Total Synthesis of Antitumor Antibiotic FR900482

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Abstract: This account describes our first total synthesis of an enantiomeric pair of FR900482 (1 and ent-1), which was accomplished in a convergent manner utilizing the aromatic segment 14 and the aliphatic segment 15 and ent-15 accessible from commercially available 5-hydroxyisophthalic acid (16) and each enantiomer of diethyl tartrate (17 and ent-17), respectively. The method for the total synthesis involves the following four key steps: (i) coupling reaction of 14 with 15 (14+15→32, Scheme 5); (ii) intramolecular aldol reaction of the highly functionalized dialdehyde 13 (13→36, Scheme 6); (iii) epimerization at the C-8 position of the hydroxy ketone 40 (40→41, Scheme 7); (iv) internal hemiacetal formation of the N-hydroxylamino ketone 11 in situ generated from the ketone 45 (45→11→10, Scheme 10). The in vitro cytotoxicity assay of the synthesized compounds (1, ent-1, 50, ent-50, 51, and ent-51) against P388 murine leukemia cells disclosed that 1 and its congeners 50, 51 possessing natural absolute configuration are ca. 100 times more cytotoxic than the corresponding unnatural enantiomers (ent-1, ent-50, ent-51).

1. Introduction

FR900482 (1) isolated from the culture broth of Streptomyces sandaensis No.6897 at Fujisawa Pharmaceutical Co. Ltd. in 1987, exhibits exceptionally potent antitumor activity against various types of mammalian solid tumors (ref. 1). FK973 (2), the more stable semisynthetic triacetyl derivative of 1, has been reported to display ca. three times more potent antitumor activity than mitomycin C (3) with a significantly low toxicity (ref. 2). FR66979 (4), the dihydro derivative of 1 isolated from the same culture broth, also exhibits antitumor activity similarly to 1 (ref. 3).

The stereostructure of 1, except for its absolute configuration, was revealed by extensive spectroscopic studies and chemical correlation with 2 to have a unique 3,9-epoxy-3H-azirino[2,3-c][1]benzazocine skeleton. The relative stereochemistry of 2 was established by single crystal X-
ray analysis (ref. 4). The absolute configuration of 1 pictured in Figure 1 was suggested based on the biogenetic studies demonstrating that the aliphatic portion in 1 is derived from D-glucosamine (ref. 5). This novel natural product exists as a 2:1 mixture of two tautomers due to its unique hydroxylamine hemiacetal functionality. Similarly to 3, 1 possesses an aziridine ring and a carbamoyloxymethyl group, but lacks a quinoid structure.

It has been shown that 1 inhibits DNA synthesis in preference to RNA and protein synthesis in cultured L1210 murine leukemia cells and forms DNA-DNA interstrand cross-links in the cells (ref. 6). The mode of antitumor action for 1 shown in Scheme 1, which is similar to that of 3, has been previously proposed (ref. 7). Thus, 1 is activated in vivo by bioreduction of the hydroxylamine hemiacetal function to produce the azocinone 5. Subsequent cyclization of 5 occurs spontaneously to generate the mitosene-like compound 6, which undergoes DNA-DNA interstrand cross-linking. Analogous formation of the DNA-DNA interstrand cross-link is well preceded for the mitosene derivative in situ produced from 3 (ref. 8). This speculation is strongly supported by the successful isolation of the interstrand cross-linked product 8 from the reaction mixture of 4 and a synthetic DNA duplex in the presence of a reducing agent (ref. 9). Structural elucidation of this substance was achieved for the corresponding heptaacetate 9 by extensive spectroscopic studies (ref. 9).

Its remarkable antitumor activity as well as its unique structural features make 1 an exceptionally intriguing and timely target for total synthesis. A number of synthetic approaches toward 1 have been reported to date (ref. 7a,10), and the two total syntheses of racemic 1 were accomplished by Fukuyama et al. (ref. 11) in 1992 and by Schkeryantz and Danishefsky (ref. 12) in 1995, respectively. In 1990, we embarked on a project directed at the total synthesis of 1, its enantiomer ent-1, and their congeners in enantiomerically pure forms with the aim of exploring the structure-activity relationships. In 1995, our earnest endeavors culminated in completing the first total synthesis of natural 1 (ref. 13). In the following year, we also succeeded in synthesizing unnatural enantiomer ent-1 by employing the explored synthetic method. This article describes our efforts toward successful enantioselective total synthesis of an enantiomeric pair of 1. Furthermore, we present the in vitro cytotoxicity of 1, its synthetic intermediates (50 and 51), and their enantiomers (ent-1, ent-50, and ent-51) against P388 murine leukemia cells, disclosing some novel aspects of the structure-activity relationships for 1.
2. Synthetic Strategies

The retrosynthetic plan for natural (+)-FR900482 (1) is outlined in Scheme 2. The most crucial step in this scheme is envisaged to be the intramolecular aldol reaction of the highly functionalized dialdehyde 13 to construct the eight-membered 1H-azirino[2,3-c][1]benzazocine system 12 representing the core skeleton of 1 (13→12). It is noteworthy that this aldol cyclization involves an interesting possibility for controlling the stereochemistry at the C-8 position in 1. At the time when this synthetic plan was devised, whether the aldol cyclization might control the C-8 stereochemistry in 12 was quite ambiguous due to the flexible conformation of the eight-membered transition state. However, since 1 possesses the (8R)-configuration in nature, we expected that the C-8 position can be epimerized to the desired configuration in a later stage even if the undesired (8S)-epimer is produced as the sole product or as a mixture with the desired (8R)-isomer. The cyclization product 12 would be transformed to the advanced key intermediate 10 having the requisite tetracyclic skeleton with correct stereochemistries via the internal hemiacetalization of the N-hydroxylamine 11. Compound 10 could be converted to the target molecule 1 by sequential functional group manipulations and deprotection or vice versa.

The cyclization precursor 13, in turn, may be elaborated by coupling of the aromatic segment 14 and the optically active aliphatic segment 15, both of which can be prepared from commercially available 5-hydroxyisophthalic acid (16) and natural diethyl L-tartrate (17), respectively. Taking into account the well-known chemical instability of 1 (ref. 1c,d), benzyl (Bn), benzyloxymethyl (BOM), and p-toluenesulfonyl (Ts) groups may be selected as promising protective groups P1, P2, and P3, respectively, because they are expected to be removed under almost neutral conditions such that the delicate core structure and functional groups involved in 1 could survive.

By employing diethyl D-tartrate (ent-17) instead of 17, unnatural (−)-FR900482 (ent-1) would be also synthesized in a similar manner to that described above.

Scheme 2. Retrosynthetic analysis of FR900482 (1)

3. Synthesis of the Aromatic Segment 14

At first, we pursued the synthesis of the aromatic segment 14 starting with commercially available 5-hydroxyisophthalic acid (16) as shown in Scheme 3. Thus, the known allyl ether 18 was prepared from 16 (98%, 2 steps) according to the reported methods (ref. 15) with several improvements of the
reaction conditions. After Claisen rearrangement of 18, benzylation of the resulting phenol and subsequent alkaline hydrolysis of the two methyl ester groups provided the dicarboxylic acid 19 in 83% yield from 18. Compound 19 was then converted to the bromolactone 20 in 58% overall yield through a three-step sequence involving bromolacozonization, reduction of the remaining carboxyl group via the mixed acid anhydride, and protection of the resulting hydroxy group as its BOM ether. Reductive cleavage of the bromolactone moiety in 20 followed by modified Curtius rearrangement (ref. 16) of the liberated carboxylic acid furnished the N-tert-butoxycarbonyl (Boc) aniline 21 in 71% yield for the two steps. Oxidative cleavage of the terminal olefin in 21 was carried out by employing the Lemieux-Johnson procedure (ref. 17), resulting in the formation of the cyclic hemiaminal 22 in 73% yield. Reduction of 22 with sodium borohydride followed by protection of the primary hydroxy group in the resulting alcohol furnished the tert-butyldimethylsilyl (TBDMS) ether 23 in 97% yield for the two steps. Finally, exchange of the Boc group in 23 with an allyloxycarbonyl (Alloc) group gave the requisite aromatic segment 14 in 90% overall yield.

4. Synthesis of the Optically Active Aliphatic Segment 15

Next, the synthesis of the optically active aliphatic segment 15 was investigated as shown in Scheme 4. Thus, the known benzyl ether 24 (ref. 18) prepared from commercially available diethyl L-tartarate (17), was converted to the mesylate 25 (90%, 3 steps) via a three-step sequence involving protection of the hydroxy group as its p-methoxyphenylmethyl (MPM) ether, catalytic hydrogenolysis of the benzyl group over Raney nickel, and mesylation of the resulting secondary alcohol. Acidic hydrolysis of the acetonide moiety in 25 followed by treatment with potassium carbonate in methanol led to the formation of the epoxide 26 in 85% yield for the two steps. The optical purity of 26 was estimated to be more than 98% ee by comparison of the 400MHz 1H-NMR spectra of the corresponding (R)- and (S)-MTPA esters (ref. 19). While 26 could be prepared more directly from commercially available cis-2-butene-1,4-diol (27) via the Sharpless asymmetric epoxidation (ref. 20), the optical purity of this epoxidation product was found to be approximately 85% ee (ref. 21). Furthermore, in a large-scale experiment (>50 mmol), the enantiomeric excess of epoxidation product reduced to 75% ee,
moreover, a longer reaction time (>90 h) was required for completion of the reaction. Therefore, the sequence starting from 17 was selected to prepare a large quantity of 26 in an enantiomerically pure form.

Nucleophilic epoxide ring-opening of 26 with an azide anion was next carried out. Thus, treatment of 26 with sodium azide in the presence of ammonium chloride in refluxing ethanol resulted in the formation of an inseparable mixture of regioisomers 28 and 29 in a ratio of ca. 3 : 2 in 92% combined yield. After exposure of this mixture to sodium periodate, the desired azide alcohol 28 could be readily isolated by column chromatography on silica gel in 55% yield from 26. Sequential selective protection of the primary hydroxy group in 28 as its tert-butyldiphenylsilyl (TBDPS) ether, reduction of the azide moiety with triphenylphosphine in THF-H$_2$O, and selective protection of the amino group in the resulting amino alcohol provided the N-2,2,2-trichloroethoxycarbonyl (Troc) amine 31 in 94% overall yield.

Finally, 31 was converted to the requisite optically active aliphatic segment 15 (89%, 3 steps) (ref. 22) by a three-step operation involving acetonide formation, deprotection of the MPM group with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), and triflation of the resulting alcohol (ref. 23).

By employing unnatural diethyl D-tartrate (ent-17) instead of 17, the enantiomeric aliphatic segment ent-15 required for the total synthesis of unnatural (-)-FR900482 (ent-1) was prepared in a similar manner to that described above.

5. Synthesis of the Substrate 13 for the Key Intramolecular Aldol Reaction

Having obtained both the aromatic segment 14 and the enantiomerically pure aliphatic segment 15, our next efforts were directed toward elaboration of the substrate 13 for the key intramolecular aldol
reaction. As shown in Scheme 5, the critical coupling reaction of 14 with 15 was carried out by treating a mixture of these segments in THF with sodium hydride at -78°C followed by warming to room temperature, furnishing the desired coupling product 32 in a quantitative yield. After simultaneous removal of the Troc group and the acetone moiety in 32, the resulting amino alcohol was further converted to the mesylate 33 (72% from 32) by chemoselective tosylation of the amino group followed by mesylation of the secondary hydroxy group. Treatment of 33 with sodium hydride in the presence of imidazole in refluxing THF cleanly provided the aziridine 34 in 92% yield. Simultaneous removal of the TBDMS and the TBDPS groups in 34 by exposure to a hydrogen fluoride-pyridine complex followed by Dess-Martin oxidation (ref. 24) of the resulting diol yielded the key cyclization precursor 13 in 97% yield for the two steps.

**Scheme 5.** Synthesis of the substrate 13 for the key intramolecular aldol reaction

![Scheme 5](image)

6. The Key Intramolecular Aldol Reaction of the Dialdehyde 13

With the key cyclization precursor 13 in hand, we next focused our attention on the crucial intramolecular aldol reaction to construct the desired eight-membered ring system 36 (Scheme 6). In general, eight-membered ring formation by intramolecular ring closure reaction is often difficult due to the steric strain as well as an unfavorable entropy factor. However, in the case of 13, the fused aromatic and aziridine rings can be expected to overcome these barriers. In the event, the crucial intramolecular aldol cyclization could be achieved by treating a dilute solution of 13 in THF (0.01 M) with 1.0 equiv of lithium bis(trimethylsilyl)amide [LiN(TMS)₂] at -78°C followed by warming to -5°C. After treatment of this mixture with sodium borohydride in H₂O, the requisite cyclization product 36 was readily isolated as the sole product in 48% yield along with recovery of the uncyclized diol 37 (33%). No other cyclized stereoisomers were obtained from the reaction mixture. The stereostructure of 36 wherein the C-8 configuration is incorrect, was determined by spectroscopic studies and chemical correlation with 12 whose stereostructure was rigorously assigned by X-ray diffraction analysis of the related bis(p-bromobenzoate) derivative (vide infra) (ref. 25). This cyclization might proceed through the transition state such as six-membered chelation intermediate 13A, wherein the eight-membered ring takes a twist-boatt conformation and the C-8 enolate moiety is oriented in a quasi-axial position to avoid an unfavorable allylic 1,3-strain between the enolate moiety and the benzyloxy (BnO) group present at the aromatic ring.

7. Synthesis of the Eight-membered Key Intermediate 12

Since the configuration at the C-8 position in the cyclization product 36 turned out to be incorrect, the inversion of the C-8 stereochemistry was next investigated as shown in Scheme 7. Thus, selective
Scheme 6. The key intramolecular aldol reaction of the dialdehyde 13

\[
13 \xrightarrow{\text{LIN(TMS)\textsubscript{2} (1.0 equiv)}} \xrightarrow{\text{THF, -78 \rightarrow -5°C}} 13A \xrightarrow{\text{NaBH\textsubscript{4}-H\textsubscript{2}O, -5 \rightarrow 0°C}} 35
\]

\[
36 \ (48\%)
\]

Scheme 7. Epimerization at the C-8 position of the cyclization product 36 and the synthesis of the eight-membered key intermediate 12

\[
36 \xrightarrow{\text{TBDPSCI, Et\textsubscript{3}N, DMAP}} 79\% \xrightarrow{\text{Dess-Martin periodinane}} 39 : R = \text{TBDPS} \ (93\%) \xrightarrow{(HF)\textsubscript{n}-Py} 39 : R = \text{H} \ (93\%)
\]

\[
36 \xrightarrow{\text{DBU, THF, rt; separation}} 64\% \text{ for } 41 \ (31\% \text{ for } 40) \xrightarrow{\text{NaBH\textsubscript{4}}} 87\% \xrightarrow{\text{H\textsuperscript{+}}} 41A
\]

\[
42 \ (30\%)
\]

silylation of the primary hydroxy group in 36 followed by Dess-Martin oxidation of the resulting TBDPS ether 38 furnished the ketone 39 in 73% yield for the two steps. Initial attempts to achieve the epimerization at the C-8 position of 39 under various basic conditions met with failure, resulting in the formation of the useless exocyclic enone 42 by elimination of the siloxy moiety in an almost quantitative yield. However, success was eventually realized by employing the hydroxy ketone 40 prepared by desilylation of 39, as the substrate for the epimerization (ref. 26). Thus, 40 was treated with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in THF at room temperature for 2 h, leading to an equilibrium mixture of 40 and 41 in a ratio of ca. 1:2. After separation of this mixture by column chromatography on silica gel, the desired product 41 possessing the correct configuration at the C-8 position was isolated in 64% yield along with the starting material 40 (31% recovery). Since prolonged reaction times caused dehydration of both 40 and 41 to the same enone 42, the reaction was terminated when an approximately 1:2 mixture of 40 and 41 was generated. Finally, reduction of the carbonyl group in 41 with sodium borohydride furnished the eight-membered key intermediate 12 as a single diastereoisomer in 87% yield. The stereochemistry of 12 was unambiguously determined by single crystal X-ray analysis of the related bis(p-bromobenzoate) derivative (ref. 25). The complete stereoselectivity observed for the reduction can be explained by considering that the hydride attack occurred exclusively from the less hindered, outside of the eight-membered ring system (see, 41A).

8. Synthesis of the Bridged Tetracyclic Key Intermediate 10

We next elaborated the bridged tetracyclic key intermediate 10 from the eight-membered compound 12 as shown in Scheme 8. Thus, selective protection of the primary hydroxy group in 12 as its TBDPS ether followed by palladium (0)-catalyzed cleavage of the Alloc group furnished the amine 43 in 59% overall yield. Oxidation of the imino moiety in 43 with m-chloroperbenzoic acid (MCPBA) at low temperature afforded the corresponding N-hydroxylamine (67%), whose hydroxy group was then chemoselectively acetylated to give the acetate 44 in 69% yield. Compound 44 was further converted to the alcohol 45 (77%, 2 steps) by Dess-Martin oxidation followed by deprotection of the TBDPS group. One of the critical steps in our synthetic scheme was envisaged to be the internal hemiacetalization of the N-hydroxylamino ketone 11 generated in situ by removal of the acetyl group in 45, to furnish the requisite tetracyclic compound 10. In the event, removal of the acetyl group in 45 by reaction with potassium carbonate in methanol at room temperature cleanly produced 11, which spontaneously underwent the expected internal hemiacetalization to give 10 (ref. 27) as the sole product in 89% yield from 45.

Scheme 8. Synthesis of the tetracyclic key intermediate 10
9. Completion of the Total Synthesis of Natural (+)-FR900482 (1) and Unnatural (-)-FR900482 (ent-1)

The final route that led to completion of the total synthesis of (+)-FR900482 (1) is summarized in Scheme 9. Thus, the bridged tetracyclic hemiacetal 10 was allowed to react with phosgene dimer (trichloromethyl chloroformate), and the resulting cyclic carbonate 46 (ref. 27) was then subjected to ammonolysis, giving rise to the urethane 47 (ref. 27) in 76% yield for the two steps. Further acetylation of the hydroxy group in 47 afforded the acetate 48 (ref. 27) in 87% yield. The critical removal of the N-Ts protecting group in 48 was found to be effected by employing sodium naphthalenide in 1,2-dimethoxyethane (DME) at -70°C, leading to the aziridine 49 in 84% yield. Both the Bn and BOM protecting groups in 49 were simultaneously deleted by catalytic hydrogenolysis over palladium-carbon, furnishing the benzyl alcohol 50 in 81% yield. Oxidation of the benzyl hydroxy group in 50 to the corresponding formyl group was best accomplished by employing Swern oxidation at -78°C (ref. 28), affording the aldehyde 51 in 88% yield. Finally, deprotection of the acetyl group in 51 was carried out by treatment with ammonia in methanol, producing (+)-FR900482 (1), mp 174°C (dec) [lit., (ref. 1b) mp 175°C (dec)], [α]D23+7.9° (c 0.97, H2O) [lit., (ref. 1b) [α]D23+8.0° (c 1.00, H2O)]. The synthesized 1 was identical with a natural sample of 1 in all spectroscopic properties (IR, 1H-NMR, MS).

By employing ent-15 instead of 15 as the aliphatic segment, unnatural (-)-FR900482 [ent-(-)-1], [α]D23-10° (c 0.02, H2O) was also synthesized in the same manner as described above.

Scheme 9. Completion of the total synthesis of natural (+)-FR900482 (1)

10. In Vitro Cytotoxicity of Enantiomeric Pairs of FR900482 (1 and ent-1) and Its Congeners (50, ent-50, 51, and ent-51)

With completion of the total synthesis of an enantiomeric pair of FR900482 (1 and ent-1), the in vitro cytotoxicity assay against P388 murine leukemia was next carried out by employing 1, ent-1 and its synthetic intermediates (50, ent-50, 51, and ent-51) along with FK973 (2). The IC50 values collected
are shown in Table 1. From these results, it was revealed that the C9-O-acetyl derivatives (50 and 51) exhibit activity superior to 1. The cytotoxicity of 50 and 51 is ca. 7 and 90 times more potent than that of 1, respectively. FK973 exhibited superior cytotoxicity to 1 as expected (ref. 29). These acetyl derivatives (50, 51, and 2) might be more efficiently incorporated in the cells than 1 due to their lipophilic properties. In the cells, these compounds are hydrolyzed by esterase to generate the corresponding deacetyl products (e.g., 1 and 4) which form DNA-DNA interstrand cross-links after bioreductive activation.

Table 1. *In vitro* cytotoxicity of enantiomeric pairs of FR900482 (1) and its congeners against P388 murine leukemia cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µg/ml)*</th>
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<tbody>
<tr>
<td>FR900482(1)</td>
<td>3.3 x 10^-2</td>
</tr>
<tr>
<td>50</td>
<td>4.7 x 10^-3</td>
</tr>
<tr>
<td>51</td>
<td>3.6 x 10^-4</td>
</tr>
<tr>
<td>FK973(2)</td>
<td>9.5 x 10^-4</td>
</tr>
<tr>
<td>ent-1</td>
<td>3.1</td>
</tr>
<tr>
<td>ent-50</td>
<td>3.0 x 10^-1</td>
</tr>
<tr>
<td>ent-51</td>
<td>4.0 x 10^-2</td>
</tr>
</tbody>
</table>

a) Concentration required for 50% inhibition of the cell growth after incubation for 96h at 37°C (initial cell density: 1 x 10^6 cells/ml).

It is noteworthy that 1, 50, and 51 bearing natural absolute configurations were found to be approximately 100 time more cytotoxic than the corresponding enantiomers (ent-1, ent-50, and ent-51) possessing unnatural absolute configurations. This result is quite interesting when compared to the fact that the unnatural enantiomer of mitomycin C (ent-3) displays half of the cytotoxicity of natural 3 (ref. 30). This difference could be rationalized if one speculates as follows. Thus, in the case of 3, bioreduction of the quinoid part to the benzenoid structure followed by mitosene formation with a loss of methanol might occur more readily than 1 and proceed at the almost same reaction rate for both enantiomers (ref. 30). This indicates that the absolute configuration of 3 is not so responsible for the cytotoxic properties. On the contrary, in the case of 1, the natural absolute configuration might provide a key structural feature for incorporation of 1 into the cells, and/or, more possibly, for bioreduction of the N-hydroxy amine moiety of 1 to produce the benzazocine structure which spontaneously cyclized to the mitosene derivative (see, Scheme 1). Further mechanistic studies on alkylation and cross-linking of DNA by employing each enantiomer of 1 should be able to critically test this hypothesis.

11. Conclusion

We have succeeded in completing the first enantioselective total synthesis of both natural (+)- and unnatural (−)-FR900482 (1 and ent-1) in a convergent manner starting from commercially available 5-hydroxyisophthalic acid (16) and each enantiomer of diethyl tartrate (17 and ent-17). The explored synthetic pathway should hold promise for preparing various structural types of FR900482 congeners in enantiomerically pure forms due to its generality and flexibility. The cytotoxicity assay of 1, its synthetic intermediates (50 and 51), and their enantiomers (ent-1, ent-50, and ent-51) disclosed some novel aspects of the structure-activity relationships useful for both investigating the mode of antitumor action of 1 as well as designing novel FR900482 congeners which can exhibit characteristic profiles.

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References and Notes


(22) After purification by column chromatography on silica gel, the aliphatic segment 15 was immediately used for the next coupling reaction due to its instability.
(23) When the corresponding mesylate and iodide were used as substrates for the subsequent coupling reaction with the aromatic segment 14, none of the desired product was obtained and the unreacted starting material was always recovered unchanged. To our delight, the triflate 15 was found to serve as an excellent electrophile for the objective coupling reaction. For recent examples for the usefulness of some triflate derivatives as good electrophiles, see, a) Fairbanks, A. J.; Fleet, G. W. J. Tetrahedron, 1995, 51, 3881. b) Takao, K.; Ochiai, H.; Yoshida, K.; Hasizuka, T.; Koshimura, H.; Tadano, K.; Ogawa, S. J. Org. Chem. 1995, 60, 8179-8193.
(25) In the preliminary synthetic studies on 1, we had prepared the 1H-azirino[2,3-c][1]benzazocine i protected differently from 12. The structure of i was determined by single crystal X-ray analysis of its bis (p-bromobenzoate)derivative. Comparison of the 1H-NMR spectra of 12 and i rigorously established identity of their stereostructures. Details of X-ray crystallographic study will be reported in a separate paper.

![Diagram of compound i]

(26) A related base-catalyzed epimerization has been reported for the synthesis of 9-epi-mitomycin B, see, Kasai, M.; Kono, M.; Shirahata, K. J. Org. Chem. 1989, 54, 5908.
(27) The stereochemistries at the C-9 position of the bridged tetracyclic compounds 10, 46-48 were proven by NOE measurements in their 400MHz 1H-NMR spectra. Thus, NOEs between the signals due to C9-H and C9a-H in 10, 46, 47, and 48 were found to be 3.1%, 3.2%, 3.3%, and 2.9%, respectively. Based on these results, their stereochemistries could be rigorously assigned as depicted. A related assignment of the C-9 stereochemistries has been reported for the structure determination of FR900482(1) (ref. 4).
(28) Other standard oxidizing reagents [e.g., manganese(IV) oxide (MnO2), Collins reagent (CrO3·2Py), pyridinium chlorochromate (PCC), pyridinium dichromate (PDC), Dess-Martin periodinane, dimethylsulfoxide/sulfur trioxide pyridine complex (DMSO/So3·Py), tetra-n-propylammonium perruthenate (TPAP), 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), etc.] gave complicated mixtures of the products probably due to chemical instability of the naked aziridine functionality in 50 and/or 51 under these conditions.
(29) It has been reported that the cytotoxicity of FK973 (2) against L1210 murine leukemia cells is ca. 10 time more potent than that of FR900482 (1), see, Masuda, K.; Suzuki, A.; Nakamura, T.; Takagi, S.; Noda, K.; Shinomura, K.; Noguchi, H.; Shibayama, F. Japan, J. Pharmacol. 1989, 51, 219.
(30) Studies on alkylation and cross-linking reaction of DNA by natural mitomycin C (3) and unnatural ent-mitomycin C (ent-3) have been reported, see, Gargiulo, D.; Musser, S. S.; Yang, L.; Fukuyama, T.; Tomasz, M. J. Am. Chem. Soc. 1995, 117, 9388.

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