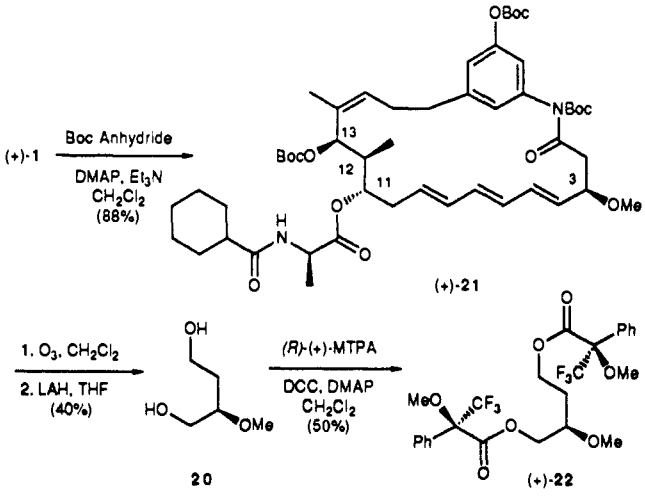
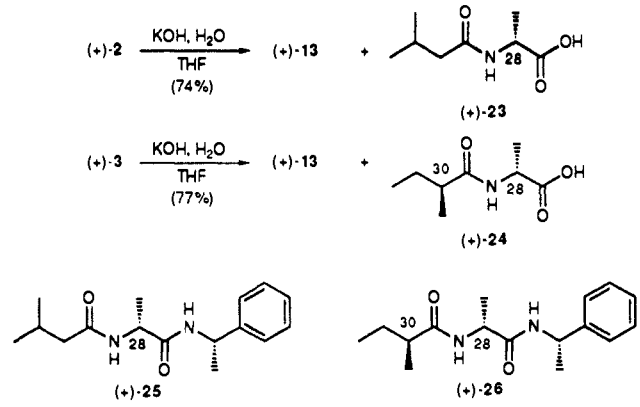
To elucidate the absolute configuration of the C(11,13) fragment, we embarked on an enantioselective synthesis of **15** (Scheme I) beginning with lactone (+)-**16**.[9] The resultant keto aldehyde [(−)-**15**][6] differed from the material obtained via degradation only in the sign of its optical rotation. This finding confirmed the relative configurations at C(11,13) and established the absolute stereochemistry of (+)-**15** as 11*S*,12*S*,13*R*.[10]

For investigation of the C(3) stereocenter of (+)-**1**, we envisioned 2-methoxy-1,4-butanediol (**20**)[11] as an attractive degradation target. Toward this end, protection of (+)-**1** as the tris-BOC derivative [(+)-**21**][6] followed by reductive ozonolysis (LAH) afforded diol **20** (40% yield),[12,13] which in turn was derivatized as the bis-Mosher ester (**22**).[6] Comparison with authentic samples of **22** and its C(3) diastereomer, prepared from (*S*)-(−)-, (*R*)-(+)-, and (±)-malic acid, permitted unambiguous assignment of the *R* absolute configuration at C(3).



We next elucidated the stereochemistry of trienomycins B and C via chemical correlation. Specifically, saponifications of (+)-**2** and (+)-**3** provided (+)-trienomycinol (**13**) and acids (+)-**23**[14] and (+)-**24**,[6] respectively. The latter furnished amides (+)-**25**[6] and (+)-**26**[6] [(*S*)-(−)-methylbenzylamine, diphenylphosphoryl azide (DPPA)], which in turn proved to be identical with authentic samples prepared from D-alanine.[15] Thus, the side chains in both (+)-**2** and (+)-**3** incorporate D-alanine moieties, and the additional C(30) stereocenter in (+)-**3** possesses the *S* configuration.

In summary, we have unambiguously assigned the complete relative and absolute configurations of trienomycins A, B, and C (**1-3**). The common absolute stereochemistry of **1-3** strongly suggests that similar features will prevail not only in trienomycins D and E but also in the closely related mycotrienins (**6** and **7**),



mycotrienols (**8** and **9**), and ansatrienins A_2-A_4 (**10-12**). Further stereochemical studies and progress toward the total synthesis of these potent antitumor/antifungal antibiotics will be reported in due course.

Acknowledgment. Support for this work was provided by the National Institutes of Health (National Cancer Institute) through Grant 19033. In addition, we thank Drs. George T. Furst and John Yates and Mr. John Dykins for their help in obtaining NMR spectra, computational data, and mass spectra, respectively. Finally, we thank Dr. David A. Johnson for his contributions to the preliminary stages of this work.

Supplementary Material Available: Calculated coupling constant data for stereoisomers of compound **15** and spectroscopic data for compounds **14**, **15**, and **17-26** and stereoisomers of **22**, **25**, and **26** (12 pages). Ordering information is given on any current masthead page.

(7) Each isomer was subjected to a Monte Carlo conformational search: Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379. The C(11,12) and C(12,13) ^{1}H coupling constants derived from the lowest energy conformations (i.e., those within 1.0 kcal/mol of the global minimum) were used for comparison.

(8) Further support for the cis-trans assignment emerged from the vicinal coupling constants reported for the twist-boat structure of *cis,trans*-2,2,4,5,6-pentamethyl-1,3-dioxane: $J_{4,5}$ = 5.3 Hz and $J_{5,6}$ = 7.9 Hz. See: Pihlaja, K.; Kellie, G. M.; Riddell, F. G. *J. Chem. Soc., Perkin Trans. 2* **1972**, 252.

(9) Hanessian, S.; Murray, P. J. *Tetrahedron* **1987**, *43*, 5055.

(10) Following the CIP sequence rules, the corresponding configuration of (+)-**1** is 11*S*,12*R*,13*R*.

(11) Lardon, A.; Reichstein, T. *Helv. Chim. Acta* **1949**, *32*, 2003.

(12) Reduction of BOC-protected secondary amides to primary alcohols has been reported previously: Fukuyama, T.; Nunes, J. J. *J. Am. Chem. Soc.* **1988**, *110*, 5196. Also see: Flynn, D. L.; Zelle, R. E.; Grieco, P. A. *J. Org. Chem.* **1983**, *48*, 2424.

(13) Compound **20** furnished high-field ^{1}H and ^{13}C (INEPT) NMR spectra and GC/MS data identical with those from a synthetic sample prepared by the method of Lardon.[11] Unfortunately, the low mass recovery of **20** precluded accurate measurement of the specific rotation.

(14) Schirlin, D.; Jung, M. Eur. Pat. Appl. EP 275,101, 1988; *Chem. Abstr.* **1989**, *110*, 173757v.

(15) The diastereomers of **25** derived from (±)- and L-alanine and three diastereomers of **26** were also prepared for comparison; see supplementary material.

Rate of Intramolecular Reduction of Ferryl Iron in Compound I of Cytochrome *c* Peroxidase

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Ferryl iron, Fe^{4+}, is the oxidation state of Fe in the enzyme intermediates of heme peroxidases[1] and possibly also heme monooxygenases[2] and cytochrome *c* oxidase.[3] However, the redox

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(1) Reczek, C. M.; Sitter, A. J.; Terner, J. *J. Mol. Struct.* **1989**, *214*, 27. Penner-Hahn, J. E.; Eble, K. S.; McMurry, T. J.; Renner, M.; Balch, A. L.; Groves, J. T.; Dawson, J. H.; Hodgson, K. O. *J. Am. Chem. Soc.* **1986**, *108*, 7819.

(2) Ortiz de Montellano, P. R. *Acc. Chem. Res.* **1987**, *20*, 289. Marnett, L. J. *Cytochrome P-450: Structure, Mechanism and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1986; pp 29-76.

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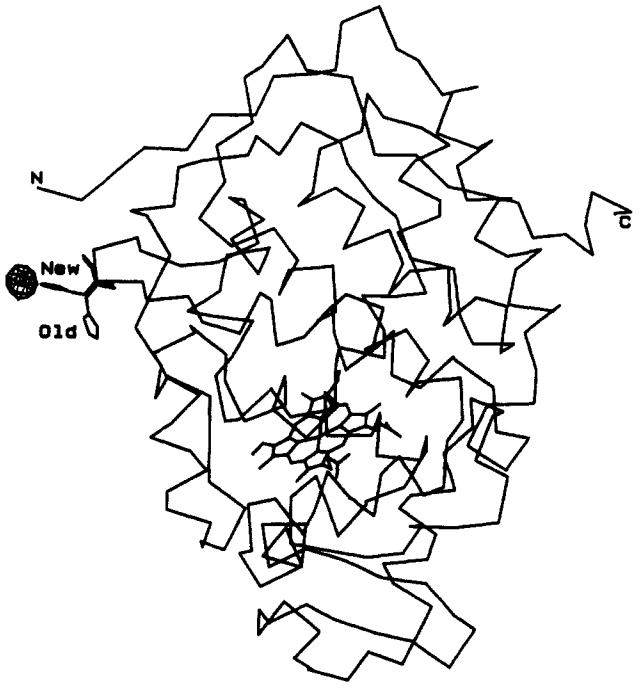

Figure 1. A 2.0-Å electron density difference map (ruthenium − parent) contoured at 5 times above background superimposed on the CCP model. The "old" position of His-60 in native CCP(Fe^{3+}) and the "new" position, pointing up toward the positive rhenium difference density, in a_5Ru^{3+}-(His-60)CCP(Fe^{3+}) are shown. Details are provided in footnote 8. The closest approach of His-60 in the "new" position to the heme edge is 21.8 Å or 20.5 Å to a heme vinyl group.

reactivity of this biologically important strong oxidant ($E° > 1$ V for peroxidases)[4] is poorly characterized.[5] To probe the electron-transfer reactivity of the Fe^{4+} center in compound I of cytochrome *c* peroxidase (CCP),[6] the most exposed histidine residue in CCP (His-60) was covalently modified with pentaammineruthenium(III) (a_5Ru^{3+}) by following standard procedures.[7] X-ray crystallography[8] of the purified derivative established that a_5Ru^{3+}(His-60)CCP(Fe^{3+}) is structurally similar to native CCP(Fe^{3+}) except for rotation of His-60 to accommodate the Ru complex as shown in Figure 1.

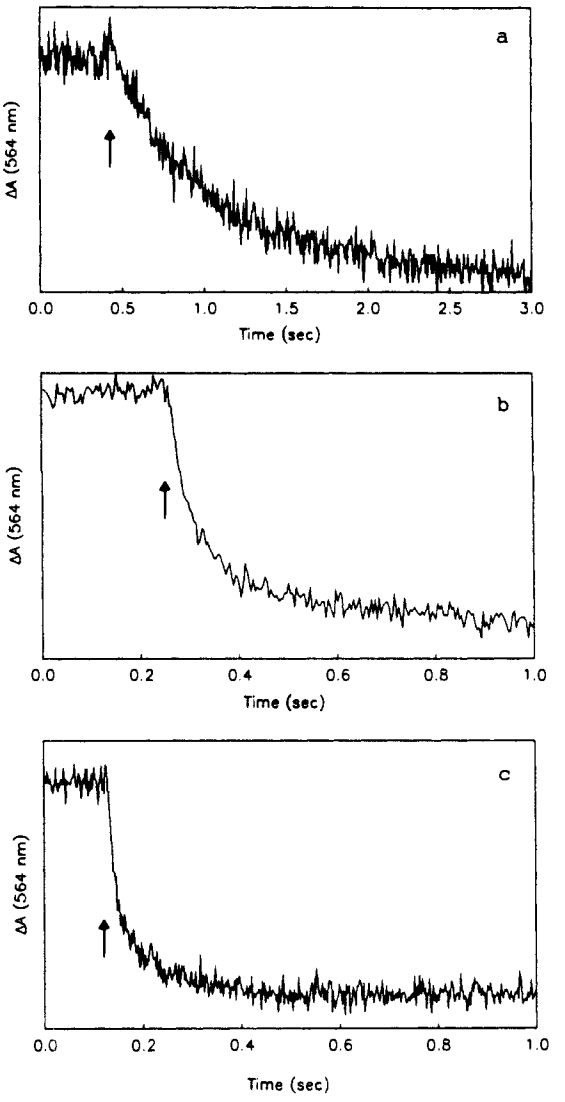

Figure 2. Absorbance change at 564 nm before and after laser flash photolysis of (a) 2.5 μM (b) 10 μM, and (c) 40 μM a_5Ru^{3+}(His-60)-CCP(Fe^{4+}), 120 mM DRF, 0.5 mM EDTA, 92 mM KCl in 4 mM phosphate (pH 7). The arrow on each trace indicates the time at which the laser flash of <1-ns duration was applied to the sample. Each trace is the *sum* of the absorbance changes following 4–6 laser flashes, which gave rise to total absorbance changes at 564 nm of ~0.01–0.03, corresponding to the reduction of 1–4 μM Fe^{4+} *in the laser beam* during each experiment.[11]

Production of a_5Ru^{2+}(His-60)CCP(Fe^{3+}) was achieved by laser flash photolysis of a solution of a_5Ru^{3+}(His-60)CCP(Fe^{3+}), 5-deazariboflavin (DRF), and EDTA.[9] The reaction kinetics of DRFH$^{\bullet}$ (produced at a concentration of <0.6 μM by the reaction of the DRF triplet with EDTA) were monitored at 518 nm, an isosbestic point for CCP(Fe^{4+}) and CCP(Fe^{3+}). In the presence of 2–20 μM a_5Ru^{3+}(His-60)CCP(Fe^{3+}), but not native CCP-(Fe^{3+}),[10] rapid decay of DRFH$^{\bullet}$ was observed corresponding to reduction of the a_5Ru^{3+} center with a rate constant of 1.3×10^9 M^{-1} s^{-1}. When a_5Ru^{3+}(His-60)CCP(Fe^{3+}) was titrated with H_2O_2 just prior to flash photolysis, a_5Ru^{2+}(His-60)CCP(Fe^{4+}) was generated following the flash, and signals due to the reduction

(3) Varotsis, C.; Babcock, G. T. *Biochemistry* **1990** In press. Ogura, T.; Takahashi, S.; Shinzawa-Itoh, K.; Yoshikawa, S.; Kitagawa, T. *J. Biol. Chem.* **1990** In press. Tatiana, V. V.; Konstantinov, A. A. *Ann. N.Y. Acad. Sci.* **1988**, *550*, 124. Blair, D. F.; Witt, S. N.; Chan, S. I. *J. Am. Chem. Soc.* **1985**, *107*, 7389.

(4) (a) Purcell, W. L.; Erman, J. E. *J. Am. Chem. Soc.* **1976**, *98*, 7033. (b) Hayashi, Y.; Yamazaki, I. *J. Biol. Chem.* **1979**, *254*, 9101.

(5) For example, electron-exchange rates of $\sim 10^2$ and 7×10^{-5} M^{-1} s^{-1} have been proposed by different workers for the CCP(Fe^{4+})/CCP(Fe^{3+}) couple[4,12] from cross-reactions with small inorganic complexes.

(6) Compound I of CCP, designated here by CCP(Fe^{4+}), results from the two-electron oxidation of CCP(Fe^{3+}) by H_2O_2, which generates Fe^{4+} and a protein cation radical ($P^{\bullet+}$). Recent evidence indicates that the $P^{\bullet+}$ site is on or near Trp-191 (Mauro, J. M.; Fishel, L. A.; Hazzard, J. T.; Meyer, T. E.; Tollin, G.; Cusanovich, M. A.; Kraut, J. *Biochemistry* **1988**, *27*, 6243. Sivaraja, M.; Goodin, D. B.; Smith, M.; Hoffman, B. M. *Science* **1989**, *245*, 738. Erman, J. E.; Vitello, L. B.; Mauro, J. M.; Kraut, J. *Biochemistry* **1989**, *28*, 7992). Since Trp-191 is on the opposite side of the heme from His-60, it is assumed that $P^{\bullet+}$ is not in the electron-transfer path between the Ru and heme centers. Future studies on the Phe-191 mutant of CCP are planned to test this supposition.

(7) An anaerobic solution of ~20:1 $a_5Ru^{2+}H_2O$ and CCP(Fe^{3+}) was maintained at room temperature for ~3 h, and a_5Ru^{3+}(His-60)CCP(Fe^{3+}) was purified from this solution by ion-exchange chromatography following procedures similar to those published (Yocom, K. M.; Shelton, J. B.; Shelton, J. R.; Schroeder, W. A.; Worosila, G.; Isied, S. S.; Bordignon, E.; Gray, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 7052). Details will be presented elsewhere (Fox, T., manuscript in preparation).

(8) Crystals of monoruthenated CCP were found to be isomorphous with native crystals. X-ray intensity data were obtained to 2.0 Å from a single crystal using a Siemens area detector. A total of 90658 observations were scaled to give a symmetry $R = 5.14\%$. A total of 23133 reflections to 2.0 Å were obtained, and the data set was 79% complete to 2.0 Å and 88% complete to 2.2 Å. The average intensity of the highest resolution shell used to 2.0 Å was 1.9 times above background. The difference map shown in Figure 1 was produced by using calculated phases obtained from the highly refined, 1.7-Å CCP structure and F_o(ruthenated) − F_c(native) amplitudes. At 5 times above background, the only difference density in the asymmetric unit was near His-60, which we assume is the location of the a_5Ru center.

(9) The laser flash photolysis system has been described previously (Simondsen, R. P.; Tollin, G. *Biochemistry* **1983**, *22*, 3008). The time resolution of the apparatus used in the present experiments is ~5 μs.

(10) Since rapid decay of DRFH$^{\bullet}$ is not observed in the presence of native CCP(Fe^{3+}) or CCP(Fe^{4+}) (Hazzard, J. T.; Poulos, T. L.; Tollin, G. *Biochemistry* **1987**, *26*, 2836), this demonstrates rapid reduction of Ru^{3+} by DRFH$^{\bullet}$.

of Fe^{4+} to Fe^{3+} were observed at 564 nm[11] on a time scale of seconds. The time course of this slow reaction (Figure 2) is dependent on the concentration of a_5Ru^{3+}(His-60)CCP(Fe^{4+}) present in the samples. At ≥ 10 μM protein, the traces are clearly biphasic and the faster, dominant phase yields a pseudo-first-order rate constant which is dependent on protein concentration in the range 10-40 μM, and is assumed to arise from *intermolecular* reduction of the Fe^{4+} center:

$$a_5Ru^{2+}(His\text{-}60)CCP(Fe^{4+}) + a_5Ru^{3+}(His\text{-}60)CCP(Fe^{4+}) \xrightarrow{k_2} a_5Ru^{3+}(His\text{-}60)CCP(Fe^{4+}) + a_5Ru^{3+}(His\text{-}60)CCP(Fe^{3+}) \quad (1)$$

where $k_2 = 1.2 \times 10^6$ M^{-1} s^{-1}. This value is comparable to that obtained for the reduction of CCP(Fe^{4+}) by free a_5Ru^{2+}py ($k_2 = 9.5 \times 10^6$ M^{-1} s^{-1}),[12] indicating that the covalently attached Ru center is highly exposed, as is evident from Figure 1.

The slower phase, which essentially disappears at 40 μM protein (Figure 2c), yields a first-order rate constant, $k_1 = 3.2 \pm 1.2$ s^{-1}, which is independent of protein concentration. This process is assigned to *intramolecular* reduction of the Fe^{4+} center by the surface-bound Ru^{2+}:

$$a_5Ru^{2+}(His\text{-}60)CCP(Fe^{4+}) \xrightarrow{k_1} a_5Ru^{3+}(His\text{-}60)CCP(Fe^{3+}) \quad (2)$$

The rate of intramolecular reduction of the Fe^{4+} is surprisingly slow considering the large driving force for this reaction ($\Delta E^\circ \sim 1$ V)[13] and the 21.8-Å separation from His-60 to the heme (Figure 1). At a lower driving force ($\Delta E^\circ \sim 0.8$ V), rates of 85-100 s^{-1} were observed for electron transfer from the Zn protoporphyrin triplet state to Ru^{3+} in three different a_5Ru^{3+} derivatives of Zn-substituted myoglobin with 19-22-Å separation between the redox centers.[14] Similarly, rapid (10^2-10^4 s^{-1}) intracomplex electron transfer has been observed in the electrostatically stabilized complexes[15] between the natural partners yeast cytochrome *c* (cyt *c*) and CCP(Fe^{4+})[16] or Zn-substituted CCP.[17] However, these rates are highly sensitive to the source (yeast, horse, etc.) of cyt *c*, as well as the ionic strength, suggesting that changes at the protein-protein interface play a dominant role in the control of the intracomplex rates.[10,16-18]

No protein-protein interface is traversed in the slow intramolecular electron transfer in eq 2. This raises the possibility that the reorganization energy associated with the conversion of 6-coordinate low-spin Fe^{4+} to 5-coordinate high-spin Fe^{3+},[19] which involves dissociation of the Fe^{4+}=O bond in the former,[1] may be quite large. It is also possible that there is no efficient electron-transfer pathway from His-60 to the heme. In fact, addition of cyt *c*(Fe^{3+}) at low ionic strength does not affect the reduction of the a_5Ru^{3+} center, indicating that cyt *c* does not interact with CCP in this region,[20] which obviates the need for electron transfer between His-60 and the heme. Furthermore, the intramolecular electron transfer rate does not change on cyt *c* binding, which eliminates the possibility of cyt *c* acting as a gating switch to allow facile electron transfer from nonspecific surface regions.

To examine the reasons for this slow electron-transfer rate, other derivatives of CCP are being prepared.[21] We also plan to prepare a_5Ru^{3+} derivatives of CCP(Zn) to compare their electron-transfer reactivities with those reported for the myoglobin derivatives.[14]

Acknowledgment. We thank Professors Alison Butler (Santa Barbara) and Harry Gray (Caltech) for their assistance in our first attempts to ruthenate CCP. This work was supported by NSERC (Canada) Grant A1530 (to A.M.E.), NSF Grant DMB-8716316 (to T.L.P.), and NIH Grant DK15057 (to G.T.).

(11) $\Delta\epsilon$ is ~ 8 mM^{-1} cm^{-1} at this wavelength (Yonetani, T. *The Enzymes Vol 18*; Boyer, P. D., Ed.; Academic Press: New York, 1976; pp 345-361), which we found to be a maximum in the CCP(Fe^{4+}) - CCP(Fe^{3+}) difference spectrum. There is no evidence to suggest that the rate of reduction of Ru^{3+} attached to CCP(Fe^{4+}) should be any different from that attached to CCP(Fe^{3+}). Thus, DRFH$^+$ reduction of the Fe^{4+} center on the slow time scale is not possible, and Fe^{3+} production must be due to electron transfer from Ru^{2+}.

(12) Yandell, J. K.; Yonetani, T. *Biochim. Biophys. Acta* **1983**, *748*, 263.

(13) $\Delta E^\circ \sim 1$ V assuming $E^\circ = 80$ mV for $Ru^{3+/2+}$, which is the value found for the free a_5RuHis complex (Nocera, D. G.; Winkler, J. R.; Yocom, K. M.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1984**, *106*, 5145), and $E^\circ = 1.087$ V for $Fe^{4+/3+}$ in CCP.[4a]

(14) Axup, A. W.; Albin, M.; Mayo, S. L.; Crutchley, R. J.; Gray, H. B. *J. Am. Chem. Soc.* **1988**, *110*, 435.

(15) The porphyrin-to-porphyrin separation in the noncovalent CCP-cyt *c* complex is not known, but a separation distance of ~ 16 Å was estimated from a computer graphics model of the complex (Poulos, T. L.; Kraut, J. *J. Biol. Chem.* **1980**, *255*, 10322. Poulos, T. L.; Finzel, B. C. *Pept. Protein Rev.* **1984**, *4*, 115).

(16) Hazzard, J. T.; McLendon, G.; Cusanovich, M. A.; Das, G.; Sherman, F.; Tollin, G. *Biochemistry* **1988**, *27*, 4445. Using the same conditions as in the present experiments, and the CCP from which a_5Ru(His-60)CCP was prepared, we obtained a limiting first-order rate constant of 175 s^{-1} for electron transfer from yeast iso-1 cyt *c*(Fe^{2+}) to CCP(Fe^{4+}).

(17) Liang, N.; Kang, C. H.; Ho, P. S.; Margoliash, E.; Hoffman, B. M. *J. Am. Chem. Soc.* **1986**, *108*, 4665.

(18) Ho, P. S.; Sutoris, C.; Liang, N.; Margoliash, E.; Hoffman, B. M. *J. Am. Chem. Soc.* **1985**, *107*, 1070.

(19) Smulevich, G.; Mantini, A. R.; English, A. M.; Mauro, J. M. *Biochemistry* **1989**, *28*, 5058.

(20) This is in agreement with Brownian dynamics computer simulations of the association of cyt *c* and CCP which do not indicate a binding site for cyt *c* in the region of His-60 (Northrup, S. H.; Boles, J. O.; Reynolds, J. C. L. *Science* **1988**, *241*, 67).

(21) An investigation of the reorganization energy by a temperature-dependence study of the intramolecular electron transfer rate is extremely difficult for the His-60 derivative because the inter- and intramolecular processes occur on overlapping time scales at the lowest protein concentration required (1 μM) to obtain a reasonable signal-to-noise ratio.

Carbocupration of Cyclopropene. Asymmetric Synthesis of Quaternary Carbon Centers

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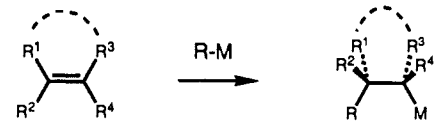
Carbometalation of a substituted olefin creates an organometallic species bearing two contiguous chiral centers (eq 1). If an effective method were available to make such a process asymmetric, then this reaction would acquire great utility as a method for the preparation of homochiral compounds.



(1)

We report that in the *cis*-carbocupration[1,2] of homochiral spirocyclic cyclopropenes **2** the asymmetric environment in the ketal moiety may be effectively transmitted through the spiro linkage to the rather distant olefinic portion of the molecule. Thus, the reaction generates highly synthetically useful[1] chiral copper reagents **3** and **4**, in which the cyclopropyl ring carbons are asymmetrically and differentially functionalized (Scheme I). Particularly notable is the regio- and stereoselective carbocupration of substituted cyclopropenes **2b,c**, which afforded cyclopropylcopper reagents **4b,c** bearing a chiral quaternary carbon center.[3]

The C_2-homochiral cyclopropene **2a** was prepared in 85% distilled yield by treatment of the ketal **1** with KNH_2 in liquid NH_3,[4] and the substituted derivatives **2b,c** were prepared by lithiation of **2a** with BuLi followed by alkylation under our previously reported conditions.[5]

(1) Nakamura, E.; Isaka, M.; Matsuzawa, S. *J. Am. Chem. Soc.* **1988**, *110*, 1297.

(2) Stoll, A. T.; Negishi, E.-i. *Tetrahedron Lett.* **1985**, *26*, 5671.

(3) Hashimoto, S.-i.; Koga, K. *Tetrahedron Lett.* **1978**, 573. Kogan, H.; Tomioka, K.; Hashimoto, S.-i.; Koga, K. *Tetrahedron Lett.* **1980**, *21*, 4005. Tomioka, K.; Cho, Y.; Sato, F.; Koga, K. *J. Org. Chem.* **1988**, *53*, 4094. Meyers, A. I.; Harre, M.; Garland, R. *J. Am. Chem. Soc.* **1984**, *106*, 1146. Caporusso, A. M.; Consoloni, C.; Lardioci, L. *Gazz. Chim. Ital.* **1988**, *118*, 857.

(4) Albert, R. M.; Butler, G. B. *J. Org. Chem.* **1977**, *42*, 674. Boger, D. L.; Brotherton, C. E.; Georg, G. I. *Org. Synth.* **1987**, *65*, 32.

(5) Isaka, M.; Matsuzawa, S.; Yamago, S.; Ejiri, S.; Miyachi, Y.; Nakamura, E. *J. Org. Chem.* **1989**, *54*, 4727.

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