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TRANSITION-METAL BINDING SITE OF BLEOMYCIN. A REMARKABLY EFFICIENT DIOXYGEN-ACTIVATING MOLECULE BASED ON BLEOMYCIN-Fe(II) COMPLEX[1]

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Summary: A synthetic model compound for the metal binding site of bleomycin (PYML-4) with a tert-butyl group as a steric environmental factor showed improved oxygen-activation up to 71% of that of bleomycin.

Bleomycin (BLM) is an antitumor antibiotic clinically used in the treatment of squamous cell carcinoma, malignant lymphoma, and testis tumors,[2] and consists of a linear hexapeptide and a disaccharide.[3] In addition to its medicinal importance, BLM is one of the most ingeniously elaborated compound by nature, because the sequence selective cleavage of DNA with BLM or the biological effect is now considered to be due to two chemical characteristics of the glycopeptide. The bithiazole-terminal amine residue

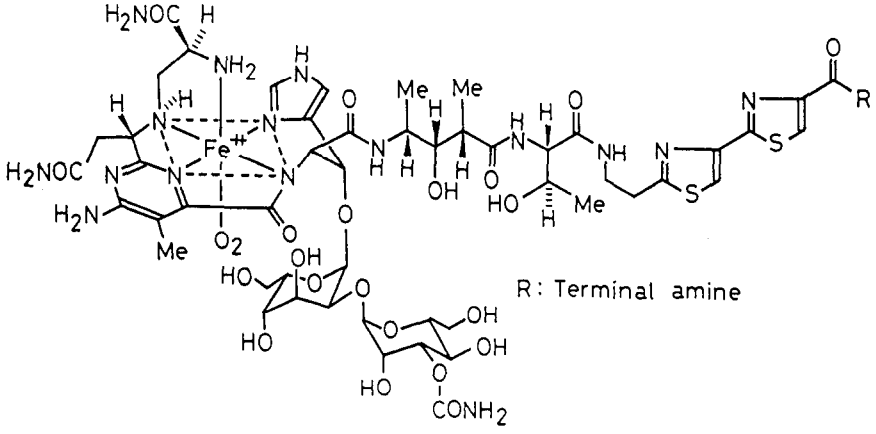
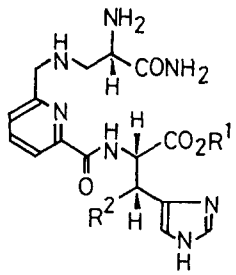


Figure 1. The proposed structure for the BLM-Fe(II)-O_2 complex.[8]

is believed to contribute to the binding to DNA[4] and the β-aminoalanine-pyrimidine-β-hydroxyhistidine moiety appears to be capable of dioxygen activation by the chelation with ferrous ion[5] (Figure 1). We first succeeded in preparing a simplified synthetic analogue (PYML-1) with a pyridine ring,[6] but PYML-1 could activate molecular oxygen only to about 20% of BLM-Fe(II) complex. The requirements for such molecules easily accesible to yield more oxygen-sensitive complexes are indeed demanding in the exploitation of sequence specific cleavage agents of DNA[7] and new anticancer compounds. We wish to report here a new synthetic analogue PYML-4 capable of binding Fe(II) to yield a remarkably oxygen-sensitive complex.

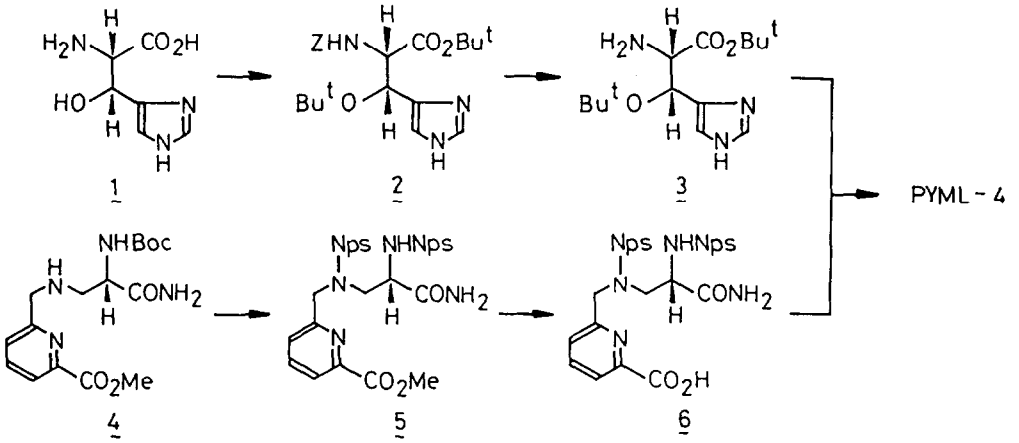
As PYML-1 is considered to be the minimum structural requirement for the metal binding and dioxygen activation of BLM,[6] we then studied the various steric and electronic factors of the PYML-1 skeleton to clarify the relationships between the designed structures and the physicochemical properties including the capability to activate dioxygen. In particular, the disaccharide moiety of BLM appears to be the main steric factor constructing a hydrophobic molecular cavity. It can be said that BLM is a kind of enzyme for dioxygen, because BLM accomodates dioxygen



PYML-1 $R^1 = R^2 = H$

PYML-4 $R^1 = Bu^t$, $R^2 = OBu^t$

Figure 2. Structures of PYMLs.



Scheme 1. Synthesis of PYML-4.

Table 1. ESR parameters for Fe(II)-^{14}NO and Fe(III) complexes of BLM and PYMLs.

	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-4	2.038	2.008	1.965	25.5	2.242	2.172	1.981	2.350	2.186	1.913
PYML-1	2.036	2.009	1.972	25.6	not detected			not detected		

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

Complex	Relative spin concentration
BLM	100
PYML-4	71
PYML-1	18

at the cavity and facilitates the activation of dioxygen to reactive oxygen radical species. A tert-butyl derivative PYML-4 (Figure 2) of β-hydroxyhistidine was considered to be a good candidate not only for the formation of a hydrophobic cavity for dioxygen but also to approach the proposed structure for BLM-Fe(II) complex.[8] Thus, PYML-4 was synthesized as shown in Scheme 1.[9] erythro-β-Hydroxy-L-histidine 1[10] was treated with CBZ-S[11] followed by isobutene to give tert-butyl derivative 2 (36% from 1) which was hydrogenated to free amine 3. On the other hand, tert-butoxycarbonyl group of the known ester 4[6] was removed with TFA and the resulting amine was again protected with Nps-Cl[12] to give bis(Nps) derivative 5 (70% from 4). The methyl ester 5 was easily hydrolyzed to carboxylic acid 6. Condensation of the acid 6 and the amine 3 was effected by using diphenyl phosphoroazidate[13] and acid treatment of the resulting dipeptide afforded PYML-4 (46% based on 2), white powder, $[\alpha]_D^{23}$+30.5° (c=0.61, MeOH), M^++1 504. ESR parameters for the iron complexes of PYML-4 agreed with those of BLM (Table 1). Particularly noteworthy is that, in contrast with PYML-1, PYML-4 exhibited an ESR signal for the transient activated Fe(III)-O_2^{2-} species for the first time as a model compound. The ESR spin trapping experiments[5] by oxygen bubbling of the PYML-4-Fe(II) complex in the presence of N-tert-butyl-α-phenylnitrone (BPN) at pH 8.3 clearly revealed the generation of hydroxyl radicals (g=2.0057, A^N=15.3 G). The dioxygen activating ability of PYML-4 was increased up to 71% of that of BLM (Table 2). Thus, the tert-butyl group was shown to exert profound steric and hydrophobic effect in improving the oxygen interaction and activation by PYML. A further study on the electronic effect of substituent is the subject of the following paper.[14]

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