## Research theme
- Chemistry, Biology and Geosciences
- Pharmaceuticals

## PI name and contact details
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## Brief summary of PI’s research area/activity/key words
Professor Mauro F. A. Adamo is the Chair of Organic and Medicinal Chemistry at RCSI. He obtained an M.Sc. in Pharmaceutical Chemistry and Technology (maximum cum Laude, 1997, Florence) and Ph.D. in enantioselective catalysis under the supervision of Prof. V.K. Aggarwal (2001, Sheffield). He has spent two years as Post-Doc in Oxford working under the supervision of Prof. J.E. Baldwin before joining RCSI at the end of 2002. The Adamo’s group research is focussed on the development of new synthetic technologies for the production of bioactive natural products and pharmaceutical active ingredients. The group built a strong reputation in the areas of: (a) organocatalysis, (b) enantioselective phase transfer catalysis, (c) multi component reactions, (d) synthesis of modified nucleosides and oligonucleotides and lately in (e) peptide-nucleic acids and their application as miRNA-based antitumor. Prof. Adamo is co-founder of Kelada Pharmachem ltd (www.keladapharmachem.com), a spin-out company which primary mission is the development of green and cost efficient processes for the manufacture of active ingredients.

## Co-PI name and contact details
n/a

## Co-PI web page
n/a

## Title of project
Phase Transfer Catalysed (PTC) Amidation, a green cost efficient procedure for the preparation of peptide drugs and Valsartan

## Brief project description
1. What evidence do you have that there is a commercial opportunity for your idea? The formation of amide bond is one of the most executed in organic synthesis, forming key linkages in peptides, peptide nucleic acids (PNAs), proteins, synthetic polymers and certain pharmaceutical active ingredients (APIs).\(^1\) According to a market report the global peptide therapeutics market was valued at USD 14.1 billion in 2011 and is estimated to reach a market worth USD 25.4 billion in 2018 at a CAGR of 8.7% from 2012 to 2018.\(^2,3\) Sales for marketed peptide therapeutics have also grown to record levels, e.g., 2010 global sales of glatiramer acetate (Copaxone\(^\circledR\); $4.0 billion), leuprolide (Lupron\(^\circledR\); $3.0 billion), octreotide acetate (Sandostatin\(^\circledR\); $1.3 billion), goserelin acetate (Zoladex\(^\circledR\); $1.1 billion), teriparatide (Forteo\(^\circledR\); $830 million), and exenatide (Byetta\(^\circledR\); $710 million).\(^3\) About 20 peptide drug candidates enter clinical trials each year, of which 1-3 gets approved. Several peptide drugs will lose patent protection in the next five years, including Fuzeon, Integrimlin and Angiomax.\(^4\) Amide bond formation is fundamental in pharmaceutical manufacturing. A recent survey conducted by the Green Chemistry Institute Roundtable identified that amide bond formation was utilized in 84 % of a set of drug candidates.\(^4\) The methodology herein proposed will be commercially competitive when considering the savings in reagent costs by not using DCC, HATU or DIPEA which are estimated at $6320 per kg of product.\(^5\) Many companies have recognised the need for new procedures. For example, Aesica, has formed a
2. What is the question that this proposal addresses? Recently, two procedures were reported using an aldehyde and an azide to make amides (Schemes 1 and 2).

In particular Moses\(^{10}\) reported the coupling of several \(\rho\)-NO\(_2\)-aniline and aldehydes under the catalysis of \(N\)-Heterocyclic Carbenes (NHC) and 2 equivalents of NaO\(_2\)Bu. Manetsch\(^{11}\) provided an even simpler condition using NaO\(_2\)Bu as the coupling reagent. In consideration of some mechanistic details provided\(^{10}\), and willing to identify a milder, e.g. less basic, media to perform this reaction we have carried out the reaction of 5 and benzaldehyde under phase transfer catalysis (Scheme 3).

Delightfully, desired compound 2 was obtained either under catalysis of 6 or 7. In our initial proposal, compound 6 could be deprotonated by solid hydroxide and acted as an \textit{in situ} generated source of alkoxyde. Similarly, we have shown that bringing in the organic solution small amounts of hydroxide reproduced the coupling of 5 and benzaldehyde. This result pointed out a nucleophilic catalysis, involving decomposition of azide to form a reactive intermediate trapped by the aldehyde. The moderate 65\% yield reported is unoptimised and should be considered as a starting point for optimisation.

The conditions highlighted in Scheme 3 are sufficiently mild to be used in the context of chiral amide synthesis. Procedures using inorganic salts and a phase transfer agent, e.g. the Merck process to prepare \(\alpha\)-aminoacids,\(^{12}\) showed this set of condition not to affect the stereochemical identity of \(\alpha\)-aminoacids. Similar considerations were not valid for the Moses and Manetsch conditions that used excess of base in homogeneous phase and dimethylformamide (DMF) particularly undesirable in industrial synthesis. In addition \(\text{OH}\), a smaller nucleophile compared to \(\text{O}^{2}\)Bu, is potentially superior in promoting the coupling of hindered azides and aldehydes, an outstanding problem using the existing protocols.\(^{11}\) The question this proposal addresses is therefore: \textit{Could the reaction in Scheme 3 be used as a cost efficient alternative for the preparation of di-peptide 10 or tri-peptides 13 and 17 (Scheme 4)?} In this answer could be answered yes, then a greener, atom economic and cost efficient method to perform peptide synthesis and amide coupling will be delivered.
3. Why is this question significant? The question introduced in Section 2 (Scheme 4) is significant for the following reasons: (i) the proposed peptide synthesis is a two steps sequence in which only Boc protection maybe required; (ii) the proposed synthesis does not require activation of the carboxylate via DCC or HATU; (iii) it produces a significant limited amount of wastes compared to current technology and therefore is greener; (iv) the proposed chemistry is conceived to run in homogeneous condition (for the purpose of this proposal); this is unique and would allow growing a peptide either from the amino or the carboxy terminus, which is impossible using conventional strategies; (v) as the synthesis produces also free carboxy peptides, it is suitable to be applied to segmental synthesis of large peptides. The reaction introduced in Scheme 3 can be used to devise a new the synthesis of API Valsartan (Scheme 5), a generic drug which annual sales raised about $1.6 billion in 2013. The synthesis will not rely on formation of acyl chlorides or DCC or HATU as activators and will therefore be cost efficient and greener compared to current industrial routes.

4. How will the question be addressed and what are the expected outcomes
WP1: Optimization of coupling conditions: the reaction in Scheme 6 involving coupling of 24 (initially X = H) and aromatic aldehydes 25 (initially p-Cl-C6H4 = Ph) will be studied by variation of standard reaction conditions: (i) solvent polarity (CH2Cl2, Et2O, CH3Ocyclopent, Toluene, THF, CHCl3, CH3CN); (ii) Catalyst loading and hydroxide source in each solvent; (iii) temperature; (iv) concentration of reactants and reagents stoichiometry (e.g. amount of base); (v) use of additives, for example substochiometric amounts of water or other protic (CH2OH or CH3OH) co-solvents; (vi) selection of best phase transfer catalysts from a range of tetra-alkyl ammonium or trialkylaryl-ammonium salts. This study will be performed using a statistical experimental design involving variation of one parameter at the time (high/low). Aim of this study is the identification of a set of conditions allowing to obtain compounds 25 in yields superior to 90% and in reaction times of 1-2h maximum. With an optimised set of conditions in hand, we will study the most general case of coupling that involves aliphatic azides and aliphatic aldehydes, as well as, implicitly, the obvious aromatic aliphatic combinations of reagents (Scheme 7). This study will define the scope of reaction and also will be preparatory to the use of aliphatic glycine derivatives 8 and 9 in WP2.

WP2: Optimization of coupling conditions for the preparation of dipeptide 10: 2-azidoacetic acid 8 is commercially available from Sigma Aldrich, although in general, azido compounds could be prepared from corresponding amines via reaction with NaN3 and (CF3SO2)2O. N-Boc-2-aminoacetaldehyde 9 is commercially available from Sigma-Aldrich, although in general, these aldehydes could be prepared from corresponding esters via DIBAL reduction. We will start the investigation of the reaction in Scheme 8 from the best conditions selected through WP2. Compound 10 is an N-Boc protected Gly-Gly dipeptide and is known.
will study the best solvent media, as sometimes dipeptides may have solubility issues, as well as the effect of key physical parameters (temperature, reaction time) on yields. We will also investigate the effect of heating and microwave irradiation. Knowledge of the effect of microwave irradiation on coupling yields and efficiency will turn useful when addressing coupling of larger peptides that may run at significant slower rates. Aim of this study is the identification of a set of conditions allowing to obtain compound 10 in yields superior to 90% and in reaction times of 1h maximum.

**WP3: optimisation of preparation of compound 11:** Preparation of compound 11 involves Boc deprotection and transformation of free primary amine to azido, an event involving nucleophilic addition of the amine to N₃ of sodium azide.⁸ Although these reactions could be performed singularly, we will optimise a one pot procedure in which the amino group will be first generated and then reacted without isolation. This involves an initial treatment with trifluoroacetic acid, an intermediate adjustment of pH to neutrality and then reaction with preformed trifluorosulfonylazide. Aim of this study is the identification of a set of conditions allowing to obtain compounds 11 in yields superior to 95% and in reaction times of 1h maximum. This will be achieved through systematic study of parameter of reactions such as solvent, concentration, temperature of exercise, ratio of reagents.

**WP4: Optimization of coupling conditions for the preparation of tripeptide 13:** The preparation of compound 13 will follow the same strategy highlighted for WP2. Noteworthy this experiment and the WP associated is designed to assess whether or not the stereochemical integrity of compound 12 will be maintained through the synthesis of 13. For this reason, not only we will define condition to obtain 13 in high yields and in short reaction time, but we will also evaluate the stereochemical identity of 13 via chiral HPLC. Compound 13 is N-Boc-Ala-Gly-Gly and is therefore known. In the unlikely case scrambling of stereochemistry will occur, we will adjust the reaction conditions, e.g. will use a less basic media. Compound 12 (BOC-L-alaninal) is commercially available from Acros. However, 12 could be prepared via DIBAL reduction of N-Boc Alanyl methyester.

**WP5: Optimization of coupling conditions for the preparation of tripeptide 15-17:** As discussed in section 3, the proposed chemistry presents the advantage of growing a peptide form the carboxy terminus, which is the objective of this working package (WP5, Scheme 11). The preparation of compound 17 will involve transformation of compound 10 to aldehyde 15. This will be achieved via preparation of 10-methyl ester and subsequent DIBAL reduction. The methyl ester of 10 could be prepared via treatment of a methanolic solution of 10 with catalytic (typically 0.05-0.1 equiv) of acetyl chloride. The reduction using DIBAL will be performed at low temperature (typically -70-78°C). Alternatively, procedures based on reduction to alcohol and re-oxidation via TEMPO could be applied. Compound 16 will be generated from Alanine using literature procedure.⁸ The coupling of 15 and 16 is analogous to coupling of 8 and 9 and its optimisation will be carried out similarly. Aims of this study are: the identification of a set of conditions allowing to obtain compound 15 and 17 in yields superior to 95%.

**WP6: Preparation of Valsartan:** Valsartan will be prepared according to Scheme 5. Hence, Valine 18 will be converted to the correspondant azide 19.¹² Then, azide 19 will be coupled to
commercially available pentanal 20. This step will be carried out initially under the conditions identified through WP2 and then further optimised by variation of solvent, concentration, reaction time and temperature. Amide 21 so obtained will be subjected to reductive amination 14 with aldehyde 22. Aldehyde 22 has been synthesised in three steps from commercially available materials. 15 Initially, the reductive amination will be carried out with benzaldehyde. Once obtained a set of suitable conditions, this step will be carried out using aldehyde 22. Should amination fail, aldehyde 22 will be replaced by benzyl chloride 24 (Figure 1). Aims of this study are the identification of a set of conditions allowing to obtain key amide 21 in yields superior to 90% and to obtain sufficient robust proof of concept for the obtainment of Valsartan 23.

(v) References


(3) http://www.peptidetherapeutics.org/


(5) The bulk price (sourced at metric ton scale, www.alibaba.com) for DCC is 30$/Kg, for HATU is 600$/kg, for DIPEA is 50$/kg. Considering making a decapeptide (9 peptide bonds), 40kg of DCC plus 40kg of HATU and 80kg of DIPEA would be required with a total cost of $7200 per kg of decapeptide. Our chemistry would use for the same decapeptide: 9kg of DIBAL (20$/Kg), 4 kg of NaN₃ (10$/Kg) and 8 kg of (CF₃SO₂)₂O (80$/Kg); therefore, under our condition, the cost of coupling will be about $880 per kg of a decapeptide, providing an forecast save of $6320 per kg of product!!


**Skills & techniques that the student will learn from the project**

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<th>Overview</th>
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<td>This project has been carefully designed to deliver innovative and valuable synthetic and analytical skills. The training components of the project include focus on a variety of specific scientific disciplines, methodologies used in the synthesis and purification of organic compounds, include, controlled atmosphere and temperature reactions, chromatography, $^1$H-NMR, $^{13}$C-NMR, Mass spectroscopy, IR, UV, GC, and HPLC.</td>
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**Transferrable skills training and acquisition.**

In addition to acquiring a complementary and synergistic set of skills in different scientific disciplines, the trainee will also be considerably enhanced through acquisition of “softer” transferrable skills which are applicable in whichever career path student ultimately undertake. Skills such as problem-solving, communication, adaptability, team-working will naturally be developed as a result of their day-to-day work on their individual projects in the context of a larger team in their host research group. It is also intended to gradually increase the responsibilities given to each of the student and thereby build their management, administrative and communication skills – e.g. tutorials, final-year undergraduate project supervision, presentations at conferences and symposia, interaction with patent agents, technology transfer officers etc. These skills will help to cement the trainee experience and ensure thus boosting their employability credentials - independent of geography or sector. |

**Key distinguishing points about this RCSI project**

| The proposed project offers the opportunity to develop a new area of organocatalysis aimed at new synthesis of amide containing bond, e.g. important peptides. |

**Which undergraduate disciplines are relevant for this project**

| Organic synthesis, analytical chemistry. |