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# Synthesis of a 3-Amino-2,3-dihydropyrid-4-one and Related Heterocyclic Analogues as Mechanism-Based Inhibitors of BioA, a Pyridoxal Phosphate-Dependent Enzyme

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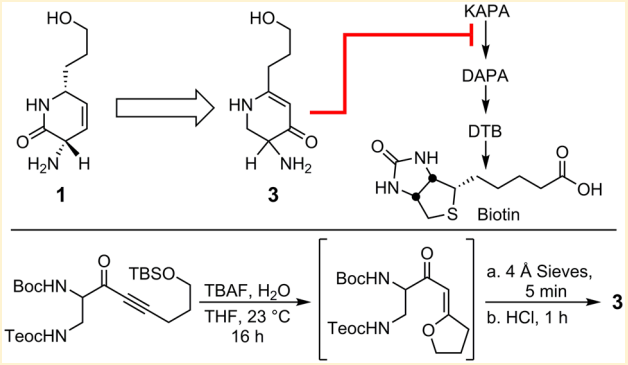
Supporting Information

**ABSTRACT:** Amiclenomycin (ACM) is a chemically unstable antibiotic with selective activity against *Mycobacterium tuberculosis* (Mtb) due to mechanism-based inhibition of BioA, a pyridoxal 5′-phosphate (PLP)-dependent aminotransferase. The first-generation ACM analogue dihydro-2-pyridone **1** maintains a similar bioactivation mechanism concluding with covalent labeling of the PLP cofactor. To improve on **1**, we report the synthesis of dihydro-4-pyranone **2**, dihydro-4-pyridone **3**, and dihydro-4-thiopyranone **13**, which were rationally designed to boost the rate of enzyme inactivation by lowering the p$K_a$ of their $\alpha$-protons. We employed a unified synthetic strategy for construction of the desired heterocycles featuring $\alpha$-amino ynone generation followed by 6-endo-dig cyclization. However, competitive 5-exo-dig cyclization, $\beta$-elimination of the ynone, and dimerization of the resultant $\alpha$-amino carbonyls all complicated the syntheses of the dihydro-4-pyranone and dihydro-4-pyridone scaffolds. These obstacles were overcome by Teoc protection of the $\beta$-amino group in the assembly of **3** and Boc-MOM protection of the $\alpha$-amino group in the synthesis of **2**, enabling the efficient construction of **2** and **3** in seven steps from commercially available starting materials. Dihydro-4-pyridone **3** possessed improved enzyme inhibition as measured by its $k_{inact}$ value against BioA.




## INTRODUCTION

Biotin (vitamin H) is an essential cofactor in all organisms and is responsible for the activation of carbon dioxide in fatty acid biosynthesis and gluconeogenesis through attachment to acyl-CoA carboxylases (ACCs) and pyruvate coenzyme A carboxylase (PCC).[1,2] *Mycobacterium tuberculosis* (Mtb), the main etiological agent of tuberculosis, is particularly sensitive to biotin deprivation. Though ACCs are required in numerous organisms, Mtb uniquely relies on three nonredundant ACCs to synthesize the unparagoned lipids found in the extraordinarily complex mycobacterial cell wall.[3] Additionally, gluconeogenesis is a vital process in Mtb to synthesize sugars needed for nucleotide and cell wall biosynthesis.[4]

Amiclenomycin (ACM, Figure 1A) is an antibiotic that was isolated in 1974 from a *Streptomyces lavendulae* strain.[5] It possesses selective antimicrobial activity against Mtb and the fast-growing *Mycobacterium smegmatis* (Msmeg).[6] ACM is an unnatural $\alpha$-amino acid containing a highly unusual amino-1,4-cyclohexadiene moiety in its side chain and has been shown to exert its activity through inhibition of mycobacterial biotin biosynthesis. The aforementioned dependence of Mtb on several biotin-containing enzymes may explain the distinct vulnerability of mycobacteria to ACM.[7] Given the paucity of new antibiotics combined with the growing threat of antimicrobial resistance in Mtb and other pathogens, efforts to reexamine old antibiotics identified during the golden-age of antibiotic discovery represents a promising and efficient strategy for antibiotic development.[8,9]

The mechanism of action of ACM was elucidated by classic complementation studies employing biotin pathway intermediates.[6b] In Mtb and Msmeg, biotin biosynthesis begins by hijacking of the fatty acid biosynthesis pathway to generate pimeloyl-ACP,[10] which is then elaborated to biotin over four enzymatic steps (Figure 1B).[11] The first step is carried out by BioF, yielding 7-keto-8-aminopelargonic acid (KAPA) from the decarboxylative condensation of pimeloyl-ACP and L-alanine. BioA then effects the reductive amination of KAPA into 7,8-diaminopelargonic acid (DAPA), which is followed by the BioD-catalyzed carboxylation of DAPA to form dethiobiotin (DTB). The pathway concludes with insertion of the sulfur atom by the iron−sulfur cluster enzyme BioB, affording biotin. The addition of exogenous DAPA, DTB, or biotin to whole-cell Msmeg antagonized the activity of ACM, whereas KAPA did not.[6a] Moreover, treatment of Msmeg with ACM led to an accumulation of KAPA.[6a] Taken together, these data

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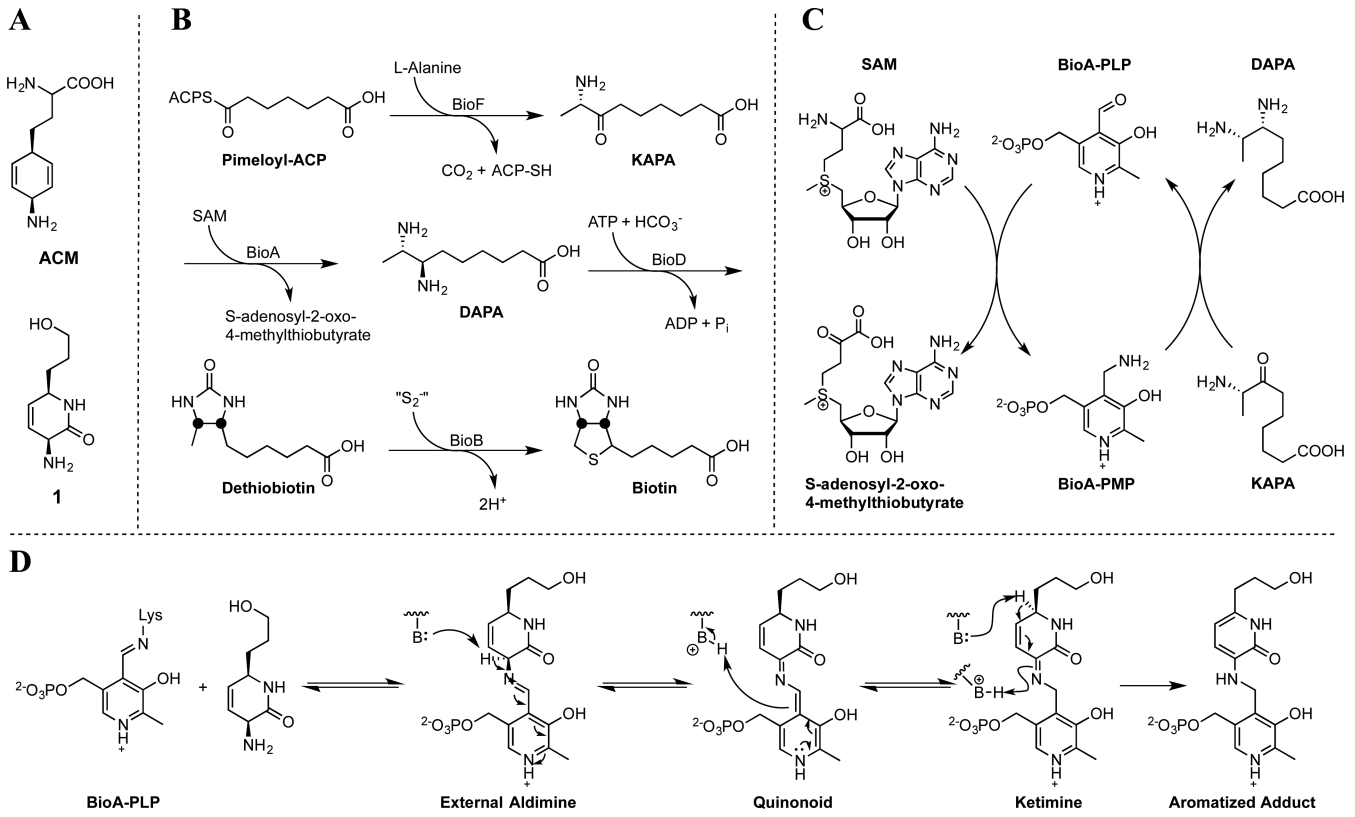


**Figure 1.** (A) Natural product ACM was the inspiration for **1**. (B) Biotin biosynthesis proceeds in four steps from pimeloyl-ACP. (C) BioA catalyzes the second step of biotin biosynthesis using SAM as the amino donor. (D) Inactivation of BioA by **1** occurs via a four-step process resulting in a stable, aromatized adduct bound to the PLP cofactor. The final tautomerizaion of the ketimine is likely mediated by the general base (Lys283) as shown.

pinpointed the antepenultimate step performed by BioA as the likely target of ACM.

BioA is a pyridoxal-5′-phosphate (PLP)-dependent aminotransferase that uses *S*-adenosylmethionine (SAM) as an amino donor when catalyzing the conversion of KAPA to DAPA (Figure 1C).[11b,d] Through a combination of elegant kinetic and structural studies, the amino-1,4-cyclohexadienyl group on ACM was shown to bind the PLP of BioA.[12] The resulting external aldimine then undergoes redox isomerization via a quinonoid intermediate to a ketimine (Figure 1D).[12b] However, rather than completing hydrolysis to generate the pyridoxamine-5′-phosphate (PMP) form of the cofactor, the ketimine intermediate tautomerizes to a stable aromatic adduct that covalently inactivates BioA.[12b]

The discovery of the mechanism of inactivation explains both the reason for and the function of the rare 1,4-cyclohexadiene moiety in ACM. However, while the 1,4-cyclohexadiene is absolutely critical for ACM's activity, it is simultaneously the primary liability of ACM, as this moiety is chemically unstable and spontaneously aromatizes to an inactive benzene nucleus. We have described the design of a simplified analogue **1** (Figure 1A) wherein the 1,3-cyclohexadiene warhead of ACM was replaced with a more stable 3,6-dihydropyrid-2-one moiety,[13] whose increased stability is derived from reduced aromatic stabilization energy of the corresponding 2-pyridone. Compound **1** was shown to inhibit BioA in a manner similar to that of ACM (Figure 1D), but further optimization of this scaffold has been hampered by the complex nature of the inactivation mechanism.

Both **1** and ACM are mechanism-based inhibitors (MBIs) because they require transformation by the inhibited enzyme's machinery to form active inhibitory species. Unlike conventional rapidly reversible inhibitors, MBIs display time-dependent inhibition and cannot be characterized by $IC_{50}$ or $K_I$ values.[14] Rather, the rate constant of inactivation ($k_{obs}$) is typically measured as a function of inhibitor concentration to furnish the global kinetic parameters $k_{inact}$ and $K_I$. $k_{inact}$ and $K_I$ are semianalogous to Michaelis–Menten parameters and describe, respectively, the maximum possible value of $k_{obs}$ at infinite inhibitor concentration and the concentration of inhibitor that produces a $k_{obs}$ that is equal to one-half of $k_{inact}$.[14] Unfortunately, both of these parameters are complex conglomerates of the individual microscopic rate constants of inactivation, and as such, neither of the parameters definitively assess binding affinity nor the identity of the rate-limiting step.[15] To rationally optimize **1**, we undertook the first detailed kinetic characterization of an MBI, using presteady-state stopped-flow kinetic experiments to determine both the mechanism and the rate of each individual step.[15] This investigation found the kinetic bottleneck is the removal of the $\alpha$-proton of the initial external aldimine to form the quinonoid intermediate. We hypothesized a simple way to facilitate this specific step would be to lower the $pK_a$ of this key proton. A dedicated synthetic program was thus devised to design and synthesize a series of analogues of **1** with reduced $pK_a$ values of the $\alpha$-proton to enhance the rate of inactivation.



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## RESULTS AND DISCUSSION

**Rational Design of Inhibitors.** 2,3-Dihydro-4-pyranone (**2**) and 2,3-dihydro-4-pyridone (**3**) scaffolds (Figure 2) were

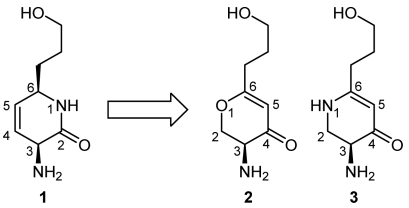


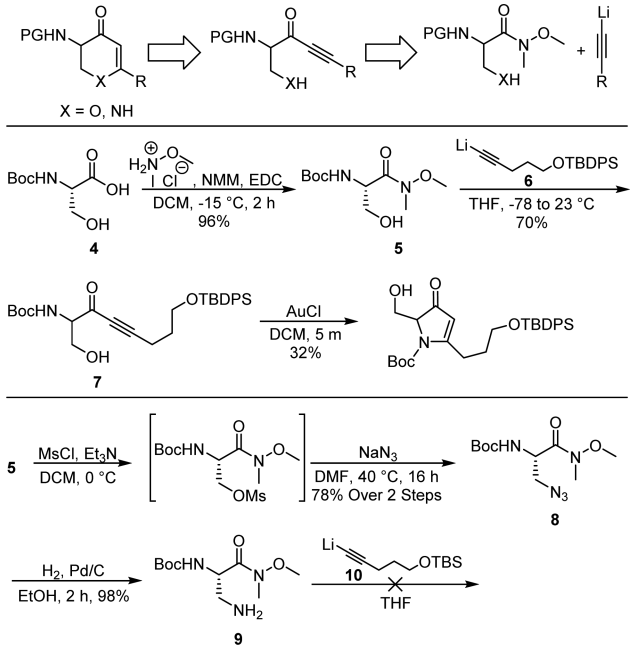
**Figure 2.** New scaffolds for BioA inhibition.

initially designed based on the computationally predicted p$K_a$ values of their $\alpha$-protons: 15.1 for **2** and 14.3 for **3**, which are nearly six p$K_a$ units less than the predicted value for **1** (p$K_a$ ~ 21.1). The greater acidity is due to conversion of the amide in **1** to a more electron-withdrawing vinylogous ester in **2** and vinylogous amide in **3**, though a difference of six p$K_a$ units between **1** and **2**/**3** is probably an overestimate. We were also attracted to the scaffolds of **2** and **3** because they remove the second stereocenter (at C6 in **1**) by shifting the position of the olefin, a change that we expected would simplify analogue synthesis.

Both ring systems have previously been reported, with 2,3-dihydro-4-pyranones commonly synthesized through hetero-Diels–Alder (HDA) reactions between Danishefsky's diene and aldehydes.[16] Unfortunately, the HDA method is generally limited to variation at C2 and C6, and modification of Danishefsky's diene to introduce an amino group appeared quite challenging. Georg and co-workers recently reported a versatile synthesis of 2,3-dihydro-4-pyridones through 6-endo-dig cyclization of an ynone derived from $\beta$-amino acids; however, the introduction of amino substituents at C3 was not disclosed.[17,18] Modifying this strategy, we envisioned that using ynones derived from either serine or 1,2-diaminopropionic acid could be cyclized to efficiently construct the desired ring systems with an amino group at C3 (Scheme 1). The ynones could be synthesized from the addition of an alkynyllithium reagent to an amino acid-derived Weinreb amide.

**First-Generation Syntheses of 2 and 3.** To begin the synthesis of **2**, commercially available Boc-serine was transformed into Weinreb amide **5** by employing EDC and NMM in $CH_2Cl_2$ (Scheme 1).[19] Using NMM in $CH_2Cl_2$ was found to be superior to the aqueous system[20] in both yield and reproducibility. Addition of 3 equiv of alkynyllithium **6** to **5** furnished $\alpha$-amino ynone **7** upon quench.[21] Unfortunately, all of the standard conditions (AgOTf,[22] AuCl,[23] Pd(MeCN)$_4$(BF$_4$)$_2$[24]) that promote 6-endo-dig cyclizations of alcohols into ynones instead provided the 5-endo-dig product. It was difficult to confirm this cyclization, as five-membered cyclic enaminones and 2,3-dihydro-4-pyranones have very similar 1D NMR resonances,[25,26] and the isolated product was unstable and polymerized readily. Thankfully, an HMBC correlation between the $\alpha$-proton and the fully substituted olefinic carbon combined with a lack of correlation between the protons on the hydroxymethyl chain and that same carbon confirmed the identity of the cyclic enaminone. It is worth noting that this synthesis was also attempted unsuccessfully with a trityl group replacing the Boc, imagining that significant increases in steric hindrance toward cyclization could be helpful. While these results were disappointing, we remained

**Scheme 1. Retrosynthesis and First-Generation Syntheses**




undismayed toward pursuing an analogous synthesis of **3**, because we surmised that the enhanced nucleophilicity of the $\beta$-amino group of **9** over the $\beta$-hydroxy group in **7** would override the unproductive competitive cyclization of the $\alpha$-Boc-amine.

A congruent route for the synthesis of **3** necessitates the conversion of the alcohol in **5** to an amine. To begin, **8** was synthesized through mesylation of **5** followed by azide displacement. Notably, the yield of the azide displacement increased by 20% when a longer reaction time was used concomitant with reduced heat (from 60 to 40 °C). Using MeOH as the solvent for the subsequent hydrogenation gave widely variable yields,[27] but switching to EtOH afforded **9**, the desired diaminopropionic acid derivative, in excellent yield. However, addition of lithium acetylide **10** to **9** only furnished the product in trace quantities, instead producing many uncharacterizable highly polar products in addition to a low yield (<10%) of the enynone that would result from addition of the alkyne and elimination of the $\beta$-amino.[28]

As the synthesis of **7** from **5** and **6** was quite facile, further investigation seemed warranted to understand the failure of **10** to add into **9**. An obvious difference in reactivity between **5** and **9** is that the alcohol of **5** gets deprotonated by alkynyllithium species, whereas the unprotected amino group of **9** does not. **5** and **9** also have highly contrasting solubility in THF: **5** is highly soluble (>0.5 M), whereas the maximum solubility of **9** is ~0.02 M. The lack of solubility of **9** appeared more likely to be causing the undesired reactivity, so we hypothesized that **9**, following mono- or dilithiation (dilithiation through reaction of the acetylide with the Weinreb amide of **9**), was forming insoluble aggregates that had perplexing reactivity.[29] Polar additives, both organic (HMPA, DMPU, TMEDA, LiHMDS) and inorganic (LiCl), were added to the reaction to break up the potential aggregates,[29] with none producing a desired effect. The use of alkynylmagensium reagents was also unproductive. Having no remaining alternatives, we chose to protect the $\beta$-amino group of **9** to enhance solubility. Common amino protecting groups orthogonal to Boc, such as Fmoc or Cbz, could not be used for this synthesis, so bis-PMB and trityl



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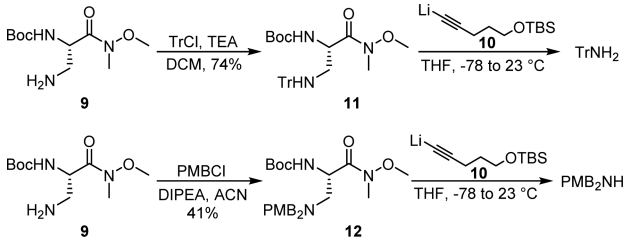
protecting groups were chosen (Scheme 2). Compounds **11** and **12** were synthesized using standard conditions in moderate

**Scheme 2. Amine Protection Reveals Elimination**

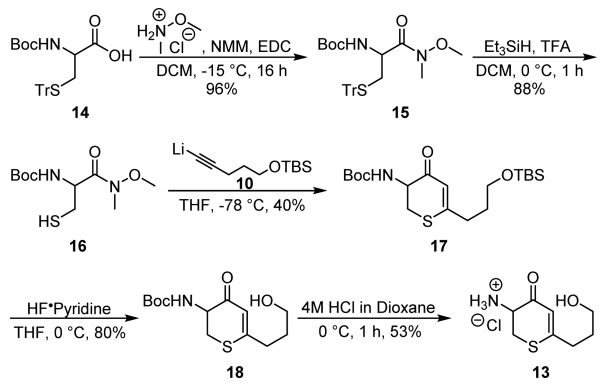



yield. When **11** and **12** were subjugated to the problematic ynone-formation reaction, isolation of the products revealed large (>40%) yields of tritylamine and bis-PMB-amine indicating that competitive $\beta$-elimination was a major side reaction. Given the extremely poor nature of these leaving groups, this is unexpected but must be facilitated by lithium coordination. These results also shed light on the failure of combining $\beta$-amino **9** and lithium acetylide **10**, suggesting that a similar elimination mechanism occurs with ejection of lithium amide, formally an endergonic process. Notably, $\beta$-elimination with **5** was not observed because the $\beta$-hydroxy group is deprotonated under the reaction conditions and elimination would formally lead to release of the dianion lithium oxide, an even more endergonic process.

**Design and Synthesis of Dihydrothiopyranone 13.** Having now uncovered the reasons for the failure of the original syntheses of both **2** and **3**, a solution came to bear. We hypothesized that replacing the ethereal oxygen in **2** with a sulfur atom, thus changing the heterocyclic core, should solve the problems with the originally designed synthesis while maintaining the molecule's ability to inactivate BioA. Thiols are more acidic than alcohols and should therefore remain deprotonated during the key Weinreb amide coupling reaction with an alkynyllithium, preventing $\beta$-elimination. Thiols are also substantially more nucleophilic; consequently, the resulting ynone should favor the desired 6-endo-dig cyclization pathway over the undesired 5-endo-dig pathway observed with **7**.[30] We thus began the synthesis of **13**, the 2,3-dihydro-4-thiopyranone analogue of **2** and **3** (Scheme 3).

Compound **15** was synthesized from (±)-**14** using our optimized conditions for Weinreb amide formation in excellent yield. *S*-Trityl deprotection proceeded smoothly with the addition of $Et_3SiH$ and a drop of TFA to afford **16**.[31] Addition

**Scheme 3. Synthesis of Dihydrothiopyranone 13**




of **10** to **16** while maintaining a temperature of −78 °C provided the desired 2,3-dihydro-4-thiopyranone ring through spontaneous cyclization of the intermediate ynone, validating our synthetic plan. The TBS group was deprotected to produce **18**, after which Boc-deprotection was attempted. The use of TFA in $CH_2Cl_2$ furnished a mixture of desired product **13** and its TFA ester,[32] a virtually unprecedented esterification under these conditions. However, switching to HCl in dioxane provided **13** in a convenient, five-step synthesis from commercially available *N*-Boc-*S*-trityl cysteine **14**.

**Second-Generation Syntheses of 2 and 3.** Bolstered by the successful synthesis of dihydrothiopyranone **13**, we envisioned two alternate approaches to tackle the synthesis of **2** and **3**. In the first of these, we recognized that bis-protection of the $\alpha$-amino group of **7** would be necessary for the synthesis of **2** to prevent competitive 5-endo-dig cyclization while for target **3**, protection of the $\beta$-amino group of **9** with a suitable protecting group would be required to prevent undesired $\beta$-elimination. Both of these strategies appeared challenging. For example, bis-Boc-$\alpha$-amino protection would certainly react with the adjacent alkoxide generated in situ from the addition of a lithium acetylide,[33] while the protecting group requirements for the $\beta$-amino group are overly restrictive due the necessity for orthogonality with the $\alpha$-amino Boc and compatibility under the strongly basic reaction conditions of the alkynyllithium addition. The second approach, that we ultimately elected to perform, involved installation of the troublesome $\alpha$-amino group postcyclization, which would considerably simplify the synthesis and avoid both of the aforementioned challenges. The feasibility of the new synthetic plan was supported by a report by Collet and co-workers,[34] who demonstrated that the reagent *N*-Boc-3-(4-cyanophenyl)oxaziridine could electrophilically transfer an NHBoc group to the carbon of an enolate. Moreover, a report by Gouault and co-workers confirmed that the required enolate could be regioselectively generated in an analogous C6-substituted *N*-Boc-protected 2,3-dihydro-4-pyridone derivative and react successfully in an aldol-type reaction.[18]

As stated above, the retrosynthetic plan calls for cyclization of an ynone followed by electrophilic amination at the C3 carbon. (Scheme 4) The second-generation synthesis of **2** begins with the mono-PMB-protection of 1,3-propanediol **19** to form **20**.[35] Subsequent Jones oxidation and Weinreb amide formation[36] afforded ynone precursor **21**. Addition of alkynyllithium **22** furnished **23** in excellent yield, which was then deprotected with DDQ to provide $\beta$-hydroxy ynone **24**. The ensuing cyclization using AgOTf afforded dihydro-4-pyranone **25** without incident, ready for the electrophilic amination. To our surprise, addition of LiHMDS to a solution of **25** resulted in a $\beta$-elimination of the heterocycle to form **26**, which was rigorously characterized. We propose that $\alpha$-enolate formation leads to $\beta$-elimination of the endocyclic ether oxygen, rupturing the dihydropyranone core and forming an alkoxy-dienone, furnishing **26** upon quench.[37] Unfortunately, the facility and rapidity of this base-catalyzed degradation pathway nullifies this synthetic route.

As mentioned previously, Gouault and co-workers had demonstrated that replacing the ethereal oxygen with a Boc-protected amino, as an analogous second-generation synthesis of **3** would necessitate, would result in stable and nucleophilic enolates for the desired electrophilic amination,[18] so we chose to proceed with this synthesis. Boc-$\beta$-alanine **27** was first transformed into Weinreb amide **28** using standard conditions



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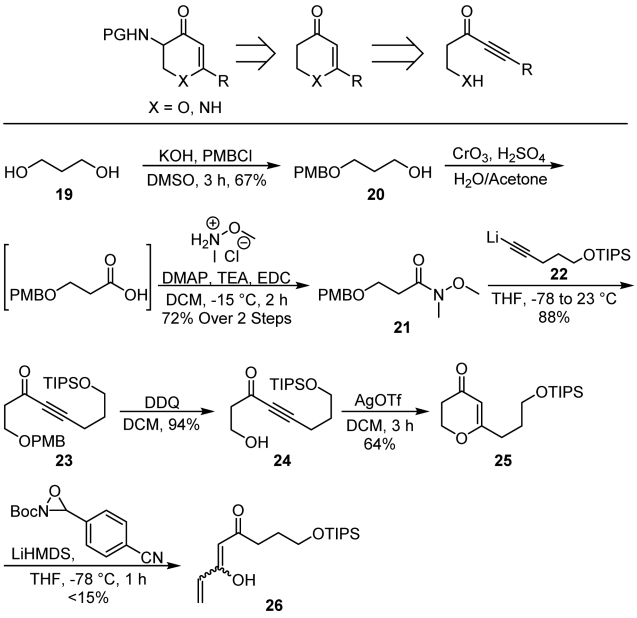
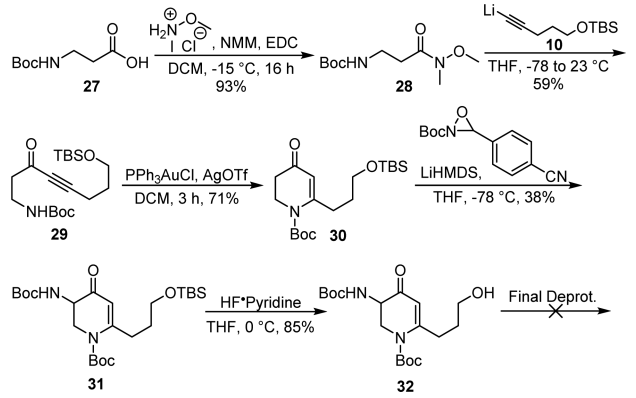
**Scheme 4. Retrosynthesis and Second-Generation Synthesis of 2**




(Scheme 5). Subsequent addition of **10** furnished Boc-protected $\beta$-amino ynone **29**, primed for cyclization. Ynone

**Scheme 5. Second-Generation Synthesis of 3**

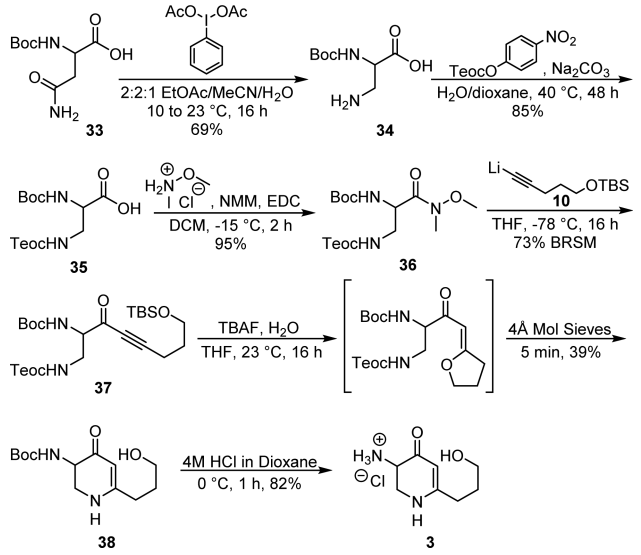



**29** was added to $PPh_3AuOTf$ generated in situ from the combination of AgOTf and $PPh_3AuCl$, producing dihydropyridone **30**.[18] Enolate formation employing LiHMDS followed by addition of *N*-Boc-3-(4-cyanophenyl)oxaziridine afforded the desired electrophilic amination product **31**. In this reaction, the byproduct *p*-cyanobenzaldehyde undergoes rapid aldol addition with the enolate, limiting the theoretical yield to approximately 50%.[34] Deprotection of the TBS group using HF·pyridine proceeded smoothly; however, the final bis-Boc deprotection of **32** proved unconquerable. Boc groups on the N1 position of dihydro-4-pyridones are much more challenging to remove than standard alkyl-amino Boc groups, requiring up to 24 h using TFA in $CH_2Cl_2$.[38] Several conditions were attempted (TFA in $CH_2Cl_2$, HCl in dioxane/EtOAc, phenol and TMSCl in $CH_2Cl_2$)[39] that provided only trace amounts of product. The Boc-amino group on C3 was cleanly deprotected within an hour applying any of these conditions, but longer reaction times to remove the second Boc group resulted in extensive decomposition. We speculate that dimerization of the resulting $\alpha$-aminoketones to a tricyclic pyrazine derivative is occurring based on the well-known propensity of $\alpha$-aminoketones to furnish dihydropyrazines, which can spontaneously oxidize.[40]

**Third-Generation Syntheses of 2 and 3.** Frustrated by the repeated failures, it was decided to return to the original retrosynthetic plans and modify them to accommodate necessary changes. As mentioned above, in the synthesis of **3**, the $\beta$-amino group on **9** needs to be protected with either an amide or carbamate group so that the addition of the lithium acetylide removes the amino proton. Most conventional amide and carbamate protecting groups are either not stable to lithiates or appeared impossible to remove without side reactivity. One that appeared adequate for our purposes is (2-trimethylsilyl)ethoxycarbonyl (Teoc), which is quite robust to lithiation and can be removed in the presence of a Boc group with TBAF.[41]

The synthesis of **3** using this revised route began with (±)-Boc-asparagine **33** (Scheme 6). A modified Hoffman

**Scheme 6. Third-Generation Synthesis of 3**




rearrangement provided mono-Boc protected **34** with only a filtration necessary for purification.[42] Subsequent addition of 4-nitrophenyl 2-(trimethylsilyl)ethyl carbonate, in a modified procedure from the one developed by Rosowski and Wright,[43] afforded differentially protected diaminopropionic acid derivative **35** following purification by simple aqueous extraction. The only previous synthesis of **35**, published by Boger and co-workers in 2000, required four steps from *N*-Boc-serine methyl ester.[44] Formation of the Weinreb amide furnished **36**, which reacted with alkynyllithium **10** to provide ynone **37**. Compared to the previous conversion of **28** to **29**, formation of **37** was quite sluggish, likely due to increased steric hindrance from the Teoc group. The most challenging point of the synthesis proved the next step, concomitant removal of the Teoc and TBS groups.

Attempted deprotection of **37** using TBAF rapidly consumed the starting material, yet only yielded **38** in a 12% yield with numerous highly polar side products. We hypothesized this was due to the strong basicity of TBAF. AcOH and water are known to attenuate both the basicity and reactivity of TBAF,[45] and as such they were each added to TBAF deprotections of **37**, attempting to remove the Teoc and TBS groups while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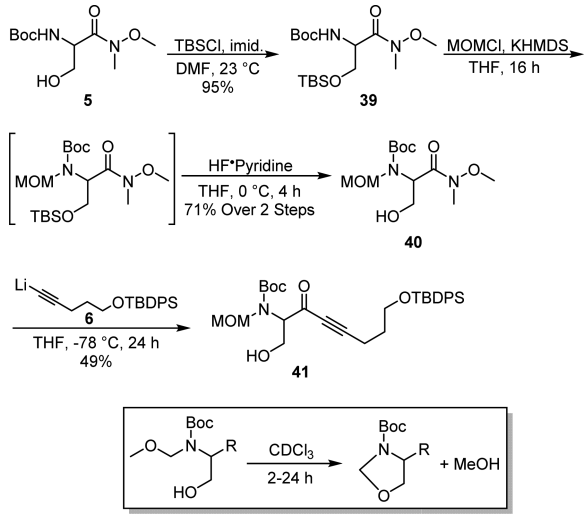
leaving the rest of the molecule intact. To our dismay their inclusion either abolished activity (AcOH, > 10 equiv $H_2O$) or slowed the reaction without improvement of the yield (<10 equiv $H_2O$). Careful monitoring by TLC showed a faint intermediate spot with an $R_f$ value slightly below the staring material that rapidly appeared following the addition of TBAF and slowly disappeared during the course of the reaction. Isolation of this spot revealed that the TBS group had been removed, and liberated alcohol cyclized into the ynone, forming a substituted tetrahydrofuran (Scheme 6). This was the sole product when >10 equiv of $H_2O$ was added to the TBAF reaction, though it appeared to degrade during chromatography, as we were never able to isolate it in yields above 50%. Realizing that the formation of the vinylogous ester in the tetrahydrofuran intermediate would tame the acidity of the $\alpha$ or $\gamma$ protons as compared to **37**, which were believed to be the source of the low yield, we developed a two-step process for this dual deprotection. First, TBAF and water (60 equiv) were stirred with **37** for 16 h, after which it had been entirely converted to the tetrahydrofuran intermediate. Then 4 Å molecular sieves were added, sequestering much of the water and causing deprotection of the Teoc group within 5 min. This procedure more than tripled the yield to 39%, a feasible yield for continuing. The speed of the second Teoc deprotection surprised us, but commercially available 1 M TBAF in THF contains 5% (w/w) $H_2O$, significantly dampening its activity.[45] Very little water remains following addition of the sieves, so the TBAF is extremely reactive and can remove even a robust group such as Teoc in under 5 min. We also tried replacing the TBS group in **38** with several common protecting groups (Bn, THP, MOM) to test whether mono-Teoc deprotection could improve the cyclization yield. Unfortunately, in all attempted cyclizations lacking the TBS group, the yield never approached 20%, further demonstrating the importance of the vinylogous ester intermediate. Finally, deprotection of **38** employing 4 M HCl in dioxane completed the synthesis of dihydropyridone **3**.

Turning our attention toward the synthesis of **2**, we deemed *N*,*N*-diprotection of the serine-derived Weinreb amide essential prior to ynone formation. The most common ways to protect both protons of an amine group are using either phthalimide or bis-Boc, both of which were eliminated from consideration for this system. Phthalimide deprotection requires relatively harsh conditions that would be incompatible with the remaining heterocycle, while the bis-Boc derivative is extremely bulky and would be highly susceptible to intramolecular cyclization with the $\beta$-alkoxy generated from alkynyllithium addition to form a stable oxazolidinone. Instead, we decided to adopt the approach reported by Devlin and Du Bois[46] which utilized dual Boc and MOM protecting groups to protect an amine. This appeared ideal for our system, as MOM and Boc share similar stability profiles as well as deprotection conditions, and a MOM group is both less electron withdrawing and smaller than a Boc group, making intramolecular cyclization to form an oxazolidinone much slower.

Beginning with **5**, standard TBS protection conditions afforded **39** in excellent yield. MOM protection using KHMDS as the base furnished the desired triprotected serine derivative,[47] whose $R_f$ was coincident with that of **39**. This intermediate was not isolated, but directly deprotected with HF·pyridine to furnish Weinreb amide **40**. Surprisingly, when **40** was left in $CDCl_3$ overnight for $^{13}C$ NMR characterization, the molecule cyclized to 1,3-oxazolidine due to residual acid (Scheme 7), an undesirable reaction that foreshadowed

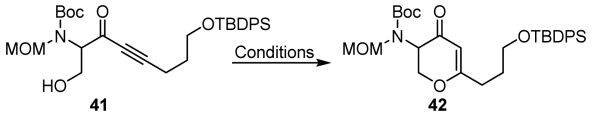
**Scheme 7. Synthesis of Dual MOM-Boc-amino Ynone 41**



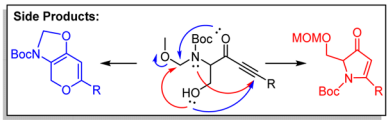

complications with the future Lewis acid-catalyzed 6-endo-dig cyclization. The use of nonacidic solvents such as acetone or $CH_2Cl_2$ was necessary for successful characterization. Addition of **6** to **40** at −78 °C furnished the desired dual Boc-MOM-protected ynone **41** in satisfactory yield. Maintaining a temperature of −78 °C proved essential to prevent the aforementioned intramolecular cyclization of the $\beta$-alkoxide into the Boc group.

To our consternation, the cyclization of **41** to dihydropyranone **42** proved problematic, as standard conditions (AgOTf, AuCl) rapidly produced **42** along with a bevy of side products (Table 1, entries 1 and 2). The reactivity of the MOM group in the presence of Ag(I) salts has been previously exploited in several synthetic transformations,[48] so the observed side reactivity was not surprising. The rapidity of these reactions suggested that dampening the activity of the Lewis acid would be necessary to improve the reaction. Lowering the temper-

**Table 1. Pyranone Cyclization Conditions**




| Entry | Conditions | Yield |
|---|---|---|
| 1 | AgOTf (1.0 equiv), 5 min, 23 °C | 15% |
| 2 | AuCl (0.1 equiv), 5 min, 23 °C | 10% |
| 3 | AgOTf (1.0 equiv), 3 h, -40 °C | N.R. |
| 4 | AgOTf (1.0 equiv), 20 min, -15 °C | ~10% |
| 5 | $PPh_3AuCl$ (0.1 equiv), 3 h, 23 °C | N.R. |
| 6 | $PPh_3AuCl$ (0.1 equiv), $AgNTf_2$ (0.05 equiv), 10 min, -78 to 0 °C | 7% |
| 7 | $PPh_3AuCl$ (0.1 equiv), $AgSbF_6$ (0.07 equiv), 16 h, -78 to 0 °C | 43% |
| 8 | $PPh_3AuCl$ (0.2 equiv), AgOTf (0.1 equiv), 3 h, -78 to 0 °C | 52% |



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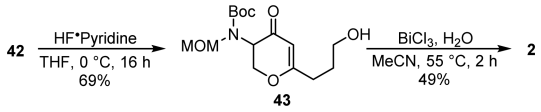
ature, while successful in completely stopping the reaction (Table 1, entry 3), did not alleviate the undesired reactivity at temperatures where the reaction proceeded (Table 1, entry 4). Pursuing alternative alkynophillic Lewis acids led to the discovery that $PPh_3AuCl$ did not promote any reaction (Table 1, entry 5), in contrast to AuCl. Surmising that the substantially increased electron density from the phosphine ligand dampened the reactivity of the gold catalyst, we attempted to see if a "sweet spot" in electron density on the gold could be attained, where cyclization would occur without the undesired side reactivity. A well-known way to modify the properties of gold catalysts is to induce ion exchange through the addition of silver salts.[18] Switching to bistriflimide (Table 1, entry 6) provided a very similar product profile to the original conditions, but the substitution of hexafluoroantimonate (Table 1, entry 7) afforded **42** in substantially increased yield, validating this approach. The use of triflate (Table 1, entry 8) proved the most successful, producing the highest yield with a modest reaction time of 3 h. The undesired side products were still present with this modification but in greatly lessened amounts.

TBDPS deprotection of **42** using HF·pyridine swiftly afforded penultimate intermediate **43** (Scheme 8). However,

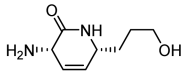
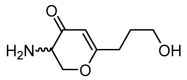
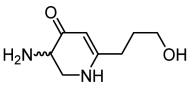
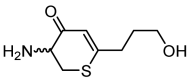
**Scheme 8. Final Deprotections To Form 2**




standard Boc deprotection conditions were unsuccessful in forming the final product **2**, instead leading to degradation. Fearing that aprotic conditions were leading to attack of the ketone onto the MOM group as seen previously (Table 1), we attempted both aqueous acid and a switch of the solvent to MeOH, with neither providing more than trace amounts of product. Employing a Lewis acid in anhydrous conditions was also fruitless, as $SnCl_4$[49] failed to produce **2**. A report by Hu and co-workers[50] using $BiCl_3$ as a Lewis acid in a partially aqueous environment appeared perfect for our system. Indeed, slight modifications to their procedure furnished **2**, completing the trio of desired scaffolds.

**Biochemical Results.** Compounds **2**, **3**, and **13** were evaluated for their ability to irreversibly inhibit BioA. A 0.5 $\mu$M BioA solution was preincubated with 0.05−1 mM inhibitor in the absence of substrates, and aliquots were removed at times between 2 and 40 min. Each of these aliquots were assayed for their remaining enzymatic activity by measuring the production of DTB following the addition of substrates and BioD. Dihydro-4-pyridone **3** displayed time-dependent inhibition of BioA, consistent with what one would expect from a mechanism-based inhibitor; however, **2** and **13** were unstable under the alkaline assay conditions and slowly degraded. Thus, we unfortunately were unable to assess their activity. The $k_{obs}$ values for **3** were obtained through plotting the observed rates of DTB formation versus preincubation time at each inhibitor concentration. Next, the $k_{obs}$ values were plotted against the concentration of inhibitor, and fitting this data to a hyperbola produced the $K_I$ and $k_{inact}$ values displayed in Table 2 (see Experimental Section). Compound **3** had a slightly improved $k_{inact}$ as compared to **1** that was not consistent with our expectations that lowering the p$K_a$ of the $\alpha$-proton would lead

**Table 2. Activity of Synthesized Compounds against BioA**

| Compound | Structure | $k_{inact}$ ($min^{-1}$) | $K_I$ (mM) |
|---|---|---|---|
| **1** |  | 0.18 ± 0.01 | 0.52 ± 0.07 |
| **2** |  | Nd[a] | nd |
| **3** |  | 0.48 ± 0.12 | 3.9 ± 1.2 |
| **13** |  | nd | nd |

[a]Nd = not determined due to instability of compound under the assay conditions.

to more rapid deprotonation by BioA. However, further computational studies on **3** suggest our initially calculated p$K_a$'s significantly overestimated the acidity of **2** and **3**.[15,51] A cocrystal structure of the final inactivated BioA-PLP adduct of **3** confirms it behaves as a covalent mechanism-based inhibitor analogous to **1**, lending support to our expectation that **3** operates via a similar four-step kinetic mechanism wherein deprotonation of the external aldimine to form the quinonoid is rate-limiting.[15] The increased $k_{inact}$ value of **3** relative to **1** indicates that the rate-limiting step is faster, but the modest 2.7-fold rate increase could be caused by other factors such as a more favorable reaction trajectory of the key general base (Lys283) or perturbation of the p$K_a$ in the active site.

## CONCLUSION

Dihydro-4-pyridone **2** and dihydro-4-pyranone **3** were designed to have $\alpha$-protons with p$K_a$ values lower than that of dihydro-2-pyridone **1**, a known mechanism-based inhibitor (MBI) of BioA. Both heterocycles have previously been synthesized through 6-endo-dig cyclization from ynone precursors. However, incorporation of a 3-amino substituent introduced a large number of unanticipated problems including competitive cyclization pathways, dimerization and oxidation, $\beta$-elimination, and multiple side reactions resulting from amine protection. We were forced to amend the syntheses of **2** and **3** and designed dihydro-4-thiopyranone **13** to facilitate construction of a heterocycle analogue, which overcame some of the initial challenges resulting from competitive 5-exo-dig cyclization and $\beta$-elimination pathways. Successful syntheses of dihydro-4-pyridone **2** featured the one-pot conversion of ynone **37** into dihydro-4-pyridone **38** by a two-stage process involving TBAF-mediated deprotection of the TBS group and intramolecular cyclization of the liberated alkoxide into the ynone to form a vinylogous ester intermediate, which served to attenuate the electrophilicity of the carbonyl group thereby minimizing side reactivity. Addition of 4 Å molecular sieves to the reaction mixture activated the TBAF, resulting in near immediate deprotection of the Teoc group that rapidly cyclized through an addition−elimination sequence of the vinylogous ester. The careful balance of reactivity needed to orchestrate this one-pot sequential transformation suggests that this strategy cannot be generally used to assess close analogues and highlights the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remarkable synthetic challenges of these relatively simple systems. Construction of the dihydro-4-pyranone **3** had its own challenges due to the requirements to prevent competitive intramolecular cyclization of the alkoxide onto the $\alpha$-amino protecting groups while being sufficiently stable to the Lewis acids needed to induce 6-exo-dig cyclization of the alkynol and not too sterically cumbersome to hinder alkynyllithium addition to the Wenireb amide. Ultimately, dual Boc-MOM protection of the $\alpha$-amino group was found to meet these requirements. Cyclization and deprotection required careful modulation of the Lewis acidity and was optimally accomplished using Au(I)OTf and $BiCl_3$ reagents, respectively. Biochemical evaluation of dihydro-4-pyranone **3** revealed that it had an increased $k_{inact}$ value against BioA, indicating its scaffold has greater potential for future inhibitor development when compared to the scaffold of **1**.

## EXPERIMENTAL SECTION

**General Methods.** All reactions were performed under an inert atmosphere of dry Ar in oven-dried (150 °C) or flame-dried glassware. $^1$H and $^{13}$C NMR spectra were recorded on a 400 or 500 MHz spectrometer. Proton chemical shifts are reported in ppm from an internal standard of residual chloroform (7.26), methanol (3.31), or acetone (2.05), and carbon chemical shifts are reported in ppm from an internal standard of residual chloroform (77.16), methanol (49.00), or acetone (29.84). Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, pent = pentet, m = multiplet, br = broad), coupling constant(s), integration. High resolution mass spectra were obtained on a TOF II TOF/MS instrument equipped with an ESI interface. TLC analyses were performed on TLC silica gel plates and were visualized with UV light, ninhydrin, 10% PMA, or $KMnO_4$ solution. Purifications were performed by flash chromatography on silica gel or with a medium-pressure flash chromatography system equipped with flash column silica cartridges.

**Materials.** An anhydrous solvent dispensing system using two packed columns of neutral alumina was used for drying THF and $CH_2Cl_2$, while two packed columns of molecular sieves were used to dry DMF, and the solvents were dispensed under argon. **5**,[25] **6**,[52] **10**,[13] **20**,[53] and **22**[35] were prepared as previously described.

*2-[(tert-Butoxycarbonyl)amino]-8-(tert-butyldiphenylsilyloxy)-1-hydroxy-oct-4-yn-3-one (**7**).* To a solution of 5-(*tert*-butyldiphenylsilyloxy)-1-pentyne[51] (11.2 g, 34.9 mmol, 3.4 equiv) in THF (110 mL) at −78 °C was added *n*-BuLi (15.7 mL of a 2.5 M solution in hexanes, 39.1 mmol 3.8 equiv). The reaction was stirred for 2 h, and then a solution of **5** (2.56 g, 10.3 mmol, 1.0 equiv) in THF (50 mL) was added slowly. The reaction was allowed to warm to 23 °C over 2 h. After 16 h, the reaction was quenched by the dropwise addition of glacial AcOH (20 mL). The reaction mixture was partitioned between saturated aqueous $NaHCO_3$ (150 mL) and EtOAc (300 mL). The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (1 × 150 mL) and saturated aqueous NaCl (1 × 100 mL), dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (500 mL of 10% EtOAc−hexanes, 1 L of 15% EtOAc−hexanes, 500 mL of 20% EtOAc−hexanes, 1.5 L of 25% EtOAc−hexanes) afforded the title compound (3.37 g, 64%) as a yellow oil: $R_f$ = 0.1 (20% EtOAc−hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.67 (d, $J$ = 7.0 Hz, 4H), 7.33−7.50 (m, 6H), 5.67 (d, $J$ = 7.0 Hz, 1H), 4.41 (br s, 1H), 3.94−4.15 (m, 2H), 3.75 (t, $J$ = 5.7 Hz, 2H), 2.59 (t, $J$ = 7.0 Hz, 2H), 1.85 (pent, $J$ = 6.3 Hz, 2H), 1.47 (s, 9H), 1.08 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 184.3, 155.7, 135.4, 133.4, 129.6, 127.6, 98.4, 80.2, 78.9, 63.4, 62.8, 61.9, 30.4, 28.2, 26.8, 19.1, 15.7; HRMS (ESI+) calcd for $C_{29}H_{39}NNaO_5Si$ $[M + Na]^+$ 532.2490, found 532.2498 (error 1.5 ppm).

*(S)-3-Azido-2-[(tert-butoxycarbonyl)amino]-N-methoxy-N-methylpropanamide (**8**).* To a solution of **5** (9.5 g, 38.2 mmol, 1.0 equiv) and $Et_3N$ (6.4 mL, 45.8 mmol, 1.2 equiv) in $CH_2Cl_2$ (150 mL) at 0 °C was added $MeSO_2Cl$ (3.2 mL, 42.0 mmol, 1.1 equiv) dropwise. The mixture was stirred for 1 h at 0 °C, and then the reaction mixture was washed with water (1 × 70 mL), saturated aqueous $NaHCO_3$ (1 × 70 mL), 10% aqueous $KHSO_4$ (1 × 70 mL), and saturated aqueous NaCl (1 × 70 mL). The organic layer was dried ($MgSO_4$), concentrated, and placed under high vacuum for 30 min. It was then redissolved in DMF (175 mL), to which $NaN_3$ (7.5 g, 115 mmol, 3.5 equiv) was added. The reaction was heated at 40 °C for 18 h and then partitioned between ice (200 g) and EtOAc (250 mL). Following melting of the ice, the layers were separated and the aqueous layer was extracted with EtOAc (2 × 250 mL). The combined organic extracts were then dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (5% EtOAc−hexanes to 30% EtOAc−hexanes, linear gradient) afforded the title compound (8.10 g, 78%) as a clear oil: $R_f$ = 0.55 (50% EtOAc−hexanes); $^1$H NMR, $^{13}$C NMR, and HRMS data matched reported values.[54]

*(S)-3-Amino-2-[(tert-butoxycarbonyl)amino]-N-methoxy-N-methylpropanamide (**9**).* To a solution of **8** (219 mg, 0.80 mmol, 1.0 equiv) in EtOH (11 mL) was added 10% Pd/C (85 mg). The reaction vessel was then sealed and flushed three times with $H_2$. After 4 h, the reaction mixture was filtered over EtOH-wetted Celite and concentrated to afford the title compound (193 mg, 98%) as a white solid: mp 106−109 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.45 (d, $J$ = 7.04 Hz, 1H), 4.70 (br s., 1H), 3.78 (s, 3H), 3.23 (s, 3H), 3.01 (dd, $J$ = 13.3, 4.7 Hz, 1H), 2.86 (dd, $J$ = 13.3, 5.8 Hz, 1H), 1.45 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 171.3, 155.7, 79.7, 61.5, 52.8, 44.0, 32.0, 28.3; HRMS (ESI+) calcd for $C_{10}H_{22}N_3O_4$ $[M + H]^+$ 248.1605, found 248.1615 (error 4.0 ppm).

*(S)-2-[(tert-Butoxycarbonyl)amino]-N-methoxy-N-methyl-3-(tritylamino)propanamide (**11**).* To a solution of **9** (1.02 g, 4.10 mmol, 1.0 equiv) and $Et_3N$ (1.27 mL, 9.03 mmol, 2.2 equiv) in $CH_2Cl_2$ (50 mL) at 23 °C was added trityl chloride (1.42 g, 4.93 mmol, 1.2 equiv). After 12 h, the reaction mixture was concentrated. Purification by flash chromatography (5% EtOAc−hexanes to 30% EtOAc−hexanes, linear gradient) afforded the title compound (1.15 g, 74%) as a clear oil: $R_f$ = 0.65 (50% EtOAc−hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.44 (d, $J$ = 7.8 Hz, 6H), 7.21−7.32 (m, 6H), 7.10−7.21 (m, 3H), 5.40 (d, $J$ = 8.6 Hz, 1H), 4.86 (br s, 1H), 3.67 (s, 3H), 3.19 (s, 3H), 2.55 (dt, $J$ = 10.8, 4.5 Hz, 1H), 2.27 (td, $J$ = 11.6, 5.7 Hz, 1H), 1.96 (br s, 1H), 1.45 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 172.2, 155.6, 145.7, 128.5, 127.8, 126.3, 79.7, 70.5, 61.5, 50.9, 45.6, 32.2, 28.4; HRMS (ESI+) calcd for $C_{29}H_{35}N_3NaO_4$ $[M + Na]^+$ 512.2520, found 512.2503 (error 3.3 ppm).

*(S)-2-[(tert-Butoxycarbonyl)amino]-3-[bis(4-methoxybenzyl)-amino]-N-methoxy-N-methylpropanamide (**12**).* To a solution of **9** (0.732 g, 2.96 mmol, 1.0 equiv) and DIPEA (1.56 mL, 8.9 mmol, 3.0 equiv) in MeCN (11 mL) at 23 °C was added PMBCl (1.0 mL, 7.4 mmol, 2.5 equiv). After 24 h, $Et_3N$ (5 mL) was added and the reaction was partitioned between saturated aqueous NaCl (70 mL) and EtOAc (80 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 80 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (5% EtOAc−hexanes to 60% EtOAc−hexanes, linear gradient) afforded the title compound (596 mg, 41%) as a clear oil: $R_f$ = 0.45 (50% EtOAc−hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.25 (d, $J$ = 8.6 Hz, 4H), 6.84 (d, $J$ = 8.6 Hz, 4H), 5.01 (d, $J$ = 8.6 Hz, 1H), 4.90 (br s, 1H), 3.61−3.84 (m, 11H), 3.41 (d, $J$ = 13.3 Hz, 2H), 3.17 (s, 3H), 2.55−2.74 (m, 2H), 1.48 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 172.0, 158.6, 155.4, 131.1, 130.1, 113.6, 79.3, 61.5, 57.5, 55.2, 54.9, 49.3, 32.0, 28.4; HRMS (ESI+) calcd for $C_{26}H_{38}N_3O_6$ $[M + H]^+$ 488.2755, found 488.2769 (error 3.1 ppm).

*(±)-N-(tert-Butoxycarbonyl)-S-trityl-cysteine N′-methoxy-N′-methylamide (**15**).* To a solution of (±)-**14** (2.5 g, 5.4 mmol, 1.0 equiv), *N*-methylmorpholine (0.66 mL, 5.9 mmol, 1.1 equiv), and *N*,*O*-dimethylhydroxylamine·HCl (0.60 g, 5.9 mmol, 1.1 equiv) in $CH_2Cl_2$ (15 mL) at −15 °C was added EDC (1.13 g, 5.9 mmol, 1.1 equiv) in four equal portions over 15 min. The mixture was stirred for 16 h at −15 °C and then partitioned between 1 N aqueous HCl (25 mL) and $CH_2Cl_2$ (40 mL). The organic layer was separated and washed with 1 N aqueous HCl (1 × 25 mL), after which the aqueous layers were combined and extracted with $CH_2Cl_2$ (1 × 50 mL) The



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combined organic extracts were washed with saturated aqueous $NaHCO_3$ (2 × 30 mL) and saturated aqueous NaCl (1 × 30 mL), dried ($MgSO_4$), filtered, and concentrated to afford the title compound (2.62 g, 96%) as a white solid: $R_f$ = 0.45 (50% EtOAc–hexanes); $^1$H NMR, $^{13}$C NMR, and HRMS data matched reported values.[55]

*(±)-N-(tert-Butoxycarbonyl)-cysteine N′-methoxy-N′-methylamide (**16**).* To a solution of **15** (2.60 g, 5.13 mmol, 1.0 equiv) and $Et_3SiH$ (0.98 mL, 6.16 mmol, 1.2 equiv) in $CH_2Cl_2$ (60 mL) at 0 °C was added TFA (2.4 mL, 31.3 mmol, 6.1 equiv), causing the solution to turn bright yellow due to the presence of the triphenylmethyl cation. The reaction was stirred for 1 h at 0 °C until the solution had again become clear and then quenched with saturated aqueous $NaHCO_3$ (50 mL). The layers were separated, and the aqueous layer was extracted with $CH_2Cl_2$ (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Purification by flash chromatography (10% EtOAc–hexanes to 40% EtOAc–hexanes, linear gradient) afforded the title compound (1.19 g, 88%) as a clear oil: $R_f$ = 0.45 (50% EtOAc–hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.44 (d, $J$ = 6.7 Hz, 1H), 4.88 (br s, 1H), 3.78 (s, 3H), 3.24 (s, 3H), 2.74–2.97 (m, 2H), 1.39–1.55 (m, 10H, $C(CH_3)_3$ and SH); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 170.6, 155.3, 80.0, 61.7, 51.9, 32.1, 28.3, 27.1; HRMS (ESI+) calcd for $C_{10}H_{20}N_2NaO_4S$ $[M + Na]^+$ 287.1036, found 287.1023 (error 4.5 ppm).

*(±)-3-N-[(tert-Butoxycarbonyl)amino]-6-[3-(tert-butyldimethylsilyloxy)propyl]-2,3-dihydro-4H-thiopyran-4-one (**17**).* To a solution of 5-(*tert*-butyldimethylsilyloxy)-1-pentyne[13] (282 mg, 1.42 mmol, 3.2 equiv) in THF (10 mL) at −78 °C was added *n*-BuLi (0.58 mL of a 2.5 M solution in hexanes, 1.45 mmol 3.3 equiv). The reaction was stirred for 1 h, and then a solution of **16** (117 mg, 0.44 mmol, 1.0 equiv) in THF (5 mL) was added dropwise. After 5 h at −78 °C, the reaction was quenched by the dropwise addition of saturated aqueous $NH_4Cl$ (5 mL). The reaction mixture was allowed to warm to 23 °C and partitioned between saturated aqueous $NH_4Cl$ (20 mL) and EtOAc (50 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 40 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (0% EtOAc–hexanes to 15% EtOAc–hexanes, linear gradient) afforded the title compound (71 mg, 40%) as a pale yellow oil: $R_f$ = 0.2 (10% EtOAc–hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.08 (s, 1H), 5.79 (br s, 1H), 4.34 (d, $J$ = 14.1 Hz, 1H), 3.61 (t, $J$ = 6.1 Hz, 2H), 3.50 (d, $J$ = 9.4 Hz, 1H), 3.01 (t, $J$ = 13.5 Hz, 1H), 2.45 (t, $J$ = 7.6 Hz, 2H), 1.71–1.83 (pent, $J$ = 6.8 Hz, 2H), 1.44 (s, 9H), 0.87 (s, 9H), 0.03 (s, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 191.1, 166.2, 155.5, 119.8, 80.1, 61.5, 54.5, 34.6, 31.73, 31.67, 28.3, 25.9, 18.2, −5.4 (2C); HRMS (ESI+) calcd for $C_{19}H_{35}NNaO_4SSi$ $[M + Na]^+$ 424.1948, found 424.1960 (error 2.8 ppm).

*(±)-3-N-[(tert-Butoxycarbonyl)amino]-6-(3-hydroxypropyl)-2,3-dihydro-4H-thiopyran-4-one (**18**).* To a solution of **17** (70 mg, 0.174 mmol, 1.0 equiv) in THF (7 mL) at 0 °C was added HF·pyridine (0.7 mL) dropwise over 30 min. The reaction was stirred at 0 °C for 3 h and then quenched with saturated aqueous $NaHCO_3$ (25 mL). The aqueous layer was extracted with EtOAc (4 × 35 mL). The combined organic extracts were dried ($MgSO_4$), concentrated, and purified by flash chromatography (15% EtOAc–hexanes to 65% EtOAc–hexanes, linear gradient), affording the title compound (40 mg, 80%) as a white powder: $R_f$ = 0.45 (80% EtOAc–hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.12 (s, 1H), 5.81 (br s, 1H), 4.37–4.40 (m, 1H), 3.69 (t, $J$ = 6.3 Hz, 2H), 3.50–3.54 (m, 1H), 3.04 (t, $J$ = 13.5 Hz, 1H), 2.51 (t, $J$ = 7.8 Hz, 2H), 2.06 (br s, 1H, OH), 1.86 (pent, $J$ = 7.0 Hz, 2H), 1.46 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 191.1, 165.8, 155.5, 119.8, 80.1, 61.3, 54.5, 34.4, 31.6, 31.4, 28.3; HRMS (ESI+) calcd for $C_{13}H_{21}NNaO_4S$ $[M + Na]^+$ 310.1083, found 310.1088 (error 1.6 ppm).

*(±)-3-Amino-6-(3-hydroxypropyl)-2,3-dihydro-4H-thiopyran-4-one hydrochloride (**13**).* To a prechilled flask containing **18** (51 mg, 0.177 mmol, 1.0 equiv) was added cold 4 M HCl in dioxane (10 mL). After 1 h at 0 °C, the reaction mixture was concentrated in vacuo. The residue was dissolved in a minimal amount of MeOH and precipitated with ether, which afforded the title compound (21 mg, 53%) as a white solid: $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 6.23 (s, 1H), 4.30 (dd, $J$ = 14.9, 4.7 Hz, 1H), 3.60 (t, $J$ = 6.1 Hz, 2H), 3.45 (dd, $J$ = 14.6, 13.0 Hz, 1H), 3.34 (dd, $J$ = 12.7, 4.7 Hz, 1H), 2.56 (dt, $J$ = 7.6, 2.4 Hz, 2H), 1.84 (pent, $J$ = 7.0 Hz, 2H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 188.0, 167.5, 119.1, 60.1, 52.7, 34.1, 31.4, 28.7; HRMS (ESI+) calcd for $C_8H_{14}NO_2S$ $[M + H]^+$ 188.0740, found 188.0740 (error 0 ppm).

*3-(4-Methoxybenzyloxy)-N-methoxy-N-methylpropanamide (**21**).* To a solution of **20** (3.78 g, 19.3 mmol, 1.0 equiv) in acetone (11 mL) at 0 °C was added 2 M Jones Reagent (23.2 mL). The mixture immediately turned green, and some precipitation was observed. After 90 min, iPrOH was added until the reaction turned deep blue, signifying a complete quench. The reaction mixture was filtered over Celite and concentrated to remove all of the acetone. The crude product was dissolved in water (30 mL) and extracted with EtOAc (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$), concentrated, and then redissolved in $CH_2Cl_2$ (130 mL). To this solution was sequentially added *N,O*-dimethylhydroxylamine hydrochloride (2.52 g, 25.1 mmol, 1.3 equiv), EDC (4.8 g, 25.1 mmol, 1.3 equiv), $Et_3N$ (3.5 mL, 25.1 mmol, 1.3 equiv), and DMAP (3.1 g, 25.1 mmol, 1.3 equiv). After 16 h at 23 °C, the reaction mixture was washed with 1 M aqueous HCl (1 × 50 mL), saturated aqueous NaCl (1 × 50 mL), saturated aqueous $NaHCO_3$ (1 × 50 mL), and saturated aqueous NaCl (1 × 50 mL). The organic layer was then dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (20% EtOAc–hexanes to 60% EtOAc–hexanes, linear gradient) afforded the title compound (2.34 g, 72%) as a clear viscous liquid: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.26 (d, $J$ = 8.5 Hz, 2H), 6.87 (d, $J$ = 8.6 Hz, 2H), 4.47 (s, 2H), 3.72–3.85 (m, 5H), 3.67 (s, 3H), 3.18 (s, 3H), 2.74 (t, $J$ = 6.5 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 172.3, 159.2, 130.4, 129.3, 113.7, 72.9, 65.6, 61.3, 55.2, 32.5, 32.0; HRMS (ESI+) calcd for $C_{13}H_{19}NNaO_4$ $[M + Na]^+$ 276.1206, found 276.1206 (error 0 ppm).

*1-(4-Methoxybenzyloxy)-8-(triisopropylsilyloxy)oct-4-yn-3-one (**23**).* To a solution of 5-(triisopropylsilyloxy)-1-pentyne[35] (2.9 g, 12.0 mmol, 1.3 equiv) in THF (40 mL) at −78 °C was added *n*-BuLi (4.4 mL of a 2.5 M solution in hexanes, 11.0 mmol 1.2 equiv). The reaction was stirred for 1.5 h, and then a solution of **21** (2.34 g, 12.0 mmol, 1.0 equiv) in THF (50 mL) was added dropwise. The reaction was allowed to warm to 23 °C over 2 h and after 16 h was quenched with AcOH (2 mL). EtOAc (300 mL) was then added, and the reaction mixture was washed with saturated aqueous $NaHCO_3$ (2 × 100 mL) and saturated aqueous NaCl (1 × 50 mL), dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (0% EtOAc–hexanes to 15% EtOAc–hexanes, linear gradient) afforded the title compound (3.51 g, 88%) as a pale yellow liquid: $R_f$ = 0.7 (20% EtOAc–hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.26 (d, $J$ = 8.5 Hz, 2H), 6.88 (d, $J$ = 8.6 Hz, 2H), 4.46 (s, 2H), 3.81 (s, 3H), 3.72–3.80 (m, 4H), 2.82 (t, $J$ = 6.3 Hz, 2H), 2.50 (t, $J$ = 7.2 Hz, 2H), 1.79 (pent, $J$ = 6.5 Hz, 2H), 0.97–1.16 (m, 21H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 185.8, 159.2, 130.1, 129.3, 113.8, 94.7, 80.8, 72.8, 64.6, 61.5, 55.3, 45.7, 31.0, 18.0, 15.5, 11.9; HRMS (ESI+) calcd for $C_{25}H_{40}NaO_4Si$ $[M + Na]^+$ 455.2588, found 455.2594 (error 1.1 ppm).

*1-Hydroxy-8-(triisopropylsilyloxy)oct-4-yn-3-one (**24**).* To a solution of **23** (3.45 g, 7.97 mmol, 1.0 equiv) in $CH_2Cl_2$ (60 mL) at 23 °C were added DDQ (2.17 g, 9.56 mmol 1.2 equiv) and $H_2O$ (6 mL). After 1.5 h, the reaction mixture was partitioned between $CH_2Cl_2$ (50 mL) and saturated aqueous $NaHCO_3$ (50 mL). The layers were separated, and the aqueous layer was extracted with $CH_2Cl_2$ (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (5% EtOAc–hexanes to 20% EtOAc–hexanes, linear gradient) afforded the title compound (2.34 g, 94%) as a pale yellow liquid: $R_f$ = 0.15 (20% EtOAc–hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.91 (q, $J$ = 5.6 Hz, 2H), 3.78 (t, $J$ = 5.9 Hz, 2H), 2.82 (t, $J$ = 5.5 Hz, 2H), 2.53 (t, $J$ = 7.0 Hz, 2H), 2.19 (t, $J$ = 6.5 Hz, 1H, OH), 1.81 (pent, $J$ = 6.5 Hz, 2H), 1.00–1.16 (m, 21H); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 187.3, 95.4, 80.8, 61.4, 57.7, 47.6, 30.9, 18.0, 15.5, 11.9; HRMS (ESI+) calcd for $C_{17}H_{32}NaO_3Si$ $[M + Na]^+$ 335.2013, found 335.2022 (error 2.7 ppm).



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*6-[3-((Triisopropylsilyl)oxy)propyl]-2,3-dihydro-4H-pyran-4-one (**25**).* To a solution of **24** (2.36 g, 7.55 mmol, 1.0 equiv) in $CH_2Cl_2$ (150 mL) was added AgOTf (1.94 g, 7.55 mmol, 1.0 equiv). After 2 h, the reaction mixture was filtered over Celite, concentrated, and purified by flash chromatography (5% EtOAc–hexanes to 20% EtOAc–hexanes, linear gradient), affording the title compound (1.46 g, 62%) as a clear oil: $R_f$ = 0.15 (20% EtOAc–hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.36 (s, 1H), 4.46 (t, $J$ = 6.9 Hz, 2H), 3.72 (t, $J$ = 6.1 Hz, 2H), 2.52 (t, $J$ = 6.9 Hz, 2H), 2.37 (t, $J$ = 7.6 Hz, 2H), 1.79 (pent, $J$ = 6.9 Hz, 2H), 1.02–1.13 (m, 21H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 192.2, 177.9, 104.6, 67.9, 62.1, 35.7, 31.3, 29.6, 18.0, 11.9; HRMS (ESI+) calcd for $C_{17}H_{32}NaO_3Si$ $[M + Na]^+$ 335.2013, found 335.2021 (error 2.4 ppm).

*6-Hydroxy-1-[(triisopropylsilyl)oxy]octa-5,7-dien-4-one (**26**).* To a solution of **25** (1.39 g, 4.0 mmol, 1.0 equiv) in THF (40 mL) at −78 °C was added LiHMDS (4.9 mL of a 1 M solution in THF, 4.9 mmol, 1.1 equiv). After 30 min at −78 °C, *N*-Boc-cyanophenyl oxaziridine (0.60 g, 2.45 mmol, 0.55 equiv) was added over 10 min as a solution in THF (10 mL). After 50 min, saturated aqueous $NH_4Cl$ (5 mL) was added, and the reaction was warmed to 23 °C. The reaction mixture was then partitioned between saturated aqueous $NH_4Cl$ (50 mL) and EtOAc (100 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (5% EtOAc–hexanes →30% EtOAc–hexanes, linear gradient) afforded the title compound (157 mg, 11%) as a clear liquid, along with several other products that were unable to be fully characterized: $R_f$ = 0.8 (20% EtOAc–hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.26 (dd, $J$ = 17.1, 1.5 Hz, 1H), 6.13 (dd, $J$ = 17.1, 10.6 Hz, 1H), 5.67 (dd, $J$ = 10.4, 1.4 Hz, 1H), 5.58 (s, 1H), 3.73 (t, $J$ = 6.1 Hz, 2H), 2.52 (t, $J$ = 7.6 Hz, 2H), 1.87 (pent, $J$ = 6.8 Hz, 2H), 0.99–1.15 (m, 21H); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 202.1, 175.4, 132.4, 125.1, 100.3, 62.4, 37.0, 28.4, 18.0, 12.0; HRMS (ESI+) calcd for $C_{17}H_{32}NaO_3Si$ $[M + Na]^+$ 335.2013, found 335.2005 (error 2.4 ppm).

*3-[(tert-Butoxycarbonyl)amino]-N-methoxy-N-methylpropanamide (**28**).* To a solution of *N*-Boc-*β*-alanine (8.5 g, 45.0 mmol, 1.0 equiv), *N*-methylmorpholine (5.5 mL, 49.5 mmol, 1.1 equiv), and *N*,*O*-dimethylhydroxylamine·HCl (4.98 g, 49.5 mmol, 1.1 equiv) in $CH_2Cl_2$ (100 mL) at −15 °C was added EDC (9.49 g, 49.5 mmol, 1.1 equiv) in four equal portions over 15 min. The mixture was stirred for 16 h at −15 °C and then washed with 1 N aqueous HCl (2 × 25 mL), saturated aqueous $NaHCO_3$ (2 × 25 mL), and saturated aqueous NaCl (1 × 25 mL). The organic layer was then dried ($MgSO_4$), filtered, and concentrated to afford the title compound (9.69 g, 93%) as a white solid: $R_f$ = 0.1 (50% EtOAc–hexanes); $^1$H NMR, $^{13}$C NMR, and HRMS data matched reported values.[56]

*1-[(tert-Butoxycarbonyl)amino]-8-(tert-butyldimethylsilyloxy)oct-4-yn-3-one (**29**).* To a solution of 5-(*tert*-butyldimethylsilyloxy)-1-pentyne[13] (4.25 g, 21.4 mmol, 2.2 equiv) in THF (35 mL) at −78 °C was added *n*-BuLi (8.6 mL of a 2.5 M solution in hexanes, 21.4 mmol 2.2 equiv). The reaction was stirred for 1.25 h, and then a solution of **28** (2.25 g, 9.7 mmol, 1.0 equiv) in THF (15 mL) was added dropwise. The reaction was allowed to warm to 23 °C after 2 h. After 16 h, the reaction was quenched by the dropwise addition of AcOH (5 mL) and then partitioned between saturated aqueous $NaHCO_3$ (50 mL) and EtOAc (150 mL). The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (1 × 50 mL) and saturated aqueous NaCl (1 × 50 mL), dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (5% EtOAc–hexanes to 25% EtOAc–hexanes, linear gradient) afforded the title compound (2.13 g, 59%) as a clear oil: $R_f$ = 0.3 (20% EtOAc–hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 4.94 (br s, 1H), 3.69 (t, $J$ = 5.7 Hz, 2H), 3.41 (q, $J$ = 5.6 Hz, 2H), 2.78 (t, $J$ = 5.5 Hz, 2H), 2.48 (t, $J$ = 7.0 Hz, 2H), 1.78 (pent, $J$ = 6.5 Hz, 2H), 1.44 (s, 9H), 0.90 (s, 9H), 0.06 (s, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 186.7, 155.7, 95.0, 80.7, 79.3, 61.1, 45.5, 35.1, 30.7, 28.4, 25.9, 18.3, 15.5, −5.4; HRMS (ESI+) calcd for $C_{19}H_{35}NNaO_4Si$ $[M + Na]^+$ 392.2228, found 392.2220 (error 2.0 ppm).

*1-tert-Butoxycarbonyl-6-[3-(tert-butyldimethylsilyloxy)propyl]-2,3-dihydro-4H-pyridin-4-one (**30**).* To a solution of **29** (2.127 g, 5.8 mmol, 1.0 equiv) and $PPh_3AuCl$ (143 mg, 0.29 mmol, 0.05 equiv) in $CH_2Cl_2$ (35 mL) at 23 °C was added AgOTf (200 mg, 0.58 mmol, 0.1 equiv). After 2 h, the reaction mixture was filtered over Celite and concentrated. Purification by flash chromatography (0% EtOAc–hexanes to 25% EtOAc–hexanes, linear gradient) afforded the title compound (1.501 g, 71%) as a clear oil: $R_f$ = 0.35 (20% EtOAc–hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.41 (s, 1H), 4.01 (t, $J$ = 6.7 Hz, 2H), 3.62 (t, $J$ = 6.1 Hz, 2H), 2.76 (t, $J$ = 7.6 Hz, 2H), 2.48 (t, $J$ = 6.7 Hz, 2H), 1.72 (pent, $J$ = 6.9 Hz, 2H), 1.54 (s, 9H), 0.90 (s, 9H), 0.05 (s, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 194.7, 161.3, 152.0, 112.3, 83.0, 62.2, 46.6, 37.3, 32.3, 31.1, 28.1, 25.9, 18.3, −5.3; HRMS (ESI+) calcd for $C_{19}H_{35}NNaO_4Si$ $[M + Na]^+$ 392.2228, found 392.2222 (error 1.5 ppm).

*(±)-1-(tert-Butoxycarbonyl)-3-[(tert-butoxycarbonyl)amino]-6-[3-(tert-butyldimethylsilyloxy)propyl]-2,3-dihydro-4H-pyridin-4-one (**31**).* To a solution of **30** (1.48 g, 4.0 mmol, 1.0 equiv) in THF (50 mL) at −78 °C was added LiHMDS (4.4 mL of a 1 M solution in THF, 4.4 mmol, 1.1 equiv). After 30 min at −78 °C, *N*-Boc-cyanophenyloxaziridine (0.54 g, 2.2 mmol, 0.55 equiv) was added over 10 min as a solution in THF (10 mL). After 50 min, saturated aqueous $NH_4Cl$ (5 mL) was added, and the reaction was warmed to 23 °C. The reaction mixture was then partitioned between saturated aqueous $NH_4Cl$ (50 mL) and EtOAc (100 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (0% EtOAc–hexanes to 25% EtOAc–hexanes, linear gradient) afforded the title compound (744 mg, 38%) as a clear oil: $R_f$ = 0.55 (20% EtOAc–hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.37 (s, 1H), 5.31 (br s, 1H), 4.78 (dd, $J$ = 12.3, 5.3 Hz, 1H), 4.22 (d, $J$ = 12.5 Hz, 1H), 3.62 (dt, $J$ = 6.1, 3.1 Hz, 2H), 3.24 (t, $J$ = 13.2 Hz, 1H), 2.99 (td, $J$ = 14.7, 7.5 Hz, 1H), 2.58 (td, $J$ = 15.0, 7.6 Hz, 1H), 1.72 (pent, $J$ = 6.9 Hz, 2H), 1.52–1.64 (m, 9H), 1.46 (s, 9H), 0.90 (s, 9H), 0.05 (s, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 191.7, 162.9, 155.5, 151.8, 108.7, 83.6, 80.0, 62.0, 53.7, 51.3, 32.1, 31.2, 28.3, 28.0, 25.9, 18.3, −5.3 (2C); HRMS (ESI+) calcd for $C_{24}H_{44}N_2NaO_6Si$ $[M + Na]^+$ 507.2861, found 507.2876 (error 3.0 ppm).

*(±)-1-(tert-Butoxycarbonyl)-3-[(tert-butoxycarbonyl)amino]-6-(3-hydroxypropyl)-2,3-dihydro-4H-pyridin-4-one (**32**).* To a solution of **31** (734 mg, 1.51 mmol, 1.0 equiv) in THF (50 mL) at 0 °C was added HF·pyridine (5 mL) over a 30 min period. After 4 h at 0 °C, the reaction mixture was partitioned between saturated aqueous $NaHCO_3$ (50 mL) and EtOAc (100 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (4 × 60 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (20% EtOAc–hexanes to 70% EtOAc–hexanes, linear gradient) afforded the title compound (478 mg, 85%) as a clear oil: $R_f$ = 0.55 (80% EtOAc–hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.38 (s, 1H), 5.29 (br s, 1H), 4.76 (dd, $J$ = 12.5, 5.5 Hz, 1H), 4.21 (d, $J$ = 12.1 Hz, 1H), 3.67 (t, $J$ = 6.0 Hz, 2H), 3.25 (t, $J$ = 13.1 Hz, 1H), 3.01 (dt, $J$ = 14.5, 7.2 Hz, 1H), 2.64 (dt, $J$ = 15.1, 7.7 Hz, 1H), 1.73–1.85 (m, 2H), 1.54 (s, 9H), 1.45 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 191.7, 162.7, 155.5, 151.8, 108.8, 83.8, 80.1, 61.7, 53.7, 51.2, 31.7, 31.1, 28.3, 28.0; HRMS (ESI+) calcd for $C_{18}H_{30}N_2NaO_6$ $[M + Na]^+$ 393.1996, found 393.2012 (error 4.1 ppm).

*(±)-3-Amino-2-[(tert-butoxycarbonyl)amino]propionic Acid (**34**).* To a slurry of (±)-*N*-Boc-asparagine (10.0 g, 43.1 mmol, 1.0 equiv) in 2:2:1 EtOAc–MeCN–$H_2O$ (120 mL) at 10 °C was added iodosobenzene diacetate (16.6 g, 51.5 mmol, 1.2 equiv). The reaction was allowed to warm to 23 °C over 2 h. After 16 h, the reaction mixture was filtered, and the filter cake was washed with EtOAc (150 mL) and then dried in vacuo to afford the title compound (6.06 g, 69%) as a white solid. $^1$H NMR, $^{13}$C NMR, and HRMS data matched reported values.[42]

*(±)-2-(tert-Butoxycarbonyl)amino-3-[2-(trimethylsilyl)-ethyloxycarbonyl]propionic Acid (**35**).* To a solution of **34** (0.41 g, 2.01 mmol, 1.0 equiv) in 1.5 M aqueous $Na_2CO_3$ (10 mL) at 23 °C



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was added 4-nitrophenyl 2-(trimethylsilyl)ethyl carbonate (Teoc-ONp) (0.68 g, 2.41 mmol, 1.2 equiv) in dioxane (5 mL) dropwise. The reaction was heated to 40 °C for 48 h, after which it was a bright yellow solution. $Na_2S_2O_4$ was then added to the reaction mixture until the solution turned white, which signifies when the *p*-nitrophenol has been reduced to the aniline. $H_2O$ (30 mL) was added, and the reaction mixture was extracted with $Et_2O$ (1 × 20 mL). The layers were separated, and the organic layer was extracted with 1 N $Na_2CO_3$ (3 × 15 mL). The combined aqueous extracts were acidified to a pH of 2 with 2 N HCl and extracted with $CH_2Cl_2$ (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated to afford the title compound (0.593 g, 85%) as a clear oil: $R_f$ = 0.1 (10% MeOH−$CH_2Cl_2$); $^1$H NMR, $^{13}$C NMR, and HRMS data matched reported values.[44]

*(±)-2-N-(tert-Butoxycarbonyl)-3-N-[2-(trimethylsilyl)-ethyloxycarbonyl]-2,3-diamino-N-methoxy-N-methylpropanamide (**36**).* To a solution of **35** (0.58 g, 1.66 mmol, 1.0 equiv), *N*-methylmorpholine (0.20 mL, 1.83 mmol, 1.1 equiv), and *N*,*O*-dimethylhydroxylamine·HCl (0.18 g, 1.83 mmol, 1.1 equiv) in $CH_2Cl_2$ (13 mL) at −15 °C was added EDC (1.13 g, 5.9 mmol, 1.1 equiv) in four equal portions over 15 min. The mixture was stirred for 2 h at −15 °C and then partitioned between saturated aqueous 1 N HCl (35 mL) and $CH_2Cl_2$ (35 mL). The layers were separated, and the aqueous layer was extracted with $CH_2Cl_2$ (1 × 50 mL), after which the organic layers were combined and washed with saturated aqueous $NaHCO_3$ (1 × 30 mL), dried ($MgSO_4$), filtered, and concentrated, affording the title compound (0.62 g, 95%) as a white solid: $R_f$ = 0.45 (50% EtOAc−hexanes); mp 92−94 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.54 (br s, 1H), 5.11 (br s, 1H), 4.75 (br s, 1H), 4.11 (t, $J$ = 8.4 Hz, 2H), 3.76 (s, 3H), 3.48 (br s, 2H), 3.19 (s, 3H), 1.42 (s, 9H), 0.94 (t, $J$ = 8.40 Hz, 2H), 0.01 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 170.6, 156.8, 155.5, 79.8, 63.1, 61.6, 50.9, 42.7, 32.3, 28.2, 17.6, −1.6; HRMS (ESI+) calcd for $C_{16}H_{33}N_3NaO_6Si$ $[M + Na]^+$ 414.2031, found: 414.2032 (error 0.2 ppm).

*(±)-2-[(tert-Butoxycarbonyl)amino]-8-(tert-butyldimethylsilyloxy)-1-[2-(trimethylsilyl)ethoxycarbonyl]-oct-4-yn-3-one (**37**).* To a solution of 5-(*tert*-butyldimethylsilyloxy)-1-pentyne[13] (0.96 g, 4.82 mmol, 3.2 equiv) in THF (15 mL) at −78 °C was added *n*-BuLi (1.99 mL of a 2.5 M solution in hexanes, 4.97 mmol 3.3 equiv). After 2 h, a solution of **36** (0.59 g, 1.51 mmol, 1.0 equiv) in THF (15 mL) was added dropwise. After 16 h at −78 C, AcOH (3 mL) was added dropwise to quench. The reaction mixture was partitioned between EtOAc (80 mL) and saturated aqueous $NaHCO_3$ (40 mL). The aqueous layer was extracted with EtOAc (1 × 80 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (5% EtOAc−hexanes to 30% EtOAc−hexanes, linear gradient) afforded the title compound (317 mg, 40%, 73% BRSM) as a pale yellow oil: $R_f$ = 0.3 (20% EtOAc−hexanes); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.62 (br s, 1H), 5.08 (br s, 1H), 4.40 (br s, 1H), 4.12 (t, $J$ = 8.4 Hz, 2H), 3.47−3.78 (m, 4H), 2.49 (t, $J$ = 7.0 Hz, 2H), 1.78 (pent, $J$ = 6.5 Hz, 2H), 1.43 (s, 9H), 0.94 (t, $J$ = 8.4 Hz, 2H), 0.87 (s, 9H), 0.04 (s, 6H), 0.01 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 184.5, 157.0, 155.5, 98.9, 80.1, 78.9, 63.4, 61.7, 61.1, 42.0, 30.6, 28.2, 25.8, 18.2, 17.6, 15.7, −1.6, −5.5; HRMS (ESI+) calcd for $C_{25}H_{48}N_2NaO_6Si_2$ $[M + Na]^+$ 551.2943, found: 551.2955 (error 2.2 ppm).

*(±)-3-[(tert-Butoxycarbonyl)amino]-6-(3-hydroxypropyl)-2,3-dihydro-1H-pyridin-4-one (**38**).* To a solution of 1 M TBAF in THF (2.72 mL, 2.72 mmol, 4.0 equiv) at 0 °C were added $H_2O$ (0.74 mL, 40.8 mmol, 60 equiv), THF (10 mL), and a solution of **37** (0.36 g, 0.68 mmol, 1.0 equiv) in THF (10 mL), respectively. The reaction was allowed to warm to 23 °C over 2 h. After 16 h, 4 Å molecular sieves (6 g) were added, and 5 min later saturated aqueous $NaHCO_3$ (30 mL) was added to quench. The reaction mixture was filtered through a thin pad of Celite and extracted with EtOAc (3 × 25 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (0% MeOH−$CH_2Cl_2$ to 10% MeOH−$CH_2Cl_2$, linear gradient) afforded the title compound (72 mg, 39%) as a pale yellow oil: $R_f$ = 0.3 (10% MeOH−$CH_2Cl_2$); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.40 (br s, 1H), 5.63 (br s, 1H), 4.99 (s, 1H), 3.93−4.18 (m, 2H), 3.68 (t, $J$ = 5.9 Hz, 2H), 3.16 (t, $J$ = 13.1 Hz, 1H), 2.37 (t, $J$ = 7.0 Hz, 2H), 1.81 (pent, $J$ = 6.5 Hz, 2H), 1.44 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 188.6, 167.7, 156.2, 95.6, 79.9, 61.2, 52.0, 46.4, 31.5, 30.2, 28.3; HRMS (ESI+) calcd for $C_{13}H_{22}N_2NaO_4$ $[M + Na]^+$ 293.1472, found: 293.1469 (error 0.2 ppm).

*(±)-3-Amino-6-(3-hydroxypropyl)-2,3-dihydro-1H-pyridin-4-one hydrochloride (**3**).* To a prechilled flask containing **38** (12 mg, 0.044 mmol, 1.0 equiv) was added cold 4 M HCl in dioxane (2 mL). After 45 min at 0 °C, the reaction mixture was concentrated. The residue was taken up in a minimal amount of methanol and precipitated with ether to afford the title compound (7.5 mg, 82%) as a white powder in equilibrium with a dimer (4:1 monomer:dimer): $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 4.52 (t, $J$ = 6.9 Hz, 1H dimer), 4.18 (dd, $J$ = 16.2, 7.2 Hz, 1H dimer), 3.98−4.08 (m, 1H dimer, 1H monomer), 3.88 (dd, $J$ = 12.9, 6.7 Hz, 1H monomer), 3.66 (t, $J$ = 5.7 Hz, 2H, dimer), 3.58 (t, $J$ = 6.2 Hz, 2H monomer), 3.51 (t, $J$ = 13.7 Hz, 1H monomer), 2.84 (t, $J$ = 7.2 Hz, 2H, dimer), 2.41 (td, $J$ = 7.6, 2.5 Hz, 2H monomer), 1.97 (pent, $J$ = 6.6 Hz, 2H, dimer), 1.79 (pent, $J$ = 7.0 Hz, 2H monomer); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 183.9, 169.0, 93.6, 59.8, 49.4, 42.8, 30.63, 30.61; HRMS (ESI+) calcd for $C_8H_{15}N_2O_2$ $[M + H]^+$ 171.1128, found: 171.1133 (error 2.9 ppm).

*N-(tert-Butoxycarbonyl)-O-(tert-butoxydimethylsilyl)-serine-N′-methoxy-N′-methylamide (**39**).* To a solution of **5** (3.0 g, 12.1 mmol, 1.0 equiv) in DMF (35 mL) at 23 °C were added imidazole (2.49 g, 36.2 mmol, 3.0 equiv) and *tert*-butyldimethylchlorosilane (2.25 g, 14.5 mmol, 1.2 equiv) sequentially. After 16 h, the reaction was partitioned between 10% $KHSO_4$ (60 mL) and EtOAc (250 mL). The layers were separated, and the organic layer was washed with 10% $KHSO_4$ (1 × 60 mL) and saturated aqueous $NaHCO_3$ (1 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (5% EtOAc−hexanes to 25% EtOAc−hexanes, linear gradient) afforded the title compound (4.16 g, 95%) as a clear oil. $^1$H NMR, $^{13}$C NMR, and HRMS data matched reported values.[57]

*N-(tert-Butoxycarbonyl)(methoxymethyl)serine-N′-methoxy-N′-methylamide (**40**).* To a solution of **39** (12.6 g, 34.8 mmol, 1.0 equiv) in THF (100 mL) at −78 °C was added solid KHMDS (13.2 g, 66.2 mmol, 1.9 equiv). After 1 h, MOMCl (8.0 g, 99.3 mmol, 2.9 equiv) was added dropwise, and the reaction was allowed to warm to 23 °C over 2 h. After 16 h, the reaction mixture was partitioned between saturated aqueous $NH_4Cl$ (200 mL) and EtOAc (250 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (1 × 250 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. To a solution of the resulting mixture in THF (130 mL) at 0 °C was added HF·pyridine (14 mL). Saturated aqueous $NaHCO_3$ (400 mL) was added after 4.5 h to quench. The product was extracted with EtOAc (2 × 300 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (25% EtOAc−hexanes to 55% EtOAc−hexanes, linear gradient) afforded the title compound (7.2 g, 71%) as a colorless oil: $R_f$ = 0.15 (50% EtOAc−hexanes); $^1$H NMR (400 MHz, acetone-$d_6$) $\delta$ 5.12 (br s, 0.5H), 4.76−4.95 (m, 2.5H), 3.91 (br s, 1H), 3.77 (s, 3H), 3.67−3.75 (m, 1H), 3.58−3.66 (m, 1H), 3.32 (br s, 3H), 3.14 (br s, 3H), 1.47 (s, 9H); $^{13}$C NMR (100 MHz, acetone-$d_6$) $\delta$ 171.5, 156.0, 81.1, 77.5, 61.9, (61.2, 61.1), (59.3, 57.7), 56.0, 32.6, 28.5; HRMS (ESI+) calcd for $C_{12}H_{24}N_2NaO_6$ $[M + Na]^+$ 315.1527, found 315.1538 (error 3.5 ppm).

*2-[(tert-Butoxycarbonyl)(methoxymethyl)amino]-8-(tert-butyldiphenylsilyloxy)-1-hydroxy-oct-4-yn-3-one (**41**).* To a solution of 5-(*tert*-butyldiphenylsilyloxy)-1-pentyne[51] (0.507 g, 1.57 mmol, 2.3 equiv) in THF (8 mL) at −78 °C was added *n*-BuLi (0.66 mL of a 2.5 M solution in hexanes, 1.64 mmol 2.4 equiv). The reaction was stirred for 1.5 h, and then a solution of **40** (200 mg, 0.68 mmol, 1.0 equiv) in THF (10 mL) was added dropwise. After 16 h at −78 °C, the reaction was quenched by the dropwise addition of AcOH (3 mL). The reaction mixture was allowed to warm to 23 °C and partitioned between saturated aqueous $NH_4Cl$ (20 mL) and EtOAc (50 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 40 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (5%



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EtOAc–hexanes to 40% EtOAc–hexanes, linear gradient) afforded the title compound (185 mg, 49%) as a pale yellow oil: $R_f$ = 0.55 (50% EtOAc–hexanes); $^1$H NMR (400 MHz, acetone-$d_6$, ratio of rotamers 1:1) $\delta$ 7.63–7.78 (m, 4H), 7.38–7.53 (m, 6H), 4.89 (dd, $J$ = 10.8, 6.0 Hz, 1H), 4.75 (dd, $J$ = 10.8, 6.0 Hz, 1H), 4.07–4.30 (m, 1.5H), 3.75–4.00 (m, 3.5H), 3.38 (s, 1.5 H), 3.36 (s, 1.5H), 2.57–2.67 (m, 2H), 1.80–1.91 (m, 2H), 1.48 (s, 4.5 H), 1.40 (s, 4.5H), 1.05 (s, 9H); $^{13}$C NMR (100 MHz, acetone-$d_6$) $\delta$ (185.2, 184.9), (155.6, 155.3), 136.1, 134.4, 130.7, 128.8, (95.3, 94.9), (81.8, 81.5), (80.48, 80.41), (80.33, 80.14), (69.7, 69.4), 63.0, (61.4, 60.9), (56.4, 56.3), (31.58, 31.53), (28.6, 28.4), 27.3, 19.8, 16.0; HRMS (ESI+) calcd for $C_{31}H_{43}NNaO_6Si$ $[M + Na]^+$ 576.2752, found 576.2744 (error 1.4 ppm).

*3-[(tert-Butoxycarbonyl)(methoxymethyl)amino]-6-[3-(tert-butyldiphenylsilyloxy)propyl]-2,3-dihydro-4H-pyran-4-one (**42**).* To a solution of **41** (23 mg, 0.042 mmol, 1.0 equiv) and $PPh_3AuCl$ (2 mg, 0.004 mmol, 0.1 equiv) in $CH_2Cl_2$ (15 mL) at −78 °C was added AgOTf (750 $\mu$g, 0.003 mmol, 0.075 equiv). The reaction was stirred for 40 min at −78 °C and then placed in an ice bath to warm to 0 °C. After 30 min, the reaction mixture was partitioned between saturated aqueous NaCl (5 mL) and $CH_2Cl_2$ (10 mL). The layers were separated, and the aqueous layer was extracted with $CH_2Cl_2$ (1 × 10 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (100 mL of hexanes, 150 mL of 10% EtOAc–hexanes, 75 mL of 15% EtOAc–hexanes, 100 mL of 20% EtOAc–hexanes) afforded the title compound (12 mg, 52%) as a pale yellow oil: $R_f$ = 0.3 (20% EtOAc–hexanes); $^1$H NMR (400 MHz, acetone-$d_6$, ~1.4:1 mixture of rotamers) $\delta$ 7.62–7.77 (m, 4H), 7.35–7.54 (m, 6H), 5.30 (s, 1H), 4.39–4.82 (m, 4.3H), 4.21 (dd, $J$ = 13.7, 6.3 Hz, 0.7H), 3.75 (t, $J$ = 6.3 Hz, 2H), 3.30 (s, 3H), 2.45 (t, $J$ = 7.4 Hz, 2H), 1.87 (pent, $J$ = 6.9 Hz, 3H), 1.47 (s, 3.7H), 1.37 (s, 5.3H), 1.05 (s, 9H); $^{13}$C NMR (100 MHz, acetone-$d_6$) $\delta$ (189.6, 189.5), (177.54, 177.47), (155.4, 155.1), 136.4, 134.5, 130.7, 128.8, (104.4, 104.1), 81.5, (79.9, 79.7), (70.4, 70.3), 63.6, (58.9, 58.2), (55.7, 55.6), 31.5, 30.2, (28.4, 28.3), 27.3, 19.8; HRMS (ESI+) calcd for $C_{31}H_{43}NNaO_6Si$ $[M + Na]^+$ 576.2752, found 576.2750 (error 0.3 ppm).

*3-[(tert-Butoxycarbonyl)(methoxymethyl)amino]-6-(3-hydroxypropyl)-2,3-dihydro-4H-pyran-4-one (**43**).* To a solution of **42** (0.48 g, 0.87 mmol, 1.0 equiv) in THF (45 mL) at 0 °C was added HF·pyridine (4.5 mL) dropwise. The reaction was stirred for 16 h at 0 °C and then quenched with saturated aqueous $NaHCO_3$ (100 mL) over 5 min. The aqueous layer was extracted with EtOAc (3 × 80 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (15% EtOAc–hexanes to 80% EtOAc–hexanes, linear gradient) afforded the title compound (0.189 g, 69%) as a pale yellow oil: $R_f$ = 0.55 (EtOAc); $^1$H NMR ($CDCl_3$, 500 MHz, 1:1 mixture of rotamers) $\delta$ 5.40 (s, 0.5 H), 5.37 (s, 0.5H), 4.80 (dd, J = 11.2, 5.9 Hz, 1H), 4.53–4.72 (m, 2.5H), 4.44–4.50 (m, 1H), 4.24 (dd, $J$ = 13.9, 6.0 Hz, 0.5H), 3.70 (br s, 2H), 3.36 (s, 1.5H), 3.32 (s, 1.5H), 2.29–2.44 (m, 2H), 1.76–1.89 (m, 2H), 1.49 (s, 4.5H), 1.44 (s, 4.5H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ (189.7, 189.4), (177.2, 176.9), (154.6, 154.5), (103.9, 103.5), (81.8, 81.5), (79.3, 79.1), (69.7, 69.6), 61.7, (57.9, 57.2), (55.7, 55.5), 31.0, 29.2, (28.2, 28.1); HRMS (ESI+) calcd for $C_{15}H_{25}NNaO_6$ $[M + Na]^+$ 338.1574, found 338.1579 (error 1.5 ppm).

*3-Amino-6-(3-hydroxypropyl)-2,3-dihydro-4H-pyran-4-one (**2**).* To a solution of **43** (9.0 mg, 0.029 mmol, 1.0 equiv) in MeCN (0.5 mL) was added $BiCl_3$ (17 mg, 0.054 mmol, 1.9 equiv). The reaction was then sealed and heated at 55 °C for 2 h. MeOH (5 mL) was then added, and the reaction mixture was filtered and then concentrated to 3 mL. $H_2O$ (10 mL) was added, and the mixture was filtered again. Removal of the solvent afforded the title compound (2.9 mg, 49%) as a white solid: $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 5.49 (s, 1H), 4.75 (dd, $J$ = 10.0, 5.0 Hz, 1H), 4.18–4.32 (m, 2H), 3.56 (t, $J$ = 6.1 Hz, 2H), 2.41 (t, $J$ = 7.6 Hz, 2H), 1.77 (pent, $J$ = 7.0 Hz, 2H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 187.9, 181.9, 103.1, 69.2, 61.9, 50.8, 32.4, 30.4; HRMS (ESI+) calcd for $C_8H_{14}NO_3$ $[M + H]^+$ 172.0968, found 172.0967 (error 0.4 ppm).

**Enzymatic Assay To Determine the Kinetic Parameters of Inhibition of BioA.** *A. Assay Procedure.* 50× DMSO solutions of inhibitor (final concentrations 0, 200 $\mu$M, 400 $\mu$M, 700 $\mu$M, and 1 mM) were added to 1× buffer solutions of 100 mM Bicine, 50 mM $NaHCO_3$, 1 mM $MgCl_2$, 5 mM ATP, pH 8.6. BioA (0.5 $\mu$M) was then added to each to well (total volume 50 $\mu$L) to initiate BioA inactivation.

To measure residual BioA activity, a coupled assay with BioD was used, which together with BioA converts 7-keto-8-aminopelargonic acid (KAPA) to dethiobiotin. This was accomplished by removing a 5 $\mu$L aliquot of the initial solution at various incubation time points (2.5, 5, 10, 20, and 40 min) and adding to 95 $\mu$L of a reaction solution, containing saturating concentrations of all substrates, and diluting the initial inhibitor 20-fold, ensuring no further inhibition. The final concentrations present in the reaction solution are 100 mM Bicine (pH 8.6), 50 mM $NaHCO_3$, 1 mM $MgCl_2$, 5 mM ATP, 5 mM SAM, 25 nM BioA, 2 $\mu$M BioD, 1 mM TCEP, and 25 $\mu$M KAPA.[58] The reaction solutions were run for 60 min (which remained under initial velocity conditions) and then quenched with a solution of 500 nM biotin in 10% trichloroacetic acid. The dethiobiotin concentration was quantified by LC-MS/MS analysis with a gradient from 0 to 100% MeCN–$H_2O$ containing 0.1% formic acid. Biotin was monitored through the $m/z$ 243 → 200 transition, and dethiobiotin was monitored through the $m/z$ 213 → 170 transition. Assays were run in duplicate on multiple days. The negative control contained no inhibitor (DMSO only), and the positive control contained no BioA.

*B. Data Analysis.* The LC-MS/MS traces were analyzed by MultiQuant 2.0.2 to obtain the area under the curve (AUC) for both dethiobiotin (analyte) and biotin (internal standard). Then the dethiobiotin AUC was divided by the biotin AUC, and this number was converted into a concentration using the standard curve. A plot was generated of preincubation time vs percentage of BioA activity remaining, and curves for each concentration of inhibitor were fit to eq 3 with Graphpad Prism to obtain values for $k_{obs}$ at each inhibitor concentration:

$$y = Ae^{-k_{obs}t} \quad (1)$$

In eq 1, $y$ is the concentration of dethiobiotin, and $A$ is the activity observed with no inhibitor. The concentration of inhibitor was then plotted against the generated $k_{obs}$ values, and this was fit to eq 2 with Graphpad Prism to determine the $K_I$ and $k_{inact}$ values.

$$k_{obs} = k_{inact}\frac{[I]}{K_I + [I]} \quad (2)$$

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b00847.

$^1$H NMR and $^{13}$C NMR spectra for all compounds (PDF)

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### Notes

The authors declare no competing financial interest.

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