

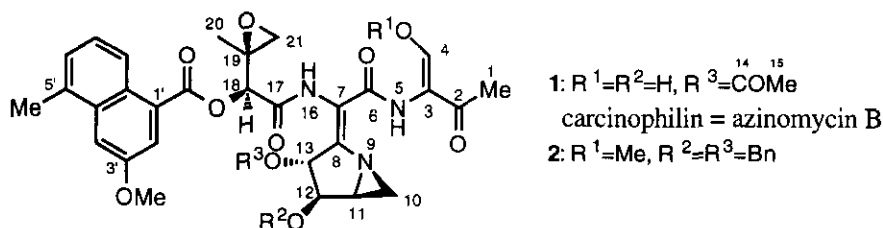
# A STEREoselective SYNTHESIS OF 4-O-METHYL-13-DESACETYL-12,13-DI-O-BENZYL CARZINOPHILIN<sup>‡</sup>

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**Abstract**- The title synthesis was achieved by the synthetic scheme featuring the following four key steps: i) coupling of the methyl thioimidate with the 2-oxazolin-5-one (**5**+**6**→**13**), ii) construction of the C17-C21 functionality by the use of Sharpless asymmetric dihydroxylation and subsequent manipulation of the functional groups (**13**→**21**), iii) activation of the 4-(pyrrolidin-2-ylidene)-2-oxazolin-5-one system by introducing an Alloc group into N9 position and subsequent ring opening with the L-threonine derivative (**21**+**7**→**26**), and iv) construction of the characteristic 2-methylidene-1-azabicyclo[3.1.0]hexane system with TBAF (**30**→**2**).

Carzinophilin (**1**) isolated from *Streptomyces sahachiroi* by Hata *et al.* in 1954, exhibits a potent antitumor activity,<sup>1</sup> and is well known as a bisalkylating agent for DNA.<sup>2</sup> The two structures distinctly different from each other were proposed for **1** by Lown *et al.*<sup>3</sup> and Onda *et al.*<sup>4</sup> in 1982 and 1983, respectively. In 1986, Yokoi *et al.* isolated azinomycins A and B (AZA and AZB) from *Streptomyces griseofuscus* S42227 as novel antitumor antibiotics<sup>5</sup> and assigned their structures based on spectral data.<sup>5</sup> Interestingly, it was reported by Yokoi *et al.* that the <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra of AZB having the structure obviously different from that of **1** previously proposed,<sup>3,4</sup> are similar to those of **1** although the molecular formula of AZB has one less oxygen.<sup>5</sup> Subsequently, Armstrong *et al.* uncovered in 1991 that **1** is identical to AZB by comparison of their spectral data.<sup>6a</sup> These unique history, novel structure, and prominent antitumor activity of **1** make this compound as an attractive target for total synthesis. While several synthetic

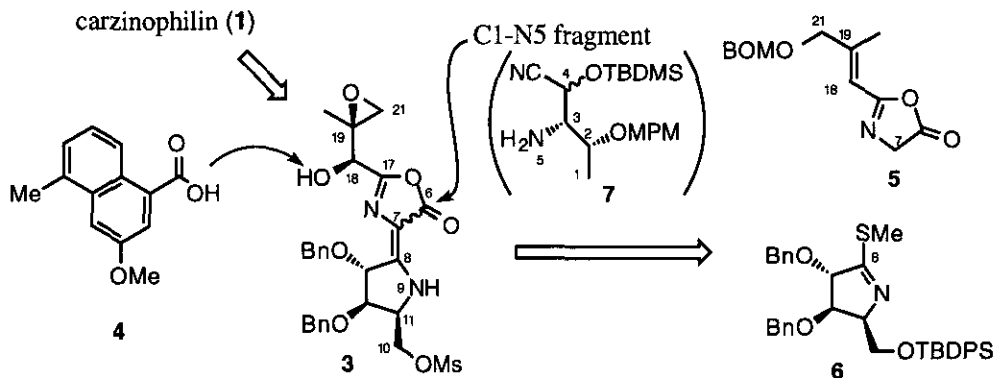


<sup>‡</sup>This paper is dedicated to Professor Koji Nakanishi on the occasion of his 75th birthday.

approaches to the compounds related to **1** have so far been reported,<sup>6-11</sup> the total synthesis of **1** has not been accomplished yet. As an extension of our synthetic studies,<sup>12</sup> we examined the total synthesis of **1** itself. Although deprotection of the 12,13-di-*O*-benzyl groups near the final synthetic stages turned out to be fruitless, we succeeded in preparing 4-*O*-methyl-13-*O*-desacetyl-12,13-di-*O*-benzylcarzinophilin (-azinomycin B) (**2**) carrying the same carbon framework as that of **1**. This report describes a stereoselective synthesis of **2** which corresponds to the compound most closely related to **1** among those hitherto reported.<sup>6-13</sup>

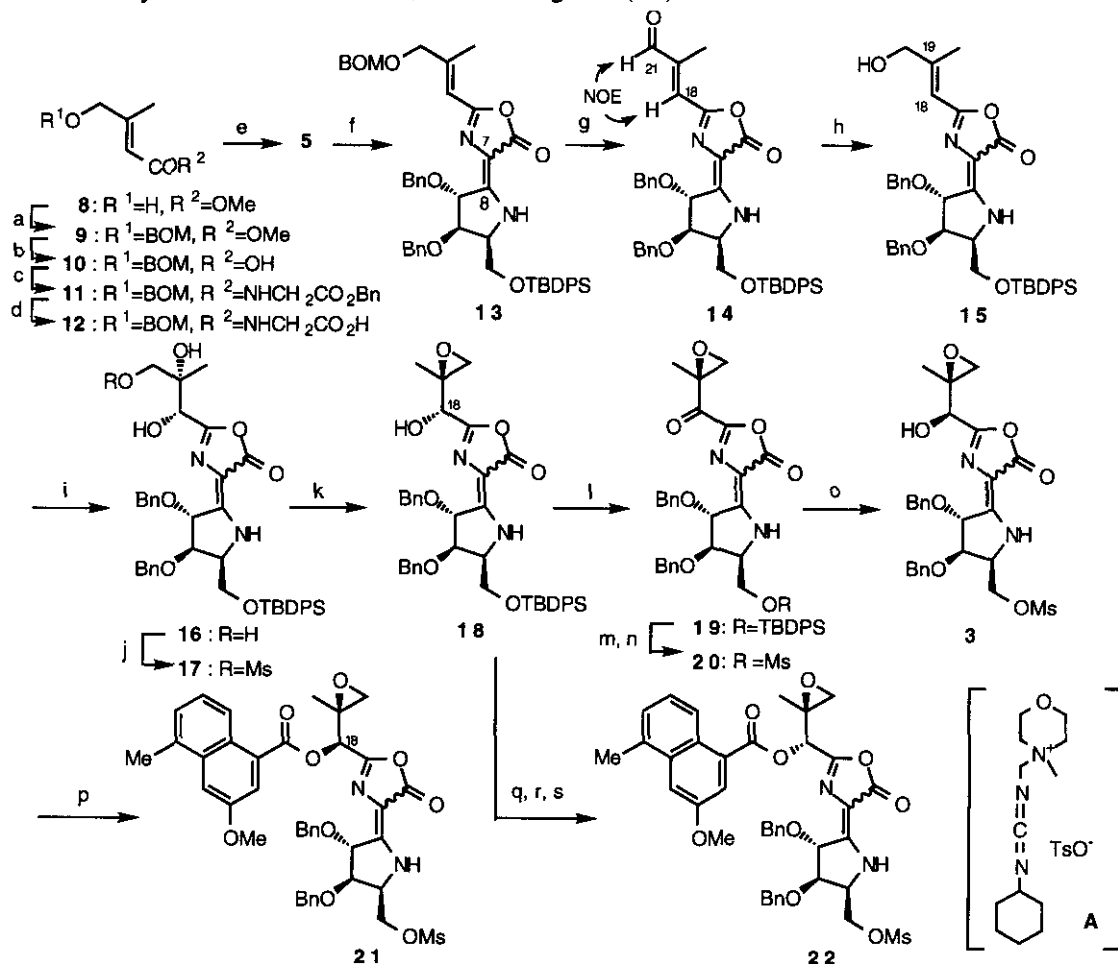
Based on our previous synthetic studies,<sup>12</sup> **1** can be retrosynthetically disconnected at the N5-C6, the N9-C10, and the C18-O-CO bonds. Cyclization between the C17-carbonyl oxygen and the C6 carbon will generate the 4-(pyrrolidin-2-ylidene)-2-oxazolin-5-one (**3**). Accordingly, it is envisioned that, as shown in **Scheme 1**, **1** can be produced by reacting **3** with the naphthalene-1-carboxylic acid (**4**) and the C1-N5 fragment and subsequent aziridine formation. Further retrosynthetic disconnection of **3** at the C7-C8 double bond provides the 2-oxazolin-5-one (**5**) and the methyl thioimidate (**6**).<sup>12c, d</sup> The L-threonine derivative (**7**) is employable as the C1-N5 fragment. Stereoselective functionalization of the C18-C21 moiety may be achieved after coupling of **5** and **6**.<sup>12c, d</sup>

**Scheme 1.** Retrosynthesis of carzinophilin (**1**)



The synthetic studies commenced with the preparation of **5**. As shown in **Scheme 2**, this was synthesized from methyl 3-hydroxymethyl-2-butenate (**8**)<sup>14</sup> in good overall yield in the five step sequence featuring i) protection of the hydroxy group, ii) basic hydrolysis, iii) condensation with benzyl glycinate, iv) basic hydrolysis, and v) dehydration to the 2-oxazolin-5-one system.<sup>15</sup> Treatment of **5** (6.8 eq) with **6**<sup>12c, d</sup> at 60 °C for 18 h (in a high concentration in toluene with argon gas bubbling) was found to give the condensation product (**13**) as a 78:22 inseparable mixture of (*E*)- and (*Z*)-isomers (*vide infra*) in 58% yield based on **6** with recovery of **6** (17%). An excess amount of **6** was employed for the condensation due to gradual decomposition of **6** under the reaction conditions. Deprotection of the BOM group in **13** was carried out by sequential oxidation with DDQ<sup>16</sup> and reduction of the produced aldehyde (**14**), giving the allyl alcohol (**15**).

With **15** in hand, the stereoselective construction of C18-C21 position was next examined. Since the (*E*)-configuration of C18-C19 double bond in **15** was obviously important for the stereoselective functionalizations, it was confirmed at the stage of **14** by observing an NOE between the signals due to the

**Scheme 2.** Synthesis of the C16-C13, N6-C21 fragment (**21**)

a) BOMCl, *i*-Pr<sub>2</sub>NEt b) aq NaOH c) GlyOBn•TsOH, 1-ethyl 3-(dimethylaminopropyl)carbodiimide hydrochloride (WSCl•HCl), Et<sub>3</sub>N, 85% (3 steps) d) aq NaOH, 92%, e) **6**, 100% f) **6**, toluene, 60°C, 58% (based on **6**), (recovery of **6** 17%) g) DDQ h) NaBH<sub>4</sub>, 97% 2 steps i) (DHQ)<sub>2</sub>PHAL, OsO<sub>4</sub>, then H<sub>2</sub>S, 59%, >95% de j) MsCl, γ-collidine, 81% k) DBU, 84% l) Dess-Martin reagent, 92% m) TBAF n) MsCl, γ-collidine, 83% 2 steps o) NaBH<sub>4</sub>, CeCl<sub>3</sub>•7H<sub>2</sub>O, 90%, 80% de p) **4**, WSCl•HCl, DMAP, 91% q) **4**, WSCl•HCl, DMAP, 78% r) (HF)<sub>n</sub>-Py, 84%, s) MsCl, Et<sub>3</sub>N, 98%

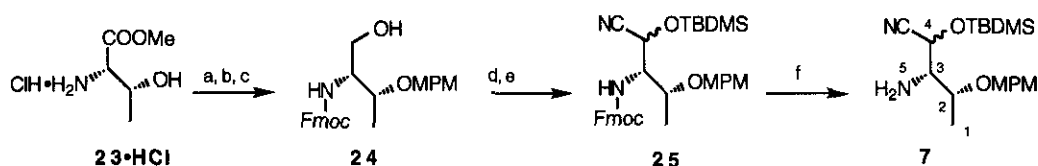
aldehyde and the C18 olefinic protons. Modified Sharpless asymmetric dihydroxylation<sup>17</sup> of **15** using stoichiometric amounts of OsO<sub>4</sub> (1.0 eq) and (DHQ)<sub>2</sub>PHAL (1.2 eq) in CH<sub>2</sub>Cl<sub>2</sub> at -15 °C followed by decomposition of the osmate with H<sub>2</sub>S, afforded triol (**16**) with >95% de<sup>18</sup> in 59% yield. After the primary hydroxy group was mesylated, treatment of mesylate (**17**) with DBU gave epoxide (**18**). In order to epimerize the remaining secondary hydroxy group in **18**, we examined to utilize the sequential oxidation and chelation controlled reduction protocol. Toward this end, **18** was first oxidized with Dess-Martin reagent<sup>19</sup> to the corresponding ketone (**19**). At this stage, the TBDPS group was replaced with a mesyl group by the two step process to afford mesylate (**20**). As expected, reduction of **20** with a combination of CeCl<sub>3</sub>•7H<sub>2</sub>O and NaBH<sub>4</sub><sup>20</sup> in MeOH at 0°C proceeded in a highly stereoselective manner, giving rise to the desired (*S*)-C18 alcohol (**3**) as a major product [(*S*):(*R*)=9:1]<sup>18</sup> in 90% yield. This was

condensed with **4**,<sup>8a</sup> furnishing ester **21**. The stereochemistry of **21** was rigorously determined by comparing its <sup>1</sup>H-NMR spectrum with the epimeric ester (**22**) prepared from **18** by sequential esterification with **4**,<sup>8a</sup> desilylation, and mesylation.

It is worthy to note that all the bicyclic compounds (**3**) and (**13-22**) were obtained as inseparable mixtures of (*E*)- and (*Z*)-isomers concerning the C7-C8 double bonds after purification by column chromatography (SiO<sub>2</sub>). These phenomena appeared evident from their <sup>1</sup>H-NMR spectra. Ratios of (*E*)- and (*Z*)-isomers are as follows: **3** (56:44), **13** (78:22, *vide supra*), **14** (81:19), **15** (71:29), **16** (56:44), **17** (66:34), **18** (68:32), **19** (94:6), **20** (77:23), **21** (58:42), **22** (62:38). Interestingly, while the <sup>1</sup>H-NMR spectrum of **19** measured right after the recrystallized sample, mp 145-147 °C (Et<sub>2</sub>O-hexane), was dissolved in CDCl<sub>3</sub>, cleanly showed the presence of pure (*E*)-isomer, gradual formation of the (*Z*)-isomer was observed by recording the <sup>1</sup>H-NMR spectrum with time course. Considering these facts, it appeared plausible that **3** and **13-22** exist as the mixtures of tautomeric (*E*)- and (*Z*)-isomers.

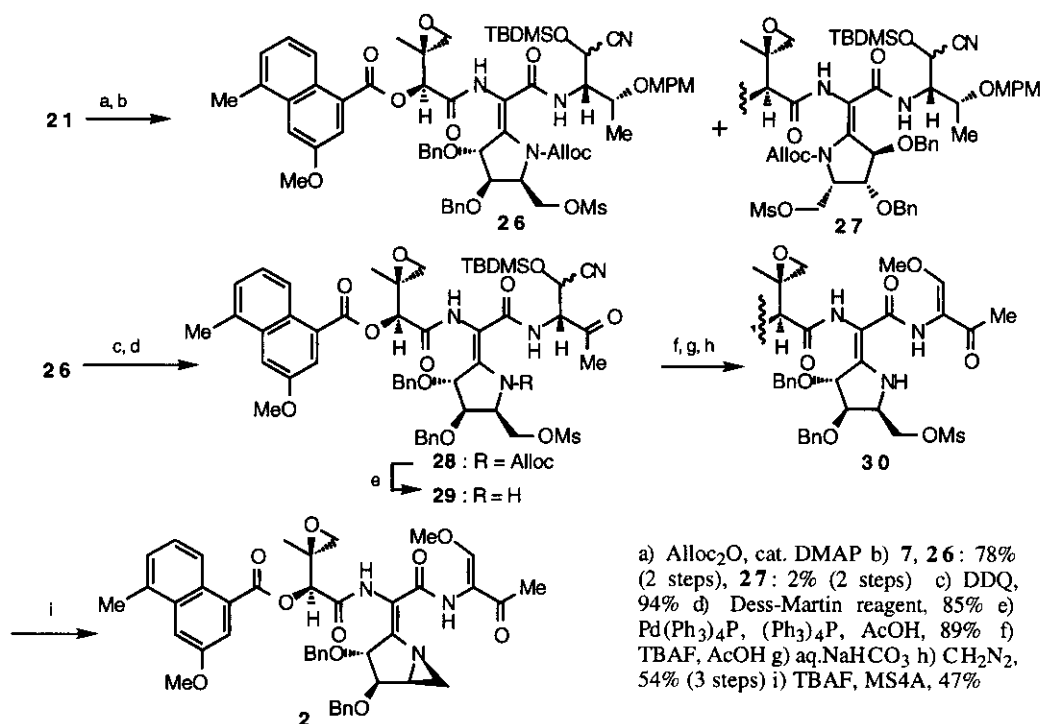
With **21** in hand, the synthesis of **7** was next attempted. As shown in **Scheme 3**, **7** was prepared from methyl L-threoninate hydrochloride (**23•HCl**) by the six step sequence employing i) protection of the amino group, ii) protection of the hydroxy group, iii) reduction, iv) oxidation, v) formation of *O*-silyl-cyanohydrin, vi) deprotection followed by separation of the major polar diastereomer. For operational and spectral simplicity, the major isomer of **7** whose stereochemistry at C4 position was not determined, was employed for the next step.

**Scheme 3.** Synthesis of the L-threonine derivative (the C1-N5 fragment) (**7**)



a) FmocCl, NaHCO<sub>3</sub>, 99% b) MPMOC(NH)CCl<sub>3</sub>, TFOH c) Zn(BH<sub>4</sub>)<sub>2</sub>, 58% (2 steps) d) Dess-Martin reagent e) TBDMSCN, 92% (2 steps) f) piperidine, 90%, then separation of the major polar diastereomer

As shown in **Scheme 4**, treatment of **21** [(*E*):(*Z*)=58:42] with Alloc<sub>2</sub>O and a catalytic amount of DMAP followed by the addition of **7** and concentration *in vacuo* at 40 °C was found to cleanly afford the desired (*E*)-amide (**26**) as a major product (78%) along with (*Z*)-isomer (**27**) (2%). Stereochemistries of **26** and **27** were definitely determined by comparing their <sup>1</sup>H-NMR spectra with those of the model compounds previously reported.<sup>12d</sup> The isomerization of (*Z*)-isomer to (*E*)-isomer might occur during or after the reaction of **21** with **7**. In this case, tautomerization between **26** and **27** was not observed. After removal of the MPM group, the produced alcohol was oxidized to give the *O*-silylated β-ketocyanohydrin **28**, whose N-Alloc group was subsequently deprotected, giving rise to pyrrolidine (**29**). At this stage, construction of the C1-C4 position was accomplished by sequential desilylation and alkaline treatment. The produced enol system was protected with a methyl group to afford methyl enol ether (**30**). Finally, treatment of **30** with TBAF in the presence of molecular sieves 4A smoothly underwent the aziridine formation, furnishing **2**,<sup>21,22</sup> [α]<sub>D</sub><sup>20</sup> +8.5° (c 0.082, MeCN), in 47% yield, which was stable enough to be separated by preparative TLC (SiO<sub>2</sub>). As shown in **Table 1**, the <sup>1</sup>H-NMR spectral data of **2** was found

**Scheme 4.** Synthesis of 4-*O*-methyl-13-*O*-desacetyl-12,13-di-*O*-benzylcarzinophilin (-azinomycin B) (**2**)**Table 1.** <sup>1</sup>H-NMR spectral data of AZB-4-*O*-Me<sup>5b</sup> and **2** (400 MHz, CDCl<sub>3</sub>)

| chemical shifts (ppm), signal patterns, and coupling constants (Hz) |                                  |                                  | chemical shifts (ppm), signal patterns, and coupling constant (Hz) |                            |                            |
|---|----------------------------------|----------------------------------|--|----------------------------|----------------------------|
| protons   | AZB-4- <i>O</i> -Me              | <b>2</b>                         | protons  | AZB-4- <i>O</i> -Me        | <b>2</b>                   |
| 1   | 2.24, <i>s</i>                   | 2.22, <i>s</i>                   | 18   | 5.08, <i>s</i>             | 5.15, <i>s</i>             |
| 4   | 7.19, <i>s</i>                   | 7.14, <i>s</i>                   | 20   | 1.51, <i>s</i>             | 1.53, <i>s</i>             |
| 4-OMe   | 3.90, <i>s</i>                   | 3.89, <i>s</i>                   | 21   | 2.80, <i>d</i> , 4.3       | 2.60, <i>d</i> , 4.6       |
| 5   | 10.89, <i>s</i>                  | 10.58, <i>bs</i>                 |  | 2.99, <i>d</i> , 4.3       | 2.90, <i>d</i> , 4.6       |
| 10  | 2.25, <i>d</i> , 3.9             | 2.27, <i>d</i> , 4.1             | 2'   | 7.94, <i>d</i> , 2.4       | 7.96, <i>d</i> , 2.6       |
|   | 2.51, <i>d</i> , 5.4             | 2.48, <i>d</i> , 5.1             | 3'-OMe   | 3.96, <i>s</i>             | 3.96, <i>s</i>             |
| 11  | 3.22, <i>ddd</i> , 3.9, 5.4, 5.8 | 3.12, <i>ddd</i> , 4.1, 4.8, 5.1 | 4'   | 7.46, <i>d</i> , 2.4       | 7.48, <i>d</i> , 2.6       |
| 12  | 4.63, <i>dd</i> , 3.9, 5.8       | 4.49, <i>dd</i> , 3.6, 4.8       | 5'-Me  | 2.66, <i>s</i>             | 2.66, <i>s</i>             |
| 13  | 5.55, <i>d</i> , 3.9             | 4.94, <i>dd</i> , 1.0, 3.6       | 6'   | 7.35, <i>dd</i> , 2.4, 7.4 | could not be assigned      |
| 15  | 2.18, <i>s</i>                   | -                                | 7'   | 7.36, <i>dd</i> , 7.4, 7.4 | could not be assigned      |
| 16  | 8.50, <i>s</i>                   | 8.21, <i>bs</i>                  | 8'   | 8.56, <i>dd</i> , 2.4, 7.4 | 8.60, <i>dd</i> , 2.4, 7.4 |

to be very similar to that reported for azinomycin B (carzinophilin) 4-*O*-methyl ether (AZB-4-*O*-Me) by Yokoi *et al.*<sup>5b</sup> except for the chemical shift of C13-H where an acetoxy group is replaced with a benzyloxy group in **2**. This spectral feature definitely supports the structure of **2**.

To further advance the synthetic scheme to **1**, we next examined the removal of two benzyl groups protecting the two hydroxy groups at C12 and C13 positions. However, all the attempts including hydrogenolysis met with failure,<sup>23</sup> disclosing that it is impossible to obtain **1** from **2** and letting us start an alternative synthetic approach to **1**.

As mentioned above, we have succeeded in preparing **2** which corresponds to the compound most closely

related to **1** among those so far reported.<sup>6-12</sup> Based on the results obtained from these studies, the total synthesis of **1** is in progress in our laboratories and will be reported in due course.

## REFERENCES AND NOTES

1. T. Hata, F. Koga, Y. Sano, K. Kanamori, A. Matsumae, R. Sunagawa, T. Shima, S. Ito, and S. Tomizawa, *J. Antibiot., Ser. A*, 1954, **7**, 107.
2. a) A. Terawaki and J. Greenberg, *Nature*, 1966, **209**, 481; b) R. W. Armstrong, M. E. Salavati, and M. Nguyen, *J. Am. Chem. Soc.*, 1992, **114**, 3144.
3. J. W. Lown and C. C. Hanstock, *J. Am. Chem. Soc.*, 1982, **104**, 3213.
4. M. Onda, Y. Konda, A. Hatano, T. Hata, and S. Omura, *J. Am. Chem. Soc.*, 1983, **105**, 6311.
5. K. Yokoi, K. Nagaoka, and T. Nakashima, *Chem. Pharm. Bull.*, 1986, **34**, 4554.
6. a) E. J. Moran and R. W. Armstrong, *Tetrahedron Lett.*, 1991, **32**, 3807; b) R. W. Armstrong and E. J. Moran, *J. Am. Chem. Soc.*, 1992, **114**, 371; c) R. W. Armstrong, J. E. Tellew, and E. J. Moran, *Tetrahedron Lett.*, 1996, **37**, 447.
7. R. S. Coleman and A. J. Carpenter, *J. Org. Chem.*, 1992, **57**, 5813.
8. a) M. Shibuya, *Tetrahedron Lett.*, 1983, **24**, 1175; b) K. Shishido, T. Omodani, and M. Shibuya, *J. Chem. Soc. Perkin Trans. I*, 1992, 2053.
9. Y. Konda, T. Machida, M. Akaiwa, K. Takeda, and Y. Harigaya, *Heterocycles*, 1996, **43**, 555.
10. H. J. Bryant, C. Y. Dardonville, T. J. Hodgkinson, M. Shipman, and A. M.Z. Slawin, *Synlett*, 1996,
11. K. Takayanagi and M. Hirama, *The 65th Annual Meeting of the Chemical Society of Japan*, 1993, *Abstract Paper II*, p. 516.
12. a) M. Hashimoto, K. Yamada, and S. Terashima, *Chem. Lett.*, 1992, 975; b) M. Hashimoto, M. Matsumoto, K. Yamada, and S. Terashima, *Tetrahedron Lett.*, 1994, **35**, 2207; c) M. Hashimoto and S. Terashima, *Chem. Lett.*, 1994, 1001; d) *idem*, *Tetrahedron Lett.*, 1994, **35**, 9409.
13. In the communication received on 8 June, 1995 (ref. 6c), Armstrong *et al.* described that they had submitted the paper in *J. Org. Chem.* reporting the synthesis of 13-*O*-desacetyl-12,13-*O*-bis-*p*-methoxybenzylazinomycin A. However, this paper has not yet appeared in *J. Org. Chem.* until now.
14. P. Garner and S. Ramakanth *J. Org. Chem.*, 1987, **52**, 2631.
15. C. F. Hoyng, M. G. McKenna, and D. L. Walters, *Synthesis*, 1982, 191.
16. R. S. Ward, P. Satyanarayana, and V.B. G. Rao, *Tetrahedron Lett.*, 1981, **22**, 3021.
17. K. B. Sharpless, W. Amberg, Y. L. Bennani, G. A. Crispino, J. Hartung, K.-S. Jeong, H.-L. Kwong, K. Morikawa, Z.-M. Wang, D. Xu, and X.-L. Zhang, *J. Org. Chem.*, 1992, **57**, 2768.
18. Determined by the <sup>1</sup>H-NMR spectrum.
19. D. B. Dess and J. C. Martin, *J. Org. Chem.*, 1983, **48**, 4155.
20. K. Li, L. G. Hamann, and M. Koreeda, *Tetrahedron Lett.*, 1992, **33**, 6569.
21. When **2** was allowed to react with thiophenol in the presence of triethylamine, the bisadduct **i** was readily produced in 80% yield. This result might mimic the reactivity of **1**, which behaves as a bisalkylating agent for DNA.
22. This compound **2** was found to exhibit IC<sub>50</sub> 0.39 µg/mL when submitted to *in vitro* cytotoxicity assay against P388 murine leukemia. We are grateful to Dr. K. Yamada (Sagami Chemical Research Center) for performing *in vitro* cytotoxicity assay.
23. Reaction of **2** over 10%Pd/C in AcOEt effected highly selective aziridine ring opening, giving rise to **ii** along with recovery of **2**.

