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Structural characteristics and optical properties of a series of solvatochromic fluorescent dyes displaying long-wavelength emission

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ABSTRACT

A series of novel, fluorescent, solvatochromic dyes (KSD-series) carrying a 4-dimethylaminophenyl moiety as π-electron donor and an acetyl moiety as π-electron acceptor, conjugated with a five-membered aromatic monoheterocyclic π-linker, were synthesized. In the cases of dyes having a pyrrole, (KSD-1) furan (KSD-2) and thiophene (KSD-3) heterocycle as π-linker, respectively, the plot of Stokes shifts in different solvents as a function of $E_T(30)$ solvent polarity value showed good linear correlation. The fluorescence of KSD-4, a π-conjugation extended derivative of KSD-3, varied from blue (491 nm) in toluene to orange (616 nm) in DMSO; KSD-4C, a carboxyl group modified derivative, displayed longer wavelength emission (619 nm) and larger Stokes shift (229 nm, 9486 cm^{-1}) than conventional dyes in methanol solution.

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1. Introduction

Fluorescence-related techniques are among the most important analytical techniques because of their non-invasive nature, their high intrinsic sensitivity and their utility for multi-analyte sensing [1]. Until now, many efforts have been made in order to develop fluorescent indicator dyes useful for bioimaging, for the detection and quantification of trace analytes (e.g. ions), and for the characterization of biological structures, among others [2].

Solvatochromic fluorescent dyes, which show matrix polarity sensitive emission wavelengths and quantum yields, are valuable tools for chemical and biochemical research (e.g. the investigation of the polarity of biological and organized media, the monitoring of biological processes) [3,4]. In the past decades, many fluorescent solvatochromic dyes have been developed and applied for these (bio)analytical purposes. *N*-Arylaminonaphthalene sulfonates (ANS) for example have been used to label macromolecules in order to estimate the polarity of the binding site for ANS [5] and to visualize hydrophobic regions of biological systems [6]. Prodan (6-propionyl-2-dimethylaminonaphthalene) and its derivatives have been used to label biomolecules [7–10]. They are useful for studies of cell membranes, for example, for imaging the phase state of membranes [11,12]. One of its derivatives, Acrylodan (6-acryloyl-2-dimethylaminonaphthalene), has been used to detect fatty acids, which are a significant source of energy. Dansyl (5-(dimethylamino)naphthalene-1-sulfonyl-) probes have been used to study the binding of organic molecules to cyclodextrins [13]. In general, solvatochromic fluorescent dyes are required to investigate and to characterize microenvironments in a variety of research fields.

However, most of the above mentioned fluorescent solvatochromic probes have drawbacks including short absorption and emission wavelengths possibly causing damage to biological samples, high background noise due to autofluorescence, low extinction coefficients and quantum yields, and small Stokes shifts.

In this context, Diwu and co-workers have developed the *Dapoxyl* dyes (*N*,*N*-dimethyl-4-(2-phenyl-5-oxazolyl)-benzenamine), possessing relatively large extinction coefficients, high quantum yields, and large Stokes shifts [14,15]. The fluorescence emission spectra of the *Dapoxyl* fluorophores are highly sensitive towards polarity: the fluorescence quantum yields show a good linear correlation with the solvent polarity quantified on the $E_T(30)$ scale, and the Stokes shift linearly increases with the solvent polarity except for protic solvents such as ethanol and methanol. A variety of *Dapoxyl* derivatives have become commercially available as acidotropic probes for lysosomes and other acidic organelles [2]. More recently, Min and co-workers

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have applied a combinatorial synthesis approach to explore *Dapoxyl*-derived dyes as fluorescent probe candidates that may show larger fluorescence spectral changes to environment polarity for the application as a human serum albumin (HSA) probe [15].

Dapoxyl dyes have a typical push–pull π-donor (D), π-acceptor (A) type molecular structure. They are synthesized via amide-coupling of electron donor substituted aminoacetophenone derivatives and electron acceptor substituted benzoyl chloride derivatives, followed by cyclization to form the central oxazole ring that connects the donor and acceptor parts of the molecule to a fully π-conjugated chromophore. While a variety of electron donor and acceptor substitutions have been investigated in order to search for superior fluorescent solvatochromic dyes, bridging hetero rings other than oxazole have to the best of our knowledge never been evaluated.

It is known that a thiophene unit can offer better effective conjugation than a benzene unit in π-conjugated donor–acceptor compounds (D–π–A), since it has a lower localization energy than benzene [16]. The photochemistry of the thiophene unit has been investigated in a number of thiophene-linked push–pull donor–acceptor chromophoric systems [17–20]. Notably, it has been reported in the literature that a thiophene-linked D–π–A derivative with arylamino (donor) and cyano (acceptor) substitution displays large solvatochromic shifts in the fluorescence emission spectra [18].

For this reason, we were interested in investigating the effect of the central hetero ring system, acting as π-linker between the donor and the acceptor, on the solvatochromic emission spectral shifts and quantum yields of D–π–A-chromophore systems. This approach might be an alternative way for the development of potential candidates with increased polarity sensitivity, longer emission wavelengths, and higher quantum yields than the *Dapoxyl*-derived dyes. In this paper, we report on the design, synthesis and optical characteristics of a series of new fluorescent solvatochromic dyes named KSD-*x*, wherein the electron-donating and accepting groups are π-linked by the pyrrole (KSD-1), furan (KSD-2), or thiophene (KSD-3, KSD-4 and KSD-4C) five-membered monoheterocyclic units.

2. Results and discussion

2.1. Molecular design and synthesis of KSD-1–3

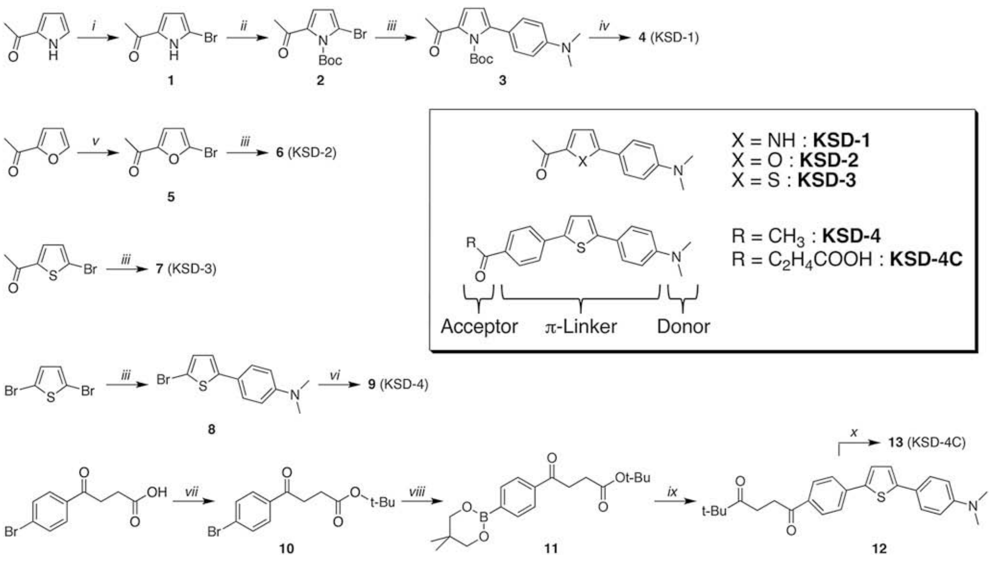
Firstly, the effect of the five-membered aromatic mono-heterocyclic unit, acting as the π-linker at the center of the D–π–A type chromophore structure, towards its fluorescence properties was investigated. In this investigation, the 4-dimethylaminophenyl moiety was chosen as the electron-donating unit because it is a well-known frequently used and relatively strong π-electron donor. The acetyl moiety was chosen as the π-electron acceptor because of its good chemical stability and because of the commercial availability of various useful molecular building blocks. In the newly designed KSD-1–3, the 4-dimethylaminophenyl donor and the acetyl acceptor were substituted at the 2- and 5-positions of pyrrole, furan and thiophene rings, respectively. The molecular structures of the compounds are shown in Scheme 1.

The synthesis of the newly designed compounds was readily achieved in good yields by Suzuki coupling of 4-(dimethylamino)-phenylboronic acid with the corresponding 2-acetyl-5-bromo-heterocycle, in one to four reaction steps starting from commercially available materials. This synthesis scheme facilitated the study of a variety of combinations of donors, acceptors and π-conjugating moieties in the D–π–A type chromophores.

2.2. Spectral properties of KSD-1–3

The wavelengths of maximum absorption and emission, Stokes shifts, and spectral bandwidths of the KSD-1–3 chromophores in different organic solvents of a wide polarity range are listed in Table 1.

For all of the dyes, no significant solvent polarity dependent spectral shifts in the absorption spectra were observed. This indicates that the chromophores in their ground states are not significantly stabilized by the coordination of solvent molecules, due to a relatively small dipole moment. On the other hand, strong solvatochromic red shifts of the emission spectra were observed with increasing solvent polarity for all three dyes (Fig. 1a–c), indicating a solvent-induced relative stabilization of the thermally



Scheme 1. Reagents and conditions. i) NaBr, oxone, MeOH, H_2O, r.t.; ii) $(Boc)_2O$, DMAP, MeCN, r.t.; iii) *N,N*-dimethylaminophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, MeOH, heat; iv) NaOMe, THF, r.t.; v) NBS, DMF, r.t.; vi) *p*-acetylphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, MeOH, heat; vii) tBuOH, EDCI, DMAP, CH_2Cl_2, 0 °C to r.t.; viii) bis-(neopentylglycolato) diboron, $PdCl_2(dppf)_2 \cdot CH_2Cl_2$, KOAc, 1,4-dioxane, 80 °C; ix) compound **8**, $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, 80 °C; x) HCOOH, r.t. Inset: molecular structures of the KSD-*x* fluorescent solvatochromic dyes.

Table 1
Spectral properties of KSD-1–3 in solvents of different polarities.

Solvent	$E_T(30)$ (kcal/mol)	KSD-1				KSD-2				KSD-3			
		λ_{Abs}[a] (nm)	λ_{Em}[b] (nm)	$\Delta\nu$[c] (cm^{-1})	fwhm[d] (nm)	λ_{Abs} (nm)	λ_{Em} (nm)	$\Delta\nu$ (cm^{-1})	fwhm (nm)	λ_{Abs} (nm)	λ_{Em} (nm)	$\Delta\nu$ (cm^{-1})	fwhm (nm)
Toluene	33.9	358	413	3708	55	368	428	3809	57	386	455	3948	57
1,4-Dioxane	36.0	352	415	4336	57	366	434	4292	56	385	466	4506	59
Ethyl acetate	38.1	352	423	4780	60	364	446	5041	60	384	479	5147	64
Chloroform	39.1	365	443	4814	57	378	465	4950	60	395	490	4908	61
Acetone	42.2	355	449	5897	65	369	474	6012	68	389	506	5928	67
DMSO	45.1	363	462	5884	67	380	499	6268	70	400	531	6182	75
Acetonitrile	45.6	355	458	6335	68	371	491	6579	71	390	519	6388	71
i-Propanol	48.4	366	479	6437	73	379	512	6839	71	394	535	6689	73
Ethanol	51.9	365	495	7195	80	380	528	7362	80	395	548	7082	81
Methanol	55.4	363	509	7917	84	381	541	7762	87	396	559	7357	88

[a] λ_{Abs} = absorption maximum.
[b] λ_{Em} = emission maximum.
[c] $\Delta\nu$ = Stokes shift.
[d] fwhm = full width at half maximum.

equilibrated excited states, due to a significant difference between the dipole moments in the ground and excited states. The solvatochromic shifts observed in the emission spectra when increasing the polarity from toluene to methanol were 96 nm, 113 nm, and 104 nm for KSD-1, KSD-2, and KSD-3, respectively. These experimental results can be interpreted by an intramolecular charge transfer (ICT) from the electron-donating dimethylaminophenyl unit to the electron accepting acetyl moiety [19]. Additionally, a characteristic broadening of the spectral bandwidths was observed.

When comparing the fluorescence properties of the three chromophores KSD-1–3, KSD-3 showed the longest emission wavelengths in all investigated solvents, as well as the largest Stokes shifts. This is likely resulting from the thiophene unit offering good effective conjugation in π-linked donor–acceptor compounds, as discussed above. It was also reported that the thiophene ring does not only increase the conjugated length between the donor and the acceptor but also acts as an auxiliary electron donor in D–π–A structures [21]. It is assumed that this auxiliary electron-donating ability contributed to the enhanced spectral properties and the increased fluorescent solvatochromism of KSD-3, compared to the pyrrole and furan derivatives.

The stokes shifts of each of the KSD-1–3 dyes in the different solvents were plotted as a function of the $E_T(30)$ values, known as a reliable and widely used scale of solvent polarity (Fig. 2) [22]. A good linear correlation is found for all three chromophores, indicating the involvement of solvent polarity dependent intramolecular charge transfer (ICT) emissive states. Moreover, also in the polar protic solvents such as ethanol and methanol that are potentially able to undergo hydrogen bonding interactions with dipolar dyes, no deviations from the linear correlation are noticed. It can therefore be concluded that solvent–dye dipole–dipole

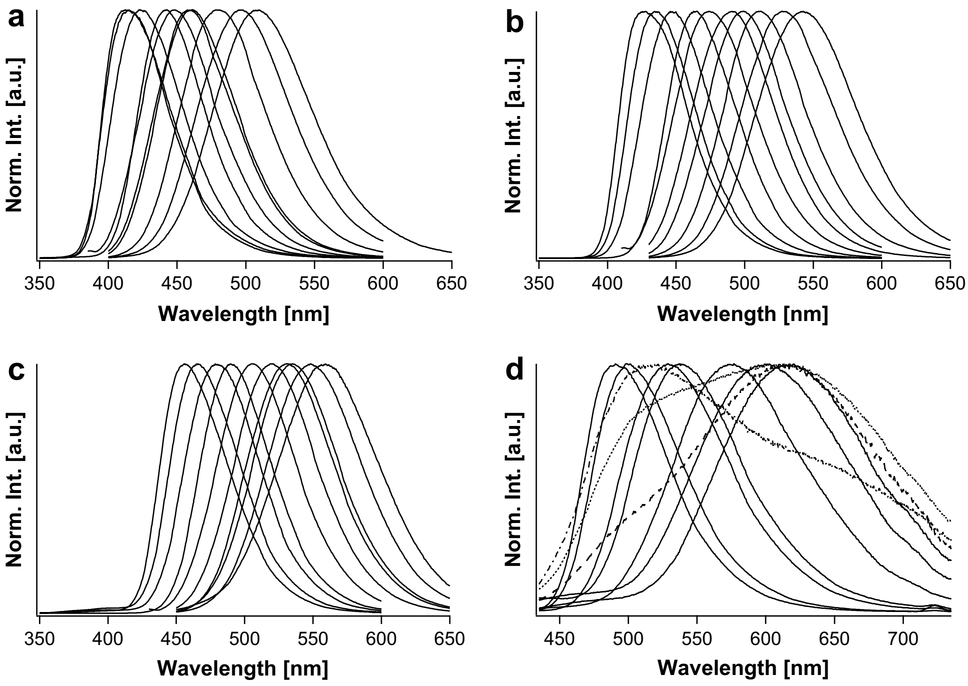


Fig. 1. Fluorescence emission spectra of KSD-*x* in different solvents; a) KSD-1 (20 μM), b) KSD-2 (20 μM), c) KSD-3 (20 μM), d) KSD-4 (3 μM). The emission peak showed red shift with increasing polarity of solvents, except for the spectra of (d) KSD-4 in i-propanol (dashed line), ethanol (dotted line) and methanol (dashed and dotted line). Solvents (from left to right except for spectra of KSD-4 in solvents as mentioned above): toluene, 1,4-dioxane, ethyl acetate, chloroform, acetone, DMSO, acetonitrile, i-propanol, ethanol and methanol.

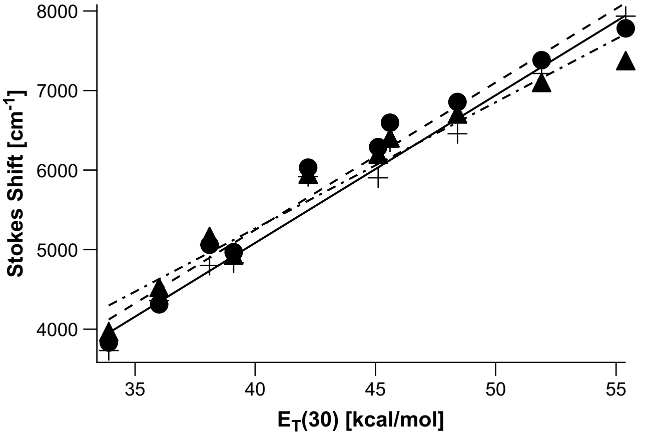


Fig. 2. Plot of the Stokes shifts of KSD-1–3 versus the $E_T(30)$ polarity parameter of different solvents; KSD-1 (cross, solid line), KSD-2 (circle, dashed line), KSD-3 (triangle, dotted and dashed line).

interactions are dominant. The corresponding data points in the plots are well fitted to the linear regression line, in contrast to the *Dapoxyl* dyes, where the Stokes shifts in ethanol and methanol are not linearly related to the polarity of the protic solvents on the $E_T(30)$ scale, but are influenced by hydrogen bonding [4].

In contrast to the *Dapoxyl* dyes, the fluorescence quantum yields of the KSD-1–3 dyes (Table 2) show no direct correlation with the solvent polarity parameter. All quantum yield values in the evaluated non-protic solvents were found to be between 0.32 and 0.82. The quantum yields remain high also in polar non-protic solvents like DMSO and acetonitrile. In the polar protic methanol solution, however, the fluorescence emission of the KSD-1–3 compounds is more strongly quenched, in particular in the case of KSD-3 ($\Phi = 0.02$), while *Dapoxyl* is still significantly fluorescent ($\Phi = 0.39$) [14].

2.3. Spectral properties of KSD-4 and KSD-4C

As reported above, the thiophene-bridged KSD-3 showed the largest Stokes shifts and the longest emission wavelengths among the KSD dyes. In the next step, we aimed at the further expansion of the π-conjugated system of KSD-3, in order to develop a fluorescent solvatochromic dye with longer emission wavelengths than the *Dapoxyl* dyes. Therefore, KSD-4 was designed and synthesized as a π-extended derivative of KSD-3 with a phenylene unit placed between the thiophene linker and the acetyl acceptor. The optical characteristics are summarized in Table 3.

Table 3
Spectral properties of KSD-4 in solvents of different polarities.

Solvent	$E_T(30)$ (kcal/mol)	λ_{Abs} (nm)	λ_{Em} (nm)	$\Delta\nu$ (cm^{-1})	fwhm (nm)	ε ($M^{-1}\ cm^{-1}$)	Φ (–)
Toluene	33.9	397	491	4822	72	26 000	0.67
1,4-Dioxane	36.0	394	499	5341	75	27 600	0.73
Ethyl acetate	38.1	392	529	6607	90	27 000	0.55
Chloroform	39.1	398	537	6853	91	19 300	0.47
Acetone	42.2	395	577	8224	113	24 400	0.41
DMSO	45.1	406	616	8397	132	25 400	0.34
Acetonitrile	45.6	393	599	8751	131	25 100	0.33
i-Propanol	48.4	397	614	8902	176	25 400	0.07
Ethanol	51.9	397	601	8550	–	22 800	0.01
Methanol	55.4	395	518	6011	–	23 800	0.005

Table 2
Molar extinction coefficients and quantum yields of KSD-1–3 in solvents of different polarities.

Solvent	ε^a ($M^{-1}\ cm^{-1}$)			Φ^b		
	KSD-1	KSD-2	KSD-3	KSD-1	KSD-2	KSD-3
Toluene	13 900	24 100	24 500	0.32	0.60	0.67
1,4-Dioxane	15 000	24 800	25 400	0.59	0.82	0.76
Ethyl acetate	15 000	24 400	24 800	0.64	0.72	0.61
Chloroform	13 200	22 000	23 200	0.33	0.62	0.54
Acetone	14 800	23 800	24 600	0.77	0.78	0.60
DMSO	14 700	22 700	23 600	0.82	0.74	0.49
Acetonitrile	14 700	23 700	24 400	0.77	0.80	0.57
i-Propanol	14 400	22 500	23 700	0.34	0.60	0.46
Ethanol	13 500	22 000	22 500	0.20	0.40	0.18
Methanol	14 300	21 900	22 300	0.11	0.13	0.02

[a] ε = molar extinction coefficient.
[b] Φ = fluorescence quantum yield.

The absorption maximum wavelengths of KSD-4 were within the range of 395–406 nm in all solvents and hardly influenced by solvent polarity, as also observed in the case of KSD-1–3. The emission color ranges from blue (491 nm, in toluene) to orange (616 nm, in DMSO). In comparison to KSD-3, larger Stokes shifts were achieved, reaching values essentially identical to *Dapoxyl* (Fig. 3). However, in the protic solvents, a short wavelength shifted shoulder appeared in the emission spectra (dashed lines in Fig. 1d). The intensity of this shoulder increased with increasing solvent polarity, and is finally observed as the main emission peak in methanol solution. As a consequence, the Stokes shifts in these solvents are no longer linearly related to the solvent polarity parameter $E_T(30)$ (Fig. 3). In the absorption spectra, similar band shapes with no shoulders were obtained in all investigated solvents. In ethanol and methanol, the fluorescence excitation spectra recorded at the two respective wavelengths of the emission peak and the shoulder are not identical and do not match exactly the absorption spectra (Fig. 4, only data for ethanol is shown). This spectral behavior is similar to the observations in a study performed by Fayed for a characteristic D–π–A type chromophore [23]. By analogy, it can therefore be considered that the dual emission in alcohols is due to contribution of two species present in the ground state, which are the free dye molecule and a hydrogen bonded dye–solvent complex, respectively. We assume that the hydrogen bonded complex, most probably between the carbonyl oxygen of the electron acceptor and the hydroxyl group of the alcohol solvent,

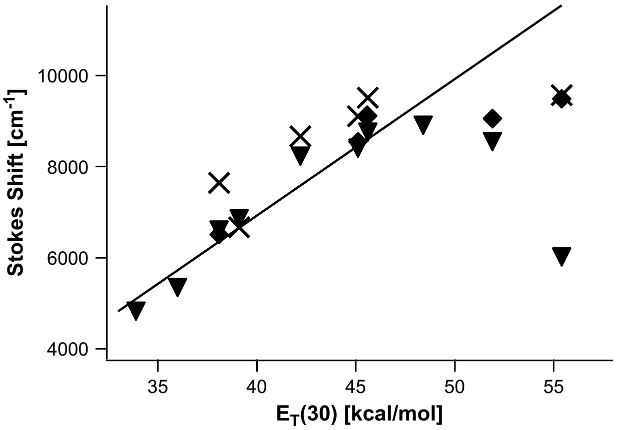


Fig. 3. Plot of the Stokes shifts of solvatochromic dyes versus the $E_T(30)$ polarity parameter of different solvents; KSD-4 (inverted triangle, solid line), KSD-4C (square), *Dapoxyl* [14] (cross).

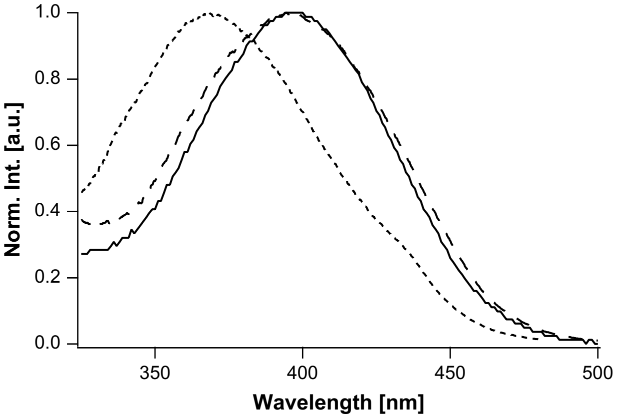


Fig. 4. Normalized excitation (em. 510 nm dotted line; em. 620 nm dashed line) and absorption spectra (solid line) for KSD-4 in methanol.

absorbs and emits at shorter wavelengths than the free dye molecule.

As can be seen in Table 3, the fluorescence quantum yield of KSD-4 increases with increasing solvent polarity and reaches a maximum value in 1,4-dioxane. Further increase in the solvent polarity leads to a sharp decrease in the quantum yield. Similar observations have also been reported by Fayed for other types of fluorescent solvatochromic dyes [23], and can be explained by several mechanisms such as biradicaloid charge transfer, proximity effect and conformational changes. The dramatical decrease of the quantum yield in the protic polar alcohols is assumed to be due to hydrogen bonding interactions between the dye and the solvent, giving rise to enhanced non-radiative losses and quenching [19,24,25]. The existence of specific dye–solvent interactions in protic solvents is also supported by the particular spectral behavior of KSD-4 in alcohols, as mentioned above (Fig. 1d).

It has to be stated that the solubility of KSD-4 in the polar solvents is generally rather low. It is known that extended conjugated compounds possessing more than two benzenoid rings are practically insoluble in most common organic solvents [16]. In order to overcome the solubility issues, the derivative dye KSD-4C was synthesized, where a carboxyl group was introduced in a position that is not directly linked to the π-conjugated chromophore system, so as not to affect the basic optical characteristics of the compound.

Table 4
Comparison of spectral properties of KSD-4C and conventional fluorescent solvatochromic dyes in methanol [14].

Dye	λ_{Abs} (nm)	ε (M^{-1} cm^{-1})	λ_{Em} (nm)
KSD-4C	390	30 300	619
Dapoxyl SEDA	373	28 000	584
Dansyl EDA	335	4 600	526
Prodan	361	16 000	498

As a result, the solubility of KSD-4C in alcohols was improved compared to the solubility of KSD-4. Furthermore, the absorption and emission spectra of KSD-4C in the lower polarity solvents such as ethyl acetate and dichloromethane correspond to those of KSD-4, indicating that the carboxyl group does not affect the energy states of the chromophore in these solvents, as expected from the molecular design. Interestingly, the short wavelength shifted shoulders observed for KSD-4 in the polar protic solvents, are significantly reduced in the case of the carboxyl-substituted derivative and the longer wavelength peak is observed as the main emission peak also in the case of methanol (Fig. 5). As a result, KSD-4C emits at longer wavelength (619 nm) and with larger Stokes shift (229 nm, 9486 cm^{-1}) in methanol than the *Dapoxyl* dyes. However, the data points for KSD-4C in ethanol and methanol in the plot of the Stokes shift versus the $E_T(30)$ polarity scale still deviate from the linear relationship obtained for the other solvents (Fig. 3). Despite of this fact, with KSD-4C, the development of a fluorescent solvatochromic dye with longer emission wavelength than conventional dyes was achieved (Table 4).

2.4. Potential applicability of KSD-4C as an analytical probe

As outlined above, fluorescent solvatochromic dyes are valuable tools for a variety of practical bioanalytical applications. Fluorescent solvatochromic dyes have been widely applied in competitive displacement assays for the study of drug–protein interactions, since the binding of a drug to blood proteins has a strong impact on the pharmacokinetic behavior of the drug in the human bloodstream [26–29]. For this reason, there is a need for highly sensitive fluorescent probes. A *Dapoxyl*-derived dye showed larger fluorescence spectral changes to increasing human serum albumin (HSA) concentrations compared to other fluorescent solvatochromic dyes, such as dansyl derivatives [15].

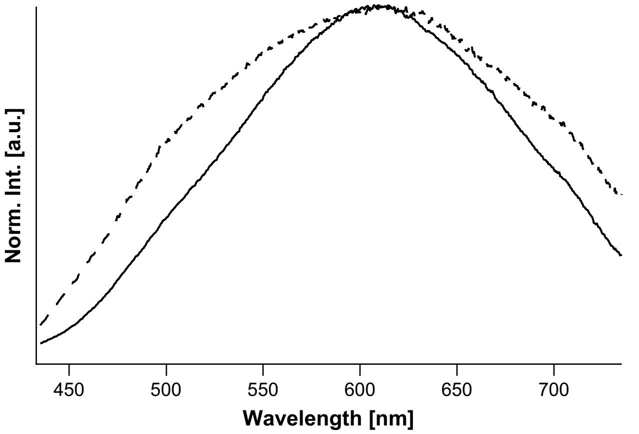


Fig. 5. Fluorescence emission spectra of KSD-4C in ethanol (solid line) and methanol (dashed line). Excited at 380 nm.

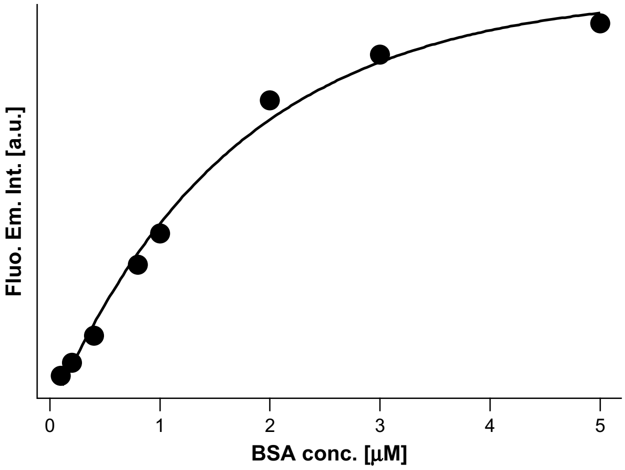


Fig. 6. BSA-dependent response curves of 5.0 μM KSD-4C in PBS (pH 7.4) containing 1% DMSO. Excited at 575 nm. Curve fitting was conducted using an exponential function.

In order to demonstrate the potential applicability of KSD-4C for fluorescence analyses, the fluorescence response of KSD-4C to protein concentration changes in phosphate buffered saline (PBS, pH 7.4) was investigated. Bovine serum albumin (BSA) was used as a protein model compound in this case. From the calibration curve in Fig. 6, the dynamic detection range is estimated to be between 0.1 and 5 μM for BSA. A strong fluorescence emission increase is observed already upon the addition of small concentrations of protein, with the emission signal in protein free buffer solution being close to the dark noise level of the fluorescence spectrophotometer. This fluorescence signal off–on behavior of KSD-4C is expected to be useful for bio-analyses including the detection and the quantification of proteins.

3. Conclusions

The effect of the central hetero ring system (pyrrole, furan, thiophene), acting as π-linker between the electron donor and the acceptor, on the solvatochromic emission spectral shifts and quantum yields of D–π–A-chromophore systems was examined. The selected synthetic approach with good yields, including Suzuki coupling from commercially available starting materials, facilitated the study of a variety of combinations of donors, acceptors and π-conjugating moieties in the D–π–A type molecules.

KSD-2, having a furan π-linking moiety, showed the largest fluorescent solvatochromic shifts, as well as the largest quantum yields in many of the solvents employed. KSD-3, based on thiophene as the π-linking moiety, showed the longest emission wavelengths in solvents of different polarity and the largest Stokes shifts. Furthermore, with KSD-4, a π-extended derivative of KSD-3, a still longer wavelength emitting solvatofluorochromic dye with larger Stokes shifts has been realized. Especially KSD-4C, a carboxyl group modified derivative of KSD-4, showed longer emission wavelength (619 nm) and larger Stokes shift (229 nm, 9486 cm^{-1}) than *Dapoxyl* dyes in methanol solution. The spectral properties of KSD-4C are, to our best knowledge, comparable or superior to those of existing solvatochromic fluorescent dyes.

The modification of heterocyclic π-conjugating units linking the electron-donating and accepting groups of fluorescent push–pull chromophores, in particular the introduction of a thiophene unit, might be an alternative way for the development of potential solvatochromic fluorescent dye candidates with increased polarity sensitivity, longer emission wavelengths, and higher quantum yields, which are required for the sensitive analysis of biological samples. Further research towards this direction is under way.

4. Experimental section

4.1. Synthesis

4.1.1. General

All reagents and solvents were purchased from commercial suppliers (Sigma–Aldrich, Tokyo Kasei Kogyo, Kanto Chemical, Wako Pure Chemical) and used without further purification. All moisture-sensitive reactions were carried out under an atmosphere of argon or nitrogen. YFLC-Al-560 chromatograph and Hi-Flash columns (Yamazen) were used in purification by flash column chromatography. Melting points were measured in open capillary tubes in a Buchi 545 melting point apparatus and were uncorrected. All ^{1}H NMR spectra were recorded on a JEOL JNM-LA 300 or VARIAN MERCURY spectrometer (300 MHz). Chemical shifts (δ, given in ppm) are relative to tetramethylsilane as an internal reference ($\delta = 0.0$). Coupling constants (J) are given in Hz. High resolution mass spectra (HRMS) were recorded on a JEOL JMS-T100LCS with MeOH as the solvent using reserpine as internal standard.

4.1.2. 1-(5-Bromo-1H-pyrrol-2-yl)-ethanone (**1**)

To a solution of 2-acetylpyrrole (2.54 g, 23.3 mmol) in a mixture of methanol (50 ml) and water (50 ml) were added oxone (7.15 g, 11.6 mmol) and NaBr (2.99 g, 29.1 mmol). After stirring at room temperature for 8 h, the resulting mixture was filtered. The filtrate was extracted with dichloromethane and the organic layer was dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (silica gel, hexane/ethyl acetate 10:1) to yield 2.49 g (13.2 mmol, 57%) of the target compound **1** as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, –C*H*$_3$, 3H), 6.90 (d, Ar–*H*, 1H, $J = 1.4$ Hz), 7.02 (d, Ar–*H*, 1H, $J = 1.5$ Hz), 9.58 (s, –N*H*–, 1H).

4.1.3. tert-Butyl 2-bromo-5-ethanoyl-1H-pyrrole-1-carboxylate (**2**)

To a solution of compound **1** (100 mg, 0.53 mmol) in acetonitrile (3 ml) were added di-*tert*-butyl dicarbonate (150 mg, 0.69 mmol) and DMAP (63 mg, 0.52 mmol) and the reaction mixture was stirred at room temperature for 1 h. After evaporation of the organic solvents, ethyl acetate was added to the residue and the mixture was washed with 1 M HCl and brine and dried over Na_2SO_4. The solvent was evaporated to yield 153 mg (0.53 mmol, 99%) of the target compound **2** as a dark orange solid. (No further purification.) ^{1}H NMR ($CDCl_3$) δ 1.57 (s, –C(C*H*$_3$)$_3$, 9H), 2.43 (s, C*H*$_3$–, 3H), 6.79 (d, Ar–*H*, 1H, $J = 1.5$ Hz), 7.31 (d, Ar–*H*, 1H, $J = 1.8$ Hz).

4.1.4. tert-Butyl 2-[4-(dimethylamino)phenyl]-5-ethanoyl-1H-pyrrole-1-carboxylate (**3**)

Compound **2** (152 mg, 0.53 mmol) and 4-(dimethylamino)-phenylboronic acid (174 mg, 1.12 mmol) were dissolved into a mixture of toluene (4 ml) and methanol (2.5 ml). Na_2CO_3 (124 mg, 1.17 mmol) was added and the mixture deaerated with argon. Then, $Pd(PPh_3)_4$ (41 mg, 35 μmol) was added, the reaction mixture was deaerated with argon again and stirred at 80 °C for 6 h. The resulting mixture was taken up in toluene, washed with water and brine, and dried over Na_2SO_4. After evaporation of the organic solvents, the residue was purified by flash column chromatography (silica gel, gradient elution:hexane/ethyl acetate 8:1–3:2) to yield 96 mg (0.29 mmol, 55%) of the target compound **3** as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.51 (s, –C(C*H*$_3$)$_3$, 9H), 2.49 (s, –C*H*$_3$, 3H), 2.97 (s, –N(C*H*$_3$)$_2$, 6H), 6.75 (d, Ar–*H*, 2H, $J = 8.7$ Hz), 7.07 (d, Ar–*H*, 1H, $J = 1.8$ Hz), 7.39 (d, Ar–*H*, 2H, $J = 9.0$ Hz), 7.47 (d, Ar–*H*, 1H, $J = 1.8$ Hz).

4.1.5. 1-[5-(4-Dimethylaminophenyl)-1H-pyrrol-2-yl]-ethanone (**4**, KSD-1)

To a solution of compound **3** (19.5 mg, 59 μmol) in tetrahydrofuran (4 ml) was added 28 wt% sodium methoxide methanol solution (0.5 ml, 2.45 mmol) and the reaction mixture was stirred at room temperature for 1.5 h. The resulting mixture was taken up in toluene, washed with water and brine, and dried over Na_2SO_4. After evaporation of the organic solvents, the residue was purified by gel permeation chromatography (eluent:chloroform) to yield 10.3 mg (45 μmol, 76%) of the target compound **4** (KSD-1) as a white solid. Mp 231.0–231.1 °C (dec.). ^{1}H NMR ($CDCl_3$) δ 2.47 (s, –C*H*$_3$, 3H), 3.00 (s, –N(C*H*$_3$)$_2$, 6H), 6.77 (d, Ar–*H*, 2H, $J = 8.8$ Hz), 7.09 (d, Ar–*H*, 1H, $J = 1.5$ Hz), 7.21 (d, Ar–*H*, 1H, $J = 1.5$ Hz), 7.41 (d, Ar–*H*, 2H, $J = 8.8$ Hz), 9.29 (br, 1H). ^{13}C NMR (DMSO) δ 25.53, 40.21, 112.70, 112.87, 120.99, 122.85, 125.45, 125.66, 132.33, 148.81, 186.79. HRMS: (ESI-TOF) *m*/*z* for $C_{14}H_{16}N_2Na_1O_1$ $[M + Na]^+$: calcd 251.1160; found 251.1179.

*4.1.6. 1-(5-Bromofuran-2-yl)-ethanone (**5**)*

To a solution of 2-acetylfuran (4.0 g, 36.3 mmol) in dimethylformamide (40 ml), *N*-bromosuccinimide (7.11 g, 40.0 mmol) was added and the reaction mixture was stirred at room temperature for 1 day. The mixture was extracted with diethyl ether and the ether layer was dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by column chromatography (silica gel, gradient elution:hexane/toluene/dichloromethane 20:20:1 to hexane/dichloromethane 1:2) to yield 2.38 g (12.6 mmol, 35%) of the target compound **5** as a brown solid. ^{1}H NMR ($CDCl_3$) δ 2.47 (s, –CH_3, 3H), 6.49 (d, Ar–*H*, 1H, $J = 3.6$ Hz), 7.12 (d, Ar–*H*, 1H, $J = 3.6$ Hz).

*4.1.7. 1-[5-(4-Dimethylaminophenyl)-furan-2-yl]-ethanone (**6**, KSD-2)*

Compound **5** (1.00 g, 5.29 mmol) and 4-(dimethylamino)phenylboronic acid (1.75 g, 10.6 mmol) were dissolved into a mixture of toluene (30 ml) and methanol (15 ml) and deaerated with argon. Then, a solution of Na_2CO_3 (1.68 g, 15.9 mmol) in water (15 ml) and $Pd(PPh_3)_4$ (306 mg, 265 μmol) was added. The reaction mixture was deaerated with argon again and stirred at 80 °C for 2 h. After evaporation of the organic solvent, the residue was extracted with ethyl acetate and the combined organic layer was dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (silica gel, hexane/dichloromethane/ethyl acetate 10:20:1) to yield 1.09 g (4.75 mmol, 90%) of the target compound **6** (KSD-2) as a yellow solid. Mp 147.7–147.8 °C. ^{1}H NMR ($CDCl_3$) δ 2.50 (s, –CH_3, 3H), 3.03 (s, –N(CH_3)$_2$, 6H), 6.56 (d, Ar–*H*, 1H, $J = 3.7$ Hz), 6.73 (d, Ar–*H*, 2H, $J = 8.8$ Hz), 7.25 (d, Ar–*H*, 1H, $J = 3.7$ Hz), 7.68 (d, Ar–*H*, 2H, $J = 8.8$ Hz). ^{13}C NMR ($CDCl_3$) δ 25.88, 40.32, 104.72, 110.03, 117.45, 120.58, 126.49, 150.95, 151.04, 159.41, 185.88. HRMS: (ESI-TOF) *m*/*z* for $C_{14}H_{15}N_1Na_1O_2$ $[M + Na]^+$: calcd 252.1001; found 252.1011.

*4.1.8. 1-[5-(4-Dimethylaminophenyl)-thiophen-2-yl]-ethanone (**7**, KSD-3)*

2-Acetyl-5-bromothiophene (1.15 g, 5.61 mmol) and 4-(dimethylamino)phenylboronic acid (1.785 g, 11.2 mmol) were dissolved into a mixture of toluene (40 ml) and methanol (20 ml) and deaerated with argon. Then, a solution of Na_2CO_3 (1.78 g, 16.8 mmol) in water (20 ml) and $Pd(PPh_3)_4$ (324 mg, 280 μmol) was added. The reaction mixture was deaerated with argon again and stirred at 70 °C for 5 h. After evaporation of the organic solvent, the residue was extracted with toluene and the combined organic layer was dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (silica gel, gradient elution: hexane/dichloromethane 5:1 to dichloromethane) to yield 1.12 g (4.57 mmol, 81%) of the target compound **7** (KSD-3) as a yellow solid. Mp 167.6–167.7 °C. ^{1}H NMR ($CDCl_3$) δ 2.54 (s, –CH_3, 3H), 3.02 (s, –N(CH_3)$_2$, 6H), 6.71 (d, Ar–*H*, 2H, $J = 8.8$ Hz), 7.16 (d, Ar–*H*, 1H, $J = 3.9$ Hz), 7.55 (d, Ar–*H*, 2H, $J = 8.8$ Hz), 7.62 (d, Ar–*H*, 1H, $J = 3.9$ Hz). ^{13}C NMR ($CDCl_3$) δ 26.50, 40.37, 112.80, 121.36, 121.48, 127.39, 134.08, 140.81, 151.00, 154.54, 190.48. HRMS: (ESI-TOF) *m*/*z* for $C_{14}H_{15}N_1Na_1O_1S_1$ $[M + Na]^+$: calcd 268.0772; found 268.0753.

*4.1.9. 4-(5-Bromothiophen-2-yl)-*N,N*-dimethylaniline (**8**)*

2,5-Dibromothiophene (1.01 g, 6.06 mmol) and 4-(dimethylamino)phenylboronic acid (1.77 g, 10.7 mmol) were dissolved into a mixture of toluene (60 ml) and ethanol (20 ml) and deaerated with argon. Then, a solution of Na_2CO_3 (3.8 g, 35.9 mmol) in water (20 ml) and $Pd(PPh_3)_4$ (370 mg, 320 μmol) was added. The reaction mixture was deaerated with argon again and stirred at 70 °C for 3.5 h. After evaporation of the organic solvent, the residue was taken up in ethyl acetate, washed with water and brine, and then dried over Na_2SO_4. After evaporation of the solvent, the residue was dissolved in chloroform and then hexane was added. The resulting precipitate was removed by filtration and the filtrate was concentrated to give a dark green solid. The crude compound was purified by flash column chromatography (silica gel, hexane/chloroform 1:1) to yield 794 mg (2.81 mmol, 46%) of the target compound **8** as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 2.98 (s, –N(CH_3)$_2$, 6H), 6.71 (d, Ar–*H*, 2H, $J = 9.0$ Hz), 6.88 (d, Ar–*H*, 2H, $J = 3.6$ Hz), 6.96 (d, Ar–*H*, 2H, $J = 3.6$ Hz), 7.39 (d, Ar–*H*, 2H, $J = 8.7$ Hz).

*4.1.10. 1-{4-[5-(4-Dimethylaminophenyl)-thiophen-2-yl]-phenyl}-ethanone (**9**, KSD-4)*

Compound **8** (21.1 mg, 128 μmol) and 4-acetylphenylboronic acid (34 mg, 120 μmol) were dissolved in toluene (2 ml) and deaerated with argon. To the solution was added $Pd(PPh_3)_4$ (14 mg, 12 μmol) followed by further toluene (4 ml), ethanol (2 ml) and Na_2CO_3 (108 mg, 1.02 mmol). The reaction mixture was deaerated once again and stirred at 50 °C for 3 h. After filtration through celite, the filtrate was concentrated under reduced pressure and the resulting residue was taken up in toluene, washed with water and brine and then dried over Na_2SO_4. After evaporation of the solvent, the residue was purified twice by flash column chromatography (silica gel, chloroform) to yield 12 mg (37 μmol, 31%) of the target compound **9** (KSD-4) as a yellow solid. Mp 248.3–250.9 °C (dec.). ^{1}H NMR ($CDCl_3$) δ 2.61 (s, –CH_3, 3H), 3.01 (s, –N(CH_3)$_2$, 6H), 6.74 (d, Ar–*H*, 2H, $J = 8.5$ Hz), 7.16 (d, Ar–*H*, 1H, $J = 3.9$ Hz), 7.38 (d, Ar–*H*, 1H, $J = 3.9$ Hz), 7.53 (d, Ar–*H*, 2H, $J = 8.5$ Hz), 7.68 (d, Ar–*H*, 2H, $J = 8.3$ Hz), 7.96 (d, Ar–*H*, 2H, $J = 8.3$ Hz). ^{13}C NMR could not be observed due to insufficient solubility of the compound. HRMS: (ESI-TOF) *m*/*z* for $C_{20}H_{19}N_1Na_1O_1S_1$ $[M + Na]^+$: calcd 344.1085; found 344.1128.

*4.1.11. 1-*tert*-Butyl-4-(4-bromophenyl)-4-oxobutanoate (**10**)*

To a solution of 3-(4-bromobenzoyl) propionic acid (1.01 g, 3.93 mmol) in dichloromethane (40 ml) was added *tert*-butanol (0.5 ml) and then cooled to 0 °C. DMAP (529 mg, 4.33 mmol) and a suspension of water soluble carbodiimide in dichloromethane (10 ml) were added and the reaction mixture was stirred at room temperature for 18 h. The resulting mixture was washed with saturated aqueous $NaHCO_3$, water and brine, and then dried over Na_2SO_4. After evaporation of the solvent, the solid residue was purified twice by flash column chromatography (silica gel, chloroform, followed by hexane/ethyl acetate 4:1) to yield 266 mg (938 μmol, 24%) of the target compound **10** as a brown oil. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, –C(CH_3)$_3$, 9H) 2.68 (t, –CH_2COOtBu, 2H, $J = 6.6$ Hz), 3.22 (t, CH_2CH$_2$COOtBu, 2H, $J = 6.6$ Hz), 7.61 (d, Ar–*H*, 1H, $J = 8.4$ Hz), 7.85 (d, Ar–*H*, 1H, $J = 8.7$ Hz).

*4.1.12. 1-*tert*-Butyl-4-[4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-phenyl]-4-oxobutanoate (**11**)*

To a solution of compound **10** (53 mg, 170 μmol) in 1,4-dioxane (6 ml) was added under argon bis-(neopentylglycolato) diboron (68 mg, 301 μmol), potassium acetate (60 mg, 610 μmol) and $PdCl_2(dppf)_2 \cdot CH_2Cl_2$ (18 mg, 22 μmol). The reaction mixture was deaerated with argon and then stirred at 80 °C for 4 h. After filtration through celite, the filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography (silica gel, gradient elution:hexane/chloroform 1:1–1:2) to yield 46.5 mg (134 μmol, 73%) of the target compound **11** as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.03 (s, –B–O–CH$_2$–C(CH_3)$_2$–, 6H), 1.45 (s, –C(CH_3)$_3$, 9H), 2.67 (t, –CH_2COOtBu, 2H, $J = 6.6$ Hz), 3.27 (t, CH_2CH$_2$COOtBu, 2H, $J = 6.6$ Hz), 3.79 (s, –B–O–CH_2–, 4H), 7.88 (d, Ar–*H*, 2H, $J = 8.1$ Hz), 7.95 (d, Ar–*H*, 2H, $J = 7.5$ Hz).

4.1.13. 1-*tert*-Butyl-4-{4-[5-(4-dimethylaminophenyl)-thiophen-2-yl]-phenyl}-4-oxobutanoate (**12**)

Compound **8** (321 mg, 1.14 mmol) was dissolved into toluene (20 ml) and deaerated with argon. To the solution was added $Pd(PPh_3)_4$ (91 mg, 79 μmol), followed by further deaerating. After stirring at room temperature for 15 min, a solution of compound **11** (260 mg, 751 μmol) in toluene (15 ml) followed by additional toluene (25 ml), ethanol (20 ml) and a solution of Na_2CO_3 (486 mg, 4.59 mmol) in water (5 ml) were added. The reaction mixture was deaerated again and stirred at 50 °C for 3 h. After evaporation of the solvent, the dark green residue was taken up in toluene, washed with water and brine and then dried over Na_2SO_4. After evaporation of the solvent, the residue was purified twice by flash column chromatography (silica gel, chloroform) to yield 159 mg (365 μmol, 49%) of the target compound **12** as a yellow solid. ^1H NMR ($CDCl_3$) δ 1.46 (s, $-C(CH_3)_3$, 9H) 2.69 (t, $-CH_2COOtBu$, 2H, $J = 6.6$ Hz), 3.01 (s, $-N(CH_3)_2$, 6H), 3.26 (t, $CH_2CH_2COOtBu$, 2H, $J = 6.6$ Hz), 6.74 (d, Ar–*H*, 1H, $J = 8.7$ Hz), 7.16 (d, Ar–*H*, 1H, $J = 3.6$ Hz), 7.38 (d, Ar–*H*, 1H, $J = 3.9$ Hz), 7.53 (d, Ar–*H*, 1H, $J = 9.0$ Hz), 7.68 (d, Ar–*H*, 1H, $J = 8.4$ Hz), 7.99 (d, Ar–*H*, 1H, $J = 8.4$ Hz). ^1H NMR (DMSO) δ 1.39 (s, $-C(CH_3)_3$, 9H), 2.57 (t, $-CH_2COOtBu$, 2H, $J = 6.3$ Hz), 2.96 (s, $-N(CH_3)_2$, 6H), 3.24 (t, $CH_2CH_2COOtBu$, 2H, $J = 6.3$ Hz), 6.77 (d, Ar–*H*, 1H, $J = 8.7$ Hz), 7.37 (d, Ar–*H*, 1H, $J = 3.9$ Hz), 7.54 (d, Ar–*H*, 1H, $J = 9.0$ Hz), 7.67 (d, Ar–*H*, 1H, $J = 3.9$ Hz), 7.80 (d, Ar–*H*, 1H, $J = 8.4$ Hz), 8.01 (d, Ar–*H*, 1H, $J = 8.7$ Hz).

4.1.14. 4-{4-[5-(4-dimethylaminophenyl)-thiophen-2-yl]-phenyl}-4-oxobutanoic acid (**13**, KSD-4C)

A solution of compound **12** in formic acid (10 ml) was stirred at room temperature for 7 h and then dried under reduced pressure overnight to give the target compound **13** (KSD-4C) as a yellow powder. (No further purification.) Mp 252.8 °C (dec.). ^1H NMR (DMSO) δ 2.59 (t, $-CH_2COOH$, 2H, $J = 6.6$ Hz), 2.96 (s, $-N(CH_3)_2$, 6H), 3.26 (t, CH_2CH_2COOH, 2H, $J = 6.6$ Hz), 6.77 (d, Ar–*H*, 1H, $J = 8.7$ Hz), 7.37 (d, Ar–*H*, 1H, $J = 3.9$ Hz), 7.54 (d, Ar–*H*, 1H, $J = 9.0$ Hz), 7.67 (d, Ar–*H*, 1H, $J = 3.9$ Hz), 7.80 (d, Ar–*H*, 1H, $J = 8.1$ Hz), 8.01 (d, Ar–*H*, 1H, $J = 8.4$ Hz). ^{13}C NMR (DMSO) δ 27.90, 33.02, 40.13, 112.41, 121.11, 122.44, 124.68, 126.32, 126.78, 128.85, 134.60, 138.17, 138.38, 145.81, 150.19, 173.81, 197.47. HRMS: (ESI-TOF) *m*/*z* for $C_{22}H_{21}N_1O_2S_1$ $[M]^+$: calcd 379.1242; found 379.1241.

4.2. Measurements

4.2.1. Instrumentation

The absorbance spectra were recorded on a Hitachi U-2001 double beam spectrophotometer (Hitachi, Ltd., Tokyo, Japan). Fluorescence emission spectra were measured at 25 ± 1 °C on a Hitachi F-4500 spectrophotometer.

4.2.2. Determination of quantum yields [30]

Quantum yields of the KSD-*x* fluorophores were determined according to the following method: Quinine sulfate ($\Phi_{ST} = 0.546$) [31] was chosen as the standard dye. Diluted solutions of the standard dye in 0.1 M sulfuric acid, and the dye to be evaluated in the various solvents were prepared at five different concentrations. The concentrations were selected, so that the absorbance of each sample was below 0.10 at the excitation wavelength and above to prevent re-absorption. Next, a graph of the absorbance (A) at a certain wavelength (363 nm) versus the integrated emission intensity (F) excited at the chosen wavelength was plotted. Then, the slope (*Grad*) of the fitting line to the plot of F versus A was calculated (intercept = 0). Finally, the quantum yield of the evaluated dye (Φ_X) was calculated using the following equation:

$$\Phi_X = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{n_X^2}{n_{ST}^2}\right),$$

where n stands for the refractive index of the used solvent [3].

Curve fitting and integration of the emission intensity was performed using the Igor Pro software (WaveMetrics Inc., Lake Oswego, OR).

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