ABSTRACT  Key studies defining the DNA alkylation properties and selectivity of a new class of exceptionally potent, naturally occurring antitumor antibiotics including CC-1065, duocarmycin A, and duocarmycin SA are reviewed. Recent studies conducted with synthetic agents containing deep-seated structural changes and the unnatural enantiomers of the natural products and related analogs have defined the structural basis for the sequence-selective alkylation of duplex DNA and fundamental relationships between chemical structure, functional reactivity, and biological properties. The agents undergo a reversible, stereoelectronically controlled adenine-N3 addition to the least substituted carbon of the activated cyclopropane within selected AT-rich sites. The preferential AT-rich non-covalent binding selectivity of the agents within the narrower, deeper AT-rich minor groove and the steric accessibility to the alkylation site that accompanies deep AT-rich minor groove penetration control the sequence-selective DNA alkylation reaction and stabilize the resulting adduct. For the agents that possess sufficient reactivity to alkylate DNA, a direct relationship between chemical or functional stability and biological potency has been defined.

Substantial progress has been made in understanding the fundamental principles responsible for the sequence-selective recognition of DNA by small organic molecules (1–4) including a range of naturally occurring antitumor antibiotics. Three fundamental issues that arise in the examination of DNA binding agents are the origin of binding affinity, binding selectivity, and reaction selectivity including DNA alkylation or cleavage. Each can independently assert levels of control on the sequence-selective recognition of DNA and the relative role and origin of these effects remain a primary objective of most investigations. The emergence of experimental techniques for the rapid sequencing of DNA (5–9), for the determination of DNA binding selectivity and affinity including footprinting and affinity cleavage techniques (10, 11), and for the determination of sites of DNA modification (12) coupled with the rapid advances made in the structural characterization of DNA complexes at defined sites by x-ray crystallography (13, 14), NMR spectroscopy (15, 16), and molecular modeling (17, 18) have advanced the understanding of the molecular interactions responsible for the sequence-selective recognition of DNA. A powerful complement to such tools in the examination of naturally derived DNA binding agents is the preparation and subsequent examination of key partial structures, agents containing deep-seated structural modifications or variations in the natural product and their corresponding unnatural enantiomers. Deliberate, well-conceived deep-seated modifications may directly address and define both the structural basis for the sequence selective recognition of DNA and fundamental relationships between structure, functional reactivity, and biological properties.

CC-1065 and the Duocarmycins. One of the newest class of agents shown to alkylate DNA includes (+)-CC-1065 (1; ref. 19), (+)-duocarmycin A (2; refs. 20–24), and (+)-duocarmycin SA (3; refs. 25 and 26). Given their remarkable cytotoxic potency, substantial efforts have been devoted to defining their properties (27–39). In these studies, the agents have been shown to exert their biological effects through a sequence-selective alkylation of DNA. The reversible, stereoelectronically controlled adenine-N3 addition to the least substituted cyclopropane carbon has been found to occur within selected AT-rich sites in the minor groove and extensive efforts have been devoted to determine the origin of the DNA alkylation selectivity, to establish the link between DNA alkylation and the ensuing biological properties (40), and to define the fundamental principles underlying the relationships between structure, chemical reactivity, and biological activity. The limited studies conducted with naturally derived duocarmycin SA (3) revealed a combination of properties that make it the most exciting of the natural products identified to date. In addition to lacking the delayed toxicity characteristic of (+)-CC-1065 (41), it is the most stable and most potent member of this class of agents. As detailed herein, it is likely that this combination of properties is not fortuitous but rather that the enhanced functional stability of 3 is directly responsible for its increased biological potency.

DNA Alkylation Selectivity. The event, sequence selectivity, quantitation, reversibility, and structure determination of the predominant DNA alkylation reaction by I–3 have been defined (42–59). The alkylation site identification and the assessment of relative selectivity were derived through thermally induced depurination and strand cleavage of labeled DNA after exposure to the agents (Scheme I). The alkylation selectivity for (+)-duocarmycin A (2) and (+)-duocarmycin SA (3) proved nearly indistinguishable (43, 45). Each alkylation site detected was adenine flanked by two 5’A or T bases and there proved to be a preference for this three-base sequence: 5’-AAA > 5’-TTA > 5’-TAA > 5’-ATA. In addition, a strong preference for the fourth 5’ base to be A or T versus G or C was observed and distinguishes the high- versus low-affinity sites. Table 1 summarizes the consensus sequence de-
The study of (+)-CC-1065 revealed a similar, but more extended, 5-bp AT-rich alkylation selectivity (42, 53–59). Table 1 summarizes the consensus sequence derived from the evaluation of (+)-CC-1065. Each site of alkylation proved to be adenine flanked by two 5' A or T bases. The sequence preference for the three-base AT-rich alkylation site was determined to follow the order 5'-AAA > 5'-TTA > 5'-TAA > 5'-ATA. In addition, the agent exhibited a strong preference for the fourth 5' base to be A or T and a weaker preference for the fifth 5' base to be A or T. The preferences for the fourth and fifth 5' bases to be A or T distinguish many of the high- versus low-affinity sites. The strict AT preference within the first 3 bp represents a combination of the initial 3' adenine-N3 alkylation site and an adjacent 5'-two-base AT site required to accommodate the central subunit binding. The weaker preferences for the fourth and fifth 5' bases to be A or T reflect, as observed with the high-affinity sites, a preferential AT-rich site for the third subunit bound in the minor groove. A study of shorter and more extended analogs of CC-1065 revealed that this AT-rich selectivity corresponds nicely to the length of the agent and, hence, binding site size surrounding the alkylation site required to accommodate the agent (42, 58).

### Table 1. Consensus sequences for the DNA alkylation reactions of the natural products and their unnatural enantiomers

<table>
<thead>
<tr>
<th>Agent</th>
<th>Base*</th>
<th>5' 4</th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0</th>
<th>-1</th>
<th>-2</th>
<th>-3</th>
<th>-4</th>
<th>3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-1</td>
<td>A/T (56)†</td>
<td>67</td>
<td>78</td>
<td>94</td>
<td>98</td>
<td>100</td>
<td>55</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Consensus</td>
<td>A/T ≥ G/C</td>
<td>A/T &gt; G/C</td>
<td>A/T</td>
<td>A/T</td>
<td>A</td>
<td>R ≥ Y</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>(+)-2 and (+)-3</td>
<td>A/T (56)†</td>
<td>58</td>
<td>79</td>
<td>100</td>
<td>100</td>
<td>69</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Consensus</td>
<td>A/T ≥ G/C</td>
<td>A/T</td>
<td>A/T</td>
<td>A</td>
<td>R ≥ Y</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>ent(-)-3</td>
<td>A/T (56)†</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>93</td>
<td>100</td>
<td>96</td>
<td>73</td>
<td>56</td>
</tr>
<tr>
<td>ent(-)-1</td>
<td>A/T (56)†</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>88</td>
<td>100</td>
<td>93</td>
<td>82</td>
<td>73</td>
</tr>
<tr>
<td>Consensus</td>
<td>A/T</td>
<td>A/T</td>
<td>A/T</td>
<td>A/T</td>
<td>A/T</td>
<td>A/T &gt; G/C</td>
<td>N</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>
| R, purine; Y, pyrimidine.

*Percentage of the indicated base located at the designated position relative to the adenine-N3 alkylation site.
†Percentage composition within the DNA examined.

### Unnatural Enantiomer DNA Alkylation Selectivity

One of the more revealing discoveries made possible through use of synthetic materials was that the unnatural enantiomers also constitute effective DNA alkytating agents (42, 43, 56, 58). ent(-)-Duocarmycin SA (3) was found to alkylate DNA but at concentrations >10× that required for the natural enantiomer (43) and Table 1 summarizes its consensus alkylation sequence. Each alkylation site detected was adenine and essentially each alkylation site was flanked by a 5' and 3' A or T base that exhibited the following sequence preference: 5'-AAA > 5'-AAT > 5'-TAA > 5'-TAT. An additional strong preference for the second 3' base from the alkylation site to be A or T was observed. In this regard, the ent(-)-duocarmycin SA alkylation proved analogous to the natural enantiomer with the exception that the binding orientation is reversed (5' → 3') over an AT-rich 3.5-bp site. However, while the bound conformation of the natural enantiomer covers an AT-rich 3.5-bp site extending from the adenine-N3 alkylation site in the 3' → 5' direction across the adjacent 2–3 5' bases (i.e., 5'-AAA), the AT-rich 3.5-bp site for the unnatural enantiomer extends in the reverse 5' → 3' direction starting at the first 5' base preceding the adenine-N3 alkylation site and extending across the alkylation site to the first and second adjacent 3' bases (i.e., 5'-AAGA). The reversed binding orientation is required to permit adenine alkylation at the least substituted cyclopropane carbon and the offset AT-rich alkylation selectivity is the natural consequence of the diastereomeric relationship of the adducts. Similarly, ent(-)-CC-1065 was found to alkylate DNA with a rate and efficiency that were comparable to the natural enantiomer (56). Table 1 summarizes the consensus alkylation sequence for ent(-)-CC-1065, which proved similar to ent(-)-duocarmycin SA but which exhibits an extended 5-bp AT-rich selectivity (42, 56). Each alkylation site proved consistent with 5' adenine-N3 alkylation with agent binding in the minor groove in the 5' → 3' direction from the alkylation site covering 5 bp across an AT-rich region. All alkylation site detected proved to be adenine and nearly all of the 3' and 5' bases flanking the adenine-N3 alkylation site proved to be A or T. Like the natural enantiomer, there proved to be a preference for this sequence that follows the order 5'-AAA > 5'-TAA > 5'-AAT, 5'-TAT. There also proved to be a substantial preference for the second and third 3' base to be A or T.

### Adenine-N3 Alkylation

The quantitation of the adenine-N3 alkylation, confirmation of its structure through isolation and characterization of the thermally released adducts, and the search for unde-
ected alkylation sites have been conducted. In these studies, the adenine-N3 alkylations illustrated in Scheme I by (+)-duocarmycin A, (+)-duocarmycin SA, ent-(-)-duocarmycin SA, and (+)-CC-1065 have been found to quantitatively account for 86–92% (46), 90–100% (43), 86–92% (43), and 80–90% (54), respectively, of their consumption in the presence of excess DNA and to constitute the near-exclusive alkylation event. The characterization of the adducts led to the unambiguous assignment of the structures 8, 9, and 10 in which adenine-N3 addition to the unsubstituted cyclopropane carbon of the agents was established.

Thus, all three agents provide predominantly or exclusively adenine-N3 adducts (≥90%). However, a minor guanine-N3 alkylation has been detected with 2 and to a lesser extent with 1 but only upon isolation of the thermally released adduct following treatment of DNA with excess agent (50), within oligodeoxynucleotides lacking a high-affinity adenine-N3 alkylation site (51), or when the adenine alkylation sites within AT-rich regions of DNA were protected from alkylation with high-affinity AT-rich minor groove binding agents (52). In contrast, duocarmycin SA (3) showed no evidence of guanine-N3 alkylation when subjected to similar or more forcing conditions (43). Even under vigorous conditions, unreacted agent was recovered and the selectivity for adenine-N3 versus guanine-N3 alkylation was >25:1. This enhanced selectivity of 3 may be attributed to its decreased reactivity. Notably, under the relevant conditions of limiting agent, even duocarmycin A provided near-exclusive adenine-N3 alkylation. Since duocarmycin SA is much more potent than A, the minor guanine-N3 alkylation cannot be uniquely relevant to the expression of the biological properties and may represent a nonproductive competitive event.

Models of the DNA Alkylation Adducts That Accommodate the Reversed Binding Orientation and Offset AT-Rich Selectivity of the Enantiomeric Agents. The examination of the natural products, their synthetic unnatural enantiomers, and key partial structures not only resulted in the unusual observation that the unnatural enantiomers constitute effective DNA alkylating agents and potent antitumor antibiotics but also has led to the emergence of a detailed model (42, 43) of the structural features responsible for their selective alkylation of DNA. The characterization of 8–10, the unambiguously established absolute configuration of the agents, and the definition of the alkylation consensus sequences for both enantiomers of 1–3 provided the necessary information for the construction of accurate models of the adenine-N3 alkylation. Fig. 1 illustrates models of the (+)- and ent-(-)-duocarmycin SA alkylation at a common site in w794 DNA, 5'-(CTAATT), which constitutes a high-affinity site for the unnatural enantiomer and a minor site for the natural enantiomer (43). Similar models of the (+)- and ent-(-)-CC-1065 alkylations have been published (42). In both instances, the hydrophobic face of the agent is imbedded deeply in the minor grove, the polar functionality lies on the outer face of the complex, and the bound helical conformation of the agent complements the topological curvature and pitch of the minor groove spanning an AT-rich 3.5- or 5-bp site, respectively. For (+)-duocarmycin SA, the binding spans 3.5 bp starting with the 3' adenine alkylation site and extends in the 3' → 5' direction over the 2–3 adjacent 5' base pair (5'-CTAA). For ent-(-)-duocarmycin SA, the binding similarly spans a 3.5-bp AT-rich site but which necessarily starts at the 5' base adjacent to the alkylation site and extends in the 5' → 3' direction over the alkylation site and the 1–2 adjacent 3' base pair (5'-AAATT). This offset alkylation selectivity within an AT-rich site is the natural
consequence of the diastereomeric relationship of the adducts and the reversed binding orientation in the minor groove with respect to the alkylation site is required to permit adenine-N3 addition to the least substituted carbon of the electrophilic cyclopropane. The importance of the fourth base (A/T > G/C) in the binding sequences is the reason this site constitutes a high-affinity site for the unnatural enantiomer but only a minor site for the natural enantiomer. Similarly, the CC-1065 models (42) accommodate the more extended 5-bp AT-rich binding site size and alkylation selectivity of the enantiomeric agents, their reversed binding orientation in the minor groove, and their offset AT-rich selectivity relative to the alkylation site.

The apparently confusing distinctions in the relative efficiency of the natural versus unnatural enantiomer DNA alkylation reactions were found to correlate with the inherent steric bulk surrounding the agent C7 center for which the unnatural enantiomers are especially sensitive, consistent with expectations based on the models (42, 43, 60).

N-Boc-DSA: A DNA Alkylating Agent Whose Selectivity and Efficiency Are Independent of Absolute Stereochemistry. The accuracy of the models was revealed when they were found to provide a beautiful explanation for the unusual observation that both enantiomers of simple derivatives of the alkylation subunit—i.e., N-Boc-DSA (11; ref. 43) or N-Boc-CPI (12; refs. 42 and 56)—alkylate the same sites in DNA. In addition to illustrating that the DNA alkylation reactions of (+) and ent-(−)-11 are substantially less efficient (×105–106) and less selective (selectivity = S′-AΔ > S″-TΔ) and proceed with an altered profile than (+)- or (−)-duocarmycin SA or CC-1065, the studies have shown that both enantiomers of 11 alkylate the same sites with essentially the same efficiency independent of the absolute stereochemistry (43). Although these observations appear unusual, they are the natural consequence of the diastereomeric relationship of the adducts. The natural enantiomer binds in the 3′ → 5′ direction from the site of alkylation extending over the adjacent 5′ base. The unnatural enantiomer binds in the reverse 5′ → 3′ orientation but with binding that also covers the same adjacent 5′ base. These alkylation site models (42, 43), which are illustrated in Fig. 2, nicely accommodate the observed selectivity of S′-AΔ > S″-TΔ for both enantiomers. The preference of S′-AΔ over S″-TΔ (2:1) is statistical rather than structural in nature since the complementary partner strand of a 5′-AT sequence contains an identical competitive alkylation site. The factor controlling alkylation is simply the depth of minor groove penetration accessible to the agent that is required to permit adenine-N3 alkylation of the electrophilic cyclopropane. For simple agents such as 11 and 12, this is possible only when the adjacent 5′ base is A or T and models of an unobserved S″-GΔ alkylation support this proposal (42, 43). For 3 or 1, a larger 3.5- or 5-bp AT-rich site surrounding the alkylation site is required to permit sufficient groove penetration for alkylation and is further enhanced by the preferential noncovalent binding of the agents within the narrower, deeper AT-rich minor groove.

Reversibility of the DNA Alkylation Reaction: Binding-Driven Bonding. Although the duocarmycin DNA alkylation have proven similar to CC-1065, one important feature distinguishes the agents. Unlike (+)-CC-1065, which irreversibly alkylates DNA, (+)-duocarmycin SA and A were found to reversibly alkylate DNA (43, 47). The ease of reversibility proved dependent upon the relative reactivity of the agent and the stability of the adduct as well as the extent of the noncovalent binding interactions. Consistent with the relative reactivity of the agents and the expected stability of the adducts, the (+)-duocarmycin A retroalkylation reaction was found to be slower than that of (+)-duocarmycin SA. In addition, a faster rate of retroalkylation and much lower degree of adduct stability was observed with (+)-11 versus (+)-3 and may be attributed to the lack of the trimethoxyindole binding stabilization. (−)-CC-1065 is less reactive than (+)-duocarmycin A but more reactive than (+)-duocarmycin SA. The lack of detection of a reversible (+)-CC-1065 alkylation reaction further indicated that the rate of reversibility is also dependent on the extent of the noncovalent binding stabilization. Consistent with this interpretation, analogs of CC-1065 possessing the same alkylation subunit but simpler and smaller binding subunits reversibly alkylate DNA (57).

Thus, a dominant force stabilizing the DNA alkylation reaction is not only the covalent bond but also the noncovalent binding derived from hydrophobic binding and van der Waals contacts. That is, the reversible nature of the alkylation reaction is rendered less reversible or irreversible by noncovalent binding stabilization. The importance of these observations becomes clear in the comparison of the properties of the agents. The exceptionally potent cytotoxic activity of the natural products versus the relatively nonpotent activity of the simple derivatives including 11 and 12 may be attributed in part to the simple event of noncovalent binding stabilization of the inherently reversible DNA alkylation reaction.

Noncovalent Binding Affinity and Selectivity. Important insights into the struc-
tural origin of the sequence-selective DNA alkylation were derived from examination of the binding affinity and selectivity of 13–16, key partial structures of 1–3 that were not capable of the subsequent covalent bond formation (61–66). Both relative and absolute DNA binding constants have been established, and the agents were found to exhibit a substantial AT-rich minor groove binding selectivity (63, 64). In addition, CDPI was found to be the optimum binding agent within the CDPI series. The minor groove binding of CDPI spans 5 bp or one-half a helix turn, which constitutes the largest site accessible for synchronous binding of both ends of the rigid agent. Partial bound forms of the larger agents—i.e., CDPI-bond CDPI4—were determined to constitute stable noncovalent complexes. The removal of the hydroxy and methoxy substituents (PDE-I, → CDPI-I) had only a small impact on the binding affinity and no apparent impact on the AT-rich binding selectivity (63).

Consequently, the studies suggested that (+)-CC-1065 is best represented as a selective alkylation group superimposed upon the rigid CDPI skeleton (63) rather than as a reactive alkylation agent (CDPI) attached to a DNA binding agent (PDE-I). The agents exhibit a substantial preference for noncovalent AT-rich minor groove binding that is optimal with the rigid trimer (CDPI3/PDE-I3). Among features that contribute to minor groove binding selectivity, two structural characteristics of an A-T base pair and of a run of A-T base pairs may prove important to agents that rely on stabilizing van der Waals contacts and hydrophobic binding. First, a G-C base pair possesses an amino substituent that extends into the minor groove and a comparable substituent is not present on an A-T base pair. This sterically more accessible region adjacent to an A-T base pair permits the deeper penetration of an agent into the minor groove and enhances the stabilization derived from van der Waals contacts. In addition, x-ray crystallographic studies of oligodeoxynucleotides that contain central to their structure a run of A-T base pairs revealed characteristic features including the constricted width of the AT-rich minor groove (13, 14). This narrower AT-rich minor groove within DNA contributes prominently to the noncovalent binding selectivity of the agents that depend on stabilizing van der Waals contacts.

More recently, the DNA binding properties of ACDPI (15; n = 1–4) and TACDPI (16; n = 1–3) have been detailed (64). Comparable to observations made with CDPI, ACDPI3 proved to be the optimal minor groove binding agent within the series and exhibited a selectivity for AT-versus GC-rich DNA. Similarly, TACDPI3 exhibited a substantial AT-rich DNA binding selectivity (n = 3; ΔΔG' = −2.7 to −3.5 kcal/mol; 1 kcal = 4.18 kJ). The comparison of CDPI3 with ACDPI3 illustrated that the introduction of a strong electronegative substituent onto the peripheral face of the agent reduced the binding affinity through introduction of destabilizing electrostatic interactions with the phosphate backbone. In contrast, the comparison with TACDPI3 revealed that the introduction of a C-5 quaternary amine substantially enhanced the binding affinity through introduction of stabilizing electrostatic interactions with the phosphate backbone (Fig. 3).

Definitive Tests of Proposed Models for the Origin of the CC-1065 and Duocarmycin DNA Alkylation Selectivity. In the course of studies on CC-1065 and the duocarmycins, the alkylation selectivity of the natural enantiomers has been attributed to a sequence-dependent activation through C4 carbonyl protonation by a strategically positioned phosphate in the DNA backbone (35–38, 55, 56), the conformational variability of DNA and alkylation at junctions of bent DNA (67–69), or preferential noncovalent binding and subsequent alkylation within the narrower, deeper minor groove of AT-rich DNA (27–34). Central to the interpretations are the perceived similarities (35–39, 53–56) or distinctions (27–34, 42, 43) in the alkylation selectivity of simple derivatives including 11–12 and the natural products 1–3. The former two proposals are based on the premise that (+)-12 and (-)-1 alkylate the same DNA sites and that the alkylation subunit or alkylation reaction controls the selectivity irrespective of noncovalent binding. In contrast, the latter proposal requires that the AT-rich noncovalent binding selectivity of the agents and their sterically accessible to the alkylation site that accompanies deep penetration into the AT-rich minor groove control the sequence selectivity. Notably, this latter model accommodates nicely the reverse and offset 3.5- or 5-bp AT-rich adenosine-N3 alkylation selectivities of the natural and unnatural enantiomers of 3 and 1, respectively, and requires that 11–12 and 1–3 exhibit distinct alkylation selectivities (42, 43, 58).

In the first key study (59, 70, 71), it was shown that 17−20 exhibited identical alkylation selectivities (5'-AA > 5'-TA) independent of their absolute configuration and identical to both enantiomers of 11 and 12. In addition, the natural enantiomers of 21–24 exhibited DNA alkylation selectivities identical to that of (+)-CC-1065 and were more selective and more efficient than 17–20. The observation that 19–20 and 23–24, which lack the C4 car-
bonyl, alkylate the same sites as 17 or 21, respectively, established that a sequence-
dependent phosphate protonation and ac-
tivation cannot be the event that deter-
mines the alkylation selectivity. In addition,
the more selective DNA alkylation by
21-24/1-3 versus 17-20/11-12 independent
of the nature of the electrophile was con-
sistent with the noncovalent binding
model and inconsistent with the proposal
that alkylation is observed preferentially
at junctions of bent DNA irrespective of
binding selectivity.

Key to the second definitive study (72)
was the disclosure that both the natural
and unnatural enantiomer of duocarmycin
SA alkylate DNA efficiently (43). One
unique feature of 3 is the C6 methyl ester
on the left-hand side of the alkylation
subunit that complements the right-hand
side linking amide. This feature provides
the ability to introduce DNA binding sub-
units on either side of the duocarmycin SA
alkylation subunit. In the studies, both the
natural and unnatural enantiomers of the
extended and reversed duocarmycin SA
analogs 25 and 26 were examined.

Both (+)-DSA-CDPI2 and ent-(-)-
DSA-CDPI2 (25) were found to alkylate
DNA with the same selectivity as (+)-
and ent-(-)-CC-1065 (1), respectively. The
natural enantiomer of the reversed agent
(+)-CDPI2-DSA (26) was found to alkylate
DNA with the same selectivity as ent-(-)-
DSA-CDPI2 and ent-(-)-CC-1065 (72).
This incorporation and conversion of a natural enantiomer of the duo-
carmycin SA alkylation subunit into an
agent that exhibits the DNA alkylation
selectivity of a typical unnatural enanti-
mer by simple reversal of the orientation
of the DNA binding subunits of the agent
are only consistent with a model where the
AT-rich noncovalent binding selectivity
and depth of minor groove penetration
surrounding the alkylation site are con-
trolling the sites of alkylation. Moreover,
the observations are inconsistent with alter-
native models based on the premise
that the natural enantiomer alkylation
subunit controls the alkylation selectivity.

Similarly, the unnatural enantiomer of the
reversed agent ent-(-)-CDPI2-DSA (26)
was found to alkylate the same sites as
(+)-DSA-CDPI2 (25) and (+)-CC-1065,
typical natural enantiomers. Thus, this
reversal of the enantiomeric alkylation
selectivity that accompanies the simple
reversal of agent orientation is general
and confirms that the same fundamental
recognition features are operative for both
the natural and unnatural enantiomers.

**Modified Alkylation Subunits: Defini-
tion of a Fundamental Relationship
Between Functional Reactivity and Biologi-
cal Activity.** In addition to the total syn-
thesis of the natural products CC-1065 (1;
refs. 73-75), duocarmycin A (2; ref. 76),
and duocarmycin SA (3; refs. 77-79), and
the extensive number of investigations
directed at the subunits of the natural prod-
ucts (33), studies that have provided ana-
logs (80, 81) and agents containing deep-
seated structural modifications have
proven unusually valuable in defining
the relationships between structure, func-
tional reactivity, and biological properties
(82-97). The acid-catalyzed activation of
the DNA alkylation reaction led to the
intuitive proposal that there may exist a
direct relationship between the reactivity
and cytotoxic activity of the agents and
established the expectation that the bio-
logical potency may be enhanced as the
electrophilic reactivity is increased (81).
However, studies conducted with the
agents 27-32, which were prepared by
synthesis employing technology de-
veloped in the natural product total synthe-
ses, revealed the reverse relationship
and that the agents possessing the greatest
stability may be expected to exhibit the
most potent cytotoxic activity. Moreover,
a well-defined direct relationship between
solvolysis stability (pH 3) and biological

![Diagram](image-url)
of the natural products themselves and further define subtle relationships between their structure, functional reactivity, and biological properties.

We gratefully acknowledge the financial support of the National Institutes of Health (CA41986 and CA55276).


