Title

A Physical Organic Approach to Tuning Reagents for Selective and Stable Methionine Bioconjugation

Journal

Journal of the American Chemical Society, 141(32)

ISSN

0002-7863

Authors

Christian, Alec H

Jia, Shang

Cao, Wendy

et al.

Publication Date

2019-08-14

DOI

10.1021/jacs.9b04744

Peer reviewed

HHS Public Access

Author manuscript

J Am Chem Soc. Author manuscript; available in PMC 2019 October 08.

Published in final edited form as:

J Am Chem Soc. 2019 August 14; 141(32): 12657–12662. doi:10.1021/jacs.9b04744.

A Physical Organic Approach to Tuning Reagents for Selective and Stable Methionine Bioconjugation

Alec H. Christian[†], **Shang Jia**[†,Δ], **Wendy Cao**[†,Δ], **Patricia Zhang**[†], **Arismel Tena Meza**[†], **Matthew S. Sigman**[‖], **Christopher J. Chang**[†,‡,§,*], **F. Dean Toste**[†,*]

[†]Department of Chemistry, University of California, Berkeley, California 94720, United States

[‡]Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, United States

[§]Howard Hughes Medical Institute, University of California, Berkeley, California 94720, United States

[‖]Department of Chemistry, University of Utah, 315 South 1400 East, Salt Lake City, Utah 84112, United States

Abstract

We report a data-driven, physical organic approach to the development of new methionine-selective bioconjugation reagents with tunable adduct stabilities. Statistical modeling of structural features described by intrinsic physical organic parameters was applied to the development of a predictive model and to gain insight into features driving stability of adducts formed from the chemoselective coupling of oxaziridine and methionine thioether partners through Redox Activated Chemical Tagging (ReACT). From these analyses, a correlation between sulfimide stabilities and sulfimide ν(C=O) stretching frequencies was revealed. We exploited the rational gains in adduct stability exposed by this analysis to achieve the design and synthesis of a bis-oxaziridine reagent for peptide stapling. Indeed, we observed that a macrocyclic peptide formed by ReACT stapling at methionine exhibited improved uptake into live cells compared to an unstapled congener, highlighting the potential utility of this unique chemical tool for thioether modification. This work provides a template for the broader use of data-driven approaches to bioconjugation chemistry and other chemical biology applications.

Graphical Abstract

[*]Corresponding Author: fdtoste@berkeley.edu; chrischang@berkeley.edu.

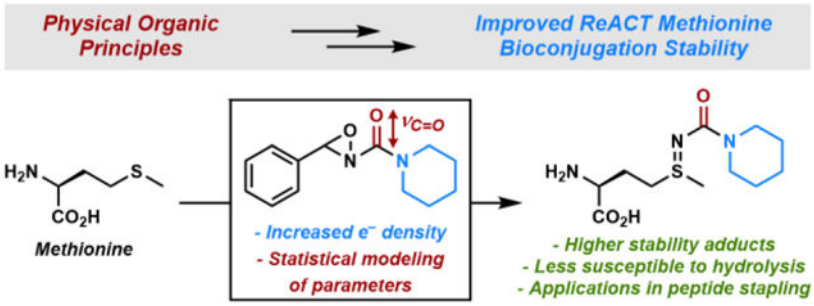
[Δ]S.J. and W.C. contributed equally.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website:

Experimental details and compound characterization data (PDF)

The authors declare no competing financial interests.



Authors are required to submit a graphic entry for the Table of Contents (TOC) that, in conjunction with the manuscript title, should give the reader a representative idea of one of the following: A key structure, reaction, equation, concept, or theorem, etc., that is discussed in the manuscript. Consult the journal's Instructions for Authors for TOC graphic specifications.

INTRODUCTION

As the second-rarest amino acid in vertebrates and one of only two naturally-occurring sulfur-containing amino acids, methionine is a nexus for redox and metal chemistry in the proteome, serving as a form of protection against oxidative stress from the cellular to organismal level as well as an endogenous regulator of cell function.[1,2] This privileged combination of relative scarcity and physiological diversity motivates the development of new chemical methods to selectively label methionines to identify and study their native properties and enhance protein function at these thioether side chains.[2,3] Traditional approaches for methionine modification utilize either strong alkylating reagents that typically require at low pH or cyanogen bromide, which can lead to instability of the adduct or cleavage the of peptide backbone, respectively.[4–6]

Inspired by the reversible post-translational oxidation of methionine to methionine sulfoxide, our laboratories developed oxaziridine reagents for selective *N*-transfer tagging to methionine to produce the isoelectronic sulfimide conjugate – a strategy coined ReACT (Redox Activated Chemical Tagging) (Scheme 1A).[2] We found that urea-derived oxaziridines can chemoselectively label methionine residues in proteins and proteomes under aqueous conditions at neutral pH. Application of this chemical platform enabled synthesis of novel antibody-drug conjugates and validation of a functional methionine motif within actin and identification of new hyperreactive methionine residues on enolase that play vital roles in protein function. Elegant recent work by the Gaunt group reported an alternative approach to methionine bioconjugation on isolated proteins and peptides to sulfoniums using hypervalent iodine reagents bearing a diazo group for further functionalization. The Gaunt methionine conjugate bears some stability considerations, as the ester of the sulfonium hydrolyzes rapidly unless the steric bulk of the alkyl group is increased, while recent reports demonstrate sulfonium instability when adjacent to nucleophilic amino acids.[7,8]

Against this backdrop, we sought to expand the scope of methionine-selective bioconjugation reagents and in the present study, we report a data-driven strategy to

predictably tune the stability of the resulting sulfimide adducts derived from ReACT to methionine. Indeed, the diverse array of potential applications of selective methionine probes requires the synthesis of methionine-payload adducts of varying stability, including biological imaging agents and therapeutics where inert or labile amino acid conjugates can play significant roles in outputs spanning signal-to-noise responses, drug efficacies, and/or pharmacokinetics and biodistributions.[9–13]

Specifically, we hypothesized that the design of new oxaziridine probes with tunable methionine adduct stabilities would be enabled by exploiting Design of Experiment (DoE) precepts to define a training set of molecules that would be interrogated by kinetic analysis and multivariate correlative tools. We envisioned that this multi-faceted approach, which has been successfully applied in the synthetic methodology space, could be adapted to research questions in chemical biology.[14] Applying this workflow, we sought to identify oxaziridine reagents that produce more stable methionine adducts as well as gain insight into structural features of the sulfimide adduct that play a role in stability through statistical modeling (Scheme 1B).[15–18] Indeed, we reasoned that an effectively designed training set would cover a sufficient diversity of chemical space using a limited number of compounds in order to probe kinetic stability, while the statistical modeling would provide a descriptive and predictive correlation to understand the underlying principles of what is controlling stability and to determine if the probe was optimal. Here we report that this multifaceted approach has enabled the design of methionine bioconjguation reagents with significantly enhanced stability for S–N bond formation and highlight the utility of this growing ReACT toolbox to introduce a new method for methionine-based peptide stapling.

RESULTS AND DISCUSSION

Design and Analysis of Training Sets to Probe Stabilities of Sulfimide Adducts Formed via ReACT.

We began our investigations by designing a training set of structurally diverse oxaziridines in order to probe the stabilities of the resultant sulfimide adducts by measuring the rate constants of decomposition to the corresponding sulfoxide congeners. We focused on exploring the electronic and steric effects of alkyl substitution on the oxaziridine as well as the contributions of the N–H group on the urea moiety in order to probe multiple possible factors that could influence S–N stability (Table 1).[19] Monosubstitution of the urea moiety was first investigated, where primary, secondary, and tertiary alkyl substituents and their effect on stability was measured. Our first-generation oxaziridine (**Ox1**) gave a sulfimide adduct (**Sulf1**) that hydrolyzed with a rate constant of 1.19×10^{-2} h^{-1} and served as our standard to compare to all other sulfimide adducts. Within this pilot data set, the trifluoroethyl substituted oxaziridine (**Ox2**) produced to the least stable sulfimide adduct (**Sulf2**) with a hydrolysis rate constant of 1.70×10^{-2} h^{-1}. In contrast, the *tert*-butyl substituted oxaziridine (**Ox9**) led to the most stable adduct (**Sulf9**), albeit with a rate constant of decomposition (0.76×10^{-2} h^{-1}) that was only modestly lower than that of the other non-fluorinated alkyl substituted sulfimide adducts. From these data, we posited that the electronic properties of the sulfimide conjugate play a major role in S–N adduct stability, with the hypothesis that increasing the electron density on the urea moiety should increase

stability of the modified methionine. To test this notion, we investigated the stabilities of sulfimide adducts derived from reactions with dialkyl substituted oxaziridines. Indeed, diethyl, piperidine, and pyrrolidine derived oxaziridines led to the most stable adducts, with **Ox10** and **Ox12** giving the most stable conjugates (**Sulf10** and **Sulf12**) with rate constants that are an order of magnitude lower (k=0.16 × 10^{-2} h^{-1}) than the parent **Ox1** reagent. To further test this model, we envisioned that replacing the urea moiety with an isostructural carbamate should make the sulfimide more electron-deficient, thus leading to a less stable, modified methionine sulfimide. In agreement with this notion, the carbamate-derived oxaziridine (**Ox13**) was observed to give the least stable adduct tested (**Sulf13**) (k=5.40 × 10^{-2} h^{-1}) by over 30-fold relative to the diethyl and piperidine derived conjugates.

To gain further insights into key structural features of the sulfimide that lead to greater stability, we employed statistical fitting analyses that relate the kinetic rate constants for the hydrolytic decomposition of the sulfimide to the sulfoxide to parameters derived from computed ground-state geometries of the urea/carbamate moiety and simplified sulfimide adducts.[15–19] A diverse set of parameters were extracted and condensed, using a Boltzmann distribution, into a representative parameter for all conformers within 2.5 kcal/mol (see SI for computational details). Using a random partition, a training set and validation set (7 TS & 6 VS) was curated from the oxaziridines used to probe the hydrolytic stability of the methionine adducts in order to identify statistically significant models. We considered several possible models, including parameters like the urea $\nu_{C=O}$ parameter, NBO charge parameter of the sulfimide nitrogen (NBO_N), and dipole moment of the urea (μ), which was accomplished by both evaluating univariate and multivariate combinations of parameters (see Supporting Information for other models). Using this method, we observed a statistically robust linear correlation between the transition state energy of decomposition ($\ln(k_{obs})$) and the carbonyl stretching frequency of the sulfimide adduct ($\nu_{C=O}$) (Figure 1).

Because lower carbonyl stretching frequencies correlate to higher S–N adduct stabilities, we reasoned that hydrolytic decomposition to the sulfoxide is electronically driven. Indeed, this model is consistent with the collected empirical data. The sulfimide adducts with the more electron-rich substitutions, **Sulf10** and **Sulf12**, were found to be the most stable within our assay conditions, whereas the more electron-poor substitutions derived from **Ox2** and **Ox13** show less stability. The correlation between the sulfimide carbonyl stretching frequency and adduct stability can be related by considering electron density donation into the **π*** orbital of the carbonyl group. In short, the more electron-rich the substitution on the sulfimide, the lower calculated carbonyl stretching frequency.

Additionally, we found that the sulfimide $\nu_{C=O}$ parameter and NBO charge parameter of the sulfimide nitrogen (NBO_N) are cross correlated, where a lower carbonyl stretching frequency correlates to more electron density residing on the sulfimide nitrogen (see Supporting Information, Figure S5). This analysis suggests that more electron-donating substituents lead to increased electron density on the nitrogen of the sulfimide bond, rendering the S−N unit less electrophilic and thus less susceptible to hydrolytic decomposition (see Supporting Information, Figure S8).

With these robust data sets and models in hand, virtual screening commenced to investigate if **Ox10** and **Ox12** were optimal probes or represented local minima. From this screen, it was clear that both **Ox10** and **Ox12** represented probes with relatively simple structural changes yet yielded notable gains in desired stability for the sulfimide adduct (see Supporting Information, Table S11).

Determination of Methionine Adduct Stabilities Under Aqueous Conditions.

With **Ox10** and **Ox12** displaying high methionine S–N adduct stabilities in our combined virtual and experimental screening assay, we next evaluated the resilience of these conjugates to hydrolytic decomposition under biologically-relevant aqueous conditions.[7] We began by exposing model peptide **1a** (250 μM) bearing two methionines to labeling conditions with **Ox1**, **Ox10**, and **Ox12** in pH 7.4 PBS buffer to yield **1b**, **1c**, and **1d**, respectively. After 120 hours at 37 °C, **1b** was fully hydrolyzed to the sulfoxide, while 56% of **1c** and 59% of **1d** remained (measured by liquid chromatography-ESI-TOF mass spectrometry; LC-MS, see Supporting Information, Figure S9) (Figure 2). Taken together, the data show that the physical organic approach can be used to develop bioconjugation reagents with significantly improved product stability.

Application of Oxaziridine Reagents with Greater Sulfimide Stabilities for Methionine-Based Peptide Stapling.

Owing to the observed increase in stability of the methionine sulfimide adduct formed using piperidine-derived **Ox12** in biologically-relevant conditions relative to the parent **Ox1** congener, we sought to establish the utility of this chemical finding in a proof-of-concept biological application. In this context, we pursued an application in peptide stapling, where bioconjugate stability plays an important role in the overall performance of the resulting macrocyclic peptide for various applications.[20–23] Peptide stapling methods often require pre-functionalization of amino acid residues, incorporation of non-natural amino acids, reactions performed in non-aqueous media, or protection of reactive residues before stapling.[20,21,24,25] We reasoned that the high chemoselectivity of the ReACT method for methionine labeling, even in the presence of competing reactive nucleophiles like tryptophan and lysine, along with gains in stability, would provide a unique and attractive technology for peptide stapling that could circumvent many of the above-mentioned limitations.

To explore this possibility, we synthesized bis-oxaziridine **Ox14** and subsequently exposed this reagent to peptide **1a**, which bears 11 amino acids and methionines positioned at the *i,i+4* distance. In our initial experiments, full conversion to **1e** at 23 °C was observed in 8 hours (Figure 3A). We then performed a set of experiments using bis-methionine peptides with varying spacing between the methionines to determine how this parameter affected the efficacy of macrocyclization. We were pleased to find that the *i,i+3, i,i+5*, and *i,i+6* distances gave 82%, 92% and 93% yield, respectively as measured by LC-MS. At the *i,i+4* distance, 74% yield of the stapled peptide adduct was obtained. To further test the limits of the ReACT method for methionine-based peptide stapling, two extreme cases were evaluated with two methionines positioned either at adjacent residues (*i,i+1*) or at the N- and C-termini (*i,i+10*). Gratifyingly, both peptide substrates gave excellent yields, with the *i,i+1* peptide affording 81% yield and the *i,i+10* peptide furnishing 94% yield of the stapled peptide

products (Figure 3B). In all cases, no oligomerization or oxygen transfer products were observed by LC-MS.

Next, we examined the stability of the stapled macrocyclic peptide **1e**.[21] Under both acidic (25 mM HCl) and basic (1.25 mM K_2CO_3) conditions after 24 hours, no decomposition of the stapled peptide was observed by LC-MS analysis. Under oxidative conditions (air, 37 °C), no decomposition was detected for the stapled peptide after 24 hours, while the unstapled analog bearing two piperidine-based sulfimides displayed modest decomposition under the same conditions. These observations suggest that the macrocyclic peptide possesses added aqueous stability compared to the acyclic, functionalized peptide (see Supporting Information, Figure S12–S14). Interestingly, under reductive conditions (pH 7.4, 1.25 mM glutathione), decomposition of the stapled peptide to starting peptide was observed after 24 hours, suggesting that this staple might serve as a reversible modification for delivery of peptide probes and/or therapeutics (see Supporting Information, Figure S15) and offers the possibility of tuning adduct stability for such applications.[26–30] Taken together, the high yields of peptide stapling over a range of distances, along with the high chemoselectivity towards the stapled product over oligomerization and other side reactions and good stability, showcases the efficiency of ReACT method for methionine-based peptide stapling to generate unique peptide macrocycles.

With a new synthetic approach to peptide stapling in hand, we sought to establish that this new technology could be used in a cellular setting. One common application of peptide stapling is to increase cell delivery and uptake, as stapled analogs are thought to increase hydrophobicity as well as stabilize alpha helices over their corresponding linear sequences.[31] To this end, we synthesized three peptides bearing a fluorescein tag, one acyclic, non-functionalized derivative (**2a**), one acyclic, bis-functionalized peptide using **Ox12** (**2b**) and one macrocyclic derivative stapled using **Ox14** (**2c**), and then tested their relative cell uptake abilities.[20,32] Incubation of HEK293T cells with 20 μM of either peptide **2a, 2b**, or **2c** for 1 hour at 37 °C showed a patent increase in the cellular uptake of the stapled peptide **2c** relative to the unstapled linear analogs **2a** and **2b** as shown by confocal microscopy and flow cytometry measurements (Figure 4). Circular dichroism (CD) experiments demonstrate that **2c** forms a well-defined secondary structure compared to its acyclic peptide counterparts, **2a** and **2b**, suggesting that macrocyclization of **2a** by **Ox14** plays a large role in the increased cell permeability (see Supporting Information, Figure S16). Additional experiments suggest that active uptake also contributes to cell permeability, as the same trends in fluorescence are observed when the three peptides are incubated with cells at 4 °C but with lower signal (see Supporting Information, Figure S19–S22). These data establish ReACT as complementary alternative method to other recently published peptide stapling techniques[20,21] and the broader potential application of methionines as amino acid partners in peptide stapling chemistry.

CONCLUDING REMARKS

In summary, we have established a workflow to understand, predict, and interrogate improved reagents for bioconjugation applications, as exemplified by optimization of methionine-based ReACT chemistry. We designed and screened a rational experimental and

virtual library based on measured stability rate constants combined with statistical modeling to identify and develop next-generation methionine bioconjugation reagents that lead to methionine sulfimide adducts with improved S-N stability. Specifically, higher stability is correlated with lower carbonyl stretching frequencies for these adducts. Most importantly, the correlation revealed key electronic and structural features driving the sulfimide adduct stability that can serve as a fundamental, predictive platform for future use. Moreover, insights gained into methionine sulfimide reactivity and stability were translated to the development of a new and unique type of peptide stapling reagent at methionine that displays chemoselective reactivity with good substrate scope and compatibility with cellular environments. Indeed, the high chemoselectivity and reactivity of ReACT toward methionine, without the need for protecting groups for other reactive amino acid side chains, offers an attractive alternative to standard cysteine- and lysine-based technologies for peptide stapling. In a broader sense, this work provides a starting point for the use of data-driven approaches that are gaining traction in the synthetic organic community to research problems in chemical biology and beyond.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

We thank Novartis Institutes for BioMedical Research and the Novartis-Berkeley Center for Proteomics and Chemistry Technologies (NB-CPACT) for supporting this work. A.H.C. thanks the NSF-GRFP (DGE 1106400) for financial support. C.J.C. is an Investigator with the Howard Hughes Medical Institute and a CIFAR Senior Fellow. We also thank the NIH (ES004705 to C.J.C. and GM121383 to M.S.S.) for funding. We thank Dr. James A. Wells and Ms. Susanna Elledge for useful discussions regarding stability. We thank Dr. John Tallarico (Novartis) for helpful suggestions, Mr. Edward Miller and Dr. Spencer O. Scholz for helpful discussions, Ms. Kristin Wucherer, Ms. Rachel Bisiewicz, Mr. Marco Lobba, and Mr. Johnathan Maza for technical advice and support on peptide chemistry, Dr. Shawn Darnall and Mr. Christopher Delre for assistance with CD experiments, Ms. Monica Neugebauer for assistance with mass spectrometry experiments, and Drs. Kathleen Durkin and David Small for computational support (NIH S10OD023532).

REFERENCES

(1). Levine RL; Mosoni L; Berlett BS; Stadtman ER Methionine Residues as Endogenous Antioxidants in Proteins. Proc. Natl. Acad. Sci 1996, 93, 15036–15040. [PubMed: 8986759]

(2). Lin S; Yang X; Jia S; Weeks AM; Hornsby M; Lee PS; Nichiporuk RV; Iavarone AT; Wells JA; Toste FD; et al. Redox-Based Reagents for Chemoselective Methionine Bioconjugation. Science 2017, 355, 597–602. [PubMed: 28183972]

(3). DeGruyter JN; Malins LR; Baran PS Residue-Specific Peptide Modification: A Chemist's Guide. Biochemistry 2017, 56, 3863–3873. [PubMed: 28653834]

(4). Bodanszky M; Bendnarek MA Experiments on the Protection of the Thioether in Methionine. Int. J. Pept. Protein Res. 1982, 20, 408–413. [PubMed: 7174203]

(5). Kleanthous C; Coggins JR Reversible Alkylation of an Active Site Methionine Residue in Dehydroquinase. J. Biol. Chem 1990, 265, 10935–10939. [PubMed: 2193028]

(6). Gross E; Witkop B Nonenzymatic Cleavage of Peptide Bonds - Methionine Residues in Bovine Pancreatic Ribonuclease. J. Biol. Chem 1962, 237, 1856–1860. [PubMed: 13902203]

(7). Taylor MT; Nelson JE; Suero MG; Gaunt MJ A Protein Functionalization Platform Based on Selective Reactions at Methionine Residues. Nature 2018, 562, 563–568. [PubMed: 30323287]

(8). Wang D; Yu M; Liu N; Lian C; Hou Z; Wang R; Zhao R; Li W; Jiang Y; Shi X; et al. A Sulfonium Tethered Peptide Ligand Rapidly and Selectively Modifies Protein Cysteine in Vicinity. Chem. Sci 2019, 10, 4966–4972. [PubMed: 31183045]

(9). Shen BQ; Xu K; Liu L; Raab H; Bhakta S; Kenrick M; Parsons-Reponte KL; Tien J; Yu SF; Mai E; et al. Conjugation Site Modulates the in Vivo Stability and Therapeutic Activity of Antibody-Drug Conjugates. Nat. Biotechnol 2012, 30, 184–189. [PubMed: 22267010]

(10). Nischan N; Chakrabarti A; Serwa RA; Bovee-Geurts PHM; Brock R; Hackenberger CPR Stabilization of Peptides for Intracellular Applications by Phosphoramidate-Linked Polyethylene Glycol Chains. Angew. Chemie - Int. Ed 2013, 52, 11920–11924.

(11). Bolm C; Müller D; Dalhoff C; Hackenberger CPR; Weinhold E The Stability of Pseudopeptides Bearing Sulfoximines as Chiral Backbone Modifying Element towards Proteinase K. *Bioorganic Med*. Chem. Lett 2003, 13, 3207–3211.

(12). Olson EJ; Lechtenberg BC; Zhao C; De La Torre ER; Lamberto I; Riedl SJ; Dawson PE; Pasquale EB Modifications of a Nanomolar Cyclic Peptide Antagonist for the EphA4 Receptor to Achieve High Plasma Stability. ACS Med. Chem. Lett 2016, 7, 841–846. [PubMed: 27660688]

(13). Spicer CD; Davis BG Selective Chemical Protein Modification. Nat. Commun 2014, 5, 1–14.

(14). Hananya N; Reid JP; Green O; Sigman MS; Shabat D Rapid Chemiexcitation of Phenoxy-Dioxetane Luminophores Yields Ultrasensitive Chemiluminescence Assays. Chem. Sci 2019, 10, 1380–1385. [PubMed: 30809354]

(15). Milo A; Neel AJ; Toste FD; Sigman MS A Data-Intensive Approach to Mechanistic Elucidation Applied to Chiral Anion Catalysis. Science 2015, 347, 737–743. [PubMed: 25678656]

(16). Neel AJ; Milo A; Sigman MS; Toste FD Enantiodivergent Fluorination of Allylic Alcohols: Data Set Design Reveals Structural Interplay between Achiral Directing Group and Chiral Anion. J. Am. Chem. Soc 2016, 138, 3863–3875. [PubMed: 26967114]

(17). Christian AH; Niemeyer ZL; Sigman MS; Toste FD Uncovering Subtle Ligand Effects of Phosphines Using Gold(I) Catalysis. ACS Catal. 2017, 7, 3973–3978. [PubMed: 29686935]

(18). Niemeyer ZL; Pindi S; Khrakovsky DA; Kuzniewski CN; Hong CM; Joyce LA; Sigman MS; Toste FD Parameterization of Acyclic Diaminocarbene Ligands Applied to a Gold(I)-Catalyzed Enantioselective Tandem Rearrangement/Cyclization. J. Am. Chem. Soc 2017, 139, 12943–12946. [PubMed: 28885017]

(19). Harper KC; Sigman MS Three-Dimensional Correlation of Steric and Electronic Free Energy Relationships Guides Asymmetric Propargylation. Science 2011, 333, 1875–1878. [PubMed: 21960632]

(20). Spokoyny AM; Zou Y; Ling JJ; Yu H; Lin YS; Pentelute BL A Perfluoroaryl-Cysteine SNAr Chemistry Approach to Unprotected Peptide Stapling. J. Am. Chem. Soc 2013, 135, 5946–5949. [PubMed: 23560559]

(21). Kubota K; Dai P; Pentelute BL; Buchwald SL Palladium Oxidative Addition Complexes for Peptide and Protein Cross-Linking. J. Am. Chem. Soc 2018, 140, 3128–3133. [PubMed: 29406701]

(22). Lau YH; De Andrade P; Quah ST; Rossmann M; Laraia L; Sköld N; Sum TJ; Rowling PJE; Joseph TL; Verma C; et al. Functionalised Staple Linkages for Modulating the Cellular Activity of Stapled Peptides. Chem. Sci 2014, 5, 1804–1809.

(23). Sun S; Compañón I; Martínez-Sáez N; Seixas JD; Boutureira O; Corzana F; Bernardes GJL Enhanced Permeability and Binding Activity of Isobutylene-Grafted Peptides. ChemBioChem 2018, 19, 48–52. [PubMed: 29105291]

(24). Blackwell HE; Sadowsky JD; Howard RJ; Sampson JN; Chao JA; Steinmetz WE; O'Leary DJ; Grubbs RH Ring-Closing Metathesis of Olefinic Peptides: Design, Synthesis, and Structural Characterization of Macrocyclic Helical Peptides. J. Org. Chem 2001, 66, 5291–5302. [PubMed: 11485448]

(25). Schafmeister CE; Po J; Verdine GL An All-Hydrocarbon Cross-Linking System for Enhancing the Helicity and Metabolic Stability of Peptides. J. Am. Chem. Soc 2000, 122, 5891–5892.

(26). Shi X; Zhao R; Jiang Y; Zhao H; Tian Y; Jiang Y; Li J; Qin W; Yin F; Li Z Reversible Stapling of Unprotected Peptides via Chemoselective Methionine Bis-Alkylation/Dealkylation. Chem. Sci 2018, 9, 3227–3232. [PubMed: 29844896]

(27). Maity SK; Jbara M; Laps S; Brik A Efficient Palladium-Assisted One-Pot Deprotection of (Acetamidomethyl)Cysteine Following Native Chemical Ligation and/or Desulfurization To Expedite Chemical Protein Synthesis. Angew. Chemie - Int. Ed 2016, 55, 8108–8112.

(28). Jbara M; Laps S; Morgan M; Kamnesky G; Mann G; Wolberger C; Brik A Palladium Prompted On-Demand Cysteine Chemistry for the Synthesis of Challenging and Uniquely Modified Proteins. Nat. Commun 2018, 9, 1–11. [PubMed: 29317637]

(29). Jbara M; Laps S; Maity SK; Brik A Palladium-Assisted Cleavage of Peptides and Proteins Containing a Backbone with Thiazolidine Linkage. Chem. - A Eur. J 2016, 22, 14851–14855.

(30). Stenton BJ; Oliveira BL; Matos MJ; Sinatra L; Bernardes GJL A Thioether-Directed Palladium-Cleavable Linker for Targeted Bioorthogonal Drug Decaging. Chem. Sci 2018, 9, 4185–4189. [PubMed: 29780549]

(31). Bird GH; Mazzola E; Opoku-Nsiah K; Lammert MA; Godes M; Neuberg DS; Walensky LD Biophysical Determinants for Cellular Uptake of Hydrocarbon-Stapled Peptide Helices. Nat. Chem. Biol 2016, 12, 845–852. [PubMed: 27547919]

(32). Zhang H; Zhao Q; Bhattacharya S; Waheed AA; Tong X; Hong A; Heck S; Curreli F; Goger M; Cowburn D; et al. A Cell-Penetrating Helical Peptide as a Potential HIV-1 Inhibitor. J. Mol. Biol 2008, 378, 565–580. [PubMed: 18374356]

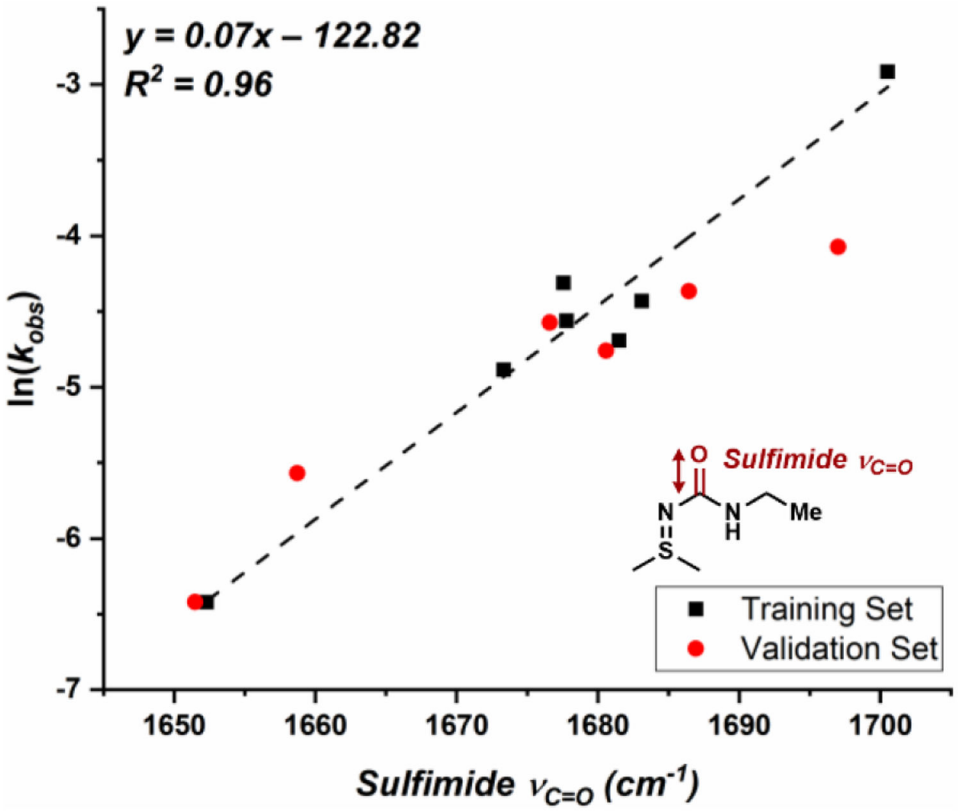


Figure 1.
Linear correlation between sulfimide $\nu_{C=O}$ and $\ln(k_{obs})$

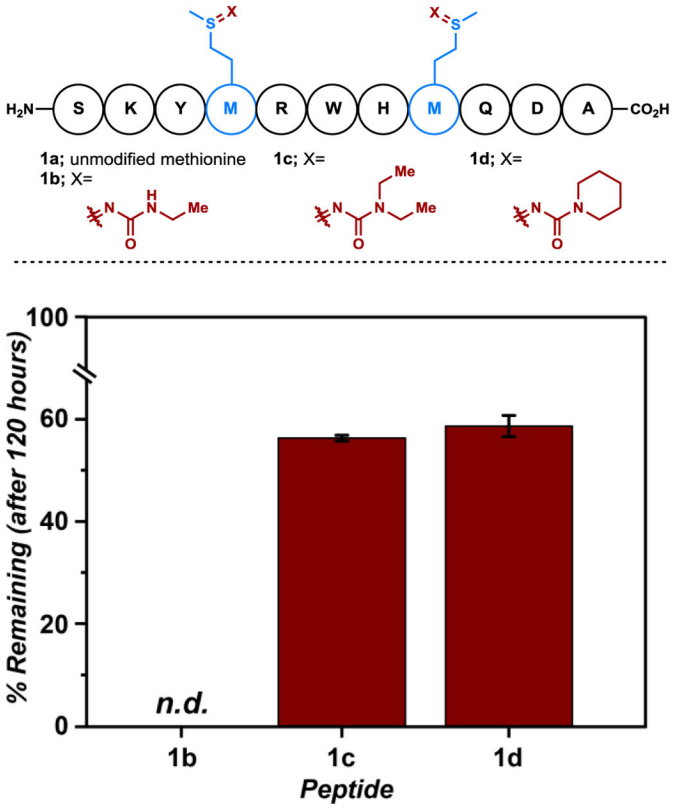


Figure 2.

In vitro stability of sulfimides derived from oxaziridine reagents on **1a**

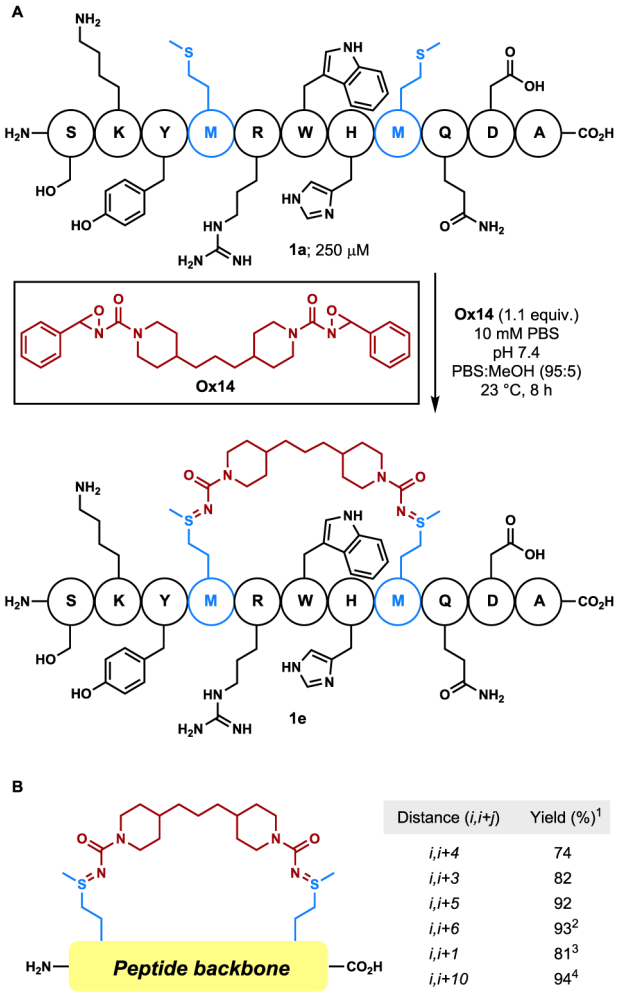


Figure 3.

(A) Application of higher stability adducts in peptide stapling (B) Efficacy of macrocyclization. Standard conditions: 250 μM peptide, 1.1 equiv. **Ox14**, pH 7.4, 95:5 PBS buffer:MeOH, 23 °C, 8 hours 1. Yields measured by LC-MS; 2. 18 hours; 3. 10 hours; 4. 16 hours

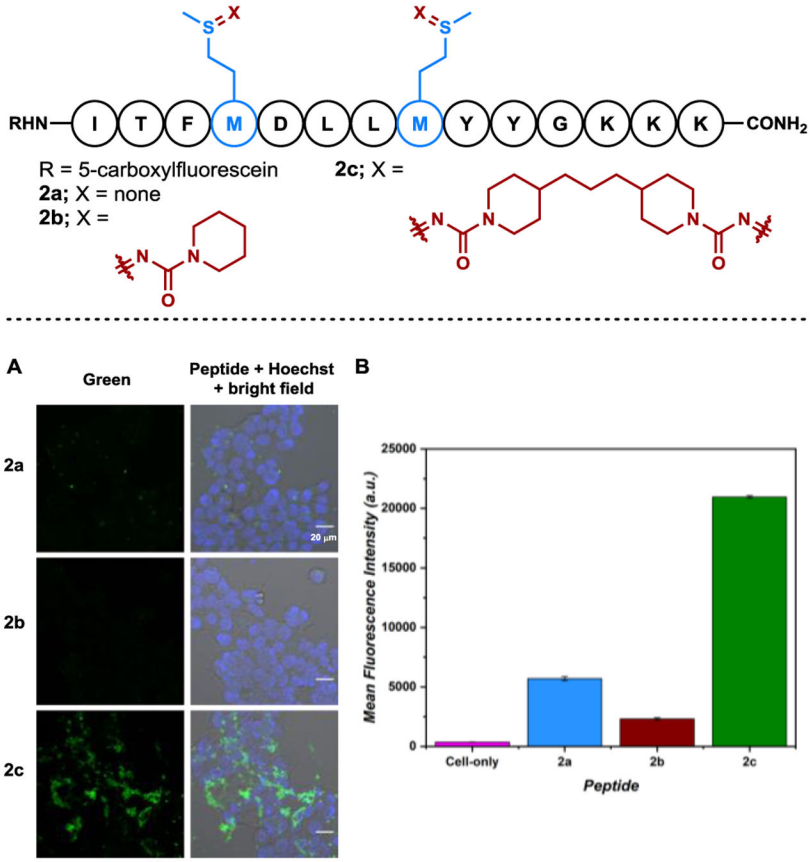
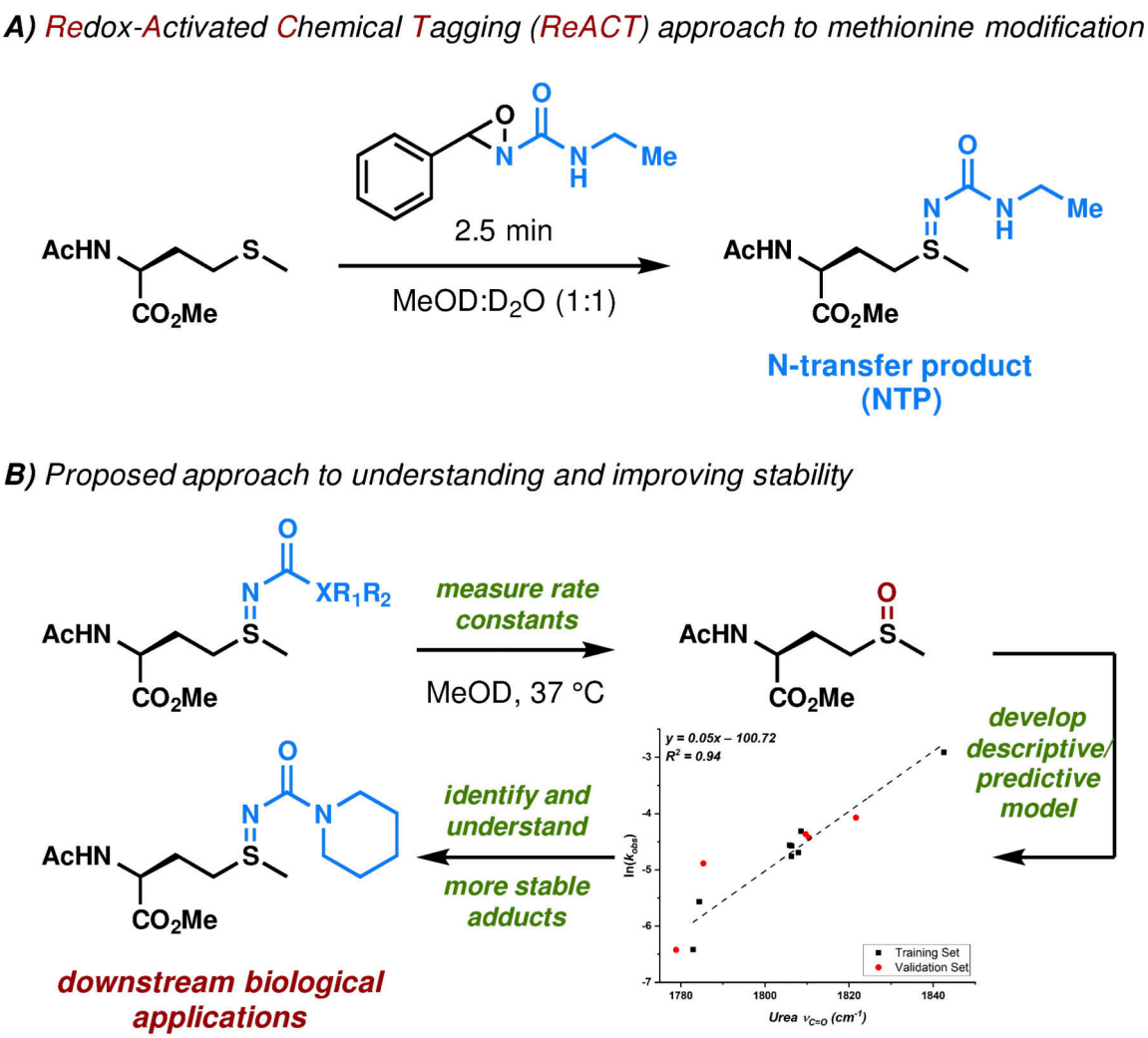


Figure 4.

Cell uptake experiment with unstapled and stapled peptides modified with ReACT reagents. (A) Confocal microscopy images of HEK293T cells incubated with 20 μM peptide for 1 hour and stained with Hoechst 33342. Scale-bars: 20 μm (B) Flow cytometry data for unfunctionalized peptide **2a**, linear bis-modified peptide **2b**, and stapled peptide **2c**.

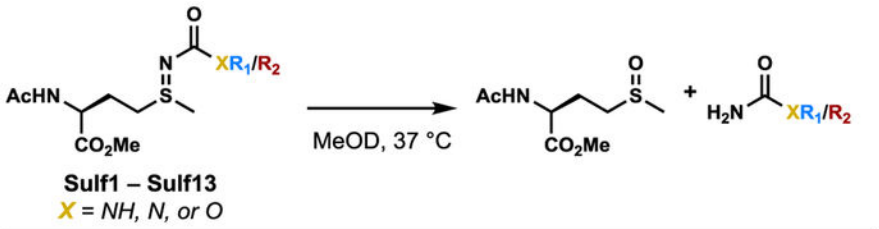


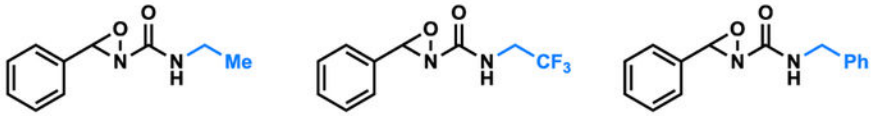
Scheme 1.

Development of Methionine Modification with Oxaziridines and Stable Sulfimide Adducts

Table 1.

Oxaziridines Used to Probe Stability of Sulfimide Adducts.

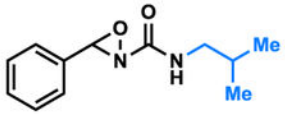




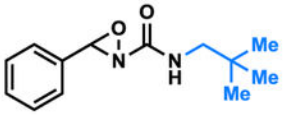
Ox1
Sulf1; $k = 1.19\times10^{-2}\ h^{-1}$
$k_{rel} = 1.00$

Ox2
Sulf2; $k = 1.70\times10^{-2}\ h^{-1}$
$k_{rel} = 1.43$

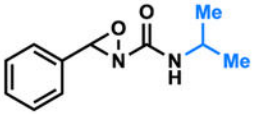
Ox3
Sulf3; $k = 1.34\times10^{-2}\ h^{-1}$
$k_{rel} = 1.13$



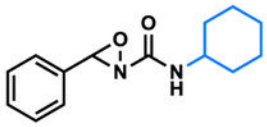
Ox4
Sulf4; $k = 0.92\times10^{-2}\ h^{-1}$
$k_{rel} = 0.77$



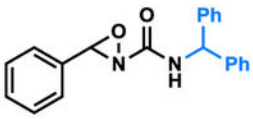
Ox5
Sulf5; $k = 0.86\times10^{-2}\ h^{-1}$
$k_{rel} = 0.72$



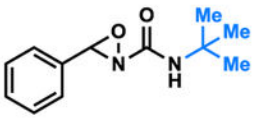
Ox6
Sulf6; $k = 1.05\times10^{-2}\ h^{-1}$
$k_{rel} = 0.88$



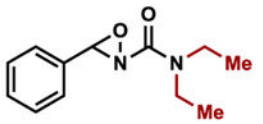
Ox7
Sulf7; $k = 1.03\times10^{-2}\ h^{-1}$
$k_{rel} = 0.87$



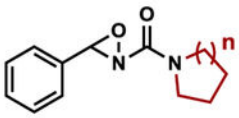
Ox8
Sulf8; $k = 1.27\times10^{-2}\ h^{-1}$
$k_{rel} = 1.07$



Ox9
Sulf9; $k = 0.76\times10^{-2}\ h^{-1}$
$k_{rel} = 0.64$

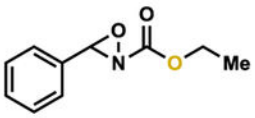


Ox10
Sulf10; $k = 0.16\times10^{-2}\ h^{-1}$
$k_{rel} = 0.13$



Ox11; n=1
Sulf11; $k = 0.38\times10^{-2}\ h^{-1}$
$k_{rel} = 0.32$

Ox12; n=2
Sulf12; $k = 0.16\times10^{-2}\ h^{-1}$
$k_{rel} = 0.13$



Ox13
Sulf13; $k = 5.40\times10^{-2}\ h^{-1}$
$k_{rel} = 4.54$