Simple Synthesis of Unnatural Amino Acids via Ni/Ag-Electrocatalytic Cross-Coupling

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ABSTRACT: A simple protocol is outlined herein for rapid access to enantiopure unnatural amino acids from trivial glutamate and aspartate precursors. The method relies on Ag/Ni-electrocatalytic decarboxylative coupling and can be rapidly conducted in parallel (24 reactions at a time) to ascertain coupling viability followed by scale-up for the generation of useful quantities of UAAs for exploratory studies.

Unnatural amino acids (UAAs) are widely employed as pharmacophores, spectroscopic probes, reagents for chemical biology, and starting materials for peptidomimetics (Figure 1A).[1-4] Historically, the synthesis of UAAs relies on polar bond disconnections, which are generally characterized by multistep sequences as summarized in Figure 1B. In general, most classic approaches require the use of organometallic reagents as starting materials, unstable intermediates, multiple protecting group manipulations and laborious reaction set ups.[5] In some cases, structurally simple/trivial UAAs needed to be synthesized in as much as 10 steps with only one of those steps leading to a core modification of the structure (i.e. forming a C–C bond).[6-15] Over the past decade, substantial progress towards developing more direct approaches to UAAs has been made. For instance, palladium-catalyzed activation of inert β-C(sp^3)–H bonds that can capitalize on readily available amino acid starting materials have been described.[16] A new appealing strategy is emerging that takes advantage of the native functionality of amino acids such as aspartate, glutamate, and lysine wherein carboxylic acid and amino side chains can be activated for decarboxylative and deaminative radical cross coupling, respectively.[17-21] Building on our expertise on decarboxylative cross-couplings that leverage Ag functionalized electrode and Ni-electrocatalysis,[22-24] we were poised to demonstrate how the arylation of inexpensive and commercially available natural amino acids like aspartic and glutamic derivatives could be achieved in an operationally simple fashion.[25, 26] Disclosed herein is a useful exemplification of this strategy laying out how a parallel reaction system can be used to rapidly access libraries of UAAs.

The current study was pursued with the goal of accessing UAAs through a parallel synthesis protocol for use in an ongoing medicinal chemistry program. As illustrated in Table 1, the commercial "E-hive" module from IKA was enlisted, which conveniently attaches to ElectraSyn 2.0. The application of this module in miniaturizing reactions and rapid assay generation has been demonstrated in multiple occasions. The device can run 24 parallel reactions at a constant potential, whereas the reaction vessel itself operates as the anode (stainless steel), and a small graphite rod (part of the cap) acts as the cathode. To showcase the setup's utility for library synthesis, a set of 4 redox-active esters (RAEs, Boc and Fmoc protected aspartic and glutamic acid **A1-A4**) were selected and screened against 20 arenes (**B1-E2**, 80 reactions total) that were of particular interest (Table 1A). The reactions were easily set-up using stock solutions of the respective starting materials and reagents on a 0.03-0.07 mmol scale and electrolyzed for 12h. Subsequently, product formation was analyzed via UPLC-DAD using 10 mol% terphenyl as an internal standard and the success of the corresponding reactions was divided into 3 categories: (Green) Product vs. internal standard ratio is higher than 0.5; (Yellow) Product vs. internal standard ratio is lower than 0.5, and (Red): Desired Product was not detected. Out of the 80 reactions, 54 were highly successful (green), 22 were modestly successful (yellow), and 4 products were not detected (red). This reactivity assay showcases great modularity and high functional group tolerance of the utilized decarboxylative Ag functionalized electrode Ni-electrocatalytic cross-coupling towards diversly substituted (hetero)aryl halides.

Electron-rich (**6**, **8**, **9**) as well as electron-poor (**1**, **7**) aryl iodides, imidazoles (**25**), pyridines (**18**, **23**), pyrimidines (**2**, **4**, **16**, **24**), protected and unprotected azaindoles (**5**, **11**, **12**, **20**, **21**, **26**), unprotected pyridinones (**19**), chromenones (**3**, **14**, **15**, **27**), imidazopyridazines (**17**, **22**), indazoles (**13**) and benzothiophenes (**10**) were successfully coupled with at least one amino acid. Additionally, free alcohols (**6**, **8**, **9**, **14**, **27**) and amines (**18**), alkyl fluorides (**7**, **17**, **22**), thioethers (**2**, **16**, **24**) and esters (**2**, **16**, **24**) were tolerated.

More importantly the applied method shows high chemoselectivity for electron-poor (hetero)aryl iodides, hence tolerating more electron-rich bromides and chlorides (**1**, **4**, **17**, **22**, **23**). This allows for further substitution of the (hetero)arene by using canonical methods such as Pd-catalyzed Suzuki cross-coupling and therefore opens up to even more diversly designed UAAs.

With a robust reactivity assay in hand, a library of selected UAAs by synthesizing the corresponding UAAs on a 0.2-0.3 mmol scale (Table 1B). Thus, 5 Boc protected (**1-5**) and 13 Fmoc protected (**6-18**) aspartic acid analogs as well as 4 Boc (**19-22**) and 5 Fmoc protected (**23-27**) glutamic acids were successfully scaled-up and isolated (27 examples total), without any loss in enantiopurity. All of the 20 arenes applied in the previous reactivity assay were isolated in at least one example. Notably, the electrode materials, concentration, and current density applied in the standardized procedure of the scale-up deviated significantly from the conditions applied in the initial reactivity assay, proving the robustness and translatability for E-hive screening in this reaction.

To further demonstrate the ability of this method to simplify UAA synthesis, six that were studied during the reactivity assay and subsequently scaled-up have been previously synthesized using 2e$^-$ methodologies (Figure 3). Those prior approaches required multi-step sequences requiring the use of rare and expensive transition metals as well as toxic and pyrophoric reagents. Several examples were synthesized using traditional enolate chemistry (**3**, **8**, **10**, **13**, **18**, **25**). Hence, UAA **8** was obtained after 4 steps in 0.3% overall yield (following Lipase resolution) and was in contrast obtained as UAA **8a** in 34% via DCC.[27] UAA **10** was previously obtained in 40% overall yield in racemic form after 5 steps.[11] Despite obtaining UAA **10a** in only 25% via DCC, labor intensive processes and time can be saved and a single enantiomer was obtained. Racemic UAA **13** was obtained in 7 steps and 3% overall yield, whereas DCC delivered compound **13a** in 52% as a single enantiomer in one step.[28] By using a chiral auxiliary (Schöllkopf), UAA **18** was obtained after 6 steps in 2% yield.[29] In contrast, the current method afforded UAA **18a** in 47%. UAA **3** was obtained in 10-15% yield and in 6 steps via ring construction of the respective chromenone.[30] Instead, bromination of the respective hydroxy chromenone followed by DCC delivered UAA **3a** in 39% yield. Finally, racemic UAA **25** was obtained after 7 steps in 12% overall yield utilizing Strecker chemistry,[31] whereas DCC afforded enantiopure compound **25a** in 30% yield after a single step.

This work demonstrates how a library of UAAs can be easily constructed in two stages from inexpensive aspartate and glutamate-based RAE precursors: parallel screening on small scale using a commercial potentiostat followed by preparative scale reactions. Even though no substantial attempts were made to optimize individual reactions beyond the originally published conditions, yields for these one-step processes are reasonable given the rapid access to UAAs that is enabled for exploratory studies.

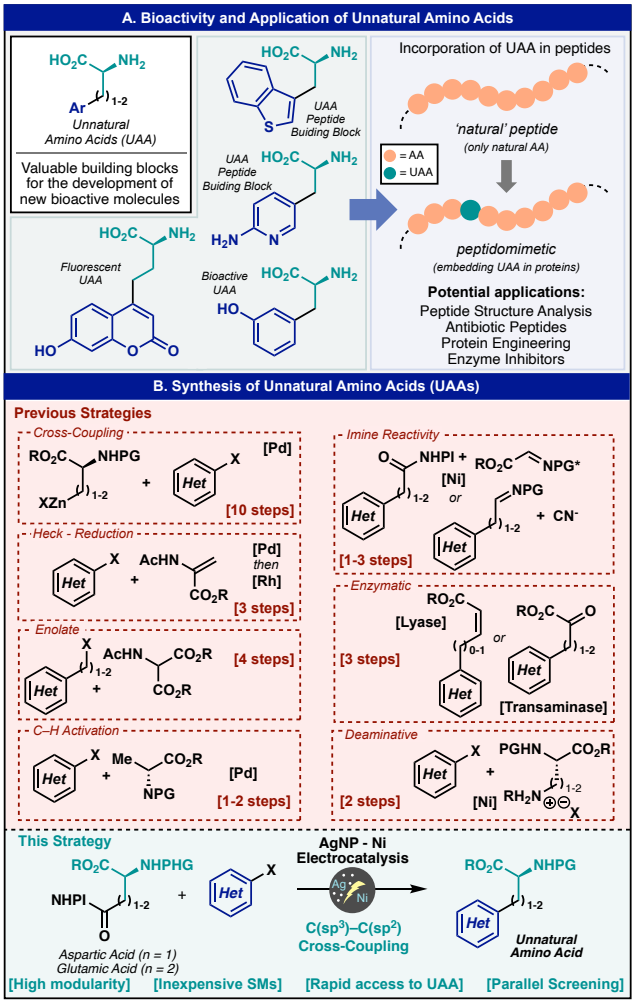

FIGURE 1. (A) Bioactivity and application of unnatural amino acids. (B) Prior synthetic approaches for the preparation of unnatural amino acids and the proposed radical decarboxylative arylation strategy.

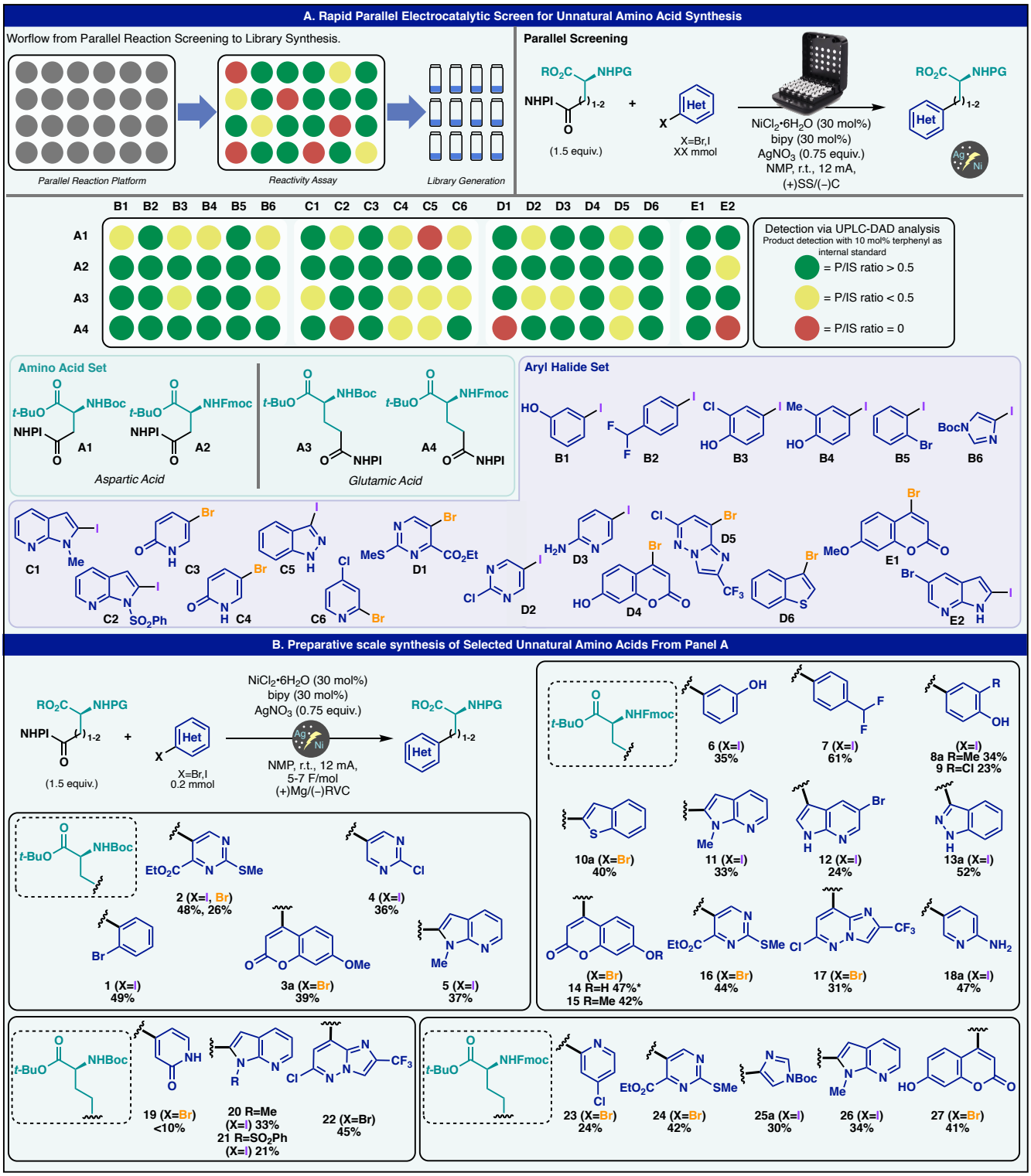

FIGURE 2 (A) Parallel reaction screen for unnatural amino acid synthesis. Accurate reaction conditions and description of the setup are reported in the Supporting Information. (B) Preparative scale synthesis of the unnatural amino acids previously tested in the parallel synthesis platform.

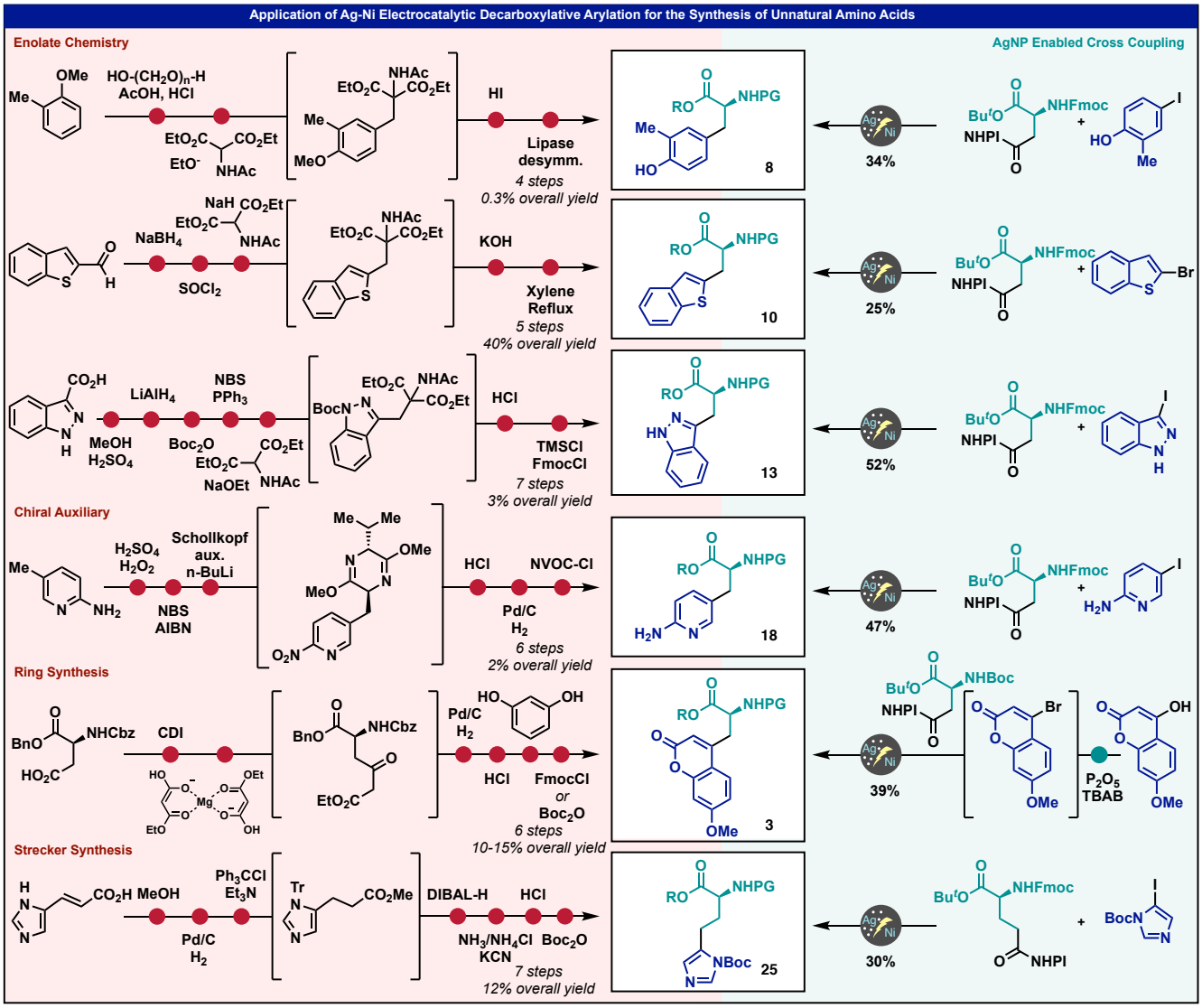

Figure 3. Electrochemical synthesis of relevant unnatural amino acids compared to previous routes reported in literature.

ASSOCIATED CONTENT

Supporting Information

Additional experimental details, materials, methods, and characterization data for all new compounds (PDF)

LCMS chromatograms of every entry of the parallel platform screening (PDF)

Data sheet with all the HPLC-DAD, and LC-MS raw data associated with SMILES structures. (excel file)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Wang, X.; Yang, X.; Wang, Q.; Meng, D. Unnatural amino acids: promising implications for the development of new antimicrobial peptides. *Critical Reviews in Microbiology* **2023**, *49* (2), 231-255. DOI: 10.1080/1040841X.2022.2047008.

(2) Drienovská, I.; Roelfes, G. Expanding the enzyme universe with genetically encoded unnatural amino acids. *Nat. Catal.* **2020**, *3* (3), 193-202. DOI: 10.1038/s41929-019-0410-8.

(3) Narancic, T.; Almahboub, S. A.; O'Connor, K. E. Unnatural amino acids: production and biotechnological potential. *World J. Microbiol. Biotechnol.* **2019**, *35* (4), 67. DOI: 10.1007/s11274-019-2642-9.

(4) Dougherty, D. A. Unnatural amino acids as probes of protein structure and function. *Curr. Opin. Chem. Biol.* **2000**, *4* (6), 645-652. DOI: https://doi.org/10.1016/S1367-5931(00)00148-4.

(5) Sardina, F. J.; Rapoport, H. Enantiospecific Synthesis of Heterocycles from α-Amino Acids. *Chem. Rev.* **1996**, *96* (6), 1825-1872. DOI: 10.1021/cr9300348.

(6) Suhartono, M.; Weidlich, M.; Stein, T.; Karas, M.; Dürner, G.; Göbel, M. W. Synthesis of Non-Natural Aromatic α-Amino Acids by a Heck Reaction. *Eur. J. Org. Chem.* **2008**, *2008* (9), 1608-1614. DOI: https://doi.org/10.1002/ejoc.200701124.

(7) Besset, T.; Kuhl, N.; Patureau, F. W.; Glorius, F. RhIII-Catalyzed Oxidative Olefination of Vinylic C□H Bonds: Efficient and Selective Access to Di-unsaturated α-Amino Acid Derivatives and Other Linear 1,3-Butadienes. *Chem. Eur. J.* **2011**, *17* (26), 7167-7171. DOI: https://doi.org/10.1002/chem.201101340.

(8) Doi, T.; Fujimoto, N.; Watanabe, J.; Takahashi, T. Palladium(0)-catalyzed Mizoroki–Heck reaction and Rh(I)-catalyzed asymmetric hydrogenation of polymer-supported dehydroalanine system. *Tetrahedron Lett.* **2003**, *44* (10), 2161-2165. DOI: https://doi.org/10.1016/S0040-4039(03)00149-7.

(9) Deboves, H. J. C.; Montalbetti, C. A. G. N.; Jackson, R. F. W. Direct synthesis of Fmoc-protected amino acids using organozinc chemistry: application to polymethoxylated phenylalanines and 4-oxoamino acids. *J. Chem. Soc., Perkin Trans. 1* **2001**, (16), 1876-1884, 10.1039/B103832J. DOI: 10.1039/B103832J.

(10) Sabat, M.; Johnson, C. R. Synthesis of Unnatural Amino Acids via Suzuki Cross-Coupling of Enantiopure Vinyloxazolidine Derivatives. *Org. Lett.* **2000**, *2* (8), 1089-1092. DOI: 10.1021/ol005645i.

(11) Paizs, C.; Katona, A.; Rétey, J. The Interaction of Heteroaryl-Acrylates and Alanines with Phenylalanine Ammonia-Lyase from Parsley. *Chem. Eur. J.* **2006**, *12* (10), 2739-2744. DOI: https://doi.org/10.1002/chem.200501034.

(12) Ni, S.; Garrido-Castro, A. F.; Merchant, R. R.; de Gruyter, J. N.; Schmitt, D. C.; Mousseau, J. J.; Gallego, G. M.; Yang, S.; Collins, M. R.; Qiao, J. X.; et al. A General Amino Acid Synthesis Enabled by Innate Radical Cross-Coupling. *Angew. Chem. Int. Ed.* **2018**, *57* (44), 14560-14565. DOI: https://doi.org/10.1002/anie.201809310.

(13) Wang, J.; Liu, X.; Feng, X. Asymmetric Strecker Reactions. *Chem. Rev.* **2011**, *111* (11), 6947-6983. DOI: 10.1021/cr200057t.

(14) Park, E.-S.; Dong, J.-Y.; Shin, J.-S. Biocatalytic Asymmetric Synthesis of Unnatural Amino Acids through the Cascade Transfer of Amino Groups from Primary Amines onto Keto Acids. *ChemCatChem* **2013**, *5* (12), 3538-3542. DOI: https://doi.org/10.1002/cctc.201300571.

(15) Turner, N. J. Ammonia lyases and aminomutases as biocatalysts for the synthesis of α-amino and β-amino acids. *Curr. Opin. Chem. Biol.* **2011**, *15* (2), 234-240. DOI: https://doi.org/10.1016/j.cbpa.2010.11.009.

(16) Chen, G.; Zhuang, Z.; Li, G.-C.; Saint-Denis, T. G.; Hsiao, Y.; Joe, C. L.; Yu, J.-Q. Ligand-Enabled β-C–H Arylation of α-Amino Acids Without Installing Exogenous Directing Groups. *Angew. Chem. Int. Ed.* **2017**, *56* (6), 1506-1509. DOI: https://doi.org/10.1002/anie.201610580.

(17) Yousif, A. M.; Colarusso, S.; Bianchi, E. Katritzky Salts for the Synthesis of Unnatural Amino Acids and Late-Stage Functionalization of Peptides. *Eur. J. Org. Chem.* **2023**, *26* (12), e202201274. DOI: https://doi.org/10.1002/ejoc.202201274.

(18) Palkowitz, M. D.; Emmanuel, M. A.; Oderinde, M. S. A Paradigm Shift in Catalysis: Electro- and Photomediated Nickel-Catalyzed Cross-Coupling Reactions. *Acc. Chem. Res.* **2023**, *56* (20), 2851-2865. DOI: 10.1021/acs.accounts.3c00479.

(19) Laudadio, G.; Palkowitz, M. D.; El-Hayek Ewing, T.; Baran, P. S. Decarboxylative Cross-Coupling: A Radical Tool in Medicinal Chemistry. *ACS Med. Chem. Lett.* **2022**, *13* (9), 1413-1420. DOI: 10.1021/acsmedchemlett.2c00286.

(20) Aguilar Troyano, F. J.; Merkens, K.; Anwar, K.; Gómez-Suárez, A. Radical-Based Synthesis and Modification of Amino Acids. *Angew. Chem. Int. Ed.* **2021**, *60* (3), 1098-1115. DOI: https://doi.org/10.1002/anie.202010157.

(21) Rahman, M.; Mukherjee, A.; Kovalev, I. S.; Kopchuk, D. S.; Zyryanov, G. V.; Tsurkan, M. V.; Majee, A.; Ranu, B. C.; Charushin, V. N.; Chupakhin, O. N.; et al. Recent Advances on Diverse Decarboxylative Reactions of Amino Acids. *Adv. Synth. Catal.* **2019**, *361* (10), 2161-2214. DOI: https://doi.org/10.1002/adsc.201801331.

(22) 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.; et al. Overcoming Limitations in Decarboxylative Arylation via Ag–Ni Electrocatalysis. *J. Am. Chem. Soc.* **2022**, *144* (38), 17709-17720. DOI: 10.1021/jacs.2c08006.

(23) Laudadio, G.; Neigenfind, P.; Péter, Á.; Rubel, C. Z.; Emmanuel, M. A.; Oderinde, M. S.; Ewing, T. E.-H.; Palkowitz, M. D.; Sloane, J. L.; Gillman, K. W.; et al. Nickel-Electrocatalytic Decarboxylative Arylation to Access Quaternary Centers**. *Angew. Chem. Int. Ed.* **2024**, *n/a* (n/a), e202314617. DOI: https://doi.org/10.1002/anie.202314617.

(24) Neigenfind, P.; Massaro, L.; Péter, Á.; Degnan, A.; Emmanuel, M. A.; Oderinde, M. S.; He, C.; Peters, D.; Ewing, T. E.-H.; Kawamata, Y.; et al. Simplifying Access to Targeted Protein Degraders via Ni-Electrocatalytic Cross-Coupling. *ChemRxiv* **2023**. DOI: https://doi.org/10.26434/chemrxiv-2023-xgt42.

(25) Bombonato, E.; Fasano, V.; Pecorari, D.; Fornasari, L.; Marcaccio, M.; Ronchi, P. Electrochemical Synthesis of Unnatural Amino Acids Embedding 5- and 6-Membered Heteroaromatics. *ChemRxiv* **2023**. DOI: https://doi.org/10.26434/chemrxiv-2023-nps5t-v2.

(26) Pavlovic, B.; Heubel, C.; Kurz, M.; Oehl, E.; Waldvogel, S. R.; Méndez, M.; Ruf, S. Single step synthesis of β– and γ– aryl-substituted β– and γ–amino acid derivatives by electrochemistry. *Bioorg. Med. Chem. Lett.* **2024**, 129614. DOI: https://doi.org/10.1016/j.bmcl.2024.129614.

(27) Seisser, B.; Zinkl, R.; Gruber, K.; Kaufmann, F.; Hafner, A.; Kroutil, W. Cutting Long Syntheses Short: Access to Non-Natural Tyrosine Derivatives Employing an Engineered Tyrosine Phenol Lyase. *Adv. Synth. Catal.* **2010**, *352* (4), 731-736. DOI: https://doi.org/10.1002/adsc.200900826.

(28) Pasquale, E. B.; Dawson, P.; Riedl, S. J.; Olson, E. Epha4 cyclic peptide antagonists for neuroprotection and neural repair. WO2016011201A2, 2015.

(29) Bai, X.; Talukder, P.; Daskalova, S. M.; Roy, B.; Chen, S.; Li, Z.; Dedkova, L. M.; Hecht, S. M. Enhanced Binding Affinity for an i-Motif DNA Substrate Exhibited by a Protein Containing Nucleobase Amino Acids. *J. Am. Chem. Soc.* **2017**, *139* (13), 4611-4614. DOI: 10.1021/jacs.6b11825.

(30) Brun, M.-P.; Bischoff, L.; Garbay, C. A Very Short Route to Enantiomerically Pure Coumarin-Bearing Fluorescent Amino Acids. *Angew. Chem. Int. Ed.* **2004**, *43* (26), 3432-3436. DOI: https://doi.org/10.1002/anie.200454116.

(31) Pirrung, M. C.; Pei, T. Synthesis of (±)-Homohistidine. *J. Org. Chem.* **2000**, *65* (7), 2229-2230. DOI: 10.1021/jo991630q.