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SYNTHETIC STUDY TOWARDS MAN-DESIGNED BLEOMYCINS. SYNTHESIS OF A DNA CLEAVING MOLECULE BASED ON BLEOMYCIN.[1]

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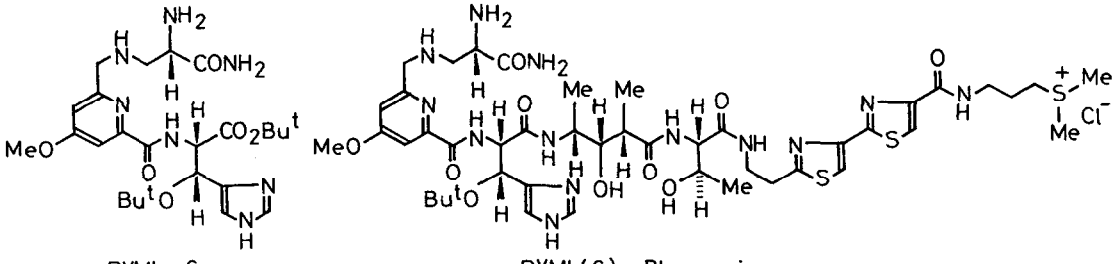
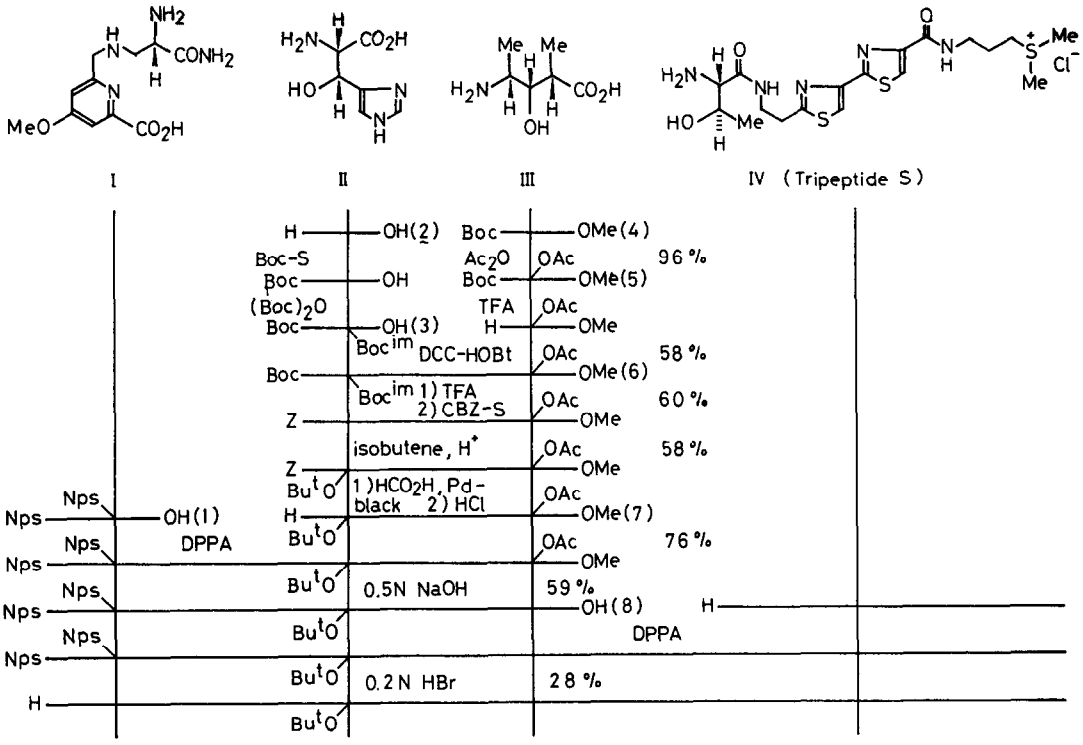
Summary: A synthetic analogue of bleomycin, PYML(6)-Bleomycin, having a bithiazole moiety as well as a 4-methoxypyridine ring and a tert-butyl group showed efficient DNA cleaving activity.

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] The DNA cleavage with BLM or the biological effect is considered to be due to two chemical characteristics; the DNA binding by the bithiazole-terminal amine moiety[4] and the dioxygen activation by the β-aminoalanine-pyrimidine-β-hydroxyhistidine moiety.[5] In our model study on the metal binding site of BLM we demonstrated that the 4-aminopyrimidine and the disaccharide moiety of BLM could be replaced by a 4-methoxypyridine and a tert-butyl group respectively and such model compound, PYML-6, was shown to be the best in terms of oxygen activation.[6] This finding provided a basis for the exploitation of sequence specific cleaving agents of DNA and new anticancer compounds.



PYML-6 PYML(6)-Bleomycin

Figure 1. Structures of PYML-6 and PYML(6)-Bleomycin.



Scheme 1. Synthesis of PYML(6)-Bleomycin.

We then introduced a DNA binding site, the tetrapeptide S moiety of BLM, into the PYML-6 skeleton and succeeded in efficient cleavage of DNA.

Thus, PYML(6)-Bleomycin was synthesized as shown in Scheme 1. The strategy of the synthesis was based on the coupling of three fragments, I, II-III, and IV. As for the fragment I, methoxypyridine derivative 1 has already been prepared.[6] For the synthesis of the fragment II-III, the amino group and the imidazole nitrogen of erythro-β-hydroxy-L-histidine 2 were succesively tert-butoxycarbonylated with Boc-S[7] and $(Boc)_2O$[8] respectively. On the other hand, methyl (2S, 3S, 4R)-4-[(tert-butoxycarbonyl)-amino]-3-hydroxy-2-methylpentanoate 4[9] was treated with acetic anhydride to give the corresponding acetate 5 (96%), which was treated with TFA followed by a coupling with the acid component 3 (DCC-HOBt). Thus, dipeptide 6 was obtained in 58% yield based on 2. The tert-butoxycarbonyl amino protection of 6 was replaced by a benzyloxycarbonyl group (TFA followed by Cbz-S[7], 60%), before introducing the O-tert-butyl group under acidic condition (isobutene, H^+, 58%). The fragment II-III (7) obtained by the removal of the amino protective group (10% HCO_2H, Pd-black) was coupled with the fragment I acid 1 (DPPA, 76%). The resulting tripeptide I-II-III

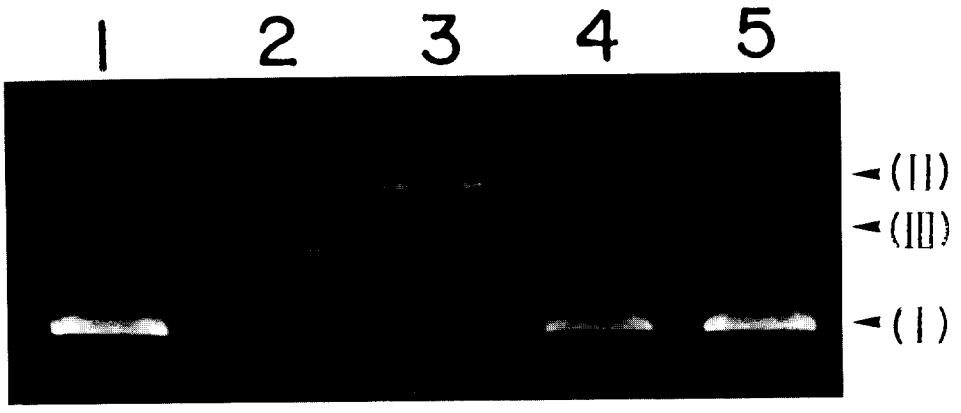


Figure 2. Strand scission of ϕX 174 RF DNA with PYML(6)-Bleomycin-Fe(II) complex. The samples contained 0.5 μg of DNA, 20 mM tris·HCl buffer (pH 8.3), and the following additions: lane 1, none; lane 2, 1 μM BLM A2-Fe(II) complex plus 100 μM dithiothreitol (DTT); lane 3, 1 μM PYML(6)-Bleomycin-Fe(II) complex plus 100 μM DTT; lane 4, 5 μM Fe(II) plus 100 μM DTT; lane 5, 5 μM PYML(6)-Bleomycin plus 100 μM DTT. The reaction samples were incubated at 20°C for 20 min under aerobic condition.

was hydrolyzed to the acid 8 (0.5N NaOH, 59%), which was then coupled with the fragment IV (tripeptide S)[10] (DPPA). The two Nps groups of the hexapeptide were carefully removed with 0.25N HCl-EtOAc and 2-methylindole. To facilitate an efficient purification PYML(6)-Bleomycin thus obtained was converted into a Cu(II) complex and subjected to CM-Sephadex C-25 chromatography (eluted with a linear gradient of 0.05M sodium phosphate, pH 6.8 and 1.05M NaCl). The blue eluate was then treated with excess EDTA at pH 2-4.5 and purified by a column of Amberlite XAD-2 (washed with 3% EDTA-2% NaCl, 2%NaCl, and H_2O, then eluted with 0.002N HCl-MeOH), affording pure PYML(6)-Bleomycin (28% from 8). The sample was proved to be homogeneous by HPLC (SSC-ODS-171 column, 4% $AcONH_4$:MeOH=6:4, flow rate 1 ml/min, retention time 10.5 min), and the structure of PYML(6)-Bleomycin was fully supported by Mass (m/e 1047, M^+) and ^1H NMR spectra.

PYML(6)-Bleomycin was shown to possess a potent DNA cleaving activity.[11] As shown in Figure 2 the DNA cleavage activity was determined on purified ϕX174 RF DNA by using 0.8% agarose gel electrophorasis which contained ethidium bromide (0.5 μg/ml).[12] Typical reaction mixtures containing 20 mM Tris HCl buffer (pH 8.3) and 0.5 μg of ϕX174 RF DNA were treated, as indicated in the figure captions, with 1 μM BLM A2-Fe(II) plus 100 μM dithiothreitol (DTT), 1 μM PYML(6)-Bleomycin-Fe(II) plus 100 μM DTT under aerobic conditions. The reaction samples were incubated at 20°C for 20 min, and then the DNA was precipitated by addition of cold ethanol. Thus, PYML(6)-Bleomycin-Fe(II) cleaved a phage ϕX174 RF DNA by an aerobic reaction

in the presence of a reducing agent at a low concentration (1 μM). The sequence specificity of the DNA cleavage with PYML(6)-Bleomycin is currently under active investigation.

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