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Mechanistic Insights into Oxidative Oligomerization of *p*-Phenylenediamine and Resorcinol

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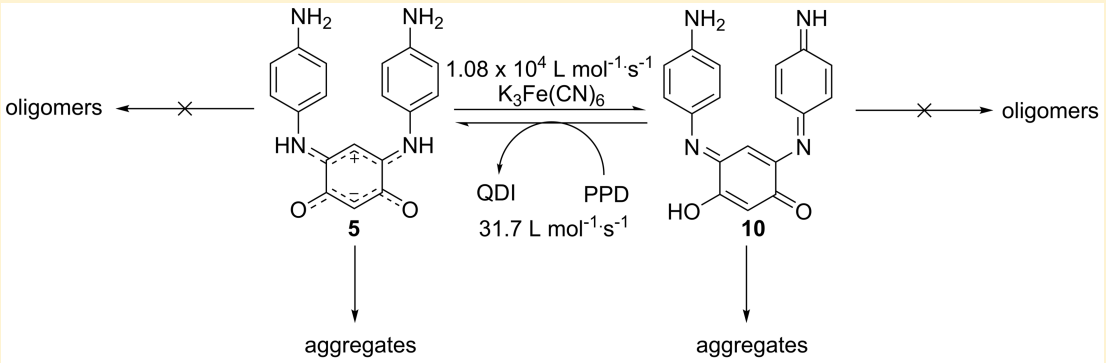
Supporting Information



ABSTRACT: An efficient synthesis of a green dye from oxidative coupling of *p*-phenylenediamine (PPD) and resorcinol (in a 2:1 ratio) has been developed. Reactivity studies of this dye molecule with a variety of reagents (PPD, resorcinol, the oxidized form of the green dye itself, and a dinuclear indo dye) demonstrate that it cannot be the key reactive intermediate in reported oxidative oligomerization of PPD and resorcinol. However, the trinuclear species does form large aggregates. At least one viable pathway of oligomerization has been demonstrated with the dinuclear indo dye.

INTRODUCTION

Permanent hair coloring relies on the reactivity of small precursors that form dyes inside the hair to enhance its natural color. The most frequently used materials, *p*-phenylenediamine (PPD, **1**) or *p*-aminophenol, known in the hair color industry as primary intermediates, are oxidized by alkaline hydrogen peroxide to yield *p*-quinonediimine (QDI, **2**) or *p*-quinonemonoimine, respectively. These electrophiles in turn attack couplers such as meta-disubstituted benzenes, in which the two substituents generally are amino or hydroxyl or the combination of thereof.[1,2] As exemplified in Scheme 1 for the oxidative coupling of PPD and resorcinol, the resulting leuco form (**3**) is oxidized to the indo dye (**4**), which is likely to undergo further coupling reactions.

Given the complexity of the dye forming process, historically two strategies have been adopted to simplify mechanistic investigation. First, some of the nucleophilic sites are blocked so that the couplers can only react once with an oxidized primary intermediate. Second, potassium ferricyanide is used as a substitute oxidant for hydrogen peroxide.[2−10] Although potassium ferricyanide is not typical of an ingredient in commercial hair products, it does simplify the kinetics when compared to the more consumer-relevant oxidant, hydrogen peroxide.[11] Rapid oxidation of the primary intermediate by potassium ferricyanide renders the coupling step rate-limiting. As a result, the coupling rate constant can be determined readily. Since the dyes produced with both oxidants are identical, this has been accepted as a valid substitution.

It has been much more challenging to generate complete kinetic profiles for reactions that use unblocked couplers such as resorcinol, *m*-aminophenol, or *m*-phenylenediamine. One of the earliest clues that additional reactions occur beyond the dinuclear indo dye was the observation of a short-lived intermediate with a different color from what would be suggested by the final dye chromophore.[12] In these cases, kinetic studies have been focused on determining the rates for the disappearance of the starting materials. A full understanding of the details of these reactions and the identity of their products is still lacking.

In early work, the greenish pigment from the PPD−resorcinol reaction was isolated from a complex mixture (eight different color bands) by preparative thin-layer chromatography and identified as a trinuclear species that incorporates two PPD moieties and one resorcinol.[13,14] It was speculated that this trinuclear species might be formed through a transient dinuclear intermediate, although such a compound has never been isolated.[12] Furthermore, based on derivatization experiments it was proposed that an oligomer of at least 18 PPD−resorcinol repeating units was produced under the

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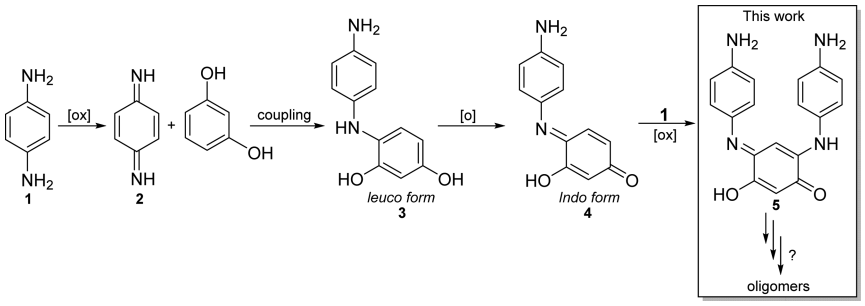
Scheme 1. Mechanism of Oxidative Coupling



oxidative conditions.[15] This oligomerization was believed to occur from additional oxidative coupling of monomers (PPD, resorcinol), or dinuclear species (**3** or **4**) to the trinuclear dye (**5**).[12,15] Despite extensive efforts, no efficient, large-scale preparation of the trinuclear species is known prior to this work, nor are the identities of the proposed oligomeric products and the mechanisms of their formation. Yet, these details are crucial for the design and improvement of commercial products.

Our goals for this project were to (a) understand in greater detail one of these key reaction pairs, PPD and resorcinol, (b) provide an efficient synthesis of the PPD−resorcinol trinuclear species, and (c) determine the role and reactivity of the trinuclear species in generation of the previously proposed oligomers.

EXPERIMENTAL SECTION

Materials. 1,4-Phenylenediamine (PPD; **1**) and resorcinol were purchased from Jos. H. Lowenstein and Sons and were sublimed prior to use. All other commercial chemicals were used as received. Water refers to deionized water. Compressed O_2 and H_2 were purchased from Wright Brothers, Inc.

Instruments. ^{1}H and $^{13}C\{^1H\}$ NMR were recorded on a 600 MHz Bruker spectrometer. Spectra were referenced internally to solvent residual peaks. Liquid chromatography/mass spectrometry (LC/MS) analysis was carried out on an Agilent Hewlet Packard series 1100 high-performance liquid chromatograph (HPLC) equipped with a UV detector using a SunFire C18 5 μm column and tandem MS analysis using electrospray ionization (ESI) by a Micromass ZQ spectrometer. Preparative HPLC was conducted on a Waters 2535 quaternary gradient module with a Waters 2489 UV−vis detector. Thin-layer chromatography (TLC) was performed using Analytech silica gel plates with fluorescent indicator. Flash chromatography was performed using a Teledyne Isco CombiFlash chromatography system equipped with a UV detector.

Stopped-flow experiments and absorbance spectra were collected on an Olis-RSM 1000 with USA stopped-flow accessory using 120 μm slit widths and gratings of 400 lines/mm. The sample cell has a path length of 2 cm and a volume of 35 μL. All reactions were performed by flowing 0.25 mL of each reagent through the cell using a pneumatic piston pressurized by 80 psig of compressed air. Temperature was controlled using a Julabo CF31 cryo-compact circulator.

Synthesis of Trinuclear Species 5. To a 250 mL round-bottom flask was added PPD (1.995 g, 18.45 mmol), resorcinol (1.016 g, 9.23 mmol), and water (60 mL). The pH was adjusted to 10.4 by adding NH_4OH (29% NH_3 in water) dropwise. A pipet attached to a compressed gas was submerged into the flask and oxygen was bubbled through the solution at a steady stream with stirring. After 8 h, the desired product was collected by vacuum filtration and washed with water (500 mL). The filtrate contained unreacted starting materials; therefore, it was collected and placed under oxygen to react further. The collected solid was washed with EtOAc (500 mL) and ethanol (100 mL). The 8 h coupling/filtration cycle was repeated until the purity of the solid collected from the recycled filtrate was below 90%. The combined solids were triturated with ethanol and the resulting powder was dried under reduced pressure to afford 1.33 g (45% yield) of dark powder of >95% purity. ^{1}H NMR (DMSO, 600 MHz) δ 10.41 (br, 2H, NH), 7.06 (d, J = 8.6 Hz, 4H, CH_{Ar}), 6.56 (d, J = 8.6 Hz, 4H, CH_{Ar}), 5.88 (s, 1 H, CH), 5.41 (s, 4H, NH_2), 5.11 (s, 1H, CH). $^{13}C\{^1H\}$ NMR (DMSO, 151 MHz) δ 171.6, 152.5, 147.4, 124.5, 123.8, 112.9, 97.4, 88.6. HRMS (ESI): m/z calcd for $[C_{18}H_{17}N_4O_2]^+$ 321.13515; found 321.13396. λ_{max} (EtOH/H_2O, pH 10.4), nm (ε/dm^3 mol^{-1} cm^{-1}): 420 (9491), 532 (sh, 3871), 600 (3085).

Synthesis of 2,4-Bis(benzyloxy)-1-nitrobenzene (7).[16] To a dry round-bottom flask with a magnetic stirbar was added benzyl alcohol (2.719 g, 25.14 mmol) and MeCN (24 mL). The solution was stirred at 0 °C while potassium *tert*-butoxide (2.962 g, 26.40 mmol) was added in one portion. The addition caused a rapid rise in temperature, and the reaction mixture was allowed to cool back to 0 °C. 2,4-Difluoronitrobenzene (2.000 g, 12.57 mmol) was then added dropwise over 15 min so that the temperature of the reaction mixture did not exceed 15 °C. The reaction mixture was slowly warmed to room temperature and stirred overnight. After 14 h, the resulting mixture was filtered and the filtrate was concentrated in vacuo to afford a dark solid. Recrystallization of the solid from dichloromethane and diethyl ether afforded an orange solid. The mother liquors were collected, concentrated, and the residue was also subjected to the recrystallization conditions. After three recrystallizations of the material isolated from the mother liquors, the final combined yield for the product was 2.33 g (55% yield). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.99 (d, 1H, J = 9.0 Hz, CH_{Ar}), 7.47 (d, 2H, J = 4.8 Hz, CH_{Ar}), 7.42−7.32 (m, 8H, CH_{Ar}), 6.66 (d, 1H, J = 2.4 Hz, CH_{Ar}), 6.59 (dd, 1 H, J = 9.0, 2.4 Hz, CH_{Ar}), 5.19 (s, 2H, CH_2), 5.10 (s, 2 H, CH_2). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 151 MHz) δ 163.7, 154.6, 135.6, 135.6, 133.7, 128.9, 128.8, 128.6, 128.5, 128.3, 127.7, 127.0, 106.2, 102.1, 71.3, 70.8. HRMS (ESI): m/z calcd for $[C_{20}H_{17}NO_4Na]^+$ 358.10553; found 358.10444.

Synthesis of 2,4-Bis(benzyloxy)aniline (8). To a 500 mL round-bottom flask with a magnetic stir bar and a reflux condenser was added zinc powder (5.69 g, 87.03 mmol), NH_4Cl (7.71 g, 144.13 mmol), acetone (208 mL), and water (42 mL). The suspension was stirred while a 2,4-bis-(benzyloxy)-1-nitrobenzene (2.335 g, 6.963 mmol) was added

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in one portion, after which the mixture was heated to reflux and stirred for 6 h. After cooling to room temperature, the reaction mixture was filtered and the solid was washed with acetone. The filtrate was concentrated in vacuo to afford a dark solid, which was treated with CH_2Cl_2 and washed with a pH 10 carbonate buffer solution. The precipitated zinc salts were filtered off and the filtrate was washed with brine. The resulting organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated to afford a dark oil. The crude product was purified by flash chromatography using hexane/EtOAc as the mobile phase on a 120 g SiO_2 column. The pure product was isolated in 66% yield (1.4 g). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.46−7.33 (m, 10H, CH_{Ar}), 6.69 (d, 1H, J = 8.4 Hz, CH_{Ar}), 6.65 (d, 1H, J = 2.5 Hz, CH_{Ar}), 6.49 (dd, 1H, J = 8.4, 2.5 Hz, CH_{Ar}), 5.06 (s, 2H, CH_2), 5.00 (s, 2H, CH_2), 3.60 (br, 2H, NH_2). ^{13}C{^{1}H} NMR ($CDCl_3$, 151 MHz) δ 152.2, 147.4, 137.5, 137.0, 130.5, 128.7, 128.6, 128.1, 127.9, 127.7, 127.6 115.4, 106.4, 101.9, 70.9, 70.6. HRMS (ESI): m/z calcd for $[C_{20}H_{20}NO_2]^+$ 306.14940; found 306.14831.

Synthesis of 2,4-Bis(benzyloxy)-*N*-(4-nitrophenyl)-aniline (9). To an oven-dried round-bottom flask equipped with a magnetic stir bar, a reflux condenser, and a N_2 inlet was charged $Pd(OAc)_2$ (140 mg, 0.624 mmol, 5 mol %), Ph_3P (491 mg, 1.87 mmol, 15 mol %), Cs_2CO_3 (12.21 g, 37.47 mmol), and 1-bromo-4-nitrobenzene (2.523 g, 12.49 mmol). The flask was evacuated and refilled with N_2 three times prior to the addition of **8** (4.00 g, 13.1 mmol) in dry toluene (125 mL). The mixture was heated to reflux using an oil bath and stirred at reflux. After 24 h, the reaction mixture was cooled to room temperature and filtered through a pad of SiO_2. The solid was washed with EtOAc, and the combined filtrate was concentrated in vacuo to yield an oil. The crude product was crystallized by dissolving in minimal CH_2Cl_2 followed by the addition of hexane. The desired product was isolated as an orange solid (4.2 g, 79% yield). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.07 (d, 2H, J = 9.2 Hz, CH_{Ar}), 7.42−7.24 (m, 11H, CH_{Ar}), 6.81 (d, 2H, J = 9.2 Hz, CH_{Ar}), 6.70 (d, 1H, J = 2.7 Hz, CH_{Ar}), 6.59 (dd, 1H, J = 8.7, 2.7 Hz, CH_{Ar}), 6.18 (br, 1H, NH), 5.04 (s, 2H, CH_2), 5.03 (s, 2H, CH_2). ^{13}C{^{1}H} NMR ($CDCl_3$, 151 MHz) δ 156.9, 152.4, 151.4, 139.3, 136.8, 136.2, 128.8, 128.4, 128.3, 127.7, 127.6, 126.3, 123.8, 122.5, 113.3, 106.0, 102.2, 70.9, 70.6. HRMS (ESI): calcd for $[C_{26}H_{22}N_2O_4Na]^+$ 449.14773; found 449.14677.

Synthesis of 4-((4-Aminophenyl)amino)benzene-1,3-diol (3). To a Fisher-Porter bottle with a magnetic stir bar was added **9** (500 mg, 1.17 mmol), methanol (10 mL), and 10% wt Pd on activated carbon (70 mg). The vessel was sealed and purged seven times with H_2 before the H_2 pressure was set to 75 psig. The reaction mixture was stirred at room temperature for 12 h, after which H_2 was vented and the bottle was refilled with N_2. The vessel was taken into a drybox, and the contents were filtered through a pad of dried Celite, which was rinsed with ethanol. The combined filtrate was concentrated in vacuo to afford 240 mg (95% yield) of dark solid. The solid was transferred to a vial and stored under vacuum. ^{1}H NMR (DMSO, 600 MHz) δ 9.14 (br, 1H, OH), 8.75 (br, 1H, OH), 6.71 (d, J = 8.5 Hz, 1H, CH_{Ar}), 6.66 (d, J = 8.8 Hz, 2H, CH_{Ar}), 6.43 (d, J = 8.8 Hz, 2H, CH_{Ar}), 6.31 (d, J = 2.7 Hz, 1H, CH_{Ar}), 6.09 (dd, J = 8.5, 2.7 Hz, 1H, CH_{Ar}), 6.01 (br, 1H, NH) 4.39 (br, 2H, NH_2). ^{13}C{^{1}H} NMR (DMSO, 151 MHz) δ 151.8, 148.9, 141.6, 135.7, 124.8, 119.3, 118.4, 113.8, 105.5, 103.1. MS (ES+, m/z): 217.21 ($[M + 1]^+$, 100%).

Oxidative Oligomerization Following Hydrogenolysis of 9. Compound **9** (60.8 mg, 143 μmol), 10% wt Pd on activated carbon (70 mg), and ethanol (10 mL) were added to a Fisher-Porter bottle. The vessel was sealed and flushed with H_2 seven times before the H_2 pressure was set to 70 psig. The mixture was stirred under H_2 for 12 h, after which H_2 was vented and the vessel was backfilled with N_2. The mixture was filtered (using a 0.45 μm glass syringe filter) into a flask containing 5 mL of ethanol. O_2 was bubbled through the solution until the solvent had evaporated, resulting in the isolation of 50 mg of dark solid. MS (ESI, m/z): 321.20 ($C_{18}H_{17}N_4O_2$, $[M + H]^+$ of **5**), 429.19 ($C_{24}H_{21}N_4O_4$), 643.31 ($C_{36}H_{31}N_6O_6$), 855.27 ($C_{48}H_{39}N_8O_8$).

Resorufamine Generated from 9. Compound **9** (61.6 mg, 145 μmol), 10% wt Pd on activated carbon (70 mg), and ethanol (10 mL) were added to a Fisher-Porter bottle. The vessel was sealed and flushed with H_2 seven times before the H_2 pressure was set to 70 psig. After stirring at this pressure for 10 h, the excess hydrogen was vented, and the colorless solution was filtered into a pH 10.4 aq solution containing $K_3Fe(CN)_6$ (119 mg, 361 μmol). The resulting mixture was stirred for 30 min before filtering through a 0.45 μm glass filter. Removal of the solvent from the filtrate afforded a dark solid, which was treated with EtOH and filtered again. The filtrate was concentrated under vacuum, and the resulting pink solid was dissolved in 1 M HCl and purified by preparative HPLC (reversed-phase C_{18}-capped silica, MeCN−water as the mobile phase) to afford 15 mg (49% yield) of dark solid. ^{1}H NMR (DMSO, 600 MHz) δ 7.50 (d, J = 8.8 Hz, 1H, CH), 7.43 (d, J = 9.6 Hz, 1H, CH_{Ar}), 7.02 (s, 2H, NH_2), 6.71 (dd, J = 8.8, 2.4 Hz, 1H, CH), 6.62 (dd, J = 9.7, 2.1 Hz, 1H, CH_{Ar}), 6.49 (d, J = 2.4 Hz, 1H, CH), 6.16 (d, J = 2.1 Hz, 1H, CH_{Ar}).

Initial Rate Study of the Oxidation of 5 by $K_3Fe(CN)_6$ To Generate 10. To a 25 mL volumetric flask was added **5** (9.2 mg, 28.7 μmol), which was diluted to the line with pH 10.4 buffer and sonicated in a 30 °C bath for 5 min. An aliquot (435 μL) was taken from stock solution and diluted with phosphate buffer to 10 mL in a volumetric flask so that the final concentration was 50 μM. The final solution was loaded into a 10 mL disposable syringe and placed at stopped-flow injection slot A. The buffer solution was loaded into another disposable 10 mL syringe and placed at injection slot B. A background scan was collected by injecting 0.25 mL of each syringe driven by a pneumatic piston. The final solution of **5** in the observation cell was 25 μM. This spectrum of **5** was used as the absorbance baseline so that kinetic run would record the change in absorbance relative to **5**. To a 5 mL volumetric flask was added $K_3Fe(CN)_6$ (21.9 mg, 66.5 μmol), which was diluted to the line with pH 10.4 buffer and sonicated in a 30 °C bath for 5 min. An aliquot (75.1 μL) was taken from the stock solution and diluted with phosphate buffer to 10 mL in a volumetric flask so that the final concentration was 100 μM. The solution was loaded into a syringe and replaced the buffer syringe located in injection slot B. The Julabo water circulator was set to 25 °C, and the solutions were kept in the loading syringes for 5 min to allow for temperature equilibration. The kinetic runs occurred by injecting 0.25 mL of each syringe through the observation cell bringing the final concentrations of the reagents as follows: [**5**] = 25 μM and $[K_3Fe(CN)_6]$ = 50 μM. The rate was determined by observing the change in absorbance at 532 nm (used due to greatest observable change in absorbance from **5** to **10** without interference of the ferricyanide absorbance). Product concentration was calculated

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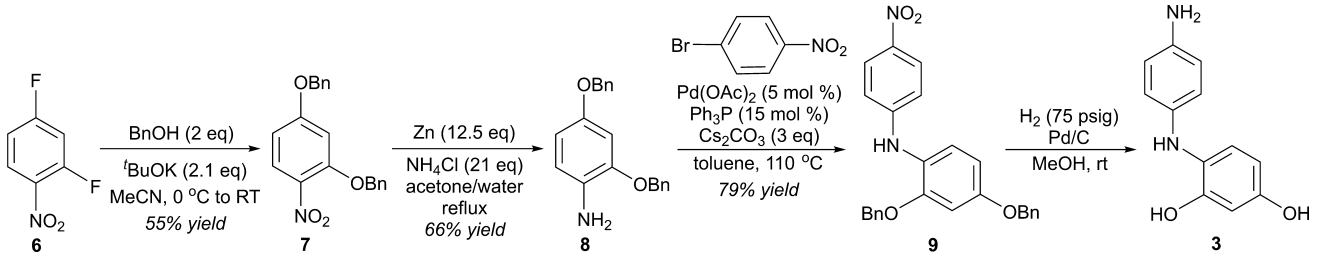
J. Phys. Chem. A XXXX, XXX, XXX−XXX

Scheme 2. Synthesis of the Leuco Dye 3



by using ε = 1249 dm^3 mol^{-1} cm^{-1} at 532 nm and Beer's Law. The initial rate was calculated by taking the slope of the line of [**10**] versus time for the first 10% conversion. Characterization data of **10**: HRMS (positive ion, m/z) [M + H]$^+$ calcd for $C_{18}H_{15}N_4O_2$ 319.119501; found 319.12000. λ_{max} (EtOH/H_2O), nm (ε/dm^3 mol^{-1} cm^{-1}): 450 (6295), 532 (sh, 5167).

General Procedure for Stopped-Flow Reaction of 10 with PPD. Similar stock solutions of **5** and $K_3Fe(CN)_6$ were prepared as above and mixed in a 10 mL volumetric flask so that the final concentrations of the reagents were 50 μM for **5** and 100 μM for $K_3Fe(CN)_6$. After 2 min, the reaction to generate **10** was complete and the resulting solution was transferred to a 10 mL disposable syringe and placed at stopped-flow injector slot A. A background scan was taken for the oxidized trinuclear dye **10** using the buffer as a baseline. A 50 μM solution of PPD in pH 10.4 buffer was added to a 10 mL syringe and replaced the buffer solution at injector slot B. The rate at 25 °C was determined by observing the change in absorbance at 532 nm. For the starting material **10** and the product **5**, concentrations were calculated by using ε_5 = 3871 dm^3 mol^{-1} cm^{-1} and ε_{10} = 5167 dm^3 mol^{-1} cm^{-1} at 532 nm and Beer's Law. The initial rate was calculated by taking the slope of the line of [**10**] versus time for the first 10% conversion.

RESULTS AND DISCUSSION

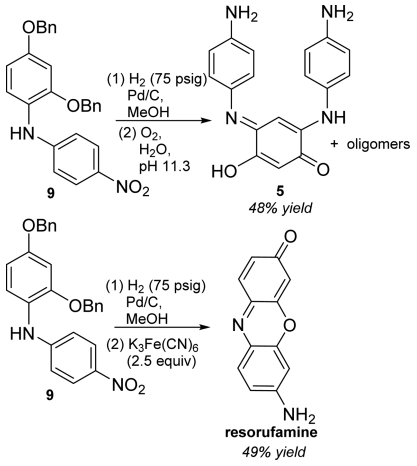
To understand potential oligomerization pathways and mechanistic details, syntheses of pure **3**, **4**, and **5** needed to be developed. The following approaches provide either isolated or in situ generated material of sufficient purity for the mechanistic investigations.

Preparation of Leuco Dye 3. Leuco dye **3** has been proposed as the first coupling product en route to trinuclear species **5** and oligomers (Scheme 1).[12] Attempts to isolate pure **3** directly from oxidative coupling of PPD and resorcinol were unsuccessful. Thus, an independent synthesis of **3** via coupling of two aromatic moieties followed by reduction/deprotection was pursued (Scheme 2).

Preparation of the cross-coupling precursor **8** was accomplished in two steps: reaction of 2,4-difluoronitrobenzene with benzyl alcohol followed by zinc-promoted reduction of the nitro group.[16,17] Subsequent palladium-catalyzed C−N coupling of **8** with 1-bromo-4-nitrobenzene afforded **9** in good yield. Compound **9** was converted readily to leuco dye **3** via palladium-catalyzed reduction of the nitro group and hydrogenolysis of the benzyl ethers in one pot. However, the obtained material or its protonated form (following acidic workup) is oxidatively unstable, thus preventing the isolation of a pure sample. Nevertheless, NMR and mass spectral data of the crude product strongly support the proposed structure. For convenience, the material generated in situ was used for the mechanistic studies.

Attempted Isolation of Indo Dye 4. It was found that the best way to generate **4** for reactivity studies was under stopped-flow conditions. All other attempts resulted in additional products. Oxidation of **3** in alkaline solution by atmospheric O_2 gave trinuclear species **5** along with a small amount of oligomers (Scheme 3). Presumably, base-promoted hydrolysis

Scheme 3. Attempted Synthesis of Indo Dye 4 from 9



of **4** generated PPD/QDI, which subsequently reacted with the remaining dinuclear species to form **5**. Using a one-electron oxidant also was ineffective. Concentrating the reaction of potassium ferricyanide with **3** yielded a cyclization product resorufamine (Scheme 3).[2,6,18−22]

Preparation of Trinuclear Species 5. Trinuclear species **5** is the final compound needed to study the kinetics and mechanisms of oligomerization. This compound was previously isolated from a small-scale synthesis that used repeated preparative TLC to purify the final product.[13,23] Purification of the compound was complicated by the appearance of several byproducts potentially arising from oligomerization of the desired product.[13]

Several other oxidation methods were tried but most had side reactions that led to low purity of the desired dye. For example, although $K_3Fe(CN)_6$ gave the desired product, as verified by mass spectrometry, removal of the Fe^{2+} salts proved difficult (Table 1, entry 1). Alternatively, using an aqueous H_2O_2 solution resulted in slow conversion of PPD and resorcinol to the desired compound with very low purity (Table 1, entry 2). Although adding catalytic amounts of redox metals drives the reaction,[24] it was not possible to isolate the product in sufficient purity (Table 1, entries 3−5).

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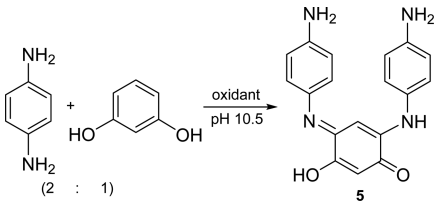
Table 1. Synthetic Attempts to Access the Trinuclear Dye 5



entry	oxidant (equiv)	additive (equiv)	time[a]	purity (yield)[b]
1[c]	$K_3Fe(CN)_6$ (4)	none	2.5 h	<80%
2	H_2O_2 (40)	none	8 h	<10%
3	H_2O_2 (40)	$K_3Fe(CN)_6$ (0.05)	3 h	~10%
4	H_2O_2 (40)	$FeCl_3$ (0.05)	1 h	30%
5	H_2O_2 (40)	$CuCl_2$ (0.05)	1 h	25%
6	oxone (0.5)	none	6 h	40%
7	oxone (1.0)	none	3 h	30%
8	oxone (1.5)	none	2 h	20%
9	oxone (2.0)	none	2 h	20%
10	O_2	none	2.5 d	>98% (45%)

[a]Based on disappearance of the starting materials from TLC analysis (eluent: ethyl acetate). [b]Based on ^{1}H NMR. [c]Product could not be isolated from the Fe(II) salts.

Likewise, the water-soluble oxidant oxone gave a material with low purity (Table 1, entries 6–9). As with all other attempts that gave an impure material, the desired product and the byproducts have limited solubility in water and organic solvents even polar ones such as DMSO, DMF, and NMP, making purification difficult. However, by bubbling O_2 through a buffered (pH 10) solution of PPD and resorcinol, the desired product was obtained in good yield and high purity, albeit slowly (Table 1, entry 10).

Compound **5** was characterized by a variety of analytical methods. High-resolution mass spectrometry (HRMS) gave the expected mass for **5**. Both ^{1}H and $^{13}C\{^1H\}$ NMR spectra of the product (in DMSO-d_6) support a C_2-symmetric molecule as illustrated in Figure 1. This type of symmetry has been observed in related systems and the core structure has been proposed to be zwitterionic based on density functional theory (DFT) calculations.[25−27]

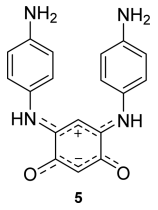


Figure 1. Zwitterionic form of **5**.

Reactivity of Dye Products. With pure **3**, **4**, and **5** in hand or generated in situ, their roles in oligomerization through the reactions with the monomeric reactants (PPD and resorcinol) were probed. Pathways to larger oligomers begin to bifurcate as the oligomer grows, and multiple possibilities can be proposed. Our interest was to examine the initial steps and determine the key intermediates for oligomer growth.

Trinuclear Dye 5 as a Potential Intermediate for Oligomerization. In the absence of an exogenous oxidant, no reaction was observed between either PPD or resorcinol and **5**. It is thus clear that oligomerization does not occur through monomer addition to **5**. However, the addition of the dinuclear species **4** to **5** presents an alternative oligomerization mechanism. Compound **4** was generated in situ under dilute conditions in a stopped-flow apparatus because of its high reactivity and inability to be isolated. Mixing PPD and resorcinol (100 μM each) with 400 μM of $K_3Fe(CN)_6$ in pH 10.4 water−ethanol (1:1) generated a new species with an absorbance maximum at 492 nm, which closely matches the absorbance of similar dinuclear dyes.[3] Mass spectrometry suggested that this species has a mass number of 215.1, further confirming the formation of **4** ($[M + H]^+$ calcd for $C_{12}H_{11}N_2O_2$ m/z 215.08). Adding 100 μM of **5** to the aforementioned mixture gave an absorbance spectrum that is simply a combination of the spectra of **4** and **5** (Figure 2), suggesting that no reaction occurred between these two compounds.

Oxidized Trinuclear Dye 10 as a Potential Intermediate for Oligomerization. The inertness of **5** toward other species present in the reaction mixture indicates that it is not directly involved in oligomer formation. However, since **5** is a major product in the oxidative coupling of PPD and resorcinol, it is reasonable to assume that it is either a precursor to the key reactive intermediate or it is a saddle point, as described by More O'Ferrall and Jencks, off the reaction coordinate.[28,29] Alternatively, oligomerization could merely be a side reaction and the oligomers could be impurities in the major dye (**5**) forming reaction. To differentiate these possibilities, oxidation of **5** to **10** was explored using potassium ferricyanide. The reaction went to completion within 30 s, and the absorbance spectrum contained two isosbestic points (Figure 3). High-resolution mass spectrometry revealed a new product with a mass corresponding to an oxidized species. However, the oxidized product (**10**) could not be isolated, and all attempts led to the recovery of **5**. Hence the reaction of **10** with **5** would not be expected to be important in oligomer formation. Interpretation of initial rates showed oxidation of **5** to be of first-order dependence on both **5** and potassium ferricyanide, with an overall second-order rate constant of $(1.08 \pm 0.10) \times 10^4$ M^{-1} s^{-1} at pH 10.40 ± 0.05 (see Supporting Information for details).

Addition of PPD or resorcinol to the oxidized trinuclear species **10** was examined next. Oligomerization potentially could occur through the nucleophilic attack of PPD on **10**; similar reactivity has been observed in the formation of the Bandrowski's base.[2] However, when PPD was added to an in situ generated solution of **10**, no coupled product was formed; instead, the resulting absorbance spectrum matched that of the reduced trinuclear dye **5**, and ^{1}H NMR analysis showed the presence of QDI **2**. Additionally, mass spectrometry confirmed that no coupled products were present. These results support a redox process occurring between **10** and PPD (Scheme 4). Evidently, the order of the reaction depends on the initial concentration of **10**. It showed zero-order dependence on PPD with 50 μM of $[\mathbf{10}]_0$, while first-order dependence on PPD was observed with 10 μM of $[\mathbf{10}]_0$. In the latter case, the reaction was determined to be second order overall (first order with respect to both **10** and PPD) and the calculated rate constant was 31.7 ± 2.8 M^{-1} s^{-1}.

For the 50 μM reaction, a radical chain mechanism could be used to rationalize the zero-order dependence on PPD, assuming a steady-state concentration of a radical intermediate derived from **10** is maintained in solution. However, the reaction of **10** and PPD in the presence of 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) failed to yield a spin-trapped

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J. Phys. Chem. A XXXX, XXX, XXX−XXX

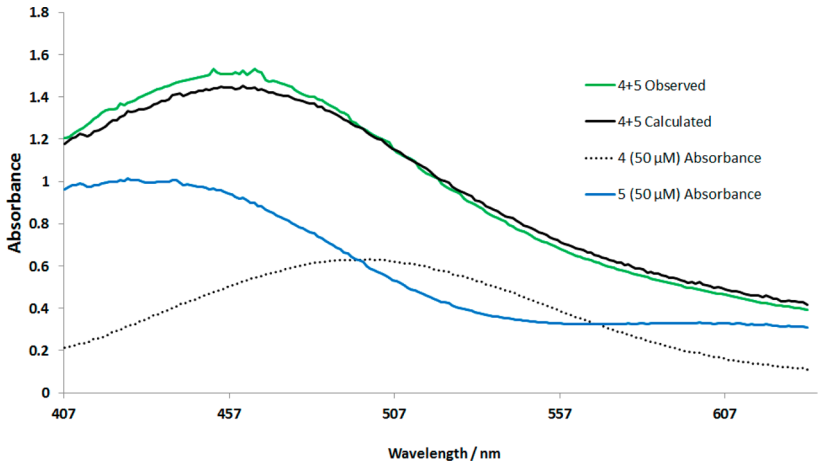


Figure 2. Comparison of calculated additive spectrum of **4** + **5** and observed spectrum of **4** + **5**.

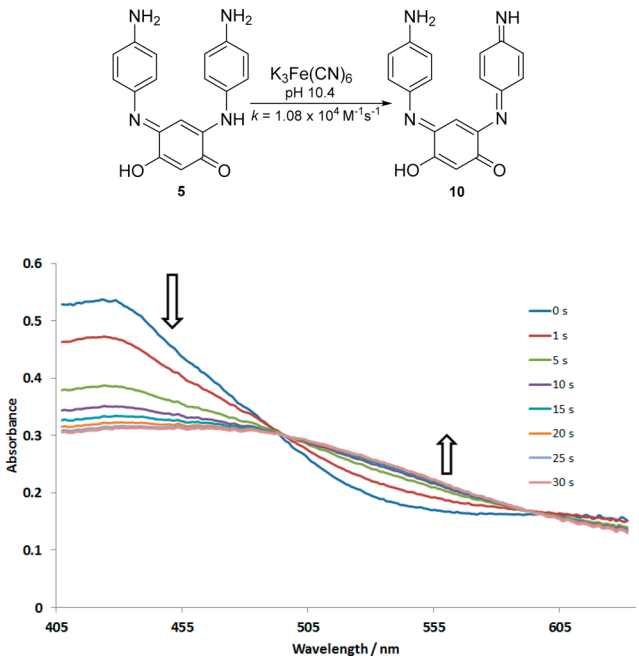
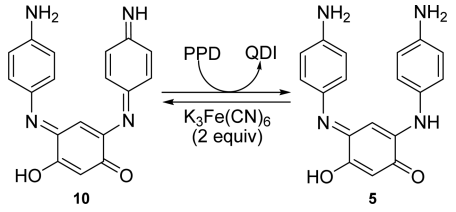


Figure 3. Oxidation of **5** by $K_3Fe(CN)_6$.

Scheme 4. Redox Reaction of 10 with PPD



product, suggesting that the radical mechanism is unlikely to be operative.

Previous reports have documented hydrogen-bonding and π-stacking behavior in molecules with similar core structures.[26,27] Using dynamic light scattering (DLS) method, particles sizes of 334 and 529 nm were observed for 50 μM solutions of **5** and **10**, respectively. Formation of aggregates under these concentrations may limit the amount of free **10** available to react in solution. The redox reaction with PPD can only occur after sufficient dissociation of the aggregates occurs. If the dissociation step were rate-limiting, PPD would not be expressed in the rate law. This can be explained mathematically using eqs 1–9.

$$\mathbf{10} + \mathbf{10} \overset{K_1}{\rightleftharpoons} \mathbf{10}_2 \quad \text{where} \quad \mathbf{10}_2 = K_1[\mathbf{10}]^2 \tag{1}$$

$$\mathbf{10}_2 + \mathbf{10} \overset{K_2}{\rightleftharpoons} \mathbf{10}_3 \quad \text{where} \quad \mathbf{10}_3 = K_1K_2[\mathbf{10}]^3 \tag{2}$$

If the aggregation follows isodesmic growth,[13,30] then $K_1 = K_2 = K_3 = \ldots = K_{i-1}$ and for the reaction with PPD:

$$\mathbf{10}_i \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathbf{10}_{i-1} + \mathbf{10} \quad \text{where} \quad K_{i-1} = k_{-1}/k_1 \tag{3}$$

$$\mathbf{10} + \text{PPD} \overset{k_2}{\rightarrow} \mathbf{5} + \text{QDI} \tag{4}$$

$$\frac{\mathrm{d}[\mathbf{5}]}{\mathrm{d}t} = k_2[\mathbf{10}][\text{PPD}] \tag{5}$$

assuming steady state for $[\mathbf{10}]$

$$\frac{\mathrm{d}[\mathbf{10}]}{\mathrm{d}t} = k_1[\mathbf{10}_i] - k_{-1}[\mathbf{10}_{i-1}][\mathbf{10}] - k_2[\text{PPD}][\mathbf{10}] \approx 0 \tag{6}$$

$$[\mathbf{10}] = \frac{k_1[\mathbf{10}_i]}{k_{-1}[\mathbf{10}_{i-1}] + k_2[\text{PPD}]} \tag{7}$$

then

$$\frac{\mathrm{d}[\mathbf{5}]}{\mathrm{d}t} = \frac{k_1k_2[\mathbf{10}_i][\text{PPD}]}{k_{-1}[\mathbf{10}_{i-1}] + k_2[\text{PPD}]} \tag{8}$$

assuming $k_2 \gg k_{-1}$, the observed rate becomes

$$\frac{\mathrm{d}[\mathbf{5}]}{\mathrm{d}t} = k_1[\mathbf{10}_i] \tag{9}$$

Following the isodesmic growth model, PPD would not appear in the rate law (eq 9) unless $[\mathbf{10}]_0$ were low enough to prevent significant aggregation from occurring. Future work will be carried out to further elucidate the aggregate growth mechanism.[31]

In the final attempt to form oligomers, **10** was reacted with resorcinol. Under stopped-flow conditions with diluted solutions, no reaction occurred using a 1:1 stoichiometric ratio. Even with a large excess of resorcinol (100 equiv), only minor amounts (<5%) of unidentifiable products were

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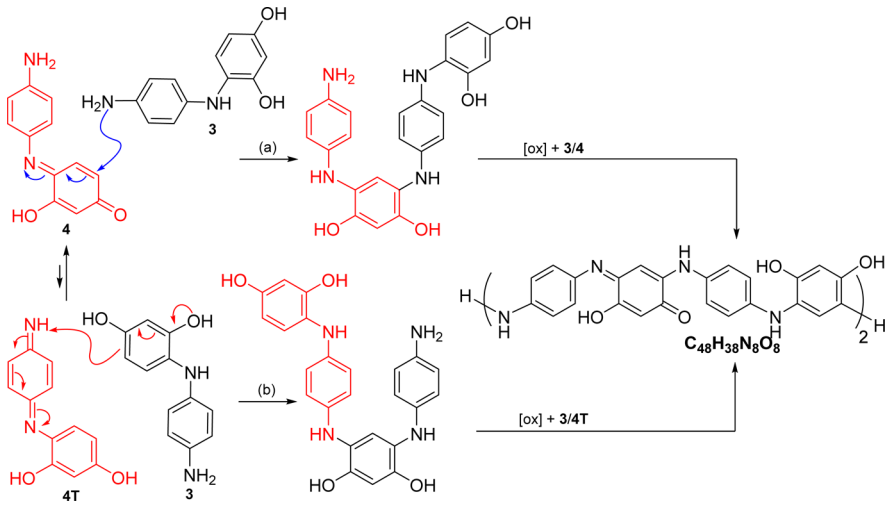
Scheme 5. Proposed Mechanisms for the Oligomerization from 3/4[a]



[a]Hartree-Fock (6-31G* in EtOH) calculations (refs 32 and 33) show that **4** is 9.8 kcal/mol more stable than its tautomer **4T**.

detected. Scaling up the reaction and performing it in the presence of hydrogen peroxide resulted in only minor amounts of products (<5%) with molecular weight of <350 Da. Isolation of these minor products was unsuccessful, but the absence of oligomeric species, as confirmed by matrix-assisted laser desorption ionization (MALDI) or ESI, eliminated resorcinol addition to **10** as a viable oligomerization route.

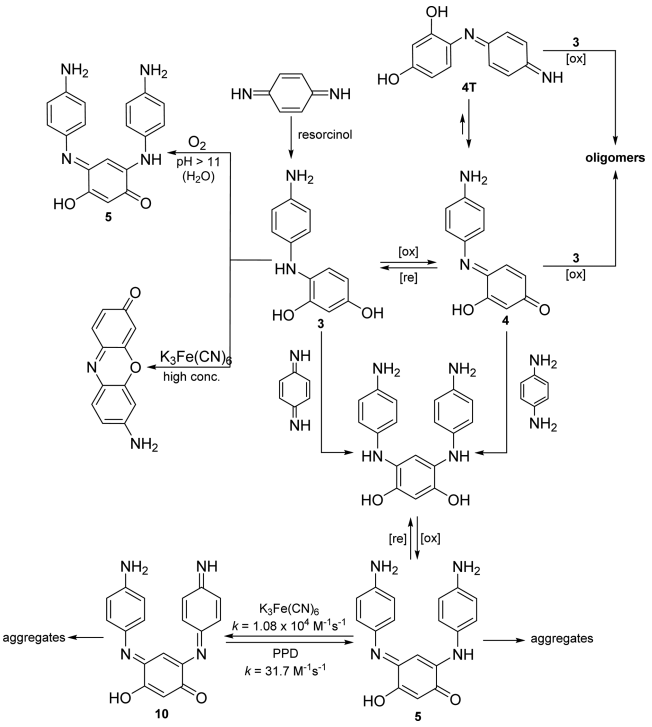
Dinuclear Dyes 3 and 4 as Potential Intermediates For Oligomerization. At this point, neither **5** nor **10** has been demonstrated as the key intermediate in oxidative oligomerization of PPD−resorcinol, as suggested by their limited or no reactivity under a variety of conditions that are representative of hair color products. Our attention was then focused on reactions of the two dinuclear species, **3** and **4**.

Bubbling O_2 through an ethanol solution of **3** resulted in a highly insoluble dark powder. Mass spectrum (MS-ESI) of the product indicated the presence of an octanuclear species (m/z 855.27 consistent with a formula of $C_{48}H_{39}N_8O_8$). The observation of larger molecules arising from oxidation of the dinuclear species has not been shown previously. Our result here provides strong evidence that **3** and **4** are key reactive intermediates for the oligomerization process. Two oligomerization pathways can be proposed: (a) aza-Michael addition of **3** to **4**, or (b) attack on tautomer **4T** by the electron-rich resorcinol moiety of **3** (Scheme 5). Although other oligomerization pathways are possible (e.g., reaction with **10** with **3**), mechanisms depicted in Scheme 5 represent two most likely paths to the proposed oligomers. We propose that the rate of coupling is slow; therefore, slow oxidative conditions are needed so sufficient quantities of **3** are available to react with **4** for oligomerization. Reactions that have a fast rate of oxidation of **3** (potassium ferricyanide or alkaline O_2) will not generate a significant amount of oligomers due to the relatively low concentrations of the redox pairs. Additional work is required to deepen our understanding of oxidative oligomerization of PPD and resorcinol. Among our future directions are (a) investigation of the pH profile for the rates of the reactions listed in Scheme 5, (b) understanding of reactivity of **10** with **3**, and (c) determination of rate constants for the individual steps once the main operative pathway is identified.

CONCLUSIONS

The success in developing a large-scale synthesis of trinuclear dye **5** has allowed us to investigate its reactivity (or lack thereof) toward oligomerization. Overall the reactivity of **5** is limited. It does not react with PPD, resorcinol, or the dinuclear dye **4**. Upon oxidation by potassium ferricyanide, **5** is converted to **10** following a second-order reaction (first order in both potassium ferricyanide and **5**). The oxidized trinuclear dye **10** is also unreactive toward resorcinol or **5**, ruling out these reactions as possibilities for oligomerization. Compound **10**, however, reacts with PPD to give **5** and QDI via a redox

Scheme 6. Current Understanding of PPD−Resorcinol Dye Reactivity



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J. Phys. Chem. A XXXX, XXX, XXX−XXX

process. Both kinetics and DLS experiments suggest that **5** and **10** readily undergo significant aggregation or agglomeration.

In situ generation of intermediate **4** using two independent routes (via PPD−resorcinol oxidative coupling, or via synthesis of the precursor leuco dye **3**) has allowed us to observe previously unknown reaction pathways from this unstable species. Scheme 6 outlines our current understanding of the PPD−resorcinol dye reactivity, which has been expanded significantly from the original mechanistic profile. Our results suggest that oligomerization through this intermediate is a viable route, and further work will be carried out to understand the mechanistic details and to determine whether other routes are possible.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpca.6b08571.

Details on coupling optimization to generate **9**, hydrogenolysis optimization to generate **3**, kinetics of oxidation of **5**, kinetics of reduction of **10**, dynamic light scattering table, and NMR data (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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