H2020-ITN THERACAT (765497)

Work Package Number	WP3	**Task Number**	T3.1	**Deliverable Number**	D3.1	**Lead Beneficiary**	GRO
Deliverable Title	Novel metal complexes for bio-orthogonal catalysis						
Contractual Delivery Date	30/06/2019 (approved extension: 30/09/2019)	**Nature**	Report			**Dissemination Level**	CO
Actual Delivery Date	14/10/2019	**Contributors**	GRO and TAU				

Overview/Abstract

This deliverable entails the synthesis of novel palladium and ruthenium complexes for catalytic uncaging of cancer drugs. The synthesis of N4Py palladium complexes was attempted, but unsuccessful. N4py ruthenium complexes were successfully prepared and tested in model uncaging reactions. It was found that these complexes were inactive in alloc deprotections, but promising activity was found in photocatalytic deprotections. Currently, new ruthenium complexes are investigated for alloc deprotections.

Micellar Pd systems have been prepared and evaluated in catalysis. These were active in depropargylations, but no beneficial effect was observed from the ligand to date. Further studies are in progress.

Explanation for large delay in submitting deliverable

N/A

Led by

Name	Gerard Roelfes	**Partner**	GRO	**Date**	30/09/2019
Name	Michela Vargiu (ESR1)	**Partner**	GRO	**Date**	27/09/2019

Reviewed by

Name	Shreyas Wagle (ESR2)	**Partner**	TAU	**Date**	27/09/2019
Name	Rosa Miralles	**Partner**	IBEC	**Date**	02/10/2019
Name	Roey Amir	**Partner**	TAU	**Date**	04/10/2019
Name	Lorenzo Albertazzi	**Partner**	IBEC	**Date**	09/10/2019

Document Control

Issue #	Date	Changed Pages	Cause of Change	Implemented by
1	27/09/2019	9-17	Contribution of TAU to D3.1	Shreyas Wagle

Novel metal complexes for bio-orthogonal catalysis

In this report are shown the researches carry out by University of Groningen and Tel Aviv University in the development of novel metal catalysts for biorthogonal uncaging reactions of prodrugs. These studies are focused on different approaches (synthesis of complexes or delivering through micelles) based on the use of ruthenium and palladium.

1. Lead Institution: University of Groningen (ESR1 Michela Vargiu)

1.1 Introduction

Bioorthogonal catalysis is a powerful method for obtaining new-to-nature reactions in a biological environment without involving endogenous functionalities of the cells. This method provides advanced tools for both the study of biological processes and develop new strategies in medicinal chemistry and in chemical biology. This is possible through the development of suitable bond-forming reactions (such as Staudinger ligation,[1] Huisgen cycloaddiction,[2] inverse electron-demand Diels-Alder reaction with tetrazines[3]) but also through the opposite approach, bond-breaking reactions. This latter approach is useful for designing new anti-cancer drugs, composed of a catalytic system that includes a biologically inactive prodrug (that differs from active drug due to a functionality that could be easily removed from the molecule) and a bioorthogonal organometallic catalyst (that could be able to uncage the functionality just mentioned). In this way is possible to free the active drug in situ, in order to limit unwanted side effects. Transition metal catalysts act in highly efficient way in uncaging of prodrug,[4-9] but maintaining their activity and simultaneously controlling their localization remains a critical issue.[10] Furthermore, because of the physiological environment in which these catalysts will have to work, the transition metal species need to perform a delicate balance between activity and stability.[11] A plethora of conditions, such as presence of air, water, cellular components required an high stability of the catalyst, but, on the other hand, also high activity is necessary because of physiological conditions (*e.g.* temperature, pH, low cellular concentrations). Both transition metal complexes [12] and transition metal nanoparticles,[13] as well as a combination between the two,[14] have been reported in the literature as catalysts in uncaging reaction. Currently the most investigated transition metals in uncaging reactions are: Ruthenium, Palladium and Gold.

1.2 Objective

The aim of this project consists in developing transition metal complexes (with a particular focus on Ruthenium and, later, on Palladium) of pyridine and polypyridine ligands for catalytic uncaging of antitumor drugs in cancer cells. Metal complexes of polypyridil ligands such as phenanthroline, terpyridine, TPA and N4Py are of interest because of their broad catalytic scope

and, as recently shown in our group, are efficiently taken up by cancer cells. We want to investigate these complexes in uncaging of prodrugs, first in model reactions and then *in vitro*. When required, the complexes will be incorporated in delivery vehicles such as single chain polymer nanoparticles, micelles and lipidic nanoparticles developed in WP3. Finally, light activable Ru complexes will be prepared and tested by ligation of nitrile ligand to open coordination sites, which can be dissociated by light irradiation.

1.3 Results and discussion

Initially, we chose to focus on N4Py ligand. N4Py was born from the collaboration between the groups led by Feringa and Que in 1995,[15] and originally was trough as synthetic mimic of binding demine of bleomycin. It is a pentadentate ligand which consist of four pyridyl group that are connected to a central nitrogen atom (Fig 1a). Complex $[(N4Py)Fe(CH_3CN)](ClO_4)$ (Fig 1b) in presence of H_2O_2 in methanol or acetone gives the low-spin Fe(III)-OOH species (Fig 1c) which has similar spectroscopic characteristics as activated bleomycin[16-20] and, in presence of H_2O_2, is capable to oxidize a lot of organic species.[21] Due to its resemblance to BLM was investigated for its the DNA cleavage capability. [22-23] In our group was also demonstrated that the DNA cleavage capability just mentioned works even in cultured cells.[24]
Moreover, further studies carried out in our group lead to modification of N4Py ligand able to modulate intracellular localization.[25]
This brief introduction about N4Py explains our interest in this ligand and for this reason selected as starting point for the creation of new uncaging catalysts.
In our laboratory were already present four N4Py complexes: $[(N4Py)Fe(CH_3CN)](ClO_4)$ (Fig 1b), $[(N4Py)Mn(CH_3CN)](ClO_4)x2H_2O$ (Fig 1d), $[(N4Py)Cu(CH_3CN)](ClO_4)$ x H_2O (Fig 1e), $[(N4Py)Fe(CH_3CN)](ClO_4)x2H_2O$ (Fig 1f), and we synthesized two Ruthenium(II) complexes (Fig 2).[26]

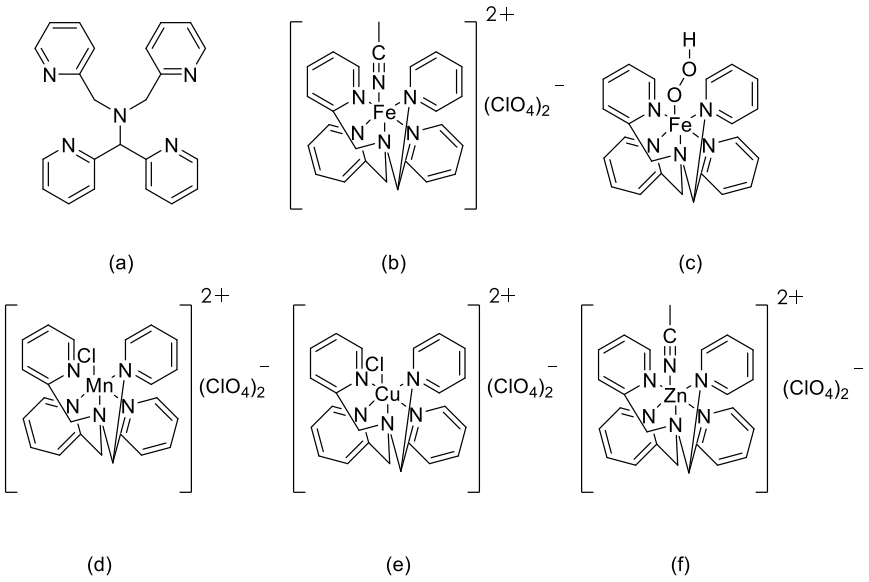


Fig.1: (a) N4Py; (b) $[(N4Py)Fe(CH_3CN)](ClO_4)$; (c) Fe(III)-OOH species; (d) $[(N4Py)Mn(CH_3CN)](ClO_4)x2H_2O$; (e) $[(N4Py)Cu(CH_3CN)](ClO_4)$ x H_2O; (f) $[(N4Py)Zn(CH_3CN)](ClO_4)x2H_2O$

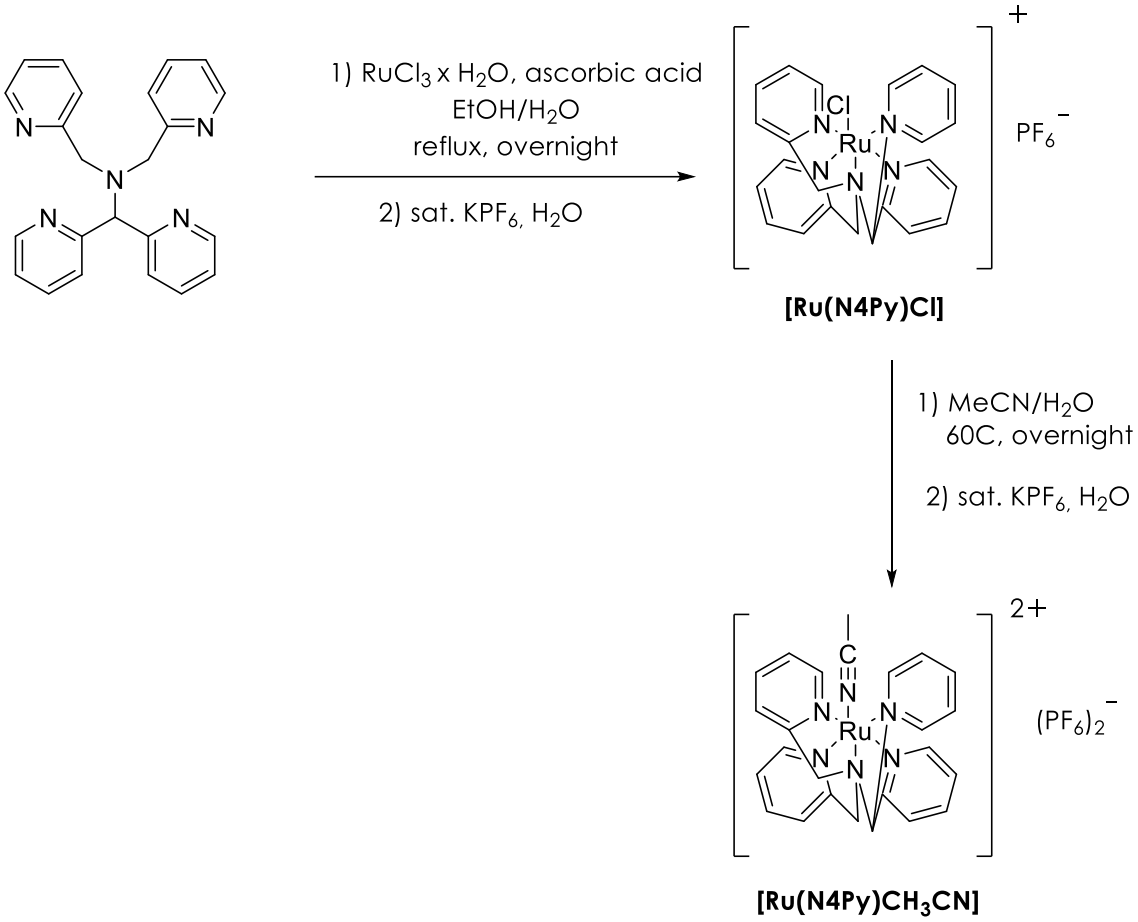


Fig.2: Synthesis of Ru(Cl)N4Py and Ru(CH_3CN)N4Py complexes.

The synthesis of the Palladium-N4Py catalyst was unsuccessful. We, first of all, synthesized $PdCl_2DMSO_2$,[27] and then we tried to modify a literature proceeding for the synthesis of a Platinum-N4Py (Fig.3):[28]

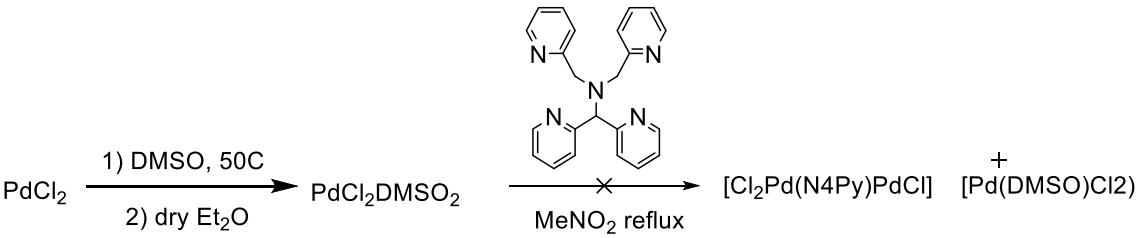
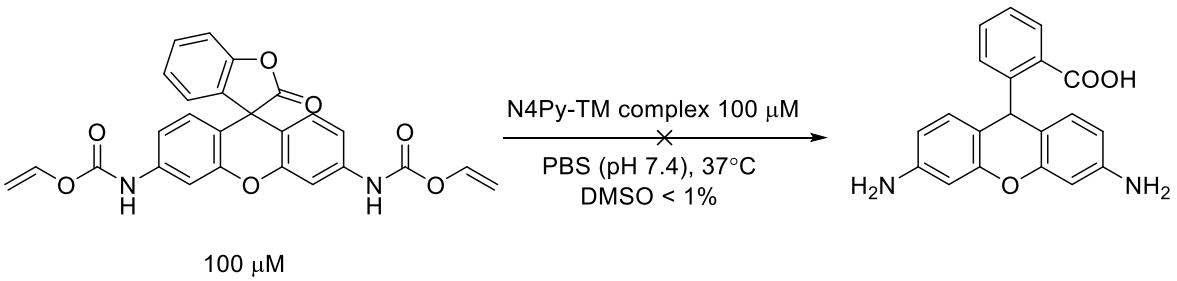


Fig.3: Synthetic path for Pd-N4Py complex.

After the synthesis of different prodrugs (propargyl-Floxuridine, allyl-Floxuridine, benzyl-Floxuridine, 1-propargyl-5FU, 3-propargyl-5FU, 1,3-dipropargyl-5FU) [29-32] and prodyes (alloc-Rhodamine110),[33] we tested all the N4Py complexes present in our library, unfortunately without any positive result. As an example, in Fig.4 is shown the reaction between an alloc-protected Rhodamine110 and all the N4Py complexes present in our library



Complexes: [Ru(N4Py)Cl] [Ru(N4Py)(CH_3CN)] [Zn(N4Py)(CH_3CN)] [Cu(N4Py) (CH_3CN)] [Fe(N4Py) (CH_3CN)] [Ru(N4Py) (CH_3CN)][a]

[a]200 μM

Fig.4: Reaction between alloc-Rhodamine110 and different N4Py catalysts.

In Fig.5 are shown results regarding the reaction just mentioned obtained with a microplate reader. With this experiment we confront the fluorescence derived from the reactions with the one emitted by a calibration curve, made with ten sample of free Rhodamine110 at different concentration (10 μM, 20 μM, 30 μM, 40 μM, 50 μM, 60 μM, 70 μM, 80 μM 90 μM, 100 μM). As you can see, the machine is not able to discriminate concentrations over 50 μM, but was not a significative problem because from the traces it can be concluded that uncaging did not occur.

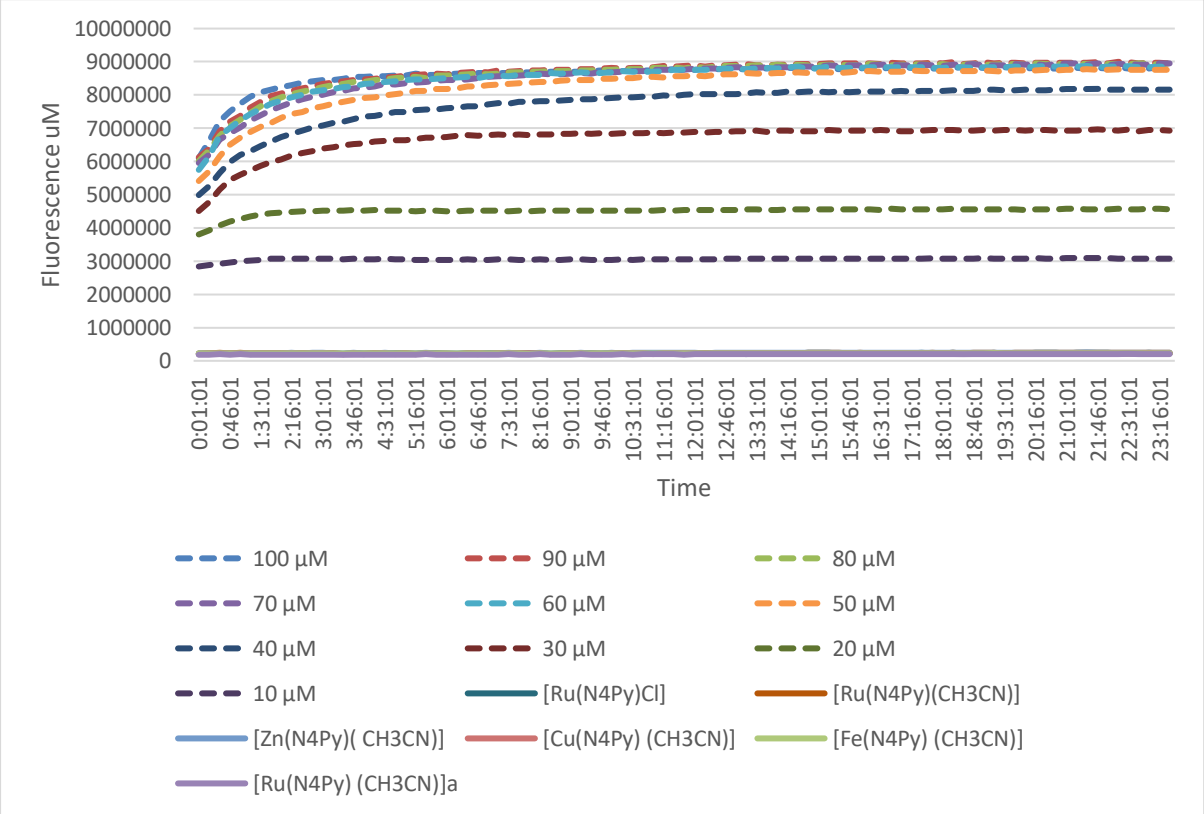


Fig.5: Uncaging experiment carried out in microplate reader. Dotted lines indicate the sample of calibration curve, continuous lines indicate reactions with different catalysts.

The same screening method was used for different concentration of catalyst (200 μM and 300 μM), with the adding of 1μL of PhSH,[12] and using methanol as solvent;[12] unfortunately in every case uncaged product was not obtained. Also, analysis on different prodrug using HPLC and UPLC gave the same results.
Once the poor activity of N4Py-metal complexes in uncaging reaction was established, we decided to re-adjust the project.

We chose to split the project in two different parts:

- SUB-PROJECT #1: USE OF A NEW CATALYST FOR THE OLD REACTION.
  Here, we chose to focus on development of novel complexes of uncaging reaction based on complexes that show activity in Tsuji-Trost reactions.

The first catalyst we chose to synthesize is the one reported in Fig.6:[34]

Fig.6: Synthesis of [RuCp(PPh3)(p-methoxy-bipyrydil)] complex.

The analogue of that catalyst without the two methoxy group was already tested from Meggers group in uncaging reaction and it showed a low activity,[35] but an electro-donating group could increase his catalytic power in Tsuji-Trost reactions.[36]
Another complex we would like to test is reported in Fig.7, which was synthesized by Kitamura et al;[37] has provided itself to be an efficient catalyst for the allyl protection/deprotection of alcohol.

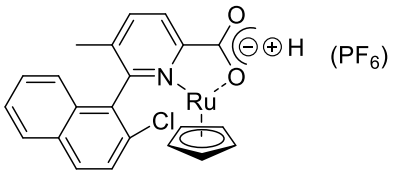


Fig.7: Synthesis of Kitamura complex.

- SUB-PROJECT #2: USE OF THE OLD CATALYST FOR A NEW REACTION.
  This is the new approach that could enable us to exploit the Ruthenium catalyst already synthesized. We call this approach "Photo to Release", and is schematized in Fig.8

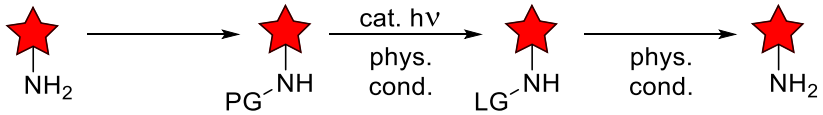


Fig.8: Photo-to-release approach

We would like to protect the biological active group of a drug with a group that could be easily converted, in physiological conditions, into a good leaving group that will abandon spontaneously the molecule, restoring the active drug.
[Ru(N4Py)(CH_3CN)] already showed a photocatalytic activity,[26] so, having in mind the reaction pathway reported in Fig.9, that was inspired by the work of Winssingeret al.,[38] we chose to test our Ru-complex in azide photoreduction reactions (Fig.10).[39]

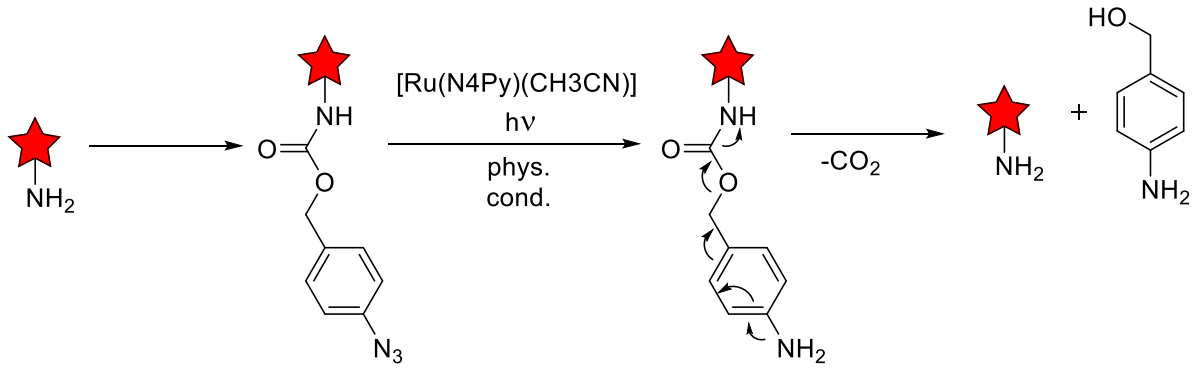


Fig.9: Caging group designed for Photo-to-release approach.

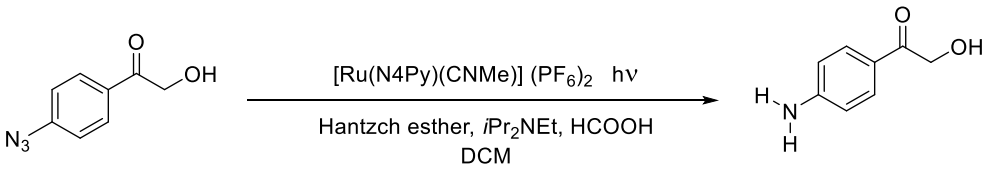


Fig.10: Reduction of azide by Ru(II)N4Py photo-catalysts in organic media.

From this reaction we obtained good results, with the complete conversion of the substrate and the formation of the desired product. We started studying this reaction in organic *media*, then we will pass at the aqueous media and, in the end, in physiological relevant conditions. This could be a very interesting method for drug containing secondary amine as biological active site. In an alternative approach, a primary amine functionality in a drug could be converted in an azide. This inactive azide containing prodrug can then be activated by photocatalytic reduction of the azide to obtain the amine. The advantage of this approach is that only dinitrogen is formed as byproduct.

1.4 Conclusions

Our initial approach to develop N4Py ruthenium and palladium complexes for uncaging proved unsuccessful. Currently we are developing new ruthenium catalysts for the uncaging reaction. Moreover, we are pursuing application of N4Py-Ru complexes in photocatalytic uncaging of azide containing prodrugs. The activity of the N4Py complex in this type of reaction has been demonstrated.

1.5 References

[1] Saxon, E. & Bertozzi, C.R.; *Science*, **2000**, 287, 2007.
[2] Gold, B.; Shevchenko, N. E.; Bonus, N.; Dudley, G. B.; Alabugin, I. V.; *J. Org. Chem.*, **2012**, 77, 75.
[3] Devaraj, N. K.; Weissleder, R.; *Acc. Chem. Res.*, **2011**, 44, 816.
[4] Völker, T.; Meggers, E.; *Curr. Opin. Chem. Biol.*; **2015**, 25, 48.
[5] Sasmal, P. K.; Streu, C. N.; Meggers, E.; *Chem. Comm.*, **2013**, 49, 1581.
[6] Völker, T.; Meggers, E.; *ChemBioChem*, **2017**, 18, 1083.
[7] Zheng, Y.; Tan, Y.; Harms, K.; Marsch, M.; Riedel, R.; Zhang, L.; Meggers, E. *J. Am. Chem. Soc.*, **2017**, 139, 4322.
[8] Li, J.; Yu, J.; Zhao, J.; Wang, J.; Zheng, S.; Lin, S.; Chen, L.; Yang, M.; Jia, S.; Zhang, X.; Chen, P.; *Nature Chemistry*, **2014**, 6, 352.
[9] Yang, M.; Li, J.; Chen, P. R.; *Chem. Soc. Rev.*, **2014**, 43, 6511.
[10] Jeschek, M.; Reuter, R.; Heinisch, T.; Trindler, C.; Klehr, J.; Panke, S.; Ward, T. R.; *Nature*, **2016**, 537, 661.
[11] Slatten, E. M.; Bertozzi, C. R.; *Angew. Chem. Int. Ed.*, **2009**, 48, 6974.
[12] Streu, C.; Meggers, E.; *Angew. Chem. Int. Ed.*, **2006**, 45, 5645.
[13] Yusop, R. M., Unciti-Broceta, A., Johansson, E. M. V., Sánchez-Martín, R. M.; Bradley, M.; *Nature Chemistry*, **2011**, 3, 239.
[14] Tonga, G. Y.; Jeong, Y.; Duncan, B.; Mizuhara, T.; Mout, R.; Das, R.; Kim, S. T.; Yeh, Y. C.; Yan, B.; Hou, S.; Rotello, V. M.; *Nature Chemistry*, **2015**, 7, 597.
[15] Lubben, M.; Meetsma, A.; Wilkinson, E. C.; Feringa, B.; Que, L. *Angew. Chem. Int. Ed.,* **1995**, 34, (13-14), 1521.
[16] Lehnert, N.; Neese,F.; Ho, R. Y. N.; Que, L.; Solomon, E. I.; *J. Am. Chem. Soc.*, **2002**, 124 (36), 10810.
[17] Roelfes, G.; Lubben, M.; Chen, K., Ho, R. Y. N.; Meetsma, A.; Genseberger, S.; Hermant, R. M.; Hage, R.; Mandal, S. K.; Young, V. G. Jr.; Zang, Y.; Kooijman, H.; Spek, A. L.; Que, L.; Feringa, B. L.; *Inorg. Chem.*, **1999**, 38 (8)., 1929.
[18] Ho, R. Y. N.; Roelfes, G.; Feringa, B. L.; Que, L.; *J. Am. Chem. Soc.*, **1999**, 121 (1), 264.
[19] Roelfes, G.; Vrajmasu, V.; Chen, K.; Ho, R. Y. N.; Rohde, J. U.; Zondervan, C.; la Crois, R. M.; Schudde, E. P.; Lutz, M.; Spek, A. L.; Hage R.; Feringa, B. L.; Munck, E.; Que, L.; *Inorg. Chem.*, **2003**, 42 (8), 26389.
[20] Kaizer, J.; Klinker, E. J.; Oh, N. Y.; Rohde, J. U.; Song, W. J.; Stubna, A.; Kim, J.; Münk, E; Nam, W.; Que, L.; *J. Am. Chem. Soc.*, **2004**, 126 (2), 472.
[21] Roelfes, G.; Lubben, M.; Hage, R.; Que, L.; Feringa, B. L.; *Chem. Eur. J.*, **2000**, 6 (12), 2152.
[22] Roelfes, G.; Branum, M. E.; Wang, L.; Que, L.; Feringa, B. L.; *J. Am. Chem. Soc.*, **2000**, 122 (46), 11517
[23] Li, Q.; van den Berg, T. A.; Feringa, B. L.; Roelfes, G.; *Dalton Trans.*, **2010**, 39 (34), 8012.
[24] Li, Q.; van der Wijst, M. G.; Kazemier, H. G.; Rots, M. G.; Roelfes, G.; *ACS Chem. Biol.*, **2014**, 9 (4), 1044.
[25] Greesing, A.; de Vries, R. H.; Jansen, G.; Rots, M. G.; Roelfes, G.; *Bioorganic & Medicinal Chemistry Letters*, **2019**, 29, 1922.
[26] Unjaroen, D.; Chen, J.; Otten, E.; Browne, W. R.; *Inorg. Chem.*, **2017**, 56, 900.
[27] Romeo, R.; Monsú Scolaro, L.; *Inorganic Syntheses*, **1998**, 32, 153.
[28] Lo, W. K. C.; Huff, G. S.; Preston, D.; McMorran, D. A.; Giles, G. I.; Gordon, K. C.; Crowley, J. D.; *Inorg. Chem.*, **2015**, 54, 6671.

[29]Weiss, J. T.; Dawson, J. C.; Macleod, K. G.; Rybski, W.; Fraser, C.; Torres-Sánchez, C; Patton, E. E.; Bradley, M.; Carragher, N. O.; Unciti-Broceta, A.; *Nature Communicatiation,* **2014**, 5, 3277.
[30] Weiss, J. T.; C. Fraser, C.; Rubio-Ruiz, B.; Myers, S. H.; Crispin,R.; Dawson, J. C.; Brunton, V. G.; Patton, E. E.; Carragher, N. O.; Unciti-Broceta, A.; *Frontiers in Chemistry*, **2014**, 2 (56), 1.
[31] Weiss, J. T.; Carragher, N. O.; Unciti-Broceta, A.; *Scientific Reports*, **2015**, 5, 9329.
[32] Rubio-Ruiz, B.; Weiss, J. T.; Unciti-Broceta, A.; *J. Med. Chem.*, **2016**, 59, 9974.
[33] Das, R,; Landis, R. F.; Tonga, G. Y.; Cao-Milán, R.; Luther, D. C.; Rotello, V. M.; *ACS Nano*, **2019**, 13, 229.
[34] Côrte-Real, L.; Karas, B.; Gírio, P.; Moreno, A.; Avecilla, F.; Marques, F.; Buckley, B. T.; Cooper, K. R.; Doherty, C.; Falson, P.; Garcia, M. H.; Valente, A.; *European Journal of Medicinal Chemistry*, **2019**, 163, 853.
[35] Vçlker, T.; Dempwolff, F.; Graumann, P. L.; Meggers, E.; *Angew. Chem. Int. Ed.*, **2014**, 53, 10536.
[36] Bruneau, C.; Renaud, J. L.; Demerseman, B.; *Pure Appl. Chem.*, **2008**, 80 (5), 861.
[37] Tanaka, S.; Suzuki, Y.; Kimura, T.; Kitamura, M.; *Bull. Chem. Soc. Jpn.*, **2019**, 92, 1707.
[38]Angerani, S,; Winssinger, N.; *Chem. Eur J.*, **2019**, 25, 6661.
[39] Chen, Y.; Kamlet, A. S.; Steinman, J. B.; Liu D. R.; *Nature Chemistry*, **2011**, 3, 146.

2. Other Contributor: Tel Aviv University (ESR2, Shreyas Wagle)

Micellar catalysts

2.1 Introduction

Bio-orthogonal chemistry is a powerful tool to selectively conduct non-natural reactions in biological environments with a high degree of precision,[1] especially the transformation of biologically inert prodrugs to active compounds. Using bio-orthogonal reactions to activate prodrugs in proximity to tumour cells could be an effective way to use these biologically inert reactions to avoid the side effects due to non-selective damage to healthy cells and tissues, which is one of the most notorious drawbacks of current chemotherapy.

While the use of non-human enzymes has been explored as orthogonal approach to allow selective activation of prodrugs, it may lead to immune response against the foreigner protein.[2] Thus, using catalytic systems based on synthetic transition metals as an alternative to conduct these reactions could partially solve the undesirable enzymatic drug activation and the next question would be to ensure the targeted drug activation with this chemistry. Many transition metals are excellent candidates as catalysts for such reactions,[3] but generally lack the specificity in prodrug activation at the required biological site.

To circumvent this drawback, we decided to work on improving the site-specificity of the drug activation by creating a *catalyst delivery system* where polymeric micelle with a covalently attached catalyst activates the prodrug within its hydrophobic core at the tumour site. [4,5] The bio-orthogonal reaction would be activated only by the intravenously introduced micellar catalysts and the reactions is non-susceptible to any biologically available catalysts or enzymes.

Our polymeric micelles are amphiphilic block-copolymers based on monofunctional PEG-dendron hybrids, which are covalently linked to bi- or multidentate ligands that form complexes with transition metals Cu (I) and Pd (II), to serve as catalytic sites for cleaving propargyl groups.

Previous studies[5] show that presence of hydrophobic moieties should significantly enhance the catalytic activity of the metal centres as compared to their molecular counterparts. Thus, using micelles will help in creating a hydrophobic environment within the biological environment for conducting these biorthogonal reactions.

Our focus here, is to understand the structure-activity relationship of our micellar catalysts with regards to their catalytic and micellar properties by varying their hydrophobic parts, the type of metals and the coordinating ligands.

The micellar system has the potential to be a game changer by bringing us a step closer to make enzyme-like catalysis possible.

2.2 Objective

Our main goal is to understand the structure-activity relationship of the catalytic micelles by varying the hydrophobic end-groups, metals and the coordinating ligands. Thus, we have synthesized monofunctional PEG-dendron hybrids, which we covalently linked to the ligands for coordinating the metal ions, and used them to prepare polymeric micelles.

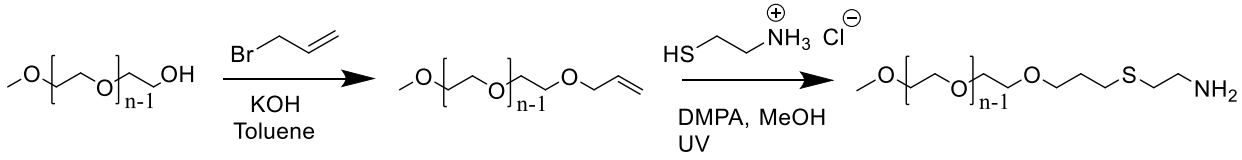
The monofunctional PEGs forming the hydrophilic segment of the micelles, will stabilize the micelle in the biological environment and increase the retention time in the blood stream without generating an immune response. The hydrophobic segment will be based on dendritic structures due to their high structural precision and modularity, which allow tuning of the amphiphilicity of the polymers and their micellar stability.[6]

The hydrophobic core of the micelles should provide a more efficient environment for the catalytic activity of the transition metals than otherwise available in biological conditions.[7] Additionally, the polymeric shell can protect the catalysts and provides a platform for further functionalization.

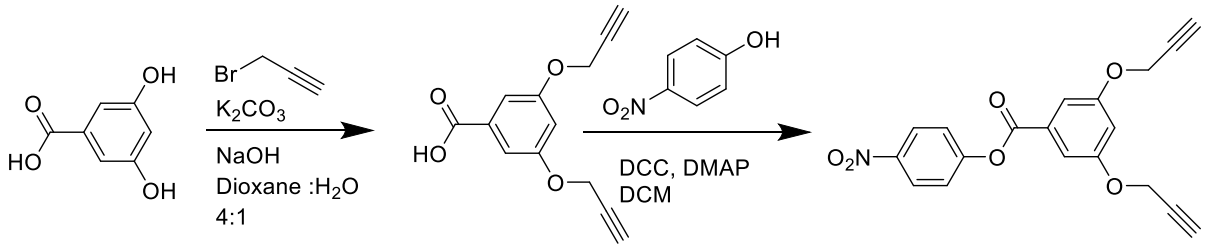
This micellar catalytic system has the potential to enhance the therapeutic effect by improving the specificity of bio-orthogonal catalysts and essentially bring us a step closer to make enzyme-like catalysis possible.

2.3 Results and discussion

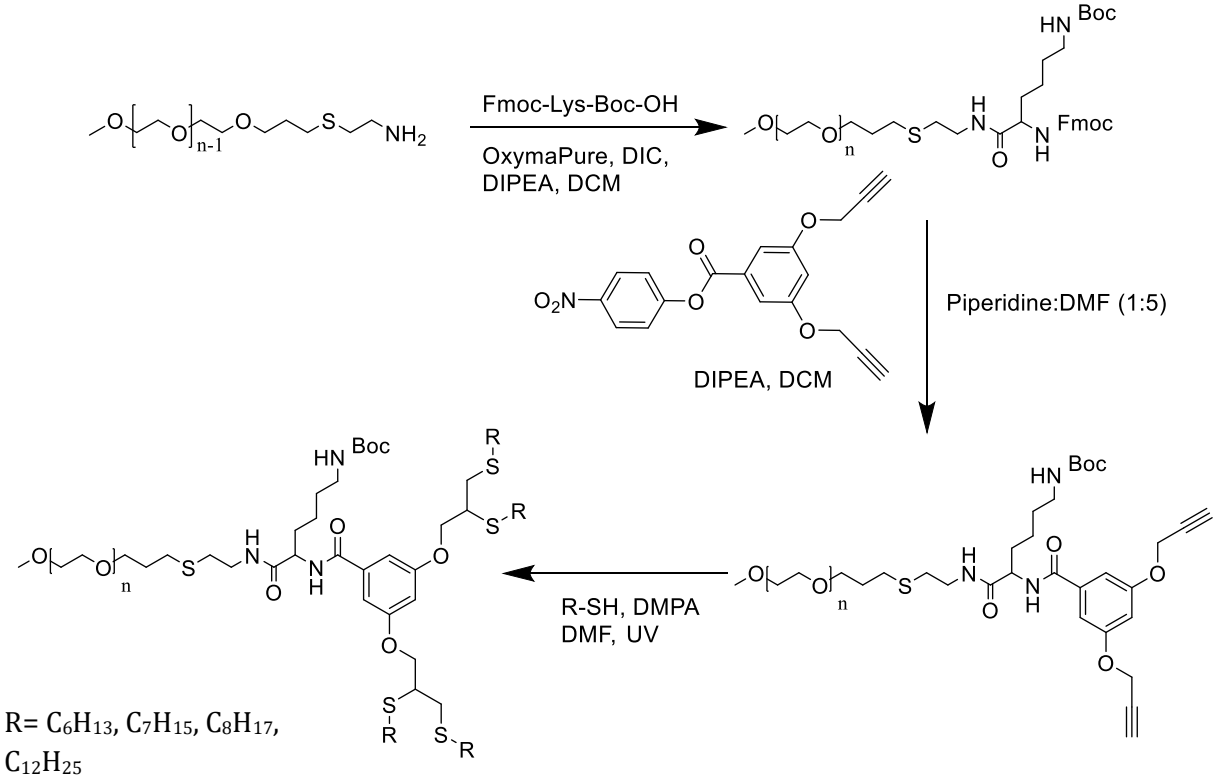
The polymeric micelles were synthesized with dendrons made of alkyl chains containing 6, 7, 8 and 12 carbon atoms to form a library of polymers with different hydrophobicities. The polymers have been synthesized according to the pathway shown in **Schemes 1, 2, 3**. All the polymers were synthesized on 500 mg scale and the approximate yield for each step was in the range 92-95%.



Scheme 1: Synthesis of mPEG-NH_2



Scheme 2: Synthesis of Branching Unit



Scheme 3: Synthesis of PEG-Dendron hybrids

Before starting with the micellar catalysis experiments, we conducted the depropargylation reaction in organic medium to understand whether the selected substrate undergoes

depropargylation in the presence of the selected palladium complex. Hence, the reaction was carried out in DMF:H_2O mixture at 80°C and the reaction was over in 5 hours. The reaction was followed by HPLC and the overlay of chromatograms at different times can be seen in **Figure 11.**

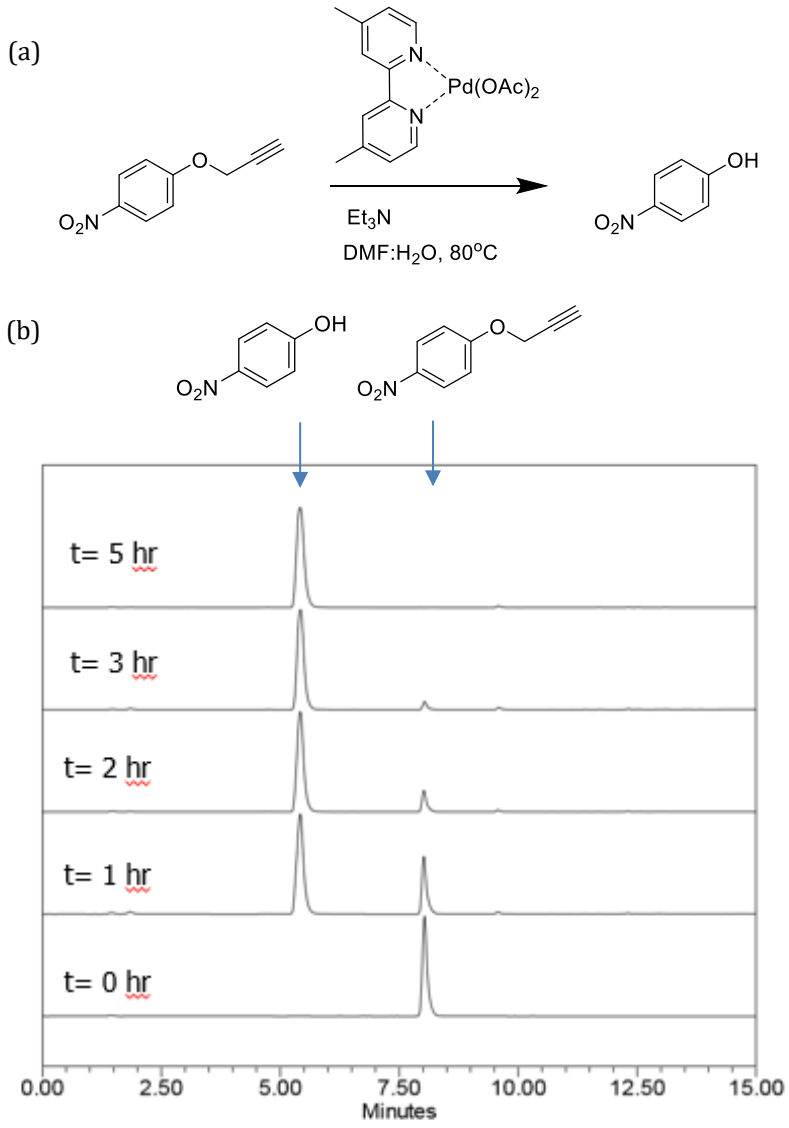
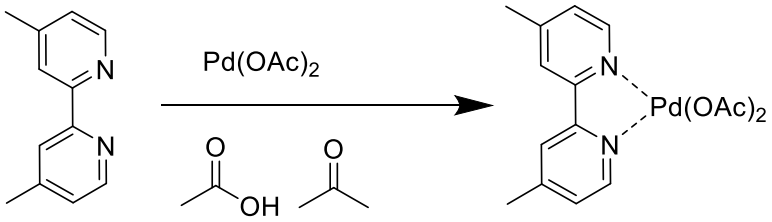


Fig.11: (a) Reference reaction and (b) HPLC overlay of the depropargylation reaction

This reaction gave us the indication that forming this complex in situ within our micellar environment would help us achieve depropargylation reaction in aqueous medium as well.

The polymeric micelles with dendron made with four octyl groups (mPEG-D-(C8)) were selected for starting the micellar catalysis experiments. Initially, the complex of the bipyridine and palladium acetate was formed and encapsulated in the mPEG-D-(C8) based micelles to act as a control experiment in order to compare with polymers where the bipyridine is covalently linked. The formation of the complex is sown in **Scheme 4**.



Scheme 4: Formation of Palladium (dimethyl bipyridine) diacetate complex

After encapsulating the complex in the mPEG-D-(C8) micelles, we conducted catalysis experiments in PBS using 1-nitro-4-(prop-2-yn-1-yloxy) benzene as the substrate, which will give 4-nitrophenol as the product post depropargylation reaction. The reaction was followed using HPLC and three control experiments run in parallel to the complex containing micelles. The three control experiments were checking the depropargylation just with mPEG-D-(C8), ligand encapsulated within mPEG-D-(C8) and $Pd(OAc)_2$ in mPEG-D-(C8). The first two control showed no depropargylation whatsoever while we observed significant depropargylation with the $Pd(OAc)_2$ containing mPEG-D-(C8), which gave us an indication that there is no reaction in the absence of metal.

We are comparing the rates of the depropargylation using the $Pd(OAc)_2$ complex that is encapsulated with mPEG-D-(C8) and just the $Pd(OAc)_2$ complex in PBS. As seen in **Fig 12**, we observed that in absence of micelles there is hardly any uncaging reaction that takes place as compared to the encapsulated complex. The kinetics show that the rate for the depropargylation for the complex encapsulated within our micelles is around four times as fast as that of the complex alone in PBS. This highlights the importance of the having an organic pocket within the aqueous environment to help conduct the organic reaction, which our micelles can provide quite efficiently.

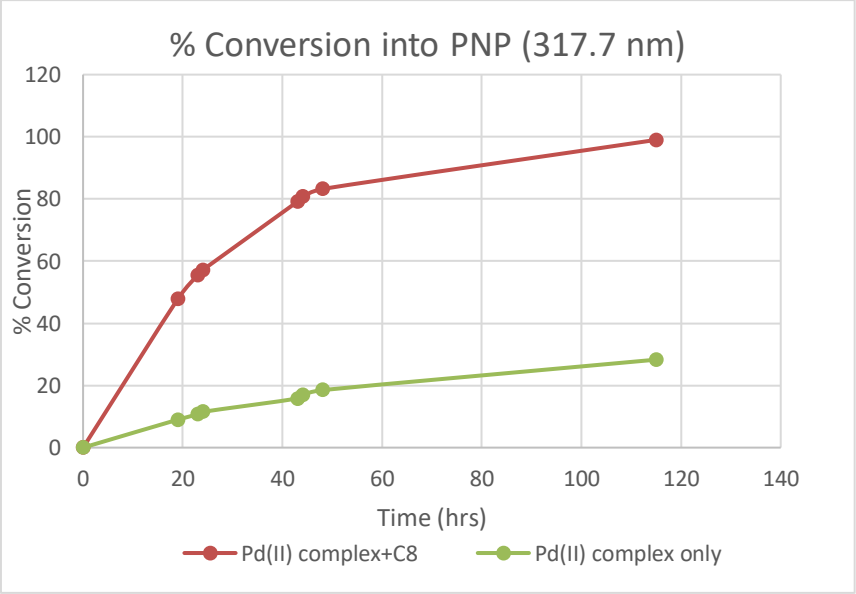
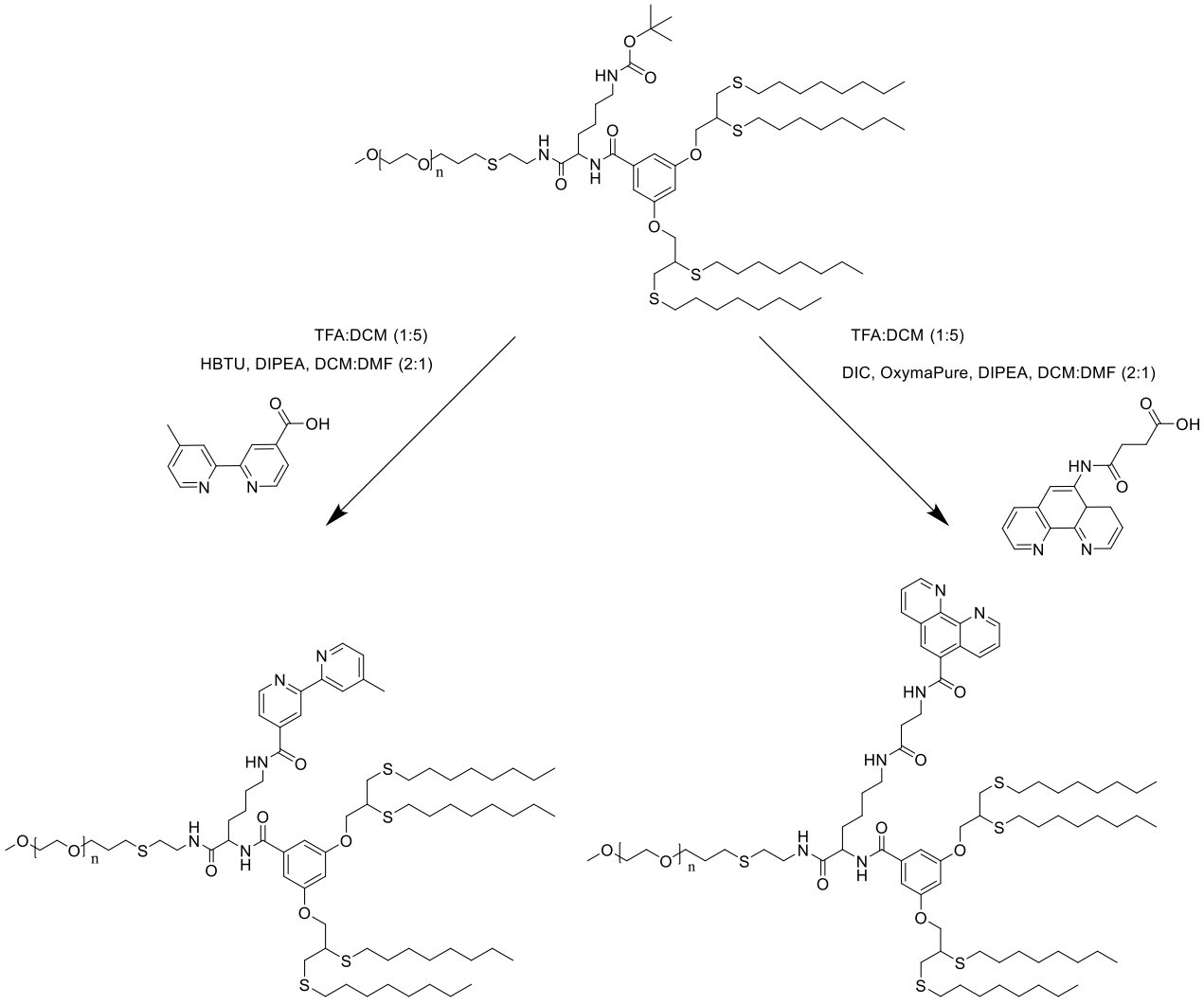


Fig.12: Reaction kinetics of Pd(II) complex encapsulated with mPEG-D-C8 vs just Pd(II) complex in PBS

Next, after studying the catalytic activity of the encapsulated complex, we set to prepare polymers with covalently linked ligands. Thus, PEG-dendron amphiphiles with four hydrophobic octyl end-groups (mPEG-D-(C8)) were synthesized and conjugated with either bipyridine or phenanthroline ligand as shown in **scheme 5**. The polymers were dissolved in buffer to form micelles and we conducted catalysis experiments for depropargylation of 1-nitro-4-(prop-2-yn-1-yloxy) benzene to give 4-nitrophenol (PNP) (See **Fig. 13**).



Scheme 5: Synthesis of bipyridine functionalized mPEG-D(BiPyridine)-C8 and mPEG-D(Phenanthroline)-C8

For the purpose of this experiment, 1:1 ratio between the palladium salt and the conjugated ligands was used. Palladium loaded mPEG-D-C8 micelles were prepared by incubating the polymers with palladium diacetate in acetone overnight before evaporating the acetone and adding PBS to form the metal loaded micelles. HPLC was used to follow the reaction.

The experiment showed that even without the conjugated ligand, the metal containing micelles, which we used as control, could catalyze the reaction as effectively as micelles containing the

covalently linked ligands (See **Fig. 14**). The reason for this could be the formation of complex between the palladium and the sulfur atoms present within the dendron forming a pincer complex, which is able to efficiently catalyze the reaction in the hydrophobic environment. We are currently working on ways to determine the nature of complex formed within these micelles. Furthermore, we are planning to conduct the catalysis with micelles having more hydrophobic dendrons to determine the effect of the hydrophobicity of the dendrons on the catalytic capabilities of these catalytic micelles.

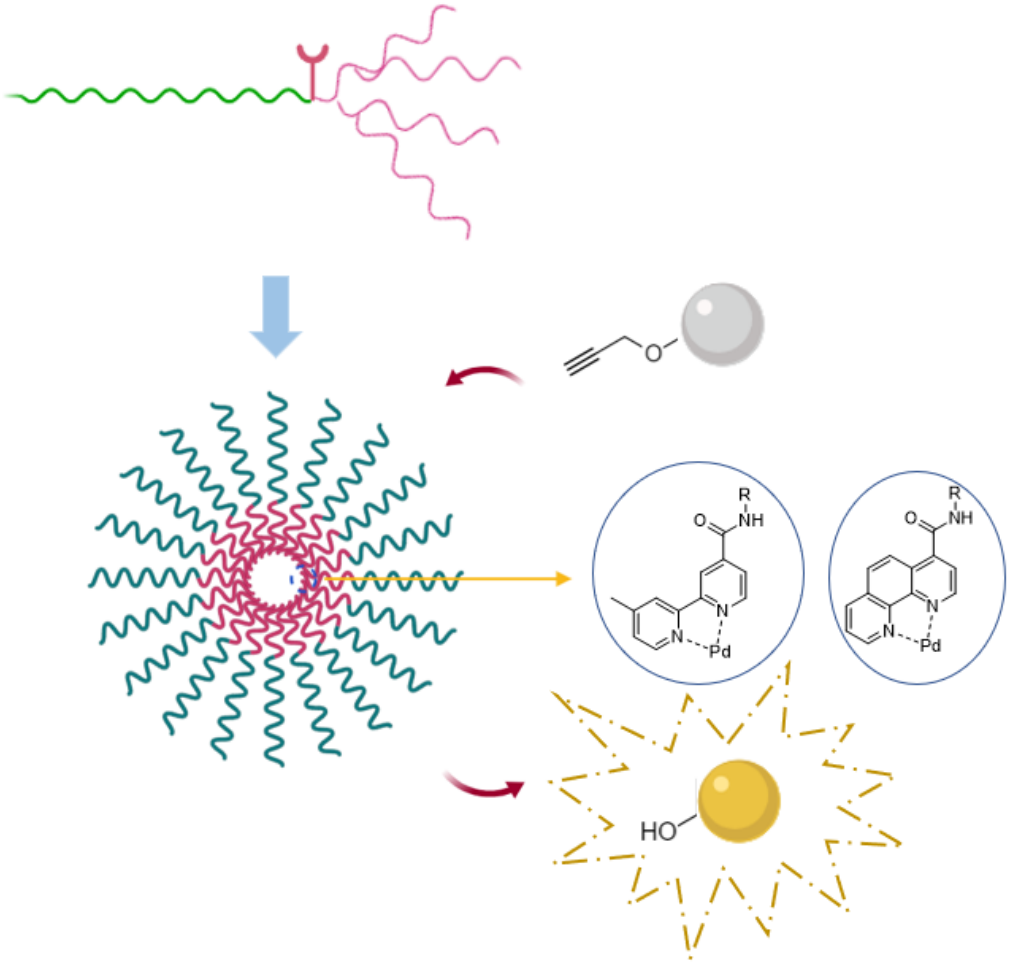


Fig.13: Schematic depression of depropargylation reaction with micellar system.

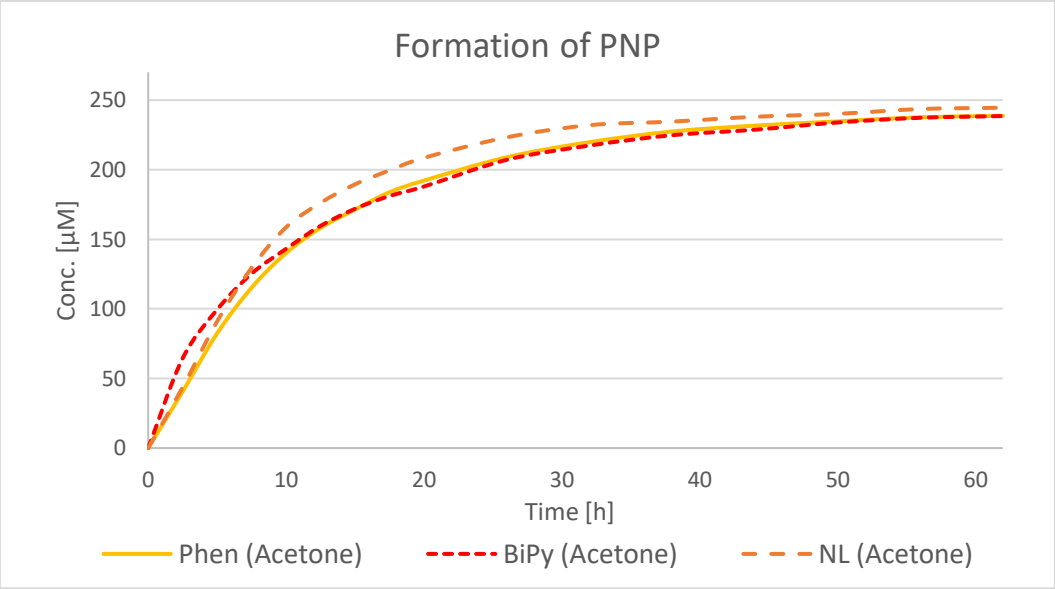


Fig.14: Formation of PNP using the Palladium metal with mPEG-D(Bipyridine)-C8 (red), mPEG-D(phenanthroline)-C8 (yellow), mPEG-D-C8 (orange).

2.4 Conclusions

Our initial experiments have shown that our micelles can carry out the uncaging reactions even without the presence of the complexing ligand within the micellar system. We suspect that the sulfur atoms present in the branching unit of the polymer can act as a chelating agent for the palladium metal and perform even slightly better than the complex formed via bipyridine and phenanthroline for the uncaging reaction. Thus, it would be interesting to know the binding behavior of palladium metal to these ligands and understand where exactly the metal binding is taking place.

The bipyridine containing mPEG-D(Bipyridine)-C12 has also been synthesized to see if there is acceleration in the depropargylation reaction due to the change in hydrophobicity of the micelles. It would be interesting to see if the comparable activity of the native amphiphiles without the ligand is observed also in the case of the more hydrophobic C12 based micelles or is it peculiar to mPEG-D(Bipyridine)-C8 and mPEG-D(Phenanthroline)-C8 hybrids.

2.5 References

1. Prescher, J. A.; Bertozzi, C. R. *Nat. Chem. Biol.* **2005**, 1, 13– 21.
2. Bagshawe K.D. *Expert Rev Anticancer Ther*. 6 (10): 1421–31.
3. Streu, C.; Meggers, E. *Angew. Chem., Int. Ed.* **2006**, 45, 5645–5648.
4. Tonga, G. Y.; Jeong, Y.; Duncan, B.; Mizuhara, T.; Mout, R.; Das, R.; Kim, S. T.; Yeh, Y.-C.; Yan, B.; Hou, S.; Rotello, V. M. *Nat. Chem*. **2015**, 7, 597–603.

5. Yusop, R. M.; Unciti-Broceta, A.; Johansson, E. M. V.; Sánchez-Martín, R. M.; Bradley, M. *Nat. Chem*. **2011**, 3, 239.
6. Segal, M; Avinery, R; Buzhor M; Shaharabani, R; Harnoy, A.J.; Tirosh, E; Beck, R; Amir, R.J. *J. Am. Chem. Soc*. (2017), 139, 803-810.
7. Liu, Y.; Pujals, S.; Stals, P. J. M.; Paulöhrl, T.; Presolski, S.I.; Meijer, E.W.; Albertazzi, L.; Palmans, A.R.A.. *J. Am. Chem. Soc.* **2018**, *140* (9), 3423–3433.