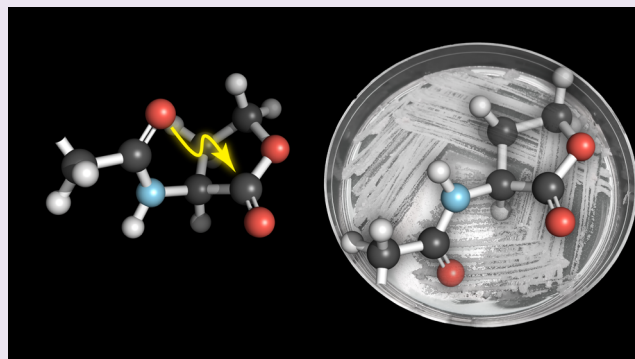


A Key $n \rightarrow \pi^*$ Interaction in *N*-Acyl Homoserine LactonesRobert W. Newberry[†] and Ronald T. Raines^{*,†,‡}[†]Department of Chemistry and [‡]Department of Biochemistry, University of Wisconsin–Madison, Madison, Wisconsin 53706, United States

Supporting Information

ABSTRACT: Many Gram-negative bacteria employ *N*-acyl homoserine lactones (AHLs) as signal molecules for quorum sensing. The binding of AHLs to their target LuxR-type receptor proteins can effect changes in growth, virulence, and other phenotypes. LuxR-type receptors therefore present attractive pharmaceutical targets for control of bacterial pathogenesis. Here, we present X-ray crystallographic and computational evidence that the conformation of free AHLs is biased away from the conformation observed when bound to their cognate receptor due to the influence of an $n \rightarrow \pi^*$ interaction. In this $n \rightarrow \pi^*$ interaction, the *p*-type lone pair (*n*) of the *N*-acyl oxygen overlaps with the π^* orbital of the lactone carbonyl group. This overlap results in the release of approximately 0.64 kcal/mol of energy. We also show that this interaction can be attenuated by installing electron-withdrawing groups on the *N*-acyl chain. Modulating this previously unappreciated interaction could present a new avenue toward effective inhibitors of bacterial quorum sensing.



Despite being unicellular organisms, bacteria have evolved mechanisms of chemical communication that regulate various physiological processes in response to cell density, a phenomenon known as quorum sensing.^{1,2} This process is receiving much attention because of its influence on biofilm formation and virulence. The principle mediators of these communication events in Gram-negative bacteria are the *N*-acyl homoserine lactones (AHLs, Figure 1).^{3,4} AHLs act by binding

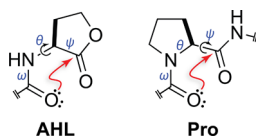


Figure 1. Structure of an *N*-acyl homoserine lactone (AHL) and a proline residue (Pro). Arrows indicate freely rotatable bonds (black) and putative $n \rightarrow \pi^*$ interactions that would constrain those bonds (red).

to intracellular LuxR-type receptors, which are then activated as transcription factors.^{5,6} Physiological transitions in bacterial colonies are induced when AHL concentrations cross particular thresholds. The strong influence of AHLs on bacterial behavior has attracted the attention of chemical biologists, as modulators of AHL-binding could impart exquisite control of bacterial pathogenesis.⁷ Toward this end, we noticed that AHLs have proximal carbonyl groups (Figure 1). We have shown that the conformations of molecules with proximal carbonyl moieties can be influenced by an $n \rightarrow \pi^*$ interaction (Figure 2).^{8,9} Here,

we sought to determine if an $n \rightarrow \pi^*$ interaction could influence the conformation of an AHL.

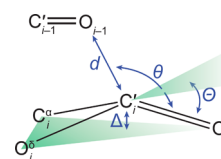


Figure 2. Structural parameters characterizing an $n \rightarrow \pi^*$ interaction.

In an $n \rightarrow \pi^*$ interaction, the filled lone pair (*n*) of one carbonyl group interpenetrates the empty π^* orbital of another. The mixing of these orbitals releases energy, thereby causing attraction between the two groups. This overlap is most effective when the oxygen of the electron-pair donor forms a sub-van der Waals contact ($d < 3.22$ Å) with the carbon of the acceptor carbonyl group along the Bürgi–Dunitz trajectory for nucleophilic addition ($95^\circ < \theta < 125^\circ$).¹⁰ We have estimated that such an interaction between adjacent amides in a polypeptide contributes 0.27 kcal/mol of stabilizing energy per occurrence.¹¹ As these are relatively weak interactions, their influence is often realized only in systems in which carbonyl groups are in close proximity, as they are in proteins,^{12–15} peptides,¹⁶ peptoids,^{17–20} polyesters,²¹ and some small molecules.^{22,23}

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The preorganization of two carbonyl groups due to the constraint of an intervening ring can enhance an $n \rightarrow \pi^*$ interaction.^{8,9,12,14,22–33} We realized that the γ -lactone of an AHL restricts its ψ dihedral angle ($N_i-C_i^{\alpha}-C'_{i-1}-N_{i+1}$) and that amidic resonance restricts its ω dihedral angle ($C'_{i-1}-C'_{i-1}-N_i-C_i^{\alpha}$),^{34,35} leaving only a single unconstrained bond between the two carbonyl groups. In this sense, an AHL is analogous to a proline residue, which has a restricted ϕ dihedral angle ($C'_{i-1}-N_i-C_i^{\alpha}-C'_i$) and has a strong tendency to form an $O_{i-1} \cdots C'_i=O_i$ $n \rightarrow \pi^*$ interaction (Figure 1). Thus, we suspected that AHLs, like proline residues, could be predisposed to form an $n \rightarrow \pi^*$ interaction.

To begin, we sought evidence for a putative $n \rightarrow \pi^*$ interaction by using X-ray diffraction analysis. Natural AHLs can have long alkyl chains that are resistant to crystallization, so we began by examining *N*-acetyl homoserine lactone. When this molecule did not afford crystals of sufficient quality for diffraction, we appended minimal precipitating groups to the *N*-acyl chain in attempts to drive crystal formation. Gratifyingly, we found the *N*-trimethylacetyl appendage to be suitable, affording crystals that diffracted to high resolution (Figure 3A).

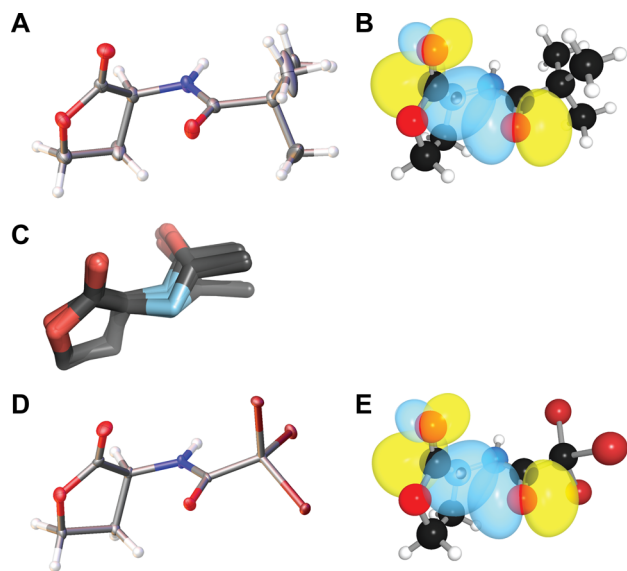


Figure 3. Structures of AHLs. (A) Crystal structure of *N*-trimethylacetyl homoserine lactone drawn with 50% probability ellipsoids. (B) Rendering of the n and π^* orbitals of *N*-trimethylacetyl homoserine lactone in its optimized geometry. (C) Overlap of the crystal structures of the acetyl homoserine lactone moieties in the 14 ligands listed in Table 2. (D) Crystal structure of *N*-tribromoacetyl homoserine lactone drawn with 50% probability ellipsoids. (E) Rendering of the n and π^* orbitals of *N*-tribromoacetyl homoserine lactone in its optimized geometry.

In this molecular structure, we noted the presence of a short contact between the *N*-acyl carbonyl oxygen and the lactone carbonyl carbon ($d = 2.73$ Å), which is 15% below the sum of

the van der Waals radii (Table 1). We also found the angle of approach of the donor oxygen to the acceptor carbonyl ($\theta = 90.6^\circ$) to be consistent with an $n \rightarrow \pi^*$ interaction.¹² As these structural features were strongly suggestive of an $n \rightarrow \pi^*$ interaction, we were motivated to search for characteristic structural deviations that result from $n \rightarrow \pi^*$ donation. In particular, we have shown that the presence of $n \rightarrow \pi^*$ interaction engenders pyramidalization of the acceptor carbonyl carbon toward the donor oxygen, as measured by the distortion parameter Θ .^{9,12,14–16,22,28} In *N*-trimethylacetyl homoserine lactone, we observed substantial pyramidalization ($\Theta = 2.7^\circ$) of the acceptor carbonyl toward the donor, in accord with that observed for other molecules with confirmed $n \rightarrow \pi^*$ interactions. Distortion of the carbonyl carbon toward the $n \rightarrow \pi^*$ is strong evidence of an attractive interaction; otherwise, distortion would likely occur away from the short contact so as to reduce unfavorable Pauli repulsion.²⁷

Confident that AHLs have a preference for forming an $n \rightarrow \pi^*$ interaction, we wished to evaluate the energy of this interaction. To do so, we performed natural bond orbital (NBO) analysis of the *N*-trimethylacetyl homoserine lactone structure optimized by density functional theory (DFT) calculations *in vacuo* at the B3LYP/6-311+G(2d,p) level of theory.³⁶ Using second-order perturbation theory, as implemented by NBO 5.9,^{37,38} we observed significant overlap of the n and π^* orbitals (Figure 3B), with an estimated energy of $E_{n \rightarrow \pi^*} = 0.64$ kcal/mol. This value is larger than that observed with a proline residue,¹¹ consistent with the carbonyl group of an ester being a better acceptor than that of an amide (Figure 1).

As the biological activity of an AHL relies upon its binding to its target LuxR-type receptor, we sought to compare the structure of a free AHL to that observed in a receptor-AHL complex. The Protein Data Bank (PDB)³⁹ currently houses the atomic coordinates of ten LuxR-type receptor structures with bound AHLs, reflecting four distinct receptors as well as four structures of two different AHL-lactonases with bound AHLs. Remarkably, the conformation of the bound AHL ligand is nearly identical in all of these complexes (Table 2; Figure 3C), and the conformation differs dramatically from the conformation in the unbound state. In particular, each of these proteins prefers to bind the AHL ligand with a ϕ dihedral angle between -100° and -160° , a nearly 180° -reorientation from that observed in the unbound state ($\phi \sim 50^\circ$; Table 1). The dichotomy in the conformation of the free and bound forms indicates that the receptor must reorganize the ligand for binding. The conformation of a bound AHL is enforced by hydrogen bonds with its receptor. In particular, the amide oxygen of a bound AHL forms a hydrogen bond with the phenolic hydroxyl group of a conserved tyrosine residue.⁶ This $C'_{i-1}=O_{i-1} \cdots H$ hydrogen bond competes with the $O_{i-1} \cdots C'_i=O_i$ $n \rightarrow \pi^*$ interaction of the free ligand. Accordingly, attenuating the basal $n \rightarrow \pi^*$ interaction could preorganize an AHL for binding to its receptor.

Table 1. Conformational Parameters of *N*-Acyl Homoserine Lactones Used in This Work

<i>N</i> -acyl group	ϕ (deg) ^a	ψ (deg) ^a	d (Å) ^a	θ (deg) ^a	Θ (deg) ^a	Δ (Å) ^a	$E_{n \rightarrow \pi^*}$ (kcal/mol) ^b
<i>N</i> -trimethylacetyl	51.51(18)	-144.65(15)	2.732(2)	90.62(12)	2.7(2)	0.023(2)	0.64
<i>N</i> -tribromoacetyl	48.50(30)	-148.20(30)	2.801(4)	88.00(20)	1.6(5)	0.014(4)	0.55

^aFrom X-ray diffraction analysis of the crystalline compound. Structural parameters are defined in Figures 1 and 2. ^bFrom second-order perturbation theory.

Table 2. Conformational Parameters of Protein-Bound *N*-Acyl Homoserine Lactones

PDB entry	resolution (Å)	protein	<i>N</i> -acyl group	ϕ (deg)	ψ (deg)
4g8b ^a	1.30	AidH	butyryl	-110	-136
3qp1	1.55	CviR	hexanoyl	-103	-139
3qp2	1.64	CviR	octanoyl	-108	-138
3qp4	1.55	CviR	decanoyl	-106	-139
3qp6	2.00	CviR	hexanoyl	-102	-140
3qp8 ^b	1.60	CviR	decanoyl	-108	-139
3ojg	1.60	GKL	butyryl	-159	-137
4h9t ^a	2.10	GKL	butyryl	-139	-135
4h9x ^a	2.20	GKL	butyryl	-100	-145
2uv0 ^b	1.80	LasR	3-oxo-dodecanoyl	-114	-144
3ix3 ^a	1.40	LasR	3-oxo-dodecanoyl	-110	-145
3szt ^a	2.55	QscR	3-oxo-dodecanoyl	-109	-125
1l3l ^b	1.66	traR	3-oxo-octanoic	-107	-129
2q0o ^a	2.00	traR	3-oxo-octanoic	-108	-128

^aMean for two molecules in the asymmetric unit. ^bMean for four molecules in the asymmetric unit.

We reasoned that electron-withdrawing groups in the *N*-acyl chain would reduce the nucleophilicity of the donor oxygen and thereby reduce the influence of the $n \rightarrow \pi^*$ interaction.^{20,40} To control for the influence of sterics, we replaced the three methyl groups in *N*-trimethylacetyl homoserine lactone with nearly isosteric bromo groups. Again using X-ray diffraction analysis, we found *N*-tribromoacetyl homoserine lactone adopts a conformation nearly identical to that of *N*-trimethylacetyl homoserine lactone (Table 1; Figure 3D). Moreover, the *N*-tribromoacetyl compound has a longer oxygen-carbon distance and diminished acceptor pyramidalization than does its *N*-trimethylacetyl analogue. These attributes are indicative of a weaker $n \rightarrow \pi^*$ interaction in the *N*-tribromoacetyl compound.^{9,14} We confirmed this conclusion with NBO analysis of the optimized geometry of this compound, which reported that the energy associated with this $n \rightarrow \pi^*$ interaction was 0.55 kcal/mol (Table 1; Figure 3E), approximately 14% lower than that in the parent compound. Although the *N*-trimethylacetyl and *N*-tribromoacetyl AHLs *per se* might not be ideal quorum sensing inhibitors due to steric concerns, our observations demonstrate not only that the $n \rightarrow \pi^*$ interaction contributes to the conformation of these important signal molecules but also that the conformation can be modulated by an appropriate choice of *N*-acyl substituents.

We note that the presence of an $n \rightarrow \pi^*$ interaction could have another important pharmacological implication. γ -Lactones are susceptible to hydrolysis,⁴¹ which eliminates the activity of an AHL.⁴² An $n \rightarrow \pi^*$ interaction increases the energy of the acceptor π^* orbital^{22,23} and thereby reduces the electrophilicity of the carbonyl group of the γ -lactone.^{29,43,44} Thus, the $n \rightarrow \pi^*$ interaction of AHLs could protect their γ -lactones against hydrolysis.

We conclude that an $O_{i-1} \cdots C'_i = O_i$ $n \rightarrow \pi^*$ interaction plays a key role in the conformation and, potentially, the biological activity of AHLs. Modifications that weaken this $n \rightarrow \pi^*$ interaction should increase the affinity of AHLs to their cognate receptors. Indeed, the propensity of electron-withdrawing substituents to attenuate the $n \rightarrow \pi^*$ interaction in AHLs could be contributing to the efficacy observed for certain synthetic AHLs as modulators of quorum sensing.⁴⁵ Conversely, modifications that strengthen this interaction should

decrease the rate of hydrolysis and endow AHLs with a longer biological half-life. We encourage exploration of this strategy for modulating bacterial quorum sensing with tailored small molecules.

■ ASSOCIATED CONTENT

Supporting Information

Procedures for the synthesis and conformational analysis of *N*-trimethylacetyl homoserine lactone and *N*-tribromoacetyl homoserine lactone and data for their structural analysis with X-ray crystallography. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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