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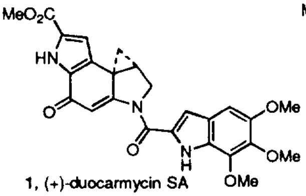
Examination Of The Role Of The Duocarmycin SA Methoxy Substituents: Identification Of The Minimum, Fully Potent DNA Binding Subunit

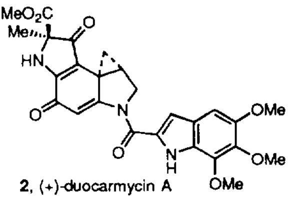
Dale L. Boger, Bernd Bollinger, and Douglas S. Johnson
Department of Chemistry, The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, California 92037, U.S.A.

Abstract. The preparation and examination of **4**–**7** revealed that (+)-**5** and (+)-duocarmycin SA were indistinguishable. In contrast, **6** and **7** exhibited properties more analogous to **4** illustrating that the C6 and C7 methoxy substituents of duocarmycin SA contribute little or nothing to its properties. Thus, the C5 methoxy substituent of the 5,6,7-trimethoxyindole subunit of duocarmycin SA is necessary and sufficient for observation of the full potency of the natural product. Copyright © 1996 Elsevier Science Ltd

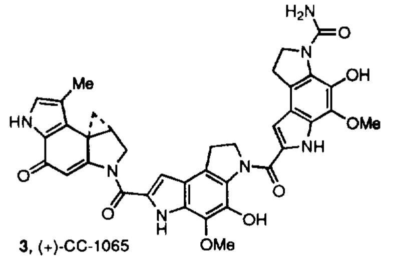
Duocarmycin SA (**1**)[1] and duocarmycin A (**2**)[2] constitute the parent members of a class of potent antitumor antibiotics[3] related to CC-1065 (**3**)[4] that derive their biological properties through a sequence selective alkylation of duplex DNA.[5-12] Since their disclosure, substantial efforts have been devoted to defining the characteristics of their DNA alkylation reactions, to determining the origin of their DNA alkylation selectivity, to establishing the link between DNA alkylation and the ensuing biological properties, and to defining fundamental principles underlying the relationships between structure, functional reactivity, and biological properties.[5,6] One important structural component of the natural products is the right-hand subunits linked to the alkylation subunit through an N^2 amide which has been shown to increase the DNA alkylation rate, efficiency, and selectivity and to increase biological potency 10^3–10^4×. Herein, we examine in detail the trimethoxyindole-2-carboxylate subunit of duocarmycin SA with the intention of defining the importance and potential role of each of the three methoxy substituents. The results of the study clearly highlight the unique importance of the C5 methoxy substituent that is imbedded deeply in the minor groove upon DNA alkylation, and a previously unrecognized role of this substituent is proposed.



1, (+)-duocarmycin SA



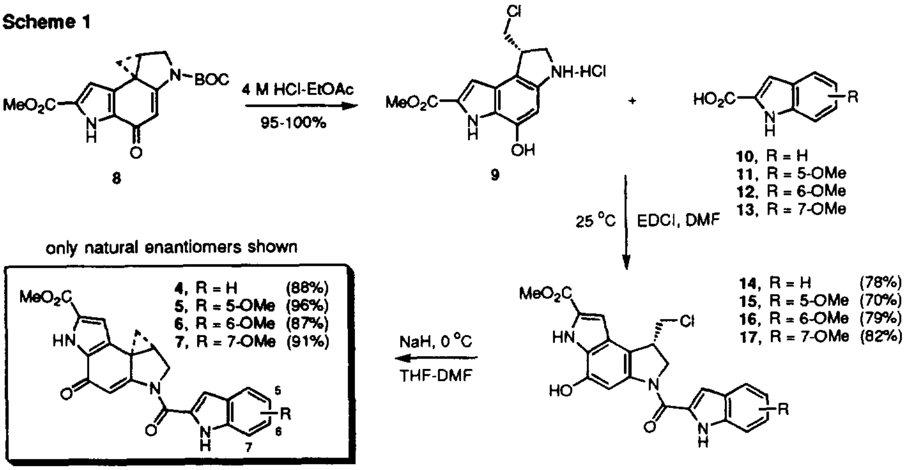
2, (+)-duocarmycin A



3, (+)-CC-1065

Synthesis. The agents **4**–**7** were targeted for evaluation since **5**–**7** provide an assessment of the individual contribution of the three methoxy groups of **1** in their comparisons with **1** and **4**. The optically active duocarmycin SA alkylation subunit, *N*-BOC-DSA (**8**), was prepared as previously disclosed.[13] Resolution of **8** was effected by direct chromatographic separation on a semipreparative ChiralCel OD HPLC column (10 μm 2 × 25 cm, 30% 2-propanol/hexane, 7 mL/min, $\alpha = 1.24$, ≥99.9% ee).[14] This proved more effective and convenient than our previously reported method of *bis*-(*R*)-*O*-acetylmandelate derivatization of an immediate precursor and chromatographic separation of the corresponding diastereomers followed by mandelate ester hydrolysis with regeneration of the pure enantiomers.[13] Acid-catalyzed deprotection of **8** (4 M HCl–EtOAc, 25 °C, 30 min, 95–100%) that was accompanied by clean addition of HCl to the activated cyclopropane provided the *seco* HCl salt **9** (Scheme 1). Immediate coupling (3 equiv EDCI, DMF, 25 °C, 4–15 h) of **9** with the indole-2-carboxylic acids **10–13** (1.1 equiv) in the deliberate absence of added base[15] provided the penultimate precursors **14–17** in excellent conversions (70–82%). Spirocyclization was effected by treatment with NaH (3 equiv, THF–DMF 4–2:1, 0 °C, 30 min) to provide both enantiomers of the agents **4–7** in excellent conversions (87–96%). Coupling of **9** in the presence of mild base including NaHCO$_3$ leads to competitive spirocyclization and the presence of adventitious moisture in the following spirocyclization reaction mixture will lead to subsequent hydrolysis of the linking N^2 amide.

Scheme 1



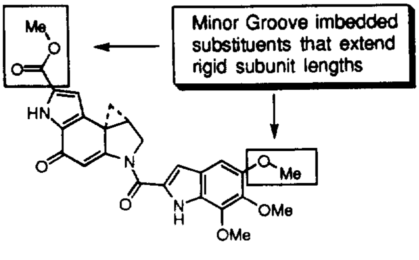
In Vitro Cytotoxic Activity. The in vitro cytotoxic activity of both enantiomers of **4–7** along with that of **1** is summarized in Table 1. For the natural enantiomers, removal of the three methoxy groups led to a 6.5-fold reduction in potency. The 5-methoxy derivative (+)-**5** was indistinguishable from duocarmycin SA illustrating that its C6 and C7 methoxy groups are not contributing significantly to its properties. Consistent with this, (+)-**7** was equipotent with (+)-**4** indicating that C7 methoxy group does not contribute to the properties of (+)-**1** while (+)-**6** exhibited intermediate activity suggesting it may potentiate the effects of the C5 methoxy substituent. Similar, but more pronounced effects were observed with the unnatural enantiomers. The unnatural enantiomers of **4**, **6**, and **7** were essentially equipotent and 13–18× less potent than *ent*-(–)-**1** while *ent*-(–)-**5** more closely approached the full activity of *ent*-(–)-**1**.

DNA Alkylation Selectivity, Efficiency, and Rate. The DNA alkylation selectivity and efficiency of the natural enantiomers of **4–7** were compared with that of **1** in w794 DNA.[11] All five agents exhibited nearly identical DNA alkylation selectivities and the distinctions observed were found in the rates and overall efficiencies of DNA alkylation. When the incubation with w794 DNA was conducted at 25 °C for 24 h, **5** was found to be essentially indistinguishable from **1** itself, **6** and **7** (**6** > **7**) were 5–10× less efficient than **1** or **5**, and **4** was the least effective of the agents being 20× less efficient than **1** or **5** (Table 1). These trends in the overall efficiency of DNA alkylation parallel the relative trends in cytotoxic potency. Similarly, the relative rates of DNA alkylation for **1**, **5**, and **4** were also examined within w794 (10^{-5} M, 25 °C, 1–72 h) at the single high affinity site of 5′-d(AATTA). (+)-Duocarmycin SA (**1**) and **5** were nearly indistinguishable with **1** exhibiting a slightly faster rate (k_{rel} = 1.3–2.3) and both were substantially faster than that of **4** (k_{rel} = 18–33).

Table 1.

Agent	IC_{50} (pM, L1210)	Rel. DNA Alkyl. Efficiency	Agent	IC_{50} (pM, L1210)
natural enantiomers			unnatural enantiomers	
(+)-**1**	10	1.0	(-)-**1**	100
(+)-**5**	10-12	1.0	(-)-**5**	300
(+)-**6**	25	0.2	(-)-**6**	1300
(+)-**7**	60	0.1	(-)-**7**	1800
(+)-**4**	65	0.05	(-)-**4**	1700

Discussion. The C7 and C6 methoxy groups, which lie on the outer face of the DNA-agent complex,[5,7] individually contribute little (C6 > C7) to the properties of duocarmycin SA. In contrast, the C5 methoxy group that is deeply imbedded in the minor groove contributes prominently to its properties. The agent containing a single C5 methoxy substituent proved indistinguishable from duocarmycin SA indicating that it alone is sufficient for observation of the full potency of the natural product. This is consistent with a role in which the C5 methoxy group provides further noncovalent binding stabilization for the inherently reversible DNA alkylation reaction[7,8] by virtue of its placement deep in the minor groove and the lack of such an effect for the C6/C7 methoxy substituents is consistent with quantitative modeling studies.[5] In addition, the C5 methoxy group of duocarmycin SA extends the rigid length of the DNA binding subunit. Its presence results in an increase in the inherent twist in the helical conformation of the DNA bound agent with the helical rise of the agent adjusted at the site of linking N^2 amide.[16] This twist in the conformation at the N^2 amide disrupts the vinylogous amide conjugation in the alkylation subunit and increases the inherent reactivity of the agent contributing to the catalysis of the DNA alkylation reaction.[16] Removing the C5 methoxy substituent shortens the length of the right-hand subunit, decreases the inherent twist in the linking N^2 amide in the DNA bound conformation, and less effectively activates the agent for DNA alkylation.


Minor Groove imbedded substituents that extend rigid subunit lengths

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