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Design and Evaluation of Ambiphilic Aryl Thiol−Iminium-Based Molecules for Organocatalyzed Thioacyl Aminolysis

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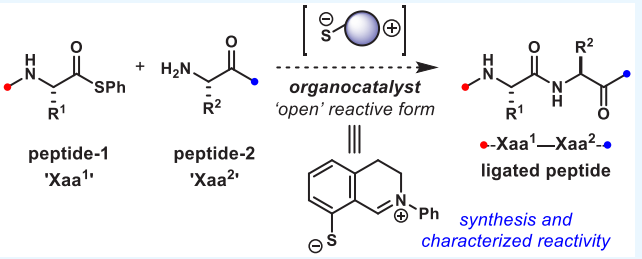
ABSTRACT: Progress toward the design and synthesis of ambiphilic aryl thiol−iminium-based small molecules for organocatalyzed thioacyl aminolysis is reported. Here we describe the synthesis of a novel tetrahydroisoquinoline-derived scaffold, bearing both thiol and iminium functionalities, capable of promoting the transthioesterification and subsequent amine capture reactions necessary to achieve organocatalyzed thioacyl aminolysis. Model studies demonstrate the ability of this designed organocatalyst to deliver critical intermediates capable of undergoing these individual reactions necessary for the proposed process. Future design improvements and directions toward cysteine-independent organocatalyzed native chemical ligation are discussed.



1. INTRODUCTION

Over the past several decades, advances in chemical protein synthesis (CPS) have enabled the evaluation of uniquely synthetic proteins among those accessible by traditional biochemical and recombinant technologies.[1] As the applicability of CPS methods broadens, especially due to growing interest in mirror-image and site-specifically modified proteins that are exclusive to chemical synthesis, there is an aligned need for the discovery and development of orthogonal peptide ligation strategies.[2] These advances should be chemoselective, providing alternative retrosynthetic ligation disconnects aimed at improving the overall efficiency of CPS.[3] The ubiquity of native chemical ligation (NCL)[4] and Ala-ligation methods[5] used in CPS endeavors has proven their robustness, yet residue-specific limitations (i.e., the need for Cys or Ala residues) have motivated innovative developments.[3b,6,7] A few recent NCL-inspired technologies that have enabled Cys-independent access to proteins with total atomic control include the Staudinger,[8] α-keto acid−hydroxylamine (KAHA),[9] and Ser/Thr ligations.[10] Auxiliary-based strategies,[6] including aldehyde-capture ligation (ACL)[11] and related methods,[12,13] as well as *N*-terminal auxiliary ligations[14,15] have significantly extended the scope of NCL. Although enabling, such strategies can be limited to residue-specific ligation junctions, may rely on the synthesis of reactive *C*- and *N*-terminal auxiliaries, and furthermore can involve postligation modification steps, restricting their overall generality to skilled practitioners.

To streamline protein retrosynthesis and develop tools to improve CPS user access, we hypothesized that a rationally designed ambiphilic organocatalyst combining nucleophilic (e.g., thiol)[4,14,15] and electrophilic (e.g., aldehyde, iminium)[11−13,16−18] functionalities could catalyze an aqueous thioester aminolysis reaction between solid-phase peptide synthesis accessible peptide partners (Figure 1a).[19] Inspired by the mechanisms of NCL and auxiliary-based ligation reactions,[4,6,20] we envisioned an organocatalytic process to achieve Cys-independent thioacyl aminolysis between any *C*-terminal thioester peptide-1 and *N*-terminal peptide-2. Overall, the ambiphilic organocatalyst could enable transthioesterification, amine capture, and *S*-to-*N* acyl transfer events to form a ligated peptide.

In a recent report, we demonstrated the dynamic properties of a series of electronically perturbed cyclic *N*,*S*-acetals (Figure 1b).[20] Under acidic conditions **1** undergoes C1−S bond ionization to form a ring-opened zwitterionic intermediate bearing aryl thiol(ate) and benzylic iminium functionalities that satisfied our ambiphilic organocatalyst design features. Due to the efficient synthetic access to these cyclic *N*,*S*-acetals and promising early observations that suggested transthioesterification and amine capture events could occur in a dynamic process, we explored their competency in model organocatalyzed thioacyl aminolysis reactions (Figure 1). Unfortunately, despite extensive efforts with **1**, we were unable to achieve organocatalyzed thioacyl aminolysis reactions. We hypothesized that the process inefficiency may be due in part to the poor water solubility of **1**. Furthermore, the proposed catalytic cycle may be hindered by the requirement for an *eight-*

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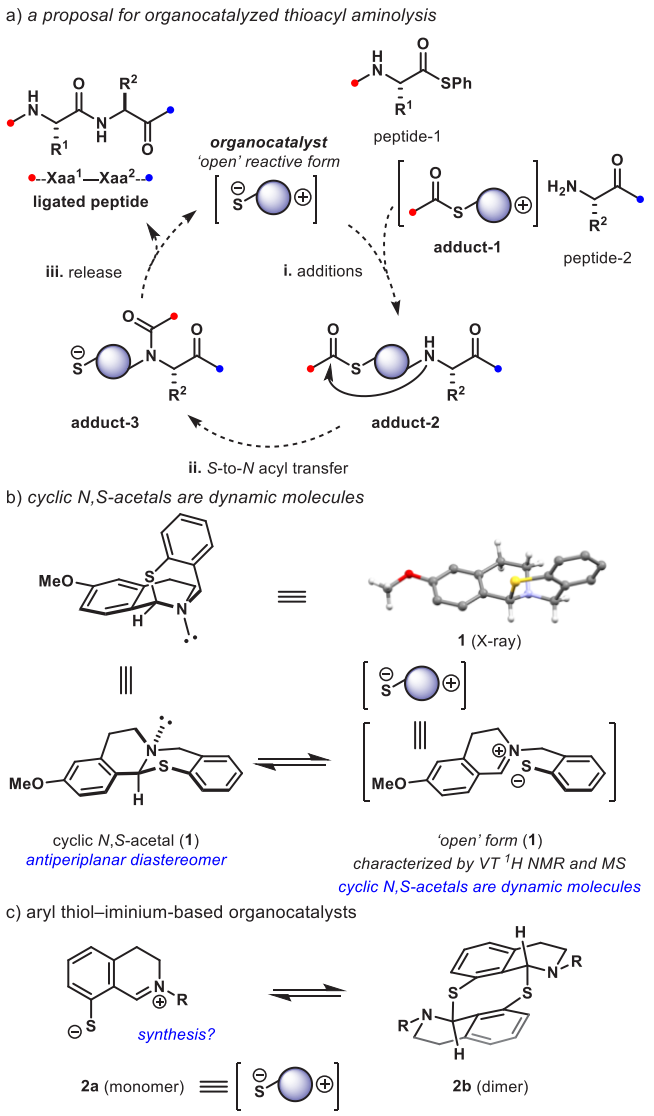

Figure 1. Organocatalyzed peptide ligation: will ambiphilic molecules promote thioacyl aminolysis reactions?

membered tetrahedral intermediate, which may be unfavorable for efficient *S*-to-*N* acyl transfer events.[6b,21] Preliminary observations from the evaluation of cyclic *N,S*-acetals informed key features to include in a revised organocatalyst design (Figure 1c). Ongoing efforts have focused on an organocatalyst design that might exhibit improved aqueous solubility due to greater ionic character while preferentially enabling *S*-to-*N* acyl transfer events via a more favorable *six-membered tetrahedral intermediate*. We therefore envisioned the design and synthesis of aryl thiol–iminium-based organocatalyst **2a** (Figure 1c). We find that **2a** exists in dynamic equilibrium with its dimeric form (**2b**). Guided by our knowledge of the dynamic reactivity of *N,S*-acetals, we herein describe our design logic, synthesis, and evaluation efforts toward the development of an aryl thiol–iminium-based ambiphile for organocatalyzed thioacyl aminolysis.

2. RESULTS AND DISCUSSION

2.1. Reactivity of Cyclic *N,S*-Acetals from Initial Thioacyl Aminolysis Studies. To better understand the reactivity of aryl thiol–iminium-based ambiphiles as designed organocatalysts for NCL, we directed our efforts to evaluate the reactions of **1** with model peptide thioesters. The *N,S*-acetal moiety of **1** provides the latent nucleophilic thiol and electrophilic iminium[18] functionalities required for respective transthioesterification and amine capture events (Figure 1a). Ideally, once both peptide fragments are captured, an *S*-to-*N* acyl transfer would occur to complete the peptide ligation process. Interestingly, we discovered that **1** incorporated several dynamic properties that allowed us to develop and evaluate its ability to undergo transthioesterification (Figure 2). The dynamic reactivity observed in our first-generation *N,S*-acetals includes ring-opening and ring-closing behavior.[20] The treatment of **1** in excess CF_3CO_2D resulted in rapid and quantitative C1–S bond ionization, enabling interchangeable ring-opened and ring-closed forms under equilibrium control by titration with triethylamine (Figure 2a). With an operationally reversible system in hand, we proposed that *in situ* formed ring-opened species (e.g., **3**) could participate in transthioesterification reactions with model peptide thioesters (**4**), followed by a subsequent amine capture and *S*-to-*N* acyl transfer event to achieve an overall organocatalyzed thioacyl aminolysis process (Figure 2b). Despite the observed production of transthioesterification intermediates such as adduct-1 (**5**), these intermediates do not appear to undergo efficient amine capture events. Therefore, informed by shortcomings with **1**, we set out to prepare revised organocatalyst **2a** (Scheme 1).

2.2. Synthesis of Organocatalyst 2a and Its Corresponding *N,S*-Acetal Dimer 2b. In the design stage, we reasoned that improving the likelihood of the amine capture step would be integral to balance the relative rates of transthioesterification, amine capture, and *S*-to-*N* acyl transfer events. In NCL reactions the transthioesterification step is rate-limiting, whereas in *N*-terminal auxiliary-based NCLs, the rate-limiting step is the *S*-to-*N* acyl transfer event.[22] We presume that the tethered nature of the thiol in **1**, which predominately exists in the ring-closed state, precludes amine capture events. Building from this evaluation, we revised our synthetic efforts to design a system that would avoid trapping by an intramolecular thiol and improve the likelihood of *S*-to-*N* acyl transfer events via a hypothesized *six-membered tetrahedral intermediate*. These informed observations resulted in the design of the second-generation organocatalyst **2a** and its corresponding *N,S*-acetal dimer **2b**.

We developed an efficient route to prepare organocatalyst **2a** in six steps from commercially available 8-bromoisoquinoline (Scheme 1). We found that the proposed monomeric form **2a** exists in equilibrium with the functional *N,S*-acetal dimer **2b**. The synthesis begins with the chemoselective reduction of 8-bromoisoquinoline **6** upon treatment with sodium cyanoborohydride in the presence of boron trifluoride diethyl etherate to yield 8-bromo-1,2,3,4-tetrahydroisoquinoline **7**.[23] The resultant amine **7** is arylated with phenylboronic acid using Chan–Lam coupling conditions to provide 8-bromo-2-phenyl-1,2,3,4-tetrahydroisoquinoline **8** in 39% yield over two steps.[24] Initial observations suggest the *N*-phenyl tertiary amine **8** is susceptible to aerobic benzylic oxidation under ambient conditions. Therefore, a careful two-step procedure to convert aryl bromide **8** into dithiocarbamate **10** was developed. Using Buchwald's copper-catalyzed halogen exchange chemistry,[25] the aryl bromide **8** can be efficiently converted into the requisite aryl iodide **9** in 92% isolated yield. This aryl iodide (**9**) provides a handle to examine conditions for the installation

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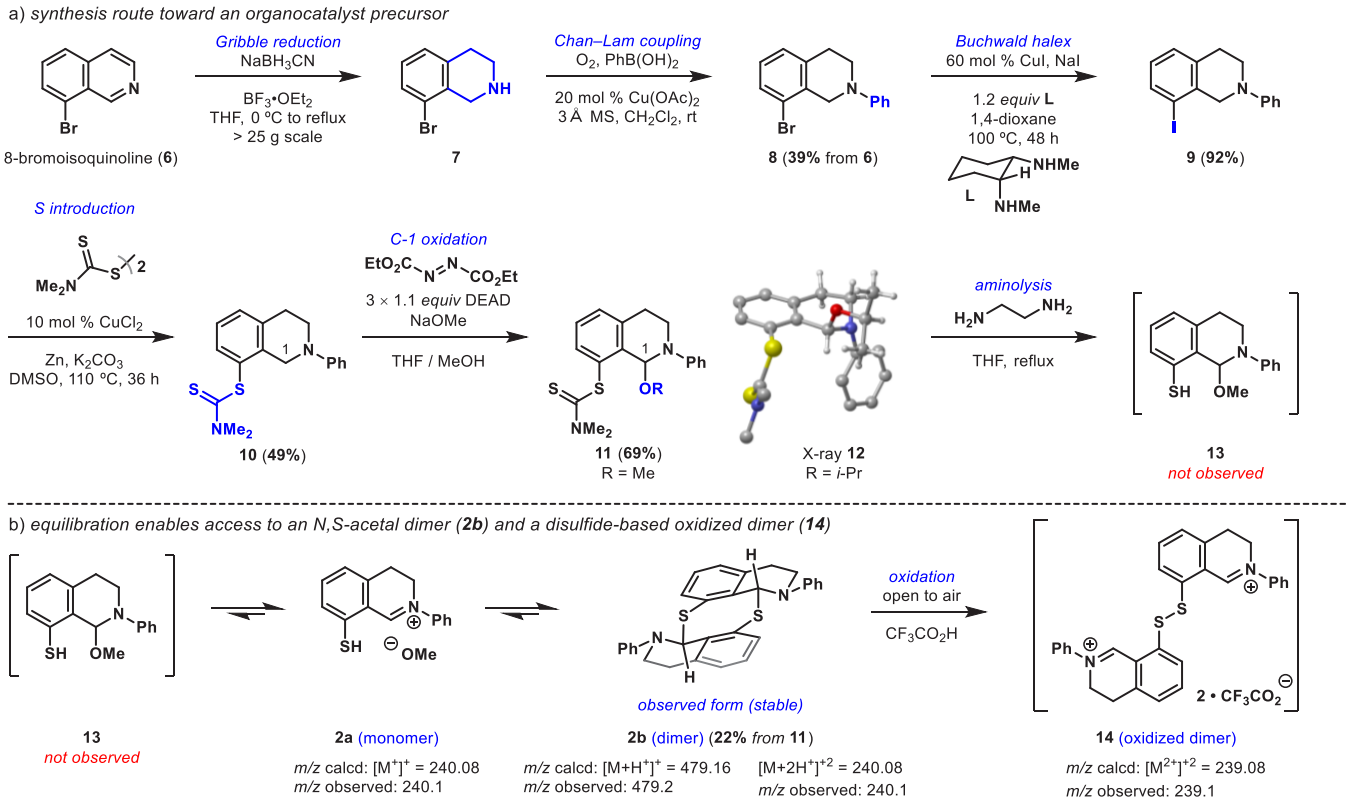
Scheme 1. Synthesis of a Functional Aryl Thiol−iminium Organocatalyst: (a) Synthesis Route toward an Organocatalyst Precursor; (b) Equilibration Enabling Access to an *N,S*-Acetal Dimer (2b) and an Alternative Disulfide-Based Oxidized Dimer (14)



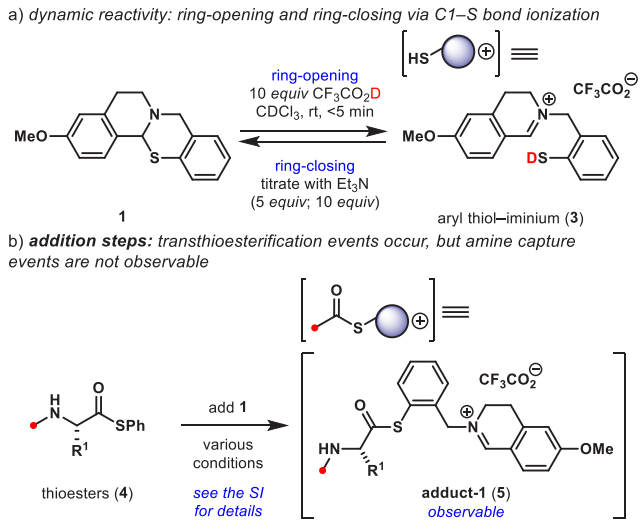


Figure 2. Cyclic *N,S*-acetals are dynamic in solution and reactive toward thioesters: (a) C1−S bond ionization of **1**; (2) addition steps. Transthioesterification events occur, but amine capture events are not observable.

of a sulfur-containing functionality at the 8-position. We found that **9** can be successfully converted into dithiocarbamate **10** in 49% yield using tetramethylthiuram disulfide and zinc in the presence of catalytic copper(II) chloride.[26] Notably, these reductive conditions prevent any undesired aerobic benzylic oxidation from occurring prematurely. Controlled benzylic oxidation (C1) using diethyl azodicarboxylate (DEAD) converts **10** into *N,O*-acetal **11**.[27] This benzylic oxidation may proceed via iminium ion formation followed by trapping with methanol. Optimization efforts led to the development of an efficient procedure to convert **10** into **11** in 69% isolated yield. The use of DEAD to affect this oxidation is superior to other oxidation chemistries that were specifically developed for 2-aryl-1,2,3,4-tetrahydroisoquinoline systems. The crystallization of **11** in 2-propanol produced derivative **12**, which was suitable for structure determination using X-ray diffraction (CCDC 2222419).[28] This suggests that **11** readily engages in a dynamic equilibrium exchange process, via the intermediate iminium ion, to provide **12**. Next, we evaluated a variety of conditions to cleave the dithiocarbamate moiety (**11**) and anticipated the isolation of aryl thiol **13**. We found that it was difficult to purify the cleavage byproducts away from presumed product **13**. We hypothesized using ethylenediamine would facilitate purification, as the cleavage byproducts would be acyclic or cyclic thioureas. Upon workup, we observed both the acyclic and cyclic thiourea byproducts, and their polarity differences allowed us to routinely isolate a stable product, first presumed to be **13**, in 22% yield by column chromatography. A thorough characterization of the stable product revealed its constitution to be most consistent with an *N,S*-acetal dimer **2b**, which we presume to be derived via the intermediacy of reactive monomer **2a** (Scheme 1b). The presumed aryl thiol **13** is not observed and likely converts into **2b** via reactive monomer **2a**.

2.3. Evaluation of *N,S*-Acetal Dimer 2b in a Dipeptide-Forming Model System. Intrigued by the preference for a dimeric state, we further studied the equilibria of *N,S*-acetal **2b** in aqueous solution (Scheme 1b). Under aqueous acidic conditions (3:1 CH_3CN:H_2O, 0.1%

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CF_3CO_2H), the monomeric **2a** and dimeric **2b** forms likely exist in equilibrium and are mostly indistinguishable by standard ESI mass spectrometry due to a similar dissociation to their preferred iminium states. However, ^1H and ^{13}C NMR spectroscopic data are most consistent with *N,S*-acetal dimer **2b**. The prolonged exposure of **2b** under aqueous conditions leads to the formation of an oxidized species that we assign to disulfide dimer **14**. We reason that **14** could be used as a precatalyst for organocatalyzed thioacyl aminolysis reactions in the presence of a suitable disulfide reductant (e.g., tris(2-carboxyethyl)phosphine). However, we find **2b** to be a more convenient, latent form of the designed reactive organocatalyst **2a**.

To elucidate the reactivity of **2a**, we combined **2b** with a single residue thioester Ac-Ala-SPh (**15**) (Figure 3a). An adduct, **16** (t_r = 4.2 min), consistent with transthioesterification is observable by UPLC-MS analysis. Encouraged by this, we added H-Gly-OMe (4 equiv) and allowed the aqueous mixture to react for 18 h. Analysis by UPLC-MS showed three distinct intermediates that we tentatively assign as *N,N*-acetal adducts (**17a,b**) (Figure 3b). These adducts are observed in iminium ion forms with different retention times (**17a**, t_r = 3.9 min; **17b**, t_r = 4.0 min; see the Supporting Information). Their distinct elution times suggest that each adduct is a unique *N,N*-acetal (**17a,b**), where attack of **16** by H-Gly-OMe yields diastereoisomers **17a,b**. We note that attack by water (X = OH) or thiophenol (X = SPh) may yield other possible adducts (**17c**). While the preference for ionization of **17a,b** to the common iminium ion (**16**) masks their structural identity, this result is consistent with our previous studies using **1** and supports the efficiency of the transthioesterification event.

To better understand the presumed formation of the two amine capture products **17a,b**, we considered an earlier spectroscopic observation where *N,O*-acetal **11** exhibits solvent-dependent interconversion of anti- and synperiplanar diastereomeric forms in chloroform-d_1. Interestingly, the ^1H NMR spectrum of **11** in benzene-d_6 shows a preference for the one diastereomeric form. This type of structural dynamicity via C1−O bond ionization is akin to similar observations made for cyclic *N,S*-acetal **1**, where interconversion occurs via C1−S bond ionization.[20] Taken together, we assign **17a,b** as diastereomeric Gly-OMe adducts and assign the third peak to the iminium ion intermediate **16**. The poor solubility of **2b** under buffered aqueous conditions limited our ability to quantify this complex process. Further studies are needed to understand the interconversion of **17a,b** as well as the implications of stereochemistry on the efficiency of productive *N,S*-acyl transfer events. We anticipate that solvent-dependent effects will play a critical role in tuning the reactivity of **2b**, as well as downstream reactivities of adduct-1 and adduct-2 type intermediates.

2.4. Evaluation of *N,S*-Acetal Dimer 2b in a Peptide-Forming Model System. Analytical and solubility complications with the dipeptide system led us to evaluate a more compatible model system using **2a**, peptide thioester **18**, and benzyl amine (Figure 4a). These experiments allowed us to

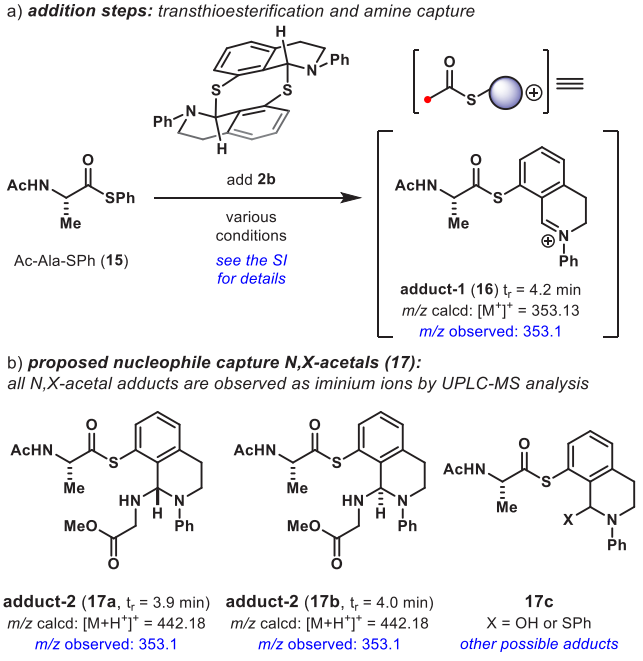

Figure 3. *N,S*-acetal dimer (**2b**) reacts with thioesters to form adduct-1 (**16**) and adduct-2 (**17a,b**).

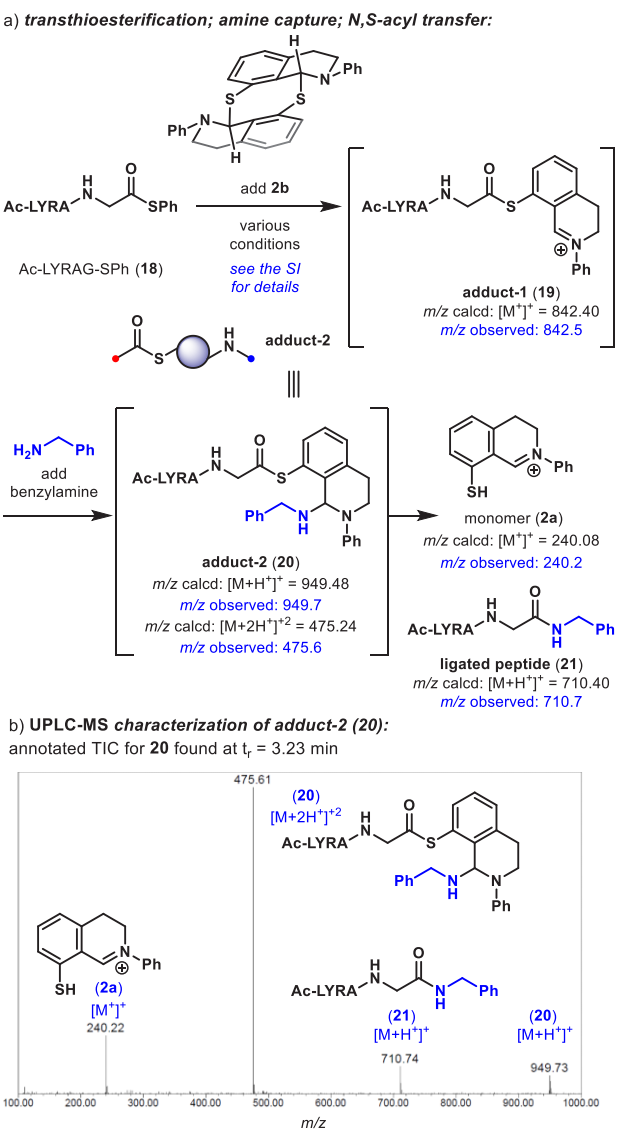

Figure 4. *N,S*-acetal dimer (**2b**) reacts with thioester peptide (**18**) to form adduct-1 (**19**) and adduct-2 (**20**).

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observe transthioesterifcation and amine capture events. The reaction of **2b** and Ac-LYRAG-SPh (**18**) yields transthioesterification product **19** in good conversion. We added an excess of benzylamine to this reaction mixture and observed a ternary adduct (**20**) that is consistent with a stepwise process involving transthioesterification followed by amine capture. The adduct (**20**) was characterized using UPLC-MS (Figure 4b). Again, TIC extraction (m/z 475) shows two peaks with distinct elution times (**20a**, t_r = 3.1 min; **20b**, t_r = 3.2 min; see the Supporting Information), which we attribute to the two diastereomeric benzyl amine adducts. When examining the MS data for each unique peak, we observe four ions (Figure 4b). Two ions correspond to the $[M + 2H^+]^{2+}$ and $[M + H^+]^+$ ions of **20**. Interestingly, the other ions correspond to ligated peptide **21** ($[M + H^+]^+$) and monomer **2a** ($[M^+]^+$). It is unusual to detect the product (**21**) and monomer (**2a**) in this region ($t_r \approx 3.2$ min), as they are respectively found at t_r = 0.7 min (**21**) and t_r = 4.4 min (**2a**). Therefore, we propose that the ionization of ternary adduct **20** produces **21** and regenerates monomer **2a**. Despite the encouraging observation of these adducts, organocatalyzed and organopromoted experiments using **2b** did not show an indication of improved reactivity when compared with the background aminolysis experiments without **2b**.[29] Ongoing efforts are focused on the development of improved analytical methods to better characterize stepwise events toward organocatalyzed NCL.

3. CONCLUSIONS

Our objective to understand the reactivity of *N*,*S*-acetals for use in organocatalyzed thioacyl aminolysis has led to several discoveries. These underexplored ambiphilic molecules are dynamic in solution and exhibit the ability to reversibly exchange adducts via C1−S bond ionization to produce observable reactive benzylic iminium ion intermediates. With our first-generation design (**1**) we characterized dynamic C1−S bond ionization and transthioesterification reactivity. However, amine capture and *N*-to-*S* acyl transfer events remained elusive, leading us to prepare the dimeric *N*,*S*-acetal system **2b** that can undergo transthioesterification and amine capture reactions via the monomeric intermediate **2a**. The transthioesterification process using **2b** is more efficient than the corresponding reactivity of **1**. While amine adducts at C1 are observable and are supported by an amine capture experiment using benzylamine, they do not appear to be the predominate species under the evaluated conditions and are elusive when using amino ester model systems under buffered conditions.[30,31] Although further development of conditions and organocatalyst refinement are needed, we are encouraged by the initial reactivity of these novel *N*,*S*-acetal-based systems toward realizing organocatalyzed thioacyl aminolysis procedures.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c07586.

Materials and methods, general experimental procedures, characterization data and spectra for associated molecules, and reaction analyses (PDF)

Crystallographic data for compound **12** (CIF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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