Supporting Information

Phthalocyanine-Peptide Conjugates for Epidermal Growth Factor Receptor Targeting

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Subcellular localization in A431 cells

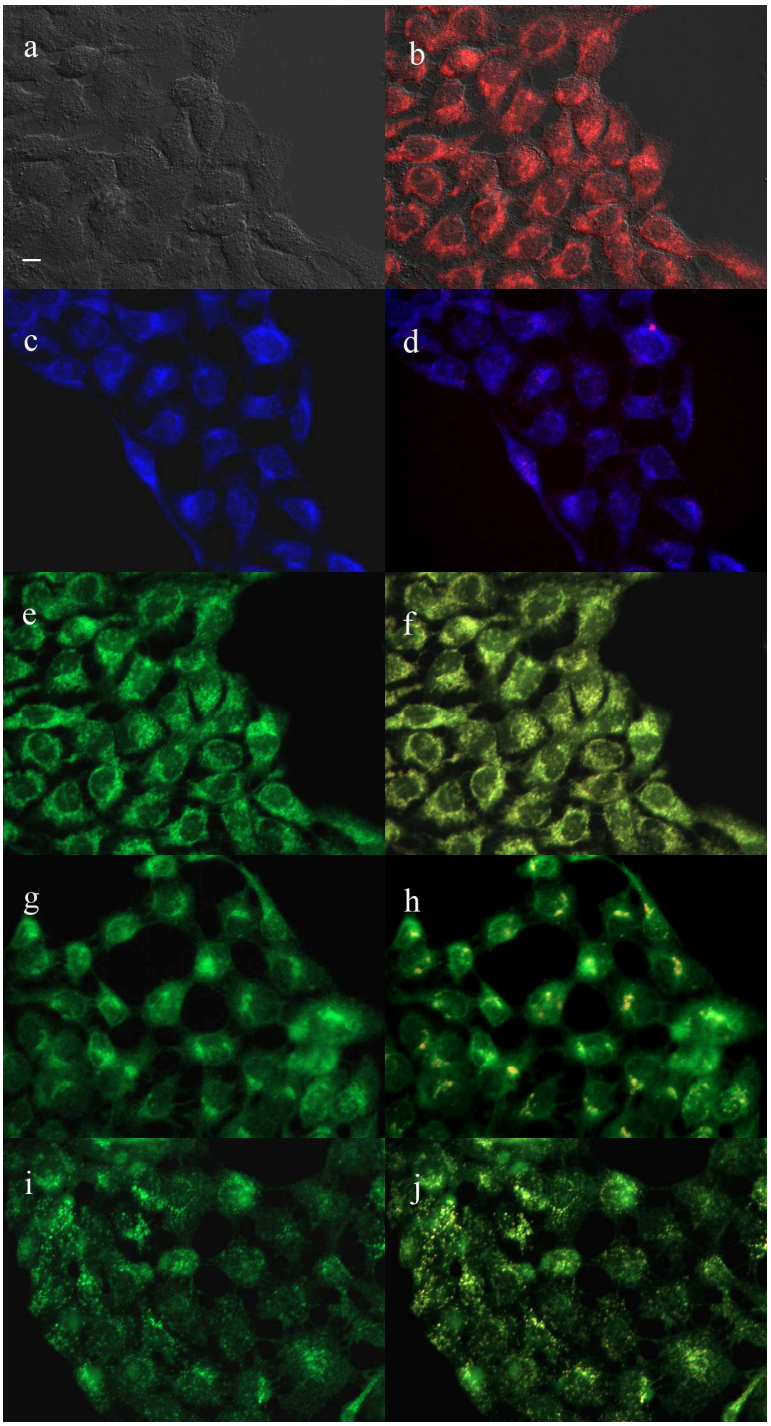


Figure S1. Subcellular localization of Pc **3a** in A431 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **3a** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **3a** fluorescence. Scale bar: 10 μm.

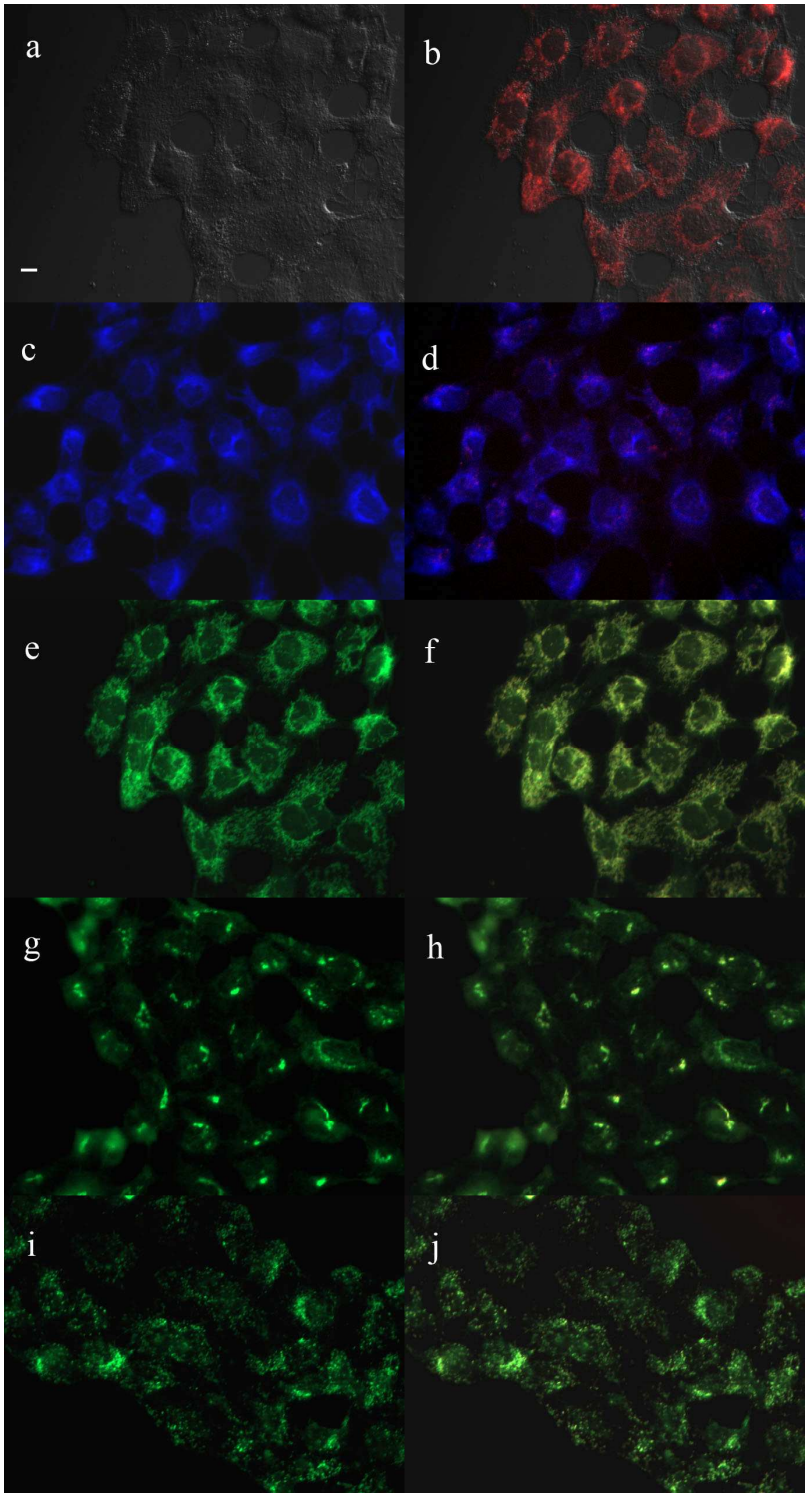


Figure S2. Subcellular localization of Pc **4b** in A431 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **4b** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **4b** fluorescence. Scale bar: 10 μm.

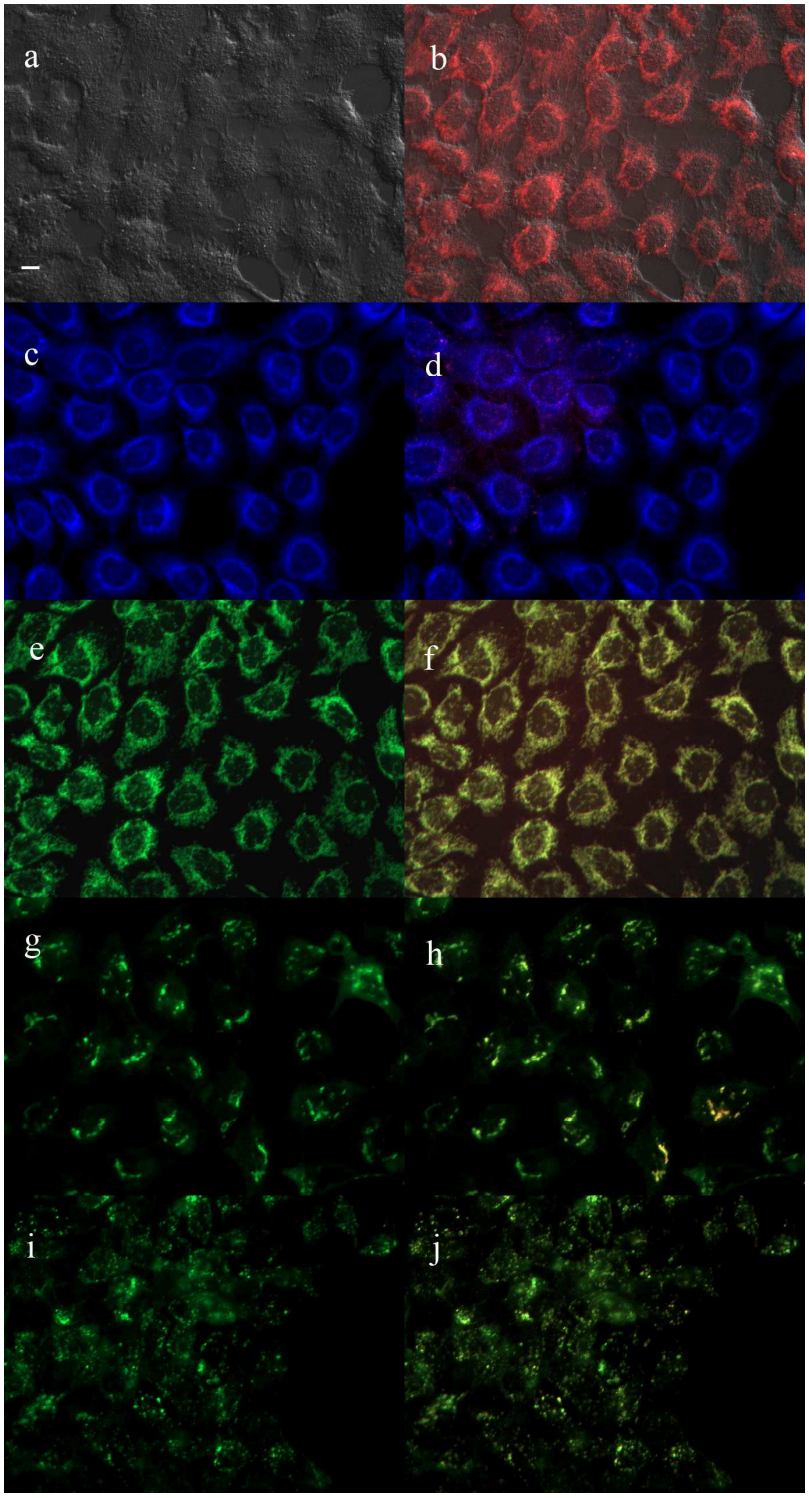


Figure S3. Subcellular localization of Pc **6a** in A431 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **6a** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **6a** fluorescence. Scale bar: 10 μm.

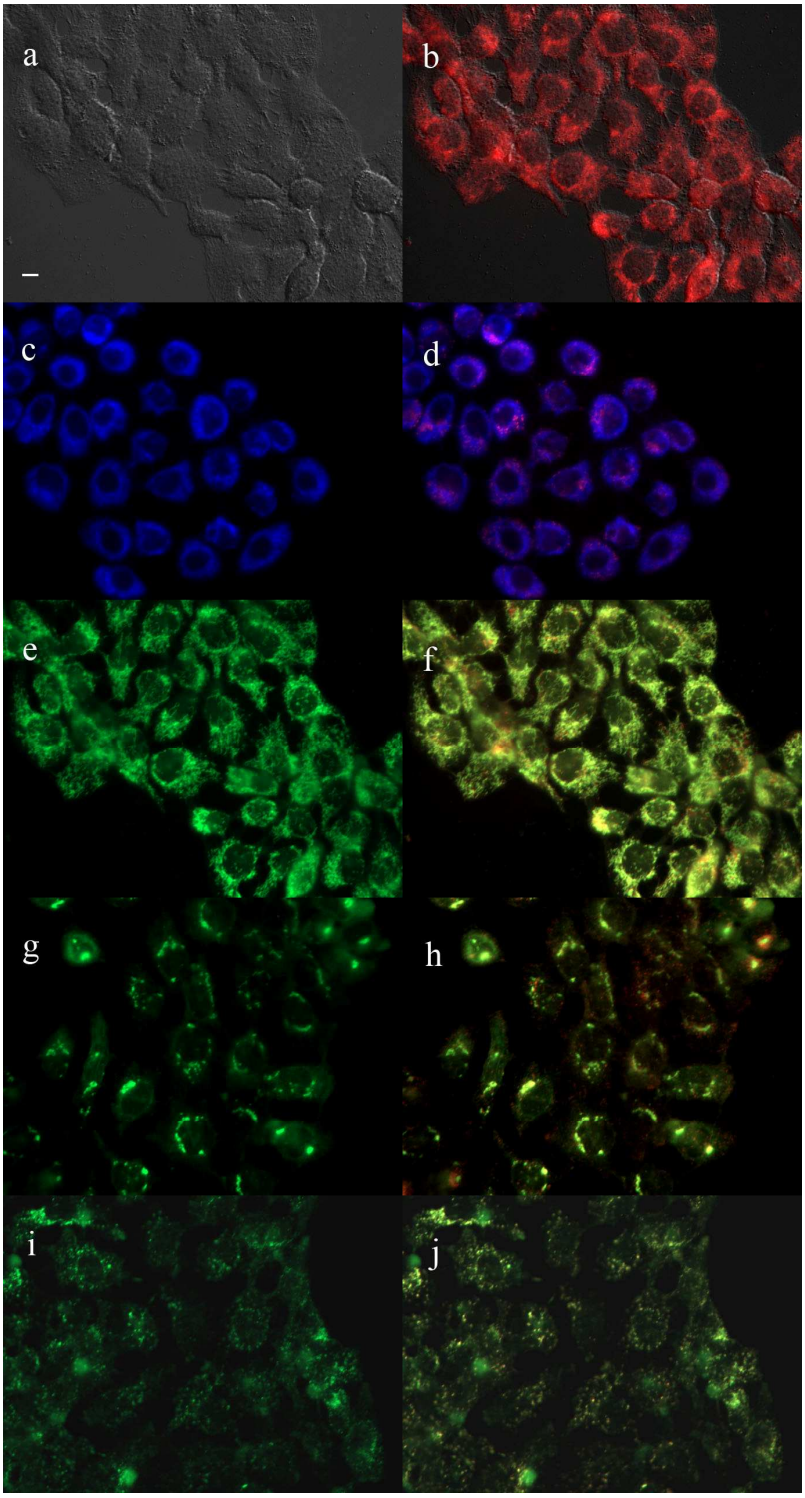


Figure S4. Subcellular localization of Pc **5a** in A431 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **5a** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **5a** fluorescence. Scale bar: 10 μm.

Subcellular localization in HEp2 cells

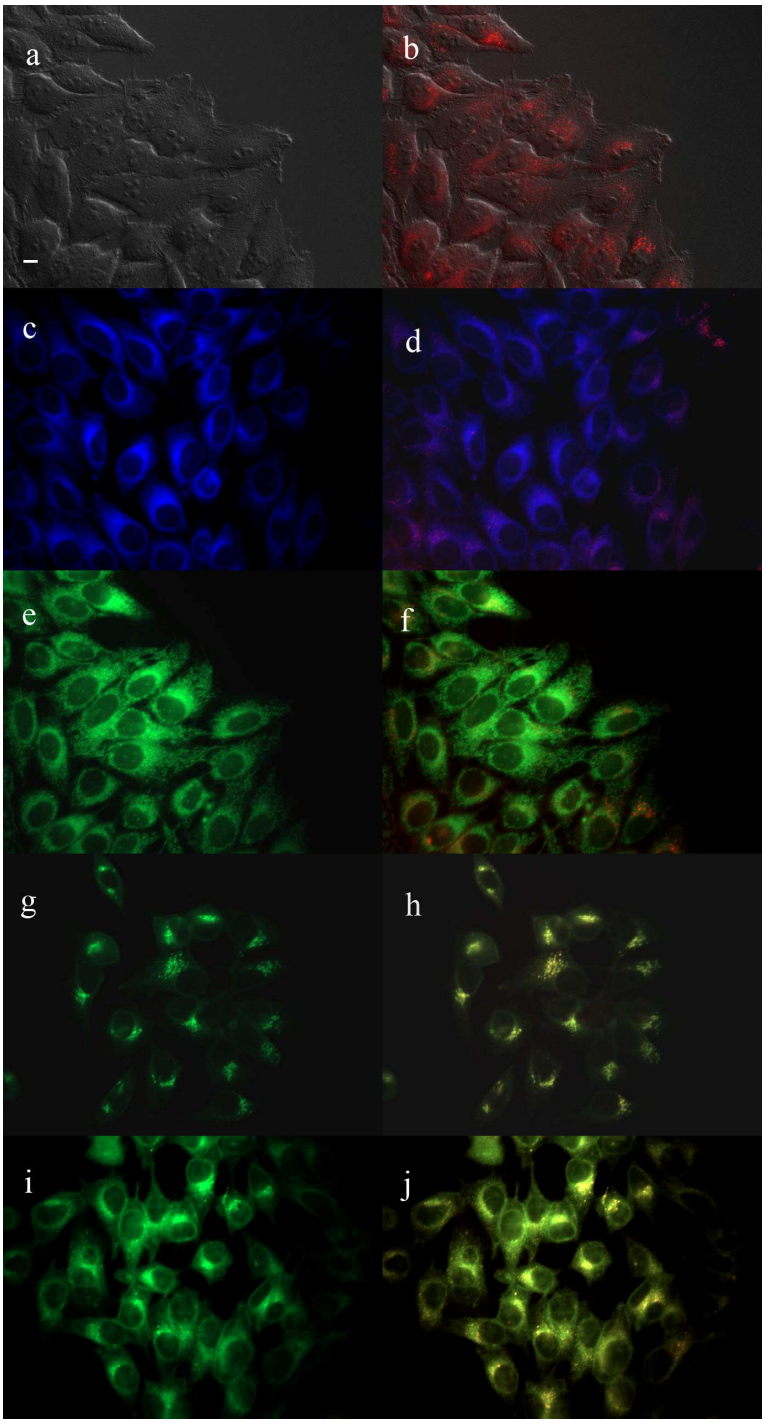


Figure S5. Subcellular localization of Pc **3a** in HEp2 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **3a** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **3a** fluorescence. Scale bar: 10 μm.

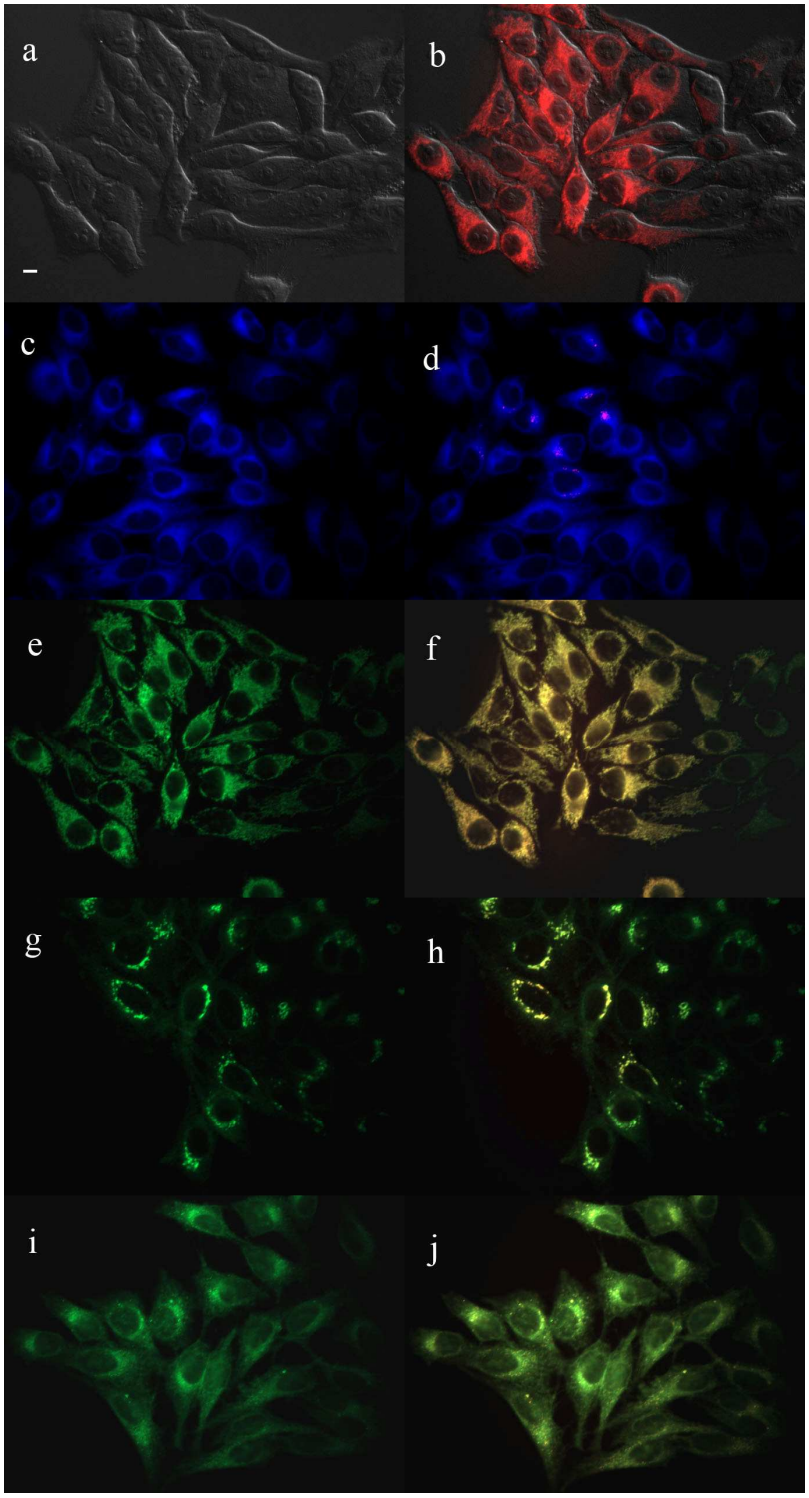


Figure S6. Subcellular localization of Pc **4b** in HEp2 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **4b** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **4b** fluorescence. Scale bar: 10 μm.

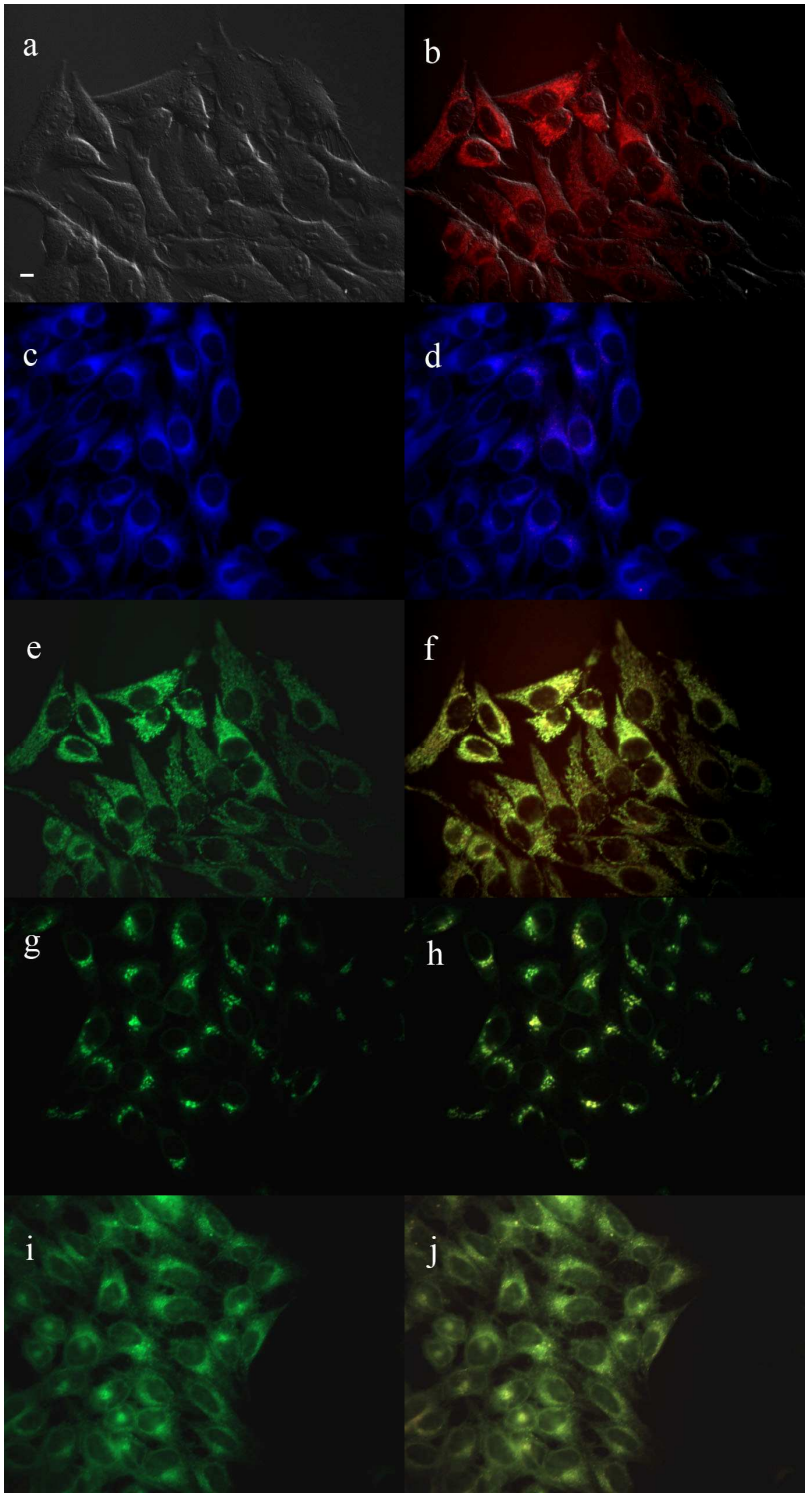


Figure S7. Subcellular localization of Pc **6a** in HEp2 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **6a** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **6a** fluorescence. Scale bar: 10 μm.

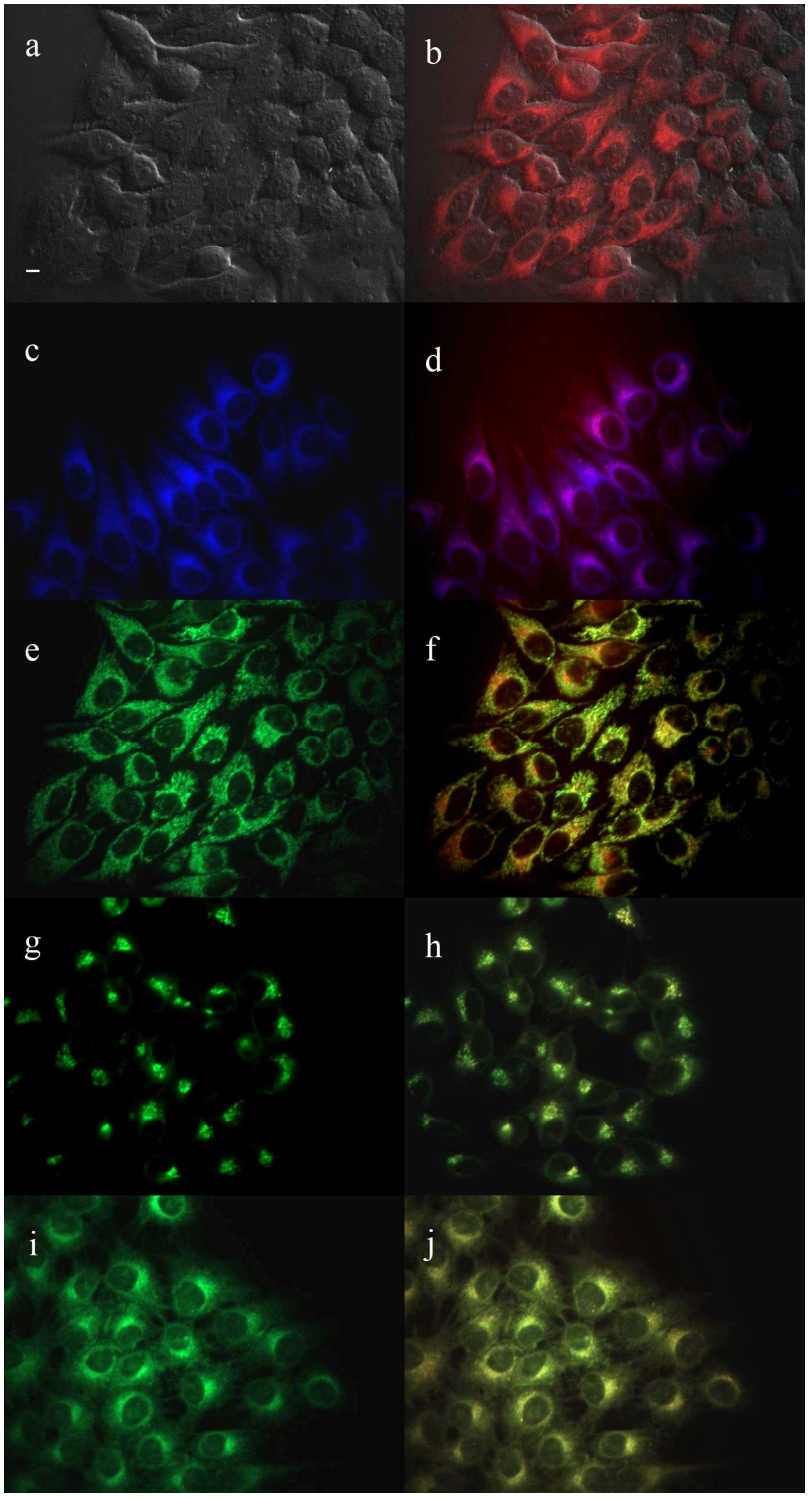


Figure S8. Subcellular localization of Pc **5a** in HEp2 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **5a** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **5a** fluorescence. Scale bar: 10 μm.

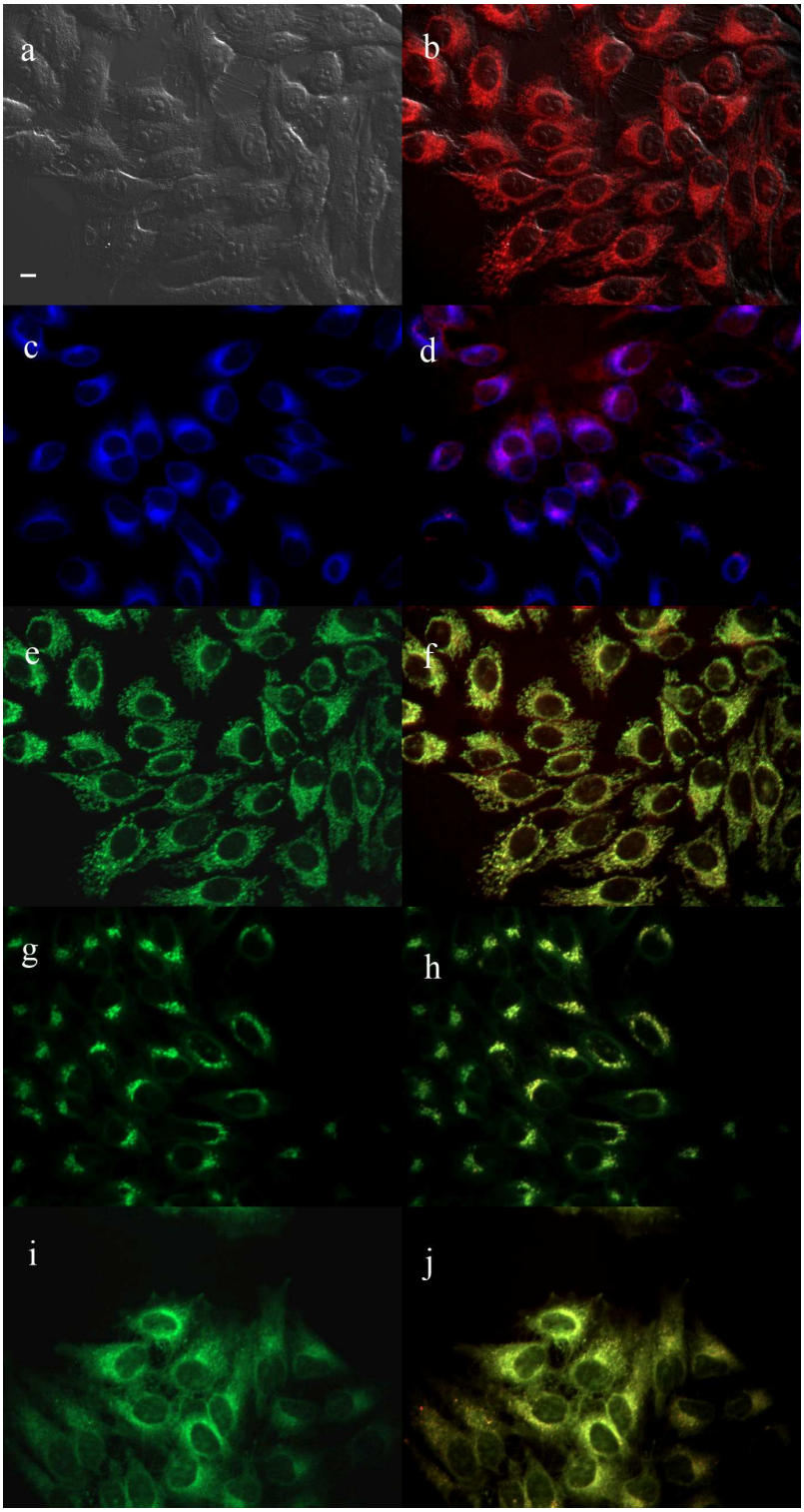


Figure S9. Subcellular localization of Pc **5b** in HEp2 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **5b** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **5b** fluorescence. Scale bar: 10 μm.

Subcellular localization in HT-29 cells

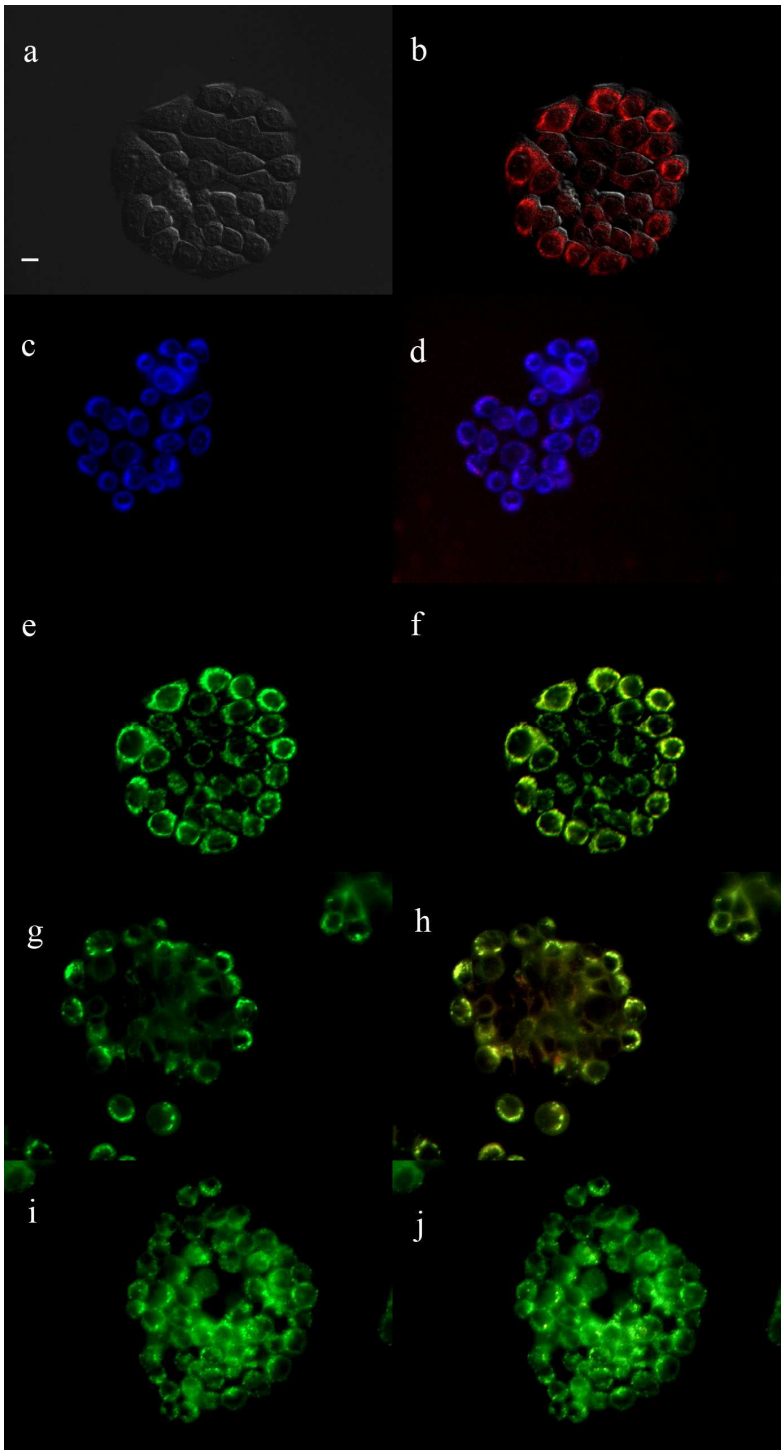


Figure S10. Subcellular localization of Pc **4b** in HT-29 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **4b** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **4b** fluorescence. Scale bar: 10 μm.

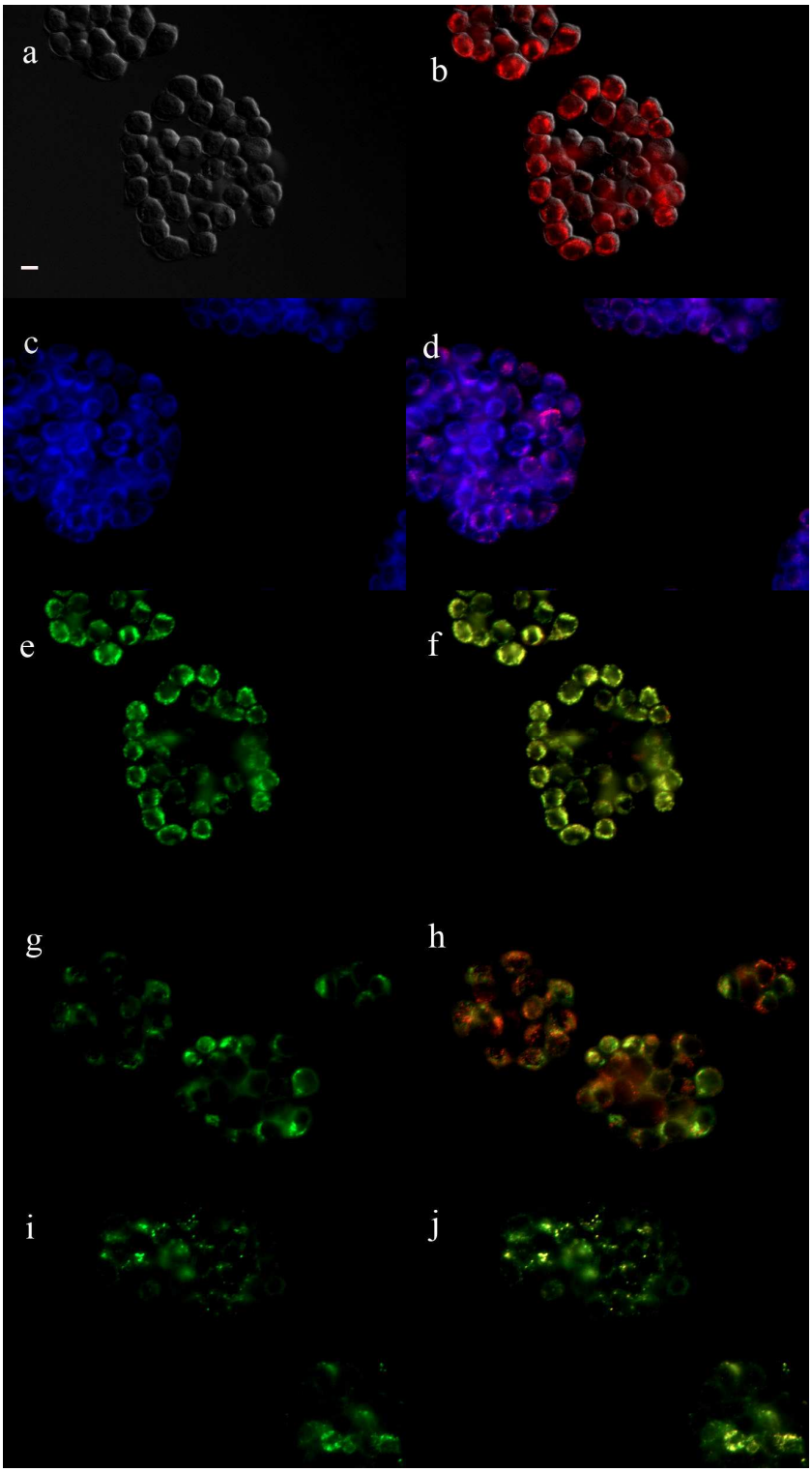


Figure S11. Subcellular localization of Pc **5a** in HT-29 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **5a** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **5a** fluorescence. Scale bar: 10 μm.

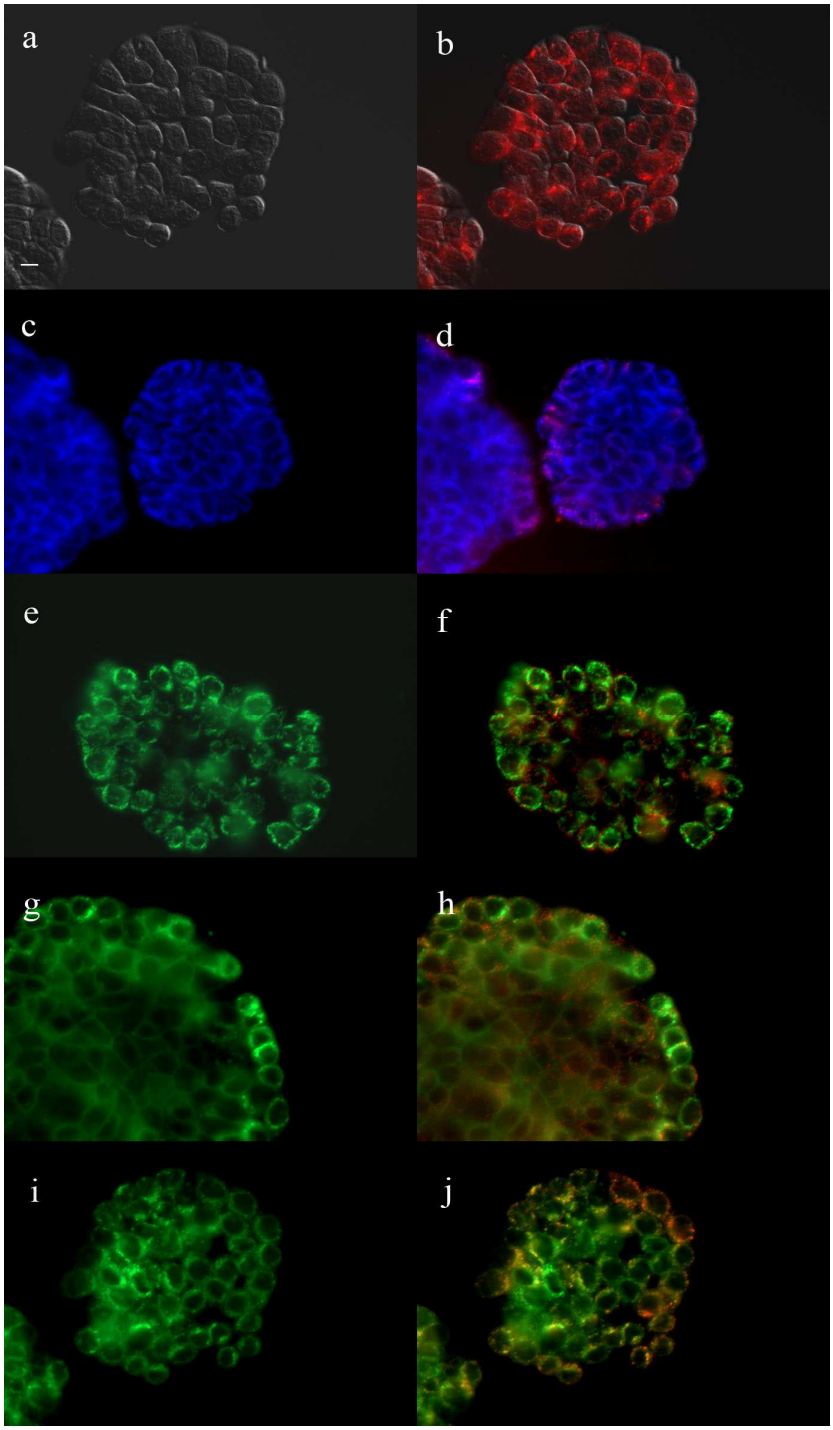


Figure S12. Subcellular localization of Pc **3a** in HT-29 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **3a** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **3a** fluorescence. Scale bar: 10 μm.

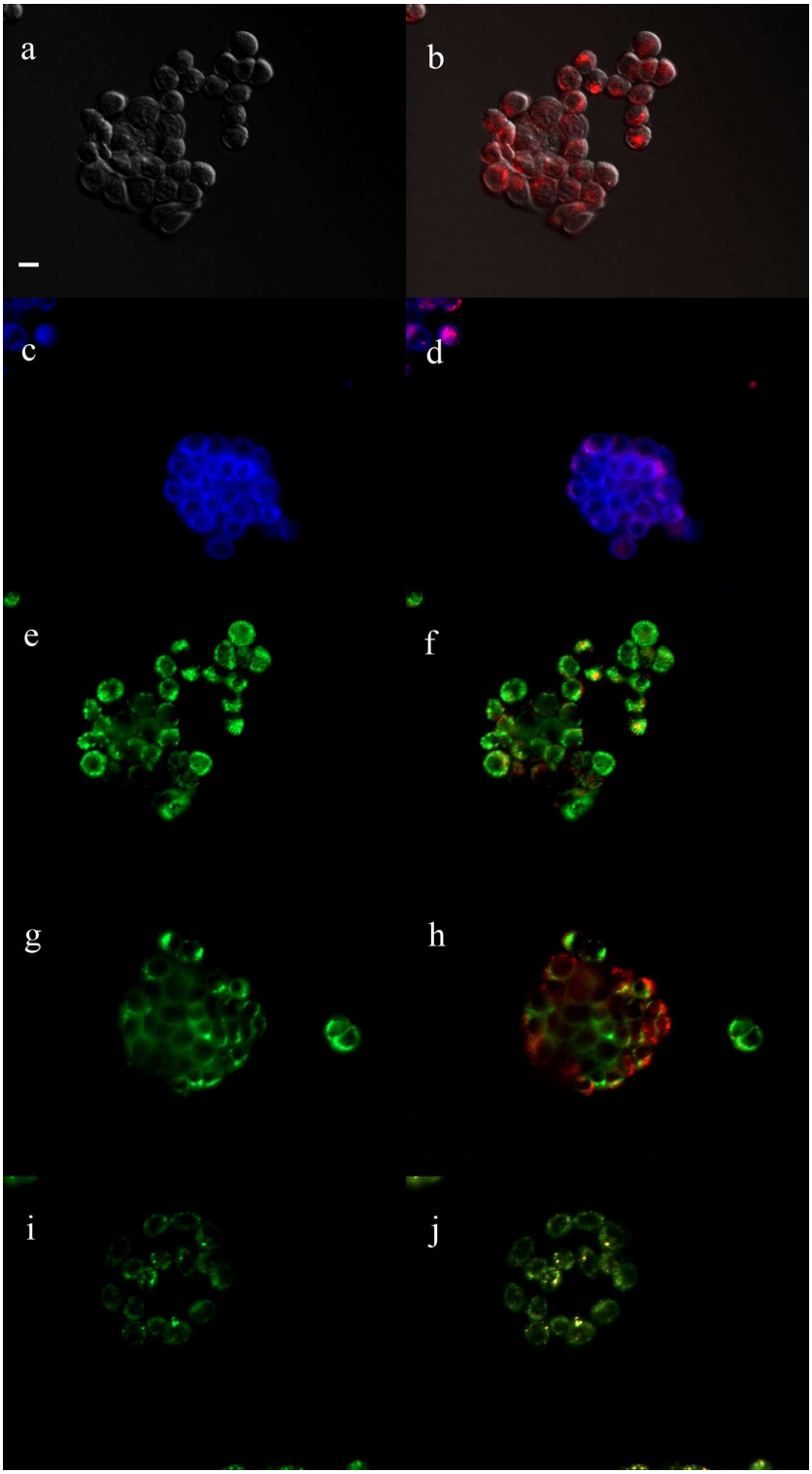


Figure S13. Subcellular localization of Pc **6a** in HT-29 cells at 10 μM for 6 h. (a) Phase contrast, (b) Overlay of **6a** fluorescence and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTrack green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor green fluorescence, and (d, f, h, j) overlays of organelle tracers with **6a** fluorescence. Scale bar: 10 μm.

Subcellular fluorescence of Pcs in Tumor cells

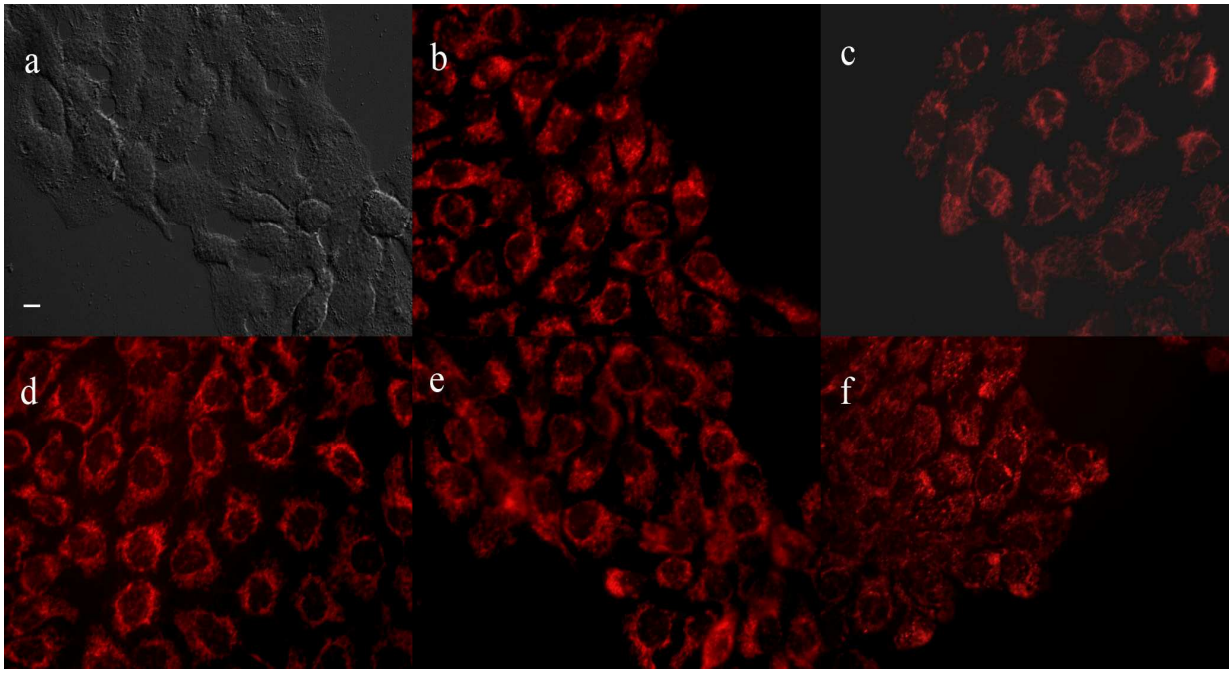


Figure S14. Subcellular fluorescence of Pcs in A431 cells at 10 μM for 6 h. (a) Phase contrast, (b) Pc **3a**, (c) Pc **4b**, (d) Pc **6a**, (e) Pc **5a**, (f) Pc **5b**. Scale bar: 10 μm.

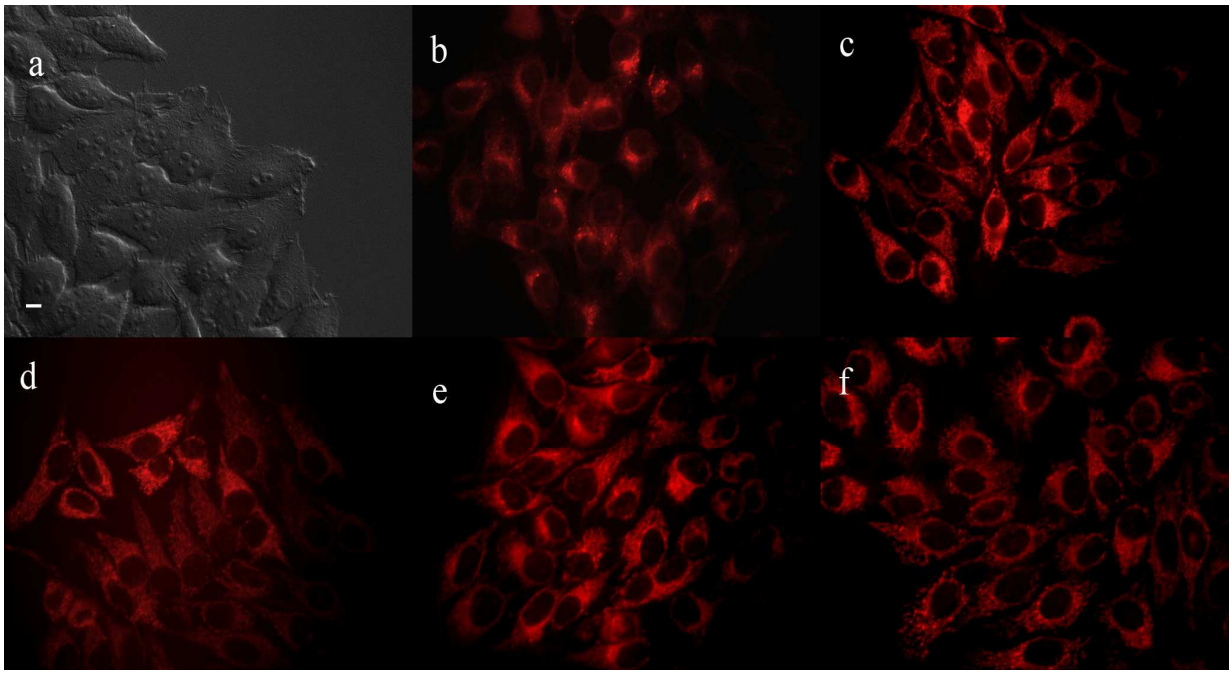


Figure S15. Subcellular fluorescence of Pcs in HEp2 cells at 10 μM for 6 h. (a) Phase contrast, (b) Pc **3a**, (c) Pc **4b**, (d) Pc **6a**, (e) Pc **5a**, (f) Pc **5b**. Scale bar: 10 μm.

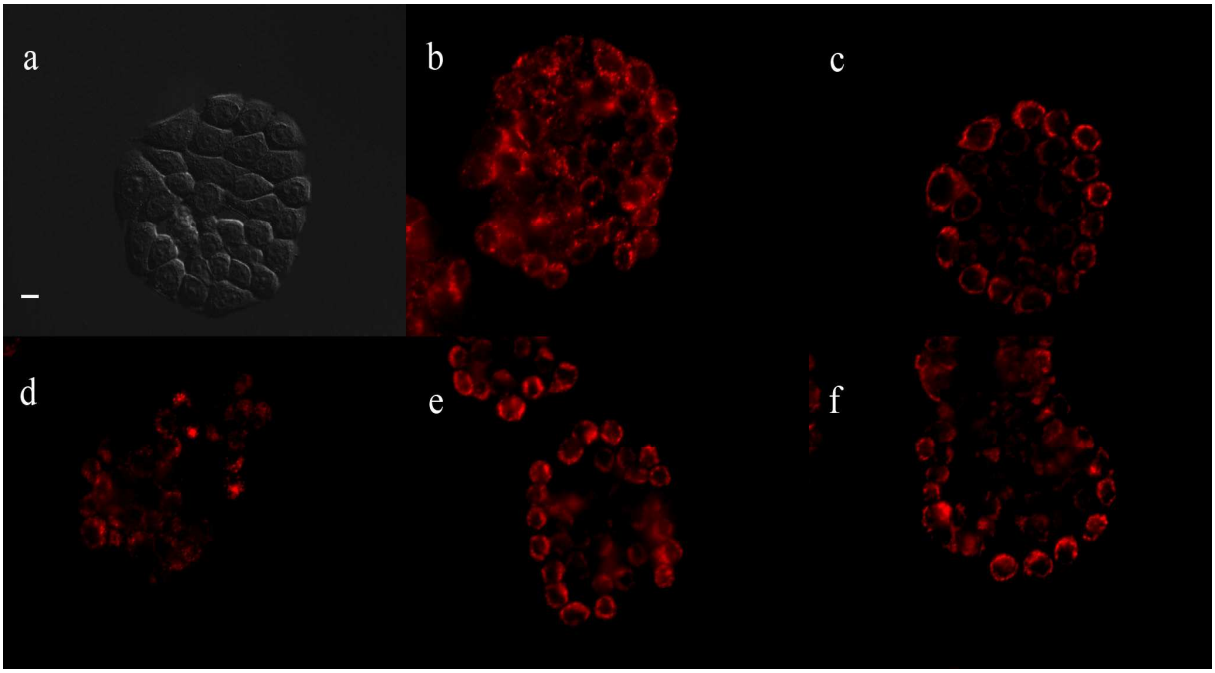


Figure S16. Subcellular fluorescence of Pcs in HT-29 cells at 10 μM for 6 h. (a) Phase contrast, (b) Pc **3a**, (c) Pc **4b**, (d) Pc **6a**, (e) Pc **5a**, (f) Pc **5b**. Scale bar: 10 μm.

HPLC Traces, Absorption and Emission Spectra for Pc-conjugates

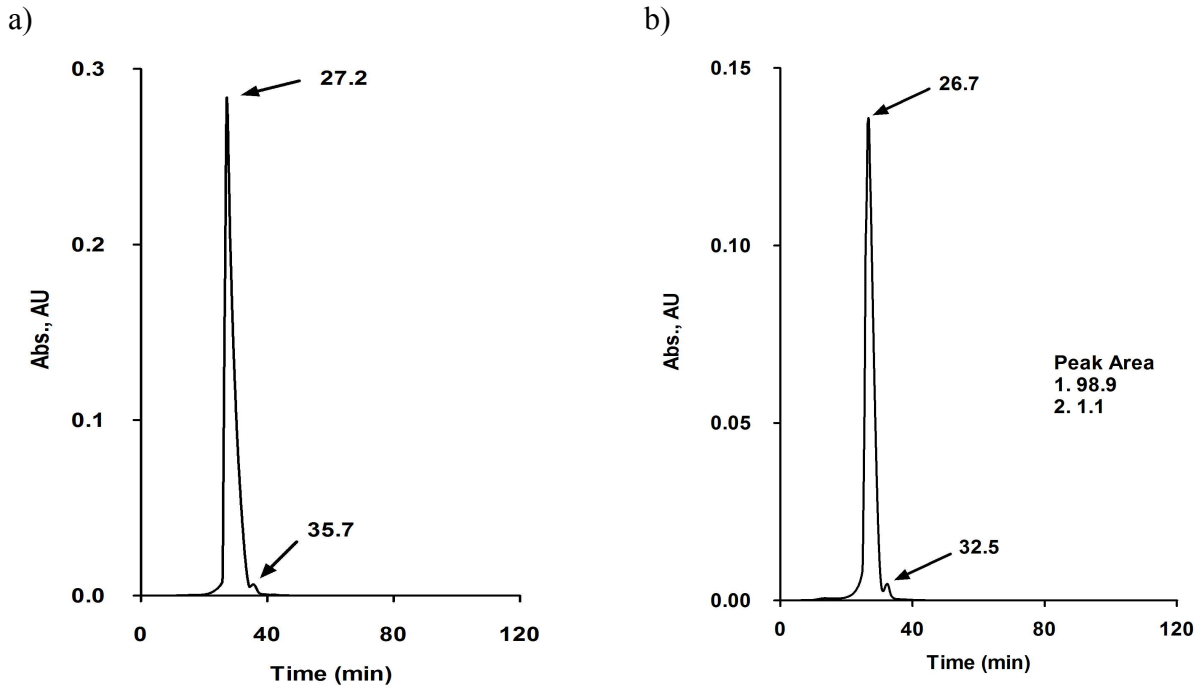


Figure S17. HPLC spectra for (a) Pc-conjugate **5a** and, (b) Pc-conjugate **5b**.

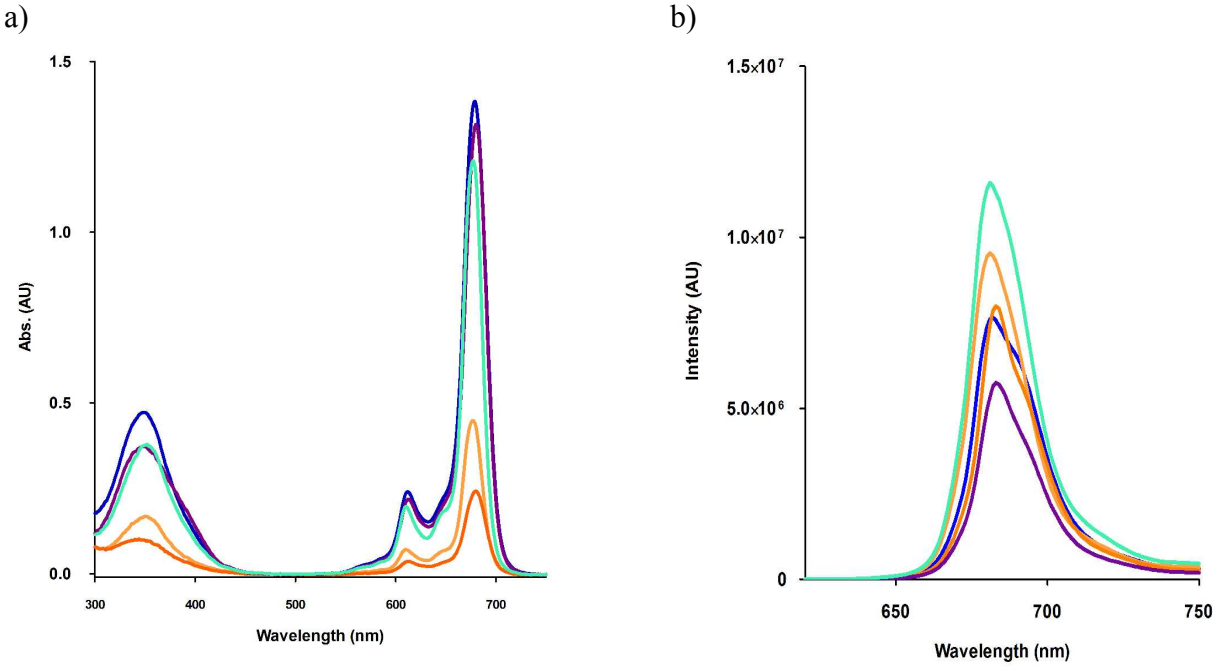


Figure S18. (a) Absorption spectra for Pc conjugates at 8.0 μM and, (b) Emission spectra for Pc conjugates. Pc-conjugates **3a** (blue), Pc **4b** (tangerine), Pc **6a** (red), Pc **5a** (purple), and Pc **5b** (turquoise) in DMF.

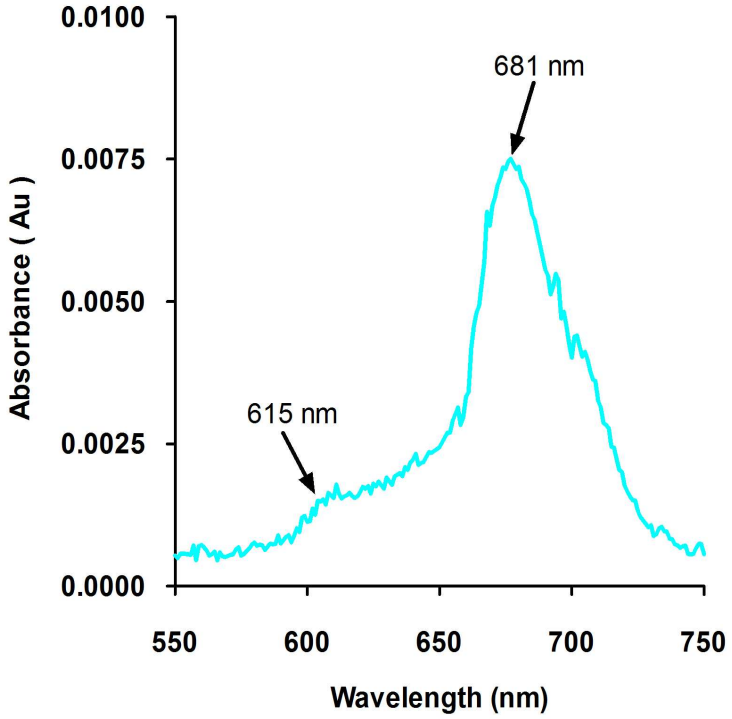


Figure S19. Absorption spectrum for Pc **5b** conjugate in methanol, extracted from HT-29 mice-tumor.

Dark-toxicity and Phototoxicity in cells

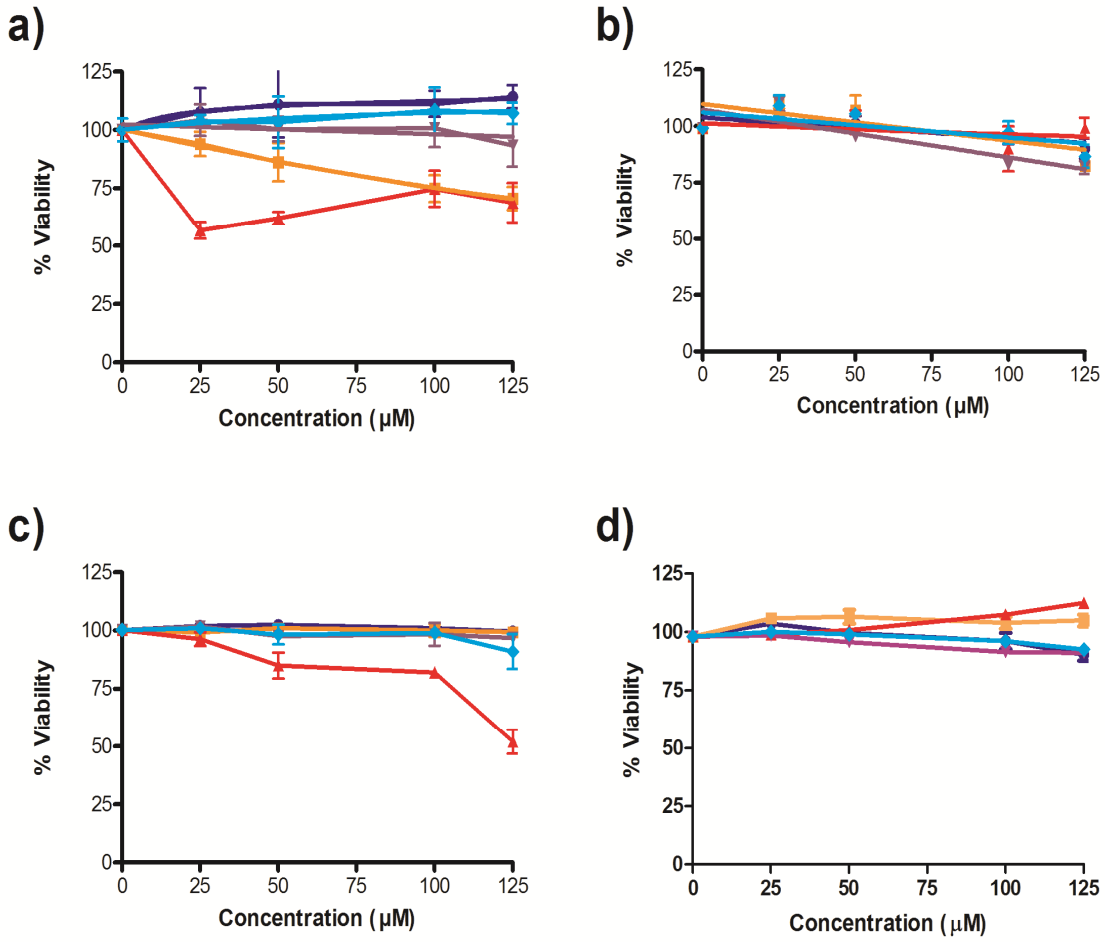


Figure S20. Dark toxicity of conjugates Pc **3a** (blue), Pc **4b** (tangerine), Pc **6a** (red), Pc **5a** (purple), and Pc **5b** (turquoise) at 10 μM toward (a) A431, (b) Vero, (c) HEp2 and (d) HT-29 cells, using the Cell Titer Blue assay.

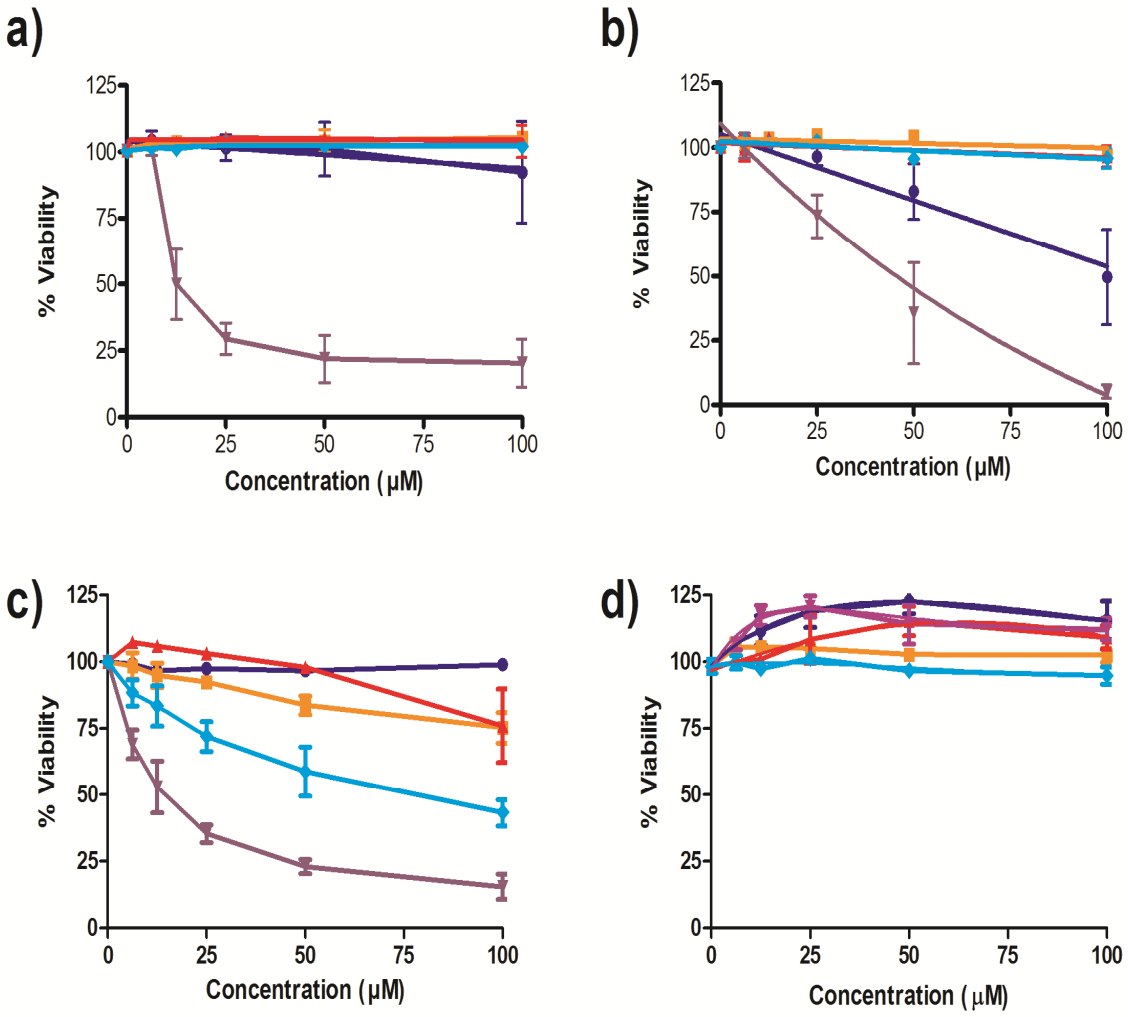


Figure S21. Phototoxicity of conjugates Pc **3a** (blue), Pc **4b** (tangerine), Pc **6a** (red), Pc **5a** (purple), and Pc **5b** (turquoise) at 10 μM toward (a) A431, (b) Vero, (c) HEp2 and (d) HT-29 cells, using the Cell Titer Blue assay.

Mass Spectra for Pcs and their Conjugates

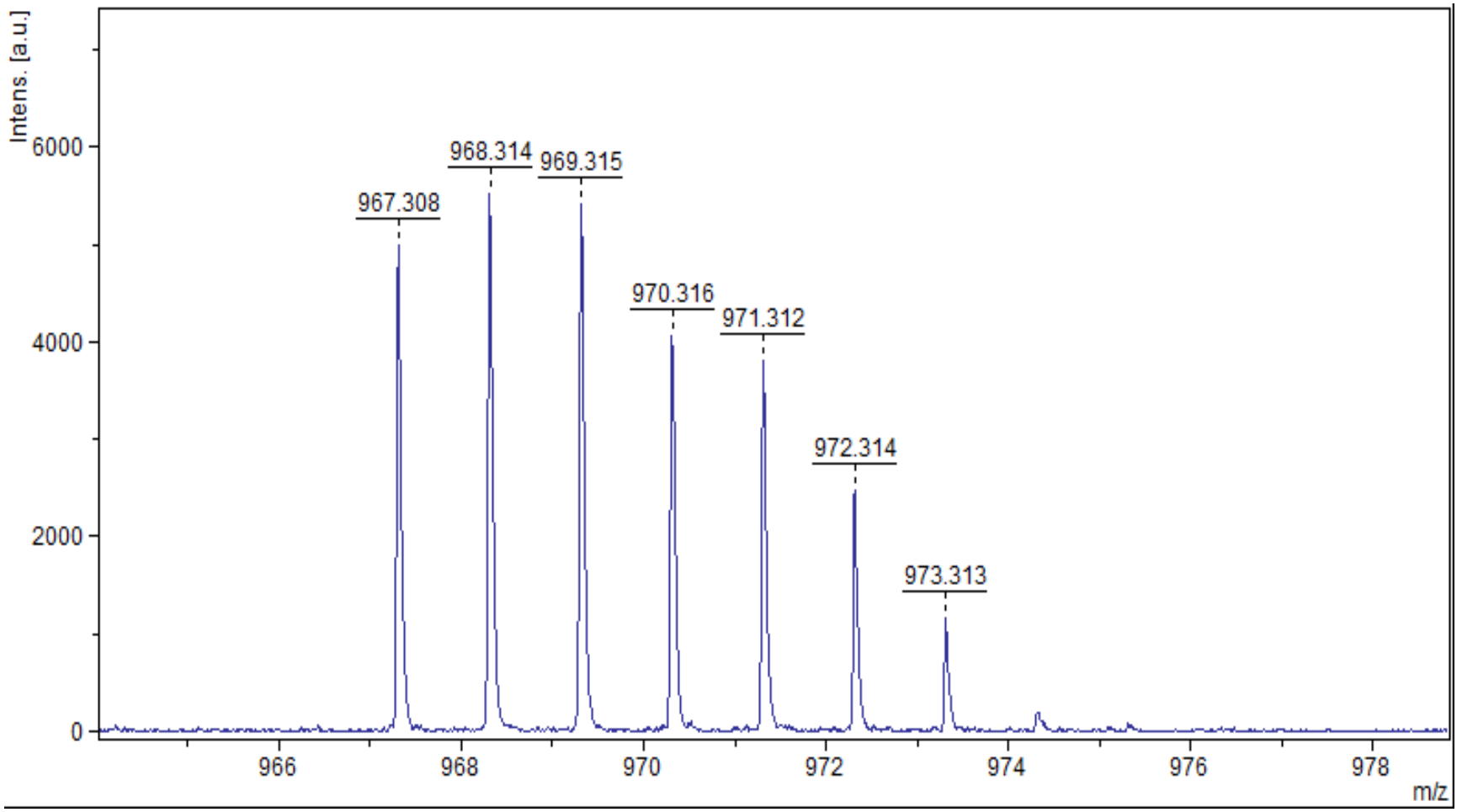


Figure S22. MS (MALDI-TOF) spectrum for Pc **2a**.

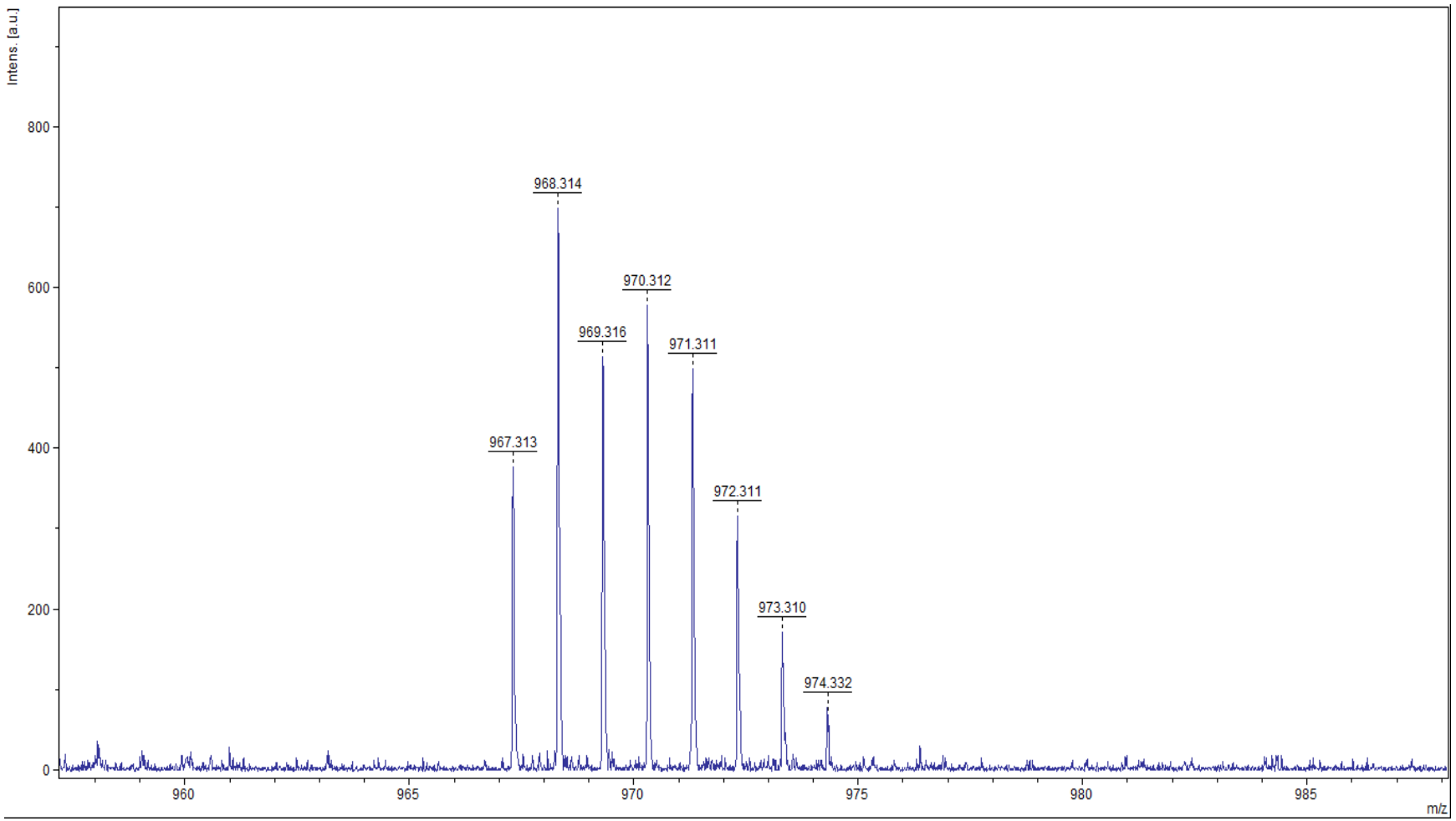


Figure S23. MS (MALDI-TOF) spectrum for Pc **2b**.

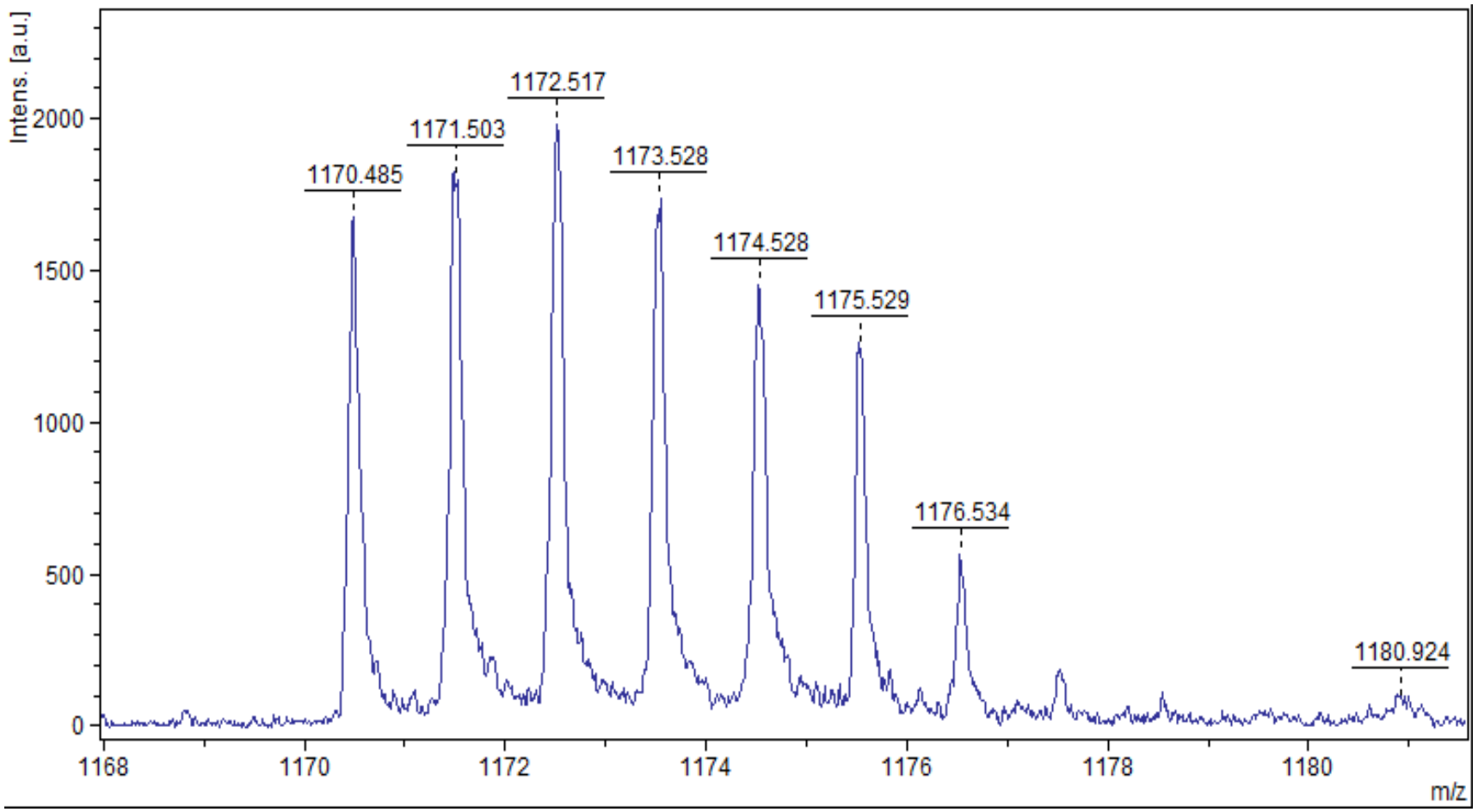


Figure S24. MS (MALDI-TOF) spectrum for tert-butyl protected Pc **3a** $[M\text{-}^{t}Bu+H]^{+}$.

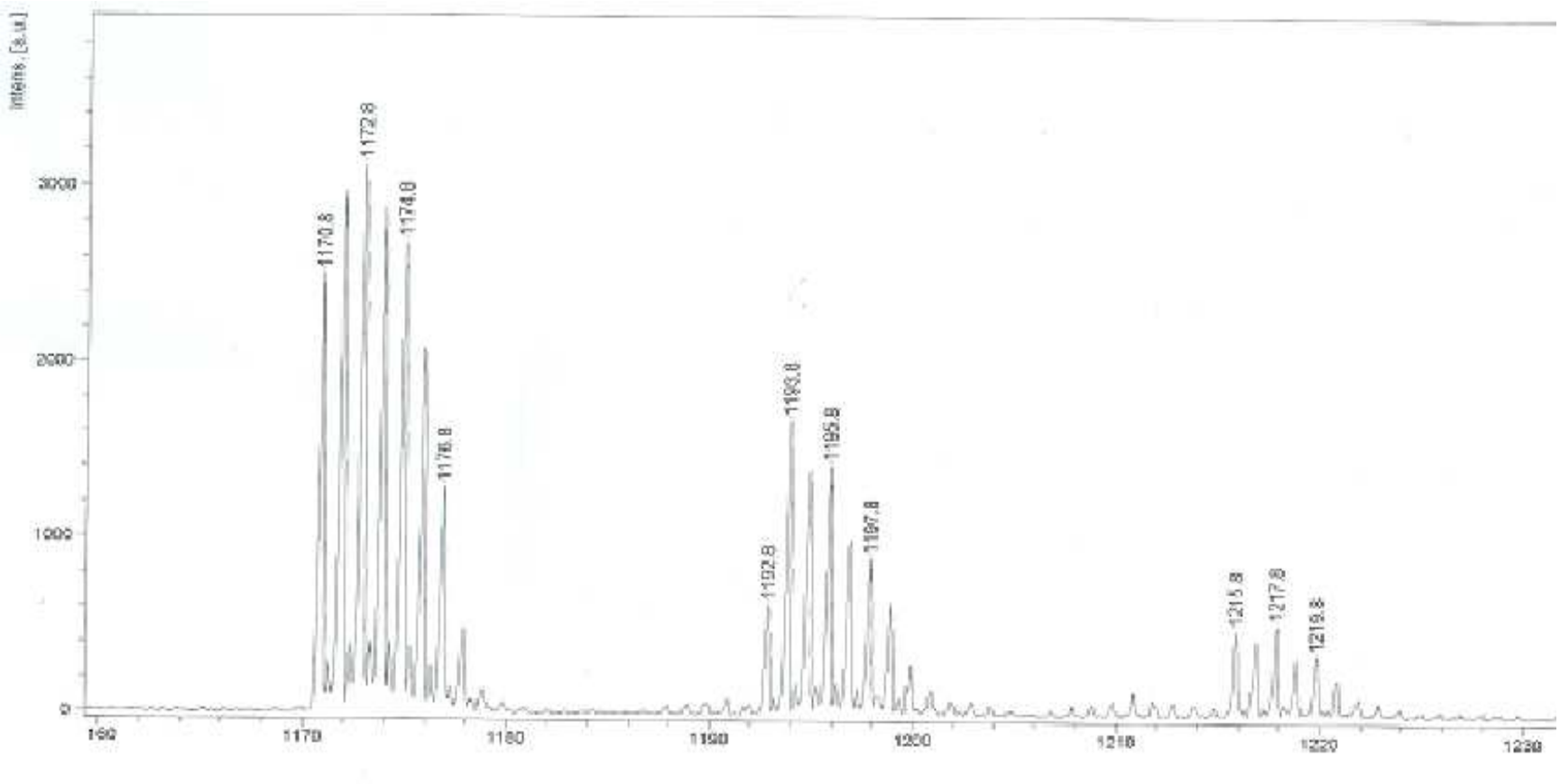


Figure S25. MS (MALDI-TOF) spectrum for deprotected Pc **3a** $[M\text{-}^{t}Bu+H]^{+}$.

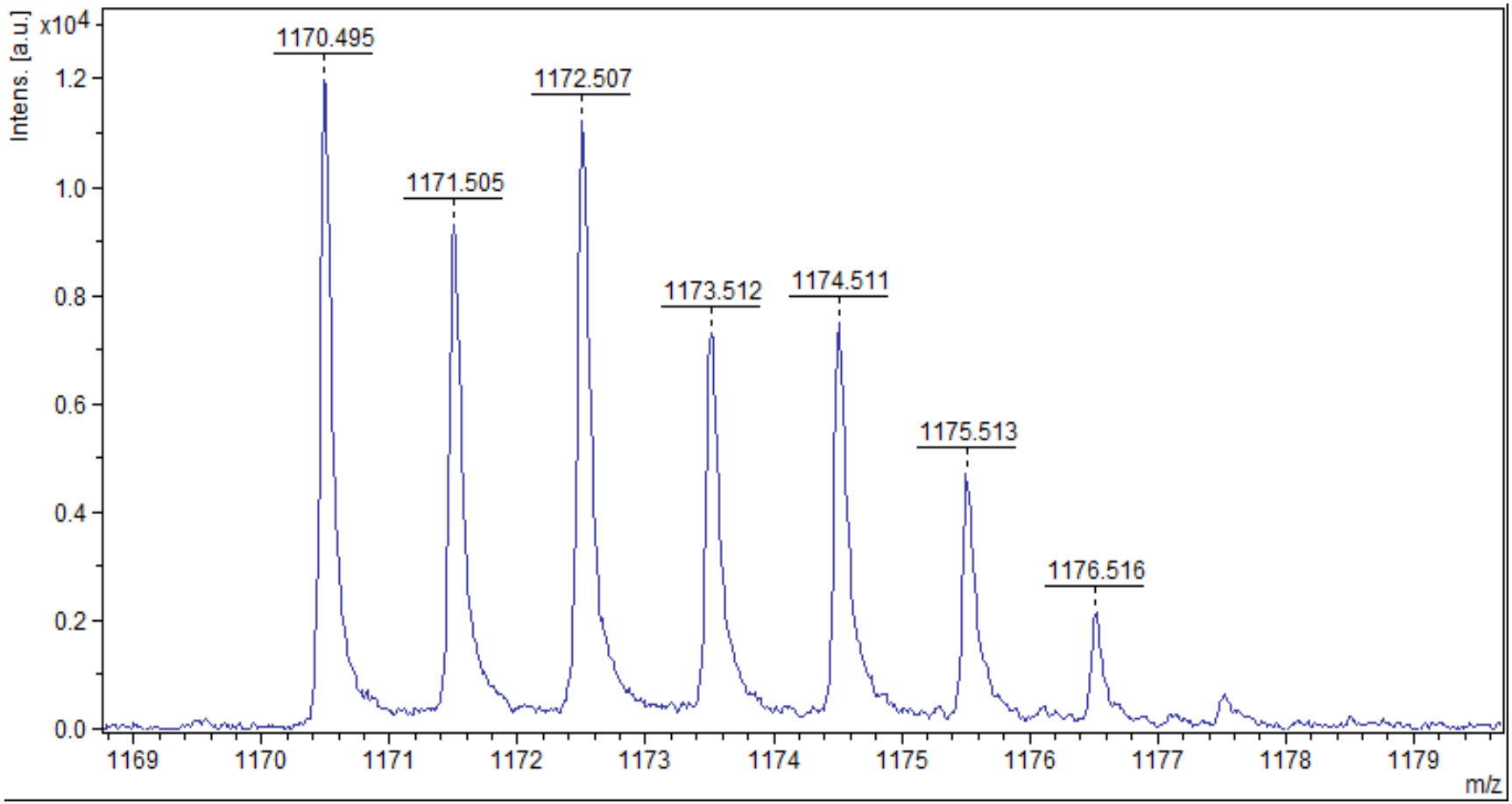


Figure S26. MS (MALDI-TOF) spectrum for tert-butyl protected Pc **3b** $[M\text{-}^{t}Bu+H]^{+}$.

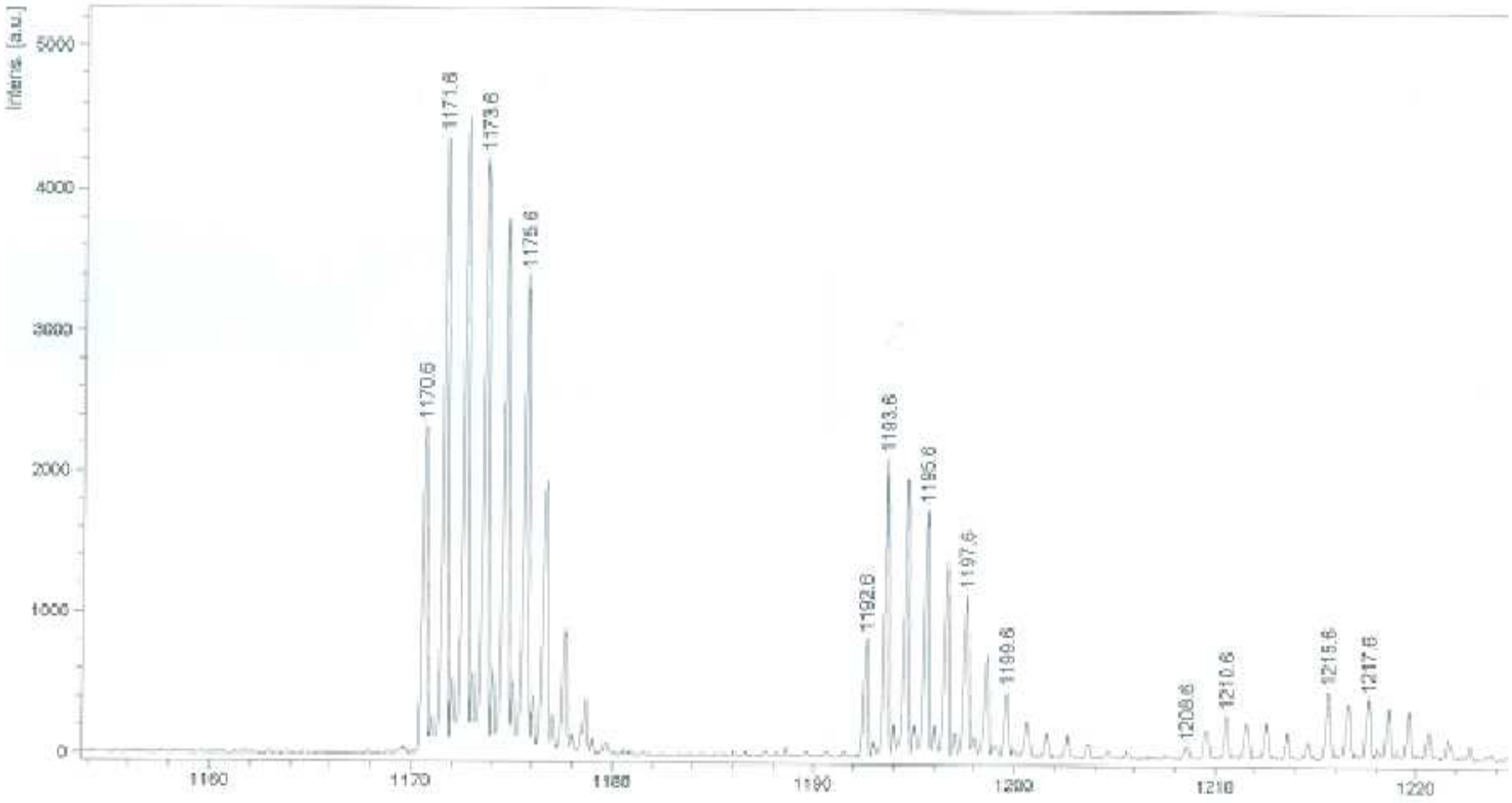


Figure S27. MS (MALDI-TOF) spectrum for deprotected Pc **3b** $[M\text{-}^{t}Bu+H]^{+}$.

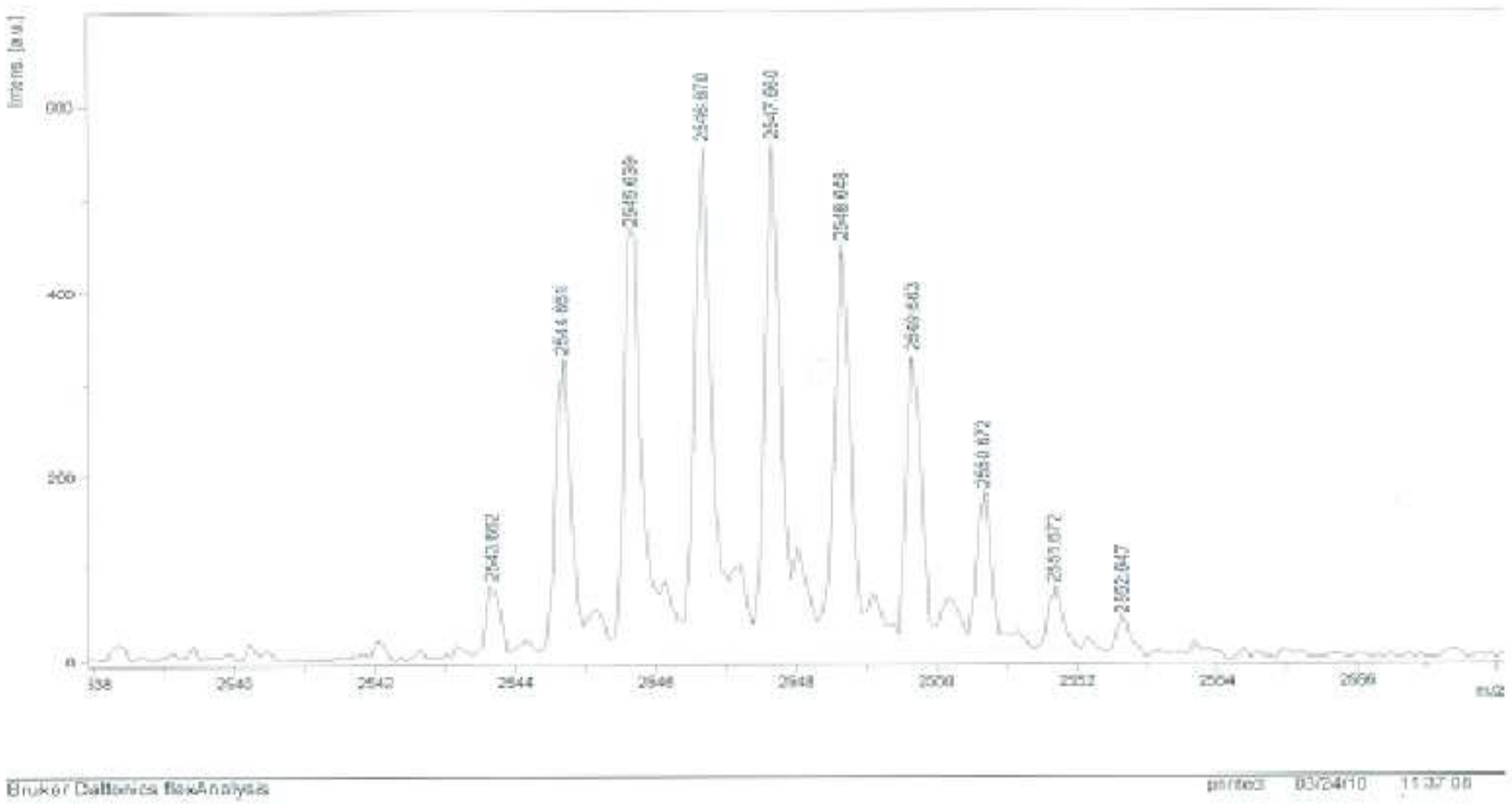


Figure S28. MS (MALDI-TOF) spectrum for Pc **4b** $[M+H]^+$.

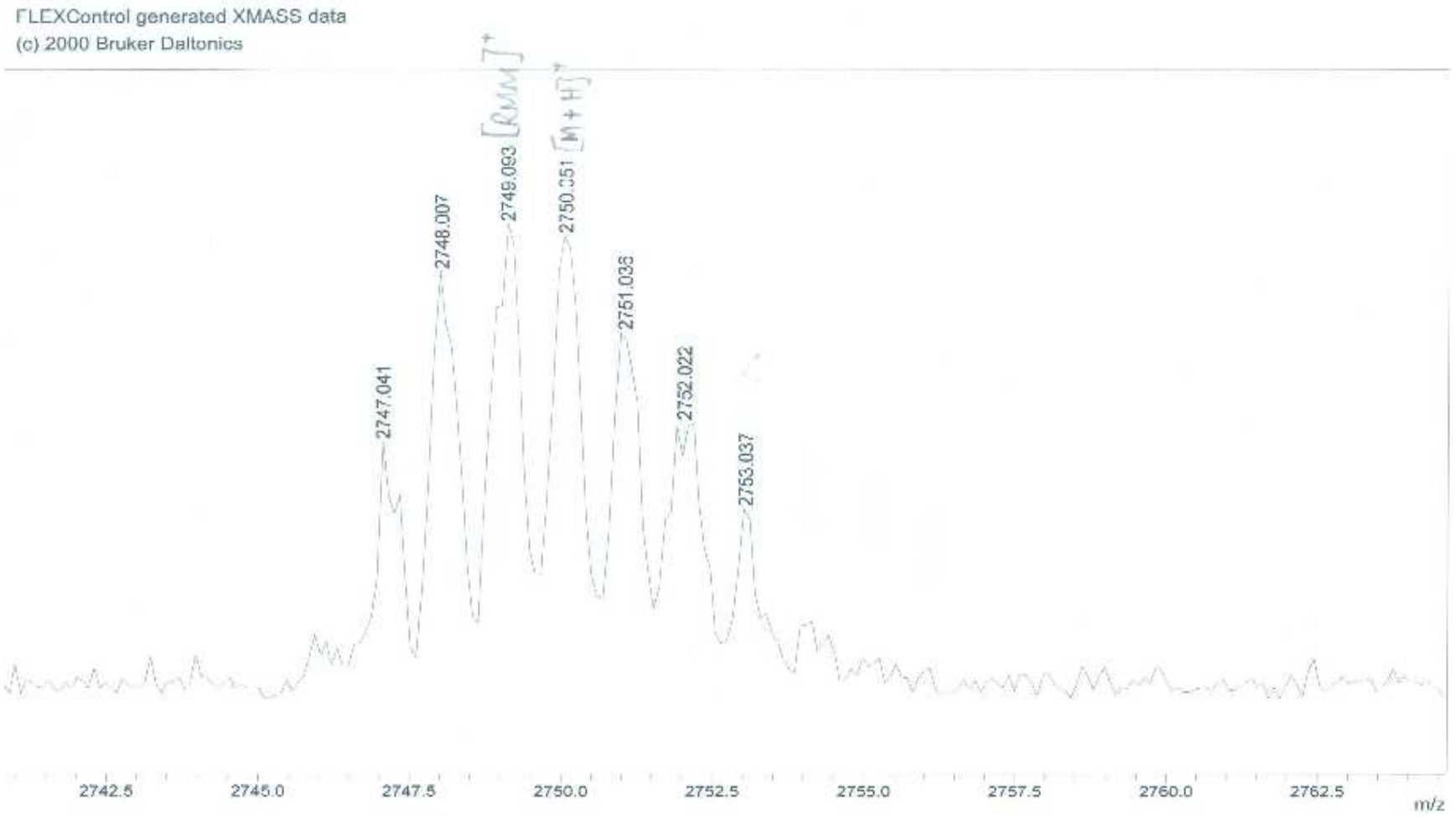


Figure S29. MS (MALDI-TOF) spectrum for Pc **6a** $[M+H]^+$.

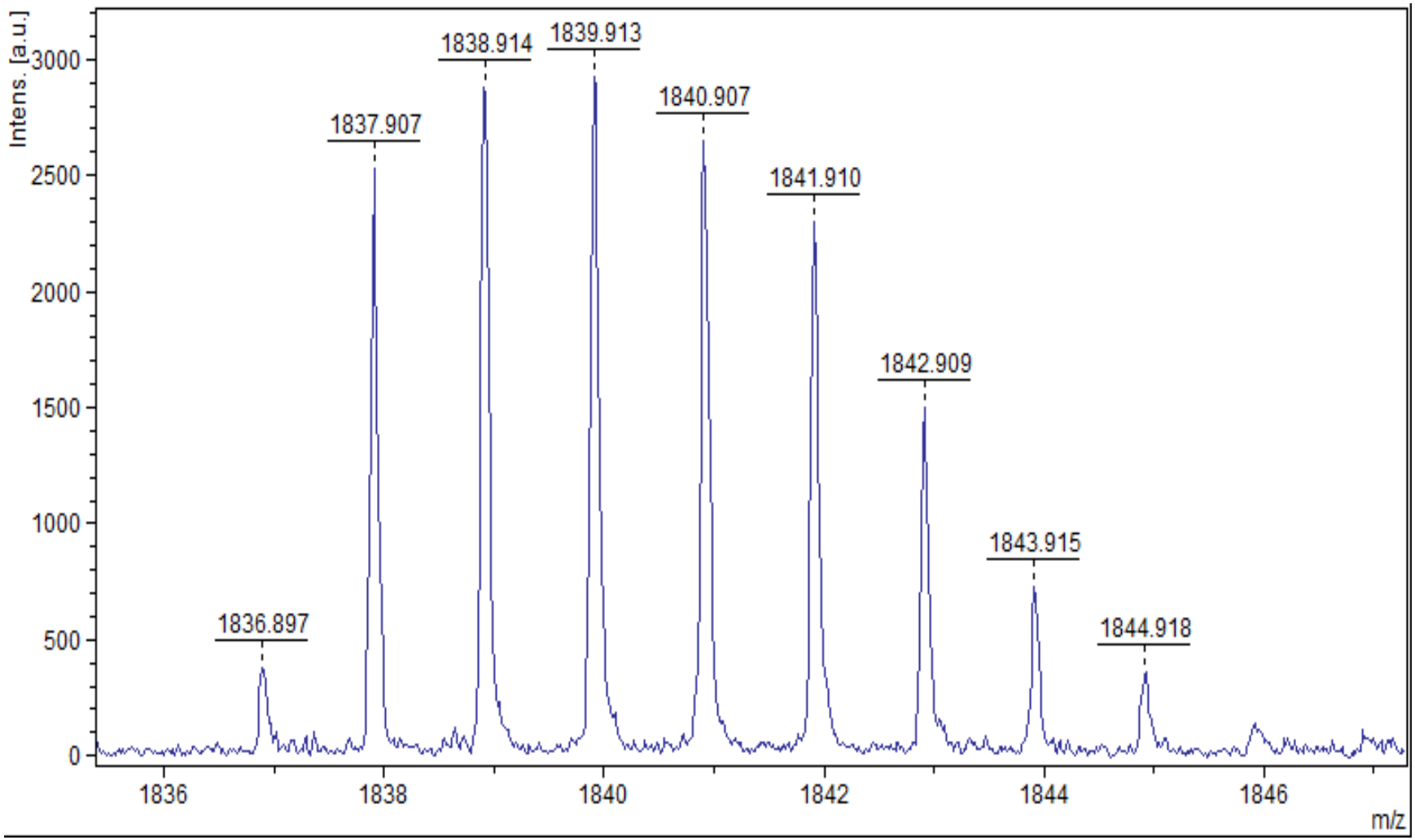


Figure S30. MS (MALDI-TOF) spectrum for Pc **5a** $[M+H]^+$.

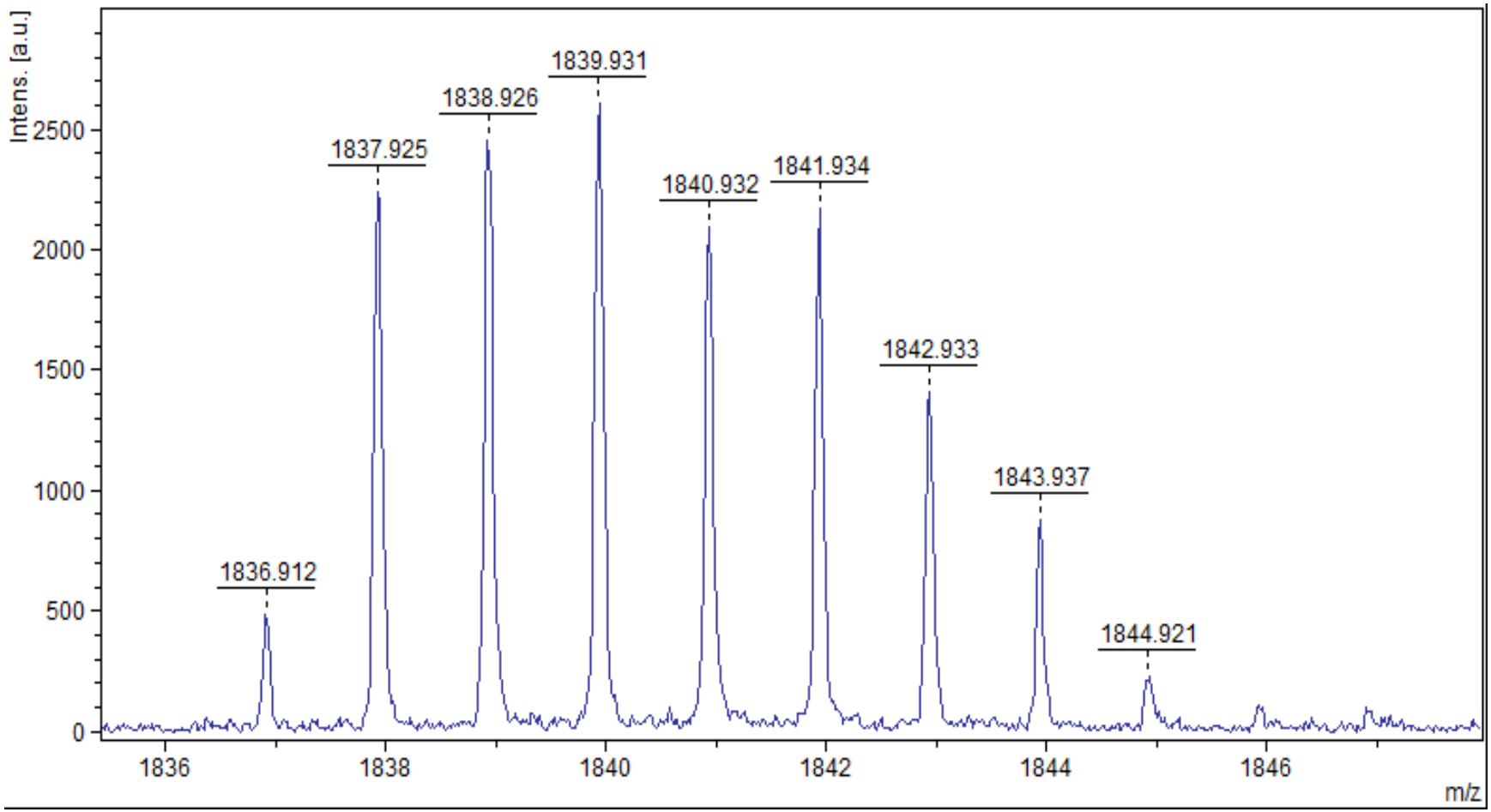


Figure S31. MS (MALDI-TOF) spectrum for Pc **5b** $[M+H]^+$.

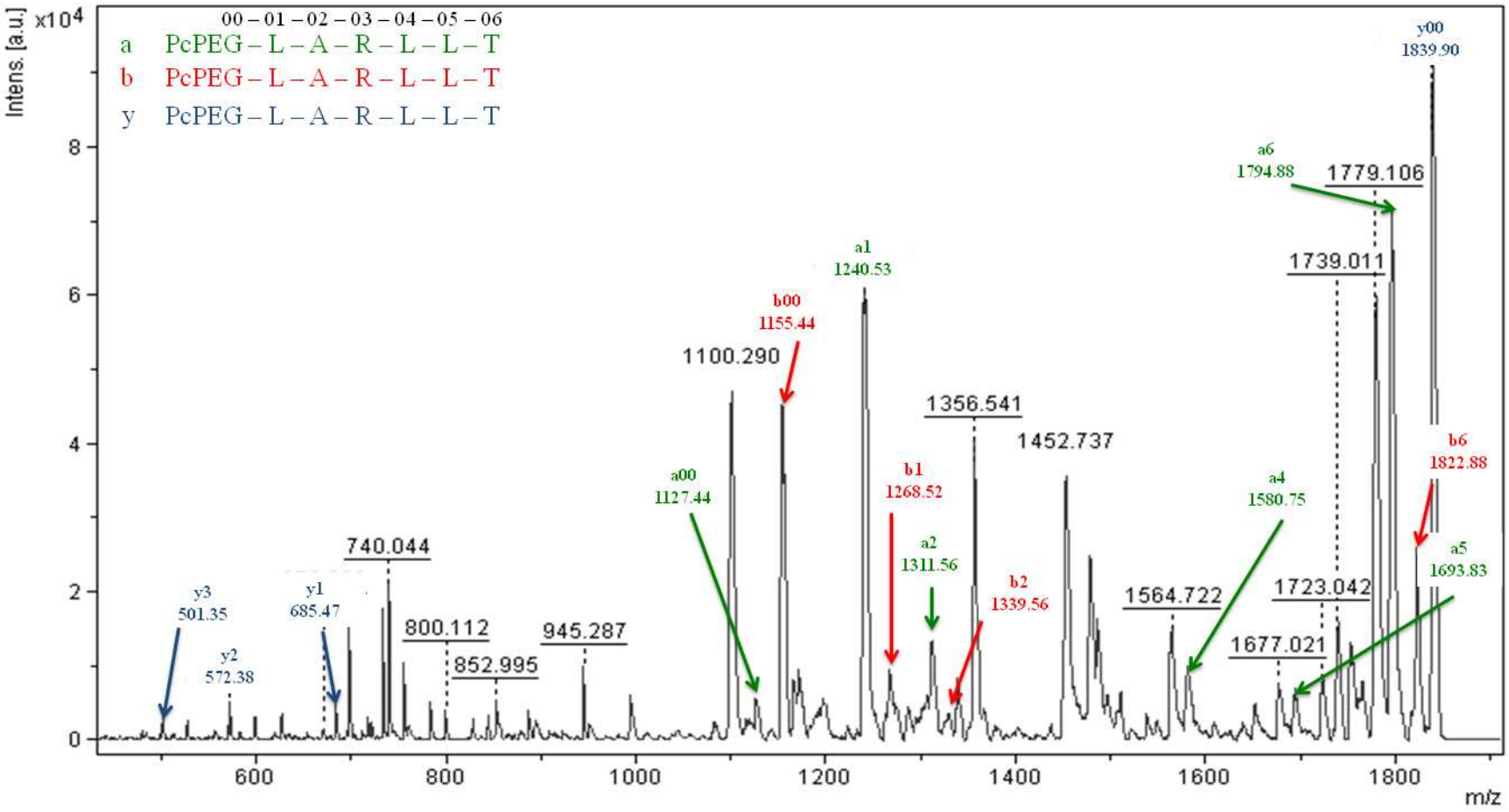


Figure S32. MALDI-TOF-TOF (MS-MS) spectrum for Pc-conjugate **5a.**

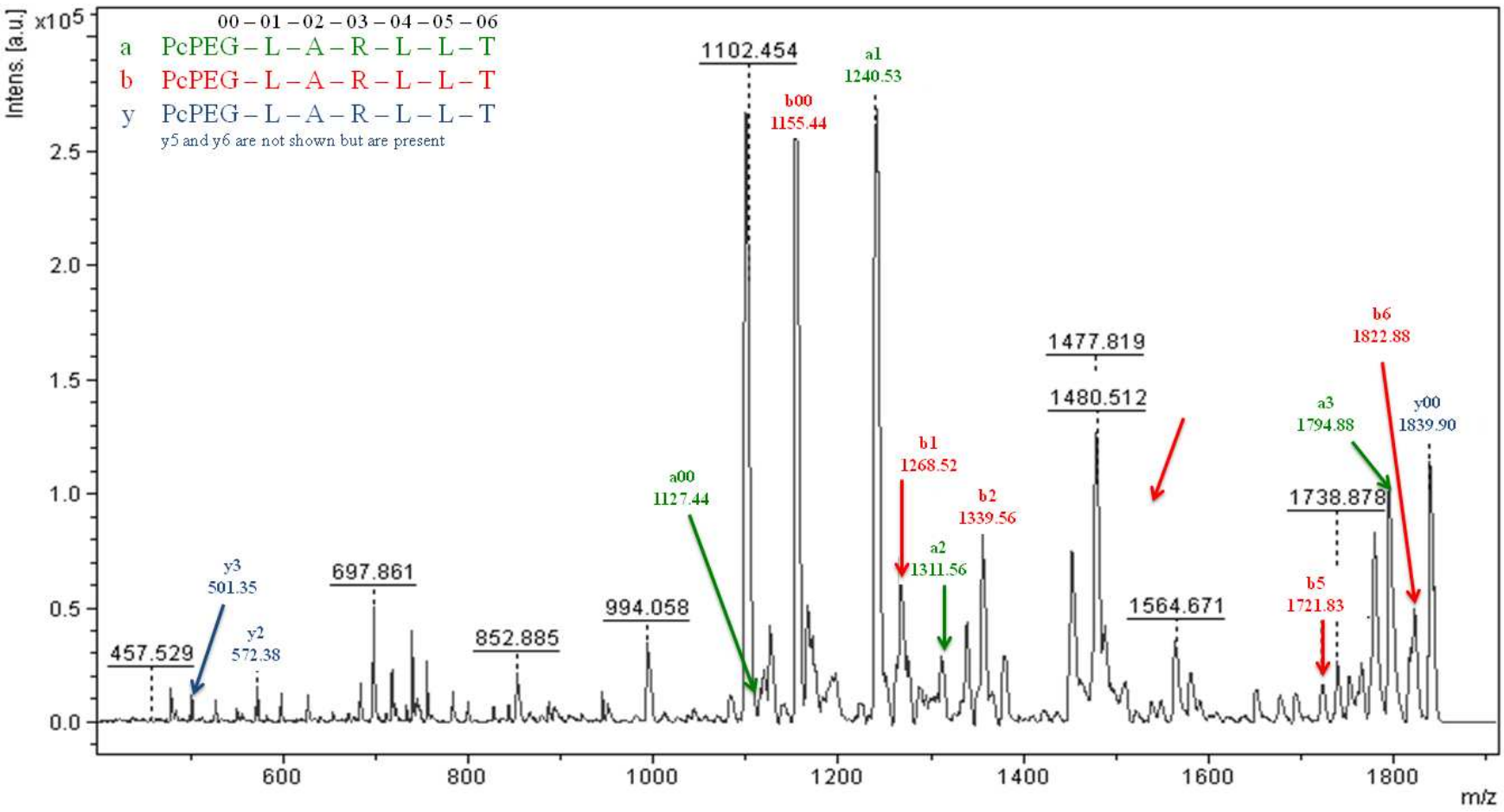


Figure S33. MALDI-TOF-TOF (MS-MS) spectrum for Pc-conjugate **5b**

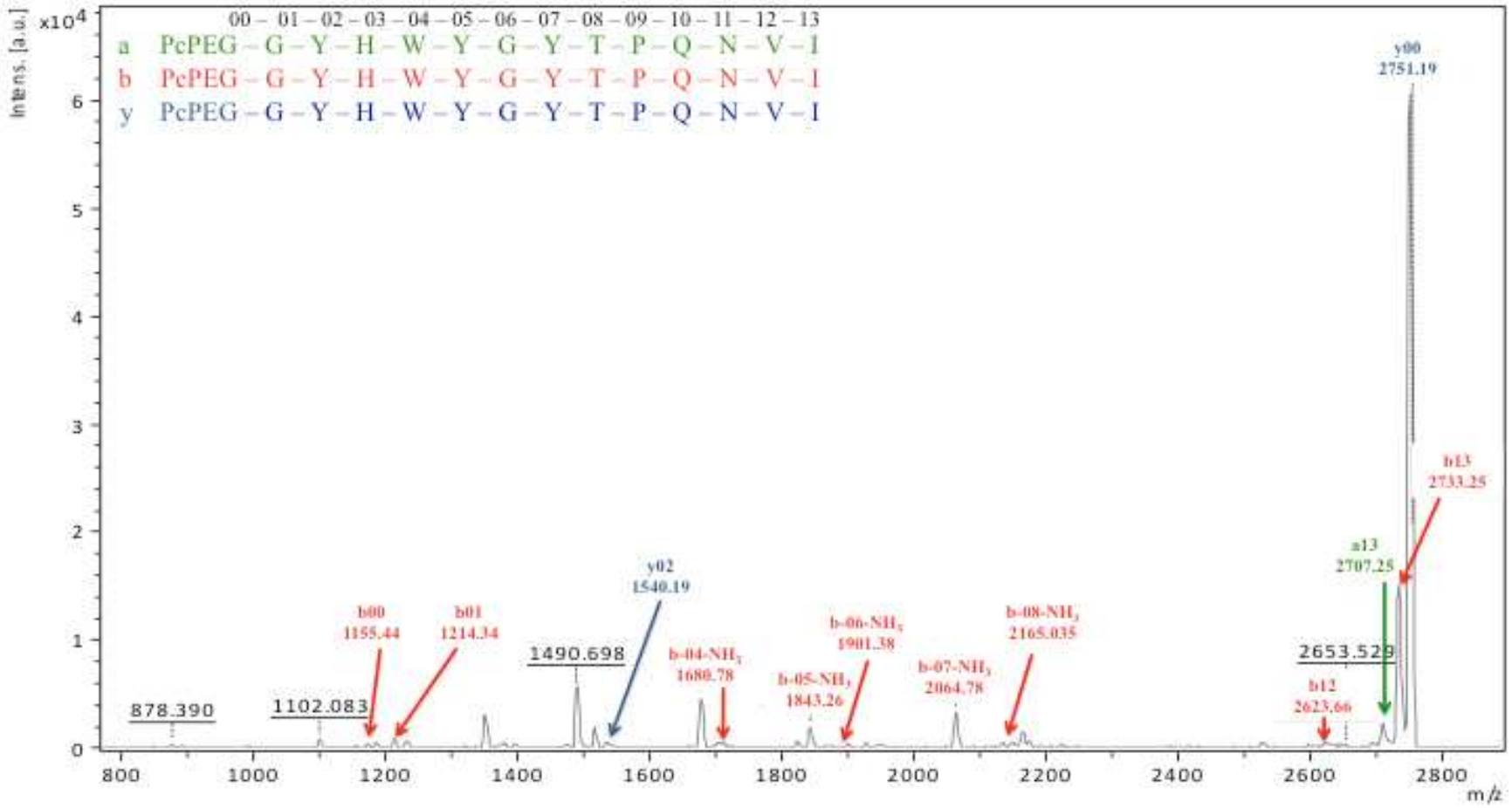


Figure S34. MALDI-TOF-TOF (MS-MS) spectrum for Pc-conjugate **6a**

[1]HNMR and [13]CNMR Spectra for Pcs and their Conjugates

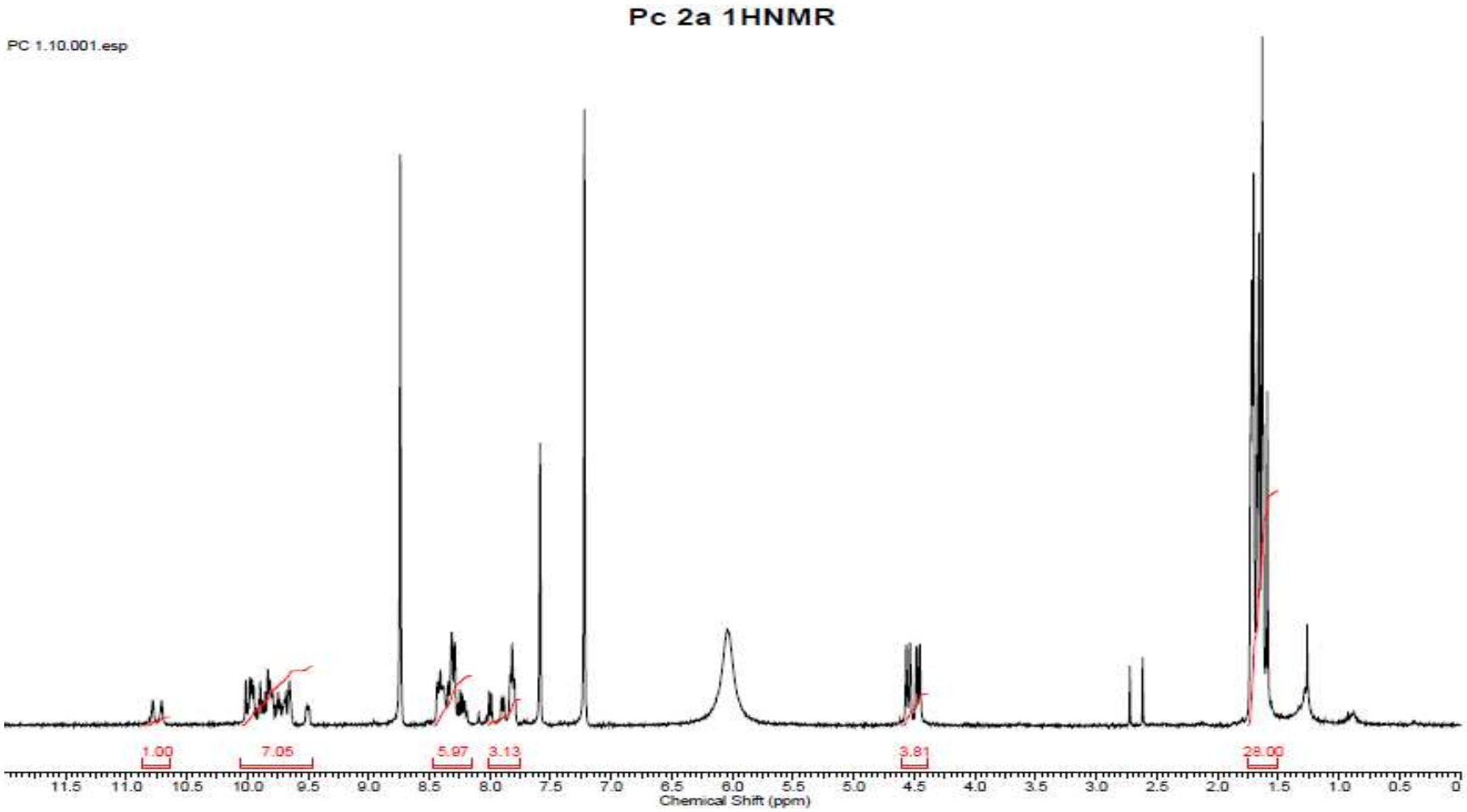


Figure S35. [1]HNMR spectrum for Pc **2a**

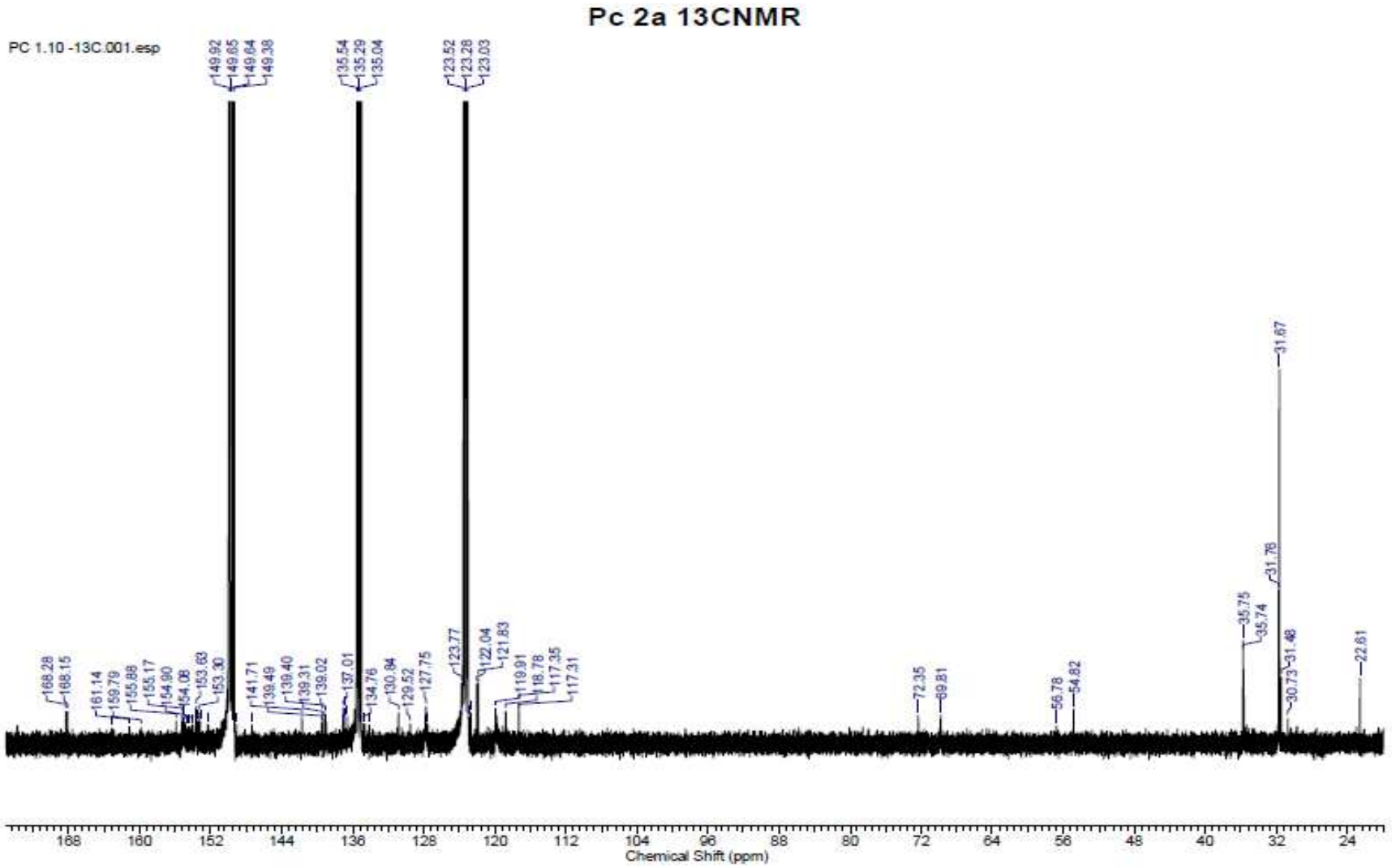


Figure S36. ^{13}CNMR spectrum for Pc **2a**

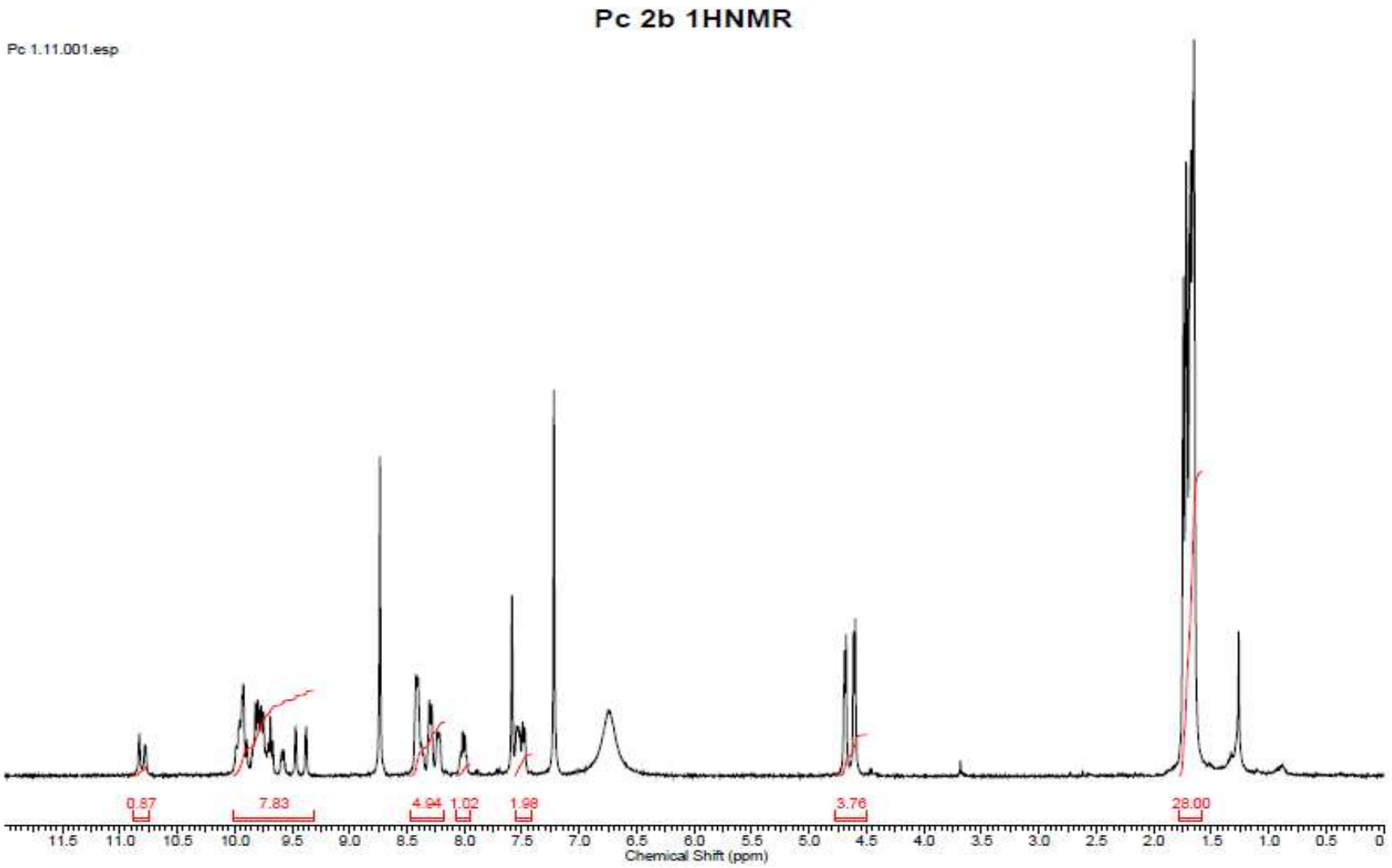


Figure S37. [1]HNMR spectrum for Pc **2b**

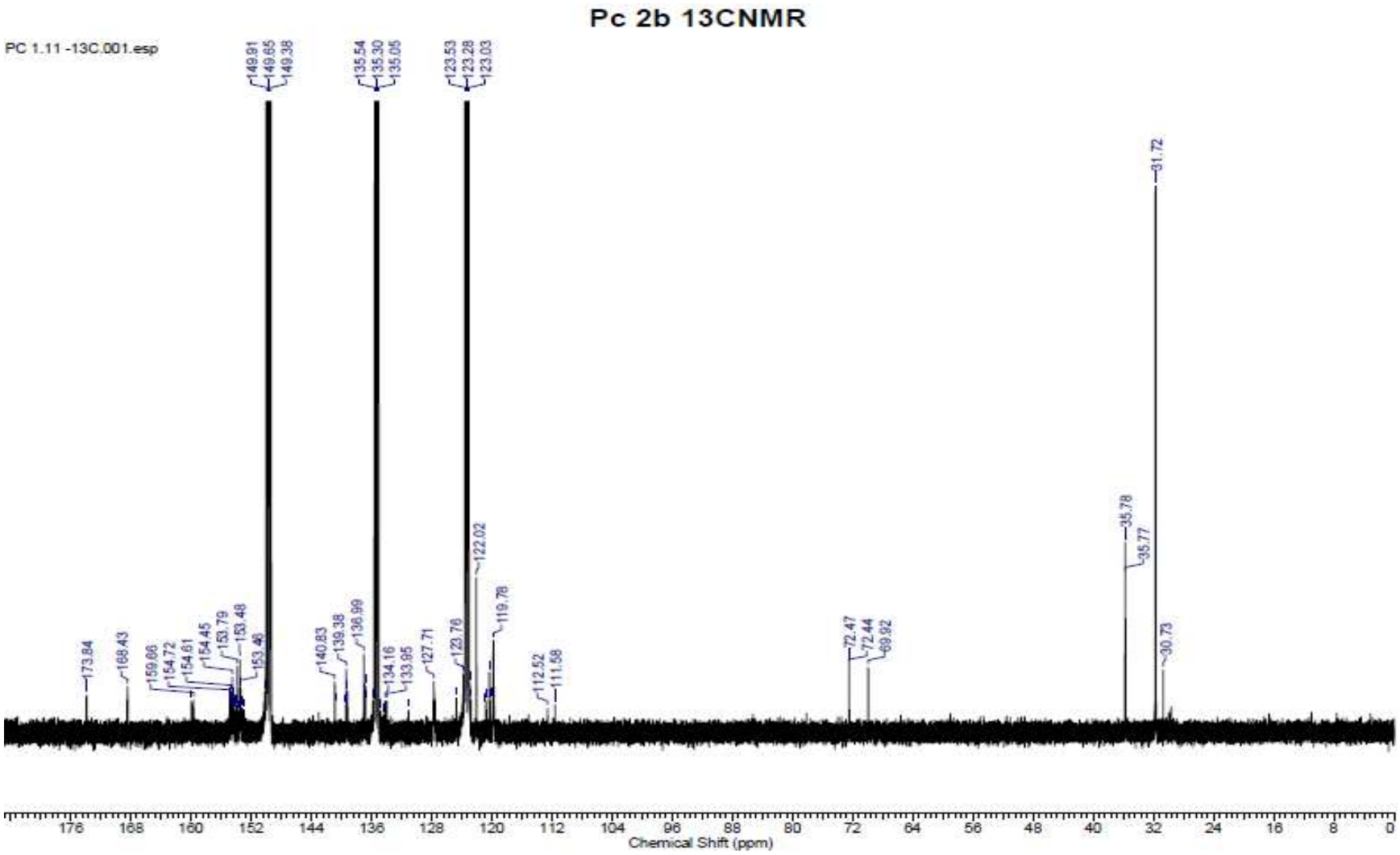


Figure S38. ^{13}CNMR spectrum for Pc **2b**

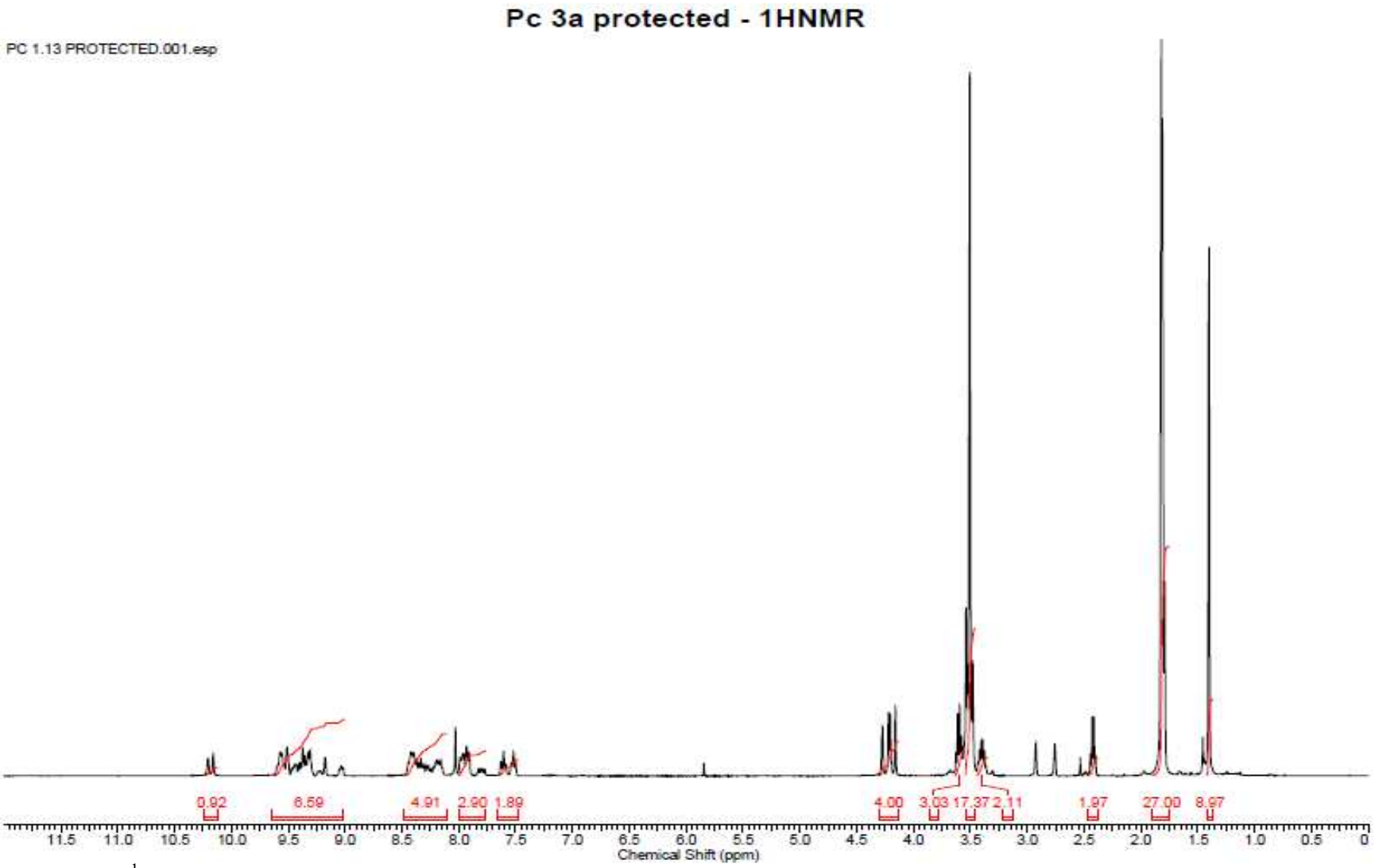


Figure S39. [1]HNMR spectrum for protected Pc **3a**

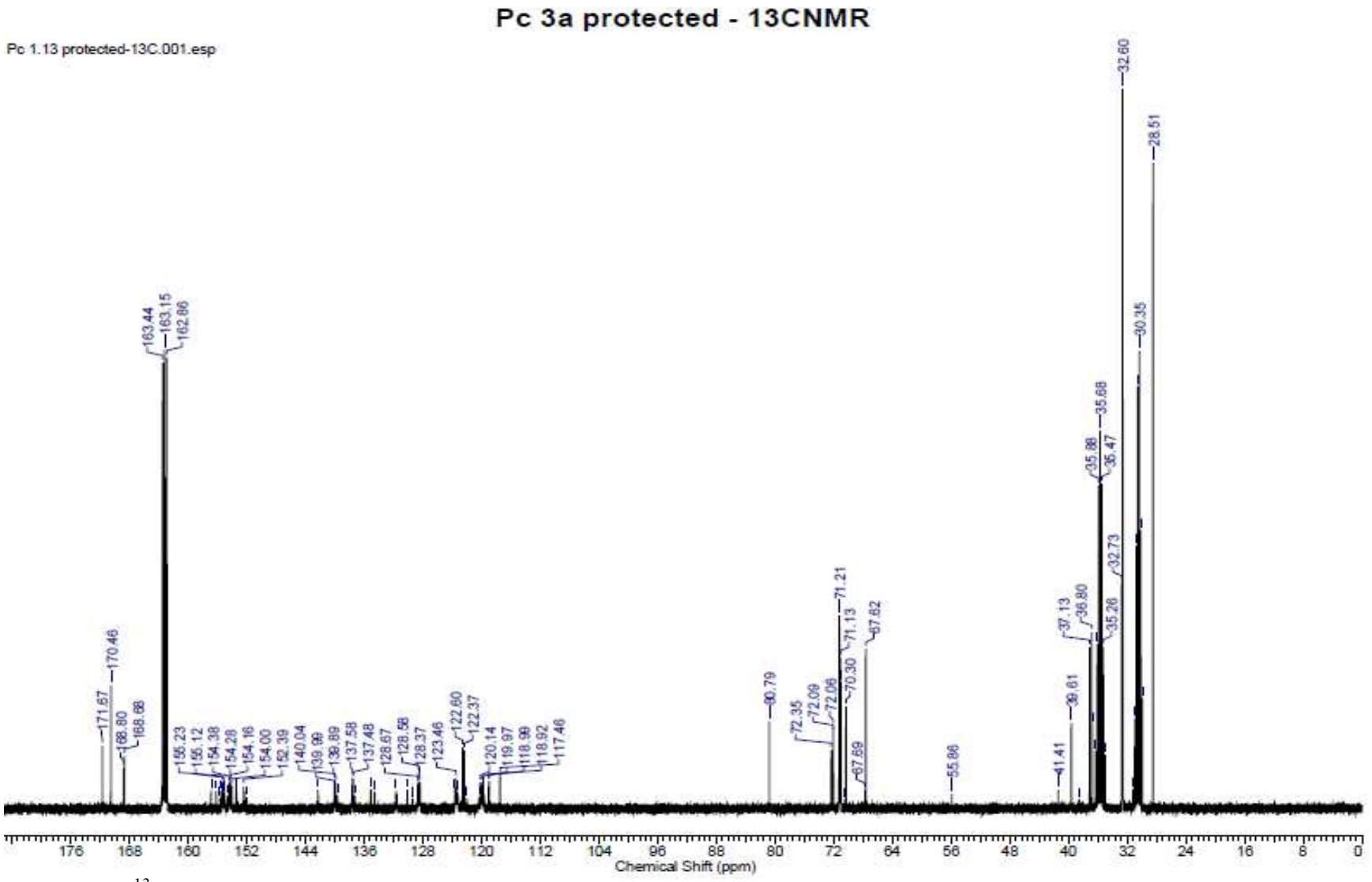


Figure S40. ^{13}CNMR spectrum for protected Pc **3a**

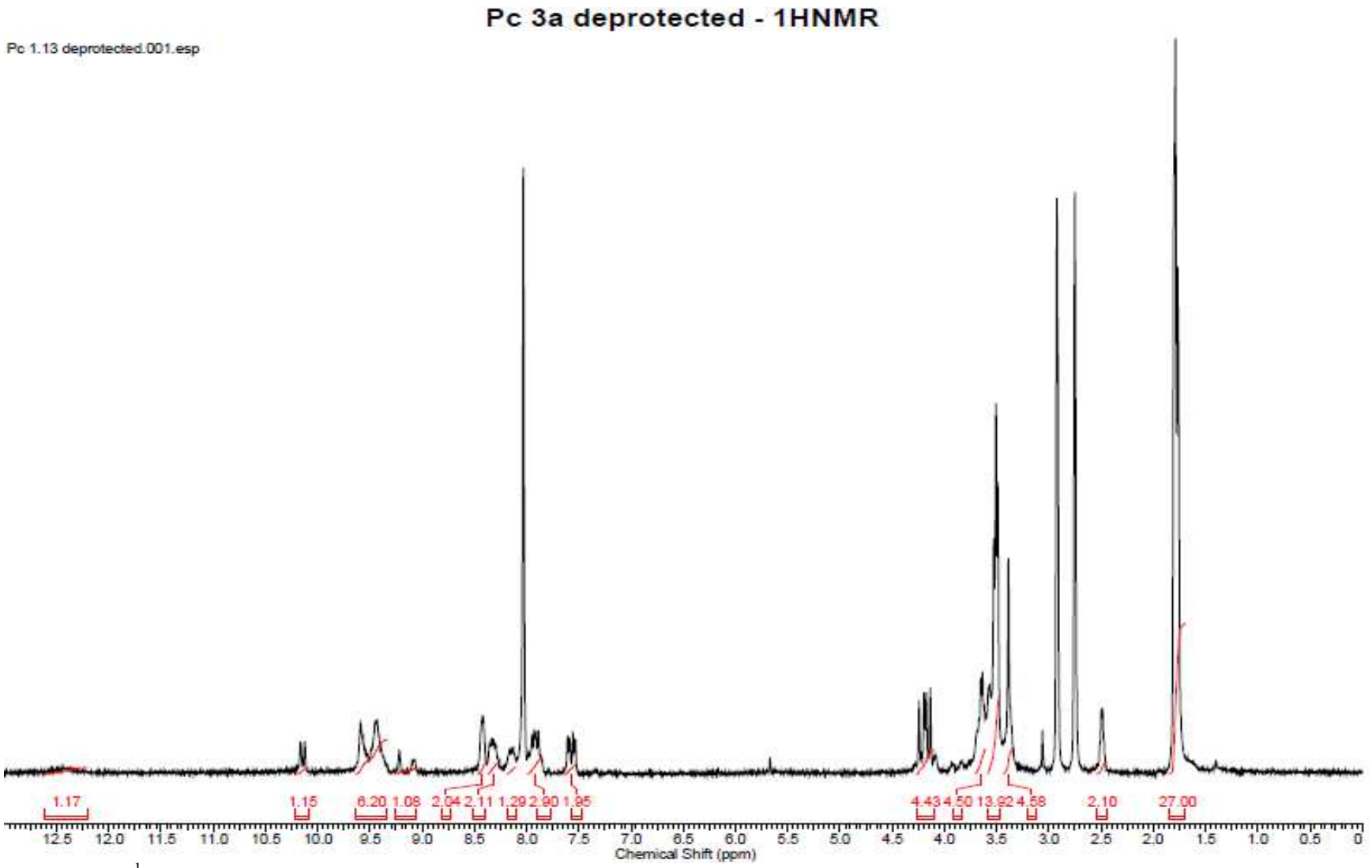


Figure S41. [1]HNMR spectrum for deprotected Pc **3a**

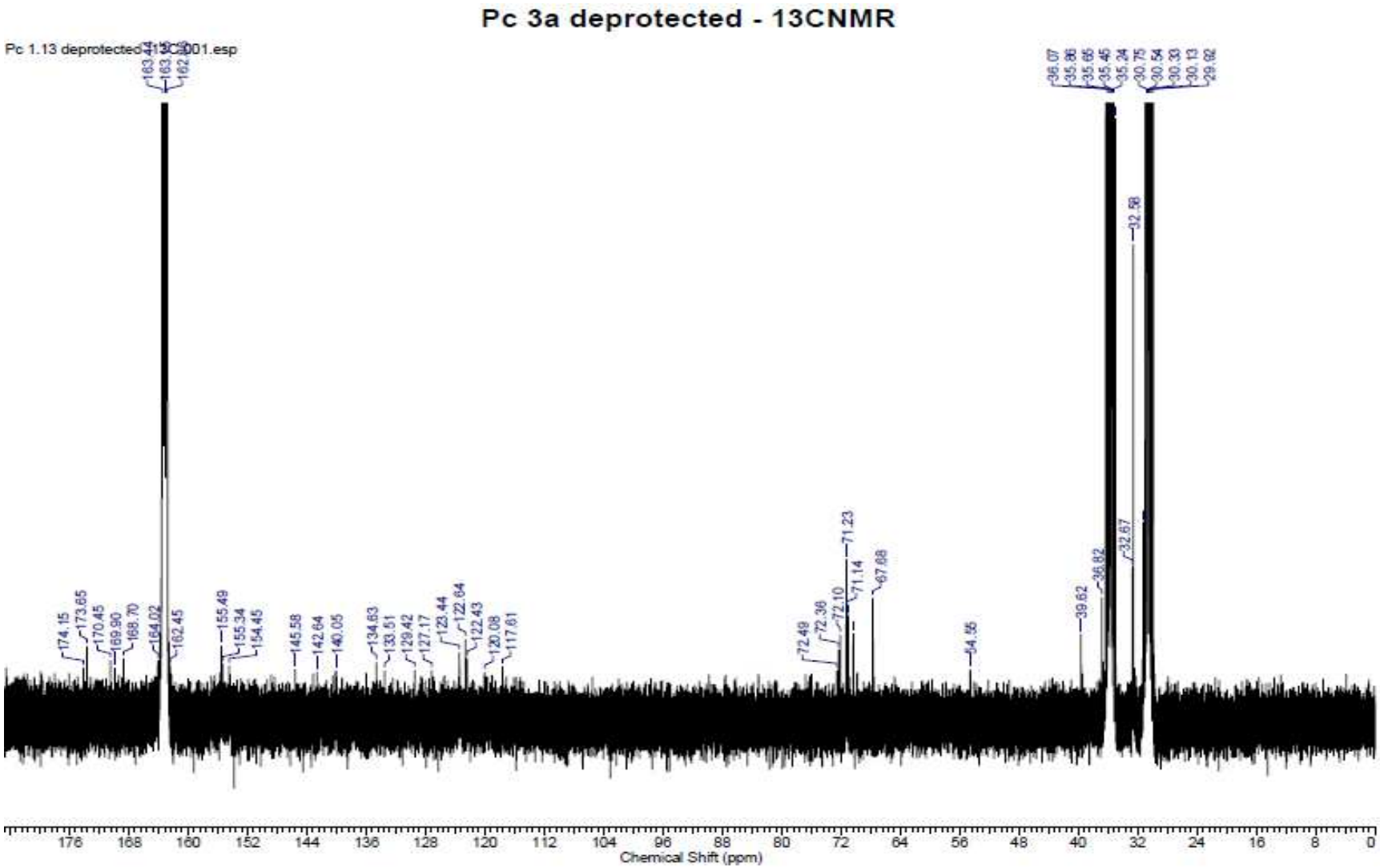


Figure S42. ^{13}CNMR spectrum for deprotected Pc **3a**

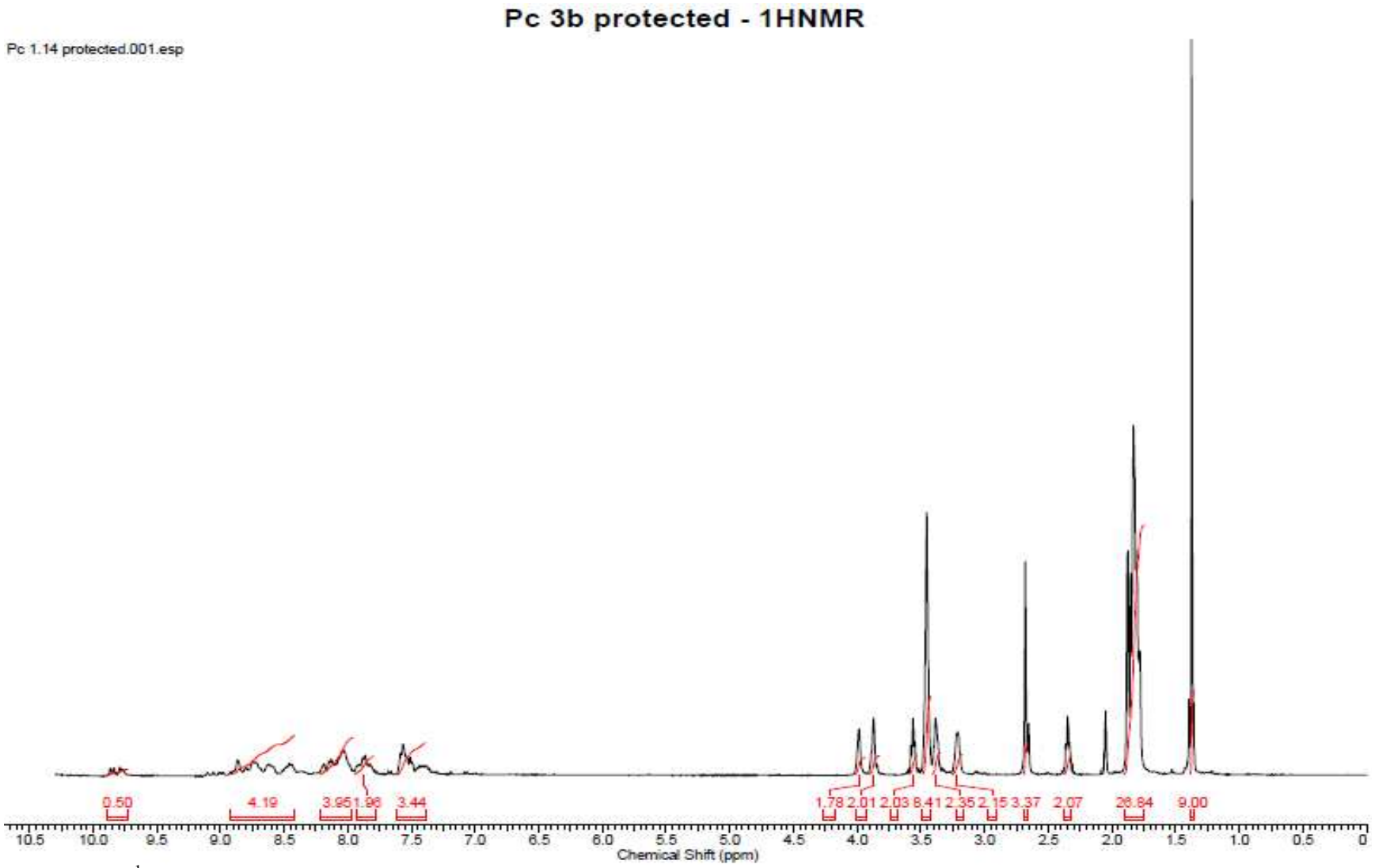


Figure S43. [1]HNMR spectrum for protected Pc **3b**

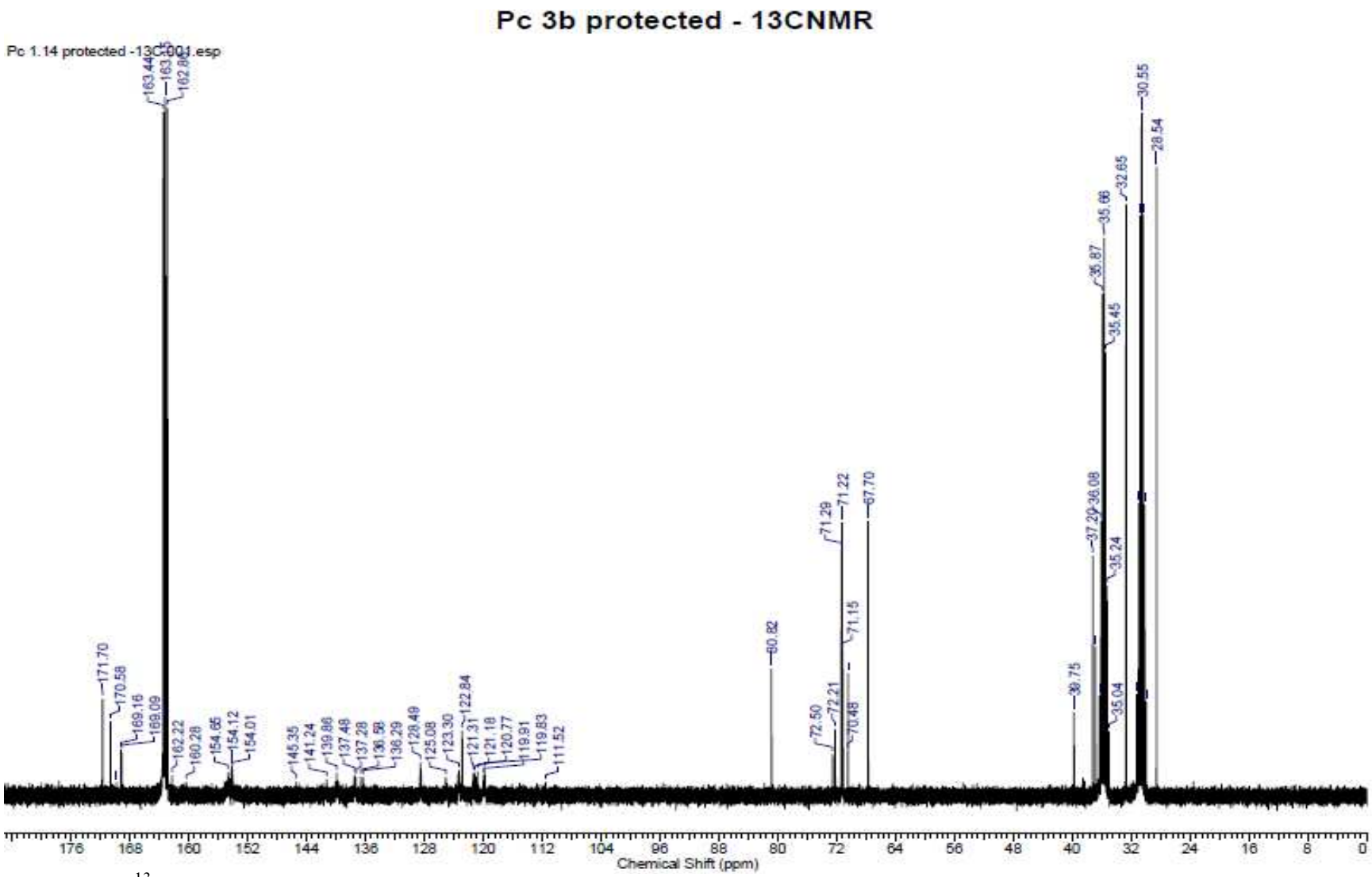


Figure S44. ^{13}CNMR spectrum for protected Pc **3b**

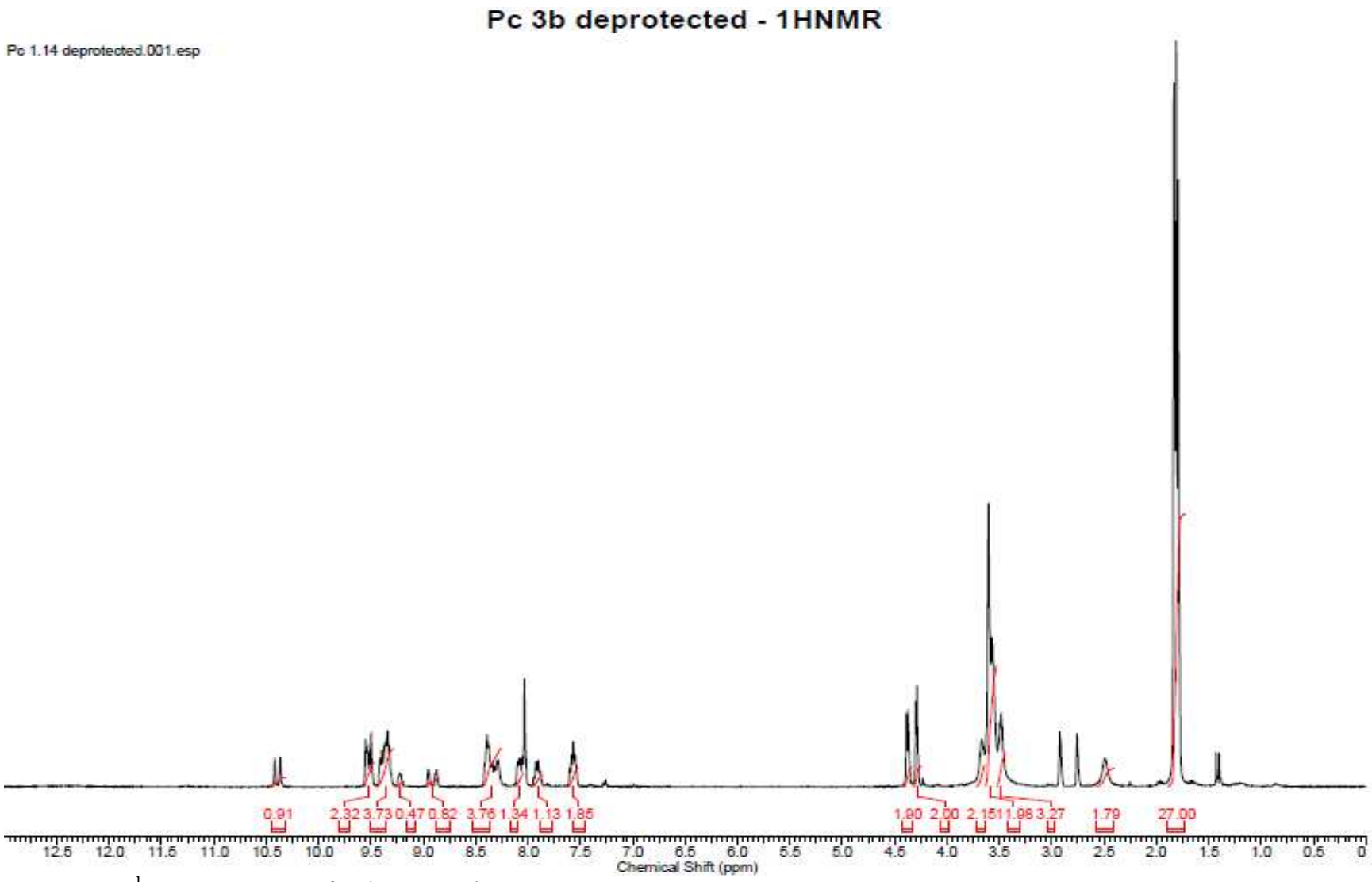


Figure S45. [1]HNMR spectrum for deprotected Pc **3b**

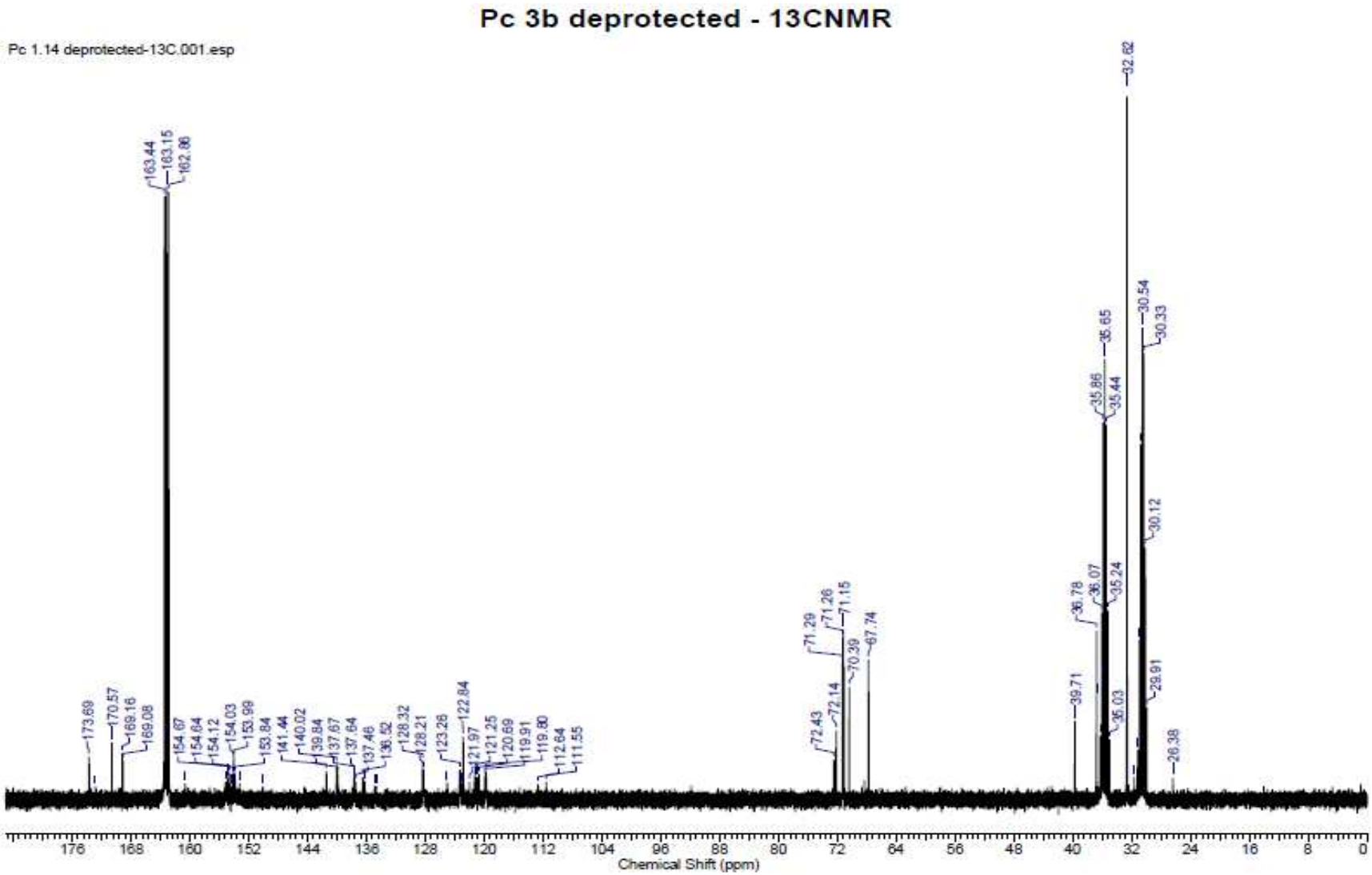


Figure S46. ^{13}CNMR spectrum for deprotected Pc **3b**

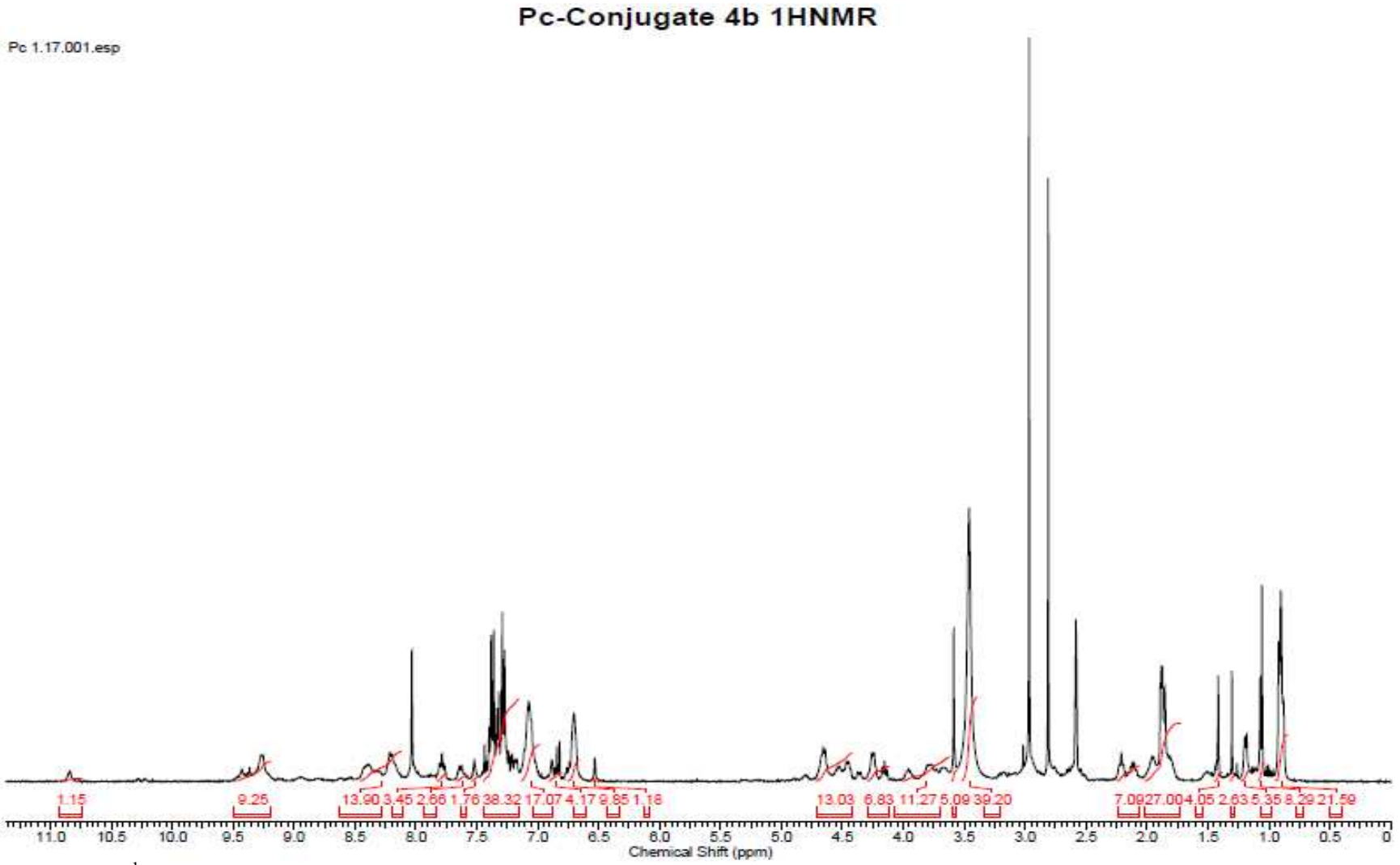


Figure S47. [1]HNMR spectrum for Pc-conjugate **4b**

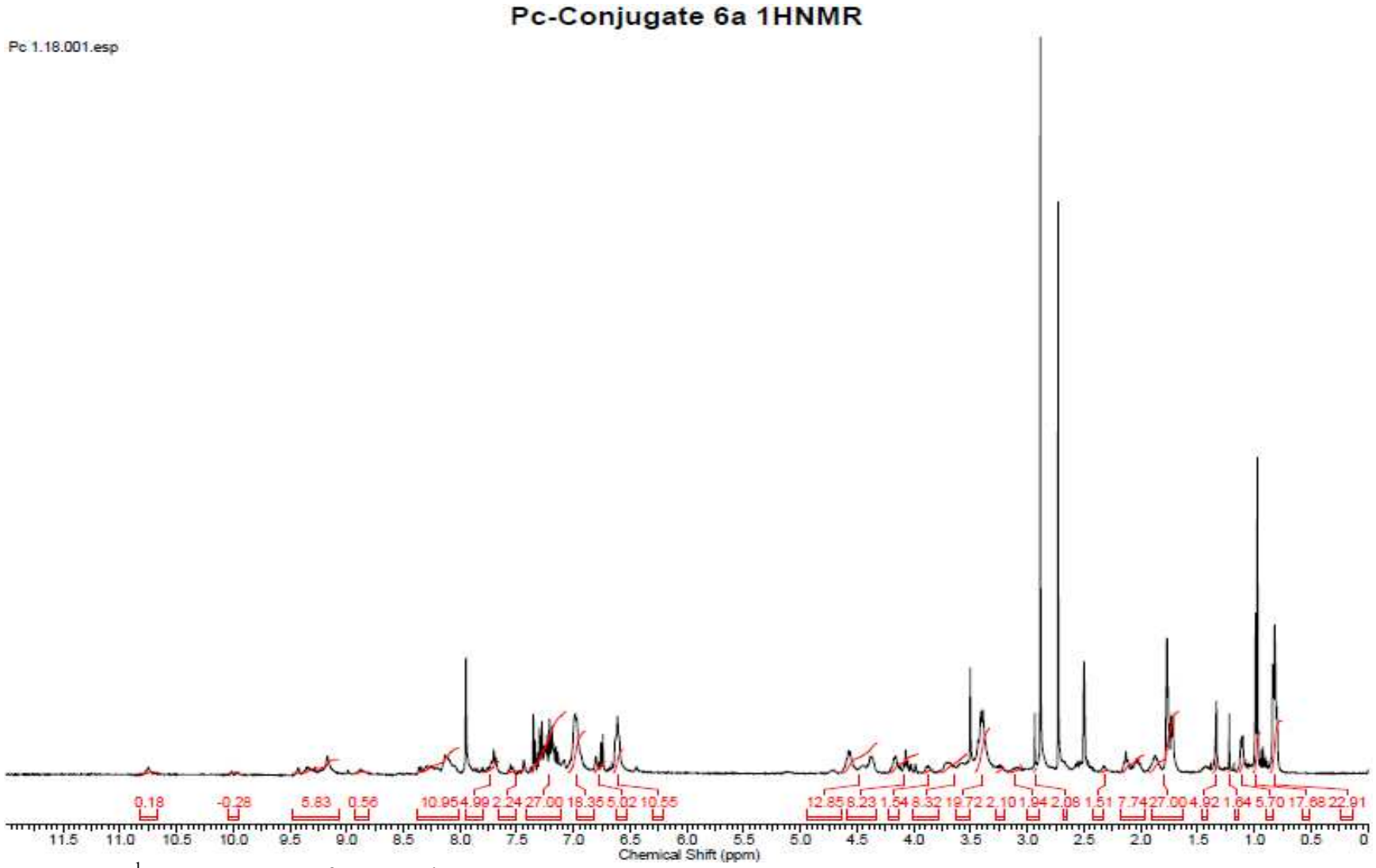


Figure S48. [1]HNMR spectrum for Pc-conjugate **6a**

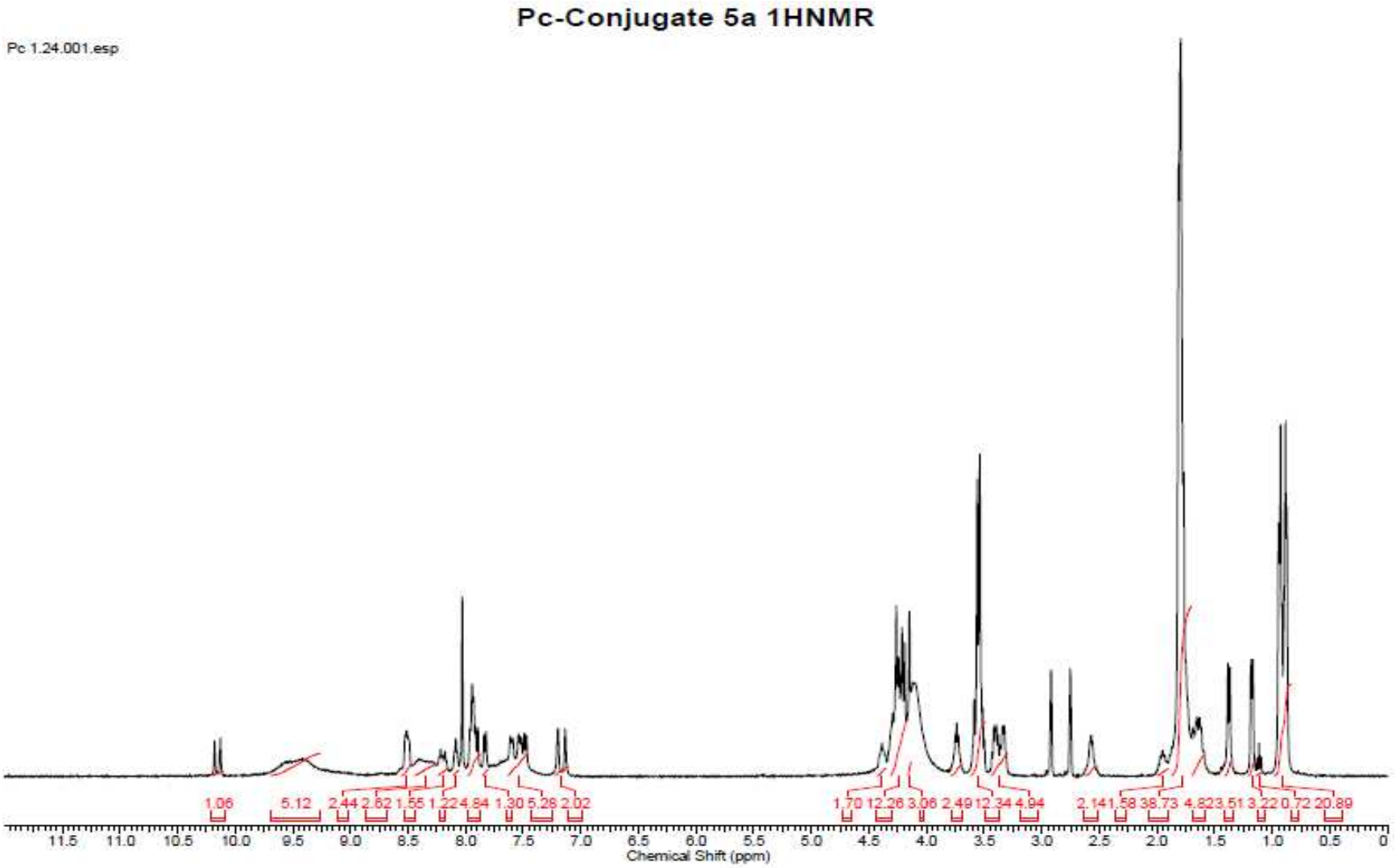


Figure S49. [1]HNMR spectrum for Pc-conjugate **5a**

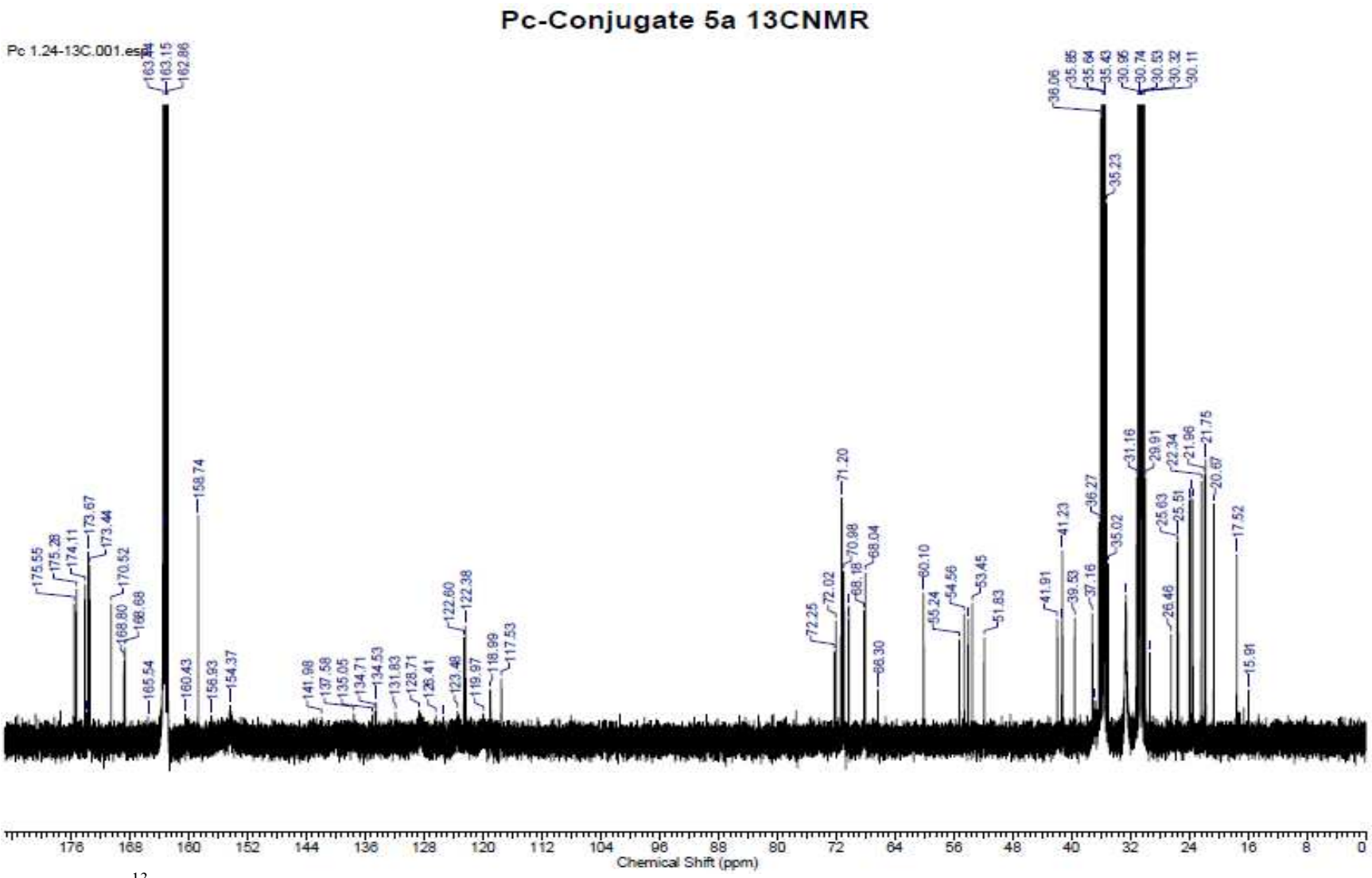


Figure S50. ^{13}CNMR spectrum for Pc-conjugate **5a**

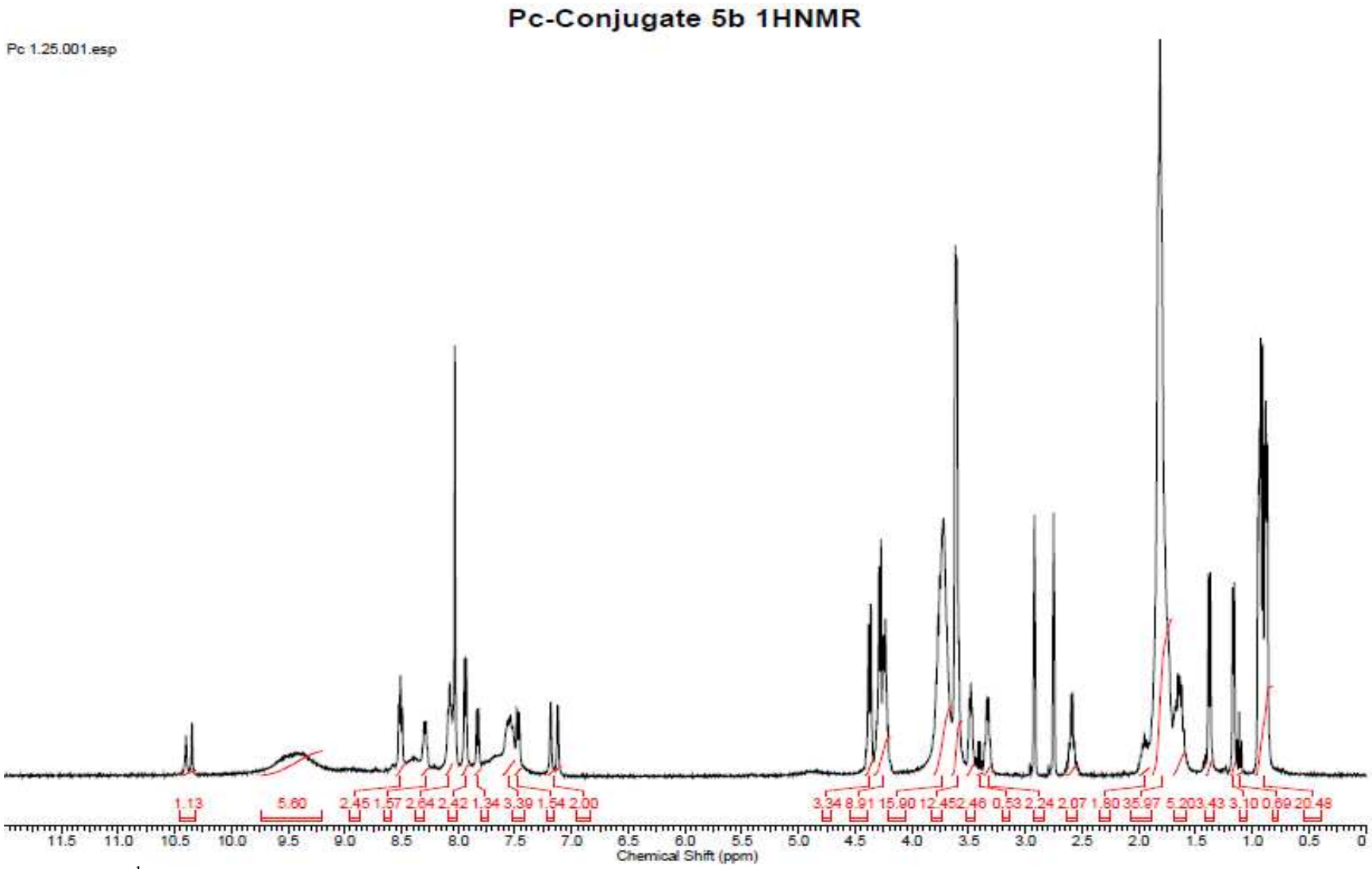


Figure S51. [1]HNMR spectrum for Pc-conjugate **5b**

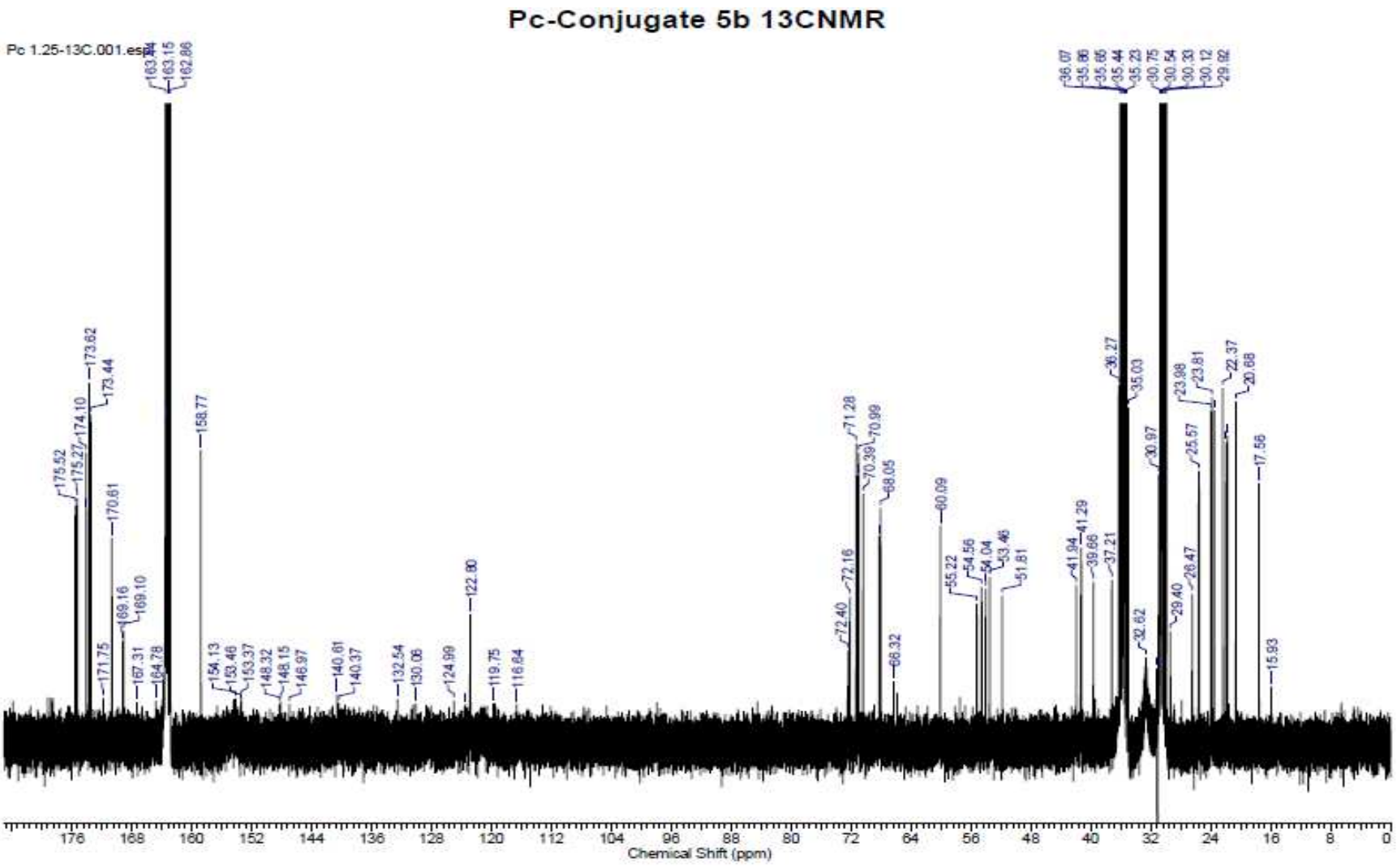


Figure S52. ^{13}CNMR spectrum for Pc-conjugate **5b**

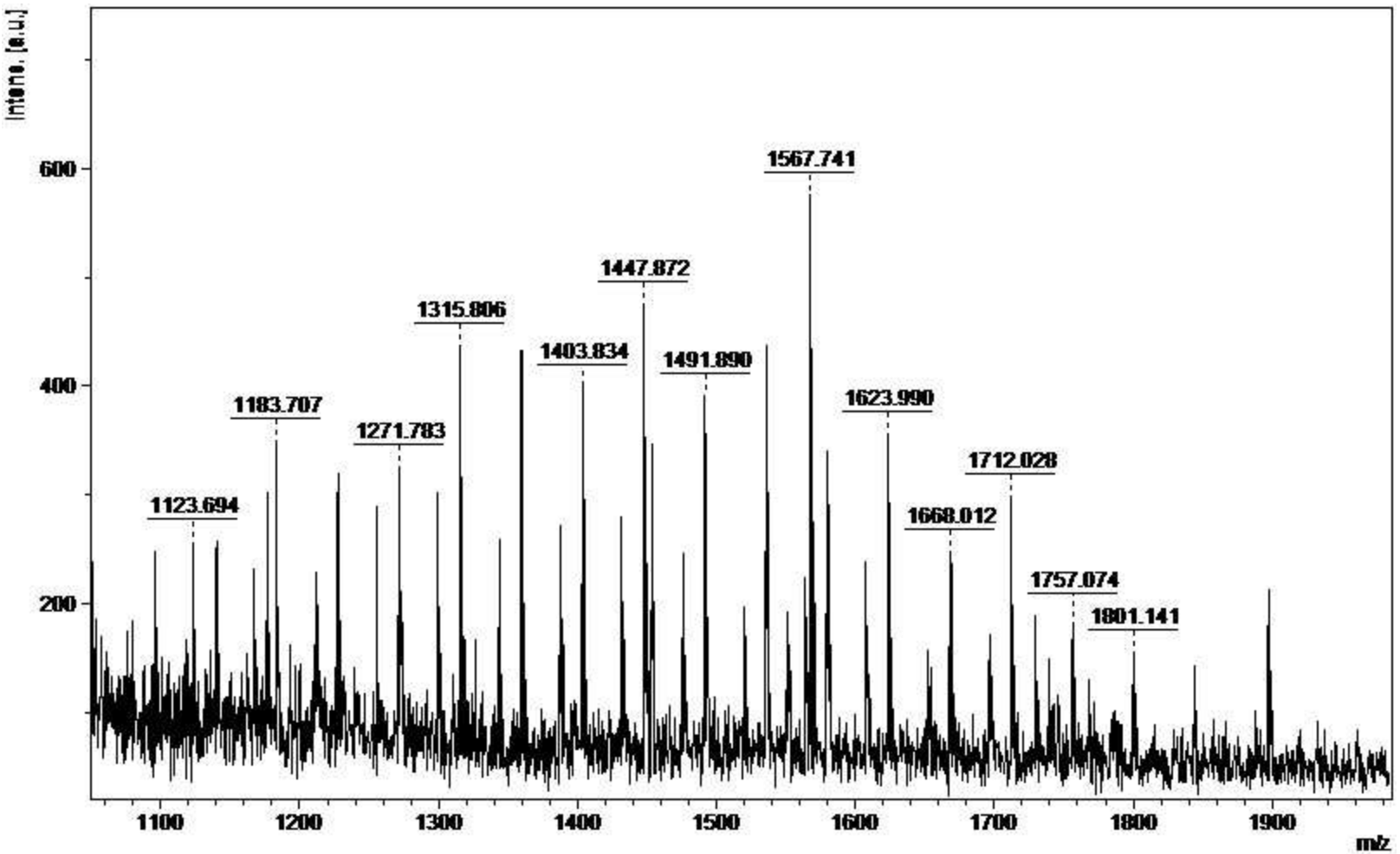


Figure S53. MALDI-TOF (MS) spectrum for Pc-conjugate **5b** extract from HT-29 mouse-tumor after one day

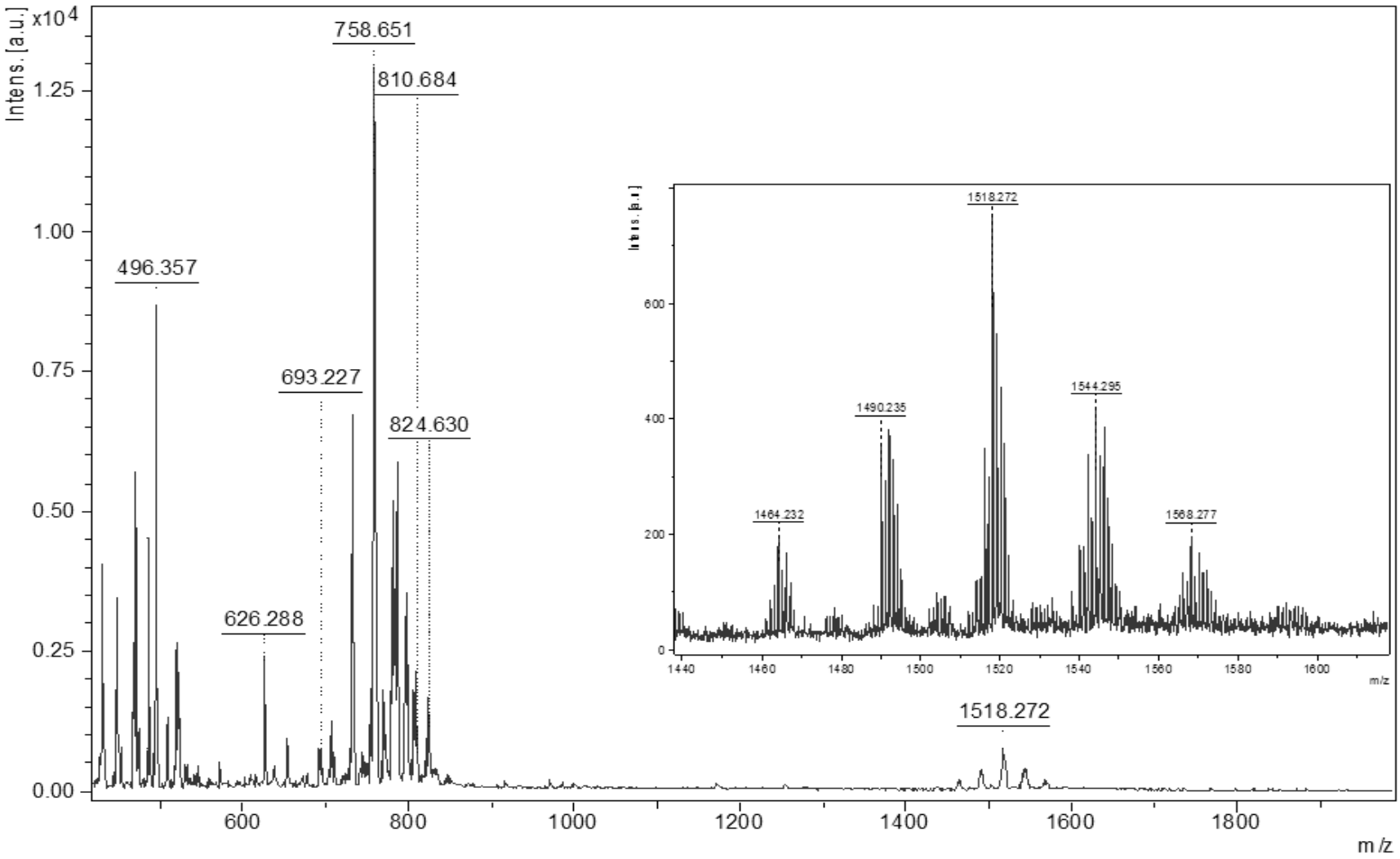


Figure S54. MALDI-TOF (MS) spectrum for Pc-conjugate **5b** extract from HT-29 mouse-tumor after four days

Low Energy Docked Structures

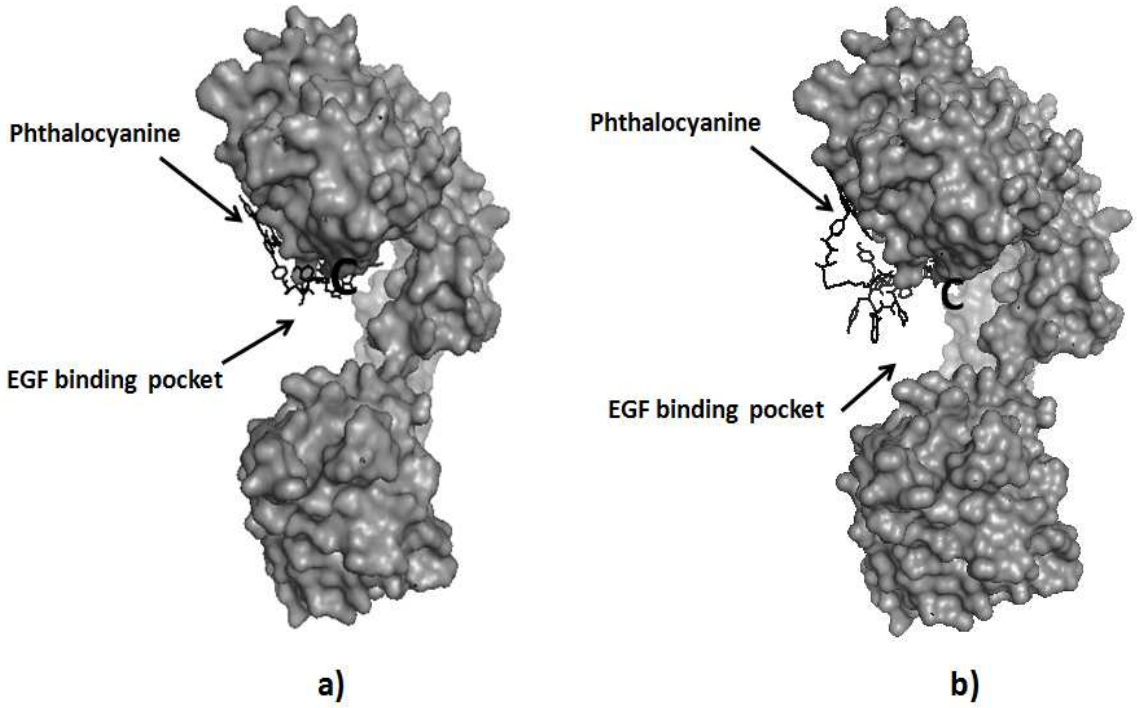


Figure S55. Low energy docked structures of a) **4b**, and b) **6a**. Notice that the peptide part of the conjugates are bound near EGF binding pocket of EGFR whereas, pthalocyanine part is anchored to the hydrophobic region outside EGF binding pocket.

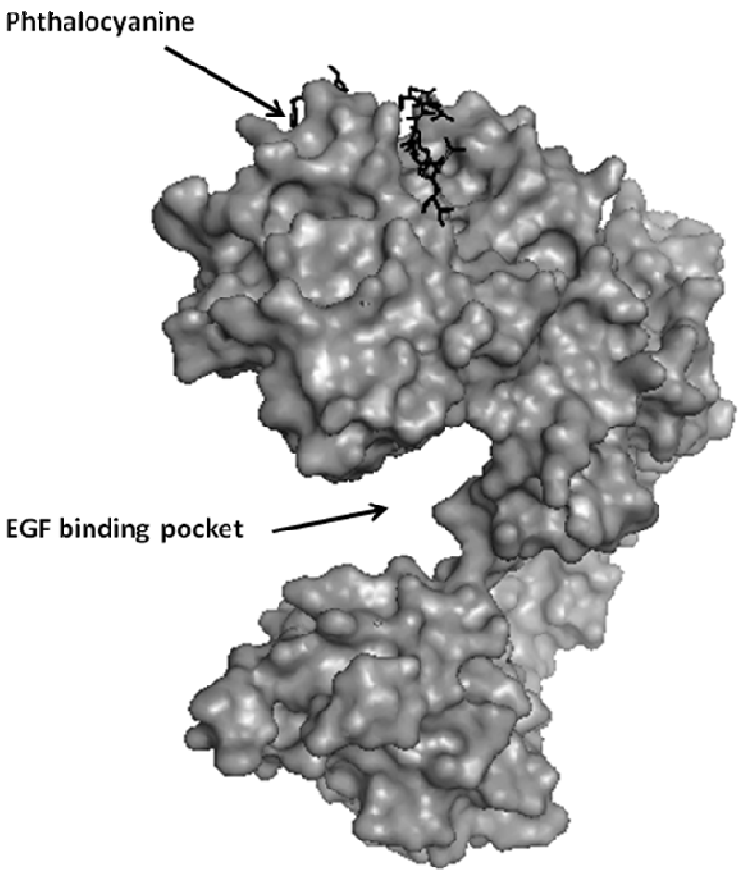
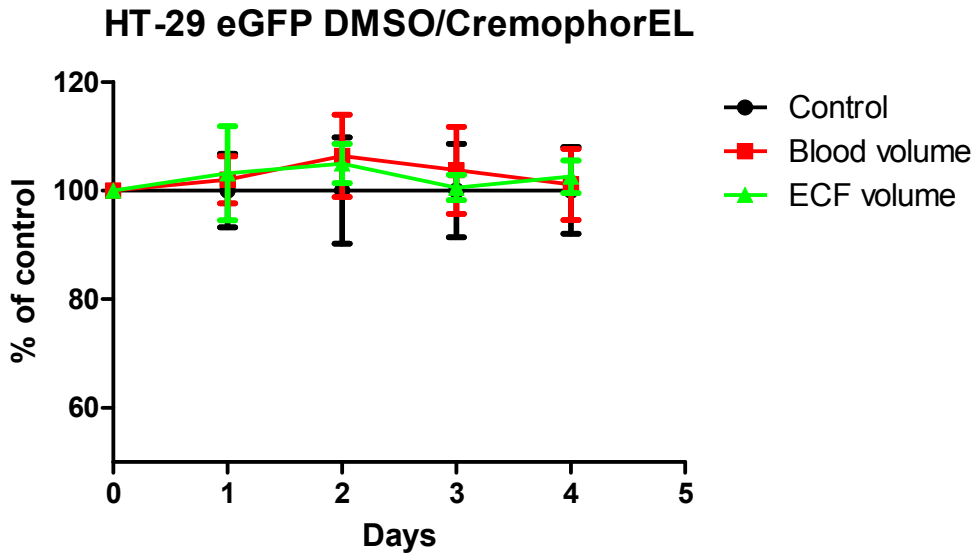


Figure S56. Low energy docked structure of **5b**. Notice that the peptide part of the conjugates are bound away from EGF binding pocket in a grove on EGFR whereas, phthalocyanine part is outside the grove.

Effect of DMSO and Cremophor on HT-29 eGFP and A431 cells

a)



b)

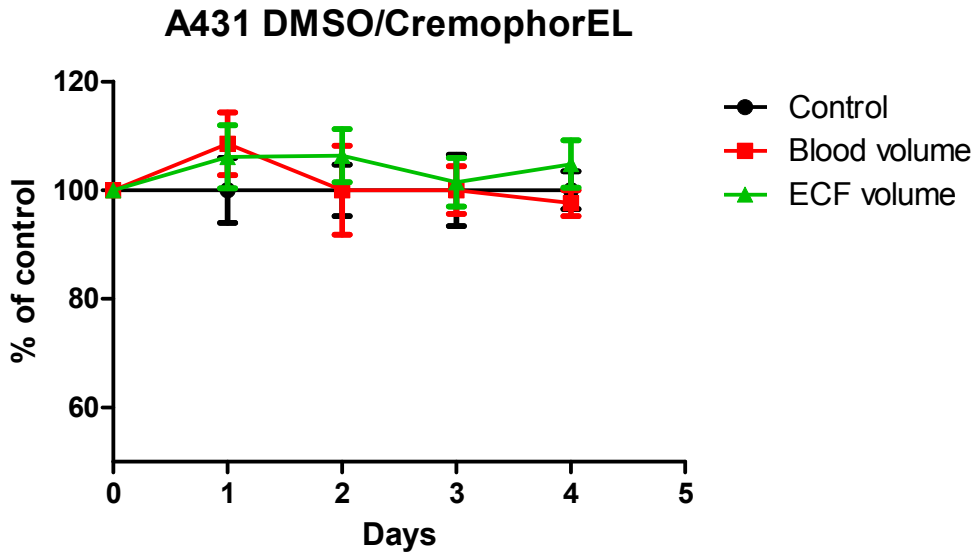


Figure S57. Effect of DMSO and Cremophor on a) HT-29 eGFP and b) A431 cells

Methods: To rule out toxicity related to the concentration of DMSO (10%) and Cremophor EL (5%) used to solubilize the conjugates, an additional viability assay was performed in vitro to estimate cancer tissue exposure in vivo. In vivo, the intravenous injection bolus (10 mg of Pc conjugate/Kg b.wt.) is immediately diluted first, by the mouse's blood volume and later by distribution into the extracellular fluid. Therefore 10% DMSO/5% Cremophor EL was added to media to a dilution matching that of a 20 µl injection bolus in the total blood volume of a mouse (based on body weight) and to a dilution matching that of a 20 µl injection in the extracellular fluid volume. The volume of 20 µL was used since this volume was the average quantity of conjugate administered IV to mice which averaging 35 grams each. These dilutions likely overestimate the level of DMSO and Cremophor exposure to cells since they assume no metabolism or intracellular uptake.

Addition of DMSO and Cremophor to culture media was tested on HT-29 eGFP and A431 cells. Each cell line was plated into 24-well plates at a seeding density of 5000 cells/cm2 and allowed to attach overnight. Cell cultures were then washed with D-PBS and cultured with DMEM (without serum). The DMSO and Cremophor EL mixture was then diluted into the media at 1:148 representing the level of dilution into total blood volume of the mouse (84.7 ml/Kg, C. Riches, J. G. Sharp, D. Brynmor Thomas, and S. Vaughan Smith. Blood volume determination in the mouse. J Physiol. **1973** January; 228(2): 279–284) and 1:350 representing dispersion throughout the extracellular fluid volume (20% body weight, Neil E Rowland. Food or Fluid Restriction in Common Laboratory Animals: Balancing Welfare Considerations with Scientific Inquiry. Comparative Medicine. Vol 57, No 2, April **2007**. Pages 149–160). Viability of each culture was measured over 4 days (96 h) using Calcein blue AM (purchased from Invitrogen) loaded cells. Calcein is a viability dye taken and metabolized into a fluorophor by live cells. The fluorescence of treated cells was determined as a percent of control (untreated) cells each day.

Results: Addition of DMSO and Cremophor to culture media (Figure **S57**) at either blood volume or extracellular fluid volume dilutions showed no effect on cell viability over a 96 h period suggesting it is unlikely that these agents resulted in toxicity *in vivo* after dilution and distribution. In addition, the concentration of Cremophor in the blood volume dilution exceed that used for the uptake, dark toxicity and phototoxicity culture assays suggesting that it did not contribute to toxicity in these assays.

SPR Experiments

Table S1. Summary of measured resonance units (RU) for binding of peptides and Pc conjugates to EGFR using SPR (according to W. D. Wilson, Science **2002**, *295*, 2103), using 100 μM concentrations of all analytes.

Table 1: Binding of Conjugates to EGFR			
#	analyte	RU after 1 s	RU after 2 s
1	Pc **3b**	159.2 RU	52.6 RU
2	EGFR-L1	149.5 RU	35RU
3	Pc **5b**	172.3 RU	92.2 RU
4	EGFR-L2	209.3 RU	56.3U
5	Pc **6a**	215.3 RU	64 RU

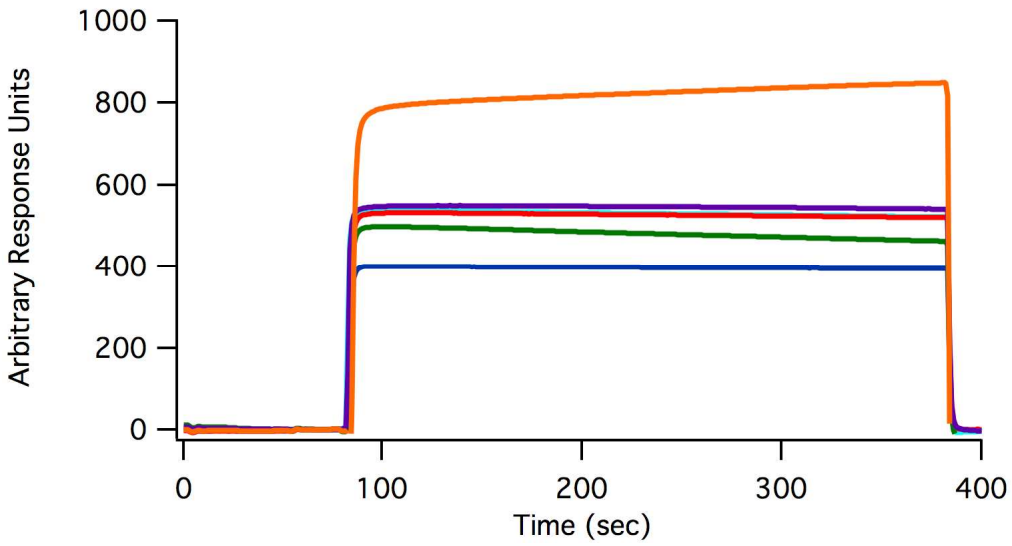


Figure S58. Sensogram showing the interaction of EGF (orange), peptides EGFR-L1 (green), EGFR-L2 (purple) and Pc conjugates **3b** (blue), **5b** (turquoise) and **6a** (red) with EGFR immobilized on a CM5 sensor chip (see Table S1 above for RU values). Background (solvent/buffer) not subtracted.