

## Solar and Visible Light Assisted Peptide Synthesis

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With the growth in the use of peptides in modern medicine and materials,<sup>1</sup> the development of novel amide- and peptide-bond-forming reactions have been deemed amongst the most important goals for modern organic synthesis.<sup>2</sup> Current peptide coupling methods are extremely powerful and selective, but rely heavily on water-sensitive, high-molecular-weight coupling agents that are often prepared in multiple steps and can lead to large amounts of unwanted co-products.

Transition-metal-catalyzed visible-light photocatalysis has been a mainstay in state-of-the-art synthetic chemistry in the last decade and a half.<sup>3</sup> In many of these reactions, an amine is oxidized to give an imine which may undergo further transformations.<sup>4</sup> This oxidation takes place via a nitrogen radical cation intermediate.

The groups of Professor Alex Szpilman at Ariel University (Ariel, Israel) and Professor Yoav Eichen at the Technion – Israel Institute of Technology (Haifa, Israel) have been interested in visible-light-assisted synthetic chemistry for some time. Professor Szpilman explained: “We started with the pioneering work by e.g. Lautenberger that showed that visible light may activate amine-halo-alkane charge-transfer complexes, resulting in the formation of halogen radicals, nitrogen radical cations, and ultimately imines.<sup>5</sup> Our initial excursion into the use of this principle led to the development of a visible-light-assisted amide bond formation between tri-substituted amines and carboxylic acids.<sup>6</sup> However, in this process one of the three alkyl substituents of the amine was unavoidably lost, via imine formation, as part of the required formal dehydration condensation. The reaction was also very slow. This work was carried out by Dr. Irit Cohen, a former joint Ph.D. student, together with two of the co-authors of this paper, Galit Parvari and Abhaya K. Mishra.”

Professor Szpilman recalls that during this previous research campaign,<sup>6</sup> a second key component that would eventually lead to the present work was the shift from using blue LED as a convenient light source to using the sun instead. “In the course of a discussion on this project, we basically looked out of the window and realized the folly of limiting our work to artificial light sources,<sup>7</sup>” said Professor Szpilman. One of the key advantages of using sunlight is that it is freely available. Its intensity may be easily measured in a given location and may even be predicted for any given day and area. “While some parts of the world have less intense light or short winter days this may be compensated for by choosing the right time of

day or substituting a powerful white LED that generates light in the 330–450 nm region where the 4-dimethylaminopyridine (DMAP)-Br-CCl<sub>3</sub> complex absorbs,” explained Professor Szpilman. He continued: “Importantly, the energy-rich shorter-UV components of sunlight that could lead to decomposition of the reactants or byproducts could be filtered off simply by using a standard Pyrex glass vessel that has a cutoff around 330 nm.”

Despite these attractive features, it was painfully obvious to the researchers that the original process could not be applied to peptide synthesis. Loss of one alkyl substituent from the amine reactant and the long reaction times that might cause epimerization were the main challenges that needed to be overcome. Furthermore, only trialkylamine and dialkylamine charge-transfer complexes with tetrachloromethane absorbed in the near UV-VIS part of the electromagnetic spectrum at synthetically relevant concentrations. “Thus, we arrived at the idea of using DMAP as both the amine responsible for forming the charge-transfer complex that would absorb in the desirable part of the spectrum, and as the precursor for the peptide coupling reagent (Scheme 2),” explained Professor Szpilman.

“The fact that DMAP is a low-molecular-weight and inexpensive amine were important considerations,” remarked Professor Szpilman. He continued: “Furthermore, the fact that DMAP is always included as an acylation catalyst in most peptide coupling methods meant that the number of reagents in our proposed method would actually be reduced compared to standard methods. Indeed, DMAP formed charge-transfer complexes with tetrachloromethane, that when irradiated with sunlight allowed coupling of standard *N*-protected amino acids with methyl and ethyl esters of a second amino acid. However, the reaction was still rather slow. Furthermore, tetrachloromethane was used as a co-solvent in order to compensate for the equilibrium for the charge-transfer complex formation being heavily shifted towards the un-complexed reactants. By substituting bromotrichloromethane for tetrachloromethane, it was possible to reduce the amount of both DMAP and alkyl halide to 10 equivalents and the peptide coupling reactions could now be carried out typically in an hour or less using sunlight. Two equivalents of the amino acid serving as the free amine component were required for optimal yield, but the low cost of amino acid methyl and ethyl ester hydrochlorides, especially when aiming at longer-chain peptides, reduced the significance of this issue. Excess of one

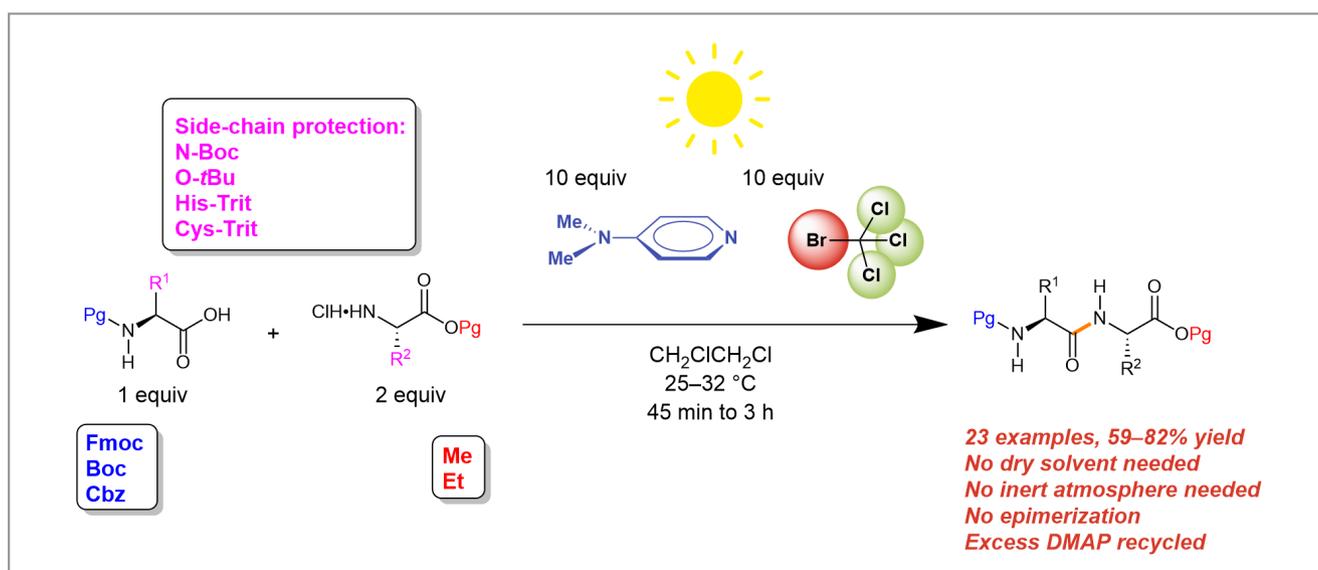
of the peptide-coupling partners is also common in other methods.”

Professor Szpilman advised that the reaction is run best at around 30 °C. The internal temperature at the end of one hour sunlight irradiation was typically 32 °C, but it was found that when the reaction was run on colder days where the internal temperature reached only 20° the reaction was slower and the yields lower. Therefore, the authors recommend using a water bath if the reaction is run using sunlight in cold weather or in the lab using a LED lamp.

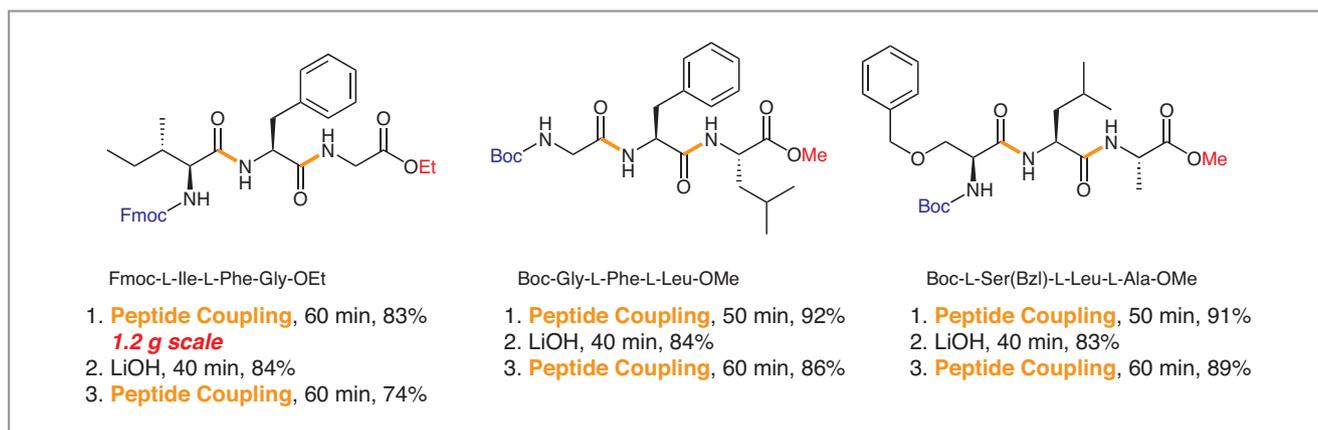
Under such conditions, the reaction is compatible with all the standard protecting groups (Scheme 1 and Figure 1) and all standard amino acids with the exception of methionine.

Professor Szpilman said: “An additional advantage of the weakly absorbing DMAP-BrCCl<sub>3</sub> charge-transfer complex is that it can be scaled up to gram scale (Figure 1) without the need for flow systems that are typically necessary for strongly absorbing transition-metal-catalyzed visible light mediated reactions. On gram-scale, excess DMAP is easily recovered during chromatography.”

“To support our mechanistic hypothesis, we carefully studied the intermediates formed and were lucky to be able to isolate the DMAP-imine adduct shown in Scheme 2,” said Professor Szpilman. He continued: “The proposal was further supported by computational studies on the charge-transfer-complex formation, including the barrier for formation as well



**Scheme 1** Overview of the sunlight-assisted peptide coupling reaction



**Figure 1** Selected examples

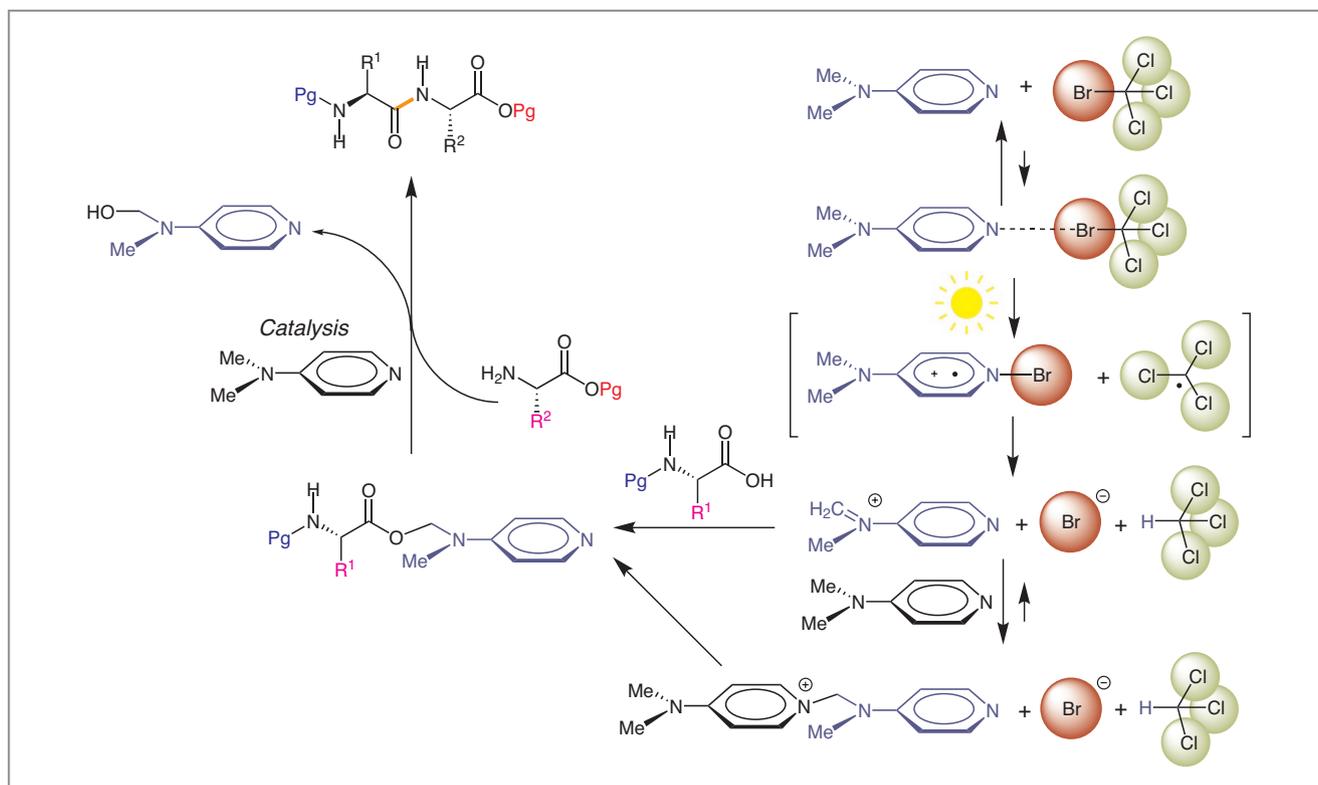
as its lowest-energy triplet state. The two calculation levels of theory chosen for this study were MP4SDTQ/6-31G(2df,p) and m062x/6-31G(2df,p) with an SMD continuum solvation model. The MP4 functional<sup>8</sup> was selected due to findings on a similar system (phosphorus–bromine bonding) and was shown to be considerably superior over MP2.<sup>9</sup> The m062x functional<sup>10</sup> was shown<sup>11</sup> to be among the best available hybrid functionals for calculating halogen-bound systems. Our addition of the solvation model was due to non-negligible interaction between the components, persisting even over large distances (120 Å). These calculations allowed us to establish the structural geometries for the complexes and allowed us to propose that the reaction proceeds through formation of a DMAP–Br radical cation and a trichloromethane radical as intermediates which are subsequently converted into the observed DMAP-imine cation and chloroform, as shown in Scheme 2.”

Professor Szpilman said: “Future developments and applications include developing better charge-transfer-complex partners for DMAP that may allow for reduction of the amounts of these components, as well possibly the amount of the amine-coupling partner. Application of the method to larger scale, namely kilo- or ton-scale peptide coupling, will

likely require use of flow chemistry technology<sup>12</sup> as well as the development of product recovery techniques that do not require chromatographic separation and purification of the products. Nonetheless, the use of an endergonic charge-transfer complex as the photoactive intermediate implies that the absorbing species is always at a low but constant concentration, allowing for deep light penetration to the reacting medium, which is highly advantageous in flow photochemistry.

The application of this method to solid phase would also be important to allow it to be compatible with existing automated peptide synthesis systems, but also for forming well-defined peptide sequences on surfaces in a spatially resolved manner, much like the way DNA chips are made.” He concluded: “Due to the possibility of activating the charge-transfer complex using light sources with spatial control, this method may find use in the preparation of materials, including in 3D printing technologies.”

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**Scheme 2** Proposed mechanism

## REFERENCES

- (1) *Peptide Synthesis and Applications*; K. J. Jensen, P. T. Shelton, S. L. Pedersen (Eds.); Springer: New York, **2013**.
- (2) M. C. Bryan, P. J. Dunn, D. Entwistle, F. Gallou, S. G. Koenig, J. D. Hayler, M. R. Hickey, S. Hughes, M. E. Kopach, G. Moine, P. Richardson, F. Roschangar, A. Steven, F. J. Weiberth *Green Chem.* **2018**, *20*, 5082–5103.
- (3) C. K. Prier, D. A. Rankic, D. W. C. MacMillan *Chem. Rev.* **2013**, *113*, 5322–5363.
- (4) J. W. Beatty, C. R. J. Stephenson *Acc. Chem. Res.* **2015**, *48*, 1474–1484.
- (5) W. J. Lautenberger, E. N. Jones, J. G. Miller *J. Am. Chem. Soc.* **1968**, *90*, 1110–1115.
- (6) (a) I. Cohen, A. K. Mishra, G. Parvari, R. Edrei, M. Dantus, Y. Eichen, A. M. Szpilman *Chem. Commun.* **2017**, *53*, 10128–10131. (b) A. K. Mishra, G. Parvari, I. Cohen, N. Fridman, Y. Eichen, A. M. Szpilman *J. Coord. Chem.* **2018**, *71*, 2082–2089.
- (7) D. M. Schultz, T. P. Yoon *Science* **2014**, *343*, 1239176.
- (8) R. Krishnan, M. J. Frisch, J. A. Pople *J. Chem. Phys.* **1980**, *72*, 4244–4245.
- (9) H. C. Georg, E. E. Fileti, T. Malaspina *J. Mol. Model.* **2013**, *19*, 329–336.
- (10) Y. Zhao, D. G. Truhlar *Theor. Chem. Acc.* **2008**, *120*, 215–241.
- (11) S. Kozuch, J. M. L. Martin *J. Chem. Theory Comput.* **2013**, *9*, 1918–1931.
- (12) (a) Z. J. Garlets, J. D. Nguyen, C. R. J. Stephenson *Isr. J. Chem.* **2014**, *54*, 351–360. (b) N. Hartrampf, A. Saebi, M. Poskus, Z. P. Gates, A. J. Callahan, A. E. Cowfer, S. Hanna, S. Antilla, C. K. Schissel, A. J. Quartararo, X. Ye, A. J. Mijalis, M. D. Simon, A. Loas, S. Liu, C. Jessen, T. E. Nielsen, B. L. Pentelute *Science* **2020**, *368*, 980–987.

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