stituent facing the same open space as 5-methyl, also exhibited a high-field shift (FOS = 1.8 ppm rather than 4–8 ppm for pigments of normal hydrophobic shifts).[8,9] The low-field signal, therefore, corresponds to the $CF_3$ occupying the 1,1-dimethyl position of the parent retinal. The presence of a recognition site for the latter moiety in the opsin binding site is well known.[5] Close nonbonding interaction of the $CF_3$ group with those amino acid residues constituting the recognition site probably caused this increased protein (down field) shift.

It is interesting to note that the low-field signal exhibited a broader line width (88 vs 72 Hz) consistent with a more crowded environment. From 0 to 25 °C (limited by thermal stability of the sample and freezing point of the medium), the relative line width of the two peaks remained the same.

For the fluoro(trifluoromethyl)phenyl sample (**1b**), the pigment was characterized by the appearance of two peaks of a 3:1 ratio in intensity, both at a higher field than the corresponding signals in the free chromophore (Figure 2). The appearance of only one set of such signals indicates that the pigment is conformationally homogeneous. The high-field shift of the $CF_3$ group is consistent with the above interpretation that the $CF_3$ group occupies the more open 5-methyl position, and the similar shift of the F signal (rather than the downfield shift for the bis-$CF_3$) is consistent with its smaller size, no longer in close contact with the recognition site for the 1,1-dimethyl groups, and consequently reduced van der Waals interactions.

In summary, $^{19}F$ NMR spectroscopy has provided direct evidence for the suspected conformational restriction of the cyclohexenyl ring and the polyene chain of the retinyl chromophore in visual pigment analogs.[10] Studies of analogs with fluorine labels directed toward the remaining regions of the hydrophobic pocket are in progress.

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(9) See, for example: Gerig, J. *Methods Enzymol.* **1989**, *77*, 3–22.
(10) A similar observation was made in a preliminary experiment with the *m*,*m*-bis(trifluoromethyl)phenyl retinal analog of BR: Mead, D. Ph.D. Thesis, University of Hawaii, Honolulu, HI, 1985.

## A Broad-Substrate Analogue Reaction System of the Molybdenum Oxotransferases

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Previously, we have developed reaction system I, $MoO_2(L\text{-}NS_2) + X \rightleftarrows MoO(L\text{-}NS_2)(DMF) + XO$,[2-5] as a functional model for the molybdenum oxotransferases,[6] which catalyze the overall transformation $X + H_2O \rightleftarrows XO + 2H^+ + 2e^-$. Others have also

**ANALOGUE REACTION SYSTEM**

$Mo^{VI}O_2(t\text{-}BuL\text{-}NS)_2 + X \rightleftharpoons Mo^{IV}O(t\text{-}BuL\text{-}NS)_2 + XO$ (II)

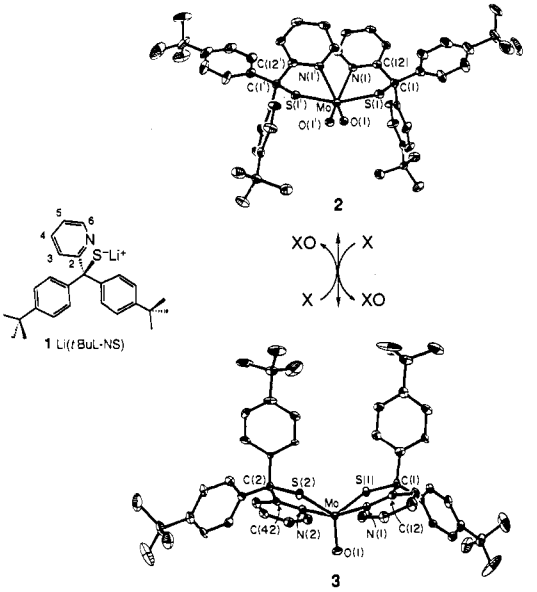


**Figure 1.** Schematic representation of analogue reaction system II of molybdenum oxotransferases, including the formula of Li(*t*-BuL-NS) (**1**) and the structures of $Mo^{VI}O_2(t\text{-}BuL\text{-}NS)_2$ (**2**) and $Mo^{IV}O(t\text{-}BuL\text{-}NS)_2$ (**3**). Substrates X/XO are given in the text. For **2**, primed and unprimed atoms are related by a 2-fold axis. Selected bond distances (Å) and angles (deg) are noted. Compound **2**: Mo–O(1), 1.696 (4); Mo–S(1), 2.418 (2); Mo–N(1), 2.411 (5); O(1)–Mo–O(1′), 107.7 (3); S(1)–Mo–S(1′), 159.8 (1); N(1)–Mo–N(1′), 76.2 (2); S(1)–Mo–N(1), 73.6 (2); O(1)–Mo–N(1), 89.8 (2). Compound **3**: Mo–O(1), 1.681 (5); Mo–S, 2.313 (3), 2.330 (3); Mo–N, 2.175 (7), 2.173 (7); S(1)–Mo–S(2), 124.3 (1); S(1)–Mo–O(1), 116.2 (3); S(2)–Mo–O(1), 119.5 (3); N–Mo–S, 80.3 (2)–92.1 (2); N(1)–Mo–N(2), 160.5 (3).

advanced pertinent model systems.[7,8] Under the oxo transfer hypothesis,[5] the substrate oxidation/reduction step involves atom transfer from/to the molybdenum center, as in $Mo^{VI}O_2 + X \rightleftarrows Mo^{IV}O + XO$. Other redox steps in the cycle restore the enzyme to its catalytic state.[5,6,9] While system I has most effectively demonstrated oxo transfer as a possible enzymatic pathway,[5] it does not fulfill several important criteria for optimal utility as an analogue reaction system. Its complexes are not stable in the presence of certain substrates, the structure of the reduced complex has not been established by X-ray diffraction, and rate constants and activation enthalpies for substrate reduction (nitrate, *N*-oxide, *S*-oxide) are sufficiently similar (ca. 1.5 (1) × $10^{-3}$ $s^{-1}$ (298 K), 23 (1) kcal/mol)[3,5] as to indicate a common early transition state without appreciable X–O bond weakening. Such a situation is uninformative with respect to the atom transfer event itself. Given the demonstration of the oxo transfer pathway for one enzyme (xanthine oxidase[10]) and its likelihood for others, we have sought an improved reaction system. As before, we utilize an anionic nitrogen–thiolate ligand to maintain an extent of consistency with Mo EXAFS results.[11]

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(2) Abbreviations: acac, acetylacetonate(1−); *t*-Bu-LNS, bis(*p*-*tert*-butylphenyl)-2-pyridylmethanethiolate(1−); $Et_2$dtc, *N*,*N*-diethyldithiocarbamate(1−); L-$NS_2$, 2,6-bis(2,2-diphenyl-2-thioethyl)pyridinate(2−); X/XO, oxygen atom (oxo) acceptor/donor.
(3) (a) Berg, J. M.; Holm, R. H. *J. Am. Chem. Soc.* **1985**, *107*, 925. (b) Harlan, E. W.; Berg, J. M.; Holm, R. H. *J. Am. Chem. Soc.* **1986**, *108*, 6992. (c) Caradonna, J. P.; Reddy, P. R.; Holm, R. H. *J. Am. Chem. Soc.* **1988**, *110*, 2139. (d) Craig, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1989**, *111*, 2111.
(4) Holm, R. H. *Chem. Rev.* **1987**, *87*, 1401.
(5) (a) Holm, R. H.; Berg, J. M. *Acc. Chem. Res.* **1986**, *19*, 363. (b) Holm, R. H. *Coord. Chem. Rev.* **1990**, *100*, 183, and references therein.
(6) Bray, R. C. *Q. Rev. Biophys.* **1988**, *21*, 299.
(7) (a) Nakamura, A.; Ueyama, N.; Okamura, T.-A.; Zaima, H.; Yoshinaga, N. *J. Mol. Catal.* **1989**, *55*, 276. (b) Ueyama, N.; Yoshinaga, N.; Okamura, T.-A.; Zaima, H.; Nakamura, A. *J. Mol. Catal.* **1991**, *64*, 247.
(8) (a) Kaul, B. B.; Enemark, J. H.; Merbs, S. L.; Spence, J. T. *J. Am. Chem. Soc.* **1985**, *107*, 2885. (b) Dowerah, D.; Spence, J. T.; Singh, R.; Wedd, A. G.; Wilson, G. L.; Farchione, F.; Enemark, J. H.; Kristofzski, J.; Bruck, M. *J. Am. Chem. Soc.* **1987**, *109*, 5655. (c) Roberts, S. A.; Young, C. G.; Cleland, W. E., Jr.; Ortega, R. B.; Enemark, J. H. *Inorg. Chem.* **1988**, *27*, 3044. (d) Roberts, S. A.; Young, C. G.; Kipke, C. A.; Cleland, W. E., Jr.; Yamanouchi, K.; Carducci, M. D.; Enemark, J. H. *Inorg. Chem.* **1990**, *29*, 3650.
(9) Wilson, G. L.; Greenwood, R. J.; Pilbrow, J. R.; Spence, J. T.; Wedd, A. G. *J. Am. Chem. Soc.* **1991**, *113*, 6803.
(10) Hille, R.; Sprecher, H. *J. Biol. Chem.* **1987**, *262*, 10914.
(11) George, G. N.; Kipke, C. A.; Prince, R. C.; Sunde, R. A.; Enemark, J. H.; Cramer, S. P. *Biochemistry* **1989**, *28*, 5075 and references therein.

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Reaction of *tert*-butylbenzene (2 equiv) with pyridine-2-aldehyde in concentrated $H_2SO_4$ gave, after neutralization with NaOH, bis(*p-tert*-butylphenyl)-2-pyridylmethane (85%).[12] Treatment of this compound with equimolar *n*-butyllithium and sulfur in THF at −65 °C followed by warming to room temperature afforded after standard workup Li(*t*-BuL-NS) (59%; **1**, Figure 1). Reaction of **1** (2 equiv) with $MoO_2(acac)_2$[13] gave yellow $MoO_2$(*t*-BuL-NS)$_2$ (98%, **2**, Figure 1; $\nu_{MoO}$ 901 cm$^{-1}$, $\lambda_{max}$ ($\epsilon_M$) 371 (6220) nm). Reaction of **2** with $Et_3P$ (1.5 equiv) in refluxing THF for 5 h yielded brown MoO(*t*-BuL-NS)$_2$ (69%; **3**, Figure 1; $\nu_{MoO}$ 940 cm$^{-1}$, $\lambda_{max}$ ($\epsilon_M$) 328 (5210), 430 (3840), 518 (780), 700 (460) nm). Compound **2** has a distorted octahedral structure with cis oxo and trans thiolate ligands, Mo–O = 1.696 (4) Å, O–Mo–O = 107.7 (3)°, and S(1)–Mo–S(1′) = 159.8 (1)°. Compound **3** possesses a distorted trigonal bipyramidal structure with an $MoOS_2$ equatorial plane, axial nitrogen ligands, Mo–O = 1.681 (5) Å, and N(1)–Mo–N(2) = 160.5 (3)°. It is an uncommon example of a five-coordinate $Mo^{IV}O$ complex with physiological-type ligation. Structures and metric parameters[14] are given in Figure 1 from which it is found that the $Mo^{VI}O_2 \rightarrow Mo^{IV}O$ conversion primarily involves large deformations of two bond angles (S–Mo–S by −36°, N–Mo–N by +84°) and significant compression of one bond length (Mo–N by −0.24 Å). It is further evident that the frontside steric hindrance of two *p-tert*-butylphenyl groups in the **2**, and to a lesser extent in **3**, obviates the undesirable formation of a stable, abiological $Mo^V$–O–$Mo^V$ bridge.[15]

In reaction system II (Figure 1), complex **3** in DMF solution (0.4–1.6 mM) cleanly reduces a variety of substrates. Reactions were monitored spectrophotometrically by the diminution of intensity at 328 and 430 nm and the increase in the 371-nm band of **2**; tight isosbestic points were observed at 341 and 404 nm. Some reactions were also examined by $^1$H NMR utilizing the chemical shift differences between the 6-H resonances of **3** ($\delta$ 9.52) and **2** ($\delta$ 9.37). *Stoichiometric* quantities of the biologically relevant substrates nicotinamide *N*-oxide, adenine *N*-oxide, $Me_3NO$, and $Me_2SO$, and also the abiological substrates 3-fluoropyridine *N*-oxide, $(PhCH_2)_3NO$, $Ph_2SO$, $Ph_2SeO$, $Ph_3AsO$, and $NaIO_4$ were reduced. Yields determined in situ from the formation of **2** were in the 81–95% range and can be increased with excess substrate. As already noted, in the reverse reaction **2** is reduced to **3** by $Et_3P$ in good yield.

The intermetal atom transfer reaction **3** + $MoO_2(Et_2dtc)_2 \rightleftarrows$ **2** + $MoO(Et_2dtc)_2$ lies essentially completely to the right, establishing that **3** is a stronger oxo acceptor than $MoO(Et_2dtc)_2$. Given that to date $Ph_3AsO$ is the weakest oxo donor to oxidize **3** and $Ph_3P$ is the weakest oxo acceptor to reduce **2**, the reaction couple **3** + $^1/_2O_2$ = **2** occurs between the couples $Ph_3As + {}^1/_2O_2 = Ph_3AsO$ ($\Delta H$ = −43 kcal/mol[16]) and $Ph_3P + {}^1/_2O_2 = Ph_3PO$ ($\Delta H$ = −70 kcal/mol[4]) in the thermodynamic scale of oxo transfer reactivity.[4] Thus reaction system II is thermodynamically competent to oxidize or reduce all enzymatic substrates (except those requiring the enzymatic state $Mo^{VI}OS$ as the oxidant). Its stability to the strong oxo donors $Me_3NO$ and $IO_4^-$ is notable.

The kinetics of substrate oxidation in system II have been examined under pseudo-first-order conditions in DMF solutions. Linear plots of rate constants $k_1$ vs [XO] indicate second-order reactions, and thus a different reaction mechanism from system I,[3,4] and rates sensitive to substrate. For example, at 298 K $k_2$ = 5.6 (2) × 10$^{-2}$ M$^{-1}$ s$^{-1}$ for XO = $Ph_3AsO$ and $k_2$ = 1.01 (2) × 10$^{-4}$ M$^{-1}$ s$^{-1}$ for XO = $Me_2SO$. From the enthalpy[4,16] of the reaction $Ph_3As_{(g)} + Me_2SO_{(g)} \rightarrow Ph_3AsO_{(g)} + Me_2S_{(g)}$, the As–O bond is 16 kcal/mol stronger than the S–O bond, yet the rate of reduction of $Ph_3AsO$, the more hindered substrate, is 560 times faster.

The synthesis of complexes **2** and **3** facilitates the development of a reaction system whose oxidized and reduced complexes are of known structure, in which the complicating factor of $\mu$-oxo Mo(V) dimer formation is absent in coordinating solvents, and which will transform a broad range of enzymatic and abiological substrates in second-order reactions whose rates are clearly substrate-dependent. System II appears to be the most useful analogue reaction system thus far devised; experiments utilizing it to probe the mechanistic aspects of oxygen atom transfer continue.

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**Supplementary Material Available:** Listing of crystal data and atom positional and isotropic thermal parameters and bond angles and distances for compounds **2** and **3** (9 pages). Ordering information is given on any current masthead page.

(12) *Experimental.* Preparations of **2** and **3** were carried out under anaerobic conditions. IR spectra were measured in Nujol or KBr and UV/visible spectra in DMF solutions. All new compounds gave satisfactory elemental analyses and appropriate parent ions in FAB–MS.

(13) Jones, M. M. *J. Am. Chem. Soc.* **1959**, *81*, 3188.

(14) Crystallographic data for compounds **2**·$CH_2Cl_2$/**3**·3MeCN: $a$ = 23.663 (6)/14.998 (3) Å, $b$ = 17.993 (3)/23.345 (4) Å, $c$ = 13.620 (3)/15.892 (3) Å, $\beta$ = 118.41 (1)°/93.96 (2)°, $T$ = 173/198 K, space group $C2/c/P2_1/n$, $Z$ = 4 (both), $2\theta_{min/max}$ = 3°–45° (both), unique data ($F_o^2 > 3\sigma F_o^2$) 3652/8078, $R$, $R_w$ = 5.69, 6.21%/6.38, 6.11%. Data were collected with Mo K$\alpha$ radiation. Structures were solved by standard procedures; empirical absorption corrections were applied.

(15) Reaction of equimolar **2** and **3** in dichloromethane followed by the addition of ether affords [Mo(*t*-Bu-LNS)$_2$]$_2$O as a dark blue solid, which was identified by the $^1$H NMR criterion for a $\mu$-oxo bridged species: Craig, J. A.; Harlan, E. W.; Snyder, B. S.; Whitener, M. A.; Holm, R. H. *Inorg. Chem.* **1989**, *28*, 2024. This compound is fully dissociated in polar solvents such acetonitrile and DMF at the concentrations used in this work.

(16) Barnes, D. S.; Burkinshaw, P. M.; Mortimer, C. T. *Thermochim. Acta* **1988**, *131*, 107.

## Driving-Force Effects on the Rates of Bimolecular Electron-Transfer Reactions

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The prediction that electron-transfer (ET) rates maximize when the reaction driving force ($-\Delta G°$) equals the reorganization energy ($\lambda$) is one of the most intriguing features of ET theory.[1] Consequently, observation of the inverted region ($-\Delta G° > \lambda$) in which rates *decrease* with *increasing* driving force has been a major objective in ET research.[2-10] On the basis of estimated reorg-

(1) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265–322.
(2) (a) Calcaterra, L. T.; Closs, G. L.; Miller, J. R. *J. Am. Chem. Soc.* **1983**, *105*, 670–671. (b) Miller, J. R.; Beitz, J. V.; Huddleston, R. K. *J. Am. Chem. Soc.* **1984**, *106*, 5057–5068. (c) Closs, G. L.; Miller, J. R. *Science* **1988**, *240*, 440–447.
(3) Wasielewski, M. R.; Niemczyk, M. P.; Svec, W. A.; Pewitt, E. B. *J. Am. Chem. Soc.* **1985**, *107*, 1080–1082.
(4) McLendon, G.; Miller, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 7811–7816.
(5) (a) Gould, I. R.; Ege, D.; Mattes, S. L.; Farid, S. J. *J. Am. Chem. Soc.* **1987**, *109*, 3794–3796. (b) Gould, I. R.; Moody, R.; Farid, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 7242–7244. (c) Gould, I. R.; Moser, J. E.; Armitage, B.; Farid, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 1917–1919.
(6) (a) Ohno, T.; Yoshimura, A.; Shioyama, H.; Mataga, N. *J. Phys. Chem.* **1987**, *91*, 4365–4370. (b) Asahi, T.; Mataga, N. *J. Phys. Chem.* **1989**, *93*, 6578–6581.
(7) (a) Chen, P.; Duesing, R.; Tapolsky, G.; Meyer, T. J. *J. Am. Chem. Soc.* **1989**, *111*, 8305–8306. (b) Chen, P.; Duesing, R.; Graff, D. K.; Meyer, T. J. *J. Phys. Chem.* **1991**, *95*, 5850–5858.
(8) (a) Macqueen, D. B.; Schanze, K. S. *J. Am. Chem. Soc.* **1991**, *113*, 7470–7479. (b) MacQueen, D. B.; Eyler, J. R.; Schanze, K. S. *J. Am. Chem. Soc.* **1992**, *114*, 1897–1898.

0002-7863/92/1514-6935$03.00/0 