Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Contents

1. Preparation of Zn-TPP-Ald

TPP-Ald was solubilized in refluxing THF, and $Zn(OAc)_2$ (5 equivalents) was added. After 3 hours, the solvent was removed and the crude material was solubilized in dichloromethane and filtered on silica gel to obtain the desired **Zn-TPP-Ald**. ^{1}H NMR (400 MHz, Acetone) δ 10.43 (s, 4H), 8.87 (s, 8H), 8.45 (d, J = 7.9 Hz, 8H), 8.37 (d, J = 8.3 Hz, 8H). MS (ESI) m/z calculated $C_{48}H_{28}N_4O_4Zn$ $[M]^+$: 788.14; observed: 788.10.

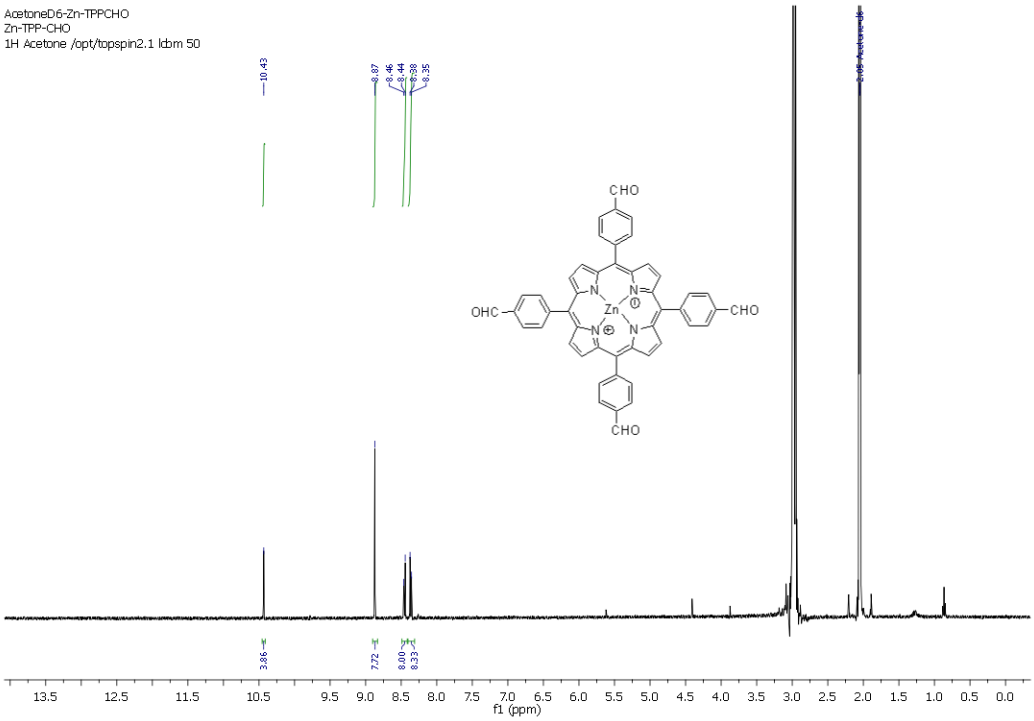


Figure S1: ^{1}H NMR spectrum of **Zn-TPP-Ald**.

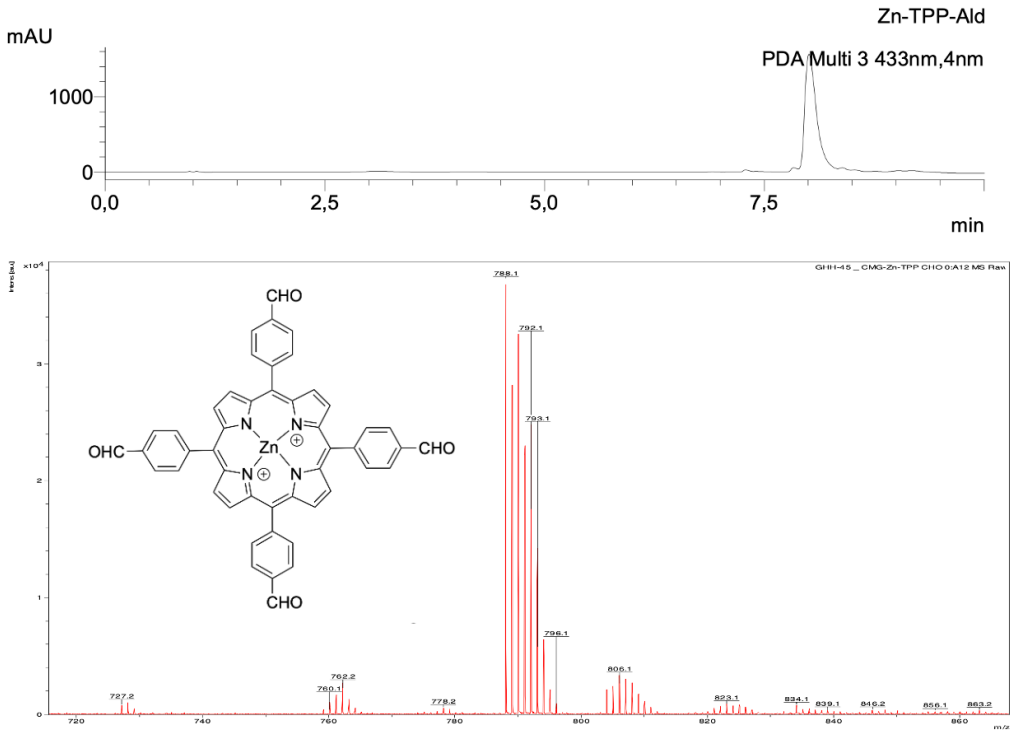
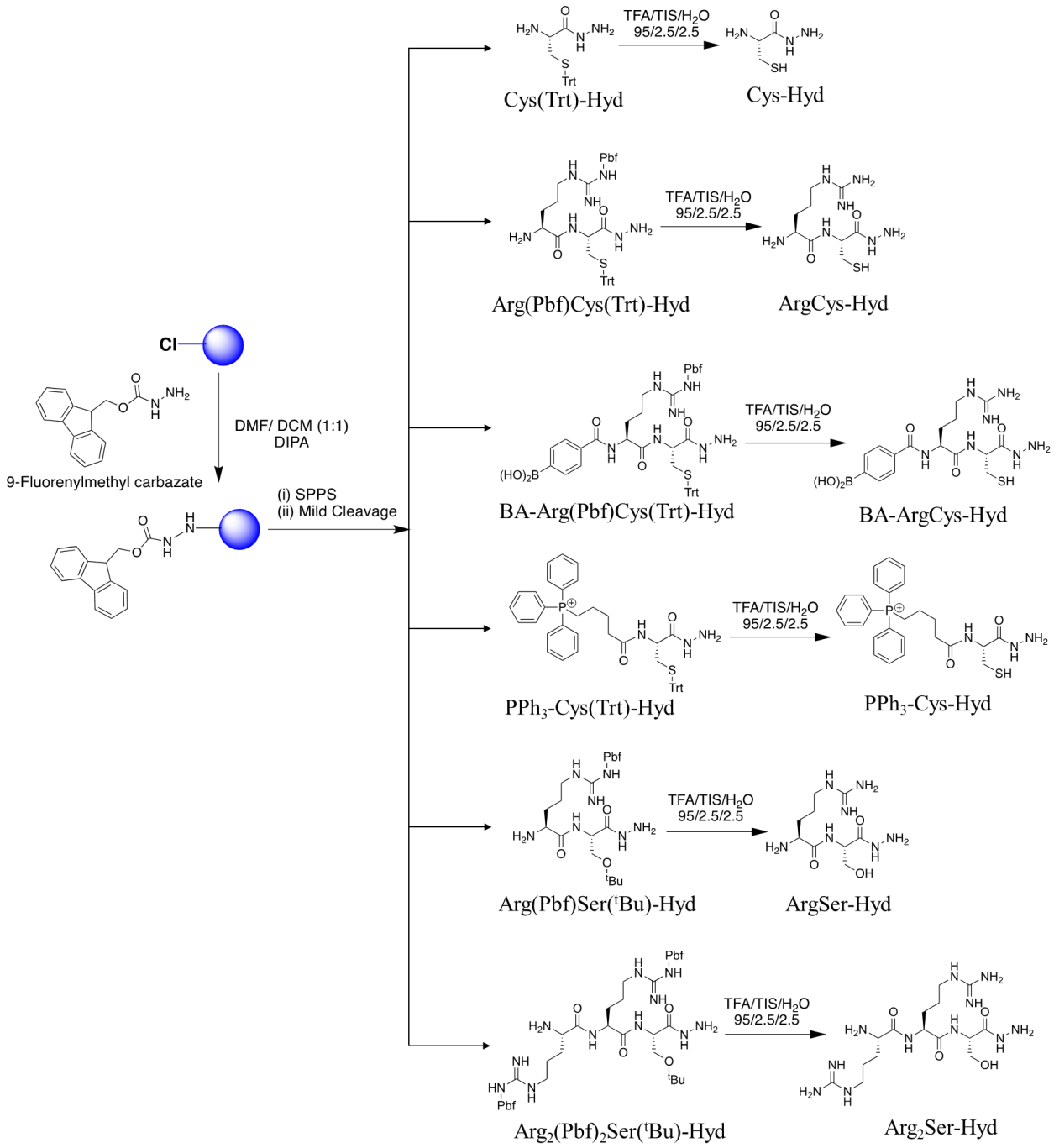


Figure S2: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **Zn-TPP-Ald**.

2. Peptide syntheses

2.1. Synthetic route



Scheme S1: Synthetic route used for preparing the protected and deprotected peptide hydrazide building blocks.

2.2. Characterization data

Cys(Trt)-Hyd: Isolated by preparative reverse-phase HPLC (linear gradient from 95/5 A/B eluents to 5/95 A/B eluents in 45 min). ^{1}H NMR (400 MHz, MeOD) δ 7.44 – 7.40 (m, 6H), 7.34 (dd, *J* = 8.7, 6.7 Hz, 6H), 7.29 – 7.25 (m, 3H), 3.59 (dd, *J* = 6.0, 3.9 Hz, 1H, H_α), 2.69 (dd, *J* = 12.4, 7.3 Hz, 1H, H_β), 2.58 (dd, *J* = 12.4, 6.5 Hz, 1H, H_β). MS (ESI) m/z calculated $C_{22}H_{23}N_3OS$ $[M+H]^+$: 378.168; observed: 378.10.

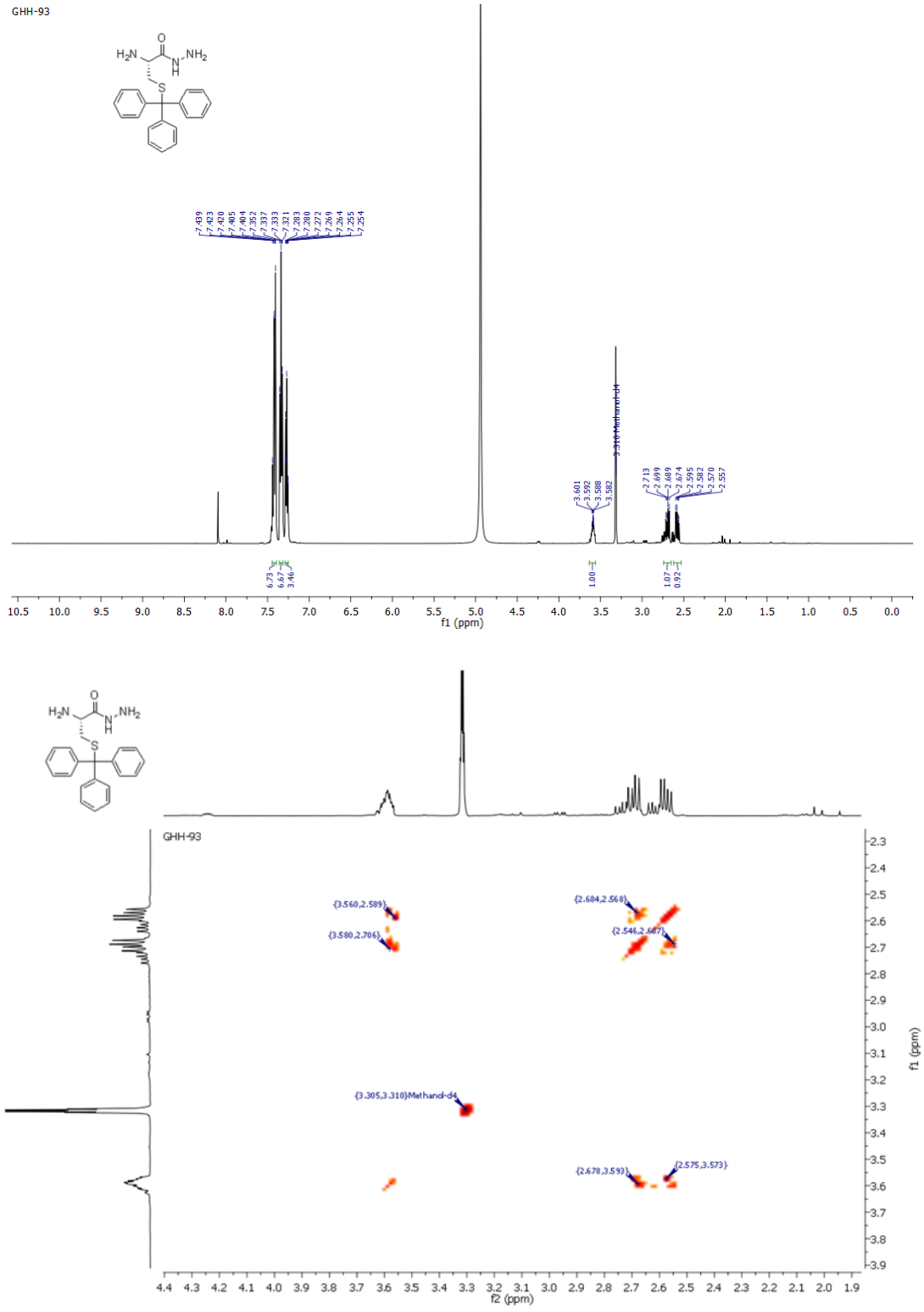


Figure S3: ^{1}H NMR (top) and COSY NMR (bottom) spectra of **Cys(Trt)-Hyd**.

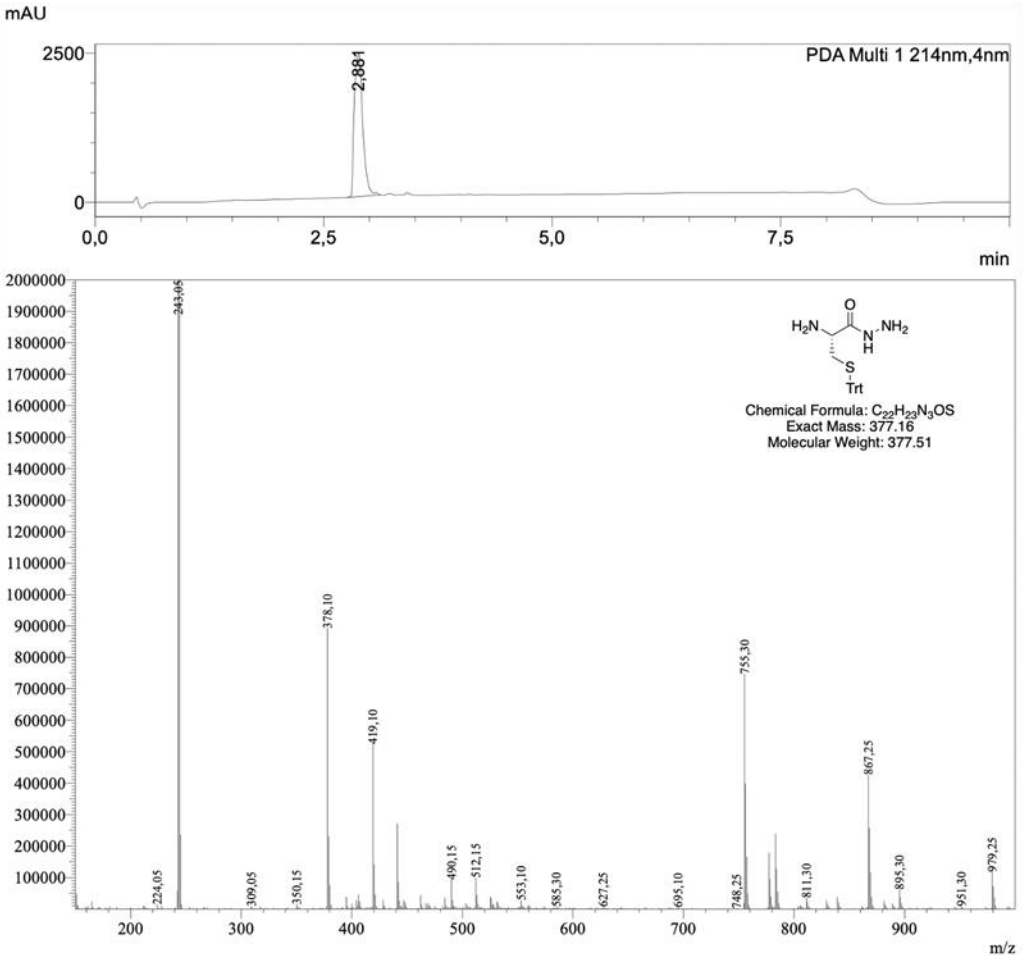


Figure S4: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **Cys(Trt)-Hyd**.

Cys-Hyd: ^{1}H NMR (400 MHz, D_2O) δ 4.35 (t, *J* = 5.7 Hz) & 4.17 (t, *J* = 6.0 Hz) (1H), 3.18 – 2.98 (m, 2H). MS (ESI) m/z calculated $C_3H_9N_3OS$ $[M+H]^+$: 136.058; observed: 136.06.

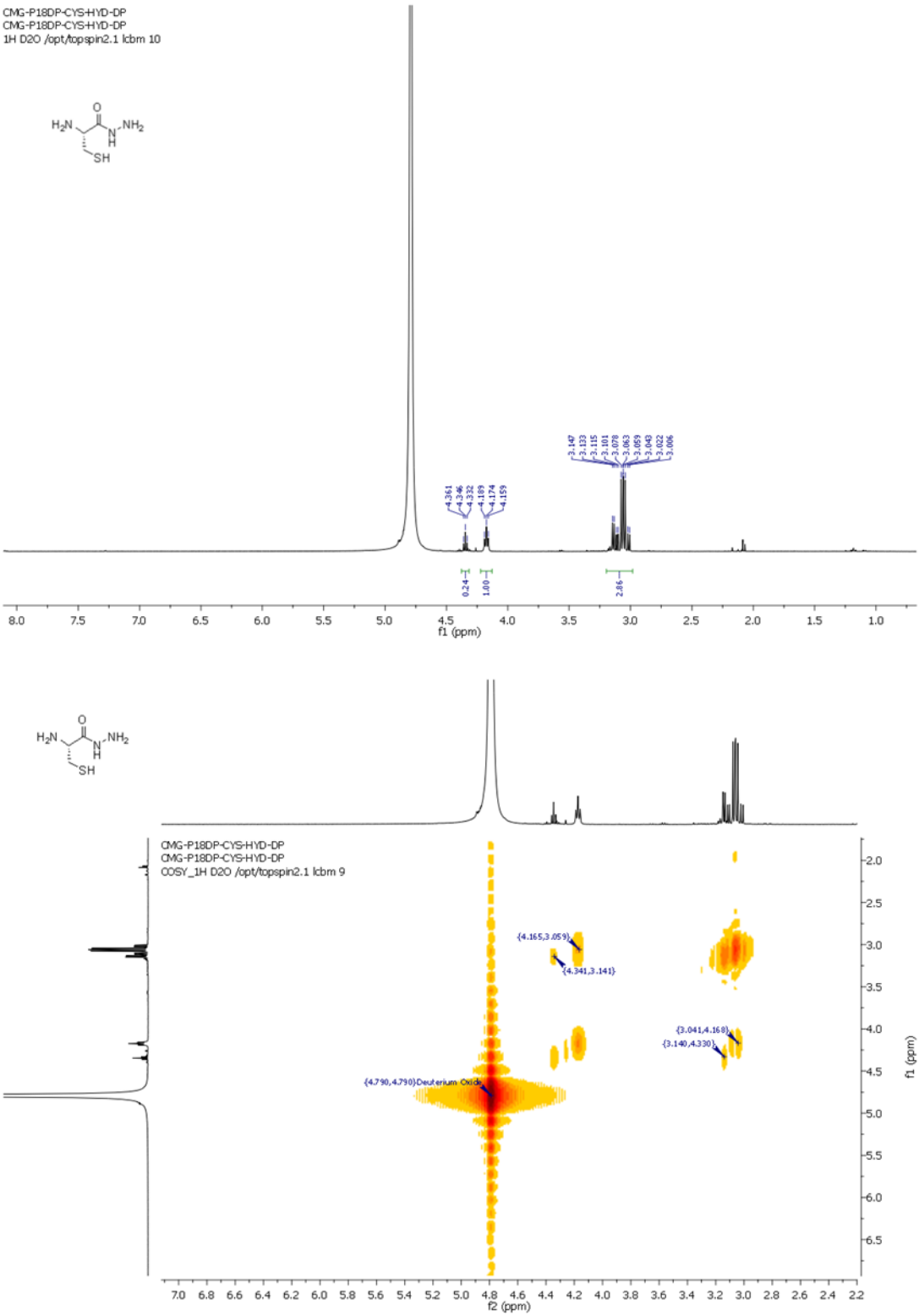


Figure S5: ^{1}H NMR (top) and COSY NMR (bottom) spectra of **Cys-Hyd**.

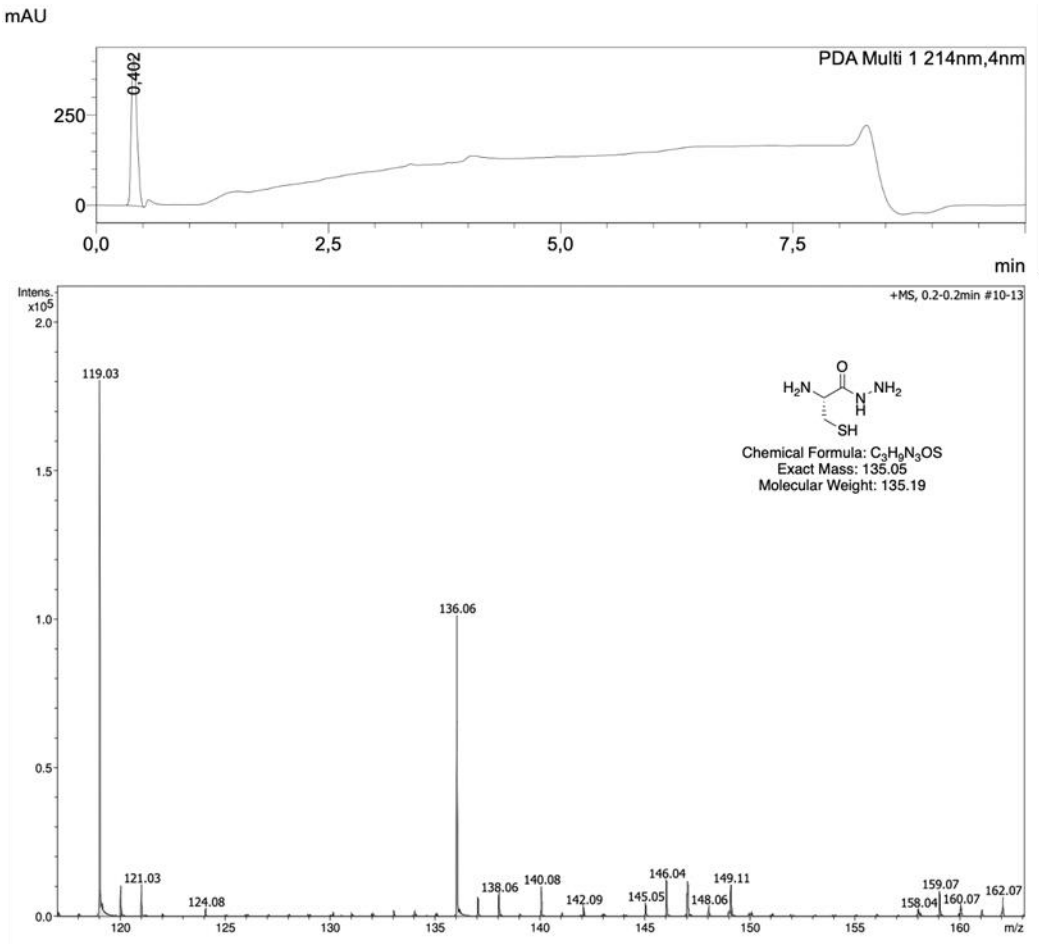


Figure S6: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **Cys-Hyd**.

Arg(Pbf)Cys(Trt)-Hyd: Isolated by preparative reverse-phase HPLC (linear gradient from 95/5 A/B eluents to 5/95 A/B eluents in 30 min, keeping this composition for 10 more min). ^{1}H NMR (400 MHz, CD_3OD) δ 7.35 (t, *J* = 1.8 Hz, 2H), 7.33 (dd, *J* = 1.5, 0.9 Hz, 4H), 7.28 (t, *J* = 1.5 Hz, 2H), 7.26 (t, *J* = 2.0 Hz, 2H), 7.25 – 7.23 (m, 2H), 7.21 (t, *J* = 1.4 Hz, 1H), 7.19 (t, *J* = 2.4 Hz, 1H), 7.18 – 7.17 (m, 1H), 4.22 (t, *J* = 7.5 Hz, 1H), 3.86 (t, *J* = 6.4 Hz, 1H), 3.20 – 3.09 (m, 2H), 2.95 (s, 2H), 2.65 – 2.55 (m, 2H), 2.53 (s, 3H), 2.46 (s, 3H), 2.04 (s, 3H), 1.87 – 1.75 (m, 2H), 1.65 – 1.50 (m, 2H), 1.41 (s, 6H). MS (ESI) m/z calculated $C_{41}H_{51}N_7O_5S_2$; $[M+H]^+$: 786.348; observed: 786.40.

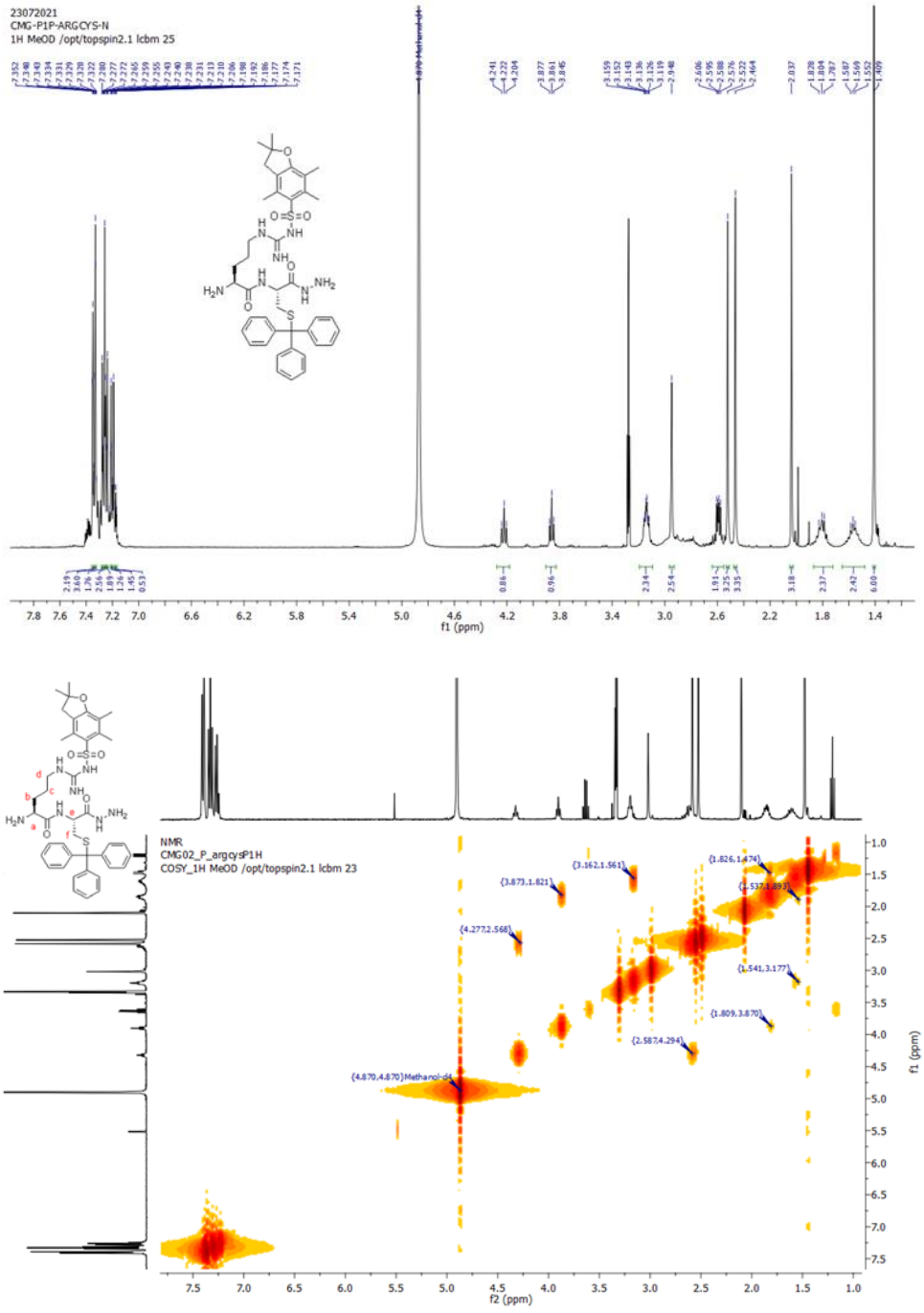


Figure S7: ^{1}H NMR (top) and COSY NMR (bottom) spectra of **Arg(Pbf)Cys(Trt)-Hyd**.

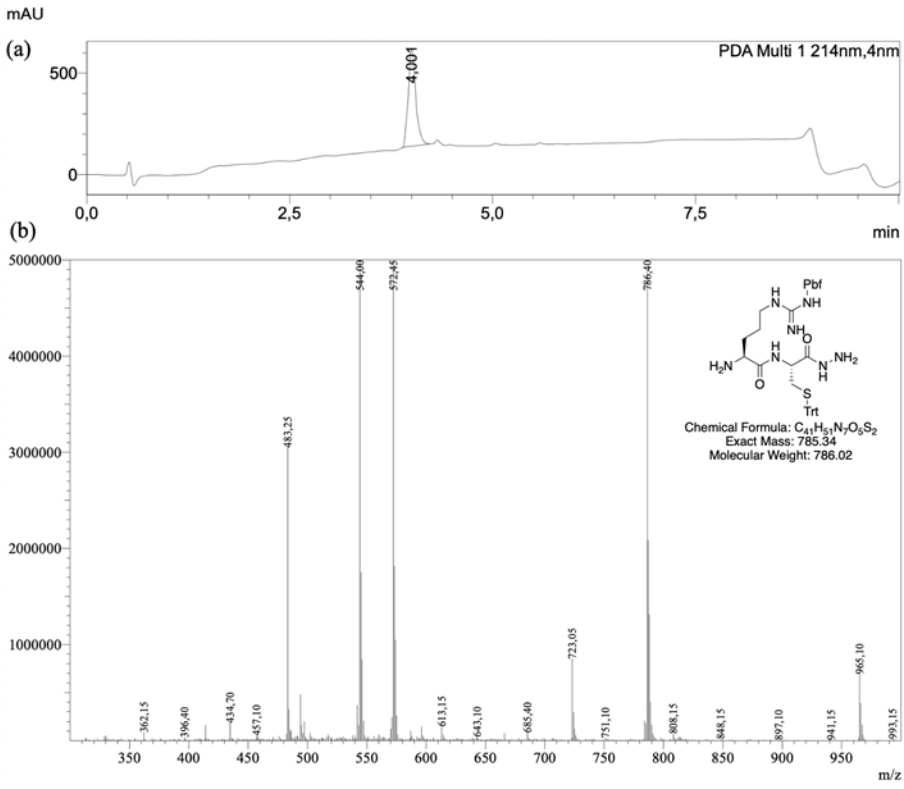


Figure S8: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **Arg(Pbf)Cys(Trt)-Hyd**.

Arg-Cys-Hyd: ^{1}H NMR (400 MHz, D_2O) δ 4.63 (t, *J* = 5.1 Hz) & 4.50 (t, *J* = 6.7 Hz) (1H), 4.09 (t, *J* = 5.4 Hz, 1H), 3.22 (t, *J* = 5.9 Hz, 2H), 3.05 – 2.81 (m, 2H), 1.94 (m, 2H), 1.65 (m, 2H). MS (ESI) m/z calculated $C_9H_{21}N_7O_2S$; $[M+H]^+$: 292.155; observed: 291.70.

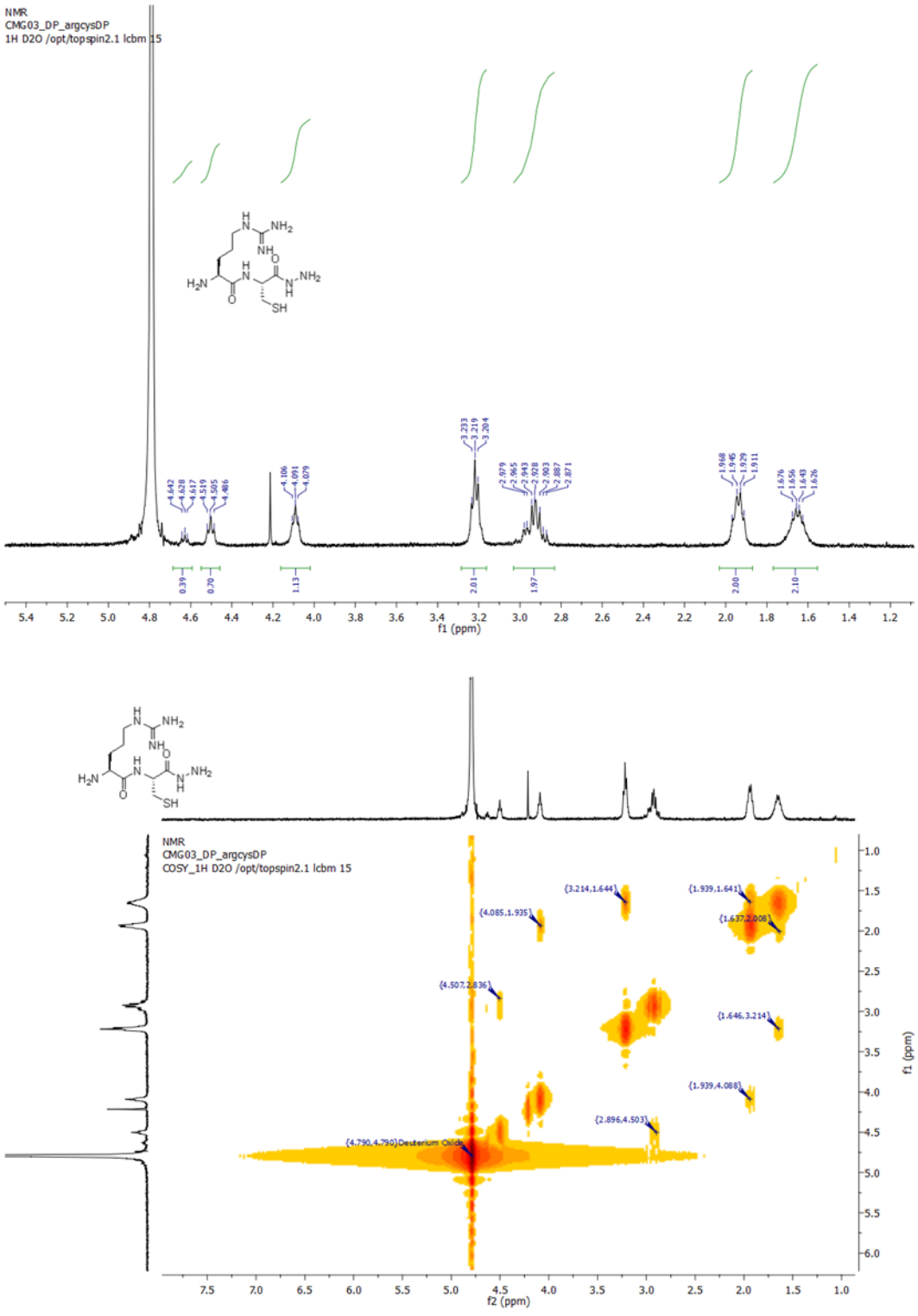


Figure S9: ^{1}H NMR (top) and COSY NMR (bottom) spectra of **ArgCys-Hyd**.

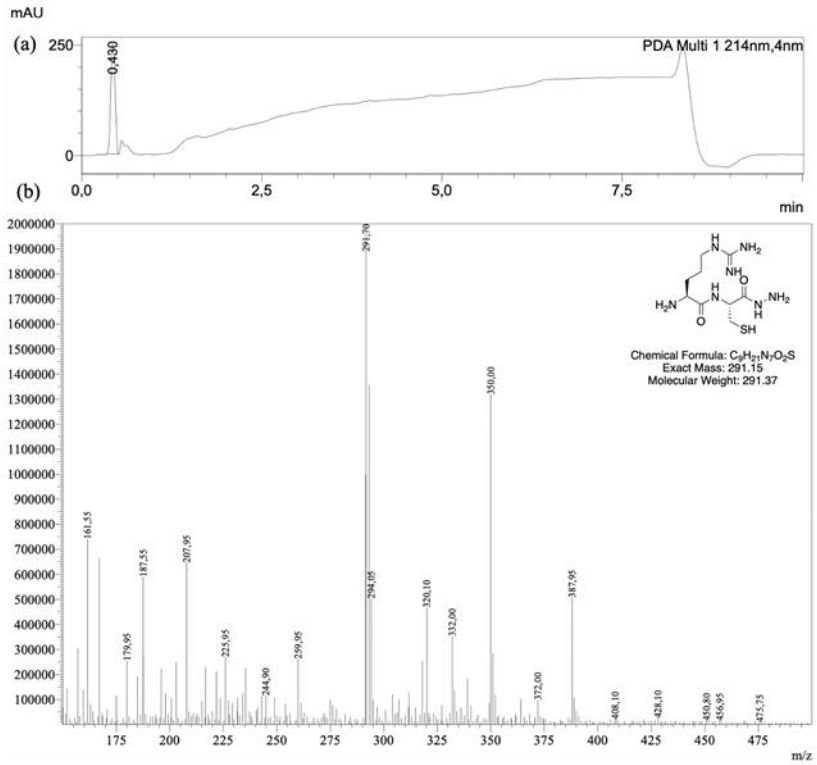


Figure S10: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **ArgCys-Hyd**.

BA-Arg(Pbf)Cys(Trt)-Hyd : Isolated by preparative reverse-phase HPLC (linear gradient from 80/20 A/B eluents to 5/95 A/B eluents in 30 min, keeping this composition for 10 more min). ^{1}H NMR (400 MHz, CD_3OD) δ 7.82 (d, *J* = 8.2 Hz, 2H), 7.75 (br, 2H), 7.36 – 7.33 (m, 6H), 7.27 – 7.17 (m, 9H), 4.51 (dd, *J* = 8.6, 5.9 Hz, 1H), 4.10 (t, *J* = 7.2 Hz, 1H), 3.27 – 3.13 (m, 2H), 2.95 (s, 2H), 2.71 – 2.61 (m, 2H), 2.55 (s, 3H), 2.49 (s, 3H), 1.95 – 1.75 (m, 2H), 1.71 – 1.55 (m, 2H), 1.42 (s, 6H). MS (ESI) m/z calculated $C_{48}H_{56}BN_7O_8S_2$; $[M+H]^+$: 934.378; observed: 934.25.

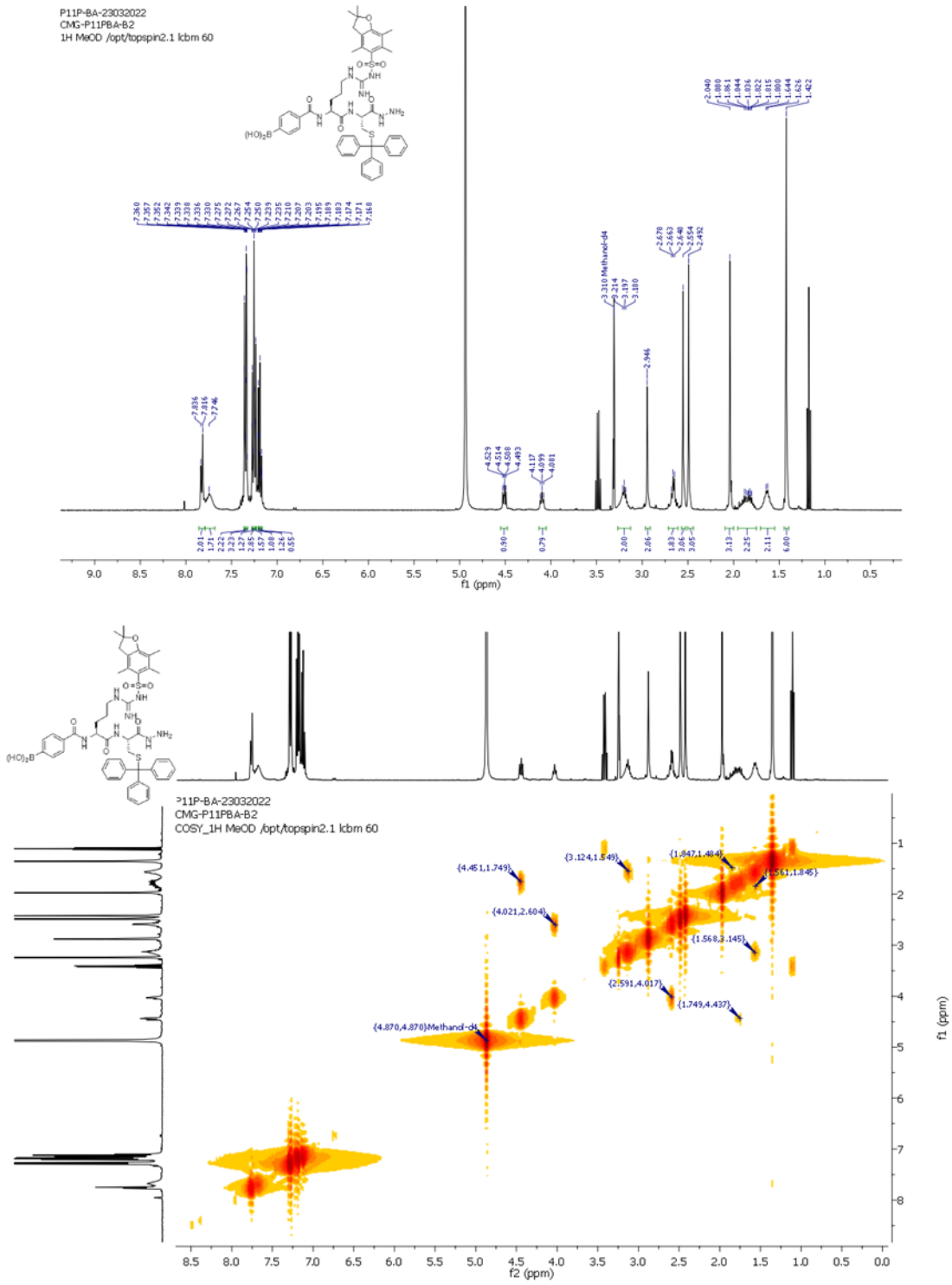


Figure S11: ^{1}H NMR (top) and COSY NMR (bottom) spectra of **BA-Arg(Pbf)Cys(Trt)-Hyd**.

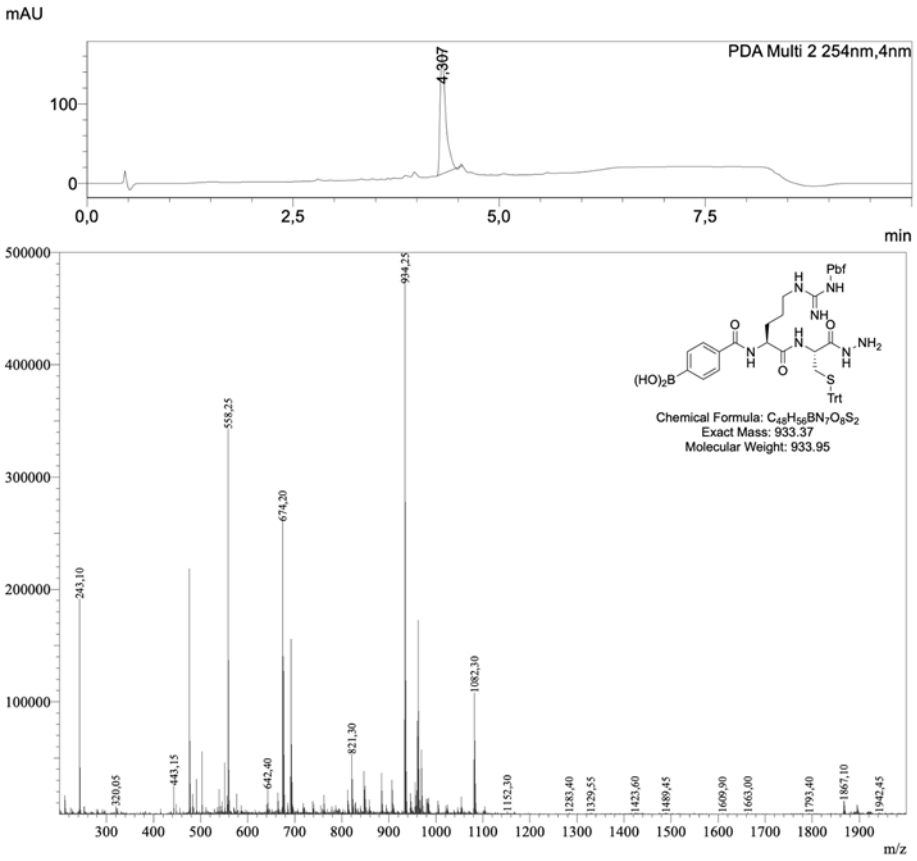


Figure S12: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **BA-Arg(Pbf)Cys(Trt)-Hyd**.

BA-ArgCys-Hyd: ^{1}H NMR (400 MHz, D_2O) δ 7.85 (dt, *J* = 6.4, 2.0, 2H), 7.78 (dt, *J* = 8.4, 2.0, 2H), 4.55 (ddd, *J* = 8.7, 6.0, 3.6 Hz, 2H), 3.24 (t, *J* = 6.9 Hz, 2H), 2.95 (dq, *J* = 14.1, 6.7 Hz, 2H), 2.01 – 1.83 (m, 2H), 1.80 – 1.64 (m, 2H). MS (ESI) m/z calculated $C_{16}H_{26}BN_7O_5S$ $[M+H]^+$: 440.18; observed: 441.15.

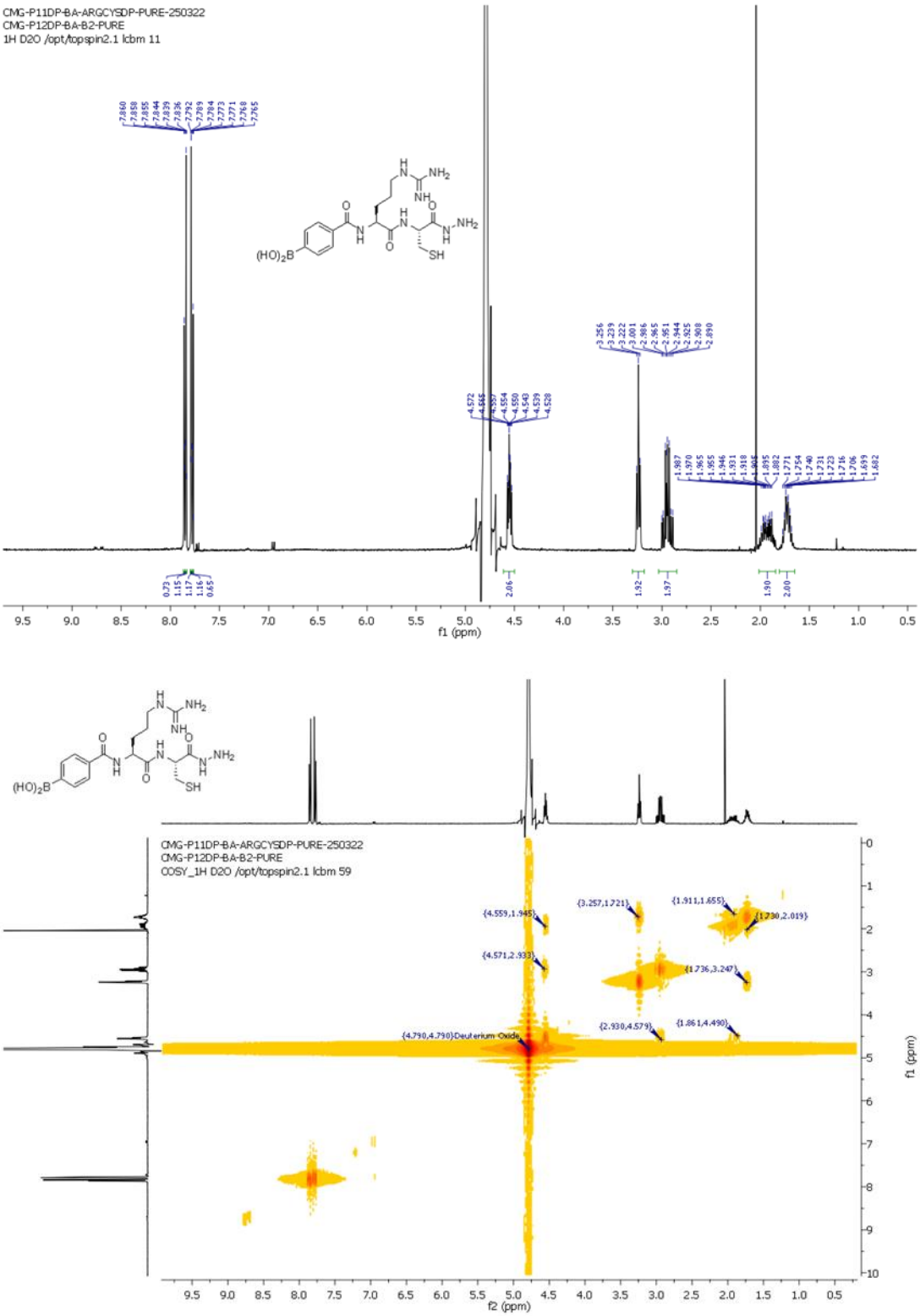


Figure S13: ^{1}H NMR (top) and COSY NMR (bottom) spectra of **BA-ArgCys-Hyd**.

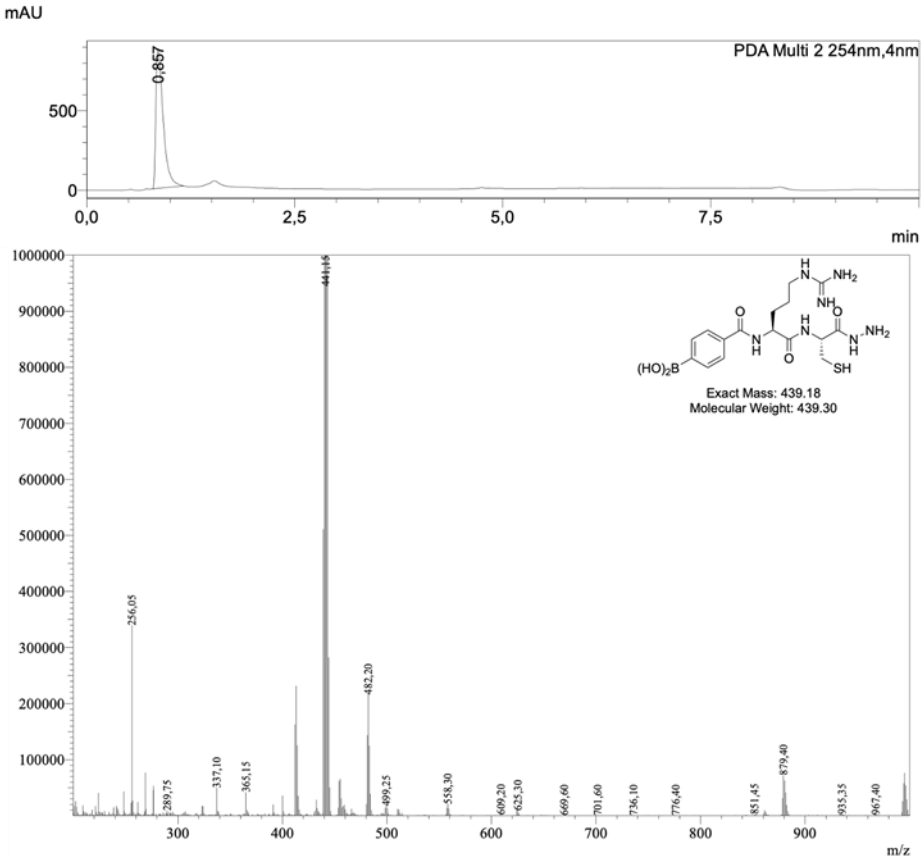


Figure S14: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **BA-ArgCys-Hyd**.

PPh_3-Cys(Trt)-Hyd: Isolated by preparative reverse-phase HPLC (linear gradient from 95/5 A/B eluents to 5/95 A/B eluents in 40 min). ^{1}H NMR (400 MHz, CD_3OD) δ 7.89 – 7.83 (m, 3H), 7.80 – 7.76 (m, 4H), 7.76 – 7.69 (m, 8H), 7.37 – 7.33 (m, 6H), 7.25 (dt, *J* = 13.8, 4.8 Hz, 6H), 7.21 (dt, *J* = 9.5, 4.2 Hz, 3H), 4.16 (dd, *J* = 7.9, 6.7 Hz, 1H), 3.51 – 3.33 (m, 2H), 2.66 – 2.54 (m, 2H), 2.29 (q, *J* = 7.7 Hz, 2H), 1.84 (m, 2H), 1.70 (m, 2H). MS (ESI) m/z calculated $C_{45}H_{45}N_3O_2PS$ $[M+H]^+$: 723.308; observed: 722.15.

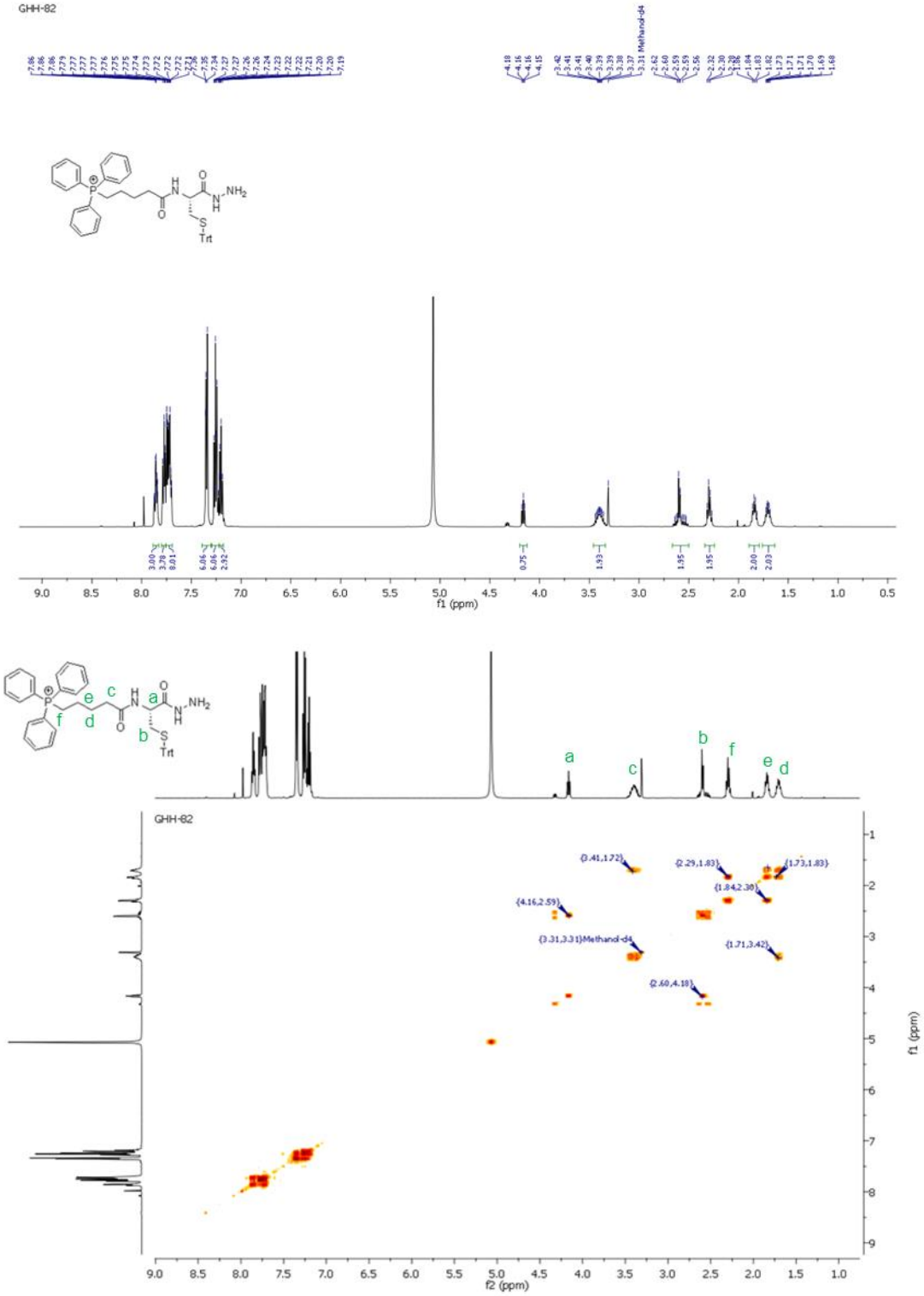


Figure S15: ^{1}H NMR (top) and COSY NMR (bottom) spectra of **PPh_3-Cys(Trt)-Hyd**.

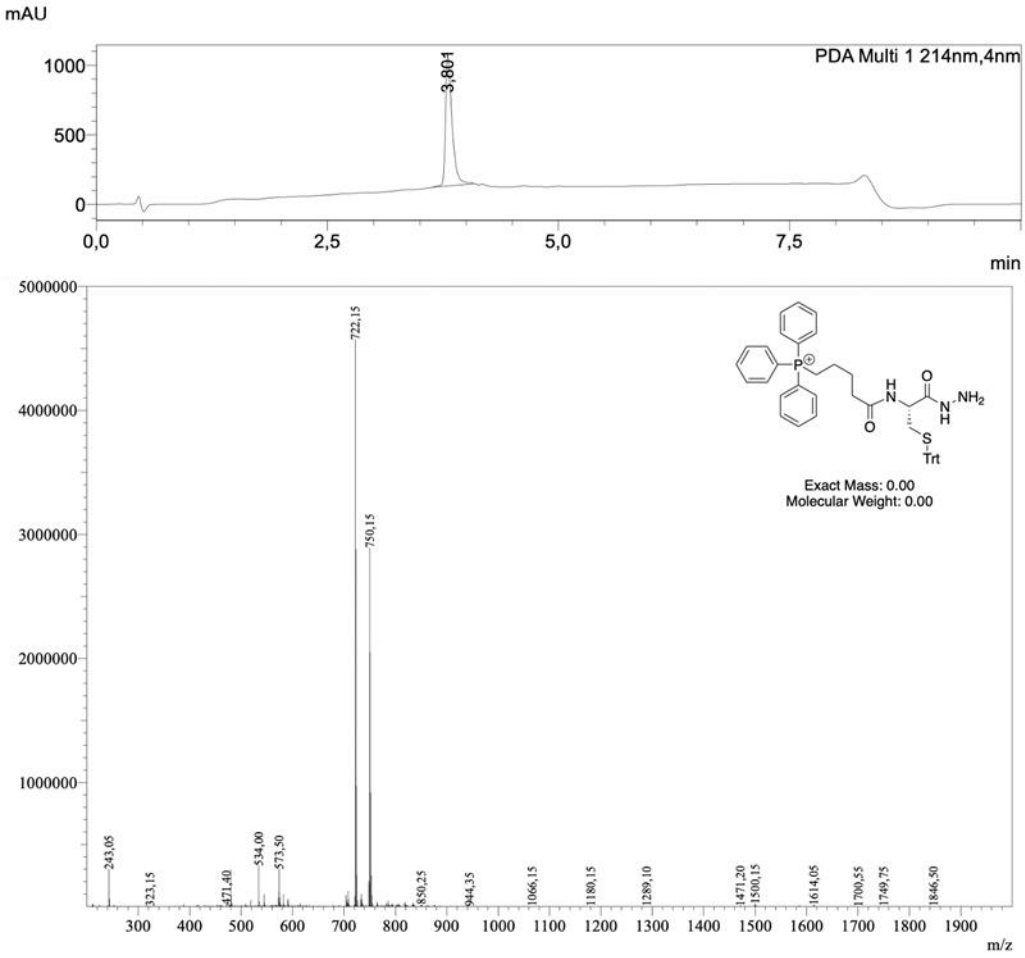


Figure S16: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **PPh_3-Cys(Trt)-Hyd**.

PPh_3-Cys-Hyd: ^{1}H NMR (400 MHz, D_2O) δ 7.70 (dd, *J* = 5.5, 1.7 Hz, 3H), 7.63 – 7.49 (m, 12H), 4.38 (dd, *J* = 8.1, 5.2 Hz, 1H), 3.17 (br, 2H), 2.72 (ddd, *J* = 22.3, 14.2, 6.7 Hz, 2H), 2.25 (m, 2H), 1.71 (br, 2H), 1.58 (br, 2H). MS (ESI) m/z calculated $C_{26}H_{31}N_3O_2PS$ $[M+H]^+$: 481.198; observed: 480.15.

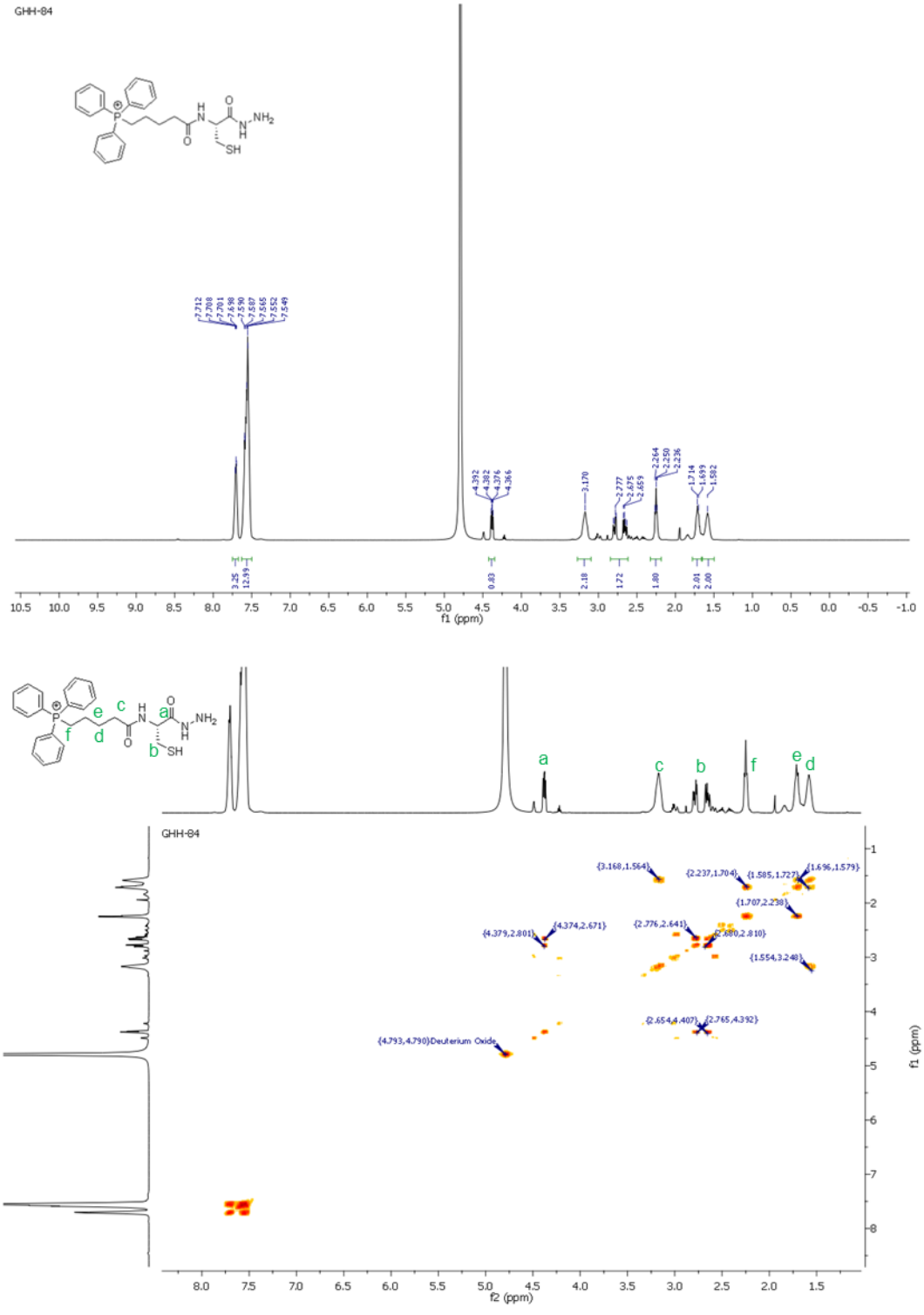


Figure S17: ^{1}H NMR (top) and COSY NMR (bottom) spectra of **PPh_3-Cys-Hyd**.

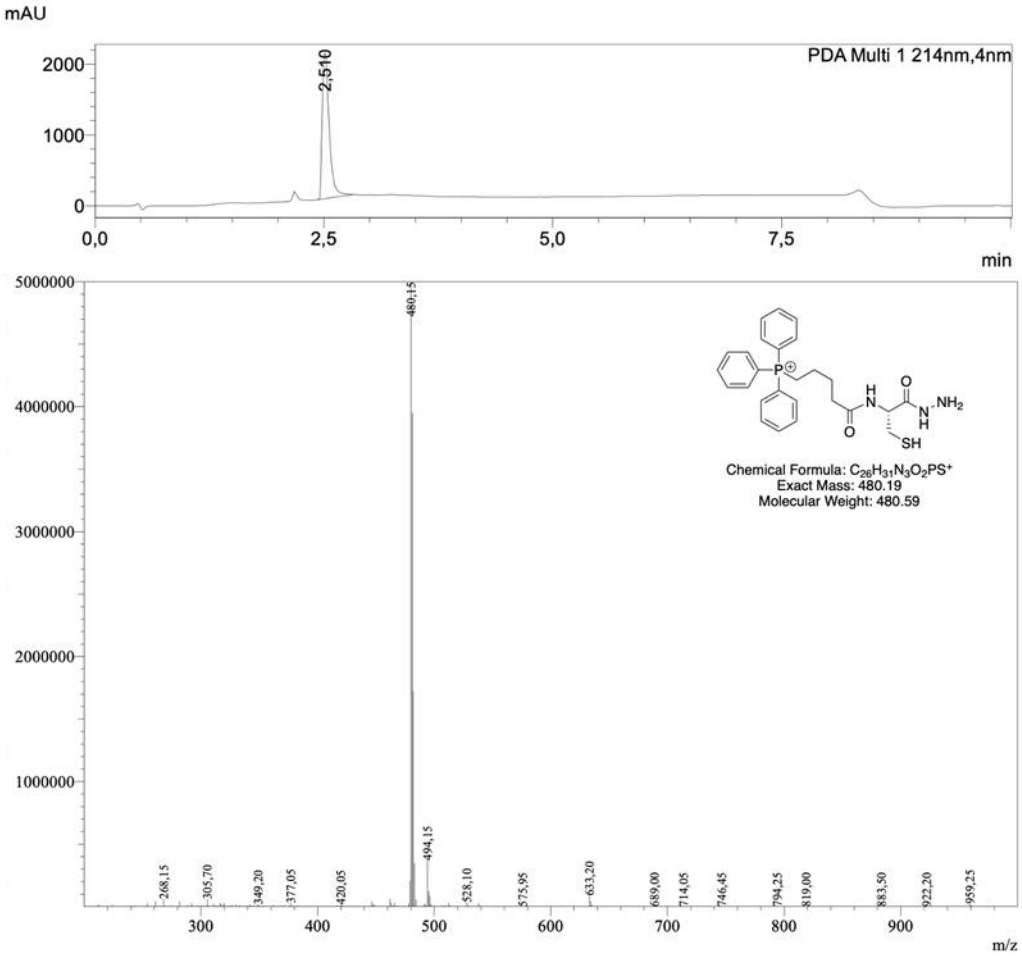


Figure S18: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **PPh_3-Cys-Hyd**.

Arg(Pbf)Ser(ᵗBu)-Hyd: Isolated by preparative reverse-phase HPLC (linear gradient from 95/5 A/B eluents to 5/95 A/B eluents in 30 min, keeping this composition for 10 more min). ^{1}H NMR (400 MHz, CD_3OD) : δ 4.56 (t, *J* = 5.8 Hz, 1H), 4.01 (t, *J* = 6.4 Hz, 1H), 3.67 (qd, *J* = 9.2, 5.8 Hz, 2H), 3.21 (t, *J* = 6.6 Hz, 2H), 3.00 (s, 2H), 2.57 (s, 3H), 2.51 (s, 3H), 2.08 (s, 3H), 1.91 (m, 2H), 1.64 (m, 2H), 1.45 (s, 6H), 1.20 (s, 9H). MS (ESI) m/z calculated $C_{26}H_{45}N_7O_6S$; $[M+H]^+$: 584.328; observed: 584.55.

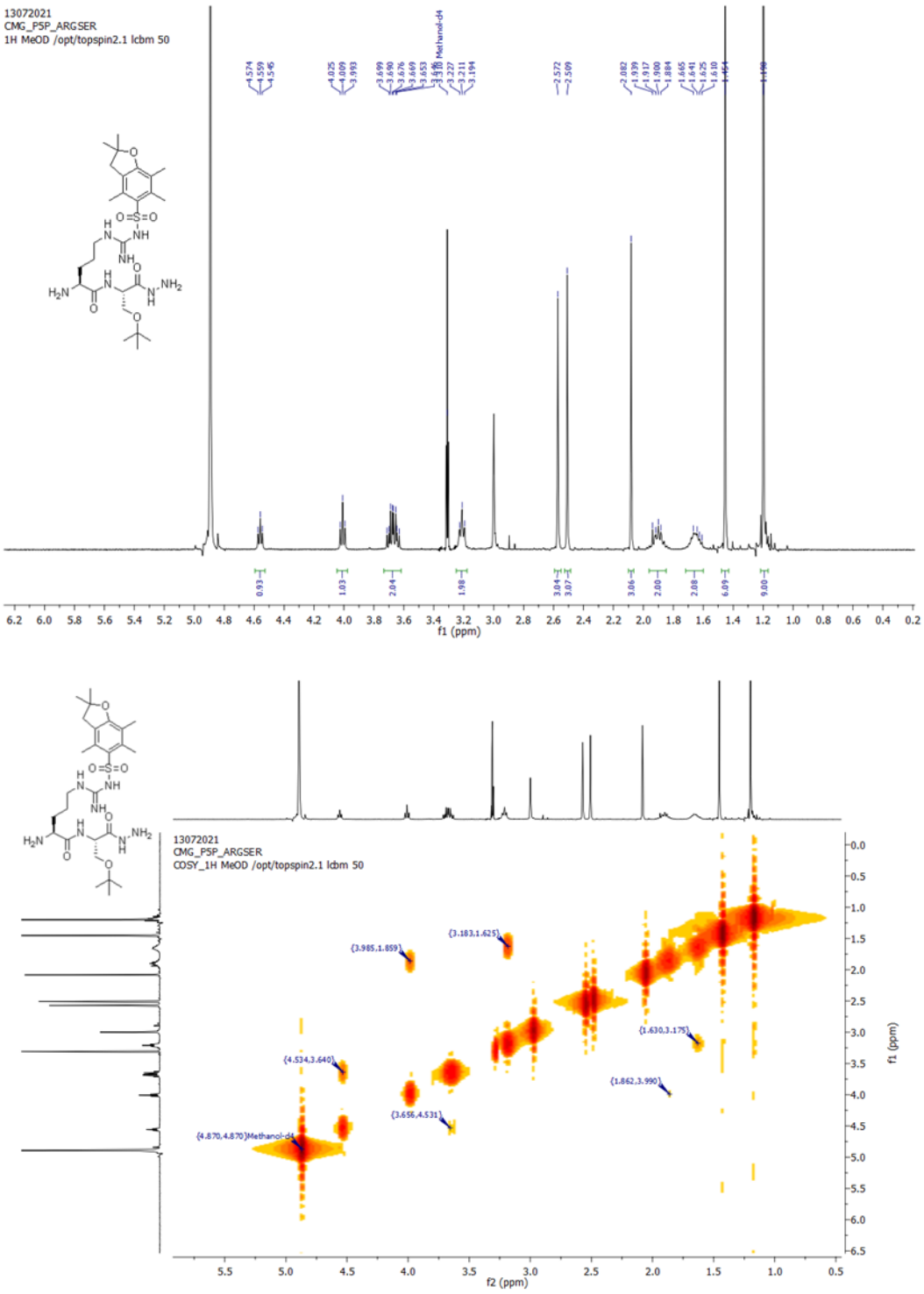


Figure S19: ^{1}H NMR (top) and COSY NMR (bottom) spectra of **Arg(Pbf)Ser(tBu)-Hyd**.

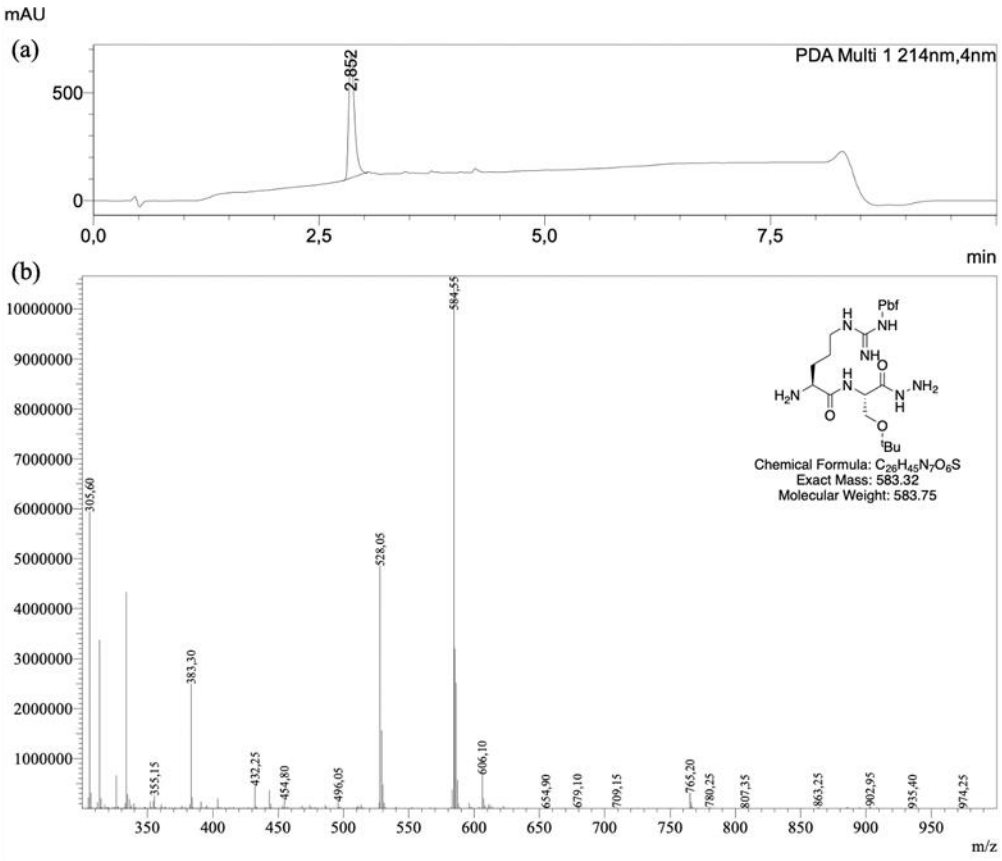


Figure S20: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **Arg(Pbf)Ser(ᵗBu)-Hyd**.

ArgSer-Hyd: ^{1}H NMR (400 MHz, D_2O): δ 4.65 (t, *J* = 5.5 Hz) & 4.54 (t, *J* = 5.4 Hz) (1H), 4.14 (d, *J* = 2.5 Hz, 1H), 3.98 – 3.86 (m, 2H), 3.26 (t, *J* = 6.6 Hz, 2H), 2.05 – 1.91 (m, 2H), 1.79 – 1.61 (m, 2H). MS (ESI) m/z calculated $C_9H_{21}N_7O_3$ $[M+H]^+$: 276.178; observed: 276.00.

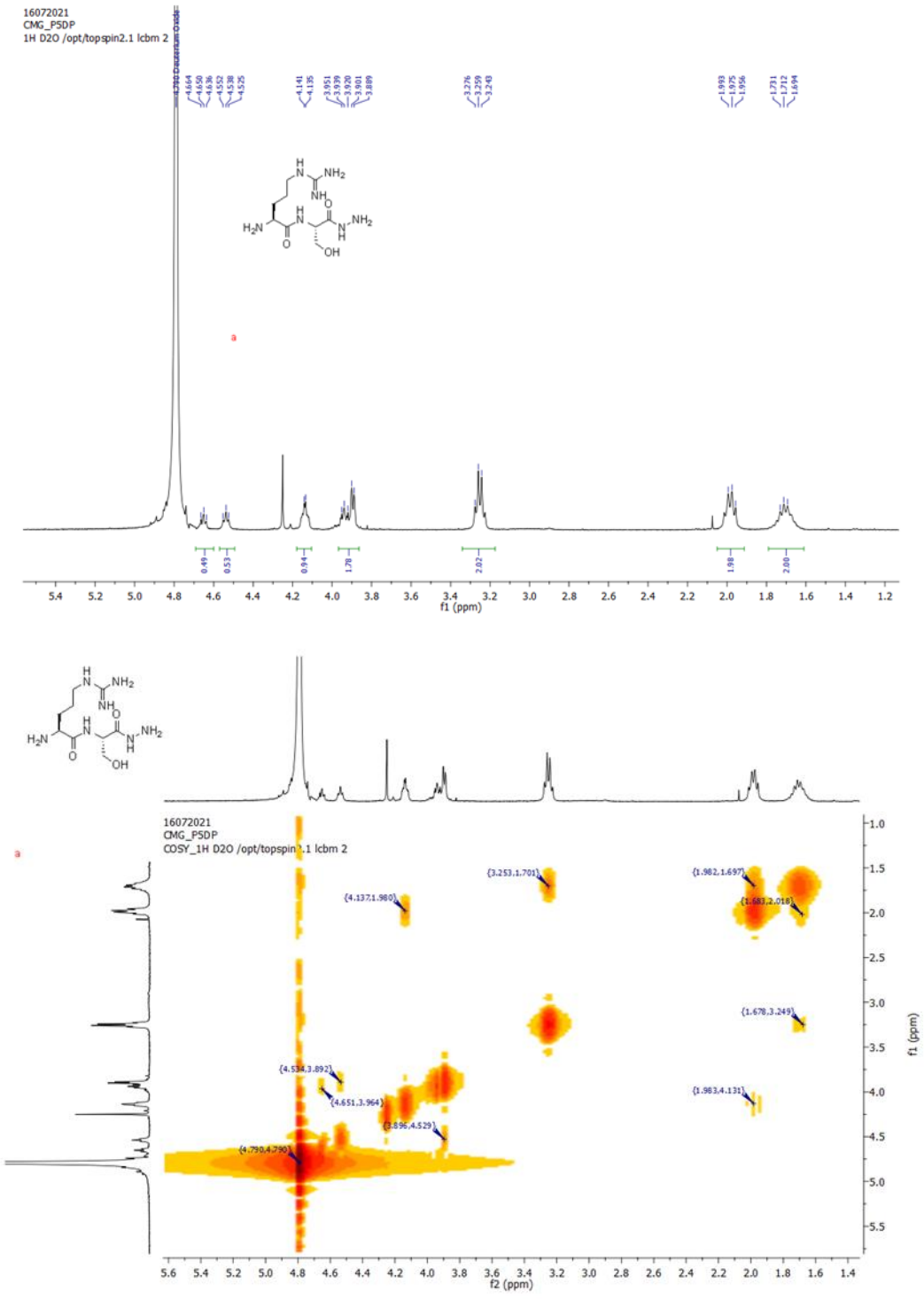


Figure S21: ^{1}H NMR (top) and COSY NMR (bottom) spectra of **ArgSer-Hyd**.

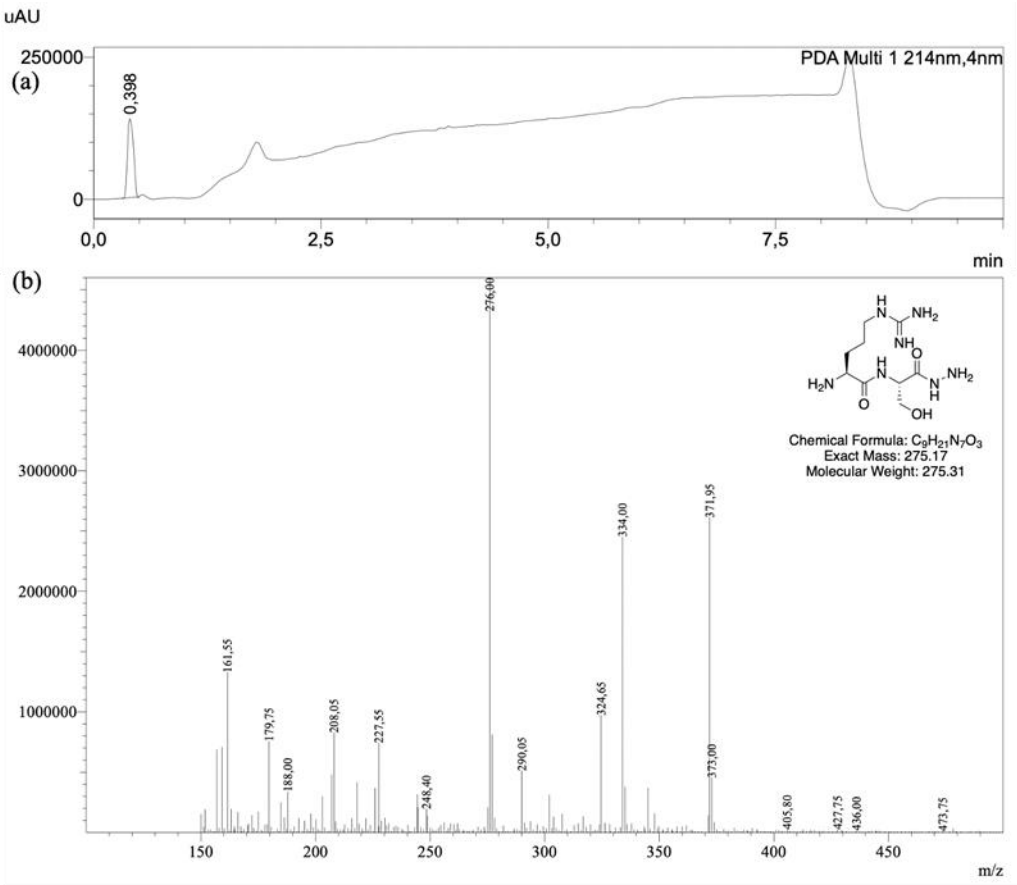


Figure S22: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **ArgSer-Hyd**.

$Arg_2(Pbf)_2Ser(^tBu)$-Hyd: Isolated by preparative reverse-phase HPLC (linear gradient from 80/20 A/B eluents to 5/95 A/B eluents in 30 min, keeping this composition for 10 more min). ^{1}H NMR (400 MHz, CD_3OD) δ 4.24 (t, *J* = 5.5 Hz, 1H), 4.20 (dd, *J* = 8.4, 5.1 Hz, 1H), 3.74 (t, *J* = 6.2 Hz, 1H), 3.37 (ddd, *J* = 15.3, 9.2, 5.6 Hz, 2H), 2.93 (t, *J* = 6.5 Hz, 4H), 2.70 (s, 4H), 2.28 (s, 6H), 2.22 (s, 6H), 1.79 (s, 6H), 1.60 (m, 3H), 1.40 (m, 5H), 1.16 (s, 12H), 0.88 (s, 9H). MS (ESI) m/z calculated $C_{45}H_{74}N_{11}O_{10}S_2$ $[M+H]^+$: 992.506; observed: 992.40.

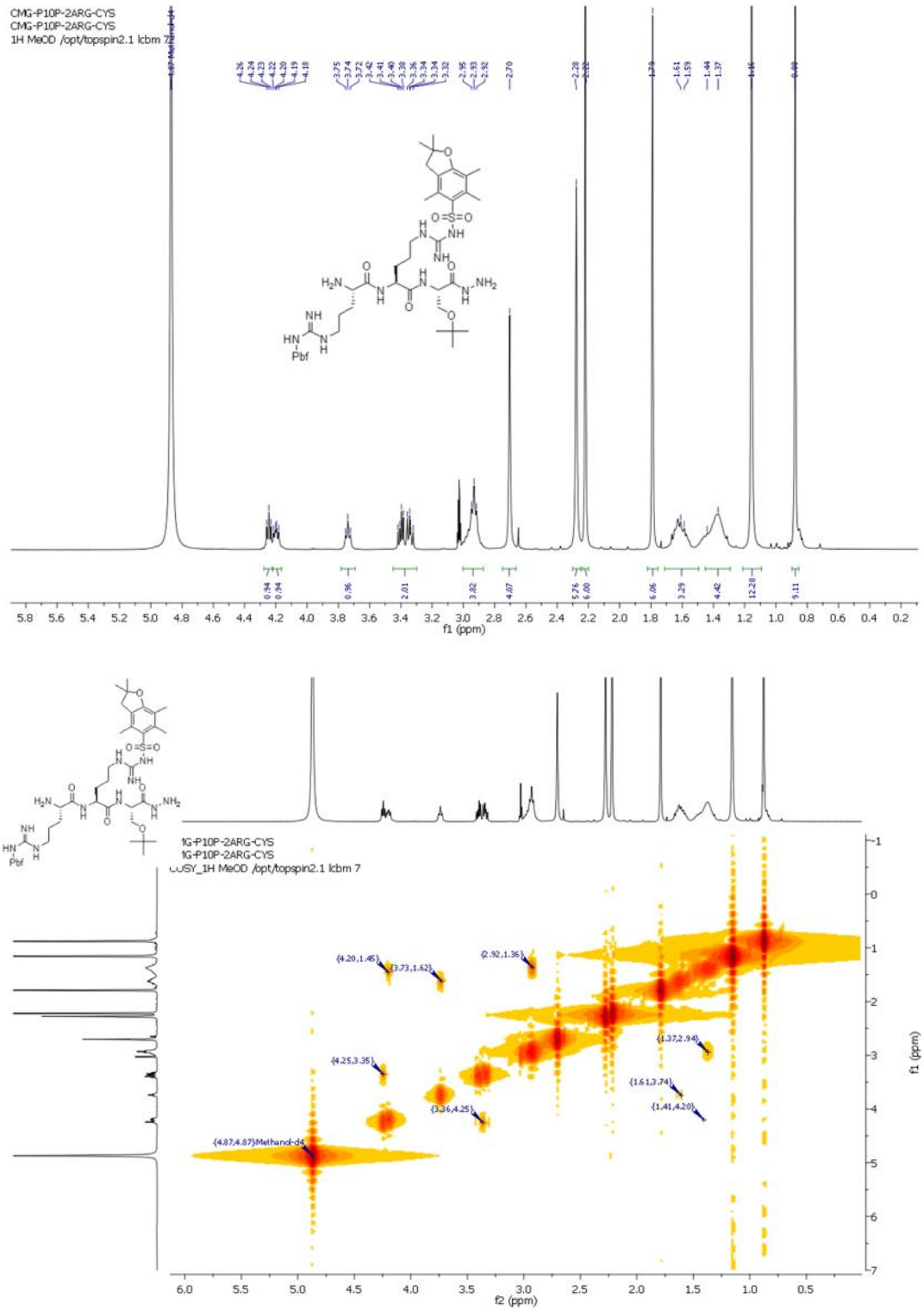


Figure S23: ^{1}H NMR (top) and COSY NMR (bottom) spectra of **$Arg_2(Pbf)_2Ser(^tBu)$-Hyd**.

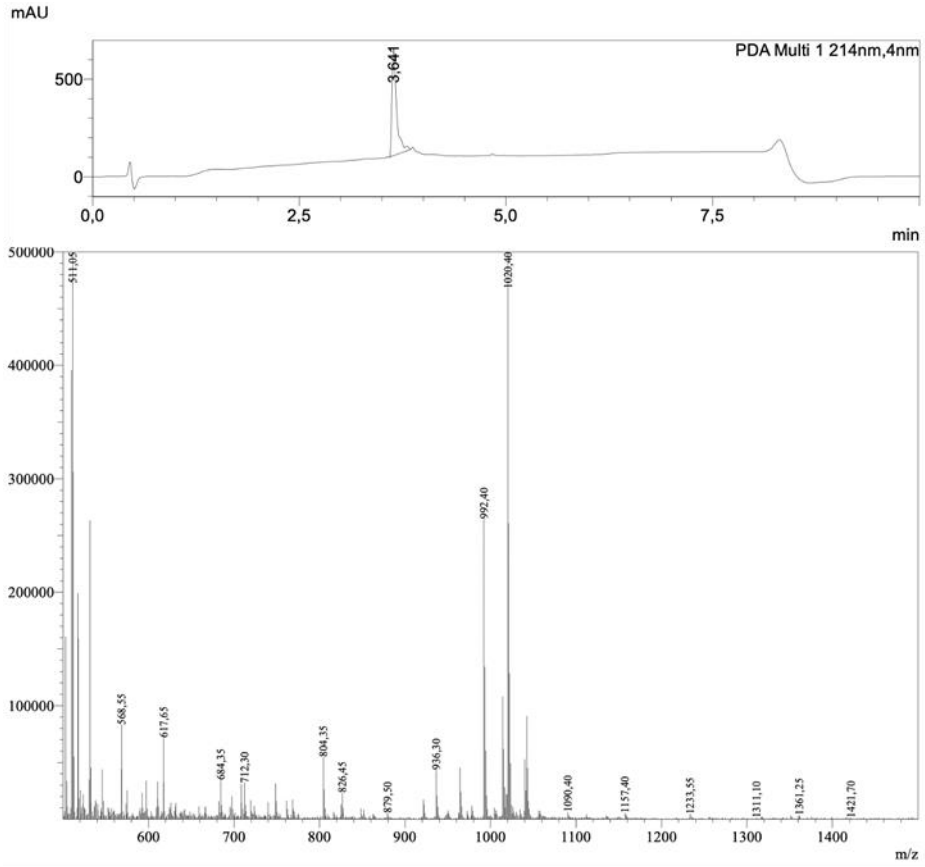


Figure S24: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **$Arg_2(Pbf)_2Ser(^tBu)$-Hyd**.

Arg$_2$Ser-Hyd: ^{1}H NMR (400 MHz, D_2O) δ 4.49 (br, 1H), 4.39 (br, 1H), 4.04 (br, 1H), 3.86 (br, 2H), 3.19 (br, 4H), 1.87 (br, 4H), 1.64 (br, 4H). MS (ESI) m/z calculated $C_{15}H_{33}N_{11}O_4$ $[M+H]^+$: 432.278; observed: 432.20.

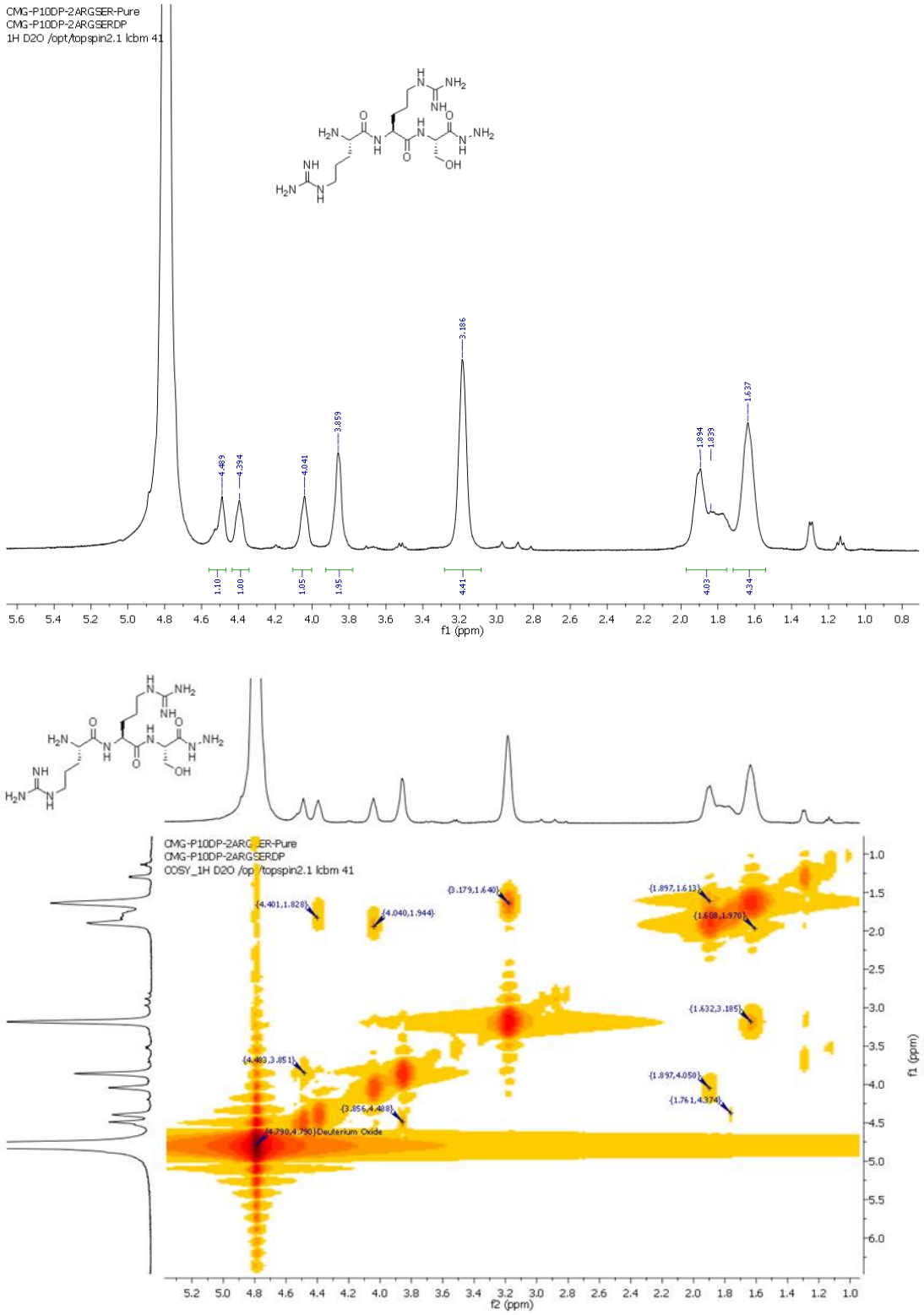


Figure S25: ^{1}H NMR (top) and COSY NMR (bottom) spectra of **Arg$_2$Ser-Hyd**.

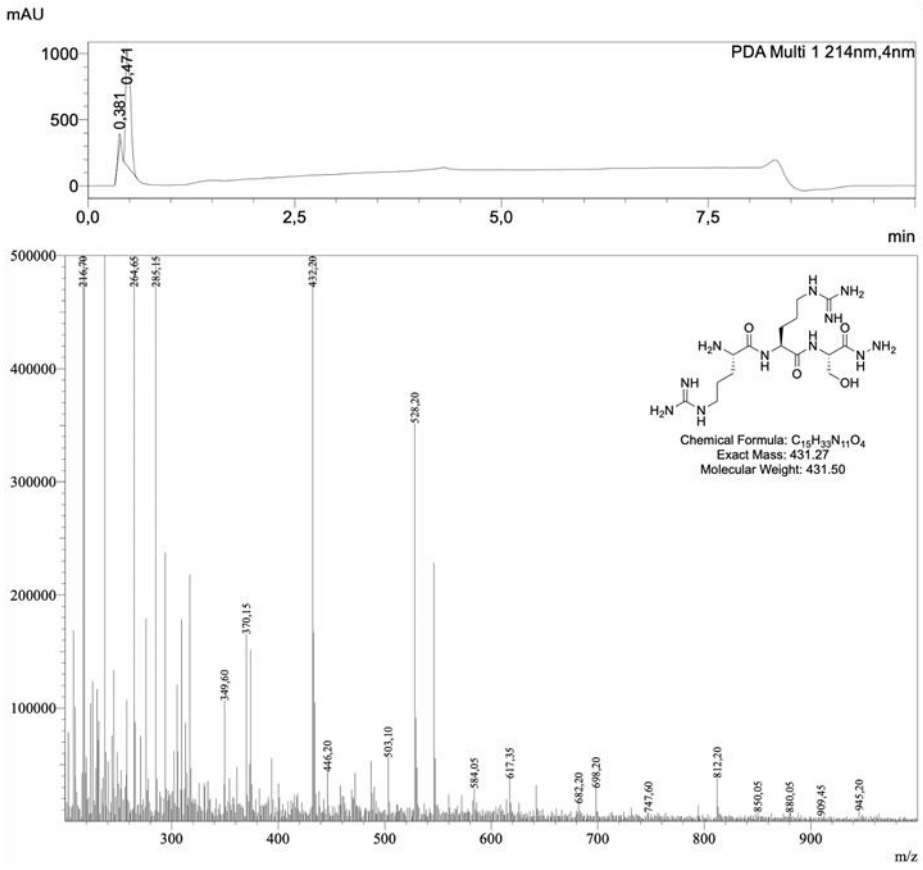


Figure S26: HPLC chromatogram (top) and mass spectrometry analysis (bottom) of **Arg_2Ser-Hyd**.

3. Characterization of TPP conjugates and cages

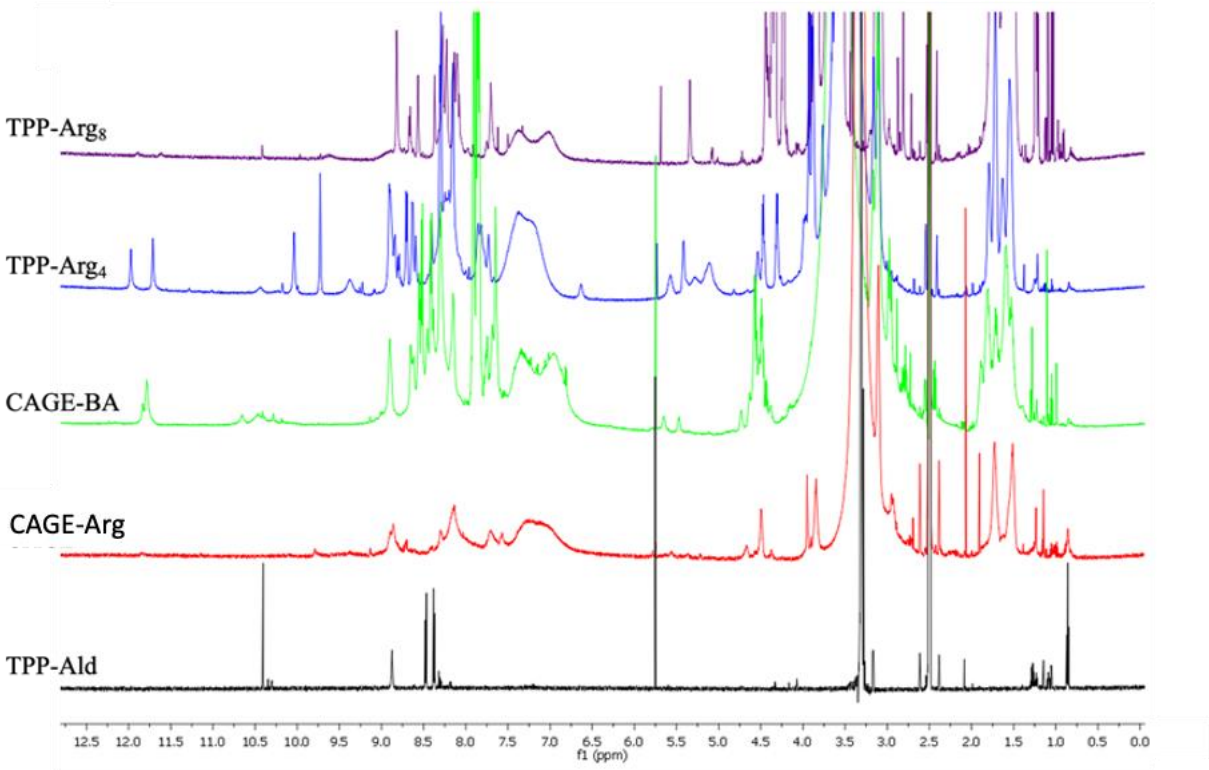


Figure S27: Representative ^{1}H NMR spectra of TPP Conjugates and TPP Cages in DMSO-d_6.

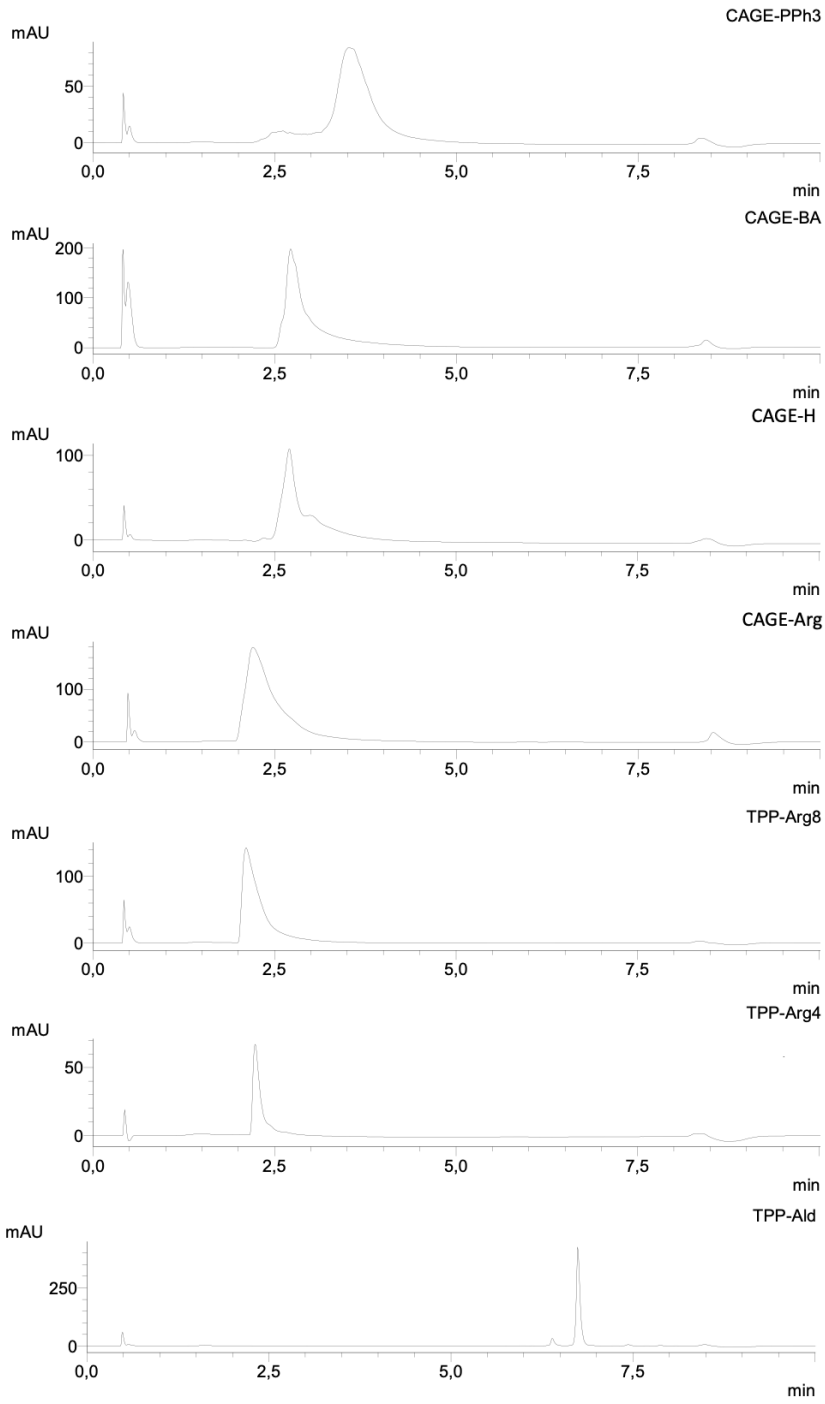


Figure S28: Representative HPLC chromatograms of TPP Conjugates and TPP Cages.

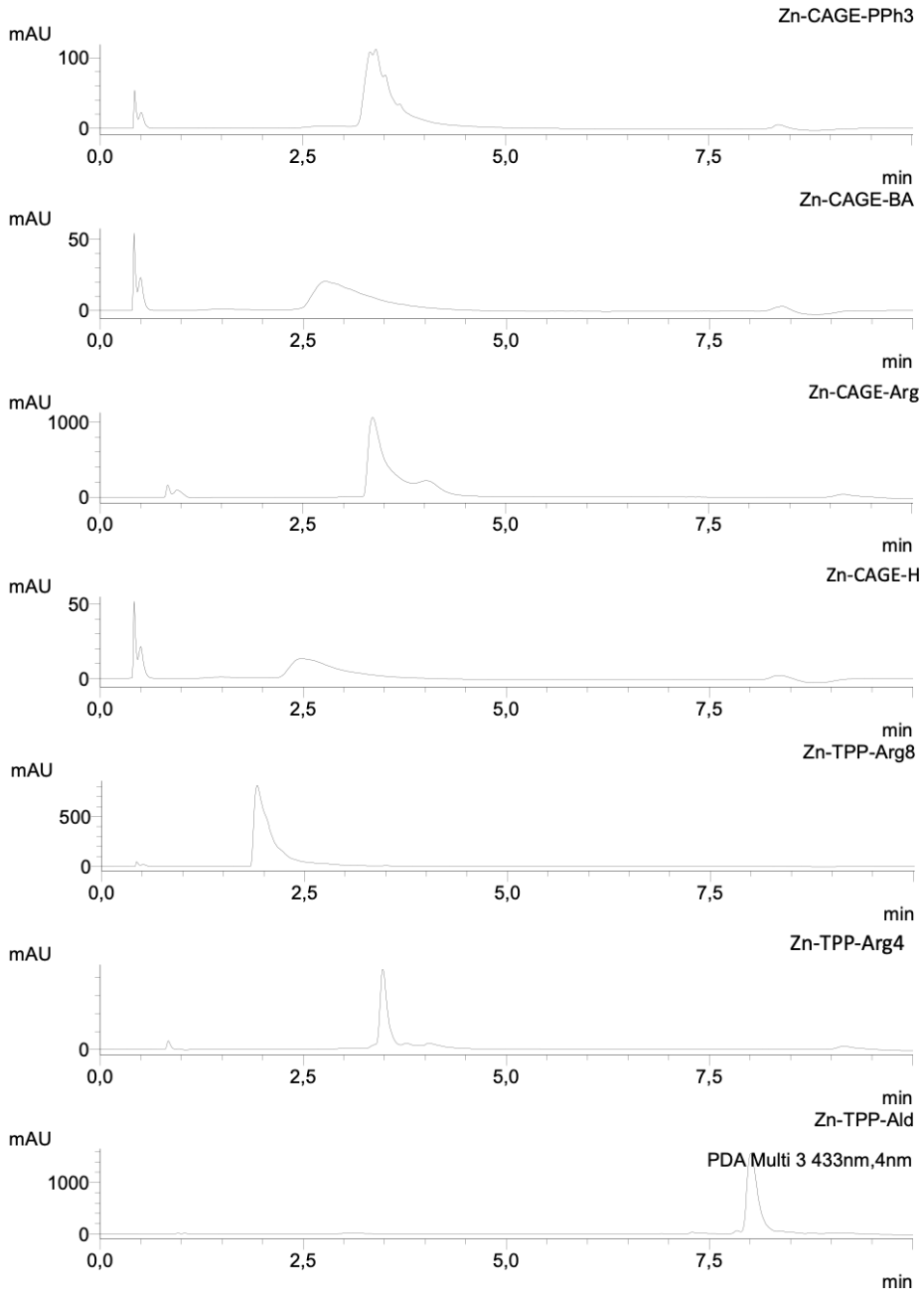


Figure S29: Representative HPLC chromatograms of Zn-TPP Conjugates and Zn-TPP Cages.

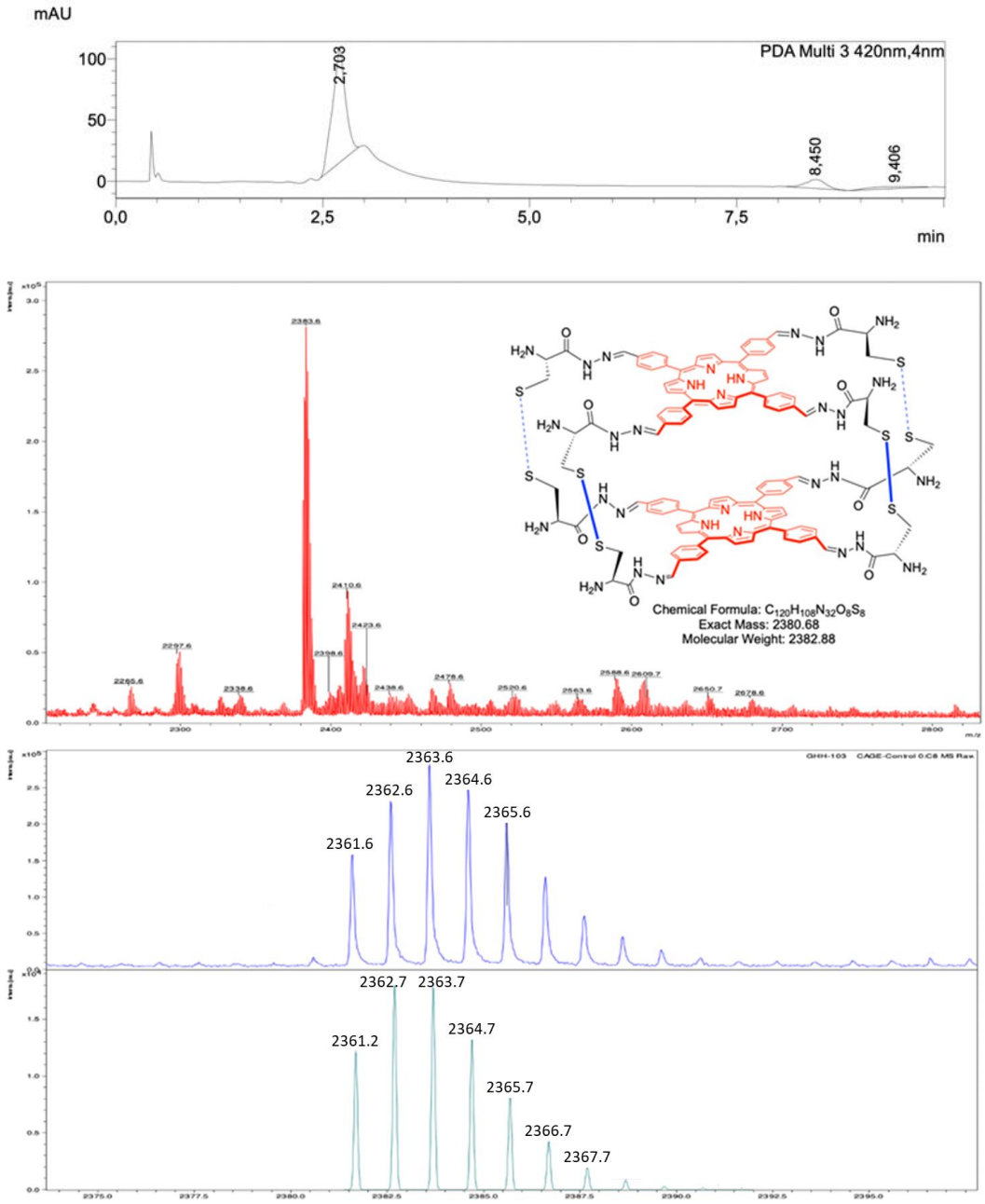


Figure S30: HPLC chromatogram (top) and MALDI-TOF mass spectrometry analysis (bottom) of **CAGE-H**.

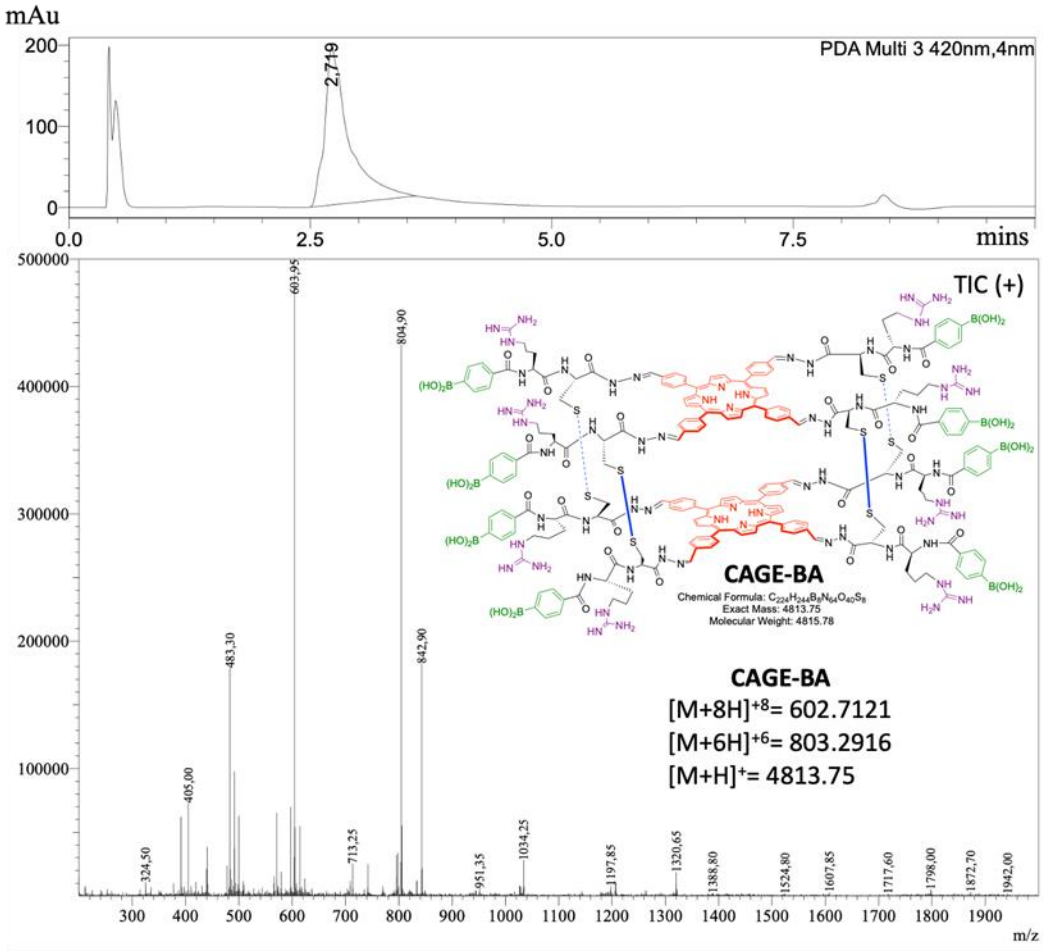


Figure S31: HPLC chromatogram (top) and MALDI-TOF mass spectrometry analysis (bottom) of **CAGE-BA**.

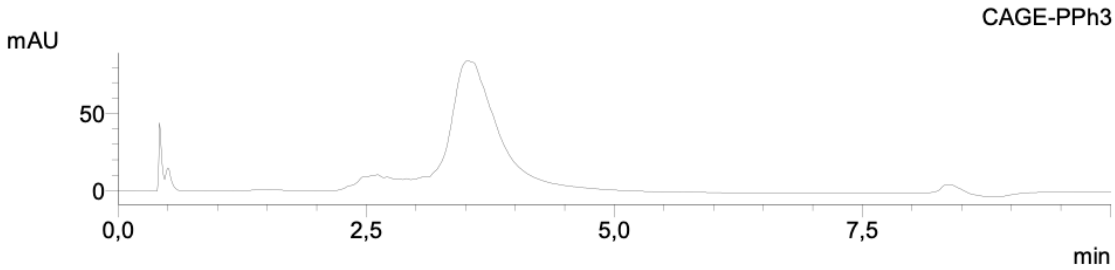


Figure S32: HPLC chromatogram of **CAGE-PPh$_3$**.

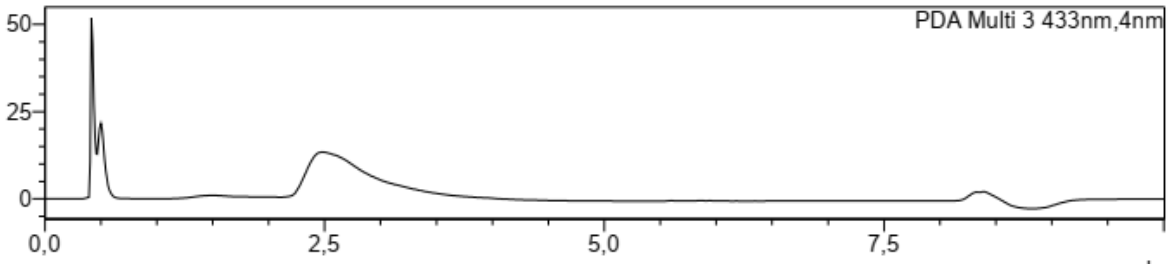


Figure S33: HPLC chromatogram of **Zn-CAGE-H**.

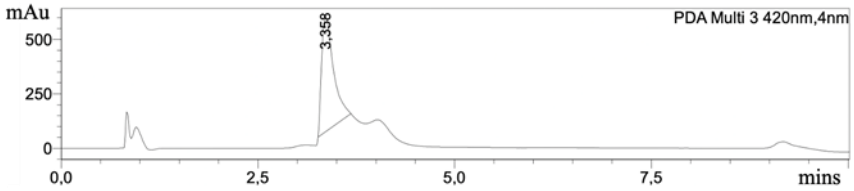


Figure S34: HPLC chromatogram analysis of **Zn-CAGE-Arg**.

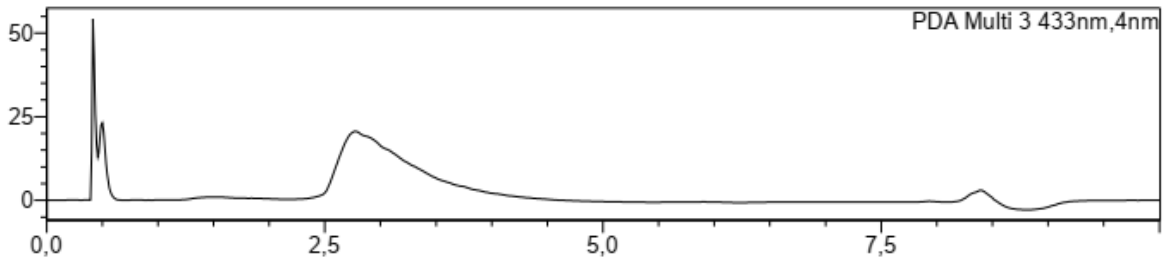


Figure S35: HPLC chromatogram of **Zn-CAGE-BA**.

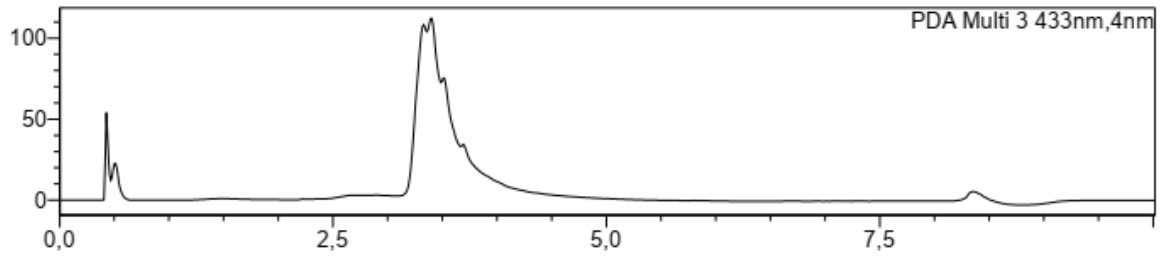


Figure S36: HPLC chromatogram of **Zn-CAGE-PPh$_3$**.

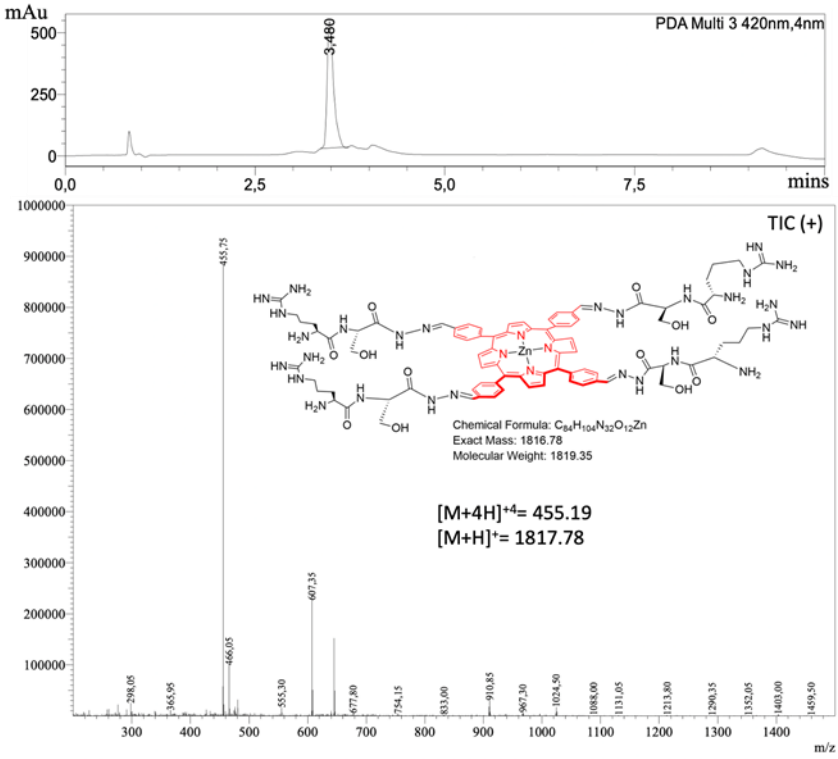


Figure S37: HPLC chromatogram (top) and MALDI-TOF mass spectrometry analysis (bottom) of **Zn-TPP-Arg$_4$**.

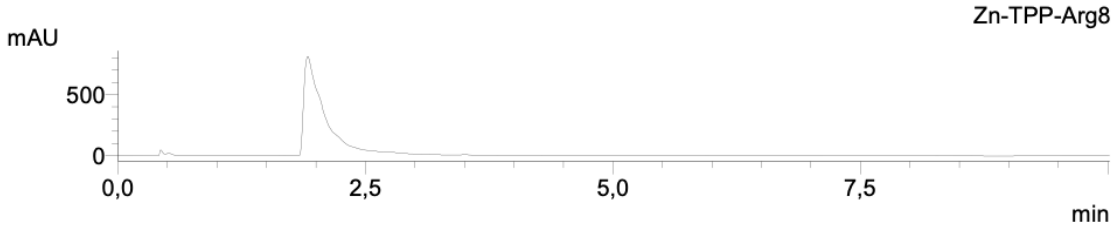


Figure S38: HPLC chromatogram of **Zn-TPP-Arg$_8$**.

3. DOSY NMR

Entry	*Compounds*	*log(D/m²s⁻¹)*	*D (m²s⁻¹)*	*R_{hyd} [Å]*	*V_{sph} [Å³]*
1.	***TPP-Ald***	*-9.64*	*2.239×10^{-10}*	*4.9*	*492.5*
2.	***CAGE-H***	*-10.23*	*9.484×10^{-11}*	*11.5*	*6367.39*
3.	***TPP-Arg₄***	*-10.167*	*6.807×10^{-11}*	*16.1*	*17472.1*
4.	***TPP-Arg₈***	*-10.336*	*4.613×10^{-11}*	*23.7*	*52279.61*
5.	***Zn-CAGE-H***	*-9.82*	*1.513×10^{-10}*	*7.2*	*1560.17*
6.	***Zn-TPP-Arg₄***	*-9.897*	*1.267×10^{-10}*	*8.6*	*2681.57*
7.	***Zn-TPP-Arg₈***	*-10.07*	*8.511×10^{-11}*	*12.8*	*8766.09*

Table S1: Hydrodynamic radii and spherical volumes calculated using the Stokes-Einstein equation.

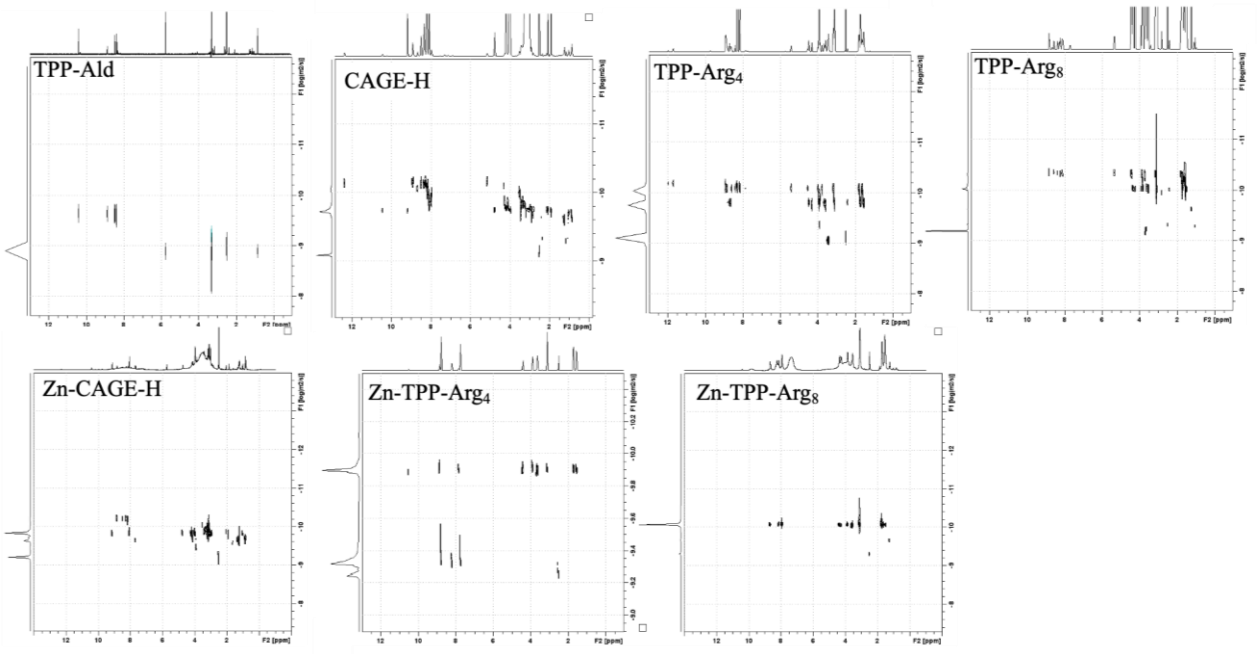


Figure S39: DOSY NMR spectra.

4. UV and Fluorescence analyses

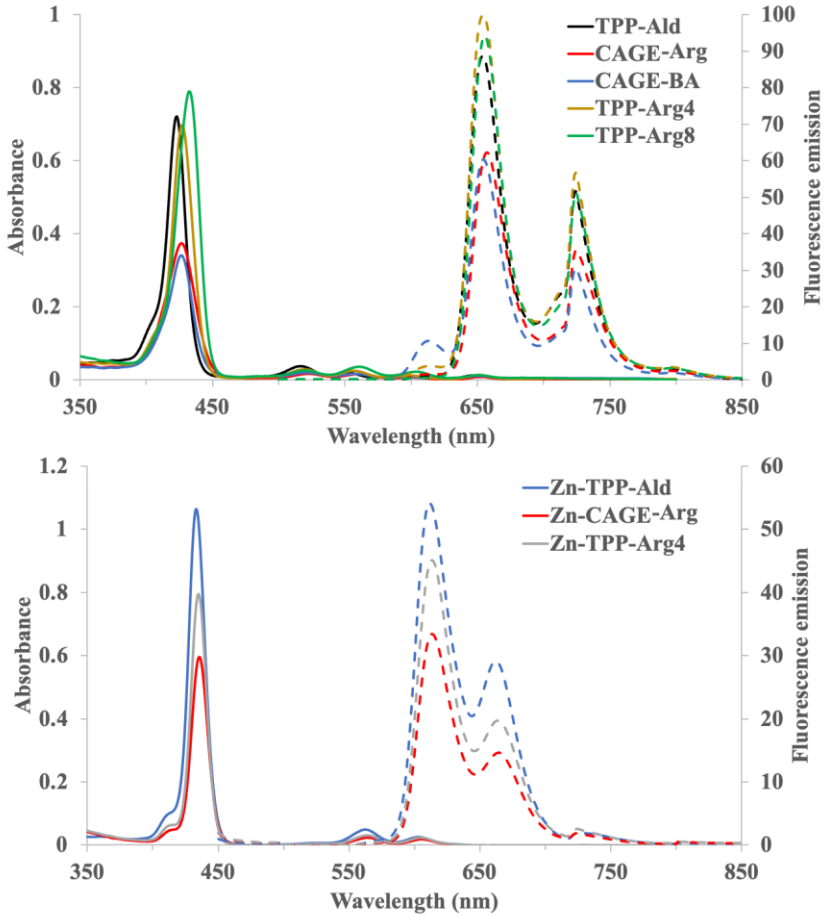


Figure S40: UV-Vis absorption (solid lines) and fluorescence (dashed lines) spectra in DMSO of representative free base (M=2H) (top) and metallated (M=Zn) (bottom) porphyrin derivatives.

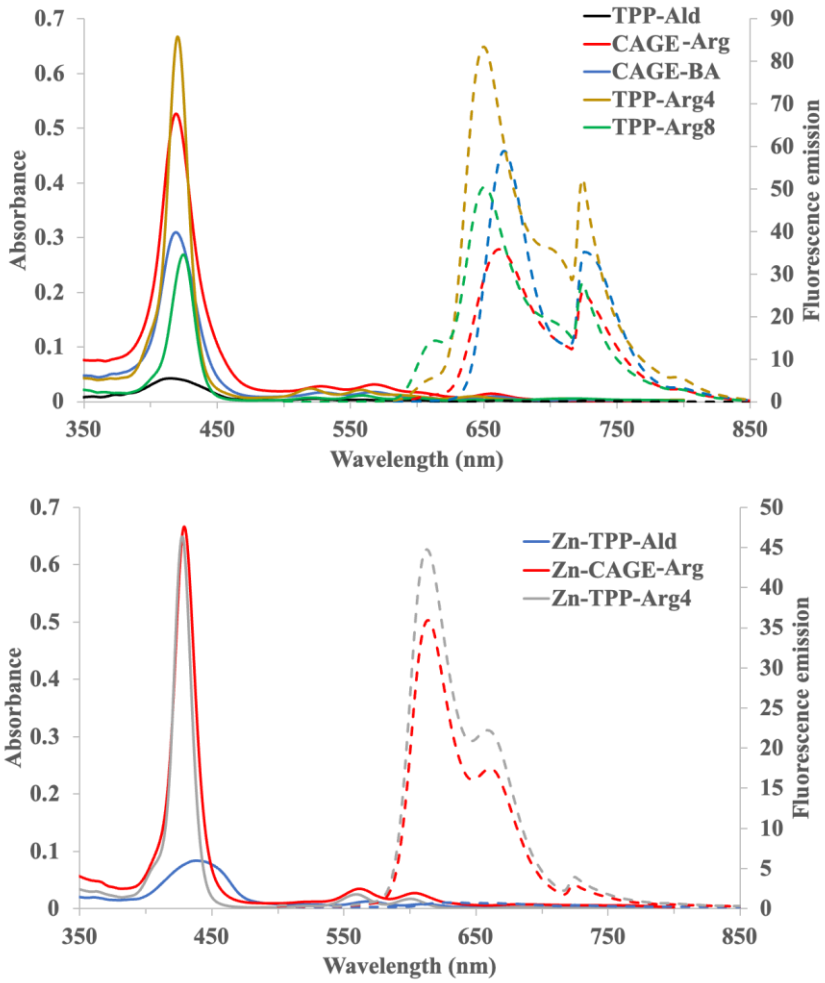


Figure S41: UV-Vis absorption (solid lines) and fluorescence (dashed lines) spectra in H_2O of representative free base (M=2H) (top) and metallated (M=Zn) (bottom) porphyrin derivatives .

5. Singlet oxygen generation

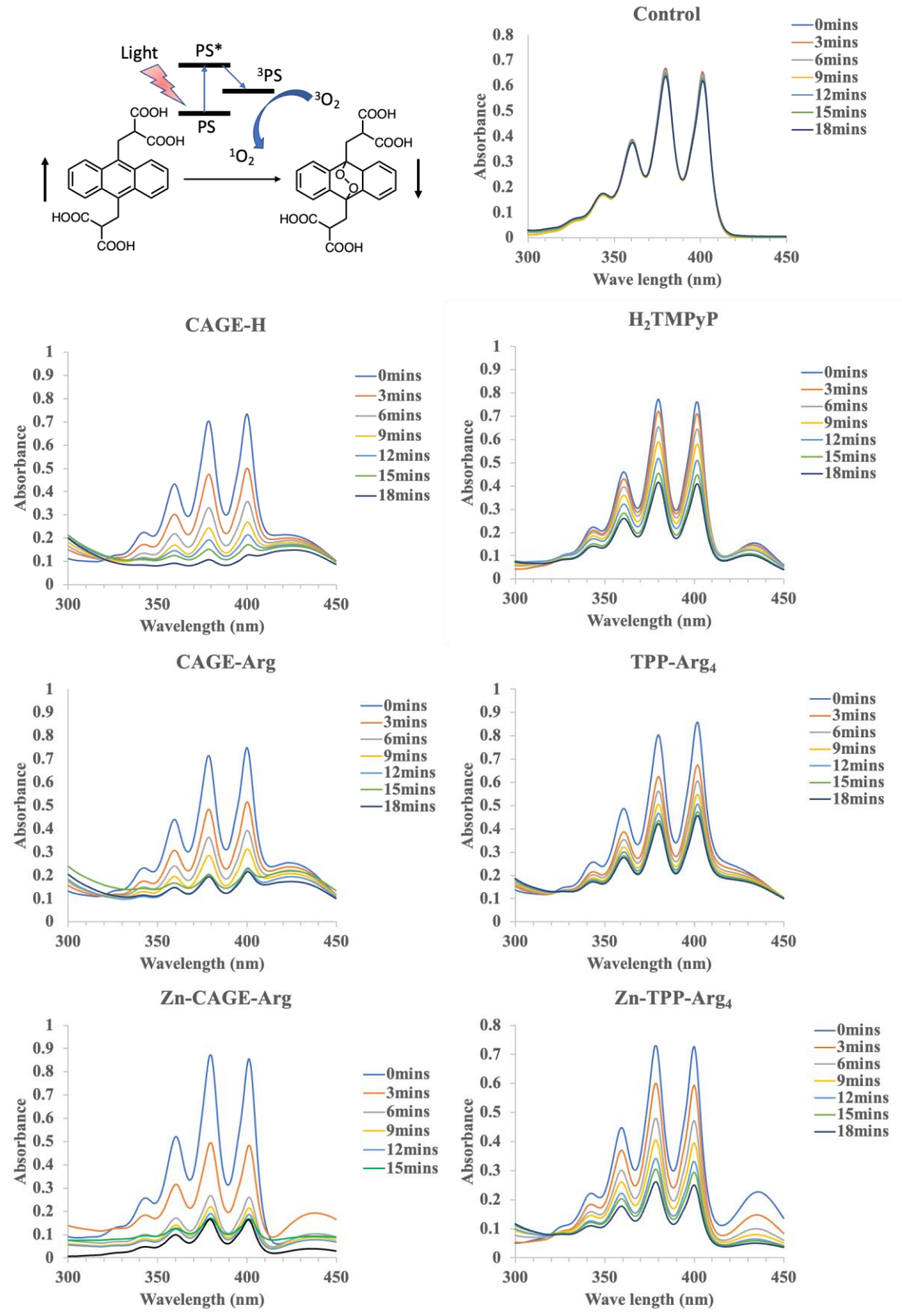


Figure S42: Singlet oxygen generation probed by UV absorption spectroscopy using ABDA as a probe at varying light irradiation times (green light, λ = 525 nm).

6. PDT on cells

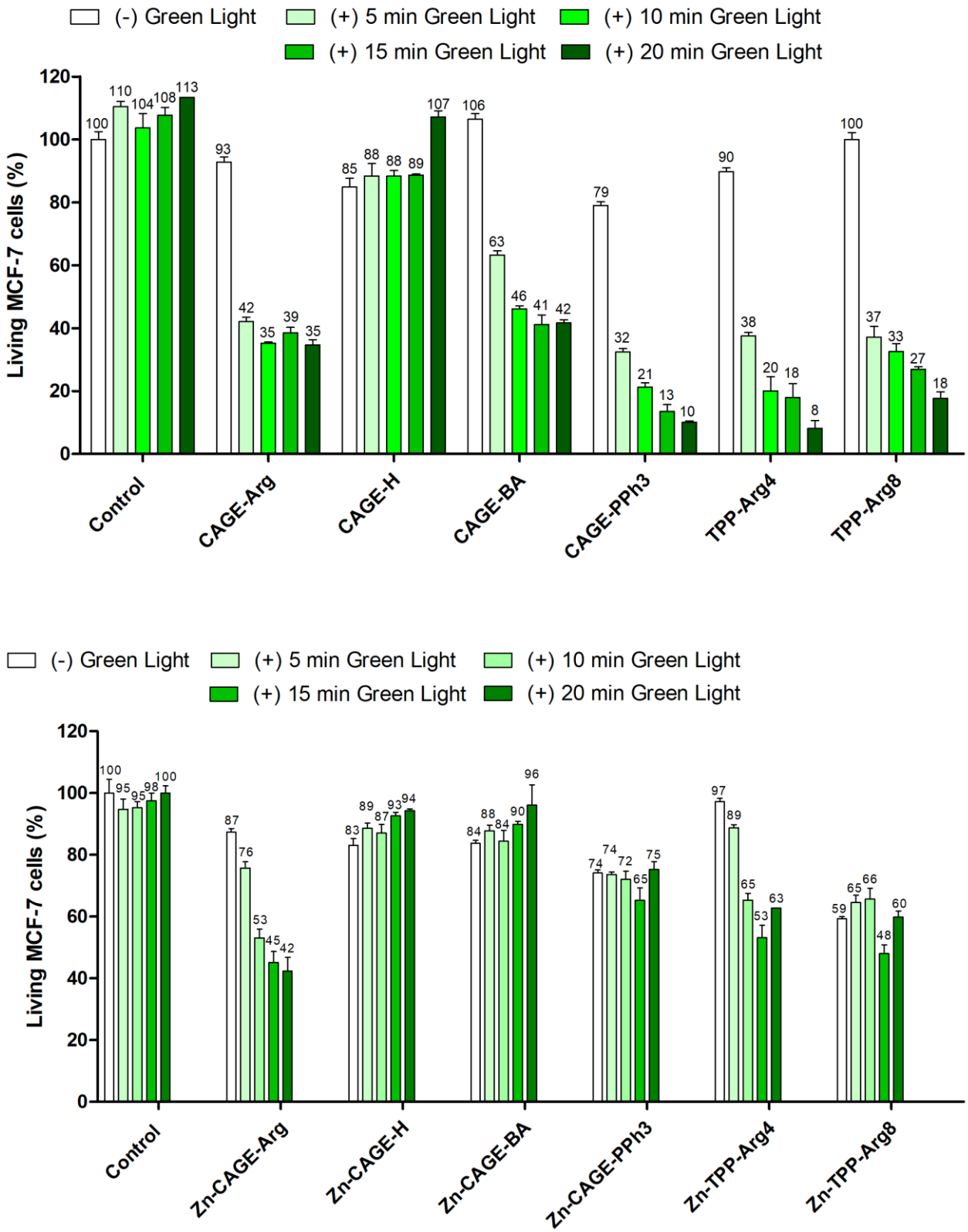


Figure S43: PDT study in human breast cancer (MCF-7) cells incubated with 1.31 µM of compounds for 24 h then exposed to green light for different irradiation times. Data are presented as mean ± SEM.

7. ROS production in cells

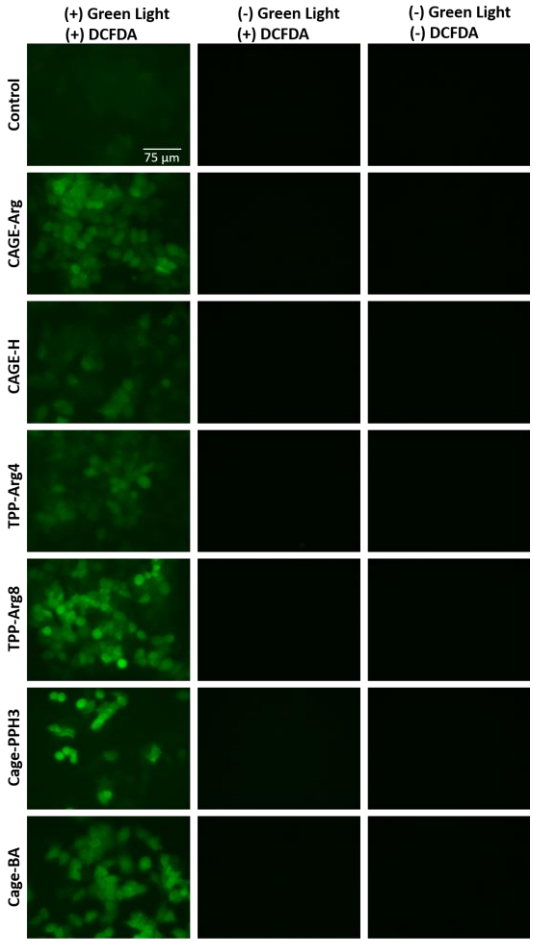


Figure S44: Fluorescence microscopy imaging of ROS using DCFDA assay in MCF-7 cells treated (or not) with 1.31 µM of compounds for 24 h, then, exposed (or not) to green light irradiation for 10 min.

8. Cellular uptake kinetics

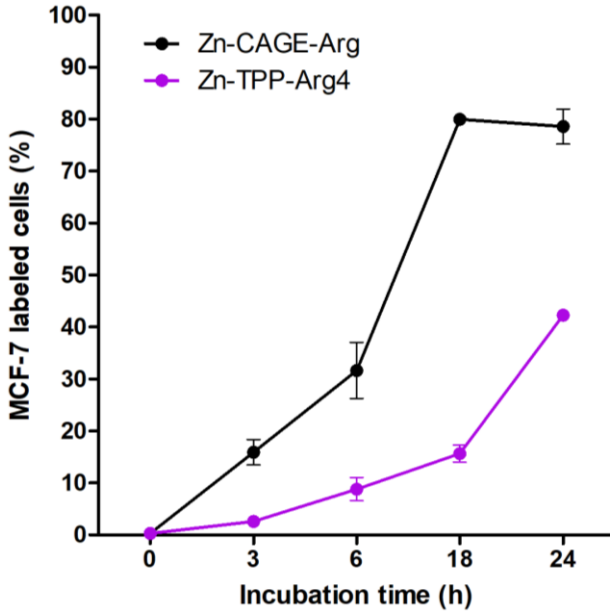


Figure S45: Uptake kinetics study of compounds incubated with human breast cancer (MCF-7) cells at 1.31 µM concentration for different time intervals. Data are presented as mean ± SEM.