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# Synthesis and Photophysical Properties of 4'-5' Disubstituted CinNapht Dyes Accessible through Double SNAr Late-Stage Functionalization

Eléonore Tacke,<sup>[a]</sup> Lilian Estaque,<sup>[b]</sup> Minh-Duc Hoang,<sup>[a]</sup> Philippe Durand,<sup>[a]</sup> Gilles Clavier,<sup>[c]</sup> Grégory Pieters,<sup>[b]</sup> and Arnaud Chevalier\*<sup>[a]</sup>

This article describes the synthesis of a difluorinated CinNapht derivative in the 4' and 5' positions allowing the easy access to two new families of fluorophores by late-stage functionalization using SNAr. The first one comprises derivatives incorporating hindered aromatic amines in the 4' and 5' positions, which show red-emission in apolar solvents. The second one is obtained through the use of dinucleophiles. Among them,

Tetrahydroquinoxaline (THQ) and tetrahydrobenzodiazepine (THB) compounds show strongly redshifted emission. The photophysical properties of all the fluorophores in these two families are studied and rationalized by DFT and TDDFT calculations. The most promising compounds have been used to image living cells by confocal microscopy.

## Introduction

Fluorescence has a wide range of applications in many fields, from imaging to materials. The progress made over the last few decades has provided us with a deeper understanding of the impact of structural modifications on the photophysical properties of a molecule. Whatever the mechanism involved, the fluorescence is impacted by the functionalization of the electron-donating atom, also known as the auxochrome atom.<sup>[1]</sup> This can lead to a modulation of fluorescence performances by either enhancing the fluorescence quantum yield,<sup>[2]</sup> tuning the absorption and emission wavelengths,<sup>[3]</sup> or improving the photostability of the fluorophore.<sup>[4]</sup> The literature also provides detailed insight into the impact of introducing several donor atoms rather than a single one. This frequently brings new properties to a conventional fluorescent chromophore, such as delayed fluorescence (DF) exploited in thermally activated DF emitters.<sup>[5]</sup> In some cases, the two electron-donating atoms can be linked together through an aliphatic linker, as in the case of tetrahydroquinoxaline derivatives (THQ).<sup>[6]</sup> In this case, THQ

moieties can rigidify the structures, thus limiting the formation of twisted intramolecular charge transfer (TICT). The result is a synergistic effect that enhances the fluorophore's overall performance by boosting brightness, enhancing photostability and widening the Stokes shift.<sup>[7]</sup> According to these reports, we can safely assume that introducing two electron-donating groups can bring further helpful features to a wide variety of fluorophores. Among the numerous families of organic dyes, 4-Amino-1,8-naphthalimide (4-ANI) is one of the most widely used.<sup>[8]</sup> The fluorescence properties of this green-emitting push-pull chromophore are also closely related to the functionalization of the auxochromic atom.<sup>[2c,9]</sup> Our group has been interested for several years in a family of red-shifted fused hybrid fluorophores<sup>[10]</sup> derived from these naphthalimides named CinNaphts.<sup>[11]</sup> The work we have carried out has provided a better understanding of the impact of auxochrome functionalization in the 4' position.<sup>[12]</sup>

Given the evident potential to provide structural diversity at this position, we recently developed a late-stage functionalization method to readily obtain multiple fluorophores for various applications, including live-cell imaging,<sup>[13]</sup> solid-state fluorescence and AIEgens for selective imaging of lipid droplets.<sup>[14]</sup>

However, the effects of introducing a second electron-donating group on the CinNapht backbone have never been reported to our knowledge. In this context, this article describes the synthesis of a 4',5'-Difluorinated CinNapht (Figure 1). Its use in a double SNAr reaction enables the late introduction of two auxochrome moieties on the CinNapht backbone. This yields a set of 4'-5' disubstituted CinNaphts whose properties are investigated for the first time in this manuscript.

[a] E. Tacke, M.-D. Hoang, P. Durand, A. Chevalier  
Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles,  
UPR 2301, 91198, Gif-sur-Yvette, France  
E-mail: arnaud.chevalier@cnr.fr

[b] L. Estaque, G. Pieters  
Département Médicaments et Technologies pour la Santé (DMTS), SCBM,  
Université Paris-Saclay, CEA, INRAE, 91191 Gif-sur-Yvette, France

[c] G. Clavier  
Université Paris-Saclay, ENS Paris-Saclay, CNRS, PPSM, 91190, Gif-sur-Yvette,  
France

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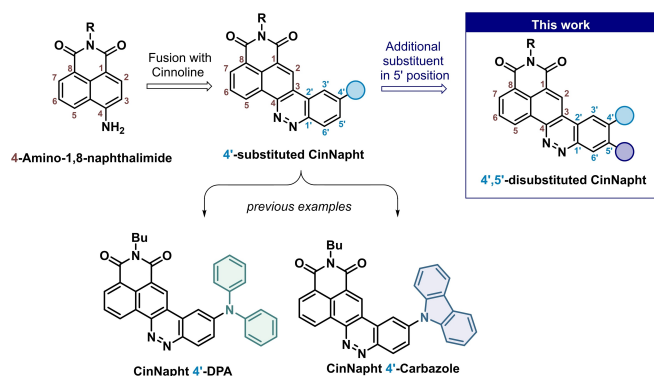


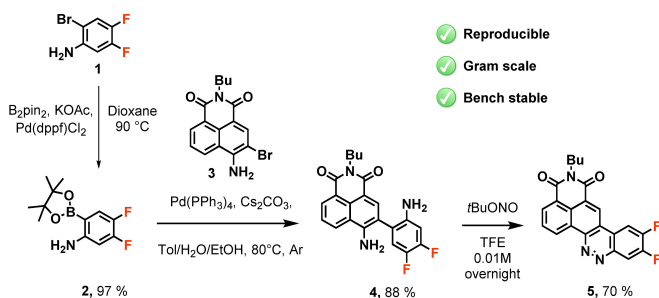
Figure 1. Structure of 4'-5' di substituted CinNapht dyes compared with previously reported 4'-ANI and 4'-monosubstituted CinNapht.

## Results and Discussion

### Synthesis of Di-Fluorinated CinNapht and Investigation of its Reactivity towards SNAr Reaction

Our study started with the synthesis of a 4'-5'-Difluorinated CinNapht designed to support a late introduction of two electron-donating groups through SNAr reactions.

This was achieved in 3 steps starting from 2-Bromo-4,5-difluoroaniline **1** (Scheme 1). The first step involves a Suzuki-



Scheme 1. Synthesis of 4',5'-difluorinated CinNapht 5.

Miyaura borylation to form the pinacol-borane derivative **2** with a 97% yield. The latter is then subjected to a Suzuki cross-coupling reaction with the naphthalimide **3** to give the bis-Aryl adduct **4** in 88% yield. The subsequent use of *tert*-butyl nitrite then leads to the oxidation of aniline into nitroso, which undergoes a Mills reaction to provide the 4'-5'-Difluorinated CinNapht **5** in 70% yield. This synthesis can be scaled up to the gram scale, providing a stable and versatile chromophore that can be stored over time without specific caution. We then investigated the reactivity of CinNapht **5** through a double SNAr reaction using multiple aliphatic and aromatic amines. Surprisingly, only the carbazole double adduct **6f** was obtained with a 40% yield. In most cases, only traces of mono-adduct were noticed, and the mixture deteriorated over time. In contrast, the mono-addition intermediate could be isolated using *N*-Methylbutylamine (**6a**) or diphenylamine (**6b**), respec-

tively, with 67% and 15% yield. Several attempts using a stronger base, such as sodium hydride, failed to yield further di-addition products. 2D COSY and NOESY <sup>1</sup>H NMR analyses were performed on compound **6b**, indicating that the first addition of diphenylamine occurred in the 4' position, and therefore supporting the structure presented in Scheme 2.

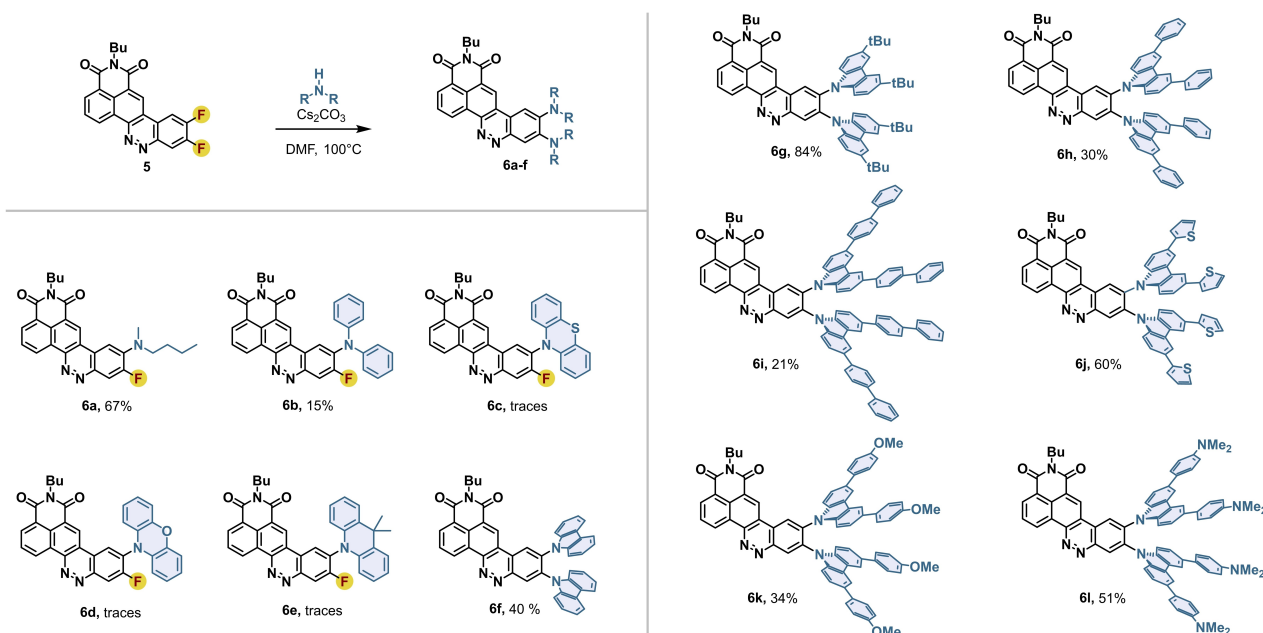
We then explored the scope of double carbazole addition reaction using a number of derivatives substituted in positions 3 and 6 with various substituents. (Details of the synthesis of these carbazoles are available in Supporting Information). As shown in Scheme 2, in addition to compound **6f**, we isolated six new fluorophores (**6g-l**) in yields ranging from 21% to 84%. All these fluorophores have been fully characterized and all spectral data are available in Supporting Information. We noted similar conversion rates, suggesting that reactivity does not depend on the nature of the carbazole. The variable yields observed here are mainly due to purification difficulties. The SNAr reaction using carbazole can also be carried out on intermediate **6b**. This result led us to consider the preparation of mixed diphenylamine/carbazole fluorophores (Scheme 3).

We thus prepared three additional CinNaphts (**7a-c**) incorporating a diphenylamine in the 4' position and several carbazole in the 5'-position with yields ranging from 17% to 60% starting from 4'-diphenylamine-5'-fluoro-CinNapht **6b**.

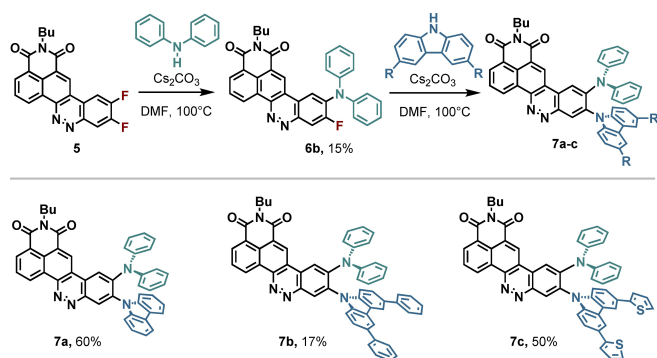
### Study of Photophysical Properties

All the 4'-5'-disubstituted CinNaphts **6f-l** and **7a-c** were characterized by absorption and fluorescence spectroscopies. Only apolar solvents such as toluene or chloroform allowed the observation of significant fluorescence from these compounds. These compounds display very low solubility in polar organic solvents. The absorption, emission and excitation spectra were thus recorded in chloroform and toluene. We also determined the relative fluorescence quantum yield (QY<sub>FL</sub>) and measured the molar extinction coefficient (ε<sub>max</sub>). The results are summarized in Table 1.

Compounds **6f-l** display absorption maxima centered around 470 nm, which are only slightly dependent on the nature of the substituents carried by the carbazole moiety. Only compound **6l**, featuring dimethylamino electron-donating groups, reveals a significant bathochromic shift of its absorption maximum of approximately 50 nm compared to the other compounds. Molar absorption coefficients are generally relatively low, never surpassing 1 000 M<sup>-1</sup>cm<sup>-1</sup>. Overall, chromophores **6f-l** exhibit very poor fluorescence in chloroform. Only compound **6f** produced a detectable signal centered at 650 nm, but its very low intensity prevented us from determining a meaningful QY<sub>FL</sub>. In contrast, toluene, which is much less polar, led to much higher fluorescence intensities. In this case, emission maxima varied from 590 nm for compound **6f** to 658 nm for compound **6j**. Nevertheless, fluorescence efficiency remains low, with QY<sub>FL</sub> never rising above 10%. Surprisingly, compound **6l** generated no fluorescence in either chloroform or toluene. These low fluorescence efficiencies, combined with low ε<sub>max</sub> values, place compounds **6f-l** among poor-quality



**Scheme 2.** Double SNAr reactions on 4'-5'-difluorinated CinNapht **5**: Investigation of amine reactivity and scope of double carbazole addition



**Scheme 3.** Synthesis of 4'-Diphenylamine-5'-carbazole-disubstituted CinNaphts dyes.

fluorophores with very limited brilliance. Nevertheless, this study provides an insight into the effect of the addition of a substituent in the 5' position. If we compare the optical properties of fluorophore **6f** with the monosubstituted in position 4' (4'-carbazole), we observe that the impact on the absorption properties is limited. On the other hand, the presence of a second carbazole unit in the 5' position results in a bathochromic shift in emission wavelengths in both toluene and chloroform. Also, the hindrance caused by the presence of two bulky aromatic amines may result in a twist in the C<sup>Ar</sup>-N bond. This torsion can result in a spatial separation of the frontier orbitals (HOMO-LUMO), thereby promoting Inter-System Crossing between the singlet state (S1) and triplet state (T1), which is essential for obtaining delayed fluorescence. To investigate this, we measured fluorescence lifetimes. Unfortunately, despite efforts to prevent the presence of oxygen, which can scavenge the triplet state, we never observed any poly-exponential decay, which could indicate a long lifetime

component (Fig. S13). These fluorophores' half-lives range from 2 ns to 9.4 ns, which are quite common values for organic fluorophores. The 4'-Diphenylamine-5'-carbazole-CinNaphts analogs (**7a-c**), exhibit similar optical properties. The absorption maxima of compounds **7a-c** are centered around 480 nm in toluene and 500 nm in chloroform, which are values strikingly similar to those of the 4-DPA 4'-monosubstituted analog. Adding a carbazole unit in the 5' position mainly results in a red shift of emission wavelengths by approximately 30 nm. Once again, no long fluorescence lifetimes were found. Taken together, the data presented here suggest that the introduction of carbazole units in the 5'-position of 4'-mono-substituted derivatives has little impact on the optical properties of the fluorophore. Nevertheless, we can emphasize that this strategy systematically induces a bathochromic shift in emission wavelength by about 30 nm. Note that preliminary studies conducted in our lab did not reveal any AIEgen behavior for these fluorophores (data not shown).

### Use of Di-Amines and Amino-Alcohols as Di-Nucleophiles

In previous work in the literature, synthetic building blocks incorporating THQ units were synthesized prior to the construction of the fluorescent polyaromatic backbone. The late functionalization approach proposed in this study should allow the introduction of the two nucleophiles through double SNAr, thus facilitating modification of the fluorophore structure. We therefore investigated the suitability of dinucleophiles, such as diamines. We have extended our approach to the use of amino alcohols and amino acids.

Initial assays readily afforded the THQ-diMe derivative **8a** from two successive amine additions in 67% yield (Scheme 4).

**Table 1.** Photophysical properties of CinNapht **6f–l** and **7a–c** compared with previously reported 4' monosubstituted analogs.

CinNapht Dye	Solvent	$\lambda_{\max}$ Abs <sup>[a]</sup> (nm)	$\epsilon_{\max}$ <sup>[b]</sup> (M <sup>-1</sup> .cm <sup>-1</sup> )	$\lambda_{\max}$ Em (nm)	Stokes Shift (cm <sup>-1</sup> )	QY <sub>FL</sub> <sup>[c]</sup>	Brightness <sup>[d]</sup>	Lifetime (ns)
<b>6f</b>	toluene	449	8 400	590	5 323	0.04	335	3.8
	CHCl <sub>3</sub>	454	7 200	650	6 642	< 0.01	–	–
<b>6g</b>	toluene	477	6 400	618	4 783	0.10	639	3.4
	CHCl <sub>3</sub>	489	3 800	–	–	–	–	–
<b>6h</b>	toluene	475	7 300	624	5 027	0.09	652	4.5
	CHCl <sub>3</sub>	481	7 300	–	–	–	–	–
<b>6i</b>	toluene	478	3 900	636	5 197	0.10	394	4.6
	CHCl <sub>3</sub>	484	5 700	–	–	–	–	–
<b>6j</b>	toluene	485	6 800	658	5 421	0.05	340	4.9
	CHCl <sub>3</sub>	489	6 600	–	–	–	–	–
<b>6k</b>	toluene	487	4 900	650	5 149	0.04	196	2.2
	CHCl <sub>3</sub>	493	5 900	–	–	–	–	–
<b>6l</b>	toluene	532	4 300	–	–	–	–	–
	CHCl <sub>3</sub>	529	3 500	–	–	–	–	–
4'-Carbazole[e]	toluene	442	8 400	553	4 541	0.01	84	n.d.
	CHCl <sub>3</sub>	452	6 700	625	6 124	0.10	670	n.d.
<b>7a</b>	toluene	481	11 000	622	4 713	0.26	2 865	3.5
	CHCl <sub>3</sub>	490	11 600	695	6 110	< 0.01	–	–
<b>7b</b>	toluene	481	13 600	619	4 635	0.24	3 272	3.4
	CHCl <sub>3</sub>	498	14 300	689	5 567	< 0.01	–	–
<b>7c</b>	toluene	482	10 700	621	4 644	0.24	2 568	3.4
	CHCl <sub>3</sub>	498	11 100	686	5 503	< 0.01	–	–
4'-DPA[f]	toluene	479	15 200	591	3 200	0.21	3 192	–
	CHCl <sub>3</sub>	496	15 100	665	5 124	0.04	604	–

[a] Values corresponding to S<sub>0</sub>-S<sub>1</sub> transition but strong S<sub>0</sub>-S<sub>2</sub> transition is also observed (see Supporting Information Figure S1 to S4), [b] Molar extinction coefficient related to S<sub>0</sub>-S<sub>1</sub> transition (Figure S5–S8). [c] Relative QY determined at 25 °C using 4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran, "DCM" (QY = 0.43 in EtOH),<sup>[15]</sup> [d] Brightness was calculated using the formula : Brightness = QY<sub>FL</sub> ×  $\epsilon_{\max}$  [e] The synthesis of this compounds was reported in a previous study.<sup>[14]</sup> [f] The synthesis of this compounds was reported in a previous study.<sup>[13]</sup>

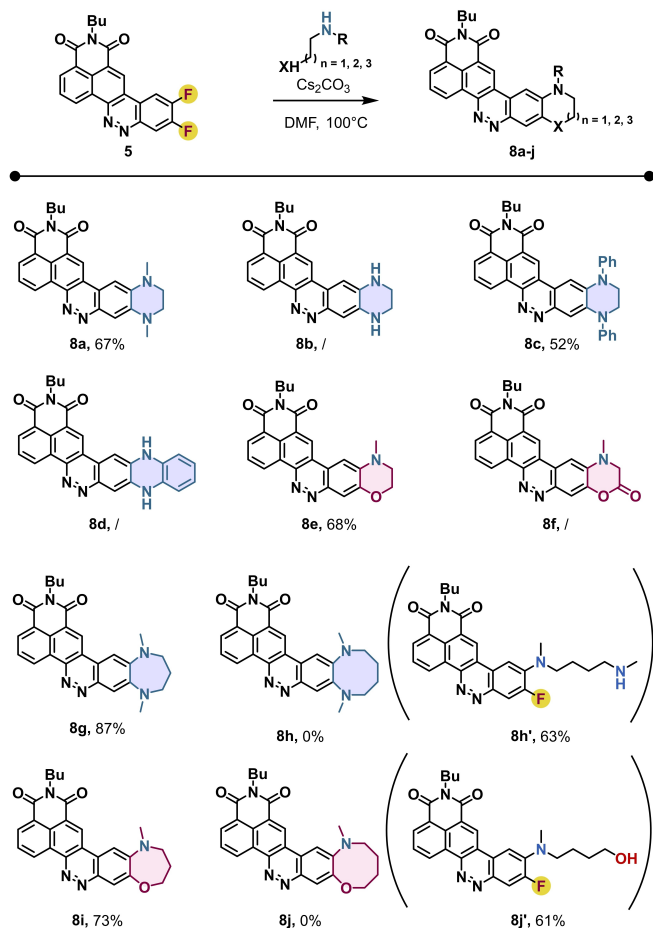
The introduction of phenyl groups does not significantly alter the reactivity, and THQ-diPhe analog **8c** could also be isolated with 52% yield. On the other hand, the use of primary diamines failed (**8b**). This suggests that the use of secondary amines as nucleophiles is here necessary. This hypothesis is all the more plausible as the use of *ortho* phenylene diamine also failed in obtaining the desired compound **8d**. Using amino alcohols such as *N*-methyl ethanolamine afforded derivative **8e** in 68% yield. In this case, amine addition occurs first on the 4' position followed by subsequent addition of the hydroxyl moiety in the 5' position. Finally, our experiments involving various amino acids such as sarcosine (**8f**) never led to the desired compound. We then explored the influence of the linker between the two nucleophilic atoms. We found that the use of a 3-carbon aliphatic linker did not affect the reactivity, even significantly increasing the efficiency of the reaction by affording compounds tetrahydrobenzodiazepine **8g** and tetrahydrobenzoxazepine **8i**, with 87% and 73% yields respectively.

On the other hand, further carbon elongation prevented the second addition. Only the amine mono-addition intermediates **8h'** and **8j'** were isolated in 63% and 61% yield respectively.

### Study of Photophysical Properties

We then evaluated the photophysical properties of the resulting fluorophores. The absorption, emission and excitation spectra of all compounds were recorded, and the fluorescence quantum yield as well as the molar extinction coefficient were determined. The results are shown in the Table 2. As expected, introducing the THQ moiety results in a pronounced bathochromic shift in fluorescence emission. Derivatives **8a** and **8c** thus exhibit identical emission maxima in chloroform, centered at 643 nm, i.e. a shift of +77 nm compared with the emission of the reference CinNapht 4'-diMe. However, in this case, there is a noticeable drop in QY<sub>FL</sub>, reaching 18% and 14% respectively. THQ **8a** also features the highest emission wavelength achieved for a CinNapht in DMSO at 765 nm. Unfortunately, the fluorescence intensity is too low to determine a QY<sub>FL</sub>. Interestingly, the elongation of the carbon chain separating the two nitrogen atoms causes a further redshift in emission.

Thus, the tetrahydrobenzodiazepine derivative **8g** displays an emission maximum centered at 673 nm and a QY<sub>FL</sub> of 17% in chloroform. To our knowledge, this is the highest known



**Scheme 4.** Use of amino alcohols and diamines for the synthesis of red-shifted CinNaphTs dyes.

emission for a CinNaphT in chloroform. The optical properties of compounds dihydrobenzoxazine **8e** and tetrahydrobenzoxazine **8i** were also investigated. Replacement of the nitrogen atom by an oxygen atom induces a strong hypsochromic shift in both absorption and emission wavelengths, resulting in yellow-orange emitting dyes with similar behavior to those of 4'-diMe reference CinNaphT. Similarly to nitrogen analogs, elongation of the carbon chain separating the nitrogen and oxygen atoms results in a bathochromic shift of the emission maximum. However, this effect is less pronounced. On the other hand, measurements in DMSO disclosed an instead preserved fluorescence efficiency that distinguishes these dihydrobenzoxazine **8e** and tetrahydrobenzoxazine **8i** derivatives from tetrahydroquinoxaline **8a** and tetrahydrobenzodiazepine **8g** derivatives. Compound **8e** shows near-infrared emission at 672 nm in DMSO with a fluorescence quantum yield of 20%, properties compatible with its use for fluorescence imaging in cells. The superposition of the emission spectra found in Table 2 summarizes the observations outlined above. It provides a better picture of how functionalization in 5' position impacts the optical properties of CinNaphTs. A computational study was carried out to understand better the mechanisms involved in these behavioral changes.

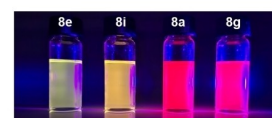
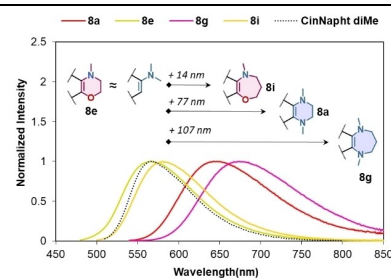
#### DFT and TD-DFT Calculations

Compounds **8a**, **8e**, **8g** and **8i** have been studied by quantum chemistry in order to better understand their photophysical properties. Both ground state ( $S_0$ ) and first singlet excited state ( $S_1$ ) geometries have been optimized, and the theoretical photophysical properties calculated using a solvation model (chloroform).

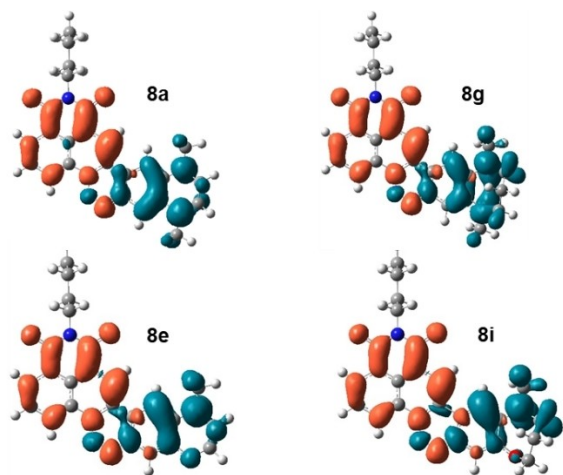
**Table 2.** Photophysical properties of CinNaphT **8a**, **8c**, **8e** **8g**, and compared with previously reported 4' mono substituted dimethylamino analog.

CinNaphT Dye	Solvent	$\lambda_{\max}$ Abs <sup>[a]</sup> (nm)	$\epsilon_{\max}$ <sup>[b]</sup> (M <sup>-1</sup> .cm <sup>-1</sup> )	$\lambda_{\max}$ Em (nm)	Stokes Shift (cm <sup>-1</sup> )	QY <sub>FL</sub> <sup>[c]</sup>	Brightness <sup>[d]</sup>
<b>8a</b>	CHCl <sub>3</sub>	513	10 100	643	3 941	0.18	1 818
	DMSO	527	7 300	765	5 903	< 0.01	n.d.
<b>8c</b>	CHCl <sub>3</sub>	509	7 350	643	4 094	0.14	1 029
	DMSO	516	8 600	745	5 957	< 0.01	n.d.
<b>8e</b>	CHCl <sub>3</sub>	475	4 800	569	3 478	0.12	576
	DMSO	485	5 300	672	5 738	0.20	1 060
<b>8g</b>	CHCl <sub>3</sub>	516	8 200	673	4 521	0.17	1 394
	DMSO	530	7 150	–	–	–	–
<b>8i</b>	CHCl <sub>3</sub>	476	11 400	580	3 767	0.17	1 938
	DMSO	486	8 900	682	5 913	0.12	1 068
CinNaphT 4'-di-Me <sup>[e]</sup>	CHCl <sub>3</sub>	489	15 700	566	2 782	0.25	3 925
	DMSO	496	15 500	682	5 499	0.06	930

[a] Values corresponding to  $S_0$ - $S_1$  transition but strong  $S_0$ - $S_2$  transition is also observed (Figure S9 and S10), [b] Molar extinction coefficient related to  $S_0$ - $S_1$  transition (Figure S11 and S12). [c] Relative QY determined at 25 °C using 4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran, "DCM" (QY = 0.43 in EtOH),<sup>[16]</sup> [d] Brightness was calculated using the formula: Brightness = QY<sub>FL</sub> ×  $\epsilon_{\max}$  [e] The synthesis of this compounds was reported in a previous study.<sup>[11]</sup>



Similarly to previously reported 4' mono substituted derivatives, the CinNapht core is mainly planar in the ground state. In all cases the cyclic substituents in 4', 5' position are out of the plane, but this doesn't prevent good participation of the nitrogen atoms lone pair to the conjugation (Figure S14). A comparison of the  $S_0$  and  $S_1$  geometries reveals very minimal modifications (Figure S15 overlay of  $S_0$  and  $S_1$ ). The charge density difference has been calculated and represented in Figure 2. It highlights the charge transfer nature of the



CinNapht Dye	$\lambda_{\max}$ Abs (nm)	$\lambda_{\max}$ Em (nm)	$\Delta r$ (Å)
8a	518	609	4.91
8e	466	525	4.30
8g	499	647	4.93
8i	486	544	4.39

**Figure 2.** Top. Charge density difference between the excited state and the ground state at the  $S_1$  geometry for **8a**, **8e**, **8i** and **8g** (right) (orange positive corresponding to the electron and blue negative corresponding to the hole). Bottom. Table of Calculated maximum absorption and emission and  $\Delta r$  index in chloroform.

transition from the substituted cinnoline part to the naphthalimide one. To get a more quantitative view of the fluorescence differences, theoretical maximum emission are reported in table of figure 2. They follow the same trend as the experimental ones ( $8e < 8i < 8a < 8g$ ) and can be related to the strength of the charge transfer which was evaluated by the  $\Delta r$  index, which is a measure of the CT length during electron excitation.<sup>[17]</sup> This calculation thus demonstrate that in our case, tetrahydrobenzoxazepine act as a stronger donor than THQ.

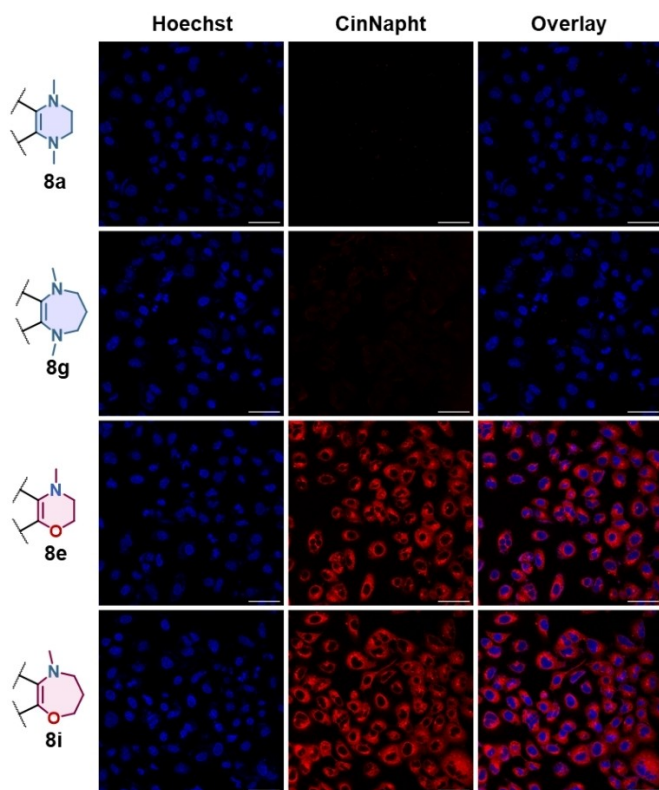
### Live Cell Imaging

We have tested the suitability of the various CinNaphts described in this article in live cell imaging. Due to their very low solubility combined with negligible fluorescence in polar solvents, CinNapht **6f–l** and **7a–c** are unlikely to be suitable for biological applications.

The better solubility of derivatives **8a–i** is more advantageous, which prompted us to focus on these compounds. Before employing these fluorophores in confocal microscopy experiments, their toxicity has been assessed. To this end, compounds **8a**, **8e**, **8g**, and **8i** were incubated on A549 cells over an increasing concentration range from 0.5  $\mu\text{M}$  to 10  $\mu\text{M}$  for 72 h, and cell viability was quantified using a Celltiter-Glo® assay (Figure S16). The results reveal only slight toxicity observed at 10  $\mu\text{M}$  for compound **8e** after 72 h of incubation. In contrast, no apparent effects were detected up to 5  $\mu\text{M}$  for any of the 4 compounds. For these reasons, it was decided to carry out the imaging experiments at a concentration of 2  $\mu\text{M}$ . Fluorophores **8a**, **8e**, **8g** and **8i** were thus incubated for 2 h at a concentration of 2  $\mu\text{M}$  in living A549 lung cancer cells, then observed by confocal microscopy. The results are presented in Figure 3. Considering the obvious difference of  $QY_{\text{Fl}}$  observed in  $\text{CHCl}_3$  and DMSO (Table 2) for CinNaphts **8a** and **8g**, we might expect a turn-ON effect in response to the lipophilic environment of certain specific cell compartments, as we witnessed in previous studies.<sup>[9,14]</sup> Unfortunately, only a weak signal is observed for those CinNaphts. Moreover, traces of precipitation observed during sample preparation in the culture medium indicate that a lack of solubility may also be at the root of this outcome. On the other hand, a significant signal was observed from the CinNaphts **8e** and **8i** with no obvious subcellular compartmentalization. This result is coherent with the encouraging optical properties of these fluorophores in DMSO. Furthermore, no prior treatment was applied to the cells to facilitate their permeabilization. This suggests the fair ability of these fluorophores to enter the cells. These results demonstrate that **8e** and **8i** fluorophores can be used for fluorescence imaging of living cells.

### Conclusions

In conclusion, we have described the efficient synthesis of a 4',5'-Difluorinated CinNapht **5**. This highly stable chromophore can be used in a double aromatic nucleophilic substitution reaction providing easy access to multiple analogs of CinNaphts substituted with donor groups in the 4' and 5' positions. Using this late-stage strategy, we could access to two distinct families of fluorophores. The first, incorporating two hindered aromatic amines, comprises of a set of red-emitting fluorophores in apolar media. However, this strategy does not appear to be suitable for creating TADF CinNaphts analogs, as we initially expected. The second family is obtained using aliphatic dinucleophilic molecules. In addition to the tetrahydroquinoxaline (THQ) derivative inspired by previous work in the literature, our "late-stage functionalization" strategy yielded a further series of derivatives: dihydrobenzoxazine, tetrahydrobenzoxazepine and tetrahydrobenzodiazepine. To our knowledge, this is the first report examining the influence of these four different functionalizations on the photophysical properties of an organic fluorophore. It reveals that the dihydrobenzodiazepine moiety is particularly powerful for redshifting the emission wavelength of CinNaphts. From the computational studies we've carried



**Figure 3.** Confocal microscopy images of live A549 cancer cell treated CinNapht dyes using a 40 $\times$  oil immersion objective. The nucleus was stained with 1  $\mu\text{g mL}^{-1}$  Hoechst 33342 for 15 min at 37  $^{\circ}\text{C}$  ( $\lambda_{\text{Exc}}$ : 405 nm,  $\lambda_{\text{Em}}$ : 425 to 480 nm). CinNapht **8a** and **8g** were incubated at 2  $\mu\text{M}$  for 2 h at 37  $^{\circ}\text{C}$  ( $\lambda_{\text{Exc}}$ : 510 nm,  $\lambda_{\text{Em}}$ : 600 to 750 nm). CinNapht **8e** and **8i** were incubated at 2  $\mu\text{M}$  for 2 h at 37  $^{\circ}\text{C}$  ( $\lambda_{\text{Exc}}$ : 475 nm,  $\lambda_{\text{Em}}$ : 510 to 700 nm). Scale bar: 30  $\mu\text{m}$ .

out, this can be attributed to variations of the strength of the charge transfer, as calculated  $\Delta r$  index values suggest. Overall, this work reflects the impact of a double functionalization of CinNaphts in positions 4' and 5'. It thus contributes to a better understanding of the structure/photophysical properties relationship of CinNaphts. In addition, it extends the known strategy of extension by THQ motifs to other heterocycles, which could be applied to other families of fluorophores in the future.

## Experimental Section

**4,5-Difluoro-2-pinacol borane-aniline 2:** 2-bromo-4,5-difluoroaniline (2.00 g, 9.61 mmol, 1.00 eq), KOAc (2.83 g, 28.8 mmol, 3.00 eq) and bis(pinacolato)diboron (3.70 g, 14.4 mmol, 1.50 eq) were dissolved in dioxane (85.0 mL) in a Schlenk tube. The resulting mixture was desoxygenated with freeze-pump-thaw cycling before adding of Pd(dppf)Cl<sub>2</sub> (703 mg, 0.96 mmol, 0.10 eq). The reaction was heated overnight at 90  $^{\circ}\text{C}$  with constant stirring under argon. The mixture was cooled to room temperature and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (Hept/ACoEt 9:1 v/v) to afford compound **2** as a transparent oil (3.18 g, 97%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): 7.35 (dd,  $J$ =10.9, 9.7 Hz, 1H), 6.35 (dd,  $J$ =12.4, 6.3 Hz, 1H), 4.68 (s, 2H), 1.33 (s, 12H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  154.6, 152.4, 151.0, 143.9, 124.2 (d,  $J$ =15.9 Hz), 103.2 (d,  $J$ =19.3 Hz), 84.1, 83.6, 25.2 (2 C), 25.0 (2 C) ppm. <sup>19</sup>F NMR

(282 MHz, CDCl<sub>3</sub>)  $\delta$  -132.44 (dt,  $J$ =23.0, 10.9 Hz), -154.00 (dt,  $J$ =20.6, 8.4 Hz). IR (neat):  $\nu$ =2979, 1619, 1593, 1514, 1440, 1392, 1366, 1323, 1300, 1279, 1233, 1199, 1165, 1139, 1122, 1037, 960, 896, 858, 847, 769, 741, 732, 703, 669  $\text{cm}^{-1}$ . ESI-HRMS calculated for C<sub>12</sub>H<sub>17</sub>BF<sub>2</sub>NO<sub>2</sub> [M+H]<sup>+</sup> 256.1320, found 256.1309.

**Bis-Aryl adduct 4:** The brominated 4-ANI **3** (2.00 g, 5.76 mmol, 1.00 eq), 4,5-Difluoro-2-pinacol borane-aniline **2** (2.20 g, 8.64 mmol, 1.50 eq), and Na<sub>2</sub>CO<sub>3</sub> (1.83 g, 17.3 mmol, 3.00 eq) were dissolved in a mixture of H<sub>2</sub>O, EtOH, and toluene (40.0 mL, 3:3:10 v/v) in a Schlenk tube. The resulting mixture was desoxygenated with freeze-pump-thaw cycling before the addition of Pd(PPh<sub>3</sub>)<sub>4</sub> (666 mg, 0.58 mmol, 0.10 eq.). The reaction was heated overnight at 80  $^{\circ}\text{C}$  with constant stirring under argon. The mixture was cooled to room temperature, then diluted with DCM, and the crude residue was purified by flash chromatography on silica gel (DCM/EtOAc 95:5 v/v) to afford compound **4** as a yellow orange solid (2.00 g, 88%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  8.62 (d,  $J$ =7.2 Hz, 1H), 8.34 (s, 1H), 8.16 (d,  $J$ =8.2 Hz, 1H), 7.71 (t,  $J$ =7.7 Hz, 1H), 7.02 (dd,  $J$ =10.4, 8.5 Hz, 1H), 6.66 (dd,  $J$ =11.7, 6.9 Hz, 1H), 5.01 (s, 2H), 4.16 (t,  $J$ =7.5 Hz, 2H), 3.65 (s, 2H), 1.76 – 1.66 (m, 2H), 1.44 (sx,  $J$ =7.4 Hz, 2H), 0.97 (t,  $J$ =7.4 Hz, 3H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): 164.5, 164.0, 152.1 (d,  $J$ =13.1 Hz), 150.2 (d,  $J$ =13.6 Hz), 146.5, 144.9 (d,  $J$ =13.6 Hz), 142.9 (d,  $J$ =13.6 Hz), 141.3 (d,  $J$ =6.8 Hz), 135.6, 131.7, 127.1, 125.8, 123.5, 120.4, 119.8 (d,  $J$ =17.6 Hz), 117.7, 112.6, 104.7 (d,  $J$ =20.3 Hz), 40.2, 30.5, 20.5, 14.0 ppm. <sup>19</sup>F NMR (282 MHz, CDCl<sub>3</sub>)  $\delta$  -136.35 (dt,  $J$ =22.0, 10.1 Hz), -149.92 (dt,  $J$ =19.4, 8.1 Hz) Rf (DCM/ACoEt 8:2)=0.6. IR (neat):  $\nu$ =3368, 3338, 2970, 2901, 1679, 1630, 1611, 1578, 1522, 1512, 1472, 1455, 1430, 1394, 1341, 1297, 1262, 1242, 1229, 1200, 1188, 1171, 1145, 1127, 1075, 970, 933, 905, 856, 820, 796, 778, 749, 689, 657  $\text{cm}^{-1}$ . ESI-HRMS calculated for C<sub>22</sub>H<sub>20</sub>F<sub>2</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup> 396.1524 found 396.1507.

**4',5'-Di-fluorinated CinNapht 5:** To a stirring mixture of compound Bis-Aryl adduct **4** (800 mg, 2.02 mmol, 1.00 eq) in 200 mL of TFE (0.01 M) at 0  $^{\circ}\text{C}$  was added dropwise tBuONO (722  $\mu\text{L}$ , 6.06 mmol, 3.00 eq). The mixture was stirred overnight. After the reaction was completed, the solvent was removed and the crude product was purified by flash chromatography on silica gel (DCM 100%) to afford the 4'-5'-difluorinated CinNapht **5** as a pale yellow solid (553 mg, 70%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  10.01 (dd,  $J$ =8.4, 1.2 Hz, 1H), 9.44 (s, 1H), 8.77 (dd,  $J$ =7.4, 1.2 Hz, 1H), 8.59 (dd,  $J$ =9.5, 7.6 Hz, 1H), 8.49 (dd,  $J$ =10.1, 7.4 Hz, 1H), 8.11 (t,  $J$ =7.9 Hz, 1H), 4.20 (t,  $J$ =7.6 Hz, 2H), 1.76 – 1.65 (m, 2H), 1.43 (sx,  $J$ =7.5 Hz, 2H), 0.95 (t,  $J$ =7.4 Hz, 3H) ppm. <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  163.9, 163.3, 155.6 (d,  $J$ =13.1 Hz), 153.4 (d,  $J$ =13.6 Hz), 151.4 (d,  $J$ =13.6 Hz), 145.29 (d,  $J$ =13.6 Hz), 141.9, 132.2, 130.7, 129.9, 129.8, 127.8, 126.1, 125.3, 123.1, 119.7, 118.7 (d,  $J$ =17.6 Hz), 109.4 (d,  $J$ =20.3 Hz), 40.8, 30.4, 20.5, 14.0 ppm. <sup>19</sup>F NMR (282 MHz, CDCl<sub>3</sub>)  $\delta$  -123.43 (d,  $J$ =20.3 Hz), -128.44 (d,  $J$ =20.4 Hz). Rf (DCM)=0.8 IR (neat):  $\nu$ =3016, 2967, 2927, 2861, 1699, 1658, 1631, 1617, 1581, 1557, 1525, 1503, 1483, 1453, 1436, 1421, 1405, 1376, 1368, 1328, 1318, 1287, 1229, 1178, 1158, 1124, 1094, 1058, 1039, 1025, 993, 977, 966, 927, 919, 884, 857, 839, 808, 798, 784, 750, 733, 712, 696, 652  $\text{cm}^{-1}$ . ESI-HRMS calculated for C<sub>22</sub>H<sub>16</sub>F<sub>2</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup> 392.1211, found 392.1194.

**General procedure for double SNAr substitution with aromatic amines (6a–l):** The 4'-5'-Difluorinated CinNapht **5** (20.0 mg, 0.05 mmol, 1.00 eq), Cs<sub>2</sub>CO<sub>3</sub> (70.0 mg, 0.21 mmol, 4.00 eq) and the corresponding amine (0.15 mmol, 3.00 eq) were dissolved in DMF (0.50 mL, 0.10 M) and stirred at 100  $^{\circ}\text{C}$ . After the reaction was completed, the mixture was cooled down to room temperature and diluted in DCM. The mixture was washed with brine and the combined organic layers were dried over MgSO<sub>4</sub> and filtered and concentrated in vacuo. The residue was purified by flash chromatography or preparative TLC plate on silica gel using an appropriate eluting solvent mixture to afford the desired pure product. Details



of purification and characterization of each of the CinNapht 6f–l are available in Supporting Information.

**General procedure for the synthesis of CinNaphts (8a–i):** The 4'-5'-Difluorinated CinNapht 5 (20.0 mg, 0.05 mmol, 1.00 eq), Cs<sub>2</sub>CO<sub>3</sub> (70.0 mg, 0.21 mmol, 4.00 eq) and the corresponding diamine/aminoalcohol (0.11 mmol, 2.00 eq) were dissolved in DMF (0.50 mL, 0.10 M) and stirred at 100 °C. After the reaction was completed the mixture was cooled down to room temperature and diluted in DCM. The mixture was washed with brine and the combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel or on preparative TLC plate using an appropriate eluting solvent mixture to afford the desired pure product. Details of purification and characterization of each of the CinNapht 8a–i are available in Supporting Information.

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## Conflict of Interests

The authors declare no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

**Keywords:** Organic fluorophore · Late-stage functionalization · SNAr · CinNapht

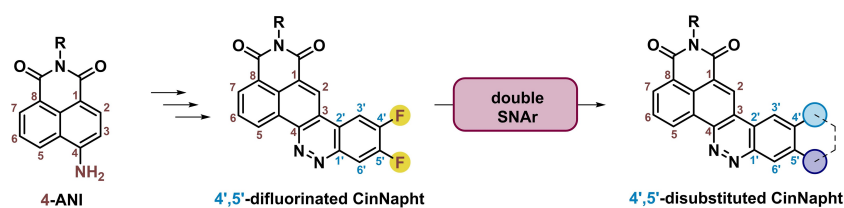
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## RESEARCH ARTICLE



The synthesis of 4', 5' di-substituted CinNapht dyes is reported. Two new families have been obtained using either double addition of aromatic amines or addition of inner di-nucleophiles. The photophysical properties

of all the fluorophores have been investigated and rationalized thanks to DFT and TD/DFT calculations. The most promising compounds have been used in live cell imaging experiments

*E. Tacke, L. Estaque, M.-D. Hoang, P. Durand, G. Clavier, G. Pieters, A. Chevalier\**

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**Synthesis and Photophysical Properties of 4'-5' Disubstituted CinNapht Dyes Accessible through Double SNAr Late-Stage Functionalization**

