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Structure−Activity Relationships of 6- and 8-Gingerol Analogs as Anti-Biofilm Agents

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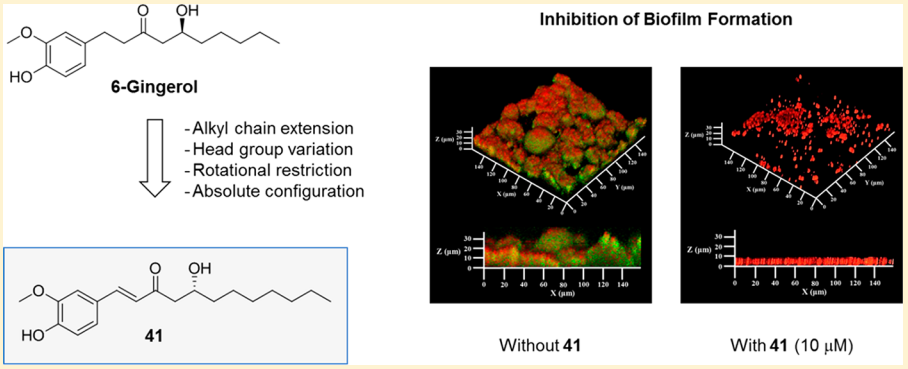
Supporting Information



ABSTRACT: *Pseudomonas aeruginosa* is a causative agent of chronic infections in immunocompromised patients. Disruption of quorum sensing circuits is an attractive strategy for treating diseases associated with *P. aeruginosa* infection. In this study, we designed and synthesized a series of gingerol analogs targeting LasR, a master regulator of quorum sensing networks in *P. aeruginosa*. Structure−activity relationship studies showed that a hydrogen-bonding interaction in the head section, stereochemistry and rotational rigidity in the middle section, and optimal alkyl chain length in the tail section are important factors for the enhancement of LasR-binding affinity and for the inhibition of biofilm formation. The most potent compound **41**, an analog of (*R*)-8-gingerol with restricted rotation, showed stronger LasR-binding affinity and inhibition of biofilm formation than the known LasR antagonist (*S*)-6-gingerol. This new LasR antagonist can be used as an early lead compound for the development of anti-biofilm agents to treat *P. aeruginosa* infections.

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic pathogen that causes infections in people with a weakened immune system, e.g., patients with cystic fibrosis, chronic wounds, pneumonia, AIDS, sepsis, or cancer.[1] It is one of the six most dangerous bacterial species according to the Infectious Diseases Society of America.[2] In particular, *P. aeruginosa* infection is the main cause of mortality in patients with cystic fibrosis.[3,4] *P. aeruginosa* can form a surface-associated community, a so-called biofilm. Biofilm cells are embedded in a self-produced exopolysaccharide matrix that confers antibiotic resistance.[5] Biofilms are involved in most of microbial infections of humans (∼80% of such bacterial infections).[6,7] Biofilms retard penetration of antibiotics and reduces the antibiotic activity, thus reducing treatment efficacy.[8]

A biofilm is an aggregation of microbial cells encapsulated by self-produced extracellular polymeric substances on the surface.[9] During biofilm formation, bacterial cells communicate with one another by means of quorum sensing (QS) network.[10] QS is a cell-to-cell communication system in which bacteria release and recognize chemical signals (autoinducers), and QS enables bacteria to behave as a group to adapt to environmental changes.[11] In general, Gram-negative bacteria including *P.*

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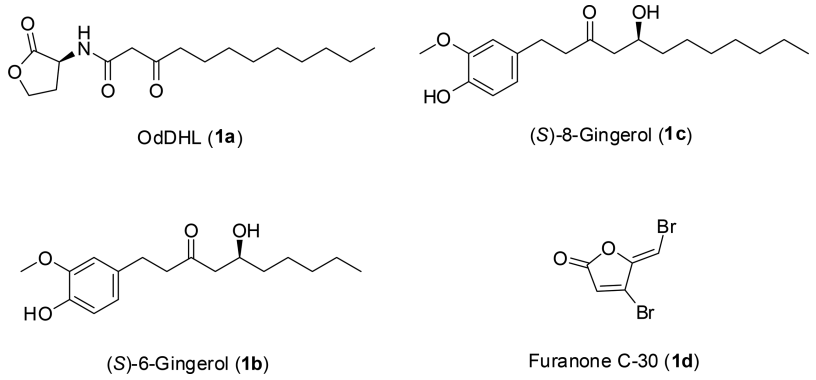

Figure 1. Small molecules interacting with LasR of *P. aeruginosa*.

aeruginosa produce and release *N*-acylhomoserine lactone (AHL) as a QS signal molecule.[12] The QS mechanism of *P. aeruginosa* is tightly regulated by the three main signal production and recognition systems: LasI-LasR, RhlI-RhlR, and PQS-MvfR.[13] LasI in *P. aeruginosa* produces an extracellular diffusible *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL, **1a** in Figure 1), which activates expression of genes responsible for group behaviors including biofilm formation and production of virulence factors. When OdDHL reaches a threshold concentration, the OdDHL-LasR complex binds to the promoter regions of multiple genes affecting RhlI-RhlR and 2-heptyl-3-hydroxy-4(1*H*)-quinolone (PQS)-MvfR systems. Similarly, RhlI produces *N*-butyryl-L-homoserine lactone (BHL), which is recognized by the transcriptional regulator RhlR.[14] In the PQS-MvfR system, PQS and its precursors bind to the transcriptional regulator MvfR, resulting in transcription of target genes.[15] Among the three systems, LasI-LasR is considered to be a master regulator of QS networks and a key system in the biofilm formation by *P. aeruginosa*.

P. aeruginosa forms biofilms and produces virulence factors through QS pathways. Therefore, disruption of these signal production and recognition systems is an attractive strategy for attenuating the virulence of *P. aeruginosa*.[16] One of the antivirulence approaches is to interrupt the interaction between chemical signals (e.g., OdDHL, BHL, and PQS) and their cognate receptors (e.g., LasR, RhlR, and MvfR).[17] For instance, halogenated furanones from the marine alga *Delisea pulchra* have a structure similar to AHL and can bind to LasR by competing with OdDHL.[18] In addition, (*Z*)-4-bromo-5-(bromomethylene)furan-2(5*H*)-one (furanone C-30, **1d** in Figure 1), a synthetic molecule, inhibits the expression of virulence factors by interfering with *P. aeruginosa* QS systems.[19]

We previously demonstrated that (*S*)-6-gingerol (**1b**, Figure 1), the main component of ginger, reduces biofilm formation and production of virulence factors by competing with OdDHL for LasR of *P aeruginosa*. RT-qPCR analyses revealed that **1b** reduces the expression of genes (e.g., *las*, *rhl*, *pqs*, and *phz* genes) in the QS system and suppresses the production of virulence factors (e.g., exoprotease, pyocyanin, and rhamnolipid), indicating that it interferes with the interaction between OdDHL and LasR, at the top of the hierarchical QS network tree of *P. aeruginosa*. Molecular modeling studies of the interaction of **1b** with LasR (PDB code 2UV0) indicates that the 3′-hydroxyl-4′-methoxyphenyl moiety engages in a hydrogen-bonding interaction with hydrophilic amino acids, while the alkyl side chain forms a hydrophobic bond.[20] In this study, we aimed to investigate the effect of each functional group of **1b** on LasR-binding affinity and on biofilm formation by *P. aeruginosa*. We herein describe the structure−activity relationship (SAR) studies of 6- and 8-gingerol analogs and the development of novel LasR antagonists based on the chemical structure of **1b**. Novel potent biofilm inhibitors targeting LasR of *P. aeruginosa* have been identified from the comprehensive SAR studies.

RESULTS AND DISCUSSION

Structural modification of **1b** was attempted based on key interactions between **1b** and LasR of *P. aeruginosa*. Chemical structure of **1b** was subdivided into three parts (head, middle, and tail sections) as shown in Figure 2. We aimed to investigate the effect of each section on LasR-binding affinity and on biofilm formation.

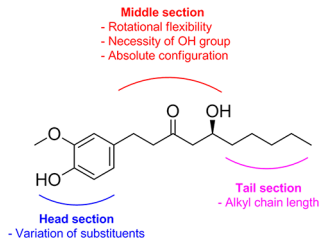

Figure 2. Strategy for structural modification of gingerol derivatives.

In the head section, we tried to determine whether the hydrogen-bonding interaction is necessary or not by replacing the methoxy group at the 3′-position and/or the hydroxyl group at 4′-position of the phenyl moiety with other functional groups. Regarding the modification of the middle section, we introduced a double bond between the phenyl moiety and the carbonyl group to assess the influence of rotational flexibility. We also evaluated the necessity of the hydroxyl group and the effect of stereochemistry of the chiral center on the affinity for LasR and on biofilm formation. In the tail section, we tried to find the optimal alkyl chain length for a maximized the van der Waals interaction with the LasR hydrophobic subpocket, which is formed by lipophilic amino acid residues (Leu36, Leu40, Ala50, Ile52, Ala70, Val76, and Leu125).

First, we synthesized gingerol analogs with various alkyl chain length from 4-gingerol to 10-gingerol to find the optimal carbon length in the tail section. As shown in Scheme 1, compound **2** was synthesized from commercial vanillin by treatment with 10% NaOH in acetone at 25 °C for 16 h in 71% yield. Compound **3** was obtained by reacting **2** with hydrogen

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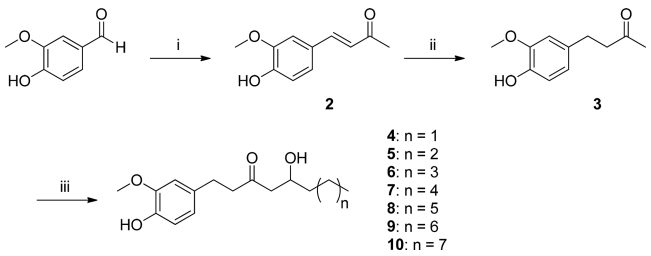
Scheme 1. Synthesis of Gingerol Derivatives with Various Alkyl Chain Lengths in the Tail Section[a]



[a]Reagents and conditions: (i) 10% NaOH, acetone, rt, 16 h, 71%; (ii) H_2, Pd/C, MeOH, rt, 2 h, 97%; (iii) LDA, appropriate aldehydes, THF, −78 °C, 2 h, 30−47%.

gas in methanol in the presence of 10% Pd/C at 25 °C for 2 h in 97% yield. Treatment of **3** with lithium diisopropylamide (LDA) at −78 °C, followed by the addition of appropriate aldehydes (butanal for **4**, pentanal for **5**, hexanal for **6**, heptanal for **7**, octanal for **8**, nonanal for **9**, and decanal for **10**), afforded the final gingerol compounds in 30−47% yield.[21−23] By-products such as condensated or dimerized compounds were produced when excess LDA was used and the reaction time was longer than 2 h.

LasR-binding affinity of the synthesized gingerols with various alkyl chain lengths was determined by measuring luminescence of an *E. coli* reporter strain. The reporter strain carried two plasmids, pJN105L (LasR expression plasmid)[24] and pSC11 (*lasI::lacZ* fusion plasmid),[25] which enabled us to assay competitive binding of OdDHL with each gingerol derivative **4**−**10**. Antagonistic activities of the synthesized compounds at 1 μM or 10 μM were determined by measuring luminescence in the presence of 1 μM OdDHL (**1a**) and presented as relative luminescence unit (RLU) ratio. Three compounds (**1b**, **1c**, and **1d**) served as positive controls.[16,19,26] As shown in Figure 3, LasR-antagonistic activities increased as the alkyl chain lengthened, indicating that the longer alkyl group contributed to the affinity for LasR via the van der Waals interaction in the hydrophobic subpocket of LasR. Just as the LasR inhibition, inhibition of biofilm formation also strengthened as the carbon chain was extended at 10 μM (Figure 3B). However, inhibition of biofilm formation decreased with 9-gingerol (**9**) and 10-gingerol (**10**) at 100 μM (Figure 3C) because of increased bacterial growth inhibition. We previously reported that 2*H*-pyran-2-one analogs show increased inhibition of LasR as the carbon chain lengthens, with the highest potency corresponding to the dodecyloxy group.[27] Park et al. reported that 10- and 12-gingerol have antibacterial activity against periodontal bacteria.[28] As shown in Figure 3D, compounds **9** and **10** with a longer alkyl chain inhibited bacterial growth significantly at 100 μM as compared with the other compounds. This effect may be due to the fact that **9** and **10** act as a surfactant, which inhibits bacterial growth. We next examined agonistic activities of the synthesized gingerols (**4**−**10**) in an *E. coli* reporter assay system in the absence of OdDHL. None of them showed agonistic activity to LasR at 10 μM (see Supporting Information).

On the basis of results of LasR antagonism and biofilm formation of compounds **4**−**10**, 6- and 8-gingerol were selected

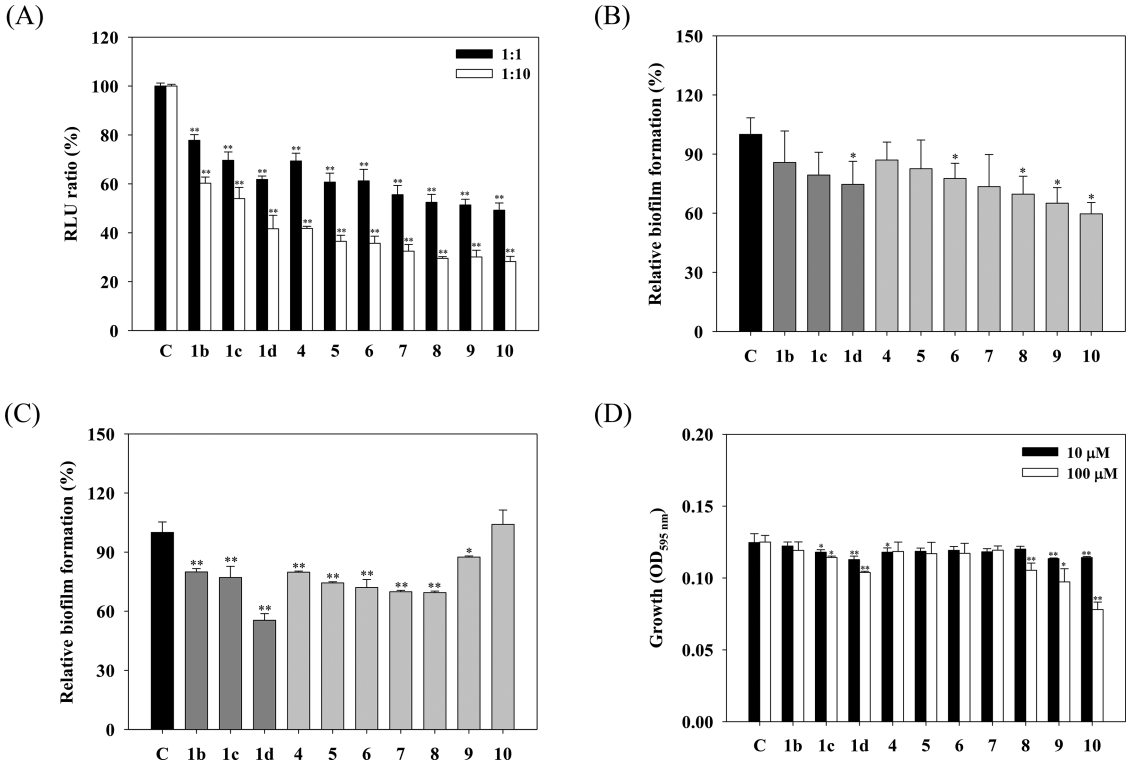


Figure 3. Effects of alkyl chain length variation in gingerol derivatives. DMSO (C, negative control) and **1b**, **1c**, and **1d** (positive controls) were used. (A) LasR binding activity of gingerol derivatives (**4**−**10**) at different ratios of **1a** to the compound (1:1 or 1:10). (B) Biofilm formation at 10 μM gingerol derivatives (**4**−**10**). (C) Biofilm formation with gingerol derivatives (**4**−**10**) at 100 μM. (D) Growth inhibition by gingerol derivatives (**4**−**10**) at 10 or 100 μM for 24 h. (**) $P < 0.005$ and (*) $P < 0.05$ as compared with the control. RLU ratio (%) in the *Y* axis is the relative luminescence unit ((luminescence/OD_{595}) × 100).

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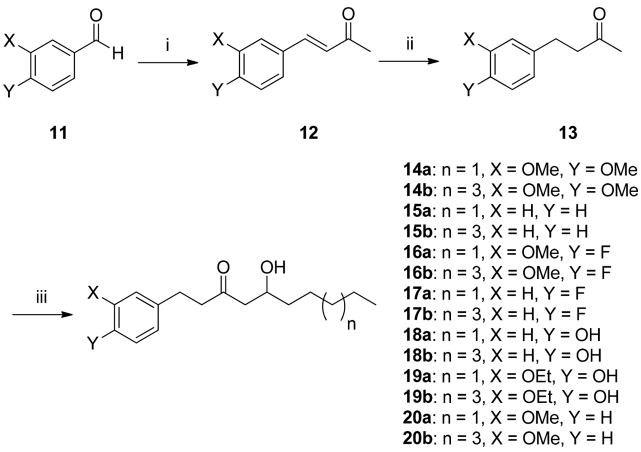
for the structural modification in the head section. A similar synthetic strategy was applied to prepare 6- and 8-gingerol derivatives with various functional groups in the head section. 6-Gingerol derivatives (**14a**−**20a**) were prepared from commercial vanillin in three steps. Briefly, treatment of various benzaldehydes with acetone afforded analogs of compound **12** in 35−75% yield. Catalytic hydrogenation of the unsaturated alkene group produced analogs of **13** in 80−97% yield. 6-Gingerol analogs (**14a**−**20a**) with various substituents in the head section were obtained by reacting **13** with *n*-hexanal as shown in Scheme 2, whereas 8-gingerol derivatives (**14b**−**20b**) were done with *n*-octanal instead of *n*-hexanal at the final step.

Scheme 2. Synthesis of 6- and 8-Gingerol Derivatives with Variations in the Head Section[a]



[a]Reagents and conditions: (i) acetone, 10% NaOH, rt, 48 h, 35−75%; (ii) H_2, Pd/C, MeOH, rt, 2 h, 80−97%; (iii) LDA, hexanal (n = 1) or octanal (n = 3), THF, −78 °C, 2 h, 25−47%.

LasR-antagonistic activities of 6- and 8-gingerol derivatives modified in the head section were evaluated. Hydrogen-bonding effects of the methoxy group at the 3′-position and the hydroxyl group at the 4′-position of the phenyl moiety in 6-gingerol analogs were evaluated by introducing other functional groups. As shown in Figure 4A, removal of the methoxy and hydroxyl group together (compound **15a**) significantly decreased the LasR-antagonistic activity, implying that at least one hydrogen-bonding interaction is required for binding to LasR. After that, we removed only the methoxy group at the 3′-position (compound **18a**); this change increased the LasR-antagonistic activity. In contrast, removal of the hydroxyl group at the 4′-position (compound **20a**) decreased the LasR-antagonistic activity. These results suggested that substituents capable of hydrogen bonding at the 4′-position were more favorable for binding to LasR. Replacement of OH at the 4′-position with F preserved the LasR-inhibitory activity (**16a** vs **1b** and **17a** vs **18a**), suggesting that the functional group at 4′-position may act as a hydrogen-bonding acceptor rather than a hydrogen-bonding donor.

LasR inhibition patterns of 8-gingerol derivatives were similar to those of 6-gingerol. As shown in Figure 4C, compounds **17b** and **18b** with a substitution by a hydrogen-bond acceptor at the 4′-position were the most potent among the synthesized compounds. Activities of compounds **15b** and **20b** without any hydrogen-bonding acceptor at the 4′-position were relatively weaker than those of the other compounds. In general, LasR inhibition by 8-gingerol derivatives was stronger than the corresponding 6-gingerol ones. The static biofilm formation assay of 6- and 8-gingerol derivatives with variation in the head section showed a tendency similar to that in the LasR inhibition assay (Figure 4B and Figure 4D). Compounds with a hydrogen-bonding acceptor at the 4′-position (**16a,b**, **17a,b**, and **18a,b**) were the most potent in the series. This result was consistent with the hypothesis that derivatives with stronger affinity for LasR can inhibit biofilm formation more effectively. Two novel compounds (**16b** and **17b**) and one known compound (**18b**) exerted stronger inhibition of biofilm formation than the known anti-biofilm agent **1b**.

To assess the effect of rotational flexibility between the head section and the carbonyl group, several compounds (**21a,b** and **22a,b**) were prepared in 30−35% yield via crossed aldol condensation (Scheme 3). In addition, compounds **24a** and **24b** were prepared to determine the necessity of the β-hydroxy group for the LasR-binding affinity and for the inhibition of biofilm formation. Reaction of vanillin with 2-nonanone or 2-dodecanone in the presence of L-proline gave compounds **23a** and **23b** in 45% and 60% yield, respectively. Compounds **24a** and **24b** were prepared in 80% and 97% yield by subjecting **23a** and **23b** to hydrogenation conditions (H_2, Pd/C) for 2 h.[29]

As shown in Figure 5, compounds **21a** and **21b** (restricted rotation) showed slightly stronger LasR affinity than did the corresponding compounds **6** and **8** with flexible rotation. The derivatives (**23a,b** and **24a,b**) without the β-hydroxyl group showed significantly weaker LasR-binding affinity and less inhibition of biofilm formation than the ones with the β-OH group. These data suggested that the OH group at the β-position of the carbonyl group may play a pivotal role in the binding to the LasR protein as well as in the inhibition of *P. aeruginosa* biofilm formation.

Results on in vitro LasR-binding and inhibition of biofilm formation indicated that 8-gingerol analogs were more potent than 6-gingerol analogs. Furthermore, a racemic mixture of 6- and 8-gingerol (**6** and **8**) was slightly more potent than the pure (*S*)-enantiomer of 6-gingerol (**1b**) or 8-gingerol (**1c**), which are natural forms and commercially available. Therefore, we hypothesized that the pure (*R*)-enantiomer of gingerol would possess stronger LasR-binding affinity and inhibition of biofilm formation than the corresponding (*S*)-enantiomer. Enantiomerically enriched (*R*)- and (*S*)-enantiomers of 8-gingerol were synthesized by means of chiral catalysts such as D-proline and Salen's catalyst.

Scheme 4 shows the synthetic approach to enantiomerically enriched (*R*)-8-gingerol (**29**) using D-proline as a chiral catalyst. Compound **25** was synthesized from 1-octanal by treatment with D-proline in acetone at room temperature for 48 h. Acetone served as a reagent and solvent in the reaction. The β-hydroxyl group in compound **25** was protected by treatment with *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in dichloromethane to give compound **26** (87% yield). Silyl enol ether compound **27** was obtained by treating **26** with trimethylsilyl trifluoromethanesulfonate (TMSOTf) and *N,N*-diisopropylethylamine (DIPEA). The Mukaiyama aldol reaction between silyl enol ether **27** and vanillin, with simultaneous removal of the TBDMS group using boron trifluoride (BF_3),[30] afforded compound **28** (65% yield) in two steps. Catalytic hydrogenation of **28** produced the final compound **29** in 97% yield. The % enantiomeric excess (ee) value of compound **29** was determined by chiral HPLC analysis.[30]

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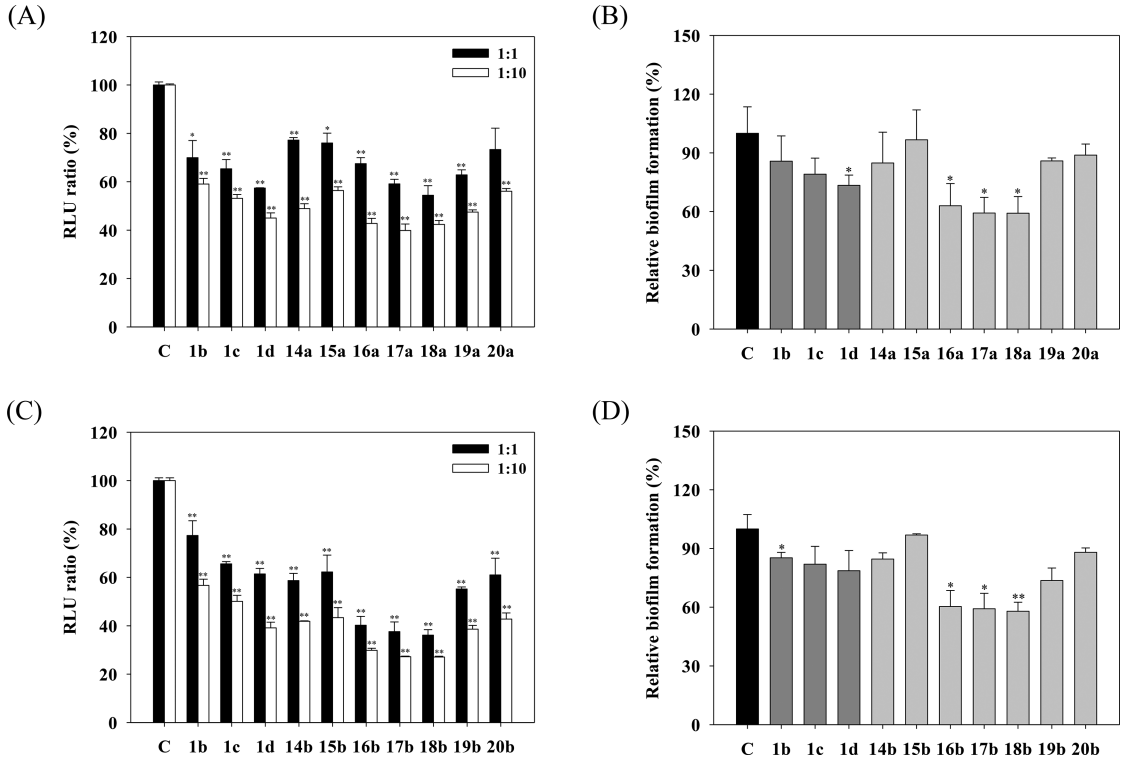
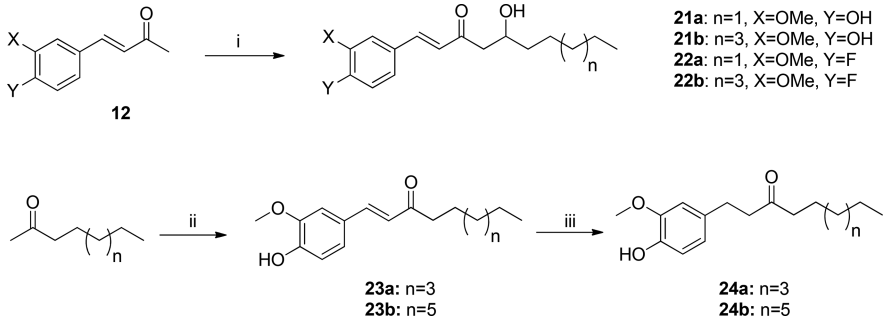


Figure 4. Effects of head group variation in 6- and 8-gingerol derivatives. DMSO (C, negative control) and **1b**, **1c**, and **1d** (positive controls) were used. (A) LasR binding activity of **14a−20a** at different ratios of **1a** to the compound (1:1 or 1:10). (B) Biofilm formation at 10 μM 6-gingerol derivatives (**14a−20a**). (C) LasR activity of **14b−20b** at different ratios of **1a** to the compound (1:1 or 1:10). (D) Biofilm formation at 10 μM 8-gingerol derivatives (**14b−20b**). (**) $P < 0.005$ and (*) $P < 0.05$ as compared with the control.

Scheme 3. Synthesis of 6- and 8-Gingerol Derivatives with Restricted Rotation or without the β-Hydroxyl Group[a]



[a]Reagents and conditions: (i) LDA, hexanal (n = 1) or octanal (n = 3), THF, −78 °C, 1 h, 30−35%; (ii) L-proline, vanillin, TEA, MeOH, rt, 48 h, 45−60%; (iii) H_2, Pd/C, MeOH, rt, 2 h, 80−97%.

The synthetic strategy for enantiomerically enriched (*R*)-8-gingerol (**42**) using Salen's catalyst is described in Scheme 5. Briefly, compound **31** was synthesized from commercial 3-buten-1-ol via treatment with sodium hydride and benzyl bromide in THF at 0 °C for 16 h.[22,31] Reaction of **31** with *m*-CPBA and $NaHCO_3$ in CH_2Cl_2 at 0 °C for 16 h gave a racemic mixture of epoxide **32** in 72% yield. The (*S*)-epoxide **33** was obtained by reacting **32** with (*S,S*)-(+)-*N,N′*-bis(3,5-di-*tert*-butylsalicyclidene)-1,2-cyclohexanediaminocobalt(II) (Salen's catalyst). The (*R*)-epoxide of **32** was transformed into (*R*)-1,2-diol, whereas the (*S*)-epoxide remained intact, as determined by ^{1}H NMR analysis during the reaction (see Supporting Information). Lithiation of the terminal alkyne of 1-hexyne with *n*-BuLi, followed by the addition of **33**, afforded compound **34** in 57% yield via an epoxide ring-opening reaction. The hydroxyl group of **34** was protected with TBDMSCl to obtain compound **35**. Debenzylation of **35** by means of H_2 and Pd/C provided primary alcohol **36** in 89% yield. Treatment of **36** with $NaIO_4$ and $RuCl_3$ oxidized the primary alcohol to the carboxylic acid, thus producing compound **37** in 67% yield.[32] The carboxylic acid was transformed into Weinreb amide **38** by using *N,O*-dimethylhydroxylamine under peptide-coupling conditions (HOBt and EDC). Reaction of **38** with methylmagnesium bromide in THF afforded compound **39** in 90% yield.[32] Silylenol ether **40** was generated by reacting **39** with TMSOTf and DIPEA in dichloromethane. The Mukaiyama aldol reaction between **40** and vanillin, with simultaneous deprotection of the TBDMS group using boron trifluoride (BF_3), afforded compound **41** (65% yield) in two steps. The final compound **42** was obtained in 97% yield by reducing the double bond of α,β-unsaturated ketone **41** under catalytic hydrogenation conditions. The (*S*)-

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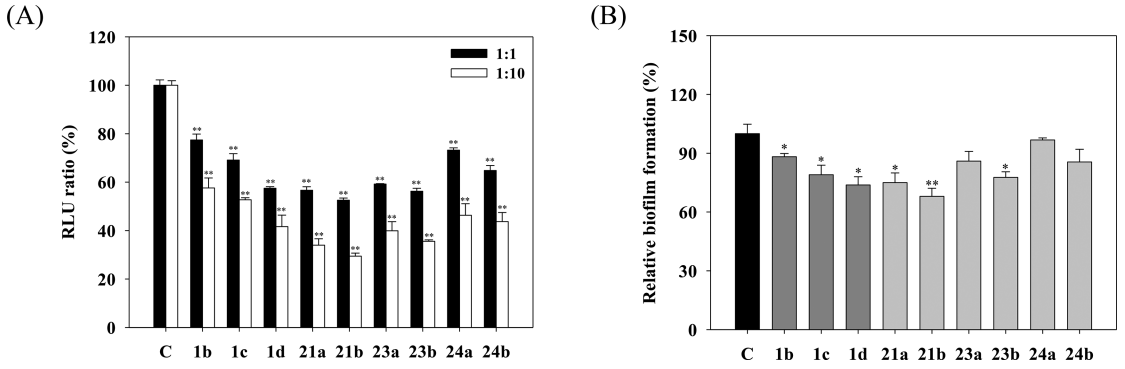
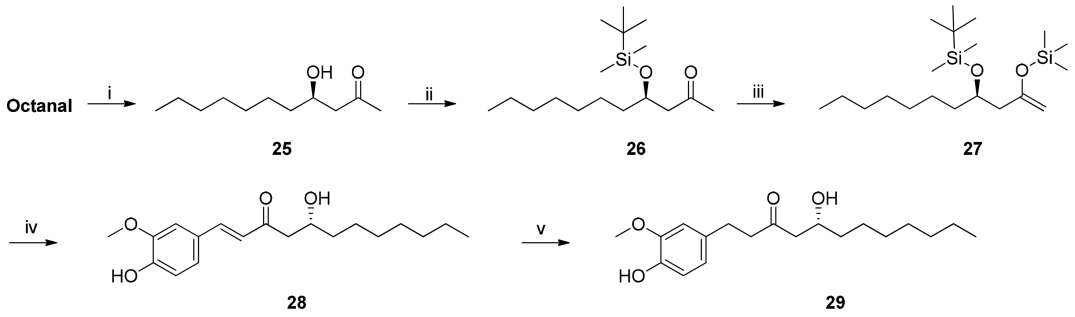


Figure 5. Rotational flexibility and necessity of the β-hydroxyl group for LasR-binding affinity and for inhibition of biofilm formation. DMSO (C, negative control) and **1b**, **1c**, and **1d** (positive controls) were used. (A) LasR binding activity of **21a−24b** at different ratios of **1a** to the compound (1:1 or 1:10). (B) Biofilm formation at 10 μM concentration of **21a−24b**. (**) $P < 0.005$ and (*) $P < 0.05$ as compared to the control.

Scheme 4. Synthesis of Enantiomerically Enriched (*R*)-8-Gingerol (29) by Means of D-Proline as a Catalyst[a]



[a]Reagents and conditions: (i) acetone, (D)-proline, rt, 48 h, 48%; (ii) TBDMSCl, imidazole, CH_2Cl_2, rt, 10 h, 87%; (iii) TMSOTf, DIPEA, CH_2Cl_2, 0 °C, 4 h; (iv) vanillin, $BF_3{\cdot}OEt_2$, TEA, CH_2Cl_2, 0 °C, 2 h (65% over 2 steps); (v) H_2, Pd/C, MeOH, rt, 2 h, 97%.

isomer of 8-gingerol (**42S**) was prepared in a similar way, where (*R*,*R*)-(+)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicyclidene)-1,2-cyclohexanediaminocobalt(II) was used at the third step (see Supporting Information).

As shown Figure 6, Scheme 1 without a chiral catalyst produced a racemic mixture of 8-gingerol (**8**) at an almost 1:1 ratio in a chiral HPLC experiment (Figure 6A). Scheme 4, in which D-proline served as a chiral catalyst, generated **29** with a 70% ee value (Figure 6B) similar to that in a previously reported reaction.[29] As expected, Scheme 5 by means of Salen's catalyst afforded (*S*)-8-gingerol (**42S**) and (*R*)-8-gingerol (**42**) with ee value of >95% (Figure 6C and Figure 6D).

LasR-binding affinity of the synthesized (*R*)- and (*S*)-8-gingerol compounds was evaluated in a luminescent reporter assay. The activity of (*S*)-8-gingerol (**42S**) synthesized using Salen's catalyst was almost the same as that of commercial (*S*)-8-gingerol (**1c**). As the proportion of the (*R*)-enantiomer of 8-gingerol increased, LasR-binding affinity was strengthened accordingly. The enantiomerically enriched (*R*)-8-gingerol **42** showed much stronger LasR-binding affinity than **1c**, as was the case for a racemic mixture of 8-gingerol (**8**). Compound **29** with an ee value of 70% had the intermediate LasR-affinity between **8** and **42** (Figure 7A). As shown in Figure 7B, the results of the static biofilm formation assay indicated a trend similar to that of the affinity for LasR. Compound **42** yielded 72% biofilm formation when the effect of **1c** was set to 100%. Effects of absolute configuration on the interaction between QS chemical signals and their cognate receptors have been investigated elsewhere.[33−35] Recently, Blackwell and co-workers studied *N*-(3-hydroxydodecanoyl)-L-homoserine lactone (OH-DHL) analogs and their putative cognate receptor AbaR in *Acinetobacter baumannii*. The (*R*)-isomer of OH-DHL was found to be a more active AbaR receptor agonist than (*S*)-isomer of OH-DHL.[35] In our study, the (*R*)-enantiomer of 8-gingerol **42** manifested stronger LasR-binding affinity than the synthesized (*S*)-enantiomer **42S** and the commercial **1c**.

Because compound **21b** (a racemic mixture) with restricted rotation between the carbonyl group and phenyl moiety was more potent than **1c** (Figure 5), compound **41** was assumed to be more potent than compound **42**. As expected, compound **41** showed stronger LasR-binding affinity and greater inhibition of biofilm formation than compound **42**, as shown in Figure 7. However, bacterial growth inhibition was not observed even at 100 μM concentration of **41** and **42** (see Supporting Information). In order to evaluate the binding reversibility of compound **41** to LasR, LasR binding activities of **41** (1 μM) were measured for different concentrations of **1a** (0, 0.1, 1, 10, and 100 μM). By increasing concentration of **1a**, the differences in LasR binding activities between the treatment group and the control (no treatment of **41**) decreased (see Supporting Information). At 100 μM **1a**, LasR-agonistic activity in the treatment group was completely recovered and almost the same as the control one. This result suggests that **41** binds reversibly to LasR by competing with **1a**.

A dynamic biofilm formation assay of the two most potent compounds (**41** and **42**) was performed in a drip-flow reactor. Compound **1c** served as a positive control, and DMSO served as a negative control. After 48 h drip-flow reactor operation, the biofilm was stained with Ruby and concanavalin A (ConA). Ruby (red) is a reagent for staining protein, and ConA (green)

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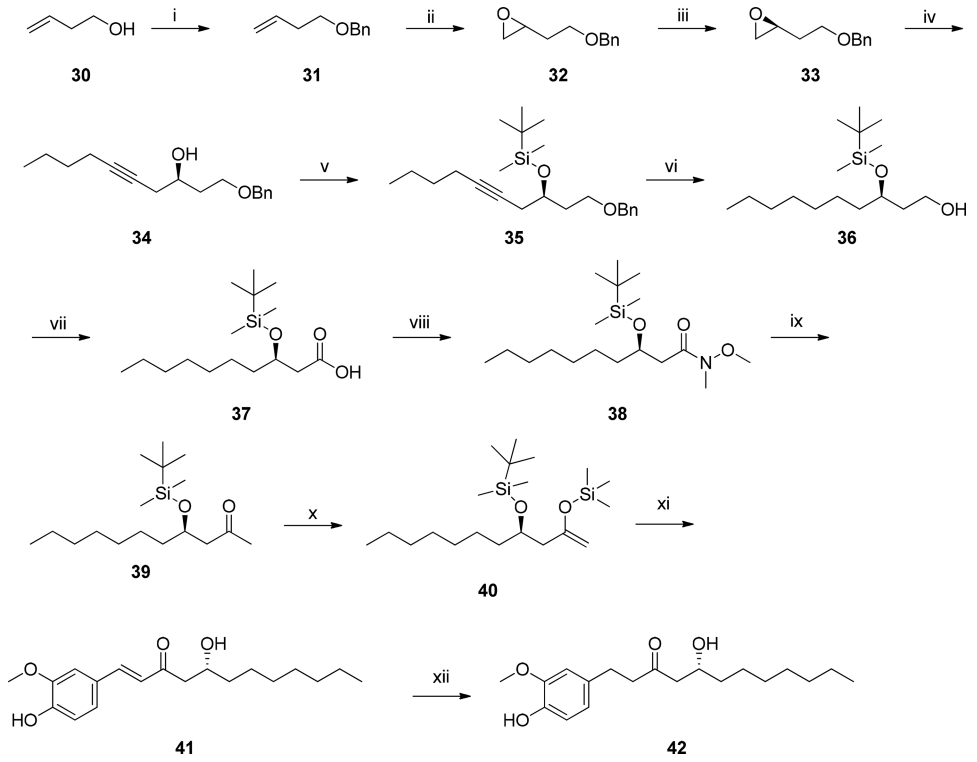
Scheme 5. Synthesis of Enantiomerically Enriched (*R*)-8-Gingerol (42) by Means of Salen's Catalyst[a]



[a]Reagents and conditions: (i) NaH, BnBr, THF, 0 °C to rt, 16 h, 90%; (ii) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, 0 °C to rt, 14 h, 72%; (iii) (*S*,*S*)-(+)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicyclidene)-1,2-cyclohexanediaminocobalt(II), AcOH, H_2O, THF, 0 °C to rt, 16 h, 50%; (iv) hex-1-yne, *n*-BuLi, $BF_3{\cdot}OEt_2$, THF, −78 °C, 2 h, 57%; (v) TBDMSCl, imidazole, CH_2Cl_2, rt, 10 h, 87%; (vi) H_2, Pd/C, MeOH, rt, 2 h, 89%; (vii) $NaIO_4$, Ru(III)Cl_3, EtOAc, CH_3CN, H_2O, rt, 4 h, 67%; (viii) *N*,*O*-dimethylhydroxylamine·HCl, HOBt, EDC, DIPEA, THF, rt, 8 h, 93%; (ix) CH_3MgBr, THF, −78 °C, 3 h, 90%; (x) TMSOTf, DIPEA, CH_2Cl_2, 0 °C, 4 h; (xi) vanillin, $BF_3{\cdot}OEt_2$, TEA, CH_2Cl_2, 0 °C, 2 h (65% in two steps); (xii) H_2, Pd/C, MeOH, rt, 2 h, 97%.

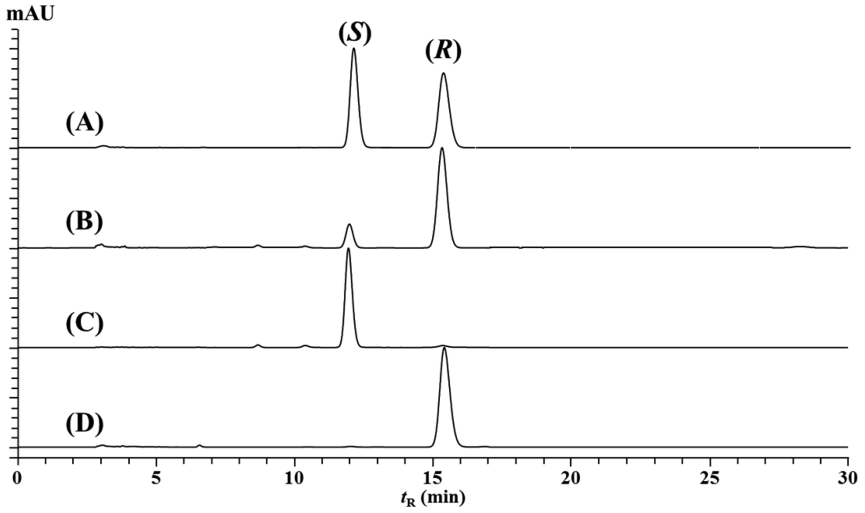


Figure 6. Chiral resolution of 8-gingerol: (A) 8-gingerol (**8**) by means of LDA from Scheme 1; (B) (*R*)-8-gingerol (**29**) by means of D-proline; (C) (*S*)-8-gingerol (**42S**) by (*R*,*R*) Salen's catalyst; (D) (*R*)-8-gingerol (**42**) by (*S*,*S*) Salen's catalyst.

is for carbohydrate of biofilm. As shown in Figure 8A, the biofilm in the presence of DMSO formed with typical mushroom-like morphology. By contrast, the biofilms treated with **1c** (Figure 8B), **42** (Figure 8C), or **41** (Figure 8D) were relatively thin and sparse as compared with the negative control. Biofilm volume and thickness with **41** were the lowest among the three groups (Table 1). Furthermore, the biofilm treated with **41** showed a relatively smaller volume of carbohydrates (47−74%) and proteins (23−56%) as compared to the other groups. Comprehensive analysis of confocal laser scanning microscopy (CLSM) images of biofilms indicated that the (*R*)-8-gingerol analogs **41** and **42** inhibited biofilm formation more effectively than **1c** did.

To explain why compounds **41** and **42** showed strong LasR-binding affinity and potent inhibition of biofilm formation, we conducted molecular docking analyses of compounds **41** and

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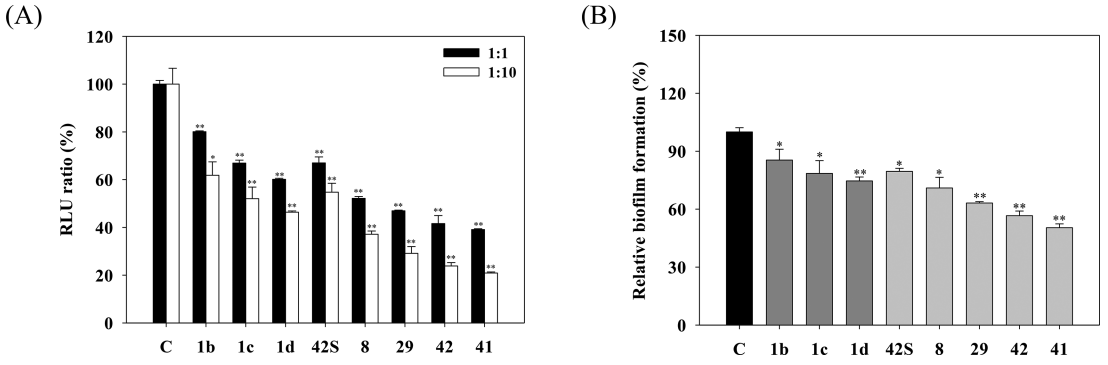

Figure 7. Effect of absolute configuration of 8-gingerol derivatives. DMSO (C, negative control) and **1b**, **1c**, and **1d** (positive controls) were used. (A) LasR binding activity of synthetic 8-gingerol derivatives (**8**, **29**, **41**, **42**, and **42S**) at different ratios of **1a** to the compound (1:1 or 1:10). (B) Biofilm formation of synthetic 8-gingerol derivatives (**8**, **29**, **41**, **42**, and **42S**) at 10 μM. (**) $P < 0.005$ and (*) $P < 0.05$ as compared to the control.

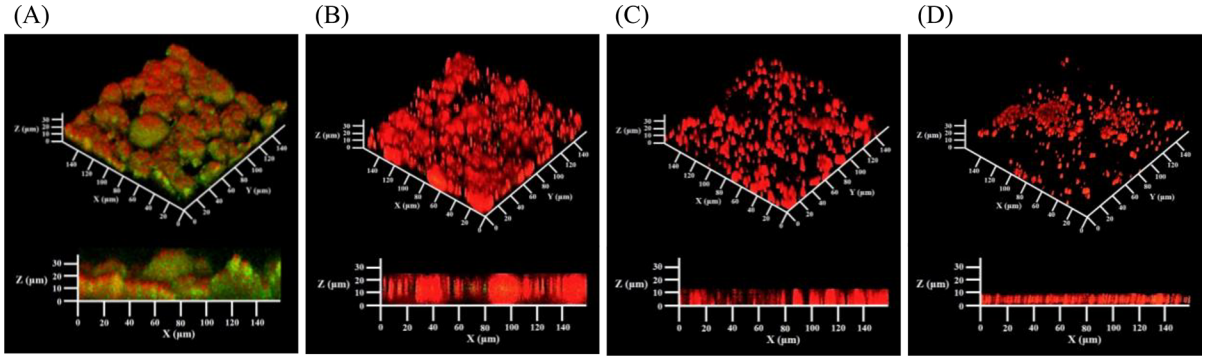

Figure 8. Confocal laser scanning microscopy (CLSM) images of *P. aeruginosa* biofilm formation. DMSO (negative control) and **1c** (positive control) were used. (A) Biofilm formation in the presence of DMSO only. (B) Biofilm formation at 10 μM concentration of **1c**. (C) Biofilm formation at 10 μM concentration of **42**. (D) Biofilm formation at 10 μM concentration of **41**. The biofilms were stained with ConA (carbohydrate, green) and Ruby (protein, red).

Table 1. Biofilm Volume and Thickness with DMSO, 1c, 41, and 42[a]

characteristic	DMSO	1c	41	42
biofilm volume ($\mu m^3/\mu m^2$)	23.1 ± 1.8	17.0 ± 0.4**	7.4 ± 0.2**	9.7 ± 1.1**
biofilm thickness (μm)	34.5 ± 1.3	17.0 ± 0.4**	10.3 ± 0.1**	13.9 ± 0.1**

[a]Average ± standard deviation of three measurements was estimated using the ImageJ software. (**) $P < 0.005$ as compared to the control.

42 and their (*S*)-enantiomers (**41S** and **42S**) by using the crystal structure of LasR (PDB code 2UV0).[36] The ligands were docked to the LasR active site by means of the CDOCKER module of Discovery Studio (Accelrys Inc., San Diego, CA, USA). The best-docked pose of each ligand in the active site coincided well with the crystal ligand OdDHL. Moreover, compound **41** engaged in a much greater number of hydrogen-bonding interactions with LasR than the other three ligands did. As shown in Figure 9A, compound **41** participated in hydrogen-bonding interactions with Tyr47, Arg61, Asp65, Asp73, and Tyr93. In particular, the OH group at the 4′-position of the phenyl moiety was deeply projected toward Tyr93 and formed polar interactions, which were not observed in the other ligands. Furthermore, it was noteworthy that the β-hydroxyl group of **41** participated in strong hydrogen-bonding interactions with the guanidinium group of Arg61. In addition, the lipophilic alkyl group made hydrophobic contacts with lipophilic amino acid residues including Leu39, Leu40, and Leu125. Tight packing between **41** and the surrounding amino acid residues contributed substantially to the stability of the protein−ligand complex; this phenomenon may explain the strongest potency of compound **41** among the synthesized compounds. In contrast, compounds **42** (Figure 9B), **41S**, and **42S** (see Supporting Information) had a relatively small number of hydrogen-bonding interactions with LasR.

CONCLUSIONS

On the basis of the chemical structure of (*S*)-6-gingerol, which is a potent anti-biofilm agent demonstrated by us, a variety of 6- and 8-gingerol analogs were designed and synthesized. These compounds were designed to evaluate the effects of the head, middle, and tail sections of 6-gingerol on LasR-binding affinity and on biofilm formation. Regarding modification of the tail section, affinity for LasR and inhibition of biofilm formation increased as the alkyl chain lengthened up to 8-gingerol. As for modification of the head section, compounds with a substitution by a hydrogen-bonding acceptor group (e.g., F or OH) at the 4′-position were the most potent, indicating that the hydrogen-bonding interaction was essential for binding to LasR. In the variants of the middle section, β-OH of the carbonyl group was necessary, whereas rotational rigidity between the head section and carbonyl group was favorable for LasR-binding affinity and for inhibition of biofilm formation. To evaluate the effects of stereochemistry,

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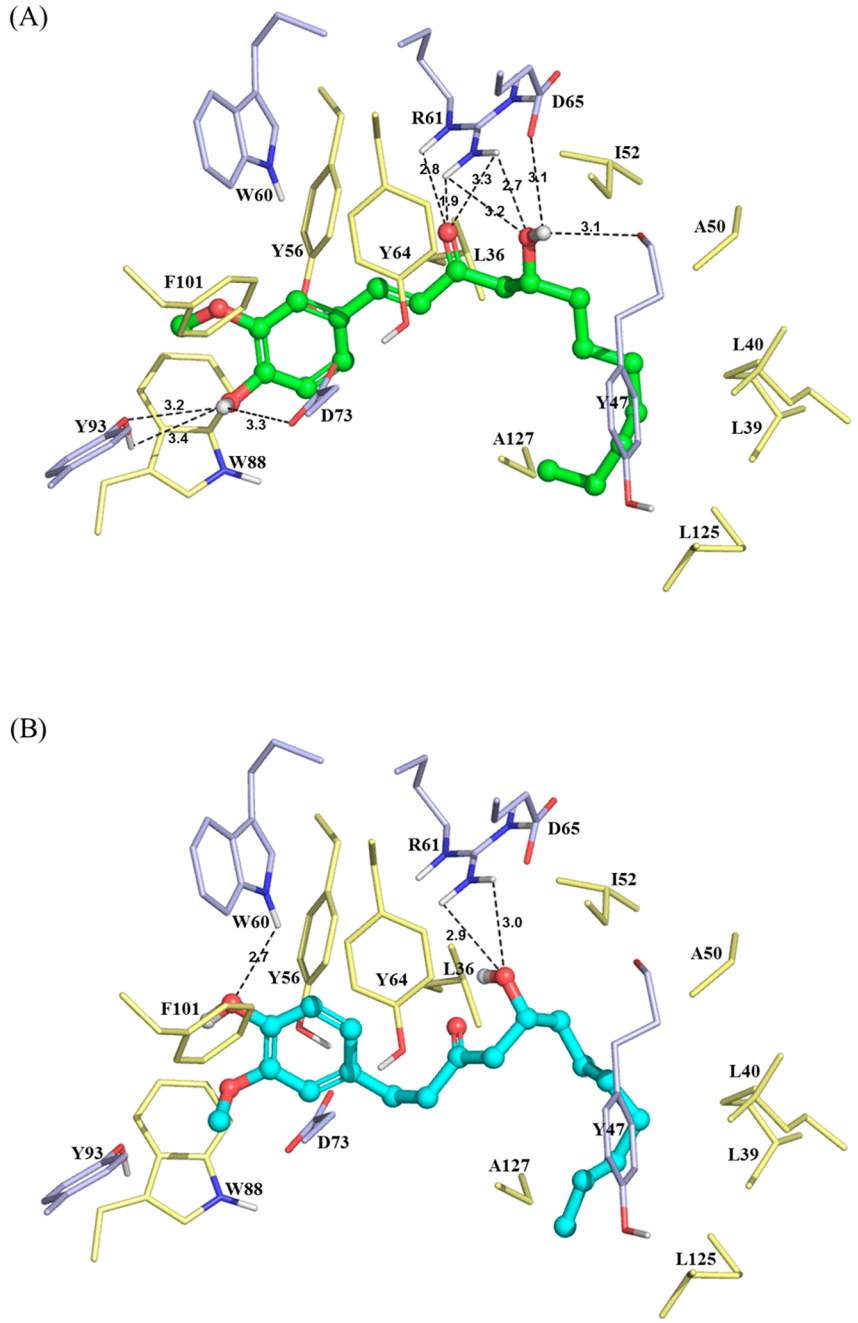

Figure 9. (A) Hydrogen-bonding interactions between **41** and LasR (PDB code 2UV0). (B) Hydrogen-bonding interactions between **42** and LasR. The hydrogen-bonding distance cutoff is 3.5 Å.

enantiomerically enriched (*R*)-8-gingerol was synthesized by means of chiral catalysts such as D-proline and Salen's catalyst. D-Proline yielded (*R*)-8-gingerol (**29**) with an ee value of 70% in five synthetic steps, whereas Salen's catalyst afforded enantiomerically enriched (*R*)-8-gingerol (**42**) with ee values of >95% in 12 steps. Comprehensive SAR studies identified compounds **41** and **42** as strong candidates for anti-biofilm agents, suggesting that the stereochemistry of 8-gingerol is one of the important factors for the enhancement of LasR-binding affinity and inhibition of biofilm formation. According to the results of SAR studies and molecular modeling, compound **41** holds promise as an early lead compound for further structural optimization in the development of anti-biofilm agents to combat *P. aeruginosa* infections.

EXPERIMENTAL SECTION

General. All the chemicals and solvents used in the reaction were purchased from Sigma-Aldrich, TCI, or Alfa Aesar and were used without further purification. Reactions were monitored by TLC on 0.25 mm Merck precoated silica gel plates (60 F_{254}). Reaction progress was monitored by TLC analysis using a UV lamp and/or $KMnO_4$ staining for detection purposes. Column chromatography was performed on silica gel (230−400 mesh, Merck, Darmstadt, Germany). NMR spectra were recorded at room temperature on either Bruker BioSpin Avance 300 MHz NMR or Bruker Ultrashield 600 MHz Plus spectrometer. Chemical shifts are reported in parts per million (ppm, δ) with TMS as an internal standard. Coupling constant are given in hertz. ^{13}C NMR spectra were obtained by using the same NMR spectrometers and were calibrated with $CDCl_3$ (δ = 77.16 ppm). Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q,

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quartet; m, multiplet; br, broad for ^{1}H NMR data. Mass spectra were obtained on a Shimadzu (MALDI-TOF) mass spectrometer or an Agilent 6530 Accurate mass Q-TOF LC/MS spectrometer or an electrospray ionization PE Biosystems Sciex Api 150 EX mass spectrometer single quadruple equipped with a turbo ion spray interface. The purity of all final compounds was measured by analytical reverse phase HPLC on an Agilent 1260 Infinity (Agilent) with a C18 column (Phenomenex, 150 mm × 4.6 mm, 3 μm, 110 Å). RP-HPLC was performed using the following isocratic conditions: for method A, mobile phase was acetonitrile and water (50:50, v/v); for method B, mobile phase was acetonitrile and water (55:45, v/v); for method C, mobile phase was acetonitrile and water (70:30, v/v). All compounds were eluted with a flow rate of 0.7 mL/min and monitored at UV detector: 254 nm. Purity of the tested compounds was >95%.

Synthesis. *(E)-4-(4-Hydroxy-3-methoxyphenyl)but-3-en-2-one (**2**).* To a solution of 4-hydroxy-3-methoxybenzaldehyde (1.25 g, 8.2 mmol) in acetone (50 mL) was added 10% NaOH (3.28 mL, 8.2 mmol) dropwise. The reaction mixture was stirred at 25 °C for 48 h and then was quenched with water and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (eluting with a mixture of hexane−EtOAc, 10:1 to 3:1) to furnish compound **2** (1.20 g, 71%) as yellow oil.[37,38] R_f = 0.25 (hexane/EtOAc = 4:1, v/v).

*4-(4-Hydroxy-3-methoxyphenyl)butan-2-one (**3**).* To a solution of compound **2** (1.2 g, 6.2 mmol) in MeOH (20 mL) was added 10% Pd/C (200 mg, 0.187 mmol). The solution was then stirred in an atmosphere of H_2 gas for 4 h. The reaction mixture was filtered through a Celite pad and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 5:1, v/v) to furnish compound **3** (1.17 g, 97%) as colorless oil.[37,38]

*5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)octan-3-one (**4**).* To a solution of compound **3** (200 mg, 1.0 mmol) in THF (5 mL) was added LDA (2.30 mL, 2.2 mmol) dropwise at −78 °C. The solution was stirred for 1 h at the same temperature. Butanal (0.74 mL, 8.3 mmol) was then added dropwise. The reaction mixture was stirred for 3 h at the same temperature, quenched with aqueous NH_4Cl (10 mL), and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (toluene/EtOAc = 10:1 to 5:1, v/v) to furnish compound **4** (15 mg, 13%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (d, J = 7.8 Hz, 1H), 6.69 (s, 1H), 6.66 (d, J = 8.7 Hz, 1H), 5.51 (s, 1H), 4.06 (brs, 1H), 3.89 (s, 3H), 2.96 (brs, 1H), 2.86 (t, J = 6.9 Hz, 2H), 2.75 (t, J = 6.9 Hz, 2H), 2.54 (t, J = 6.6 Hz, 2H), 1.51−1.27 (m, 4H), 0.93 (t, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.5, 146.4, 143.9, 132.6, 120.7, 114.4, 110.9, 67.4, 55.9, 49.4, 45.5, 38.6, 29.3, 24.0, 18.7, 14.0. MS (MALDI-TOF) m/z calculated for $C_{15}H_{22}O_4^+$ $[M]^+$, 266.2; found, 266.1. >98% purity (as determined by RP-HPLC, method B, t_R = 3.49 min).

Compounds **5**−**10** were prepared by a method similar to the one described for compound **4**.

*5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)nonan-3-one (**5**).* Compound **5** was prepared in 32% yield as colorless oil, following the same procedure as described for the synthesis of **4** but with pentanal instead of butanal. R_f = 0.15 (toluene/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 (d, J = 8.1 Hz, 1H), 6.69 (s, 1H), 6.68 (d, J = 9.0 Hz, 1H), 5.50 (s, 1H), 4.04 (brs, 1H), 3.89 (s, 3H), 2.80 (s, 1H), 2.83 (t, J = 6.6 Hz, 2H), 2.75 (t, J = 6.6 Hz, 2H), 2.72−2.42 (m, 2H), 1.55−1.23 (m, 6H), 0.91−0.89 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.5, 146.4, 143.9, 132.6, 120.7, 114.3, 110.9, 67.6, 55.8, 49.3, 45.4, 36.1, 29.3, 27.6, 22.6, 14.1. MS (MALDI-TOF) m/z calculated for $C_{16}H_{24}O_4^+$ $[M]^+$, 280.2; found, 280.1. >98% purity (as determined by RP-HPLC, method B, t_R = 4.20 min).

*5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one (**6**).* Compound **6** was prepared in 47% yield as colorless oil, following the same procedure as described for the synthesis of **4** but with hexanal instead of butanal. R_f = 0.14 (toluene/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 (d, J = 7.8 Hz, 1H), 6.69 (s, 1H), 6.68 (d, J = 8.7 Hz, 1H), 5.52 (s, 1H), 4.04 (brs, 1H), 3.89 (s, 3H), 2.96 (s, 1H), 2.85 (t, J = 6.6 Hz, 2H), 2.75 (t, J = 6.6 Hz, 2H), 2.68−2.43 (m, 2H), 2.68−2.43 (m, 8H), 0.98−0.81 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.5, 146.4, 144.0, 132.6, 120.7, 114.4, 110.1, 67.7, 55.9, 49.4, 45.5, 36.4, 31.7, 29.3, 25.1, 22.6, 14.0. MS (MALDI-TOF) m/z calculated for $C_{17}H_{26}O_4^+$ $[M]^+$, 294.2; found, 294.1. >98% purity (as determined by RP-HPLC, method B, t_R = 5.30 min).

*5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)undecan-3-one (**7**).* Compound **7** was prepared in 28% yield as colorless oil, following the same procedure as described for the synthesis of **4** but with heptanal instead of butanal. R_f = 0.15 (toluene/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (d, J = 7.8 Hz, 1H), 6.69 (s, 1H), 6.68 (d, J = 8.7 Hz, 1H), 5.50 (brs, 1H), 3.89 (s, 3H), 2.96 (brs, 1H), 2.86 (t, J = 6.6 Hz, 2H), 2.75 (t, J = 6.6 Hz, 2H), 2.72−2.41 (m, 2H), 1.71−1.21 (m, 10H), 0.98−0.89 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.5, 146.4, 144.0, 132.7, 120.7, 114.4, 110.9, 67.7, 55.9, 49.4, 45.5, 36.5, 31.8, 29.3, 29.2, 25.4, 22.6, 14.1. MS (MALDI-TOF) m/z calculated for $C_{18}H_{28}O_4^+$ $[M]^+$, 308.2; found, 308.2. >98% purity (as determined by RP-HPLC, method B, t_R = 6.97 min).

*5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one (**8**).* Compound **8** was prepared in 32% yield as colorless oil, following the same procedure as described for the synthesis of **4** but with octanal instead of butanal. R_f = 0.15 (toluene/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.83 (d, J = 8.1 Hz, 1H), 6.68 (s, 1H), 6.66 (d, J = 9.0 Hz, 1H), 5.52 (s, 1H), 4.02 (brs, 1H), 3.87 (s, 3H), 2.95 (s, 1H), 2.84 (t, J = 6.9 Hz, 2H), 2.73 (t, J = 6.9 Hz, 2H), 2.52 (t, J = 7.5 Hz, 2H), 1.61−1.12 (m, 12H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.5, 144.0, 132.6, 120.7, 114.4, 111.0, 67.7, 55.9, 49.3, 45.4, 36.5, 31.8, 29.5, 29.2, 29.2, 25.5, 22.7, 14.1. MS (MALDI-TOF) m/z calculated for $C_{19}H_{30}O_4^+$ $[M]^+$, 322.2; found, 322.2. >98% purity (as determined by RP-HPLC, method B, t_R = 9.56 min).

*5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)tridecan-3-one (**9**).* Compound **9** was prepared in 32% yield as colorless oil, following the same procedure as described for the synthesis of **4** but with nonanal instead of butanal. R_f = 0.15 (toluene/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (d, J = 7.8 Hz, 1H), 6.69 (s, 1H), 6.68 (d, J = 8.7 Hz, 1H), 5.50 (brs, 1H), 4.04 (brs, 1H), 3.89 (s, 3H), 2.91 (brs, 1H), 2.85 (t, J = 6.6 Hz, 2H), 2.75 (t, J = 6.6 Hz, 2H), 2.71−2.39 (m, 2H), 1.71−1.15 (m, 14H), 0.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.5, 144.0, 132.6, 120.7, 114.4, 111.0, 67.7, 55.9, 49.3, 45.4, 36.5, 31.8, 29.5, 29.2, 29.2, 25.5, 22.7, 14.1. MS (MALDI-TOF) m/z calculated for $C_{20}H_{32}O_4^+$ $[M]^+$, 336.2; found, 336.2. >98% purity (as determined by RP-HPLC, method B, t_R = 13.56 min).

*5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)tetradecan-3-one (**10**).* Compound **10** was prepared in 32% yield as colorless oil, following the same procedure as described for the synthesis of **4** but with decanal instead of butanal. R_f = 0.16 (toluene/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (d, J = 7.8 Hz, 1H), 6.69 (s, 1H), 6.68 (d, J = 8.7 Hz, 2H), 5.50 (brs, 1H), 4.04 (brs, 1H), 3.89 (s, 3H), 2.90 (brs, 1H), 2.85 (t, J = 6.6 Hz, 2H), 2.75 (t, J = 6.6 Hz, 2H), 2.56−2.34 (m, 2H), 1.49−1.18 (m, 16H), 0.98−0.79 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.6, 178.4, 146.5, 144.2, 132.6, 121.5, 114.57, 111.5, 67.7, 59.3, 45.5, 36.4, 29.7, 26.1, 25.4, 24.7, 22.7, 14.1. MS (MALDI-TOF) m/z calculated for $C_{21}H_{34}O_4^+$ $[M]^+$, 350.2; found, 350.2. >98% purity (as determined by RP-HPLC, method B, t_R = 19.78 min).

*1-(3,4-Dimethoxyphenyl)-5-hydroxydecan-3-one (**14a**).* To a solution of 4-(3,4-dimethoxyphenyl)butan-2-one (200 mg, 1.0 mmol) in THF (5 mL) was added LDA (2.3 mL, 2.2 mmol) at −78 °C. The solution was stirred for 1 h at the same temperature. Hexanal (0.72 mL, 8.2 mmol) was added dropwise. The reaction mixture was stirred for 3 h at the same temperature, quenched with aqueous NH_4Cl (10 mL), and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (toluene/EtOAc = 10:1 to 5:1) to furnish compound **14a** (97 mg, 32%) as colorless oil. R_f = 0.26 (hexane/EtOAc = 7:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.81 (t, J = 8.7 Hz, 1H), 6.69 (d, J = 12.3 Hz, 2H), 4.04 (brs, 1H), 3.86 (s, 6H), 2.90−2.74 (m, 4H), 2.63−2.36 (m, 2H), 1.65−1.28 (m, 8H), 0.90 (s,

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3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.5, 148.9, 147.4, 133.3, 120.0, 112.0, 67.7, 55.9, 49.3, 45.3, 36.4, 31.7, 29.2, 25.2, 22.6, 14.0. MS (MALDI-TOF) m/z calculated for $C_{18}H_{28}O_4^+$ $[M + H]^+$, 309.2; found, 309.2. >98% purity (as determined by RP-HPLC, method A, t_R = 9.86 min).

*1-(3,4-Dimethoxyphenyl)-5-hydroxydodecan-3-one (**14b**).* Compound **14b** was obtained in 32% yield as colorless oil, following the same procedure as described for the synthesis of **14a** but with octanal instead of hexanal. R_f = 0.26 (hexane/EtOAc = 7:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.81 (t, J = 8.7 Hz, 1H), 6.70 (d, J = 12.3 Hz, 2H), 4.02 (brs, 1H), 3.86 (s, 6H), 2.90−2.74 (m, 4H), 2.68−2.32 (m, 2H), 1.75−1.28 (m, 10H), 0.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.5, 148.9, 147.4, 133.3, 120.0, 111.6, 111.3, 67.7, 55.9, 49.3, 45.3, 36.4, 33.8, 29.7, 25.7, 24.5, 22.6, 14.0. MS (MALDI-TOF) m/z calculated for $C_{20}H_{32}O_4^+$ $[M]^+$, 336.2; found, 336.2. >98% purity (as determined by RP-HPLC, method B, t_R = 14.10 min).

*5-Hydroxy-1-phenyldecan-3-one (**15a**).* Compound **15a** was prepared in 40% yield as colorless oil, following the same procedure as described for the synthesis of **14a** but with 4-phenylbutan-2-one instead of 4-(3,4-dimethoxyphenyl)butan-2-one. R_f = 0.43 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (t, J = 7.5 Hz, 2H), 7.21 (d, J = 7.2 Hz, 3H), 4.05 (brs, 1H), 2.99 (brs, 1H), 2.93 (t, J = 7.5 Hz, 2H), 2.77 (t, J = 7.5 Hz, 2H), 2.62−2.46 (m, 2H), 1.49−1.30 (m, 8H), 0.95−0.82 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.3, 140.7, 128.6, 128.3, 126.2, 67.6, 49.3, 45.1, 36.4, 31.7, 29.5, 25.2, 22.6, 14.1. MS (MALDI-TOF) m/z calculated for $C_{16}H_{24}O_2^+$ $[M + H]^+$, 249.2; found, 249.9. >98% purity (as determined by RP-HPLC, method A, t_R = 16.57 min).

*5-Hydroxy-1-phenyldodecan-3-one (**15b**).* Compound **15b** was prepared in 38% yield as colorless oil, following the same procedure as described for the synthesis of **15a** but with octanal instead of hexanal. R_f = 0.43 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (t, J = 7.5 Hz, 2H), 7.20 (d, J = 7.2 Hz, 3H), 4.04 (brs, 1H), 2.98 (brs, 1H), 2.93 (t, J = 7.5 Hz, 2H), 2.79 (t, J = 7.5 Hz, 2H), 2.57−2.46 (m, 2H), 1.60−1.28 (m, 10H), 0.96−0.83 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.0, 152.6, 149.4, 147.3, 137.1, 120.3, 115.9, 67.7, 56.2, 49.4, 45.1, 36.5, 31.8, 29.5, 29.2, 25.5, 22.7, 14.1. MS (MALDI-TOF) m/z calculated for $C_{18}H_{28}O_2^+$ $[M + Na]^+$, 299.2; found, 299.1. >98% purity (as determined by RP-HPLC, method B, t_R = 23.28 min).

*1-(4-Fluoro-3-methoxyphenyl)-5-hydroxydecan-3-one (**16a**).* Compound **16a** was prepared in 33% yield as colorless oil, following the same procedure as described for the synthesis of **14a** but with 4-(4-fluoro-3-methoxyphenyl)butan-2-one instead of 4-(3,4-dimethoxyphenyl)butan-2-one. R_f = 0.32 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (t, J = 9.8 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 6.71 (brs, 1H), 4.05 (s, 1H), 3.89 (s, 3H), 2.94−2.84 (m, 3H), 2.77 (t, J = 7.3 Hz, 2H), 2.57−2.46 (m, 2H), 1.49−1.30 (m, 8H), 0.98−0.81 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.0, 152.6, 149.4, 147.5, 137.1, 120.3, 116.1, 113.6., 67.7, 56.2, 49.4, 45.1, 36.4, 34.5, 32.8, 31.7, 29.1, 25.1, 22.6, 14.1. MS (MALDI-TOF) m/z calculated for $C_{17}H_{25}FO_3^-$ $[M - H]^-$, 295.2; found, 295.0. >98% purity (as determined by RP-HPLC, method A, t_R = 15.63 min).

*1-(4-Fluoro-3-methoxyphenyl)-5-hydroxydodecan-3-one (**16b**).* Compound **16b** was prepared in 38% yield as colorless oil, following the same procedure as described for the synthesis of **16a** but with octanal instead of hexanal. R_f = 0.32 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (t, J = 9.8 Hz, 1H), 6.80 (d, J = 8.1 Hz, 1H), 6.70 (brs, 1H), 4.04 (s, 1H), 3.88 (s, 3H), 3.02−2.81 (m, 3H), 2.76 (t, J = 6.9 Hz, 2H), 2.57−2.49 (m, 2H), 1.48−1.28 (m, 10H), 0.97−0.80 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.0, 152.6, 149.4, 147.5, 137.1, 120.9, 116.1, 114.0, 67.7, 56.2, 49.4, 45.1, 36.4, 34.5, 32.8, 31.7, 29.1, 26.1, 25.1, 22.6, 14.0. MS (MALDI-TOF) m/z calculated for $C_{19}H_{29}FO_3^+$ $[M + H]^+$, 325.2; found, 325.3. >98% purity (as determined by RP-HPLC, method B, t_R = 21.19 min).

*1-(4-Fluorophenyl)-5-hydroxydecan-3-one (**17a**).* Compound **17a** was prepared in 40% yield as colorless oil, following the same procedure as described for the synthesis of **14a** but with 4-(4-fluorophenyl)butan-2-one instead of 4-(3,4-dimethoxyphenyl)butan-2-one. R_f = 0.29 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22−7.13 (m, 2H), 6.97 (d, J = 8.7 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 4.03 (s, 1H), 2.99 (s, 1H), 2.88 (t, J = 6.6 Hz, 2H), 2.75 (t, J = 6.6 Hz, 2H), 2.68−2.39 (m, 2H), 1.64−1.40 (m, 8H), 0.90 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.0, 148.6, 147.4, 132.5, 115.1, 112.9, 116.1, 67.7, 63.2, 49.4, 45.1, 36.4, 34.8, 32.2, 31.0, 29.3, 26.1, 25.4, 22.7, 14.0. MS (MALDI-TOF) m/z calculated for $C_{16}H_{23}FO_2^-$ $[M - H]^-$, 265.4; found, 264.9. >98% purity (as determined by RP-HPLC, method A, t_R = 17.31 min).

*1-(4-Fluorophenyl)-5-hydroxydodecan-3-one (**17b**).* Compound **17b** was prepared in 45% yield as colorless oil, following the same procedure as described for the synthesis of **17a** but with by using octanal instead of hexanal. R_f = 0.29 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18−7.08 (m, 2H), 6.97 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 8.7 Hz, 1H), 4.03 (brs, 1H), 3.00 (s, 1H), 2.88 (t, J = 6.6 Hz, 2H), 2.75 (t, J = 6.6 Hz, 2H), 2.68−2.39 (m, 2H), 1.63−1.28 (m, 10H), 0.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.5, 145.7, 144.1, 132.5, 120.6, 114.4, 114.3, 111.8, 67.7, 64.4, 49.3, 45.5, 36.4, 31.8, 29.8, 29.3, 25.5, 22.7, 14.9, 14.1. MS (MALDI-TOF) m/z calculated for $C_{18}H_{27}FO_2^+$ $[M]^+$, 294.2; found, 294.0. >98% purity (as determined by RP-HPLC, method B, t_R = 23.81 min).

*5-Hydroxy-1-(4-hydroxyphenyl)decan-3-one (**18a**).* Compound **18a** was prepared in 40% yield as colorless oil, following the same procedure as described for the synthesis of **14a** but with 4-(4-hydroxyphenyl)butan-2-one instead of 4-(3,4-dimethoxyphenyl)butan-2-one. R_f = 0.23 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 (d, J = 7.2 Hz, 2H), 6.75 (d, J = 7.2 Hz, 2H), 5.77 (brs, 1H), 4.05 (s, 1H), 3.19 (brs, 1H), 2.84 (t, J = 6.6 Hz, 2H), 2.74 (t, J = 6.6 Hz, 2H), 2.68−2.41 (m, 2H), 1.69−1.12 (m, 8H), 0.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.2, 159.7, 142.4, 129.5, 120.6, 114.1, 111.5, 67.7, 55.1, 49.3, 45.0, 36.5, 31.7, 29.6, 25.1, 22.6, 22.2, 14.0. MS (MALDI-TOF) m/z calculated for $C_{16}H_{24}O_3^+$ $[M + H]^+$, 265.2; found, 265.9. >98% purity (as determined by RP-HPLC, method A, t_R = 6.60 min).

*5-Hydroxy-1-(4-hydroxyphenyl)dodecan-3-one (**18b**).* Compound **18b** was prepared in 25% yield as colorless oil, following the same procedure as described for the synthesis of **18a** but with octanal instead of hexanal. R_f = 0.23 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (d, J = 8.1 Hz, 2H), 6.76 (d, J = 8.1 Hz, 2H), 5.09 (brs, 1H), 4.04 (s, 1H), 3.15 (brs, 1H), 2.85 (t, J = 6.6 Hz, 2H), 2.74 (t, J = 6.6 Hz, 2H), 2.68−2.45 (m, 2H), 1.58−1.15 (m, 10H), 0.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.2, 159.7, 142.4, 129.7, 129.5, 129.4, 120.6, 114.1, 111.5, 67.7, 55.2, 49.4, 45.0, 36.4, 31.8, 29.5, 29.2, 25.5, 22.6, 14.1. MS (MALDI-TOF) m/z calculated for $C_{18}H_{28}O_3^+$ $[M + H]^+$, 293.2; found, 293.1. >98% purity (as determined by RP-HPLC, method B, t_R = 9.05 min).

*1-(3-Ethoxy-4-hydroxyphenyl)-5-hydroxydecan-3-one (**19a**).* Compound **19a** was prepared in 35% yield as colorless oil, following the same procedure as described for the synthesis of **14a** but with 4-(3-ethoxy-4-hydroxyphenyl)butan-2-one instead of 4-(3,4-dimethoxyphenyl)butan-2-one. R_f = 0.32 (hexane/EtOAc = 4:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 (d, J = 7.8 Hz, 1H), 6.65 (s, 1H), 6.82−6.61 (m, 1H), 5.59 (brs, 1H), 4.10 (q, J = 7.2 Hz, 2H), 2.84 (t, J = 6.6 Hz, 2H), 2.76 (t, J = 6.6 Hz, 2H), 2.67−2.45 (m, 2H), 1.45 (t, J = 7.2 Hz, 3H), 1.58−1.15 (m, 8H), 0.90 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.8, 148.1, 146.8, 142.6, 127.1, 124.2, 123.4, 114.8, 109.3, 56.0, 45.7, 40.7, 31.9, 29.5, 29.3, 29.3, 24.6, 22.7, 14.1. MS (MALDI-TOF) m/z calculated for $C_{16}H_{24}O_3^+$ $[M + H]^+$, 265.4; found, 265.9. >98% purity (as determined by RP-HPLC, method A, t_R = 9.25 min).

*1-(3-Ethoxy-4-hydroxyphenyl)-5-hydroxydodecan-3-one (**19b**).* Compound **19b** was prepared in 27% yield as colorless oil, following the same procedure as described for the synthesis of **19a** but with octanal instead of hexanal. R_f = 0.32 (hexane/EtOAc = 4:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (d, J = 7.8 Hz, 1H), 6.68 (s, 1H), 6.66 (d, J = 7.2 Hz, 1H), 5.56 (brs, 1H), 4.10 (q, J = 7.2 Hz, 2H), 2.84 (t, J = 6.6 Hz, 2H), 2.74 (t, J = 6.6 Hz, 2H), 2.91−2.45 (m, 2H), 1.46 (t, J = 6.9 Hz, 3H), 1.84−1.19 (m, 10H), 0.90 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.1, 159.7, 142.4, 129.7, 129.5, 129.4, 120.6, 114.0, 111.5 67.7, 55.2, 49.4, 45.0, 36.4, 31.8, 29.8, 29.5, 29.2, 25.5, 22.6, 14.0; MS (MALDI-TOF) m/z calculated for $C_{18}H_{28}O_3^+$ $[M + H]^+$, 293.2;

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found, 293.1. >98% purity (as determined by RP-HPLC, method B, t_R = 12.91 min).

*5-Hydroxy-1-(3-methoxyphenyl)decan-3-one (**20a**).* Compound **20a** was prepared in 32% yield as colorless oil, following the same procedure as described for the synthesis of **14a** but with 4-(3-methoxyphenyl)butan-2-one instead of 4-(3,4-dimethoxyphenyl)-butan-2-one. R_f = 0.34 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (t, J = 7.8 Hz, 1H), 6.79−6.72 (m, 3H), 4.04 (brs, 1H), 3.81 (s, 3H), 2.96 (s, 1H), 2.90 (t, J = 7.2 Hz, 2H), 2.78 (t, J = 7.2 Hz, 2H), 2.61−2.49 (m, 2H), 1.68−1.21 (m, 8H), 0.90 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.2, 159.7, 142.4, 129.5, 120.6, 114.1, 111.5, 67.7, 55.1, 49.3, 45.0, 36.5, 31.7, 29.6, 25.1, 22.6, 14.0. MS (MALDI-TOF) m/z calculated for $C_{17}H_{26}O_3^+$ $[M]^+$, 278.2; found, 278.0. >98% purity (as determined by RP-HPLC, method A, t_R = 15.50 min).

*5-Hydroxy-1-(3-methoxyphenyl)dodecan-3-one (**20b**).* Compound **20b** was prepared in 34% yield as colorless oil, following the same procedure as described for the synthesis of **20a** but with octanal instead of hexanal. R_f = 0.34 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (t, J = 7.8 Hz, 1H), 6.79−6.73 (m, 3H), 4.04 (brs, 1H), 3.81 (s, 3H), 2.95−2.86 (m, 3H), 2.82−2.74 (m, 2H), 2.52 (t, J = 9.0 Hz, 2H), 1.61−1.18 (m, 10H), 0.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.2, 159.7, 142.4, 129.7, 120.6, 114.1, 111.5, 67.7, 55.2, 49.3, 45.0, 36.5, 31.8, 29.6, 29.2 25.5, 22.7, 14.1. MS (MALDI-TOF) m/z calculated for $C_{19}H_{30}O_3^+$ $[M + H]^+$, 307.2; found, 307.2. >98% purity (as determined by RP-HPLC, method B, t_R = 21.69 min).

*(E)-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)dec-1-en-3-one (**21a**).* To a solution of compound **12** (150 mg, 0.7 mmol) in THF (5 mL) was added LDA (1.7 mL, 1 M in THF/hexanes) at −78 °C under Ar. The solution was stirred for 1 h at the same temperature. Hexanal (0.52 mL, 6.2 mmol) was slowly added. The reaction mixture was stirred for 3 h at the same temperature. The reaction mixture was quenched with aqueous NH_4Cl (10 mL) and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (eluting with a mixture of hexane/EtOAc, 10:1 to 3:1, v/v) to furnish compound **21a** (70 mg, 31%). R_f = 0.25 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 16.2 Hz, 1H), 7.12 (d, J = 8.1 Hz, 1H), 7.07 (s, 1H), 6.95 (d, J = 8.1 Hz, 1H), 6.61 (d, J = 16.2 Hz, 1H), 4.15 (brs, 1H), 3.95 (s, 3H), 2.93−2.71 (m, 1H), 2.39 (s, 1H), 1.82−1.25 (m, 8H), 0.92 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.0, 148.6, 146.9, 143.9, 126.7, 124.1 123.8, 114.9, 109.5, 68.1, 56.0, 46.5, 36.5, 31.8, 25.2, 22.6, 14.1. MS (MALDI-TOF) m/z calculated for $C_{17}H_{24}O_4^+$ $[M + H]^+$, 293.2; found, 293.1. >98% purity (as determined by RP-HPLC, method A, t_R = 6.91 min).

*(E)-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodec-1-en-3one (**21b**).* Compound **21b** was prepared in 31% yield, following the same procedure as described for the synthesis of **21a** but with octanal instead of hexanal. R_f = 0.25 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 16.2 Hz, 1H), 7.11 (d, J = 8.1 Hz, 1H), 7.08 (s, 1H), 6.94 (d, J = 8.1 Hz, 1H), 6.60 (d, J = 16.2 Hz, 1H), 4.15 (brs, 1H), 3.91 (s, 3H), 2.93−2.68 (m, 1H), 2.35 (s, 1H), 2.75−1.21 (m, 10H), 0.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.1, 148.5, 146.9, 143.8, 126.7, 124.1, 123.7, 114.9, 109.5, 68.0, 56.0, 46.5, 36.6, 31.8, 29.6, 29.2, 25.5, 22.6, 14.0. MS (MALDI-TOF) m/z calculated for $C_{19}H_{28}O_3^+$ $[M + Na]^+$, 343.4; found, 343.2. >98% purity (as determined by RP-HPLC, method B, t_R = 11.97 min).

*(E)-1-(4-Fluoro-3-methoxyphenyl)-5-hydroxydec-1-en-3-one (**22a**).* Compound **22a** was prepared in 40% yield, following the same procedure as described for the synthesis of **21a** but with (*E*)-4-(4-fluoro-3-methoxyphenyl)but-3-en-2-one instead of compound **12**. R_f = 0.32 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 16.2 Hz, 1H), 7.15 (d, J = 8.1 Hz, 2H), 7.11 (s, 1H), 6.66 (d, J = 16.2 Hz, 1H), 4.16 (brs, 1H), 3.95 (s, 3H), 3.2 (s, 1H), 2.93−2.72 (m, 2H), 1.63−1.33 (m, 8H), 0.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.1, 155.7, 152.3, 148.2, 148.0, 142.6, 130.9, 126.2, 122.2, 116.5, 112.3, 67.9, 58.0, 46.9, 36.6, 31.8, 25.6, 25.3, 22.7, 22.6, 14.0. MS (MALDI-TOF) m/z calculated for $C_{17}H_{25}FO_3^+$ $[M]^+$, 294.2; found, 294.0. >98% purity (as determined by RP-HPLC, method A, t_R = 16.32 min).

*(E)-1-(4-Fluoro-3-methoxyphenyl)-5-hydroxydodec-1-en-3-one (**22b**).* Compound **22b** was prepared in 31% yield, following the same procedure as described for the synthesis of **22a** but with octanal instead of hexanal. R_f = 0.32 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, J = 16.2 Hz, 1H), 7.27 (s, 1H), 7.12 (d, J = 8.1 Hz, 2H), 6.66 (d, J = 16.2 Hz, 1H), 4.15 (brs, 1H), 3.95 (s, 3H), 3.2 (s, 1H), 2.93−2.79 (m, 2H), 1.60−1.30 (m, 10H), 0.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.1, 155.7, 152.3, 148.2, 148.0, 142.6, 130.9, 126.2, 122.2, 116.5, 112.3, 67.9, 58.0, 46.9, 36.6, 31.8, 29.5, 29.3 25.6, 25.3, 22.7, 22.6, 14.0. MS (MALDI-TOF) m/z calculated for $C_{19}H_{27}FO_3^+$ $[M]^+$, 323.2; found, 323.2 >98% purity (as determined by RP-HPLC, method B, t_R = 22.13 min).

*(E)-1-(4-Hydroxy-3-methoxyphenyl)dec-1-en-3-one (**23a**).* To a solution of 4-hydroxy-3-methoxybenzaldehyde (913 mg, 6.0 mmol) in MeOH (10 mL) was added L-proline (86 mg, 0.75 mmol) and nonan-2-one (0.87 mL, 5.0 mmol) at 25 °C under Ar. After 30 min, triethylamine (0.21 mL, 1.5 mmol) was introduced. The reaction mixture was stirred 25 °C for 48 h and then quenched with water and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 10:1 to 3:1, v/v) to furnish compound **23a** (746 mg, 45%) as a fluffy white solid. R_f = 0.32 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, J = 16.2 Hz, 1H), 7.12 (d, J = 7.8 Hz, 1H), 7.08 (s, 1H), 6.94 (d, J = 8.1 Hz, 1H), 6.63 (d, J = 16.2 Hz, 1H), 5.94 (s, 1H), 3.95 (s, 3H), 2.66 (t, J = 7.2 Hz, 2H), 1.78−1.56 (m, 2H), 1.54−1.19 (m, 8H), 0.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.0, 148.4, 147.0, 142.8, 126.9, 123.9, 123.3, 114.9, 109.6, 55.9, 40.6, 31.7, 29.3, 29.1, 24.6, 22.6, 14.0. MS (MALDI-TOF) m/z calculated for $C_{14}H_{24}O_3^+$ $[M]^+$, 276.2; found, 276.2. >98% purity (as determined by RP-HPLC, method A, t_R = 24.61 min).

*(E)-1-(4-Hydroxy-3-methoxyphenyl)dodec-1-en-3-one (**23b**).* Compound **23b** was prepared in 60% yield as a fluffy white solid, following the same procedure as described for the synthesis of **23a** but with octanal instead of hexanal. R_f = 0.32 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, J = 16.2 Hz, 1H), 7.12 (d, J = 7.8 Hz, 1H), 7.08 (s, 1H), 6.94 (d, J = 8.1 Hz, 1H), 6.63 (d, J = 16.2 Hz, 1H), 5.92 (s, 1H), 3.95 (s, 3H), 2.66 (t, J = 7.2 Hz, 2H), 1.78−1.56 (m, 2H), 1.51−1.13 (m, 12H), 0.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.1, 148.1, 146.8, 142.6, 127.1, 124.2, 123.4, 114.8, 109.3, 55.9, 45.7, 40.7, 31.9, 29.5, 29.3, 29.2, 24.6, 22.7 14.1. MS (MALDI-TOF) m/z calculated for $C_{19}H_{28}O_3^+$ $[M]^+$, 304.2; found, 304.2. >98% purity (as determined by RP-HPLC, method C, t_R = 10.56 min).

*1-(4-Hydroxy-3-methoxyphenyl)decan-3-one (**24a**).* To a solution of compound **23a** (100 mg, 0.36 mmol) in MeOH (10 mL) was added 10% Pd/C (1.9 mg, 0.02 mmol) carefully. The reaction mixture was purged with H_2 gas and stirred for 2 h and then was filtered through a Celite pad and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 12:1, v/v) to furnish compound **24a** (76 mg, 75%) as a white solid. R_f = 0.31 (hexane/EtOAc = 7:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 (d, J = 7.8 Hz, 1H), 6.70 (s, 1H), 6.69 (d, J = 10.2 Hz, 1H), 5.51 (s, 1H), 3.91 (s, 3H), 2.84 (t, J = 6.6 Hz, 2H), 2.79 (t, J = 6.6 Hz, 2H), 2.39 (t, J = 7.2 Hz, 2H), 1.71−1.52 (m, 2H), 1.49−1.21 (m, 8H), 0.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.7, 146.6, 143.9, 132.9, 120.7, 114.5, 111.2, 55.8, 44.5, 42.9, 31.6, 29.5, 29.1, 29.0, 23.8, 22.6, 14.0. MS (MALDI-TOF) m/z calculated for $C_{17}H_{26}O_3^+$ $[M]^+$, 278.2; found, 278.2. >98% purity (as determined by RP-HPLC, method A, t_R = 24.76 min).

*1-(4-Hydroxy-3-methoxyphenyl)dodecan-3-one (**24b**).* Compound **24b** was prepared in 97% yield as a fluffy white solid, by following the same procedure as described for the synthesis of **24a** but with **23b** instead of **23a**. R_f = 0.33 (hexane/EtOAc = 7:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 (d, J = 7.8 Hz, 1H), 6.70 (s, 1H), 6.69 (d, J = 10.2 Hz, 1H), 5.49 (s, 1H), 3.89 (s, 3H), 3.21 (s, 1H), 2.84 (t, J = 6.6 Hz, 2H), 2.74 (t, J = 6.6 Hz, 2H), 2.39 (t, J = 7.2 Hz, 2H), 1.58−1.52 (m, 2H), 1.31−1.21 (m, 10H), 0.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.7, 146.3, 143.8, 133.1, 120.8, 114.3, 111.0, 55.9,

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44.6, 43.2, 31.9, 29.5, 29.4, 29.2, 29.2, 23.8, 22.7, 14.1. MS (MALDI-TOF) m/z calculated for $C_{19}H_{30}O_3^+$ $[M]^+$, 306.2; found, 306.2. >98% purity (as determined by RP-HPLC, method C, t_R = 10.51 min).

*(R)-4-Hydroxyundecan-2-one (**25**).* To a suspension of D-proline (0.23 g, 2.0 mmol) in acetone (100 mL) was added 1-octanal (3.1 mL, 20 mmol) in one portion at 25 °C. The reaction mixture was stirred for 48 h and then was quenched with brine (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 7:1 to 3:1, v/v) to furnish compound **25** as colorless oil (1.8 g, 48%). R_f = 0.23 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.05 (s, 1H), 2.96 (s, 1H), 2.68−2.50 (m, 2H), 2.19 (s, 3H), 1.51−1.29 (m, 12H), 0.90 (s, 3H). ^{1}H NMR data were in complete agreement with those previously reported in the literature.[30]

*(R)-4-((tert-Butyldimethylsilyl)oxy)undecan-2-one (**26**).* To a solution of compound **25** (900 mg, 5.69 mmol) in CH_2Cl_2 (50 mL) were added imidazole (1.16 g, 17.1 mmol) and TBDMSCl (1.29 g, 8.5 mmol). The reaction mixture was stirred for 10 h at room temperature and then was quenched with water and extracted with diethyl ether. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 15:1 to 7:1, v/v) to furnish compound **26** (1.35 g, 87%) as a fluffy white solid. R_f = 0.75 (hexane/EtOAc = 8:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.15 (t, J = 6.3 Hz, 1H), 2.67−2.18 (m, 2H), 2.18 (s, 3H), 1.44 (brs, 2H), 1.32−1.22 (m, 10H), 0.97−0.76 (m, 12H), 0.07 (s, 3H), 0.04 (s, 3H). ^{1}H NMR data were in complete agreement with those previously reported in the literature.[30]

*(R)-6-Heptyl-2,2,8,8,9,9-hexamethyl-4-methylene-3,7-dioxa-2,8-disiladecane (**27**).* To a solution of compound **26** (272 mg, 1.0 mmol) in CH_2Cl_2 were added DIPEA (388 mg, 3.0 mmol) and TMSOTf (0.271 mL, 1.5 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h and then was quenched with saturated aqueous $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was dissolved in diethyl ether (20 mL) and washed with water and brine, and dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide compound **27** which was used in the next step without further purification. R_f = 0.89 (hexane/EtOAc = 8:1, v/v).

*(R,E)-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodec-1-en-3-one (**28**).* To a solution of compound **27** and vanillin (152 mg, 1.0 mmol) in CH_2Cl_2 (10 mL) was added $BF_3{\cdot}OEt_2$ (0.19 mL, 1.5 mmol) for 10 min at 0 °C. The reaction mixture was stirred at 0 °C for additional 30 min, followed by the addition of triethylamine (0.84 mL, 6.0 mmol) in one portion. The reaction mixture was stirred for 20 min at the same temperature and then was quenched with saturated aqueous $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 5:1 to 2:1, v/v) to furnish compound **28** (82 mg, 30% over 2 steps) as a yellow solid. R_f = 0.29 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J = 16.5 Hz, 1H), 7.09 (t, J = 8.1 Hz, 2H), 6.94 (d, J = 8.1 Hz, 1H), 6.60 (d, J = 16.5 Hz, 1H), 4.15 (brs, 1H), 3.94 (s, 3H), 3.41 (s, 1H), 2.91−2.71 (m, 2H), 1.61−1.24 (m, 12H), 0.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.0, 148.6, 146.9, 143.8, 126.7, 124.2, 123.7, 114.9, 109.5, 68.0, 56.0, 46.5, 36.6, 31.8, 29.6, 29.3, 25.6, 22.7, 14.1. ^{1}H and ^{13}C NMR data were in complete agreement with those previously reported in the literature.[30]

*(R)-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one (**29**).* To a solution of compound **28** (60 mg, 0.19 mmol) in MeOH (10 mL) was added 10% Pd/C. The reaction mixture was purged with H_2 gas and stirred for 2 h. The reaction mixture was filtered through a Celite pad and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 3:1, v/v) to prepare compound **29** (57 mg, 0.18 mmol) as colorless oil. R_f = 0.32 (hexane/EtOAc = 5:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82 (d, J = 7.8 Hz, 1H), 6.66 (s, 1H), 6.65 (d, J = 9.0 Hz, 2H), 5.52 (s, 1H), 4.02 (brs, 1H), 3.87 (s, 3H), 2.95 (s, 1H), 2.85−2.70 (m, 4H), 2.55−2.49 (m, 2H), 1.47−1.26 (m, 12H), 0.86 (d, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.0, 148.3, 147.0, 142.8, 127.0, 123.9, 123.4, 115.0, 109.6, 55.9, 40.6, 31.7, 29.3, 29.1, 24.6, 22.6, 14.1. ^{1}H and ^{13}C NMR data were in complete agreement with those previously reported in the literature.[30] >98% purity (as determined by RP-HPLC, method B, t_R = 9.53 min).

*[(But-3-en-1-yloxy)methyl]benzene (**31**).* To a suspension of sodium hydride (1.9 g, 48 mmol) in dry THF (60 mL) was added 3-buten-1-ol (2.3 mL, 27.0 mmol) dropwise at 0 °C. The solution was stirred 1 h at the same temperature. Benzyl bromide (3.5 mL, 29.1 mmol) was added dropwise to the solution. The reaction mixture was stirred for 16 h and quenched with brine (50 mL), followed by the extraction with diethyl ether (3 × 50 mL). The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give compound **31** as colorless oil (3.9 g, 90%). R_f = 0.89 (hexane/EtOAc = 8:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49−7.23 (m, 5H), 5.91−5.82 (m, 1H), 5.10 (t, J = 15.2 Hz, 2H), 4.55 (s, 3H), 3.55 (t, J = 6.7 Hz, 2H), 2.41 (q, J = 6.7 Hz, 2H). ^{1}H NMR data were in complete agreement with those previously reported in the literature.[39]

*2-[2-(Benzyloxy)ethyl]oxirane (**32**).* To a solution of compound **32** (3.0 g, 19.0 mmol) in dry CH_2Cl_2 (100 mL) was added $NaHCO_3$ (2.1 g, 25.0 mmol) at 0 °C, followed by the addition of *m*-CPBA (70−75% w/w, 8.3 g, 38.0 mmol). The reaction mixture was stirred for 16 h and then was filtered through a Celite pad and concentrated under reduced pressure. The crude residue was dissolved in water (50 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layer was washed with 3 N NaOH (3 × 50 mL), brine (50 mL), dried over $MgSO_4$, and concentrated. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 4:1) to furnish compound **32** (racemate) as colorless oil (1.56 g, 66% over 2 steps). R_f = 0.51 (hexane/EtOAc = 7:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49−7.23 (m, 5H), 4.56 (s, 2H), 3.65 (q, J = 5.6 Hz, 2H), 3.10 (brs, 1H), 2.78 (brs, 1H), 2.55 (brs, 1H), 1.97−1.76 (m, 2H). ^{1}H NMR data were in complete agreement with those previously reported in the literature.[39]

*(S)-2-[2-(Benzyloxy)ethyl]oxirane (**33**).* To a solution of (±)-**32** (3.1 g, 17.0 mmol) in THF (1 mL) were added (*S,S*)-(+)-*N,N′*-bis(3,5-di-*tert*-butylsalicyclidene)-1,2-cyclohexanediaminocobalt(II) (0.21 g, 0.4 mmol) and AcOH (80 μL, 1.4 mmol). The reaction mixture was cooled to 0 °C, and H_2O (0.17 mL, 9.5 mmol) was added in one portion. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was quenched with H_2O and extracted with EtOAc. The combined organic layer was washed with water (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 12:1 to 3:1) to furnish compound **33** (1.5 g, 50%) as a pale yellow oil. R_f = 0.51 (hexane/EtOAc = 7:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43−7.23 (m, 5H), 4.56 (s, 2H), 3.65 (q, J = 5.6 Hz, 2H), 3.10 (brs, 1H), 2.78 (brs, 1H), 2.55 (brs, 1H), 1.97−1.76 (m, 2H). ^{1}H NMR data were in complete agreement with those previously reported in the literature.[39]

*(R)-1-(Benzyloxy)dec-5-yn-3-ol (**34**).* To a solution of 1-hexyne (349 mg, 4.3 mmol) in dry THF (6 mL) was added *n*-BuLi (1.6 M in hexanes, 2.7 mL, 4.3 mmol) at −78 °C. The reaction mixture was stirred for 0.5 h, followed by the addition of $BF_3{\cdot}Et_2O$ (0.54 mL, 4.3 mmol). Compound **33** (500 mg, 2.8 mmol) dissolved in dry THF (6 mL) was added to the reaction solution at −78 °C. The reaction mixture was stirred for 2 h at −78 °C and then was quenched with saturated NH_4Cl (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 10:1 to 6:1, v/v) to furnish compound **34** (418 mg, 57%) as colorless oil. R_f = 0.42 (hexane/EtOAc = 6:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48−7.21 (m, 5H), 4.55 (s, 2H), 3.94 (brs, 1H), 3.78−3.64 (m, 2H), 2.97 (d, J = 3.3 Hz, 1H), 2.38 (brs, 2H), 2.18 (brs, 2H), 1.89−1.62 (m, 2H), 1.48−1.35 (m, 4H), 0.92 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.1, 128.5, 127.8, 127.7, 82.9, 76.2,

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73.4, 69.8, 68.7, 35.5, 31.2, 27.6, 22.0, 18.5, 13.7; MS (ESI) m/z calculated for $C_{17}H_{24}O_2$ $[M + Na]^+$, 283.2; found, 283.1.

*(R)-{[1-(Benzyloxy)dec-5-yn-3-yl]oxy}(tert-butyl)dimethylsilane (**35**).* To a solution of compound **34** (418 mg, 1.6 mmol) in CH_2Cl_2 (12 mL) were added imidazole (328 mg, 4.8 mmol) and TBDMSCl (363 mg, 2.4 mmol) slowly. The reaction mixture was stirred at 25 °C for 10 h and then was quenched with water (50 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 15:1 to 7:1, v/v) to furnish compound **35** (538 mg, 87%) as colorless oil. R_f = 0.95 (hexane/EtOAc = 6:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48−7.21 (m, 5H), 4.52 (s, 3H), 3.96 (brs, 1H), 3.58 (t, J = 6.3 Hz, 2H), 2.33 (brs, 1H), 2.16 (brs, 1H), 2.07−1.98 (m, 2H), 1.58−1.4 (m, 4H), 0.98−0.81 (m, 12H), 0.09 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.7, 128.4, 127.7, 127.5, 82.1, 76.7, 72.9, 68.7, 67.0, 36.7, 31.2, 28.2, 25.9, 22.0, 18.6, 18.1, 13.7, −4.4, −4.7. MS (MALDI-TOF) m/z calculated for $C_{23}H_{38}O_2Si$ $[M + H]^+$, 375.3; found, 375.4.

*(R)-3-[(tert-Butyldimethylsilyl)oxy]decan-1-ol (**36**).* To a solution of compound **35** (538 mg, 1.4 mmol) in MeOH (10 mL) was added 10% Pd/C (76 mg, 0.1 mmol). The reaction mixture was purged with H_2 and stirred for 3 h. The reaction mixture was filtered through a Celite pad and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 8:1, v/v) to furnish compound **36** (370 mg, 89%) as colorless oil. R_f = 0.64 (hexane/EtOAc = 6:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.93−3.83 (m, 2H), 3.76−3.70 (m, 1H), 2.50 (t, J = 5.1 Hz, 1H), 1.98−1.78 (m, 1H), 1.75−1.61 (m, 1H), 1.61−1.48 (m, 2H), 1.41−1.21 (m, 10H), 0.98−0.82 (m, 12H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 72.1, 60.4, 37.8, 37.0, 31.9, 29.8, 29.4, 26.0, 25.5, 22.7, 18.1, 14.2, −4.3, −4.6. MS (ESI) m/z calculated for $C_{16}H_{36}O_2Si^+$ $[M + H]^+$, 289.2557; found, 289.2563.

*(R)-3-[(tert-Butyldimethylsilyl)oxy]decanoic Acid (**37**).* Sodium periodate (1.6 g, 7.7 mmol) was added to a solution of compound **36** (370 mg, 1.3 mmol) in EtOAc (4 mL), acetonitrile (4 mL), and water (6 mL). The solution was stirred for 5 min. Ruthenium trichloride (53 mg, 0.3 mmol) was added to the solution. The reaction mixture was stirred for 6 h and then was filtered through a Celite pad and washed with EtOAc (2× 50 mL). The excess solvent was removed under reduced pressure, and the residue was partitioned between EtOAc (50 mL) and water (50 mL). The aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with brine (50 mL), dried over $MgSO_4$, and concentrated. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 8:1 to 4:1, v/v) to furnish compound **37** (260 mg, 67%) as colorless oil. R_f = 0.38 (hexane/EtOAc = 6:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.11 (t, J = 5.5 Hz, 1H), 2.53 (t, J = 4.9 Hz, 2H), 1.55 (brs, 1H), 1.42−1.19 (brs, 10H), 0.99−0.81 (m, 12H), 0.12 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.9, 69.6, 42.1, 37.4, 31.9, 29.7, 29.3, 25.88, 25.3, 22.8, 18.1, 14.2, −4.4, −4.7. MS (ESI) m/z calculated for $C_{16}H_{34}O_3Si$ $[M + H]^+$, 303.2; found, 303.1.

*(R)-3-[(tert-Butyldimethylsilyl)oxy]-N-methoxy-N-methyldecanamide (**38**).* To a solution of compound **37** (260 mg, 0.9 mmol) in THF (10 mL) were added *N*,*O*-dimethylhydroxylamine hydrochloride (168 mg, 1.7 mmol), HOBt (158 mg, 1.0 mmol) and EDC hydrochloride (198 mg, 1.0 mmol). The solution was stirred for 5 min. Diisopropylethylamine (0.45 mL, 2.6 mmol) was added. The reaction mixture was stirred at room temperature until the disappearance of the acid, as determined using TLC. The reaction mixture was concentrated, and the residue was partitioned between EtOAc (50 mL) and water (50 mL). The aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with brine (50 mL), dried $MgSO_4$, and concentrated. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 10:1 to 6:1, v/v) to furnish compound **38** (276 mg, 93%) as colorless oil. R_f = 0.44 (hexane/EtOAc = 6:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.21 (t, J = 5.5 Hz, 1H), 3.69 (s, 3H), 3.18 (s, 3H), 2.81−2.62 (m, 2H), 2.39 (dd, J = 4.5 and 14.6 Hz, 1H), 1.54−1.41 (m, 2H), 1.44−1.13 (m 10H), 0.94−0.83 (m, 12H), 0.07 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7, 69.6, 61.4, 39.7, 38.0, 31.9, 29.8, 29.4, 26.0, 25.2, 22.7, 18.1, 14.2, −4.6, −4.6. MS (MALDI-TOF) m/z calculated for $C_{18}H_{39}NO_3Si$ $[M + H]^+$, 346.3; found, 346.2.

*(R)-4-[(tert-Butyldimethylsilyl)oxy]undecan-2-one (**39**).* To a solution of compound **38** in THF (10 mL) was added methylmagnesium bromide (3 M solution in ether, 0.8 mL, 2.4 mmol) at −78 °C. The reaction mixture was stirred for 2 h at the same temperature and then was poured into saturated aqueous NH_4Cl (25 mL) and diluted with EtOAc. The aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with brine (25 mL), dried $MgSO_4$, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 10:1, v/v) to furnish compound **39** (216 mg, 90%) as colorless oil. R_f = 0.53 (hexane/EtOAc = 8:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.13 (t, J = 5.5 Hz, 1H), 2.60 (dd, J = 6.9 and 15.0 Hz, 1H), 2.46 (dd, J = 4.8 and 14.9 Hz, 1H), 2.16 (s, 3H), 1.49−1.38 (m, 2H), 1.49−1.18 (m, 10H), 0.98−0.82 (m, 12H), 0.06 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.1, 69.3, 51.0, 37.8, 31.9, 31.8, 25.9, 24.8, 22.7, 18.1, 14.1, −4.5, −4.7. MS (MALDI-TOF) m/z calculated for $C_{17}H_{36}O_2Si$ $[M + Na]^+$, 323.2; found, 323.2.

*(R)-6-Heptyl-2,2,8,8,9,9-hexamethyl-4-methylene-3,7-dioxa-2,8-disiladecane (**40**).* To a solution of compound **39** (200 mg, 0.7 mmol) in CH_2Cl_2 (10 mL) were added diisopropylethylamine (258 mg, 2.0 mmol) and TMSOTf (0.27 mL, 1.0 mmol) at 0 °C. The reaction mixture was stirred for 3 h at 0 °C and then was quenched with saturated aqueous $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue dissolved in diethyl ether (20 mL) was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide compound **40** which was used in the next step without further purification. R_f = 0.89 (hexane/EtOAc = 8:1, v/v).

*(R,E)-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodec-1-en-3-one (**41**).* To a mixture of compound **40** and vanillin (108 mg, 0.7 mmol) in CH_2Cl_2 (10 mL) was added $BF_3{\cdot}OEt_2$ (0.312 mL, 1.2 mmol) over 10 min at 0 °C. The reaction mixture was stirred for 30 min at 0 °C. Triethylamine (0.494 mL, 6.0 mmol) was added in one portion to the reaction mixture. The reaction mixture was stirred for 20 min and then was quenched with saturated aqueous $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 5:1 to 2:1, v/v) to furnish compound **41** (41 mg, 18% over 2 steps). R_f = 0.32 (hexane/EtOAc = 4:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 16.1 Hz, 1H), 7.10 (d, J = 8.1 Hz, 1H), 7.06 (s, 1H), 6.94 (d, J = 8.1 Hz, 1H), 6.61 (d, J = 16.2 Hz, 1H), 5.93 (s, 1H), 4.15 (brs, 1H), 3.96 (s, 3H), 3.31 (s, 1H), 2.90 (d, J = 16.5 Hz, 1H), 2.75 (dd, J = 9.3 and 17.3 Hz, 1H), 1.63−1.21 (m, 12H), 0.98−0.82 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.0, 148.6, 146.9, 143.8, 126.7, 124.2, 123.7, 114.9, 109.5, 68.0, 56.0, 46.5, 36.6, 31.8, 29.6, 29.3, 25.6, 22.7, 14.1. MS (MALDI-TOF) m/z calculated for $C_{19}H_{28}O_4^+$ $[M + H]^+$, 321.2; found, 321.4. >99% purity (as determined by RP-HPLC, method B, t_R = 9.51 min).

*(R)-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one (**42**).* To compound **41** (20 mg, 0.06 mmol) dissolved in MeOH (5 mL) was added 10% Pd/C (1.2 mg, 0.01 mmol). The reaction mixture was purged with H_2 gas and stirred for 1 h. The reaction mixture was filtered through a Celite pad and then was concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc = 3:1, v/v) to frunish compound **42** (19 mg, 95%) as colorless oil. R_f = 0.29 (hexane/EtOAc = 4:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 (d, J = 7.9 Hz, 1H), 6.69 (s, 1H), 6.68 (d, J = 7.9 Hz, 1H), 5.50 (s, 1H), 4.04 (brs, 1H), 3.89 (s, 3H), 2.95 (brs, 1H), 2.93−2.81 (m, 2H), 2.81−2.72 (m, 2H), 2.61−2.45 (m, 2H), 1.51−1.22 (m, 12H), 0.88 (d, J = 6.7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.6, 146.6, 144.1, 132.8, 120.9, 114.5, 111.1, 67.8, 56.0, 49.5, 45.6, 36.6, 31.9, 29.6, 29.4, 29.4, 25.6, 22.8, 14.2. MS (MALDI-TOF) m/z calculated for $C_{19}H_{30}O_4^+$ $[M]^+$, 322.2; found,

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322.2. >99% purity (as determined by RP-HPLC, method B, t_R = 9.53 min).

Kinetic Resolution by Chiral HPLC. The ee values of 8-gingerol were determined by chiral HPLC analyses on a chiral column (CHIRALPAK IG; 4.6 mm i.d. × 250 mm). Chromatographic analyses were carried out on an HPLC system (Agilent 1260 series) for 30 min at a flow rate of 1 mL/min with an isocratic solution of 20% ethanol in hexane. The autosampler and the column compartment temperatures were set to 25 °C. UV detection was conducted at a wavelength of 230 nm; 5 μL of the sample was injected with three repeats at each concentration.

LasR Reporter Gene Assay. This assay was conducted by modifying a previously reported method.[20] *E. coli* DH5α co-transformed with two plasmids, pJN105L (LasR expression plasmid) and pSC11 (*lasI::lacZ* fusion plasmid), was used as a bioassay reporter strain. Overnight culture of the reporter strain with 10 μg/mL gentamicin and 50 μg/mL ampicillin was diluted in the Luria−Bertani (LB) medium (1:100). Then, the reporter strain (optical density at 595 nm [OD_{595}] was 0.3) mixed with either a positive control or the compound under study was incubated with OdDHL (Sigma-Aldrich, St. Louis, MO, USA) and 0.4% arabinose (Sigma-Aldrich). After incubation at 37 °C for 1.5 h, OD_{595} was measured on a VICTOR x5 multimode plate reader (PerkinElmer, Waltham, MA, USA). The β-galactosidase activity was determined using a Tropix plus kit (Applied Biosystems, USA), and luminescence was measured on the VICTOR x5 multimode plate reader. RLU ratio was quantified by dividing luminescence with OD_{595}.

Static Biofilm Formation Assay. Overnight culture of *P. aeruginosa* PA14 (OD_{595} = 1.0) was diluted (1:20) with the AB medium (300 mM NaCl, 50 mM $MgSO_4$, 0.2% vitamin-free casamino acids, 10 mM potassium phosphate, 1 mM L-arginine, and 1% glucose, pH 7.5)[40] (1:20) containing with either positive controls or compounds (0−100 μM). The dilutions were aliquoted into borosilicate bottles, and the bottles were incubated at 37 °C for 24 h without agitation. After that, OD_{595} of the cell suspension was measured on the VICTOR x5 multimode plate reader. The biofilm cells attached to the bottle were washed two times with phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4, pH 7.2) and stained with 0.1% crystal violet for 10 min. Next, the stained biofilm cells were eluted with 100% ethyl alcohol, and OD_{540} was measured on the VICTOR x5 multimode plate reader. The biofilm formation was quantified by dividing OD_{540} with OD_{595}.

Dynamic Biofilm Formation Assay. Glass slides were dipped into a Petri dish containing 2 mL of a *P. aeruginosa* PA14 suspension (OD_{595} = 1.0) and 18 mL of the AB medium, followed by incubation at 37 °C for 24 h without agitation to let the cells adhere to the slides. The slides were then inserted into a drip-flow reactor (DFR-110, BioSurface, MT, USA). The AB medium with either a positive control or the compound under study (0−10 μM) was fed into the reactor continuously via a peristaltic pump (Masterflex C/L tubing pumps, Cole-Parmer, IL, USA) at 0.3 mL/min. After operation of the reactor at 37 °C for 48 h, the cells on the slides were washed two times with PBS. The biofilm cells were stained with fluorescein isothiocyanate-labeled type IV ConA (Sigma-Aldrich) and SYPRO Ruby (Ruby, Invitrogen, Carlsbad, CA, USA) for 15 min, respectively. CLSM images were captured via a 20× objective lens (W N-Achroplan 20×/0.5 W [DIC] M27) with green fluorescence (ConA, excitation wavelength of 490 nm, emission wavelength of 525 nm) and red fluorescence (Ruby, excitation wavelength of 470 nm, emission wavelength of 618 nm) and were analyzed in the Zen 2011 software (Carl Zeiss, Jena, Germany). For quantification, biofilm volume ($\mu m^3/\mu m^2$) and average thickness (μm) were measured by means of Comstat2 in ImageJ.[41]

Growth Inhibition Assay. A 5% dilution of overnight culture of *P. aeruginosa* PA14 (OD_{595} = 1.0) containing either a positive control or the compound under study (0−100 μM) was inoculated into wells of a 96-well polystyrene microtiter plate (Sigma-Aldrich). The plate was incubated at 37 °C for 24 h. OD_{595} of the suspension culture was measured on the VICTOR x5 multimode plate reader.

In Silico Docking Study of Compounds 41 and 42 with LasR. The processes of ligand preparation and optimization were conducted by means of the Prepare Ligands module, a protocol of Discovery Studio 3.0 (Accelrys Inc.). The prepared ligands were converted to the SD file format. LasR Protein structure in PDB format was downloaded from the RCSB Web site (http://www.pdb.org). Before the docking procedure, the original crystal ligand OdDHL and water molecules were removed from the protein−ligand complexes. Hydrogen atoms were added by application of CHARMm force field and the Momany−Rone partial charge as default settings in Discovery Studio 3.0. The ligand-binding site was extracted from PDB site records and designated as active site 1. Docking analyses of compound **41** or **42** with the LasR protein in the presence of OdDHL were performed by means of the CDOCKER module. The number of generated poses was set to 100 for each ligand, and default settings were selected for other parameters.

Statistical Analysis. *P* values were estimated by Student's *t* test (SigmaPlot version 10, Systat Software Inc., San Jose, CA, USA).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.7b01426.

Analytical spectra (^{1}H NMR and ^{13}C NMR) of compounds **4**−**42** and additional figures (PDF)

Molecular formula strings and some data (CSV)

Three-dimensional coordinates of the molecular model of LasR (PDB code 2UV0) with compound **41** presented in Figure 9 (PDB)

Three-dimensional coordinates of the molecular model of LasR (PDB ID: 2UV0) with compounds **42** presented in Figure 9 (PDB)

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Author Contributions

#H.C. and S.-Y.H. contributed equally. H.C., S.-Y.H., S.-H.S., H.-D.P., and Y.B. were involved in the initial stages of the project, including design of gingerol analogs and development of LasR reporter gene assay. H.C., Y.S., S.-H.S., and J.K.B. synthesized and analyzed gingerol analogs. S.-Y.H., E.C., and H.-S.K. performed LasR reporter gene, static biofilm formation, and dynamic biofilm formation assay. H.C., S.-Y.H., S.-H.S., H.-D.P., and Y.B. analyzed the data and wrote the paper. All authors contributed to editing the final manuscript.

Notes

The authors declare no competing financial interest.

Authors will release the atomic coordinates and experimental data upon article publication.

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■ ABBREVIATIONS USED

AHL, *N*-acylhomoserine lactone; BHL, *N*-butyryl-L-homoserine lactone; CLSM, confocal laser scanning microscopy; ConA, concanavalin A; DIPEA, *N*,*N*-diisopropylethylamine; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOBt, *N*-hydroxybenzotriazole hydrate; LDA, lithium diisopropylamide; OdDHL, *N*-(3-oxododecanoyl)-L-homoserine lactone; PQS, 2-heptyl-3-hydroxy-4(1*H*)-quinolone; QS, quorum sensing; RLU, relative luminescence unit; RT-PCR, reverse transcription polymerase chain reaction; SAR, structure–activity relationship; TBDMSCl, *tert*-butyldimethylsilyl chloride; TMSOTf, trimethylsilyl trifluoromethanesulfonate

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