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Antitumor Activity of the Mixed Phosphine Gold Species Chlorotriphenylphosphine-1,3-bis(diphenylphosphino)propanegold(I)

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The title compound has been designed for antitumor activity based on structural features of related known antitumor gold agents, that is, gold-monophosphine and gold-diphosphine derivatives. It is a gold complex that contains both types of phosphine ligands, thus suggesting a possible synergistic action. The results of a single crystal X-ray structure determination of this molecule show the metal surrounded by 3 P atoms and one Cl anion in a distorted tetrahedral arrangement. The chloro anion, however, is weakly bound to the metal and so the species shows ionic character. The P NMR study, performed in solution, confirms the structural features observed in the solid and, in addition, indicates partial formation of other known gold-(I)-diphosphine antitumor agents. The ionic character and strong Au−P bonds of this novel gold(I) species are similar to those of the most active antitumor gold compounds so far studied. The former feature contributes to solubility in biological fluids, and the latter prevents fast biomolecular attack. In addition, the title compound is less lipophilic, a feature recently correlated to lower liver toxicity. The title compound shows in vitro antitumor activity in the two initial National Cancer Institute protocols against human tumors. In the first screening, a unique dose (0.10 mM) of the title compound reduced cell growth of MCF7 (breast cancer), NCI−H460 (lung cancer), and SF-268 (Central Nervous System cancer-CNS) to 5, 8, and 11%, respectively. In the second protocol a 60-cell line panel was analyzed with the title compound concentration in the 0.1 mM−0.01 μM range. The highest activity was for the breast tumor cell line MCF7 with a LC_{50} less than 0.01 μM. LC_{50} values in the micromolar range were obtained for 29 cell lines. With the exception of leukemia, these micromolar activities were observed in at least one cell line for each subgroup tumor (non small lung, colon, CNS, melanoma, renal, prostate, breast, and ovarian). The leukemia inactivity was unexpected, as all antitumor gold(I) phosphine compounds in the literature described thus far are active. Melanoma was the most sensitive subgroup screened (five out of seven cell lines).

Introduction

For many years the coordination chemistry of gold(I) seemed confined almost completely to linear two-coordinate species,[1] and only a few three-coordinate compounds were known.[2] Four-coordination was demonstrated to be feasible around 1980, although most complexes were unstable.[3] Later, remarkably stable four-coordinate Au(I) complexes containing diphosphine donor ligands were reported and chelation of diphosphine was considered important for the stability of species such as $[Au(diphosphine)_2]X$, X = anion.[4] The study of these compounds received increased attention because of their in vitro and in vivo antineoplastic activity toward some transplantable tumor models, such as P388 leukemia, L1210 leukemia, M5076 reticulum cell sarcoma, B16 melanoma, Lewis lung carcinoma, Madison lung carcinoma, and sc mammary carcinoma 16/c.[5]

Gold metabolism is likely to go through interactions with cysteine, as gold shows an affinity for sulfur. However, not all Cys groups have the same affinity for the metal; for instance albumin Cys34 can sequester Au when bound to hemoglobin Cysβ93 in vitro.[6] Gold antitumor agents have been reviewed recently.[7] Au-diphosphine antitumor compounds act on the mitochondria that undergo uncoupled oxidative phosphorylation with consequent permeability to cations and protons through the inner mitochondrial membrane.[8] Therefore, gold(I) diphosphine compounds are interesting antitumor agents whose mechanism of action differs from that of cisplatin, and an increase of life span is observed when cisplatin and Au-diphosphines are administrated concurrently.[5a] Although the exact mechanism of action is not known, two features can be distinguished:

(1) The most potent antitumor Au(I) compounds are formed with $R_2P-(CH_2)_n-PR_2$ diphosphines having n = 2 or 3, i.e., 1,2-bis(diphenylphosphino)ethane (DPPE) and 1,3-bis(diphenylphosphino)propane (DPPP). These are $[Au(diphosphine)_2]X$ ionic species whose antitumor activity is not influenced by the nature of anion X.[5f] Au(I) seems to function as a carrier for the diphosphine ligand which could be released, for example, after interaction of Au(I) with thiols. This also would explain the fact that antitumor activity is observed when gold is replaced by Ag(I) or Cu(I) in $[Au(DPPE)_2]Cl$.[5e,h,9]

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A proposed mechanism for this cytotoxicity is that Au(I) protects the diphosphine ligand from oxidation reactions, enabling it to be delivered to cells. Once there, diphosphines can display their activity since it is known that DPPE and DPPP are themselves effective antitumor agents (although at high doses,[5b,10] rendering them pharmacologically useless). Another mechanism suggests that the diphosphine can coordinate Cu(I) affording a species likely involved in the cytotoxic mechanism.[5e] In this process, a redox reaction could be established so that one P atom of diphosphine gets oxidized [P(III) → P(V)] while Cu(II), the copper ion found in the body, is reduced to Cu(I). Since this ion has affinity for soft ligands such as diphosphines, a Cu(I)-diphosphine species would be formed. In any case, a more direct involvement of gold in antitumor action is feasible, as some Au(III) compounds without any phosphine bound to the metal are also antitumor active.[6,11] Recently, a specific regulation of a transcriptional Cu activator by gold was described. It was suggested that Au can activate the removal of excess levels of copper that buildup in arthritic patients.[12] This novel feature provides evidence that the function of gold in the body may be closely related to copper and will prompt further studies on gold antitumor mechanism.

(2) Au(I) derivatives of monophosphine ligands, on the other hand, also display antitumor activity.[13] For example, the orally active antiarthritic drug "auranofin" (1-thio-β-D-glucopyranosato-2,3,4,6-tetraacetato-S)(triethylphosphine)gold(I), shows in vitro antiproliferative effects against P388 leukemia, B16 melanoma, and cultured human cancer cells, although activity in vivo is shown only for the P388 leukemia.[14]

These features prompted us to design and synthesize Au(I) complexes containing both mono- and diphosphine ligands simultaneously and to evaluate their antitumor activity which could be potentially increased due to synergistic action. Here we report an in vitro biological study and the synthesis and complete chemical characterization, both in the solid state and in solution, of chlorotriphenylphosphine-1,3-bis(diphenylphosphino)-propanegold(I), a mixed mono- and diphosphine gold(I) derivative.

Results and Discussion

The title compound is obtained by mixing ethyl ether solutions of the linear compound chlorotriphenylphosphinegold(I), $Au(PPh_3)Cl$, and the diphosphine ligand 1,3-bis(diphenylphosphino)propane (DPPP) in stoichiommetric amounts (1:1).

$$Au(PPh_3)Cl + Ph_2P(CH_2)_3PPh_2 \rightarrow Au(PPh_3)(Ph_2P(CH_2)_3PPh_2)Cl$$

An air- and moisture-stable precipitate was obtained and studied with spectroscopic methods (IR and NMR). The IR spectrum shows the bands due to the phosphine ligands to be close to those of analogous phosphine gold(I) complexes.[3,4] A weak band at ca. 357 cm^{-1} likely corresponds to ν(Au−Cl) vibration.[3,4,15] The $^{31}P\{^1H\}$ NMR spectrum of Au{P(Ph)$_3$}(DPPP)Cl, taken at room temperature in $CDCl_3$, shows three signals that can be assigned to the two different phosphorus environments in the complex (PPh_3 and DPPP) and to $[Au(DPPP)_2]Cl$ formed in solution. When the experiment is carried out at −55 °C, four sets of signals are seen: two of them are broad resonances with lower intensity due to formation of $[Au(DPPP)_2]Cl$ and $[Au_2Cl_2](DPPP)$ species,[16] whereas the other two sets, assigned to the title compound, are multiplets typical of AB2 (the more intense) and AA′B spin systems. The magnitude of the ^2J(P−P) coupling constants for these signals is in the range 100−140 Hz, as expected for P donors linked to three- or four-coordinate gold(I) or other geometrically similar metal centers.[4b,d,17−19]

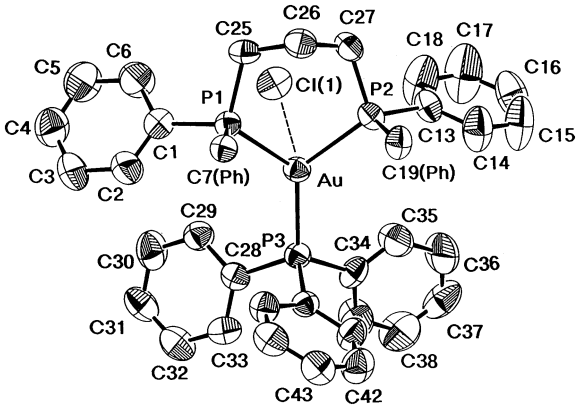

Figure 1. X-ray molecular structure of $Au(DPPP)(PPh_3)Cl$. Five C atoms of two phenyls (C8−C12 and C20−C25) and all H atoms are omitted for clarity. The weak Au−Cl(1) bond is shown with dashed line.

Table 1. Selected Structural Parameters for $Au(DPPP)PPh_3Cl$

Distances (Å)	
Au−P1	2.390(2)
Au−P2	2.386(2)
Au−P3	2.305(2)
Au−C1(1)	2.928(2)
Angles (deg)	
P1−Au−P2	97.66(7)
P1−Au−P3	129.78(7)
P2−Au−P3	128.71(7)
Cl(1)−Au−P1	83.31(7)
Cl(1)−Au−P2	87.61(7)
Cl(1)−Au−P3	112.95(7)

The titration of the complex $Au(PPh_3)Cl$ with free DPPP was also monitored by $^{31}P\{^1H\}$ spectroscopy. The addition of 1 equiv of the free ligand DPPP to a $CDCl_3$ solution containing $Au(PPh_3)Cl$ gave rise to the same ^{31}P NMR resonances found in the spectrum of the title compound.

The crystal structure of the title compound is constructed from well-separated gold complex molecules and a dichloromethane solvent molecule, in a 1:1 ratio. No intermolecular contact exists between the molecules nor is there any imposed symmetry on the molecule. Figure 1 depicts the molecular structure, and Table 1 contains a selection of geometrical parameters.

The coordination sphere shows a distorted tetrahedral gold(I) atom bound to triphenylphosphine, the diphoshine DPPP, and the Cl atom. Due to its small bite, the chelating DPPP ligand subtends a P1−Au−P2 bond angle of 97.66(7)°, smaller than the normal tetrahedral value (109.2°), which induces larger values for the other two P−Au−P angles, 129.78(7)° and 128.71(7)°. The effect of DPPP chelation on the structure can be seen when comparing with the related compound[20] $Au(PPh_3)_3$-

Table 2. In Vitro Most Sensitive Obtained LC_{50} Values (μM) in the 60-Cell Line Panel[a]

Non Small Lung Cancer							
A549/ATCC	EKVX	HOP-62	HOP-92	NCIH226	NCI−H322M	NCI−H460	NCI−H522
4.70	2.00		4.27				
Colon Cancer							
COLO-205	HCC-2998		HCT-116	HCT-15	HT29	KM12	SW-620
7.24	4.68		4.17	5.50			>[b]
CNS Cancer							
SF-268	SF-295		SF-539	SNB-19	SNB-75		U251
6.02	3.98		4.17	5.50			>
Melanoma							
LOX IMVI	MALME−3M		M14	SK-MEL-2	SK-MEL-28	UACC-257	UACC-62
4.17	6.31		5.89		2.29	1.48	5.37
Ovarian Cancer							
IGROVI	OVCAR-3		OVCAR-4	OVCAR-5	OVCAR-8		SKOV-3
	2.69		5.37	6.46			4.37
Renal Cancer							
786−0	A498	ACHN	CAKI-1	RXF 393	SN12C	TK-10	UO-31
	6.17		6.17	5.25		8.32	4.17
Prostate Cancer							
	PC-3				DU-145		
					5.02		
Breast Cancer							
MCF7	MBA-MB-231/ATC		HS 578T	MDA-MB-435	MDA-N	T-47D	
<[c]			>	5.89	7.86		
Leukemia							
CCRF−CEM	HL-60(TB)		K-562	MOLT-4	RPMI-8226	SR	
>	>		>	>	>	>	

[a] Analyzed range is 0.1mM−0.01 μM. [b] > indicates that LC_{50} is undetermined because higher than 0.1 mM. [c] < indicates an active compound with LC_{50} undetermined because lower than 0.01 μM.

Cl that has Au−P bonds of 2.431(2), 2.404(2), and 2.395-(2) Å. The title compound has a shorter Au−P(triphenylphosphine) bond length (2.305(2) Å) and a shorter average Au−P bond (2.36 Å versus 2.41 Å). Therefore, the presence of a chelating diphosphine generates stronger Au−P bonds on the title compound. A structural consequence of this feature is a lengthening of the Au−Cl bond, 2.928(2) Å, in comparison with 2.710(2) Å in $Au(PPh_3)_3Cl$. In $Au(PPh_3)_2Cl$, where the metal is bound to one Cl and two P atoms, the fact that Au−Cl bond is longer than Au−P bond is also seen, 2.500(4) Å and 2.331(4) Å (average), respectively.[2a] This difference is enhanced in the title compound, as the Au−Cl length (2.928(2) Å) is much longer than Au−P bonds (average 2.36 Å), indicating that the Cl atom is weakly bound to the metal. That the sum of the three P−Au−P bond angles (356.1°) is close to 360°, corresponding to a hypothetical "AuP_3" three-coordinate species, confirms this. For comparison, in the four-coordinate tetrahedral cation $[Au\{P(Ph_2)(CH_3)\}_4]^+$ the sum of three P−Au−P angles range from 328 to 342° and Au−P bond lengths are 2.449 Å,[3a] whereas in the title compound they are shorter. We conclude that the title compound has ionic character. This feature is present in $[Au(DPPE)_2]Cl$, where a cationic bis-phosphine-gold moiety (i.e., the chloro is out of the coordination sphere) has strong Au−P bonds (useful to limit detoxification from biomolecules of the body) and shows antitumor activity. Therefore antitumor activity for the title compound is suggested and is confirmed below.

Antitumor Activity. The title compound, assigned code NCS708069, was studied at the National Cancer Institute. The starting protocol consists of an in vitro screening using three tumor cell lines, MCF7 (breast cancer), NCI−H460 (lung cancer), and SF-268 (Central Nervous System cancer-CNS), with a single agent dose of concentration 0.10 mM. These tumor cells were incubated and treated with NCS708069, obtaining reduction of cell viability to 5, 8, and 11%, respectively, and is indicative of high activity, as the minimum condition for satisfying this test is reduction to at least 32% of cell growth in any one of the three lines. These results ensured NCS706089 access to the second level protocol that checks in vitro cell viability in a 60-cell line screening through progressive 10-fold dilution of the agent. Table 2 shows the corresponding LC_{50} parameters for the most sensitive tumors; for 29 cell lines these are in the micromolar range.

The most interesting result is the activity shown against the breast cell line MCF7, with LC_{50} lower than 0.01 μM, but undetermined because the lowest concentration tested in the protocol produces more than 50% supression. Particularly sensitive are melanoma tumors with LC_{50} in the micromolar range for five out of seven cell lines. Activity in the micromolar range was shown in at least one cell line for each subgroup tumor (non small lung, colon, CNS, melanoma, renal, prostate, breast, and ovarian) except for leukemia. The leukemia inactivity was unexpected, as all antitumor gold(I) phosphines in the literature so far are active against leukemia.[9] This suggests that the antitumor activity of our novel mixed mono- and diphosphine gold compound differs from that of related cytotoxic Au-phosphine species. This may be important since the development of the promising compound $[Au(DPPE)_2]Cl$ was stopped due to liver toxicity in dogs.[21] Interest in this field is reemerging, however, as seen by two recent patents,[22] and a study[17] showing that lower lipophilicity of cationic

bis-diphosphine-gold(I) compounds is correlated to a decrease of liver toxicity. Reduced lipophilicity in the title compound, compared to $[Au(DPPP)_2]Cl$, is achieved by replacing a DPPP ligand with a PPh_3, thus removing one Ph and three CH_2 groups. Interestingly, these new agents[17] show LC_{50} values for ovarian cancer similar to those of the title compound. In addition, increasing the hydrophilic/lipophilic ratio in $[Au(R_2P(CH_2)_2PR_2)_2]X$ (R = 2-, 3- and 4-pyridyl) improves selectivity, although some potency is lost as shown by increased IC_{50} values.[17] The possibility exists for tuning the antitumor activity of gold-diphosphine cationic compounds between these two extremes: (a) Very lipophilic compounds which are more cytotoxic and have increased side effects on mitochondria; (b) more hydrophilic compounds which are more selective, less cytotoxic, and have fewer side effects.

Compounds between these limits may be good candidates for clinical use. The title compound appears well equipped with these features: it is mostly cationic (the Cl anion is almost out of the coordination sphere), it is selective (showing useful activity on 29 out of 60 tumor cell lines), and it has reduced lipophilicity in comparison with the corresponding $[Au(DPPP)_2]Cl$ active species.

A cationic gold species with chemical properties between a and b, $[Au(Et_2P(CH_2)_2PEt_2)_2]Cl$, i.e., with lower lipophilicity than $[Au(Ph_2P(CH_2)_2PPh_2)_2]Cl$, resulted in a decrease or loss of antitumor activity.[5e,f] This was associated to the higher reactivity of alkyl phosphines (yielding phosphine oxides) in comparison with aryl phosphines. On the other hand, compound $[Au(R_2P(CH_2)_2PR_2)_2]X$ (R = 2-pyridyl) has a LC_{50} of 1 μM against the cisplatin-resistant ovarian tumor SKOV-3 (slightly more potent than the title compound) and is being further investigated.[17]

We outline the following structure−activity relationship for the role of triphenylphospine, which was not previously described in antitumor Au compounds. A modification in the gold coordination of auranofin is made by the replacement of its tetraacetylthioglucose anion by Cl. The resulting compound, $(PEt_3)AuCl$, still shows potent activity in vitro but less activity in vivo. Binding of DNA to $(PEt_3)AuCl$ was detected but inhibited upon addition of thioglucose in 1:1 ratio.[23] Additional tests indicated the possibility of lipid peroxidation for $(PEt_3)AuCl$.[24] The pattern that emerges is the protective role of thioglucose for the metal in auranofin since the Au−S bond is stronger than Au−Cl. Auranofin is more protected and not allowed to act in the lipid peroxidation as happens for $(PEt_3)AuCl$ where the concentration of the effective $(PEt_3)Au$ moiety decreases. In the title compound the protective role is provided by P atoms that almost completely surround the metal; that is, P atoms have strong affinity for gold and allow the title compound to express marked activity and selectivity. Therefore, one specific role of PPh_3 in the title compound is to protect the metal from biological modification. We also expect to analyze whether additional functions for PPh_3 exist.

Conclusions

The ionic character of the title compound is only slightly lower than that of active bis-diphosphine gold(I) compounds resulting in almost equivalent solubility, which is useful for transport. Also, since membrane crossing is generally favored for less ionic species, its partial ionic character could be useful. Its lower lipophilicity matches those observed in recent Au(I)-phosphine compounds characterized by having antitumor activity with increased selectivity and fewer side effects.[17] Furthermore, the P NMR study shows that dissolving NCS708069 in chloroform results in three species: NCS708069 itself, $(AuCl)_2(DPPP)$, and $[Au(DPPP)_2]Cl$, the latter two are known cytotoxic agents. Therefore, NCS708069 could provide a combination of active species having effective antitumor activity as shown by the interesting results in the NCI screening tests on the 60-cell panel: activity against MCF7 breast tumor (with LC_{50} less than 0.01 μM) and 29 tumor cells showing LC_{50} in the micromolar range. Additional tests will be performed by another laboratory as, unfortunately, current NCI policy excludes screening of metal derivatives.

Experimental Section

Synthesis and Spectroscopic Characterization of Chloro-triphenylphosphine-1,3-bis(diphenylphosphino)propane-gold(I). DPPP and $(PPh_3)AuCl$ were purchased from Aldrich and used without further purification. All solvents were dried, degassed, and distilled prior to use. Elemental analyses (C,H) were performed with a Fisons Instruments 1108 CHNS−O Elemental analyzer. IR spectra were recorded from 4000 to 100 cm^{-1} with a Perkin-Elmer System 2000 FT-IR instrument. ^1H and $^{31}P\{^1H\}$ NMR spectra, referenced to $Si(CH_3)_4$ and external 85% H_3PO_4 respectively, were recorded on a VXR-300 Varian spectrometer (300 MHz for ^1H, and 121.4 MHz for ^{31}P). Relative intensity of signals is given in square brackets and *J* in hertz.

Chloro-triphenylphosphine-1,3-bis(diphenylphosphino)propane-gold(I), $Au(PPh_3)(DPPP)Cl$, was synthesized upon addition of 0.83 g of 1,3-bis(diphenylphosphino)propane (ca 2.0 mmol) to a suspension (50 mL) of chloro-triphenylphosphine-gold(I) (1.000 g, ca. 2.0 mmol) in diethyl ether, forming a colorless precipitate. The suspension was then stirred for 3 days and filtered and the precipitate washed with diethyl ether (20 mL) to give 1.41 g (1.54 mmol, 77%) of the title compound, mp 189−191 °C. Anal. ($C_{45}H_{41}AuClP_3$) C, 59.42; H, 4.32. IR (Nujol mull, cm^{-1}): 3060w, 3039w, 1990w, 1906w, 1826w, 1770w, 1671w, 1582m, 1569m, 1436m, 1313m, 1196br, 1094s, 1070w, 1043w, 1026w, 998w, 971m, 824m, 794m, 761m, 747m, 738m, 722m, 710m, 697s, 648m, 617w, 547m, 530m, 515s, 508s, 491m, 470m, 429m, 398w, 357w, 329w, 302w, 280w, 252sh, 248w, 227w. ^1H NMR ($CDCl_3$, 295 K, δ ppm): 2.2 br (CH_2, 2H), 2.6br (CH_2, 4H), 7.0−7.7m br (CH_{Ph}, 35H).^{31}P NMR ($CDCl_3$, 293K, δ ppm): −1.7br [1], 26.4br [1], 32.7br [1]. ^{31}P NMR ($CDCl_3$, 218K δ ppm): −2.7br [100], 31.2d [90], 34.3t [5] 41.9d [100], 45.1.

This compound also can be prepared by slow diffusion of a dichloromethane solution (50 mL) of chloro-triphenylphosphine-gold(I) (ca 2.0 mmol) into a diethyl ether suspension (30 mL) containing the ligand DPPP (2.0 mmol) (56%).Crystalline material was obtained by slow evaporation of a solution obtained after mixing a dichloromethane solution (5 mL) of 1,3-bis(diphenylphosphino)propane (0.014 g, 0.033 mmol) and a dichloromethane solution (5 mL) of chloro-triphenylphosphine-gold(I) (0.011 g, 0.033 mmol).

X-ray Diffraction Study. Crystals were plate shaped, and the one selected displayed faces of the form (1 0−2), (−1 0 2), (−1 0−1), (1 0 1), (0 1 0), (1−1 0) with a calculated volume of 0.00220 mm^3. Data were collected with a Syntex $P2_1$ diffractometer and corrected for Lorentz, polarization, and absorption effects (a psi-scan showed absorption anisotropy). The structure was solved with the combination of Patterson and Fourier methods using CAOS.[25] Reflections collected: *h* [(−18)−16], *k* [0−11], *l* [0−32]. H atoms were riding on attached C atoms

at 0.96 Å and hydrogen *B*(iso) fixed. The crystallographic unit also contains a molecule of dichloromethane, which was the solvent used in the reaction mixture.

Antitumor Analysis. The in vitro study was performed at the National Cancer Institute using a published procedure[26] following two protocol levels. The first consisted of a three cell line panel, MCF7 (breast), NCI−H460 (lung), and SF-268 (central nervous system). Each cell line was inoculated and preincubated on a microtiter plate. The title compound (NCS708069) was then added at a single concentration (0.10 mM) and the culture incubated for 48 h. End-point determinations were made with sulforodanine B, a protein-binding dye. Results for the antitumor agent were reported as the percent of growth of the treated cells compared to the untreated control cells. The title compound reduced cell growth to 5, 8, and 11%, respectively. The compound was considered active since reduction of growth in at least one of the three cell lines to 32% or less (negative numbers indicate cell kill) defines activity. It was then passed on for further evaluation in the full 60-cell line panel, which uses the same end-point procedure and progressive dilution of the tested agent, over a 5-log dose in the range 0.1 mM−0.01 μM.

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Supporting Information Available: Data from diffraction study for NSC708069: Fractional coordinates; anisotropic displacement parameters; full list of distances and angles; a table containing a summary of crystal data and refinement details. This material is available free of charge via the Internet at http://pubs.acs.org.

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