JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Synthesis and Molecular Modeling Provide Insight into a *Pseudomonas aeruginosa* Quorum Sensing Conundrum

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Supporting Information

ABSTRACT: The triphenyl amide/ester **12** was originally reported to be a potent mimic of the natural 3-oxo-dodecanoyl homoserine lactone quorum sensing molecule in *Pseudomonas aeruginosa*. However, explicit synthesis/chemical characterization was lacking, and a later report providing protein crystallographic data inferred **12** to be incorrect, with **9** now being the surmised structure. Because of these inconsistencies and our interest in quorum sensing molecules utilized by Gram-negative bacteria, we found it necessary to synthesize **9** and **12** to test for agonistic activity in a *P. aeruginosa* reporter assay. Despite distinct regiochemical differences, both **9** and **12** were found to have comparable EC_{50} values. To reconcile these unanticipated findings, modeling studies were conducted, and both compounds were revealed to have comparable properties for binding to the LasR receptor.

Quorum sensing (QS) is the process in which bacteria communicate intercellularly through chemical signals termed autoinducers.[1] QS, a population-density-dependent phenomenon, allows bacterial populations to regulate a variety of physiological processes such as bioluminescence, antibiotic biosynthesis, biofilm differentiation, and production of virulence factors. *Pseudomonas aeruginosa*, an opportunistic pathogen, is found in many immunocompromised patients suffering from diseases such as cystic fibrosis, AIDS, burns, or neutropenic cancer.[2] *P. aeruginosa*, like many other Gram-negative bacteria, regulates QS through the exchange of acyl homoserine lactone-based autoinducers. One of the major autoinducers of *P. aeruginosa*, 3-oxo-dodecanoyl homoserine lactone (3OC12-HSL), along with its cognate receptor LasR, has been the subject of intense investigation.[3] Accordingly, and due to the dire effects of *P. aeruginosa* on human health, a myriad of approaches to the modulation of QS have been examined,[4] including the construction of structural analogues of 3OC12-HSL to act as QS agonists and antagonists.[5]

Recently, a library of 200 000 compounds was screened by Greenberg and co-workers in the hopes of discovering potent inhibitors or activators of the LasR-dependent QS pathway.[6] Both activators and inhibitors of QS were uncovered, and, excitingly, one compound exhibited more potent QS activation than the natural 3OC12-HSL signal. This compound, triphenyl **12** (termed TP-1P), was structurally unrelated to 3OC12-HSL but had an activity greater than that of the natural ligand (EC_{50} = 14 vs 140 nM).[7] However, the chemical identity of this novel activator was never explicitly confirmed and was subsequently proven incorrect in an X-ray crystallography study of LasR by Zou and Nair. According to the electron density maps of TP-1 bound to LasR, Zou and Nair proposed that the chlorine atom and the nitro group were reversed in the actual structure of TP-1, **9** (herein referred to as TP-1R).[8] Thus, the proposed potency of TP-1P, coupled with the quandary of its chemical structure, provided an impetus to synthesize both previous and revised compounds to be fully characterized and tested in a *P. aeruginosa* QS reporter assay system. Herein, we report these findings and the unexpected activity of both compounds.

Synthesis of both TP-1R and TP-1P began from commercially available 3,5-dibromosalicylaldehyde **1**, which was protected as the MOM ether **2** (Scheme 1). Reduction of the aldehyde using $NaBH_4$, followed by conversion of alcohol **3** to the mesylate, afforded compound **4**. At this point, Gabriel synthesis was invoked to provide primary amine **6**, which serves as the common intermediate for both TP-1R and TP-1P. Toward TP-1R, amide **7** was formed in the presence of 2-nitrobenzoic acid, EDC, and HOBt, followed by cleavage of the MOM ether to achieve phenol **8**. Finally, esterification of **8** with 2-chlorobenzoic acid yielded TP-1R (**9**).

The synthesis of TP-1P from **6** follows the same sequence with the following modifications: amide formation was performed in the presence of 2-chlorobenzoic acid to afford **10**, followed by MOM deprotection and ester formation in the presence of 2-nitrobenzoic acid to provide TP-1P (**12**).

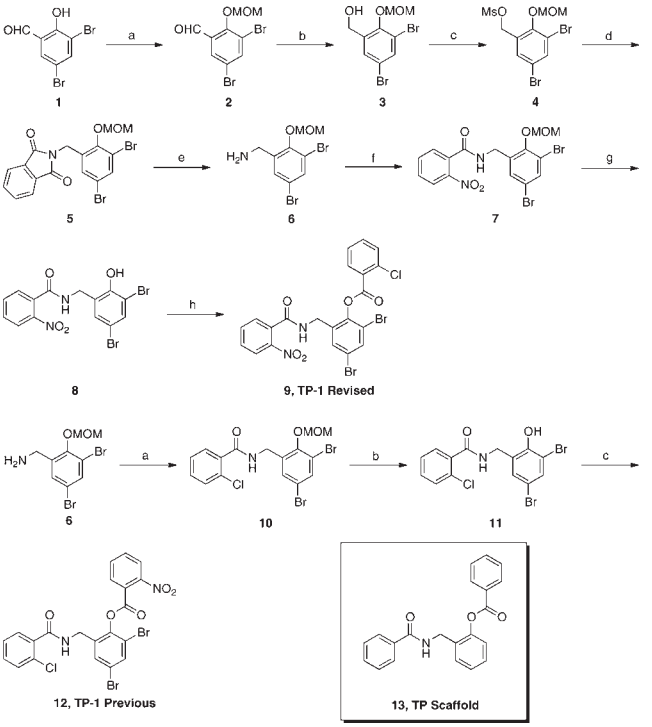
Before biological evaluation, the solubility and stability of TP-1P and TP-1R were measured to ensure optimal conditions for the cell-based QS assays. This is particularly important due to the presence of a seemingly labile *o*-nitro ester in TP-1P. Nevertheless, in agreement with previous reports,[7] TP-1P and TP-1R were stable in MES and TRIS buffer systems, with half-lives of 59.91 and 607.5 h at pH 8 (Supporting Information, Figure S1−S4 and Table S1). Furthermore, TP-1P and TP-1R were found to have a maximum solubility of 5 μM in 10% DMSO and a minimum tested solubility of 3.12 nM in 0.16% DMSO, thus ensuring the integrity of each compound under the assay conditions.

A reporter strain of *P. aeruginosa* based on the *luxCDABE* gene cassette was used to test agonistic activity of TP-1P and TP-1R

Received: December 10, 2010
Published: February 24, 2011

dx.doi.org/10.1021/ja111138y | *J. Am. Chem. Soc.* 2011, 133, 3840–3842

Scheme 1. Synthesis of TP-1R (top)[a] and TP-1P (bottom)[b]



[a] Conditions: (a) MOMCl, K_2CO_3, DMF, rt, 16 h, 93%; (b) $NaBH_4$, MeOH, THF, rt, 14 h, 96%; (c) MsCl, Et_3N, CH_2Cl_2, rt, 3 h, 65%; (d) potassium phthalimide, DMF, rt, 19 h, 80%; (e) $H_2NNH_2-H_2O$, EtOH, reflux, 17 h, 85%; (f) (2-NO_2)$PhCO_2H$, EDC−HCl, HOBt, DMF, rt, 19 h, quant.; (g) TFA, CH_2Cl_2, 0 °C to rt, 3 h, 61%; (h) (2-Cl)$PhCO_2H$, EDC−HCl, DMAP, THF, rt, 13 h, 50%. [b] Conditions: (a) (2-Cl)$PhCO_2H$, EDC−HCl, HOBt, rt, 19 h, 66%; (b) TFA, CH_2Cl_2, 0 °C to rt, 3 h, quant.; (c) (2-NO_2)$PhCO_2H$, EDC−HCl, DMAP, THF, rt, 13 h, 52%.

Table 1. Biological Activity and Binding Energies of TP-1R, TP-1P, Tp scaffold, and 3OC12-HSL

compound	EC_{50} (nM)	ΔG (kcal/mol)[a]
9, TP-1R	28.3 ± 4.3	−11.5
12, TP-1P	42.8 ± 4.0	−11.7
13	>10 000	nd[b]
3OC12-HSL	30.9 ± 1.3	nd[b]

[a] Represents relative free energy of binding between ligand and LasR. [b] Not determined.

compared to that of the natural autoinducer 3OC12-HSL.[9] TP-1P, TP-1R, and 3OC12-HSL all demonstrated similarly potent agonistic activity of LasR-dependent signaling (Table 1). Interestingly, maximal luminescence was nearly equal for TP-1R and 3OC12-HSL, but TP-1P induced only 50% luminescence relative to the former compounds (SI, Figure S5). Nevertheless, the finding of similar EC_{50} values between the two triphenyl compounds was unforeseen, in light of the perceived effect of the regiochemical change of the chloro and nitro positioning in the two agonists. This was even more puzzling considering that the LasR receptor protein has evolved to bind a vastly dissimilar ligand in 3OC12-HSL.

Seeking to delineate the similar QS activity of TP-1P and TP-1R on a molecular level and to elucidate the structural

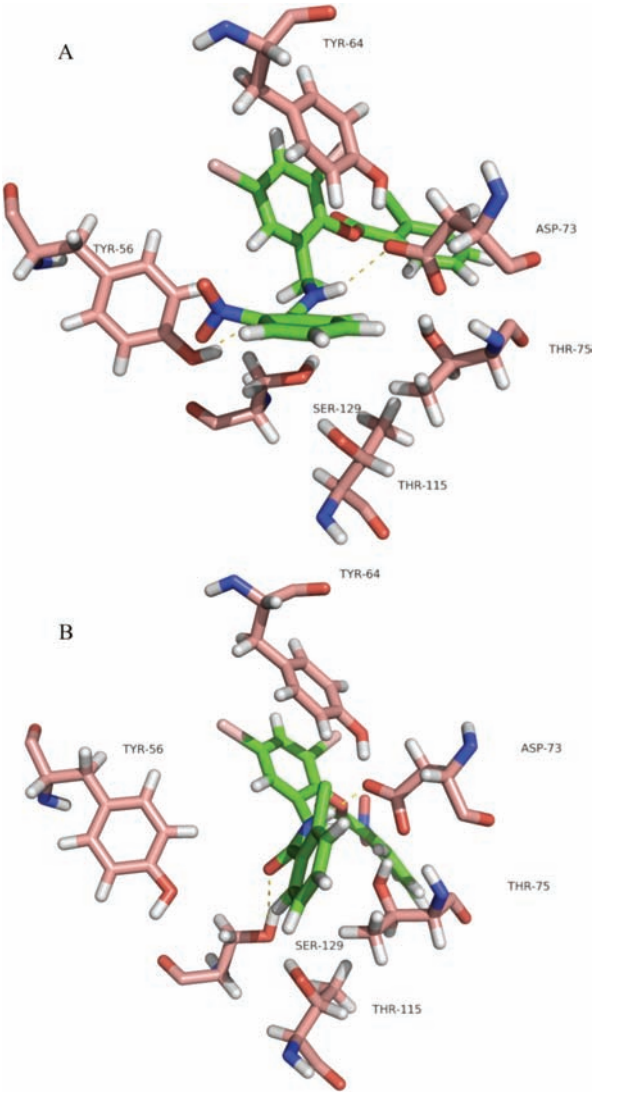


Figure 1. Simulations from the 3IX4 structure show the amide in TP-1R forming hydrogen bonds with Asp73 and Tyr56 (A), whereas with TP-1P, Tyr56 has moved away and no longer interacts with TP-1P. In TP-1P, the amide is rotated compared to that in TP-1R and makes hydrogen bonds with Asp73 and Ser129 (B). In both simulations, Asp73 is caged through interactions with Tyr64 and two interactions with Thr75.

requirements of binding, computational studies were initiated to model the TP ligands into the LasR binding domain. Studies of TP-1P and TP-1R were simulated using Gromacs v4.0.7 to determine the necessity of the nitro and chloro substituents. Simulations of the 3IX4 complex[8] did not show any highly occupied hydrogen bonds between TP-1R and LasR. The most consistent interactions were between the amide proton of TP-1R and Asp73 and the amide carbonyl of TP-1R and Tyr56, but these interactions were transient, as Asp73 also hydrogen bonds with Thr75 and Tyr64 throughout the simulation and Tyr56 also hydrogen bonds with Ser129 (Figure 1). The exchange of the nitro and chloro groups in TP-1R and TP-1P does not affect the occupancy of either the hydrogen bond with Asp73 or that with Tyr56 in TP-1R. The only significant difference in the simulations was that, with TP-1P, the amide carbonyl occasionally forms a hydrogen bond with Ser129, which is unseen with TP-

dx.doi.org/10.1021/ja111138y |J. Am. Chem. Soc. 2011, 133, 3840–3842

1R. Pleasingly, truncated Alchemical FEP simulations (Table 1) show only a small difference in the relative binding energy $\Delta\Delta G$ = 0.2 kcal/mol between TP-1R and TP-1P.

Due to the high degree of shape similarity of TP-1R and TP-1P, both compounds' ability to make hydrogen bonds through their amide moiety to nearby residues, and the low $\Delta\Delta G$ of binding between the two molecules, it is not surprising from our simulations that they are both of similar potency. The positioning of the nitro and chloro groups within the binding pocket of LasR with no consistent hydrogen-bonding or electrostatic interactions with nearby residues sheds light on the similar activity observed with TP-1P and TP-1R. To investigate the effect of the scaffold alone, 2-(benzamidomethyl)phenyl benzoate (**13**) was synthesized in one step from 2-hydroxybenzyl amine and benzoic acid and tested in the *P. aeruginosa* reporter assay. This compound failed to give any QS activation at concentrations up to 10 μM (Table 1). Thus, while the regiochemical interchange of the nitro and chloro substituents does not seem paramount for activity, the presence of an electronegative substituent appears to be critical for LasR binding, dimerization, and ultimately gene expression.

In summary, through chemical synthesis, we have firmly established the chemical identities of TP-1R/TP-1P, and through modeling, we have ascertained the minimal necessary chemical architecture for LasR activation. Lastly, we highlight the potential synthetic interchangeable pieces found within the 2-(benzamidomethyl)phenyl benzoate. The ester and amide units could readily serve as a viable grounding for the diversity and development of additional agonists and antagonists against LasR-dependent QS in *P. aeruginosa*.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures, spectral data, and biological protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We gratefully acknowledge the NIH (AI077644) for support and Prof. Michael Surette (University of Calgary) for providing the luminescent PAO-JP2 *P. aeruginosa* strain.

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