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OXIDATION OF ALKENES BY A CHIRAL NON-PORPHYRINIC OXIDIZING CATALYST BASED ON THE BLEOMYCIN-Fe(II) COMPLEX[1]

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Abstract - A synthetic model for the metal binding site of bleomycin with 4-dimethylaminopyridine nucleus, namely PYML-8, shows dioxygen activation up to 125% of that of bleomycin. β-Methylstyrene is oxidized with the Fe(III)-H_2O_2, Fe(III)-PhIO, or Fe(II)-O_2 complex systems of PYML-8 to give a set of products including optically active epoxide. The product composition is dependent on iron, oxygen source, and reducing agent employed, suggesting varied active species generated from each complex system.

Bleomycins (BLMs) are chemotherapeutic agents used for the clinical treatment of Hodgkin's lymphoma, carcinomas of skin, head, and neck, and tumors of testis.[2] The drug was isolated from *Streptomyces verticillus* as a copper chelate by Umezawa and his co-workers in 1966 and the

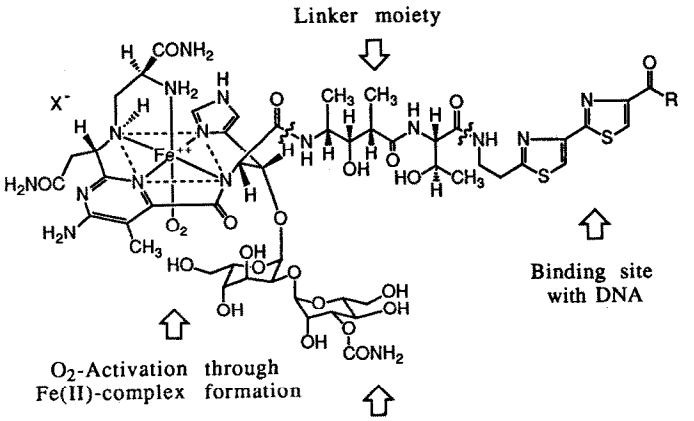


Figure 1. Proposed structure of BLM-Fe(II)-O_2 complex and assumed role of each part. X^- is considered to be ^-OH in aqueous solution.

structure was shown to be a glycopeptide consisting of an unusual hexapeptide and a disaccharide [3] It has been well documented that BLM activates dioxygen by the formation of a unique iron complex of the amine-pyrimidine-imidazole region to generate active species closely related to BLM-Fe(III)-O_2^{2-}, and binds to guanine base of DNA by the bithiazole-terminal amine region (Figure 1) [4] BLM thus cleaves DNA specifically at GC and GT sequences to exert the antitumor activity In our continuing study toward man-designed BLMs, we designed several non-porphyrinic ligands based on the metal binding site of BLM and demonstrated that the 4-aminopyrimidine nucleus and the disaccharide of BLM can be replaced by a simplified pyridine ring and a *tert*-butyl group, respectively, i e , a synthetic model PYML-4 showed remarkably efficient dioxygen activating capability (Figure 2) [5,6,7] Furthermore, a synthetic model PYML-6 with a 4-methoxypyridine nucleus was found to be almost equivalent to BLM in dioxygen activating capability (Figure 2) [8,9] We now achieved a dioxygen activation superior to BLM by a newly designed model ligand PYML-8 and examined olefin epoxidation using PYML-8-iron complex systems [10]

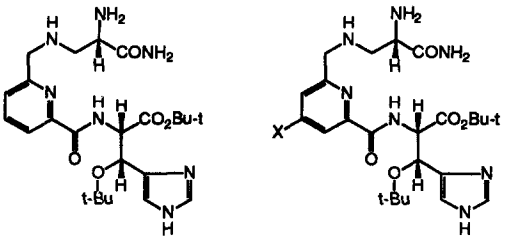


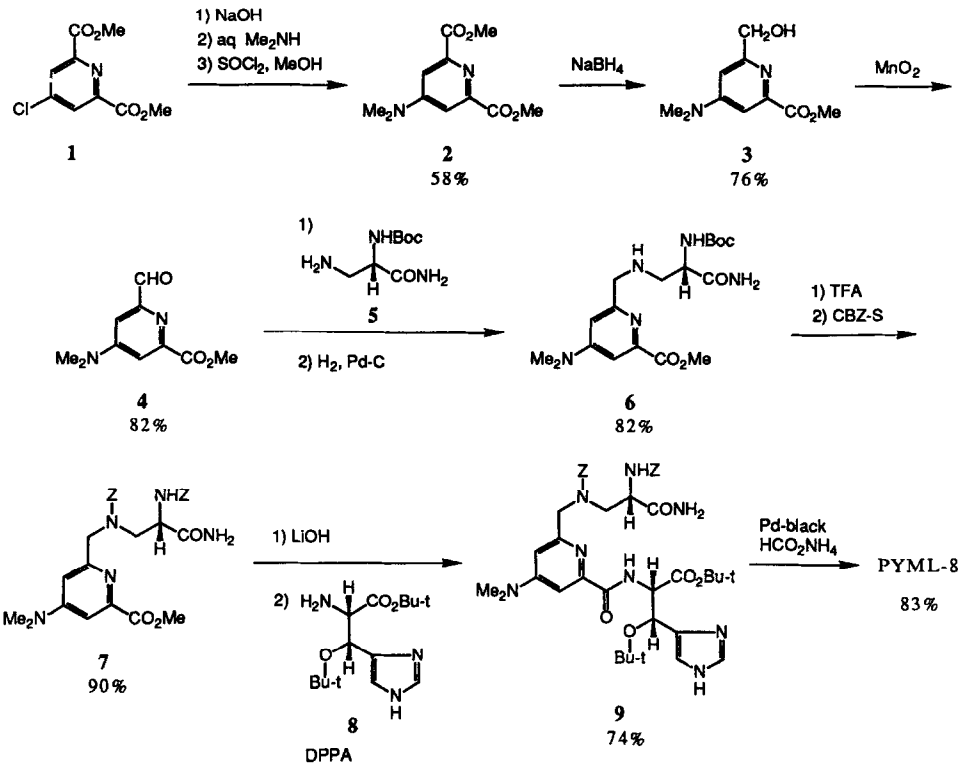
Figure 2 Synthetic analogues of BLM

Design and Synthesis of PYML-8

As the dioxygen activation process of BLM-Fe(II) complex involves electron transfer from iron to oxygen, electronic property of the ligand surrounding the metal must have a profound influence on the oxygen activation Therefore, we assumed that increased electron density of the ligand would increase the capability to activate dioxygen for the Fe(II) complex, and *vice versa* The result of Huckel molecular orbital calculation indicated that π-electron density of the N-atom of various 4-substituted pyridines are considerably dependent on the nature of the substituents [11] This was in accordance with the enhanced dioxygen activation by PYML-6 which has an electron-donating methoxyl substituent on the pyridine ring In order to attain a more efficient oxygen activation, we designed a new ligand PYML-8 containing a more strongly electron-donating 4-dimethylaminopyridine moiety (Figure 2)

PYML-8 was synthesized as follows (Scheme 1) Dimethyl 4-chloropyridine-2,6-dicarboxylate (1) was easily prepared from chelidamic acid [9] It was saponified to give the corresponding dicarboxylic acid which was treated with aqueous dimethylamine in a sealable bulb and then re-

esterified, affording 4-dimethylamino derivative **2** in 58% overall yield Aldehyde **4** was obtained by borohydride reduction of ester **2** (76% yield) followed by manganese dioxide oxidation of alcohol **3** (82% yield) Condensation of aldehyde **4** with (*S*)-3-amino-2-[(*tert*-butoxycarbonyl)amino]propionamide (**5**)[12] gave a Schiff base, which was hydrogenated over palladium on charcoal, furnishing secondary amine **6** in 82% yield We found that the choice of an appropriate amino-protecting group was crucial for the rest of the synthesis Although Nps (*o*-nitrophenylthio) group[13] was successfully applied to the synthesis of PYML-6, it was found not to be useful for the dimethylamino derivatives because of an unexpected difficulty in the final deprotection Therefore, we converted mono-Boc derivative **6** into bis-Z derivative **7** by the use of benzyl *S*-(4,6-dimethylpyrimidin-2-yl) thiocarbonate (CBZ-S)[14] in 90% yield Hydrolysis of the ester **7** was facilitated by lithium hydroxide and the subsequent coupling with *erythro*-β-*tert*-butoxy-L-histidine (**8**)[7] by diphenyl phosphorazidate (DPPA)[15] afforded peptide **9** in 74% yield The use of palladium black and ammonium formate was found to be most effective for the removal of the Z group of **9** and PYML-8 was obtained in 83% yield



Scheme 1

PYML-8 showed metal-binding properties remarkably similar to those of BLM Cu(II) complexes of PYML-8 exhibited ESR spectra characterized by axially symmetric g- and A-tensor components (Table 1) The oxygen-analogous nitric oxide adduct complex was easily obtained by addition of sodium nitrite to the Fe(II) complex of PYML-8 (Table 2) Moreover, PYML-8 apparently produced two low-spin Fe(III) complex species, similar to those observed in the course of dioxygen activation of BLM One ferric complex is presumed to be the transient Fe(III)-O_2H^- species and another ferric complex seems to be the stable Fe(III)-OH^- species, as indicated by the ESR parameters The oxygen activation of the iron complex of the model was demonstrated by an ESR spin trapping experiment using *N-tert*-butyl-α-phenylnitrone It was noteworthy that the spin concentration of hydroxyl radicals generated from the PYML-8-Fe(II)-O_2 system was estimated to be about 125% of that of the corresponding BLM system (Table 3) Thus, oxygen-activating power of a man-designed peptide finally exceeded that of natural BLM, presumably owing to the substantial electron donation by the dimethylamino group

Table 1 ESR parameters for Cu(II) complexes of BLM and PYML-8

ligand	$g_{//}$	$g_{\perp}$	$A_{//}$(G)
BLM	2 211	2 055	183 0
PYML-8	2 217	2 062	168 9

Table 2 ESR parameters for iron complexes of BLM and PYML-8

ligand	Fe(II)-^{14}NO complex				transient Fe(III) complex			stable Fe(III) complex		
	g_1	g_2	g_3	A^N (G)	g_1	g_2	g_3	g_1	g_2	g_3
BLM	2 041	2 008	1 976	23 8	2 254	2 171	1 937	2 431	2 185	1 893
PYML-8	2 039	2 008	1 970	24 8	2 247	2 173	1 940	2 366	2 185	1 910

Table 3 Spin concentration of hydroxyl radicals from Fe(II)-O_2 complex systems of BLM and synthetic analogues

ligand	relative spin concentration
BLM	100
PYML-4	71
PYML-6	97
PYML-8	125

Epoxidation of Olefin with PYML-8

In addition to the DNA cleaving activity, BLM is known to oxidize a range of olefinic substrates So far reported are oxidation of stilbene, styrene, chalcone, cinnamic acid, cyclohexene, norbornene, and indene using Fe(II), Fe(III), Cu(II), Mn(III), or Zn(III) complexes of BLM in the presence of O_2, PhIO, or $KHSO_2$.[16] We also reported epoxidation of stilbene with Fe(III)-H_2O_2 complex systems of PYMLs [8,9] Furthermore, *cis*-β-methylstyrene was found to be affected by the chiral environment around the iron nucleus of PYML-6 complex, resulting in some asymmetric induction in epoxidation reaction, as reported preliminarily [17] We now studied enantioselective epoxidation of alkenes with iron complex systems of PYML-8 in detail As BLM-Fe(II)-O_2 complex affords active species such as Fe(III)-O_2^{2-} or Fe(V)=O, we investigated alkene oxidation with PYML-8-Fe(III)-O_2^{2-}, PYML-8-Fe(V)=O, and PYML-8-Fe(II)-O_2 systems to examine the reactivity of each complex systems

Oxidation with PYML-8-Fe(III)-H_2O_2. We first investigated oxidation of stilbene and β-methylstyrene with PYML-8-Fe(III)-H_2O_2 complex system PYML-8 (1 eq) was allowed to react with alkene substrate (213 eq) and oxidant (30 eq) in degassed methanol for 30 minutes at room temperature under argon and the products were analyzed by gas chromatography (Table 4)

Oxidation of *cis*-stilbene afforded *cis*-stilbene oxide in ~35% yield based on H_2O_2 along with concomitant formation of *trans*-epoxide and deoxybenzoin Oxidation of *trans*-stilbene exclusively afforded *trans*-epoxide in 31% yield [18] The same tendency was seen in the reaction of β-methylstyrene with PYML-8-Fe(III)-H_2O_2 That is, oxidation of *cis*-β-methylstyrene afforded *cis*-epoxide (30% yield) and phenylacetone with virtually no concomitant formation of *trans*-epoxide On the other hand, treatment of *trans*-β-methylstyrene with PYML-8-Fe(III)-H_2O_2 afforded *trans*-epoxide as a sole product (32% yield) without any formation of phenylacetone [18]

Asymmetric induction was observed in the epoxidation of β-methylstyrene The epoxide was isolated by silica gel column chromatography and the enantiomeric excess (ee) of the material obtained was determined based on the ^1H NMR measurement using chiral shift reagent [19] There was a marked difference in the enantioselectivity between the *cis*-substrate and the *trans*-substrate (Table 5) Whereas (-)-*cis*-epoxide with 57% ee was obtained from *cis*-β-methylstyrene, *trans*-β-methylstyrene gave racemic *trans*-epoxide These results suggest that, in the active species generated from PYML-8-Fe(III)-H_2O_2 complex system, oxygen is bound tightly to the central iron and the stereochemical environment around the iron-oxygen center influenced the transition state of the oxidation reaction These are in accordance with the result previously reported for PYML-6 [17]

Oxidation with PYML-8-Fe(III)-PhIO. PhIO is known as a one-oxygen donor for iron complex to generate oxidizing Fe=O species [20] In fact, reaction of PYML-8-Fe(III)-PhIO with *cis*-stilbene or *cis*-β-methylstyrene actually gave *cis*-stilbene oxide or *cis*-β-methylstyrene oxide, and *trans*-alkenes produced the corresponding *trans*-epoxides (Table 4) Concomitant formation of deoxybenzoin or phenylacetone was also observed in the case of *cis*-substrates but not observed for *trans*-substrates However, this reaction was characterized by predominant formation of benzaldehyde either from *cis*-alkenes or from *trans*-alkenes The formation of benzaldehyde was

Table 4 Oxidation of stilbene and β-methylstyrene with PYML-8-Fe(III) complex systems

substrates	products	yields %*			
		Fe(III)-H_2O_2		Fe(III)-PhIO	
		PYML-8	none	PYML-8	none
		34 9 (992)	trace	22 3 (666)	trace
		3 31 (94 1)	n d	n d	n d
		11 2 (318)	n d	5 43 (162)	n d
	PhCHO	trace	trace	66 9 (1997)	17 4 (519)
		n d	n d	n d	n d
		31 4 (925)	trace	18 4 (549)	1 89 (56 5)
		n d	n d	n d	n d
	PhCHO	trace	trace	67 1 (2003)	21 6 (644)
		30 7 (916)	n d	22 5 (672)	n d
		trace	n d	trace	trace
		+ (n m)	n d	+ (n m)	n d
	PhCHO	trace	trace	66 8 (1993)	32 7 (976)
		n d	n d	n d	n d
		32 1 (958)	n d	31 0 (925)	2 55 (76 2)
		n d	n d	n d	n d
	PhCHO	trace	trace	64 2 (1916)	32 1 (957)

* Yields based on H_2O_2 or PhIO (Yields based on Fe(III))
n d not detected n m not measured

Table 5 Ee of products of epoxidation of β-methylstyrene with PYML-8-Fe(III)-H_2O_2

substrates	epoxides	ee (%)
		57
		0

also reported by Hecht *et al* in the oxidation of *cis*-stilbene with BLM-Fe(III)-PhIO system in the presence of oxygen, demonstrating that the oxygen atom of benzaldehyde originates from molecular oxygen [16f,16g] However, in the case of our PYML-8-Fe(III)-PhIO complex system, benzaldehyde was obtained either under aerobic condition or under anaerobic condition in almost the same yields, indicating that benzaldehyde was produced *via* a route independent of molecular oxygen In the mechanistic study on the reaction of alkenes with the hypervalent iron-oxo porphyrins by Bruice *et al*, they referred to the following possible intermediates, metallaoxetane (A), radical (B), cation (C), cation radical (D), and an intermediate of concerted insertion (E) (Figure 3) [20] These intermediates seem to be relevantly applied also to the case of BLM or PYMLs, because a considerable similarity was found between BLM and heme-iron complexes [4] The observed formation of benzaldehyde, being independent of molecular oxygen, may be explained by invoking the cleavage of ferroxetane (A) formed by cycloaddition of Fe(V)=O species to the double bond, as recently discussed by Barton *et al* [21]

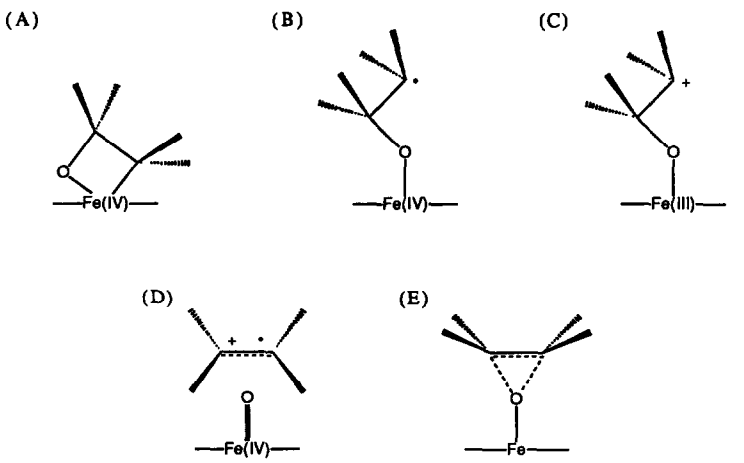


Figure 3 Proposed intermediates in the reaction of alkene with hypervalent iron-oxo species [20]

Oxidation with PYML-8-Fe(II)-O_2. As BLM-Fe(II)-O_2 produces a catalytically active complex, it must be possible to oxidize low molecular weight substrates by the Fe(II)-O_2 system Thus, PYML-8-Fe(II) (1 eq) was allowed to react with β-methylstyrene (177 eq) in the presence of oxygen and reducing agents (46 eq) [2-mercaptoethanol (2-ME), 1,4-dithiothreitol (DTT), or sodium L-ascorbate] for 2 hours The result was largely dependent on the reducing agent (Table 6) When 2-ME or DTT was used, the main product was benzaldehyde and epoxides were produced in low yields The reaction was found to be enantioselective, *cis*-olefin afforded (-)-*cis*-epoxide and *trans*-olefin gave racemic *trans*-epoxide (Table 7) On the other hand, the use of L-ascorbate afforded entirely different products, i e, benzaldehyde was not produced and both *cis*-olefin and *trans*-olefin uniformly gave *trans*-epoxide

In contrast with the results of PYML-8-Fe(III)-PhIO system, the formation of benzaldehyde was dependent on the molecular oxygen When the most of molecular oxygen was excluded from the reaction system of *cis*-β-methylstyrene oxidation, the formation of benzaldehyde was markedly suppressed and, instead, considerable amount of *trans*-β-methylstyrene was produced This can be accounted for by the mechanism involving a cation radical intermediate (Figure 3, D),

Table 6 Oxidation of β-methylstyrene with PYML-8-Fe(II)-O_2

reducing agent	substrate	ligand	products, yields (%)*		
					PhCHO
2-ME		PYML-8	1 6 (71 0)	0 4 (17 7)	14 6 (647)
		none	n d	n d	~2 1 (~93 1)**
		PYML-8	n d	2 8 (124)	13 3 (590)
		none	n d	0 8 (35 5)	~4 0 (~177)**
DTT		PYML-8	1 6 (72 6)	trace	21 5 (975)
		none	n d	trace	2 9 (132)
		PYML-8	n d	3 1 (141)	21 8 (989)
		none	n d	0 6 (27 2)	3 7 (168)
L-ascorbate		PYML-8	trace	2 8 (129)	trace
		none	trace	n d	trace
		PYML-8	n d	3 6 (165)	1 6 (73 5)
		none	n d	0 8 (36 7)	1 6 (73 5)

* Yields based on the reducing agent (Yields based on Fe(III))

** Exact yield could not be obtained due to the partial overlapping of the GC signals of 2-ME and benzaldehyde

Table 7 Ee of products of epoxidation of β-methylstyrene with PYML-8-Fe(II)-O_2-(2-ME)

substrates	epoxides	ee (%)
		33
		0

i e, a small amount of dioxygen present in the reaction system was activated by the PYML-8-Fe(II) complex, converting the substrate alkene into a cation radical by one-electron oxidation As the amount of oxygen was limited, the aerobic transformation of the cation radical species to benzaldehyde was suppressed Instead, a facile isomerization of the cation radical from *cis*-configuration to the more stable *trans*-configuration and subsequent acceptance of one electron produced *trans*-β-methylstyrene

Conclusion

We designed a non-porphyrinic oxidizing catalyst based on the metal binding site of BLM, namely PYML-8, which is characterized by a highly electron-donating 4-dimethylaminopyridine moiety Synthesis of PYML-8 was carried out by introducing the 2,3-diaminopropionamide side chain into the 4-dimethylaminopyridine nucleus followed by peptide coupling with *erythro*-β-*tert*-butoxy-L-histidine moiety Cu(II) and iron complexes of PYML-8 exhibited ESR characteristic of those of BLM In particular, PYML-8-Fe(II) was shown to be the first man-designed peptide which exceeded natural BLM in oxygen activation

Active species generated from PYML-8-Fe(III) and H_2O_2 or PhIO were capable of epoxidize stilbene and β-methylstyrene Notably, asymmetric induction observed in the oxidation of *cis*-β-methylstyrene suggested that the alkene was placed and oxidized under an asymmetric environment around the iron-oxygen center Similar asymmetric induction was observed in the case of PYML-8-Fe(II)-O_2 Oxidation of alkene using PYML-8-Fe(II)-O_2 and 2-ME or DTT afforded benzaldehyde as a major product along with a small amount of epoxide and ketone On the other hand, PYML-8-Fe(II)-O_2-L-ascorbate system showed different reactivity toward alkene, resulting in the trace amount of benzaldehyde Thus, varied oxygen species were shown to be generated depending on iron, oxidant, and reducing agents Reactivity of active species of PYML-8-Fe(II)-O_2-2-ME (or DTT) is evidently different from that of PYML-8-Fe(III)-H_2O_2 PYML-8-Fe(III)-PhIO appears to generate species possessing reactivity between those of PYML-8-Fe(II)-O_2 and PYML-8-Fe(III)-H_2O_2 Efforts are continuing to explore a new class of oxidizing catalyst based on BLM

EXPERIMENTAL

Melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected ^{1}H NMR spectra were recorded on a JEOL GX-400 (400 MHz) or JEOL FX-100 (100 MHz) spectrometer Abbreviations are as follows s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet) IR spectra were recorded on a Perkin Elmer 1600 FTIR spectrometer Mass spectra (MS) and fast atom bombardment mass spectra (FABMS) were recorded on a JEOL MS-300 and JEOL JMA DX-300, respectively Gas chromatography was carried out on a Shimadzu GC-4CM equipped with a flame ionization detector (column, PEG 20M 30 mmϕ x 200 cm, N_2 as carrier gas) X-band ESR spectra were recorded on a JEOL JES FE-3X spectrometer Reagents and solvents were purified by standard procedures MeOH used for the epoxidation reaction was distilled and degassed immediately before use

Dimethyl 4-dimethylaminopyridine-2,6-dicarboxylate (2). A suspension of dimethyl 4-chloropyridine-2,6-dicarboxylate (1)[9] (7 82 g, 34 1 mmol) in 1 N NaOH (85 3 ml) was stirred at 80°C for 2 h The mixture was cooled with ice and acidified to pH 4 with 1 N HCl White precipitate deposited was collected and dried *in vacuo* for 12 h to give crude 4-chloropyridine-2,6-dicarboxylic acid (7 49 g) A suspension of crude 4-chloropyridine-2,6-dicarboxylic acid (6 87 g, 34 1 mmol) in 33% aqueous dimethylamine (100 ml) was stirred at 140°C for 12 h in a sealable tube The tube was cooled with ice and opened After addition of conc H_2SO_4 (5 ml) to the mixture, white precipitate deposited was collected and dried *in vacuo* for 12 h to give crude 4-dimethylaminopyridine-2,6-dicarboxylic acid as a white powder (6 32 g) Thionyl chloride (27 ml) was added to MeOH (100 ml) at -10°C After the mixture was stirred for 10 min, crude 4-dimethylaminopyridine-2,6-dicarboxylic acid (6 32 g, 30 1 mmol) was added to the solution The solution was refluxed for 5 h and concentrated *in vacuo* The residue was partitioned between brine and AcOEt The aqueous layer was further extracted with AcOEt The AcOEt layers were combined, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo* The residue was purified by chromatography on silica gel (eluted with hexane AcOEt = 1 1) to give 2 as colorless powder (4 72 g, 19 8 mmol, 58% yield based on 1) Recrystallization from AcOEt - hexane gave colorless needles mp 167 - 168°C, IR (KBr) 3098, 2997, 2954, 1708, 1604, 1509, 1427, 1338 cm^{-1}, 100 MHz ^{1}H NMR ($CDCl_3$) δ 3 14 (6H, s), 3 98 (6H, s), 7 49 (2H, s), MS *m/e* 238 (M^+), Anal calcd for $C_{11}H_{14}O_4N_2$, C55 45, H5 92, N11 76, found C55 34, H5 91, N11 58

Methyl 6-hydroxymethyl-4-dimethylaminopyridine-2-carboxylate (3). $NaBH_4$ (461 mg, 12 2 mmol) was added to a solution of ester 5 (1 45 g, 6 09 mmol) in MeOH (30 ml) and CH_2Cl_2 (6 ml) at 0°C The solution was stirred at 0°C for 30 min then at room temperature for 2 h, neutralized with 1 N HCl, and concentrated *in vacuo* The residue was partitioned between saturated aqueous $NaHCO_3$ and CH_2Cl_2 The aqueous layer was further extracted with CH_2Cl_2 The CH_2Cl_2 layers were combined, washed with water and brine, 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 3 as white powder (972 6 mg, 4 63 mmol, 76% yield) IR (neat) 3362, 2989, 2926, 1922, 1609, 1509, 1433, 1389, 1349 cm^{-1}, 100 MHz ^{1}H NMR ($CDCl_3$) δ 3 08 (6H, s), 3 97 (3H, s), 4 67 (2H, s), 6 66 (1H, d, J = 3 Hz), 7 26 (1H, d, J = 3 Hz), MS *m/e* 210 (M^+)

Methyl 6-formyl-4-dimethylaminopyridine-2-carboxylate (4). MnO_2 (995 6 mg, 11 5 mmol) was added to a solution of alcohol 3 (248 9 mg, 1 18 mmol) in CH_2Cl_2 (4 ml) under argon After being stirred overnight at room temperature, the mixture was filtered through celite and the filtrate was concentrated *in vacuo* The residue was purified by chromatography on silica gel (eluted with hexane AcOEt = 1 2) to give 4 as white powder (201 mg, 0 965 mmol, 82% yield) Recrystallization from AcOEt - hexane gave colorless needles mp 117 - 117 5°C, IR (KBr) 3091, 2988, 2860, 1705, 1606, 1509, 1431, 1397, 1354 cm^{-1}, 100 MHz ^1H NMR ($CDCl_3$) δ 3 15 (6H, s), 4 04 (3H, s), 7 28 (1H, d, J = 3 Hz), 7 54 (1H, d, J = 3 Hz), 10 04 (1H, s), MS *m/e* 208 (M^+), Anal calcd for $C_{10}H_{12}O_3N_2$, C57 68, H5 81, N13 46, found C57 66, H5 87, N13 18

Methyl 6-[[*N*-[(2*S*)-2-carbamoyl-2-[(*tert*-butoxycarbonyl)amino]ethyl]amino]-methyl]-4-dimethylaminopyridine-2-carboxylate (6). A mixture of aldehyde 4 (114 mg, 0 547 mmol), amine 5[12] (132 mg, 0 469 mmol), activated molecular sieves 3A (1 g) and acetonitrile (5 ml) was stirred at room temperature for 12 h under argon The mixture was filtered through celite and the filtrate was concentrated to dryness *in vacuo* The residue was dissolved in MeOH (5 ml) and 10% Pd-C (25 mg) was added to the solution The mixture was vigorously stirred overnight under hydrogen The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* The residue was purified by chromatography on silica gel (eluted with MeOH CH_2Cl_2 25% aqueous NH_3 = 5 100 1) to give 6 as a colorless foam (176 3 mg, 0 466 mmol, 82% yield) $[\alpha]_D^{21\,0}$ +35 0 ° (c = 1 73, $CHCl_3$), IR (neat) 3355, 2976, 1677, 1509, 1436, 1391, 1366 cm^{-1}, 400 MHz ^1H NMR ($CDCl_3$) δ 1 44 (9H, s), 2 77 (1H, dd, J = 8 1, 12 2 Hz), 3 07 (6H, s), 3 14 (1H, dd, J = 3 7, 12 2 Hz), 3 90 (1H, d, J = 14 3 Hz), 3 96 (3H, s), 3 97 (1H, d, J = 14 3 Hz), 4 15 (1H, br m, J = 5 1 Hz), 5 64 (1H, br s), 5 82 (1H, br d, J = 5 1 Hz), 6 63 (1H, br d, J = 2 2 Hz), 7 29 (1H, d, J = 2 2 Hz), 7 86 (1H, br s), FABMS *m/z* 396 (MH^+)

Methyl 6-[[*N*-[(2*S*)-2-carbamoyl-2-[(benzyloxycarbonyl)amino]ethyl]-*N*-(benzyloxycarbonyl)amino]methyl]-4-dimethylaminopyridine-2-carboxylate (7) Trifluoroacetic acid (3 ml) was added to a solution of Boc derivative 6 (157 4 mg, 0 398 mmol) in CH_2Cl_2 (3 ml) at 0°C After being stirred at 0°C for 30 min then at room temperature for 2 h, the solution was concentrated *in vacuo* C_6H_6 (5 ml) was added to the solution and the resulting mixture was concentrated *in vacuo* The residue was dissolved in CH_2Cl_2 (3 ml) Et_3N (0 249 ml, 1 79 mmol) and benzyl *S*-(4,6-dimethylpyrimidin-2-yl) thiocarbonate (CBZ-S)[14] (273 0 mg, 0 99 mmol) were successively added to the solution at 0°C After being stirred at 0°C for 30 min then at room temperature overnight, the solution was concentrated *in vacuo* The residue was purified by chromatography on silica gel (eluted with CH_2Cl_2 followed by MeOH CH_2Cl_2 = 1 20) to give 7 as faint yellow foam (200 7 mg, 0 356 mmol, 90% yield) $[\alpha]_D^{23\,0}$ +34 8 ° (c = 1 025, $CHCl_3$), IR (neat) 3320, 2950, 1714, 1606, 1514, 1454, 1434, 1415, 1392, 740, 699 cm^{-1}, 400 MHz ^1H NMR (DMSO-d_6) δ 2 95 (3H, s), 2 96 (3H, s), 3 47 (1H, m), 3 75 (1H, m), 3 82 (3H, s), 4 31 (1H, m), 4 43 (1H, dd, J = 6 6, 16 9 Hz), 4 51 (1H, d, J = 16 9 Hz), 5 00 ~ 5 09 (4H, m), 6 46 (1H, br) 7 13 (2H, s), 7 19 ~ 7 44 (10H, m), 7 54 (2H, br s), FABMS *m/z* 564 (MH^+)

N^{α}-[6-[[*N*-[(2*S*)-2-Carbamoyl-2-[(benzyloxycarbonyl)amino]ethyl]-*N*-(benzyloxycarbonyl)amino]methyl]-4-dimethylaminopyridine-2-carbonyl]-*erythro*-β-*tert*-

butoxy-L-histidine *tert*-butyl ester (9). 0 2 N LiOH (3 7 ml) was added to a solution of ester 7 (350 2 mg, 0 621 mmol) in MeOH (6 ml) at 0°C The solution was stirred at 0°C for 30 min then at room temperature overnight, neutralized with 0 2 N HCl, and concentrated *in vacuo* The residue and amine 8•2HCl[7] (216 2 mg, 0 607 mmol) were dissolved in DMF (8 ml) under argon DPPA[15] (0 20 ml, 0 932 mmol) and Et_3N (0 32 ml, 2 26 mmol) were successively added to the solution at 0°C After being stirred at 0°C for 2 h then at room temperature for 2 days, the solution was concentrated *in vacuo* The residue was partitioned between water and AcOEt and the aqueous layer was further extracted with AcOEt The AcOEt solutions were combined, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* The residue was purified by chromatography on silica gel (eluted with MeOH CH_2Cl_2 = 1 20) to give **9** as colorless foam (366 5 mg, 0 45 mmol, 74% yield) $[\alpha]_D^{21.0}$ +48 2 ° (c = 1 455, $CHCl_3$), IR (neat) 3323, 2976, 1682, 1608, 1520, 1392, 1368, 1155, 1126, 754 cm^{-1}, 400 MHz ^1H NMR (DMSO-d_6) δ 1 10 (9H, s), 1 30 (9H, s), 2 89 (3H, s), 2 95 (3H, s), 3 4 ~ 3 7 (1H, br m), 3 80 (1H, br m), 4 40 (2H, m), 4 72 (1H, br m), 4 96 (1H, d, J = 4 8 Hz), 5 04 (4H, m), 5 12 (1H, s), 6 41 (1H, br), 7 02 (1H, br), 7 13 (2H, s), 7 22 ~ 7 38 (13H, m), 7 54 (1H, s), 7 59 (1H, d, J = 11 8 Hz), 9 07 (1H, br), FABMS *m/z* 815 (MH^+)

***N*$^{\alpha}$-[6-[[*N*-[(2*S*)-2-Amino-2-carbamoylethyl]amino]methyl]-4-dimethylamino-pyridine-2-carbonyl]-*erythro*-β-*tert*-butoxy-L-histidine *tert*-butyl ester (PYML-8).** Pd-black (130 mg) and ammonium formate (254 7 mg, 4 04 mmol) were successively added to a solution of bis-Z derivative **9** (329 1 mg, 0 404 mmol) in MeOH (5 ml) at 0°C The solution was allowed to gradually warm to room temperature, stirred for 70 min at the same temperature, and filtered through celite The filtrate was concentrated *in vacuo* The residue was purified by chromatography on silica gel (eluted with MeOH CH_2Cl_2 25% aqueous NH_3 = 20 180 1) to give PYML-8 as white powder (182 5 mg, 0 334 mmol, 83 % yield) $[\alpha]_D^{24.5}$ +17 2 ° (c = 1 115, MeOH), IR (KBr) 3384, 2976, 2928, 1734, 1670, 1609, 1522, 1392, 1366, 1154 cm^{-1}, 400 MHz ^1H NMR (CD_3OD) δ 1 18 (9H, s), 1 42 (9H, s), 2 77 (1H, dd, J = 7 3, 12 1 Hz), 2 92 (1H, dd, J = 5 1, 12 1 Hz), 3 05 (6H, s), 3 52 (1H, dd, J = 5 1, 7 3 Hz), 3 82 (1H, d, J = 14 3 Hz), 3 86 (1H, d, J = 14 3 Hz), 4 78 (1H, d, J = 5 9 Hz), 5 17 (1H, d, J = 5 9 Hz), 6 71 (1H, d, J = 2 6 Hz), 7 06 (1H, s), 7 23 (1H, d, J = 2 6 Hz), 7 64 (1H, s), FABMS *m/z* 547 (MH^+)

Oxidation of *cis*-β-methylstyrene with PYML-8-Fe(III)-H_2O_2 A solution of PYML-8 (1 1 mg, 2 01 μmol) in MeOH (1 ml), *cis*-β-methylstyrene (55 μl, 428 μmol), and 1 20 M H_2O_2 (50 μl, 60 0 μmol) were successively added to a solution of $Fe(ClO_4)_3$•$6H_2O$ (1 0 mg, 2 16 μmol) in MeOH (2 ml) under argon After being stirred at room temperature for 30 min, the solution was partitioned between brine and CH_2Cl_2 The aqueous layer was further extracted with CH_2Cl_2 The organic layers was combined, dried over Na_2SO_4, and concentrated *in vacuo* below 25°C The residue was dissolved in AcOEt and the insoluble material was removed by filtration The AcOEt solution was analyzed by gas chromatography to determine the yields of the oxidation products (column 120 °C, injector 160 °C)

Ee of the epoxide was determined as follows A solution of PYML-8 (11 mg, 20 1 μmol) in MeOH (5 ml), *cis*-β-methylstyrene (250 μl, 1 946 mmol), and 1 14 M H_2O_2 (0 50 ml, 570 μmol) were successively added to a solution of $Fe(ClO_4)_3$•$6H_2O$ (9 5 mg, 20 5 μmol) in MeOH (5 ml) under argon After being stirred at room temperature for 30 min, the reaction mixture was worked up as

described above *cis*-β-Methylstyrene oxide was isolated by chromatography on silica gel (eluted with hexane followed by Et_2O hexane = 1 30) Ee of the epoxide was determined by ^1H NMR measurement using tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium (III) [18]

Oxidation of *cis*-β-methylstyrene with PYML-8-Fe(III)-PhIO. A solution of PYML-8 (1 1 mg, 2 01 μmol) in MeOH (1 ml), *cis*-β-methylstyrene (55 μl, 428 μmol), and PhIO (13 2 mg, 60 0 μmol) were successively added to a solution of $Fe(ClO_4)_3 \cdot 6H_2O$ (1 0 mg, 2 16 μmol) in MeOH (2 ml) under argon After the misture was stirred at room temperature for 30 min, a material obtained by the extractive work up was analyzed by gas chromatography (column 200 °C, injector 200 °C)

Oxidation of *cis*-β-methylstyrene with PYML-8-Fe(II)-O_2 A 23 0 μM aqueous solution of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (0 1 ml), *cis*-β-methylstyrene (50 μl, 389 μmol), and a 0 102 mM solution of 2-mercaptoethanol in MeOH (1 ml) [a 99 8 μM solution of 1,4-dithiothreitol in MeOH (1 ml) or sodium L-ascorbate (20 mg)] were succesively added to a solution of PYML-8 (1 2 mg, 2 20 μmol) in MeOH (3 ml) After being stirred at room temperature for 2 h in the presence of air, a material obtained by the extractive work up was analyzed by gas chromatography (column 120 °C, injector 160 °C)

Ee of the epoxide was determined as follows *cis*-β-Methylstyrene (200 μl, 1 56 mmol) and 2-mercaptoethanol (85 8 mg, 1 10 mmol) were successively added to a solution of PYML-8 (30 mg, 54 9 μmol) and $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (26 mg, 56 2 μmol) in MeOH (10 ml) After the mixture was stirred at room temperature for 2 h in the presence of air, epoxide isolated as described above was subjected to ^1H NMR measurement using tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium (III) [18]

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REFERENCES AND NOTES

1 Synthetic Studies on Antitumor Antibiotic, Bleomycin XXXI For Part XXX, see Owa, T, Sugiyama, T , Otsuka, M , Ohno, M, Maeda, K *Tetrahedron Lett* , in press

2 Umezawa, H , Maeda, K , Takeuchi, T , Okami, Y *J Antibiot Ser A*, **1966**, *19*, 20

3 Takita, T , Muraoka, Y , Nakatani, T , Fujii, A , Umezawa, Y , Naganawa, H , Umezawa, H *J Antibiot* **1978**, *31*, 801

4 (a) Sugiura, Y , Takita, T , Umezawa, H In "Metal Ions in Biological Systems", Sigel, H , Ed , Marcel Dekker New York, 1985 pp81-108 (b) Hecht, S M *Acc Chem Res* , **1986**, *19*, 383 (c) Stubbe, J , Kozarich, J W *Chem Rev* , **1987**, *87*, 1107

5 Kittaka, A , Sugano, Y , Otsuka, M , Ohno, M , Sugiura, Y , Umezawa, H *Tetrahedron Lett* , **1986**, *27*, 3631

6 Ohno, M , Otsuka, M , Kittaka, A , Sugano, Y , Sugiura, Y , Suzuki, T , Kuwahara, J , Umezawa, K , Umezawa, H *Int J Exp Clin Chemotherapy*, **1988**, *1*, 12

7 Kittaka, A , Sugano, Y , Otsuka, M , Ohno, M *Tetrahedron*, **1988**, *44*, 2811

8 Sugano, Y , Kittaka, A , Otsuka, M, Ohno, M , Sugiura, Y , Umezawa, H *Tetrahedron Lett* , **1986**, *27*, 3635

9 Kittaka, A , Sugano, Y , Otsuka, M , Ohno, M *Tetrahedron*, **1988**, *44*, 2821

10 Synthesis of PYML-8 was preliminarily reported, see Suga, A , Sugiyama, T , Sugano, Y , Kittaka, A , Otsuka, M , Ohno, M , Sugiura, Y , Maeda, K *Synlett*, **1989**, 70

11 Sugano, Y M Sc Dissertation, University of Tokyo, 1985

12 Otsuka, M , Kittaka, A , Iimori, T , Yamashita, H , Kobayashi, S , Ohno, M *Chem Pharm Bull* , **1985**, *33*, 509

13 Zervas, L , Borovas, D , Gazis, E *J Am Chem Soc* , **1963**, *85*, 3660

14 Nagasawa, T , Kuroiwa, K , Narita, K , Isowa, Y *Bull Chem Soc Japan*, **1973**, *46*, 1269

15 Shioiri, T , Ninomiya, K , Yamada, S *J Am Chem Soc* , **1972**, *94*, 6203

16 (a) Murugesan, N, Ehrenfeld, G M , Hecht, S M *J Biol Chem* **1982**, *257*, 8600 (b) Aoyagi, Y , Suguna, N , Murugesan, N , Ehrenfeld, G M , Chang, L H , Ohgi, T , Shenkhai, M , Kirkup, M P , Hecht, S M *J Am Chem Soc* , **1982**, *104*, 5237 (c) Ehrenfeld, G M , Murugesan, N , Hecht, S M *Inorg Chem* , **1984**, *23*, 1496 (d) Murugesan, N , Hecht, S M *J Am Chem Soc* , **1985**, *107*, 493 (e) Moriarty, R M , Penmasta, R , Prakash, I *Tetrahedron Lett* , **1985**, *26*, 4699 (f) Heimbrook, D C , Moulholland Jr , R L , Hecht, S M *J Am Chem Soc* , **1986**, *108*, 7839 (g) Heimbrook, D C , Carr, S A , Mentzer, M A , Long, E C , Hecht, S M *Inorg Chem* , **1987**, *26*, 3835 (h) Girardet, M , Meunier, B , *Tetrahedron Lett* , **1987**, *28*, 2955 (i) E C Long, Hecht, S M *Tetrahedron Lett* , **1988**, *29*, 6413

17 Kaku, Y , Otsuka, M , Ohno, M *Chem Lett* , **1989**, 611

18 Duration of this reaction was 30 minutes Oxidation of *trans*-alkene gave results apparently dependent on the reaction time When the reaction was continued for 2 hours, *trans*-epoxide could hardly be detected presumably due to the breakdown of the epoxide during the prolonged reaction time under this condition [8,16] In the oxidation of *cis*-alkene, the yields and composition of products were not affected by the reaction time

19 (a) Fraser, R R , Petit, M A , Saunders, J K *J Chem Soc , Chem Commun* , **1971**, 1450 (b) Castedo, L , Castro, J L , Eiguera, R *Tetrahedron Lett* , **1984**, *25*, 1206

20 (a) Castellino, A J , Bruice, T C *J Am Chem Soc* , **1988**, *110*, 158 (b) Ostovic, D , Bruice, T C *J Am Chem Soc* , **1989**, *111*, 6511

21 Barton, D H R , Lee, K W , Mehl, W , Ozbalik, N , Zhang, L *Tetrahedron*, **1990**, *46*, 3753