Dyes and Pigments xxx (2013) 1–5

Contents lists available at SciVerse ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Short communication

# Synthesis and characterization of new rhodamine dyes with large Stokes shift

Zhidan Tian, Baozhu Tian, Jinlong Zhang*

*Key Laboratory for Advanced Materials and Institute of Fine Chemicals, East China University of Science and Technology, Shanghai 200237, China*

ARTICLE INFO

*Article history:*
Received 14 March 2013
Accepted 14 June 2013
Available online xxx

*Keywords:*
Rhodamine
1, 4-Diethyl-1, 2, 3, 4-tetrahydroquinoxaline
Fluorescence
Stokes shift
Synthesis
Intramolecular charge transfer

ABSTRACT

Two new rhodamine dyes (Rh Q-H, Rh Q-Me) containing 1, 4-diethyl-1, 2, 3, 4-tetrahydroquinoxaline as an effective electron donor are designed and synthesized. The structures of the novel compounds are confirmed by $^{1}H$ NMR, $^{13}C$ NMR and ESI. Due to an excited-state intramolecular charge transfer (ICT), the new dyes exhibit longer absorption (>580 nm) and emission (>640 nm) compared with the model compounds, rhodamine 101 and rhodamine 6G. The new rhodamine dyes show large Stokes shift of 40–50 nm in commonly used solvents. Notably, when measured in a mixture of $H_2O$/EtOH solution, significant stokes shift of 65–68 nm are achieved, which is among the largest Stokes shifts ever reported for rhodamine dyes.



## 1. Introduction

Rhodamine dyes are widely used as laser dyes [1], fluorescence standards [2], single-molecule imaging agents [3], fluorescent markers in biological studies [4,5] and chemosensors [6,7] due to their excellent photophysical properties, such as long absorption and emission wavelength, high fluorescence quantum yield, large extinction coefficient and high photostability. The absorption and emission of the classic rhodamine dyes are only in the range of 500–600 nm and have a small Stokes shift of 20–40 nm. Their fluorescence detection sensitivity is severely compromised by background signals caused by biological autofluorescence. This limitation renders their application for biological imaging in living systems. Thus, it is highly desirable to develop rhodamine analogues with absorption and emission beyond 600 nm. It is known that the addition of π-conjugation system, as well as the formation of rigid rings in organic dyes can shift their absorption and emission maxima to longer wavelength. Based on this hypothesis, some groups have reported a novel class of highly fluorescent rhodamine derivatives with absorption beyond 600 nm [8,9]. In another important respect, the substituents on the amino group profoundly affect the absorption and emission wavelength of the traditional rhodamine dyes. Rhodamine with strong electron-donating amino group has pronounced bathochromic shift of absorption and emission bands, the emission wavelengths of these classic dyes are in the order of rhodamine 101 > rhodamine B > rhodamines 6G. Besides, the absorption and emission bands are considerably shifted to the red if tetrafluorophthalic anhydride is used for rhodamine synthesis instead of phthalic anhydride [10]. Moreover, amidation [11] and esterification [12] of benzoic acid in rhodamine dyes also result in a red shift in the absorption and emission bands. Unfortunately, these typical chemical modifications of the rhodamine dyes which lead to red-shifted absorption and emission are not helpful in increasing the Stokes shift. Design and synthesis of new rhodamine dyes simultaneously with long emission and large Stokes shift is a challenging issue.

1, 4-Diethyl-1, 2, 3, 4-tetrahydroquinoxaline has been reported as an effective electron donor in styryl [13], coumarin [14] and squaraine dyes [15], which surprisingly shifts their absorption and emission to longer wavelength and greatly increases their thermal stability and Stokes shift. In this work, we present the synthesis of two new rhodamine dyes based on 1, 4-diethyl-1, 2, 3, 4-tetrahydroquinoxaline. Their UV–vis absorption and fluorescence emission spectroscopic behavior in different solvents have been investigated. Compared with the traditional rhodamine dyes, these new dyes exhibit unique properties.

## 2. Experimental

### 2.1. Chemicals and instruments

4-Methoxy-2-nitroaniline was purchased from Aladdin. The other chemicals were of the highest grade available and were used

* Corresponding author. Tel./fax: +86 21 64252062.
*E-mail address:* jlzhang@ecust.edu.cn (J. Zhang).

0143-7208/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.dyepig.2013.06.013

without further purification. All employed solvents were analytically pure and were employed without any further drying or purification.

Reactions were monitored by TLC. Flash chromatography separations were carried out using silica gel (200–300 mesh). $^1$H NMR and $^{13}$C NMR spectra were recorded on Brucker AM-400 MHz instruments with tetramethylsilane as internal standard. ESI was performed using a Waters LCT Premier XE spectrometer. Absorption spectra were carried out on an SHIMADZU UV–Vis spectrophotometer. Fluorescence spectra were measured on an SHIMADZU RF-5301PC Fluorescence spectrophotometer.

### 2.2. Synthesis

The synthesis of target compounds, Rh Q-H and Rh Q-Me were achieved by the route outlined in Scheme 1.

#### 2.2.1. Synthesis of 6-methoxyquinoxaline (**3**)

4-Methoxy-2-nitroaniline **1** (6.70 g, 25 mmol) and Raney Nickel (1.00 g) in methanol (140 mL) were mixed and heated to 60 °C. After that, hydrazinehydrate (85%, 8.00 mL) was added dropwise into the solution within 30 min. The reaction mixture was then stirred at 60 °C for 2 h. After cooling, the reaction mass was filtered to separate the catalyst and then the filtrate concentrated with a rotavapor to get 4-Methoxy-1, 2-phenylenediamine **2.** Compound **2** dissolved in acetonitrile (100 mL) and glyoxal (40%, 13.00 mL) was added to this solution. The reaction mixture was then stirred at 60 °C for 6 h and cooled. The solvent was removed in a rotary evaporator and the dark brown sticky solid obtained was purified by silica gel chromatography (EtOAc) to get white crystals (5.40 g, 85%), m.p. 59–61 °C.

#### 2.2.2. Synthesis of 1, 4-diethyl-6-methoxy-1, 2, 3, 4-tetrahydroquinoxaline (**4**)

1, 4-diethyl-6-methoxy-1, 2, 3, 4-tetrahydroquinoxaline **4** was synthesized according to reported method [16].

#### 2.2.3. Synthesis of 1, 4-diethyl-6-hydroxy-1, 2, 3, 4-tetrahydroquinoxalin (**5**)

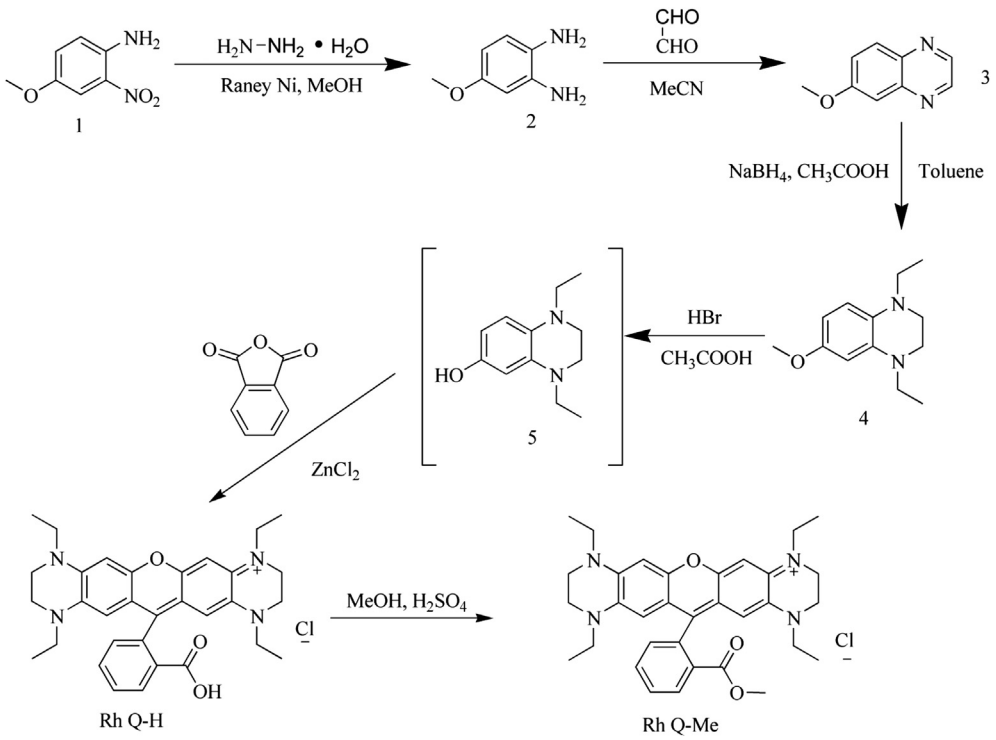
1, 4-diethyl-6-methoxy-1, 2, 3, 4-tetrahydroquinoxaline **4** (2.20 g, 0.01 mol), acetic acid (10 mL), hydrobromic acid (45%, 5 mL) were mixed, and then the ensuing mixture was stirred under an inert atmosphere at reflux for 6 h. The solvent was evaporated under reduced pressure. The crude product was unstable and directly used for the next step without further purification.

#### 2.2.4. Synthesis of Rh Q-H

1, 4-diethyl-6-hydroxy-1, 2, 3, 4-tetrahydroquinoxalin **5** (1.03 g, 5 mmol), $ZnCl_2$ (0.68 g, 5 mmol) and phthalic anhydride (0.74 g, 5 mmol) were heated at 160 °C for 2 h. After cooling, the reaction mixture was dissolved in DMF (10 mL), and then the mixture was added dropwise into the water (50 mL (10% NaCl, 1% HCl) w%) while stirring. The resulting dark precipitate was collected and dried in vacuo to give crude product. Rh Q-H furnished as a dark powder was purified by silica gel chromatography (DCM/MeOH = 20:1, V/V), and then recrystallized from ethanol to get golden crystals (0.25 g, 17.8% yield). $^1$H NMR (400 MHz, DMSO-d6, TMS): $\delta$ 8.12 (s, 1H), 7.64 (m, 2H), 7.23 (d, $J$ = 6.56 Hz, 1H), 6.87 (s, 2H), 5.93 (s, 2H), 3.60 (m, 8H), 3.24 (m, 4H), 3.01 (m, 4H), 1.21 (t, $J$ = 6.99 Hz, 6H), 0.86 (t, $J$ = 6.70 Hz, 6H). $^{13}$C NMR (100 MHz, DMSO-d6): $\delta$ 151.63, 144.58, 133.96, 130.57, 130.05, 129.08, 114.30, 103.05, 93.97, 47.11, 46.08, 44.77, 43.64, 10.46, 8.98. ESI found 525.3$[M - Cl]^+$ calculated for $C_{32}H_{37}N_4O_3^+$: 525.29.

#### 2.2.5. Synthesis of Rh Q-Me

Rh Q-H (0.10 g, 0.19 mmol), methanol (3 mL) and 0.1 mL $H_2SO_4$ were refluxed under an inert atmosphere for 24 h. After cooling, the reaction mixture was added dropwise into the water (20 mL, 10% NaCl w%) while stirring. The resulting golden precipitate was collected and dried in vacuo to give the pure product (0.08 g, 78.0%). $^1$H NMR (400 MHz, DMSO-d6, TMS): $\delta$ 8.23 (d, $J$ = 7.01 Hz, 1H), 7.91 (t, $J$ = 7.52 Hz, 1H),




**Scheme 1.** Synthetic route for Rh Q-H and Rh Q-Me.

Please cite this article in press as: Tian Z, et al., Synthesis and characterization of new rhodamine dyes with large Stokes shift, Dyes and Pigments (2013), http://dx.doi.org/10.1016/j.dyepig.2013.06.013

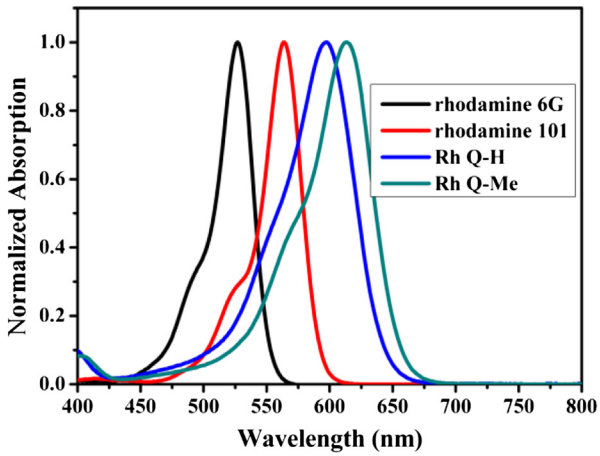



**Fig. 1.** The normalized UV–Vis absorption spectra of Rh Q-H, Rh Q-Me, rhodamine 101 and rhodamine 6G (ca. $10^{-5}$ mol/L) in EtOH.

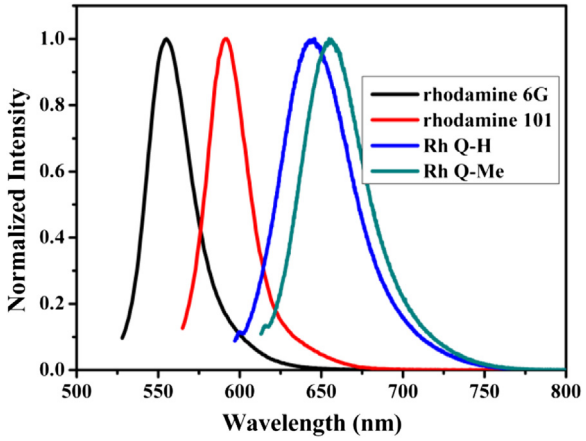



**Fig. 2.** The normalized fluorescence emission spectra of Rh Q-H, Rh Q-Me, rhodamine 101 and rhodamine 6G (ca. $10^{-5}$ mol/L) in EtOH excited with 597 nm, 613 nm, 594 nm, 527 nm respectively.

7.82 (t, $J$ = 7.66 Hz, 1H), 7.48 (d, $J$ = 7.56 Hz, 1H), 6.78 (s, 2H), 5.84 (s, 2H), 3.66 (m, 8H), 3.33(s, 3H), 3.29 (m, 4H), 3.06 (m, 4H), 1.24 (t, $J$ = 7.05 Hz, 6H), 0.88 (t, $J$ = 7.00 Hz, 6H). $^{13}$C NMR (100 MHz, DMSO-d6): $\delta$ 165.34, 151.80, 149.26, 144.95, 134.43, 134.34, 133.07, 130.56, 130.48, 130.09, 129.72, 114.20, 101.48, 94.18, 52.26, 47.09, 46.29, 44.74, 43.62, 10.52, 8.60. ESI found 539.3[M − Cl]$^+$ calculated for C33H39N4O3$^+$: 539.29.

## 3. Results and discussion

### 3.1. UV–Vis absorption and fluorescence spectra

The UV–vis absorption spectra of Rh Q-H and Rh Q-Me in EtOH are studied (Fig. 1). Close examination reveals that the shape of the absorption spectrum of Rh Q-H is highly resemble to that of rhodamine 6G and rhodamine 101. The intense absorption band with maximum at 597 nm of Rh Q-H is assigned to the 0–0 band of the S1 ← S0 transition, and a (less pronounced) shoulder peak on the high-energy side (at around 560 nm) is attributed to the 0–1 vibrational band of the same transition. Rh Q-Me shows an intense absorption band with maximum at 613 nm, which is assigned to the 0–0 band of the S1 ← S0 transition. A less pronounced shoulder peak on the high-energy side at around 570 nm is also observed, which is attributed to the 0–1 vibrational band of the same transition. Compared to Rh Q-H, although the absorption spectrum is similar, the absorption of Rh Q-Me shows 15 nm red shift, which is attributed to the esterification of benzoic acid. Rh Q-H and Rh Q-Me show a more red-shifted absorption than rhodamine 101 and rhodamine 6G. Rh Q-H shows absorption maxima at 597 nm, which is 33 nm red-shifted compared with rhodamine 101. The absorption wavelength of rhodamine dyes is highly dependent on the substituents on the amino group, and the absorption maxima is also increased with an increasing electron-donating ability. In Rh Q-H and Rh Q-Me, the electron-donating ability of the 1, 2, 3, 4-tetrahydroquinoxaline substituent is much stronger than julolidine.

The fluorescence emission profiles of Rh Q-H and Rh Q-Me in EtOH are shown in Fig. 2. Rh Q-H exhibits an intense emission peak with maximum at 644 nm, which extends to an onset of 780 nm. Compared with rhodamine 101, the emission spectrum of Rh Q-H is drastically bathochromic shifted for 50 nm while the spectrum shape is maintaining the same. Similar emission bands are observed for Rh Q-Me and maximum peak is 656 nm and further extends to 790 nm. Both Rh Q-H and Rh Q-Me show a more red-shifted absorption than rhodamine 101, which is attributed to a much stronger electron-donating ability of the 1, 2, 3, 4-tetrahydroquinoxaline substituent than julolidine.

Table 1 shows the Stokes shifts of Rh Q-H, Rh Q-Me, rhodamine 101 and rhodamine 6G. The traditional rhodamine dyes have small Stokes shifts, as shown in Table 1, 30 nm for rhodamine 101 and 28 nm for rhodamine 6G. The Stokes shifts of Rh Q-H and Rh Q-Me are increased to 47 nm and 43 nm respectively. This might be attributed to an excited-state intramolecular charge transfer (ICT). ICT effect is greatly enhanced in these new rhodamine dyes because of the strong electron donors. To the best of our knowledge, this is among the largest Stokes shifts ever reported for the rhodamine dyes, without resorting to the FRET effect [17,18] and TBET effect [19,20].

**Table 1**
$\lambda_{ab}$ and $\lambda_{em}$ values of Rh Q-H, Rh Q-Me, rhodamine 101 and rhodamine 6G in various solvents.

| Solvent | Rh Q-H | | | Rh Q-Me | | | Rhodamine 101 | | | Rhodamine 6G | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\lambda_{ab}$ (nm) | $\lambda_{em}$ (nm) | $\Delta\lambda$ (nm)[a] | $\lambda_{ab}$ (nm) | $\lambda_{em}$ (nm) | $\Delta\lambda$ (nm) | $\lambda_{ab}$ (nm) | $\lambda_{em}$ (nm) | $\Delta\lambda$ (nm) | $\lambda_{ab}$ (nm) | $\lambda_{em}$ (nm) | $\Delta\lambda$ (nm) |
| $CH_2Cl_2$ | 603 | 652 | 49 | 616 | 655 | 39 | 578 | 603 | 25 | 520 | 546 | 26 |
| MeCN | 604 | 653 | 49 | 614 | 660 | 46 | 576 | 603 | 27 | 523 | 552 | 28 |
| EtOH | 597 | 644 | 47 | 613 | 656 | 43 | 564 | 594 | 30 | 527 | 555 | 28 |
| MeOH | 598 | 645 | 47 | 612 | 656 | 44 | 565 | 592 | 27 | 525 | 555 | 30 |
| DMF | 601 | 651 | 50 | 622 | 664 | 42 | 573 | 588 | 15 | 532 | 562 | 30 |
| DMSO | 603 | 652 | 49 | 626 | 668 | 42 | 585 | 612 | 27 | 537 | 567 | 30 |
| $H_2O$/EtOH | 588 | 656 | 68 | 597 | 662 | 65 | 574 | 601 | 15 | 525 | 555 | 30 |

[a] $\Delta\lambda$(nm) = Stokes shift.

Please cite this article in press as: Tian Z, et al., Synthesis and characterization of new rhodamine dyes with large Stokes shift, Dyes and Pigments (2013), http://dx.doi.org/10.1016/j.dyepig.2013.06.013

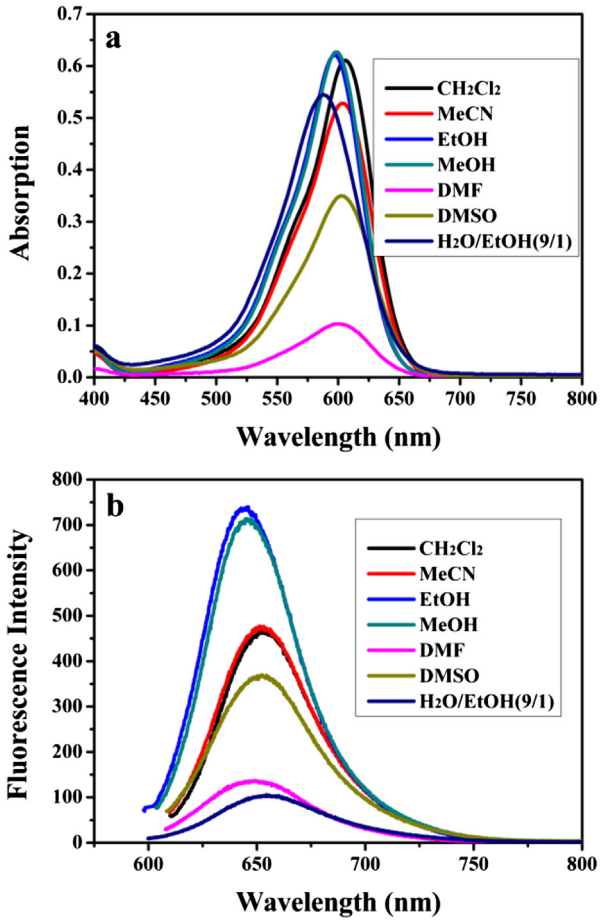



**Fig. 3.** UV–vis absorption (a) and fluorescence emission spectra (b) of Rh Q-H (ca. $10^{-5}$ mol/L) in different solvents.

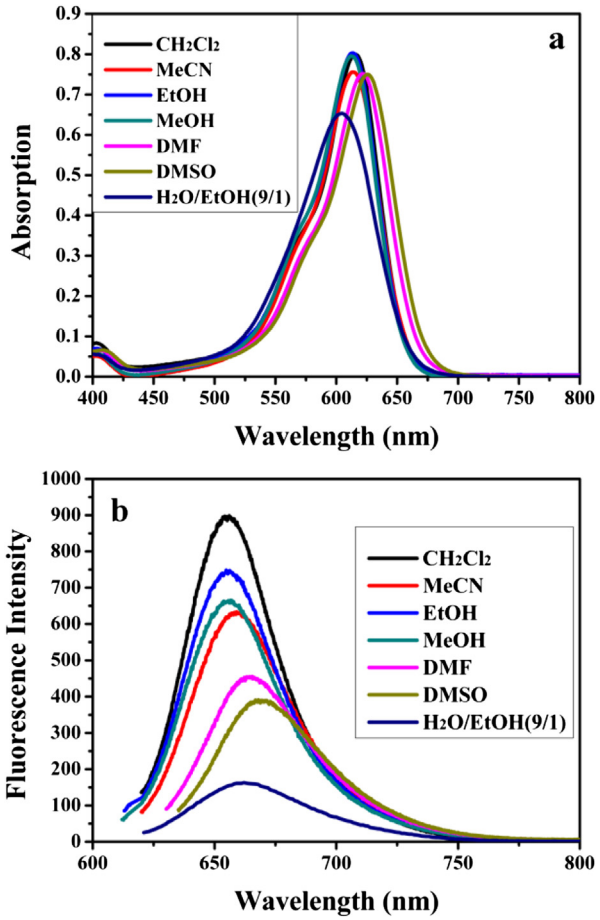



**Fig. 4.** UV–vis absorption (a) and fluorescence emission spectra (b) of Rh Q-Me (ca. $10^{-5}$ mol/L) in different solvents.

### 3.2. Polarity sensitivity of the absorption and fluorescence

The absorption and fluorescence emission profiles of Rh Q-H in distinct solvents ($CH_2Cl_2$, MeCN, EtOH, MeOH, $H_2O$/EtOH (9/1, V/V)) are shown in Fig. 3. The absorption and emission of Rh Q-H depend greatly on the polarity of solvents. In DMF, Rh Q-H shows very low absorption and fluorescence for parts of Rh Q-H molecules transfer into spirocyclic form which is essentially colorless and nonfluorescent. The transformation mechanism in DMSO consists with that in DMF, but to a less degree than in DMF. In water solution, Rh Q-H shows a strong absorption but very weak fluorescence, while in EtOH and MeOH, Rh Q-H shows stronger absorption and fluorescence than those in other solvents. These properties might be due to hydrogen-bonding interaction between the solvents and the dye molecules [21]. The Stokes shift in these solutions is 47–50 nm except for $H_2O$/EtOH in which 68 nm shift is achieved. Collectively, it proved that an excited-state intramolecular charge transfer (ICT) should take place in this case.

The absorption and fluorescence emission profiles of Rh Q-Me in distinct solvents ($CH_2Cl_2$, MeCN, EtOH, MeOH, $H_2O$/EtOH (9/1, V/V)) are also studied (Fig. 4). Different from Rh Q-H, the absorption of Rh Q-Me is not sensitive to the polarity of the solution. In particular, Rh Q-Me still keep high absorption in DMF and DMSO, due to that the esterification of benzoic acid restrains rhodamine change into spirocyclic form which is essentially colorless. Extinction coefficient and fluorescence intensity of Rh Q-Me are higher than those of Rh Q-H, which could be attributed to the esterification of benzoic acid. Fluorescence intensity in DMF, MDSO, $H_2O$/EtOH is lower than in other solvents. The emission can be quenched by the ICT effect, which is more significant in polar solvents. The Stokes shift of Rh Q-Me is 3–10 nm smaller than that of Rh Q-H, but the Stokes shift still have 65 nm in $H_2O$/EtOH.

## 4. Conclusions

We have synthesized and characterized two new rhodamine dyes (Rh Q-H, Rh Q-Me). This investigation constitutes the first use of 1, 4-diethyl-1, 2, 3, 4-tetrahydroquinoxaline as an amino auxochrome group in rhodamine dyes. These dyes show more excellent photophysical performance than the traditional rhodamine dyes with both absorption and emission in the long region. Especially, the Stokes shifts of these new dyes are 40–68 nm in different solvents, which are significantly larger than those of the traditional rhodamine dyes. The introduction of the two amino derivatives in the same benzene of the rhodamine is an effective method to change the character of the dyes.

## Acknowledgements

This work was supported by Science and Technology Commission of Shanghai Municipality (11142201100, 10520709900), and the Fundamental Research Funds for the Central Universities.

## References

[1] Drexhage KH. What's ahead in laser dyes. Laser Focus 1973;9:35–9.

[2] Karstens T, Kobs K. Rhodamine B and rhodamine 101 as reference substances for fluorescence quantum yield measurements. J Phys Chem 1980;84:1871–2.
[3] Bossi M, Folling J, Belov VN, Boyarskiy VP, Medda R, Egner A, et al. Multicolor far-field fluorescence nanoscopy through isolated detection of distinct molecular species. Nano Lett 2008;8:2463–8.
[4] Zhou ZG, Yu MX, Yang H, Huang KW, Li FY, Yi T, et al. FRET-based sensor for imaging chromium(III) in living cells. Chem Commun 2008:3387–9.
[5] Liu W, Howarth M, Greytak AB, Zheng Y, Nocera DG, Ting AY, et al. Compact biocompatible quantum dots functionalized for cellular imaging. J Am Chem Soc 2008;130:1274–84.
[6] Dujols V, Ford F, Czarnik AW. A long-wavelength fluorescent chemodosimeter selective for Cu (II) ion in water. J Am Chem Soc 1997;119:7386–7.
[7] Beija M, Afonso CAM, Martinho JMC. Synthesis and applications of rhodamine derivatives as fluorescent probes. Chem Soc Rev 2009;38:2410–33.
[8] Liu JX, Diwu ZJ, Leung WY, Lu Y, Patcha B, Hauglanda RP. Rational design and synthesis of a novel class of highly fluorescent rhodamine dyes that have strong absorption at long wavelengths. Tetrahedron Lett 2003;44:4355–9.
[9] David E, Lejeune J, Pellet-Rostaing S, Schulz J, Lemaire M, Chauvin J, et al. Synthesis of fluorescent rhodamine dyes using an extension of the Heck reaction. Tetrahedron Lett 2008;49:1860–4.
[10] Nakata E, Nazumi Y, Yukimachi Y, Uto Y, Maezawa, Hashimoto T, et al. Synthesis and photophysical properties of new SNARF derivatives as dual emission pH sensors. Bioorg Med Chem Lett 2011;21:1663–6.
[11] Nguyen T, Francis MB. Practical synthetic route to functionalized rhodamine dyes. Org Lett 2003;5(18):3245–8.
[12] Chen X, Wu QZ, Henschke L, Weber G, Weil T. An efficient and versatile approach for the preparation of a rhodamine B ester bioprobe library. Dyes Pigm 2012;94:296–303.
[13] Satam V, Rajule R, Bendre S, Bineesh P, Kanetkar V. Synthesis and application of novel styryl dyes derived from 1, 4-diethyl-1, 2, 3, 4-tetrahydro-6-methoxy-quinoxaline. J Heterocyclic Chem 2009;46:221–5.
[14] Jagtap AR, Satam VS, Rajule RN, Kanetkar VR. Synthesis of highly fluorescent coumarinyl chalcones derived from 8-acetyl-1, 4-diethyl-1, 2, 3, 4-tetrahydro-7H-pyrano-[2, 3-g]quinoxalin-7-one and their spectral characteristics. Dyes Pigm 2011;91:20–5.
[15] Chandrasekaran Y, Dutta GK, Kanth RB, Patil S. Tetrahydroquinoxaline based squaraines: synthesis and photophysical properties. Dyes Pigm 2009;83:162–7.
[16] Jagtap AR, Satam VS, Rajule RN, Kanetkar VR. The synthesis and characterization of novel coumarin dyes derived from 1, 4-diethyl-1, 2, 3, 4-tetrahydro-7-hydroxy-quinoxalin-6-carboxaldehyde. Dyes Pigm 2009;82:84–9.
[17] Long LL, Lin WY, Chen BB, Gao WS, Lin Y. Construction of a FRET-based ratiometric fluorescent thiol probe. Chem Commun 2011;47:893–5.
[18] Yu HB, Xiao Y, Guo HY, Qian XH. Convenient and efficient FRET platform featuring a rigid biphenyl spacer between rhodamine and BODIPY: transformation of 'Turn-On' sensors into ratiometric ones with dual emission. Chem-Eur J 2011;7(11):3179–91.
[19] Lin WY, Yuan L, Cao ZM, Feng YM, Song JZ, Song JZ. Through-bond energy transfer cassettes with minimal spectral overlap between the donor emission and acceptor absorption: coumarin-rhodamine dyads with large pseudo-Stokes shifts and emission shifts. Angew Chem Int Ed 2010;49(2):375–9.
[20] Jiao GS, Thoresen LH, Burgess K. Fluorescent, through-bond energy transfer cassettes for labeling multiple biological molecules in one experiment. J Am Chem Soc 2003;125(48):14,668–14,669.
[21] Peng XJ, Song FL, Lu EH, Wang YN, Zhou W, Fan JL, et al. Heptamethine cyanine dyes with a large Stokes shift and strong fluorescence: a paradigm for excited-state intramolecular charge transfer. J Am Chem Soc 2005;127:4170–1.

Please cite this article in press as: Tian Z, et al., Synthesis and characterization of new rhodamine dyes with large Stokes shift, Dyes and Pigments (2013), http://dx.doi.org/10.1016/j.dyepig.2013.06.013