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REVIEW ARTICLE

Water-soluble BODIPY and aza-BODIPY dyes: synthetic progress and applications

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Abstract In recent years, boron-dipyrromethene (BODIPY) and boron-azadipyrromethene (aza-BODIPY) dyes have attracted considerable multidisciplinary attention due to their diverse applications. By introducing various hydrophilic groups, such as quaternary ammonium, sulfonate or oligo-ethyleneglycol moieties into the BODIPY core, the solubilities of these dyes in aqueous solution can be greatly improved while maintaining their high fluorescence quantum yields. Accordingly, applying these fluorescent dyes in aqueous systems to areas such as chemosensors, biomacromolecule labeling, bio-imaging and photodynamic therapy has been achieved. In this article, the recent progress on the synthesis, optical properties and application of water-soluble BODIPY dyes and aza-BODIPY dyes is reviewed.

Keywords boron-dipyrromethene, boron-azadipyrromethene, synthetic progress, applications

1 Introduction

Water is one of the most essential substances for sustaining life and the environment on earth. Water-soluble fluorescent dyes are widely used to identify, label and detect targets in aqueous systems [1]. Compared to conventional fluorescent dyes such as fluorescein and rhodamine, boron-dipyrromethene (BODIPY) and boron-azabiprromethene (aza-BODIPY) dyes [2] have unique features such as strong absorption in the visible region, narrow emission bands with high quantum yields, and excellent physiological stability [3–6]. More interestingly, the absorption and emission maxima of these dyes can be shifted into the near infrared (NIR) region with appropriate chemical modifications. Thus, these dyes have served as valuable *in vivo* imaging probes [2].

Since the first BODIPY dye was synthesized by Treibs et al. in the 1960s [7], a large number of these types of dyes with different various functional groups have been synthesized [2]. Most of these BODIPY dyes are well-soluble in organic solvents, but not in water [8]. However, many applications like fluorescence imaging and metal ion detection [9–13] are performed in aqueous environments and are often hampered by the poor solubility of these dyes in water. To overcome this problem, the synthesis and characterization of new water-soluble BODIPY dyes is currently receiving a great deal of attention. The recent advances in this area are summarized in this review and the application potentials for BODIPY dyes are discussed.

2 Synthesis and optical properties of water-soluble BODIPY dyes

The boron-dipyrromethene core can be considered as a derivative of "rigidified" monomethine cyanine dyes [6] and is formed by the complexation of a dipyrromethene unit to boron trifluoride. In general, boron-dipyrromethenes are synthesized by using one of three strategies (Scheme 1) each of which starts from different compounds. The first strategy starts from a reaction between pyrroles and acid chlorides. In this reaction, an unstable dipyrromethene hydrochloride salt intermediate is formed by the condensation of the pyrrole and the acid chloride which subsequently complexes with boron trifluoride-diethyl etherate under basic conditions.

The second strategy starts from pyrroles and aldehydes. In this method, the pyrrole undergoes an acid catalyzed reaction with the aldehyde. Further oxidation with *p*-chloranil is then necessary to form the intermediate which then complexes with boron trifluoride-diethyl etherate.

The third strategy uses ketopyrroles. This approach is appropriate for the synthesis of unsymmetrical BODIPY

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dyes. The ketopyrrole intermediate undergoes a Lewis acid mediated condensation with another pyrrole moiety [14], and subsequently complexes with boron trifluoride-diethyl etherate in basic conditions.

In 2008, Wu and Burgess developed a new method for the preparation of symmetrical BODIPY dyes that involved treating pyrrole-2-carbaldehyde with a slight overdose of $POCl_3$ [15]. In some cases, yields of over 90% were achieved by this method.

In order to obtain water-soluble BODIPY dyes, hydrophilic groups need to be introduced into the chemical structures. BODIPY dyes containing ionic and neutral hydrophilic groups, such as quaternary ammonium salts, sulfonates, phosphonates and oligo-ethyleneglycol moieties have been reported. Water-soluble BODIPY sulfonates have been obtained through an electrophilic substitution reaction (e.g. sulfonation reaction) at the 2- and 6-positions (Scheme 1). Halogenation at the 2- and 6-positions or the 3- and 5-positions followed by a palladium-catalyzed coupling reaction has proven to be effective for introducing various hydrophilic groups. Alkynyl Grignard reagents can be also utilized to attach hydrophilic groups by substituting fluorine atoms onto the boron atom. Another effective method to introduce hydrophilic groups is a Knoevenagel condensation between a 3,5-dimethyl-substituted BODIPY and an aldehyde.

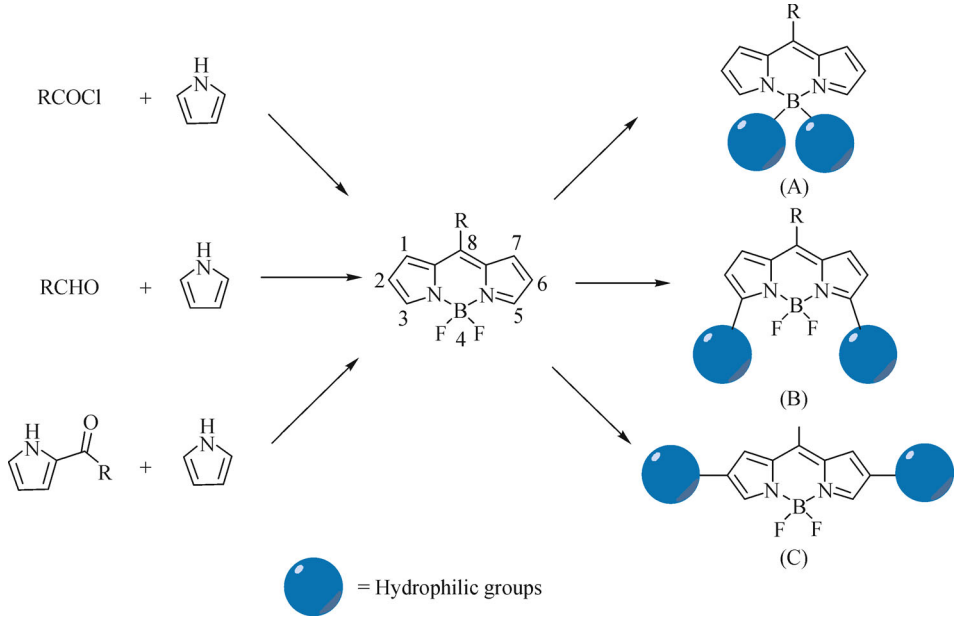
2.1 Water-soluble BODIPY dyes containing sulfonate groups

The first water-soluble BODIPY sulfonate derivative **1** (Fig. 1) was developed by Worries et al. in 1985 [16]. The 1,3,5,7-tetramethyl-substituted BODIPY was sulfonated with chlorosulfonic acid and subsequently neutralized with sodium hydroxide to give **1**. This pioneering work is of great significance for the preparation and application of water-soluble BODIPY dyes. Dye **1** has a high fluorescence quantum yield (Φ_{fl} = 0.85) in water (Table 1). The absorption and emission maxima of dye **1** are almost identical with those of the non-sulfonated precursor (with hydrogen atoms at the 2- and 6-positions) [17]. When the sulfonation reaction was performed with equimolar amounts of chlorosulfonic acid and the precursor, a mono-sulfonated dye was obtained.

Other sulfonated BODIPY dyes that have been synthesized include the water-soluble dyes **2** and **3** which bear a methyl or ethyl group at their *meso*-positions respectively [18]. These dyes have slightly lower fluorescence quantum yields in water compared to that of dye **1**. Dye **4** can be coupled with biomolecules via acylation reactions. Through a diazotization-azide reaction sequence, dye **4** was converted to the derivative **5**. A "click" reaction between azide **5** and hexynoic acid produced dye **6** which can be further coupled with an amino group on a protein or DNA-derivative. The syntheses of other sulfonate containing BODIPY derivatives like **7** and **8** have also been reported. All these dyes display strong fluorescence in water, methanol and other polar solvents, and are applicable for biological labeling [18–20].

2.2 Water-soluble BODIPY dyes containing quaternary ammonium groups

Niu and coworkers have reported the synthesis of water-soluble BODIPY **9** (Fig. 2) which contains quaternary



Scheme 1 Synthetic strategies for water-soluble BODIPY dyes. (A) Hydrophilic modification at the boron atom; (B) Hydrophilic modification at the 3- and 5-positions; (C) Hydrophilic modification at the 2- and 6-positions

Table 1 Absorption and emission spectroscopic data for BODIPYs (**1**–**22**)

Compound	Solvent	λ_{abs} [a] /nm	ε_{max} [b] /(L·mol^{-1}·cm^{-1})	λ_{em} [c] /nm	Φ_{fl} [d]	Ref.
1	H_2O	491	n.a.[e]	510	0.85	[16]
2	MeOH	492	n.a.	533	0.73(H_2O)	[17]
3	MeOH	498	n.a.	530	0.44(H_2O)	[17]
4	H_2O	496	115000	511	0.01	[18]
5	H_2O	498	78000	509	0.15	[18]
6	H_2O	498	79000	511	0.49	[18]
7	H_2O	497	67000	510	0.01	[19]
8	MeOH	565	69000	579	0.65	[20]
9	PBS[f]	522	n.a.	530	0.61	[21]
10	PBS	521	n a.	540	0.78	[21]
11	PBS	601, 649	n.a.	655	0.04	[22]
12	PBS	642	55100	657	0.22	[22]
13	PBS	588, 650	n.a.	658	0.05	[22]
14	H_2O	630	76600	643	0.30	[22]
15	PBS	641	58000	657	0.25	[22]
16	H_2O	500	64500	509	0.61	[23]
17	H_2O	638	102000	655	0.42	[23]
18	H_2O	651	118500	667	0.23	[23]
19	PBS/DMSO[g]	510	n.a.	525	0.66	[24]
20	CCBK[h]	655	n.a.	670	0.0002	[25]
21	HEPES[i]	495	58000	507	0.002	[26]
22	HEPES[i]	495	n.a.	520, 600	n.a.	[27]

a) λ_{abs}: absorption maximum; b) ε_{max}: molar extinction coefficients at absorption maximum; c) λ_{em}: emission maximum; d) Φ_{fl} : fluorescence quantum yields; e) n.a.: not available; f) PBS (1X, pH = 7.5); g) PBS (20% DMSO, 100 mmol·L^{-1}, pH = 7.4); h) CCBK: Calcium calibration buffer kit; i) HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 20 mmol·L^{-1} buffered to pH 7.1

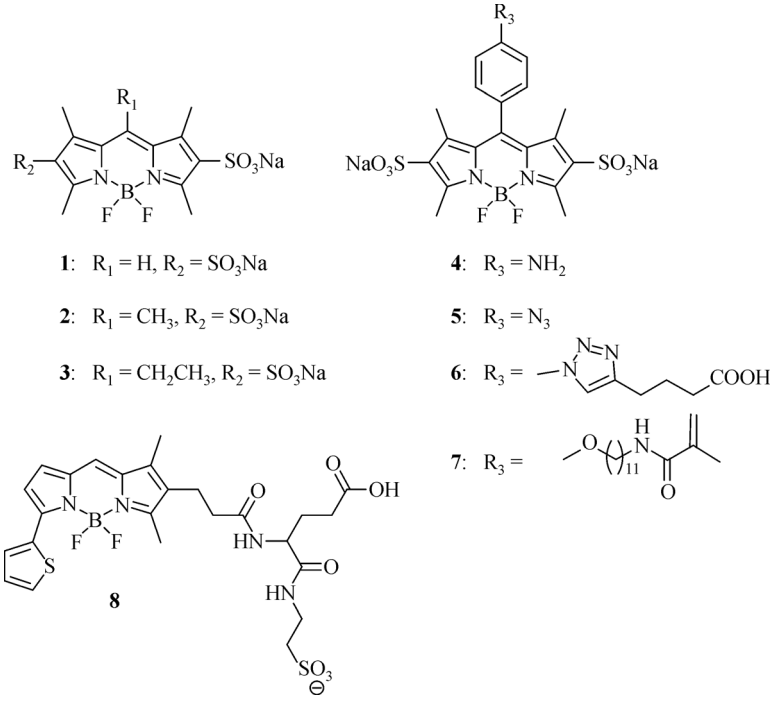


Fig. 1 Chemical structures of water-soluble BODIPY derivatives (**1**–**8**) containing sulfonate groups

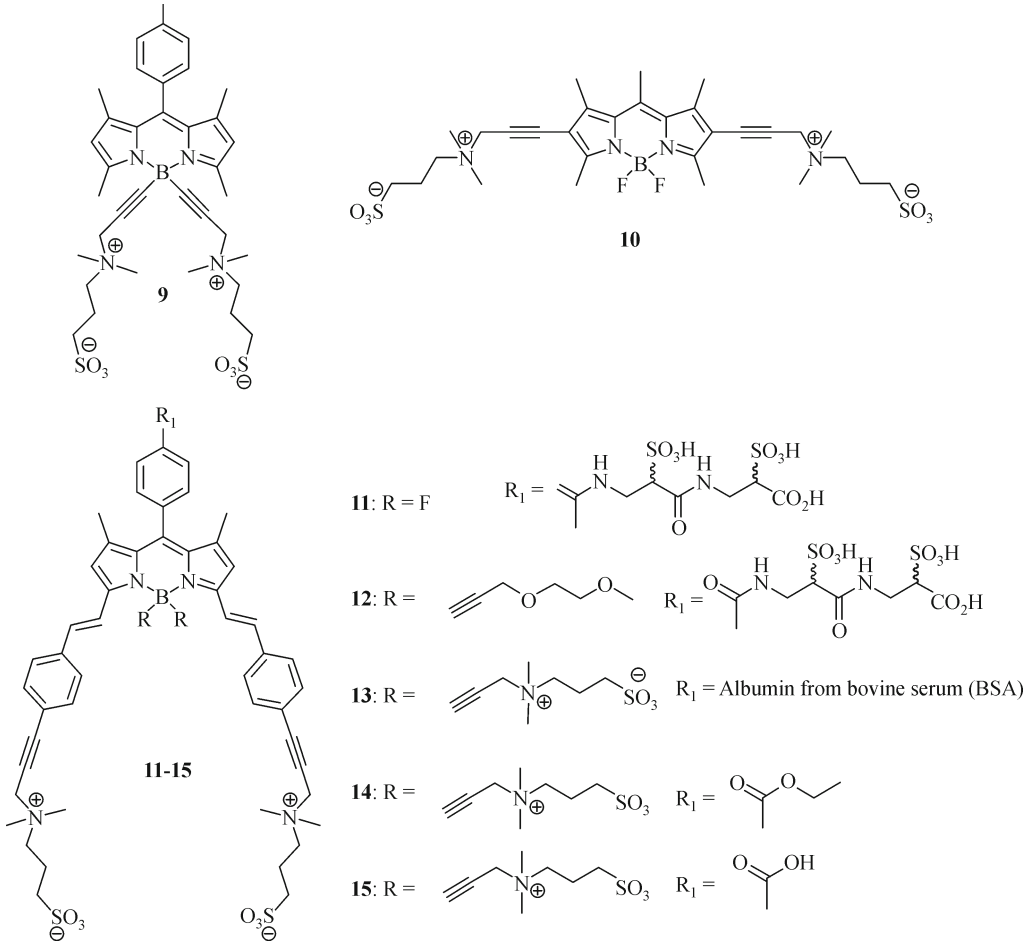


Fig. 2 Chemical structures of water-soluble BODIPYs (**9**–**15**) containing quaternary ammonium groups

ammonium groups [21]. The water-solubilizing groups were introduced on the boron by first adding fluorine atoms with an alkynyl Grignard reagent and then forming quaternary ammonium groups with 1,3-propanesultone. Dye **9** is highly fluorescent (Φ_{fl} = 0.61) in water (Table 1). More interestingly, the absorption spectrum of this dye in phosphate buffer saline (PBS, 1X) exhibited two absorption bands around 522 and 567 nm indicating the formation of the aggregate of **9** in the PBS. When ethanol was added to the PBS solution, the aggregate band disappeared [21]. Another quaternary ammonium containing dye **10** was obtained through a Sonogashira coupling reaction between a 2,6-diiodo-substituted BODIPY precursor and 3-dimethylamino-1-propyne, and then a subsequent reaction with 1,3-propanesultone. Both the absorption and emission bands of dye **10** red shifted with respect to those of dye **9** owing to the extended π-conjugated system of **10**.

Bura and Ziessel have reported another series of water-soluble red-emitting BODIPY dyes (compounds **11**–**15**) [22]. They introduced different types of water-solubilizing groups (i.e., quaternary ammonium, oligo-ethyleneglycol and sulfonated peptide chains) into the dye molecule in order to reduce or completely suppress the self-aggregation of the dyes in water (Fig. 2).

2.3 Water-soluble BODIPY dyes containing phosphonate groups

Incorporating phosphonate groups into the scaffold of the dye molecules can significantly improve the solubility of BODIPY dyes in water. Bura and coworkers synthesized a series of dye intermediates containing alcoholic or phenolic hydroxyl groups [22]. These hydroxyl groups were then further modified with phosphonate groups to give dyes **16**–**18** (Fig. 3). For dye **16**, first oligo-ethyleneglycol hydrophilic chains with triisopropylsilyloxy were introduced onto the boron atom and then the siloxy groups were cleaved with 2 mol·L^{-1} HCl.

Similarly, the precursors of dyes **17** and **18** were synthesized using Knoevenagel condensation reactions at the 3- and 5-methyl groups and subsequently deprotected with base (Fig. 3). Dyes **16**–**18** all have good solubilities in water and are fluorescent with quantum yields in the range of 0.23–0.61 and emission wavelengths from 509 nm to 667 nm (Table 1).

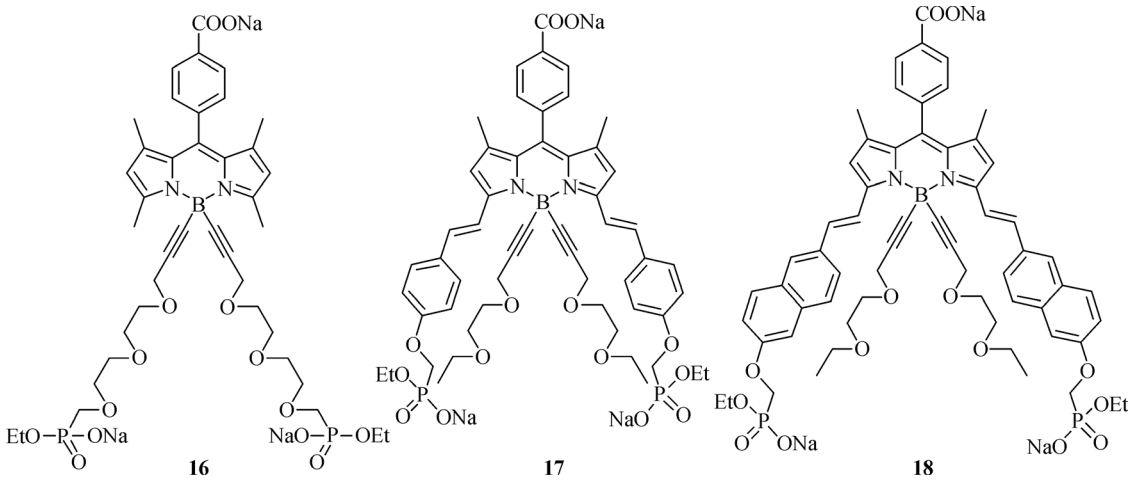


Fig. 3 Chemical structures of water-soluble BODIPY dyes (**16**–**18**) containing phosphonate groups

2.4 Water-soluble BODIPY dyes containing carboxylate groups

The water-soluble 2,6-dicarboxylate BODIPY **19** and its amide and ester derivatives were first synthesized by Komatsu et al. (Fig. 4) [24]. Dye **19** was obtained from its benzyl ester derivative by catalytic hydrogenation. Hydrolysis of the alkyl ester derivative under basic conditions results in destruction of the fluorophore. Dye **19** has strong green fluorescence. Its emission band undergoes a bathochromic shift of 20 nm in comparison to those of its ester derivatives, owing to the lower electron-withdrawing capacity of the COO^- group.

Matsui et al. developed a red fluorescent calcium probe (compound **20**) with an extended π-conjugated system [25]. The emission maximum of this dye is at 670 nm, which is a much longer wavelength than that of dye **19**. Dye **21** combines a BODIPY core and a N/O/S receptor group which can recognize ions and **21** has been used as a chemosensor for Ni^{2+} [26]. A more complicated water-soluble dye, **22**, was synthesized by Han et al. [27]. This dye has a conjugated donor-acceptor-donor (D-A-D) structure and was prepared via Sonogashira coupling of alkynyl fluorescein building blocks and a 2,6-diiodo-substituted BODIPY precursor. This triad molecule displayed two emission bands at 520 and 600 nm which

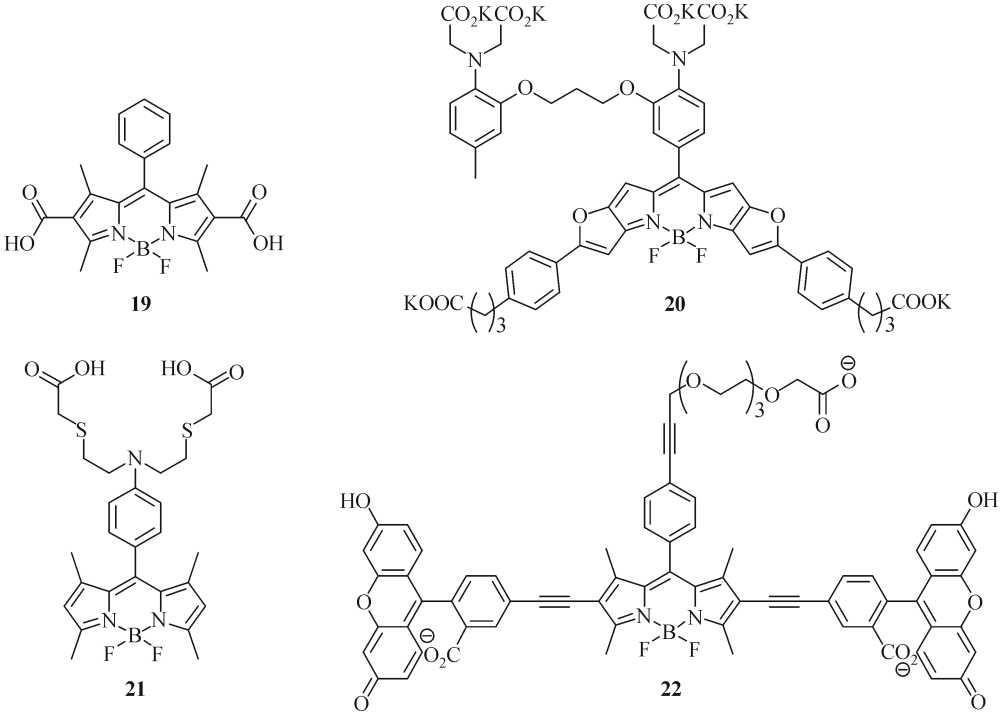


Fig. 4 Chemical structures of water-soluble BODIPY dyes (**19**–**22**) containing carboxylate groups

correspond to the fluorescence of the donor and acceptor moieties, respectively.

2.5 Water-soluble BODIPY dyes containing oligo-ethyleneglycol chains

In addition to the BODIPY dyes containing charged hydrophilic groups, water-soluble BODIPY dyes with neutral oligo-ethyleneglycol hydrophilic chains as water-solubilizing moieties have also been widely reported (Fig. 5).

Zhu et al. reported a series of BODIPY dyes (**23** to **29**) containing linear or branched oligo-ethyleneglycol chains [28]. These hydrophilic chains not only greatly increased the water-solubility of the dyes, but also effectively reduced the aggregation of the dyes which is due to their steric hindrance effect. As a result, some of these dyes are strongly fluorescent in water. For example, dye **23** has a fluorescence quantum yield of 0.68 in PBS (Table 2). For dyes **23**, **25**, and **26**, the alkynyl groups on the boron atoms have little effect on the absorption and emission maxima of the compounds. In contrast, the attachment of alkynyl groups at the 2- and 6-positions of the BODIPY core extended the π-conjugated system and led to bathochromic shifts in the absorption and emission maxima, as observed for dyes **24** and **27**. Dyes **28** and **29** were synthesized by Knoevenagel condensations between dye **26** and the corresponding aldehyde. Due to their extended π-con-

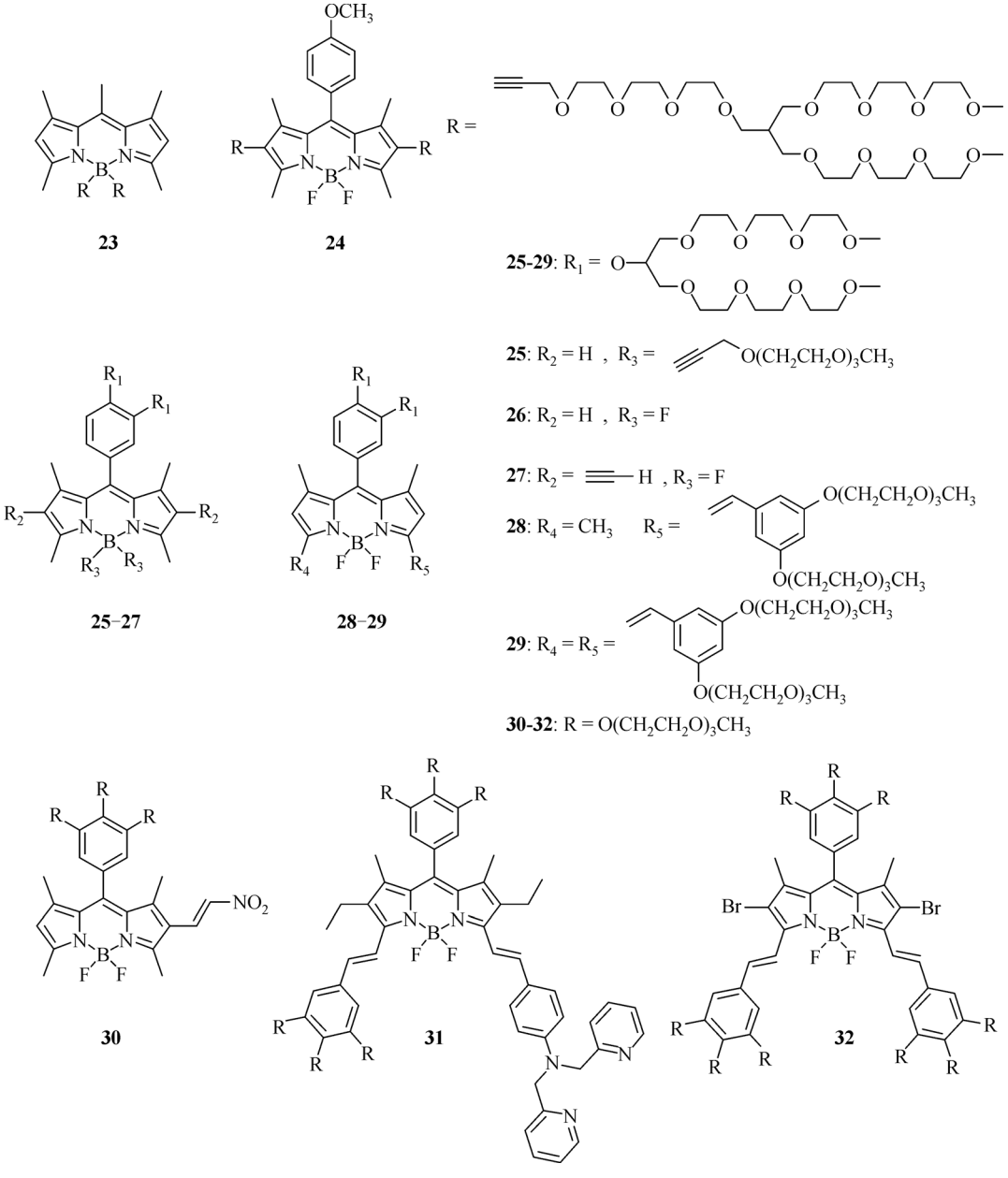


Fig. 5 Chemical structures of BODIPYs containing oligo-ethyleneglycol hydrophilic groups (**23**–**32**)

Table 2 Absorption and emission spectroscopic data of the BODIPY derivatives (**23**–**32**)

Compound	Solvent	λ_{abs} [a] /nm	ε_{max} [b] /(L·mol^{-1}·cm^{-1})	λ_{em} [c]/nm	Φ_{fl} [d]	Ref.
23	PBS [e]	491	n.a.[d]	501	0.68	[28]
24	PBS	543	n.a.	563	0.34	[28]
25	PBS	496	n.a.	507	0.36	[28]
26	PBS	499	n.a.	510	0.04	[28]
27	PBS	536	n.a.	555	0.09	[28]
28	PBS	577	n.a.	598	0.21	[28]
29	PBS	660	n.a.	676	0.01	[28]
30	HEPES[g]	525	n.a.	540	0.056	[29]
31	EtOH	655	102000	697	n.a.	[30]
32	HEPES[h]	680	72000	726	n.a.	[31]

a) λ_{abs}: absorption maximum; b) ε_{max}: molar extinction coefficients at absorption maximum; c) λ_{em}: emission maximum; d) Φ_{fl}: fluorescence quantum yields; e) PBS: 1X, pH = 7.5; f) n.a.: not available; g) 20% HEPES in CH_3CN, 50 mmol·L^{-1}, pH = 7.2; h) 5% in ethanol, 100 mmol·L^{-1}, pH = 7.2

jugated systems, these dyes had larger bathochromic shifts of their absorption and emission bands than dyes **25**–**27**.

In addition, water-soluble dyes **30**–**32** containing water-solubilizing oligo-ethyleneglycol chains as well as other functional groups [29–31], such asbiological thiols sensors, ion recognition units, have also been reported.

3 Synthesis and optical properties of water-soluble aza-BODIPY dyes

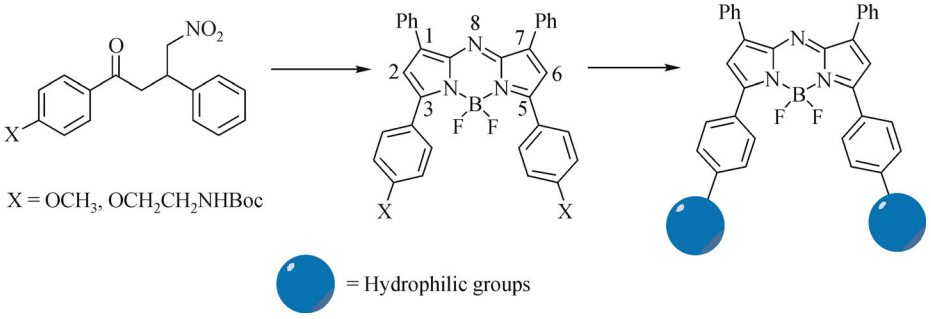
The importance of NIR fluorescent dyes in biological applications has been widely recognized in recent years [32–36]. The most attractive feature of these dyes is that NIR light can penetrate biological tissues more deeply and noninvasively than visible light, which is favorable in fields such as intracellular labeling and photodynamic therapy (PDT) [34]. Aza-BODIPY dyes (Scheme 2) possess a similar core structure to that of the BODIPYs except that a nitrogen atom replaces the carbon atom at the *meso*-position. This class of dyes has attracted increasing attention recently owing to their high NIR extinction coefficients and their moderate fluorescence quantum yields [37].

Several aza-BODIPY dyes have been prepared from γ-nitro-β-phenyl-butyrophenone (prepared through a Michael addition of nitromethane and chalcone) [32]. The intermediate was then condensed in either melted ammonium acetate or in refluxed alcoholic solvents containing ammonium acetate followed by complexation with boron trifluoride-diethyl etherate. Several water-soluble aza-BODIPY deriveatives **33**–**37** that have been reported are illustrated in Fig. 6. Chemical modifications of aza-BODIPY dyes with water-solubilizing groups have been conducted on precursors with hydroxyl groups (R_2= R_3= OH). Dyes **33**–**37** exhibited absorption and emission in the NIR range with moderate quantum yields (Table 3).

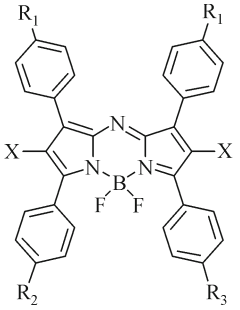
4 Applications of water-soluble BODIPY and aza-BODIPY dyes

4.1 Fluorescent labeling and imaging

Water-soluble BODIPYs have been widely used to label nanoparticles as well as biomarcromolecules. Sauer et al. developed a new BODIPY dye based on a fluorescent surfactant and monomer **7** (surfmer) which was used for emulsion copolymerization to prepare fluorescent nano-particles (Fig. 7(A)) [38]. Owing to the surfactant proper-

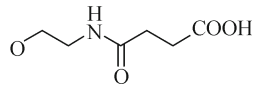


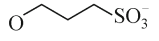
Scheme2 Synthesis of water-soluble aza-BODIPY dyes



33–**37**

33: $R_1 = X = H$, $R_2 = O(CH_2CH_2O)_3CH_3$, $R_3 = OCH_2CH_2NHBoc$

34: $R_1 = X = H$, $R_2 = O(CH_2CH_2O)_3CH_3$, $R_3 =$ 

35: $R_1 = X = H$, $R_2 = R_3 =$ 

36: $R_1 = X = H$, $R_2 = R_3 = OCH_2COO^-$

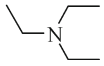
37: $R_1 =$  , $R_2 = R_3 = p\text{-}CH_3OC_6H_4$, $X = Br$

Fig. 6 Aza-BODIPYs (**33**–**37**) with various hydrophilic chains

Table 3 Absorption and emission spectroscopic data of the aza-BODIPY derivatives (**33**–**37**)

Compound	Solvent	λ_{abs} [a] /nm	ε_{max} [b] /(L·mol^{-1}·cm^{-1})	λ_{em} [c] /nm	Φ_{fl} [d]	Ref.
33	$CHCl_3$	688	87100	717	0.36	[32]
34	$CHCl_3$	689	n.a.[e]	717	n. a.	[32]
35	$CHCl_3$	694	n.a.	726	0.31	[32]
36	$CHCl_3$	681	n.a.	711	0.30	[32]
37	$CHCl_3$	684	70800	n.a.	n.a.	[33]

a) λ_{abs}: absorption maximum; b) ε_{max}: molar extinction coefficients at absorption maximum; c) λ_{em}: emission maximum; d) Φ_{fl} : fluorescence quantum yields; e) n.a.: not available

ties of dye **7**, the fluorophore mainly distributed on the surface of the particles. Accordingly, these nanoparticles could be used to quantify synthetic or natural macromolecules that absorb onto the particles.

In addition to nanoparticle tagging, water-soluble BODIPYs are excellent candidates for protein labeling [19,39]. For example, Dilek and coworkers successfully synthesized water soluble BODIPY **8** for labeling immunoglobulin [19]. The absorption and emission maxima of the labeled proteins were almost the same as those for dye **8**. However, a significantly drop in the fluorescence quantum yield was observed which is partially due to the interactions between the BODIPY and the proteins or to self-quenching.

The pioneering works of Monsma et al., Pagano et al., Knaus et al. and Oliver et al. demonstrated that water-soluble BODIPY derivatives are excellent candidates for cell and organelle imaging probes [40–43]. For example, dye **19** has been used as an imaging probe to study esterase activities in both *in vitro* and live cells [24]. This ratiometric fluorescent probe was developed to change its fluorescence wavelength in response to changes in the electron-withdrawing character of the 2- and 6- substituents. When cultured with HeLa cells, the probe immediately entered the cells and was subsequently hydrolyzed (Fig. 7(B)).

In another work, dye **20** was used as a novel red fluorescent probe for real-time and dual-color intracellular Ca^{2+} imaging of cervical cancer (HeLa) cells (Fig. 7(C)) [25]. This dye contains a *O*,*O*′-bis(2-amino-phenyl) ethyleneglycol-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) unit, which is known to strongly chelate to Ca^{2+}. The fluorescence of the dye molecules was quenched in the absence of Ca^{2+} due to a photoinduced electron transfer (PET) process from the BAPTA ion group to the BODIPY core. While chelating with Ca^{2+}, the electron-donating ability of the BAPTA group is weakened and consequently the PET process is hampered. Thus, the emission of the dye **20** is turned on. An *in vivo* study demonstrated that the fluorescent signal of dye **20** clearly reflected the intracellular calcium behavior.

In another study, the NIR fluorescent aza-BODIPY dyes **33** and **34** were used to label nano-beads which can be used as calibrants for fluorescence in analytical instrumentation such as flow cytometry [32].

4.2 Chemosensors

The applications of water-soluble BODIPY dyes as chemosensors have also been investigated extensively [30–31,44–46]. Isik et al. designed and synthesized water-soluble BODIPY dye **30** which contains three hydrophilic oligo-ethyleneglycol chains and a nitrovinyl moiety for the detection of thiols in biological systems (Fig. 8(A)) [30]. The BODIPY core of dye **30** functions as a signal generating unit (fluorescence or color change) whereas the nitrovinyl group reacts with thiols through a Michael addition reaction. The addition of a thiol to the vinyl group blocks the PET process between the oligo-ethyleneglycol group and the core, thereby, resulting in a blue shift in the

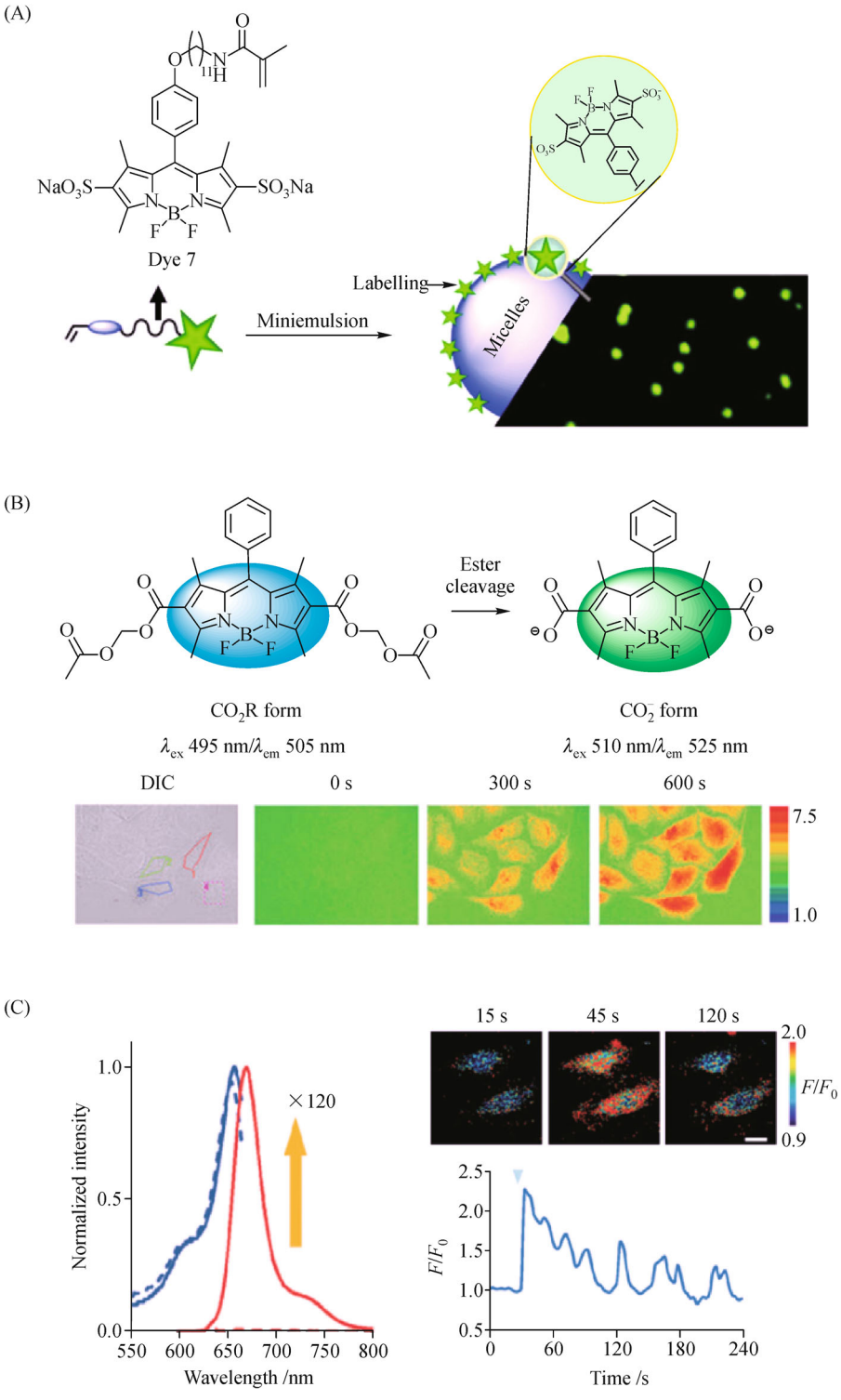


Fig. 7 (A) Left: Schematic representation of fluorescent surface-labeled polymeric nanoparticles via miniemulsion polymerization with a BODIPY surfactant and monomer (surfmer). Right: Probable orientation of dual-sulfonated BODIPY dye **7** on the surface of nanoparticles after miniemulsion polymerization. Reprinted with permission from [38]. (B) Ratiometric measurements of esterase activity using dye **19**. Inset picture: bright-field transmission and fluorescence ratiometric images of HeLa cells treated with probes. Reproduced from [24] with permission of the Royal Society of Chemistry. (C) Left: Normalized absorption (blue) and fluorescence emission (red) spectra of **20** in the absence (0 mmol·L^{-1}, dotted line) and presence (39 mmol·L^{-1}, solid line) of Ca^{2+} and the time course of fluorescence change of dye **20**; λ_{ex} = 635 nm. Right: Real-time single-color Ca^{2+} imaging; Up, pseudo-colored images of dye-loaded HeLa cells with ATP stimulation (100 mmol·L^{-1}) at 15, 45, and 120 s; scale bar, 20 mm; The arrowhead indicates the timing of ATP addition. Reproduced from [25] with permission of The Royal Society of Chemistry

absorption maxima and greatly enhanced fluorescence intensity. The significant change in the color and fluorescence of the dye can be distinguished even by the naked eye, demonstrating its high sensitivity for thiol detection.

Another water-soluble dye **31** was synthesized by Atilgan et al. for Zn^{2+} detection (Fig. 8(B)) [31]. This dye contains six hydrophilic triethyleneglycol groups and dipicolylamine (DPA), a well-known Zn^{2+} specific ligand. The absorption maximum of dye **31** is at 680 nm and its emission maximum is at 726 nm in an aqueous buffer solution (5% in ethanol, HEPES 0.1mol·L^{-1}, pH = 7.2). As the concentrations of Zn^{2+} increased in an ethanol-water mixture, the fluorescence intensity of the dye also greatly increased. As shown by the photographs of a series of dye solutions containing different metal ions under daylight or UV illumination (Fig. 8(B)), only the Zn^{2+} containing solution had intense red fluorescence, indicating that the probe is highly specific for Zn^{2+}.

Recently, Zhu et al. synthesized a series of water-soluble BODIPY-based probes bearing multiple ethylene glycol chains and dipicolylamine residues [46]. These displayed specific fluorescence ratiometric responses for Zn^{2+}.

4.3 pH indicators

Han et al. have designed a D-A-D triad **22** (Fig. 9(A)) for the detection of small pH changes in live cells [27]. In this probe, two xanthenes donors were conjugated with a BODIPY acceptor through an alkyne triple bond. When the xanthene donor was protonated (pH < 6) and excited at 495 nm, an energy transfer between the donor and acceptor occurred and the probe emitted red light (600 nm). On the other hand, at pH > 6.5, the energy transfer is hindered and probe **22** emits green light (around 520 nm). This red-green color change which is induced by small changes in pH was used for detecting different pH environments of organelles in live cells.

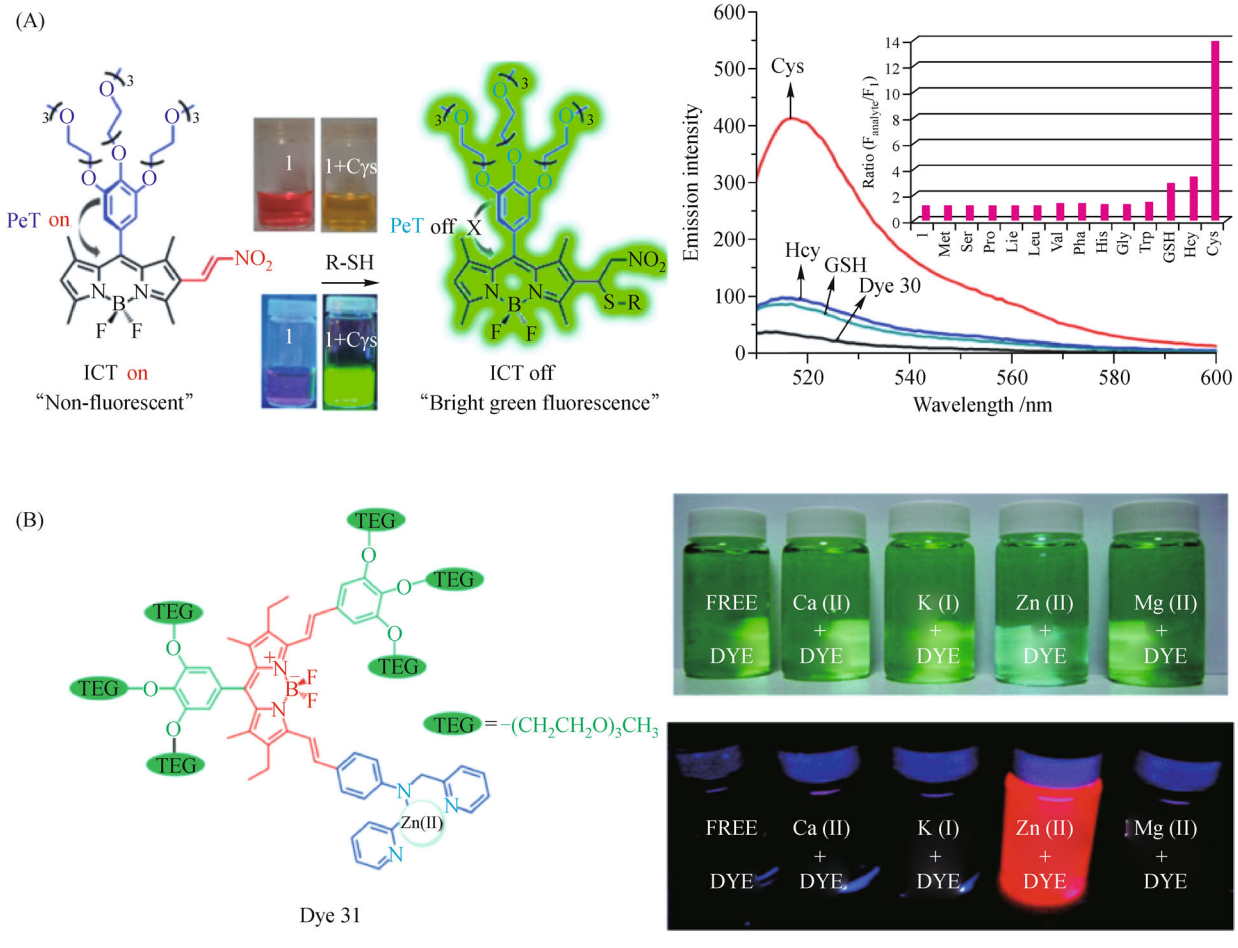


Fig. 8 (A) Left: Design elements of the nitroethenyl-BODIPY conjugate thiol probe (Dye **30**). Right: Fluorescence response of the dye **30** toward biothiols (Cys: Cysteine; Hcy: 2-Amino-4-sulfanylbutanoic acid; GSH: (2S)-2-amino-4-{[(1R)-1-[(carboxymethyl) carbamoyl]-2-sulfanylethyl]carbamoyl} butanoic acid) and other natural amino acids. Reprinted with permission from [30]. (B) Left: Chemical structure of Dye **31**. Right: The fluorescence emission with different ions. In the presence of Zn^{2+} the complex is bright red. Reprinted with permission from [31]

4.4 Photodynamic therapy

The next-generation of BODIPY dyes are being investigated as photodynamic therapy (PDT) agents, for treating malignant tumors (e.g. multidrug-resistant tumors) and macular degeneration [47–49]. Bromo-substituted water-soluble BODIPY dye **32** has been synthesized by Atilgan et al. and works as a photosensitizer for PDT (Fig. 9(B)) [29]. The solubility of this dye in water was ensured by the presence of several oligo-ethyleneglycol moieties and the dye can be readily absorbed by tumor cells. The bromo-substitutions in the molecule effectively promote spin-orbit coupling and enhance the intersystem crossing process of dye **32**. Thus, the dye has a high generation efficacy for singlet oxygen (by the rapid bleaching of 1,3-diphenylisobenzofuran) even at concentration as low as 9 n mol·L^{-1}. The cell-cytoxicity assay showed that the EC_{50} value (the concentration required for 50% of the maximum possible effect) was less than 200 nmol·L^{-1}.

Water-soluble aza-BODIPY dyes that have absorption maxima in the NIR region are attractive PDT reagent candidates [50]. The aza-BODIPY dye **37** reported by McDonnell et al. has been used for PDT studies of cancer cells. At pH values of 6.5–6.8 (the pH environment around cancerous cells), the amine group of **37** was protonated, which resulted in an enhancement of the intersystem crossing of dye **37** to the triplet state. Consequently, the cytotoxic effect of the dye increased more around tumor tissues than healthy ones [33]. Dye **37** showed a higher singlet oxygen quantum yield in acidic environments than in neutral ones (a 10.6-fold increase). The EC_{50} value of **37** was 5.8 nmol·L^{-1} for MRC5-SV40 transformed fibroblast cells (illuminated under a power density of 16 J·cm^{-2}), which is one of the highest values reported so far. Moreover, the aqueous solubility of the dye also increased under protonation and the dye was readily internalized by cancer cells.

5 Conclusions and outlook

The synthesis and application of water-soluble BODIPY and aza-BODIPY dyes have attracted considerable attention in the last decade. Numerous water-soluble BODIPYs and aza-BODIPYs dyes containing charged as well as neutral hydrophilic groups, including quaternary ammo-

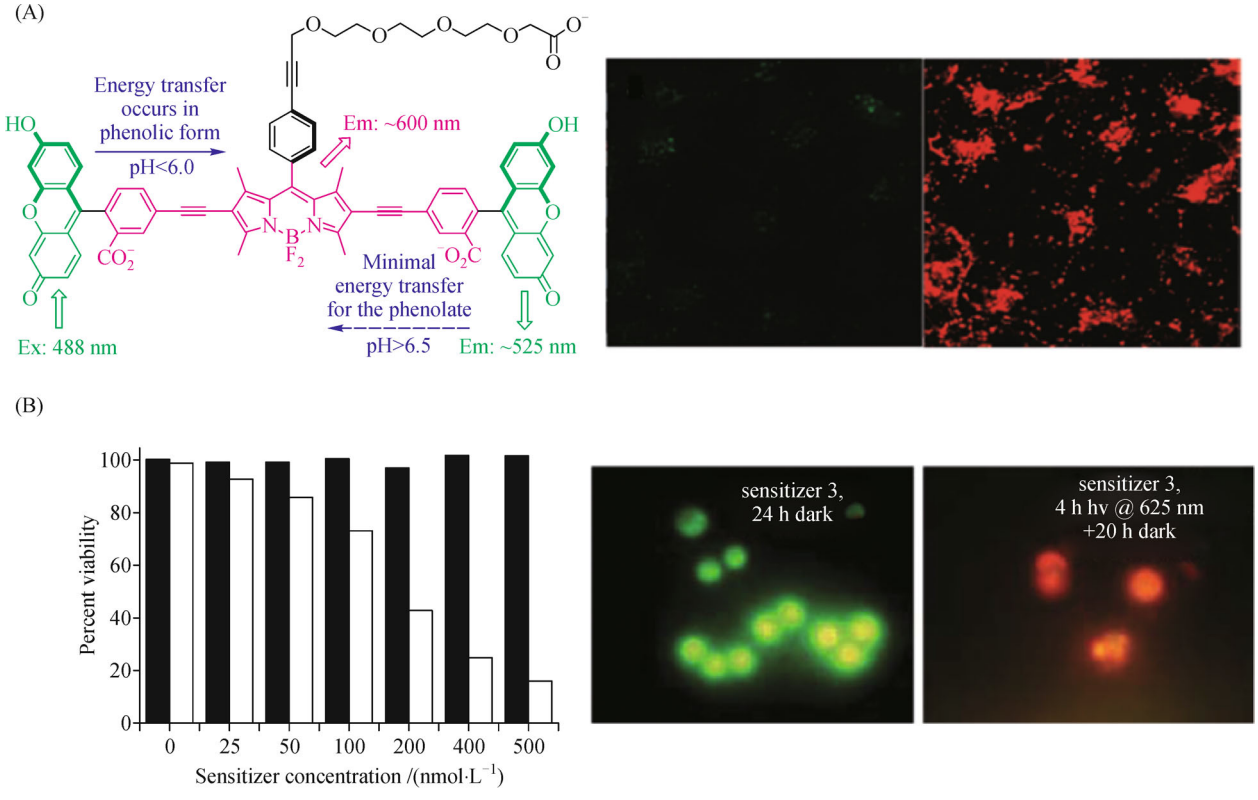


Fig. 9 (A) Schematic representation of a ratiometric pH sensor **22** that can be used in living cells. Inset picture: cellular uptake of pH-probe (1 μmol·L^{-1}) into fibroblast-like (COS-7) cells after 1 h incubation at 37 °C. Reprinted with permission from [27]. (B) Left: Percent viability as determined by a standard MTT assay with K562 cells kept in full medium at 37 °C in an incubator, in the presence of varying concentrations of compound **32**. Right: Fluorescence microscope images of acridine orange (AO) and propidium iodide (PI) stained K562 cells, incubated in full medium with 500 nmol·L^{-1} sensitizer 3 in the dark (the left panel); irradiated with red LED at 625 nm for 4 h, followed by 20 h incubation at 37 °C in the presence of 500 nmol·L^{-1} **32** (right panel). Reproduced from [29] with permission of the

nium, sulfonates, phosphonates, carboxylates, and oligo-ethyleneglycols, have been synthesized and characterized. The absorption and emission properties of these dyes can be tuned by attaching different electron-withdrawing or electron-donating groups or by extending the length of the π-conjugation of the BODIPY core. The absorption maxima of these dyes span from 490 to 700 nm and their emission maxima span from 500 to 730 nm. To improve the fluorescence quantum yields of these dyes in water, the aggregation of the dye in water can be diminished by attaching hydrophilic sterically hindered groups. Fluorescence quantum yields up to 0.8 in water have been obtained for these dyes.

These water-soluble dyes have application potentials in cell imaging, labeling of bio-macromolecules, chemosensors, pH indicators, and photodynamic therapy. However, for further improvement of the performance of these dyes in applications, the optical properties and biocompatibility of the water-soluble BODIPY dyes need to be optimized through rational molecular design. Developing water-soluble BODIPYs and aza-BODIPYs which are highly fluorescent in the NIR range is an important research topic and of particular interest due to the unique advantages of NIR in biological applications.

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