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

Manabu Horikawa,[a,*] Kazuhiro Tateda,[b] Etsu Tuzuki,[b] Yoshikazu Ishii,[b] Chihiro Ueda,[c] Tohru Takabatake,[c] Shinichi Miyairi,[c] Keizou Yamaguchi[b] and Masaji Ishiguro[a]

[a]*Suntory Institute for Bioorganic Research, Mishima-gun, Osaka 618-8503, Japan*
[b]*Department of Microbiology, Toho University School of Medicine, Tokyo 143-8540, Japan*
[c]*Laboratory of Bio-organic Chemistry, College of Pharmacy, Nihon University, Funabashi, Chiba 274-8555, Japan*

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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-

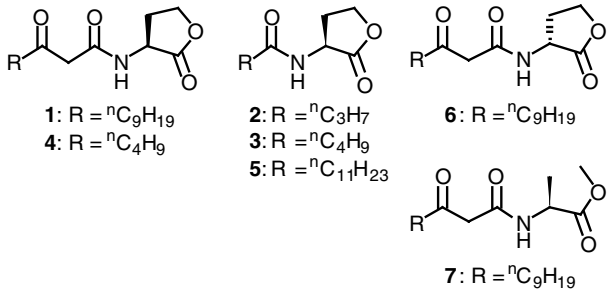

Figure 1. *Pseudomonas* autoinducers (**1**,**2**) and their previously synthesized analogs (**3**–**7**).

Keywords: Apoptosis induction; Quorum-sensing; Autoinducer; *N*-3-Oxododecanoyl-L-homoserine lactone; *Pseudomonas aeruginosa*; Macrophage; P388D1.
* Corresponding author. Tel.: +81 75 962 3742; fax: +81 75 962 2115; e-mail: horikawa@sunbor.or.jp

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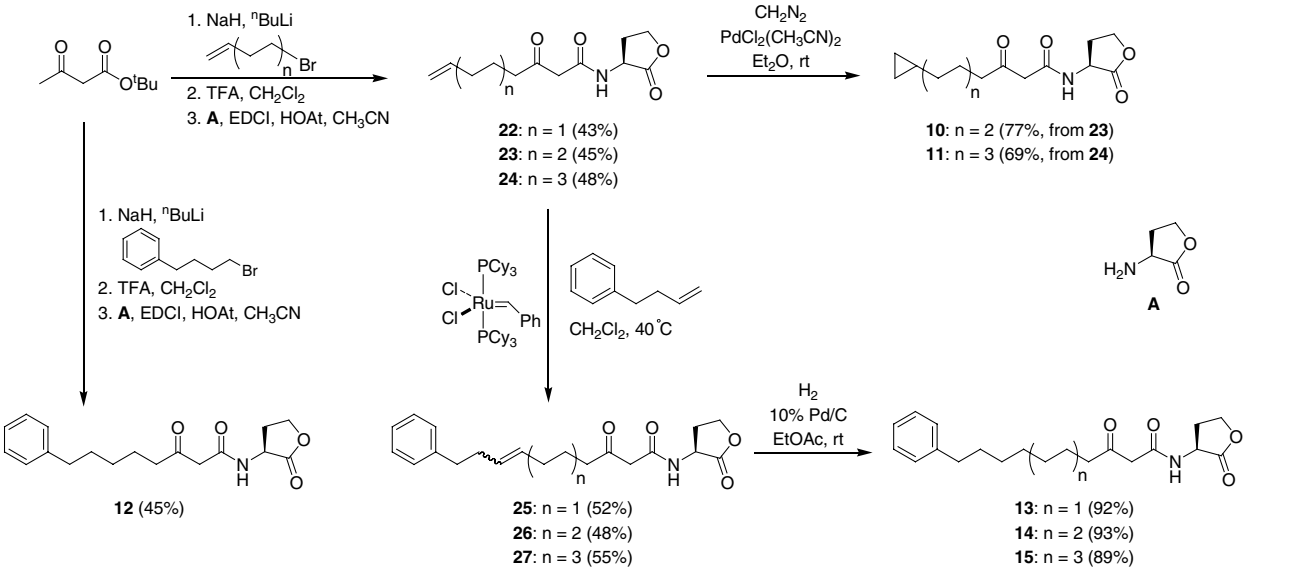
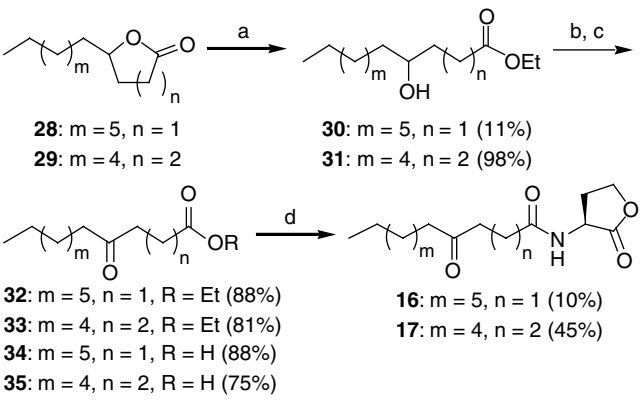


Scheme 1. Synthesis of analogs **10**–**15**.

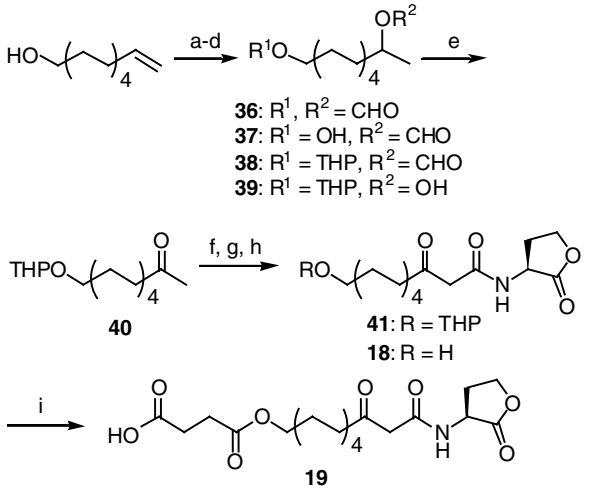
HSL **22**. For this coupling reaction, 1-hydroxy-7-azabenzotriazole (HOAt) was used instead of HOBt because a more convenient procedure is required for removal of HOAt, that is, by washing with a 5% $CuSO_4$ aqueous solution. The same procedure employed for 1-bromo-5-hexene or 1-bromo-7-octene gave 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%).