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From Bifunctional Nucleophilic Behavior of DBU to a New Heterocyclic Fluorescent Platform

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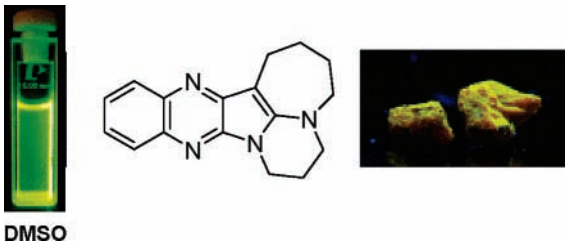
ABSTRACT



An unexpected discovery of a novel cyclocondensation reaction of 1,8-diazabicyclo[5.4.0]undec-8-ene (DBU) with activated 1,2-dichloro compounds is described. The 2-aminopyrrole skeleton is generated through the concomitant formation of new nitrogen–carbon and carbon–carbon bonds. A new pentacyclic derivative formed upon the reaction of 2,3-dichloroquinoxaline with DBU exhibits strong fluorescence both in solutions (Φ in hexane = 0.4) and in the solid state.

The development of new molecular fluorescent sensor platforms for in vivo and in vitro analysis has emerged as an actively investigated research field in recent years.[1] As the applications for fluorescent probes continue to increase, so does the need for dyes with diverse spectral and physicochemical properties. Despite the multitude of available fluorophores, new fluorophoric systems are hotly sought for more challenging applications including single molecule imaging and other areas.[2]

Herein, we report an unprecedented cascade reaction for the elaboration of a complex heterocyclic scaffold from simple building blocks in only one synthetic operation.

During the course of our recent attempts to improve the synthesis of the fluoflavine (5,12-dihydroquinoxalino[2,3-*b*]quinoxaline)[3,4] from 2,3-dichloroquinoxaline (**1**) and *o*-phenylenediamine, we used 1,8-diazabicyclo[5.4.0]undec-8-ene (DBU) (**2**) as a nonnucleophilic base for neutralization of the HCl formed in this reaction. When neat DBU was used as the solvent for this reaction at 150 °C, a small amount

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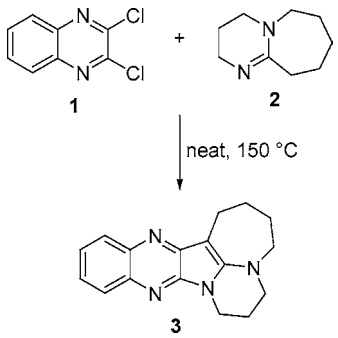
of the expected product along with a bright-yellow strongly fluorescent compound were detected. To determine whether DBU itself could react with one of the substrates, two additional experiments were carried out: 2,3-dichloroquinoxaline + DBU (Scheme 1) and DBU + *o*-phenylenediamine. The formation of a new fluorescent compound was observed only in the first reaction. This led to the conclusion that apparently nonnucleophilic DBU participates as a substrate in this reaction. After straightforward purification, the yellow compound was obtained in 7.4% yield.

Scheme 1



Extensive analysis of this compound was performed to elucidate the structure. To our surprise, mass spectrometry indicated that this product resulted from the elimination of 2 equiv of HCl between DBU and **1**. Elemental analysis confirmed this finding. The ^{1}H NMR spectrum was clean and exhibited seven completely resolved signals derived from methylene groups. The connectivity of these groups was correlated by a ^{1}H−^{1}H COSY spectrum to two isolated spin systems with three and four methylene groups, respectively. These facts along with the ^{13}C NMR spectrum, yellow color, and strong fluorescence led us to propose the pentacyclic structure **3**. The presence of three conjugated aromatic rings along with the bathochromic effect of the amino group would be responsible for the strong absorption in the blue region. This hypothesis was eventually confirmed by a single-crystal X-ray diffraction study (Figure 1) which demonstrated that compound **3** was almost completely flat. A literature search revealed only a handful of papers describing the synthesis and properties of amino-pyrrolo[2,3-*b*]quinoxalines.[5]

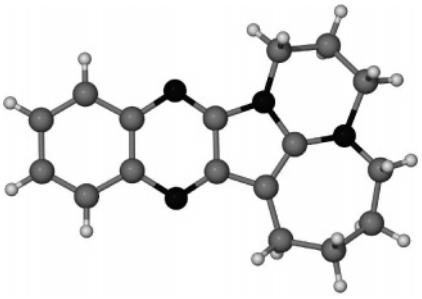


Figure 1. X-ray structure of compound **3**.

There are a few examples of DBU acting as a C- and N-nucleophile,[6] in reactions with esters of α-chloroacids,[5a] diethyl maleate,[5b] heptafluorobut-2-ene,[5c] dimethyl acetylenedicarboxylate,[5d] and polynitroaromates.[5e] However, there is no report describing the reaction of DBU with 1,2-dichlorobenzene or its analogues. The literature data are not clear regarding the order in which the above-mentioned reactions occur. We assumed that the first arylation of nitrogen precedes bond rearrangement and the second nucleophilic substitution (Scheme 1 in Supporting Information).

Because the yield of the adduct **3** was low, short optimization studies were undertaken. Optimal conditions for the condensation were identified after examining various reaction parameters (time, temperature, excess of DBU, and solvent) (Table 1). Because 2 equiv of HCl is formed in this reaction, among other modifications, the reaction was performed in the presence of a solid inorganic base (NaOH). The use of this additional proton scavenger led to a sharp decrease in the yield of compound **3** (Table 1, entry 4). The reaction performed in the neat always led to a very sticky, honey-like mixture which was difficult to stir. Consequently, we also carried out the reaction of compound **1** with DBU in a solvent. Ethyl acetate was chosen due to its ability to dissolve both substrates and the product. No product formed in this reaction (Table 1, entry 7). Finally, we found that the highest yield (35%) was obtained when 3 equiv of DBU was heated with **1** for 45 min at 150 °C (Table 1, entry 5). No other products were isolated from this process. The remaining of the reaction mixture consists of intractable, highly polar, and highly colored materials.

Table 1. Optimization of Conditions for the Transformation of 2,3-Dichloroquinoxaline (**1**) into Compound **3**[a]

entry	**2**/**1** (mM)	time of reaction (h)	*T* (°C)	yield of **3** (%)[b]
1	2	0.5	150	7.4
2	1	0.5	150	2
3	2	16	150	21
4[c]	1.2	3	150	3.5
5	3	0.75	150	35
6	3	3	150	27
7[d]	3	16	60	0

[a] All reactions were performed in the neat unless otherwise noted. [b] Isolated yields. [c] 2 equiv of powdered NaOH was added. [d] Reaction was performed in EtOAc.

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The spectral characteristics of **3** were then examined and compared to those of the known simple aminopyrrolo[2,3-*b*]quinoxalines. The most notable feature of **3** is an intense absorption band at $\lambda_{max} = 419$ nm with $\epsilon = 12\,400$. The emission maximum of **3** occurs at $\lambda_{max} = 470$ nm with $\Phi = 0.40$ (in hexane). The absorption and emission spectra of **3** are presented in Figure 2. The Stokes shift is moderate (3480 cm^{-1} in DMSO). Compound **3** is also fluorescent in the solid state.

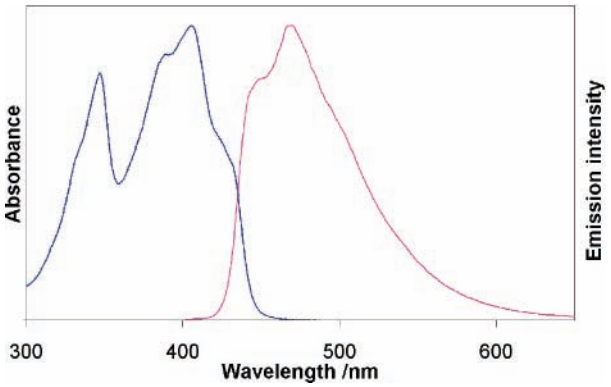

Figure 2. Normalized absorption (blue line) and emission (pink line) spectra of pyrroloquinoxaline **3** (hexane).

None of the publications concerning 2-amino-pyrrolo[2,3-*b*]quinoxalines reported any fluorescent properties of this class of compounds. The dramatic increase in the fluorescence yield in compound **3** can be attributed to a substantial decrease in nonradiative relaxation to the ground state caused by hindered internal rotation of the amino group with respect to the aromatic ring. Reduction of the internal rotation is caused via inclusion of the nitrogen atom in rings. Interestingly, the fluorescence quantum yield for **3** is sensitive to solvent polarity: $\Phi_{hexane} = 0.40$, $\Phi_{DMSO} = 0.28$, $\Phi_{MeOH} = 0.08$. It is well established that hydrogen bonding formation or protonation in 1,4-diazines leads to enhanced nonradiative depopulation of the lowest excited singlet state.[7]

Compound **3** has several characteristics that make it potentially superior to conventional fluorophores (such as dansyl, fluorescein, rhodamine, or BODIPY) in some applications. These include: (a) high fluorescence quantum yields; (b) a relatively long excited-state lifetime, making this dye useful for fluorescence polarization-based assays; (c) a relatively large Stokes shift; (d) a lack of ionic charge. In addition, many 1,2-diaminoaromatic compounds can be transformed in two steps into respective 2,3-dichloroquinoxaline analogues (crucial substrates in the described synthesis), which can be utilized in the construction of fluorophores bearing additional functionalities or possessing an expanded π-aromatic system.

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Recognizing that the scope and versatility of the discovered reaction could be considerably enhanced if the library of starting materials was expanded to other effortlessly available compounds, we employed analogous substrates. First, the reaction of **1** with 1,5-diazabicylo[4.3.0]non-5-ene (DBN) was performed, but unexpectedly, no analogous product was formed. This result can be rationalized by possible steric strain in the structure of the respective product. We envisaged that compound **1** could be replaced by other 1,2-dichloro compounds in which chlorine atoms are susceptible to nucleophilic substitution. 2,3-Dichloropyrazine and 3,4-dichloro-1,2,5-thiadiazole were chosen as the most promising substrates. The reaction of 2,3-dichloropyrazine with DBU performed under previously optimized conditions led to the formation of the expected 4,7-diaza-2-aminoindole derivative **4** in 42% yield (Figure 3). As anticipated, both the absorption

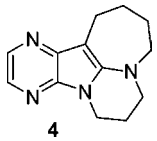

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Figure 3. Structure of compound **4**.

($\lambda_{max} = 352$ nm) and the emission ($\lambda_{max} = 416$ nm, $\Phi = 0.36$) were blue shifted (~50 nm) in respect to compound **3** (Table 2). Both compounds (**3** and **4**) reveal the same fluorescence decay times in *n*-hexane solutions, 4.8 ± 0.1 ns.

Table 2. Spectroscopic Data for Compounds **3**, **4**, and **6**−**8**

compd	solvent	λ_{abs}/nm	$\epsilon \times 10^{-3}$	λ_{em}/nm	Φ^a
3	hexane	408		470	0.40
	CH_3CN	419	12.4		
	DMSO	441		521	0.28
	MeOH			533	0.08
4	hexane	352		416	0.36
	CH_3CN	370	12.8		
	DMSO			473	0.20

[a] Determined using quinine sulfate in 1 N H_2SO_4 as a standard.

Exposure of 3,4-dichloro-1,2,5-thiadiazole to DBU both in the neat and in solutions led to the formation of highly polar tar materials only.

Our serendipitous discovery directly led to the development of a new method for the construction of 2-amino-pyrrole-fused heterocycles, while unearthing a new paradigm for DBU reactivity as a C- and N-nucleophile. This reaction is sensitive to the structure of the 1,2-dichloro derivative, with the highest yields obtained for moderately reactive substrates. Polyheterocyclic compounds obtained from 2,3-dichloropyrazine and its analogues, bearing a 4,7-diazaindole skeleton, display fluorescent properties. These fluorophores

can be characterized by the following advantages: (1) one-step synthesis from commercially available materials, (2) inexpensive substrates, and (3) high-fluorescence quantum yield. The rationale for the intense fluorescence, both in solution and in the solid state, lies in hindered internal rotation of the amino group, which in our case is achieved with no extra synthetic steps. The expansion of this synthetic methodology for the preparation of other fluorophores is currently underway.

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Supporting Information Available: Full experimental procedures for the synthesis of compounds **3** and **4** as well as NMR spectra for compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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