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Reaction of Guanidines with α-Diketones. V.[1] Mechanism of the Fluorescence Reaction of Monosubstituted Guanidines with 9,10-Phenanthraquinone

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Mechanism of the fluorescence reaction of monosubstituted guanidines with 9,10-phenanthraquinone was investigated. Benzylguanidine reacted with 9,10-phenanthraquinone in an alkaline solution to give 2-benzylideneamino-1*H*-phenanthro[9,10-*d*]-imidazole (II) as an intermediate of the fluorescence reaction. II easily underwent hydrolysis with HCl to give a fluorescent product, 2-amino-1*H*-phenanthro[9,10-*d*]imidazole hydrochloride monohydrate (I), and benzaldehyde. II also underwent partial hydrolysis in an alkaline solution to give the free base of I, and the free base reacted with 1-naphthol and 9,10-phenanthraquinone to give an indophenol pigment, N-(1*H*-phenanthro[9,10-*d*]-imidazolyl)-1,4-naphthoquinone monoimine (IV).

Amidines also reacted with 9,10-phenanthraquinone in the presence of alkali to afford 2′-(substituted)spiro[9*H*-fluorene-9,4′-[4*H*]imidazol]-5′(3′*H*)/5′(1′*H*)-ones (VI and VII) which underwent an intramolecular rearrangement, as a non-fluorescent product.

Keywords——fluorescence reaction of monosubstituted guanidines; 2-benzylideneamino-1*H*-phenanthro[9,10-*d*]imidazole; Schiff base; reaction of amidines with 9,10-phenanthraquinone; 2′-(substituted)spiro[9*H*-fluorene-9,4′-[4′*H*]imidazol]-5′(3′*H*)/5′(1′*H*)-ones; reaction mechanism; IR and NMR spectra of phenanthroimidazole and spiro[9*H*-fluorene-9,4′-[4*H*]imidazol]-5′(3′*H*)/5′(1′*H*)-ones

In the previous paper,[1] we reported a fluorometric method for the determination of guanidine employing the fluorescence reaction of monosubstituted guanidines with 9,10-phenanthraquinone found by Yamada and Itano.[3,4] The structure of final fluorescent product of this reaction derived from acidic solution was already elucidated by Itano and Yamada[4] to be 2-amino-1*H*-phenanthro[9,10-*d*]imidazole hydrochloride monohydrate (I), but an intermediate of the fluorescence reaction derived from alkaline solution has not yet been isolated.

In the present work, we isolated an intermediate (II) of I from benzylguanidine in alkaline solution and its chemical structure and reaction mechanism were investigated. Reaction products of amidines with 9,10-phenanthraquinone, which exhibited no fluorescence, were prepared and the difference between their structures and that of II is also discussed.

1) Part IV: T. Sakaguchi, S. Tanabe, H. Yagi, T. Miyawaki (née Iijima), and A. Saito, *Yakugaku Zasshi*, **97**, 1053 (1977).
2) Location: *1–33, Yayoi-cho, Chiba, 280, Japan.*
3) S. Yamada and H.A. Itano, *Biochim. Biophys. Acta*, **130**, 538 (1966).
4) H.A. Itano and S. Yamada, *Anal. Biochem.*, **48**, 483 (1972).

Table I. Reaction Products

Compound	Formula	Mol. Wt.[a] M^+ (m/e)	mp (°C)	Appearance	Analysis (%) Calcd. (Found) C	H	N
II	$C_{22}H_{15}N_3 \cdot 1/3C_6H_6 \cdot 1/2H_2O$	321	140—142	Golden yellow plates	80.87 (80.69)	5.09 (5.33)	11.79 (11.73)
III[b]	$C_{13}H_{10}N_4O_4$	286	234—235	Reddish orange needles	54.55 (54.25)	3.52 (3.44)	19.58 (19.57)
V[b]	$C_7H_6N_4O_4$	210	166—167	Reddish orange needles	40.00 (40.44)	2.88 (2.95)	26.66 (26.51)
VI	$C_{20}H_{19}N_3O \cdot 1/2H_2O$	317	218—220	Colorless needles	73.59 (73.85)	6.18 (6.14)	12.88 (12.92)
VII	$C_{21}H_{14}N_2O$	310	277—279	Colorless needles	81.26 (80.97)	4.55 (4.61)	9.03 (9.01)

a) Molecular weight was measured from mass spectra.
b) 2,4-Dinitrophenylhydrazone derivatives.

Structure of Intermediate (II) obtained from 9,10-Phenanthraquinone and Benzylguanidine

The reaction of benzylguanidine with an equimolar amount of 9,10-phenanthraquinone in the presence of alkali gave II as golden yellow plate crystals. The high resolution mass spectrum of II corresponded to $C_{22}H_{15}N_3$ (M^+: m/e 321. Calcd. 321.1266. Found 321.1237). The elemental analysis of II suggested involvement of one-third of benzene and one-half of water, as shown in Table I.

The nuclear magnetic resonance (NMR) spectrum of II indicated eight protons due to the phenanthrene ring at 7.7 (4H, m), 8.14 (2H, d–d) and 8.8 ppm (2H, m) similar to that of I, five protons due to phenyl group in the benzylidene group at 7.7 (3H, m) and 8.5 ppm (2H, m), one singlet azomethine signal[5] at 9.56 ppm, and the phenanthroimidazole NH proton at 13.6 ppm (exchangeable with deuterium), as shown in Fig. 1. The singlet signal at 7.36 ppm was assigned as two protons corresponding to one-third benzene molecule (Fig. 1). Furthermore, the infrared (IR) spectrum of II exhibited the characteristic bands of NH and C=N cor-

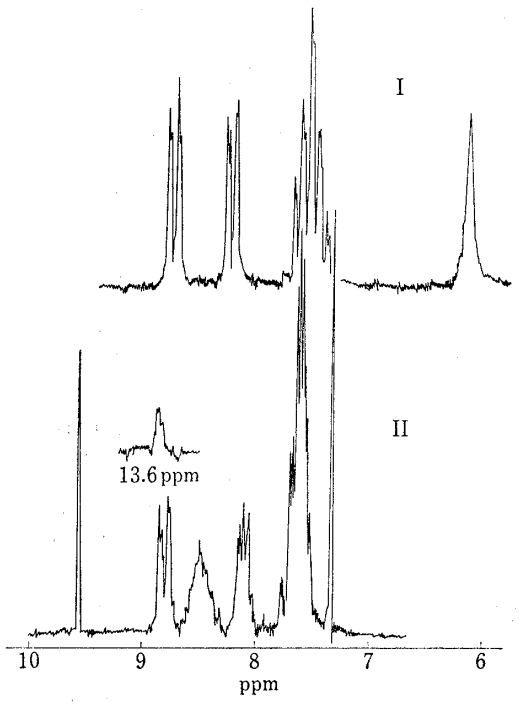


Fig. 1. NMR Spectra of II and 2-Amino-1*H*-phenanthro[9,10-*d*]imidazole·HCl·H_2O (I) in $(CD_3)_2SO$

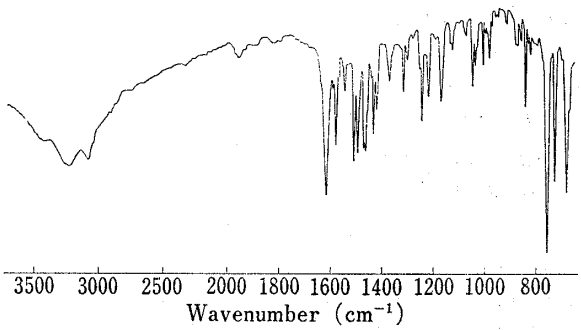
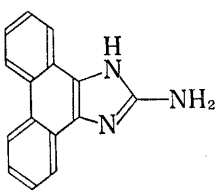


Fig. 2. IR Spectrum of II in KBr Disk

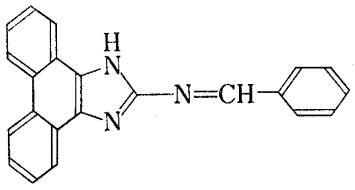
5) K. Tabei and E. Saito, *Bull. Chem. Soc. Jpn.*, **42**, 1140 (1969).

responding to phenanthroimidazole and azomethine[5] at 3225 and 1620 cm^{-1}, respectively, as shown in Fig. 2.

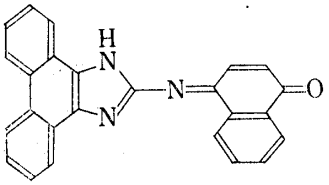
On the basis of these data, the structure of II was determined as 2-benzylideneamino-1*H*-phenanthro[9,10-*d*]imidazole which is a Schiff base.[6]



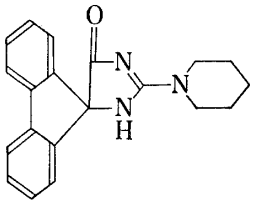
I



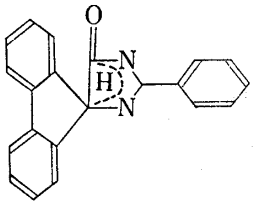
II



IV



VI



VII

Formation of Fluorescent Product (I) from the Intermediate (II), and Its Reaction Mechanism

When ethanol solution of II was acidified with HCl, yellow color of the solution faded immediately and a fluorescent product (I) and benzaldehyde were isolated. Benzaldehyde was identified as 2,4-dinitrophenylhydrazone derivative (III) (Table I). II also reacted with 9,10-phenanthraquinone and 1-naphthol in the presence of alkali to give N-(1*H*-phenanthro[9,10-*d*]imidazolyl)-1,4-naphthoquinone monoimine[7] (IV) as an indophenol pigment. The reaction velocity derived from II to IV with 1-naphthol, however, was slower than that from I, as shown in Fig. 3.

From these results, it may be postulated that monosubstituted guanidines first react with 9,10-phenanthraquinone in the alkaline medium to give a Schiff base, 2-alkylideneamino-1*H*-phenanthro[9,10-*d*]-imidazoles, as the intermediate of the fluorescent product. The intermediate (II) isolated from benzylguanidine is probably comparatively stable alkali because of its conjugated structure, but intermediates

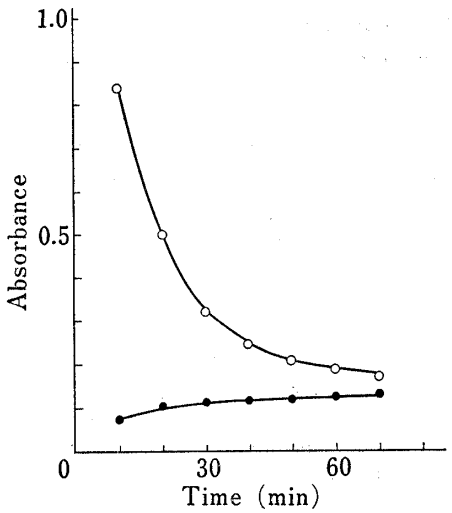


Fig. 3. Effect of Standing Time on Color Developments of I and II

To 1 ml of samples, 2 ml of EtOH mixture of 0.03% 9,10-phenanthraquinone and 1.5% 1-naphthol and 2 ml of 0.5 N KOH were added.
Absorbance was measured at 655 nm
○—○, 0.28 mM ethanolic solution of I.
●—●, 0.28 mM ethanolic solution of II.

6) After this paper submitted, 2-benzylamino-1*H*-phenanthro[9,10-*d*]imidazole, which was the reduction product of II, was isolated by Itano, *et al.* from a reaction mixture of 9,10-phenanthraquinone and benzylguanidine reduced with sodium borohydride. They deduced the intermediate of the fluorescent product in the reaction of 9,10-phenanthraquinone with benzylguanidine from the reduction product to be II and published the same mechanism of this fluorescence reaction as our result. H.A. Itano, K. Hirota, I. Kawasaki, and S. Yamada, *Anal. Biochem.*, **76**, 134 (1976).

7) T. Sakaguchi, S. Tanabe, and T. Ohya, *Yakugaku Zasshi*, submitteel.

from monosubstituted guanidines such as methylguanidine, arginine, *etc.*, are unstable and they were immediately hydrolyzed in the alkaline solution to give the free base of I[4] and aldehydes. Actually, formaldehyde was isolated from the reaction mixture of 9,10-phenanthraquinone and methylguanidine (Table I).

These results are illustrated in Chart 1.

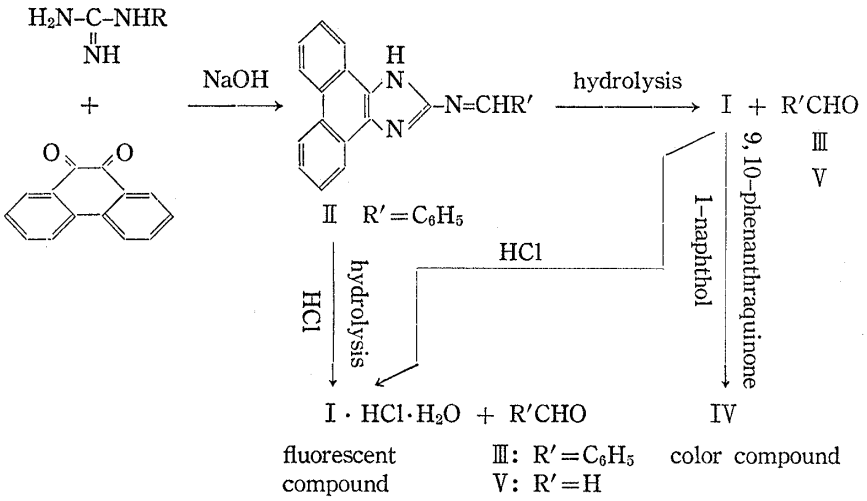


Chart 1

Reaction Products of Amidines with 9,10-Phenanthraquinone

1,1-Pentamethyleneguanidine (piperidinoamidine) and benzamidine having a free amidino group exhibited no fluorescence. Therefore, the reaction products from these amidines with 9,10-phenanthraquinone were prepared in alkaline medium and the structural difference between their products and II was examined.

When 1,1-pentamethyleneguanidine and benzamidine were reacted with an equivalent amount of 9,10-phenanthraquinone in the presence of alkali, colorless needle crystals of VI and VII were respectively obtained. The elemental analyses and mass data of both products suggested that the products were formed through the condensation of one equivalent each of amidines and 9,10-phenanthraquinone with elimination of water, as shown in Table I.

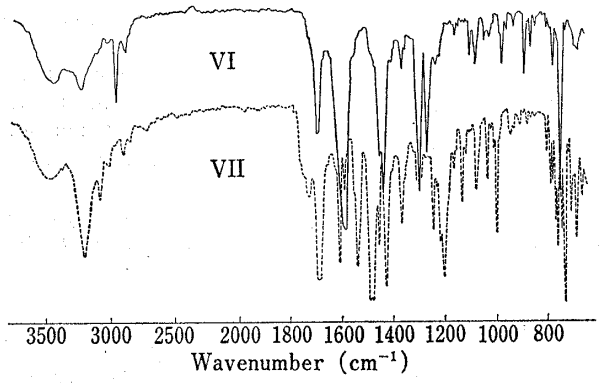


Fig. 4. IR Spectra of VI and VII in KBr Disk

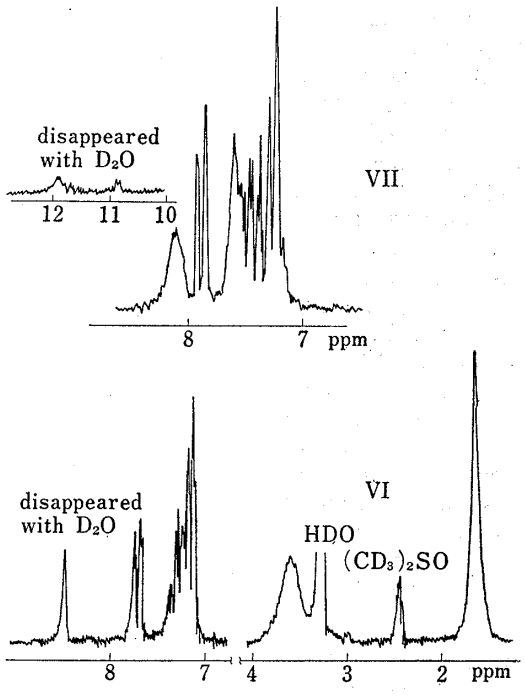


Fig. 5. NMR Spectra of VI and VII in $(CD_3)_2SO$

In the IR spectra of both products, the frequencies of the carbonyl and C=N absorptions of VI (C=O, 1695 cm^{-1}, C=N, 1595 cm^{-1}) were similar to those of 2′-(dimethylamino)spiro-[9*H*-fluorene-9,4′-[4*H*]imidazol]-5′(3′*H*)-one isolated from 1,1-dimethylguanidine and 9,10-phenanthraquinone as a non-fluorescent product by Itano,[8] while the frequencies of the carbonyl and C=N absorptions of VII were similar to those of 4,4/5,5-disubstituted-2-phenyl-imidazoline-5/4-one (C=O, 1710, 1650 cm^{-1}, C=N, 1610 cm^{-1}, as shown in Fig. 4).[9] In the NMR spectra of VI and VII, eight protons corresponding to the fluorene ring at 7.2—7.9 ppm were also similar to those of the non-fluorescent product, as shown in Fig. 5. The NH proton of imidazole ring of VII, however, exhibited a splitting at 10.9 and 11.9 ppm, while VI exhibited a singlet at 8.5 ppm.

On the basis of these results, the structures of VI and VII were respectively elucidated as 2′-(piperidino)spiro[9*H*-fluorene-9,4′-[4*H*]imidazol]-5′(3′*H*)-one and 2′-(phenyl)spiro[9*H*-fluorene-9,4′-[4*H*]imidazol]-5′(3′*H*)/5′(1′*H*)-one

Thus, the reaction of amidines with 9,10-phenanthraquinone gave non-fluorescent products in which the phenanthrene ring underwent an intramolecular rearrangement, as did 1,1-disubstituted guanidines.[8]

Experimental

Apparatus——Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Visible absorption spectra and intensity were measured with Hitachi autorecording spectrophotometer EPS-3T and Hitachi 181 spectrometer. IR spectra were taken with Hitachi IR-215. NMR spectra were taken at 100 MHz with JMN 4H-100 spectrometer. The chemical shifts are given in ppm from tetramethylsilane used as an internal standard. Abbreviations: s=singlet, m=multiplet, d-d=double doublet, b=broad. Mass spectra were taken with Hitachi RMU-6E spectrometer. High-resolution mass spectra were measured with JMS-OISG spectrometer connected with JAM-IC-O system. TLC on silica gel (500 μm thickness, Wako-gel B-5) was performed with solvent system 50: 1 $CHCl_3$–pyridine.

Materials——All chemicals used were of analytical reagent grade. Benzylguanidine sulfate and 1,1-pentamethyleneguanidine sulfate, were prepared according to the methods described in the literature.[10] 9,10-Phenanthraquinone was recrystallized from dioxan.

Isolation of Intermediate (II) from Benzylguanidine and 9,10-Phenanthraquinone——To a suspension of 0.416 g of 9,10-phenanthraquinone in 150 ml of EtOH were added, first 0.396 g of benzylguanidine sulfate dissolved in 11 ml of H_2O, and then 20 ml of 2 M NaOH. The reaction mixture was stirred for 3 hr at room temperature and H_2O was added until a yellow precipitate appeared. The precipitate was collected by filtration and recrystallized from benzene to give golden yellow plates (II), mp 140—142°; yield 0.124 g. High-resolution mass spectrum: Calcd. for $C_{22}H_{15}N_3$ 321.1266. Found: 321.1237. IR ν_{max}^{KBr} cm^{-1}: 3225 (imidazole NH), 1620 (azomethine C=N). NMR in $(CD_3)_2SO$ ppm: 7.36 (2H, s, 1/3 benzene), 7.7 (4H, m, phenanthrene ring), 7.7 (3H, m, phenyl ring), 8.14 (2H, d-d, phenanthrene ring), 8.5 (2H, m, phenyl ring), 8.8 (2H, m, phenanthrene ring), 9.56 (1H, s, azomethine), 13.6 (1H, b, imidazole NH).

Isolation of Fluorescent Product (I) from II——To a solution of II (0.1 g) in EtOH 1 N HCl was added till the solution changed from yellow to colorless. The reaction mixture was distilled until a white precipitate appeared and the precipitate was collected by filtration and recrystallized from EtOH to give 2-amino-1*H*-phenanthro[9,10-*d*]imidazole hydrochloride monohydrate (**I**) as colorless needles, mp above 300°; yield 75 mg. *Anal.* Calcd. for $C_{15}H_{11}N_3 \cdot HCl \cdot H_2O$: C, 62.61; H, 4.90; N, 14.60. Found: C, 62.56; H, 4.76; N, 14.65. NMR in $(CD_3)_2SO$ ppm: 6.15 (2H, b, NH_2), 7.5 (4H, m, phenanthrene ring), 8.2 (1H, d-d, phenanthrene ring), 8.7 (2H, d-d, phenanthrene ring).

Isolation of Benzaldehyde (III) from II——The filtrate and distillate obtained from the above experimental were combined. When 10 ml of 2.5% 2,4-dinitrophenylhydrazine (DNP) solution was added to this combined solution, reddish orange precipitate appeared. The precipitate was collected by filtration and recrystallized from EtOH to give benzaldehyde-DNP derivative (III) as reddish orange needles, mp 234—235°; yield 20 mg.

Isolation of Formaldehyde (V) from Methylguanidine and 9,10-Phenanthraquinone——To a suspension of 0.52 g of 9,10-phenanthraquinone in 150 ml of EtOH were added, first 0.273 g of methylguanidine hydro-

8) H.A. Itano, *J. Heterocycl. Chem.*, 11, 1071 (1974).
9) T. Sakaguchi and S. Tanabe, *Yakugaku Zasshi*, 97, 223 (1977).
10) T. Nishimura, S. Tanabe, H. Toku, T. Kono, Y. Sakabe, and T. Sakaguchi, *Chem. Pharm. Bull.* (Tokyo), 17, 639 (1969).

chloride dissolved in 11 ml of H_2O and then 20 ml of 2 M NaOH. The reaction mixture was stirred for 1 hr at room temperature, and the resulting solution was neutralized with 1 N HCl and distilled. To 100 ml of the distillate was added 10 ml of 2.5% DNP solution. The mixture was evaporated *in vacuo* until a reddish orange precipitate appeared. The precipitate was collected by filtration and recrystallized from EtOH to give formaldehyde-DNP derivative (V) as reddish orange needles, mp 166°; yield 147 mg.

Identification of IV from II——To a solution of 71 mg of II 42 mg of 9,10-phenanthraquinone, and 29 mg of 1-naphthol in 50 ml of EtOH, was added 10 ml of 2 M NaOH and the mixture was stirred for 2 hr at room temperature. The reaction mixture was neutralized with 1 N HCl until a blue precipitate appeared. The precipitate was collected by filtration and dried *in vacuo*. The precipitate was identified with authentic N-(1*H*-phenanthro[9,10-*d*]imidazolyl)-1,4-naphthoquinone monoimine[7] (IV) on TLC. This precipitate was located at $Rf=0.45$, identical with that of the authentic sample.

2′-(Piperidino)spiro[9*H*-fluorene-9,4′-[4*H*]imidazol]-5′(3′*H*)-one (VI)——To a suspension of 0.52 g of 9,10-phenanthraquinone in 150 ml of EtOH were added, first 0.44 g of 1,1-pentamethyleneguanidine sulfate dissolved in 11 ml of H_2O, and then 20 ml of 2 M NaOH. The reaction mixture was stirred for 3 hr at room temperature, and 1 N HCl was added until a white precipitate appeared. The precipitate was collected by filtration and recrystallized from H_2O–EtOH to give VI as colorless needles, mp 218—220°; yield 0.364 g. IR ν_{max}^{KBr} cm^{-1}: 3200 (NH), 1695 (C=O), 1595 (C=N). NMR in $(CD_3)_2SO$ ppm: 1.6 (6H, s, piperidine ring), 3.6 (4H, m, piperidine ring), 7.2 (6H, m, fluorene ring), 7.7 (2H, d-d, fluorene ring), 8.5 (1H, s, imidazole NH).

2′-(Phenyl)spiro[9*H*-fluorene-9,4′-[4*H*]imidazol]-5′(3′*H*)/5′(1′*H*)-one (VII)——To a suspension of 0.416 g of 9,10-phenanthraquinone in 150 ml of EtOH were added, first 0.312 g of benzamidine hydrochloride and then 20 ml of 2 M NaOH. The reaction mixture was stirred for 3 hr at room temperature and 1 N HCl was added until a white precipitate appeared. The precipitate was collected by filtration and recrystallized from EtOH to VII as colorless needles, mp 277—279°; yield 0.3 g. IR ν_{max}^{KBr} cm^{-1}: 3200 (NH), 1710, 1650 (C=O), 1605 (C=N). NMR in $(CD_3)_2SO$ ppm: 7.3 (6H, m, fluorene ring), 7.58 (3H, m, phenyl ring), 7.9 (2H, d-d, fluorene ring), 8.1 (2H, m, phenyl ring), 10.9 and 11.9 (1H, b, imidazole NH).

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