Synthetic Model of a Bleomycin Metal Complex

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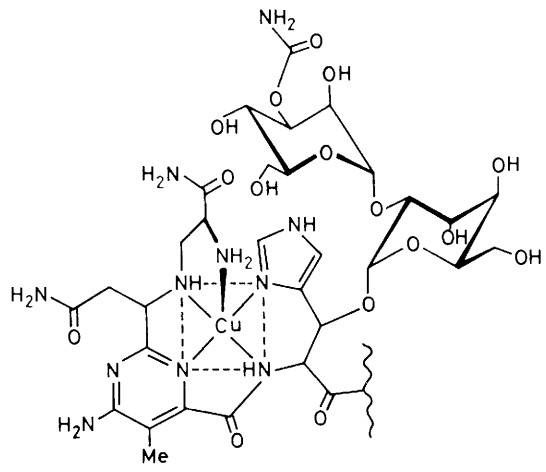
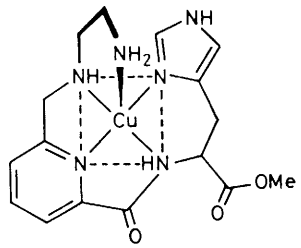
A simple analogue of the metal-complexing pseudopeptide bleomycin has been synthesised and the e.s.r. spectral parameters of its Cu^{II}-complex were found to correspond to the characteristics of the Cu^{II}-bleomycin system.

Metal-binding properties of the glycopeptide antibiotic antitumor agent bleomycin have been the subject of extensive investigation.[1] The structures of different metallobleomycins have been studied, in particular that of the cupric complex,



(a)



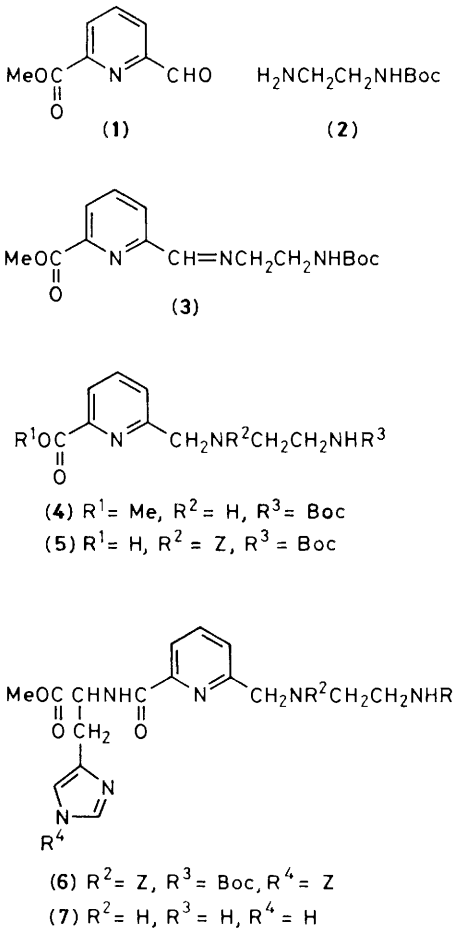
(b)

Figure 1

which is the natural form of the drug that is isolated,[2] and the ferrous complex, which has been proposed as the form responsible for the DNA strand scission.[3] On the basis of spectroscopic determinations[4] on transition-metal complexes, in particular on Cu^{II}-bleomycin, it has been postulated that the co-ordinating atoms consist of four nitrogen atoms of the pseudopeptide chain in a square plane, with a nitrogen atom of the terminal amine as a fifth axial ligand (Figure (1a)]. However, alternative structures for Cu^{II} or Fe^{II}-bleomycin systems have been proposed involving other atoms.[5,6]

We decided to synthesise a simplified model containing the five assumed metal binding sites but excluding all the nitrogen atoms not involved in the chelation.[4] For this purpose, the pyrimidine moiety was replaced with a pyridine ring, substituted by an aminomethyl group instead of by the β-aminopropionamide chain, and the terminal alaninamide group of bleomycin was changed to an aminoethyl side chain. Moreover, the sugar moiety was omitted and β-hydroxyhistidine was simplified to a histidine residue [Figure (1b)].

Our synthetic strategy for the elaboration of this pseudopeptide was based on the key compound methyl 6-formylpyridine-2-carboxylate[7] (**1**) which was combined in ethyl ether at room temperature for 3 h with the appropriate monoprotected ethylenediamine (**2**) to give the corresponding imine (**3**). Starting material (**2**) was prepared in 77% yield by catalytic hydrogenation of (t-butoxycarbonyl)aminoacetonitrile [β-aminoacetonitrile treated with di-t-butyl dicarbonate $(Boc)_2O$ under the classical conditions[8]], in the presence of 5% Raney Ni [b.p. at 0.1 mmHg 72—80 °C, ν_{max} 1680 cm^{-1}, δ ($CDCl_3$) 1.32 (2H, s, NH_2), 1.46 (9H, s, CH_3), 2.67—3.37 (4H, m, CH_2), and 5.70 (1H, m, NH)].



(**4**) R^1 = Me, R^2 = H, R^3 = Boc
(**5**) R^1 = H, R^2 = Z, R^3 = Boc

(**6**) R^2 = Z, R^3 = Boc, R^4 = Z
(**7**) R^2 = H, R^3 = H, R^4 = H

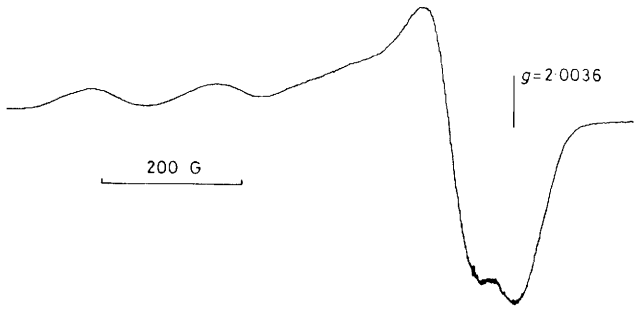


Figure 2. E.s.r. spectrum of the model Cu^{II}-complex.

Catalytic reductive conversion of the Schiff base (**3**) [92%, ν_{max} 1690 cm^{-1}, δ (CCl_4) 1.39 (9H, s, CH_3), 3.10—3.90 (4H, m, CH_2), 3.96 (3H, s, CH_3), 7.67—8.30 (3H, m, pyridyl), and 8.41 (1H, s, CH)] in the presence of Pd–C gave (**4**) [95%, ν_{max} 1705 cm^{-1}, δ ($CDCl_3$) 1.39 (9H, s, CH_3), 3.10—3.92 (6H, m, CH_2), 4.00 (3H, s, CH_3), and 7.57—8.30 (3H, m, pyridyl)] whose secondary amino-function was protected with the benzyloxycarbonyl group Z (by treatment with benzyl chloroformate in the presence of 1 M NaOH in dichloromethane at 0 °C) and whose methyl carboxylate function was hydrolysed (NaOH in methanol–water medium for 2 h at room temperature and neutralization by HCl). The resulting *N*,*N*′-diprotected free acid (**5**) {58% from (**4**), ν_{max} 1700 and 1720 cm^{-1}, δ [$(CD_3)_2SO$] 1.39 (9H, s, CH_3), 3.15—4.05 (6H, m, CH_2), 5.14 (2H, s, CH_2), 7.37 (5H, s, arom.), and 7.74—8.29 (3H, m, pyridyl)} was coupled with N(Im)-carbonyl-L-

histidine methyl ester[9] in the presence of dicyclohexylcarbodiimide in dimethylformamide at 0 °C for 3 h and at room temperature for 12 h, affording the protected pseudopeptide (**6**) {70%, ν_{max} 1640 and 1720 cm^{-1}, δ [$(CD_3)_2SO$] 1.40 (9H, s, CH_3)}. The *N*-protecting groups were removed by action of an HBr–acetic acid solution, followed by neutralization with $NaHCO_3$, extraction with dichloromethane, and purification on silica-gel. The ester (**7**) [63%, ν_{max} 1630 and 1750 cm^{-1}] was then complexed by adding $CuCl_2$ and the e.s.r. spectrum of the Cu^{II}-complex was recorded (Figure 2).

The *g*-values ($g_{\parallel} = 2.21$ and $g_{\perp} = 2.05$) and the hyperfine constant ($a_{\parallel} = 177$ G) are consistent with values expected for Cu^{II} chelated to a square planar array of ligands[10] and with values obtained with the Cu^{II}-bleomycin complex.[11] It can be concluded that our simplified synthetic pseudopeptide is a quite satisfactory model for the study of the metal binding sites of bleomycin. They support the previous results of Iitaka[4] and Dabrowiak[11] and refute the structure proposed by Bereman.[6] The model may be a useful tool in the study of the mechanism of bleomycin.

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