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Bifunctional Alkylating Agents Derived from Duocarmycin SA: Potent Antitumor Activity with Altered Sequence Selectivity

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Abstract—The series of four dimers derived from head to tail coupling of the two enantiomers of the duocarmycin SA alkylation subunit are described.

Duocarmycin SA (**1**), is a potent antitumor antibiotic which derives its biological activity from a reversible, sequence-selective alkylation of DNA in the minor groove and is a member of the class of agents that includes CC-1065 and duocarmycin A (Fig. 1).[1–9] For the natural enantiomer, the stereoelectronically controlled adenine N^3 addition to the unsubstituted cyclopropane carbon of the left-hand subunit has been shown to occur within selected AT-rich regions of duplex DNA with a binding orientation that extends in a 3′ to 5′ direction from the site of alkylation. The unnatural enantiomers similarly alkylate adenine N^3 but with a binding orientation that extends in the opposite, 5′ to 3′, direction from the alkylation site. Extensive studies of agents containing deep-seated structural modifications[10–16] have served to determine the fundamental structural features contributing to polynucleotide recognition and functional reactivity.[17–20]

We have recently described the mechanism of activation of the duocarmycins by a binding-induced conformational change which disrupts vinylogous amide stabilization and activates the cyclopropane for alkylation.[21,22] Key to these studies was the synthesis of reversed analogues of duocarmycin SA, in which the methyl ester of the alkylation subunit was hydrolyzed and coupled to the right-hand amine of DNA binding subunits.[23] The alkylation pattern (involving a switch in the inherent enantiomer sequence selectivities) and reactivity (1000×reduced) showed both that the sequence selectivity is derived from noncovalent interactions with

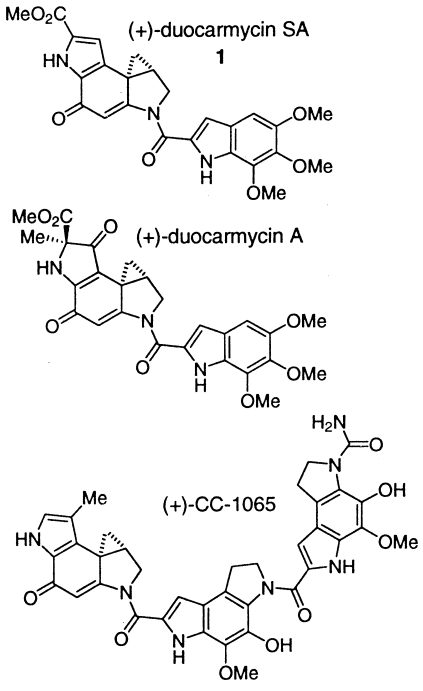

Figure 1. Structures of CC-1065 and the duocarmycins.

the narrow, deep AT-rich minor groove, and that the activation for alkylation requires an extended, rigid heteroaryl amide linked to the right-hand side of the alkylation subunit.

One consequence of the ability to couple through amide bond formation at either side of the duocarmycin SA subunit is the possibility to assess the effects of incor-

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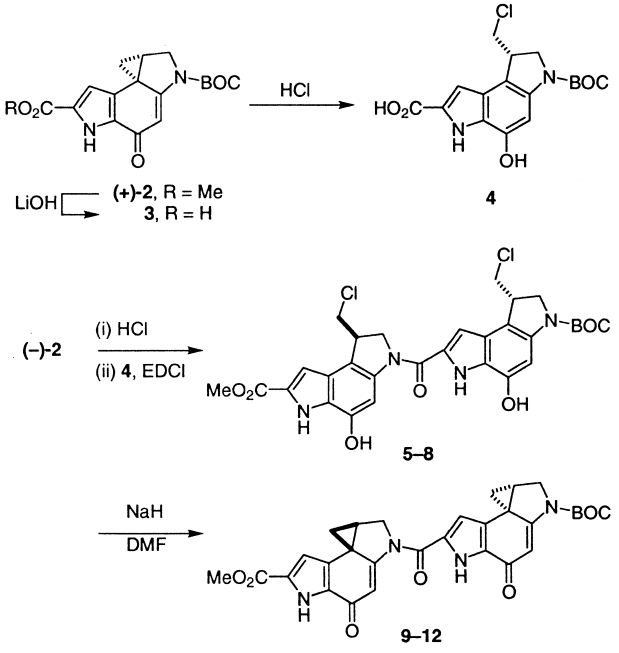
poration of multiple alkylating groups in a single structure by head to tail coupling. The arrangement of alkylating groups of either enantiomer allows for the study of both inter- and intramolecular cross-linking agents, the effects of the polyfunctionalization on biological activity and on the sequence selectivity of DNA alkylation.

Herein, we provide the first synthesis of bifunctional alkylating agents based upon the duocarmycin SA subunit, their potent cytotoxic activity, and preliminary observations on their DNA alkylation properties and sequence selectivities.

Synthesis

The synthesis of the four agents is illustrated with **10**, (+)(−)-DSA_2, in Scheme 1. The hydrolysis of *N*-BOC-DSA (**(+)-2**), the alkylation subunit of duocarmycin SA has been described,[10] (LiOH, 60 °C, THF:MeOH:H_2O 3:2:1, 80–90%). Coupling occurs efficiently only with the ring opened acid **4** and this was achieved by treatment of **3** with dilute acid (1 M HCl:EtOAc, 0 °C, 30 min) which served to cleave the cyclopropane, but not deprotect the *N*-BOC group. Direct coupling of **4** (EDCI, DMF, 25 °C, 16 h, typically 43–52%) with the unstable free amine released by deprotection of *N*-BOC-DSA (**(−)-2**) (3.3 M HCl/EtOAc, 25 °C, 30 min) provided the precursors. The resulting *seco* agents were spirocyclized to the DSA dimers under basic conditions (NaH, DMF, 25 °C, 30 min, 99–100%).

Using this approach, all four possible diastereomers were prepared incorporating all combinations of the natural and unnatural enantiomers of the DSA alkylation subunit.[24]



Scheme 1.

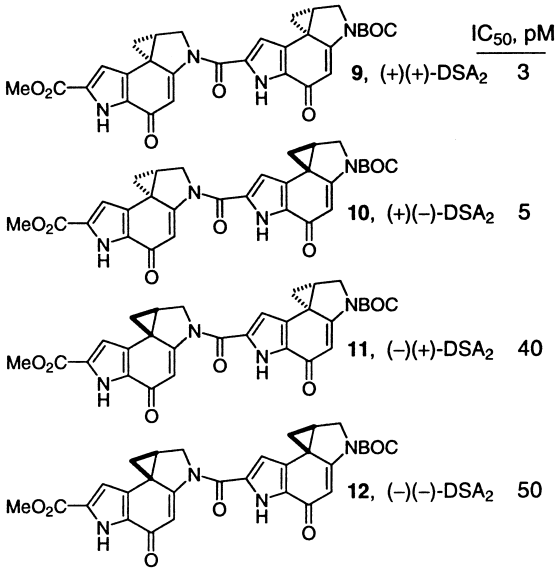
Cytotoxic Activity

All four agents proved to be potent in cytotoxicity assays against the L1210 cell line (Table 1), with the (+)(+)-DSA_2 (**9**, 3.0 pM) and (+)(−)–DSA_2 (**10**, 5.0 pM) displaying a 2–3 fold higher activity than duocarmycin SA (10 pM).

The properties of the agents appear to follow well-defined and, in retrospect, predictable potency orders. Those possessing a natural enantiomer left-hand alkylation subunit (i.e. **9** and **10**) were found to be ca. 10× more potent than the corresponding unnatural enantiomers (i.e. **11** and **12**). Given the requirement for a rigid, extended heteroaryl amide on the N^2 nitrogen (versus BOC) for effective DNA alkylation catalysis, the left-hand subunit can predictably be expected to alkylate DNA first and dominate the characteristics of the compounds. This is born out in the comparisons of **9** and **10** versus **11** and **12**, of which the former pair are more active. Just as **9** and **10** are 2–3× more potent than (+)-duocarmycin SA (IC_{50} = 10 pM), **11** and **12** are 2–3× more potent than *ent*-(−)-duocarmycin SA (IC_{50} = 100 pM). Thus, the dimerization head-to-tail linkage of the duocarmycin SA alkylation subunit provides compounds which are 2−3× more potent than ducarmycin SA and approximately 2000× more potent than *N*-BOC-DSA. Moreover, the enantiomeric configuration of the right-hand subunit also impacts the potency, albeit not as substantially (1–2×), and those containing the natural enantiomer (**9** and **11**) were more potent than those containing the unnatural enantiomer (**10** and **12**).

The increased biological activity of the agents relative to *N*-BOC-DSA is analogous to the change observed with respect to duocarmycin SA and is derived from the

Table 1. In vitro cytotoxic activity, L1210 IC_{50}, pM



enhanced DNA binding affinity of the dimer-like structure and the DNA alkylation catalysis resulting from the incorporation of a rigid, extended right-hand heteroaromatic linking amide. In this manner, the right-hand DSA alkylation subunit serves the same function as the duocarmycin SA trimethoxyindole subunit. The 2–3-fold increase in potency of **9** and **10** or **11** and **12** over the corresponding DSA enantiomer can be attributed to the subsequent behavior of the additional right-hand subunit. Notably **9** and **12** are capable of only providing intrastrand DNA cross-links while **10** and **11** can only provide interstrand DNA cross-links. Apparently, this distinction does not contribute to the cytotoxic potency of the resulting agents. It may be that the increased potency of **9**–**12** compared with the monoalkylating analogues may be a consequence of a change in the stability of the DNA adduct, with the bisalkylated adduct being more stable than the monoalkylated analogue. It may also reflect a change in DNA alkylation sequence selectivity, or the formation of more lethal adducts at different sites within the DNA. In order to probe these effects, we investigated their ability to alkylate DNA.

DNA Alkylation

The left-hand subunit was found to dominate the characteristics of the agents being responsible for the first and primary site of DNA alkylation. Thus, **9** and **10** alkylated terminal 3′ adenines within a 3–5 base-pair AT-rich site (e.g. 5′- AAA<u>A</u>) analogous to (+)-duocarmycin SA. Similarly, **11** and **12** alkylated terminal 5′ adenines within a 3–5 base pair AT-rich site (e.g. 5′-A<u>A</u>AA) analogous to *ent*-(−)-duocarmycin SA and with an analogous one base-pair offset relative to the natural enantiomers within the AT-rich site.

This corresponds to a 3′ to 5′ binding orientation across an AT-rich site for **9** and **10** analogous to (+)-duocarmycin SA or a 5′ to 3′ binding orientation for **11** and **12** analogous to *ent*-(−)-ducarmycin SA indicating that the left-hand subunit of the agents dominate the primary DNA alkylation reaction. Fundamentally more interesting and predictably, **9** and **12** showed no evidence of interstrand DNA cross-linking consistent with the fact that both cyclopropanes are oriented toward the same strand of DNA in their DNA-bound conformation. In contrast, both **10** and **11** are expected to display interstrand DNA cross-links consistent with the orientation of the cyclopropanes toward the opposite, or complimentary, DNA strands. These and related characteristics of the DNA alkylation properties of **9**–**12** are under further investigation and will be disclosed in due time.

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24. Spectroscopic Data: **(−)(−)-DSA$_2$** ^{1}H NMR (DMF-d_7, 400 MHz) δ 12.67 (br s, 1H), 12.35 (br s, 1H), 6.83 (s, 1H), 6.80 (s, 1H), 6.66 (br s, 1H), 6.48 (br s, 1H), 4.43 (dd, J = 10.6, 5.0 Hz, 1H), 4.32 (d, J = 10.8 Hz, 1H), 4.01 (dd, J = 11.0, 4.7 Hz, 1H), 3.98 (d, J = 11.0 Hz, 1H), 3.84 (s, 3H), 3.08 (m, 1H), 2.95 (m, 1H), 1.85 (dd, J = 7.6, 3.7 Hz, 1H), 1.78 (dd, J = 7.6, 3.7 Hz, 1H), 1.74 (t, J = 5.0 Hz, 1H), 1.51 (s, 9H), 1.48 (t, partially obscured, 1H); FABHRMS (NBA/CsI) m/z 689.1029 (M^+ + Cs, $C_{30}H_{28}N_4O_7$ requires 689.1012). **(+)(−)-DSA$_2$** ^{1}H NMR (DMF-d_7, 400 MHz) δ 12.66 (br s, 1H), 12.35 (br s, 1H), 6.84 (s, 1H), 6.80 (s, 1H), 6.66 (br s, 1H), 6.54 (br s, 1H), 4.44 (dd, J = 10.6, 5.0 Hz, 1H), 4.30 (d, J = 10.8 Hz, 1H), 4.05 (dd, J = 11.0, 4.7 Hz, 1H), 3.98 (d, J = 11.0 Hz, 1H), 3.84 (s, 3H), 3.05 (m, 1H), 2.97 (m, 1H), 1.85 (dd, J = 7.6, 3.7 Hz, 1H), 1.76

(dd, $J=7.6$, 3.7 Hz, 1H), 1.72 (t, $J=5.0$ Hz, 1H), 1.51 (s, 9H), 1.47 (m, partially obscured, 1H); FABHRMS (NBA/CsI) m/z 689.1030 ($M^+ + Cs$, $C_{30}H_{28}N_4O_7$ requires 689.1012). **(+)(+)-DSA$_2$** ^{1}H NMR (DMF-d_7, 400 MHz) δ 12.68 (br s, 1H), 12.32 (br s, 1H), 6.83 (s, 1H), 6.80 (s, 1H), 6.66 (br s, 1H), 6.48 (br s, 1H), 4.44 (dd, $J=10.6$, 5.0 Hz, 1H), 4.32 (d, $J=10.8$ Hz, 1H), 4.04 (dd, $J=11.0$, 4.7 Hz, 1H), 3.99 (d, $J=11.0$ Hz, 1H), 3.84 (s, 3H), 3.05 (m, 1H), 2.97 (m, 1H), 1.84 (dd, $J=7.6$, 3.7 Hz, 1H), 1.79 (dd, $J=7.6$, 3.7 Hz, 1H), 1.76 (t, $J=5.0$ Hz, 1H), 1.51 (s, 9H), 1.48 (m, partially obscured, 1H); FABHRMS (NBA/CsI) m/z 689.1030 ($M^+ + Cs$, $C_{30}H_{28}N_4O_7$ requires 689.1012). **(−)(+)-DSA$_2$** ^{1}H NMR (DMF-d_7, 400 MHz) δ 12.72 (br s, 1H), 12.33 (br s, 1H), 6.84 (s, 1H), 6.80 (s, 1H), 6.66 (br s, 1H), 6.54 (br s, 1H), 4.44 (dd, $J=10.6$, 5.0 Hz, 1H), 4.29 (d, $J=10.8$ Hz, 1H), 4.04 (dd, $J=11.0$, 4.7 Hz, 1H), 3.98 (d, $J=11.0$ Hz, 1H), 3. 84 (s, 3H), 3.05 (m, 1H), 2.97 (m, 1H), 1.84 (dd, $J=7.6$, 3.7 Hz, 1H), 1.76 (dd, $J=7.6$, 3.7 Hz, 1H), 1.71 (t, $J=5.0$ Hz, 1H), 1.51 (s, 9H), 1.47 (m, partially obscured, 1H); FABHRMS (NBA/CsI) m/z 689.1029 ($M^+ + Cs$, $C_{30}H_{28}N_4O_7$ requires 689.1012).