Two-Step Synthesis of α-Aryl-α-diazoamides as Modular Bioreversible Labels

Joomyung V. Jun and Ronald T. Raines*

ABSTRACT: α-Aryl-α-diazoamides were synthesized in two steps under mild conditions. This expeditious route employs Pd-catalyzed C–H arylation of N-succinimidyl 2-diazoacetate to obtain N-succinimidyl 2-aryl-2-diazoacetates, followed by aminolysis. The ensuing diazo compounds can esterify carboxyl groups in aqueous solution, and the ester products are substrates for an esterase. The broad scope of the synthetic route enables the continued development of diazo compounds in chemical biology.

Since the discovery of diazomethane by von Pechmann in 1894,1 diazo compounds have become important reagents in synthetic organic chemistry. Often, diazo groups are utilized via thermal, photochemical, or transition-metal-mediated carbenoid formation for constructing new C–C, C–O, or C–N bonds.2 Recently, the utility of diazo compounds has been extended into the realm of chemical biology.3,4 Recent work has shown that α-aryl-α-diazoacetamides can esterify carboxyl groups in proteins (ribonuclease A,5 green fluorescent protein,6 and ribonuclease 1 7), enabling their delivery across cellular membranes (Figure S1).6,7 This strategy bears analogy to the use of ester prodrugs of small-molecule carboxylic acids.8 The critical attribute of efficacious diazo compounds is their basicity,9 which leads to abstraction of a proton from a carboxylic acid but not water and thereby to the esterification of carboxyl groups in aqueous solution.5,10 Moreover, the ensuing esters are substrates for intracellular esterases.6,7 This bioreversibility11 provides a unique means to “cloak” protein carboxyl groups in a traceless manner (Figure 1A).

Although this application of α-aryl-α-diazoamides has demonstrated promise, synthetic accessibility (e.g., a lengthy preparation time and a lack of scalability) has been a major deterrent to progress. Previously, such diazo compounds have been accessed via deimidogenation of the corresponding azide (Figure S1).12 This approach has a high tolerance for functional groups, but access to the azide typically required lengthy low-yielding synthetic routes.13 Additionally, the deimidogenation reaction was not compatible with 2-aryl-2-azidoacetamides containing bulky N-substituents.6

We sought a facile and general route to the modular α-aryl-α-diazoamide scaffold. Known synthetic routes can provide access to α-diazo carbonyl compounds. Most, however, focus on stable diazoketones, diazoesters, or aryl diazomethanes14 and employ explosive diazo-transfer reagents, high temperature, or strong base,15 conditions that can be incompatible with applications in chemical biology. Routes to α-aryl-α-diazoamides are underdeveloped and have limited substrate scope.14,16 Their preparation and isolation is challenging.
because of insolubility and functional group incompatibility.\textsuperscript{3,12,15,17}

Here, we report on the mild, efficient, and versatile synthesis of α-aryl-α-amides in two steps from a commercially available\textsuperscript{18} and highly scalable precursor, N-succinimidyl 2-diazoacetate (1).\textsuperscript{19} Desired α-aryl-α-diazoamides are accessed via palladium-catalyzed C–H arylation followed by aminolysis under mild and safe conditions (Figure 1B). This route encompasses multiple benefits for applications in chemical biology: (1) facility, (2) broad applicability because of available building blocks (i.e., aryl iodides and amines), and (3) compatibility with diverse functionality (e.g., azido and alkynyl groups) that can be useful for late-stage bioconjugation.

Metal-catalyzed C–H arylation in the presence of a diazo group has been reported only sporadically due to the undesired competitive formation of metal–carbene species (Figure 1B).\textsuperscript{20} Wang and coworkers reported on the C–H functionalization of ethyl diazoacetate using Pd(PPh\textsubscript{3})\textsubscript{4} and Ag\textsubscript{2}CO\textsubscript{3}, prevent product decomposition.\textsuperscript{21} The product, however, required the use of a strong base or metal catalyst to effect amidation.\textsuperscript{22} Mendoza and coworkers used another catalytic system, Pd(II) acetate and tris(2-furyl)phosphine (P(2-Fu)\textsubscript{3}), for the C–H arylation of N-phthalimidoyl diazoacetate to couple (hetero)aryl groups but encountered incompatibility with p-substituted electron-rich aryl groups (e.g., 4-iodoanisole).\textsuperscript{20c} Recently, Nelson and coworkers reported a suite of methods for the synthesis of α-diazoamides, including the C–H arylation of α-diazo N,N-disubstituted acetamides.\textsuperscript{15} Similarly, this method failed in the coupling of electron-rich substrates (e.g., 4-iodoanisole) and provided no examples of C–H arylation with α-diazo N-monosubstituted acetamides.

To access a large number of target compounds under mild conditions, we investigated diazo compound 1 as a coupling partner for C–H arylation. Diazo compound 1 has been used to install a diazo group via acyl transfer reactions with amines, phenols, thio phenol, and peptides.\textsuperscript{19} We envisioned that the C–H arylation of diazo compound 1 could enable a rapid entry into more complex succinimidyl diazo compounds (2) and ultimately into diverse α-aryl-α-diazoamides (3).

We found that diazo compound 1 can undergo arylation with aryl iodides containing a wide variety of functional groups (Scheme 1). To do so, we prepared diazo compound 1 on a gram scale (Figure S2)\textsuperscript{19,23} and employed a Pd(OAc)\textsubscript{2}/P(2-Fu)\textsubscript{3} catalytic system. Two additives, triethylamine (Et\textsubscript{3}N) and silver carbonate (Ag\textsubscript{2}CO\textsubscript{3}), prevent product decomposition and scavenge iodide, respectively.\textsuperscript{21,24} The reaction mixture was stirred in EtOAc at room temperature for 6 h. A range of aryl iodides, spanning electron-donating to -withdrawing p-substituted phenyl iodides, bulky m-substituted phenyl iodides, and a heteroaryl iodide, were subjected to the same reaction conditions. Notably, the electron-rich (2a), electron-neutral (2c), and electron-poor (2d) phenyl iodides all afforded high isolated yields (≥77%). Of the sterically hindered phenyl iodides, methoxy (2f) and trifluoromethyl (2h) functional groups at the m-position resulted in >80% isolated yields, whereas the smaller hydroxy group (2g) gave an even higher yield of 90%. We note too that 3-iodophenol (2g) proved to be orthogonal to the N-succinimidyl diazoester moiety, whereas 1-(4-iodophenyl)piperazine did not (Figure S11). The cross-coupling condition was compatible with heteroaryl substrate (2i) and a variety of fluoro groups, including trifluoromethoxy (2b), which is an important functional group for medicinal chemistry because of its high metabolic stability and cell permeability.\textsuperscript{25} We effected C–H arylation in the presence of a TMS-protected alkynyl (2e) or azido (2j) group in 95 and 66% yields, respectively. Further, compound 2j highlights a convenient means of diversification. This compound was accessed by a condensation reaction with 4-iodophenyl acetic acid. Lastly, we note that previously reported routes failed in arylation with 4-iodoanisole,\textsuperscript{15} whereas our route provided 2a

\textsuperscript{19}Reaction conditions: 10 mol % Pd(OAc)\textsubscript{2} 20 mol % P(2-Fu)\textsubscript{3}.
in 65% yield. Overall, we successfully demonstrated metal-catalyzed C–H arylation in the presence of N-succinimidyldiazoo groups, both of which will serve as important functionality for instilling diversity.

Next, we examined the aminolysis of representative N-succinimidyldiazoo groups 2b–2f and 2h. We first tested the aminolysis of analogous N-phthalimidoyldiazoo esters S5–S7, which were synthesized by a method reported previously (Figure 2A). Those diazoesters yielded only a

trace amount of diazoamide product based on liquid chromatography–mass spectrometry (LC–MS) analysis. Even after an extensive screening of reactant concentrations, solvents, and additives, aminolysis at N-phthalimidoyldiazoo esters proved to be unattainable, possibly due to rapid decarboxylation (Table S1). An initial evaluation of aminolysis with 2c showed that the use of 1,8-diazabicycloundec-7-ene (DBU) led to degradation, whereas Et3N afforded the desired product (Figure 2A). In these reactions, a solution of the N-succinimidyl diazoester was treated with a secondary amine and Et3N in tetrahydrofuran (THF) at 0 °C to yield the corresponding α-diazoamide (3e–3l) in up to 76% yield. An excess of Et3N was used to prevent product decomposition. Most of the reactions showed quantitative conversion based on analysis with TLC (Figure S12). Due to their apparent degradation on silica, the isolated yields for diazoamide compounds 3a, 3c, 3e, 3i, and 3k were low. Still, a wide range of aryl diazoesters was converted into N,N-disubstituted diazoamides.

Then, we demonstrated that the aminolysis of N-succinimidyldiazooacetates is also effective with various primary amines (Scheme 2). Those containing a

pyridinyl (4a), arylhalo (4b), Boc-protected amino (4c), alkynyl (4d), or azido (4e) group displaced the N-hydroxy succinimide moiety of 2c to yield the desired N-monosubstituted diazoamides. Additional scope for this reaction includes 6 N-succinimidyldiazooacetates × 4 primary amines = 24 α-aryl-α-diazoacetamides (see: Scheme S1).

Having accomplished the facile synthesis of α-aryl-α-diazoamides, we turned our attention to their esterification of carboxylic acids. Specifically, we screened for the O-alkylation of five structurally diverse small molecules, pivalic acid, rhodamine B, coumarin-3-carboxylic acid, biotin, and HGlUOMe, by three representative diazo compounds (3b, 3j, and 3l) in 1:1 acetonitrile:MES–NaOH buffer, pH 6.0, at 37 °C for 19 h (Figure S5). Each of the reactions was analyzed by LC–MS to quantify the esterified product as well as the hydrolyzed byproduct, α-aryl-α-hydroxyamide (Table S2). Though hydrolysis is unavoidable due to the excess of water, esterification was successful regardless of the steric and electronic nature of the carboxylic acid or diazo compound.

Finally, we tested the bioreversibility of esterification by our diazo reagents. As a model acid, we used AcGluNH2 (5), which we derived from L-glutamic acid and which represents the most common residue for protein esterification and the residues in human proteins. In compound 5, the N-terminal amino group is acetylated to prevent aminolysis of a side-chain ester, and the C-terminal carboxyl group is amidated to prevent main-chain esterification (Scheme S2). Compound 5 was treated with 3j to yield ester 6, which was then subjected to hydrolysis in the presence or absence of pig liver esterase (PLE) under biomimetic conditions at 37 °C for 19 h (Figure S6). Each of the reactions was analyzed by LC–MS to quantify the esterified product as well as the hydrolyzed byproduct, α-aryl-α-hydroxyamide (Table S2). Though hydrolysis is unavoidable due to the excess of water, esterification was successful regardless of the steric and electronic nature of the carboxylic acid or diazo compound.
will catalyze the hydrolysis of a nascent ester to reveal the native carboxylic acid of a protein.

Figure 3. LC−MS analysis of the enzyme-catalyzed hydrolysis of γ-glutamyl ester 6 at pH 7.2 and 37 °C.

In conclusion, we demonstrated a facile two-step synthesis of α-aryl-α-diazoamides, which are modular reagents. This route will expedite the ongoing exploration of diazo compounds as reagents in chemical biology. We anticipate that the bioreversibility of our modification will enable applications in chemical biology, including the cellular delivery of proteins.

■ ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/10.1021/acs.orglett.1c00793.

Experimental procedures, Tables S1−S3, Figures S1−S12, Schemes S1 and S2, NMR spectra, and additional compound characterization (PDF)

■ AUTHOR INFORMATION

Corresponding Author
Ronald T. Raines—Department of Chemistry and Koch Institute for Integrative Cancer Research at MIT, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States; orcid.org/0000-0001-7164-1719; Email: rtraines@mit.edu

Author
Joomyung V. Jun—Department of Chemistry and Koch Institute for Integrative Cancer Research at MIT, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States; orcid.org/0000-0002-7276-4806

Complete contact information is available at:

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Notes
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