Synthesis and Investigation of the DNA-Binding and DNA-Photodamaging Properties of Indolo[2,3-*b*]quinolizinium Bromide

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Dedicated to Prof. Manfred Regitz on the occasion of his 65th birthday

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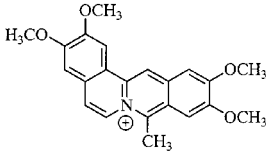
The readily available cationic dye **3-Br** binds to DNA as was shown by UV, fluorescence and CD spectroscopy. The electronic spectra of the quinolizinium salt **3** exhibit significant bathochromic shifts and a decrease of the absorbance and emission intensity on addition of calf thymus DNA. Moreover, fluorometric titration of $[poly(dCdG)]_2$ with salt **3** resulted in a significant decrease of the emission intensity, whereas addition of $[poly(dAdT)]_2$ led only to a marginal perturbation of the emission spectrum. Analysis of the binding constants with the different polynucleotides revealed that quinolizinium **3-Br** binds preferentially to GC base pairs. Irradiation of DNA in the presence of quinolizinium **3-Br** resulted in efficient single-strand cleavage of the nucleic acid.

Introduction

Since it was first shown that cationic aromatic dyes exhibit a high propensity to bind to DNA[1−5] and lead to photoinduced DNA damage[6,7] there has been a growing interest in the design and synthesis of novel representatives of such compounds. In most cases, the interaction of the dye with DNA leads to changes of the absorption and emission properties of the dye[1−5] and may be monitored by UV/Vis or emission spectroscopy.[8] Furthermore, the intentional photoinduced damage of DNA in the presence of cationic dyes may be applied in phototherapy.[9−11]

It has been reported that cationic aromatic heterocycles such as coralyne (**1**)[12,13] and related quinolizinium salts[14] bind efficiently to DNA. Moreover, we have observed that the readily available benzo[*b*]-quinolizinium salts (acridizinium salts) **2a**,**b** exhibit pronounced DNA-binding properties.[15] To investigate further the photobiological features of quinolizinium derivatives we have synthesized the novel indolo[2,3-*b*]-quinolizinium bromide **3-Br**. The interactions of this aromatic dye with DNA seemed to be of special interest since it closely resembles the structure of the known anti-tumor drug Céliptium® (**4**) and that of the antibacterial and antimalarial alkaloid cryptolepine (**5**).[16] Although a derivative of quinolizinium salt **3-Br**, namely 5,6-dimethyl-5*H*-indolo[2,3-*b*]quinolizinium iodide, has been known for a long time,[17] no further photophysical or photobiological studies have been performed with this derivative. Herein, we present the synthesis of the quinolizinium dye **3-Br** and the preliminary results of its interactions with DNA.[18]



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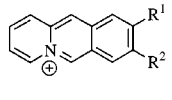
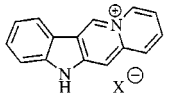
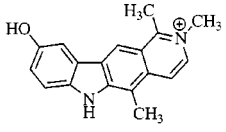


2a: $R^1 = NH_2$; $R^2 = H$
2b: $R^1 = H$; $R^2 = NH_2$



3-Br (X = Br)
3-BF$_4$ (X = BF_4)



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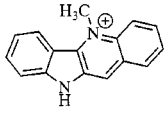

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Results and Discussion

Synthesis

Indoloquinolizinium **3-Br** was synthesized from the pyridinium derivative **8** (Scheme 1), which was obtained by quaternization of 2-(1,3-dioxolan-2-yl)pyridine[19] (**7**) with the bromomethylindole **6**.[20] Thus, cyclization of the tetrafluoroborate salt **8-BF$_4$** with polyphosphoric acid afforded the quinolizinium **3-BF$_4$** in 39% yield after crystallization from methanol.[21] Under the conditions employed complete deprotection of the pyrrole-nitrogen atom took place. Counterion exchange of the salts **3-BF$_4$** and **8-Br** was achieved according to the procedure by Hünig et al.[22] The structural assignment of the pyridinium salt **8** and the

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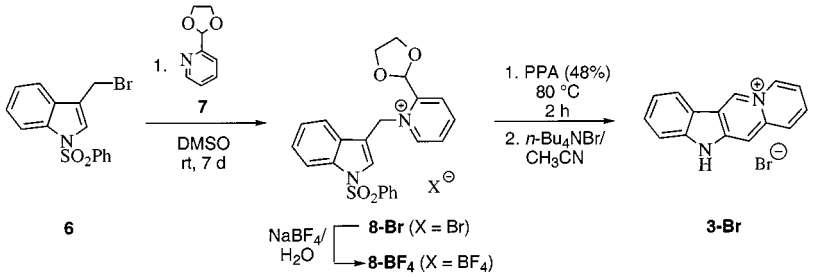


Scheme 1

quinolizinium salt **3-Br** is based on their 1D- and 2D NMR spectral and ESI-mass spectral data and combustion analyses.

Absorption and Emission Properties

The quinolizinium salt **3-Br** is a pale yellow solid and exhibits an absorption maximum in methanol at $\lambda = 352$ nm (Table 1), along with a broad absorption band and a zero onset at $\lambda = 420$ nm. A broad fluorescence band at $\lambda = 452$ nm was also observed. The fluorescence quantum yield depends on the solvent and ranges from 0.05 in water or DMF to 0.15 in methanol. With an increasing donor number[23] (DN) of the solvent a slight red-shift ($\Delta\lambda = 5$ nm) of the absorption and emission maxima was detected (Table 1). Thus, in acetonitrile (DN = 14.1) a fluorescence maximum at $\lambda = 450$ nm was observed whereas in DMSO (DN = 29.8) the quinolizinium salt **3-Br** emits at $\lambda = 455$ nm. Such a solvatochromic behavior may be explained by an increased dipole moment in the excited state which is better stabilized by a solvent with a higher electron-donating ability.[23] This stabilization of the excited state results in a red shift of the absorption and emission spectra.

Table 1. Absorption and emission data of quinolizinium bromide **3-Br**

Solvent	λ_{abs} [nm][a]	ε [$M^{-1}cm^{-1}$]	λ_{em} [nm][b]	φ_{em}[c]
buffered H_2O[d]	350	11776	452	0.05
MeOH	352	11520	452	0.15
CH_3CN	350	11048	450	0.10
DMF	355	9728	454	0.05
DMSO	356	8771	455	0.09

[a] $c = 10^{-4}$ mol/L, S_0-S_1 transition. – [b] $\lambda_{ex} = 370$ nm; $c = 10^{-5}$ M, V = 2 mL. – [c] Relative to quinine sulfate in 1 N H_2SO_4; error: ± 0.02. – [d] 10 mM phosphate buffer (pH = 7).

Interaction of the Quinolizinium salt 3-Br with DNA

The addition of calf thymus (ct) DNA to an aqueous solution of **3-Br** was monitored by absorption and emission spectroscopy (Figure 1 and 2). With an increasing DNA concentration a decrease of the absorbance was observed, along with a red shift of 4 nm and a broadening of the linewidth. Moreover, isosbestic points were detected which indicate that one type of quinolizinium-DNA complex is formed almost exclusively. Furthermore, the fluorescence intensity of **3-Br** is significantly quenched by addition of DNA, and the emission maximum exhibits a bathochromic shift of 4 nm. Such perturbations of the electronic spectra on DNA addition usually indicate the association of a cationic dye with DNA;[1–5,24] thus, the results of the spectrophotometric and fluorometric titrations of quinolizinium salt **3-Br** provide evidence that this dye also binds to DNA.

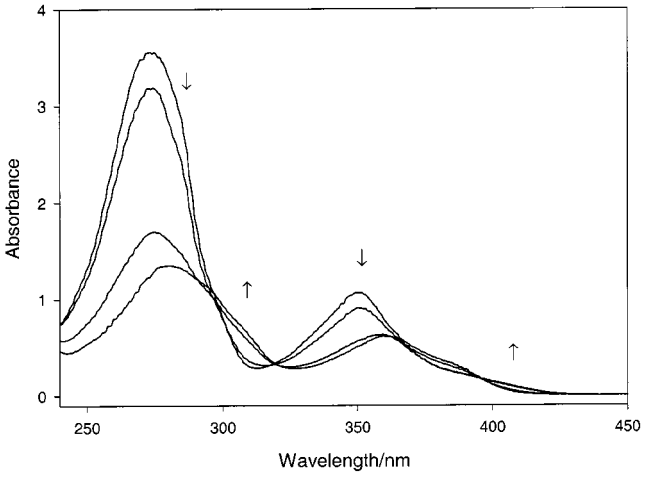


Figure 1. Spectrophotometric titration of quinolizinium **3-Br** with ct DNA in aqueous solution [c(**3-Br**) = 10^{-4} M, V = 2 mL; dye/DNA ratio at the respective titration steps: without DNA, 1.6, 0.3, 0.2]

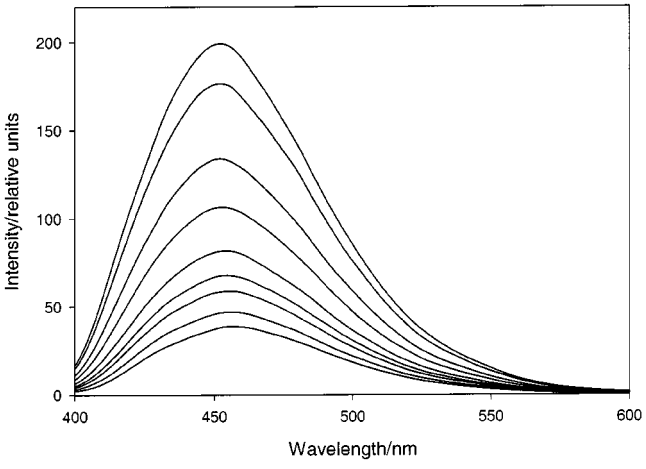


Figure 2. Fluorometric titration of quinolizinium **3-Br** with ct DNA in aqueous solution [$\lambda_{ex} = 319$ nm; c(**3-Br**) = 10^{-5} M, V = 2 mL; titration interval: 1.0 molar equivalents of DNA]

To study the influence of the different DNA base pairs on the emission quenching of **3-Br**, fluorometric titrations were performed with the synthetic polynucleotides [poly(dCdG)]$_2$ and [poly(dAdT)]$_2$ (Figure 3). Whereas the addition of [poly(dCdG)]$_2$ to the dye **3-Br** led to a significant quenching (95%) of the emission intensity without a shift of the fluorescence maximum, the titration of **3-Br** with [poly(dAdT)]$_2$ resulted in a slight increase (ca. 15%) of the intensity along with a small bathochromic shift of 5 nm. These results show that the emission quenching of quinolizinium **3-Br** upon DNA addition is predominantly caused by the GC-rich regions of the nucleic acid. Since guanine is the nucleic base with the lowest one-electron redox potential ($E^0 = 1.29$ vs. NHE),[25] it was assumed that the quenching is due to an electron-transfer reaction of the excited state of **3-Br** with guanine. Interestingly, however, the fluorescence intensity of **3-Br** was not quenched by guanosine monophosphate (GMP), even with a 100-fold excess of GMP. Thus, the binding interaction of **3-Br** with DNA may cause a tight contact between the dye and the guanine base in the DNA matrix, leading to an efficient quenching process. In solution, such a close proximity between GMP and the dye does not exist and no quenching takes place.

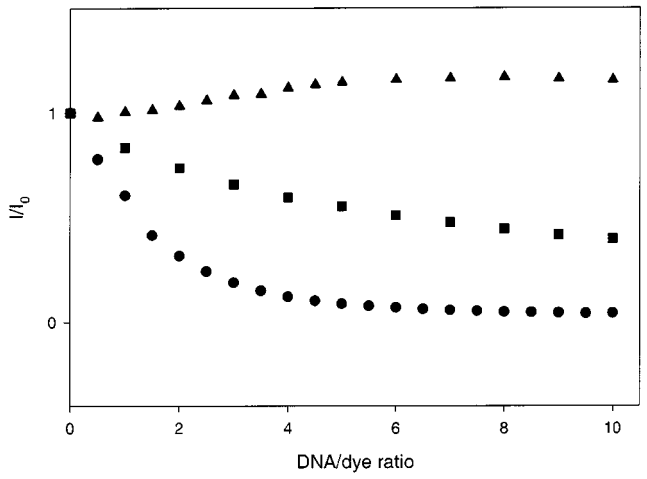


Figure 3. Fluorometric titration of quinolizinium **3-Br** with calf thymus DNA (λ_{ex} = 318 nm), [poly(dAdT)]$_2$ (λ_{ex} = 319 nm), and [poly(dCdG)]$_2$ (λ_{ex} = 316 nm) in 10 mM phosphate buffer [c(**3-Br**) = 10^{-5} M, V = 2 mL]

Furthermore, from the data of the fluorometric titrations, the association constants of **3-Br** with the different nucleic acids were determined.[26] The binding constant of **3-Br** with ct DNA was found to be 2×10^5 M^{-1} and 1.0×10^6 M^{-1} with [poly(dCdG)]$_2$. The binding constant of **3-Br** with [poly(dAdT)]$_2$ could not be determined because of the small change of the emission intensity during titration. The obtained association constants are in good agreement with those of cryptolepine (**5**)[16] and elipticines, which possess similar structures.[27] Moreover, these data clearly show that the quinolizinium dye **3-Br** binds preferentially to the GC moiety of the DNA.

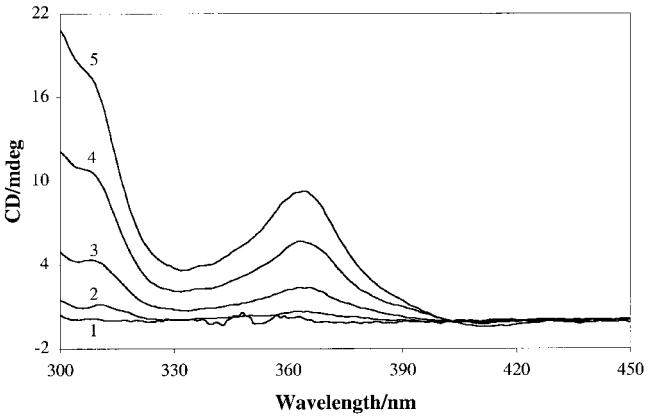


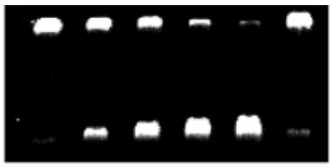
Figure 4. CD spectra of quinolizinium **3-Br** with ct DNA in aqueous solution ($c_{DNA} = 5.7 \times 10^{-3}$ M); 1: without DNA; 2: [dye]/[DNA] = 0.05; 3: [dye]/[DNA] = 0.1; 4: [dye]/[DNA] = 0.15; 5: [dye]/[DNA] = 0.2

The DNA-dye interaction was also monitored by CD spectroscopy. Because of its achiral structure, **3-Br** does not exhibit circular dichroism in solution. On addition of ct DNA, however, a distinct positive induced CD (ICD) signal appeared in the absorption region of the chromophore (Figure 4). Such an ICD signal results from nondegenerate coupling of the achiral dye chromophore with the DNA-base transitions when the molecule is bound to DNA.[28] Thus, the present CD-spectral investigation provides further proof for pronounced binding interactions between the quinolizinium salt **3-Br** and DNA.

Furthermore, we observed with preliminary flow-LD-spectroscopic experiments[29] that **3-Br** intercalates preferentially into DNA as shown by a negative LD and LD$_r$ signal for the long wavelength absorption of the dye in the presence of DNA (dye/DNA ratios: 0.01, 0.02, 0.04, 0.08, 0.2, and 0.4). The intercalation of **3-Br** into DNA was also indicated by a significant increase of the LD signal of the DNA bases on addition of the dye: the stiffening of the DNA upon intercalation results in a better orientation in the flow field which gives rise to the observed enhanced LD absorption.[29]

DNA Photocleavage

Since a binding interaction between the quinolizinium salt **3-Br** and DNA has been demonstrated, it was of further interest to determine whether the complex formation may be used for photoinduced DNA damage. Irradiation of supercoiled DNA (pBR 322) in the presence of the **3-Br** at λ = 350 nm under aerobic conditions resulted in single strand breaks (Figure 5). After 1 h, 80% DNA damage was detected, whereas after further irradiation (2 h), linear DNA fragments were also observed. Under anaerobic conditions, essentially the same results were obtained. These results demonstrate that **3-Br** damages DNA with high efficiency on irradiation.



Lane	1[a]	2	3	4	5	6[b]
Irradiation time [min]	0	15	30	45	60	60
Strand breaks[c] [%]	10	35	50	80	90	10

[a] Blank sample – [b] DNA sample irradiated in the absence of dye – [c] error: ± 10%

Figure 5. Photocleavage of plasmid DNA pBR 322 with quinolizinium salt **3-Br** (gel-electrophoresis pattern: top spots are supercoiled DNA; bottom spots are open-cirular DNA)

Conclusion

In summary, it has been demonstrated in the present work that the novel indolo-annelated quinolizinium salt **3-Br** exhibits promising photobiological features including base-pair-selective binding. Since the parent compound **3-Br** is readily available and offers attractive opportunities for further derivatization, a new class of DNA-binding and DNA-photodamaging drugs may be developed on the basis of this class of dye.

Experimental Section

Materials: Distilled water was deionized by employing a Millipore MilliQ apparatus. Calf thymus DNA (Merck), $[poly(dAdT)]_2$, $[poly(dCdG)]_2$ (Sigma), and plasmid pBR 322 (AP BioTech) were used as purchased and dissolved in deionized water (ca. 1 mg/mL) or phosphate buffer solution (10 mM, pH = 7). The actual concentrations (in nucleic base) were determined by UV spectroscopy (ct DNA and $[poly(dAdT)]_2$: ε_{260} = 6600 $M^{-1}cm^{-1}$, $[poly(dCdG)]_2$: ε_{254} = 8400 $M^{-1}cm^{-1}$).

NMR Spectroscopy: ^1H NMR and ^{13}C NMR: Bruker AC 200 (^1H NMR: 200 MHz; ^{13}C NMR: 50.3 MHz); Bruker Avance 400 (^1H NMR: 400 MHz, COSY/HETCOR experiments). C_q, CH, CH_2 and CH_3 were determined by using the DEPT pulse sequence. ^1H NMR chemical shifts refer to δ_{TMS} = 0.0 and ^{13}C NMR chemical shifts to solvent signals ([D$_6$]DMSO: δ = 39.5).

Absorption, Emission and CD Spectroscopy: UV spectra: Hitachi U3200. Emission spectra: Perkin–Elmer LS50. Unless noted otherwise, the solution concentrations were 10^{-4} M for absorption spectra and 10^{-5} M for emission spectra. Emission spectra were recorded with an excitation wavelength at the corresponding isosbestic point, which was determined by UV spectroscopy (ct DNA: λ_{ex} = 318 nm; $[poly(dAdT)]_2$: λ_{ex} = 319 nm; $[poly(dCdG)]_2$: λ_{ex} = 316 nm). The relative fluorescence quantum yields were determined by the standard method[30] with quinine sulfate in 1 N H_2SO_4 as reference (φ_{Fl} = 0.546[31]). CD spectra: JASCO J715.

Binding isotherms were obtained from fluorometric titrations and represented as Scatchard plots. They were evaluated according to the model of McGhee and von Hippel[26] to obtain the intrinsic binding constant (K). To avoid large systematic inaccuracies due to experimental errors, the range of bound-drug fraction[26] to be analyzed was 0.85–0.15.

DNA-Photocleavage Experiments: DNA/dye samples (10 μL) were prepared from 2 μL of pBR 322 (100 mgL^{-1}), 1 μL of phosphate buffer (5 mM KH_2PO_4; pH = 7.4), 5 μL of dye (10^{-3} M), and 2 μL of water. Irradiations were performed with a Rayonet photoreactor at λ = 350 nm at 0 °C. DNA strand breaks were determined by agarose-gel electrophoresis with ethidium bromide as the indicator. Spots were detected by exposure on a UV transilluminator (366 nm) and recorded with a Herolab EASY 429 K camera. The ratio of relaxed DNA relative to the total amount of DNA was determined from the light intensities of the spots.

2-(1,3-Dioxolan-2-yl)-1-{[1-(phenylsulfonyl)-1*H*-indol-3-yl]-methyl}pyridinium (8): A solution of 3-bromomethylindole (**6**)[20] (1.40 g, 4.0 mmol) and 2-(1,3-dioxolan-2-yl)pyridine[19] (**7**; 0.69 g, 4.6 mmol) in DMSO (6 mL) was stirred under an argon atmosphere at room temperature for 7 days. With rapid stirring, the pyridinium derivative **8-Br** was precipitated by slow addition of ethyl acetate. The precipitate was separated and washed four times with ethyl acetate (100 mL). Recrystallization from methanol/ethyl acetate gave the salt **8-Br** (1.20 g, 2.4 mmol, 60%) as white cubic crystals. The bromide salt **8-Br** was transformed almost quantitatively to the tetrafluoroborate salt **8-BF$_4$** by dissolving it in methanol and slowly adding a saturated aqueous solution of sodium tetrafluoroborate. Recrystallization from ethanol gave the analytically pure salt **8-BF$_4$** as white needles.

8-Br: M.p. 116–117 °C. – ^1H NMR (200 MHz, [D$_6$]DMSO): δ = 4.20 (s, 4 H), 6.04 (s, 2 H), 6.43 (s, 1 H), 7.29–8.96 (m, 14 H). – ^{13}C NMR (50 MHz, [D$_6$]DMSO): δ = 54.1, 67.6, 99.2, 115.1, 115.3, 120.5, 125.6, 127.2, 127.6, 128.4, 129.8, 130.0, 130.3, 131.0, 136.0, 136.8, 139.1, 146.9, 148.5, 154.4. – $C_{23}H_{21}BrN_2O_4S{\cdot}H_2O$ (519.4): calcd. C 53.19, H 4.46, N 5.39, S 6.17; found C 53.63, H 4.26, N 5.50 S 6.27.

8-BF$_4$: M.p. 168–170 °C (dec.). – $C_{23}H_{21}BF_4N_2O_4S$ (508.3): calcd. C 54.35, H 4.16, N 5.51, S 6.31; found C 54.13, H 4.32, N 5.55 S 6.40.

Indolo[2,3-b]-quinolizinium bromide (3-Br): A sample of pyridinium salt **8-BF$_4$** (194 mg, 0.38 mmol) was added in small portions to polyphosphoric acid (2.5 g, 84%) at 80 °C. The viscous solution was slowly stirred at 100 °C for 3 h. After careful addition of water (20 mL), the solution was stirred at 90 °C for 45 min. and the solution was cooled to room temperature (ca. 20 °C). Saturated aqueous $NaBF_4$ (5 mL) was slowly added and a peach-colored solid precipitated, which was collected by filtration and additionally washed with water (2 × 25 mL) and diethyl ether (20 mL). The solid was extracted with acetonitrile. The solvent was evaporated in vacuo (40 °C, 5 mbar) and the remaining solid was crystallized from methanol to give the quinolizinium salt **3-BF$_4$** as a yellow solid (46 mg, 0.15 mmol, 39%). The tetrafluoroborate salt **3-BF$_4$** was transformed almost quantitatively to the bromide salt **3-Br** by precipitation with a saturated solution of tetrabutylammonium bromide in acetonitrile. Recrystallization from methanol gave analytically pure salt **3-Br** as a yellow solid.

3-Br: M.p. 290–295 °C (dec.). – ^1H NMR (200 MHz, CD_3OD): δ = 7.26 (ddd, J = 8 Hz, J = 7 Hz, J = 1 Hz, 1 H), 7.42 (dd, J = 8 Hz, J = 1 Hz, 1 H), 7.48–7.58 (m, 2 H), 7.80 (ddd, J = 9 Hz, J = 7 Hz, J = 1 Hz, 1 H), 8.03 (s, 1 H), 8.11–8.18 (m, 2 H), 8.89 (d, J = 7 Hz, 1 H), 9.88 (s, 1 H). – ^{13}C NMR (50 MHz, [D$_6$]DMSO): δ = 104.7, 113.3, 120.3, 120.5, 123.6, 123.6, 123.7, 127.1, 131.5, 132.2, 132.4, 135.6, 141.7, 144.6, 145.0. –

$C_{15}H_{11}BrN_2 \cdot 2H_2O$ (335.2): calcd. C 53.75, H 4.51, N 8.33; found C 54.38, H 4.32, N 8.33.

Acknowledgments

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