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# Synthesis and cytotoxicity of natural (+)-duocarmycin A and its three possible stereoisomers

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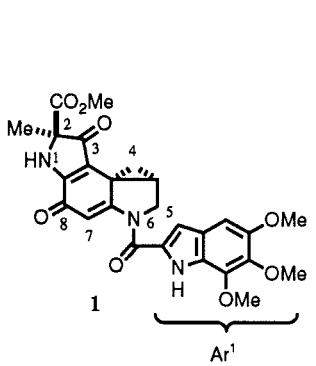
**Abstract**: The title synthesis was accomplished by featuring 1) novel methoxycarbonylation of the C-4 position of the 5-aminoindoline (*dl*-**13**) by way of the isatin (*dl*-**15**), 2) the Dieckmann cyclization of diester (*dl*-**19**) to the methyl 2-methylindoxyl-2-carboxylates (*dl*-**20** and *dl*-**21**), 3) the Wierenga-Kelly-Winstein Ar-3' cyclization as key steps and by employing optical resolutions of various key synthetic intermediates (*dl*-**26**, *dl*-**28**, *dl*-**34**, and *dl*-**36**). *In vitro* cytotoxicity assay of the produced title compounds obviously disclosed that their cytotoxicity is closely related to absolute stereochemistries of cyclopropane moieties.

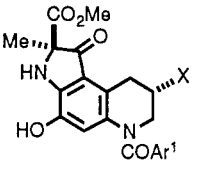
Duocarmycins A (**1**), $C_1$ (**2**), $C_2$ (**3**), $B_1$ (**4**) and $B_2$ (**5**) isolated from *Streptomyces sp.* by a research group at Kyowa Hakko are novel antitumor antibiotics which are effective against various strains of murine cancers (ref. 1 and 2). Almost the same time, workers at Meiji Seika also reported pyrindamycins A and B being identical with **3** and **2**, respectively (ref. 1 and 2). Following to **1** – **5**, the isolation of duocarmycin SA (**6**) which is more stable than **1** was recently reported (ref. 1 and 2). The structures of **1** – **6** have been established by X-ray diffraction analysis of **3**, chemical derivation of **1** to **2** and **3** or **4** and **5**, and consideration of analogy of the structure and biosynthetic process of **6** to those of **1** (ref. 1 - 3).

The structural feature of **1** holding a central position of the duocarmycin family is its close resemblance to the potent antitumor antibiotic CC-1065 (**7**) (ref. 4). Recent extensive studies performed by Boger *et al.* have disclosed that **1** can alkylate DNA in the mechanism very similar to that for **7** (ref. 1).

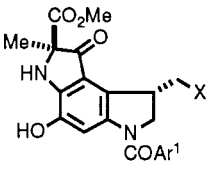
This report concerns with the first total synthesis and *in vitro* cytotoxicity assay of natural (+)-**1** and its three possible stereoisomers [(–)-duocarmycin A ((–)-**1**), (+)-2-*epi*-duocarmycin A ((+)-2-*epi*-**1**), and (–)-2-*epi*-duocarmycin A ((–)-2-*epi*-**1**)] (ref. 5 and 6). The latter studies obviously suggested that cytotoxicity of these compounds is closely related to their absolute stereochemistries of cyclopropane moieties (ref. 6 and 7). Recently, total synthesis of (+)-**6** was also reported (ref. 8).




**1**




**2** : X = Cl
**4** : X = Br




**3** : X = Cl
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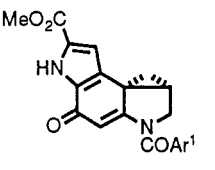
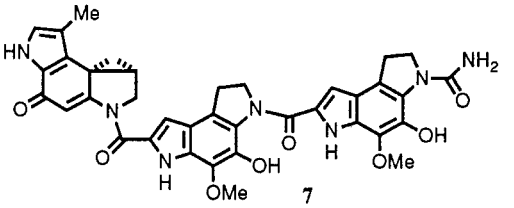



**6**




**7**

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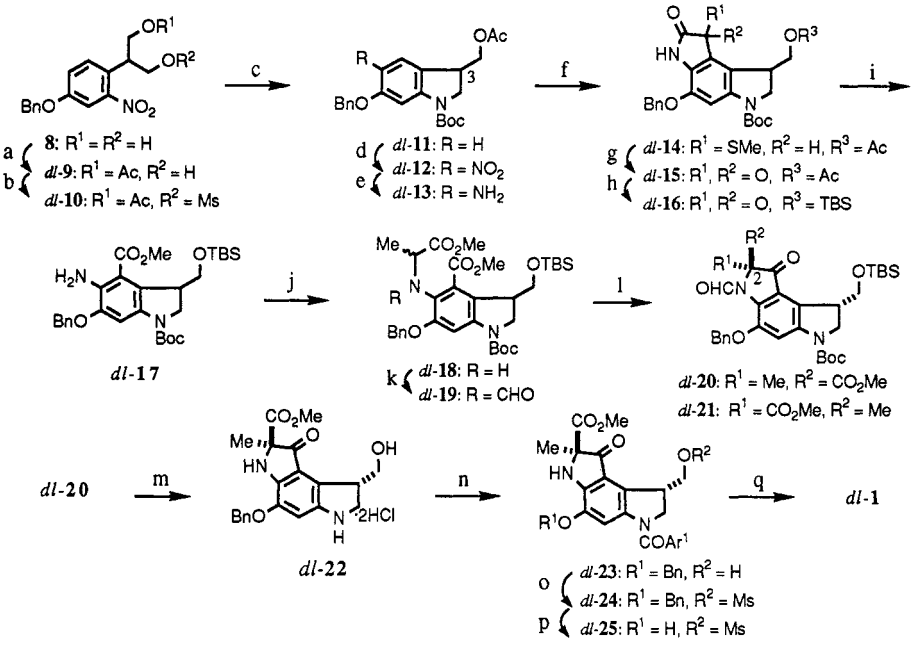
**Synthesis of *dl*-1 and *dl*-2-*epi*-1** (ref. 5):

Prior to synthesize (+)-**1**, (–)-**1**, (+)-2-*epi*-**1**, and (–)-2-*epi*-**1**, the preparation of *dl*-**1** and *dl*-2-*epi*-**1** was first attempted to explore an efficient synthetic scheme. As shown in **Scheme 1**, the 5-aminoindoline (*dl*-**13**) was prepared from diol (**8**) following the procedure well investigated in the synthetic studies on **7** (ref. 9). Introduction of one carbon unit into the C-4 position of *dl*-**13** could be accomplished by employing the Gassman's oxindole synthesis followed by oxidation of the 3-methylthiooxindole (*dl*-**14**), giving rise to the isatin (*dl*-**15**). Sequential exchange of the protective group for primary alcohol and ring opening of an isatin moiety by way of the isatoic anhydride furnished the 5-amino-4-methoxycarbonylindoline (*dl*-**17**). Alkylation of *dl*-**17** with methyl 2-bromopropionate followed by formylation afforded diester (*dl*-**19**). The Dieckmann cyclization of *dl*-**19** cleanly produced a diastereomeric mixture of the methyl 2-methylindoxyl-2-carboxylates (*dl*-**20** and *dl*-**21**) which could be separated by preparative TLC. Successful derivation of more polar *dl*-**20** to *dl*-**1** established the stereochemistries of *dl*-**20** and *dl*-**21** (*vide infra*). Deprotection of *dl*-**20** under acidic conditions gave rise to diamino alcohol dihydrochloride (*dl*-**22**). Condensation of *dl*-**22** with 5,6,7-trimethoxyindole-2-carboxylic acid followed by mesylation and debenzylation afforded the substrate (*dl*-**25**) for the Wierenga-Kelly-Winstein Ar-3' cyclization. As expected, treatment of *dl*-**25** with sodium hydride cleanly produced *dl*-**1**. By employing the same reaction sequence, less polar *dl*-**21** could be derived to *dl*-2-*epi*-**1**.

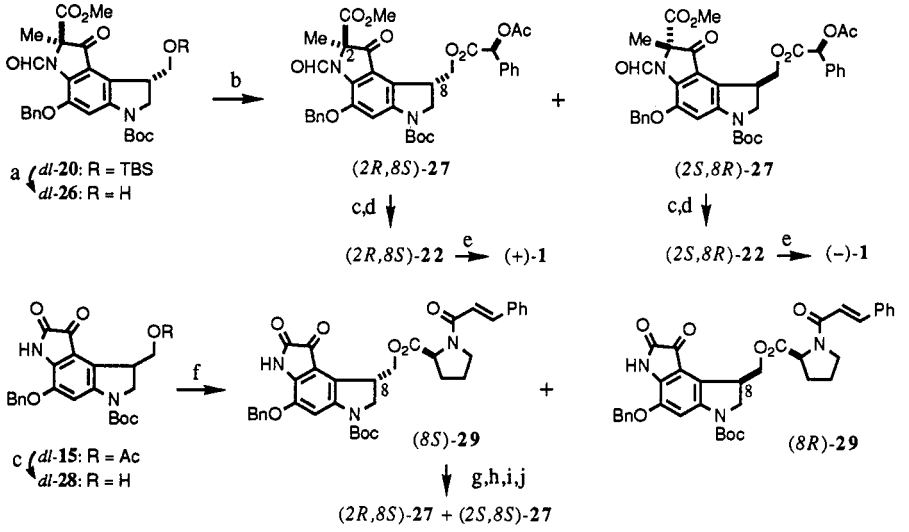



**Scheme 1** : a) $Ac_2O$, $Et_3N$, 74% b) MsCl, $Et_3N$, 100% c) 1) $H_2$–$PtO_2$–$Et_3N$ 2) $Boc_2O$, 95% (2 steps) d) $AcONO_2$, 77% e) $H_2$–$PtO_2$, 95% g) 1) $MeS^+(Cl)CH_2CO_2Et{\cdot}Cl^-$–1,8-bis(dimethylamino)naphthalene 2) $Et_3N$ 3) AcOH 4) $CuCl_2$–CuO, 77% (4 steps) h) 1) $K_2CO_3$–MeOH 2) TBSCl–ImH, 78% (2 steps) i) 1) m-CPBA–$NaHCO_3$ 2) $K_2CO_3$–MeOH 94% (2 steps) j) $MeCHBrCO_2Me$–1,8-bis(dimethylamino)naphthalene, 88% k) $HCO_2H$–$Ac_2O$, 93% l) 1) LDA 2) TLC, 28% (*dl*-**20**), 28% (*dl*-**21**) m) HCl–MeOH, 100% n) 5,6,7-trimethoxyindole-2-carboxylic acid–1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide· HCl–$NaHCO_3$, 57% o) MsCl–$Et_3N$, 99% p) $H_2$–Pd/C, 85% q) NaH, 60% .

**Synthesis of (+)-1, (–)-1, (+)-2-*epi*-1, and (–)-2-*epi*-1** (ref. 6):

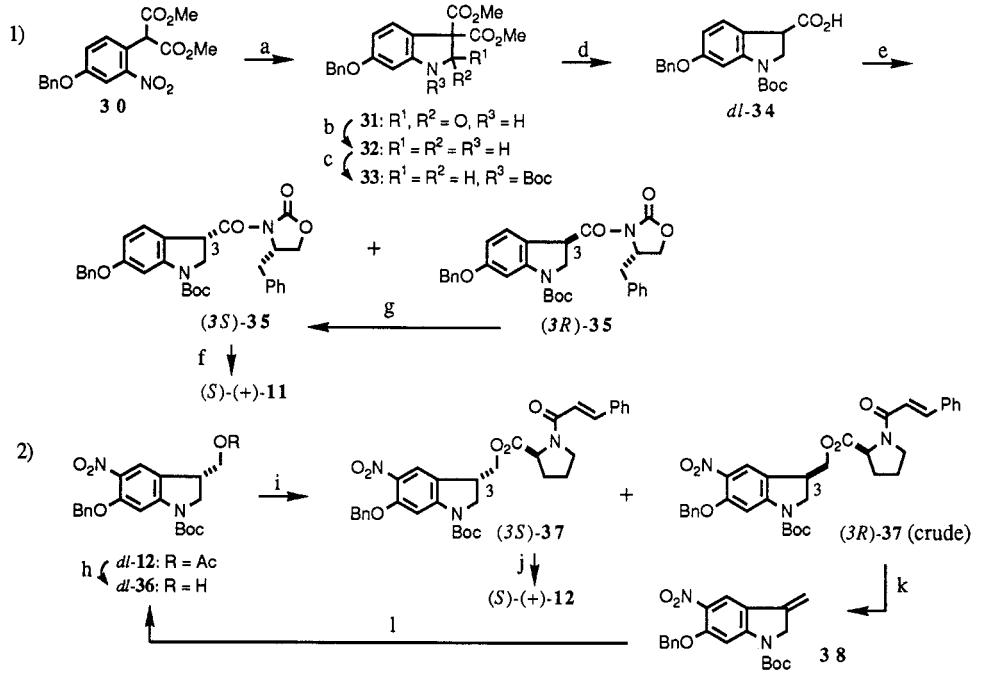
With completion of the synthetic scheme to *dl*-**1** and *dl*-2-*epi*-**1**, the total synthesis of (+)-**1**, (–)-**1**, (+)-2-*epi*-**1**, and (–)-2-*epi*-**1** was next examined. As shown in **Scheme 2**, the silyl group of the more polar product (*dl*-**20**) from the Dieckmann cyclization was selectively removed to afford alcohol (*dl*-**26**). This was condensed with (*S*)-*O*-acetylmandelic acid, affording a mixture of the diastereomeric esters which was separated by HPLC to give less polar (2*R*,8*S*)-**27** and more polar (2*S*,8*R*)-**27**. The less polar ester [(2*R*,8*S*)-**27**] was sequentially treated under the conditions for transesterification and acidic hydrolysis to give (2*R*,8*S*)-**22**, from which natural (+)-**1** could be prepared similarly to *dl*-**1**. In complete the same manner, more polar (2*S*,8*R*)-**27** was derived to unnatural (–)-**1** by way of (2*S*,8*R*)-**22**.

The less polar product (*dl*-**21**) from the Dieckmann cyclization could be similarly resolved, and (+)- and (–)-2-*epi*-**1** were obtained from the less polar and more polar diastereomeric (*S*)-*O*-acetylmandelates [(2*S*,8*S*)- and (2*R*,8*R*)-**27**], respectively (*vide infra*). On the other hand, transesterification of *dl*-**15** readily produced the corresponding alcohol (*dl*-**28**) bearing a single asymmetric center. This was resolved



**Scheme 2 :** a) AcOH–aq.citric acid, 95% b) 1) (*S*)-*O*-acetylmandelic acid–1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide·HCl–DMAP 2) HPLC, 49% [(*2R, 8S*)-**27**], 49% [(*2S, 8R*)-**27**] c) $K_2CO_3$–MeOH, 98% [for (*2R, 8S*)- and (*2S, 8R*)-**27**], 97% (*dl*-**28**) d) see **Scheme 1** m e) see **Scheme 1** n-q f) 1) (*S*)-*N*-cinnamolyproline–DCC–DMAP 2) column chromatography, 38% [(*8S*)-**29**], 29% [(*8R*)-**29**] g) 1) m-CPBA–$NaHCO_3$ 2) $K_2CO_3$–MeOH 3) TBSCl–ImH, 63% (3 steps) h) see **Scheme 1** j-l i) see a j) see b.

by way of the diastereomeric esters of (*S*)-*N*-cinnamoylproline [(*8S*)- and (*8R*)-**29**]. The less polar ester [(*8S*)-**29**] separated could be elaborated to a mixture of (*2R,8S*)- and (*2S,8S*)-**27** by sequential synthetic operations. Based on these results, the absolute configurations of (*2S,8S*)- and (*2R,8R*)-**27** obtained by sequential desilylation and resolution, that is, those of (+)- and (–)-2-*epi*-**1**, could be definitely established.



**Scheme 3 :** a) 1) $H_2$–$PtO_2$, 2) $ClOCOCCl_3$–$Et_3N$, 77% (2 steps) b) $BH_3$·THF, 51% c) $Boc_2O$, 93% d) 1) aq KOH, 2) aq AcOH, 97% (2 steps) e) 1) $SOCl_2$ 2) n-BuLi–(*S*)-4-benzyl-2-oxazolidinone 3) column chromatography, 27% (3 steps) [(*3S*)-**35**], 22% (3 steps) [(*3R*)-**35**] f) 1) $LiBH_4$, 95% ii) $Ac_2O$–$Et_3N$, 99% g) DBU, (*3S*)-**35**:(*3R*)-**35**=1.6:1 h) aq KOH, 99% i) 1) (*S*)-*N*-cinnamoylproline–DCC–DMAP 2) recrystallization, 36% [(*3S*)-**37**] j) 1) aq KOH ii) $Ac_2O$–$Et_3N$, 97% (2 steps) k) 1) aq NaOH, 83% 2) MsCl–$Et_3N$, 87% 3) aq NaOH, 88% l) 1) $BH_3$·$Me_2S$ 2) $H_2O_2$–aq NaOH, 82% (2 steps).

**Novel Methods for Preparing the Optically Active Key Synthetic Intermediates of (+)-1 and (+)-2-*epi*-1:**

In order to produce a large quantity of (+)-**1** and (+)-2-*epi*-**1**, novel resolution methods were sought which could afford their optically active key intermediates more effectively. *In vitro* cytotoxicity assay had disclosed that (+)-**1** and (+)-2-*epi*-**1** exhibit a stronger activity than antipodal (–)-**1** and (–)-2-*epi*-**1** (*vide infra*). As shown in **Scheme 3**, the indoline-3-carboxylic acid (*dl*-**34**) readily accessible from diester (**30**) by way of the oxindole (**31**) was found to give a mixture of the diastereomeric amides [(*3S*)- and (*3R*)-**35**], which are cleanly separable by column chromatography. The more polar amide [(*3S*)-**35**] could be elaborated to (*S*)-(+)-**11** by successive reduction and acylation. Base-catalyzed isomerization of less polar (*3R*)-**35** afforded a mixture of (*3S*)- and (*3R*)-**35** usable for further separation. On the other hand, the alcohol (*dl*-**36**) derived from *dl*-**12** gave a diastereomeric mixture of the esters of (*S*)-*N*-cinnamoylproline [(*3S*)- and (*3R*)-**37**], from which desired (*3S*)-**37** could be readily isolated by recrystallization. From the well-crystalline amide [(*3S*)-**37**], (*S*)-(+)-**12** was produced by sequential hydrolysis and acetylation. The undesired amide [(*3R*)-**37**] was recycled to *dl*-**36** by way of the achiral 3-methyleneindoline (**38**). These methods seem to be more efficient than those initially explored (see **Scheme 2**) due to optical resolution at the early synthetic stage and possible recycle of the undesired diastereomer.

***In Vitro* Cytotoxicity of (+)-1, (–)-1, (+)-2-*epi*-1, and (–)-2-*epi*-1** (ref. 6 and 7):

*In vitro* cytotoxicity assay against three types of murine cancers was performed by employing (+)-**1**, (–)-**1**, (+)-2-*epi*-**1**, and (–)-2-*epi*-**1**. $IC_{50}$ values (ng/ml) collected are shown in **Table 1** along with the absolute configurations and optical rotations. It appeared that the compounds having natural configuration at the cyclopropane ring [(+)-**1** and (+)-2-*epi*-**1**] are more cytotoxic than those bearing unnatural configuration [(–)-**1** and (–)-2-*epi*-**1**]. These observations obviously indicate that the cyclopropane moiety of (+)-**1** not only plays a key role for alkylation of DNA but also provides a key structural feature for molecular recognition. The latter interaction might occur when (+)-**1** is incorporated into a minor groove of duplex DNA prior to the alkylation (ref. 1).

Taking into account the above results, the synthesis of duocarmycin congeners carrying only the cyclopropane moiety or its equivalents is being examined with an aim of exploring more effective anticancer agents.

**Table 1.** *In vitro* cytotoxicity of (+)-**1**, (–)-**1**, (+)-2-*epi*-**1**, and (–)-2-*epi*-**1** against various murine tumors [ $IC_{50}$ (ng/ml) ]

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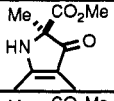
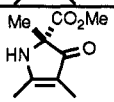
|---|---|---|---|---|
|  | (+)-**1**<br>$[\alpha]_D^{25}$ +332°<br>(c=0.14, $CHCl_3$) | 0.002 (P388)<br>0.1 (L1210)<br>0.2 (B16) | (-)-2-*epi*-**1**<br>$[\alpha]_D^{25}$ -160°<br>(c=0.21, $CHCl_3$) | 0.3 (P388)<br>>12 (L1210)<br>>3 (B16) |
|  | (+)-2-*epi*-**1**<br>$[\alpha]_D^{25}$ +161°<br>(c=0.07, $CHCl_3$) | 0.007 (P388)<br>0.8 (L1210)<br>0.8 (B16) | (-)-**1**<br>$[\alpha]_D^{25}$ -327°<br>(c=0.28, $CHCl_3$) | 0.3 (P388)<br>>11 (L1210)<br>>3 (B16) |

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