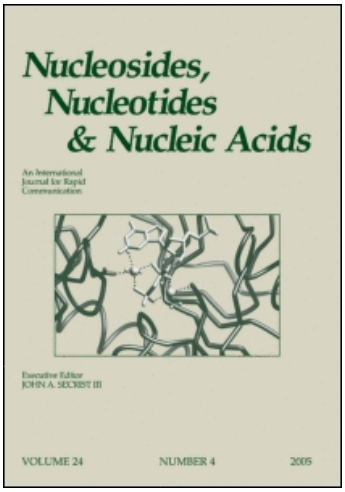

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Novel Inhibitor-Probes of DNA Polymerase III Based on DGTP Analogues of the H_2·HPURA Type: Redesign of the Inhibitor Structure Using an N^2-Benzylguanine Format

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NOVEL INHIBITOR-PROBES OF DNA POLYMERASE III BASED ON DGTP ANALOGUES OF THE H_2·HPURA TYPE:. REDESIGN OF THE INHIBITOR STRUCTURE USING AN N^2-BENZYLGUANINE FORMAT.

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6-(Arylhydrazino)uracils, exemplified by 6-(p-hydroxyphenylhydrazino)uracil (H_2·HPUra; cf., structure in Fig. 1) are potent and highly selective inhibitors of the replicative DNA synthesis of $Gram^+$ bacteria such as *Bacillus, Streptococcus, and Staphylococcus.*[1] The specific inhibitor target in this class of organisms is the replication-specific DNA polymerase, pol III.[2,3] Although formally pyrimidines, the HPUra class of inhibitors are in fact enzyme-specific dGTP analogues which contain a guanine-like H-bonding domain (denoted by "HB" in FIG. 1) and an enzyme-specific, aryl moiety. The HB domain endows the polymerase-bound molecule with the capacity to form a guanine-like base-pair with template cytosine and, thus, sequester the enzyme in a protein:inhibitor:DNA complex (cf., review of inhibitor mechanism in ref 4).

We seek to define, at the 3-D, molecular level, the structure of the dNTP binding site of a $Gram^+$-specific pol III and the basis for its susceptibility to these unique dGTP mimics. To facilitate this definition we have sought to redesign the inhibitor prototype in a guanine format to permit the synthesis of a *bona fide* dNTP form. Our approach to redesign has produced the N^2-substituted guanine, N^2-(3,4-dichlorobenzyl)guanine (DCBG) and its dNTP form, DCBdGTP (cf., FIG. 1 for structures). DCBG was synthesized from 2-Br Hx and 3,4-dichlorobenzylamine (Fig.2 and ref. 5 for this and the subsequent synthetic steps). Its 6-chloro derivative was employed in the synthesis of DCBdG *via* the sodium salt glycosylation, followed by hydrolysis. DCBdGMP was obtained by the phosphorylation of unprotected nucleoside with $POCl_3$ in triethyl phosphate, and DCBdGTP was produced by coupling of DCBdGMP imidazolate with inorganic pyrophosphate in HMPA.

Using *B. subtilis* and its DNA pol III as the model host/target system, we investigated the mechanism of DCBG and DCBdGTP relative to that of the H_2·HPUra prototype; the results are summarized as follows:

1) **DCBG penetrates cells and selectively inhibits DNA synthesis and cell division;** the addition of DCBG to exponentially growing cells immediately inhibits replicative DNA synthesis with no significant effect on the formation of RNA and protein.

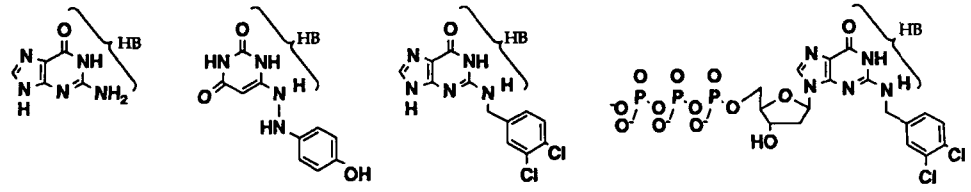

Figure 1

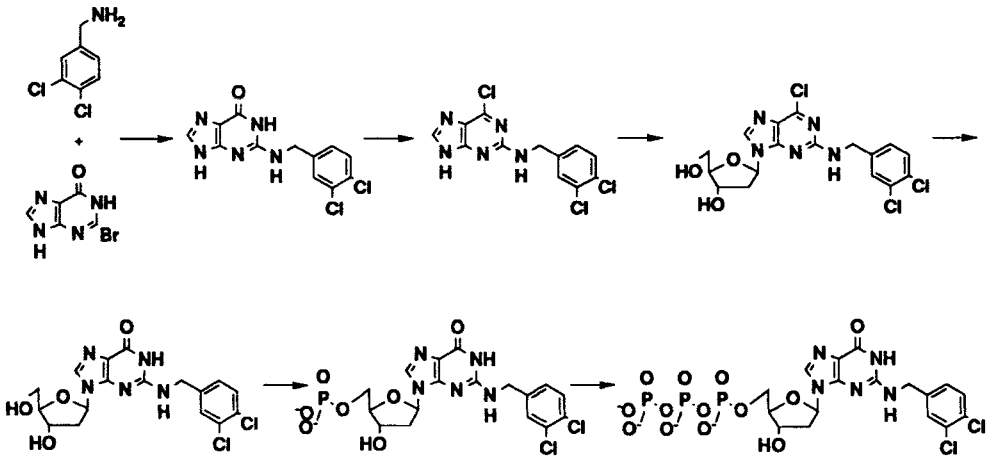

Figure 2. Synthesis of DCBG and DCBdGTP

2) **DCBG acts on isolated pol III with a potency and mechanism essentially identical to that of H_2-HPUra**; it: (a) displays a K_i of 0.5 μM; (b) is specifically competitive with dGTP; (c) requires template cytosine, and, (d) typically induces sequestration of the enzyme in a ternary complex with primer-template.

3) **Relative to its parent base, DCBdGTP acts by an indistinguishable mechanism.** DCBdGTP: (a) with a K_i of 0.1μM, is 5 times more potent than DCBG; (b) is specifically competitive with dGTP; and, (c) sequesters enzyme by the conventional mechanism.

4) **DCBdGTP, despite its strong potential for polymerization, is a very poor substrate for pol III.** Experiments specifically designed to assess pol III-catalysed primer extension of primer DNA with dNTP indicated limited polymerization of DCBdGMP. At a concentration 100 times its K_i, DCBdGTP was at least 50 times less efficient than dGTP as a substrate.

CONCLUSIONS AND FUTURE DIRECTIONS.

We have developed, in the form of DCBG, a guanine formatted, pol III inhibitor which, mechanistically, is the equivalent of the prototypic 6-substituted uracil forms. We have successfully used DCBG to construct DCBdGTP (FIG.2), a potent and non-polymerizable dNTP form displaying an identical mechanism. DCBdGTP will be a unique and powerful tool in the exploration of the structure of the dNTP binding site of its pol III target.

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