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[Au(dien)(N-heterocycle)]$^{3+}$: Reactivity with Biomolecules and Zinc Finger Peptides

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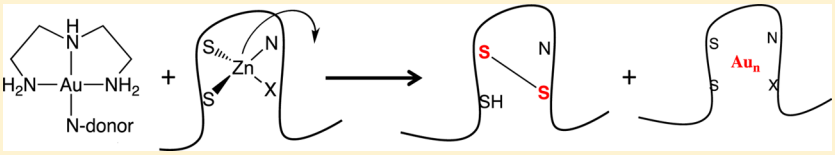
Supporting Information



ABSTRACT: The reaction of [Au(dien)(N-heterocycle)]$^{3+}$ (AuN_4) coordination compounds with simple amino acids and zinc finger proteins is reported. Compared to [AuCl(dien)]$^{2+}$, NMR studies show that the presence of a more substitution-inert N-donor as the putative leaving group slows the reaction with the sulfur-containing amino acids *N*-acetylmethionine (NAcMet) and *N*-acetylcysteine (NAcCys). Lack of ligand dissociation upon reaction with NAcCys indicates, to our knowledge, the first long-lived N-heterocycle−Au−S species in solution. Reactions with zinc finger proteins show a higher reactivity with the Cys_3His zinc finger than with Cys_2His_2, likely due to the presence of fewer aurophilic cysteines in the latter. Of the Au(III) compounds studied, [Au(dien)(DMAP)]$^{3+}$ (DMAP = 4-dimethylaminopyridine) appears to be the least reactive, with ESI-MS studies showing the presence of intact zinc fingers at initial reaction times. These results, in combination with previously reported characterization and pH dependency studies, will further aid in optimizing the structure of these AuN_4 species to obtain a substitution-reactive yet selective compound for targeting zinc finger proteins.

INTRODUCTION

Zinc finger (ZF) proteins are comprised of amino acids with a combination of cysteine and histidine residues coordinated to a tetrahedral zinc ion core.[1−4] They are critical for a variety of biological functions, including transcription, DNA repair, and apoptosis. Displacement of the central zinc ion, along with mutation of coordinated amino acids, can result in a loss of biological function.[5] There is increasing interest in synthesizing small molecules to selectively target zinc finger proteins to in turn inhibit DNA(RNA)/ZF interactions and therefore result in loss of protein function. A zinc finger of special interest is the HIV nucleocapsid HIVNCp7 (NC) protein, a significant potential target for HIV intervention.[6,7] A key interaction between RNA and NC is the π stacking between the guanine bases of RNA and the planar aromatic amino acid tryptophan37 (Trp37) of the protein.[8,9] Mutation and/or deletion of this residue from the zinc finger significantly decreases the chaperone activity of HIVNCp7 and inhibits viral replication.[10]

Coordination compounds such as *cis*-[$PtCl_2(NH_3)_2$] (cisplatin), *trans*-[$PtCl(9\text{-EtG})(py)_2$]$^+$ (9-EtG = 9-ethylguanine; py = pyridine), and [PtCl(dien)]$^+$ (dien = diethylenetriamine) act as electrophiles toward zinc fingers, disrupting protein conformation with zinc ejection.[5,11−13] An approach to target specifically the C-terminal finger of HIVNCp7 is to use platinated nucleobases, such as [Pt(dien)(nucleobase)]$^{2+}$, as electrophiles capable of tryptophan molecular recognition and eventual attack on the highly nucleophilic zinc-cysteinate residues.[12,14−16] Pt(II)−N-heterocycle compounds, with 4-dimethylaminopyridine (DMAP) for example, may also interact with the HIVNCp7 C-terminal zinc finger through noncovalent interactions between ZF tryptophan and the N-heterocycle ligand.[17]

Given the isoelectronic and isostructural relationship of Pt(II) and Au(III), we have been exploring analogous Au(III) chemistry. The properties of [Au(III)(dien)(nucleobase/N-heterocycle)]$^{3+}$ show that the N-donor ligand stabilizes the Au(III) oxidation state, affects the acidity of the central secondary amine proton of the dien ligand, and significantly enhances π-π stacking with the simple amino acid tryptophan more than their Pt(II) analogues.[18]

The "parent" [AuCl(dien)]Cl_2 reacts rapidly with the C-terminal finger of HIV NCp7 with Zn^{2+} ejection and formation of $AuF/Au_2F/Au_4F$ "gold fingers".[13] The Au(III) compound also reacts much faster than its Pt(II) analogue, attributed to the "soft" nature and high thiol affinity of Au(III).[5,13] The presence of a more substitution-inert ligand such as a nucleobase or planar amine, producing AuN_4 rather than $AuClN_3$ coordination spheres, may allow for tuning of the Au(III) reactivity, aiding in the overall selectivity of the reaction with peptides. Following our description of the basic chemistry of [Au(III)(dien)(nucleobase/N-heterocycle)]$^{3+}$, we now report on the reactivity of these compounds with relevant biomolecules, specifically with the sulfur-containing amino acids *N*-acetylmethionine (NAcMet) and *N*-acetylcysteine (NAcCys) and zinc finger peptides with differing zinc coordination spheres, including the Cys_2His_2 finger-3 of the Sp1 transcription factor (Sp1(F3)) and the Cys_3His C-terminal

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finger of the HIVNCp7 nucleocapsid protein (NCp7(F2)) (Figure 1). The Au(III)−N-heterocycle compounds reacted

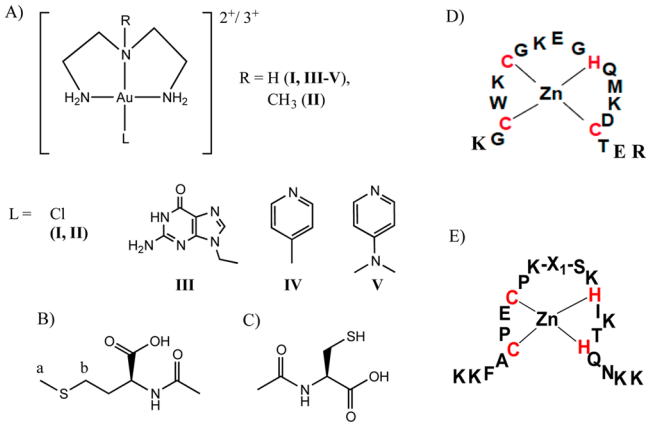

Figure 1. Structures of (A) Au(III) complexes studied, (B) NAcMet, (C) NAcCys, (D) NCp7(F2), and (E) Sp1(F3) ZF (X_1 = RFMSDHL).

more slowly than did $[AuCl(dien)]Cl_2$ with both NAcMet and NAcCys, the latter producing an N-heterocycle−Au−S species, unlike previous results seen for Au(I)−N-heterocycle compounds.[19] Differences in reactivity of Cys_3His and Cys_2His_2 zinc coordination cores are also discussed.

EXPERIMENTAL METHODS

Materials. $HAuCl_4$ was purchased from Strem Chemicals. 2,2′-Diamino-*N*-methyldiethylamine (N-Medien) was purchased from TCI America. The NCp7(F2) and Sp1(F3) apo-peptides were purchased from GenScript Corporation. All other reagents were purchased from Sigma-Aldrich.

Synthesis. Synthesis and characterization of $[AuCl(dien)]Cl_2$ **I**, $[AuCl(N\text{-}Medien)]Cl_2$ **II**, $[Au(dien)(9\text{-}EtG)](NO_3)_3{\cdot}2H_2O$ **III**, and $[Au(dien)(DMAP)]Cl_3{\cdot}1.5H_2O$ **V** have been previously published.[18,20]

Synthesis of [Au(dien)(4-picoline)](NO$_3$)$_3$·H$_2$O (IV). Added to a solution of **I** in water were 0.98 equiv of 4-picoline and 2.98 equiv of $AgNO_3$. The reaction mixture was stirred at room temperature in the dark for 1 h. The solution was filtered to remove AgCl, and the yellow filtrate was evaporated to dryness, resulting in an orange film. Acetone was added to precipitate an orange hygroscopic crystalline product, which may be recrystallized from H_2O/acetone. Anal. Calcd for $C_{10}H_{21}AuCl_3N_5$: C, 20.11; H, 3.71; N, 16.42%. Found: C, 19.67; H, 3.25; N, 16.01%. At 1 mM: λ_{max1} = 206 nm, log ε1 = 4.63; λ_{max2} = 247 nm, log ε2 = 3.92; λ_{max3} = 357 nm, log ε1 = 2.87. ^{1}H NMR in D_2O at pH* 3.69 (* = reading of pH meter in deuterated solvent): δ 8.59 (t, 2H), 7.87 (d, 1.11H), 7.70 (d, 0.96H), 3.61 (broad t, 6.68H), 3.09 (broad s, 1.31H), 2.97 (broad s, 1.80H), 2.65 (s, 1.77H), 2.54 (s, 1.46H).

Ethidium Bromide (EtBr) Fluorescence Assay. Calf thymus (CT)-DNA was prepared in 1 mM phosphate buffer (50 mM NaCl, pH 7.4). The concentrations of both DNA and EtBr were determined by absorption measurements at 260 and 338 nm, respectively, using ε_{260} = 6000 M^{-1} cm^{-1} for CT-DNA and ε_{338} = 5680 M^{-1} cm^{-1} for EtBr. Stock solutions of the CT-DNA incubated with the Au(III) compounds at a starting r_i of 0.1 were prepared and incubated at 37 °C for 24 h for **I** and **II** (covalent binding) and for 1 h for **III** and **V** (noncovalent binding). Varying amounts of EtBr in the range of r_i = 0−0.25 were added to control CT-DNA and CT-DNA treated with all compounds (concentration of DNA at 100 μM). Fluorescence emission spectra were recorded on a Cary Eclipse (Varian) fluorimeter at 25 °C. Fluorescence excitation was at λ_{ex} = 525 nm and registered at λ_{em} = 600 nm. Spectra were recorded in triplicate and averaged.

NMR Spectroscopy with *N*-Acetylmethionine (NAcMet) and *N*-Acetylcysteine (NAcCys). Added to a solution containing 2.5 equiv of NAcMet or 1 equiv of NAcCys were 0.006−0.009 mmol solutions of compounds **I**−**V**. The reaction was followed over time by ^{1}H NMR spectroscopy. NMR spectra were referenced to the residual signal of D_2O (δ(^{1}H) 4.80).

Zinc Finger Preparation. Free peptide was dissolved in water (concentration was dependent on the experiment/technique being performed). The pH was adjusted to 7.2−7.4 with a concentrated solution of NH_4OH. A concentrated solution of zinc acetate ($Zn(O_2CCH_3)_2$) was used to add 1.3 equiv of zinc. For both the Sp1 and NCp7 cases, the ZF formed immediately, and formation was confirmed by both circular dichroism (CD) and mass spectrometry (MS). The data obtained were in agreement with values previously reported in the literature.[13,19,21−23]

Tryptophan Quenching by Fluorescence Spectroscopy. For fluorescence quenching studies, methods were adapted from those previously published.[12] A 3 mL solution of *N*-acetyltryptophan (NAcTrp) or NCp7(F2) (5 μM) was titrated with aliquots of the corresponding quenching compound (7.5 mM) in the range of 1−10 for [quencher]/[NAcTrp]. For time studies performed with zinc finger proteins, 1 equiv of Au compound was added to 5 μM NCp7(F2), and fluorescence was monitored over time.

Circular Dichroism Spectroscopy. Methods were adapted from those previously published.[13,19] A 0.2 mg/mL solution of peptide in water was prepared. CD spectra were run before and after adding zinc (1.3 equiv) to ensure correct formation of the zinc finger. The reaction with 1 equiv of ZF and 1.3 equiv of drug was performed and followed over time.

Mass Spectrometry. Methods were adapted from those previously published.[13,19] Electrospray ionization mass spectrometry (ESI-MS) experiments were performed on an Orbitrap Velos mass spectrometer from Thermo Electron Corporation. Instrument parameters were as follows: electrospray voltage = 2.3 kV, capillary temperature = 230 °C, and flow rate = 0.7 μL/min. A concentrated ZF solution was prepared in water (450 μM), and 1 equiv of Au(III) compound was added. Samples were diluted as follows for analysis by mass spectrometry: 20 μL of sample, 20 μL of methanol, and 200 μL of 20 μM acetic acid.

RESULTS AND DISCUSSION

Reactivity with Biomolecules. Competition assays of the DNA−ethidium bromide interaction showed little fluorescent quenching of the intercalator in the presence of Au(III) compounds (Figure S1, Supporting Information), confirming that DNA is unlikely to be the principal target of these compounds.[24] $[Au(dien)(4\text{-picoline})]^{3+}$ appears to slightly bind to DNA, resulting in a loss of fluorescence intensity. On the basis of these overall results, little competitive binding between the drug and DNA is likely, which is a necessity if we are to target zinc finger proteins.

The soft nature of Au(III) predicts reactivity with sulfur-containing amino acids. Reactions of $[AuCl_4]^-$ with L-Met and L-Cys, and the influence of tridentate N-donors varying in π-donor ability on the stability of the Au(III) oxidation state, have been reported.[25,26] The dien chelate affords the least reactive compounds of the N-donors studied. Given that MN_4 compounds stabilize the Au(III) oxidation state more than $AuClN_3$ compounds,[18] we compared the reactivity of the AuN_4 species with both NAcMet and NAcCys to verify if this stabilization would further retard the reaction with sulfur-containing amino acids.

The reaction of $[AuCl_4]^-$ with L-Met results in oxidation of the thioether and concomitant Au(III) → Au(I) reduction.[25,26] Indeed, the oxidation of methionine to methionine sulfoxide has been shown to be stereospecific.[27] Little or no reaction has been reported for $[AuCl(dien)]^{2+}$ and L-Met based on UV−vis

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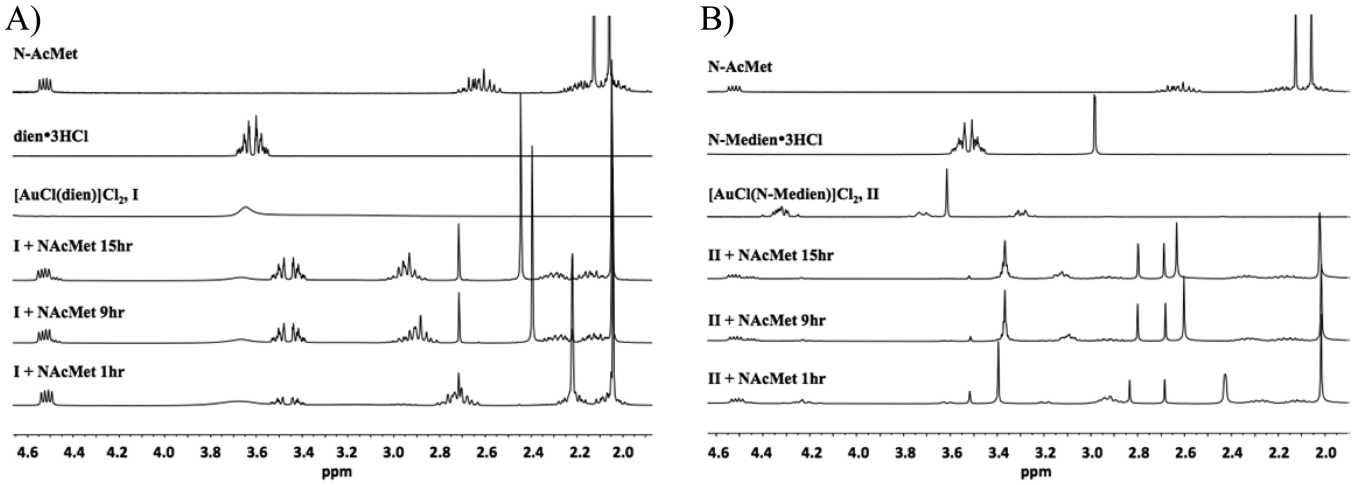

Figure 2. NMR spectra of (A) $[AuCl(dien)]Cl_2$ and (B) $[AuCl(N\text{-}Medien)]Cl_2$ reacted with 2.5 equiv of NAcMet.

spectra.[25] In this study, we used a 2.5-fold excess of NAcMet, and ^{1}H NMR spectral changes were noticed over time (Figure 2). The initial pH of the solutions was ∼3.0. Clear downfield shifts of the S-CH_3 H_a and S-CH_2 H_b protons indicate binding of NAcMet to gold (Figure 2). Multiple peaks appear for the S-CH_3 singlet shifted downfield from the free ligand at 2.13 ppm. In the case of $[AuCl(dien)]^{2+}$, two major new singlet peaks arise at 2.45 and 2.72 ppm, which increase slightly in intensity over time. The S-CH_2 (b) protons (Figure 1) appear as a multiplet, which are also deshielded upon metalation. The dien ligand dissociates as shown by the loss of the broad singlet at 3.65 ppm indicative of $[AuCl(dien)]^{2+}$ and the appearance of a multiplet centered at ∼3.46 ppm for free dien (Figure 2).[18,28] Selected time points are shown in Figure 2, and a detailed time course is shown in Figure S2 of the Supporting Information. For $[AuCl(N\text{-}Medien)]^+$, the situation is somewhat different with up to three S-CH_3 species clearly distinguishable (Figure 2B). In this case, the singlet of the N-Medien ligand undergoes an upfield shift to ∼3.4 ppm, which is consistent with the formation of Au−Met trans to the Au−N-Me bond. No evidence for the production of free N-Medien was seen throughout the reaction; however, it is possible that the primary amines on N-Medien are deprotonated allowing further ligand substitution, as noted previously in the solution behavior of $[AuCl(N\text{-}Medien)]^{2+}$.[18] It is noteworthy that in excess NAcMet (2.5 equiv), all free S-ligand disappears for both cases, suggesting that the S-CH_3 peaks may reflect the presence of oxidized methionine sulfone or sulfoxide as well as those of Au-SCH_3.[25] Conversion of one Met to Met═O and coordination of the remaining Met to the Au(I) center formed by the reduction reaction could explain the absence of free methionine even when used in excess. The details of these reactions, especially for the N-Medien derivative, are worthy of further detailed investigation.

NMR studies showed that the presence of the more inert N-donor ligands 9-EtG and DMAP results in reduced reactivity with the absence of downfield shifts for the S-CH_3 protons, indicating no significant reaction with NAcMet. Further, no evidence for significant loss of the dien ligand or release of the N-heterocycle is observed (Figure S3, Supporting Information). For $[Au(N\text{-}Medien)(DMAP)]^{3+}$, small peaks corresponding to NAcMet substitution can be observed after 15 h.

The thiol-containing amino acid L-cysteine quickly reduces Au(III) in aqueous solution with the reaction being complete within the first minute.[25,26] The initial rapid substitution reaction results in an unstable Au(III)-S complex that is reduced to Au(I/0) in a slower electron-transfer process.[25,29] The addition of a tridentate N-donor ligand such as dien somewhat stabilizes the Au(III) metal center,[30] and addition of the more inert N-donor leaving group is expected to stabilize it even further.[18] Both $Au(III)ClN_3$ compounds are very reactive with NAcCys compared to NAcMet due to the greater nucleophilicity of thiol(ate). The reactions are similar, resulting in loss of dien/N-Medien ligands, and precipitation of white solids as has been observed previously[29] (Figure S4, Supporting Information). The final pH of all reaction mixtures upon addition of NAcCys is ∼3. For AuN_4 compounds, the dien ligand also dissociates, but there is no evidence for concomitant release of the DMAP N-heterocycle ligand indicated by a lack of ligand peak shifts in the NMR spectra (Figure 3). The broad peak at ∼3.6 ppm (Figure 3) represents the dien protons when bound to Au. This broad peak decreases over time, whereas the multiplet at 3.4 ppm (free dien) increases, again showing loss of the chelate over time. In contrast, as shown in Figure 3, the DMAP ligand does not dissociate over time; the presence of free DMAP would be indicated by peaks at 8.15 and 6.7 ppm. This situation is in contrast to that of Au(I), in which the reaction of $[Au(I)(PPh_3)(DMAP)]^+$ with 1 equiv of NAcCys results in DMAP displacement and formation of a P−Au−S coordination unit.[19]

Similar spectra are seen for 9-EtG. The pH of the $[Au(dien)(9\text{-}EtG)]^{3+}$ solution increases from 2.7 to 3.0 upon addition of NAcCys. The H8 peaks of the 9-EtG ligand shift upfield upon addition of NAcCys as a result of this pH change (Figure S5, Supporting Information). This shift is not due to ligand dissociation as the spectrum is identical to that of $[Au(dien)(9\text{-}EtG)]^{3+}$ alone at pH ∼3.0.[18] Despite the

Table 1. ^{1}H Chemical Shifts of NAcMet and N-Me Protons upon Reaction with AuCl(dien) Species[a]

compound	S-CH_3 (ppm)	S-CH_2 (ppm)	N-Me (ppm)
I + NAcMet	2.45 (+0.32), 2.72 (+0.59)	2.93 (+0.31)	N/A
II + NAcMet	2.64 (+0.51), 2.69 (+0.24), 2.8 (+0.67)	2.93 (+0.31)	3.37 (−0.25)

[a]Values in parentheses refer to the change in the chemical shift from free, unreacted ligand (S-CH3 = 2.13 ppm, S-CH_2 = 2.62, and N-Me = 3.62 ppm).

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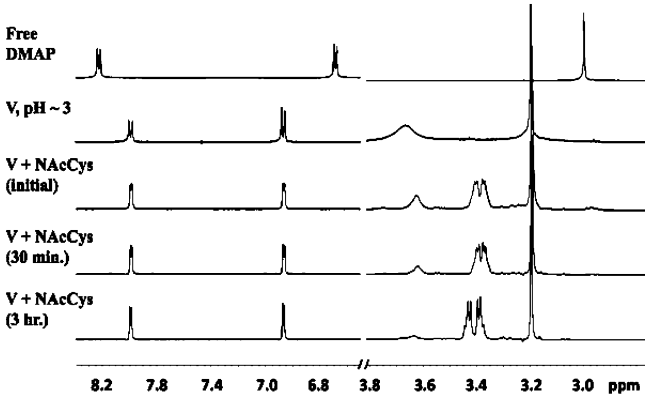

Figure 3. ^{1}H NMR spectra of $[Au(dien)(DMAP)]^{3+}$ **V** and NAcCys over time. Spectra of free DMAP and $[Au(dien)(DMAP)]^{3+}$ alone are shown for reference.

complexity of the reaction, it does appear that stabilization of the Au(III) oxidation state in $[Au(dien)(N\text{-heterocycle})]^{3+}$ extends to reactions with biomolecules, and overall, the results suggest that AuN_4 systems might be capable of fine-tuning to distinguish substitution from redox reactions.

Reactivity with Zinc Finger Proteins. The rationale for the design of the $[M(N_3)(nucleobase/N\text{-heterocycle})]^{n+}$ species is to allow for targeting of the essential tryptophan of the HIVNCp7 peptide.[12,17] Fluorescence studies using free NAcTrp show that Au(III) compounds exhibit a greater stacking interaction compared to that of their Pt(II) analogues (Table S1, Supporting Information).[18] Further, compounds containing DMAP as the N-heterocycle have K_a values significantly higher than those of their 9-EtG analogues, which reflects the greater basicity of the DMAP ligand.[12,17,18] N-donor planar amines are good σ-donor and π-acceptor groups, and the kinetics of L(N-heterocycle) substitution by Cl^- in $AuCl_3L + Cl^- \rightarrow [AuCl_4]^- + L$ shows a linear relationship between the basicity of the leaving group and the reactivity with respect to chloride substitution.[31] To examine this relationship with respect to stacking, we studied the complex $[Au(dien)(4\text{-picoline})]^{3+}$ because 4-picoline shows intermediate basicity with respect to DMAP and 9-EtG; literature values show that 4-picoline has a pK_a of 6.05, which is intermediate between the pK_a values of 2.7 and 9.1 for N7 of 9-EtG and DMAP, respectively.[32,33] The measured association constants for the Au(III) and Pt(II) 4-picoline compounds with NAcTrp are much lower than those of all other previous compounds studied, however, with the K_a of the free ligand being too low to determine. Nevertheless, there was a more than 2-fold increase in the K_a values for Au(III) compared to Pt(II) as similarly seen for the $[M(dien)(9\text{-EtG})]^{n+}$ compounds.[18]

Earlier mass spectrometric studies of the reaction of $[AuCl(dien)]Cl_2$ with NCp7(F2) show immediate formation of Au_2F and Au_4F species.[13] Upon reaction of $[AuCl(dien)]Cl_2$ or its N-heterocycle analogues with NCp7(F2), the fluorescence from the tryptophan residue decreased significantly over time with little difference between the "control" $[AuCl(dien)]^{2+}$ and the N-heterocycle ligands (Figure 4A). Given this point, it is likely that the decrease in fluorescence represents loss of zinc and a change in peptide structure rather than quenching through π-π stacking, and therefore no association constants were calculated.

Circular dichroism spectroscopy experiments show that the Au(III) compounds resulted in some degree of zinc ejection and conformational change when reacted with the C-terminal finger of the NCp7 zinc finger protein. The CD spectrum of NCp7(F2) is characterized by a positive band at ~220 nm and a negative band at 195−200 nm.[13] The Au compounds cause a decrease in intensity of the positive band and a significant increase in negative ellipticity, with a slight blue shift of the 195−200 nm band, which is indicative of a conformational change from ordered structure to random coil.[21−23] After a 15 min incubation, $[AuCl(dien)]^{2+}$ resembles the free peptide spectrum. $[Au(dien)(9\text{-EtG})]^{3+}$ and $[Au(dien)(DMAP)]^{3+}$ both result in loss of ordered structure, though to a lesser degree than $[AuCl(dien)]^{2+}$ (Figure 4B). After a 1 h incubation, the spectrum of $[Au(dien)(9\text{-EtG})]^{3+}$ + NCp7(F2) resembles that of the free peptide; however, the spectrum of $[Au(dien)(DMAP)]^{3+}$ + NCp7(F2) does not change to the same extent with a clear shoulder appearing at <200 nm, indicating that $[Au(dien)(DMAP)]^{3+}$ may be the least reactive of the three gold compounds studied.

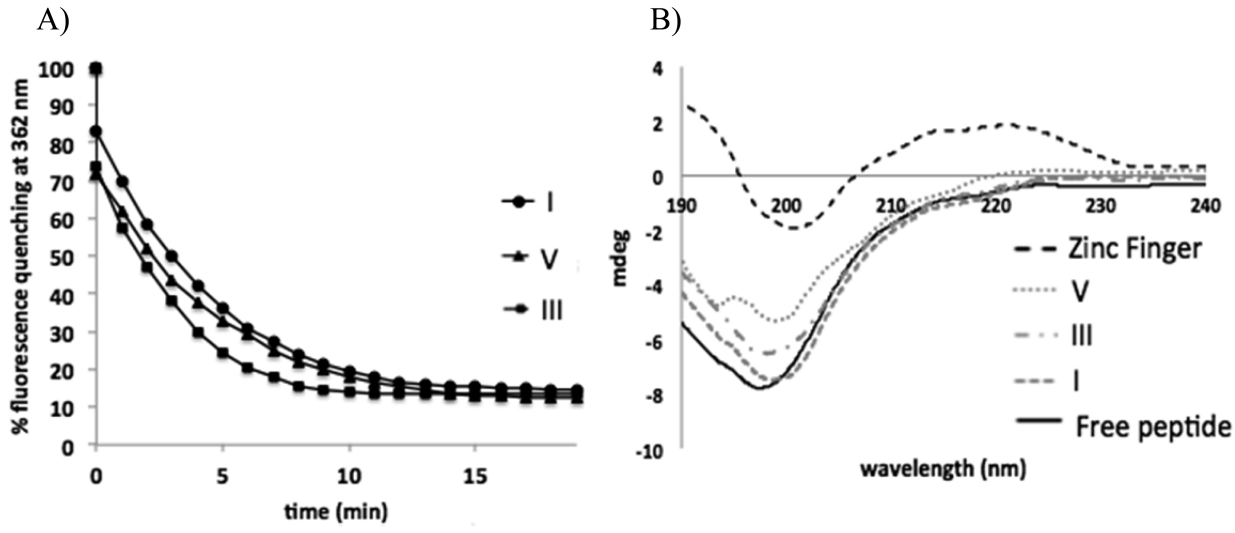

Figure 4. (A) Fluorescence quenching over time upon addition of $[AuCl(dien)]Cl_2$ **I**, $[Au(dien)(9\text{-EtG})]^{3+}$ **III**, and $[Au(dien)(DMAP)]^{3+}$ **V**. (B) CD spectrum of **I**, **III**, and **V** with NCp7(F2) after a 15 min incubation.

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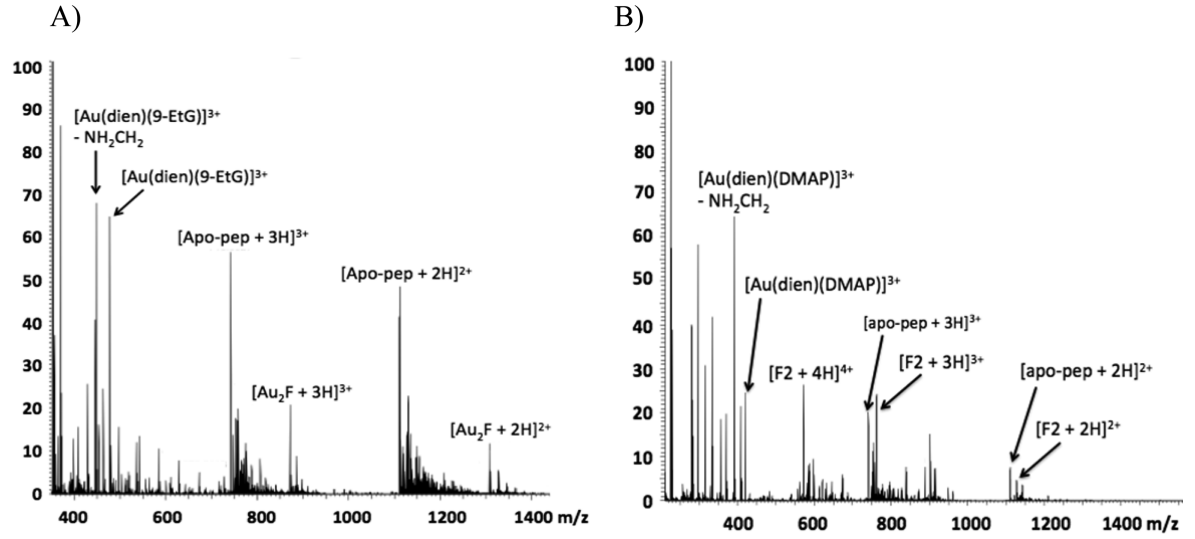

Figure 5. Initial ESI-MS spectra of (A) $[Au(dien)(9\text{-EtG})]^{3+}$ and (B) $[Au(dien)(DMAP)]^{3+}$ with NCp7(F2).

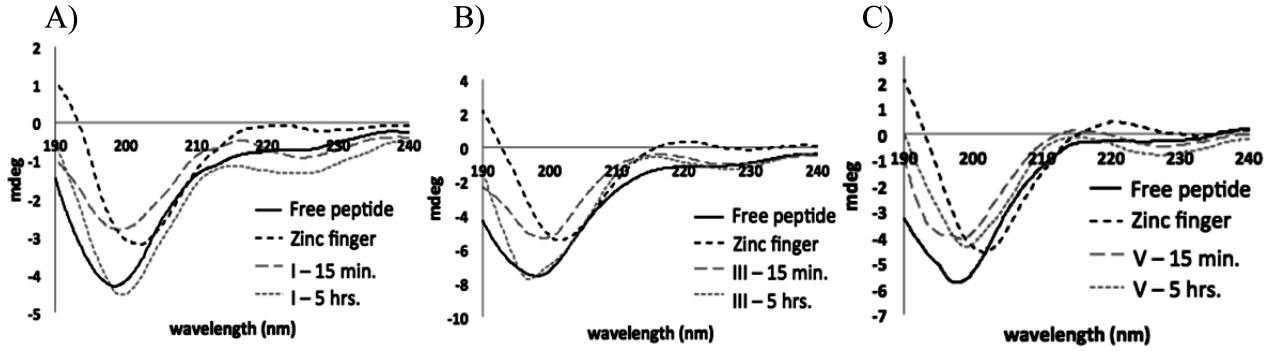

Figure 6. CD spectra of (A) $[AuCl(dien)]^{2+}$ **I**, (B) $[Au(dien)(9\text{-EtG})]^{3+}$ **III**, and (C) $[Au(dien)(DMAP)]^{3+}$ **V** with Sp1(F3) at 15 min, 1 h, and 5 h incubation times.

As determined by mass spectrometry, the ESI-MS spectrum of $[AuCl(dien)]^{2+}$ + NCp7(F2) shows loss of ligand and complete zinc ejection immediately upon incubation with incorporation of two and four Au ions into the peptide.[13] ESI-MS spectra of both $[Au(dien)(9\text{-EtG})]^{3+}$ and $[Au(dien)(DMAP)]^{3+}$ (Figure 5) show the presence of Au(III) starting material at initial time points. The compounds themselves undergo fragmentation (corresponding to an $-NH_2CH_2-$ group) resulting in partial loss of the dien ligand. This feature was also observed in the recorded ESI-MS spectrum of the compounds alone. The most prominent peaks for the $[Au(dien)(9\text{-EtG})]^{3+}$ + NCp7(F2) reaction are 740.9927 (3+) and 1110.9861 m/z (2+), which correspond to apo-peptide. Smaller peaks at 872.3034 (3+) and 1307.9520 m/z (2+) represent Au_2F. Therefore, like $[AuCl(dien)]$, $[Au(dien)(9\text{-EtG})]^{3+}$ results in the formation of gold fingers, but incorporation of four Au ions was not observed. The ESI-MS spectrum of $[Au(dien)(DMAP)]^{3+}$ + NCp7(F2), however, shows peaks corresponding primarily to an intact zinc finger at 571.7282 (4+) and 762.6356 m/z (3+). There is some reaction accompanied by zinc ejection, as indicated by a peak at 1111.4963 m/z (2+), which represents oxidized apo-peptide. At 4 h reaction time, the starting-material zinc finger peaks are no longer present. The spectra are difficult to interpret at later time points; however, it does not appear that Au_2F is present, as is the case for the 9-EtG analogue. Nevertheless, the results are consistent with the CD data, indicating that $[Au(dien)(DMAP)]^{3+}$ results in slightly less reaction than $[Au(dien)(9\text{-EtG})]^{3+}$ and certainly less than $[AuCl(dien)]Cl_2$.

Zinc finger cysteinates vary in chemical reactivity; the Zn-Cys49 thiolate of NC is considered one of the most susceptible to electrophilic attack.[34,35] The Cys_2His_2 zinc finger motif is less nucleophilic, and therefore, it is expected that it would be less reactive toward the Au(III) compounds.[34,35] To examine this point further, we compared the reactivity of ZFNCp7(F2) with that of Sp1(F3), the third zinc finger of the Sp1 transcription factor. As indicated by CD spectroscopy (Figure 6), there is a difference in reactivity between the Cys_3His and Cys_2His_2 ZFs, the latter taking ∼5 h to result in complete loss of ordered structure, in contrast to the 15 min required for Cys_3His(F2) (Figure 4B). $[AuCl(dien)]Cl_2$ and $[Au(dien)(9\text{-EtG})]^{3+}$ appear to react similarly, resulting in complete Zn ejection and loss of structure at 5 h. Further, the early time point indicated production of apo-peptide followed by a further slower reaction resulting in gold-peptide formation.[36,37] $[Au(dien)(DMAP)]^{3+}$ again appears to have less reactivity than $[AuCl(dien)]Cl_2$ or $[Au(dien)(9\text{-EtG})]^{3+}$. The typical free peptide spectrum with an increase in negative ellipticity is not seen in this case, and though a slight blue shift occurs at ∼200 nm compared to that of the zinc finger, the peak does not significantly diminish in intensity over time. However, upon further examination, the CD spectrum in Figure 6C is similar to that of oxidized Cys_2His_2 peptide as reported by Franzman et al.[36] This suggests that zinc is being ejected, and disulfide bonds are being formed.

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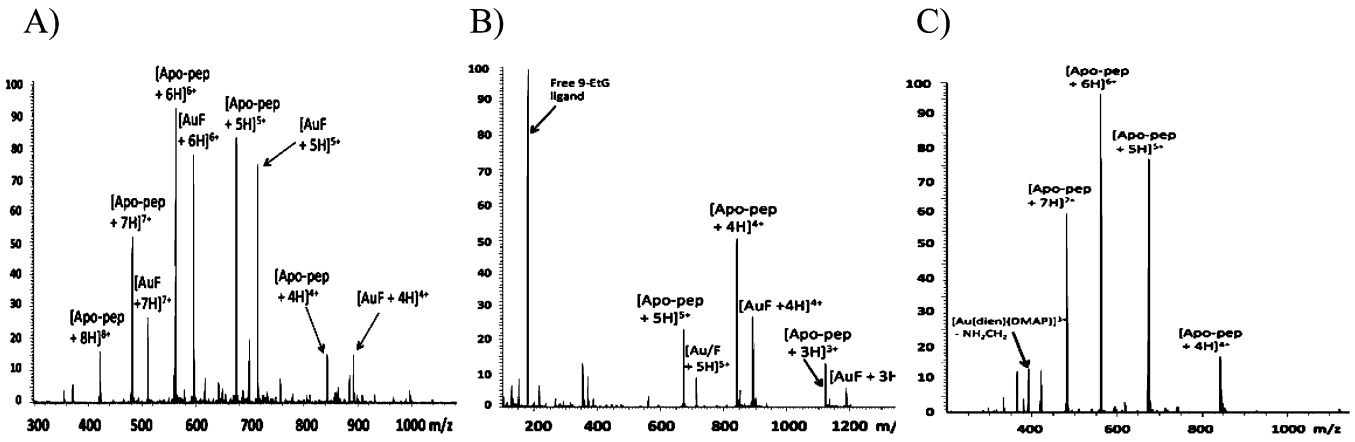

Figure 7. ESI-MS spectra of (A) $[AuCl(dien)]^{2+}$ **I**, (B) $[Au(dien)(9\text{-EtG})]^{3+}$ **III**, and (C) $[Au(dien)(DMAP)]^{3+}$ **V** with Sp1(F3) at initial time points.

After 24 h, the final spectra for all three compound species are similar.

The ESI-MS spectrum of $[AuCl(dien)]^{2+}$ and Sp1(F3) shows prominent peaks for apo-peptide (674.3635 m/z, 5+) and AuF (713.7566 m/z, 5+) (Figure 7A). Differences relative to the reaction with NCp7(F2) are that incorporation of more than one gold ion was not observed initially, though small peaks representing Au_2F appeared after 1 h of incubation, and that there was no evidence for intact $[Au(dien)(9\text{-EtG})]^{3+}$ at early time points (free 9-EtG ligand was observed instead). Immediately following addition of the compound, the prominent peaks in the mass spectrum are apo-peptide (842.4415 m/z, 4+) and AuF (891.9345 m/z, 4+) (Figure 7B). Over time, the AuF peaks increase in intensity; however, Au_2F is not present as seen for $[AuCl(dien)]^{2+}$. Unlike both the −Cl and −9EtG species, the Au-DMAP species does not result in immediate formation of AuF. The initial ESI-MS spectrum shows a small peak at 391.1222 m/z, indicating a fragment of $[Au(dien)(DMAP)]^{3+}$ with loss of $-CH_2NH_2$ from the dien ligand. Other peaks present represent apo-peptide (Figure 7C). The peak at 561.9996 m/z (6+) refers to the mass of apo-peptide minus two protons, indicating the formation of a disulfide bond, which agrees with the CD results in Figure 6C. After 1 h, AuF begins to appear at 713.3511 m/z (5+) and increases in intensity over 24 h; however, oxidized apo-peptide remains the prominent species. An equimolar reaction of the Au(I) drug aurothiomalate and preformed Sp1 zinc finger (excess Zn present) resulted in 60% AuF and 40% zinc finger.[23] Similar to our results, binding of more than one metal ion was not observed. $[Au(dien)(DMAP)]^{3+}$ appears to be the least reactive of the Au(III) compounds studied for both the Cys_2His_2 and Cys_3His zinc finger proteins. Though zinc ejection and formation of gold fingers still occur, the extent of the reaction was decreased with the presence of DMAP as the putative leaving group.

Upon binding to the peptide, the gold compound formed may formally contain Au(III) with preferred square-planar geometry or Au(I) with preferred linear geometry.[36,37] Table 2 shows the expected and observed m/z values for the reaction of the Au(III) compounds with Sp1(F3). To calculate the expected values, we assumed that Au would bind in a 4-coordinate geometry similar to that of Zn^{2+}.[37,38] When the expected and observed m/z values are compared, they are found to be within 0.1 m/z of their expected value, suggesting that when bound to the Cys_2His_2 zinc finger (Sp1(F3)), Au remains in the 3+ charge state. CD spectra of AuF formed from the reaction of aurothiomalate (Au^{1+}) and Sp1 showed a slight red shift of the minimum, indicating a difference in conformational features, such as possibly a less α-helical nature.[23] On the basis of the difference in CD spectra between the Au(III) compounds studied here (large increase in negative ellipticity) and various Au(I) compounds in the literature (decrease in negative ellipticity),[19,23] it is likely that Au(III) binding results in conformational changes different from those of either Au(I) or Zn(II). This change in conformation can then presumably result in inhibition of DNA binding and therefore loss of function. Goldaurothiomale (AuTM) interaction with a TFIIIA zinc finger transcription factor can inhibit the transcription factor's binding to DNA in the presence of high levels of thiol.[23]

Table 2. Observed and Expected Relative Intensity Values for AuF in the Reaction of Au(III) Compounds with Sp1(F3)

charge state	observed relative intensity (m/z)	expected relative intensity (m/z)[a]	Δ
3+	1188.255	1188.24	0.015
4+	891.432	891.430	0.002
5+	713.347	713.344	0.003
6+	594.624	594.620	0.004
7+	509.679	509.817	−0.138

[a]Calculated values assume Au binds in the same 4-coordinate number as Zn^{2+}.

It is slightly more complicated to determine how the Au atoms are binding in the Cys_3His zinc finger NCp7(F2) due to the fact that multiple gold ions bind. Expected values match most closely to the observed values when the first gold is assumed to bind as 3+, and the second gold is assumed to bind as Au(I) with concomitant loss of a proton (Table 3). This situation is similar to that observed for the reaction of Au(III) compounds with the Cys_3His zinc finger present in PARP-1.[37,38]

CONCLUSIONS

The Au(III) compounds studied are the first isolated Au(III)−dien−N-heterocycle compounds. We have recently shown the effect of the nature of the N-heterocycle on the acidity of the Au-dien ligand, the stabilization of the Au(III) metal center, and the π-stacking ability with free *N*-acetyltryptophan

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Table 3. Observed and Expected Relative Intensity Values for Au_2F in the Reaction of Au(III) Compounds with NCp7(F2)

charge state	observed relative intensity (m/z)	expected relative intensity (m/z)[a]	Δ
2+	1307.952	1307.964	−0.012
3+	872.303	872.309	−0.006

[a]Calculated values assume Au binds in the same 4-coordinate number as Zn^{2+}.

compared to that of their Pt(II) analogues.[18] Expanding on that work, we report here the reactivity of these compounds with model amino acids and Cys_3His and Cys_2His_2 zinc finger protein cores. NMR studies show a decreased reaction rate for AuN_4 species with both NAcMet and NAcCys compared to that of $AuClN_3$ species. Upon reaction with NAcCys, the dien ligand completely dissociates, whereas N-Medien dissociates more slowly. The N-heterocycle ligand remains bound, suggesting formation of an N-heterocycle−Au−S species. This is in contrast to the reaction of $[Au(I)(PPh_3)(N\text{-heterocycle})]^+$ compounds with NAcCys, which resulted in loss of the N-heterocycle ligand and formation of a P−Au−S species.[19]

Many studies have been reported on the reaction of Au(I)/Au(III) compounds with various zinc finger proteins. Such reactions uniformly result in rapid displacement of Zn^{2+} with concomitant replacement of Zn^{2+} by Au(I)/(III) in which all gold-coordinated ligands are lost.[13,23,36,37] The Au(III) drugs discussed herein were designed to selectively target the NCp7 ZF through π-stacking interactions with the planar amino acid tryptophan. Though fluorescence experiments show a significant enhancement of π-stacking with free NAcTrp for Au(III) compounds over that of their Pt(II) analogues, studies with NCp7(F2) suggested rapid zinc ejection. The initial mode of recognition may result from the noncovalent interaction of the N-heterocycle ligand with the tryptophan residue; however, the reaction is still too fast to observe any intermediate species.

In agreement with reactions with model amino acids, ESI-MS and CD experiments with zinc finger proteins also showed a decrease in the rate of reactivity for AuN_4 species, albeit with overall profiles similar to that of the "parent" $[AuCl(dien)]^{2+}$ species. CD and ESI-MS studies showed $[Au(dien)(DMAP)]^{3+}$ to be the least reactive, with evidence of intact zinc finger present at early time points, and CD spectra indicating that the Cys_2His_2 zinc finger Sp1(F3) reacted slower than the Cys_3His zinc finger NCp7(F2). Further, the nuclearity (Au_nF) of the gold clusters formed is dependent on both the nature of the Au(III) compound and the nature of the zinc finger. No evidence for the formation of $[Zn(dien)X]^{n+}$ (X = Cl or H_2O) was seen in any spectra, in agreement with previous studies.[13] Previous pH dependency studies showed $[Au(dien)(DMAP)]^{3+}$ to be the most stable of the Au(III)−N-heterocycle compounds studied with the DMAP ligand stabilizing the dien ligand, and the ligand itself does not dissociate at low or high pH values.[18] The studies reported herein overall support the hypothesis that Au(III) compound reactivity, especially that of the $Au(III)N_4$ chemotype, can be fine-tuned to result in slower substitution reactions on biomolecules with concomitant opportunities for selectivity between different ZF coordination spheres. Further efforts to produce Au(III) compounds that are more stable under physiological conditions are warranted.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of $[Au(dien)(N\text{-heterocycle})]^{3+}$ interactions with small amino acids, DNA binding assays, and a table of tryptophan-association constants. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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