Quantitative evaluation of the N_{cap} effect requires introducing position-dependent σ values into models of the helix–coil equilibrium.[6,14] A rough estimate can be obtained by assuming that the N_{cap} interaction affects σ, the helix nucleation constant, rather than s, the helix propagation constant,[6] and fitting a new σ value to the CD data.[14] Substitutions in the middle of the sequence are taken to reflect changes in s values, rather than σ.[14] Table I shows that Ala stabilizes α helix more than Ser by 0.55 kcal/mol in the middle, as in other models,[2b,c] while Ser is 0.74 kcal/mol more stable than Ala at the N_{cap} position. The availability of σ and s values specific for the N_{cap}, C_{cap}, and middle positions of α-helices should lead to improved predictions of helix structure.[1]

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Aberrantly Methylated DNA: Site-Specific Introduction of *N*7-Methyl-2′-deoxyguanosine into the Dickerson/Drew Dodecamer

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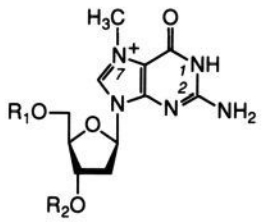
Whereas the products of enzymatic DNA methylation are essential for proper genetic function, most products of nonenzymatic DNA methylation are genotoxic.[1] Adducts arising from aberrant methylation are believed to present a persistent challenge, since most organisms maintain high concentrations of a methylating agent[2,3]—*S*-adenosyl-L-methionine—in the solution containing their DNA.[4] The predominant adduct, *N*7-methyl-2′-deoxyguanosine (m^7dG),[5,6] remains poorly understood at the



m^7dG: R_1, R_2 = H or DNA
m^7dGTP: $R_1 = HP_3O_9$, R_2 = H

molecular level, in part because it has heretofore not been incorporated into DNA site-specifically. M^7dG is not only encountered frequently as a biological lesion but is also widely used as a probe for specific protein–DNA interactions;[7,8] this has intensified the need for its chemical and structural characterization in DNA. Here we report the site-specific insertion of m^7dG into a prototypical duplex oligodeoxynucleotide, the Dickerson/Drew dodecamer.[9]

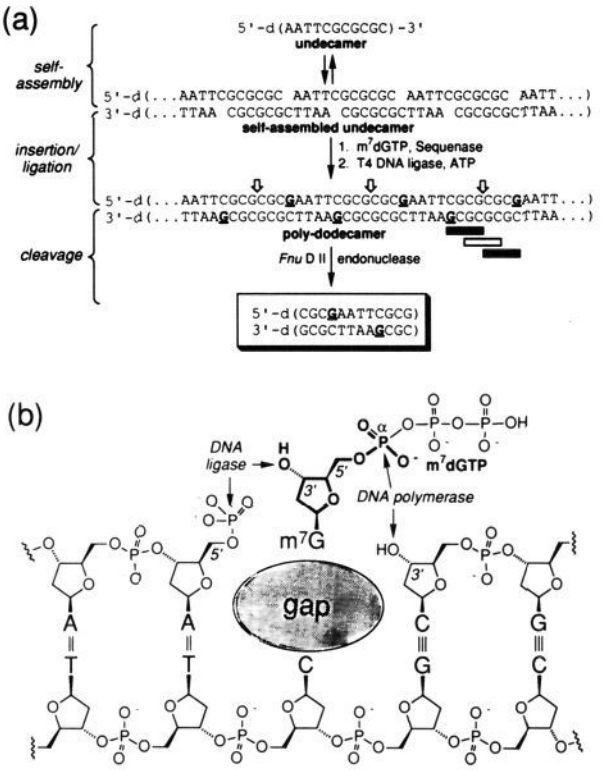


Figure 1. Synthesis of a Dickerson/Drew dodecamer specifically modified with m^7dG. (a) Reaction scheme. Arrows denote sites at which blunt-ended cleavage yields the desired product. Boxes directly under sequence denote overlapping *Fnu*DII sites: unmodified site (unshaded); m^7dG-containing sites (shaded). All oligonucleotides shown in this scheme and elsewhere in the text possess a 5′-phosphoryl substituent; 3′-phosphoryl substituents, if any, are shown explicitly. (b) Structure of the single-nucleotide gaps into which an m^7dG nucleotide unit (bold structure) is inserted enzymatically. The orientation of the strands is reversed from that shown in part a. Arrows denote atoms that are joined by the enzymes indicated.

Table I. Thermal Denaturation of Duplex Oligonucleotides[a]

2 ssDNA ⇄ dsDNA

sequence	T_m (°C)	$\Delta G°$	$\Delta H°$	$T\Delta S°$
5′-d(CGCGAATTCGCG)	63.0	−14.7	−66.8	−52.1
5′-d(CGCGAATTCGCG)	58.5	−14.9	−74.5	−59.6

[a] $\Delta G°$, $\Delta H°$, and $T\Delta S°$ are in units of kcal mol^{-1}. T = 298 K.

An undecamer, 5′-d(AATTCGCGCGC), was designed to self-assemble (by base-pairing) into a multimeric array with single-nucleotide gaps (Figure 1a). The gaps possess all of the functionality required for enzymatic insertion of a single m^7dG residue: 3′-hydroxyl and 5′-phosphate groups, to participate in phosphodiester linkages with an m^7dG nucleotide unit (in bold); and a cytosine (on the opposite strand) to Watson–Crick pair with m^7dG (Figure 1b). Incubation of 5′-d(AATTCGCGCGC) with m^7dGTP[10] and modified T7 polymerase[11] ("Sequenase") afforded the dodecamer 5′-d(AATTCGCGCGCG) (G = m^7dG). This was ligated to yield a polydodecamer (Figure 1a) containing repeating units of the desired product; all that remained was to cleave the

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polydodecamer at a specific site in each repeating unit (Figure 1a, open arrows). The restriction endonuclease *Fnu*DII catalyzes blunt-ended cleavage at 5'-d(CGCG) sites, three of which overlap in each repeating unit (Figure 1a) of the polydodecamer: two possess m^7dG residues (shaded), whereas one is native (unshaded). *Fnu*DII cleavage took place only at the unmodified site, thereby generating the Dickerson/Drew dodecamer, 5'-d(CGCGAATTCGCG).[12-14] The presence and amount of m^7dG in the dodecamer were confirmed by nucleoside composition analysis, and its location within the sequence was confirmed by specific cleavage at the m^7dG site with piperidine.[14]

In thermal denaturation experiments, the duplex half-melting transition (T_m) of the Dickerson/Drew dodecamer was lowered slightly by the presence of m^7dG (Table I). However, analysis in terms of $\Delta G°$, $\Delta H°$, and $\Delta S°$ revealed that, at room temperature, duplex stability in 5'-d(CGCGAATTCGCG) is indistinguishable from that in 5'-d(CGCGAATTCGCG). Moreover, the enthalpy ($\Delta H°$) of duplex formation was made significantly more favorable by the presence of m^7dG, suggesting that the m^7dG·dC base-pair is more stable than dG·dC.[15] The less favorable entropy change ($\Delta S°$) for denaturation of 5'-d(CGCGAATTCGCG) is more difficult to interpret, but may arise from effects on solvation and stacking interactions.[16] Whatever their origins, the small magnitude of these thermodynamic effects strongly suggests that m^7dG does not significantly perturb duplex DNA structure. By way of comparison, N^6-methylation of adenines in DNA reduced T_m by 6-18 °C,[17] yet was shown by X-ray crystallography to have a negligible effect on duplex structure.[18]

We have found that m^7dG is stable for days in duplex DNA at 25 °C (half-life > 1000 h; K.E.-N., unpublished), which contrasts with the reported instability of the free nucleoside.[5,10] This observation leads us to conclude that the probe moiety in dimethyl sulfate[7] and template-directed[8] interference footprinting is m^7dG and not a product of its decomposition. Furthermore, these findings are consistent with the observed persistence of m^7dG residues in genomic DNA.[19] Repair proteins specific for m^7dG are ubiquitous[20,21] although poorly understood mechanistically. The modified dodecamer reported here now provides a homogeneous substrate for studies of recognition and repair by these proteins.

The methodology reported herein should be useful for generating specifically modified DNA molecules that could not be accessed by prior methods.[22]

Supplementary Material Available: Detailed experimental procedures, polyacrylamide gel electrophoretic analysis of reaction products, and an HPLC trace showing the results of nucleoside composition analysis on 5'-d(CGCGAATTCGCG) (6 pages). Ordering information is given on any current masthead page.

(12) This synthesis has been carried out on a ~5-mg scale using overproduced enzymes; this scale is sufficient to service NMR and X-ray crystallography studies.

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A [4 + 3] Transition State for a [4 + 2] Cycloaddition. A New Secondary Orbital Interaction in Diels-Alder Reactions

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Recent reports from this laboratory have detailed the exceptional and unusual characteristics of Diels-Alder reactions of vinylboranes.[1] We describe here some surprising results of ab initio calculations of transition structures for these reactions which shed light on the unusual reactivity and selectivity of vinylboranes and suggest the importance of a new secondary orbital interaction for Diels-Alder reactions in general.

Fully optimized endo and exo transition structures for the reactions of butadiene with vinylborane and vinyldimethylborane were located in ab initio RHF and MC-SCF[2] calculations as shown in Table I. The most striking result of these calculations is the [4 atom + 3 atom] character of the endo transition structures (**1-3**). In each case, C_1 is closer to the boron atom (B) than to C_6. The bonding of C_1 and B in **1-3** is apparent from positive Mulliken overlap populations and pyramidalization of C_1 toward B instead of C_6.[3] In sharp contrast, the exo transition structures (**4** and **5**) are [4 + 2] in character: C_1 is closer to C_6 than to B, and the C_1-C_4-C_5-C_6 dihedral angles are only -5.9° and -4°, compared to 19.8°, 18.0°, and 16.7° for **1-3**, respectively.

Although the activation energies at the 6-31G*//3-21G level are high by ~25 kcal, the predicted endo selectivity and *relative* reactivity of vinyldimethylborane correlate well with experimental observations. Calculations correctly predict that butadiene should react faster with vinyldimethylborane than with acrolein ($\Delta\Delta G^{\ddagger}_{calcd}$ = 2.9 kcal,[4] $k_{rel}^{298} \approx 40$[5]), but slower than with maleic anhydride ($\Delta\Delta G^{\ddagger}_{calcd}$ = -1.6 kcal,[6] $k_{rel}^{298} \approx 0.06$[7]) or Lewis acid-acrolein complexes,[8] despite no allowance for solvent and entropy effects or zero-point energies. The calculations also predict well the endo selectivity of vinyldimethylborane ($\Delta\Delta G^{\ddagger}_{calcd}$ = 2.3 kcal, endo:exo ≈ 95:5 with piperylene). These results suggest that the structural factors responsible for the relative reactivity and endo selectivity of vinyldimethylborane are reflected in the calculations (Chart I).[9]

The preference for endo [4 + 3] and exo [4 + 2] transition structures seems fairly rigid. When the C_3-C_4-C_5-C_6 dihedral angle of the exo vinylborane transition structure was fixed at 15°, the resulting [4 + 3]-like structure (after reoptimization) was 3.3 kcal higher in energy at the RHF-3-21G level. An attempt to obtain a [4 + 2]-like endo transition structure by fixing the C_3-C_4-C_5-C_6 angle at 57° failed; considerable C_1-B interaction

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