PREPARATION AND NUCLEIC ACID BINDING OF ELLIPTICINE DERIVATIVES

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Abstract: Two series of 2-substituted and 6-substituted ellipticine derivatives have been prepared. Several derivatives show a significant increase in the ability to bind nucleic acids compared to the parent ellipticine.

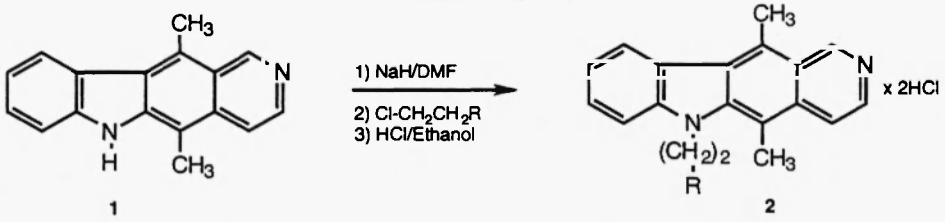
Introduction

Retroviruses have proven to be the causative agent in a number of diseases such as AIDS and leukemia[1]. Therefore, there exists a great need to develop agents that possess antiviral properties. Wilson has suggested that compounds which target viral genomic RNA could be good antiviral agents[2]. We have chosen to examine ellipticine derivatives because it has been reported that ellipticine binds to both RNA and DNA as well as inhibits RNA synthesis [3]. We have prepared two series of ellipticine derivatives that are substituted in either the 2-position or the 6-position with an amine containing side chain which will be protonated at physiological pH and should enhance the binding with the negatively charged phosphate backbone of nucleic acids.

Results

The preparation of 6-substituted ellipticines **2** was accomplished by deprotonation of the indole nitrogen of ellipticine with sodium hydride in dimethylformamide and subsequent alkylation with a series of amino substituted alkyl halides[4]. The crude product was chromatographed and the desired hydrochloride salts were formed in ethanolic HCl. The 6-substituted derivatives and the results of melting studies of their complexes with RNA and DNA are listed in Table 1.

Reaction 1



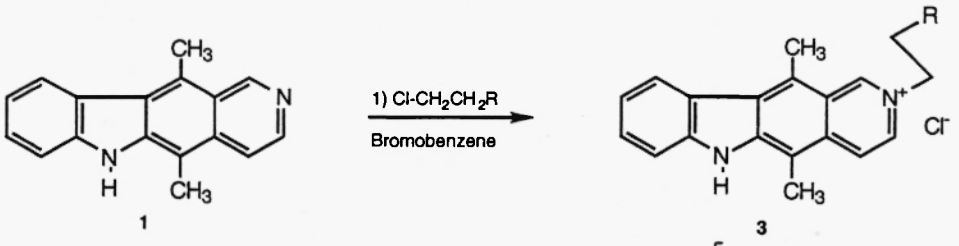
1 2

Table 1; Nucleic Acid Melting Temperatures Changes With 6-Substituted Ellipticines[5]

Compound (R-Group)	RNA ΔTm °C	DNA ΔTm °C
Ellipticine x HCl	10	11
2a (-diethylamino)	13	14
2b (-pyrrolidyl)	19	20
2c (-piperidyl)	18	14
2d (-morpholinyl)	12	10
2e (-azapinyl)	4	6

The 2-substituted ellipticines **3** were prepared by refluxing ellipticine with two equivalents of amino substituted alkyl chloride in bromobenzene as solvent. The compounds and results from their melting studies with RNA and DNA is presented in Table 2.

Reaction 2



1 3

Table 2: Nucleic Acid Melting Temperatures Changes With 2-Substituted Ellipticines[5]

Compound (R-Group)	RNA ΔTm °C	DNA ΔTm °C
3a (-diethyl amino)	11	8
3b (-pyrrolidyl)	13	12
3c (-piperidyl)	12	14

Conclusion

Our goal in preparation of these two series of compounds was to examine their nucleic acid binding ability and to attempt to identify the structural features which enhance RNA binding. We note that the 6-substituted ellipticines **2** show better binding to both DNA and RNA than the 2-substituted ones **3** compared to ellipticine. It is also noted that 6-substituted derivatives have almost doubled the binding ability of the parent ellipticine with both nucleic acids. Based on this information our laboratory is now actively pursuing new 6-substituted compounds.

Experimental

Dimethylformamide was stored over 4A sieves for 72 hours and then distilled. Commercial bromobenzene was used without additional purification. ^{1}H and ^{13}C NMR spectra were recorded on either a JEOL GX-270 NMR spectrometer or a Varian VXR-400 NMR spectrometer. IR spectra were recorded on a Perkin-Elmer System 2000 FTIR spectrometer. Melting points were taken in a Thomas Hoover Uni-melt apparatus and are uncorrected. Elemental analyses were performed by Atlanta Microlab, Atlanta, Georgia. All MS data were obtained at the Georgia Institute of Technology.

The following procedure is representative for the 6-substituted ellipticines **2**. NaH (1.2, eq., 0.00024 mol, 0.007 g) was suspended in anhydrous DMF (20 ml) under a nitrogen atmosphere. Ellipticine (0.05 g, 0.0002 mol) was added and the mixture was stirred at room temperature for 1 hr. Diethylaminoethyl chloride (1.2 eq., 0.00024 mol, 0.033 g, generated from hydrochloride salt) was added to the mixture via addition funnel over 10 min. The mixture was stirred for 24 hrs. The DMF was removed under high vacuum. The crude product was chromatographed on silica gel (95:5 v/v $CHCl_3$:MeOH). The free base (0.032 g) was mixed with ethanolic HCl and precipitated hydrochloride salt **2a** was washed with ether and dried.

(2a): Yield , 30%; m.p. 294°C (dec.); Anal. Calcd. for $C_{23}H_{29}N_3Cl_2$•2 1/2 H_2O, C 59.61, H 7.34, N 9.07; Found: C 59.43, H 7.08, N 8.83; ^{1}H NMR (270 MHz, DMSO/65°C): 9.89 (s, 1H), 8.48 (m, 3H), 8.07 (d, 1H, J = 8 Hz, 7.71 (t, 1H, J = 7 Hz), 7.45 (t, 1H, J = 7 Hz), 5.27 (t, 2H, J = 7.8 Hz), 3.45 (t, 2H, J = 7.3 Hz), 3,31 (s, 3H), 3.26 (q, 2H, J = 7.3 Hz), 3.13 (s, 3H), 1.29 (t, 3H, J = 7.3 Hz); ^{13}C NMR (270 MHz, D_2O, DMSO ref.): 144.6, 144.2, 143.5, 137.0, 134.3, 130.9, 128.4,

128.0, 125.9, 123.8, 123.2, 121.7, 121.4, 111.7, 110.7, 50.8, 49.6, 41.5, 15.8, 14.7, 9.7; MS (EI): 346 (M^+ + 1), 345, 247, 163, 146, 124, 111, IR (KBr, cm^{-1}): 3429, 3026, 2982, 1641, 1594, 1483, 1406, 1333, 1219, 1112, 1034, 814, 746, cm^{-1}.

(2b): Yield, 24%; m.p. 282-84° C (dec.); ^1H NMR (270 MHz, D_2O): 8.81 (s, 1H), 7.97 (d, 1H, J = 7 Hz), 7.91 (d, 1H, J = 7 Hz), 7.51 (d, 1H, J = 8.5 Hz), 7.40 (t, 1H, J = 7 Hz), 7.13 (d, 1H, J = 8.5 Hz), 7.05 (t, 1H, J = 7 Hz), 4.20 (br, 2H), 3.51 (br, 2H), 3.29 (br, 2H), 2.94 (br, 2H, 2.47 (s, 3H, 2.15 (s, 3H), 2.02-1.91 (br m, 4H); ^{13}C NMR (D_2O, 270 MHz): 144.5, 144.4,137.0, 134.1, 130.7, 130.3, 127.9, 126.1, 123.6, 122.2, 122.1, 121.0, 111.6, 110.8, 56.25, 53.7, 42.7, 24.2, 15.9, 14.7; IR (KBr, cm^{-1}): 3074, 3043, 2961, 1644, 1591, 1455, 1403, 1237, 1215, 1101, 1041, 801, 748; MS (HR): Exact mass observed 344.2128 , calculated 344.2127.

(2c): Yield, 39%; m.p. = >300°C; Anal. Calcd. for $C_{24}H_{29}N_3Cl_2 \cdot 2\ 1/2\ H_2O$, C= 60.63, H = 7.15, N = 8.81. Found: C = 60.86, H = 6.98, N = 8.84; ^1H NMR (400 MHz, D_2O): 9.24 (s, 1H), 8.21 (d, 1H, J = 7.5 Hz), 8.10 (d, 1H, J = 7.5 Hz), 8.0 (d, 1H, J = 7.5 Hz), 7.59 (t, 1H, J = 7.5 Hz), 7.33 (m, 2H), 4.6 (2H, t , under HOD peak), 3.47 (m, 2H), 3.25 (t, 2H, J = 8 Hz), 2.94 (m, 2H), 2.73 (s, 3H), 2.71 (s, 3H), 1.89-1.75 (m, 6H); ^{13}C NMR (400 MHz, D_2O, 1 drop DMSO for ref. at 39.6 ppm): 144.9, 144.5, 143,7,137.2, 134.6, 130.9, 129.0, 128.3, 126.1, 123.8, 123.4, 121.9, 121.5, 111.9, 110.8, 55.3, 55.0, 41.2, 24.3, 22.4, 15.9, 14.7; Mass spec. (EI): 357 (M^+), 259, 243, 229, 217, 98 (base), 70, 55, 41; IR (KBr, cm^{-1}): 3032, 2940, 1639, 1578, 1459, 1407, 1325, 1221, 811, 760.

(2d): Yield, 54%; m.p. 300°C (dec.). (Lit. m.p. = >300°C), J. Med. Chem., **29**, 1321-1322 (1986); Anal. Calcd. for $C_{23}H_{27}N_3OCl_2$ 1.8 H_2O, C= 59.43, H = 6.54, N = 9.04. Found: C = 59.59, H = 6.33, N = 8.79; MS (EI): 359 (M^+), 246, 231, 217, 100; ^1H NMR (D_2O, 400 MHz): 9.04 (s, 1H), 8.11 (d, 1H, J = 7 Hz), 8.01 (d, 1H, J = 7 Hz), 7.78 (d, 1H, J = 8 Hz), 7.51 (t, 1H, J = 8 Hz), 7.27 (d, 1H, J = 8 Hz), 7.19 (t, 1H, J = 8 Hz), 4.55 (t, 2H, J = 8 Hz), 3.95 (br, 4H), 3.34 (br, 4H), 3.30 (t, 2H, J = 7 Hz), 2.65 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (400 MHz, D_2O): 144.6, 144.2, 143.4, 136.9, 134.2, 130.9, 128.9, 127.9, 125.8, 123.8, 123.1, 121.7, 121.3, 111.7, 110.7, 65.3, 55.3, 53.7, 40.9, 15.8, 14.7; IR (KBr, cm^{-1}): 2984, 2930, 1644, 1591, 1471, 1403, 1343, 1215, 1102, 989, 884, 808, 741.

(2e): Yield, 28%; m.p. 300°C; Anal. Calcd. for $C_{25}H_{31}N_3Cl_2$ 3.1 H_2O, C= 60.02, H = 7.44, N = 8.40. Found: C = 59.62, H = 6.89, N = 8.35; MS (HR): observed = 372.2440, Calculated = 372.2440; IR (KBr, cm^{-1}): 3029, 2939, 1644, 1576, 1456, 1403, 1335, 1223, 1042, 824, 748; ^1H NMR (400 MHz, D_2O): 9.30 (s, 1H), 8.25 (d, 1H, J = 7 Hz), 8.15 (d, 1H, J = 7 Hz), 8.05(d, 1H, J = 8 Hz), 7.62 (t, 1H, J = 8 Hz), 7.40 (d, 1H, J = 8 Hz), 7.33 (t, 1H, J = 8 Hz), 4.56 (t, 2H, J = 7 Hz), 3.85 (br m, 2H), 3.30 (t, 3H, J = 7 Hz), 3.15 (br.m, 2H), 2.78 (s, 3H), 2.76 (s, 3H), 1.85 (br, 4H), 1.65 (br, 4H); ^{13}C NMR (400 MHz, D_2O): 144.6, 144.2, 143.4,136.9, 134.1, 130.9, 128.9, 127.8, 125.8, 123.8, 123.0, 121.6, 121.3, 111.7, 110.8, 57.2, 55.7, 41.6, 27.0, 25.1, 15.8, 14.7.

The following procedure is representative for the 2-substituted ellipticines **3**: To a suspension of ellipticine (0.025 g, 0.0001 mol) in 5 ml of MeOH was added a solution of (N,N-diethylamino)ethylchloride hydrochloride (0.021 g, 0.00012 mol) in 1 ml MeOH followed by Et_3N (0.012 g, 0.00012 mol) in MeOH (1 ml) and the mixture was stirred for 48 hrs. Additional amine hydrochloride (0.005 g, 0.00003 mol) in 1 ml methanol and Et_3N (0.005 g, 0.00003) in 1 ml MeOH were added and stirring was continued for 18 hrs at which time another identical addition was made and stirring was continued for 12 hrs. The solvent was removed under reduced pressure to yield a yellow gum. It was dissolved in EtOH (5 ml) and kept in a freezer overnight to yield crystals which were filtered, washed with hexane and dried.

(3a): Yield, (82%; m.p. 274°C (dec.); Anal. Calcd. for $C_{23}H_{29}N_3Cl_2 \cdot 3\ H_2O$, C = 58.48, H = 7.41, N = 8.89. Found: C = 58.52, H = 7.43, N = 8.93; ^1H NMR (270 MHz, D_2O): 8.94 (s, 1H), 7.88 (d, 1H, J = 7 Hz), 7.74 (d, 1H, J = 7 Hz), 7.53 (d, 1H, J = 7.8 Hz), 7.21 (t, 1H, J = 7 Hz), 6.97 (d, 1H, J = 7 Hz), 6.90 (d, 1H, J = 7.8 Hz), 4.74 (t, 2H, J = 7 Hz), 3.58 (t, 2H, J = 7 Hz), 3.24 (q, 2H, J = 7 Hz), 2.49 (s, 3H), 2.21 (s, 3H), 1.34 (t, 3H, J = 7 Hz); IR (KBr, cm^{-1}): 3433, 3366, 2988,

2655, 2477, 1644, 1600, 1572, 1466, 1422, 1244, 1183, 1022, 805, 744, 716, 600, 461; MS (EI): 345, 246, 231, 217, 123, 100, 86, 56; ^{13}C NMR (D_2O, 400 MHz): 145.73, 144.30, 142.04, 133.18, 133.08, 130.47, 129.36, 125.68, 125.58, 121.94, 121.86, 121.55, 120.55, 111.65, 110.75, 54.78, 52.35, 49.82, 15.45, 12.38, 10.04.

(3b): Yield, 90%; m.p. >300°C; Anal. Calcd. for $C_{23}H_{26}N_3Cl \cdot 5\ H_2O$, C = 58.78, H = 7.65, N = 8.95. Found: C = 58.96, H = 7.09, N = 9.30; MS (HR): Exact mass observed = 344.2125, calculated = 344.2126; ^1H NMR (400 MHz, DMSO-d_6): 12.40 (s, 1H), 10.23 (s, 1H), 8.57 (d, 1H, J = 6 Hz), 8.47 (d, 1H, J = 6 Hz), 8.44 (d, 1H, J = 6 Hz), 7.66 (m, 2H), 7.39 (t, 1H, J = 6 Hz), 5.16 (t, 2H, J = 7 Hz), 3.83 (m, 4H), 3.40 (2H, under water peak), 3.30 (3H, under water peak), 2.83 (s, 3H), 2.05 (m, 4H); ^{13}C NMR (400 MHz, D_2O): 147.9, 144.7, 142.7, 133.9, 132.7, 130.9, 128.8, 125.9, 124.5, 122.2, 120.9, 120.6, 120.2, 111.8, 110.5, 54.9 (br), 53.7 (br), 53.6 (br), 22.9, 15.31, 12.2; IR (KBr, cm^{-1}): 3420, 3165, 2958, 1601, 1458, 1424, 1327, 1247, 1186, 1115, 1071, 1034, 957, 906, 804, 755, 717, 610.

(3c): Yield,68%; m.p. 256°C (dec.); Anal. Calcd for $C_{24}H_{28}N_3ClO \cdot 9\ H_2O$. C = 70.29, H = 7.27, N = 10.25. Found: C = 70.41, H = 7.22, N = 10.22; ^1H NMR (270 MHz, D_2O, 40°C): 8.56 (s, 1H), 7.63 (d, 1H, J = 7 Hz), 7.37 (d, 1H, J = 8 Hz), 7.05 (m, 2H), 6.65 (m, 2H), 4.38 (t, 2H, J = 7 Hz), 3.02 (t, 2H, J = 6 Hz), 2.78 (br m, 4H), 2.05 (s, 3H), 1.87 (s, 3H, 1.63-1.79 (m, 6H). ^{13}C NMR (270 MHz, DMSO, 85°C): 148.5, 146.3, 144.5, 134.8, 134.3, 132.8, 130.1, 127.8, 125.9, 123.9, 122.4, 121.5, 121.5, 113.3, 112.1, 54.3, 58.5, 55.3, 26.9, 25.1, 16.6, 13.6; MS (HR): Exact mass observed = 358.2285, calculated = 358.2283. IR (KBr, cm^{-1}): 3057, 2932, 1654, 1602, 1465, 1421, 1246, 1039, 742, 610, 597.

(3d): Yield, 70%; m.p. 272-274°C (dec.); Anal. Calcd. for $C_{25}H_{30}N_3ClO \cdot 0.6\ H_2O$. C = 67.93, H = 6.69, N = 10.33. Found: C = 67.71, H = 6.54, N = 10.21; ^1H NMR (400 MHz, D_2O): 8.28 (s, 1H), 7.39 (d, 1H, J = 7 Hz), 7.05 (d, 1H, J = 7 Hz), 6.74 (d, 1H, J = 7 Hz), 6.71 (d, 1H, J = 7 Hz), 6.42 (t, 1H, J = 7 Hz), 6.34 (d, 1H, J = 7 Hz), 4.11 (t, 2H, J = 7 Hz, 3.69 (t, 4H, J = 6 Hz), 2.74 (t, 2H, J = 7 Hz), 2.55 (t, 4H, J = 7 Hz), 1.76 (s, 3H), 1.57 (s, 3H). ^{13}C NMR (400 MHz, D_2O, DMSO ref. at 39.5 ppm): 144.7, 143.6, 141.8, 132.7, 132.2, 130.9, 129.2, 125.2, 124.1, 121.6, 121.4, 120.9, 120.2, 111.5, 110.1, 67.8, 59.1, 57.2, 54.2, 15.1, 12.1; MS (FB): 360, 325, 307, 273, 257, 247,234; IR (KBr, cm^{-1}): 3059, 2976, 1651, 1591, 1576, 1463, 1425, 1403, 1327, 1297, 1245, 1184, 1147, 1117, 1064, 1026, 936, 853, 815, 763.

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(1) (a) A.S. Fauci, *Science* **239**, 617-622 (1988); (b) E. De Clercq, in *Design of Anti-AIDS Drugs*, Ed. E. De Clercq, Elsevier Publishing Company: Amsterdam, pp. 1-24 (1990)

(2) W.D. Wilson, G.D. Kennedy, D. Boykin, L. Ratmeyer, M.T. Cegla, J. Spychala, M. Demeunyck, J. Lhomme, G. Krishnan, R. Vinayak and G. Zon, *The New Journal of Chemistry* **18**, 41, 1994

(3) (a) C. Auclair, *Arch. Biochem. Biophys.* **259**. 1-14 (1987): (b) M. Schwaller, J. Aubard, G. Dodin, *Anti-cancer Drug Design* 5, 77-87 (1990); (c) C. Auclair, B. Meunier, B. Dugue and C. Paoletti, *Biochemistry* **25**, 1240-1245 (1986); (d) G. Meunier, D. DeMontauzon, J. Bernadou, G. Grassy, M. Bonnafous, S. Cros and B. Meunier, *Mol. Pharmacol.* **33**, 93-102 (1988)

(4) L.M. Werbel, M. Angelo, D.W. Fry and D.F. Worth, *J. Med. Chem.* **29**, 1321-1322 (1986)

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