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Sydnone-Cyanines as clickable Probes for Fluorescent Labelling

Kim Anh Nguyen,^a Emilie Lesur,^a Maxime Ribéraud,^a Antoine Sallustrau,^a Davide Audisio^a and Frédéric Taran*^a

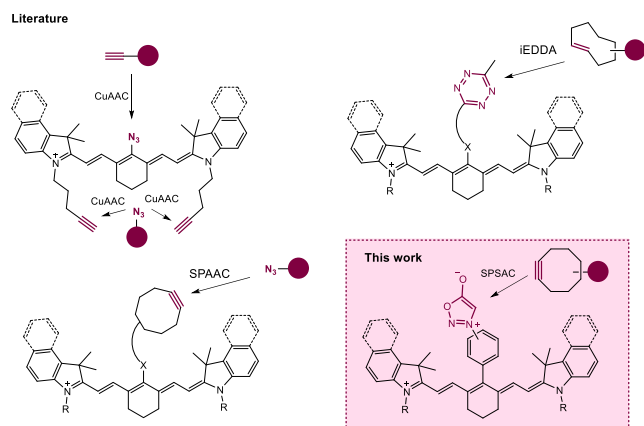
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The synthesis of four clickable sydnone-heptamethine cyanine derivatives is described in this article. The synthetic route is based on a palladium-cross coupling reactions of sydnone boronates affording the desired sydnone-cyanine conjugates in only 5 steps. These compounds were shown to react smoothly with cyclooctynes to form the corresponding pyrazoles clicked products quantitatively at room temperature and with kinetic constants up to $18 \text{ M}^{-1} \cdot \text{sec}^{-1}$, affording new interesting tools for biorthogonal fluorescent labelling of (bio)molecules. Fluorescence properties of both sydnone- and pyrazole-cyanines are described, as well.

Keywords: Click chemistry • Mesoionics • Iminosydnones • Cyanine

Introduction

Cyanines are major family of dyes for *in vivo* imaging applications due to their outstanding photophysical properties, excellent biocompatibility, and low toxicity to living systems.^[1] Among them, near-infrared fluorescence heptamethine cyanine (Cy7) dyes are of particular interest because of their ability to generate strong fluorescence emission at the range of 700 to 1000 nm, offering the possibility of biomedical imaging, especially in the field of fluorescence-guided tumor surgery.^[2] In addition, some Cy7 have been reported to display photosensitizing properties, which could be potentially exploited in photodynamic therapy for cancer treatments^[3] and their ability to bioaccumulate in tumors has been mainly used to deliver anticancer drugs.^[4] All of these applications require to connect the Cy7 to the desired ligands, drugs or biomolecules, a task which could be challenging. Click chemistry is the ideal solution to link two compounds together under smooth conditions and thus has been widely used to attach cyanines to small as well as large molecules such as antibodies.^[5] The main clickable Cy7 described in literature are derivatized with azide or alkyne functions to allow copper-catalyzed^[6] or strained promoted^[7] azide-alkyne cycloaddition click reactions (CuAAC, SPAAC respectively, Scheme 1) or with tetrazines to allow fast coupling with trans-cyclooctenes (iEDDA, Scheme 1).^[8]

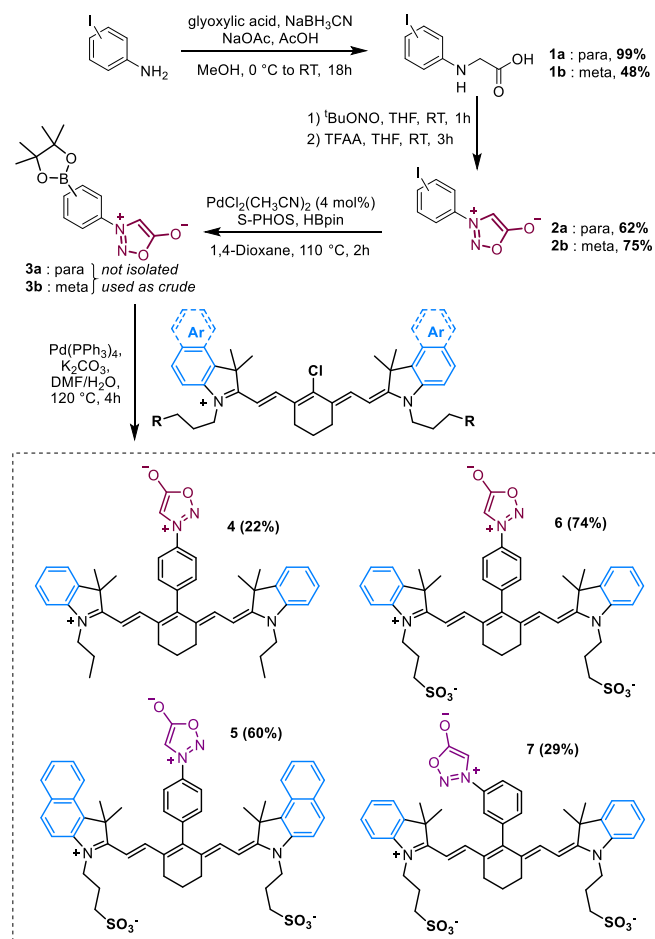


Scheme 1. Examples of known clickable Cy7, Cy7-sydnones envisaged in this work.

Besides these click reactions, the strained promoted sydnone-cycloalkyne cycloaddition (SPSAC)^[9] appears as an interesting alternative to connect Cy7 to cyclooctyne-containing (bio)molecules. In the present article, we describe the synthesis and properties of Cy7-sydnone derivatives that may complete the choice of available click tools to prepare Cy7 conjugates.

Results and Discussion

Based on our own expertise in sydnone chemistry,^[10] we designed four new clickable Cy7: **4**, **5**, **6** and **7** taking advantage of the well-known ability of the chloride on the polyene core of Cy7 to undergo Pd-catalyzed cross-coupling reactions (Scheme 2).^[11] At first, we carried out the synthesis of pinacolboronate-sydnones **3a** and **3b** in 4 steps starting from *meta*- or *para*-iodoaniline. Reaction of the anilines in the presence of glyoxylic acid and sodium cyanoborohydride allowed smooth reductive amination at room temperature in methanol leading to phenylglycines **1a** and **1b** in 99% and 48% yield respectively (Scheme 2). 4-iodophenyl-sydnones **2a** and **2b** were obtained in 62 and 75% yields by a two-steps one-pot procedure involving nitrosylation of the secondary amine function by *tert*-butyl nitrite followed by cyclisation in the presence of trifluoroacetic anhydride. Subsequent palladium cross-coupling between iodo-sydnones **2a** and **2b** with pinacolborane afforded the expected pinacolboronate-sydnones **3a** and **3b**. These products were found not stable enough to be stored and were thus used directly in the next step after rapid filtration on celite. The sydnone moiety was incorporated on Cy7 scaffold *via* a Pd-catalyzed Suzuki reaction between **3a** and **3b** and the chloride on the polyene scaffold of three different Cy7. Four clickable Cy7 were thus obtained in low to high yields (Scheme 2). Once these cyanine-sydnone probes synthesised, we characterised their physico-optical properties in organic solvent and in aqueous media (Table 1). At the outset, we were aware that the hydrophobicity of the heptamethine cyanine scaffold may cause potential aggregation in aqueous media, a phenomenon which is minimised by the presence of hydrophilic sulfonate moieties and which can be further influenced by the presence of the sydnone moiety.



Scheme 2. Synthesis of Sydnone-Cyanine **4**, **5**, **6** and **7**.

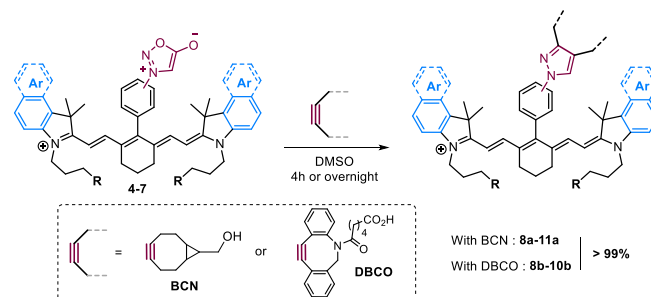
In fact, cyanines **4** and **5**, the most hydrophobic among the four cyanine-sydnones synthesised, were poorly soluble and formed aggregates in water. On the other hand, they displayed strong fluorescence properties in organic solvents. For solubility reasons, compound **5** was solubilized in DMSO, while others were solubilized in methanol. In addition, compound **5** showed the lowest Stock shift among the four cyanines tested, the highest (60 nm) being obtained with cyanine **7** in organo-aqueous media (Table 1). Additional experiments were carried out in other solvents (see supporting information); the high molar attenuation coefficients are in the common range of cyanine derivatives.

Table 1. Physico-optical properties of cyanine-sydnones (Cy7-Syd).

Cy7-Syd	Solvent	λ_{exc} (nm)	λ_{em} (nm)	ϵ (L·mol ⁻¹ ·cm ⁻¹)
4	MeOH	768	781	212000
	PBS/MeOH	aggregate	aggregate	-
5	DMSO	820	823	207000
	PBS/DMSO	aggregate	aggregate	-
6	MeOH	771	785	270000
	PBS/MeOH	764	777	-
7	MeOH	771	785	172000
	PBS/MeOH	763	823	-

PBS : Phosphate Buffer (0.1 M); 1% of organic solvent were used.

We then studied the click reactions between cyanines **4-7** with two different cyclooctynes, the bicyclononyne BCN and the dibenzocyclooctyne DBCO. Cyclooctynes were added to a solution of cyanine-sydnones and the reaction mixture was stirred for 4 hours with DBCO or overnight with BCN at room temperature to reach completion (Scheme 3). LC/MS analysis of all crude reactions proved complete conversion of the cyanine-sydnones **4-7** into the corresponding cyanine-pyrazoles **8-11**, and no byproduct was detected.



Scheme 3. SPSAC reactions between compounds **4**, **5**, **6** or **7** and cyclooctynes DBCO and BCN. Reactions were carried out with 1.05 equiv of cyclooctyne at room temperature for 4h with DBCO or overnight with BCN, and analyzed by LC-MS.

The optical properties of the clicked products resulting from the SPSAC click reactions were then studied to compare them to those of the starting sydnones (Table 2). In order to do so, the cyanine-pyrazoles were solubilised in the same solvents as those used to characterise the cyanine-sydnones precursors.

Table 2. Physico-optical properties of cyanine-pyrazoles (Cy7-Pyr).

SPSAC	Cy7-Pyr	Solvent	λ_{exc}	λ_{em}	Turn-on
4 + BCN	8a	MeOH	762	825	12.3
			762	850	67
4 + DBCO	8b	MeOH	763	777	1.1
5 + BCN	9a	DMSO	816	823	1.3
5 + DBCO	9b	DMSO	806	823	1.7
6 + BCN	10a	MeOH	763	777	1.5
		PBS/MeOH	758	771	1.4
6 + DBCO	10b	MeOH	764	779	1.4
		PBS/MeOH	758	771	1.3
7 + BCN	11a	MeOH	765	779	1.6
		PBS/MeOH	760	772	1.3

The results showed that the optical properties of the cyanine-pyrazoles **9**, **10** and **11** are almost identical to those of the corresponding cyanine-sydnones **5**, **6** and **7** independently from the cyclooctyne used for the click reaction (see supporting information). On the contrary, cyanine-sydnones **4** and cyanine-pyrazole **8a** presented quite different fluorescent properties in MeOH resulting in a significant turn-on effect after click reaction. At 825 nm, the fluorescence is 12 times higher for cyanine-pyrazole than for cyanine-sydnones and at 850 nm it is 67 times higher. The absorbance and fluorescence spectra of both compounds are indicated in Figure 1.

Surprisingly, this turn-on phenomenon is much lower when the reaction was carried out with DBCO as pyrazole **8b** presented similar optical properties as sydnone **4**. Furthermore, the turn-on effect observed with **8a** is limited to organic solvent as shown in Table 2 indicating possible positive solvatochromism of pyrazole **8a**. Hydrophobicity of the cyanine **4** and DBCO may induce aggregation in organo-aqueous solvents explaining the absence of turn on effect in that case.

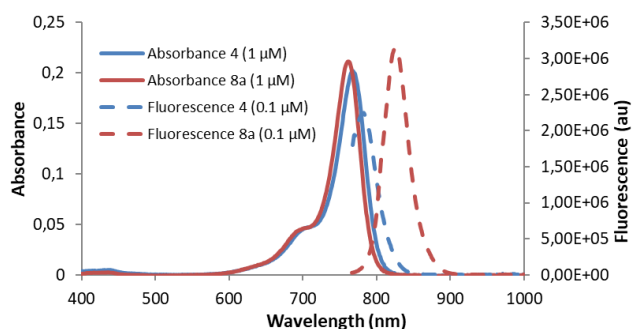
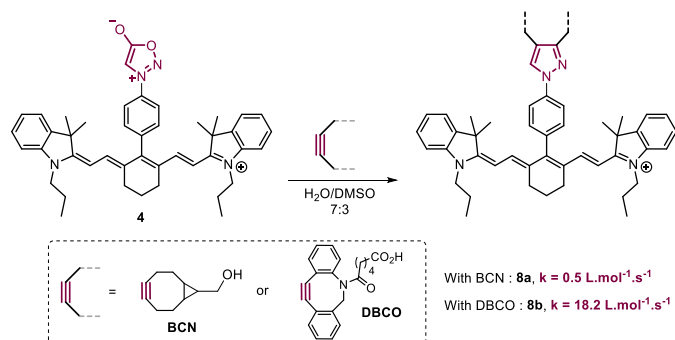


Figure 1. Absorbance and fluorescence excitation and emission spectra of sydnone **4** and pyrazole **8a**. Absorbance and fluorescence spectra were obtained in MeOH with 1 μM and 0.1 μM concentrations of compounds respectively.

We were then interested in the kinetics of the reaction between the cyanine-sydnone **4** and the two cyclooctynes BCN and DBCO. Kinetics of click reactions are indeed of prime importance to allow coupling at low concentrations. SPSAC reactions of **4** with BCN and DBCO were monitored by HPLC. Their kinetics is of order 2 following the equation:

$$\frac{\ln\left(\frac{[\text{Syd}]}{[\text{cyclooctyne}]}\right)}{[\text{Syd}]_0 - [\text{cyclooctyne}]_0} = kt + a$$

As expected, DBCO was more than 30 times more reactive than BCN (Scheme 5). With a kinetic constant of $18.2 \text{ mol}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$, DBCO afforded a fast SPSAC reaction, which will allow easy attachment of Cy7 on biomolecules even when used at low concentrations. Reaction with BCN is in the same range as SPAAC and can be also useful if concentrations of reactants are not below $1 \mu\text{M}$.



Scheme 3. Click reactions between cyanine-sydnone **4** and two cyclooctynes.

Conclusions

In this work we described the synthesis, reactivity and physico-optical properties of four Cy7 derivatised with clickable sydnones. All of these clickable cyanines reacted smoothly with cyclooctynes to afford the corresponding pyrazoles quantitatively at room temperature and with good kinetics. These compounds represent valuable additional biorthogonal

tools permitting the attachment of cyanine moieties into complex molecules or biomolecules.

Supplementary Material

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/MS-number>. Complete analytical data of compounds, kinetics, chemical and optical characterization of cyanine probes.

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Author Contribution Statement

Kim Anh Nguyen - performed the majority of the experiments described in this manuscript.

Emilie Lesur - completed the experiments, summarized and formatted all the results, write a part of the manuscript.

Maxime Ribéraud – participated to the writing of the manuscript.

Antoine Sallustrau - completed the experiments.

Davide Audisio - supervised the experimental work and participated to the writing of the manuscript.

Frédéric Taran - had the original idea of the paper, supervised the experimental work and participated to the writing of the manuscript.

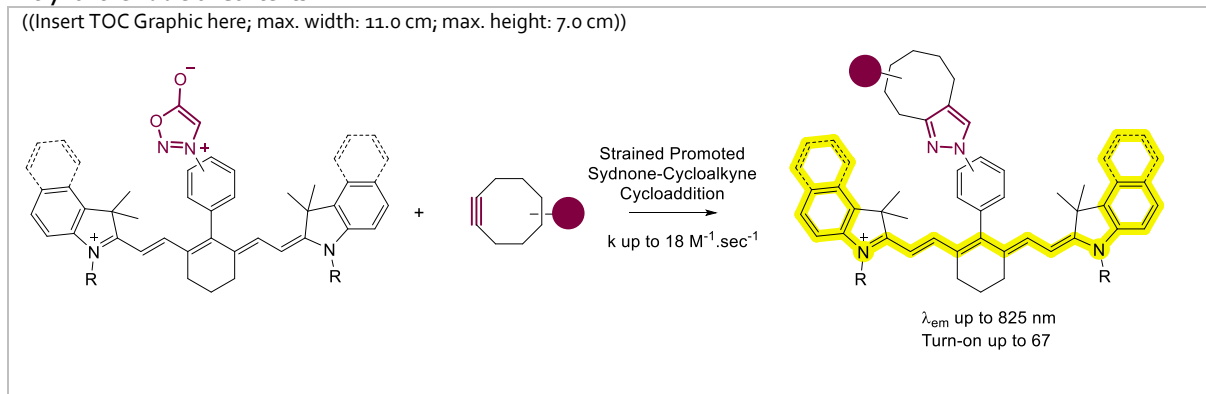
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Clickable sydnone-cyanine derivatives allow fast and quantitative coupling of Cy7 scaffold to molecules or biomolecules.