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RESEARCH ARTICLE

Ligand Exchange/Scrambling Study of Gold(I)-Phosphine Complexes in the Solid Phase by DESI-MS Analysis

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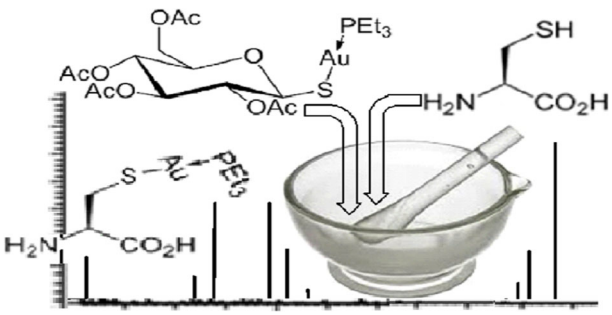

Abstract. Only a few analytical techniques are available for the characterization of mechanochemical synthetic reaction products. We demonstrate here that DESI-MS is a powerful technique for this purpose, combining the selectivity of MS-based assays with the simplicity and in situ analysis capability of ambient ionization methods. In this work, we report that auranofin, a gold-based drug, and its precursor triethylphosphine gold(I) chloride undergo a complex array of ligand exchange/scrambling reactions with thiol-containing amino acids in the solid state. The products were readily characterized by DESI-MS analysis from the solid-phase reaction, clearly exhibiting ligand exchange and scrambling, with independent confirmation by solid state ^{13}C-NMR. The thioglucose and triethylphosphine moieties exchanged with cysteine and its derivatives, whereas the glutathione replaced 2,3,4,6-tetra-*O*-acetyl-β-1-D-glucopyranose only. It was concluded that ligand exchange and scrambling reactions can be carried out in the solid state, and some of the unique products reported in this study can be conveniently prepared through mechanochemical synthesis in good yields (> 98%), as demonstrated by synthesis of (L-cysteinato-*S*)-triethylphosphine gold(I) from triethylphosphine gold(I) chloride and L-cysteine.

Keywords: Gold complexes, Auranofin, Ligand exchange, Ligand scrambling, DESI-MS, Ambient mass spectrometry

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Introduction

Gold complexes are known to exhibit medicinal properties, [1, 2] and their modern clinical use dates back to 1929. Various gold(I) thiolates including sodium aurothiomalate and auranofin, (2,3,4,6-tetra-*O*-acetyl-β-1-D-glucopyranosato-*S*)-triethylphosphine gold(I), have been used to treat conditions such as rheumatoid arthritis. Auranofin is now under clinical trials as an anti-cancer drug, and it has also shown great promise for treatment of parasitic diseases [3]. Numerous other gold(III) species exhibiting anti-tumor activity are also currently under investigation as therapeutics. [4, 5]

Gold complexes are conventionally synthesized through solution-phase chemistry, which typically result in lower yield, with a propensity for the reduction of gold(I) or gold(III) to elemental gold and of ligands to their oxidized products unless the reactions are carried out under inert atmosphere. An alternative to solution-based syntheses is to carry out chemical reactions in the solid phase by grinding the reactants together in mortar and pestle—a technique generally known as mechanochemistry.

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Mechanochemical synthesis is an emerging methodology through which it has been demonstrated that coordination complexes can be easily produced in very high yields, and in some cases, side reactions can be avoided [6, 7]. The reactions in the solid state could be more appropriate for synthesis of metal complexes, which are often more rigorous to synthesize in solution due to rapid redox reactions stemming from the presence of dissolved oxygen. For example, reactions of $M(en)(NO_3)_2$ (M=Pd, Pt; en=ethan-1,2-diamine) with 4,4′-bipyridine (bipy) form tetranuclear square $[M(bipy)(en)]_4(NO_3)_8]$ complexes during mechanochemical synthesis [8]. This reaction illustrates the benefits that solid-state reactions offer over those in solution; here the formation of the platinum complex takes 4 weeks at 100 °C in solution, whereas it can be synthesized in ~ 10 min by grinding the reactants together.

However, a major challenge faced by mechanochemical techniques is accurate characterization of the product in the solid state. Only a few analytical techniques, such as solid-state NMR and X-ray diffraction, are available for this purpose. Gold(I) complexes possess few distinctive features in their UV-visible spectra, and ^{197}Au-NMR signals are typically very broad and uninformative. On the other hand, electrospray ionization-mass spectrometry (ESI-MS) and multi-nuclear ^{1}H, ^{13}C, and ^{31}PNMR methods provide useful data regarding the speciation and reactivity of gold compounds [9–14]. Selectivity and structural elucidation capabilities of MS methods, specifically tandem MS analyses, proved advantageous for these assays. Furthermore, these species and the environments in which they reside have the potential to be directly probed when utilizing next-generation, ambient ionization techniques [15–17], allowing investigation in the native and biomimetic condensed phases.

Besides application in areas such as forensics [18–21] and biomedical imaging [22], desorption electrospray ionization–mass spectrometry (DESI-MS) has shown the capability to probe native chemical reactions in solution, [23] as demonstrated for reactions in low-volume droplets [24] and for rapid electrochemical reactions in thin films [25, 26]. While appearing specifically useful for these systems, the application of DESI-MS in solid-phase reaction monitoring is relatively less explored, with the exception of monitoring Baeyer-Villiger solid-phase reactions by Xie and co-workers [27]. Application of DESI-MS to the analysis of gold-containing products in solid-phase, mechanochemical reactions present an interesting opportunity. In this work, auranofin, a gold-based drug, and its precursor triethylphosphine gold(I) chloride were reacted in the solid phase with thiol-containing amino acids, utilizing DESI-MS to probe the complex array of cognizant ligand exchange/scrambling reactions.

Experimental Section

Samples and Materials

The chemicals used in this study were the following: acetic acid (Fisher Scientific, Hampton, NH, USA); auranofin (a gift from GlaxoSmithKline, USA); L-cysteine (Millipore Sigma, St. Louis, MO, USA); cysteine methyl ester hydrochloride (Fisher); glutathione reduced (Millipore Sigma); *N*-acetylcysteine (Fisher); triethylphosphine gold(I) chloride (Millipore Sigma). Deionized water (resistivity ≈ 18.0 MΩcm) generated by a Barnstead Nanopure® system and HPLC-grade methanol (Millipore Sigma) were used for all DESI-MS spray solvents. Some materials utilized in the study were abbreviated for brevity, as follows: auranofin: $Et_3PAuStagl$; L-cysteine: CySH; *O*-methylcysteine: *O*-MeCySH; *N*-acetylcysteine: *N*-AcCySH; glutathione: GSH; triethylphosphine-gold(I) chloride: Et_3PAuCl.

Mechanochemical Syntheses

Accurately measured equimolar quantities of the gold compound and the target thiol were mixed and ground vigorously with an agate mortar and pestle for 7–10 min. Product mixtures were then analyzed, as is.

DESI-MS and Sample Preparation

Samples for DESI-MS analysis were prepared by placing a small deposit (~ 1–2 mg) of the ground solid mixture onto the surface of double-sided tape (Scotch™ brand, Avon, OH) mounted onto a glass slide. The powder was pressed into the adhesive with a glass stir rod, and excess sample was removed using a stream of nitrogen gas. No significant background ions attributed to the adhesive were observed during direct analysis via DESI-MS.

Positive and negative ion spectra were recorded on a Thermo Fisher LCQ Fleet ion trap MS using an Ominspray DESI source from Prosolia, Inc. (Indianapolis, IN, USA). DESI-MS parameters utilized for this experiment were similar to those commonly reported in the literature, [28] which included a spray solvent flow rate of 3.0 μl min^{-1}, spray voltage of 3.5 kV, a spray angle of 45°, and a nebulizing nitrogen gas pressure of 120 psi. Spectral data were collected using an average of 5 μsec/MS scan and an ionization time of 250 msec. Spray solvent compositions examined during this study were (i) water, (ii), methanol, or (iii) 1:1 methanol:water.

Spectroscopic Methods

FTIR spectra were recorded by placing the solid product on the ATR crystal of an Agilent Cary 630 FTIR spectrophotometer, collecting spectra in the 4000–400 cm^{-1} range. Powder X-ray diffraction (pXRD) patterns were collected at room temperature on a Bruker D8 Advance diffractometer equipped with a Vantec 2D-detector using Cu-K_α radiation in reflection mode, utilizing open sample cups for analysis. Collected data were analyzed and fitted to database patterns using the Evaluation (EVA) Application 7.001 software from SOCABIM SAS (1996–2001). Solid-state ^{13}C-NMR was used to verify the presence of ligand exchange after grinding in the absence of any solvent, as C moieties directly attached to -SH groups are shifted significantly downfield after coordination of thiolate ion with gold, whereas there is little change in the ^{1}H-NMR

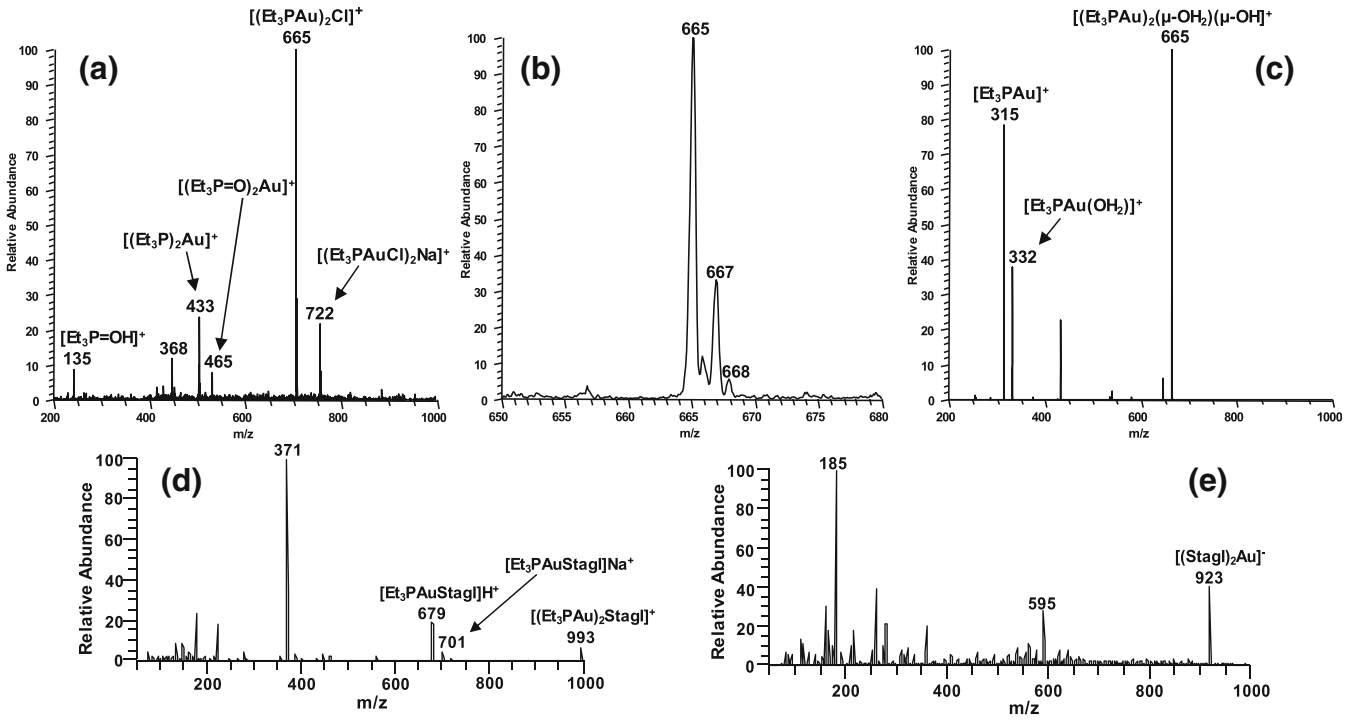
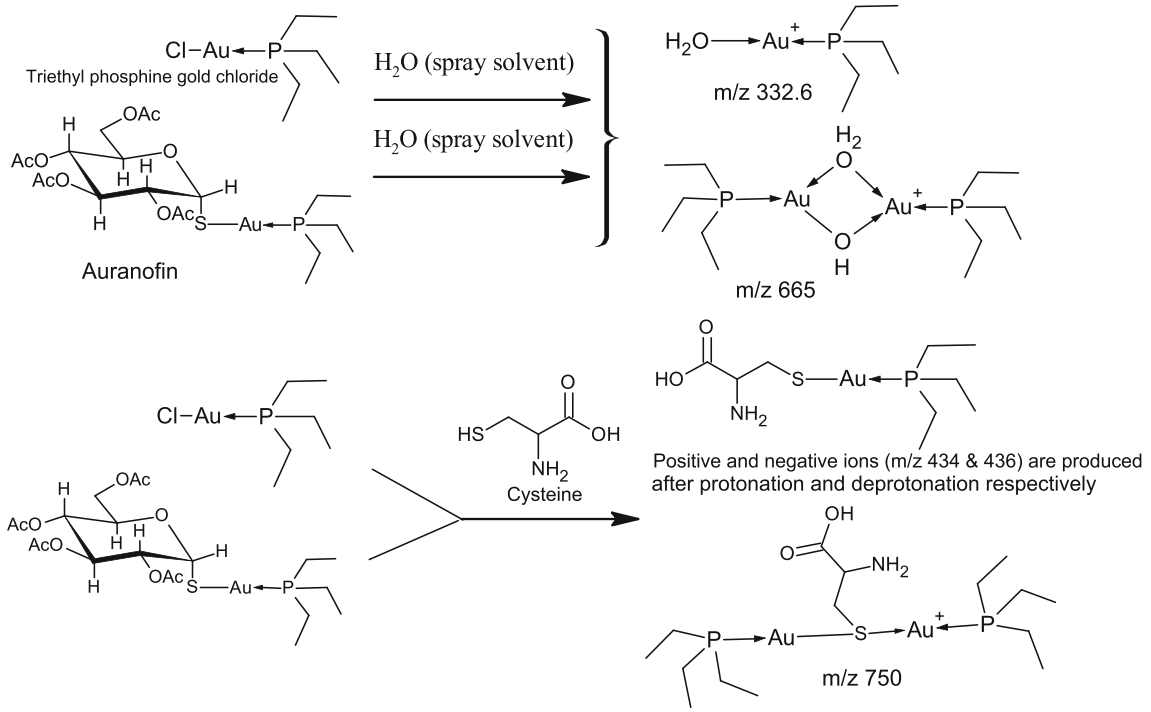


Figure 1. (**a**) Positive ion DESI-MS spectrum for Et_3PAuCl using a methanol-only spray solvent, exhibiting a base peak at *m/z* 665 for $[(Et_3PAu)_2Cl]^+$. (**b**) Isotopic distribution for *m/z* 665 moiety, confirming the presence of chlorine. (**c**) Positive ion DESI-MS spectrum for Et_3PAuCl using a methanol:water (1:1) spray solvent system, showing a base peak lacking the chloride isotopic distribution at *m/z* 665, corresponding to $[(Et_3PAu)_2(\mu\text{-}OH_2)(\mu\text{-}OH)]^+$. (**d**) Representative positive ion DESI-MS spectrum for auranofin ($Et_3PAuStagl$), showing protonated (*m/z* 679), sodiated (*m/z* 701), and an adduct (*m/z* 993) of the drug. (**e**) Representative negative ion DESI-MS spectrum for auranofin ($Et_3PAuStagl$), showing only a gold-bound adduct of the molecule at *m/z* 923 and is marked by overall low intensity



Scheme 1. Ligand exchange reactions of auranofin using water as spray solvent in the DESI-MS experiment (top) and with L-cysteine (bottom).

Table 1. Elemental (CHNS) analysis of (L-cysteinato-*S*)-triethylphosphine gold(I)

% Carbon found (calculated)	% Hydrogen found (calculated)	% Nitrogen found (calculated)	% Sulfur found (calculated)	% Gold found (calculated)
24.87 (24.83)	4.95 (4.86)	3.30 (3.22)	7.19 (7.37)	45.10 (45.25)

spectra. To confirm exchange reactions of target thiols with auranofin, cross-polarized magic angle spinning (CPMAS) spectra were recorded using a three-channel, 11.7 T Varian 500 MHz spectrometer operating at 125.7 MHz for ^{13}C with a sample spinning frequency of 11.111 kHz.

Results and Discussion

Auranofin ($Et_3PAuStagl$) and its precursor Et_3PAuCl were systematically reacted via mechanochemical processing with thiols differing in net charge and molecular weight. As gold(I) compounds are known to undergo facile ligand exchange reactions in aqueous solution, it was reasonable to expect that finely divided reactants might also, under the pressure of grinding, undergo ligand exchange reactions. Utilizing DESI-MS, mass/charge ratios (*m/z*) of various reaction products, complemented by the unique isotopic signatures of chlorine, oxygen, sulfur, and other ligand constituents, were helpful in exact speciation of ionic species observed.

Representative DESI-MS spectra for individual gold-based reactants can be seen in Fig. 1. Collected spectra for Et_3PAuCl are relatively simplistic but dependent on the spray solvent system for the desorption/ionization processes of DESI-MS. Et_3PAuCl is a neutral linear molecule, wherein the chloride ligand is weakly bound to gold compared to 2,3,4,6-tetra-*O*-acetyl-β-1-D-glucopyranose (taglS$^-$) in $Et_3PAuStagl$.

In methanol-only solvent conditions (Fig. 1a), Et_3PAuCl produced a single dominant signal for $[(Et_3PAu)_2Cl]^+$ (m/z = 665), exhibiting a chloride isotopic pattern (exemplified in Fig. 1B) that results from ligand exchange, and lower intensity peaks for $[Et_3P{=}OH]^+$, $[(Et_3P)_2Au]^+$, $[(Et_3P{=}O)_2Au]^+$ and $[(Et_3PAuCl)_2Na]^+$ at *m/z* 135, 433, 465 and 722, respectively;

Table 2. DESI-MS data collected from examined solid-phase reactions

Reactants	DESI-MS signals showing ligand exchange	DESI-MS signals showing ligand scrambling
$Et_3PAuStagl$ + CySH	$[Et_3PAuSCy]^-$ (*m/z* 434) [b][c]; $[Et_3PAuSCy]^+$ (*m/z* 436)[b][c]; $[TaglSAuSCy]^-$ (*m/z* 680) [b][c]; $[(Et_3PAu)_2SCy]^+$ (*m/z* 750) [a][b][c]	$[Et_3PH]^+$ (*m/z* 119) [b][c] [CySH] $^-$(*m/z* 120) [b] [CySH] $^+$ (*m/z*122) [b][c][$Et_3POH]^+$ (*m/z* 135) [b][c] [$Et_3PONa]^+$ (*m/z* 157) [b][c] $[(Et_3P)_2Au]^+$ (*m/z* 433) [b][c] [$Et_3PAuStagl]H^+$(*m/z* 679) [a][b][c]; [$Et_3PAuStagl]Na^+$(*m/z* 701) [a][b][c]; $[Et_3PAuStagl]K^+$(*m/z* 716)[a][b][c]; $[(Stagl)_2Au]^-$(*m/z* 923) [b]; $[(Et_3PAu)_2Stagl]^+$(*m/z* 993) [a][b][c]
$Et_3PAuStagl$ + *O*-Methyl CySH	$[Et_3PAuSCyOMe]^+$ (*m/z* 450[b][c]; $[TaglSAuSCyOMe]^-$ (*m/z* 694) [b][c]; $[(Et_3PAu)_2SCyOMe]^+$ (*m/z* 764) [a][b][c]	$[Et_3PH]^+$ (*m/z* 119); $[Et_3POH]^+$ (*m/z* 135) [b][c]; $[Et_3PONa]^+$ (*m/z* 157) [b][c]; $[(Et_3P)_2Au]^+$ (*m/z* 433)†; $[Et_3PAuStagl]H^+$(*m/z* 679) [b][c]; $[Et_3PAuStagl]Na^+$(*m/z* 701) [b][c]; $[Et_3PAuStagl]K^+$(*m/z* 716); $[(Stagl)_2Au]^-$(*m/z* 923) [a][b]; $[(Et_3PAu)_2Stagl]^+$(*m/z* 993) [b][c]
$Et_3PAuStagl$ + *N*-Acetyl CySH	$[TaglSAuSNAcCy]^-$ (*m/z* 722) [b]; $[(Et_3PAu)_2SCyOAc]^+$ (*m/z* 792) [a][b]	$[Et_3PH]^+$ (*m/z* 119); $Et_3POH]^+$ (*m/z* 135); $[Et_3PONa]^+$ (*m/z* 157); $[(Et_3P)_2Au]^+$ (*m/z* 433) [b]; $[Et_3PAuStagl]H^+$(*m/z* 679) [b][c]; $[Et_3PAuStagl]Na^+$ (*m/z* 701) [b][c]; $[Et_3PAuStagl]K^+$ (*m/z* 716) [b][c]; $[(Stagl)_2Au]^-$ (*m/z* 923) [b][c]; $[(Et_3PAu)_2Stagl]^+$ (*m/z* 993) [b][c]
$Et_3PAuStagl$ + GSH	$[Et_3PAuSG]^-$ (*m/z* 620) [a][b][c]; $[Et_3PAuSGH]^+$ (*m/z* 622) [a][b][c]; $[(Et_3PAu)_2SG]^+$ (*m/z* 936) [b][c]	$[Et_3PH]^+$ (*m/z* 119); $[Et_3POH]^+$ (*m/z* 135) [b][c]; $[Et_3PONa]^+$ (*m/z* 157) [b][c]; $[(Et_3P)_2Au]^+$ (*m/z* 433) [a][b]; $[Et_3PAuStagl]H^+$(*m/z* 679) [a][b][c]; $[Et_3PAuStagl]Na^+$(*m/z* 701) [a][b][c]; $[Et_3PAuStagl]K^+$(*m/z* 716); $[(Stagl)_2Au]^-$(*m/z* 923) [a][b]; $[(Et_3PAu)_2Stagl]^+$(*m/z* 993) [a][b][c]
Et_3PAuCl + CySH	$[Et_3PAuSCy]^+$ (*m/z* 436); $[Et_3PAu)_2SCy]^+$ (*m/z* 750) [a][b][c]	$[Et_3PH]^+$ (*m/z* 119) [a][b][c]; $[Et_3POH]^+$ (*m/z* 135) [a][b][c]; $[Et_3PONa]^+$ (*m/z* 157) [b][c]; $[Et_3PAu]^+$ (*m/z* 315) [a][c]; $[Et_3PAu(OH_2)]^+$ (*m/z* 333) [a]; $[(Et_3P)_2Au]^+$ (*m/z* 433) [a][b][c]; $[(Et_3P{=}O)_2Au]^+$ (*m/z* 465)[a][b][c]; $[(Et_3PAu)_2Cl]^+$ (*m/z* 665)[a][b][c]; $[(Et_3PAu)_2(\mu OH_2)(\mu\ OH)]^+$ (*m/z* 665); $[(Et_3PAuCl)_2Na]^+$ (*m/z* 722)[a]
Et_3PAuCl + *O*-Methyl CySH	$[Et_3PAuSCyOMe]^+$ (*m/z* 450) [a][b][c]; $[Au(SCyOMe)_2]^-$ (*m/z* 465) [a][b]; $[(Et_3PAu)_2SCyOMe]^+$ (*m/z* 764) [a][b]	$[Et_3PH]^+$ (*m/z* 119); $[Et_3POH]^+$ (*m/z* 135) [a][c]; $[Et_3PONa]^+$ (*m/z* 157) [c]; $[Et_3PAu(OH_2)]^+$ (*m/z* 333)[a][b][c]; $[(Et_3P)_2Au]^+$ (*m/z* 433) [a][b]; $[(Et_3P{=}O)_2Au]^+$(*m/z* 465) [a][b][c]; $[(Et_3PAu)_2Cl]^+$(*m/z* 665)[a][b][c]; $[(Et_3PAu)_2(\mu OH_2)(\mu\ OH)]^+$ (*m/z* 665)
Et_3PAuCl + *N*-Acetyl CySH	$[(Et_3PAu)_2SCyNAc]^+$ (*m/z* 792) [a]	$[Et_3PH]^+$ (*m/z* 119)*; $[Et_3POH]^+$ (*m/z* 135) [a][c]; $[Et_3PONa]^+$ (*m/z* 157) [a][c]; [N-AcCySH]$^-$ (*m/z* 162) [a]; $[Et_3PAu(OH_2)]^+$ (*m/z* 333); $[(Et_3P)_2Au]^+$ (*m/z* 433) [a][b][c]; $[(Et_3P{=}O)_2Au]^+$ (*m/z* 465)[a][c]
Et_3PAuCl + GSH	$[Et_3PAuSGH]^+$ (*m/z* 622) [b]; $[(Et_3PAu)_2SG]^+$ (*m/z* 936) [a][b][c]	$[Et_3PH]^+$ (*m/z* 119) [a][c]; $[Et_3POH]^+$ (*m/z* 135) [a][b][c]; $[Et_3PONa]^+$ (*m/z* 157) [b]; [GSH] $^-$ (*m/z* 306); $[GSH]^+$ (*m/z* 308) [b]; $[Et_3PAu]^+$ (*m/z* 315) [a]; $[Et_3PAu(OH_2)]^+$ (*m/z* 333) [a][c]; $[(Et_3P)_2Au]^+$ (*m/z* 433) [a][b][c]; $[(Et_3P{=}O)_2Au]^+$(*m/z* 465)[a][b][c]; $[(Et_3PAu)_2Cl]^+$ (*m/z* = 665)[a][b][c]; $[(Et_3PAu)_2(\mu OH_2)(\mu\ OH)]^+$ (*m/z* = 665)

[a] water; [b] methanol; [c] methanol:water (1:1) used as solvents

alkali earth metal adducts are commonly seen in spray-based ambient MS methods, attributed to trace metals in examined samples and commercial solvents. The species distribution is markedly different in a 1:1 methanol-water solvent system (Fig. 1C), where signals were observed for $[Et_3PAu]^+$ (*m/z* 315), $[Et_3PAu(OH_2)]^+$ (*m/z* 332), and $[(Et_3PAu)_2(\mu\text{-}OH_2)(\mu\text{-}OH)]^+$ (*m/z* 665, no Cl^-isotopic signal). The $Et_3P{=}O$ forms by air oxidation, $[(Et_3P)_2Au]^+$ by ligand scrambling, and $[(Et_3PAuCl)_2Na]^+$ by ion-pairing with trace sodium. Water is a more effective ligand than methanol and is able to displace chloride to form an aqua complex, $[Et_3PAu(OH_2)]^+$; this complex appears to be formed in the water-methanol system, which aggregates to form $[(Et_3PAu)_2(\mu\text{-}OH_2)(\mu\text{-}OH)]^+$ due to the presence of methanol that in turn dissociates to $[Et_3PAu]^+$ during desolvation. Representative DESI-MS spectra for auranofin can be seen in Fig. 1d (positive ion mode) and e (negative ion mode).

Data suggest that both $Et_3PAuStagl$ and Et_3PAuCl undergo significant ligand exchange and ligand scrambling reactions in the solid state, represented in Scheme 1. In fact, sufficiently pure (L-cysteinato-*S*)-triethylphosphine gold(I) was able to be obtained from grinding accurately weighed triethylphosphine gold(I) chloride and L-cysteine in a stoichiometric ratio with a practical yield (> 98%); this offers a marked improvement over early attempts to isolate this compound with purity from solutions, which was unsuccessful due to it's instability in the solution-phase [2, 29]. The elemental analysis (CHNS) and gold composition (by atomic absorption) of the product reasonably matched theoretical calculated values (Table 1).

Positive ion DESI-MS of $Et_3PAuStagl$ in the absence of added thiols (as seen in Fig. 1d) exhibited signals for protonated, sodiated and potassiated cations of the compound at *m/z* 679, 701, and 717 (low intensity), respectively. Additional minor signals observed at *m/z* = 119, 433 and 993 were assignable to $[Et_3PH^+]$, $[(Et_3P)_2Au]^+$ and $[(Et_3PAu)_2(\mu\text{-}Stagl)]^+$, respectively. The single dominant peak in the negative ion spectrum (Fig. 1e) was $[Au(Stagl)_2]^-$ (*m/z* 923). $Et_3PAuStagl$ has been characterized via crystallographic means as a linear complex with $d_{Au\text{-}S} = 2.29$ and $d_{Au\text{-}P} = 2.26$ Å [29]. As the Au–S and Au–P bond lengths are close to each other, there is possibility of ligand exchange/scrambling at both sites when $Et_3PAuStagl$ is allowed to react with other soft ligands. Thus, it was expected that various species would be formed according to the scrambling equilibria accounting for these species as:

$$2Et_3PAuStagl \rightleftharpoons \left[(Et_3P)_2Au\right]^+ + \left[Au(Stagl)_2\right] \quad (1)$$

$$3Et_3PAuStagl + H^+ \rightleftharpoons \left[(Et_3PAu)_2Stagl\right]^+ + \left[Au(Stagl)_2\right]^- + Et_3PH^+ \quad (2)$$

These species have previously been observed in ESI-MS [14], where they exist in a stable equilibrium with the neutral $Et_3PAuStagl$ molecule, but were not observed in methanolic solution by NMR-based methods.

Mechanochemical reaction products identified via DESI-MS for either $Et_3PAuStagl$ or Et_3PAuCl with a selection of individual thiols (i.e., CySH, *O*-MeCySH, *N*-AcCySH, and GSH, can be seen in Table 2, with indication of the cognizant spray solvent

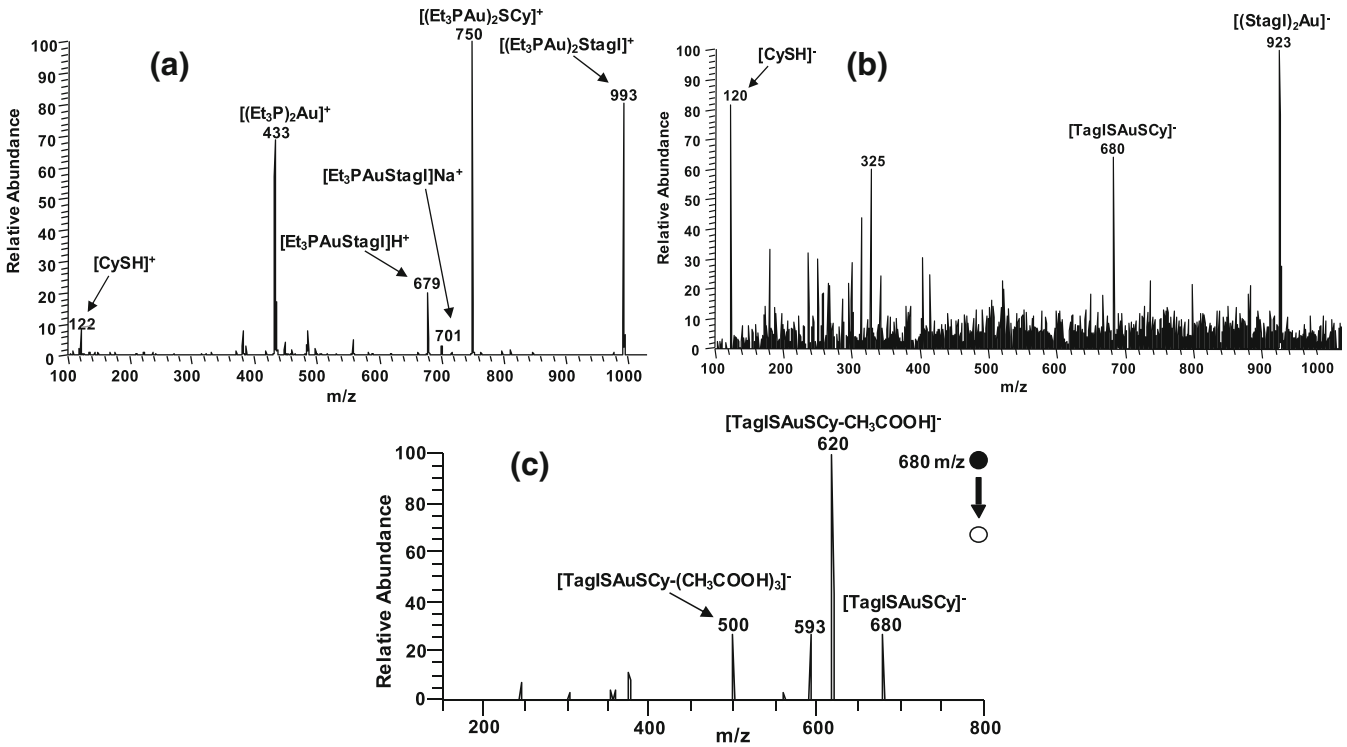


Figure 2. Representative DESI-MS mass spectrum collected from a mixture of auranofin ($Et_3PAuStagl$) with L-cysteine (CySH) in (**a**) positive ion mode, showing reaction-related signatures such as $[(Et_3PAu)_2SCy]^+$ at *m/z* 750, and (**b**) negative ion mode, exhibiting $[taglSAuSCy]^-$ at *m/z* 680. DESI-MS/MS of the *m/z* 680 precursor, yielding product ions corresponding to losses of the CH_3COOH moiety at *m/z* 500 (*n* = 3) and 620 (*n* = 1)

system included. Collision-induced dissociation (CID) MS/MS spectra of target precursor ions were used to assist in the assignment of molecular formula and deducing ligand exchange and scrambling mechanisms. Representative DESI-MS and MS/MS spectra from the mixture of auranofin ($Et_3PAuStagl$) with L-cysteine can be seen in Fig. 2. For this combination, positive mode DESI-MS (Fig. 2a) yields characteristic products such as $[(Et_3PAu)_2SCy]^+$ ($m/z = 750$), with negative mode exhibiting $[taglSAuSCy]^-$ ($m/z = 680$); additional signatures stemming from $Et_3PAuStagl$ and CySH were observed in methanol and methanol-water (1:1) spray solvents, whereas only one signal due to $[(Et_3PAu)_2SCy]^+$ ($m/z = 750$) appeared in water. (Table 2). DESI-MS/MS spectra from the m/z 680 negative ion precursor yielded fragment ions corresponding to losses of the CH_3COOH moiety at m/z 500 ($n = 3$) and 620 ($n = 1$), indicative of a cysteine-containing reaction product.

Formation of $[Et_3PH^+]$ was also confirmed by FTIR spectra (seen in Fig. S5), where the band at 2341 cm^{-1} (ν_{PH}) appeared for reaction products of various thiols with $Et_3PAuStagl$ and Et_3PAuCl. For Et_3PAuCl reactions, only chloride exchanged with the thiol ligands, as indicated by the DESI-MS signals due to $[(Et_3PAu)_2SCy]^+$ ($m/z = 750$). In these products, the band around 2550 cm^{-1} due to ν_{SH} in thiol ligands in corresponding FTIR spectra were absent, which confirmed the ligand exchange through thiol groups. Complimentary pXRD spectra, as seen in Fig. 3, also suggested the presence of phase changes stemming from the mechanochemical reaction. While spectra were not well resolved, changes in intensity and position of diffraction peaks were observed.

Similarly, $Et_3PAuStagl$ was ground in equimolar amounts with *O*-MeCysH, *N*-AcCySH, and GSH and subjected to DESI-MS analysis, with representative MS and MS/MS spectra seen in Figs. S1 through S3 in the Supplementary Materials; tabulated ion assignments can also be seen in Table 2. Analogous ionic species, except $[Et_3PAuSR]^-$, were found to be present in the reaction product of $Et_3PAuStagl$ with *O*-MeCysH (Fig. S1). In case of GSH, the mixed dithiolato anion $[taglSAuSG]^-$ was not observed (Fig. S3). Of these thiols, *N*-AcCySH exhibited the slowest reactivity, as very weak signals were observed after grinding the mixture for ~ 1 h in contrast to 7–10 min in other reactions. The signals at $m/z = 792$ $[(Et_3PAu)_2SNAcCy]^+$ (Fig. S2A) and $m/z = 722$ $[taglSAuSNAcCy]^-$ (Fig. S2B) were observed only when methanol was used as spray solvent. This may be due to the high pK_{SH} (9.5) value of *N*-acetylcysteine, decreasing its reactivity.

For comparison purposes, all of the above experiments were repeated using physical mixtures of separately ground reactants (i.e., no mechanochemical grinding of mixed reactants). Very weak signals, some approaching detection limit ($S/N = 3$), for some of the ionic species listed in Table 2 could be observed, which clearly demonstrates that ligand scrambling and exchange readily takes place as a result of grinding the reactants together. This was further verified by collecting DESI-MS spectra after grinding the reactants for differing time periods, where it was found that the observed intensity of target ions increased correspondingly with reaction time, plateauing at the maxima after ~ 10 min for most thiols.

To assist in independently validating the DESI-MS results presented herein, solid-state ^{13}C-NMR spectroscopy proved useful, using the ligand exchange reaction of auranofin with L-cysteine as a representative system. The CPMAS spectra

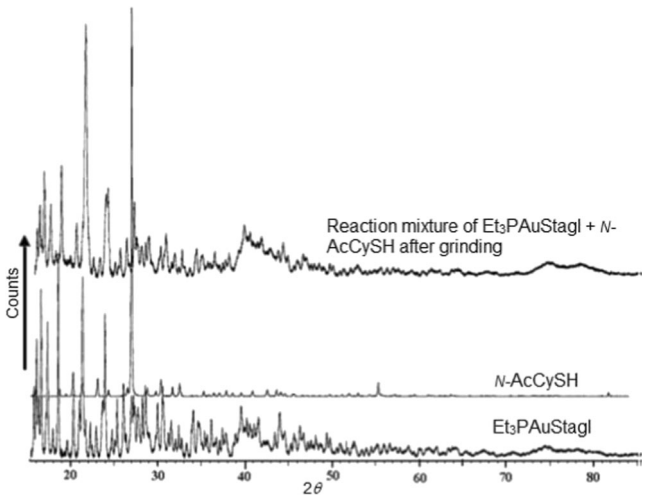


Figure 3. Powder X-ray diffraction pattern of $Et_3PauStagl$ (bottom), *N*-AcCySH (middle), and reaction mixture of $Et_3PAuStagl$ + *N*AcCySH after grinding (top)

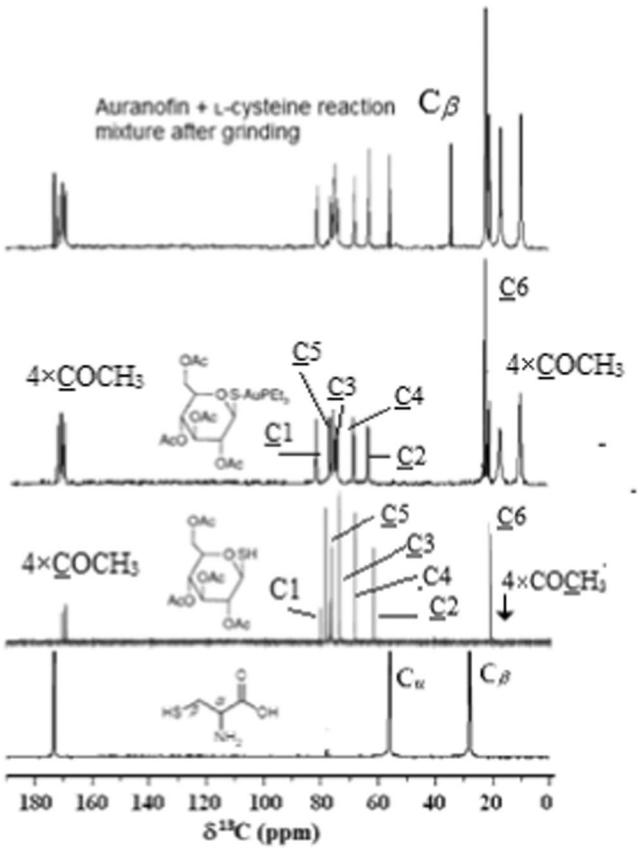


Figure 4. $^{13}C\{^1H\}$ CPMAS spectra of L-cysteine (bottom), 2,3,4,6-tetra-*o*-acetyl-β-1-d-glucopyranose (bottom middle), auranofin (top middle) and the ground reaction mixture between auranofin and L-cysteine (top)

collected from L-cysteine (only), 2,3,4,6-tetra-*o*-acetyl-β-1-D-glucopyranose (only), auranofin (only), and the overall reaction mixture (auranofin with L-cysteine) are shown in Fig. 4. A coordination shift of approx. 8 ppm in C_β of L-cysteine, i.e., the carbon attached to sulfur, was observed that unambiguously verified bonding of L-cysteine with gold through the sulfur atom. No such shift was observed on simple mixing (without grinding) of the reactants. Thus, the NMR results help to support the observations from DESI-MS analysis of mechanochemical reactions.

Conclusions

The results of this study demonstrate that the DESI-MS technique can be successfully employed in monitoring the ligand exchange and scrambling reactions of coordination compounds in the solid state for gold-based therapeutic drugs. Further, the dependence of the observable reaction products on the utilized spray solvent suggests that this could be a tuneable variable, increasing the sensitivity and/or selectivity towards select targets. [30] The simplicity of mechanochemical syntheses coupled with the direct analysis capability of DESI-MS creates a rapid and impactful experimental design. In the iteration reported here, the full experiment (i.e., synthesis and analysis) was able to be accomplished in less than 20 min. This approach could prove useful towards accelerated screening of ligand targets for novel active pharmaceutical ingredients in gold-based drugs, particularly when isolation from the solution-phase is problematic. [2, 29]. With the increased availability of reduced-scale MS systems, [20, 31] performing similar investigations in the proximity of synthetic apparatus, potentially in a fume hood and/or in inert atmosphere, [32] is now conceivable.

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