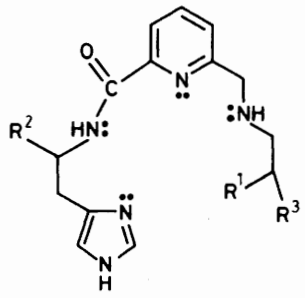
Bleomycin Metal Site Models with Apical Imidazole or Sulphydryl Donors

Thomas J. Lomis,[a] Jerome F. Siuda,[a] and Rex E. Shepherd[b]*

[a]*Department of Pharmaceutical Sciences and* [b]*Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, U.S.A.*

New synthetic models for the bleomycin (BLM) metal ion binding site which contain the terminal binding group of imidazole (HAPH) or S–Me, $SCH_2C_6H_4OMe$, and –SH (SAPH 1—3) derivatives have been prepared; Fe^{II}(HAPH)/O_2 yielded 53%, and Fe^{II}(HPAH)/H_2O_2 63%, of the HO· generated by Fe^{II} (BLM)/O_2.

Bleomycin (BLM) is an antibiotic glycopeptide recognized as an antitumour drug.[1] The Fe^{II}(BLM) complex is an oxygen sensitive complex which is capable of DNA-nicking; the rapid activation of O_2 by Fe^{II}(BLM) is believed to account for its antitumour activity.[2] The structures of Zn^{II}, Fe^{II}, Cu^{II}, and Co^{III} metallobleomycins have been studied previously.[3] Synthetic methodology has been recently applied to constructing functional metal-ion binding core units which mimic the metal-ion binding site of bleomycin. These efforts have previously included the Fe^{II} and Cu^{II} complexes of PYML



(1)

	R^1	R^2	R^3
AMPHIS	$-NH_2$	-C(O)OMe	H
PYML	$-NH_2$	-C(O)OH	$-C(O)NH_2$
HAPH	5-Imidazolyl	H	H
SAPH-1	-SMe	H	H
SAPH-2	$-SCH_2C_6H_4OMe$	H	H
SAPH-3	-SH	H	H

(PYML = *N*-[6-({[(*S*)-2-amino-2-(carbamoyl)-ethyl]amino}-methyl)pyridine-2-carbamoyl]-L-histidine)[4] and AMPHIS [AMPHIS = methyl 2-(2-aminoethyl)aminomethyl-pyridine-6-carboxylhistidinate][5] which contain the bleomycin core donors of imidazole, amide nitrogen, pyridine, and secondary amine joined to a terminal primary amine as in (**1**). We have successfully prepared a related series of compounds possessing a terminal imidazole functionality (HAPH) or terminal sulphydryl or alkylated sulphur (SAPH 1—3). The synthetic methods for preparing HAPH and SAPH 1—3, ^1H and ^{13}C n.m.r. spectra, and high resolution mass spectral data will be presented in detail elsewhere.[6—8] Representative data are provided below for HAPH and SAPH-3.†

The new series provides interesting modifications of the bleomycin metal ion binding site in that both HAPH and SAPH 1—3 Fe^{II} complexes are even closer to the cytochromes which Fe^{II}(BLM) is known to mimic,[1,9] *e.g.* inner-sphere binding of O_2 and CO; generation of oxygen radical species including O_2^-, HO·, and ferryl oxygen ($Fe^{IV}O$), much in the manner of the cytochrome P-450, catalase, peroxidase, and O_2 storage metalloproteins.

Fe^{II}(HAPH) and Fe^{II}(SAPH-3) exhibit amide deprotonation with a pK_a of *ca.* 5.5 compared to 5.2 for Fe^{II}(BLM). Solutions appear cherry to yellow-orange in colour upon loss of the amide hydrogen. The Fe^{II} complexes exhibit a resolved

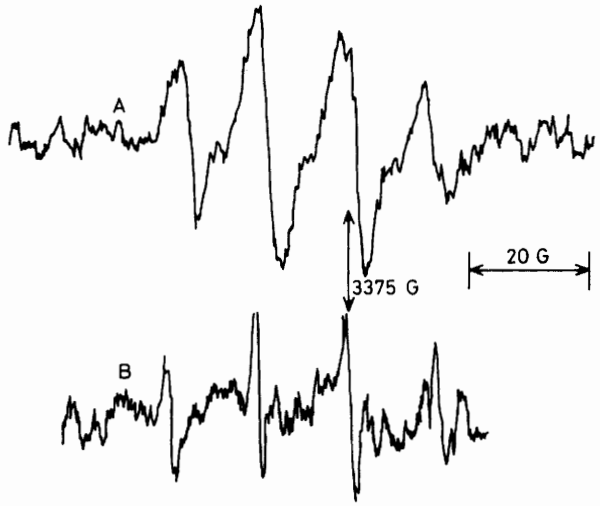


Figure 1. HODMPO· formed during Fe^{II}(HAPH) oxidations: (A) $[H_2O_2]$ = 0.49 M, [Fe(HAPH)] = 8.33 × 10^{-4} M, pH = 7.50; (B) O_2 saturated, [Fe(HAPH)] = 1.34 × 10^{-4} M, pH = 7.50; 15.0 mW, 9.482 GHz, 1.60 G mod. amp., 8.0 min scans, 3.0 s time constant; (A) RG = 5.0 × 10^4, (B) RG = 8.0 × 10^4.

shoulder band in the visible region of the spectrum (λ_{max}, ε, pH): Fe^{II}(HAPH), 445 nm, 278 dm^3 mol^{-1} cm^{-1}, 7.75; Fe^{II}(SAPH-3), 475, 200, 8.00. These values are comparable to the literature values for Fe^{II}(BLM), 476 nm, 380 dm^3 mol^{-1} cm^{-1};[2a] Fe^{II}(PYML), 465, 300.[9a] Like Fe^{II}(BLM), Fe^{II}-(HAPH) and Fe^{II}(SAPH-3) bind CO reversibly. Fe^{II}-(HAPH)CO and Fe^{II}(SAPH-3)CO exhibit a strong transition with a flat u.v. maximum at 360—390 nm with ε_{360} = 968 for Fe^{II}(HAPH)CO and near 400 nm, ε_{400} = 715, for Fe^{II} (SAPH-3)CO, compared to Fe^{II}(PYML)CO at 390, ε_{390} = 2000.[9a] The equilibrium constants for CO binding are approximately 2.7 × 10^3 dm^3 mol^{-1} for Fe^{II}(HAPH), Fe^{II}(SAPH-3) and Fe^{II}(BLM).[7]

The ability of Fe^{II}(HAPH) and Fe^{II}(SAPH-3) to activate O_2 as a potential drug functional unit which forms radicals that escape the solvent cage of the Fe(L)O_2 complex was examined using DMPO (DMPO = 5,5-dimethyl-1-pyrroline 1-oxide) and PBN (PBN = N-t-butyl-α-phenylnitrone) radical traps.[5a,9a,10—12] Fe^{II}(BLM) was prepared from blenoxane as a control to evaluate the efficiency of radical production.‡ The amount of HO·, trapped as the spin-adduct HODMPO· (a_N = a_H = 15.0 G), was determined at [DMPO] ≃ 0.24 M. Autoxidation of 1.34 × 10^{-3} M Fe^{II}(HAPH) proceeds in *ca.* 5 seconds upon O_2 saturation (pH 7—8) with loss of the tangerine solution colour. A yellow solution of Fe^{III}(HAPH) is formed. Autoxidation of Fe^{II}(SAPH-3) (2.00 × 10^{-3} M) proceeds under similar conditions with the appearance of an olive green intermediate for the first 10 seconds; the intermediate fades to the yellow colour of Fe^{III}(SAPH-3) in *ca.* 5 seconds. No oxygen-centred radical (HO·, O_2^-, HO_2·) is trapped for the Fe^{II}(SAPH-3)/O_2 reaction. The protected S–Me complex, Fe^{II}(SAPH-1), yielded a small amount of trappable radical with PBN in the presence of ethanol as a mediator.[8] The presence of HODMPO· and a small amount of HO_2DMPO· is readily detected for the Fe^{II}(BLM)/O_2 control. HO· originates as the dismutation or further reduction product of O_2^-. Fe^{II}(HAPH) reacts with H_2O_2 in the presence

† SAPH-3: (trihydrochloride salt) ^1H n.m.r. (D_2O) δ 9.02 (s, 1H, Imid-C-2-*H*) 8.6—8.2 (m, 2H, PyrC-3- and C-5-*H*), 8.2—7.9 (m, 1H, PyrC-4-*H*), 7.70 (s, 1H, Imid-C-5-*H*), 4.98 (s, 2H, PyrCH_2N), 4.2 (m, 2H, CONHCH_2CH_2), 3.84 (t, 2H, ImidCH_2CH_2), 3.6—3.1 (m, 4H, SCH_2CH_2N); ^{13}C n.m.r. (D_2O) δ 167.0 (CONH), 8 ArC at δ 151.3, 149.5, 140.9, 134.4, 132.0, 127.4, 123.3, 117.4, 51.2, 50.9 (Pyr$CH_2NH_2^+CH_2$), 39.4 (CONHCH_2), 25.4 (ImidCH_2), 21.2 (SCH_2CH_2N); i.r. (KBr) 3425 (N–H), 1640 cm^{-1} (amide C=O); mass spectrum *m/z*, 305 (M^+), 272 (100%), 258, 245; M^+ 305.1313 (calc. 305.1317).

HAPH: ^1H n.m.r. (CD_3OD) δ 7.92, 7.87 (d, s, Imid-C-2-*H*), 7.59 (m, 3H, Py ring-H), 6.85 (d, 2H, Imid-C-5-*H*), 3.98 (s, PyrCH_2N), 3.65 (m, 2H, Imid-CH_2CH_2NHCO), 2.9 [br., 6H, Imid(1)CH_2, Imid(2)CH_2CH_2]; ^{13}C n.m.r. (CD_3OD) δ 166.42 (CO), 8 ArC at δ 150.61, 139.39, 126.41, 121.73, 117.94, 117.45, 53.90 (PyrCH_2N), 51.79 (CONHCH_2CH_2Imid), 50.87 [NHCH_2Imid(2)], 49.89, 49.46 [CH_2Imid(1),(2)]; mass spectrum *m/z* 339 (M^+), 258, 230 (100%), 95; (M^+) 339.1806 (calc. 339.1808).

‡ Blenoxane was kindly provided by the Bristol-Myers Company.

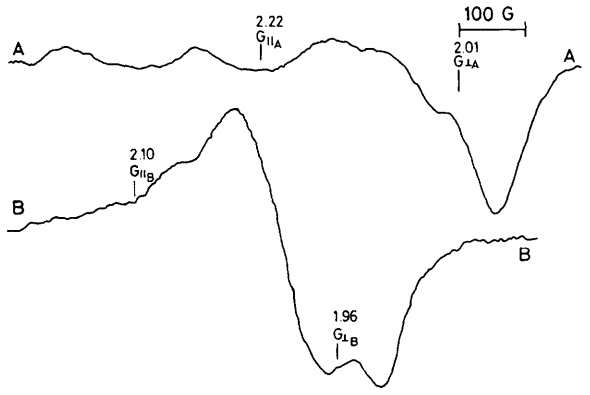


Figure 2. E.s.r. spectrum for Cu^{II}(HAPH) and Cu^{II}(SAPH-3) at pH 9.6: (A) [Cu^{II}(HAPH)] = 2.20×10^{-3} M, 20.0 mW, 9.019 GHz, 1.60 mod. amp., 8.0 min scan, 1.0 s time constant, in liquid N_2, 77 K. (B) [Cu^{II}(SAPH-3)] = 8.85×10^{-3} M, 20.0 mW, 9.090 GHz, 1.25 mod. amp., 4.0 min scan, 1.0 s time constant, in Varian temperature controller, 113 K.

of DMPO to yield HODMPO· (Figure 1A). The Fe^{II}-(HAPH)/O_2 reaction also forms HODMPO· (Figure 1B; signal has been averaged from 4 scans). Based on a per mole production of HO· relative to the Fe^{II}(BLM)/O_2 control, Fe^{II}(HAPH)/O_2 generates HO· at 53% the efficiency of Fe^{II}(BLM)/O_2. The production of HO· *via* Fe^{II}(HAPH)/H_2O_2 is 63% as efficient. Absence of a trappable oxygen radical, together with the formation of the olive green intermediate for the Fe^{II}(SAPH-3)/O_2 system and minor amount of reactivity for the protected Fe^{II}(SAPH-1)/O_2 system, implicates the rapid $2e^-$ reduction of the initial O_2 adduct *via* oxidation of both the Fe^{II} and sulphydryl groups within the solvent cage of Fe^{II}(SAPH-3).

Cu^{II} derivatives of the HAPH and SAPH 1—3 series exhibit amide deprotonation with a concomitant increase in ligand field strength with pK_a values near 6.5. Cu^{II}(HAPH) possesses a visible maximum at 625 nm (pH 3.18) shifting to 570 nm above pH 7.0. The co-ordination geometry of Cu^{II} complexes with ligands similar to the bleomycin core changes from trigonal bipyramidal below pH~3, but adopts the pseudo-square-planar co-ordination of the primary core donors (with co-ordinated deprotonated amide) above pH ~ 6.5.[6] The e.s.r. spectra in 50% dmso (dmso = dimethyl sulphoxide) –H_2O glasses[13] of Cu^{II}(HAPH) and Cu(SAPH-3) are shown in Figures 2A and B, respectively, at pH 9.6. A detailed pH-dependent study of the e.s.r. spectra for Cu^{II}(SAPH 1—3) in 50:50 H_2O–dmso frozen glasses is reported elsewhere.[6] The Cu^{II} complexes of HAPH and SAPH-3 retain their respective structures from pH 6.5 to 9.6. The spectra shown in 2A for Cu^{II}(HAPH) and 2B for Cu^{II}(SAPH-3) are quite similar to the square-pyramidal co-ordination reported for Cu^{II}(AMPHIS) ($g_\parallel$ 2.21, $g_\perp$ 2.05, $A_\perp$ 178 G)[5] and Cu^{II}(BLM) ($g_\parallel$ 2.21, $g_\perp$ 2.06, $A_\parallel$ 183 G)[5,14] compared to Cu^{II}(HAPH) ($g_\parallel$ 2.22, $g_\perp$ 2.01, $A_\parallel$ 201 G) and Cu^{II}(SAPH-3) ($g_\parallel$ 2.10, $g_\perp$ 1.96, $A_\parallel$ = 144 G) (G = 10^{-4} T); the slightly weaker ligand field due to the sulphydryl (or H_2O) as the axial ligand in Cu^{II}(SAPH-3) is evident from the reduced values of the e.s.r. parameters. The similarity of axial N-donors in Cu^{II}(HAPH) and Cu^{II}(AMPHIS) or Cu^{II}(BLM) is apparent. Further investigations into oxygen activation, by systems related to HAPH and SAPH 1—3 are currently in progress.

Received, 25th August 1987; Com. 1253

References

1 Y. Sugiura, T. Takita, and H. Umezawa, 'Bleomycin Antibiotics: Metal Complexes and Their Biological Action' in *Metal Ions in Biological Systems*, 1985, **19**, 81—108.

2 (a) E. A. Sausville, J. Peisach, and S. B. Horwitz, *Biochem. Biophys. Res. Commun.*, 1976, **73**, 814; (b) J. W. Lown, and S. K. Sim, *ibid.*, 1977, **77**, 1150; (c) E. A. Sausville, R. W. Stein, J. Peisach, and S. B. Horwitz, *Biochemistry* 1978, **17**, 2746; (d) T. Takita, Y. Muraoka, T. Nakatani, A. Fujii, Y. Iitaka, and H. Umezawa. *J. Antibiot.*, 1978, **31**, 1073; (c) H. Umezawa, *Prog. Biochem. Pharmacol.*, 1976, **11**, 18.

3 N. J. Oppenheimer, L. O. Rodriguez, and S. M. Hecht, *Proc. Nat. Acad. Sci. USA*, 1979, **76**, 5616; R. E. Lenkinski and J. L. Dallas, *J. Am. Chem. Soc.*, 1979, **101**, 5902; M. Tsukayama, C. R. Randall, F. S. Santillo, and J. C. Dabrowiak, *ibid.*, 1981, **103**, 458; J. C. Dabrowiak, F. T. Greenaway, F. S. Santillo, and S. T. Crooke, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 721; J. C. Dabrowiak and M. Tsukayama, *J. Am. Chem. Soc.*, 1981, **103**, 7543; Y. Iitaka, H. Nakamura, T. Nakatani, Y. Murata, A. Fujii, T. Takita, and H. Umezawa, *J. Antibiot.*, 1978, **31**, 1070.

4 M. Otsuka, M. Yoshida, S. Kobayashi, M. Ohno, Y. Sugiura, T. Takita and H. Umezawa, *J. Am. Chem. Soc.*, 1981, **103**, 6986.

5 (a) J.-P. Henichart, J.-L.Bernier, R. Houssin, M. Lohez, A. Kenani, and J.-P. Catteau, *Biochem. Biophys. Res. Commun.*, 1985, **126**, 1036; (b) J.-P. Henichart, R. Houssin, J.-L. Bernier, and J.-P. Catteau, *J. Chem. Soc., Chem. Commun.*, 1982, 1295.

6 T. J. Lomis, J. Martin, B. McCloskey, S. Siddiqui, R. E. Shepherd, and J. F. Siuda, *J. Am. Chem. Soc.*, 1988, submitted for publication.

7 T. J. Lomis, M. G. Elliott, and R. E. Shepherd, *Inorg. Chem.*, to be submitted for publication.

8 T. J. Lomis, M.Sc. Thesis, University of Pittsburgh 1987; Univ. Microfilms, Ann Arbor, 1987.

9 (a) Y. Sugiura, *J. Am. Chem. Soc.*, 1980, **102**, 5208; (b) Y. Sugiura, J. Kuwahara, and T. Suzuki, *J. Chem. Soc., Chem. Commun.*, 1982, 908; (c) Y. Sugiura, T. Suzuki, J. Kuwahara, and H. Tonaka, *Biochem. Biophys. Res. Commun.*, 1982, **105**, 1511.

10 E. G. Janzen, *Acc. Chem. Res.*, 1971, **4**, 31; C. A. Evans, *Aldrichimica Acta*, 1979, **12**, 23.

11 T. K. Myser and R. E. Shepherd, *Inorg. Chem.*, 1987, **26**, 1544.

12 C. R. Johnson, T. K. Myser, and R. E. Shepherd, *Inorg. Chem.*, 1988, in the press; R. E. Shepherd, T. K. Myser, and M. G. Elliott, *ibid.*, 1988, in the press.

13 S. Siddiqui and R. E. Shepherd, *Inorg. Chem.*, 1986, **25**, 3869.

14 T. Sugiura, M. Suzuki, S. Otsuka, M. Kobayashi, M. Ohno, T. Takita, and H. Umezawa, *J. Biol. Chem.*, 1983, **258**, 1328; J. C. Dabrowiak, F. T. Greenaway, W. F. Longo, M. Van Husen, and S. T. Crooke, *Biochim. Biophys. Acta*, 1978, **517**, 517.