

Fluorescent Dyes with 2-Amino-4,7-diazaindole Skeleton: Synthesis and Spectroscopy

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The reaction of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) with compounds possessing two vicinal chlorine atoms activated toward nucleophilic substitution has been studied. All derivatives bearing a 2,3-dichloropyrazine moiety react with DBU leading to fluorescent dyes. Among others, only 2,3-dichloro-1,4-naphthoquinone reacts giving the expected pentacyclic product albeit in a very low yield and accompanied by the product of hydrolysis. Spectroscopic properties of the synthesized compounds were studied. The dye formed from 5,6-dichloro-2,3-dicyanopyrazine exhibits a very high Stokes shift and strong dependence of the fluorescence quantum yield on solvent polarity.

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 fluorophores, new fluorophoric systems are hotly sought for more challenging applications including single molecule imaging and others.[2] The majority of fluorescent compounds used are derivatives of heterocyclic systems.[3]

Recently, we have revealed a novel cyclocondensation reaction of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) with 2,3-dichloroquinoxaline.[4] A 2-aminopyrrole skeleton is generated through the concomitant formation of new nitrogen–carbon and carbon–carbon bonds. New pentacyclic derivative **1** (Figure 1), exhibits strong fluorescence both in solutions ($\Phi = 0.4$) and in the solid state.[4]

To improve (or change) the properties of fluorescent compounds toward longer emission wavelength and higher quantum yield, two methods are often used: a) expansion of the π-conjugated system and b) the introduction of either an electron-withdrawing or an electron-donating group(s) into a π-conjugated system. Though some helpful experimental principles are known (like intramolecular charge transfer [ICT], photoinduced energy transfer [PET], etc.),[5] it is still quite difficult to predict the accurate position to attach a functional group for achieving satisfactory fluorescent properties. Annulation has proven to be quite successful for effecting bathochromic shifts through extension of the conjugated system of various dyes architectures.[6] Our serendipitous discovery[4] prompted us to study the scope and limitations of this cyclocondensation reaction along these lines.

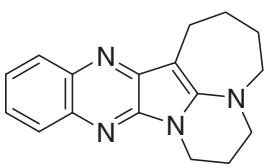

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Figure 1. The structure of compound **1**.

Results and Discussion

Assuming that 2,3-dichloroquinoxaline could be replaced with other molecules possessing two chlorine atoms susceptible to nucleophilic substitution in vicinal position, we initially focused on its simple analogs. Compounds **2**–**4** were chosen as the most promising substrates from the point of view of spectroscopic properties of expected products. In particular we expected strong intramolecular charge transfer for product prepared from 2,3-dichloro-5,6-dicyanopyrazine (**2**) and significant bathochromic shift of both absorption and emission for product synthesized from benzoquinoxaline **4**. Compounds **2** and **3** are commercially available, while 2,3-dichlorobenzo[*g*]quinoxaline (**4**) was prepared using literature methods from 2,3-diaminonaphthalene.[7] The reactions of compounds **2**–**4** with DBU performed under previously optimized conditions (neat, 115–150 °C) led to the expected 4,7-diaza-2-aminoindole derivatives **6**–**8** in 28–70% yield (Scheme 1). Reaction with compound **2** which, due to the presence of additional electron-withdrawing groups, is the most reactive substrate studied, led to derivative **6** in 67% yield. We also carried out reactions of **2**–**4** with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) but they failed (as previous attempts with 2,3-dichloroquinoxaline).[4] The heating of all compounds studied with DBN led to vigorous reaction and formation of black and very polar products. Different ring size in DBN versus DBU is most probably responsible for the different course of this reaction. Presumably ring opening starts after the first nucleophilic attack.

One can expect that reaction of DBU with derivatives of dichloroquinoxaline lacking C_2 symmetry will lead to the mixture of regioisomeric products, although not necessarily in 1:1 ratio. To test this 2,3-dichlorobenzo[*f*]quinoxaline (**10**) was chosen as a substrate, which was expected to give rise to two

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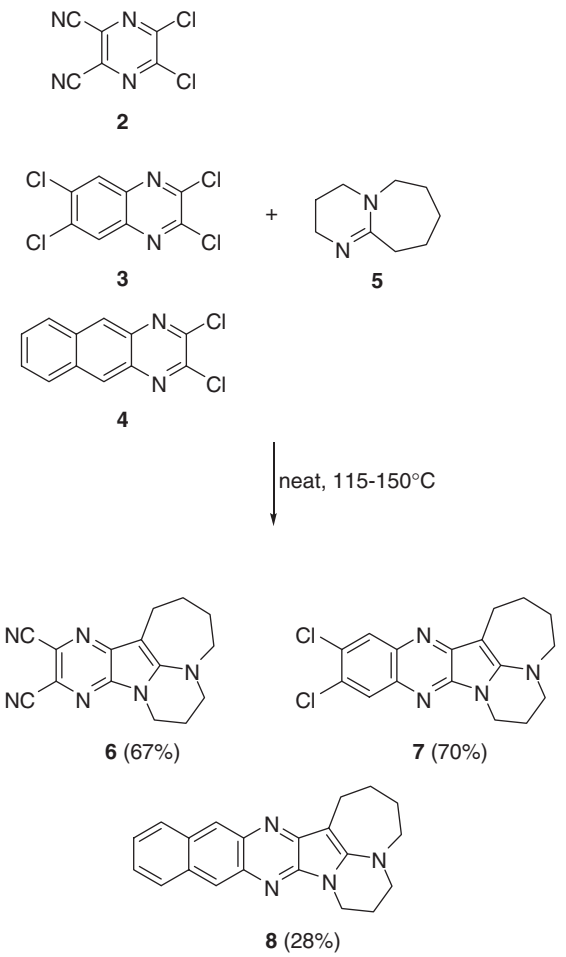
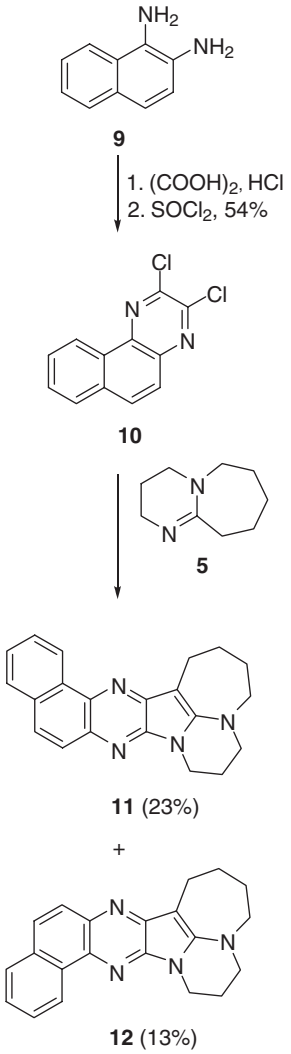


Scheme 1. The synthesis of compounds **6**–**8**.

products being regioisomers of compound **8**. The preparation of dyes **11** and **12** (Scheme 2) should allow for detailed spectroscopic comparison of these three regioisomers. It was recently reported that the direction of annulation plays an important role in influencing the spectral properties of a series of different benzannulated coumarins.[8] Our synthesis started from the preparation of 1,2-diaminonaphthalene (**9**)[9] as a building block and we transformed it into corresponding benzoquinoxaline **10** using literature procedures (Scheme 2).[10] The reaction of **10** with DBU gave two yellow products which were carefully separated by column chromatography. They formed in the ratio ≈2:1. Conventional analyses confirmed the identity of regioisomeric products **11** and **12**, but did not allow for the structure assignment. Consequently, X-ray analysis of a more polar product was performed revealing its structure as **11** (Figure 2). It showed that, in analogy to compound **1**,[4] the molecular structure is almost completely flat. However, contrary to **1** which displayed no aggregation due to the antiparallel arrangement in crystal packing, molecules of **11** stack in columns in parallel orientation. This also leads to fluorescence in the solid state[11] of neither **11** nor **6**–**8** and **10**.

Finally other types of compounds possessing two activated chlorine atoms in vicinal position were studied. As was previously reported, 3,4-dichloro-1,2,5-thiadiazole failed to give any isolable material. The reactions of DBU with pentafluorobenzaldehyde, 3,6-dinitro-1,2,4,5-tetrachlorobenzene and with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone were performed under various conditions (neat, 150 °C; CH_3CN, 25 °C; and CH_3CN, −30 °C), but in all cases the desired product was not formed. Pentafluorobenzaldehyde has been chosen since its fluorine atoms are highly activated toward nucleophilic substitution.[12]



Scheme 2. The synthesis of dyes **11** and **12**.

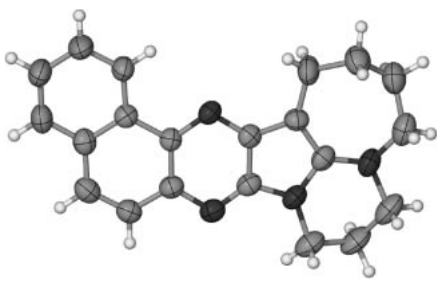
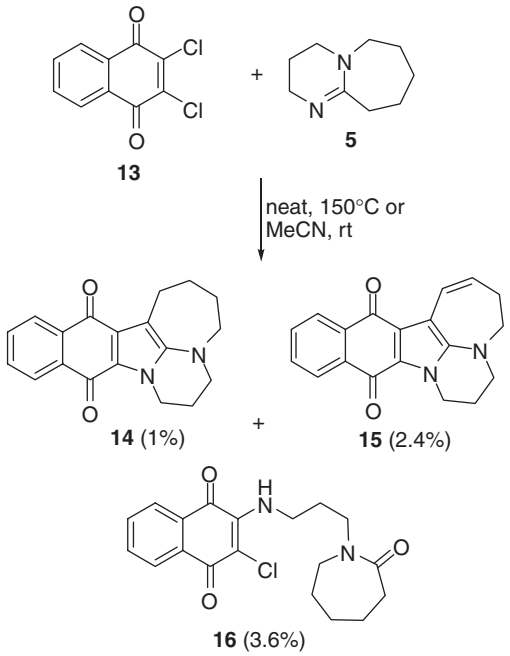


Figure 2. X-ray structure of compound **11**.

On the other hand, the reaction of 2,3-dichloro-1,4-naphthoquinone (**13**) with DBU was first performed at 150 °C in the neat to give a complex mixture of products. Two relatively non-polar compounds with distinct colors were separated and purified (violet: 1.0% and blue: 2.4%) (Scheme 3). Low yields



Scheme 3. Products of reaction of DBU with 2,3-dichloro-1,4-naphthoquinone.

and the presence of a significant amount of tar material prompted us to try less harsh conditions. Reaction of quinone **13** with DBU at rt in MeCN resulted in the formation of blue and violet compounds accompanied by an additional orange one (yields: 0.6%, 0.6%, and 3.6% respectively). The analysis by thin-layer chromatography (TLC) showed complete consumption of compound **13** under both conditions. Complex, inseparable mixtures of very polar, brown compounds was the main output in these reactions.

These three products (violet, blue, and orange) were separated by column chromatography and analyzed by various spectroscopic techniques. The structure of the violet product turned out to be analogous to that of **6**–**8**. The assignment (i.e., structure **14**) was based on ^{1}H NMR spectrum, MS and 2D NMR techniques. ^{1}H NMR spectrum of the blue compound revealed the presence of only five methylene groups instead of seven. Furthermore, two alkene-like signals were clearly visible. Given that the molecular mass was only two units smaller than the molecular mass of compound **14**, we came to the conclusion that the blue compound is the product of dehydrogenation of **14** and has the structure **15** (Scheme 3). This conclusion is additionally supported by a red-shift in the absorption spectrum, caused by the elongated conjugation. The source of an oxidant necessary to transform **14** into **15** is oxygen from air or a second molecule of quinone. The least polar orange compound was shown to have structure **16**. Molecular mass suggests that the orange compound is the product of substitution of only one chlorine and addition of one molecule of H_2O. ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra allowed us to identify all methylene groups and respective carbon atoms. Long-range ^{13}C–^{13}C correlation indicated that the amidine bond is broken. This information allowed us to propose structure **16**. This structure was further confirmed by correlation between NH and one of the CH_2 groups in ^{1}H–^{1}H COSY

Table 1. Spectroscopic Properties of Compounds **1**, **6**–**8**, **11**, **12**, and **14**–**16**

Compound	Solvent	λ_{abs}/nm	λ_{em}/nm	$\Phi^{a)}$/%	τ/ns
1	CH_3CN	421	515	23	5.2
	Toluene	416	493	42	6.4
6	CH_3CN	396	530	1.5	0.9
	Toluene	390	500	18	4.4
7	CH_3CN	438	535	16	3.7
	Toluene	432	512	46	6.5
8	CH_3CN	478	564	14	4
	Toluene	469	555	22	3.8
11	CH_3CN	423	503	35	5.5
	Toluene	418	480	48	5
12	CH_3CN	420	507	35	5.4
	Toluene	414	483	47	4.7
14	CH_3CN	542			
15	CH_3CN	582			
16	CH_3CN	465			

a) Determined using quinine sulphate in 0.5 M H_2SO_4 as a standard.

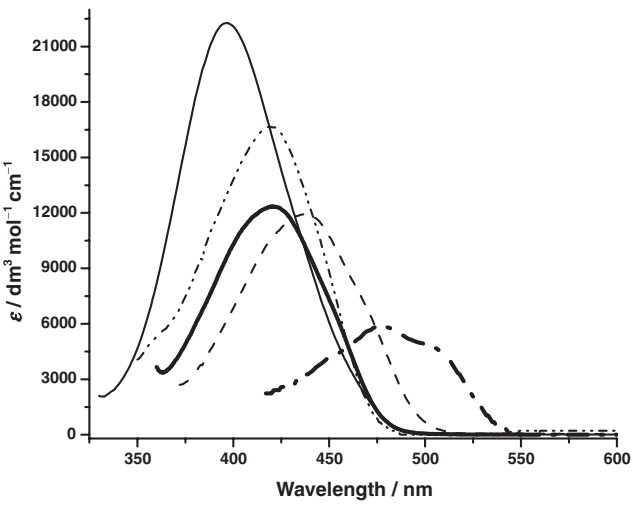


Figure 3. Normalized absorption spectra of compounds **6** (solid line), **7** (dashed line), **8** (dashed-dotted line), **11** (dotted-dashed-dotted line), and **1** (bold solid line) in acetonitrile.

spectra. The presence of compound **16** in the reaction mixture explained the low yield of desired pentacyclic products **14** and **15**. Water present in DBU probably induced the hydrolysis of the product of the first nucleophilic N-substitution.

Spectral characteristics of products **6**–**8**, **11**, **12**, and **14**–**16** were then examined and compared to those of the parent compound **1** (Table 1). The most notable feature was the bathochromic shift of absorption when going from **1** to **8** (Table 1, Figure 3, λ_{max} (**1**) = 421 nm, λ_{max} (**8**) = 478 nm). On the other hand, according to expectations, fusion of **1** with a benzene ring in nonlinear manner (i.e., **1** → **11**, **12**) resulted in almost no bathochromic shift (Table 1).

Fluorescence quantum yields of products **6**–**8**, **11**, and **12** were found to be moderate, but they vary from case to case. High fluorescence quantum yields of these compounds could be attributed to a substantial decrease in nonradiative relaxation

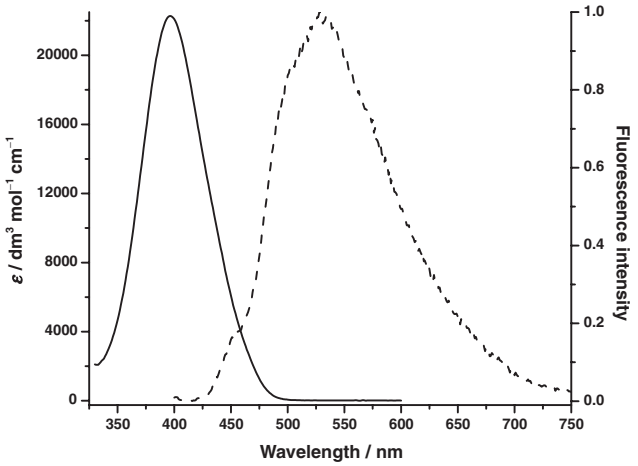


Figure 4. Absorption (solid line) and normalized fluorescence (dashed line) spectra of compound **6**, in acetonitrile.

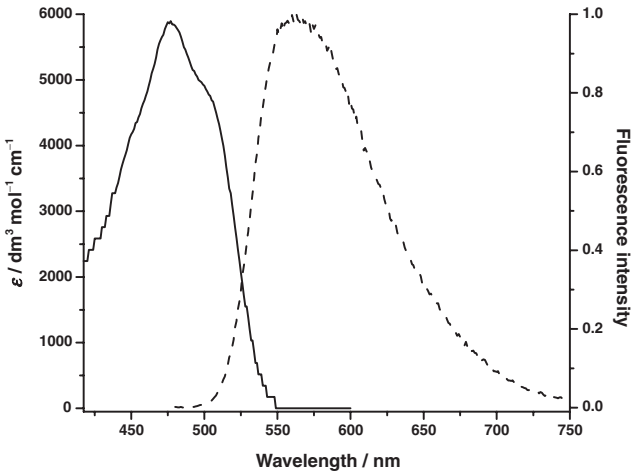


Figure 5. Absorption (solid line) and normalized fluorescence (dashed line) spectra of compound **8**, in acetonitrile.

to the ground state caused by hindered internal rotation of the amino group with respect to the aromatic ring.[1c] Reduction of the internal rotation is caused via inclusion of the nitrogen atom in rings. Regioisomers **11** and **12** had significantly higher fluorescence quantum yields (Φ_{fl} (**11**) = 48%, Φ_{fl} (**12**) = 47% in toluene) than linearly fused **8** (22%). The characteristic feature of **11** and **12** was a very small dependence of Φ_{fl} on the polarity of the solvent used in comparison to compounds **1**, **7**, and **8**. Compound **6** was a very intriguing case (Table 1). Due to the smaller system of conjugated rings (versus **1**) its absorption was hipsochromically shifted ($\lambda_{abs} = 396$ nm). However Stokes shift was very high (6400 cm^{-1} in CH_3CN and 5640 cm^{-1} in toluene) (Figure 4). The Stokes shifts for compounds **1**, **7**, **8**, **11**, and **12** were moderate (3200–4430 cm^{-1}) (Figures 4 and 5). This difference could be explained by ICT[13] which might occur in nitrile **6**, due to the presence of electron-withdrawing cyano groups and an electron-donating aminopyrrole moiety. Although the Stokes shift ($\Delta\nu/cm^{-1}$) of the compound **6** was largely dependent on the solvent polarity, the Lippert–Mataga analysis showed very poor correlation, i.e., $\Delta\nu = 7370\ cm^{-1}$ (in DMSO); $\Delta\nu = 6400\ cm^{-1}$ (in CH_3CN); $\Delta\nu = 6680\ cm^{-1}$ (in acetone); $\Delta\nu = 7630\ cm^{-1}$ (in THF); $\Delta\nu = 7690\ cm^{-1}$ (in EtOAc); $\Delta\nu = 5640\ cm^{-1}$ (in toluene). It is notable that Φ_{fl} of products **1**, **6–8**, **11**, and **12** was always higher in non-polar solvents (Table 1). For dinitrile **6** fluorescent quantum yield in CH_3CN is only 1.5%, while in non-polar toluene it is 18%. This phenomenon can find use in recently developed hydrophobic sensors working by the introduction of an environment-sensitive fluorophore into stimulus-responsive macromolecules such as proteins and synthetic polymers.[14] The maximum emission wavelength (λ_{em}) of **1**, **6–8**, **11**, and **12** was also sensitive to the environment (always 10–30 nm longer in polar solvents, Table 1). The longer emission wavelength in a hydrophilic solvent (CH_3CN) compared with that in a hydrophobic solvent (toluene) was due to the ICT character of the excited state.[5b] Fluorescence lifetimes are generally on the level of a few ns. Again, compound **6** represents a special case with a significantly higher (4.4 ns) fluorescence lifetime in toluene than in CH_3CN. The spectroscopic properties of compounds **14–16** differ significantly from compounds **6–8**, **11**, and **12**. They did not display any fluorescence and their absorption was found to be very weak ($\varepsilon \approx 5000$) but bathochromically shifted (Table 1).

Conclusion

In conclusion we proved that reaction of DBU with derivatives and analogs of 2,3-dichloroquinoxaline is general. On the other hand attempts to expand the scope of this condensation to include other compounds bearing two vicinal chlorine atoms prone to nucleophilic substitution, failed. The yields of polyheterocyclic products formed were moderate to good. Reactions of unsymmetrically substituted analogs of 2,3-dichloropyrazine led to the formation of both possible products, but not in a 1:1 ratio. Reactions of some highly reactive substrates like DDQ were very vigorous and yielded only polar tar materials. The examination of spectroscopic properties of all compounds bearing 4,7-diazaindole skeleton showed that they display intense fluorescence, usually dependent on solvent polarity. 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. We found that chemical modifications result in significantly altered spectroscopic properties relative to compound **1** important with respect to biosensing. These include (1) red-shifted absorption maxima; (2) low or high dependance of Φ_{fl} on solvent polarity. We demonstrated that tetracyclic compound **6** exhibited intense yellowish-green fluorescence with a notably large Stokes shift and high photostability and is a suitable candidate for applications in biosensing.

Experimental

All chemicals were used as received unless otherwise noted. Reagent grade solvents (CH_2Cl_2, hexane, and cyclohexane) were distilled prior to use. All reported ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AM 500 MHz or Varian 400 MHz spectrometers. Chemical shifts (δ) were determined with TMS as the internal reference; J values are given in Hz. UV–vis spectra were recorded in toluene (Cary). Chromatography was performed on silica (Kieselgel 60, 200–400 mesh). Mass spectra were obtained via EI or electrospray MS (ESI-MS). The following molecules were prepared according to literature procedures: **4**,[7] **9**,[9] and **10**.[10]

2,3,4,5,6,7-Hexahydro-1*H*-3a,8,11,11b-tetraazacyclohepta-[1,2,3-*jk*]fluorene-9,10-dicarbonitrile (6). A tube containing 5,6-dichloro-2,3-pyrazinedicarbonitrile (**2**) (0.2 g, 1 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (**5**) (250 µL, 3 mmol) was placed in a pre-heated oil bath (125 °C). After 3 min CH_2Cl_2 was added to hot residue, which resulted in a yellow suspension. After cooling down filtration gave pure product **6** (186 mg, 67%) as yellow crystals, $R_f = 0.70$ (silica, CH_2Cl_2/MeOH 99:1); mp (dec) 290–300 °C; Anal. Found: C, 64.7; H, 5.2; N, 30.2%. Calcd for $C_{15}H_{14}N_6$: C, 64.7; H, 5.1; N, 30.2%; λ_{max} (acetonitrile)/nm 267 ($\varepsilon \times 10^{-3} = 25.9$), 302 (20.0), and 397 (22.3); ^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ 1.83–1.90 (2H, m, CH_2CH_2), 1.99–2.03 (2H, m, CH_2CH_2), 2.26–2.31 (2H, m, CH_2CH_2), 2.87 (2H, t, $J = 6.0$ Hz, $ArCH_2$), 3.48–3.53 (4H, m, $2 \times NCH_2$), and 4.16 (2H, t, $J = 6.0$ Hz, NCH_2); ^{13}C NMR (125 MHz, $CDCl_3$, Me_4Si): δ 21.2, 22.6, 26.3, 29.2, 38.4, 49.6, 56.1, 94.9, 115.8, 116.5, 117.3, 126.5, 138.8, 141.5, and 153.7; *m/z* (EI) 278.12701 [M^+] calcd 278.12799 ($C_{15}H_{14}N_6$).

10,11-Dichloro-2,3,4,5,6,7-hexahydro-1*H*-3a,8,13,13b-tetraazabenzo[*b*]cyclohepta[1,2,3-*jk*]fluorene (7). A tube charged with 2,3,6,7-tetrachloroquinoxaline (**3**) (0.1 g, 0.37 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (**5**) (166 µL, 1.11 mmol) was placed in an oil bath. The reaction mixture was heated with stirring until the temperature reached 150 °C, while stirring (ca. 30 min). Subsequently, CH_2Cl_2 was added to the hot residue, which resulted in a yellow suspension. After cooling down filtration gave pure product **7** (90 mg, 70%) as yellow crystals, $R_f = 0.31$ (silica, CH_2Cl_2/MeOH 98:2); mp (dec) 240–250 °C; λ_{max} (acetonitrile)/nm 235 ($\varepsilon \times 10^{-3} = 24.9$), 291 (32.7), 343 (5.1), 358 (7.9), and 437 (11.9); ^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ 1.83–1.89 (2H, m, CH_2CH_2), 1.96–2.01 (2H, m, CH_2CH_2), 2.23–2.29 (2H, m, CH_2CH_2), 2.94 (2H, t, $J = 6.0$ Hz, $ArCH_2$), 3.41 (2H, t, $J = 5.5$ Hz, NCH_2), 3.45 (2H, t, $J = 5.7$ Hz, NCH_2), 4.15 (2H, t, $J = 6.0$ Hz, NCH_2), 7.97 (1H, s, ArH), 8.07 (1H, s, ArH); ^{13}C NMR (125 MHz, $CDCl_3$, Me_4Si): δ 21.8, 21.9, 23.0, 26.9, 29.6, 38.0, 49.9, 56.4, 90.4, 127.0, 127.7, 128.0(2), 128.9, 136.0, 142.3, 152.9; *m/z* (EI) 346.07637 [M^+], calcd 346.07520 ($C_{17}H_{16}N_4Cl_2$).

2,3,4,5,6,7-Hexahydro-1*H*-3a,8,15,15b-tetraazabenz[*c,d*]azuleno[1,2-*b*]anthracene (8). A tube containing 2,3-dichlorobenzo-[*g*]quinoxaline (**4**) (0.1 g, 0.4 mmol) and 1,8-diazabicyclo[5.4.0]-undec-7-ene (**5**) (180 µL, 1.2 mmol) was placed in a pre-heated oil bath (115 °C). After 10 min, CH_2Cl_2 was added to hot residue, which resulted in an orange suspension. After cooling down filtration gave pure product **8**, (36.7 mg, 28%), as orange crystals $R_f = 0.60$ (silica, CH_2Cl_2/MeOH 8:2); mp (dec) 185–230 °C; λ_{max} (acetonitrile)/nm 248 ($\varepsilon \times 10^{-3} = 18.8$), 296 (51.0), 359 (1.7), 377 (6.3), 398 (6.9), and 477 (2.8); ^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ 1.85–1.91 (2H, m, CH_2CH_2), 1.96–2.02 (2H, m, CH_2CH_2), 2.24–2.29 (2H, m, CH_2CH_2), 3.00 (2H, t, $J = 6.0$ Hz, $ArCH_2$), 3.41–3.46 (4H, m, $2 \times NCH_2$), 4.17 (2H, t, $J = 6.0$ Hz, $2 \times NCH_2$), 7.37–7.45 (2H, m, $2 \times$ ArH), 7.97–8.02 (2H, m, $2 \times$ ArH), 8.44 (1H, s, ArH), 8.52 (1H, s, ArH); ^{13}C NMR (125 MHz, $CDCl_3$, Me_4Si): δ 21.9, 23.0, 27.0, 29.6, 37.9, 49.9, 56.3, 89.7, 123.8, 124.1, 124.4, 124.6, 126.9, 127.6, 127.7, 127.8, 128.6, 130.7, 131.8, 136.1, 143.7; *m/z* (EI) 328.16758 [M^+], calcd 328.16880 ($C_{21}H_{20}N_4$).

Synthesis of Compounds 11 and 12. A tube containing 2,3-dichlorobenzo[*f*]quinoxaline (**10**) (0.2 g, 0.8 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (**5**) (360 µL, 2.4 mmol) was placed in a pre-heated oil bath (115 °C) and stirred for 10 min. Subsequently CH_2Cl_2 was added and the mixture was chromatographed to obtain two regioisomers (silica, CH_2Cl_2:acetone 99:1 then 93:7 then 9:1). Pure dark yellow products were obtained after recrystallization (CH_2Cl_2): **11** (61 mg, 23%), $R_f = 0.56$ (silica, CH_2Cl_2/MeOH 9:1) and **12** (35 mg, 13%), $R_f = 0.70$ (silica, CH_2Cl_2/MeOH 96:4).

2,3,4,5,6,7-Hexahydro-1*H*-3a,8,15,15b-tetraazabenz[*c,d*]azuleno[2,1-*b*]phenanthrene (11): Mp (dec) 203–232 °C; λ_{max} (acetonitrile)/nm 273 ($\varepsilon \times 10^{-3} = 22.9$), 281 (22.3), 295 (18.8), 313 (12.5), 325 (11.4), and 419 (13.2); ^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ 1.81–1.86 (2H, m, CH_2CH_2), 1.91–1.96 (2H, m, CH_2CH_2), 2.19–2.24 (2H, m, CH_2CH_2), 3.01 (2H, t, $J = 6.0$ Hz, $ArCH_2$), 3.24–3.29 (2H, m, NCH_2), 3.32 (2H, t, $J = 5.6$ Hz, NCH_2), 4.26 (2H, t, $J = 6.0$ Hz, NCH_2), 7.54 (1H, t, $J = 7.3$ Hz, ArH), 7.64 (1H, t, $J = 7.5$ Hz, ArH), 7.81 (1H, d, $J = 9.0$ Hz, ArH), 7.88 (1H, d, $J = 7.9$ Hz, ArH), 8.00 (d, 1H, $J = 9.0$ Hz, ArH), 9.19 (1H, d, $J = 8.2$ Hz, ArH); ^{13}C NMR (125 MHz, $CDCl_3$, Me_4Si): δ 22.0, 23.3, 27.2, 30.0, 38.1, 50.0, 56.3, 90.9, 123.1, 125.6, 125.8, 126.2, 127.3, 127.5, 131.3, 131.8, 133.2, 138.8, 139.9, 142.8, 150.9; *m/z* (EI) 328.16723 [M^+], calcd 328.16880 ($C_{21}H_{20}N_4$).

2,3,4,5,6,7-Hexahydro-1*H*-3a,8,15,15b-tetraazabenz[*c,d*]azuleno[1,2-*b*]phenanthrene (12): Mp (dec) 192–201 °C; λ_{max} (acetonitrile)/nm 242 ($\varepsilon \times 10^{-3} = 28.3$), 261 (21.3), 269 (17.7), 299 (33.1), 328 (10.1), and 424 (16.5); ^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ 1.86–1.91 (2H, m, CH_2CH_2), 1.96–2.02 (2H, m, CH_2CH_2), 2.24–2.30 (2H, m, CH_2CH_2), 3.12 (2H, t, $J = 5.7$ Hz, $ArCH_2$), 3.32–3.36 (2H, m, NCH_2), 3.41 (2H, t, $J = 5.7$ Hz, NCH_2), 4.25 (2H, t, $J = 6.0$ Hz, NCH_2), 7.56–7.60 (1H, m, ArH), 7.64–7.68 (1H, m, ArH), 7.67–7.91 (2H, m, $2 \times$ ArH), 7.76 (1H, d, $J = 8.9$ Hz, ArH), 9.35–9.42 (1H, m, ArH); ^{13}C NMR (125 MHz, $CDCl_3$, Me_4Si): δ 22.1, 23.3, 27.2, 30.2, 38.2, 49.9, 56.5, 91.7, 124.0, 125.1, 125.8, 126.2, 126.9, 127.4, 131.6, 132.0, 134.9, 137.7, 140.7, 142.0, 150.8; *m/z* (EI) 328.16778 [M^+], calcd 328.16880 ($C_{21}H_{20}N_4$).

Reaction of 2,3-Dichloro-1,4-naphthoquinone (13) with 1,8-Diazabicyclo[5.4.0]undec-7-ene (4). Method A: 2,3-Dichloro-1,4-naphthoquinone (**13**) (300 mg, 1.3 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (**5**) (600 µL, 4 mmol) were heated at 150 °C for 45 min. Subsequently, the warm residue was dissolved in MeOH and cooled down. The MeOH was partially evaporated and residue was filtrated through a silica pad (silica, hexane/EtOAc 8:2). Next, the collected fractions were chromatographed (silica, hexane/EtOAc 8:2 then 7:3 then 6:4 then 1:1) to obtain two pure products: blue (**15**) (9.8 mg, 2.4%) and violet (**14**) (3.9 mg, 1%).

Method B: 2,3-Dichloro-1,4-naphthoquinone (**12**) (300 mg, 1.3 mmol) was dissolved in CH_3CN (4 mL). Then 1,8-diazabicyclo[5.4.0]undec-7-ene (**5**) (600 µL, 4 mmol) was added and the resulting mixture was stirred at rt for 6 h. The residue was partially evaporated and chromatographed (silica, hexane/EtOAc, 8:2 then 7:3 then 6:4 then 1:1) to obtain three pure products: blue (**15**) (2.4 mg, 0.6%): $R_f = 0.45$ (silica, hexane/EtOAc 2:3), violet (**14**) (2.5 mg, 0.6%): $R_f = 0.59$ (silica, hexane/EtOAc 3:2) and orange (**16**) (17.1 mg, 3.6%): $R_f = 0.39$ (silica, hexane/EtOAc 1:9).

2,3,4,5,6,7-Hexahydro-1*H*-3a,13b-diazabenzo[*b*]cyclohepta-[1,2,3-*jk*]fluorene-8,13-dione (14): λ_{max} (acetonitrile)/nm 243 ($\varepsilon \times 10^{-3} = 20.4$), 308 (23.1), and 542 (4.1); ^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ 1.70–1.76 (2H, m, CH_2CH_2), 1.88–1.93 (2H, m, CH_2CH_2), 2.12–2.18 (2H, m, CH_2CH_2), 3.14–3.19 (4H, m, $ArCH_2$ and NCH_2), 3.27 (2H, t, $J = 5.5$ Hz, NCH_2), 4.48 (2H, t, $J = 6.0$ Hz, NCH_2), 7.53 (1H, ddd, $J_1 = 1.4$ Hz, $J_2 = 7.5$ Hz, $J_3 = 7.5$ Hz, ArH), 7.58 (1H, ddd, $J_1 = 1.4$ Hz, $J_2 = 7.2$ Hz, $J_3 = 7.5$ Hz, ArH), 8.05 (2H, ddd, $J_1 = 1.4$ Hz, $J_2 = 4.1$ Hz, $J_3 = 7.6$ Hz, $2 \times$ ArH); ^{13}C NMR (125 MHz, $CDCl_3$, Me_4Si): δ 21.9, 24.6, 26.4, 30.4, 43.6, 49.7, 56.0, 110.9, 123.6, 125.3, 125.4, 125.8, 131.4,

132.7, 133.8, 135.0, 146.9, 171.7, 182.3; *m/z* (EI) 306.1375 [M^+], calcd 306.1368 ($C_{19}H_{18}N_2O_2$).

2,3,4,5-Tetrahydro-1*H*-3a,13b-diazabenzo[*b*]cyclohepta[1,2,3-*jk*]fluorene-8,13-dione (15): λ_{max} (acetonitrile)/nm 252 ($\varepsilon \times 10^{-3} = 18.9$), 305 (12.6), and 582 (4.2); ^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ 2.19–2.25 (2H, m, CH_2CH_2), 2.63–2.67 (2H, m, CH_2CH_2), 3.36 (2H, t, $J = 5.6$ Hz, NCH_2), 3.40 (2H, t, $J = 4.8$ Hz, NCH_2), 4.50 (2H, t, $J = 6.1$ Hz, NCH_2), 5.78 (1H, dt, $J_1 = 5.6$ Hz, $J_2 = 11.3$ Hz, CH_2CH), 7.44 (1H, dt, $J_1 = 1.4$ Hz, $J_2 = 11.3$ Hz, CH_2CH), 7.54 (1H, ddd, $J_1 = 1.4$ Hz, $J_2 = 7.5$ Hz, $J_3 = 7.5$ Hz, ArH), 7.59 (1H, ddd, $J_1 = 1.4$ Hz, $J_2 = 7.5$ Hz, $J_3 = 7.5$ Hz, ArH), 8.05 (2H, ddd, $J_1 = 1.4$ Hz, $J_2 = 4.4$ Hz, $J_3 = 7.6$ Hz, 2 × ArH); ^{13}C NMR (125 MHz, $CDCl_3$, Me_4Si): δ 22.1, 32.3, 43.0, 48.6, 51.8, 106.1, 122.2, 123.5, 124.0, 125.3, 125.8, 126.6, 131.5, 132.7, 133.7, 134.8, 145.7, 172.1, 182.9; *m/z* (EI) 304.1225 [M^+], calcd 304.1212 ($C_{19}H_{16}N_2O_2$).

2-Chloro-3-{[3-(2-oxoazepan-1-yl)propyl]amino}-1,4-naphthoquinone (16): λ_{max} (acetonitrile)/nm 275 ($\varepsilon \times 10^{-3} = 16.6$) and 465 (2.2); ^{1}H NMR (500 MHz, $CDCl_3$, Me_4Si): δ 1.63–1.78 (6H, m, 3 × CH_2), 1.82–1.88 (2H, m, CH_2), 2.58–2.62 (2H, m, CH_2), 3.33–3.39 (2H, m, CH_2), 3.49 (2H, t, $J = 6.1$ Hz, CH_2), 3.80–3.85 (2H, m, CH_2), 7.32 (1H, br s, NH), 7.60 (1H, ddd, $J_1 = 1.2$ Hz, $J_2 = 7.5$ Hz, $J_3 = 7.5$ Hz, ArH), 7.70 (1H, ddd, $J_1 = 1.2$ Hz, $J_2 = 7.6$ Hz, $J_3 = 7.6$ Hz, ArH), 8.04 (1H, d, $J = 7.6$ Hz, ArH), 8.14 (1H, dd, $J_1 = 0.9$ Hz, $J_2 = 7.7$ Hz, ArH); ^{13}C NMR (125 MHz, $CDCl_3$, Me_4Si): δ 23.4, 28.6, 29.6, 29.9, 37.0, 40.9, 45.1, 49.9, 126.7, 126.8, 130.0, 132.2, 132.8, 134.7, 140.0, 144.7, 176.7, 177.1, 180.5; *m/z* (EI) 360.1244 [M^+], calcd 360.1241 ($C_{19}H_{21}N_2O_3Cl$).

Optical Measurements. Electronic absorption spectra were measured on a Shimadzu UV 3100 spectrophotometer. Stationary fluorescence spectra were recorded and corrected for instrumental response using an Edinburgh FS 900 CDT spectrofluorometer. The solvents *n*-hexane, DMSO, and methanol were checked for the presence of fluorescent impurities. For the determination of quantum yields, quinine sulfate in 0.5 M H_2SO_4 was used as a standard ($\Phi_{fl} = 0.55$).

Crystal data for compound **11**: $C_{21}H_{20}N_4$, $M_r = 328.41$, bronze block, $0.80 \times 0.60 \times 0.40\,mm^3$, monoclinic, space group $P2_1/n$ (No. 14), $a = 14.2124(6)$, $b = 14.6403(6)$, $c = 15.9418(7)$ Å, $\beta = 92.641(4)°$, $V = 3313.5(2)$ Å^3, $Z = 8$, $D_{calcd} = 1.317\,g\,cm^{-3}$, $F_{000} = 1392$, Bruker APEX-II CCD, Cu Kα radiation, $\lambda = 1.54178$ Å, $T = 296(2)$ K, $2\theta_{max} = 100.9°$, 7831 reflections collected, 3355 unique ($R_{int} = 0.0416$). Final $GOF = 1.054$, $R1 = 0.0545$, $wR2 = 0.1282$, R indices based on 2128 reflections with $I > 2\sigma(I)$ (refinement on F^2), 452 parameters, 0 restraints. Lp and absorption corrections applied, $\mu = 0.626\,mm^{-1}$.

Crystallographic data have been deposited with Cambridge Crystallographic Data Centre: Deposition number CCDC-746166 for compound No. **11**. Copies of the data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, U.K.; Fax: + 44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

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