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Design, synthesis, and evaluation of DNA minor groove binding agents

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Abstract. The design, synthesis, and evaluation of DNA minor groove binding agents related to (+)-CC-1065 and (+)-duocarmycin A are detailed in continuing efforts to define the structural origin of the sequence-selective DNA alkylation reaction.

(+)-CC-1065 (**1**)[1] and the duocarmycins,[2-4] Fig. 1, constitute exceptionally potent antitumor-antibiotics that have been shown to be related through their common participation in a characteristic minor groove adenine N3 alkylation of duplex DNA.[5-9] For the natural enantiomers, the stereoelectronically-controlled adenine N3 addition to the agent unsubstituted cyclopropane carbon has been shown to occur

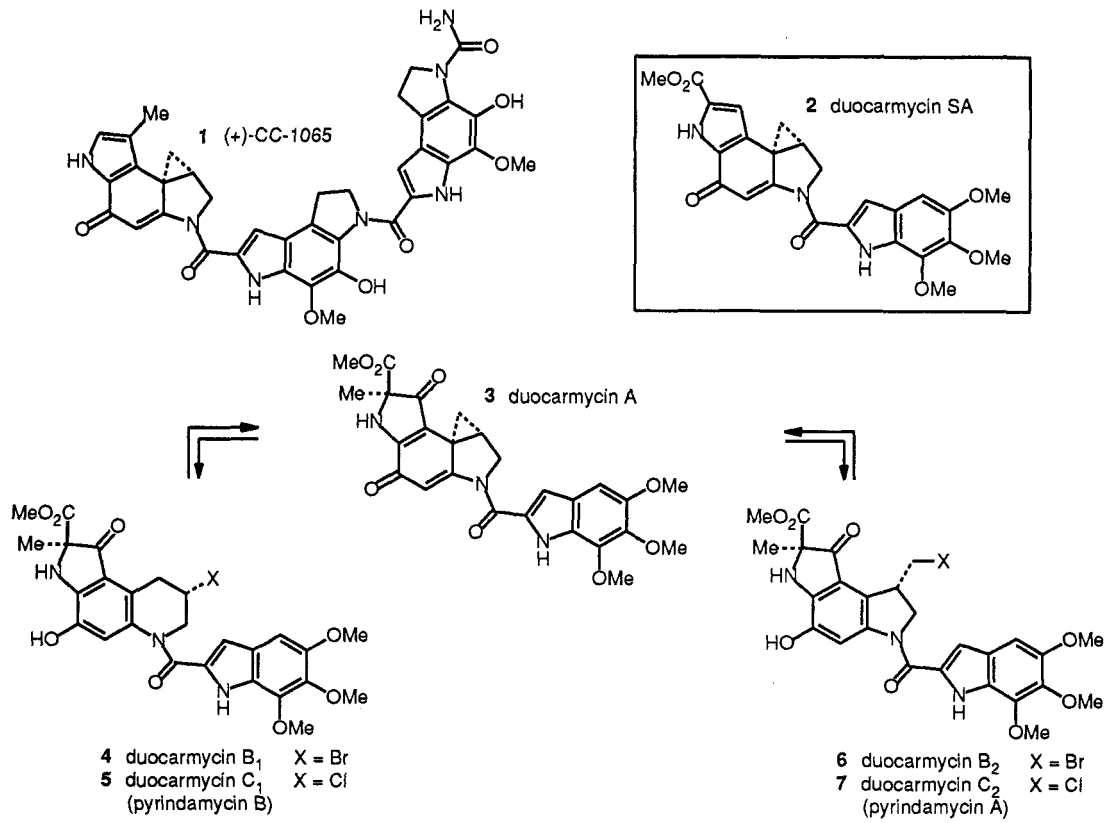


Fig. 1

within selected AT-rich regions of duplex DNA with a binding orientation that extends in the 3′ to 5′ direction from the site of alkylation. In past efforts, we have detailed the preparation of agents incorporating deep-seated changes in the alkylation subunit with the intent of determining the fundamental structural features contributing to polynucleotide recognition and functional reactivity.

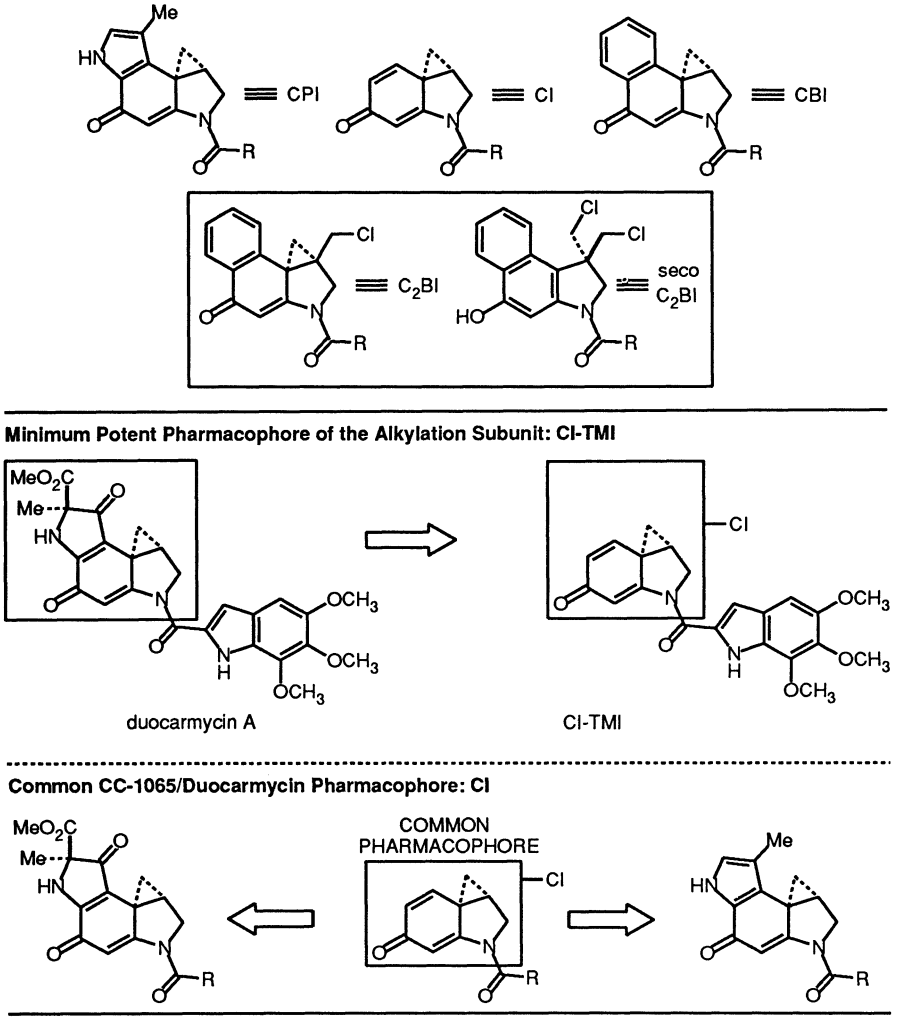


Fig. 2

In these studies in which the properties of the CPI, CI and CBI based agents have been examined, Fig. 2, the electrophilic cyclopropane proved not to be obligatory to observation of the (+)-CC-1065 alkylation selectivity[10] and the noncovalent binding selectivity of the agents[11] has been shown to exhibit a pronounced effect on the DNA alkylation selectivity.[12,13] The CI subunit was shown to constitute the minimum potent pharmacophore of the (+)-CC-1065 and (+)-duocarmycin alkylation subunits and the common pharmacophore relating the two classes of agents.[14,15] Agents incorporating the simplified and chemically more stable CBI alkylation subunit have proven especially interesting. Such agents have displayed more potent cytotoxic activity than the corresponding CPI-based agents and selected agents within the series, i.e. (+)-CBI-indole$_2$, have displayed efficacious antitumor activity.[16] Despite the decreased reactivity of the CBI-based agents, they have been found to participate in the characteristic DNA alkylation at a greater rate and with a higher efficiency than the corresponding agents possessing

the authentic CPI alkylation subunit.[16,17] As a consequence of the comparison of the CPI, CI, and CBI agents, the sequence-dependent conformational variability of duplex B-DNA was implicated in contributing to the agent DNA alkylation selectivity[14] and a fundamental relationship between agent chemical stability and cytotoxic potency was proposed.[18]

C_2BI-CDPI$_n$: Achiral DNA Crosslinking Agents. The most recent extension of these studies has addressed the synthesis of agents incorporating the 9a-chloromethyl-1,2,9,9a-tetrahydrocycloprop[*c*]benz[*e*]indol-4-one (C_2BI) alkylation subunit, Fig. 3.[19] In addition to the observations that the parent CBI-based agents have proven chemically more stable, biologically more potent, and synthetically more accessible than the authentic CPI alkylation subunit, the precursor acyclic *seco*-C_2BI agents could be anticipated to display properties comparable to the C_2BI agents and are inherently achiral. Consequently, they provided attractive candidates for synthesis free of the technical considerations of resolution or asymmetric synthesis. Since both (+)-CC-1065 and *ent*-(-)-CC-1065 display potent biological and effective DNA alkylation properties, the potential in vivo closure of the achiral *seco*-C_2BI agents to both enantiomers of the C_2BI agents may not prove to be of consequence.

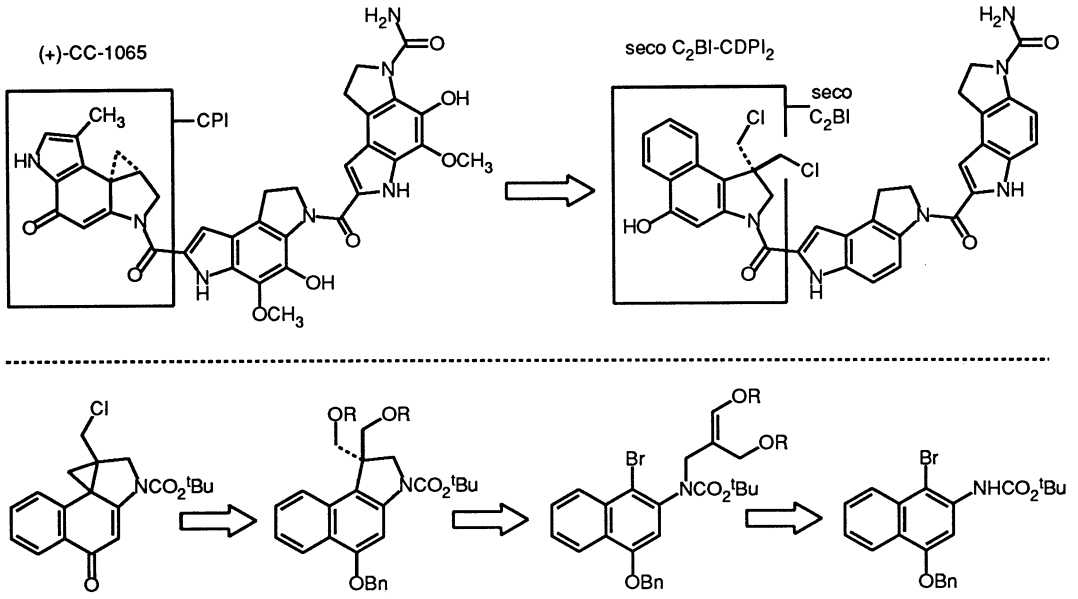


Fig. 3. C_2BI-CDPI$_n$: Achiral DNA Cross-linking Agents

Finally, the bis-alkylating capabilities of the C_2BI-based agents provide for the opportunity of DNA cross-linking, Fig. 4. Pertinent to the potential cross-linking capabilities of the C_2BI-based agents, the precursor *seco*-C_2BI agents possessing the capabilities for ring closure to the cyclopropane can be expected to possess the biological and DNA alkylation properties of the parent cyclopropane bearing agents. That such a cross-linking event may be reasonable was established in modeling studies of the high affinity alkylation site of w794 DNA [5′-d(AATTA)] which provides for a potential adenine-adenine cross-link with the second alkylation occurring on the complementary strand one base-pair removed in the 5′ direction from the initial alkylation site, Fig. 4.

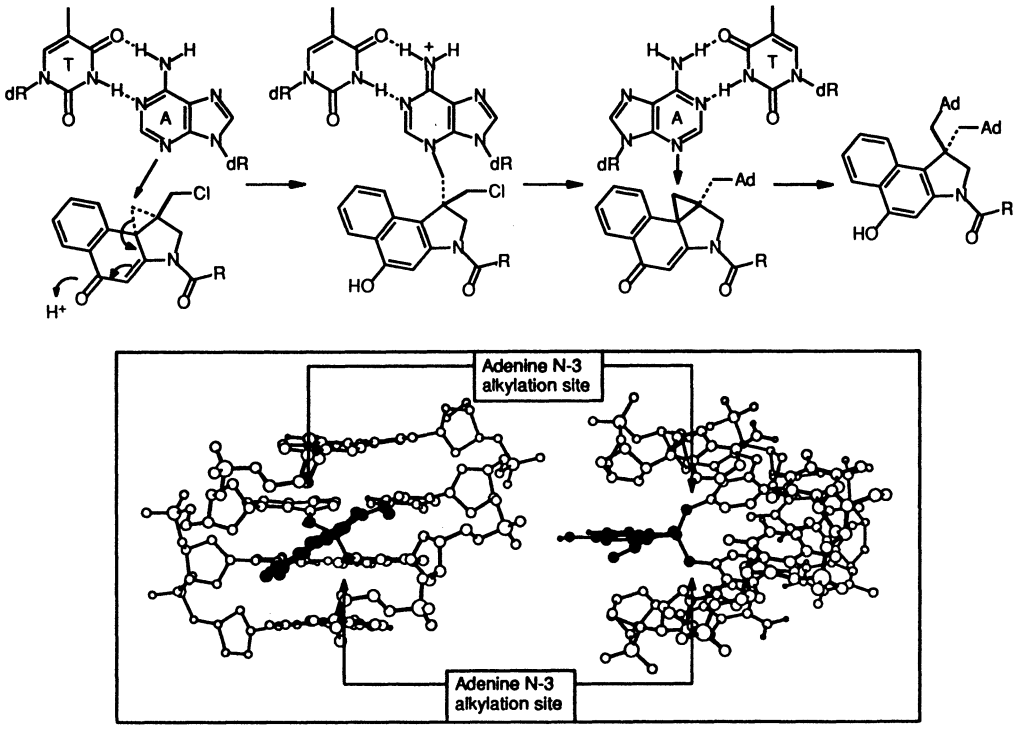


Fig. 4 Proposed Adenine-Adenine Cross-linking Reaction of C_2BI-$CDPI_n$

Synthesis of C_2BI-$CDPI_n$. The synthesis of the C_2BI alkylation subunit will be summarized and is based on the implementation of a key 5-*exo-trig* aryl radical-alkene cyclization with direct introduction of an appropriately functionalized 3,3-bis(hydroxymethyl)indoline, Fig. 3.

Evaluation of C_2BI-$CDPI_n$. Important characteristics of the alkylation subunit of CC-1065 and related analogs are the relative solvolysis reactivity and the site of cyclopropane cleavage. Past agents including those bearing the CPI, CI, or CBI subunits undergo a stereoelectronically-controlled ring opening with exclusive addition of a nucleophile to the least substituted cyclopropane carbon. With the additional C-9a chloromethyl substitution of the C_2BI agents, it remained to be determined if the solvolytic ring opening reaction would proceed under stereoelectronic control with nucleophilic addition to the unsubstituted cyclopropane carbon or through a stable tertiary carbocation with ring expansion, Fig. 5. Treatment of *N*-Ts-C_2BI with anhydrous HCl at -78°C or 0°C in EtOAc provided exclusively *seco-N*-Ts-C_2BI with no trace of the ring expansion product indicating that the C-9a substitution of the C_2BI agents does not alter the solvolytic behavior of the agents. Like the preceding examples, this may be attributed to the near perfect alignment of the σ C8b-C9 cyclopropane bond with the cyclohexadienone π-system versus the near orthogonal alignment of the σ C8b-C9a cyclopropane bond which leads to preferential C8b-C9 bond cleavage and nucleophilic addition at C9. In addition, the inductive effect of the electron deficient chloromethyl substituent should destabilize the tertiary carbocation and may contribute to the deceleration of the cyclopropane cleavage with ring expansion. At a pH of 3, *N*-BOC-C_2BI ($t_{½}$ = 433 h) proved to be substantially more stable than *N*-BOC-CBI ($t_{½}$ = 133 h) or *N*-BOC-CPI ($t_{½}$ = 37 h) to solvolysis. Presumably this may be attributed to the steric or inductive electronic effect of the added chloromethyl substituent.

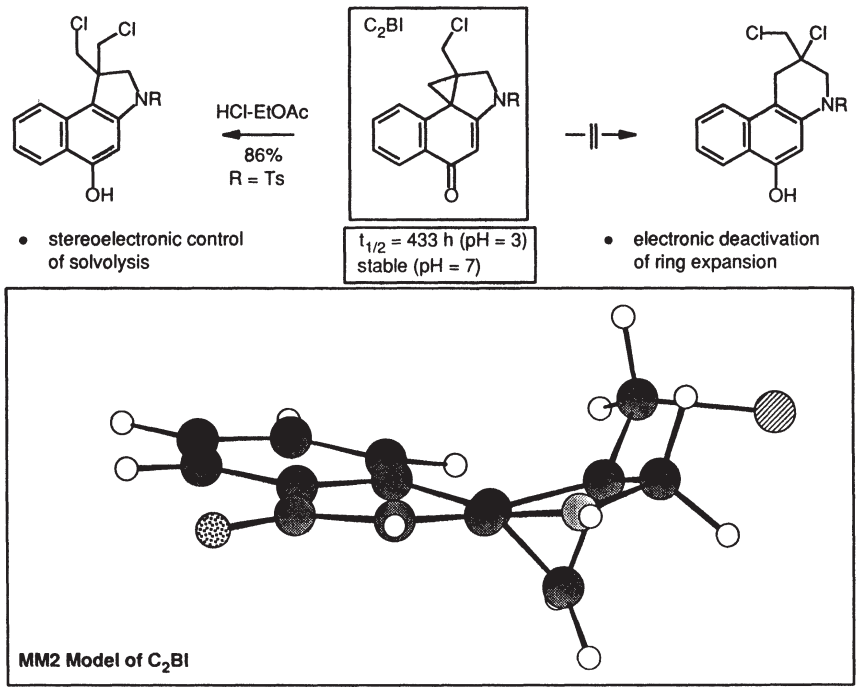
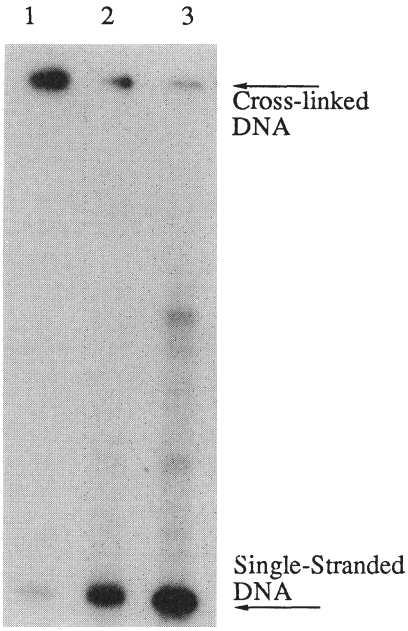


Fig. 5

A preliminary examination of the DNA alkylation properties of C_2BI-$CDPI_2$ has been conducted in efforts to demonstrate the event and selectivity of DNA alkylation and the DNA cross-linking capabilities. The C_2BI-based agents were shown to alkylate DNA within the minor groove in a fashion analogous to CC-1065 or duocarmycin. The stereoelectronically-controlled adenine N3 addition to the least substituted cyclopropane carbon occurs with a selectivity that represents a composite of the two enantiomers of the corresponding CBI-based agents. Additional high affinity alkylation sites were detected



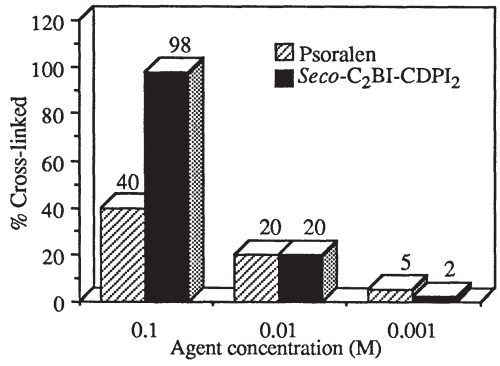
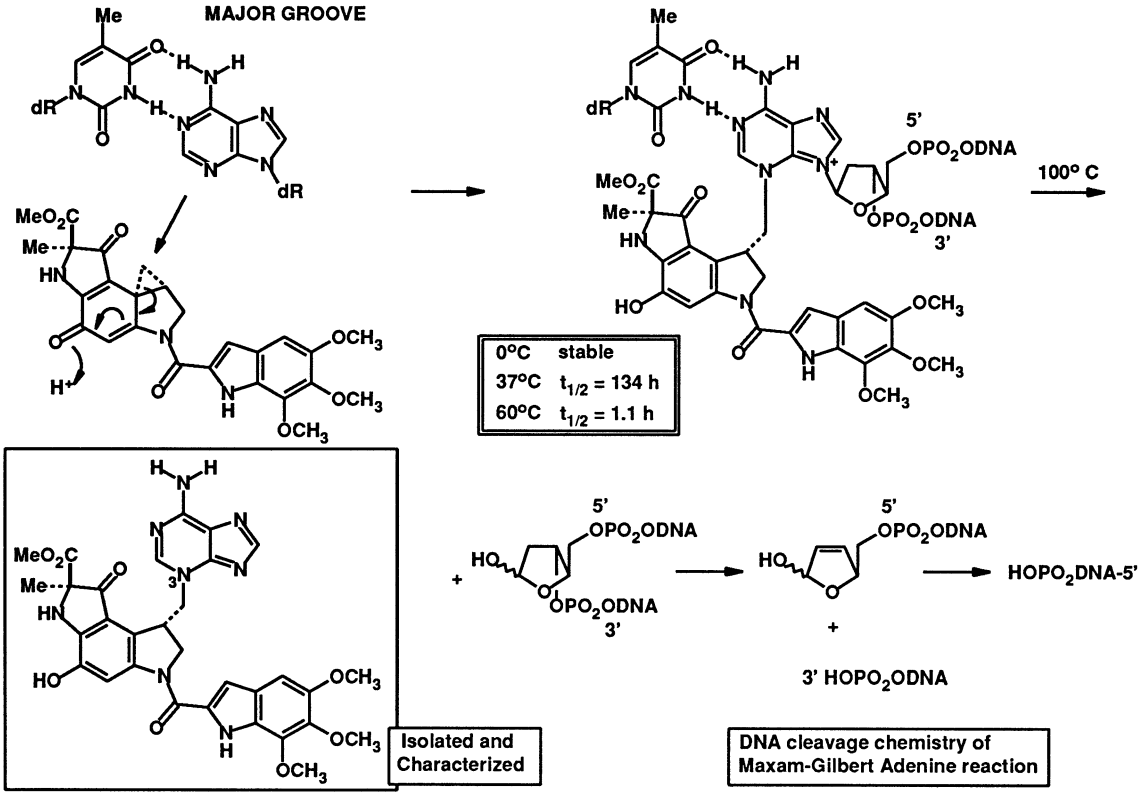
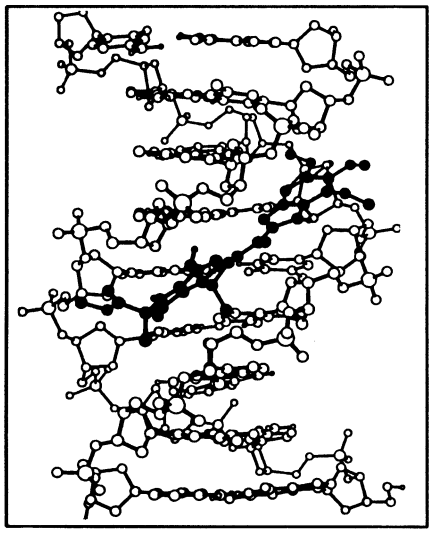


Fig. 6. Cross-linking study of *seco*-C_2BI-$CDPI_2$ with w794 duplex DNA. DNA-agent incubation at 25°C (24 h, pH 6), removal of unbound agent followed by denaturing 8% PAGE and autoradiography. Lanes 1-3, 0.1, 0.01, and 0.001 M *seco*-C_2BI-$CDPI_2$. Quantification of the extent of cross-linking was conducted using a scanning laser densitometer and run adjacent to psoralen (hv 365 nm, 1h) as a positive cross-linking control (data not shown).

which were not prominent alkylation sites for either enantiomer of the CBI-based agents. Such sites have been interpreted to represent induced high affinity alkylation sites resulting from DNA cross-linking subsequent to complementary strand alkylation at a high affinity alkylation site. Each such site detected proved consistent with predicted models of an adenine-adenine cross-linking event, Fig. 4. Consistent with this interpretation, the C_2BI agents were shown to constitute efficient DNA cross-linking agents. The DNA cross-linking of *seco*-C_2BI-$CDPI_2$ was observed at the same concentrations that DNA alkylation was detected and with an efficiency that exceeded that of psoralen, Fig. 6.





Duocarmycin DNA Alkylation Properties

Selectivity:

5'-d(AAA)-3' > 5'-d(TTA)-3' >> 5'-d(TAA)-3' > 5'-d(ATA)-3'

5'-d(T/AXXA)-3' >> 5'-d(G/CXXA)-3'

5'-d(XXXAPu)-3' > 5'-d(XXXAPy)-3'

(+)-Duocarmycin A = C_2 = C_1

(+)-Duocarmycin A = epi-(+)-Duocarmycin A

High Affinity Sequences:

5'-d(T/AAAA)-3' and 5'-d(T/ATTAPu)-3'

Intensity:

(+)-Duocarmycin A > C_2 > C_1 (4° C)

Natural enant. >> unnatural enant.
(+)-Duocarmycin A > epi-(+)-Duocarmycin A (3 - 6x) >>
ent-(-)-Duocarmycin A, epi,ent-(-)-Duocarmycin A (> 100x)

(+)-CC-1065 > (+)-Duocarmycin A (1 - 5x)

Fig. 7

Duocarmycins: DNA Alkylation Properties. The DNA alkylation properties of the naturally-derived and synthetic duocarmycins will be reviewed and details of the establishment of a model for the adenine N3 alkylation discussed,[8,15,20-22] Fig. 7.

Total Synthesis of (+)-Duocarmycin SA. (+)-Duocarmycin SA (**2**)[2] represents the newest and most potent naturally occurring member of the growing class of duocarmycins[2-4] that derive their biological properties through the characteristic, stereoelectronically-controlled adenine N3 alkylation of the electrophilic cyclopropane within the minor groove of duplex DNA at selected four base-pair AT-rich sites.[8] Because of its enhanced solvolytic stability relative to (+)-CC-1065 or (+)-duocarmycin A, the examination of **2**, which is currently available in limited quantities from natural sources, promises to be especially interesting. In recent efforts, we have completed a total synthesis of (+)-duocarmycin SA[23] based on sequential, regioselective nucleophilic substitution reactions[24] of the unsymmetrical *p*-quinone diimide **8** in the preparation of a functionalized dihydropyrroloindole precursor to the duocarmycin SA alkylation subunit, Fig. 8. In addition to constituting a new synthetic strategy for the preparation of natural or synthetic members of this growing class of agents, both enantiomers of *N*-BOC-DSA and its immediate precursors have been made available through the approach.

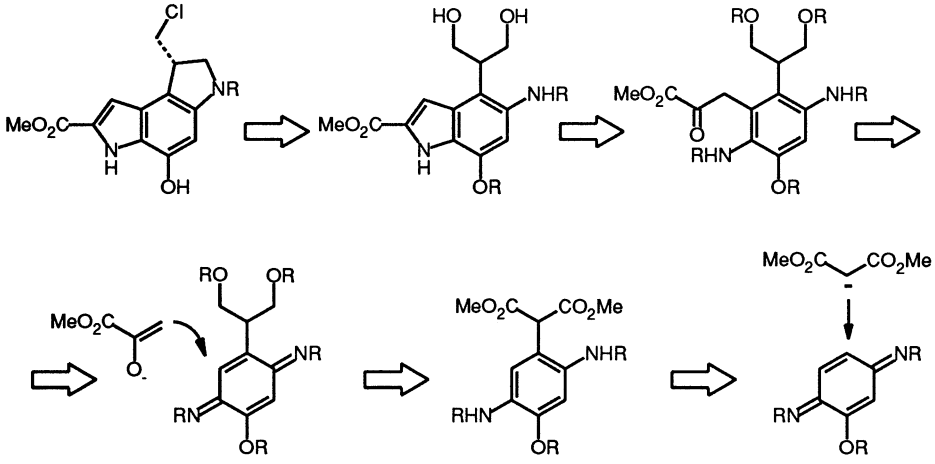


Fig. 8

Treatment of the unsymmetrical *p*-quinone diimide **8**[24] with dimethyl malonate provided **9** derived from regioselective C5 nucleophilic substitution attributable to steric and electronic deactivation of addition at C3 or C6 by the C2 benzyloxy substituent, Scheme 1. Sodium borohydride reduction of the methyl esters, protection of the diol as the acetonide **10**, and oxidation provided the *p*-quinone diimide **11** and a suitable acceptor substrate for a second nucleophilic substitution reaction. Clean regioselective C6 nucleophilic addition of the pyrrolidine enamine of pyruvaldehyde dimethyl acetal was achieved if followed immediately by mild acid treatment of the initial adduct under carefully defined reaction conditions (40 mL THF - 10 mL pH 4 phosphate buffer/mmol, 25°C, 4 h) to provide **12** cleanly in good yield. Competitive or predominant C1 or C4 imide addition or *p*-quinone diimide reduction was observed with a number of alternative nucleophiles and other enamine hydrolysis conditions examined generally provided complex mixtures of products derived from **12** including **13**. Notably, alternative diol protecting groups for **11** (e.g. TBDMS, MOM) proved less satisfactory in the nucleophilic substitution reaction

presumably due to increased steric interactions with the incoming nucleophile. Acid-catalyzed indole formation and concurrent acetonide hydrolysis provided **13** without observation of competitive indole *N*-benzoyl deprotection or acetal hydrolysis, Scheme 2. Completion of the preparation of the functionalized dihydropyroloindole skeleton of the duocarmycin alkylation subunit was accomplished by direct cyclization of diol **13** under Mitsunobu alkylation conditions. Deprotection of the benzamides followed by selective protection of the more reactive indoline C3-amine provided **15**. Mild acid-catalyzed hydrolysis of the dimethyl acetal under carefully prescribed reaction conditions (DMSO-pH 4 phosphate buffer-dioxane, 1:2:12, reflux, 15 h) provided the corresponding aldehyde in excellent yield *without* competitive BOC deprotection. Subsequent oxidation of the aldehyde provided the methyl ester **16** directly and two phase, transfer catalytic hydrogenolysis served to remove the benzyl ether to provide **17**. Conversion of the primary alcohol **17** to the chloride **18** followed by treatment of **18** with NaH provided *N*-BOC-DSA, the *t*-butyloxycarbonyl derivative of the duocarmycin SA alkylation subunit.

Acid-catalyzed deprotection of **18** followed by immediate coupling of the unstable indoline hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid provided **19**, Scheme 3. Final intramolecular Ar-3′ alkylation of **19** with closure of the cyclopropane ring provided racemic duocarmycin SA in excellent yield and identical in all compared respects with natural material (^{1}H NMR, ^{13}C NMR, IR, UV, $[\alpha]_D$, MS/HRMS).

Resolution of intermediate **17** through chromatographic separation of the corresponding *bis*-(*R*)-*O*-acetylmandelate ester diastereomers (SiO_2, α = 1.31) followed by incorporation of the resulting enantiomers of **17** derived from their hydrolysis into the preparation of (+)-duocarmycin SA and *ent*-(-)-duocarmycin SA completed the synthesis of the natural product and its enantiomer. The approach has provided the optically active materials in suitable quantities for a full examination and comparison of their properties.

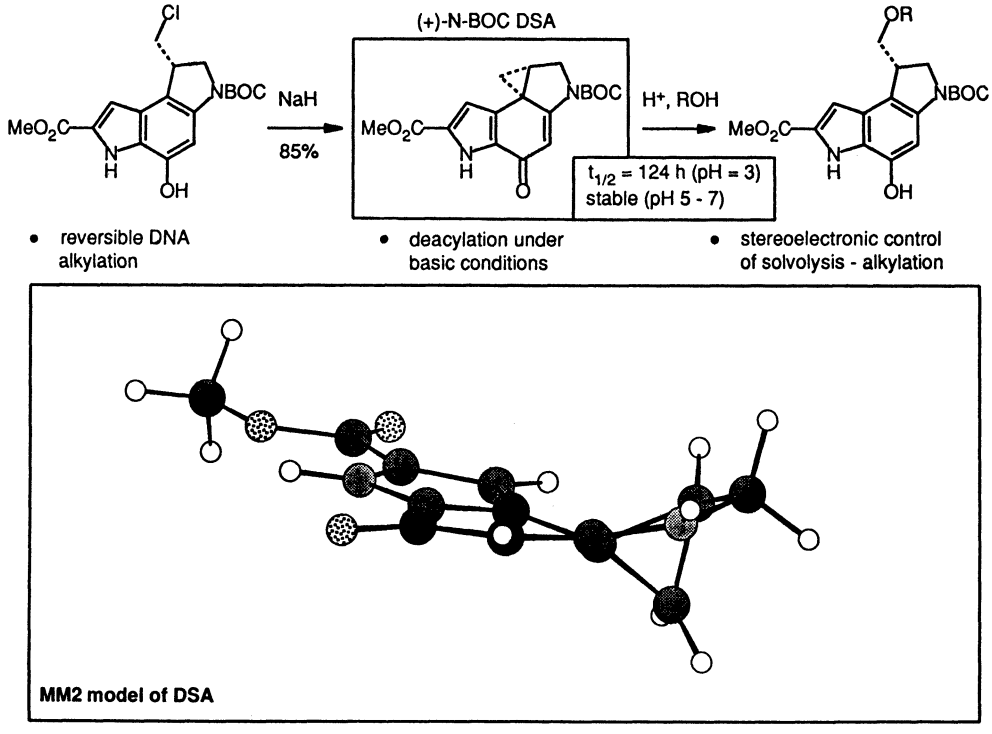
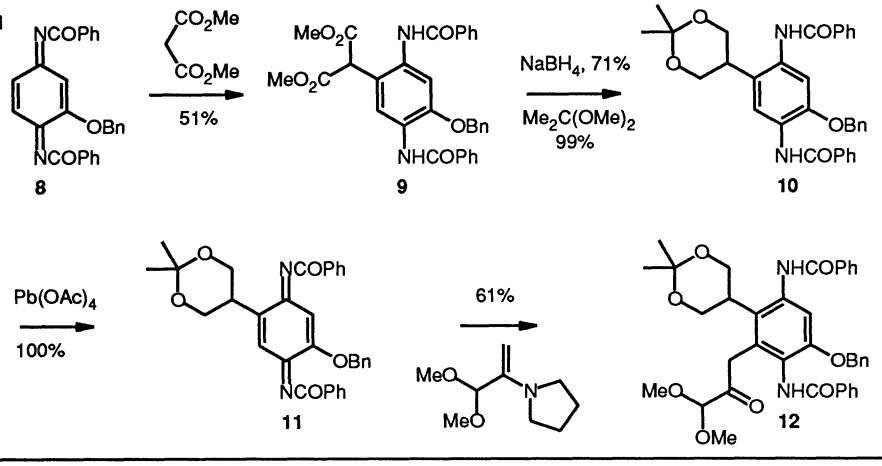


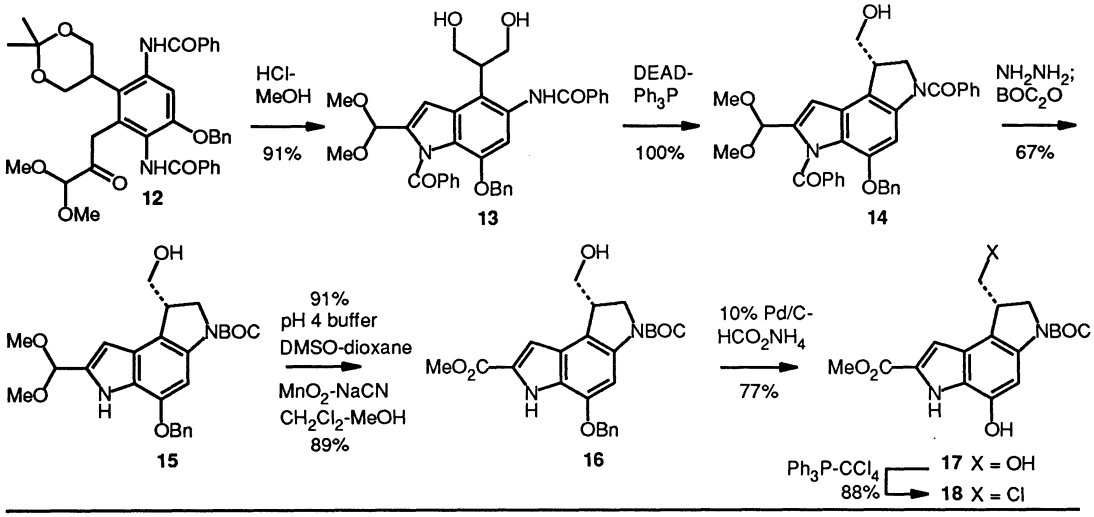
Fig. 9

In preliminary studies, racemic duocarmycin SA has proven to be approximately 2-10x more potent than (+)-CC-1065 in *in vitro* L1210 cytotoxic assays and *N*-BOC-DSA has proven to be approximately 4x more stable than N-BOC-CPI, the *t*-butyloxycarbonyl derivative of the authentic alkylation subunit of (+)-CC-1065, Fig. 9.

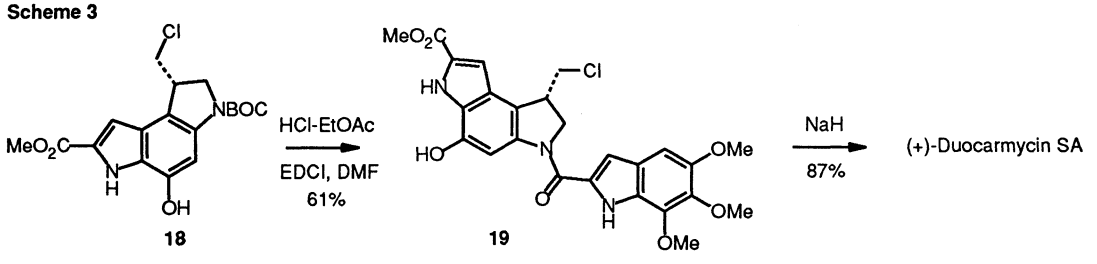
Scheme 1



Scheme 2



Scheme 3



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