Umpolung Amide Synthesis Using Free Amino Acids

By

Jonathan Witt

Thesis

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

in

Chemistry

August, 2014

Nashville, Tennessee

Approved:

Jeffrey N. Johnston, Ph.D.

Craig W. Lindsley, Ph.D.

ACKNOWLEDGMENTS

This work would not have been possible without numerous funding sources. I would like to thank the Vanderbilt Institute of Chemical Biology, the Vanderbilt Chemistry Department, and the NIH. I would also like to thank Dr. Jeffrey Johnston for support on an intellectual and personal level. We spoke at length concerning scientific and philosophical issues and these conversations contributed to my overall development. I hope to continue to strive for the same excellence in science that he embodies.

I would also like to thank the members of my preliminary exam committee, Dr. Gary Sulikowski and Dr. Piotr Kaszynski, as well as Dr. Craig Lindsley for his generous examination of this document. Additionally, I would like to thank the NMR staff of Vanderbilt University, in particular Dr. Don Stec and Dr. Markus Voehler, for help in interpreting and developing NMR experiments. I would like to acknowledge Dr. Jonathan Karty and his staff at Indiana University for performing the mass spectrometry experiments that were vital to my research.

I would like to thank the other members of the Johnston lab for their constant support and encouragement. In particular, I thank Jessica Shackleford and Dr. Bo Shen for paving the way in developing UmAS as a viable synthetic methodology.

Lastly, I wish to thank my family and friends, without whom this truly would have been impossible. Thank you for all of the conversations during the hard times and for always believing in me.

TABLE OF CONTENTS

LIST OF SCHEMES

LIST OF TABLES

LIST OF CHARTS

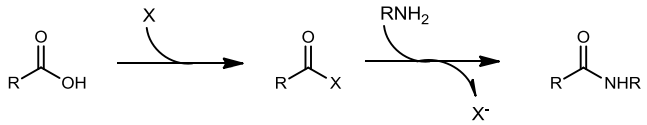
Chapter 1

Free Amino Acid Umpolung Amide Synthesis

1.1 Background

Amides are central linking components of biological and synthetic molecules. They can be found throughout biological systems but are central to peptide structure. They are equally essential to modern drug discovery, with 25% of known drugs containing at least one amide linkage.[1] Increasingly, peptide drugs (molecules greater than 500 Da but less than 50 amino acids) have become clinically useful. It has been estimated that there are currently 100 such drugs on the market, with others constantly forthcoming.[2] These longer peptide drugs continue to push the boundaries of current synthetic methods.

In nature, condensative methods are employed to assemble long chains of amino acid polymer with amide linkages. A free amino acid is coupled with a carboxylic acid via a thioester intermediate, with the formal loss of H_2O. The use of RNA templates allows for an accurate molecule-by-molecule approach to amide synthesis.[3] Enzymatic catalysis enables this inherently kinetically disfavored reaction to proceed smoothly and without epimerization. Synthetic chemists are faced with the challenge of selectively and quantitatively assembling amide linkages without the advantage of such precise catalytic methods.



Scheme 1. Activation of a carboxylic acid followed by aminolysis.

[1] Montalbetti, C.; Falque, V. *Tetrahedron.* **2005**, *61*, 10827–10852.
[2] Craik, D.J.; Fairlie, D.P.; Liras, S.; Price, D. *Chem. Biol. Drug. Des.* **2013**, *81*, 136-147.
[3] Pattabiraman, V.R.; Bode, J.W. *Nature*. **2011**, *480*, 471-479.

The primary strategy for laboratory amide bond synthesis has been the transformation of a carboxylic acid into a more reactive form, for which coupling with an amine becomes kinetically favorable. This is accomplished by replacing the hydroxyl moiety of the carboxylic acid with a better leaving group. Broadly speaking, this strategy can be subdivided based upon whether the reactive intermediate is used immediately, used upon isolation, or formed in the same pot with the amine before undergoing subsequent aminolysis (Scheme 1).[4]

A virtually endless selection of reagents exists for each paradigm. For the first and second type of reaction, in which a discrete intermediate is isolated or used immediately, nearly a century of innovation has created a range of active ester derivatives and a myriad of reagents to make them. These include the formation of acyl halides (most typically acyl chlorides), acyl azides, symmetric and mixed anhydrides, carbonic anhydrides, cyclic anhydrides, and active esters.[5] Though each approach has benefits, two universal drawbacks have increasingly limited their utility: the need to form the activated species in a separate reaction, and the inclination of these species to promote epimerization alpha to the carbonyl.

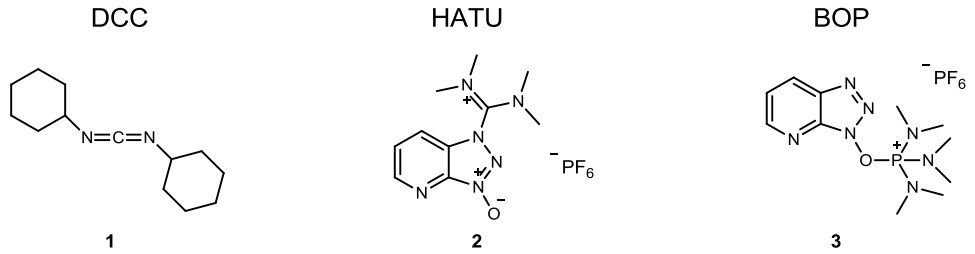


Chart 1. Common reagents for the activation of carboxylic acids.

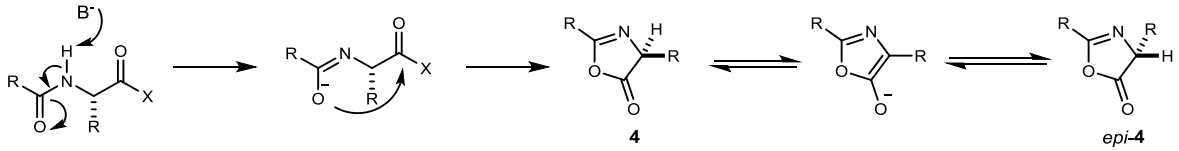
Several classes of reagents have now addressed the inconvenient requirement to isolate the activated acid (Chart 1). Among these are the carbodiimides (DCC **1**, EDC, DIC), uronium/aminium reagents (HATU **2**, HBTU), and phosphonium salts (BOP **3**, PyBOP).[6] Each of these reagents forms an activated ester with the target carboxylic acid *in situ* before undergoing aminolysis. This inherent efficiency helps to limit chemical waste and provides an

[4] Jones, J. *Amino acid and Peptide Synthesis*, Oxford Science Publications: Oxford, 1992.
[5] For general reviews on amide coupling: Montalbetti, C.A.G.N.; Falque, V. *Tetrahedron.* **2005**, *61*, 10827-10852. El-Faham, A.; Albericio, F. *Chem. Rev.* **2011**, *111*, 6557-6602.
[6] For reviews on *in situ* coupling reagents see: Han, S.; Kim, Y. *Tetrahedron.* **2004**, *60*, 2447-2467.; Valeur, E.; Bradley, M.; *Chem. Soc. Rev.* **2009**, *38*, 606-631,

appealing step economy. However, these newer reagents continue to allow some degree of epimerization.

Any amide coupling reagent that operates via activation of a carboxylic acid towards nucleophilic attack necessarily risks epimerization. This risk is brought on by the mechanism of epimerization, which can occur through several routes. The simplest mechanism for this transformation is the deprotonation of an α-proton. Activation of the carboxylic acid with a leaving group increases the acidity of this proton. Though α-deprotonation is still limited under common peptide coupling conditions, the addition of an activating group to the acid increases this possibility.

In the context of peptide synthesis, the formation of an intermediate oxazolone has been identified as the primary cause of epimerization (Scheme 2).[4] After the carboxylic acid becomes activated it is susceptible to attack by various nucleophiles, in particular the most proximal amide oxygen. The resulting oxazolone ring **4** is a competent electrophile that readily leads to the desired amide product. However, the oxazolone is also more prone to deprotonation than the open chain compound. Deprotonation can then lead to epimerization and loss of stereochemical purity.



Scheme 2. The mechanism for the formation and deprotonation of an oxazolone ring in a peptide.

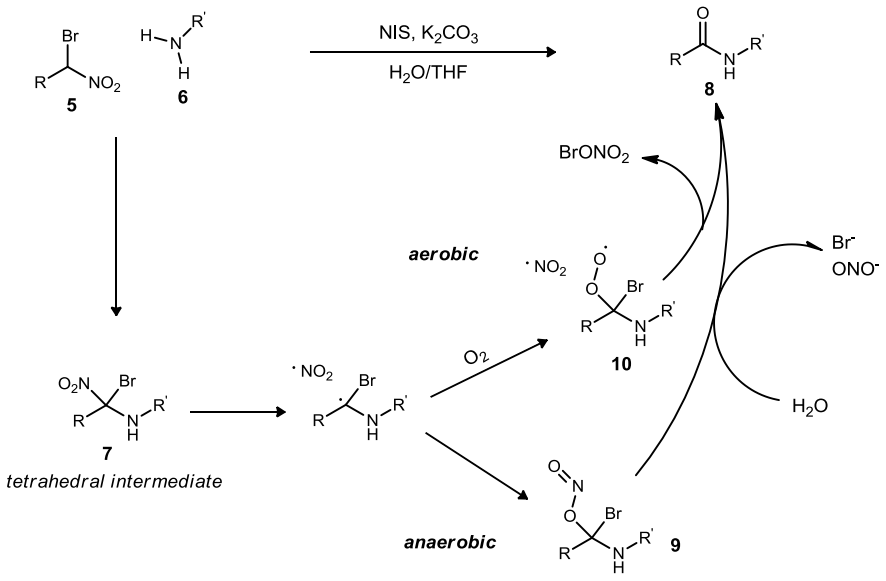
Recently, a novel reaction for amide synthesis was reported that circumvents the problem of epimerization.[7] It was reported that electrophilic amines combine with α-bromonitronates to yield amides. While working on the stereoselective addition of α-bromonitromethane to imines, colleagues in the Johnston lab had postulated that the resulting α-bromonitroalkanes may be readily converted to amides in the presence of an amine. This hypothesis was based on the similar oxidation state of the α-bromonitroalkane and the targeted

[7] Shen, B.; Makley, D.; Johnston, J.N.; *Nature*. **2010**, *464*, 1027.

amide, and knowledge of the Nef reaction. The Nef reaction, which can be used to convert nitroalkanes to aldehydes, is believed to proceed via the hydrolysis of an intermediate nitronate. It was postulated that an amine could serve as the nucleophile in this process and that the resulting tetrahedral intermediate could hydrolyze to furnish the desired amide product.

A small amount of amide product was observed after combining α-bromonitroalkane and amine for several days. The observation of debromonated nitroalkane in the reaction mixture led to the critical hypothesis that an electrophilic halogen source may be necessary for the reaction. Subsequent experiments incorporating stoichiometric amounts of halogenating reagents confirmed this conclusion. Optimum yields of amide were obtained using stoichiometric amounts of NIS in ethereal solvents (particularly THF).

Extensive mechanistic studies have suggested a novel umpolung mechanism for the transformation (Scheme 3).[8] Contrary to the initial hypothesis of a Nef related pathway, experimental results indicated that the amide product arises instead from the nucleophilic attack of the nitronate formed from nitroalkane **5** on an iodoamine formed from amine **6**. The resulting tetrahedral intermediate **7** could then undergo a radical-mediated transformation to provide the



Scheme 3. The suggested competing aerobic and anaerobic mechanisms for the umpolung amide coupling of an α-bromonitroalkane and an amine.

[8] Shackleford, J.; Shen, B.; Johnston, J.N. *Proc. Natl. Acad. Sci.* **2012**, *109*, 44.

amide **8**. Previous labeling studies indicate the nature of this process depends on whether the reaction is under anaerobic or aerobic conditions. Under argon the amide likely arises from rearrangement of the nitro group to give nitrite **9**. This process has been compared to examples of nitro-nitrite isomerization in the literature.[9] Under an oxygen atmosphere a notably different pathway has been identified. Beginning at the same tetrahedral intermediate, a stabilized radical is formed either via homolysis or another process. This radical is captured by molecular oxygen resulting in an intermediate **10** that collapses to provide amide.

The unique polarity with which the key carbon-nitrogen bond is formed inspired the term Umpolung Amide Synthesis (UmAS) for the reaction. In contrast with traditional methods for amide formation, epimerization does not readily occur with UmAS. β-Carbon epimerization of the α-bromonitroalkane is unlikely, particularly when in the nitronate form. The amine component is similarly stable even when an amino acid or other carbonyl containing compound is used. This lack of epimerization has been evidenced by a series of previous experimental results.[10] In combination with methods of asymmetric addition of bromonitromethane to imines[11] and aldehydes[9] UmAS represents a powerful method for the stereoselective synthesis of chiral amides.

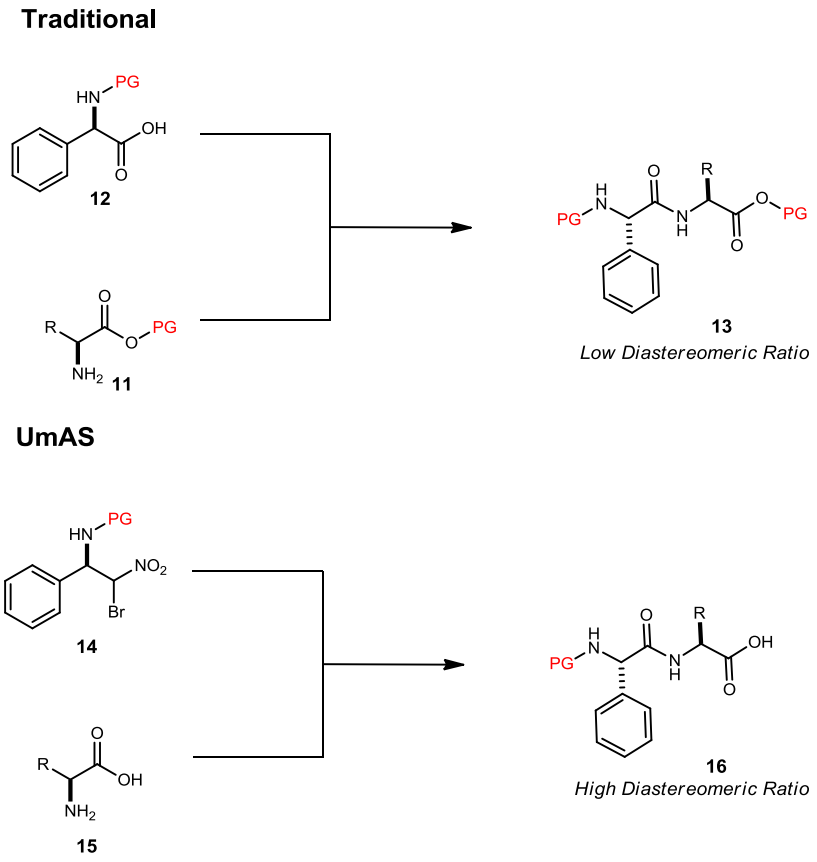
1.2 UmAS Couplings Using Unprotected Amino Acids

Traditional amide coupling techniques for peptide synthesis involve the use of protecting groups. In order to promote only the desired coupling reaction, all other amines and carboxylic acids present in the reaction must be masked (Scheme 4). During dipeptide synthesis, the carboxylic acid of the amino acid **11** is protected. The carboxy-protected amino acid **11** is then coupled via its free amine with a second amino acid **12** which has been amino-protected. The result of the coupling reaction is a dipeptide **13** that bears a protecting group at each end. Failure to follow the necessary protecting group scheme can result in undesired coupling products. However, use of two protecting groups in each reaction entails additional material and time.

We reasoned that UmAS could provide a means of partially avoiding this issue, granting access to peptides without a *C*-terminal protecting group (Scheme 4). Since carboxylic acids do

[9] Ketari, R.; Foucaud, A. *J. Org. Chem.* **1981**, *46*, 4498-4501.; Hartshorn, M.P.; Robinson, W.T.; Wright, G.J.; Yong, C.L. *Aust. J. Chem.* **1989**, *42*, 1569-78.
[10] Leighty, M.; Shen, B.; Johnston, J.N. *J. Am. Chem. Soc.* **2012**, *134*, 15233.
[11] Dobish, M.; Villalta, F.; Waterman, M.R.; Lepesheva, G.I.; Johnston, J.N.; *Org. Lett.* **2012**, *14*, 6322.

not take part in the UmAS reaction, it should be possible to use unprotected carboxylic acids as reagents. We envisioned that a protected α-amino-bromonitroalkane **14** could be reacted with an unprotected amino acid **15** to give dipeptides **16** containing an aryl glycine in high diastereomeric excess and without a protecting group at the carboxy terminus. In addition to reducing the number of protecting groups, this approach greatly reduces the risk of epimerization during coupling. This fact is especially relevant when arylglycines are used as coupling partners, as they are highly prone to epimerization under traditional coupling conditions.[12]



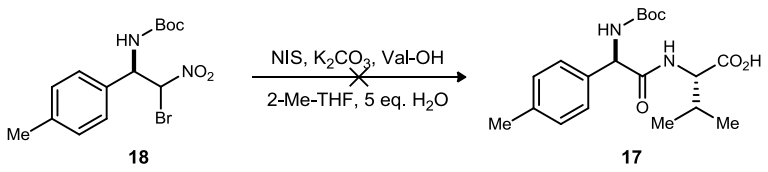
Scheme 4. Traditional vs. UmAS approaches to dipeptide synthesis.

Previous members of the Johnston laboratory have demonstrated the feasibility of coupling unprotected amino acids with α-bromonitroalkanes. However, the success of their attempts has varied considerably depending upon reaction conditions and the amino acid chosen for coupling. Previous successful attempts using phenylalanine and valine demonstrated that free

[12] Willians, R.M.; Hendrix, J.A. *Chem. Rev.* **1992**, 889-917.

amino acids can furnish the expected amide products, albeit in modest yields.[13] However, reactions using the relatively less hydrophobic amino acid glycine were unsuccessful.[14]

Though a precedent had been set for the targeted transformation, initial attempts to produce amide **17** under standard UmAS conditions from an α-bromonitroalkane **18** and free valine proved unsuccessful (Scheme 5). These reactions typically resulted in complex reaction mixtures. Adjustments to solvent or other reaction conditions had little effect on the results. While others had shown that α-bromonitroalkane substrates could be successfully coupled with free amino acids, it was apparent that the same methodology could not be applied universally.



Scheme 5 Initial attempts to couple valine with an α-amino bromonitroalkane were unsuccessful.

Though the electronic nature of the α-bromonitroalkane may be significant, a larger role was discovered for solvent. It was surmised that the insolubility of the amino acids in organic solvents may be the predominant issue in these types of reactions. A review of the literature revealed frequent notations of the insolubility of peptides and free amino acids in organic solvents, and a general trend towards increased solubilities in mixtures of organic solvents with water. The standard conditions for UmAS called for THF or DME as solvent with a small amount of water added (5 equiv). These mixtures were tested and failed to solubilize unprotected amino acids. These results suggested the need for an alternate solvent system designed to solvate both α-bromonitroalkane and free amino acid simultaneously.

Returning to previous work done in order to explore UmAS conditions, we discovered that a variety of solvent systems are tolerated by the reaction. Large amounts of water were initially included in these reactions, but it was later noted that 5 equivalents of water was sufficient to produce similar results. However, inclusion of greater amounts of water is not

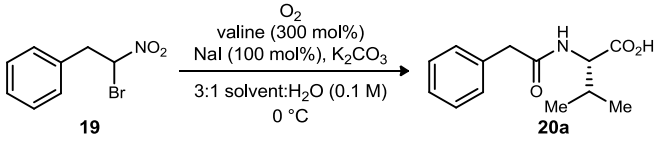
[13] Matt Leighty and Bo Shen. *Unpublished results.*
[14] Jessica Shackleford. *Unpublished results.*

precluded. It was previously determined that 3:1 mixtures of a variety of organic solvents with water resulted in moderate conversions to a standard amide.[15]

It occurred to us that some mixture of organic solvent and water may meet our need to solvate both the α-bromonitroalkane and amino acid (Table 1). Applying a solvent screen to the reaction of a standard bromonitroalkane **19** with valine, we were able to establish the promise of this strategy. In contrast to earlier attempts, solvents such as toluene and dichloromethane, which have limited miscibility with water, completely failed to promote any reaction. Alcoholic solvents such as methanol also failed to produce adequate yields. However, we were gratified to observe that THF, DME and dioxane were successful in furnishing the desired amide **20a** in low to moderate yields. A further screen of solvent ratios revealed that 3:1 THF:H_2O was the ideal system for the reaction. It was also determined that extra equivalents of base had little or no positive impact on isolated yield.

With the fundamental issue of solubility addressed, we turned to further optimization (Table 2). The practicality of UmAS reactions using substoichiometric amounts of NIS in

Table 1. Results of a solvent screen of the reaction between an α-bromonitroalkane and valine.

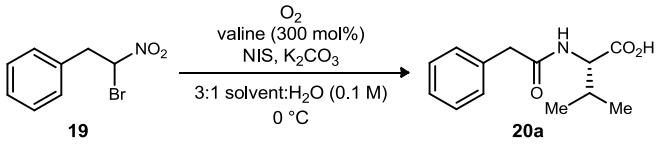


19 **20a**

entry	solvent	yield (%)[a]
1	DCM	0
2	Toluene	0
3	EtOH	Trace
4	MeOH	Trace
5	DMF	Trace
6	Acetone	Trace
7	DME	41
8	Dioxane	46
9	THF	57

[a] Isolated yield.

[15] Bo Shen. *Unpublished results*.



19 **20a**

entry	NIS (eq)	yield (%)
1	0.2	50
2	0.6	61
3	1.0	70
4	NaI (1.0)	57
5	I_2 (1.0)	65

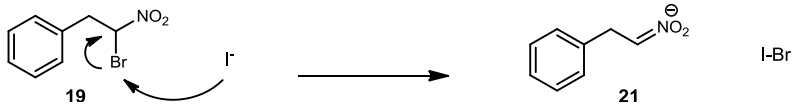
Table 2. Comparison of different halogen sources in the reaction between an α-bromonitroalkane and valine.

combination with oxygen was established in prior work. Though the mechanism of turnover has not yet been clarified, the basic observation was documented by several investigators. This finding became useful when it was discovered that succinimide, a product formed by deiodination of NIS, is often inseparable from the desired amide product. Though both 20 and 60 mol % catalyst loadings gave similar yields to the stoichiometric reaction, separation of even a small amount of succinimide from the reaction proved difficult.

The ultimate solution to the problem of purification came while exploring alternate sources of iodine for the reaction. It was found that both I_2 and NaI were competent in this role, a fact previously established by others.[15] Though NaI appeared moderately less efficient than I_2 for the purposes of the model reaction, it was selected as the most convenient alternative. Starving the reaction of iodide catalyst resulted in a reduced yield (trace by crude NMR), confirming the pivotal role of this additive in promoting the reaction. This result closely mirrors background reaction rates recorded in previous studies of UmAS. It has been speculated that the amine can be converted to a reactive *N*-bromo amine through bromination by the α-bromonitroalkane, providing the small amount of product typically noted in cases where a halogenating agent is not added.

The effectiveness of NaI in promoting the amide coupling suggested a mechanistic question, which is perhaps closely related to the issue of the observed background reaction rate. If I^+ is necessary for the activation of amine substrate, an explanation must be offered for the oxidation of I^- derived from sodium iodide. Two immediate possibilities can be considered:

direct oxidation of NaI by oxygen, and formation of I-Br resulting from nucleophilic attack of I^- upon the α-bromonitroalkane **19** to form nitronate **21** (Scheme 6). Though direct oxidation by oxygen seems logical, no such literature precedent exists. The latter suggestion of an electrophilic bromine is more consistent with experimental as well as theoretical considerations. This mechanistic notion also closely mirrors the mechanism that has been suggested for the background reaction, in which an electrophilic bromine serves to activate the amine.



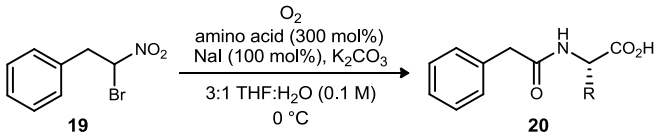
Scheme 6. Possible mechanism for the formation of I^+.

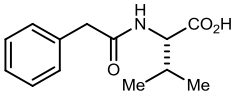
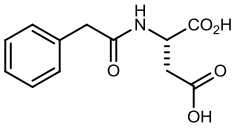
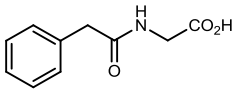
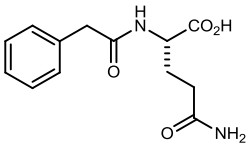
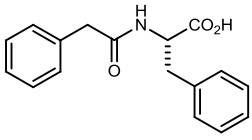
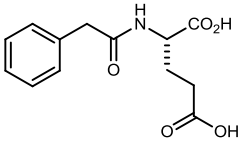
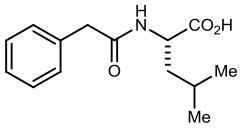
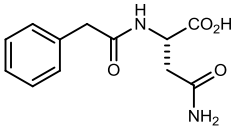
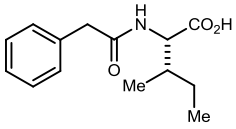
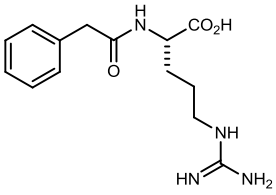
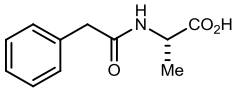
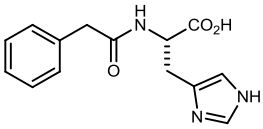
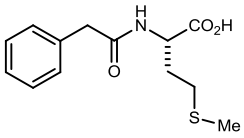
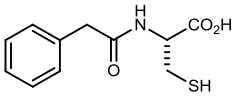
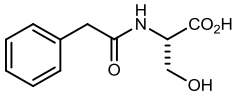
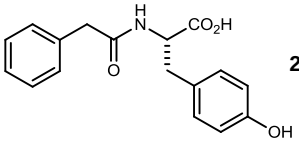
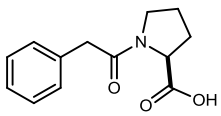
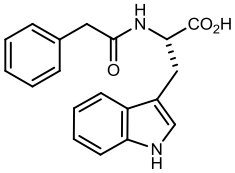
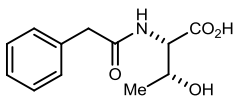
It should be noted that desbromonitroalkane has been observed by others in prior work. Though this fact does not prove a mechanistic pathway for I^- oxidation, it suggests that debromination to form the elements of I-Br is theoretically viable. Furthermore, catalytic studies have revealed that under an oxidative environment a small amount of I^+ is sufficient to match the yield when stoichiometric NIS is used.[16] Therefore, the oxidation of a small amount of I^- by this pathway may be sufficient to effect full conversion.

Following reaction optimization, these reaction conditions were applied to the twenty natural amino acids (with the exception of lysine) using the standard α-bromonitroalkane **19** (Table 3). The canonical amino acids were used as readily available examples exhibiting a wide range of functionalization. The amino acids with alkyl side chains as well as glycine produced amides **20a**-**20f** in moderate to good yields. It was speculated that the relative hydrophobicity of these amino acids made them better candidates for the reaction. Methionine, serine, and proline gave amides **20g**, **20h**, and **20i** in slightly lower yields of 27-30%. This set of results emphasized the difficulty of using more polar or secondary amino acids in the reaction, but demonstrated the viability of certain examples. Threonine, aspartic and glutamic acid, asparagine and glutamine appeared to show products **20j**-**20n** by NMR analysis of the crude reaction mixtures. However, the products were not isolated. Arginine, histidine, cysteine, tyrosine and tryptophan did not

[16] "Umpolung Amide Synthesis Using Substoichiometric NIS and Oxygen as a Terminal Oxidant" Schwieter, K. E.; Shen, B.; Shackleford, J. P.; Leighty, M. W.; Johnston, J. N. submitted.

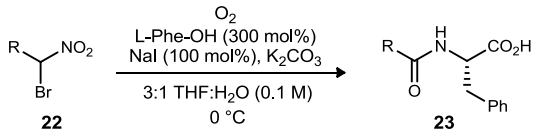
Table 3. Substrate scope of the UmAS reaction between a standard α-bromonitroalkane and various amino acids.

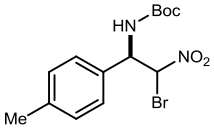
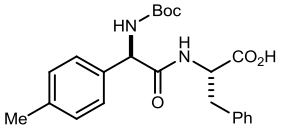
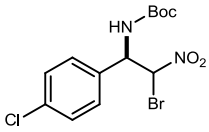
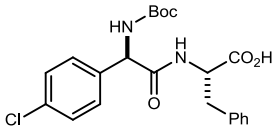
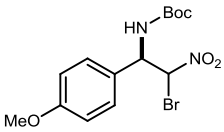
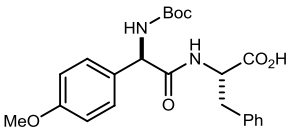
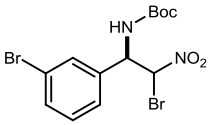
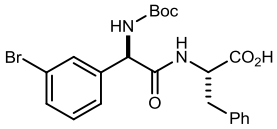
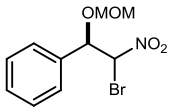
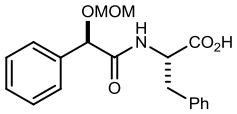
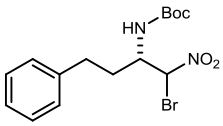
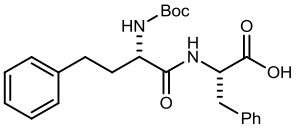


entry	amino acid	product	product number	yield[a] (%)	entry	amino acid	product	product number	yield[a] (%)
1	Val-OH		**20a**	57	11	Asp-OH		**20k**	trace[b]
2	Gly-OH		**20b**	58	12	Gln-OH		**20l**	trace[b]
3	Phe-OH		**20c**	68	13	Glu-OH		**20m**	trace[b]
4	Leu-OH		**20d**	54	14	Asn-OH		**20n**	trace[b]
5	Ile-OH		**20e**	51	15	Arg-OH		**20o**	0
6	Ala-OH		**20f**	41	16	His-OH		**20p**	0
7	Met-OH		**20g**	30	17	Cys-OH		**20q**	0
8	Ser-OH		**20h**	31	18	Tyr-OH		**20r**	0
9	Pro-OH		**20i**	27	19	Trp-OH		**20s**	0
10	Thr-OH		**20j**	trace[b]					

[a] See experimental section for general conditions. [a] Isolated yields. [b] Product observed by ^{1}H NMR.

Table 4. Substrate scope of free acid UmAS utilizing various α-bromonitroalkanes.



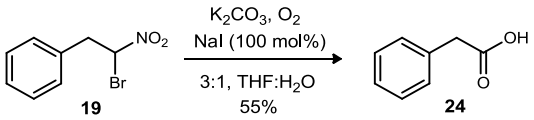
entry	bromonitroalkane	starting material	product	product number	isolated yield (%)
1		**22a**		**23a**	68
2		**22b**		**23b**	41
3		**22c**		**23c**	48
4		**22d**		**23d**	46
5		**22e**		**23e**	57
6		**22f**		**23f**	59

produce the desired amides **20o**-**20s**. In some cases these reactions generated known side products of the UmAS reaction. In other cases complex reaction mixtures were detected by crude NMR analysis. It should be noted that unprotected side chains were used in each case. It is possible that these results could be improved by the use of common side chain protected amino acid derivatives.

After confirming the compatibility of UmAS with many of the natural amino acids, we considered the role of the bromonitroalkane. A brief scope (Table 4) demonstrates the wide

applicability of this methodology. As previously mentioned, we were particularly interested in the application of UmAS to aryl glycine amino acid surrogates. Therefore, we tested the reaction in four such cases. Two electron rich aryl glycine surrogates **22a** and **22c** provided moderate to good yields of desired amides **23a** and **23c**. Electron poor substrates **22b** and **22d** resulted in similar yields of the corresponding amides **23b** and **23d**. We also demonstrated the compatibility of the reaction with α-oxy bromonitroalkane **22e**. Free acid UmAS resulted in a moderate yield of amide **23e**. To show that the reaction is compatible with additional alkyl substrates beyond the prototypical bromonitroalkane **19**, we tested homophenylalanine surrogate **22f**. This once again resulted in a reasonable yield of the desired compound **23f**. It is important to note that these yields should be compared with the traditional two-step approach to accessing these compounds, which typically involves the coupling of a protected amino acid followed by deprotection of the carboxy terminus.

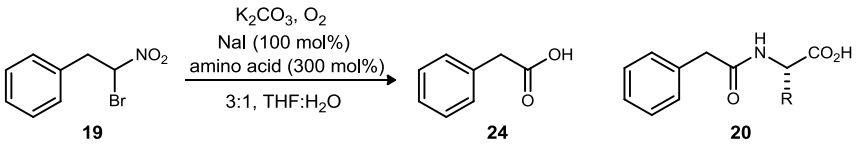
1.3 A Novel Pathway for the Formation of Carboxylic Acids



Scheme 7. Synthesis of a carboxylic acid from an α-bromonitroalkane.

In the course of our investigation of amino acids in the UmAS reaction, a unique side reaction was uncovered. Under the amide coupling reaction conditions, in cases where UmAS was relatively slow, moderate yields of a carboxylic acid **24** were generated (Scheme 7). In many cases, analysis of crude reaction mixtures by NMR indicated complete conversion of starting material to a mixture of the desired amide along with the corresponding carboxylic acid. The original studies of UmAS included experiments indicating that although the amide did not form from an active ester, the formation of carboxylic acid or an active ester precursor were not precluded. The ratio of the two products in several cases, as measured by integration of benzylic (^{1}H NMR of crude reaction mixtures), is detailed in Table 5. Higher relative abundances of carboxylic acid were formed in reactions such as that with aspartic acid, where formation of amide **20k** was slow. In this case a ratio of amide:acid of 1:5.6 was recorded. Conversely,

Table 5. Relative abundance of amide and carboxylic acid in a series of UmAS reactions.



19 **24** **20**

entry	amino acid	amide product	isolated yield amide (%)	ratio amide:acid
1	Phe-OH	**20b**	68	8.6:1
2	Val-OH	**20a**	57	2.0:1
3	Ser-OH	**20h**	31	1:1.3
4	Pro-OH	**20i**	27	1:1.5
5	Asp-OH	**20k**	0	1:5.6

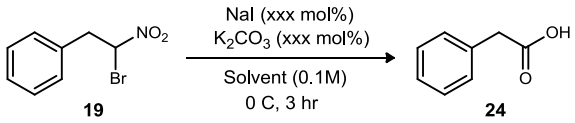
efficient amide coupling reactions such as that with phenylalanine coincided with lower observed amounts of carboxylic acid and a high isolated yield of amide **20b**. In this case the ratio of amide:acid favorably increased to 8.6:1. These experiments indicate a direct competition between carboxylic acid and amide formation. At this point we postulated that one key to increasing the yield and consistency of free acid UmAS reactions would be to understand and control the rate of carboxylic acid formation.

It was determined that under the standard conditions for free acid UmAS (Table 6, entry 3), no starting material remains after only 3 hours, with the primary product being carboxylic acid **24**. This leads to the conclusion that the free acid amides under investigation must also be formed within this timeframe. Indeed, with cases such as phenylalanine coupling, which proceeds with little phenylacetic acid formation, the conversion to free acid amide must occur in much less than three hours. Though significant evidence has already been presented in our group that UmAS coupling is complete in several hours, the current practical paradigm calls for overnight reaction time. The current study of carboxylic acid formation from α-bromonitroalkanes only provides further evidence that this practice is unnecessary.

A series of experiments determined the necessary conditions for the formation of carboxylic acid **24** from α-bromonitroalkane **19** (Table 6). It was established that the observed reaction is not the result of decomposition of the starting material, as α-bromonitroalkane stirred for 3 hours in solvent is recovered intact (Table 6, entry 1). Next, we probed the role of sodium

iodide and K_2CO_3 in the reaction (Table 6, entry 2). The reaction without sodium iodide but including K_2CO_3 proceeds well, indicating that K_2CO_3 is necessary and sufficient for the conversion and that NaI is not necessary. However, these reactions consisistently resulted in slightly lower isolated yields and messier crude reaction mixtures as judged by NMR when compared with reactions including K_2CO_3 *and* sodium iodide (Table 6, entry 3). This fact indicates that sodium iodide may accelerate the reaction. The exact mechanistic underpinnings of this observation are not yet understood.

Table 6. Isolated yields for the conversion of α-bromonitroalkane to carboxylic acid under a variety of conditions.



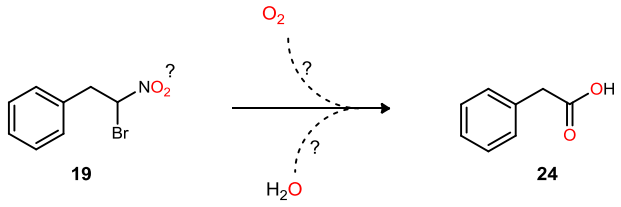
entry	NaI (mol%)	K_2CO_3 (mol%)	atmosphere	solvent	Isolated Yield (%)
1	0	0	O_2	3:1 THF:H_2O	0
2	0	400	O_2	3:1 THF:H_2O	46
3	100	400	O_2	3:1 THF:H_2O	64
4	100	100	O_2	3:1 THF:H_2O	61
5	100	400	O_2	THF	7
6	100	400	capped	3:1 THF:H_2O	60
7	100	400	argon	3:1 THF:H_2O	3

We subsequently examined the role of water in the reaction. Forgoing the addition of water to the reaction results in greatly reduced conversion and isolated yield. It is unclear whether this is due to the direct participation of water in the reaction, or the increased stability of some crucial intermediate in a mixture of water and an organic solvent. It should be noted that previous experiments in our lab have revealed that esters can be formed under UmAS conditions when 10 equivalents of ethanol are added to reaction mixtures in the absence of amine.[16] This

result in combination with the observation that water is necessary to form carboxylic acid **24** from α-bromonitroalkane **19** suggests the possibility of a common active ester intermediate.

Finally, we turned to the possible involvement of molecular oxygen in the reaction. A trial which reduced the amount of available oxygen by using a capped vial instead of one with an oxygen balloon maintained the conversion and yield observed previously (Table 6, entry 6). However, degassing of the reaction solvent coupled with use of an inert atmosphere resulted in low conversion and yield. This result strongly indicates the importance of molecular oxygen to the reaction.

In an effort to further uncover the mechanism for generating the carboxylic acid, a series of labeling experiments were conducted. Following the model established by other labeling experiments in our lab, we conducted experiments using ^{18}O-labeled starting materials.[8] This approach should allow us to track the contribution of each starting material to the carboxylic acid oxygens of the product. We reasoned that these oxygens were most likely derived from three possible sources: the nitro group of the α-bromonitroalkane starting material **19**, from water and/or from atmospheric oxygen (Scheme 8).

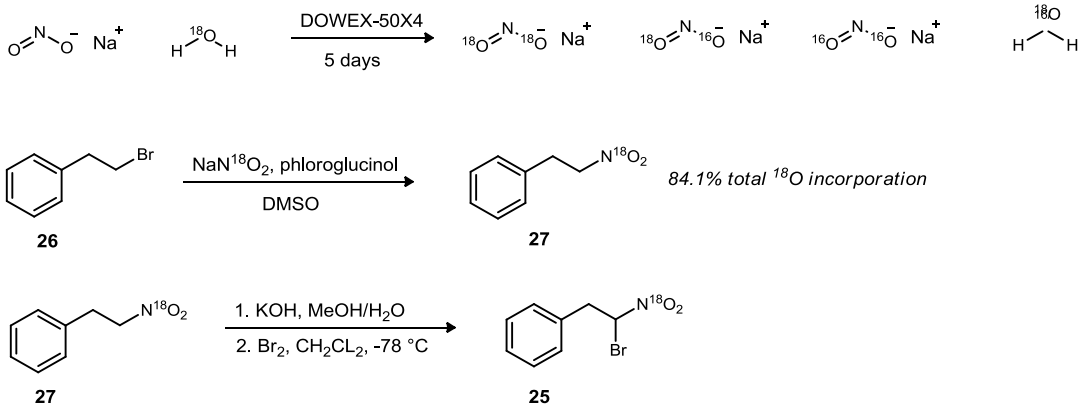


Scheme 8. Potential sources for the carboxylic acid oxygens.

The first step was to obtain the labeled starting materials. $^{18}O_2$ and $H_2{}^{18}O$ are available from commercial sources. We were able to synthesize the labeled α-bromonitroalkane **25** using a previously established process (Scheme 9).[8] $N^{18}O_2$ was generated according to literature precedent by exposing unlabeled $NaNO_2$ to slightly acidic (pH 3-5) $H_2{}^{18}O$.[17] Bromide **26** was then converted to nitroalkane **27** under S_N2 conditions using the labeled $NaN^{18}O_2$. At this point, high resolution mass spectroscopy (HRMS) was used to confirm the incorporation of ^{18}O into the

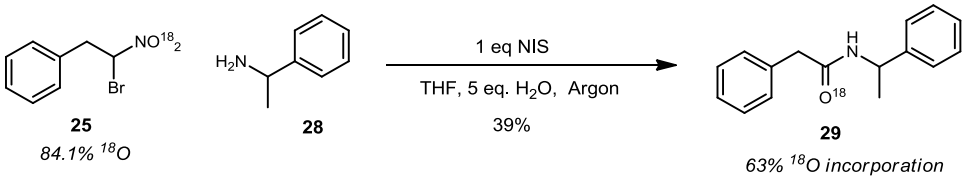
[17] Yang, C.C.; Goldberg, I.H. *J. Labelled Compd. Radiopharm.* **1989**, *27*, 423.

product. The analysis showed that the labeling procedure had generated 70.6% doubly labeled nitroalkane, 26.9% singly labeled nitroalkane, and 2.8% unlabeled nitroalkane. The total incorporation of ^{18}O into **27** was therefore determined to be 84.1%. This result is closely aligned with the previous reported attempt to label the same nitroalkane, which resulted in a total ^{18}O incorporation of 83.6%.[8] After a high level of ^{18}O incorporation was determined, nitroalkane **27** was brominated to give labeled α-bromonitroalkane **25**.



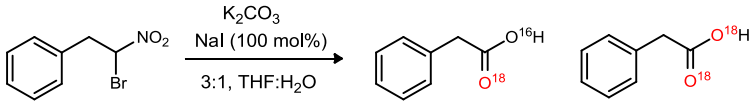
Scheme 9. Synthesis of ^{18}O labeled α-bromonitroalkane **25**.

Before any new labeling experiments were attempted, an experiment that had been previously performed (Scheme 10) was replicated.[8] Labeled α-bromonitroalkane **25** was stirred with 5 equivalents of amine **28** in the presence of NIS in degassed THF/H_2O to give a 39% yield of amide **29**. ^{13}C NMR analysis of the product revealed 63% labeling of the amide oxygen, which compared favorably with the previous result of 66% incorporation. However, it should be noted that the yield of 39% was significantly lower than the 70% yield previously reported. The discrepancy notwithstanding, the result demonstrated the soundness of our technique, obtaining reproducible levels of ^{18}O incorporation.



Scheme 10. UmAS labeling experiment using labeled α-bromonitroalkane .

Table 7. Results of labeling experiments to determine the mechanism of carboxylic acid formation.



19 **24a** **24b**

entry	aerobic/ anaerobic	^{18}O Source	singly labeled (%)	doubly labeled (%)	yield (%)[a]
1	aerobic	$H_2{}^{18}O$	8.1	0	40
2	anaerobic	$H_2{}^{18}O$	7.2	0	15
3	anaerobic	$RN^{18}O_2$	20.6	0	40
4	aerobic	$^{18}O_2$	23.5	1.7	75

Having synthesized the needed starting material and confirmed the accuracy of our technique, we turned to a series of experiments to probe the nature of the formation of carboxylic acid (Table 7). It should be noted that two oxygens are present in the product, therefore three compounds can result from a labeling experiment: unlabeled carboxylic acid **24**, carboxylic acid with incorporation of one ^{18}O **24a**, and carboxylic acid with incorporation of two ^{18}O's **24b**.

The first set of experiments was conducted using $H_2{}^{18}O$. A mixture of α-bromonitroalkane **19**, K_2CO_3, and NaI in THF/ $H_2{}^{18}O$ was stirred under anaerobic conditions. A separate reaction examined the oxygen contribution from $H_2{}^{18}O$ under aerobic conditions. These experiments resulted in 8.1% singly labeled product **24a** under aerobic conditions and 7.2% singly labeled product **24a** under anaerobic conditions. No doubly labeled product **24b** was detected in either case.

Subsequent experiments examined the contributions of labeled α-bromonitroalkane **25** and labeled $^{18}O_2$. When subjected to the standard reaction conditions under argon, labeled α-bromonitroalkane provided 20.6% singly labeled product **24a**. Again, no doubly labeled product **24b** was detected. Finally, when the standard reaction was run under an $^{18}O_2$ environment, 23.5% singly labeled product **24a** and 1.7% doubly labeled product **24b** were detected.

It should be noted that all analyses of ^{18}O incorporation were performed by HRMS. During the course of our investigation, we discovered that IR and ^{13}C NMR analyses of the carboxylic acid products were insufficiently sensitive or reliable for this purpose, unlike their efficacy when analyzing ^{18}O content in amide products.[8] The fact that three possible products can

result from each labeling experiment necessitates an analytical technique which is sufficiently sensitive to differentiate between them.

Though the contribution from each source is small, it can be concluded from these studies that all of the oxygen sources tested may play a role in the formation of the carboxylic acid. Without further experimental results it is impossible to speculate on a specific mechanism. It should be noted that the results reported here represent one trial of each reaction. Further studies would be required to definitively identify all the sources of oxygen that contribute to the final product. Thorough study of the necessary components of the reaction coupled with tentative labeling results strongly indicate that molecular oxygen is incorporated into the final product. Further studies should address this hypothesis.

1.4 Conclusion

Initial results have shown the promise of using free amino acids in UmAS. Out of the nineteen canonical amino acids subjected to the optimized reaction conditions, nine examples furnished the desired product in low to moderate yield (27%-68%). Furthermore, the reaction has been shown to be compatible with secondary amines as well as unprotected alcohols. In addition, several examples seemed to produce some product by analysis of crude reaction mixtures by NMR, but not enough to isolate.

In each case, a clear competition between formation of amide and carboxylic acid was identified. More facile amide couplings resulted in higher yields of amide and reduced presence of carboxylic acid in crude reaction mixtures. These findings indicate that conditions may exist that can increase the rate of amide coupling while simultaneously maintaining or lowering the rate of carboxylic acid formation. If these conditions can be identified, it is likely that further examples of free carboxylic acid amides will be isolated.

Another focus was the identification of the mechanism of carboxylic acid formation under conditions typical of UmAS. Initial results indicated that the oxygens found in the product may originate from the nitroalkane starting material, water, and atmospheric oxygen. Further experiments are needed to critically evaluate levels of incorporation from each of these sources. Once the mechanism for carboxylic acid formation is identified, this information this information might be used to reduce the impact of this pathway on the yield of desired products.

Chapter 2

Experimental

General Experimental Details

All reagents and solvents were commercial grade and purified prior to use when necessary. Tetrahydrofuran (THF) was dried by passage through a column of activated alumina as described by Grubbs.[18] In cases where water is used as cosolvent, this was done to accurately quantitate the amount of water in each reaction. NIS was recrystallized from dioxane/CCl_4.

Thin layer chromatography (TLC) was performed using glass-backed silica gel (250 μm) plates, and flash chromatography utilized 230-400 mesh silica gel from Scientific Adsorbents. Products were visualized by UV light, potassium iodoplatinate, and/or the use of $KMnO_4$ solution.

IR spectra were recorded on a Thermo Nicolet IR100 spectrophotometer and are reported in wavenumbers (cm^{-1}). Compounds were analyzed as neat films on a NaCl plate (transmission). Nuclear magnetic resonance spectra (NMR) were acquired on a Bruker DRX-400 (400 MHz) or a Bruker AVIII-600 (600 MHz) spectrometer. Chemical shifts are measured relative to residual solvent peaks as an internal standard set to 7.26 and 77.0 for $CDCl_3$ and 2.50 and 39.52 for d_6-DMSO. Mass spectra were recorded on a Thermo Electron Corporation MAT 95XP-Trap mass spectrometer by use of chemical ionization (CI), electron impact ionization (EI) or electrospray ionization (ESI) by the Indiana University Mass Spectrometry Facility. Optical rotations were measured on a Perkin Elmer-341 polarimeter.

^{18}O Percentage Mass Spectrometry Calculation

Contributions to the [M+2] and [M+4] mass peak include singly and doubly labeled M(^{18}O) and M($^{18}O_2$) carboxylic acid and combinations of all naturally occurring heavy atoms with combined masses of [M+2] and [M+4]. Their contribution is removed from the final ^{18}O percentage by the following calculations:

[18] Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics*. **1996**, 15, 1518-1520.

([M] ion intensity) x (predicted [M+2] ion natural abundance in unlabeled compound) = **[M+2] ion intensity expected in unlabeled compound**

([M+2] ion intensity) – ([M+2] ion intensity expected in unlabeled compound) = **corrected ^{18}O ion intensity**

([M+2] ion intensity) x (predicted [M+2] ion natural abundance in unlabeled compound) = **[M+4] ion intensity expected from one ^{18}O label combined with natural heavy atoms**

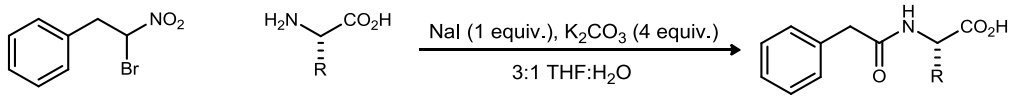
([M] ion intensity) x (predicted [M+4] ion natural abundance in unlabeled compound) = **[M+4] ion intensity expected from unlabeled compound with natural heavy atoms**

([M+4] ion intensity) – ([M+4] ion intensity expected from one ^{18}O label combined with natural heavy atoms) – ([M+4] ion intensity expected from unlabeled compound with natural heavy atoms) = **corrected $^{18}O_2$ ion intensity**

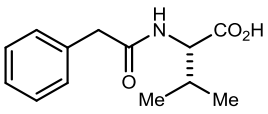
(corrected ^{18}O ion intensity) / (([M] ion intensity) + (corrected ^{18}O ion intensity) + (corrected $^{18}O_2$ ion intensity)) = **corrected % ^{18}O ion**

(corrected $^{18}O_2$ ion intensity) / (([M] ion intensity) + (corrected ^{18}O ion intensity) + (corrected $^{18}O_2$ ion intensity)) = **corrected % $^{18}O_2$ ion**

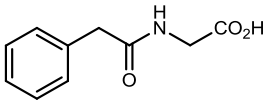
General Procedure for UmAS Free Acid Peptide Synthesis



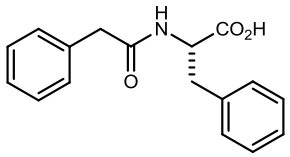
To a round-bottomed flask equipped with a stir bar were added α-bromonitroalkane (1.0 equiv) and amino acid (3.0 equiv), followed by THF and water (3:1, 0.1 M). The reaction was cooled to 0 °C and NaI (1.0 equiv) and K_2CO_3 (4.0 equiv) were added. An O_2 balloon was attached and the reaction was allowed to stir at 0 °C for 1-2 days. The reaction mixture was quenched with excess 3 M HCl and concentrated to remove THF. The reaction mixture was transferred to a separatory funnel and extracted with ethyl acetate. The organic fractions were combined and washed with satd aq sodium thiosulfate, dried, filtered, and concentrated. The crude residue was then purified by column chromatography, recrystallization, or trituration.



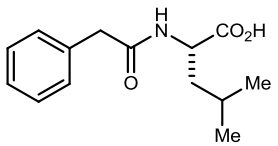
(*S*)-3-Methyl-2-(2-phenylacetamido)butanoic acid (20a). The α-bromonitroalkane (50.0 mg, 217 μmol) and L-valine (76.3 mg, 651 μmol) were subjected to the general coupling procedure. Purification by flash chromatography (SiO_2, 10-40% ethyl acetate in dichloromethane with 1% AcOH) gave the amide as a white powder (29.2 mg, 57%). $[\alpha]_D^{20}$ -5.6 (*c* 0.13, DMSO); mp 126-130 °C; R_f = 0.15 (30% EtOAc/DCM, 1% AcOH); IR (film) 3316, 2922, 1710, 1597, 1551 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 8.21 (d, *J* = 8.6 Hz, 1H), 7.32-7.26 (m, 4H), 7.22-7.20 (m, 1H), 4.15 (dd, *J* = 8.6, 5.8 Hz, 1H), 3.56 (d, *J* = 13.8 Hz, 1H), 3.48 (d, *J* = 13.8 Hz, 1H), 2.05 (dqq, *J* = 13.6, 6.8 Hz, 1H), 0.87 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 6.8 Hz, 3H)[19]; ^{13}C NMR (100 MHz, d_6-DMSO) ppm 173.1, 170.4, 136.6, 129.0, 128.1, 126.3, 57.2, 41.9, 29.9, 19.1, 18.0; HRMS (ESI) Exact mass calcd for $C_{13}H_{17}NO_3$ $[M+Na]^+$ 258.1095, found 258.1106.



2-(2-Phenylacetamido)acetic acid (20b). The α-bromonitroalkane (50.0 mg, 217 μmol) and L-glycine (48.9 mg, 651 μmol) were subjected to the general coupling procedure. Washing of the crude residue with carbon tetrachloride gave the amide as an off-white solid (20.4 mg, 49%). Mp 131-134 °C; R_f = 0.11 (30% EtOAc/DCM, 1% AcOH); IR (film) 3283, 3063, 2920, 2522, 1729, 1658, 1543 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 8.36 (t, *J* = 5.7, 1H), 7.31-7.25 (m, 4H), *7*.23-7.19 (m, 1H), 3.76 (d, *J* = 5.9 Hz, 2H), 3.47 (s, 2H)[19]; ^{13}C NMR (100 MHz, d_6-DMSO) ppm 171.3, 170.5, 136.2, 129.1, 128.2, 126.3, 42.0, 40.7; HRMS (ESI) Exact mass calcd for $C_{10}H_9NO_3Na$ $[M+Na]^+$ 215.1813, submitted for analysis. Prepared previously by a different method and characterized by NMR (300 MHz, d6-acetone) and LRMS.[20]



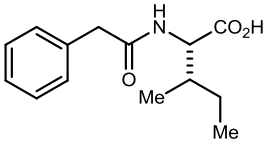
(*S*)-3-Phenyl-2-(2-phenylacetamido)propanoic acid (20c). The α-bromonitroalkane (50.0 mg, 217 μmol) and L-phenylalanine (107.5 mg, 651 μmol) were subjected to the general coupling procedure. Purification by flash chromatography (SiO_2, 10-40% ethyl acetate in dichloromethane with 1% AcOH) gave the amide as a light-yellow oil (41.0 mg, 68%). $[\alpha]_D^{20}$ +5.8 (*c* 0.38, DMSO); R_f = 0.26 (30% EtOAc/DCM, 1% AcOH); IR (film) 3286, 3062, 3030, 2927, 2522, 2362, 1727, 1655, 1540 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 8.39 (d, *J* = 8.0 Hz, 1H), 7.27-7.16 (m, 8H), 7.13-7.11 (m, 2H), 4.44 (m, 1H), 3.44 (d, *J* = 14.0 Hz, 1H), 3.39 (d, *J* = 14.0 Hz, 1H), 3.07 (dd, *J* = 13.8, 4.8 Hz, 1H), 2.87 (dd, *J* = 13.8, 9.6 Hz, 1H)[19]; ^{13}C NMR (100 MHz, d_6-DMSO) ppm 173.0, 170.0, 137.5, 136.2, 129.1, 129.0, 128.2 , 128.1, 126.4, 126.2, 53.5, 42.0, 36.8; HRMS (ESI) Exact mass calcd for $C_{17}H_{17}NO_3$ $[M]^+$ 284.1287, found 284.1280.



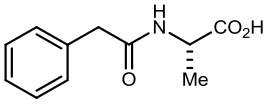
(*S*)-4-(Methylthio)-2-(2-phenylacetamido)butanoic acid (20d). The α-bromonitroalkane (150 mg, 652 μmol) and L-leucine (257 mg, 1.96 mmol) were subjected to the general coupling procedure. Purification by flash chromatography (SiO_2, 10-40% ethyl acetate in dichloromethane with 1% AcOH) gave the amide as a yellow solid (87.8 mg, 54%). Mp 131-134

[19] The CO_2H proton was not observed by ^1H NMR.
[20] Jin, H.J.; Lu, J.; Wu, X. *Bioorganic and Medicinal Chemistry*. **2012**. 20, 2465-3469.

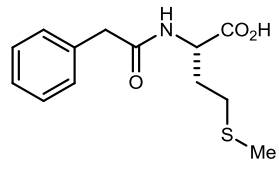
°C; $[\alpha]_D^{20}$ -20.2 (*c* 0.52, DMSO); R_f = 0.27 (30% EtOAc/DCM, 1% AcOH); IR (film) 3330, 2954, 1703, 1619, 1548 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 12.50 (br s, 1H), 8.34 (d, *J* = 8.0, 1H), 7.30-7.24 (m, 4H), *7*.22-7.19 (m, 1H), 4.21 (ddd, *J* = 8.9, 8.9, 5.6 Hz, 1H), 3.48 (d, *J* = 14.0, 1H), 3.44 (d, *J* = 14.0, 2H), 1.67-1.56 (m, 1H), 1.54-1.49 (m, 2H), 0.88 (d, *J* = 6.5, 3H), 0.81 (d, *J* = 6.4, 3H); ^{13}C NMR (100 MHz, d_6-DMSO) ppm 174.2, 170.1, 136.4, 129.0, 128.1, 126.3, 50.3, 41.9, 40.03, 24.3, 22.8, 21.3; HRMS (ESI) Exact mass calcd for $C_{14}H_{20}NO_3$ $[M]^+$ 250.1443, found 250.1431.



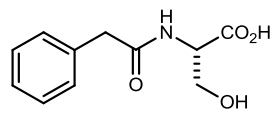
(*2S,3S*)-3-Methyl-2-(2-phenylacetamido)pentanoic acid (20e). The α-bromonitroalkane (49.8 mg, 216 μmol) and L-isoleucine (85.4 mg, 651 μmol) were subjected to the general coupling procedure. Purification by flash chromatography (SiO_2, 10-40% ethyl acetate in dichloromethane with 1% AcOH) gave the amide as a clear oil (27.5 mg, 51%). $[\alpha]_D^{20}$ +0.7 (*c* 1.9, DMSO); R_f = 0.3.8 (30% EtOAc/DCM, 1% AcOH); IR (film) 3332, 3030, 2963, 2876, 2529, 2361, 2342, 1716, 1653, 1618, 1543 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 8.23 (d, *J* = 8.2, 1H), 7.30-7.25 (m, 4H), *7*.22-7.18 (m, 1H), 4.19 (dd, *J* = 8.5, 6.0 Hz, 1H), 3.54 (d, *J* = 13.8, 1H), 3.48 (d, *J* = 13.8, 2H), 1.78 (m, 1H), 1.45-1.35 (m, 1H), 1.22-1.13 (m, 1H), 0.83 (m, 6H)[19]; ^{13}C NMR (100 MHz, d_6-DMSO) ppm 173.1, 170.3, 136.6, 129.0, 128.1, 126.3, 56.3, 41.9, 36.5, 24.7, 15.6, 11.3; HRMS (ESI) Exact mass calcd for $C_{14}H_{20}NO_3$ $[M]^+$ 250.1443, found 250.1440.



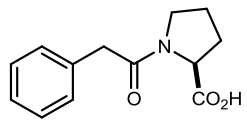
(*S*)-2-(2-Phenylacetamido)propanoic acid (20f). The α-bromonitroalkane (50.0 mg, 217 μmol) and L-alanine (58.0 mg, 651 μmol) were subjected to the general coupling procedure. Subsequent recrystallization from carbon tetrachloride gave the amide as a clear yellow oil (18.3 mg, 41%); $[\alpha]_D^{20}$ -16.3 (*c* 0.24, DMSO); R_f = 0.23 (50% EtOAc/DCM, 1% AcOH); IR (film) 3279, 2920, 2361, 1726, 1649, 1542 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 12.50 (br s, 1H), 8.39, (d, *J* = 7.2 Hz, 1H), *7*.30-7.24 (m, 4H), 7.24-7.21 (m, 1H), 4.19 (dq, *J* = 7.3, 7.3 Hz, 1H), 3.45 (s, 2H), 1.27 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, d_6-DMSO) ppm 174.2, 169.9, 136.3, 129.0, 128.2, 126.3, 47.6, 41.8, 17.3; HRMS (ESI) Exact mass calcd for $C_{11}H_{13}NO_3Na$ $[M+Na]^+$ 230.0793, found 230.0801.



(*S*)-4-(Methylthio)-2-(2-phenylacetamido)butanoic acid (20g). The α-bromonitroalkane (149 mg, 648 μmol) and L-methionine (293 mg, 1.96 mmol) were subjected to the general coupling procedure. Purification by flash chromatography (SiO_2, 10-40% ethyl acetate in dichloromethane with 1% TFA) gave the amide as a clear oil (52.0 mg, 30%). $[\alpha]_D^{20}$ -9.7 (*c* 0.34, DMSO); R_f = 0.61 (30% EtOAc/DCM, 1% AcOH); IR (film) 3286, 3062, 2918, 2556, 1953, 1728, 1651, 1544 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 12.55 (br s, 1H), 8.39 (d, *J* = 7.8, 1H), 7.30-7.24 (m, 4H), *7*.22-7.20 (m, 1H), 4.30 (ddd, *J* = 8.3, 8.3, 4.6 Hz, 1H), 3.50 (d, *J* = 14.0, 1H), 3.45 (d, *J* = 14.0, 1H), 2.48-2.39 (m, 2H), 2.00 (s, 3H), 1.97-1.91 (m, 1H), 1.90-1.81 (m, 1H); ^{13}C NMR (100 MHz, d_6-DMSO) ppm 173.4, 170.3, 136.4, 129.0, 128.2, 126.3, 51.0, 42.0, 30.9, 29.7, 14.6; HRMS (ESI) Exact mass calcd for $C_{13}H_{17}NO_3SNa$ $[M+Na]^+$ 290.0827, found 290.0833.

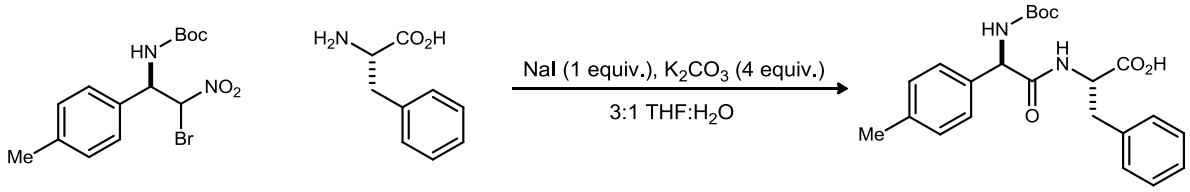


(*S*)-3-Hydroxy-2-(2-phenylacetamido)propanoic acid (20h). The α-bromonitroalkane (150 mg, 652 μmol) and L-serine (206 mg, 1.96 mmol) were subjected to the general coupling procedure. Recrystallization from ethyl acetate and hexanes gave the amide as a clear oil (45.7 mg, 31%). $[\alpha]_D^{20}$ -5.6 (*c* 0.27, DMSO); R_f = 0.07 (1% AcOH in ethyl acetate); IR (film) 3299, 3030, 2921, 1730, 1647, 1539 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 8.23 (d, *J* = 7.9 Hz, 1H), 7.31-7.26 (m, 4H), *7*.23-7.20 (m, 1H), 4.27 (ddd, *J* = 9.3, 4.6, 4.6 Hz, 1H), 3.71 (dd, *J* = 10.9, 5.4 Hz, 1H), 3.62 (dd, *J* = 10.9, 4.3 Hz, 1H), 3.51 (s, 2H)[19]; ^{13}C NMR (100 MHz, d_6-DMSO) ppm 172.0, 170.2, 136.4, 129.1, 128.1, 126.3, 61.4, 54.7, 41.8; HRMS (ESI) Exact mass calcd for $C_{11}H_{13}NNaO_4$ $[M+Na]^+$ 246.0742, found 246.0737.

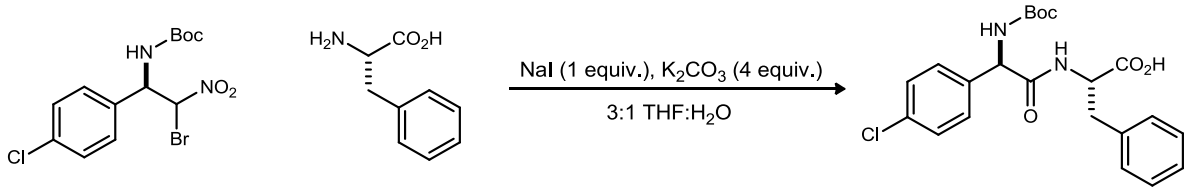


(*S*)-3-Hydroxy-2-(2-phenylacetamido)propanoic acid (20i). The α-bromonitroalkane (50.0 mg, 217 μmol) and L-proline (75.0 mg, 651 μmol) were subjected to the general coupling procedure. Purification by flash chromatography (SiO_2, 40-80% ethyl acetate in hexanes with 1% AcOH) gave the amide as a clear oil (13.6 mg, 27%). $[\alpha]_D^{20}$ -116.5 (*c* 0.26, $CHCl_3$); R_f =

0.10 (60% EtOAc/Hexanes, 1% AcOH); IR (film) 3029, 2955, 2882, 1734, 1645, 1597 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36-7.26 (m, 4H), *7*.25 (br s, 1H), 4.63 (dd, *J* = 8.2, 3.0 Hz, 1H), 3.75 (s, 2H), 3.61-3.57 (m, 1H), 3.52-3.46 (m, 1H), 2.48-2.44 (m, 1H), 2.05-1.92 (m, 3H)[19]; ^{13}C NMR (150 MHz, $CDCl_3$) ppm 173.4, 171.9, 133.2, 128.92, 128.87, 127.3, 60.3, 48.1, 41.7, 27.1, 24.8; HRMS (ESI) Exact mass calcd for $C_{13}H_{15}NNaO_3$ $[M+Na]^+$ 256.0950, found 256.0938.

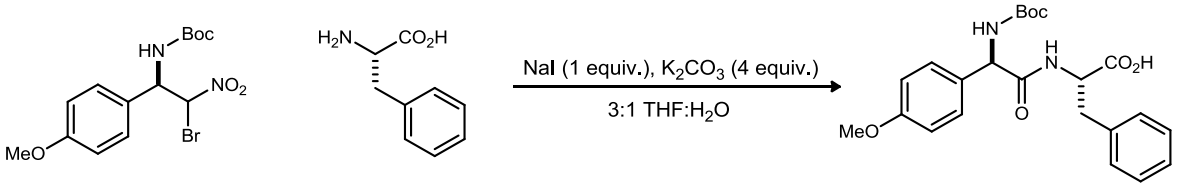


(*S*)-2-((*R*)-2-((tert-butoxycarbonyl)amino)-2-(p-tolyl)acetamido)-3-phenylpropanoic acid (23a). The α-bromonitroalkane (50.0 mg, 139 μmol) and L-phenylalanine (68.8 mg, 417 μmol) were subjected to the general coupling procedure. Purification by flash chromatography (SiO_2, 10-20% ethyl acetate in dichloromethane with 1% AcOH) gave the amide as a clear oil (39.1 mg, 68%). $[\alpha]_D^{20}$ +12.2 (*c* 0.90, $CHCl_3$); R_f = 0.10 (20% EtOAc/DCM, 1% AcOH); IR (film) 3317, 3030, 2979, 2931, 1720, 1662 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.20-7.15 (m, 5H), 7.04 (d, *J* = 7.4, 2H), 6.70 (d, *J* = 7.7 Hz, 2H), 6.62 (d, *J* = 6.8 Hz, 1H), 6.08 (d, *J* = 7.8 Hz, 1H), 5.58 (d, *J* = 8.1 Hz, 1H), 4.88 (dd, *J* = 10.8, 4.5 Hz, 1H), 3.06 (dd, *J* = 13.4, 4.5 Hz, 1H), 2.99 (dd, *J* = 13.3, 4.9 Hz, 1H), 2.41 (s, 3H), 1.41 (s, 9H)[19]; ^{13}C NMR (150 MHz, $CDCl_3$) ppm 173.3, 170.1, 155.9, 138.0, 135.3, 135.1, 129.6, 129.5, 128.2, 127.1, 126.7, 80.9, 57.1, 52.9, 37.2, 28.3, 21.2; HRMS (ESI) Exact mass calcd for $C_{23}H_{29}N_2O_5$ $[M+H]^+$ 413.2076, found 413.2096.

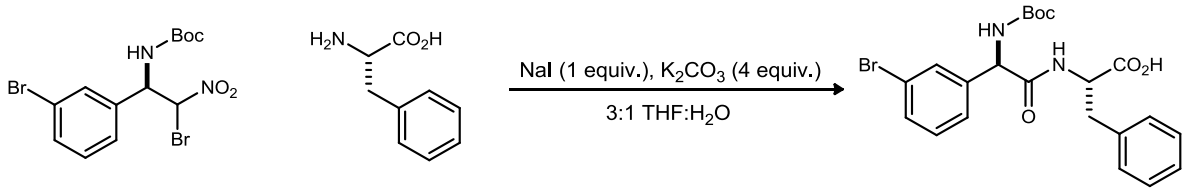


(*S*)-2-((*R*)-2-((tert-Butoxycarbonyl)amino)-2-(4-chlorophenyl)acetamido)-3-phenylpropanoic acid (23b). The α-bromonitroalkane (50.0 mg, 132 μmol) and L-phenylalanine (65.0 mg, 395 μmol) were subjected to the general coupling procedure.

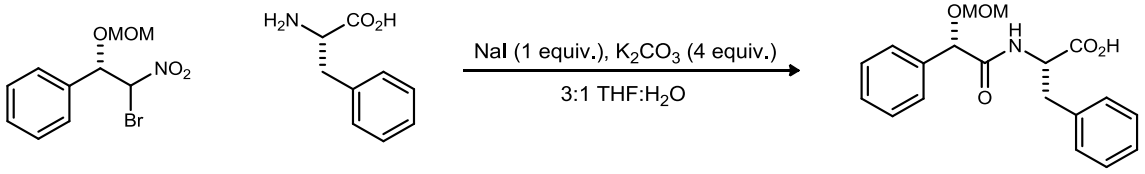
Purification by flash chromatography (SiO_2, 20-40% ethyl acetate in dichloromethane with 1% AcOH) gave the amide as a clear oil (23.4 mg, 41%). $[\alpha]_D^{20}$ +4.0 (*c* 0.59, $CHCl_3$); R_f = 0.20 (30% EtOAc/DCM, 1% AcOH); IR (film) 3313, 2926, 1717, 1662 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33-7.28 (m, 2H), 7.21-7.17 (m, 3H), 7.06 (dd, *J* = 7.4 Hz, 2H), 6.76 (d, *J* = 6.1 Hz, 1H), 6.71 (d, *J* = 7.2 Hz, 2H), 6.12 (d, *J* = 7.8 Hz, 1H), 5.60 (d, *J* = 7.8 Hz, 1H), 4.84 (dd, *J* = 12.5, 5.9 Hz, 1H), 3.08 (dd, *J* = 13.9, 4.6 Hz, 1H), 2.94 (dd, *J* = 14.1, 6.1 Hz, 1H), 1.39 (s, 9H)[19]; ^{13}C NMR (100 MHz, $CDCl_3$) ppm 173.8, 169.8, 156.1, 136.8, 135.4, 129.5, 129.2, 128.7, 128.5, 127.0, 81.3, 56.9, 53.2, 37.4, 29.8, 28.4; HRMS (ESI) Exact mass calcd for $C_{22}H_{25}ClN_2NaO_5$ $[M+Na]^+$ 455.1350, found 455.1361.



(*S*)-2-((*R*)-2-((tert-butoxycarbonyl)amino)-2-(4-methoxyphenyl)acetamido)-3-phenylpropanoic acid (23c). The α-bromonitroalkane (50.0 mg, 133 μmol) and L-phenylalanine (66.0 mg, 400 μmol) were subjected to the general coupling procedure. Purification by flash chromatography (SiO_2, 10-20% ethyl acetate in dichloromethane with 1% AcOH) gave the amide as a yellow oil (27.4 mg, 48%). $[\alpha]_D^{20}$ -0.3 (*c* 0.96, $CHCl_3$); R_f = 0.08 (20% EtOAc/DCM, 1% AcOH); IR (film) 3319, 2978, 2934, 1719, 1662, 1511 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.21 (d, *J* = 8.1 Hz, 2H), 7.15 (t, *J* = 5.5 Hz, 1H), 7.04 (t, *J* = 7.2 Hz, 2H), 6.87 (d, *J* = 7.7 Hz, 2H), 6.73 (d, *J* = 5.3 Hz, 2H), 6.62 (br s, 1H), 6.03 (d, *J* = 5.7 Hz, 1H), 5.52 (d, *J* = 5.5 Hz, 1H), 4.86 (br s, *J* = 1H), 3.84 (s, 3H), 3.05 (dd, *J* = 12.4, 12.4, 1H), 2.97 (dd, *J* = 10.3, 10.3, 1H), 1.40 (s, 9H)[19]; ^{13}C NMR (150 MHz, $CDCl_3$) ppm 173.4, 170.3, 159.6, 155.8, 135.3, 130.2, 129.4, 128.5, 128.3, 126.7, 114.3, 80.8, 56.9, 55.3, 52.9, 37.3, 28.3; HRMS (ESI) Exact mass calcd for $C_{23}H_{29}N_2O_6$ $[M+H]^+$ 429.2026, found 429.2044.

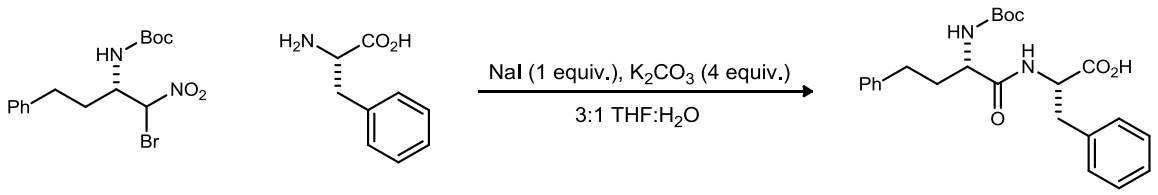


(*S*)-2-((*R*)-2-(3-bromophenyl)-2-((tert-butoxycarbonyl)amino)acetamido)-3-phenylpropanoic acid (23d). The α-bromonitroalkane (50.0 mg, 118 μmol) and L-phenylalanine (58.0 mg, 354 μmol) were subjected to the general coupling procedure. Purification by flash chromatography (SiO_2, 20-40% ethyl acetate in dichloromethane with 1% AcOH) gave the amide as a clear oil (25.9 mg, 46%). $[\alpha]_D^{20}$ -1.6 (*c* 0.96, $CHCl_3$); R_f = 0.13 (20% EtOAc/DCM, 1% AcOH); IR (film) 3316, 2979, 1718, 1662 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.50-7.48 (m, 2H), 7.23-7.21 (m, 2H), 7.17-7.14 (m, 1H), 7.10-7.08 (m, 2H), 6.74 (d, *J* = 7.0 Hz, 2H), 6.70 (d, *J* = 6.7 Hz, 1H), 6.09 (d, *J* = 7.9 Hz, 1H), 5.60 (d, *J* = 8.1 Hz, 1H), 4.87 (dd, *J* = 14.1, 8.4 Hz, 1H), 3.08 (dd, *J* = 14.4, 4.7 Hz, 1H), 2.97 (dd, *J* = 13.6, 5.5, 1H), 1.40 (s, 9H)[19]; ^{13}C NMR (150 MHz, $CDCl_3$) ppm 173.3, 169.2, 155.8, 140.5, 135.1, 131.4, 130.5, 130.2, 129.3, 128.3, 126.9, 125.8, 122.9, 81.2, 56.8, 53.0, 37.4, 28.3; HRMS (ESI) Exact mass calcd for $C_{22}H_{26}BrN_2O_5$ $[M+H]^+$ 477.1025, found 477.1021.



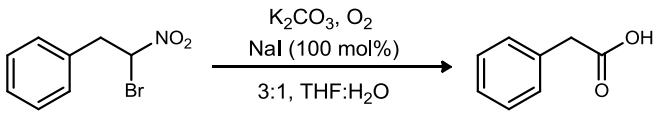
(*S*)-2-((*S*)-2-(Methoxymethoxy)-2-phenylacetamido)-3-phenylpropanoic acid (23e). The α-bromonitroalkane (49.9 mg, 172 μmol) and L-phenylalanine (85.2 mg, 516 μmol) were subjected to the general coupling procedure. Purification by flash chromatography (SiO_2, 10-50% ethyl acetate in dichloromethane with 1% AcOH) gave the amide as a yellow oil (33.5 mg, 57%). $[\alpha]_D^{20}$ +133.0 (*c* 0.64, $CHCl_3$); R_f = 0.43 (40% EtOAc/DCM, 1% AcOH); IR (film) 3401, 3063, 3031, 2926, 2853, 1735, 1661, 1526 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.67 (br s, 1H), 7.33-7.21 (m, 9H), 7.06-7.04 (m, 2H), 5.07 (s, 1H), 4.90 (dd, *J* = 13.3, 6.6 Hz, 1H), 4.63 (d, *J* = 6.8 Hz, 1H), 4.59 (d, *J* = 6.6 Hz, 1H), 3.30 (s, 3H), 3.22 (dd, *J* = 14.1, 5.3 Hz, 1H), 3.09 (dd, *J* =

13.9, 6.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm 174.8, 171.2, 136.5, 135.6, 129.5, 128.74, 128.71, 128.69, 127.4, 127.3, 94.6, 77.7, 56.2, 52.8, 37.3; HRMS (ESI) Exact mass calcd for $C_{19}H_{21}NNaO_5$ $[M+Na]^+$ 366.1317, found 366.1312.



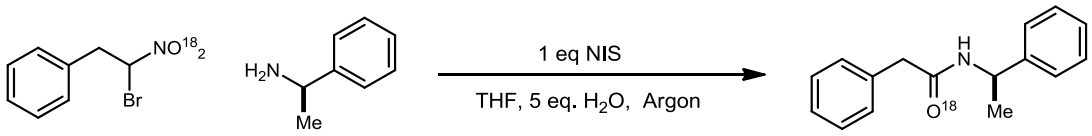
(*S*)-2-((*S*)-2-((tert-butoxycarbonyl)amino)-4-phenylbutanamido)-3-phenylpropanoic acid (23f). The α-bromonitroalkane (21.4 mg, 57 μmol) and L-phenylalanine (28.0 mg, 172 μmol) were subjected to the general coupling procedure. Purification by flash chromatography (SiO_2, 10-20% ethyl acetate in dichloromethane with 1% AcOH) gave the amide as a clear oil (14.4 mg, 59%). $[\alpha]_D^{20}$ +36.8 (*c* 0.25, $CHCl_3$); R_f = 0.07 (20% EtOAc/DCM, 1% AcOH); IR (film) 3317, 2929, 1658, 1525 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23-7.18 (m, 5H), 7.15-7.11 (m, 5H), 6.71 (d, *J* = 7.4, 1H), 5.03 (d, *J* = 5.1, 1H), 4.82 (dd, *J* = 13.6, 6.7 Hz, 1H), 4.12 (br s, 1H), 3.18 (br d, *J* = 3.18 Hz, 1H), 3.03 (dd, *J* = 14.0, 6.4 Hz, 1H), 2.61 (t, *J* = 7.5 Hz, 2H), 2.06 (m, 1H), 1.83 (br s, 1H), 1.44 (s, 9H)[19]; ^{13}C NMR (150 MHz, $CDCl_3$) ppm 173.8, 171.8, 155.7, 140.7, 135.8, 129.4, 128.54, 128.48, 128.4, 127.1, 126.1, 80.6, 54.0, 53.2, 37.4, 33.8, 31.7, 28.3; HRMS (ESI) Exact mass calcd for $C_{24}H_{30}N_2O_5$ $[M+Na]^+$ 449.2052, found 449.2056.

2.4 Carboxylic Acid Synthesis and Labeling Experiments



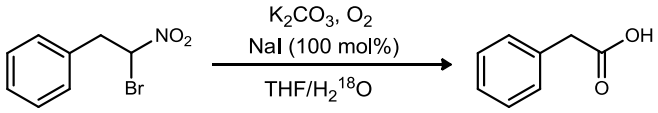
Phenylacetic acid (24). To a vial equipped with a stir bar were added α-bromonitroalkane (25.0 mg, 109 μmol), THF (750 μL) and water (250 μL). The reaction was cooled to 0 ˚C and K_2CO_3 (60.0 mg, 436 μmol) and NaI (16 mg, 109 μmol) were added. An O_2 balloon was attached and

the reaction was allowed to stir at 0 ˚C for 3 hours. The reaction mixture was quenched with excess 3 M HCl and concentrated to remove THF. The reaction mixture was transferred to a separatory funnel and extracted with ethyl acetate. The organic fractions were combined and washed with satd aq sodium thiosulfate, dried, filtered, and concentrated. Purification of the residue using flash chromatography (SiO_2, 5-10% ethyl acetate in hexanes with 1% AcOH) gave the acid as a white solid (13.4 mg, 64%); spectroscopic data (^{1}H NMR) was in complete accord with that previously reported.[21]

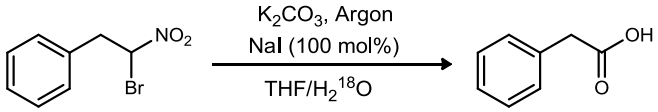


2-Phenyl-N-(1-phenylethyl)acetamide (29) Prepared Using 84% ^{18}O-Labeled A-bromonitroalkane. The labeled α-bromonitroalkane (25.0 mg, 107 μmol) was dissolved in THF (0.5 mL) and H_2O (10 μL, 535 μmol) in a two-necked round-bottomed flask. The solvent was then degassed using three 30 minute freeze-pump-thaw cylcles. The solvent was refrozen and NIS (24.0 mg, 107 μmol) and amine (69 μL, 535 μmol) were added. The flask was then evacuated and backfilled with argon three times. The reaction was then thawed before a final 10 minute freeze-pump-thaw cycle was performed. The reaction was then warmed to 0 ˚C for 20 hours. The crude reaction mixture was diluted with dichloromethane, dried over $MgSO_4$, and filtered through celite. Purification by flash chromatography (SiO_2, 10-30% ethyl acetate in hexanes with 1% AcOH) gave the amide as a yellow solid (10.1 mg, 39%); spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR) was in complete accord with that previously reported for ^{16}O-amide,[22] but two carbonyl peaks were visible in the ^{13}C NMR spectrum. It was previously reported that the ^{18}O peak is shifted upfield approximately 0.03 ppm.[23] Integration of the two peaks indicated around a 63% ^{18}O incorporation.

[21] Milne, J.E. *et. al. J. Org. Chem.* **2011**. 76, 9519-9524; Leon, T.; Correa, A.; Martin, R. *J. Am. Chem. Soc.* **2013**. 135, 1221-1224.
[22] Nordstrøm, L. U.; Vogt, H.; Madsen, R. J. Am. Chem. Soc. **2008**. 130, 17672.
[23] Shackleford, J.; Shen, B.; Johnston, J.N. *Proc. Natl. Acad. Sci.* **2012**. 109, 44.

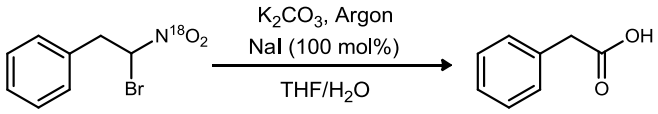


Phenylacetic acid (24) Prepared Using 95% $H_2{}^{18}O$. The α-bromonitroalkane (50.0 mg, 217 μmol) was dissolved in dry THF (2.0 mL) and $H_2{}^{18}O$ (200 μL) in a flame-dried two-necked round-bottomed flask. The solvent was then degassed using three 30 minute freeze-pump-thaw cylcles. The solvent was refrozen and NaI (32.5 mg, 217 μmol) and K_2CO_3 (120 mg, 868 μmol) were added. The flask was then evacuated and backfilled with argon three times. The reaction was then thawed before a final 10 minute freeze-pump-thaw cycle was performed. An O_2 balloon was then attached and the reaction was then warmed to 0 ˚C for 24 hours. The reaction mixture was quenched with excess 3 M HCl and concentrated to remove THF. The reaction mixture was transferred to a separatory funnel and extracted with ethyl acetate. The organic fractions were combined and washed with satd aq sodium thiosulfate, dried, filtered, and concentrated. Purification by flash chromatography (SiO_2, 2-20% ethyl acetate in hexanes with 1% AcOH) gave the acid as a white solid (12.1 mg, 40%); spectroscopic data (^{1}H NMR) was in complete accord with that previously reported.[21] HRMS (EI): Exact mass calcd for $C_8H_8O_2$ $[M]^+$ 136.0519, $C_8H_8{}^{18}O{}^{16}O$ $[M]^+$ 138.0561, and $C_8H_8{}^{18}O_2$ $[M]^+$ 140.0604, found 136.0524, 138.0579, and 140.0634. The relative intensities of these three peaks and their natural abundances were used to determine 8.1% singly labeled and 0% doubly labeled carboxylic acid.



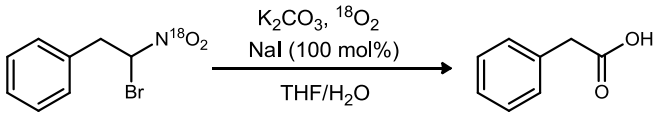
Phenylacetic acid (24) Prepared Using 95% $H_2{}^{18}O$ and Degassed Solvent. The α-bromonitroalkane (50.0 mg, 217 μmol) was solvated in dry THF (2.0 mL) and $H_2{}^{18}O$ (200 μL) in

a flame-dried two-necked round-bottomed flask. The solvent was then degassed using three 30 minute freeze-pump-thaw cylcles. The solvent was refrozen and NaI (32.5 mg, 217 μmol) and K_2CO_3 (120 mg, 868 μmol) were added. The flask was then evacuated and backfilled with argon three times. The reaction was then thawed before a final 10 minute freeze-pump-thaw cycle was performed. The reaction was then warmed to 0 ˚C for 24 hours, quenched with excess 3 M HCl and concentrated to remove THF. The reaction mixture was transferred to a separatory funnel and extracted with ethyl acetate. The organic fractions were combined and washed with satd aq sodium thiosulfate, dried, filtered, and concentrated. Purification by flash chromatography (SiO_2, 2-20% ethyl acetate in hexanes with 1% AcOH) gave the acid as a white solid (4.4 mg, 15%); spectroscopic data (^{1}H NMR) was in complete accord with that previously reported.[21] HRMS (EI): Exact mass calcd for $C_8H_8O_2$ $[M]^+$ 136.0519, $C_8H_8{}^{18}O^{16}O$ $[M]^+$ 138.0561, and $C_8H_8{}^{18}O_2$ $[M]^+$ 140.0604, found 136.0524, 138.0579, and 140.0634. The relative intensities of these three peaks and their natural abundances were used to determine 7.2% singly labeled and 0% doubly labeled carboxylic acid.



Phenylacetic acid (24) Prepared Using 84% $^{18}O_2$ α-Bromonitroalkane and Degassed Solvent. The α-bromonitroalkane (50.0 mg, 214 μmol) was solvated in THF (1.5 mL) and H_2O (0.5 mL) in a two-necked round-bottomed flask. The solvent was then degassed using three 30 minute freeze-pump-thaw cylcles. The solvent was refrozen and NaI (32.0 mg, 214 μmol) and K_2CO_3 (118 mg, 856 μmol) were added. The flask was then evacuated and backfilled with argon three times, and then thawed before a final 10 minute freeze-pump-thaw cycle was performed. The reaction mixture was then warmed to 0 ˚C for 24 hours, quenched with excess 3 M HCl and concentrated to remove THF. The reaction mixture was transferred to a separatory funnel and extracted with ethyl acetate. The organic fractions were combined and washed with satd aq sodium thiosulfate, dried, filtered, and concentrated. Purification by flash chromatography (SiO_2, 2-20% ethyl acetate in hexanes with 1% AcOH) gave the acid as a white solid (11.7 mg, 40%);

spectroscopic data (^{1}H NMR) was in complete accord with that previously reported.[21] HRMS (EI): Exact mass calcd for $C_8H_8O_2$ $[M]^+$ 136.0519, $C_8H_8{}^{18}O^{16}O$ $[M]^+$ 138.0561, and $C_8H_8{}^{18}O_2$ $[M]^+$ 140.0604, found 136.0524, 138.0579, and 140.0634. The relative intensities of these three peaks and their natural abundances were used to determine 20.6% singly labeled and 1.7% doubly labeled carboxylic acid.



Phenylacetic acid (24) Prepared Using 99% $^{18}O_2$. NaI (16.0 mg, 109 μmol) and K_2CO_3 (60 mg, 435 μmol) were added to a 1 mL HPLC screw cap vial (vial A), followed by the addition of THF (200 μL). The vial was subsequently sealed with the screw cap (containing a silicone septum). The α-bromonitroalkane (25.0 mg, 109μmol) was added to THF (200 μL) in a second 1 mL glass screw cap HPLC vial (vial B) and sealed with the silicone septum screw cap and parafilm. Both flasks were degassed using three 80 minute freeze-pump-thaw cycles. Once degassing was complete, vial A was refrozen in liquid nitrogen. The α-bromonitroalkane solution in vial B was transferred to vial A via a dry microsyringe in one portion. Once the transferred solution had frozen, the $^{18}O_2$ gas regulator needle was inserted through the septum, and the entire system was placed under high vacuum. The vacuum was turned off, and the $^{18}O_2$ regulator was opened to allow its entry to the system under static vacuum. The regulator was then closed and the reaction was warmed to 0 ˚C overnight. The crude reaction mixture was quenched with excess 2 M HCl and concentrated to remove THF. The reaction mixture was transferred to a separatory funnel and extracted with ethyl acetate. The organic fractions were combined and washed with satd aq sodium thiosulfate, dried, filtered, and concentrated. Purification by flash chromatography (SiO_2, 100% ethyl acetate with 1% AcOH) gave the acid as a white solid (11.0 mg, 75%); spectroscopic data (^{1}H NMR) was in complete accord with that previously reported.[21] HRMS (EI): Exact mass calcd for $C_8H_8O_2$ $[M]^+$ 136.0519, $C_8H_8{}^{18}O^{16}O$ $[M]^+$ 138.0561, and $C_8H_8{}^{18}O_2$ $[M]^+$ 140.0604, found 136.0524, 138.0567, and 140.0611. The relative intensities of these three peaks and their natural abundances were used to determine 23.5% singly labeled and 1.7% doubly labeled carboxylic acid.