Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Toxic Dopamine Metabolite DOPAL Forms an Unexpected Dicatechol Pyrrole Adduct with Lysines of α-Synuclein

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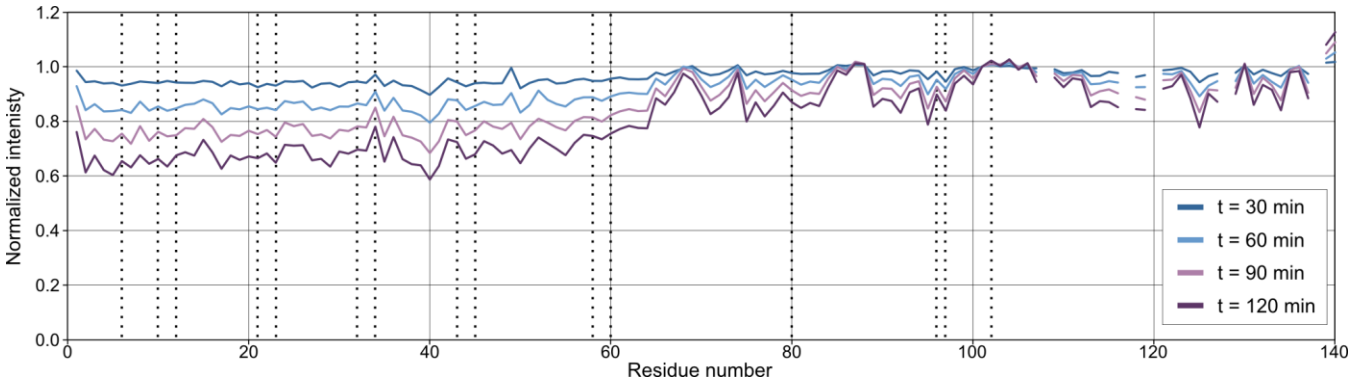


Figure S1. DOPAL reactivity toward α-synuclein monitored by backbone amide ^{1}H-^{15}N crosspeak intensity in 2D HSQC spectra. Samples of ^{1}H-^{15}N labeled Ac-WT aS were extracted from reactions with DOPAL at different time points, and the loss of native amide signal intensity was used to probe for reactive sites along the protein sequence. The intensity for each residue was normalized by its intensity in the initial time point sample (t=0). Dashed lines indicate the positions of Lys residues. Discontinuities in the C-terminal region correspond to Pro residues. The attenuation profile seen here is qualitatively similar to the one observed by Follmer et al. at lower temperature and over a more extended incubation time.[1]

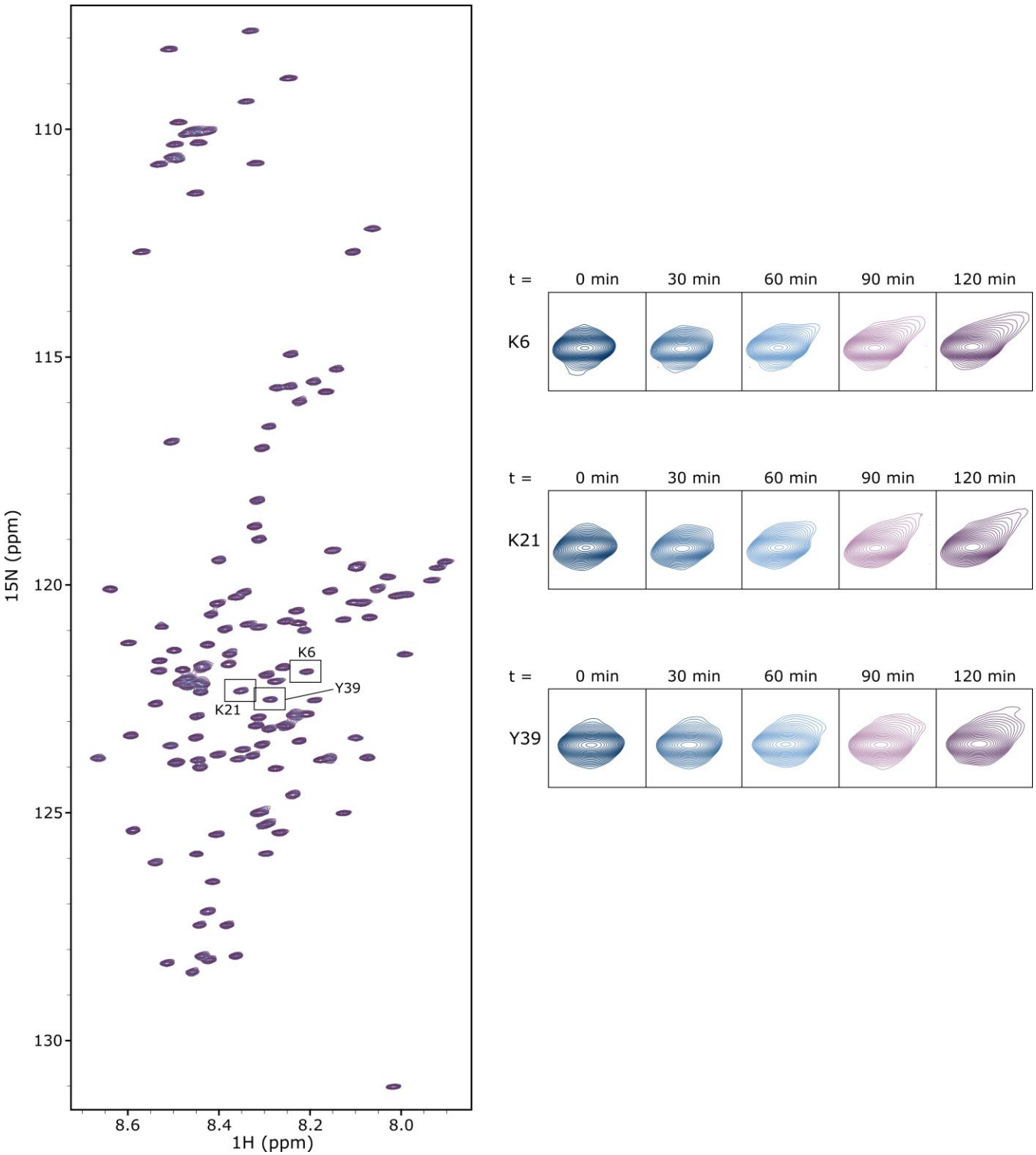


Figure S2. ^{1}H-^{15}N HSQC spectra of DOPAL-reacted N-terminally-acetylated, wild-type α-synuclein. Spectra were collected as described in the Methods section of the Supporting Information and plotted using exponential contours with a multiplication factor of 1.3. The left panel is an overlay of full spectra for the five measured time points (0, 30, 60, 90, and 120 minutes). In the panels on the right, the amide signals of three representative residues are shown.

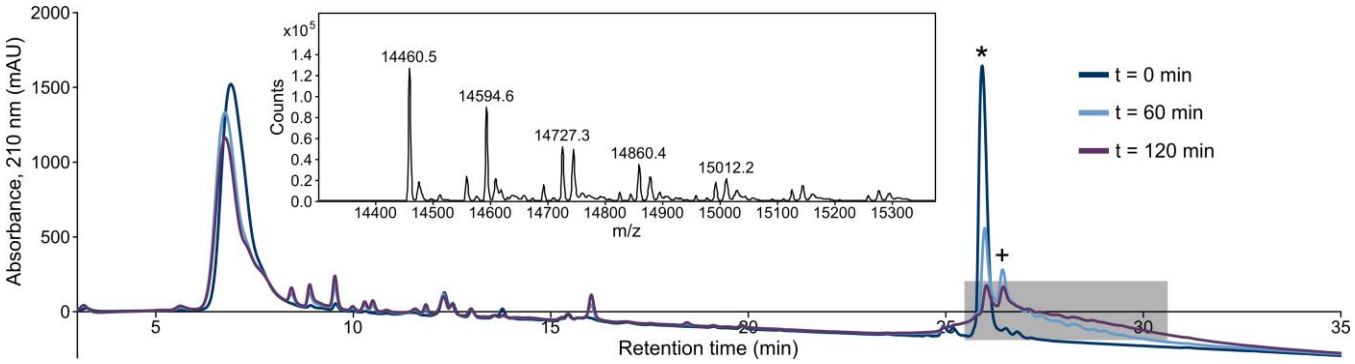


Figure S3. LC-MS analysis of the reaction between DOPAL and non-acetylated WT aS. Samples were withdrawn at 60 minute intervals and subjected to reverse-phase chromatography and ESI-MS. As with Ac-WT aS, there is a progressive loss of the native protein (marked by an asterisk in the chromatogram, m/z = 14460); however, there is a unique product formed (marked by a plus sign), featuring a well-defined elution peak and a mass corresponding to a single Schiff base adduct. The inset shows the deconvoluted mass spectrum of the products in the last reaction time point, from the LC region indicated by a shaded square, which include mass differences of +134 Da, +266 Da and +400 Da over the native protein.

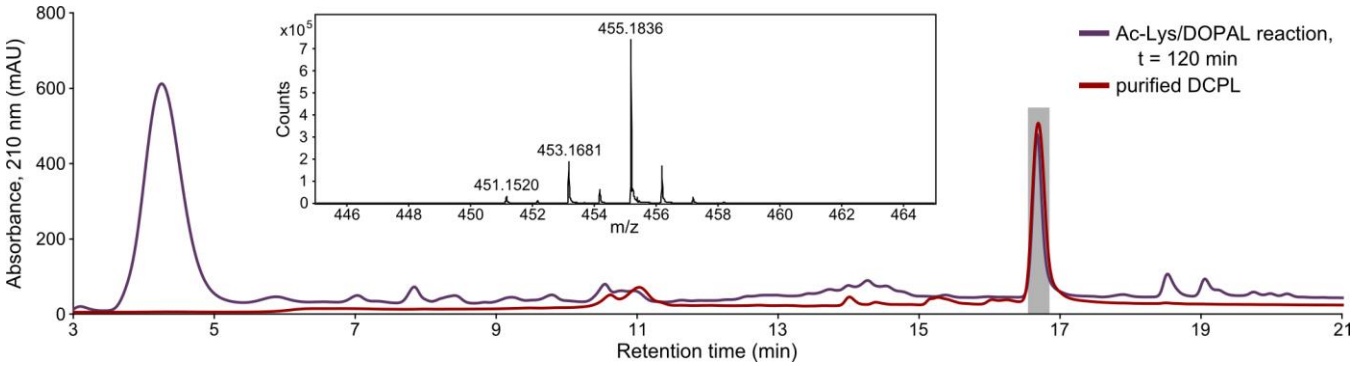


Figure S4. LC-MS confirms the purification of the desired product from the reaction between DOPAL and Ac-Lys. An overlay of the chromatographic traces of the 120 minute time point from the reaction (in purple) and the purified material (red) illustrates a conserved elution profile for the DCPL adduct. The absorbance values for the purified adduct were scaled uniformly in order to match the intensities of the product peak between traces. The inset shows the deconvoluted mass spectrum of the purified material from the LC region indicated by the shaded square, which gives the expected mass of 454 Da, a +266 Da increase over unmodified Ac-Lys.

Table S1. Summary of NMR data for dicatechol pyrrole lysine product and two reactants, DOPAL and Ac-Lys.

	Dicatechol pyrrole lysine						DOPAL						Nα-acetylated lysine			
	^{1}H (ppm)	^{13}C (ppm)	J_{HH} (Hz)	J_{HC} (Hz)	integral		^{1}H (ppm)	^{13}C (ppm)	J_{HH} (Hz)	J_{HC} (Hz)	integral		^{1}H (ppm)	^{13}C (ppm)	integral
ω	1.91	22.7			1.52							ω'	1.98	22.7	1.40
ψ		172.3										ψ'		172.3	
χ		178.9										χ'		178.5	
α	4.29	55.9			0.57							α'	4.26	55.6	0.43
β	1.70, 1.90	33.3			ND[a]							β’	1.69, 1.83	33.2	ND[a]
γ	1.39	23.7			1.06							γ'	1.42	23.2	1.06
δ	1.81	32.2			ND[a]							δ’	1.66	27.9	ND[a]
ε	3.88	50.0			0.96							ε'	2.90	40.4	1.00[b]
1	6.65	120.4		184	0.98	1’	4.58	100.7	5.5	164	0.98				
2		123.9				2’	2.65, 2.73	43.8	13.9, 5.5	126	1.96				
3		130.0				3’		130.1							
4	6.68	116.7	2.0	157	0.96	4’	6.68	117.7	2.0	156	0.97				
5		145.5				5’		145.8							
6		143.9				6’		144.6							
7	6.63	116.0	8.1	158	1.02	7’	6.66	116.0	8.0	156	0.99				
8	6.56	120.9	8.1, 2.0	160	1.00[b]	8’	6.54	121.8	8.0, 2.0	158	1.00[b]				

[a]Not determined due to overlap with other signals

[b]Integrals for the rest of the molecule normalized to the volume of this peak

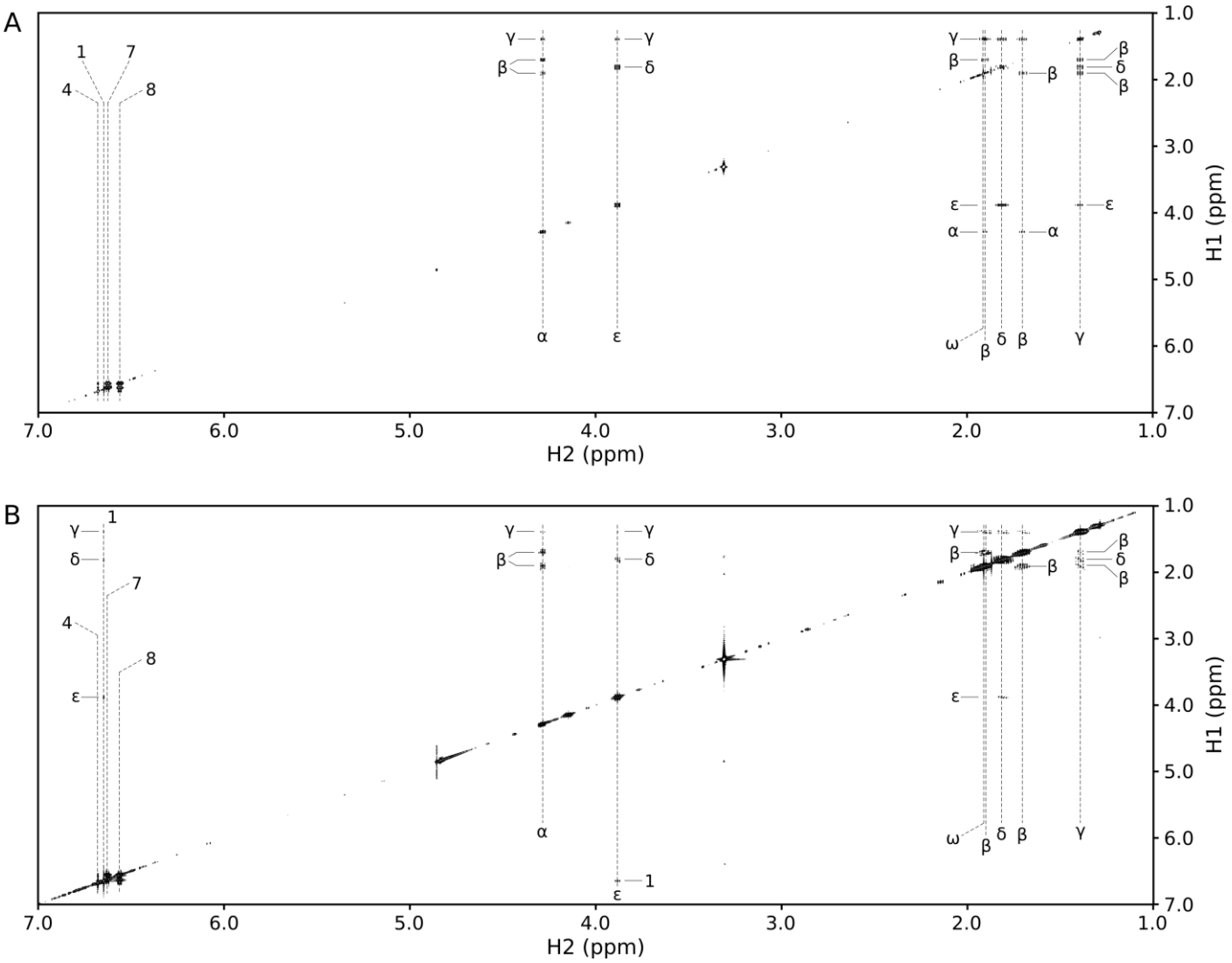


Figure S5. Full 2D ^{1}H-^{1}H spectra for purified dicatechol pyrrole lysine. (**A, B**) ^{1}H-^{1}H TOCSY (A) and ^{1}H-^{1}H ROESY (B) spectra are shown with signal labels corresponding to the structure in Figure 2A.

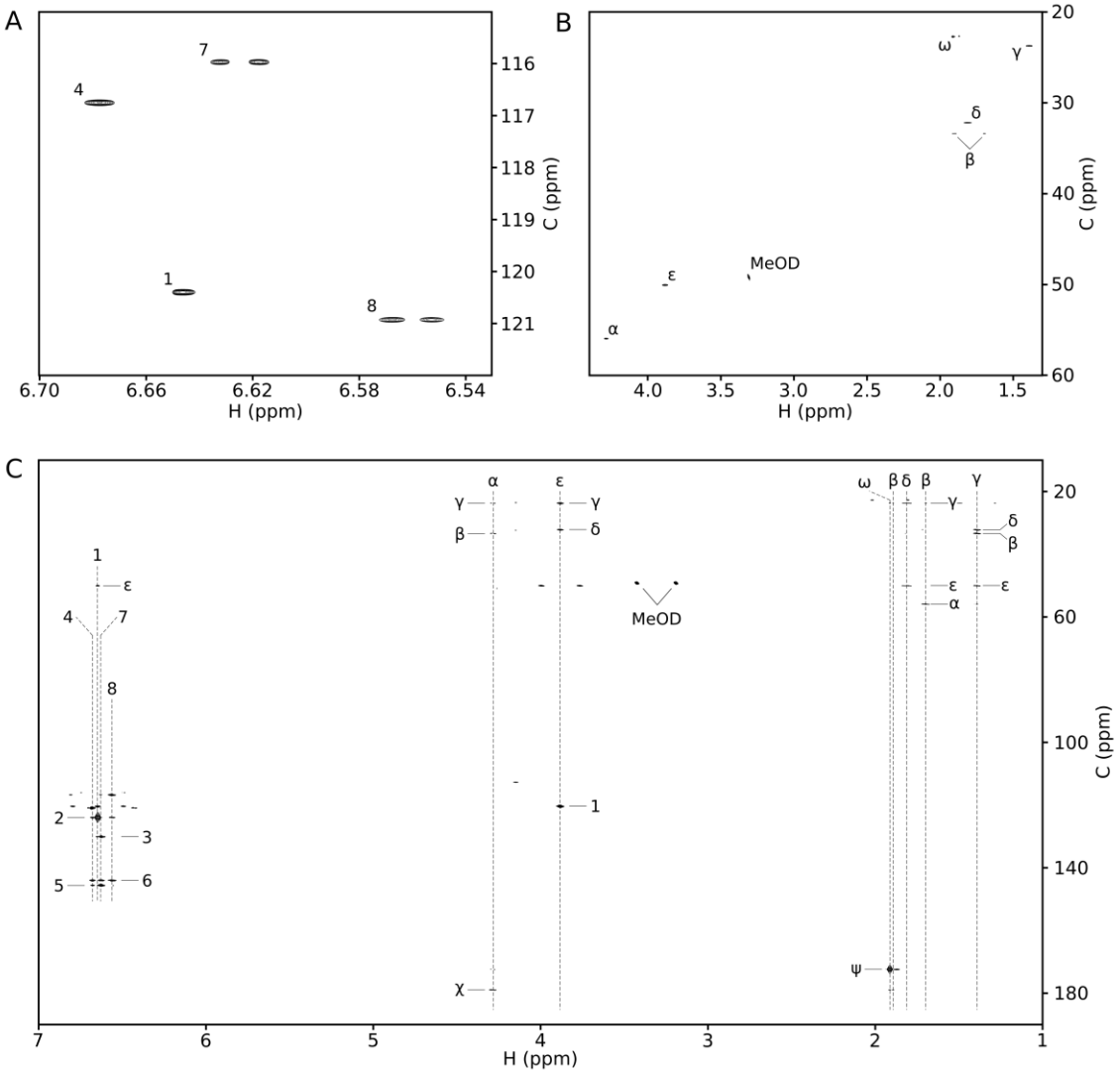


Figure S6. Full 2D ^{1}H-^{13}C spectra for purified dicatechol pyrrole lysine. (**A-C**) ^{1}H-^{13}C HSQC spectra for the aromatic (A) and aliphatic (B) resonances and the ^{1}H-^{13}C HMBC spectrum (C) are shown with signal labels corresponding to the structure in Figure 2A.

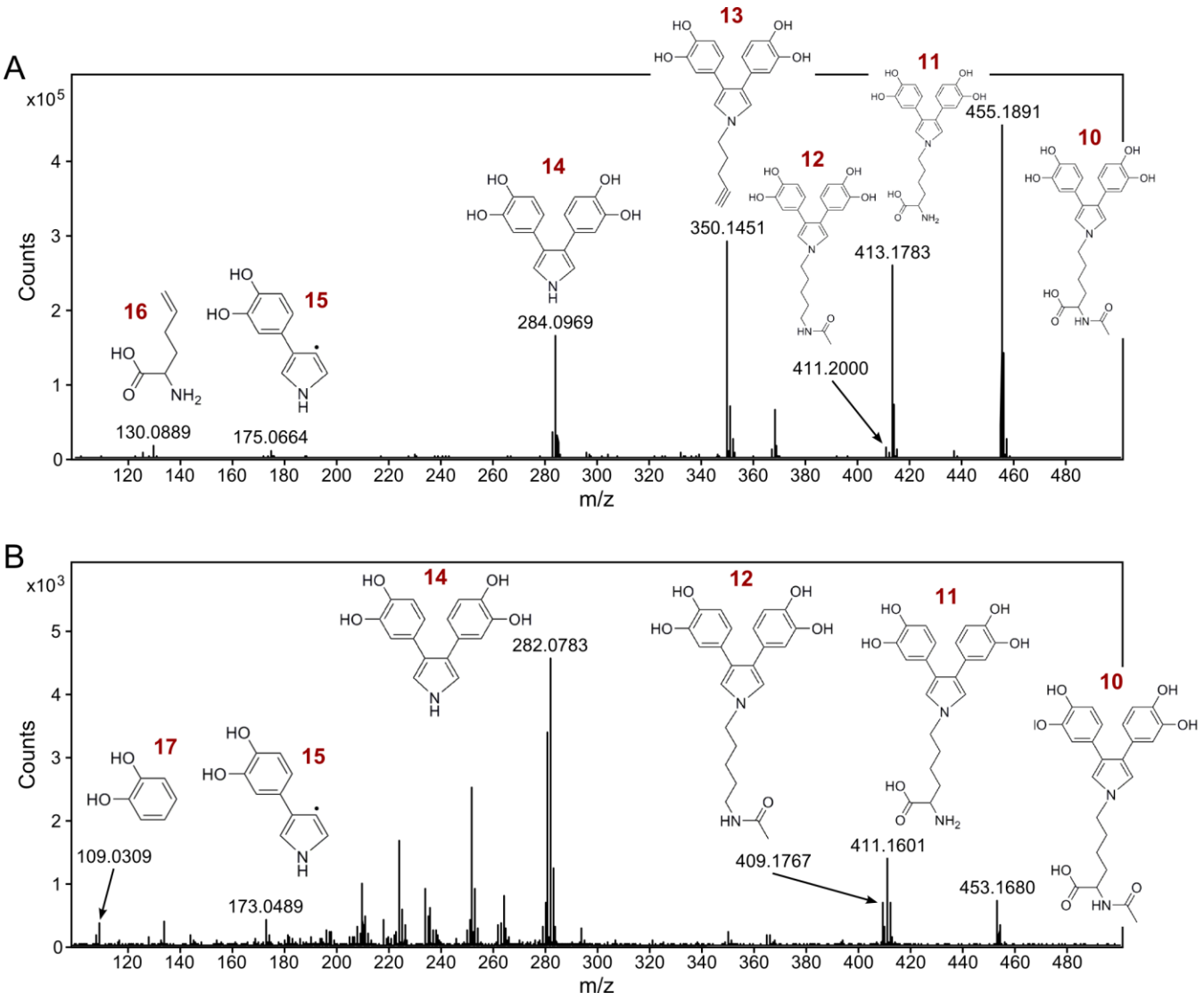


Figure S7. MS-MS fragment analysis for purified DCPL. Spectra were collected in either positive (A) or negative (B) ion mode. Charge symbols are omitted from the assigned fragment structures. (**A**) The observed compounds and their calculated m/z ratios are compound 10, DCPL, m/z = 455.1891; compound 11, loss of N-acetyl group, m/z = 413.1713; compound 12, loss of carboxyl group, m/z = 411.1920; compound 13, loss of N-acetyl group and carboxyl group, m/z = 350.1392; compound 14, dicatechol pyrrole fragment, m/z = 284.0917; compound 15, catechol pyrrole neutral radical, m/z = 175.0633; compound 16, lysine fragment, m/z = 130.0868. (**B**) The observed compounds and their calculated m/z ratios are compound 10, DCPL, m/z = 453.1680; compound 11, loss of N-acetyl group, m/z = 411.1556; compound 12, loss of carboxyl group, m/z = 409.1764; compound 14, dicatechol pyrrole fragment, m/z = 282.0783; compound 15, catechol pyrrole neutral radical, m/z = 173.0477; compound 17, catechol, m/z = 109.0290.

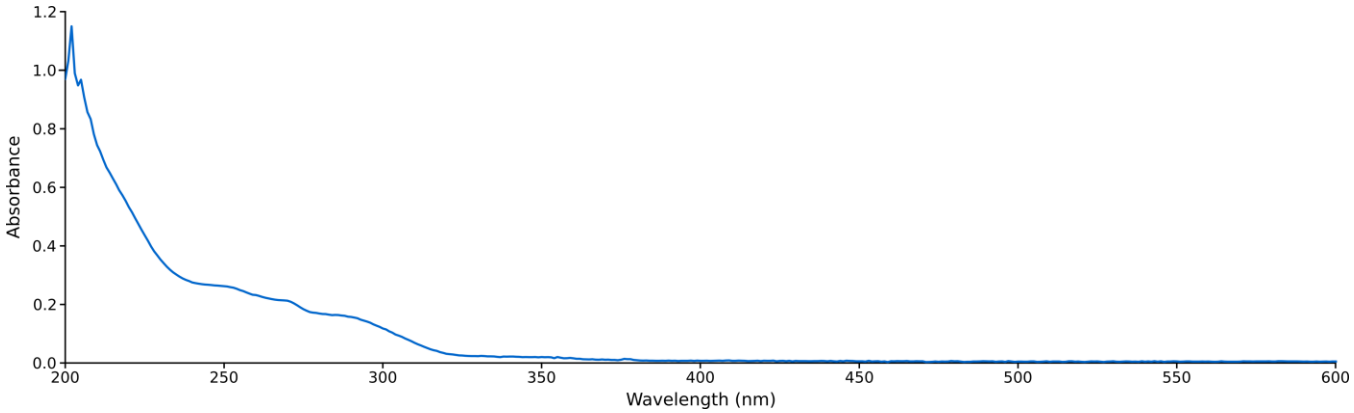


Figure S8. UV-Vis spectrum of purified dicatechol pyrrole lysine. DCPL was purified as described in the main text, resuspended in deuterated methanol, and its concentration measured by comparison of catechol signal volumes in the 1D ^{1}H NMR spectrum with a standard sample of dopamine. The sample was then diluted to a concentration of 10 µM in methanol and its UV-Vis spectrum was collected on an Agilent 8453 spectrophotometer using a cuvette with a 1 cm path length after blanking with methanol.

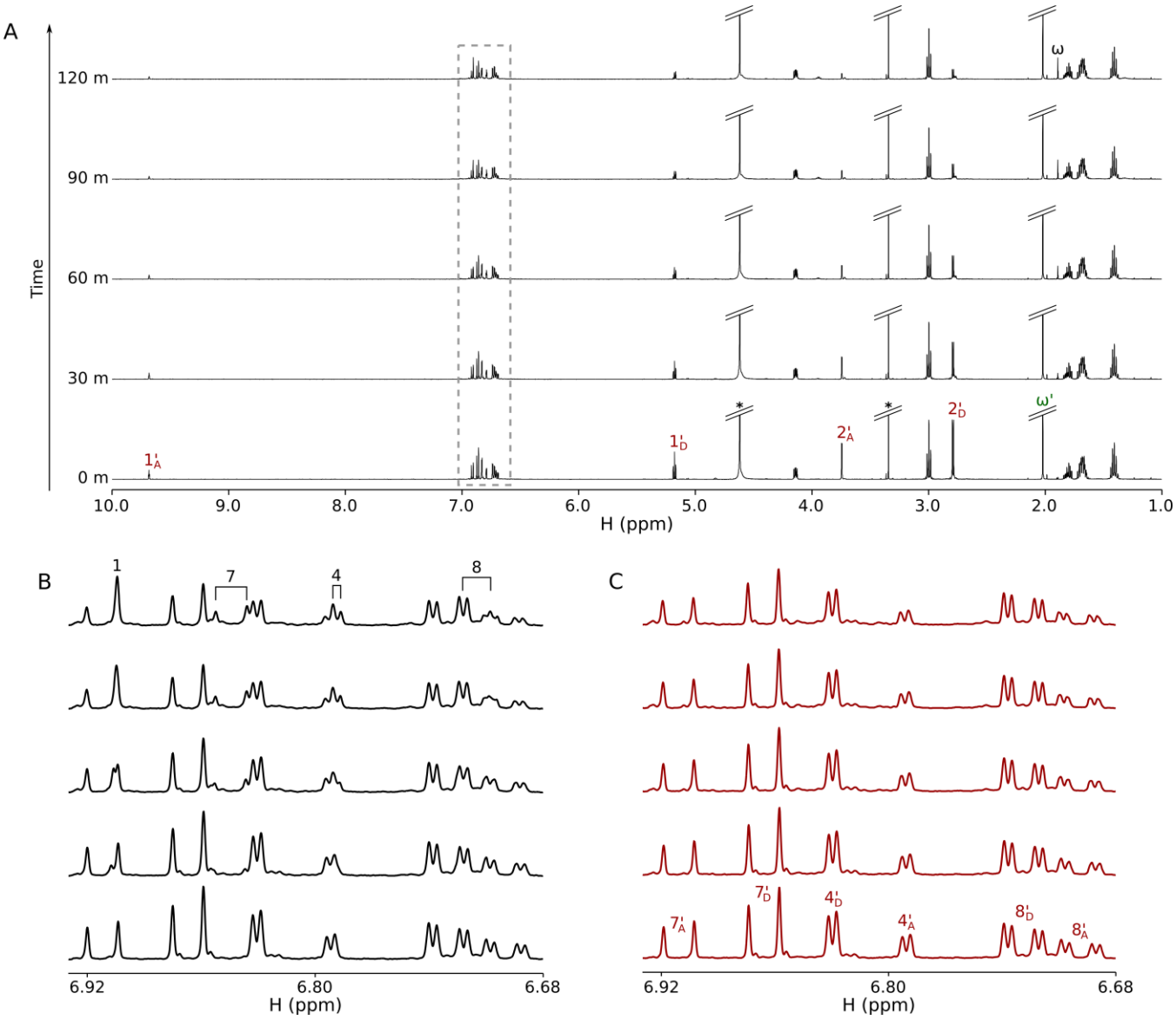
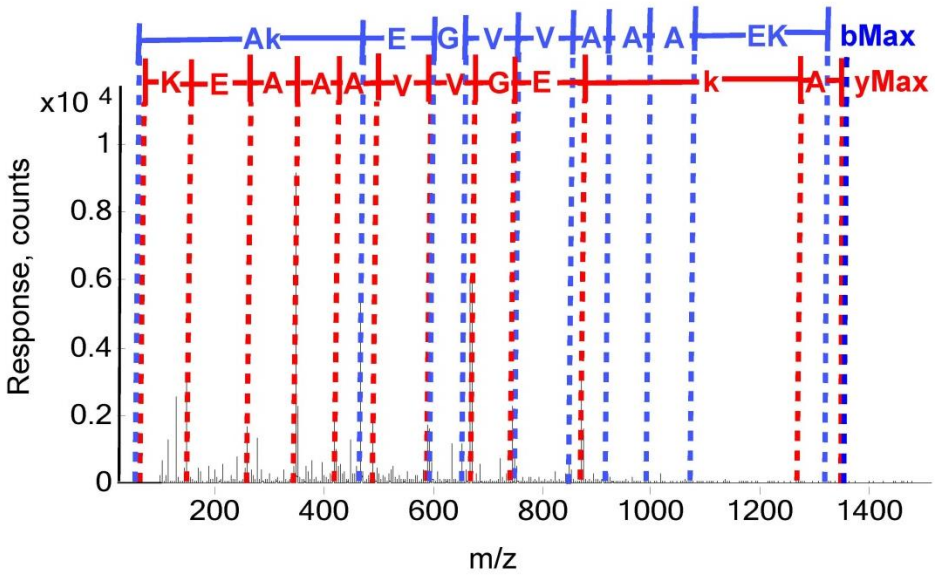


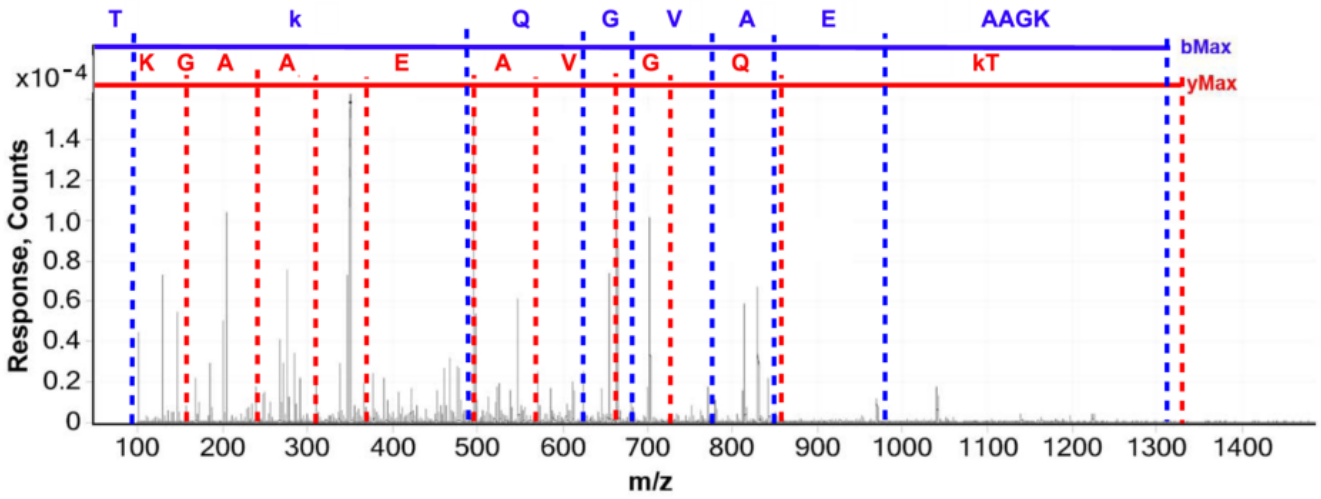
Figure S9. DCPL formation monitored by 1D ^{1}H NMR. (**A**) Stacked trace of a representative time course with 2 mM DOPAL and 1.5 mM Ac-Lys. Under aqueous conditions, the aldehyde group of DOPAL is in equilibrium between the hydrated and non-hydrated forms, with populations of 70% and 30%, respectively. The non-catechol DOPAL signals are annotated in the first time point, with numbering corresponding to the structures in Figure 2A and subscripts A and D indicating the aldehyde and hydrated, geminal diol forms, respectively. Asterisks denote solvent signals from the DOPAL stock, which was made in deuterated methanol for these reactions, and the acetyl methyl signal of unmodified lysine is labeled in green. The equivalent acetyl methyl signal for the DCPL product, which appears over the course of the reaction, is labeled in black in the spectrum of the last time point. (**B, C**) The aromatic region of the 1D ^{1}H NMR spectrum for the reaction containing DOPAL and Ac-Lys (B) and a control with DOPAL alone (C). This region of the spectrum in (A) is denoted by a dashed box. The DOPAL catechol signals are annotated in (C). The chemical shifts and assignments of the DOPAL signals

are in excellent agreement with previously reported values.[2] While the auto-oxidation of DOPAL alone in (C) proceeds without the observable accumulation of any predominant product, in the reaction with Ac-Lys in (B) there is progressive formation of the DCPL adduct, the signals of which are numbered in the spectrum for the last time point. We are unable to detect any intermediates in the DCPL pathway, suggesting that they are short-lived species.



	b-ions			y-ions		
Residue	**Calculated**		**Observed**	**Calculated**		**Observed**
Ala11	b1	72.045	ND	-	-	
Lys12+DCPL	b2	466.200	466.197	y10	1267.623	1267.569
Glu13	b3	595.242	595.246	y9	873.468	873.466
Gly14	b4	652.264	652.268	y8	744.425	744.422
Val15	b5	751.332	751.342	y7	687.404	687.408
Val16	b6	850.401	850.389	y6	588.335	588.331
Ala17	b7	921.438	921.430	y5	489.267	489.263
Ala18	b8	992.475	992.470	y4	418.230	418.227
Ala19	b9	1063.512	ND	y3	347.193	347.193
Glu20	b10	1192.555	1192.541	y2	276.155	276.158
Lys21	b11	-	-	y1	147.113	147.115

Figure S10. MS/MS sequencing of Ac-WT aS peptide 11-21 with dicatechol pyrrole adduct at Lys12. The parent peptide's mass was measured at 1338.6520 Da. The expected mass of each b and y ion was calculated by GPMAW and compared to the observed mass, demonstrating that Lys12 was modified to DCPL (represented by a lower case k in the figure). ND, not detected.



	b-ions			y-ions		
Residue	**Calculated**		**Observed**	**Calculated**		**Observed**
Thr22	b1	102.056	102.057	-	-	
Lys23+DCPL	b2	496.208	496.210	y10	1224.590	ND
Gln24	b3	624.267	624.268	y9	830.437	830.408
Gly25	b4	681.288	ND	y8	702.378	702.377
Val26	b5	780.357	780.350	y7	645.357	645.353
Ala27	b6	851.394	851.392	y6	546.288	546.288
Glu28	b7	980.437	980.430	y5	475.251	475.252
Ala29	b8	1051.474	ND	y4	346.208	346.208
Ala30	b9	1122.511	ND	y3	275.171	275.171
Gly31	b10	1179.532	ND	y2	204.134	204.136
Lys32	b11	-	-	y1	147.113	147.116

Figure S11. MS/MS sequencing of Ac-WT aS peptide 22-32 with dicatechol pyrrole adduct at Lys23. The parent peptide's mass was measured at 1324.6322 Da, and the calculated mass for the DCPL derivative of the peptide is 1324.6299. The expected mass of each b and y ion was calculated by GPMAW and compared to the observed mass, demonstrating that Lys23 was modified to DCPL (represented by a lower case k in the figure). ND, not detected.

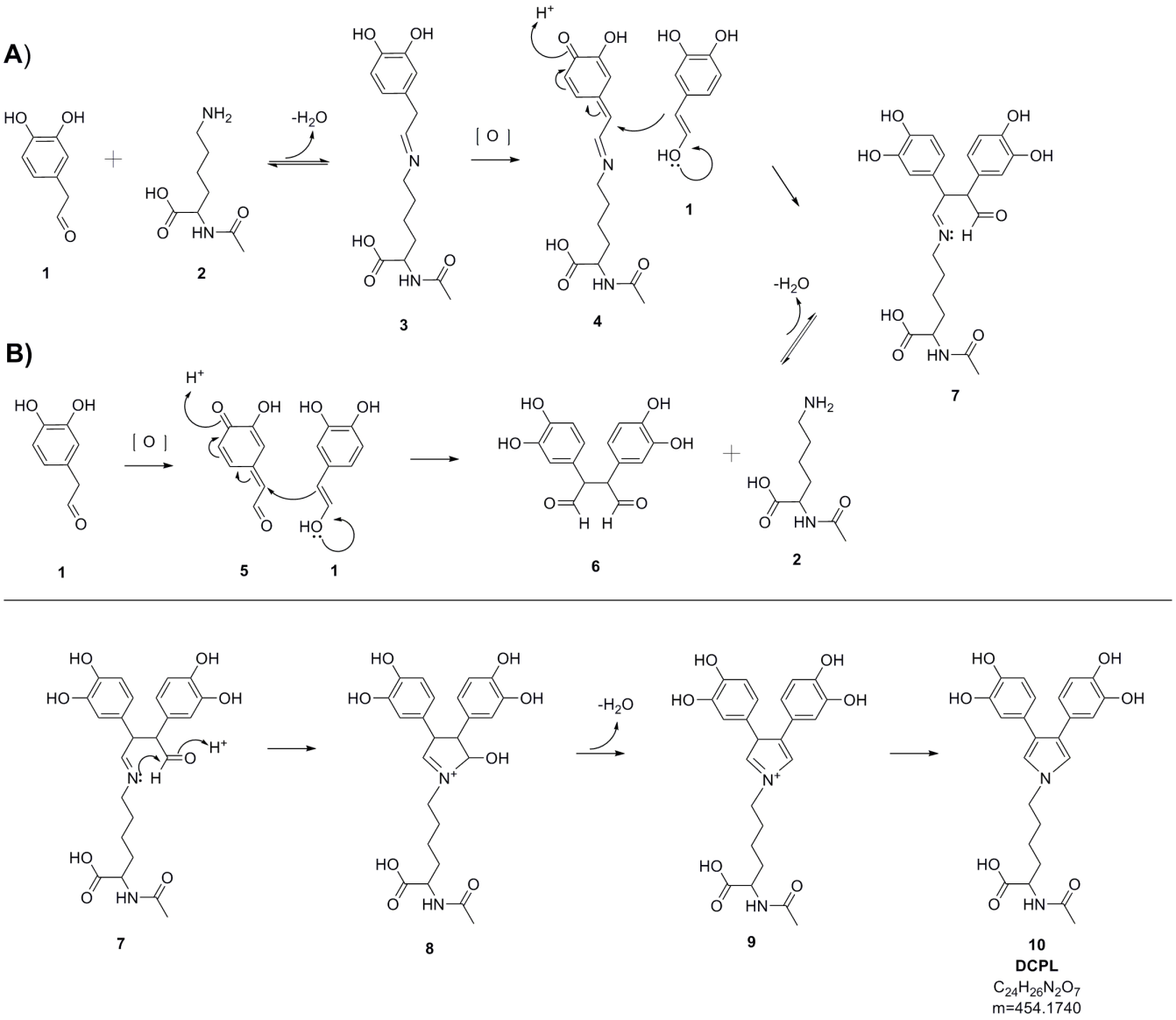


Figure S12. Proposed reaction mechanism for dicatechol pyrrole lysine formation. In pathway A, the first step is a condensation of DOPAL with Ac-Lys to yield a Schiff base intermediate (labeled 3). In the second step, the catechol auto-oxidizes to a quinone, which then isomerizes to a quinone methide (labeled 4). This oxidation and isomerization has been described previously for catechols with alkyl groups in a para position,[3] and the resulting quinone methide is stabilized by extended pi conjugation.[4-5] The quinone methide activates the ring-proximal alkene carbon for nucleophilic attack by a second DOPAL, with formation of the pyrrole carbon-carbon bond leading to the reduction of the quinone back to a catechol (labeled 7). At this stage, the molecule is analogous to a Paal-Knorr condensation intermediate, and closure of the ring and isomerization to the pyrrole (labeled 10) proceed by the Paal-Knorr mechanism.[6] Our finding that a Schiff base is the primary adduct at non-acetylated α-synuclein's N-terminus is consistent with this pathway, as the addition of the second DOPAL to the amine requires the nitrogen to hold a positive charge, a significantly more favorable pathway for Lys given the greater basicity of its amine group. In pathway B, the initial steps are different, such that the carbon-carbon bond of the pyrrole group is formed before the reaction with

Ac-Lys. First, oxidation of DOPAL to the quinone methide is followed by the attack of a second DOPAL to form a dicatechol molecule that is linked through the alkyl chains (labeled 6), similar to the oxidative dimerization of dopamine reported by Sugumaran and colleagues.[7] The dicatechol molecule then reacts with Ac-Lys to yield the Paal-Knorr condensation intermediate (labeled 7). In support of this pathway, we were able to detect the dicatechol mass by LC-MS in the auto-oxidation products from incubation of DOPAL alone and to verify its alkyl-linked structure with MS/MS (data not shown). However, we have been unable to observe the significant accumulation of any intermediates in the Ac-Lys reaction by monitoring with 1D ^{1}H NMR spectra (Figure S9), indicating that they are highly transient species. We also note the possibility that oxidation of the catechol in pathway A of our proposed mechanism occurs before the initial Schiff base formation. This would agree with 1) the increased susceptibility to nucleophilic attack of the aldehyde group in the quinone species compared to the catechol and 2) the observation that the DOPAL auto-oxidation rate is comparable in the presence and absence of Ac-Lys (Figure S9). In either case, the formation of the DCPL pyrrole ring through a quinone methide is consistent with both the DOPAL catechol group and aldehyde groups being required for DOPAL reactivity and the inhibition of that reactivity by ascorbate.[8] Indeed, we find full inhibition of DCPL formation in the presence of ascorbate.

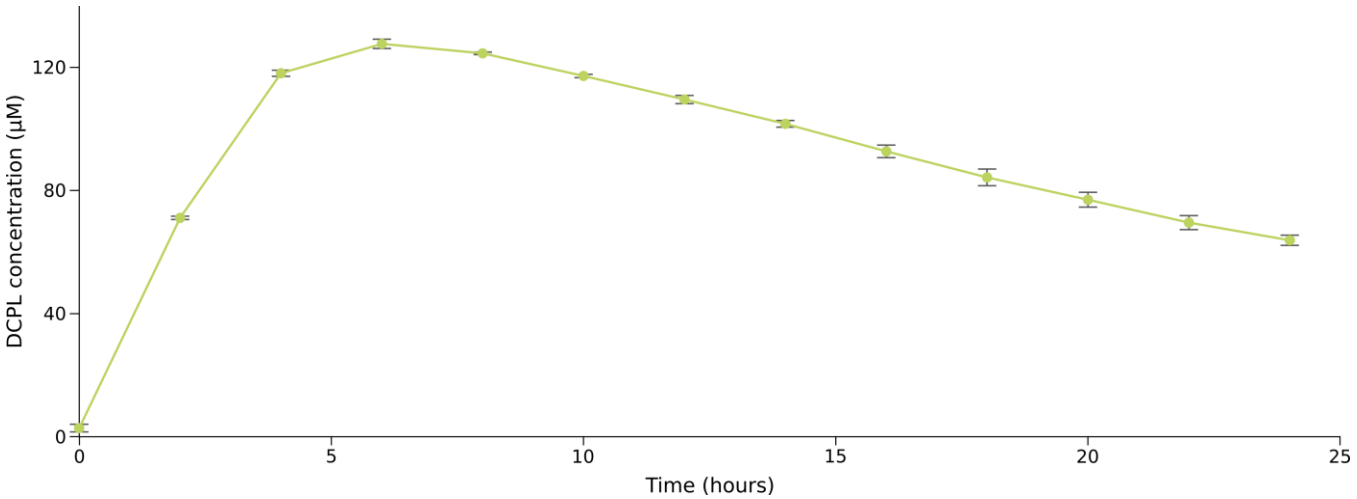


Figure S13. Dicatechol pyrrole lysine is not stable over extended incubations. The production and decay of DCPL in reactions with 2 mM DOPAL and 1.5 mM Ac-Lys at 37 °C were followed by ^{1}H NMR. Error bars represent standard deviations from three independent reactions. The DCPL concentration reaches a maximum of 128 μM at 6 hours, then declines steadily to 64 μM over the following 18 hours.

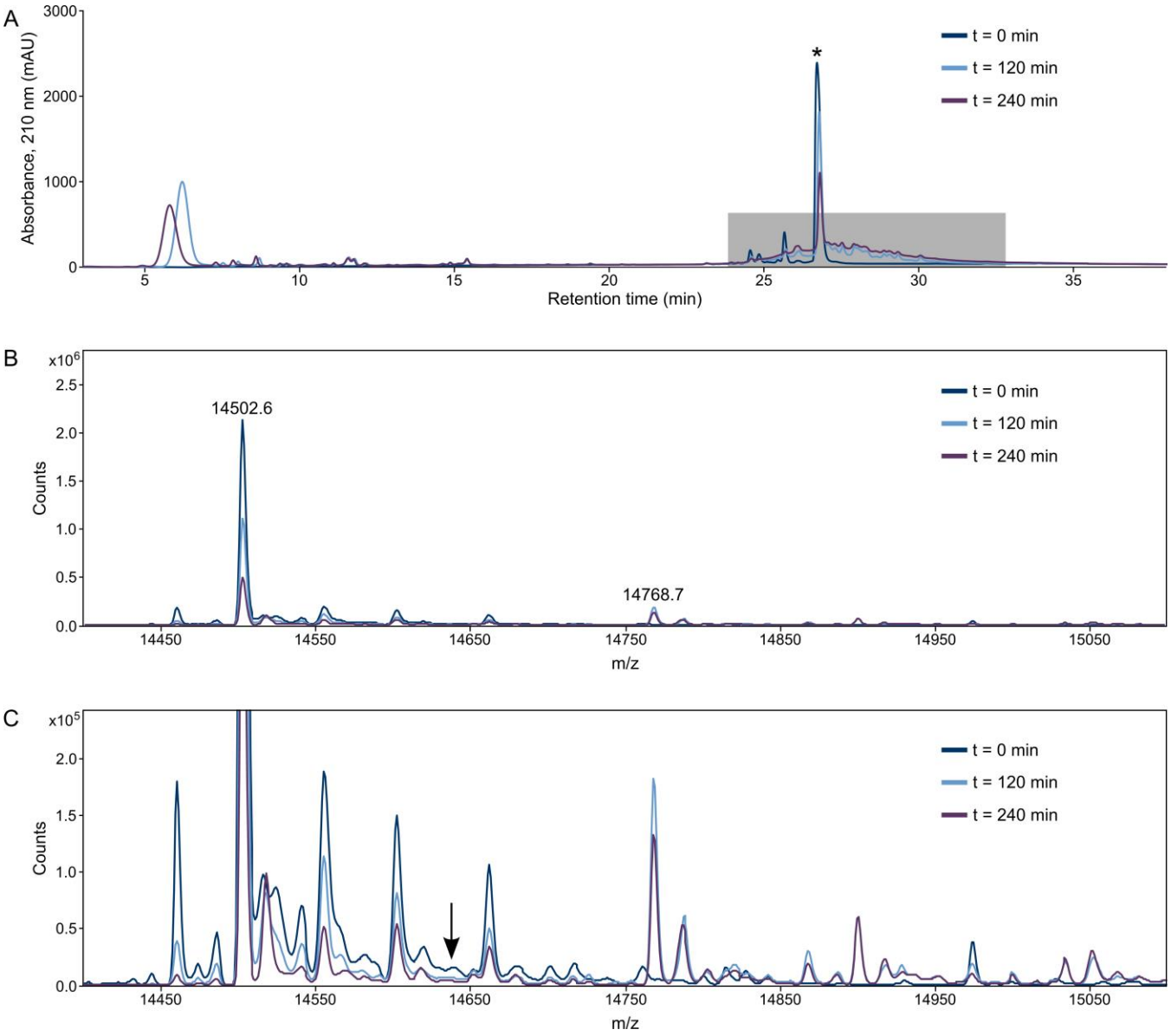


Figure S14. LC-MS analysis of the reaction between DOPAL and acetylated WT aS in 10 mM sodium phosphate buffer at pH 7.4. 100 µM Ac-WT aS was incubated with 2 mM DOPAL and aliquots were subjected to reverse-phase chromatography and ESI-MS as described for the samples in Figure 1A in the main text. Note that the first time point shown (t = 0 min) was taken *before* the addition of DOPAL and represents the unmodified protein. (**A**) Overlaid chromatographic traces for the indicated time points. An asterisk marks the elution peak of the native protein, which progressively decreases over the course of the reaction. (**B**) Overlay of deconvoluted mass spectra at full scale extracted from the chromatographic region denoted by the shaded box in (A). Native and DCPL-modified protein masses are labeled. (**C**) The deconvoluted mass spectra in (B) are shown on a vertically expanded scale. An arrow marks the expected position of a Schiff base adduct, which is not observed in the initial reaction period monitored here.

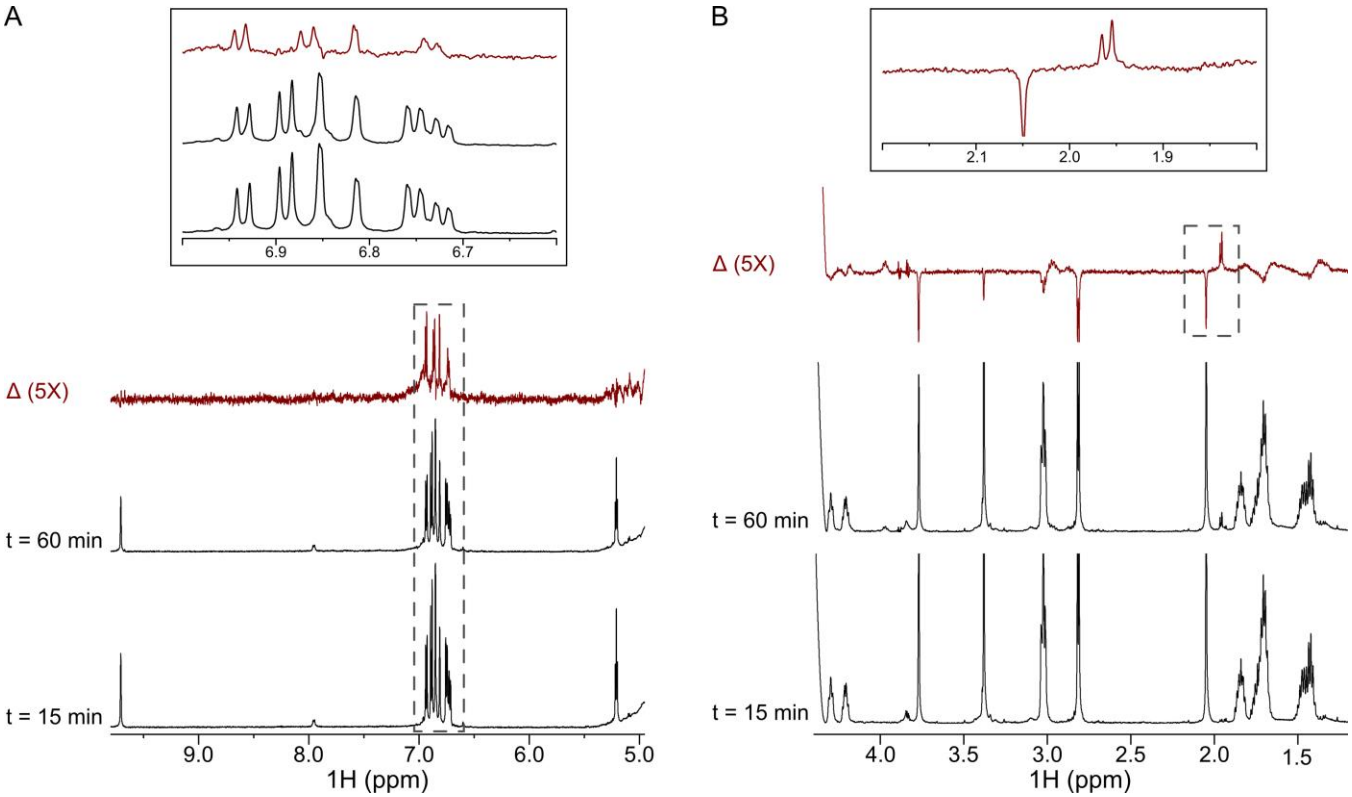


Figure S15. 1D ^{1}H NMR spectra of Nα-acetylated Lys-Lys before and after reaction with DOPAL in 10 mM sodium phosphate buffer at pH 7.4. Spectra were collected at 600 MHz with 2 mM DOPAL and 1 mM dipeptide in 95% H_2O, 5% D_2O at 37°C. (**A**) Aromatic region of the spectrum at 15 and 60 minutes, and in red the NMR difference spectrum (after scaling the 15 minute time point spectrum by 0.78 to remove the signals of the unreacted DOPAL). The difference spectrum is shown at 5-fold magnification. Whereas the two DCPL adducts have nearly overlapping catechol signals, the pyrrole singlets (at 6.93 and 6.94 ppm) are resolved. (**B**) Aliphatic region of the same spectrum. The difference spectrum (unscaled) shows loss of DOPAL methylene intensity (aldehyde form at 3.8 ppm; hydrated form at 2.8 ppm). The 3.37 ppm signal corresponds to 0.7% methanol (v/v) from the DOPAL stock. The loss in intensity of the acetyl methyl group (2.04 ppm) returns as new intensity at 1.96 and 1.97 ppm, corresponding to the peptide modified at Lys-1 and Lys-2. The relative intensities of these two methyl groups (see boxed expansion at top of figure) and of the pyrrole resonances in (A) indicates that the level of adduct formation is comparable (to within 25%) for the N- and C-terminal Lys residues. Dispersive-appearing resonances for the Lys methylene groups in the difference spectrum result from the small changes in chemical shifts for reacted and unreacted Lys residues.

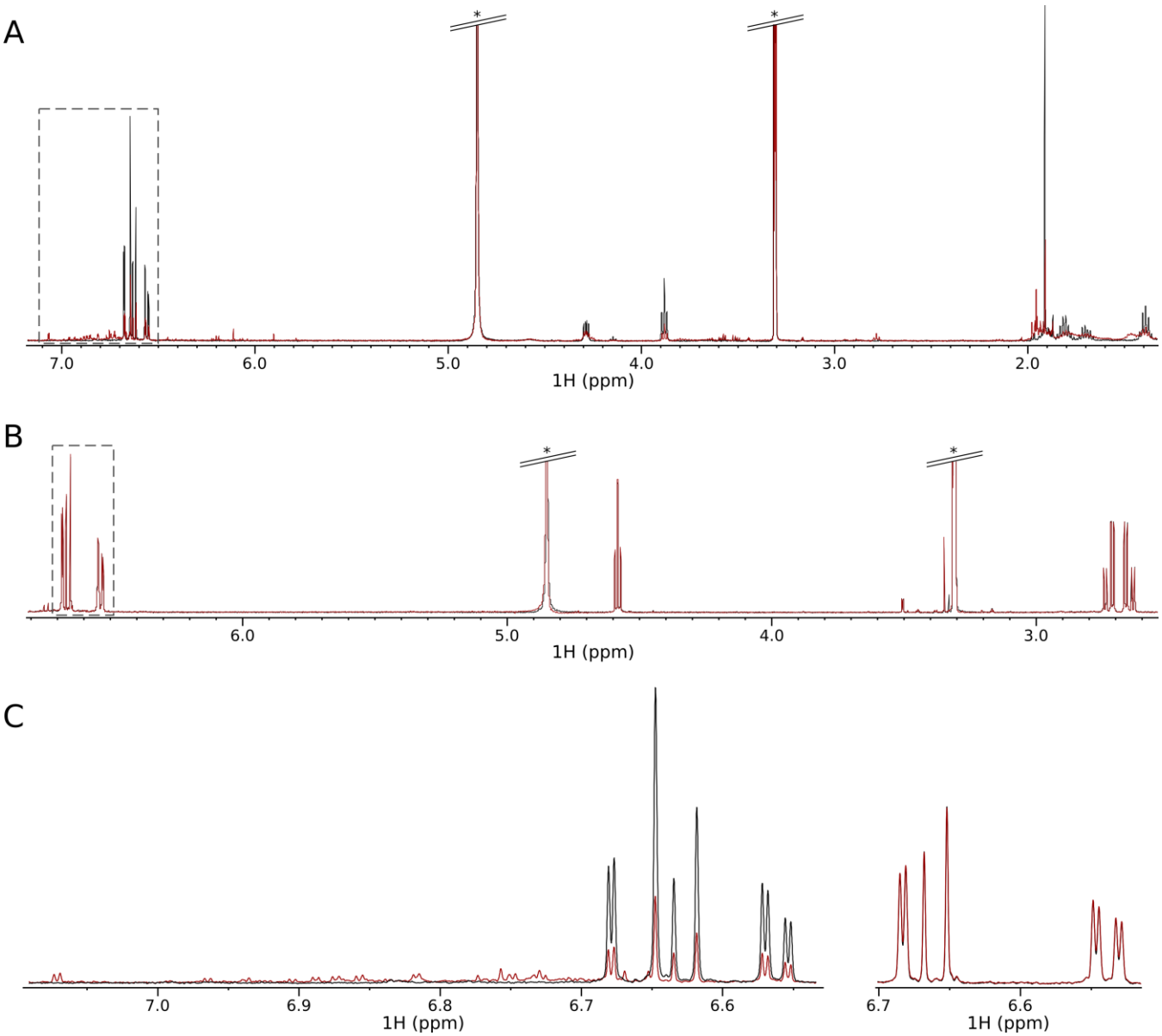


Figure S16. Dicatechol pyrrole lysine is sensitive to light. (**A, B**) Samples of DCPL (A) and DOPAL (B) were prepared in deuterated methanol and 1D ^{1}H spectra were collected before (black) and after (red) simultaneous exposure of the two samples to natural light for 5 minutes. (**C**) Aromatic regions for DCPL (left panel) and DOPAL (right panel) marked by dashed boxes in (A) and (B). Light exposure has no measurable effect on DOPAL. In contrast, for DCPL there is a 71% loss of signal and the appearance of heterogeneous degradation products, which contain aromatic signals that are shifted downfield of the DCPL signals as illustrated in (C). Asterisks denote the methanol solvent signals.

METHODS

Preparation of reactants. Ac-WT aS was prepared by bacterial co-expression of a codon-optimized synuclein construct and the NatB acetyltransferase as described previously.[9-10] To achieve complete acetylation, 1 g/L of ^{15}N-labeled IsoGro (Sigma-Aldrich) was added to standard ^{15}N-labeled M9 media. Cultures were grown at 37 °C with shaking to an OD600 of 0.6-0.8, induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) for 3 hours, and cells were then harvested by centrifugation. Cell pellets were resuspended in 50 mM Tris-HCl pH 7.4, 500 mM NaCl, at 10 mL buffer/liter of culture, and heated at 90 °C for 10 minutes. Lysate was cleared by centrifugation for 45 minutes at 64,000 x g, and the supernatant was diluted 5-fold with 50 mM Tris-HCl pH 7.4 and loaded on a HiTrap Q FF ion exchange column (GE Healthcare). Protein was eluted over a NaCl gradient from 0 to 500 mM. Protein-containing fractions were pooled, and ethylenediaminetetraacetic acid (EDTA) was added to a concentration of 10 mM. The pooled fractions were concentrated to a volume of ~5 mL using Amicon Ultra spin filters (EMD Millipore) with 3 kDa cutoff membranes, loaded on a 26 mm/60 cm HiLoad Superdex 75 size exclusion column (GE Healthcare), and eluted in 20 mM sodium phosphate pH 7.4. The desired fractions were pooled and concentrated with spin filters to a final protein concentration of ~500 µM as measured by A280, then divided into single-use aliquots, flash frozen in liquid nitrogen, and stored at -20 °C. Ac-Lys was purchased from Sigma-Aldrich and stored at -20 °C in stocks at 10 times the working concentration.

DOPAL was purchased from VDM Biochemicals and dissolved in methanol at a concentration of 330 mM. 1D ^{1}H NMR spectra were used to judge the purity of the material, which often contained residual organic solvents. These were easily removed by lyophilizing overnight, and the pure DOPAL was then resuspended in methanol. Exact concentrations were determined by comparison of catechol signal volumes in the 1D ^{1}H NMR spectrum with a standard sample of dopamine. As illustrated in Figure 2B, in methanol the DOPAL aldehyde exists entirely in the hydrated, geminal diol form, with only a single set of catechol proton resonances, whereas in aqueous solution the aldehyde and geminal diol forms are in equilibrium with relative populations of 30% and 70%, respectively (Figure S9), as previously reported.[2] Final DOPAL stocks were aliquoted and stored at -80 °C. DOPAL was found to be stable under these conditions for a period of several months as judged by 1D ^{1}H NMR spectra.

DOPAL reactions. Reactions with α-synuclein were performed in PBS buffer (KD Medical) in the dark at 37 °C, with concentrations of 100 μM Ac-WT aS and 2 mM DOPAL. For the NMR samples used in Figure S1, aliquots were withdrawn at the indicated times and protein was extracted using ethanol precipitation. Four volumes of ice-cold ethanol were added to each reaction aliquot, which was then incubated at -20 °C for 10 minutes and centrifuged at 10,000 x g for 5 minutes. The supernatant was removed and the protein pellet was dried briefly in vacuo. The pellet was resuspended in PBS with 1 mM EDTA and 1,000X exchanged into 20 mM sodium phosphate pH 6.2 with 10% D_2O using an Amicon Ultra spin filter. ^{1}H-^{15}N HSQC spectra were collected at 15 °C using a conventional pulse sequence on an 800 MHz Bruker Avance III spectrometer equipped with a z-axis pulsed field gradient cryogenic probe. Acquisition times in the ^{15}N and ^{1}H dimensions were 243 and 106 ms, respectively. The amide intensity ratios in Figure S1 were scaled using residue Gly101, which appeared unaffected during the reaction, as a reference to correct for protein concentration variability between samples. No aggregation or proteolysis of samples was detected by SDS-PAGE and NMR over the reaction times used here.

DOPAL reactions subjected to LC-MS were also performed in PBS buffer at 37 °C, with concentrations of 2 mM DOPAL and either 100 μM Ac-WT aS or 1.5 mM Ac-Lys. As Ac-WT aS contains 15 Lys residues, the total lysine concentration is the same in the two reactions. Although the +266 Da mass observed for DCPL-modified Ac-WT aS could potentially arise from modification of two lysines on a single protein by Schiff base-like adducts, there is no detectable protein adduct with a mass corresponding to a single such adduct (+132 Da or +134 Da, see Figure 1 and SI Figure S14). Chromatography was performed on an Agilent 1200 series HPLC system equipped with an autosampler that allowed timed incubations at 37 °C (Agilent Technologies), and samples were withdrawn directly from the reaction without prior purification of products. Separations were affected by a Zorbax 300Å StableBond C18 column (1.0 x 50 mm, 3.5 μm particle size, Agilent catalog #865630-902). For the chromatography of α-synuclein, the solvents were water/0.05% trifluoroacetic acid and acetonitrile/0.05% trifluoroacetic acid, and a gradient of acetonitrile was developed from 0-70% at 2%/min with a flow rate of 20 μL/min. For reactions of DOPAL with amino acids, the initial solvent was water/0.1 % formic acid and elution was

by a gradient with acetonitrile/0.1 % formic acid developed from 0-45% acetonitrile at 2%/min with a flow rate of 20 µL/min.

ESI-MS was performed on an Agilent Model 6520 accurate mass quadrupole-time of flight instrument. For reactions with synuclein, the chromatographic effluent was mixed in a tee with 20 µL/min acetic acid to displace the bound trifluoroacetic acid and generate internal standards for the mass spectra.[11-12] Spectra were recorded for the mass range of 500-2500 m/z. The drying gas temperature was 350°C with a flow rate of 10L/min and a nebulizer pressure of 2 bar. The voltages were: capillary 3500V, fragmentor 235V, skimmer 65V, and octopole 750V. For reactions with Ac-Lys, separate experiments were used to obtain positive and negative ion electrospray ionization analyses, as catechols ionize well in negative ion mode and poorly in positive ion mode, and spectra were recorded for the mass range of 100-1000 m/z. The drying gas temperature was 300°C with a flow rate of 10L/min and a nebulizer pressure of 2 bar, and the voltages were: capillary 3500V, fragmentor 175V, skimmer 65V, and octopole 750V. MS/MS fragmentation employed a collision energy of 30 V with a data collection range of 20-500 m/z. All mass spectra were analyzed using Agilent MassHunter version B.05.00.

DCPL Purification and Characterization by NMR. Product purification was carried out on an Agilent 1100 Series HPLC system. DCPL was purified from a 5 mL reaction containing 2 mM DOPAL and 1.5 mM Ac-Lys in PBS after incubation at 37 °C for two hours. The column was a Zorbax 300Å StableBond C18 column, which has the same resin as the column used in the LC-MS experiments but a larger diameter (2.1 x 50 mm, 3.5 µm particle size, Agilent catalog #885750-902). The flow rate was 0.5 mL/min and the initial solvent was water. Following loading of the sample, the column was washed with 2.5 mL water and then a gradient of acetonitrile was developed from 0 to 30% at 1%/min. Fractions containing pure product were pooled, flash frozen in liquid nitrogen, and lyophilized to dryness. NMR samples were prepared by resuspending the product in deuterated methanol (Sigma-Aldrich), and the typical yield of purified DCPL was ~90 µg. Samples of Ac-Lys and DOPAL used to collect the couplings and chemical shifts listed in Table 1 were also prepared in deuterated methanol.

NMR data were collected for the DCPL adduct, Ac-Lys, and DOPAL at 25 °C on a 600 MHz Bruker Avance III spectrometer equipped with a z-axis cryogenic probe using standard experiments and parameter sets, and ^{1}H and ^{13}C chemical shifts were referenced to the methanol solvent signal at 3.31 and 49.15 ppm, respectively. ^{1}H-^{1}H TOCSY, ^{1}H-^{1}H ROESY, and CT ^{1}H-^{1}H COSY spectra were collected with acquisitions times in the indirect and direct ^{1}H dimensions of 56 and 500 ms, respectively, giving datasets with 400*3605 complex points. The ^{1}H-^{1}H TOCSY experiment utilized a 30 ms DIPSI2 mixing sequence, and the ^{1}H-^{1}H ROESY experiment was run with a 150 ms mixing period using a 2.5 kHz spin lock pulse and the ^{1}H carrier position at 4.78 ppm. The indirect ^{1}H evolution period of the CT ^{1}H-^{1}H COSY experiment was set to 150 ms. Carbon chemical shifts were measured from ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra. The ^{1}H-^{13}C HMBC experiment was run with a long-range coupling dephasing delay of 70 ms and acquisition times of 26 and 164 ms for the ^{13}C and ^{1}H dimensions, respectively.

Quantification of DCPL. For quantification of DCPL by NMR, reactions with 1.5 mM Ac-Lys, 1 mM diethylene triamine pentaacetic acid (DTPA), and varying DOPAL concentrations were set up in D_2O PBS and incubated at 37 °C in a 500 MHz Bruker Avance III spectrometer equipped with a triple-axis gradient probe. The time courses in Figure 3A were monitored by 1D ^{1}H spectra collected at 30 minute intervals, with 5 minute total data collection time per FID, comprising 92 scans, a 1 second recycle delay, and a 2 second acquisition time. The amount of DCPL present at each time point was calculated by integration of the well-resolved DCPL acetyl methyl signal (see Figure S9). The DCPL concentrations in Figure 3C were taken from the 5 hour time points in Figure 3A for DOPAL concentrations from 200 µM to 10 mM. For DOPAL concentrations from 25 µM to 100 µM, DCPL was measured with a single 1D ^{1}H spectrum comprised of 1198 scans, with a 1 hour collection time centered at 5 hours of incubation at 37 °C, and concentrations were calculated by integration of the pyrrolic singlet.

For quantification of DCPL by Ehrlich's assay, aliquots from reactions were mixed 1:1 with reagent solution containing 5% dimethylaminobenzaldehyde (DMAB) in glacial acetic acid and developed at room temperature for 30 minutes. This produced a blue color with maximum absorbance at 598 nm that was read on a Cary 8454 UV-Vis spectrophotometer (Agilent). The extinction coefficient of the DCPL-DMAB product was calculated by relating the absorbances of time points taken from the reaction

with 2 mM DOPAL and 1.5 mM Ac-Lys shown in Figure 3A to the concentrations of DCPL measured by NMR. The linearity of the correlation was excellent (R^2 = 0.995) and gave an extinction coefficient of 26,300 $M^{-1}cm^{-1}$ that was used to quantify DCPL formation in reactions with Ac-WT aS.

Samples for peptide mapping of DCPL adducts in Ac-WT aS were prepared by ethanol precipitation of 100 µM protein after reaction with 2 mM DOPAL for 2 hours at 37 °C, followed by resuspension in 100mM Tris pH 8.5, 1mM DPTA, with 20 mM ascorbate to prevent oxidative degradation of adducts. Samples were digested with trypsin at either a 1:20 molar ratio of protease:synuclein overnight or a 1:1 molar ratio for 1 hour at 37 °C. Digests were then acidified with 10% trifluoroacetic acid, and peptide mapping was performed by LC MS/MS as described above. Peptides were identified using Agilent MassHunter version B.05.00 and PEAKS versions 6 and 7 (Bioinformatics Solutions).

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