Dottorato di Ricerca in Chemistry & Biology - XXIX Ciclo - Anno I

A versatile method for the oxidation of Pt(II) antitumour drugs

Stefano Tinello

Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale,
Viale Teresa Michel 11, 15121 Alessandria (Italy).

e-mail: stefano.tinello@unipmn.it

Introduction

The platinum(II) complexes are the most important drugs in anticancer chemotherapy. In recent years more attention has been paid to Pt(IV) complexes as anticancer pro-drugs [1]. These complexes can be reduced *in vivo*, through a two electron reduction, in the hypoxic, reducing environment of the tumour tissue so that the octahedral Pt(IV) complexes are transformed into the active square-planar Pt(II) metabolites by loss of the axial ligands. Pt(IV) complexes exhibit greater chemical inertness than their Pt(II) counterparts and undergo fewer side reactions with biomolecules. The choice of the ligands is essential to modulate their lipophilicity (and related cellular uptake) and their redox properties. The axial ligands may be biologically active molecules themselves or simply only chemical linker from the Pt core to an active molecule [2]. Therefore, it is useful to have two different axial ligands: the former can be used for the coupling with drug delivery vectors, while the latter may be used to modulate the reduction potential (e.g. chlorides can facilitate the reduction respect to alkoxide ligands). It is important for a potential antitumor drug targeted molecule to show different kind of functional groups that can be used for the coupling to an active vector.

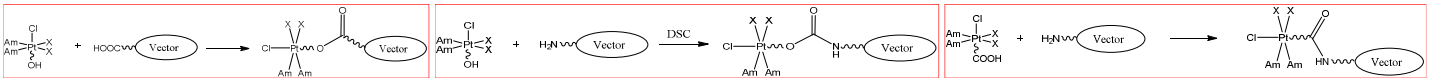


Fig 1 : General coupling reaction of a Pt(IV) complex to an appropiate vector

Synthesis

Pt(IV) complexes are usually prepared by oxidation of the corresponding Pt(II) counterparts, typically using hydrogen peroxide or chlorine. A different way to oxidize the Pt(II) compounds is represented by the use of N-chlorosuccinimide [3]. The reaction between Pt(II) complexes and this reagent in different coordinating solvents was set up to get the final asymmetric complex $[PtA_2ClX_2(Solv)]$.

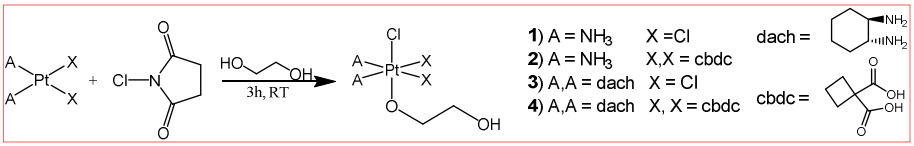


Fig 2 : General oxidation reaction for Pt(II) complexes

This method was developed to produce the asymmetric Pt(IV) octahedral complexes $[PtA_2Cl(glyc)X_2]$ (A = amine; X = chlorido or carboxylato; glyc = ethylene glycol) (Figure 2) but it proved to be quite versatile towards the synthesis of other asymmetric complexes depending on the conditions of the synthesis.

Characterization

All of these complexes have been characterized by HPLC-MS, mono- and bi-dimensional NMR spectroscopy (^1H, ^{13}C, ^{195}Pt, COSY, HSQC) and, only for Pt(dach)Cl(glyc)(cbdc), by single crystal X-rays diffraction, to confirm the formation of Pt(IV) complexes with solvent moiety as axial ligands.

1D and 2D NMR

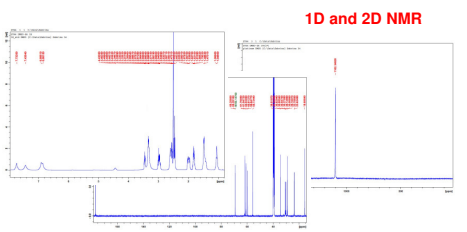


Fig 3: NMR spectra of compound **4** in DMSO-d_6

MASS SPECTROMETRY

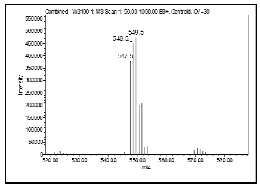


Fig.4: ESI-MS spectrum of **4**

X-RAY DIFFRACTION

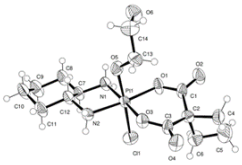
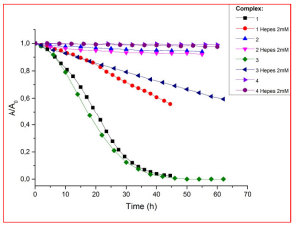


Fig.5 : X-ray structure of **4**

Stability

The stability of complexes **1**-**4** was studied both in carbonated water (pH = 6.4) and in HEPES buffer (pH 7.5) by monitoring the decrease of the area of the Pt(IV) HPLC peaks as function of time. The chlorido complexes **1** and **3** undergo fast hydrolysis, while the cbdc complexes **2** and **4** are 100% recovered after 3 days. The stability of **1** and **3** is higher at neutral pH (**1** $t_{½}$ = 50 h, **3** $t_{½}$ = 70 h) than at slightly acidic pH ($t_{½}$ *ca.* 21 h for both complexes) (Figure 6). ESI-MS spectra (positive ion mode) of the aged solutions show that the first hydrolysis product contains water instead of glyc; this intermediate further loses chlorides that are replaced by water/hydroxyl groups (Figure 7).



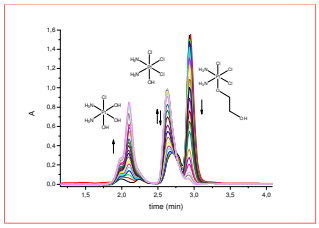
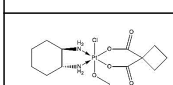
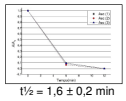
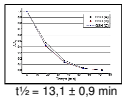
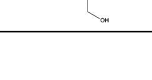
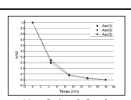
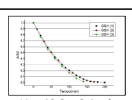


Fig 6:Normalized area (A_t / A_0) of the HPLC chromatographic peaks *vs.* aging time (*t*) in water (pH = 6.4, solid symbols) and HEPES buffer (pH = 7.5, empty symbols).

Fig 7: HPLC chromatograms of complex **1** *vs.* aging time (0 - 45 h) in water

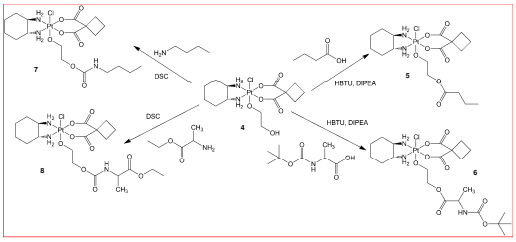
Reductions

The stable complexes **2** and **4** were challenged with the two most common biological reductants, i.e. ascorbic acid and glutathione (γ-glutamylcysteinylglycine, GSH). All kinetic measurements were performed with a 10-fold excess of AsA or GSH in HEPES buffer by monitoring the decrease of the area of the Pt(IV) chromatographic peaks (Figure 2. HPLC data were elaborated to obtain a pseudo-first-order rate constant *k* and the corresponding $t_{½}$, as previously reported for several Pt(IV) complexes.

Compound	Ascorbic Acid	Glutathione (GSH)
	 t½ = 1,6 ± 0,2 min	 t½ = 13,1 ± 0,9 min
	 t½ = 2,4 ± 0,2 min	 t½ = 46,3 ± 3,1 min

Coupling Reactions

The low propensity of **4** towards hydrolysis prompted us to set up the reaction conditions for the esterification of **4**, butanoic acid was used as a model for carboxylic acids. The best results were obtained with the uronium salts as coupling agents and, in particular, with HBTU in dry DMF to give complex **5** (Figure 8). The coupling reaction was then performed using *t*-butoxycarbonyl-protected L-alanine as a model for the coupling with α-COOH of peptides to give complex **6** (Scheme 3). Hydroxyl group has been made react with the modification agent, *N,N*-disuccinimidylcarbonate (DSC), which is able to temporarily activate it as succinimidyl carbonate group, that can be further reacted with an amine to form a stable carbamate bond. Also in this case, a simple model amine (i.e., n-butylamine) and a protected amino acid (i.e., an esterified alanine) were used to test the reactivity of **4** with two kinds of -NH_2 groups (complexes **7** and **8**, Figure 8).



Scheme of the reaction between complex **4** and acids, amines or aminoacids. HBTU = *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, DIPEA = diisopropylethylamine, DSC = *N,N*-disuccinimidylcarbonate.

References

[1] Hall, M.D.; Hambley, T.W. Coord. Chem. Rev. 2002, 232, 49-67.
[2] Graf, N.; Lippard, S.J. Adv. Drug. Deliv. Rev. 2012, 64, 993-1004.
[3] Mailliet, P.; Bourrie, B.; Normand, A. SANOFI AVENTIS, FR 2954321 – A1

Acknowledgments

Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici (Bari)

COST Action CM1105 Functional metal complexes that bind to biomolecules

FONDAZIONE CASSA DI RISPARMIO DI ALESSANDRIA

FONDAZIONE CRT Cassa di Risparmio di Torino