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Tunable fluorescent sensing of cysteine and homocysteine by intramolecular charge transfer

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An amphiphilic oligo *p*-phenylene derivative (**DCHO**) bearing electron-donating group (—$NH(CH_2)_2OH$) and electron-withdrawing group (—CHO) has been synthesised and characterised. The sensing characteristics of this probe (**DCHO**) for cysteine (Cys) and homocysteine (Hcy) are studied in a mixture solution of DMSO–HEPES by UV–vis and fluorescence spectra. ^{1}H NMR, MALDI-TOF and UV–vis titration experiments proved that thiazolidine and thiazinane derivatives were formed. The highly Cys/Hcy-selective fluorescence hypsochromic shift (>110 nm) can be observed due to the switching of intramolecular charge transfer, leading to potential fabrication of ratiometric fluorescent detection of Cys/Hcy.

Keywords: fluorescence sensor; amino acid; molecular recognition

Introduction

The design and construction of chemosensors with high selectivity and sensitivity for amino acids has received particular interest because amino acids play the essential biological roles in living systems. Their deficiency might cause a lot of severe human health problems (*1*) such as slow growth, hair depigmentation, muscle and fat loss, skin lesions and weakness (*2*). Specially, deficiency in homocysteine (Hcy) is more dangerous and is believed to cause Alzheimer's, cardiovascular diseases and osteoporosis (*3*). Therefore, it is very important to develop highly sensitive and selective assays for cysteine (Cys) or Hcy. In recent years, several groups have reported that Cys/Hcy analyses could be performed with high-performance liquid chromatography, capillary electrophoresis, electrochemical detection, Fourier transform infrared (FT-IR) detection and mass spectrometry identification (*4*). Moreover, there are a few chemosensors with UV–vis and fluorescence turn-on sensing and bioimaging of Cys/Hcy in mixed solvents (*5*). Although many techniques and reaction mechanisms have been developed for assaying Cys and Hcy, there is still much room for improvement in terms of rapidity, sensitivity, selectivity and cost-effectiveness to recognise those biologically important organic molecules such as amino acids.

A molecule containing conjugated electron donor (D) and electron acceptor (A) usually undergoes an intramolecular charge transfer (ICT) upon electronic excitation (*6*). The ICT and hence an elongation of the π electron conjugation occurring upon Franck–Condon exciton contributes to the absorption profile. The process of ICT fluorescence playing a key role in the biological systems such as photosynthesis was also studied based on the D–π–A compounds, which led to increasing significance (*7*). Oligo-, poly-*p*-phenylene and their derivatives are the subjects of tremendous investigations because of their interesting optical and electrochemical properties. These compounds often have high extinction coefficients, high fluorescence quantum yields and high stability against light and chemical reactions, and can be modified by various substituents, which might tune their absorption and emission spectra (*8*). Thus, the study of the compounds has attracted significant interest due to their potential applications on catalysts and optoelectronic devices (*9*). To date, many studies on recognition of analytes have been focused on poly-*p*-phenylene derivatives (*10*). In order to efficiently recognise the biologically important amino acids, we are encouraged to develop a new D–π–A probe based on oligo *p*-phenylene chromophores.

In this paper, we describe the design and construction of a class of colorimetric and fluorometric probe to specifically detect the presence of Cys/Hcy over a wide range of other amino acids and glucose. To achieve this goal, a modular molecule (**DCHO**, Scheme 1) is synthesised that is composed of oligo *p*-phenylene fluorophore which possesses interesting spectroscopic properties. Electron-donating group (—$NH(CH_2)_2OH$) and electron-withdrawing unit (—CHO) that graft into the chromophore through a covalent bond to create a D–π–A system, which makes the ICT from the donor to the acceptor, proceed upon excitation. This process might induce changes in two-channel output signals (colour change and fluorescence variation), which is convenient for practical utilisation.

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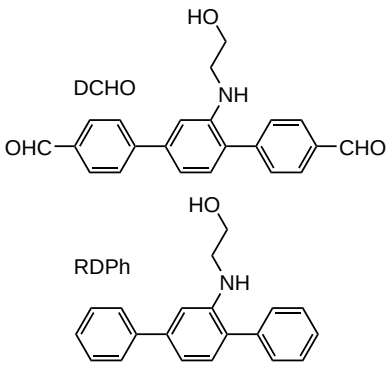
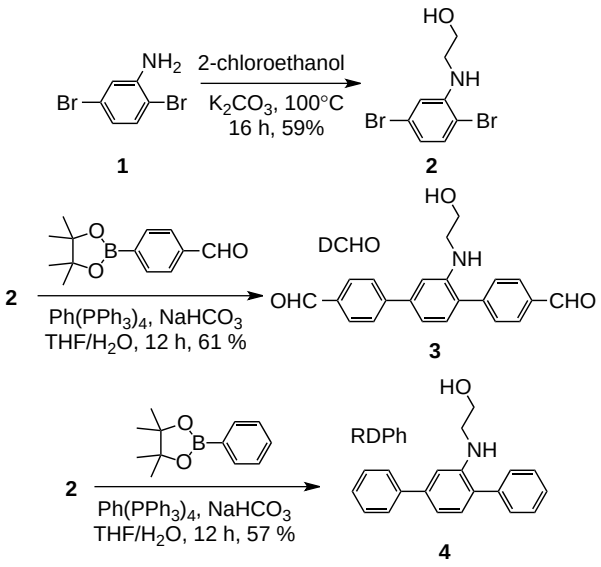


Scheme 1. Chemical structure of **DCHO** and the reference compound **RDPh**.

Results and discussion

Compound **DCHO** and the reference compound **RDPh** were readily prepared via Suzuki cross-coupling reactions of *N*-hydroxyethyl-2,5-dibromoaniline and 4-formyl-benzeneboronic acid pinacol ester or benzeneboronic acid pinacol ester, respectively, in the presence of $Pd(PPh_3)_4$ and $NaHCO_3$ in THF/H_2O solution according to the reported method (Scheme 2) (*11*). It should be noted that the hydroxyethyl group was introduced in **DCHO** to increase the hydrophile. The resulting molecules were characterised by FT-IR, ^1H NMR, ^{13}C NMR and mass spectroscopy, which were shown to be in full agreement with the structures presented.

Compounds **DCHO** and **RDPh** exhibit good solubility in CH_2Cl_2, $CHCl_3$, THF, CH_3CN, EtOH, DMF and DMSO. Typical UV–vis absorption spectra of **DCHO** and **RDPh** in different solvents at room temperature are shown in



Scheme 2. Synthesis of conjugated compounds **DCHO** and **RDPh**.

Figure 1 and Table 1. The electron-withdrawing substituent (—CHO) can induce a remarkable red shift of the absorption maximum, the origin of which can be attributed to the ICT state for a strong push–pull system. The broader absorption band of **DCHO** might be assigned to electronic transitions delocalised throughout the whole molecule (*12*). The solvatochromic shift of **DCHO** is observed due to the large difference in dipole moment of the Franck–Condon excited state and the ground state, which is induced by the formyl group (*7d*). In addition, the fluorescence spectrum of **DCHO** in DMSO exhibits pronounced red shift, indicative of an ICT characteristic of the fluorescence states. The emission maximum of **DCHO** with two formyl substituents extends from 483 nm in toluene to 565 nm in DMSO, and the solvatochromic shift becomes as large as 82 nm on change from toluene to DMSO, while the fluorescence maximum of **RDPh** ranges from 419 nm (in toluene) to 443 nm (in DMSO). The results show that the magnitude of the solvatochromism effect also depends on electron-withdrawing groups, and that **DCHO** has low dipole moments in the ground state, but much higher dipole moments in the first excited singlet state, which was better stabilised by polar solvents. It should be noted that it was difficult to measure the fluorescence quantum yields in ethanol due to the low intensity. Considering the solvatochromic effect and the strong fluorescence quenching in protonic solvent (ethanol), a mixture of DMSO and HEPES (v/v, 19:1) was chosen for the further study.

DCHO in $DMSO/H_2O$ (v/v, 19:1) solution (2.2×10^{-5} M) at room temperature exhibited absorption spectra with λ_{max} at 386 nm and other two peaks at 280 and 304 nm, respectively. The absorption maximum of **DCHO** has about a 47 nm red shift in comparison to the reference compound **RDPh**, which was attributed to the efficient ICT process from the donor nitrogen atom – the one that conjugated to oligo *p*-phenylene – to the acceptor group (Figure 2(A)). In comparison, the emission spectra of **DCHO** (2.2×10^{-5} M) in $DMSO/H_2O$ (v/v, 19:1) solution at room temperature were showed at 442 and 565 nm with excitation wavelength at 386 nm (Figure 3(A)), and the quantum yield (Φ_f) of **DCHO** in DMSO was measured as $\Phi_f = 0.02$ with quinine sulphate as standard at 25°C (Table 1). Figure 2(A) shows the UV–vis spectral changes of **DCHO** as a function of the Cys concentration in a $DMSO/H_2O$ solution (v/v, 19:1, 10 mM HEPES buffer, pH 6.7) at room temperature. The addition of Cys (0–3.2×10^{-3} M) to **DCHO** solution (2.2×10^{-5} M) led to a decrease in the intensities of the initial bands at 310 and 386 nm with the concomitant growth of intensity at 343 nm (Figure 2(A)). These results suggest that thiazolidine **DCHO-a** was formed (Scheme 3). The coordination of the Cys to **DCHO** reduced the electron-withdrawing ability of the formyl group, thus the ICT progress is not possible any more and the red shift in absorption spectra is suppressed. A similar blue shift was also found for Hcy. As shown

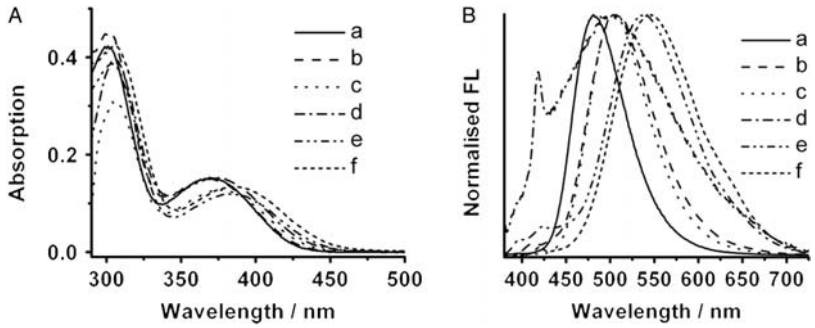


Figure 1. (A) UV–vis absorption and (B) normalised fluorescence spectra of **DCHO** (2.2×10^{-5} M) in (a) toluene, (b) $CHCl_3$, (c) THF, (d) EtOH, (e) DMF and (f) DMSO.

in Figure 2(B), upon addition of Hcy, the absorption intensity of **DCHO** at 386 nm decreased with a blue shift band at 354 nm, which is indicative of the formation of thiazinane **DCHO-b**.

Note that a fluorometric detection of Cys/Hcy is also possible for compound **DCHO**. In the fluorescence spectra, **DCHO** exhibits characteristic emission bands at 443 and 565 nm when excited at 386 nm (Figure 3(A),(B)). Upon addition of Cys/Hcy to the solution of **DCHO**, a significant decrease in the 565 nm emission band was observed with the concomitant growth of the blue-shifted band centred at 443 nm. The obvious blue shift and the resolved emission peaks make it possible to obtain a clear I_{449}/I_{565} 46-fold enhancement for Cys and I_{449}/I_{565} 220-fold enhancement for Hcy. The highly Cys/Hcy-sensitive UV–vis and fluorescence hypsochromic shift showed more obvious than those in the reported methods (*13*). These observations might also suggest that reaction of Cys/Hcy with the formyl group that was conjugated to the oligo-phenylene core suppressed the ICT progress (Scheme 3). It should be noted that the low concentration of Cys/Hcy did not affect distinctly the ICT emission due to its unstable formation of thianolidine or thiazinane derivatives from the reaction ability of **DCHO** to Cys/Hcy in the emission spectral analysis. Importantly, the large Cys/Hcy-induced blue shift in emission spectra resulted in a colour change from yellow to blue as shown in Scheme 3. This visible emission allows **DCHO-a** or **-b** to be readily distinguished by the naked eye under UV light. No obvious colour changes were observed upon addition of other amino acids or glucose.

Table 1. Photophysical properties of **DCHO** and **RDPh**.

Sample	Solvent	UV–vis (λ_{max})	PL (λ_{max})	Stokes shift (nm)	Φ_f
DCHO	Toluene	370	483	113	0.23
	$CHCl_3$	370	503	133	0.36
	THF	377	510	133	0.46
	EtOH	373	513	140	–[a]
	DMF	383	555	172	0.08
	DMSO	386	565	179	0.02
RDPh	Toluene	329	419	80	0.49
	DMSO	339	443	104	0.50

[a]It was difficult to measure this value due to the low intensity. All emission spectra were measured at each UV–vis (λ_{max}, nm). The Φ_f values for **DCHO** and **RDPh** are relative to those of quinine sulphate (0.53 in 0.1 N H_2SO_4) (*14*).

To verify specificity, the optical response experiments were investigated with other amino acids and glucose, such as Phe, Pro, Gly, Lys, glucose (Glu), Ser, Thr, Asp, Val and His. As shown in Figure 4(A), under the identical condition to Cys/Hcy, no changes were observed in the UV–vis spectra of **DCHO** (2.2×10^{-5} M) upon addition of 90 equiv. of Phe, Pro, Gly, Lys, Glu, Ser, Thr, Asp, Val and His. This might result from the specific reaction of the aldehyde group and the β- or γ-aminoalkylthio moieties. In good agreement with the changes in the absorption, the emission spectra also displayed a significant change upon addition of Cys/Hcy (Figure 4(B)). In comparison, no visible variations were observed upon addition of other amino acids or glucose. To further explore the utility of **DCHO** as a selective fluorescence probe for Cys/Hcy, the competition experiments were conducted. Figure 5 depicts the fluorescence responses of **DCHO** to the presence of 90 equiv. of Cys or Hcy and 40 equiv. of Phe, Pro, Gly, Lys, Glu, Ser, Thr, Asp, Val and His. The addition of other amino acids and glucose showed nearly no changes in emission spectra, indicative of the sensitive and selective detection of Cys/Hcy under competition from other related analytes.

The formation of thiazolidine **DCHO-a** and thiazinane **DCHO-b** was demonstrated by ^{1}H NMR spectroscopic studies in DMSO-d_6. Figure 6 shows the ^{1}H NMR spectra in the absence and presence of Cys. The concentration of **DCHO** (2.9×10^{-2} M) was kept constant, and the aldehyde signal at 10.05 ppm disappeared after 30 min upon addition of 10 equiv. Cys to **DCHO**. Meanwhile, the new peaks centred at 5.74 and 5.54 ppm (for Cys) and 5.32 ppm (for Hcy) (Figure S1 of the Supplementary Material) appeared, which might be assigned to the methine protons of the thiazolidine and thiazinane diastereomer, respectively. The MALDI-TOF

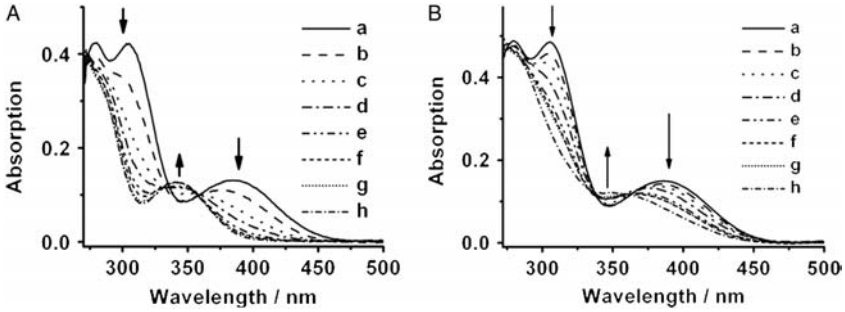


Figure 2. UV–vis absorption spectral changes of **DCHO** (2.2×10^{-5} M) upon addition of increasing concentration (0–140 equiv.) of (A) Cys and (B) Hcy in DMSO/H_2O (v/v, 19:1): (a) 0, (b) 4×10^{-4} M, (c) 8×10^{-4} M, (d) 1.2×10^{-3} M, (e) 1.6×10^{-3} M, (f) 2×10^{-3} M, (g) 2.4×10^{-3} M, (h) 3.2×10^{-3} M; pH 6.7. Each spectrum is recorded after 30 min incubation in a 45°C water bath after Cys/Hcy addition.

mass spectrograms of **DCHO** with Cys and Hcy also showed clear dimeric complex of the peaks at 552.3 and 580.3, respectively (Figures S2 and S3 of the Supplementary Material, available online). The results also strongly suggest that the thiazolidine **DCHO-a** and thiazinane **DCHO-b** are formed through the interaction of the aldehyde group and Cys or Hcy.

Conclusion

In summary, we have developed a new class of colorimetric and fluorometric probe of **DCHO** for Cys/Hcy detection with high selectivity and sensitivity on the basis of the tuning of the ICT process. The **DCHO** molecule with *N*-hydroxyethylaniline component renders a straightforward preparation and good solubility in aqueous media. Meanwhile, a highly Cys/Hcy fluorescence enhancing in conjunction with remarkable blue-shift property can be observed. This D–π–A constitution demonstrated in this study might serve as a protocol for the future development of a multi-stage chemosensor for the possible analyte.

Experimental section

Materials and methods

Most of the chemicals were purchased from Alfa Aesar (Karlsruhe, Germany) or Aldrich Corporation (Steinheim, Germany), and used as received. Column chromatography was performed on silica gel (200–300 mesh). Melting points were taken on a TX-4 hot-stage apparatus and are uncorrected. IR spectra were measured on a Nicolet 380 instrument. UV–vis spectra were recorded on a Shimadzu UV-2550 spectrometer, and fluorescence spectra were measured on an RF5300PC spectrofluorometer. ^{1}H and ^{13}C NMR spectra were obtained on Bruker 400 spectrometers using $CDCl_3$ or DMSO-d_6 as a solvent with tetramethylsilane (TMS) as the internal standard. MALDI-TOF mass spectrometric measurements were performed on Bruker Biflex MALDI-TOF. Elemental analyses were carried out on a Carlo Erba 1106 elemental analyser.

*N-(2-Hydroxyethyl)-2,5-dibromoaniline (**2**)*

A mixture of 2,5-dibromoaniline (1.50 g, 6.0 mmol), 2-chloroethanol (36 ml) and K_2CO_3 (2.48 g, 18.0 mmol) was

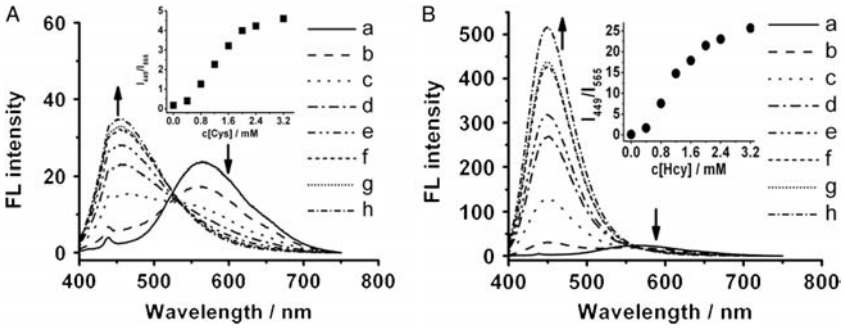
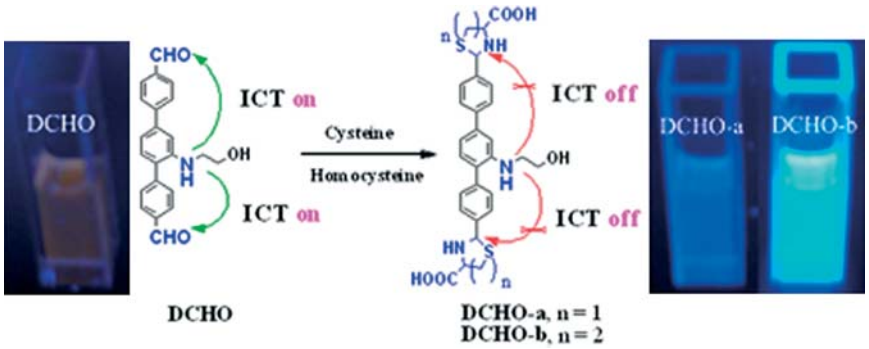


Figure 3. Fluorescence spectral changes (excitation at 386 nm) of **DCHO** (2.2×10^{-5} M) upon addition of increasing concentration (0–140 equiv.) of (A) Cys and (B) Hcy in DMSO/H_2O (v/v, 19:1): (a) 0, (b) 4×10^{-4} M, (c) 8×10^{-4} M, (d) 1.2×10^{-3} M, (e) 1.6×10^{-3} M, (f) 2×10^{-3} M, (g) 2.4×10^{-3} M, (h) 3.2×10^{-3} M; pH 6.7. Each spectrum is recorded after 30 min incubation in a 45°C water bath after Cys/Hcy addition. The inset shows the curves I_{449}/I_{565} with the addition of Cys and Hcy (colour online).



Scheme 3. Reaction of **DCHO** with Cys or Hcy to afford thiazolidine (**DCHO-a**) or thiazinane (**DCHO-b**) and the change in fluorescent colour on excitation at 365 nm using UV light at room temperature.

stirred at 100°C for 16 h under an atmosphere of nitrogen. The reaction was then cooled to ambient temperature, brine was added (100 ml) and the solution was extracted with CH_2Cl_2 (3× 50 ml). The combined organic phase was collected and dried over Na_2SO_4, and filtered. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (silica gel) with petroleum–CH_2Cl_2 (v/v, 10:3) as the eluent to give white solid **2** (1.03 g, 59%).

Mp: 59–60°C; IR (KBr): 3376, 3242, 2930, 2879, 1588, 1504, 1456, 1408, 1286, 1060, 825, 782 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 298 K, TMS): $\delta = 7.25$ (d, $J = 8.0$ Hz, 1H), 6.76 (s, 1 H), 6.99 (d, $J = 8.0$ Hz, 1H), 4.69 (br, 1H), 3.86 (s, 2H), 3.32 (s, 2H), 1.89 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 298 K, TMS): 146.0, 133.4, 122.3, 120.6, 114.1, 108.4, 60.7, 45.6; MS (EI): 295 $[M]^+$.

*N-(2-Hydroxyethyl)-2,5-di(4-formylphenyl)aniline (**3**)*

A mixture of *N*-(2-hydroxyethyl)-2,5-dibromoaniline (**2**, 238 mg, 0.8 mmol), 4-formylbenzeneboronic acid pinacol ester (428 mg, 1.84 mmol), $Pd(PPh_3)_4$ (45 mg, 0.04 mmol) and $NaHCO_3$ (400 mg, 4.76 mmol) in THF/H_2O (44 ml/4 ml, v/v, 10:1) was stirred for 12 h at refluxing temperature under nitrogen. After cooling to room temperature, the mixture was filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with CH_2Cl_2–CH_3CO_2Et (v/v, 50:8) as the eluent to give white solid **3** (170 mg, 61%).

Mp: 170–171°C; IR (KBr): 3413, 2957, 2872, 2729, 1727, 1693, 1599, 1553, 1386, 1291, 1209, 1165, 1069, 834, 802, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 298 K, TMS): $\delta = 10.01$ (s, 2H), 7.98 (t, $J = 8.8$ Hz, 4H), 7.79 (d, $J = 7.6$ Hz, 2H), 7.67 (d, $J = 7.6$ Hz, 2H), 7.22 (d, $J = 7.6$ Hz, 1H), 7.08 (d, $J = 7.6$ Hz, 1H), 6.99 (s, 1H), 4.36 (br, 1H), 3.86 (t, $J = 4.0$ Hz, 2H), 3.40 (t, $J = 4.0$ Hz, 2H), 1.59 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 298 K, TMS): 192.2, 147.7, 145.8, 145.7, 141.5, 135.8, 135.7, 131.3, 130.8, 130.6, 130.2, 128.0, 127.2, 117.2, 110.3, 61.4, 46.4; MS (EI): 345 $[M]^+$; Anal. Calcd for $C_{22}H_{19}NO_3$ (345.14): C, 76.50; H, 5.54; N, 4.06. Found: C, 75.82; H, 5.69; N, 3.88.

*N-(2-Hydroxyethyl)-2,5-diphenylaniline (**4**)*

A mixture of *N*-(2-hydroxyethyl)-2,5-dibromoaniline (**2**, 100 mg, 0.33 mmol), benzeneboronic acid pinacol ester (135 mg, 0.66 mmol), $Pd(PPh_3)_4$ (15 mg, 0.01 mmol) and $NaHCO_3$ (200 mg, 2.38 mmol) in THF/H_2O (44 ml/4 ml,

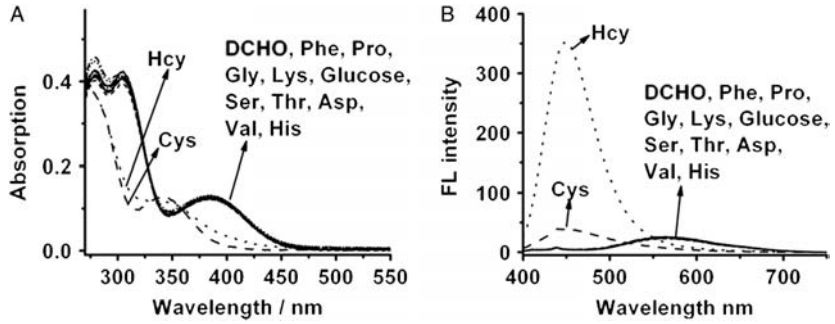


Figure 4. (A) UV–vis absorption and (B) fluorescence spectra (excitation at 386 nm) of **DCHO** (2.2×10^{-5} M) with or without amino acids, and glucose (90 equiv.) in DMSO/HEPES (v/v, 19:1); pH 6.7.

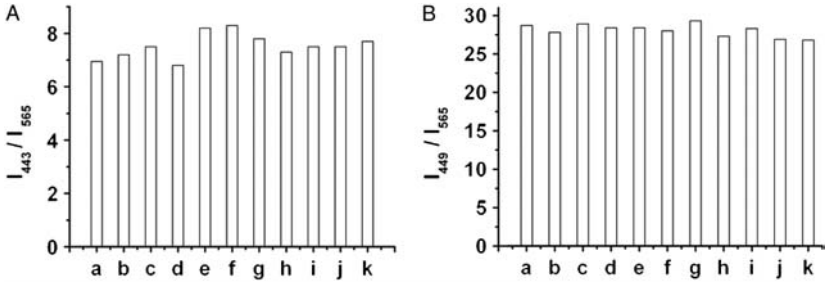


Figure 5. Fluorescence spectral changes of **DCHO** (1.1×10^{-5} M) upon addition of (A) Cys (90 equiv.), (a) Cys, (b) Cys + Phe, (c) Cys + Pro, (d) Cys + Gly, (e) Cys + Lys, (f) Cys + Glu, (g) Cys + Ser, (h) Cys + Thr, (i) Cys + Asp, (j) Cys + Val, (k) Cys + His; (B) Hcy (90 equiv.), (a) Hcy, (b) Hcy + Phe, (c) Hcy + Pro, (d) Hcy + Gly, (e) Hcy + Lys, (f) Hcy + Glu, (g) Hcy + Ser, (h) Hcy + Thr, (i) Hcy + Asp, (j) Hcy + Val, (k) Hcy + His and subsequent addition of different amino acids and glucose (40 equiv.) in DMSO/HEPES (v/v, 19:1); pH 6.7.

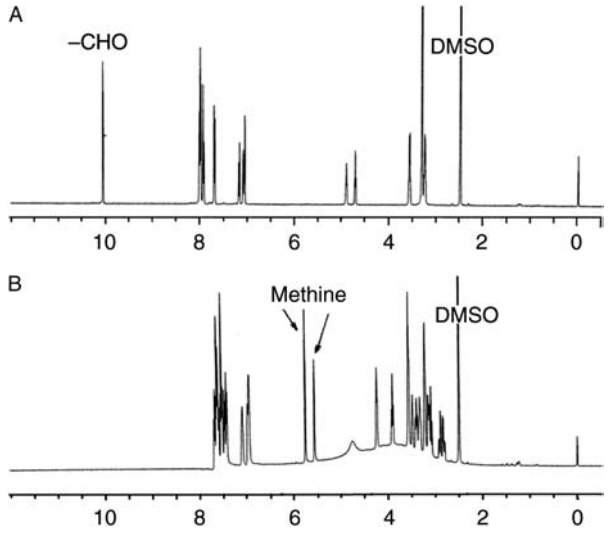


Figure 6. ^{1}H NMR spectra of **DCHO** in DMSO-d_6 in the (A) absence and (B) presence of Cys at 298 K. The concentration of **DCHO** was kept constant at 2.9×10^{-2} M.

v/v, 10:1) was stirred for 12 h at refluxing temperature under nitrogen. After cooling to room temperature, the mixture was filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with petroleum–CH_2Cl_2 (v/v, 1:2) as the eluent to give yellow solid **4** (56 mg, 57%).

Mp: 113–114°C; IR (KBr): 3405, 3254, 3025, 2928, 1606, 1557, 1485, 1415, 1257, 1041, 848, 758, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, 298 K, TMS): $\delta = 7.63$ (d, $J = 7.2$ Hz, 2H), 7.45 (t, $J = 7.2$ Hz, 6H), 7.36 (t, 2H), 7.19 (d, $J = 7.6$ Hz, 1H), 7.04 (d, $J = 7.6$ Hz, 1H), 6.97 (s, 1H), 4.41(br, 1H), 3.81 (t, $J = 4.0$ Hz, 2H), 3.38 (t, 2H), 1.61 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 298 K, TMS): 142.1, 141.8, 138.7, 131.1, 129.4, 129.2, 128.9, 127.7, 127.5, 127.3, 61.5, 47.3; MS (EI): 289 $[M]^+$; Anal. Calcd for $C_{20}H_{19}NO$ (289.15); C, 83.01; H, 6.62; N, 4.84. Found: C, 83.01; H, 6.75; N, 4.63.

Acknowledgements

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