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SYNTHESIS AND CYTOTOXICITY OF ENANTIOMERIC PAIRS OF DUOCARMYCIN A AND ITS 2-EPIMER

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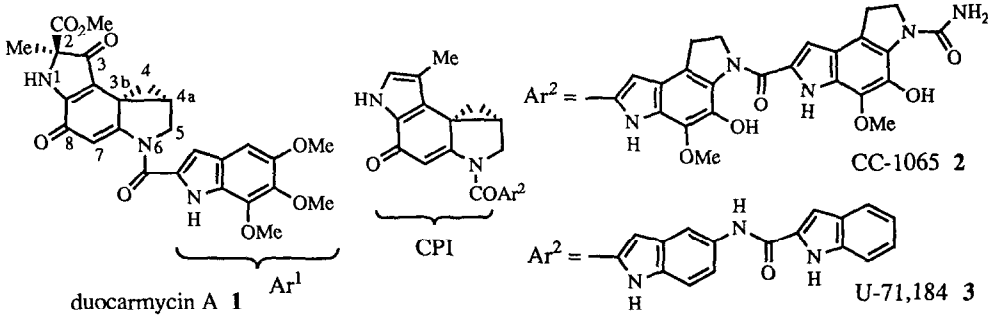
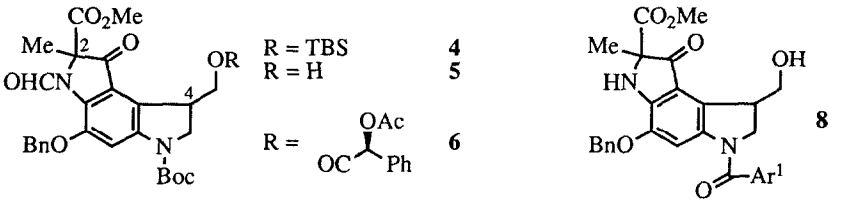
Abstract. The synthesis of the four possible diastereomers of duocarmycin A was achieved through optical resolution of a tricyclic synthetic intermidiate. The stereochemical configuration of the cyclopropane ring was found to be closely related with their cytotoxicity against P388 murine leukemia.

Duocarmycin A (**1**) isolated from *Streptomyces sp.* is a novel antitumor antibiotic which is effective against various strains of murine tumors.[2] The striking structural feature of **1** is its close resemblance to that of the potent antitumor antibiotic, CC-1065 (**2**). The cyclopropapyrroloindole ring (CPI) system of **2** is similar to that of **1** and has been revealed to be a site of nucleophilic attack by adenine N-3 in DNA.[3] Since **2** showed unusual delayed lethality,[4] a less toxic analogue of **2**, U-71,184 (**3**), has been developed.[3a] Interestingly, the cytotoxic potency of the enantiomer of **3** to L1210 cells *in vitro* was two orders of magnitude less than that of **3**. It was suggested that the absolute configuration of cyclopropane ring might be an important factor for their cytotoxic and other biological activities. In a previous communication reporting the first total synthesis of *dl*-duocarmycin A and its *dl*-2-epimer,[5] we disclosed that they showed a comparable level of cytotoxicity against P388 murine leukemia *in vitro*. These observations suggested that the stereochemistry at the C-2 quaternary center is not an important factor in their cytotoxicities. In this communication, we would like to report the synthesis of (+)-duocarmicin A as well as the three additional possible diastereomers to explore the role of the absolute configuration of cyclopropane ring on their cytotoxic activity. Upon examining their *in vitro* cytotoxicities, the compounds with unnatural absolute configurations at the cyclopropane rings were found to exhibit substantially weaker level of cytotoxicity.

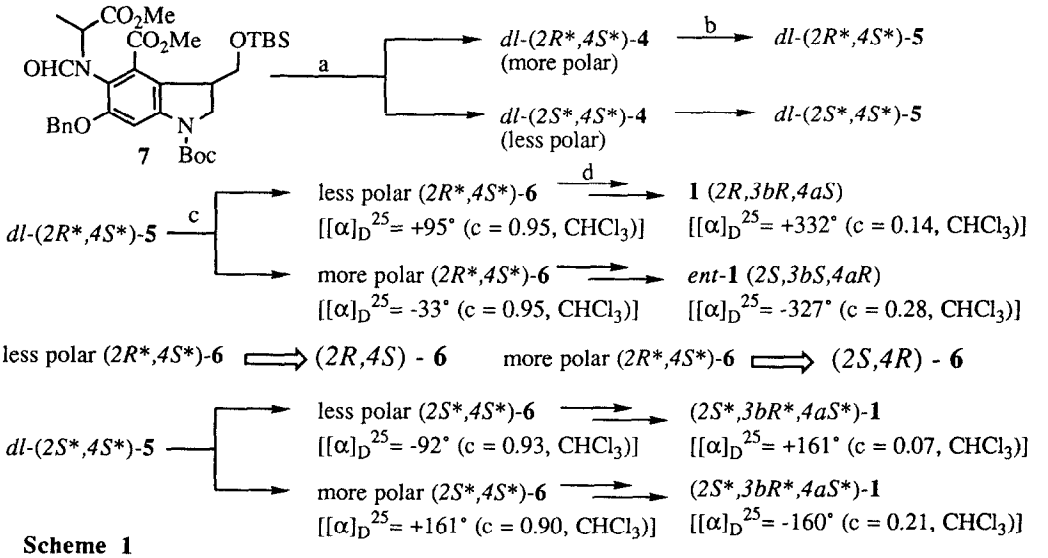
For the synthesis of four possible diastereomers of **1**, we first examined optical resolution of the tricyclic synthetic intermediate *dl*-**5** and subsequent conversion to natural (+)-**1** to determine the absolute





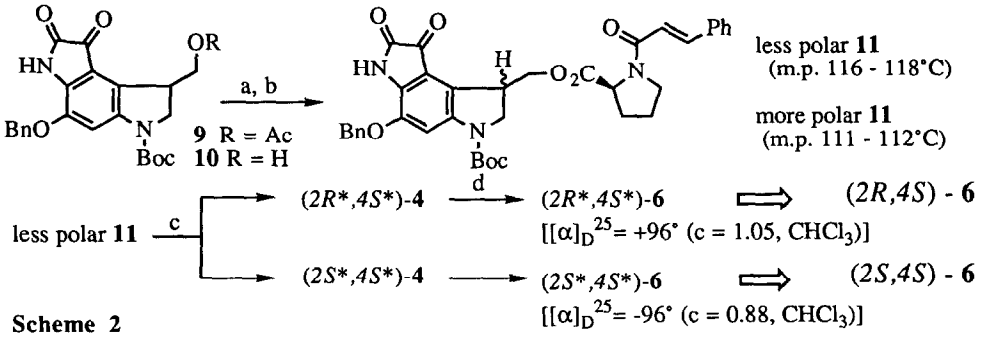
configurations of **5** and *ent*-**5** (**Scheme 1**).

Dieckmann cyclization of the dimethyl ester (**7**)[5] followed by separation of the resulting racemic diastereomers gave the more polar and less polar β-ketoester [*dl*-(*2R*,4S**)-**4** and *dl*-(*2S*,4S**)-**4**], respectively.[6] Acid hydrolysis of TBS ether of each isomer afforded the racemic primary alcohols [*dl*-(*2R*,4S**)-**5** and *dl*-(*2S*,4S**)-**5**]. The primary alcohol in *dl*-(*2R*,4S**)-**5** was acylated with (*S*)-O-acetylmandelic acid to give a mixture of diastereomeric mandelates, which was separated by HPLC (YMC-PAK S-043, CH_2Cl_2 : EtOAc = 21 : 2, 25 ml / min.) to give the less polar [(*2R*,4S**)-**6** (49%, Rt = 20.99 min., $[\alpha]_D^{25}$ = +95° (c = 0.95, $CHCl_3$)] and more polar diastereomers (*2R*,4S**)-**6** [(49%, Rt = 22.68 min., $[\alpha]_D^{25}$ = -33° (c = 0.95, $CHCl_3$)], respectively. Although the relative stereochemistry of each diastereomer was not determined at this stage, they were elucidated by their conversions to **1** and *ent*-**1**, respectively, according to the sequence employed in the synthesis of *dl*-**1**.[5] Thus, acid hydrolysis of the enantiomeric pair of (*2R*,4S**)-**5** obtained by methanolysis of less and more polar (*2R*,4S**)-**6**, and subsequent coupling with 5,6,7-trimethoxyindole-2-carboxylic acid (Ar^1CO_2H) gave the amides [(*2R*,4S**)- and *ent*-(*2R*,4S**)-**8**], respectively. By sequential mesylation of the primary alcohol, hydrogenolysis of the benzyl ether, and formation of the cyclopropadienone system, they were successfully converted to **1** and *ent*-**1**, respectively. Upon comparing the sign of the optical rotations of the synthetic **1** with authentic **1** $[[\alpha]_D^{25}$ =



Scheme 1

Conditions a) see ref. 5, b) AcOH, 10% citric acid, rt, 8h, 95% c) i) O-acetylmandelic acid, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, DMAP, DMF, rt, 4hr, ii) HPLC separation (see text) d) i) MeOH, K_2CO_3, 98% for each isomers, ii) see ref. 5



Scheme 2

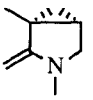
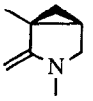
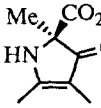
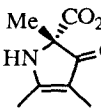
Condition a) K_2CO_3, MeOH, rt, 3hr, 97% b) (*S*)-N-cinnamoylproline, DCC, DMAP, THF, 0°C, 30min, rt, 1 hr, less polar **11** 38%, more polar **11** 29% c) i) m-CPBA, $NaHCO_3$, CH_2Cl_2, rt, 5h, ii) K_2CO_3, MeOH, rt, 1.5h, iii) TBDMSCl, imidazole, DMF, rt, 12h, 63% from less polar **11**, iv) $CH_3CHBrCO_2Me$, 1,8-bis(dimethylamino)naphthalene, CH_3CONMe_2, 70°C, 35h, 88%, v) HCO_2H, Ac_2O, rt, 9h, 93%, vi) LDA, THF, -78°C, 5.5h, (*2R*,4S**)-**4** 28%, (*2S*,4S**)-**4** 28% d) see footnotes b) and c) in **scheme 1**.

+332° (c = 0.05, $CHCl_3$)], it was confirmed that the compound derived from the less polar (*2R*,4S**)-**6** possesses the natural (*2R,3bR,4aS*)-configuration. Accordingly, the less and more polar (*2R*,4S**)-**6** diastereomers have (*2R,4S*)- and (*2S,4R*)-configurations, respectively, as shown in **Scheme 1**.

Similarly, the optical resolution of *dl*-(*2S*,4S**)-**5** was achieved by HPLC separation of less polar (*2S*,4S**)-**6** [$[\alpha]_D^{25}$ = -92° (c = 0.93, $CHCl_3$)] and more polar (*2S*,4S**)-**6** diastereomers [$[\alpha]_D^{25}$ = +161° (c = 0.90, $CHCl_3$)] followed by methanolysis of each isomer. Following the same sequences detailed above, both enantiomers of 2-*epi*-**1** were obtained from the less polar (*2S*,4S**)- and more polar (*2S*,4S**)-**6** by way of (*2S*,4S**)- and *ent*-(*2S*,4S**)-**5**, respectively. However, the absolute configurations of enantiomeric pair of synthetic 2-*epi*-**1** were not able to be assigned at this stage because the optical resolution was carried out on *dl*-(*2S*,4S**)-**5** bearing two asymmetric centers. We then focused on determining the absolute configuration of 2-*epi*-series by optical resolution of the racemic isatin *dl*-**10** prior to generation of the second asymmetric center at C-2.

After considerable effort to identify promising resolving agents for *dl*-**10**, we eventually found that the diastereomeric (*S*)-N-cinnamoylprolyl esters **11** prepared from *dl*-**10** and (*S*)-N-cinnamoylproline[7] were readily separated by silica gel column chromatography ($CHCl_3$: acetone = 5:1) to afford the less (m.p. 116-118 °C) and more polar diastereomers **11** (m.p. 111-112 °C) (Rf: 0.59 and 0.46) (**Scheme 2**). The task remaining for elucidation of the configuration of synthetic 2-*epi*-**1** was to correlate less or more polar diastereomers **11** with the mandelate **6** [(*2R,4S*)- or (*2S,4R*)-**6**]. Thus, oxidation of less polar **11** with m-CPBA followed by methanolysis under basic conditions gave rise to the primary alcohol, which was converted to the TBS ether in 63% yield from less polar **11**. It was then elaborated to optically active **7** (a mixture of diastereoisomers) by alkylation and protection in 82% combined yield. Dieckmann cyclization of **7** followed by separation afforded the β-ketoester and its 2-epimer [(*2R*,4S**)-**4** and (*2S*,4S**)-**4**], respectively. Hydrolysis of TBS ethers of each compounds and acylation with (*S*)-O-acetylmandelic acid afforded the mandelate (*2R*,4S**)-**6** [$[\alpha]_D^{25}$ = +96° (c = 1.05, $CHCl_3$)] and its 2-epimer (*2S*,4S**)-**6** [$[\alpha]_D^{25}$ = -96° (c = 0.88, $CHCl_3$)]. Comparing the sign and value of optical rotations of each mandelate with the four diastereomeric mandelates previously obtained, they were determined to be identical with less polar (*2R*,4S**)-**6** [thus (*2R,4S*)-**6**] and less polar (*2S*,4S**)-**6**, respectively. Since both mandelates derived from less polar **11** possess the same absolute configurations at C-

Table 1 Absolute configurations and cytotoxicity of duocarmycin A and its diastereomers

		
	**1** $[\alpha]_D^{25}$ + 332° (c = 0.14) IC_{50} 0.002	*ent*-2-*epi*-**1** $[\alpha]_D^{25}$ - 160° (c = 0.21) IC_{50} 0.3
	2-*epi*-**1** $[\alpha]_D^{25}$ + 161° (c = 0.07) IC_{50} 0.007	*ent*-**1** $[\alpha]_D^{25}$ - 327° (c = 0.28) IC_{50} 0.3

*Optical rotaion was measured in $CHCl_3$ ** IC_{50} (P388) (ng/mL)

4, the latter was assigned as (*2S,4S*)-**6**. Thus, the compounds derived from less polar (*2S*,4S**)-**6** and more polar (*2S*,4S**)-**6** were 2-*epi*-**1** and *ent*-2-*epi*-**1**, respectively.

With the four possible diastereomers of **1** in hand, their *in vitro* cytotoxicity against P388 murine leukemia was studied. IC_{50} values (ng/ml) collected are shown in **Table 1**. It appeared that level of cytotoxicity obviously depends upon the absolute configuration of cyclopropane moiety. Thus, the compounds bearing natural configurations at the cyclopropane ring (**1** and 2-*epi*-**1**) were found to be about two orders of magnitude more toxic than those possessing the unnatural configuration (*ent*-**1** and *ent*-2-*epi*-**1**).

These results clearly suggest that the cyclopropane moiety of **1** at least plays two roles, providing a site of nucleophilic attack of DNA and a site (or shape) for molecular recognition. In this respect , the relationship between the absolute configuration at cyclopropane ring and the effect of alternating amide side chain as seen in the case of CC-1065 (**2**) has become one of the attractive problems in light of producing more efficient and less toxic analogues of **1**. Studies along this line will be reported in due course.

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References and Notes

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5. Fukuda, Y.; Nakatani, K.; Ito, Y.; Terashima, S. *Tetrahedron Lett.*, **1990**, *31*, 6699.
6. In our previous report,[5] the stereochemistry of more and less polar **4** were erroneously assigned. They should be corrected as described in the text.
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