	-2.23390200	-1.06869300
H	3.67083700	-2.52114800	0.66460500
H	2.92320400	-3.70733800	-0.42607000
C	-1.98292000	0.03888500	0.17189100
H	-2.09706100	0.47892600	1.16604000
H	-2.05846300	0.84525700	-0.56119300
H	1.46167800	4.14255400	-0.23627000
H	0.72728700	4.13232700	-1.84204900
H	2.81753700	0.94147600	0.60025200
H	1.58805500	1.95302700	-1.30144800
N	-3.07473700	-0.93117100	-0.05961900
N	-4.18935200	-0.42658300	0.02662500
N	-5.24257200	-0.04861500	0.08660600

PL-COOH 2 + methyl amine

E	G
-799.422921	-799.234829

C	-0.78202200	2.32655000	-0.16238500
C	-1.33218100	1.06094100	0.07881400
C	-0.50779400	-0.05247300	0.19793300
C	0.87640100	0.13780600	0.09050900
C	1.32968900	1.42692200	-0.14148600
N	0.53133400	2.49243000	-0.27126200
H	2.39161300	1.62511100	-0.23159100
C	-1.09839600	-1.43388900	0.37644100
H	-0.35473700	-2.12124700	0.77880200
N	-1.52784100	-1.95256000	-0.89687100
O	-2.14819800	-1.31252300	1.35383300
C	-2.09720200	-3.29731800	-0.80080300
H	-1.35713300	-3.96520200	-0.35757500
O	-2.69623200	0.99650800	0.16191400
H	-2.48609200	-2.19347400	1.55944200
C	-1.68595700	3.51127800	-0.30571500
H	-2.38853400	3.36759800	-1.12953500
H	-2.27885300	3.65823200	0.59973400
H	-1.09641400	4.40597300	-0.49423800
C	1.83565200	-1.02660500	0.22079000
H	1.71576500	-1.48159700	1.20606000
H	1.59654800	-1.78203600	-0.52972100
H	-3.01270200	-3.34698500	-0.20290500
H	-2.32840000	-3.66225000	-1.79988200
H	-2.94004400	0.14729000	0.57215100
H	-2.21660000	-1.31693100	-1.29331800
O	3.76976000	-0.61503200	-1.11393200
C	3.29379600	-0.60156400	0.04985600
O	3.91707200	-0.24688600	1.08265800

PL-COOH 3 + methyl amine

E	G
-838.728476	-838.513177

C	2.14545200	-1.95030900	-0.11848500
C	2.11123300	-0.56574200	0.09472600
C	0.89526400	0.10016100	0.18689000
C	-0.28488800	-0.65170000	0.07955100
C	-0.15060200	-2.01665700	-0.12249200
N	1.02400600	-2.65254600	-0.22512200
H	-1.02653300	-2.64558200	-0.21124100
C	0.85711300	1.60517200	0.33617200
H	-0.10951700	1.93146400	0.71746400
N	1.05443300	2.23133400	-0.94573400
O	1.84756900	1.94963300	1.32353700
C	1.01454000	3.69288500	-0.87639800
H	0.05932200	4.00136200	-0.44892900
O	3.32167400	0.06819000	0.17832600
H	1.78376100	2.89486100	1.51051200
C	3.46406100	-2.65035500	-0.23360300
H	4.04819700	-2.24714200	-1.06385100
H	4.05733600	-2.51200000	0.67292100
H	3.30519500	-3.71430900	-0.39602800
C	-1.63894700	0.01508500	0.17633600
H	-1.72607900	0.49344900	1.15598800
H	-1.68679200	0.82720300	-0.55340200
H	1.82079900	4.12893400	-0.27812000
H	1.08395700	4.10277800	-1.88241600
H	3.18129700	0.94556200	0.57827000
H	1.95029500	1.93004800	-1.32322100
C	-2.83131500	-0.90280300	-0.03307600
H	-2.85253200	-1.69850800	0.71666800
H	-2.77724900	-1.40119100	-1.00456400
C	-4.18542500	-0.19771700	0.03249400
O	-4.22598500	1.03209200	0.28726700
O	-5.19818900	-0.91948600	-0.17997500

S9. Cartesian coordinates of each compound at transition state

PLP + methyl amine

E	G
-1253.344841	-1253.159379

C	2.50931200	-2.03645400	-0.16088500
C	2.58531600	-0.63270900	0.04466400
C	1.37384800	0.07287800	0.08062800
C	0.15650300	-0.60719600	-0.04239700
C	0.20416800	-1.97414100	-0.23215900
N	1.35391100	-2.66852000	-0.29887400
H	-0.70913900	-2.54261700	-0.33928800
C	1.37895100	1.55551500	0.19669500
H	0.64295700	2.02783200	0.83677600
N	1.73829000	2.25527200	-0.86118800
O	2.72540500	1.71528300	1.53808400
C	1.70918500	3.71076000	-0.86774600
H	0.88735200	4.04770600	-0.23881400
O	3.74598100	-0.03429100	0.22349500
H	3.09698200	2.60097700	1.42485700
C	3.78475400	-2.81818400	-0.22070900
H	4.43456400	-2.44346600	-1.01519000
H	4.34154500	-2.72201500	0.71478100
H	3.57319700	-3.87039500	-0.40121200
C	-1.14391000	0.15191500	0.02087700
H	-1.22403400	0.64562100	0.99725600
H	-1.14927900	0.93763900	-0.74305500
O	-2.24000000	-0.71646700	-0.17519600
P	-3.75384200	-0.06117900	0.07359200
O	-4.65773100	-1.21302600	-0.33092100
O	-3.80673700	0.29283500	1.55355000
O	-3.82749600	1.15499300	-0.83873800
H	2.64679300	4.11830900	-0.48628500
H	1.55343900	4.06393600	-1.88397800
H	3.36117800	1.01949100	1.01025600
H	2.34252100	1.79846000	-1.53555900

PL-N_3 + methyl amine

E	G
-774.843824	-774.659249

C	1.76960300	-1.94193600	-0.13536700
C	1.74310400	-0.55886600	0.09540700
C	0.53068600	0.11271100	0.18801200
C	-0.64427500	-0.63946400	0.06372100
C	-0.52667400	-2.00014000	-0.15575300
N	0.64622900	-2.63772600	-0.25933800
H	-1.41065000	-2.61425100	-0.25619500
C	0.49325300	1.61654700	0.35364800
H	-0.47348800	1.94285000	0.73757400
N	0.69238900	2.25300800	-0.92214000
O	1.48105700	1.94624700	1.34561700
C	0.65530500	3.71416200	-0.83970900
H	-0.29991000	4.02080200	-0.41092100
O	2.95441900	0.06535100	0.19602800
H	1.42600100	2.89114900	1.53782200
C	3.08527000	-2.64638200	-0.24877500
H	3.67735500	-2.23390200	-1.06869300
H	3.67083700	-2.52114800	0.66460500
H	2.92320400	-3.70733800	-0.42607000
C	-1.98292000	0.03888500	0.17189100
H	-2.09706100	0.47892600	1.16604000
H	-2.05846300	0.84525700	-0.56119300
H	1.46167800	4.14255400	-0.23627000
H	0.72728700	4.13232700	-1.84204900
H	2.81753700	0.94147600	0.60025200
H	1.58805500	1.95302700	-1.30144800
N	-3.07473700	-0.93117100	-0.05961900
N	-4.18935200	-0.42658300	0.02662500
N	-5.24257200	-0.04861500	0.08660600

PL-COOH 2 + methyl amine

E	G
-799.391852	-799.208388

C	-0.76324900	2.31722800	-0.20637800
C	-1.34981100	1.04215300	0.00795800
C	-0.47706200	-0.05194900	0.11737700
C	0.90965500	0.13515500	0.05861300
C	1.36233600	1.42647800	-0.14373900
N	0.54844000	2.48562900	-0.28220400
H	2.42409800	1.63516800	-0.20424300
C	-1.02609400	-1.42777700	0.24252700
H	-0.54411700	-2.11807000	0.92430900
N	-1.56623600	-1.97618200	-0.82817900
O	-2.40325400	-1.05552200	1.51127300
C	-2.08106500	-3.33815500	-0.81765700
H	-1.46768500	-3.93979100	-0.14959600
O	-2.65529500	0.90684100	0.12843700
H	-3.06197600	-1.75148800	1.38199500
C	-1.66421600	3.50420900	-0.34886200
H	-2.36557600	3.36383700	-1.17484900
H	-2.26361600	3.64538800	0.55406500
H	-1.07729400	4.40278500	-0.52928300
C	1.85152100	-1.04263700	0.19133000
H	1.68246900	-1.52981000	1.15453800
H	1.63588600	-1.76946000	-0.59453600
H	-3.11642300	-3.35746200	-0.47353700
H	-2.03068500	-3.75045200	-1.82222200
H	-2.71744200	-0.19248000	0.94272200
H	-1.93082500	-1.34724200	-1.53535500
O	3.85324800	-0.63578300	-1.04004900
C	3.31895500	-0.62535200	0.09812400
O	3.88929100	-0.27718200	1.16320300

PL-COOH 3 + methyl amine

E	G
-838.697512	-838.487807

C	2.11965100	-1.96758500	-0.13374900
C	2.11967700	-0.55807000	0.03896000
C	0.87074300	0.07747300	0.09533400
C	-0.31618100	-0.66641500	0.02495600
C	-0.18628300	-2.03421500	-0.13781100
N	0.99806600	-2.66531500	-0.22363100
H	-1.06079500	-2.66728700	-0.20851900
C	0.79772000	1.56023100	0.17859200
H	0.05661200	2.00655600	0.83039800
N	1.08947900	2.25550300	-0.90282400
O	2.17545500	1.83261700	1.47429400
C	0.98675700	3.70723700	-0.93237000
H	0.16743500	4.01357900	-0.28475100
O	3.24951000	0.10959900	0.17068700
H	2.49080100	2.73323300	1.31740700
C	3.43473900	-2.67884500	-0.21441700
H	4.03989700	-2.28833900	-1.03611000
H	4.01185800	-2.52990400	0.70168000
H	3.27708700	-3.74519500	-0.36474700
C	-1.65581700	0.02724200	0.11738600
H	-1.71301700	0.54350500	1.07993200
H	-1.70203800	0.81398100	-0.64170700
H	1.91328200	4.16839800	-0.58619200
H	0.78390100	4.03451100	-1.94901000
H	2.83030100	1.15618100	0.94104500
H	1.69993600	1.81820300	-1.58441000
C	-2.86724900	-0.87704800	-0.03410600
H	-2.86718500	-1.65623100	0.73457000
H	-2.85516100	-1.39523100	-0.99531200
C	-4.20753300	-0.15334100	0.08744700
O	-5.23014800	-0.80955600	-0.25145300
O	-4.22770200	1.02268300	0.53016900

References

1 N. L. Morozowich, A. L. Weikel, J. L. Nichol, C. Chen, L. S. Nair, C. T. Laurencin and H. R. Allcock, *Macromolecules*, 2011, **44**, 1355.

2 C. Iwata and D. E. Metzler, *J. Heterocycl. Chem.*, 1967, **4**, 319.

3 A. Adronov, R. Chadwick, S. Van Gyzen and S. Liogier, *Synthesis*, 2014, **46**, 669.

4 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, Williams, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman and D. J. Fox, *Gaussian 16*, 2016.

5 (a) K. K. Baldridge and M. W. Schmidt, *Theor. Chem. Acc.*, 2008, **120**, 1; (b) A. V. Marenich, C. J. Cramer and D. G. Truhlar, *J. Phys. Chem. B*, 2009, **113**, 6378; (c) S. Grimme, S. Ehrlich and L. Goerigk, *J. Comput. Chem.*, 2011, **32**, 1456.

6 A. Dirksen, S. Dirksen, M. Tilman, Hackeng and P. E. Dawson, *J. Am. Chem. Soc.*, 2006, **128**, 15602.

7 W. Li, J. Zou, S. Zhu, X. Mao, H. Tian and X. Wang, *Chem. Eur. J.*, 2019, **25**, 10328.

8 Y. M. Li, Y. T. Li, M. Pan, X. Q. Kong, Y. C. Huang, Z. Y. Hong and L. Liu, *Angew. Chem. Int. Ed.*, 2014, **53**, 2198.