A synthetic strategy towards S, N, and O-heterocyclooctynes facilitates bioconjugation using multifunctional handles

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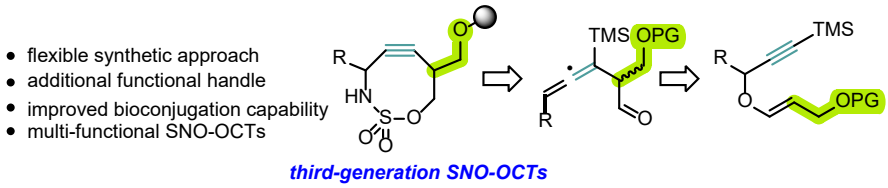
TOC graphic



Abstract

Over the past two decades, the introduction of bioorthogonal reactions has transformed the ways in which chemoselective labelling, isolation, imaging, and drug delivery are carried out in a complex biological milieu. A key feature of a good bioorthogonal probe is the ease with which it can be attached to a target compound through bioconjugation. This paper describes the expansion of the utility of a class of unique S, N, and O-containing heterocyclooctynes (SNO-OCTs), which show chemoselective reactivity with Type I and Type II dipoles and divergent reactivities in response to electronic tuning of the alkyne. Currently, bioconjugation of SNO-OCTs to a desired target is achieved through an inconvenient aryl- or amide-linker at the sulfamate nitrogen. Herein, a new synthetic approach towards general SNO-OCT scaffolds is demonstrated that enables the installation of functional handles at both propargylic carbons of the heterocycloalkyne. This capability increases the utility of SNO-OCTs as labeling reagents through the design of bifunctional bioorthogonal probes with expanded capabilities. NMR kinetics also revealed up to

six-fold improvement in cycloaddition rates of the second-generation analogs compared to first-generation SNO-OCTs.

Introduction

Over the past few decades, diverse and chemoselective bioorthogonal reactions have been developed to perform reactions in complex biological milieu that achieve selective bioconjugation, labelling, isolation, imaging, and drug delivery.[1-5] Cyclooctynes remain the most popular and widely adopted partners for bioorthogonal labelling; however, the synthesis and design of new cycloalkynes requires a multi-variate balancing act of stability, bioorthogonality, synthetic accessibility and ease of bioconjugation. For example, dibenzylcyclooctyne (DBCO) and its derivatives are widely used due to their commercial availability and good rates (**Figure 1A**), as in an observed $k_2 = 0.31\ M^{-1}\bullet s^{-1}$ in the reaction of DBCO with benzyl azide. While appending aryl groups to the cycloalkyne core of DBCO accelerates reactivity, it also renders the compound susceptible to nucleophilic addition of thiols to the alkyne. Rates can be improved using electronic effects, as demonstrated by thiocycloalkynes such as tetramethylthiacycloheptyne (TMTH), which show enhanced reaction rates of up to $4\ M^{-1}\bullet s^{-1}$.[6] While this scaffold contains no aromatic rings, it displays no convenient functional handles for bioconjugation, limiting its utility in bioorthogonal labeling. To address this issue, Liskamp and coworkers recently developed a TMTH sulfoximine derivative to facilitate bioconjugation.[7] Despite this advance, there remains a need for an expanded toolbox of convenient, multifunctional cycloalkyne labelling tools that are accessible using robust syntheses and are easy to deploy in biological applications.

Our group previously developed a new class of heterocyclooctynes containing a sulfamate group in the ring. These compounds, termed 'SNO-OCTs',[8-10] were prepared via

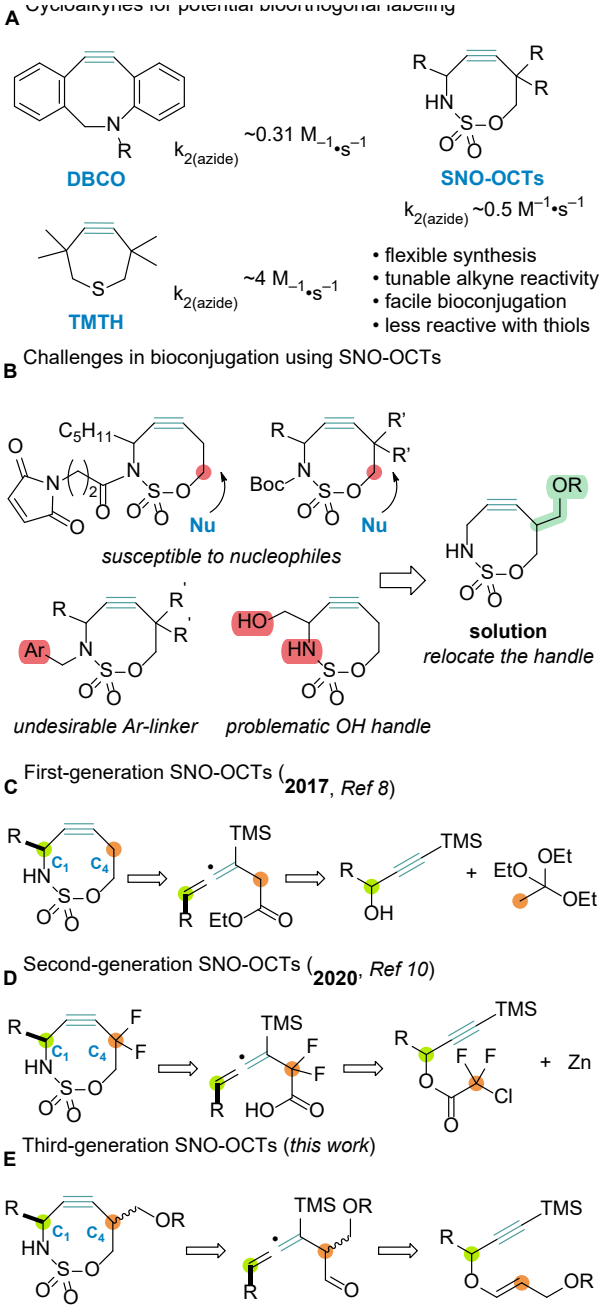


Figure 1. (A) Cycloalkynes for potential bioorthogonal labeling. (B) Challenges in bioconjugation using SNO-OCTs. (C) First-generation SNO-OCTs. (D) Second-generation SNO-OCTs. (E) Third-generation SNO-OCTs.

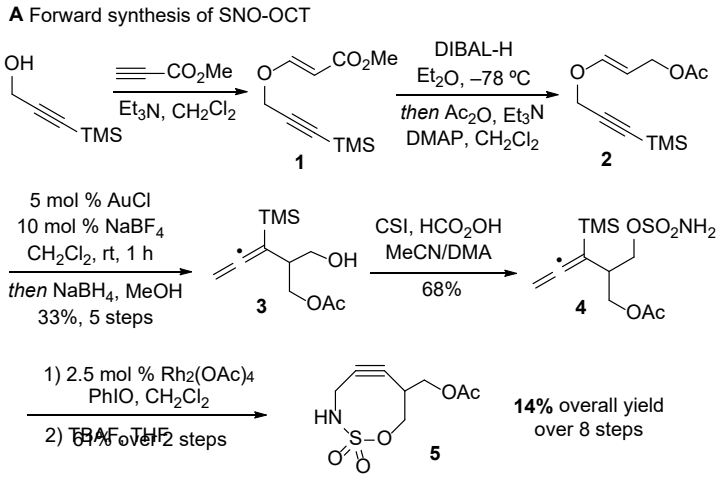
a highly selective Rh-catalyzed silyl allene aziridination/elimination strategy. This allows for the facile installation of substituents on the ring and ensures that the alkyne moiety remains relatively inert to nucleophilic additions by glutathione and other exogenous nucleophiles in the biological milieu.[8] However, a general strategy for facile bioconjugation to SNO-OCTs remains underdeveloped (**Figure 1B**); previous attachment to RNase required a reaction at the sulfamate nitrogen, resulting in susceptibility of the SNO-OCT to potential competing ring-opening.[9,11] Attachment of proteins or a cell surface to the SNO-OCT sulfamate nitrogen required the use of aryl linkers, which increased the lipophilicity of the cyclooctyne. Finally, attempted functionalization of exocyclic hydroxyl groups handles on our first-generation SNO-OCTs using a carbonate-based linker gave significant decomposition, perhaps due to interference by the adjacent sulfamate nitrogen (**Figure 1B**). To fully realize the potential of SNO-OCTs as powerful tools for bioorthogonal labelling, an alternative strategy was needed to introduce useful synthetic handles for bioconjugation of this scaffold to molecules of interest. Herein, we describe a key gold(I)-catalyzed Claisen rearrangement that furnishes SNO-OCTs with a previously inaccessible propargylic handle to unlock diverse possibilities for facile bioconjugations.[12]

Our first-generation approach towards SNO-OCT scaffolds (**Figure 1C**) employed an acid-catalyzed Claisen rearrangement of a propargyl alcohol with an orthoester to install alkyl substitution at the α-amino carbon (C1, highlighted in green), yielding products that showed similar reactivities in click reactions with azides and diazoalkanes.[8] A second-generation approach (**Figure 1D**) focused on the preparation of SNO-OCT derivatives bearing substituents to electronically tune cycloaddition rates and achieve divergent chemoselectivity in reactions with diverse dipoles.[10] A Zn-mediated Reformatsky-Claisen rearrangement successfully furnished

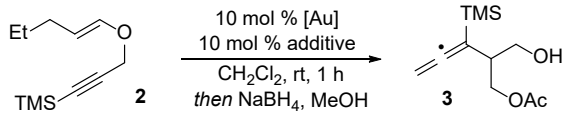
SNO-OCTs with *gem*-difluoro substitution at the C4 propargyl position (highlighted in orange); these SNO-OCTs engage in chemoselective cycloadditions with diazoacetamides in preference to azides or tetrazines. While this approach enabled us to functionalize the second propargyl position (C4) on the SNO-OCT, it was limited to a single example. To expand the scope of functional handles that can be installed at C4, a tertiary allenic carbon center must be introduced (**Figure 1E**, highlighted in orange). Retrosynthetic analysis suggested this motif could be accessed through a Claisen rearrangement of a propargyl vinyl ester, which is readily prepared via conjugate addition to a propiolate. This paper describes the development of third-generation SNO-OCTs with versatile functional handles at both propargylic carbons of the heterocycloalkyne.

Results and discussion

The synthesis commenced with conjugate addition of the commercially available TMS-protected propargyl alcohol to methyl propiolate to form the acrylate **1** (**Scheme 1**). A DIBAL-H reduction delivers an allylic alcohol that is protected with acetic anhydride to furnish **2** as the precursor for an Au(I)-catalyzed Claisen rearrangement. Toste and co-workers[13-15] provided precedent for this transformation, although optimization (**Scheme 1B**) studies showed a simple AuCl catalyst with no ligand successfully promotes Claisen rearrangement (Entry 1). A $NaBF_4$ additive increased the yield to 81% (entry 2), but surprisingly, other Au(I) catalysts and additives gave no desired product **3** (entries 3-8). It appears all three ions (sodium, chloride, BF_4) play roles in the rearrangement, although the reasons are unclear. The rearrangement occurred rapidly, with completion resulting in catalyst decomposition, as noted by the presence of dark insoluble solids and a metallic coating on the flask. A gram-scale synthesis of **3** from the commercially available propargyl alcohol gave a 33% yield over five steps using only simple silica plug purifications. Formation of the homoallenic sulfamate **4** in 68% yield employed an in situ-generated sulfamoyl chloride from



B Optimization of the AuCl-catalyzed Claisen rearrangement



entry	[Au]	additive	product **3** (%)	RSM **2** (%)
1	AuCl	none	65	0
2	AuCl	$NaBF_4$	81	0
3	AuCl	$AgBF_4$	0	0
4	AuCl	$AgPF_6$	0	0
5	AuCl	$AgSbF_6$	0	0
6	Au(PPh_3)Cl	none	0	0
7	Au(PPh_3)Cl	$NaBF_4$	0	quant
8[a]	AuCl	$NaBF_4$	0	50

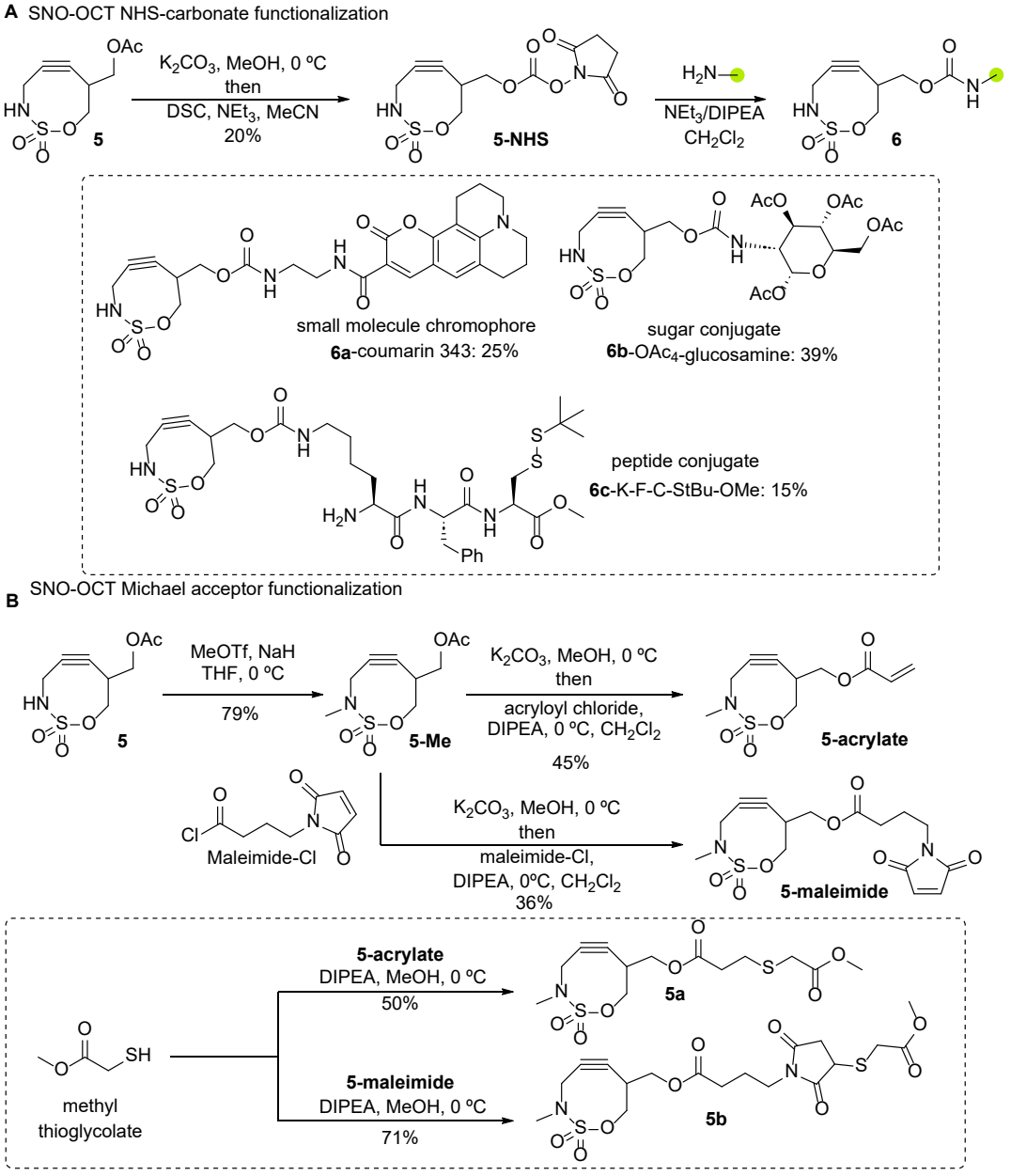
[a] Reaction was performed in MeOH instead of CH_2Cl_2. The yield was determined using ^1H NMR with 1 equiv of mesitylene as the internal standard.

Scheme 1. (A) Synthesis of SNO-OCT **5.** (B) Optimization of the Au(I)-catalyzed Claisen rearrangement.

chlorosulfonyl isocyanate (CSI) and formic acid. The SNO-OCT **5** was prepared according to reported conditions[8] with an overall yield of 14% over 8 steps.

With SNO-OCT **5** in hand, two common bioconjugation strategies were examined (**Scheme 2**): use of an NHS-carbonate handle for nucleophilic amines and a Michael acceptor for thiol-based conjugations. The synthesis of the NHS-modified SNO-OCT **5-NHS** was achieved by acetyl deprotection under basic conditions, followed by treatment with disuccinimidyl carbonate (DSC).

The viability of SNO-OCT conjugation to diverse molecules was explored, including the small molecule chromophore **6a**, a protected sugar conjugate **6b** and the peptide conjugate **6c**, all of which can be further employed for imaging,[16-17] metabolic labelling,[18-20] and targeted therapeutic delivery.[21] The formation of **6a** and **6b** provided satisfactory yields, but formation and isolation



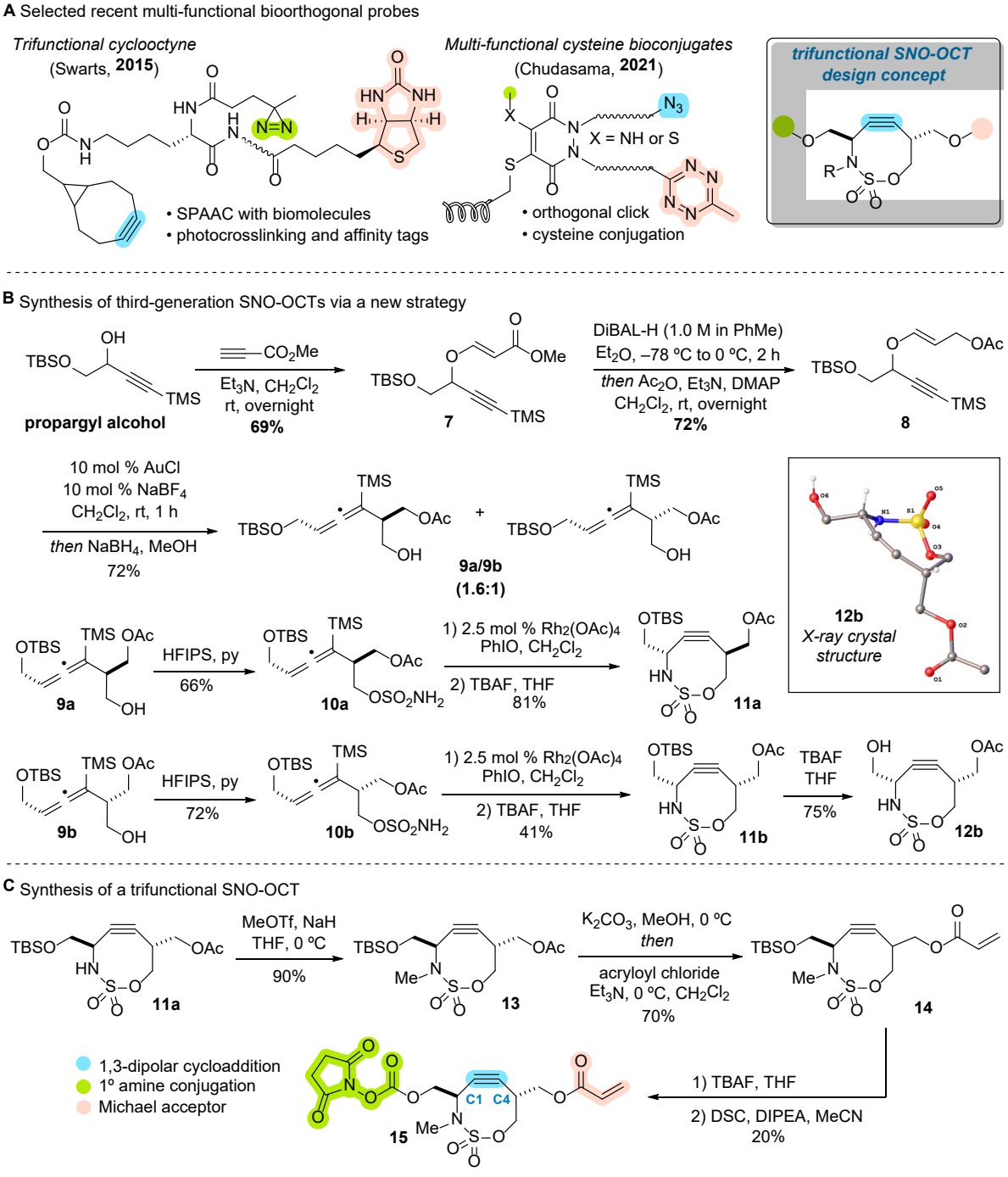
Scheme 2. (A) SNO-OCT NHS-carbonate functionalization. (B) SNO-OCT Michael acceptor functionalization.

of **6c** proved challenging due to competing oxidation of the protected cysteine residue that resulted in a low yield.

Formation of the SNO-OCT Michael acceptor derivative **5-acrylate** was initially unsuccessful due to product instability and significant decomposition in the presence of a free sulfamate N–H group. However, methylation using MeOTf to avoid competitive SNO-OCT ring-opening furnished **5-acrylate** in 45% yield and **5-maleimide** in 36% yield. In addition, we also examined the viability of both SNO-OCT Michael acceptor conjugates in the presence of a commercially available methyl thioglycolate. As expected, both reacted rapidly to result desired products **5a** and **5b** with maleimide-thiol reaction proving more robust. We have not observed the thiol adduct of the SNO-OCT alkyne via NMR and LC-MS.

Encouraged by the successful synthesis of SNO-OCT **5** and its bioconjugation, we next explored whether it would be possible to prepare a trifunctional SNO-OCT bearing synthetic handles at both propargylic carbons. If this could be achieved, the SNO-OCT could be orthogonally functionalized with both a Michael acceptor and an NHS-carbonate (**Scheme 3A**). There continues to be significant interest and a steady development of multi-functional bioorthogonal probes that allow for the enrichment of biomolecules[22] and multiple chemoselective protein modifications.[23]

The synthesis commenced from the reported propargyl alcohol (**Scheme 3B**),[8] which engaged in conjugate addition to methyl propiolate to deliver **7** in 69% isolated yield (**Scheme 3B**). Reduction and acetate protection proceeded to give **8** in a satisfying 72% yield. Application of optimized conditions for Au(I)-catalyzed Claisen rearrangement to **8** gave two diastereomeric homoallenic alcohols **9a** and **9b** that are separable by flash column chromatography. The relative stereochemistry was established via X-ray crystallographic studies on the final product **12b**. An HFIP-based sulfamate precursor (HFIPS) was utilized in the formation of the homoallenic



Scheme 3. (A) Selected recent multi-functional bioorthogonal probes. (B) Synthesis of third-generation SNO-OCTs via a new strategy. (C) Synthesis of a trifunctional SNO-OCT.

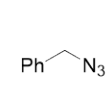
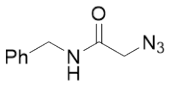
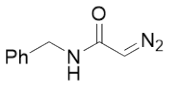
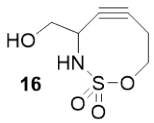
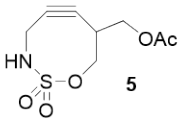
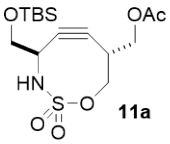
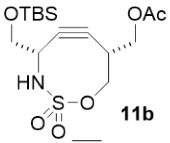
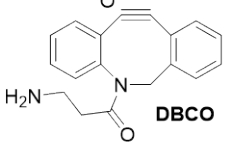
sulfamate as an alternative to the standard CSI condition, as this can lead to acid-promoted allene rearrangement into an undesired diene or loss of TBS-protecting group. The formation of SNO-

OCTs **11a** and **11b** was achieved under standard conditions and the relative stereochemistry determined by removal of the TBS-protecting group on **12b** to give a white crystalline solid. The crystal structure revealed a *cis* relationship between the two propargyl substituents of **12b**. This information was used to derive the relative stereochemistry of all precursors (See the SI, **Scheme S1** for details). The relative stereochemistry of **11a** was not determined through a crystal structure.

With the successful preparation of SNO-OCTs **11a** and **11b** in hand, efforts to synthesize a new trifunctional SNO-OCT that combines the ability to perform 1,3-dipolar cycloaddition, amine conjugation, and thiol Michael addition into a single probe (**Scheme 3C**) were undertaken. The synthesis commenced with methylation of the sulfamate nitrogen to prevent undesired SNO-OCT decomposition; product **13** was obtained in 90% yield. Acetyl deprotection and acrylate formation resulted in **14** in 70% yield. While synthesis of the final NHS-ester gave a low 20% yield of **15** over two steps, it solved our previous problem of installing a C1-OH handle due to the lack of product stability when the adjacent sulfamate nitrogen remained unprotected.

Lastly, the reaction kinetics of each novel SNO-OCT were compared to the known SNO-OCT analogue **16**. The SNO-OCT **5** demonstrated the fastest reaction kinetics, with a second-order rate constant k_2 up to 0.563 ± 0.018 $M^{-1}\bullet s^{-1}$ in reactions with benzyl diazoacetamide **19**, a nucleophilic Type I dipole. Despite their general structural similarities, **5** demonstrated much faster kinetics than **16** in reactions with all three dipoles **17-19**, and it is comparable to the kinetic rate of commercially available DBCO (**Table 1**).[24] We partially attribute this to the electron-withdrawing effects of the acetyl group, but other potential factors include the solvent and hydrogen-bonding effects. Steric effects play the biggest role in k_2 when comparing SNO-OCT **11a** and **11b** across all three dipoles (**17b**, **18**, **19**), with the *cis* isomer **11b** having a k_2 roughly double that of the *trans* isomer, due to having a more accessible alkyne.

Table 1. 1,3-Dipolar cycloaddition kinetic rates with SNO-OCTs and DBCO in CD_3CN.

k_2 ($M^{-1}•s^{-1}$) in CD_3CN	 **17**	 **18**	 **19**
 **16**	0.087 ± 0.001 [a]	0.0469 ± 0.0007	0.087 ± 0.011 [b]
 **5**	0.1065 ± 0.0054	0.1757 ± 0.0059	0.563 ± 0.018
 **11a**	0.0422 ± 0.0011	0.0467 ± 0.0015	0.1636 ± 0.0031
 **11b**	0.0668 ± 0.0016	0.0969 ± 0.0027	0.3095 ± 0.0035
 **DBCO**	n/a	0.23 ± 0.06 [c]	0.45 ± 0.09 [c]

[a] Reference 8. [b] Reference 10. [c] Reference 24.

Conclusions

In this study, we report a third-generation SNO-OCT synthesis that enables either functionalization at the C4 or the C1-C4 propargylic carbons of the heterocycloalkyne. These new labelling tools enable facile bioconjugation of diverse molecules to SNO-OCT and the chemistry is readily scalable, including a key gold-catalyzed Claisen rearrangement. Novel SNO-OCT derivatives include those bearing NHS-carbonate handles for amine attachment and a Michael acceptor acrylate for reactions with thiols. An expansion of this approach resulted in the preparation of a bifunctional SNO-OCT containing both NHS-carbonate and acrylate handles, which had not been previously possible without post-methylation of the sulfamate nitrogen. We envision these bi-, and trifunctional SNO-OCTs will find utility in assisting chemoselective

bioconjugation, while substitution on the sulfamate can be extended to include aryl linkers[10] to furnish unique and functionally dense heterocycloalkynes for a host of biological applications.

Experimental section

General information

All reactions were carried out in either round bottom flasks or in 1 ½ dram vials that were flame-dried under a stream of dry nitrogen before use. Reagents were used as obtained from the vendor without further purification unless otherwise specified. All reactions were performed under a nitrogen atmosphere unless specified otherwise. The mobile phases for column chromatography varied depending on the substrate as pentane/ether (Et_2O), hexanes/ethyl acetate (EA), dichloromethane (CH_2Cl_2)/methanol (MeOH), or CH_2Cl_2/acetonitrile (MeCN) were used. For reactions producing products without a UV signature, potassium permanganate ($KMnO_4$) was employed to visualize the reaction progress. ^{1}H NMR and ^{13}C NMR spectra were obtained using Bruker Avance III 500, Bruker Avance III 400, and Bruker Avance Neo 500 spectrometers. For ^{1}H NMR, chemical shifts are reported relative to residual protiated solvent peaks (δ 7.26, 1.94, and 4.80 ppm for $CDCl_3$, CD_3CN and CD_3OD, respectively). ^{13}C NMR spectra were measured at either 125 MHz or 101 MHz on the same instruments noted above for recording ^{1}H NMR spectra. Chemical shifts were again reported according to the deuterium signal of the solvent (δ 77.1, 118.69, and 49.0 ppm for $CDCl_3$, CD_3CN, and CD_3OD, respectively). When necessary, structural assignments were made with additional information from gHSQC experiments. Accurate mass measurements were acquired at the University of Wisconsin, Madison using a Thermo Q Exactive™ Plus (electrospray ionization or atmospheric solids analysis probe (ASAP-MS) methods).

Methyl (*E*)-3-((3-(trimethylsilyl)prop-2-yn-1-yl)oxy)acrylate (1). A flame-dried 250 mL round bottom flask equipped with a stir bar was charged with TMS-propargyl alcohol (10.0 mmol, 1.48 mL), followed by dilution with diethyl ether (20 mL). Triethylamine (2.79 mL, 2 equiv., 20.0 mmol) was added. The mixture was cooled to 0 ºC, and then methyl propiolate (0.98 mL, 11.0 mmol, 1.1 equiv.) was added dropwise over 5 min. The mixture was allowed to warm to ambient temperature overnight. The next day, the crude mixture was concentrated under reduced pressure in a well-ventilated fume hood. The crude material was diluted with 100 mL of dichloromethane and filtered through a pad of silica (3 cm in a 60 mL fritted funnel). The silica was washed with 200 mL of dichloromethane. The filtrate was concentrated in vacuo. The material is a clear, colorless liquid that was carried forward without further purification. Note: the crude material should be concentrated on a rotary evaporator in a well-ventilated hood. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (dd, J = 12.6, 1.2 Hz, 1H), 5.31 (dd, J = 12.7, 1.2 Hz, 1H), 4.51 (d, J = 1.2 Hz, 2H), 3.71 (d, J = 1.3 Hz, 3H), 0.19 (d, J = 1.3 Hz, 9H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 167.9, 161.0, 98.0, 97.9, 94.6, 59.2, 51.3, -0.3. HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_{10}H_{16}O_3SiNa$ 235.0761; Found, 235.0757.

(*E*)-3-((3-(trimethylsilyl)prop-2-yn-1-yl)oxy)allyl acetate (2). A flame-dried 250 mL round bottom flask equipped with a large stir bar (note: sufficient stirring is important) was charged with

the ester **1** (10.0 mmol) and diluted with diethyl ether (17 mL, 0.6 M). The mixture was cooled to –78 ºC and di-isobutylaluminum hydride in toluene (22 mL, 1.0 M) was added with a slow and steady flow over 10 min. The reaction was stirred at –78 ºC for 30 min, warmed to 0 ºC and stirred for an additional 30 min. The reaction mixture was diluted with 50 mL of diethyl ether and slowly quenched at 0 ºC with 5 mL of saturated potassium sodium tartrate solution. The crude solution was dried with $MgSO_4$, sonicated for 10 min, and then filtered through a pad of silica (4 cm of silica in 250 mL fritted funnel). The silica pad was washed with 100 mL of diethyl ether and concentrated under reduced pressure. The crude material was carried forward without further purification.

A flame-dried round bottom flask equipped with a stir bar was charged with alcohol above (10.0 mmol) and diluted with dichloromethane (50 mL, 0.2 M). Triethylamine (2.8 mL, 20.0 mmol, 2.0 equiv) and DMAP (122 mg, 1.0 mmol, 0.1 equiv) were added. Acetic anhydride (1.4 mL, 15.0 mmol, 1.5 equiv) was added slowly over 5 min and stirred overnight at ambient temperature. The next day, the reaction was quenched with 20 mL of a saturated NH_4Cl solution. The aqueous layer was extracted three times with dichloromethane. The pooled organics were washed with brine, dried over MgSO4, and filtered through a pad of silica (3 cm in a 60 mL fritted funnel). The silica was washed with 50 mL of dichloromethane and concentrated under reduced pressure. The crude was carried forward without further purification as a light-yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.57 (d, *J* = 12.6 Hz, 1H), 5.06 (dt, *J* = 12.6, 7.8 Hz, 1H), 4.49 (dd, *J* = 7.8, 1.0 Hz, 2H), 4.39 (s, 2H), 2.04 (s, 3H), 0.18 (s, 9H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 171.1, 150.8, 99.8, 99.3, 93.3, 62.4, 57.9, 21.3, -0.2. HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_{11}H_{18}O_3SiNa$ 249.0917; Found, 249.0914.

2-(Hydroxymethyl)-3-(trimethylsilyl)penta-3,4-dien-1-yl acetate (3). A flame-dried round bottom flask equipped with a stir bar was charged with AuCl (116 mg, 0.50 mmol, 0.05 equiv) and $NaBF_4$ (110 mg, 1.0 mmol, 0.1 equiv). An aliquot of 10 mL of dichloromethane was added and the mixture stirred for 10 min. The allyl acetate **2** (10.0 mmol) as a solution in 10 mL of dichloromethane was added to the stirring catalyst mixture. The reaction mixture turned from a light champagne-colored solution to a heterogeneous mixture with black solids over 30 min, indicating complete reaction conversion with concomitant death of the gold catalyst. The reaction was cooled to 0 ºC, and $NaBH_4$ (454 mg, 12.0 mmol, 1.2 equiv) was added in one portion, followed by 10 mL of MeOH. After 1 h, the crude mixture was diluted with more dichloromethane (~200 mL) and transferred to a separatory funnel. The organic layer was washed with water, then brine and dried over $MgSO_4$. The crude mixture was filtered through a pad of silica (3 cm in a 50 mL fritted funnel) and washed with several portions of dichloromethane. The resulting product was a viscous clear yellow oil (751.2 mg, 3.29 mmol, 33% overall yield over 4 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.33 (t, *J* = 1.5 Hz, 2H), 4.11 (dd, *J* = 11.1, 5.1 Hz, 1H), 3.96 (dd, *J* = 11.1, 7.8 Hz, 1H), 3.57 (ddd, *J* = 11.2, 7.4, 5.2 Hz, 1H), 3.50 (dt, *J* = 11.3, 5.7 Hz, 1H), 2.33 – 2.22 (m, 1H), 1.92 (s, 3H), 1.75 (dd, *J* = 7.5, 5.7 Hz, 1H), 0.00 (s, 9H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 209.1, 171.4, 92.8, 77.4, 76.9, 70.8, 64.8, 63.5, 40.5, 21.1, -1.4. HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_{11}H_{20}O_3SiNa$ 251.1074; Found, 251.1071.

2-((Sulfamoyloxy)methyl)-3-(trimethylsilyl)penta-3,4-dien-1-yl acetate (4). A flame-dried 250 mL three-neck flask equipped with a stir bar was charged with chlorosulfonyl isocyanate (CSI, 0.26 mL, 3.0 mmol, 1.5 equiv) and then cooled to 0 ºC. Formic acid (0.11 mL, 3.0 mmol, 1.5 equiv) was slowly added dropwise over 5 min under vigorous stirring (note: this step is exothermic and

generates gas). Once a white solid formed in the round bottom flask, it was diluted with acetonitrile (6.0 mL). The stirring solution was allowed to warm up to ambient temperature overnight. The next day, the solution was then cooled again to 0 ºC. A solution of homoallenic alcohol (457 mg, 2.0 mmol, 1 equiv) in DMA (3.3 mL, 0.6 M) was added slowly over 5 min. The reaction was warmed to room temperature. After 2 h, the reaction was quenched with an equal amount of water. The mixture was extracted 3 times with diethyl ether. The pooled organics were washed 5 times with water and once with brine. The crude mixture was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The desired product was purified on silica via flash column chromatography with a gradient of 0% ethyl acetate (EA) in hexanes to 40% EA with a 10% increment. The product was an amorphous viscous light-yellow oil (415.2 mg, 1.35 mmol, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.78 (s, 2H), 4.54 – 4.48 (m, 2H), 4.25 (dd, *J* = 9.8, 5.7 Hz, 1H), 4.22 – 4.13 (m, 3H), 2.65 (dddd, *J* = 7.8, 6.7, 5.5, 4.2 Hz, 1H), 2.06 (s, 3H), 0.15 (s, 9H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 209.5, 171.2, 91.9, 71.7, 71.2, 64.3, 37.4, 21.0, -1.5. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{12}H_{21}O_5NSSi$, 308.0982; Found, 308.0977.

(2,2-Dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl)methyl acetate (5). A flame-dried round bottom flask equipped with a stir bar was charged with sulfamate **4** (548.2 mg, 1.8 mmol, 1 equiv) and diluted with dichloromethane (18 mL, 0.1 M). The $Rh_2(OAc)_4$ catalyst (2.5 mol%, 19.7 mg, 0.045 mmol) was added and the mixture stirred for 5 min. The iodosobenzene oxidant (784.6 mg, 3.6. mmol, 2.0. equiv) was added and the reaction mixture stirred under ambient temperature for 1 h or until TLC indicated complete consumption of the starting material and the formation of the methylene aziridine intermediate. The reaction mixture was then filtered through a pad of silica (2cm in 15 mL fritted funnel), the silica with several portions of dichloromethane and concentrated under reduced pressure. The intermediate was then re-dissolved in 18 mL THF and treated with 2.1 mL TBAF (1.0 M in THF, 1.2 equiv). After 30 min, the crude material was diluted with EA and transferred to a separatory funnel. The organic layer was washed twice with water and once with brine, dried over $MgSO_4$, and filtered through a fritted funnel. The filtrate was concentrated under reduced pressure and purified on silica using 0% to 30% EA in hexanes with a 5% increment to yield a colorless viscous oil (255.1 mg, 1.78 mmol, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.44 (t, *J* = 6.0 Hz, 1H), 4.87 (dd, *J* = 11.3, 4.1 Hz, 1H), 4.63 (dd, *J* = 11.3, 4.0 Hz, 1H), 4.21 (dd, *J* = 11.1, 6.0 Hz, 1H), 4.13 (dd, *J* = 11.1, 8.4 Hz, 1H), 3.95 (ddd, *J* = 17.7, 6.7, 2.1 Hz, 1H), 3.74 (dd, *J* = 17.6, 5.8 Hz, 1H), 3.10 – 3.00 (m, 1H), 2.09 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.5, 98.9, 94.7, 61.8, 36.0, 34.3, 20.7. HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_8H_{11}NO_5SNa$ 256.0250; Found, 256.0248.

Methyl (*E*)-3-((1-((tert-butyldimethylsilyl)oxy)-4-(trimethylsilyl)but-3-yn-2-yl)oxy)acrylate (7). A round bottom flask equipped with a stir bar was charged with the propargyl alcohol (4.840 g, 17.76 mmol, prepared from a previously reported procedure)[1] and diluted with 35.5 mL dichloromethane (0.5 M). Triethylamine (2.7 mL, 1.98 mmol, 1.1 equiv) was added and the mixture cooled to 0 ºC. Methyl propiolate (3.2 mL, 35.5 mmol, 2.0 equiv) was added dropwise via a syringe. The reaction was warmed to ambient temperature and stirred overnight. The next day, the reaction was concentrated under reduced pressure. The crude material was purified on silica using a gradient of 100% hexanes to 10% ethyl acetate in hexanes with 2.5% increment of ethyl acetate to yield a clear colorless oil 4.4 g (12.3 mmol, 69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, *J* = 12.4 Hz, 1H), 5.38 (d, *J* = 12.4 Hz, 1H), 4.59 (dd, *J* = 6.9, 4.4 Hz, 1H), 3.88 – 3.79 (m, 2H), 3.70 (s, 3H), 0.89 (s, 9H), 0.18 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$)

δ 168.2, 161.4, 98.8, 98.4, 94.4, 73.9, 65.8, 51.2, 25.9, 18.4, -0.2, -5.1, -5.3. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{17}H_{33}O_4Si_2$ 357.1912; Found, 357.1911.

(*E*)-3-((1-((tert-butyldimethylsilyl)oxy)-4-(trimethylsilyl)but-3-yn-2-yl)oxy)allyl acetate (8). A flame-dried round bottom flask equipped with a stir bar was charged with the ester **7** (4.400 g, 12.34 mmol) and diluted with 21 mL of diethyl ether (0.6 M). The mixture was cooled to –78 ºC and 27 mL of DiBAL-H (1.0 M in toluene, 27.0 mmol, 2.2. equiv) was added slowly over 10 min. The reaction was stirred at –78 ºC for 1 h, then warmed to 0 ºC and stirred for an additional hour. The mixture was cooled to 0 ºC and slowly quenched with 2 mL of saturated Rochelle salt. The reaction was diluted with more diethyl ether to double the reaction volume. The mixture was warmed up to room temperature for over 30 min. The reaction was dried over $MgSO_4$, filtered over a pad of silica (4 cm in a 60 mL fritted funnel), and concentrated under reduced pressure to yield a yellow oil (72% yield). The product was carried forward without further purification.

A flame-dried round bottom flask equipped with a stir bar was charged with the alcohol **S2** (2.9204 g, 8.89 mmol) and diluted with dichloromethane (18 mL, 0.5 M). Triethylamine (2.5 mL, 1.78 mmol, 2.0. equiv), DMAP (108 mg, 0.89 mmol, 0.1 equiv), and lastly, acetic anhydride (1.3 mL, 13.33 mmol, 1.5 equiv) were added sequentially. The reaction was stirred under ambient temperature overnight. The next day, the reaction was quenched with saturated NH_4Cl solution and diluted with EA. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified on silica with a gradient of 0% to 10% EA in hexanes with a 2.5% increment to yield a light-yellow oil (3.31 g, 8.93 mmol, with a quantitative yield). The yield of **8** over 2 steps was 72%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.58 (d, *J* = 12.5Hz, 1H), 5.16 (dt, *J* = 12.4, 7.8 Hz, 1H), 4.49 (dd, *J* = 7.8, 0.9 Hz, 2H), 4.46 (t, *J* = 5.8 Hz, 1H), 3.80 (d, *J* = 5.7 Hz, 2H), 2.03 (s, 3H), 0.89 (s, 9H), 0.17 (s, 8H), 0.08 (s, 3H), 0.07 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 171.1, 150.4, 100.7, 100.4, 93.2, 72.1, 66.0, 62.6, 26.0, 21.3, 18.5, -0.1, -5.1, -5.2. HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_{18}H_{34}O_4Si_2Na$ 393.1888; Found, 393.1882.

(2*R,4*S**)-6-((*tert*-butyldimethylsilyl)oxy)-2-(hydroxymethyl)-3-(trimethylsilyl)hexa-3,4-dien-1-yl acetate (9a) and (2*S*,4*S*)-6-((*tert*-butyldimethylsilyl)oxy)-2-(hydroxymethyl)-3-(trimethylsilyl)hexa-3,4-dien-1-yl acetate (9b).** A flame-dried round bottom flask was charged with AuCl (85.5 mg, 0.37 mmol, 10 mol %) and diluted with 27 mL dichloromethane. Sodium tetrafluoroborate (80.1 mg, 0.74 mmol, 20 mol %) was added and the reaction mixture stirred for 10 min. Compound **8** in 10 mL dichloromethane was added as a solution and stirred for 45 min. (Note: at the end of the reaction, the reaction mixture turns into a heterogenous dark grey mixture.) $NaBH_4$ (403.5 mg, 10.7 mmol, 1.2 equiv) was added, followed by 35 mL of MeOH. After 1 h, the crude mixture was filtered through a pad of celite and washed with portions of EA. The filtrate was washed with water twice and once with brine. The crude mixture was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified on silica with a gradient of 0% to 50% EA in hexanes to yield diastereomer **9a** 567.3 mg and diastereomer **9b** 423.0 mg (an overall yield of 72%).

*Spectral information of diastereomer **9a**:* ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.11 – 5.05 (dd, *J* = 4.0, 0.7 Hz, 1H), 4.23 – 4.11 (d, *J* = 0.1 Hz, 3H), 4.09 – 4.02 (dd, *J* = 10.9, 8.8 Hz, 1H), 3.79 – 3.70 (ddd, *J* = 11.4, 9.1, 5.0 Hz, 1H), 3.61 – 3.54 (ddd, *J* = 11.4, 5.3, 3.3 Hz, 1H), 3.29 – 3.21 (dd, *J* = 9.3, 5.5 Hz, 1H), 2.47 – 2.36 (ttd, *J* = 7.7, 4.3, 3.8, 1.8 Hz, 1H), 2.10 – 1.99 (s, 3H), 0.97 – 0.86 (s, 8H), 0.14 – 0.11 (s, 8H), 0.11 – 0.08 (s, 5H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 204.8, 171.0,

97.8, 88.0, 64.4, 62.0, 59.6, 40.7, 25.9, 20.9, 18.5, -1.6, -5.5, -5.6. HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_{18}H_{36}O_4Si_2Na$ 395.2044; Found, 395.2043.
Spectral information of diastereomer ***9b****:* ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.11 – 5.06 (td, *J* = 6.2, 1.6 Hz, 1H), 4.30 – 4.22 (dd, *J* = 11.1, 5.0 Hz, 1H), 4.19 – 4.10 (m, 2H), 4.10 – 4.02 (dd, *J* = 11.1, 8.2 Hz, 1H), 3.75 – 3.61 (dhept, *J* = 11.2, 5.3 Hz, 2H), 2.53 – 2.41 (dqd, *J* = 8.3, 5.1, 1.6 Hz, 1H), 2.14 – 2.07 (t, *J* = 6.8 Hz, 1H), 2.07 – 2.04 (s, 3H), 0.95 – 0.86 (s, 9H), 0.18 – 0.11 (s, 9H), 0.09 – 0.06 (s, 6H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 206.8, 172.5, 97.5, 89.6, 66.0, 64.4, 62.6, 42.6, 27.3, 22.30 19.7, 0.1, -3.9, -3.9. HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_{18}H_{36}O_4Si_2Na$ 395.2044; Found, 395.2040.

(2*S,4*S**)-6-((*tert*-butyldimethylsilyl)oxy)-2-((sulfamoyloxy)methyl)-3-(trimethylsilyl)hexa-3,4-dien-1-yl acetate (10a).** A vial equipped with a stir bar was charged with the homoallenic alcohol **9a** (262.1 mg, 0.703 mmol) and diluted with pyridine (1.4 mL, 0.5 M). HFIPS (260.7 mg, 1.06 mmol, 1.5. equiv) was added and the reaction was stirred overnight. The next day, the mixture was concentrated under reduced pressure. Toluene was added to assist in the removal of pyridine. The crude mixture was purified on silica with a gradient of 0% to 40% EA in hexanes with a 10% increment to yield a viscous oil (208.2 mg, 0.46 mmol, 66% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 (s, 2H), 5.05 (ddd, *J* = 8.7, 5.2, 1.4 Hz, 1H), 4.30 – 4.20 (m, 3H), 4.19 – 4.13 (m, 2H), 4.03 (dd, *J* = 11.3, 8.3 Hz, 1H), 2.70 (ddtd, *J* = 8.2, 6.4, 4.8, 1.4 Hz, 1H), 2.07 (s, 3H), 0.91 (s, 9H), 0.13 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 206.3, 170.9, 127.9, 116.4, 94.7, 88.0, 70.0, 63.8, 62.0, 37.8, 25.9, 20.8, 18.4, -1.4, -5.2, -5.3. HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_{18}H_{37}NO_6Si_2Na$ 474.1772; Found, 474.1765.

(2*R,4*S**)-6-((*tert*-butyldimethylsilyl)oxy)-2-((sulfamoyloxy)methyl)-3-(trimethylsilyl)hexa-3,4-dien-1-yl acetate (10b).** A vial equipped with a stir bar was charged with the homoallenic alcohol **9b** (119.0 mg, 0.534 mmol) and diluted with pyridine (1.1 mL, 0.5 M). HFIPS was added (197.9 mg, 0.801 mmol, 1.5. equiv) and the reaction was stirred overnight. The next day, the reaction mixture was concentrated under reduced pressure. Toluene was added to assist in the removal of pyridine. The crude mixture was purified on silica with a gradient of 0% to 40% EA in hexanes with a 10% increment to yield a viscous oil (174.8 mg, 0.39 mmol, 72% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.25 (ddd, *J* = 7.6, 6.1, 1.6 Hz, 1H), 5.06 (s, 2H), 4.53 – 4.17 (m, 6H), 2.91 – 2.79 (m, 1H), 2.21 (s, 3H), 1.04 (s, 9H), 0.29 (s, 10H), 0.22 (s, 6H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 205.8, 170.9, 94.9, 88.7, 70.6, 64.0, 61.5, 53.4, 37.9, 25.9, 20.9, 18.4, -0.0, -1.3, -5.3. HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_{18}H_{37}NO_6Si_2Na$ 474.1772; Found, 474.1765.

[(4*R,7*R**)-4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl]methyl acetate (11a).** A flame-dried round bottom flask was charged with the sulfamate **10a** (161.5 mg, 0.36 mmol) and then diluted with dichloromethane (3.6 mL, 0.1 M). $Rh_2(OAc)_4$ (7.9 mg, 17.88 μmol, 5 mol %) was added, and the mixture was stirred for 5 min. PhIO (157.3 mg, 0.72 mmol, 2.0 equiv) was added and the mixture stirred at room temperature for 1 h. The crude mixture was filtered through a pad of silica (2 cm in a 15 mL fritted funnel) and rinsed with several portions of dichloromethane. The filtrate was concentrated under reduced pressure and the residue was then treated with 3.6 mL of dichloromethane and cooled to 0 ºC. TBAF (1.0 M in THF, 0.39 mL, 0.39 mmol, 1.1 equiv) was added and the reaction was stirred at 0 ºC for 30 min. The crude mixture was diluted with dichloromethane and water, extracted three times with dichloromethane, and dried over $MgSO_4$. After filtration, the crude was concentrated

under reduced pressure, and then purified on silica (0 to 40% EA in hexanes with 10% increment) to yield a white crystalline solid 109.1 mg (0.289 mmol) with 81% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.66 (d, *J* = 7.0 Hz, 1H), 5.02 (dd, *J* = 11.4, 3.7 Hz, 1H), 4.58 (dd, *J* = 11.4, 1.4 Hz, 1H), 4.31 (dtd, *J* = 7.1, 3.5, 1.7 Hz, 1H), 4.25 (dt, *J* = 10.3, 5.2 Hz, 1H), 4.13 (dd, *J* = 11.0, 9.0 Hz, 1H), 3.73 (dd, *J* = 10.4, 3.5 Hz, 1H), 3.68 (dd, *J* = 10.4, 3.8 Hz, 1H), 2.89 (dtt, *J* = 9.4, 4.1, 1.7 Hz, 1H), 2.07 (s, 2H), 0.91 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.3, 99.4, 95.3, 62.1, 62.0, 50.5, 34.3, 25.7, 20.7, 18.3, -5.4, -5.4. HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_{18}H_{37}NO_6Si_2Na$ 474.1772; Found, 474.1765.

[(4*S,7*R**)-4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl]methyl acetate (11b).** A vial equipped with a stir bar was charged with the sulfamate **10b** (174.8 mg, 0.39 mmol) and diluted with dichloromethane (3.9 mL, 0.1 M). $Rh_2(OAc)_4$ (8.6 mg, 19.4 μmol, 5 mol%) was added, and the reaction was stirred for 5 min. PhIO (170.3 mg, 0.77 mmol, 2.0 equiv) was added and the reaction stirred at rt for 1 h. The crude mixture was then filtered through a pad of silica (2 cm in a 15 mL fritted funnel) and washed with portions of dichloromethane. The mixture was concentrated under reduced pressure to yield the methylene aziridine intermediate. The crude material was then transferred to a vial with a stir bar and re-dissolved in 3.9 mL of dichloromethane. The reaction was cooled to 0 ºC, and TBAF (0.39 mL, 0.39 mmol, 1.0. equiv) was added. The reaction was stirred for 30 min, diluted with water and EA, washed with water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified on silica with a gradient of 0% to 25% EA in hexanes (with a 5% increment) to yield a white solid (60.2 mg, 0.16 mmol, 41% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.69 – 5.57 (d, *J* = 7.0 Hz, 1H), 5.12 – 4.97 (dd, *J* = 11.3, 3.8 Hz, 1H), 4.82 – 4.65 (dd, *J* = 11.3, 1.4 Hz, 1H), 4.36 – 4.29 (dtd, *J* = 7.1, 3.6, 1.9 Hz, 1H), 3.81 – 3.76 (dd, *J* = 7.3, 5.4 Hz, 2H), 3.74 – 3.71 (dd, *J* = 10.4, 3.5 Hz, 1H), 3.70 – 3.65 (dd, *J* = 10.3, 3.9 Hz, 1H), 2.82 – 2.69 (dddt, *J* = 7.3, 5.5, 3.8, 1.8 Hz, 1H), 1.67 – 1.56 (t, *J* = 5.5 Hz, 1H), 0.95 – 0.87 (s, 9H), 0.11 – 0.10 (s, 3H), 0.10 – 0.09 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 100.1, 94.8, 62.0, 61.4, 50.4, 37.2, 25.7, 18.2, -5.5, -5.5. HRMS (ESI) m/z: $[M + NH_4]^+$ Calcd for $C_{15}H_{31}N_2O_6SSi$ 395.1667; Found, 395.1661.

[(4*S*,7*R*)-4-(hydroxymethyl)-2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl]methyl acetate (12b). An oven-dried vial equipped with a stir bar was charged with the SNO-OCT **11b** (20.3 mg, 53.8 μmol) and diluted with THF (0.5 mL, 0.1 M). TBAF (1.0 M in THF, 81 μL, 80.7 μmol, 1.5 equiv) was added and the reaction was stirred for 30 min. The reaction was then diluted with dichloromethane and water, extracted three times with dichloromethane, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified on silica (0% to 100% EA in hexanes) to yield a crystalline solid (10.6 mg, 40.3 μmol, 75%). *Recrystallization condition:* the purified product was diluted with 0.6 mL of $CDCl_3$, sonicated for a few minutes, and transferred to an NMR tube. After two days, colorless crystals grew inside the NMR tube that was left at ambient temperature. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.00 (dd, *J* = 11.4, 3.9 Hz, 1H), 4.61 (dd, *J* = 11.4, 2.4 Hz, 1H), 4.34 (ddd, *J* = 4.4, 3.6, 1.9 Hz, 1H), 4.31 (dd, *J* = 11.0, 6.3 Hz, 1H), 4.17 (dd, *J* = 11.0, 8.5 Hz, 1H), 3.79 (dd, *J* = 11.6, 4.5 Hz, 1H), 3.71 (dd, *J* = 11.6, 3.6 Hz, 1H), 2.97 (dddt, *J* = 8.4, 6.2, 4.1, 2.1 Hz, 1H), 2.10 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.9, 100.5, 94.7, 77.4, 62.3, 61.8, 50.8, 34.5, 20.9. HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_9H_{13}NO_6SNa$ 286.0356; Found, 286.0353.

(3-Methyl-2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl)methyl acetate (5-Me). A flame-dried 5 mL round bottom flask equipped with a stir bar was charged with SNO-OCT **5** (23.3 mg, 0.1 mmol) and diluted with diethyl ether (1.0 mL, 0.1 M). NaH (60% in mineral oil, 6.0 mg, 0.15 mmol, 1.5 equiv) was added and the reaction was stirred at 0 ºC for 20 min. Neat MeOTf (13.6 µL, 0.12 mmol, 1.2 equiv) was added via an Eppendorf pipette and the mixture stirred at 0 ºC for 30 min, then quenched with water and diluted with EA. The reaction mixture was transferred to a separatory funnel and washed the organic layer with water. The organics were dried over $MgSO_4$, filtered through a fritted funnel, and concentrated under reduced pressure. The crude mixture was purified on silica with a gradient of 0% to 30% EA in hexanes with a 10% increment to yield a white solid (19.6 mg, 0.079 mmol, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.86 (dd, *J* = 11.0, 3.7 Hz, 1H), 4.63 (dd, *J* = 11.2, 3.8 Hz, 1H), 4.24 (dd, *J* = 11.1, 5.9 Hz, 1H), 4.12 (dd, *J* = 11.0, 8.3 Hz, 1H), 3.06 (s, 3H), 3.01 (s, 1H), 2.09 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.4, 96.2, 94.7, 76.5, 61.8, 44.7, 37.3, 34.5, 20.7. HRMS (ESI) m/z: [M + H]$^+$ Calcd for $C_9H_{14}NO_5S$ 248.0587; Found, 248.0582.

(3-Methyl-2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl)methyl prop-2-enoate (5-acrylate). A 4 mL vial equipped with a stir bar was charged with SNO-OCT **5** (12.8 mg, 0.052 mmol) and diluted with 0.9 mL of MeOH and 0.1 mL of water. The mixture was cooled to 0 ºC and K_2CO_3 (13.0 mg, 0.094 mmol, 2.0. equiv) was added. After stirring at 0 ºC for 1 h, the mixture was diluted with EA, washed with water followed by brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The alcohol intermediate was then transferred to another 4 mL vial equipped with a stir bar and diluted with CH_2Cl_2 (1.0 mL, 0.05 M). After cooling to 0 ºC, diisopropyl ethylamine (90.2 µL, 0.52 mmol, 10.0 equiv) and acryloyl chloride (6.3 µL 0.078 mmol, 1.5 equiv) were added. After 30 min, the reaction was quenched with water, diluted with EA, washed with water, and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude mixture was purified on silica with a gradient 0% to 40% EA in hexanes with a 10% increment to yield a white solid (6.0 mg, 23 µmol, 45%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.48 (dd, *J* = 17.3, 1.3 Hz, 1H), 6.16 (dd, *J* = 17.3, 10.5 Hz, 1H), 5.93 (dd, *J* = 10.4, 1.3 Hz, 1H), 4.91 (dd, *J* = 11.4, 3.7 Hz, 1H), 4.68 (dd, *J* = 11.2, 3.7 Hz, 1H), 4.35 (dd, *J* = 11.1, 5.8 Hz, 1H), 4.25 (dd, *J* = 11.1, 8.5 Hz, 1H), 3.08 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 165.5, 132.0, 127.6, 96.1, 94.8, 76.5, 61.8, 44.7, 37.3, 34.6. HRMS (ESI) m/z: [M + NH_4]$^+$ Calcd for $C_{10}H_{17}N_2O_5S$ 277.0853; Found, 277.0848.

(3-Methyl-2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl)methyl 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoate (5-maleimide). A 4 mL vial equipped with a stir bar was charged with SNO-OCT **5** (12.8 mg, 0.052 mmol) and diluted with 0.9 mL of MeOH and 0.1 mL of water. The mixture was cooled to 0 ºC and K_2CO_3 (14.3 mg, 0.104 mmol, 2.0. equiv) was added. After stirring at 0 ºC for 1 h, the mixture was diluted with EA, washed with water followed by brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The alcohol intermediate was then transferred to another 4 mL vial equipped with a stir bar and diluted with CH_2Cl_2 (1.0 mL, 0.05 M). After cooling to 0 ºC, diisopropyl ethylamine (90.2 µL, 0.52 mmol, 10.0 equiv) and maleimide-Cl (15.7 mg, 0.078 mmol, 1.5 equiv) were added. After 30 min, the reaction was quenched with water, diluted with EA, washed with water, and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude mixture was purified on silica with a gradient 0% to 50% EA in hexanes with a 10% increment to yield a white solid (6.9 mg, 19 µmol, 36%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (s, 2H), 4.88 (dd, *J* = 11.3, 4.0 Hz, 1H),

4.64 (dd, J = 11.3, 3.7 Hz, 1H), 4.26 (dd, J = 11.0, 5.8 Hz, 1H), 4.14 (dd, J = 11.1, 8.5 Hz, 2H), 3.72 – 3.52 (m, 3H), 3.07 – 2.97 (m, 4H), 2.35 (t, J = 7.5 Hz, 2H), 1.93 (p, J = 7.0 Hz, 2H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 172.1, 170.9, 134.3, 96.3, 94.9, 76.7, 62.0, 44.8, 37.4, 37.0, 34.6, 31.2, 23.8. HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_{15}H_{18}N_2O_7SNa$ 388.1173; Found, 388.1172.

(3-Methyl-2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ⁶,3-oxathiazocin-7-yl)methyl 3-[(2-methoxy-2-oxoethyl)sulfanyl]propanoate (5a). In a vial, a solution of SNO-OCT 5-acrylate (6.0 mg, 23 μmol, 1 equiv), and methyl thioglycolate (2.5 mg, 2.1 μL, 23 μmol, 1 equiv) were prepared in MeOH (0.05 M, 0.46 mL each). Diisopropyl ethylamine (DIPEA, 4.5 mg, 6.0 μL, 35 μmol, 1.5 equiv) was added to the SNO-OCT solution and cooled to 0ºC. The two solutions were combined and vortexed. After 2.5 h in an ice bath, the solution was concentrated under reduced pressure and purified on silica with a gradient of 0% to 40% EA in hexanes using a 10% increment to yield the product as an amorphous colorless solid (4.2 mg, 11 μmol, 50%). ^1H NMR (500 MHz, $CDCl_3$) δ 4.87 (dd, J = 11.0, 3.0 Hz, 1H), 4.64 (dd, J = 11.3, 3.8 Hz, 1H), 4.28 (dd, J = 11.1, 5.8 Hz, 1H), 4.17 (dd, J = 11.0, 8.5 Hz, 1H), 4.11 (broad singlet, 1H), 3.75 (s, 3H), 3.61 (broad singlet, 1H), 3.26 (s, 2H), 3.09 – 2.98 (m, 3H), 2.92 (t, J = 7.1 Hz, 2H), 2.69 (t, J = 7.1 Hz, 2H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 171.2, 170.8, 96.2, 94.9, 76.6, 62.1, 52.7, 44.8, 37.1, 34.6, 34.2, 33.6, 27.6. HRMS (ESI) m/z: $[M + NH_4]^+$ Calcd for $C_{13}H_{23}N_2O_7S_2$ 383.0941; Found, 383.0940.

(3-Methyl-2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ⁶,3-oxathiazocin-7-yl)methyl 4-{3-[(2-methoxy-2-oxoethyl)sulfanyl]-2,5-dioxopyrrolidin-1-yl}butanoate (5b). In a vial, a solution of SNO-OCT 5-acrylate (6.9 mg, 19 μmol, 1 equiv), and methyl thioglycolate (2.0 mg, 1.7 μL, 19 μmol, 1 equiv) were prepared in MeOH (0.05 M, 0.37 mL each). Diisopropyl ethylamine (DIPEA, 3.6 mg, 4.9 μL, 28 μmol, 1.5 equiv) was added to the SNO-OCT solution and then cooled to 0 ºC. The two solutions were combined and vortexed. After 1 hour in an ice bath, the solution was concentrated under reduced pressure and purified on silica with a gradient of 0% to 50% EA in hexanes using a 10% increment to yield the product as an amorphous colorless solid (6.3 mg, 13 μmol, 71%). ^1H NMR (500 MHz, $CDCl_3$) δ 4.87 (dd, J = 11.5, 3.8 Hz, 1H), 4.64 (dd, J = 11.3, 3.7 Hz, 1H), 4.26 (ddd, J = 11.0, 5.8, 2.2 Hz, 1H), 4.18 – 4.07 (m, 2H), 4.03 (dd, J = 9.2, 3.9 Hz, 1H), 3.92 (dd, J = 15.7, 1.0 Hz, 1H), 3.77 (s, 3H), 3.65 – 3.52 (m, 3H), 3.39 (d, J = 15.8 Hz, 1H), 3.14 (ddd, J = 18.8, 9.2, 1.9 Hz, 1H), 3.07 – 2.98 (m, 4H), 2.52 (ddd, J = 18.8, 3.9, 1.8 Hz, 1H), 2.37 (t, J = 7.2 Hz, 2H), 1.93 (p, J = 7.0 Hz, 2H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 176.5, 176.5, 174.5, 174.5, 172.1, 170.2, 96.3, 94.9, 76.7, 62.0, 52.9, 44.8, 38.4, 38.4, 38.2, 37.4, 35.4, 34.6, 33.0, 33.0, 31.2, 31.2, 22.8, 22.7. HRMS (ESI) m/z: $[M + NH_4]^+$ Calcd for $C_{18}H_{28}N_3O_9S_2$ 494.1262; Found, 494.1260.

2,5-Dioxopyrrolidin-1-yl (2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ⁶,3-oxathiazocin-7-yl)methyl carbonate (5-NHS). A vial equipped with a stir bar was charged with SNO-OCT **5** (25.0 mg, 0.11 mmol) and diluted with MeOH (2.5 mL) and water (0.5 mL). After cooling to 0 ºC, K_2CO_3 was added. After 1 h, the crude mixture was diluted with EA, washed with 1 M HCl, dried over $MgSO_4$, filtered through a fritted funnel, and concentrated under reduced pressure. The mixture was treated with 0.2 mL MeCN. Triethylamine (45 μL, 3.0 equiv, 0.33 mmol) and disuccinimidyl carbonate (DSC, 22 mg, 1.5 equiv, 0.16 mmol) were added. After stirring at room temperature for 30 min, the mixture was concentrated under reduced pressure. The crude was purified on silica with a gradient 0 to 20% MeCN in CH_2Cl_2 with 5% increment to result a white

solid 6.9 mg in 20% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.59 (t, *J* = 6.3 Hz, 1H), 4.84 (dd, *J* = 11.6, 4.3 Hz, 1H), 4.60 (dd, *J* = 11.6, 4.0 Hz, 1H), 4.45 (dd, *J* = 10.8, 6.6 Hz, 1H), 4.37 (dd, *J* = 10.8, 5.8 Hz, 1H), 3.84 (ddd, *J* = 17.9, 6.7, 2.2 Hz, 1H), 3.76 – 3.63 (m, 1H), 3.23 (s, 1H), 2.78 (s, 4H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.7, 152.3, 98.1, 97.4, 77.6, 69.4, 36.1, 34.9, 26.3. HRMS (ESI) m/z: $[M + NH_4]^+$ Calcd for $C_{11}H_{16}N_3O_8S$ 350.0653; Found, 350.0651.

(2*R*,3*R*,4*R*,5*S*,6*R*)-6-[(acetyloxy)methyl]-3-({[(2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl)methoxy]carbonyl}amino)oxane-2,4,5-triyl triacetate (6b-OAc$_4$-glucosamine). A vial equipped with a stir bar was charged with **5-NHS** conjugate (8.3 mg, 0.025 mmol) and amino sugar (12 mg, 0.03 mmol) and diluted with 0.25 mL of CH_2Cl_2. Triethylamine (10 µL, 0.075 mmol) was added, and then the reaction was stirred at room temperature overnight. The next day, the crude reaction mixture was concentrated under reduced pressure and purified on silica with a gradient of 0 to 75% EA in hexanes with 5% increment, resulting in a white solid 5.5 mg in 39% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.22 (t, *J* = 3.4 Hz, 1H), 5.60 (dt, *J* = 26.3, 6.4 Hz, 1H), 5.27 – 5.07 (m, 2H), 4.95 (d, *J* = 9.0 Hz, 1H), 4.85 (td, *J* = 12.1, 4.2 Hz, 1H), 4.58 (td, *J* = 11.8, 3.8 Hz, 1H), 4.39 – 3.84 (m, 7H), 3.72 (td, *J* = 17.0, 5.8 Hz, 1H), 3.02 (s, 1H), 2.20 (s, 3H), 2.13 – 2.03 (m, 9H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 171.6, 170.8, 169.3, 169.2, 155.1, 98.7, 95.2, 90.8, 77.0, 70.7, 69.9, 67.7, 62.9, 61.7, 53.3, 36.1, 34.8, 21.1, 20.9, 20.8, 20.7. HRMS (ESI) m/z: $[M + NH_4]^+$ Calcd for $C_{21}H_{31}N_3O_{14}S$ 582.1599; Found, 582.1598.

Methyl (9*S*,12*S*,15*R*)-9-amino-12-benzyl-15-[(*tert*-butyldisulfanyl)methyl]-1-(2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl)-3,10,13-trioxo-2-oxa-4,11,14-triazahexadecan-16-oate (6c-K-F-C-StBu-OMe). A vial equipped with a stir bar was charged with **5-NHS** (6.6 mg, 0.02 mmol), peptide (12 mg, 0.025 mmol), triethylamine (8.4 µL, 0.06 mmol), and 0.2 mL of CH_2Cl_2. After stirring at room temperature overnight, the crude mixture was concentrated under reduced pressure and purified on Prep-HPLC (see Supporting Information for more details) to produce 2.2 mg of the desired SNO-OCT peptide conjugate in 15% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 8.53 (s, 2H), 7.28 (d, *J* = 4.4 Hz, 5H), 7.25 – 7.15 (m, 1H), 4.80 (dd, *J* = 11.2, 4.0 Hz, 1H), 4.76 – 4.70 (m, 3H), 4.60 (dd, *J* = 11.3, 4.3 Hz, 1H), 4.17 – 4.01 (m, 2H), 3.80 (d, *J* = 17.7 Hz, 1H), 3.71 (s, 4H), 3.67 (d, *J* = 17.8 Hz, 1H), 3.57 (d, *J* = 6.4 Hz, 1H), 3.24 – 3.13 (m, 3H), 3.10 (t, *J* = 6.9 Hz, 3H), 2.99 (dd, *J* = 13.7, 8.4 Hz, 1H), 2.92 (dd, *J* = 14.0, 8.9 Hz, 1H), 1.70 (dp, *J* = 21.7, 6.8 Hz, 2H), 1.49 (p, *J* = 7.1 Hz, 2H), 1.38 – 1.31 (m, 11H). ^{13}C{^{1}H} NMR (126 MHz, CD_3OD) δ 173.3, 172.2, 158.3, 138.2, 130.4, 129.5, 127.9, 101.2, 99.6, 96.8, 77.7, 76.6, 63.4, 55.7, 54.9, 53.2, 53.1, 42.4, 41.3, 39.0, 36.0, 35.9, 33.8, 30.5, 30.2, 23.1. HRMS (ESI) m/z: $[M + NH_4]^+$ Calcd for $C_{30}H_{49}N_6O_9S_3$ 716.2452; Found, 716.2460.

(2,2-Dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl)methyl (2-{[(11-oxo-2,3,6,7-tetrahydro-1*H*,5*H*,11*H*-pyrano[3,2-*g*]pyrido[3,2,1-*ij*]quinolin-10-yl)carbonyl]-amino}ethyl)carbamate (6a-coumarin 343). A vial equipped with a stir bar was charged with Boc-coumarin (4.8 mg, 0.011 mmol, prepared based on a previously reported procedure)[6] and diluted with CH_2Cl_2 (0.11 mL). Trifluoroacetic acid (29 µL, 0.38 mmol) was added and the rection mixture stirred at room temperature for 1.5 h. The crude mixture was concentrated under reduced pressure and diluted with 0.11 mL of CH_2Cl_2. Diisopropyl ethylamine (20 µL, 0.11 mmol) and 5-NHS (2.5 mg, 0.0075 mmol) were added. After stirring at room temperature for 4 hours, the crude was concentrated under reduced pressure. The crude was purified on Prep-HPLC (see Supporting

Information for more details), resulting in a bright green solid (10.8 mg) in 25% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.16 (t, *J* = 6.2 Hz, 1H), 8.59 (s, 1H), 7.03 (s, 1H), 6.45 (broad singlet, 1H), 5.69 (d, *J* = 4.7 Hz, 1H), 4.94 (dd, *J* = 11.3, 4.1 Hz, 1H), 4.63 (dd, *J* = 11.3, 2.9 Hz, 1H), 4.28 – 4.14 (m, 2H), 4.09 – 3.96 (m, 1H), 3.71 (d, *J* = 17.9 Hz, 1H), 3.67 – 3.53 (m, 2H), 3.49 – 3.36 (m, 2H), 3.34 (dd, *J* = 7.5, 3.8 Hz, 4H), 2.95 (s, 1H), 2.89 (t, *J* = 6.5 Hz, 2H), 2.78 (t, *J* = 6.2 Hz, 2H), 1.98 (h, *J* = 6.1 Hz, 4H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 165.5, 163.3, 156.1, 153.0, 148.7, 148.7, 127.5, 120.0, 108.4, 108.2, 105.8, 98.8, 96.0, 78.0, 62.8, 50.5, 50.0, 42.6, 39.8, 36.1, 35.1, 27.6, 21.3, 20.3, 20.3, 20.2. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{25}H_{29}N_4O_8S$ 545.1701; Found, 545.1698.

[(4*R,7*R**)-4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-3-methyl-2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl]methyl acetate (13).** A 5 mL flame-dried round bottom flask was charged with **11a** (37.8 mg, 0.1 mmol) and diluted with diethyl ether (2.0 mL, 0.05 M). After cooling to 0 ºC, NaH (60% in mineral oil, 4.4 mg, 0.11 mmol, 1.1 equiv) was added and the mixture was stirred for 15 min. MeOTf (13.2 µL, 0.12 mmol, 1.2 equiv) was added via an Eppendorf pipette. After stirring at 0 ºC for 30 min, the reaction was quenched with water and diluted with EA. The reaction mixture was transferred to a separatory funnel and washed the organic layer with water. The organics were dried over $MgSO_4$, filtered through a fritted funnel, and concentrated under reduced pressure. The crude mixture was purified on silica with a gradient of 0% to 30% EA in hexanes with a 10% increment to yield a white solid (35.1 mg, 0.090 mmol, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.94 (dd, *J* = 11.4, 3.9 Hz, 1H), 4.58 (dd, *J* = 11.4, 2.2 Hz, 1H), 4.25 (dd, *J* = 11.0, 6.0 Hz, 1H), 4.12 (dd, *J* = 11.0, 8.7 Hz, 1H), 4.00 (dd, *J* = 10.1, 7.5 Hz, 1H), 3.90 (t, *J* = 6.9 Hz, 1H), 3.81 (dd, *J* = 10.1, 6.1 Hz, 1H), 3.10 (s, 3H), 2.89 (ddt, *J* = 8.9, 6.1, 2.5 Hz, 1H), 2.09 (s, 3H), 0.89 (s, 9H), 0.09 (s, 6H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.6, 95.3, 95.0, 77.4, 76.5, 64.0, 62.5, 59.6, 34.4, 26.0, 20.9, 18.4, -5.1, -5.2. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{16}H_{30}NO_6SSi$ 392.1558; Found, 392.1560.

[(4*R,7*R**)-4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-3-methyl-2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl]methyl prop-2-enoate (14).** A vial equipped with a stir bar was charged with SNO-OCT **13** (9.8 mg, 25 µmol) and diluted with 450 µL methanol and then 50 µL water. The mixture was cooled to 0 ºC and K_2CO_3 (6.9 mg, 50 µmol, 2.0 equiv) was added and the reaction was stirred for 1 h. The reaction was diluted with EA and water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to produce a white crystalline solid. The alcohol intermediate was redissolved in 1.0 mL of dichloromethane (0.025 M). At 0 ºC, diisopropyl ethylamine (43.5 µL, 250 µmol, 10 equiv), acryloyl chloride (3.0 µL, 37.5 µmol, 1.5 equiv) were added. After 30 min, the reaction was quenched with water, diluted with EA, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified on silica (gradient 0% to 30% EA in hexanes with 10% increment) to yield a white solid (7.1 mg, 18 µmol, 70% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.45 (dd, *J* = 17.3, 1.3 Hz, 1H), 6.13 (dd, *J* = 17.3, 10.5 Hz, 1H), 5.89 (dd, *J* = 10.4, 1.3 Hz, 1H), 4.96 (dd, *J* = 11.4, 3.9 Hz, 1H), 4.61 (dd, *J* = 11.4, 2.1 Hz, 1H), 4.34 (dd, *J* = 11.0, 5.8 Hz, 1H), 4.22 (dd, *J* = 11.1, 8.8 Hz, 1H), 4.01 (dd, *J* = 10.1, 7.5 Hz, 1H), 3.90 (t, *J* = 6.8 Hz, 1H), 3.81 (dd, *J* = 10.1, 6.1 Hz, 1H), 3.11 (s, 3H), 2.94 (p, *J* = 4.3 Hz, 2H), 0.89 (s, 9H), 0.09 (s, 6H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 165.5, 131.9, 127.6, 95.3, 94.8, 76.4, 63.9, 62.4, 59.5, 39.0, 34.3, 25.8, 18.2, -5.3, -5.3. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{17}H_{30}NO_6SSi$ 404.1558; Found, 404.1551.

{(4*R,7*R**)-4-[({[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}oxy)methyl]-3-methyl-2,2-dioxo-5,6-didehydro-3,4,7,8-tetrahydro-2*H*-1,2λ^6,3-oxathiazocin-7-yl}methyl prop-2-enoate (15).** A vial equipped with a stir bar was charged with **14** (5.0 mg, 0.012 mmol) and diluted with THF (0.6 mL, 0.02 M). TBAF (1.0 M in THF, 19 µL, 1.5 equiv) was added and the mixture was stirred for 1 h. The crude reaction mixture was diluted with EA and washed with brine. The organics were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The intermediate was then diluted with MeCN (0.6 mL, 0.02M). Diisopropyl ethylamine (6.5 µL, 37µmol, 3.0 equiv), and DSC (4.9 mg, 15 µmol, 1.2 equiv) were added. After one hour, the reaction was concentrated under reduced pressure. The crude was purified on silica 0% to 100% EA in hexanes with 10% increment to result product 0.9 mg in 20% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.46 (dd, *J* = 17.3, 1.3 Hz, 1H), 6.13 (dd, *J* = 17.3, 10.5 Hz, 1H), 5.91 (dd, *J* = 10.5, 1.3 Hz, 1H), 5.04 (dd, *J* = 11.5, 3.8 Hz, 1H), 4.76 (dd, *J* = 11.0, 8.1 Hz, 1H), 4.62 (dd, *J* = 11.4, 1.2 Hz, 1H), 4.51 (dd, *J* = 11.0, 6.1 Hz, 1H), 4.37 (dd, *J* = 11.0, 6.1 Hz, 1H), 4.25 (dd, *J* = 11.0, 8.7 Hz, 1H), 4.08 (t, *J* = 7.1 Hz, 1H), 3.15 (s, 3H), 2.95 (dt, *J* = 9.4, 5.1 Hz, 1H), 2.85 (s, 4H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 168.5, 165.6, 151.3, 132.3, 127.6, 97.4, 92.3, 76.6, 69.6, 62.4, 55.6, 39.6, 34.4, 25.6. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{16}H_{19}N_2O_{10}S$ 469.0314; Found, 469.0309.

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Data availability statement

The data underlying this study are available in the published article and its Supporting Information section.

Supporting information

The supporting information is available free of charge.

- Additional experimental procedures, compound isolation conditions, NMR kinetic procedures, NMR kinetics data, NMR spectra, LC separation gradients, characterization of cycloaddition products and X-ray crystallographic data for **12b**.

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