Registry No. **11b**, 104706-47-0; **12b**, 100858-33-1; **13b**, 122536-68-9; **14a**, 122536-70-3; **15a**, 122536-72-5; **16a**, 122536-74-7; (±)-**17**, 140629-77-2; **17a**, 122536-76-9; **17b**, 122536-77-0; **18**, 51-35-4; **19a**, 140412-80-2; **20a**, 122536-66-7; **20b**, 140412-68-6; **20c**, 122537-11-5; **20d**, 140412-67-5; **21a** (free base), 140629-71-6; **21b** (free base), 127967-03-7; **21b**·HCl, 138668-47-0; **21c** (free base), 140412-10-8; (*S,R*)-**21e** (free base), 122536-21-4; (*S,R*)-**21e**·*x*HCl, 140412-41-5; (*R,R*)-**21e** (free base), 140412-12-0; (*R,R*)-**21e**·*x*HCl, 140412-42-6; **21f** (free base), 122536-20-3; **21f**·HCl, 122536-48-5; **21g** (free base), 140412-11-9; **21g**·HCl, 122536-42-9; (*S,S*)-**21h** (free base), 122536-95-2; (*S,S*)-**21h**·*x*HCl, 140412-43-7; (*R,S*)-**21h** (free base), 122536-38-3; (*R,S*)-**21h**·*x*HCl, 140412-44-8; **21i** (free base), 140412-13-1; **21i**·*x*HCl, 140412-45-9; (*S,S*)-**21j** (free base), 140438-08-0; (*S,S*)-**21j**·2HCl, 140412-46-0; (*R,S*)-**21j** (free base), 140412-14-2; (*R,S*)-**21j**·2HCl, 140412-47-1; (*S,S*)-**21k** (free base), 140412-15-3; (*S,S*)-**21k**·HCl, 140412-48-2; (*R,S*)-**21k** (free base), 140412-16-4; (*R,S*)-**21k**·HCl, 140412-49-3; (*S,S*)-**21l** (free base), 140412-17-5; (*R,S*)-**21l** (free base), 140412-18-6; **21p**, 122536-91-8; **22a** (free base), 140629-72-7; **22a**·HCl, 140629-75-0; **22b** (free base), 133298-78-9; **22b**·HCl, 140412-50-6; **22c** (free base), 140412-19-7; **22c**·HCl, 133298-73-4; (*S,R*)-**22e** (free base), 140412-21-1; (*S,R*)-**22e**·HCl, 122536-44-1; (*R,R*)-**22e** (free base), 140412-22-2; (*R,R*)-**22e**·HCl, 122536-43-0; **22f** (free base), 122548-08-7; **22f**·3/2HCl, 140412-51-7; **22g** (free base), 140412-20-0; **22g**·HCl, 122536-36-1; (*S,S*)-**22h** (free base), 140412-23-3; (*S,S*)-**22h**·HCl, 122537-08-0; (*R,S*)-**22h** (free base), 140412-24-4; (*R,S*)-**22h**·HCl, 122536-60-1; **22i** (free base), 140412-25-5; **22i**·HCl, 140412-52-8; (*S,S*)-**22j** (free base), 140412-26-6; (*S,S*)-**22j**·2HCl, 140412-53-9; (*R,S*)-**22j** (free base), 140412-27-7; (*R,S*)-**22j**·2HCl, 140412-54-0; **23a** (free base), 140629-73-8; **23g**·2HCl, 140629-76-1; (*S,S*)-**23b** (free base), 140412-28-8; (*S,S*)-**23b**·*x*HCl, 140412-55-1; (*R,S*)-**23b** (free base), 140412-29-9; (*R,S*)-**23b**·*x*HCl, 140412-56-2; (*S,R*)-**23c** (free base), 140412-30-2; (*S,R*)-**23c**·*x*HCl, 140412-57-3; (*R,R*)-**23c** (free base), 140412-31-3; (*R,R*)-**23c**·*x*HCl, 140412-58-4; (*S,S*)-**23d** (free base), 140412-32-4; (*S,S*)-**23d**·*x*HCl, 140412-59-5; (*R,S*)-**23d** (free base), 140412-33-5; (*R,S*)-**23d**·*x*HCl, 140412-60-8; **23e** (free base), 140412-34-6; **23e**·*x*HCl, 140412-61-9; (*S,S*)-**23f** (free base), 140412-35-7; (*S,S*)-**23f**·*x*HCl, 140412-62-0; (*R,S*)-**23f** (free base), 140412-36-8; (*R,S*)-**23f**·*x*HCl, 140412-63-1; **24a** (free base), 140629-74-9; **24b** (free base), 133298-74-5; **24b**·HCl, 140412-64-2; **24c** (free base), 140412-37-9; **24c**·HCl, 140412-65-3; **24d** (free base), 140412-38-0; **24d**·HCl, 122536-56-5; **24e** (free base), 140412-39-1; **24e**·HCl, 122536-55-4; **24f** (free base), 140412-40-4; **24f**·HCl, 140412-66-4; **25**, 100361-18-0; 8-deaza-**25**, 86393-33-1; **26**, 122536-80-5; (*R*)-**26**, 122536-81-6; (±)-**26**, 140630-88-2; (±)-8-aza-**2b**, 140412-77-7; (±)-**26** (6-monofluoro derivative), 140438-09-1; 7-chloro-**26** (free acid), 140412-78-8; 5-amino-7-chloro-**26** (free acid), 140412-79-9; **27**, 127934-52-5; Boc-Ala, 15761-38-3; Boc-Gly, 4530-20-5; Boc-Lys, 13734-28-6; Boc-Phe-(*R*)-NH(3-pyrrolidinyl), 122536-86-1; Boc-Phe-(*S*)-NH(3-pyrrolidinyl), 140412-69-7; Boc-Gly-(±)-NH(3-pyrrolidinyl), 140412-70-0; Boc-Lys-(*R*)-NH-(3-pyrrolidinyl), 140412-71-1; Boc-Lys-(*S*)-NH(3-pyrrolidinyl), 140412-72-2; Boc-Val-(*R*)-NH(3-pyrrolidinyl), 140412-73-3; Boc-Val-(*S*)-NH(3-pyrrolidinyl), 140412-74-4; Boc-Gln-(*R*)-NH(3-pyrrolidinyl), 140412-75-5; Boc-Gln-(*S*)-NH(3-pyrrolidinyl), 140412-76-6.

Structure–Activity Relationships of (+)-CC-1065 Analogues in the Inhibition of Helicase-Catalyzed Unwinding of Duplex DNA†

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(+)-CC-1065 is a potent antitumor antibiotic produced by *Streptomyces zelensis*. Previous studies have shown that the potent cytotoxic and antitumor activities of (+)-CC-1065 are due to the ability of this compound to covalently modify DNA. (+)-CC-1065 reacts with duplex DNA to form a (*N*3-adenine)–DNA adduct which lies in the minor groove of DNA overlapping with a five base-pair region. As a consequence of covalent modification with (+)-CC-1065, the helix bends into the minor groove and also undergoes winding and stiffening. In the studies described here, we have constructed templates for helicase-catalyzed unwinding of DNA that contain site-directed (+)-CC-1065 and analogue DNA adducts. Using these templates we have shown that (+)-CC-1065 and select synthetic analogues, which have different levels of cytotoxicity, all produce a significant inhibition of unwinding of a 3′-tailed oligomer duplex by helicase II when the displaced strand is covalently modified. However, the extent of helicase II inhibition is much more significant for (+)-CC-1065 and an analogue which also produced DNA winding when the winding effects are transmitted in the opposite direction to the helicase unwinding activity. This observed pattern of inhibition of helicase-catalyzed unwinding of drug-modified templates was the same for a 3′-T-tail, for different duplex region sequences, and with the *Escherichia coli rep* protein. Unexpectedly, the gel mobility of the displaced drug-modified single strand was dependent on the species of drug attached to the DNA. Last, strand displacement by helicase II coupled to primer extension by *E. coli* DNA polymerase I showed the same pattern of inhibition when the lagging strand was covalently modified. In addition, the presence of helicase II on single-stranded regions of templates caused the premature termination of primer extension by DNA polymerase. These results are discussed from the perspective that (+)-CC-1065 and its analogues have different effects on DNA structure, and these resulting structural changes in DNA molecules are related to the different in vivo biological consequences caused by these drug molecules.

Introduction

In order for DNA to serve as an efficient substrate for polymerases, the duplex DNA must be unwound. The task of unwinding DNA rapidly is carried out by a special class of enzymes called helicases.[1,2] These enzymes progress in a unidirectional or bidirectional manner through the helix utilizing energy derived from hydrolysis of nucleotide triphosphates to fuel this process. The precise mechanisms of helicase-catalyzed unwinding of DNA are not known, but in general they require a single-stranded region to which the protein initially binds. Helicase-catalyzed unwinding of DNA is important in replication, transcription, recombination, and repair.[3-5] While most in vitro studies

† Abbreviations: DDW, double-distilled water; EDTA, ethylenediaminetetraacetic acid; N, any nucleotide; pu, purine nucleotide; py, pyrimidine nucleotide; ss, single-stranded; TEMED, *N,N,N′,N′*-tetramethylethylenediamine; Tris, tris(hydroxymethyl)aminomethane.

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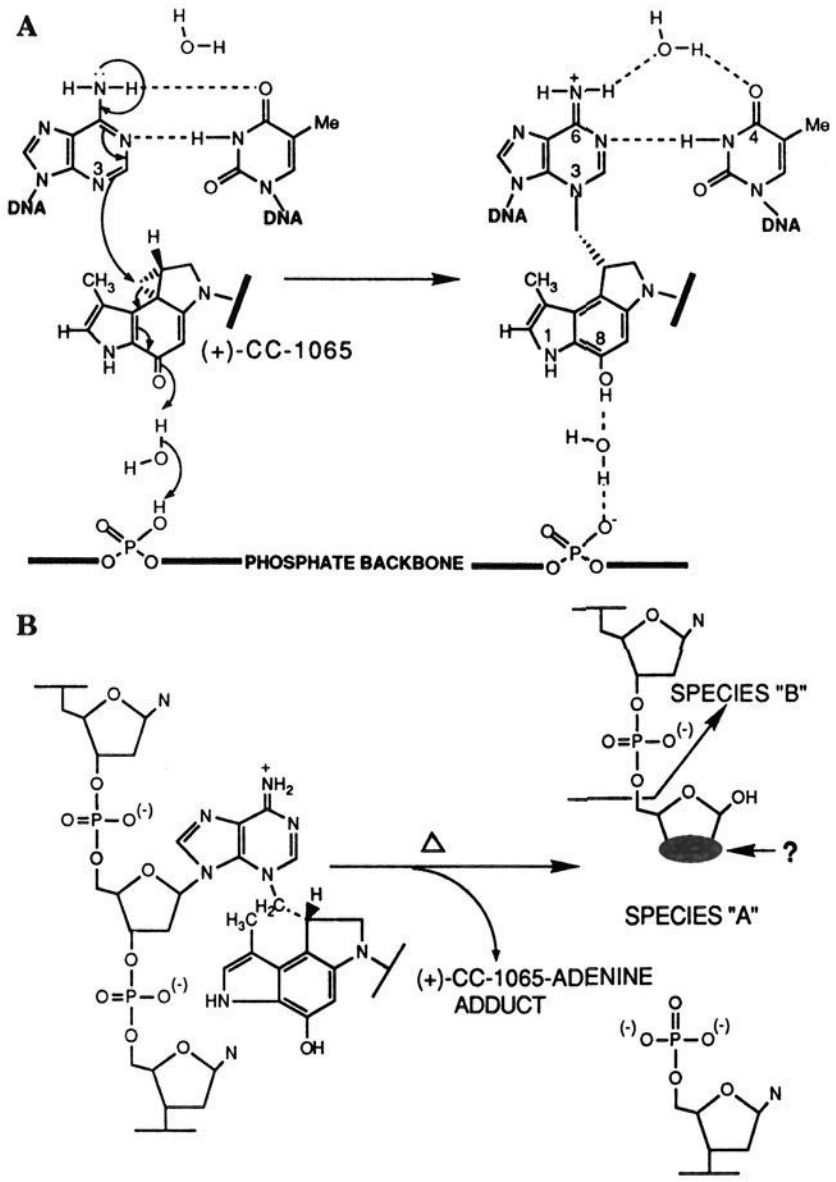

Figure 1. (A) Reaction of (+)-CC-1065 with N3 of adenine in DNA to form the (+)-CC-1065–(*N*3-adenine)–DNA duplex adduct.[15,18] (B) Products of the thermal strand breakage assay.

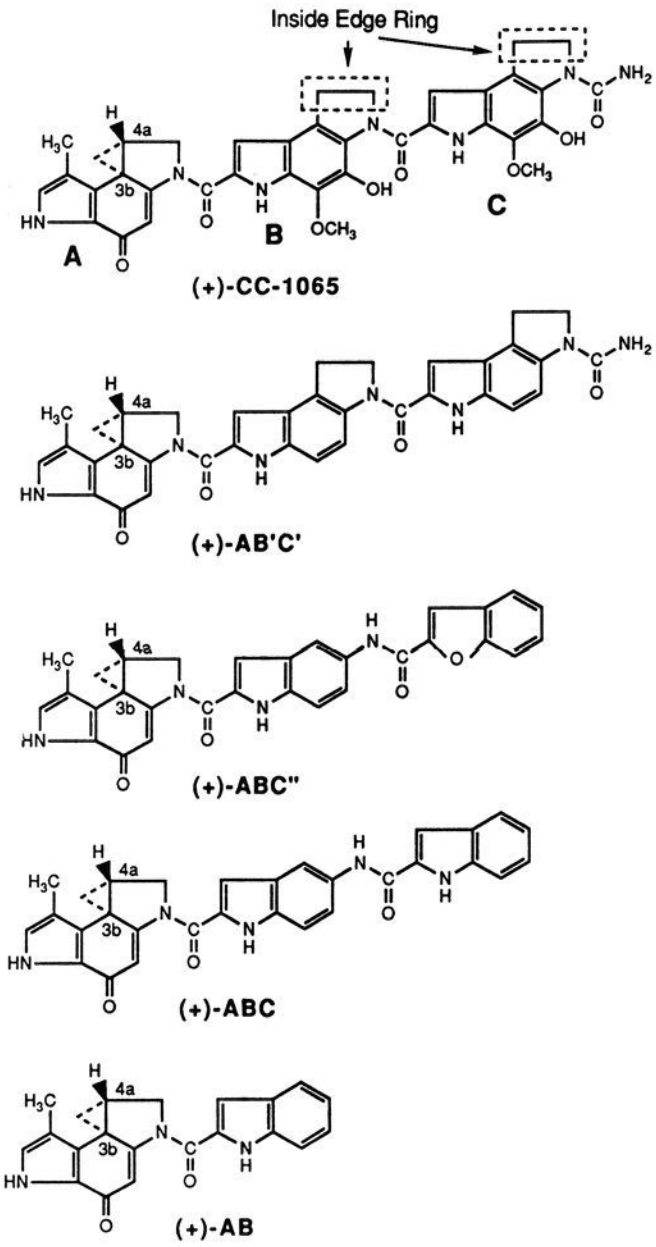

Figure 2. Structures of (+)-CC-1065 and its analogues used in this study.

of DNA helicases involve naked duplex DNA with single-stranded tails, in vivo, the DNA is probably rarely presently as naked DNA, but rather as protein-bound templates.[6]

Both in studies of the molecular mechanisms of carcinogenesis and antitumor drug action, there is a considerable body of published work on recognition and repair of covalently modified DNA and the effect of covalent drug modification of DNA on DNA or RNA polymerase activity.[7-9] In contrast to these extensive studies, very little has so far appeared in the literature on how covalent modification on DNA effects helicase-catalyzed unwinding of duplex DNA. We believe this is a serious omission, since, according to current models of replication and recombination, the helicase is likely to be the first component of the "protein machines" to encounter the modified DNA.[1-3] Consequently, if the covalently modified DNA stalls the helicase and subsequently inhibits unwinding of duplex DNA, the direct effect of the DNA lesion on DNA polymerase activity becomes irrelevant.

As an initial approach to addressing this deficiency, we have constructed stable DNA templates for helicase-catalyzed unwinding that contain site-directed (+)-CC-1065 and related drug–DNA adducts. (+)-CC-1065 is an extremely potent antitumor antibiotic produced by *Streptomyces zelensis*.[10] It is active against several experimental murine tumors in vivo and is about 100 times more potent than adriamycin against a broad spectrum of tumors in the cloning assay.[11-13] There is overwhelming

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Table I. Sequences Used in This Study[a]

TD1	
5′-AATCCGTAATCATGGTCTCAGTTACGTAGATCACGAGATCAGCGCTAGCAGT-3′	
3′-TTAGGCATTAGTACCAGAG-5′	
TD2	
5′-GCGGGATTAGGGGCGGGATGGAGTTACGTAGATCGAGATCAGCGCTAGCATG-3′	
3′-CGCCCTAATCCCCGCCCTACC-5′	
TD3	
5′-TGAATCCGTAATCATGGTCATTTTTTTTTTTTTTT-3′	
3′-ACTTAGGCATTAGTACCAGT-5′	
TD4	
5′-TGACCATGATTACGGATTCATTTTTTTTTTTTTTT-3′	
3′-ACTGGTACTAATGCCTAAGT-5′	
GD1	
5′-AATCCGTAATCATGGTCTCAGTTACGTAGATCACGAGATCAGCGCTAGCAGT-3′	
3′-TTAGGCATTAGTACCAGAG-5′	3′-CTCTAGTCGCGATCGTCA-5′
GD2	
5′-ACCATGATTACGGATTGAGAGTTACGTAGATCACGAGATCAGCGCTAGCAGT-3′	
3′-TGGTACTAATGCCTAACTC-5′	3′-CTCTAGTCGCGATCGTCA-5′

[a] Covalent modification sites are shown in boldface.

evidence that DNA is the principal biological target of (+)-CC-1065 and that the alkylating properties of this drug molecule are believed to be responsible for its potent cytotoxic effect.[13,14] Previously we have shown that (+)-CC-1065 forms a covalent adduct with duplex DNA through N3 of adenine[15] in a highly sequence specific manner,[15,16] in which the drug molecules lie within the minor groove covering a three and one base-pair region to the 5′- and 3′-sides, respectively, of the covalently modified adenine (Figure 1).[17] (+)-CC-1065 is the first DNA-reactive drug in which the involvement of critically ordered water molecules has been demonstrated and catalytic activation of the covalent reaction involving phosphate has been implicated (Figure 1).[18] However, in addition to DNA-alkylating properties, these drug molecules induce local bending, winding, and helix stiffening of DNA molecules, which can be attributed to both the covalent bonding and associated binding interactions.[19]

In previous studies we have examined how (+)-CC-1065 and the structurally related drugs shown in Figure 2 affect DNA polymerases and T4 DNA ligase.[20,21] The activity of these enzymes is highly sensitive to changes on DNA structures, so that it is possible to differentiate between the subtle difference induced in DNA structure by different drug molecules in the (+)-CC-1065 group of antitumor agents. We have recently demonstrated that the DNA-helix stabilizing and stiffening effect of select analogues of (+)-CC-1065 induces both proximal and distal inhibition of the DNA ligation reaction mediated by T4 DNA ligase, and that the winding effect of (+)-CC-1065 exerts a more significant inhibitory effect on the proximal ligation reaction.[20] In addition, we have found that (+)-CC-1065 and its structurally related analogue–DNA adducts act as strong blocks against the progression of DNA polymerase through the adduct site, which may partly explain the molecular mechanism of their cytotoxic effects.[21] We have also recently shown that (+)-CC-1065 strongly inhibits the DNA-unwinding process that is mediated by helicase II and T4 dda helicases, because the covalently modified DNA template traps the helicases.[22] To determine which of the structural consequences of covalent bonding or binding interactions produces this effect, we have now compared (+)-CC-1065 with the four analogues shown in Figure 2 and also examined how helicase II unwinding of gapped duplex adducts can effect the DNA polymerase I primer extension reaction. These (+)-CC-1065 analogues are available to us through a highly successful synthetic program at the Upjohn Co. in which one of these analogues [(+)-ABC″ or adozelesin] has been introduced into Phase I clinical trials.[25] This compound has superior in vivo antitumor activity and lacks the de-

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layed lethality associated with (+)-CC-1065 and (+)-AB'C'.[23-25]

In accord with our previous studies,[22] the results described in this paper clearly demonstrate that unwinding of a 3'-tailed oligomer duplex was significantly inhibited when (+)-CC-1065 was covalently bound to the displaced strand. The amount of helicase inhibition is much more significant for (+)-CC-1065- and (+)-AB'C'-modified templates than with the other templates modified with other analogues that lack the DNA-winding effects. Also, strand displacement coupled to primer extension by *Escherichia coli* DNA polymerase showed the same pattern of inhibition when the lagging strand was covalently modified. We discussed the possibility that because (+)-CC-1065 and its analogues have different effects on DNA structure, some of these drug-induced structural changes in DNA molecules (i.e., winding) can be associated with unique biological consequences (i.e., delayed lethality) caused by some of these drug molecules.

Experimental Section

Chemicals and Enzymes. (+)-CC-1065 and the synthetic analogues (Figure 2) used in this study were provided by J. Patrick McGovren at the Upjohn Co. (Kalamazoo, MI). Electrophoretic reagents [acrylamide, TEMED, ammonium persulfate, and bis-(acrylamide)] were purchased from Bio-Rad. Other chemicals for chemical DNA sequencing were from Aldrich Chemical Co.; [γ-^{32}P]ATP was from ICN; and X-ray film, intensifying screens, and developing chemicals were from Kodak. UvrD gene product (helicase II) and *E. coli rep* protein were generously provided by Professor Kodadek at the University of Texas (Austin, TX). The Klenow fragment, *E. coli* DNA polymerase I, T4-polynucleotide kinase, and reagents for dideoxy-DNA sequencing were from United States Biochemical.

Oligonucleotide Synthesis. The oligonucleotides that were annealed to form the tailed or gapped duplexes (Table I) were synthesized on an automated DNA synthesizer (Applied Biosystem 381A) by the phosphoramidite method. The oligomers were then deprotected separately by heating at 55 °C overnight with saturated ammonium hydroxide, dried under vacuum, and redissolved in DDW.

Construction of Tailed and Gapped Duplexes. 3'-Single-stranded-tailed duplexes (TD1, TD2, TD3, and TD4 in Table I) were designed to have a unique drug bonding site (5'-GATTA*-3'; * indicates drug-modified adenine) within the duplex region. Drug molecules can form covalent adducts with either the short (TD1 and TD3) or the long (TD2 and TD4) strand. For the gapped duplexes (GD1 and GD2), the drug bonding site was designed to be within the left duplex region so that either the short (GD1) or the long (GD2) strand can be selectively covalently modified with drug molecules.

To construct 5'-^{32}P labeled substrates, the long strands of TD2 and TD4, the short strands of TD1 and TD3, or both short strands of GD1 and GD2 were kinated separately for 1 h at 37 °C in 25 μL of solution containing 70 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM dithrothreitol, 30 μCi of [γ-^{32}P]ATP, and 10 units of T4 polynucleotide kinase. Reaction mixtures were heated at 95 °C for 10 min to inactivate the remaining T4 polynucleotide kinase and annealed to the complementary cold strand to generate the desired tail or gapped duplexes. The resulting tailed and gapped duplexes were purified from 8% nondenaturing polyacrylamide gel after electrophoresis, according to the method previously described.[19]

Construction of Drug-Modified Templates and Thermal Strand Breakage Assay. To construct drug-modified substrates for helicase assay, tailed or gapped duplexes were modified with the drug molecules, shown in Figure 2, as previously described.[19] Unbound drug molecules were removed by ethanol precipitation. For the thermal strand breakage reaction, drug-modified substrates were heated in DDW for 2 h at 95 °C, dried under vacuum, and redissolved in sequencing dye [90% (w/v)] formamide in 0.1 M Tris-HCl (pH 7.8)]. After heating of the DNA samples for 3 min at 95 °C, samples were applied to a 12% denaturing polyacrylamide gel electrophoresis and run in parallel with Maxam and Gilbert DNA sequencing reaction.

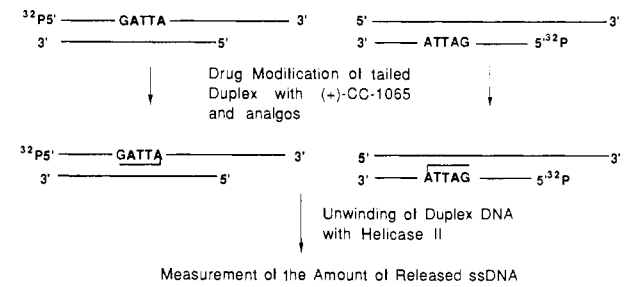


Figure 3. Strategy for the construction of two differently oriented tailed drug–DNA duplex adducts to evaluate the effect of drug modification on the DNA unwinding catalyzed by DNA helicases.

Helicase II-Catalyzed Unwinding of Drug-Modified or Non-Drug-Modified Tail or Gapped Duplexes. Each reaction was carried out in 10 μL of helicase II unwinding buffer containing 10 ng of DNA substrate and the indicated amount of helicase II or *E. coli rep* helicase. After preincubation for 5 min, an 8 molar excess of unlabeled ss DNA (the same strand as the labeled strand) was added to the reaction as a trap to prevent the released labeled strand from reannealing. Each reaction was incubated for 45 min at 30 °C and then quenched with helicase stop buffer. Helicase II and *E. coli rep* protein unwinding buffer consists of 25 mM Tris-HCl (pH 7.5), 25 mM NaCl, 10% glycerol, 5 mM β-mercaptoethanol, 25 mM $MgCl_2$, 100 μg/mL bovine serum albumin, and 1.5 mM ATP. An ATP-regeneration system including 10 mM creatine phosphate and 1 unit/mL creatine phosphokinase was also present. Helicase stop buffer is 10 mM EDTA and 2% sodium dodecylsulfate.

DNA Polymerase Reaction. Template DNA (gapped duplex, 20 ng) was incubated with 1 unit of Klenow or *E. coli* DNA polymerase I in 20 μL of helicase II unwinding buffer in the presence of the indicated amount of dNTPs. Reactions were quenched by the addition of a half-volume of sequencing dye [90% formamide, 100 mM Tris-HCl (pH 7.8), and 10 mM EDTA].

Results

Design and Construction of Tailed Duplex Adducts To Evaluate the Effect of Drug Modification on the Unwinding Reaction Catalyzed by Helicase II. *E. coli* helicase II is a ss DNA-dependent ATPase with helicase activity that translocates progressively along ss DNA in a 3'- to 5'-direction.[26] In order to evaluate the effect of drug modification of DNA on the efficiency of helicase II, partial duplexes were constructed to have a 3' ss tail and to contain a unique drug bonding site (5'-GATTA*-3', where * represents the drug modification site) on either the long or the short strand. The rationale for using both substrates (i.e., drug modification on either the long or short strand) was gleaned from our previous study,[20] which showed that the drug-induced winding and helix-stabilizing effects on DNA molecules occurred predominantly to the 5'-side of the covalently modified adenine (i.e., toward the drug overlap site). Thus, if drug molecules are positioned in both orientations relative to the direction of helicase translocation, it is possible to test the differential effect of drug orientation on helicase II-catalyzed unwinding of DNA. In this study, we have also included select analogues of (+)-CC-1065 (see Figure 2) to attempt to relate these results to the differential biological activities of these drug molecules. In order to measure in the same experiment both the helicase unwinding activity and the extent and selectivity of drug modification at the desired site (using the thermal strand breakage assay), only the drug-modified strands were labeled with γ-^{32}P at the 5'-end. The overall

(26) Matson, S. *Escherichia Coli* Helicase II (Uvr D Gene Product) Translocates Unidirectionally in a 3' to 5' Direction. *J. Biol. Chem.* **1986**, *261*, 10169–10175.

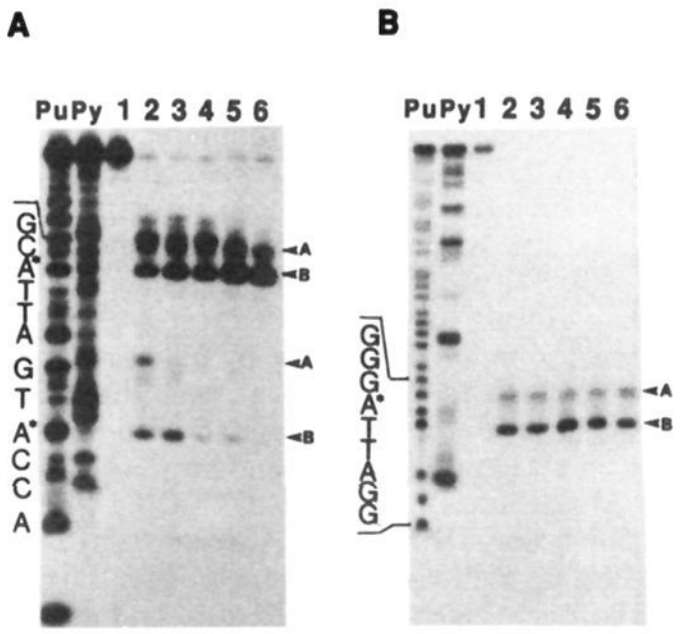


Figure 4. Autoradiograms showing the thermally induced strand breakage assay[15] for determination of the location and extent of drug modification of oligomers TD1 (panel A) and TD2 (panel B). Pu and Py represent purine-specific and pyrimidine-specific reactions, respectively. For panels A and B, lane 1 contains the non-drug-modified oligomer and lanes 2–6 contain (+)-CC-1065-, (+)-AB′C′-, (+)-ABC″-, (+)-ABC-, and (+)-AB-modified oligomers, respectively. The 5′-^{32}P-labeled DNA samples were prepared and subjected to thermally induced strand breakage as described in Experimental Section. The drug-bonding sequences are shown to the left of each panel and the (*) pinpoints the covalently modified adenine. The higher and lower molecular weight material corresponds to species A and B in Figure 1.

strategy for the construction and execution of this experiment is shown in Figure 3.

Characterization of Drug–Oligonucleotide Adducts Using the DNA Thermal Strand Breakage Assay.[15] Upon thermal treatment of (+)-CC-1065 and its analogue (*N*3-adenine)–DNA adducts, cleavage of the *N*-glycosidic bond and subsequent backbone cleavage occurs to the 3′-side of the covalently modified adenine to leave a 5′-phosphate on the 3′-side of the break and, presumably, a modified deoxyribose on the 5′-side. Further thermal treatment or piperdine treatment of 5′-labeled substrates causes release of a modified deoxyribose to produce a product identical to that of a Maxam and Gilbert adenine sequencing reaction (Figure 1).[15] This strand breakage assay was used to identify the drug-modified adenine and to monitor the extent of drug modification at the desired site. As shown in Figure 4A, thermal treatment induced almost quantitative strand cleavage of drug-modified TDI oligomers (lanes 2–6) at the expected site (5′-GATTA*-3′) on the DNA backbone, while non-drug-modified single-stranded DNA (lane 1) remained intact after this treatment. In oligomer TD2 (Figure 4B), exclusive bonding at 5′-GATTA* was observed for each of the drugs except for (+)-CC-1065 or (+)-AB′C′ (lanes 2 and 3, respectively), where minor bonding of these drug molecules to 5′-GACCA*-3′ occurred. However, for the latter cases, the possibility that these two drug molecules were bound to the same DNA molecule can be excluded, since the two bonding sites are too close together for both drug molecules to form covalent adducts on the same template molecule.

Determination of the Optimum Time To Add Chase DNA To Exhibit Maximum Helicase Unwinding Activity. The enzymatically released ss DNA induced by the unwinding activity of helicase II will be readily reannealed to the complementary strand to reform duplex molecules. To circumvent this problem, an 8 molar excess of nonlabeled ss DNA (the same strand as the labeled strand) was included to act as a trap to compete with the released labeled strand for reannealing. The optimum time to add cold chase DNA to show the maximum amount of unwinding activity was determined by the addition of ss DNA at 5, 10, 20, 30, and 50 min. The results show the optimum time to be 5 min after mixing helicase II and substrate (Figure 5).

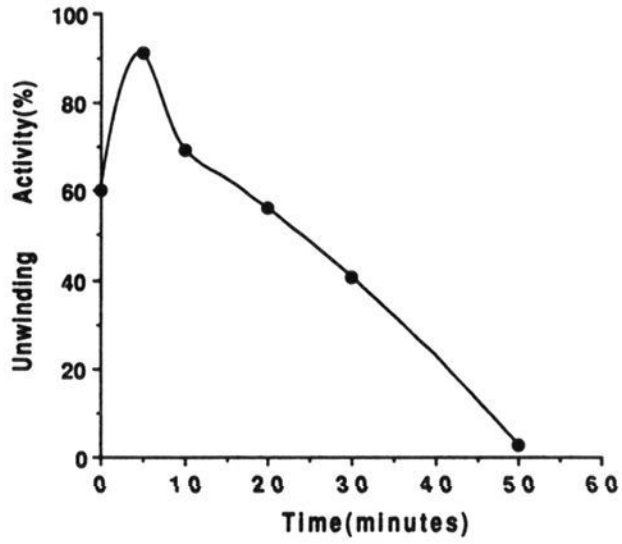
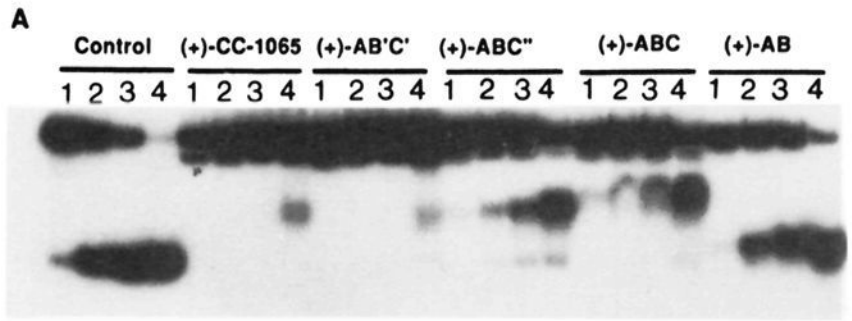
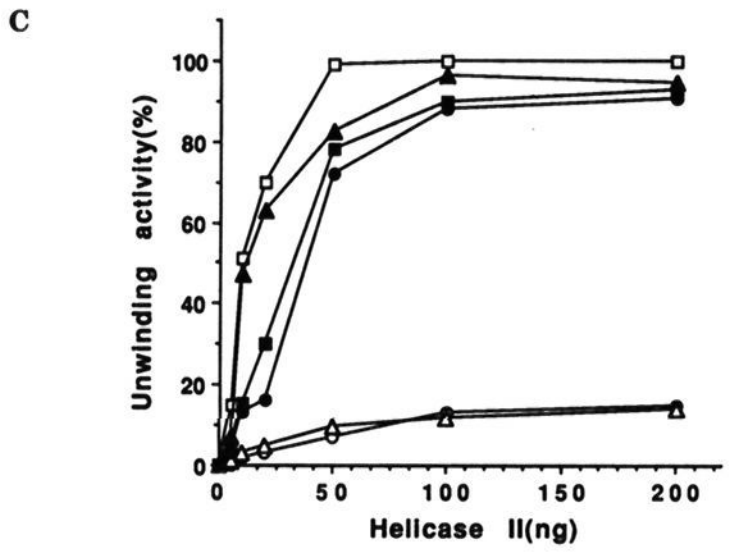


Figure 5. Determination of the optimum time to add chase DNA after addition of helicase II to show maximum helicase unwinding activity. Five nanograms of chase DNA was added after 0, 5, 10, 20, 30, and 50 min to the reaction mixture containing 10 ng of helicase II and 10 ng of substrate TD3, and the amount of unwinding was determined as described in Experimental Section.

Helicase II-Catalyzed Unwinding of Drug-Modified or Non-Drug-Modified Oligomers. The effect of (+)-CC-1065 and related drugs on helicase II-catalyzed unwinding of DNA following covalent modification of TD1 and TD2 is shown in Figure 6A,B. Figure 6A,B shows the gel analysis using increasing amounts of helicase II, and these results are also graphically represented using the densitometric scanning results of the gels, which are plotted in Figure 6C,D. Parts A and C of Figure 6 show the results from TD1, in which the drug winding and stiffening effects are orientated in the opposite direction to helicase II unwinding, while parts B and D represent the drug-induced effects oriented in the same direction relative to the helicase II unwinding direction. Irrespective of orientation, all of the drugs produced a pronounced inhibition of helicase II unwinding of DNA; however, (+)-CC-1065 and (+)-AB′C′ produced a more significant inhibitory effect in TD1 than in TD2 and than with the other analogues (Figure 6A,B). This more pronounced effect of (+)-CC-1065 and (+)-AB′C′ in inhibition of helicase II-catalyzed unwinding of TD1 is also revealed by the densitometric analysis (Figure 6C), where it is also evident that (+)-ABC″ and (+)-ABC produce moderate inhibitory effects at the lower helicase II levels. In the case of the drug orientation where the winding and stiffening effects are in the *same direction* of helicase II unwinding (Figure 6B,D), and drug-induced effects are more uniform (i.e., no pronounced effect of (+)-AB′C′ and (+)-CC-1065, relative to (+)-ABC and (+)-AB), but like the results in Figure 6A,C the drugs produce a moderate inhibition at low helicase II levels. Overall, what is particularly striking about these results is the selective inhibitory effect of (+)-CC-1065 and (+)-AB′C′ on helicase II when they are orientated so that the winding effect of the drug molecules is *opposite* to the helicase II unwinding direction. It is unlikely that the thermal stabilizing effect of drug molecules on duplex DNA (i.e., Δ Tm) is primarily responsible for this inhibitory effect, since both (+)-AB′C′ and (+)-ABC″, which have quantitatively *different* inhibitory effects on helicase II unwinding, produce relatively *the same* increase in Tm of duplex DNA.[16]

Gel Mobility of the Released Drug-Modified Single-Stranded DNA following Helicase II-Catalyzed





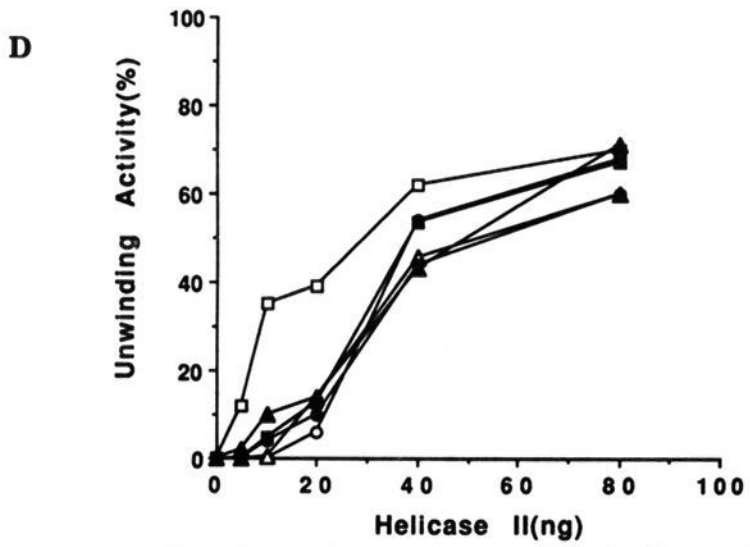


Figure 6. Gel electrophoresis (panels A and B) and kinetics (panels C and D) of the helicase II-catalyzed unwinding of drug-modified and non-drug-modified oligomers. The DNA substrates used in the helicase reaction were oligomers TD1 (panel A and graph C) and TD2 (panel B and graph D), prepared as shown in Figure 3 and described in Experimental Section. In panel A, lane 1 is without helicase II and lanes 2, 3, and 4 contain 10, 50, and 100 ng of helicase II, respectively; and in panel B, lane 1 is without helicase II and lanes 2, 3, and 4 contain 20, 40, and 80 ng of helicase II, respectively. DNA samples were electrophoresed on 8% nondenaturing polyacrylamide gel (acrylamide, bisacrylamide 29:1) for 3 h at 0.7 V/cm. Graph C shows the kinetic analysis of unwinding of non-drug-modified and drug-modified oligomer TD1 using 0, 10, 20, 50, 100, and 200 ng of helicase II. Graph D shows the same experiment as shown in C, but using oligomer TD2 (□ = control, ○ = (+)-CC-1065, △ = (+)-AB′C′; ● = (+)-ABC″, ■ = (+)-ABC, ▲ = (+)-AB).

Unwinding Is Dependent on the Species of Drug Attached to DNA. Following unwinding of drug-modified DNA by helicase II, it was observed that the ss DNA showed a gel mobility on nondenaturing polyacrylamide gel electrophoresis that was dependent on the species of drug attached (panel B of Figure 7). This drug dependency of gel mobility could be eliminated by heating the samples at 95 °C for 1 min (panel C of Figure 7, lanes 1–6). Further thermal treatment induced the expected strand breakage[15] at the drug modification site (panel D of Figure 7, lanes 2–6). We propose that the drug-modified ss DNA is converted into a drug-dependent secondary structure during unwinding of the duplex by helicase II, which is disrupted at high temperature, so that the unique secondary structure, which is associated with abnormal gel mobility, is no longer drug dependent.

Drug Modification of Oligo-dT-Tailed Partial Duplexes Showed a Similar Pattern of Inhibition of Unwinding by Helicase II. To investigate the generality of the inhibitory effects of (+)-CC-1065 and analogues on helicase II-catalyzed unwinding of DNA, two further tailed duplexes each having oppositely orientated drug molecules were constructed. These tailed duplexes (TD3 and TD4 in Table I) differed from TD1 and TD2 in that they had 3′-T-tails and different duplex region sequences. Overall, the results (Figure 8A,B) show the same pattern of drug-induced inhibition of helicase II-catalyzed unwinding of DNA. In particular, the most marked inhibition was found with (+)-CC-1065 and (+)-AB′C′ when the drug-induced winding effects were orientated in the *opposite* direction to helicase II unwinding (Figure 8A). This parallels the observation made with the other pair of tailed duplexes (see before). It is also important to note that the 3′-T-tailed partial duplexes are more favorable substrates for helicase II-catalyzed unwinding than partial duplexes containing random sequences on the 3′-tailed region. In the case of 3′-T-tailed partial duplexes, the extent of the inhibitory effect on helicase II-catalyzed unwinding caused by drug modification is also dependent on the orientation of drug molecules in the duplex region.

Duplex Unwinding Catalyzed by *E. coli rep* Protein Also Shows the Same Pattern of Inhibition Produced by Drug Modification of DNA. Like helicase II, the *E. coli rep* protein also translocates in a 3′- to 5′-direction with respect to the DNA strand to which it is bound.[27] *rep* protein was also evaluated in this investigation, since this protein has an important function in replication of *E. coli* DNA. The results show that when *rep* protein was used to catalyze the unwinding of drug-modified substrates, the same general pattern of inhibition as previously shown for helicase II was produced by each of the drug molecules (Figure 9). Taken together, these results suggest that the pattern of (+)-CC-1065-mediated inhibition of unwinding of duplex DNA drug may be common to other DNA unwinding proteins such as phage T7 gene 4 protein, phage gene 41 protein, and SV40 T antigen.

Helicase II-Catalyzed Unwinding of Gapped Duplexes Is Also Inhibited by Drug Modification. The gapped duplexes GD1 and GD2 (Table I and Figure 10) were designed to determine whether primer extension by polymerase I would be facilitated by the combined action of helicase II. Drug-modification sites are within the left duplex region so that either the short (GD1) or the long (GD2) strand can be selectively covalently modified with drug molecules. After drug modification, the drug-bonding sites were confirmed by the strand-breakage assay.[15] In oligomers GD1 and GD2, exclusive drug bonding at 5′-GATTA*-3′ was achieved for all of the drugs except

(27) Yarranton, G. T.; Gefter, M. L. Enzyme-Catalyzed DNA Unwinding: Studies on *Escherichia coli rep* Protein. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 1658–1662.

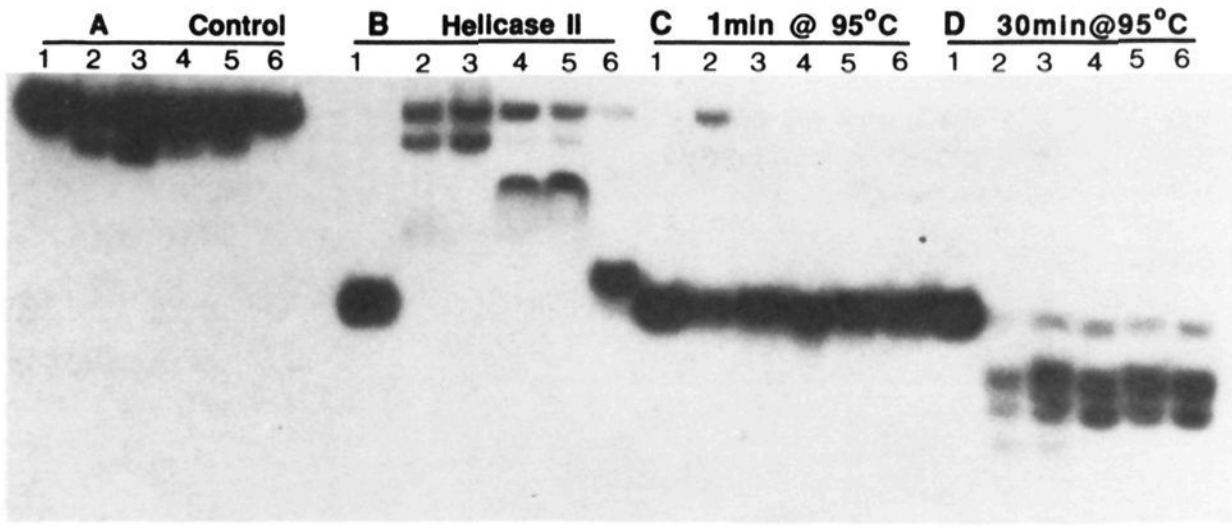


Figure 7. Demonstration of dependence of the gel mobility of the enzymatically released drug-modified ss DNA on the species of drug attached to DNA. Reactions in panels A–D contain 10 ng of non-drug-modified, (+)-CC-1065-, (+)-AB′C′-, (+)-ABC″-, (+)-ABC-, and (+)-AB-modified oligomer TD1 in lanes 1–6, respectively. Panel A is without helicase II. Panels B–D were incubated with 100 ng of helicase II at 30 °C for 50 min, and then the reaction mixtures were electrophoresized on 8% nondenaturing polyacrylamide gel without further treatment (panel B), with heating at 95 °C for 1 min (panel C) or with heating at 95 °C for 30 min. Duplicate bands at the top of the gel for (+)-CC-1065-, (+)-AB′C′-, (+)-ABC″-, and (+)-ABC-modified oligomer TD1 in panels A and B appeared to be interconvertible, since isolated DNA from both bands show the same pattern of gel mobility on 8% nondenaturing gel electrophoresis. The lower molecular weight material in panel D is due to the thermal strand breakage species A and B (see Figure 1B).

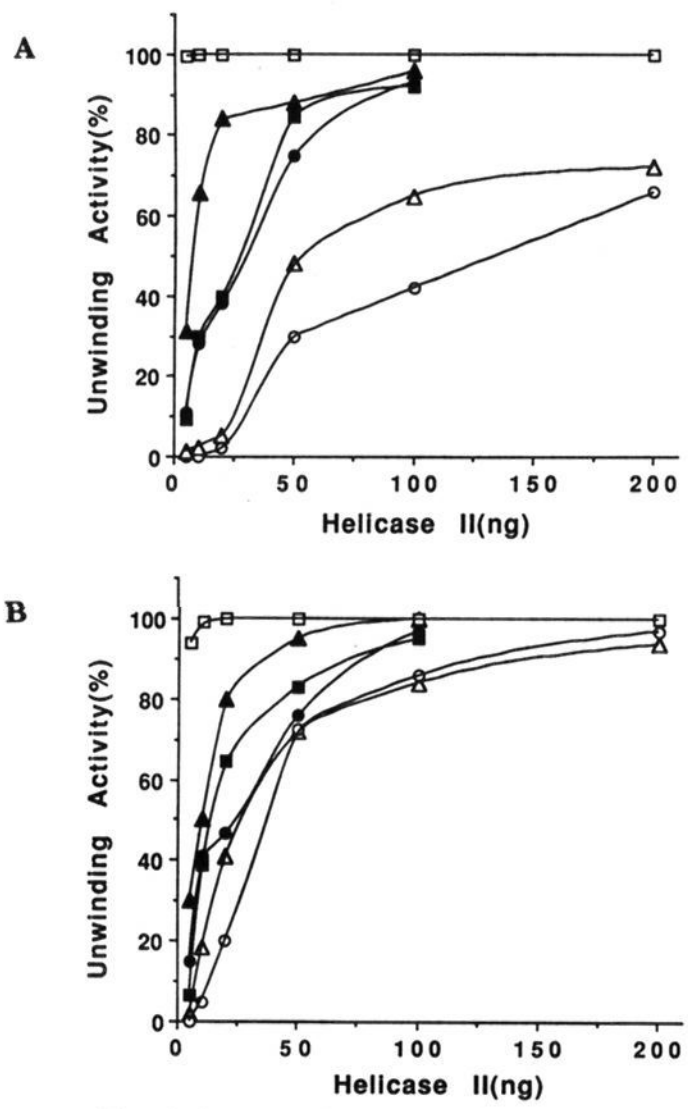


Figure 8. Unwinding reaction of non-drug-modified and drug-modified oligomer TD3 (graph A) and TD4 (graph B) at various helicase II concentrations. Reaction mixture contains 10 ng of DNA substrate with the indicated amount of helicase II (□ = control, ○ = (+)-CC-1065, △ = (+)-AB′C′, ● = (+)-ABC″, ■ = (+)-ABC, ▲ = (+)-AB).

(+)-CC-1065- and (+)-AB′C′-modified GD2, in which minor bonding of these drug molecules to 5′-GACCA*-3′ occurred at about 15% (data not shown). The possibility that two drug molecules are bound to the same DNA molecule can be excluded, since the two bonding sites are too close together for two drug molecules to form covalent adducts on the same DNA molecule. As anticipated, the unwinding of GD1 by helicase II was virtually completely blocked by (+)-CC-1065 and (+)-AB′C′ modification (see lanes 8 and 9 of Figure 10A), while (+)-ABC″ and (+)-ABC

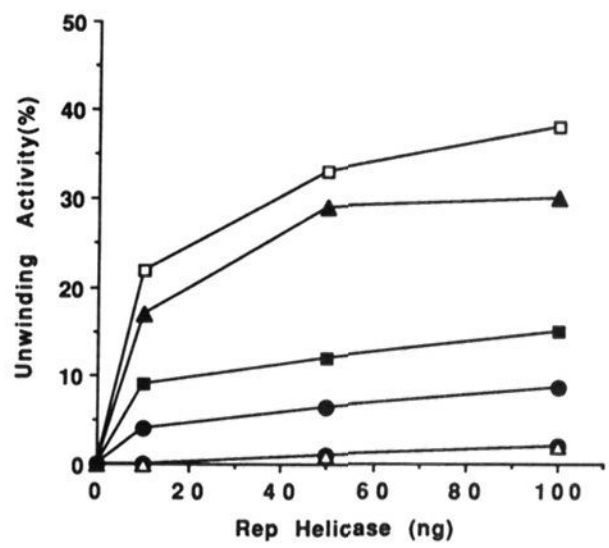


Figure 9. Unwinding reaction of non-drug-modified and drug-modified oligomer catalyzed by *E. coli rep* protein at various helicase II concentrations. Reaction mixture contains 10 ng of DNA substrate (TD1) with the indicated amount of *rep* protein (□ = control, ○ = (+)-CC-1065, △ = (+)-AB′C′, ● = (+)-ABC″, ■ = (+)-ABC, ▲ = (+)-AB).

showed a moderate inhibition (lanes 10 and 11 of Figure 10A). In contrast, in the case of the drug-modified GD2 substrate, irrespective of the analogue tested, only a weak inhibition of unwinding was observed at the concentration of helicase II utilized in this experiment (Figure 10B).

Helicase II Stalled on a Single-Stranded DNA Template Prevents Primer Extension by Polymerase I. To assess the combined action of helicase II and DNA polymerase I, DNA polymerase I and a four dNTP mixture were added to a drug-modified GD1 substrate (Table I and Figure 11), which had been preincubated with helicase II. Primer extension was measured using 12% denaturing polyacrylamide gel electrophoresis. As a control, drug-modified gapped templates were incubated with DNA polymerase I and a four dNTP mixture in the absence of helicase II (lanes 1–6 in Figure 11). The results show that, unexpectedly, even without drug modification preincubation in the presence of helicase II prevents DNA polymerase from extending primers by DNA synthesis (compare lanes 1 and 7 in Figure 11). Band "c" in lane 7 shows the position of stalled DNA synthesis. Presumably, the presence of helicase II stalled on the template after unwinding of the left region of the gapped duplex prevents full extension of the primer. In the case of (+)-CC-1065- and (+)-AB′C′-modified GD1 substrate, primer extension

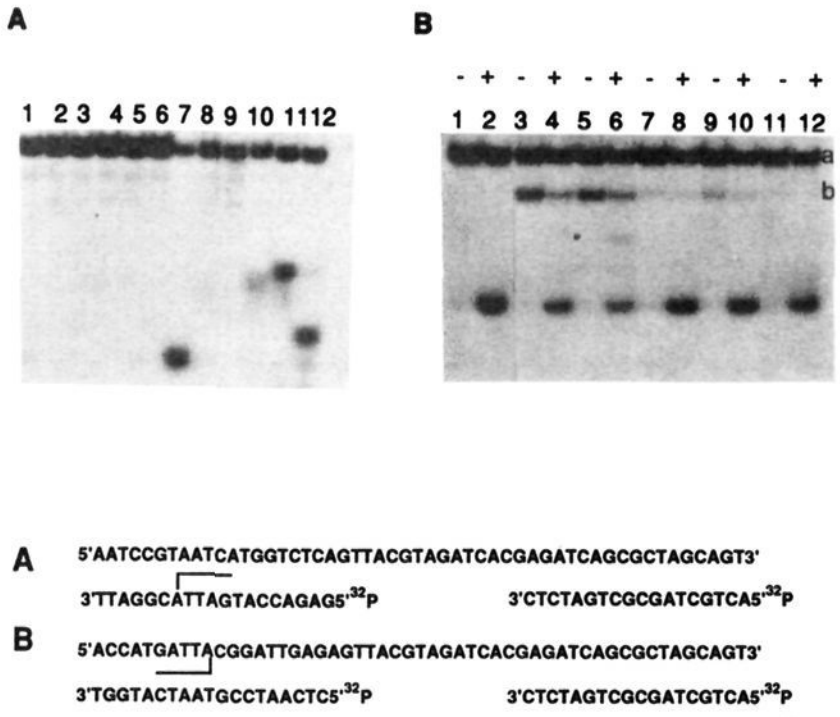


Figure 10. Helicase II-catalyzed unwinding of gapped duplexes GD1 and GD2 modified with the drug molecules, shown in Figure 2. Panels A and B represent unwinding reactions of non-drug-modified and drug-modified gapped duplexes GD1 and GD2, respectively. In panel A, each reaction contains 10 ng of either control DNA (lanes 1 and 7), (+)-CC-1065-modified DNA (lanes 2 and 8), (+)-AB′C′-modified DNA (lanes 3 and 9), (+)-ABC″-modified DNA (lanes 4 and 10), (+)-ABC-modified DNA (lanes 5 and 11), or (+)-AB-modified DNA (lanes 6 and 12). For panel A, lanes 1–6 are without helicase II (control) and lanes 7–12 are with 100 ng of helicase II. DNA substrate was preincubated with helicase II for 5 min, and an 8 molar excess of the unlabeled, displaced DNA strand was added and further incubated for 45 min at 30 °C. In panel B, the amount of DNA-substrate and helicase II used in this experiment is the same as in panel A. However, in this case lanes 1, 3, 5, 7, 9, and 11 represent reactions without helicase containing the non-drug-modified (+)-CC-1065-, (+)-AB′C′-, (+)-ABC″-, (+)-ABC-, and (+)-AB-modified DNA, respectively, while lanes 2, 4, 6, 8, 10, and 12 represent the reactions containing 100 ng of helicase II and the same DNA substrates as in the preceding lanes. Bands "a" and "b" are most likely two different conformations of the same drug-modified template GD2.

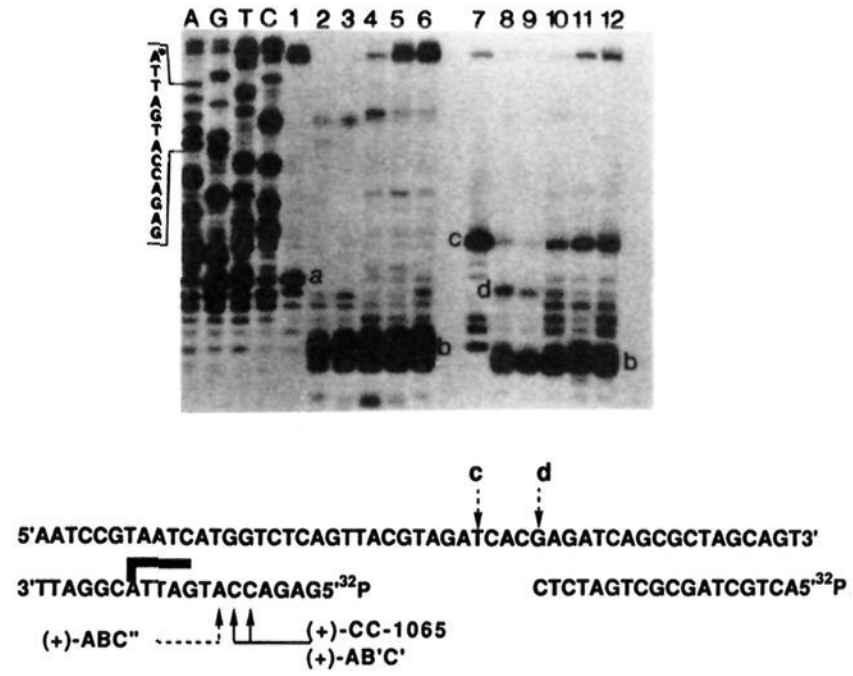


Figure 11. Primer extension of the gapped duplex (GD1) by DNA polymerase I alone (lanes 1–6) or by the combined action of helicase II and polymerase I (lanes 7–12). Reactions in lanes 1–12 contain 20 ng of DNA substrate, 50 μM of the four dNTPs, and 1 unit of DNA polymerase I in helicase II unwinding buffer. Reactions were carried out at 30 °C for 30 min. For reactions in lanes 7–12, where helicase was included, DNA substrates were unwound by 100 ng of helicase II for 15 min and then further incubated for 30 min with the addition of 1 unit of DNA polymerase I and 50 μM of dNTPs. Reactions contain unmodified DNA in lanes 1 and 7, (+)-CC-1065-modified DNA in lanes 2 and 8, (+)-AB′C′-modified DNA in lanes 3 and 9, (+)-ABC″-modified DNA in lanes 4 and 10, (+)-ABC-modified DNA in lanes 5 and 11, and (+)-AB-modified DNA in lanes 6 and 12. Band "a" represents ss DNA displaced by DNA polymerase I coupled to DNA synthesis. Band "b" represents the strand breakage product of drug-modified strands. Bands "c" and "d" represent prematurely terminated DNA synthesis. The four lanes on the left-hand side are the dideoxy-DNA sequencing lanes. The sequence complementary to the covalent modification site is shown on the left. The sequence of GD1 is shown at the bottom of the figure, identifying the drug bonding site and the position of bands "c" and "d", as well as the pausing sites for DNA polymerase near the adduct sites for (+)-ABC″, (+)-CC-1065, and (+)-AB′C′.

was terminated even more prematurely than in the control and in the other drug-modified template lanes (see band "d" in lanes 8 and 9 of Figure 11). We propose this is because helicase II is prematurely blocked from progression to the end of the template due to the presence of drug molecules. In contrast, DNA polymerase alone can fully extend the primer by using intrinsic strand-displacement activity coupled with DNA synthesis (lane 1 in Figure 11). For drug-modified GD1, premature termination of DNA synthesis occurred about seven to nine base pairs away from the drug modification site (lanes 2–6 in Figure 11), even though it is not the template DNA that is covalently modified with drug molecules. Moreover, by using nondenaturing polyacrylamide gel electrophoresis, we have shown that DNA polymerase can produce unwinding ss DNA from duplex in the presence of dNTPs, but this is also inhibited by the (+)-CC-1065- and (+)-AB′C′-modified substrates (unpublished data). The results of these experiments imply that DNA polymerase I alone cannot dissociate helicase II from template DNA, and other proteins might participate in the turnover of the helicase II bound to template DNA.

Discussion

DNA plays a vital role in maintaining the biological activities of all living cells, since the cellular processes necessary for life depend ultimately on the precise regulation of gene expression and genome replication. These DNA processes are mediated by the DNA itself together with its interactions with DNA binding proteins. Therefore, it is to be expected that DNA-interactive small molecules, such as intercalators, groove binders, and alkylating agents, will have profound effects on the function of DNA molecules, resulting in potent cytotoxicity. Physical studies on DNA–drug complexes have been carried out extensively in the past few years in attempts to correlate the biological activities of drugs with the manner and extent to which drug molecules interact with the DNA double helix.[28-30]

(+)-CC-1065 is the lead candidate for a group of largely synthetic drugs that alkylate DNA, in this case, through N3 of adenine in a surprisingly highly sequence-selective manner.[15] The DNA alkylating properties of (+)-CC-1065 and related drug molecules are believed to be primarily

(28) Hurley, L. H. DNA and Associated Targets for Drug Design. *J. Med. Chem.* **1989**, *32*, 2027–2033.
(29) Covey, J. M.; Kohn, K. W.; Kerrigan, D.; Tilchen, E. J.; Pommier, Y. Topoisomerase II-Mediated DNA Damage Produced by 4′-(9-acridinylamino)methanesulfon-*m*-anisidide and Related Acridines in L1210 Cells and Isolated Nuclei: Relation to Cytotoxicity. *Cancer Res.* **1988**, *48*, 860–865.
(30) Lambert, B.; Jones, B. K.; Roques, B. P.; Le Pecq, J.-B. The Noncovalent Complex between DNA and the Bifunctional Intercalator Ditercalinium Is a Substrate for the UvrABC Endonuclease of *Escherichia Coli*. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 6557–6561.

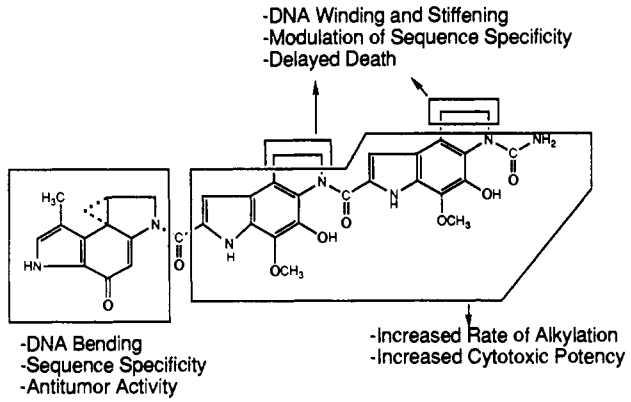


Figure 12. Structure–activity relationship of (+)-CC-1065 and related compounds.[16,19,20,23]

responsible for the cytotoxicity and antitumor activity shown in in vivo studies.[11,16,24] However, it has also been demonstrated that (+)-CC-1065 induces DNA bending, winding, and stiffening as a result of covalent modification of DNA.[19,20] Furthermore, the DNA-winding effect is unique to (+)-CC-1065 and (+)-AB′C′, while the DNA-stiffening effect is only observed for (+)-CC-1065, (+)-AB′C′, and (+)-ABC″. Thus, in previous studies the inclusion of select (+)-CC-1065 analogues in addition to (+)-CC-1065 has made it possible to define quite precisely the structure–activity relationship of (+)-CC-1065 and related molecules (Figure 12). As an extension of these studies, we are presently examining the biochemical consequences of these drug-induced structural changes in DNA molecules by using defined in vitro systems, including DNA-metabolizing proteins such as DNA polymerase, T4 DNA ligase, and other DNA-binding proteins.[20-22] These studies have shown that (+)-CC-1065 and its analogues are strong blocks to in vitro DNA synthesis mediated by the Klenow fragment, *E. coli* DNA polymerase I, and T4 DNA polymerase as a result of covalent modification of adenine, and that the DNA winding and stiffening effects of (+)-CC-1065 and its select analogues cause the proximal and distal inhibition of oligomer ligation mediated by T4 DNA ligase, respectively.[20,21]

In this present study, we have primarily focused on the helix-stabilizing and -winding effects of these drug molecules on DNA and their resulting consequences on the DNA-unwinding processes mediated by DNA helicases such as helicase II (UvrD *E. coli*) and *E. coli rep* protein. These drug-induced effects will presumably inhibit the physical separation of DNA duplex by increasing the stabilization of the DNA duplex or changing the helical periodicity of the DNA duplex by virtue of their DNA-winding effects. The results of this study clearly demonstrate that the extent of inhibition of DNA unwinding mediated by both helicase II and *E. coli rep* protein is partially correlated with the drug-induced DNA-stabilizing effect. However, drug-induced stabilization of DNA is in itself insufficient to explain all the helicase inhibition. For example, while the effect of (+)-AB′C′ on the Δ Tm of DNA is almost the same as that of (+)-ABC″,[16] (+)-AB′C′ produced a dramatically increased inhibition of DNA unwinding compared to (+)-ABC″. The additional extent of helicase II inhibition common to (+)-CC-1065 and (+)-AB′C′ appears to be correlated with their unique winding effects.[19] Significantly, the increase in the amount of helicase inhibition is also dependent upon the orientation of the winding effect of drug molecules, which has to be opposite with respect to the direction of helicase II translocation. There is a striking correlation of the orientation specificity of the winding effect of (+)-CC-1065 and a similar orientation specificity of the *ter*-binding protein, which also produces contra-helicase activity.[31] It is interesting to speculate whether the mechanisms for helicase inhibition might be related. The (+)-CC-1065 molecule has its major interactions with DNA to the 5′-side of the covalently modified adenine, and the drug-induced winding and helix-stiffening effects occur predominantly to the 5′-side of the covalently modified adenine (i.e., toward the drug overlap site). Our results show that helicases have more difficulty in unwinding a duplex, having a drug adduct on the displaced strand compared to that on the helicase-bound strand. We speculate that the presence of drug molecules may prevent helicases from entering the drug modification site by stabilizing or winding the duplex when drug molecules were positioned on the displaced strand, whereas helicases have difficulty in translocation through the covalently modified strand because of steric interaction due to the drug–adenine adduct when drug molecules are present on the helicase-bound strand.

The inclusion in this study of gapped duplexes (GD1 and GD2) as substrates for helicase II and *E. coli* polymerase I has provided to us important insights into understanding the effect of drug-induced inhibition of DNA unwinding mediated by helicase II and the possible further impact on DNA metabolism, such as the DNA-repair process. It is well-known that one pathway for repair of oligonucleotides containing DNA damage is through the combined actions of the UvrD protein (helicase II) and DNA polymerase after the UvrA, UvrB, and UvrC gene products of *E. coli* have identified the damaged site and produced 3′- and 5′-incisions.[5,26,32] These proteins recognize DNA damaged by bulky adducts, such as pyrimidine–pyrimidine cyclobutane dimers, and produce incision at the fourth or fifth phosphodiester bond on the 3′-side of the damage and at the eighth phosphodiester bond on the 5′-side.[32] Our results reported here indicate that the (+)-CC-1065- and (+)-AB′C′-damaged nucleotide might be resistant to excision by the combined action of UvrD and DNA polymerase, resulting in persistent DNA strand breaks after incision by UvrAB and C protein. The real possibility of generation of persistent DNA strand breaks during the repair process was suggested in a previous study using a eukaryotic system, in which it was shown that (+)-CC-1065 produces depletion of NAD levels in repair-proficient and -deficient (xeroderma pigmentosum) human cells, which appears to be related to poly(ADP)ribosylation and persistent DNA strand breakage.[32] If persistent DNA strand breaks are generated as a result of the normal repair processes, we can speculate that drug molecules such as (+)-CC-1065 and (+)-AB′C′ are likely to have much more potent biological effects in vivo compared to other analogues lacking these DNA-winding effects. This leads us to pose the question as to whether the unique winding effects of (+)-CC-1065 and (+)-AB′C′ may be related to the delayed lethality produced in mice by these same compounds.[23] To further evaluate this hypothesis, we are carrying out experiments using the *E. coli* repair system and the normal and xeroderma pigmentosum human cell

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lines with the analogues utilized in this investigation.

The results of our recent study concerning the mechanistic aspects of the inhibition of DNA-unwinding mediated by helicase II suggest that drug modification of DNA slows down the turnover rate of helicase II, which results in an increase of retention time of helicase II on drug-damaged DNA.[22] Likewise, the stalling of helicase II on drug-damaged DNA, particularly (+)-CC-1065- and (+)-AB'C'-modified DNA, was also observed in the primer extension experiment with the combined action of helicase II and polymerase I (see Figure 11). The distance between the drug modification site and termination of primer extension (about 20 nucleotides) appears to represent a critical length of coverage of DNA by helicase II, which is constant from one drug-modified sample to another, and is also about the same length of helical coverage revealed in the nonmodified gapped duplex. A similar observation was made in the previous study.[22]

Conclusively, the results presented in this paper clearly define some of the biochemical consequences of drug-induced winding and helix-stabilizing of DNA molecules on the activity of unwinding enzymes such as helicase II and *E. coli rep* protein. Studies are in progress that use a DNA transcription system in combination with eukaryotic transcriptional factors (e.g., Sp1) to determine the biochemical consequences of structural changes in DNA molecules that result from covalent adduct formation with (+)-CC-1065 and its analogues.

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4'-Modified Analogues of Aristeromycin and Neplanocin A: Synthesis and Inhibitory Activity toward *S*-Adenosyl-L-homocysteine Hydrolase

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The carbocyclic adenosine analogues aristeromycin and neplanocin A both display significant *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase inhibitory activity and broad-spectrum antiviral effects. Since phosphorylation of the 4'-hydroxymethyl substituent has been implicated with the cytotoxicity of these compounds, various analogues modified at this position were synthesized utilizing a key cyclopentenone intermediate **3** which can be derived from several members of the natural chiral pool. Cyclopentenone **3** underwent a highly stereoselective conjugate addition with organocuprate reagents, and the 1,4-adducts so formed were then readily elaborated to the corresponding 4'-modified aristeromycin analogues. Alternatively, quenching the enolate intermediate of the organocuprate conjugate addition with methanesulfinyl chloride followed by pyrolytic syn elimination resulted in the formation of 4'-modified neplanocin A intermediates. Three of the final compounds (**1b**, **1c**, and **1e**) displayed inhibitory activity toward AdoHcy hydrolase in the nanomolar range.

Introduction

The cellular enzyme *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) plays an important regulatory role in *S*-adenosyl-L-methionine (AdoMet)-dependent methylation reactions.[1] AdoMet serves as a methyl donor for a variety of biomolecules, including macromolecules such as mRNA,[2] and the byproduct of these methylations, AdoHcy, functions as a feedback inhibitor for the plethora of methyltransferases that catalyze these reactions. Since AdoHcy hydrolase provides the only known mechanism for AdoHcy catabolism in eukaryotes, catalyzing its hydrolysis to adenosine and homocysteine, the action of this enzyme is thought to allow the methylation process to continue at its normal physiological rate.

In recent years, AdoHcy hydrolase has become of interest as a target for antiviral chemotherapy.[3] There are several reasons for this: (1) most plant and animal viruses require a methylated cap structure at the 5'-terminus of their mRNA for viral replication;[4] (2) virus-encoded methyltransferases that are involved in the formation of this methylated cap structure are inhibited by AdoHcy;[5] (3) undermethylation of the viral mRNA cap structure induced by the inhibition of AdoHcy hydrolase has been correlated with the inhibition of viral replication;[6] (4) a close correlation exists between the antiviral potency of adenosine analogues and their inhibitory effects on AdoHcy hydrolase;[7] and (5) a close correlation exists be-

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