relative magnitudes of the $^1J_{C_\alpha N}$ and $^2J_{C_\alpha N}$ couplings,[5] the specific detection of this cross peak in the HCA(CO)NNH spectrum allows its unambiguous identification. In the next step, the cross peak due to the intraresidue correlation for E24, in the HCANNH spectrum, is located by searching at the $^1H_\alpha$ and $^{13}C_\alpha$ chemical shifts determined for the previous interresidue correlation; the cross peak is found in the f_2f_1 plane shown in Figure 2c. In this case, the next interresidue correlation to I23 is missing from the HCANNH spectrum. However, this correlation is easily detected in the corresponding f_2f_1 plane of the HCA(CO)NNH spectrum, allowing one to proceed with the sequential assignment (Figure 2d). The comparisons shown in Figure 2 indicate that the detection of the interresidue connectivity in the HCA(CO)NNH experiment is at least as sensitive as the detection of the intraresidue connectivity in the HCANNH experiment; we have found this to be true in all cases in ubiquitin.

In summary, the two 4D NMR experiments allow us to determine unambiguously intra- and interresidue connectivities between the backbone ^1H_N, ^{15}N, $^{13}C_\alpha$, and $^1H_\alpha$ nuclei. Because both experiments rely solely on one bond magnetization transfer steps, they are insensitive to the secondary structure of the protein. Our approach requires just two 4D NMR experiments, each of which can be recorded in a similar time to one or two of the (five or more) 3D NMR experiments used in the earlier approach. As with the 4D HCACON spectrum,[4] the higher dimensionality of our spectra reduces problems caused by overlap of $^1H_\alpha$-$^{13}C_\alpha$ cross peaks. Both of our 4D NMR experiments can be recorded on a protein sample dissolved in H_2O using an identical experimental set-up, which is a significant additional benefit. It avoids difficulties in comparing spectra with differing digital resolution or differing chemical shifts due to isotope effects. This will be particularly important for automated assignment procedures presently being developed.

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Copper(I) and Copper(II) Complexes of New Chelating Pterins

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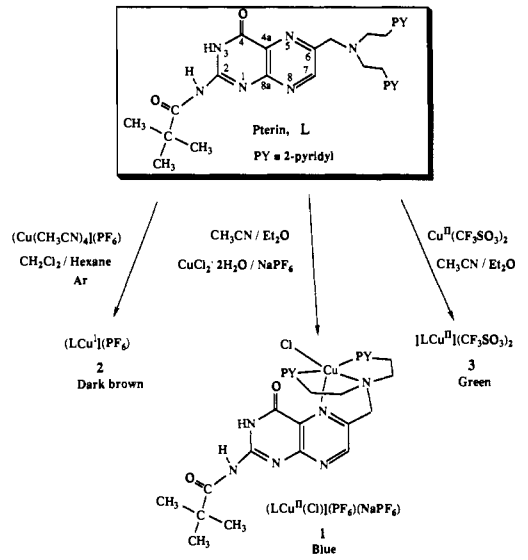
In this report, we describe a new class of pterin-derived ligands and copper ion complexes (Scheme I), designed to permit the controlled investigation of pterin/Cu/O_2 interactions and redox chemistry.[2-4] Such studies may be relevant to the active site

Scheme I



chemistry of a phenylalanine hydroxylase (PAH) from *Chromobacterium violaceum*, described recently by Benkovic and co-workers.[5,6] In mammalian systems, this is a non-heme iron, pterin, and dioxygen dependent enzyme which effects the hydroxylation of phenylalanine, giving tyrosine. Our current activities in bioinorganic copper–dioxygen chemistry[7] have interested us in PAH since, in this bacterial form, reaction of a reduced tetrahydropterin cofactor and O_2 occurs in the presence of a required copper ion cofactor. Since a 4a-hydroxypterin moiety can also be detected as a reaction product, the copper-mediated formation and/or reaction of a previously formed 4a-peroxypterin intermediate has been proposed.[5b,8]

To study the basic chemistry of such systems, we wanted to (1) synthetically modify pterins, placing a ligating group in the 6-position. Placement here would allow favorable chelation to the neighboring pterin N5 nitrogen and also allow proximity to the 4a-position where O_2/peroxide chemistry is suggested to occur.[5] EPR[9] and EXAFS[10] spectroscopic approaches implicate protein copper (as Cu(II)) coordination to N5, in addition to two or three protein-derived imidazole ligand donors. Substituents at the pterin 6-position should not inhibit the desired chemistry, since a variety of 6-substituted pterins (e.g., Me, $CH(OH)CH(OH)CH_3$, biopterin) act as substrates for PAH.[5] We also wanted to (2) use a nitrogen-containing chelate group, one that might mimic aspects of imidazole coordination but, more importantly, be known to effect Cu(I)/Cu(II) redox and O_2 chemistry,[6,7] and to (3) solubilize the pterin moiety (known to generally be very insoluble),[11]

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(1) (a) The Johns Hopkins University. (b) Syracuse University.

(2) Recent studies of copper complexes using pterin ligands include the following: (a) Perkinson, J.; Brodie, S.; Yoon, K.; Mosny, K.; Carroll, P. J.; Morgan, T. V.; Nieter Burgmayer, S. J. *Inorg. Chem.* **1991**, *30*, 719–727. (b) Kohzuma, T.; Masuda, H.; Yamauchi, O. *J. Am. Chem. Soc.* **1989**, *111*, 3431–3433. (c) Kohsuma, T.; Odani, A.; Morita, Y.; Takani, M.; Yamauchi, O. *Inorg. Chem.* **1988**, *27*, 3854–3858. (d) Bessenbacher, C.; Vogler, C.; Kaim, W. *Inorg. Chem.* **1989**, *28*, 4645–4648.

(3) A pterin cofactor is required in a variety of oxo-molybdenum enzymes, and molybdenum–pterin complexes are known: (a) Burgmayer, S. J. N.; Stiefel, E. I. *J. Am. Chem. Soc.* **1986**, *108*, 8310–8311. (b) Nieter Burgmayer, S. J.; Baruch, A.; Kerr, K.; Yoon, K. *J. Am. Chem. Soc.* **1989**, *111*, 4982–4984. (c) Gruber, S.; Kilpatrick, L.; Bastian, N. R.; Rajagopalan, K. V.; Spiro, T. G. *J. Am. Chem. Soc.* **1990**, *112*, 8179–8180. (d) Lehnen, J.; White, B. M.; Kendrick, M. J. *Inorg. Chim. Acta* **1990**, *167*, 257–259. (e) Soricelli, C. L.; Szalai, A.; Nieter Burgmayer, S. J. *J. Am. Chem. Soc.* **1991**, *113*, 9877–9878.

(4) For other examples of transition metal complexes with pterin ligands or analogues, see ref 2d and the following: (a) Abelleira, A.; Galang, R. D.; Clarke, M. J. *Inorg. Chem.* **1990**, *29*, 633–639. (b) Bhattacharya, S.; Boone, S. R.; Pierpont, C. G. *J. Am. Chem. Soc.* **1990**, *112*, 4561–4562.

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(6) Pember, S. O.; Johnson, K. A.; Villafranca, J. J.; Benkovic, S. J. *Biochemistry* **1989**, *28*, 2124–2130.

(7) (a) Tyeklár, Z.; Karlin, K. D. *Acc. Chem. Res.* **1989**, *22*, 241–248. (b) Paul, P. P.; Tyeklár, Z.; Jacobson, R. R.; Karlin, K. D. *J. Am. Chem. Soc.* **1991**, *113*, 5322–5332. (c) Sanyal, I.; Strange, R. W.; Blackburn, N. J.; Karlin, K. D. *J. Am. Chem. Soc.* **1991**, *113*, 4692–4693. (d) Karlin, K. D.; Tyeklár, Z.; Zuberbühler, A. D. In *Bioinorganic Catalysis*; Reedijk, J., Ed.; Marcel Dekker: New York, in press.

(8) The 4a-peroxy adduct has strong precedent in flavin chemistry and biochemistry. For example, see discussion in ref 5b and the following: (a) Bruice, T. C. *Acc. Chem. Res.* **1980**, *13*, 256–262. (b) Ballou, D. R. In *Flavins and Flavoproteins*; Bray, R. C., Engel, P. C., Mayhew, S. G., Eds.; de Gruyter: Berlin, 1984; pp 605–618.

(9) (a) Pember, S. O.; Benkovic, S. J.; Villafranca, J. J.; Pasenkiewicz-Gierula, M.; Antholine, W. E. *Biochemistry* **1987**, *26*, 4477–4483. (b) McCracken, J.; Pember, S.; Benkovic, S. J.; Villafranca, J. J.; Miller, R. J.; Peisach, J. *J. Am. Chem. Soc.* **1988**, *110*, 1069–1074.

(10) Blackburn, N. J.; Strange, R. W.; Carr, R. T.; Benkovic, J. S. Submitted for publication.

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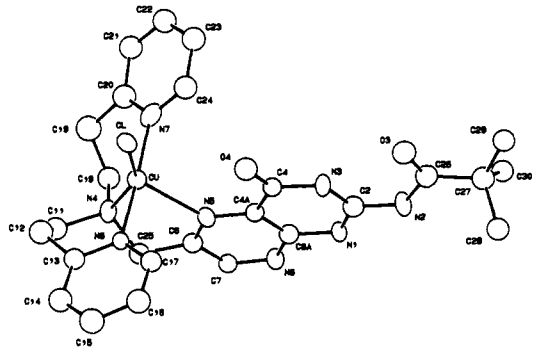

Figure 1. ORTEP diagram of the cationic part of the complex, $[LCu^{II}(Cl)](PF_6)(NaPF_6)\cdot 3CH_3CN$, showing the atom labeling scheme. Selected bond lengths (angstroms) and angles (deg) are as follows: Cu–N4, 2.129 (8); Cu–N5, 2.328 (3); Cu–N6, 1.985 (7); Cu–N7, 2.019 (8); Cu–Cl, 2.296 (3); N4–Cu–N5, 78.8 (3); N4–Cu–N6, 87.3 (3); N4–Cu–N7, 94.7 (3); N4–Cu–Cl, 151.6 (2); N5–Cu–N6, 88.7 (3); N5–Cu–N7, 94.6 (3); N5–Cu–Cl, 128.7 (2); N6–Cu–N7, 176.4 (3); N6–Cu–Cl, 86.7 (2); N7–Cu–Cl, 90.2 (2).

especially in organic solvents.[12] This was accomplished by placing an R group in a position which would not be likely to affect Cu/O_2 chemistry. Here, we describe the synthesis of a new class of pterin compounds, which give the desired highly soluble ligands and copper ion complexes possessing N5 coordination. A chloro–copper(II) derivative has been structurally characterized. Also, the *first* copper(I)–pterin complex has been isolated, and it can be reversibly oxidized to a Cu(II) species.

Pivalic anhydride was used to acylate[13] the 2-amino group of 6-methylpterin,[14] and bromination (NBS, acetic acid) gave a 6-(bromomethyl)pterin, to which a variety of chelate groups could be readily attached.[15] We first reacted bis[2-(2-pyridyl)-ethyl]amine (PY2),[16] affording gram quantities of L (Scheme I) after column chromatography on silica gel (CH_2Cl_2/MeOH, 9:1).[16b]

The pterin ligand L was reacted with cupric chloride, and after addition of $NaPF_6$, a blue complex $[LCu(Cl)](PF_6)(NaPF_6)\cdot 3CH_3CN$ (**1**) was isolated.[17] This was amenable to X-ray crystallographic analysis,[18,19] and the structure (Figure 1) confirmed the ligand synthesis design, with the tridentate PY2 chelate attached through the 6-position. The mononuclear Cu(II) complex displays a coordination geometry distorted from perfect square-based pyramidal, consistent with the EPR spectrum observed [DMF (77 K): $g_{\parallel} = 2.23$, $A_{\parallel} = 147 \times 10^{-4}$ cm^{-1}, $g_{\perp} = 2.04$]. The pterin N5 nitrogen is in the axial position, with Cu–N5 = 2.33 Å. The Cu···O4 distance is long (3.29 Å) and essentially noninteracting, and Cu···C4a = 3.41 Å. The coordination in **1** contrasts with that seen in the ternary species of the type $[L_xCu^{II}(\text{pterinate})_y]^{n+}$ (L is 2,2′-bipyridine, 1,10-phenanthroline, tris(3-phenylpyrazolyl)hydroborate, or acetate), recently studied by Nieter Burgmayer[2a] and Yamauchi.[2b] In these complexes, strong in-plane coordination provides $Cu\text{–}N5_{\text{pterinate}}$ bond lengths ranging from 2.01 to 2.06 Å; Cu(II) also binds to the C4 anionic oxygen atom, where the pterin moiety *is* a deprotonated anion. Pterin L is coordinated in its un-deprotonated form in **1**, as evidenced by (i) the requirements of total charge on the Cu(II) complex **1** and (ii) the presence of multiple N–H IR absorptions for **1**,[17] assigned to the N–H N3 bond and/or N2 amide N–H bond.

To begin probing the redox properties of this fully oxidized copper(II)–pterin_{ox} system, we sought to prepare a Cu(I) analogue. Thus, reaction of L with $[Cu(CH_3CN)_4]PF_6$ in CH_2Cl_2 under Ar followed by precipitation with hexane gave a dark brown Cu(I) complex, $[LCu](PF_6)$ (**2**) (Scheme I).[20] This species displays a sharp ^{1}H NMR spectrum,[20] is EPR silent, and shows no visible absorptions ascribable to *d–d* bands, consistent with its formulation as a copper(I)–pterin_{ox} compound. The absorption seen at 470 nm ($\epsilon = 3100$) in **2** is therefore tentatively assigned as a copper(I)-to-pterin MLCT transition, suggesting that it is indeed coordinated to the pterin moiety through N5.[20b] Cyclic voltammetric (CV) experiments indicate that **2** displays a reversible one-electron (confirmed by bulk electrolysis) oxidation in acetonitrile with $E_{1/2} = +0.063$ V vs $Ag/AgNO_3$. This exactly matches the CV behavior of a Cu(II) complex not having a chloride ligand, $[LCu](CF_3SO_3)_2$ (**3**),[21] independently prepared by reaction of copper(II) triflate with L (Scheme I). Thus, we suggest **2** and **3** have closely related tetracoordinate (e.g., PY2 and pterin N5) structures, differing from **1** by the additional chloride ligation.

Thus, we have succeeded in derivatizing pterin moieties and have isolated and characterized highly soluble[22] Cu(I) and Cu(II) complexes containing a chelate at the 6-position of the oxidized pterin. This makes these compounds potentially suitable for studies of copper/pterin/O_2 interactions and reactivity. Complex **2** is not sensitive to dioxygen, and further studies will focus upon variation of the 6-substituted chelate group and, more importantly, generation of reduced dihydro and/or tetrahydropterin–copper complexes.

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Supplementary Material Available: Tables I–V of crystal data and experimental conditions, positional and displacement parameters, bond lengths, bond angles, and anisotropic displacement parameters (12 pages). Ordering information is given on any current masthead page.

(11) Pfeiderer, W. In *Folates and Pterins*: Blakley, R. L.; Benkovic, S. J., Eds.; John Wiley & Sons: New York, 1985; Vol. 2, pp 43–114.

(12) Solubility in organic solvents affords the opportunity to employ low-temperature solution studies of unstable intermediates, an approach which has been found to be very useful in the characterization of Cu_2O_2 complexes derived from Cu(I) and dioxygen.[7]

(13) Modification of a published procedure: Taylor, E. C.; Ray, P. S. *J. Org. Chem.* **1987**, *52*, 3997–4000.

(14) Waring, P.; Armarego, W. L. F. *Aust. J. Chem.* **1985**, *38*, 629–623.

(15) We synthesized and characterized several chelating pterin ligands, such as those obtained by reaction of the 6-(bromomethyl)pterin with bis-[2-(2-pyridyl)ethyl]amine,[16] bis(2-pyridylmethyl)amine, and 2-[2-(methylamino)ethyl]pyridine.

(16) (a) Nelson, S. M.; Rodgers, J. *Inorg. Chem.* **1967**, *7*, 1390–1395. (b) Romary, J. K.; Zachariasen, R. D.; Garger, J. D.; Schiesser, H. *J. Chem. Soc. C* **1968**, 2884–2887. (c) ^{1}H NMR (400 MHz) ($CDCl_3$): δ 8.48 (d, 2 H), 8.43 (s, 1 H), 7.55 (m, 2 H), 7.10 (m, 2 H), 7.02 (d, 2 H), 4.03 (s, 2 H), 3.05 (t, 4 H), 2.95 (t, 4 H), 1.35 (s, 9 H). UV/vis [CH_3CN, λ_{max}, nm (ϵ, M^{-1} cm^{-1})]: 278 (1570), 329 (750). FAB mass spectrum (m/z): 487 $(M + H)^+$.

(17) Compound **1** loses acetonitrile solvate upon evacuation. Data for $[LCu(Cl)](PF_6)(NaPF_6)$ (**1**): UV/vis [CH_3CN, λ_{max}, nm (ϵ, M^{-1} cm^{-1})] 345 (6600), 640 (190), 914 (90); IR (Nujol, cm^{-1}) 3650, 3450 (br), 3250 (br), 1680, 1620, 1580, 840 (PF_6); molar conductivity (DMF) 155.40 Ω^{-1} cm^2 mol^{-1}; μ_{eff} (room temperature) = 1.86; FAB mass spectrum (m/z) 584 [CuLCl], 549 [CuL]. Anal. Calcd for $C_{26}H_{29}ClCuF_{12}N_8NaO_2P_2$: C, 34.78; H, 3.23; N, 12.48. Found: C, 34.32; H, 3.57; N, 12.39.

(18) X-ray quality crystals of $[LCu(Cl)](PF_6)(NaPF_6)\cdot 3CH_3CN$ (**1**) were obtained by recrystallization from CH_3CN/Et_2O. The complex crystallizes in the monoclinic space group, $P2_1/n$, with $a = 15.301$ (3) Å, $b = 12.520$ (4) Å, $c = 22.781$ (4) Å, $\beta = 95.15$ (2)°, $V = 4346$ (2) Å^3, and $Z = 4$. A Rigaku AFC5S diffractometer was used in the ω scan mode to collect 8058 unique reflections, of which 2818 reflections with $F_o \geq 6\sigma|F_o|$ were used in the solution and refinement. The positional parameters of the copper atom were determined by direct methods. The remaining non-hydrogen atoms were located on subsequent difference Fourier maps. The structure was refined to the current residual values of $R = 0.064$ and $R_w = 0.062$ (Mo Kα, $\lambda = 0.71073$ Å).

(19) Supplementary material.

(20) (a) Data for $[LCu](PF_6)$ (**2**): ^{1}H NMR (400 MHz) (CD_2Cl_2) δ 8.90 (d+s, 3 H), 7.75 (m, 2 H), 7.30 (m, 4 H), 4.2 (s, 2 H), 2.8 (m, 2 H), 2.95 (m, 2 H), 3.10 (m, 2 H), 3.35 (m, 2 H), 1.4 (s, 9 H); UV/vis [CH_3CN (Ar), λ_{max}, nm (ϵ, M^{-1} cm^{-1})] 324 (9500), 470 (3100); IR (Nujol, cm^{-1}) 3200 (br, NH), 1680, 1620, 1570, 840 (PF_6); molar conductivity (DMF) 78.0 Ω^{-1} cm^2 mol^{-1}. Anal. Calcd for $C_{26}H_{29}CuF_6N_8O_2P$: C, 44.97; H, 4.48; N, 16.09. Found: C, 44.98; H, 4.18; N, 16.14. (b) The downfield shift of the pterin C-7 proton is consistent with Cu(I) coordination to the pterin.

(21) Data for $[LCu](CF_3SO_3)_2$ (**3**): UV/vis [CH_3CN, λ_{max}, nm (ϵ, M^{-1} cm^{-1})] 345 (6560), 630 (120), 870 (80); IR (Nujol, cm^{-1}) 3400 (br, NH), 1660, 1620, 1560, 1250 (CF_3SO_3); mass spectrum (m/z) 549 [CuL]; molar conductivity (DMF) 170.40 Ω^{-1} cm^2 mol^{-1}; EPR [DMF (77 K)] $g_{\parallel} = 2.24$, $A_{\parallel} = 156 \times 10^{-4}$ cm^{-1}, $g_{\perp} = 2.02$. Anal. Calcd for $C_{28}H_{30}CuF_6N_8O_2S_2$: C, 39.63; H, 3.42; N, 13.21. Found: C, 39.41; H, 3.87; N, 12.99.

(22) For example, the Cu(I) complex **2** and Cu(II) species **3** are quite soluble in solvents such as MeOH, CH_3CN, CH_2Cl_2, THF, and $CHCl_3$.