gel electrophoresis (SDS-PAGE); the gels showed no major impurities, only a multitude of very minor impurity bands. Routine assays were conducted by monitoring tritium wash-out from [2,3-^{3}H]-γ-butyrobetaine by a modification of the method of Englard et al.[34] Assay mixtures (pH 7.0) contained an aliquot of enzyme solution, 0.5 μmol of [2,3-^{3}H]-γ-butyrobetaine, 0.75 μmol of α-ketoglutarate, 4.3 μmol of ascorbate, 0.5 μmol of ferrous sulfate, 1 mg of catalase, and 12.5 μmol of potassium phosphate in a final volume of 0.25 mL. All assay components except the radiolabeled substrate were mixed in open test tubes (13 × 100 mm) and preincubated for 3 min at 37 °C with rotary shaking (160 rpm). The substrate was added, and the incubation was continued for 45 min. The reaction was terminated by addition of 2,2'-bipyridyl (50 μL of a 0.5 M ethanolic solution). The mixture was applied to a small column (1-mL total volume) of Dowex 50X8-400 (H^+), and the column was eluted with 1.5 mL of water. Aliquots of the effluent were then analyzed for tritium radioactivity. In determination of the enzyme activity, two correction factors must be applied to account for the fact that (a) only one-fourth of the tritium in the labeled substrate is in the 3*R* position, which is the site of hydroxylation, and (b) there is a small primary tritium kinetic isotope effect for the hydroxylation, $^T(V/K) = 1.5$.[10] Under these conditions our most active preparations of BB hydroxylase showed a specific activity of 6.1 μmol min^{-1} mg^{-1}. More typically, the specific activity was about 2 μmol min^{-1} mg^{-1}, but there was no difference in the purity of the various preparations as judged by SDS-PAGE.

Reversible Inhibition Studies. For the determination of reversible inhibition of BB hydroxylase by substrate analogues, a series of routine assays were performed that contained various concentrations of [2,3-^{3}H]-γ-butyrobetaine and various concentrations of the potential inhibitor. For these assays, all components except the substrate and inhibitor were mixed and preincubated as described above, and then the inhibitor was added, followed immediately by the substrate to initiate the reaction. In this way the inhibitors could have no effect on the activation of the enzyme during the preincubation period. The remainder of the assay procedures were as above, and the inhibition data were analyzed in the form of Lineweaver-Burk plots.[19]

Time-Dependent Inactivation Studies. For the determination of time-dependent inactivation of BB dioxygenase by substrate analogues, the following procedure was used. A series of reaction mixtures were prepared, each of which contained (at pH 7.0) 100 μg of BB dioxygenase, 1.2 μmol of α-ketoglutarate, 4.5 μmol of ascorbate, 0.6 μmol of ferrous sulfate, 1 mg of catalase, and 50 μmol of potassium phosphate in a final volume of 0.28 mL. After preincubation for 3 min at 37 °C with rotary shaking (160 rpm), 20-μL aliquots of inhibitor stock solutions of appropriate concentrations were added. The incubations were continued, and at 10-min intervals aliquots were taken from the mixtures for the assay of remaining enzyme activity by using the standard routine assay procedure.

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Registry No. [^{3}H]-**1**, 124201-55-4; **3**, 24499-80-7; [^{2}H]-**3**, 124201-56-5; [^{3}H]-**3**, 124201-57-6; **4**, 124201-52-1; **5**, 124201-53-2; **6**, 124201-54-3; D_2, 7782-39-0; T_2, 10028-17-8; γ-amino[2,3-^{3}H]butyric acid, 13048-68-5; diethyl malonate, 105-53-3; 3,3-dimethylbutanal, 2987-16-8; methyl 5-methyl-4-hexenoate, 35901-76-9; BB hydroxylase, 9045-31-2.

(34) Englard, S.; Horwitz, L. J.; Mills, J. T. *J. Lipid Res.* **1978**, *19*, 1057.

Man-Designed Bleomycin with Altered Sequence Specificity in DNA Cleavage†

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Abstract: The synthetic approach to the concerted antitumor mechanism of bleomycin is studied by introducing a dynamic change into the O_2-activation moiety and DNA-binding site. A model PYML(6)-bleomycin previously reported, possessing an oxygen-activating methoxypyridine moiety and a DNA-binding bithiazole moiety, exhibits a nucleotide cleavage mode virtually identical with that of bleomycin. Herein reported is a newly designed bleomycin analogue, PYML(6)-(4*R*-APA)-distamycin, wherein the 4-methoxypyridine moiety and a DNA-binding distamycin component are connected through an (*R*)-4-aminopentanoic acid linker moiety. Synthesis of PYML(6)-(4*R*-APA)-distamycin is carried out by condensation of the hydroxyhistidine-pentanoic acid fragment with the methoxypyridine moiety, followed by introduction of the distamycin moiety. PYML(6)-(4*R*-APA)-distamycin cleaves a G4 phage DNA fragment (100 base pairs) at 1 μM concentration in the presence of Fe(II), oxygen, and dithiothreitol and induces dramatically altered adenine/thymine specificity. It is indicated that the specific recognition of base sequences for the cleavage is mainly controlled by the DNA affinity site and that the (*R*)-4-aminopentanoic acid linker seems to determine the proper arrangement of the iron-oxygen site and the distamycin moiety on DNA.

Bleomycins (BLMs) are chemotherapeutic agents used for the clinical treatment of Hodgkin's lymphoma, carcinomas of the skin, head, and neck, and tumors of the testis.[1] The drug was isolated from *Streptomyces verticillus* as a copper chelate by Umezawa and his co-workers in 1966, and the structure was shown to be a glycopeptide consisting of an unusual hexapeptide and a disaccharide.[2] In addition to its clinical usefulness, BLM attracts current interest because of its unique biochemical functions. It has been well documented that BLM cleaves DNA preferentially at G-C (5′ → 3′) and G-T (5′ → 3′) sequences in the presence of oxygen and ferrous ion.[3] The biochemical capability of BLM

† Synthetic Studies on Antitumor Antibiotic, Bleomycin. 27. Part 26: Kaku, Y.; Otsuka, M.; Ohno, M. *Chem. Lett.* **1989**, 611.

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(1) Umezawa, H.; Maeda, K.; Takeuchi, T.; Okami, Y. *J. Antibiot., Ser. A* **1966**, *19*, 20.

(2) Takita, T.; Muraoka, Y.; Nakatani, T.; Fujii, A.; Umezawa, Y.; Naganawa, H.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 801.

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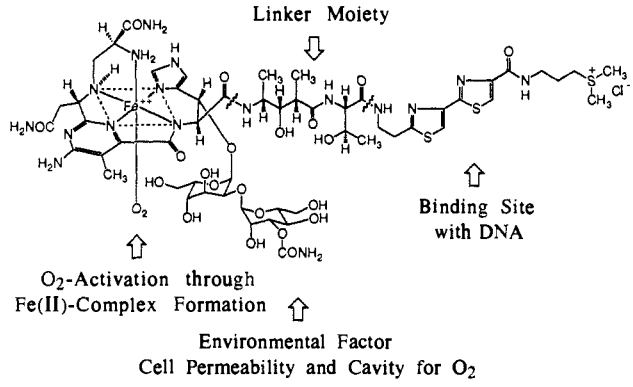


Figure 1. Proposed structure of BLM–Fe(II)–O_2 and assumed role of each functional moiety.

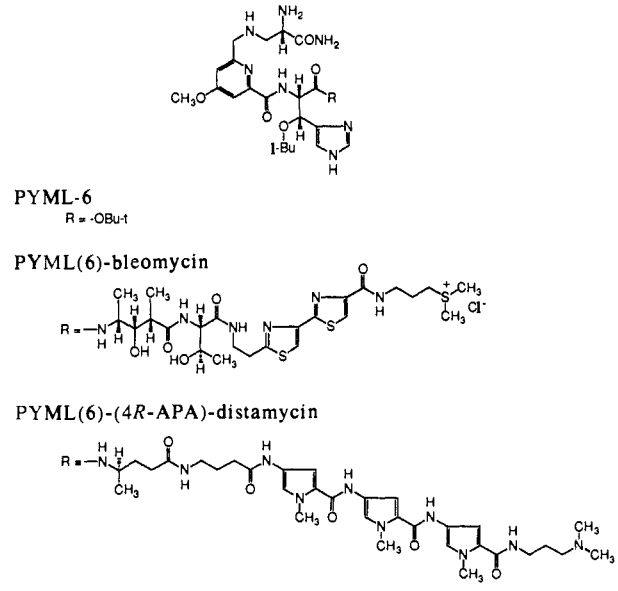


Figure 2. Synthetic analogues of BLM.

appears to be associated with the functions of the following structural units: (i) an iron-binding site (amine–pyrimidine–imidazole region) to activate dioxygen, (ii) a disaccharide moiety to facilitate the iron–oxygen complex formation and membrane transport, (iii) a DNA affinity site (bithiazole–terminal amine region) to interact with certain sequences of nucleotides, and (iv) a linker moiety to combine each part of BLM at an appropriate distance and in a good orientation (Figure 1).

In our continuing study toward man-designed BLMs, we demonstrated that the 4-aminopyrimidine nucleus and the disaccharide of BLM can be replaced by a 4-methoxypyridine and a *tert*-butyl group, respectively, and that such a synthetic model, PYML(6), was able to activate molecular oxygen as efficiently as BLM (Figure 2).[4] Furthermore, a BLM-type DNA affinity site was coupled with PYML(6) to afford a DNA-cleaving molecule, PYML(6)–bleomycin, which showed a nucleotide sequence cleavage mode virtually identical with that of BLM in vitro (Figure 2).[5] Since PYML(6) showed no significant DNA-cleaving activity, the bithiazole moiety seemed to contribute mainly to the binding to DNA by recognizing the base sequence, whereas

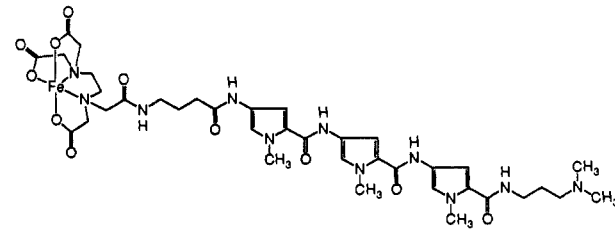
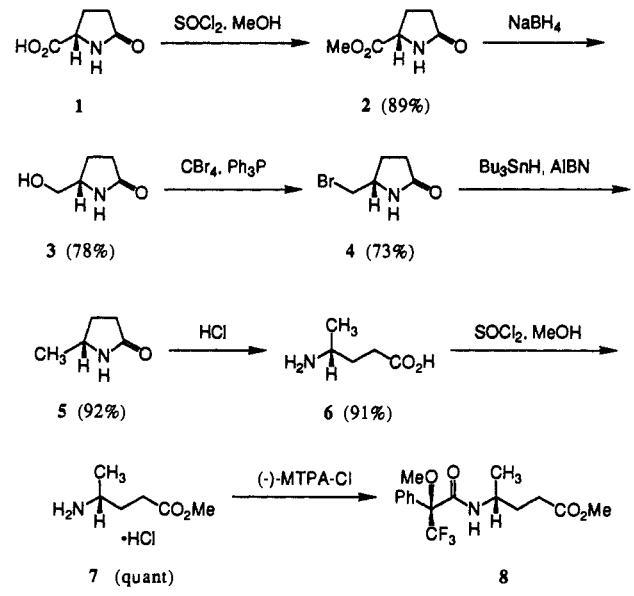


Figure 3. Distamycin–EDTA–Fe(II).

Scheme I



the sugar moiety is not directly concerned with recognition of the base sequence. In fact, a DNA-cleavage inhibition analysis and computer model-building study indicated that metallo-BLM binds in the minor groove of B-DNA, probably by the formation of hydrogen bonds between the nitrogen atoms in the bithiazole moiety and the 2-amino group of the guanine base.[6] On the other hand, a DNA-cleaving molecule of entirely different structure, hemin–intercalator, reported by Shudo et al., also exhibited a DNA-cleaving pattern remarkably similar to that of BLM.[7] Thus it remains an alternate possibility that certain nucleotide residues are intrinsically vulnerable and sensitive to the activated oxygens generated by such iron complexes. In order to clarify whether the sequence specificity is actually dependent upon the DNA-binding site or not, we studied the relationships between the DNA-cleavage pattern and the DNA-binding moiety by modifying our synthetic model.

First, we considered the possibility that the G specificity of PYML(6)–bleomycin could be altered by replacing its bithiazole moiety with repeating *N*-methylpyrrole subunits, as envisaged in the structures of distamycin or netropsin, whose binding to A/T-rich nucleotide sequences has been unequivocally demonstrated by X-ray studies.[8] The base specificity of distamycin or netropsin was explained by van der Waals interaction between the CH group of the *N*-methylpyrrole ring and the adenine C2 hydrogens.[8] Furthermore, distamycin serves as a potential DNA affinity site with AT specificity, as seen in the ingenious design of distamycin–EDTA–Fe(II) by Dervan (Figure 3).[9] Distamycin–EDTA–Fe(II) reagent was shown to cleave double-helical nucleotide sequences adjacent to 5-base-pair AT recognition sites, and the

(3) (a) Sugiura, Y.; Takita, T.; Umezawa, H. In *Metal Ions in Biological Systems*; Sigel, H., Ed.; Marcel Dekker: New York, 1985; pp 81–108. (b) Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 383. (c) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107.

(4) (a) Sugano, Y.; Kittaka, A.; Otsuka, M.; Ohno, M.; Sugiura, Y.; Umezawa, H. *Tetrahedron Lett.* **1986**, *27*, 3635. (b) Kittaka, A.; Sugano, Y.; Otsuka, M.; Ohno, M. *Tetrahedron* **1988**, *44*, 2811.

(5) (a) Otsuka, M.; Kittaka, A.; Ohno, M.; Suzuki, T.; Kuwahara, J.; Sugiura, Y.; Umezawa, H. *Tetrahedron Lett.* **1986**, *27*, 3639. (b) Ohno, M.; Otsuka, M.; Kittaka, A.; Sugano, Y.; Sugiura, Y.; Suzuki, T.; Kuwahara, J.; Umezawa, K.; Umezawa, H. *Int. J. Exp. Clin. Chemother.* **1988**, *1*, 12. (c) Kittaka, A.; Sugano, Y.; Otsuka, M.; Ohno, M. *Tetrahedron* **1988**, *44*, 2821.

(6) Kuwahara, J.; Sugiura, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 2459.

(7) Hashimoto, Y.; Iijima, H.; Nozaki, Y.; Shudo, K. *Biochemistry* **1986**, *25*, 5103.

(8) (a) Coll, M.; Frederick, C. A.; Wang, A. H.-J.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8385. (b) Kopka, M. L.; Yoon, C.; Goodshell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376.

(9) (a) Dervan, P. B. *Science* **1986**, *232*, 464. (b) Sluka, J. P.; Horvath, S. J.; Bruist, M. F.; Simon, M. I.; Dervan, P. B. *Science* **1987**, *238*, 1129.

cleavage takes place at contiguous multiple bases, presumably because of the diffusible nature of the oxidizing species.

On the other hand, natural BLM has (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylpentanoic acid as a linker between the metal-binding site and the bithiazole moiety. Although the exact role of the linker moiety remains to be clarified, it is considered that the preferred conformation of this moiety may contribute to making the flexible BLM molecule rather inflexible in the twisted minor groove of DNA. Umezawa et al. extensively studied modification of the linker moiety and clearly demonstrated that a modified BLM possessing a simplified (*R*)-4-aminopentanoic acid linker [4*R*-APA (**6**); see Scheme I] shows outstanding DNA-cleaving activity comparable to that of natural BLM.[10] Their results indicated that the 4*R* configuration is particularly important among the three consecutive 2*S*,3*S*,4*R* asymmetric centers in the linear amino acid.

Therefore, we designed PYML(6)–(4*R*-APA)–distamycin, which has a PYML(6) metal core, a distamycin moiety, and a 4*R*-APA linker (Figure 2).

Results

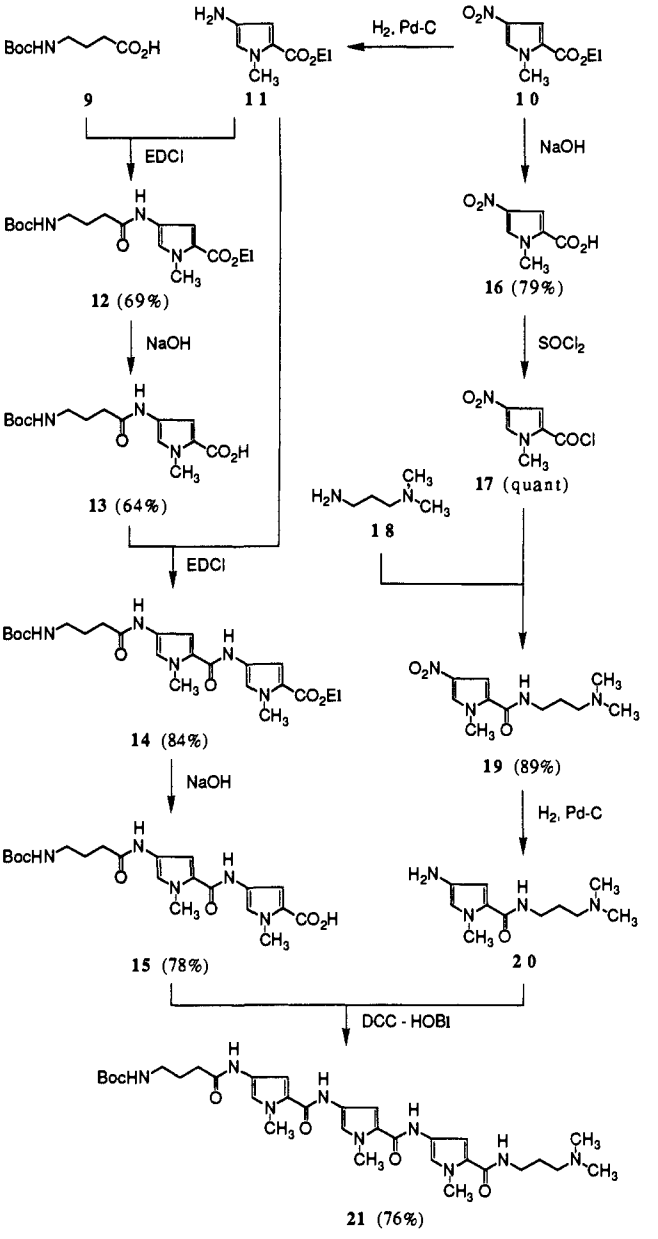
Synthesis of PYML(6)–(4*R*-APA)–Distamycin. The 4-methoxypyridine moiety and *erythro*-β-hydroxy-L-histidine part were previously prepared in the synthesis of PYML(6) and PYML(6)–bleomycin.[4,5]

4*R*-APA was previously synthesized by Saito by Wittig reaction of an aldehyde derived from D-alanine.[11] We prepared 4*R*-APA (**6**) by a different and practical route involving a chiral γ-lactam **5**, easily obtained according to the procedure of Ringdahl,[12] starting with commercially available L-pyroglutamic acid (**1**; Scheme I). The ring opening of γ-lactam **5** was accomplished by Saito's procedure.[11] The optical purity of 4*R*-APA (**6**) thus obtained was shown to be better than 99% ee on the basis of NMR measurement of the corresponding MTPA[13] derivative **8**.

The distamycin moiety was prepared by a modification of Dervan's synthesis[14] (Scheme II). Ethyl 4-nitro-1-methylpyrrole-2-carboxylate (**10**) was hydrogenated to give amine **11**, which was coupled with γ-aminobutyric acid derivative **9** mediated by 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDCI).[15] The ester **12** obtained in 69% yield was saponified and again coupled with amine **11** by the EDCI method, affording the bispyrrole ester **14** in 84% yield. On the other hand, the starting ester **10** was saponified to give acid **16** in 79% yield. The subsequent peptide condensation with 3-(*N*,*N*-dimethylamino)propylamine (**18**) was accomplished by the acyl chloride method, and peptide **19** was obtained in 89% yield. The nitro group of **19** was reduced to afford amine component **20** in 89% yield. The bispyrrole acid **15**, obtained in 78% yield by saponification of **14**, and the monopyrrole amine **20** were coupled by the DCC–HOBt method to give the Boc-protected distamycin moiety **21** in 76% yield.

The components thus obtained were connected to each other as follows (Scheme III). *erythro*-β-Hydroxy-L-histidine (**22**)[16] was treated with di-*tert*-butyl dicarbonate[17] to give the corresponding bis-Boc derivative **23**[4b] in 91% yield. Coupling with 4*R*-APA methyl ester (**7**) was effected by the DCC–HOBt procedure, furnishing dipeptide **24** in 67% yield. The Boc protection in **24** was replaced by a Z group by standard procedures in 31% yield, and a *tert*-butyl group was introduced by isobutene to give fragment **26** in 83% yield. Removal of the Z group of **26** was accomplished quantitatively with Pd-black and formic acid. Condensation of the 4-methoxypyridine moiety **28**[5c] with fragment **27** was effected with diphenyl phosphoroazidate (DPPA)[18] to give **29** in 77% yield, and subsequent saponification afforded fragment **30** in 56% yield. On the other hand, the distamycin amine **31** was quantitatively prepared by treatment of the Boc derivative **21** with trifluoroacetic acid. Coupling of the amine component **31** with the acid fragment **30** was facilitated by DPPA to give the fully protected precursor **32**. Conventional conditions for removal of the (*o*-nitrophenyl)thio (Nps) group[19] (e.g., dilute HCl or HBr in the presence of skatole) were not successfully applied to **32** due to the facile decomposition of the distamycin residue. After extensive attempts, the deprotection was finally accomplished with 5% trifluoroacetic acid in dichloromethane to give PYML-

Scheme II



(10) (a) Umezawa, H.; Takita, T.; Saito, S.; Muraoka, Y.; Takahashi, K.; Ekimoto, H.; Minamide, S.; Nishikawa, K.; Fukuoka, T.; Nakatani, T.; Fujii, A.; Matsuda, A. In *Bleomycin Chemotherapy*; Sikic, B. I., Rozenweig, M., Carter, S. K., Eds.; Academic Press: Orlando, FL, 1985; p 289. (b) Muraoka, Y.; Saito, S.; Nogami, T.; Umezawa, K.; Takita, T.; Takeuchi, T.; Umezawa, H.; Sakata, N.; Hori, M.; Takahashi, K.; Ekimoto, H.; Minamide, S.; Nishikawa, K.; Kuramochi, H.; Motegi, A.; Fukuoka, T.; Nakatani, T.; Fujii, A.; Matsuda, A. In *Horizons on Antibiotic Research*; Davis, B. D., Ichikawa, T., Maeda, K., Mitscher, L. A., Eds.; Japan Antibiotic Research Association: Tokyo, 1988; p 88.

(11) Saito, S. Dissertation, University of Tokyo, 1984.

(12) Amstutz, R.; Ringdahl, B.; Karlèn, B.; Roch, M.; Jenden, D. J. *J. Med. Chem.* **1985**, *28*, 1760.

(13) (a) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2453. (b) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.

(14) Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457.

(15) Sheehan, J. C.; Preston, J.; Cruickshank, P. A. *J. Am. Chem. Soc.* **1965**, *87*, 2492.

(16) (a) Owa, T.; Otsuka, M.; Ohno, M. *Chem. Lett.* **1988**, *83*. (b) Owa, T.; Otsuka, M.; Ohno, M. *Chem. Lett.* **1988**, 1873.

(17) Tarbell, D. S.; Yamamoto, Y.; Pope, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 630.

(18) Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203.

(19) Zervas, L.; Borovas, D.; Gazis, E. *J. Am. Chem. Soc.* **1963**, *85*, 3660.

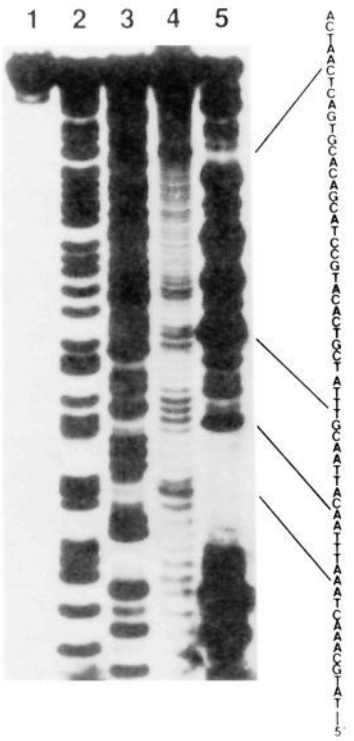


Figure 4. Nucleotide-specific cleavage of the 5′-end-labeled G4 phage DNA fragment (100 base pairs) by PYML(6)–(4*R*-APA)–distamycin (lane 4) and natural BLM (lane 5). Lane 1 shows the DNA blank. Lanes 2 and 3 show the Maxam–Gilbert sequencing reactions for A + G and C + T, respectively.

(6)–(4*R*-APA)–distamycin in 30% yield.

DNA Cleavage. Sequence-specific cleavage with PYML-(6)–(4*R*-APA)–distamycin was examined by using a 5′-end ^{32}P-labeled G4 phage DNA fragment (100 base pairs) which contains AT bases in relatively high frequency. The cleavage was carried out aerobically in the presence of dithiothreitol, and the result is shown in the autoradiogram of the Maxam–Gilbert gel (Figure 4). Lanes 4 and 5 show the cleavage pattern of PYML(6)–(4*R*-APA)–distamycin and natural BLM, respecively. Whereas BLM mainly cleaved non-AT sites, PYML(6)–(4*R*-APA)–distamycin evidently cleaved A/T-rich regions preferentially.

The same G4 phage DNA fragment was labeled at the 3′-end and cleaved with PYML(6)–(4*R*-APA)–distamycin. AT specificity was observed in the autoradiogram of the Maxam–Gilbert gel (Figure 5). A histogram was taken from the autoradiogram, and the cleavage pattern for the 3′-end-labeled fragment was compared to that for the 5′-end-labeled strand. Figure 6 clearly shows asymmetric cleavage patterns on opposite strands, with the cleaved sites of each strand shifted to the 3′ side.

Discussion

It is evident that the DNA cleavage is not an outcome of random molecular collision between BLM and DNA. The efficiency of the DNA cleavage should be regarded as a sum of both the oxygen-activating power and the magnitude of DNA affinity. DNA-cleaving activity of PYML(6)–(4*R*-APA)–distamycin was found to be more potent than that of PYML(6)–bleomycin, and a dramatic change in the base sequence specificity was observed. Obviously, PYML(6)–(4*R*-APA)–distamycin predominantly cleaved A/T-rich regions that were not cleaved by BLM (Figures 4 and 5), and the cleavage pattern is in accordance with the finding by Dervan et al. that distamycin covers 5 base pairs at A/T-rich regions.[9] In addition, the present asymmetric cleavage pattern to the 3′ side on opposite strands suggests interaction of the compound PYML(6)–(4*R*-APA)–distamycin with the minor groove of DNA (Figure 6).[9b] It should also be noted that, whereas iron is placed *next to* the site of DNA binding in the DNA scission with Dervan's distamycin–EDTA–Fe(II),[9] it appears that

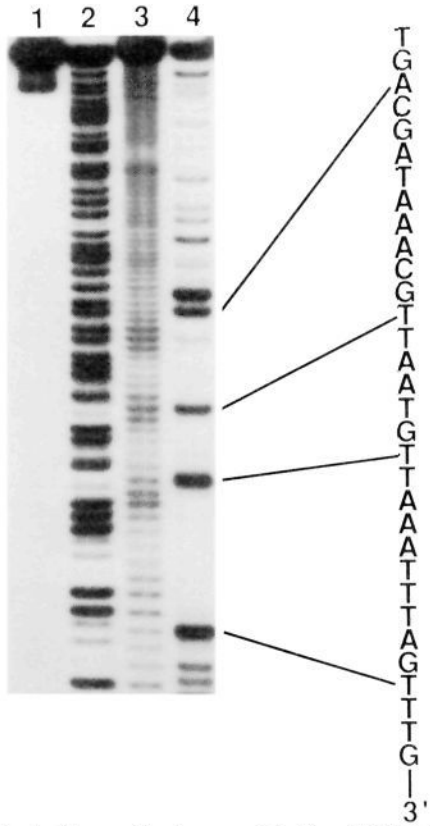


Figure 5. Nucleotide-specific cleavage of the 3′-end-labeled G4 phage DNA fragment (100 base pairs) by PYML(6)–(4*R*-APA)–distamycin (lane 3) and natural BLM (lane 4). Lanes 1 and 2 show the DNA blank and the Maxam–Gilbert sequencing reaction for A + G, respectively.

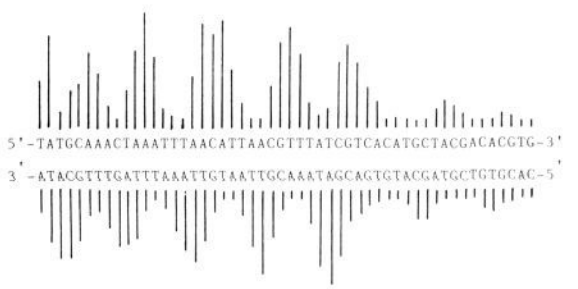


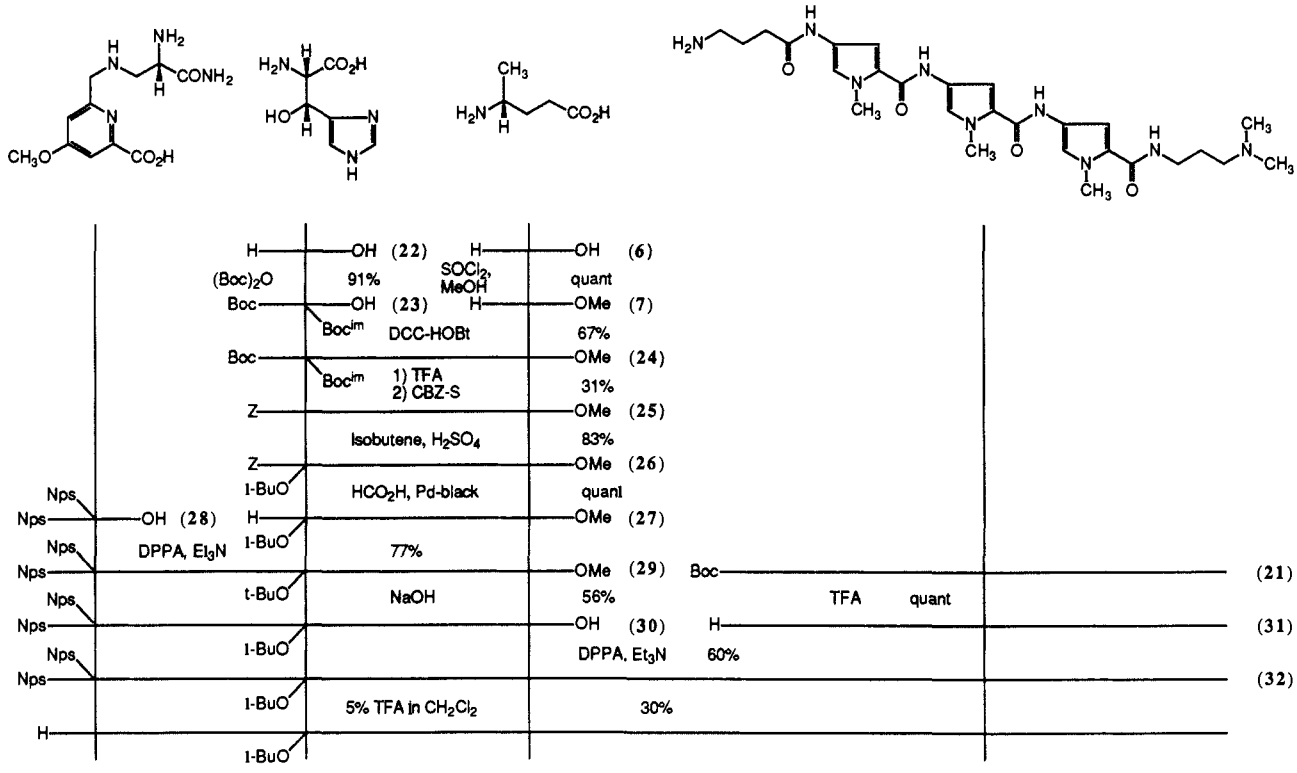
Figure 6. Histogram of cleavage sites by PYML(6)–(4*R*-APA)–distamycin for the G4 phage DNA fragment (100 base pairs). Relative DNA cleavage frequencies were obtained from densitometric scans of the gel autoradiograms.

PYML(6)–(4*R*-APA)–distamycin cleaves the site of DNA binding itself (Figure 5). This seems to be due to the conformation and the chain length of the linker moiety, which determine the placement of the metal–oxygen site relative to the location of the distamycin moiety on DNA. In fact, inspection of a molecular model of man-made BLM strongly suggests that the molecule is bending at the 4*R*-APA linker to orient the metal center just at the observed cleavage site. As the oxygen species activated by BLM appears to be nondiffusible and bound to iron, the sites of cleavage are indicative of the location of the iron–oxygen moiety.

Hénichart et al.[20] and Saito et al.[21] independently reported simpler BLM models consisting of an ethylenediamine–pyridine–histidine chelation site, a γ-aminobutyric acid–glycine linker, and a DNA affinity site. Although nicking of supercoiled DNA by an iron complex and photochemical cleavage of DNA by a

(20) Kenani, A.; Lohez, M.; Houssin, R.; Helbecque, N.; Bernier, J. L.; Lemay, P.; Hénichart, J. P. *Anti-Cancer Drug Des.* **1987**, *2*, 47.
(21) Saito, I.; Morii, T.; Obayashi, T.; Sera, T.; Sugiyama, H.; Matsuura, T. *J. Chem. Soc., Chem. Commun.* **1989**, 360.

Scheme III



cobalt complex were demonstrated, the Maxam–Gilbert sequencing analysis showed that all bases were cleaved without any selectivity.[20,21] Considering that the structures of these models are much simplified compared with our man-made BLMs, it appears that a highly advanced structure with complicated stereochemistry is necessary to attain specificity in DNA cleavage. We have demonstrated that the DNA affinity site with the proper linker moiety is crucial for the PYML ligand to manifest sequence specificity in DNA scission and believe that the present study provides a basis for the molecular design of novel BLM homologues with altered antitumor properties.

Experimental Section

Melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP 140 instrument. ^{1}H NMR (400 MHz) spectra were recorded on a JEOL GX-400 spectrometer. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). IR spectra were recorded on a JASCO DS-402G or JASCO A-102 spectrometer. Fast atom bombardment mass spectra (FABMS) were recorded on a JEOL JMS DX-300 spectrometer. Reagents and solvents were purified by standard procedures.

L-Pyroglutamic Acid Methyl Ester (2). A solution of L-pyroglutamic acid (**1**) (25.37 g, 196 mmol) in MeOH (220 mL) was cooled in a dry ice–MeOH bath. Thionyl chloride (16 mL, 221 mmol) was added over 15 min to the solution at −25 to −20 °C. The mixture was allowed to warm to room temperature and stirred for further 1.5 h. The solvent of the resulting mixture was removed in vacuo, and the residue was distilled under reduced pressure to give **2** as a colorless oil (25.07 g, 89.1%): bp 139–140 °C (2 mmHg) [lit. bp 148 °C (1 mmHg),[12] 136–138 °C (2 mmHg)[22]]; IR (neat) 3230, 1735, 1690 cm^{-1}; NMR ($CDCl_3$) δ 2.00–2.70 (4 H, m, CH_2CH_2), 3.80 (3 H, s, CH_3O), 4.20–4.40 (1 H, m, CH), 7.13 (1 H, br s, NH).

(*S*)-5-(Hydroxymethyl)-2-pyrrolidinone (3). $NaBH_4$ (6.72 g, 178 mmol) was added to an ice-cooled solution of **2** (25.04 g, 175 mmol) in EtOH (250 mL). After being stirred for 2 h at room temperature, the solution was cooled with ice, acidified with concentrated HCl, and concentrated in vacuo. The residue was suspended in $CHCl_3$ and filtered through celite, and the filtrate was concentrated in vacuo. The resulting pale yellow oil was purified by chromatography on silica gel (eluted with $CHCl_3$:MeOH = 20:1) to give **3** as colorless prisms (15.71 g, 78.0%): mp 86–87 °C (lit. mp 65–68 °C,[12] 72–73 °C,[22] 66–68 °C[23]); $[\alpha]^{20.0}_D$ +34.5° (c = 1.04, EtOH) {lit. $[\alpha]^{22}_D$ +29° (c = 1, EtOH),[12] $[\alpha]^{20}_D$ +32.4° (c = 1.76, EtOH),[22] $[\alpha]^{20}_D$ +29° (c = 5, EtOH)[23]}; IR (KBr) 3202, 1663 cm^{-1}; NMR ($CDCl_3$) δ 1.50–2.60 (4 H, m, CH_2CH_2), 3.10–4.00 (3 H, m, $HOCH_2CH$), 4.60 (1 H, br t, J = 5 Hz, OH), 7.50 (1 H, br s, NH).

(*S*)-5-(Bromomethyl)-2-pyrrolidinone (4). A solution of CBr_4 (45.37 g, 137 mmol) in CH_3CN (120 mL) was added to an ice-cooled suspension of **3** (15.01 g, 130 mmol) and Ph_3P (35.88 g, 137 mmol) in CH_3CN (250 mL) under an argon atmosphere. The mixture was allowed to warm to room temperature, stirred overnight at the same temperature, and concentrated in vacuo. Water (400 mL) and hexane (400 mL) were added to the residue, and the colorless crystals deposited were removed by filtration. NaCl was added to the aqueous layer, which was extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated in vacuo. The residual colorless crystals were purified by chromatography on silica gel (eluted with $CHCl_3$:MeOH = 20:1) to afford **4** as colorless prisms (16.89 g, 72.8%): mp 73–76 °C (lit. mp 69–72 °C,[12] 71–74 °C[23]); $[\alpha]^{20.0}_D$ −28.3° (c = 1.02, EtOH) {lit. $[\alpha]^{22}_D$ −32° (c = 1, EtOH),[12] $[\alpha]^{22}_D$ −33° (c = 5, EtOH)[23]}; IR (KBr) 3264, 1686 cm^{-1}; NMR ($CDCl_3$) δ 1.70–2.70 (4 H, m, CH_2CH_2), 3.42 (2 H, d, J = 6 Hz, $BrCH_2CH$), 4.00 (1 H, distorted q, J = 6 Hz, CH), 7.25 (1 H, br s, NH).

(*R*)-5-Methyl-2-pyrrolidinone (5). Tributyltin hydride (29 mL, 108 mmol) was added to a soluton of **4** (16.02 g, 90.0 mmol) and AIBN (0.1414 g, 0.861 mmol) in toluene (140 mL) under an argon atmosphere. The solution was stirred at 80 °C for 5 h and concentrated in vacuo. The oily residue was purified by chromatography on silica gel (eluted with CH_2Cl_2:MeOH = 10:1). The colorless oil obtained was distilled under reduced pressure to afford **5** as a colorless oil (8.18 g, 91.7%): bp 89 °C (6 mmHg) [lit. bp 66 °C (3 mmHg)[12]]; $[\alpha]^{22.0}_D$ +17.2° (c = 1.02, EtOH) {lit. $[\alpha]^{22}_D$ +16.6° (c = 1, EtOH)[12]}; IR (neat) 3244, 1685 cm^{-1}; NMR ($CDCl_3$) δ 1.24 (3 H, d, J = 6 Hz, CH_3CH), 1.40–2.50 (4 H, m, CH_2CH_2), 3.81 (1 H, distorted q, J = 6 Hz, CH), 6.90 (1 H, br s, NH).

(*R*)-4-Aminopentanoic Acid (4*R*-APA) (6). A solution of **5** (7.88 g, 79.5 mmol) in 6 N HCl (120 mL) was heated at reflux for 8 h and concentrated in vacuo. The residue was purified by chromatography on Amberlite IR 120B (120 mL), washed with water, and then eluted with 2 N NH_3. Ninhydrin-positive fractions were collected and concentrated in vacuo to give colorless crystals that were recrystallized from MeOH, affording **6** as colorless prisms (8.44 g, 90.6%): mp 212 °C (lit. mp 209 °C[11]); $[\alpha]^{22.0}_D$ +13.6° (c = 1.05, H_2O) {lit. $[\alpha]^{21}_D$ +11.8° (c = 1, H_2O)[11]}; IR (KBr) 3043, 1577 cm^{-1}; NMR (D_2O) δ 1.30 (3 H, d, J = 7 Hz, CH_3CH), 1.60–2.50 (4 H, m, CH_2CH_2), 3.40 (1 H, sextet, J = 7 Hz, CH).

Methyl (*R*)-4-Aminopentanoate Hydrochloride (7). Thionyl chloride (0.04 mL, 0.55 mmol) was added dropwise to MeOH (1 mL) cooled with

(22) Saijo, S.; Wada, M.; Himizu, J.; Ishida, A. *Chem. Pharm. Bull.* **1980**, *28*, 1449.

(23) Silverman, R. B.; Levy, M. A. *J. Org. Chem.* **1980**, *45*, 815.

ice-NaCl. To the resulting solution was added **6** (20.0 mg, 0.171 mmol). The mixture was allowed to warm to room temperature, stirred for 3 days, and concentrated in vacuo to give **7** as a colorless oil (30.2 mg, quantitative): NMR ($CDCl_3$) δ 1.47 (3 H, distorted d, J = 4 Hz, CH_3CH), 1.70–2.80 (4 H, m, CH_2CH_2), 3.30–3.80 (1 H, m, CH), 3.71 (3 H, s, CH_3O), 8.45 (3 H, br s, NH_3^+).

Methyl (*R*)-4-[*N*-[(*S*)-2-Methoxy-2-(trifluoromethyl)-2-phenylacetyl]amino]pentanoate (8). (*R*)-2-Methoxy-2-(trifluoromethyl)-phenylacetyl chloride[13] (0.05 mL, 0.27 mmol) was added to a solution of **7** (30.2 mg, 0.171 mmol) in pyridine (0.5 mL) under an argon atmosphere. After the solution was stirred overnight, 3-(*N*,*N*-dimethylamino)propylamine (0.04 mL) and ether were successively added. The resulting mixture was washed with cold 2 N HCl, water, and saturated $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo to afford **8** as colorless crystals (81.4 mg, quantitative): NMR ($CDCl_3$) δ 1.22 (3 H, d, J = 6.6 Hz, CH_3CH), 1.70–1.90 (2 H, m, CH_2), 2.20–2.40 (2 H, m, CH_2), 3.43 (3 H, distorted s, OCH_3), 3.65 (3 H, s, CO_2CH_3), 4.00–4.15 (1 H, m, CH), 6.78 (1 H, br d, J = 6.6 Hz, NH), 7.35–7.65 (5 H, m, arom).

Determination of Optical Purity of 4-Aminopentanoic Acid (6). Methyl (*RS*)-4-[*N*-[(*S*)-2-methoxy-2-(trifluoromethyl)-2-phenylacetyl]amino]pentanoate was prepared from racemic 4-aminopentanoic acid according to the same procedure as described above: NMR ($CDCl_3$) δ 1.18 ($^3/_2$ H, d, J = 6.6 Hz, *S*-CH_3CH), 1.22 ($^3/_2$ H, d, J = 6.6 Hz, *R*-CH_3CH), 1.70–1.95 (2 H, m, CH_2), 2.20–2.45 (2 H, m, CH_2), 3.40 ($^3/_2$ H, distorted s, *S*-OCH_3), 3.43 ($^3/_2$ H, distorted s, *R*-OCH_3), 3.65 ($^3/_2$ H, s, *R*-CO_2CH_3), 3.70 ($^3/_2$ H, s, *S*-CO_2CH_3), 4.00–4.15 (1 H, m, CH), 6.70 ($^1/_2$ H, br d, J = 6.6 Hz, *S*-NH), 6.77 ($^1/_2$ H, br d, J = 6.6 Hz, *R*-NH), 7.35–7.65 (5 H, m, arom). The optical purity of (*R*)-4-aminopentanoic acid (**6**) prepared from L-pyroglutamic acid (**1**) was determined to be >99% ee on the basis of the integration ratio of the 5-methyl signals.

Ethyl 4-[[4-[*N*-(*tert*-Butoxycarbonyl)amino]butyryl]amino]-1-methyl-2-pyrrolecarboxylate (12). A solution of ethyl 1-methyl-4-nitro-2-pyrrolecarboxylate (**10**)[24] (300 mg, 1.51 mmol) in DMF (10 mL) was hydrogenated over 10% Pd–C (30 mg) overnight at room temperature and atmospheric pressure. The catalyst was removed by filtration to afford a solution of crude amine **11** in DMF. 4-[*N*-(*tert*-Butoxycarbonyl)amino]butyric acid (**9**)[25] (305 mg, 1.50 mmol) and EDCI[15] (345 mg, 1.80 mmol) were added to the solution. After being stirred for 4 h at room temperature, the solution was concentrated in vacuo. The residue was dissolved in CH_2Cl_2, washed with aqueous $NaHCO_3$, 5% aqueous citric acid, and saturated NaCl, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on silica gel (eluted with CH_2Cl_2:MeOH = 20:1) to give **12** as a colorless amorphous material (365 mg, 69%): IR ($CHCl_3$) 3452, 1697 cm^{-1}; NMR ($CDCl_3$) δ 1.33 (3 H, t, J = 7 Hz, CH_3CH_2), 1.43 (9 H, s, CH_3C × 3), 1.70–2.10 (2 H, m, $CH_2CH_2CH_2$), 2.38 (2 H, t, J = 7 Hz, CH_2CH_2CO), 3.21 (2 H, q, J = 7 Hz, $NHCH_2CH_2$), 3.87 (3 H, s, NCH_3), 4.27 (2 H, q, J = 7 Hz, CH_3CH_2O), 5.35 (1 H, t, J = 7 Hz, NH), 6.84 (1 H, d, J = 2 Hz, arom), 7.38 (1 H, d, J = 2 Hz, arom), 9.15 (1 H, br s, NH); FABMS m/z 354 (MH^+), 353 (M^+).

4-[[4-[*N*-(*tert*-Butoxycarbonyl)amino]butyryl]amino]-1-methyl-2-pyrrolecarboxylic Acid (13). A solution of **12** (360 mg, 1.02 mmol) in MeOH (7 mL) and 1 N NaOH (2 mL) was stirred at 50 °C for 10 h. Water was added to the reaction mixture and the MeOH was removed by evaporation in vacuo. The resulting aqueous solution was cooled with ice and acidified to pH 3 with 4 N HCl. The precipitate deposited was collected and dried over P_2O_5 to give **13** as a colorless amorphous material (211 mg, 64%), which was used for the next step without further purification.

Ethyl 4-[[[4-[[4-[*N*-(*tert*-Butoxycarbonyl)amino]butyryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolecarboxylate (14). A solution of **10**[24] (100 mg, 0.505 mmol) in DMF (3 mL) was hydrogenated over 10% Pd–C (20 mg) at room temperature and atmospheric pressure for 7 h. The catalyst was removed by filtration to afford a solution of crude amine **11** in DMF. Carboxylic acid **13** (130 mg, 0.400 mmol) and EDCI (96 mg, 0.50 mmol) were added to the solution. After being stirred overnight at room temperature, the solution was concentrated in vacuo. The residue was dissolved in CH_2Cl_2, washed with aqueous $NaHCO_3$, 5% aqueous citric acid, and saturated NaCl, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on silica gel (eluted with CH_2Cl_2:MeOH = 12:1) to give **14** as a colorless amorphous material (160 mg, 84%): IR (KBr) 3422, 1685, 1654 cm^{-1}; NMR ($CDCl_3$) δ 1.34 (3 H, t, J = 7 Hz, CH_3CH_2), 1.46 (9 H, s, CH_3C × 3), 1.60–2.10 (2 H, m, $CH_2CH_2CH_2$), 2.36 (2 H, t, J = 7 Hz, CH_2CH_2CO), 3.21 (2 H, q, J = 7 Hz, $NHCH_2CH_2$), 3.90 (6 H, s, NCH_3 × 2), 4.20 (2 H, q, J = 7 Hz, CH_3CH_2O), 5.12 (1 H, t, J = 7 Hz, NH), 6.76 (1 H, d, J = 2 Hz, arom), 6.92 (1 H, d, J = 2 Hz, arom), 7.17 (1 H, d, J = 2 Hz, arom), 7.48 (1 H, d, J = 2 Hz, arom), 8.38 (1 H, br s, NH), 8.83 (1 H, br s, NH); FABMS m/z 476 (MH^+), 475 (M^+).

4-[[[4-[[4-[*N*-(*tert*-Butoxycarbonyl)amino]butyryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolecarboxylic Acid (15). A solution of **14** (160 mg, 0.336 mmol) in EtOH (3 mL) and 1 N NaOH (0.7 mL) was stirred at 55 °C for 8 h. Water was added to the reaction mixture and the EtOH was removed by evaporation in vacuo. The resulting aqueous solution was cooled with ice and acidified to pH 3 with 4 N HCl. The precipitate deposited was collected and dried over P_2O_5 to give **15** as a colorless amorphous material (117 mg, 78%): IR (KBr) 3422, 1685, 1654 cm^{-1}; NMR ($CDCl_3$) δ 1.43 (9 H, s, CH_3C × 3), 1.65–2.00 (2 H, m, $CH_2CH_2CH_2$), 2.10–2.60 (2 H, m, CH_2CH_2CO), 3.00–3.40 (2 H, m, $NHCH_2CH_2$), 3.83 (3 H, s, NCH_3), 3.87 (3 H, s, NCH_3), 5.14 (1 H, br, NH), 6.88 (1 H, distorted s, arom), 6.98 (1 H, distorted s, arom), 7.11 (1 H, distorted s, arom), 7.45 (1 H, distorted s, arom), 8.45 (1 H, br s, NH), 8.81 (1 H, br s, NH); FABMS m/z 447 (M^+).

1-Methyl-4-nitro-2-pyrrolecarboxylic Acid (16). NaOH (1 N, 20 mL) was added to an ice-cooled solution of **10**[24] (2.00 g, 10.1 mmol) in dioxane (30 mL) and EtOH (50 mL) over 10 min. After being stirred for 2 h at room temperature, the solution was concentrated in vacuo. The residue was partitioned between water and ether. The aqueous layer was cooled with ice, acidified to pH 5 with 2 N HCl, and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and concentrated in vacuo to give **16** as colorless crystals (1.36 g, 79%): mp 196–199 °C (lit. 195–197 °C[24]); IR (KBr) 3000, 1705, 1515, 1315 cm^{-1}; NMR (DMSO-d_6) δ 3.93 (3 H, s, NCH_3), 7.31 (1 H, d, J = 2 Hz, arom), 8.26 (1 H, d, J = 2 Hz, arom).

1-Methyl-4-nitro-2-pyrrolecarbonyl Chloride (17). Thionyl chloride (3.0 mL, 41 mmol) was added to **16** (1.0031 g, 5.90 mmol). The solution was heated at reflux for 3 h and concentrated in vacuo to give **17** as pale brown crystals (1.1115 g, quantitative), which was used for the next step without further purification.

***N*-[3-(*N*,*N*-Dimethylamino)propyl]-1-methyl-4-nitro-2-pyrrolecarboxamide (19).** A solution of **17** (1.1115 g, 5.89 mmol) in CH_2Cl_2 (10 mL) was added to an ice-cooled solution of 3-(*N*,*N*-dimethylamino)propylamine (**18**; 0.9 mL, 7.3 mmol) and Et_3N (1.25 mL, 8.96 mmol) in CH_2Cl_2 (10 mL) over 5 min. After being stirred for 2 days at room temperature, the solution was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo to give **19** as colorless prisms (1.3357 g, 89.1%): mp 129–131 °C; IR (KBr) 3300, 3140, 1655, 1500, 1305 cm^{-1}; NMR ($CDCl_3$) δ 1.73 (2 H, quintet, J = 6 Hz, $CH_2CH_2CH_2$), 2.33 (6 H, s, NCH_3 × 2), 2.52 (2 H, t, J = 6 Hz, CH_2CH_2N), 3.50 (2 H, q, J = 6 Hz, $CONHCH_2CH_2$), 4.02 (3 H, s, NCH_3), 6.98 (1 H, d, J = 2 Hz, arom), 7.58 (1 H, d, J = 2 Hz, arom), 8.63 (1 H, br, NH); FABMS m/z 255 (MH^+).

4-[[[4-[[[4-[[4-[*N*-(*tert*-Butoxycarbonyl)amino]butyryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (21). A solution of **19** (127.1 mg, 0.500 mmol) in DMF (1.5 mL) was hydrogenated over 10% Pd–C (40 mg) at room temperature and atmospheric pressure for 2 days. The catalyst was removed by filtration, and the filtered catalyst was washed with DMF (1 mL). The DMF solutions were combined to afford a solution containing amine **20**, which was added to **15** (200.9 mg, 0.449 mmol) and $HOBt·H_2O$ (75.8 mg, 0.495 mmol) under an argon atmosphere. The resulting solution was cooled with ice and DCC (106.7 mg, 0.517 mmol) was added. The mixture was allowed to warm to room temperature, stirred overnight, and concentrated in vacuo. The residue was purified by chromatography on silica gel (eluted with CH_2Cl_2:MeOH = 5:1) and subsequent preparative TLC (developed four times with $CHCl_3$:MeOH:Et_3N = 50:5:1) to give **21** as a colorless amorphous material (223.6 mg, 76%): IR (KBr) 3309, 1638 cm^{-1}; NMR ($CDCl_3$) δ 1.45 (9 H, s, CH_3C × 3), 1.60–2.10 (4 H, m, CH_2 × 2), 2.20–2.90 (4 H, m, CH_2 × 2), 2.30 (6 H, s, NCH_3 × 2), 3.00–3.60 (4 H, m, CH_2 × 2), 3.90 (9 H, s, NCH_3 × 3), 5.12 (1 H, br t, J = 7 Hz, NH), 6.68 (2 H, distorted s, arom), 6.80 (1 H, distorted s, arom), 7.11 (1 H, distorted s, arom), 7.25 (1 H, distorted s, arom), 7.30 (1 H, distorted s, arom), 7.73 (1 H, br t, J = 7 Hz, NH), 8.16 (1 H, br s, NH), 8.45 (1 H, br s, NH), 8.93 (1 H, br s, NH); FABMS m/z 654 (MH^+).

N^{α},N^{im}-Bis(*tert*-butoxycarbonyl)-*erythro*-β-hydroxy-L-histidine (23).[5] To an ice-cooled solution of *erythro*-β-hydroxy-L-histidine hydrate (197.2 mg, 1.04 mmol) in water (4 mL) were successively added Na_2SO_4 (305.9 mg, 2.89 mmol) and a solution of $(Boc)_2O$ (557.8 mg, 2.56 mmol) in dioxane (3.2 mmol). The solution was allowed to warm to room tem-

(24) Weiss, M. J.; Webe, J. S.; Smith, J. M., Jr. *J. Am. Chem. Soc.* **1957**, *79*, 1266.

(25) (a) Schnabel, E. *Justus Liebigs Ann. Chem.* **1967**, *702*, 88. (b) Kricheldorf, H. R. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 550.

perature and stirred overnight. The dioxane was removed in vacuo. The resulting solution was acidified to pH 3 with citric acid and, after adding NaCl, extracted with AcOEt. The extract was washed with water, dried with $MgSO_4$, and concentrated in vacuo to give **23** as a colorless amorphous material (351.5 mg, 91%), which was used for the next step without further purification.

Methyl (*R*)-4-[[N^{α},N^{im}-Bis(*tert*-butoxycarbonyl)-*erythro*-β-hydroxy-L-histidyl]amino]pentanoate (24). To an ice-cooled solution of **23** (351.5 mg, 0.946 mmol) in CH_2Cl_2 (3 mL) were successively added a solution of **7** (161.6 mg, 0.964 mmol) and Et_3N (0.15 mL, 1.08 mmol) in CH_2Cl_2 (3 mL), $HOBt·H_2O$ (160.7 mg, 1.05 mmol), and DCC (215.8 mg, 1.05 mmol). The solution was allowed to warm to room temperature, stirred overnight, and concentrated in vacuo. AcOEt was added to the residue and insoluble material was removed by filtration. The remaining solution was concentrated in vacuo. The residue was purified by chromatography on silica gel (eluted with $CHCl_3$:MeOH = 40:1 → 30:1) to give **24** as a colorless amorphous material (307.3 mg, 67%): $[\alpha]^{22.5}_D$ −17.9° (*c* = 1.00, $CHCl_3$); IR (KBr) 3350, 1760, 1740, 1710, 1650 cm^{-1}; NMR ($CDCl_3$) δ 1.05 (3 H, d, *J* = 7 Hz, CH_3CH), 1.43 (9 H, s, CH_3C × 3), 1.60 (9 H, s, CH_3C × 3), 1.40–1.90 (2 H, m, $CHCH_2CH_2$), 2.27 (2 H, t, *J* = 7 Hz, $COCH_2CH_2$), 3.33 (1 H, br, OH), 3.67 (3 H, s, OCH_3), 3.70–4.20 (1 H, m, CH_3CH), 4.53 (1 H, dd, *J* = 8 and 5 Hz, NHCHCO), 4.97 (1 H, d, *J* = 5 Hz, ArCHOH), 5.97 (1 H, d, *J* = 8 Hz, NH), 6.60 (1 H, br d, *J* = 8 Hz, NH), 7.43 (1 H, s, arom), 8.09 (1 H, s, arom); FABMS *m/z* 485 (MH^+).

Methyl (*R*)-4-[[N^{α}-(Benzyloxycarbonyl)-*erythro*-β-hydroxy-L-histidyl]amino]pentanoate (25). Trifluoroacetic acid (15 mL, 202 mmol) was added to ice-cooled **24** (1.4597 g, 3.01 mmol) over 5 min under an argon atmosphere. The solution was stirred at the same temperature for 10 min and then at room temperature for 1 h and concentrated in vacuo. The pale brown oily residue was dissolved in CH_2Cl_2 (20 mL) under an argon atmosphere and cooled with ice. Et_3N (1.9 mL, 13.6 mmol) and CBZ-S[26] (1.0019 g, 3.65 mmol) were successively added to the solution. After being stirred for 3 h, the solution was poured into CH_2Cl_2, washed with saturated $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by chromatography on silica gel (eluted with $CHCl_3$:MeOH = 100:1 → 20:1) to give colorless crystals, which were further washed with hexane to give **25** as colorless crystals (392.4 mg, 31%): mp 108–111 °C; $[\alpha]^{23.0}_D$ +1.8° (*c* = 0.90, MeOH); IR (KBr) 3270, 1725, 1685, 1640 cm^{-1}; NMR ($CDCl_3$ + CD_3OD) δ 1.08 (3 H, d, *J* = 7 Hz, CH_3CH), 1.50–1.90 (2 H, m, $CHCH_2CH_2$), 2.26 (2 H, t, *J* = 7 Hz, $COCH_2$), 3.64 (3 H, s, OCH_3), 3.70–4.10 (1 H, m, CH_3CH), 4.40–4.70 (1 H, m, NHCHCO), 4.90–5.10 (1 H, m, ArCHOH), 5.05 (2 H, s, $C_6H_5CH_2$), 6.60–6.90 (1 H, m, NH or OH), 7.10 (1 H, s, arom), 7.35 (5 H, s, $C_6H_5CH_2$), 7.90 (1 H, s, arom); FABMS *m/z* 419 (MH^+).

Methyl (*R*)-4-[[N^{α}-(Benzyloxycarbonyl)-*erythro*-β-*tert*-butoxy-L-histidyl]amino]pentanoate (26). A suspension of **25** (101.9 mg, 0.244 mmol) in CH_2Cl_2 (30 mL) in a sealable tube was cooled with dry ice–acetone under an argon atmosphere. Isobutene (ca. 20 mL) was added dropwise, and then concentrated H_2SO_4 (0.3 mL) was added to the suspension. The tube was sealed, allowed to warm to room temperature, and stirred for 11 days. The tube was again cooled with dry ice–acetone and opened. After addition of Et_3N (5 mL), the solution was stirred at room temperature to remove the excess isobutene. The resulting mixture was partitioned between AcOEt and saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by chromatography on silica gel (eluted with $CHCl_3$:MeOH = 30:1) to give **26** as a colorless wax (94.3 mg, 82%): $[\alpha]^{23.0}_D$ +32.8° (*c* = 1.06, $CHCl_3$); IR (KBr) 3300, 1725, 1650 cm^{-1}; NMR ($CDCl_3$) δ 1.08 (3 H, d, *J* = 7 Hz, CH_3CH), 1.15 (9 H, s, CH_3C × 3), 1.50–1.90 (2 H, m, $CHCH_2CH_2$), 2.13 (2 H, t, *J* = 7 Hz, $COCH_2CH_2$), 3.63 (3 H, s, OCH_3), 3.65–4.20 (1 H, m, CH_3CH), 4.52 (1 H, dd, *J* = 8 and 5 Hz, NHCHCO), 5.12 (3 H, distorted s, ArCHOH and $C_6H_5CH_2$), 6.60–6.90 (2 H, m, NH × 2), 6.97 (1 H, s, arom), 7.38 (5 H, s, $C_6H_5CH_2$), 7.60 (1 H, s, arom); FABMS *m/z* 475 (MH^+).

Methyl (*R*)-4-[[N^{α}-[[6-[[*N*-[(*S*)-2-Carbamoyl-2-[*N*-[(*o*-nitrophenyl)thio]amino]ethyl]-*N*-[(*o*-nitrophenyl)thio]amino]methyl]-4-methoxy-2-pyridinyl]carbonyl]-*erythro*-β-*tert*-butoxy-L-histidyl]amino]pentanoate (29). A solution of **26** (275.6 mg, 0.581 mmol) in 5% HCO_2H–MeOH (7 mL) was added to a mixture of Pd-black and 5% HCO_2H–MeOH (14 mL) under an argon atmosphere. After the solution was stirred for 1.5 h at room temperature, the catalyst was removed by filtration and then washed with MeOH. The combined methanolic solution was concentrated in vacuo and the residue was treated with 0.2 N HCl (7 mL). Toluene was added to the solution, and the solvent was azeotropically removed in vacuo to give **27** as a colorless amorphous material (251.9 mg, quantitative). DPPA[18] (0.16 mL, 0.744 mmol) and Et_3N (0.30 mL, 2.15 mmol) were successively added to an ice-cooled solution of the amine salt **27** (251.9 mg) and acid **28**[5] (400.2 mg, 0.697 mmol) in DMF (15 mL) under an argon atmosphere. The solution was allowed to warm to room temperature, stirred overnight, and concentrated in vacuo. The residue was partitioned between AcOEt and aqueous $NaHCO_3$. The AcOEt extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by chromatography on silica gel (eluted with CH_2Cl_2:MeOH = 20:1) followed by gel filtration on Sephadex LH-20 (eluted with MeOH) to give **29** as a yellow amorphous material (398.8 mg, 77%): $[\alpha]^{23.5}_D$ +41.1° (*c* = 0.84, $CHCl_3$); IR ($CHCl_3$) 3460, 3330, 1730, 1675, 1665, 1510, 1340 cm^{-1}; NMR (DMSO-d_6) δ 1.03 (3 H, d, *J* = 7.3 Hz, CH_3CH), 1.05 (9 H, s, CH_3C × 3), 1.50–1.60 (2 H, m, CH_2), 2.10–2.30 (2 H, m, CH_2), 3.30–3.50 (3 H, m), 3.53 (3 H, s, OCH_3), 3.55–3.70 (2 H, m), 3.70–3.85 (1 H, m), 3.81 (3 H, s, $ArOCH_3$), 4.40–4.65 (4 H, m), 4.95 (1 H, distorted d, *J* = 6.6 Hz), 5.18 (1 H, br s), 6.97 (1 H, br s), 7.10–7.25 (1 H, m), 7.25–7.45 (4 H, m), 7.54 (1 H, br s), 7.60–7.80 (2 H, m), 7.90–8.10 (2 H, m), 8.29 (2 H, d, *J* = 9.4 Hz), 9.05 (1 H, br); FABMS *m/z* 897 (MH^+).

(*R*)-4-[[N^{α}-[[6-[[*N*-[(*S*)-2-Carbamoyl-2-[*N*-[(*o*-nitrophenyl)thio]amino]ethyl]-*N*-[(*o*-nitrophenyl)thio]amino]methyl]-4-methoxy-2-pyridinyl]carbonyl]-*erythro*-β-*tert*-butoxy-L-histidyl]amino]pentanoic Acid (30). To an ice-cooled solution of **29** (390.8 mg, 0.436 mmol) in MeOH (15 mL) was added 0.5 N NaOH (2.2 mL) dropwise over 30 min. After being stirred for 72 h at 5–10 °C, the solution was cooled with ice and 0.5 N NaOH (1.0 mL) was added dropwise over 15 min. After being stirred for 24 h at 5–10 °C, the solution was cooled with ice and acidified to pH 3–4 with citric acid. NaCl was added and the solution was extracted with AcOEt. The extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by chromatography on silica gel (eluted with CH_2Cl_2:MeOH = 5:1) followed by gel filtration on Sephadex LH-20 (eluted with MeOH) to give **30** as a yellow amorphous material (214.2 mg, 56%): $[\alpha]^{23.0}_D$ +33.0° (*c* = 1.05, MeOH); IR (KBr) 3448, 1654, 1508, 1338 cm^{-1}; NMR (DMSO-d_6) δ 1.01 (3 H, d, *J* = 5.9 Hz, CH_3CH), 1.06 (9 H, s, CH_3C × 3), 1.45–1.70 (2 H, m, CH_2), 1.90–2.15 (2 H, m, CH_2), 3.30–4.05 (6 H, m), 3.80 (3 H, s, $ArOCH_3$), 4.40–4.60 (4 H, m), 4.96 (1 H, distorted d, *J* = 7.0 Hz), 5.28 (1 H, d, *J* = 7.7 Hz), 5.40 (1 H, br s), 6.96 (1 H, br s), 7.05–7.25 (1 H, m), 7.25–7.45 (4 H, m), 7.51 (1 H, br s), 7.60–7.75 (2 H, m), 7.75–8.30 (2 H, m), 8.25 (2 H, d, *J* = 8.4 Hz), 9.03 (1 H, br); FABMS *m/z* 884 (MH^+ + 1).

4-[[[4-[[[4-[[4-[[(*R*)-4-[[N^{α}-[[6-[[*N*-[(*S*)-2-Carbamoyl-2-[*N*-[(*o*-nitrophenyl)thio]amino]ethyl]-*N*-[(*o*-nitrophenyl)thio]amino]methyl]-4-methoxy-2-pyridinyl]carbonyl]-*erythro*-β-*tert*-butoxy-L-histidyl]amino]pentanoyl]amino]butyryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (32). Trifluoroacetic acid (1 mL, 13 mmol) was added dropwise to an ice-cooled solution of **21** (90.1 mg, 0.138 mmol) in CH_2Cl_2 (1 mL) over 5 min under an argon atmosphere. The solution was stirred at the same temperature for 30 min and then at room temperature for 1.5 h and concentrated in vacuo. The residue was dried over KOH in vacuo to give **31** (118.0 mg, quantitative) as a pale brown amorphous material. DPPA[18] (0.04 mL, 0.19 mmol) and Et_3N (0.10 mL, 0.72 mmol) were successively added to an ice-cooled solution of the amine TFA salt **31** (118.0 mg) and acid **30** (99.8 mg, 0.113 mmol) in DMF (4 mL) under an argon atmosphere. The solution was allowed to warm to room temperature, stirred for 3 days, and concentrated in vacuo. The residue was purified by chromatography on silica gel (eluted with CH_2Cl_2:MeOH = 5:1) followed by gel filtration on Sephadex LH-20 (eluted with MeOH) to give **32** as a yellow amorphous material (96.9 mg, 60%): $[\alpha]^{23.0}_D$ +11.7° (*c* = 0.97, MeOH); IR (KBr) 3422, 1648, 1508, 1338 cm^{-1}; NMR (DMSO-d_6) δ 1.04 (3 H, d, *J* = 6.6 Hz, CH_3CH), 1.06 (9 H, s, CH_3C × 3), 1.55–1.75 (6 H, m), 2.00–2.15 (2 H, m), 2.15–2.30 (2 H, m), 2.21 (6 H, s, NCH_3 × 2), 2.30–2.45 (2 H, m), 2.50–2.65 (2 H, m), 2.95–3.15 (2 H, m), 3.20–3.30 (2 H, m), 3.55–3.95 (4 H, m), 3.80 (6 H, s, $ArOCH_3$ or NCH_3), 3.81, 3.83 (each 3 H, s, $ArOCH_3$ or NCH_3), 4.35–4.65 (4 H, m), 4.97 (1 H, d, *J* = 6.6 Hz), 5.05–5.20 (1 H, m), 6.75–6.80 (3 H, m), 6.82 (1 H, d, *J* = 1.8 Hz), 6.84 (1 H, d, *J* = 1.8 Hz), 7.00 (1 H, d, *J* = 1.5 Hz), 7.12 (1 H, d, *J* = 1.5 Hz), 7.15–7.30 (1 H, m), 7.18 (1 H, d, *J* = 1.8 Hz), 7.21 (1 H, d, *J* = 1.5 Hz), 7.30–7.35 (3 H, m), 7.54 (1 H, br s), 7.60–8.20 (4 H, m), 8.20–8.35 (3 H, m), 8.95 (1 H, br), 9.85 (1 H, s), 9.87 (1 H, s), 9.89 (1 H, s); FABMS *m/z* 1418 (MH^+).

PYML(6)–(4*R*-APA)–Distamycin. Trifluoroacetic acid (0.40 mL, 5.4 mmol) was added dropwise to an ice-cooled solution of **32** (40.5 mg, 0.0285 mmol) and skatole (79.7 mg, 0.608 mmol) in CH_2Cl_2 (8 mL) over 4 min under an argon atmosphere. The solution was stirred for 1.5 h at room temperature, treated with Et_3N (0.76 mL, 5.4 mmol) with ice cooling, and concentrated in vacuo. The residue was purified by repeated gel filtration on Sephadex LH-20 (eluted with MeOH) to give PYML-

(26) Nagasawa, T.; Kuroiwa, K.; Narita, K.; Isowa, Y. *Bull. Chem. Soc. Jpn.* **1973**, *69*, 1269.

(6)-(4*R*-APA)-distamycin as a colorless amorphous material (9.5 mg, 30%): $[\alpha]^{22.5}_D$ +7.9° (c = 0.365, MeOH); IR (KBr) 3446, 1654, 1647 cm^{-1}; NMR (CD_3OD) δ 1.16 (9 H, s, $CH_3C \times 3$), 1.24 (3 H, d, J = 6.6 Hz, CH_3CH), 1.65-1.90 (4 H, m), 1.90-2.10 (2 H, m), 2.15-2.40 (4 H, m), 2.92 (6 H, s, $NCH_3 \times 2$), 3.00-3.30 (4 H, m), 3.35-3.45 (2 H, m), 3.80-4.20 (6 H, m), 3.87, 3.886, 3.894, 3.91 (each 3 H, s, $ArOCH_3$ and $NCH_3 \times 3$), 4.60-4.70 (1 H, m), 5.26 (1 H, d, J = 9.2 Hz), 6.79 (1 H, d, J = 1.8 Hz), 6.90 (1 H, d, J = 1.8 Hz), 6.95 (1 H, d, J = 1.8 Hz), 7.06 (1 H, d, J = 2.2 Hz), 7.13 (1 H, d, J = 1.8 Hz), 7.17 (2 H, distorted s), 7.35-7.45 (1 H, m), 7.38 (1 H, d, J = 2.6 Hz), 7.44 (1 H, distorted s); FABMS m/z 1112 (MH^+).

DNA-Cleavage Experiment. Nucleotide sequence cleavage was investigated on the 5'- and 3'-end-labeled strands of a 100-base-pair DNA restriction fragment (*Alu*I-*Hae*III) from the phage R199/G4ori. The reaction mixtures contained 10 mM Tris-HCl buffer (pH 7.4), the 5'- or 3'-end ^{32}P-labeled G4 phage DNA fragment, 1 μg of carrier calf thymus DNA, 1 mM dithiothreitol, and 1 μM PYML(6)-(4*R*-APA)-distamycin (or natural BLM)-iron complex. After the reaction solutions were incubated at 37 °C for 10 min, the DNA samples were subjected to electrophoresis on a 10% polyacrylamide/7 M urea slab gel. The autoradiogram was scanned with a microdensitometer.

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Registry No. 1, 98-79-3; **2**, 4931-66-2; **3**, 17342-08-4; **4**, 72479-05-1; **5**, 21395-93-7; **6**, 5937-83-7; (±)-**6**, 627-61-2; **7**, 123993-04-4; (*R*,*S*)-**8**, 123993-05-5; (*S*,*S*)-**8**, 123993-18-0; **9**, 57294-38-9; **10**, 2853-29-4; **11**, 40889-84-7; **12**, 123993-06-6; **13**, 123993-07-7; **14**, 123993-08-8; **15**, 123993-09-9; **16**, 13138-78-8; **17**, 28494-51-1; **18**, 109-55-7; **19**, 65361-30-0; **20**, 78486-14-3; **21**, 123993-10-2; **22**, 41215-80-9; **23**, 82692-03-3; **24**, 123993-11-3; **25**, 123993-12-4; **26**, 123993-13-5; **27**·HCl, 124020-63-9; **28**, 108998-85-2; **29**, 124020-64-0; **30**, 123993-14-6; **31**·TFA, 123993-15-7; **32**, 123993-16-8; (*R*)-MTPA-Cl, 39637-99-5; PYML-(6)-(4*R*-APA)-distamycin, 123993-17-9.

Proton Exchange and Epimerization of Co(III) Chelated Amino Acids via Carbanion Intermediates

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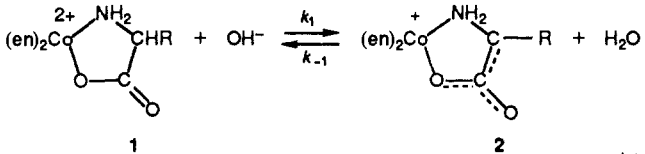
Abstract: An earlier investigation into proton exchange and epimerization of amino acids chelated to Co(III)[1] has been improved and extended to include the amino acids (AA) Phe, Val, Ala, Gly, Glu, and Asp in the complex cations Δ,Λ-$[Co(en)_2(S\text{-AA})]^{2+,+}$. Equilibrium concentrations of the diastereomers measured in H_2O (K_C(Δ-*S*/Λ-*S*) = 1.15 (Phe), 2.0 (Val), 1.0 (Ala, Gly), 0.85 (Glu), 0.67 (Asp)) vary little with ionic strength and are the same in D_2O. Rate constants for OD^--catalyzed proton exchange at the 2-CH centers differ for the Δ-*S* and Λ-*S* diastereomers and can be related to the rate constant for epimerization provided the concept of a common carbanion intermediate is used. There is no correlation between the rate data and the overall charge on the complex. Selectivity differences are demonstrated in the reprotonation process (Λ-*R*/Λ-*S* = 1.6 (Phe), 1.6 (Val), 0.9 (Ala), 0.8 (Gly), 0.75 (Glu), 0.5 (Asp)), and these are shown to be thermodynamically driven. This corrects previous investigations on the AA = Asp and Glu complexes. ^3H rate studies show a kinetic isotope difference of ~8 for reprotonation in favor of ^1H, but no selectivity difference between ^3H and ^1H in forming the Λ-*R*, Λ-*S* epimers.

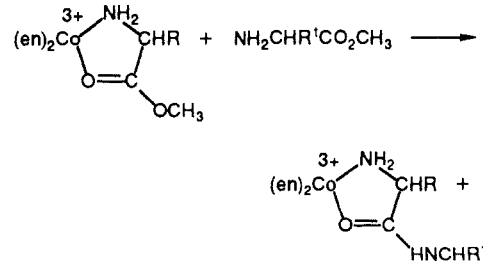
This paper extends our earlier investigation into proton exchange and epimerization of chelated amino acid anions of type **1**[1] by examining in greater detail the properties of carbanion **2** generated by deprotonation of **1** by OH^- ions in aqueous solution (eq 1).



(1)

It is well-known that metal ions enhance the carbon acidity of chelated amino acids,[2] but the influence of the additional asymmetric metal center (structures such as **1** are diastereotopic) on the thermodynamic and kinetic stereochemical preferences for electrophilic addition to the resulting prochiral carbanion **2** is little investigated or understood.

This new study came about for two reasons. First, an investigation of epimerization during peptide synthesis using Co(III)-activated amino acid esters[3] (eq 2) has revealed that carbanions **3**, generated under the conditions of the coupling reaction, have decided diastereomeric preferences for reprotonation, and it was of interest to compare these preferences with those of the somewhat more conjugated amino acid carbanions **2**. Such information could lead to an appreciation of the steric properties, and possibly lifetimes, of such intermediates. Second, subsequent



(2)

(1) Buckingham, D. A.; Marzilli, L. G.; Sargeson, A. M. *J. Am. Chem. Soc.* **1967**, *89*, 5133.

(2) (a) Sato, M.; Okawa, K.; Akabori, S. *Bull. Chem. Soc. Jpn.* **1957**, *30*, 937. (b) Williams, D. H.; Busch, D. H. *J. Am. Chem. Soc.* **1965**, *87*, 4644. (c) Yoshikawa, S.; Saburi, M.; Yamaguchi, M. *Pure Appl. Chem.* **1978**, *50*, 915. (d) Pasini, A.; Casella, L. *J. Inorg. Nucl. Chem.* **1974**, *36*, 2133.

(3) Buckingham, D. A.; Sutton, P. A. *Acc. Chem. Res.* **1987**, 20, 357. A detailed account of the epimerization during the peptide synthesis by the Co(III)-active ester method is being prepared for publication.

0002-7863/90/1512-845$02.50/0