TRANSITION-METAL BINDING SITE OF BLEOMYCIN. SIGNIFICANCE OF THE β-AMINOALANINAMIDE APPENDAGE IN REGULATING OXYGEN ACTIVATION†

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Abstract - A synthetic model study on the iron-binding site of bleomycin demonstrated that the dioxygen-activating properties of ligands largely depend on the characteristics of functional groups contained in the β-aminoalaninamide moiety.

It has been well documented that antitumor antibiotics bleomycins (BLMs) form iron-complex by the β-aminoalaninamide-pyrimidine-β-hydroxyhistidine region to activate molecular oxygen (Figure 1A).[3] BLM is inactivated by an enzyme BLM hydrolase which hydrolyzes the carbamoyl group of the β-aminoalaninamide moiety.[4] The resulting deamido-BLM shows remarkably lowered dioxygen activating capability due to the formation of a high spin iron-complex in which the fifth coordination site is presumably occupied by the carboxylate at the physiological pH region (Figure 1B).[5] This exemplified the significance of the β-aminoalaninamide moiety in regulating the oxygen-

† Dedicated to Professor Alan R. Katritzky on the occasion of his 65th birthday.

activating power of the iron complex. We further studied the function of the fifth ligand moiety by synthetic model approach as described below.

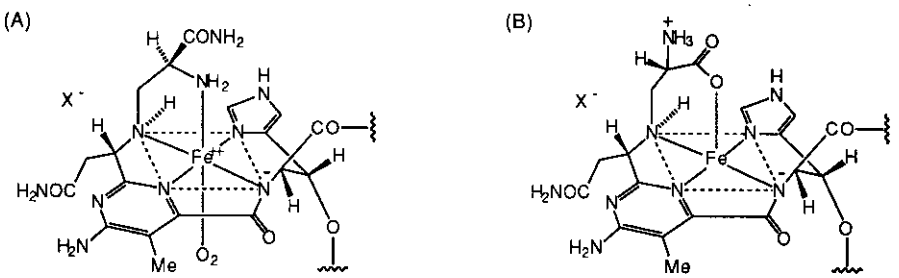
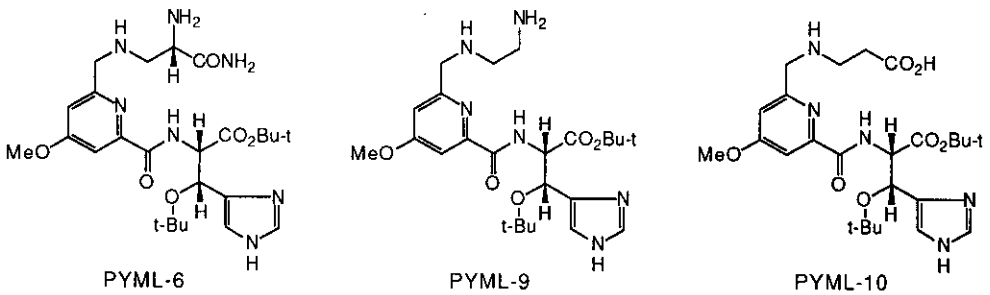


Figure 1. Probable structure of (A) BLM-Fe(II)-O_2 and (B) deamido-BLM-iron complexes.



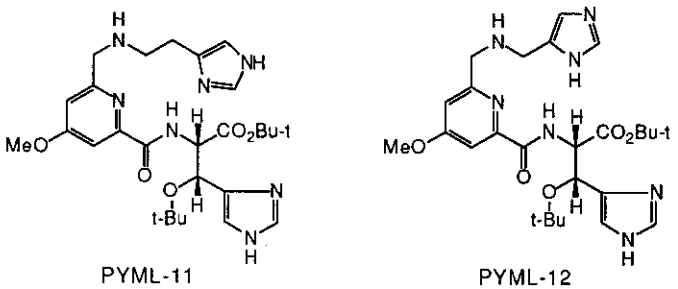


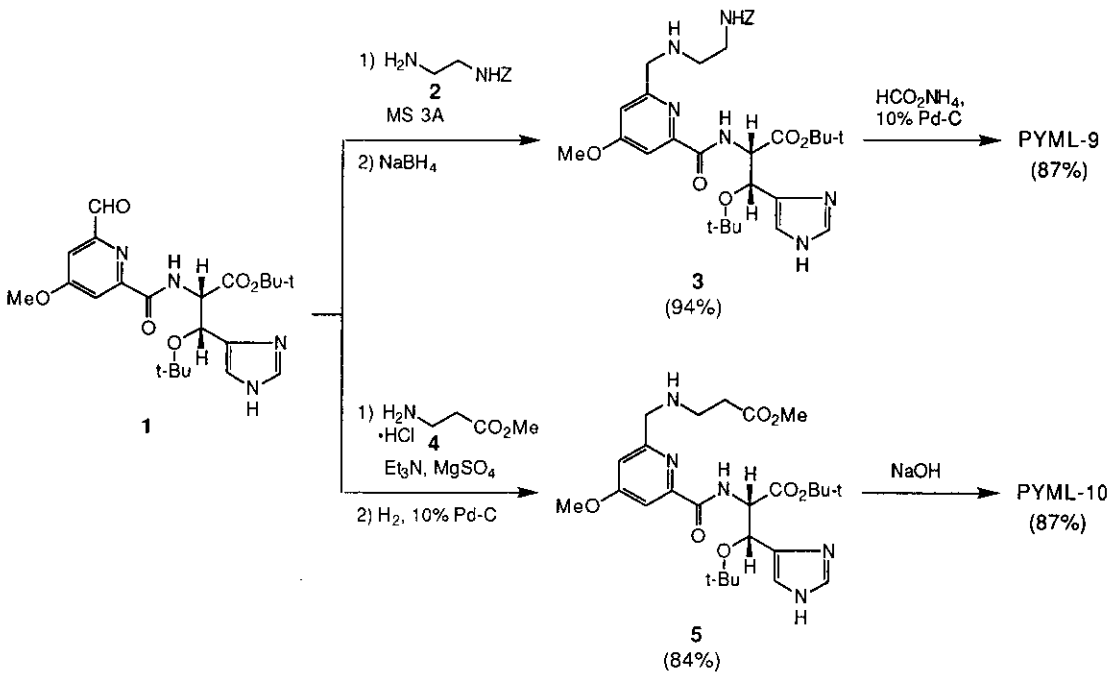
Figure 2. Synthetic models for the metal binding site of bleomycin.

In our continuing synthetic effort toward man-designed BLMs, we have developed several models including PYML-6 (Figure 2), which exhibited metal-binding and dioxygen-activating properties well in accordance with those of BLM in every respect.[6-12] Herein we report several new entries of model ligands based on PYML-6 in which the axial side chain was modified (Figure 2). PYML-9

and PYML-10 are amino and carboxyl derivatives, respectively, designed to separate and examine the function of substituents contained in the axial ligand moiety of deamido-BLM. Furthermore, in order to study the influence of the electronic characteristics of the axial donor on the oxygen activating property, we also prepared imidazole derivatives PYML-11 and PYML-12.

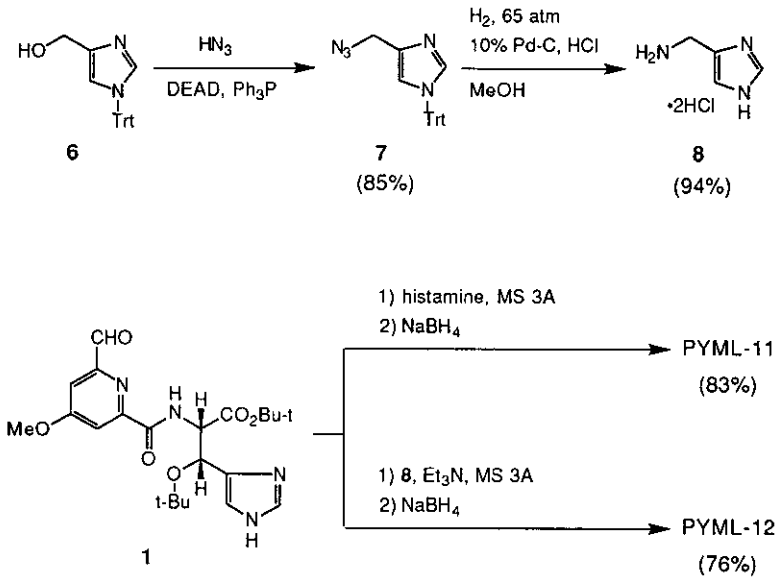
Previously we prepared PYML-6 and other model ligands by introducing the β-aminoalaninamide moiety into the pyridine aldehyde followed by coupling with β-hydroxyhistidine part.[6-12] Now we developed a new synthetic route involving a key aldehyde (**1**), which may allow us to conveniently introduce various axial side chain (Scheme 1). Thus, aldehyde (**1**) we previously synthesized[13] was treated with amine (**2**)[14] in the presence of molecular sieves 3A and the resulting Schiff base was reduced with sodium borohydride and secondary amine (**3**) was obtained in 94% yield. The removal of the benzyloxycarbonyl (Z) group of **3** was best achieved with ammonium formate and palladium on carbon, furnishing PYML-9[15] in 87% yield. On the other hand, condensation of aldehyde (**1**) and β-alanine methyl ester (**4**)[16] and reduction of the resulting Schiff base gave secondary amine (**5**) in 84% yield and the subsequent ester hydrolysis afforded PYML-10[17] in 87% yield.

Scheme 1



Synthesis of imidazole model ligands was straightforward. Previously we prepared 4-hydroxymethylimidazole derivative (**6**) as a synthetic intermediate for *erythro*-β-hydroxy-L-histidine.[18] Alcohol (**6**) was converted into azide (**7**) in 85% yield by the Mitsunobu condition using hydrazoic acid. Hydrogenation of the azide (**7**) gave amine (**8**) in 94% yield. Histamine or amine (**8**) was coupled with aldehyde (**1**) in the presence of molecular sieves 3A. The resulting Schiff bases were reduced with sodium borohydride to afford PYML-11[19] (83% yield) and PYML-12[20] (76% yield).

Scheme 2



In the catalytic process of oxygen activation by the BLM-iron complex system, two distinct low spin Fe(III) species have been observed by ESR spectroscopy.[5] These includes the transient Fe(III) species, which is regarded as the actual catalytically active species closely related to BLM-Fe(III)-O_2H^-- complex, and the stable BLM-Fe(III)-OH^- species. Table 1 shows the ESR parameters for the Fe(III) complexes of the synthetic model ligands and BLM. As reported previously, ESR parameters of PYML-6-Fe(III) complex were well in accordance with those of BLM-Fe(III) complex, demonstrating that PYML-6 constitutes an excellent model system containing all the functional groups necessary for reproducing the metal-binding characteristics of BLM.[7,9] Now we found that PYML-9 exhibited both the transient and the stable low-spin ferric species. Despite PYML-9 lacks

the carbamoyl group of the β-aminoalaninamide moiety of PYML-6, the formation of catalytically active transient species, presumably PYML-9-Fe(III)-O_2H^-, was particularly notable. This exemplified that the presence of the axial amino group is essential for the generation of active species. On the other hand, PYML-10, which has only a carboxyl group in the axial appendage, could exhibit ESR signals for the low-spin Fe(III) complex no longer and, instead, showed a signal (g = 4.4) characteristic for the catalytically inert high-spin ferric species.

Table 1. ESR parameters for the Fe(III) complexes of BLM and synthetic analogues.

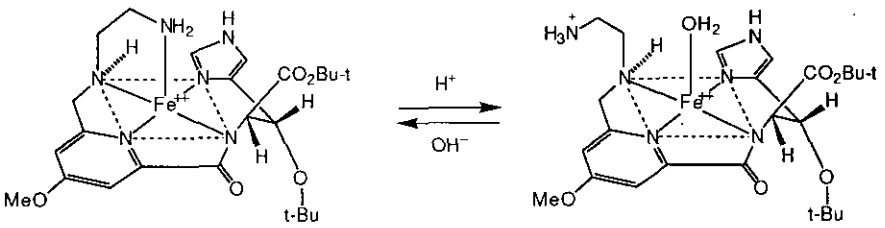
	Transient Fe(III) Complex			Stable Fe(III) Complex		
Ligand	g_1	g_2	g_3	g_1	g_2	g_3
BLM	2.254	2.171	1.937	2.431	2.185	1.893
PYML-6	2.243	2.173	1.940	2.357	2.187	1.918
PYML-9	2.254	2.172	1.940	2.360	2.184	1.912
PYML-10			g = 4.4			
PYML-11			g = 4.4			
PYML-12			g = 4.4			

Table 2 lists the results of ESR spin trapping experiment, *i. e.*, relative spin concentration of hydroxyl radicals generated from Fe(II)-complex system, which represent the oxygen activating power of synthetic models relative to that of BLM. It should be noted that pH-dependence in oxygen activation was observed for PYML-9. Whereas the efficiency of oxygen activation by PYML-9 was 46% of that of BLM at neutral condition, it increased up to 85% in pH 9.8 buffer. Among the donor atoms contained in PYML-9, the primary amino group is thought to be most susceptible to the change of pH of the solution. The pH-dependent duality of the oxygen activation by PYML-9-Fe(II) complex can be explained by assuming the equilibrium between amino- and aquo-complexes (Scheme 3). In other words, the oxygen-activating power is dependent upon whether the primary amino group coordinates to the iron or not. The fact that PYML-6 efficiently activated dioxygen even at neutral pH region demonstrated the effect of the carbamoyl group on the basicity and the

Table 2. Spin concentrations of hydroxyl radicals from Fe(II)-O_2 complex systems of BLM and synthetic analogues.

Complex	Relative spin concentration
BLM	100
PYML-6	97
PYML-9	46 (pH 7.4), 85 (pH 9.8)
PYML-10	<4
PYML-11	77
PYML-12	73

Scheme 3



coordinating property of the primary amino group. Although various ethylenediamine-containing models have been reported by several groups, pH-dependent behavior has scarcely been studied in detail.[21-23] We believe that the successful observation of the pH-dependence was owing to the presence of the bulky *tert*-butyl group in PYML-9 which favors the formation of iron-oxygen adduct complex. On the other hand, PYML-10 showed virtually no dioxygen activation, in consistent with the above mentioned observation that carboxy-coordination gives high-spin catalytically inert complex. Thus, it seemed that a carboxyl group does not effectively participate in the oxygen activation. Whereas Akkerman *et al.* claimed that the primary amino group does not participate in the metal coordination based on their nmr study,[24] the present results demonstrated the significance of the primary amino group as the ligand controlling the oxygen activation.

On the other hand, the imidazole model ligands PYML-11 and PYML-12 exhibited the formation of high-spin ferric species. Both activated moelcular oxygen in the efficiency of about 75% of that of BLM. The iron complex was extremely sensitive to oxygen and the ferrous complex was immediately oxidized to the ferric state upon contact with oxygen. The active transient ferric species could not be detected due to the very short life time of the species. Thus, PYML-11 and PYML-12 were markedly different from BLM as metal complexes.

Thus, we demonstrated that the dioxygen-activating property of BLM models largely depends on the characteristics of the functional group in the axial ligand moiety.

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REFERENCES AND NOTES

1. Present address: Department of Chemistry, College of Arts and Sciences, The University of Tokyo, Komaba, Meguro-ku, Tokyo 153, Japan.
2. Present address: Eisai Co., Ltd., Research and Development, 1-3, Tokodai 5-chome, Tsukuba-shi, Ibaraki 300-26, Japan.
3. Y. Sugiura, T. Takita, and H. Umezawa, 'Metal Ions in Biological Systems,' ed. by H. Sigel, Marcel Dekker, New York, 1985, pp. 81 - 88; S. M. Hecht, *Acc. Chem. Res.*, 1986, **19**, 383; J. Stubbe and J. W. Kozarich, *Chem. Rev.*, 1987, **87**, 1107; M. Otsuka and Y. Sugiura, 'Handbook in Metal-Liagnd Interaction in Biological Fluids,' ed. by G. Berthon, Marcel Dekker, in press.
4. H. Umezawa, S. Hori, T. Sawa, T. Yoshioka, and T. Takeuchi, *J. Antibiot.*, 1974, **27**, 419.
5. Y. Sugiura, *J. Am. Chem. Soc.*, 1980, **102**, 5208.
6. A. Kittaka, Y. Sugano, M. Otsuka, M. Ohno, Y. Sugiura, and H. Umezawa, *Tetrahedron Lett.*, 1986, **27**, 3631.
7. Y. Sugano, A. Kittaka, M. Otsuka, M. Ohno, Y. Sugiura, and H. Umezawa, *Tetrahedron Lett.*, 1986, **27**, 3635.
8. A. Kittaka, Y. Sugano, M. Otsuka, and M. Ohno, *Tetrahedron*, 1988, **44**, 2811.

9. A. Kittaka, Y. Sugano, M. Otsuka, and M. Ohno, *Tetrahedron*, 1988, **44**, 2821.
10. A. Suga, T. Sugiyama, Y. Sugano, A. Kittaka, M. Otsuka, M. Ohno, Y. Sugiura, and K. Maeda, *Synlett*, **1989**, 70.
11. A. Suga, T. Sugiyama, M. Otsuka, M. Ohno, Y. Sugiura, and K. Maeda, *Tetrahedron*, 1991,**47**, 1191.
12. M. Otsuka, T. Nishio, T. Oshitari, T. Owa, Y. Sugiura, K. Maeda, M. Ohno, and S. Kobayashi, *Heterocycles*, 1993, **33**, 27.
13. T. Owa, T. Sugiyama, M. Otsuka, M. Ohno, and K. Maeda, *Tetrahedron Lett.*, 1990, **31**, 6063.
14. W. G. Lawson, M. D. Leafer, A. Tewas, and G. J. S. Rao, *Hoppe-Seyler's Z. Physiol. Chem.*, 1968, **349**, 251.
15. PYML-9: $[\alpha]_D^{27}$ +50.9° (c = 1.05, $CHCl_3$).
16. W. J. Hale and E. M. Honan, *J. Am. Chem. Soc.*, 1919, **41**, 770.
17. PYML-10: $[\alpha]_D^{26}$ +30.0° (c = 0.87, $CHCl_3$).
18. T. Owa, M. Otsuka, and M. Ohno, *Chem. Lett.*, **1988**, 1873.
19. PYML-11: $[\alpha]_D^{23}$ +22.8° (c = 3.35, MeOH).
20. PYML-12: $[\alpha]_D^{23}$ +27.2° (c = 1.01, MeOH).
21. J.-P. Hénichart, R. Houssin, J.-L. Bernier, and J.-P. Catteau, *J. Chem. Soc., Chem. Commun.*, **1982**, 1295.
22. H. Kurosaki, H. Anan, and E. Kimura, *Nippon Kagaku Kaishi*, **1988**, 691.
23. S. J. Brown, P. K. Mascharak, and D. W. Stephan, *J. Am. Chem. Soc.*, 1988, **110**, 1996.
24. M. A. J. Akkerman, E. W. J. F. Neijman, S. S. Wijmenga, C. W. Hilbers, and W. Bermel, *J. Am. Chem. Soc.*, 1990, **112**, 7462.

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