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TRANSITION-METAL BINDING SITE OF BLEOMYCIN. A SYNTHETIC ANALOGUE EQUIVALENT TO BLEOMYCIN IN ACTIVATING MOLECULAR OXYGEN[1]

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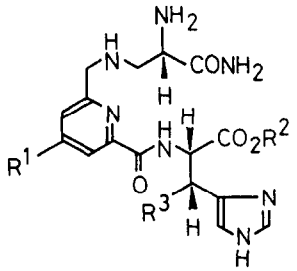
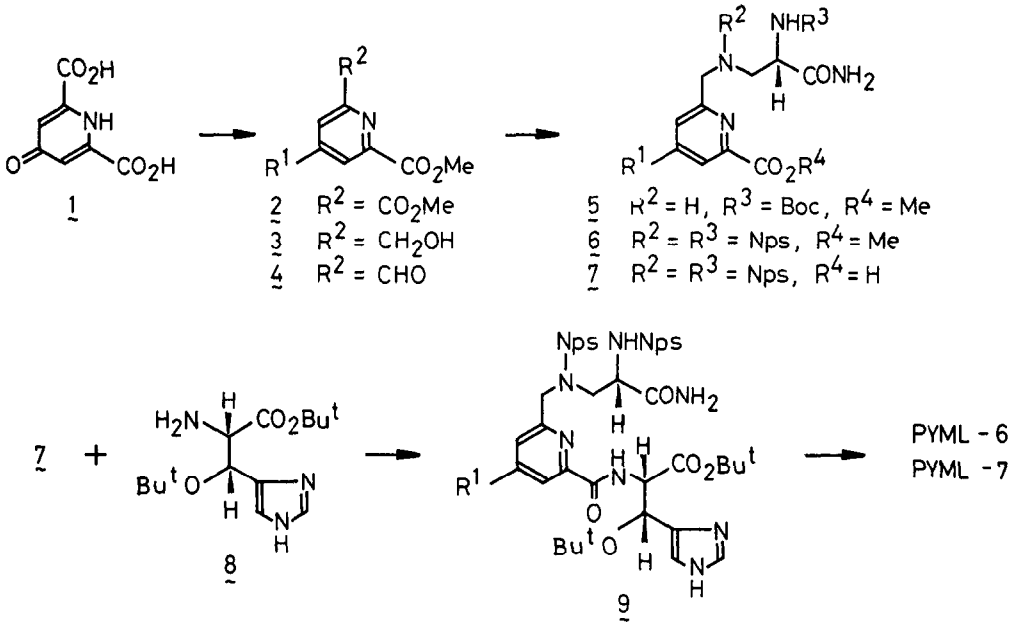
Summary: A synthetic model compound for the metal binding site of bleomycin (PYML-6) with an electron donating methoxy substituent showed remarkably efficient oxygen activation comparable to bleomycin.

The dioxygen activation with an antitumor antibiotic bleomycin (BLM) was attributed to the Fe(II) binding by the pyrimidine moiety and the hydrophobic effect of the disaccharide moiety was suggested.[2] In the preceding paper we reported an efficient oxygen activating molecule, PYML-4, with a tert-butyl group as a hydrophobic factor.[3] We now assumed that increased π-electron density of the N-atom of the pyridine ring would increase the capacity to activate dioxygen for the Fe(II) complex, and vice versa. The result of molecular orbital calculation using Streitwieser's parameters[4] indicated that π-electron density of the N-atom of various 4-substituted pyridines are considerably dependent upon the substituents and 4-methoxypyridine seemed most closely in accordance with 4-aminopyrimidine of BLM (Table 1). Thus, PYML-6 and -7 were synthesized as shown in Scheme 1.[5] Chelidamic acid 1 was converted into methoxypyridine 4 (R^1=OMe) by



	R^1	R^2	R^3
PYML-1	H	H	H
PYML-4	H	Bu^t	OBu^t
PYML-6	OMe	Bu^t	OBu^t
PYML-7	Cl	Bu^t	OBu^t

Figure 1. Structures of PYMLs.



Scheme 1. Synthesis of PYML-6 and -7.

Table 1. π-Charge density (PRS), self polarizability (PAIRR), and frontier electron density (FRE) for N-1 of substituted pyrimidine and pyridines.

Compound	PRS	PAIRR	FRE
4-Aminopyrimidine	1.26	0.50	0.61
4-Methoxypyridine	1.24	0.51	0.72
4-Chloropyridine	1.21	0.51	0.00

Table 2. ESR parameters for Fe(II)-^{14}NO and Fe(III) complexes of BLM and PYMLs.

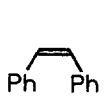
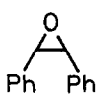
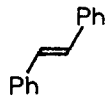
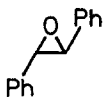
	Fe(II)-^{14}NO complex				Transient Fe(III) complex			Stable Fe(III) complex		
	g_1	g_2	g_3	A^N,G	g_1	g_2	g_3	g_1	g_2	g_3
BLM	2.041	2.008	1.976	23.8	2.254	2.171	1.937	2.431	2.185	1.893
PYML-6	2.037	2.009	1.967	25.2	2.243	2.173	1.980	2.357	2.185	1.918
PYML-7	2.037	2.008	1.963	26.2	2.240	2.173	1.981	2.341	2.190	1.910

Table 3. Spin concentrations of hydroxyl radicals from Fe(II)-O_2 complex systems of BLM and PYMLs.

Complex	Relative spin concentration
BLM	100
PYML-6	97
PYML-4	71
PYML-7	55
PYML-1	18

treatment with CH_2N_2 (79%), then $NaBH_4$ (85%) followed by the Swern oxidation (78%). Chloropyridine 4 (R^1=Cl) was also obtained from 1 by successive treatment with i) PCl_5 followed by MeOH (26%), ii) $NaBH_4$ (89%), and iii) the Swern oxidation (99%). Aldehyde 4 (R^1=OMe) was treated with (S)-3-amino-2-[(tert-butoxycarbonyl)amino]propionamide[6] and the resulting Schiff base was hydrogenated over Pd-C, affording amine 5 (R^1=OMe, 89%). Treatment of 5 with TFA followed by Nps-Cl[7] gave bis(Nps) derivative 6 (R^1=OMe, 86%). The ester 6 (R^1=OMe) was hydrolyzed with 0.1N LiOH and then treated with erythro-β-hydroxy-L-histidine derivative 8[3] in the presence of DCC-HOBt to afford dipeptide 9 (R^1=OMe, 36%). Treatment of 9 (R^1=OMe) with aq HBr (4 eq) gave PYML-6 (57%), white powder, $[\alpha]^{22}_D$+1.0° (c=0.53, MeOH), M^++1 534. PYML-7 was obtained by the same procedure starting with 4 (R^1=Cl), white powder, $[\alpha]^{22}_D$+11.4° (c=0.78, MeOH), M^++1 540. The ESR parameters of iron complexes were remarkably close to those of BLM and the signal for the transient Fe(II)-O_2^{2-} species was most clearly observed for PYML-6 (Table 2). It was gratifying to find that PYML-6 with a methoxy substituent showed oxygen activation virtually equivalent to that of BLM (Table 3).

Table 4. Epoxidation of cis- and trans-stilbene with Fe(III)-H_2O_2 complex systems of BLM and PYMLs.

Fe(III) complex	Substrate	Product	Relative yield
BLM			100
PYML-6			95
PYML-4			71
PYML-7			39
PYML-1			50
BLM			trace
PYML-6			3
PYML-4			7
PYML-7			5
PYML-1			38

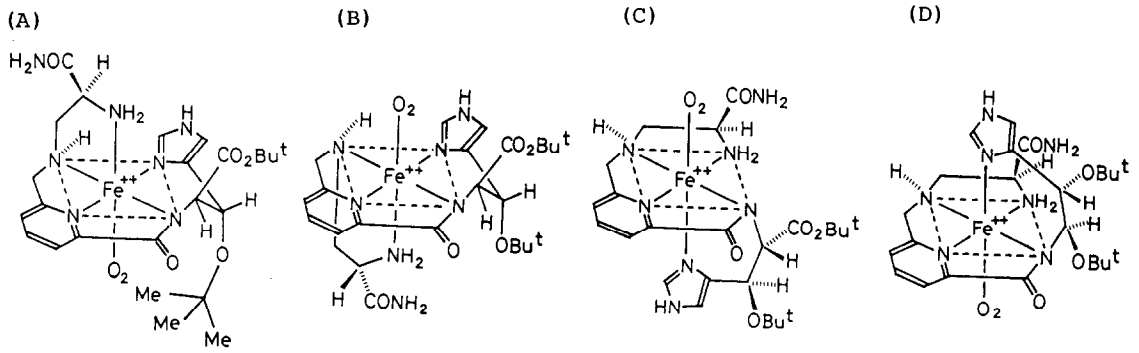


Figure 2. Possible structures for PYML-Fe(II)-O_2 complex.

On the other hand, an electron withdrawing chloro group induced a reverse effect in PYML-7 less active than unsubstituted PYML-4. The epoxidation reaction of cis- and trans-stilbene was carried out by using Fe(III)-H_2O_2 complex system of PYMLs (Table 4).[8,9] Whereas the yield of cis-epoxide from cis-stilbene was almost proportional to the oxygen activating ability of the ligands, the epoxidation of trans-stilbene appeared to be suppressed, suggesting that the steric environment of the iron complex of the tert-butyl substituted PYMLs is highly crowded to support one of the four possible structures of the complex (i.e., (A) in Figure 2) similar to the previously proposed structure.[2] Work is continuing to explore new man-designed BLMs and efficient DNA cleaving molecules by introducing DNA binding site.[10]

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