

treatment a π -interaction between the heteroatom and the square metal array was identified as crucial for the heteroatom to lie in the plane of the four-metal base. Carbon and nitrogen atoms achieve this π -interaction because the p_z heteroatom energy is close to the metal atom d-orbital energies and $d\pi$ - $p\pi$ overlap is favorable. By contrast, the more electronegative sulfur and oxygen atoms have p-orbital energies well below those of the metals, which localizes an electron pair on the heteroatom. An unfavorable nonbonding interaction between the filled heteroatom p_z orbital and the filled metal d- π orbitals of the same symmetry is avoided by displacement of the heteroatom out of the basal plane of the cluster.

The μ_4 environment has been observed for oxygen on the low index faces of metals, $c(2 \times 2)O$ on Fe(100) being one example.²³ The present results indicate that the oxygen might take a capping position above the surface of this solid. In one report the interpretation of LEED data placed the oxygen atom 0.48 Å out of the Fe_4 plane,²⁴ and this agrees with low-energy ion scattering which places the oxygen 0.56 (5) Å out of the iron plane.²⁵ Thus the surface oxo ligand appears to be at a somewhat intermediate position and certainly not as far from the metal plane as observed in the present study of a cluster oxo complex.

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Supplementary Material Available: Tables of crystal data, positional parameters, anisotropic thermal parameters, and bond distances and angles for $[BzMe_3N]_2[Fe_2Ru_3(CO)_{14}(\mu_4-O)] \cdot [BzMe_3N][BPh_4] \cdot THF$ (15 pages). Ordering information is given on any current masthead page.

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Studies on the Molybdenum Cofactor. Determination of the Structure and Absolute Configuration of Form A

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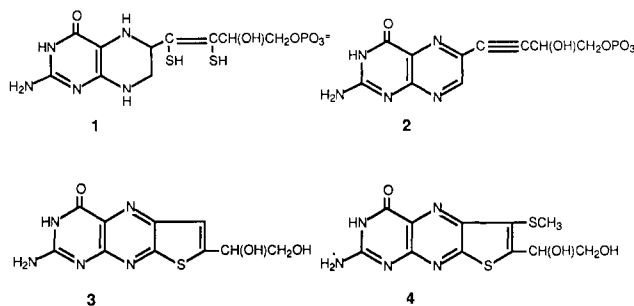
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The molybdenum cofactor, which is present in all but one (nitrogenase) of the known molybdenum-containing enzymes, is a complex of Mo^{VI} with a unique sulfur-containing reduced pterin (molybdopterin) for which structure **1** has been suggested.^{1,2} Several oxidative degradation products of molybdopterin have been isolated. Form A, derived from molybdopterin by oxidation of the pyrazine ring and removal of both sulfur atoms, has been assigned structure **2** on the basis of spectroscopic evidence, while Form B, which possesses a fused thiophene ring, retains one of



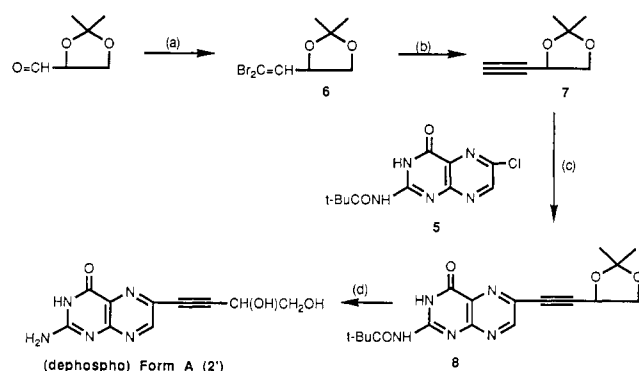
the original sulfur atoms (**3**).³⁻⁶ The structure of urothione, the urinary metabolite of the molybdenum cofactor, has recently been confirmed as **4** by an unequivocal total synthesis.⁷ Despite this array of synthetic, degradative, and spectroscopic structural evidence, however, there has been to date no evidence bearing on the absolute configuration of the side chain secondary hydroxyl group in molybdopterin. We now describe an unequivocal, *stereospecific* synthesis of (dephospho) Form A (**2'**) which establishes the absolute chirality of its secondary hydroxyl group, and we present spectroscopic evidence which places the phosphate grouping on the primary side chain hydroxyl group (C-4'), as depicted in **2**.

Synthetic Studies: Determination of the Absolute Configuration of (Dephospho) Form A (2'**).** Our synthetic strategy was based upon a general procedure recently reported by us for the preparation of 6-alkynylpterins which employs a palladium-catalyzed coupling between 2-pivaloyl-6-chloropterins (**5**) and monosubstituted acetylenes. The pterins themselves can then be obtained by hydrolytic removal of the 2-pivaloyl protecting group.⁸⁻¹⁰ (*R*)-Glyceraldehyde acetonide (from lead tetraacetate cleavage of 1,2,5,6-di-*O*-isopropylidene-D-mannitol)¹¹ was converted to the acetylene **7** by treatment with carbon tetrabromide and triphenylphosphine in methylene chloride to give the dibromoalkene **6** (55% yield), followed by reaction with 2 equiv of *n*-BuLi in THF (58% yield).¹² The resulting (*S*)-3,4-dihydroxybutyne acetonide (**7**) proved to be extremely difficult to purify and was coupled directly with 2-pivaloyl-6-chloropterins (**5**) in the presence of palladium acetate/tri-*o*-tolylphosphine/CuI to give **8**.¹³ Hydrolysis of **8** with 0.5 N HCl in aqueous dioxane at gentle reflux removed both the acetonide and pivaloyl groups to give (*S*)-**2'** (Scheme I).¹⁴ An analogous sequence of reactions starting from

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(13) Representative coupling procedure: A mixture of **5** (0.5 g, 1.8 mmol), Pd(OAc)₂ (50 mg, 0.2 mmol), tri-*o*-tolylphosphine (136 mg, 0.4 mmol), CuI (42 mg, 0.4 mmol), Et₃N (3 mL), and (*S*)-**7** (0.5 g, 4 mmol) in MeCN (10 mL) was heated at 100 °C in a sealed tube for 16 h. Solvent was removed in vacuo, and the residue was chromatographed on silica gel, eluting with 1% MeOH in CHCl₃. The residual solid from evaporation of the fractions containing the product was recrystallized from ethanol to give 145 mg (20%) of **8** as a cream-colored microcrystalline powder, mp 240–241 °C: NMR (CDCl₃) δ 12.48 (br s, 1 H), 8.88 (s, 1 H), 8.37 (br s, 1 H), 4.99 (t, 1 H, J = 6.3 Hz), 4.28 (dd, 1 H, J = 6.6 Hz, 8.4 Hz), 4.12 (dd, 1 H, J = 6.3 Hz, 8.4 Hz), 1.55 (s, 3 H), 1.44 (s, 3 H), 1.36 (s, 9 H). Acceptable microanalytical data were obtained for C₁₈H₂₁N₅O₆. HRMS calcd m/z 371.1593, found 371.1604.
(14) (*R*)- and (*S*)-(dephospho) Form A: pale yellow solids, mp > 250 °C dec; ¹H NMR (DMSO-*d*₆) δ 8.71 (s, 1 H), 4.44 (t, 1 H, J = 6.1 Hz), 3.53 d, 2 H, J = 6.1 Hz; UV λ_{max} (0.1 N NaOH) nm (e) 270 (33 300), 380 (13 200).

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Scheme 1^a

^a (a) CBr_4 , PPh_3 (2 equiv), CH_2Cl_2 , 0 °C to room temperature, (b) $n\text{-BuLi}$ (2 equiv), THF, -78 °C to room temperature, (c) $\text{Pd}(\text{OAc})_2$, (*o*-tolyl) $_3\text{P}$, CuI , Et_3N , CH_3CN , 100 °C, (d) 0.5 N HCl, dioxane, reflux.

(*S*)-glyceraldehyde acetonide (from L-ascorbic acid)¹⁵ gave the (*R*)-enantiomer of 2'.¹⁴

A comparison of the CD spectra of concentrated samples of these enantiomers with the CD spectrum of naturally derived 2' showed convincingly that the spectrum of the latter was essentially superimposable with the spectrum of synthetic (*S*)-2' and was the mirror image of the spectrum derived from synthetic (*R*)-2' (Figure 1). We conclude that Form A of the molybdenum cofactor, which therefore has the same chirality as (*R*)-glyceraldehyde, can be assigned the (*S*) configuration.

Spectroscopic Studies: Determination of the Position of Phosphorylation in Form A (2). The presence of a phosphate monoester functionality in naturally derived 2⁵ was indicated by mass spectral studies and by chemical analysis. In preliminary studies, the phosphate ester grouping was placed on the primary (4') hydroxyl group in the side chain on the basis of proton NMR data which compared the chemical shifts of the methine (C-3') and methylene (C-4') protons in the phospho and dephospho derivatives.² It had been observed that both methine and methylene resonances were shifted downfield in the phospho species but that the effect was more pronounced in the case of the C-4' proton resonance. Similar but more dramatic shifts were noted in the corresponding resonances in the phospho and dephospho carboxamidomethyl derivatives of molybdopterin.²

We have now obtained direct evidence for placement of the phosphate monoester grouping on C-4' by examination of the ³¹P NMR spectrum of naturally derived Form A (Figure 2). A well-resolved triplet (pH 8.4 buffer) collapsed to a singlet at 4.30 ppm when proton decoupled; the chemical shift and the splitting pattern are as expected for the resonance of a phosphate monoester on C-4', where the phosphorus interacts with two equivalent methylene protons. Lowering the pH of the sample to 5.2 results in an upfield shift of the resonance to 1.15 ppm, providing further evidence that the phosphate in Form A is a monoester with the characteristic *pK* near neutral pH.¹⁶

Conclusion. The assignment of structure 2 ((*S*) configuration) to Form A of the molybdenum cofactor can now be made with confidence. A synthetic assault on molybdopterin itself is now underway utilizing synthetic (dephospho) (*S*)-2' as a key intermediate.

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(17) All spectra were recorded on samples at a concentration of 0.5 mM in 0.3 mL of 1 N HCl using cells with a path length of 1 mm. The sample concentration was calculated by using a molar extinction coefficient of 24 200 at 272 nm and 14 100 at 344 nm for Form A in 1 N HCl. Each spectrum reported represents the computer average of three scans. θ represents molar ellipticity in units of $\text{deg cm}^2 \text{dmol}^{-1}$. The arrows on the y axis indicate the positions of the base lines for each of the three spectra.

(18) Spectra were acquired with a digital resolution of 0.372 Hz per point and are plotted with 2 Hz line broadening.

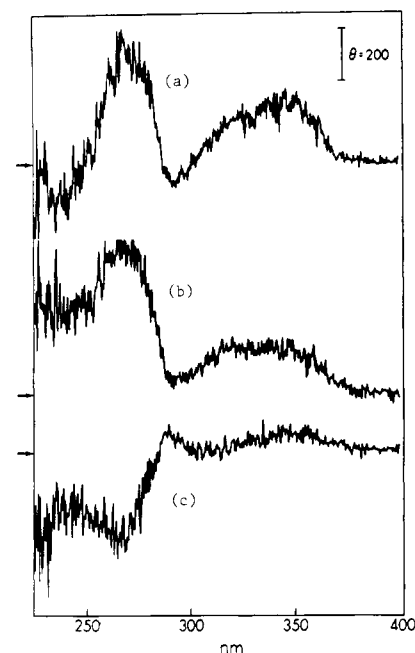


Figure 1. CD spectra of Form A (dephospho):¹⁷ (a) from the natural product, (b) from the synthetic (*S*) isomer, and (c) from the synthetic (*R*) isomer.

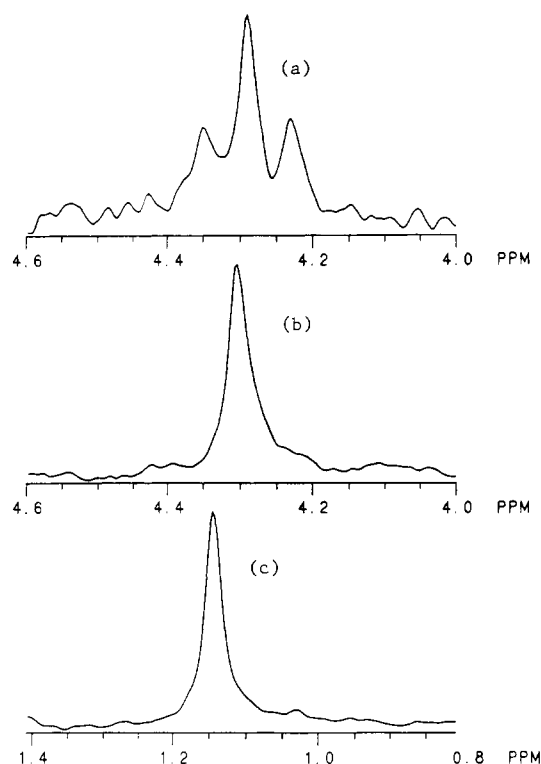


Figure 2. ³¹P NMR spectra of Form A:¹⁸ (a) from 9300 scans of a sample in 50 mM Tris-HCl, pH 8.4, containing 1 mM EDTA and 10% D₂O (Data were acquired with no proton decoupling), (b) from 10 000 scans of the same sample acquired with broad band proton decoupling, and (c) from 20 000 scans of the same sample, acquired with broad band proton decoupling, after adjusting the pH to 5.2 with HCl.

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