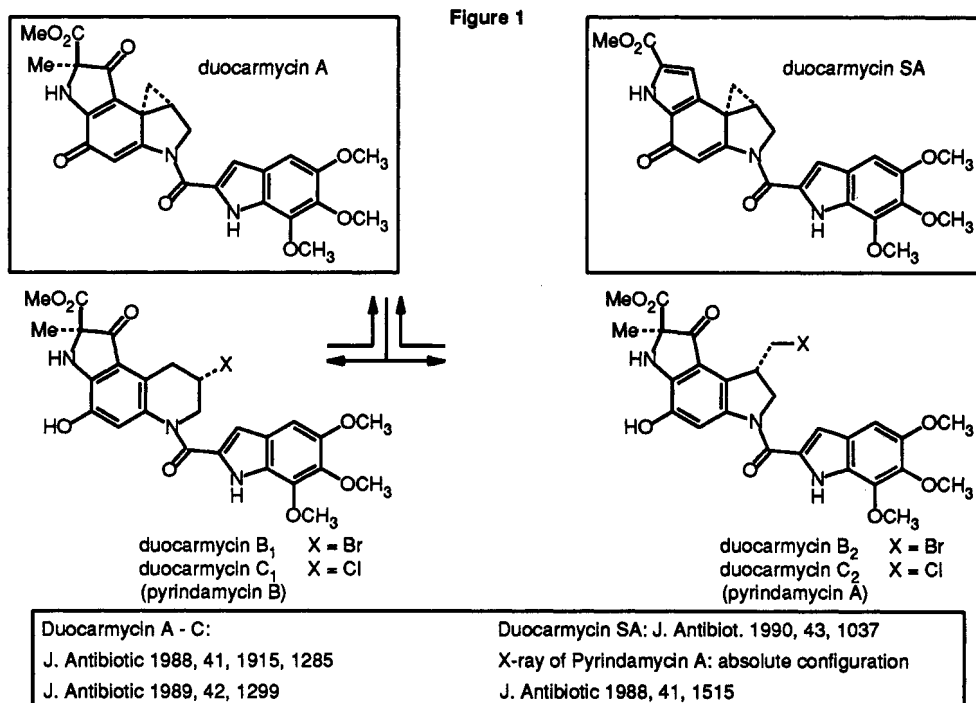# Design, synthesis, and evaluation of DNA minor groove binding agents: the duocarmycins

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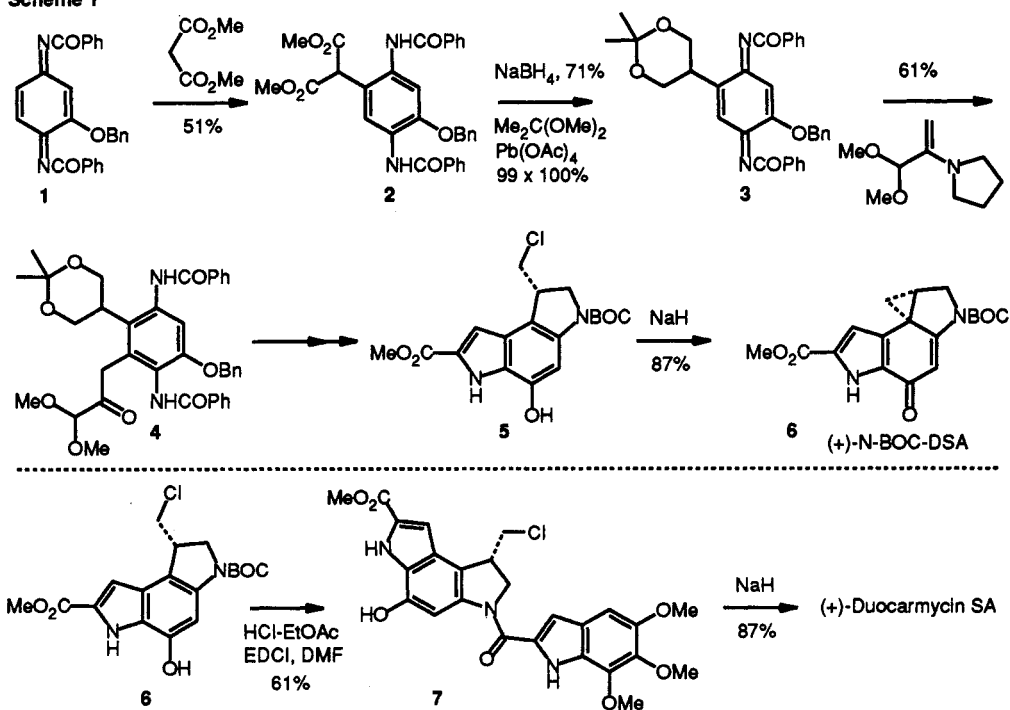
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Abstract. A summary of recent investigations of the duocarmycins is provided including the total synthesis of (+)-and *ent*-(-)-duocarmycin SA, the preparation and evaluation of the minimum potent pharmacophore of the alkylation subunit constituting the common pharmacophore of duocarmycin A and SA, the definition and characterization of the DNA alkylation properties of the agents and their correlation with biological properties, and studies to define the fundamental reactivity and molecular recognition principles relating the agents structure and properties.

The duocarmycins[1-3] constitute exceptionally potent antitumor-antibiotics that exert their biological effects through participation in a characteristic minor groove adenine N3 alkylation of duplex DNA,[4-9] Fig. 1.

Figure 1

duocarmycin A

duocarmycin SA

duocarmycin B₁ X = Br
duocarmycin C₁ X = Cl
(pyrindamycin B)

duocarmycin B₂ X = Br
duocarmycin C₂ X = Cl
(pyrindamycin A)

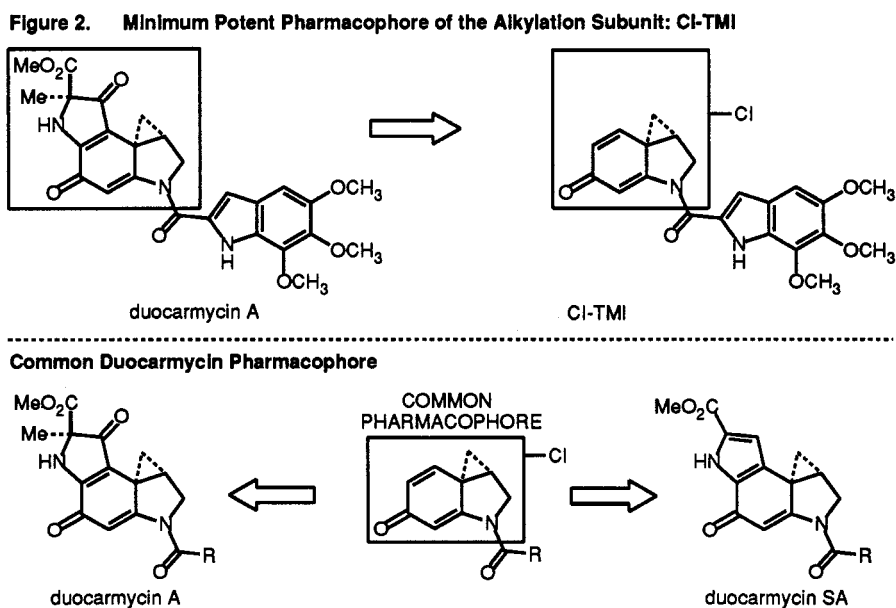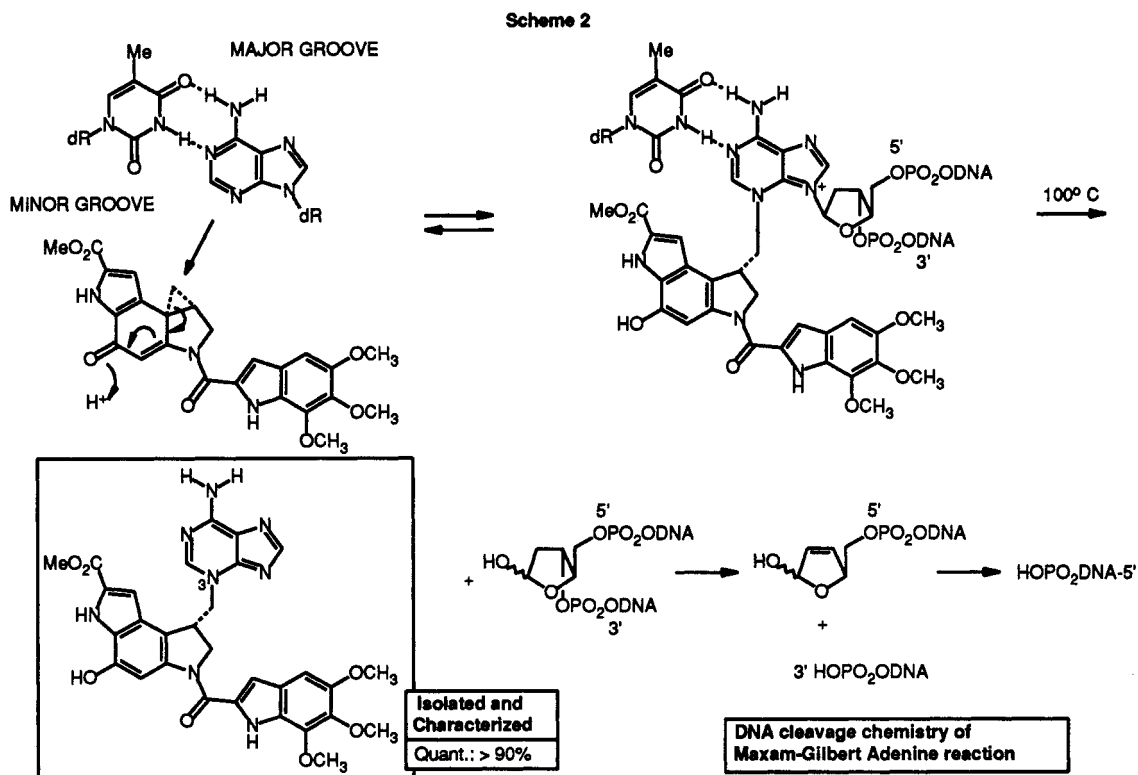| Duocarmycin A - C:	Duocarmycin SA: J. Antibiot. 1990, 43, 1037
J. Antibiotic 1988, 41, 1915, 1285	X-ray of Pyrindamycin A: absolute configuration
J. Antibiotic 1989, 42, 1299	J. Antibiotic 1988, 41, 1515

Scheme 1

Total Synthesis of (+)- and ent-(-)-Duocarmycin SA.[10] Duocarmycin SA[1] represents the newest and most potent naturally occurring member of this new structural class of agents. Because of its enhanced solvolytic stability relative to duocarmycin A, its examination has proven especially interesting. The total synthesis of (+)- and ent-(-)-duocarmycin SA which provided the necessary material for our subsequent evaluations was based on two, sequential regioselective nucleophilic substitution reactions[11] of the unsymmetrical p-quinone diimide 1, Scheme 1. In addition to constituting a new synthetic strategy for the preparation of the natural or synthetic members of this growing class of agents, both enantiomers of N-BOC-DSA (6) and their immediate precursors have been made available through the approach.

Minimum Potent Pharmacophore of the Duocarmycin Alkylation Subunit.[7,8] In early studies, the CI alkylation subunit[12] was shown to constitute the common, minimum potent pharmacophore of duocarmycin A and SA, Fig. 2. The preparation and evaluation of CI-TMI provided an exceptionally reactive agent ($t_{1/2}$= 5.2 h, pH 7; $t_{1/2}$= 35 s, pH 3) that displayed a DNA alkylation profile comparable to that of duocarmycin A or SA. Alkylation at the same adenine N3 sites was observed with CI-TMI but

with less efficiency and less selectivity among the available alkylation sites. In the conduct of these studies, the electrophilic cyclopropane proved not to be obligatory to observation of the characteristic adenine N3 alkylation and additional electrophilic centers within structurally related agents were shown to provide an identical alkylation profile.[13,14] Within this structurally simplified class of agents, definitive evidence for the important contribution of the noncovalent binding selectivity[15] to the DNA alkylation selectivity[12,13] was unambiguously defined and the initial observation of a relationship between agent chemical stability and biological potency reported.[6]

(+)-Duocarmycin A and (+)-Duocarmycin SA DNA Alkylation Properties. Duocarmycin A[6] and duocarmycin SA[16] participate in a now characteristic stereoelectronically-controlled adenine N3 alkylation reaction (minor groove) with addition to the least substituted cyclopropane carbon of the agent, Scheme 2. Our studies have defined the event, sequence selectivity, quantitation, and reversibility[17] of this predominant adenine N3 DNA alkylation reaction under conditions of limiting agent (86-92% for duocarmycin A, > 90% for duocarmycins SA). The isolation, quantitation, and characterization of the thermally released adenine-duocarmycin A[8] and adenine-duocarmycin SA[18] adducts unambiguously established the structural nature and relative importance of the minor groove adenine N3 alkylation reaction. (+)-Duocarmycin A and SA exhibit an identical DNA alkylation selectivity in which each adenine alkylation site was always found to be flanked by two 5′ A or T bases and there proved to be a preference for the three base-pair sequence that follows the order of 5′- AA<u>A</u>
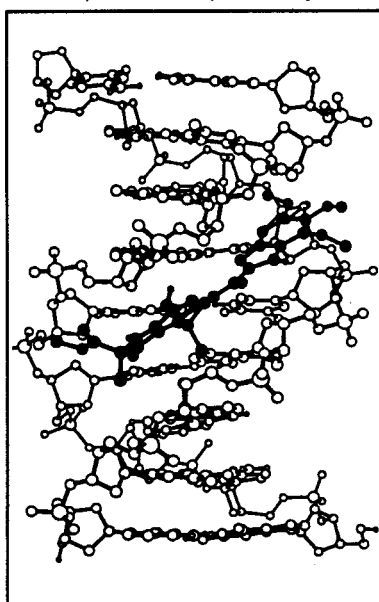
Figure 2. Minimum Potent Pharmacophore of the Alkylation Subunit: CI-TMI

duocarmycin A   CI-TMI

Common Duocarmycin Pharmacophore

duocarmycin A   duocarmycin SA

Scheme 2

> 5'-TT**A** > 5-AT**A**. There also proved to be preference, but not absolute requirement, for the fourth 5'

base to be A or T and a weak preference for the 3' base preceding the alkylation site to be purine versus

pyrimidine base. The distinction in the DNA alkylation properties of (+)-duocarmycin SA and A lies

in the relative efficiency or intensity of DNA alkylation and the less reactive but more stable agent, (+)-

duocarmycin SA, proved to be approximately 10x more effective at alkylation of duplex DNA. Similar

studies have been conducted with *ent*-(-)-duocarmycin SA,[16] *epi*-(+)-duocarmycin A, *ent*-(-)-

duocarmycin A, and *epi,ent*-(-)-duocarmycin A[9] and the seminal results are summarized in Fig. 3.

From these studies, a model of the natural enantiomer DNA alkylation was reconstructed and is

illustrated in Fig. 3 with high affinity alkylation site in w794 duplex DNA. The bound helical

conformation of the agent complements the topological curvature and pitch of the minor groove with

agent binding spanning 3.5 base-pairs in the 5'-direction from the adenine N3 alkylation site. The

hydrophobic concave face of the agent is deeply imbedded in the minor groove and the polar

functionality of the agent lies on the outer face of the complex. This nicely explains the requirement

for the first three base-pairs of the alkylation sites to be A or T which extends less rigidly to the fourth base-pair.

(+)- and *ent*-(-)-*N*-BOC-DSA DNA Alkylation Properties. The examination and definition of the DNA alkylation properties of *N*-BOC-DSA, a simple derivative of the duocarmycin SA alkylation subunit, proved especially revealing. Both enantiomers of *N*-BOC-DSA displayed an identical profile and efficiency of DNA alkylation. The DNA minor groove adenine N3 alkylation reaction proved analogous to that observed with the natural products themselves except that it proceeds with a significantly reduced sequence selectivity and substantially reduced efficiency (ca. 10000x), Fig. 4. In each instance under the conditions of limiting agent, the detected alkylation sites proved to be adenine flanked by a single 5′ A or T base and the preference for DNA alkylation proved to be 5′-AA > 5′-TA. From these and related studies,[19-23] we suggest that it is the simple event of the depth of minor groove penetration by the agent and steric accessibility to the adenine N3 alkylation site that are determining factors controlling the alkylation selectivity. For simple agents such as *N*-BOC-DSA, a single 5′ A or T base is sufficient for agent accessibility to the adenine N3 alkylation site. For duocarmycin A and SA, two to three additional 5′ A-T base-pairs are required for binding accessibility to the adenine N3 alkylation site. In addition to enhancing the DNA alkylation selectivity, the trimethoxyindole subunit of the duocarmycins serves to stabilize the inherently reversible nature of the alkylation reaction. We

Figure 3.
5′-d(CAATTAGTC):duocarmycin A   **Summary of Duocarmycin DNA Alkylation**

Binding Directionality: 3′ to 5′
   bound conformation spans 3.5 base-pairs
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 SA ≅ 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 SA >
   Duocarmycin A > C$_2$ > C$_1$ (4° C)
(+)-CC-1065 = (+)-Duocarmycin SA
(+)- > (-)-Duocarmycin SA (10 - 100x)
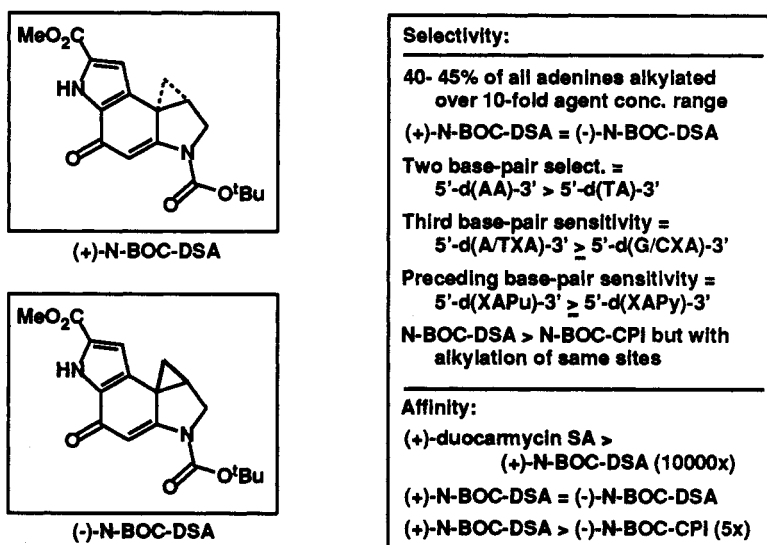**(+)-Duocarmycin SA > A (10x)**
(+)- > epi-(+)-Duocarmycin A (3-6x) >>
   ent-(-)- and epi,ent-(-)-Duocarmycin A

have suggested that it is the simple event of the noncovalent binding stabilization of the reversible DNA alkylation reaction provided by the trimethoxyindole subunit that leads to the enhanced, potent biological properties of duocarmycin SA versus *N*-BOC-DSA.
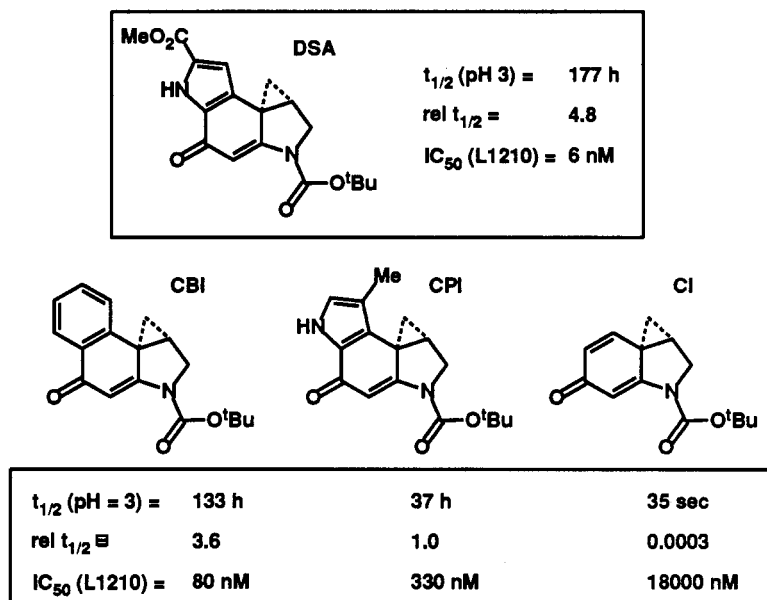
Biological Properties: A Fundamental Relationship Between Functional Reactivity and Biological Potency. Consistent with past studies in which a direct relationship between agent chemical stability and in vitro cytotoxic potency was defined,[23] agents bearing the (+)-duocarmycin SA alkylation subunit have proved to be the most potent and most stable agents defined to date. Representative examples of the general relationship are shown in Fig. 5. More advanced analogs of the natural products, (+)-duocarmycin SA (IC_{50} = 6 pM), (+)-CC-1065 (IC_{50} = 20 pM), and (+)-duocarmycin A (IC_{50} = 100-40 pM), exhibit the same general relationship except they have proven to be 1000-10000x more potent that the simple derivatives of the alkylation subunits. For the range of agents that possess sufficient reactivity to participate in the characteristic adenine N3 alkylation reaction within duplex DNA, presumably the direct relationship between agent chemical stability and biological potency may be attributed to the effectiveness with which the agent reaches its biological target.

Figure 4.   DNA Alkylation Properties of (+)- and ent-(-)-N-BOC-DSA

(+)-N-BOC-DSA

(-)-N-BOC-DSA

Selectivity:

40- 45% of all adenines alkylated over 10-fold agent conc. range
(+)-N-BOC-DSA = (-)-N-BOC-DSA

Two base-pair select. =
  5'-d(AA)-3' > 5'-d(TA)-3'

Third base-pair sensitivity =
  5'-d(A/TXA)-3' ≥ 5'-d(G/CXA)-3'

Preceding base-pair sensitivity =
  5'-d(XAPu)-3' ≥ 5'-d(XAPy)-3'

N-BOC-DSA > N-BOC-CPI but with alkylation of same sites

Affinity:
(+)-duocarmycin SA >
           (+)-N-BOC-DSA (10000x)
(+)-N-BOC-DSA = (-)-N-BOC-DSA
(+)-N-BOC-DSA > (-)-N-BOC-CPI (5x)

Figure 5.

DSA		
$t_{1/2}$ (pH 3) =	177 h	
rel $t_{1/2}$ =	4.8	
IC_{50} (L1210) =	6 nM	

	CBI	CPI	CI
$t_{1/2}$ (pH = 3) =	133 h	37 h	35 sec
rel $t_{1/2}$ =	3.6	1.0	0.0003
IC_{50} (L1210) =	80 nM	330 nM	18000 nM

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