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Synthesis and photophysical properties of a new amino acid possessing a BODIPY moiety

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ABSTRACT

The synthesis of a new amino acid possessing a BODIPY fluorophore, which is of use in peptide synthesis, is described. The influence of the amino acid as well as of benzoxazole moieties on the BODIPY spectral and photophysical properties is discussed. The photophysical properties of this fluorophore were modified only to a small extent compared to those of the parent compound.

4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) derivatives have become popular fluorophores as a result of their valuable spectroscopic and photophysical properties. These include elevated photostability, narrow absorption and emission bands in the visible region, a high absorption coefficient ($\varepsilon > 50{,}000\ M^{-1}\ cm^{-1}$) and high fluorescence quantum yields (often approaching 1.0). Recently, a review on the synthesis and spectroscopic properties of BODIPY and its derivatives was published by Loudet and Burgess.[1] Both the absorption and emission spectra of BODIPY derivatives are relatively insensitive to the polarity and pH of their environment (except those of derivatives carrying an amino[2a,b] or hydroxy substituent[2c]). Also, these compounds are reasonably stable under physiological conditions. As a result, they have found many applications in numerous fields of science and medicine. They are used as sensitizers for solar cells,[2,3] as molecular photonic wires,[4] fluorescence sensing materials,[2,5] probes for biolabelling[6] as well as for laser applications[7] and labelling of nanoparticles.[1]

BODIPY derivatives can be modified easily by introducing suitable substituents at the 3- and 5-positions.[2b,5b,6a,8] Alkylation or arylation at C-8 does not affect the positions of the absorption and emission maxima significantly,[9] except for 4-amino[2a] or 4-hydroxyphenyl derivatives.[2c] BODIPY derivatives with substituent(s) at C-3 and/or C-5, carrying an amino or carboxy group are used to label biomolecules.[6b] Also, it is possible to attach them to the side chain of an amino acid[6c] which allows them to be incorporated into a peptide chain.

In this Letter we describe the synthesis and photophysical properties of an alanine derivative, with a side chain containing a 2-(4-BODIPY-phenyl)-benzoxazole moiety (Scheme 1). The presence of an amino acid moiety allows this derivative to be incorporated directly into a peptide chain enabling a broadening of the range of its possible application, especially taking into account that a benzoxazole moiety may serve as a type of extension arm for BODIPY. Moreover, the presence of an additional chromophore absorbing at a shorter wavelength than BODIPY results in the excitation of the new compound also being possible in the UV range (the absorption spectrum covers the range from 220 nm to 550 nm). Also, the introduction of such a large substituent as the benzoxazole moiety extends the π-electron system of this compound significantly and as a result should modify the spectral and photophysical properties of the BODIPY fluorophore. Hence, its influence on these properties was studied.

The synthesis of the BODIPY-containing 2-phenyl-benzoxazol-5-yl-alanine derivative is outlined in Scheme 1. First, *N*-Boc-3-[2-(4-formylphenyl)benzoxazol-5-yl]alanine methyl ester ([(4-CHO)Ph]Box-Ala) (**1**) was synthesized according to the procedure published previously.[10,11] Product **1** was isolated in 10% yield following silica gel column chromatography. *N*-Boc-3-[2-(4-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene)phenyl)benzoxazol-5-yl]alanine methyl ester ((BODIPY)Box-Ala) (**2**) was synthesized according to the published procedure for such derivatives[2,6a,8b] by condensing **1** with 2 equiv of 2,4-dimethyl-1*H*-pyrrole in CH_2Cl_2 under an argon atmosphere at room temperature in the presence of one drop of TFA as the catalyst, followed by oxidation with *p*-chloranil. The product was further reacted with $BF_3{\cdot}OEt_2$ in the presence of triethylamine to afford **2**. In addition, the synthesis

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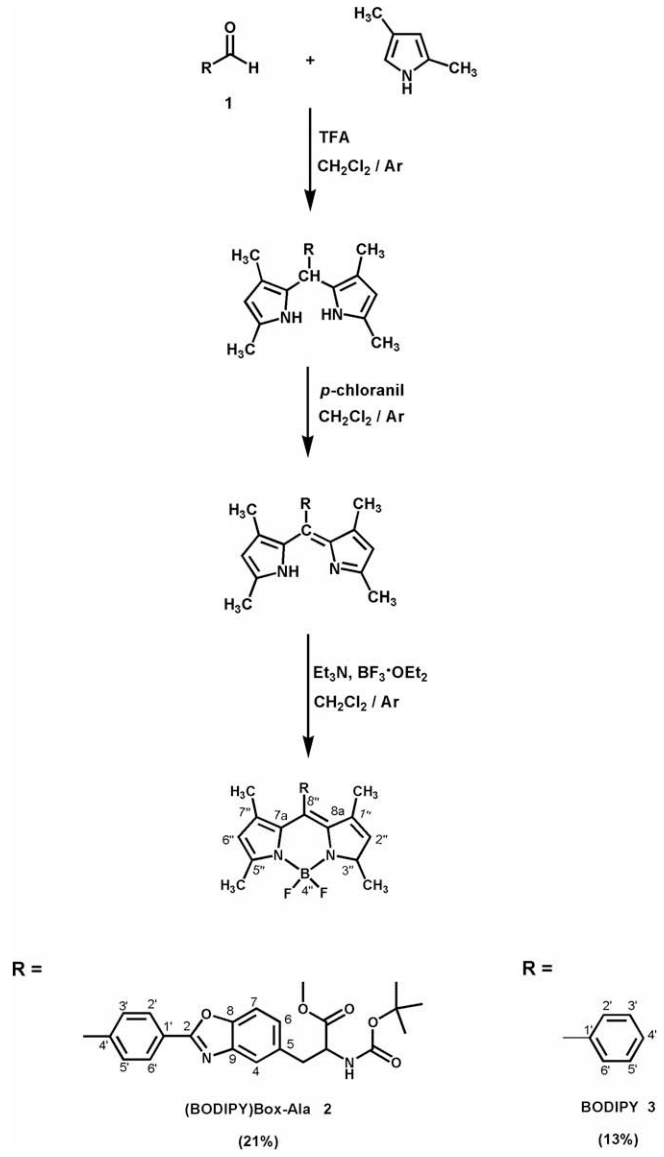

Scheme 1. Synthesis of *N*-Boc-3-[2-(4-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene)phenyl)benzoxazol-5-yl]alanine methyl ester ((BODIPY)-Box-Ala **2**).

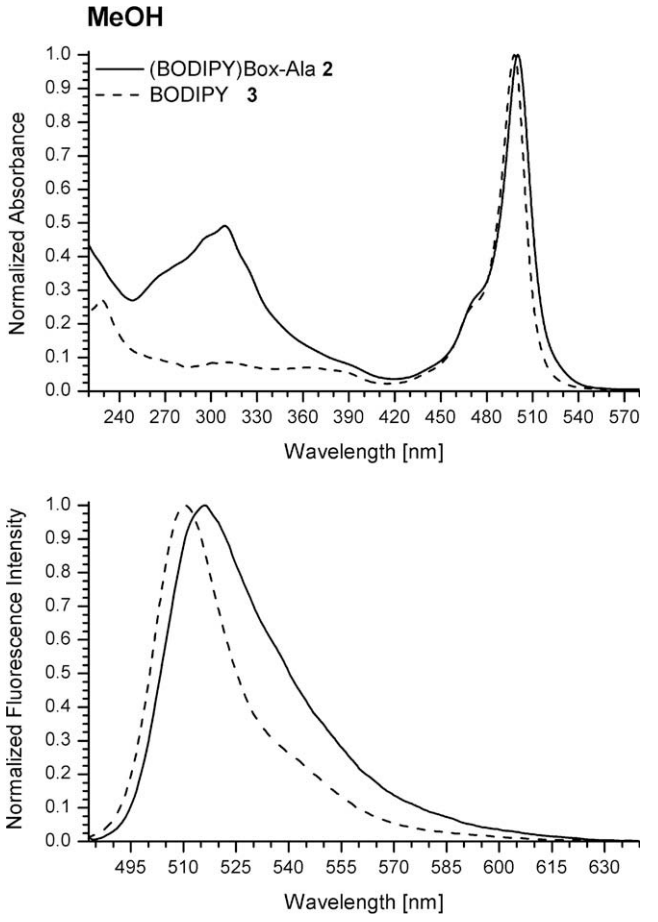

Figure 1. Normalized absorption (top) and emission (bottom) spectra of *N*-Boc-3-[2-(4-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene)phenyl)benzoxazol-5-yl]alanine methyl ester ((BODIPY)Box-Ala) **2** and 8-phenyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) **3** in methanol.

of 8-phenyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (**3**) was performed using the same procedure as in the case of compound **2** with 2,4-dimethyl-1*H*-pyrrole and benzaldehyde as substrates. The purified and fully characterized (^{1}H and ^{13}C NMR, IR and mass spectra) BODIPY derivatives were dissolved in various solvents in order to study their spectral properties.

Figure 1 shows the absorption and steady-state emission spectra of compounds **2** and **3** dissolved in methanol.

The shape of the absorption spectra is similar to those of previously described BODIPY dyes with a strong absorption band around 500 nm and a shoulder at shorter wavelength. However, the absorption spectrum of **2** is bathochromically shifted slightly (~2 nm) compared to that of **3**. Also, it is a little wider [fwhm (full width at half maximum) of **2** = 867 cm^{-1}, **3** = 791 cm^{-1}] and possesses an additional band around 310 nm as a result of the benzoxazole moiety absorption.[10b] The absorption spectra are only affected slightly by the solvent polarity; the absorption maximum is only marginally hypsochromically shifted on increasing the polarity (Table 1). These results are consistent with the general behaviour of the BODIPY chromophore.[1]

The molar absorption coefficients for the new BODIPY analogues are relatively high [in the range 46,000–73,000 dm^3 mol^{-1} cm^{-1} (Table 1)]. Also, the emission features of the studied compounds are typical for BODIPY fluorophores.[1] The bands are narrow and slightly Stokes-shifted with a mirror image shape. The fluorescence quantum yields (φ_f) are high and solvent polarity independent. The fluorescence band of **3** is hypsochromically shifted in contrast to that of **2** which is shifted slightly bathochromically with increased solvent polarity. The features that differentiate (BODIPY)Box-Ala **2** from the model compound **3** are the smaller solvent polarity dependence of the absorption and emission bands and its higher values of full width at half maximum as well as the twofold lower fluorescence quantum yield (**2** φ_f = 0.26, **3** φ_f = 0.52 being comparable to the published value[5h]). In addition, more distinct differences between these two compounds are observed for their fluorescence intensity decays. For compound **3**, the fluorescence intensity decay in all three solvents studied is described by a mono-exponential function. The fluorescence lifetime is about 3.2 ns in methanol and acetonitrile whereas in cyclohexane it is slightly lower (2.42 ns). The presence of the Box-Ala moiety strongly modifies the fluorescence intensity decay of the BODIPY core. Only in cyclohexane is the fluorescence intensity decay mono-exponential with a short lifetime (1.36 ns) whereas in polar solvents, a bi-exponential function is necessary to describe the fluorescence intensity decay correctly (Table 1) and the fluorescence lifetimes depend on the solvent polarity. In methanol, the fluorescence lifetimes are 1.57 ns with a contribution in the decay of α = 0.940 and 3.08 ns with α = 0.060, whereas in acetonitrile, the

Table 1
Photophysical properties of BODIPY compounds **2** and **3** in cyclohexane, methanol and acetonitrile

BODIPY	Solvent	λ_{abs}[a] (nm)	ε[b] ($dm^3\ mol^{-1}\ cm^{-1}$)	λ_{em}[c] (nm)	$fwhm_{abs}$[d] (cm^{-1})	$fwhm_{em}$[e] (cm^{-1})	$\Delta\nu$[f] (cm^{-1})	φ_f[g]	τ[h] (ns)	α[i]	χ^2_R[j]
2	Cyclohexane	—[k]	—	515	—	1155	—	0.25 ± 0.02	1.36 ± 0.055	1.000 ± 0.080	1.02
	Methanol	501	72,600 ± 700	516	867	1343	600	0.26 ± 0.01	1.57 ± 0.043 3.08 ± 0.277	0.940 ± 0.022 0.060 ± 0.026	1.08
	Acetonitrile	500	49,500 ± 400	517	899	1432	680	0.25 ± 0.01	1.66 ± 0.056 3.36 ± 0.253	0.894 ± 0.023 0.106 ± 0.032	1.01
3	Cyclohexane	503	50,600 ± 900	515	716	908	464	0.49 ± 0.02	2.42 ± 0.004	1.000 ± 0.003	1.08
	Methanol	498	46,300 ± 900	511	791	932	492	0.52 ± 0.03	3.21 ± 0.004	1.000 ± 0.003	1.12
	Acetonitrile	497	47,600 ± 900	511	818	980	520	0.52 ± 0.03	3.27 ± 0.004	1.000 ± 0.003	1.04

[a] Absorption maximum.
[b] Molar absorption coefficient.
[c] Emission maximum.
[d] Full width at half maximum of the absorption band.
[e] Full width at half maximum of the emission band.
[f] Stokes shift.
[g] Fluorescence quantum yield.
[h] Fluorescence lifetime.
[i] Pre-exponential factor obtained from the fluorescence intensity decay analysis.
[j] The statistical parameter defining the adequacy of the exponential function fitting to the fluorescence intensity decay.
[k] Solubility was too low for correct measurement of the absorption spectrum.

lifetimes are 1.66 ns and 3.36 ns and $\alpha = 0.894$ and $\alpha = 0.106$, respectively. The wider fluorescence band of **2** than that of **3**, and the lower fluorescence quantum yield and dependence on solvent polarity indicated that for **2**, an interaction between the two parts of the molecule (Box-Ala and BODIPY) exists in spite of the nearly perpendicular position of both moieties. Also, it seems that this interaction has a charge transfer (CT) nature,[2,5b,5c,8] however, the formation of intermolecular π-stacking cannot be excluded, taking into account that the fluorescence spectrum is slightly hypsochromically shifted on dilution of the solution (~2.5 nm in methanol and ~4.5 nm in acetonitrile, Fig. 4 in Supplementary data) and also the contributions of the fluorescence lifetimes change according to analysis (the contribution of the shorter lifetime increases whereas the contribution of the longer lifetime decreases with solution dilution, see Table in the Supplementary data). Intramolecular π-stacking interactions were observed for BODIPY derivatives by Bergström et al.[12]

In conclusion, the described new compound **2** has spectral and photophysical properties similar to BODIPY derivatives, however, its advantage over the types of compound previously described in the literature is the presence of an amino acid moiety allowing its direct incorporation into a peptide chain.[13–15]

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Supplementary data

Supplementary data (Experimental details of the synthesis [procedures, characterization of the compounds] and spectroscopic measurements [absorption and fluorescence spectra]) associated with this Letter can be found, in the online version, at doi:10.1016/j.tetlet.2009.03.195.

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