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对甲酰基苯氧乙酸和 2-氨基苯并噻唑的新型三元铜配合物的合成、晶体结构及抗微生物活性

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摘要: 以对甲酰基苯氧乙酸和 2-氨基苯并噻唑为原料,合成了一种三元铜配合物,并通过元素分析,红外光谱以及 X-射线衍射单晶结构分析对其进行了表征。该配合物晶体属单斜晶系,$P2_1/c$ 空间群,中心铜(Ⅱ)离子的配位数为 4,分别与 2 个对甲酰基苯氧乙酸配体分子的羧基和 2 个 2-氨基苯并噻唑配体分子的噻唑环上的氮原子发生配位。抑菌实验结果表明,配合物对新型隐球菌、粘性红圆酵母、桔青霉和黑曲霉具有很好的抑制作用,而对大肠杆菌和金黄色葡萄球菌的抑制效果一般。

关键词: 苯并噻唑; 苯氧乙酸; 三元铜配合物; 晶体结构; 抗微生物活性
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Synthesis, Crystal Structure and Antimicrobial Activity of A Ternary Copper(Ⅱ) Complex with Para-Formyl Phenoxyacetic Acid and 2-Amino Benzothiazole

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Abstract: A ternary complex of $Cu(pfpa)_2(aben)_2$ (pfpa=para-formyl phenoxyacetic acid anion, aben=2-amino benzothiazole) was synthesized by the reaction of copper acetate, 2-amino benzothiazole and para-formyl phenoxyacetic acid. Elemental analysis, IR spectra and X-ray single-crystal diffraction were carried out to determine the composition and crystal structure. The complex crystallizes in the monoclinic system, space group $P2_1/c$ with a=1.319 7(5) nm, b=0.738 8(4) nm, c=1.743 3(9) nm, β=110.42(4)°, $C_{32}H_{26}CuN_4O_8S_2$, M_r=722.23, V=1.592 7(13) nm^3, Z=2, D_c=1.506 $Mg\cdot m^{-3}$, μ=0.875 mm^{-1}, F(000)=742, the final R=0.067 7, wR=0.143 0 for 1 842 observed reflections [$I>2\sigma(I)$]. The central copper atom is four-coordinated with two carboxylate oxygen atoms of the two pfpa ligands and two nitrogen atoms in thiazole rings of the two aben ligands. The crystal structure shows that there are intermolecular and intramolecular hydrogen bonding between amino-nitrogen atoms of the two aben ligands and carboxylate oxygen atoms of the two pfpa ligands. The antimicrobial properties of the complex and its two ligands were tested against representative bacterial and fungal strains. The copper complex exhibited an excellent antifungal activity, better than that of its two free ligands, but for bacteria, its inhibitory effect is less than that of 2-amino benzothiazole. CCDC: 739398.

Key words: benzothiazole; phenoxyacetic acid; ternary copper complex; crystal structure; antimicrobial activity

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In all ages, all kinds of microorganisms always represent a dreadful menace to men′s health and a tremendous destruction to men′s substantial wealth, and therefore, for a more efficient control, the steady development of novel and more powerful antimicrobial compounds is required in many industries such as medicine, agricultural chemicals, cosmetic, food, paint, leather, textile, and so on. The purpose of our research program is to synthesize new compounds bearing different active structures, evaluate in vitro their antimicrobial activities, and estimate their potential as a leather antimicrobial agent.

This study reports a new ternary complex designed to combine the benzothiazole ring and phenoxyacetic acid structure with antimicrobial copper (Ⅱ) ion. In general, benzothiazole and its derivatives have many good biological activities, such as antimicrobial action, antiviral action, anticancer action, and antiinflammation[1~4]. In medicine, agriculture and many other industrial fields, they are widely used as antimicrobial agents, bactericide or mold preventive. For example, in leather industry, 2-(thiocyanomethylthio) benzothiazole (TCMTB) is the commonest fungicide used to inhibit the growth of moulds on chrome tanned leather[5]. Besides, phenoxyacetic acid and its derivatives also possess good biological activities, and they are often used to produce bactericides, insecticides, herbicides, and so on[6~8]. So, compounds with the two active structures maybe have improved antimicrobial activities. Here, using 4-formyl phenol and chloroacetic acid, para-formyl phenoxyacetic acid was first synthesized, and then, it reacted with 2-amino benzothiazole and copper acetate to prepare the title complex. Elemental analysis, IR spectra and X-ray single-crystal diffraction were used to characterize its structure, and the twofold serial dilution method was adopted to evaluate its antimicrobial activity.

1 Experimental

1.1 General

All the reagents used in this experimental were research grade. Elemental analyses (C, H, N) were performed on a Carlo-Erba 1106 analyser and copper ion content was determined at a ICP-AES. Infrared spectra were obtained on a FTIR spectrophotometer (MAGNA.IR506, Nicolet Ltd., USA) by using KBr disk in the range 4 000~400 cm^{-1}. Measurement of the crystal was carried out on a four-circle single crystal X-ray diffractometer (Enraf-Nonius CAD-4, Holland). ^1H NMR spectra were recorded on a Bruker Avance 600 instrument in deuterated dimethyl sulfoxide solutions. Chemical shifts were reported as δ (ppm) relative to TMS as internal standard. Melting point was recorded by the capillary method.

1.2 Synthesis of para-formyl phenoxyacetic acid

To a stirred solution of 4-formyl phenol (12.212 g, 0.1 mol) and chloroacetic acid (14.175 g, 0.15 mol) in water (50 mL) was slowly added 50 mL water solution of sodium hydroxide (12.00 g, 0.30 mol) within 30 minutes at room temperature. The solution was stirred at 98 ℃ for 4 hours and then acidified with concentrated hydrochloric acid to pH 2.0. The resulting solid was filtered and purified by recrystallization from water to give para-formyl phenoxyacetic acid (9.110 g, 50.61%). m.p. 202~203 ℃ [203~204 ℃][9]. Anal. Calcd.(%) for $C_9H_8O_4$: C, 60.00; H, 4.48. Found(%): C, 60.63; H, 4.88. IR (KBr, cm^{-1}): 3 490.60(m), 2 920.92(s), 2 851.52(s), 1 755.81(s), 1 719.60(s), 1 648.02(m), 1 591.48(s), 1 575.49 (s), 1 513.30 (m), 1 269.09 (s), 1 074.85(s), 845.76(vs). ^1H NMR (600 MHz, CD_3SOCD_3): δ 13.0847 (s, 1H, -COOH); 9.8583(s, 1H, 9-CHO); 7.8575, 7.8388 (d, 2H); 7.0994, 7.0849(d, 2H); 4.8082(s, 2H, $-OCH_2-$).

1.3 Synthesis of the title complex

To a stirred solution of 4-formyl phenoxyacetic acid (1.80 g, 0.01 mol) and 2-amino benzothiazole (1.50 g, 0.01 mol) in 70 mL mixed solvent of ethanol and distilled water (1∶1, *V*/*V*) was slowly added 15 mL water solution of copper acetate (0.998 3 g, 0.005 mol) within 10 minutes at 70 ℃. The solution was stirred at 70 ℃ for 2 h and a green powdered solid was obtained after cooling. Then the solid was filtrated, washed with distilled water and ethanol, respectively. After vacuum drying at 50 ℃, 2.813 g solid product was obtained. When the powdered solid was recrystallized in mixed solvent of ethanol and distilled water (1∶1, *V*/*V*), dark green crystals of the copper complex were obtained

after seven days. m.p. 178~179 ℃. Anal. Calcd(%) for $C_{32}H_{26}CuN_4O_8S_2$: C, 53.21; H, 3.63; N, 7.78; Cu, 8.80. Found(%): C, 53.24; H, 3.65; N, 7.70; Cu, 8.94. IR (KBr, cm^{-1}): 3 383.70(m), 3 274.79(m), 2 938.02(s), 2 843.69(s), 1 675.21(s), 1 602.81(s), 1 623.96(m), 1 577.54(s), 1 533.92(s), 1 508.64(m), 1 263.43(s), 1 065.82(s), 842.38(vs), 755.00(vs).

1.4 Crystal structure determination

A single crystal of the title compound with dimensions of 0.42 mm×0.32 mm×0.09 mm was selected for the structure analysis. X-ray diffraction intensity data were collected on an Enraf-Nonius CAD-4 diffractometer equipped with a graphite-monochromatized Mo $K\alpha$ radiation (λ=0.071 073 nm) by using the ω-2θ scan technique at 291(2) K. In the range of $1.65° \leqslant \theta \leqslant 25.50°$ ($-15 \leqslant h \leqslant 14$, $0 \leqslant k \leqslant 8$, $-6 \leqslant l \leqslant 20$), a total of 3 280 reflections were collected, of which 2 847 are independent (R_{int}=0.009 3) and 1 842 with $I>2\sigma(I)$ were considered as observed and used in the succeeding structure calculations. The NRCVAX program was used for the data reduction and absorption correction[10]. The structure was solved by direct methods using SHELXS-97 and refined by full matrix least squares methods for all the non-hydrogen atoms using SHELXL-97[11]. All the hydrogen atoms were added geometrically and refined using a riding model. The final cycle of refinement gave R=0.067 7, wR=0.143 0 (ω=1/[$\sigma^2(F_o)^2$+(0.091 7P)^2], where P=(F_o^2+2F_c^2)/3), S=0.976, $(\Delta\rho)_{max}$=833 e·nm^{-3} and $(\Delta\rho)_{min}$=−824 e·nm^{-3}. A summary of the crystal data and structure refinement parameters for the title complex are given in Table 1.

CCDC: 739398.

Table 1 Crystal data and structure refinement for the copper complex

Empirical formula	$C_{32}H_{26}CuN_4O_8S_2$	Crystal size / mm	0.42×0.32×0.09
Formula weight	722.23	θ range for data collection / (°)	1.65~25.50
Temperature / K	291(2)	Index ranges	$-15 \leqslant h \leqslant 14$, $0 \leqslant k \leqslant 8$, $-6 \leqslant l \leqslant 20$
Wavelength / nm	0.071 073	Reflections collected	3 280
Crystal system	Monoclinic	Independent reflections (R_{int})	2 847 (0.009 3)
Space group	$P2_1/c$	Observed reflections	1 842
a / nm	1.319 7(5)	Absorption correction	Sphere
b / nm	0.738 8(4)	Max. and min. transmission	0.925 4 and 0.710 2
c / nm	1.743 3(9)	Refinement method	Full-matrix least-squares on F^2
β / (°)	110.42(4)	Data / restraints / parameters	2 847 / 6 / 214
V / nm^3	1.592 7(13)	Goodness-of-fit on F^2	0.976
Z	2	Final R indices [$I>2\sigma(I)$]	R_1=0.067 7, wR_2=0.143 0
D_c / (Mg·m^{-3})	1.506	R indices (all data)	R_1=0.184 9, wR_2=0.183 1
F(000)	742	Largest diff. peak and hole / (e·nm^{-3})	883 and −824
μ / mm^{-1}	0.875		

1.5 Antimicrobial activity

The evaluation of the inhibitory effect of the title complex and its two free ligands (4-formyl phenoxyacetic acid and 2-amino benzothiazole) on the bacterial and fungal growth was carried out by the twofold serial dilution method. The following test microorganisms were used: *Escherichia coli* (Gram positive bacterium), *Staphylococcus aureus* (Gram negative bacterium), *Rhodotorula mucilaginosa* and *Cryptococcus albidosimilis* (yeasts), *Penicillium citrinum* and *Aspergillus niger* (moulds).

Nutrient Agar and Potato medium (PDA) were employed as culture media for bacteria and fungi, respectively. The compounds were dissolved dimethyl sulfoxide (DMSO) and tested at concentrations ranging from 0.01 to 100 μg·mL^{-1}. Test inoculum of 5 × 10^4 bacteria per 1 mL and 10^3 yeasts or spores per 1 mL was applied. The criterion of effectiveness was taken in the absence of microbial growth after an incubation period of 24 h at 37 ℃ for bacteria or of 48 h at 30 ℃ for fungi. In every case, the lowest concentration (μg·mL^{-1}) of compound, which inhibits the growth of bacteria after

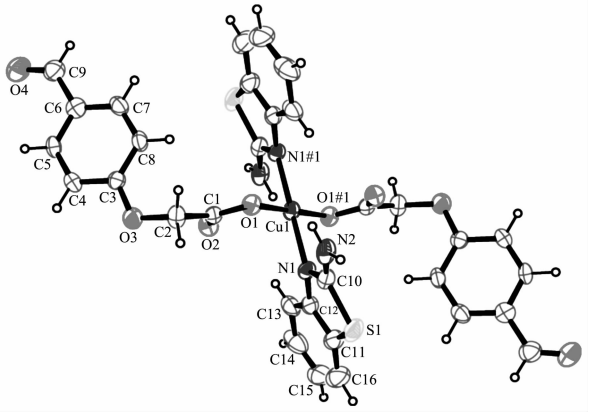
24 h incubation at 37 ℃, and of fungi after 48 h incubation at 30 ℃ was taken as the minimum inhibitory concen-tration (MIC).

The concentration of DMSO in the medium did not affect the growth of any of the microorganisms tested. All experiments were made in duplicate and the results were confirmed in three independent experiments.

2 Results and discussion

2.1 Structure description

The crystal structure and crystal group accumulate chart of the complex are shown in Fig.1 and Fig.2, respectively. The selected bond lengths and bond angles are listed in Table 2. As depicted in Fig.1, the central Cu1 ion in the complex is four-coordinated with two carboxylate oxygen atoms (O1and O1#1) from two para-formyl phenoxyacetic acid ligands and two nitrogen atoms (N1 and N1#1) from thiazole rings of the two 2-amino benzothiazole ligands. In the complex, the bond length of Cu1-O1 is equal to that of Cu1-O1#1, both are 0.195 5(4) nm. Similarly, both bond lengths of Cu1-N1 and Cu1-N1 #1 are 0.199 0(5) nm. The bond angle of O1#1-Cu1-O1 is 180°, indicating the three atoms lie in one straight line. The N1, Cu1 and N1#1 atoms are also in one straight line, their included angle of N1#1-Cu1-N1 is 180°. The bond angles of O1-Cu1-N1 and O1#1-Cu1-N1#1 are 89.39(19)°, and the bond angles of O1#1-Cu1-N1 and O1-Cu1-N1#1 are 90.61(19)°. Above data of bond lengths and angles show that the central Cu1 atom and the four coordinating atoms of N1, N1 #1, O1 and O1#1 are coplanar, and



Symmetry code #1: $-x$, $-y$, $-z$

Fig.1　Molecular structure of the copper complex

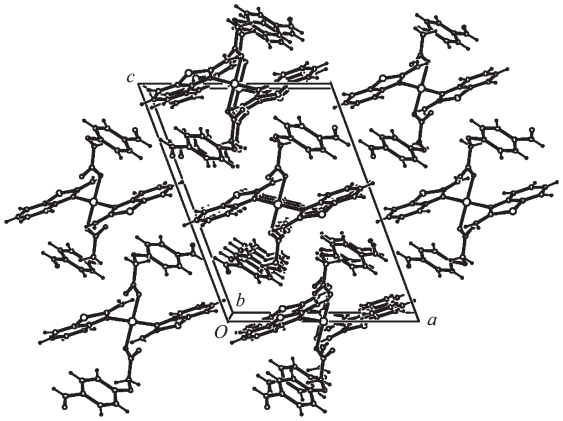


Fig.2　Crystal group accumulate chart of the copper complex

Table 2　Selected bond lengths (nm) and angles (°) for the copper complex

Cu1-O1	0.195 5(4)	O1-C1	0.128 1(7)	N1-C10	0.129 3(9)
Cu1-O1#1	0.195 5(4)	O2-C1	0.120 7(7)	N1-C12	0.140 6(8)
Cu1-N1	0.199 0(5)	O3-C3	0.134 6(8)	N2-C10	0.131 8(8)
Cu1-N1#1	0.199 0(5)	O3-C2	0.145 2(9)	N2-H2A	0.086 00
S1-C10	0.175 0(78)	O4-C9	0.119 5(10)	N2-H2B	0.086 00
S1-C11	0.175 8(11)				
O1#1-Cu1-O1	180.0	C1-O1-Cu1	105.7(4)	O1-C1-C2	113.3(6)
O1-Cu1-N1	89.39(19)	C3-O3-C2	117.4(5)	O3-C2-C1	111.7(6)
O1#1-Cu1-N1	90.61(19)	C10-N1-C12	112.2(6)	N1-C10-N2	125.1(7)
O1-Cu1-N1#1	90.61(19)	C10-N1-Cu1	121.6(5)	N1-C10-S1	114.9(6)
O1#1-Cu1-N1#1	89.39(19)	C12-N1-Cu1	124.1(5)	N2-C10-S1	119.9(6)
N1#1-Cu1-N1	180.0	O2-C1-O1	122.4(6)	C12-C11-S1	109.8(7)
C10-S1-C11	89.0(4)	O2-C1-C2	124.0(7)	C16-C11-S1	128.7(9)

Symmetry transformations used to generate equivalent atoms: #1: $-x+1$, $-y$, $-z$.

they forms a parallelogram. The crystal structure indicates that the nitrogen atom of amino group and sulfur atom of thiazole rings from 2-amino benzothiazole ligand do not take part in coordination. In addition, from Fig.1, it can be seen that the two benzothiazole rings are coplanar, too.

The intermolecular and intramolecular hydrogen bonds are listed in Table 3. There is intramolecular hydrogen bonding between the amino-nitrogen atom (N2) of 2-amino benzothiazole and carboxyl oxygen atom (O1, which is coordinated to Cu1 at the same time) of para-formyl phenoxyacetic acid ligand. Thereinto, the bond lengths of N2-H2A, H2A…O1 and N2…O1 are 0.086, 0.224 and 0.289 1(8) nm, respectively. The bond angle of N2–H2A…O(1) is 132.2°. Besides, there exist intermolecular bonding between the amino-nitrogen atom (N2) of 2-amino benzothiazole and carboxyl oxygen atom (O2#2, which is not coordinated to Cu1) of para-formyl phenoxyacetic acid. The bond lengths of N2-H2B, H2B…O2#2 and N2…O2#2 are 0.086, 0.200 and 0.283 3(8) nm, respectively. The bond angle of N2–H2B…O2#2 is 164.2°.

Table 3 Specified hydrogen bonds (with esds except fixed and riding H) for the copper complex

D–H…A	D-H / nm	H…A / nm	D…A / nm	∠(DHA) / (°)
N2–H2A…O1	0.086	0.224	0.289 1(8)	132.2
N2–H2B…O2#2	0.086	0.200	0.283 3(8)	164.2

2.2 IR spectra

The most significant IR bands of the title complex and para-formyl phenoxyacetic acid are listed in the experimental section. For the other ligand, 2-amino benzothiazole, its infrared spectrum was also determined by using KBr disk in the range 4 000~400 cm^{-1}, and the main peaks are 3 396.70 (m), 3 273.63 (m), 1 644.56(m), 1 589.30(s), 1 528.63(s), 1 446.33(s) and 741.55(vs) cm^{-1}. Compared with IR spectra of the two ligands, the spectrum of the copper complex has the following significant differences which indicate the coordinate mode of copper(Ⅱ) and the coordinating atom of the ligands:

(1) In the IR spectrum of the copper (π) complex, the two absorption peaks at 3 383.70 cm^{-1} and 3 274.79 cm^{-1} are similar to the characteristic peaks at 3 396.70 cm^{-1} and 3 273.63 cm^{-1} attributed to $\nu(NH_2)$ in the IR spectrum of 2-amino benzothiazole, which shows there is no coordination between the copper ion and nitrogen atom of amido group in the complex molecule. For the characteristic absorption peaks of ν(C=N) in the benzothiazole ring, the complex and free 2-amino benzothiazole ligand have different wave number, which are 1 623.96 and 1 644.56 cm^{-1}, respectively. The shift should be attributed to the coordination between the copper ion and the nitrogen atom of thiazole ring.

(2) The coordination of carboxylic group ($-COO^-$) of para-formyl phenoxyacetic acid to the copper ion is suggested by the disappearance of the band of ν(C=O) in IR spectrum of the copper complex. The peaks at 1 602.81 cm^{-1} and 1 348.47 cm^{-1} are assigned to antisymmetric and symmetric vibration absorption of $-COO^-$ group, respectively. The value of $\Delta(COO^-)$ is 254.34 cm^{-1}, indicating that the carboxylic group is coordinated to the Cu(Ⅱ) atom in the monodentate coordinated mode. The antisymmetric and symmetric vibration absorption of (C-O-C) bands are located at 1 263.43 and 1 065.82 cm^{-1}, respectively. Compared with the corresponding absorption peaks in the IR spectrum of para-formyl phenoxyacetic acid, there is no obvious shift, which shows that there is no coordination between the oxygen atom of (C-O-C) group and Cu(Ⅱ) atom.

2.3 Antimicrobial activity

The *in vitro* antimicrobial properties of the copper complex and its two ligands were evaluated against Gram-positive and Gram-negative bacteria, yeasts and moulds. The obtained results are reported in Table 4. The complex shows a moderate inhibitory effect against bacteria (MIC 10~20 $\mu g \cdot mL^{-1}$), which is lower than that of 2-amino benzothiazole (aben), but higher than that of para-formyl phenoxyacetic acid (pfpa). Noticeably, for the tested yeasts and moulds, a significant inhibitory activity (MIC 1~5 $\mu g \cdot mL^{-1}$), higher than that of the both ligands, is displayed by the copper complex. It is

Table 4 Antimicrobial activity expressed as MIC of the complex and its two ligands

$\mu g \cdot mL^{-1}$

Microoganisms	Compounds		
	pfpa	aben	$Cu(pfpa)_2(aben)_2$
Bacteria			
Escherichia coli	75	5	20
Staphylococcus aureus	50	1	10
Yeasts			
Rhodotorula mucilaginosa	100	20	5
Cryptococcus albidosimilis	100	20	5
Moulds			
Penicillium citrinum	>100	25	1
Aspergillus niger	>100	50	5

evident that the complexation with copper enhances the antifungal activity of the two ligands, namely, the corresponding two active structures (benzothiazole ring and phenoxyacetic acid structure) in the title complex play a synergistic inhibitory effect against the yeasts and moulds. Although this kind of complexation is not effective to the inhibition of the growth of bacteria, the copper complex still has some potential application. For example, it may be used as fungicide in the tanning industry, where moulds are often the troublesome microorganism species. Besides, the structure-activity relationship should be strengthened by combining some antimicrobial groups with the two ligands, or extended by the complexation with other metal ions such as nickel(Ⅱ), zinc(Ⅱ), cobalt(Ⅱ), and so on.

References:

[1] CHEN Jin-Can(陈锦灿), QIAN Li(钱　力), SHEN Yong(沈勇), et al. *Sci. China Ser. B-Chem.* (*Zhongguo Kexue B Ji*: *Huaxue*), **2008**,**51**(2):111~119

[2] XU Yun-Gen(徐云根), HUA Wei-Yi(华维一), LIU Xiao-Yan(刘晓燕), et al. *Chin. J. Org. Chem.*(*Youji Huaxue*), **2004**,**24**(10):1217~1222

[3] El-Gazzar A R B A, Hafez H N. *Acta Chim. Slov.*, **2008**,**55**(2):359~371

[4] Akhtar T, Hameed S, Al-Masoudi N A, et al. *Acta Pharmaceutica*, **2008**,**58**(2):135~149

[5] Muthusubramanian L, Mitra R B, Rao V S S. *J. Soc. Leather Technol. Chem.*, **1998**,**82**(1):22~23

[6] Moanta A, Radu S. *Rev. Chim.*, **2008**,**59**(6):708~711

[7] Fracchiolla G, Laghezza A, Piemontese L, et al. *Chem. Med. Chem.*, **2007**,**2**(5):641~654

[8] Carbonara G, Fracchiolla G, Loiodice F, et al. *Farmaco*, **2001**, **56**(10):749~754

[9] HU Qiao-Fei(胡巧斐), HONG Yong-Yu(洪镛裕), LIAO Ben-Ren(廖本仁). *Chemical Reagent*(*Huaxue Shiji*), **2004**,**26**(5):309~310

[10]Gabe E J, Le Page Y, Charland J P, et al. *J. Appl. Cryst.*, **1989**,**22**(5):384~387

[11]Sheldrick G M. *SHELXS*-97, *Program for X-ray Crystal Structure Solution, and SHELXL*-97, *Program for X-ray Structure Refinement*, University of Göttingen, Germany, **1997**.

[12]*SHELXTL Version* 5.0, *Siemens Industrial Automation*, Analytical Instruments, Madison, WI, **1995**.