Tetrahedron Letters 51 (2010) 1269–1272

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Convenient synthesis of water-soluble nitrilotriacetic acid (NTA) BODIPY dyes

Marie Brellier [a,1], Guy Duportail [b], Rachid Baati [a,*]

[a] University of Strasbourg, Faculty of Pharmacy, CNRS/UMR 7199, Laboratory of Functional ChemoSystem, 74 route du Rhin BP 60024, 67401 Illkirch, France
[b] University of Strasbourg, Faculty of Pharmacy, CNRS/UMR 7213, Laboratory of Biophotonics and Pharmacology, 74 route du Rhin BP 60024, 67401 Illkirch, France

ARTICLE INFO

Article history:
Received 2 November 2009
Revised 16 December 2009
Accepted 21 December 2009
Available online 6 January 2010

In memory of Dr. C. Mioskowski

Keywords:
Nitrilotriacetic acid (NTA)
bis-NTA
BODIPY dyes
Water soluble probes

ABSTRACT

Mono- and bis-NTA-BODIPY dyes have been efficiently synthesized by amide bond formation between hepta-alkylated *meso*-aryl-BODIPY derivatives and the free amine of *mono*- and bis-NTA binder surrogates. These new fluorescent hybrids exhibited valuable photophysical properties under buffered aqueous conditions, thus expanding the arsenal of water-soluble BODIPY dyes for potential labeling and monitoring of biological systems and processes.

Since their discovery in 1968 by Treibs and Kreuzer,[1] 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene or BODIPY® (boron dipyrromethene) dyes have been extensively investigated as novel functional materials owing to their fascinating photophysical, chemical, and electronic properties.[2–6] Especially appealing is their use either as molecular probes for intracellular and cell membrane imaging[7,8] or in the construction of diverse conjugated devices with unrivaled electronic properties for energy transfer purposes.[9] Among the most recently reported technological advances involving BODIPY properties is the development of water-soluble BODIPY probes for protein labeling and imaging in living cells or whole organisms.[10] To date, the most widely used methodology for increasing the water solubility of BODIPY dyes relies on the incorporation of sulfonate groups in the hydrophobic BODIPY scaffold.[11–13] This post-functionalization of the BODIPY platform can be achieved by virtue of the unique and versatile chemistry exhibited by the difluoroboradipyrrin systems.[4–6] Indeed, S_NAr reactions of 3- or 5-dichloro systems,[11] electrophilic sulfonations of 2- and 6-positions, and Heck-type coupling have been used successfully for the synthesis of useful sulfonated water-soluble BODIPY dyes.[12] Quite recently two other strategies have been developed for the introduction of sulfonate functions on BODIPY dyes.[13a] The first one relies on the connection onto the BODIPY core of di- and trisulfonated peptides using an acylation reaction with an adequately functionalized *meso*-hepta-alkylated BODIPY. The second original approach is based on the formation of polar zwitterion by reacting suitable propargyl amine BODIPY derivatives with sultones. Alternatively, the incorporation of neutral triethyleneglycol moieties in the BODIPY core also leads to water-soluble amphiphilic distyrylboradiazaindacenes with photosensitization and cell membrane permeability properties.[14] Notwithstanding these achievements, among the issues that still await solution are the design and synthesis of hydrophilic BODIPY dyes that could potentially bind proteins selectively and reversibly via ternary metallic complexes. Since the nitrilotriacetic acid (NTA) anchoring system is well known to have a huge potential in the labeling of his-tagged proteins,[15] we envisaged introducing this hydrophilic binder onto a BODIPY core in order to provide new stable and specific fluorescent probes with broad scope for diverse applications (Fig. 1).[16]

It was also of paramount importance to target organic dyes with new properties, in terms of solubility under physiologic conditions, photostability attributes, large molar absorption coefficients and fluorescence quantum yields, and narrow absorption and emission bands with high peak intensities.[17] In continuation of our research program devoted to the elaboration of new NTA multivalent fluorescent probes,[18] we wish to report the efficient syntheses of new symmetrical acidic NTA-BODIPY dyes (Fig. 1) that could potentially be used for the labeling and monitoring of biological systems.[15] As shown in Figure 2, our synthetic approach relies on the introduction, at a late stage via an amide bond-forming reaction, of the fully protected NTA binder **4** or **5**[18a,19] with the symmetrical *meso*-functionalized hepta-alkylated acidic BODIPY **3**.[13a]

The preparation of compound **3** was envisaged from the corresponding dipyrromethene **6**. *Meso*-substituted BODIPY **3** dyes can

* Corresponding author. Fax: +33 368 854 306.
E-mail address: baati@bioorga.u-strasbg.fr (R. Baati).
[1] Present address: NovAlix, Building A: Chemistry, BioParc, bld Sébastien Brant BP 30170, 67405 Illkirch, France.

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doi:10.1016/j.tetlet.2009.12.126

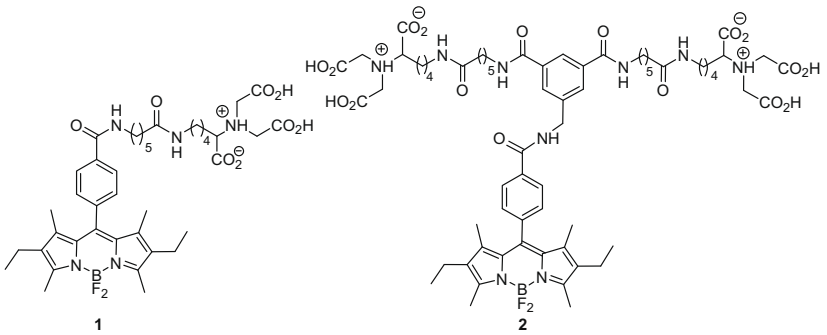


Figure 1. Structure of the targeted NTA-BODIPY dyes **1** and **2**.

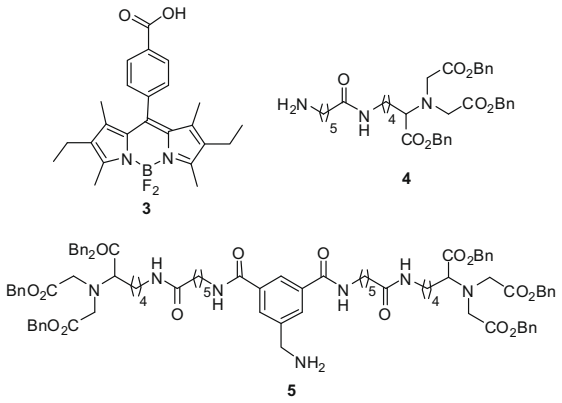


Figure 2. Structure of the building blocks required for the preparation of **1** and **2**.

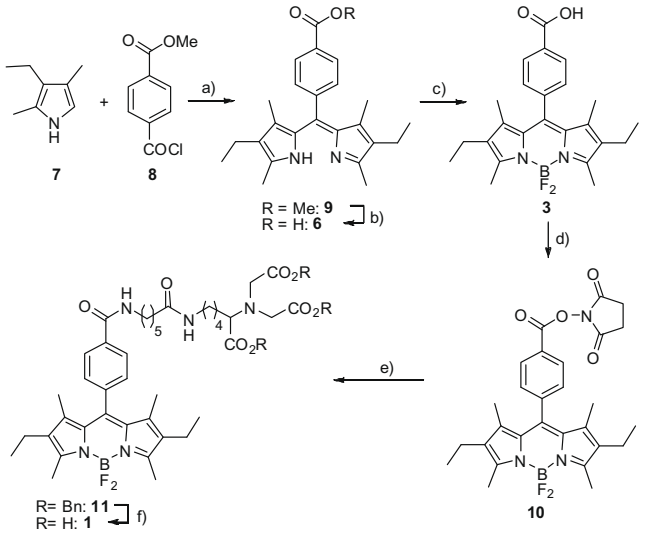
be accessed in three steps from the direct condensation of aromatic aldehydes and pyrroles. Nowadays, such scaffolds tend to be synthesized through a straightforward two-step process via the condensation of acyl chlorides or anhydrides with pyrroles or ketopyrroles, followed by the complexation reaction with $BF_3{\cdot}Et_2O$.[6]

Our syntheses of **1** and **2** started with the construction of dipyrromethene **9** by condensing directly the commercially available trialkylated pyrrole **7** with the freshly prepared acyl chloride **8**, in DCM as the solvent (Scheme 1). The sensitive methyl ester **9** was isolated after column chromatography on silica gel with a reproducible yield (27%). Subsequent methyl ester hydrolysis upon treatment with LiOH in a 1:1 THF/H_2O mixture (v/v) at room temperature afforded cleanly the carboxylic acid **6** in 99% yield. This intermediate was used as such without further purification in the complexation reaction with $BF_3{\cdot}Et_2O$ under reported conditions using toluene as the solvent and triethylamine (TEA) at 80 °C.[6] The standard procedure was effective and gave acidic BODIPY **3** in 70% yield, after column chromatography on silica gel. This key intermediate was then converted to the corresponding *N*-hydroxysuccinimidyl ester **10** in 78% yield, upon reaction with *N*-hydroxysuccinimide (NHS) in the presence of *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC) in dichloromethane (DCM) at room temperature. The subsequent coupling of the activated acid **10** with amine **4** bearing benzyl protected NTA functions in the presence of diisopropylethylamine (DIPEA) as the base yielded BODIPY **11** in good yield (70%).[13a] Smooth benzyl deprotection by hydrogenolysis under Pd/C catalysis in EtOH, afforded mono-NTA-BODIPY **1** in 29% yield over two steps, after purification on C_{18} reverse phase.[21]

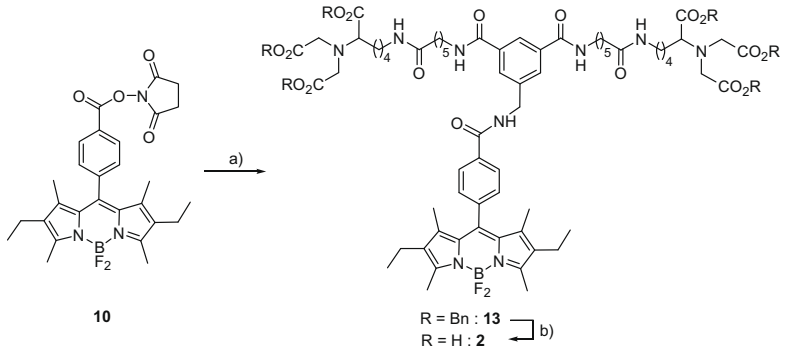
Similarly, bis-NTA-BODIPY **2** was synthesized from the same activated BODIPY **10** by coupling with the functionalized benzylamine **5** followed by hydrogenolysis of the benzyl ester groups in 40% yield over the two steps (Scheme 2).[22]

A very appealing feature of this chemistry is that the free highly polar NTA binders are delivered at the last step of the synthesis, thus facilitating the handling and the purification of all the intermediates.

We next turned our attention to the determination of the intrinsic photophysical parameters of these new dyes (see the Supplementary data). Table 1 summarizes the spectroscopic properties recorded in organic solvents (MeOH and $CHCl_3$) for dyes **3**, **10**, and the NTA-protected precursors **11** and **13**, and in protic polar solvent (MeOH, H_2O) for NTA-BODIPY dyes **1** and **2**. All the compounds analyzed in $CHCl_3$ (**3**, **10**, **11**, and **13**) exhibited absorption maxima in the range of 529–539 nm, suggesting that the presence of benzylated NTA binders on the dyes **11** and **13** does not interfere with the intrinsic spectral characteristics of the common BODIPY parent molecules **3** and **10**. Also, the molar extinction coefficients at λ_{max} are ranging from 57,000 M^{-1} cm^{-1} (**11** and **13**) to 97,600 M^{-1} cm^{-1} (**3**), in agreement with other BODIPY dyes.[8a] The emission maxima of the molecules **3**, **10**, **11**, and **13** are in the range of 544–549 nm, while maintaining a relatively high quantum yield $_{exp}$ between 0.58 and 0.80. Due to the high polarity of dyes **1** and **2**, their photophysical characteristics were measured in a protic polar organic solvent and in aqueous medium. Absorption spectra of the dyes in MeOH solutions are in accordance with our expectations, with a notable slightly lower absorption and emission maxima in the range of 4–5 nm. This difference might be ascribed most likely to the lower refractive index of MeOH compared with $CHCl_3$.[20] Finally, the photophysical parameters of **1** and **2** measured in water did not vary significantly com-



Scheme 1. Chemical synthesis of dye **1**. Reagents and conditions: (a) DCM, reflux, 3 h, 27%; (b) LiOH, THF/H_2O (1:1, v/v), 40 °C, 20 h, 99%; (c) toluene, TEA, $BF_3{\cdot}Et_2O$, 80 °C, 45 min, 70%; (d) DCM, NHS, EDC, rt, 3 h, 78%; (e) DCM, DIPEA, **4**, 20 h, 50%; (f) EtOH, Pd/C (10 mol %), rt, 4 h, 57%.



Scheme 2. Chemical synthesis of dye **2**. Reagents and conditions: (a) DCM, DIPEA, **5**, rt, 18 h, 70%; (b) EtOH, Pd/C (10%, w/w), rt, 4 h, 57%.

Table 1
Spectroscopic properties of fluorescent BODIPYs

Dye	Solvent	λ_{max}(abs) ±0.25[a] (nm)	λ_{max}(em) ±0.5[a] (nm)	Stokes shift $\Delta\nu$ (cm^{-1})	Φ_{exp}[b] ±0.02	τ (ns) ±0.05
3	$CHCl_3$	529.5	544	504	0.73	4.85
10	$CHCl_3$	531.25	549.5	625	0.58	4.40
11	$CHCl_3$	529.0	544.5	538	0.80	5.10
13	$CHCl_3$	529.5	544	504	0.74	5.00
1	MeOH	523.75	540	574	0.79	5.30
2	MeOH	524	540	565	0.34	5.15
1	H_2O[c]	524.25	541	591	0.53	5.25
2	H_2O[c]	524	542	634	0.28	5.50

[a] Determined at a 2 μM concentration in the indicated solvent.
[b] Rhodamine 6G in ethanol was used as standard at 20 °C.[23]
[c] 15 mM phosphate-citrate buffer, pH 7.0.

pared with those recorded in MeOH, except for the quantum yield $_{exp}$ which decreased slightly, while maintaining a high value for the fluorescence lifetime τ. Few photophysical properties of hepta-substituted *meso*-aryl BODIPY systems bearing *para*-electron-withdrawing carboxyl groups are reported in the literature. The experimental spectroscopic data found for dyes **1** and **2** in aqueous conditions are comparable to those recently reported for bis-sulfonic BODIPY dyes (λ_{max} (abs) = 523, λ_{max} (em) = 539 nm).[13a]

For all molecules, the Stokes shifts ($\Delta\nu$ = 504–625 cm^{-1}) are in agreement with those reported elsewhere for *meso*-aryl-substituted BODIPY dyes.[24] These characteristics make dyes **1** and **2** valuable probes not only for cellular imaging under physiological conditions but also for the selective labeling of histidine-tagged proteins. These studies are under investigation in our laboratories.

In summary, a convergent and highly effective method has been developed for the preparation of symmetrical NTA-BODIPY hybrids. Two new water-soluble *mono*- and bis-NTA-BODIPY dyes have been successfully synthesized and fully characterized. The incorporation of NTA functionalities in the BODIPY core did not impact the photophysical properties of the dyes at all. Further efforts are currently being devoted to the development of non-symmetrically substituted, water-soluble NTA-BODIPY derivatives. Construction of a library of NTA as well as poly-NTA-based BODIPY dyes is also under investigation in our laboratories.

Acknowledgments

We acknowledge Dr. Bernard Barlaam from Astra-Zeneca (Reims) for scientific discussions, and the CNRS for financial support of MB.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.126.

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21. *Procedure for the preparation of* **1**:
The compound **11** (30 mg, 29 μmol, 1.0 equiv) is dissolved in ethanol (2 mL) under an argon atmosphere in the dark. The solution is degassed for 5 min before adding dry Pd/C (6 mg, 6 μmol, 0.2 equiv, 10%). The reaction mixture is purged four times with hydrogen and stirred under an atmospheric pressure of hydrogen for 4 h, at room temperature in the dark. Palladium is then filtered off over Celite® and washed several time with HPLC-grade ethanol. The resulting filtrate is evaporated to dryness in vacuo and the resulting residue is purified by column chromatography on silica gel using a chloroform/methanol/water gradient (6:4:0.1→6:4:1, v/v/v). Residual impurities are then eliminated on a C_{18} reverse phase column (cartridge C_{18} SEP-PAK) using a water/ethanol mixture (1:1, v/v) to afford pure **1** (13 mg, 17 μmol, 57%) as a red solid which is stored in the dark. TLC: R_f = 0.45 (chloroform/methanol/water 6:4:0:1, v/v/v); ^{1}H NMR ($CD_3OD/CDCl_3$ (1:1, v/v), 300 MHz) δ 8.96 (d, J = 7.1 Hz, 2H), 8.20 (d, J = 7.1 Hz, 2H), 3.40 (s, 4H), 3.11 (m, 5H), 2.28 (s, 6H), 2.07 (m, 6H), 1.60–0.70 (m, 18H), 0.46 ppm (m, 6H); ^{13}C NMR ($CD_3OD/CDCl_3$ (1:1, v/v)), 75.5 MHz) δ 175.2, 168.7, 154.9, 140.3, 139.8, 139.2, 136.0, 134.0, 131.3, 129.5, 129.0, 58.1, 49.6, 48,4, 40.8, 40.2, 36.9, 30.4, 30.0, 27.6, 26.4, 17.6, 15.0, 12.7, 12.2 ppm; MS (ESI): m/z = 805 [(M+Na)$^+$, 100]. IR (KBr): ν = 3434, 2930, 1596, 1542, 1473, 1413, 1189, 1099, 970, 796, 466 cm^{-1}.
22. *Procedure for the preparation of* **2**:
To an ethanolic solution (3.5 mL) of compound **13** (30 mg, 16 μmol, 1 equiv), palladium on charcoal 10% (13 mg, 12 μmol, 0.75 equiv) is added. The reaction mixture is degassed several times and purged four times with hydrogen. The reaction mixture is stirred at atmospheric pressure of hydrogen for 4 h at room temperature in the dark. The palladium catalyst is removed by filtration on Celite® and washed several time with HPLC-grade ethanol. The filtrate is concentrated in vacuo and the resulting red oil is purified by column chromatography on silica gel using a chloroform/methanol/water gradient (6:4:0.1→6:4:1, v/v/v). The residual impurities are finally removed on a C_{18} reverse phase cartridge (C_{18} SEP-PAK) using a methanol/water mixture (3:2, v/v) to give pure compound **2** (12 mg, 9 μmol, 57%) as a red solid which is stored in the dark. TLC: R_f = 0.40 (chloroform/methanol/water 6:4:1, v/v/v); ^{1}H NMR (CD_3OD, 300 MHz) δ 8.15 (s, 1H), 8.00 (s, 2H), 7.96 (m, 2H), 7.50 (m, 2H), 4.71 (br s, 2H), 3.62 (s, 8H), 3.47 (m, 2H), 3.39, 3.15 (m, 8H), 2.21 (s, 6H), 1.70–1.16 (m, 38H), 0.90 ppm (m, 6H); ^{13}C NMR (CD_3OD, 50 MHz) δ 175.1, 174.7, 168.3, 160.2, 140.4, 135.7, 129.6, 128.5, 125.0, 66.1, 54.8, 44.3, 39.9, 39.0, 36.0, 33.8, 29.4, 28.9, 26.5, 25.7, 25.1, 23.9, 17.3, 11.6 ppm; HRMS (Maldi-TOF/TOF, HCCA matrix): m/z = calcd for $C_{65}H_{88}BFN_9O_{17}$ [M−·F]$^+$: 1296.6375; found 1296.6459; IR (KBr) ν = 3421, 2926, 2859, 1635, 1602, 1546, 1416, 1189, 1125, 804, 731, 615, 469 cm^{-1}.
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