of light dispersion and software that allows scanning of the spectrometer was prepared. Details of this Raman system will be reported elsewhere.[15] Purification procedures of beef heart cytochrome *c* oxidase has been described previously,[16a] but the final step of purification adopted was crystallization,[16b] which removed the fluorescence background in Raman scattering. A deuterated sample was prepared by repeated (three times) filtration through a membrane filter (Amicon YM-5) and dilution by 10-fold (v/v) $D_2O$ buffer.

Figure 1 shows the 840-nm-excited RR spectra of the resting enzyme (A) and its fully reduced form (B). They are distinct from the 600-nm-excited RR spectra reported by others[12] and contain protein modes such as amide I (1656 $\mathrm{cm}^{-1}$), $CH_2$ scissoring (1447 $\mathrm{cm}^{-1}$), and the side-chain modes of Phe residues (1003 $\mathrm{cm}^{-1}$). A heme mode also appears at 1556 $\mathrm{cm}^{-1}$ for the resting form and at 1545 $\mathrm{cm}^{-1}$ for the reduced form. This band became stronger when the excitation wavelength was shifted to 735 nm. Although amide II is expected in this frequency region, it is generally weak in Raman scattering and, moreover, the 1556-$\mathrm{cm}^{-1}$ band does not disappear in $D_2O$ contrary to the property of amide II. Besides these protein and heme modes, a broad and intense band is observed at 330 $\mathrm{cm}^{-1}$ for the resting form. This band disappears in the reduced form while the protein modes show little change upon the redox change. Although a fluorescence band appears in the reduced form in the low-frequency region and the background becomes higher, a weak protein band at 471 $\mathrm{cm}^{-1}$ is identified similarly to the oxidized form. Therefore, if the 330-$\mathrm{cm}^{-1}$ band were present in the reduced form, it could be observed under this background level. The 330-$\mathrm{cm}^{-1}$ band is observable with the mixed-valence enzyme in which cytochrome *a* and $\mathrm{Cu_A}$ are oxidized, but not with one with the opposite redox combination. Therefore, it is reasonable to assign the band at 330 $\mathrm{cm}^{-1}$ to the moiety associated with the 830-nm absorption, that is, the $\mathrm{Cu_A}^{\mathrm{II}}$ center.

Figure 2 compares the RR spectra in the 500–300-$\mathrm{cm}^{-1}$ region of the resting enzyme in $H_2O$ (A) and $D_2O$ (B). Although this was excited at 845 nm to examine another excitation wavelength, a spectrum similar to Figure 1A, obtained with 840-nm excitation, was observed. The difference spectrum (trace C), which shows one derivative pattern around 353 $\mathrm{cm}^{-1}$ and one positive peak at 335 $\mathrm{cm}^{-1}$, indicates the presence of at least three components in the broad band; the 356, 335, and 330-$\mathrm{cm}^{-1}$ bands show a frequency shift, an intensity change, and no change in $D_2O$, respectively.

The visible absorption of blue copper proteins around 600 nm is considered to arise from a sulfur (Cys) to $\mathrm{Cu^{II}}$ charge-transfer transition,[17] and accordingly, RR spectra obtained with 600-nm excitation can be attributed mainly to the $\mathrm{Cu^{II}}$–S stretching mode.[8b,10,11] The 830-nm absorption of $\mathrm{Cu_A}$ is also considered to arise from a similar transition of the $\mathrm{Cu_A}$ center,[13] and hence we tentatively assign the most intense and deuteration insensitive band at 330 $\mathrm{cm}^{-1}$ to a mode mainly associated with a $\mathrm{Cu^{II}}$–S(Cys) stretching vibration of $\mathrm{Cu_A}$ and the weak and deuteration sensitive band at 356 $\mathrm{cm}^{-1}$ to the Cu–N(His) stretching vibration. The Cu–S stretching bands of blue copper proteins with a single cysteine ligand exhibit complicated patterns of $D_2O$ shifts,[8b,11b] which are explained in terms of vibrational mixing of the Cu–S with Cu–N stretching modes. Normal coordinate calculations of the Cu center of blue copper proteins indicate significant mixing of the $\mathrm{Cu^{II}}$–S with the $\mathrm{Cu^{II}}$–N stretching modes.[8b,18] Therefore, it is highly likely that $\mathrm{Cu_A}$-associated RR bands are mixed modes of the Cu–N and Cu–S stretching vibrations. Recently, coordinations of Cys-196, Cys-200, His-204, and His-161[19] or Met-207[20] of subunit II to $\mathrm{Cu_A}$ have been suggested.

The frequency of the Cu–S stretching band of $\mathrm{Cu_A}$ is distinctly low compared with the corresponding frequencies of blue copper proteins (350–450 $\mathrm{cm}^{-1}$). This might be explained by the longer $\mathrm{Cu_A}$–S bond as revealed by EXAFS.[6] It is observed from the MCD, EXAFS, and sequence analysis[21] that, among various copper proteins so far characterized, only nitrous oxide reductase has the $\mathrm{Cu_A}$ type copper,[21] and in fact, the present RR spectrum bears close similarity to that of nitrous oxide reductase.[22]

The function of $\mathrm{Cu_A}$ of cytochrome *c* oxidase is considered to be delivery of electrons, together with Cyt *a*, from cytochrome *c* to a Cyt $a_3$–$\mathrm{Cu_B}$ center,[1,23] and possibly service as a gate of a proton pump.[24] However, the mechanism still remains to be experimentally clarified. The present technique combined with the flow apparatus for time-resolved measurements[25] is expected to provide a new means for studying the $\mathrm{Cu_A}$ site of cytochrome *c* oxidase.

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## Photosensitized Decarboxylative Michael Addition through *N*-(Acyloxy)phthalimides via an Electron-Transfer Mechanism

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Although photosensitized electron-transfer reactions have recently attracted considerable attention in organic photochemistry, application of this methodology to organic synthesis has been limited to fewer examples. We have recently reported a method of photodecarboxylation using *N*-(acyloxy)phthalimides which can be readily obtained from carboxylic acids.[1] This method may be applicable to decarboxylative radical type Michael addition. Hitherto, an elegant decarboxylative radical addition to electron-deficient olefins has been reported by Barton and his coworkers.[2] However, in their method, the 2-pyridylthio group is always introduced to the α-position of the electron-deficient group because of the high affinity of alkyl radicals toward the sulfur atom of the thiocarbonyl group. In order to introduce hydrogen to this position (Michael addition), we have examined several redox systems using *N*-(acyloxy)phthalimides. We now report a new and efficient radical chain method for this conversion using the redox combination of $\mathrm{Ru(bpy)_3Cl_2}$ and 1-benzyl-1,4-dihydronicotinamide (BNAH) in aqueous solvents with visible-light excitation.

Irradiation of a THF–water (7:3, 40 mL) solution of $\mathrm{Ru(bpy)_3Cl_2}$ (ca. 10 mg), *N*-(acyloxy)phthalimide (**1a**, 0.58 mmol),

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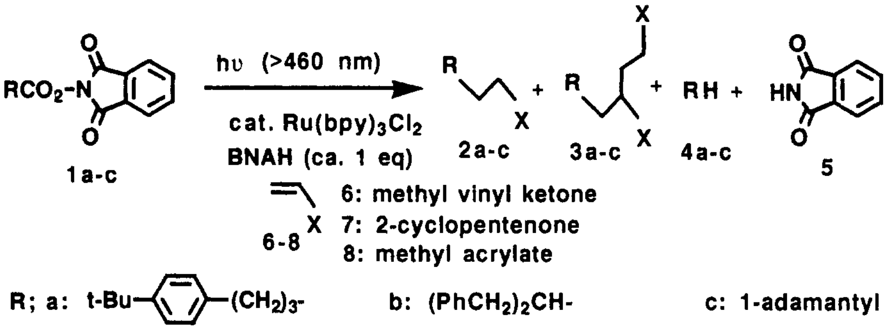
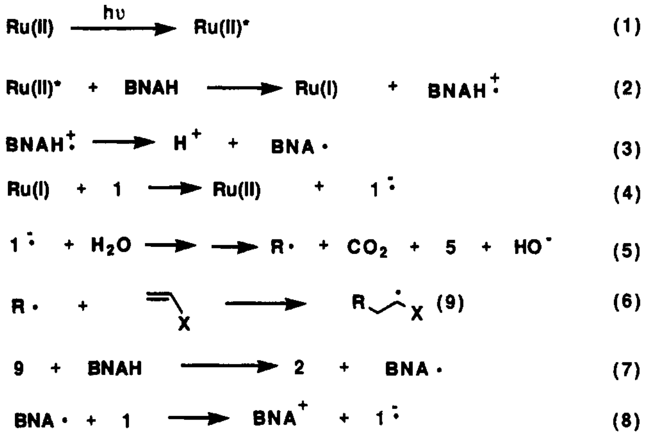
**Table I.** Photosensitized Decarboxylative Michael Addition of *N*-(Acyloxy)phthalimides to Electron-Deficient Olefins



| run | R | olefin (equiv) | solvent[a] | 2 (%) | 3 (%) | 4 (%) |
|---|---|---|---|---|---|---|
| 1 | **a** | **6** (1.0) | THF-$H_2O$ | 68 | 5 | 18 |
| 2 | **b** | **6** (1.0) | THF-$H_2O$ | 68 | 3 | 11 |
| 3 | **c** | **6** (1.9) | THF-$H_2O$ | 68 | 10 | 0 |
| 4 | **a** | **7** (5.4) | *t*-BuOH-$H_2O$ | 41 | 0 | 16 |
| 5 | **b** | **7** (4.8) | *t*-BuOH-$H_2O$ | 58 | 0 | 17 |
| 6 | **c** | **7** (5.0) | *t*-BuOH-$H_2O$ | 69 | 0 | 1 |
| 7 | **a** | **8** (1.1) | *t*-BuOH-$H_2O$ | 49 | 6 | 7 |
| 8 | **b** | **8** (1.1) | THF-$H_2O$ | 45 | 0 | 11 |
| 9 | **c** | **8** (1.1) | THF-$H_2O$ | 50 | 0 | 1 |

[a] In aqueous solvents containing 30% of water.

**Scheme I**



BNAH (0.63 mmol), and methyl vinyl ketone (0.60 mmol) with visible light (>460 nm from a 500-W Xenon lamp) for 2 h under a nitrogen atmosphere produced the expected Michael addition product (**2a**, 68%) and phthalimide (89%) along with a small amount of the hydrocarbon (**4a**, 18%) and the 1:2 adduct (**3a**, 5%) of the alkyl radical and methyl vinyl ketone.[3] Under the conditions, incident light is absorbed only by the Ru sensitizer and no reaction takes place without $Ru(bpy)_3Cl_2$ and/or BNAH.[4] The reaction similarly proceeds for secondary (**1b**) and tertiary (**1c**) carboxylic acid derivatives with several electron-deficient olefins (Table I). The quantum yields for addition to methyl vinyl ketone are over unity (1.3 for **1a**, 2.3 for **1b**, and 1.2 for **1c**)[5] and clearly demonstrate the participation of radical chain processes.

We envision the reaction mechanism shown in Scheme I. The initial process of this reaction would be electron transfer from BNAH to the triplet state of $Ru(bpy)_3^{2+}$ (eqs 1 and 2).[6,7] The oxidized BNAH deprotonates to give BNA$^•$ (eq 3).[8] The produced $Ru(bpy)^+$ can reduce **1** to give the anion radical (eq 4),[9] which gives alkyl radical according to our previous studies (eq 5).[1] The alkyl radical adds to the electron-deficient olefins to give radical **9**, which has a radical center at the $\alpha$-position of the electron-withdrawing group (eq 6). The radical **9** may abstract hydrogen from BNAH (eq 7) or may be reduced by BNA$^•$.[6a,b] The occurrence of the former process was established by a deuterium-labeling experiment. Thus, similar irradiation using **1a** and BNAH-*4,4*-$d_2$ in the presence of methyl acrylate in THF-$H_2O$ or $CH_3CN$-$H_2O$ (7:3) produced **2a** with deuterium incorporation at the $\alpha$-position of the carbomethoxy group in 68% or 79% yield, respectively.[10] The BNA$^•$ would reduce **1** to regenerate the anion radical of **1** (eq 8),[9] and the radical chain process is initiated.

This method is applicable to several functionalized substrates and radical cyclizations (supplementary material).

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**Supplementary Material Available:** Expanded Table I recording photosensitized decarboxylation Michael additions of *N*-(acyloxy)phthalimides to electron-deficient olefins (2 pages). Ordering information is given on any current masthead page.

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[3] 1-Benzyl-3-carbamoylpyridinium chloride was isolated (64%) by recrystallization of the acidified (hydrochloric acid) water-soluble components of the photolysate.

[4] Irradiation with a shorter wavelength (>330 nm) of BNAH ($\lambda_{max}$ = 352 nm) without $Ru(bpy)_3Cl_2$ ($\lambda_{max}$ = 453 nm) under similar conditions slowly gave the same products in similar yield. The mechanism of this uncatalyzed reaction would involve electron transfer of the short-lived excited state of BNAH to **1**. See: Martens, F. M.; Vorhoeven, J. W.; Gase, R. A.; Pandit, U. K.; de Boer, Th. J. *Tetrahedron* **1978**, *34*, 443.

[5] Quantum yields were determined by using a U-shaped glass tube in which solid BNAH (corresponding to 50 mM) and an aqueous THF solution of **1** (10 mM), **6** (10 mM), and $Ru(bpy)_3Cl_2$ (4.5 mM) were separately degassed and mixed in the dark before irradiation; Reinecke's salt actinometer at 545 nm: Wegner, E. E.; Adamson, A. W. *J. Am. Chem. Soc.* **1966**, *88*, 394.

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[9] Although the free energy changes of these electron-transfer processes are 0 to a little endothermic,[1a,7b,c] the processes would be feasible at room temperature.

[10] Similar irradiation using BNAH in THF-$D_2O$ gave no deuterated adduct.