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"Guanidine switch" enabled intermolecular OPA-amine-thiol three-component reactions for modular constructions

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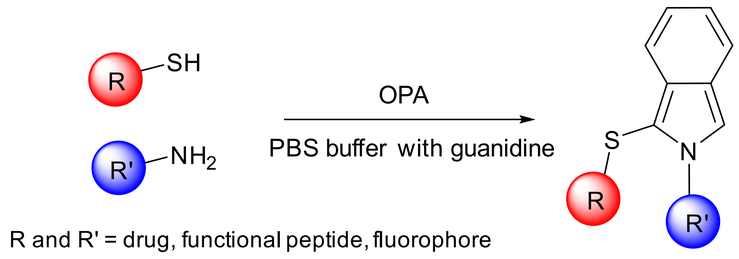
Introduction

Recently, ortho-phthalaldehyde (OPA) is gaining a renascence for modifying proteins and peptides, via OPA-amine two-component reaction (2CR) for bioconjugation[1] and intramolecular OPA-amine-thiol three-component reaction (3CR) for cyclization[2]. Historically, small thiol molecules were used in large excess to allow for the intermolecular OPA-amine-thiol reaction forming 1-thio-isoindole derivatives[3]. In this report, we discovered that guanidine could serve as an effective additive to switch the intermolecular OPA-amine-thiol three-component reactions to a stoichiometric process and enable the modular construction.



R and R' = drug, functional peptide, fluorophore

Conditions screening to achieve stoichiometric intermolecular OPA-amine-thiol 3CR

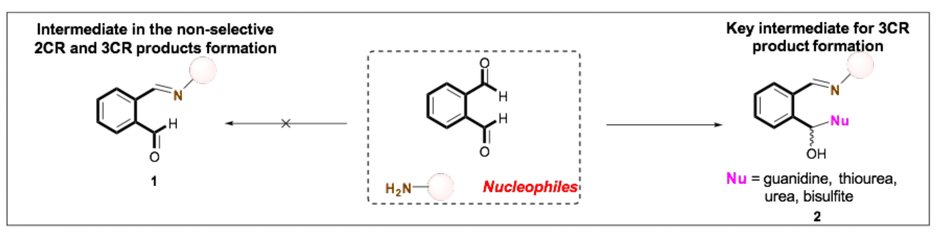
Various aldehyde-reacting nucleophiles were tested and guanidine appeared to be a promising additive to "switch off" the 2CR pathway.

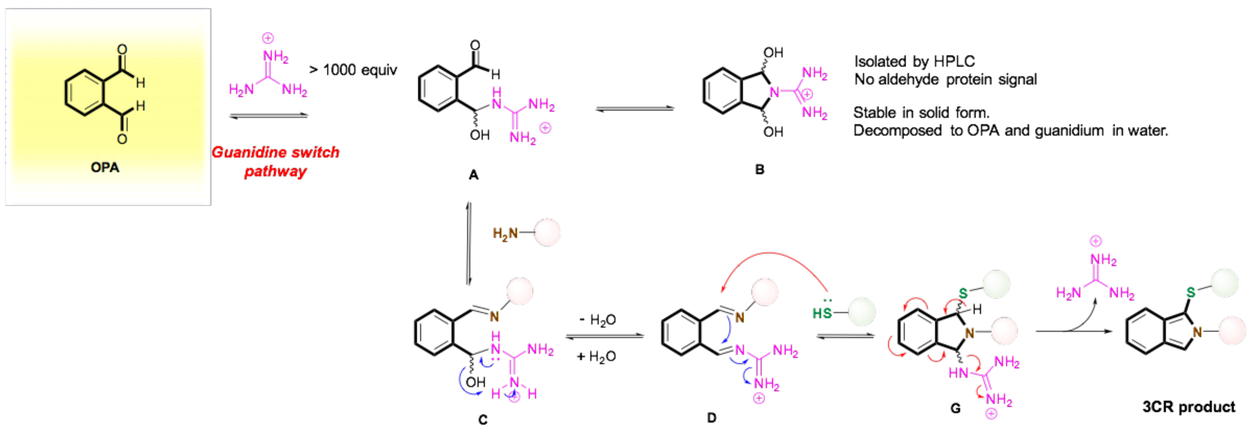
Entry	Buffer	Additive	Conc. (mM)	Temp.	Reaction time[a]	Conversion[b] (selectivity[c])
1	PBS buffer pH 7.4	/	0.5	RT	1 h	>99% (40%)
2		6 M guanidine			> 7 h	77% (>99%)
3		6 M urea			1 h	94% (68%)
4		4 M urea + 2 M thiourea			1 h	89% (61%)
5		6 M urea + 6 M guanidine			> 7 h	28% (>99%)
6		Sodium bisulfite (2equiv.)			> 7 h	54% (>99%)
7		6 M guanidine		40°C	> 3 h	31%[d] (>99%)
8	Borate buffer pH 8.5	6 M guanidine		RT	> 3 h	45%[d] (>99%)
9	PBS buffer pH 7.4	1 M guanidine			2 h	86% (87%)
10		3 M guanidine			> 2 h	80% (98%)
11			2		1 h	86% (93%)

The additives (Guanidine HCl / urea / thiourea / sodium bisulfie) were first dissolved in the buffer and adjusted to the desired pH before use. The thiol-carrying peptide Ac-QSQQTFSNLWRLLCQN-NH2 (1.0 equiv) and OPA (1.2 equiv) were then dissolved in the buffer prepared, followed by the addition of the amine-carrying peptide Ac-QSQQTFKNLWRLLPQN-NH2 (1.0 equiv). The reactions were monitored by LCMS. [a] Time required for the reaction to complete. [b] Percentage of conversion calculated based on the LCMS analysis of the crude reaction mixture. [c] Selectivity towards the 3CR product. [d] Side product F.

Proposed mechanism

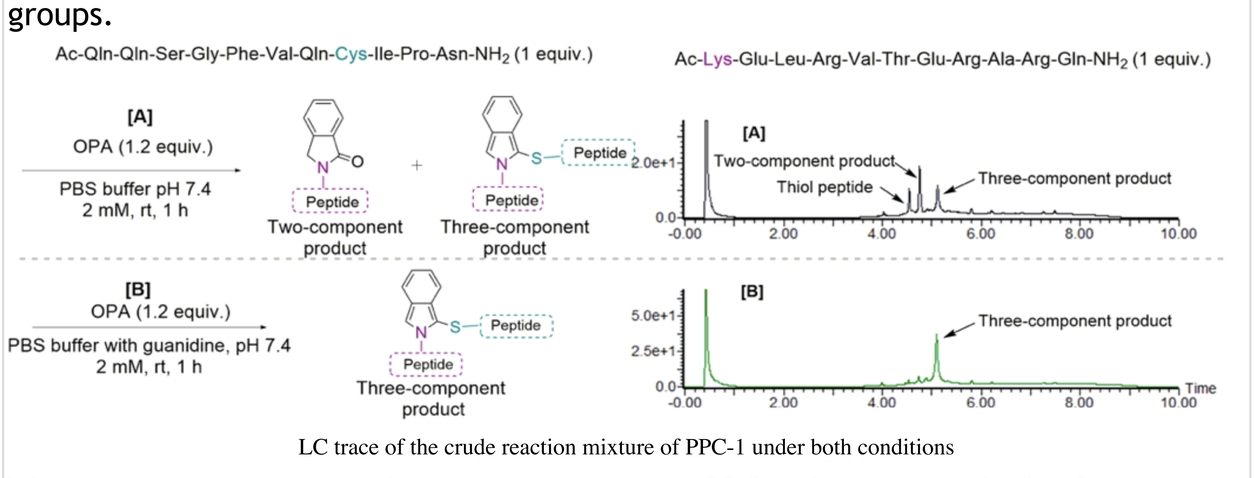
We proposed that guanidine could serve as an effective additive to realize the stoichiometric intermolecular OPA-amine thiol 3CR by reversibly blocking the aldehyde groups in the key intermediates and switching off the 2CR pathway.





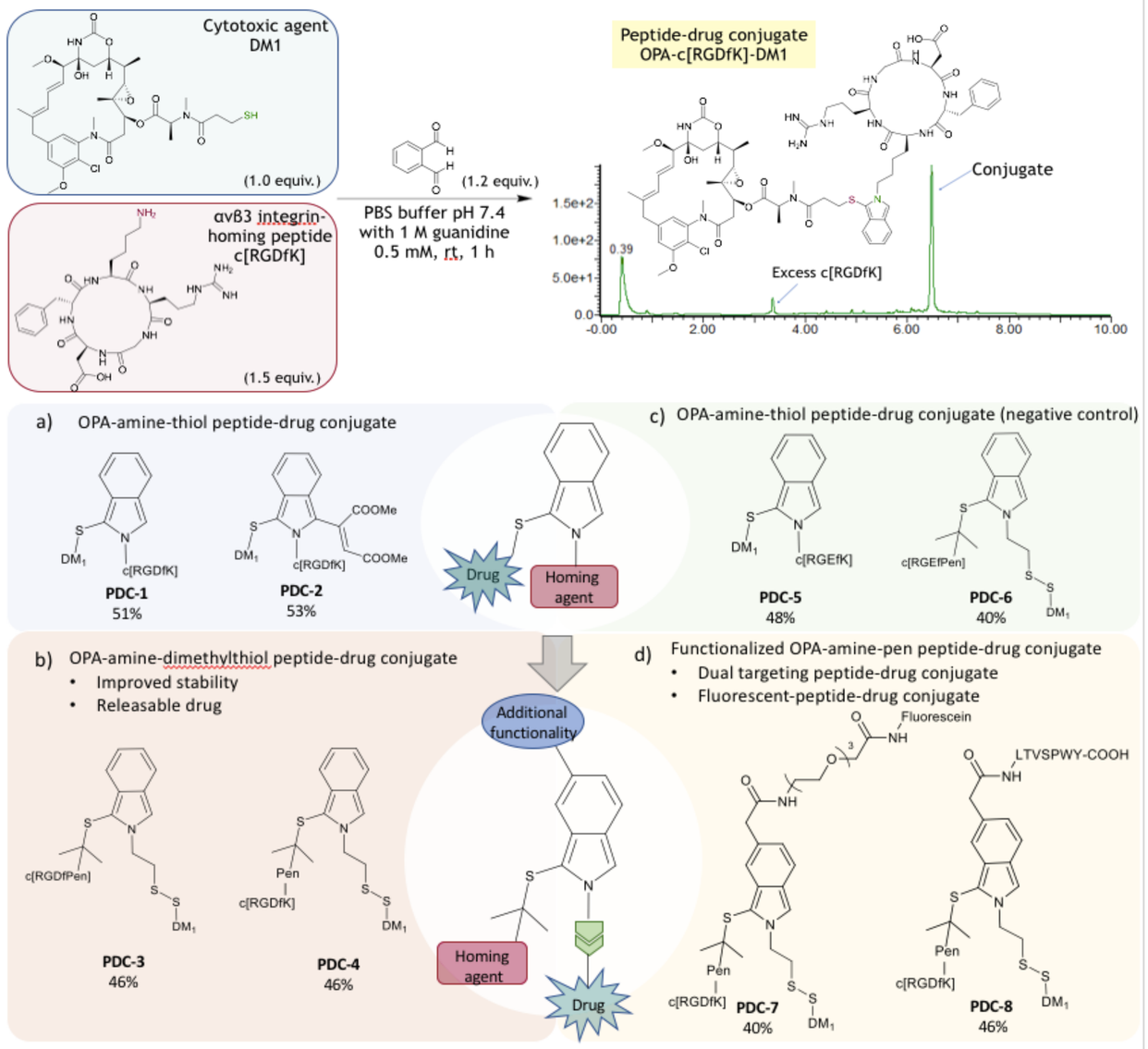
Construction of peptide-peptide conjugates (PPCs) and peptide-drug conjugates (PDCs)

Twelve PPCs were smoothly constructed. All side chain functionalities and S-StBu (Cys) were fully compatible. The 3CR product were isolated by HPLC in all tested cases without observation of the 2CR product (or too minimal to be isolated). On the contrary, an average ratio of 56 : 44 with the 2CR products favoured in the control groups.



LC trace of the crude reaction mixture of PPC-1 under both conditions

We have also extended this method to prepare PDCs with maytansinoid (DM1) as the cytotoxic agent and cyclic RGD peptide c[RGDfK] as the drug delivery vector targeting $\alpha_v\beta_3$ integrin. On glioblastoma U87-MG cell line that expresses high level of $\alpha_v\beta_3$ integrin, our designed PDC has shown up to an 5.95-fold increase in cytotoxicity.



(Top) LC trace of the crude reaction mixture of PDC-1. (Bottom) Structures of the PDCs synthesized and respective isolated yield.

References

1. Tung, C. L., Wong, C. T., Fung, E. Y. & Li, X. *Org. Lett.* **18**, 2600–2603 (2016)
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Acknowledgements

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