REDUCTIVE CYCLIZATION OF 8-(2-NITROPHENYL)-1-AZAAZULENE DERIVATIVES; FORMATION OF 6a,7-DIAZANAPHTH[3,2,1-*cd*]AZULENE AND 7*H*-1,7-DIAZAINDENO[1,2-*e*]AZULENE SYSTEMS, A NEW DNA INTERCALATER †

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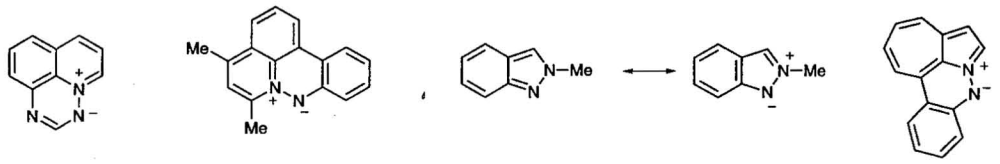
Abstract - Reaction of ethyl 2-dimethylamino-8-(2-nitrophenyl)-1-azaazulene-3-carboxylate with hexamethylphosphorous triamide gave ethyl 6-dimethylamino-6a,7-diazanaphth[3,2,1-*cd*]azulene-5-carboxylate (**7b**) and ethyl 2-dimethylamino-7*H*-1,7-diazaindeno[1,2-*e*]azulene-3-carboxylate (**8b**). The structures of **7b** and **8b** were determined by X-Ray structure analyses. Compound (**8b**) was intercalated to Calf-Thymus DNA and a 12-mer DNA with binding constants K_D = 1.55 x 10^6 and 1.75 x 10^6 M^{-1}, respectively.

INTRODUCTION

Because of the versatile synthetically utility, heteroaromatic *N*-imines, being considered as azomethine imine, have been investigated extensively.[1] The conjugated heterocyclic mesomeric betaine,[2] such as the tricyclic 1,3-dipole (**1**)[3] and the tetracyclic mesomeric betaine (**2**),[4] in which heteroaromatic *N*-imine moieties are incorporated in the aromatic ring, also attracted attention, since these systems are isoconjugate

† Dedicated to Prof. Shô Itô on the occasion of his 77th birthday.

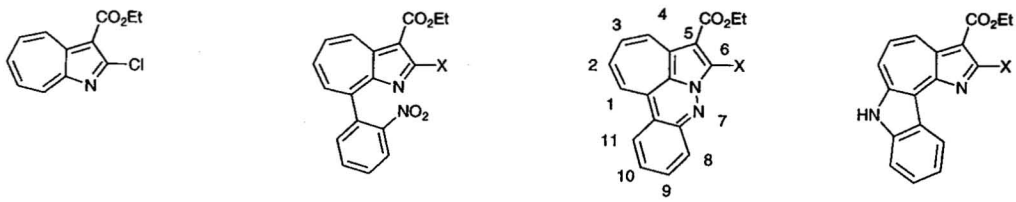
with odd alternant phenalenide anion and its benzolog.[5] Individually, because of the large resonance contribution of benzene ring, 2-methylindazole (**3A**) can be considered to have an azomethine imine moiety as resonance form (**3B**), although it is completely incorporated in an aromatic ring.[6] As a continuation of our investigation about 1-azaazulenium *N*-imines,[7] we are interested in the synthesis and the properties of 6a,7-diazanaphth[3,2,1-*cd*]azulene system (**4**), which is a non-alternant isomeric system of **2**. It is expected that the large resonance contribution of benzene ring would bring about the preferential formation of azomethine imine form. For synthetic approach, we adopted the well-established methodology for the formation of azomethine imines by nitrogen insertion by nitrene, which would be generated by deoxygenation of the corresponding 8-(2-nitrophenyl)-1-azaazulene.



1 **2** **3A** **3B** **4**

RESULTS AND DISCUSSION

For synthetical convenience, we adopted ethyl 2-chloro-8-(2-nitrophenyl)-1-azaazulene-3-carboxylate (**6a**) as *o*-nitrophenyl compound. The desired compound (**6a**) was prepared by the treatment of ethyl 2-chloro-1-azaazulene-3-carboxylate (**5**) with 2-nitrophenyllithium[8] and successive dehydrogenation with tetrachloro-1,2-benzoquinone in 66% yield. Ethyl 2-dimethylamino-8-(2-nitrophenyl)-1-azaazulene-3-carboxylate (**6b**) was obtained by treatment of **6a** with dimethylamine in 80% yield.

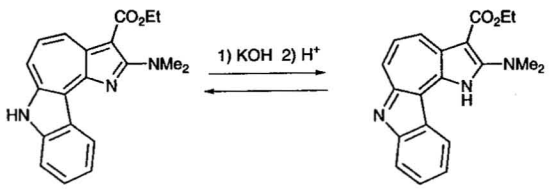


5 **6a** : X = Cl, **6b** : X = NMe_2 **7a** : X = Cl, **7b** : X = NMe_2 **8a** : X = Cl, **8b** : X = NMe_2

Triethyl phosphite is usually employed for the generation of a nitrene from a nitro-substituted compound.[9] Therefore we treated **6a** with triethyl phosphite in boiling benzene for 3 h at first (Table 1, run 1). In the reaction, ethyl 6-chloro-6a,7-diazanaphth[3,2,1-*cd*]azulene-5-carboxylate (**7a**) was obtained only low yield (2%) together with recovered **6a** (58%). To modify the reaction, we next performed the reaction of

6a with hexamethylphosphorous triamide (HMPT). When **6a** was treated with HMPT in refluxing xylene for 3 h, the expected compound (**7a**) was obtained only 4% yield; **6b** (6%), ethyl 6-dimethylamino-6a,7-diazanaphth[3,2,1-*cd*]azulene-5-carboxylate (**7b**) (4%), and ethyl 2-dimethylamino-7*H*-1,7-diazaindeno[1,2-*e*]azulene-3-carboxylate (**8b**) (18%) were obtained, where a chloro-substituent was replaced by a dimethylamino-substituent (run 3). Therefore we next treated ethyl 2-dimethylamino-8-(2-nitrophenyl)-1-azaazulene-3-carboxylate (**6b**) with HMPT. Results were listed in Table 1. A moderate result was obtained by treatment of **6b** with HMPT in xylene in a sealed tube at 165 ℃ for 1.5 h under an argon atmosphere, where **7b** and **8b** were obtained in 10% and 24% yields, respectively, together with recovered **6b** (run 9). Prolonged heating of **6b** with HMPT gave **7b** and **8b** in low yield (run 7).

When compound (**6b**) was treated with triethyl phosphite in boiling xylene, ethyl 2-dimethylamino-1*H*-1,7-diazaindeno[1,2-*e*]azulene-3-carboxylate (**9b**), an isomer of **8b**, was obtained in 63% yield together with **7b** (10%) (run 5).



8b **9b**

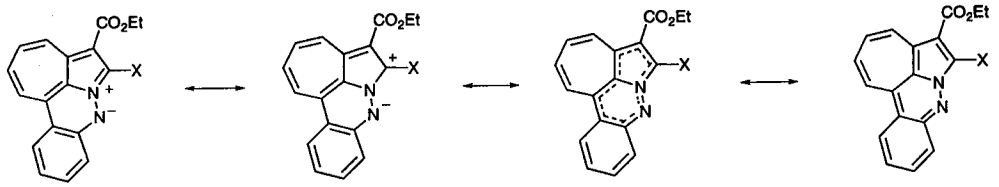
Table 1. Reductive cyclization of **6a** and **6b**.

run	Substrate	Reaction conditions	Products (Yield / %)
1	**6a**	$P(OEt)_3$ benzene, reflux, 3 h	**7a** (2), **6a** (58)
2	**6a**	$P(OEt)_3$ xylene, reflux, 3 h	**7a** (2)
3	**6a**	$P(NMe_2)_3$ xylene, reflux, 3 h	**7a** (4), **7b** (4), **8b** (18), **6b** (6)
4	**6a**	$P(NMe_2)_3$ neat, 160 ℃, 3 h	**8b** (11)
5	**6b**	$P(OEt)_3$ xylene, reflux, 3 d	**7b** (10), **9b** (63)
6	**6b**	$P(NMe_2)_3$ toluene, reflux, 3 h	**7b** (3), **8b** (5), **6b** (86)
7	**6b**	$P(NMe_2)_3$ xylene, reflux, 3 d	**7b** (5), **8b** (9), **6b** (3)
8	**6b**	$P(NMe_2)_3$ *t*-butylbenzene, 150 ℃, 2 h	**7b** (8), **8b** (13), **6b** (57)
9	**6b**	$P(NMe_2)_3$ xylene, 165 ℃, 1.5 h	**7b** (10), **8b** (24), **6b** (38)

Compound (**9b**) was obtained by treatment of **8b** with potassium hydroxide in 94% yield. Compound (**9b**) was slightly unstable and converted to **8b** with a half-life period of 20 days in $CDCl_3$.

The structures of **7b** and **8b** were confirmed by a single-crystal X-Ray structural analysis and the structures of the other products were deduced by a comparison of the spectroscopic data with **7b** and **8b**. ORTEP drawings[10] are shown in Figures 1 and 2.

In ^{1}H NMR spectrum of **7b**, ring proton signals are observed at rather high resonance field with a large divergence of coupling constants, such as δ 5.12 (dd, J 11.0 and 8.1, H-3), 5.74 (dd, J 12.1 and 8.1, H-2), 5.84 (d, J 12.1, H-1), and 6.93 (d, J 11.0, H-4) for the seven-membered ring protons and 6.31 (dd, J 8.5 and 6.4, H-10), 6.67 (d, J 8.5, H-11), 6.69 (d, J 8.7, H-8), and 6.84 (dd, J 8.7 and 6.4, H-9) for the six-membered ring protons. These suggest that the compound (**7b**) has a bond alternation in the seven-membered ring and the benzene ring and the contribution of azaindolizine moiety would be large than that of benzene moiety in its resonance form. The results were supported by the inspections from the X-Ray analysis and the MO calculation using RHF/6-31G* of **7b**, where a divergence of the bond-lengths was large in the seven-memberd ring and the benzene ring, whereas that was rather small in the azaindolizine moiety (Figure 3). These results showed that the resonance form (**D**) is most preferred among other resonance forms (**A**, **B**, and **C**). Expected contribution of the azomethine imine forms (**A** and **B**) by the aid of the resonance of benzene ring would be rather small.



The compound (**8b**) showed strong fluorescence with a peak at 549.0 nm. Remarkable quenching of fluorescence was observed on addition of calf thymus DNA to a solution of **8b**, indicating that it interacted with DNA (Figure 4). Figure 5 shows the absorption spectra of **8b**, and the spectral changes induced by addition of DNA. Bathochromic shift and hypochromic effects were observed with obvious isosbestic points. These should indicate that the intercalation of **8b** with DNA is simple and only one species of bound form is formed. In Figure 6, Scatchard plot[11] was made on the basis of the change of fluorescence emission intensity at 549.0 nm shown in Figure 4. The apparent binding constants K_D of **8b** with calf thymus DNA and the double helical DNA 12mer (5'-d(CATCCCGGGATG)-3')$_2$ are 1.55 x

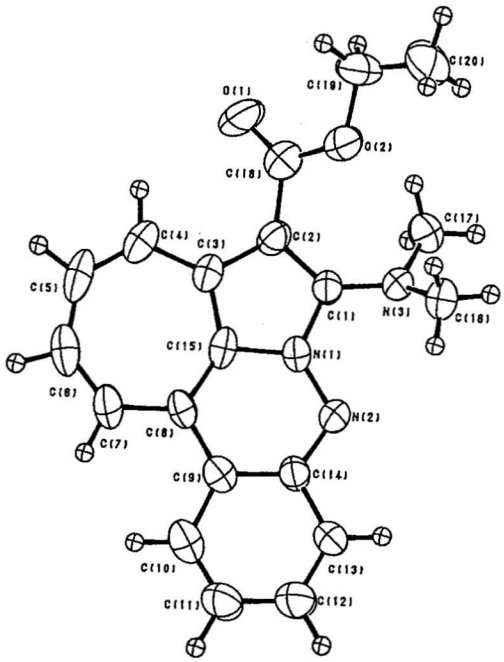


Figure 1. ORTEP drawing of **7b** showing with thermal ellipsoid plot (50% probability).

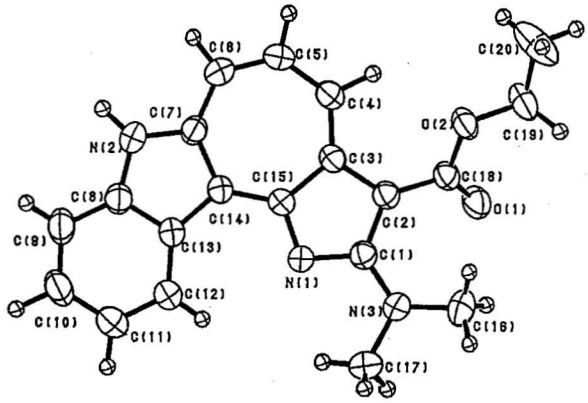


Figure 2. ORTEP drawing of **8b** showing with thermal ellipsoid plot (50% probability).

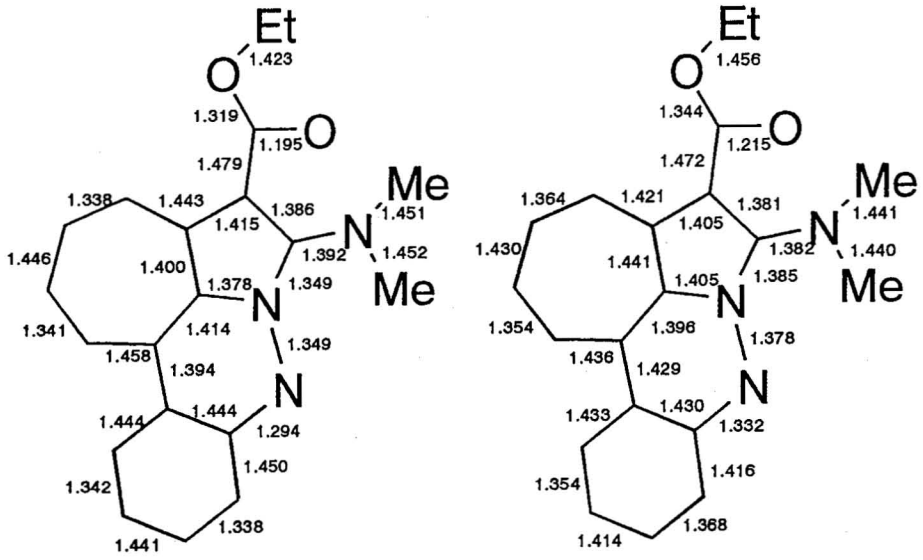


Figure 3. Calculated (left, RHF/6-31G* level) and observed (right, X-Ray analysis) bond lengths (Å) of **7b**.

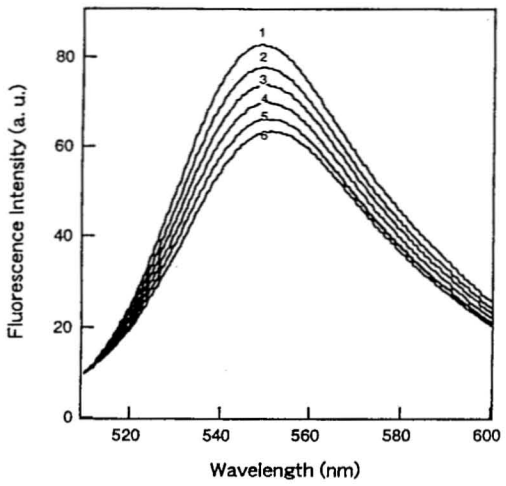


Figure 4 Optical titration of **8b** with DNA. Fluorescence spectrum of **8b** and its quenching on titration with calf thymus DNA. Solutions were excited at 350.0 nm, and emission spectra were recorded. Curve 1, **8b** (1.68×10^{-6} M); curve 2 to 6, 3 mL solution of **8b** plus 21, 39, 63, 78, and 100 μL of 1.04×10^{-5} M DNA.

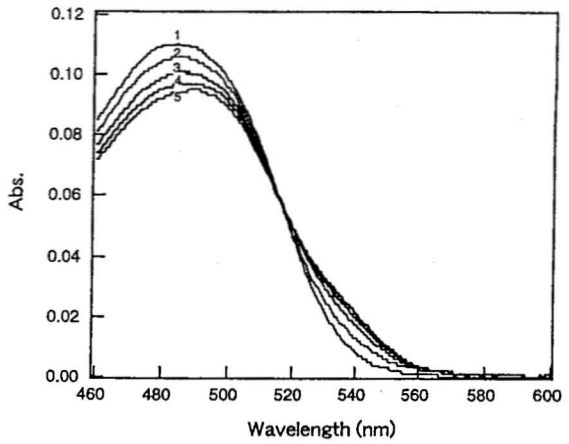


Figure 5 Absorption spectra of 1.68×10^{-5} M **8b** (3 mL) plus 0, 5, 10, 15, 20 μL of 2.08×10^{-6} M calf thymus DNA solution for curves 1 to 5.

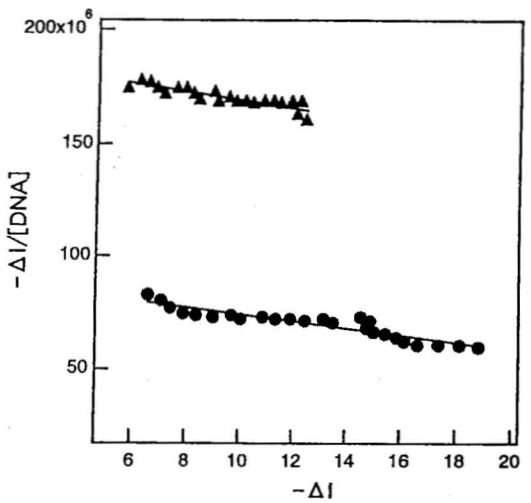


Figure 6 Scatchard plots of **8b**-calf thymus DNA and **8b**-12mer DNA (5'-d(CATCCCGGGATG)-3')$_2$ complexes. Fluorescence emission at 549.0 nm (excitation at 350.0 nm) of the complexes were used for the calculation; ●, calf thymus DNA; ▲, the 12mer DNA.

10^6 and 1.55 x 10^6 M^{-1} at 25 °C, respectively. These values are comparable to that of ethidium bromide, which strongly intercalates to DNA (K_D = 8.9 x 10^6 M^{-1} at 25 °C)[12]

EXPERIMENTAL

Mps are measured using Yanagimoto micro-melting apparatus and uncorrected. ^{1}H NMR spectra were recorded on Bruker AVANCE 400S (400 MHz) using deuteriochloroform as a solvent with tetramethylsilane as an internal standard unless otherwise stated; *J* values are recorded in Hz. Electronic spectra were taken with Hitachi 220A spectrophotometer using ethanol as a solvent. IR spectra were recorded for KBr pellets on a Nicolet FT-IR Impact 410. Kieselgel 60 and alumina activated 300 were used for column chromatography and kieselgel 60G was used for thin layer chromatography. In optical titration, electronic or fluorescence spectra were taken with a JASCO V-550 or a Hitachi F-2500 spectrophotometer. Calf-thymus DNA (Type I) was purchased from Sigma, and the double helical DNA 12mer (5'-d(CATCCCGGGATG)-3')$_2$ was prepared using a PE Applied Biosystems 393 DNA/RNA synthesizer.

Synthesis of ethyl 2-chloro-8-(2-nitrophenyl)-1-azaazulene-3-carboxylate (6a)

To the solution of *o*-nitrobenzene (1.112 g, 4.00 mmol) in dry THF (8 mL) at –100 ℃ (liquid N_2 / Et_2O) under argon was introduced slowly a solution of phenyllithium (4.5 mL of a 0.86 M solution in ether—cyclohexane, 3.87 mmol), and was stirred for 50 min at –100 ℃. Then the solution of ethyl 2-chloro-1-azaazulene-3-carboxylate (**5**) (0.468 g, 1.99 mmol) in dry THF (10 mL) was added to the mixture and stirring was continued for 50 min at –100 ℃. To the mixture methanol (5 mL) was added, and then reaction temperature was raised to rt. Tetrachloro-1,2-benzoquinone (1.041 g, 4.23 mmol) was added to the mixture. After stirring for 2 h, the mixture was poured to water and extracted with chloroform. The extract was dried over sodium sulfate and evaporated. Chromatography of the residue on alumina with benzene gave ethyl 2-chloro-8-(2-nitrophenyl)-1-azaazulene-3-carboxylate (**2a**) (0.470 g, 66%), which was recrystallized from hexane—dichloromethane to give pale yellow prisms, mp 163.5—164.5 ℃; δ_H 1.47 (3H, t, *J* 7.1), 4.48 (2H, q, *J* 7.1), 7.53 (1H, d, *J* 7.6), 7.67 (1H, dd, *J* 8.1 and 7.5), 7.77 (1H, dd, *J* 7.6 and 7.5), 7.96 (1H, d, *J* 10.2), 8.00 (1H, dd, *J* 10.2 and 10.0), 8.09 (1H, dd, *J* 10.2 and 10.0), 8.21 (1H, d, *J* 8.1), and 9.66 (1H, d, *J* 10.2) ; δ_C 14.8, 61.1, 112.7, 125.0, 130.1, 132.3, 132.9, 133.7, 134.5, 136.1, 137.9, 139.3, 147.4, 149.4, 154.2, 159.1, and 163.8; ν_{max} / cm^{-1} 1704 (C=O), 1525 and 1356 (NO_2); λ_{max} nm (log ε) 224 (4.52), 237 (4.44), 286 (4.62), 342 (3.93), and 452 (3.14). MS *m/z* (rel. intensity) 356 (M^+, 9), 312 (33), 311 (26), 310 (97), 284 (33), 282 (100), 238 (36), and 237 (31). *Anal.* Calcd for $C_{18}H_{13}N_2O_4Cl$: C, 60.60; H, 3.67; N, 7.85. Found: C, 60.59; H, 3.64; N, 7.91.

Synthesis of ethyl 2-dimethylamino-8-(2-nitrophenyl)-1-azaazulene-3-carboxylate (6b)

A solution of **6a** (0.970 g, 2.72 mmol) and 50% dimethylamine (8.0 mL) in ethanol (100 mL) was refluxed for 24 h then cooled. Filtration of the precipitate gave ethyl 2-dimethylamino-8-(2-nitrophenyl)-1-azaazulene-3-carboxylate (**6b**) (0.796 g, 80%), which was recrystallized from hexane—dichloromethane to give pale yellow prisms, mp 166.5—167.0 ℃; δ_H 1.43 (3H, t, *J* 7.1), 3.16 (6H, s), 4.48 (2H, q, *J* 7.1), 7.45 (1H, dd, *J* 9.9 and 9.6), 7.51 (1H, d, *J* 7.6), 7.54 (1H, dd, *J* 8.1 and 7.6), 7.56 (1H, dd, *J* 10.0 and 9.6), 7.64 (1H, t, *J* 7.6), 7.71 (1H, d, *J* 9.9), 8.03 (1H, d, *J* 8.1), and 8.97 (1H, d, *J* 10.0) ; δ_C 15.0, 41.6, 60.6, 101.0, 124.0, 128.1, 130.4, 132.0, 132.7, 133.1, 134.2, 137.0, 137.5, 150.0, 151.0, 156.2, 165.9, and 168.3; ν_{max} / cm^{-1} 1690 (C=O), 1523 and 1352 (NO_2); λ_{max} nm (log ε) 217 (4.41), 262 (4.43), 299 (4.45), 358 (3.99), 411(4.01), and 452 (3.14). MS *m/z* (rel. intensity) 365 (M^+, 17), 336 (17), 319 (19), 288 (24), 272 (100), 264 (39), 247 (22), 231 (30), 217 (26), and 203 (33). *Anal.* Calcd for $C_{20}H_{19}N_3O_4$: C, 65.74; H, 5.24; N, 11.50. Found: C, 65.79; H, 5.22; N, 11.57.

Reaction of 6a with triethyl phosphite

Typical procedure — Under an argon atmosphere, a mixture of **6a** (0.191 g, 0.535 mmol) and triethyl phosphite (3.0 mL) in benzene (5 mL) was refluxed for 3 h. The solution was evaporated and the residue was chromatographed on silica gel with chloroform to give ethyl 6-chloro-6a,7-diazanaphth[3,2,1-*cd*]azulene-5-carboxylate (**7a**) (0.004 g, 2%) and recovered **6a** (0.111 g, 58%).

7a: Brown needles (from hexane–dichloromethane) mp 128—132 ℃; δ_H 1.36 (3H, t, *J* 7.3), 4.31 (2H, q, *J* 7.3), 5.31 (1H, dd, *J* 12.2 and 8.5), 5.89 (1H, dd, *J* 12.2 and 8.5), 6.08 (1H, d, *J* 12.2), 6.55 (1H, dd, *J* 9.2 and 6.1), 6.88 (1H, d, *J* 9.2), 6.89 (1H, d, *J* 7.9), 7.00 (1H, dd, *J* 7.9 and 6.1), and 7.29 (1H, d, *J* 12.2); ν_{max} / cm^{-1} 1697 (C=O).

Reaction of 6b with hexamethylphosphorous triamide (HMPT)

Typical procedure — Under an argon atmosphere, a mixture of **6b** (0.359 g, 0.981 mmol) and HMPT (0.631 g, 3.76 mmol) in xylene (20 mL) was heated in a sealed tube at 165 ℃ for 1.5 h. The mixture was evaporated and the residue was chromatographed on silica gel with hexane—ethyl acetate (5 : 1) then ethyl acetate to give recovered **6b** (0.138 g, 38%), ethyl 2-dimethylamino-7*H*-1,7-diazaindeno[1,2-*e*]azulene-3-carboxylate (**8b**) (0.078 g, 24%) and ethyl 6-dimethylamino-6a,7-diazanaphth[3,2,1-*cd*]azulene-5-carboxylate (**7b**) (0.031 g, 10%).

7b: Dark brown prisms (from acetonitrile) mp 108.5—109.0 ℃; δ_H 1.38 (3H, t, *J* 7.1), 2.60 (6H, s), 4.34 (2H, q, *J* 7.1), 5.12 (1H, dd, *J* 11.0 and 8.1), 5.74 (1H, dd, *J* 12.1 and 8.1), 5.84 (1H, d, *J* 12.1),

6.31 (1H, dd, *J* 8.5 and 6.4), 6.67 (1H, d, *J* 8.5), 6.69 (1H, d, *J* 8.7), 6.84 (1H, dd, *J* 8.7 and 6.4), and 6.93 (1H, d, *J* 11.0); δ_C 12.5, 38.2, 58.7, 101.8, 118.1, 118.4, 120.9, 121.4, 122.1, 122.3, 130.3, 136.6, 136.7, 136.8, 138.9, 143.4, 152.1, and 162.4; ν_{max} / cm^{-1} 1697 (C=O); λ_{max} nm (log ε) 241 (4.46), 295 (4.41), 388 (3.66), 410 (3.64, sh), 512 (3.87), 637 (2.69), 709 (2.90), 796 (3.00), and 911 (2.39). MS *m/z* (rel. intensity) 333 (M^+, 100), 304 (16), 288 (20), 276 (27), and 272 (23). *Anal.* Calcd for $C_{20}H_{19}N_3O_2 \cdot 1/4H_2O$: C, 69.25; H, 5.96; N, 12.11. Found: C, 69.45; H, 5.82; N, 12.06.

8b: Red prisms (from acetonitrile) mp 186.0—186.5 ℃; δ_H 1.47 (3H, t, *J* 7.1), 3.45 (6H, s), 4.46 (2H, q, *J* 7.1), 7.41—7.56 (5H, m), 8.74 (1H, d, *J* 9.6), 8.99 (1H, br s), and 9.39 (1H, d, *J* 7.9); δ_C 15.2, 42.4, 60.0, 93.5, 110.5, 117.1, 120.3, 121.7, 123.2, 126.3, 127.5, 128.2, 128.3, 140.2, 143.5, 148.6, 156.4, 166.5, and 170.7; ν_{max} / cm^{-1} 3224 (NH) and 1645 (C=O); λ_{max} nm (log ε) 248 (4.24), 276 (4.28), 308 (4.52), 316 (4.50), 347 (4.52), 361 (4.66), 410 (3.99), 429 (4.20), and 487 (3.86). MS *m/z* (rel. intensity) 333 (M^+, 94), 332 (100), 286 (32), 272 (48), 271 (50), 232 (78), and 231 (70). *Anal.* Calcd for $C_{20}H_{19}N_3O_2$: C, 72.05; H, 5.74; N, 12.60. Found: C, 72.23; H, 5.69; N, 12.71.

Conversion of 8b to ethyl 2-dimethylamino-1*H*-1,7-diazaindeno[1,2-*e*]azulene-3-carboxylate (9b)

A mixture of **8b** (0.038 g, 0.112 mmol) in ethanol (15 mL) and potassium hydroxide (0.344 g, 6.14 mmol) in water (10 mL) was refluxed for 1 h, then poured into water. The mixture was neutralized with 5%. hydrochloric acid, and extracted with water. The extract was dried over sodium sulfate and evaporated. Preparative chromatography of the residue with chloroform—ethanol (4 : 1) gave ethyl 2-dimethylamino-1*H*-1,7-diazaindeno[1,2-*e*]azulene-3-carboxylate (**9b**) (0.035 g, 94%) as a red oil, which was converted to **8b** in NMR tube ($CDCl_3$) with 20 days of a half-life period.

9b: Red oil, δ_H1.47 (3H, t, *J* 7.1), 3.43 (6H, s), 4.47 (2H, q, *J* 7.1), 7.36—7.47 (4H, m), 7.51 (1H, dd, *J* 7.1 and 6.0), 8.73 (1H, d, *J* 9.5), 9.17 (1H, br s), and 9.38 (1H, d, *J* 7.9); ν_{max} / cm^{-1} (neat) 3230 (NH) and 1650 (C=O). *Anal.* Calcd for $C_{20}H_{19}N_3O_2$: C, 72.05; H, 5.74; N, 12.60. Found: C, 72.38; H, 5.82; N, 12.45.

Crystal data for 7b: Dark red prism, $C_{20}H_{19}N_3O_2$, *M*=333.39, orthorhombic, space group Pcca, *a*=20.996(9), *b*=7.041(9), *c*=23.22(1) Å^3, V=3428(5) Å^3, Z=8, D_{calcd}=1.292 g/cm^3, crystal dimensions 0.24 x 0.60 x 0.88 mm. Data were measured on a Rigaku AFC 5S radiation diffractometer with graphite-monochromated Mo-Kα radiation. A total 4226 reflections were collected using the ω scan technique to a maximum 2θ value of 55.0°. All calculations were performed using TEXAN structure analysis software.[13] The structure was solved by direct methods and refined by a full-matrix least-

squares method using SIR[14] using 226 variables and 1347 observed reflections ($I > 2\sigma(I)$). The non-hydrogen atoms were refined anisotropically. The weighting scheme $\omega = 4Fo^2/\sigma^2(Fo^2)$ gave satisfactory agreement analyses. The final R and Rw values were 0.076 and 0.078. The maximum peak and the minimum peak in final difference map were 0.33 e$^-$/Å^3 and –0.35 e$^-$/Å^3.

Crystal data for 8b: Violet needle, $C_{20}H_{19}N_3O_2$, M=333.39, monoclinic, space group $P2_1/c$, a=8.269(5), b=12.239(6), c=16.956(4) Å^3, β=94.46(4)° , V=5656(23) Å^3, Z=4, D_{calcd}=1.294 g/cm^3, crystal dimensions 0.40 x 0.64 x 0.98 mm. Data were measured on a Rigaku AFC 5S radiation diffractometer with graphite-monochromated Mo-Kα radiation. A total 4383 reflections were collected using the ω scan technique to a maximum 2θ value of 54.9°. All calculations were performed using TEXAN structure analysis software.[13] The structure was solved by direct methods and refined by a full-matrix least-squares method using MITHRIL[15] using 303 variables and 2329 observed reflections ($I > 2\sigma(I)$). The non-hydrogen atoms were refined anisotropically. The weighting scheme $\omega = 4Fo^2/\sigma^2(Fo^2)$ gave satisfactory agreement analyses. The final R and Rw values were 0.049 and 0.054. The maximum peak and the minimum peak in final difference map were 0.17 e$^-$/Å^3 and –0.18 e$^-$/Å^3.

Optical titration of 8b with DNA

A 3 mL solution (1.68 x 10^{-5} M) of **8b** in 1 mM Na_2-phosphate / 1 mM Na_2-EDTA (pH 7.0) buffer including 50 mM NaCl is titrated with each 3 μL portion of a calf thymus or 12mer DNA solution (1.04 x 10^{-5}, 8.91 x 10^{-6} M, respectively) in a cuvette. Fluorescence emission was measured at 549.0 nm (10.0 nm slit width) under excitation at 350.0 nm (10.0 nm slit width).

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