

Development of a click reaction approach for nitrosyl ruthenium complex as strategy to increase cellular uptake

Ana P. S. Gaspari¹ (PG), Marcelo R. Carvalho² (TM), Ivone Carvalho² (PQ), Roberto S. da Silva^{*1,2}(PQ)

*silva@usp.br

¹Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto – FFCLRP/USP

²Faculdade de Ciências Farmacêuticas de Ribeirão Preto – FCFRP/USP

Keywords: ruthenium nitrosyl, click chemistry

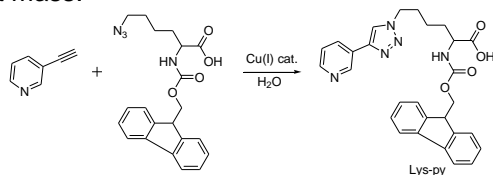
Introduction

Click Chemistry has been transformed into convenient and versatile chemical procedure that is widely used in organic chemistry, especially in drug discovery [1]. We have extended this application for coordination compounds by applying this concept in inorganic chemistry and open new directions in the preparation of complexes with bioinorganic application.

On the other hand, labeling biomolecule in nitrosyl ruthenium complexes seems to be a good strategy to increase specificity and chemotherapy properties of nitric oxide (NO) delivery agents. Based on this, the goal of this project is to synthesize a series of new compounds by click approach by alkyne-azide coupling to functionalize ruthenium complex with amino acid as biomolecule. The chemical reaction was studied by several physical chemistry properties and biological assays are conducting in order to understand the cellular uptake in B16F10 and MCF-7 cell lines. This subject is a remarkable example of interplay between organic and inorganic chemistry.

Results and Discussion

Lysine (Lys-R) was modified by adding azide as terminal substituent. Coupling reaction with 3-ethynylpyridine (3-Etpy) generating new specie (Scheme 1), which was characterized by ¹H NMR (Figure 1) and mass spectrometry. The mass spectrum carried out in negative mode showed the peak of *m/z* in 496,1978, consistent with expected exact mass.



Scheme 1. Coupling reaction pathway of azide (Lys-R) and ethynilpyridine, using Click Chemistry.

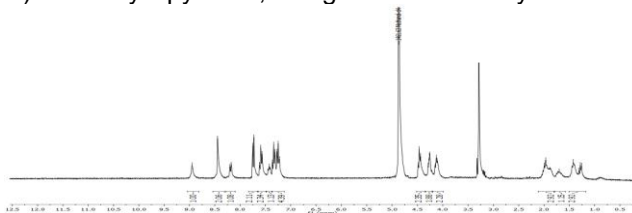


Figure 1. ¹H NMR spectra of the coupling product Lys-py containing a triazole bridge.

Similar product was also found by reacting AR1 with Lys-R, originating AR2 (Figure 2). All the compounds were characterized by FTIR, UV-vis and

cyclic voltammetry as described on Table 1.

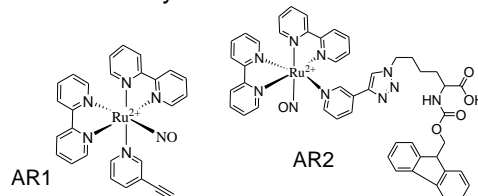


Figure 2. Structures for complex *cis*-[Ru(bpy)₂(3-Etpy)(NO)]³⁺ (AR1) and for *cis*-[Ru(bpy)₂(Lys-py)(NO)]³⁺ (AR2).

Table 1. Physical chemistry properties of some ruthenium complexes.

Complex	V _{no}	UV-Vis (nm)	E _{1/2} (NO ⁺⁰) *
AR1 ^a	1942 ^d	206 (IL), 235 (IL), 291 (IL), 332 (MLCT)	0,105 V
AR2 ^a	1946 ^d	212 (IL), 250 (IL) 297 (IL), 330 (MLCT)	-
AR3 ^b	-	238 (IL), 285 (IL), 416 (MLCT)	-
AR4 ^b	-	243 (IL), 283 (IL), 405 (MLCT)	-
AR5 ^c	-	238 (IL), 287 (IL), 348 (MLCT), 425 (MLCT)	-

^apH ~2,8, ^bpH ~7,8, ^cwater, ^dKBr, * TBAH 0,10 mol L⁻¹. AR3 = *cis*-[Ru(bipy)₂(NO₂)(3-Etpy)]⁺; AR4 = *cis*-[Ru(bipy)₂(NO₂)(Lys-py)]⁺ e AR5 = *cis*-[Ru(bipy)₂(H₂O)(3-Etpy)]²⁺

AR1 and AR2 were stable in pH ≤ 4,0 and above this value the correspondent nitro specie *cis*-[Ru(bipy)₂(NO₂)(3-Etpy)]⁺ or *cis*-[Ru(bipy)₂(NO₂)(Lys-py)]⁺ complexes were readily generated. The evaluation of both nitro derivatives obtained from the ruthenium complexes shows no fluorescent characteristics. Under reduction, NO release was observed by NO-sensor measurement undergoing to *cis*-[Ru(bipy)₂(H₂O)(3-Etpy)]²⁺, a luminescent specie, with emission in 574 nm (excitation in 550 nm). Preliminary results show enhance of cellular uptake in B16F10 and MCF-7 cell lines by amino acid modification bonded to nitrosyl ruthenium fragment.

Conclusions

The application of click chemistry on nitrosyl ruthenium complexes has proven to be a powerful tool to obtain new molecules with site binding specificity. The bioconjugation proceed rapidly and in large yield. Cellular uptake was advantageous with the modified specie in comparison to non-modified ones.

Acknowledgments

CAPES, FAPESP, CNPq and NAP-fotochem.

[1] Thirumurugan, P. Dariusz M. and Jozwiak, K. Chemistry for Drug Development and Diverse Chemical- Biology Applications. *Chem. Rev.* 2013, 113, 4905-4979.