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Catalytic Arylhydroxylation of Dehydroalanine in Continuous Flow for Simple Access to Unnatural Amino Acids

R. Kashif M. Khan,[a]‡ Yang Zhao,[a]‡ Tal D. Scully,[a] and Stephen L. Buchwald*[a]

Abstract: This report discloses the first example of catalytic arylhydroxylation of dehydroalanine with aryldiazonium salts. Aryldiazonium salts, which are generated from aniline precursors under partially aqueous conditions in continuous flow, efficiently react with dehydroalanine in the presence of 10-15 mol% ferrocene to furnish α–hydroxyarylalanine derivatives (up to 82% yield). The reactions proceed with regioselectivity, broad functional group tolerance, and without polymerization of the dehydroalanine. Furthermore, the products can be used to access α–unnatural amino acids, important targets with application in drug development.

Dehydroalanine (dha), a terminal alkene-based amino acid, is found in a large number of naturally occurring peptides.[1] Owing to the high propensity of dha to undergo conjugate addition,[2] it serves as an excellent electrophile in cysteine-mediated peptide cyclization, a key step during posttranslational modification (PTM) of peptides to form lantibiotics.[3] In addition to its role as a natural synthon, dha is routinely used as a building block in various synthetic schemes for the preparation of α–unnatural amino acids (α–UAAs)[4] — an important class of molecules employed in the development of peptide-based pharmaceuticals [5] and PTM of proteins. [6] Although dha functionalization strategies permit variation in the α–UAA architecture, there exists a dearth of methods to access the related α,α–UAA subclass. Notably, the discoveries of Cα–S and Cα–O linked residues in antimicrobial peptides (i.e., **I** and **II,** respectively)[7] as well as their relevance to drug development[8] have underlined the need for techniques to access heteroatom-substituted α,α–UAAs (Figure 1a). Within the aforementioned category, the O-substituted variants can be readily transformed into several types of UAAs (**IV–VI,** Figure 1b).[9] Despite the high potential for synthetic utility, structures similar to **III**, only a few methods for their preparation are available and they are mainly accessed through the chemical [10] and electrochemical [11] oxidation of the corresponding phenylalanine derivatives. In this context, the development of robust catalytic methods for the facile conversion of dha to the corresponding α,α–UAAs (**VII** to **IX**, Figure 1c) would be of high value.[12]

Our group previously reported that treatment of enol ethers with ArN_2Cl in the presence of a catalytic amount of ferrocene generates α–acetaldehydes.[13] Similarly, we reasoned that dha (**VII)** would rapidly undergo coupling with an aryl radical accessed from the 1e-reduction of the aryldiazonium partner to form a highly stabilized intermediate (**VIII**). It is noteworthy that a related arylation step (in an overall reductive arylation process) has been reported with aryldiazonium salts in the presence of excess $TiCl_3$ reducing agent. [14] Upon the 1e-oxidation of **VIII** and subsequent addition of H_2O, the desired product **IX** would be formed. However, a major complication could arise if free radical-based polymerization of **VII** were to occur. Indeed, polymerization of dha has been reported in the literature. [15] Moreover, the use of ArN_2Cl reagents, which are prone to spontaneous and violent decomposition, could pose serious safety hazards.[16]

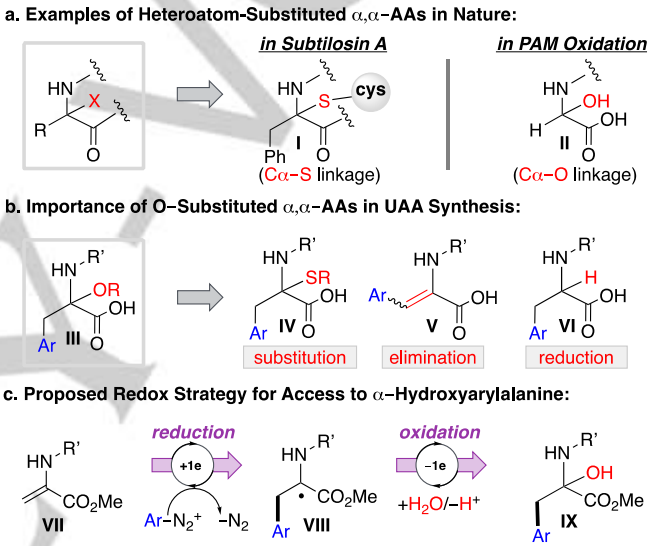

Figure 1. Significance of O-based α,α–UAAs and proposal for their formation through catalytic arylhydroxylation of dehydroalanine (**VII**).

In order to provide synthetically useful and operationally viable access to α,α–UAAs, we set out to develop an integrated continuous flow reactor (CFR) setup (Figure 2). We anticipated some challenges associated with the use of a CFR to perform this transformation. For instance, the potential precipitation of ArN_2Cl in a multistep protocol and the release of $N_{2\ (g)}$ could hamper the overall efficiency as well as the safety profile of the process.

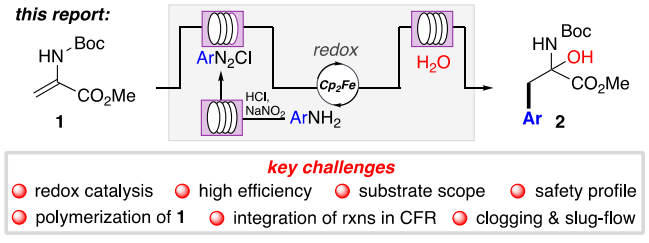

Figure 2. Catalyzed arylhydroxylation of dehydroalanine (**1**) with aryldiazonium salts promoted by ferrocene in continuous flow.

Our studies commenced with the development of homogeneous reaction conditions in batch on a small scale. In a one-pot procedure under air,[17] the aryldiazonium salt **3b** was generated from anline **3a** *in situ* at 0 °C and subsequently allowed to react with alkene **1** (2.5 equivalents) in the presence of 10 mol% ferrocene for one hour (entry 1, Table 1). Upon

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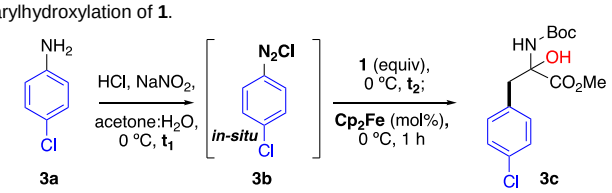
workup with aqueous bicarbonate, 10% of the desired product **3c** was detected. Notably, the ^{1}H NMR analysis of the unpurified reaction mixture revealed >98% consumption of **3a** and the generation of *p*-chlorobenzene (7%) along with an unknown colored side-product.[18] Furthermore, no polymerization of dha **1** was detected. The diazotization time (**t_1**) was increased, which led to the improved formation of **3c** (i.e., 17% with **t_1** = 15 min, entry 3). Nonetheless, the overall transformation continued to suffer from low efficiency. Remarkably, the admixture of the aryldiazonium salt with **1** (at **t_2** = 5 min) prior to the addition of ferrocene resulted in a 71% yield of **3c** (entry 4). The reaction was further improved by increasing the pre-mixing time (i.e., 81% with **t_2** = 20 min, entry 7). Moreover, whereas the reaction efficiency was slightly improved using 15 mol% ferrocene (84%, entry 8), significant attenuation with 5 mol% loading was observed (50%, entry 9).

Table 1. Optimization of a single-pot ArN_2Cl generation and catalytic arylhydroxylation of **1**.

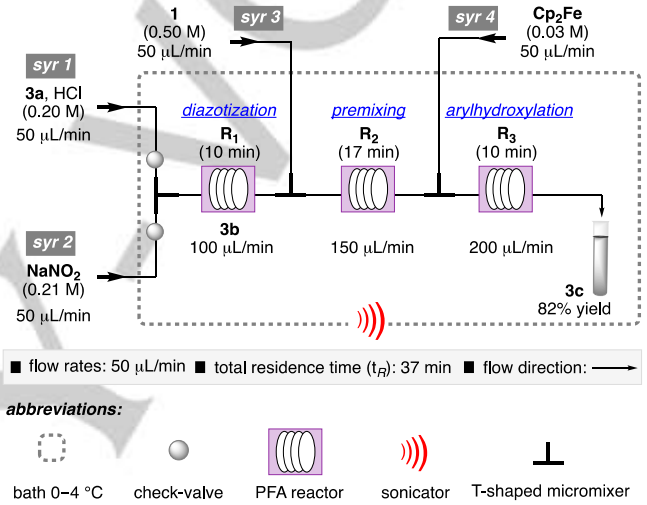


3a **3b** **3c**

entry[a,b]	t_1 (min)	1 (equiv)	t_2 (min)	Cp_2Fe (mol%)	conv (%); yield (%)[c]
1	5	2.5	0	10	>98; 10
2	10	2.5	0	10	>98; 13
3	15	2.5	0	10	>98; 17
4	15	2.5	5	10	>98; 71
5	15	2.5	10	10	>98; 77
6	15	2.5	15	10	>98; 78
7	15	2.5	20	10	>98; 81
8	15	2.5	20	15	**>98; 84**
9	15	2.5	20	5	>98; 50

[a]Reactions were performed (in batch) at 200 μmol scale. [b]Overall solvent ratio: (acetone/water = 3/1). See supporting information for details. [c]Aniline disappearance (conv %) and **3c** formation (yield %) were determined by the ^{1}H NMR (400 MHz) analysis of the unpurified reaction mixture.

With the batch-scale conditions in hand, adaptation to a continuous flow protocol was begun. As shown in Scheme 1, we designed an integrated flow setup involving three reactors (R_1, R_2, and R_3) composed of 0.04'' inner-diameter PFA tubing to carry out the diazotization-premixing-arylhydroxylation sequence, respectively. The reagent delivery was conducted through four syringes (each at 50 μL/min flow rate) connected with 0.02'' inner-diameter PFA feeding lines. In order to maximize the homogeneity of the reaction mixture which is necessary for efficient flow at 0–4 °C, the CFRs were placed in a constantly sonicated ice-bath. Under the CFR setup, mixing of the acidic solution of **3a** (syringe 1) with aqueous sodium nitrite (syringe 2, 1.05 equiv) in R_1 led to complete diazotization within 10 minutes. Two check-valves were employed to prevent any backward flow of **3b**, which could potentially occur due to N_2 gas evolution in the arylhydroxylation step. Subsequently, the solution was mixed with a stream of **1** (syringe 3, 2.5 equiv) in R_2 for 17 minutes. At this point, ferrocene (syringe 4, 15 mol%) was introduced and allowed to react over 10 minutes in R_3. After the flow-equilibration over two total residence times (i.e., 2 x t_R = 74 min), the resulting stream of the crude product mixture was collected under air in a pre-cooled (0 °C) test tube followed by the treatment with aqueous bicarbonate.[17] Upon purification by silica gel chromatography, **3c** was delivered in 82% yield on 1.0 mmol scale. It is important to note that while 10 mol% ferrocene was sufficient for 81% yield in batch (*cf.* entry 7 in Table 1), 15 mol% loading was necessary in the CFR protocol. The difference may arise from a low effective concentration of the catalyst caused by being in a slug-flow regime (i.e., liquid-gas segmentation) upon N_2 evolution in the CFR. Furthermore, the employment of a 20 psi back-pressure regulator to discourage the slug-flow behavior led to significantly diminished yield (36% **3c**).

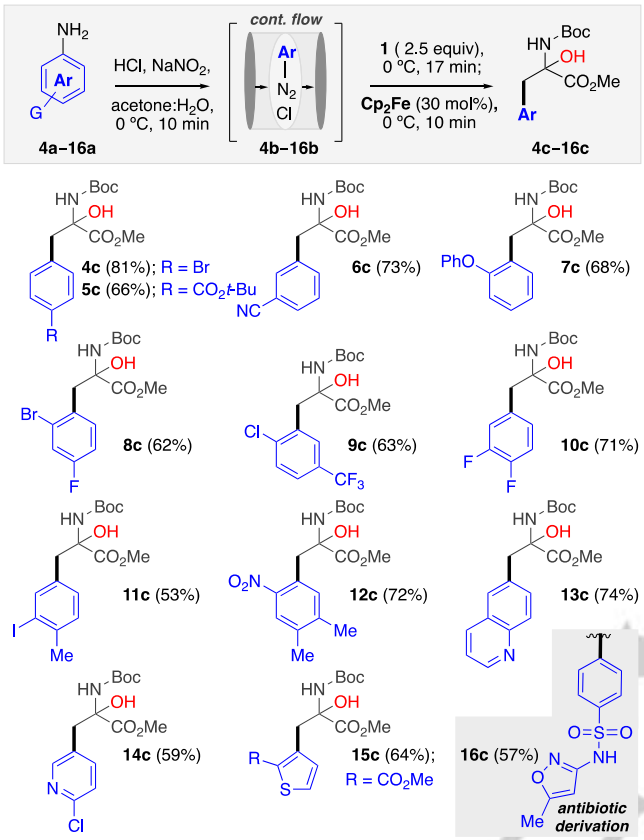


Scheme 1. Rapid ArN_2Cl generation and catalyzed arylhydroxylation of dehydroalanine in CFR. See supporting information for details.

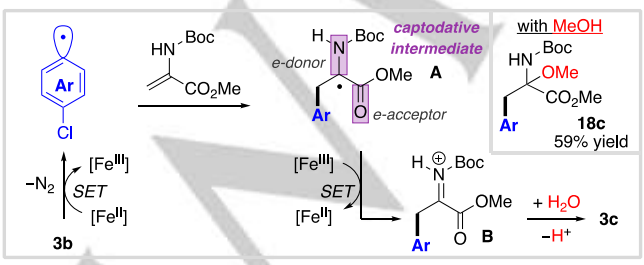
Next, we examined the scope with anilines involving various substitution patterns and containing functional groups (**4a-16a**, Table 2). In line with our results in Scheme 1, the transformation with 4-bromoaniline furnished **4c** in 81% yield. Similarly, the use of a 4-*tert*-butyl ester based substrate led to 66% of **5c**. The relative decrease in efficiency could be attributed to the lability of $-CO_2t$-Bu under the acidic reaction conditions. Moreover, the reactions with 3-CN and 2-OPh based anilines proceeded readily to give the corresponding products (**6c:** 73% and **7c:** 68%). Subsequently, arylamines with multiple substitution patterns were tested. In this regard, we noticed that the lack of *ortho*-substitution led to relatively higher yield in **10c** (vs **8c** and **9c**). Furthermore, reaction with the mildly electron-releasing 3-iodo-4-methylaniline took place with lower efficiency (**11c:** 53%). The CFR protocol also permitted the facile formation of products derived from 4,5-dimethyl-2-nitroaniline and 6-aminoquinoline (**12c:** 72% and **13c:** 74%). The related heteroarylamine-derived α,α–UAAs (**14c** and **15c**) were also assembled in 59% and 64% yield, respectively. Most notably, sulfamethoxazole, an aniline-based antibiotic, was successfully employed in this procedure (**16c:** 57%). We then examined the reaction efficiency with *N*–Cbz protected dha (vs *N*–Boc in **1**). Under the same conditions used for **3c** (*cf.* Scheme 1), the *N*–Cbz protected α–hydroxy-(4-chlorophenyl)alanine derivative (**17c**)[17] was isolated in 78% yield. This result is potentially useful for

protection group alterations that are occasionally necessary in the enantioselective hydrogenation of dehydroarylalanine precursors (*cf.* Scheme 3).

Table 2. Substrate scope with complex aryl and heteroaryl primary amines for α,α–UAAs.

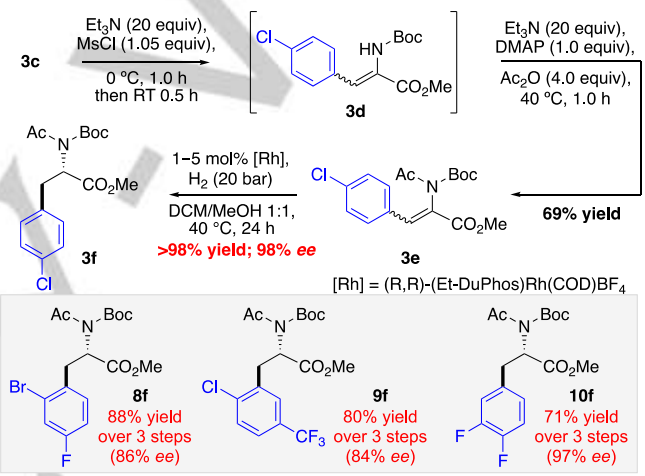


A preliminary mechanistic proposal for the catalytic transformation is put forth in Scheme 2. The aryldiazonium **3b** is reduced through single-electron transfer (SET) arising from oxidation of Fe^{II} to Fe^{III}.[19] The resulting aryl radical rapidly reacts with dehydroalanine to form a captodative[20] intermediate **A**. Subsequently, the radical polar-crossover,[21] which occurs through SET with mildly oxidizing $[Fe^{III}]$[22] to regenerate $[Fe^{II}]$, provides a reactive iminium **B** thus allowing the addition of water[23] to form the corresponding α,α–UAA (**3c**). The proposed scenario involving the intermediacy of iminium is supported by the isolation of **18c** in the presence of methanol. A radical chain mechanism has not been ruled out.[24]



Scheme 2. Plausible mechanism involving catalyzed arylhydroxylation of dehydroalanine with Cp_2Fe.

With a simple and reliable method to make α,α–UAAs in a CFR, we next explored their conversion to the valuable α–UAAs. In this context, a straightforward dehydration/enantioselective-hydrogenation protocol was developed. As shown in Scheme 3, **3c** was converted to the corresponding α,β–dehydroarylalanine (**3e**) in 69% yield over two steps. The Rh-(*R,R*)-Et-Duphos catalyzed enantioselective hydrogenation of **3e** under H_2–pressure (20 bar) furnished **3f**[25] in high efficiency and selectivity (i.e., >98% yield, >98% ee). The absolute stereochemical configuration of the α–UAA **3f** was determined through stereochemical correlation with authentic enantiomerically pure sample. Similarly, high yields were attained even with the bulkier and electron-deficient **8f** and **9f** (i.e., 3 steps: 88% and 80% yields, respectively). However, the attenuation in ee values (**8f:** 86% and **9f:** 84%) could be due to the *ortho*-substitution in both cases. Consequently, higher



enantioselectivity with **10f** (97% ee) was achieved.

Scheme 3. Efficient and enantioselective derivatization protocol for conversion to α–UAAs.

In summary, we report the first examples of a continuous flow process for the synthesis of α–hydroxyarylalanines. The strategy features a catalytic arylhydroxylation of dehydroalanine with transiently generated aryldiazonium salts in a controlled and safe manner. The reactions proceed with a broad range of electron-deficient aryl and heteroaryl based anilines in high yield, and do not require inert conditions. The CFR setup, which consists of three reactors powered by two syringe pumps, is simple to assemble and allows product generation within 37 minutes. Furthermore, a functionalization strategy for accessing α–UAAs in high enantioselectivity is also described. In light of this investigation, efforts are ongoing for the development of new peptide based conjugation strategies. Detailed studies to understand the mechanism are also underway.

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Keywords: captodative • dehydroalanine • aryldiazonium salts • arylhydroxylation • α,α–UAAs • redox catalysis • continuous flow • α–UAAs

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