Electrochemical Synthesis of Unnatural Amino Acids Embedding 5- and 6-Membered Heteroaromatics

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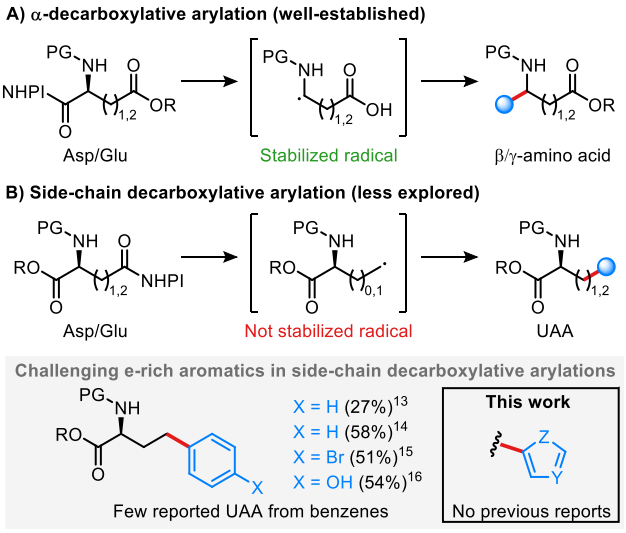
ABSTRACT: Using a commercially available potentiostat, the electrochemical synthesis of unnatural amino acids bearing heteroaromatics on the lateral chain has been accomplished. This strategy exploits the side-chain decarboxylative arylation of aspartic/glutamic acid, a reaction that becomes challenging with electron-rich coupling partners such as 5- and 6-membered heteroaromatics. These rings are underrepresented in unnatural amino acids, therefore it allowed a wider exploration of the chemical space, also given the abundance of the aryl bromides employable in this reaction.

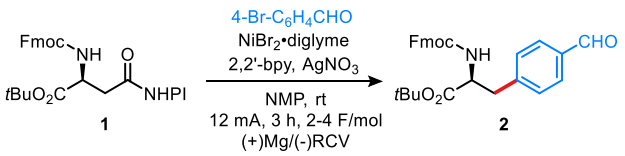
The past decade has witnessed spectacular progress in the field of unnatural amino acids (UAAs), molecular entities widely employed in proteomics, protein engineering, and peptidomimetics.[1-4] Many methodologies have been devised for the *de novo* synthesis of UAAs.[5-7] An alternative approach is instead based on the lateral chain modification of natural amino acids, a useful strategy to retain the α-stereocentre. This is particularly true for aspartic acid (Asp) and glutamic acid (Glu) whose side-chain carboxylic group offers a strategic handle for derivatizations, especially those based on radical decarboxylations of the corresponding redox-active esters (RAEs).[8-11] However, side-chain decarboxylation of these amino acids is usually more challenging than decarboxylation of the α-CO_2H since only the latter provides a stabilized intermediate, that is an α-amino radical (Scheme 1).[12] While many radical partners (e.g. alkenes, alkynes, CO_2) have been engaged in lateral-chain decarboxylations, decarboxylative arylation of these amino acid residues remains elusive, with only a few examples reported in the literature.[13-16] Indeed, decarboxylative arylations are limited by the H-atom abstraction or dimerization of the transient radicals as well as by unproductive N–O bond heterolysis. This limitation is further hampered by the fact that electron-rich heteroaromatics are less suitable as coupling partners in these arylations.[16] Not surprisingly, side-chain decarboxylative arylations of Asp/Glu have not been reported using *N*, *S*, or *O*-containing 5-membered aromatics. Given that these heterocycles are ubiquitous in drugs,[17-19] herein we report our efforts to develop lateral-chain decarboxylative heteroarylations of aspartic acid and glutamic acids by means of electrochemistry. By exploiting the new opportunities offered by the renaissance of radical chemistry, our approach allows the one-pot creation of novel unnatural glutamic acid by means of C(sp^2)–C(sp^3) linkages with 5- and 6-membered aromatic heterocycles.



Scheme 1. State-of-the-art decarboxylation of Asp and Glu for the synthesis of β/γ-amino acids (A) or unnatural amino acids (B). NPHI = *N*-Hydroxyphthalimide.

Our work takes inspiration from the decarboxylative arylation of RAEs and halogenated aromatics recently reported by Baran and co-workers.[16] The coupling of these reagents was achieved using sub-stoichiometric amounts (20 mol%) of $NiCl_2 \cdot 6H_2O$ and 2,2′-bipyridine (2,2′-bpy), in the presence of the additive $AgNO_3$ (0.5 equiv.) in dimethylformamide (DMF) under constant current electrolysis with a reticulated vitreous carbon (RVC) cathode and sacrificial magnesium anode. This methodology was successfully applied to the decarboxylation arylation of many carboxylic acids, including a couple of examples employing glutamic acid. Using 4-iodophenol, this reaction allowed the multigram scale synthesis of homotyrosine in 43%, a classic UAA usually synthesized *via* multistep syntheses, but no other examples with electron-rich halides (especially 5-membered aromatics) were further investigated. Building on this precedence, we set off developing an electrochemical method that could allow the synthesis of UAAs containing the challenging 5- and 6-membered heteroaromatics coupling partners. This method should also be cheap and transferable to flow-chemistry to increase its appeal to pharmaceutical companies. Our investigation thus started with the search for alternative electrochemical conditions for the coupling of redox-active ester **1** (derived from Glu) and 4-bromobenzaldehyde, an electron-poor aromatic whose coupling should be easier compared to 5-membered heterocycles (Table 1).

Table 1. Optimization of conditions for direct side-chain decarboxylative arylation of RAE 1.



Entry	Modification	Yield[a]
1	None[b]	42% (28% iso)
2	ArI instead of ArBr	28%
3	No $NiBr_2$•diglyme	0%
4	No $AgNO_3$	0%
5	$Fe(OAc)_2$ instead of $AgNO_3$	20%
6	(+)GC instead of (+)Mg	0%
7	(+)Al instead of (+)Mg	40%
8	(-)$Pt_{ceramic}$, (-)Pt_{foil}, or (-)graphite instead of (-)RCV	0%
9	(-)Ni_{foam} instead of (-)RCV	20%
10	Using (-)Ni_{foam} and (+)Al	39%

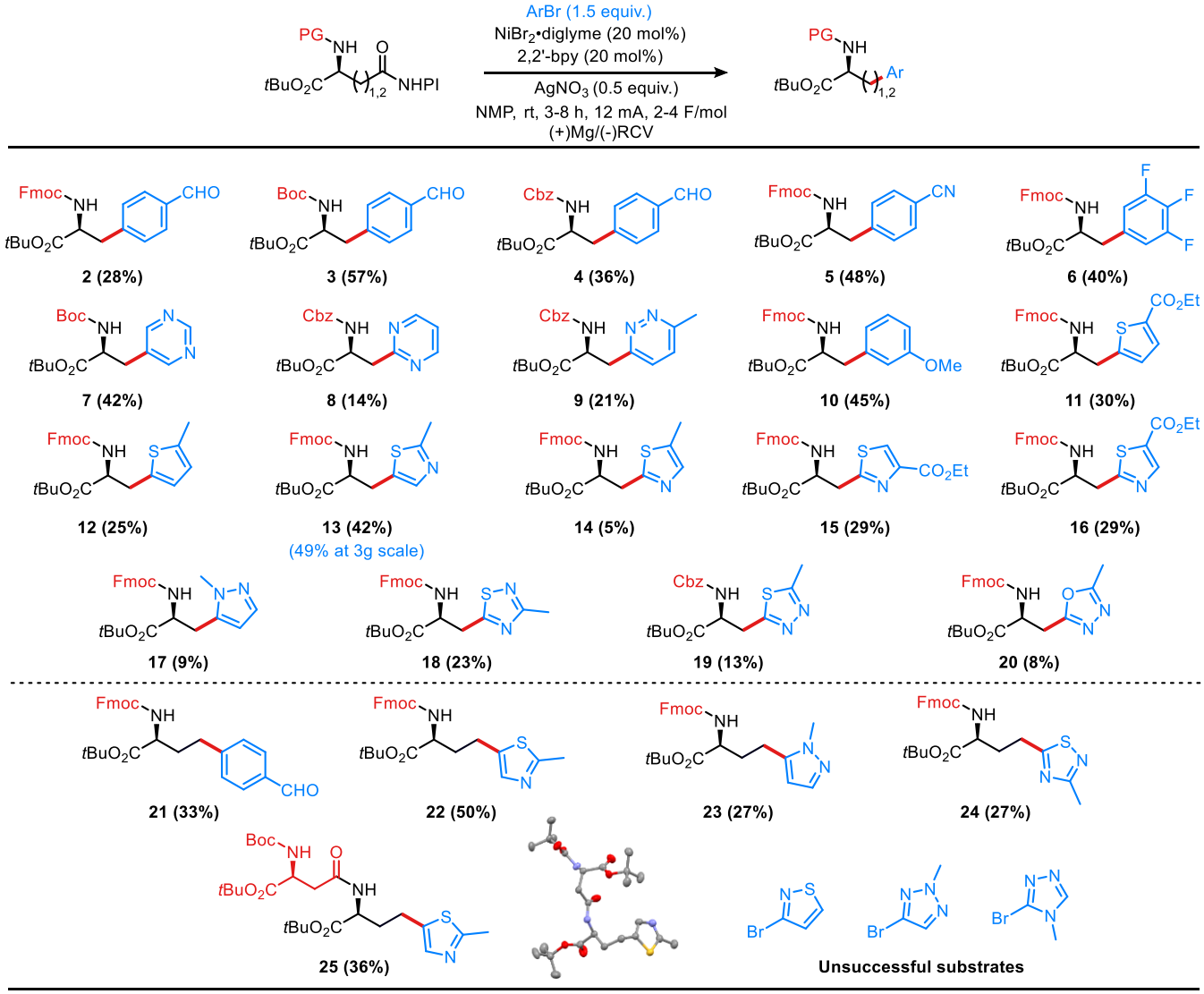
[a]Yield determined by UPLC with 1-chloro-trifluorometilbenzene as an internal standard. [b]Standard conditions: 4-Br-C_6H_4CHO (1.5 equiv.), $NiBr_2$·diglyme (20 mol%), 2,2-bpy (20 mol%), $AgNO_3$ (0.5 equiv.), NMP (0.1 M).

Initially, toxic $NiCl_2 \cdot 6H_2O$ was replaced by safer $NiBr_2$·diglyme, while *N*-methyl-2-pyrrolidone (NMP) was preferred to DMF as the solvent. Under these conditions, UAA **2** was observed by quantitative UPLC in 42% yield (entry 1), a value in agreement with the yield reported for homotyrosine by Baran and co-workers (54%).[16] As reported also in their work, dimerization of the unstable primary radical accounted for the rest of the mass balance. Isolation of **2** required two purifications by flash chromatography, thus causing a reduced isolated yield (28%). Replacing the aryl bromide with 4-iodobenzaldehyde furnished **2** in a lower yield (entry 2), a result in apparent disagreement with the work of Baran and co-workers.[16] We believe that the sluggish oxidative addition of these substrates and the different solubility of AgBr *vs* AgCl could favor the aryl bromide over the aryl iodide depending on the substrate under investigation. Performing the reaction in the absence of the nickel catalyst or without $AgNO_3$ resulted in no productive coupling (entry 3 and 4, respectively), whereas replacing $AgNO_3$ with $Fe(OAc)_2$ gave **2** in 20% UPLC yield (entry 5). This is particularly important since highlights that silver can act both forming *in situ* Ag-functionalized electrodes (as previously determined),[16] and as a mediator, thus making it replaceable by iron which does not form nanoparticles at the electrode. Attempts to replace the sacrificial magnesium anode with a glassy carbon (GC) electrode gave no reaction (entry 6), supporting the role of magnesium as a source of electrons. Notably, the magnesium sacrificial anode could be replaced with aluminium which increases the sustainability of the process (entry 7). Attempts to replace the counter electrode with platinum- or graphite-based ones were unsuccessful (entry 8), therefore pointing out how a large surface area is key in these decarboxylative arylations. Nevertheless, the use of nickel foam as the counter electrode showed reactivity to some extent (entry 9). This, in combination with aluminium as the sacrificial anode gave product **2** in 39% (entry 10), thus providing a cheaper and safer alternative to previously reported working conditions. Based on the optimization study and in analogy with previous results,[16,21] a nickel-based catalytic mechanism system is believed to be responsible for the $C(sp^2)$-$C(sp^3)$ coupling, with the deposition of silver nanoparticles on the electrode having a key role in the reactivity. However, in addition to this established mechanism, a concomitant pathway involving silver or other additives as a mediator could occur too, as observed when performing the reaction with FeOAc and using brand-new electrodes.

With the optimized conditions in hand, the scope of UAAs was explored performing the reactions under air and at room temperature, using technical-grade solvents and a commercially available potentiostat (Scheme 2). (+)Mg/(-)RCV electrodes were chosen since slightly more performing than (+)Al/(-)Ni_{foam}, although these still represent a valid option for economical reasons. Moreover, aryl bromides were employed as coupling partners since their greater availability (relative to aryl iodides) would allow a wider exploration of the chemical space. First, other protective groups on the starting aspartic acid were evaluated. To our delight, Boc and Cbz could both be tolerated under the reaction conditions, thus providing isolated UAA **3** and **4** in 57% and 36%, respectively. Despite the higher yield obtained with the Boc group, the exploration of the scope was continued using Fmoc- or Cbz-protected starting materials since this would give access to orthogonally protected UAAs. Secondly, electron-poor (hetero)aromatics other than benzaldehyde were also amenable to this chemistry, including pharmaceutically relevant diazines (products **5**-**9**). Notably, the stereocentre remains intact during this decarboxylative arylation as demonstrated by the chiral UPLC of compound **7** (see supporting information). This is quite

relevant since alternative approaches starting from dehydroalanine would furnish a racemic product.[22] Moving toward electron-rich benzenes and 5-membered heterocycles, a slightly lower reactivity was observed, in agreement with the reduced ability of these aromatics to engage in decarboxylative arylations. Yet, a wide scope of heterocycles could be obtained in one-pot using the methodology described. For instance, thiophene-containing UAAs **11** and **12**, potentially useful as fluorescent chemosensors,[23] were obtained up to 30%. Thiazole, another ubiquitous 5-membered ring in medicinal chemistry, could be embedded too, as reported for UAAs **13**-**16**. Interestingly, the relative position of the nitrogen atom within the structure can dramatically impact the outcome, as observed comparing regioisomeric UAAs **13** and **14**. This could be attributed to the different stereoelectronic properties of the starting methylthiazole bromide which affects the catalytical cycle (e.g. irreversible coordination to nickel).[24] Other 5-membered heterocycles incorporated for the first time in a UAA were *N*-methyl-pyrazole (unsuccessful in previous studies),[16] thiadiazole, and oxadiazole (entries **17**-**20**), with the last three representing examples of an aromatic ring containing three heteroatoms. Isothiazole- and triazole-based coupling partners were instead unsuccessful in the decarboxylative arylation, with the mass balance given by the dimerization product of the unstable primary radical. The decarboxylative arylation could be extended also to glutamic acid, thus obtaining UAAs **21**-**24** bearing in the side-chain benzaldehyde (33%), 2-methylthiazole (50%), *N*-methyl-pyrazole (27%), and thiadiazole (27%).

Using an RAE derived from a dipeptide, UAA **25** was isolated in 36%, thus opening possibilities for late-stage functionalization of small proteins.[25] Single-crystal X-ray crystallography of **25** confirmed its structure, hence highlighting that the developed method is a useful strategy to retain the α-stereocentre. Finally, using the six-reactors carousel, this methodology can be scaled up to 3 grams of starting material in one batch, as demonstrated with compound **13** isolated in 1.2 grams (49% yield).



Scheme 2. Synthesis of UAAs by means of electrochemical lateral-chain decarboxylative arylations. In brackets isolated yield after two purifications by flash chromatography.

In conclusion, the synthesis of UAAs by means of side-chain decarboxylation of aspartic/glutamic acid has been presented. This simple protocol, requiring short reaction times and working at room temperature, allowed the incorporation of challenging electron-rich heterocycles on the lateral chain of novel UAAs, thus providing a more intuitive retrosynthesis of high-value targets. These aromatics, especially 5-membered ones, are underrepresented in previously reported UAAs, so novel building blocks are now available for usage in proteomics. Finally, other relevant features of this methodology are the use of readily available aryl bromides as well as the possibility to use cheaper variants based on (+)Al/(-)Ni_{foam} electrodes.

ASSOCIATED CONTENT

Supporting Information. Procedures including preparation of substrates, optimization of reaction conditions, characterization data, and NMR Spectra. This material is available free of charge via the Internet at http://pubs.acs.org."

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

UAA, unnatural amino acid; Asp, aspartic acid; Glu, glutamic acid; RAE, redox-active ester; DMF, dimethylformamide; RCV, reticulated vitreous carbon; NHPI, *N*-Hydroxyphthalimide, NMP, *N*-methyl-2-pyrrolidone; UPLC, Ultra-performance liquid chromatography; GC, glassy carbon; Ni_{foam}, Nichel foam.

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