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

Supporting Information

Photoswitcable Anticancer Activity via *trans-cis* Isomerization of a Combretastatin A-4 Analog

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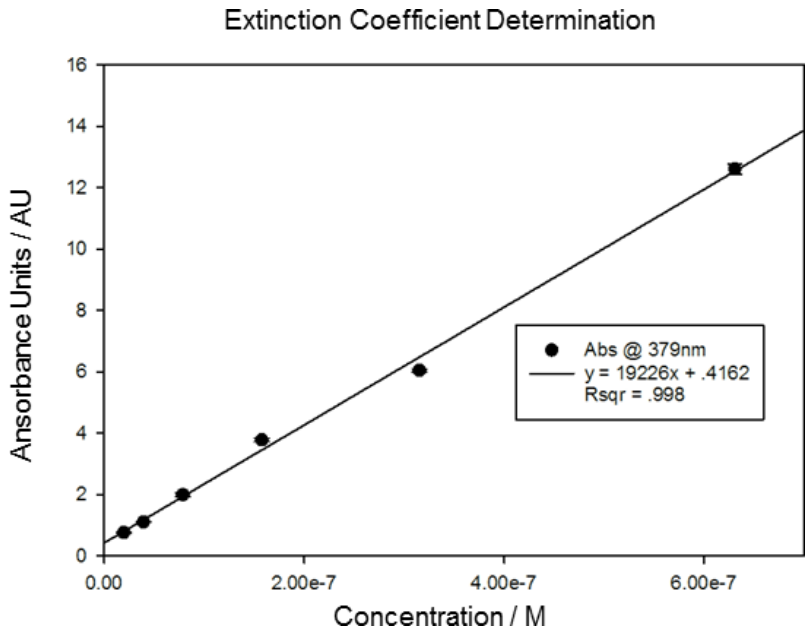


Figure S1. Determination of the molar absorptivity of Azo-CA-4. Absorbance's of Azo-CA4 (1.26 mM) and 2-fold serial dilutions were measured on a NanoDrop ND-1000 spectrophotometer with a 1 mm pathlength and were multiplied by 10 to convert to a 1 cm pathlength.

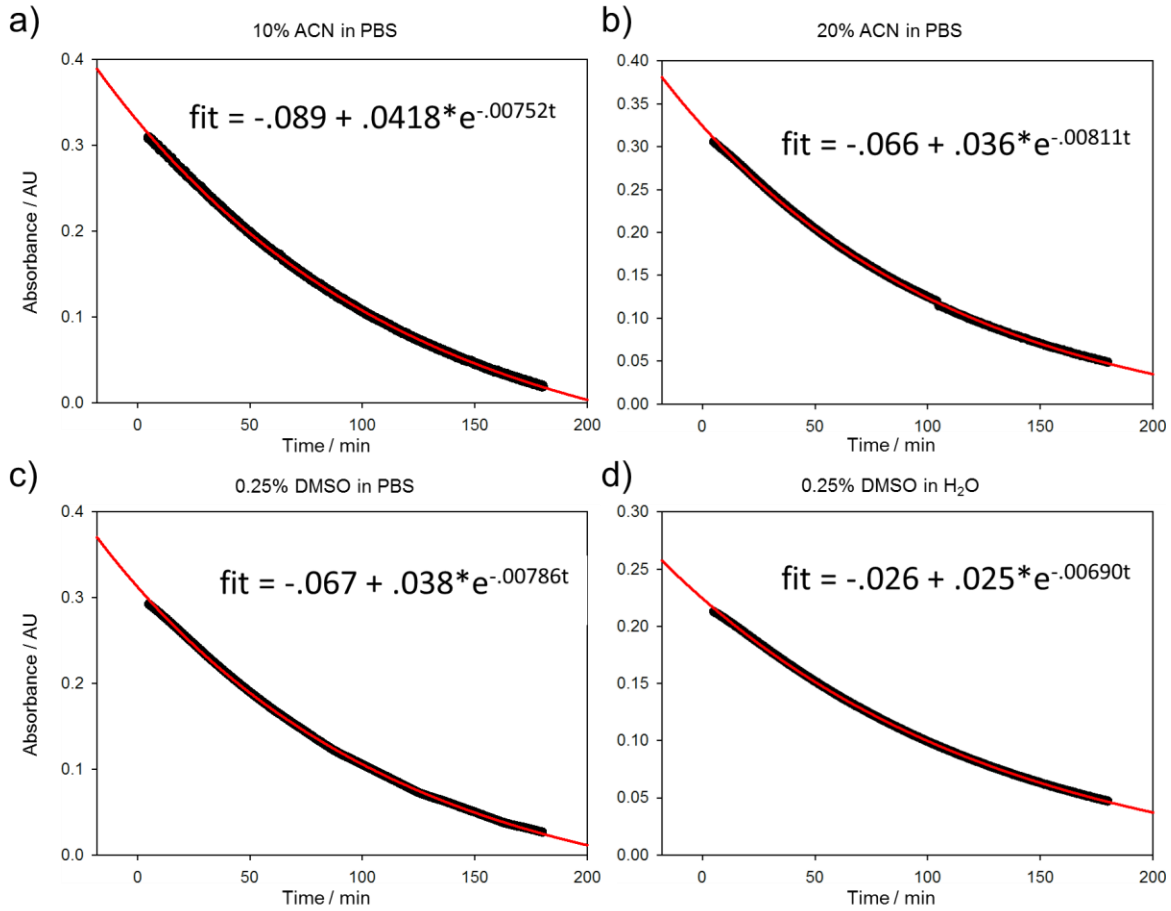


Figure S2. Exponential decay of absorbance of Azo-CA4. A solution of 300 µM Azo-CA4 in a) 10% acetonitrile in PBS, b) 20% acetonitrile in PBS, c) 0.25% DMSO in PBS and d) 0.25% DMSO in H_2O was irradiated with 380 nm light (4.4 mW/cm2) for 1 minute. Absorbance readings were taken from triplicate wells every 1 min for 3 h and subtracted from the fully relaxed absorbance reading. Data was fitted to a 3-parameter exponential curve and equation is shown on corresponding plots. The curve fit is shown in red.

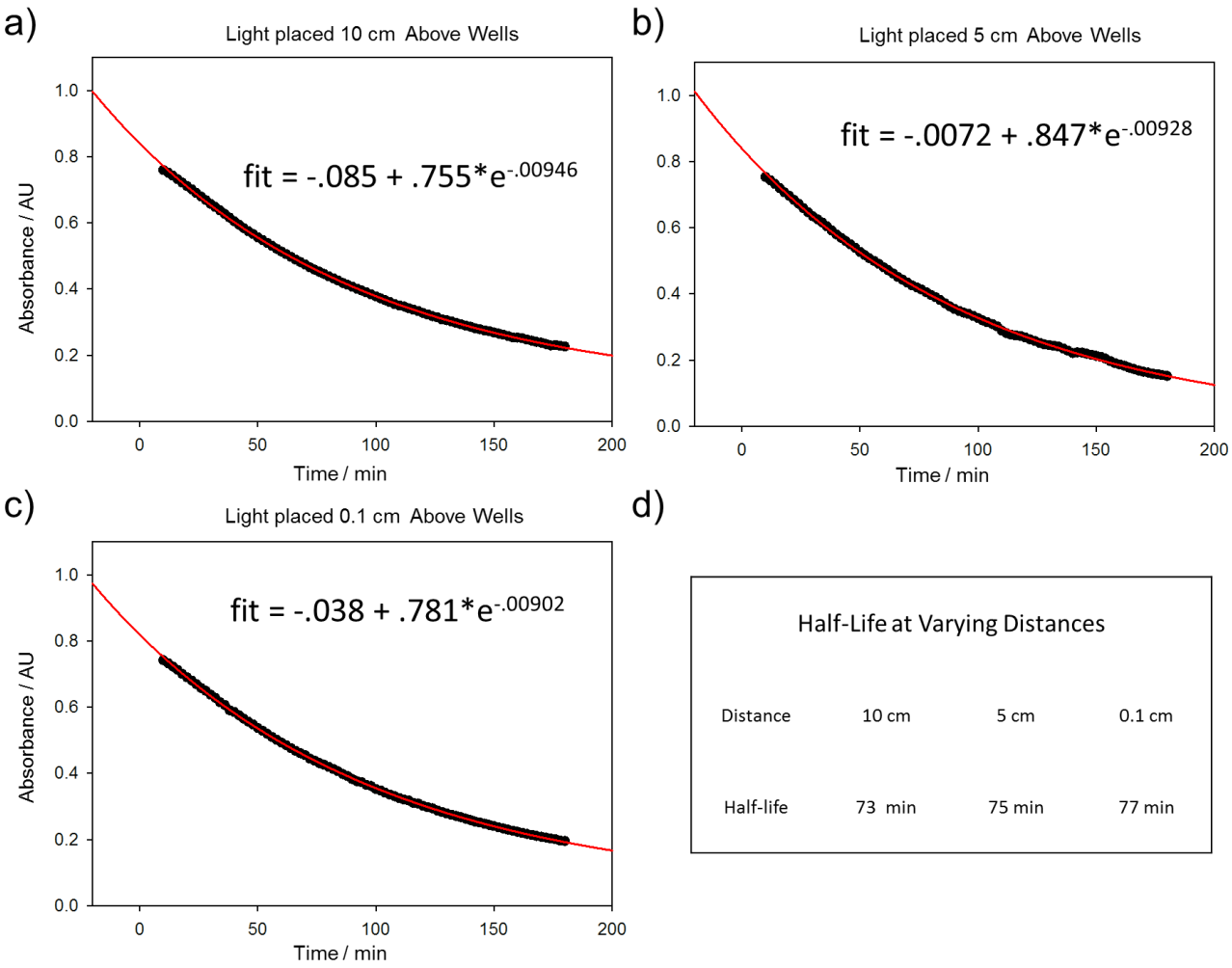


Figure S3. Effect of initial light intensity on the half-life of thermal relaxation. A solution of 300 μM Azo-CA4 in 0.9 % DMSO in PBS were measured for absorbance after 1 minute of irradiation at 380 nm at a) 10 cm height above wells, b) 5 cm above wells, and c) 0.1 cm above wells. Absorbance readings were taken from triplicate wells every 2 min for 3 h and subtracted from the fully relaxed absorbance reading. Data was fitted to a 3-parameter exponential curve and equation is shown on corresponding plots.

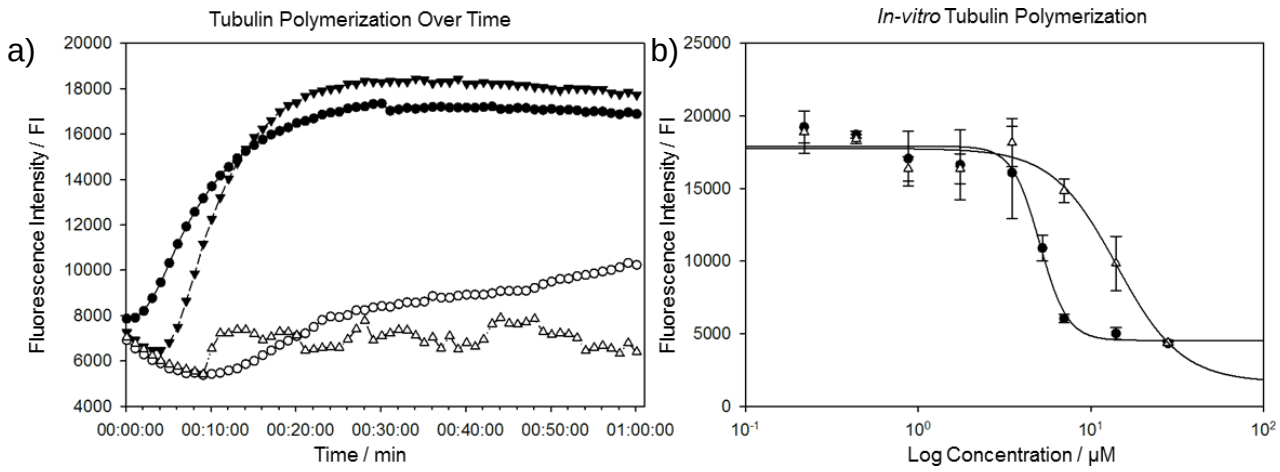


Figure S4. Effect of Azo-CA4 on tubulin polymerization. (a) Effect of Azo-CA4 on polymerization of tubulin monitored over time as measured by a fluorescence polymerization assay. Closed triangles—Vehicle control; Closed circles--Azo-CA4 in the dark (20µM); Open circles—Azo-CA4 (20 µM) + light, and Open triangles CA4 (20 µM). Experiments were performed in triplicate. (b) Measurement of the IC_{50} for tubulin polymerization inhibition by Azo-CA4. Fluorescence was measured 30 minutes after the start of the assay. Closed circles represent tubulin containing wells irradiated with 380 nm light (4.4 mW/cm^2) for 1 min in the presence Azo-CA4 prior to the start of the assay. Open triangles represent tubulin exposed to Azo-CA4 without illumination.

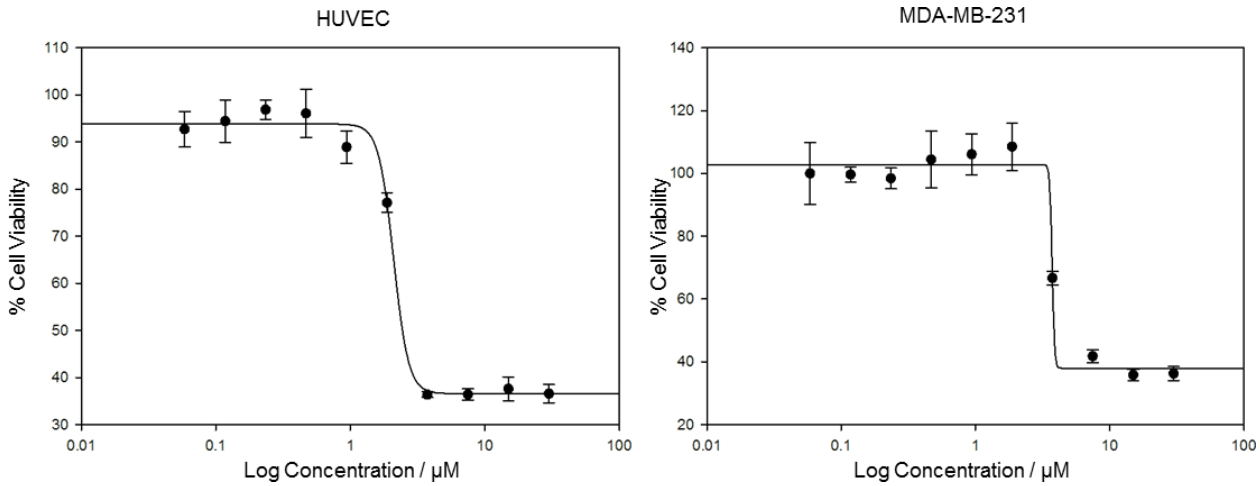


Figure S5. Cell viability assays with Combretastatin A4 (CA4). HUVEC cells were seeded at 1500 cells per well (left) and MDA-MB-231 were seeded at 10,000 cells per well (right) for six hours. CA4 (Sigma) was added to the wells in concentrations from 0.06 – 30 nM and incubated at 37°C for 48 hours. At the end of the incubation period 20µL cell titer blue was added to the wells and incubated for an additional 2 hours. Fluorescence was normalized against controls.

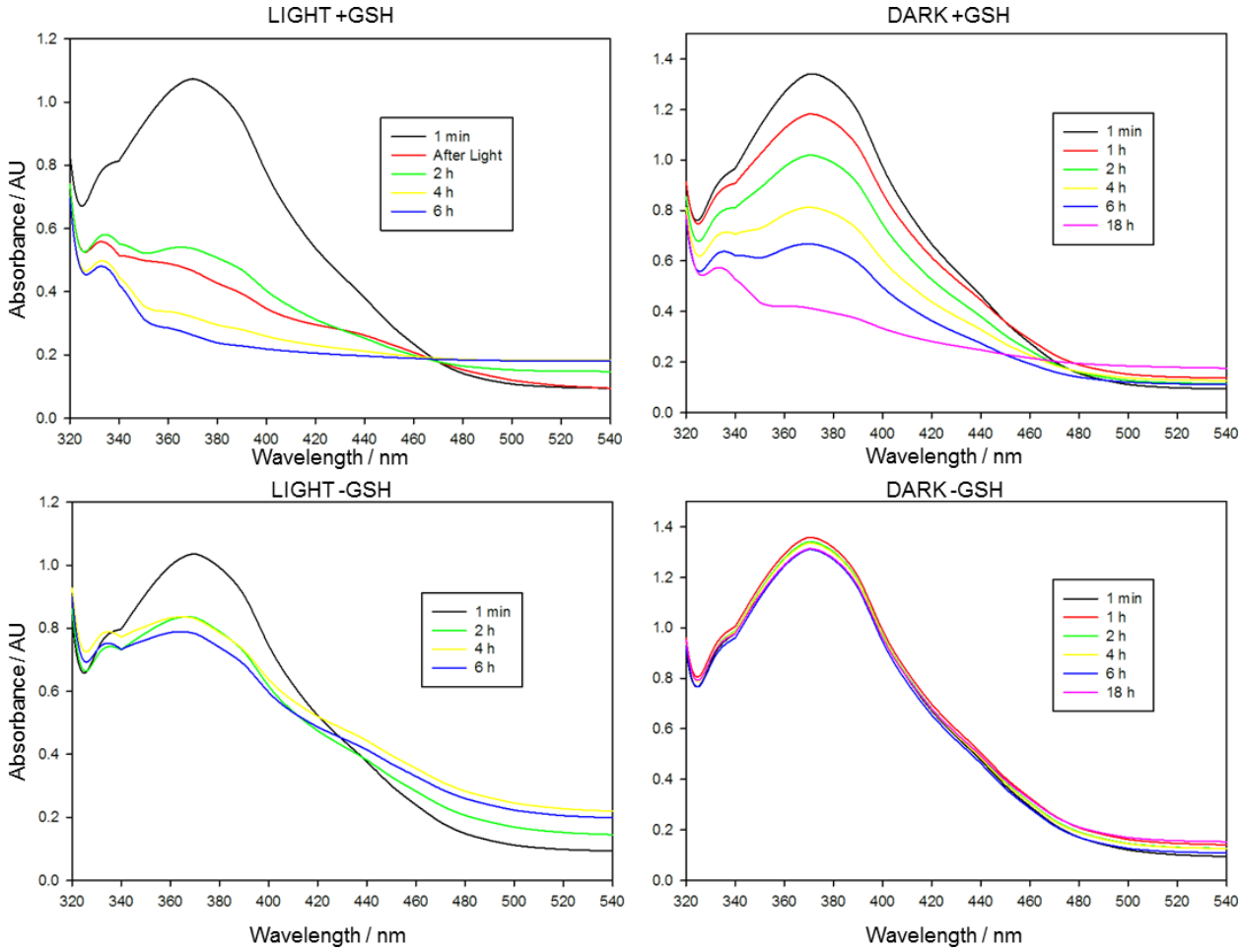


Figure S6. Stability of Azo-CA4 to glutathione. Azo-CA4 (300 μM in 5.7% DMSO / PBS pH 7.2) in the presence (top) or absence (bottom) of fully reduced glutathione (10 mM in PBS pH 7.2) and TCEP (5 mM in PBS pH 7.2) was placed in 3 separate wells (100 μL / well) of a 96 well plate at 37°C and exposed to light (380 nm, 4.4 mW/cm^2) for 1 min every 2 hours (top left and bottom left) or protected from light (top right and bottom right). Absorbance spectra were taken at different time points directly preceding exposure to light and are expressed as an average of triplicate readings.

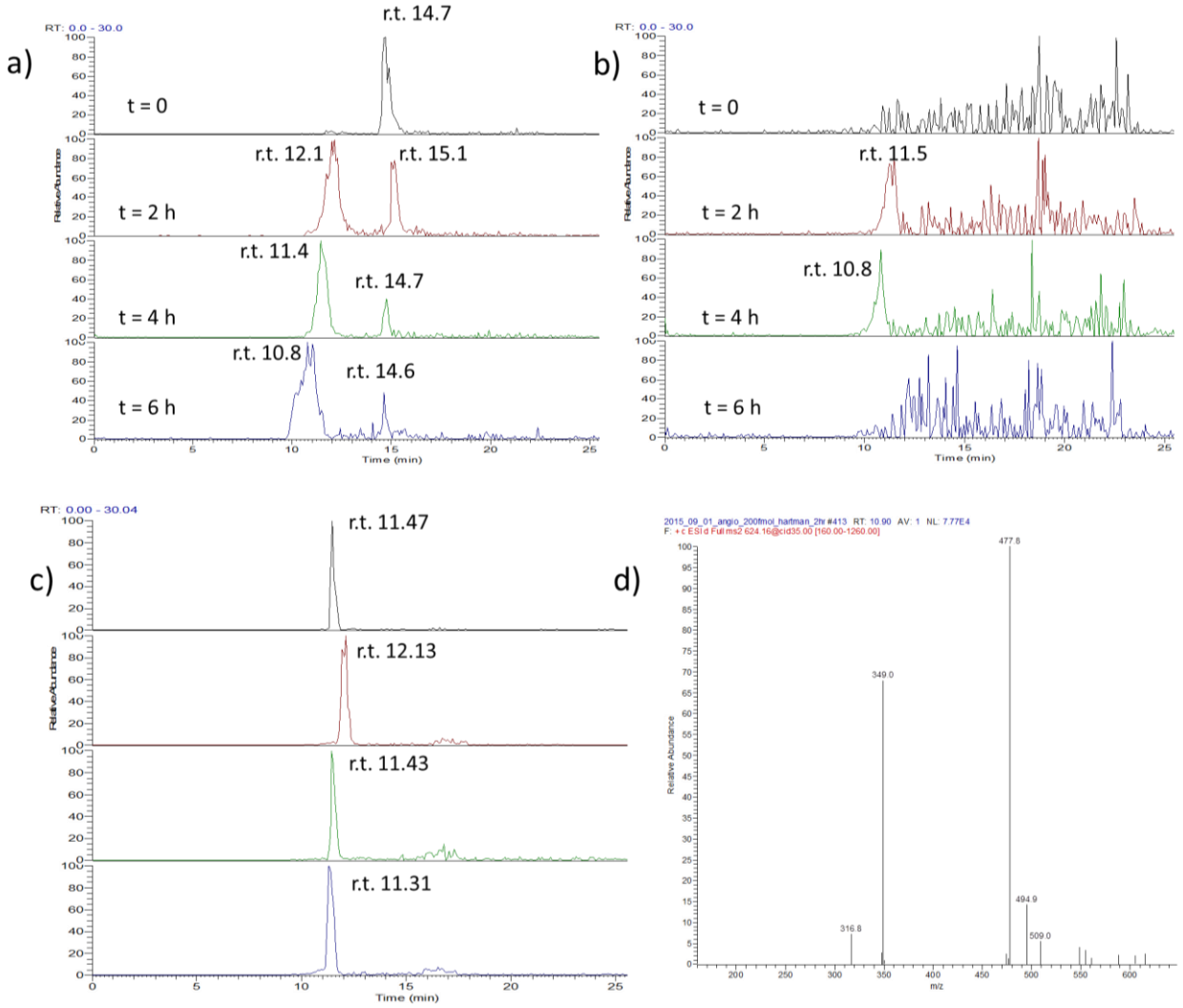


Figure S7. LC-MS Analysis of Azo-CA4 reaction with glutathione. (a) and (b) are repeated from Figure 5 in the manuscript and highlight the 319 and 614 Da peaks. Part (c) shows the retention time of the angiotensin standard added to each ESI-MS sample, highlighting the slight retention time drift due to manual loading of the capillary containing sample onto the instrument. (d) MS/MS spectrum of the 319 Da peak sampled at 11.88 min (t = 4 h). (c) MS/MS spectrum of the 624 Da peak sampled at 10.90 min (t = 2 h). Peaks at 317 (neutral loss of 307), 349 (neutral loss of 275), and 495 (neutral loss of 129) are all characteristic for glutathione adducts.

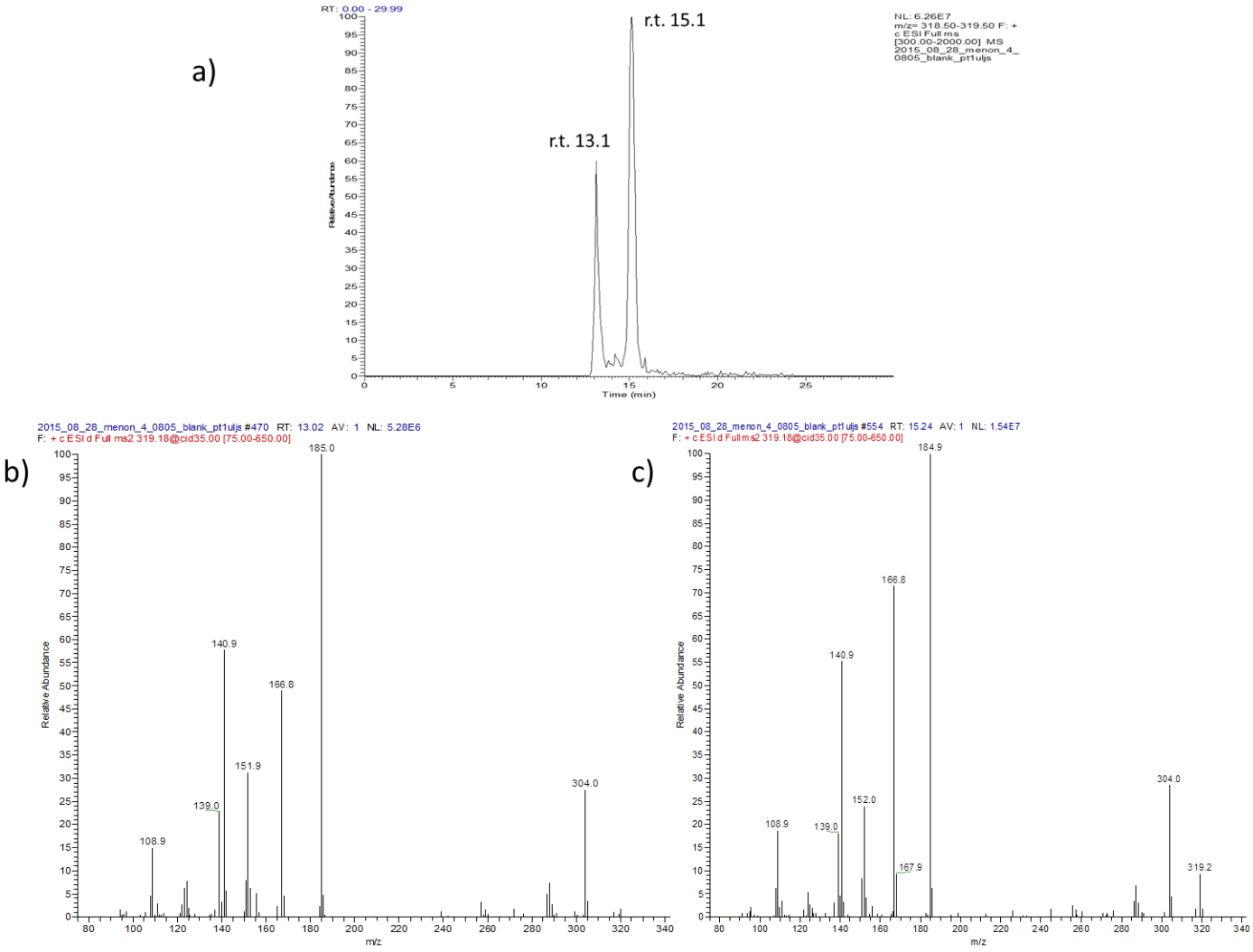


Figure S8. Analysis of *cis* and *trans* azo-CA4 by LC-MS. (a) LC-MS chromatogram of a mixture of *cis* and *trans* Azo-CA4. Peaks of corresponding to a mass of 319 Da are shown. Based on comparisons with other LC-MS experiments, the *trans* isomer has a retention time of 13.1 and the *cis* isomer has a retention time of 15.1. (b) MS/MS spectrum of the 319 Da peak with retention time of 13.1 min. (c) MS/MS spectrum of the 319 Da peak with retention time of 15.1 min.

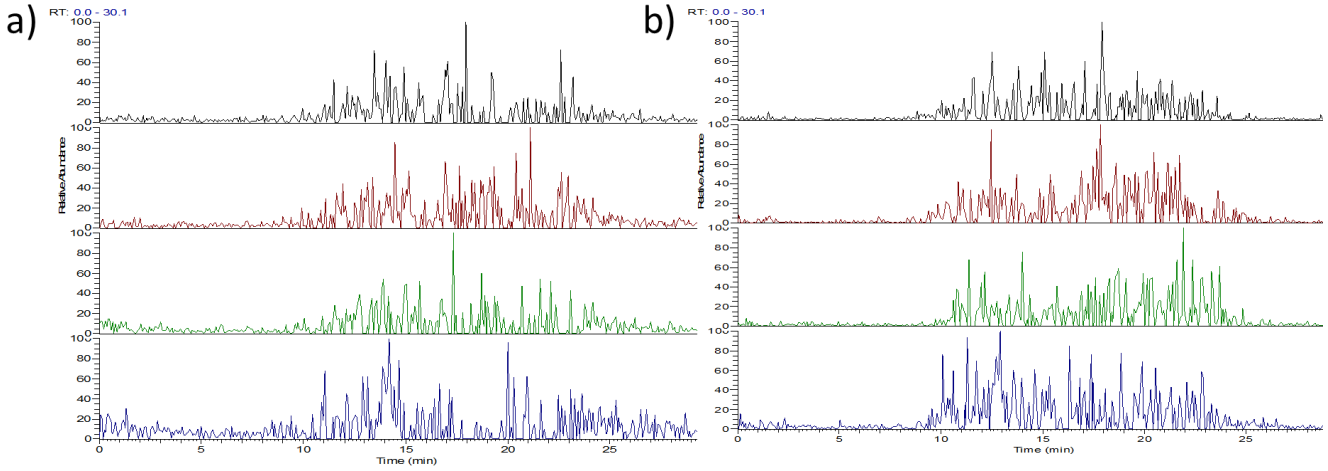


Figure S9. Evidence that the 319 and 624 Da peaks require Azo-CA4. Reactions were identical to those described in Figure 5, except they lacked Azo-CA4. From top to bottom the reactions were t = 0, 2, 4, and 6 h respectively. No significant peaks at 319 or 624 nm were observed.

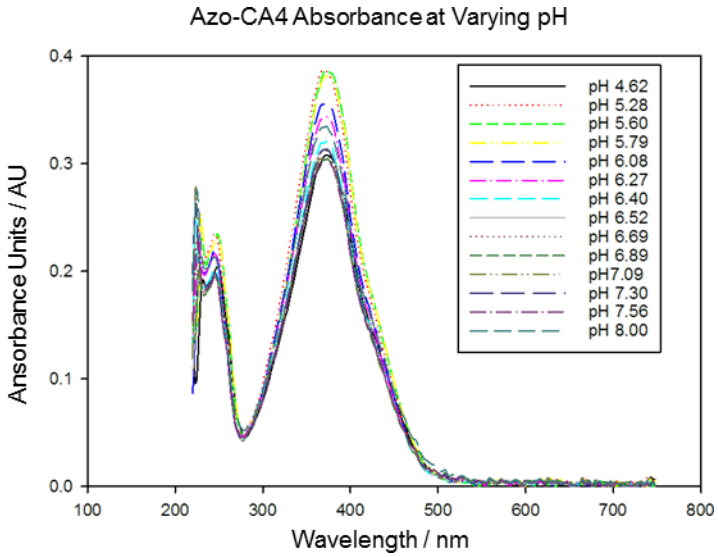
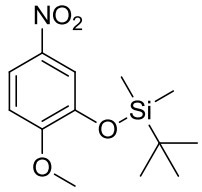


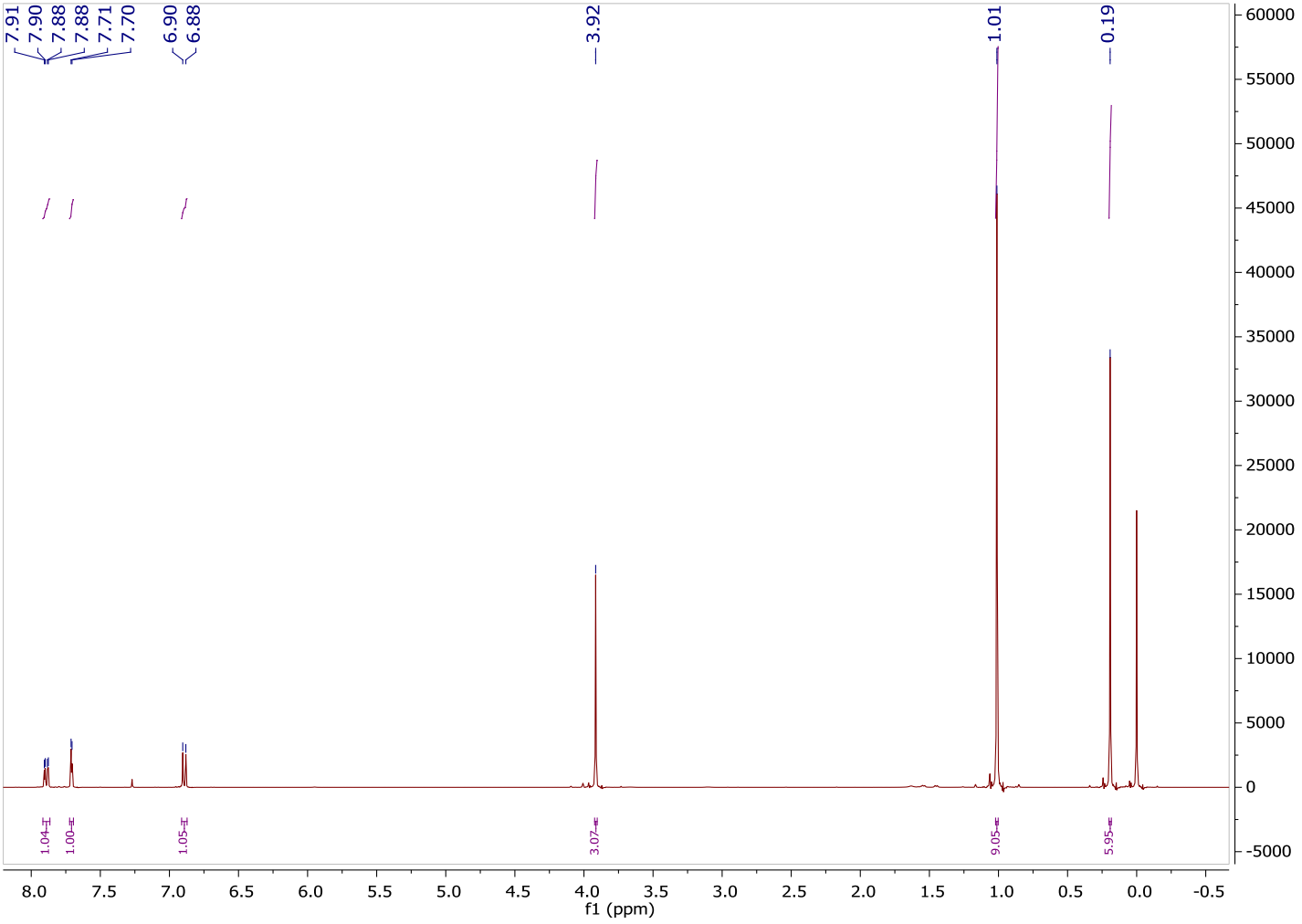
Figure S10. Absorbance of Azo-CA4 at different pH values. Absorbance spectra from 200-700 nm at different pH values (left). Absorbance spectra at 380 nm versus pH (right). All experiments were done in duplicate.

Table S1

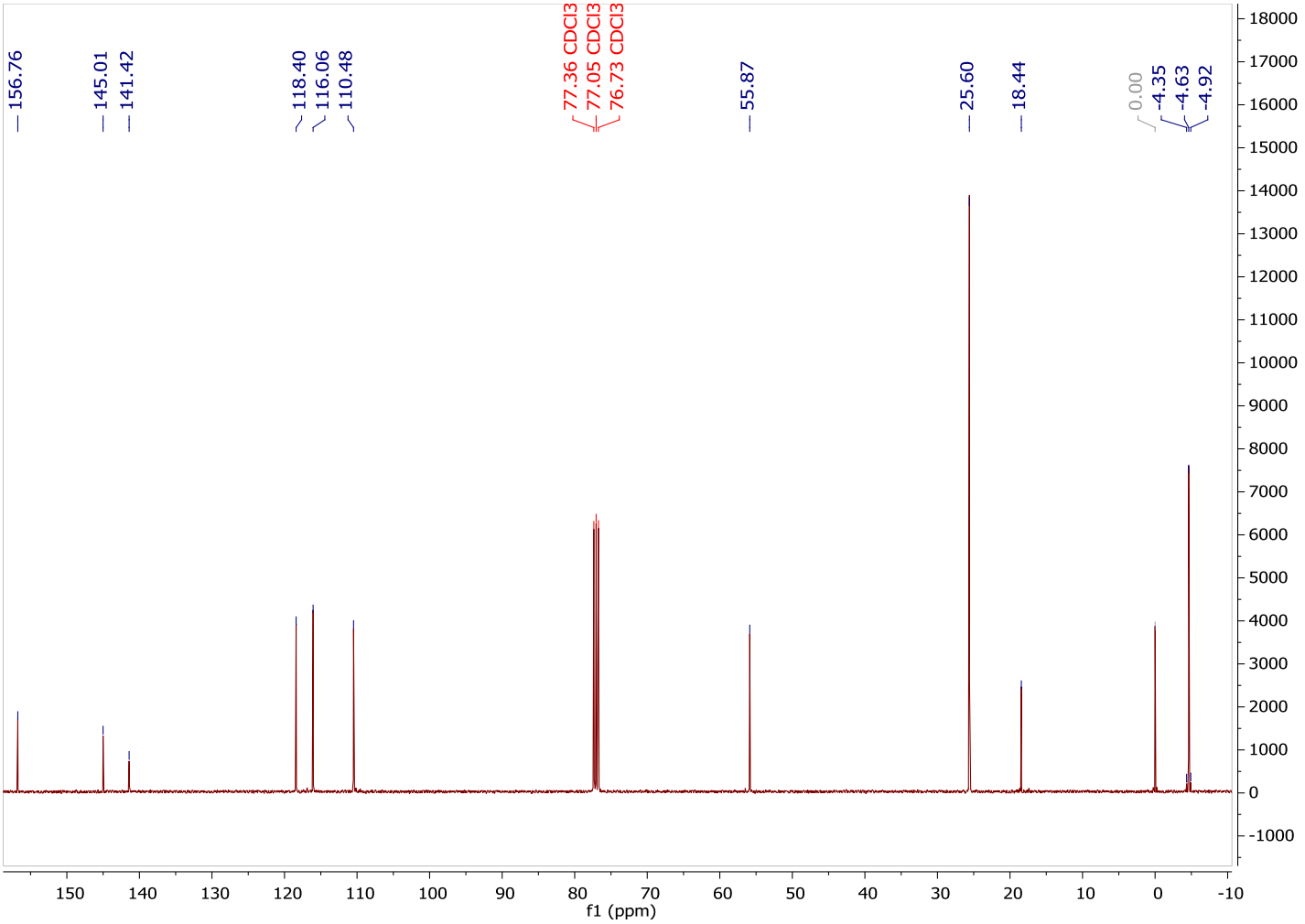
Conditions of Half-Life Measurement					
Media	0.25% DMSO in H_2O	0.25% DMSO in PBS	0.9% DMSO in PBS	10% ACN in PBS	20% ACN in PBS
Half-Life	100 min	88 min	75 min	92 min	85 min

^{1}H NMR of 3-((*tert*-butyldimethylsilyl)oxy)-4-methoxynitrobenzene, 1.

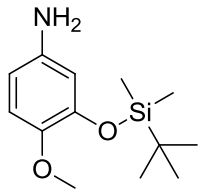


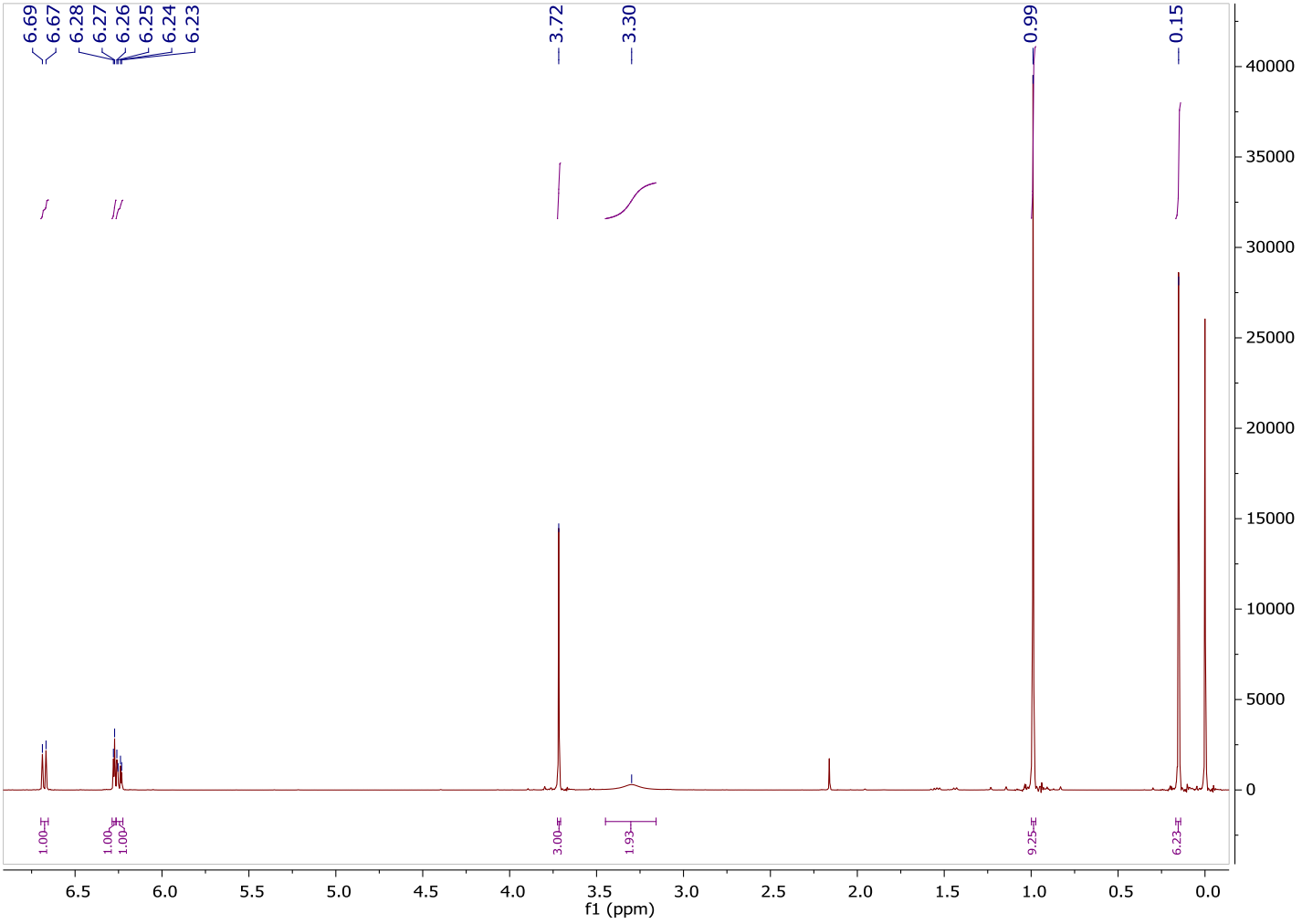


^{13}C NMR of 3-((*tert*-butyldimethylsilyl)oxy)-4-methoxynitrobenzene, 1.



^{1}H NMR of 3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyaniline, 2.





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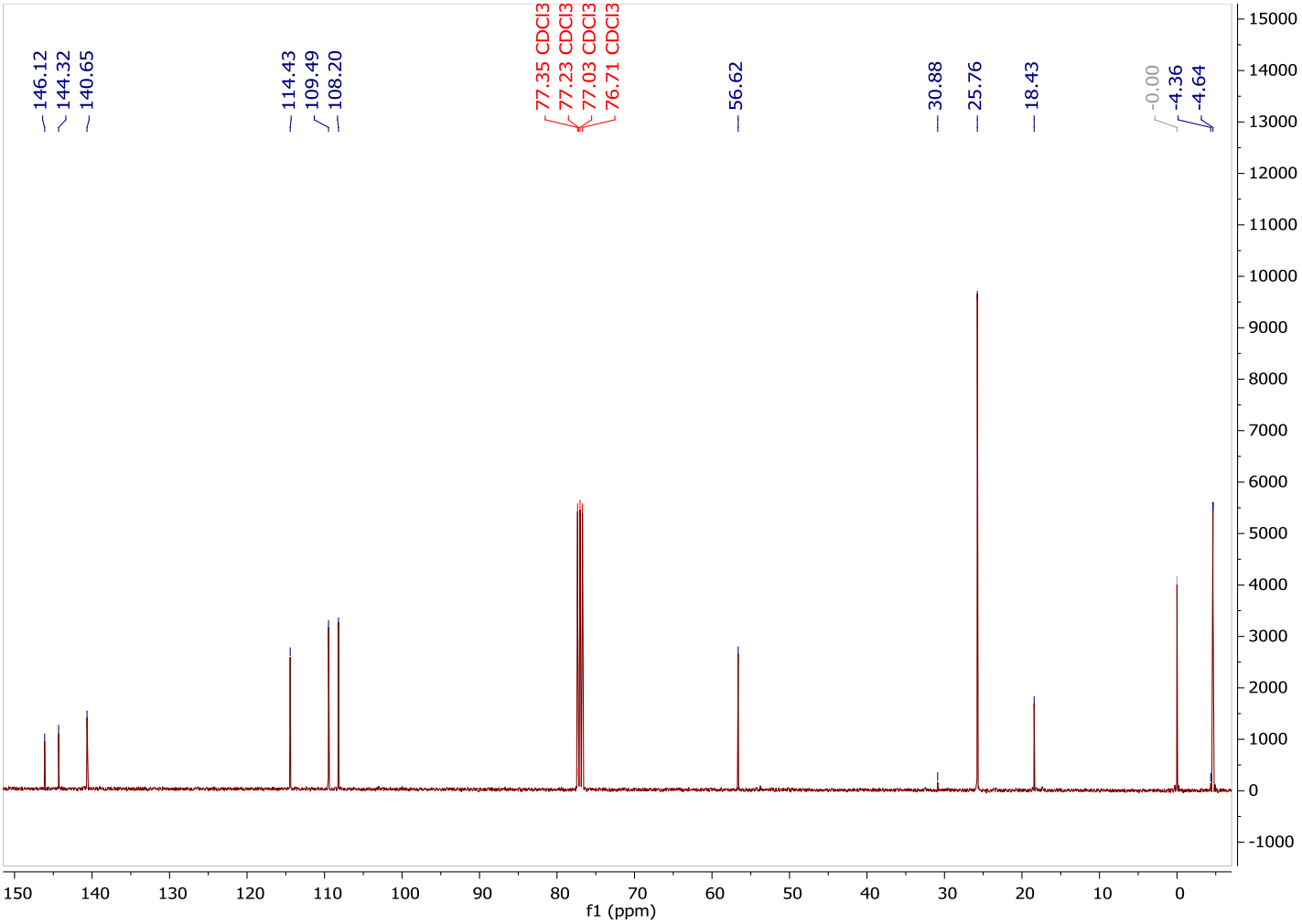
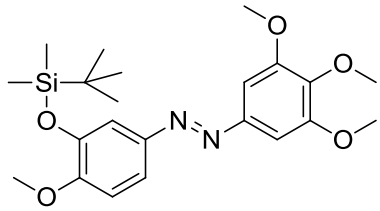
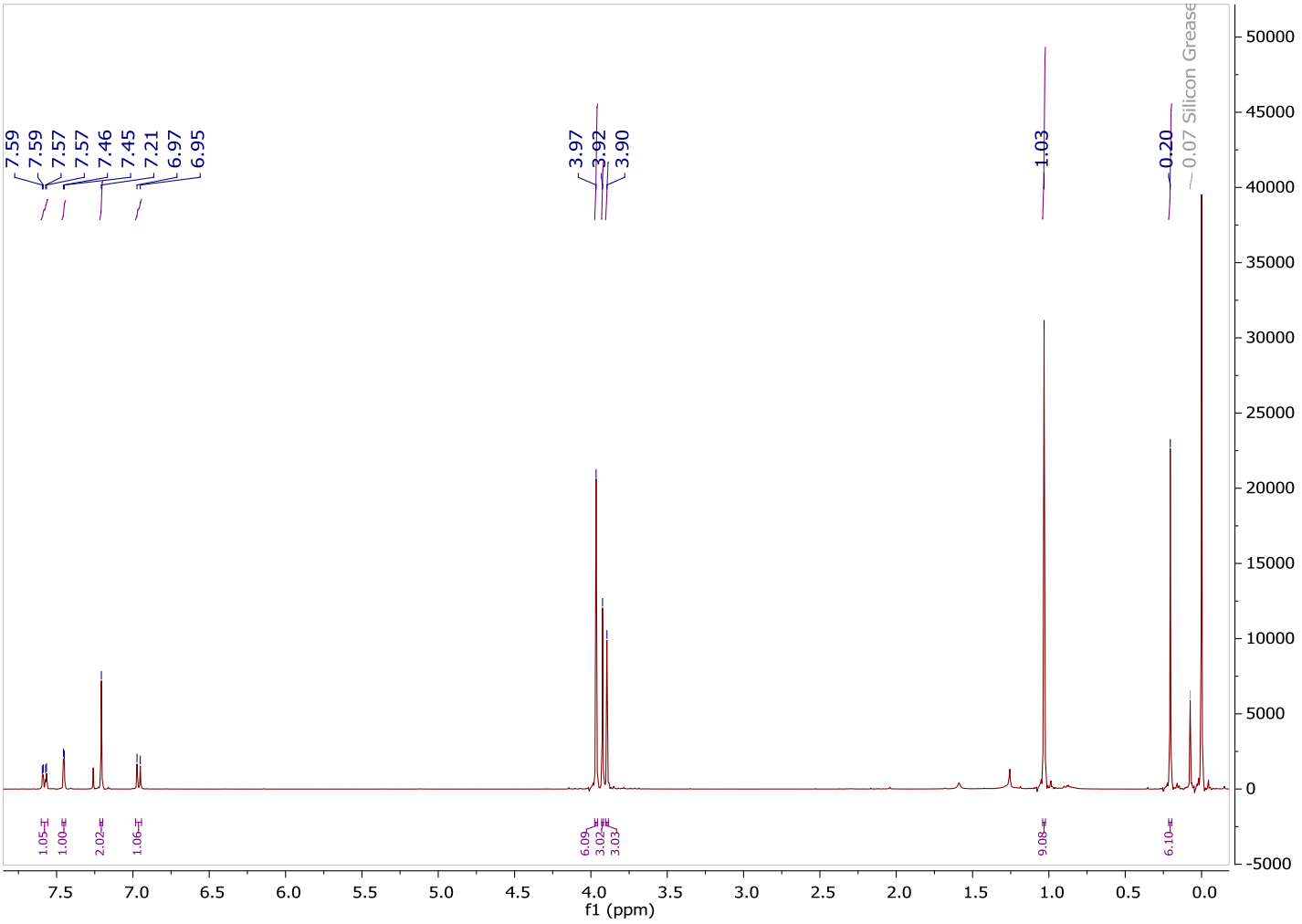


^{1}H NMR of (*E*)-1-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenol)-2-(3,4,5-trimethoxyphenyl)diazene, 3.





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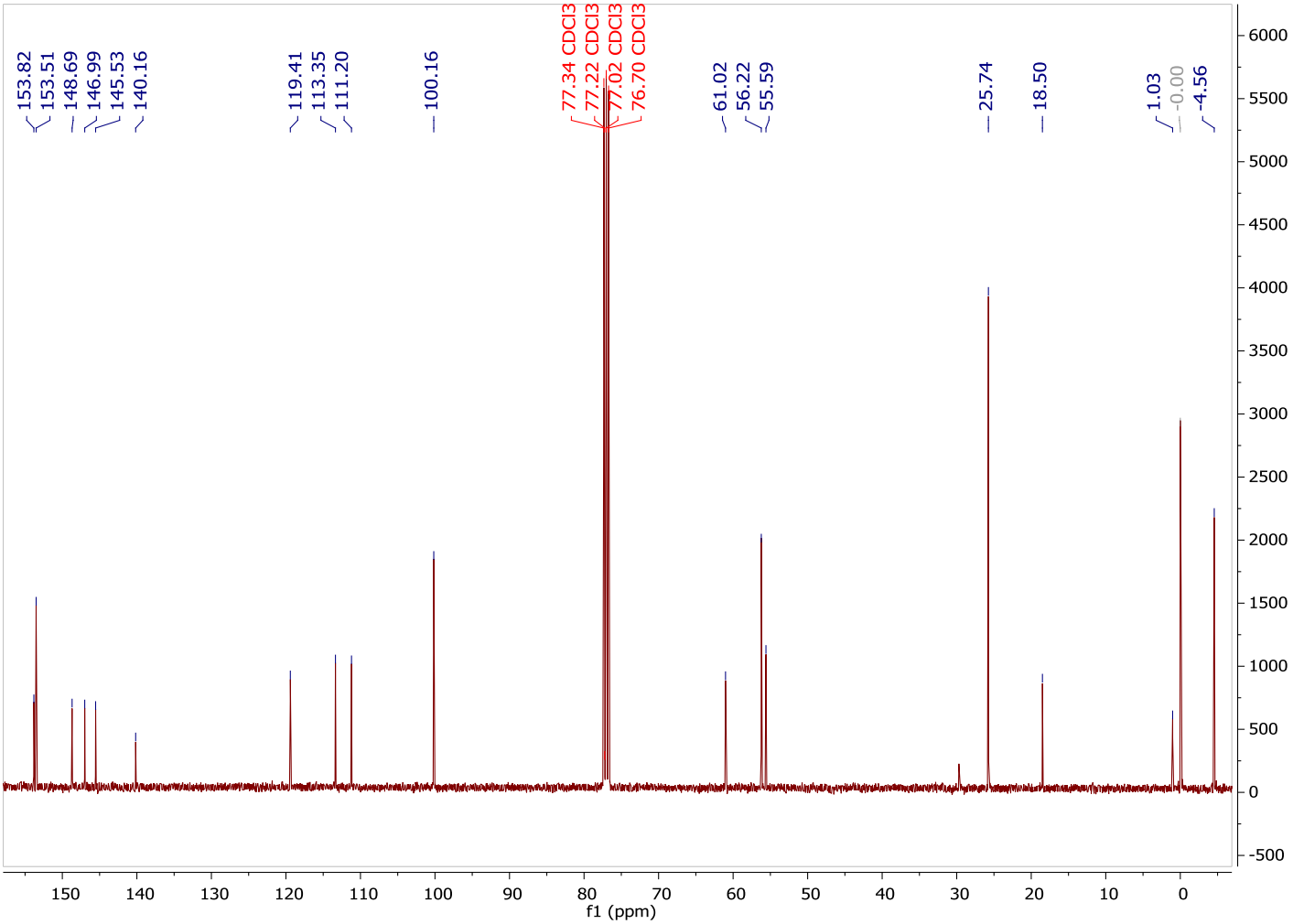
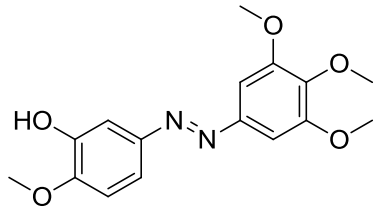
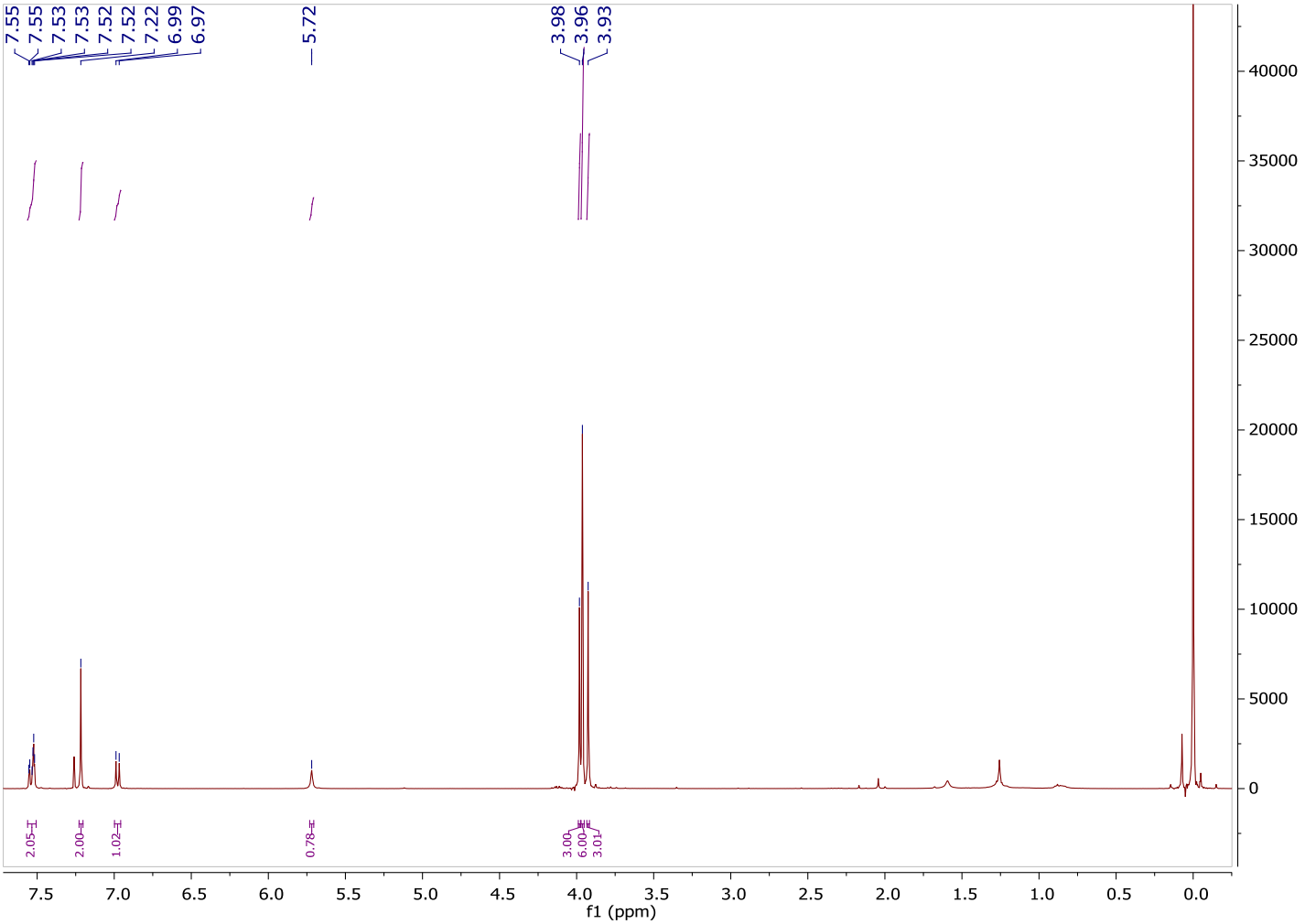


^{1}H NMR of Azo CA-4 (*E*)-2-methoxy-5-((3,4,5-trimethoxyphenyl)diazenyl)phenol, 4.





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