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Water-Soluble Phosphonate-Substituted BODIPY Derivatives with Tunable Emission Channels

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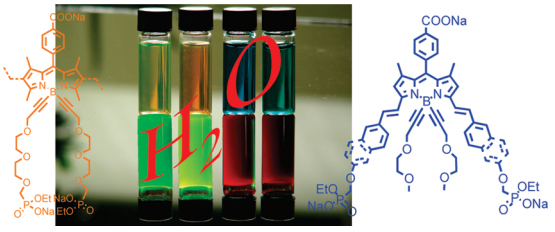
ABSTRACT



Water-soluble BODIPY dyes have been readily obtained by introduction of phosphonate fragments either on the boron for the green and yellow emitting dyes or on the side chain for the red emitting dyes. Hydrolysis of the phosphonate is realized at the end of the reaction sequence and allows isolation of the targets by precipitation. All these novel dyes are soluble and fluorescent in water with quantum yields in the 23−59% range and emission wavelength spanning from 667 to 509 nm.

Over the past decade there has been a renewed interest in the design of highly luminescent dyes for use as probes in biological systems.[1] The major drawbacks with neutral organic dyes are their low solubility in polar solvents and their tendency to decrease solvent contact by forming nonemissive aggregates.[2] Furthermore, most of these dyes display fluorescence features sensitive to environmental factors such as solvent polarity or association with hydrophobic biomolecules or with organized matter such as micelles, vesicles and thin films.[3] Numerous fluorescent molecules (e.g., fluorescein, rhodamine, acridine, anthracene, phenanthrene, pyrene, quinoline, benzofuran, dansyl, naphthalimide, squaraines, cyanines and indacene) display a rich variety of optical properties but challenges remain concerning: (i) water solubility, (ii) facility of synthesis and purification procedures, (iii) control of nonradiative decay pathways and (iv) conversion of often fragile organic frameworks to more robust species.

To engender water solubility and other requirements imposed for use in bioassays, sophisticated functionalization of basic dye skeletons is usually necessary. Currently, propelled by scientific reviews highlighting their exceptional chemical stability and promising spectroscopic features, there is a major interest in the engineering of difluoro-bora-diaza-s-indacene (BODIPY) dyes.[4] Advantages of the basic BODIPY core are its absorptive properties in the visible region and the absence of deactivation complications due to triplet state population. However, the synthetic methodologies for construction of water-soluble dyes are limited, and the provision of larger quantities may represent a bottleneck for further studies and development in biomedical analysis. Various strategies leading to water-soluble BODIPY dyes have been envisaged by introduction of oligo(ethyleneglycol) chains,[5] α-galactosylceramide,[6] nucleotides,[7]

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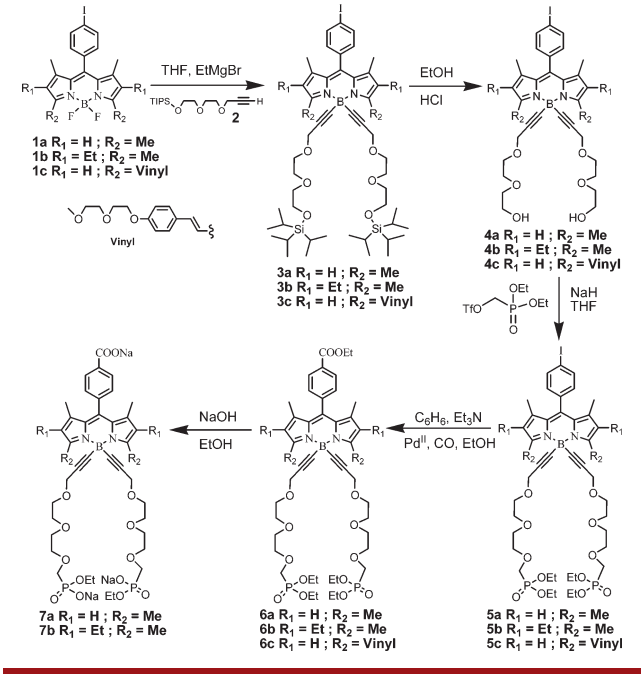
sulfonated peptides,[8] *N*,*N*-bis(2-hydroxyethyl)amines,[9] carboxylates,[10] sulfonates,[11] or betaine[12] units. In most cases, relatively good solubility in water was reached but the absence of aggregates and strong fluorescence were only observed with the less conjugated dyes emitting in the 500−540 nm range. Despite great synthetic effort, little success was achieved for the more extended dyes emitting in the 650−700 nm window and synthetic methodologies remain elusive and purification procedures extremely tedious.[7,13]

In this light, it may be noted that most key natural biomolecules (ATP, DNA, etc.) are constructed with phosphate residues, which ensure good solubility and stability in water under daylight conditions. Herein, we report a convenient strategy based on the grafting of alkylphosphonate residues onto the boron center or the styryl side arms of BODIPY dyes to achieve water solubility and fluorescence properties without the formation of aggregates even with the dyes emitting in the red part of the electromagnetic spectrum.

Our first goal was the efficient preparation of a variety of dyes bearing an ethyleneglycol chain with a TIPS-protected alcohol at one end and tethered to the boron center via an ethynyl bond (**3a**−**c** in Scheme 1). The pivotal derivative **2**[14] was prepared in 83% yield from the TIPS-monoprotected diethyleneglycolate[15] and propargyl bromide. The Grignard derivatives of **2** readily react with the fluoro derivatives **1a**−**c** in excellent yields applying a protocol previously developed by us.[16] In the second step, 2 M HCl can be used to deprotect the alcohol. The corresponding alkoxides were prepared with NaH or *t*-BuOK and allowed to react with diethoxy(phosphonate) trifluoromethylsulfonate[17] providing a mixture of mono- and disubstituted compounds **5a**−**c**. Despite the use of an excess of the triflate, complete substitution could not be attained. The disubstituted derivatives **5a**−**c** were isolated in only 35% yields but were efficiently transformed to the corresponding ethylcarboxylic esters **6a**−**c** via a carboalkoxylation promoted by $[Pd(PPh_3)_2Cl_2]$ under a flow of CO at atmospheric pressure and in the presence of EtOH as the nucleophile and TEA as base to quench the nascent acid.[18]

Hydrolysis of both the carboxylic ester and the phosphonate to the corresponding monophosphonic acid was brought about in one step using NaOH. The water-soluble dyes **7a** and **7b** were isolated by crystallization from the reaction mixture without the need to purify them by reverse-phase chromatography. Notice that the presence of the carboxylate group certainly facilitate water solubility and prevent dye aggregation through charge repulsion. Surprisingly, the distyryl derivatives **6c** could not be properly hydrolyzed under the given conditions and provided an intractable mixture of dyes.

Scheme 1. Synthesis of BODIPY Phospohnates



To circumvent this problem and to further extend the methodology to blue dyes, an alternative suggested by earlier work of Lindsey on chlorins[19] is to link the phosphonate to the side arm and to graft a simple ethyleneglycol chain onto the boron center. In practice, it was found that the method outlined in Schemes 3 and 4 must be adapted to the nature of the aryl residues carrying the alkylphosphonate fragments. We were fortunate first to be able to prepare blue dyes **8** and **9** with a TIPS-protected phenol or naphthol aldehydes using established procedures (Scheme 2).[20,21]

From these building blocks, it was possible to substitute the fluoro ligands by alkyne derivatives, leading to dyes

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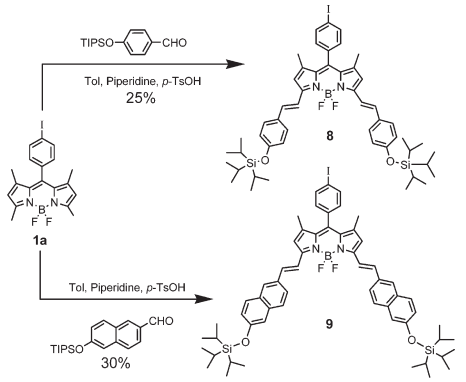
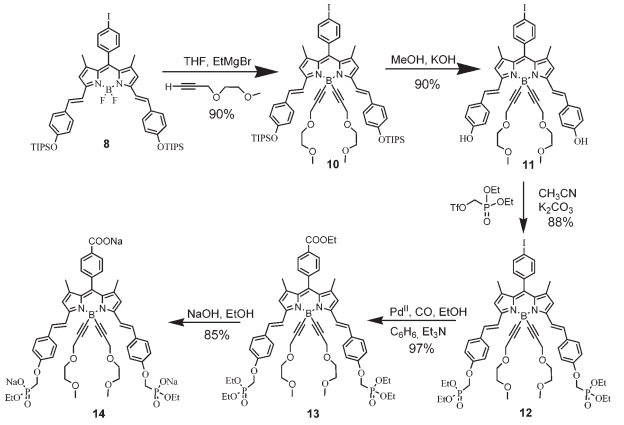
Scheme 2. Synthesis of TIPS Protected Phenol- and Naphtol-Based Distyryl BODIPY Dyes



10 and **15** in excellent yields. At this stage, the reaction sequence for the phenol and naphthol dyes must diverge due to solubility and reactivity reasons. For the phenol-based protocol depicted in Scheme 3, after deprotection of the TIPS in basic conditions and nucleophilic substitution using diethoxy(phosphonate) trifluoromethylsulfonate, the desired phosphonated derivative **12** was easily prepared.

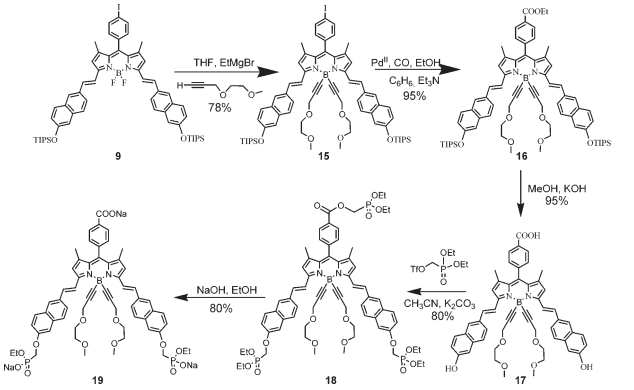
Conversion of the phenyliodo residue to the corresponding ethylester **13** is feasible using the carboalkoxylation reaction referred to above. Finally, concomitant hydrolysis of the carboxy- and phosphonate esters gave rise to the target derivative **14** which precipitated during the course of the reaction and appeared to be perfectly soluble and highly fluorescent in water (*vide infra*). Complete hydrolysis of the phosphonate to the phosphonic acid moieties was not achievable at this stage even when using a high concentration of base in different solvents or using TMS-Br as an activator.

Scheme 3. Synthesis of Phenol-Based BODIPY Phosphonates



For the naphthol derivative **15**, an alternative route had to be devised involving first the formation of the carboxyester **16** and then the simultaneous deprotection of the TIPS and saponification of the ester, leading to dye **17** in excellent yields (Scheme 4).

Scheme 4. Synthesis of Naphtol-Based BODIPY Phosphonate



Alkylation of the naphthol hydroxyl in **17** was accomplished with diethoxy(phosphonate)trifluoromethylsulfonate. Simultaneously, the carboxylic acid was esterified, as clearly seen from the ^{1}H and ^{31}P NMR spectra, the latter displaying two phosphorus signals at 18.9 and 19.1 ppm (in $CDCl_3$) for **18** whereas a single signal at 20.1 ppm (in CD_3OD) was observed for dye **13**. As would be expected in light of previous observations, hydrolysis under basic conditions leads to the monophosphonic and carboxylic acids in dye **19**. This dye precipitated during the course of the reaction and could be isolated pure by centrifugation (see Figure 1b). It appeared to be highly soluble and fluorescent in water.

The proton NMR of the prototypic dyes **7b** and **19** are shown in Figure 1. In both cases, integration of the ethyl signal at 3.96 and 1.24 ppm versus the AB quartet of the phenyl-carboxylate is diagnostic for the monohydrolysis of

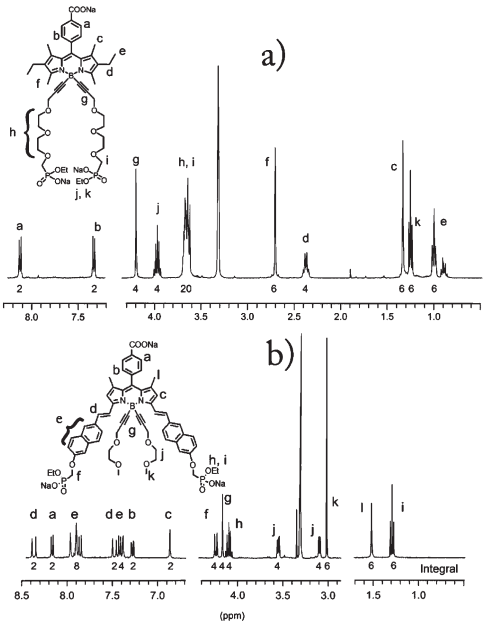


Figure 1. ^{1}H NMR of dyes (a) **7b** and (b) **19** in d_4-MeOH.

the phosphonate. For **7b**, the presence of two type of ethyl groups confirms this statement. For **19**, the doublet at 4.23 ppm with a J_{PH} coupling constant of 10.2 Hz is easly identifiable and the doublet at 8.35 with a J_{HH} = 16.4 Hz confirms the *trans* conformation of the vinyl functions. Furthermore, the presence of a singlet at 6.86 ppm assigned to the β-pyrrolic protons of the dipyrromethene core confirms the symmetry of the molecule. The well-defined NMR spectra and expected integrales confirm the absence of aggregates and impurities as well as the molecular structures.

The photophysical properties of the new dyes were measured in alcohols and water and the data are collected in Table 1. The absorption spectra of the final compounds **7a**–**b**, **14** and **19** exhibit a major absorption peak at the expected wavelength with extinction coefficients in the range 60 000 to 66 000 $M^{-1}cm^{-1}$ for the classical BODIPY and 100 000 to 120 000 $M^{-1}cm^{-1}$ for the more extended dyes. This transition corresponds to the $S_0 \rightarrow S_1$ transition.[22]

Table 1. Selected Optical Data Measured in Polar Solution

dye	λ_{abs} (nm)	ε $M^{-1}cm^{-1}$	λ_F (nm)	τ_F (ns)	Φ_F[a]	k_r[b] $10^8\ s^{-1}$	k_{nr}[b] $10^8\ s^{-1}$	solvent
7a	500	66 000	509	4.8	0.59	12.2	8.5	EtOH
7a	500	64 500	509	5.0	0.61	12.1	7.8	H_2O
7b	518	60 000	531	7.5	0.73	9.7	3.6	EtOH
7b	516	60 500	529	6.4	0.68	10.7	5.0	H_2O
14	638	106 000	654	5.3	0.45	8.5	10.4	MeOH
14	638	102 000	655	3.5	0.42	12.0	16.6	H_2O
19	649	120 500	664	4.3	0.35	8.1	15.0	MeOH
19	651	118 500	667	2.8	0.23	5.3	29.9	H_2O

[a] Using Rhodamine 6G as reference, Φ = 0.78 in water,[23] λ_{ex} = 480 nm for **7a,b** and using tetramethoxydiisoindomethene-difluoroborate (ϕ_f = 0.51 in methanol)[24] λ_{ex} = 570 nm for **14** and λ_{ex} = 580 nm for **19**. [b] Calculated using the following equations: $k_r = \Phi_f/\tau_f$; $k_{nr} = (1-\Phi_f)/\tau_f$, assuming that the emitting state is produced with unit quantum efficiency.

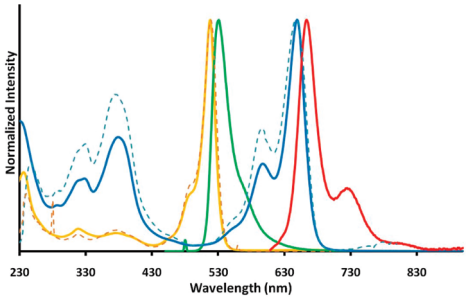


Figure 2. Absorption, emission and excitation spectra measured in water: green and violet lines, dye **7b**; blue and red lines, dye **19**. For the excitation spectra (dashed lines), the emission is plotted at 560 nm for **7b** and at 730 nm for **19**.

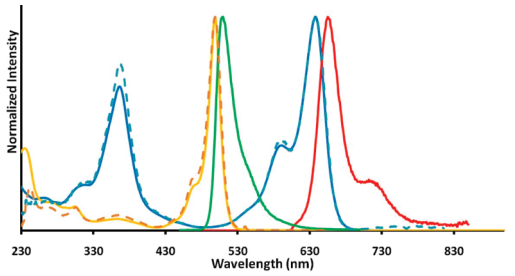


Figure 3. Absorption, emission and excitation spectra measured in water: green and violet lines, dye **7a**; blue and red lines, dye **14**. For the excitation spectra (dashed lines), the emission is plotted at 560 nm for **7a** and at 700 nm for **14**.

Note that the weaker absorption in the UV window corresponds to an $S_0 \rightarrow S_2$ transition and to an internal charge transfer band (ICTB) for dyes **14** and **19** (Figures 2 and 3).[21] By irradiation in the lower energy absorption bands, strong fluorescence is observed and the shape of the emission bands shows excellent mirror symmetry with the lowest-energy absorption band, suggesting that the chromophore has a similar geometry in the ground and first excited states. Furthermore, the high quantum yields, the weak Stokes shift around 400 cm^{-1} and the nanosecond excited state lifetime regime are in keeping with a singlet excited state. The radiative deactivation rates are higher than the nonradiative deactivation rates for dyes **7a**–**b** and conversely for the red emitting dyes **14** and **19**. Note also that these dyes entail a significant red-shift of the emission extending to 830 nm in case of dye **19**. It is also interesting that the spectroscopic properties of dyes **7a**–**b**, **14** and **19** are very similar in EtOH, MeOH and pure water, excluding the formation of aggregates.[8] As shown in Figures 2 and 3, the excitation spectra (in dashed lines) match the absorption spectra fairly well over the entire absorption window (250–700 nm). Finally, it appears that the aqueous solutions of the dyes are stable for weeks without significant degradation or flocculation of the dyes.

In short, a convergent method has been successfully developed for the synthesis of water-soluble BODIPY dyes emitting light in the 490 to 750 nm range. The proposed method uses alkylphosphonate as a tool to carry out the synthesis allowing purification of each synthetic intermediate by conventional methods. At the end of the procedure, hydrolysis led to the free carboxylate as well as the ethylphosphonate as substituents. The desired dyes were obtained purely by precipitation without the need for column chromatography.

Supporting Information Available. Synthetic procedures and analytical data reported herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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