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ORIGINAL PAPER

Ferric hydrogensulphate as a recyclable catalyst for the synthesis of fluorescein derivatives

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Polycondensation reactions of phenols with phthalic anhydride were carried out in the presence of ferric hydrogensulphate under melt conditions. The reactions proceeded in short reaction times by using a catalytic amount of $Fe(HSO_4)_3$ and the corresponding fluorescein derivatives were obtained in high yields. The simplicity, scale-up, along with the use of an inexpensive, non-toxic, recyclable catalyst of an environmentally benign nature, are other remarkable features of the procedure. The absorption and emission properties of these fluorescein derivatives were studied.

Keywords: fluorescein, ferric hydrogensulphate, eosin, rhodamines

Introduction

Among the many fluorescent compounds, fluorescein is known to have a high quantum yield of fluorescence in aqueous solution and to be excitable at long wavelength (490 nm) (Sun et al., 1997). Fluorescein and other xanthene dyes are the most widely used fluorophores for labelling and sensing biomolecules (Eshghi et al., 2011; Kojima et al., 1998; Mugherli et al., 2006; Zaikova et al., 2001). Long-wavelength light does not cause severe cell damage (Tsien et al., 1995), and is convenient for fluorescence confocal microscopy using an Ar/ion laser. Furthermore, due to the enhanced optical transparency of tissue from approximately 650 nm to 900 nm (Weissleder et al., 2003), probes based on these far-red emitting dyes may find a use in a variety of in vivo imaging applications (Peng & Yang, 2010; Mizukami et al., 1999). There is a need for further development of new routes for the preparation of xanthene dyes that are water-soluble, have long wavelength absorption and emission, and are amenable to modification into fluorescence-based analyte sensors.

Fluoresceins and rhodamines are commonly synthesised by acid-catalysed condensation of resorcinol or 3-aminophenol with the substituted phthalic acid species. $MeSO_3H$ (Woodroofe et al., 2005; Tanaka et al., 2001), H_3PO_4 (Hilderbrand et al., 2007), $ZnCl_2$ (Gronowska & Dabkowska-Naskret, 1981; Sun et al., 1997; Woodroofe et al., 2005), $CaCl_2$ and $FeCl_3$ (Fatima et al., 2009), and microwave irradiation (Heller, et al., 2010; Cihelník et al., 2002) catalysed these condensations. However, some of these methods suffer from one or more disadvantages such as use of stoichiometric excess amounts of the reagents, long reaction times, high temperatures and low yields.

Continuing our findings on acid-catalysed reactions by ferric hydrogensulphate $Fe(HSO_4)_3$ (Eshghi, 2006; Eshghi et al., 2008a, 2008b, 2009a, 2009b; Rahimizadeh et al., 2009), in this paper we report on an efficient and simple method for the synthesis of fluorescein derivatives by condensation of phenols with phthalic anhydride (Fig. 1).

Experimental

The chemicals were either prepared in our laboratories or purchased from Merck (Germany), Fluka (Switzerland), and Aldrich (USA) chemical companies. All yields refer to isolated products. The products were characterised by comparison of their physical data with those of known compounds or by their spectral data. IR spectra were recorded on

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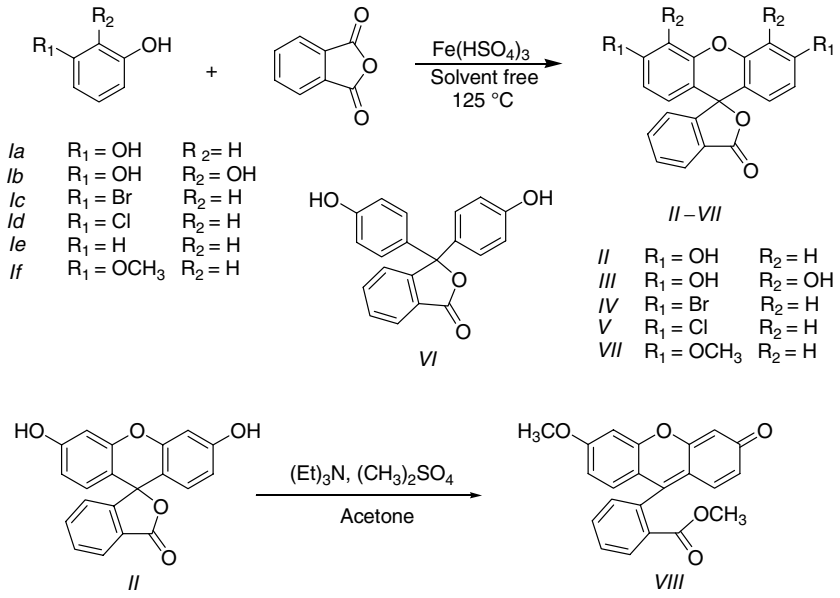

Fig. 1. Synthesis of fluorescein derivatives.

a Shimadzu-IR 470 (Japan) spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker 100-MHz (Germany) spectrometer in $CDCl_3$ (compounds *IV*, *V*, and *VIII*) and d_6-acetone (compounds *II*, *III*, and *VI*) solvents and TMS as internal standard. Ferric hydrogensulphate was prepared according to procedures reported previously (Eshghi, 2006; Eshghi et al., 2008b).

General procedure for synthesis of fluorescein derivatives II–VI

A well-ground mixture of phthalic anhydride (0.15 g, 1.0 mmol), phenols (2.0 mmol) and $Fe(HSO_4)_3$, (0.10 g, 0.30 mmol) was heated in an oil bath (125 °C) to melt. The mixture was maintained in this state for the periods of time specified in Table 2. After completion of the reaction (which was checked by TLC), the mixture was cooled and treated with methanol (5 mL). The catalyst was recovered by filtration, and washed with CH_2Cl_2 (10 mL). Water (10 mL) was added to the combined organic solutions and the products were extracted with CH_2Cl_2 (3 × 10 mL). The solvent was dried with Na_2SO_4 and evaporated to give the fluorescein derivatives. The final product was purified by recrystallisation from methanol (70 % yield). For example, a solution of crude fluorescein *IV* (417 mg, 0.91 mmol) was dissolved in 2.5 mL of hot MeOH. Fluorescein *IV* was obtained as yellowish–brown needle crystals (321 mg, 0.70 mmol, 70 % isolated yield) upon cooling of this solution to room temperature. The spectroscopic data reported were obtained using these crystalline samples.

Preparation of fluorescein VIII

Triethylamine (0.7 mL, 5 mmol) was added to a cooled solution of fluorescein *II* (2 mmol) in acetone (10 mL). After stirring for 5 min, dimethyl sulphate (0.5 mL, 5 mmol) was added drop-wise. The reaction mixture was refluxed for 2 h. Then the mixture was cooled and treated with water (10 mL). The product was extracted with CH_2Cl_2 (3 × 10 mL) and washed with water (2 × 10 mL). The solvent was dried with Na_2SO_4 and evaporated to give fluorescein *VIII* in 80 % yield. A solution of this crude material (290 mg, 0.80 mmol) was dissolved in 1.5 mL of hot MeOH. Crystalline fluorescein *VIII* was obtained as orange needles (238 mg, 0.66 mmol, 66 % isolated yield) upon cooling to room temperature. M.p. = 204–205 °C (M.p. = 206 °C, Miura et al. (2003)).The spectroscopic data reported were obtained using this crystalline sample.

Results and discussion

Condensation of phthalic anhydride with phenols in the presence of $Fe(HSO_4)_3$ under melt conditions gave fluorescein derivatives in high yields. To find optimum conditions, the one-pot reaction of phthalic anhydride and resorcinol (*Ia*) was carried out at different conditions. As shown in Table 1, the highest yield of fluorescein (*II*) was obtained after 30 min at 125 °C using 15 % molar equivalents of ferric hydrogensulphate as a catalyst under melt conditions.

Reusability of the catalyst was studied in the reaction of phthalic anhydride and resorcinol in the presence of $Fe(HSO_4)_3$. After each run was completed, the catalyst was simply recovered. As shown in Fig. 2, the catalyst could be reused without significant loss of its catalytic activity up to at least 4 times.

Various phenols (*Ia–Ie*) were allowed to react with phthalic anhydride under melt conditions to obtain the corresponding fluorescein derivatives in good

Table 1. Optimisation of reaction conditions and catalyst reusability in the reaction of phthalic anhydride and resorcinol

Entry	Temperature	Time	Catalyst equivalents	Yield[a]
	℃	min	%	(%)
1	110	30	15	63
2	125	30	15	75
3	135	30	15	75
4	125	10	15	45
5	125	20	15	65
6	125	45	15	75
7	125	30	5	60
8	125	30	10	67
9	125	30	20	76
10[b]	125	30	15	73
11[b]	125	30	15	72
12[b]	125	30	15	70

a) Isolated yields; *b*) reusability of recovered catalyst in new runs.

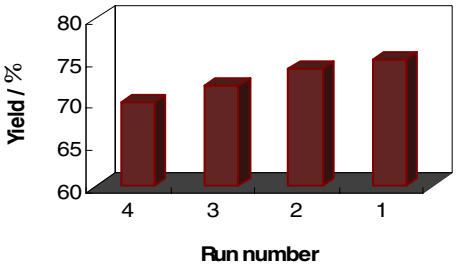

Fig. 2. Reusability of catalyst in the synthesis of fluorescein in new runs.

yields using a catalytic amount of $Fe(HSO_4)_3$. The results and properties of the corresponding products are summarized in Tables 2 and 3. Fluorescein (*II*), gallein (*III*), and phenolphthalein (*VI*) are water-soluble dyes under alkali conditions; halofluoresceins (*IV*) and (*V*) are water-insoluble dyes. Although phenols bearing electron-donating groups reacted successfully and afforded the products in high yields, reac-

Table 2. Results of polycondensation of phthalic anhydride and phenols in the presence of ferric hydrogensulphate[a]

Entry	Phenol	Product	Time	Temperature	Yield	Color and shape	M.p.
			h	℃	%		℃
1	*Ia*	*II*	0.5	125	75	Red powder	312–315, 314–316[b]
2	*Ib*	*III*	0.17	100	68	Brownish-red powder	> 300, > 300[b]
3	*Ic*	*IV*	13	160	70	Yellowish-brown needles	280–281, 277–280[c]
4	*Id*	*V*	13	160	70	Pale green needles	255–257, 257.6–258.1[d]
5	*Ie*	*VI*	1	125	77	Colourless powder	256, 256–262[b]

a) Isolated yields; *b*) Windholz (1976); *c*) Woodroofe et al. (2005); *d*) Gronowska and Dabkowska-Naskret (1981).

Table 3. Spectral data of prepared compounds

Compound	Spectral data
II	IR, $\tilde{\nu}$/cm^{-1}: 3400, 1645, 1620, 1470, 1275, 1250, 1220, 1120 ^{1}H NMR (acetone-d_6), δ: 6.5–6.8 (m, 6H), 7.25 (dd, 1H, J_1 = 7.5 Hz, J_2 = 1.5 Hz), 7.75 (m, 2H), 8.00 (dd, 1H, J_1 = 7.5 Hz, J_2 = 1.5 Hz), 9.2 (b, 2H, D_2O exchangeable) MS, m/z (I_r/%): 332 (28.5) (M^+)
III	IR, $\tilde{\nu}$/cm^{-1}: 3400, 1625, 1465, 1210, 1165, 1120, 1100 ^{1}H NMR (acetone -d_6), δ: 6.1 (d, 2H, J = 7.5 Hz), 6.6 (d, 2H, J = 7.5 Hz), 7.3 (dd, 1H, J_1 = 7.5 Hz, J_2 = 1.5 Hz), 7.5–7.9 (m, 6H, 4H, D_2O exchangeable), 7.9 (dd, 1H, J_1 = 7 Hz, J_2 = 1.5 Hz)
IV	IR, $\tilde{\nu}$/cm^{-1}: 1750, 1600, 1465, 1250, 1105, 1060, 950 ^{1}H NMR ($CDCl_3$), δ: 6.6 (d, 2H, J = 7.5 Hz), 7.15 (dd, 2H, J_1 = 7.5 Hz, J_2 = 1.5 Hz), 7.25 (t, 1H, J = 7.5 Hz), 7.5 (d, 2H, J = 1.5 Hz), 7.7 (m, 2H), 8.05 (dd, 1H, J_1 = 7.5 Hz, J_2 = 1.5 Hz)
V	IR, $\tilde{\nu}$/cm^{-1}: 1765, 1635, 1475, 1260, 1095, 1075 ^{1}H NMR ($CDCl_3$), δ: 6.9 (d, 2H, J = 8.5 Hz), 7.1 (dd, 2H, J_1 = 7.5 Hz, J_2 = 1.5 Hz), 7.2 (t, 1H, J = 7.5 Hz), 7.4 (d, 2H, J = 1.5 Hz), 7.6–7.8 (m, 2H), 8.05 (dd, 1H, J_1 = 7.5 Hz, J_2 = 1.5 Hz)
VI	IR, $\tilde{\nu}$/cm^{-1}: 3300, 1730, 1625, 1450, 1225, 1175, 1125 ^{1}H NMR (acetone -d_6), δ: 6.8 (d, 4H, J = 8 Hz), 7.1 (d, 4H, J = 8 Hz), 7.55 (dd, 1H, J_1 = 7.5 Hz, J_2 = 1.5 Hz), 7.70 (dt, 1H, J_1 = 7.5 Hz, J_2 = 1.5 Hz), 7.8 (t, 1H, J = 7.5 Hz), 7.90 (dd, 1H, J_1 = 7.5 Hz, J_2 = 1.5 Hz), 8.65 (bs, 2H, D_2O exchangeable)
VIII	IR, $\tilde{\nu}$/cm^{-1}: 1725, 1645, 1450, 1245, 1215, 1115, 1080 ^{1}H NMR ($CDCl_3$), δ: 3.63 (s, 3H), 3.91 (s, 3H), 6.5–6.9 (m, 6H), 7.2 (dd, 1H, J_1 = 7.5 Hz, J_2 =1.5 Hz), 7.5–7.9 (m, 2H), 8.20 (dd, 1H, J_1 = 7.5 Hz, J_2 = 1.5 Hz)

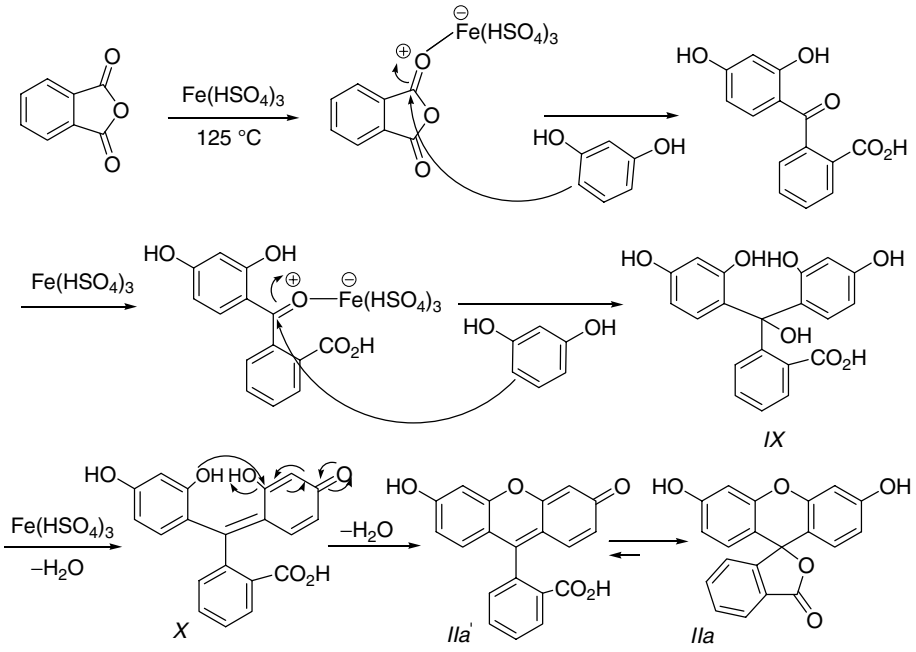

Fig. 3. Proposed mechanism of condensation reaction of phthalic acid and phenols.

Table 4. Synthesis of fluorescein derivatives catalysed by different catalysts in the one-pot polycondensation reaction of phthalic acid and phenols

Entry	Derivative	Catalyst	Conditions		Yield
			T/℃	t/h	%
1	*II*	$ZnCl_2$	150	–[a]	37.9[a]
2	*II*	$CaCl_2$	150	–[a]	–[a]
3	*II*	$FeCl_3$	150	–[a]; 0.5	58.4[a]; 61[b]
4	*II*	H_3PO_4	MW	0.0083	97[c]
5	*II*	$Fe(HSO_4)_3$	125	0.5	75[d]
6	*II*	–	125	1.0	12[d]
7	*IV*	CH_3SO_3H	140	16	43[e]
8	*IV*	$ZnCl_2$	120–125	9	35[f]
9	*IV*	$Fe(HSO_4)_3$	160	13	70[d]

a) Reaction times were not reported (Fatima et al., 2009); *b*) repeated for comparison with our catalyst; *c*) Cihelník et al. (2002); *d*) present work; *e*) Woodroofe et al. (2005); *f*) Gronowska and Dabkowska-Naskret (1981).

tion times varied profoundly depending on the phenol activities. As expected, the phenols with electron-withdrawing groups (e.g. 3-nitrophenol) failed in this polycondensation reaction unless allowed long reaction times (24 h). Phenol (*Ie*) gave phenolphthalein (*VI*) in good yield. *m*-Cresol failed to give the corresponding fluorescein derivative. 3-Methoxyphenol (*If*) was not available, so we tried to prepare the corresponding fluorescein (*VII*) by methylation of fluorescein (*II*). Alkylation of fluorescein (*II*) by dimethyl sulphate in the presence of triethylamine in acetone gave fluorescein derivative (*VIII*) in 80 % yield instead of fluorescein (*VII*) (Fig. 1).

The mechanism proposed for the synthesis of fluorescein is outlined in Fig. 3. Depending on the phenol derivatives which could reach the state of the quinone-like intermediate (*X*), it dehydrated rapidly to form the fluorescein derivatives. However, phenols gave intermediates (*IX*) and (*X*) with no *ortho*-hydroxyl groups, which did not dehydrate to fluorescein-type products and converted to phenolphthalein.

Comparison of this method with other similar methods, including the polycondensation reaction of phthalic anhydride and phenols, demonstrates that the ferric hydrogensulphate catalyst has appropriate effects on the efficiency of this reaction and is an efficient catalyst for the fluorescein derivatives synthesis (Table 4).

Next we examined the absorption and emission properties of fluorescein derivatives (*II*–*VI* and *VIII*) as chromophore and/or fluorophore species via UV-VIS spectroscopy and fluorimetric emission. Fig. 4 shows the UV-VIS absorption spectrum of these compounds at a concentration of 1×10^{-4} M in methanol

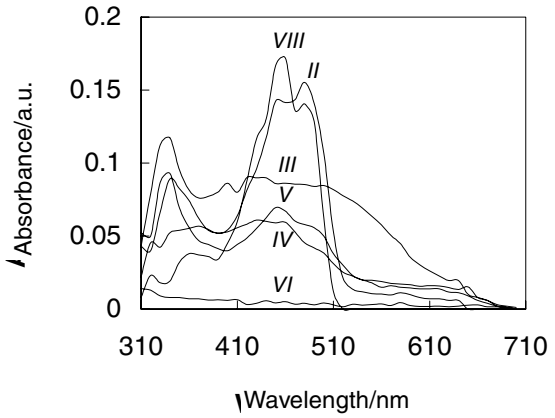

Fig. 4. UV-VIS spectra of fluorescein derivatives (*II*–*VI* and *VIII*) in methanol solution, $c = 0.1$ mM.

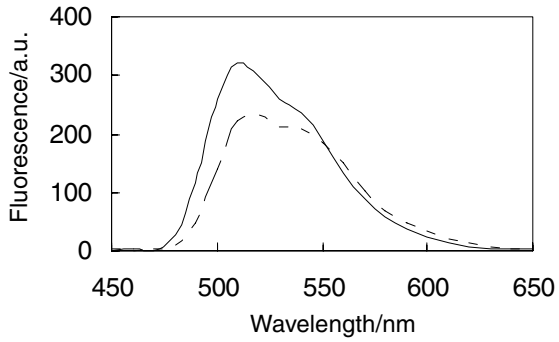

Fig. 5. Emission spectra of (— *II*) and (– – *VIII*), solution in methanol, $c = 5$ μM.

solution. Phenolphthalein (*VI*) had no important absorbance in the neutral environment; all the fluorescein derivatives studied showed visible absorption in the range of wavelengths examined.

Fig. 5 shows the fluorescence emission spectra of compounds *II* and *VIII* at $c = 5 \times 10^{-6}$ M in methanol as the solvent. The fluorescence excitation (λ_{ex}) wavelength at 456 nm was used for all compounds. Although compounds *II* and *VIII* showed intense emission spectra at 510 nm and 520 nm respectively, the intensity of this emission decreased in the other derivatives, which exhibited low-intensity emission spectra (not shown).

Conclusion

In conclusion, a convenient and efficient procedure has been developed for the synthesis of fluorescein derivatives by condensation of some phenols and phthalic anhydride using $Fe(HSO_4)_3$ under solvent-free conditions. The simplicity, scale-up, along with the use of an inexpensive, non-toxic, and recyclable catalyst of an environmentally benign nature, are other remarkable features of the procedure which can be extended to the synthesis of other organic compounds. Whereas compounds *II*–*V* and *VIII* showed visible absorption, only compounds*II* and *VIII* showed intense emission spectra in 510 nm and 520 nm respectively.

References

Cihelník, S., Stibor, I., & Lhoták, P. (2002). Solvent-free synthesis of sulfonephthaleins, sulfonefluoresceins and fluoresceins under microwave irradiation. *Collection of Czechoslovak Chemical Communications*, *67*, 1779–1789. DOI: 10.1135/cccc20021779.

Eshghi, H. (2006). Ferric hydrogensulfate catalysed Schmidt reaction of ketones to amides under solvent-free conditions. *Journal of the Chinese Chemical Society*, *53*, 987–990.

Eshghi, H., Bakavoli, M., & Moradi, H. (2009a). Ferric hydrogensulfate catalyzed aerobic oxidative coupling of 2-naphthols in water or under solvent free conditions. *Chinese Chemical Letters*, *20*, 663–667. DOI: 10.1016/j.cclet.2008.12.045.

Eshghi, H., Bakavoli, M., & Moradi, H. (2008a). $Fe(HSO_4)_3$: An efficient, heterogeneous and reusable catalyst for the synthesis of 14-aryl- or alkyl-14*H*-dibenzo[*a*,*j*]xanthenes. *Chinese Chemical Letters*, *19*, 1423–1426. DOI: 10.1016/j.cclet.2008.09.048.

Eshghi, H., Bakavoli, M., Moradi, H., & Davoodnia, A. (2009b). $Fe(HSO_4)_3$ and $Fe(HSO_4)_3$/DMSO as efficient, heterogeneous, and reusable catalyst systems for the oxidative coupling of thiols. *Phosphorus, Sulfur and Silicon and the Related Elements*, *184*, 3110–3118. DOI: 10.1080/10426500802704654.

Eshghi, H., Mirzaie, N., & Asoodeh, A. (2011). Synthesis of fluorescein aromatic esters in the presence of P_2O_5/SiO_2 as catalyst and their hydrolysis studies in the presence of lipase. *Dyes and Pigments*, *89*, 120–126. DOI: 10.1016/j.dyepig.2010.09.013.

Eshghi, H., Rahimizadeh, M., & Saberi, S. (2008b). $Fe(HSO_4)_3$ as an inexpensive, eco-friendly, heterogeneous and reusable catalyst for acetal/ketal formation and their facile regeneration. *Catalysis Communications*, *9*, 2460–2466. DOI: 10.1016/j.catcom.2008.06.015.

Fatima, K., Nosheen, S., Azhar, H., & Azhar, M. (2009). Synthesis and application of eosin. *Pakistan Journal of Agricultural Sciences*, 46, 1–7.

Gronowska, J., & Dabkowska-Naskret, H. (1981). Fluoran derivatives. Part IX. Synthesis of halofluorans. *Polish Journal of Chemistry*, *55*, 2151–2163.

Heller, E., Klöckner, J., Lautenschläger, W., & Holzgrabe, U. (2010). Online monitoring of microwave-enhanced reactions by UV/Vis spectroscopy. *European Journal of Organic Chemistry*, *2010*, 3569–3573. DOI: 10.1002/ejoc.201000441.

Hilderbrand, S. A., & Weissleder, R. (2007). One-pot synthesis of new symmetric and asymmetric xanthene dyes. *Tetrahedron Letters*, *48*, 4383–4385. DOI: 10.1016/j.tetlet.2007.04.088.

Kojima, H., Nakatsubo, N., Kikuchi, K., Kawahara, S., Kirino, Y., Nagoshi, H., Hirata, Y., & Nagano, T. (1998). Detection and imaging of nitric oxide with novel fluorescent indicators: diaminofluoresceins. *Analytical Chemistry*, *70*, 244–2453. DOI: 10.1021/ac9801723.

Miura, T., Urano, Y., Tanaka, K., Nagano, T., Ohkubo, K., & Fukuzumi, S. (2003). Rational design principle for modulating fluorescence properties of fluorescein-based probes by photoinduced electron transfer. *Journal of the American Chemical Society*, *125*, 8666–8671. DOI: 10.1021/ja035282s.

Mizukami, S., Kikuchi, K., Higuchi, T., Urano, Y., Mashima, T., Tsuruo, T., & Nagano, T. (1999). Imaging of caspase-3

activation in HeLa cells stimulated with etoposide using a novel fluorescent probe. *FEBS Letters*, *453*, 356–360. DOI: 10.1016/S0014-5793(99)00755-3.

Mugherli, L., Burchak, O. N., Chatelain, F., & Balakirev, M. Y. (2006). Fluorogenic ester substrates to assess proteolytic activity. *Bioorganic & Medicinal Chemistry Letters*, *16*, 4488–4491. DOI: 10.1016/j.bmcl.2006.06.037.

Peng, T., & Yang, D. (2010). HKGreen-3: A rhodol-based fluorescent probe for peroxynitrite. *Organic Letters*, *12*, 4932–4935. DOI: 10.1021/ol102182j.

Rahimizadeh, M., Eshghi, H., Bakhtiarpoor, Z., & Pordel, M. (2009). Ferric hydrogensulfate as a recyclable catalyst for the synthesis of some new bis(indolyl)methane derivatives. *Journal of Chemical Research*, *2009*, 269–270. DOI: 10.3184/030823409X430194.

Sun, W.-C., Gee, K. R., Klaubert, D. H., & Haugland, R. P. (1997). Synthesis of fluorinated fluoresceins. *The Journal of Organic Chemistry*, *62*, 6469–6475. DOI: 10.1021/jo9706178.

Tanaka, K., Miura, T., Umezawa, N., Urano, Y., Kikuchi, K., Higuchi, T., & Nagano, T. (2001). Rational design of fluorescein-based fluorescence probes. Mechanism-based design of a maximum fluorescence probe for singlet oxygen. *Journal of the American Chemical Society*, *123*, 2530–2536. DOI: 10.1021/ja0035708.

Tsien, R. Y., & Waggoner, A. (1995). Fluorophores for confocal microscopy. In J. B. Pawley (Ed.), *Handbook of biological confocal microscopy* (2nd ed., pp. 267–279). New York, NY, USA: Plenum Press.

Weissleder, R., & Ntziachristos, V. (2003). Shedding light onto live molecular targets. *Nature Medicine*, *9*, 123–128. DOI: 10.1038/nm0103-123.

Windholz, M. (1976). *The Merck index* (9th ed.). Rahway, NJ, USA: Merck.

Woodroofe, C. C., Lim, M. H., Bu. W., & Lippard, S. J. (2005). Synthesis of isomerically pure carboxylate- and sulfonate-substituted xanthene fluorophores. *Tetrahedron Letters*, *61*, 3097–3105. DOI: 10.1016/j.tet.2005.01.024.

Zaikova, T. O., Rukavishnikov, A. V., Birrell, G. B., Griffith, O. H., & Keana, J. F. W. (2001). Synthesis of fluorogenic substrates for continuous assay of phosphatidylinositol-specific phospholipase C. *Bioconjugate Chemistry*, *12*, 307–313. DOI: 10.1021/bc0001138.