## Water-soluble benzo- and naphtho-thiadiazole-based bistriazoles and their metal-binding properties<sup>†</sup>

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## A click reaction furnishes water-soluble acenothiadiazole-based bistriazoles with red-shifted absorption and emission characteristics.

The 1,3-dipolar cycloaddition of alkynes to azides was first investigated by Szeimies, Huisgen *et al.*,<sup>1</sup> and was later retooled as a copper catalyzed process for the easy access towards 1,4-disubstituted triazoles.<sup>2</sup> While intended by Kolb and Sharpless<sup>3</sup> for the construction of biologically active molecules, the "click" reaction is now also popular for the construction of polymers and materials.<sup>4</sup>

A generally attractive but less explored aspect of the triazole formation is their incorporation, as functional modules, into fluorophores and/or chromophores; the triazole group could either work as an auxochromic group or a conjugative bridge between two chromophores or  $\pi$ -systems.<sup>5</sup> Herein we report that triazoles are quite powerful auxochromes, *i.e.*, functional groups that red-shift the absorption and emission of acenothiadiazole-types.

We<sup>6,7</sup> and others<sup>8</sup> are interested in metallo-responsive fluorophore systems, and we have recently disclosed that a dipolar 1,3-cycloadduct containing a 2-pyridyl residue leads to turn-on fluorescence when exposed towards metal cations. In this communication we present attractive, novel water-soluble and fluorescent bis-cycloadducts **4** and **5** that display binding pockets for metal cations.

The TMS-protected diethynylbenzothiadiazole  $(1)^9$  is deprotected *in situ* by KF; the azide **3**, CuSO<sub>4</sub> (2.5 eq.) and sodium ascorbate (2.5 eq.) are added to give **4** in 55% yield (Scheme 1). The same approach works for **5** which is formed analogously in 60% yield by reaction of **2** with **3** under identical conditions. The use of a large excess of copper sulfate was necessary to catalyze this reaction, as the products **4** and **5** bind copper salts quite efficiently. We selected the oligoethylene azide **3** as substituent, as it (a) confers water solubility and (b) is known for its "biophobic" properties, *i.e.*, it suppresses non-specific interactions with proteins, *etc.* Table 1 summarizes the relevant photophysical properties of **1**, **2**, **4** and **5**, while Fig. 1 shows the absorption and emission spectra of **1**, **2**, **4** and **5**.

There are several noteworthy trends. In dichloromethane (DCM) the emissive quantum yields of all investigated species (1, 2, 4, and 5) are high. Generally, the quantum yields of the precursor alkynes are somewhat higher than those of their



Scheme 1 Synthesis of compounds 4 and 5.

1,3-dipolar azide adducts. As the precursor alkynes do not dissolve in water, only the quantum yields of **4** and **5** were obtained for aqueous solutions. While **4** displays quite a robust quantum yield of 13% in water, in the case of **5** the quantum yield in water drops to 1.3%. With its emission wavelength of 519 nm and a quantum yield of 13%, **4** is potentially a useful fluorophore core as it is stable towards photobleaching. In addition, the emissive lifetimes of **4** in DCM (14 ns) and in water (7 ns) solutions are unusually high. The larger congener **5** displays similarly large fluorescence lifetimes of 18 ns and 5 ns in DCM and water, respectively. This is testimony to the photophysical properties of the benzothiadiazole core, as the precursor alkynes also display relatively long emissive lifetimes, although not as long as those observed for the 1,3-cycloadducts.

The long fluorescence lifetime of the cycloadduct **4** is promising for potential applications as a bio-fluorophore. As complex biological matrices such as cells, serum, *etc.* are fraught with background fluorescence<sup>10</sup> with an emissive half-life of around 3 ns, fluorophores such as **4** should shine if time-gated detection is used. Quite unusual is also that the emission and absorption wavelengths of **4** and **5** are not very solvent dependent, and that we actually see a slight blue-shift in the absorption features for both **4** and **5** when going from DCM into water as a result of the change of the refractive index (Table 1).

The emission of **4** displays a slight red-shift when going from DCM to water, while **5** displays a slight blue-shift upon the same solvent change (Table 1). The similarity of the spectral properties in water and in DCM suggests that these fluorophores do not exhibit a large degree of charge transfer character in the ground or excited states; **4** and **5** are electron poor as both of their constituent modules (triazoles and benzothiadiazole) are electron accepting.

Surprising are the quite significant red-shifts in the absorption onset and particularly emission wavelengths of the 1,3-diploar cycloadducts 4 and 5 in comparison to their respective diyne precursors 1 and 2.<sup>11</sup> Quantum chemical calculations (B3LYP  $6-31G^{**}//6-31G^{**}$  as implemented by SPARTAN for Windows) support this trend (Table 1) and show that the HOMO and the LUMO positions are both raised, however the destabilization of the HOMO is more pronounced than the

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Table 1	Photophysical	properties of	compounds	1, 2,	4, and 5	5 in	dichloromethane and water
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Cpd	Abs $\lambda_{\max}$		Emission $\lambda_{\max}$		${\it \Phi}^a$		Fluorescence lifetime (ns, $\tau$ )		Stokes shift/cm <sup>-1</sup>		Gap calcd <sup>b</sup> eV (nm)	FMO positions calculated <sup>b</sup> /eV	
	DCM	$H_2O$	DCM	$H_2O$	DCM	$H_2O$	DCM	$H_2O$	DCM	$H_2O$		НОМО	LUMO
1 <sup>c</sup> 4 2 <sup>c</sup> 5	386 406 528 505	NA 385 NA 475	443 508 534 610	NA 519 NA 602	0.85 0.72 0.28 0.09	NA 0.13 NA 0.013	7.9 14.0 15.2 18.4	NA 7.0 NA 5.3	3334 4946 212 3409	NA 6706 NA 4442	3.41 (364) 3.10 (399) 2.50 (496) 2.35 (527)	-6.15 -5.64 -5.55 -5.13	-2.74 -2.54 -3.05 -2.78

<sup>&</sup>lt;sup>*a*</sup> Quinine sulfate in 0.1 M H<sub>2</sub>SO<sub>4</sub> (aq.) used as a reference standard. <sup>*b*</sup> B3LYP 6-31G\*\*//6-31G\*\* SPARTAN. <sup>*c*</sup> In the case of 1 and 2, the TIPS protected analogue was utilized due to its greater stability than the corresponding TMS or TES analogues.



Fig. 1 Normalized absorption and emission spectra of 1 (blue), and 2 (green, both top) and 4 (blue) and 5 (green, both bottom) in dichloromethane. Solid lines depict absorption spectra while broken lines depict emission spectra. In the case of 1 and 2, the TIPS protected analogue was utilized due to its greater stability than the corresponding TMS or TES analogues.

destabilization of the LUMO when going from alkyne to triazole. The absorption and emission profiles for **6a** (DCM;  $\lambda_{\text{max abs}} = 409 \text{ nm}, \lambda_{\text{max emission}} = 507 \text{ nm}$ ) are very similar to those obtained for **4** and Spartan (B3LYP 6-31G\*\*//B3LYP 6-31G\*\*) computes HOMO and LUMO of the model compound **6b** at -5.69 eV and -2.66 eV. Consequently, while the triazole unit has a strong auxochromic effect, it is poor at transmitting electronic communication between two  $\pi$ -systems.



Adducts 4 and 5 display a binding pocket that should readily bind to metal analytes of appropriate charge and



Fig. 2 Representative absorption titration of 4 (blue trace;  $151.5 \,\mu M$ ) with CuSO<sub>4</sub> in water. [Cu] ranges from 0–6.26 ×  $10^{-1}$  M (red trace 4–Cu<sup>2+</sup> complex).



Fig. 3 Emission data (black dots) of the titration of 4 (151.5  $\mu$ M) with CuSO<sub>4</sub> in water. Red line indicates the fitted equation used to determine binding constant. [Cu] ranges from 0–6.26 × 10<sup>-1</sup> M. Inset: representative emission titration of 4 with CuSO<sub>4</sub> in water.

atomic radius as previously demonstrated by us and by Xie *et al.* for similar types of 1,3-dipolar cycloadducts, but only in non-aqueous solutions.<sup>12</sup>

Our adducts (4 and 5) allow the screening of metal binding in water. Upon addition of the triflate salts of  $Li^+$ ,  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ ,  $Zn^{2+}$ ,  $Mg^{2+}$ , and  $Sn^{2+}$  to an aqueous solution of 4, no change in the absorption or emission spectra is observed. Upon addition of  $Hg^{2+}$  and trifluoroacetic acid (TFA) no change in the absorption spectra is observed, however the emission of 4 becomes quenched (see ESI<sup>†</sup>). Upon the addition

 Table 2
 Binding data of 4 and 5 with copper(II) and nickel(II) in water

Compound Metal <sup>a</sup>	<b>4</b> Cu(11)	<b>4</b> Ni(11)	5 Cu(II)
log K from absorption <sup>b</sup> log K from emission <sup>c</sup>	$\begin{array}{c} 2.83\\ 2.70\pm0.01\end{array}$	$\begin{array}{c} 3.18\\ 3.17\pm0.01\end{array}$	$2.96 \\ 2.71 \pm 0.03$
<sup><i>a</i></sup> The sulfate salt was use deconvolution of the abs <sup><i>c</i></sup> Values obtained from fi	ed in all cases. orption spectra	<sup>b</sup> Values obtain a utilizing Data whing of the em	ined from the an software. <sup>14</sup>

of  $Cu^{2+}$  or  $Ni^{2+}$  the charge transfer band in the absorption spectra red-shifts (~20–30 nm) and the emission also quenches (Fig. 2 and 3). Titrations of **4** and **5** with copper sulfate and **4** with nickel sulfate in water were performed to determine the strength of the binding.

Attempting to plot the fluorescence quenching spectra according to the standard Stern–Volmer equation resulted in significant deviation from linearity (see ESI<sup>†</sup>). However, the data were well correlated when eqn (1) is employed,<sup>12,13</sup>

$$I_{q} = I_{o} + \frac{I_{\text{final}} - I_{o}}{2} \times \left\{ 1 + \frac{[Q]}{[F]} + \frac{1}{K_{\text{SV}}[F]} - \left[ \left( 1 + \frac{[Q]}{[F]} + \frac{1}{K_{\text{SV}}[F]} \right)^{2} - 4 \frac{[Q]}{[F]} \right]^{\frac{1}{2}} \right\}$$
(1)

where  $I_q$  is the intensity of the fluorescence at a given quencher concentration,  $I_o$  is the initial fluorescence intensity of the fluorophore,  $I_{\text{final}}$  is the final intensity of the fluorescence of the quenched fluorophore, [Q] is the concentration of the quencher added, [F] is the concentration of the fluorophore and  $K_{\text{sv}}$  is the apparent Stern–Volmer constant.

The results of the titration are summarized in Table 2. The binding of **4** to Ni(II) in water resulted in a binding constant of log  $K = 3.17 \pm 0.01$ . The lower binding constant in comparison to Xie's (log  $K = 4.48 \pm 0.03$ ) is expected as the titration was performed in water, which is a more competitive ligating solvent than acetonitrile. The binding constant for Cu(II) in water was slightly smaller in magnitude than that of nickel with log  $K = 2.70 \pm 0.01$ .

The binding constant for the binding of copper to 5 was determined to be  $\log K = 2.71 \pm 0.03$ . This binding constant was nearly identical to that of 4 which demonstrated the independence of the binding upon the size of the aceno portion of the core. Interestingly, the necessity of a stoichiometric amount of copper in the synthesis of 4 and 5 can be attributed to this high binding constant as once the triazole group is formed, the effective concentration of free copper available to catalyze the reaction is drastically reduced.

Binding constants were also obtained from the deconvolution of the absorption spectra from the titration of the metal utilizing Datan software.<sup>14</sup> In all cases, the constants obtained from the absorption spectra were in good to excellent agreement with the values obtained from the Stern–Volmer plots of the emission spectra. Assuming a 1 : 1 complex agrees very well with the obtained data. Apparently upon coordination to one copper ion, the second binding pocket becomes too electron poor to effectively bind another copper ion in aqueous solution; it is not clear in how far the oligoethylene glycol units partake in the binding of the copper.

In conclusion, we have prepared water-soluble bistriazoles 4 and 5 and the model compound 6a. From the combination of spectroscopic and computational data, we can conclude that the triazole-ring has a strong auxochromic effect and leads to red-shifted spectroscopic features for the connected arene in the 4-position, but at the same time is a poor electronic conduit, as the spectroscopic properties of 6a are almost identical to that of 4. The adducts 4 and 5 do not show large solvent dependencies of their spectroscopic properties, and 4 is surprisingly fluorescent in water and binds both copper and nickel in aqueous solution. Overall, the 1,3-dipolar cycloaddition of alkynes to azides is a superb tool to prepare functional, metallo-responsive fluorophores.

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with eqn (1).