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Thiourea-catalyzed aminolysis of *N*-acyl homoserine lactones†

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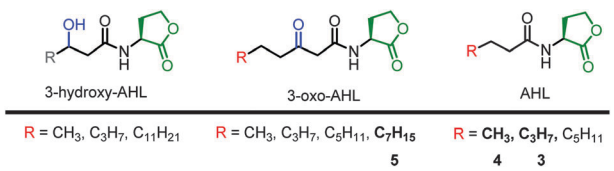
Thiourea catalysts accelerate aminolysis of *N*-acyl homoserine lactones (AHLs), molecules integral to bacterial quorum sensing. The catalysts afford rate enhancement of up to 10 times the control in CD_3CN. Mild catalysis in other polar aprotic solvents is still observed, while the activity is attenuated in polar protic solvents.

Translating well-established organic reactions to physiological conditions is one approach for solving contemporary problems in biology. Bacterial virulence mediated by a density-dependent communication pathway known as quorum sensing (QS) is one such problem.[1] For instance, QS pathways in *P.aeruginosa* and *B. cepacia* are responsible for opportunistic infections in immunocompromised patients.[2]

In most gram-negative bacteria, *N*-acyl homoserine lactones (AHLs) are integral to this multicellular communication network (Scheme 1).[3] Efforts to inhibit QS have focused on small molecule inhibitors of the AHL synthases and receptors, while methods of disabling the AHL messenger directly have been less extensively investigated.[4–6] In fact, beyond the physiological degradation of the AHLs, their fundamental reactivity is not well documented.



Scheme 1 AHLs implicated in quorum sensing; sites for interspecies variability include the length of the *N*-acyl chain (red) and the oxidation state (blue). The C_6-AHL **3**, the C_4-AHL **4**, and 3-oxo-C_{12}-AHL **5** were each tested for amenability to thiourea catalysis.

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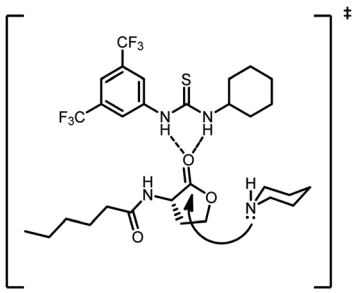
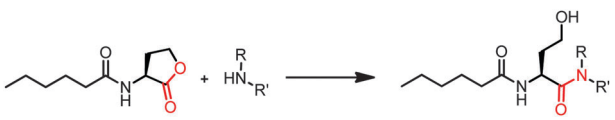
† Electronic supplementary information (ESI) available: Synthetic procedures, ^1H and ^{13}C NMR spectra, and catalyst screening procedure. See DOI: 10.1039/c3cc00268c



Scheme 2 The proposed mode of activation of an AHL through H-bonding with a thiourea catalyst.

To begin addressing this deficiency, we sought to determine the susceptibility of AHLs to nucleophilic ring opening, specifically by amines to form stable amides (eqn (1)).

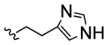
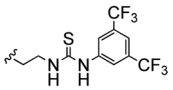
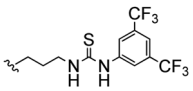
Traditionally, reactions of amines with butyrolactones are sluggish and require a large excess of the nucleophile and/or heat.[7] The recalcitrant nature of this simple acyl transfer reaction offered the opportunity to develop catalysts for the aminolysis of AHLs. Use of thiourea organocatalysts appeared fitting as they have proven effective in the supramolecular activation of esters (Scheme 2).[8] This H-bond activation mechanism has been showcased in a variety of classic organic methodologies, including the polymerization of cyclic esters.[9] Of relevance to potential biological applications are examples of thiourea catalysis in water.[10] In this communication, the utility of thiourea catalysts for the aminolysis of AHLs is assessed to determine if these catalysts can be utilized for irreversible AHL derivitization.



(1)

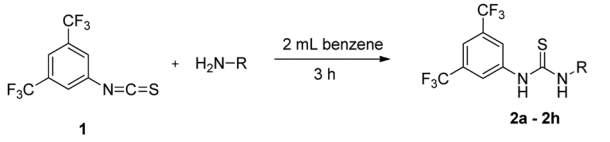
To evaluate the ability of thiourea organocatalysts to accelerate the aminolysis of AHLs, we synthesized a small library of catalysts (Table 1). The thioureas were assembled

Table 1 Yields for the synthesis of each thiourea catalyst and relative rate constants for 10 mol% of the catalysts in aminolysis of the C_6-AHL with piperidine[a]

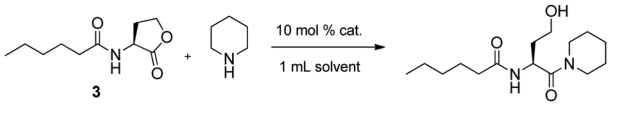
Catalyst	R	Yield (%)	k_{rel} CD_3CN	k_{rel} DMF-d_7
2a		90	4.5	3.4
2b		84	5.6	3.6
2c		82	4.3	3.4
2d		87	4.4	3.3
2e		38	5.5	3.2
2f		11	4.5	—
2g		79	7.9	5.2
2h		83	10	5.3

[a] For further details on the screening procedure, see ESI. The reactions were monitored until 50% conversion (t_{50}) was reached. See Table 3 for the t_{50} values of the uncatalyzed reaction.

through the reaction of a primary amine with 3,5-bis(trifluoromethyl) phenylisothiocyanate (**1**) to form **2** (eqn (2)). The electron withdrawing ability of the 3,5-bis(trifluoromethyl) phenyl functionality is known to enhance the H-bond donating ability and consequent catalytic potency of the thiourea.[11] Eight potential organocatalysts were synthesized; monofunctional catalysts **2a** and **2b** from simple alkyl amines, and bifunctional catalysts **2c–2f** from alkylated diamines and histamine. The scope of bifunctional catalysts ranged from catalysts hypothesized to assist in nucleophile activation by H-bonding interactions (**2c** and **2d**) to those conceivably providing anionic transition state stabilization (**2f**).[9,12] The reaction of simple diamines with **1** yielded dithiourea compounds **2g** and **2h**.



(2)



(3)

The catalysts were then screened for activity in a C_6-AHL (**3**) (50 mM) and piperidine (20 eq.) control reaction (eqn 3).‡ AHL conversion in each catalytic trial was monitored by ^{1}H NMR spectroscopy in CD_3CN. Prior to the addition of piperidine, a slight downfield shift in the N–H resonances of the thiourea was observed, supporting an AHL–catalyst interaction. The entire library of thioureas proved active in accelerating the aminolysis of the AHL. The monofunctional and bifunctional thioureas enhanced the nucleophilic ring opening up to 5.6 times the background reaction while the dithioureas reached a rate 10-fold faster than the control. Notably, the bifunctional catalysts did not provide any noticeable advantage compared to the monofunctional catalysts. This lack of secondary activation may be due to an inherent weakness of the anticipated N–H···N bonding interaction between the amine nucleophile and the nitrogen containing appendages of the catalysts.

Given the structural variety in the AHL family, two other AHLs were tested. The C_4-AHL (**4**) and 3-oxo-C_{12}-AHL (**5**) are both utilized by the human pathogen *P.aeruginosa*, but vary in alkyl chain length; the 3-oxo-C_{12}-AHL is also oxidized at C_3 of the alkyl chain (Scheme 1).[3] Both AHLs were screened in CD_3CN in the presence of catalyst **2a** and our best performing catalyst **2h** (10 mol%) (Table 2). The C_4-AHL performed comparably to the C_6-AHL. On the other hand, the barely soluble 3-oxo-C_{12}-AHL was less amenable to thiourea catalysis with **2a** and **2h**, displaying a rate increase only 2.4 and 4.0 times the control, respectively. Though lipophilic interactions could be leading to substrate aggregation in the markedly nonpolar 3-oxo-C_{12}-AHL, the decrease in relative rate may also be attributed to the extra carbonyl moiety's competitive affinity for the thiourea.

The effect of solvent on catalysis and thermal background reactivity was also assessed (Table 3). In general, more polar solvents decelerated the aminolysis rate (entries 1–5), though catalytic rate accelerations were still observed. Catalyst **2h** was quite effective in DMF-d_7 (entry 4), a solvent that we expected might deactivate the catalyst by competitive H-bonding.

To progress closer to physiological conditions, a variety of protic co-solvents (10% v/v) were tested. These protic additives

Table 2 Relative rates of aminolysis of different AHLs in CD_3CN[a]

AHL	k_{rel} (**2a**)	k_{rel} (**2h**)
C_6-AHL (**3**)	4.5	10
C_4-AHL (**4**)	4.1	7.3
3-oxo-C_{12}-AHL (**5**)	2.7	4.0

[a] With piperidine as the amine nucleophile in 20-fold excess.

Table 3 Relative rates of C_6-AHL aminolysis in the presence of catalysts **2a** and **2h** in aprotic solvents and protic solvent mixtures[a]

Entry	Solvent (% v/v)	t_{50}[b] uncat ($\times 10^3$ s)	k_{rel} (**2a**)	k_{rel} (**2h**)
1	C_6D_6	5.1	2.0	4.3
2	CD_2Cl_2	3.6	1.0	1.1
3	CD_3CN	35.5	4.5	10
4	DMF-d_7	100.7	3.4	5.3
5	DMSO-d_6	123.2	1.9	2.2
6	10% MeOH-d_4–CD_3CN	14.3	0.9	1.5
7	10% D_2O–DMSO-d_6	21.0	1.3	1.0
8	10% TFE-d_3–CD_3CN	9.7	1.1	1.0
9	10% D_2O–CD_3CN	4.2	1.0	0.9

[a] With piperidine as the amine nucleophile in 20-fold excess. [b] t_{50} represents the reaction time to reach 50% conversion.

dramatically accelerated the aminolysis reactions, but negated the effects of the added catalysts (entries 6–9, Table 3). Overall, increasing solvent polarity served to decrease background reaction rates, while protic additives increased them. It seems reasonable to suggest that in addition to accelerating the background reaction, the protic nature of the co-solvents simultaneously deactivate the catalyst by inhibiting the necessary supramolecular events that have been proposed for thiourea catalysis (Scheme 2).[9–12]

Taking simple, well-establish synthetics methodologies and applying them to biologically relevant targets helps assess the utility of those methods in a physiological setting. In the present case, thioureas have been demonstrated to provide up to 10-fold rate accelerations for the aminolysis of AHLs. However, attempts to transition these observations to conditions that are more physiological (polar protic) reveal the significant challenge of using small molecule catalysts in such environments. Nevertheless, information of AHL fundamental reactivity has emerged from these studies.

We wish to acknowledge Dr Masa Matsumoto, Dr Silvia Bezer-Bratu and Dr Mee-Kyung Chung for their valuable insights and offer a special thanks to Dr Marcey L. Waters and the US Army Research Office for their support.

Notes and references

‡ Piperidine was chosen during a screen of several amines for reaction with C_6-AHL. The timescale of the reaction was appropriate for monitoring aminolysis by ^{1}H NMR.

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