acquisition and relaxation delay (G. Lippens, C. Dhalluin, C. Wieruszeski, *J. Biomol. NMR* **1995**, *5*, 327–331).

[12] K. Wüthrich, *NMR of Proteins and Nucleic Acids*, Wiley, New York, **1986**, p. 162, and references therein.

[13] J. D. Baleja, J. Moult, B. D. Sykes, *J. Magn. Res.* **1990**, *87*, 375–384.

[14] The modeling of MK2 was carried out with the program Discover 95.0 and the CFF95 force field (Molecular Simulations Inc. San Diego, CA, USA). All calculations were performed without nonbonded or coulombic cutoffs and a dielectric constant of 1.0. Twenty-five NMR-derived distance restraints were generated from the NMR data using a method similar to that of Sykes et al.[13] The restraints were applied as strong (1.8–2.7 Å), medium (1.8–3.5 Å), or weak (1.8–5.0 Å) flat-bottomed potentials. A single, high-temperature unrestrained dynamics run was performed at 1000 K using a timestep of 1 fs, with 40 structures collected at intervals of 10 ps to generate a starting set of conformations. Each structure was cooled and minimized using the following simulated annealing protocol: The temperature was lowered to 500 K at a rate of 50 $\mathrm{K\,ps^{-1}}$, where strong restraints were applied, and then to 250 K (5 $\mathrm{K\,ps^{-1}}$). The remaining restraints were added, and cooling to 50 K (0.5 $\mathrm{K\,ps^{-1}}$) was performed followed by restrained minimization to a final gradient of 0.01 $\mathrm{kcal\,mol^{-1}\,Å^{-1}}$. Twenty-nine low-energy structures were isolated (average total restraint violation: 0.11 $\mathrm{kcal\,mol^{-1}}$; root mean square deviation for all heavy atoms: 0.241), and a representative consistent with the NMR data was selected.

[15] L. Tong, C. Qian, M.-J. Massariol, R. Déziel, C. Yoakim, L. Lagacé, *Nature Struct. Biol.*, in press.

Calixarene-Based Copper(I) Complexes as Models for Monocopper Sites in Enzymes**

Sébastien Blanchard, Loïc Le Clainche, Marie-Noëlle Rager, Benoît Chansou, Jean-Pierre Tuchagues, Arthur F. Duprat, Yves Le Mest, and Olivia Reinaud*

Metalloenzymes are fascinating natural "factories" adept at transforming organic molecules with high efficiency and selectivity.[1] A good chemical model for metalloenzymes is a key to the understanding of the fundamental mechanisms of the chemistry involved in the catalytic cycles and to the design of efficient and selective new tools for the synthetic chemist. Although mononuclear copper enzymes constitute an important class of proteins,[2] few, if any, chemical systems allow the specific chemistry at a monocopper site to be modeled. The first step for the elaboration of a biomimetic system is the conception of a synthetic model for the active copper(I) center (since the copper center in this oxidation state is the active form for the activation of small molecules).[2] For this purpose, three important elements are required:

- A polydentate ligand to coordinate to the cuprous ion and reproduce the geometry and the chemical properties of the amino acid residues involved in the coordination sphere of the metal.
- An appropriate environment to prevent the formation of binuclear species.
- A vacant site to allow the coordination and exchange of an external ligand.

The classic strategy to protect a redox metal center, is based on tuning the sterical environment provided by a tripodal ligand. This approach presents a dilemma: high steric hindrance will prevent the cuprous ion from reacting with an external substrate molecule; but on the other hand, a decrease in the steric bulk will drive the reaction with O_2 (or any other oxidant) to produce binuclear copper(II) complexes.[3]

Here we describe a new supramolecular model of the mononuclear cuprous sites, which takes advantage of the conic shape of a calix[6]arene that is appropriately functionalized with three pyridine (Py) moieties. The role of the pyridine groups is to provide an N_3 donor set for the copper ion, thereby mimicking the imidazole residues which coordinate the type II cuprous ion in enzymes such as dopamine β-hydroxylase, peptidylglycine α-amidating monooxygenase, copper amine oxidases, as well as nitrate reductases.[2] The geometry of the system is designed to orient the fourth coordination site toward the inside of the conic cavity provided by the calixarene skeleton. The role of the calixarene is thus to protect the metal center from any undesired interaction with another metal center, to facilitate the approach of an organic molecule, and finally to operate a means of selection based on its size and nature.

The new tridentate N-ligand $X_6Me_3Pic_3$ was synthesized in two steps from *p-tert*-butylcalix[6]arene (X_6H_6). The first step involved the alternate protection of three out of the six phenolic OH functions of the calixarene by a known selective methylation procedure.[4] Treatment of this key intermediate $X_6Me_3H_3$ with picolyl chloride in the presence of K_2CO_3 in DMF provided the desired tripod $X_6Me_3Pic_3$ in 87% yield (Scheme 1). The ^{1}H NMR spectrum of this ligand recorded at 297 K is characteristic of a major flattened *cone* conformation with C_3 symmetry. The methoxy groups are projected toward the inside of the cavity as indicated by the high field shift of the methyl protons in the spectrum. However, a low-temperature study revealed the presence of a minor conformer of lower symmetry, which is in equilibrium with the conic conformation.[5]

The addition of 1.1 equivalents of $[Cu(MeCN)_4]PF_6$ to a solution of $X_6Me_3Pic_3$ in $CDCl_3$ resulted in the formation of a

---

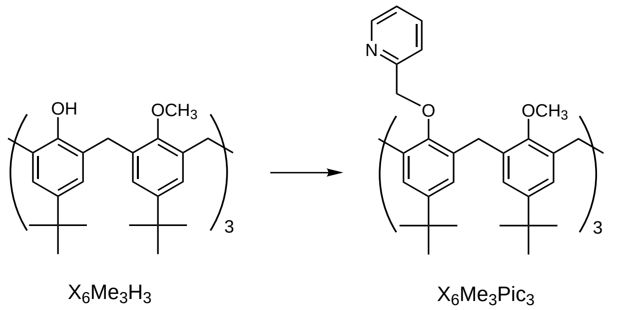
[*] Dr. O. Reinaud, S. Blanchard, L. Le Clainche, M.-N. Rager
Laboratoire de Structure et Réactivité des Complexes Moléculaires,
U.M.R. CNRS 7576
Ecole Nationale Supérieure de Chimie de Paris
11 rue Pierre et Marie Curie, F-75231 Paris Cedex 05 (France)
Fax: (+33) 1-43-26-07-70
E-mail: reinaud@ext.jussieu.fr

Dr. B. Chansou, Prof. J. P. Tuchagues
Laboratoire de Chimie de Coordination du CNRS
205 route de Narbonne, F-31077 Toulouse cedex (France)

Dr. A. F. Duprat
Laboratoire de Recherches Organiques associé au CNRS
Ecole Supérieure de Physique et Chimie Industrielles
10 rue Vauquelin, F-75231 Paris Cedex 05 (France)

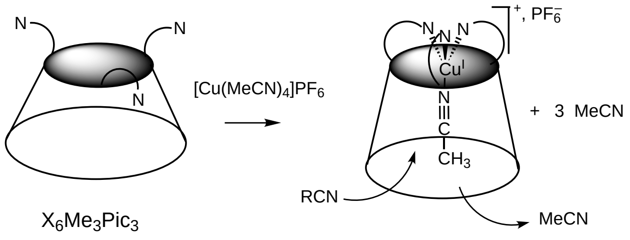
Dr. Y. Le Mest
Laboratoire de Chimie, Electrochimie Moléculaires et
Chimie Analytique, U.M.R. CNRS 6521
Département de Chimie, Université de Bretagne Occidentale U.F.R.
Sciences, B.P. 809
6 Avenue Le Gorgeu, F-29285 Brest Cedex (France)

[**] We thank Dr. A. van Dorsselaer and H. Nierengarten (LSMBO, Strasbourg, France) for the mass spectra of our complex.



Scheme 1. Synthesis of $X_6Me_3Pic_3$. Conditions: $PyCH_2Cl \cdot HCl$, K_2CO_3, DMF, reflux 3 h; 87%.

new compound as shown by ^1H NMR spectroscopy. The simplicity of the overall profile excludes the presence of oligomeric species and is in good agreement with a mononuclear complex with C_3 symmetry. The large chemical shifts observed for the protons on the pyridine ring, together with the IR stretches of the corresponding C_5N skeleton attest to the coordination of the copper(I) ion to the nitrogen atoms on the pyridine rings. The ^1H NMR signal for the methoxy protons now appears at a lower field in the spectrum, thus indicating that the calixarene conformation has changed upon complexation. There is also a new broad resonance at $\delta = -1.2$ that does not belong to the calixarene-based ligand and vanished upon addition of deuterated acetonitrile. All these observations strongly suggest that the cuprous ion is four coordinate surrounded by: a) three pyridine groups, which are projected toward the inside of the cone of the calixarene, thus pushing out the methoxy groups, and b) an acetonitrile molecule, which sits inside the π-basic conic cavity as a consequence of the steric hindrance induced by the capping at the lower rim of the calixarene (Scheme 2).



Scheme 2. Synthesis and ligand exchange of cuprous $X_6Me_3Pic_3$ complexes.

The acetonitrile ligand was easily exchanged by the addition of 20 molar equivalents of propionitrile as shown in the ^1H NMR spectrum by the appearance of two new signals at $\delta = -0.7$ (q, 2H) and -1.7 (t, 3H). Under the same experimental conditions, allyl nitrile was also effective for this substitution process, whereas neither benzonitrile nor benzylnitrile were effective, even after two days. These observations may be attributed to steric crowding, which causes unfavorable interactions between these large molecules and the calixarene cavity.

Single crystals of the cuprous complex were obtained from a $CHCl_3$ solution containing 25 molar equivalents of propionitrile. The structure of $[Cu(X_6Me_3Pic_3)(NCEt)]PF_6$ was established by X-ray crystallographic analysis (Figure 1).[6] The Cu^I center resides in a regular tetrahedral N_4 ligand environment, with close to C_{3v} symmetry, provided by the three pyridine groups and the propionitrile ligand. The

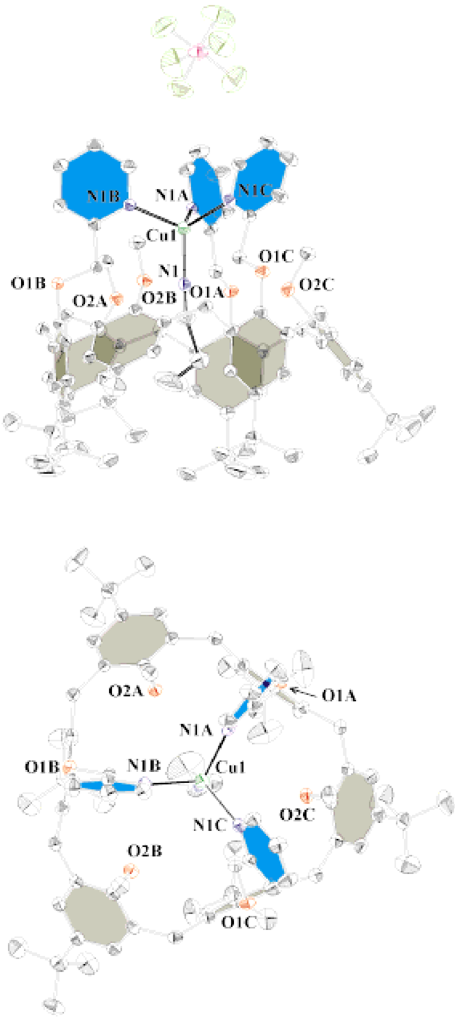


Figure 1. Crystal structure of $[Cu(X_6Me_3Pic_3)(NCEt)]PF_6$. Top: side view; bottom: plan view. The aromatic rings of the calixarene macrocycle and pyridine ligands are shaded in light gray and blue, respectively. Selected bond lengths [Å] and angles [°]: Cu–N1 1.949(7), Cu–N1A 2.090(7), Cu–N1B 2.097(7), Cu–N1C 2.127(6); N1-Cu-N1A 115.0(3), N1-Cu-N1B 111.6(3), N1A-Cu-N(1B) 107.3(2), N1-Cu-N1C 111.8(2), N1A-Cu-N1C 102.6(2), N1B-Cu-N1C 108.1(2).

average value for the three $Cu-N_{Py}$ distances of 2.105 Å is similar to those for four-coordinate Cu^I compounds with aromatic N-donor ligands.[7] The propionitrile ligand (Cu–N 1.949(7) Å) is buried deep inside the cavity of the *tert*-butylcalix[6]arene macrocycle in a *cone* conformation. The diameters of the openings at the two ends of the cavity can be estimated by the distances between two opposite O atoms (7.91 Å) and C_{tBu} atoms (11.28 Å). The unit cell includes two enantiomeric molecules: the metal-centered chirality results from the helical shape provided by the three pyridine groups wrapping around the metal ion. In solution, conformational exchange occurs between the two enantiomeric forms.

The complex is surprisingly stable: it was not oxidized under an atmosphere of O_2 in most common solvents and the acetonitrile remained coordinated to the cuprous ion after chromatography on silica gel in air. This lack of reactivity towards O_2 contrasts the facile exchange of the acetonitrile ligand with molecules as large as allyl nitrile. These observations can be explained by either the intrinsic instability of the CuO_2 adduct or by the metal center having a high redox potential.[8] Indeed, preliminary cyclic voltammetric experiments in CH_2Cl_2 revealed essentially an irreversible oxidation of Cu^I to Cu^{II}, at $E_p(ox) = +1.05$ V (versus ferrocene/ferrocenium), associated with a very broad and cathodically shifted reduction peak ($\Delta E_p = E_p(ox) - E_p(red) = 1.0$ V). The particularly high value of this oxidation potential is best explained by the tetrahedral environment in which the copper ion is constrained, which strongly stabilizes Cu^I relative to oxidation to Cu^{II}.[7]

Although calix[6]arenes have long been utilized for the construction of supramolecular assemblies, their ability to accommodate a transition metal has scarcely been explored.[10] The work herein demonstrates that although it is very flexible,[5, 10] a calix[6]arene can participate both in the formation of a stable mononuclear transition metal complex and in the construction of a receptacle which interacts with an external molecule. Indeed, the novel ligand $X_6Me_3Pic_3$ appears to fulfill three essential requirements for playing the role of a metalloenzyme active site pocket by providing: a) a N_3 donor set that allows the formation of a stable cuprous complex upon coordination of a fourth ligand at the remaining binding site, b) a cavity that acts not only as protection for the metal center but also as a funnel for external organic molecules that compete for binding to the metal center, and c) a geometrical constrain that confers to the Cu^+ ion an unusually high oxidation potential. We are currently investigating the catalytic properties of these copper(I) complexes as well as developing the synthesis of complexes with divalent transition metal ions and other tridentate calix[6]arene-based ligands.

Experimental Section

$X_6Me_3Pic_3$: Under an argon atmosphere, dry K_2CO_3 (2.86 g, 20.7 mmol, 15 equiv), X_6Me_3 (1.40 g, 1.38 mmol), and 2-chloromethylpyridine chlorohydrate (1.36 g, 8.28 mmol, 6 equiv) were suspended in dry DMF (70 mL). The reaction mixture was heated to reflux for 3 h. Water (350 mL) was poured into the warm light orange solution and then stored for 12 h at 4 °C. The resulting white precipitate was collected by filtration over a glass frit, washed with water, dried under vacuum over KOH, recrystallized from $MeCN/CH_2Cl_2$, and finally dried under vacuum overnight at 40 °C. Yield: 1.65 g (87 %). M.p. 270 °C (loss of solvent molecules at 191 °C); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 0.83$ (s, 27H; *t*Bu), 1.37 (s, 27H; *t*Bu), 2.33 (s, 9H; OCH_3), 3.43 (d, $J = 15$ Hz, 6H; Ar-αCH_{eq}), 4.61 (d, $J = 15$ Hz, 6H; Ar-αCH_{ax}), 5.1 (s, 6H; Py-αCH_2), 6.72 (s, 6H; *Ar*-H), 7.25 (t, $J = 4.8$ Hz, 3H; *Py*-H), 7.26 (s, 6H; *Ar*-H), 7.80 (t, $J = 3$ Hz, 3H; *Py*-H), 7.91 (d, $J = 3$ Hz, 3H; *Py*-H), 8.56 (d, $J = 4.8$ Hz, 3H; *Py*-H); IR (KBr): $\tilde{\nu} = 1595$ (m), 1575 (w), 1480 (s), 1460 (sh), 1430 (s) cm^{-1}: (C=N, C=C (Py)); elemental analysis calcd for $C_{87}H_{105}N_3O_6 \cdot 1.5H_2O \cdot DMF$: C 77.85, H 8.31, N 4.09; found: C 77.82, H 8.33, N 3.96.

$[Cu(X_6Me_3Pic_3)(NCMe)]PF_6$: Dioxygen-free $CDCl_3$ (1 mL) was added to a schlenk flask containing $X_6Me_3Pic_3$ (200 mg, 0.155 mmol) and $[Cu(NCMe)_4]PF_6$ (64 mg, 0.17 mmol, 1.1 equiv) under an argon atmosphere. The mixture was stirred for 4 h, filtered over celite, and an aliquot (0.1 mL) of the resulting homogeneous tan solution was diluted in an NMR tube with $CDCl_3$ (0.4 mL). ^1H NMR (250 MHz, $CDCl_3$): $\delta = -1.2$ (br s, 3H; coordinated CH_3CN); 0.81 (s, 27H; *t*Bu), 1.41 (s, 27H; *t*Bu), 2.0 (br s, 9H, free CH_3CN); 3.01 (s, 9H; OCH_3), 3.37 (d, $J = 15$ Hz, 6H; Ar-αCH_{eq}), 4.32 (d, $J = 15$ Hz, 6H; Ar-αCH_{ax}), 5.54 (s, 6H; Py-αCH_2), 6.76 (s, 6H; *Ar*-H), 7.30 (s, 6H; *Ar*-H), 7.67 (t, $J = 5$ Hz, 3H; *Py*-H), 7.96 (t, $J = 7$ Hz, 3H; *Py*-H), 8.06 (d, $J = 7$ Hz, 3H; *Py*-H), 8.94 (d, $J = 5$ Hz, 3H; *Py*-H). Exchange of the acetonitrile ligand was accomplished through the addition of 20 equivalents of another nitrile RCN to the NMR tube. The rest of the solution was chromatographed directly on silica gel (THF/CH_2Cl_2 (2.5/97.5) as eluent) to give $[Cu(X_6Me_3Pic_3)(NCMe)]PF_6$ as a white solid ($R_f = 0.4$) which was dried under vacuum for 24 h at room temperature. M.p. > 280 °C (decomp); IR (KBr) $\tilde{\nu} = 1610$ (m), 1575 (w), 1485 (s), 1465 (sh), 1490 (s) cm^{-1} (C=N, C=C (Py)); elemental analysis calcd for $C_{89}H_{108}N_4O_6CuPF_6 \cdot THF \cdot 0.5CH_2Cl_2$: C 67.93, H 7.13, N 3.38, Cu 3.84; found: C 68.01, H 7.06, N 3.07, Cu 3.60; (the presence of solvent molecules was confirmed by NMR spectroscopy); ESI-MS (CH_2Cl_2): 1392.4 ($[Cu(X_6Me_3Pic_3)(NCCH_3)]^+$. This peak was isolated and fragmented (MS–MS) and shown to lose a mass of 43 Da (CH_3CN).

Received: March 10, 1998 [Z11569IE]
German version: *Angew. Chem.* **1998**, *110*, 2861–2864

Keywords: bioinorganic chemistry · calixarenes · coordination chemistry · copper · N ligands

---

[1] Reviews of bioinorganic enzymology: *Chem. Rev.* **1996**, *96*, 2237–3042.

[2] a) J. P. Klinman, in ref. [1], 2541–2561; b) W. Kaim, J. Rall, *Angew. Chem.* **1996**, *108*, 47–64 ; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 43–60.

[3] a) N. Kitajima, Y. Moro-oka, *Chem. Rev.* **1994**, *94*, 737–757; b) W. B. Tolman, *Acc. Chem. Res.* **1997**, *30*, 227–237; c) K. D. Karlin, S. Kaderli, A. D. Zuberbühler, *Acc. Chem. Res.* **1997**, *30*, 139–147.

[4] R. G. Janssen, W. Verboom, D. N. Reinhoudt, A. Casnati, M. Freriks, A. Pochini, F. Ugozzoli, R. Ungaro, P. M. Nieto, M. Carramolino, F. Cuevas, P. Prados, J. de Mendoza, *Synthesis*, **1993**, 380–386.

[5] Such a conformational behaviour has already been described in detail for some closely related $X_6Me_3Bn_3$ calixarenes: a) S. Kanamathareddy, C. D. Gutsche, *J. Org. Chem.* **1994**, *59*, 3871–3879; b) J. P. M. van Duynhoven, R. G. Janssen, W. Verboom, S. M. Franken, A. Casnati, A. Pochini, R. Ungaro, J. de Mendoza, P. M. Nieto, P. Prados, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1994**, *116*, 5814–5822.

[6] Crystal structure analysis: $[C_{90}H_{110}N_4O_6Cu]PF_6 \cdot 7CHCl_3$, $M_r = 2387.9$, colorless crystal ($0.6 \times 0.6 \times 0.4$ mm), triclinic, space group $P\bar{1}$, $a = 16.195(2)$, $b = 18.249(3)$, $c = 22.435(3)$ Å, $\alpha = 108.20(2)$, $\beta = 110.88(2)$, $\gamma = 93.57(2)°$, $V = 5771.5(2)$ Å^3, $Z = 2$, $\rho_{calcd} = 1.374$ g cm^{-3}, $\mu(Mo_{K\alpha}) = 7.48$ cm^{-1}. Of the 39919 reflections collected at 160 K (STOE-IPDS diffractometer) in the $\theta = 1.85$–$22.72°$ range, 14724 were unique; 1468 parameters were refined on F^2 with all data [SHELX-97][11] to give the final R indices: $R1$ [$I > 2\sigma(I)$] = 0.0916 and $wR2$ (all data) = 0.2321. The crystals were poorly shaped and weakly diffracting, thus giving low-resolution data and relatively high R values. One reason for these effects is the high amount of disorder in the crystal structure. The *t*Bu groups of the calixarene ligand and the CH_2 group of the propionitrile are disordered and could be refined onto two positions using distance and ADP restraints. In addition, the lattice bound $CHCl_3$ molecules are disordered. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-101179. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[7] S. M. Carrier, C. E. Ruggiero, R. P. Houser, W. B. Tolman, *Inorg. Chem.* **1993**, *32*, 4889–4899, and references therein.

[8] Although several superoxo Cu^{II} complexes have been characterized at low temperature[3] (for an X-ray structure, see K. Fujisawa, M. Tanaka, Y. Moro-oka, N. Kitajima, *J. Am. Chem. Soc.* **1994**, *116*, 12079–12800), their room temperature stability seems to be precluded for entropic reasons. In the specific case of a neutral N_3 ligand, these species have been proposed as transient intermediate in the

formation of binuclear peroxo adducts but never observed.[3, 9] Hence, the possibility of a thermodynamically unfavored equilibrium with $[Cu(X_6Me_3Pic_3)(O_2)]^+$ cannot be ruled out, even if it has not yet been observed under these experimental conditions.

[9] A. D. Zuberbühler in *Bioinorganic Chemistry of Copper*, (Eds.: K. D. Karlin, Z. Tyeklár), Chapman & Hall, New York, **1993**, pp. 264–276, and references therein.

[10] a) C. D. Gutsche, *Calixarenes*, *Monographs in Supramolecular Chemistry*, (Ed.: J. F. Stoddart), The Royal Society of Chemistry, Cambridge, **1989**; b) *Calixarenes. A Versatile Class of Macrocyclic Compounds,* (Eds.: J. Vicens, V. Böhmer), Kluwer Academic Publishers, Dordrecht **1991**; c) V. Böhmer, *Angew. Chem.* **1995**, *107*, 785–818 ; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 713–745; d) C. Wieser, C. B. Dieleman, D. Matt, *Coord. Chem. Rev.* **1997**, *165*, 93–161; e) A. Ikeda, S. Shinkai, *Chem. Rev.* **1997**, *97*, 1713–1734.

[11] G. M. Sheldrick, SHELX-97, Program for X-ray structure analysis, Universität Göttingen, **1997**.

Deposition of Data from X-Ray Structure Analyses

In order to make life easier for authors and referees the Cambridge Crystallographic Data Centre (CCDC) and the Fachinformationszentrum Karlsruhe (FIZ) have unified their procedures for the depostion of data from single-crystal X-ray structure analyses.

Prior to submitting a manuscript please deposit the data for your compound(s) **electronically** at the appropriate date base, that is, at the CCDC for organic and organometallic compounds and at the FIZ for inorganic compounds. Both data bases will be pleased to provide help (see our *Notice to Authors* in the first issue of this year). In general, you will receive a depository number from the data base within two working days after electronic deposition; please include this number with the appropriate standard text (see our Notice to Authors) in your manuscript. This will enable the referees to retrieve the structure data quickly and efficiently if they need this information to reach their decision.

This is now the uniform procedure for manuscripts submitted to the journals *Advanced Materials, Angewandte Chemie, Chemistry–A European Journal, the European Journal of Inorganic Chemistry, and the European Journal of Organic Chemistry.*

1433-7851/98/3719-2735 $ 17.50+.50/0