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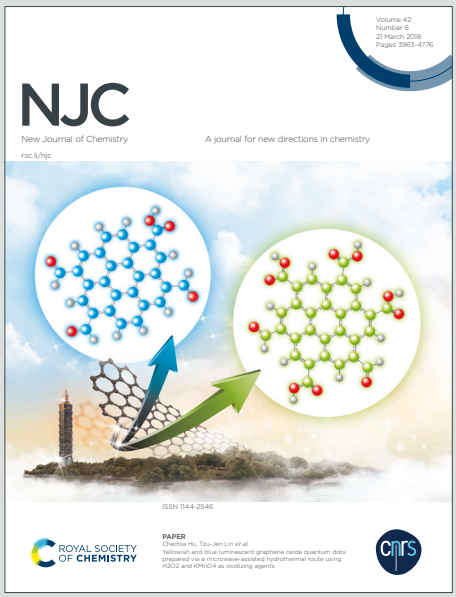

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New Journal of Chemistry

View Article Online
DOI: 10.1039/C9NJ03362A

ARTICLE

Facile synthesis of oligo anilines as permanent hair dyes: How chemical modifications impart colour and avoid toxicity

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Many dyes for long-lasting hair coloring contain aromatic amines that undergo oxidative polymerizations, resulting in allergic contact dermatitis, with potential to develop serious toxic effects. Among these amines, para-phenylenediamine (PPD) is a small molecule form of aniline commonly used in beauty products despite being a known allergen to humans. Hence, in this study we designed and synthesized safer PPD analogues through the synthesis of oligomeric PPD and the introduction of bulky side chains on PPD to overcome the PPD dye's toxicity. We hypothesized that a) increase in molecular size of PPD by addition of the PPD monomer unit on free amines and b) strategic functionalizations at the ortho position of PPD with strong electron-donating bulky groups are able to maintain the hair coloring properties, increase the resistance to binding to skin proteins and therefore decrease the chance of skin sensitization. 13 oligomers were synthesized, with the aim to produce safer hair dyes while minimising eventual toxicity to humans. In particular, oligomers with bulky sidechain, PPD 6 (13), PPD 7 (14) & PPD 8 (15) displayed a weak-to-moderate (27.1%, 24.1% & 34.0%) sensitization potential. The results confirmed the importance of having bulky and strong electron donating O-alkyl substituents in order to decrease the reactivity of PPD analogues towards skin proteins and thus preventing the interaction with immune cells and providing safer hair dyes.

Introduction

Many dyes for long-lasting and intense hair coloring contain unstable di–/tri-functional aromatic amines that have to undergo oxidative polymerizations to provide the desired pigmentation.[1] However, oxidative and enzymatic alterations of these amines generate potentially harmful and sensitizing metabolites which may cause serious irritant and allergic contact dermatitis.[2,3] *Para*-phenylenediamine (PPD) (**Figure 1**) is a common amine-containing compound used in beauty products.[4] In the year 2006 it was declared as "allergen of the year".[5] This small molecule (*MW* = 108.1 Da) is able to penetrate skin and, in clinical studies, has been detected in plasma, urine and faeces of volunteers upon topical application of PPD-containing hair dyes.[6,7,8] Systemic PPD Hair dye poisoning

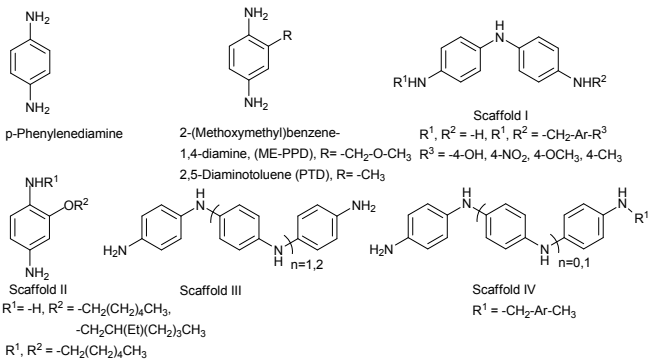

Figure 1. Structures of PPD derivatives.

incidents such as cervico-facial oedema, severe rhabdomyolysis, acute tubular necrosis and renal failure have also been reported.[9] Till now, it has not been clearly established yet if PPD itself or its oxidized metabolites, which include a *spectrum* of known and unknown electrophiles, are responsible for the toxic, irritant or allergic reactions and how these compounds affect the keratinocytes in the skin and the immune response.[10]

Recently, hair dye products containing structurally-similar PPD alternatives, such as *para*-toluenediamine sulfate (PTD) and 2-(methoxymethyl)benzene-1,4-diamine (ME-PPD), have been

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

proposed as safer alternatives to PPD (**Figure 1**). While approximately half of PPD-sensitive individuals still reacted to PTD,[11] ME-PPD showed lower sensitization potential than PPD.[12] Nevertheless, small cohort studies still yielded a sensitizing response to ME-PPD in more than 30% of PPD-allergic subjects.[12] Hence, more research and development to achieve less irritant and sensitizing stable hair dyes are warranted.

Polyanilines are electro-conducting polymers and are the model compounds to prepare oligoanilines. They have been synthesized as nanofibers, nano-ribbons polymer materials for detection of electro-conductance and for improved cell-biomaterial interaction in tissue engineering applications.[13-15] To our knowledge, polyanilines so far have been tested for their material properties only and not as hair dyes.

In this study we designed and synthesized a series of oligomeric structures of PPD by adding monomeric units of PPD and introducing functionalized side chains or substituents in order to increase the molecular size, lipophilicity and hydrogen bonding properties of the hair dyeing molecule. We further postulated that these structural modifications would render the PPD derivatives more resistant to oxidative changes compared to PPD, thereby decreasing their ability to interact with skin proteins and induce skin sensitization. **Figure 1** illustrates the modifications made to PPD. We increased the number of 1° and 2° amine groups by a) introducing novel head groups and the conjugate system into a dimeric, trimeric or tetrameric structure; b) adding long hydrocarbon chains c) increasing the sum of hydrogen bond accepting and hydrogen bong donating groups and d) increasing the molecular weight of the compounds. Modifications specifically included symmetrically amine (NH_2/NH_2) capped and aryl (Ph/NH_2) capped oligoanilines (Scaffold I), C-substituted and *C,N*-substituted PPD derivatives (Scaffold II), symmetrically amine (NH_2/NH_2) capped oligoanilines (Scaffold III), unsymmetrically aryl(Ph/NH_2) capped oligoanilines (Scaffold IV).

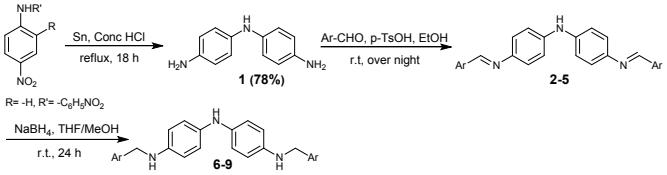
The design of dimer and trimer structures (**Figure 1,** Scaffolds I, III and IV) was aimed at assessing how the increase in size and lipophilicity affected the properties of the compounds, especially when compared with the main product of oxidation of PPD, Bandrowski's base (BB). For the *C*-substituted and *C,N*-substituted derivatives (Scaffold II), monomeric or dimeric PPD was substituted with hexyl chain to investigate the effect of electron donating chain on PPD. The long chain compounds were designed to investigate the effect of the substitution at the *ortho* position of PPD: as it was reported that the development of skin allergy decreases in the order PPD < PTD < ME-PPD, [16] we reasoned that electron-donating alkyl functional chains at the *ortho* position of PPD would decrease the oxidation potential of the benzene ring in PPD, thereby decreasing its conversion into a reactive quinonediamine; at the same time, by increasing the nucleophilic property of the benzene ring through electron donating resonance effects, we would have further decreased PPD's reactivity towards nucleophilic skin proteins. This idea was taken from studies in which the introduction of one or two electron-withdrawing chains at the *ortho* position to the main reactive functional group in a molecule (e.g. the 'OH' group in acetaminophen) resulted in a decreased analgesic effect due to enhanced oxidation of the drug.[17-18] Hence, by introducing functionalized sidechains or substituents at the *ortho* position of the PPD, our PPD analogues could decrease the benzene's reactivity towards oxidation and overcome the toxicity of PPD.

Our results confirm the hypothesis that a suitable combination of increased molecular size and strong electron-donating side chains minimize the skin sensitization potential and preserve the desired hair nuance properties, making these oligomeric series of PPD analogues potentially much safer in comparison to existing products in the market.

Result and Discussion

Synthesis of PPD derivatives

All compounds were successfully synthesized, with yields in the 50-99% range, and characterized by ^1H and ^{13}C NMR spectroscopy and High Resolution Mass Spectroscopy (HRMS) (**Figures S1-S14**). The PPD precursor dinitro derivative was obtained by the coupling of *p*-nitroaniline with nitrobenzene in good yield (refer to experimental section of SI for details). The secondary amine proton in 4,4′-dinitrodiphenylamine resonates at δ 9.98 ppm, showing a significant upfield shift (Δδ 3.22 ppm) of the secondary amine proton in the ^1H NMR spectrum of 4,4′-diaminodiphenylamine (1). This upfield shift of the NH proton is due to the increase of the electron density in the aromatic ring upon the conversion of the nitro group into the amine. This is further supported by the upfield shift of aromatic protons from the ranges δ 8.2-7.3 to δ 6.6-6.4. The dimeric compound 1 (PPD 1) was synthesized by the reduction of 4,4′-dinitrodiphenylamine with tin powder in strongly acidic medium (**Scheme 1**).



Entry	Reagent (ArCHO)	Ar	Yield
1	4-hydroxy benzaldehyde	C_6H_4(4-OH)	67% (**6**)
2	4-nitro benzaldehyde	C_6H_4 (4-NO_2)	82% (**7**)
3	4-methoxy benzaldehyde	C_6H_4(4-OCH_3)	88% (**8**)
4	4-methyl benzaldehyde	C_6H_4 (4-CH_3)	90% (**9**)

Scheme 1. Synthesis of symmetrically amine (NH_2/NH_2) capped PPD 1(**1**), aryl (Ph/NH_2) capped oligoanilines PPD 2-5 (**6-9**).

A host of higher oligomers (MW > 400 Da) of symmetric aryl capped derivatives (PPD 2-5 (**6-9**)) was synthesized by functionalizing the synthesized PPD 1. First, an imine (**2-5**) was synthesized by the reaction of the symmetric PPD 1 with an aldehyde in presence of *p*-toluenesulphonic acid.

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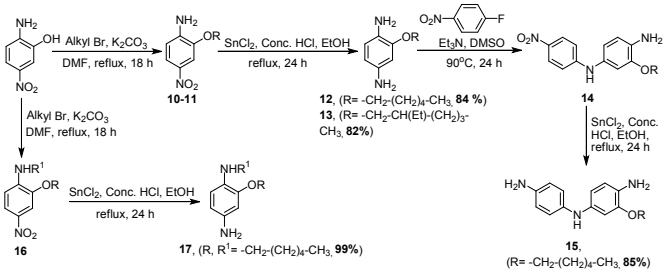
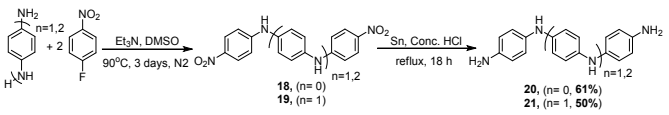
Then, the imine was reduced to provide the corresponding amines (**6-9**) (**Scheme 1**) in very good yield (67-90%). Irrespective of the nature of substituents, the imine proton signals appeared in the range of δ 8.9 to 8.50 ppm, with the nitro derivative showing the highest chemical shift. Upon reduction, the imine signal disappeared and two new signals, corresponding to the NH–CH_2 group, appeared in the range δ 5.9 – 5.4 ppm (triplet) for the amine and δ 4.3 – 4.0 ppm (doublet) for the methylene group, respectively. As usual, all aromatic proton signals underwent an upfield shift upon imine reduction.



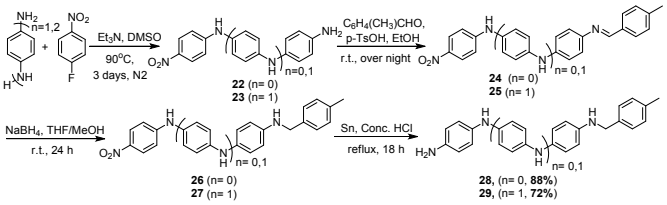
Scheme 2. Synthesis of C-substituted derivatives PPD 6 (**12**), 7 (**13**) and 9 (**17**) and *C,N*-substituted PPD 8 **(15**).

Alkoxy derivatives of PPD such as PPD 6-7 (**12-13**), 8(**15**) and PPD 9(**17**) (**12-13, 15 and 17**) were synthesized by nucleophilic substitution of the bromo group of 1-bromoalkane with the *in situ* generation of phenoxy derivative of 2-amino-5-nitrophenol. Subsequent $SnCl_2$ reduction of the nitro group afforded the 2-alkoxy PPD 6 (**12**) in high yield (**Scheme 2**). Although *O*-alkylation was the major product (**12**), for the 1-bromohexane *N,O*-alkylation was also observed. *N,O*-hexyl-4-aminobenzene (**17**) was isolated as minor product along with the *O*-alkylated product. Further, the newly synthesized product **12** was further functionalized to get higher oligomers. The substituted PPD derivative (**12**) was treated with 1-fluoro-4-nitrobenzene in presence of triethylamine to get the nitro derivatives, which were then reduced by $SnCl_2$/Conc HCl to get the corresponding dimer with O-hexyl substituted PPD 8 (**15**). In the *O,N* alkyl functional series, PPD 9 (**17**) was obtained in a quantitative yield by reacting 2-amino-5-nitrophenol with 2 equivalents of 1-bromohexane, and subsequent reduction of nitro compound.



Scheme 3. Synthesis of symmetrically amine (NH_2/NH_2) capped oligoanilines PPD 10(**20**) and 11(**21**).

The higher oligomers (PPD 10 and 11 (**20-21**)) were conveniently synthesized *via* S_NAr type substitution reactions with aromatic amines.[19] PPD 10 (**20**) was synthesized by coupling PPD with two equivalents of 1-fluoro-4-nitrobenzene to the dinitro compound *N¹,N⁴*-bis(4-nitrophenyl)-1,4-benzenediamine (**18**), followed by Sn/HCl reduction. Similarly, the reaction of compound **1** with two equivalents of 1-fluoro-4-nitrobenzene and subsequent reduction of resulting nitro compound 19 afforded 4'-bis[(4 aminophenyl) amino] diphenylamine (PPD 11 (**21**)) (**Scheme 3**).



Scheme 4. Synthesis of unsymmetrically aryl(Ph/NH_2) capped oligoanilines PPD 12(**28**) and 13(**29**)).

A different synthetic approach was adopted for the synthesis of the unsymmetrically aryl capped oligoanilines (**Scheme 4**). Non-symmetric intermediate oligomers (**22-23**) were synthesized with amino and nitro groups as terminal substituents. The free amino terminal group was substituted with *para*-tolyl methyl substituent and the nitro terminal group was reduced to obtain non-symmetric oligomers **28** and **29** (PPD 12 and 13). In our initial effort, we synthesized a dimeric compound 4-amino-4'-nitrodiphenylamine (**22**) by the coupling of PPD with one equivalent of 1-fluoro-4-nitrobenzene in the presence of triethylamine. The secondary amine signal appeared at δ 8.88 ppm. The corresponding imine (**24**) was then synthesized by the reaction with *para*-toluenecarboxaldehyde in the presence of *para*-toluenesulphonic acid. Sodium borohydride reduction of the imine, followed by Sn/HCl reduction of the nitro group, afforded non-symmetrically substituted PPD dimer 4-amino-4'-[(4-tolylaminomethyl)phenyl]diphenylamine (PPD 12 (**28**)). The imine proton signal of compound **24** appeared at δ 9.37 ppm, and then disappeared upon sodium borohydride reduction. A triplet and a doublet appeared at δ 6.24 and 4.22 ppm corresponding to the amine and methylene proton, respectively, of the newly formed NH–CH_2 group in compound **26**. Simultaneously, compound **28** was coupled with 1-fluoro-4-nitrobenzene to get the nitro compound *N¹*-[4-(4-tolylaminomethyl)phenyl]-*N⁴*-(4-aminophenyl)-1,4-benzenediamine (**27**) with 26% yield, which was then reduced with $SnCl_2$/HCl to get corresponding trimer *N¹*-[4-(4-tolylaminomethyl)phenyl]-*N⁴*-(4-aminophenyl)-1,4-benzenediamine (PPD 13 (**29**)).

An alternative method was also developed for the synthesis of the trimeric PPD 13 (**29**) with higher yield and involving less number of steps. Similar to the synthesis of compound **22**, non-symmetrically substituted trimeric structure *N¹*-(4-aminophenyl)-*N⁴*-(4-nitrophenyl)-1,4-benzenediamine (**23**) was synthesized from the NH_2/NH_2 type dimeric compound **1**.

Compound **23** was then condensed with *para*-toluenecarboxaldehyde to get the corresponding imine (**25**). Sequential reduction of the imine by sodium borohydride yielded compound **27** with high yield (95%) and the nitro groups of **27**

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afforded PPD 13 (**29**) upon reduction with Tin powder in acidic medium. A general synthetic pathway is presented in **Scheme 4.**

Direct peptide reactivity assay (DPRA) for *in chemico* skin sensitization

Table 1 summarizes our classification results for PPD, ME-PPD, PTD and our PPD derivatives, following the Organisation for Economic Development's (OECD) guidelines.[20]

S. N	Compound	Cysteine % (1:10)	Lysine % (1:50)	Mean Peptide depletion % Cysteine (1:10) / (Lysine1:50)	Sensitizing potential
1	PPD	77.7	33.2	55.5	Strong
2	**1** (PPD 1)	69.4	23.7	46.6	Strong
3	**6** (PPD 2)	51.2	15.6	33.4	Moderate
4	**7** (PPD 3)	48.0	11.3	29.7	Moderate
5	**8** (PPD 4)	47.1	8.5	27.8	Moderate
6	**9** (PPD 5)	40.9	12.8	26.9	Moderate
7	**12** (PPD 6)	40.8	13.4	27.1	Moderate
8	**13** (PPD 7)	32.7	15.4	24.1	Weak-moderate
9	**15** (PPD 8)	53.8	14.3	34.0	Moderate
8	**17** (PPD 9)	37.1	10.8	23.9	Weak-moderate
9	**20** (PPD 10)	64.1	9.0	36.5	Moderate
10	**21** (PPD 11)	78.8	10.2	44.5	Strong
11	**28** (PPD 12)	27.6	8.1	17.9	Weak
12	**29** (PPD 13)	35.2	10.9	23.0	Weak-moderate
14	Bandrowski's Base	70.9	6.9	38.9	Moderate
13	ME-PPD	62.9	24.8	43.7	Moderate
14	PTD	71.3	25.3	48.3	Strong
15	Control (2,4 dinitro 1-chloro benzene (DNCB))	83.7	44.6	64.1	Strong
16	Control (Cinnamaldehyde)	35.4	12.7	24.0	Moderate
17	Control (Lactic acid)	4.8	0.3	2.5	Weak

The two model heptapeptide-containing nucleophilic amino acids cysteine and lysine are used in the DPRA to simulate the protein reactivity event within the skin sensitization adverse outcome pathway. OECD guidelines recommend the use of mean cysteine and

lysine percent peptide depletion values as a way of discriminating between skin sensitizers and non-sensitizers.[20] The average peptide reactivities determined for 24 h place the derivatives in the weakly or moderately reactive categories (**Table 1**).

They display lower reactivity compared to PPD, ME-PPD and PTD due to suitable substituents possessing different electronic properties. PPD undergoes auto-oxidation to form an electrophilic imine intermediate[21] that is reactive to nucleophilic amino acids. Similarly, for our PPD derivatives to be reactive towards nucleophilic amino acids, such an imine intermediate is expected to form. However, by modifying and substituting the PPD at the *ortho* position (as in PPD 6-9) with stronger electron donating chain than PTD (*ortho* methyl) and ME-PPD (*ortho* methoxy methyl), the electron density of the benzene ring is expected to increase through electron donating resonance effects of the *ortho O*-alkyl functional chain. Stronger donating chain like *O*-hexyl increase electron density at *ortho* and *para* positions to *O*-hexyl chain (such as positions 3, 5 and 6 in PPD 6 and 7) in pi system of benzene ring and make it a more nucleophilic site than PTD and ME-PPD. This causes less interaction of our PPD derivatives with nucleophilic protein or peptides, as confirmed by the DPRA.

In addition, we aimed to decrease the oxidation potential by affecting the resonance mechanism on benzene ring and amine chain, as previously reported.[16-18] This was achieved by blocking the extension of conjugation with either substituted benzyl chain (as in PPD 2-5, PPD 12 and PPD 13) or long aliphatic chain at the one end of the amine functionality (as in PPD 9): these substitutions made PPD amine unavailable for the electronic oxidation, which minimizes the formation of reactive imines and, thus, becomes less reactive towards peptides in comparison to PPD.

Among the synthesized derivatives, PPD 12 and PPD 13 showed the lowest mean peptide depletion, with values of 17.9% and 23.0%, respectively, suggesting the least expected side effects in the whole PPD derivatives series. In both derivatives, one end of the amine functionality was blocked by a benzyl substituent, which resulted in the unavailability of the amine for oxidation to form the imine conjugate intermediate, and this could have contributed to the lower extent of binding with the model peptides. PPD 6, 7 and 9 showed depletion values of 27.1%, 24.1 and 23.9%, respectively, i.e. 2 times less binding than PPD. These derivatives have strong electron donating substituents at the *ortho* position of PPD. This could render the benzene ring electron rich by electron donating resonance effect, making it less available for nucleophilic protein interaction. A lower value of mean depletion observed for PPD 7 *vs*. PPD 6 was likely due to a stronger donating effect of branched ethyl hexyl chain in PPD 7. Conversely, the lower depletion of PPD 9 was

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due to an additional *N*-hexyl substitution, which made the compound less susceptible to oxidation than PPD 6 by blocking the amine functionality, in addition to making the system less nucleophilic with electron donating resonance effect of the *O*-hexyl chain.

The other derivatives (PPD 1, 10 and 11) displayed high mean peptide depletion values. These molecules are dimeric, trimeric and tetrameric structures of PPD and are un-substituted. They are expected to form electrophilic conjugated imine intermediates, e.g. indamine dye in case of dimeric PPD 1 and emeraldine dye-like compound in case of PPD 10 and 11 upon auto oxidation, as benzoquinone diamine (BQDI) observed for PPD. Hence, it is not surprizing that these derivatives naturally have higher tendency to make binding with nucleophiles of tested peptides. Interestingly, the dimeric form of PPD 1 displayed higher peptide depletion than trimer PPD 10 and tetramer PPD 11. Another trimeric form of PPD, i.e. Bandrowski's base, showed similar mean peptide depletion (38.9%) to PPD 10 (**Table 1**). Interestingly, another dimeric PPD derivative (PPD 8), with *ortho* hexyl substitution, showed slightly lower peptide depletion (34%) as compared to its unsubstituted counterpart PPD 1 (46.6%), proving the fact that addition of the O-hexyl side chain at *ortho* position is beneficial in decreasing the peptide depletion due to electron donating resonance effect.

Another series of PPD derivatives, symmetrically aryl capped oligo anilines PPD 2-5 are moderate-to-weak sensitizers according to the DPRA. Among this series of derivatives, PPD 2 displayed higher depletion (33.4%) as compared to rest of the compounds, because of the presence of hydrophilic hydroxyl head group of the benzyl chain at both the end of the amine cap, which could be involved in hydrogen bonding interactions with heptapeptides. Other commercially available dye precursors of PPD derivative such as ME-PPD and PTD, showed a mean peptide depletion of 43.7%, 48.3 %, respectively. This suggests that the addition of weaker electron donating chain in ME-PPD and PTD, at the *ortho* position of PPD did not affect the electron density of the benzene ring. As expected, the *ortho* substitutions in these compounds caused weak electron donating inductive effects to the benzene ring, and made *ortho* and *para* positions sites (such as position 3, 5 and 6) of ME-PPD and PTD weaker nucleophiles than PPD 6, 7 and 9. Hence, these compounds were still moderate and strong sensitizers in the DPRA assay.

All PPD derivatives tested (PPD 2-5, 6-9 and 12-13) showed significantly lower mean peptide depletion percentages than PPD. This corroborates the hypothesis that their structural diversity (with increased electron density and/or protection of terminal amine by benzyl substituents) might indeed be associated with improved safety in cosmetic products. In fact the most promising compounds from the DPRA assay were also shown to minimally penetrate through the skin using porcine ear skin inserted in Franz diffusion cells (data not shown). The results also signify the importance of having strong electron donating substituents at the *ortho* position of PPD, with large chains able to minimize the reactivity towards peptides and potential skin-related side effects. It should be noted that the classification according to DPRA assays is a preliminary screening tool to estimate the potentially undesired

Table 1. DPRA classification results of PPD derivatives

biological activities of test compounds based on peptide depletion.[20] However, it is not known
if and what kind of reactions to living cells this DPRA test is predicting. It seems that it can predict to a certain extent the binding and interaction of chemical structures to skin cells. This interaction can induce damage to skin cells in the form of irritant dermatitis and eventual allergic dermatitis through release of cytokines and proliferation of the slightly damaged skin cells.[22]

Hair dyeing

Figure 2 shows the hair dyeing results obtained from PPD, PPD 1, 6, 7, 8, 10, PTD and ME-PPD applied as formulation A (aqueous solution of derivative alone). The derivatives dyes impart a vibrant color in contrast to the bleached hair tone.

Hair coloring results of the same derivatives in formulations B (with the oxidant H_2O_2) and C (with the coupler resorcinol) are shown in the Supplementary Information (**Figure S15**) along with the measured color tone and color difference values (**Table S2**)**.** The results of the colour measurement are represented as the L, a, b values and further these values are converted into delta H, delta E values using the formula presented in the supplementary section. As noticed in the **Figure 2**, PPD, PTD and ME-PPD showed no colour without oxidizing agent. Whereas the oxidized PPD, PTD and ME-PPD showed black or brown colour (**Figure S15**). It was observed that ΔH & ΔE value of PPD, PTD and ME-PPD type A is comparable to the bleached hair, which indicates that PPD, PTD and ME-PPD have no intrinsic dyeing efficiency. As expected, the oxidized PPD type B (upon treatment with H2O2) showed similar ΔH & ΔE value as the original black hair, indicating that PPD oxidized product is black

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in colour. PTD and ME-PPD type B showed similar ΔH & ΔE value responsible for brown colour. PPD 1 showed black colour and its ΔH value (**Table S2**) is comparable to the natural hair shaft. PPD 6 type

A showed intense

PPD (No color)	PPD 1 (Black)	PPD 6 (Magenta)	PPD 7 (Purple)	PPD 8 (Black)	PPD 10 (Bluish Purple)

PTD (No color)	ME-PPD (No color)

Figure 2. Hair colors obtained from PPD derivatives 1, 6, 7, 8, 10, PTD and ME-PPD in aqueous solution (formulation A).

magenta colour with high ΔE, ΔH value and the oxidation type displayed black colour nuance. PPD 7 displayed a purple colour with higher ΔH & ΔE value higher than PPD 6. PPD 8 displayed black colour for both types A & B, which resembled the natural black hair. PPD 10 showed higher ΔH value for all 3 types indicating grey colour dye.

Our hair dye experiments confirmed that our PPD derivatives were intrinsically colored and able to provide hair nuance even in the absence of the *in situ* oxidation with a developer (i.e. strong oxidizing agent). This interesting result, although confined to bleached hair samples, indicates that our PPD derivatives can in principle avoid the usage of oxidizers like H_2O_2, which is known to damage the hair cuticle.[23,24] Moreover, the color on the hair was stable for several weeks upon repeated washes with shampoo and conditioner (**Table S3**), indicating that our PPD derivatives were "locked" stably in the form of permanent hair dyes. It could be argued that traces of H_2O_2 could have been responsible for this observation, but this hypothesis was rejected as it would have shown a black color for PPD; instead it remained colorless during the first 30 minutes of application (**Figure 2**, PPD, PTD and ME-PPD samples). The presence of the dyes on the hair was also confirmed by the quantitative evaluation of hair samples through LC-MS/MS and UV spectroscopy, confirming that the dyeing compounds are retained on the hair over time (data not shown). This opens up the possibility for PPD 1, 6, 7, 8 and 10 to be incorporated into hair dye preparations to provide persistent and bright colors to hair.

Conclusions

We have synthesized and characterised novel oligomeric PPD derivatives with functionalized side chains for use as safer, less toxic hair dyes. Among the PPD derivatives, PPD 6 (**13**), PPD 7(**14**) and the dimeric form of oligo aniline, PPD 8 (**15**), PPD 10(**20**), showed a reduced sensitizing potential in the DPRA assay and stably colored bleached hair samples without staining the skin. Although PPD 9, 12 and 13 showed weak sensitizing potential, their poor coloring properties preclude them as hair dyes. Our work represents a major step in the development of novel hair dyes, together with recently reported structures that are safer than currently marketed products. The potential for chemical structures such as hair dye ingredients to cause sensitization depends, among other interactions with skin, on their ability to penetrate the skin barrier and to covalently modify epidermal proteins.

Experimental Section

Materials

All the commercial materials for the synthesis was obtained from Sigma Aldrich (Singapore) and were used without further purification. For hair dyeing experiment, PPD (98.0%) and resorcinol (≥ 99.0%) were purchased from Sigma-Aldrich (Singapore). Schwarzkopf BlondMe Premium Lift 9+ bleaching powder and Schwarzkopf BlondMe 12%/40 vol developer solution, Silkpro VitAir series daily balance shampoo, and Silkpro VitAir series daily treatment Masque (hair conditioner) were purchased from Amazon and used without further purification. 30% hydrogen peroxide solution was purchased from Merck (Singapore). For DPRA experiment, HPLC grade ACN (99.0%), methanol (99.0%) from Fisher Chemical (Fischer Scientific, Belgium), leucine enkephalin acetate salt hydrate (95.0%), deferoxamine Mesylate (92.5%), lysine-containing (Ac-RFAAKAA-COOH, 95.5%) and cysteine-containing (Ac-RFAACAA-COOH, 96.05%) heptapeptides were purchased from Peptide 2.0 Inc (Chantilly, VA). All other chemicals were purchased from Sigma Aldrich, Singapore. 1,4-dithiothreitol (DTT) (98.0%), monobasic sodium phosphate (99.0%), dibasic sodium phosphate (98.5%), ammonium acetate (98.0%), cinnamaldehyde (98.0%), 2,4 dinitro-1-chloro benzene (DNCB) (97.0%), ethyl acrylate (99.0%), glutaraldehyde (50 wt % in H_2O), lactic acid (97.5%), lactose (98.0%), methyl salicylate (> 99.0%), 3-methylcatechol (98.0%) were purchased from Sigma-Aldrich (Singapore).

Instrumentation

NMR spectra were recorded on a Bruker Ultrashield 300MHz system, Bruker Ultrashield Plus 400MHz system and Bruker Ultrashield 500MHz system, at room temperature with DMSO-d_6 as the solvent and TMS as the internal standard. ^{1}H and ^{13}C chemical shifts are reported in ppm downfield of tetramethylsilane with reference to the residue solvent peak. Coupling constant (J) were reported in Hertz (Hz) and signal couplings are reported using the following abbreviations: s, singlet; d, doublet; t, triplet; m, multiplet; p, pentet; q, quartet; br, broad resonance. High

DOI: 10.1039/C9NJ03362A

resolution mass spectra (HRMS) were obtained on a Finnigan/MAT 95XL-T spectrometer system equipped with electrospray ionization (ESI) and, or Atmospheric Pressure Chemical Ionization (APCI) mode.

4,4'-dinitrodiphenylamine

Solid sodium hydroxide (1.65 g, 41 mmol) was added to a DMSO solution of *p*-nitroaniline (1.4 g, 10 mmol). To the greenish brown mixture, nitrobenzene (3.8 mL, 37 mmol) was added dropwise and the mixture was stirred at 80°C temperature for 12 hours with a slow stream of air bubbling through. After complete consumption of nitrobenzene, as evident from TLC analysis, the reaction mixture was cooled to room temperature and was added dropwise to water with vigorous stirring, which resulted in a yellow precipitate. The precipitate was collected by filtration, washed thoroughly with water to remove traces of NaOH and then with hexane to remove excess nitrobenzene. The precipitate was dissolved in minimum amount of DCM/acetone mixture and added dropwise to hexane with vigorous stirring. Yellow precipitate was filtered, washed with hexane and dried under suction to provide 2 g of pure product.

Yield: 2.0 g (76.6%). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 9.98 (s, 1H), 8.21 (d, *J* = 9 Hz, 4H), 7.36 (d, *J* = 9 Hz, 4H). ^{13}C NMR (125 MHz, DMSO-*d6*): δ 147.6, 140.5, 125.8, 117.1.

General method for the synthesis of imines of 4,4'-diaminodiphenylamine (2-5)

General procedure. Substituted aromatic aldehyde (1.1 mmol) and *p*-toluene sulfonic acid (5 mol %) were added to an ethanolic suspension of 4,4'-diaminodiphenylamine (1 mmol). Resulting mixture was stirred at room temperature overnight. Resulting crystalline precipitate was filtered and washed with a 1:1 mixture of ethanol and diethyl ether and then finally with diethyl ether. Drying under suction afforded the desired product as crystalline solid. Pure imine was obtained by the recrystallization from a mixture of dichloromethane and hexane.

4-[(*E*)-[4-[4-[(*E*)-(4-hydroxyphenyl)methyleneamino]anilino] phenyl]iminomethyl] phenol (2)

Using the reagents in **Scheme 1** and general procedure A, the title compound, was isolated as a yellow-orange crystalline powder.

Yield: 0.3 g (75%). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.03 (s, 2H), 8.51 (s, 2H), 8.31 (s, 1H), 7.75 (d, *J* = 8 Hz, 4H), 7.25 (d, *J* = 6.5 Hz, 4H), 7.11 (d, *J* = 7 Hz, 4H), 6.88 (d, *J* = 8 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 160.6, 157.2, 144.2, 142.0, 130.7, 128.4, 122.6, 117.7, 116.1.

4-[(*E*)-(4-nitrophenyl)methyleneamino]-*N*-[4-[(*E*)-(4-nitrophenyl) methyleneamino] phenyl] aniline (3)

Using the reagents in **Scheme 1** and general procedure A, the title compound was isolated as a green powder.

Yield: 0.44 g (95%). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.87 (s, 2H), 8.71 (s, 1H), 8.36 (d, *J* = 8 Hz, 4H), 8.16 (d, *J* = 8 Hz, 4H), 7.42 (d, *J* = 8 Hz, 4H), 7.20 (d, *J* = 8 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 155.1,148.8, 143.1, 142.8, 142.7, 129.6, 124.5, 123.6, 117.8.

4-[(*E*)-(4-methoxyphenyl)methyleneamino]-*N*-[4-[(*E*)-(4-methoxy phenyl) methylene-amino]phenyl]aniline (4)

Using the reagents in **Scheme 1** and general procedure A, the title compound, was isolated as a golden yellow crystalline powder.

Yield: 0.36 g (84.0%). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.57 (s, 2H,), 8.34 (s, 1H), 7.86 (d, *J* = 8 Hz, 4H), 7.24 (d, *J* = 8 Hz, 4H), 7.12 (d, *J* = 8 Hz, 4H), 7.06 (d, *J* = 8 Hz, 4H), 3.83 (s, 6H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 161.4, 156.4, 143.5, 141.6, 129.9, 129.4, 122.2, 117.2, 114.2, 55.3.

4-[(*E*)-p-tolylmethyleneamino]-*N*-[4-[(*E*)-p-tolylmethylene amino] phenyl]aniline (5)

Using the reagents in **Scheme 1** and general procedure A, the title compound was isolated as a golden yellow crystalline powder.

Yield: 0.32 g (80%). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.62 (s, 2H), 8.40 (s, 1H), 7.81 (d, 4H, *J* = 8 Hz), 7.32 (d, 4H, *J* = 8 Hz), 7.28 (d, 4H, *J* = 8 Hz), 7.13 (d, 4H, *J* = 8 Hz), 2.38 (s, 6H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 157.3, 143.8, 142.3, 142.3, 134.5, 129.8, 128.8, 122.8, 117.5,21.6.

Synthesis of symmetrically amine capped oligoanilines (1). 4,4'-Dinitrodiphenylamine (1) (PPD 1) (1 g, 3.86 mmol) was suspended in 20 mL of concentrated hydrochloric acid under nitrogen atmosphere. Fine tin powder (2.46 g, 20.72 mmol) was added portion wise to the reaction mixture. After complete addition of tin, the reaction mixture was refluxed for 18 h. The reaction mixture was cooled to room temperature and diluted with 50 mL of water. The pH of the mixture was set at 12 by dropwise addition of 20% aqueous NaOH solution. Greyish white precipitate formed and was filtered and washed with water to get rid of traces of NaOH. After prolonged drying the product was collected as greyish white powder. Further, this compound was dissolved in 2 mL of methanol, to which 0.5 mL of conc. HCl was added dropwise slowly over 20 min and stirred at 0 °C for 6 h. The title product was precipitated out as hydrochloride salt. The spectral data is consistent with literature data.[19] Yield: 0.6 g (78.1%); Grey powder, decompose at 220.5°C (±0.1). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 6.74 (s, 1H), 6.66 (d, *J* = 6.8 Hz, 4H), 6.47 (d, *J* = 8.8 Hz, 4H), 4.47 (s, 4H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 141.4, 135.6, 118.5, 1s15.0. HRMS (ESI): calcd for $C_{12}H_{14}N_3$ $[M+H]^+$: 200.1182, found: 200.1185.

Synthesis of symmetrically aryl capped oligoanilines (6-9).

General synthetic method for the reduction of imines of 4,4'-diaminodiphenylamine (6-9). The imine (1 mmol) was dissolved in mixture of 12 mL of dry THF/MeOH in a two-necked round bottom flask under nitrogen atmosphere. To the solution excess sodium borohydride (4 mmol) was added and the mixture was stirred at room temperature for 24 h. Excess water was added to quench the reaction as well as to quench any unreacted sodium borohydride. THF was removed under vacuum and the residue was extracted with dichloromethane. The DCM extract was washed thrice with water and then with brine solution. After drying over anhydrous Na2SO4 and removal of solvent under vacuo afforded the desired amines as crystalline solid.

4,4'-(((azanediylbis(4,1 phenylene))bis(azanediyl))Bis(methylene)) diphenol (6). The title compound was synthesized from the corresponding imine using general procedure. Yield: 0.27 g (67%): Red powder of mp 145.4-146.4°C (±0.1-0.2). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.21 (s, 2H), 7.15 (d, *J* = 8 Hz, 2H), 6.80 (s, 1H), 6.73-6.67 (m, 9H), 6.47 (d, *J* = 8.8 Hz, 4H), 5.45 (s, 2H), 4.05 (d, *J* = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 156.0, 142.3, 135.4, 130.6, 128.5, 122.1, 118.3, 114.9, 113.4, 47.0. HRMS (ESI): calcd for $C_{26}H_{25}N_3O_2$ [M+H]$^+$: 411.1941 found: 411.1931.

N^1-(4-nitrobenzyl)-N4-(4-((4-nitrobenzyl) amino)phenyl) benzene-1,4-diamine (7). Yield: 0.37 g (82%). Reddish brown powder of mp 153.1-155.1°C (±0.1-0.3). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.18 (d, *J* = 8.8 Hz, 4H), 7.61 (d, *J* = 8.8 Hz, 4H), 6.87 (s, 1H), 6.67 (d, *J* = 8.8 Hz, 4H), 6.43 (d, *J* = 8.8 Hz, 4H), 5.91 (s, 2H), 4.34 (s, 4H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 149.6, 146.3, 141.5, 135.7, 128.1, 123.4, 118.4, 113.4, 46.7. HRMS (ESI): calcd for $C_{26}H_{23}N_5O_4$ [M+H]$^+$: 469.1745, found: 469.1747.

***N*^{1}-(4-methoxybenzyl)-*N*^{4}-(4-((4-methoxybenzyl)amino) phenyl)benzene-1,4-diamine (8).** Yield: 0.38 g (88%). Golden brown powder of mp 160.0-162.0°C (±0.4-0.5). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.27 (d, *J* = 8.8 Hz, 4H), 6.87 (d, *J* = 8.8 Hz, 4H), 6.81 (s, 1H), 6.67 (br, 4H), 6.47 (d, *J* = 7.6 Hz, 4H), 5.54 (s, 2H), 4.11 (s, 4H), 3.72 (s, 6H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 158.0, 142.2, 135.5, 132.5, 128.4, 118.3, 113.6, 113.4, 55.0, 46.8. HRMS (ESI): calcd for $C_{28}H_{29}N_3O_2$ [M+H]$^+$: 439.2254,found: 439.2253.

***N*^{4}-(*4*-tolylmethyl)-*N*^{1}-[4-(p-tolylmethylamino) phenyl] benzene-1,4-diamine (9).** Yield: 0.36 g (90%).: Reddish brown powder of mp 155.5-157.5°C (±0.2-0.3). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.24 (d, *J* = 8.0 Hz, 4H), 7.11 (d, *J* =8.0 Hz, 4H), 6.81 (s, 1H), 6.67 (br, 4H), 6.46 (d, *J* = 7.2 Hz, 4H), 5.59 (s, 2H), 4.14 (s, 4H), 2.27 (s, 6H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 144.3, 137.4, 135.5, 135.4, 128.7, 127.2, 122.2, 113.1, 46.7, 20.6. HRMS (ESI): calcd for $C_{28}H_{29}N_3$ [M+H]$^+$: 407.2356, found: 407.2363.

Synthesis of *C*-substituted PPD derivatives (12-13 &15).

2-hexyloxy-4-nitroaniline (10)

2-Amino-5-nitrophenol (3.08 g, 20 mmol) was dissolved in 10 mL of DMF kept under an inert atmosphere and 1-bromohexane (2.87 ml, 20.5 mmol) and K_2CO_3 (2.83 g, 20.5 mmol) were added to the solution. The resulting red mixture was refluxed for 18 hours, after which the resulting dark brown mixture was added dropwise to saturated $NaHCO_3$ solution with vigorous stirring. After 30 minutes, the mixture was extracted with DCM and the organic layer was washed 3 times with $NaHCO_3$ solution, 3 times with saturated LiCl solution and finally with brine solution. After drying over anhydrous Na_2SO_4, the solvent was removed under reduced pressure and the resulting mixture oil dried *in vacuo*. The oily mixture was then purified by gradient column chromatography with ethyl acetate and hexane mixture as eluent. The compound was eluted with 50% ethyl acetate and hexane mixture and solvent evaporation afforded the product as a bright yellow solid.

Yield: 3.2 g (67.3%). ^{1}H NMR (300 MHz, DMSO-*d6*) δ 7.72 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 7.55 (d, *J* = 2.4 Hz, 1H), 6.66 (d, *J* = 8.8 Hz, 1H), 6.34 (s, 2H), 4.04 (t, *J* = 6.4 Hz, 2H), 1.75 (p, *J* = 7 Hz, 2H), 1.52-1.39 (m, 2H), 1.31 (m, 4H), 0.88 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, DMSO) δ 146.4, 144.2, 136.0, 119.9, 111.2, 106.7, 68.6, 31.4, 28.8, 25.4, 22.4, 14.2.

2-(2-ethylhexyloxy)-4-nitroaniline (11)

2-amino-5-nitrophenol (1.54 g, 10 mmol) was dissolved in 20 mL of DMF kept under an inert atmosphere and 1.39 g (10 mmol) was added to the stirring solution. After five minutes of stirring at room temperature, 1-bromo-2-ethylhexane (2.3 ml, 12.93 mmol) was added. Resulting red mixture was refluxed for 18 hours. The dark brown mixture was added dropwise to saturated $NaHCO_3$ solution with vigorous stirring. After 30 minutes the mixture was extracted with DCM and the organic layer was washed 3 times with $NaHCO_3$ solution, 3 times with saturated LiCl solution and finally with brine solution. After drying over anhydrous Na_2SO_4, solvent was removed under reduced pressure and dried under vacuo. The title compound was isolated as yellowish-green viscous liquid.

Yield: 0.8g (52%). ^{1}H NMR (300 MHz, DMSO-*d6*) δ 7.72 (dd, *J* = 8.8 Hz, 2.2 Hz, 1H), 7.57 (d, *J* = 2.3 Hz, 1H), 6.67 (d, *J* = 8.8 Hz, 1H), 6.30 (s, 2H), 3.94 (d, *J* = 5.3 Hz, 2H), 1.74 (dt, *J* = 12.0 Hz, 5.9 Hz, 1H), 1.53-1.38 (m, 4H), 1.33-1.26 (m, 4H), 0.92-0.85 (m, 6H).

2-hexyloxy-1,4-diaminobenzene (12). Excess tin(II) chloride (7.14 g, 37.6 mmol) was added to an ethanolic solution of 1.31 g (5.5 mmol) of 2-hexyloxy-4-nitroaniline under inert atmosphere. Concentrated HCl (2.5 ml) was added and the mixture was refluxed for 24 hours. The resulting off white cloudy mixture was poured into ice-water mixture and the pH of the mixture was set at 12 with 20% aqueous NaOH solution. The mixture was extracted with ethyl acetate and the organic layer was quickly washed with water and finally with brine solution. After solvent evaporation, the crude residue was purified by column chromatography eluting with ethylacetate and petroleum ether (3:7) to give a purple residue. Further, this residue was dissolved in 2 mL of methanol, to which 0.5 mL of conc. HCl was added dropwise slowly over 20 min and stirred at 0 °C for 6 h. The title product was precipitated out as hydrochloride salt.

Yield: 0.96 g (84%).: purple powder decompose at 204.2°C (±0.2). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 6.38 (d, *J* = 8.0 Hz, 1H), 6.18 (d, *J* = 2.0 Hz, 1H), 5.98 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 4.04 (br, 4H), 3.83 (t, *J* = 6.4 Hz, 2H), 1.73 – 1.66 (m, 2H), 1.44 – 1.40 (m, 2H), 1.32-1.30 (m, 4H), 0.88 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 146.7, 139.9, 127.6, 115.4, 106.6, 100.4, 67.4, 31.0, 28.9, 25.2, 22.1, 13.9. HRMS (ESI): calcd for $C_{12}H_{21}N_2O$ [M+H]$^+$: 209.1648, found: 209.1650.

2-((2-ethylhexyl)oxy)benzene-1,4-diamine (13). The title product was synthesized same manner as the procedure described for 2-hexyloxy-1,4-diaminobenzene (12). Yield: 0.6 g (82%).: pale purple powder of melting point 225-226°C (±0.2-0.3). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 6.38 (d, *J* = 8.0 Hz,1H), 6.20 (d, *J* = 2.4Hz, 1H), 5.98 (dd, *J* = 8.0 Hz, 2.0 Hz, 1H), 4.04 (s, 4H), 3.73 (d, *J* = 5.6 Hz, 2H), 1.70-1.65 (m, 2H), 1.47-1.39 (m, 4H), 1.38 -1.29 (m, 4H), 0.88 (t, *J* = 6.0 Hz,

6H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 146.9, 139.9, 127.5, 115.3, 106.5, 100.2, 69.8, 30.0, 28.5, 23.4, 22.5, 13.9, 10.9. HRMS (ESI): calcd for $C_{14}H_{25}N_2O$ $[M+H]^+$: 237.1961, found: 237.1958.

4-Amino-(3-hexyloxy)-4′-nitrodiphenylamine (14)

Triethyl amine (0.635 ml, 4.6mmol, 1.5 equivalent) was added to a DMSO (7 mL) solution of the equimolar mixture of 1-Fluoro-4-nitrobenzene (0.323 ml, 3.04 mmol) and 2-hexyloxy-1,4-diaminobenzene (0.63 g, 3.04 mmol). After 24 hours of stirring at 90°C, reaction mixture was cooled and poured into water to induce precipitation. The sticky precipitate was extracted with ethyl acetate and the organic layer was washed thoroughly with water and brine solution. Evaporation of solvent afforded the crude product as red liquid which was purified by silica gel column chromatography.

Yield: 0.89 g (89%).^{1}H NMR (300 MHz, DMSO-*d6*) δ 8.94 (s, 1H), 8.00 (d, *J* = 9.2, 2H), 6.81 (d, *J* = 9.3, 2H), 6.71 – 6.64 (m, 2H), 6.59 (dd, *J* = 8.3, 1.9, 1H), 4.66 (s, 2H), 3.92 (t, *J* = 6.4, 2H), 1.77 – 1.67 (m, 2H), 1.42 (dd, *J* = 13.2, 6.0, 2H), 1.34 – 1.26 (m, 4H), 0.87 (t, *J* = 6.8, 3H). ^{13}C NMR (75 MHz, DMSO-*d6*) δ 153.3, 146.3, 136.8, 135.9, 128.8, 126.6, 116.4, 114.3, 112.3, 108.3, 68.2, 31.4, 29.1, 25.6, 22.4, 14.2.

4-Amino-(3-hexyloxy)-4′-aminodiphenylamine (15). The title product was synthesized by same manner as the procedure described for 2-hexyloxy-1,4-diaminobenzene (12). Yield: 0.85 g (85%).: dark green powder decompose at 182.5°C (±0.5). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 6.66 (d,8.0 Hz 1H), 6.61 (d, *J* = 8.8 Hz, 2H), 6.43 (d, *J* = 8.4 Hz, 2H), 6.27 (d, *J* = 2.4, 1H), 4.58 (s, 2H), 4.45 (s, 2H), 3.85 (t, *J* = 6.4 Hz, 2H), 1.70 – 1.64 (m, 2H), 1.40 – 1.33 (m, 2H), 1.28-1.23 (m, 4H), 0.87 (t, *J* = 4.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 149.8, 143.6, 131.6, 125.1, 123.9, 122.9, 118.5, 117.3, 115.4, 108.1, 68.5, 31.0, 28.4, 25.0, 22.0, 13.9. HRMS (ESI): calcd for $C_{18}H_{26}N_3O$ $[M+H]^+$: 300.2070, found: 300.2073.

Synthesis of *C,N*-substituted PPD derivatives (17).

(*N*^{1}-hexyl)(2-hexyloxy)-4-nitroaniline (16)

The title compound was obtained by analogy to the process to make 2-hexyloxy-4-nitroaniline (**10**) above, except that 41 mmol of 1-bromohexane and K_2CO_3 were used.

The compound was isolated from the reaction mixture by elution with a 20% ethyl acetate and hexane mixture. A bright yellow viscous liquid was obtained upon evaporation of the effluents.

Yield: 4.90 g (76%). ^{1}H NMR (300 MHz, DMSO) δ 7.81 (dd, *J* = 9.0 Hz, 2.4 Hz, 1H), 7.53 (d, *J* = 2.5 Hz, 1H), 6.61 (d, *J* = 9.0 Hz, 1H), 6.35 (t, *J* = 5.4 Hz, 1H), 4.07 (t, *J* = 6.4 Hz, 2H), 3.23 (dd, *J* = 13.6 Hz, 6.6 Hz, 2H), 1.82 – 1.70 (m, 2H), 1.54 (m, 2H), 1.44 (m, 2H), 1.38-1.23 (m, 10H), 0.87 (q, *J* = 6.8 Hz, 6H). ^{13}C NMR (75 MHz, DMSO) δ 145.3, 144.4, 135.4, 120.2, 106.8, 105.6, 68.8, 42.43, 31.3, 28.7, 28.6, 26.4, 25.5, 22.4, 14.2.

(*N*^{1}-hexyl)(2-hexyloxy)- 1,4-diaminobenzene (17). Following the method for the synthesis of 2-hexyloxy-1,4-diaminobenzene (12), reduction of (*N*^{1}-hexyl)(2-hexyloxy)-4-nitroaniline (1.05 g, 3.1 mmol) was performed using 3 g (15.8 mmol) tin(II) chloride in presence of 2 mL of conc. HCl to give title compound as black solid. Yield: 0.94 g (99.0%).: black solid of mp 161.2-162.3°C (±0.1-0.3). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 6.30 (d, *J* = 8.4 Hz, 1H), 6.21 (d, *J* = 2.4 Hz, 1H), 6.05 (dd, *J* = 8.0 Hz, 2.0 Hz, 1H), 4.28 (br, 2H), 3.84 (t, *J* = 6.4 Hz, 2H), 2.92 (t, *J* = 6.7 Hz, 2H), 1.72 - 1.65 (m, 2H), 1.52 -1.45 (m, 2H), 1.42-1.39 (m, 3H), 1.31-1.26 (m, 10H), 0.86 (q, *J* = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 146.9, 139.5, 129.5, 111.6, 106.4, 100.3, 67.6, 44.3, 31.1, 31.0, 29.0, 28.8, 26.3, 25.3, 22.1, 22.0, 13.8. HRMS (APCI): calcd for $C_{16}H_{25}N_2O_3$ $[M+H]^+$: 293.2587, found: 293.2587.

Synthesis of symmetrically amine (NH_2/NH_2) capped oligoanilines (20) and (21).

***N*^{1},*N*^{4}-bis(4-nitrophenyl)- 1,4-benzenediamine (18)**

1-Fluoro-4-nitrobenzene (2.2 mL, 20.7 mmol) and triethylamine (3 mL, 21.5 mmol) was added successively to 20 ml DMSO solution of p-phenylenediamine (1.08 g, 10 mmol) under inert atmosphere. The dark red solution was stirred at 90°C for three days, after which the mixture was cooled to room temperature and was dropwise added to 200 ml of chilled water with vigorous stirring. Dark brown precipitate was collected by filtration and washed thoroughly with distilled water. Drying under suction afforded the crude product which was purified by silica gel column chromatography using 20% acetone/DCM mixture to afford dark green solid. Alternatively, the product can be isolated by washing the crude mixture with 70% DCM/hexane mixture. The spectral data is consistent with literature data. [19]

Yield: 1.40 g (40%). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 9.31 (s, 2H), 8.10 (d, *J* = 9.3 Hz, 4H), 7.28 (s, 4H), 7.04 (d, *J* = 9.3 Hz, 4H). ^{13}C NMR (125 MHz, DMSO-*d6*): δ 150.0, 137.6, 135.6, 126.2, 122.4, 113.0.

4,4'-Bis[(4-nitrophenyl)amino]diphenylamine (19)

1-Fluoro-4-nitrobenzene (0.8 mL, 7.5 mmol) and triethylamine (1 mL, 7.2 mmol) was added successively to 7 ml DMSO solution of 4,4′-diaminodiphenylamine (0.497 g, 2.5 mmol) under inert atmosphere. The dark red solution was stirred at 90°C for three days, after which the mixture was cooled to room temperature and was dropwise added to 200 ml of chilled water with vigorous stirring. Dark brown sticky precipitate was extracted with methanol and the supernatant with DCM. Combined extract was evaporated to dryness and the black sticky residue was dissolved in DMF and triturated with a 1:1 mixture of Et_2O and hexane to remove excess 1-fluoro-4-nitrobenzene. Further repeated trituration from DCM and hexane afforded the title compound as dark brown crystalline solid. The spectral data is consistent with literature data. [19]

Yield: 0.96 g (87%). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 9.13 (s, 2H), 8.23 (s, 1H), 8.06 (d, *J* = 9.3 Hz, 4H), 7.14 (q, *J* = 8.9 Hz, 8H), 6.92 (d, *J* = 9.3 Hz, 4H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 152.0, 140.3, 137.0, 131.7, 126.2, 123.6, 117.6, 112.3.

***N*^{1},*N*^{4}-bis(4-aminophenyl)-1,4-benzenediamine (20).** This compound was synthesized by the reduction of corresponding dinitro compound *N*^{1},*N*^{4}-bis(4-nitrophenyl)- 1,4-benzenediamine (18) (0.6 g, 1.7 1 mmol) with tin powder (2 g, 16 mmol) following the method described for the synthesis of 4,4′-

diaminodiphenylamine (section 1.2) to give title product as purple oil. Further, this oil was dissolved in 2 mL of methanol, to which 0.5 mL of conc. HCl was added dropwise slowly over 20 min and stirred at 0°C for 6 h. The title product was precipitated out as hydrochloride salt. The spectral data is consistent with literature data.[19] Yield: 0.3 g (61%).: purple solid of mp >300°C. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 6.99 (s, 2H), 6.73-6.71 (m, 8H), 6.48 (d, *J* = 8.3 Hz, 4H), 4.56 (s, 4H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 142.1, 138.1, 134.5, 119.7, 117.1, 115.1. HRMS (ESI): calcd for $C_{18}H_{18}N_4$ $[M+H]^+$: 290.1526, found: 290.1518.

***N*^{1}-(4-aminophenyl)-*N*^{4}-(4-nitrophenyl)-1,4-benzenediamine (21)**

The title compound was obtained by analogy to the process to make compound **20** above, except that 4,4′-diaminodiphenylamine (0.5948 g, 2.98 mmol) was used instead of p-phenylenediamine.
Black solid. Yield: 0.89 g (65.8%). ^{1}H NMR (300 MHz, DMSO-*d6*): δ 8.98 (s, 1H), 8.01 (d, *J* = 9.1 Hz, 2H), 7.53 (s, 1H), 7.01 (d, *J* = 8.6 Hz, 2H), 6.83 (dd, *J* = 11.7 Hz, 4.9 Hz, 6H), 6.55 (d, *J* = 8.5 Hz, 2H), 4.76 (s, 2H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 152.7, 144.0, 143.8, 136.6, 131.7, 129.1, 124.3, 122.3, 114.7, 114.4, 111.9.

Synthesis of unsymmetrically aryl(Ph/NH_2) capped oligoanilines (28) and (29).

4-Amino-4′-nitrodiphenylamine (22)
Excess potassium carbonate (2.8 g, 20 mmol) was added to a DMSO (10 mL) solution of p-phenylenediamine (1.087g, 10 mmol), 1-fluoro-4-nitrobenzene (1.1 mL, 10.4 mmol) and kept under inert atmosphere. The dark red solution was stirred at 90°C for 3 days, after which the mixture was cooled to room temperature and was dropwise added to 200 mL of chilled water with vigorous stirring. Resulting red precipitate was collected by filtration. Washing of the filter cake with water and drying under suction afforded the crude product which was purified by silica gel column chromatography. The title compound was eluted with 5% ethyl acetate/DCM mixture.
Yield: 0.99 g (43%). ^{1}H NMR (300 MHz, DMSO-*d6*): δ 8.88 (s, 1H), 8.00 (d, *J* = 9.2 Hz, 2H), 6.92 (d, *J* = 8.5 Hz, 2H), 6.76 (d, *J* = 9.2 Hz, 2H), 6.61 (d, *J* = 8.5 Hz, 2H), 5.08 (s, 2H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 153.1, 146.3, 136.4, 127.8, 126.3, 124.7, 114.5, 111.7.

***N*^{1}-(4-aminophenyl)-*N*^{4}-(4-nitrophenyl)-1,4-benzenediamine (23)**
1-Fluoro-4-nitrobenzene (0.31 mL, 2.9 mmol) and triethylamine (0.8 mL, 5.74 mmol) was added successively to 7 ml DMSO solution of 4,4′-diaminodiphenylamine (0.5948 g, 2.98 mmol) under inert atmosphere. After 3 days of stirring at 90°C, reaction mixture was cooled and poured into water to induce precipitation. The dark precipitate was collected by filtration, washed with water and dried under suction. The crude product was purified by gradient column chromatography while the desired product eluted with 2% DCM/ethyl acetate mixture.
Yield: 0.89 g (65.8%). ^{1}H NMR (300 MHz, DMSO-*d6*): δ 8.98 (s, 1H), 8.01 (d, *J* = 9.1 Hz, 2H), 7.53 (s, 1H), 7.01 (d, *J* = 8.6 Hz, 2H), 6.83 (dd, *J* = 11.7 Hz, 4.9 Hz, 6H), 6.55 (d, *J* = 8.5 Hz, 2H), 4.76 (s, 2H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 152.7, 144.0, 143.8, 136.6, 131.7, 129.1, 124.3, 122.3, 114.7, 114.4, 111.9.

(*E*)-*N*^{1}-(4-methylbenzylidene)-*N*^{4}-(4-nitrophenyl)benzene-1,4 diamine (24)
p-Toluenecarbox aldehyde (0.39 mL, 3.27 mmol) and 5 mol% of p-toluenesulphonic acid was added to an ethanolic solution (10 mL) of 4-amino-4′-nitrodiphenylamine (0.682 g, 2.97 mmol) and the mixture was stirred overnight at room temperature under nitrogen atmosphere. Resulting orange precipitate was collected by filtration and washed with 20 mL of 20% ethanol/hexane mixture and finally with hexane. Drying over suction afforded pure crystalline solid.
Yield: 0.97 g (98%). ^{1}H NMR (300 MHz, DMSO-*d6*): δ 9.37 (s, 1H), 8.62 (s, 1H), 8.10 (d, *J* = 9.0 Hz, 2H), 7.83 (d, *J* = 7.9 Hz, 2H), 7.36 – 7.26 (m, 6H), 7.07 (d, *J* = 9.1 Hz, 2H), 2.38 (s, 3H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 159.1, 150.6, 146.6, 141.3, 138.1, 137.9, 133.6, 129.3, 128.5, 126.1, 122.2, 121.5, 113.4, 21.1.

***N*^{1}-[(4-imino-p-tolyl) phenyl]-*N*^{4}-(4-nitrophenyl)-1,4 benzenediamine (25)**
p-Toluenecarboxaldehyde (0.11 mL, 0.96 mmol) and 5 mol% of p-toluenesulphonic acid was added to an ethanolic solution (10 mL) of *N*^{1}-(4-aminophenyl)-*N*^{4}-(4-nitrophenyl)-1,4-benzenediamine (0.278 g, 0.87 mmol) and the mixture was stirred overnight at room temperature under nitrogen atmosphere. Resulting orange precipitate was collected by filtration and washed with 20 mL of 20% ethanol/hexane mixture and finally with hexane. Drying over suction afforded pure crystalline solid.
Yield: 0.31 g (86%). ^{1}H NMR (300 MHz, DMSO-*d6*): δ 9.13 (s, 1H), 8.61 (s, 1H), 8.31 (s, 1H), 8.06 (d, *J* = 9.2 Hz, 2H), 7.80 (d, *J* = 8.0 Hz, 2H), 7.29 (dd, *J* = 13.4 Hz, 8.4 Hz, 4H), 7.17 – 7.08 (m, 6H), 6.93 (d, *J* = 9.2 Hz, 2H), 2.37 (s, 3H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 156.6, 151.9, 143.0, 142.2, 140.7, 139.8, 137.0, 133.9, 131.9, 129.3, 128.2, 126.2, 123.5, 122.3, 118.0, 116.7, 112.4, 21.05.

4-Nitro-4′-[(4-tolylaminomethyl)phenyl]diphenylamine (26)
Excess sodium borohydride (0.25 g, 6.61 mmol) was added to a solution of the imine (0.45 g, 1.36 mmol) in a 2:1 mixture of THF and methanol. The mixture was stirred at room temperature for 24 hours before quenching with water. Volatiles were removed under reduced pressure and the solution pH was adjusted to 9 with careful addition of conc. HCl and the mixture was extracted with DCM. The organic layer was washed with water and then with brine solution, finally dried over anhydrous Na_2SO_4. Solution was concentrated and pure product was precipitated with the addition of excess hexane. Precipitate was dried under vacuum to get brown powder.
Yield: 0.4 g (88%). ^{1}H NMR (300 MHz, DMSO-*d6*): δ 8.90 (s, 1H), 7.99 (d, *J* = 9.1 Hz, 2H), 7.26 (d, *J* = 7.8 Hz, 2H), 7.13 (d, *J* = 7.8 Hz, 2H), 6.95 (d, *J* = 8.5 Hz, 2H), 6.76 (d, *J* = 9.1 Hz, 2H), 6.61 (d, *J* = 8.5 Hz, 2H), 6.24 (t, *J* = 5.9 Hz, 1H), 4.22 (d, *J* = 5.9 Hz, 2H), 2.27 (s, 3H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 152.9, 146.3, 137.0, 136.4, 135.6, 128.8, 127.8, 127.1, 126.2, 124.4, 112.8, 111.7, 46.4, 20.6.

***N*^{1}-[4-(4-tolylaminomethyl)phenyl]-*N*^{4}-(4-nitrophenyl)-1,4 benzenediamine (27)**
1-Fluoro-4-nitrobenzene (0.12 mL, 1.1 mmol) and triethylamine (0.23 mL, 1.2 mmol) was added successively to 7 mL DMSO solution

of 4-amino-4′-[(4-tolylaminomethyl)phenyl]diphenylamine (**28**) (0.303 g, 1 mmol) under inert atmosphere. The dark red solution was stirred at 90°C for two days, after which the mixture was cooled to room temperature and was dropwise added to 200 ml of chilled water with vigorous stirring which afforded colloidal suspension. Addition of brine solution produced dark brown precipitate which was collected by f iltration, washed with water and dried under suction. The crude product was purified by silica gel column chromatography by elution with pure DCM. Yield: 0.11 g (26 %).

Alternatively, this compound was also synthesized by reducing the imine group of *N*^{1}-[(4-imino-p-tolyl)phenyl]-*N*^{4}-(4-nitrophenyl)-1,4-benzenediamine (**21**) (0.2 g, 0.47 mmol) with sodium borohydride (0.1 g, 2.64 mmol) in a 1:2 mixture of methanol/THF. Work up was performed following the method used for the synthesis of 4-nitro-4′-[(4-tolylaminomethyl)phenyl]diphenylamine (**22**) to isolate product as red solid.

Yield: 0.19 g (95 %). ^{1}H NMR (300 MHz, DMSO-*d6*): δ 8.98 (s, 1H), 8.01 (d, *J* = 9.1 Hz, 2H), 7.55 (s, 1H), 7.26 (d, *J* = 7.8 Hz, 2H), 7.12 (d, *J* = 7.8 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 2H), 6.90-6.80 (m, 6H), 6.56 (d, *J* = 8.6 Hz, 2H), 5.90 (t, *J* = 5.9 Hz, 1H), 4.19 (d, *J* = 5.8 Hz, 2H), 2.27 (s, 3H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 152.6, 144.2, 143.8, 137.3, 136.6, 135.4, 131.8, 129.2, 128.7, 127.1, 126.2, 124.2, 122.1, 114.5, 113.1, 111.9, 46.8, 20.6.

Synthesis of unsymmetrically aryl capped oligoanilines: 4-Amino-4′-[(4 -tolylaminomethyl)phenyl]diphenylamine (28). Tin powder (0.612 g, 5 mmol) was added to a 15 mL conc. HCl suspension of 4-nitro-4′-[(4-tolylaminomethyl)phenyl]diphenylamine (0.334 g, 1 mmol) and the mixture was refluxed for 18 hours. Upon completion of the reaction, the colourless solution was diluted with 50 mL of water and placed in an ice bath and the pH of the mixture was adjusted to 12. White precipitate appeared was filtered, quickly washed with water and dried under vacuo. Yield: 0.27 g (88%). Reddish brown powder of mp 110.6-112.6°C (±0.3-0.4). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.24 (d, *J* = 7.6 Hz, 2H), 7.11 (d, *J* = 7.6 Hz, 2H), 6.78 (s, 1H), 6.68 - 6.64 (m, 5H), 6.47 - 6.45 (m, 5H), 5.58 (s, 1H), 4.48 (s, 2H), 4.14 (d, *J* = 5.6 Hz, 2H), 2.27 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 142.1, 141.5, 137.6, 135.7, 135.4, 135.3, 128.7, 127.2, 118.6, 118.2, 115.0, 113.4, 47.1, 20.6. HRMS (ESI): calcd for $C_{20}H_{21}N_3$ [M+H]$^+$: 303.1730, found: 303.1731.

***N*^{1}-[4-(4-tolylaminomethyl)phenyl]-*N*^{4}-(4-aminophenyl)-1,4-benzenediamine (29).** To the dark red solution of nitro compound (0.505 g, 1.2 mmol) in 20 mL ethanol, 1.5 g (7.91 mmol) stannous chloride was added followed by 5 mL conc. HCl. Resulting mixture was refluxed for 18 hours. After cooling the reaction mixture was diluted with water and pH of the solution was adjusted to 10 by addition of 20% aqueous NaOH solution. The cloudy mixture was extracted with ethyl acetate and organic layer was washed with water, brine and dried over anhydrous sodium sulphate and evaporation of solvent afforded the title compound as dark green powder. Yield: 0.34 g (72%).: dark green powder of mp 143.5-145.5 °C (±0.1-0.3). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.26 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 6.87-6.75 (m, 10H), 6.51(d, *J* = 8.4 Hz, 4H), 5.67 (br, 1H), 4.59 (br, 2H), 4.17 (s, 2H), 2.27 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 142.7, 142.1, 138.1, 137.8, 137.6, 135.5, 134.4, 128.8, 127.2, 119.7, 119.4, 117.1, 116.9, 115.0, 113.4, 47.1, 20.7. HRMS (ESI): calcd for $C_{26}H_{26}N_4$, [M+H]$^+$: 394.2152, found: 394.2157.

Synthesis of Bandrowski's base. PPD (5g, 0.0462 mol) was dissolved in 150 mL of water and to which 1.5 mL of 28% ammonium hydroxide solution (pH 9.5) and 62.5 mL of 3% H_2O_2 were added. The resulting reaction mixture was maintained for 24 hours at room temperature. The insoluble products were separated by filtration to obtain a pure title compound. For further purification, the compound was quickly dissolved in hot pyridine and slow addition of water to this solution resulted in a precipitation of Bandrowski's base as black solid.[25] Yield: 2.2 g (15%).: black solid of mp 236.4-237.4°C (±0.2-0.3). ^{1}H NMR (400 MHz, DMSO-*d6*); 6.61-6.56 (m, 8H), 6.0 (s, 4H), 5.69 (s, 2H), 4.94 (br, 4H,), ^{13}C NMR (100 MHz, DMSO-*d6*); 153.3, 148.3, 144.9, 139.9, 122.3, 114.3, 90.7; HRMS (ESI): calcd for $C_{18}H_{19}N_6$ [M+H]$^+$: 319.1666, found: 319.1663.

Direct peptide reactivity assay

Incubation mixtures were formulated in glass auto sampler vials using peptide to test compound ratios of both 1:10 for cysteine peptide and 1:50 for lysine peptide.[20,26,27] Stock solutions of cysteine (Ac-RFAA**C**AA-COOH,) and lysine (Ac-RFAA**K**AA-COOH,) containing peptides was prepared fresh immediately before use. Reactivity of PPD derivatives towards the peptides was determined using 20 µM of cysteine and lysine in 0.1 M phosphate buffer (pH 7.5) and 0.1 M ammonium acetate buffer (pH 10.2) respectively, containing 10 µM deferoxamine mesylate. PPD derivatives were prepared in HPLC-grade ACN with 20 mM stock concentration. Reactions were initiated by adding 3 µL of either PPD, PPD derivatives, ME-PPD, PTD or control stock solutions for cysteine and 15 µL stock solutions for lysine peptide for a total incubation mixture volume of 300 µL. These mixtures were then placed in a temperature-controlled shaking incubator at 25°C.[28,29] At each time point of cysteine and lysine peptides (24 h), the incubation mixtures were quenched with 300 µL of 95% ACN in water mixture containing 3 µg/mL of internal standard leucine encephalin. For cysteine DPRA, 10 µL of each sample mixture was diluted in 380 µL of 2% ACN in water. Then 10 µL of 16 mM 1,4-dithiothreitol solution (freshly prepared in Milli-Q water) was added to prevent dimerization of the thiol groups and degradation of di-adducts to mono-adducts.[26] Thereafter, the samples were incubated at 45°C for 30 min, then allowed to cool to room temperature prior to analysis LC-MS/MS. For lysine DPRA, DTT solution is unnecessary and 10 µL of above quenched mixture was diluted into 390 µL 2% ACN in water mixture before the analysis. A standard calibration curve was prepared for the cysteine and the lysine peptides. Cysteine peptide standards was prepared in a solution of 20% ACN:buffer using phosphate buffer (pH 7.5) for the cysteine peptide and ammonium acetate buffer (pH 10.2) for the lysine peptide.

Analysis was performed to detect cysteine, lysine and internal standard leucine enkephalin selectively. The MRM of cysteine (m/z 750.3-120.1), lysine (m/z 389.0-129.30) and leucine encephalin (m/z 555.6-120.2) were optimized to detect analytes and IS with high selectivity and sensitivity.

Hair dyeing

Dye preparation. Hair dye formulations were prepared from PPD and the derivatives PPD 1, 6, 7, 8, 10, PTD and ME-PPD. Three types of formulations were prepared, following the methods of Shah *et al* [29] and Tucker *et al*[30], to differentiate the dyeing efficacy of PPD derivatives with or without using hydrogen peroxide. Formulation A is an aqueous solution prepared without an oxidizing agent. Formulation B contains 3% H_2O_2. Formulation C contains the coupler resorcinol and 3% H_2O_2. Derivatives were dissolved in deionized water and the pH of the dye was then adjusted to pH 9.5-10 using 20% aqueous Na_2CO_3 solution, before the addition of H_2O_2. Hair samples approximately 35 cm in length and weighing 2-5 g were used for dyeing. The dye formulations were applied to swatches of naturally black Asian hair samples bleached for 20 min. Each hair swatch was then removed from the dye preparation, air dried for 20 min, and washed with deionized water to remove any excess dye. They were then washed twice with Silkpro VitAir Series daily balance shampoo, further treated with Silkpro VitAir Series daily treatment Masque (hair conditioner) for 5 min and air dried. Hair colors were measured 1 day later as well as once a week following washing for 4 weeks and then continued every month for 6 months.

Hair color measurement. The SkinColorCatch (Delfin Technologies Ltd, Kuopio, Finland) was used to measure the CIELAB tristimulus L^*, a^* and b^* values of the dyed hair samples. L^* indicates brightness from black (L^* = 0) to white (L^* = 100), a^* is the green (negative, to -128) to red scale (positive, to +127) and b^* the blue (negative, to -128) to yellow scale (positive, to +127). L_0, a_0 and b_0 are baseline values measured from natural, untreated hair. The difference in the color tone is given by $\boldsymbol{\Delta H}$ $\sqrt{(a^* - a^0)^2 + (b^* - b^0)^2}$ and the total color difference by $\boldsymbol{\Delta E} = \sqrt{(L^* - L^0)^2 + (a^* - a^0)^2 + (b^* - b^0)^2}$.

Conflicts of interest

The hair dyes included in this article are covered by a patent (PCT/SG2018/050571) filed by the National university of Singapore in November 2018.

Acknowledgements

This work was supported by the National University of Singapore (NUS), Department of Pharmacy (AcRF Tier 1 FRC grant R-148-000-267-114), A-STAR SERC (grant number: 152 80 00046 (R-148-000-222-305)), and R-148-000-227-720.

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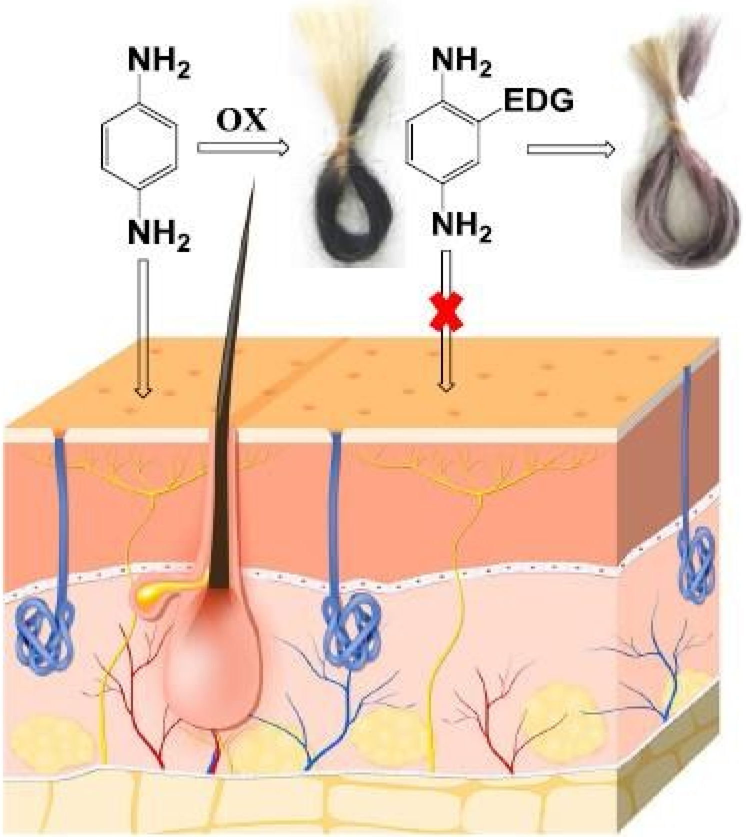

Chemical modification of the hair dye para-phenylenediamine results in colors that avoid *in situ* oxidation and toxicity