Published in final edited form as:
*Bioorg Med Chem Lett*. 2011 May 1; 21(9): 2702–2705. doi:10.1016/j.bmcl.2010.11.122.

# Synthesis of "clickable" acylhomoserine lactone quorum sensing probes: unanticipated effects on mammalian cell activation

**Amanda L. Garner**[a,b,c], **Jing Yu**[a,b], **Anjali Kumari Struss**[a,b], **Colin A. Lowery**[a,b,c], **Jie Zhu**[a,b], **Sook Kyung Kim**[a,b], **Junguk Park**[a,b,c], **Alexander V. Mayorov**[a,b,c], **Gunnar F. Kaufmann**[a,b,c], **Vladimir V. Kravchenko**[b], and **Kim D. Janda**[a,b,c,d,*]

[a]Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

[b]Department of Immunology and Microbial Sciences, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

[c]The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, USA

[d]Worm Institute of Medical Research (WIRM), The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

## Abstract

Alkynyl- and azido-tagged 3-oxo-$C_{12}$-acylhomoserine lactone probes have been synthesized to examine their potential utility as probes for discovering the mammalian protein target of the *Pseudomonas aeruginosa* autoinducer, 3-oxo-$C_{12}$-acylhomoserine lactone. Although such substitutions are commonly believed to be quite conservative, from these studies, we have uncovered a drastic difference in activity between the alkynyl- and azido-modified compounds, and provide an example where such structural modification has proved to be much less than conservative.

### Keywords

Acylhomoserine lactones; Click chemistry; *Pseudomonas aeruginosa*; Quorum sensing

Quorum sensing is a diffusion-based process whereby bacteria secrete various signaling molecules, or autoinducers, as a population sensor and a means to control gene expression in response to changes in cell number; thus, allowing the bacteria to behave as a multicellular organism.1 Importantly, this process has been correlated with the expression of virulence factors and biofilm formation,1 and a "deafening" of bacteria to these signaling molecules should provide a mechanism by which to target the bacteria without exerting a selective



*Corresponding author. Tel.: +1 858 784 2515; fax: +1 858 784 2595; kdjanda@scripps.edu.



**Supplementary Material**

Supplementary data associated with this article can be found, in the online version, at doi:…

pressure thereby decreasing the possibility of resistance development.[2,3] As a result, much effort has been invested in developing small molecule probes,[4,5] autoinducer analogues[4,5] and protein biologics[6–8] for studying quorum sensing.

In particular, quorum sensing in the gram-negative bacteria *Pseudomonas aeruginosa* has received significant attention due to the species' role as an opportunistic pathogen in humans.[9] Most prominent is the role of *P. aeruginosa* in patients suffering from cystic fibrosis.[10] Affected individuals suffer from frequent bacterial lung infections due to impaired lung defense function, and these infections are often a cause of morbidity.[10] With respect to quorum sensing, it has now been established that this signaling process is required for *P. aeruginosa* to cause disease, including lung infections in cystic fibrosis patients.[11–14] More specifically, the ability of the bacteria to grow into sessile, drug-resistant biofilms at high cell densities makes such infections difficult to treat, and resistance is beginning to be observed with currently available antibiotics.[15]

To conduct its quorum sensing-controlled activities, including biofilm formation, *P. aeruginosa* uses *N*-acyl L-homoserine lactones (AHLs) as its primary autoinducers (Figure 1).[1] AHL **1** activates LasR[16] and AHL **2** activates RhlR,[17] and activation of both receptor proteins is required for quorum sensing.[1] Since LasR activation by AHL **1** is required to initiate the quorum-sensing cascade in this species,[1] many studies have been conducted toward deciphering the role of this chemical signal in both quorum sensing and its resulting human pathologies. Recently, our group[18,19] and others[20,21] have explored the impact of AHL **1** in mammalian cells. For example, AHL **1** inhibits innate immune responses through modulation of NF-κB signaling induced by pro-inflammatory stimuli such as tumor necrosis factor and the Toll-like receptor (TLR) agonists, thereby potentially promoting persistent infection.[19] Notably, this molecule directly activates mammalian cells through a mechanism distinct from the TLR and other canonical pathogen-associated receptor pathways.[18] Because of the potential significance of these findings, our group and others[22] have been actively involved in identifying the biochemical target(s) of this important quorum sensing signaling molecule.

Click chemistry, more specifically $Cu^{I}$-catalyzed azide-alkyne 1,3-dipolar cycloaddition chemistry,[23–25] has emerged as a powerful method for bioconjugation.[26,27] Coupled with activity-based protein profiling (ABPP), this method grants the ability to probe the molecular interactions of a molecule of choice by modifying the compound with a bioorthogonal azide or alkyne tag.[28,29] While this substitution may seem conservative, in fact, this modification can have drastic impact on the activity of the small molecule, although such findings are often not reported. Moreover, as high affinity is a requirement for use of a probe in target identification studies, particularly in cases when the unknown target may be of low abundance,[30] choice of this modification may prove critical for such future studies. Herein, we describe our efforts to synthesize clickable AHL probes for target identification studies and demonstrate the critical nature that the choice of an alkyne or azide modification has on AHL activity in mammalian cells.

Although much is known about the structural requirements for AHL-mediated activation in *P. aeruginosa*,[4,5] very little has been concluded about the requirements for biological activity in mammalian cells. Previously, our group demonstrated that the L-homoserine lactone, 3-oxo substituent and an alkyl chain of 10–14 carbons were necessary to activate mammalian cells.[18] To begin our studies, we first synthesized alkynyl analogues **5a** and **5b**, as an alkyne seemed to be the most conservative modification since it is composed of only carbons similar to the natural tail of AHL **1**. Additionally, previous studies have showed that detection with rhodamine-azide compounds yields lower background signals than detection with the corresponding rhodamine-alkyne tags in click chemistry-mediated ABPP.[29]

Although a diazirine moiety was used in a previously reported AHL-based probe,22 we hypothesized that the acylhomoserine lactone could act as a covalent tag on its own, particularly since lactone natural products are well known to covalently modify protein targets *in vivo*.31 The synthesis of alkynyl analogues **5a** and **5b** is shown in Scheme 1, and proceeded via a straightforward route starting from the commercially available alkynyl fatty acids **3a** and **3b**. Briefly, alkynyl acids **3** were first activated with DCC followed by nucleophilic displacement of the resultant activated esters with Meldrum's acid and coupling with L-homoserine lactone **4** to yield alkynyl AHL analogues **5**. For carbon chain lengths, we chose to synthesize both a shorter 9 carbon tail and a longer 13 carbon tail for comparison.

To assess a potential utility of these alkynyl AHL analogues as probes for target identification studies, we tested their biological activities in primary macrophages. In brief, bone marrow-derived macrophages (BMDM) were treated with an alkynyl AHL derivative (50 μM) over a period of 90 minutes, and cellular extracts were examined by Western blot analysis for the biochemical markers indicative of AHL **1**-mediated signaling events including the phosphorylation of the protein kinase p38, the phosphorylation of the eukaryotic translation initiation factor 2α(eIF2α) and the cleavage of the poly(ADP)-ribose) polymerase (PARP), as previously described.18 AHL **1** (3-oxo-$C_{12}$-AHL) and $C_{12}$-AHL (no 3-oxo group, structure not shown) were used as positive and negative controls, respectively. As Figure 2 shows, only alkynyl analogue **5a** exhibited activity; however, its activity was greatly reduced compared to that of the parent compound **1**.

Knowing that the diminished activity of alkynyl analogue **5a** and apparent lack of activity in analogue **5b** would damper any efforts toward identifying the mammalian target(s) of AHL **1**, we next synthesized an azide derivative in hope of identifying a more viable AHL analogue for our desired future studies. Since the shorter alkyne probe showed no activity, we chose to examine only a long alkyl chain. Azido analogue **8a**, which contains 12 carbons in its tail, was synthesized in an analogous manner as the alkynyl derivatives (Scheme 2). In this case, however, azido-carboxylic acid **7** was used as a starting material and was obtained via nucleophilic substitution of bromo-acid **6** with sodium azide (Scheme 2). We then tested the activity of this azido derivative in BMDM. Interestingly, unlike the alkynyl derivatives, the azido AHL analogue exhibited activity very similar to that of the parent AHL (Figure 3). Thus, substitution at the tail terminus clearly has a drastic impact on AHL activity. This finding is unanticipated since an azido group, although overall neutral, is polar and carries charged nitrogen atoms, while an alkynyl group is fully neutral and nonpolar similar to the natural AHL tail. Although purely speculative, it is possible that the azido moiety makes additional hydrogen bond or van der Waal contacts32 that aids in its binding of the putative AHL target(s). Importantly, this potential probe exhibits much higher activity in mammalian cells than a previously reported diazirine-modified alkynyl AHL that was used to label and isolate *P. aeruginosa* LasR.22 As an additional control compound, we also synthesized the corresponding D-homoserine lactone derivative (**8b**, Scheme 2), and similar to previous experiments with this enantiomer,18 no activity was observed (Figure 3). Thus, **8b** could prove useful as a structurally similar negative control for potential target identification strategies.

In addition to testing these AHL derivatives in mammalian cells, we also examined their impact as potential autoinducers in *P. aeruginosa*. Autoinducer assays were conducted using *P. aeruginosa* luminescence reporter strain PAO-JP2, a PAO1 *lasI/rhlI* double mutant (PAO-JP2-*luxABCDE*).33,34 As Figure 4 shows, only alkynyl AHL analogue **5a** showed autoinducer activity similar to that of the parent AHL **1**. These data indicate that the structural requirements for autoinducer activity and activity in mammalian cells are distinct,

and nonpolar, all carbon alkynyl probes may be more useful for identifying bacterial AHL receptors.

In conclusion, we have synthesized alkynyl- and azido-tagged 3-oxo-$C_{12}$-AHL analogues as potential ABPP probes for discovering the mammalian target of this important *P. aeruginosa* quorum sensing signaling molecule. Of particular significance, we have found a drastic difference in activity between the alkynyl- and azido-tagged AHL derivatives in that only the azide-containing analogue possessed activity similar to that of the natural compound. Thus, this data demonstrates that click chemistry-amenable substitutions may not be as conservative as previously thought, and that no simple guidelines can exist for such click chemistry-mediated ABPP endeavors. Both alkynyl- and azido-modified compounds should be initially examined for activity before examination as potential probes. Additionally, the discovery that polar, yet neutral substituents at the tail are accepted for mammalian cell activity may open up the possibility for the synthesis of new, more potent AHL derivatives. Target identification and additional synthetic studies are currently ongoing and will be reported in due course.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We thank M. M. Meijler (Ben Gurion University) for kindly providing *P. aeruginosa* strain PAO-JP2. We gratefully acknowledge the financial support of the NIH (Grants AI079503 and DE018452 to K.D.J., and grant AI085324 to G.F.K.), the Skaggs Institute for Chemical Biology, and a NIH postdoctoral fellowship (F32-DK083179, A.L.G.).

## References and notes

1. Waters CM, Bassler BL. Annu. Rev. Cell. Dev. Biol. 2005; 21:319. [PubMed: 16212498]
2. Rasmussen TB, Givskov M. Int. J. Med. Microbiol. 2006; 296:149. [PubMed: 16503194]
3. Suga H, Smith KM. Curr. Opin. Chem. Biol. 2003; 7:586. [PubMed: 14580562]
4. Lowery CA, Salzameda NT, Sawada D, Kaufmann GF, Janda KD. J. Med. Chem. doi:10.1021/jm901742e.
5. Mattmann ME, Blackwell HE. J. Org. Chem. 2010; 75:6737. [PubMed: 20672805]
6. Kaufmann GF, Sartorio R, Lee SH, Mee JM, Altobell LJ III, Kujawa DP, Jeffries E, Clapham B, Meijler MM, Janda KD. J. Am. Chem. Soc. 2006; 128:2802. [PubMed: 16506750]
7. De Lamo Marin S, Xu Y, Meijler MM, Janda KD. Bioorg. Med. Chem. Lett. 2007; 17:1549. [PubMed: 17254784]
8. Kaufmann GF, Park J, Mee JM, Ulevitch RJ, Janda KD. Mol. Immunol. 2008; 45:2710. [PubMed: 18304641]
9. Lyczak JB, Cannon CL, Pier GB. Microbes Infect. 2000; 2:1051. [PubMed: 10967285]
10. Lyczak JB, Cannon CL, Pier GB. Clin. Microbiol. Rev. 2002; 15:194. [PubMed: 11932230]
11. Pearson JP, Feldman M, Iglewski BH, Prince A. Infect. Immun. 2000; 68:4331. [PubMed: 10858254]
12. Tang HB, DiMango E, Bryan R, Gambello M, Iglewski BH, Goldberg JB, Prince A. Infect. Immun. 1996; 64:37. [PubMed: 8557368]
13. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. Science. 1998; 280:295. [PubMed: 9535661]
14. Calfee MW, Coleman JP, Pesci EC. Proc. Natl. Acad. Sci. U. S. A. 2001; 98:11633. [PubMed: 11573001]
15. Drenkard E. Microbes Infect. 2003; 5:1213. [PubMed: 14623017]

16. Pearson JP, Gray KM, Passador L, Tucker KD, Eberhard A, Iglewski BH, Greenberg EP. Proc. Natl. Acad. Sci. U. S. A. 1994; 91:197. [PubMed: 8278364]
17. Pearson JP, Passador L, Iglewski BH, Greenberg EP. Proc. Natl. Acad. Sci. U. S. A. 1995; 92:1490. [PubMed: 7878006]
18. Kravchenko VV, Kaufmann GF, Mathison JC, Scott DA, Katz AZ, Wood MR, Brogan AP, Lehmann M, Mee JM, Iwata K, Pan Q, Fearns C, Knaus UG, Meijler MM, Janda KD, Ulevitch RJ. J. Biol. Chem. 2006; 281:28822. [PubMed: 16893899]
19. Kravchenko VV, Kaufmann GF, Mathison JC, Scott DA, Katz AZ, Grauer DC, Lehmann M, Meijler MM, Janda KD, Ulevitch RJ. Science. 2008; 321:259. [PubMed: 18566250]
20. Thomas GL, Bohner CM, Williams HE, Walsh CM, Ladlow M, Welch M, Bryant CE, Spring DR. Mol. Biosyst. 2006; 2:132. [PubMed: 16880931]
21. Li L, Hooi D, Chhabra SR, Pritchard D, Shaw PE. Oncogene. 2004; 23:4894. [PubMed: 15064716]
22. Dubinsky L, Jarosz LM, Amara N, Krief P, Kravchenko VV, Krom BP, Meijler MM. Chem. Commun. 2009:7378.
23. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. Angew. Chem. Int. Ed. 2002; 41:2596.
24. Tornoe CW, Christensen C, Meldal M. J. Org. Chem. 2002; 67:3057. [PubMed: 11975567]
25. Meldal M, Tornoe CW. Chem. Rev. 2008; 108:2952. [PubMed: 18698735]
26. Wang Q, Chan TR, Hilgraf R, Fokin VV, Sharpless KB, Finn MG. J. Am. Chem. Soc. 2003; 125:3192. [PubMed: 12630856]
27. Best MD. Biochemistry. 2009; 48:6571. [PubMed: 19485420]
28. Speers AE, Adam GC, Cravatt BF. J. Am. Chem. Soc. 2003; 125:4686. [PubMed: 12696868]
29. Speers AE, Cravatt BF. Chem. Biol. 2004; 11:535. [PubMed: 15123248]
30. Sato S, Murata A, Shirakawa T, Uesugi M. Chem. Biol. 2010; 17:616. [PubMed: 20609411]
31. Konaklieva MI, Plotkin BJ. Mini-Rev. Med. Chem. 2005; 5:73. [PubMed: 15638793]
32. Petsalakis EI, Chrysina ED, Tiraidis C, Hadjiloi T, Leonidas DD, Oikonomakos NG, Aich U, Varghese B, Loganathan D. Bioorg. Med. Chem. 2006; 14:5316. [PubMed: 16616506]
33. Duan K, Surette MG. J. Bacteriol. 2007; 189:4827. [PubMed: 17449617]
34. Amara N, Mashiach R, Amar D, Krief P, Spieser SAH, Bottomley MJ, Aharoni A, Meijler MM. J. Am. Chem. Soc. 2009; 131:10610. [PubMed: 19585989]

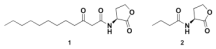


**Figure 1.**
Autoinducers used by *Pseudomonas aeruginosa*.

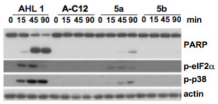



**Figure 2.**

Biological activities of AHL **1**, $C_{12}$-HSL (A-C12; negative control) and its alkynyl analogues **5a** and **5b** in mammalian cells. BMDM were treated as indicated, and cellular extracts were analyzed by Western blot for the cleavage of PARP, phosphorylated forms of eIF2α(p-eIF2α) or p38 (p-p38) and actin as a loading control.

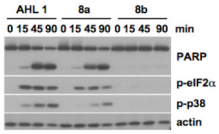



**Figure 3.**
Biological activities of azido analogues **8a** and **8b** in mammalian cells.

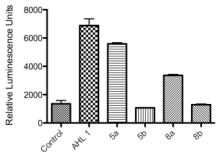
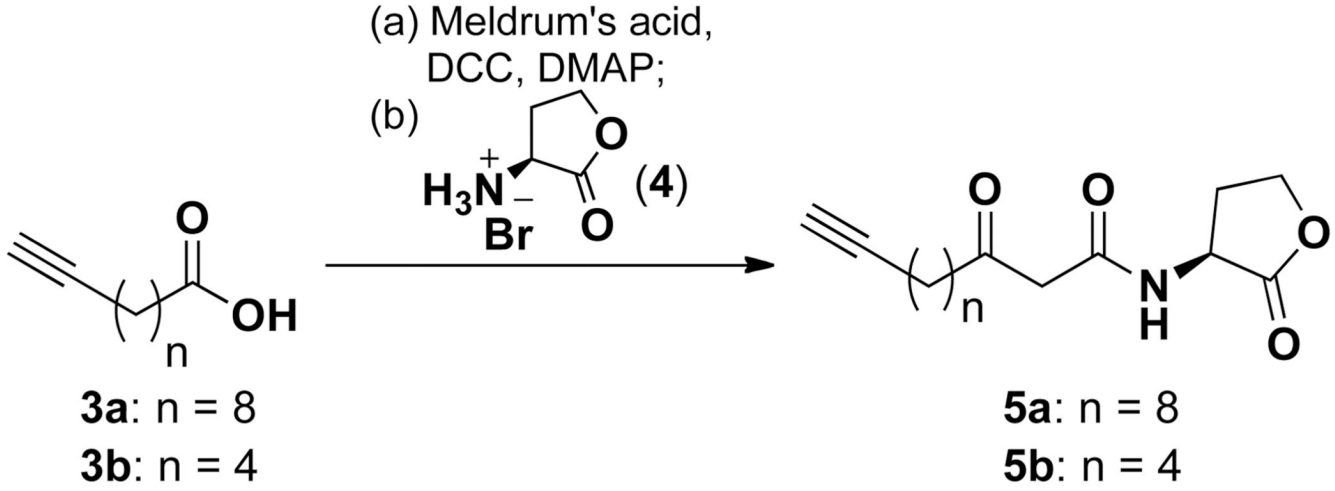
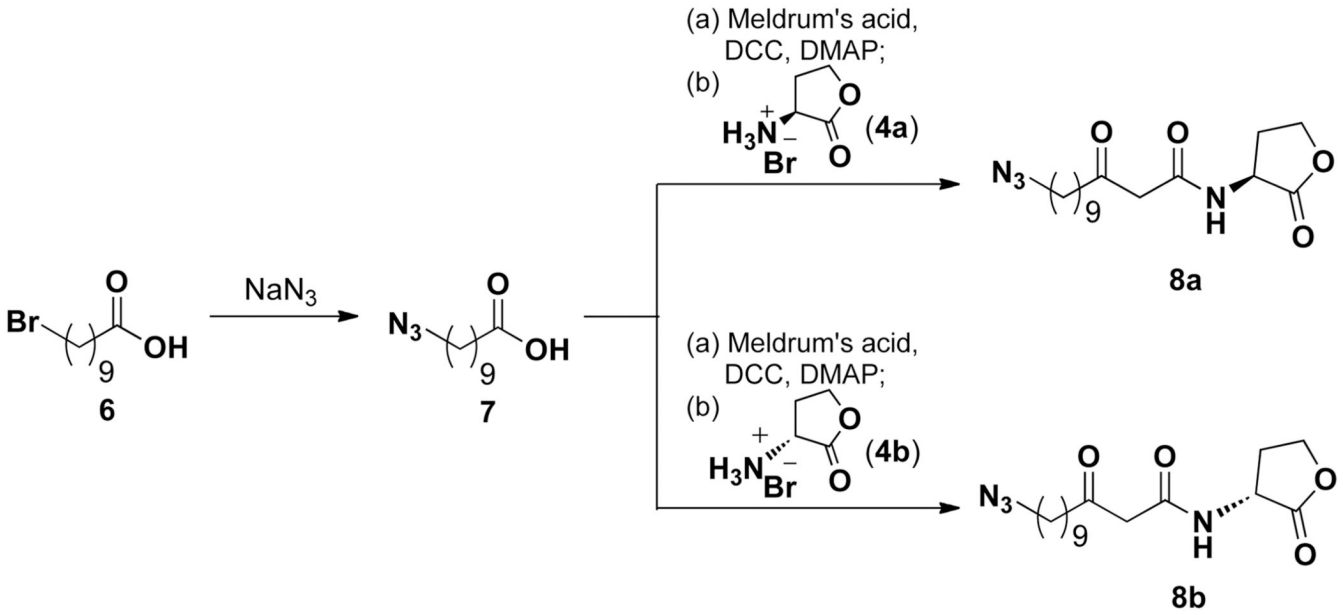



**Figure 4.**

Autoinducer activities of alkynyl- and azido-AHL analogues in *P. aeruginosa*. Relative luminescence units were normalized with respect to cell viability ($OD_{600}$).




**Scheme 1.**
Synthesis of alkynyl AHL analogues.




**Scheme 2.**
Synthesis of azido AHL analogues.