$[MoOS]^{2+}$ complexes is highly desirable.

Stabilization of enzymatic $[MoOS]^{2+}$ centers through an active site interaction, possibly with cysteine sulfur or molybdopterin, provides an attractive reconciliation of the extreme reactivity of such groups and their presence in nature. A potential interaction between the $[MoOS]^{2+}$ center and the dithiolene moiety of Mo–co is reflected in recently reported ene-1-perthiolate-2-thiolate-molybdenum(IV) complexes.[15b] We have demonstrated that the stabilization of a $[MoOS]^{2+}$ fragment by a sulfur–sulfur interaction only slightly perturbs the Mo=S bond; it is especially significant that the Mo–S(1) distance in **3** falls within the range of Mo=S distances found in molybdoenzymes and that this range, in turn, does not extend into that established crystallographically for *cis*-oxothio- or monothio-molybdenum complexes.[10,21] Given an O=Mo=S···X fragment in the active site, it would be possible to account for other enzyme behavior by postulating the severing of the S···X interaction upon reduction or initiation of catalysis (through population of Mo=S π^* to produce a nucleophilic S center). Finally, **3** reacts quantitatively with cyanide to produce **1** and SCN^- via a short-lived intermediate; in the presence of water and oxygen this reaction yields **2** in a process which mimics the deactivation of xanthine oxidase upon cyanolysis.[22]

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Supplementary Material Available: Tables of positional parameters, anisotropic thermal parameters, bond distances, and bond angles for **3** (3 pages); tables of observed and calculated structure factors for **3** (7 pages). Ordering information is given on any current masthead page.

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Template-Directed Synthesis: Use of a Reversible Reaction

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DNA replication is fundamental to the storage, transfer, and enactment of the genetic information which defines living organisms. Template-directed reactions which model DNA replication have received a great deal of attention[1-5] and have generally involved the irreversible coupling of activated constituents to form complementary products. In an effort to better define essential features of the DNA polymerization reaction, we have developed a template-directed reaction in which the irreversible coupling step is preceded by an equilibrium between the transiently coupled and uncoupled substrates.[6] This equilibrium allows the thermodynamics of substrate–template association to direct product formation.

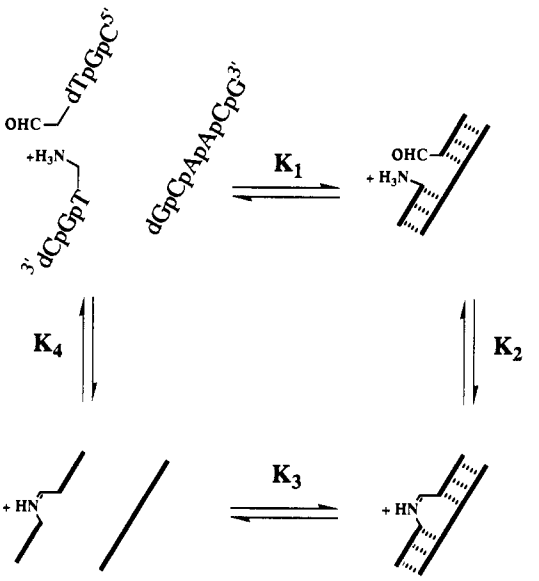
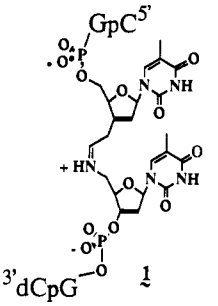


Figure 1. Thermodynamic cycle.

3′-Allyl-3′-deoxythymidine[8] was incorporated through solution-phase synthesis[9] at the 3′-terminus of a DNA trimer and 5′-amino-5′-deoxythymidine[10,11] via solid-phase synthesis[12] at the 5′-end of a separate trimer. The aldehyde was unmasked by oxidation of the allyl group with OsO_4 and $NaIO_4$.[13] The trimers were synthesized and allowed to equilibrate[7] with their imine hexamer product, **1**, under aqueous conditions, both in the absence and presence of the complementary hexamer template. The



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reactants, template, and product can be assigned to a thermodynamic cycle (Figure 1) where the DNA association equilibria were estimated from literature data for the corresponding all-phosphate-linked oligomers:[14] $K_1 = 0.8$ (DNA ternary complex);[14,15] $K_3 = 5 \times 10^5$ (DNA duplex); $K_4 = 10^{-4}$ (imine con-

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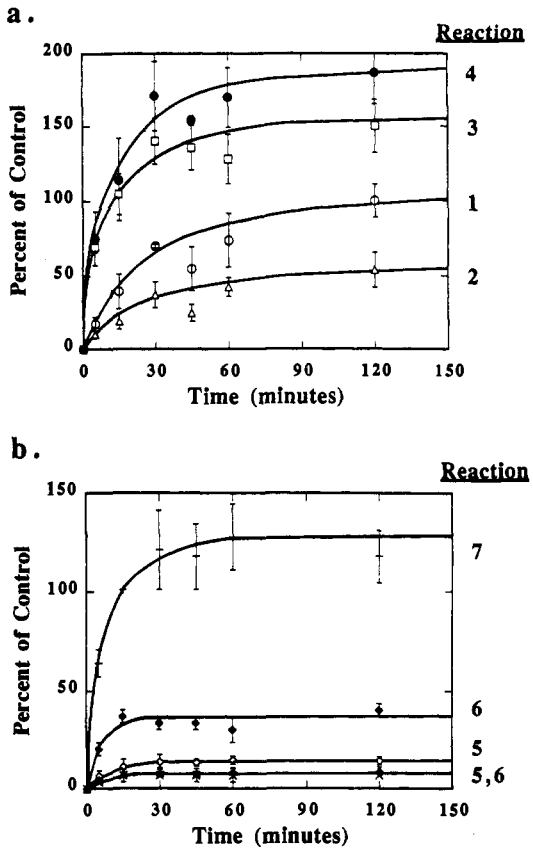

Figure 2. Amine-linked DNA product formation. Reaction conditions: 1 mM in trimers dCGT-CHO (A) and H_2N-dTGC (B) and 1 mM tetramer H_2N-dTTTT (C) and/or template dGCAACG (T) where indicated; 100 mM NaCl, 10 mM NaH_2PO_4, pH 6; 20 equiv of $NaBH_3CN$. (a) Reaction 1 (○): A + B, room temperature. Reaction 2 (△): A + B, 0 °C. Reaction 3 (□): A + B + T, room temperature. Reaction 4 (●): A + B + T, 0 °C. (b) Reaction 5 (◇, hexamer; ▲, heptamer): A + B + C, room temperature. Reaction 6 (◆, hexamer; ×, heptamer): A + B + C + T, room temperature. Reaction 7 (+, hexamer): A + B + C + T, 0 °C, the heptamer product was below the limits of detection. The reactions are normalized against the extent of reaction in the absence of template (reaction 1). All reactions were run in triplicate, and the average normalized concentrations are indicated along with their standard deviations.

densation);[16] the equilibrium constant K_2 for imine formation in the ternary complex was estimated to be ca. 40.[17,18] Due to the kinetic instability of the imine functionality in water, the imine-linked product was reduced with $NaBH_3CN$[19] to yield the corresponding secondary amine-linked DNA hexamer, and the products were monitored by reversed-phase HPLC (Figure 2).

A series of reactions with the modified DNA trimers in the absence and presence of template was used to determine the effects upon amine formation; these reactions involve a competition between imine and aldehyde reduction. Figure 2a shows the production of amine-linked hexamer over a 2-h period. At room temperature approximately 30% of the reactants were converted to the hexamer product, while at 0 °C 15% conversion was attained. In the presence of template at room temperature the amine hexamer yield was 45%, whereas at 0 °C conversion to product was *enhanced* to 65%. Increasing yields of hexamer with decreasing temperature in the presence of template mirrors the increased stability of DNA duplexes with lower temperature;[20,21] the template increased hexamer production at 0 °C by 4-fold.

The increasing stability of the substrate–template complex at lower temperature should then result in increased discrimination against incorporation of noncomplementary substrates into products.[22] We have addressed this possibility by introducing a 5′-amine-DNA tetramer of noncomplementary sequence, 5′-^+H_3N-dTTTT, to compete with the 5′-amine trimer for the available 3′-aldehyde trimer in formation of amine-linked products. As shown in Figure 2b, in the presence of 1 equiv of the competing tetramer the template biased the production of the complementary hexamer over that of the noncomplementary heptamer by approximately 2-fold at room temperature; at 0 °C the bias was increased to greater than 30-fold. Even in the presence of 5 equiv of tetramer at 0 °C the template favored hexamer formation by 20-fold over that of the heptamer product (data not shown).

The utilization of binding energy to enhance reaction rates is one of the central tenets of enzyme catalysis[23] and has been the focus of many model studies.[24-26] Template-directed reactions function in much the same way as these enzyme models by binding and destabilizing the substrates relative to the bound products. In our system the presence of template alters the relative ground-state energies of the substrates to that of the imine such that imine formation becomes more favorable, resulting in the increased rates of complementary amine-linked hexamer formation.[27] The magnitude of the effect in this case is not clear since K_3 is estimated from the all-phosphate duplex,[14] whereas melting curves with the amine-linked hexamer[20,21] suggest that this association is much weaker. Nevertheless, a level of discrimination of better than 0.03 per trimer incorporated was found at 0 °C. By comparison, the replication model of Hong et al.[5a] shows a 2-fold degree of selectivity among competing substrates, whereas DNA polymerase has evolved to utilize the thermodynamics of substrate–template association in a sophisticated manner to provide a level of discrimination approaching 10^{-8}–10^{-12} per nucleotide incorporated into product.[28] The extent and selectivity of reaction in this template-directed model exhibit significant sensitivity to the thermodynamics of the substrate–template association. The introduction of a reversible step therefore holds promise for enhancing thermodynamic control in template-directed synthesis.

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Supplementary Material Available: Details of the estimation of DNA duplex equilibrium constants (2 pages). Ordering information is given on any current masthead page.

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