The Synthesis of a Potential Bisintercalating Ethidium Bromide Analog

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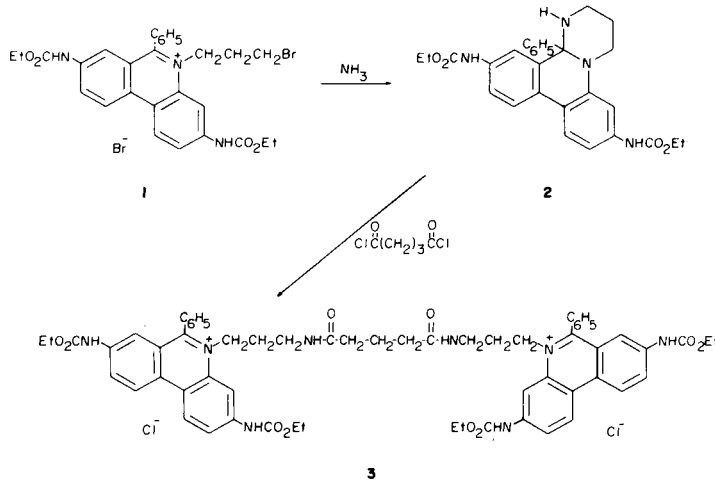
Received October 6, 1981

The synthesis of diethyl-1,3,4,13b-tetrahydro-13b-phenyl-2*H*-pyrimido[1,2-*f*]phenanthridine-7,12-dicarbamate and *N,N'*-3,3'[5,5'-Bis(3,8-biscarbethoxyamino-6-phenyl)phenanthridinium]propylglutarylamide dichloride are reported starting from 3,8-biscarbethoxyamino-5-(3'-bromopropyl)-6-phenylphenanthridinium bromide.

J. Heterocyclic Chem., **19**, 695 (1982).

Molecules which bind to DNA by intercalation are of considerable interest to both molecular biologists studying DNA structure and function as well as to chemists designing and synthesizing chemotherapeutic agents. Synthetic bisintercalating molecules, two intercalating aromatic ring systems linked together by variable length chains, have recently received attention as probes to test DNA binding models (1) and as potential drugs (2). Our interest (3) in both these fields led us to synthesize a potential bisintercalating analog of ethidium bromide; which is perhaps the most widely studied and best understood intercalating molecule. Previous synthetic efforts to modify ethidium bromide in order to make bisintercalators have focused attention upon attachment of the linking chain through the 6-aryl group of ethidium (1d, 2a), connection of the linking chain through the 8-amino function (1c) and through a chain attached to the 5-nitrogen atom (1a, 1b). The latter bisintercalators contained an alkyl diamine function as the connecting unit. We report the synthesis of an ethidium bromide type bisintercalator which has the two phenanthridinium moieties linked through a diamide group bound to the heterocyclic nitrogen atom (5-position).

The synthesis of the bis-phenanthridinium compound of interest was achieved starting with the previously reported bromopropylphenanthridinium bromide **1** (4). Reaction of **1**, with a saturated ammoniacal ethanol solution proceeded smoothly to give the tetracyclic system **2** in excellent yields. The long wavelength absorption maximum in the uv-visible absorption spectrum of **2** is shifted approximately 100 nm towards the ultraviolet (357 versus 451 nm) compared to its phenanthridinium product, **3**, and this is consistent with the structure assigned to **2**. Likewise the **13**C nmr spectrum for **2** is in accord with that expected for the structure shown in Scheme 1. The lack of a signal near 159 ppm [C-6 in ethidium bromide (5)] is consistent with structure **2**, arising from intramolecular ring closure at the immonium carbon. The carbon-13 signal at 76.0 ppm is a reasonable position for carbon 13-b in **2**. The three signals at 42.7, 39.7 and 26.7 are in the region expected for the three piperazine carbons **2**, **3** and **4** (6). The tetracyclic compound II is a result of nucleophilic displacement of the alkyl bromide by ammonia followed by intramolecular



addition to the immonium functional group of the phenanthridinium ring. Such an intramolecular ring closure is not unexpected since it follows well known principles of ring-chain tautomerism (7). Apparently, only two previous reports have appeared describing synthetic approaches to tetrahydropyrimido[1,2-*f*]phenanthridines (8); both of which started with phenanthridones. Thus the approach described here provides a new alternative synthetic method for the synthesis of this rarely reported ring system.

Under acidic conditions the tetracyclic compound **2** would be expected to be in equilibrium with its acyclic tautomer and consequently capable of reacting with an acid chloride. Compound **2** on reaction with glutaryl chloride in dimethylformamide gave the desired bisphenanthridinium **3**. The uv-visible spectrum of **3** is similar to its phenanthridinium starting material **1** and consistent with the structure assigned as opposed to a bistetracyclic structure. Carbon-13 nmr results are also in accord with the structure assigned.

EXPERIMENTAL

Diethyl 1,3,4,13b-Tetrahydro-13b-phenyl-2*H*-pyrimido[1,2-*f*]phenanthridine-7,12-dicarbamate (**2**).

A solution of 3,8-biscarbethoxyamino-5-(3'-bromopropyl)-6-phenylphenanthridinium bromide (**1**) (0.69 g, 0.0011 mole) dissolved in 85 ml of ethanol was saturated with ammonia and the mixture was allowed to stir

for 24 hours; the ethanol was removed under reduced pressure and an orange residue remained. The residue was dissolved in aqueous hydrobromic acid, filtered, the filtrate was made basic with aqueous ammonia and fine yellow crystals were collected by filtration. The acidification, filtration basification process was repeated and the resulting pale yellow solid was filtered, washed with water and dried over phosphorous pentoxide *in vacuo* overnight and 0.48 g (90%) of solid was obtained. The compound, which is slightly hydroscopic, was recrystallized from ethanol, mp 220-222 dec; λ max (ethanol): 298 nm (21,500), 357 (14,300). ^{13}C nmr (deuteriochloroform): 153.6, 146.0, 143.0, 139.5, 138.9, 136.9, 128.4, 127.1, 126.6, 125.4, 123.6, 122.2, 118.4, 116.5, 115.5, 108.2, 101.4, 76.0, 61.1, 42.7, 39.7, 26.7, 14.5 ppm.

Anal. Calcd. for $C_{28}H_{30}N_4O_4$: C, 69.11; H, 6.22; N, 11.52. Found: C, 69.10; H, 6.04; N, 11.21.

N,N'-{3,3'[5,5'-Bis(3,8-biscarbethoxyamino-6-phenyl)phenanthridinium)]-propyl}glutarylamide dichloride (**3**).

A solution of 0.18 g (0.00037 mole) of **2** in 4 ml of dry dimethylformamide under a nitrogen atmosphere was treated with 0.03 g (0.00017 mole) of glutaryl chloride and the mixture was allowed to stir overnight. The solution was filtered and the collected solid washed with diethyl ether and dried *in vacuo*. The yield was 0.08 g (40%), mp 229-239 dec; λ max (ethanol): 317 (46,000), 341 (10,800), 451 (11,300); ^{13}C nmr (DMSO-d_6): 171.9, 163.0, 153.6, 153.3, 142.3, 139.8, 133.9, 131.2, 130.0, 129.2, 128.8, 128.1, 125.6, 125.2, 123.4, 120.9, 117.5, 106.5, 53.3 (peaks appearing at 37.3 and 36.4 in CD_3OH are obscured by the DMSO signal) 29.0, 21.1, 14.3 ppm.

Anal. Calcd. for $C_{61}H_{66}Cl_2N_8O_{10}$: C, 64.13; H, 5.82; N, 9.81. Found: C, 64.07; H, 5.84; N, 9.64.

Acknowledgement.

This work was supported in part by NIH (Grants CA 24454 and RR09201).

REFERENCES AND NOTES

(1a) B. Gaugain, J. Barbet, R. Oberlin, B. P. Roques, and J-B. Le Pecq, *Biochemistry* **24**, 5071 (1978); (b) B. Gaugain, J. Barbet, N. Capelle, B. P. Roques and J-B. Le Pecq, *ibid.*, **24**, 5078 (1978); (c) J. W. Lown, B. C. Gunn, K. C. Majumdar and E. McGoran, *Can. J. Chem.*, **57**, 2305 (1979); (d) M. M. Becker and P. B. Dervan, *J. Am. Chem. Soc.*, **101**, 3664 (1979).

(2a) K. F. Kuhlman, N. J. Charbeneau and C. W. Mosher, *Nucleic Acids Res.*, **5**, 2629 (1978); (b) K. F. Kuhlman, C. W. Mosher and R. F. Hammen, *Biochem. Biophys. Res. Commun.*, **92**, 1172 (1980).

(3a) W. D. Wilson and R. L. Jones., "Intercalating Drugs: DNA Binding and Molecular Pharmacology", in "Advances in Pharmacology and Chemotherapy", F. Hawkins, ed, Academic Press, 1981, pp 177-222; (b) R. L. Jones and W. D. Wilson, *J. Am. Chem. Soc.*, **102**, 7776 (1980); (c) B. P. Das, R. A. Wallace and D. W. Boykin, *J. Med. Chem.*, **23**, 578 (1980).

(4) T. I. Watkins, *J. Chem. Soc.*, 3059 (1952).

(5) B. G. Griggs, M. W. Davidson, W. D. Wilson and D. W. Boykin, *Org. Magn. Reson.*, **14**, 371 (1980).

(6) E. Wenkert, J. S. Bindra, C-J. Chang, D. W. Cochran and F. M. Schell, *Acc. Chem. Res.*, **7**, 46 (1974).

(7a) P. R. Jones, *Chem. Rev.*, **63**, 461 (1963). (b) R. E. Lutz and C. E. Griffen, *J. Org. Chem*, **25**, 928 (1960).

(8a) H-L. Pan and T. L. Fletcher, *J. Heterocyclic Chem.*, **9**, 859 (1972); (b) R. F. Cookson and R. E. Rodway, *J. Chem. Soc., Perkin Trans. I*, 1850 (1975).