Bioorthogonal Chemistry

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An Alkyne-Appended, Click-Ready Pt^{II} Complex with an Unusual Arrangement in the Solid State**

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Abstract: *To better understand the range of cellular interactions of Pt^{II}-based chemotherapeutics, robust and efficient methods to track and analyze Pt targets are needed. A powerful approach is to functionalize Pt^{II} compounds with alkyne or azide moieties for post-treatment conjugation through the azide–alkyne cycloaddition (click) reaction. Herein, we report an alkyne-appended cis-diamine Pt^{II} compound, cis-[Pt(2-(5-hexynyl)amido-1,3-propanediamine)Cl_2] (**1**), the X-ray crystal structure of which exhibits a combination of unusual radially distributed CH/π(C≡C) interactions, Pt–Pt bonding, and NH:O/NH:Cl hydrogen bonds. In solution, **1** exhibits no Pt–alkyne interactions and binds readily to DNA. Subsequent click reactivity with nonfluorescent dansyl azide results in a 70-fold fluorescence increase. This result demonstrates the potential for this new class of alkyne-modified Pt compound for the comprehensive detection and isolation of Pt-bound biomolecules.*

Platinum(II) chemotherapeutics are used ubiquitously in cancer treatments today. An estimated 50–70% of all regimes include one of the first- and second-generation FDA-approved Pt-based drugs cisplatin, carboplatin, and oxaliplatin.[1] Drug binding to the purine bases of DNA is an accepted cause of anticancer activity; however, the effects of binding to alternative cellular targets remain difficult to study and poorly understood.[2] Indeed, less than 10% of the Pt (in the case of cisplatin) accumulates within genomic DNA.[3] It is known that Pt also binds to RNA, proteins, and other small molecules (e.g., glutathione) in cells; however, the downstream consequences of Pt binding to these alternative targets are far less understood, especially with respect to how they relate to cytotoxicity and drug resistance.[4] Novel methods of analyzing alternative Pt binding and target interactions are needed to better understand and thus design Pt drugs and drug treatments.

To broadly study cellular Pt interactions, we aim to functionalize Pt complexes with bioorthogonally reactive handles. Two azide-functionalized Pt^{II} compounds have recently been described. Our group has reported the synthesis and utility of an azide-functionalized picoplatin derivative for reaction through copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC).[5] Furthermore, an extended acridine-functionalized, monofunctional Pt^{II} compound has been used in fluorescence post-labeling experiments.[6] While the utility of these Pt-azide reagents is clearly demonstrated, Pt-alkyne reagents capable of click chemistry would provide additional advantages. These include reduced hydrophilicity (vs. azide analogues) and access to complementary azide-modified partner reagents. Bifunctional Pt^{II} compounds with accessible alkyne modifications are, however, rare.

As an alternative to azide-functionalized Pt analogues, we present herein a Pt-alkyne species for use with "turn-on" azide-containing fluorophores (Figure 1). We report the synthesis and characterization of alkyne-functionalized Pt complex **1** and demonstrate its click reactivity with the "turn-on" dansyl azide fluorophore in a model Pt-binding and CuAAC click reaction. Additionally, X-ray structure analysis of **1** reveals a remarkable arrangement within the crystal lattice that contains both CH/π(C≡C) and Pt–Pt bonding interactions. The availability of Pt-alkyne reagents permits the future use of click conjugation to a variety of azide-modified molecular probes for Pt target elucidation.

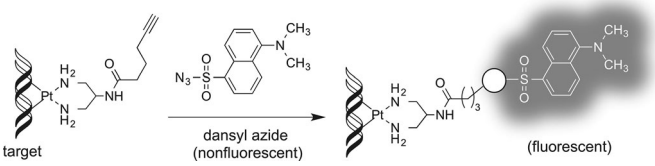

Figure 1. Target DNA bound by compound **1** undergoing click ligation with the "turn-on" fluorophore dansyl azide.

Compound **1** was designed to mimic the difunctional reactivity and *cis* geometry of cisplatin. Reduction of *tert*-butyl 2-azidopropane-1,3-diyldicarbamate with tin(II) chloride yielded the Boc-protected amine,[7] which was treated with 5-hexynoic acid to form the peptide-coupled product (Scheme 1). The Boc-protected amide was reacted with *cis*-[$Pt(DMSO)_2Cl_2$] after deprotection to afford **1**. Crystals were deposited from the crude reaction mixture upon the addition

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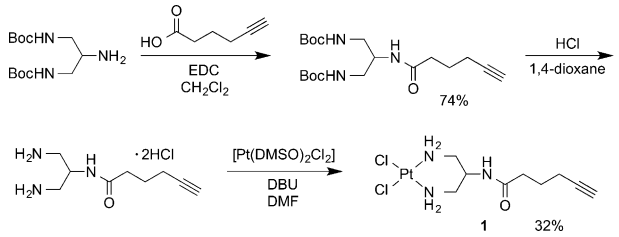

Scheme 1. Synthesis of Pt^{II} complex **1**. Boc = *tert*-butoxycarbonyl, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, DMSO = dimethyl sulfoxide, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DMF = *N*,*N*-dimethylformamide.

of water (see the Supporting Information). While alkyne–Pt complexes comprise a diverse, well-known field, Pt^{II} complexes in which a terminal alkyne moiety is attached through organic linkers and not directly bound to the Pt metal are far less common. These usually incorporate bulkier aromatic or exchange-inert ligands around the Pt coordination sphere to discourage Pt–alkyne interactions.[8] De Sousa et al. reported a moderately unhindered propargyl-containing chloroquinoline derivative coordinated to Pt^{II}.[9] By contrast, Parker and co-workers reported on the incompatibility of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ with a small alkyne-containing *O*,*O*′-bidentate ligand in aqueous solution.[10] It was assumed that the insoluble brown solid that formed was a result of Pt^{II}-catalyzed polymerization reactions with the alkyne. In the case of **1**, formation of the desired complex through a route avoiding the highly reactive Pt–H_2O-containing species was desired and proved successful. Despite concerns that **1** might yet undergo unwanted side reactions in solution, such as Pt-catalyzed hydration or hydroamination across the triple bond, no such reactivity of **1** was observed over several hours (in d_7-DMF) by ^1H, ^{13}C and ^{195}Pt NMR spectroscopy (see the Supporting Information).

The X-ray crystal structure of **1** reveals a network of six CH/π(C≡C) bonds with the terminal alkyne group acting as both hydrogen-bond donor and hydrogen-bond acceptor (Figure 2).[11,12] It has been shown that the acidity of the terminal alkyne proton and electron-rich π orbitals of the alkyne group give rise to a relatively weak hydrogen-bonding interaction. In the case of **1**, the CH/π(C≡C) hydrogen bonds form a radially symmetric wheel or spoke-type arrangement, with alkyne–hydrogen distances of 2.83 Å and bond angles of less than 180°, which are within the range of previously reported values for CH/π(C≡C) interactions.[13,14] While Steiner, Boese, and others have reported several "zig-zag" formations in the solid state,[13c,14] a cyclic arrangement of CH/π(C≡C) interactions has rarely been described.[13] The crystal structure of **1** also includes a Pt–Pt distance of 3.34 Å, which is indicative of Pt–Pt orbital overlap.[15] Hydrogen bonds involving the carbonyl oxygen and halide acceptors with the amine ligand donors are also observed. In all, three very different types of intermolecular interactions in the crystal structure of **1** provide for an unusual solid-state arrangement.

To confirm its target binding and subsequent click reactivity, **1** was bound to a 5′-GG-3′ DNA hairpin sequence, followed by click conjugation with the dansyl azide fluorophore (Figure 3). A suspension of **1** in 20% *v*/*v* DMF was added to folded hairpin DNA in two-fold molar excess and allowed to react at 37 °C for 18 h. After removal of unbound **1** with Sephadex G25 resin, the **1**-bound DNA was reacted with dansyl azide (1 equiv) with $CuSO_4$ and sodium ascorbate in 0.6% *v*/*v* CH_3CN. Fluorescence of the **1**-bound DNA strand is revealed only upon click conjugation, since unreacted dansyl azide is nonfluorescent.[16] Despite widespread use of the dansyl group in fluorescent labeling and detection methods,[17] to our knowledge, the ability of dansyl azide to become a "turn-on" fluorophore upon azide–alkyne cycloaddition has not been reported. This turn-on fluorescence capability of **1** with azide-containing fluorophores demonstrates its potential utility for cellular localization studies, as well as other imaging studies.

While the usual click reaction between azides and alkynes produces the thermodynamically stable 1,2,3-triazole, click reactions with sulfonyl azides may produce additional products upon subsequent rearrangement and elimination of N_2. Upon Cu-catalyzed formation of the sulfonyl triazole, rearrangement and elimination of N_2 may lead to the formation of stable *N*-sulfonyl amides.[18] Owing to the reduced stability of *N*-sulfonyl triazoles and the use of aqueous $CuSO_4$ as the catalytic Cu source,[18c] we hypothesize that the product formed in the reaction between **1**-bound DNA and dansyl azide is the *N*-sulfonyl amide conjugated species. To confirm the fluorescent properties of the *N*-sulfonyl amide, dansyl *N*-sulfonyl amide was synthesized through click ligation with

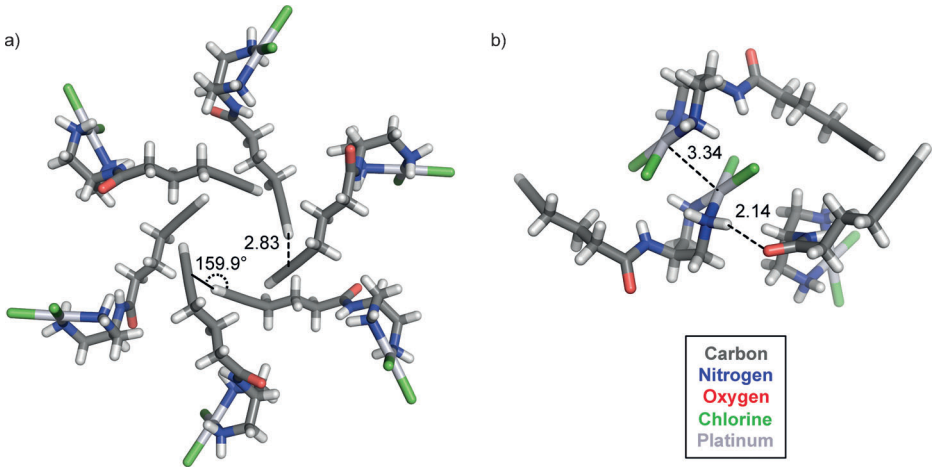

Figure 2. a) Crystal structure arrangement of **1** showing the radial distribution of alkyne "tails." b) An alternate view of the crystal structure showing Pt–Pt stacking. Selected bond lengths (Å) and angles (°): Pt–Cl 2.3137(11), 2.3028(9); Pt–N 2.042(3), 2.047(3); C≡C 1.174(9); Pt···Pt 3.3367(5), H(N)···O (as shown) 2.14(3); C–C≡C 178.0(8). NH:O/NH:Cl hydrogen bonds are also present (not shown). See the Supporting Information and deposited structure[11] for detailed crystallographic information.

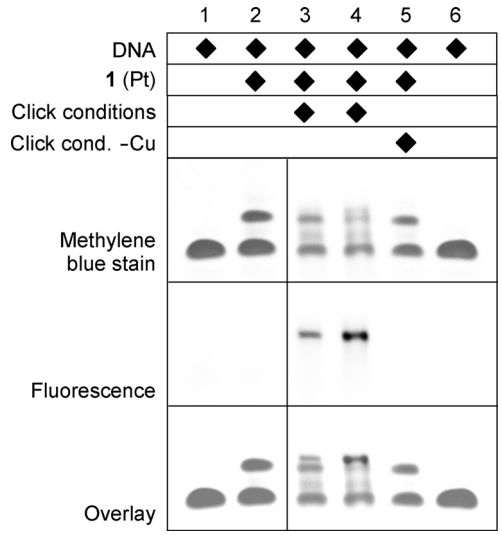

Figure 3. Polyacrylamide gel electrophoresis (PAGE) experiment showing the binding of **1** to a DNA hairpin (Lane 2, methylene blue stain), followed by click conjugation to dansyl azide (Lanes 3 and 4, appearance of fluorescent band). No fluorescence is observed under the Cu-free conditions of Lane 5 (control). Click conditions: 90 mM Na_2HPO_4, pH 7.0, $CuSO_4$ (50 μM), dansyl azide (50 μM), and sodium ascorbate (200 μM), 2 h 50 °C (Lane 3) or 18 h RT (Lane 4). DNA = TATGG-TATTTTTATACCATA, typically 5 nmol (50 μM).

methyl propargyl ether in $CHCl_3$ with CuI as the catalyst, and characterized by ^1H and ^{13}C NMR, mass spectrometry, fluorescence spectroscopy, and UV/Vis spectroscopy (see the Supporting Information). Importantly, the dansyl *N*-sulfonyl amide exhibits a fluorescence quantum yield of 0.35 (60% *v*/*v* CH_3CN/H_2O, 40 mM TRIS buffer pH 8.0) upon reaction with methyl propargyl ether, an approximately 70-fold fluorescence increase over the nonfluorescent dansyl azide (Figure 4).

In summary, we report the unusual, bifunctional alkyne-derivatized Pt^{II} compound **1** for post-binding analysis of Pt-bound targets through click chemistry. The crystal structure of **1** exhibits CH/π(C≡C) interactions in a rare six-fold radially symmetrical chair-like arrangement, in addition to containing Pt–Pt stacking and multiple hydrogen-bonding interactions. In solution, **1** binds readily to DNA, showing target binding akin to known Pt drugs and drug analogues. Finally, DNA-bound **1** undergoes covalent ligation with dansyl azide, demonstrated here to be a convenient turn-on fluorophore upon alkyne–azide cycloaddition. Together, these experiments highlight the unique ability of **1** to report on the target binding and localization of Pt compounds, thus advancing high-throughput and comprehensive studies on the cellular distributions, pathways, and mechanisms of action of metallodrugs.

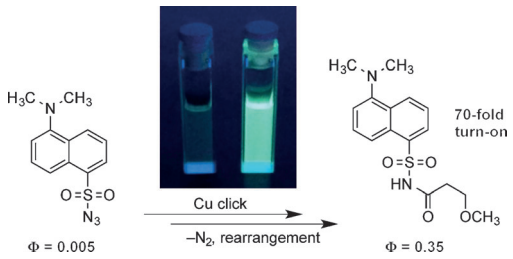

Figure 4. Solutions of dansyl azide and *N*-sulfonyl amide (0.20 mM, 60% *v*/*v* CH_3CN, 40 mM TRIS pH 8) formed upon click reaction with methyl propargyl ether, revealing an approximately 70-fold fluorescence increase.

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[11] X-ray data for **1**: $C_9H_{17}N_3Cl_2O_3Pt$, $M = 449.25$, $0.08 \times 0.04 \times 0.03$ mm, $T = 150$ K, Hexagonal, space group *R*-3, $a = b = 29.893(4)$ Å, $c = 7.6143(9)$ Å, $V = 5892.7(12)$ Å^3, $Z = 18$, $\rho_{calcd} = 2.279$ Mg m^{-3}, $\mu = 11.107$ mm^{-1}, $F(000) = 3816$, $2\theta_{max} = 56.00°$, 22294 reflections, 3158 independent reflections [$R_{int} = 0.0418$],

$R1 = 0.0221$, $wR2 = 0.0446$ and GOF = 1.051 for 3158 reflections (165 parameters) with $I > 2\,\sigma(I)$, $R1 = 0.0288$, $wR2 = 0.0467$ and GOF = 1.053 for all reflections, max/min residual electron density $+1.081/-1.228$ e Å^{-3}. CCDC 1012565 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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