[3] a) G. Kaupp in *Comprehensive Supramolecular Chemistry*, *Vol. 8* (see also color plates 3–22) (Ed.: J. E. D. Davies, J. A. Ripmeester), Elsevier, Oxford, **1996**, pp. 381–423; b) G. Kaupp, *Chemie Unserer Zeit* **1997**, *31*, 129–139 (English translation in http://kaupp.chemie.uni-oldenburg.de); c) G. Kaupp, A. Kuse, *Mol. Cryst. Liq. Cryst.* **1998**, *313*, 361; d) G. Kaupp, J. Schmeyers, M. Haak, T. Marquardt, A. Herrmann, *Mol. Cryst. Liq. Cryst.* **1996**, *276*, 315; e) G. Kaupp, A. Herrmann, *J. Prakt. Chem.* **1997**, *339*, 256; f) A. Herrmann, G. Kaupp, T. Geue, U. Pietsch, *Mol. Cryst. Liq. Cryst.* **1997**, *293*, 261.

[4] The frequently used terms "tandem" (no time sequence!) or "domino" (table game with 28 divided plates with differing numbers of points) are less appropriate for sequential reactions that do not mutually interfere. On the other hand "cascades" describe sequential or stepwise events.

[5] a) G. Kaupp, K. Sailer, *J. Prakt. Chem.* **1996**, *338*, 47; G. Kaupp, K. Sailer, *Angew. Chem.* **1990**, *102*, 917; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 933; b) G. Kaupp, U. Pogodda, A. Atfah, H. Meier, A. Vierengel, *Angew. Chem.* **1992**, *104*, 783; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 768; c) G. Kaupp, in *Photochemical Key Steps in Organic Synthesis* (Eds.: J. Mattay, A. Griesbeck), VCH, Weinheim, **1994**, pp. 224–225; d) S. N. Denisenko, E. Pasch, G. Kaupp, *Angew. Chem.* **1989**, *101*, 1397; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 1381; e) G. Kaupp, *Top. Curr. Chem.* **1989**, *146*, 57–98; f) G. Kaupp, H. Voss, H. Frey, *Angew. Chem.* **1987**, *99*, 1327; *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 1280; g) G. Kaupp, M. Stark, *Angew. Chem.* **1977**, *89*, 555; *Angew. Chem. Int. Ed. Engl.* **1977**, *16*, 552; h) G. Kaupp, H. Rösch, *Angew. Chem.* **1976**, *88*, 185; *Angew. Chem. Int. Ed. Engl.* **1976**, *15*, 163; i) G. Kaupp, U. Pogodda, J. Schmeyers, *Chem. Ber.* **1994**, *127*, 2249.

[6] a) F. Tietze, U. Beifuss, *Angew. Chem.* **1993**, *105*, 137; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 131; b) F. Tietze, *Chem. Rev.* **1996**, *96*, 115; c) S. E. Denmark, A. Thorarensen, *Chem. Rev.* **1996**, *96*, 137; d) J. D. Winkler, *Chem. Rev.* **1996**, *96*, 167; e) I. Ryu, N. Sonoda, D. P. Curran, *Chem. Rev.* **1996**, *96*, 177; f) P. J. Parsons, C. S. Penkett, A. J. Shell, *Chem. Rev.* **1996**, *96*, 195; g) K. K. Wang, *Chem. Rev.* **1996**, *96*, 207; h) A. Padwa, M. D. Weingarten, *Chem. Rev.* **1996**, *96*, 223; i) M. Malacria, *Chem. Rev.* **1996**, *96*, 289; j) G. A. Molander, C. R. Harris, *Chem. Rev.* **1996**, *96*, 307; k) B. B. Snider, *Chem. Rev.* **1996**, *96*, 339; l) E. Negishi, C. Coperet, S. Ma, S. Y. Liou, F. Liu, *Chem. Rev.* **1996**, *96*, 365.

[7] Recently, *cis*-1,2-diacetylethene and **1a** were allowed to react in benzene with analogous result (80% yield): G. Adembri, A. M. Celli, A. Sega, *J. Heterocycl. Chem.* **1997**, *34*, 541.

[8] All new compounds gave correct IR, ^{1}H, ^{13}C NMR, and (highly resolved) mass spectra, for example **3b**: IR (KBr): $\tilde{\nu} = 1691$ cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.00$ (d, 2H), 7.35 (m, 8H), 4.22 (s, 2H), 3.54 (s, 3H), 3.41 (s, 3H), 2.61 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 199.12$, 165.93, 137.43, 136.91, 133.13, 132.53, 131.33, 130.66 (2C), 128.43 (2C), 128.36 (2C), 128.01 (2C), 127.87, 115.10, 110.13, 50.24, 36.53, 31.77, 12.01; HR-MS: calcd. for $C_{22}H_{21}NO_3$: 347.1521; found: 347.1521. **5**: IR (KBr): $\tilde{\nu} = 1695$ (C=O), 1652 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.03$ (d, 2H), 7.51 (m, 3H), 7.30 (m, 3H), 7.11 (m, 7H), 4.42 (s, 2H), 2.69 (t, $J = 6.05$ Hz, 2H), 2.48 (t, $J = 6.6$ Hz, 2H), 2.14 (quint, $J = 6.05$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 198.69$, 195.12, 144.55, 137.67, 137.51, 132.71, 130.71, 130.31 (2C), 129.07 (3C), 128.48 (2C), 128.39 (2C), 128.19 (2C), 127.92, 127.80 (2C), 127.43, 119.16, 114.19, 38.33, 35.95, 23.70, 23.26; HR-MS: calcd. for $C_{28}H_{23}NO_2$: 405.1667; found: 405.1698. **11**: IR (KBr): $\tilde{\nu} = 3305$ (NH, sharp), 1680 (sh, C=O), 1658 (C=O), 1270, 1218 cm^{-1} (C=S); ^{1}H NMR ($CDCl_3$/[D_6]DMSO ca. 4/1, 300 MHz): $\delta =$ 8.08 (2NH), 7.52 (d, 2ArH), 7.01 (d, 2ArH), 6.40 (s, 1H), 2.82 (s, 3H); ^{13}C NMR ($CDCl_3$/[D_6]DMSO, 75 MHz): $\delta = 180.2$, 164.3, 152.7, 141.2, 131.2 (2C), 130.6 (2C), 124.3, 119.6, 101.4, 25.3; HR-MS: calcd. for $C_{12}H_{10}BrN_3O_2S$: 340.9684; found: 340.9687.

[9] SpecTool for Windows, Version 2.1, Chemical Concepts GmbH, Weinheim, **1994**.

[10] a) G. Kaupp, J. Schmeyers, J. Boy, *Eur. J. Chem.* **1998**, *4*, 2467; b) G. Kaupp, J. Boy, J. Schmeyers, *J. Prakt. Chem.* **1998**, *340*, 346.

[11] J. C. J. Bart, G. M. J. Schmidt, *Recl. Trav. Chim. Pays-Bas* **1978**, *97*, 231.

[12] V. Bertolasi, P. Gilli, V. Ferretti, G. Gilli, *Acta Crystallogr. Sect. B* **1998**, *54*, 50.

[13] P. C. Thieme, E. Hädicke, *Justus Liebigs Ann. Chem.* **1978**, 227.

Novel Fluorescent Probes for Singlet Oxygen**

Naoki Umezawa, Kumi Tanaka, Yasuteru Urano, Kazuya Kikuchi, Tsunehiko Higuchi, and Tetsuo Nagano*

Singlet oxygen ($^1O_2$), an excited state of molecular oxygen, has aroused much interest as a chemical and biological oxidant. The chemical reactivity of $^1O_2$ is well characterized since $^1O_2$ is useful for organic synthesis and has unique reactivity.[1] Singlet oxygen is thought to be an important toxic species in vivo[2] since it can oxidize various kinds of biological molecules such as DNA, proteins, and lipids, and its reactivity toward DNA bases has been especially well characterized by Foote et al.[3] Furthermore, Sies et al. and other researchers have reported that $^1O_2$ plays a role as an activator of gene expression.[4]

Although many $^1O_2$ traps have been reported,[5] it is still difficult to detect $^1O_2$ generated in biological systems because of its short lifetime. The most widely used $^1O_2$ trap is 9,10-diphenylanthracene (DPA), which reacts rapidly with $^1O_2$ specifically to form a thermostable endoperoxide at a rate of $k = 1.3 \times 10^6\,\text{M}^{-1}\,\text{s}^{-1}$.[6] The decrease in absorbance at 355 nm is used as a measure of the formation of the endoperoxide. Many water-soluble DPA derivatives have been developed,[7] but the quenching of $^1O_2$ by water means that they are difficult to apply to biological systems. Steinbeck et al. have achieved the direct detection of $^1O_2$ generation from phagocytes with DPA by adapting the method to avoid $^1O_2$ quenching.[8]

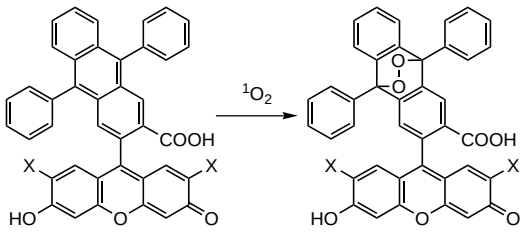
DPA derivatives are not very sensitive as probes because the detection is based on the measurement of absorbance. Hence, we designed and synthesized novel fluorometric probes for $^1O_2$ in order to improve the sensitivity. In general, fluorescence measurement is more sensitive, and so is easier to use in imaging studies, for example, fura-2 is used in Ca^{2+} imaging.

We designed 9-[2-(3-carboxy-9,10-diphenyl)anthryl]-6-hydroxy-3*H*-xanthen-3-one (DPAX-1, Scheme 1) as a suitable fluorescent probe. We chose fluorescein as a fluorophore since it has a high fluorescence quantum yield in aqueous solution and is able to be excited at long wavelength. Excitation by visible light is preferable for biological applications as it minimizes cell damage and autofluorescence. We then fused this fluorophore with the reactive moiety of DPA. When DPAX reacts with $^1O_2$ to yield DPAX-endoperoxide (DPAX-EP) the conjugation between the DPA structure and xanthene ring is greatly altered, so we expected a change in fluorescence properties.

[*] Prof. T. Nagano, Dr. N. Umezawa, K. Tanaka, Dr. Y. Urano, Dr. K. Kikuchi, Dr. T. Higuchi
Graduate School of Pharmaceutical Sciences
The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, 113-0033 (Japan)
Fax: (+81) 3-5841-4855
E-mail: tlong@mol.f.u-tokyo.ac.jp

[**] This work was supported in part by a research grant from the Ministry of Education, Science, Sports, and Culture of Japan. N.U. gratefully acknowledges the Japanese Society for the Promotion of Science for the JSPS Research Fellowship for Young Scientists.

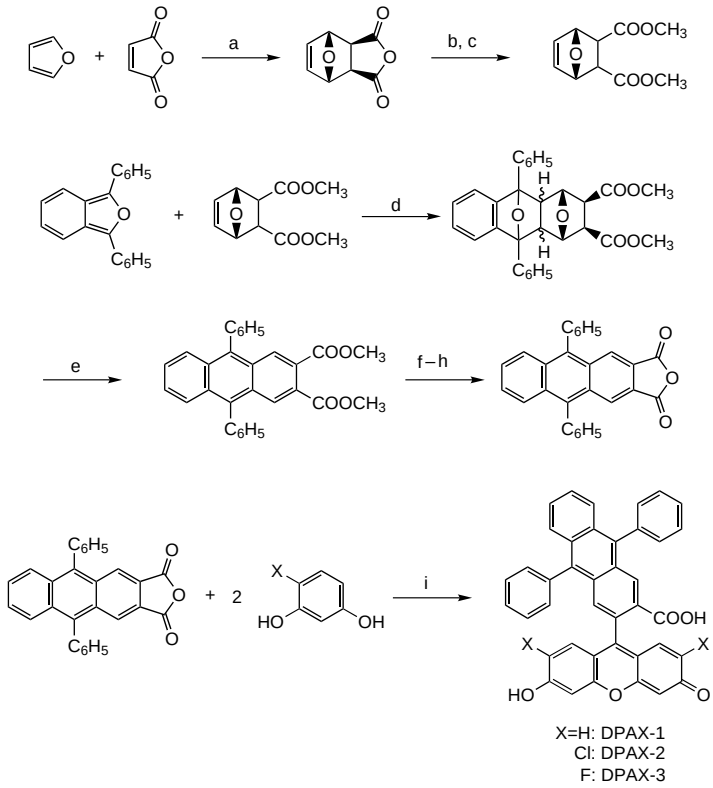
  1433-7851/99/3819-2899 $ 17.50+.50/0



Scheme 1. Reaction of DPAXs with $^1O_2$.

The fluorescence intensity of fluorescein derivatives is known to be decreased under acidic conditions as a consequence of the protonation of the phenoxide oxygen atom. In order to stabilize the fluorescence intensity at physiological pH we incorporated electron-withdrawing groups, Cl (DPAX-2) and F (DPAX-3), at the 2- and 7-positions of the xanthene chromophore. This modification lowered the pK_a value of the phenolic oxygen atom.

DPAX-1, DPAX-2, and DPAX-3 were synthesized according to Scheme 2. The corresponding endoperoxides were synthesized with chemically generated $^1O_2$ (MoO_4^{2-}/H_2O_2). The fluorescence parameters are shown in Table 1; these values were obtained under basic conditions, as the compounds are highly fluorescent when deprotonated. Absorbance maxima (A), molar absorption coefficients (ε), and emission maxima (E_m) were not altered much between DPAXs and DPAX-EPs. However, the quantum efficiencies of fluorescence (Φ_f) were greatly changed: DPAXs themselves are almost nonfluorescent and DPAX-EPs are highly fluorescent. The maximum absorbance and emission of the chlorinated compound DPAX-2-EP were shifted to longer wavelengths than the other compounds.



Scheme 2. Synthesis of DPAXs. a) THF, room temperature, 46%; b) H_2O, room temperature, 98%; c) CH_3I, Cs_2CO_3, acetone, room temperature, 56%; d) $CHCl_3$, reflux, quantitative yield; e) H_2SO_4, CH_2Cl_2, reflux, 41%; f) dioxane, KOH, CH_3OH, reflux, 91%; g) HCl, H_2O, room temperature, quantitative yield; h) Ac_2O, reflux, 97%; i) CH_3SO_3H, 80–85 °C (DPAX-1 (43%) and DPAX-2 (59%)) or 150 °C (DPAX-3 (63%)).

Table 1. Absorbance and fluorescence properties of DPAXs and DPAX-EPs.[a]

	A [nm]	ε [$\times 10^4$ M^{-1}cm^{-1}]	E_m [nm]	Φ_f
DPAX-1	493	6.1	516	0.007
DPAX-1-EP	494	7.9	515	0.53
DPAX-2	507	5.7	524	0.006
DPAX-2-EP	506	8.9	527	0.66
DPAX-3	493	7.6	514	0.006
DPAX-3-EP	494	6.7	515	0.70

[a] All data were obtained at 20 °C in 0.1 M NaOH (0.1% DMSO).

We examined the pH profiles of fluorescence intensity for the DPAX-EPs (Figure 1). The fluorescence intensities were greatly decreased under acidic conditions. The pK_a values of DPAX-1-EP, DPAX-2-EP, and DPAX-3-EP were 6.6, 5.7, and 5.3, respectively. In contrast to the rapid decrease of fluorescence intensity of DPAX-1-EP below pH 8, the intensity was stable above pH 7 in the cases of DPAX-2-EP and DPAX-3-EP. These profiles showed that DPAX-2 and DPAX-3 should be useful as probes under neutral conditions.

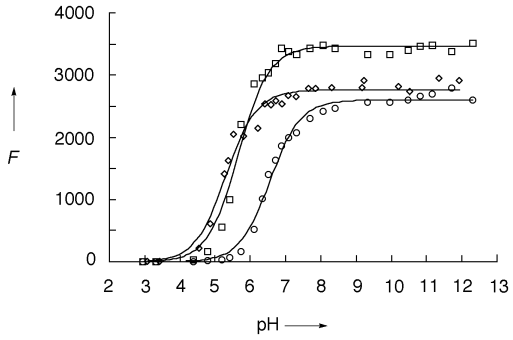


Figure 1. Effect of pH on the fluorescence intensity of DPAX-EPs. DPAX-EPs (1 μM; 0.1% DMSO as a cosolvent) were added to sodium phosphate solution (0.1 M) adjusted to various pH values. The fluorescence intensity of the DPAX-EPs was measured at 515, 530, and 515 nm with excitation at 495, 505, and 494 nm, respectively. ○: DPAX-1-EP, □: DPAX-2-EP, ◇: DPAX-3-EP. F = fluorescence intensity (in arbitrary units).

Then, we tried to detect chemical $^1O_2$ production using DPAX-2. The MoO_4^{2-}/H_2O_2 system was again used as a chemical source of $^1O_2$. The reaction was performed at pH 10.5, since the MoO_4^{2-}/H_2O_2 system only works effectively under basic conditions.[9] Hydrogen peroxide (final concentration: 20 mM) was added to a buffer solution of DPAX-2 (10 μM; 0.1% DMSO) and MoO_4^{2-} (1 mM) every hour; the reaction of MoO_4^{2-} with H_2O_2 is slow and excess H_2O_2 inhibits the reaction. The excitation and emission spectra of DPAX-2 are shown in Figure 2. Each spectrum was measured one hour after the addition of H_2O_2. The fluorescence intensity

  1433-7851/99/3819-2900 $ 17.50+.50/0

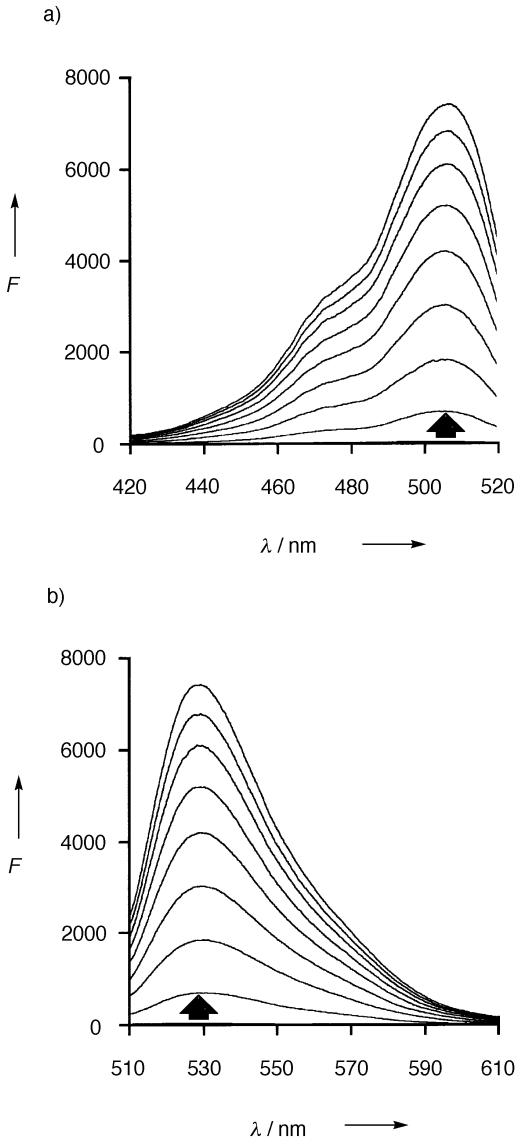


Figure 2. a) Excitation spectra recorded at 530 nm and b) emission spectra recorded at 505 nm of DPAX-2 in the reaction with $^1O_2$ generated from a MoO_4^{2-}/H_2O_2 system. The reaction was performed at 25 °C in 0.1 M sodium phosphate buffer at pH 10.5 containing 0.1 mM EDTA.

increased with the generation of $^1O_2$ in a dose-dependent manner. An increase in fluorescence was also observed at pH 7.4 using 3-(4-methyl-1-naphthyl)propionic acid endoperoxide (EP-1) as a $^1O_2$ source.[10] These results showed that DPAX-2 is a useful probe in both basic and neutral aqueous solutions. In addition, we confirmed the production of DPAX-2-EP in these reactions by HPLC. The fluorescence intensity did not change upon reaction with H_2O_2, superoxide, and nitric oxide, which are reported to be produced in biological samples. The specificity of DPAX-2 for $^1O_2$ is therefore confirmed.

The detection of $^1O_2$ in biological samples was also investigated. For this we prepared DPAX-2 diacetate (DPAX-2DA), which is thought to be more permeable to the cell. DPAX-2DA will be hydrolyzed by intracellular esterases to generate DPAX-2. We have tried both DPAX-2 and DPAX-2DA, however, cells were stained similarly in both cases. This observation means that DPAX-2 itself is membrane-permeable.

Received: April 19, 1999 [Z13290IE]
German version: *Angew. Chem.* **1999**, *111*, 3076 – 3079

Keywords: dyes · endoperoxides · fluorescence spectroscopy · singlet oxygen

[1] a) M. Prein, W. Adam, *Angew. Chem.* **1996**, *108*, 519 – 538; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 477 – 494; b) *Singlet Oxygen* (Eds.: H. H. Wasserman, R. W. Murray), Academic Press, New York, **1979**.
[2] a) K. Briviba, L. O. Klotz, H. Sies, *Biol. Chem.* **1997**, *378*, 1259 – 1265; b) B. Epe, *Chem. Biol. Interact.* **1991**, *80*, 239 – 260; c) J. R. Wagner, P. A. Motchnik, R. Stocker, H. Sies, B. N. Ames, *J. Biol. Chem.* **1993**, *268*, 18502 – 18506.
[3] a) C. Sheu, C. S. Foote, *J. Am. Chem. Soc.* **1993**, *115*, 10446 – 10447; b) C. Sheu, C. S. Foote, *J. Am. Chem. Soc.* **1995**, *117*, 474 – 477.
[4] a) S. Basu-Modak, R. M. Tyrrell, *Cancer Res.* **1993**, *53*, 4505 – 4510; b) K. Scharffetter-Kochanek, M. Wlaschek, K. Briviba, H. Sies, *FEBS Lett.* **1993**, *331*, 304 – 306; c) M. Wlaschek, K. Briviba, G. P. Stricklin, H. Sies, K. Scharffetter-Kochanek, *J. Invest. Dermatol.* **1995**, *104*, 194 – 198; d) M. Wlaschek, J. Wenk, P. Brenneisen, K. Briviba, A. Schwarz, H. Sies, K. Scharffetter-Kochanek, *FEBS Lett.* **1997**, *413*, 239 – 242; e) S. Grether-Beck, S. Olaizola-Horn, H. Schmitt, M. Grewe, A. Jahnke, J. P. Johnson, K. Briviba, H. Sies, J. Krutmann, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 14586 – 14591.
[5] a) C. S. Foote, F. C. Shook, R. A. Abakerli, *J. Am. Chem. Soc.* **1980**, *102*, 2503 – 2504; b) V. Nardello, N. Azaroual, I. Cervoise, G. Vermeersch, J. M. Aubry, *Tetrahedron* **1996**, *52*, 2031 – 2046; c) M. Botsivari, D. F. Evans, *J. Chem. Soc. Chem. Commun.* **1979**, 1114 – 1117; d) B. A. Lindig, M. A. J. Rodgers, A. P. Schaap, *J. Am. Chem. Soc.* **1980**, *102*, 5590 – 5593; e) J. M. Aubry, J. Rigaudy, N. K. Cuong, *Photochem. Photobiol.* **1981**, *33*, 149 – 153.
[6] a) E. J. Corey, W. C. Taylor, *J. Am. Chem. Soc.* **1964**, *86*, 3881 – 3882; b) H. H. Wasserman, J. R. Scheffer, J. L. Cooper, *J. Am. Chem. Soc.* **1972**, *94*, 4991 – 4996; c) N. J. Turro, M. F. Chow, J. Rigaudy, *J. Am. Chem. Soc.* **1981**, *103*, 7218 – 7224.
[7] a) C. Schmitz, J. M. Aubry, J. Rigaudy, *Tetrahedron* **1982**, *38*, 1425 – 1430; b) V. Nardello, J. M. Aubry, *Tetrahedron Lett.* **1997**, *38*, 7361 – 7364.
[8] a) M. J. Steinbeck, A. U. Khan, M. J. Karnovsky, *J. Biol. Chem.* **1992**, *267*, 13425 – 13433; b) M. J. Steinbeck, A. U. Khan, M. J. Karnovsky, *J. Biol. Chem.* **1993**, *268*, 15649 – 15654.
[9] a) J. M. Aubry, B. Cazin, *Inorg. Chem.* **1988**, *27*, 2013 – 2014; b) J. M. Aubry, B. Cazin, F. Duprat, *J. Org. Chem.* **1989**, *54*, 726 – 728.
[10] I. Saito, T. Matsuura, K. Inoue, *J. Am. Chem. Soc.* **1983**, *105*, 3200 – 3206.

  1433-7851/99/3819-2901 $ 17.50+.50/0