Synthetic Spectroscopic Models Related to Coenzymes and Base Pairs, VII.* Stacking Interactions in tRNA; the “Bend” at Dimethylguanosine†

Hajime Iwamura, Nelson J. Leonard, and Josef Eisinger

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF ILLINOIS, URBANA, AND BELL TELEPHONE LABORATORIES, MURRAY HILL, NEW JERSEY

Communicated January 9, 1970

Abstract. We have examined the stacking interactions of \( N_2 \)-dimethylguanosine with the nucleosides, e.g., adenosine and cytidine, found adjacent to it in certain tRNA’s, by the use of model compounds in which the trimethylene bridge was substituted for the ribose-phosphate-ribose linkage. From the hypochromism exhibited by synthetic \( 9-[3-(\text{aden}-9-y1)\text{propyl}]-2\text{-dimethylaminopurin-6-one} \) (IV) and by \( 9-[3-(\text{cytos}-1-y1)\text{propyl}]-2\text{-dimethylaminopurin-6-one} \) in aqueous solution it is apparent that the interaction is at least as great between the \( N’ \)-dimethylguanine moiety and adenine or cytosine as between guanine and these two bases. The fluorescence and phosphorescence emission spectra were obtained in ethylene glycol–water glass at 80°C. The exciplex fluorescence observed for both bi-molecules (IV and VI) containing the \( N_2 \)-dimethylguanine unit provides further evidence for stacked chromophores.

Modified nucleotides play a role in determining the conformations available to tRNA’s.\(^5\) It is therefore of interest to establish, if possible, the physical basis for the secondary structure-determining role of each of the modified bases. Among these, \( N_2 \)-dimethylguanosine (2Me2Guo or m2G)\(^1\) occupies an important position in many of the yeast tRNA’s for which the base sequences are known. It is the ninth nucleoside component from the first letter of the anticodons,\(^2\) and it occurs at a bend\(^2\) or bulge\(^2\) between adjacent hydrogen-bonded, base-paired, helical regions or “arms” in the clover leaf model. In the extended conformation the helix is not interrupted at m2G.\(^4\) With the exception of torula and baker’s yeast tRNA\(_{\text{Val}}\), \( N_2 \)-dimethylguanosine is contained in all the yeast tRNA’s of presently established structure: tRNA\(_{\text{Ser}}\), tRNA\(_{\text{Hs}}\), tRNA\(_{\text{Le}}\), tRNA\(_{\text{Ph}}\), tRNA\(_{\text{Tyr}}\), tRNA\(_{\text{Ala}}\), tRNA\(_{\text{FMet}}\) also in rat liver tRNA\(_{\text{Ser}}\).\(^13\) By contrast, this dimethylated guanosine has not been found in the corresponding \( E. \text{coli} \) B tRNA’s of presently known sequences.\(^14\–17\) The conformational contribution of \( N_2 \)-dimethylguanosine to a tRNA molecule may be expressed in terms of its lacking the ability to base-pair with cytidine\(^18\) or in a modification of its stacking ability with the nucleosides adjacent to it, i.e., adenosine and cytidine.\(^2,\ 3,\ 7–13\) We have examined the interaction of the modified guanosine with each of these two bases spectroscopically, using model compounds.

Materials and Methods. 2-Dimethylamino-5-nitro-6-propylamino-4(3H)-pyrimidinone: 6-Chloro-2-dimethylamino-5-nitro-4(3H)-pyrimidinone (I)\(^19\) (200 mg
0.92 mmole) was added in portions to 5 ml of n-propylamine with stirring and external cooling. The stirring was continued for 18 hr at 25°, excess n-propylamine was removed in vacuo, and the residual pale yellow solid was suspended in 10 ml of water. The slightly alkaline suspension was neutralized to about pH 6.5 by acetic acid and stirred at room temperature for 10 hr. The resulting pale yellow solid, 207 mg (94%), was recrystallized from ethanol, melting point (mp) 257–259° (dec.); nuclear magnetic resonance (nmr) (TFA) \( \tau \) 6.29 (br, 2, NCH\(_2\)), 6.61 (s, N(CH\(_3\))\(_2\)), 8.20 (m, 2, CCH\(_3\)), and 8.93 (t, 2, CH\(_3\)) \( \delta_{\text{H}}^{\text{N}} \max \) 219 nm (e 12,160), 340 (19,570), \( \lambda_{\text{max}} \) 262 (2680); \( \delta_{\text{N}}^{\text{OCH}} \max \max \) 219 (2612), 339 (19,620), \( \lambda_{\text{max}} \) 262 (2860); \( \delta_{\text{O}} \max \) 356 (22,110), \( \lambda_{\text{min}} \) 258 (1970). \( \text{Anal.} \) Caled. for C\(_{14}H\(_{18}\)N\(_{6}\)O\(_3\): C, 44.80; H, 6.27; N, 29.03. Found: C, 45.07; H, 6.32; N, 28.76.

2-Dimethylamino-9-propylurin-6-one (II, 2Me\(_{3}\)Gu-C\(_6\)) : A solution of 1.0 gm (4.15 mmole) of 2-dimethylamino-5-nitro-6-propylamino-4(3H)-pyrimidinone in 150 ml of 98–100% formic acid to which was added 23 gm of zinc dust was stirred at room temperature for 1 hr and filtered. The filtrate was refluxed for 1 hr under nitrogen and evaporated to dryness in vacuo. The oily residue was dissolved in 250 ml of dry dimethylformamide, and the solution was reduced in vacuo to about 160 ml. Potassium carbonate (1.70 gm) was added, and the resulting suspension was refluxed under nitrogen for 25 hr. After removal of the solvent in vacuo, the residue was dissolved in 50 ml of water and 1 ml of concentrated aqueous ammonia. The deep green solution was neutralized to pH 6.5 by acetic acid and allowed to stand at 5° overnight. The solid which separated was discarded. The mother liquor was evaporated to dryness, and the residue was dissolved in 20 ml of chloroform–methanol (90:10). On cooling at 5° inorganic material was deposited, and the treatment was repeated three times. The residue following solvent removal was dissolved in 2 ml of chloroform–ethanol (95:5) and applied to a column of 35 gm of silica gel packed with the same solvent. The desired product was eluted with chloroform–ethanol (95:5) as a pale yellow solid which was recrystallized from ethyl acetate and twice from water, fine rods, mp 258–259°, yield 255 mg (28%); nmr (CDCl\(_3\) \( \tau \) 2.51 (s, 1, purine H), 6.03 (t, 2, Gu-CH\(_2\)), 6.73 (s, 6, N(CH\(_3\))\(_2\)), 8.13 (m, 2, CCH\(_3\)), and 9.07 (t, 3, CH\(_3\)) \( \lambda_{\text{max}} \) 258 nm (e 16,120), 281 (sh) (9070), \( \lambda_{\text{min}} \) 230 (3660); \( \delta_{\text{N}}^{\text{HCl}} \max \) 261 (16,830), 293 (6620), \( \lambda_{\text{min}} \) 235 (4120), 281 (6000); \( \delta_{\text{O}}^{\text{NaOH}} \max \) 258 (11,580), 279 (9600), \( \lambda_{\text{min}} \) 241 (7560), 273 (9480). \( \text{Anal.} \) Caled. for C\(_{19}\)H\(_{26}\)N\(_{6}\)O: C, 54.28; H, 6.83; N, 31.66. Found: C, 54.62; H, 6.69; N, 31.38.

6-3-(Aden-9-y)propylamino-2-dimethylamino-6-chloropyrimidine (I)\(^{18}\) in 150 ml of absolute methanol prepared by heating was cooled to room temperature, and a solution of 221 mg (2.20 mmole) of triethylamine and 351 mg (1.83 mmole) of 9-(3-aminopropyl)adenine (III)\(^{16}\) in 50 ml of absolute methanol was added. The initially homogeneous solution was heated to 50–60° for 23 hr, at which time 449 mg (64%) of an amorphous, pale yellow powder had precipitated, mp 320–322° (dec.), nmr (TFA) \( \tau \) –1.05 (br, 1, NH), 0.37 (br, 2, NH\(_2\)), 0.74 and 1.23 (2s, 2, purine H\(_2\)), 5.25 (br, 2, Ad-CH\(_2\)), 6.08 (br, 2, Py-CH\(_3\)), 6.58 (s, 6, N(CH\(_3\))\(_2\)), and 7.43 (br, 2, CCH\(_3\)C); \( \lambda_{\text{N}}^{\text{HCl}} \max \) 261 nm (e 13,050), 339 (17,060), \( \lambda_{\text{min}} \) 258 (12,940), 290 (4240); \( \delta_{\text{N}}^{\text{HCl}} \max \) 257 (13,880), 338 (16,280), \( \lambda_{\text{max}} \) 251 (13,820), 291 (4970); \( \delta_{\text{O}}^{\text{NaOH}} \max \) 262 (12,890), 302 (19,830), \( \lambda_{\text{min}} \) 236 (9700), 286 (3550). \( \text{Anal.} \) Caled. for C\(_{19}\)H\(_{26}\)N\(_{6}\)O\(\cdot\)H\(_2\)O: C, 43.86; H, 5.00; N, 36.54. Found: C, 43.98; H, 4.85; N, 36.79.

9-[3-(Aden-9-y)propyl]-2-dimethylaminopurin-6-one (IV, 2Me\(_{3}\)Gu-C\(_6\)-Ad) : The preparation of IV was effected by using the method employed for II, starting with 1.37 gm (3.57 mmole) of 6-[3-(aden-9-y)propylamino]-2-dimethylamino-5-nitro-4(3H)-pyrimidinone. The crude yield was 455 gm (36%), and final purification was effected by recrystallization from methanol and from water with decolorization, mp 327–328°; nmr (TFA) \( \tau \) 0.55, 1.04, and 1.33 (3s, 3, purine H\(_3\)'), 5.29 (br, 4, 2 \( \times \) Pu-CH\(_2\)), 6.63 (s, 6, N(CH\(_3\))\(_2\)), 7.12 (br, 2, CCH\(_3\)C); nmr (0.1–0.2 \( \times \) NaOH, D\(_2\)O) \( \tau \) 2.10, 2.43 (s and 2 overlapping, s, s, purine H\(_3\)'), 5.95 (br, 4, 2 \( \times \) Pu-CH\(_2\)) 7.14 (s, 6, N(CH\(_3\))\(_2\)), and 7.43 (br, 2, CCH\(_3\)C); \( \lambda_{\text{N}}^{\text{HCl}} \max \) 257 nm (e 24,440), \( \lambda_{\text{min}} \) 231 (6300); \( \delta_{\text{N}}^{\text{HCl}} \max \) 259 (26,310), \( \lambda_{\text{min}} \) 234 (7300); \( \delta_{\text{O}}^{\text{NaOH}} \max \) 239 (21,450), \( \lambda_{\text{min}} \) 239 (12,020). \( \text{Anal.} \) Caled. for C\(_{20}\)H\(_{26}\)N\(_{6}\)O\(\cdot\)H\(_2\)O: C, 50.83; H, 5.12; N, 39.52. Found: C, 50.55; H, 5.11; N, 39.64.
6 - [3-(Cytos-1-yl)propylamino]-2-dimethylamino-5-nitro-4(3H)-pyrimidine: This compound was made from I and 1-(3-aminopropyl)cytosine (V) (see paper V of this series*) by the method employed with I and III, yield 53%; pale yellow powder, mp 288-291° (dec.), nmr (TFA) 7.49 (br, 1, NH), 1.90 (br, 2, NH₂), 2.09 and 3.58 (2d, 1 each, Cy-H), 5.9 and 6.1 (overlapping, m, 4, Cy-CH₂, Py-CH₂), 6.58 (s, 6, N(CH₃)₂), and 7.65 (br m, 2, CCH₂C); λ max 277 nm (ε 10,950), 340 (17,840), λₘᵦᵣ 257 (7830), 300 (6060); λ 0.1 N HCl 287 (16,180), 339 (17,770), λₘᵦᵣ 256 (6780), 310 (8870); λ max 276 (10,310), 357 (20,170), λₘᵦᵣ 254 (6310), 302 (6160). Anal. Calcd. for C₁₃H₁₈N₈O₄: C, 51.12; H, 5.59; N, 33.92. Found: C, 51.12; H, 5.59; N, 33.66.

Electronic absorption spectra: The measurements were obtained on a Cary model 15 spectrophotometer as described previously, using dilute aqueous solutions (see paper V of this series*). The spectra of 2Me₂Gu-C₄ (II), 2Me₂Gu-C₄-Ad (IV), Ad-C₄, 2Me₂Gu-C₄-Cy (VI), and Cy-C₄ were determined three times and averaged values of ε were used. The electronic absorption spectra were digitized at intervals of 2.5 nm with a Benson-Lehner Corp. decimal converter model F. Oscillator strengths were calculated by an IBM 7094 computer using a program based on Simpson’s rule.

Emission spectra: These were measured at 80°K in a polar glass of ethylene glycol-water (1:1, v/v), which has been shown to preserve the aqueous room temperature conformation of nucleic acids at low temperatures. Fluorescence and phosphorescence spectra were obtained by means of an apparatus that has been described previously. The experimental uncertainties in the fluorescence quantum yield (Φₑ) and phosphorescence quantum yield (Φₚ) were about 30%. The excitation wavelength was 265 nm.

Results and Discussion. The applicability of trimethylene-bridged heterocyclic bases, B-C₃B', for the evaluation of intramolecular base-base interaction in aqueous solution has been amply demonstrated (see papers I–VII in this series).
The trimethylene bridge is of sufficient length to allow (but not dictate) vertical ring stacking, or partial overlapping, similar to that found in nucleic acids, since the rings can lie in parallel or near-parallel planes. Model compounds in which the trimethylene bridge was substituted for the ribose-phosphate-ribose linkage of adenyl-(3'-5')-N²-dimethylguanylate and cytidylyl-(3'-5')-N²-dimethylguanylate were prepared from a common precursor, 6-chloro-2-dimethylamino-5-nitro-4(3H)-pyrimidinone (I). We turned from previously reported methods of making N²-dimethylguanines in favor of a procedure that relegated the potentially low-yield reaction to the final step in the sequence. Thus, the product of reaction of the substituted pyrimidine I with propylamine was reduced with zinc and acetic acid, and ring closure was effected with potassium carbonate in dimethylformamide to give 2-dimethylamino-9-propylpurin-6-one (II), 2Me²Gu-C³, the model for the N²-dimethylguanine "half" of IV and VI. The latter compounds were made by essentially the same synthetic route, involving initial condensation of I with the appropriate amine, either 9-(3-aminopropyl)-adenine (III) or 1-(3-aminopropyl)cytosine (V). The purity and identity of the compounds were established by analytical and spectroscopic means as indicated in the section on Materials and Methods.

The ultraviolet spectra of 2Me²Gu-C³-Ad (IV) and of the two half molecules, 2Me²Gu-C³ (II) and Ad-C³, 9-propyladenine (see paper V of this series*) were determined in triplicate at concentrations 2.4–5.4 × 10⁻⁵ M in aqueous solution (Fig. 1). A computer-calculated difference curve (ΔΔ) relating the spectrum of IV to the summation of the 2Me²Gu-C³ and Ad-C³ curves indicated a hypochromatic effect for IV extending over the range 230–300 nm, with troughs at 262
and 287 nm, and barely detectable hyperchromism in the region above 300 nm. The hypochromic effect integrated over the entire band as the quantity hypochromism (%H), defined in terms of oscillator strengths, was determined for 2Me2Gu-C3-Ad and the corresponding N-propyl half molecules by computer integration of the electronic absorption spectra from a cutoff wavelength in the vicinity of the absorption minimum to a point of zero absorption at long wavelength (see paper V of this series*).28 The values of the hypochromism are given in Table 1 for dilute aqueous solution: neutral, acidic, and basic. By comparison with the corresponding percentages for Gu-C3-Ad (Table 1) (see paper V of this series*) it is apparent that the N²-dimethyl derivative exhibits at least as much, and probably greater, intramolecular interaction between the guanine and adenine rings. This is in line with findings that introduction of alkyl groups on a purine or pyrimidine favors association,29-32 but is not consistent with the finding that poly N²-dimethylguanylate has lower thermal stability than does poly G.18

The type of association suggested by the magnitude of the hypochromism of 2Me2Gu-C3-Ad in aqueous solution is that in which the relative orientation of the transition moments are parallel.28 When 95 per cent ethanol was used as a solvent, the hypochromism was drastically reduced to 3.3 per cent, corresponding to a reduction of π-electron interaction between the rings in this denaturing solvent.32 In the case of 9,9'-trimethyleisadenine, Ad-C3-Ad, virtually no interaction was detectable in 95 per cent ethanol (see paper V of this series*). The residual hypochromism of 2Me2Gu-C3-Ad in ethanol recalls the observation that poly N²-dimethylguanylate has single strandedness in methanolic solution.18

The ultraviolet spectra of 2Me2Gu-C3-Cy (VI) and of the two half molecules, 2Me2Gu-C3 (II) and Cy-C3, 1-propylectosine, were also determined in aqueous solution (3.9-6.2 × 10⁻⁵ M) (Fig. 2). The hypochromism values (Table 1), indicated that there was greater interaction between the N²-dimethylguanine and cytosine than there was between guanine and cytosine, as in Gu-C3-Cy (see paper V of this series*). The contribution of the modified guanine (or guanosine) to stacking is apparent with both adenine (or adenosine) and cytosine (or cytidine), as judged on the basis of the ultraviolet absorption spectra alone. It is therefore conceivable that dimethylated guanosine plays a role in defining the tertiary structure of tRNA despite the fact that it will be ineffectual for hydrogen-bonded base-pairing.18

This contention is borne out by the character of fluorescence and phosphorescence spectra of the model bi-molecules 2Me2Gu-C3-Cy and 2Me2Gu-C3-Ad. While the absorption spectra of these compounds are the same as those of equimolar mixtures of the constituent molecules to within a few nanometers, their

### Table 1. Hypochromism values (%H).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Water</th>
<th>0.1 N HCl</th>
<th>0.1 N NaOH</th>
<th>95% EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>2Me2 Gu-C3-Ad (IV)</td>
<td>17.6</td>
<td>9.8</td>
<td>15.3</td>
<td>3.3</td>
</tr>
<tr>
<td>2Me2 Gu-C3-Cy (VI)</td>
<td>17.5</td>
<td>10.2</td>
<td>16.6</td>
<td>...</td>
</tr>
<tr>
<td>Gu-C3-Ad</td>
<td>14.9</td>
<td>6.2</td>
<td>10.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Gu-C3-Cy</td>
<td>13.9</td>
<td>6.5</td>
<td>12.4</td>
<td>...</td>
</tr>
</tbody>
</table>

Vol. 65, 1970 BIOCHEMISTRY: IWAMURA ET AL. 1029
fluorescence spectra are red shifted by several tens of nanometers (see Table 2 and Fig. 3). This is indicative of the formation of a complex between the constituent bases in the excited state which is commonly referred to as an exciplex. The appearance of exciplex fluorescence can be taken as evidence for stacked

Table 2. *Emission spectral parameters at 80°K in ethylene glycol-water.*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fluorescence, X_{max}(nm)</th>
<th>Phosphorescence, X_{thr}(nm)*</th>
<th>X_{thr}(nm)*a</th>
<th>T_{p}(sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad-C_{3}</td>
<td>329</td>
<td>0.01</td>
<td>373</td>
<td>0.02</td>
</tr>
<tr>
<td>Cy-C_{3}</td>
<td>316</td>
<td>0.02</td>
<td>353</td>
<td>~0.002</td>
</tr>
<tr>
<td>2Me_{2}Gu-C_{3}</td>
<td>338</td>
<td>0.27</td>
<td>373</td>
<td>0.14</td>
</tr>
<tr>
<td>2Me_{2}Gu-C_{3}-Ad</td>
<td>360*</td>
<td>0.13</td>
<td>375</td>
<td>0.11</td>
</tr>
<tr>
<td>2Me_{2}Gu-C_{3}-Cy</td>
<td>400*</td>
<td>0.08</td>
<td>373</td>
<td>0.10</td>
</tr>
</tbody>
</table>

* X_{thr} is the wavelength of the phosphorescence threshold and is determined by the (0,0) triplet energy level.

a About 20% of 2Me_{2}Gu monomer emission is present.

b About 30% of 2Me_{2}Gu monomer emission is present.

c Depending upon emission wavelength.

chromophores, 21, 22, 34 and while the fluorescence can only be obtained in glasses at low temperature (near 80°K in the present case) this presents evidence for stacking being the preferred relative orientation of 2Me_{2}Gu and Ad or Cy where these chromophores are linked by a trimethylene bridge. On the basis of the fluorescence emission results methylation of the 2-amino group of guanine appears not to alter greatly its stacking properties with Ad and Cy, since exciplexes with similar abundance were observed for Gu-C_{3}-Ad and Gu-C_{3}-Cy (see paper V of this series*).

Triplet energy transfer 34 results from an overlap between the wave functions of donor and acceptor molecules (exchange interaction) and has been estimated to occur at rates greater or comparable to the triplet decay rates (about 1 sec^{-1}) for intermolecular distances less than roughly 8 Å. 35 This explains the fact that the phosphorescence of stacked dinucleotides 36 and of models of dinucleotides similar to the ones being considered here (see paper V of this series*) has been found to be virtually the same as that of the component chromophore with the lower
lying triplet state.‡ Figure 4 and Table 2 show that the phosphorescence of 2Me₂G-C₅-C₃ is indeed the same as that of 2Me₂G-C₅ whose triplet energy, as judged by the short wavelength threshold of the phosphorescence spectrum, lies well below that of Cy-C₅. The energies of the triplet levels of Ad-C₅ and 2Me₂G-C₅ on the other hand are seen to be practically degenerate so that triplet energy transfer between these two chromophores can occur in either direction at comparable rates.³⁴ The phosphorescence spectrum of 2Me₂G-C₅-Ad is indeed found to be virtually the same as the superposition of the spectra of 2Me₂G-C₅ and of Ad-C₅. 

In summary, all of the spectroscopic evidence points to strong stacking interaction of the dimethylguanine unit with either adenine or cytosine, and, while normal base-pair hydrogen-bonding is ruled out for dimethylguanine, there need be no "bend" at m₂G in a tRNA.


† The work at the University of Illinois was supported by a research grant (GM-05829) from the National Institutes of Health, U.S. Public Health Service.

‡ Ad-C₅-ipAd is an exception to this rule. The phosphorescence of the bi-molecule appears to originate from a triplet state complex (see paper III of this series).

³ Abbreviations used in this paper are as follows: 2Me₂Goor m₂G, N⁺-dimethylguanosine; 2Me₂Gu-C₅, N⁺-dimethyl-9-propylguanine; 2Me₂Gu-C₅-Ad, 9-[3-aden-9-yl]propyl]2-dimethylaminopurin-6-one; 2Me₂Gu-C₅-Cy, 9-[3-(cytos-1-yl)propyl]-2-dimethylaminopurin-6-one; Ad-C₅, 9-propyladenine; poly C, poly cytidylic acid; Ad-C₅-ipAd, 9-[3-(aden-9-yl]propyl]-6-(3-methyl-2-butenyl)purine.


23 Davis, R. C., and I. Tinoco, Jr., Biopolymers, 6, 223 (1968), and references therein.
35 Eisinger, J., B. Feuer, and A. A. Lamola, Biochemistry, in press.