On-resin Photochemical Decarboxylative Arylation of Peptides

Sunit Pal[a], Joseph Openy[a], Adrian Krzyzanowski[b]†, Anaïs Noisier[c], Peter 't Hart*[a]

[a]Chemical Genomics Centre, Max Planck Institute of Molecular Physiology, 44227, Dortmund, Germany

[b]Department of Chemical Biology, Max Planck Institute of Molecular Physiology, 44227, Dortmund, Germany

[c]Medicinal Chemistry, Research and Early Development Cardiovascular, Renal and Metabolism BioPharmaceutical R&D, AstraZeneca, Gothenburg, Sweden

ABSTRACT: Here we describe the application of photochemical decarboxylative arylation as a late-stage modification reaction for peptides. The reaction uses redox active esters of aspartic acid and glutamic acid on solid phase to provide analogues of aromatic amino acids. By using aryl bromides as arylation reagents a wide variety of amino acids can be accessed without having to synthesize them individually in solution. The reaction is compatible with proteinogenic amino acids and was used to perform a structure-activity relationship study on a PRMT5 binding peptide.

The increasing interest in peptides as a modality in drug discovery creates a demand for optimized synthetic methods to prepare unnatural amino acids (UAAs).[1,2] UAAs can enhance the affinity of a peptide for its target by making new interactions that cannot be achieved with proteinogenic amino acids as well as provide proteolytic stability.[3–9] Traditionally, UAAs suitably protected for Fmoc SPPS are prepared via multistep organic synthetic routes which often require thorough optimization to produce high optical purity.[10] Instead, by leveraging the existing chirality of commercially available natural amino acid, late-stage functionalization (LSF) approaches allow to rapidly generate an array of modified peptides in enantiomerically pure form. In the past decade, several protocols have been developed for the LSF of peptides.[11–15] While solution phase LSF was quite successful for short peptides, only exquisitely selective methods can be employed with more complex peptides. Furthermore, peptides longer than 5-6 amino acids face solubility problems in organic solvents.[16,17] Therefore, methodologies to perform LSF on solid-phase open up the possibility to modify larger peptides. In addition, using on-resin approaches, site-selectivity is easily achieved via use of orthogonally protected natural amino acids which, after deprotection, are readily converted to the desired functionality. While cumbersome in solution phase, these steps are rendered straightforward on solid-phase as the removal of reagents and high-boiling solvents only require simple filtration.

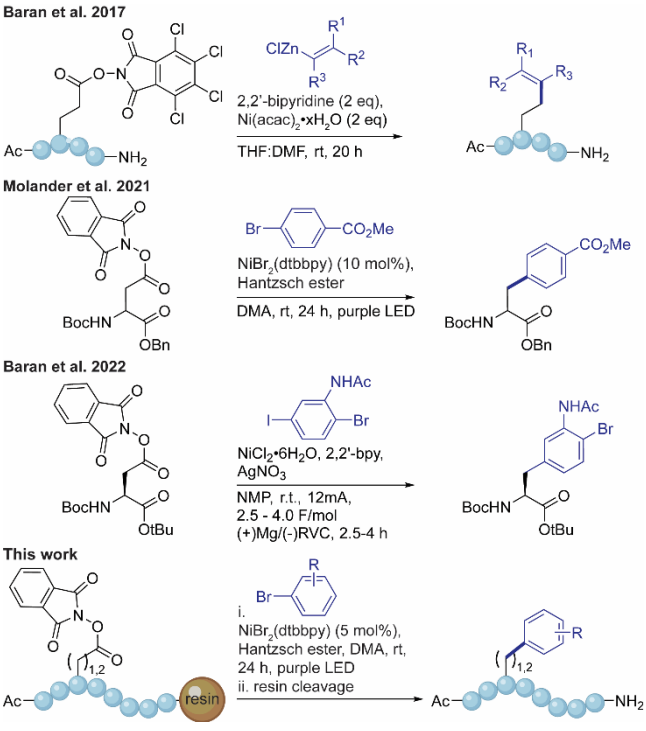
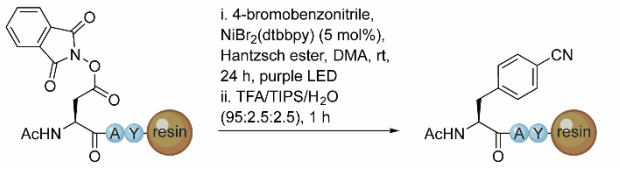


Figure 1. Previously reported and current decarboxylative strategies of redox active esters to functionalize amino acids and peptides.

One class of organic transformations that can be exploited for peptide LSF are decarboxylative Csp^3-Csp^2 cross-coupling (DCC) reactions of redox active esters (RAE) (Figure 1).[18–20] In the context of peptides DCC of aspartic acid (Asp) or glutamic acid (Glu) provides straightforward access to phenylalanine and homophenylalanine analogues.[21] Optimization of these residues is often beneficial for peptide affinity and stability as is illustrated by the clinically approved degarelix as well as the tricyclic peptidic Proprotein convertase subtilisin-like/kexin type 9 (PCSK9) inhibitor currently in clinical trials.[6,7] The Baran group reported a Csp^3-Csp^2 DCC reaction using nickel catalysis and organozinc reagents.[22] Later the Molander group reported electron donor-acceptor (EDA) formation between a RAE and the inexpensive Hantzsch ester (HE) which allowed them to undergo a photoinduced radical mediated Csp^3-Csp^2 DCC to functionalize single amino acids.[23] More recently, the Baran group demonstrated the use of electrochemistry on RAE of Asp and Glu to yield aromatic amino acids.[24] We hypothesized that from these protocols the photochemical method from the Molander group was most applicable to derivatize peptides directly on solid-phase. However, a limitation of the activation of Asp as a RAE is that it will rapidly undergo aspartimide formation with the neighbouring amino acid in the peptide sequence. Here, we first provided optimized reaction conditions to mitigate aspartimide or pyroglutamate (from Glu) forming side reactions. Thereafter, the key solid-phase photochemistry reaction conditions were screened and improved conditions were identified which enabled the functionalization of peptides in good yields. The substrate scope was also investigated by reacting a broad selection of aryl bromides (Ar-Br) with a resin-bound model peptide. In addition, we tested the compatibility of amino acids and their side chain protecting groups as well as various linker-resins commonly employed in Fmoc SPPS with the photochemistry conditions. Finally, we modified biologically relevant peptides, including a PRMT5 binding peptide to demonstrate the broad applicability of the current methodology.

Table 1. Optimization of on-resin decarboxylative photochemistry.



Entry	Catalyst (mol%)	Ar-Br (eq.)	HE (eq.)	Yield (%)[a]
1	40	4	4	19
2	20	4	4	23
3	10	4	4	30
4	5	4	4	32
5	5	2	4	21
6	5	4	2	19
7	5 ($NiBr_2 \cdot 3H_2O$)	4	4	NR
8	5 (No cat.)	4	4	NR
9	5 (No LED)	4	4	NR
10	5 (Blue LED)	4	4	NR
11	5 (Rink PEG resin)	4	4	13
12[b]	5 (Wang PS resin)	4	4	19

[a]: NMR yields calculated over 10 steps starting from tyrosine loading.
[b]: Provides peptide **1-OH**. NR: No reaction.

Initially, we designed a resin-bound model tripeptide to test the photochemical LSF. The tripeptide contains the Asp to be modified at the *N*-terminus and a tyrosine (Tyr) residue at the *C*-terminus for easy detection during HPLC analysis and purification. Briefly, the *N*-terminally acetylated tripeptide was synthesized on Rink amide polystyrene (PS) resin, followed by removal of the allyl side chain protecting group of Asp by treatment with $Pd(PPh_3)_4$ and phenylsilane (Scheme S1). Next, *N*-hydroxypthalimide (NHPI) was to be coupled to the free acid to obtain the activated ester but this was complicated by aspartimide formation.[25,26] Solid-phase activation of Asp and Glu side chain carboxylic acids with NHPI has previously been performed using excesses of a dicarbodiimide or hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) and various bases.[27,28] However, in these reports, this was performed with proline as the neighbouring amino acid which eliminates the possibility for aspartimide formation but limits the applicability to peptide sequences with an Asp-Pro pair. Instead, we wanted to create a general protocol that avoids this side reaction irrespective of the nature of the next amino acid. We hypothesized that the equivalents of base used in this reaction could be fine-tuned and therefore used NHPI (8 eq), HATU (4 eq) and varying amounts of diisopropylethylamine (DIPEA). Interestingly, 8 eq led to the substantial aspartimide formation, but we could not observe any when using 6 eq (Figure S2). Using 4 eq of DIPEA led to incomplete conversion of the free carboxylic acid. Note that, isolation of the activated ester was not possible due to its instability during prolonged exposure to HPLC buffers during preparative purification.

With reliable conditions for the formation of the starting material in hand, we tested the EDA-complex mediated cross-coupling reaction on the resin-bound peptide with 4-bromobenzonitrile by adapting the protocol by Molander *et al*.[23] The inherent reduced mass transfer effect of the solid support prompted us to use slight excess amounts of Ar-Br and HE. We observed satisfactory conversion of the starting material to **1** by HPLC chromatography and the highest yields when using 4 eq of Ar-Br and HE with 5 mol % Ni catalyst (Table 1, entry 1-4, Figure S3). The yields for the reaction were calculated over all 10 steps after Tyr loading. These are similar or higher than previously reported cross-coupling reactions directly on peptides on solid-phase.[22,27,29] Interestingly, the cross-coupling reaction was catalytic and a reduced yield was observed at higher catalyst loadings unlike other reports where an equivalent amount of nickel catalyst or more was required to successfully convert the resin-bound peptide.[22,27] Lowering the amount of either Ar-Br or HE reduced the overall yield of the reaction (entries 5/6). Performing the reaction using $NiBr_2{\cdot}3H_2O$ salt instead of the $NiBr_2$(dtbbpy) catalyst or removing the catalyst altogether led to a complete loss of conversion (entries 7/8). The light source also played a key role in generating the radical of the resin-bound peptide since only purple LEDs could promote the reaction while the use of blue LED light or absence of light did not yield any product (entry 9/10). To investigate the compatibility with alternative linker-resins, we also tested Rink amide polyethyleneglycol (PEG) resin as well as Wang PS to provide a free *C*-terminus (Table 1, entries 11/12). Both alternatives were less efficient but still afforded the desired products.

After determining the optimal reaction conditions for the photochemical arylation, a variety of aryl bromides with different substituents were tested to explore the scope of the methodology (Figure 2). Substrates with electron-withdrawing groups in para position (**1**, **2**, **6**), provided good yields of 23-36% over 10 steps. Substitutions at both ortho and meta position were also successful for the cross-coupling reaction providing compound **3** and **4**. Additionally, the activated ester underwent cross-coupling with aryl bromides containing electron-donating groups or completely unactivated bromobenzene to obtain **5, 9**, **14**, and **15** respectively in moderate to good yields. Di-substitution of the phenyl ring was possible and provided molecule **7**. However, a reduced yield (16%) was obtained possibly due to steric encumbrance during *in-situ* formation of the activated Ni-complex as proposed in the literature.[23] Using the methodology our model peptide could be efficiently functionalized with polycyclic aromatic bromides to obtain peptide **8**, **10,** and **13**. These results demonstrated that the electronics at the C-Br bond of the aryl bromide reactant do not extensively influence the Ni-catalysed cross-coupling reaction.

Our next aim was to evaluate our decarboxylative arylation protocol on a resin-bound peptide containing glutamic acid (Glu), as well as the D-enantiomers of Asp and Glu. Such transformations would provide access to analogues of D-phenylalanine and both L- and D-analogues of homophenylalanine, all of which are often not commercially available or very costly but can be valuable in the SAR study of a peptide.[30–32] A subset of aryl bromides was tested and found to successfully afford the functionalized peptides (**17**-**34**) with good yields (Figure 2). Surprisingly, D-Glu performed better in various cases suggesting that it was the least affected by steric occlusion in the context of this model peptide.

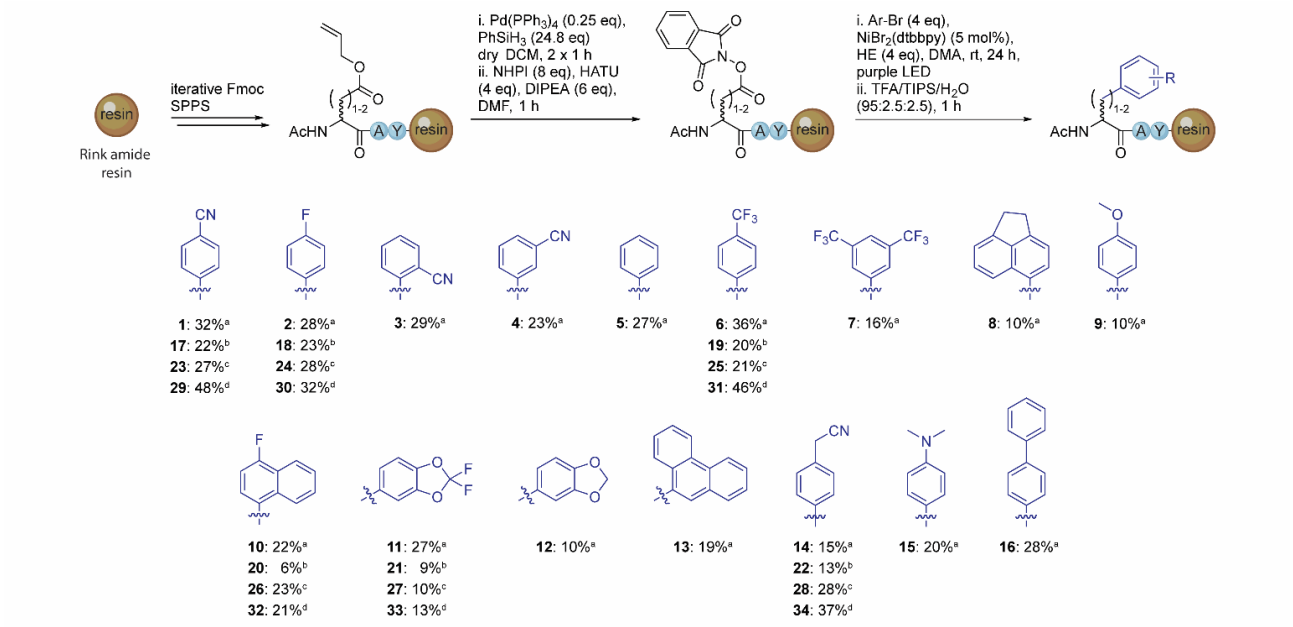


Figure 2. Scope of the decarboxylative photochemical reaction of the resin-bound peptides for the Csp^3-Csp^2 cross-coupling reactions. Yields were determined by ^{1}H-NMR calculated over 10 steps starting from tyrosine loading. [a]: from L-Asp, [b]: from D-Asp, [c]: from L-Glu, [d]: from D-Glu.

Various proteinogenic amino acids (eg. Cys, Trp, Met, His) were utilized in the past for photochemical diversification due to their reactive side-chains functionalities.[14] To probe whether our method was compatible with these we synthesized model tetrapeptides where one of these amino acids was placed at the *N*-terminus (Figure 3A). After RAE formation and photochemical arylation the desired modified peptides were obtained in all cases without affecting the added side-chain functionality. These results indicated the compatibility with these amino acids but also that the amino acid to be modified does not need to be *N*-terminal. Note that the yields were calculated over 12 steps from first amino acid loading.

To further demonstrate the applicability of the methodology we modified three biologically active peptides. The NA-1 peptide **39** was synthesized on Wang resin and the *N*-terminal Fmoc-group was maintained (Figure 3B).[33] The on-resin photochemistry was found to be compatible with the Fmoc group which would also allow further extension of a peptide after the decarboxylative arylation step. The APC-Asef inhibitor peptide **40** and ACE2 derived peptide **41** were synthesized on Rink amide resin prior to LSF and both of them underwent decarboxylative arylation with good yields (Figure 3B).[34,35]

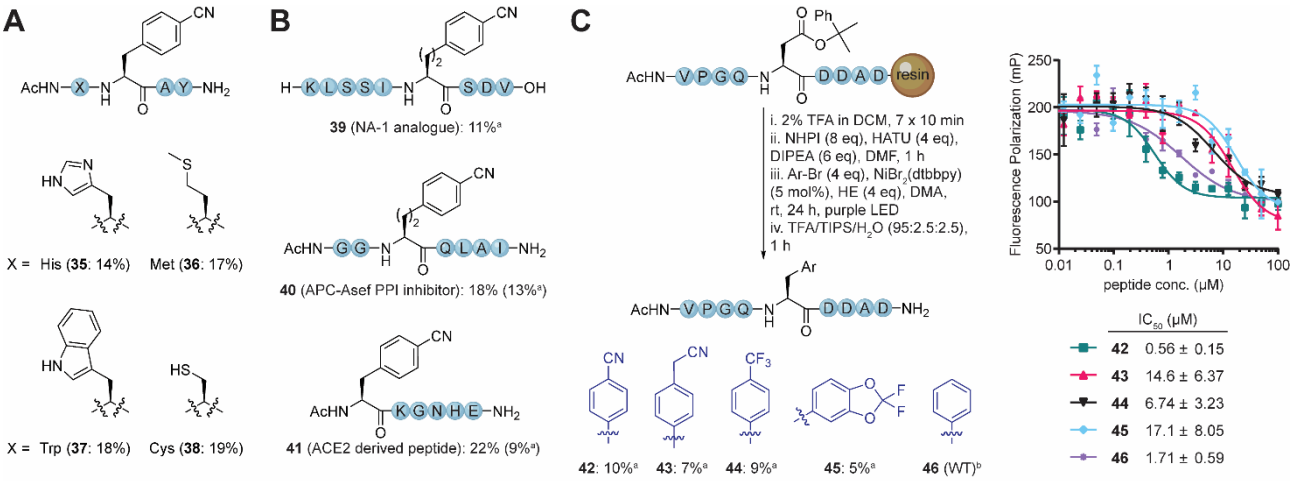


Figure 3: A) Compatibility of sensitive amino acids with the photochemical decarboxylation and arylation reaction. Yields were determined by ^{1}H-NMR over 12 steps. B) Modification of biologically active peptides. Yields were determined by ^{1}H-NMR over all steps starting from first amino acid loading C) SAR study of a PRMT5 binding peptide and evaluation via competitive fluorescence polarization assay. [a]: isolated yield, [b]: peptide prepared using regular SPPS.

Next, we modified a peptide that we previously described as an inhibitor of PRMT5 protein-protein interactions to demonstrate the use of the described method in a SAR study.[36] The peptide is derived from the PRMT5 binding protein RioK1 and contains a phenyl alanine that is important for high affinity binding. We prepared a peptide with an Asp residue installed in its place, split the batch, and reacted each portion with various aryl bromides to rapidly generate analogues with diverse phenylalanine derivatives (Figure 3C). For these peptides Asp was protected using the 2-phenylisopropyl group to avoid aspartimide formation during peptide extension which occurs while using the allyl group.[37] The prepared peptides were then tested in a competitive fluorescence polarization assay against a tracer peptide binding to the PRMT5/MEP50 complex.[36] Satisfyingly, peptide **42** showed a three-fold improvement in IC_{50} in comparison to the wildtype peptide **46**.

We demonstrated that EDA driven decarboxylative Ni-catalyzed Csp^3-Csp^2 cross-coupling reactions can be translated to solid-phase peptide chemistry. First, we refined the protocol for the activation of the side-chain carboxylic group with NHPI, that was previously plagued by undesired aspartimide/pyroglutamate formation. Thus, we provide a methodology which alleviates the restrictions in terms of neighbouring amino acids and is therefore suitable for any peptide sequence. Next, we optimized conditions for the cross-coupling using a simple photochemical set-up making it applicable in general peptide chemistry labs. The obtained yields are typical for peptide synthesis providing sufficient material for biological evaluation. The method widely expands access to diverse phenylalanine and homophenylalanine analogues by using low-cost aryl bromides. By avoiding multistep solution phase amino acids synthesis, it saves on large amounts of reagents and solvents providing a greener alternative. Furthermore, the approach does not require any directing groups and is independent of the peptide sequence making it applicable not only to biologically active peptides, but also to other areas of peptide chemistry such as materials science.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, synthesis of peptides, purity, and characterization data, as well as the description of the FP assay are included in the supporting information. This material is available free of charge via the internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

E-mail: peter.t-hart@mpi-dortmund.mpg.de

Present Addresses

† GSK Medicines Research Centre, Gunnels Wood Road, SG1 2NY Stevenage, U.K.

Notes

The authors declare no competing financial interests.

ACKNOWLEDGMENT

We kindly acknowledge the Protein Chemistry Facility of the Max Planck Institute for expression of the PRMT5:MEP50 complex. We would like to acknowledge Christiane Heitbrink (TU Dortmund) for measuring HRMS data.

REFERENCES

(1) Henninot, A.; Collins, J. C.; Nuss, J. M. The Current State of Peptide Drug Discovery: Back to the Future? *J. Med. Chem.* **2018**, *61* (4), 1382–1414. https://doi.org/10.1021/acs.jmedchem.7b00318.

(2) Guillemard, L.; Kaplaneris, N.; Ackermann, L.; Johansson, M. J. Late-Stage C–H Functionalization Offers New Opportunities in Drug Discovery. *Nat. Rev. Chem.* **2021**, *5* (8), 522–545. https://doi.org/10.1038/s41570-021-00300-6.

(3) Vlieghe, P.; Lisowski, V.; Martinez, J.; Khrestchatisky, M. Synthetic Therapeutic Peptides: Science and Market. *Drug Discov. Today* **2010**, *15* (1), 40–56. https://doi.org/10.1016/j.drudis.2009.10.009.

(4) Blaskovich, M. A. T. Unusual Amino Acids in Medicinal Chemistry. *J. Med. Chem.* **2016**, *59* (24), 10807–10836. https://doi.org/10.1021/acs.jmedchem.6b00319.

(5) Gentilucci, L.; De Marco, R.; Cerisoli, L. Chemical Modifications Designed to Improve Peptide Stability: Incorporation of Non-Natural Amino Acids, Pseudo-Peptide Bonds, and Cyclization. *Curr. Pharm. Des.* **2010**, *16* (28), 3185–3203. https://doi.org/10.2174/138161210793292555.
(6) Tucker, T. J.; Embrey, M. W.; Alleyne, C.; Amin, R. P.; Bass, A.; Bhatt, B.; Bianchi, E.; Branca, D.; Bueters, T.; Buist, N.; Ha, S. N.; Hafey, M.; He, H.; Higgins, J.; Johns, D. G.; Kerekes, A. D.; Koeplinger, K. A.; Kuethe, J. T.; Li, N.; Murphy, B.; Orth, P.; Salowe, S.; Shahripour, A.; Tracy, R.; Wang, W.; Wu, C.; Xiong, Y.; Zokian, H. J.; Wood, H. B.; Walji, A. A Series of Novel, Highly Potent, and Orally Bioavailable Next-Generation Tricyclic Peptide PCSK9 Inhibitors. *J. Med. Chem.* **2021**, *64* (22), 16770–16800. https://doi.org/10.1021/acs.jmedchem.1c01599.
(7) Doehn, C.; Sommerauer, M.; Jocham, D. Degarelix and Its Therapeutic Potential in the Treatment of Prostate Cancer. *Clin. Interv. Aging* **2009**, *4*, 215–223. https://doi.org/10.2147/cia.s3503.
(8) Drucker, D. J. Advances in Oral Peptide Therapeutics. *Nat. Rev. Drug Discov.* **2020**, *19* (4), 277–289. https://doi.org/10.1038/s41573-019-0053-0.
(9) Ruttekolk, I. R.; Witsenburg, J. J.; Glauner, H.; Bovee-Geurts, P. H. M.; Ferro, E. S.; Verdurmen, W. P. R.; Brock, R. The Intracellular Pharmacokinetics of Terminally Capped Peptides. *Mol. Pharm.* **2012**, *9* (5), 1077–1086. https://doi.org/10.1021/mp200331g.
(10) A. King, T.; Kandemir, J. M.; J. Walsh, S.; R. Spring, D. Photocatalytic Methods for Amino Acid Modification. *Chem. Soc. Rev.* **2021**, *50* (1), 39–57. https://doi.org/10.1039/D0CS00344A.
(11) Sengupta, S.; Mehta, G. Late Stage Modification of Peptides via CH Activation Reactions. *Tetrahedron Lett.* **2017**, *58* (14), 1357–1372. https://doi.org/10.1016/j.tetlet.2017.02.069.
(12) Zhan, B.-B.; Jiang, M.-X.; Shi, B.-F. Late-Stage Functionalization of Peptides via a Palladium-Catalyzed C(Sp 3)–H Activation Strategy. *Chem. Commun.* **2020**, *56* (90), 13950–13958. https://doi.org/10.1039/D0CC06133F.
(13) Romero, E.; Jones, B. S.; Hogg, B. N.; Rué Casamajo, A.; Hayes, M. A.; Flitsch, S. L.; Turner, N. J.; Schnepel, C. Enzymatic Late-Stage Modifications: Better Late Than Never. *Angew. Chem. Int. Ed.* **2021**, *60* (31), 16824–16855. https://doi.org/10.1002/anie.202014931.
(14) Hu, C.; Chen, Y. Biomolecule-Compatible Chemical Bond-Formation and Bond-Cleavage Reactions Induced by Visible Light. *Tetrahedron Lett.* **2015**, *56* (7), 884–888. https://doi.org/10.1016/j.tetlet.2014.12.034.
(15) Openy, J.; Amrahova, G.; Chang, J.-Y.; Noisier, A.; Hart, P. 't. Solid-Phase Peptide Modification via Deaminative Photochemical Csp3-Csp3 Bond Formation Using Katritzky Salts. *Chem Eur J* **2022**, *28* (39), e202201121. https://doi.org/10.1002/chem.202201121.
(16) Narita, M.; Honda, S.; Umeyama, H.; Obana, S. The Solubility of Peptide Intermediates in Organic Solvents. Solubilizing Potential of Hexafluoro-2-Propanol. *Bull. Chem. Soc. Jpn.* **1988**, *61* (1), 281–284. https://doi.org/10.1246/bcsj.61.281.
(17) Narita, M.; Ishikawa, K.; Chen, J.-Y.; Kim, Y. Prediction and Improvement of Protected Peptide Solubility in Organic Solvents*. *Int. J. Pept. Protein Res.* **1984**, *24* (6), 580–587. https://doi.org/10.1111/j.1399-3011.1984.tb03163.x.
(18) Dasgupta, A.; Stefkova, K.; Babaahmadi, R.; Yates, B. F.; Buurma, N. J.; Ariafard, A.; Richards, E.; Melen, R. L. Site-Selective Csp3–Csp/Csp3–Csp2 Cross-Coupling Reactions Using Frustrated Lewis Pairs. *J. Am. Chem. Soc.* **2021**, *143* (11), 4451–4464. https://doi.org/10.1021/jacs.1c01622.
(19) Tabassum, S.; Zahoor, A. F.; Ahmad, S.; Noreen, R.; Khan, S. G.; Ahmad, H. Cross-Coupling Reactions towards the Synthesis of Natural Products. *Mol. Divers.* **2022**, *26* (1), 647–689. https://doi.org/10.1007/s11030-021-10195-6.
(20) Hutchinson, J. H.; Rowbottom, M. W.; Lonergan, D.; Darlington, J.; Prodanovich, P.; King, C. D.; Evans, J. F.; Bain, G. Small Molecule Lysyl Oxidase-like 2 (LOXL2) Inhibitors: The Identification of an Inhibitor Selective for LOXL2 over LOX. *ACS Med. Chem. Lett.* **2017**, *8* (4), 423–427. https://doi.org/10.1021/acsmedchemlett.7b00014.
(21) Zuo, Z.; Cong, H.; Li, W.; Choi, J.; Fu, G. C.; MacMillan, D. W. C. Enantioselective Decarboxylative Arylation of α-Amino Acids via the Merger of Photoredox and Nickel Catalysis. *J. Am. Chem. Soc.* **2016**, *138* (6), 1832–1835. https://doi.org/10.1021/jacs.5b13211.
(22) Edwards, J. T.; Merchant, R. R.; McClymont, K. S.; Knouse, K. W.; Qin, T.; Malins, L. R.; Vokits, B.; Shaw, S. A.; Bao, D.-H.; Wei, F.-L.; Zhou, T.; Eastgate, M. D.; Baran, P. S. Decarboxylative Alkenylation. *Nature* **2017**, *545* (7653), 213–218. https://doi.org/10.1038/nature22307.
(23) Kammer, L. M.; Badir, S. O.; Hu, R.-M.; Molander, G. A. Photoactive Electron Donor–Acceptor Complex Platform for Ni-Mediated C(Sp 3)–C(Sp 2) Bond Formation. *Chem. Sci.* **2021**, *12* (15), 5450–5457. https://doi.org/10.1039/D1SC00943E.
(24) Palkowitz, M. D.; Laudadio, G.; Kolb, S.; Choi, J.; Oderinde, M. S.; Ewing, T. E.-H.; Bolduc, P. N.; Chen, T.; Zhang, H.; Cheng, P. T. W.; Zhang, B.; Mandler, M. D.; Blasczak, V. D.; Richter, J. M.; Collins, M. R.;

Schioldager, R. L.; Bravo, M.; Dhar, T. G. M.; Vokits, B.; Zhu, Y.; Echeverria, P.-G.; Poss, M. A.; Shaw, S. A.; Clementson, S.; Petersen, N. N.; Mykhailiuk, P. K.; Baran, P. S. Overcoming Limitations in Decarboxylative Arylation via Ag–Ni Electrocatalysis. *J. Am. Chem. Soc.* **2022**, *144* (38), 17709–17720. https://doi.org/10.1021/jacs.2c08006.
(25) Malins, L. R. Decarboxylative Couplings as Versatile Tools for Late-Stage Peptide Modifications. *Pept. Sci.* **2018**, *110* (3), e24049. https://doi.org/10.1002/pep2.24049.
(26) Zhang, Y.; Ma, D.; Zhang, Z. Utilization of Photocatalysts in Decarboxylative Coupling of Carboxylic N-Hydroxyphthalimide (NHPI) Esters. *Arab. J. Chem.* **2022**, *15* (7), 103922. https://doi.org/10.1016/j.arabjc.2022.103922.
(27) Qin, T.; Cornella, J.; Li, C.; Malins, L. R.; Edwards, J. T.; Kawamura, S.; Maxwell, B. D.; Eastgate, M. D.; Baran, P. S. A General Alkyl-Alkyl Cross-Coupling Enabled by Redox-Active Esters and Alkylzinc Reagents. *Science* **2016**, *352* (6287), 801–805. https://doi.org/10.1126/science.aaf6123.
(28) Elbaum, M. B.; Elkhalifa, M. A.; Molander, G. A.; Chenoweth, D. M. Solid-Phase Photochemical Peptide Homologation Cyclization. *Org. Lett.* **2022**, *24* (28), 5176–5180. https://doi.org/10.1021/acs.orglett.2c02012.
(29) Twitty, J. C.; Hong, Y.; Garcia, B.; Tsang, S.; Liao, J.; Schultz, D. M.; Hanisak, J.; Zultanski, S. L.; Dion, A.; Kalyani, D.; Watson, M. P. Diversifying Amino Acids and Peptides via Deaminative Reductive Cross-Couplings Leveraging High-Throughput Experimentation. *J. Am. Chem. Soc.* **2023**, *145* (10), 5684–5695. https://doi.org/10.1021/jacs.2c11451.
(30) Solanas, C.; de la Torre, B. G.; Fernández-Reyes, M.; Santiveri, C. M.; Jiménez, M. Á.; Rivas, L.; Jiménez, A. I.; Andreu, D.; Cativiela, C. Therapeutic Index of Gramicidin S Is Strongly Modulated by D-Phenylalanine Analogues at the β-Turn. *J. Med. Chem.* **2009**, *52* (3), 664–674. https://doi.org/10.1021/jm800886n.
(31) Drygała, P.; Olejnik, J.; Mazur, A.; Kierus, K.; Jankowski, S.; Zimecki, M.; Zabrocki, J. Synthesis and Immunosuppressive Activity of Cyclolinopeptide A Analogues Containing Homophenylalanine. *Eur. J. Med. Chem.* **2009**, *44* (9), 3731–3738. https://doi.org/10.1016/j.ejmech.2009.03.037.
(32) Kuhn, D. J.; Chen, Q.; Voorhees, P. M.; Strader, J. S.; Shenk, K. D.; Sun, C. M.; Demo, S. D.; Bennett, M. K.; van Leeuwen, F. W. B.; Chanan-Khan, A. A.; Orlowski, R. Z. Potent Activity of Carfilzomib, a Novel, Irreversible Inhibitor of the Ubiquitin-Proteasome Pathway, against Preclinical Models of Multiple Myeloma. *Blood* **2007**, *110* (9), 3281–3290. https://doi.org/10.1182/blood-2007-01-065888.
(33) Milani, D.; Cross, J. L.; Anderton, R. S.; Blacker, D. J.; Knuckey, N. W.; Meloni, B. P. Neuroprotective Efficacy of Poly-Arginine R18 and NA-1 (TAT-NR2B9c) Peptides Following Transient Middle Cerebral Artery Occlusion in the Rat. *Neurosci. Res.* **2017**, *114*, 9–15. https://doi.org/10.1016/j.neures.2016.09.002.
(34) Jiang, H.; Deng, R.; Yang, X.; Shang, J.; Lu, S.; Zhao, Y.; Song, K.; Liu, X.; Zhang, Q.; Chen, Y.; Chinn, Y. E.; Wu, G.; Li, J.; Chen, G.; Yu, J.; Zhang, J. Peptidomimetic Inhibitors of APC–Asef Interaction Block Colorectal Cancer Migration. *Nat. Chem. Biol.* **2017**, *13* (9), 994–1001. https://doi.org/10.1038/nchembio.2442.
(35) Odolczyk, N.; Marzec, E.; Winiewska-Szajewska, M.; Poznański, J.; Zielenkiewicz, P. Native Structure-Based Peptides as Potential Protein–Protein Interaction Inhibitors of SARS-CoV-2 Spike Protein and Human ACE2 Receptor. *Molecules* **2021**, *26* (8), 2157. https://doi.org/10.3390/molecules26082157.
(36) Krzyzanowski, A.; Esser, L. M.; Willaume, A.; Prudent, R.; Peter, C.; ‘t Hart, P.; Waldmann, H. Development of Macrocyclic PRMT5–Adaptor Protein Interaction Inhibitors. *J. Med. Chem.* **2022**, *65* (22), 15300–15311. https://doi.org/10.1021/acs.jmedchem.2c01273.
(37) Yue, C.; Thierry, J.; Potier, P. 2-Phenyl Isopropyl Esters as Carboxyl Terminus Protecting Groups in the Fast Synthesis of Peptide Fragments. *Tetrahedron Lett.* **1993**, *34* (2), 323–326. https://doi.org/10.1016/S0040-4039(00)60578-6.