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Synthetic Studies on Duocarmycin. 2.[1] Synthesis and Cytotoxicity of Natural (+)-Duocarmycin A and Its Three Possible Stereoisomers.[2]

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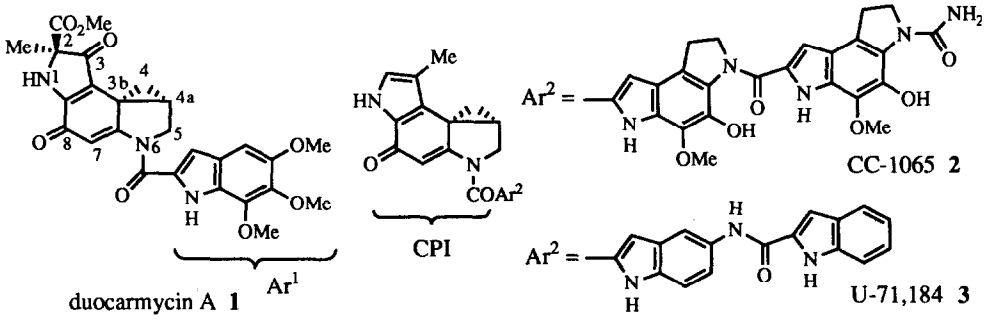
Abstract. The title synthesis was achieved by featuring the optical resolution of two types of the tricyclic intermediates and the synthetic scheme established in the synthesis of racemic compounds. *In vitro* cytotoxicity assay against P388 murine leukemia obviously showed that the absolute configuration of cyclopropane moiety in (+)-1 is closely related to its cytotoxicity.

Duocarmycin A (**1**) isolated from *Streptomyces sp.* is a novel antitumor antibiotic which is effective against various strains of murine tumors.[4] The structural similarity of cyclopropadienone moiety in **1** to the cyclopropapyrroloindole (CPI) ring system in the potent antitumor antibiotic CC-1065 (**2**) as well as its prominent antitumor activity made **1** the exceptionally attractive target not only for total synthesis but also for exploration of novel anticancer agents designed based on **1**. The cyclopropadienone systems present in **1** and **2** have been postulated to be the sites of nucleophilic attack by adenine N-3 in DNA, inducing their potent antitumor activity.[5]

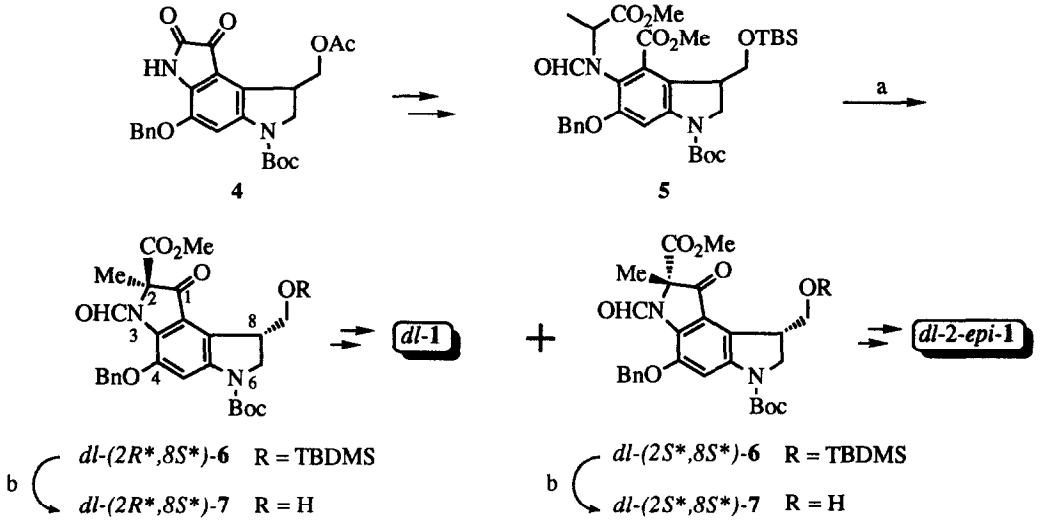
Since **2** caused unusual delayed lethality in clinical trials,[6] a less toxic analogue U-71,184 (**3**) involving modified middle and left hand segments has been developed.[5a,7] Of most interest was that *in vitro* cytotoxicity of the enantiomer of **3** (*ent*-**3**) to L1210 cells was three orders of magnitude less than that of **3**.[5a] As described in the preceding paper,[1] we have disclosed that our synthetic racemic **1** (*dl*-**1**) exhibits *in vitro* cytotoxicity being almost half of that of natural **1** [(+)-**1**] against P388 murine leukemia. Thus, it was hypothesized that there



could be a significant difference of the cytotoxic potency between natural (+)-**1** and its enantiomer [(-)-**1**] and that the absolute configuration of cyclopropane moiety in (+)-**1** could control its cytotoxicity. To investigate the relationship between the absolute configuration of cyclopropane moiety and cytotoxicity, we have embarked on the synthesis of (+)-**1** and its three possible stereoisomers [(-)-duocarmycin A ((-)-**1**), (+)-2-epiduocarmycin A ((+)-2-epi-**1**), (-)-2-epiduocarmycin A ((-)-2-epi-**1**)]. We herein disclose the details of synthesis and cytotoxicity of these compounds, suggesting that the above mentioned hypothesis is correct.[2]

Preparation of (+)-duocarmycin A and its three possible stereoisomers

As described in the preceding paper,[1] we have succeeded in the synthesis of *dl*-**1** and *dl*-2-*epi*-**1** by way of two sorts of racemic β-keto esters [*dl*-(*2R*,8S**)- and *dl*-(*2S*,8S**)-(**6**)], respectively. To achieve the synthesis of (+)-**1**, (-)-**1**, (+)-2-epi-**1**, (-)-2-epi-**1**, the optical resolution of *dl*-(*2R*,8S**)-**6** and subsequent conversion of optically active (*2R,8S*)-**6** into natural (+)-**1** was first examined. The latter elaboration was anticipated to determine the absolute configurations of each enantiomer of (*2R*,8S**)-**6** (**Scheme 1**).

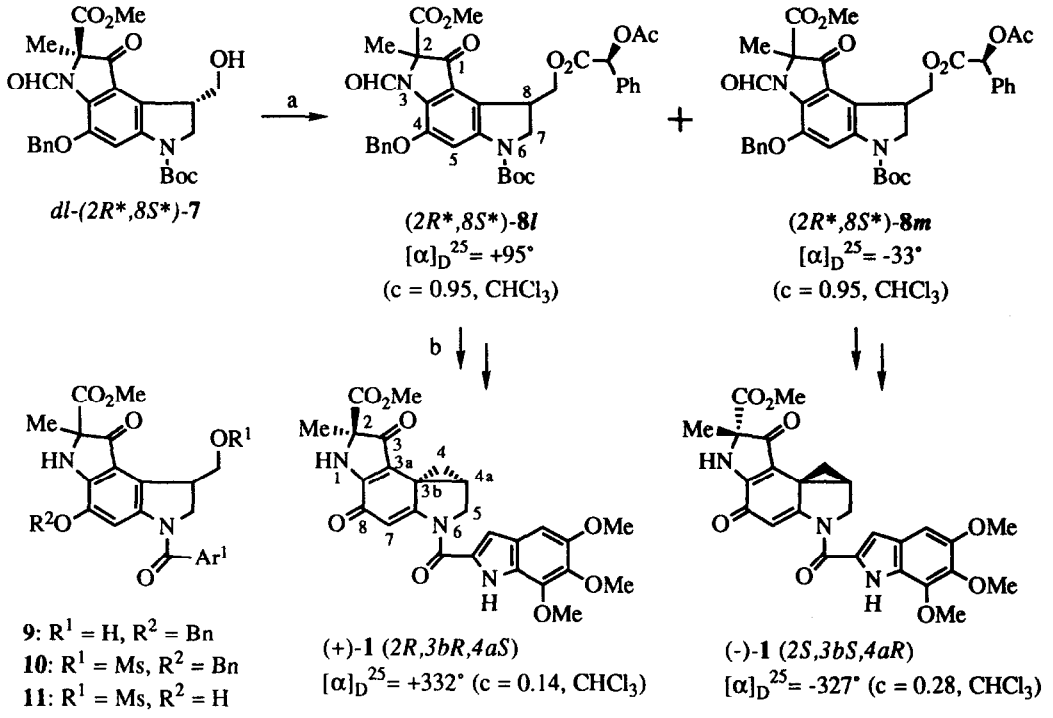


Scheme 1
Conditions: a) see ref. 1 b) AcOH, 10% citric acid, rt, 8h, 99% for *dl*-(*2R*,8S**)-**7**, 99% for *dl*-(*2S*,8S**)-**7**.

According to the method detailed in the preceding paper,[1] racemic β-keto ester [*dl*-(*2R*,8S**)-**6**] was prepared by way of isatin derivative (**4**) and dimethyl ester (**5**). Thus, the Dieckmann cyclization of **5** followed by separation of the resulting diastereomeric products afforded more polar *dl*-(*2R*,8S**)-**6** and less polar *dl*-(*2S*,8S**)-**6**, respectively. The relative stereochemistries of both compounds had been established by successful conversion of the former into *dl*-**1**.[1] Acid hydrolysis of the *t*-butyldimethylsilyl (TBDMS) ether of each diastereoisomer gave two sorts of racemic primary alcohols [*dl*-(*2R*,8S**)-**7** and *dl*-(*2S*,8S**)-**7**], respectively.

With these primary alcohols in hand, we examined their optical resolution by converting them into mixtures of the diastereomeric esters by condensation with an optically active carboxylic acid. After experimentation, it was found that the primary alcohol of *dl*-(*2R*,8S**)-**7** was readily acylated with (*S*)-*O*-acetylmandelic acid, giving a mixture of the diastereomeric mandelates [(*2R*,8S**)-**8***l* and (*2R*,8S**)-**8***m*]. This could be cleanly

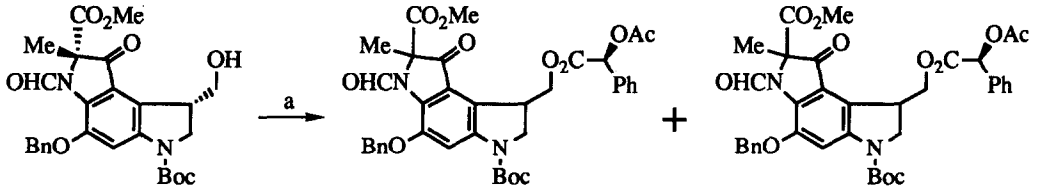
separated by HPLC (YMC-PAK S-043, CH_2Cl_2:EtOAc = 21:2, 2.5 ml/min.) to afford less polar (*2R*,8S**)-**8*l***, Rt = 22.58 min and $[\alpha]_D^{25}$ = +95° ($CHCl_3$), in 49% yield and more polar (*2R*,8S**)-**8*m***, Rt = 27.06 min and $[\alpha]_D^{25}$ = -33° ($CHCl_3$), in 49% yield, respectively (**Scheme 2**). To avoid any confusions to differentiate optically active diastereomers bearing unidentified absolute configurations, suffixes ***l*** (for less polar isomer on tlc analysis) and ***m*** (for more polar isomer) were used at this moment. Although the relative stereochemistries of tricyclic portion to (*S*)-*O*-acetylmandelate group in these diastereomers were not determined at this stage, those were established by their conversions to (+)-**1** and (-)-**1** according to the sequence developed in the synthesis of *dl*-**1**.[1]



Scheme 2
Conditions: a) i) (*S*)-*O*-acetylmandelic acid, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, 4-dimethylaminopyridine (DMAP), CH_2Cl_2, rt, 4h, ii) HPLC separation (see text), (*2R*,8S**)-**8*l*** 49%, (*2R*,8S**)-**8*m*** 49% b) i) MeOH, K_2CO_3, 98% for (*2R*,8S**)-**8*l*** and (*2R*,8S**)-**8*m***, ii) see ref. 1 and experimental section.

Thus, acid hydrolysis of (+)- and (-)-(*2R*,8S**)-**7** independently obtained by methanolysis of (*2R*,8S**)-**8*l*** and -**8*m***, and subsequent coupling with 5,6,7-trimethoxyindole-2-carboxylic acid (Ar^1CO_2H) gave enantiomeric amides [(+)- and (-)-(*2R*,8S**)-**9**], respectively. By sequential mesylation of the primary alcohol, hydrogenolysis of the benzyl ether, and formation of the cyclopropadienone system, (+)- and (-)-(*2R*,8S**)-**9** were successfully converted to (+)- and (-)-**1**, $[\alpha]_D^{25}$ = +332° ($CHCl_3$) and $[\alpha]_D^{25}$ = -327° ($CHCl_3$), respectively. Upon comparing the signs of optical rotations of synthetic samples with natural (+)-**1**, $[\alpha]_D^{25}$ =

+332° ($CHCl_3$), it was unambiguously confirmed that the compound derived from (*2R*,8S**)-**8*l*** possesses natural (*2R,3bR,4aS*)-configuration. Accordingly, it appeared evident that (*2R*,8S**)-**8*l*** and (*2R*,8S**)-**8*m*** have (*2R,8S*)- and (*2S,8R*)-configurations, respectively.



dl-(*2S*,8S**)-**7**

(*2S*,8S**)-**8*l***
$[\alpha]_D^{25}$= -92°
(c = 0.93, $CHCl_3$)

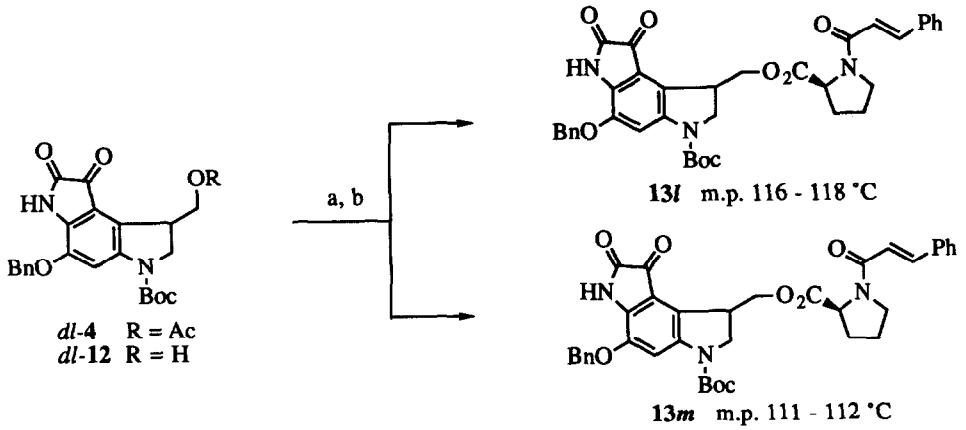
(*2S*,8S**)-**8*m***
$[\alpha]_D^{25}$= +161°
(c = 0.90, $CHCl_3$)

Scheme 3
Conditions: a) i) (*S*)-*O*-acetylmandelic acid, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, DMAP, CH_2Cl_2, rt, 4h ii) HPLC separation, 48% for (*2S*,8S**)-**8*l*** and 46% for (*2S*,8S**)-**8*m*** .

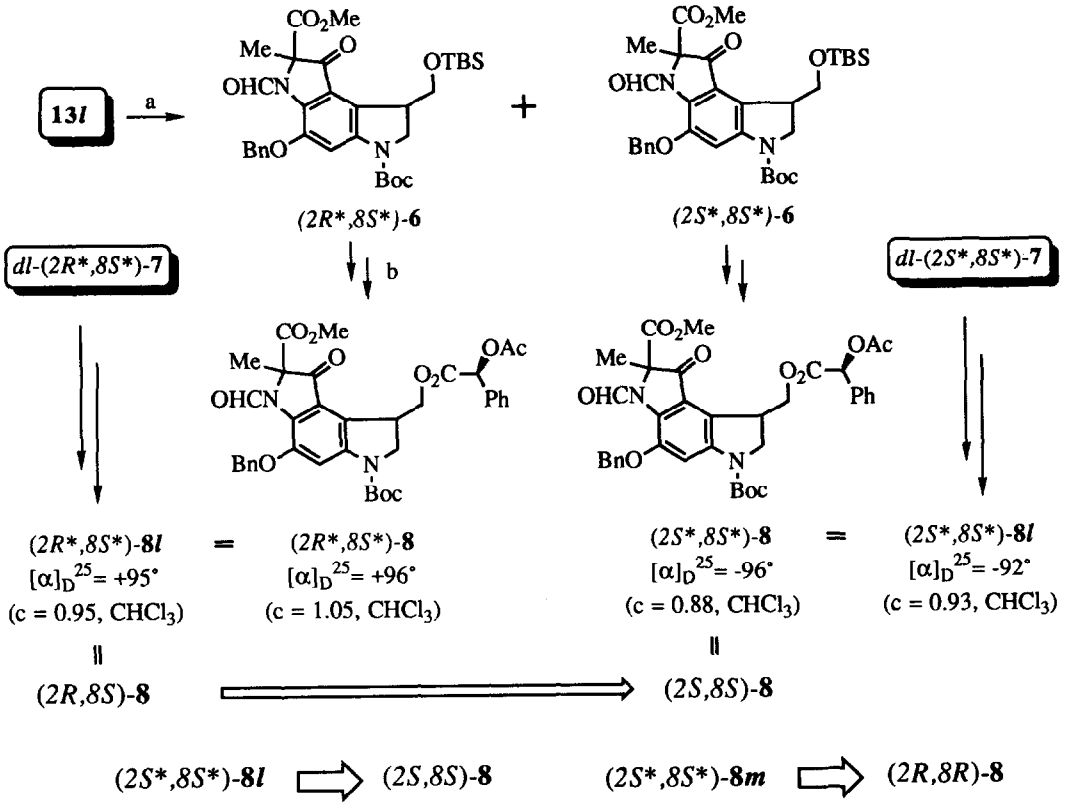
Having completed the synthesis of (+)-**1** and (-)-**1**, the optical resolution of *dl*-(*2S*,8S**)-**7** was next examined to obtain two other diastereomers of (+)-**1**, (+)-2-*epi*-**1** and (-)-2-*epi*-**1**. According to the same procedure as mentioned for the optical resolution of *dl*-(*2R*,8S**)-**7**, the separation of the diastereomeric mandelates [(*2S*,8S**)-**8*l*** and (*2S*,8S**)-**8*m***] derived from *dl*-(*2S*,8S**)-**7** was achieved by HPLC, giving (*2S*,8S**)-**8*l***, Rt = 22.80 min and $[\alpha]_D^{25}$ = -92° ($CHCl_3$), in 48% yield and (*2S*,8S**)-**8*m***, Rt = 27.68 min and $[\alpha]_D^{25}$ = +161° ($CHCl_3$), in 46% yield, respectively (**Scheme 3**). The absolute configurations of synthetic (+)-**1** and (-)-**1** were assigned by correlating the signs of their optical rotations to that of natural (+)-**1**. However, we could not determine the absolute configurations of synthetic (+)-2-*epi*-**1** and (-)-2-*epi*-**1** derived from (*2S*,8S**)-**8*l*** and -**8*m***, respectively, by the same protocol because of the lack of an authentic sample of optically active (+)- or (-)-2-*epi*-**1**. In order to assign the absolute configurations of (+)- and (-)-2-*epi*-**1**, our efforts were next focussed on the determination of absolute configurations of (*2S*,8S**)-**8*l*** and -**8*m*** by correlating one of them to (*2R,8S*)- or (*2S,8R*)-**8** which had previously been derived to (+)- or (-)-**1**. Accordingly, we examined the optical resolution of the racemic isatin (*dl*-**12**) obtainable from **4** and subsequent transformation of one of the enantiomers of **12** into two diastereomers of **8**, both of which bear the same absolute configurations at the C_8-positions.

After exhaustive efforts to find out a promising resolving agent effective for *dl*-**12**, it was finally found that diastereomeric (*S*)-*N*-cinnamoylprolyl esters (**13**) prepared from *dl*-**12** and (*S*)-*N*-cinnamoylproline[8] were readily separated with column chromatography on silica gel ($CHCl_3$: acetone = 5:1), affording less polar **13*l***, Rf = 0.59 and mp. 116-118 °C, in 38% yield and more polar **13*m***, Rf = 0.46 and mp. 111-112 °C, in 29% yield, respectively (**Scheme 4**).

Tasks remaining for elucidating the absolute configurations of (*2S*,8S**)-**8*l*** and **8*m*** were to correlate **13*l*** or **13*m*** with (*2R,8S*)-**8** or (*2S,8R*)-**8**, both of which involve the known absolute configurations. Thus, according to the procedure mentioned in the preceding paper,[1] **13*l*** was transformed into optically active diester (**5**). The Dieckmann cyclization of optically active **5** followed by separation of two sorts of the diastereomeric products



Scheme 4
Conditions: a) K_2CO_3, MeOH, rt, 3h, 97% b) i) (*S*)-*N*-cinnamoylproline, dicyclohexylcarbodiimide, DMAP, THF, 0 °C, 30min, rt, 1h, ii) separation by column chromatography (see text), **13*l*** 38%, **13*m*** 29%.



Scheme 5
Conditions: a) i) m-chloroperbenzoic acid (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 **13*l*** 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*,8S**)-**6** 28%, (*2S*,8S**)-**6** 28% b) see footnote b) in **Scheme 1** and a) in **Scheme 2**.

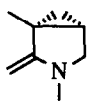
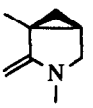
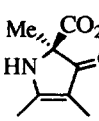
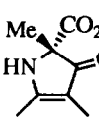
gave rise to diastereomeric optically active β-keto esters [(*2R*,8S**)- and (*2S*,8S**)-**6**]. Hydrolysis of the TBDMS ethers of (*2R*,8S**)- and (*2S*,8S**)-**6** followed by acylation with (*S*)-*O*-acetylmandelic acid yielded the mandelate [(*2R*,8S**)-**8**], $[\alpha]_D^{25}$ = +96° (c = 1.05, $CHCl_3$), and its 2-epimer [(*2S*,8S**)-**8**], $[\alpha]_D^{25}$ = -96° (c = 0.88, $CHCl_3$), respectively. Comparing the signs and values of optical rotations of these mandelates with four diastereomers, [(*2R*,8S**)-**8*l*** and **8*m*** and (*2S*,8S**)-**8*l*** and **8*m***] previously obtained by the optical resolutions of *dl*-(*2R*,8S**) and *dl*-(*2S*,8S**)-**6**, (*2R*,8S**)- and (*2S*,8S**)-**8** were found to be identical with (*2R*,8S**)-**8*l*** [thus (*2R,8S*)-**8**] and (*2S*,8S**)-**8*l***, respectively. Since both mandelates (*2R*,8S**)-**8** [now determined as (*2R,8S*)-**8**] and (*2S*,8S**)-**8** [determined as (*2S*,8S**)-**8*l***] derived from **13*l*** possess the same absolute configurations at the C_8-position, the absolute stereochemistry of (*2S*,8S**)-**8*l*** was assigned to be (*2S,8S*)-configuration. Accordingly, (*2S*,8S**)-**8*m*** was determined to belong to (*2R,8R*)-configuration (**Scheme 5**).

Having established the absolute configurations of (*2S*,8S**)-**8*l*** and **8*m***, both enantiomer of (+)-2-*epi*-**1** and (-)-2-*epi*-**1** were synthesized from (*2S*,8S**)-**8*l*** [now defined as (*2S,8S*)-**8**] and (*2S*,8S**)-**8*m*** [now defined as (*2R,8R*)-**8**], respectively, following the same sequences as detailed for the synthesis of (+)-**1** from (*2R,8S*)-**8**. **Scheme 6** schematically summarizes the results of the optical resolutions of *dl*-(*2R*,8S**)-**6** and *dl*-(*2S*,8S**)-**6** to give four diastereomers of **8**, the chemical correlation of two diastereomers of **8** by the use of optical resolution of *dl*-**12**, and subsequent conversion of four diastereomers of **8** into (+)-, (-)-, (+)-2-*epi*- and (-)-2-*epi*-**1**.

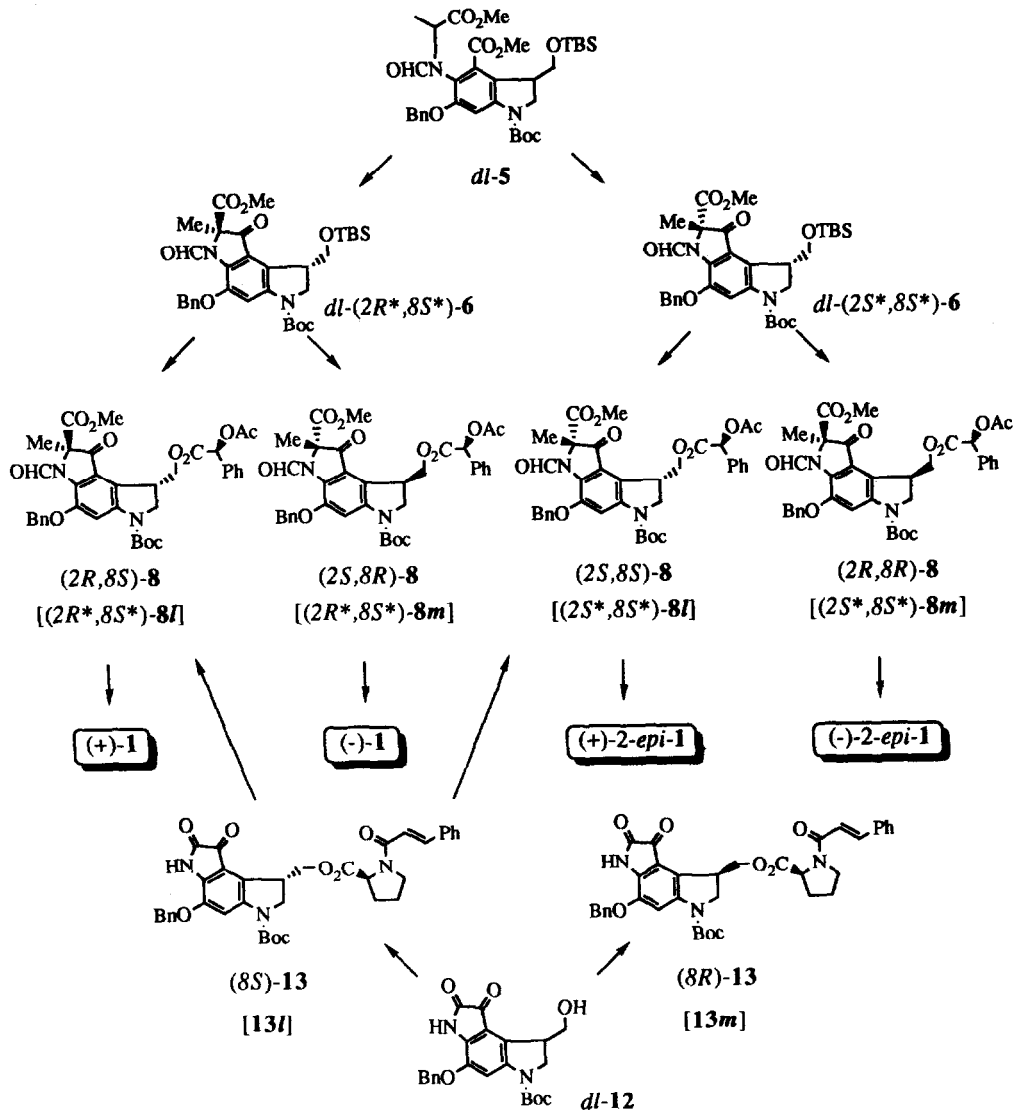
In vitro cytotoxicity of (+)-duocarmycin A and its three possible stereoisomers

With (+)-, (-)-, (+)-2-*epi*- and (-)-2-*epi*-**1** in hand, their *in vitro* cytotoxicities against P388 murine leukemia were next studied. IC_{50} values (ng/ml) collected are shown in **Table 1**. It appeared that the level of cytotoxicity obviously depends upon the absolute configuration of cyclopropane moiety. Thus, the compounds bearing natural (*3bR, 4aS*) configurations at the cyclopropane ring [(+)-**1** and (+)-2-*epi*-**1**] were found to be about two orders of magnitude more toxic than those possessing unnatural (*3bS, 4aR*) configurations [(-)-**1** and (-)-2-*epi*-**1**]. On the other hand, the absolute configurations at the C_2-position had little effects on their cytotoxicity. These results were found to be quite similar to those collected by cytotoxicity assay against other types of murine cancers such as L1210 and B16.[9]

Table 1 Absolute configurations and cytotoxicity of (+)-duocarmycin A and its three stereoisomers

		
	(+)-**1** $[\alpha]_D^{25}$ + 332° (c = 0.14) IC_{50} 0.002	(-)-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	(-)-**1** $[\alpha]_D^{25}$ - 327° (c = 0.28) IC_{50} 0.3

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



Scheme 6 Schematic summary of the synthesis of (+)-, (-)-, (+)-2-*epi*-, and (-)-2-*epi*-**1**

Boger *et al.* have studied DNA alkylation properties of our synthetic duocarmycins and found that those involving natural configurations at the cyclopropane ring [(+)-**1** and (+)-2-*epi*-**1**] were at least 10 times more potent alkylating ability than those with unnatural configurations [(-)-**1** and (-)-2-*epi*-**1**].[9] In these studies, it was pointed out based on the features of DNA cleavage that both (-)-**1** and (-)-2-*epi*-**1** might be contaminated with a trace amount of (+)-**1** and (+)-2-*epi*-**1**, respectively. Accordingly, the actual difference of alkylating ability between two configurations at the cyclopropane ring should be much more significant than those observed. Of particular interest was that differences between cytotoxicity of (+)-**1** and (-)-**1** and that of (+)-2-*epi*-**1** and (-)-2-

epi-**1** well reflect the results of their DNA alkylation ability. Thus, in these particular cases, the growth of tumor cells might be efficiently inhibited by (+)-**1** and (+)-2-*epi*-**1** through suppression of DNA replication induced by effective alkylation of purine bases.

Conclusion

We have synthesized natural (+)-**1** and its three possible stereoisomers by optical resolutions of *dl*-(*2R*,8S**)- and *dl*-(*2S*,8S**)-**7**. Upon examining their cytotoxicity, the compounds bearing natural configurations at the cyclopropane moiety were found to be obviously more potent than those having unnatural configurations. It is apparent from these results that the cyclopropane moiety of (+)-**1** at least plays two roles, providing the site of nucleophilic attack by DNA and the 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 **2** has become one of the attractive problems in light of producing more efficient and less toxic analogues of (+)-**1**.

Experimental Section

All melting points were determined with a Yamato MP-500D melting point apparatus and were uncorrected. Optical rotations were measured with a JASCO DIP-360 polarimeter. Infrared (IR) spectra were recorded on a Hitachi 260-10 spectrometer. ^{1}H-NMR spectra were measured with a JEOL JNM-EX400 (400 MHz) spectrometer. The chemical shifts were expressed in parts per million downfield from tetramethylsilane, using tetramethylsilane (δ=0) and/or residual solvents such as chloroform (δ=7.26) and benzene (δ=7.20) as internal standards. Splitting pattern were indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak. Mass spectra were taken with a Hitachi RMU-6MG, a Hitachi M-80A, a Hitachi M-80B and a Hitachi M-2000 mass spectrometer. Unless otherwise noted, all experiments were carried out using anhydrous solvents under an atmosphere of dry argon. Especially, tetrahydrofuran and diethyl ether (ether) were distilled from sodium benzophenone ketyl. Throughout this work, Merck pre-coated TLC plates (silica gel 60 F_{254}, 0.25 mm, Art 5715) were used for thin layer chromatographic (TLC) analyses. Wako Gel C-200 and C-300 were used as an adsorbent for flash column chromatography.

Methyl (*2R*,8S**)-4-benzyloxy-6-t-butoxycarbonyl-3-formyl-1,2,3,6,7,8-hexahydro-8-hydroxymethyl-2-methyl-1-oxobenzo[1,2-b;4,3-b']dipyrrole-2-carboxylate [*dl*-(*2R*,8S**)-**7**] and Its *dl*-(*2S*,8S**)-Isomer [*dl*-(*2S*,8S**)-**7**]

To a solution of *dl*-(*2R*, 8S**)-**6**[1] (16.0 mg, 26 μmol) in AcOH (3 ml) was added 10 % citric acid, and the mixture was stirred for 10 hr at room temperature. After dilution with H_2O, the resulting mixture was extracted with EtOAc. The organic layer was washed with H_2O and 10% $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, then concentrated *in vacuo*. Preparative thin layer chromatography (SiO_2; benzene: EtOAc=3:1) of the residue gave *dl*-(*2R*, 8S**)-**7** as a pale yellow caramel (13.0 mg, 99%). ^{1}H-NMR ($CDCl_3$): δ = 1.57 (s, 9H -*t-Bu*), 1.82 (s, 3H, -*Me*), 2.02 (br, 1H, -*OH*), 3.72 (s, 3H, -CO_2*Me*), 3.71-3.83 (m, 3H), 4.07 (m, 2H), 5.22 (s, 2H, -OCH_2Ph), 7.43 (s, 5H, C_6H_5), 8.23 (br s, 1H, C_5-*H*), 9.47 (s, 1H, *CHO*). IR ($CHCl_3$): 3750, 1760, 1700, 1660, 1330, 1140 cm^{-1}. MS (SIMS): 511 $(M+H)^+$. HRMS for $C_{27}H_{30}N_2O_8$ (M^+); Calcd 510.2003. Found 510.1980. Treatments of *dl*-(*2S*, 8S**)-**6**[1] (15.5 mg, 25 μmol) in the same manner as described for the preparation of *dl*-(*2R*, 8S**)-**7** gave *dl*-(*2S*, 8S**)-**7** as a pale yellow caramel (12.6 mg, 99%) after separation with preparative thin layer chromatography. ^{1}H-NMR ($CDCl_3$): δ = 1.56 (s, 9H -*t-Bu*), 1.80 (s, 3H, -*Me*), 1.96 (br, 1H, -*OH*), 3.74 (s, 3H, -CO_2*Me*), 3.73-3.88 (m, 3H), 4.08 (m, 2H), 5.22 (s, 2H, -OCH_2Ph), 7.42 (s, 5H, C_6H_5), 8.22 (br s, 1H, C_5-*H*), 9.45 (s, 1H, *CHO*). IR ($CHCl_3$): 3700, 1755, 1700, 1670, 1335, 1140 cm^{-1}. MS (SIMS): 511 $(M+H)^+$. HRMS for $C_{27}H_{30}N_2O_8$ (M^+); Calcd 510.2003. Found 510.1994.

Methyl (*2R,8S*)-8-[(*S*)-(1-acetoxy-1-phenyl)acetoxy]methyl-4-benzyloxy-6-t-butoxycarbonyl-3-formyl-1,2,3,6,7,8-hexahydro-2-methyl-1-oxobenzo[1,2-b;4,3-b']dipyrrole-2-carboxylate [(*2R,8S*)-**8**] and Its (*2S,8R*)-, (*2R,8R*)-, and (*2S,8S*)-Isomers [(*2S,8R*)-, (*2R,8R*)-, and (*2S,8S*)-**8**]

A solution of *dl*-(*2R*, 8S**)-**7** (21.4 mg, 42 μmol), (*S*)-*O*-acetylmandelic acid (12.2 mg, 63 μmol), 1-[3-dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (16.1 mg, 84 μmol), DMAP (0.5 mg, 4.2 μmol) in CH_2Cl_2 (2.5 ml) was stirred for 4 hr at room temperature. The resulting mixture was washed with H_2O, dried over anhydrous Na_2SO_4, filtered, then concentrated *in vacuo*. Preparative HPLC (YMC-PAK S-043, CH_2Cl_2:EtOAc=21:2, 2.5 ml/min, detected at 310 nm) gave less polar (*2R, 8S*)-**8** (14.2 mg, 49%) and more

polar (*2S, 8R*)-**8** (14.3 mg, 49%) both as a pale yellow caramel. (*2R, 8S*)-**8**: Rt = 22.58 min, $[\alpha]_D^{25}$ = +95° (c = 0.95, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ = 1.57 (s, 9H, *-tBu*), 1.77 (s, 3H, *-Me*), 2.15 (s, 3H, -OCO*CH*$_3$), 3.69 (s, 3H, -CO_2*Me*), 3.88-3.98 (m, 3H), 4.37 (d, 1H, J=9.3 Hz), 4.44 (dd, 1H, J=10.7, 6.8 Hz), 5.23 (br s, 2H, -O*CH*$_2$Ph), 5.81 (s, 1H), 7.29-7.44 (m, 10H, *C*$_6$*H*$_5$x2), 8.17 (br s, 1H), 9.45 (s, 1H). IR ($CHCl_3$): 1755, 1695, 1665, 1500, 1335, 1145 cm^{-1}. MS (SIMS): 687 $(M+H)^+$. HRMS for $C_{37}H_{38}N_2O_{11}$ (M^+); Calcd 686.2476. Found 686.2468. (*2S, 8R*)-**8**: Rt = 27.06 min, $[\alpha]_D^{25}$ = -33° (c = 0.95, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ = 1.55 (s, 9H, *-tBu*), 1.72 (s, 3H, *-Me*)), 2.14 (s, 3H, -OCO*CH*$_3$), 3.69 (s, 3H, -CO_2*Me*), 3.88-3.92 (m, 2H), 4.01 (m, 1H), 4.28 (dd, 1H, J=10.7, 2.9 Hz), 4.57 (dd, 1H, J=10.7, 5.4 Hz), 5.20 (br s, 2H, -O*CH*$_2$Ph), 5.79 (s, 1H), 7.27-7.45 (m, 10H, *C*$_6$*H*$_5$x2), 8.05 (br s, 1H), 9.43 (s, 1H). IR ($CHCl_3$): 1760, 1720, 1695, 1665, 1505, 1335, 1150 cm^{-1}. HRMS for $C_{37}H_{38}N_2O_{11}$ (M^+); Calcd 686.2476. Found 686.2450. The same treatments of *dl*-(*2S*, 8S**)-**7** (21.7 mg, 43 μmol) as described for the preparation of (*2R, 8S*)- and (*2S, 8R*)-**8** gave less polar (*2S, 8S*)-**8** (13.9 mg, 48%) and more polar (*2R, 8R*)-**8** (13.5 mg, 46%) both as a pale yellow caramel after separation with preparative HPLC (YMC-PAK S-043, CH_2Cl_2:EtOAc=21:2, 2.5 ml/min, detected at 310 nm). (*2S, 8S*)-**8**: Rt = 22.80 min, $[\alpha]_D^{25}$ = -92° (c = 0.93, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ = 1.57 (s, 9H, *-tBu*), 1.77 (s, 3H, *-Me*), 2.16 (s, 3H, -OCO*CH*$_3$), 3.70 (s, 3H, -CO_2*Me*), 3.85-3.96 (m, 3H), 4.32 (dd, 1H, J=10.7, 7.8 Hz), 4.48 (d, 1H, J=9.8 Hz), 5.22 (d, 2H, J=10.3 Hz), 5.86 (s, 1H), 7.37-7.43 (m, 10H, *C*$_6$*H*$_5$x2), 8.16 (br s, 1H), 9.45 (s, 1H). IR ($CHCl_3$): 1760, 1720, 1700, 1678, 1505, 1335, 1145 cm^{-1}. HRMS for $C_{37}H_{38}N_2O_{11}$ (M^+); Calcd 686.2476. Found 686.2464. (*2R, 8R*)-**8**: Rt = 27.68 min, $[\alpha]_D^{25}$ = +161° (c = 0.90, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ = 1.56 (s, 9H, *-tBu*), 1.78 (s, 3H, *-Me*), 2.15 (s, 3H -OCO*CH*$_3$), 3.73 (s, 3H, -CO_2*Me*), 3.40-4.10 (m, 3H), 4.39-4.44 (m, 2H), 5.18 (m, 2H, -O*CH*$_2$Ph), 5.83 (s, 1H), 7.27-7.44 (m, 10H, *C*$_6$*H*$_5$x2), 8.03 (br s, 1H), 9.44 (s, 1H). IR ($CHCl_3$): 1755, 1695, 1665, 1500, 1335, 1145 cm^{-1}. HRMS for $C_{37}H_{38}N_2O_{11}$ (M^+); Calcd 686.2476. Found 686.2477.

Methyl (*2R,8S*)-4-benzyloxy-6-t-butoxycarbonyl-3-formyl-1,2,3,6,7,8-hexahydro-8-hydroxymethyl-2-methyl-1-oxobenzo[1,2-b;4,3-b']dipyrrole-2-carboxylate [(*2R, 8S*)-7] and Its (*2S,8R*)-, (*2S,8S*)-, and (*2R,8R*)-Isomers [(*2S,8R*)-, (*2S,8S*)-, and (*2R,8R*)-7]

To a solution of (*2R, 8S*)-**8** (8.2 mg, 12 μmol) in MeOH (2 ml) was added K_2CO_3 (1.2 mg, 12 μmol) at room temperature, and the mixture was stirred for 1 hr at the same temperature. After dilution with EtOAc, the resulting mixture was washed with 10% citric acid, H_2O, and brine, dried over anhydrous Na_2SO_4, then concentrated *in vacuo*. Flash chromatography (SiO_2; benzene:EtOAc = 3:1) of the residue gave (*2R, 8S*)-**7** as a pale yellow caramel (6.1 mg, 100%). ^{1}H-NMR spectrum of this sample was identical with that of *dl*-(*2R*,8S**)-**7**. Similarly, (*2S, 8R*)-**7** (10.4 mg, 98%), (*2S, 8S*)-**7** (5.8 mg, 100%), (*2R, 8R*)-**7** (9.9 mg, 98%) were prepared all as a pale yellow caramel from (*2S, 8R*)-**8** (14.3 mg, 21 μmol), (*2S, 8S*)-**8** (7.8 mg, 11 μmol) and (*2R, 8R*)-**8** (13.5 mg, 20 μmol), respectively. ^{1}H-NMR spectrum of (*2S, 8R*)-**7** and those of (*2S, 8S*)- and (*2R, 8R*)-**7** were identical with those of *dl*-(*2R*,8S**)- and *dl*-(*2S*,8S**)-**7**, respectively.

Methyl (*2R,8S*)-4-benzyloxy-1,2,3,6,7,8-hexahydro-8-hydroxymethyl-2-methyl-1-oxo-6-[(5,6,7-trimethoxy-*1H*-indole-2-yl)carbonyl]benzo[1,2-b;4,3-b']dipyrrole-2-carboxylate [(*2R, 8S*)-9] and Its (*2S,8R*)-, (*2S,8S*)-, and (*2R,8R*)-Isomers [(*2S,8R*)-, (*2S,8S*)-, and (*2R,8R*)-9]

Treatments of (*2R,8S*)-**7** (7.8 mg, 11 μmol), (*2S,8R*)-**7** (10.4 mg, 20 μmol), (*2S,8S*)-**7** (8.2 mg, 12 μmol) and (*2R,8R*)-**7** (9.9 mg, 19 μmol) in the same manner as described for the corresponding racemic compounds[1] gave (*2R, 8S*)-**9** [4.2 mg, 60% (2 steps)], $[\alpha]_D^{25}$ = +101° (c=0.42, $CHCl_3$), (*2S,8R*)-**9** [10.9 mg, 85% (2 steps)], $[\alpha]_D^{23}$ = -88° (c=0.38, $CHCl_3$), (*2S,8S*)-**9** [4.3 mg, 59% (2 steps)], $[\alpha]_D^{23}$ = -108° (c=0.29, $CHCl_3$), and (*2R,8R*)-**9** [9.4 mg, 78% (2 steps)], $[\alpha]_D^{23}$ = +106° (c=0.31, $CHCl_3$), respectively, after purification by preparative thin layer chromatography. ^{1}H-NMR spectra of these samples were identical with those of the corresponding racemic compounds.[1] The absolute value of specific rotation for (*2R, 8S*)-**9** was somewhat different from that for (*2S,8R*)-**9**, although the reason is not clear at this moment. However, it is apparent that optical purity of these compounds did not cause this difference, by comparing the specific rotations of (*2R, 8S*)-**10** and (*2S,8R*)-**10** derived from (*2R, 8S*)-**9** and (*2S,8R*)-**9**, respectively.

Methyl (*2R,8S*)-4-benzyloxy-1,2,3,6,7,8-hexahydro-8-(methanesulfonyloxy)methyl-2-methyl-1-oxo-6-[(5,6,7-trimethoxy-*1H*-indole-2-yl)carbonyl]benzo[1,2-b;4,3-b']dipyrrole-2-carboxylate [(*2R, 8S*)-10] and Its (*2S,8R*)-, (*2S,8S*)-, and (*2R,8R*)-Isomers [(*2S,8R*)-, (*2S,8S*)-, and (*2R,8R*)-10]

The same treatments of (*2R,8S*)-**9** (4.2 mg, 6.8 μmol), (*2S,8R*)-**9** (10.9 mg, 18 μmol), (*2S,8S*)-**9** (4.3 mg, 7.0 μmol) and (*2R,8R*)-**9** (9.4 mg, 15 μmol) as described for the corresponding racemic compounds[1] gave (*2R, 8S*)-**10** (3.8 mg, 81%), $[\alpha]_D^{23}$ = +108° (c=0.38, $CHCl_3$), (*2S,8R*)-**10** (11.6 mg, 94%), $[\alpha]_D^{23}$ = -112°

(c=0.31, $CHCl_3$), (*2S,8S*)-**10** (4.3 mg, 90%), $[\alpha]_D^{23}$ = -77° (c=0.29, $CHCl_3$), and (*2R,8R*)-**10** (10.2 mg, 96%), $[\alpha]_D^{23}$ = +75° (c=0.49, $CHCl_3$), respectively, after purification by preparative thin layer chromatography. ^{1}H-NMR spectra of these samples were identical with those of the corresponding racemic compounds.[1]

Methyl (*2R,8S*)-1,2,3,6,7,8-hexahydro-4-hydroxy-8-(methanesulfonyloxy)methyl-2-methyl-1-oxo-6-[(5,6,7-trimethoxy-*1H*-indole-2-yl)carbonyl]benzo[1,2-b;4,3-b']dipyrrole-2-carboxylate [(*2R, 8S*)-11] and Its (*2S,8R*)-, (*2S,8S*)-, and (*2R,8R*)-Isomers [(*2S,8R*)-, (*2S,8S*)-, and (*2R,8R*)-11]

Treatments of (*2R,8S*)-**10** (3.8 mg, 5.5 μmol), (*2S,8R*)-**10** (11.6 mg, 17 μmol), (*2S,8S*)-**10** (4.3 mg, 6.2 μmol) and (*2R,8R*)-**10** (4.9 mg, 7.1 μmol) in the same manner as as described for the corresponding racemic compounds[1] gave (*2R, 8S*)-**11** (2.6 mg, 79%), $[\alpha]_D^{23}$ = +105° (c=0.26, $CHCl_3$), (*2S,8R*)-**11** (8.7 mg, 86%), $[\alpha]_D^{23}$ = -107° (c=0.36, $CHCl_3$), (*2S,8S*)-**11** (2.8 mg, 76%), $[\alpha]_D^{23}$ = -66° (c=0.19, $CHCl_3$), and (*2R,8R*)-**11** (3.8 mg, 89%), $[\alpha]_D^{23}$ = +73° (c=0.26, $CHCl_3$), respectively, after purification by preparative thin layer chromatography. ^{1}H-NMR spectra of these samples were identical with those of the corresponding racemic compounds.[1]

(+)-Duocarmycin A [(+)-1], (-)-Duocarmycin A [(-)-1], and (+)-2-Epiduocarmycin A [(+)-2-epi-1], and (-)-2-Epiduocarmycin A [(-)-2-epi-1]

The same treatments of (*2R,8S*)-**11** (2.6 mg, 4.3 μmol), (*2S,8R*)-**11** (5.4 mg, 9.0μmol), (*2S,8S*)-**11** (2.8 mg, 4.6 μmol) and (*2R,8R*)-**11** (5.6 mg, 9.3 μmol) as described for the corresponding racemic compounds[1] gave (+)-**1** (1.4 mg, 65%), $[\alpha]_D^{25}$ = +332° (c=0.14, $CHCl_3$) [lit.,[4b] $[\alpha]_D^{22}$ = +282° (MeOH), $[\alpha]_D^{25}$ = +332° (c=0.05, $CHCl_3$) measured by us], (-)-**1** (2.8 mg, 62%), $[\alpha]_D^{25}$ = -327° (c=0.28, $CHCl_3$), (+)-2-epi-**1** (1.2 mg, 51%), $[\alpha]_D^{25}$ = +161° (c=0.07, $CHCl_3$), and (-)-2-epi-**1** (2.1 mg, 45%), $[\alpha]_D^{25}$ = -160° (c=0.21, $CHCl_3$), respectively. ^{1}H-NMR spectra of these samples were superimposable on those of the corresponding racemic compounds.[1]

(*8S*)-4-Benzyloxy-6-t-butoxycarbonyl-8-[(*S*)-*N*-cinnamoylprolyloxy]methyl-1,2-dioxo-1,2,3,6,7,8-hexahydrobenzo[1,2-b;4,3-b']dipyrrole [(*S*)-13] and Its (*R*)-isomer [(*R*)-13]

To a solution of *dl*-**12** (263 mg, 0.62 mmol) obtained by methanolysis of *dl*-**4** as described in the preceding paper,[1] (*S*)-*N*-cinnamoylproline[8] (167 mg, 0.68 mmol), and dicyclohexylcarbodiimide (141 mg, 0.68 mmol) in THF (6 ml) was added DMAP (7.6 mg, 0.06 mmol) at 0 °C, and the mixture was stirred for 30 min at the same temperature and for 2 hr at room temperature. The resulting mixture was diluted with EtOAc and insoluble materials were filtered off. After concentration of the filtrate *in vacuo*, flash chromatography (SiO_2; benzene:MeCN=3:1) of the residue gave less polar (*S*)-**13** (152 mg, 38%) and more polar (*R*)-**13** (119 mg, 29%) both as a purple powder. Specific rotations of (*S*)-**13** and (*R*)-**13** could not be measured by ambiguous reasons. (*S*)-**13**: mp. 116-118 °C and Rf = 0.59 (benzene : CH_3CN). ^{1}H-NMR ($CDCl_3$): δ = 1.55 (s, 9H, -*^{t}Bu*), 2.00-2.06 (m, 3H), 2.18 (m, 1H), 3.67 (m, 1H), 3.75 (m, 1H), 3.89 (m, 1H), 3.98-4.11 (m, 2H), 4.36 (m, 1H), 4.49-4.56 (m, 2H), 4.99-5.12 (m, 2H, -*CH_2*Ph), 6.67 (d, 1H, J=15.6 Hz, *C_2'-H*), 7.35-7.53 (m, 10H, *C_6H_5*x2), 7.59 (br s, 1H, *NH*), 7.67 (d, 1H, J=15.6 Hz, *C_3'-H*), 7.97 (br s, 1H, *C_4-H*). IR (KBr): 3430, 3230, 1730, 1690, 1645, 1630, 1605, 1490, 1410, 1130 cm^{-1}. MS (SIMS): 652 $(M+H)^+$. *Anal.* Calcd. for $C_{37}H_{37}N_3O_8$: C, 68.19; H, 5.72; N, 6.45%. Found: C, 67.93; H, 5.98; N, 6.16%. (*R*)-**13**: mp. 111-112 °C and Rf = 0.46 (benzene : CH_3CN). ^{1}H-NMR ($CDCl_3$): δ = 1.55 (s, 9H, -*^{t}Bu*), 1.89-2.01 (m, 3H), 2.17 (m, 1H), 3.65 (m, 1H), 3.75 (m, 1H), 3.88 (m, 1H), 3.98 (dd, 1H, J=11.7, 4.4 Hz), 4.07 (dd, 1H, J=11.7, 10.3 Hz), 4.40-4.55 (m, 1H), 4.44 (dd, 1H, J=10.7, 4.4 Hz), 4.51 (dd, 1H, J=8.8, 4.4 Hz), 5.08 (m, 2H, -*CH_2*Ph), 6.67 (d, 1H, J=15.6 Hz, *C_2'-H*), 7.33-7.52 (m, 10H, *C_6H_5*x2), 7.61 (br s, 1H, *NH*), 7.65 (d, 1H, J=15.6 Hz, *C_3'-H*), 7.98 (br s, 1H, *C_4-H*). IR (KBr): 3430, 3190, 1735, 1700, 1655, 1635, 1605, 1495, 1410, 1140 cm^{-1}. MS (SIMS): 652 $(M+H)^+$. *Anal.* Calcd. for $C_{37}H_{37}N_3O_8$: C, 68.19; H, 5.72; N, 6.45%. Found: C, 68.13; H, 5.97; N, 6.18%.

Preparation of Methyl (*2R,8S*)-8-[(*S*)-(1-acetoxy-1-phenyl)acetyl]methyl-4-hydroxy-6-t-butoxycarbonyl-3-formyl-1,2,3,6,7,8-hexahydro-2-methyl-1-oxobenzo[1,2-b;4,3-b']dipyrrole-2-carboxylate [(*2R,8S*)-8] and Its (*2S,8S*)-Isomer [(*2S,8S*)-8] from (*8S*)-4-benzyloxy-6-t-butoxycarbonyl-8-[(*S*)-*N*-cinnamoylprolyloxy]methyl-1,2-dioxo-1,2,3,6,7,8-hexahydrobenzo[1,2-b;4,3-b']dipyrrole [(*S*)-13]

1) Methyl (*S*)-5-amino-6-benzyloxy-1-t-butoxycarbonyl-3-(t-butyldimethylsilyloxy)-methyl-2,3-dihydro-*1H*-indole-4-carboxylate: A suspension of (*S*)-**13** (108 mg, 0.16 mmol), m-CPBA (51.6 mg, 0.30 mmol) and $NaHCO_3$ (27.9 mg, 0.33 mmol) in CH_2Cl_2 (3 ml) was stirred for 5 hr at -10 to 10 °C. The resulting mixture was washed with 10% $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, then

concentrated *in vacuo*, to give crude isatoic anhydride derivative. K_2CO_3 (57.4 mg, 0.42 mmol) was then added to a MeOH (3 ml) solution of this crude product, and the mixture was stirred for 1.5 hr at room temperature. After dilution with EtOAc, the resulting mixture was washed with 10% citric acid, dried over anhydrous Na_2SO_4, filtered, then concentrated *in vacuo*, to give crude methyl (*S*)-5-amino-6-benzyloxy-1-t-butoxycarbonyl-2,3-dihydro-3-hydroxymethyl-*1H*-indole-4-carboxylate. To a solution of this crude alcohol in DMF (1 ml) was added TBDMSCl (50.1 mg, 0.33 mmol) and imidazole (33.9 mg, 0.50 mmol), and the mixture was stirred overnight at room temperature. After dilution with EtOAc, the resulting mixture was washed with sat. NH_4Cl, dried over anhydrous Na_2SO_4, filtered, then concentrated *in vacuo*. Flash chromatography (SiO_2; benzene:EtOAc=30:1) of the residue gave methyl (*S*)-5-amino-6-benzyloxy-1-t-butoxycarbonyl-3-(t-butyldimethylsilyloxy)methyl-2,3-dihydro-*1H*-indole-4-carboxylate as pale yellow crystals (56.4 mg, 63% from (*S*)-**13***l*), mp. 94-96 °C and $[\alpha]_D^{25}$= -50° (c = 1.1, THF). ^{1}H-NMR spectrum of this sample was identical with that of the corresponding racemic compounds.[1]

2) Methyl (*2R,8S*)-4-benzyloxy-6-t-butoxycarbonyl-8-(t-butyldimethylsilyloxy)methyl-3-formyl-1,2,3,6,7,8-hexahydro-2-methyl-1-oxobenzo[1,2-b;4,3-b']dipyrrole-2-carboxylate [(*2R, 8S*)-6] and Its (*2S,8S*)-Isomer [(*2S,8S*)-6]: Alkylation of methyl (*S*)-5-amino-6-benzyloxy-1-t-butoxycarbonyl-3-(t-butyldimethylsilyloxy)methyl-2,3-dihydro-*1H*-indole-4-carboxylate (56.1 mg, 103 μmol) obtained in a) with methyl 2-bromopropionate in the same manner as described for the preparation of the corresponding racemic compound[1] gave methyl (*S*)-6-benzyloxy-1-t-butoxycarbonyl-3-(t-butyldimethylsilyloxy)methyl-2,3-dihydro-5-[(*RS*)-1-methoxycarbonylethyl]amino-*1H*-indole-4-carboxylate as a diastereomeric mixture (57.4 mg, 88%) after purification by flash chromatography. ^{1}H-NMR spectrum of this sample was almost identical with that of the corresponding racemic compound.[1] This amine (57.4 mg, 91 μmol) was formylated in a similar manner to that described for the preparation of the corresponding racemic compound,[1] affording methyl (*S*)-6-benzyloxy-1-t-butoxycarbonyl-3-(t-butyldimethylsilyloxy)methyl-2,3-dihydro-5-[(*RS*)-*N*-formyl-*N*-(1-methoxycarbonylethyl)]amino-*1H*-indole-4-carboxylate as a diastereomeric mixture (55.8 mg, 93%) after purification by preparative thin layer chromatography. ^{1}H-NMR spectrum of this sample was almost identical with that of the corresponding racemic compound.[1] The Dieckmann cyclization of this formamide (55.4 mg, 84 μmol) under the same conditions as described for the preparation of the corresponding racemic compounds[1] gave (*2R, 8S*)-**6** (14.8 mg, 28%), $[\alpha]_D^{23}$ = +37° (c = 0.79, THF), and (*2S, 8S*)-**6** (14.8 mg, 28%), $[\alpha]_D^{23}$ = -152° (c = 0.66, THF), after separation by preparative thin layer chromatography. ^{1}H-NMR spectra of these samples were identical with those of the corresponding racemic compounds.[1]

3) Methyl (*2R,8S*)-8-[(*S*)-(1-acetoxy-1-phenyl)acetyl]methyl-4-hydroxy-6-t-butoxycarbonyl-3-formyl-1,2,3,6,7,8-hexahydro-2-methyl-1-oxobenzo[1,2-b;4,3-b']dipyrrole-2-carboxylate [(*2R,8S*)-8] and Its (*2S,8S*)-Isomer [(*2S,8S*)-8]: Desilylations of (*2R, 8S*)-**6** (11.8 mg, 19 μmol) and (*2S, 8S*)-6 (9.9 mg, 16 μmol) under the same conditions as described for the corresponding racemic compounds[1] gave (*2R, 8S*)-**7** (9.1 mg, 95%) and (*2S, 8S*)-**7** (7.4 mg, 91%), respectively, after purification by preparative thin layer chromatography. These samples were identified by comparisons of their ^{1}H-NMR spectra with those of the corresponding racemic compounds.[1] Acylations of (*2R, 8S*)-**7** (9.1 mg, 18 μmol) and (*2S, 8S*)-**7** (7.4 mg, 15 μmol) with (*S*)-*O*-acetylmandelic acid under the same conditions as described for the corresponding racemic compounds[1] afforded (*2R, 8S*)-**8** (10.5 mg, 86%), $[\alpha]_D^{25}$ = +96° (c=1.05, $CHCl_3$), and (*2S, 8S*)-**8** (8.8 mg, 88%), $[\alpha]_D^{25}$ = -96° (c=0.88, $CHCl_3$), respectively, after purification by preparative HPLC. These compounds showed identical ^{1}H-NMR spectra with those of the authentic samples obtained by the resolutions of *dl*-(*2R*,8S**)-**7** and *dl*-(*2S*,8S**)-**7**, respectively.

Cytotoxicity Assay

This was performed in a similar manner to that described in the preceding paper.[1] The IC_{50} values reported in this work were found to be different from those of the previous studies[1] in more than one order of magnitude. This might be due to the different sensitivity of cultivated cells since *in vitro* cytotoxicity assays in this and previous works were carried out at Sagami Chemical Research Center and Kyorin Pharmaceutical Co. Ltd., respectively, after the interval of *ca.* 1 year.

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