# Communications to the Editor

## An Ethidium Analogue That Binds with High Specificity to a Base-Bulged Duplex from the TAR RNA Region of the HIV-I Genome

The genomic RNA of retroviruses is present in cells during initial infection and during viral replication from integrated DNA.[1] The RNA of such viruses is highly folded with sections of A-form helix containing base bulges and loops.[2] Specific RNA conformations (such as the TAR sequence in HIV-1, Figure 1) and their interactions with proteins (such as tat of HIV-1) are essential for efficient viral replication.[2] Disruption of such specific RNA conformations and/or RNA–protein interactions is a potential route for retroviral chemotherapy that has not been extensively explored. Although very little is known about the general interaction of organic cations with RNA, the intercalation of ethidium (1, Figure 1) with RNA has been studied in some detail.[3] Previous work has shown that ethidium binds better to poly(A)·poly(U) than to poly(dA)·poly(dT), but binds similarly to RNA and DNA containing G·C base pairs.[3] White and Draper[4] found that

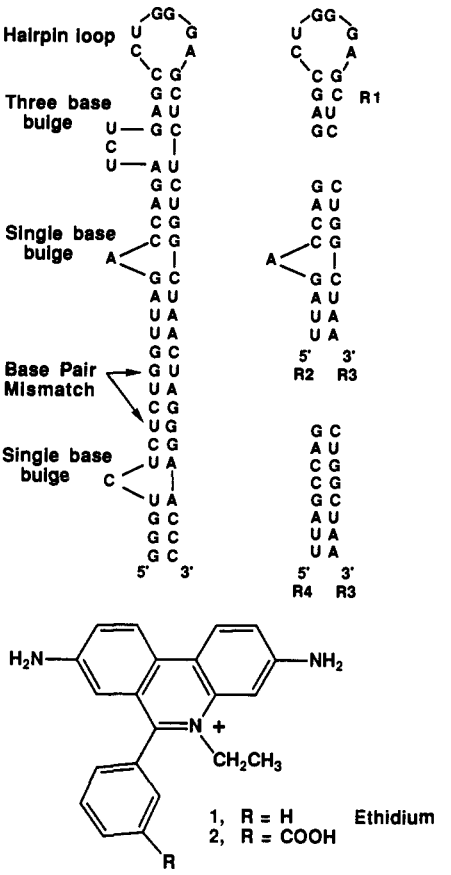


**Figure 1.** RNA models from the TAR sequence of the HIV-1 genome. R1 represents the hairpin loop of TAR, R2 and R3 form a single-base bulge duplex from TAR, R4 and R3 form a corresponding duplex without the bulge, and D4 and D3 form a deoxyribonucleotide duplex corresponding to R3·R4 for comparison. Structures for ethidium, 1, and 2 are shown.

ethidium binds with a 4–5-fold increase in affinity at CpG sites when they are 3′ to a base bulge in a ribosomal RNA hairpin, but that analogues of the hairpin, with the base bulge at other positions, did not bind ethidium with enhanced affinity. We have initiated a systematic search for molecular structures that (i) show enhanced affinity for RNA relative to DNA and (ii) bind selectively at specific RNA conformational features such as those that exist in the TAR RNA sequence of HIV-1 (Figure 1). We report here that an analogue of ethidium, with a *m*-carboxyphenyl group (2, Figure 1),[5] binds very strongly to an RNA se-

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(5) (a) Ethidium was purchased from Sigma and recrystallized from methanol. Compound 2[5b] was obtained from Dr. S. S. Berg of May & Baker LTD, Dagenham, Essex, England. (b) Kuhlmann, K. F.; Charbeneau, N. J.; Mosher, C. W. Synthesis, DNA-binding and Biological Activity of a Double Intercalating Analog of Ethidium Bromide. *Nucleic Acids Res.* **1978**, *7*, 2629–2641 and references therein.

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quence from TAR that contains a bulged base, but binds significantly more weakly to the corresponding RNA and DNA sequences without the bulge.

We have synthesized[6] several initial oligonucleotides as models for different regions of TAR (Figure 1). R-1 has a relatively broad thermal melting curve and will not be discussed here. R2·R3, R3·R4, D3·D4 exhibit reversible, cooperative melting transitions (Figure 2, top). The RNA duplex has the highest Tm, the DNA duplex the next highest, and the bulged duplex the lowest Tm. Tm vs log (sodium ion activity) plots are linear (inset, Figure 2), and have slopes for R2·R3, R3·R4, and D3·D4 of 11.1, 10.5, and 8.4, respectively, as compared with RNA and DNA polymers which have slopes near 20.[7] The lower slopes for the oligomers are as expected due to their lower charge densities.[7b] Tm values, predicted from standard data sets at 1 M NaCl,[8] are 46 and 31 °C for R3·R4 and D3·D4, respectively, in good agreement with our experimental values at that salt concentration (Figure 2, inset). The significant destabilizing effect of the bulged base is also as expected.[8a]

Figure 2 also shows Tm curves for the oligomers in the presence of saturating ratios of compound to base pair (0.6). Under these conditions, cooperative, well-defined transitions are observed, and R2·R3, R3·R4, and D3·D4 complexes have ΔTms (the Tm of the free nucleic acid subtracted from the Tm of the nucleic acid complex) of 10.1, 5.6, and 8.8 °C, respectively, with ethidium and 5.2, 1.9, and 2.5 °C, respectively, with **2**. Poly(A)·poly(U) and poly(dA)·poly(dT) have ΔTms of 17.3 and 7.2 °C, respectively, with ethidium and 6.9 and 0.6 °C, respectively, with **2**. The alternating polymers of the same sequence, poly(A-U)$_2$ and polyd(A-T)$_2$, have ΔTm values of 15.1 and 14.5 °C with ethidium and 4.1 and 3.2 °C with **2**. At ratios below saturation broader or biphasic (depending on the salt concentration) melting curves are observed.

We have, thus, confirmed the selective binding of ethidium to polyA·polyU,[3a,b] but the similar binding of ethidium to R3·R4 and D3·D4 and to the alternating AU and AT polymers supports other results[3e] that indicate that ethidium does not have a general RNA binding selectivity. Although the strong interaction of ethidium with the buldged-base RNA sequence is encouraging, ethidium retains significant interactions with other RNA and DNA sequences, and this nonspecific binding could lead to general cellular toxicity in long-term therapy. Compound **2** binds weakly to both the RNA sequence without the A bulge and to DNA as expected from its negatively charged carboxyl group and overall charge neutrality. Compound **2**, however, binds significantly more strongly to the TAR segment with the bulge, and such binding could selectively disrupt several critical steps in the life cycle of HIV-1. Ethidium is known to bind selectively to some unusual nucleic acid conformations,[4,9] and interactions of this type

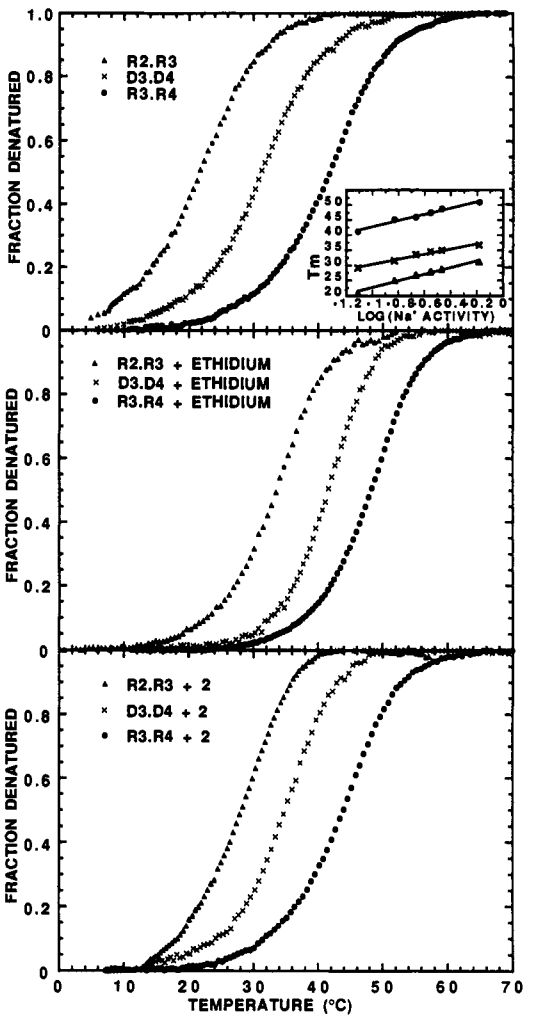


**Figure 2.** Tm curves for R2·R3, D4·D3, R4·R3 free (top), in the presence of ethidium (middle), and in the presence of **2** (bottom). A Cary 219 spectrophotometer interfaced to a microcomputer was used to obtain the thermal denaturation data, as previously described.[10] The Tm data was fitted with a nonlinear least squares computer program that includes sloping base lines in the duplex and single-strand regions.[11]. The base lines were subtracted from the experiment data, and the results plotted in the figure as fraction of duplex denatured as a function of temperature. The Tm is defined as the temperature at which the fraction denatured is 0.5. PIPES buffer (0.01 M PIPES, 0.001 M EDTA, pH 7) was used in all experiments at a 0.1 M NaCl concentration, except for the salt dependence Tm studies which were conducted at a range of salt concentrations (top, inset). The same symbols are used for the three oligomers in the figures and inset. Buffers were treated with DEPC and autoclaved to inactivate nucleases and microorganisms. All glassware was soaked in DEPC treated water and either baked for at least 8 h at 180 °C or autoclaved.

(6) (a) All oligomers were synthesized on an Applied Biosystems DNA/RNA synthesizer Model 394. The RNA oligomers were synthesized by using the fast oligonucleotide deprotection RNA phosphoramidites[6b] on polystyrene supports[6b,c] by a standard protocol.[6d] Deprotection of the base-protecting groups of RNA was complete in 1 at 55 °C in EtOH/$NH_4OH$ (1:3). The DNA oligomers were synthesized by a standard protocol.[6e] All the oligomers were purified by HPLC on Aquapore RP-300 (C8, 7 μm) column (220 × 4.6 mm) with a flow rate of 1.0 mL/min and a biphasic linear gradient of 0% acetonitrile/100% 0.1 M triethylammonium acetate–$H_2O$ to 20% acetonitrile/80% 0.1 M triethylammonium acetate–$H_2O$ in 24 min, and then to 40% acetonitrile/60% 0.1 M triethylammonium acetate–$H_2O$ in 10 min. (b) Vinayak, R.; McCollum, C.; Hampel, A. Manuscript in review. (c) McCollum, C.; Andrus, A. *Tetrahedron Lett.* **1991**, 4069–4072; U.S. Patent 5,047,524. (d) Applied Biosystems User Bulletin No. 53. (e) Applied Biosystems User Bulletin No. 13.

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may also account for the selectivity of **2** for R2·R3. We are currently conducting more detailed studies to define the molecular basis for the strong selective binding of **2** to the TAR RNA model sequence, and we are also preparing derivatives of **2** with bases (e.g. uracil) linked through the phenanthridinium nitrogen alkyl group to attempt to obtain additional binding strength and specificity for R2·R3 and other bulged base sequences in TAR.

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