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An aza-Diels–Alder route to quinoline-based unnatural amino acids and polypeptide surrogates

M. J. Umerani[1], H. Yang[2], P. Pratakshya[2], J. S. Nowick[2], A. A. Gorodetsky[1,2,3,*]

[1]Department of Materials Science and Engineering, University of California, Irvine, Irvine, CA 92697, USA

[2]Department of Chemistry, University of California, Irvine, Irvine, CA 92697, USA

[3]Department of Chemical and Biomolecular Engineering, University of California, Irvine, Irvine, CA 92697, USA

*Correspondence to: alon.gorodetsky@uci.edu

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I. General Information and Procedures.

A. Materials. All chemicals and reagents were purchased from Acros Organics, ChemPep, and/or Sigma-Aldrich, and all solvents were obtained from Fisher Scientific and used as received, unless otherwise noted. The dimethylformamide used to prepare the quinoline-based amino acids and polypeptide surrogates was dried with 3 Å molecular sieves and stored under argon. The reactions were performed under dry argon unless otherwise noted.

B. Purification of the Quinoline-based Amino Acids and Polypeptide Surrogates. The quinoline-based amino acid and polypeptide surrogates were purified via flash chromatography by using a Teledyne Isco CombiFlash Rf 200 System. When necessary, the silica gel columns/cartridges were flushed with 1/9 triethylamine/chloroform to deactivate the silica gel and were then flushed with chloroform to remove excess triethylamine.

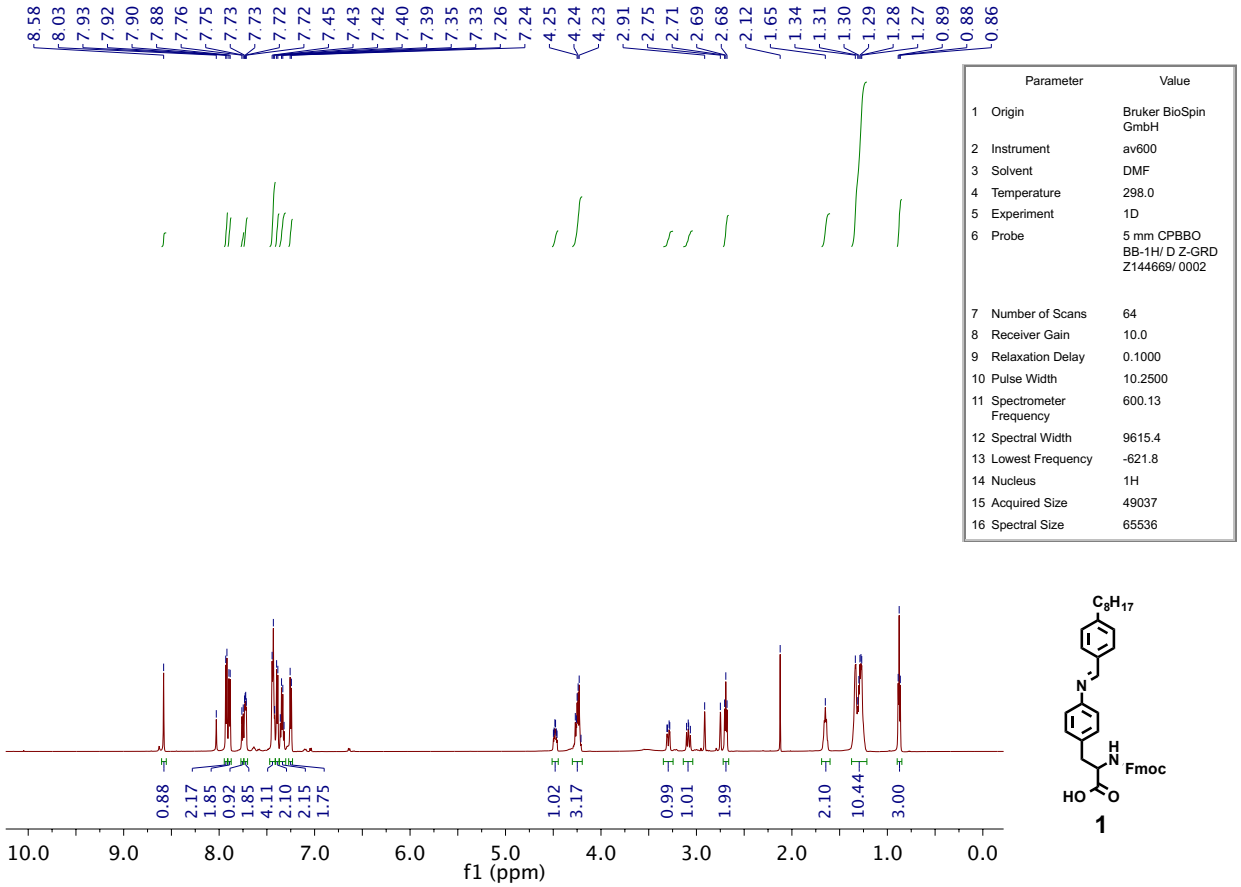
C. Spectroscopic Characterization of the Quinoline-based Amino Acid and Polypeptide Surrogates. The quinoline-based amino acids and polypeptide surrogates, as well as the intermediates necessary for their synthesis, were characterized with nuclear magnetic resonance (NMR) spectroscopy, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, ultraviolet-visible (UV-Vis) absorption spectroscopy, fluorescence spectroscopy, and/or circular dichroism (CD) spectroscopy. The ^{1}H NMR and ^{13}C NMR spectra were typically recorded on an AVANCE600 instrument in $CDCl_3$, DMF-d_7, and/or DMSO-d_6. The chemical shifts were reported in ppm for ^{1}H and ^{13}C NMR. The chemical shifts in the NMR spectra were referenced as follows: for samples in $CDCl_3$, the ^{1}H NMR signals were referenced to $CDCl_3$ at 7.26 ppm, and the ^{13}C NMR signals were referenced to $CDCl_3$ at 77.16 ppm; for samples in DMF-d_7, the ^{1}H NMR signals were referenced to the solvent peak at 8.03 ppm, and the ^{13}C NMR signals were referenced to the solvent peak at 163.15 ppm; for samples in DMSO-d_6, the ^{1}H NMR signals were referenced to the

solvent peak at 2.50 ppm, and the ^{13}C NMR signals were referenced to the solvent peak at 39.51 ppm. The data are labeled as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet), the coupling constants in Hertz, and the integration value. The MALDI mass spectra were recorded on an AB Sciex TOF/TOF 5800 series mass spectrometer using a 349 nm Nd:YAG laser and either dithranol or 2,5-dihydroxybenzoic acid (DHB) as the matrix. The UV-Vis absorption spectra were collected on an Agilent Cary 100 Series UV-Vis absorption spectrophotometer in tetrahydrofuran (THF) at room temperature. The fluorescence spectra were collected on a Cary Eclipse fluorescence spectrophotometer in tetrahydrofuran (THF) at room temperature. The CD spectra were collected on a JASCO J-810 spectropolarimeter in tetrahydrofuran (THF) at room temperature.

D. Chromatographic Characterization and Analysis of the Quinoline-based Amino Acids and Polypeptide Surrogates. The quinoline-based amino acids and polypeptide surrogates were characterized via high-performance liquid chromatography (HPLC) or via size-exclusion chromatography (SEC). The HPLC experiments were performed on an Agilent 1260 Infinity System using a reverse phase C18 column. The gradient was evolved from 95% Buffer A:5% Buffer B to 5% Buffer A:95% Buffer B at a flow rate of 1 mL/min over 35 min (Buffer A: 99.9% water, 0.1% trifluoroacetic acid; Buffer B: 95% acetonitrile, 4.9% water, 0.1% trifluoroacetic acid). The typical flow rate was 1 mL/min, typical injection volume was 50 – 100 μL, and typical sample concentration was 1 mg/mL. The SEC experiments were performed on a Malvern OMNISEC GPC/SEC system comprised of the OMNISEC RESOLVE separations module that is equipped with a Malvern single-pore T3000 column (300 x 8 mm), and the OMNISEC REVEAL multi-detector module that is equipped with a differential refractive index detector, multi-angle light scattering detector, viscometer, and UV/Vis spectrometer. Tetrahydrofuran (THF) was used

as the mobile phase. The typical flow rate was 0.8 mL/min, typical injection volume was 70 – 100 μL, and typical sample concentration was 1.0 – 2.5 mg/mL. The molecular weights (M_n and M_w) were estimated with the Omnisec V10 software using polystyrene (PS-245K) standards (Malvern).

II. Collated Characterization Data



	Parameter	Value
1	Origin	Bruker BioSpin GmbH
2	Instrument	av600
3	Solvent	DMF
4	Temperature	298.0
5	Experiment	1D
6	Probe	5 mm CPBBO BB-1H/ D Z-GRD Z144669/ 0002
7	Number of Scans	64
8	Receiver Gain	10.0
9	Relaxation Delay	0.1000
10	Pulse Width	10.2500
11	Spectrometer Frequency	600.13
12	Spectral Width	9615.4
13	Lowest Frequency	-621.8
14	Nucleus	1H
15	Acquired Size	49037
16	Spectral Size	65536

Figure S1. A ^{1}H NMR spectrum obtained for **1**.

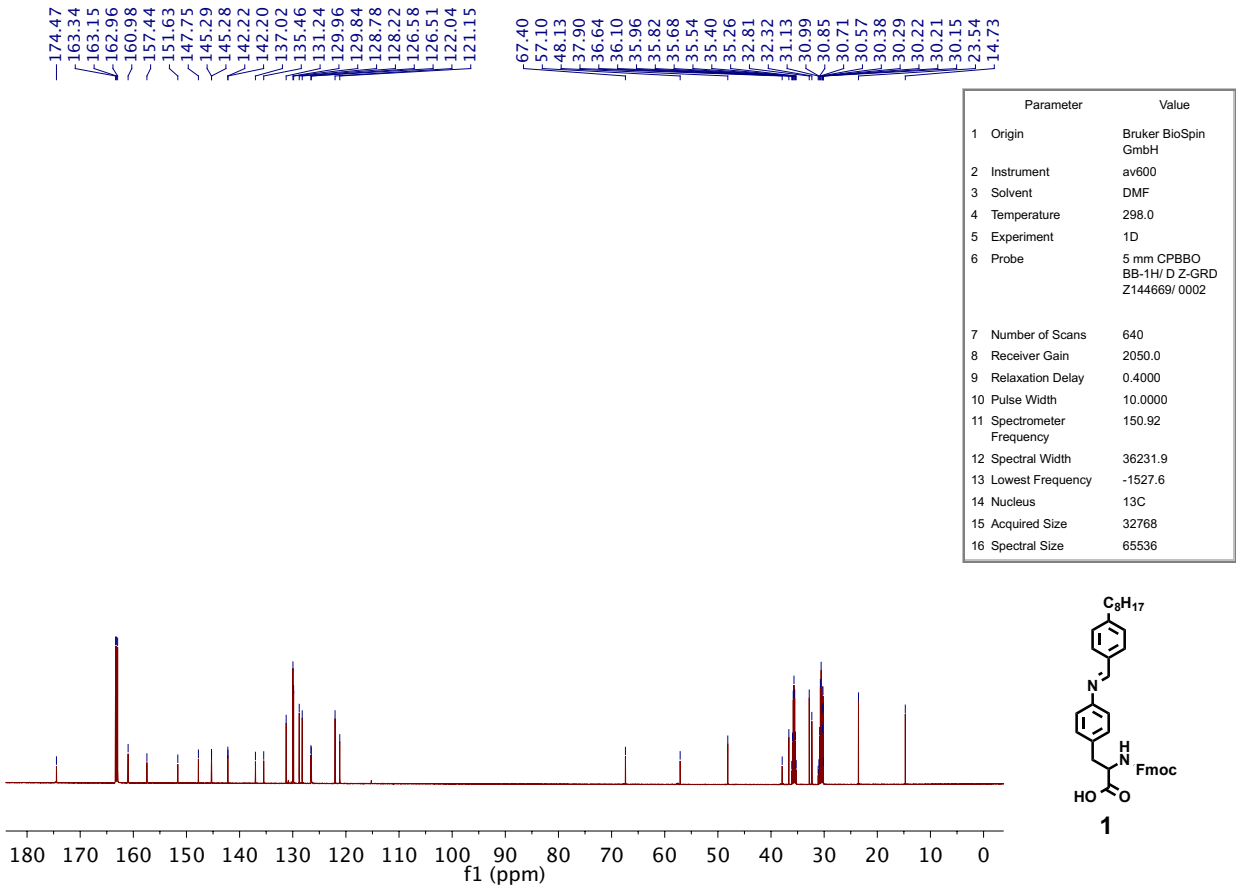


Figure S2. A ^{13}C NMR spectrum obtained for **1**.

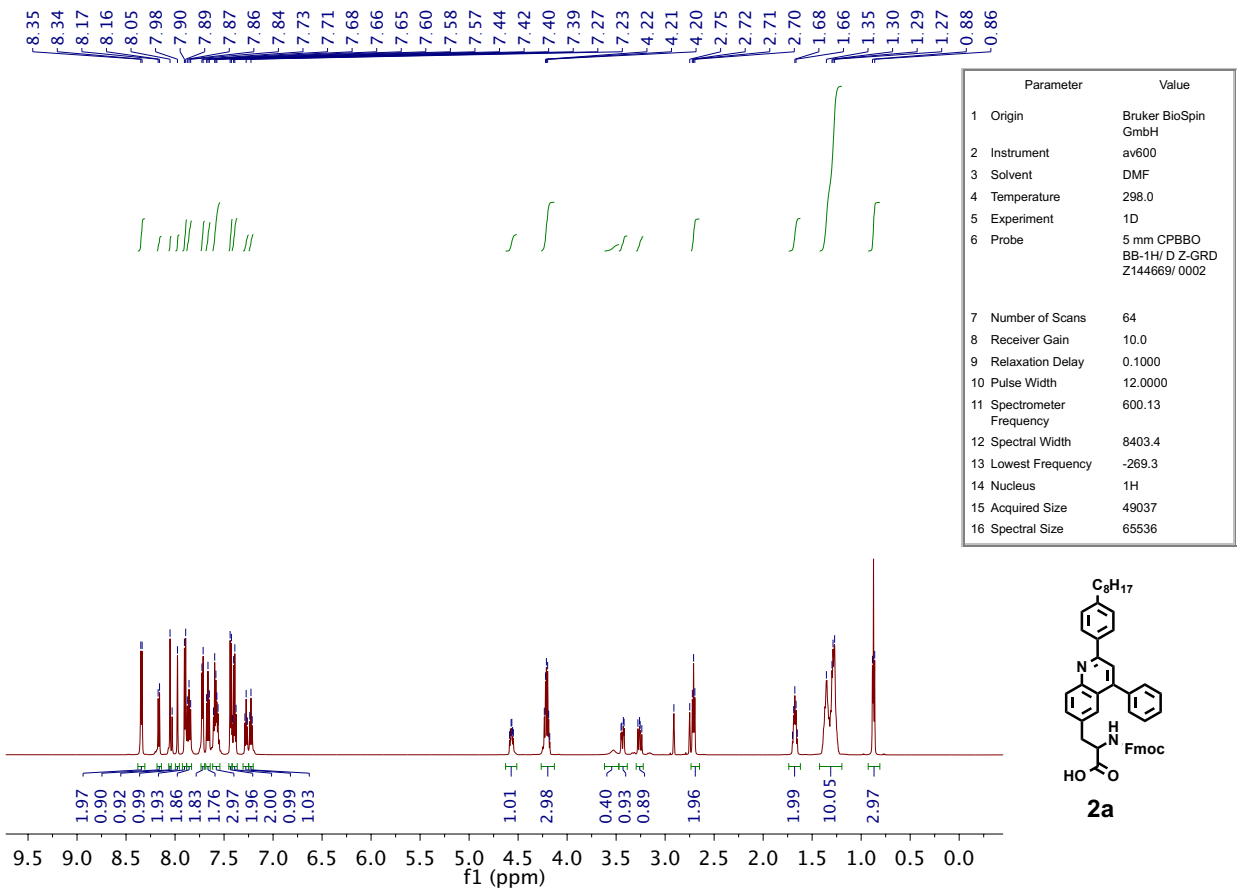


Figure S3. A ^{1}H NMR spectrum obtained for **2a**.

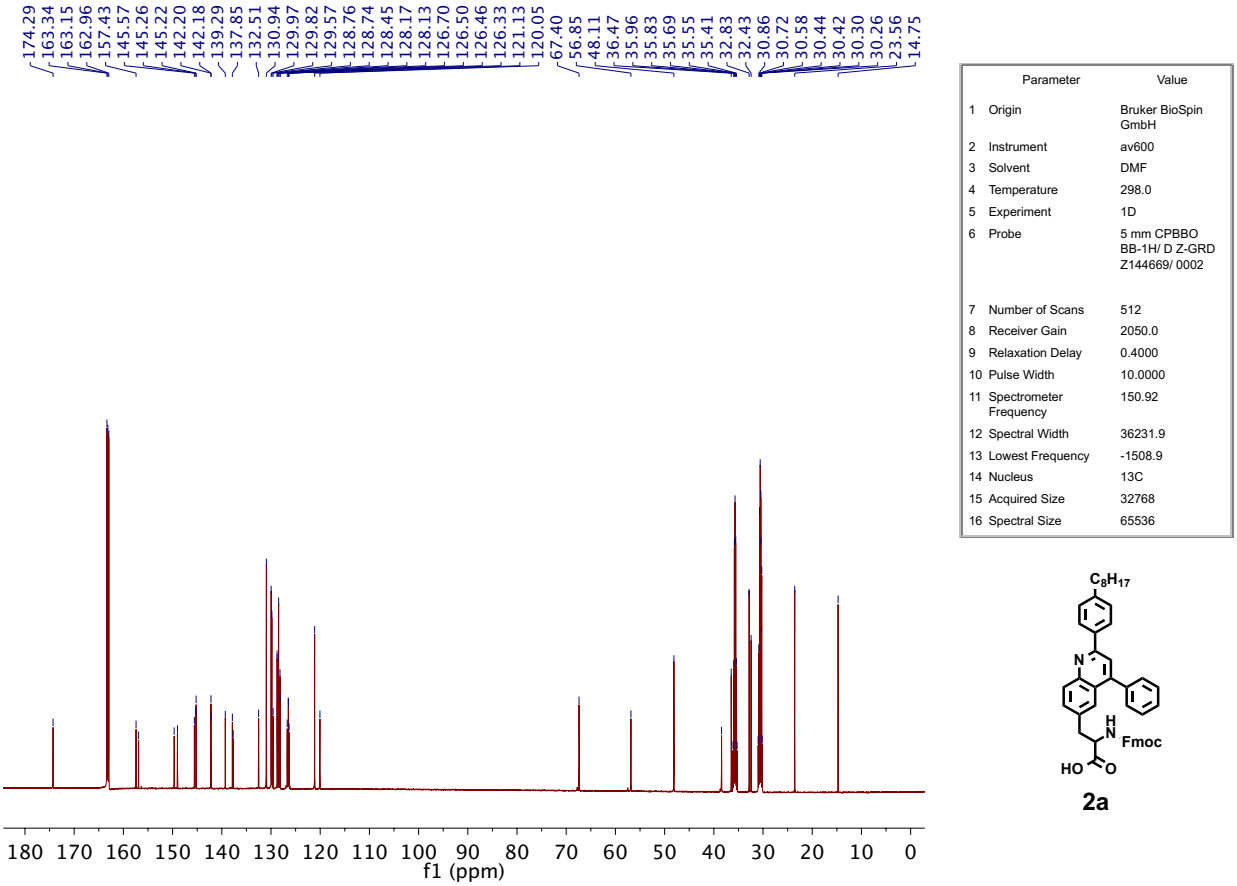


Figure S4. A ^{13}C NMR spectrum obtained for **2a**.

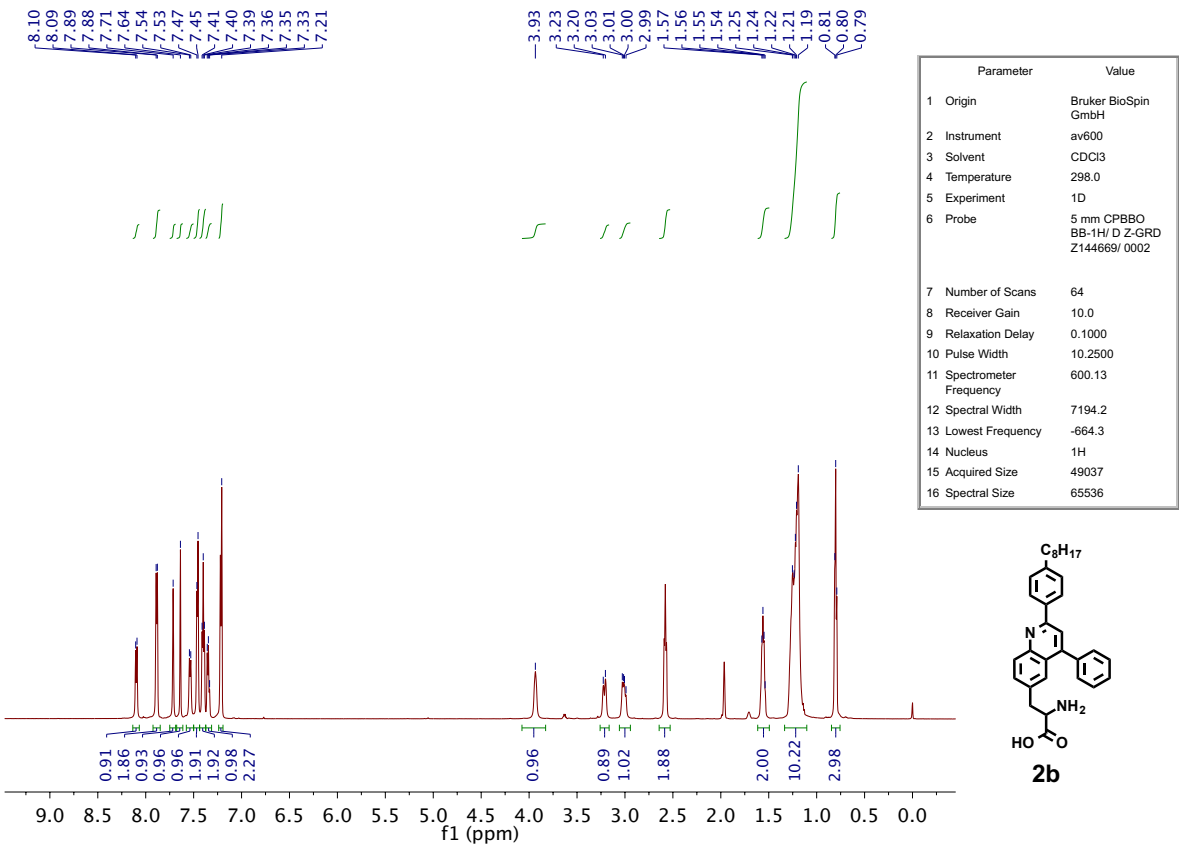


Figure S5. A ^{1}H NMR spectrum obtained for **2b**.

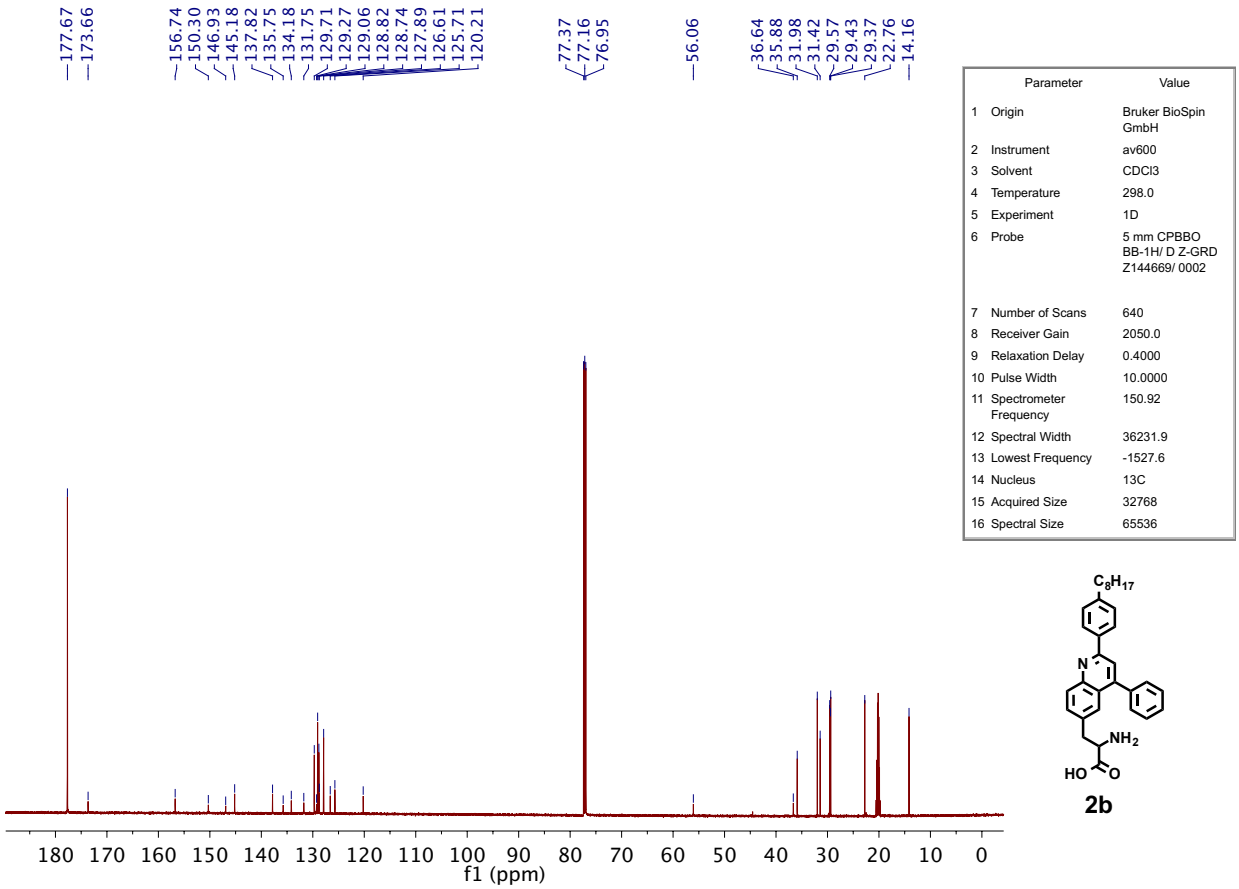


Figure S6. As ^{13}C NMR spectrum obtained for **2b**

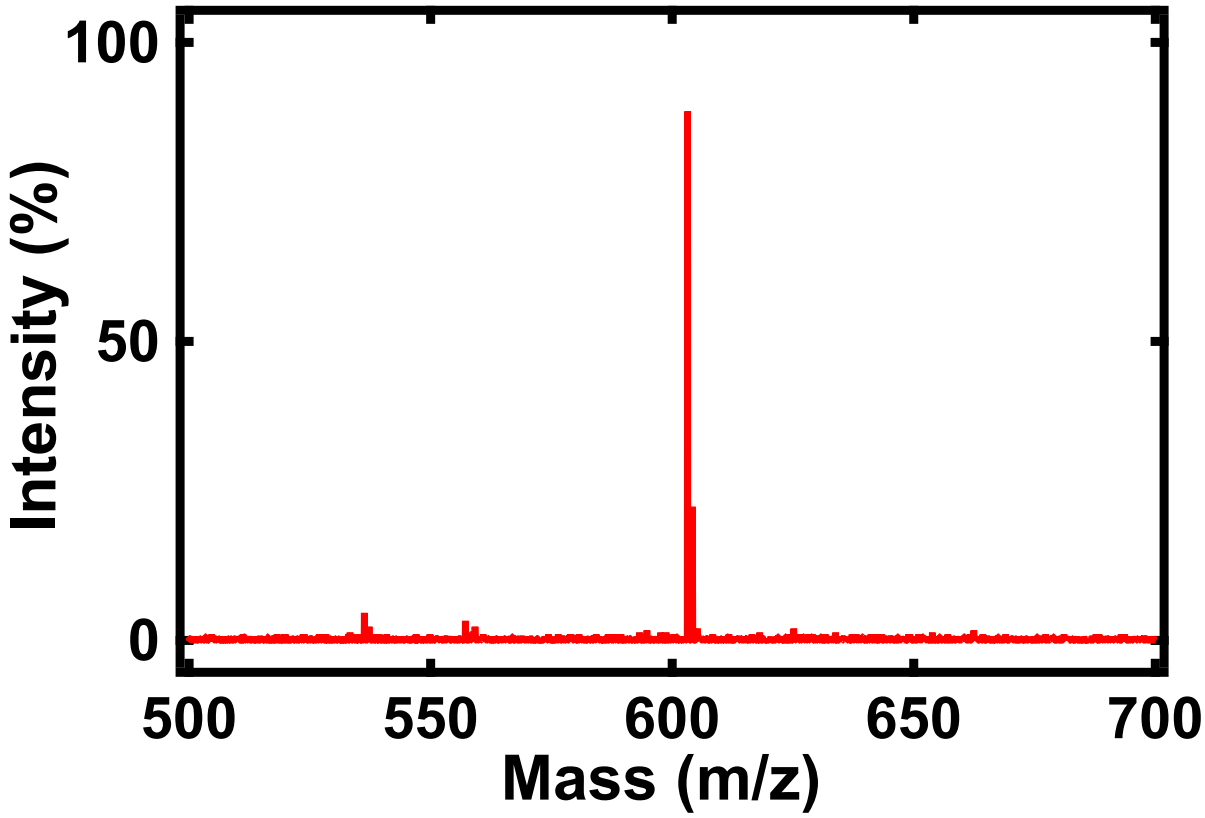


Figure S7. A MALDI mass spectrum obtained for **1**.

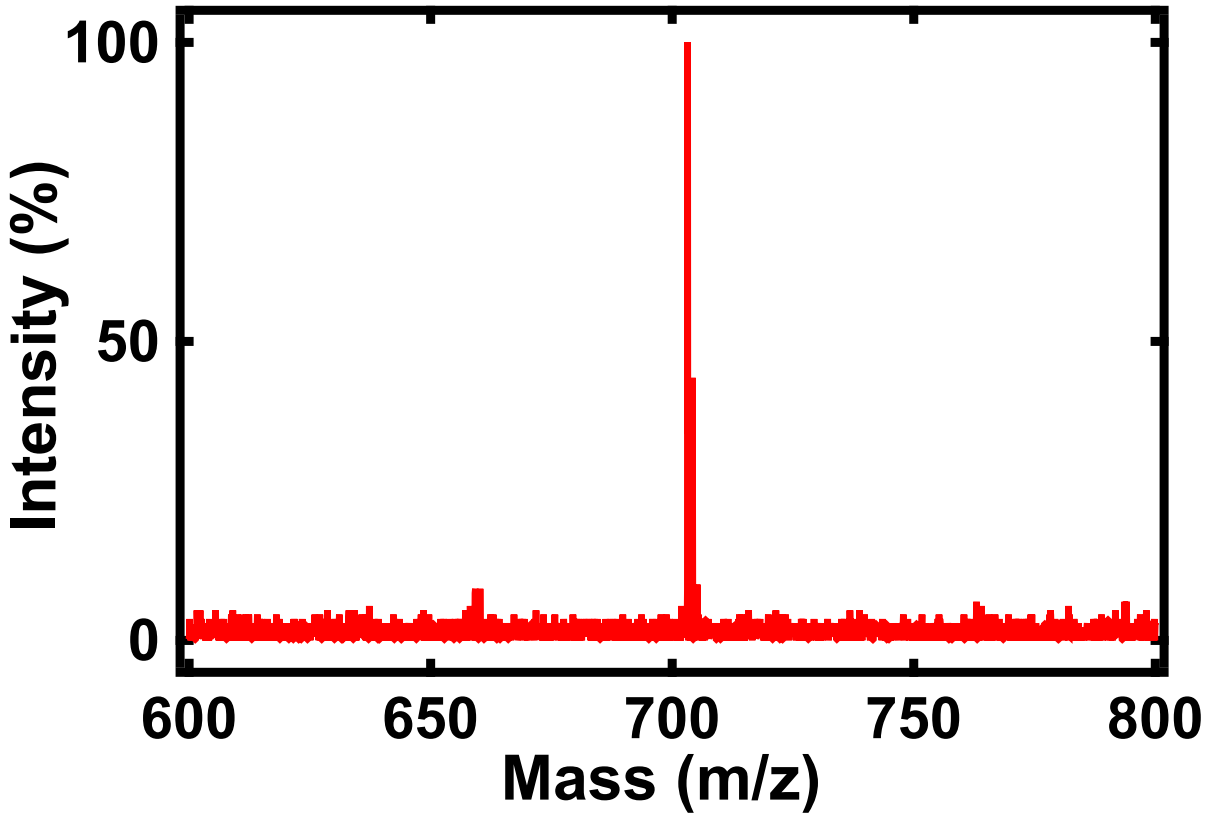


Figure S8. A MALDI mass spectrum obtained for **2a**.

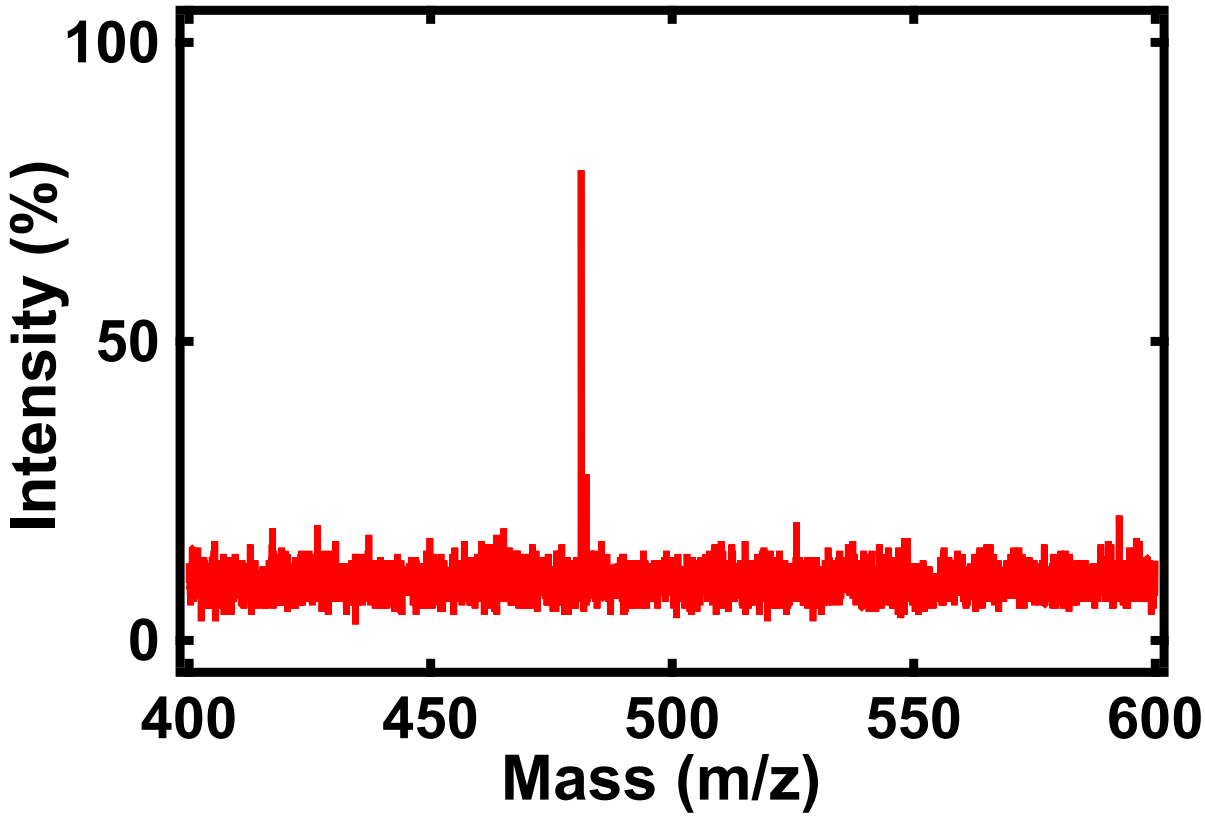


Figure S9. A MALDI mass spectrum obtained for **2b**.

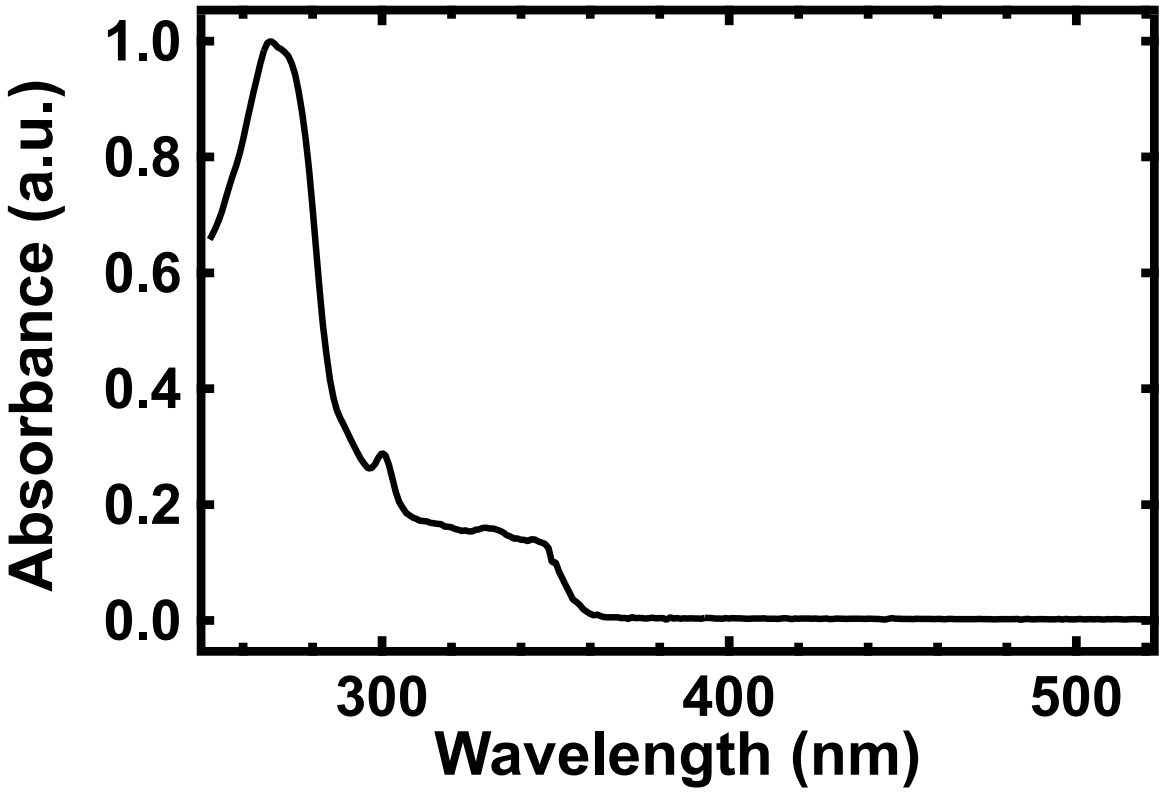


Figure S10. A UV-Vis absorption spectrum obtained for **2a**.

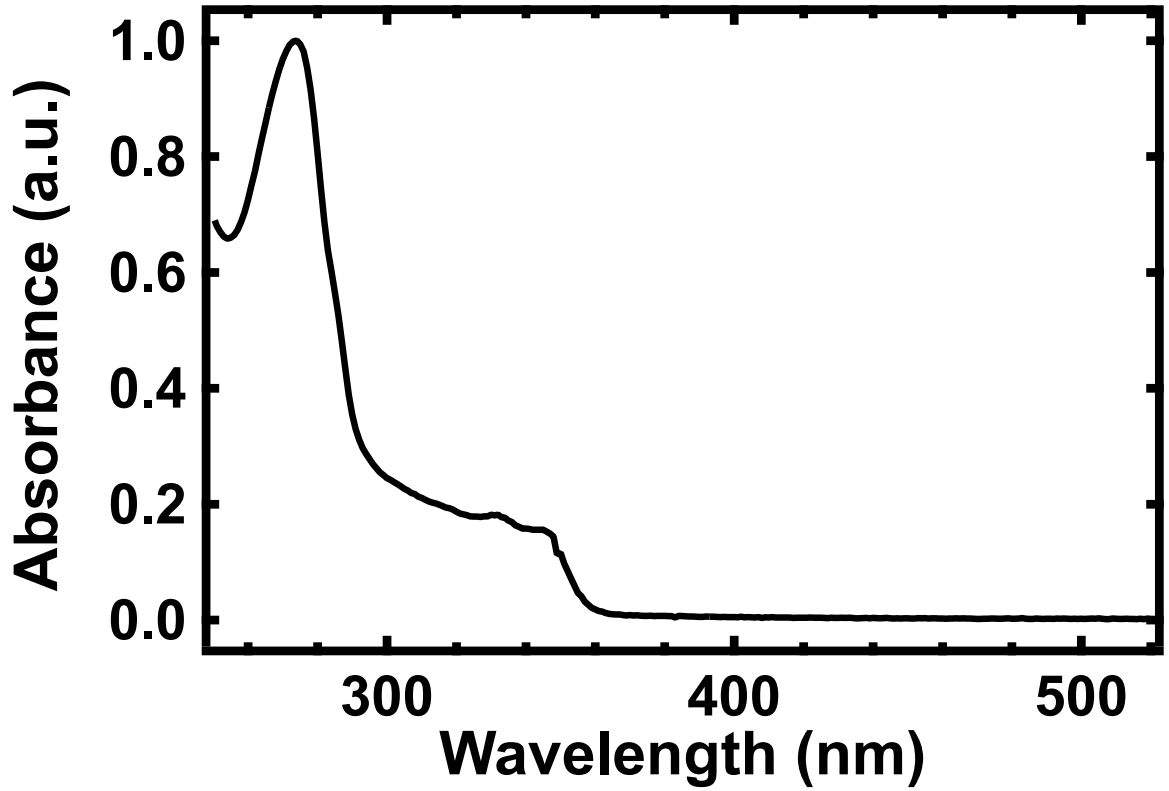


Figure S11. A UV-Vis absorption spectrum obtained for **2b**.

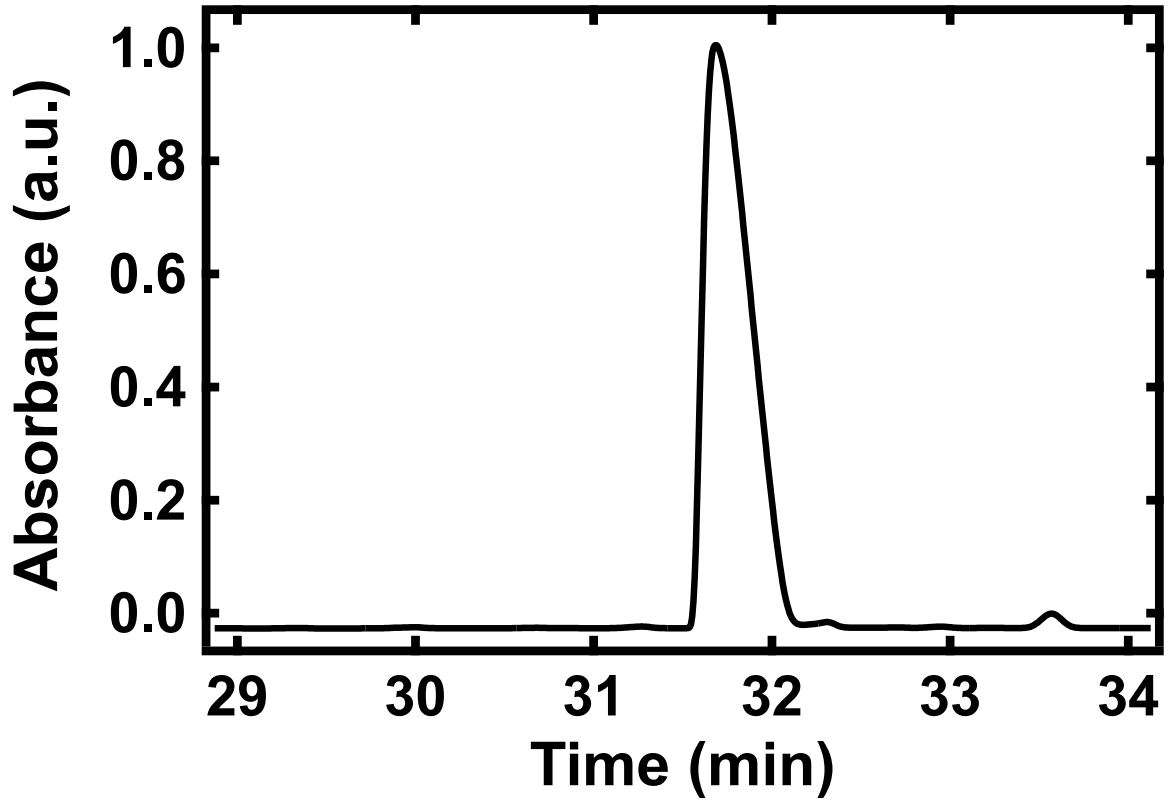


Figure S12. An HPLC chromatogram obtained for **2a**.

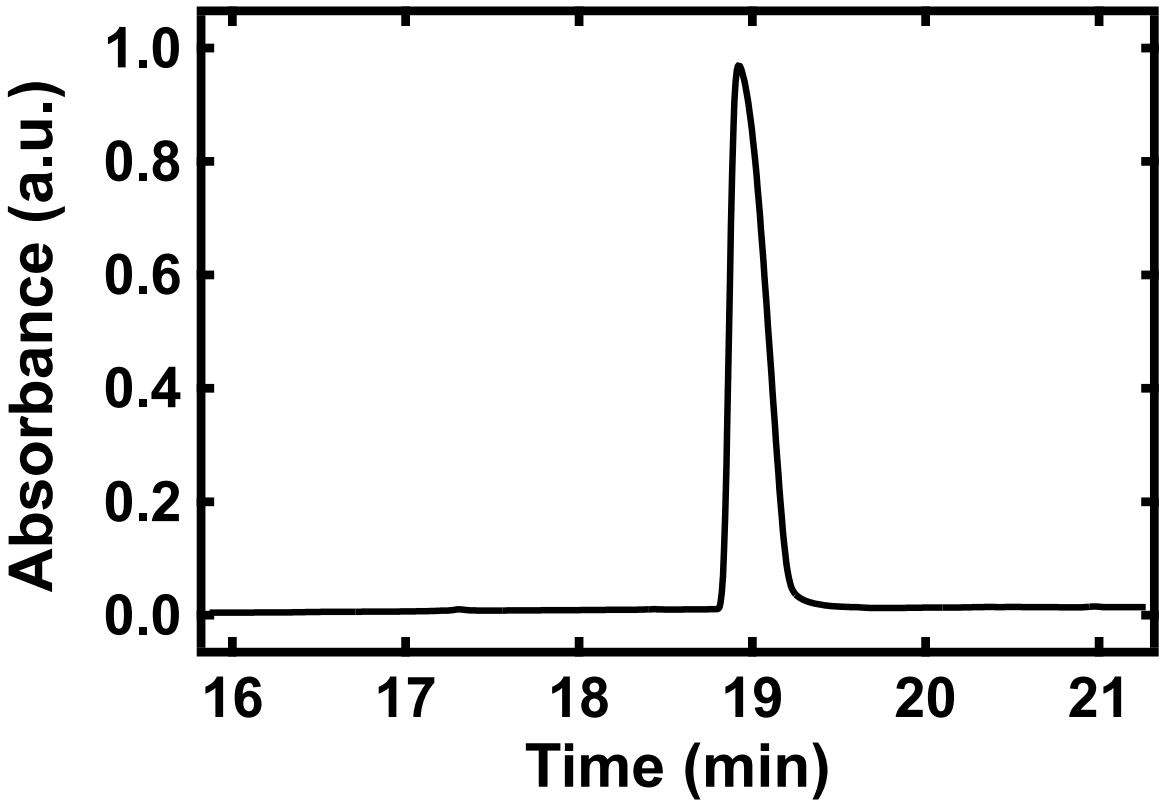


Figure S13. An HPLC chromatogram obtained for **2b**.

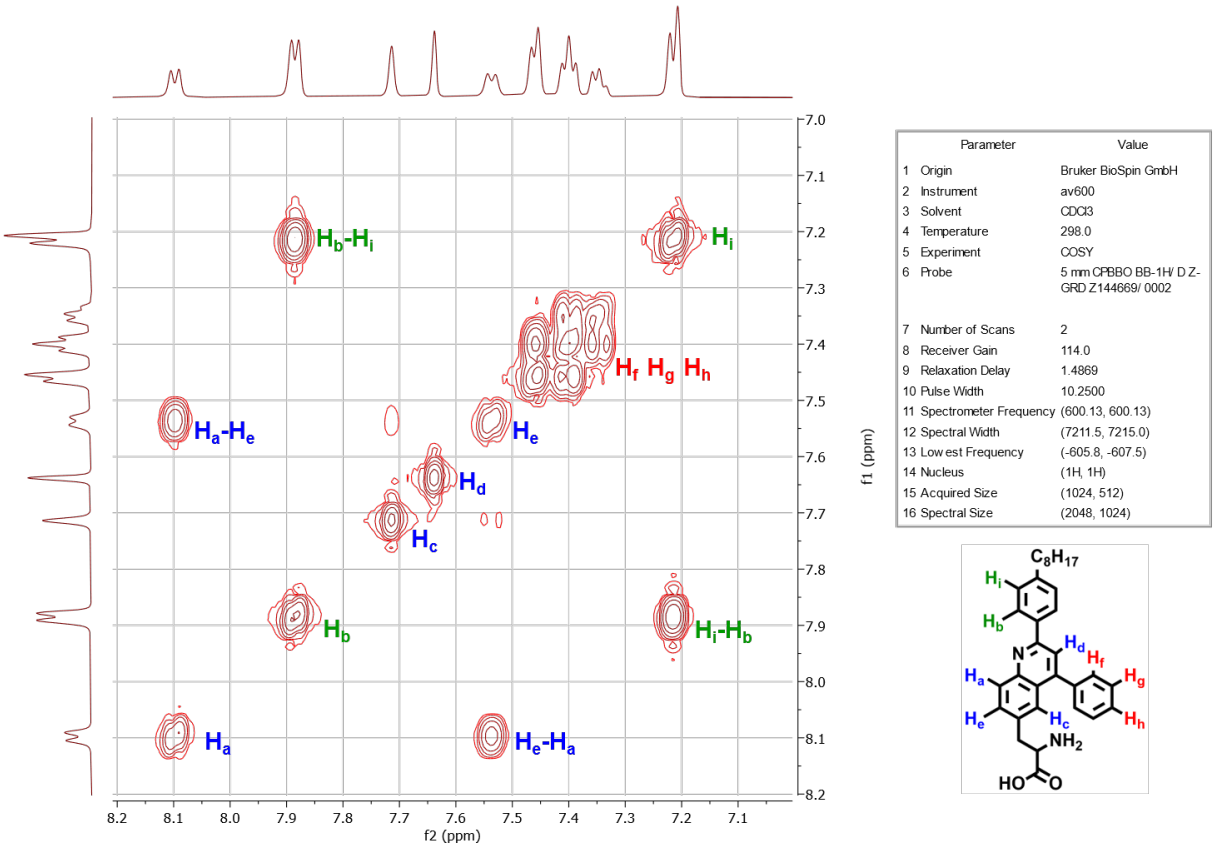


Figure S14. A ^{1}H-^{1}H COSY spectrum obtained for **2b**. The assignment of the aromatic protons is indicated.

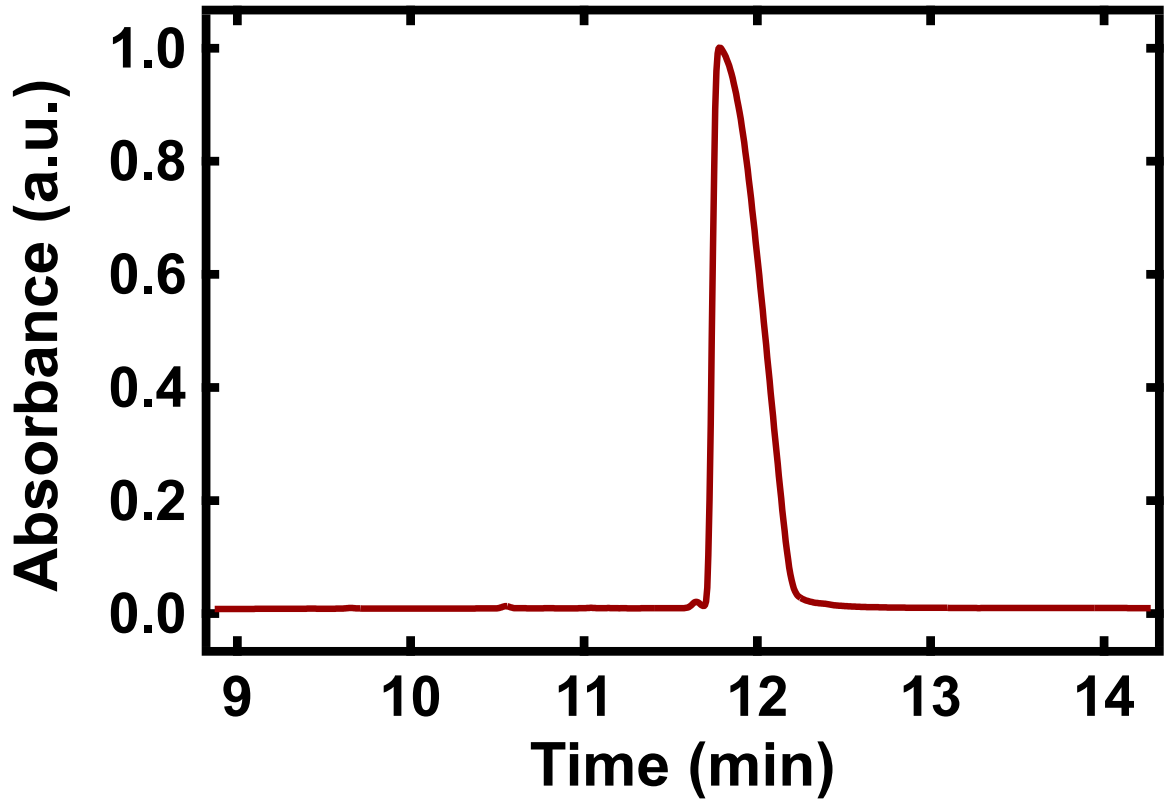


Figure S15. An HPLC chromatogram obtained for **3**.

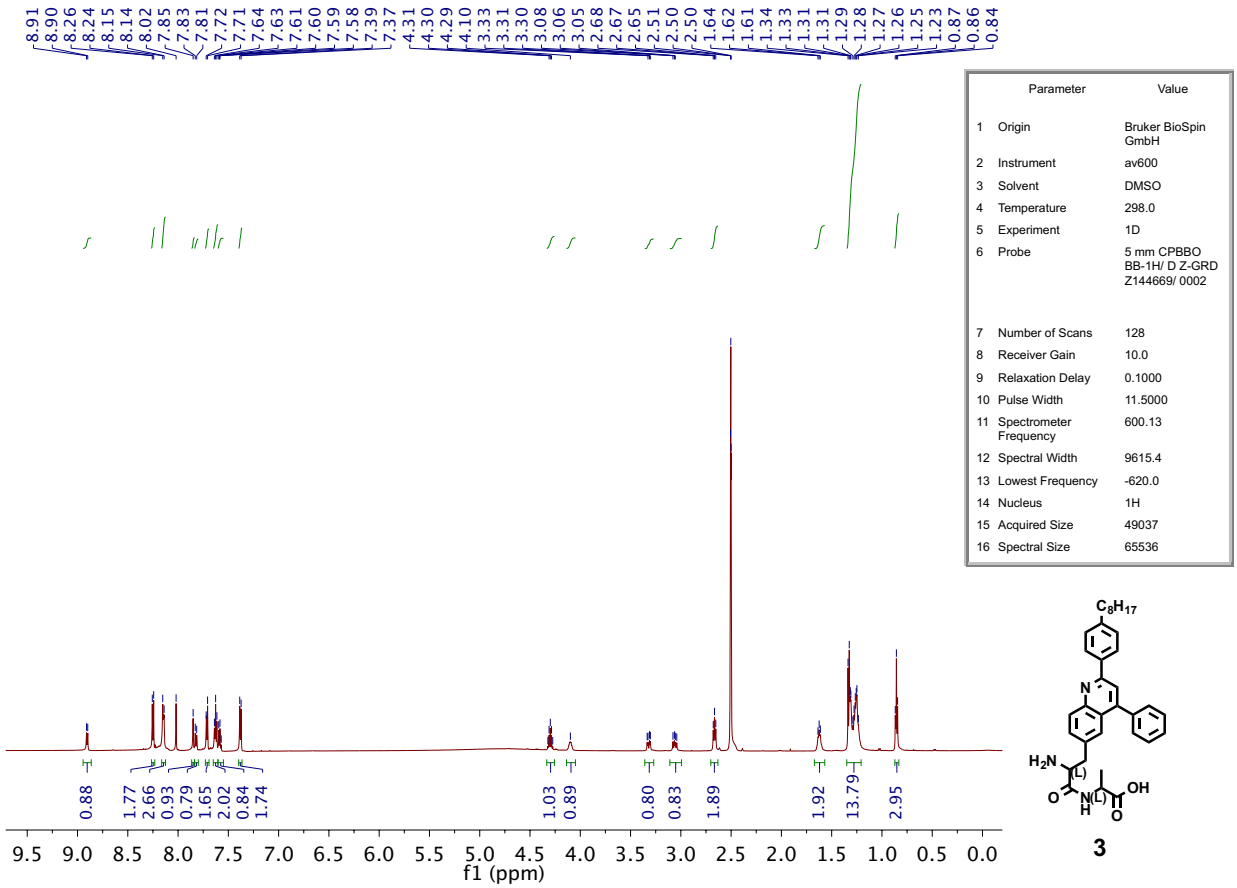


Figure S16. A ^{1}H NMR spectrum obtained for **3**.

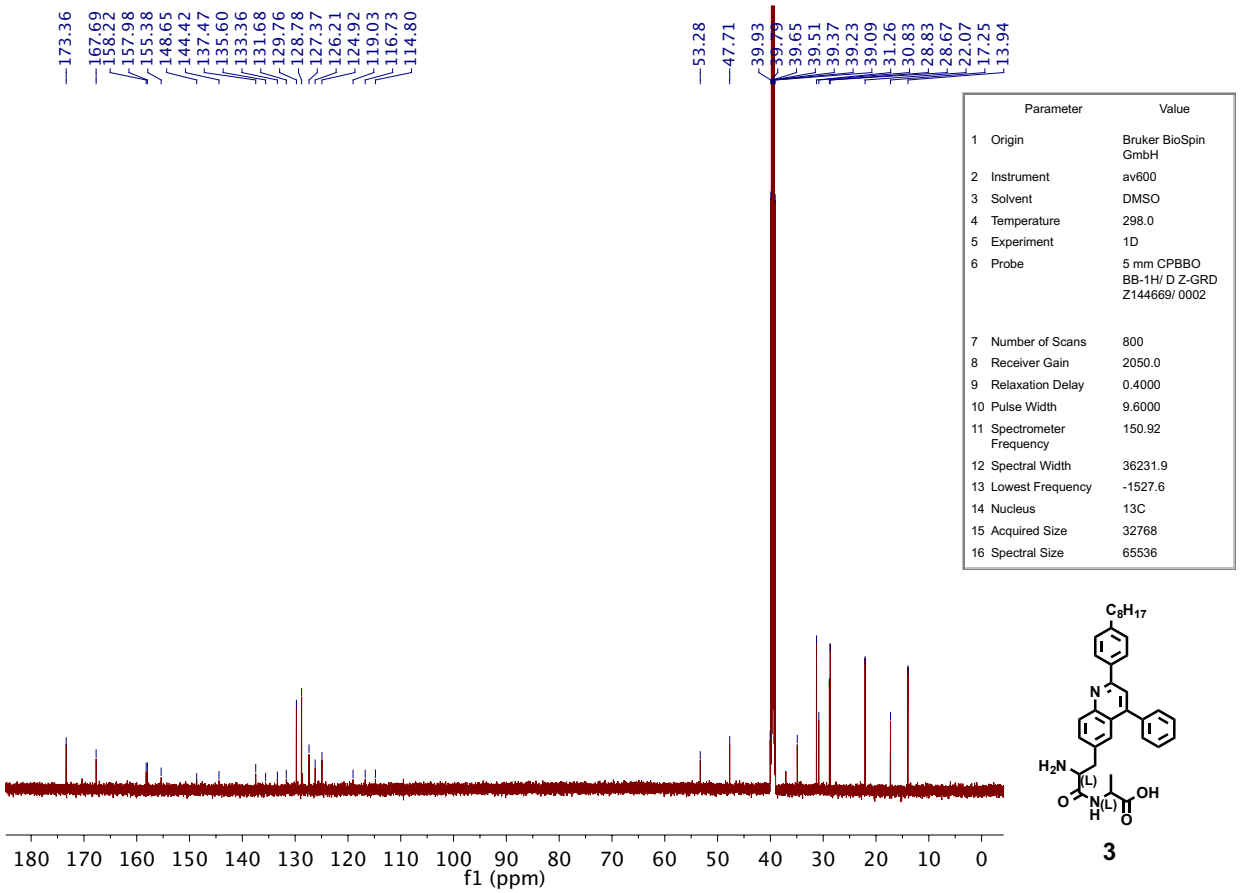


Figure S17. A ^{13}C NMR spectrum obtained for **3**.

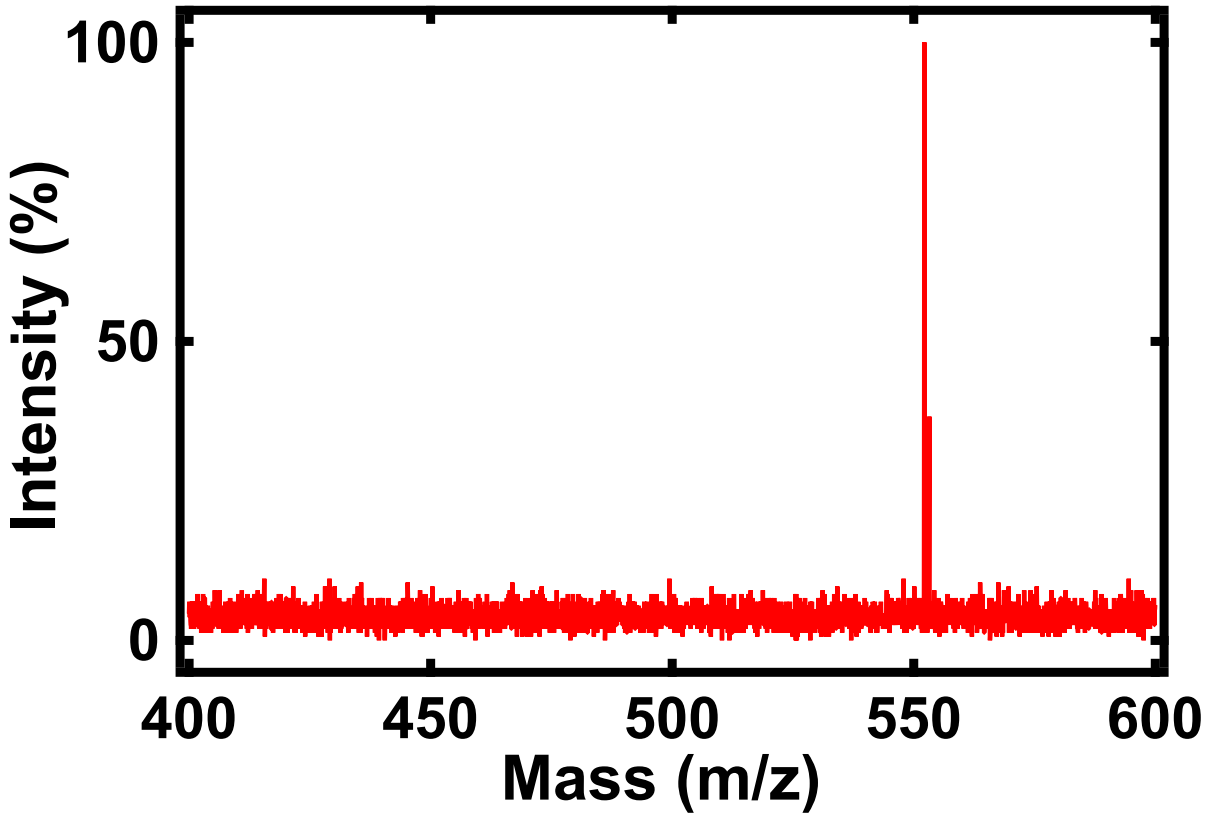


Figure S18. A MALDI mass spectrum obtained for **3**.

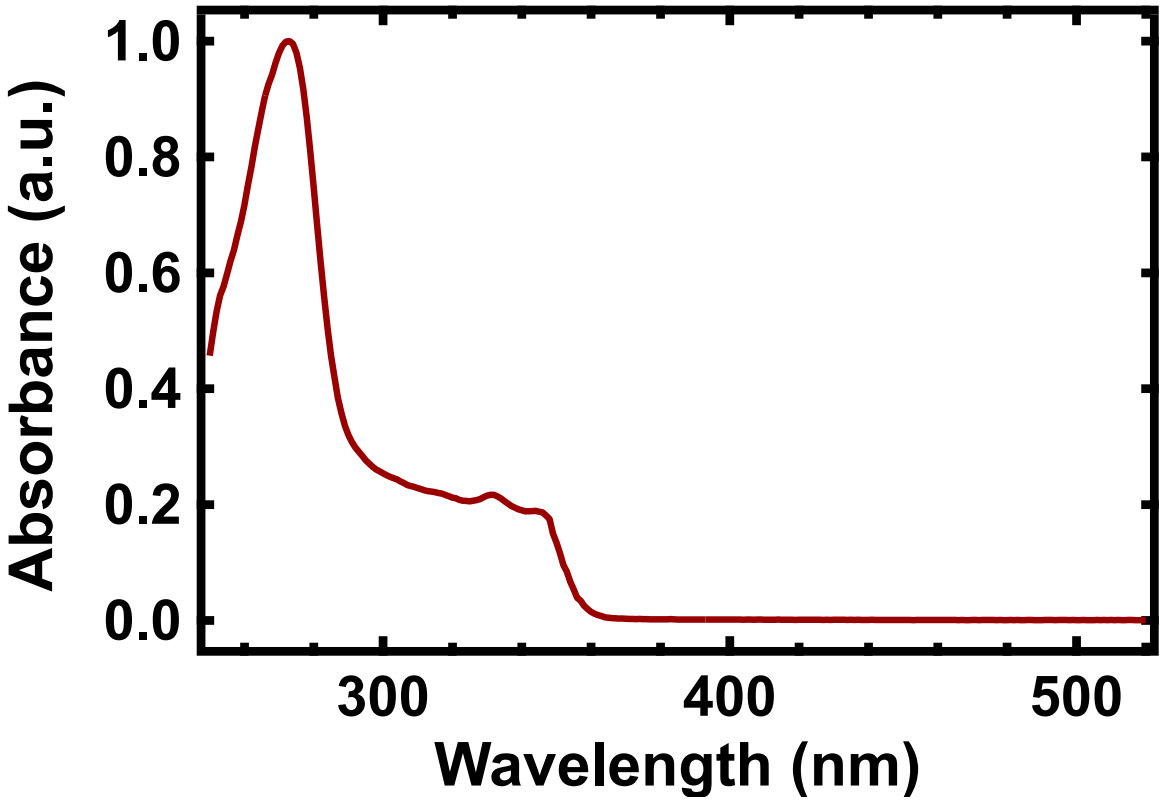


Figure S19. A UV-Vis absorption spectrum obtained for **3**.

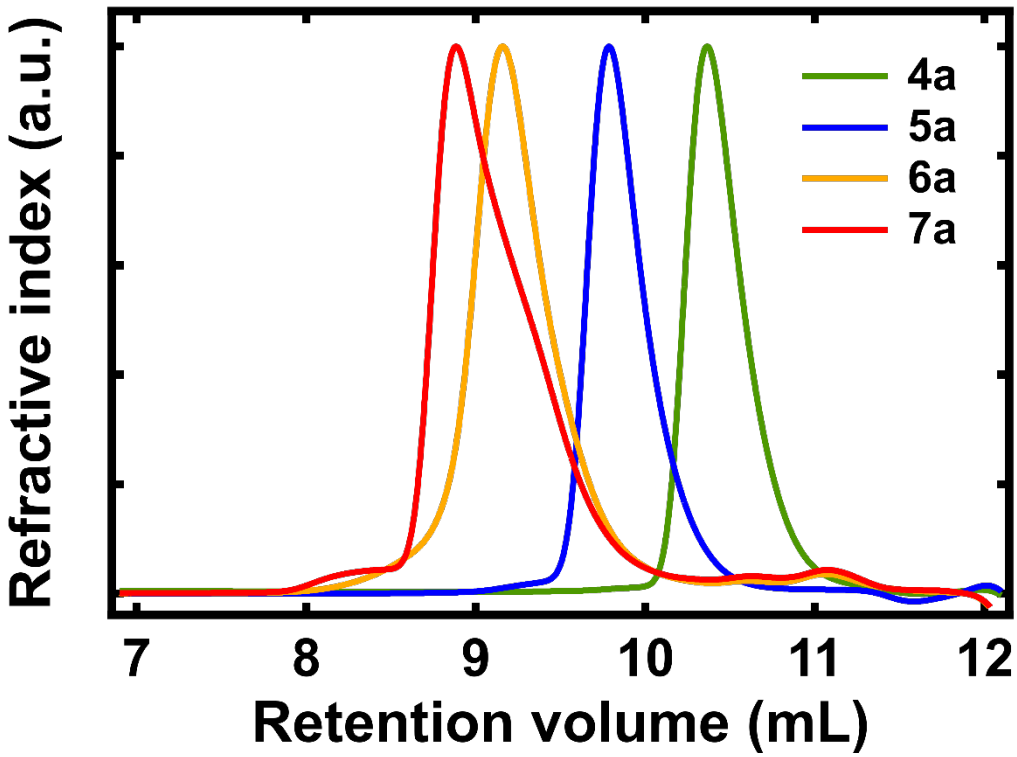


Figure S20. The SEC chromatograms obtained for **4a** (green trace), **5a** (blue trace), **6a** (orange trace), and **7a** (red trace).

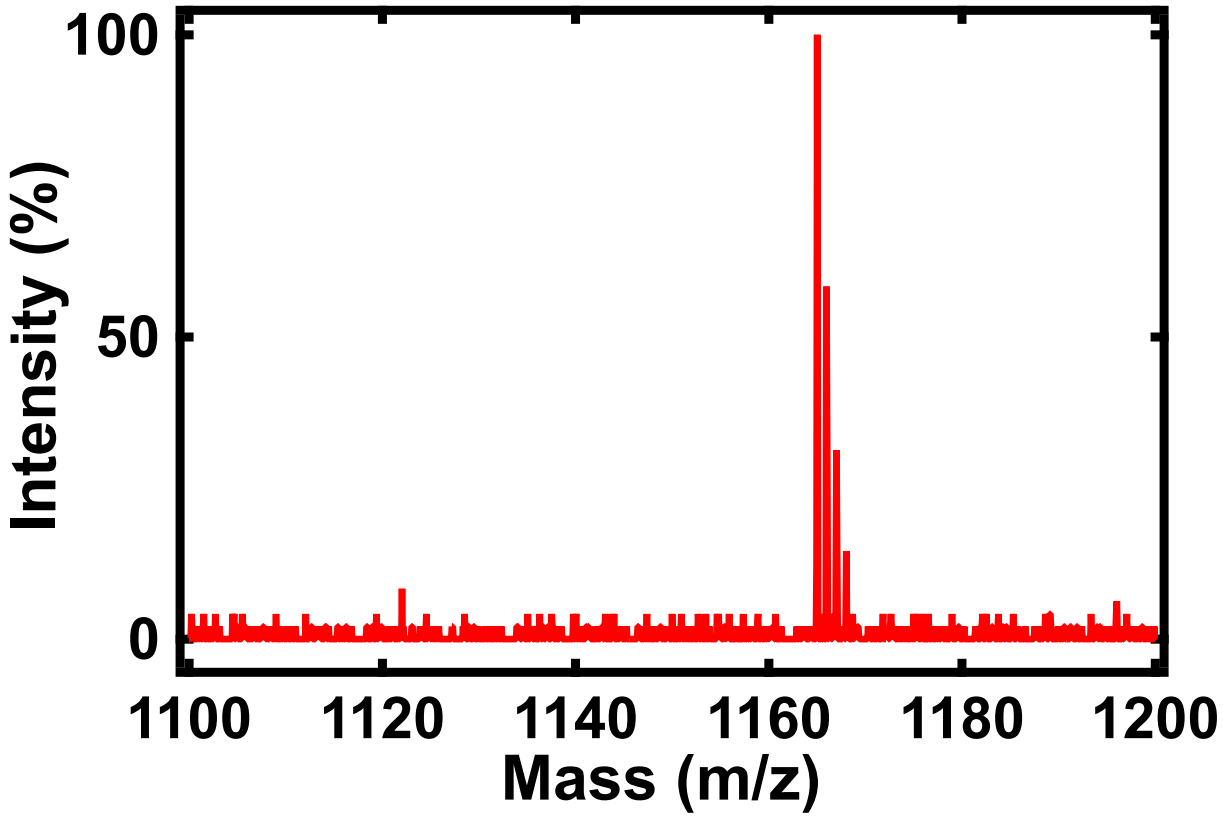


Figure S21. A MALDI mass spectrum obtained for **4a**.

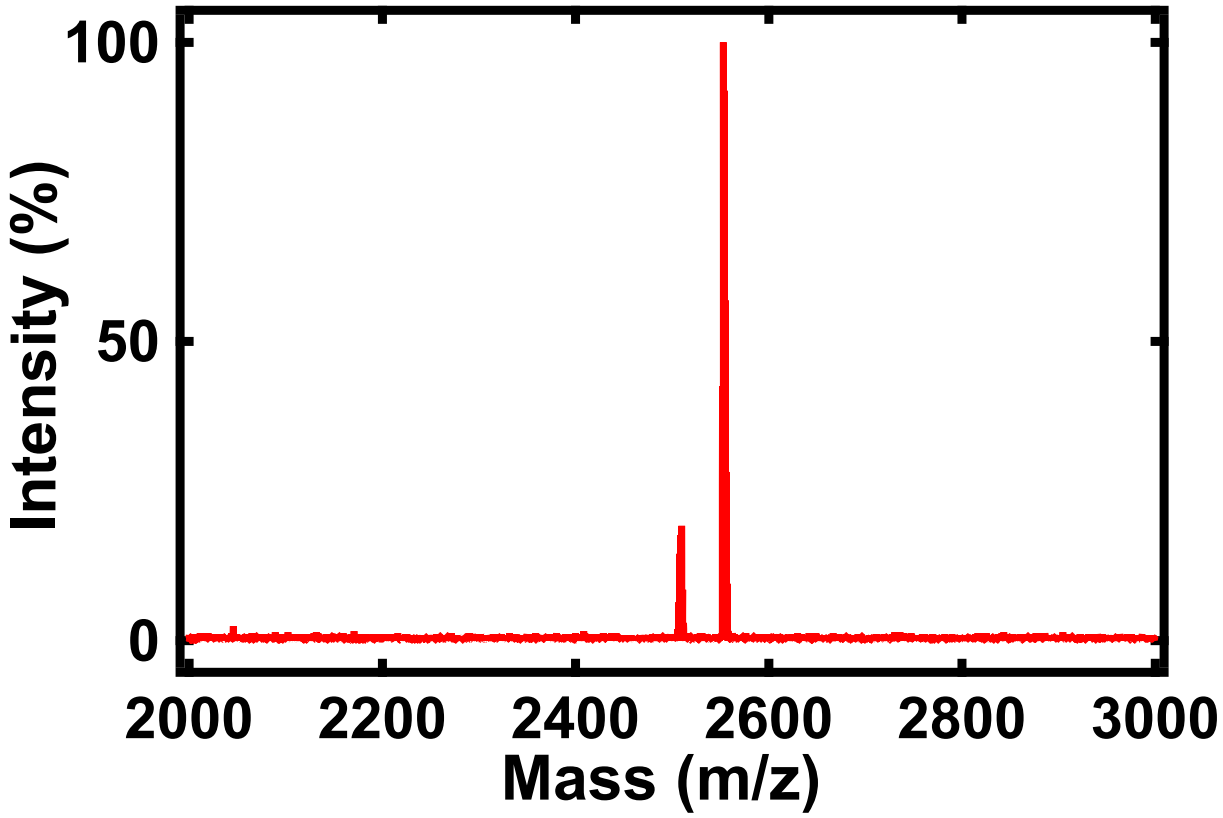


Figure S22. A MALDI mass spectrum obtained for **5a**.

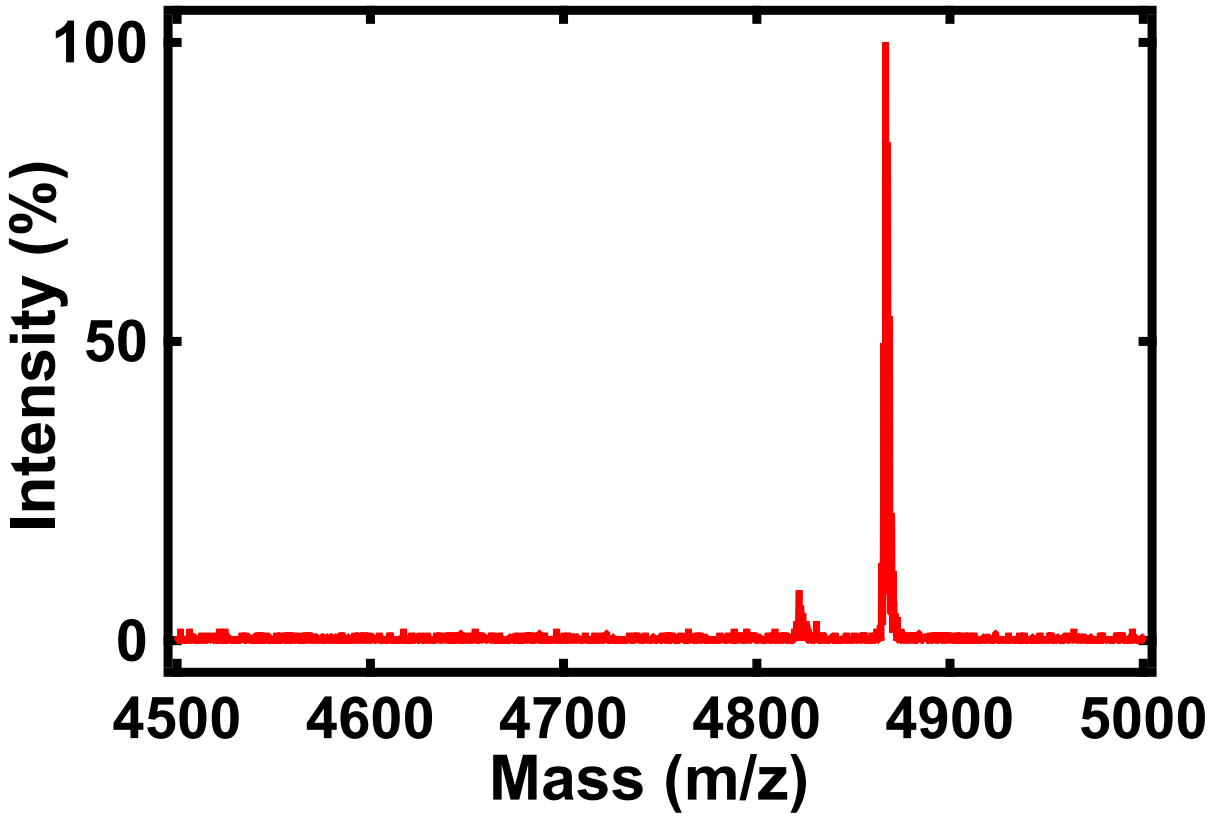


Figure S23. A MALDI mass spectrum obtained for **6a**.

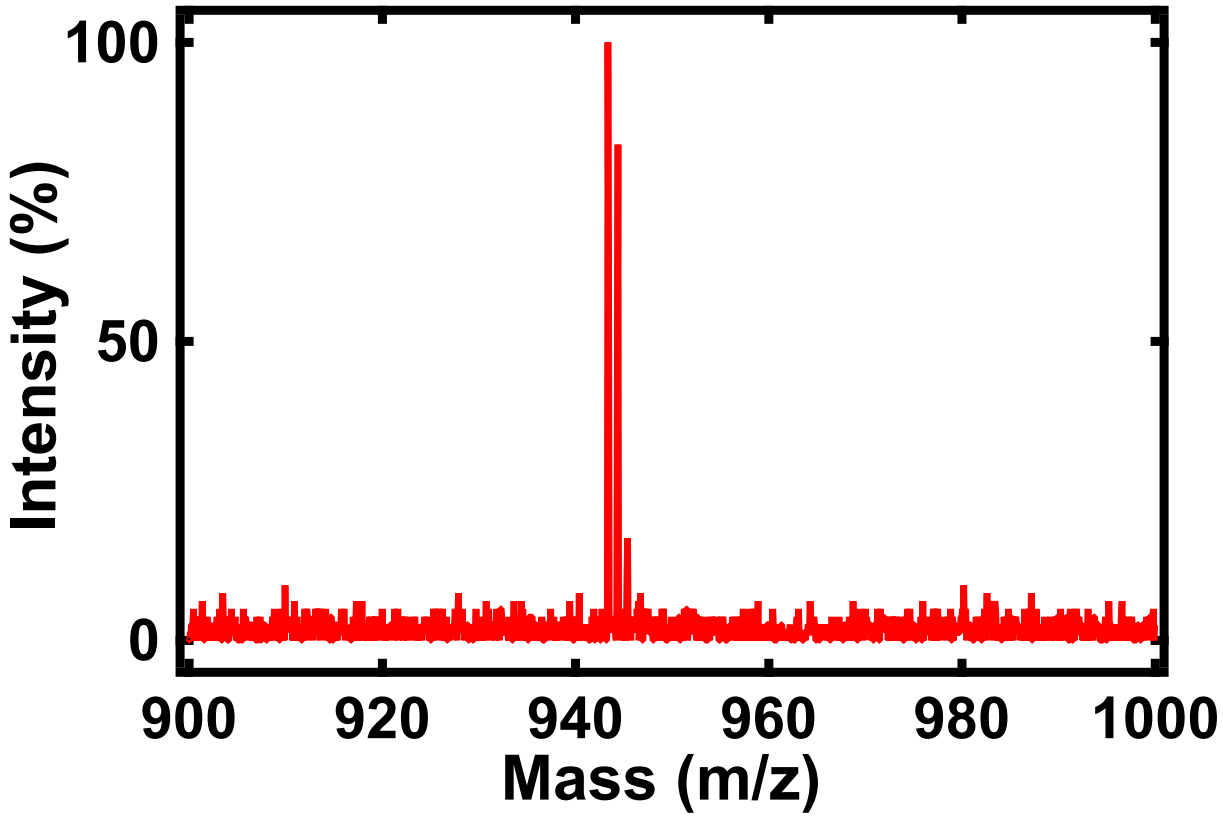


Figure S24. A MALDI mass spectrum obtained for **4b**.

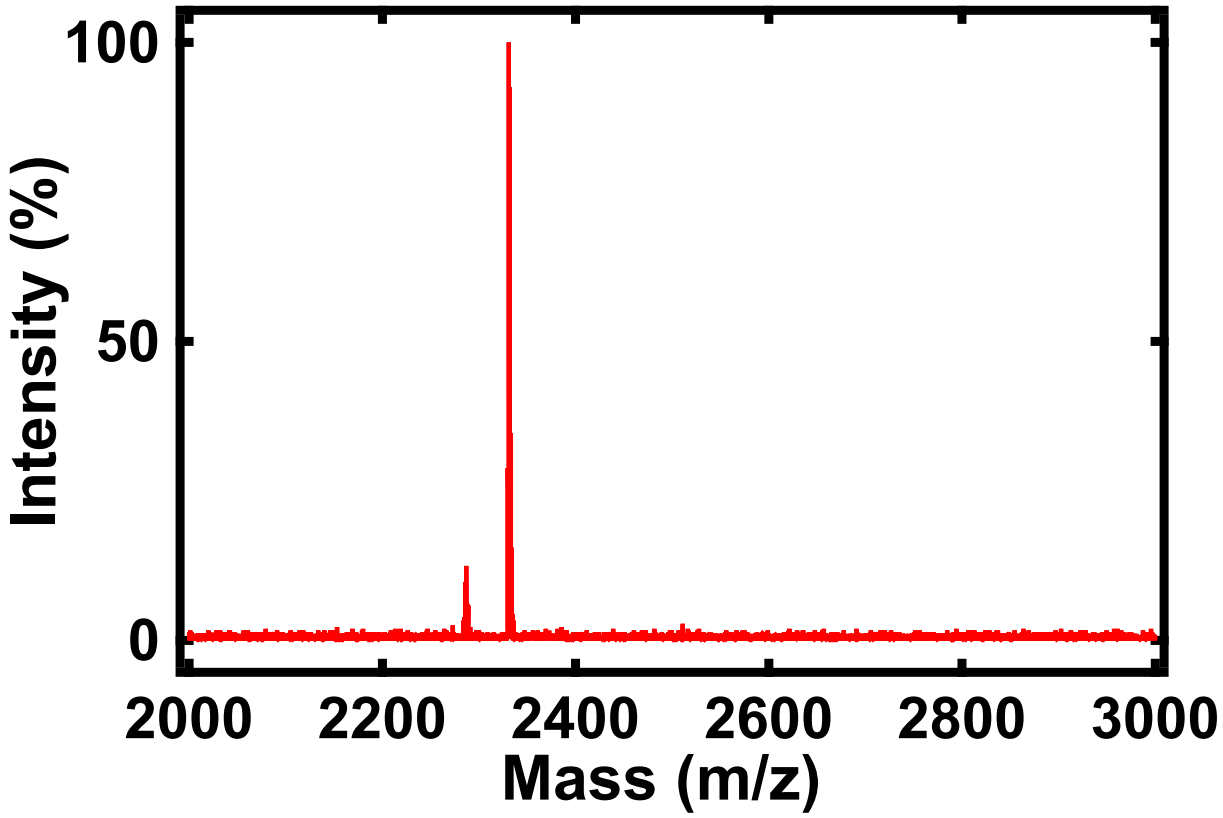


Figure S25. A MALDI mass spectrum obtained for **5b**.

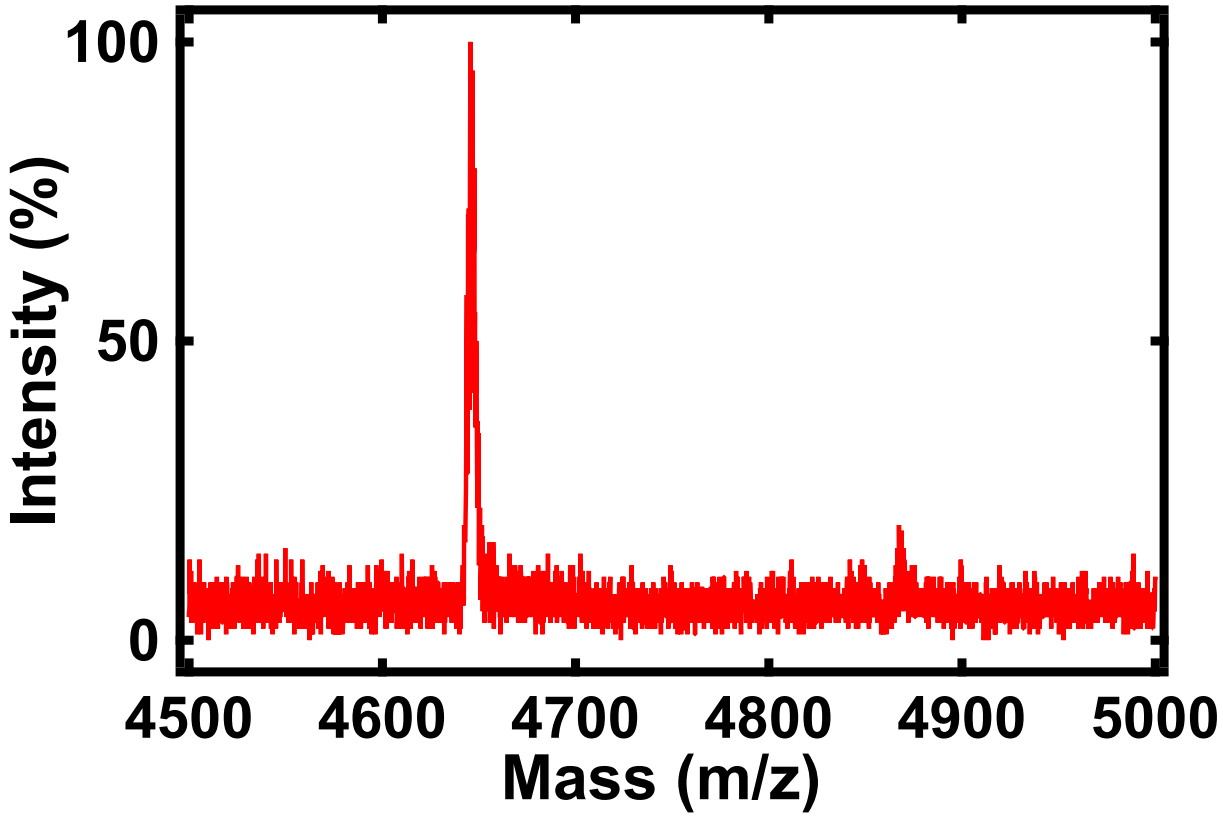


Figure S26. A MALDI mass spectrum obtained for **6b**.

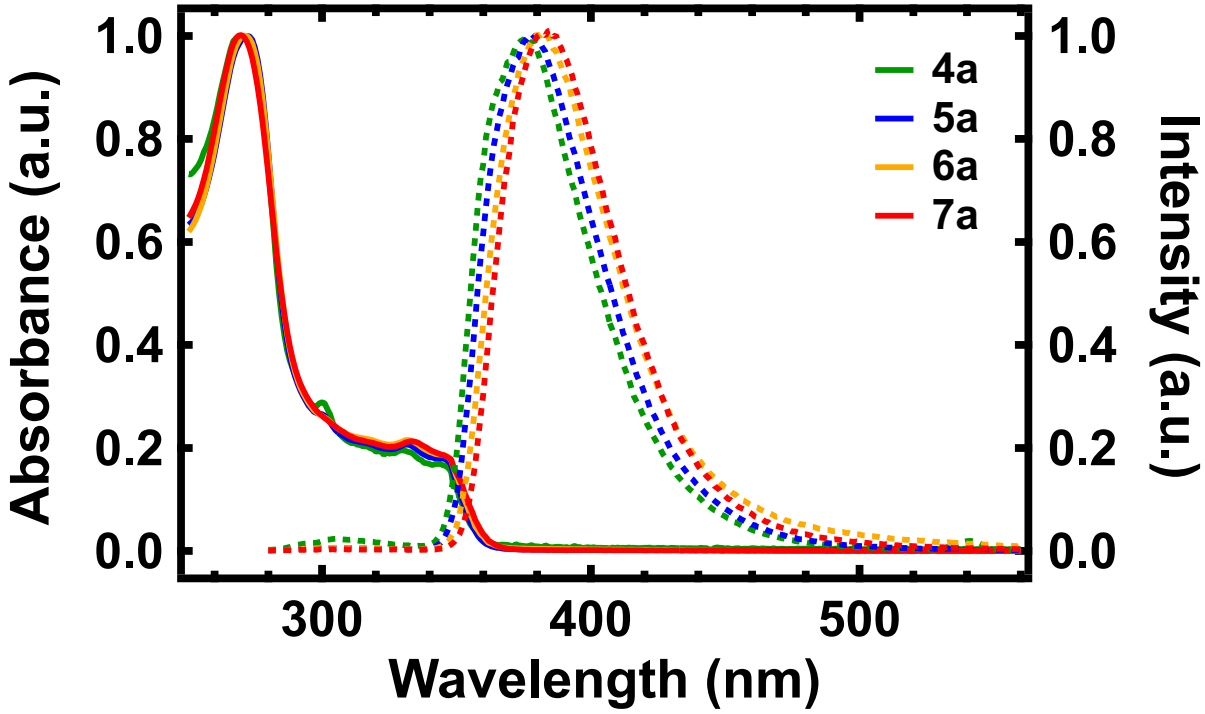


Figure S27. The. UV-Vis absorption spectra obtained for **4a** (green solid trace), **5a** (blue solid trace), **6a** (orange solid trace), and **7a** (red solid trace), and the fluorescence emission spectra obtained for **4a** (green dashed trace), **5a** (blue dashed trace), **6a** (orange dashed trace), and **7a** (red dashed trace).

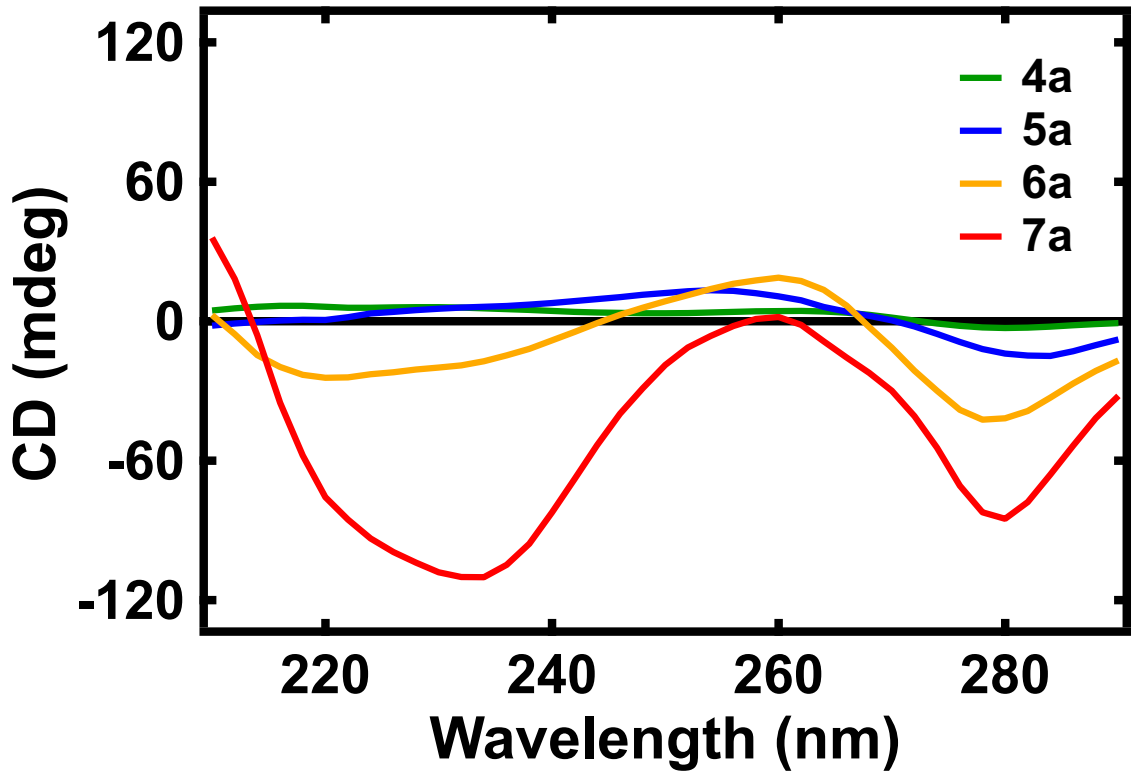


Figure S28. The CD spectra obtained for **4a** (green trace), **5a** (blue trace), **6a** (orange trace), and **7a** (red trace).