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Synthesis, Chemical Reactivity, and Cytotoxicity of 2-Bis(alkoxycarbonyl)methyliden-1-azabicyclo[3.1.0]hexane Systems Related to Antitumor Antibiotic Carzinophilin A†

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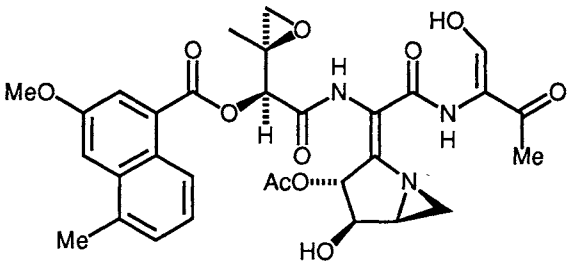
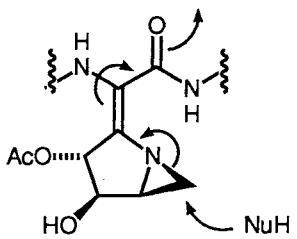
Enantiomeric pairs of the title compounds were synthesized starting from (*S*)- and (*R*)-pyroglutamic acid. They were found to be susceptible to nucleophilic ring opening of aziridine moieties and to exhibit weak *in vitro* cytotoxicity.

Carzinophilin A (Cz-A) (**1**) is an antitumor antibiotic isolated from *Streptomyces sahachiroi* by Hata *et al.* in 1954.[1] While the structure of **1** has been revised several times by plural groups over 30 years,[2] Armstrong *et al.* reported in 1991 that ^{1}H- and ^{13}C-NMR spectra of **1** were superimposable on those of azinomycin B.[3] The latter compound has been isolated as an antitumor antibiotic from other *Streptomyces* species and carries a characteristic 2-methyliden-1-azabicyclo[3.1.0]hexane ring system.[4] Since **1** is known as one of the strand cross-rinking compounds for DNA,[5] it is anticipated that, as shown in **2**, the antitumor activity of **1** is probably due to cleavage of the strained aziridine ring by attack of a nucleotide which might effect denaturation of DNA.[6]

Since Armstrong *et al.* recently reported the synthesis of the model compound of Cz-A [(Z)-isomer] (**3**),[7] we wish to report here our own results in this area, culminating in successful preparation of the enantiomeric pair



carzinophilin A (Cz-A) (**1**)
= azinomycin B (Az-B)



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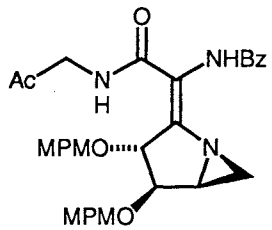

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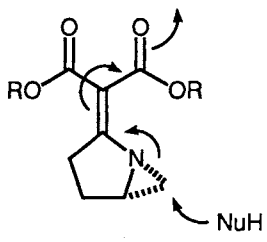

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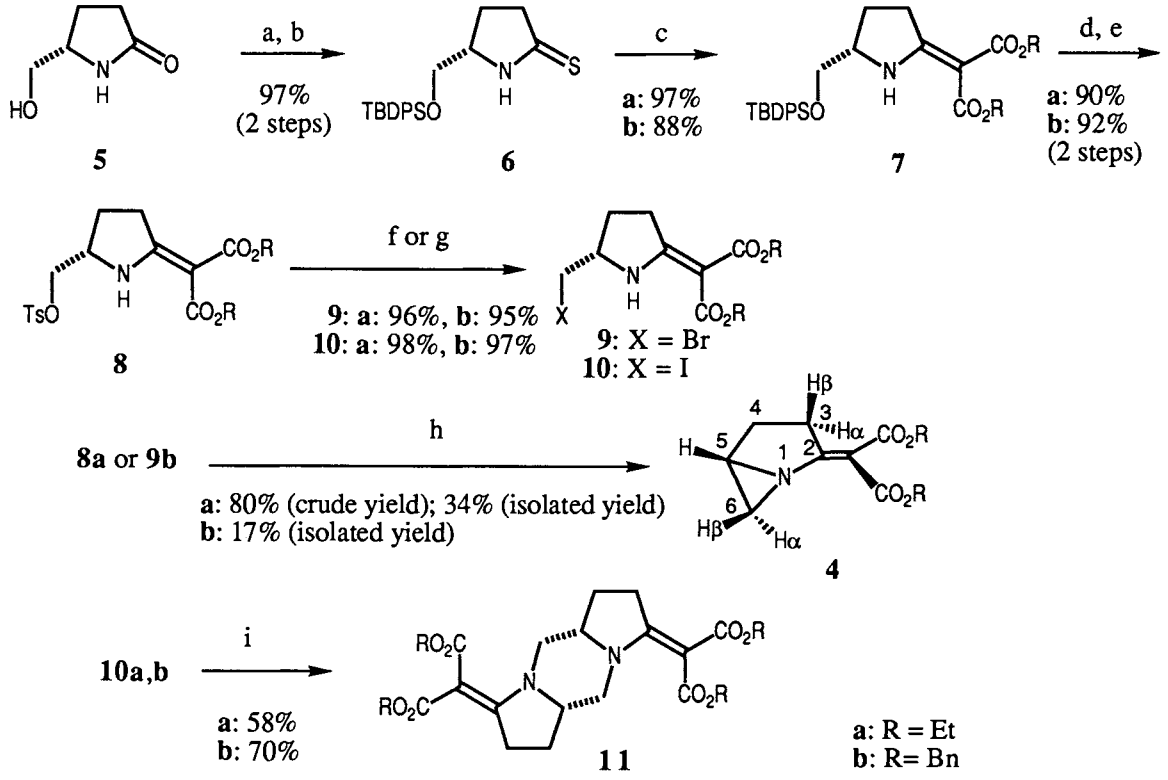
a: R = Et
b: R = Bn

†Dedicated to Professor Emeritus Osamu Simamura of The University of Tokyo on the occasion of his 80th birthday.

of novel 2-bis(alkoxycarbonyl)methyliden-1-azabicyclo[3.1.0]hexane system (**4** and *ent*-**4**) related to **1**. These bicyclic compounds (**4** and *ent*-**4**) were originally designed by expecting that their aziridine moieties could be cleaved in a similar manner to that proposed for **2**. As expected, **4** and *ent*-**4** were found to undergo facile nucleophilic ring opening of their aziridine moieties and, moreover, to exhibit weak *in vitro* cytotoxicity.

The synthesis of **4** and *ent*-**4** commences from optically pure (*S*)- and (*R*)-5-hydroxymethyl-2-pyrrolidone (**5** and *ent*-**5**) obtainable from (*S*)- and (*R*)-pyroglutamic acid according to the reported procedure.[8] For convenience, the synthetic route employing **5** is shown in Scheme 1. Thus, the hydroxyl group of **5** was first protected in a form of TBDPS ether. The 2-pyrrolidone moiety of the silyl ether was converted into a thiolactam by using Lawesson's reagent,[9] yielding the pyrrolidin-2-thione (**6**). Treatment of **6** with diethyl or dibenzyl bromomalonate followed by the addition of aqueous $KHCO_3$ solution resulted in the formation of the 2-bis(alkoxycarbonyl)methylidenpyrrolidines (**7a,b**)[10] in good yields. After the TBDPS ethers of **7a,b** were cleaved by the usual method, the generated alcohols were converted into the tosylates (**8a,b**), which were further transformed into the bromides (**9a,b**) and the iodides (**10a,b**), respectively.

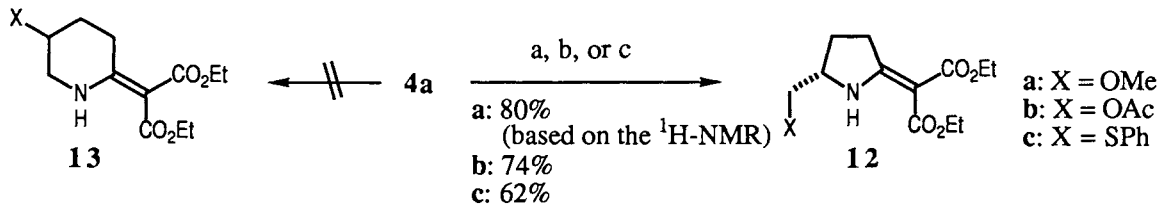
With completion of the synthesis of three types of the reaction substrates (**8-10**), the intramolecular aziridine formation producing a 2-bis(alkoxycarbonyl)methyliden-1-azabicyclo[3.1.0]hexane system was attempted. It was found that treatment of **8a** with potassium hydride in THF at room temperature for 15 min smoothly effected the construction of aziridine ring, giving rise to **4a** in 80% yield based on the ^{1}H-NMR spectrum of the crude reaction product (crude yield). Since **4a** was quite unstable under acidic conditions (*vide infra*), its isolation



a) TBDPSCl, ImH., DMF, rt, 12 h b) Lawesson's reagent, toluene, reflux, 20 min c) diethyl bromomalonate or dibenzyl bromomalonate, CH_2Cl_2, rt, 12 h, then aq. $KHCO_3$, 3-4 h d) TBAF, THF, rt, 1-2 h e) TsCl, Py., CH_2Cl_2, rt, 1-2 d f) Bu_4NBr, CH_3CN, reflux, 3-4 h g) NaI, acetone, reflux, 15-18 h h) KH, THF, rt, 15 min i) KH, THF, rt, 2 h

Scheme 1.

was able to be achieved only by quick Florisil column chromatography, affording a pure sample of **4a** as an oil, $[\alpha]_D^{20}$ +104° (c 0.750, $CHCl_3$),[11] in 34% yield (isolated yield). This rather low yield might reflect that **4a** decomposes during purification process. While **9a** also cyclized to **4a** in 70% crude yield, its isolation in a pure state turned out to be fruitless due to low separation efficiency of **4a** from **9a** by Florisil column chromatography. The cyclization of **8b** to **4b** took place in a very low yield under the same conditions as employed for **8a**. However, desired **4b**[12] was obtained in 17% isolated yield by using **9b** as a reaction substrate. Long range couplings (1.0 and 0.9 Hz, respectively) between signals of C_3-H_α and C_6-H_α observed in the ^{1}H-NMR spectra of **4a,b** as well as NOE experiments carried out on **4a** obviously established the structures of **4a,b** as depicted. It is notable that treatment of **10a,b** under the same conditions as employed for **8a,b** and **9a,b** gave the dimeric products (**11a,b**) in 57% and 70% yields, respectively, after 2 hrs' reactions, although production of a small amounts of **4a,b** was always detected by TLC analysis of the reaction mixture. Accordingly, the formation of **11a,b** might be explained by coupling of produced **4a,b** with the metaloamides of unreacted **10a,b** followed by intramolecular cyclization. Following the same procedures as described above, *ent*-**4a**, $[\alpha]_D^{20}$ -93.0° (c 0.800, $CHCl_3$), and *ent*-**4b**[12] were prepared from *ent*-**5**.



a) MeOH, rt, 7 d b) AcOH, THF, rt, 3 h c) PhSH, Et_3N, THF, rt, 3 h

Scheme 2.

With **4a,b** and *ent*-**4a,b** in hand, the chemical reactivity of 2-bis(alkoxycarbonyl)methyliden-1-azabicyclo-[3.1.0]hexane system was next studied by employing **4a** as a representative substrate. Thus, a pure sample of **4a** was found to be stable in $CDCl_3$ at 0 °C for a week. However, its slow decomposition was observed in MeOH at room temperature, producing the 5-methoxymethylpyrrolidine (**12a**). The $T_{1/2}$ value for the decomposition of **4a** was estimated as *ca.* 2.5 days. Acetic acid reacted with **4a** in THF to afford the 5-acetoxymethylpyrrolidine (**12b**) smoothly. Even under weakly basic conditions, for example, when **4a** was treated with thiophenol in the presence of triethylamine in THF at room temperature, cleavage of the aziridine ring occurred to give the 5-phenylthiomethylpyrrolidine (**12c**). In these experiments, no formation of the 5-substituted piperidine derivatives (**13a-c**) was observed. The structures of **12a-c** were definitely verified by their ^{1}H-NMR and mass spectra.[13] Highly regioselective formation of **12a-c** obviously suggests that nucleophilic ring opening of the 2-bis(alkoxycarbonyl)methyliden-1-azabicyclo[3.1.0]hexane systems takes place solely at their C_6-positions as initially expected.

Finally, *in vitro* cytotoxicity of **4a,b** and *ent*-**4a,b** was examined. Thus, when **4a,b** and *ent*-**4a,b** were subjected to *in vitro* cytotoxicity assay against P388 murine leukemia, the following very weak cytotoxicity was observed for these compounds [IC_{50} (μg/mL): 0.74 (**4a**), 11.6 (**4b**), 3.1 (*ent*-**4a**), and 7.6 (*ent*-**4b**)].

As described above, we have succeeded in preparing **4a,b** bearing a novel 2-bis(alkoxycarbonyl)-methyliden-1-azabicyclo[3.1.0]hexane system related to **1** and in exploring their chemical reactivity and *in vitro* cytotoxicity. Based on these results, synthetic studies aiming at the second generation of the model compound of **1** are in progress in these laboratories.

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5) A. Terawaki and J. Greenberg, *Nature*, **209**, 481 (1966); Hata reported that **1** was deactivated by treating with cysteine, aqueous acidic solution, thioglycolic acid, and so on. See, T. Hata, *Tanpakushitu Kakusan Koso*, **4**, 96 (1959).
6) In their studies on the isolation of azinomycins, Yokoi *et al.* reported that the naphthalene moiety of **1**, which lacks the peptide part corresponding to **2**, exibited no cytotoxicity.[4)]
7) R. W. Armstrong and E. J. Moran, *J. Am. Chem. Soc.*, **114**, 371 (1992). It was reported that the model compound (**3**) is highly unstable ($T_{1/2}$ = 4 h).
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11) ^{1}H-NMR spectral data of **4a,b** ($CDCl_3$) are as follows; **4a** δ 1.20, 1.26 (each 3H, t, *J*= 7.1 Hz, CH_3 ×2), 1.61 (1H, d, *J*= 5.1 Hz, C_6H_α), 2.14 (2H, m, C_4H_2), 2.40 (1H, dd, *J*= 1.0 and 4.3 Hz, C_6H_β), 2.61 (1H, ddt, *J*= 1.0, 17.9, and 4.3 Hz, C_3H_α), 2.88 (1H, m, C_5H), 3.25 (1H, ddd, *J*= 6.4, 8.7, and 17.9 Hz, C_3H_β), 4.11 (1H, q, *J*= 7.1 Hz, $C\underline{H}_2CH_3$), 4.22 and 4.31 (each 1H, dq, *J*= 10.8 and 7.1 Hz, $C\underline{H}_2CH_3$); **4b** δ 1.67 (1H, d, *J*= 4.3 Hz, C_6H_α), 2.19 (2H, m, C_4H_2), 2.42 (1H, dd, *J*= 0.9 and 5.0 Hz, C_6H_β), 2.70 (1H, ddt, *J*= 0.9, 20.1, and 8.0 Hz, C_3H_α), 2.98 (1H, m, C_5H), 3.33 (1H, ddd, *J*= 6.9, 7.2, and 20.1 Hz, C_3H_β), 5.15 (2H, s, CH_2Ph), 5.21, 5.35 (each 1H, d, *J*= 12.3 Hz, CH_2Ph), and 7.20-7.40 (10H, m, aromatic protons). In the spectrum of **4a**, NOEs were observed between the signals of C_3-H_α and C_6-H_α (2.6%), C_5-H and C_6-H_β (4.9%), and C_6-H_α and C_6-H_β (23%).
12) Optical rotation values of **4b** and *ent*-**4b** were not measured because of their low yields.
13) Representative ^{1}H-NMR ($CDCl_3$) and mass (EI) spectral data are as follows; **12a** δ 1.67 (1H, m, C_4H), 2.10 (1H, m, C_4H), 3.05-3.50 (7H, m, C_3H_2, C_5CH_2, CH_3O), and 4.03 (1H, m, C_5H), m/z 271 (rel. int., 15, M^+) and 180 (100, M-EtO-$MeOCH_2$-H^+); **12b** δ 1.67 (1H, dddd, *J*= 6.2, 6.8, 9.3, and 13.1 Hz, C_3H), 2.03 (3H, s, CH_3CO), 2.10 (1H, dddd, *J*= 5.8, 8.0, 9.4, and 13.1 Hz, C_3H), 3.02 (1H, ddd, *J*= 6.9, 9.4, and 18.5 Hz, C_4H), 3.13 (1H, ddd, *J*= 5.8, 9.5, and 18.5 Hz, C_4H), 3.93 (1H, dd *J*= 7.6 and 11.2 Hz, $C_2C\underline{H}H$), 4.04 (1H, m, C_2H), and 4.16 (1H, dd, *J*= 3.9 and 11.2 Hz, $C_2C\underline{H}H$), m/z 299 (rel. int., 2.7, M^+), 226 (4.1, M-$AcOCH_2^+$), and 180 (100, M-EtO-$AcOCH_2$-H^+); **12c** δ 1.78 (1H, dddd, *J*= 6.0, 6.8, 9.4, and 12.9 Hz, C_4H), 2.21 (1H, dddd, *J*= 5.7, 7.7, 9.1, and 12.9 Hz, C_4H), 2.96 (1H, dd *J*= 7.3 and 13.4 Hz, $C_5C\underline{H}H$), 3.05 (1H, ddd, *J*= 6.8, 9.5, and 18.5 Hz, C_3H), 3.07 (1H, dd *J*= 5.8 and 13.4 Hz, $C_5C\underline{H}H$), 3.23 (1H, ddd, *J*= 5.7, 9.4, and 18.5 Hz, C_3H), and 3.98 (1H, br quint, *J*= 6.0 Hz, C_5H), m/z 349 (rel. int., 2.1, M^+), 226 (11, M-$PhSCH_2^+$), 180 (100, M-EtO-$PhSCH_2$-H^+).

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