

# Molecular and vibrational structure of the extracellular bacterial signal compound *N*-butyryl-homoserine lactone (C4-HSL)

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## ABSTRACT

The molecular and vibrational structure of the title compound (C4-HSL) was studied by experimental and theoretical methods. The infrared (IR) absorption spectrum was measured in the solid state and in CCl<sub>4</sub> suspension. The observed absorption bands were compared with transitions obtained with B3LYP/cc-pVTZ density functional theory (DFT) calculations. Two stable molecular conformations were predicted, corresponding to an *endo*- and an *exo*-conformer with similar energies. Intermolecular amide–amide hydrogen bonding in the crystal state was approximated by a simple cluster model, leading to excellent agreement with the observed solid state IR spectrum. Due to the low solubility of C4-HSL in common solvents for IR spectroscopy, such as CS<sub>2</sub> and CCl<sub>4</sub>, a liquid solution spectrum of pure, monomeric C4-HSL was not obtained. However, absorbance peaks observed in oversaturated CCl<sub>4</sub> solution could be assigned to distinct contributions from suspended micro-crystalline aggregates and dissolved monomeric species. The key vibrational bands of the monomeric form of C4-HSL are reported here for the first time: 3425 cm<sup>-1</sup> [ $\nu$ (N–H)], 1784 cm<sup>-1</sup> [ $\nu$ (C=O), lactone], 1688 cm<sup>-1</sup> [amide I], and 1494 cm<sup>-1</sup> [amide II] (CCl<sub>4</sub>).

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## 1. Introduction

A special class of organic compounds, *N*-acyl-homoserine lactones (AHL), is synthesized in bacteria and takes part in the intercellular signaling designated “quorum sensing” [1–3]. This bacterial cell-to-cell communication is responsible for many of the properties that characterize colony-forming bacteria, such as adherence on surfaces, production of slime, and resistance to a range of antibiotics. Biofilm formation on surfaces and medical devices poses a threat against public health [1–3]. For instance, catheter-related infections initiated by biofilms are common, costly, and responsible for a substantial part of the morbidity and mortality in many medical treatments.

Proper characterization of *N*-acyl-homoserine lactones is thus an important task. But the spectroscopic information on these compounds in the literature is sparse, particularly in the field of vibrational spectroscopy. One of the otherwise most thoroughly investigated quorum sensing signal compounds is *N*-butyryl-homoserine lactone (*N*-[tetrahydro-2-oxo-3-furanyl]-butanamide, C4-HSL, Scheme 1); nevertheless, diverging IR data are found in the literature [4,5]. In this communication we present the results of an

IR spectroscopic study of C4-HSL. Our investigation is supported by quantum chemical model calculations, leading to characterization of the molecular structure and unambiguous assignments of the key vibrational features of C4-HSL.

## 2. Experimental details

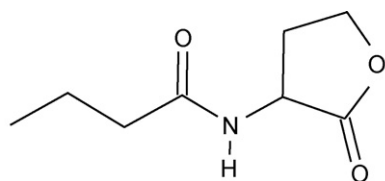
A sample of C4-HSL was purchased from Fluka BioChemika (*N*-butyryl-DL-homoserine lactone, CAS 98426-48-3, ≥97%). IR absorption spectra were recorded at room temperature with a spectral resolution of 2 cm<sup>-1</sup> and 10 scans on a PerkinElmer Spectrum 2000 FTIR spectrophotometer equipped with a FR-DTGS mid-IR (KBr) detector. A solid state spectrum was measured on a KBr tablet (Merck Uvasol) and liquid solution spectra were measured in CCl<sub>4</sub> and CS<sub>2</sub> solvents (Merck Uvasol). The solubility of the compound in the two solvents was very low, and CS<sub>2</sub> turned out to be inadequate for IR spectroscopy. The spectrum recorded in CCl<sub>4</sub> contained contributions from suspended micro-crystalline aggregates as well as from dissolved monomeric molecules (Section 4.2).

## 3. Calculations

The experimental spectra were compared with the results of quantum chemical calculations, performed with the GAUSSIAN 03 suite of programs [6] by using the B3LYP [7–9] density functional

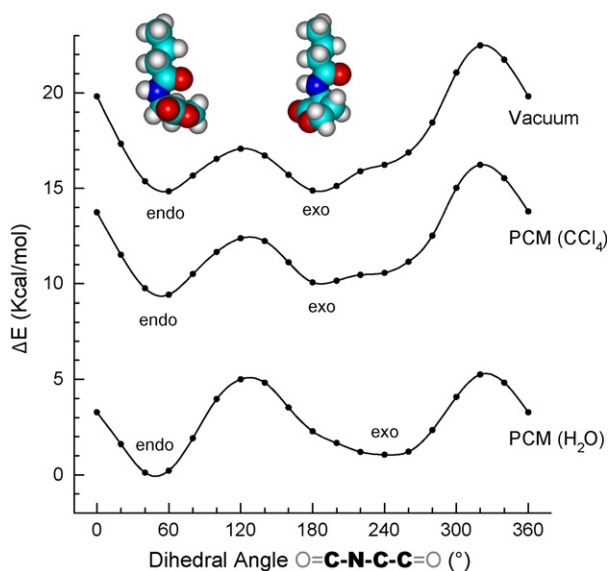
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*N*-Butyryl-homoserine lactone (C4-HSL)

Scheme 1.

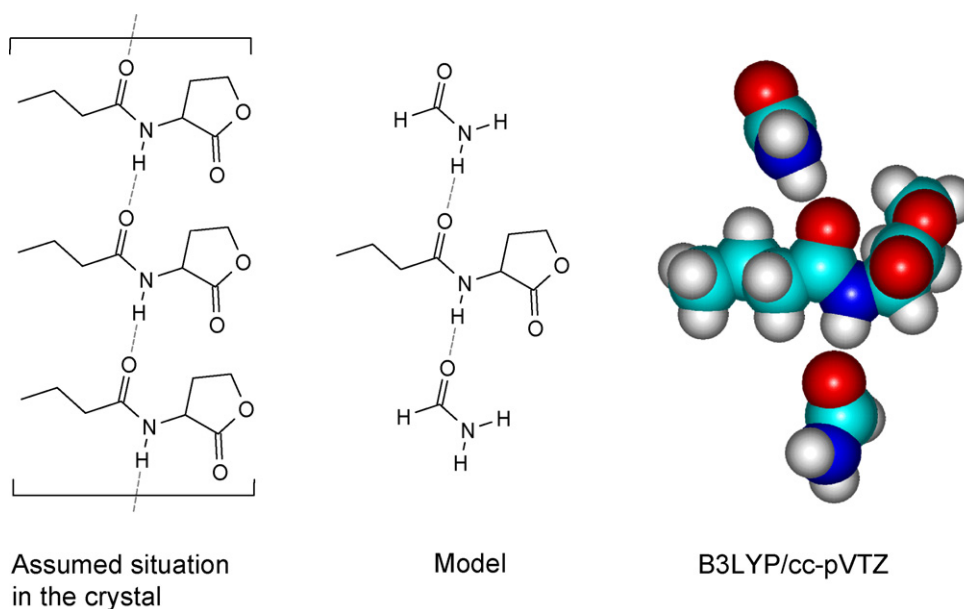


**Fig. 1.** Total molecular energy of C4-HSL computed with B3LYP/6-31G\* as a function of rotation around the N–C bond linking the butyryl and lactone moieties (1 kcal = 4.184 kJ). The curves indicate results in vacuum, and in the solvent media CCl<sub>4</sub> and H<sub>2</sub>O as represented by the polarized continuum model (PCM).

and the cc-pVTZ [10] basis set. In previous investigations, we have found that this level of theory leads to good agreement with the molecular and vibrational structure for a variety of organic compounds [11–15].

A series of preliminary B3LYP/6-31G\* [6] calculations revealed the presence of two stable conformations with respect to rotation around the N–C bond linking the butyryl and the lactone moieties of C4-HSL, corresponding to *endo*- and *exo*-conformations, as shown in Fig. 1. The pertinent dihedral angles (O=C)–N–C–C(=O) are close to 56° and 172°, respectively. B3LYP/cc-pVTZ nuclear equilibrium coordinates and other details, as well as IR and Raman transitions predicted within the harmonic approximation for both conformers are given as Supplementary data S1–S4. In the gas phase, the predicted energies of the two conformers are similar, but inclusion of a contribution from a solvent medium, such as CCl<sub>4</sub> or H<sub>2</sub>O, by means of the polarized continuum model (PCM) [6,16–18] leads to the stabilization of the *endo*-relative to the *exo*-conformer (Fig. 1). This is consistent with the much larger dipole moment of the former (S1 and S2). At the same time, increasing the polarity of the medium tends to increase the dihedral angle of the *exo*-conformer, while the structure of the *endo*-conformer is relatively unaffected. Inclusion of a PCM corresponding to CCl<sub>4</sub> had only minor influence on the calculated vibrational transitions (not reported here). In the remainder of this investigation, the PCM was not applied.

To our knowledge, the crystal structure of C4-HSL has not been resolved, but in the solid state the compound may be assumed to form polymeric chains by hydrogen bonding between neighboring amide functions, like other secondary amides [19,20]. In order to simulate the influence of intermolecular hydrogen bonding on the vibrational structure of C4-HSL, we have adopted a simple cluster model, representing first neighbor amide functions by formamide molecules, as indicated in Fig. 2. Similar models have been investigated by Guo and Karplus in their study of the hydrogen bonding of *N*-methylacetamide [21]. The hydrogen-bonded cluster with *endo*-C4-HSL and two formamide species predicted by B3LYP/cc-pVTZ is shown in Fig. 2 (right). The predicted O–N hydrogen-bond distances are close to 2.94 Å, indicating weak hydrogen bonding. The nuclear coordinates and the calculated IR and Raman



**Fig. 2.** The impact of intermolecular hydrogen bonding in the solid state is approximated by a simple cluster model, involving one C4-HSL molecule and two formamide molecules (see text).

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