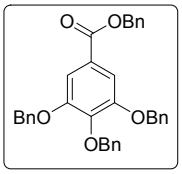
Supplementary Information

π- Stacking Assisted Redox Active Peptide-Gallol Conjugate: Synthesis Of A New Generation Low-Toxic Antimicrobial Silver Nanoparticles

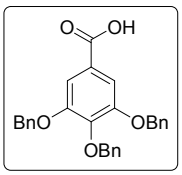
Manisit Das, Kalyan Senapati, Sayak Subhra Panda, Prabuddha Bhattacharya, Saibal Jana, Santi M. Mandal and Amit Basak**

Procedure for synthesis of compounds:

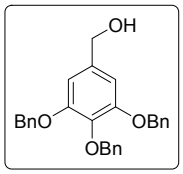
3,4,5-Tris-benzyloxy-benzoic acid benzyl ester (1A): To a solution of K_2CO_3 (9.74 g, 70.52 mmol, 6.0 eq) in dry *N*,*N*-Dimethylformamide (DMF) (20 mL), 3,4,5-Trihydroxybenzoic acid (2.00 g, 11.76 mmol, 1.0 eq) was added and allowed to stir at 0°C for 15 minutes. The reaction mixture was brought to room temperature, allowed to stir for 30 minutes further and brought back to 0°C. Benzyl bromide (12.06 g, 70.54 mmol, 6.0 eq) was subsequently added and the reaction was allowed to stir for 16 hours atrt. The reaction was quenched with water; the combined aqueous layer was extracted using EtOAc. The organic layer was washed with brine water and dried using anhydrous Na_2SO_4. The concentrated reaction mixture was washed with Petroleum ether overnight and filtered using Whatman® 41 filter paper. The colourless solid product **1A** (4.91 g, 80%) was dried and stored without further purification. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.70 – 7.11 (m, 22H), 5.34 (s, 2H), 5.14 (s, 6H).



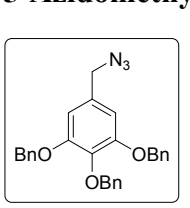
3,4,5-Tris-benzyloxy-benzoic acid (1B): The benzyl ester **1A** (4.71 g, 8.88 mmol) was taken in 1:1 EtOH: NaOH (5 M) in H_2O. The solution was refluxed for 4 hours at 100°C. The reaction mixture was concentrated, acidified using dilute HCl and was extracted using EtOAc. The concentrated reaction mixture was washed with Petroleum ether overnight and filtered using Whatman® 41 filter paper. The colourless solid product **1B** (3.75 g, 96%) was dried and stored without further purification.^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 – 7.22 (m, 17H), 5.15 (s, 6H).



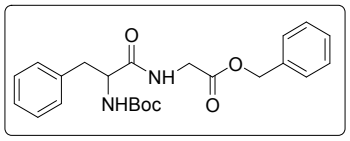
(3,4,5-Tris-benzyloxy-phenyl)-methanol (1C): The benzoic acid **1B** (3.65 g, 8.28 mmol, 1.0 eq) was taken in dry THF (40 mL) and the solution was brought to 0°C and $(CH_3)_2S{\cdot}BH_3$ [(2 M), (1.256 g, 16.56 mmol, 2.0 eq)] was added dropwise. The reaction was allowed to run for 3 hours and quenched with water at 0°C. The concentrated reaction mixture was extracted using EtOAc, washed with Petroleum ether overnight and filtered using Whatman® 41 filter paper.Thecolourless solid product **1C** (3.35 g, 95%) was dried and stored without further purification.[1]H NMR (400 MHz, Chloroform-*d*) δ 7.47 – 7.21 (m, 15H), 6.68 (s, 2H), 5.11 (s, 4H), 5.05 (s, 2H), 4.58 (s, 2H), 1.63 (s, 1H).



5-Azidomethyl-1,2,3-tris-benzyloxy-benzene (1D): The alcohol **1C** (3.52 g, 8.26 mmol, 1.0 eq) was taken in dichloromethane (50 mL) at 0°C. PBr_3 [(0.8 mL), (2.23 g, 8.26 mmol, 1.0 eq)] was added subsequently and the combined solution was stirred for 30 minutes. The reaction mixture was brought to rtandmaintained for 3 hours. The reaction was quenched using water; neutralized using $NaHCO_3$ and extracted using dichloromethane. The organic layer was concentrated and the solid, colourless product was isolated by vacuum drying which was further dissolved in *N*,*N*-Dimethylformamide (DMF) (30 mL). NaN_3 (2.38 g, 36.86 g, 5.0 eq) was subsequently added and stirred at rt for 30 minutes. The reaction was quenched using water and extracted using EtOAc. The organic phase was concentrated and the crude mixture was purified using column chromatography (silica gel, EtOAc:Petroleum Ether 1:24). The colourless solid **1D** (2.79 g, 75%) was stored for subsequent steps of the reaction. [1]H NMR (400 MHz, Chloroform-*d*) δ 7.58 – 7.18 (m, 15H), 6.62 (s, 2H), 5.13 (s, 4H), 5.08 (s, 2H), 4.24 (s, 2H).

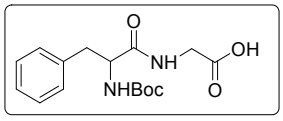


(2-*tert*-Butoxycarbonylamino-3-phenyl propionylamino)-acetic acid benzyl ester (2A): Boc-Phe-OH (2.00 g, 7.53 mmol, 1.0 eq) was taken in dry dichloromethane (10 mL) at 0°C. EDC (1.403 g, 9.04 mmol, 1.2 eq) was added to the solution and stirred for 30 minutes. TFA salt of Glycine benzyl ester (2.85 g, 9.04 mmol, 1.2 eq) was simultaneously taken in dry

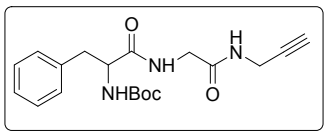


dichloromethane (10 mL) and Et_3N (1.14 g, 11.29 mmol, 1.5 eq) was added subsequently. The combined solution was dropwise added to the solution containing Boc-Phe-OH and EDC. HOBt (0.06 g, 0.37 mmol, 0.05 eq) was added in catalytic amount and the reaction mixture was allowed to stir for 24 hours. The reaction was quenched using water and extracted using EtOAc. The organic layer was washed with brine water and dried using anhydrous Na_2SO_4. The crude mixture was purified using column chromatography (silica gel, EtOAc:Petroleum Ether 1:4). The colourless solid **2A** (2.24 g, 72%) was dried and stored. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 7.16 (m, 10H), 6.48 (t, J = 5.1 Hz, 1H), 5.16 (s, 2H), 5.01 (s, 1H), 4.41 (s, 1H), 4.08 (dd, J = 18.3, 5.5 Hz, 1H), 3.97 (dd, J = 18.3, 5.1 Hz, 1H), 3.12 (dd, J = 13.9, 6.5 Hz, 1H), 3.03 (dd, J = 13.8, 7.3 Hz, 1H), 1.39 (s, 9H);^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 169.5, 155.6, 136.7, 135.2, 129.4, 128.8, 128.7, 128.5, 127.1, 80.4, 67.3, 55.8, 41.5, 38.5, 28.4; ESI-MS$^+$*m/z*Calcd. for $C_{23}H_{28}N_2NaO_5$ $[M+Na]^+$ 435.1896, found 435.1904.

(2-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-acetic acid (2B): NaOH (1N, 9.70 mmol, 2.0 eq) was taken in MeOH. The acid benzyl ester **2A** (2.00 g, 4.85 mmol, 1.0 eq) was added to the NaOH solution and allowed to stir at rt for 2 hours. The reaction was quenched with water and acidified with 1N HCl and extracted using EtOAc. The organic layer was dried and was washed with hexane overnight and filtered using Whatman® 41 filter paper. The colourless solid product **2B** (1.48 g, 95%) was dried and stored without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (s, 1H), 8.32 – 8.16 (m, 1H), 7.47 – 7.10 (m, 5H), 6.92 (d, J = 8.7 Hz, 1H), 4.20 (td, J = 10.7, 9.9, 3.7 Hz, 1H), 3.79 (dd, J = 16.0, 5.8 Hz, 2H), 3.00 (dd, J = 14.0, 3.9 Hz, 1H), 2.71 (dd, J = 13.9, 10.7 Hz, 1H), 1.28 (s, 8H); ^{13}C NMR (150 MHz, DMSO) δ 172.1, 171.2, 155.2, 138.3, 129.2, 127.9, 126.1, 77.9, 55.5, 40.7, 37.5, 28.1; ESI-MS$^+$*m/z*Calcd. for $C_{16}H_{22}N_2NaO_5$ $[M+Na]^+$ 345.1426, found 345.1434.

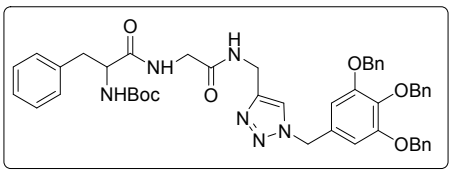


[2-Phenyl-1-(prop-2-ynylcarbamoylmethyl-carbamoyl)-ethyl]-carbamic acid *tert*-butyl ester (2C): The acid **2B** (1.40 g, 4.34 mmol, 1.0 eq) was taken in dry dichloromethane (15 mL) at 0 °C. EDC (0.82 g, 5.20 mmol, 1.2 eq) was added to the solution and stirred for 30 minutes. Et_3N (0.66 g, 6.51 mmol, 1.5 eq) was added subsequently, stirred for 10 minutes and propargylamine (0.29 g, 5.20 mmol, 1.2 eq) was added. HOBt (0.03 g, 0.22 mmol, 0.05 eq) was added in catalytic amount

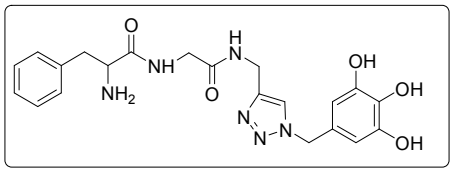


and the reaction mixture was allowed to stir for 24 hours. The reaction was quenched using water and extracted using EtOAc. The organic layer was washed with brine water and dried using anhydrous Na_2SO_4. The crude mixture was purified using column chromatography (silica gel, EtOAc:Petroleum Ether 2:3). The colourless solid **2C** (1.29 g, 83%) was dried and stored.^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 – 7.15 (m, 5H), 6.89 (s, 1H), 6.67 (s, 1H), 5.09 (d, *J* = 6.7 Hz, 1H), 4.29 (q, *J* = 6.9 Hz, 1H), 3.92-4.06 (m, 3H), 3.85 (dd, *J* = 16.8, 5.7 Hz, 1H), 3.12 (dd, *J* = 13.8, 6.6 Hz, 1H), 3.01 (dd, *J* = 13.8, 7.6 Hz, 1H), 2.19 (s, 1H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 168.6, 156.0, 136.4, 129.5, 129.3, 128.9, 128.8, 127.4, 81.0, 79.4, 71.6, 56.7, 43.2, 38.0, 29.2, 28.4; ESI-MS$^+$*m/z*Calcd. for $C_{19}H_{25}N_3NaO_4$ $[M + Na]^+$ 382.1743, found 382.1736.

{2-Phenyl-1-[({[1-(3,4,5-tris-benzyloxy-benzyl)-1*H*-[1,2,3]triazol-4-ylmethyl]-carbamoyl}-methyl)-carbamoyl]-ethyl}-carbamic acid *tert*-butyl ester (2D): The ester **2C** (0.50 g, 1.39 mmol, 1.0 eq) and the azide**1D** (0.63 g, 1.39 mmol, 1.0 eq) were taken in dichloromethane (20 mL): ^{t}BuOH (30 mL) and bubbled with N_2 gas for 1 hour to eliminate O_2 gas. $CuSO_4$ (0.35 g, 1.39 mmol, 1.0 eq) was taken in H_2O (30 mL) and bubbled similarly with N_2 gas for 1 hour. Sodium ascorbate (0.41 g, 2.08 mmol, 1.5 eq) was added to the solution and bubbled with N_2 gas for additional 15 minutes. The solution containing the ester **2C** and the azide**1D** was subsequently added dropwise to the aqueous solution and the reaction was allowed to run at rt for 24 hours. The reaction was quenched with NH_4Cl solution, concentrated and extracted using EtOAc. The crude reaction mixture was purified using column chromatography (silica gel, EtOAc:Petroleum Ether 4:1). The colourless solid **2D** (1.00 g, 89%) was dried and stored.^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 6.99 (m, 22H), 6.70 (s, 1H), 6.49 (s, 2H), 5.24 (s, 2H), 5.03 (s, 1H), 4.99 (s, 4H), 4.95 (s, 2H), 4.42 – 4.29 (m, 2H), 4.19 (q, *J* = 7.0 Hz, 1H), 3.86 (dd, *J* = 16.8, 6.1 Hz, 1H), 3.73 (dd, *J* = 16.9, 5.6 Hz, 1H), 2.98 (dd, *J* = 14.3, 6.9 Hz, 1H), 2.87 (dd, *J* = 13.8, 7.6 Hz, 1H), 1.26 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 169.1, 155.9, 153.3, 145.2, 144.5, 138.9, 137.7, 136.8, 136.4, 129.9, 129.3, 128.9, 128.7, 128.3, 128.1, 128.0, 127.6, 127.3, 122.3, 108.1, 80.9, 75.3, 71.4, 56.5, 54.4, 43.2, 38.0, 35.1, 28.3; ESI-MS$^+$*m/z*Calcd. for $C_{47}H_{61}N_6O_7$ $[M + H]^+$ 811.3819, found 811.3811.



2-Amino-3-phenyl-*N*-({[1-(3,4,5-trihydroxy-benzyl)-1*H*-[1,2,3]triazol-4-ylmethyl]-carbamoyl}-methyl)-propionamide (2E): The ester **2D** (0.10 g, 0.12 mmol, 1.0 eq) was taken



in dichloromethane (12 mL). TFA (1.8 mL) was added dropwise to the solution at 0°C. The solution was brought back to rt and allowed to run overnight. The reaction was quenched with water, neutralized using $NaHCO_3$and extracted using EtOAc. The organic layer was vacuum dried and was taken in dry MeOH (2 mL): dry tetrahydrofuran (20 mL). Pearlman's catalyst [$Pd(OH)_2$] (0.01 g, 0.06 mmol, 0.5 eq) was added subsequently and the hydrogenation reaction was performed using Parr apparatus. The reaction was allowed to run for 6 hours. The reaction mixture was filtered using celite bed and dried under reduced pressure. The crude product was washed with Petroleum ether and subsequently with Petroleum Ether: EtOAc (19:1) twice. The colourless solid product **2E** (0.047 g, 90%) was dried and stored without further purification.^{1}H NMR (600 MHz, DMSO-d_6) δ 8.27 (t, J = 5.8 Hz, 1H), 8.19 (s, 1H), 7.82 (s, 1H), 7.29 – 7.17 (m, 5H), 6.24 (s, 2H), 5.26 (s, 2H), 4.29 (d, J = 5.6 Hz, 2H), 3.70 (s, 2H), 3.46 (dd, J = 8.7, 4.6 Hz, 1H), 2.98 (dd, J = 13.5, 4.6 Hz, 1H), 2.60 (dd, J = 13.5, 8.8 Hz, 1H); ^{13}C NMR (150 MHz, DMSO) δ 170.3, 168.6, 146.1, 144.8, 138.5, 132.9, 129.3, 128.2, 126.2, 125.9, 122.6, 107.0, 55.9, 52.9, 41.9, 40.4, 34.2; ESI-MS$^+$*m/z*Calcd. for $C_{21}H_{25}N_6O_5$ $[M + H]^+$ 441.1886, found 441.1904.

Spectra

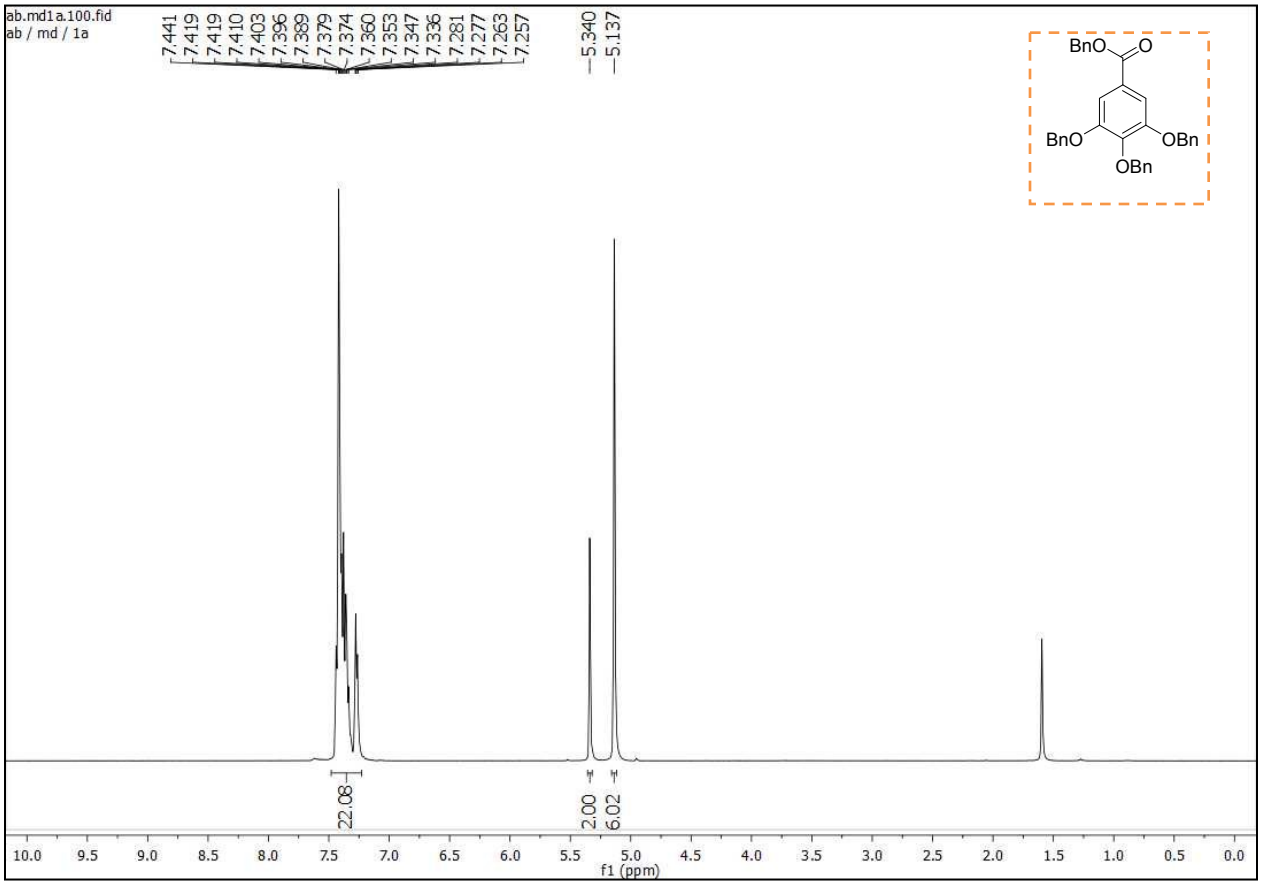


Figure S1: ^{1}H NMR spectra (400 MHz, Chloroform-*d*) of 1A

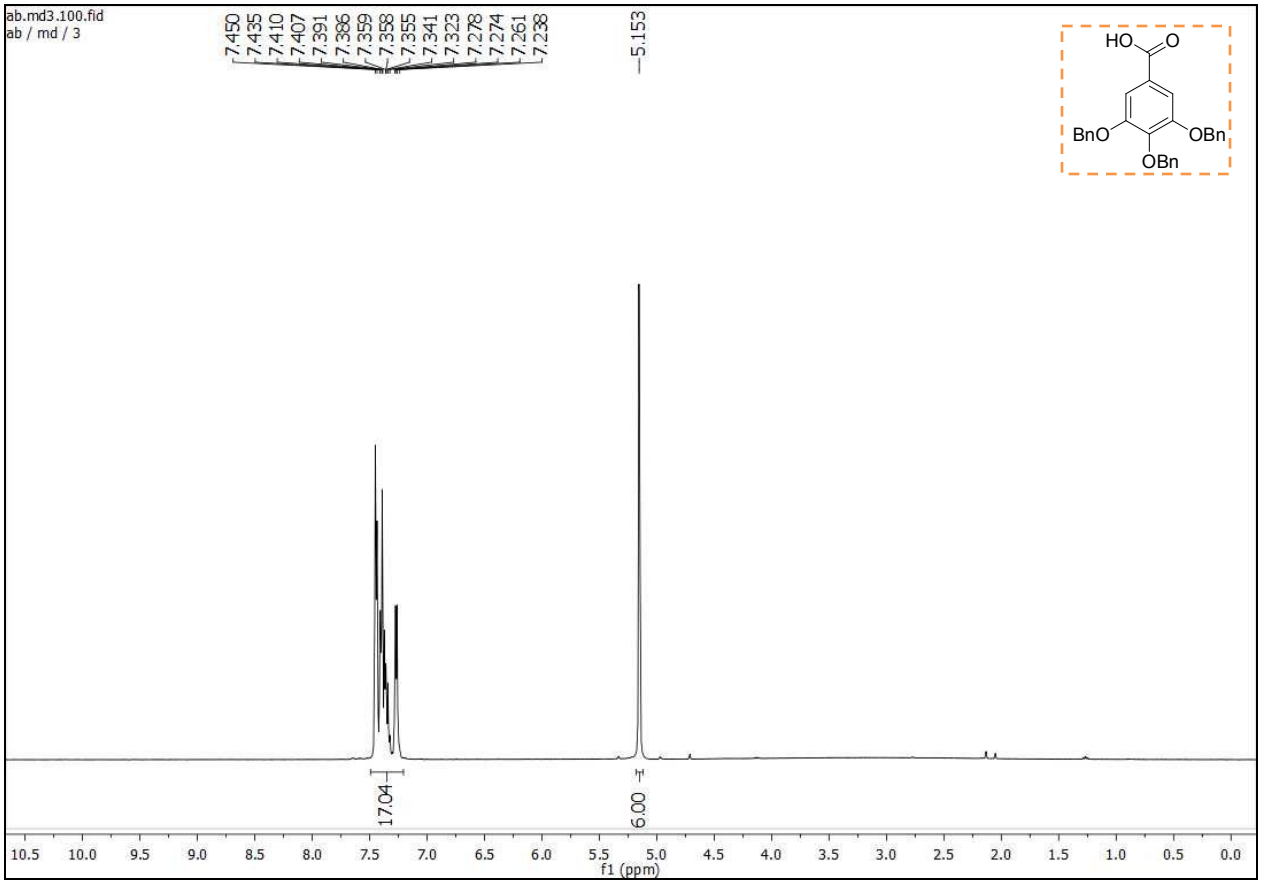


Figure S2: ^{1}H NMR spectra (400 MHz, Chloroform-*d*) of 1B

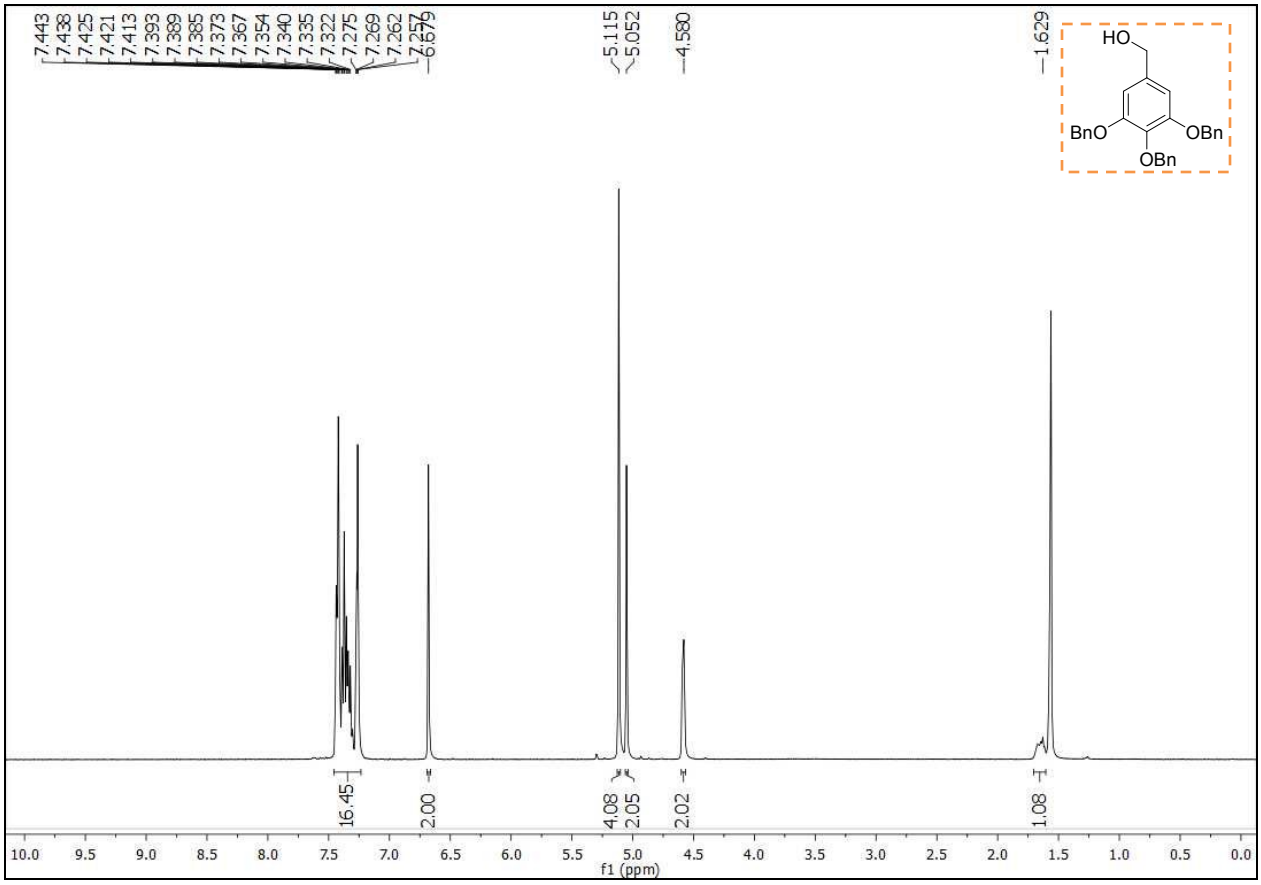


Figure S3: ^{1}H NMR spectra (400 MHz, Chloroform-*d*) of 1C

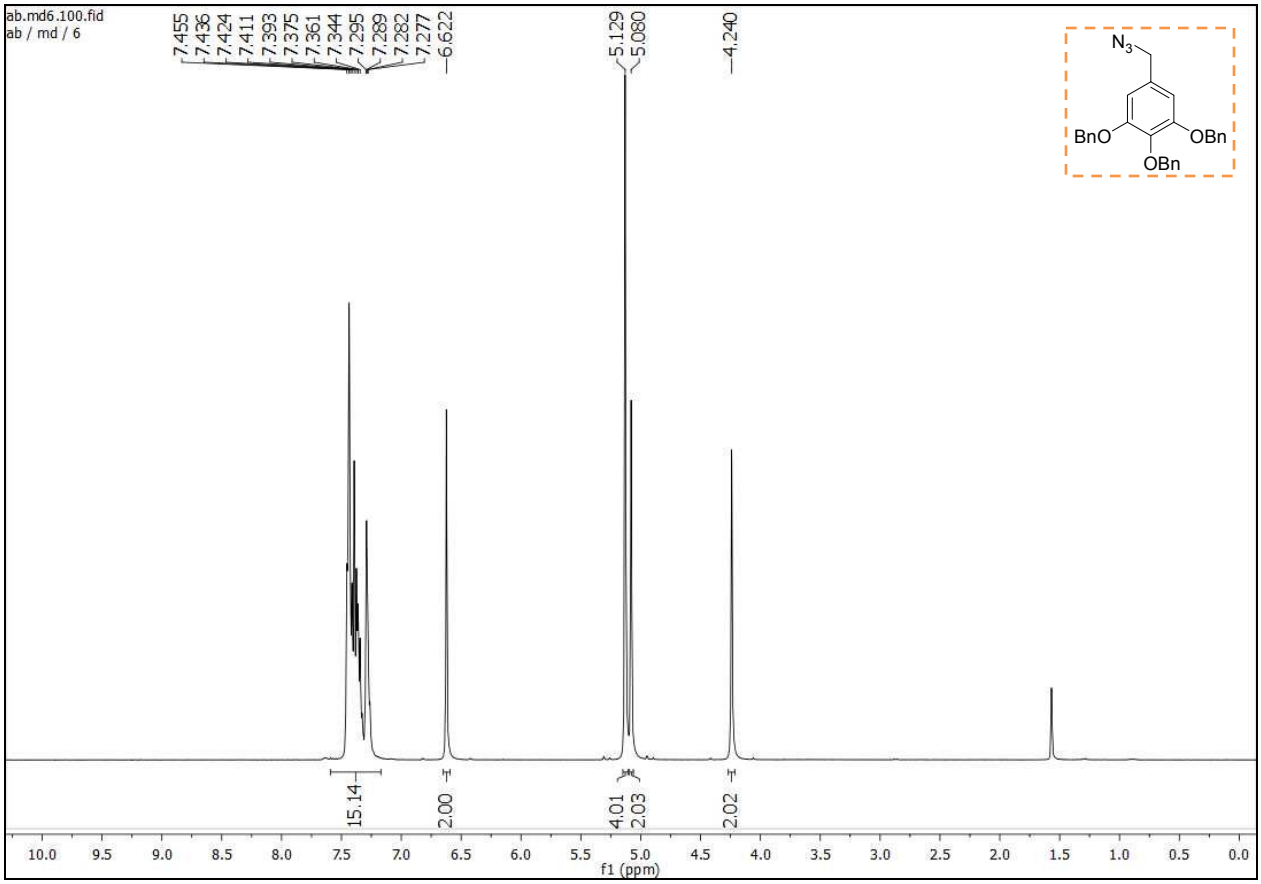


Figure S4: ^{1}H NMR spectra (400 MHz, Chloroform-*d*) of 1D

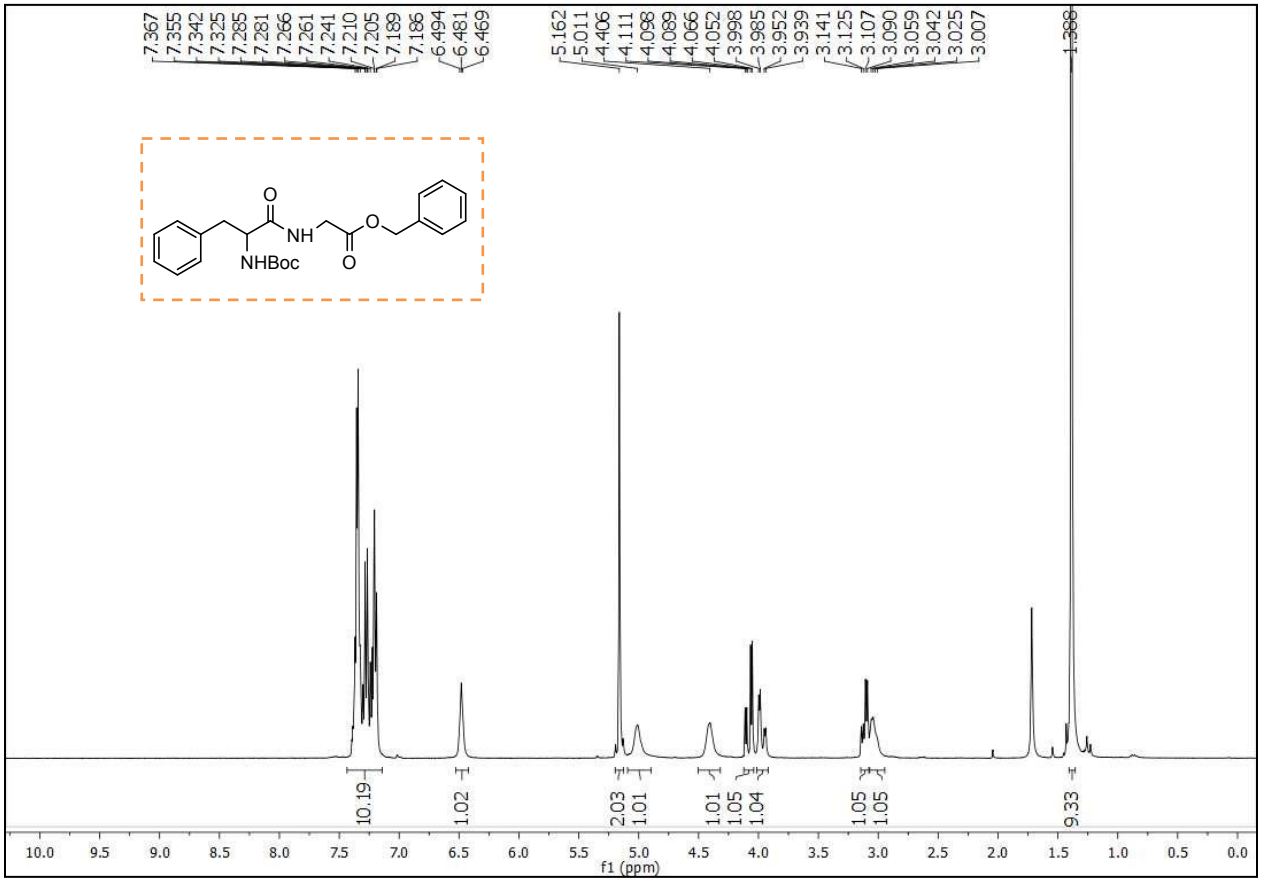


Figure S5: ^{1}H NMR (400 MHz, Chloroform-*d*) of 2A

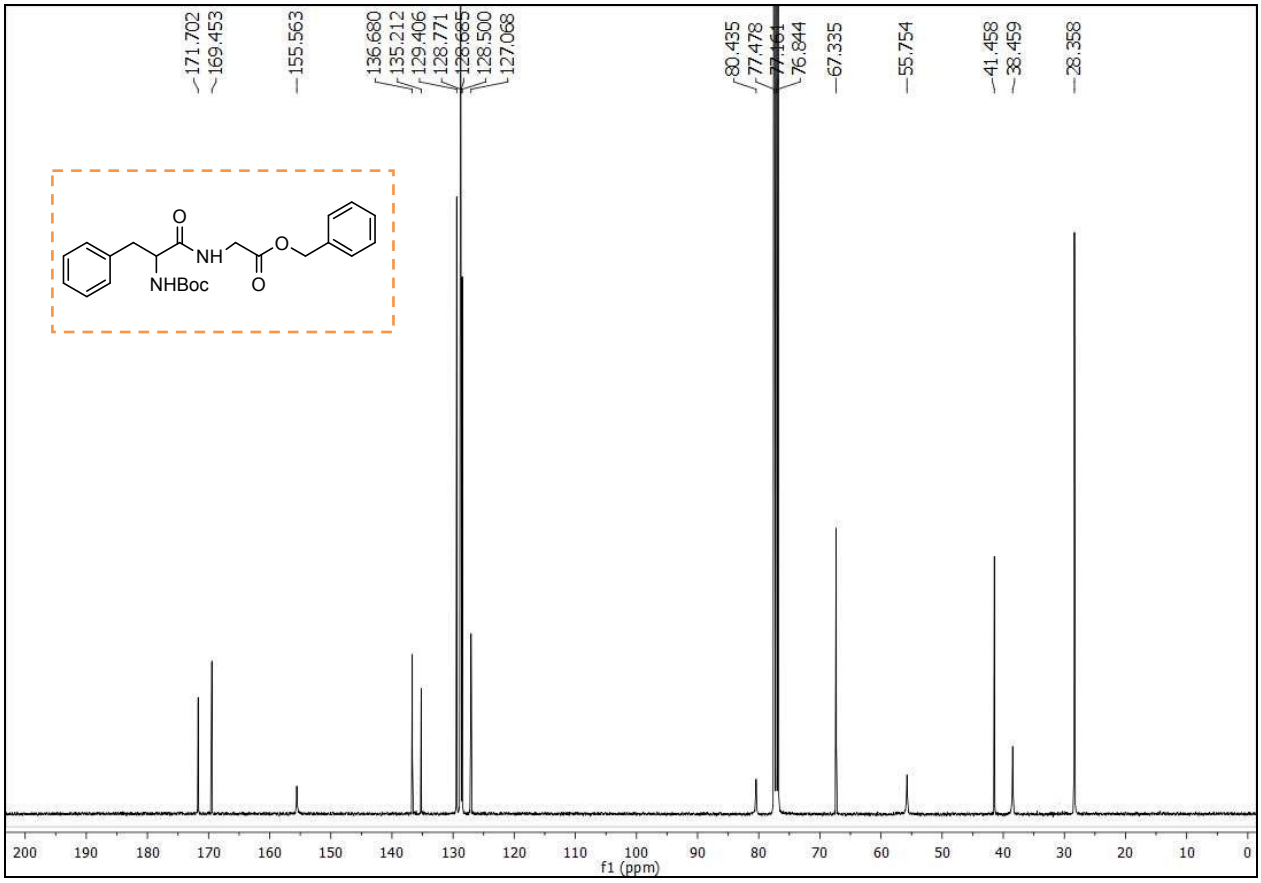


Figure S6: ^{13}C NMR (100 MHz, $CDCl_3$) of 2A

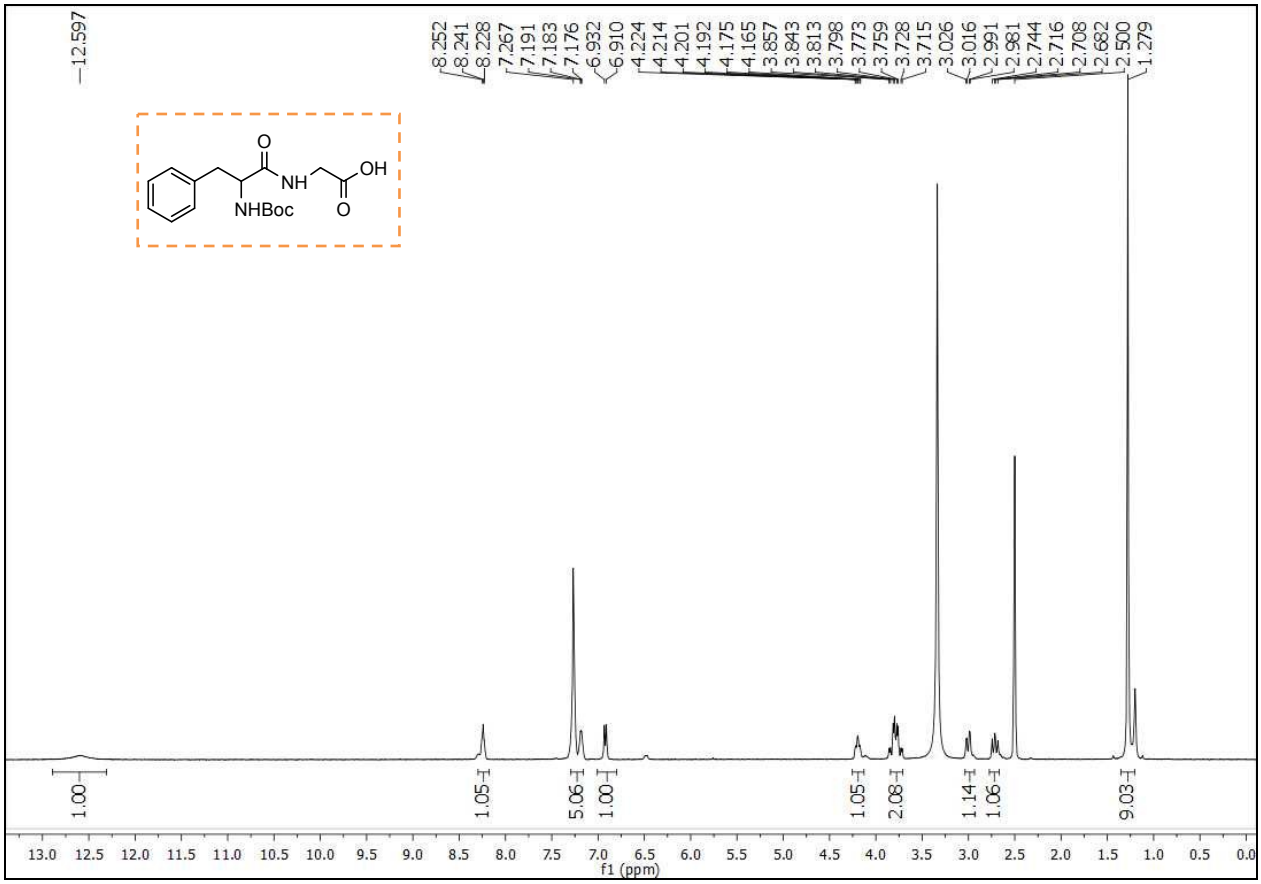


Figure S7: ^{1}H NMR (400 MHz, Chloroform-*d*) of 2B

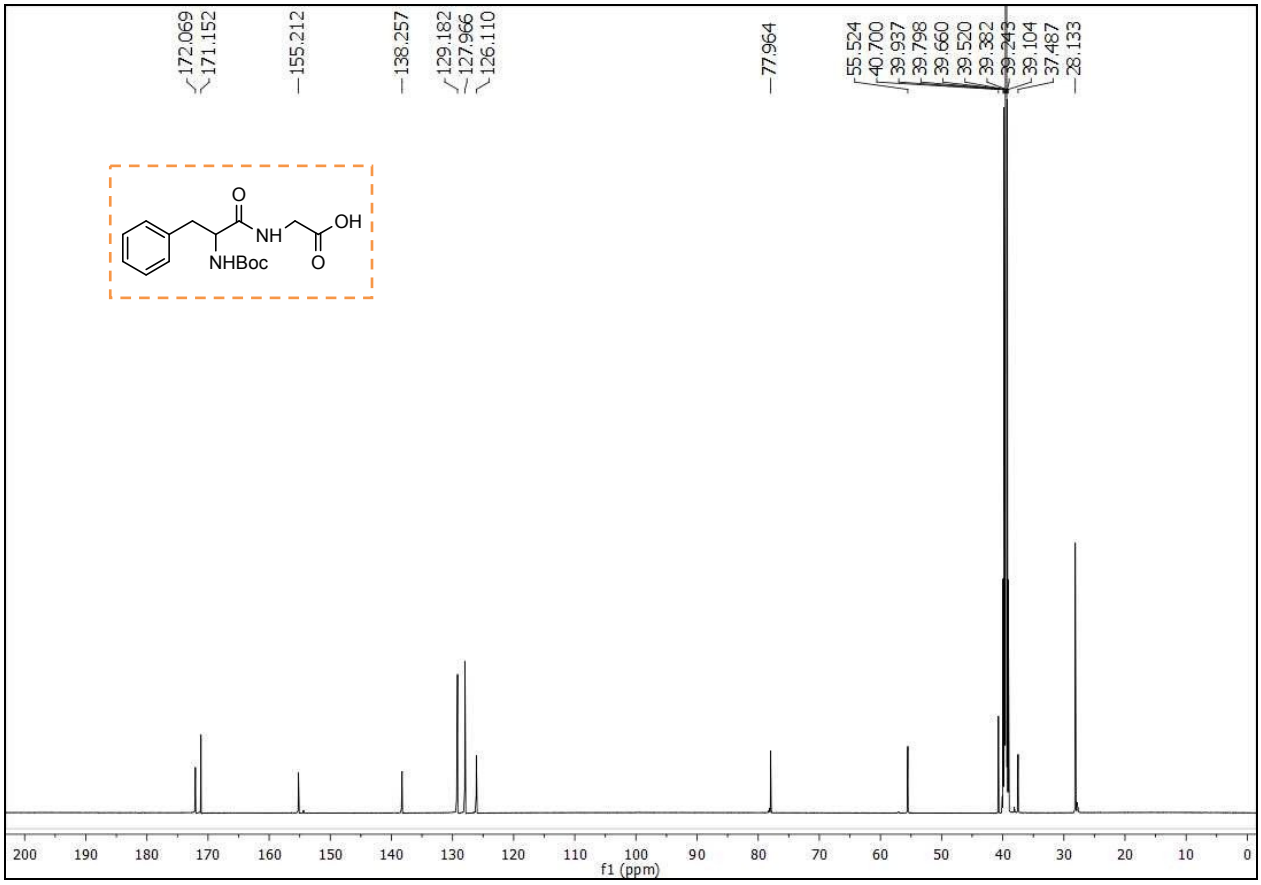


Figure S8: ^{13}C NMR (150 MHz, DMSO) of 2B

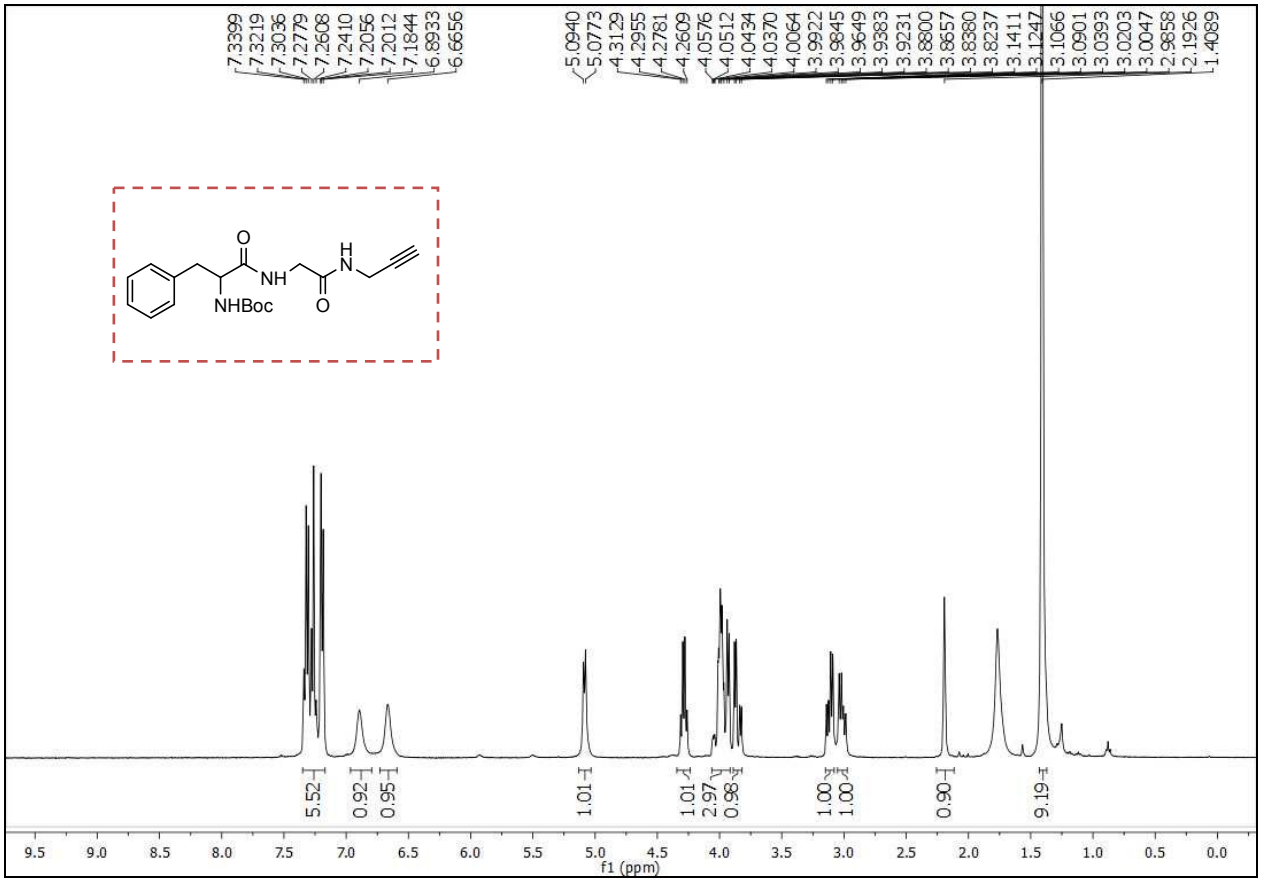


Figure S9: ^{1}H NMR (400 MHz, Chloroform-*d*) of 2C

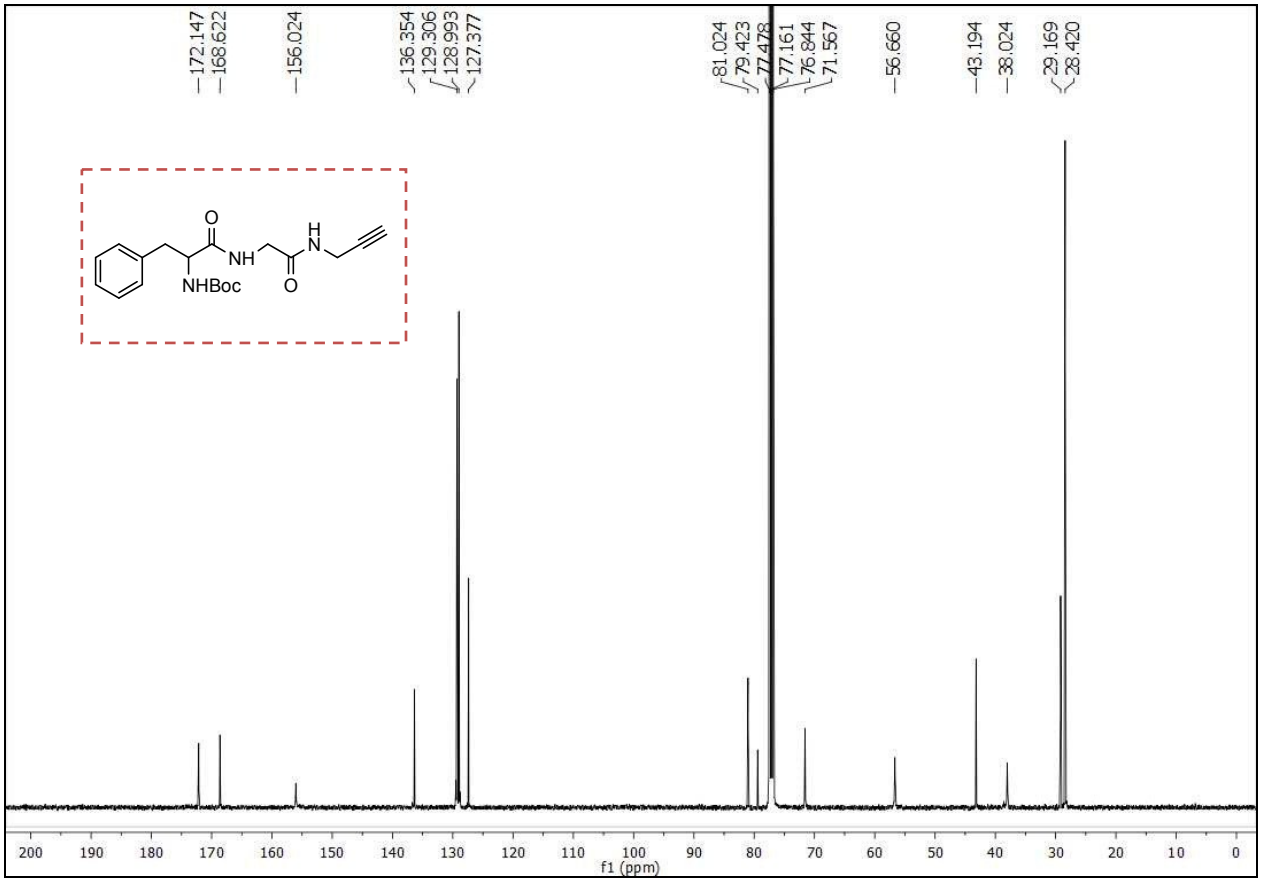


Figure S10: ^{13}C NMR (100 MHz, $CDCl_3$) of 2C

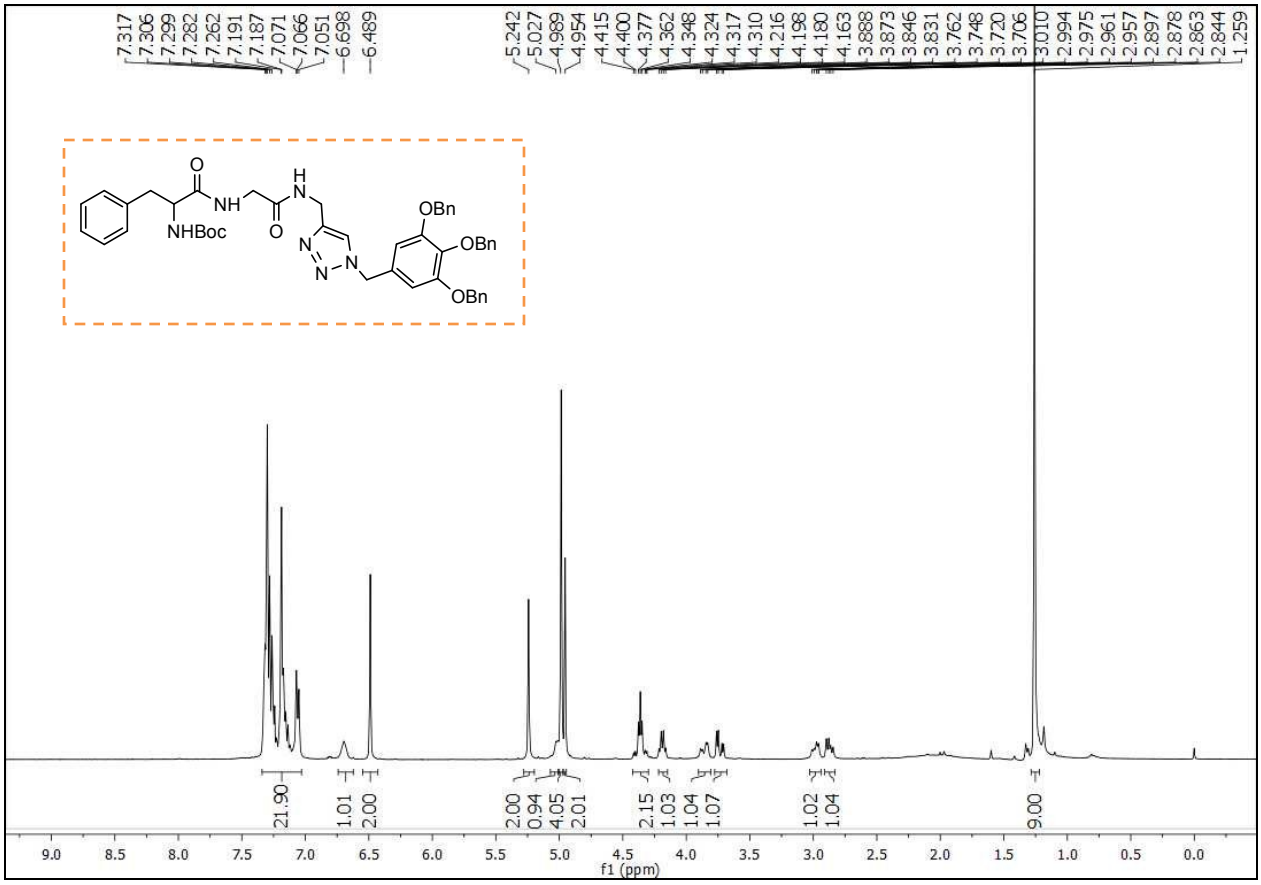


Figure S11: ^{1}H NMR (400 MHz, Chloroform-*d*) of 2D

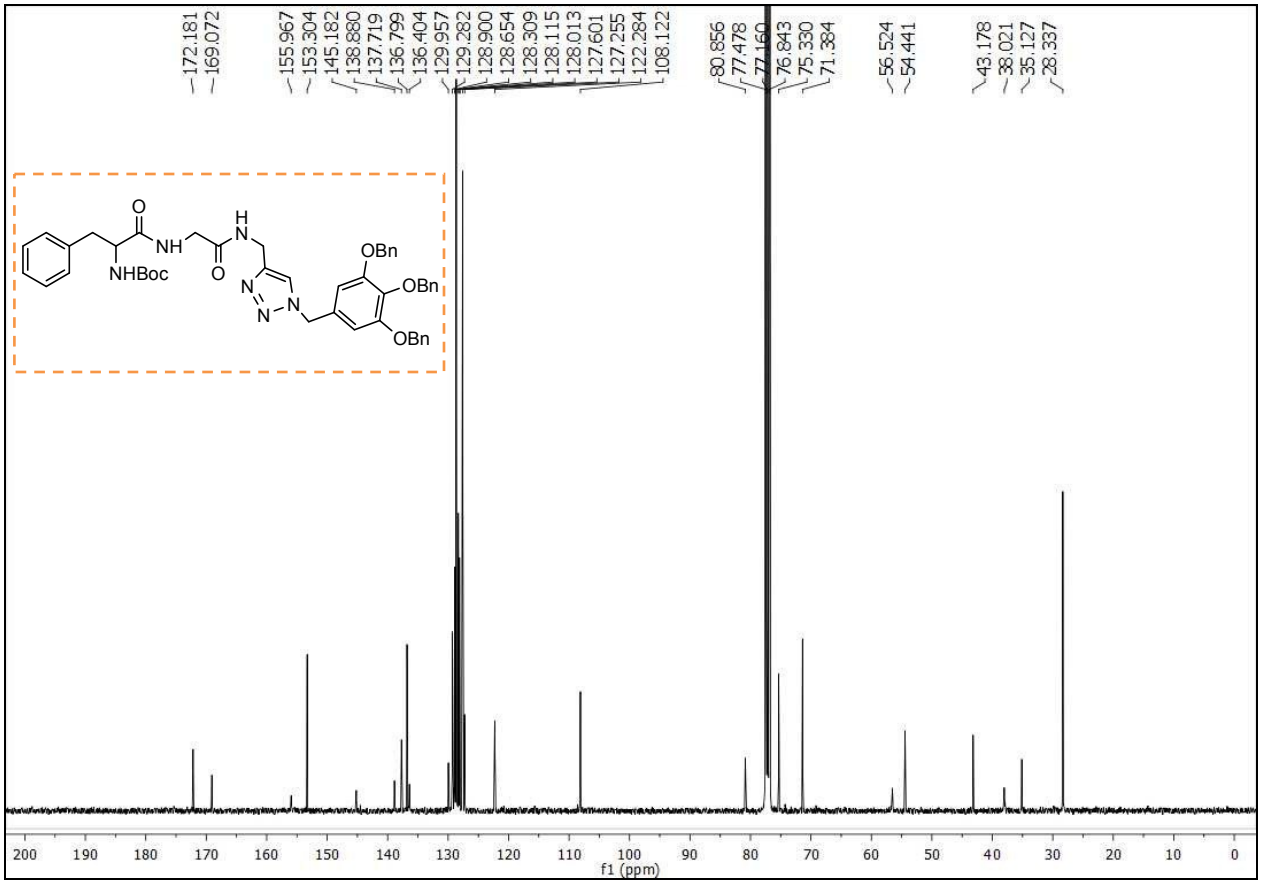


Figure S12: ^{13}C NMR (100 MHz, $CDCl_3$) of 2D

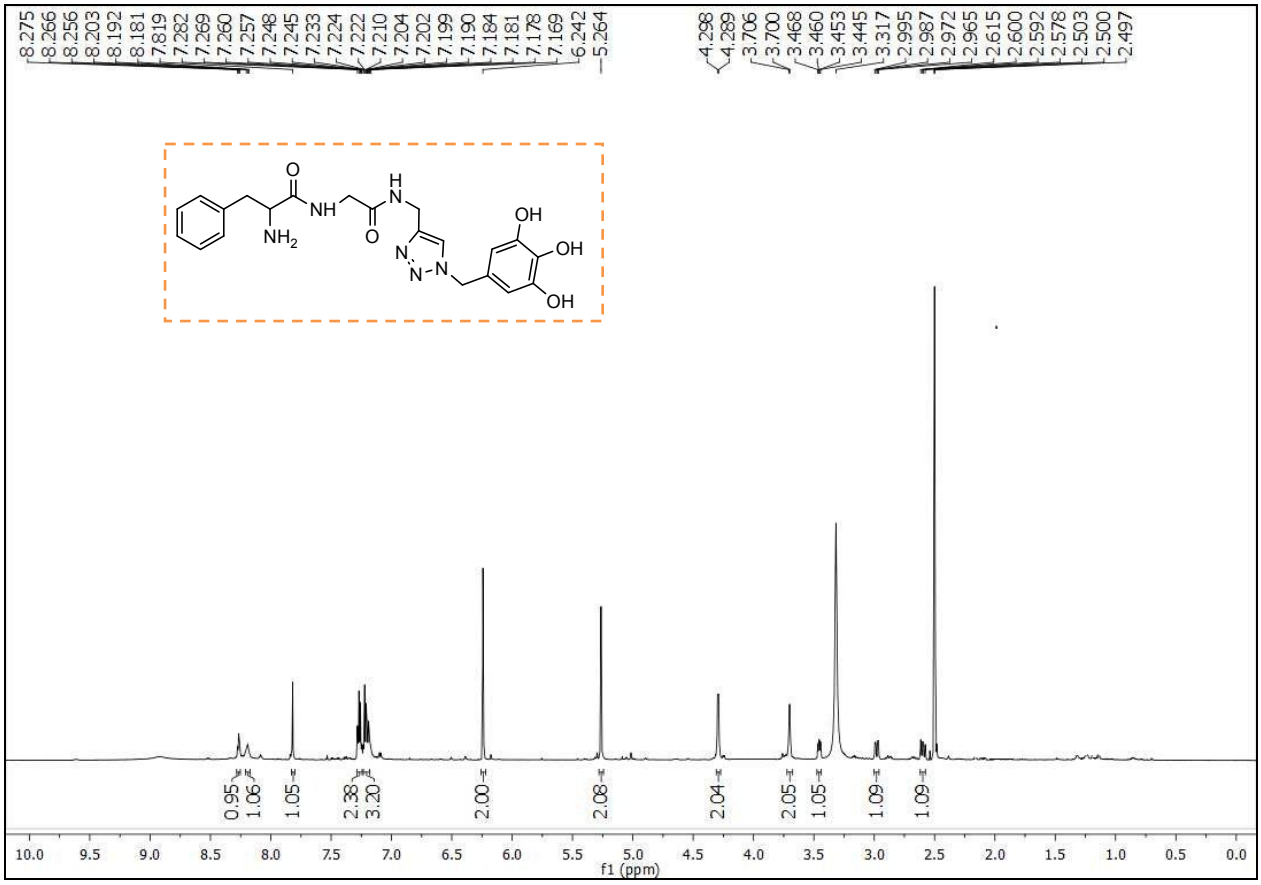


Figure S13: ^{1}H NMR (600 MHz, DMSO-d_6) of 2E

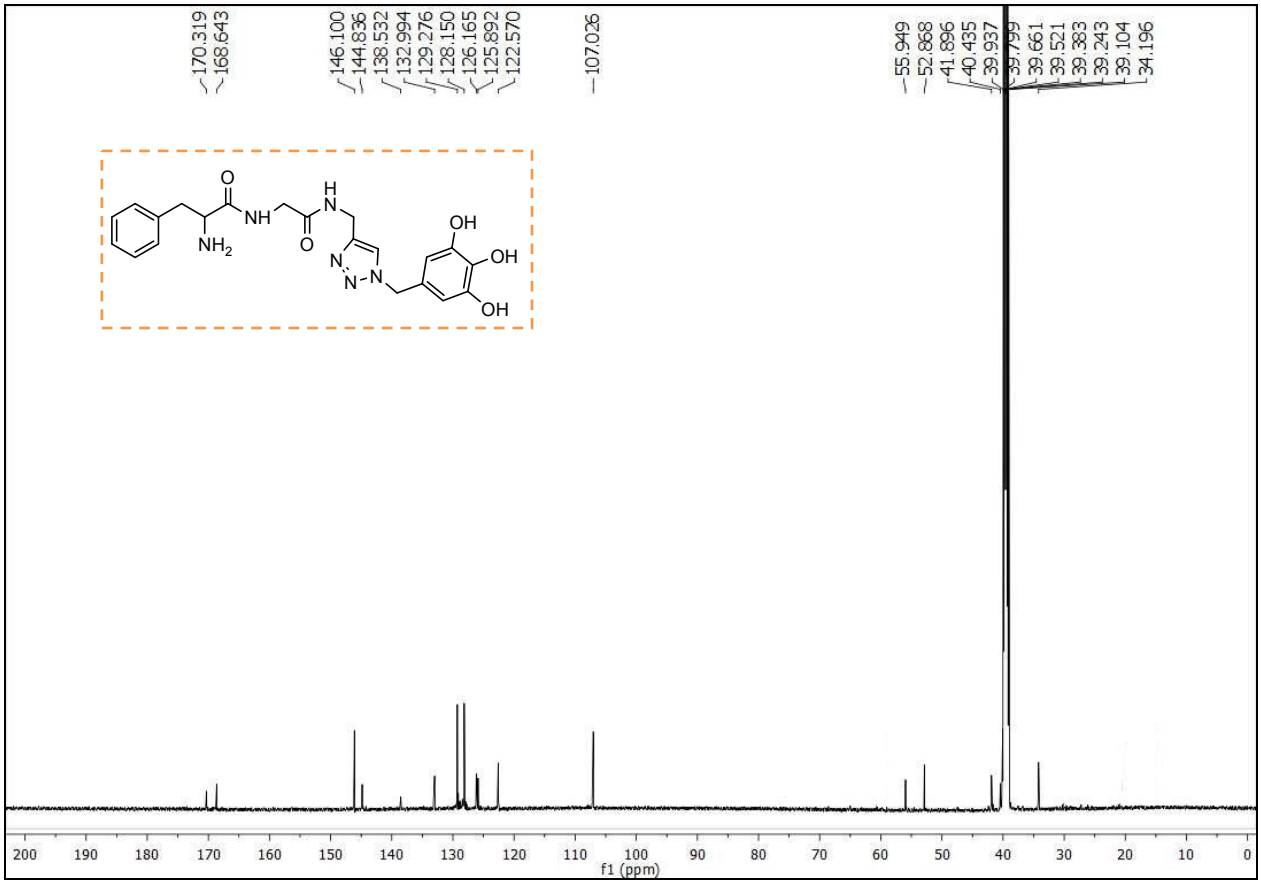


Figure S14: ^{13}C NMR (150 MHz, DMSO) of 2E

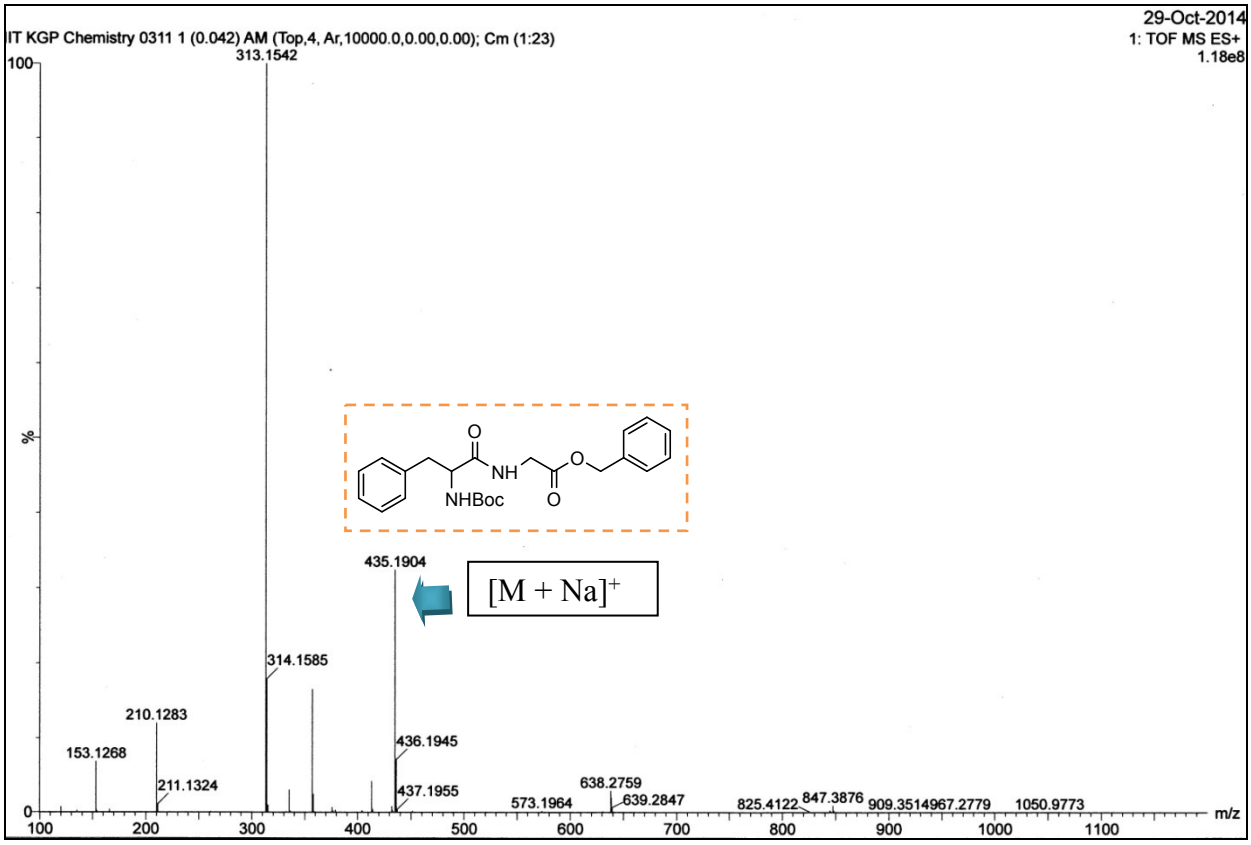


Figure S15: ESI-MS$^+$spectrum of 2A(Calcd. for $C_{23}H_{28}N_2NaO_5$ $[M+Na]^+$ 435.1896)

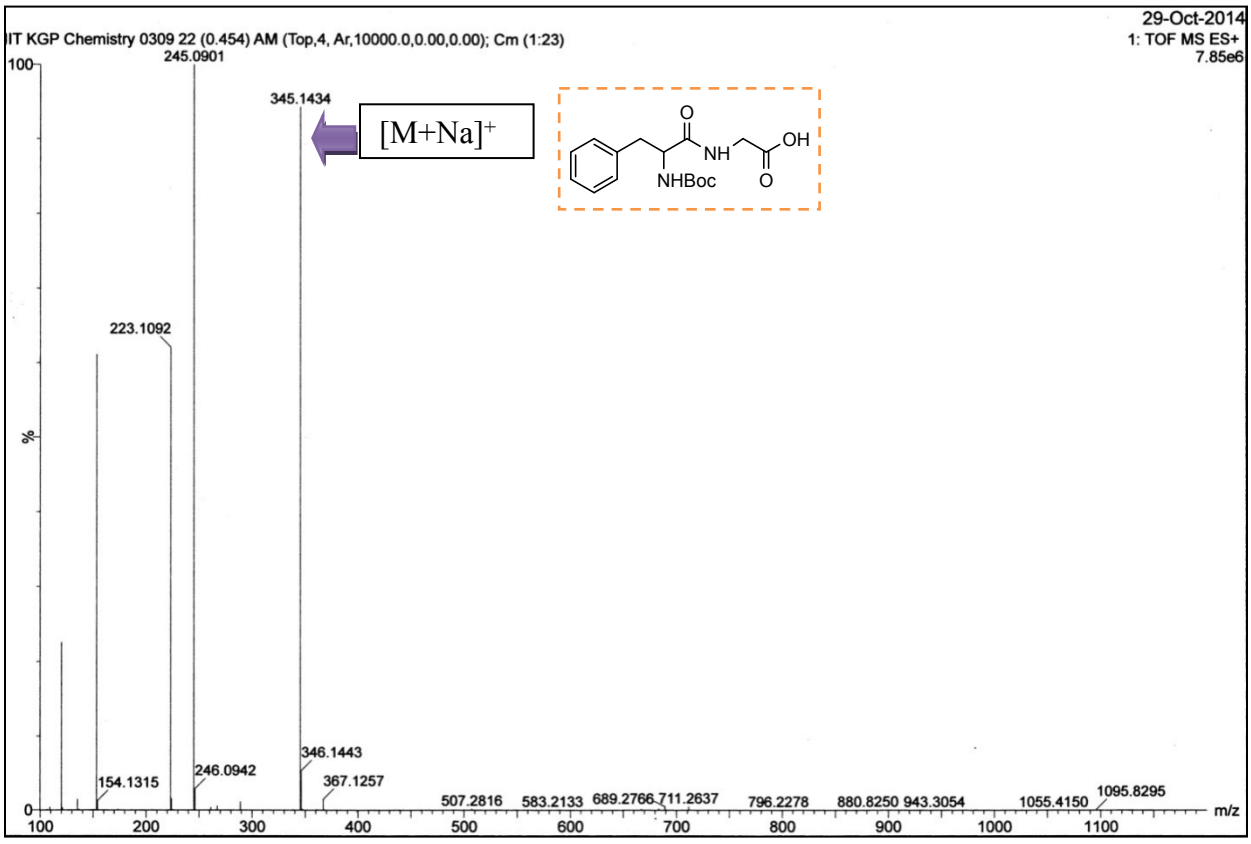


Figure S16: ESI-MS$^+$spectrum of 2B(Calcd. for $C_{16}H_{22}N_2NaO_5$ $[M+Na]^+$ 345.1426)

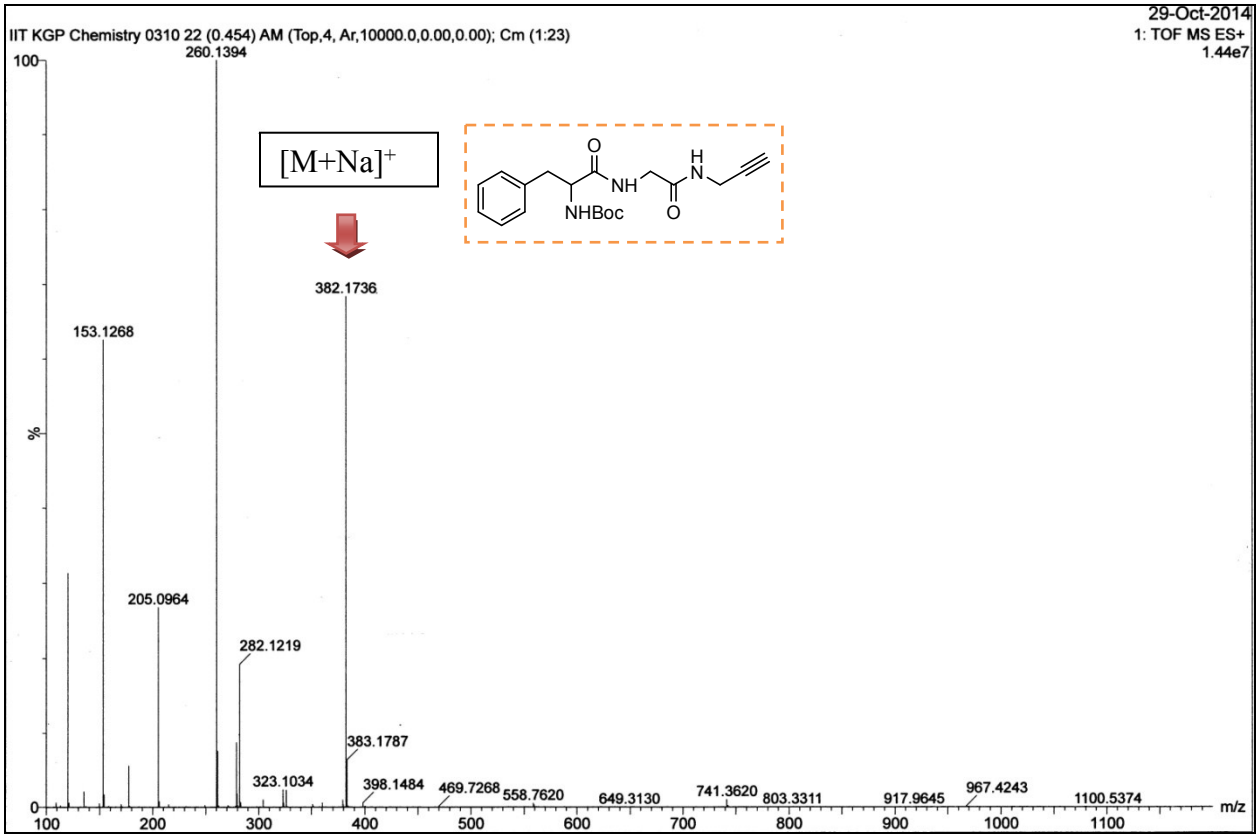


Figure S17: ESI-MS$^+$spectrum of 2C(Calcd. for $C_{19}H_{25}N_3NaO_4$ $[M + Na]^+$ 382.1743)

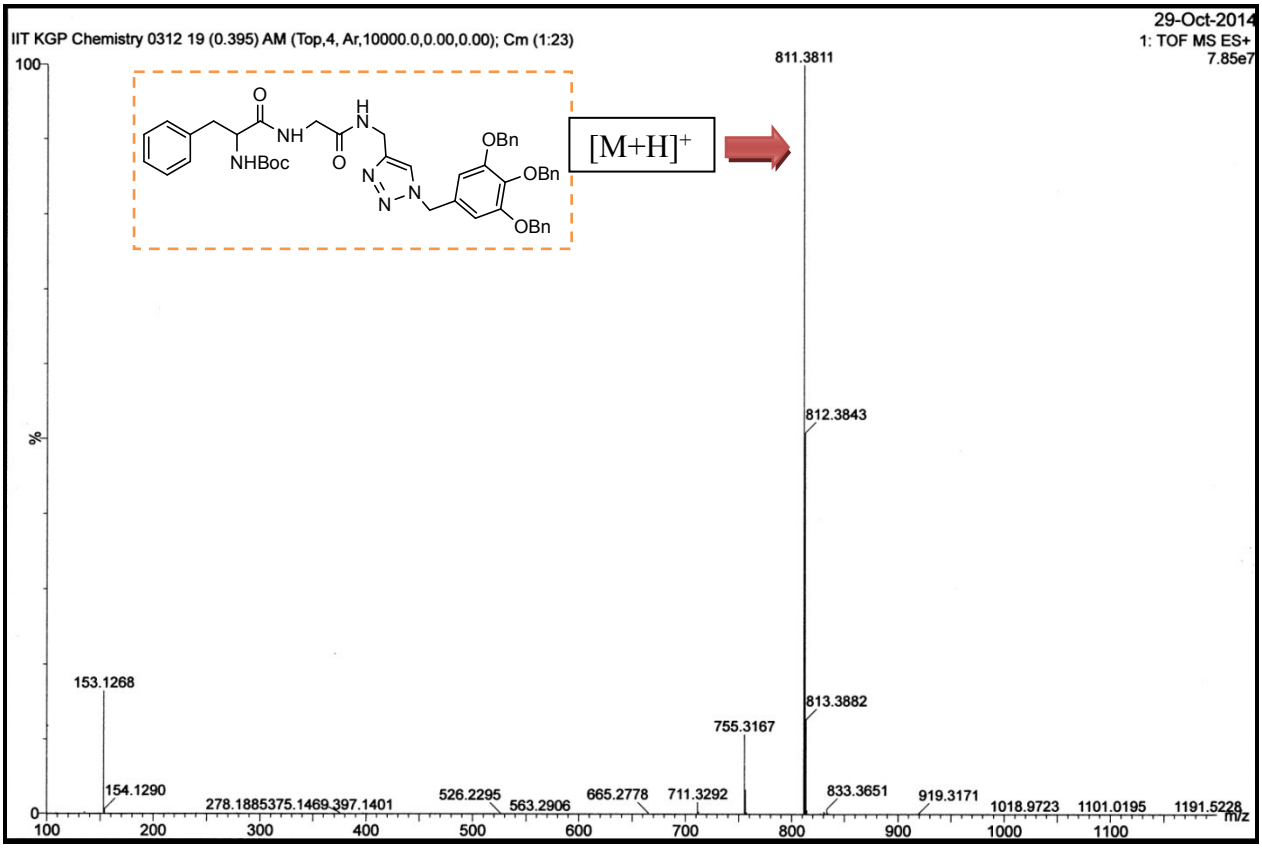


Figure S18: ESI-MS$^+$spectrum of 2D(Calcd. for $C_{47}H_{61}N_6O_7$ $[M + H]^+$ 811.3819)

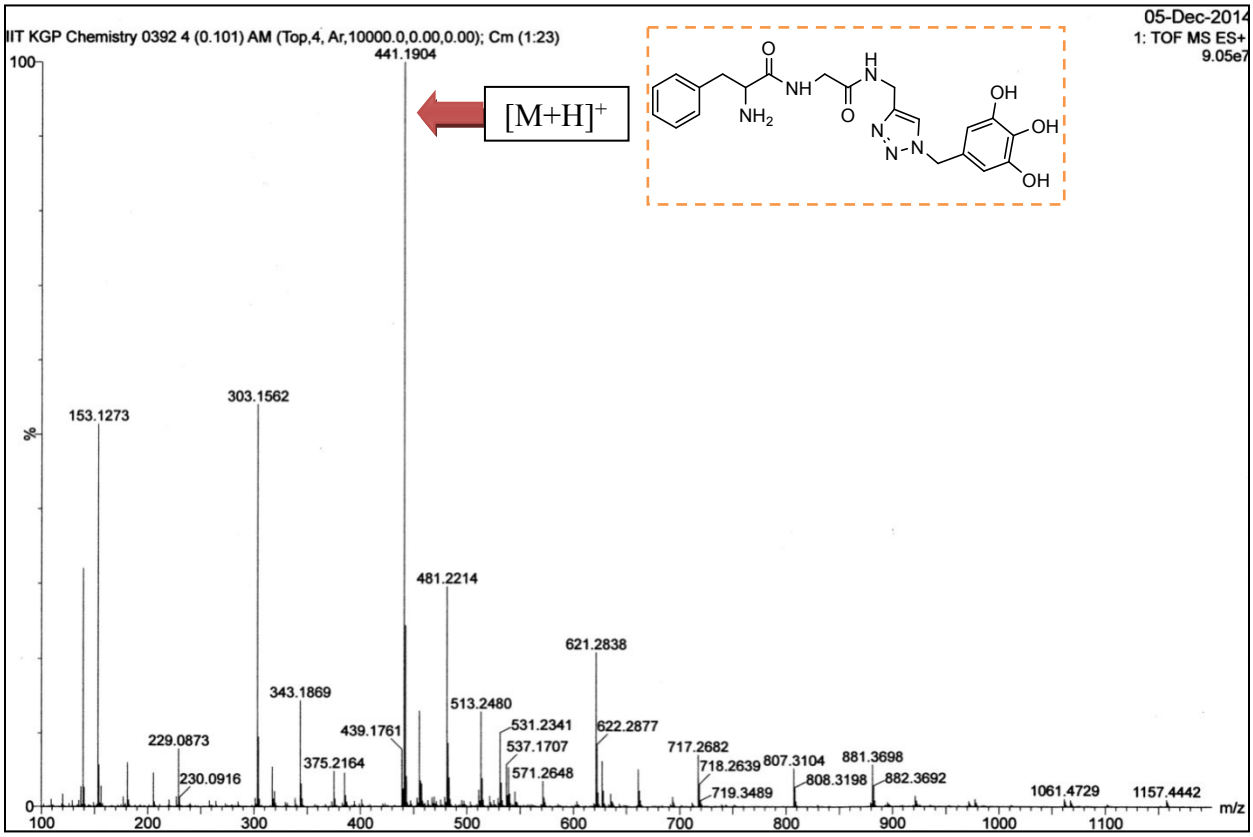


Figure S19: ESI-MS$^+$spectrum of 2E(Calcd. for $C_{21}H_{25}N_6O_5$ $[M + H]^+$ 441.1886)

Computational Details: Geometry optimizations were done in gas phase using the BP86[i] functional, and the def2-TZVP[ii] basis set. Resolution of the Identity approximation (RI) was included to speed up the calculations along with the corresponding auxiliary basis set.[iii] Empirical dispersion correction (DFT-D3BJ)[iv] was included in all calculations. The nature of the stationary points was characterized by vibrational frequency analysis. All the stationary points have zero imaginary mode. References: Neese, F. Orca 3.0.3; Max Planck Institute for Bioinorganic Chemistry: Mülheim/Ruhr, Germany. https://orcaforum.cec.mpg.de: [i] (a) Becke, A. D. *Phys. Rev. A: At., Mol., Opt. Phys.* **1988**, *38*, 3098. (b) Perdew, J. P. *Phys. Rev. B: Condens. Matter Mater. Phys.* **1986**, *33*, 8822. [ii] Schäfer, A.; Huber, C.; Ahlrichs, R. *J. Chem. Phys.* **1994**, *100*, 5829. [iii] Eichkorn, K.; Weigend, F.; Treutler, O.; Ahlrichs, R. *Theor. Chem. Acc.* **1997**, *97*, 119. [iv](a) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. *J. Chem. Phys.* **2010**, *132*, 154104. (b) Grimme, S.; Ehrlich, S.; Goerigk, L. *J. Comput. Chem.* **2011**, *32*, 1456.