molbank

MDPI

Short Note

3-Methoxy-5-methyl-12-phenylbenzacridinium Iodide

Malokhat Uktamova [1,2,†], Rintaro Koga [3,†], Fotima Mukhamedjonova [1], Tursunali Kholikov [2], Bakhtiyor Ibragimov [1], Kohei Torikai [2,3,*] and Khamid Khodjaniyazov [1,2,*]

1 A. S. Sadikov Institute of the Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, 83 Mirzo Ulugbek Str., Tashkent 100125, Uzbekistan
2 Faculty of Chemistry, National University of Uzbekistan Named after Mirzo Ulugbek, 4 University Str., Tashkent 100174, Uzbekistan
3 Department of Chemistry, Graduate School and Faculty of Science, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan
* Correspondence: torikai@chem.kyushu-univ.jp (K.T.); hamidkhodjaniyazov@yandex.ru (K.K.)
† These authors contributed equally to this work.

Abstract: *N*-alkylacridinium derivatives are some of the most popular azo dye templates. Herein, we report the synthesis of 3-methoxy-5-methyl-12-phenylbenzacridinium iodide (MMPBAI) via *N*-alkylation. The structure of MMPBAI was elucidated using ^{1}H Nuclear magnetic resonance (NMR), ^{13}C NMR, Electronspray ionization mass spectrometry (ESI-MS), and Fourier-transform infrared spectroscopy (FT-IR). MMPBAI could not visualize double-stranded DNA in agarose gel, although the structural core would still be interesting as a template for new azo dyes.

Keywords: 12-phenylbenzacridine; acridine; acridinium; *N*-methyl; methylation; alkylation; dye

Citation: Uktamova, M.; Koga, R.; Mukhamedjonova, F.; Kholikov, T.; Ibragimov, B.; Torikai, K.; Khodjaniyazov, K. 3-Methoxy-5-methyl-12-phenylbenzacridinium Iodide. *Molbank* **2022**, *2022*, M1431. https://doi.org/10.3390/M1431

Academic Editor: R. Alan Aitken

Received: 26 July 2022
Accepted: 19 August 2022
Published: 22 August 2022

1. Introduction

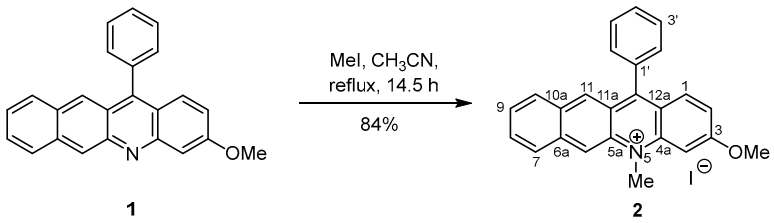
Acridines and their corresponding cationic form, acridinium, have long been used as a number of functional dyes; for example, ethidium bromide has been used to visualize electrophoretic migration behavior in double-stranded DNA in the field of molecular biology. Recently, reliable and synthetically useful acridinium-based photoredox catalysts have been developed. Fukuzumi et al. first introduced and popularized acridinium-based dyes as photoredox catalysts. In this study, the electron-transfer (ET) state of the 9-mesityl-10-methylacridinium ion was achieved successfully with a longer lifetime and higher energy than that in the neutral system and without losing energy due to multistep ET processes [1]. This chemistry allows for the generation of highly reactive intermediates via photo-induced electron transfer under operationally mild conditions that typically utilize low-energy visible light. One of the currently developed efficient and scalable methods for preparing acridinium cores is the direct conversion of xanthylium salts prepared from biaryl ethers and aromatic esters to the corresponding acridinium by treating them with amines [2]. To access the π-expanded system, we recently developed a diversity-oriented doubling strategy that can afford two 12-arylbenzoacridines from a single triarylmethanol precursor [3,4] and further studied their estrogenic, anti-estrogenic, antibacterial, and anti-oxidative activities [3–5].

Owing to our interest in *N*-methyl benzacridinium species, possessing an expanded conjugated π-electron system, for the preparation of novel azo dyes, we, herein, report the synthesis of *N*-methyl benzacridinium iodide **2** and our attempt to utilize it as a DNA-visualizing agent.

2. Results and Discussion

3-Methoxy-5-methyl-12-phenylbenzacridinium iodide (**2**) was synthesized via *N*-alkylation of the previously reported benzacridine **1** [3,4] with methyl iodide in refluxing acetonitrile (Scheme 1). The reaction proceeded in a spot-to-spot fashion to furnish **2** in

84% yield. The structure of **2** was unambiguously confirmed by spectroscopic analyses, including Fourier-transform infrared spectroscopy (FT-IR), 1D (^{1}H and ^{13}C), and 2D Nuclear magnetic resonance (NMR), including correlation spectroscopy (COSY), nuclear Overhauser effect spectroscopy (NOESY), heteronuclear multiple-bond correlation (HMBC), heteronuclear single quantum coherence (HSQC), and high-resolution electronspray ionization mass spectrometry (ESI-MS) (all spectra are shown in Supplementary Material and a complete summary of the assignments is depicted in Figure S14). The singlet ^{1}H NMR signal at 4.49 ppm was first assigned to the methoxy protons (OCH_3) because it had an HMBC correlation only with C-3 (Figure S10). Cross peaks from the OCH_3 protons in the NOESY spectrum (Figure S12) could be easily identified as H-2 (7.23 ppm) and H-4 (7.93 ppm). H-1 could be found in a multiplet at 7.79–7.73 ppm by the COSY correlation from H-2 (Figure S7). The singlet signal at 9.06 ppm was determined to be H-6 through a NOESY cross-peak with a singlet signal derived from *N*-methyl protons (5.20 ppm, Figure S12). H-7 (8.36 ppm) was also identified from the NOESY correlation with H-6 (Figure S13), whereas H-8 (7.81 ppm), H-9 (7.63 ppm), and H-10 (7.97 ppm) were easily identified by the COSY experiment. NOESY (Figure S13) from H-10 identified H-11 at 8.49 ppm, which had another NOESY cross-peak with H-2′ (7.51 ppm). By pursuing COSY correlations from H-2′, signals for H-3′ and H-4′ were found in a multiplet at 7.79–7.73 ppm. ^{13}C signals were assigned based on HSQC (Figures S8 and S9). As a result, all 1D and 2D signals agreed well with the structure of **2** (Figure S14).



Scheme 1. Synthesis of compound **2**.

The DNA visualization ability of the target compound, benzacridinium, was tested. After electrophoresis of commercially available DNA (marker 3), the agarose gel was treated separately for 25 min with compounds **2** (127 μM), **3** (127 μM) [3,4], and the most popular DNA visualizing agent, ethidium bromide (1.27 μM) (Figure 1). Unfortunately, DNA bands were only detected in the gels treated with ethidium bromide. The bulky and hydrophobic structures with fewer interacting functionalities (such as amino groups) of **2** and **3** might have interfered with DNA intercalation.

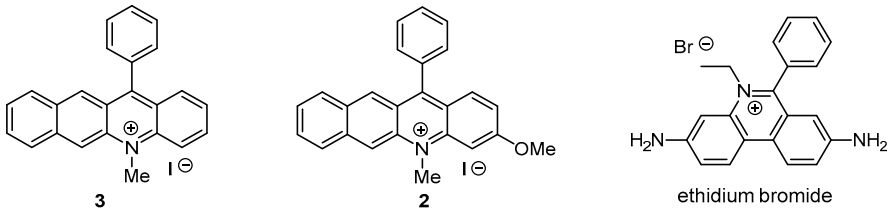


Figure 1. Compounds whose DNA visualization ability was tested.

3. Materials and Methods

3.1. Instrumentation

Melting point was recorded on a Yanaco MP-J3 (Tokyo, Japan). UV–Vis spectrum was recorded on a JASCO V-630 Bio (Tokyo, Japan). IR spectrum was recorded on a JASCO FT/IR-4000 (Tokyo, Japan). NMR spectra were recorded on JEOL JNM-ECA 600 spectrometer (Tokyo, Japan). Chemical shifts are reported in ppm from tetramethysilane (TMS) with reference to internal residual solvent [^{1}H NMR: $CHCl_3$ (7.26); ^{13}C NMR: $CDCl_3$ (77.16)]. The following abbreviations are used to designate the multiplicities: s = singlet, d = doublet,

t = triplet, m = multiplet, and br = broad. High-resolution mass spectrum (HRMS) was recorded under Bruker microTOFfocus conditions.

*3.2. Synthesis of 3-Methoxy-5-methyl-12-phenylbenzacridinium Iodide (**2**)*

3-Methoxy-12-phenylbenzacridine **1** (20.2 mg, 60.2 μmol) and methyl iodide (0.10 mL, 1.6 mmol) were dissolved in acetonitrile (1.0 mL). The reaction mixture was refluxed for 14.5 h under argon atmosphere until the completion of the reaction monitored by TLC. The crude mixture was concentrated to dryness under reduced pressure, and the residual thin film on the surface of the flask was washed with diethyl ether (2 mL × 2). The residual solid was dried in vacuo to afford **2** (24.2 mg, 50.7 μmol, 84%) as reddish-brown needles.

R_f = 0.64 (CH_2Cl_2/MeOH = 5/1); m.p. 190–194 °C; IR (neat) 3041, 2837, 1613, 1584, 1567, 1534, 1483, 1469, 1433, 1413, 1390, 1372, 1350, 1330, 1307, 1249, 1228, 1197, 1169, 1150, 1128, 1102, 1071, 1017, 1002, 957, 938, 909, 883, 863, 829, 813, 787, 754, 737, 701, 672 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 9.06 (s, ^1H, H-6), 8.49 (s, ^1H, H-11), 8.36 (d, *J* = 9.0 Hz, ^1H, H-7), 7.97 (d, *J* = 7.8 Hz, ^1H, H-10), 7.93 (d, *J* = 2.0 Hz, ^1H, H-4), 7.81 (dd, *J* = 9.0, 7.2 Hz, ^1H, H-8), 7.79–7.73 (m, ^3H, H-1, H-3′, H-4′), 7.63 (dd, *J* = 7.8, 7.2 Hz, ^1H, H-9), 7.51 (dd, *J* = 8.4, 1.8 Hz, ^1H, H-2′), 7.23 (dd, *J* = 9.6, 2.0 Hz, ^1H, H-2), 5.20 (s, ^3H, N^+-Me), 4.49 (s, ^3H, *O*-Me); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.4 (C-3), 160.3 (C-12), 147.3 (C-4a), 137.8 (C-6a), 136.2 (C-5a), 133.6 (C-1′), 132.4 (C-1), 132.0 (C-11), 131.6 (C-8), 131.0 (C-10a), 130.6 (C-4′), 130.0 (2C, C-2′), 129.2 (2C, C-3′), 129.0 (C-10), 128.7 (C-7), 127.9 (C-9), 123.2 (C-11a), 122.4 (C-2), 121.9 (C-12a), 115.8 (C-6), 97.7 (C-4), 59.7 (*O*-Me), 42.0 (N^+-Me); HRMS (ESI-TOF) *m*/*z* 350.1541 $[M - I]^+$ (calcd. for $C_{25}H_{20}ON^+$, 350.1539).

4. Conclusions

In summary, we reported the synthesis of *N*-methylbenzacridinium iodide **2** in a good yield via *N*-alkylation of 3-methoxy-12-phenylbenzacridine **1** with methyl iodide. In addition to its ease of operation, this protocol offered a clean reaction profile. Although **2** could be employed as a novel azo-dye template, DNA intercalation could not be visualized.

Supplementary Materials: The following supporting information for the characterization of **2** can be downloaded online: Molfile of Compound **2**; Figure S1: IR spectrum (neat); Figure S2: HRMS (ESI-TOF) spectrum; Figure S3: ^1H NMR spectrum (600 MHz, $CDCl_3$); Figure S4: Assignment of ^1H NMR; Figure S5: DEPT 90 (150 MHz, $CDCl_3$, top) and ^{13}C NMR spectra (150 MHz, $CDCl_3$, bottom); Figure S6: Assignment of ^{13}C NMR; Figure S7: Assignment of COSY spectrum (600 MHz, $CDCl_3$); Figure S8: Assignment of HSQC spectrum (part 1, 600/150 MHz, $CDCl_3$); Figure S9: Assignment of HSQC spectrum (part 2, 600/150 MHz, $CDCl_3$); Figure S10: Assignment of HMBC spectrum (part 1, 600/150 MHz, $CDCl_3$); Figure S11: Assignment of HMBC spectrum (part 2, 600/150 MHz, $CDCl_3$); Figure S12: Assignment of NOESY spectrum (part 1, 600 MHz, $CDCl_3$); Figure S13: Assignment of NOESY spectrum (part 2, 600 MHz, $CDCl_3$); Figure S14: Summary of the assignments of ^1H NMR, ^{13}C NMR, COSY, HMBC, and NOESY spectra.

Author Contributions: Conceptualization, K.T.; methodology, K.T.; validation, K.K.; formal analysis, M.U., R.K. and F.M.; investigation, R.K.; resources, T.K., B.I., K.T. and K.K.; data curation, K.T.; writing—original draft preparation, M.U., K.K. and K.T.; writing—review and editing, K.T.; visualization, M.U. and K.K.; supervision, K.T.; project administration, K.T. and K.K.; funding acquisition, K.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fukuoka Public Health Promotion Organization (formerly Fukuoka Foundation for Sound Health).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank Keitaro Umeno (Kyushu University, Japan) for his support with the structural analysis.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds are not available from the authors.

References

1. Fukuzumi, S.; Kotani, H.; Ohkubo, K.; Ogo, S.; Tkachenko, N.V.; Lemmetyinen, H. Electron-Transfer State of 9-Mesityl-10-methylacridinium Ion with a Much Longer Lifetime and Higher Energy than That of the Natural Photosynthetic Reaction Center. *J. Am. Chem. Soc.* **2004**, *126*, 1600–1601. [CrossRef] [PubMed]
2. White, A.R.; Wang, L.; Nicewicz, D.A. Synthesis and Characterization of Acridinium Dyes for Photoredox Catalysis. *Synlett* **2019**, *30*, 827–832. [CrossRef]
3. Koga, R.; Oishi, T.; Torikai, K. Tuned classical thermal aromatization furnishing an estrogenic benzoacridine. *Synlett* **2015**, *26*, 2801–2805.
4. Torikai, K.; Koga, R.; Liu, X.; Umehara, K.; Kitano, T.; Watanabe, K.; Oishi, T.; Noguchi, H.; Shimohigashi, Y. Design and synthesis of benzoacridines as estrogenic and anti-estrogenic agents. *Bioorg. Med. Chem.* **2017**, *25*, 5216–5237. [CrossRef]
5. Wungsintaweekul, B.; Abe, K.; Koga, R.; Katakura, Y.; Torikai, K. Antimicrobial and Anti-Oxidative Activities of 12-Arylbenzoacridines. *Indones. J. Chem.* **2020**, *20*, 1199–1205. [CrossRef]