Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

pH-dependent cross-linking of catechols through oxidation via Fe^{3+} and potential implications for mussel adhesion

Dominic E. Fullenkamp,[1,4,‡] Devin G. Barrett,[1,4,5,‡] Dusty Rose Miller,[8] Josh W. Kurutz,[7] and Phillip B. Messersmith[,1-6]*

[1]Biomedical Engineering Department, [2]Materials Science and Engineering Department, [3]Chemical and Biological Engineering Department, [4]Chemistry of Life Processes Institute, [5]Institute for Bionanotechnology in Medicine, [6]Robert H. Lurie Comprehensive Cancer Center, [7]Integrated Molecular Structure Education and Research Center, Northwestern University, Evanston, IL 60208.

[8]Biomolecular Science and Engineering Program, University of California, Santa Barbara, California 93106, USA

Author Contributions

‡DEF and DGB contributed equally.

Supplementary Methods

Oxidation of 4-methylcatechol (4MC) with Fe^{3+} and mass spec (MS) analysis: 7.4 mg 4MC (Sigma) was dissolved in 800 μL water, and 200 μL 100 mM $FeCl_3$ solution was added to produce a final concentration of 60 mM 4MC and 20 mM Fe^{3+}. The reaction was allowed to proceed for 24 h. A green precipitate developed. The sample was centrifuged on a bench top minicentrifuge, and the precipitate was dissolved in 100 μL methanol. The sample was directly injected onto an Agilent 1100 LC/MSD high performance ion trap mass spectrometer in negative ion mode. Methanol was used as the mobile phase.

Synthesis of Ac-Ser-DOPA-NH_2: Ac-Ser-DOPA-NH_2 was prepared by standard Fmoc solid phase peptide synthesis methods.[1] Reactions were carried out on a Rink amide resin (Novabiochem) using standard reagents: N,N-dimethylforamide (DMF), 20 % piperidine in DMF for cleavage of Fmoc groups, O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) as the coupling reagent, Fmoc-DOPA(Ac)-OH (Novabiochem) and Fmoc-Ser(tBu)-OH as the amino acids, acetic anhydride for capping the amine terminus, and a solution of 95 % trifluoroacetic acid (TFA), 2.5 % water and 2.5% triisopropylsilane (TIPS) for cleavage from the resin. The ninhydrin test was used to confirm each coupling step. The resin (541 mg; 0.335 mmol) was swollen in DMF for 30 min and rinsed twice with DMF. Piperidine (20 % in DMF) was incubated with the resin for 3 min, and this was repeated twice. The resin was rinsed with DMF five times. Fmoc-DOPA(Ac)-OH (461 mg, 1.00 mmol) and HBTU (381 mg, 1.00 mmol) were dissolved in a minimal amount of solvent, followed by the addition of 350 μL diisopropylethyl amine (DIPEA, 2.01 mmol). The solution was added to the resin and allowed to react for 1 h. The resin was rinsed twice with DMF, three times with 20 % piperidine (3 min), and five times with DMF. Fmoc-Ser(tBu)-OH (642 mg, 1.67 mmol) and HBTU (635 mg, 1.67 mmol) were dissolved in a minimal amount of DMF, followed by the addition of 583 μL DIPEA (3.35 mmol). The solution was added to the resin and allowed to

react for 1 h. The resin was rinsed twice with DMF, three times with 20 % piperidine (3 min), and rinsed five times with DMF. Acetic anhydride (317 µL, 3.35 mmol) was dissolved in several mLs of DMF, and 583 µL DIPEA (3.35 mmol) was added. The solution was added to the resin, and the reaction was allowed to proceed for 1 h. The resin was rinsed five times with DMF, three times with DCM, and three times with methanol, followed by drying under vacuum for 2 days. The TFA cleavage solution (10 mL) was incubated with the resin for 1 h and collected. The resin was rinsed three times with 10 mL TFA, and the eluent was collected. The solution was evaporated under reduced pressure to <1 mL and precipitated in 25 mL ether. The precipitate was dissolved in ~0.5 mL methanol and precipitated in 5 mL of ether twice. The peptide was then dried under vacuum. Peptide mass and purity were confirmed by HPLC-MS as described in the main text.

References

1. Chan, W.C. & White, P.D. *Fmoc solid phase peptide synthesis: a practical approach*. (Oxford University Press, New York, 2000).

Supplementary Results

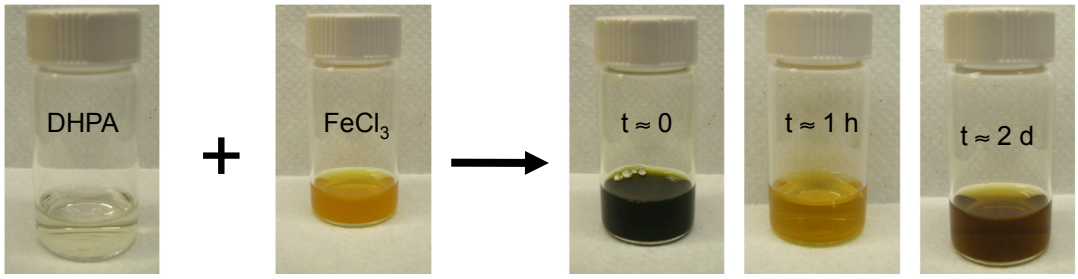

Figure S1. Digital images of DHPA oxidation. Digital images of the reaction between DHPA and Fe^{3+} performed at an Fe^{3+}:DHPA ratio of 2:3.

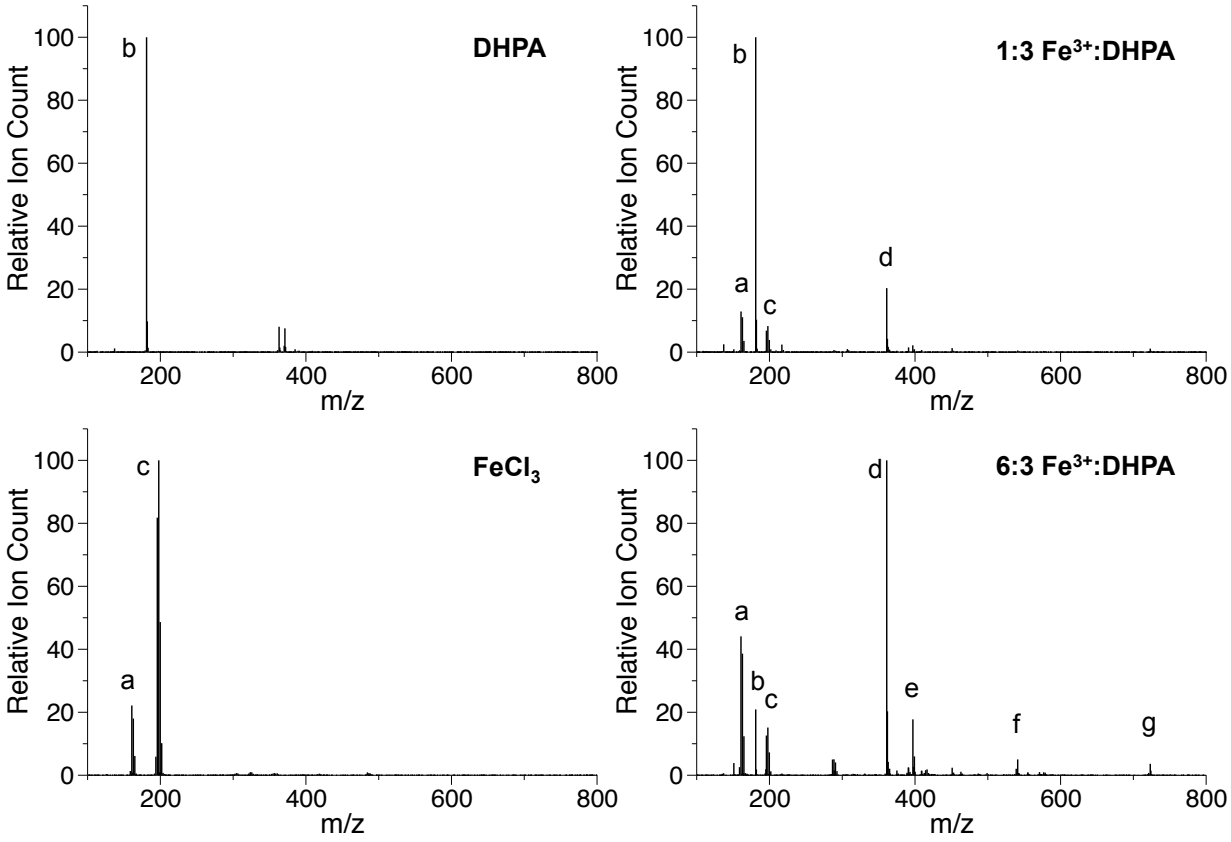

Figure S2. Mass spectra of DHPA oxidation. Representative negative MS spectra of direct injection of DHPA, $FeCl_3$, and the reaction mixture (1:3 and 6:3, Fe^{3+}: DHPA) after 7 d. The solution contained iron species (peaks a and c at m/z of 161 and 198, respectively), DHPA (peak b at m/z of 181), dimers (peak d at m/z of 361), trimers (peak f at m/z of 541), and tetramers (peak g at m/z of 721). Peak e represents dimers associating with a chloride ion (m/z of 397).

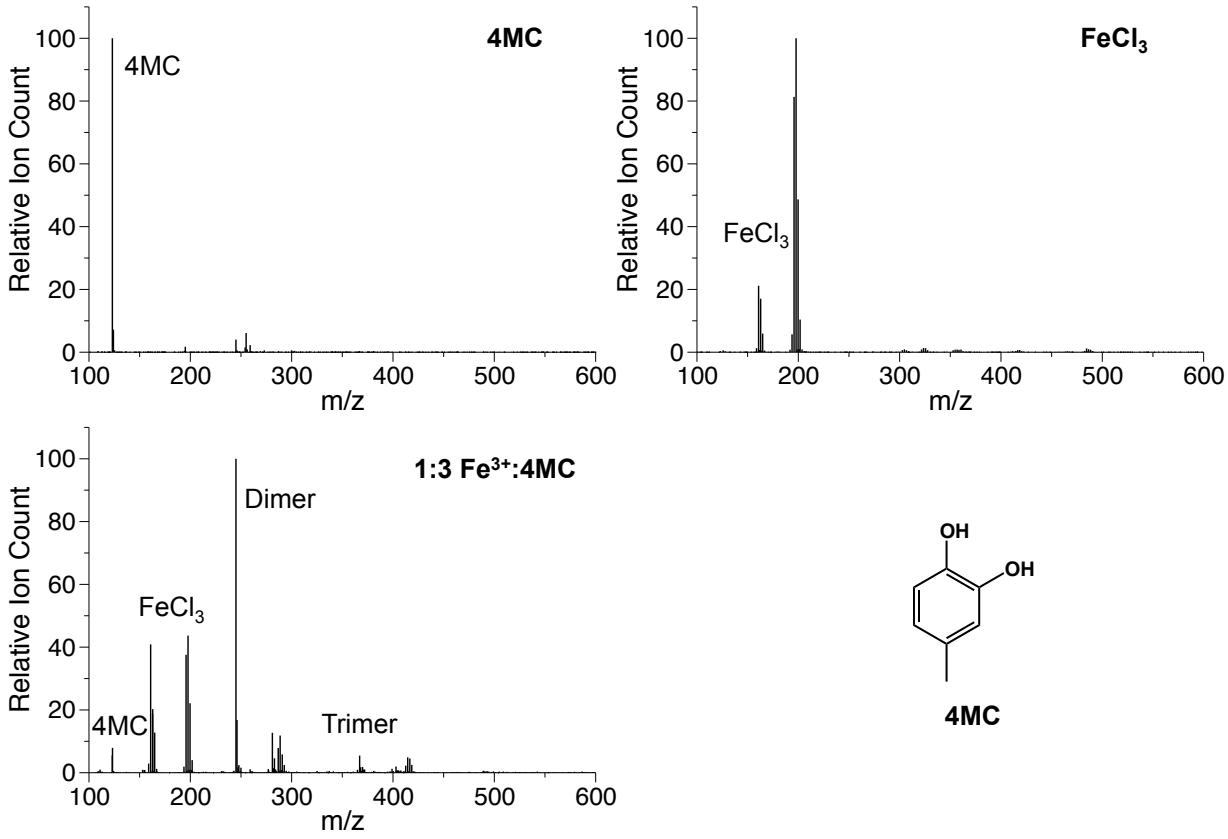

Figure S3. Mass spectra of 4MC oxidation. Negative ion MS in methanol of 4MC, $FeCl_3$, and 1:3 $FeCl_3$: 4MC. Bottom right shows structure of 4MC.

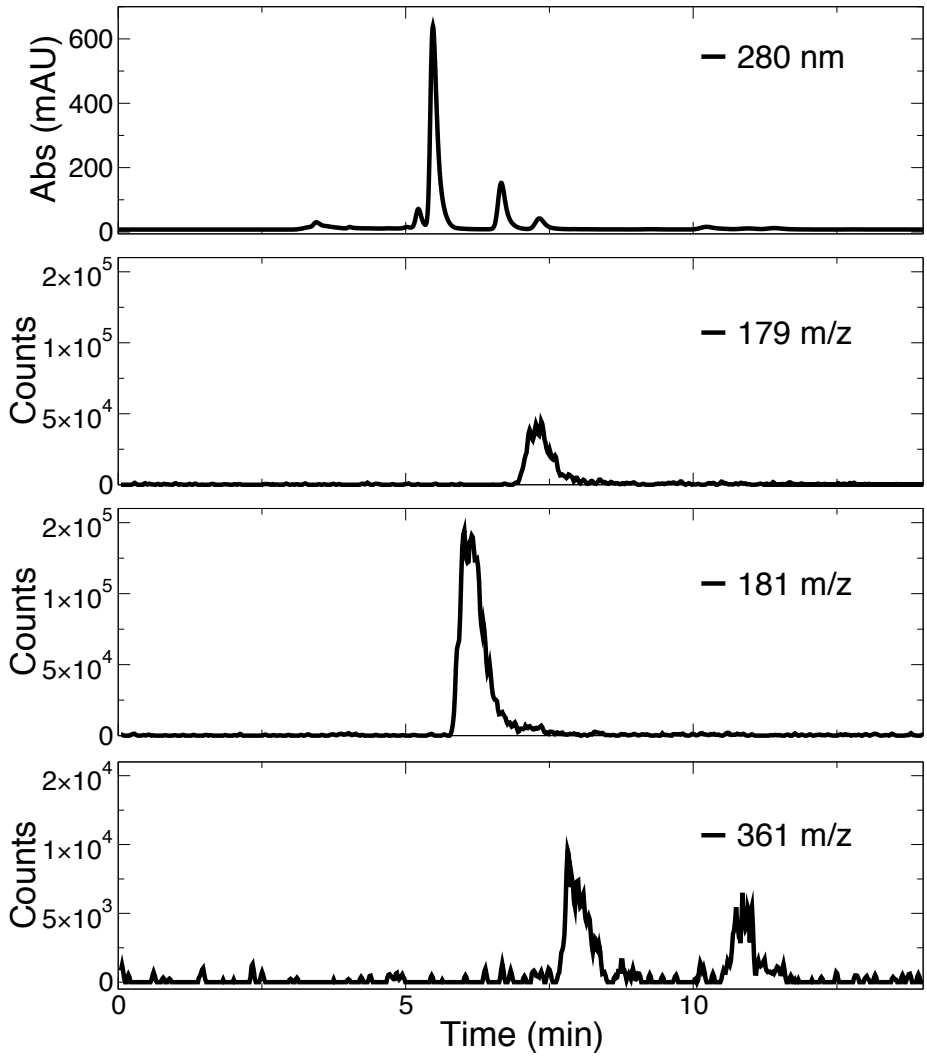

Figure S4. HPLC-MS extracted ion chromatograms (EICs) of DHPA reaction with Fe^{3+}. Representative results from HPLC-MS characterization of the reaction between DHPA (6 mM) and Fe^{3+} (6 mM) after ~100 min of reaction time. EIC in negative ion mode. Top-down: Full UV-Vis (280 nm) trace of the separation; EIC of m/z = 179, corresponding to oxidized quinone form of DHPA; EIC of m/z = 181, corresponding to DHPA; EIC of m/z = 361, corresponding to dimer of DHPA. A short delay in the MS data (~30 s), relative to the HPLC trace, was observed due to distance between the UV-Vis and MS detectors.

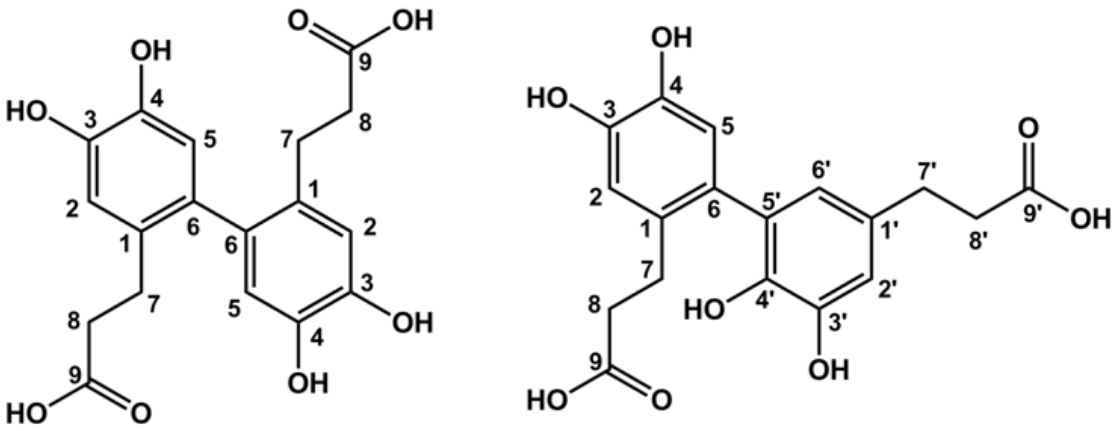

Figure S5. DHPA dimer labeling system. Labeled structures of Dimer 1 and Dimer 2. This numbering system is used to identify protons and carbons in the subsequent NMR characterization.

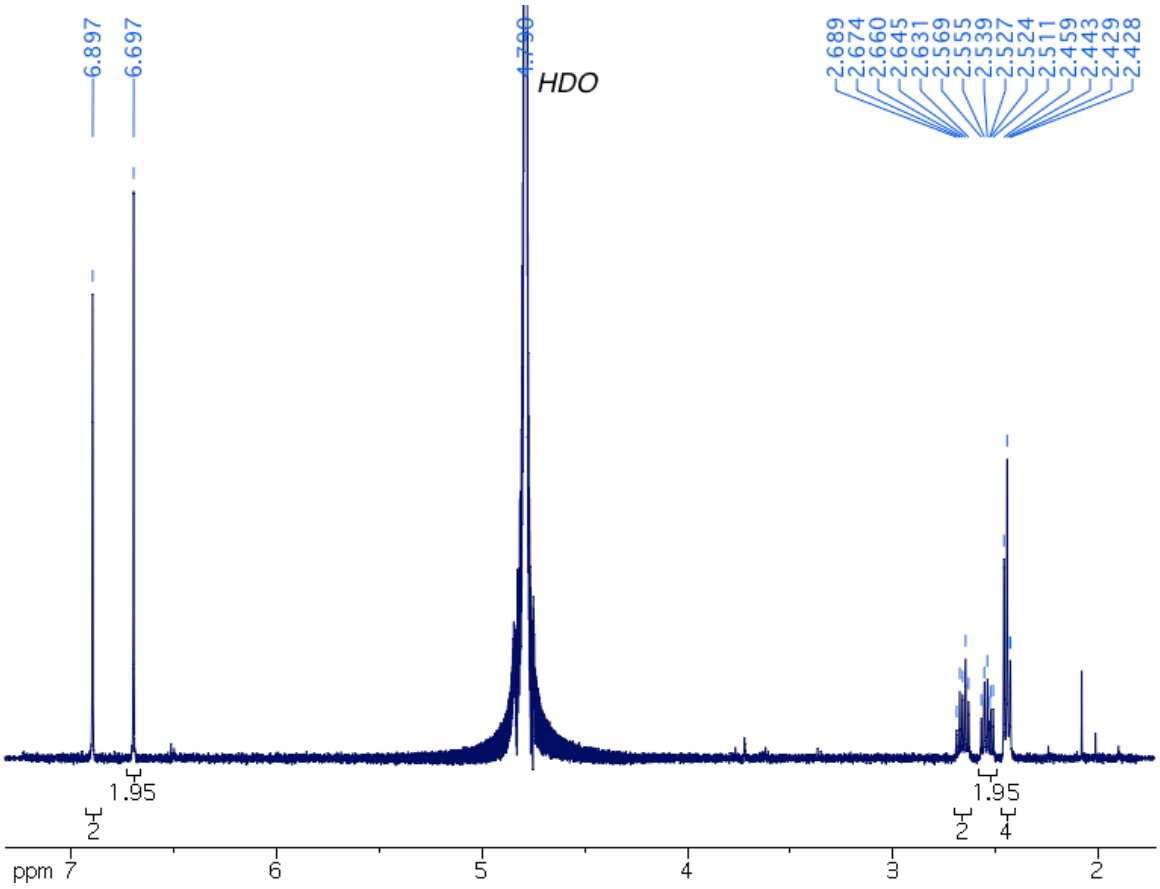

Figure S6. Dimer 1 ^{1}H NMR. 500 MHz ^{1}H spectrum of the compound isolated from the Dimer 1 peak at 7.5 min, acquired at 25 °C and referenced to residual HDO at 4.790 ppm.

^{1}H-NMR (499 MHz; D_2O): δ 6.90 (d, *J* = 0.3 Hz, H-2), 6.70 (d, *J* = 0.3 Hz, H-5), 2.66 (dt, *J* = 14.4, 7.3 Hz, H-7a), 2.57-2.51 (dt, *J* = 14.4, 7.3 Hz, H-7b), 2.46-2.43 (t, *J* = 7.3 Hz, H-8).

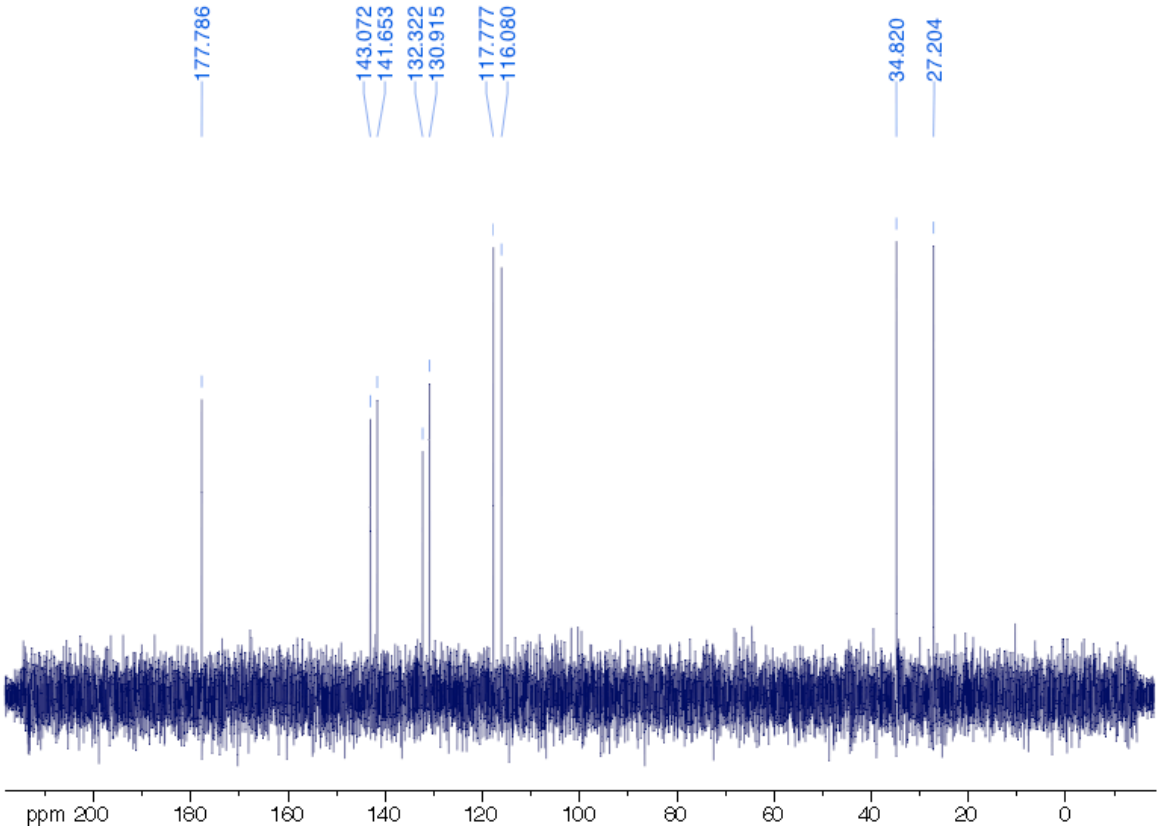

Figure S7. Dimer 1 ^{13}C NMR. ^{13}C spectrum of the compound isolated from the Dimer 1 peak at 7.5 min, acquired at 25 °C on a 500 MHz NMR spectrometer, and indirectly referenced to residual HDO.

^{13}C-NMR (126 MHz, D_2O): δ 177.79 (C-9), 143.07 (C-3 or C-4), 141.65 (C-4 or C-3), 132.32 (C-6), 130.92 (C-1), 117.78 (C-5), 116.08 (C-2), 34.82 (C-8) , 27.20 (C-7).

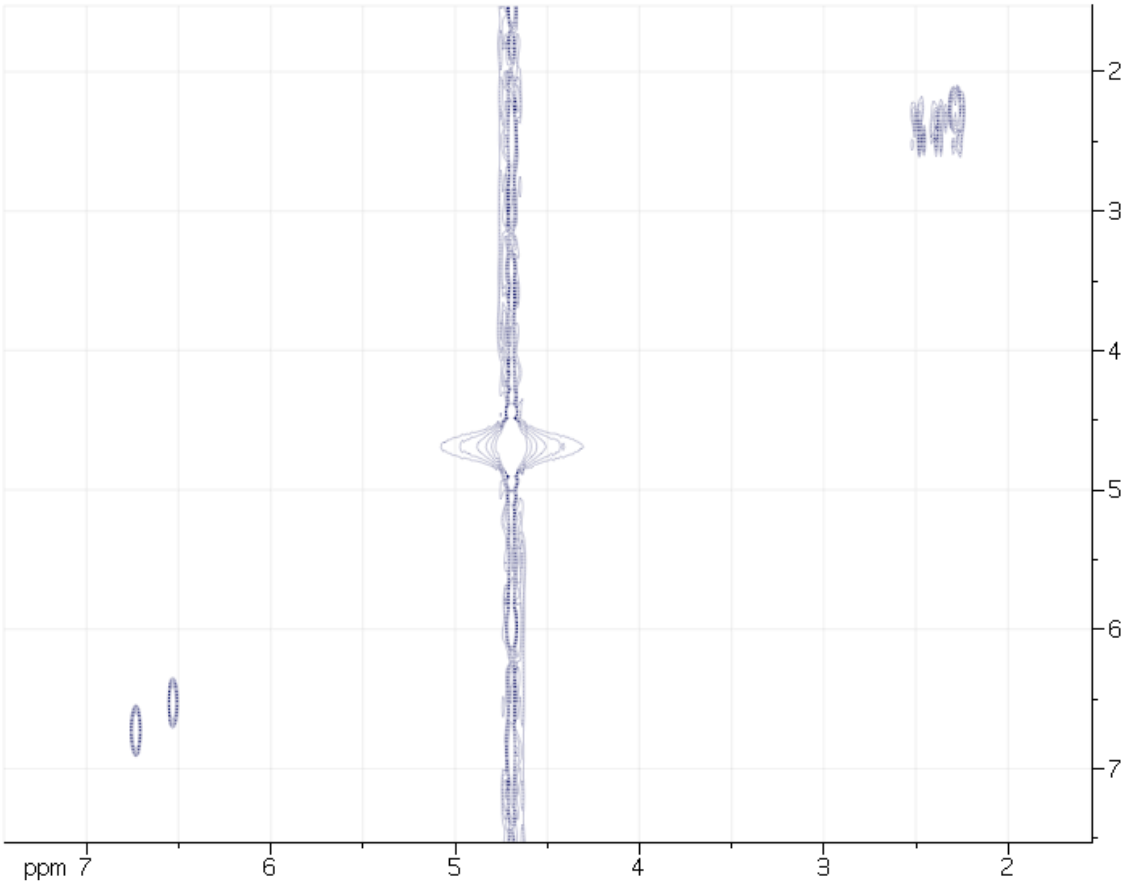

Figure S8. Dimer 1 ^{1}H-^{1}H COSY. ^{1}H-^{1}H COSY of the compound isolated from the Dimer 1 peak at 7.5 min, acquired at 25 °C on a 500 MHz NMR spectrometer, and referenced to residual HDO at 4.79 ppm.

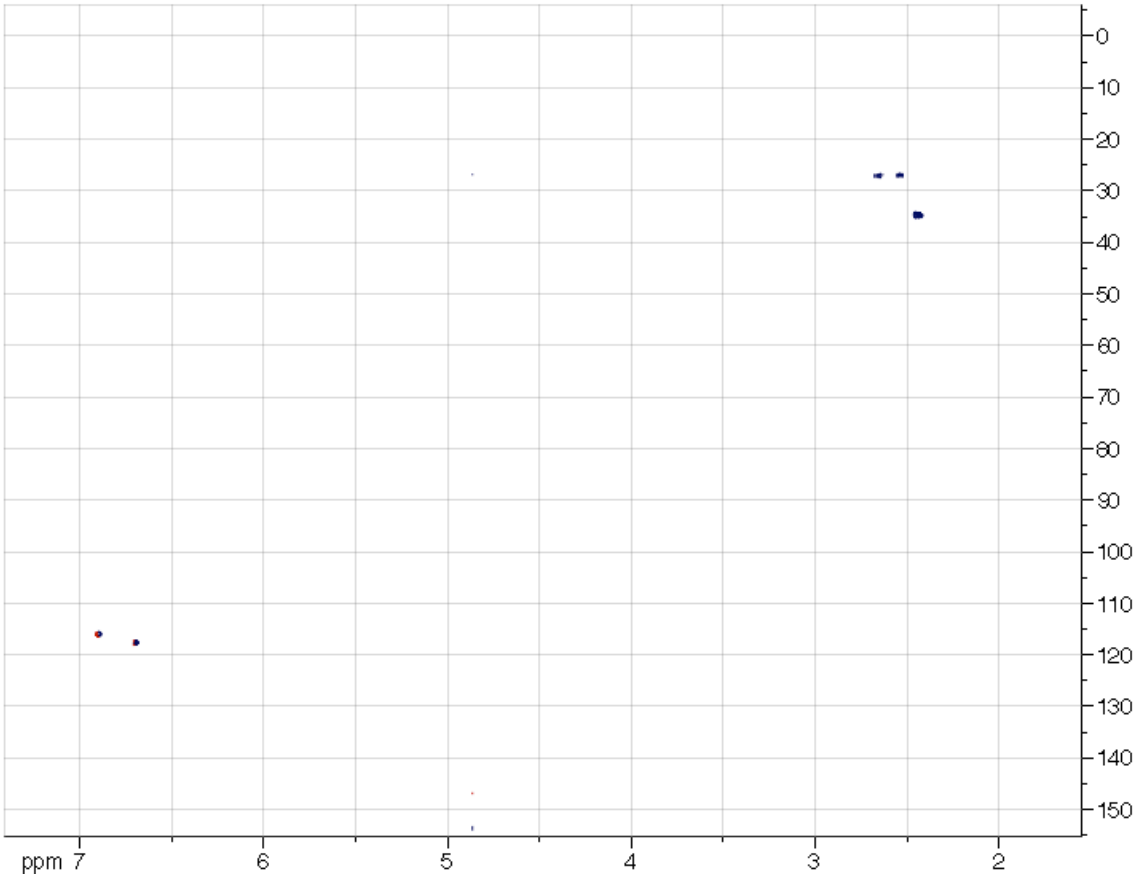

Figure S9. Dimer 1 ^{1}H-^{13}C HSQC. ^{1}H-^{13}C HSQC of the compound isolated from the Dimer 1 peak at 7.5 min, acquired at 25 °C on a 500 MHz NMR spectrometer.

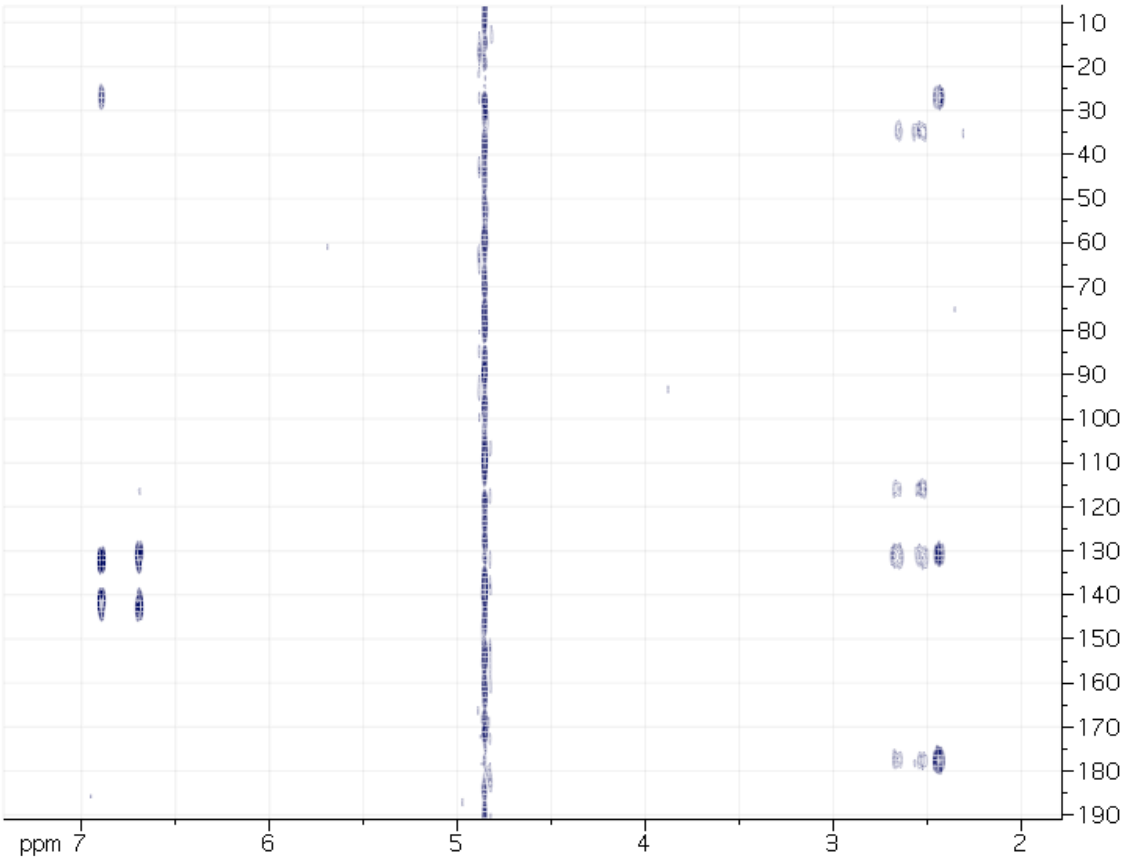

Figure S10. Dimer 1 ^{1}H-^{13}C HMBC. ^{1}H-^{13}C HMBC of the compound isolated from the Dimer 1 peak at 7.5 min, acquired at 25 °C on a 500 MHz NMR spectrometer.

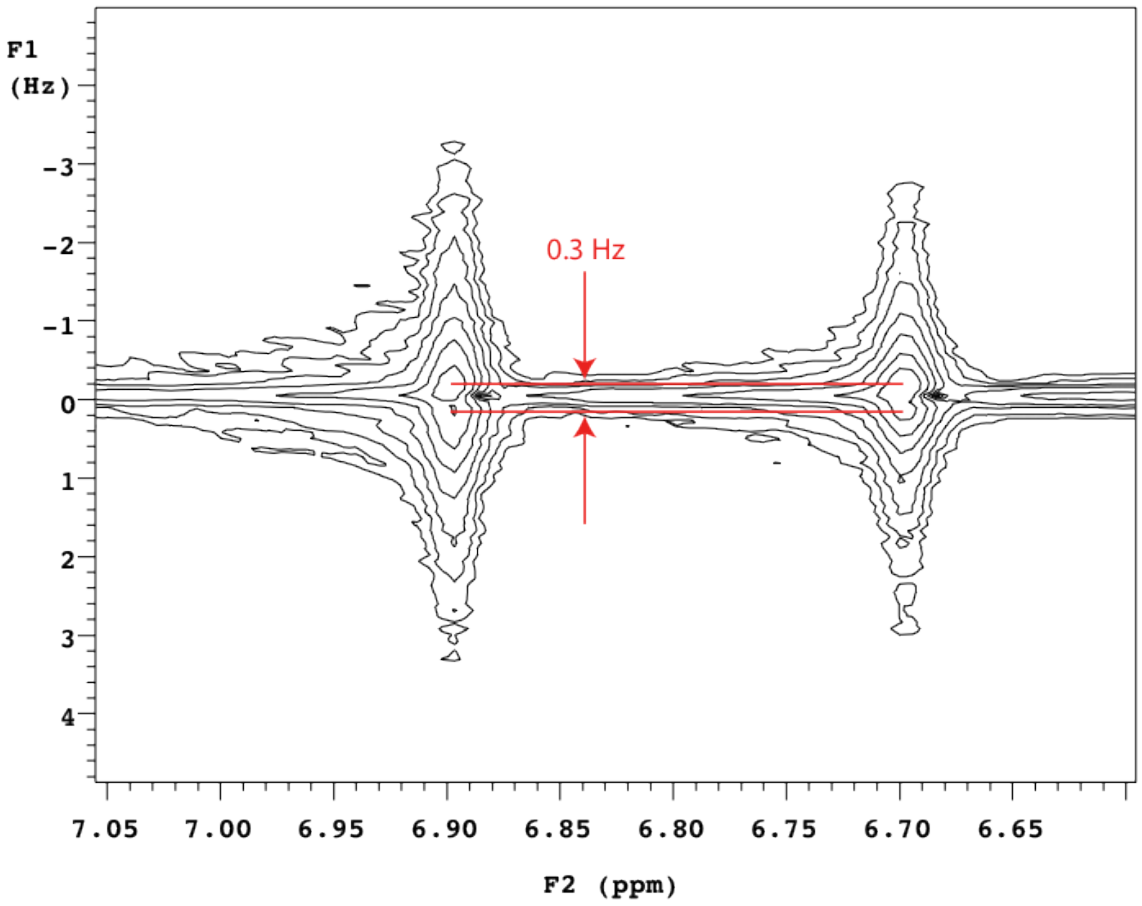

Figure S11. Dimer 1 2D-J NMR. Aromatic region of the ^{1}H-^{1}H homonuclear 2D-J correlation spectrum of the compound isolated from the Dimer 1 peak at 7.5 min, acquired at 25 °C on a 500 MHz NMR spectrometer.

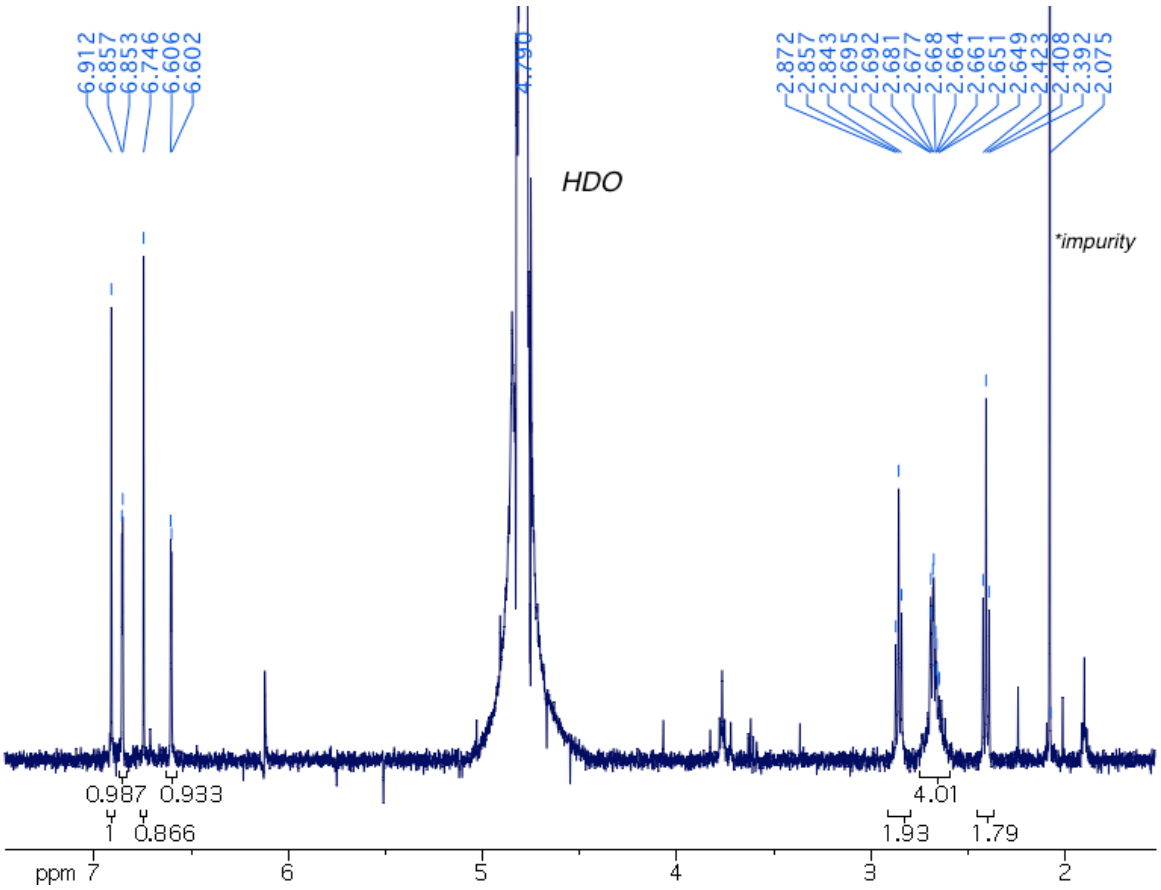

Figure S12. Dimer 2 ^{1}H NMR. 500 MHz ^{1}H NMR spectrum of the compound isolated from the Dimer 2 peak at 10.5 min, acquired at 25 °C and referenced to HDO at 4.79 ppm.

Dimer 2, ring with para protons:
1H-NMR (499 MHz; D2O): δ 6.91 (d, *J* = 0.3 Hz, H-2), 6.75 (d, *J* = 0.3 Hz, H-5), 2.65 (dt, *J* = 14.0, 7.7 Hz, H-7), 2.41 (t, *J* = 7.7 Hz, H-8).

Dimer 2, Ring with meta protons:
1H-NMR (499 MHz; D2O): δ 6.86 (d, *J* = 2.1 Hz, H-6'), 6.60 (d, *J* = 2.1 Hz, H-2'), 2.86 (t, *J* = 7.4 Hz, H-7'), 2.68 (dt, *J* = 14.0, 7.4 Hz, H-8').

Carbons assignments were derived from ^{1}H-^{13}C HSQC and HMBC data because the sample for the compound isolated from the Dimer 2 peak at 10.5 min was too dilute.

Dimer 2, ring with para protons:
^{13}C-NMR (126 MHz, D_2O): δ 177.91 (C-9), 141.3-143.8 (C-3 & C-4), 131.61 (C-1 or C-6), 129.12 (C-6 or C-1), 117.59 (C-5), 116.41 (C-2), 34.91 (C-8), 27.24 (C-7).

Dimer 2, ring with meta protons:
^{13}C-NMR (126 MHz, D_2O): δ 177.91 (C-9'), 139.22 (C-4'), 132.7 (C-1'), 121.81 (C-2'), 114.76 (C-6'), 35.56 (C-8'), 29.44 (M-C-7), (C-3' & -5' are unassigned).

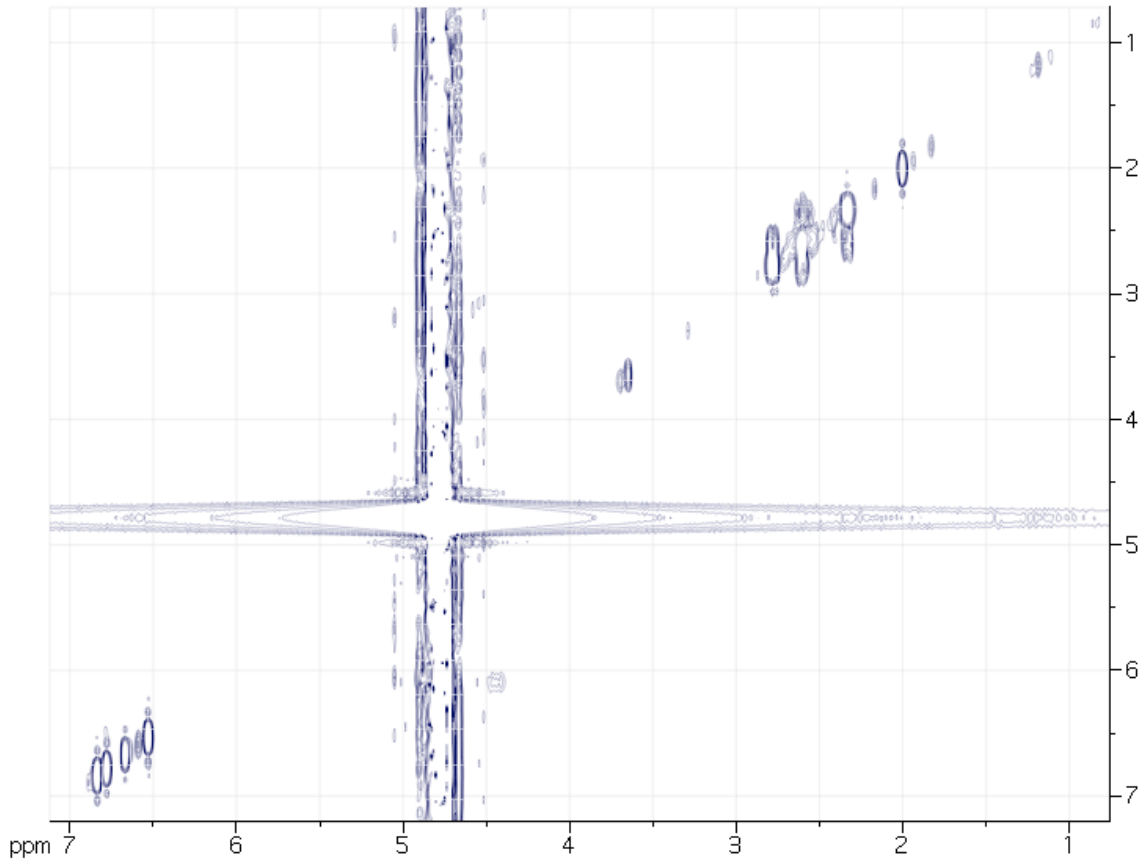

Figure S13. Dimer 2 ^{1}H-^{1}H COSY. ^{1}H-^{1}H COSY of the compound isolated from the Dimer 2 peak at 10.5 min, acquired at 25 °C on a 500 MHz NMR spectrometer.

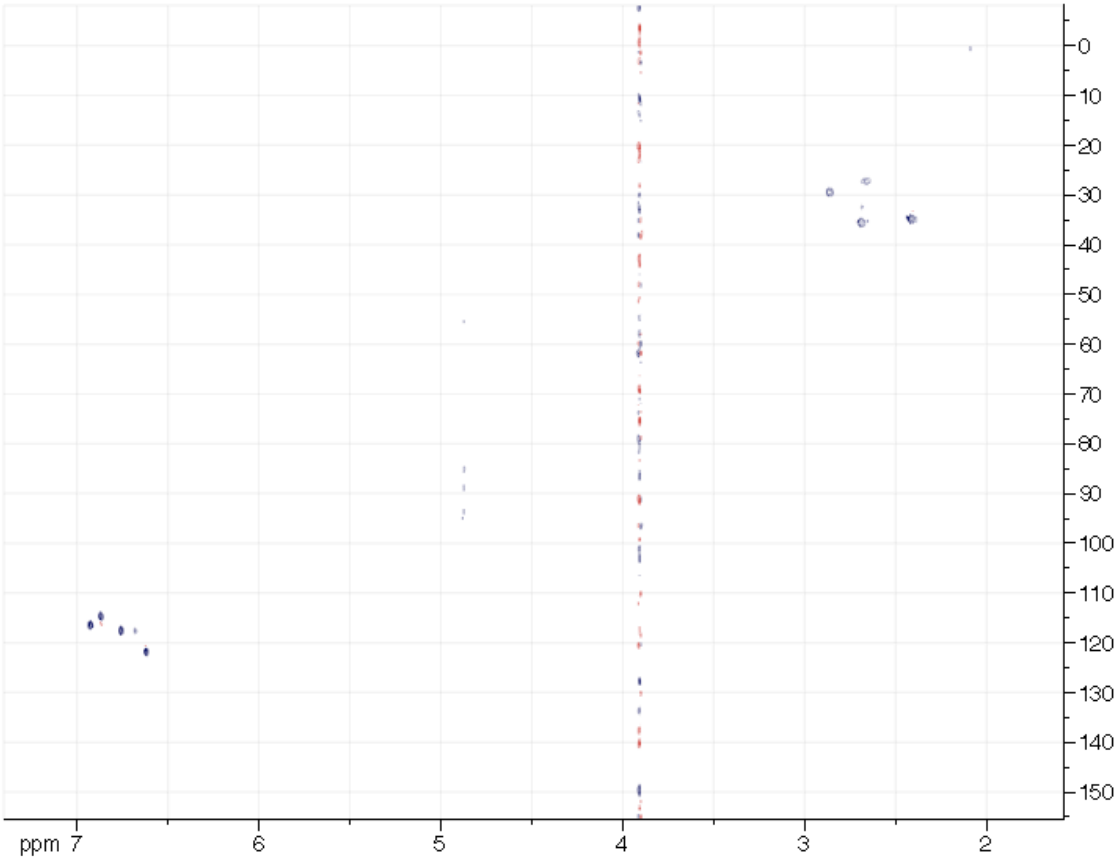

Figure S14. Dimer 2 ^{1}H-^{13}C HSQC. ^{1}H-^{13}C HSQC of the compound isolated from the Dimer 2 peak at 10.5 min, acquired at 25 °C on a 500 MHz NMR spectrometer.

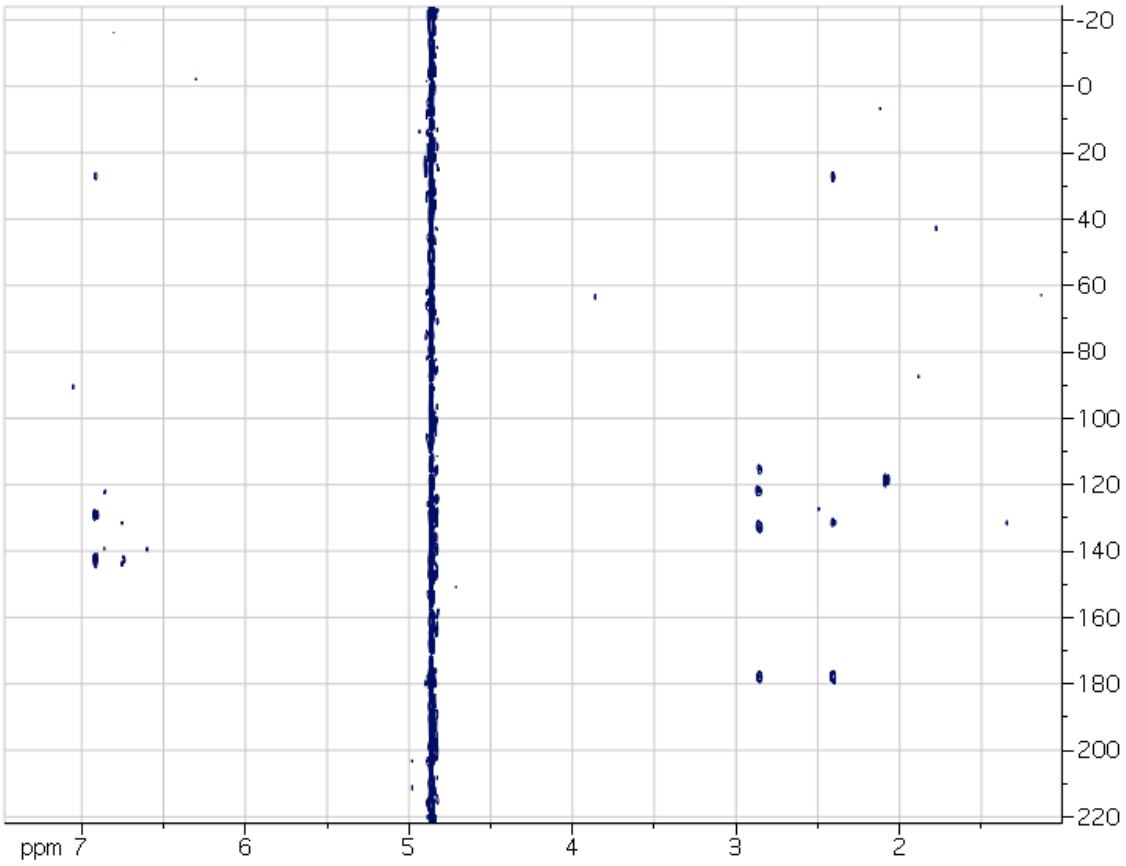

Figure S15. Dimer 2 ^{1}H-^{13}C HMBC. ^{1}H-^{13}C HMBC of the compound isolated from the Dimer 2 peak at 10.5 min, acquired at 25 °C on a 500 MHz NMR spectrometer.

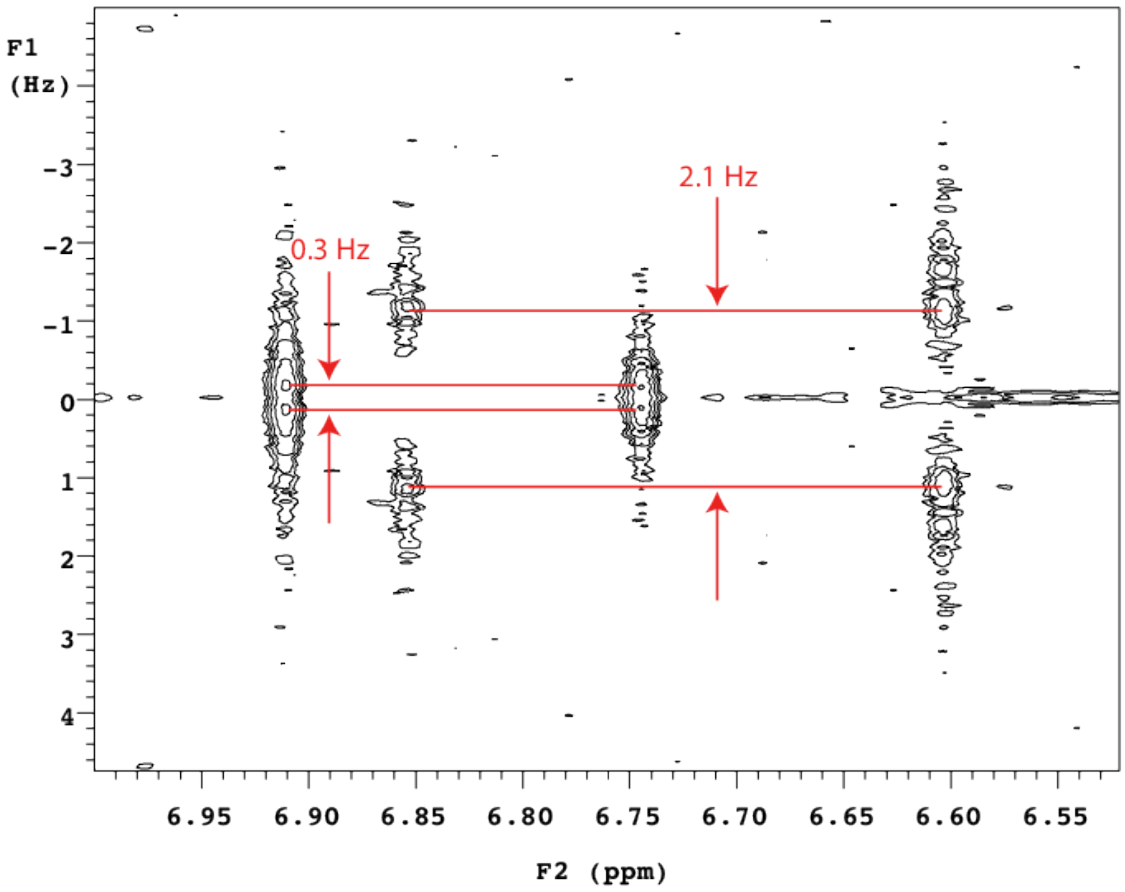

Figure S16. Dimer 2 2D-J NMR. ^{1}H homonuclear 2D-J correlation spectrum of the compound isolated from the Dimer 2 peak at 10.5 min, acquired at 25 °C on a 500 MHz spectrometer.

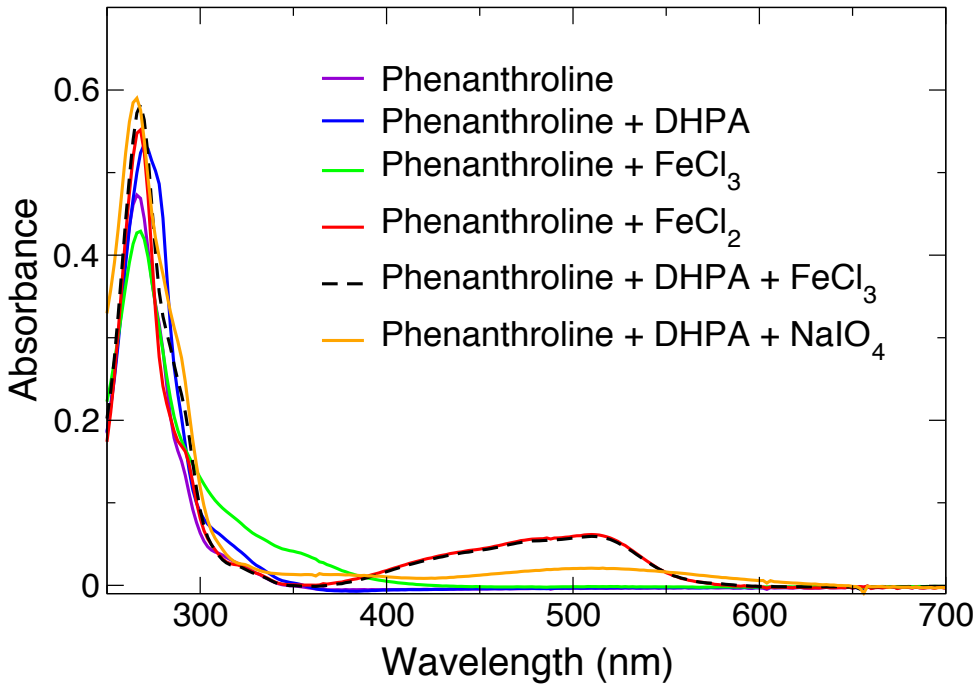

Figure S17. UV-Vis confirmation of the reduction of Fe^{3+} to Fe^{2+} in the presence of DHPA. The interaction between 1,10-phenanthroline and Fe^{2+} results in a color change from yellow/orange to deep red. The transition to a red solution was only observed for solutions containing phenanthroline and Fe^{2+} or phenanthroline, DHPA, and Fe^{3+}.

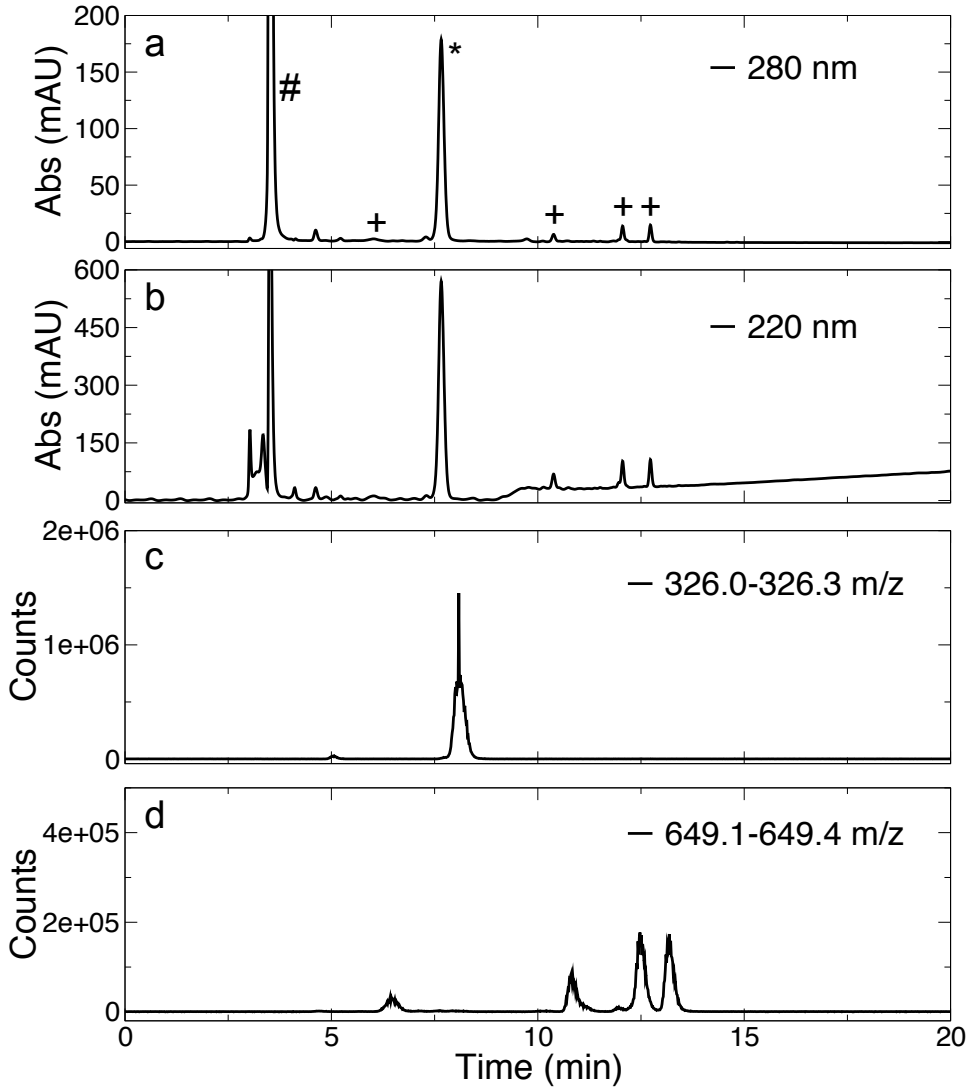

Figure S18. HPLC-MS EIC of pH 5 dipeptide with 1xFe^{3+}. Representative HPLC-MS experiment of Ac-Ser-DOPA-NH_2 at pH 5 and 1xFe^{3+}. UV chromatograms at (a) 280 and (b) 220 nm. Peaks correspond to (#) EDTA/Fe injection peak, (*) peptide, and (+) dimer of peptide. Negative ion mode EIC of (c) mass of the peptide and (d) mass of dimer of the peptide. EICs allow for identification of UV chromatogram peaks.

Table S1. Relative abundance of DHPA, *o*-quinone, and dimers[a]

$[Fe^{3+}]$: [DHPA]	$[Fe^{3+}]$ (mM)	[DHPA] (mM)	Time of Reaction	Amount DHPA (%)	Amount Quinone (%)	% of Total Dimer	
						Dimer 1 C6-C6	Dimer 2 C5-C6
0:1	0	60	NA	100	0	-	-
1:3	20	60	7 d	77	0	72	29
2:3	40	60	7 d	57	0	76	24
3:3	60	60	1 min	60	4.66	76	24
3:3	60	60	37 min	43	9.24	77	23
3:3	60	60	189 min	43	1.98	77	23
3:3	60	60	618 min	43	1.31	78	22
3:3	60	60	1 d	46	0.99	78	22
3:3	60	60	7 d	43	0	78	22
4:3	80	60	7 d	29	0	80	20
6:3	120	60	7 d	12	0	85	15

[a]As determined by the areas under the HPLC curves of Figure 3