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SYNTHETIC MODELS FOR THE TRANSITION METAL BINDING SITE OF BLEOMYCIN. REMARKABLE IMPROVEMENT OF DIOXYGEN ACTIVATING CAPABILITY[1]

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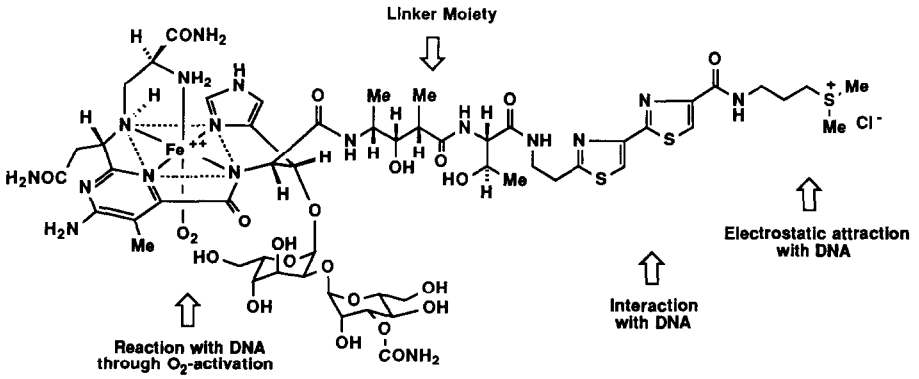
Abstract - Model compounds for the metal binding site of bleomycin with a CH_2CONH_2 group (PYML-3) or a tert-butyl group (PYML-4) as[2] steric environmental factors are synthesized. These exhibit metal binding properties similar to those of bleomycin. In particular, PYML-4 shows oxygen activation up to 71 % of that of bleomycin.

Bleomycins (BLMs) are a family of glycopeptide antitumor antibiotics discovered by H. Umezawa et al. from cultures of Streptomyces verticillus in 1966[2,3,4] and are now clinically used in the treatment of Hodgkin's lymphoma, carcinomas of the skin, head and neck, and tumors of testis.[5] BLM induces sequence specific cleavage of cellular DNA owing to the following two chemical characteristics; (1) dioxygen activation by the ferrous complex of the β-aminoalanine-pyrimidine-β-hydroxyhistidine moiety[6,7] and (2) binding to DNA by the bithiazole and the terminal amine residues[8] as shown in Figure 1. As BLM contains many heteroatoms which can coordinate iron, a number of possible structures for the BLM-metal chelate were proposed on the basis of theoretical and spectroscopic studies.[4,9] On the other hand, the disaccharide moiety and the CH_2CONH_2 group at the α-position of the pyrimidine ring seem to play an important role as the environmental factors in the effective dioxygen activation, just as the pivalimidophenyl groups in the picket fence porphyrins.[10] It was considered that such metal binding property of BLM can be

Figure 1. Proposed structure of BLM-Fe(II)-O_2 complex and implication for the mechamism of action.



best investigated by use of artificially designed models. We already have developed a strategy for the elaboration of the pyrimidine moiety,[11,12] which enabled us to prepare various BLM-like peptides. Herein we detail the design and synthesis of BLM models to study the chelation chemistry of the antibiotic.[13]

Previously we have reported the first synthetic model ligand, PYML-1 (Figure 2),[14] which has the following structural characteristics; (1) replacement of the pyrimidine nucleus of BLM by a pyridine nucleus, (2) use of a simplified side chain without CH_2CONH_2 appendage, and (3) removal of the disaccharide and the bithiazole-terminal amine moieties. The oxygen activating capability of PYML-1-Fe(II) complex was demonstrated by a ESR spin trapping experiment and the radical spin concentration of the PYML-1-Fe(II)-O_2 complex system was estimated to be approximately 18% of that of the BLM-Fe(II)-O_2 complex system, indicating PYML-1 to be the simplified structural unit for the dioxygen activation. As chelated BLM has the CH_2CONH_2 group and the disaccharide in the vicinity of the metal center (Figure 1), we assumed that these substituents may have large influence on the metal binding property. In particular, the significance of the CH_2CONH_2 group was suggested by the remarkably lowered biological activity of epi-BLM (Figure 3), which possesses the CH_2CONH_2 group with epimeric (R)-configuration.[15] Thus, we planned a synthesis of a model ligand with a CH_2CONH_2 group, namely PYML-3 (Figure 2).

Figure 2. Synthetic models for the metal binding site of BLM.

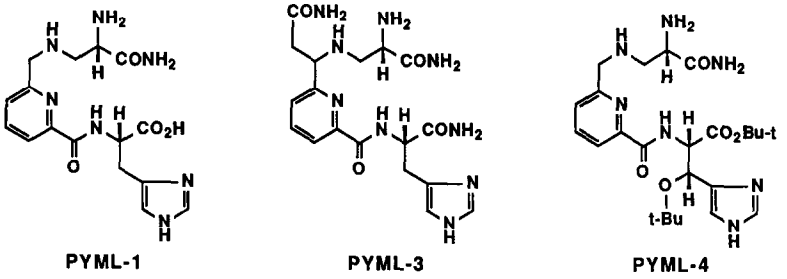
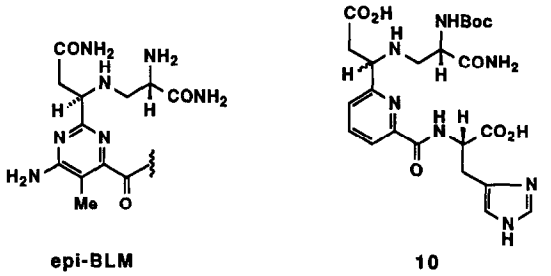
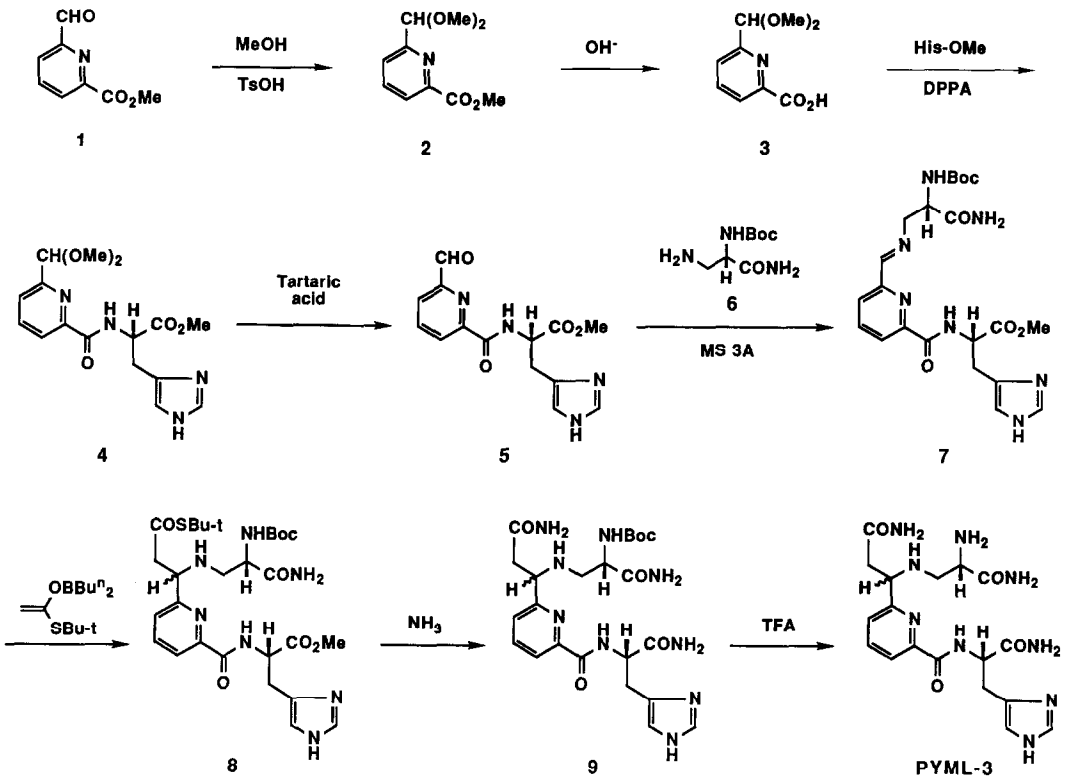


Figure 3.



In order to construct the β-aminopropionamide moiety of BLM, we reported the reaction of Schiff base with vinyloxyborane or malonic monoester.[11] The vinyloxyborane methodology was successfully applied to the synthesis of PYML-3 (Scheme 1). Thus, aldehyde **1**[16] was converted into dimethyl acetal **2** (86%), which was hydrolyzed to acid **3** (quantitative). Coupling of the acid **3** and

Scheme 1. Synthesis of PYML-3.



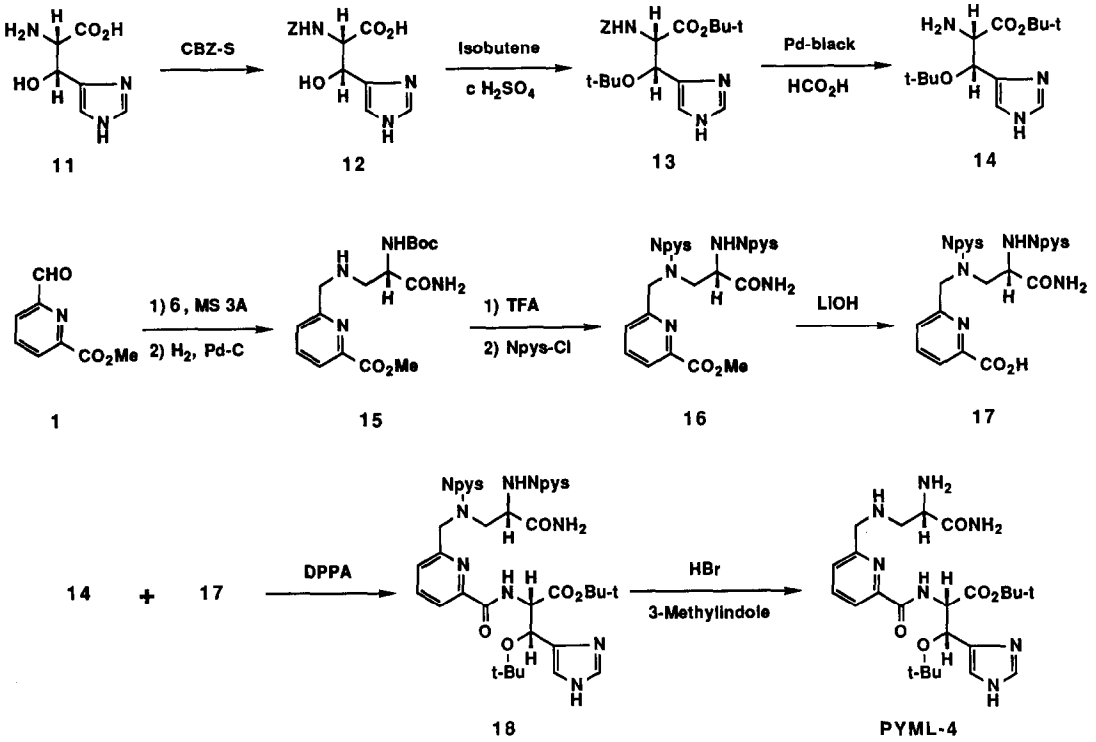
L-histidine methyl ester[17] was carried out using diphenyl phosphoroazidate (DPPA)[18] and dipeptide **4** was obtained in 93% yield. Hydrolysis of acetal **4** was effected with saturated tartaric acid, furnishing the aldehyde **5** in 76% yield. Reaction of amine **6**[19] with the aldehyde **5** in the presence of activated molecular sieves 3A in acetonitrile gave Schiff base **7** (95% yield) which was treated with vinyloxyborane[11] to give thiol ester **8** in 45% yield. Amination of **8** followed by deprotection with trifluoroacetic acid (TFA) gave PYML-3 in 48% yield. PYML-3 thus obtained seemed to be a 1:1 mixture of two inseparable isomers because of the following observations; (1) high performance TLC analysis of **8** indicated that **8** is a mixture of two products, and (2) diacid **10** (Figure 3), derived from **8** by alkaline hydrolysis, was shown to be a 1:1 mixture of two isomers by HPLC. ESR measurements of the transition metal complexes of PYML-3 were carried out according to the reported procedure.[14b] It was noteworthy that the low spin PYML-3-Fe(III)-OH^- complex was observed by ESR spectroscopy (g_1 = 2.353, g_2 = 1.196, g_3 = 1.904) whereas the corresponding PYML-1-Fe(III)-OH^- complex could not be detected. The PYML-3-Fe(II)-^{14}NO complex exhibited ESR parameters (g_1 = 2.038, g_2 = 2.008, g_3 = 1.970, A^N = 25.0 G) similar to those for the PYML-1-Fe(II)-^{14}NO complex (g_1 = 2.036, g_2 = 2.009, g_3 = 1.972, A^N = 25.6 G). The presence of two diastereomeric species of the PYML-3-Cu(II) complex was suggested by its ESR spectrum which consists of two signals, i. e., $g_{\parallel}$ = 2.402, $g_{\perp}$ = 2.074, $A_{\parallel}$ = 117.7 G, and $g_{\parallel}$ = 2.208, $g_{\perp}$ = 2.060, $A_{\parallel}$ = 182.2 G, the latter being close to the signal of the BLM-Cu(II) complex ($g_{\parallel}$ = 2.211, $g_{\perp}$ = 2.055, $A_{\parallel}$ = 183.0 G). The present results indicated that the CH_2CONH_2 group contributes

to the stabilization of the metal complex.

On the other hand, the disaccharide moiety of BLM appeared to be particularly important as a major steric factor. We designed PYML-4 (Figure 2), which is promising in forming a hydrophobic cavity to accomodate dioxygen by the bulky tert-butyl ether in place of the disaccharide of BLM.[20]

The synthesis of PYML-4 was carried out by coupling acid **17** and amine **14**, as shown in Scheme 2. erythro-β-Hydroxy-L-histidine **11**[21] was treated with CBZ-S[22] to give benzyloxycarbonyl (Z) derivative **12** quantitatively. tert-Butyl groups were successfully introduced into **12** by treatment with isobutene and **13** was obtained in 68% yield based on **11**. Removal of the Z group in **13** was effected with formic acid and Pd-black, furnishing the amine **14** quantitatively. On the other hand, the acid **17** was synthesized as follows. tert-Butoxycarbonyl (Boc) protected intermediate **15** was prepared from **1** via the procedure developed for the synthesis of PYML-1.[14] The Boc group was then replaced by a 3-nitro-2-pyridinesulfenyl (Npys) group for the selective removal at the final stage. Thus, the Boc derivative **15** was treated with TFA and the resulting amine was reprotected with Npys-Cl[23] to give bis(Npys) derivative **16** in 64% yield. The methyl ester group of **16** was smoothly hydrolyzed to carboxylic acid **17** with lithium hydroxide. Condensation of the acid **17** with the amine **14** was effected by DPPA[18] to give dipeptide **18** in 65% yield. The two Npys groups in **18** were selectively removed by acid treatment in the presence of 3-methylindole and PYML-4 was obtained in 68% yield.

Scheme 2. Synthesis of PYML-4.



The ESR spin trapping experiments by oxygen bubbling of the PYML-4-Fe(II) complex in the presence of N-tert-butyl-α-phenylnitrone at pH 8.3 clearly revealed the generation of hydroxyl radicals.[20] As expected, the dioxygen

activating ability of the PYML-4-Fe(II) complex increased up to 71% of that of BLM. The tert-butyl group was shown to exert a profound steric and hydrophobic effect in improving the interaction with oxygen. This finding allowed us to design the more advanced models equipped with other functional moieties which will be described in the following paper.[24]

EXPERIMENTAL

Melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP 140 instrument. ^1H NMR (100 MHz) spectra were recorded on a JEOL FX-100 spectrometer. Abbreviations are as follows; s (singlet), d (doublet), t (triplet) and q (quartet). IR spectra were recorded on a JASCO DS-402G or JASCO A-102 spectrometer. Low resolution and fast atom bombardment mass spectra (FABMS) were recorded on a JEOL JMS DX-300 spectrometer. Secondary ion mass spectra (SIMS) were recorded on a Hitachi RMU-6M or -7M spectrometer. HPLC was carried on a JASCO TRI ROTAR SR model equipped with a 254 nm fixed-wavelength detector. The reagents and solvents were purified by standard procedures.

Methyl 6-(Dimethoxymethyl)pyridine-2-carboxylate **2**.

A solution of aldehyde **1** (3.15 g, 1.91 mmol) and p-toluenesulfonic acid (31 mg) in MeOH (95 ml) was heated at reflux for 3 h. The solution was concentrated to 10 ml and the residual solution was partitioned between AcOEt and sat $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was crystallized from Et_2O-hexane to give **2** as colorless needles (3.47 g, 86%).

Mp 66.0-67.0°C; ^1H NMR ($CDCl_3$, TMS) δ 3.43 (6H, s), 4.01 (3H, s), 5.46 (1H, s), 7.68-8.18 (3H, m); IR ($CHCl_3$) 2990, 2950, 2830, 1725, 1590, 1440 cm^{-1}; Anal. calcd. for $C_{10}H_{13}O_4N$, C 56.86, H 6.20, N 6.63: found, C 56.76, H 6.19, N 6.74; MS m/e 210 (M^+).

N^{α}-[6-(Dimethoxymethyl)pyridine-2-carbonyl]-L-histidine Methyl Ester **4**.

A mixture of NaOH (1 M, 5 ml) and MeOH (5 ml) was added dropwise to a solution of **2** (1.06 g, 5.00 mmol) in aq MeOH (95%, 10 ml) at 0°C. After being stirred at room temperature for 1 h, the solution was concentrated in vacuo to remove MeOH. The resulting aqueous solution was acidified to pH 3 with HCl (1 M) at 0°C and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give **3** as a colorless oil (quantitative).

Et_3N (1.21 ml, 8.72 mmol), DPPA[18] (1.37 g, 4.57 mmol) and further Et_3N (0.64 ml, 4.75 mmol) were successively added to a suspension of **3** (817.9 mg, 4.15 mmol) and L-histidine methyl ester hydrochloride[17] (1.11 g, 4.57 mmol) in DMF (65 ml) at 0°C under argon with vigorous stirring. After being stirred for 4 days at room temperature, the solution was concentrated in vacuo. The residue was purified by chromatography on silica gel eluted with 5-10% MeOH/CH_2Cl_2 to give **4** as a yellow foam (1.34 g, 93%).

$[\alpha]_D^{24}$ +19.5° (c 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 3.16 (2H, d, J = 5.5 Hz), 3.39 (3H, s), 3.40 (3H, s), 3.65 (3H, s), 4.97 (1H, br m), 5.33 (1H, s), 6.73 (1H, s), 7.44 (1H, s), 7.56-8.18 (3H, m), 8.85 (1H, br d, J = 8.0 Hz); IR ($CHCl_3$) 3460, 3370, 2990, 2950, 2830, 1740, 1670, 1595, 1524, 1440 cm^{-1}; MS m/e 348 (M^+).

N^{α}-(6-Formylpyridine-2-carbonyl)-L-histidine Methyl Ester **5**.

Acetal **4** (1.65 g, 4.74 mmol) was dissolved in sat aq tartaric acid (20 ml) at 0°C. After being stirred overnight at room temperature, the solution was neutralized with sat $NaHCO_3$ and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by chromatography on silica gel eluted with 5% $MeOH/CH_2Cl_2$ to give **5** as a white foam (1.09 g, 76%).

$[\alpha]_D^{23}$ +38.3° (c 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 3.27 (2H, d, J = 5.5 Hz), 3.73 (3H,s), 5.05 (1H, br m), 6.86 (1H, s), 7.84 (1H, s), 7.70-8.44 (4H, m), 9.10 (1H, br d, J = 7.5 Hz); IR (KBr) 3370, 1745, 1665, 1520, 1440 cm^{-1}; MS m/e 302 (M^+).

N^{α}-[6-[[[(S)-2-[(tert-Butoxycarbonyl)amino]-2-carbamoylethyl]imino]-methyl]pyridine-2-carbonyl]-L-histidine Methyl Ester **7**.

A solution of amine **6**[19] (331.3 mg, 1.63 mmol) in CH_3CN (10 ml) was added dropwise to a vigorously stirred mixture of **5** (492.9 mg, 1.63 mmol) and activated molecular sieves 3A (3 g) in CH_3CN (10 ml) at room temperature. After being stirred overnight, the molecular sieves were removed by filtration. The resulting solution was concentrated in vacuo below 30°C to give **7** as a white foam (777 mg, 95%) which was used for the next step without further purification.

N^{α}-[6-[(RS)-1-[[(S)-2-[(tert-Butoxycarbonyl)amino]-2-carbamoylethyl]-amino]-2-(tert-butylthiocarbonyl)ethyl]pyridine-2-carbonyl]-L-histidine Methyl Ester **8**.

Diisopropylethylamine (2.97 ml, 1.71 mmol) and CH_3COSBu^t (2.05 g, 15.5 mmol) were successively added to a solution of Bu_2BOTf[25] (4.25 g, 15.5 mmol) in Et_2O (40 ml) at -78°C under argon. After being stirred for a further 30 min at the same temperature, a solution of **7** (777 mg, 1.55 mmol) in CH_2Cl_2:Et_2O = 1:9 was added dropwise to the reaction mixture at -78°C under argon. The solution was allowed to warm to room temperature. After being stirred vigorously for a further 1 h at the same temperature, the solution was partitioned between AcOEt (20 ml) and water (20 ml) - H_2O_2 (30%, 10 ml). The organic layer was washed with aq $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified with preparative TLC ($MeOH:CH_2Cl_2$ = 1:7) to give **8** as a pale yellow foam (430 mg, 45%).

^1H NMR ($CDCl_3$, TMS) δ 1.41 (9H, s), 1.43 (9H, s), 2.50-2.90 (2H, m), 2.84-3.08 (2H, m), 3.26 (2H, br s), 3.64 (1H, m), 3.72 (3H, s), 4.18-4.40 (1H, m), 4.80-5.10 (1H, m), 5.92-6.17 (1H, m), 6.18-6.60 (2H, br s), 6.85 (1H, s), 7.30-7.45 (1H, m), 7.42-8.08 (3H, m), 9.24 (1H, m); IR ($CHCl_3$) 3400, 2960, 1730, 1685, 1675, 1590, 1515, 1405, 1365 cm^{-1}; SIMS m/z 642 (MNa^+), 620 (MH^+).

N^{α}-[6-[(RS)-1-[[(S)-2-[(tert-Butoxycarbonyl)amino]-2-carbamoylethyl]-amino]-2-carbamoylethyl]pyridine-2-carbonyl]-L-histidinamide **9**.

A solution of **8** (120.8 mg, 0.195 mmol) in MeOH (2 ml) was bubbled with NH_3 at -78°C. After 40% increase of the volume, the solution was stirred for 2 days in a sealed bulb at room temperature. The bulb was cooled again and opened and the soloution was concentrated in vacuo. The residue was purified by preparative TLC ($AcOEt:EtOH:H_2O$ = 3:1:1) to give **9** as white powder (54.7 mg, 53%).

^1H NMR (D_2O, TPS) δ 1.24-1.50 (9H, m), 2.56-3.08(4H, m), 3.16-3.40 (3H, m), 3.80-4.48 (2H, m), 7.08 (1H, s), 7.56-8.07 (3H, m), 7.80 (1H, s); IR (KBr) 3360, 2980, 1685, 1660, 1525, 1515, 1250 cm^{-1}; SIMS m/z 554 (MNa^+), 532 (MH^+).

N^{α}-[6-[(RS)-1-[[(S)-2-[(tert-Butoxycarbonyl)amino]-2-carbamoylethyl]-amino]-2-carboxyethyl]pyridine-2-carbonyl]-L-histidine **10**.

A mixture of NaOH (1 M, 0.62 ml) and MeOH (0.62 ml) was added to a solution of **8** (174.9 mg, 0.282 mmol) in MeOH (2 ml) at 0°C. After being stirred overnight at room temperature, the solution was concentrated in vacuo to remove MeOH. The resulting aqueous solution was charged on a column of Dowex 1x2 (OH^- form, 20 ml). The column was washed with water (100 ml) and eluted with a linear gradient of water-4%AcOH. Pauly-positive fractions were collected and concentrated in vacuo to give **10** as a colorless solid (114.4 mg, 76%). A solution of **10** (5 mg) and $Cu(OAc)_2 \cdot H_2O$ (1 eq) in water (1 ml) was subjected to HPLC (Hibar RT 250-4 column, 4.5 mm x 250 mm, reverse phase; MeOH:0.1M Et_3N AcOH = 1:9; flow rate 1.0 ml/min) and the specimen was shown to be a 1:1 mixture of two isomers (retention time, 11.6 min and 12.5 min).

PYML-3.

A solution of **9** (45.6 mg, 0.0858 mmol) in TFA (1 ml) was stirred at 0°C for 40 min then at room temperature for 20 min. TFA was removed in vacuo and Et_2O (3 ml) was added to the residue. After being stirred overnight at room temperature, white precipitate was collected, dissolved in water (1 ml) and charged on a column of DIAION WK 10 (H^+ form, 10 ml). The column was washed with water (60 ml) and eluted with aq NH_3 (1%). UV-, ninhydrin-, Dragendorff-, and Pauly-positive fractions were collected and concentrated in vacuo to give PYML-3 as white powder (33.9 mg, 92%).

Rf 0.47 (MeOH:10%NH_4OAc = 1:1); ^1H NMR (CD_3OD, TMS) δ 2.50-2.80 (4H, m), 3.13-3.28 (3H, m), 3.98-4.06 (1H, m), 4.12-4.32 (1H, m), 6.86 (1H, s), 7.40-7.98 (3H, m), 7.58 (1H, s); IR (KBr) 3380, 3180, 1655, 1515 cm^{-1}; SIMS m/z 454 (MNa^+), 432 (MH^+).

Methyl 6-[[N-[(S)-2-Carbamoyl-2-[(3-nitro-2-pyridylthio)amino]ethyl]-N-(3-nitro-2-pyridylthio)amino]methyl]pyridine-2-carboxylate **16**.

TFA (3 ml) was added to an ice cooled solution of **15**[14] (120 mg, 0.341 mmol) in CH_2Cl_2 (3 ml) under argon. The mixture was stirred for 50 min at room temperature and then concentrated in vacuo. To the residual oil dissolved in CH_2Cl_2 (5 ml) were added Et_3N (0.214 ml, 1.53 mmol) and Npys-Cl[23] (123.0 mg, 0.682 mmol) at 0°C under argon. The solution was stirred overnight at room temperature and then partitioned between CH_2Cl_2 and water. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography eluted with AcOEt to give **16** as a yellow oil (121.9 mg, 64%).

$[\alpha]_D^{22}$ +13.4° (c 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 3.36-3.74 (2H, m), 3.89 (3H, s), 3.90-4.04 (1H, m), 4.80 (2H, s), 5.24 (1H, br s), 5.67 (2H, s), 7.16-8.96 (9H, m); IR ($CHCl_3$) 3510, 3470, 2990, 1725, 1680, 1585, 1510, 1435, 1335 cm^{-1}; SIMS m/z 561 (MH^+).

6-[[N-[(S)-2-Carbamoyl-2-[(3-nitro-2-pyridylthio)amino]ethyl]-N-(3-nitro-2-pyridylthio)amino]methyl]pyridine-2-carboxylic Acid **17**.

A solution of LiOH (0.1 M, 3.0 ml) was added to a vigorously stirred solution of **16** (101.1 mg, 0.180 mmol) in MeOH (4 ml) at 0°C. After being stirred at room temperature for 5 h, water (5 ml) was added to the solution and the MeOH was removed in vacuo. The remaining aqueous solution was washed with AcOEt, acidified to pH 3.5 with citric acid (1 M) at 0°C and extracted with

AcOEt. The AcOEt extract was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to yield almost pure **17** as yellow powder (84.2 mg, 86%), which was used for the next step without further purification.

N^{α}-Benzyloxycarbonyl-*erythro*-β-*tert*-butoxy-L-histidine *tert*-Butyl Ester **13**.

A solution of CBZ-S[22] (158.6 mg, 0.578 mmol) in dioxane (2.4 ml) and Et_3N (0.22 ml, 1.59 mmol) was added to a vigorously stirred solution of **11** hydrochloride[21] (100 mg, 0.482 mmol) in water (2.4 ml) at 0°C. The mixture was stirred at 0°C for 1 h then at room temperature for 12 h. The solution was concentrated *in vacuo*, and the residue was partially purified by chromatography on silica gel eluted with 35-50% MeOH/$CHCl_3$ to give **12** as white powder (148 mg, quantitative) which was used for the next step without further purification.

A mixture of **12** (148 mg, 0.482 mmol), CH_2Cl_2 (5 ml), isobutene (2 ml) and c H_2SO_4 (0.2 ml) in a sealed bulb was stirred for 12 h at room temperature. The bulb was cooled to -78°C and opened. Excess Et_3N (2 ml) was immediately added and the solution was concentrated *in vacuo*. The residue was chromatographed on silica gel eluted with 3% MeOH/$CHCl_3$ to give **13** as a colorless oil (80.5 mg, 40%). Succesive elution with 50% MeOH/$CHCl_3$ gave recovered **11** which could be recycled to afford further **13** (56.9 mg, 28%) (total yield of **13**; 68% based on **11**, 137.4 mg).

$[\alpha]_D^{18}$ +36.6° (c 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 1.18 (9H, s), 1.41 (9H, s), 4.52 (1H, dd, J = 4.0, 8.5 Hz), 5.03 (1H, d, J = 4.0 Hz), 5.09 (2H, s), 5.80 (1H, d, J = 8.5 Hz), 6.90 (1H, s), 7.13 (5H, s), 7.54 (1H, s); IR ($CHCl_3$) 3430, 2970, 1720, 1510, 1150 cm^{-1}; SIMS m/z 418 (MH^+).

erythro-β-*tert*-Butoxy-L-histidine *tert*-Butyl Ester Hydrochloride **14**.

Formic acid (99%, 0.5 ml) and Pd-black (30 mg) were added to a solution of **13** (142.8 mg, 0.342 mmol) in MeOH (15 ml). The mixture was vigorously stirred under argon at room temperature for 1 h and then the catalyst was removed by filtration. The filtrate was concentrated *in vacuo* and the residue was dissolved in water (1 ml). The solution was charged on a column of DIAION SA 10A (OH^- form, 7 ml). The column was eluted with water (15 ml). The eluant was acidified to pH 4.5 with HCl (0.5 M) and concentrated *in vacuo* to yield **14** hydrochloride as colorless wax which was used for the next step without further purification (109 mg, ca 98%).

N^{α}-[6-[[N-[(S)-2-Carbamoyl-2-[(3-nitro-2-pyridylthio)amino]ethyl]-N-(3-nitro-2-pyridylthio)amino]methyl]pyridine-2-carbonyl]-*erythro*-β-*tert*-butoxy-L-histidine *tert*-Butyl Ester **18**.

Et_3N (22 μl, 0.158 mmol) and DPPA[18] (21 μl, 0.0945 mmol) were added to a suspension of **17** (24.7 mg, 0.0441 mmol) and **14** (17.3 mg, ca 0.0485 mmol) in CH_3CN (3 ml) under argon at 0°C. The mixture was stirred overnight at room temperature and was purified by preparative TLC (AcOEt:EtOH:H_2O = 3:1:1) to give **18** as a yellow oil (23.3 mg, 65%).

$[\alpha]_D^{23}$ +39.2° (c 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$, TMS) δ 1.23 (9H, s), 1.48 (9H, s), 3.44-3.78 (2H, br s), 3.80-3.96 (1H, m), 4.64-4.84 (1H, m), 4.75 (2H, s), 4.96-5.16 (1H, m), 5.28 (2H, s), 6.60 (1H, br s), 7.00 (1H, s), 7.20-8.90 (10H, m); IR ($CHCl_3$) 3450, 2970, 1730, 1675, 1585, 1512, 1335, 1155 cm^{-1}; SIMS m/z 812 (M^+).

PYML-4.

3-Methylindole (11.7 mg, 0.0895 mmol) and HBr (0.2 M, 1 ml) were

successively added to a vigorously stirred solution of **18** (29.1 mg, 0.0358 mmol) in AcOEt (3 ml) at 0°C. The mixture was stirred at room temperature for 6 h, then partitioned between AcOEt and water. The aqueous layer was washed with AcOEt and charged on a column of Amberlite IRA 93 (OH^- form, 7 ml). The column was eluted with water (15 ml) and the eluant was concentrated in vacuo and freeze-dried to give PYML-4 as white powder (12.2 mg, 68%).

Rf 0.83 (PrOH:pyridine:water:AcOH = 15:10:12:3), 0.41 (10%NH_4OAc:MeOH = 1:1); $[\alpha]_D^{21}$ +30.5° (c 0.61, MeOH); ^1H NMR (CD_3OD, TMS) δ 1.21 (9H, s), 1.43 (9H, s), 2.82-3.16 (2H, m), 3.56-4.04 (2H, m), 4.12 (2H, s), 5.19 (1H, d, J = 6.0 Hz), 7.05 (1H, s), 7.44-8.12 (4H, m); IR (KBr) 3360, 2955, 2910, 1735, 1660, 1595, 1460, 1365, 1155, 1080 cm^{-1}; FABMS 504 (MH^+).

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