# Synthesis and Properties of Diazirinyl Organo-Platinum Compounds for Manipulations of Photoaffinity Labeled Components

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**Synthesis of diazirinyl organo-platinum complexes, which specifically interact with purine base, characterization of photoreactivity and interaction between guanosine 5′-monophosphate (GMP) were examined. The results indicated that the diazirinyl organo-platinum complex was useful for manipulations of photoaffinity labeled components.**

**Key words** photoaffinity labeling; diazirine; organo-platinum compound

Photoaffinity labeling is a useful biochemical method used to reveal structural and functional relationships between low molecular weight bioactive compounds and biomolecules[1] and for this, various photophores, such as phenyldiazirine, arylazide and benzophenone, are used. Although, comparative irradiation studies of these three photophores in living cells have indicated that a carbene precursor 3-(trifluoromethyl)-3-phenyl-3*H*-diazirine is the most promising photophore,[2] despite this, the low cross-linking yield of photoaffinity labeling experiments still hampers the purification and isolation of labeled components.[3] The tags, which can utilize specific manipulations for isolation and/or detection of photolabeled components, are very important for the design of photoaffinity labeling reagents (Chart 1). We have attempted to resolve these difficulties by using the combinational introduction of a diazirinyl photophore and an avidin–biotin system (photoaffinity biotinylation).[4] Specific interaction between organo-platinum complexes (ex. cisplatin) and purine base has been used in molecular biology[5] and may utilize as tag for photoaffinity labeling. Although benzophenone containing photoreactive organo-platinum complex has been reported to reveal DNA-protein interaction,[6—9] but no detailed data has been reported for efficiency of complex formation between the photoreactive organo-platinum compound and DNA before and after irradiation. In our best knowledge, organo-platinum complex, which contains diazirinyl moiety, as a photophore, and efficiency of complex formation between purine base and photoactivated diazirinyl organo-platinum compound have not been reported. In this paper we described diazirinyl organo-platinum compound and their properties of interaction between guanosine 5′-monophosphate (GMP) as photoaffinity labeling tags to manipulate of photoaffinity labeled components.

Chlorine at organo-platinum compound plays an important role for interaction with purine base.[5] Platinum coordinated

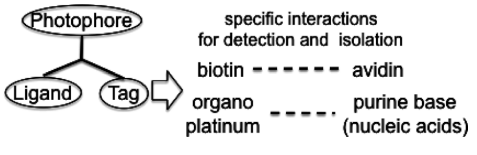


Chart 1. Schematic Illustration of Photoaffinity Labeling Reagents

*tert*-butyl 2,3-diaminopropylcarbamate **2** was selected as a mother skeleton which was synthesized from *tert*-butyl *N*-(2,3-dihydroxypropyl)carbamate **1** in a similar manner described by references.[10,11] After deprotection of *tert*-butoxycarbonyl (Boc) group with an acidic conditions, the amine derivative **3**[11] was reacted with benzoic acid *N*-succinimidyl ester (**4**) and diazirinylbenzoic acid *N*-succinimidyl esters (**5**,[12] **6**[13]) to afford desired products (**7**—**9**) in moderate yields (Chart 2).

The compounds **8** and **9** were subjected to the photoirradiation experiment. We have reported that diazo isomerization is not a serious side reaction for generation of carbene from diazirinyl compounds of less than 1 mM concentration.[14] Aqueous solutions of both **8** and **9** (55 $\mu$M) were irradiated with 15 W black light and reduction at around 350 nm adsorption was measured (Fig. 1). The half-life of each of the diazirinyl compounds was calculated as 3.5 and 2.7 min for **8** and **9**, respectively. Furthermore, a chemical shift for the trifluoromethyl group at an $^{19}$F-NMR measurement for both compounds caused a move from –67 ppm, which is the identical peak for the 3-(trifluoromethyl)-3*H*-diazirin-3-yl group, to –78 ppm after irradiation for 30 min.[15]

On the other hand, $^{195}$Pt-NMR measurement indicated the platinum still remained in the organo-platinum skeleton (–2270 ppm) after irradiation for 30 min. The results indicated that the 3-(trifluoromethyl)-3*H*-diazirin-3-yl organo-platinum derivatives easily generated carbene by black light irradiation without decomposition of organo-platinum skeleton.

The synthetic compounds were subjected to experiments of interaction with GMP as model studies to elucidate nucleic acid interaction in a similar manner described by references.[5] Organo-platinum compounds were interacted with 7 position of guanosine base preferentially. These interactions caused downfield shift of the proton at position 8 of guanosine base at $^1$H-NMR.[2] Integration of the moved H-8 proton (8.52 ppm) and the original H-8 (8.16 ppm) proton was calculated as a complex formation. In preliminary experiment compound **2** (2.5 $\mu$mol) and GMP (4 $\mu$mol) were suspended in 50 mM phosphate buffer (pH 7.0)–$D_2O$ (0.8 ml). The suspension did not become clear solution with incubation at 50 °C over 24 h and complex formation was calculated as

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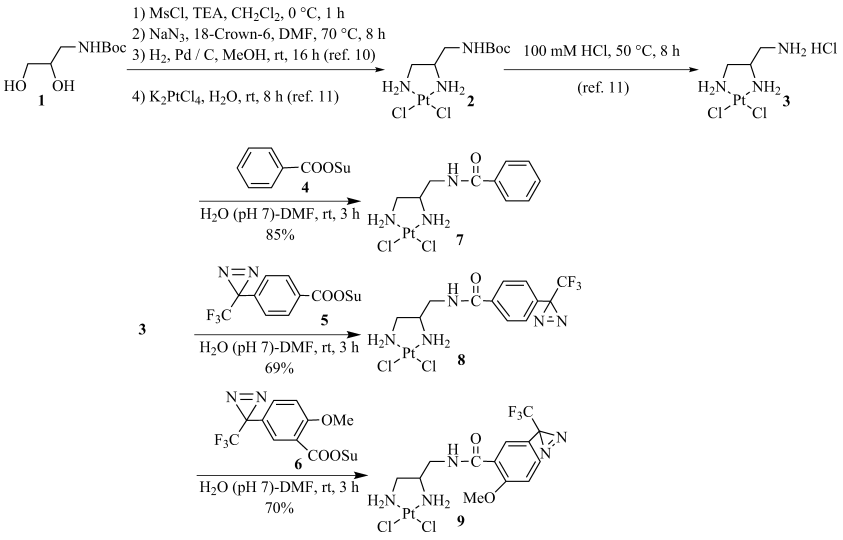


Chart 2. Synthesis of Organo-Platinum Derivatives

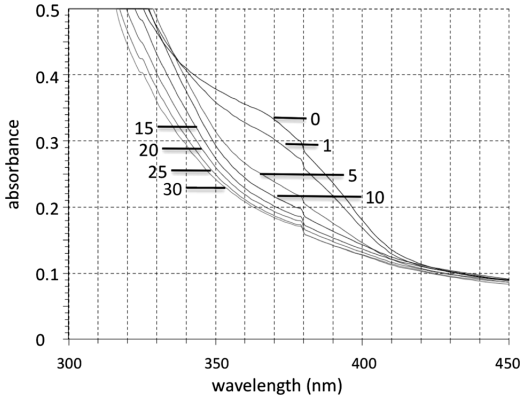


Fig. 1. Photolysis of 0.055 mM of **8** in Water with 15 W Black Light

An UV spectrum of the photolysis reaction mixture was recorded at times (in min), indicated by the numbers.

28%. To improve organo-platinum complex solubility in the assay, 0.2 ml of *N*,*N*-dimethylformamide (DMF)-$d_7$ was replaced a part of $D_2O$. The assay mixture became clear solution after incubation at 50 °C 24 h and complex formation was calculated as 44%. Incubation of the assay mixture more than 24 h did not improve for complex formation. The results indicated the solubility of the organo-platinum derivatives were important for the assay. The properties of compound **7** for complex formation with GMP was similar that of compound **2** (19 and 38% complex formation with and without DMF-$d_7$, respectively). Diazirinyl derivatives **8** and **9** were less soluble in the assay mixture. The complex formations were not observed without DMF and was increased proportional to amount of DMF-$d_7$. The assay mixtures for **8** and **9** became clear solution with 2-fold DMF-$d_7$ than for compound **2** and complex formations were calculated as 28 and 30% for **8** and **9**, respectively (Table 1). Although slightly less coordination properties to GMP than **2** and **7**, the diazirinyl organo-platinum compounds **8** and **9** can interact with GMP to form complex. The irradiated mixtures of compound **8** and **9** were also subjected to the assay and no differences were observed between before and after irradiation for complex formation of organo-platinum compounds and GMP. The results indicated the specific interactions between

Table 1. Interactions of Synthetic Organo-Platinum Compounds (**2**, **7**—**9**) with GMP

| Compound | $D_2O$ | DMF-$d_7$ | Complex formation (%) |
|---|---|---|---|
| **2** | 0.8 | 0 | 28 |
| | 0.6 | 0.2 | 44 |
| **7** | 0.8 | 0 | 19 |
| | 0.6 | 0.2 | 38 |
| **8** | 0.8 | 0 | 0 |
| | 0.7 | 0.1 | 10 |
| | 0.6 | 0.2 | 17 |
| | 0.4 | 0.4 | 28 |
| Irradiated | 0.4 | 0.4 | 25 |
| **9** | 0.8 | 0 | 0 |
| | 0.7 | 0.1 | 11 |
| | 0.6 | 0.2 | 18 |
| | 0.4 | 0.4 | 30 |
| Irradiated | 0.4 | 0.4 | 27 |

organo-platinum and purine base were slightly lower than avidin-biotin system, which can form the complex near quantitatively,[16] but it would be useful as a tag for diazirine-based photoaffinity labeling to manipulate labeled components from the photoirradiated mixtures.

## Experimental

$^1$H-, $^{13}$C- $^{19}$F- and $^{195}$Pt-NMR spectra were measured by JEOL ECA-500 spectrometers. UV spectra were obtained using Hitachi U-2800. All solvents were of reagent grade and distilled using the appropriate methods.

**(*N*$^1$-Benzoylpropane-1,2,3-triamine)dichloroplatinum(II) (7)** Dichloro[(2,3-diaminopropyl)ammonium] platinum(1+) chloride (**3**) was prepared from *tert*-butyl 2,3-dihydroxypropylcarbamate **1** by the reported procedure. *N*-Succinimidyl bezoate (**4**) (12.7 mg, 0.058 mmol) in DMF (2 ml) was added to the aqueous solution of compound **3** (13.0 mg, 0.029 mmol), which was adjusted to pH 7, at 0 °C. The reaction mixture was stirred at room temperature for 3 h and concentrated. The residue was washed with ethyl acetate and cold water, successively to afford **7** (11.0 mg, 85%) as a colorless amorphous mass.

$^1$H-NMR (DMF-$d_7$) $\delta$: 7.84 (2H, d, $J$=7.6 Hz), 7.57—7.53 (1H, m), 7.47 (2H, t, $J$=7.6 Hz), 3.70 (2H, m), 3.14 (1H, m), 2.68 (1H, m), 2.54 (1H, m), $^{13}$C-NMR (DMF-$d_7$) $\delta$: 167.0, 134.2, 128.9, 127.0, 61.0, 52.0, 40.5, $^{195}$Pt-NMR (DMF-$d_7$) $\delta$: −2268.

**(*N*$^1$-[4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl]propane-1,2,3-triamine)dichloroplatinum(II) (8)** Compound **3** (9.2 mg, 0.020 mmol)

and *N*-succinimidyl 4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzoate (**5**) (6.5 mg, 0.020 mmol) were reacted by the same procedure described above to afford **8** (7.8 mg, 0.014 mmol, 69%) as a colorless amorphous mass.

$^1$H-NMR (DMF-$d_7$) $\delta$: 7.93 (2H, d, $J$=8.0 Hz), 7.35 (2H, d, $J$=8.0 Hz), 3.65 (2H, m), 3.10 (1H, m), 2.74 (1H, m), 2.55 (1H, m), $^{13}$C-NMR (DMF-$d_7$) $\delta$: 166.0, 143.0, 131.7, 129.0, 123.9 (q, $^1J_{CF}$=273.5 Hz), 123.5, 61.0, 52.0 40.5, 29.7 (q, $^2J_{CF}$=40.0 Hz), $^{19}$F-NMR (DMF-$d_7$) $\delta$: −66.8, $^{195}$Pt-NMR (DMF-$d_7$) $\delta$: −2270.

**($N^1$-[4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]-2-methoxybenzoyl]-propane-1,2,3-triamine)dichloroplatinum(II) (9)** Compound **3** (9.2 mg, 0.020 mmol) and *N*-succinimidyl 5-[(3-(trifluoromethyl)-3*H*-diazirin-3-yl] 2-methoxybenzoate (**6**) (6.4 mg, 0.018 mmol) were reacted by the same procedure described above to afford **9** (7.5 mg, 0.013 mmol, 70%) as a colorless amorphous mass.

$^1$H-NMR (DMF-$d_7$) $\delta$: 8.65 (1H, s), 7.80 (1H, d, $J$=8.0 Hz), 7.35 (1H, d, $J$=8.0 Hz), 3.78 (2H, m), 3.15 (1H, m), 2.74 (1H, m), 2.55 (1H, m), $^{13}$C-NMR (DMF-$d_7$) $\delta$: 165.4, 159.5, 131.7, 129.9, 124.1 (q, $^1J_{CF}$=273.5 Hz), 121.9, 120.5, 114.0, 61.1, 56.9, 51.7, 40.0, 29.6 (q, $^2J_{CF}$=40.4 Hz), $^{19}$F-NMR (DMF-$d_7$) $\delta$: −67.3, $^{195}$Pt-NMR (DMF-$d_7$) $\delta$: −2275.

**Photolysis of the Diazirinyl Compounds 8 and 9** A methanolic solution of **8** and **9** (56 $\mu$M) was placed in a quartz cuvette. After replacing the inner atmosphere with nitrogen and cooling on ice, photolysis was carried out using a 15 W black light (UVP, San Gabriel, California, U.S.A.) at a distance of 2 cm from the surface of the light source. The UV spectrum was measured at the intervals indicated in Fig. 1. The half-life was calculated from a semi-log plot of the decay of the absorbance at 360 nm. After measurement of absorbance, the irradiated sample was concentrated. The residue was re-dissolved in $CD_3OD$ and subjected to $^{19}$F-NMR analysis.

**Reactivities of Synthetic Compounds towards GMP** Complex formation between organo-platinum compounds with or without irradiation and GMP were performed at a 5.0 mM GMP and 2.5 mM synthetic compounds in a deuterated 50 mM sodium phosphate buffer pH 7.0 at 50 °C. DMF-$d_7$ was used to dissolve the organo-platinum compounds. Reaction mixture was subjected to $^1$H-NMR spectroscopy analysis directly. The H-8 proton of guanosine base was drastically downfield shift before (8.16 ppm) and after (8.52 ppm) coordination platinum to guanosine. Calculations were performed by relative integration of the H8 proton signals of both reaction product and starting material during the reaction.

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