Functionalisation of fluorescent BODIPY dyes by nucleophilic substitution

Taoufik Rohand, Mukulesh Baruah, Wenwu Qin, Noël Boens and Wim Dehaen*

Received (in Cambridge, UK) 13th September 2005, Accepted 18th October 2005
First published as an Advance Article on the web 22nd November 2005
DOI: 10.1039/b512756d

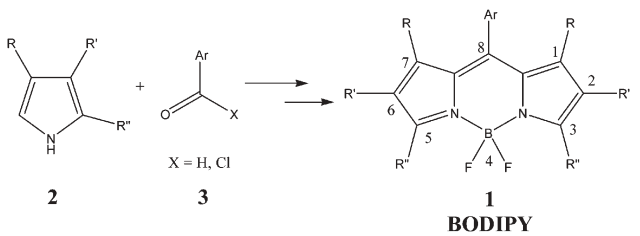
The BODIPY chromophore can be easily modified by nucleophilic mono- or disubstitution of 3,5-dichloroBODIPY with O-, N-, S- and C-nucleophiles. Absorption and fluorescence spectral data of the new BODIPY derivatives are also reported.

BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene) derivatives **1**[1] have been known for almost 40 years[2] but in the last decade the number of papers on these interesting fluorescent dyes has increased exponentially. This is due to a combination of valuable properties such as elevated chemical and photostability, relatively high absorption coefficients and fluorescence quantum yields, combined with a rather short synthesis for the simpler derivatives. Furthermore, BODIPY dyes can be optically excited with visible light, show narrow absorption and emission bands with high peak intensities, and are amenable to structural modification so that spectral shifts in the absorption and emission bands can be generated by introducing the appropriate substituent pattern. In fact, a number of BODIPY dyes are commercially available, allowing their widespread use. We will restrict ourselves to mentioning recent applications of BODIPY derivatives as fluoroionophores,[3] as probes in biochemical experiments[4] and in supramolecular photochemistry.[5] There are several positions on the chromophore where functionalisation can be carried out. Most commonly in the literature, the pyrroles are substituted with phenyl or alkyl groups, and functional groups such as ligands or biomolecules are introduced *via* the 8-aryl group. This functional group may be introduced before or after the BODIPY formation from pyrrole **2** and substituted benzaldehyde or benzoyl chloride **3** (Scheme 1).

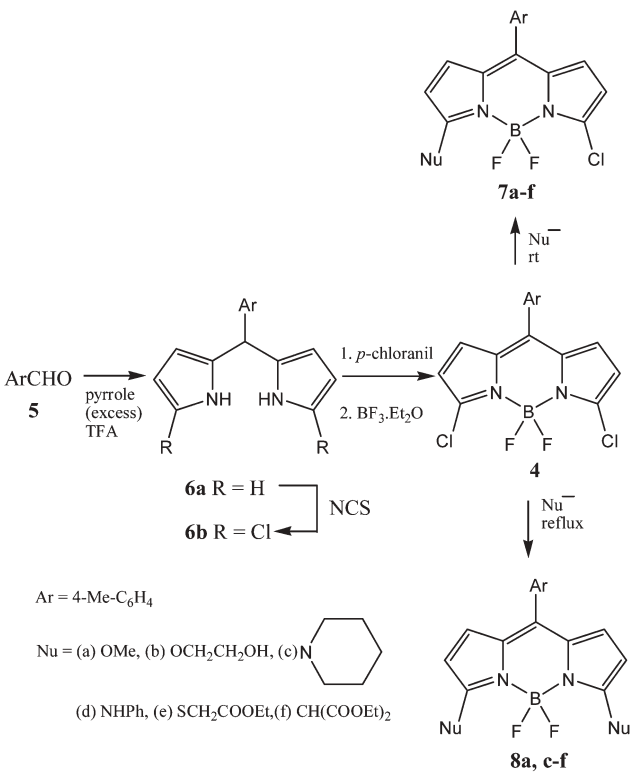
Electronic conjugation between the *meso*-aryl group and the chromophore is weak, due to the two moieties being almost perpendicular to each other.[6] A more effective way to modulate the properties of the BODIPY chromophore **1** is to work with differently substituted aryl groups at the pyrrole rings, *e.g.* the 3,5-positions.[7] However, this will involve a preceding, not always straightforward synthesis of the arylpyrrole building blocks.

Direct introduction of electron donating groups, without aryl spacer, at the 3,5-positions would also have a significant effect, but the corresponding pyrroles are not readily available for condensation reactions. Therefore, we wanted to investigate whether it would be possible to introduce these substituents on a ready-made BODIPY chromophore by nucleophilic substitution at the 3,5-positions.

Firstly, we prepared the novel 3,5-dichloro-BODIPY derivative **4** in a few simple steps adapting literature procedures,[8] starting from 4-methylbenzaldehyde **5** that was converted to the dipyrromethane **6a** (R = H) with an excess of pyrrole and trifluoroacetic acid (TFA) as a catalyst (Scheme 2). Chlorination of **6a** (R = H) with *N*-chlorosuccinimide (NCS), followed by oxidation of **6b** (R = Cl) with *p*-chloranil and complexation with $BF_3{\cdot}Et_2O$ gave our starting material **4** in a 20% overall yield for the four steps.



Scheme 1



Scheme 2

Department of Chemistry, Katholieke Universiteit Leuven, Celestijnenlaan 200F, 3001 Leuven, Belgium. E-mail: Wim.Dehaen@chem.kuleuven.be; Fax: +32 16 327990; Tel: +32 16 327390

As a first oxygen-centred nucleophile, we tried methoxide (2 equivalents in methanol as solvent) at room temperature. This reaction gave the monosubstituted product **7a** in good yield. Under more forcing conditions, namely four equivalents of methoxide at reflux temperature (again in methanol), we obtained the disubstituted derivative **8a**. Ethylene glycol with sodium hydride in acetonitrile at room temperature reacted with **4** to afford the monosubstituted **7b**, while reaction with excess ethylene glycol/sodium hydride at reflux temperature did not give the disubstituted derivative **8b** but led to the decomposition of the chromophore.

Next, several nitrogen-centred nucleophiles were tried. Without the addition of additional base, the secondary amine piperidine caused monosubstitution of **4** at room temperature yielding **7c**. Again, heating at reflux temperature (in acetonitrile) with excess nucleophile led to the disubstituted BODIPY derivative **8c**. Analogous mono- and disubstitution reactions occurred with aniline, affording respectively **7d** and **8d**. The 1,10-diaza-18-crown-6 **9** at room temperature reacted with **4** (2 equivalents) to give the disubstituted bis(BODIPY)diazacrown ether **10**.

We then used ethyl 2-thioacetate with triethylamine as base to demonstrate the reactivity of sulfur-based nucleophiles. Again, reaction at room temperature yielded the monosubstituted derivative **7e**, while disubstitution was possible at reflux temperature, yielding **8e**.

Finally, diethyl malonate together with sodium hydride as base was used as a carbon nucleophile to afford either the mono or disubstituted BODIPY derivatives **7f** and **8f**, respectively.

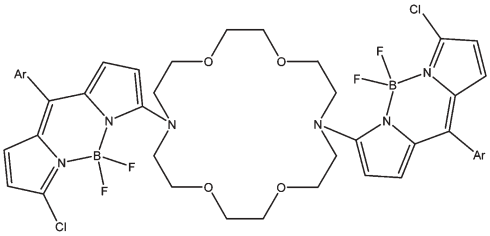
Two different nucleophiles can be introduced after each other using similar conditions. Thus, monosubstituted **7a** could be substituted with *N*-methylaniline and sodium hydride base to afford **11**. Reaction conditions and yields are summarized in Table 1

As an example, Fig. 1 shows the absorption and steady-state fluorescence emission spectra of **7a**, **8a** and **8e** dissolved in methanol. The absorption spectra of **7a** and **8a** are of similar shape as those of previously described boron dipyrromethene dyes:[9] *i.e.*, a narrow absorption band with a maximum around 500 nm, and in addition, a considerably weaker, broad absorption band with a maximum around 350 nm. Compounds **7a**, **8a** and **8e** also show the typical emission features of BODIPY:[9] *i.e.*, a narrow, slightly Stokes-shifted fluorescence emission band of mirror image shape.

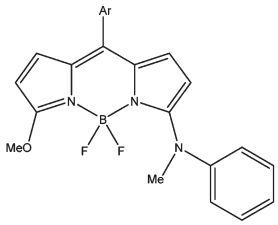
Table 1 Nucleophilic substitution of **4** (or **7a**) with O, N, S, and C centred nucleophiles. The solvent was acetonitrile, except for the reactions with NaOMe where methanol was used

Nucleophile (equiv.)	Temp.	Reaction time	Product	Yield (%)
NaOMe (2)	rt	30 min	**7a**	65
NaOMe (4)	reflux	3 h	**8a**	66
$HOCH_2CH_2OH$ (2), NaH (1)	rt	2 h	**7b**	68
Piperidine (2)	rt	15 min	**7c**	74
Piperidine (4)	reflux	2 h	**8c**	78
Aniline (2)	rt	30 min	**7d**	69
Aniline (4)	reflux	8 h	**8d**	64
$EtOOCCH_2SH$ (2), Et_3N (2)	rt	3 h	**7e**	65
$EtOOCCH_2SH$ (4), Et_3N (2)	reflux	8 h	**8e**	70
1,10-Diaza-18-crown-6 (0.5)	rt	30 min	**10**	61
Diethyl malonate (2.2), NaH (1)	rt	2 h	**7f**	67
Diethyl malonate (4), NaH (2)	reflux	5 h	**8f**	71
N-Methyl aniline (2.2), NaH (1)	reflux	4 h	**11**[a]	60

[a] starting from **7a**



10



11

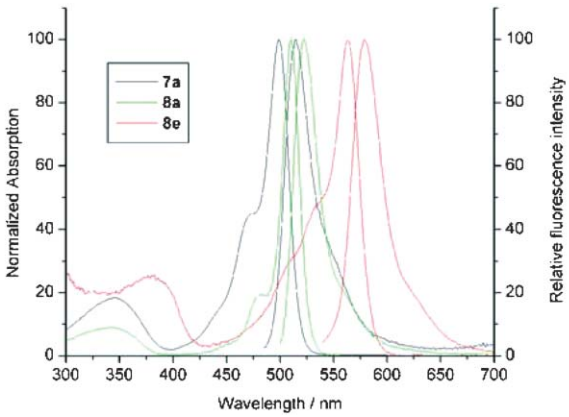


Fig. 1 Normalized absorption and fluorescence emission spectra of **7a** (black), **8a** (green) and **8e** (red) in methanol.

Table 2 summarizes the photophysical data [the position of the spectral maxima (λ_{abs}, λ_{em}), the fluorescence quantum yields (ϕ_f), and the Stokes shifts ($\Delta\bar{\nu} = \bar{\nu}_{abs} - \bar{\nu}_{em}$)] of several new BODIPY compounds in methanol and cyclohexane. The excitation maxima coincide exactly with the absorption maxima. The absorption, excitation and emission spectra can be shifted considerably by the appropriate substitution pattern at positions 3 and 5.

The photophysical properties of the new BODIPY derivatives will be described in detail elsewhere.

The easily obtained 3,5-dichloroBODIPY can be substituted with a wide range of oxygen, nitrogen, sulfur, and carbon centred nucleophiles and the reaction conditions can be adjusted to have either mono- or disubstitution. These nucleophilic addition–elimination substitution reactions of the 3,5-dichloroBODIPY core happen to be a very successful approach for preparing a variety of symmetric and asymmetric BODIPY compounds with substitution patterns that are difficult to realize otherwise. The substituents at the 3 and 5 positions have a significant effect on the

Table 2 Absorption and fluorescence emission spectral data of compounds **4**, **7a–f**, **8a**, **8c–f** in methanol and cyclohexane

BODIPY	Solvent	λ_{abs} (max/nm)	λ_{em} (max/nm)	$\Delta\bar{\nu}$ (cm^{-1})	ϕ_f
4	MeOH	508	519	417	0.27
	Cyclohexane	514	525	645	0.33
7a	MeOH	499	515	623	0.062
	Cyclohexane	508	520	738	0.085
8a	MeOH	510	523	487	0.20
	Cyclohexane	513	525	446	0.13
7b	MeOH	500	515	583	0.083
	Cyclohexane	509	520	416	0.17
7c	MeOH	479	562	3083	0.002
	Cyclohexane	517	565	1643	0.004
8c	MeOH	572	612	1143	0.011
	Cyclohexane	584	611	757	0.032
7d	MeOH	498	566	2412	0.003
	Cyclohexane	529	567	1267	0.28
8d	MeOH	588	613	694	0.45
	Cyclohexane	594	616	601	0.86
7e	MeOH	536	550	475	0.26
	Cyclohexane	542	553	367	0.24
8e	MeOH	564	579	459	0.50
	Cyclohexane	568	583	453	0.49
7f	MeOH	508	522	528	0.28
	Cyclohexane	514	526	444	0.46
8f	MeOH	509	522	489	0.35
	Cyclohexane	515	527	442	0.62

photophysics of the BODIPY fluorophore, causing shifts in the absorption and/or emission spectra (see Fig. 1), and affecting the fluorescence quantum yields.

Asymmetrically substituted BODIPY derivatives such as **7a–f**, **10** and **11** are not readily available by any reported synthetic method. For such compounds a multistep procedure would normally be needed, starting from pyrroles substituted with electron rich substituents that are unstable or difficult to obtain.

This new synthetic method allows for the easy linking of the BODIPY unit to biomolecules or other groups of interest, as demonstrated by the substitution of a diazacrown ether.

The authors thank the University Research Fund of the K.U.Leuven for grant IDO/00/001 and for postdoctoral fellowships to M.B. and W.Q. The Fonds voor Wetenschappelijk Onderzoek – Vlaanderen (FWO) and the IAP-V-03 programme are thanked for continuing support.

Notes and references

1 (*a*) R. P. Haugland, *The Handbook. A Guide to Fluorescent Probes and Labeling Technologies*, 10th edn, Molecular Probes Inc., Eugene, Oregon, 2005; (*b*) BODIPY® is a registered trademark of Molecular Probes, Inc., Eugene, Oregon, USA.
2 A. Treibs and F.-H. Kreuzer, *Justus Liebigs Ann. Chem.*, 1968, **718**, 208–223.
3 (*a*) K. Yamada, Y. Nomura, D. Citterio, N. Iwasawa and K. Suzuki, *J. Am. Chem. Soc.*, 2005, **127**, 6956–6957; (*b*) A. Coskun and E. U. Akkaya, *J. Am. Chem. Soc.*, 2005, **127**, 10464–10465; (*c*) N. Basarić, M. Baruah, W. Qin, B. Metten, M. Smet, W. Dehaen and N. Boens, *Org. Biomol. Chem.*, 2005, **3**, 2755–2761; (*d*) J.-P. Malval, I. Leray and B. Valeur, *New J. Chem.*, 2005, **29**, 1089–1094.
4 (*a*) E. V. Pozharski and R. C. MacDonald, *Anal. Biochem.*, 2005, **341**, 230–240; (*b*) Z. M. Li, X. P. Bai, H. Ruparel, S. Kim, N. J. Turro and J. Y. Ju, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 414–419.
5 G. Beer, C. Niederalt, S. Grimme and J. Daub, *Angew. Chem., Int. Ed.*, 2000, **39**, 3252–3255.
6 B. Herradón, A. Chana, M. Alonso, F. Amat-Guerri, M. Liras and M. Maestro, *J. Mol. Struct.*, 2004, **697**, 29–40.
7 (*a*) K. Rurack, M. Kollmannsberger and J. Daub, *Angew. Chem., Int. Ed.*, 2001, **40**, 485; (*b*) G. Ulrich and R. Ziessel, *Tetrahedron Lett.*, 2004, **45**, 1949–1953.
8 (*a*) B. J. Litter, M. A. Miller, C.-H. Hung, R. W. Wagner, D. F. O'Shea, P. D. Boyle and J. S. Lindsey, *J. Org. Chem.*, 1999, **64**, 1391–1396; (*b*) M. Baruah, W. Qin, N. Basaric, W. M. De Borggraeve and N. Boens, *J. Org. Chem.*, 2005, **70**, 4152–4157.
9 (*a*) E. Vos de Wael, J. A. Pardoen, J. A. van Koeveringe and J. Lugtenburg, *Recl. Trav. Chim. Pays-Bas*, 1977, **96**, 306–309; (*b*) T. A. López, F. L. Arbeloa, I. L. Arbeloa, I. Garcia-Moreno, A. Costela, R. Sastre and F. Amat-Guerri, *Chem. Phys. Lett.*, 1999, **299**, 315–321; (*c*) A. Costela, I. Garcia-Moreno, C. Gomez, R. Sastre, F. Amat-Guerri, M. Liras, F. L. Arbeloa, J. B. Prieto and I. L. Arbeloa, *J. Phys. Chem. A*, 2002, **106**, 7736–7742; (*d*) F. L. Arbeloa, J. B. Prieto, V. M. Martínez, T. A. López and I. L. Arbeloa, *ChemPhysChem*, 2004, **5**, 1762–1771; (*e*) J. B. Prieto, F. L. Arbeloa, V. M. Martínez, T. A. López, F. Amat-Guerri, M. Liras and I. L. Arbeloa, *Chem. Phys. Lett.*, 2004, **385**, 29–35; (*f*) W. Qin, M. Baruah, M. Van der Auweraer, F. C. De Schryver and N. Boens, *J. Phys. Chem. A*, 2005, **109**, 7371–7384.

