H2020-ITN THERACAT (765497)

Work Package Number	WP4	**Task Number**	T4.2	**Deliverable Number**	D4.2	**Lead Beneficiary**	TUE[1]
Deliverable Title	Set of 2-3 fluorescent pro-dyes						
Contractual Delivery Date	30/06/2020 (revised: 30/10/20)[2]	**Nature**	Report			**Dissemination Level**	CO
Actual Delivery Date	15/09/2020	**Contributors**	TUE				

[1] *Of note, this deliverable was originally led by IBEC but it has been reassigned to TUE due to the change of institution of ESR6 (this reassignment was already informed and justified in the mid-term Report, as well as through a Formal Notification at the Funding & Tenders Portal).*

[2] *A new delivery date was approved by the Project Officer due to the delays caused by COVID-19 outbreak.*

Overview/Abstract

A set of four pro-dyes were prepared as model compounds to study the in-vitro performance of the catalyst. Activation of these pro-dyes were evaluated using Pd(II) loaded single chain polymeric nanoparticles in water, PBS and DMEM medium.

Explanation for large delay in submitting deliverable

N/A

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Document Control

Issue #	Date	Changed Pages	Cause of Change	Implemented by
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Set of 2-3 fluorescent pro-dyes

1. Introduction

Pro-drug activation using bio-orthogonal cleavage reactions is a powerful approach towards targeted cancer therapy with minimal side effects. The development of bio-orthogonal catalysts for pro-drug activation in cells is challenging, which requires careful evaluation of the performance of these catalysts in both aqueous and complex medium. Pro-dyes can be used as model compounds of pro-drugs to study the activity of catalysts using fluorescence and absorption spectroscopy. The heterogeneity and stability of catalyst at a single molecule as well as the turnover number can be studied using single molecule fluorescence microscopy. Pro-dyes also help to study the trend of catalytic activity with respect to variable concentration and hydrophobicity of substrates. These studies on pro-dyes will help to improve the design of catalyst carriers and thereby best system can be developed for pro-drug activation in cells.

Palladium mediated bond cleavage reactions has shown several applications in cell surface engineering, protein and pro-drug activation both inside and outside living cell due to its unique catalytic properties and low cytotoxicity.[1] Among various bond cleavage reactions, Pd(II) mediated depropargylation reactions gained significant interest recently. Here, we developed propargyl protected pro-dyes which can be activated using Pd(II) loaded nano-carriers.

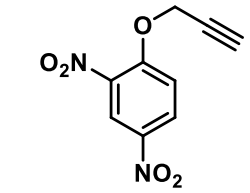
2. Objective

The aim of this deliverable was to synthesize 2-3 pro-dyes that can be activated using Pd(II) loaded single chain polymeric nanoparticles (SCPNs).

3. Results and Discussion

A set of propargyl-protected, palladium activable pro-dyes **1-4** has been synthesized, Figure 1 as reported in literature.[1-4] On activation, corresponding products can be monitored using fluorescence or absorption spectroscopy at micromolar concentration in the aqueous medium. Hydrophobicity of the substrates plays an essential role during their activation by SCPNs, especially in complex media. Greater the hydrophobicity, higher is the tendency of substrates to accumulate in hydrophobic reaction space inside the nanoparticles.

A



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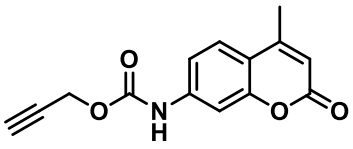

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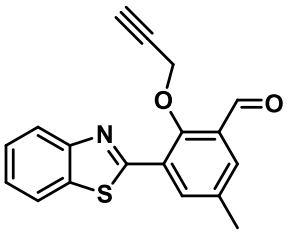

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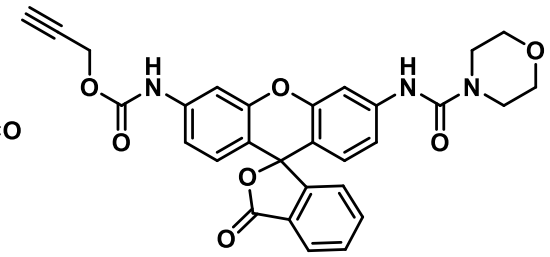

4

B

Pro-dye	Log P
1	1.97
2	2.06
3	4.2
4	3.49

Figure 1: A) Structure B) Log P value of synthesized pro-dyes

The activation of these pro-dyes were tested using Pd(II) loaded single chain polymeric nanoparticles. 2,4-dinitrophenyl propargyl ether (DNPPE) **1** on depropargylation yields 2, 4-dinitrophenol (DNP) and the reaction can be monitored using UV-Vis spectroscopy at a wavelength of 400 nm. *N*-Proc-caged Coumarin **2** activation can be monitored using fluorescence spectroscopy where the uncaged product has an excitation maximum at 370 nm and emission maximum at 440 nm when inside the SCPNs. The uncaging of **1** and **2** was successful in water and PBS but was not successful in the DMEM medium. This can be due to the low hydrophobicity which decreases the substrate accumulation in the presence of competing elements present in the DMEM medium. Propargyl protected benzothiazole derivative **3** was also synthesized however uncaging was not successful even in water. Finally, *N*-proc Rhodamine 110 **4** was prepared which on depropargylation yields fluorescent Rhodamine 110 which has an excitation maximum 495 nm and emission maximum at 520 nm. The uncaging was successful in water, PBS and DMEM medium. It was found to be the best candidate owing to its higher hydrophobicity. Therefore, N-proc Rhodamine 110 **4** can be used to study the performance of the catalysts in complex biological media by fluorescence spectroscopy and single-molecule fluorescence microscopy.

Synthesis

DNPPE **1** was synthesized from 2,4-dinitrophenol on reaction with 3-bromo-1-propyne and K_2CO_3 in DMF as yellow solid. 7-Amino-4-methylcoumarin and propargyl carbonyl chloride was reacted in the presence of pyridine in DCM to yield *N*-Proc-caged Coumarin **2.** Propargyl protected benzothiazole derivative **3** was prepared from 2-Hydroxy-5-methylbenzaldehyde in three steps. 2-Hydroxy-5-methylbenzaldehyde and 2-aminothiophenol was reacted together with sodium metabisulfite to yield 2-(2′-hydroxyphenyl -5′-methyl)benzothiazole. This product on further reaction with hexamethylenetetramine (HMT) in TFA gave 2-(2′- hydroxyphenyl -3′-aldehyde-5′-

methyl)benzothiazole which later on was allowed to react with 3-bromopropyne to yield 2-(2′-(propargyl ether) phenyl -3′-aldehyde-5′-methyl)benzothiazole **3**. Mc-Rh110 was synthesized from Rhodamine110 chloride on reaction with 4- morpholinecarbonyl chloride and NaH in DMF. Mc-Rh110 was then allowed to react with propargyl chloroformate in the presence of pyridine to yield N-proc Rhodamine 110 **4.**

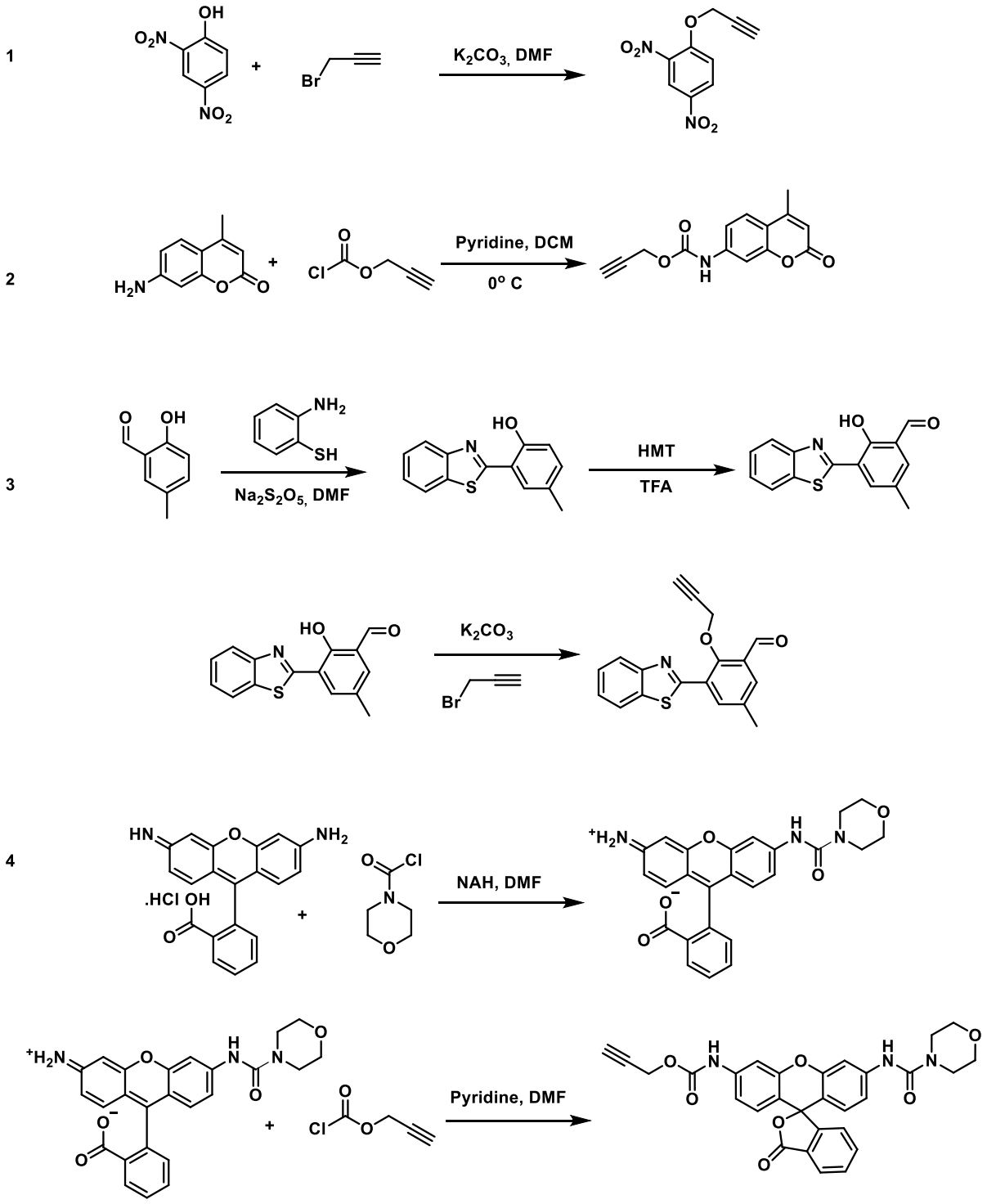

Figure 2: Synthetic route of pro-dyes **1-4**

Bipy and PPh3 functionalized amphiphilic polymers complexed with Pd(II) were used to activate pro-dyes in water, PBS, DMEM medium as shown in Figure 3. *N*-proc Rhodamine 110 **4** is the best candidate for in-vitro evaluation. Protection of one handle with morpholine makes it easier to quantify the product formed by depropargylation. It rules out the possibility of two products in reaction mixture if both handles were protected with propargyl group.

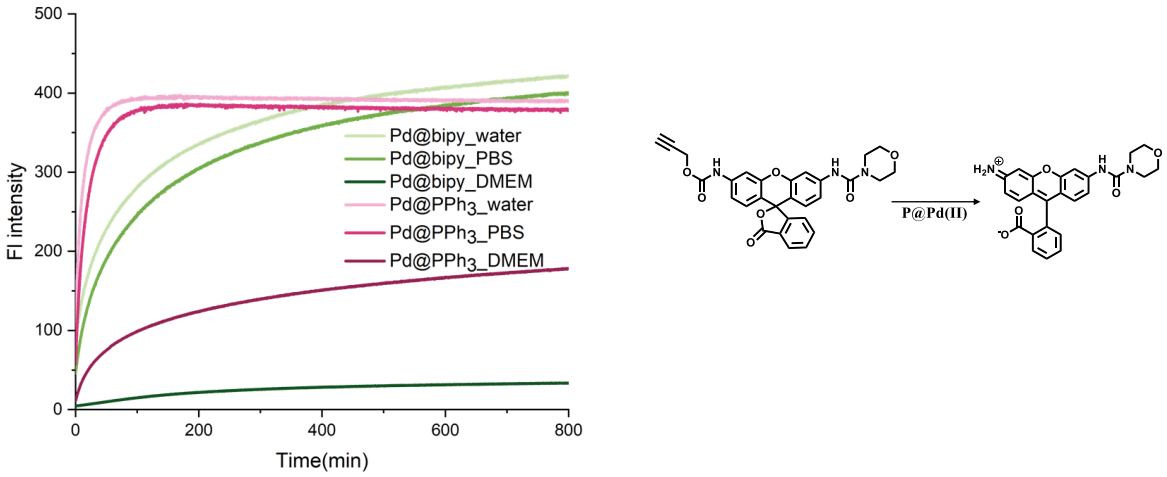

Figure 3: Pd(II) catalyzed depropargylation of **4** in water, PBS and DMEM medium monitored by fluorescence spectroscopy over time

4. Conclusion

Four pro-dyes that can be activated by bio-orthogonal Pd(II) catalysts were prepared during T4.2. Therefore, deliverable D4.2 is completed.

5. References

1. Coelho, S. E. *et al.* Mechanism of Palladium(II)-Mediated Uncaging Reactions of Propargylic Substrates. *ACS Catal.* **9**, 3792–3799 (2019).
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