Tetrahedron Letters 51 (2010) 3304–3308

Contents lists available at ScienceDirect

Tetrahedron Letters

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

# Water solubilization of xanthene dyes by post-synthetic sulfonation in organic media

Anthony Romieu [a,b,*], Delphine Tavernier-Lohr [c], Stéphane Pellet-Rostaing [d,*], Marc Lemaire [c], Pierre-Yves Renard [a,b,e]

[a] Equipe de Chimie Bio-Organique, COBRA-CNRS UMR 6014 & FR 3038, rue Lucien Tesnière, 76131 Mont-Saint-Aignan, France
[b] Université de Rouen, Place Emile Blondel, 76821 Mont-Saint-Aignan, France
[c] ICBMS, UMR 5246, Université Claude Bernard Lyon1, CPE, 43 Boulevard du 11 Novembre 1918, 69100 Villeurbanne, France
[d] ICSM, UMR 5257, Bâtiment 426, BP 17171, 30207 Bagnols Sur Ceze Cedex, France
[e] Institut Universitaire de France, 103 Boulevard Saint-Michel, 75005, Paris, France

ARTICLE INFO

*Article history:*
Received 22 March 2010
Revised 13 April 2010
Accepted 19 April 2010
Available online 24 April 2010

*Keywords:*
Fluorescein
Fluorescence
Rhodamines
Sulfonated linker
Water solubility

ABSTRACT

Highly water-soluble fluorescent fluorescein and rhodamine dyes were synthesized through amidification of their carboxylic acid functionality with original di- or tri-sulfonated amino linkers derived from taurine or α-sulfo-β-alanine. This post-synthetic derivatization was performed in organic media both to minimize the premature hydrolysis and to suppress the precipitation of the involved active ester of fluorophore, frequently encountered using standard Schotten–Baumann conditions.



## 1. Introduction

Fluorescent organic dyes are widely used as non-radioactive labels and as a key component of optical bio-probes for various biosensing and imaging applications.[1] Xanthene-based dyes such as rhodamines[2] and fluoresceins[3] (and more recently rhodols[4] and rosamines[5]) are among the most commonly used class of fluorescent detection reagents. Their spectral properties, especially in the near-infrared (NIR) range and/or in physiological conditions, are often less optimal than those exhibited by BODIPY and cyanine dyes. However, their use is preferred when the following valuable properties are required: (1) high photo- and chemical stability, (2) easy reversible formation of a colorless and non-fluorescent spirocyclic structure, and (3) modulation of fluorescence properties through reversible chemical modification (e.g., amidation or esterification) of aniline (for rhodamines) or phenol (for fluoresceins) moiety. Indeed, the latter two features are essential for the current design of spectroscopic off-on type probes (also named fluorogenic probes, latent or pro-fluorophores)[6] used as chemo- or biosensors for detecting various analytes (enzymes, heavy metal ions, etc.).[7]

For most biological applications, xanthene dyes must also exhibit both good water solubility and resistance to the formation of non-fluorescent dimers and higher aggregates, especially after conjugation to biological material.[8] Consequently, recent efforts have been devoted to the development of water-soluble analogues of rhodamines, through the introduction of some ionizable hydrophilic groups (carboxylic acid and/or sulfonic acid) within the xanthene core structure of these fluorescent dyes.[9] Most of them are commercialized by Molecular Probes (Invitrogen) and sold under the trade name Alexa Fluor®.[10] However, such chemical functionalization often requires: (1) the use of aggressive reagents such as oleum or concentrated sulfuric acid often not compatible with the stability of the fluorophore, or (2) restarting the synthesis of the fluorescent core from the beginning with building blocks bearing the water-solubilizing groups (de novo approach). Thus, these synthetic processes often lead to the desired fluorophore in a modest overall yield and are not amenable to large-scale preparation (i.e., gram scale). Recently, we have reported a straightforward method to enhance the water solubility of organic dyes (BODIPYs, cyanines and rhodamines) by a post-synthetic chemical derivatization of their carboxylic acid functionality with a poly-sulfonated peptide-based linker (i.e., α-sulfo-β-alanine di- or tripeptide) under standard Schotten–Baumann conditions.[11,12] This methodology has not yet been applied to the fluorescein derivatives and high hydrophobic extended conjugated rhodamines. However, the corresponding water-soluble analogues of these latter long-wavelength fluorophores could be useful as laser dyes and fluorescent

* Corresponding authors. Tel.: +33 2 35 52 24 15; fax: +33 2 35 52 29 71 (A.R.).
*E-mail address:* anthony.romieu@univ-rouen.fr (A. Romieu).

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.04.080

markers in numerous bio-labeling, bio-imaging, and single-molecule-based spectroscopy applications.

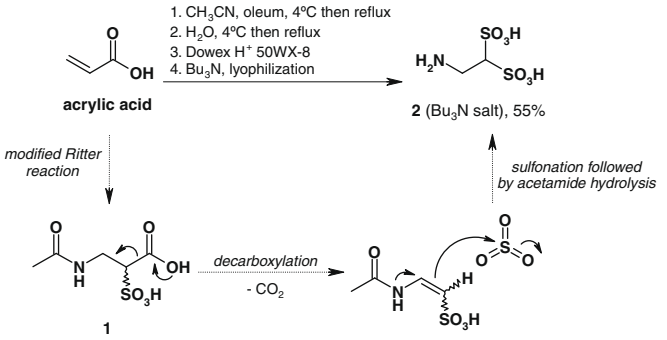
In this Letter, we report the water-solubilization of fluorescein, benzo[*b*]thiophene-, and indole-containing rhodamine dyes, through amidification of their carboxylic acid function with 2-aminoethane-1,1-disulfonic acid and tripeptide (α-sulfo-β-alanine)$_3$, respectively. The use of tributylammonium salts of these two poly-sulfonated linkers was considered in order to perform the derivatization reaction in organic media (instead of a mixture of DMF and aq buffer), especially to prevent both the premature hydrolysis and precipitation of the involved *N*-hydroxysuccinimide (NHS) active ester of hydrophobic rhodamine dyes, previously observed by us when the reaction was conducted under standard Shcotten–Baumann conditions. The spectral properties of the resulting water-soluble xanthene dyes were then evaluated under physiological conditions.

## 2. Results and discussion

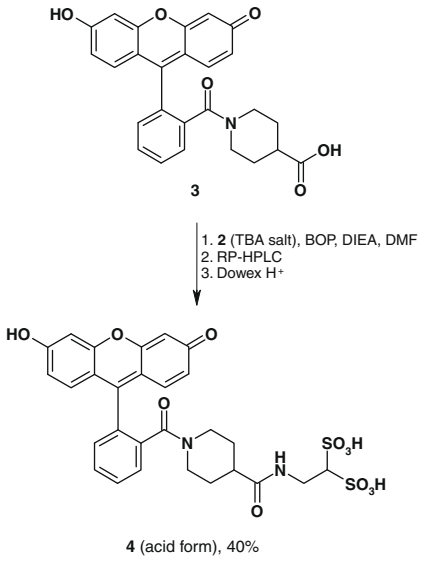
### 2.1. Synthesis of water-soluble fluorescein derivative 4

First, we focused on the water-solubilization of fluorescein. To our knowledge, only the di-sulfonated rhodamine derivative developed by Molecular Probes and named Alexa Fluor® 488 is currently used as an hydrophilic substitute of this xanthene dye. In order to offer an alternative to this expensive commercial fluorescent marker, we have explored the post-synthetic sulfonation of xanthamide derivative **3** with the original di-sulfonated amino linker **2** (Scheme 2). In the present case, it is not essential to use a water-solubilizing linker bearing a terminal reactive group for bioconjugation because the ultimate goal of this synthesis was to provide a new phenol-based fluorophore useful for the preparation of fluorogenic probes based on the pro-fluorescent concept (involving the reversible modification of its phenol moiety). The use of a fluorescein derivative bearing a tertiary amide of isonipecotic acid at the 2′-position instead of the current carboxyl group was preferred to provide steric protection and so to improve the photostability of fluorescein.[13] Furthermore, this is the most common way to avoid the undesired cyclization equilibrium leading to the formation of the colorless and non-fluorescent spirolactone (especially at pH <6). Synthesis of 2-aminoethane-1,1-disulfonic acid **2** was accomplished in one step from acrylic acid, oleum, and acetonitrile using the conditions reported by Wagner et al. for the preparation of α-sulfo-β-alanine through a modified Ritter reaction (Scheme 1).[14] Yet, in our hands, such experimental conditions did not provide this amino acid but the taurine-like di-sulfonated linker **2**. Since the heating temperature of the sulfonation reaction mixture was not indicated by Wagner et al. in their publication (dating from 1968), we chose to heat the reaction mixture under reflux and we suspect that under those harsh experimental conditions, once *N*-acetyl derivative of α-sulfo-β-alanine **1** was formed by the modified Ritter reaction, a spontaneous decarboxylation followed by the sulfonation reaction occurred, leading to **2** (Scheme 1). The structure of **2** was confirmed by detailed measurements, including ESI mass spectrometry and NMR analyses. This compound was converted into the corresponding tributylammonium salt (TBA salt) and dissolved in dry *N*-methylpyrrolidone (NMP) to yield a stock solution (0.5 M) suitable for post-synthetic derivatizations in organic media.

Thereafter, di-sulfonated amino linker **2** could be readily coupled to fluorescein derivative **3** by using BOP phosphonium salt[15] and DIEA in dry DMF to give the desired water-soluble analogue **4** in a satisfying yield (40%). Purification and desalting of **4** were sequentially achieved by semi-preparative reversed-phase HPLC (RP-HPLC) and ion-exchange chromatography (Dowex H$^+$), respectively. The introduction of the di-sulfonated linker onto the isonipecotic acid arm of fluorescein **4** was confirmed by $^1$H NMR and ESI mass analyses.




**Scheme 1.** Synthesis of 2-aminoethane-1,1-disulfonic acid and proposed mechanism for its formation.




**Scheme 2.** Synthesis of water-soluble fluorescein **4**.

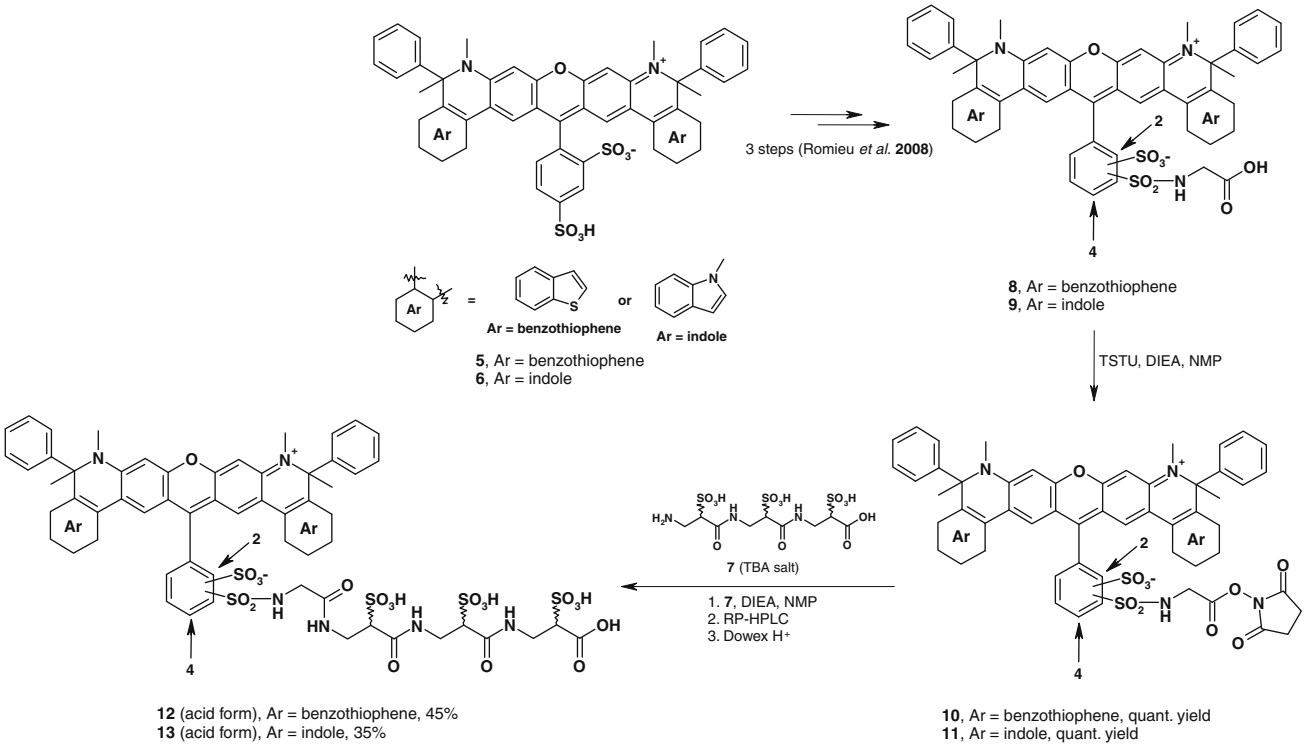
### 2.2. Synthesis of water-soluble far-red emitting rhodamine derivatives 12 and 13

To demonstrate the full potential of this water-solubilizing procedure performed in organic media, the post-synthetic sulfonation of benzo[*b*]thiophene- and indole-containing rhodamine dyes **5** and **6** was also investigated. These two compounds belong to a novel class of highly fluorescent rhodamine derivatives with long-wavelength absorption and emission, reported in 2003 by Liu et al.[16] We have recently developed an alternative pathway to these valuable far-red emitting fluorophores, requiring an aryl–aryl coupling through C–H activation followed by a Pictet–Spingler reaction, to facilitate the synthetic access to these new rigid rhodamine derivatives in fewer steps.[17] Our preliminary experiments aiming at the post-synthetic derivatization of these fluorophores with poly-sulfonated amino linkers under previously reported standard Schotten–Baumann conditions failed. This was due to the complete precipitation of the corresponding NHS esters in the mixture of DMF and aq buffer. Furthermore, the small amounts of derivatized fluorophores that we obtained showed that their great hydrophobic character could not be countered by the introduction of only two sulfonate groups.

Consequently, conjugation of these rhodamines to tri-sulfonated linker **7** in organic media was considered. This α-sulfo-β-alanine tripeptide was easily prepared on a gram scale according to standard peptide synthesis protocols (Fmoc strategy).[11] Its conversion into TBA salt was achieved using the protocol applied to 2-aminoethane-1,1-disulfonic acid **2**, and a 0.35 M solution of the organic solubilized tri-sulfonated linker in dry NMP was prepared. Since the sulforhodamine sulfonyl chlorides are of delicate use for direct conjugation to amine-containing compounds, we first considered the conversion of **5** and **6** into their carboxylic acid derivatives **8** and **9**, respectively, through the introduction of a glycine spacer. Indeed, the activation of these latter reactive dyes is more convenient and the small-scale implementation of the subsequent coupling reaction (less than 10 mg of starting reactive fluorophore) with the valuable tri-sulfonated linker **7** will be easier because the corresponding NHS ester is less sensitive to moisture than the sulfonyl chloride derivative. Thus, rhodamine carboxylic acids **8** and **9** were obtained using the three-step procedure reported by us for sulforhodamine 101[12] (Scheme 3). Thereafter, **8** and **9** were quantitatively converted into the corresponding NHS esters **10** and **11**, respectively, by treatment with the peptide coupling uronium reagent TSTU and DIEA in dry NMP. Finally, the crude mixture of NHS ester **10** (or **11**) was subjected to aminolysis with tripeptide (α-sulfo-β-alanine)$_3$ in the presence of DIEA to give the water-soluble rhodamine **12** (or **13**). RP-HPLC purification using triethylammonim bicarbonate (TEAB) buffer and acetonitrile as eluents, followed by desalting on ion-exchange resin Dowex H$^+$ provided **12** and **13** in a pure form (mixture of two regioisomers, each consisting of four racemic diastereomers, isolated yield: 45% for **12** and 35% for **13**). This non-quantitative yield for the present post-synthetic sulfonation although it was carried out in an organic medium was explained both by the moderate reactivity of NHS ester toward tri-sulfonated amino linker (steric hindrance?) and by significant losses of derivatized fluorophore during the chromatographic purification and lyophilization steps. Indeed, this is the main unavoidable drawback of reactions performed at the micromole scale. The structures of **12** and **13** were unambiguously confirmed by ESI mass analyses and their purity (>95%) was checked by RP-HPLC.

### 2.3. Photophysical properties of the synthesized water-soluble fluorescent dyes

As expected, fluorescein and rhodamine derivatives **4**, **12**, and **13** were found to be perfectly soluble in water and related aq buffers in the concentration range (1 μM to 10 mM) suitable for bio-labeling applications. The photophysical properties of these three novel water-soluble fluorophores were evaluated under simulated physiological conditions (i.e., phosphate buffered saline (PBS), pH 7.5) and collected in Table 1. As would be expected from previous studies, the presence of a hydrophilic linker on the peripheral benzene ring does not affect the spectral properties for the dyes except for the indole-based rhodamine **13** (Fig. 1). Indeed, molar absorption coefficients ($\varepsilon$) are comparable to those of the parent fluorophores, and quantum yields ($\Phi_F$) are good especially for the water-soluble rhodamine emitting beyond 600 nm. Interestingly, the value of brightness ($\varepsilon \times \Phi_F$) for **12** (39,600 $M^{-1}$ $cm^{-1}$) is of the same order as those reported for sulfoindocyanine dyes Cy 5.0[18] (50,000 $M^{-1}$ $cm^{-1}$) and Cy 5.5[19] (43,700 $M^{-1}$ $cm^{-1}$), the most popular water-soluble NIR fluorophores for bio-imaging applications. The excitation spectrum of **12** matches its absorption spectrum (see supplementary material).This confirms the absence of H-aggregates in this case. Surprisingly, despite the presence of the same tri-sulfonated tail, **13** shows a tendency to aggregate in aqueous solution, which issued in a quenching of its fluorescence (Fig. 1b). The observed blue shift in the absorption maximum at 602 nm is in keeping with the formation of H-dimers.[19] This aggregation is persistent even at concentrations as low as 1 μM in PBS. The differences in aggregation ability that we observed between **12** and **13** are probably due to the nature and orientation of




**Scheme 3.** Synthesis of water-soluble rhodamines **12** and **13**.

**Table 1**
Spectral properties of water-soluble xanthene dyes in physiological conditions

| Dye | Solvent | $\lambda_{max,\ abs}$ (nm) | $\lambda_{max,\ em}$ (nm) | Stokes shift (nm) | $\varepsilon$ ($M^{-1}\ cm^{-1}$) | $\Phi_F$[a] |
|---|---|---|---|---|---|---|
| **4** | PBS | 499 | 522 | 23 | 59,000 | 0.69 |
| **12** | PBS | 628 | 645 | 17 | 90,000 | 0.44 |
| **13** | PBS | 602, 643 | Nonfl | Nonfl | —[b] | Nonfl |
| **13** | PBS + 5% (w/v) BSA | 650 | 672 | 22 | —[b] | 0.19 |

[a] Determined at 25 °C by using either fluorescein (for **4**, $\Phi_F$ = 0.90 in 0.1 M NaOH, Ex. $\lambda$ = 488 nm) or sulfoindocyanine Cy 5.0 (for **12** and **13**, $\Phi_F$ = 0.20 in PBS, Ex. $\lambda$ = 600 nm) as standards.[18,24] All $\Phi_F$ are corrected for changes in refractive index.
[b] Quantity isolated was too small for a highly accurate measurement.

heterocycle (benzo[*b*]thiophene or *N*-methylindole) fused to the xanthene ring. The free fluorescent monomer of **13** is obtained in PBS using 5% (w/v) bovine serum albumin (BSA), an additive often used in buffers for mimicking body fluids (Fig. 1c). The strong enhancement in emission in conjunction with the red shift in the absorption maximum in the presence of BSA observed for **13** points to dye-BSA interactions. The beneficial effect of such dye-protein interactions on quantum yield has been already reported for series of cyanine labels commercialized by Dyomics.[20]

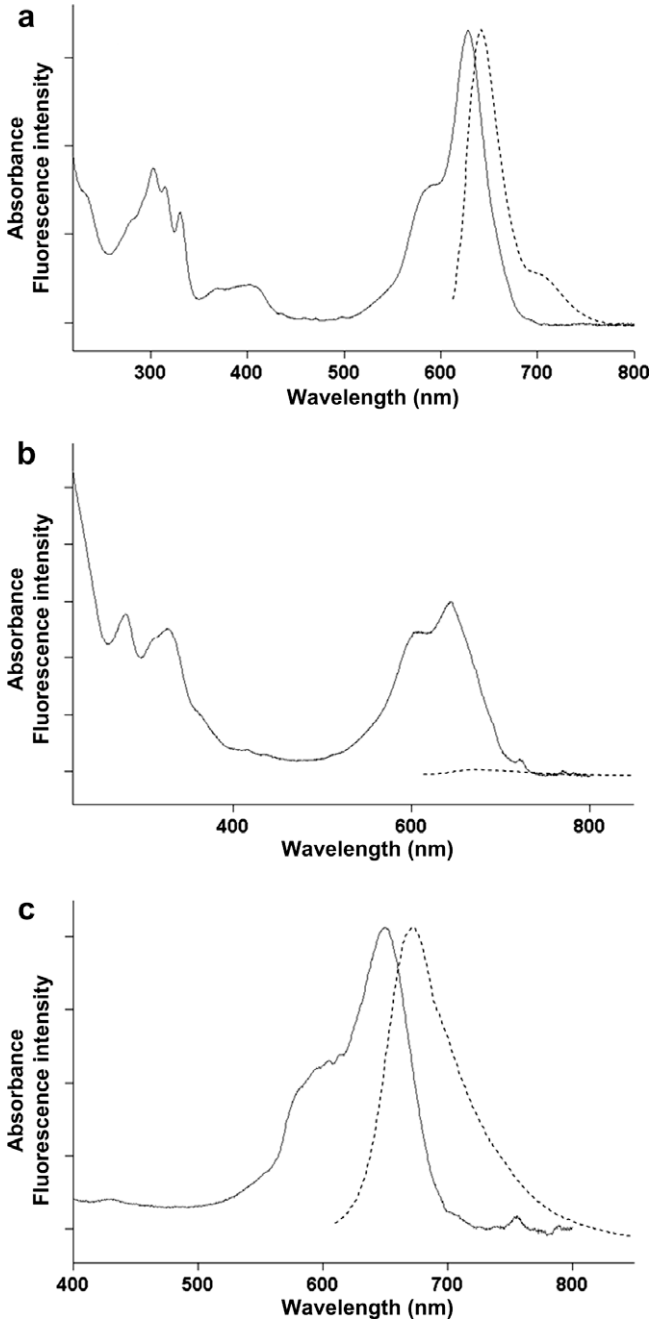
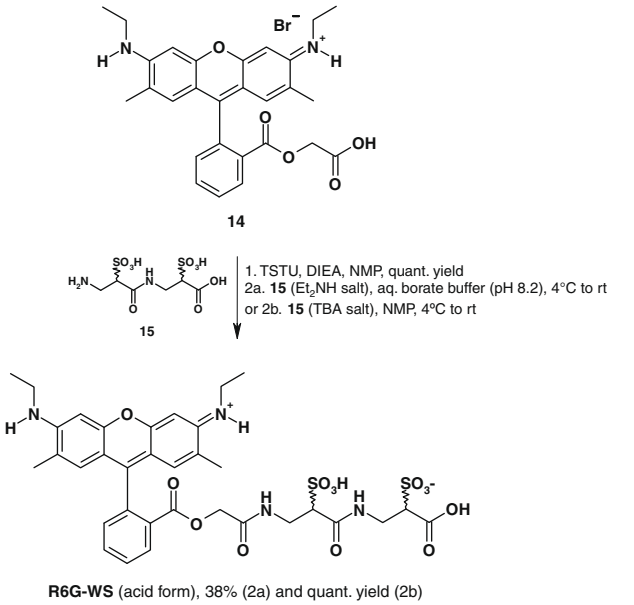



**Figure 1.** (a) Normalized UV–vis absorption (—) and emission (- - -) spectra of (Ex. $\lambda$ = 605 nm) **12** in PBS at 25 °C. (b) Normalized UV–vis absorption (—) and emission (- - -) spectra of (Ex. $\lambda$ = 605 nm) **13** in PBS at 25 °C. (c) Normalized UV–vis absorption (—) and emission (- - -) spectra of (Ex. $\lambda$ = 600 nm) **13** in PBS + 5% BSA (w/v) at 25 °C.

### 2.4. Alternative synthetic route to water-soluble rhodamine 6G (R6G-WS)

In addition to these promising results, we have also evaluated the positive effect (or not) on the reaction yield for the post-synthetic sulfonation performed in an aprotic polar solvent instead of a mixture of NMP and aq buffer as previously described. To this end, rhodamine 6G (**R6G**) carboxylic acid **14** was chosen as a model xanthene dye. Since, this compound is significantly less hydrophobic than benzo[*b*]thiophene- and indole-containing rhodamine dyes, its derivatization with dipeptide (α-sulfo-β-alanine)$_2$ **15** was considered (Scheme 4).[12] Thus, after the conversion of **14** into NHS ester, the crude mixture was divided into two equal parts and aminolysis with di-sulfonated amino linker was carried either in NMP (with the corresponding TBA salt of **15**) or in a mixture of NMP and borate buffer (0.1 M, pH 8.2). After RP-HPLC purification, desalting, and lyophilization, the recovered amount of **R6G-WS** is 2.7-fold higher for the reaction performed under non-aqueous conditions. This result clearly shows that the modifications brought to the original post-synthetic sulfonation procedure published in 2008[12] are effective to prevent competitive hydrolysis of fluorophore active ester and so to improve the yield of this water-solubilization process.




**Scheme 4.** Synthesis of **R6G-WS**.

In summary, this work highlights the utility to perform post-synthetic sulfonation reactions of sophisticated fluorescent cores in organic media, both to get valuable water-soluble fluorescent labels and to significantly improve the overall yield of this tunable and versatile synthetic method. The use of TBA salts of poly-sulfonated amino linkers enables to perform the aminolysis reaction in an anhydrous aprotic solvent (DMF, NMP, or DMSO) alone and not in mixture with an aq buffer. Thus, this improved method has a strong potential to enhance the water solubility of promising but highly hydrophobic fluorescent and/or redox active multi-component systems such as 'cascatelle' dyes[21] or through-bond energy transfer cassettes.[22] Furthermore, in this Letter, we describe for the first time the synthesis of 2-aminoethane-1,1-disulfonic acid. We believe that this original non-peptidyl di-sulfonated building block has a strong potential for water solubilization of fluorophores not suitable for bioconjugation applications but used as key components in the design of phenol-based pro-fluorophores[23] such as 7-hydroxycoumarin or (naphtho)fluorescein derivatives.

## Acknowledgments

This work was supported by La Région Haute Normandie via the CRUNCh program (CPER 2007–2013) and IUF. We thank Dr. G. Clavé for the synthesis of sulfoindocyanine dye Cy 5.0 (used as standard for QY measurements) and purification of **R6G-WS**, and QUIDD company for the open access to their HPLC instruments.

## Supplementary data

Supplementary data (detailed synthetic procedures, spectroscopic and photophysical characterizations of water-soluble xanthene dyes **4**, **12** and **13**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.04.080.

## References and notes

1. Lavis, L. D.; Raines, R. T. *ACS Chem. Biol.* **2008**, *3*, 142–155.
2. Beija, M.; Afonso, C. A. M.; Martinho, J. M. G. *Chem. Soc. Rev.* **2009**, *38*, 2410–2433.
3. For a recent example, see: Wu, X.-L.; Tian, M.; He, H.-z.; Sun, W.; Li, J.-l.; Shi, Z. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2957–2959.
4. For recent examples, see: (a) Peng, T.; Yang, D. *Org. Lett.* **2010**, *12*, 496–499; (b) Li, J.; Yao, S. Q. *Org. Lett.* **2009**, *11*, 405–408; (c) Kamino, S.; Ichikawa, H.; Wada, S.-i.; Horio, Y.; Usami, Y.; Yamaguchi, T.; Koda, T.; Harada, A.; Shimanuki, K.; Arimoto, M.; Doi, M.; Fujita, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4380–4384.
5. For recent examples, see: (a) Wu, L.; Burgess, K. *Org. Lett.* **2008**, *10*, 1779–1782; (b) Wu, L.; Burgess, K. *J. Org. Chem.* **2008**, *73*, 8711–8718; (c) Ahn, Y.-H.; Lee, J.-S.; Chang, Y.-T. *J. Am. Chem. Soc.* **2007**, *129*, 4510–4511.
6. For a review about the pro-fluorescence concept, see: Chen, X.; Sun, M.; Ma, H. *Curr. Org. Chem.* **2006**, *10*, 477–489.
7. For recent reviews, see: Reymond, J.-L.; Fluxa, V. S.; Maillard, N. *Chem. Commun.* **2009**, 34–46; Kim, H. N.; Lee, M. H.; Kim, H. J.; Kim, J. S.; Yoon, J. *Chem. Soc. Rev.* **2008**, *37*, 1465–1472; Johnsson, N.; Johnsson, K. *ACS Chem. Biol.* **2007**, *2*, 31–38.
8. For a comprehensive review about fluorescent labeling of biomolecules, see: Goncalves, M. S. T. *Chem. Rev.* **2009**, *109*, 190–212.
9. For recent examples, see: (a) Kolmakov, K.; Belov, V. N.; Bierwagen, J.; Ringemann, C.; Mueller, V.; Eggeling, C.; Hell, S. W. *Chem. Eur. J.* **2010**, *16*, 158–166; (b) Boyarskiy, V. P.; Belov, V. N.; Medda, R.; Hein, B.; Bossi, M.; Hell, S. W. *Chem. Eur. J.* **2008**, *14*, 1784–1792; (c) Bandichhor, R.; Petrescu, A. D.; Vespa, A.; Kier, A. B.; Schroeder, F.; Burgess, K. *J. Am. Chem. Soc.* **2006**, *128*, 10688–10689; (d) Bandichhor, R.; Petrescu, A. D.; Vespa, A.; Kier, A. B.; Schroeder, F.; Burgess, K. *Bioconjugate Chem.* **2006**, *17*, 1219–1225.
10. (a) Berlier, J. E.; Rothe, A.; Buller, G.; Bradford, J.; Gray, D. R.; Filanoski, B. J.; Telford, W. G.; Yue, S.; Liu, J.; Cheung, C.-Y.; Chang, W.; Hirsch, J. D.; Beechem, J. M.; Haugland, R. P. *J. Histochem. Cytochem.* **2003**, *51*, 1699–1712; (b) Panchuk-Voloshina, N.; Haugland, R. P.; Bishop-Stewart, J.; Bhalgat, M. K.; Millard, P. J.; Mao, F.; Leung, W.-Y.; Haugland, R. P. *J. Histochem. Cytochem.* **1999**, *47*, 1179–1188.
11. Niu, S. L.; Ulrich, G.; Ziessel, R.; Kiss, A.; Renard, P.-Y.; Romieu, A. *Org. Lett.* **2009**, *11*, 2049–2052.
12. Romieu, A.; Brossard, D.; Hamon, M.; Outaabout, H.; Portal, C.; Renard, P.-Y. *Bioconjugate Chem.* **2008**, *19*, 279–289.
13. Gao, J.; Wang, P.; Giese, R. W. *Anal. Chem.* **2002**, *74*, 6397–6401.
14. Wagner, D.; Gertner, D.; Zilkha, A. *Tetrahedron Lett.* **1968**, 4875–4876.
15. For a comprehensive review on peptide coupling reagents, see: Valeur, E.; Bradley, M. *Chem. Soc. Rev.* **2009**, *38*, 606–631.
16. Liu, J.; Diwu, Z.; Leung, W.-Y.; Lu, Y.; Patch, B.; Haugland, R. P. *Tetrahedron Lett.* **2003**, *44*, 4355–4359.
17. David, E.; Lejeune, J.; Pellet-Rostaing, S.; Schulz, J.; Lemaire, M.; Chauvin, J.; Deronzier, A. *Tetrahedron Lett.* **2008**, *49*, 1860–1864.
18. Mujumdar, R. B.; Ernst, L. A.; Mujumdar, S. R.; Lewis, C. J.; Waggoner, A. S. *Bioconjugate Chem.* **1993**, *4*, 105–111.
19. Mujumdar, S. R.; Mujumdar, R. B.; Grant, C. M.; Waggoner, A. S. *Bioconjugate Chem.* **1996**, *7*, 356–362.
20. Pauli, J.; Vag, T.; Haag, R.; Spieles, M.; Wenzel, M.; Kaiser, W. A.; Resch-Genger, U.; Hilger, I. *Eur. J. Med. Chem.* **2009**, *44*, 3496–3503.
21. (a) Goze, C.; Ulrich, G.; Ziessel, R. *J. Org. Chem.* **2007**, *72*, 313–322; (b) Ulrich, G.; Goze, C.; Guardigli, M.; Roda, A.; Ziessel, R. *Angew. Chem., Int. Ed.* **2005**, *44*, 3694–3698.
22. For recent examples, see: (a) Lin, W.; Yuan, L.; Cao, Z.; Feng, Y.; Song, J. *Angew. Chem., Int. Ed.* **2010**, *49*, 375–379; (b) Barin, G.; Yilmaz, M. D.; Akkaya, E. U. *Tetrahedron Lett.* **2009**, *50*, 1738–1740; (c) Jiao, G.-S.; Thoresen, L. H.; Kim, T. G.; Haaland, W. C.; Gao, F.; Topp, M. R.; Hochstrasser, R. M.; Metzker, M. L.; Burgess, K. *Chem. Eur. J.* **2006**, *12*, 7816–7826.
23. For recent examples, see: (a) Albers, A. E.; Dickinson, B. C.; Miller, E. W.; Chang, C. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5948–5950; (b) Huang, S.-T.; Peng, Y.-X.; Wang, K.-L. *Biosens. Bioelectron.* **2008**, *23*, 1793–1798; (c) Huang, S.-T.; Ting, K.-N.; Wang, K.-L. *Anal. Chim. Acta* **2008**, *620*, 120–126; (d) Meyer, Y.; Richard, J.-A.; Massonneau, M.; Renard, P.-Y.; Romieu, A. *Org. Lett.* **2008**, *10*, 1517–1520; (e) Richard, J.-A.; Massonneau, M.; Renard, P.-Y.; Romieu, A. *Org. Lett.* **2008**, *10*, 4175–4178; (f) Richard, J.-A.; Meyer, Y.; Jolivel, V.; Massonneau, M.; Dumeunier, R.; Vaudry, D.; Vaudry, H.; Renard, P.-Y.; Romieu, A. *Bioconjugate Chem.* **2008**, *19*, 1707–1718.
24. Olmsted, J., III *J. Phys. Chem.* **1979**, *83*, 2581–2584.