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Characterisation of new norcyanine dyes and their application as pH chromoionophores in optical sensors

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Abstract

New nor-based cyanine dyes have been characterised for their use as long wavelength chromoionophores in optochemical sensors. Since common quaternary cyanine dyes present optimum spectroscopic characteristics but with high basicities, three kinds of molecules (nortricarbocyanine dyes, norindosquarocyanine dyes and norindocrococyanine dyes), based on an acid–base equilibrium, where the proton attached to the indolic nitrogen is involved, have been designed, expecting to obtain lower pK_a values. For comparison purposes, every type of dye has been synthesised with a different central group and molecules with the same heterocyclic substituents have been proposed. The acid forms of the norcyanine dyes present sharp and intense absorption bands from the far visible to the NIR region, with absorption maxima between 648 and 821 nm, allowing the use of non-expensive semiconductor based optical sources and detectors. Relationships between the optical properties, the basicity and the molecular structure are discussed.

Keywords: Norcyanine dyes; NIR; pH indicators; LED laser

1. Introduction

Recent progress in optical sensor technology has considerably intensified interest in the development of new acidochromic molecules [1–6], especially with regards to their use as transduction elements in bulk optodes [7–10]. This type of membrane combines the selective recognition of an ionophore with the optical transduction of a chromoionophore, enclosed within a plasticized PVC membrane. The response mechanism is based either on an ion-exchange or a coextraction mechanism depending on the charge of the ions and the involved chromoionophores, which are typically neutral or charged lipophilic acidochromic dyes. Owing to their flexible configuration, many ion-selective optodes have been reported for the analysis of relevant cations [11–13] and anions [14,15].

Ideally, indicators should possess characteristics such as large molar extinction coefficient, selective and reversible interaction with protons, appropriate changes in absorption spectrum, adequate basicity, complete solubility in the solution matrix and photostability.

In contrast, current trends towards the miniaturisation of chemical analysis systems result in a reduction in the size and complexity of optical systems, which widens their application fields and reduces their cost. With this purpose, integrated optochemical techniques take profit of the non-expensive and mass-produced diode lasers, LEDs and photodetectors, which are fabricated using semiconductor technology. Of the available light sources, diode lasers emitting at 670 and 780 nm are preferred due to their high intensity, low cost and suitable performance when waveguides are to be used. However, the lack of commercial chromoionophores, which display absorption bands in the far vis–NIR region of the electromagnetic spectrum, is a significant hindrance to the exploitation of integrated optical sensors [16,17] and, only very few types of dyes

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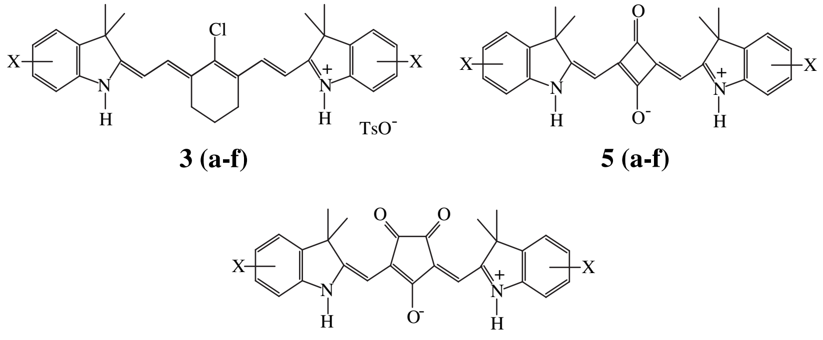

3 (a-f) **5 (a-f)** **7 (a-f)**

Fig. 1. Synthesised nor-based cyanine dyes: nortricarbocyanine dyes (**3**), norindosquarocyanine dyes (**5**) and norindocrococyanine dyes (**7**).

with such characteristics have been successfully applied in bulk optodes and integrated sensors [18]. Therefore, some promising new types of chromoionophores have been synthesised in our research group.

Although heptamethinecyanine dyes, showing appropriate absorbance wavelengths and high molar extinction coefficients, were first synthesised and characterised for this purpose, their acid–base equilibrium comprises the addition of a hydroxy group, at very basic pH values, to an electrophilic site within the dye [1,19], resulting in indicators with high pK_a values. As this may restrict their application as chromoionophores in optical membranes, in order to obtain lower pK_a values and maintain the same spectroscopic features, three new types of cyanine-based acidochromic dyes have been synthesised namely nortricarbocyanine [20], norindosquarocyanine [21] and norindocrococyanine dyes [22]. These are analogues of other known cyanine dyes, which absorb in the near-infrared region but contain non-alkylated indolenine moieties that yield chromoionophores with a different acid–base equilibrium. As the spectral properties of non-alkylated cyanine dyes only differ slightly from those of alkylated analogues [23], we also expect to obtain dyes, whose absorption maxima will be located in the near-infrared region.

Heptamethinecyanine dyes absorb infrared light and are widely employed as light absorbing media in optical recording systems [24–26]. However, the so-called norcyanine dyes have been scarcely investigated [27]. The propounding nortricarbocyanine dyes share the same molecular structure of previously studied heptamethinecyanine [1,19] dyes but with the only difference of a terminating non-alkylated indolenine nucleus. On the other hand, squarocyanine dyes and crococyanine dyes [28,29] are well-known zwitterionic polymethinic dyes with high extinction coefficients and absorption bands covering the region from the vis to the NIR. They present the same chromophoric system as the corresponding cyanine dyes, but they also contain a central squarate or croconate bridge that shifts the absorption maximum to longer wavelengths [30].

Different functionalities have been introduced into the structure of the molecules obtaining 16 acidochromic dyes with similar characteristics. Spectroscopic properties have been studied in ethanolic solution in order to determine their suitability as chromoionophores for optical membranes. In addition, the relationship between the structural parameters, the maximum absorbance wavelength and the pK_a values is discussed to enable the synthesis of other kinds of dyes with optical properties, which can be tailored for a specific application.

2. Results and discussion

Sixteen new nor-based cyanine dyes have been obtained by combining six different substituted indolenines with three polymethinic central groups. Nortricarbocyanine dyes (**3**) consist of a nor-heptamethinecyanine central group, where three carbons are included in a six-membered ring, while norindosquarocyanine dyes (**5**) and norindocrococyanine dyes (**7**) have a pentamethincyanine chain with a squaric or a croconic ring. Their structure is shown in Fig. 1 and Table 1.

Chromoionophores with different spectral characteristics but similar acid–base properties are expected to be obtained. The analytical characterisation has been performed in ethanol

Table 1
Absorption maxima, molar extinction coefficients and pK_a values for the synthesised nor-based cyanine dyes in ethanol

Dye	X	λ_{ac} (nm)	ε_{ac} (l/mol cm)	λ_{bs} (nm)	pK_a
3a	H	780.0	2.7×10^5	520.5	4.9
5a	H	648.5	1.6×10^5	508.5	11.9
7a	H	759.0	1.5×10^5	626.0	9.7
3b	[4,5]benz-	821.0	1.1×10^4	542.5	4.1
5b	[4,5]benz-	679.5	1.7×10^5	534.5	11.6
7b	[4,5]benz-	800.0	7.1×10^4	650.0	8.6
3c	5-N_2O	801.5	1.3×10^5	538.5	2.8
5c	5-N_2O	665.5	8.2×10^4	580.5	8.3
7c	5-N_2O	793.0	1.5×10^5	630.0	6.5
5d	5-$NHCOCH_3$	675.5	1.4×10^5	523.0	11.5
7d	5-$NHCOCH_3$	798.5	4.4×10^4	639.5	9.4
3e	5-C_4H_9	793.0	2.0×10^5	528.0	5.1
5e	5-C_4H_9	660.5	1.5×10^5	513.5	12.6
7e	5-C_4H_9	775.0	1.7×10^5	642.5	9.1
3f	5-CH_3O	808.0	2.0×10^5	531.0	5.2
7f	5-CH_3O	793.5	5.8×10^4	638.5	9.6

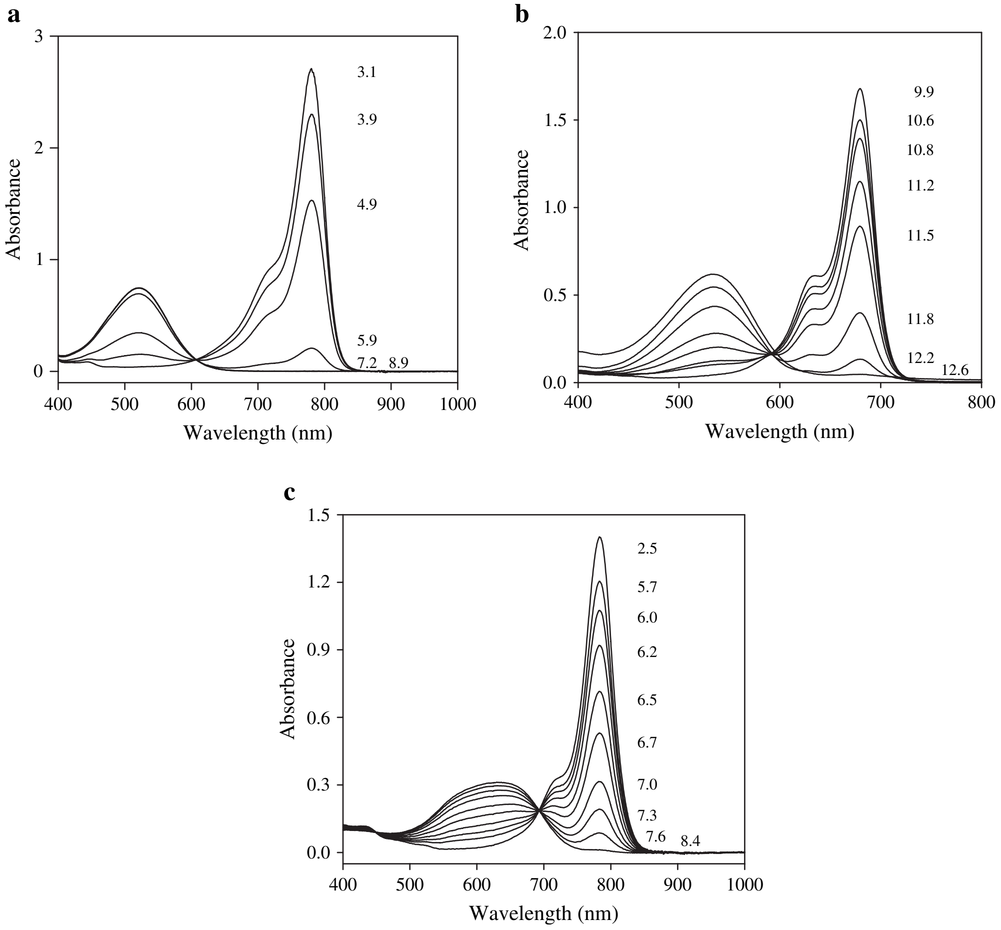

Fig. 2. Representative absorption spectra of nor-based cyanine dyes in ethanol at the indicated pH. (a) Nortricarbocyanine dye **3a**; (b) norindosquarocyanine dye **5b**; (c) norindocrococyanine dye **7c**.

and for dye numbers **3c**, **5c**, **7b**, **7c** and **7e**, a solution of 10% DMFA/EtOH has been employed to avoid aggregation. Their absorbance wavelength, acid–base behaviour and reversibility have been tested and compared.

The aim of the work has been the design and synthesis of cyanine dyes based on non-alkylated nitrogen groups, which show lower pK_a values than the alkylated cyanine dyes, but preserving the same spectroscopic properties such as high molar absorptivities and narrow absorption maxima located in the NIR region. When comparing the maximum absorbance wavelengths of the synthesised dyes with the corresponding alkylated analogues [1,19], we can observe that both, the absorbance maxima and the molar extinction coefficient, are not influenced by the nitrogen substituents. The protonated forms of the synthesised dyes present high and sharp absorption bands covering the region from the far visible to the near-infrared region (645–825 nm) and so, allowing the use of non-expensive diode lasers emitting at 670 and 780 nm as excitation light sources. The wavelengths of the maximum absorbance in acid and basic media as well as the molar absorptivities of the acid form are shown in Table 1.

The absorbance of the cyanine dyes basically depends on their chromophoric system and on the nature of its terminating groups [31]. As nortricarbocyanine dyes are based on a longer polymethinic chain than norindosquarocyanine dyes and norindocrococyanine dyes, the absorption bands of the acid forms lie at longer wavelengths in the NIR region. However, it can be also observed that the synthesised crococyanine dyes absorb in

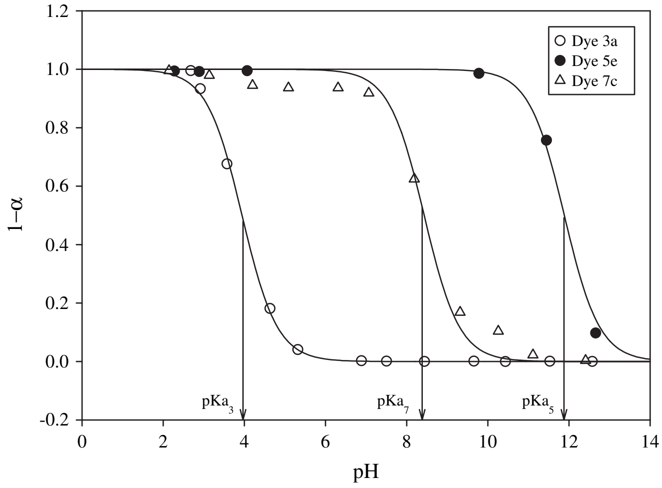

Fig. 3. Calibration curves corresponding to three examples of each type of dye. In order to normalise absorbance values, $1-\alpha$ has been calculated as the degree of deprotonation of the dye.

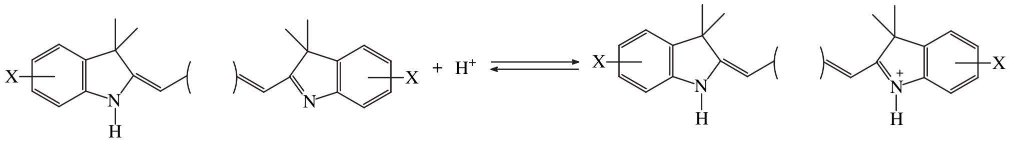

Fig. 4. Proposed acid–base equilibrium for the nor-based cyanine dyes.

the NIR region. This fact demonstrates that the substituents of the polymethinic chain influence to some extent the absorbance wavelength as well. Thus, if some of the carbon atoms of the polymethinic chain are included into a ring, this fact contributes as well to keep higher stability [32] to the molecule as to give a bathochromic shift of the absorbance bands. A comparison of the three types of dyes assigns the following order of the absorption maxima for the acid forms: λ dye type **3** > λ dye type **7** > λ dye type **5**.

In concordance with previous studies [26], the evaluation of the wavelength shift of the analogue nortricarbocyanine (**3**) and norindosquarocyanine (**5**) dyes gives a 131–142 nm bathochromic shift, while the difference between nortricarbocyanine (**3**) and norindocrococyanine (**7**) dyes is only about 8–21 nm.

It is generally known [33,34] that substituents in the heterocyclic nuclei slightly influence the absorption maxima of the dye by shifting the absorbance maxima to longer wavelengths. An increase in the maximum wavelength in all the synthesised substituted dyes (**b**–**f**) with regard to the unsubstituted ones (**a**) has been observed. Moreover, dyes with a benzoindole heterocycle (**b**) undergo the stronger change, presenting bathochromic shifts between 31.5 and 64.5 nm. No clear relationship could be found between the extent of the shift and the electron-donating or electron-withdrawing nature of the substituents.

The acid–base behaviour of the dyes has also been studied in ethanol. Fig. 2 shows the representative absorption spectra of every type of dye at different pH values. Dyes show a good performance during calibrations in each entire working pH range. The absorption bands related to the basic and acid forms appear in the visible region and the NIR region, respectively. The permanency of a well-defined isosbestic point in the spectra of the indicators demonstrates that there is only one equilibrium involving two species. The calibration curves have been obtained taking the absorption maximum of the acid form from the spectra when the equilibrium has been reached for every pH variation. Fig. 3 shows the calibration curves of an example of each type of dye, where it can be noticed that with the synthesised dyes it is possible to cover almost the entire pH range. Norindosquarocyanine dyes turn out to be the most basic chromoionophores, showing pK_a values from 8.3 to 12.6, followed by crococyanine dyes (6.5–9.7) and nortricarbocyanine dyes (1.8–5.3). According to the molecular structure and to the obtained pK_a values, the proposed acid–base equilibrium for the nor-based cyanine dyes consists of the protonation–deprotonation of the acidic nitrogen (Fig. 4). As it is observed experimentally, the deprotonation avoids the charge delocalization and therefore, gives a hypsochromic shift of the maximum wavelength for the basic form. As a result of this acid–base equilibrium, we have obtained three types of chromoionophores with the required characteristics of the common cyanine dyes and with more appropriate pK_a values for their use in pH optical sensing.

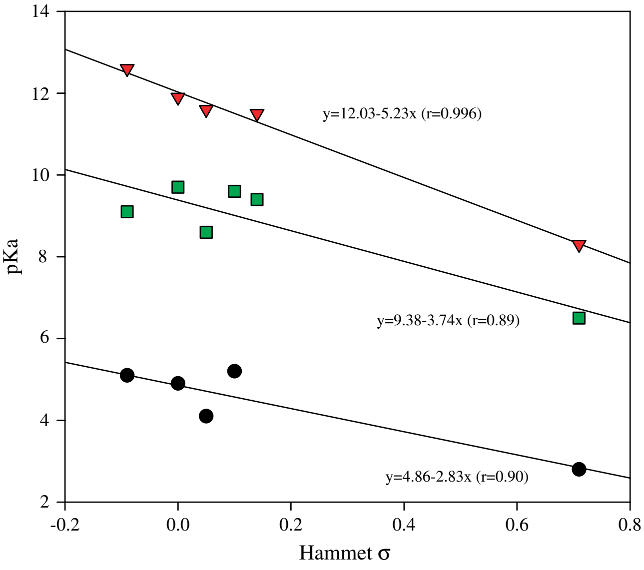

Fig. 5. Correlation of pK_a values determined in ethanol to Hammet σ substituent constants. Nortrimethinecyanine dye (●), norindosquarocyanine dye (▼), norindocrococyanine dye (■). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The nature of the heterocyclic nuclei also appears to have an important influence on the pK_a value. Large changes in basicity can be achieved by the introduction of either electron-withdrawing or electron-releasing substituents into the heterocyclic nuclei [35]. Basicity values have been correlated with the Hammet substituent constant σ [36] obtaining a lineal relationship for each type of dyes (Fig. 5). Due to the high electron-withdrawing properties of the nitro substituent, dyes of class **c** always present lower pK_a values. These results provide a useful tool for the design of future dyes with the desired basicities.

Furthermore, the experiments show good repeatability and stability of dyes (100% of the acidic and basic forms are recovered) while the pH is increased and decreased repeatedly.

3. Conclusions

Three new types of cyanine indicators, based on an acid–base equilibrium, where the proton attached to the indolic nitrogen is involved, are presented. They show intense absorption maxima in the far visible or the NIR region, which match the output of some cheap and mass-produced diode lasers and the determined pK_a values cover a wide range from 2.8 to

12.6. One of the advantages of the molecular structure of the norcyanine dyes is that modifications of the maximum absorbance wavelength and pK_a are easily achieved by varying the nature of some substituents. In particular, basicities have been correlated to the Hammet σ constants of the heterocyclic substituents, allowing the prediction of the pK_a value for newly designed dyes. Future work will focus on the combination of norcyanine dyes with commercially available ionophores for the development of ion-selective optodes. The next step will be their characterisation in integrated waveguide absorbance optodes (IWAO) fabricated in our research group.

4. Experimental

4.1. Apparatus

MALDI-TOF measurements have been performed on a BIFLEX Spectrometer (Bruker-Franzen). FABMS measurements have been made in a Hewlett Packard HP-5988A. The reported yields are of pure and isolated compounds. Proton NMR spectra have been measured on a Bruker AC250 at 250 MHz with TMS as internal standard using DMSO, CD_3OD or CD_3Cl as deuterated solvents. Spectrophotometric measurements have been performed in a UV–vis–NIR scanning spectrophotometer Shimadzu UV-3101PC. The absorption spectra have been recorded between 1000 and 400 nm. pH values have been determined with a pH-meter (Crison-micro pH 2002, Barcelona, Spain).

4.2. Reagents

Reagents such as HCl 37%, NaOH, ethanol 96%, DMSO 99.9% and CD_3OD 99.95% have been purchased from Panreac (Barcelona, Spain) and CD_3Cl 99.96% from Aldrich (Madrid, Spain). All the characterised dyes have been synthesised in our laboratory [20–22].

4.3. pK_a determination

The concentration of dye solutions is 1×10^{-5} M in ethanol except for some dyes, for which a 10% of DMSO has been required for the dye solubilization. The pH has been adjusted with concentrated solutions of HCl and NaOH. pK_a values have been determined using the absorbance maximum of the higher wavelength band as a function of pH according to the following equation:

$$pK_a = pH - \log\left[\left(A_f - A\right) - \left(A - A_0\right)\right] \qquad \text{(I)}$$

where A is the signal at any given pH, A_0 is the signal of the completely deprotonated form and A_f is the maximum signal of the totally protonated form.

4.4. Synthesis of chromoionophores

The synthesis of nortricarbocyanines (**3**) [20], norindosquarocyanine dyes (**5**) [21] and norindocrococyanine dyes (**7**) [22] was reported previously. Yields, ^1H NMR, elemental analysis, MALDI or FAMBS are given below. Table 1 and Fig. 1 show the structure of the 16 synthesised dyes.

Dye**3a**: yield 78%. Analysis: calculated for $C_{37}H_{39}O_3N_2SCl$; C, 70.85; H, 6.27; N, 4.47; S, 5.11. Found: C, 70.51; H, 6.26; N, 4.31; S, 4.89. FABMS *m/z* 456 (M^+ (Cl^{35}) + H, 100), 419 (M^+ (Cl^{35}) − HCl, 100). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.49 (s, 12H), 1.82 (quint., 2H), 2.29 (s, 3H), 2.65 (t, 4H), 6.12 (d, $J = 13.9$ Hz, 2H), 7.10–7.54 (m, 12H), 8.23 (d, $J = 13.9$ Hz, 2H).

Dye**3b**: yield 65%. Analysis: calculated for $C_{45}H_{43}O_3N_2SCl$; C, 74.31; H, 5.96; N, 3.85; S, 4.41. Found: C, 73.06; H, 6.14; N, 3.58; S, 3.31. FABMS *m/z* 519.2 (M^+ (Cl^{35}) − HCl, 100), 555.2 (M^+ (Cl^{35}) − Cl, 23). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.74 (s, 12H), 1.84 (br. quint., 2H), 2.27 (s, 3H), 2.70 (br. t, 4H), 6.23 (d, $J = 14.0$ Hz, 2H), 7.12 (d, $J = 8.0$ Hz, 2H), 7.44–8.04 (m, 12H), 8.19 (d, $J = 8.0$ Hz, 2H), 8.30 (d, $J = 14$ Hz, 2H).

Dye**3c**: yield 65%. Analysis: calculated for $C_{37}H_{37}O_7N_4SCl$; C, 61.96; H, 5.20; N, 7.81; S, 4.47. Found: C, 60.66; H, 5.08; N, 7.24; S, 4.17. FABMS *m/z* 545.1 (M^+ (Cl^{35}) + H, 100), 509.2 (M^+ (Cl^{35}) − HCl, 98). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.50 (s, 12H), 1.85 (quint., 2H), 2.32 (s, 3H), 2.69 (t, 4H), 6.25 (d, $J = 13.4$ Hz, 2H), 7.15 (d, $J = 7.8$ Hz, 2H), 7.37 (d, $J = 8.7$ Hz, 2H), 7.51 (d, $J = 7.9$ Hz, 2H), 7.96 (d, $J = 13.4$ Hz, 2H), 8.24 (dd, $J = 8.7$ Hz, $J = 2.3$ Hz, 2H), 8.37 (d, $J = 2.3$ Hz, 2H).

Dye**3e**: yield 65%. Analysis: calculated for $C_{45}H_{55}O_3N_2SCl$; C, 73.09; H, 7.50; N, 3.79; S, 4.34. Found: C, 73.24; H, 7.33; N, 3.68; S, 4.09. FABMS *m/z* 531.3 (M^+ (Cl^{35}) − Cl, 100), 567.3 (M^+ (Cl^{35}) + H, 22). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 0.89 (t, $J = 6.9$ Hz, 6H), 1.21–1.58 (m, 12H), 1.46 (s, 12H), 1.79 (quint., 2H), 2.27 (s, 3H), 2.59 (t, $J = 7.7$ Hz, 4H), 6.06 (d, $J = 13.9$ Hz, 2H), 7.08–7.12 (m, 6H), 7.33 (s, 2H), 7.45 (d, $J = 8.0$ Hz, 2H), 8.16 (d, $J = 13.9$ Hz, 2H).

Dye**3f**: yield 45%. Analysis: calculated for $C_{40}H_{47}O_6N_2SCl$; C, 66.79; H, 6.59; N, 3.89; S, 4.46. Found: C, 67.23; H, 6.47; N, 3.94; S, 4.33. FABMS *m/z* 479.2 (M^+ (Cl^{35}) − Cl, 100), 515.2 (M^+ (Cl^{35}) + H, 63). ^1H NMR (250 MHz, CD_3OD) δ (ppm): 1.56 (s, 12H), 1.91 (quint., 2H), 2.37 (s, 3H), 2.66 (t, 4H), 3.83 (s, 6H), 6.01 (d, $J = 13.9$ Hz, 2H), 6.89 (dd, $J = 8.0$ Hz, $J = 2.2$ Hz, 2H), 7.07–7.14 (m, 4H), 7.23 (d, $J = 7.3$ Hz, 2H), 7.71 (d, $J = 8.1$ Hz, 2H), 8.27 (d, $J = 14.0$ Hz, 2H).

Dye**5a**: yield 77%. Analysis: calculated for $C_{26}H_{24}O_2N_2$; C, 78.76; H, 6.10; N, 7.07. Found: C, 78.78; H, 5.98; N, 6.93. MALDI *m/z* 396.9 (M^+ + H, 100), 397.9 (M^+ + 2H, 98). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.44 (s, 12H), 5.55 (s, 2H), 7.09–7.49 (m, 8H).

Dye**5b**: yield 80%. Analysis: calculated for $C_{34}H_{28}O_2N_2$; C, 82.23; H, 5.68; N, 5.64. Found: C, 82.22; H, 5.79; N, 5.52. MALDI *m/z* 496.1 (M^+, 100), 497.1 (M^+ + H, 72). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.72 (s, 12H), 5.70 (s, 2H), 7.41–8.16 (m, 12H).

Dye**5c**: yield 80%. Analysis: calculated for $C_{26}H_{22}O_6N_4$; C, 64.19; H, 4.56; N, 11.52. Found: C, 64.39; H, 4.67; N, 11.39. MALDI *m/z* 487.0 (M^+ + H, 57), 488.0 (M^+ + 2H, 100). Low resolution of the ^1H NMR spectra due to the low solubility of the dye in DMSO-d_6 and CD_3Cl.

Dye**5d**: yield 67%. Analysis: calculated for $C_{30}H_{30}O_4N_4$; C, 70.57; H, 5.92; N, 10.97. Found: C, 69.20; H, 5.99; N, 10.61. MALDI *m/z* 510.9 (M^+, 100), 511.9 ($M^+ + H$, 80). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.42 (s, 12H), 2.03 (s, 6H), 5.51 (s, 2H), 7.17 (d, $J = 8.0$ Hz, 2H), 7.43 (dd, $J = 8.0$ Hz, $J = 1.5$ Hz, 2H), 7.69 (d, $J = 1.5$ Hz, 2H), 9.98 (s, 2H).

Dye**5e**: yield 45%. Analysis: calculated for $C_{34}H_{40}O_2N_2$; C, 80.28; H, 7.93; N, 5.51. Found: C, 79.84; H, 7.86; N, 5.18. MALDI *m/z* 509.1 ($M^+ + H$, 100), 510.2 ($M^+ + 2H$, 89). ^1H NMR (250 MHz, CD_3Cl) δ (ppm): 0.95 (t, $J = 6.3$ Hz, 6H), 1.32–1.40 (m, 4H), 1.45 (s, 12H), 1.52–1.69 (m, 4H), 2.65 (t, $J = 6.3$ Hz, 4H), 5.40 (s, 2H), 7.04–7.09 (m, 6H).

Dye**7a**: yield 76%. Analysis: calculated for $C_{27}H_{24}O_3N_2$; C, 76.40; H, 5.70; N, 6.60. Found: C, 75.60; H, 5.73; N, 6.47. MALDI *m/z* 424.1 (M^+, 100), 447.2 ($M^+ + Na$, 98). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.50 (s, 12H), 5.97 (s, 2H), 7.21–7.41 (m, 8H).

Dye**7b**: yield 64%. Analysis: calculated for $C_{35}H_{28}O_3N_2$; C, 80.13; H, 5.38; N, 5.34. Found: C, 79.85; H, 5.28; N, 5.24. MALDI *m/z* 637.1 ($M^+ + C_4H_9 - 2H$, 100). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.50 (s, 12H), 5.96 (s, 2H), 7.21–7.56 (m, 12H).

Dye**7c**: yield 70%. Analysis: calculated for $C_{27}H_{22}O_7N_4$; C, 63.03; H, 4.31; N, 10.89. Found: C, 62.80; H, 4.48; N, 10.39. MALDI *m/z* 627.4 ($M^+ + 2C_4H_9 - 2H$, 100). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.58 (s, 12H), 6.15 (s, 2H), 7.74 (d, $J = 8.7$ Hz, 2H), 8.31 (dd, $J = 8.7$ Hz, $J = 2.5$ Hz, 2H), 8.55 (d, $J = 2.5$ Hz, 2H).

Dye**7d**: yield 35%. Analysis: calculated for $C_{31}H_{30}O_5N_4$; C, 69.13; H, 5.61; N, 10.40. Found: C, 68.40; H, 5.45; N, 10.20. MALDI *m/z* 651.6 ($M^+ + 2C_4H_9 - H$, 100). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.47 (s, 12H), 2.05 (s, 6H), 5.95 (s, 2H), 7.42–7.51 (m, 4H), 7.79 (s, 2H), 10.10 (s, 2H).

Dye**7e**: yield 63%. Analysis: calculated for $C_{35}H_{40}O_3N_2$; C, 78.33; H, 7.51; N, 5.23. Found: C, 77.97; H, 7.58; N, 5.05. MALDI *m/z* 536.2 ($M^+ - H$, 100), 537.2 (M^+, 62). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 0.83–0.94 (m, 6H), 1.29–1.38 (m, 4H), 1.47 (s, 12H), 1.55–1.61 (m, 4H), 2.62 (t, $J = 7.8$ Hz, 4H), 5.92 (s, 2H), 7.18 (d, $J = 8.4$ Hz, 2H), 7.36–7.40 (m, 4H).

Dye**7f**: yield 36%. Analysis: calculated for $C_{29}H_{28}O_5N_2$; C, 71.88; H, 5.82; N, 5.78. Experimental: C, 71.52; H, 5.77; N, 5.49. MALDI *m/z* 541.4 ($M^+ + C_4H_9 - H$, 100). ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.48 (s, 12H), 3.80 (s, 6H), 5.89 (s, 2H), 6.93 (dd, $J = 8.6$ Hz, $J = 2.0$ Hz, 2H), 7.24 (d, $J = 2.0$ Hz, 2H), 7.44 (d, $J = 8.56$ Hz, 2H).

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