First bodipy–DOTA derivatives as probes for bimodal imaging†

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The synthesis and the photophysical studies of the first bodipy–DOTA and its In(III), Ga(III) and Cu(II) complexes are reported. The introduction of an isothiocyanate handle generates a new bimodal imaging agent capable of both optical and nuclear imaging.

Multimodal imaging agents can provide complementary information improving the accuracy of disease diagnosis and enhancing patient management.[1] Many clinical and preclinical imaging applications are based on positron emission tomography (PET), single-photon emission computed tomography (SPECT), fluorescence imaging, bioluminescence, magnetic resonance imaging (MRI), and ultrasound. Each imaging modality has its own strengths and weaknesses, and thus, combining different and complementary systems can overcome inherent limitations associated with any one individual technique. In particular dual-modality optical/nuclear imaging may find important preclinical and clinical applications. For example, the radionuclide component can provide early quantitative data in a target tissue, and subsequent events can be monitored longitudinally by an optical method. In intraoperative procedure, diseased tissues can be localized by nuclear imaging (ex: PET/CT) which are then biopsied for histologic validation by an optical method. Recently, elegant molecular designs have been developed to take full advantage of the unique high detection sensitivity of both optical and nuclear imaging methods. One possible approach seeks to fuse the two imaging systems into one molecule (MonOmolecular Multimodality Imaging Agent [MOMIA])[2] in order to ensure the same biodistribution of the two probes.

Despite the high sensitivity and complementary nature of radionuclear and optical methods, combinations of these two imaging modalities are rare.[3] This is attributable to a variety of reasons, one of them being the difficulty to synthesize and characterize species capable of this type of bimodal imaging.[4]

DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) is one of the most important chelators in radiochemistry as it forms very stable complexes with a large number of metal ions. It thus finds wide application in medical imaging.[5] This azamacrocycle is commonly used for *in vivo* applications such as cancer therapy and clinical diagnosis.[6] More precisely, the properties of DOTA have been explored as MRI contrast agents,[7] or for labeling biomolecules using metal radioisotopes for both diagnosis (SPECT or PET) and therapeutic purposes.[8]

Among fluorescent probes, bodipy derivatives represent a promising family due to their exceptional properties. They exhibit high stability, high extinction coefficients, sharp emission bands and high quantum yields.[9] Bodipys are involved in many applications such as donor–acceptor systems, artificial light harvesters, fluorescent sensors, laser dyes, electron-transfer reagents and light emitting devices.[10] Despite all the advantages of this dye, bimodal imaging agents containing a bodipy moiety have never been synthesized and studied. This is probably due to one major drawback which has to be solved before involving the bodipy moiety in bimodal imaging systems. Indeed, most bodipys suffer from intrinsic hydrophobicity that limits their usefulness as labels for biomolecules. While a few teams are working on the synthesis of hydrophilic bodipys, this interesting field remains largely unexplored.[11]

In this communication, we report the synthesis of new bodipy DOTA derivatives. Interestingly, the coupling of the azamacrocycle to the bodipy induced the complete solubilization of the molecule in water. The bodipy–DOTA derivative was then metallated with Cu(II), In(III) and Ga(III). Complexation of In(III) and Ga(III) didn't affect the fluorescence of the bodipy component of the molecule, while Cu(II) partially quenched the fluorescence of the ligand.

Bodipy **1** containing an activated ester[12] and ethylene diamine-functionalized cyclen **2**[13] were synthesized by adapting literature procedures (ESI†). Deprotection of the *tert*-butyl ester groups in **2** to give cyclen derivative **3** followed by coupling to the activated bodipy **1** (Scheme 1) afforded the desired water-soluble DOTA–bodipy **4**. Here the order of deprotection followed by coupling was essential to synthetic success. Indeed, the boron part of the bodipy didn't survive the *tert*-butyl ester group deprotection conditions. The synthesis of the In(III), Ga(III) and Cu(II) complexes was achieved by addition of one equivalent of the metal salt to bodipy–DOTA **4** (Scheme 2). The three neutral complexes are soluble in water. All compounds present a strong absorption transition in the 522–527 nm range, which can be attributed to S_0–S_1 transition of the bodipy (Table 1).

The molar absorption coefficients (ε) for this transition are relatively high, in the 30 000–75 000 M^{-1} cm^{-1} range, and are strongly dependent on the solvent. A second broader and weaker band at around 375 nm can be attributed to the S_0–S_2 transition of the bodipy fragment. All the compounds exhibit the typical emission features of bodipy, that is, narrow, slightly Stokes shifted bands of mirror image shape, and relatively high quantum yields. In all compounds, the excitation spectrum of the emissive species matches the absorption, confirming that the emission comes only from the S_1 energy

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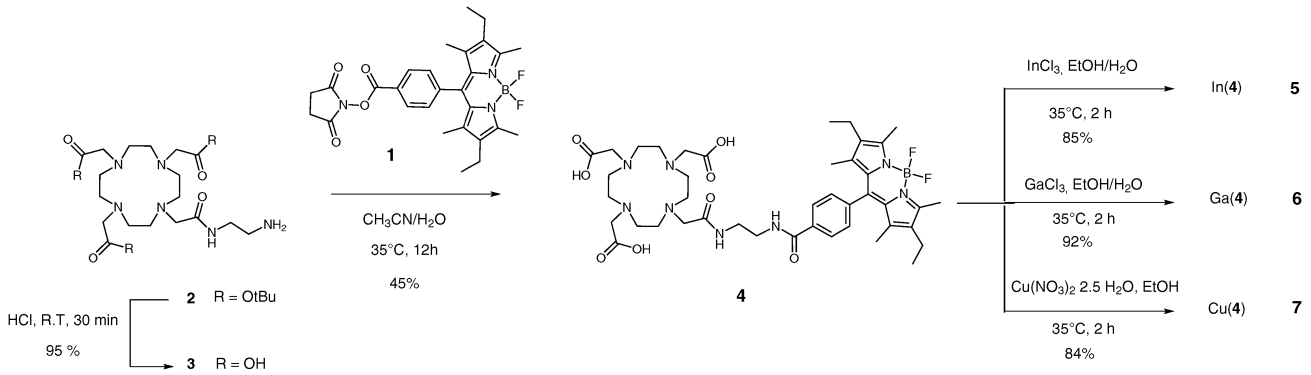
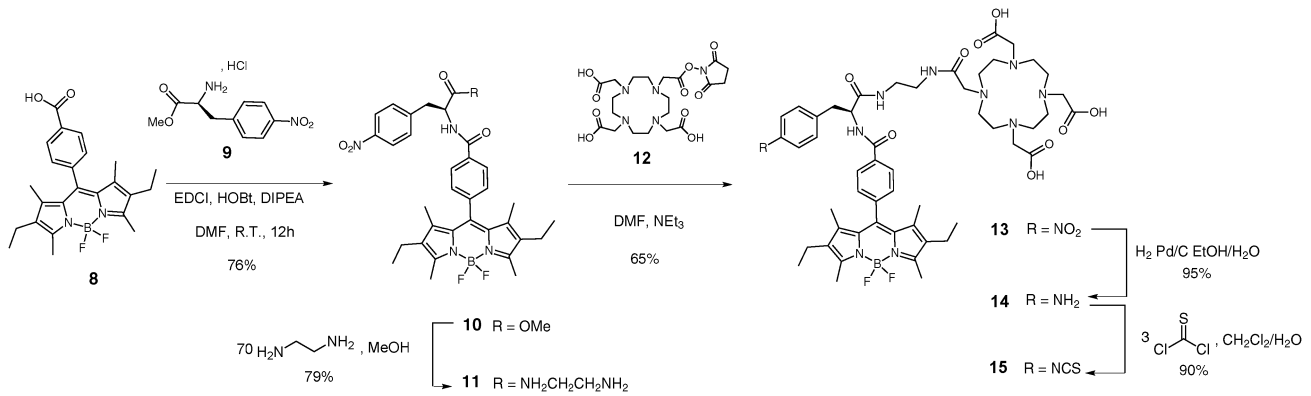


Scheme 1



Scheme 2

Table 1 Spectroscopic data of bodipy–DOTA derivatives in several solvents at 298 K

Bodipy	Solvent	λ_{abs}/nm	ε/M^{-1} cm^{-1}	λ_{em}/nm	$\Delta\nu$/cm^{-1}	Φ_f (%)[a,b]
1	MeCN	525	73 000	542	597	64
4	MeCN	522	31 000	536	400	42
	MeOH	524	59 500	536	427	58
	H_2O	524	31 000	536	427	50
	DMF	525	35 000	538	460	65
5	H_2O	527	32 000	540	457	48
	DMF	525	36 000	538	460	63
6	H_2O	523	34 000	540	602	50
	DMF	525	75 000	538	460	61
7	H_2O	523	30 400	539	567	21
	DMF	525	60 300	536	391	23

[a] λ_{exc} = 488 nm. [b] Using Rhodamine 6G as reference, Φ_f = 0.78 in water, λ_{exc} = 488 nm.[14] All Φ_f are corrected for changes in refractive index.

state of the bodipy system in the different molecules. For all molecules, the Stokes shifts ($\Delta\nu$ = 534–542 cm^{-1}) are in good agreement with those reported elsewhere for meso-aryl substituted bodipy dyes.[15]

Bodipy–DOTA **4** presents a quantum yield of 50% in water. The fluorescence is not perturbed upon complexation of Ga(III) and In(III) which fit well in the cavity of DOTA ligands (Fig. 1). Fluorescence of the Cu(II) complex **7** is only 50% as intense as the non-metalated ligand DOTA–bodipy **4**. This quenching is probably due to a partial PET (photoinduced electron transfer) mechanism between the bodipy and the redox active Cu(II) cation. However, the quantum yield of the Cu(II) complex in water remains 21%, which is still reasonably high for fluorescent probes.

With the model bimodal imaging agent **5–7** in hand we sought to add a handle that could be used to conjugate the bodipy–DOTA derivatives to a biorelevant species of interest, such as an antibody or a peptide. The isothiocyanate functional group was chosen for this purpose due to its synthetic accessibility and more importantly its proven track record in biomolecule conjugation. For instance, the isothiocyanate group reacts with free N-terminal lysine residues under mild conditions forming a thiourea linker.[16] Bodipy

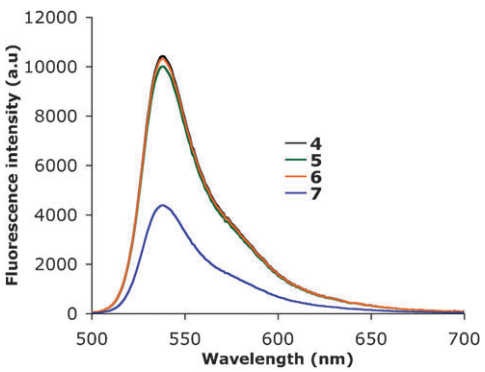


Fig. 1 Emission spectra of the bodipy–DOTA **4** and its corresponding complexes in water at 298 K.

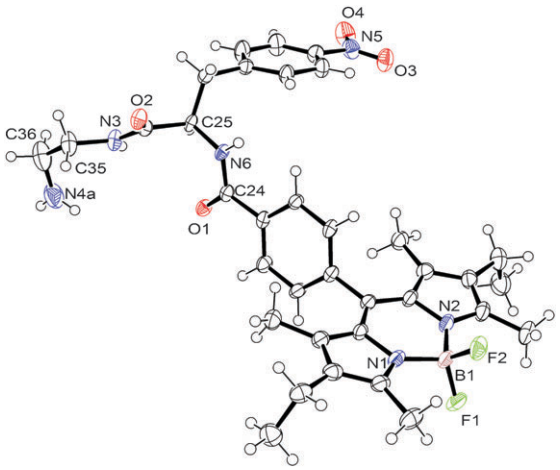


Fig. 2 ORTEP view of compound **11**, showing thermal ellipsoid at the 50% probability level. Disordered ending amine group and dichloromethane are omitted for clarity.

derivative **8**[12] was coupled with the 4-nitrophenylalanine methylester **9** to give compound **10** (Scheme 2), which was then reacted with an excess of ethylenediamine to give primary amine containing bodipy derivative **11**. Red crystals of bodipy **11** suitable for structure determination *via* X-ray diffraction were obtained from dichloromethane (Fig. 2).

Coupling of the bodipy amine **11** with the activated DOTA NHS ester‡ **12** gave nitro-bodipy DOTA **13** (Scheme 2). The advantage of this approach is the possibility to introduce at this stage any kind of macrocyclic chelating agent. Indeed, tacn or cross-bridged cyclam derivatives, which are more adapted to Ga(III) and Cu(II), respectively,[17] can be used instead of the DOTA compound **12**. Finally, the nitro functional group was converted into isothiocyanate *via* reduction to the amine and reaction with thiophosgene to give the bimodal imaging agent **15** containing a handle for biomolecules.

In conclusion, the first DOTA–bodipy system and its corresponding In(III), Ga(III) and Cu(II) complexes were prepared. The complexes exhibit promising fluorescent properties and are water-soluble, thus solving one of the problems preventing the use of bodipy agents for *in vivo* optical imaging. A second generation bodipy–DOTA containing an isothiocyanate function was prepared for use as a tag capable of simultaneous optical imaging and PET or SPECT scintigraphy. Further studies (electrochemistry, complexation constants determination, and modification of the bodipy skeleton by derivatization reaction with benzaldehyde derivatives to reach the near-IR range)[18] are currently being undertaken in our laboratory.

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Notes and references

‡ The macrocycle DOTA NHS ester was obtained from CheMatech (www.chematech-mdt.com).

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