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Synthesis of *Pseudomonas* quorum-sensing autoinducer analogs and structural entities required for induction of apoptosis in macrophages

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Abstract—The synthesis of the analogs of *N*-3-oxododecanoyl-L-homoserine lactone (**1**) and their structure–activity relationship for the apoptotic induction in macrophages, P388D1 cells, are described. It was revealed that the position of the oxo group in the acyl side chain in addition to the presence of the L-homoserine lactone unit is crucial for the apoptosis-inducing activity. Furthermore, the long acyl side chains with hydrophobic distal ends are preferable for the activity.

N-3-Oxododecanoyl-L-homoserine lactone (**1**; 3-oxo-C_{12}-HSL), a *Pseudomonas* quorum-sensing autoinducer for the bacterial cell-to-cell communication,[1,2] has been known to exhibit various immunostimulatory activities in host cells,[3–6] such as the induction of IL-8 and prostaglandin E_1 production. Recently, we reported that **1**, and not *N*-butanoyl-L-homoserine lactone (**2**; C_4-HSL), another *Pseudomonas* autoinducer, induces apoptosis in eukaryotic cells.[7] Interestingly, the induction of apoptosis with **1** was observed in macrophages (U937, P388D1) and neutrophils, but not in fibroblasts (L-cell) or epithelial cells (CCL-185, HEp-2). Although these phenomena have been intriguing in relation to the chronic respiratory infection by *Pseudomonas aeruginosa*, which is one of the opportunistic pathogens, their molecular mechanisms are largely unknown. We have previously synthesized a few analogs of **1** and shown the importance of the 3-oxo group in the acyl side chain and L-homoserine lactone unit for the apoptotic induction in the macrophages. Here, we report the synthesis of an array of acyl-HSL analogs, most of which possess different acyl side chains, and the structural characteristics required for the induction of apoptosis in macrophages, that is, the P388D1 cells (Fig. 1).

The synthetic schemes for the individual compounds are outlined below. 3-Oxo-C_{10}-HSL (**8**) and 3-oxo-C_{14}-HSL (**9**) were, respectively, prepared by the procedure[7] previously described in the synthesis of **1**.[8] The acyl-HSL analogs **10**–**15**, each possessing a cyclopropane ring or a phenyl group at the end of acyl side chain, were synthesized as outlined in Scheme 1. Treatment of a dianion of *tert*-butyl acetoacetate with 1-bromo-3-butene provided the corresponding β-ketoester. Removal of its *tert*-butyl group with TFA in CH_2Cl_2 was followed by coupling with L-HSL (**A**) to afford 7,8-dehydro-acyl-

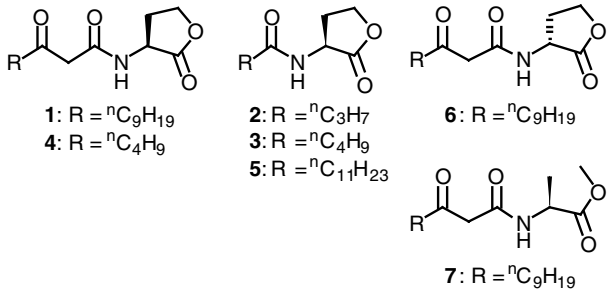

Figure 1. *Pseudomonas* autoinducers (**1**,**2**) and their previously synthesized analogs (**3**–**7**).

Keywords: Apoptosis induction; Quorum-sensing; Autoinducer; *N*-3-Oxododecanoyl-L-homoserine lactone; *Pseudomonas aeruginosa*; Macrophage; P388D1.
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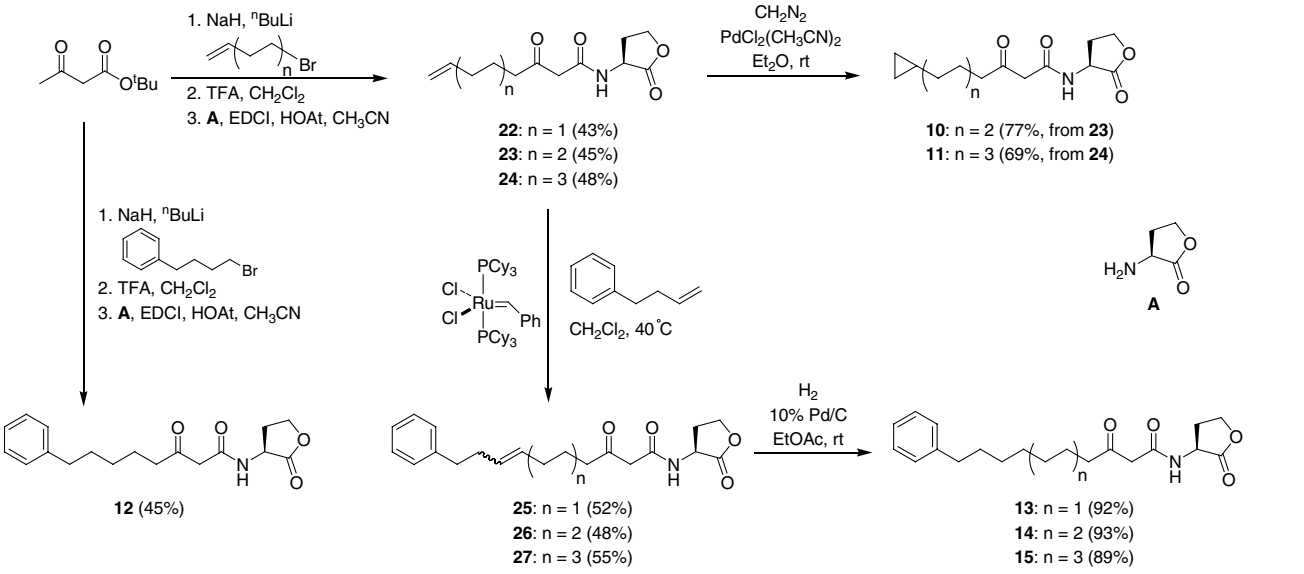
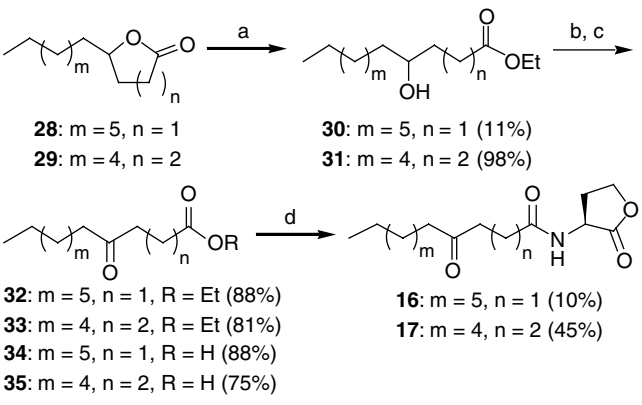


Scheme 1. Synthesis of analogs **10**–**15**.

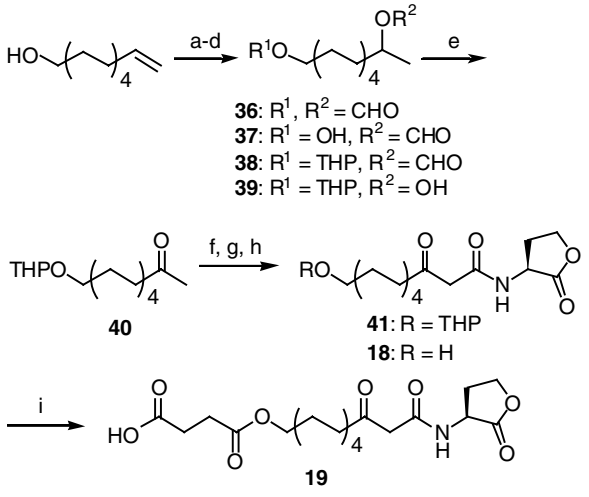
HSL **22**. For this coupling reaction, 1-hydroxy-7-azabenzotriazole (HOAt) was used instead of HOBt because a more convenient procedure is required for removal of HOAt, that is, by washing with a 5% $CuSO_4$ aqueous solution. The same procedure employed for 1-bromo-5-hexene or 1-bromo-7-octene gave the corresponding dehydro-acyl-HSLs, **23** or **24**, respectively. The cyclopropanation of **23** with diazomethane catalyzed by bis(acetonitrile)dichloropalladium (II) provided cyclopropyl-acyl-HSL **10** in a moderate yield. The conversion of **24** in a similar manner led to **11**. The olefin cross-metathesis reaction[9] of the acyl-HSLs **22**–**24** with 4-phenyl-1-butene provided the endo-olefins **25**–**27** in moderate yields, respectively. Hydrogenation of **25**–**27** gave a series of phenylacyl-HSLs **13**–**15** possessing different acyl side-chain length. In addition, another phenylacyl-HSL analog **12** possessing a side chain shorter than **13** was prepared from 1-bromo-4-phenylbutane in the same manner as that employed for **22**–**24**.

The γ- or δ-oxoacyl-HSLs **16** and **17** were synthesized using γ- or δ-dodecanolactone (**28**) as the starting materials, respectively. The ethanolysis of γ-dodecanolactone with concentrated sulfuric acid was followed by the treatment of chromium (VI) oxide to afford ketone **32**. The hydrolysis of **32** led to the acid **34**, which was condensed with L-HSL (**A**) to give the γ-ketoacyl-HSL **16**. The δ-oxoacyl-HSL **17** was also prepared from δ-dodecanolactone (**29**) in the same manner as the synthesis of **16** (Scheme 2).

12-Hydroxy-3-oxo-C_{12}-HSL (**18**) was synthesized via the methyl ketone **40**, which was prepared from 10-undecen-1-ol in five steps by a practical procedure. The methyl ketone **40** was converted to the acyl-HSL **41** in the same manner as the synthesis of **8** or **9**. Removal of the THP group provided the ω-hydroxy group-bearing HSL analog (**18**), which was treated with succinic anhydride and pyridine to give 12-succinyl-3-oxo-C_{12}-HSL (**19**) in good yield (Scheme 3).



Scheme 2. Synthesis of analogs **16** and **17**. Reagents and conditions: (a) EtOH, H_2SO_4; (b) CrO_3, acetone; (c) KOH, MeOH; (d) **A**, EDCI, dioxane.



Scheme 3. Synthesis of analogs **18** and **19**. Reagents and conditions: (a) HCO_2H, $HClO_4$ (**36**: 77%); (b) K_2CO_3, MeOH (**37**: 61%); (c) DHP, PPTS, CH_2Cl_2 (**38**: 100%); (d) NaOH, MeOH (**39**: 100%); (e) CrO_3, acetone (72%); (f) LiHMDS, THF, then crushed dry ice (92%); (g) **A**, EDCI, dioxane (39%); (h) H_2O, AcOH (42%); (i) succinic anhydride, pyridine (99%).

Table 1. Effects of acyl-HSL analogs on the viability of P388D1 cells

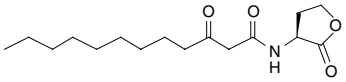
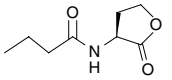
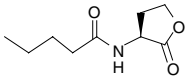
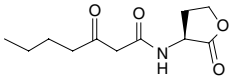
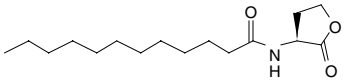
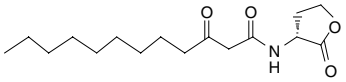
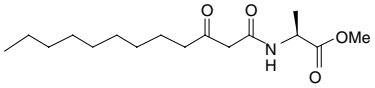
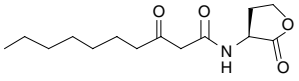
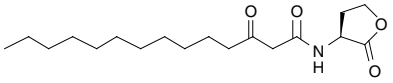
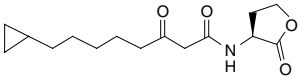
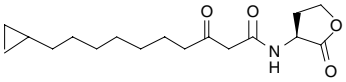
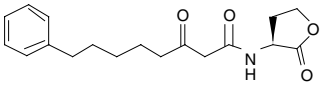
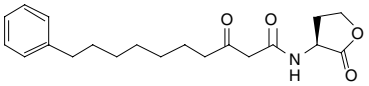
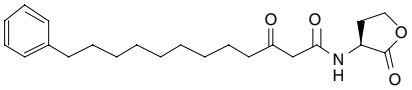
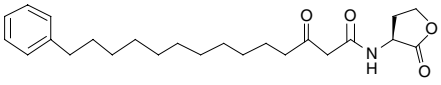
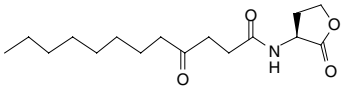
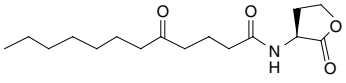
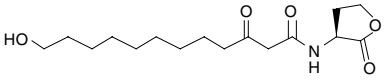
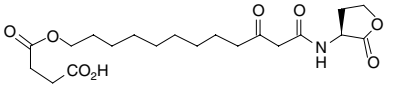
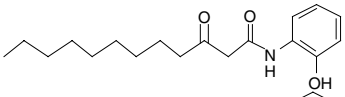
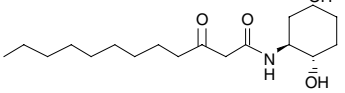
Compound		Viability (%)[a]
1		31.9 ± 1.4
2		100.4 ± 15.2
3		101.8 ± 5.0
4		111.0 ± 22.0
5		91.1 ± 3.2
6		105.0 ± 10.9
7		111.2 ± 21.7
8		107.7 ± 16.0
9		14.8 ± 0.1
10		122.9 ± 2.0
11		38.7 ± 1.9
12		122.0 ± 16.9
13		25.2 ± 1.4
14		14.5 ± 0.1
15		12.2 ± 0.6
16		115.6 ± 2.2
17		97.7 ± 4.1
18		109.5 ± 3.1
19		114.0 ± 4.5

Table 1 (*continued*)

Compound		Viability (%)[a]
20		109.6 ± 2.1
21		96.2 ± 2.8

[a] Viability values of P388D1 cells incubated with 50 μM of acyl-HSL analogs are shown as means ± SD from three determinations compared to that of the nontreated cells.

In our previous study,[7] the apoptosis activity of a limited number of compounds, **2**–**7**, was tested and compared with that of **1** using the U937 cell lines. The test with this cell line later turned out to give a poor reproducibility. In the present study, therefore, the apoptosis-inducing activities of all compounds (**1**–**21**) were measured on the macrophage P388D1 cell line, which provided reproducible and thus more reliable results.

The apoptotic activity of **1** was reconfirmed, whereas compounds **2**–**7** showed no activity in agreement with the previous result using the U937 cell lines. This again clearly proved the importance of the presence and sufficient chain length of the 3-oxo acyl group (analogs **5** and **4**) and of the homoserine lactone moiety (analog **7**) including the (*S*)-configuration at its C-2 position (analog **6**). The role of the homoserine lactone moiety was further confirmed by the lack of activity in the analogs **20** and **21**, which are a *Pseudomonas* quorum-sensing agonist and an antagonist,[10] respectively. Thus, we focused on compounds having comparable or longer 3-oxo-acyl chains, and found that compounds **9**, **11**, **13**, **14**, and **15** retained potent apoptosis-inducing activities. According to the method previously described for **1**,[7] these active analogs were also confirmed to induce active caspase-3 as one of the apoptosis markers (data were not shown). Their activities would be equal to that of **1** because of the structural similarities between **1** and them. On the other hand, **8**, **10**, and **12** possessing shorter chains lost the apoptosis activity. The change in the 3-oxo group to the 4- or 5-oxo position reduced the activity as exemplified by compounds **16** and **17**. Furthermore, the introduction of hydrophilic functional groups, such as hydroxy or carboxy groups, at the ω-position reduced the apoptosis inducing ability as exemplified by compounds **18** and **19** (Table 1).

The present structure–activity relationship study demonstrated that the 3-oxo group in the acyl side chain and the homoserine lactone moiety of the L-form are crucial for the apoptosis-inducing activity. Thus, a plausible target receptor is expected to have a specific hydrophilic pocket. Furthermore, the acyl side chains possessing the polar groups in the end eliminated the activity. In addition, the hydrophobic acyl side chains longer and bulkier than that of the natural counterpart **1** kept the activity, whereas the shorter ones lost the activity. Thus, the target receptor is also expected to have a large and flexible hydrophobic pocket.

In summary, we demonstrated the synthesis of a series of acyl-HSL analogs and their apoptosis-inducing activity. The present results revealed the structural characteristics of the acyl-HSL analogs necessary for the apoptosis-inducing activity in macrophages, that is, the P388D1 cell lines, suggesting the presence of a putative receptor in eukaryotic cells. Further investigation to discover the molecular target of 3-oxo-C_{12}-HSL for the induction of apoptosis in macrophages is under way.

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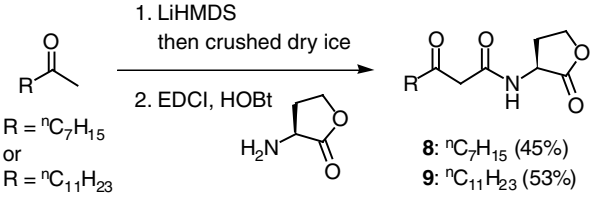
8. Compounds **8** and **9** were synthesized in moderate yields, respectively.



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