Organic & Biomolecular Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

Journal Name

RSCPublishing

COMMUNICATION

Selective transamidation of the 3-oxo-*N*-acyl homoserine lactones by hydrazine derivatives

Cite this: DOI: 10.1039/xoxxoooooox

Michael A. Bertucci,[a] Stephen J. Lee,[b] and Michel R. Gagne[a*]

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

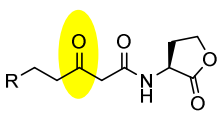
www.rsc.org/

A method for the selective transamidation of the 3-oxo subfamily of *N*-acyl homoserine lactones (3-oxo-AHLs) under physiologically relevant conditions has been developed. The reaction has the potential to serve as a strategy for selective knockdown of key autoinducers in a multicellular environment.

Chemical strategies for the manipulation of bacterial quorum sensing (QS) have advanced the understanding of this bacterial communication phenomenon while also creating approaches for its inhibition.[1] As quorum sensing has been demonstrated as a mechanism for virulence in a variety of human and plant pathogens, including *P.aeruginosa*, it has become a prominent target for the development of anti-virulence strategies.[2] Some of the chemical methods for intervening in QS include the design of small molecule analogs,[1a] use of catalytic antibodies,[3] and synthesis of abiotic polymers.[4]

The intercellular communication process that underlies quorum sensing is regulated by small signaling molecules that allow the bacteria to assess the local concentration of cells and thus regulate colony-wide function. In gram-negative bacteria, the majority of these signaling molecules are the structurally related *N*-acyl homoserine lactones (AHLs). The 3-oxo-subfamily of these molecules, contain a ketone at the β-position relative to the α-amino-γ-butyrolactone and have been identified as key autoinducers in numerous pathogens (Table 1).[1a,5] Chemical methods that specifically modify the 3-oxo-AHLs could be valuable for studying the bacteria that produce them in a multicellular environment.

Table 1. 3-oxo-AHLs in pathogenic bacteria



R	Pathogen
$-CH_3$	*S.marcescens, E. carotovora*
$-C_3H_7$	*A. tumefaciens*
$-C_7H_{13}$	*P.putida, P.aeruginosa*

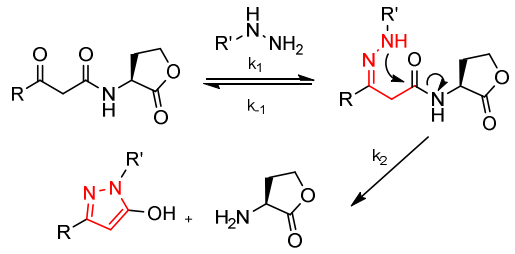
The nucleophilicity of amines in water is critical to many biochemical pathways in nature, including protein synthesis[6] and histone posttranslational modifications.[7] In a non-enzymatic environment, harnessing this nucleophilicity at physiological pH is hindered by their basicity, which ensures that they will predominately exist in their protonated form. The low concentration of the neutral, nucleophilic amine form decreases its propensity to participate in addition and acyl transfer reactions.[8]

Despite this challenge, synthetic chemists have developed strategies for eliciting nucleophilic amine behavior for modifying biomolecules in water. The widely employed method of native chemical ligation (NCL) for protein synthesis couples reversible thiol-thioester exchange with an irreversible S→N acyl shift to form amide bonds. Thioester formation favorably positions the N-terminal amine of a cysteine residue for intramolecular cyclization/acyl transfer through a low-energy five-membered transition state.[9] The preorganization of the thioester/amine pair ensures that efficient (by virtue of being intramolecular) trapping of the low concentration of amine ensues.

Alpha-effect amines such as hydrazine and hydroxylamine have also been used to affect nucleophilic addition reactions in water. Their lowered basicities but high nucleophilicity parameters have enabled their use for chemoselective condensations onto aldehydes and ketones, leading to their classification as bioorthorthogonal reagents.[10] Although innately sluggish at neutral pH, the rates of both oxime and hydrazone ligations have been enhanced with aniline-based catalysts.[11] In some applications, the reversibility of the condensation complicates their utility; hydrazones are additionally prone to hydrolysis.[12]

Even with these potential drawbacks, hydrazone and oxime ligations have been successfully employed in bioconjugation, cellular imaging, and the engineering of immunotherapies. In recent work by Rayo, *et al.*, oxime ligation was employed for live cell labeling of a receptor-tethered 3-oxo-C_{12}-AHL.[13] This example inspired us to explore the possibility of post-hydrazone cyclization/functionalization of the autoinducer in direct analogy to NCL (Scheme 1). Herein, we report the ability of hydrazine derivatives to promote 3-oxo-AHL amide cleavage under physiologically relevant conditions.

Scheme 1. 3-oxo-AHL transamidation



To investigate the envisioned reactivity, the 3-oxo-C_8 AHL (**1**) was synthesized as a control substrate, since it displays good solubility at millimolar concentrations. The autoinducer was then combined with hydrazine (20 equiv.) in pH 7.0 buffer. Previous studies on AHL hydrolysis[14] and aminolysis[15] highlight the potential for multiple routes to autoinducer derivatization, including lactone hydrazinolysis, lactone hydrolysis, and the desired reaction. Monitoring the reaction progress by LC-MS enabled the relative distribution of products to be determined. Upon consumption of the AHL, the major product observed had a mass of 155.1 m/z [M+H], indicating cleavage of the parent AHL structure (242.1 m/z [M+H]). The truncated mass was identified to be hydroxypyrazole (**2**). Masses corresponding to the aminolysis and hydrolysis products were only detected in trace amounts on the total ion mass chromatogram, indicating that the condensation/cyclization reaction mode is the dominant pathway for AHL decomposition. The hydroxypyrazole remained the predominant product when stoichiometric equivalents of hydrazine were employed and became the sole product upon acidification of the reaction media to pH = 4.5 (SI).

Our initial hypothesis, condensation of hydrazine onto the 3-oxo-position of the AHL followed by transamidation through intramolecular cyclization, provides a viable pathway for the generation of the 5-hydroxypyrazole product (Scheme 1). In analogy to NCL, reversible hydrazone formation generates an arrangement for a low energy 5-membered cyclic transition state and subsequent nucleophilic attack at the amide carbonyl. The aromaticity of the resultant pyrazole product likely provides an additional thermodynamic driving force to enable the observed AHL cleavage. This transformation also parallels the known synthesis of pyrazolones in organic solvents.[16] In such solvents, we found that the keto-form dominates (SI). As a putative intermediate in the transformation, the hydrazone (256.2 m/z [M+H]) was only observed in trace amounts when hydrazine was used, but built up more significantly when substituted hydrazines were employed *vide infra*.

The proposed reaction pathway, in conjunction with our initial screening results, suggested the possibility of using hydrazine derivatives for selective modification of the 3-oxo-AHLs in the presence of other AHLs. To test this concept, a competition experiment containing a representative example of each naturally observed AHL β-carbon functionality was conducted. The C_6-AHL (3), 3-OH-C_8-AHL (4), and **1** were combined and subjected to a single equivalent of hydrazine at pH 4.5 (Figure 1). HPLC monitoring of the reaction clearly demonstrated that the 3-oxo-C_8-AHL was the only species consumed in the mixture of AHL autoinducers. Accordingly, **2** was the only species amplified in the trace. This selective depletion of the 3-oxo-AHL supports the importance of pre-equilibrium hydrazone formation in the transamidation mechanism as the non-keto functionalized AHLs were unreactive. Further, the exclusive formation of the

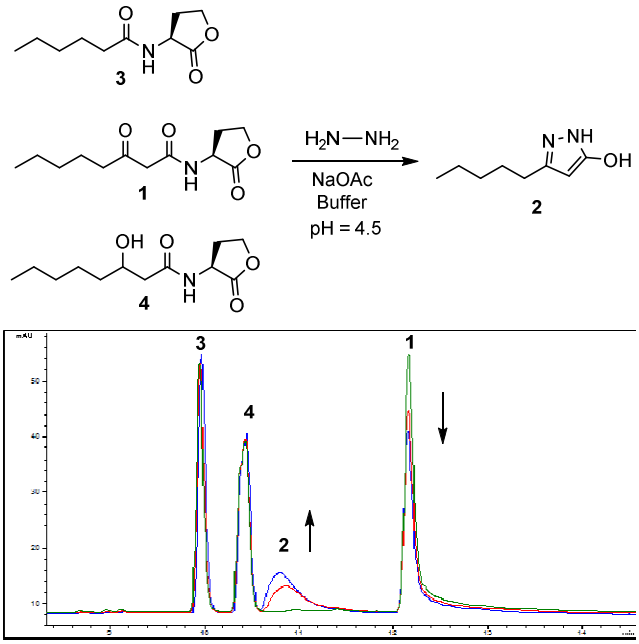


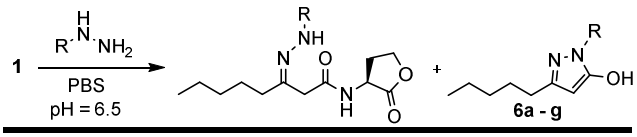
Figure 1. Selective derivatization of the 3-oxo-C_8-AHL (**1**) in the presence of the 3-OH-C_8-AHL (**4**) and the C_6-AHL (**3**) to form hydroxypyrazole (**2**). The overlaid green, red, and blue lines on the HPLC trace represent injections made at 0, 14, and 44 h, respectively. The α-amino-γ-butyrolactone is not displayed in the above trace.

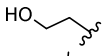
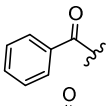
hydroxypyrazole reinforces the kinetic benefit of the condensation-cyclization pathway as compared to AHL modification by lactone or amide aminolysis.

The impact of pH on the rate of transamidation was investigated by screening the hydrazinolysis of **1** in a series of NaOAc and PBS buffers varying from pH 4.5 to 7.4. The autoinducer was converted to the hydroxypyrazole product most efficiently at pH 7.4, with a relative rate of AHL consumption close to five times the rate at pH 4.5 (SI). As hydrazone formation is known to be acid- catalyzed, the rate reversal with increasing pH suggests that the cyclization is rate determining.[17] Although hydrazone formation slows with increasing pH (smaller k_1, Scheme 1), this effect is compensated by a higher concentration of the neutral, nucleophilic hydrazone α-amine.

A series of commercial hydrazines were tested to probe substituent effects on rate and reactivity (Table 2). The selected hydrazines (5 equiv.) were screened with **1** at pH 6.5 to minimize hydrolysis. *N*-alkyl hydrazines were noticeably less reactive than hydrazine itself (**5a**), but **5b** and **5c** still generated isolable quantities of the corresponding pyrazole (SI). Reaction rates for pyrazole formation were found to decrease as size and electron withdrawing character increased. For instance, hydrazides **5e** and **5f** arrested at formation of the hydrazone and conversion to the transamidated product was not observed (SI). Consistent with a k_2 effect (Scheme 1), this is most reasonably attributed to a detrimental loss of nucleophilicity at the hydrazone state, a consequence of the strong electron withdrawing group on nitrogen. By comparison, phenylhydrazine (**5g**) proved to be the most efficient hydrazine in consuming the AHL, reaching 82% conversion after 2.5 h (Figure 2). The rapid depletion of the AHL was driven by formation of the corresponding hydrazone, the dominant product detected by LC-MS. From 2.5 to 30 h, the concentration of the hydrazone steadily declined as it was observed to cyclize to the *N*-phenylhydroxypyrazole. Thus, even the poorly nucleophilic *N*-

Table 2. Percent consumption of 1 as a function of hydrazines[a]



Entry	Hydrazine	R	% Consumption of **1** at 30 h
1	-	AHL hydrolysis	29
2	**5a**		97
3	**5b**		60
4	**5c**		87
5	**5d**		43
6	**5e**		37
7	**5f**		41
8	**5g**		100 (82 at 2.5 h)

[a]Reactions were monitored by analytical HPLC with $[\mathbf{1}]_{initial}$ = 1.0 mM and $[RNNH_2]$ = 5.0 mM. Conversion was determined by tracking the decrease in absolute AHL integration at 210 nm.

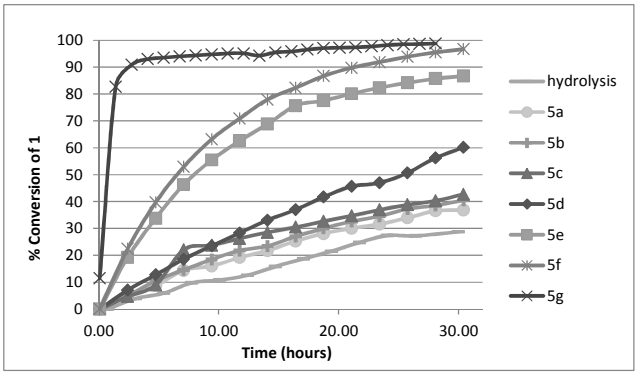
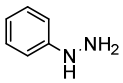
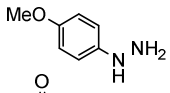
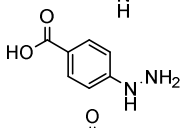
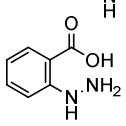


Figure 2. Percent conversion of **1** with the hydrazines **5a – 5g** from Table 2 relative to background rates of AHL hydrolysis.

phenyl amine adds to the amide under intramolecular conditions. The reaction was repeated with an AHL concentration of 10 uM and 500 nM with monitoring by LC-TOF (SI). The corresponding hydroxypyrazole and hydrazone products were observed in both trials, supporting the robustness of the transamidation at concentrations approaching that of the native signaling molecule.

The observed rate of transamidation by phenylhydrazine was furthered by screening substituted phenylhydrazines **5h, 5i,** and **5j**. AHL conversion improved in each case, with carboxyhydrazine derivatives **5i** and **5j** forwarding the most rapid degradation of the autoinducer. The similar reactivity of these nucleophiles alludes to an unexpected electronic effect or simply improved solubility that enhances transamidation as opposed to acid/base interactions previously described for hydrazone formation.[18] Second order rate

Table 3. Second order rate constants for reaction of phenylhydrazine derivatives and **1**[a]

Entry	Hydrazine		Initial Rate (M/s)	k ($M^{-1}s^{-1}$)[b]
1	**5a**		0.09	4.7×10^{-3}
2	**5g**		0.40	2.2×10^{-2}
3	**5h**		0.62	3.4×10^{-2}
4	**5i**		1.14	6.3×10^{-2}
5	**5j**		1.09	6.1×10^{-2}

[a]Reaction conditions are identical to those described in Table 2. [b]Rate constants were calculated assuming second order conditions in which the contribution of the reverse reaction (k_{-1}, Scheme 1) is negligible.

constants were calculated based on the initial rates of AHL consumption, displaying greater than ten-fold difference between the hydrazinobenzoic acids and hydrazine (Table 3). The absolute rate constant of the most effective hydrazine **5i** (k = 0.063 $M^{-1}s^{-1}$) is still two to three orders of magnitude behind those ideal for bioorthogonal transformations. However, the observed dependence of selected hydrazine on transamidation rate bodes well for identifying more efficient alpha nucleophiles for cleavage of the AHL amide bond.

Conclusions

By exploiting the unique β-ketoamide functionality of the 3-oxo-AHL, the reversible process of hydrazone formation was employed to promote the first selective and irreversible modification of these critical quorum sensing signaling molecules. The transamidation reaction proceeds at physiological pH and at nanomolar concentrations of the AHL. By continuing to enhance observed rates, the transformation has the potential to serve as a powerful method for selective knockdown of the 3-oxo-AHLs both in and beyond their intraspecies communication pathways.

We wish to acknowledge Dr. Masaomi Matsumoto and Professor Marcey L. Waters for valuable conversations and Dr. Mee-Kyung Chung for acquisition of our HRMS data. The Defense Threat Reduction Agency (DTRA) is thanked for support (HDTRA1-10-1-0030). Stephen J. Lee thanks the US Army Research Office for financial support.

Notes and references

[a] Department of Chemistry, University of North Carolina at Chapel Hill, Campus Box 3290, Caudill and Kenan Laboratories, Chapel Hill, NC, USA 27599-3290

*Email: mgagne@unc.edu

[b] US Army Research Office, P.O. Box 12211, Research Triangle Park, NC, 27709, USA

Electronic Supplementary Information (ESI) available: [Experimental procedures, characterization data, representative HPLC/LC-MS/LC-TOF traces are included]. See DOI: 10.1039/c000000x/

1. For relevant reviews see: (a) Galloway, W. R. J. D., Hodgkinson, J. T., Bowden, S. D., Welch, M. & Spring, D. R. Chem. Rev. 2011, 111, 28–67. (b) Geske, G. D., O'Neill, J. C. & Blackwell, H. E. Chem. Soc. Rev. 2008, 37, 1432 - 1447. (c) Praneenararat, T., Palmer, A. G. & Blackwell, H. E. Org. Biomol. Chem. 2012, 10, 8189 - 8199. Amara, N., Krom, B. P., Kaufmann, G. F., & Meijler, M. M. Chem Rev. 2011, 111, 195 – 208.
2. For relevant reviews see: (a) Fuqua, C. & Greenberg, E. P. Nat. Rev. Mol. Cell Biol. 2002, 3, 685–695. (b) Jayaraman, A. & Wood, T. K. Annu. Rev Biomed. Eng. 2008, 10, 145–167. (c) Miller, M. B. & Bassler, B. L. Annu. Rev. Microbiol. 2001, 55, 165–199. (d) Waters, C. M. & Bassler, B. L. Annu. Rev. of Cell Dev. Biol. 2005, 21, 319–346. (e) Antunes, L. C. M., Ferreira, R. B. R., Buckner, M. M. C. & Finlay, B. B. Microbiology 2010, 126, 2271–2282. (f) Williams, P. Microbiology 2007, 153, 3923–3938.
3. (a) Kaufmann, G. F., J. Am. Chem. Soc. 2006, 107, 2802–2803. (b) De Lamo Marin, S., Xu, Y., Meijler, M. M. & Janda, K. D. Bioorg. & Med. Chem. Lett. 2007, 17, 1549–1552.
4. (a) E. V. Piletska, G. Stavroulakis, L. D. Larcombe, M. J. Whitcombe, A. Sharma, S. Primrose, G. K. Robinson & S. A. Piletsky, Biomacromolecules, 2011, 12, 1067–1071. (b) N. Kato, T. Tanaka, S. Nakagawa, T. Morohoshi, K. Hiratani and T. Ikeda, J. Inclusion Phenom. Macrocyclic Chem., 2007, 57, 419–423.
5. Chhabra, S., Philipp, B., Eberl, L., Givskov, M., Williams, P. & Cámara, M. Topics in Current Chemistry, 2005, 240, 279–315.
6. Pattabiraman, V. R. & Bode, J. W. Nature, 2011, 480, 471–479.
7. (a) Smith, B. C. & Denu, J. M. Biochimica et Biophysica Acta, 2009, 1789, 45–57. (b) Zhang, X. & Bruice, T. C. Proc. Natl. Acad. Sci., 2008, 105, 5728–5732.
8. (a) Jencks, W. P., Haber, M. T., Herschlag, D. & Nazaretian, K. L. J. Am. Chem. Soc. 1986, 108, 479–483. (b) Harris, T. K. & Turner, G. J. IUBMB Life, 2002, 53, 85–98.
9. (a) Dawson, P. E. & Kent, S. B. H. Annu. Rev. Biochem. 2000, 69, 923–960. (b) Hackenberger, C. P. R. & Schwarzer, D. Angew. Chem. Int. Ed. 2008, 47, 10030–10074.
10. (a) Sletten, E. M. & Bertozzi, C. R. Angew. Chem. Int. Ed. 2009, 48, 6974–6998. (b) Ulrich, S., Boturyn, D., Marra, A., Renaudet, O. & Dumy, P. Chem. Eur. J. 2013, 20, 34-41.
11. (a) Dirksen, A., Hackeng, T. M. & Dawson, P. E. Angew. Chem. Int. Ed. 2006, 45, 7581–7584. (b) Dirksen, A., Dirksen, S., Hackeng, T. M. & Dawson, P. E. J. Am. Chem. Soc. 2006, 128, 15602–15603. (c) Dirksen, A. & Dawson, P. E. Bioconj. Chem. 2008, 19, 2543–2548. (d) Dirksen, A., Yegneswaran, S. & Dawson, P. E. Angew. Chem. Int. Ed. 2010, 49, 2023–2027. (e) Crisalli, P. & Kool, E. T. J. Org. Chem. 2013, 28, 1184–1189. (f) Rashidian, M., Mahmoodi, M., Shah, R., Dozier, J., Wagner, C. & Distefano, M. Bioconj. Chem. 2013, 24, 333–342. (g) Crisalli, P. & Kool, E. T. Org. Lett. 2013, 15, 1646–1649.
12. Kalia, J. & Raines, R. T. Angew. Chem. Int. Ed. 2008, 47, 7523–7526.
13. Rayo, J., Amara, N., Krief, P. & Meijler, M. M. J. Am. Chem. Soc. 2011, 133, 7469–7475.
14. Yates, E. A., Philipp, B., Buckley, C., Atkinson, S., Chhabra, S. R., Sockett, R. E., Goldner, M., Dessaux, Y., Cámara, M., Smith, H., & Williams, P. Infection and Immunity, 2002, 70, 5635–5646.
15. Bertucci, M. A., Lee, S. J. & Gagné, M. R. Chem. Commun. 2013, 49, 2055 - 2057.
16. Varvounis, G. Pyrazol-3-ones: Part IV: Synthesis and Applications. In Advances in Heterocyclic Chemistry. Katritzky, A. R.; Elsevier: The Netherlands, 2009, 98, 144-196.
17. Jencks, W. P. J. Am. Chem. Soc. 1959, 81, 475−481.
18. Kool, E. T., Park, D., Crisalli, P. J. Am. Chem. Soc. 2013, 47, 17663-17666.