

Chapter 2

Total Synthesis of Thielocin B1 and NMR Chemical Shift Perturbation Experiments with PAC3 Homodimer

2.1 Introduction

Thielocin B1 (**1**) was isolated from the fermentation broth of *Thielavia terricola* RF-143 by Yoshida et al. [1] in 1995. The high-throughput screening of a natural products library was performed by Shin-ya et al. [2] in 2009 to identify protein–protein interaction (PPI) inhibitors, and it was revealed that **1** strongly inhibited the formation of the proteasome-assembling chaperon 3 (PAC3) homodimer [3], which is a component of the α -rings of the 20S proteasome [4]. Remarkably, thielocin A1 β (**2**) and B3 (**3**) did not exhibit any inhibitory activity towards the same PPI (Fig. 2.1), which indicated that the central core in **1** is in some way critical to the expression of its activity, because the wings to the left and right of all three of these compounds are identical. Additionally, the results of glide docking experiments indicated that **1** recognized specific β -sheet structures on the interface of PAC3, although no cavities are visible in this particular area of the protein. It was envisaged that an NMR chemical shift perturbation experiment with the **1**/PAC3 complex would allow for the characterization of the rare and interesting inhibitory mechanism of **1**. However, it would be necessary to achieve the organic synthesis of **1** to conduct this experiment because the supply of this compound from natural sources is limited. Herein, we report the first total synthesis of **1** and the characterization of its inhibitory mechanism based on the results of NMR chemical shift perturbation experiments involving PAC3 homodimer with synthesized **1** and an in silico docking study.

2.2 Strategy

Thielocin B1 (**1**) is composed of five fully substituted benzene rings, which are connected to each other through one ether and three ester linkages. It is known that highly *ortho*-substituted diphenyl ether and phenyl benzoate compounds are

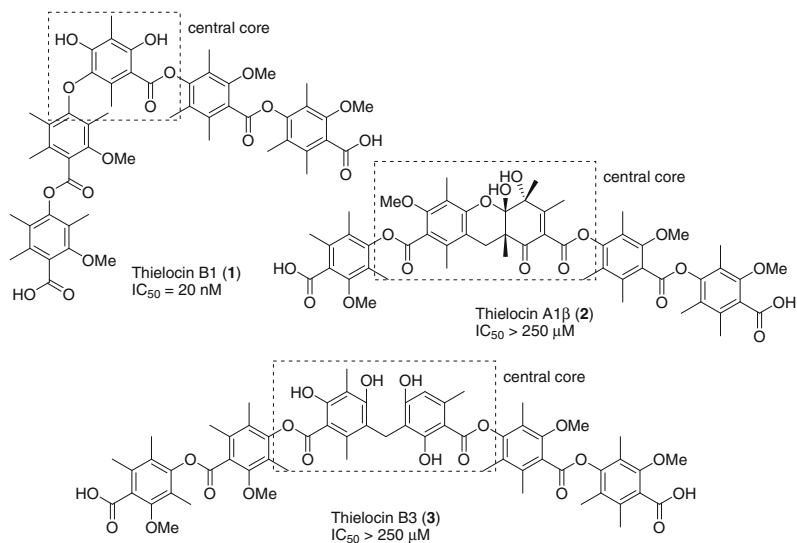


Fig. 2.1 IC₅₀ values of several thielocins towards PAC3 homodimer

difficult to form because of steric hindrance. In particular, there have been very few reports pertaining to the synthesis of electron-rich 2,2',6,6'-tetrasubstituted diphenyl ether moieties using transition metal-catalyzed intermolecular coupling reactions (e.g., Pd or Cu) [5]. It was envisaged, however, that the use of a depsidone skeleton, which is found in a number of natural products, could provide a workable solution to this critical problem. Depsidone **4** possesses a diphenyl ether moiety as part of a seven-membered lactone ring, and the reductive ring opening of the lactone in **4** could therefore provide access to the desired diphenyl ether **5** (Fig. 2.2).

The retrosynthetic strategy for the construction of **1** is shown in Scheme 2.1. Compound **1** was disconnected in three units, including a central core **6**, right wing **7** and left wing **8**. To prevent the nonselective elongation of the side wings, one of

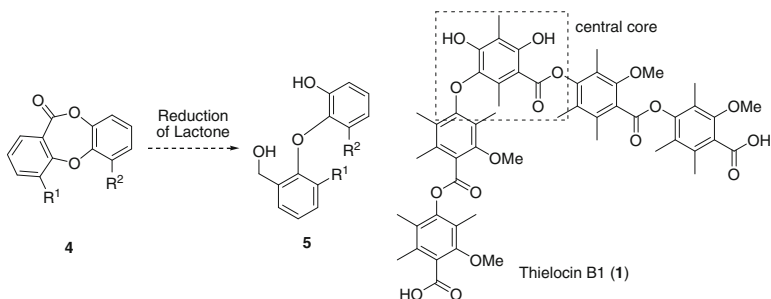
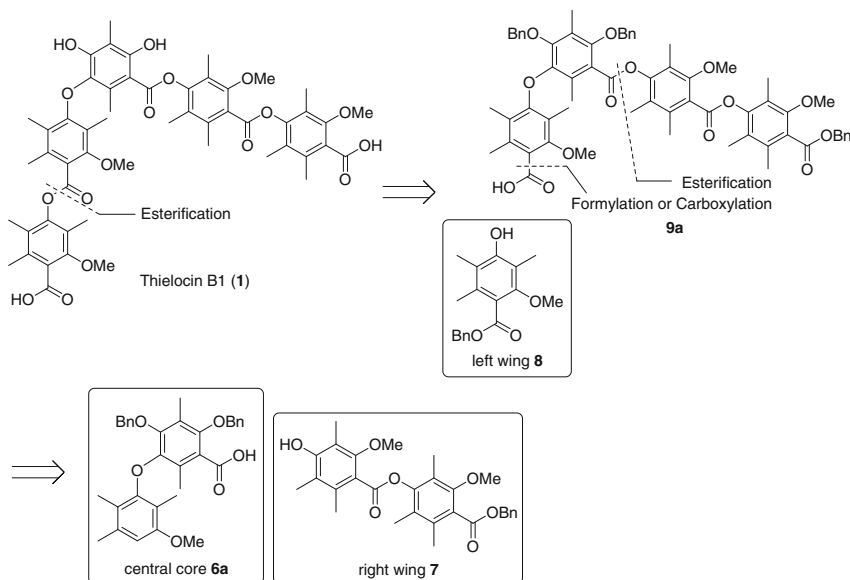


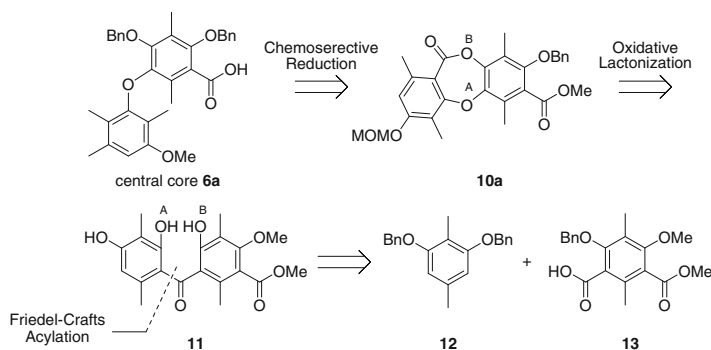
Fig. 2.2 Synthetic strategy for the construction of the 2,2',6,6'-tetrasubstituted diphenyl ether moiety



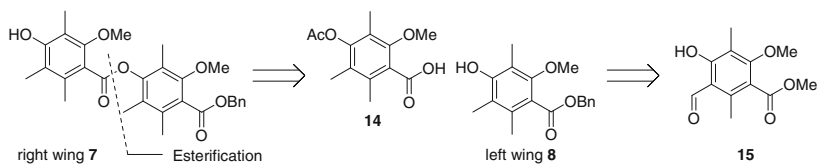
Scheme 2.1 Retrosynthesis of thielocin B1 (1)

the carboxyl groups would have to be introduced after the esterification of acid **6a** with phenol **7**. However, highly reactive formylation or carboxylation methods capable of introducing carbonyl-containing functionalities at sterically hindered positions would be required to access **9a**. Given that **1** was expected to be a highly polar compound, benzyl groups were chosen as the protecting groups for the two hydroxyl and two carboxyl groups in **1** under the assumption that they could be readily removed by hydrogenolysis under neutral conditions in the final step of the synthesis.

The synthetic strategies for the construction of the different components of **1** are described below. As mentioned above, **6a** could be synthesized by the chemoselective reduction of the lactone moiety in **10a** without losing the methoxycarbonyl group. Following on from the Friedel-Crafts acylation of **12** with acid **13**, it was envisaged that the resulting ketone **11** could be subjected to an oxidative lactonization according to the procedure reported by Sargent et al. [6] to afford **10a** (Scheme 2.2). For the right wing **7**, which is equivalent to two molecules of the left wing **8**, it was envisaged that the esterification of acid **8** with phenol **14** would give **7**. During the synthesis of these different components, aldehyde **15** was identified as a key synthetic intermediate, because it was used to access compounds **8**, **13** and **14** (Scheme 2.3).



Scheme 2.2 Retrosynthesis of central core **6a**



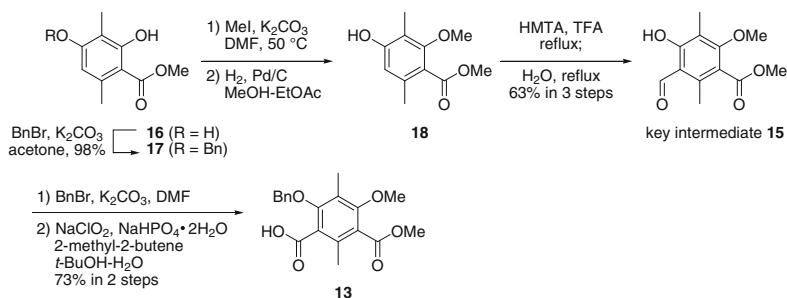
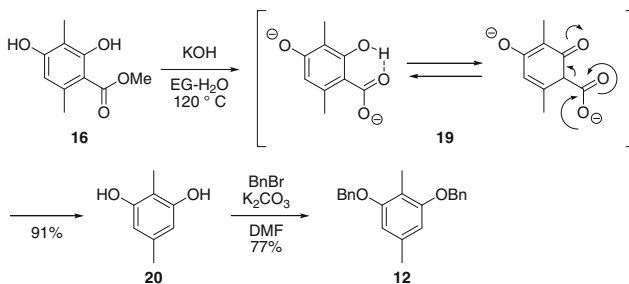
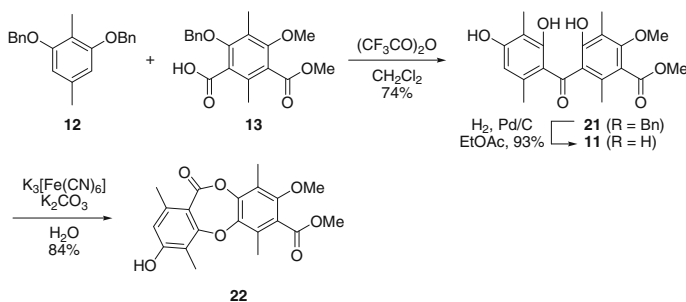
Scheme 2.3 Retrosynthesis of side wings **7** and **8**

2.3 Synthesis of the Central Core of Thielocin B1

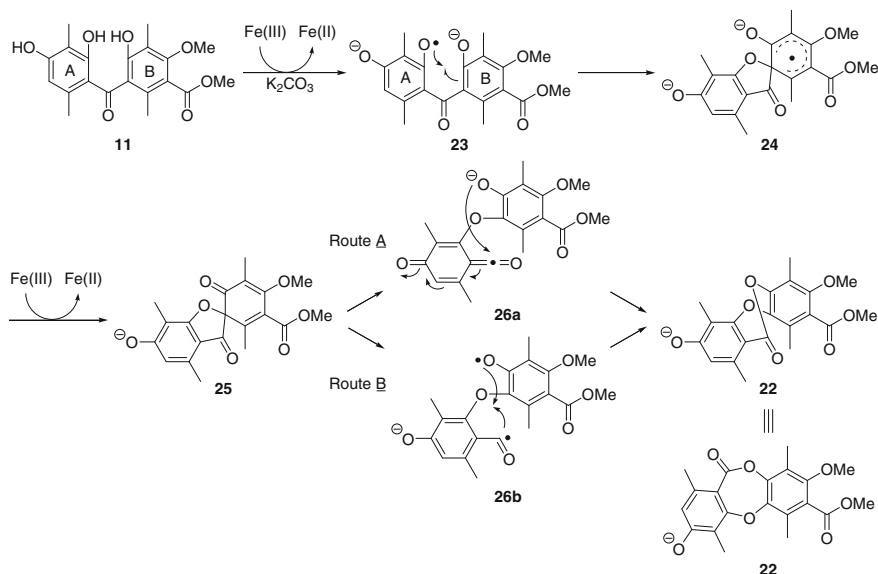
2.3.1 Construction of the Depsidone Skeleton

To provide access to the desired depsidone, it was necessary to develop scalable synthetic routes to compounds **12** and **13**. The commercially available methyl 2,4-dihydroxy-3,6-dimethylbenzoate (**16**) bearing two hydroxyl groups was selectively benzylated to afford monobenzyl ether **17**. Subsequent methylation of the other hydroxyl group followed by the cleavage of the benzyl group and a modified Duff reaction [7] afforded key intermediate **15** from **17** in 63 % yield. Protection of the free hydroxyl group as a benzyl ether, followed by a Kraus oxidation [8–10], gave the known acid **13** (Scheme 2.4) [6]. It is noteworthy that the original route developed for the synthesis of **12** had to be abandoned because commercially available **20** was too expensive. The hydrolysis of **12** with KOH proceeded with concomitant decarboxylation to give **20** in 91 % yield. Subsequent protection of the two hydroxyl groups in **20** as benzyl ethers provided the desired product **12** in good yield (Scheme 2.5).

The preparation of depsidone **22** is shown in Scheme 2.6. The Friedel-Crafts acylation of **12** with **13** in the presence of $(\text{CF}_3\text{CO})_2\text{O}$ afforded benzophenone **21**. Following the cleavage of all three of its benzyl groups by hydrogenolysis, the resulting triol **11** was oxidized with alkaline ferricyanide to give the known lactone

Scheme 2.4 Synthesis of acid **13**Scheme 2.5 Synthesis of benzyl ether **12**Scheme 2.6 Synthesis of depsidone **22**

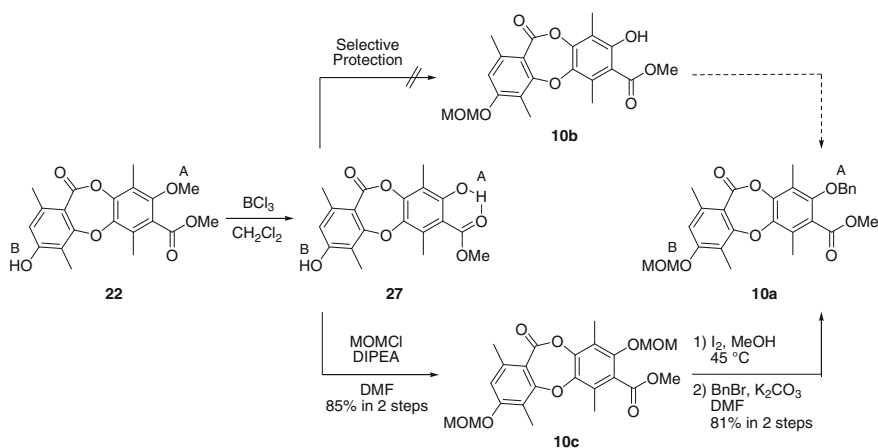
22 [6]. A plausible mechanism for this transformation is shown in Scheme 2.7, and is similar to the mechanism suggested by Sargent et al. [11]. Briefly, an initial single electron oxidation would proceed at the more electron-rich benzene ring A. The homolytic formation of a C–O bond followed by further oxidation would give **25** as an intermediate [12, 13]. Subsequent heterolytic (route A) or homolytic (route B) cleavage would result in the immediate cyclization of the resulting C–O bond of **26a** or **26b**, respectively, to give **22**.



Scheme 2.7 Plausible mechanism for the oxidative lactonization

2.3.2 Construction of Desired Diphenyl Ether Moiety

With the desired depsidone **22** in hand, it was possible to investigate the conversion of this material to the desired biphenyl ether moiety. The method used for the protection of hydroxyl groups in **22** is shown in Scheme 2.8. As shown in Scheme 2.2, it would be preferable to protect hydroxyl group A as a benzyl ether

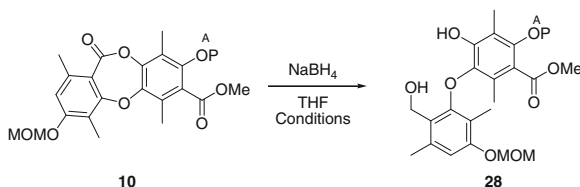


Scheme 2.8 Synthesis of key precursor **10a**

group so that it could be readily removed in the final step. In contrast, hydroxyl group **B** was protected with a MOM group, which could be easily extricated without losing the benzyl groups at a later stage to allow for the investigation of the formylation or carboxylation strategies. Following the cleavage of the methyl ether in **22** with BCl_3 , the selective protection of hydroxyl group **B** in **27** was examined. Although it was expected that hydroxyl group **A** would be less reactive than hydroxyl group **B** because it formed an intramolecular hydrogen bond to the methyl ester, the MOM protection step did not proceed in a sufficiently selective manner to give the desired mono-protected material **10b**. This lack of selectivity was attributed to the high reactivity of MOMCl , and a mixture of two mono-protected compounds was obtained even though the reaction did not reach completion. Based on this lack of selectivity, it was therefore necessary to effect the selective removal of one of the MOM ethers from the bis-MOM ether **10c** using chelating effects. Following a period of extensive investigation, it was found that the use of catalytic HI , which was generated in situ from I_2 in MeOH [14], allowed for the selective cleavage of the MOM ether at hydroxyl group **A**, which was subsequently benzylated to give key precursor **10a**.

Research into the chemoselective reduction of precursor **10** is summarized in Table 2.1. Given that a phenoxide is a better leaving group than a methoxide, it was

Table 2.1 Chemoselective reduction of lactone **10**



Entry	Substrate	P	Temp. (°C)	Time (h)	Product (yield, %)	σ_{para} of OP^{a}
1	10a	Bn	0	58	28a (52), 10a (26)	−0.42
2	10a	Bn	Rt	75	28a (<48) ^b	−0.42
3	10b	H	Rt	62	Complex mixture	−0.52 ^c
4	10c	MOM	0	94	28c (21), 10c (33)	—
5	10d	Me	Rt	44	28d (82)	−0.28
6 ^d	10e	(4-Cl)Bn	Rt	60	28e (<60) ^b , 10e (12)	—
7 ^e	10e	(4-Cl)Bn	30	103	28e (69), 10e (10)	—

^a Hammett substituent constant

^b Inseparable mixture of desired **28** and a small amount of unknown byproduct

^c Constant of O^-

^d 38 mM

^e 10 mM

envisaged that the phenyl ester could be reduced prior to the methyl ester. In an initial attempt to influence the selectivity of the reduction, the mildly reactive reducing agent NaBH_4 was used to affect the reduction of **10a** under low-temperature conditions. Although these conditions provided access to the desired **28a** in moderate yield, the reproducibility of the reaction was found to be poor (Table 2.1, entry 1). Furthermore, the reaction gave an inseparable mixture of **28a** and an unknown byproduct when it was conducted at room temperature (Table 2.1, entry 2). Several other reducing agents such as DIBAL-H were also investigated but failed to give pure **28a**. The focus of the investigation then shifted towards the nature of the protecting group for hydroxyl group **A**. Given that highly electron-donating protecting groups can lead to an increase in the electron density of the benzene ring, the more electron-rich phenol would perform poorly as a leaving group compared with the corresponding system with one less electron-donating ether (Fig. 2.3). To confirm the influence of the protecting groups, the reduction of several other substrates **10** bearing different protecting groups at hydroxyl group **A** was investigated. Although the application of the reduction conditions to the methyl esters in phenol **10b** and MOM ether **10c** gave a complex mixture or poor selectivity, respectively (Table 2.1, entries 3 and 4), methyl ether **10d** was reduced smoothly to provide the desired compound **28d** exclusively (Table 2.1, entry 5). These results for the different alkoxy groups were also found to be in agreement with their Hammett substituent parameters [15]. Considering these results, the 4-chlorobenzyl group was selected as the best protecting group for hydroxyl group **A** because it could be readily cleaved at a later stage by hydrogenolysis. Furthermore, the electron-withdrawing effect of the Cl substituent could lead to an improvement in the yield [16]. As expected, 4-chlorobenzyl ether **10e** was successfully converted to **28e** in a yield comparable with that of benzyl ether **10a** (Table 2.1, entry 6 vs. entry 2). Moreover, the formation of the inseparable unknown product was suppressed by running the reaction under dilute conditions (Table 2.1, entry 7). These dilute conditions also appeared to suppress the occurrence of unwanted intermolecular side reactions. Disappointingly, however, compound **28e** was not reproducibly obtained in a scalable reaction, and the reaction had to be carried out over several smaller batches to provide enough material to investigate the subsequent steps.

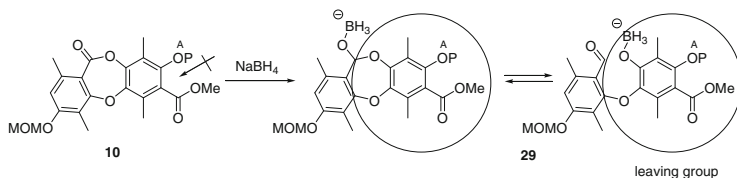
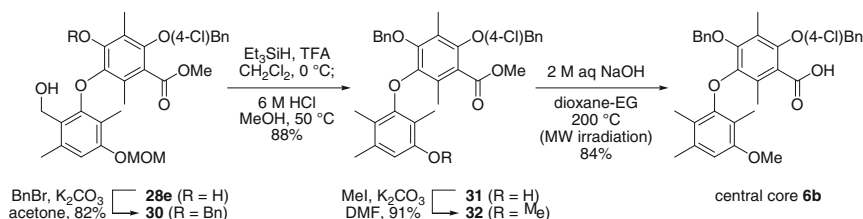


Fig. 2.3 Plausible influence of the protected phenol over the reduction of lactone **10**. The use of a stronger electron-donating protecting group (P) could lead to an increase in the electron density on the phenyl ring, which would lead to a decrease in the leaving group activity on the phenol



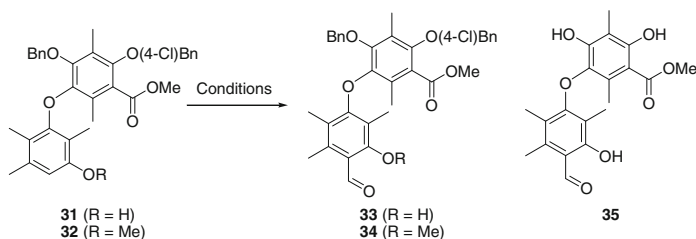
Scheme 2.9 Synthesis of central core **6b**

The central core **6b** was synthesized from **28e**, as shown in Scheme 2.9. The phenolic hydroxyl group in **28e** was selectively benzylated to afford **30**. The reduction of the benzylic alcohol with Et₃SiH [17] and removal of the MOM group under acidic conditions were performed in a one-pot reaction to give **31**. The subsequent methylation of **31** gave the corresponding methyl ether **32**. The basic hydrolysis of the methyl ester in **32** was examined, and the reaction was found to proceed smoothly under microwave irradiation conditions at 200 °C to furnish the central core **6b** in high yield [18], whereas the 2,6-disubstituents of the methyl benzoate effectively prevented the hydrolysis from occurring under conventional conditions [19, 20].

2.3.3 Introduction of the Carboxyl Group for Central Core

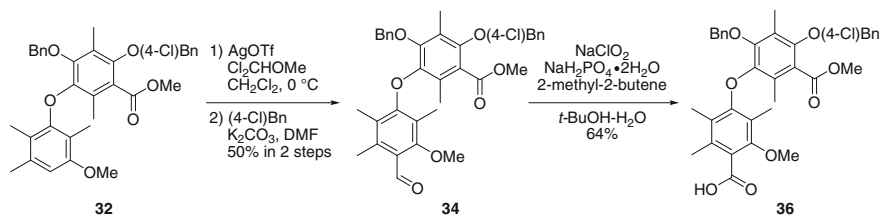
As mentioned in Sect. 2.2, the late-stage introduction of one more carboxyl group would be required at the sterically hindered position of the central core to allow it to undergo a condensation reaction with the left wing **8**. To establish a suitable method for this transformation, a preliminary investigation was conducted using compounds **31** and **32**. Considering the reactivity of the electrophile, it was envisaged that a two-step synthesis would be required for this transformation involving sequential formylation and Kraus oxidation reactions.

Several conditions were investigated for the formylation of **31** and **32**, and the results are summarized in Table 2.2. Conventional formylation methods such as the Vilsmeier-Haack reaction [21], Reimer-Tiemann reaction [22–25] and a modified version of the Duff reaction failed to provide any of the desired aldehyde product from **31**, which is the least sterically hindered of the two compounds (Table 2.2, entries 1–3). In contrast, Rieche conditions using Cl₂CHOMe–TiCl₄ [26, 27] provided aldehyde **35** in low yield (Table 2.2, entry 4). However, the use of these strongly acidic conditions also led to the debenzoylation of the product to give **35**. The use of milder Lewis acid such as BF₃·OEt₂ was investigated, but these acids could not sufficiently activate Cl₂CHOMe, and **31** was recovered quantitatively from these reactions (Table 2.2, entry 5). Given that the possibility of competition between *O*- and *C*-formylation could potentially complicate the reaction system, the

Table 2.2 Formylation of central cores **31** and **32**

Entry	Substrate	Reagents	Solv.	Temp. (°C)	Time (h)	Product (yield, %)
1	31	POCl ₃	DMF	80	7.5	N.R.
2	31	NaOH	CHCl ₃ -H ₂ O	60	24	N.R.
3	31	HMTA	TFA	50	1.5	Decomp.
4	31	Cl ₂ CHOMe	CH ₂ Cl ₂	Rt	8	35 (9)
		TiCl ₄				
5	32	Cl ₂ CHOMe	CH ₂ Cl ₂	Rt	22	N.R.
		BF ₃ ·OEt ₂				
6	32	Cl ₂ CHOMe	CH ₂ Cl ₂	0	1	34 (21)
		SnCl ₄				
7	32	Cl ₂ CHOMe	CH ₂ Cl ₂	-20	4	34 (45)
		AgOTf				
8	32	Cl ₂ CHOMe	CH ₂ Cl ₂	Rt	7.5	N.R.
		AgCl				
9	32	Cl ₂ CHOMe	CH ₂ Cl ₂	35	12	N.R.
		AgOCOCF ₃				
10	32	Cl ₂ CHOMe	CH ₂ Cl ₂	0	12	Decomp.
		AgNTf ₂				
11	32	Cl ₂ CHOMe	CH ₂ Cl ₂	Rt	4	Decomp.
		AgClO ₄				

methyl ether **32** was selected as an alternative model substrate. The formylation of **32** using Cl₂CHOMe–SnCl₄ [26, 27] gave the desired aldehyde **34** together with the debenzylated byproducts (Table 2.2, entry 6), which suggested that it would not be possible to avoid the debenylation of the product using the reported Rieche conditions with a hard Lewis acid. For this reason, the focus of the optimization work shifted towards the use of soft Lewis acids, which would have a lower affinity for the O atoms of the benzyl ethers. Following an extensive period of investigation with Ag salts, it was discovered that **32** could be successfully formylated with Cl₂CHOMe–AgOTf to afford **34** in moderate yield without losing the benzyl groups (Table 2.2, entry 7). Several other Ag salts were also tested, including AgCl,



Scheme 2.10 Scalable synthesis of acid **36**

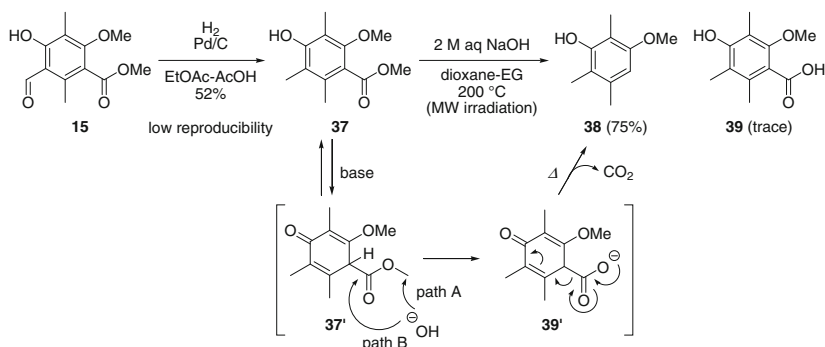
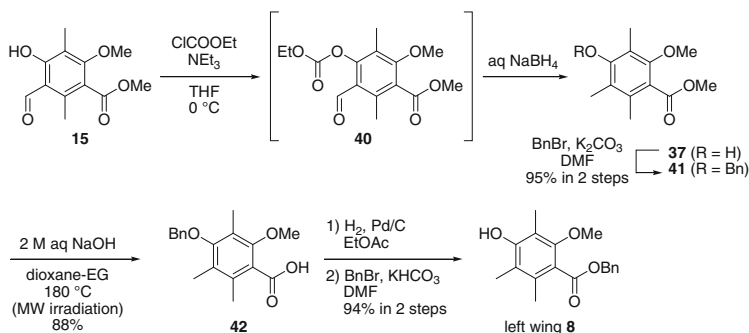
AgOCOCF_3 , AgNTf_2 and AgClO_4 , but all of these salts failed to provide any of the desired aldehyde (Table 2.2, entries 8–11) [28].

Although a new method had been successfully developed for the synthesis of **34**, the partial removal of the 4-chlorobenzyl group was observed when the reaction was conducted on a larger scale, which was attributed to the TfOH generated during the course of the reaction [29]. Given that the coexistence of bulky Lewis bases such as 2,6-di-*tert*-butylpyridine can deactivate the formylating species, the Kraus oxidation was performed to give acid **36** after the benzylation of the partially deprotected phenol with 4-chlorobenzyl chloride (Scheme 2.10). Several other C–C bond-forming reactions were also investigated for the installation of the carbonyl group, including a haloformylation reaction [30] and the rearrangement of the MOM group [31, 32], but all of these strategies failed to provide a new C–C bond at the sterically hindered position. For this reason, the AgOTf-promoted formylation followed by a Kraus oxidation was deemed to be the best route for the synthesis of **1**.

2.4 Synthesis of the Side Wings

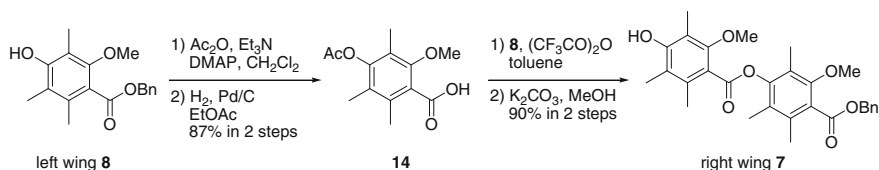
The synthesis of left wing **8**, which is also a component of the right wing **7**, was the first of the two wings to be synthesized. Hydrogenolysis of the common intermediate **15** gave **37**, although it should be mentioned that the reproducibility of this reaction was quite low. Subsequent alkaline hydrolysis under microwave irradiation conditions afforded the corresponding decarboxylated compound **38** as the major product with only a trace amount of desired product **39** being detected. The phenolic anion generated by the treatment of **37** with NaOH could readily pick up a proton on its phenyl ring prior to the hydrolysis reaction because of the steric hindrance of its *ortho*-substituents. Demethylation in a Krapcho fashion (path A) or hydrolysis at the position next to the less sterically hindered sp^3 carbon (path B) could then proceed, and further decarboxylation under heating conditions would provide **38** (Scheme 2.11).

Based on these results, the alkaline hydrolysis of the protected phenol was also examined. Benzyl ether **41** was quantitatively prepared from **15** according to the one-pot procedure for the reduction of salicylaldehyde reported by Kijima et al. [33], followed by benzylation of the resulting phenol **39**. As expected, hydrolysis of

**Scheme 2.11** Decarboxylation of **37** under heating conditions**Scheme 2.12** Synthesis of left wing **8**

the methyl ester proceeded without decarboxylation to provide acid **42**. Cleavage of the benzyl group followed by the selective benzylation of the carboxyl group afforded the desired left wing **8** (Scheme 2.12).

The route used for the synthesis of the right wing **7** from synthesized **8** is shown in Scheme 2.13. Compound **8** was converted to acid **14** by the acetylation of the phenol followed by the debenzoylation of the benzyl ester. The esterification of acid **14** with phenol **8** proceeded successfully in the presence of the condensation

**Scheme 2.13** Synthesis of right wing **7**

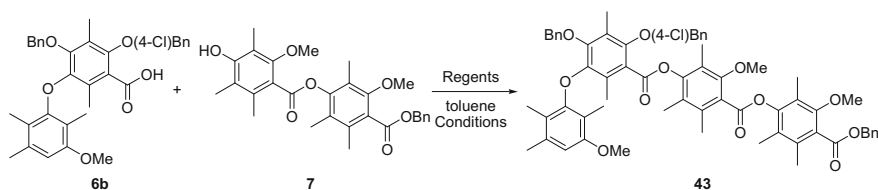
reagent $(\text{CF}_3\text{CO})_2\text{O}$ [34] despite the steric hindrance of both coupling partners. Subsequent methanolysis to remove the acetyl group furnished the desired compound **7** in high yield.

2.5 Total Synthesis of Thielocin B1

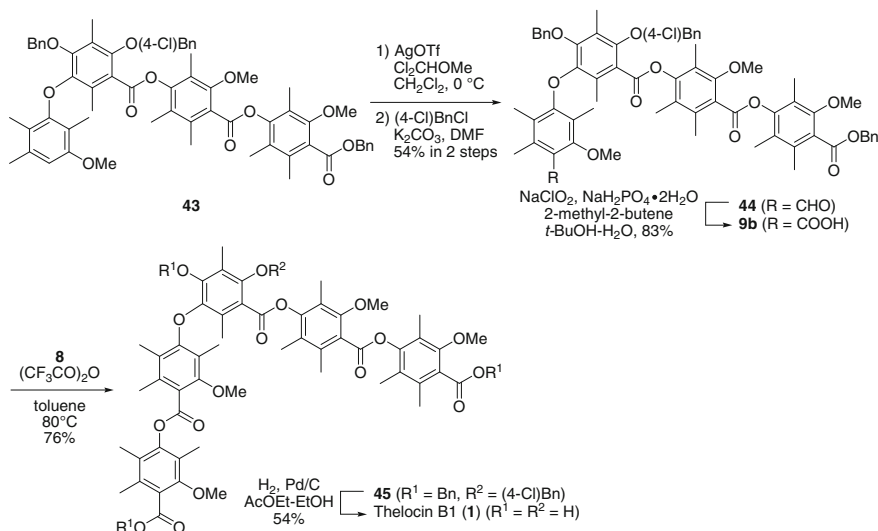
With all of the components needed for the synthesis of **1** in hand, the focus of the work shifted towards the attachment of the side wings to the central core. Several different conditions were investigated for the esterification of acid **6b** with phenol **7**, and the results are summarized in Table 2.3. Although the use of excess $(\text{CF}_3\text{CO})_2\text{O}$ at room temperature provided a poor yield of the desired product **43** (Table 2.3, entry 1), increasing the temperature to 80 °C allowed for the near quantitative formation of the desired compound (Table 2.3, entry 2). In contrast, the Corey-Nicolaou reaction [35, 36], which is also conducted under acidic conditions, failed to provide any of the desired product (Table 2.3, entry 3). The mixed anhydride containing a trifluoroacetyl group performed well as a highly active species because of its high electron-withdrawing effect and low steric hindrance.

The total synthesis of thielocin B1 (**1**) is shown in Scheme 2.14. The newly developed formylation method using Cl_2CHOMe -AgOTf was found to be particularly effective for **43**, and subsequent benzylation of the partially formed phenol with 4-chlorobenzyl chloride afforded aldehyde **44** in moderate yield. Subsequent oxidation of **44**, followed by the esterification of the resulting acid **9b** with phenol **8** in the presence of excess $(\text{CF}_3\text{CO})_2\text{O}$ at 80 °C, provided **45**. Finally, all three of the benzyl groups were removed by hydrogenolysis, thus completing the first total synthesis of thielocin B1 (**1**). A mixture of EtOH and EtOAc was identified as the best solvent for the hydrogenolysis reaction to dissolve both **45** and **1**. The spectral data for synthesized **1** were found to be in good agreement with those of the natural product.

Table 2.3 Esterification of acid **6b** with phenol **7**



Entry	Reagents	Temp. (°C)	Time (h)	Yield (%)
1	$(\text{CF}_3\text{CO})_2\text{O}$ (50 equiv)	Rt	29	28
2	$(\text{CF}_3\text{CO})_2\text{O}$ (40 equiv)	80	9	97
3	$(\text{PyS})_2$, PPh_3 , AgClO_4	80	11	N.R.



Scheme 2.14 Total synthesis of thielocin B1 (**1**)

The inhibitory activity of synthesized **1** towards PAC3 homodimer was evaluated in vitro using a protein complementation assay with monomeric Kusabira-Green fluorescent protein [2]. The IC_{50} value of the synthesized material was determined to be 40 nM, which was in good agreement with that of natural **1** (20 nM).

2.6 NMR Chemical Shift Perturbation Experiment of PAC3 with Thielocin B1

NMR chemical shift perturbation experiments were conducted with PAC3 homodimer both in the presence and absence of synthesized **1** to elucidate the characteristics of its inhibition mechanism. A solution of **1** in methanol- d_4 (10 mM) was titrated against ^{15}N -labeled PAC3 (0.2 mM for the monomer) to give final concentrations of **1** of 0.1, 0.2, 0.4 and 0.8 mM. These samples were then analyzed by ^1H - ^{15}N HSQC spectroscopy, which revealed changes in the NMR spectra of PAC3 as the concentration of **1** increased. Further increasing the concentration of **1** resulted in the formation of insoluble material, which was presumably derived from the aggregation of destabilized PAC3, and the ^1H - ^{15}N HSQC spectra of PAC3 were therefore compared in the presence (0.8 mM) and absence of **1** (Fig. 2.4a). Changes in the chemical shifts were quantified as $[(\Delta\delta_{\text{H}})^2 + (0.2\Delta\delta_{\text{N}})^2]^{1/2}$, where δ_{H} and δ_{N} are the observed chemical shift changes for ^1H and ^{15}N , respectively. As a result, notable changes were observed in the chemical shifts $[(\Delta\delta_{\text{H}})^2 + (0.2\Delta\delta_{\text{N}})^2]^{1/2} > 0.025$ ppm)

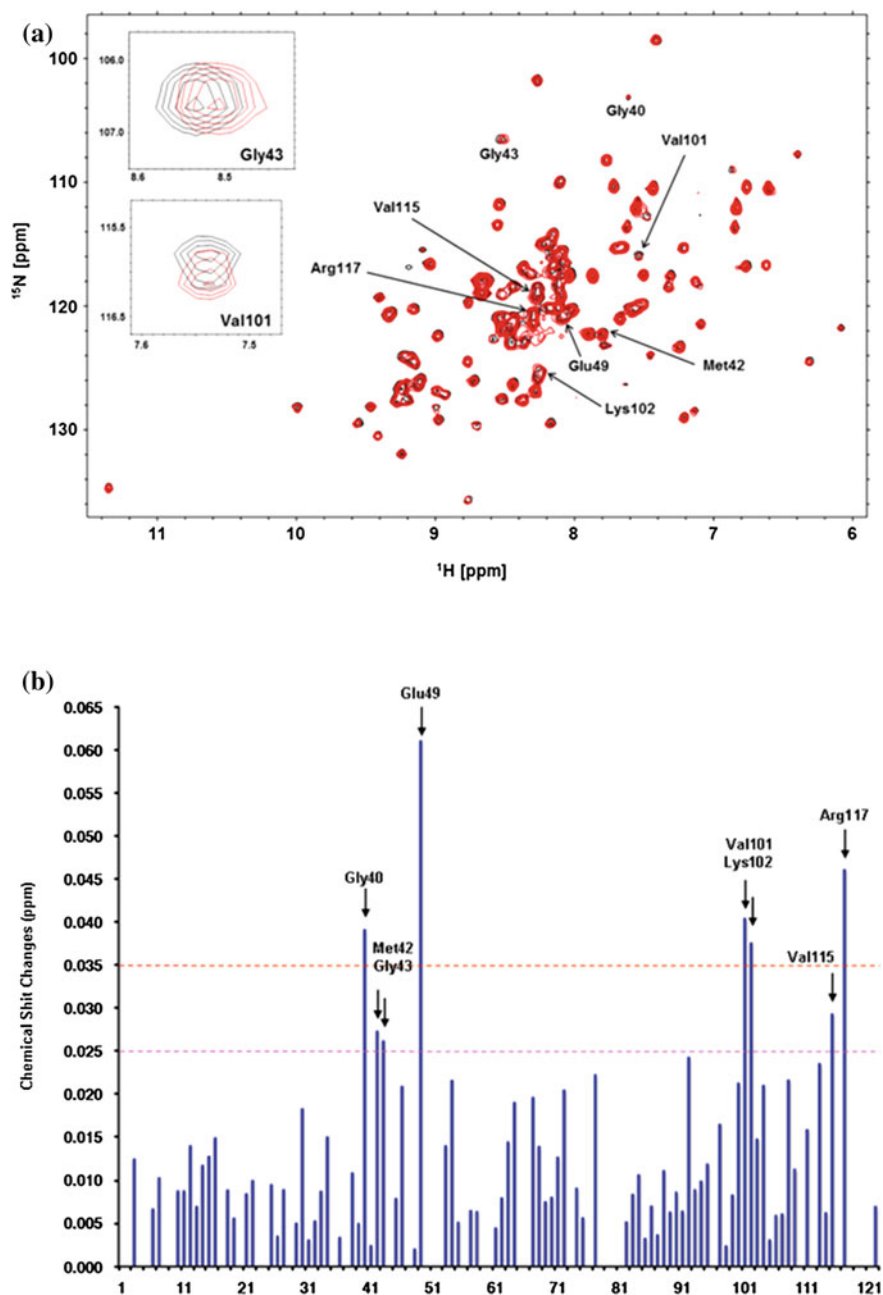
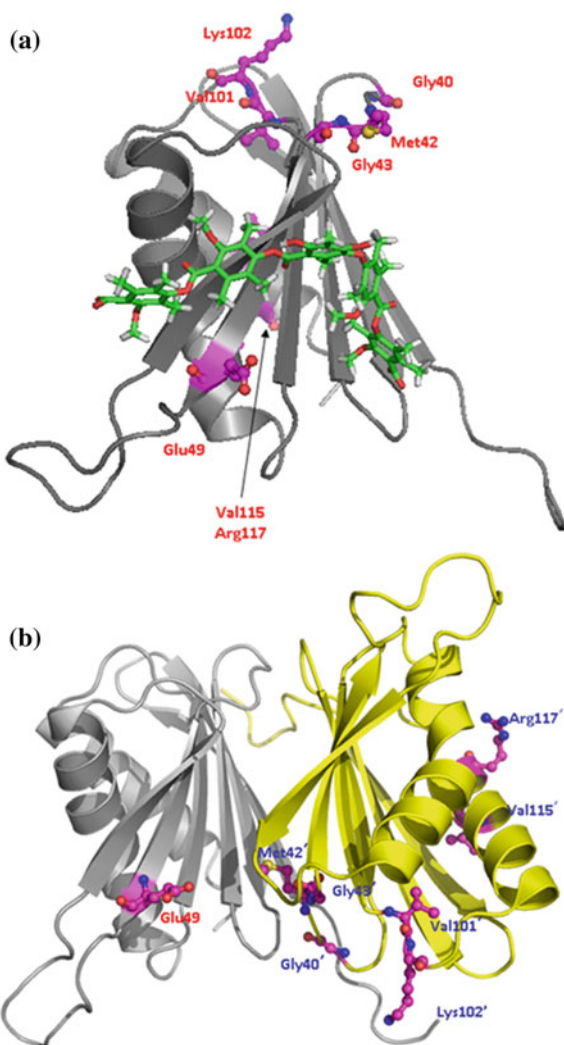


Fig. 2.4 NMR chemical shift perturbation experiment for PAC3 homodimer with **1**. Significant changes in the chemical shifts, i.e., $[(\Delta\delta_{\text{H}})^2 + (0.2\Delta\delta_{\text{N}})^2]^{1/2} > 0.025$ ppm, have been labeled. **a** ^1H - ^{15}N NSQC spectra of PAC3 homodimer both in the absence (*black*) and presence (*red*) of four molar equivalents (for the monomer) of **1**. **b** Differences in the chemical shifts according to the equation $[(\Delta\delta_{\text{H}})^2 + (0.2\Delta\delta_{\text{N}})^2]^{1/2}$

of eight amino acid residues on PAC3, including Gly40, Met42, Gly43, Glu49, Val101, Lys102, Val115 and Arg117 (Fig. 2.4b).

The interaction site of PAC3 with **1** was subsequently examined based on the results of the NMR experiments. However, it was difficult to explain the mechanism of the interaction between **1** and monomeric PAC3 (PDB code: 2Z5E_A) [3] using these data because the eight residues identified by the NMR study appeared to be scattered over the surface of monomeric PAC3 (Fig. 2.5a). In contrast, the mapping of these data onto PAC3 homodimer (PDB code: 2Z5E) [3] indicated that these residues were located in close proximity to each other at the interface of PAC3

Fig. 2.5 Mapping of the residues exhibiting significant changes in their chemical shifts on PAC3. Gly40, Met42, Gly43, Glu49, Val101, Lys102, Val115 and Arg117 are shown as carbons in magenta (ball and stick models). **a** Mapping of the residues onto monomeric PAC3 (PDB code: 2Z5E_A). **1**, located at the proposed binding site, is shown as carbons in green (stick model). **b** Mapping of the residues onto PAC3 homodimer (PDB code: 2Z5E)



homodimer (Fig. 2.5b). These results therefore suggested that the approach of **1** to PAC3 homodimer could be observed using NMR chemical shift perturbation experiments.

2.7 Docking Study for Thielocin B1 and PAC3 Homodimer

Based on the results of the NMR chemical shift perturbation experiments, an *in silico* docking study was performed for **1** and PAC3 homodimer using the Molecular Operating Environment program [37]. The results of the docking revealed that the most likely pose for **1** involved the molecule spanning the interface of PAC3 homodimer, which suggested that the PPI inhibition mechanism of **1** was as suggested in Fig. 2.6 and in conjunction with the model for the interaction of **1** with monomeric PAC3. In this way, the initial approach of **1** would bring it into close proximity to the Glu49, Gly40', Met42' and Gly43' residues, which are located on the interface of PAC3 homodimer. The insertion of the left wing of **1** into the interface would induce the dissociation of PAC3 homodimer to monomeric PAC3. Changes in the chemical shifts of the Val101' and Lys 102' residues would be affected by the movement of the nearby Gly40', Met42' and Gly43' residues. Given that Val115' and Arg117' are located at the flexible C-terminal site, they could be susceptible to structural changes on PAC3.

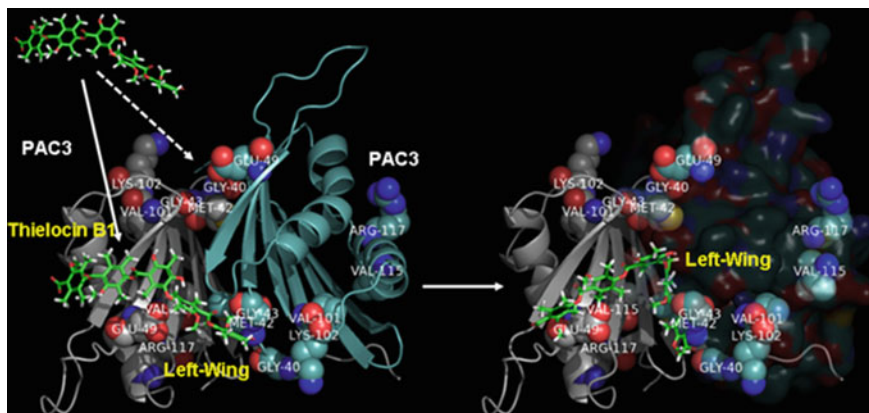


Fig. 2.6 Proposed mechanism of inhibition based on an *in silico* docking study for **1** (stick model, carbons in green)/PAC3. Residues exhibiting significant changes in their chemical shifts are shown as CPK models. The figures to the *left* and *right* show the poses of the **1**/PAC3 homodimer and **1**/monomeric PAC3 complexes, respectively

2.8 Summary

In conclusion, the first total synthesis of thielocin B1 (**1**) has been accomplished. The 2,2',6,6'-tetrasubstituted diphenyl ether moiety in **1** was synthesized from a depsidone skeleton via the chemoselective reduction of the lactone without losing the methyl ester. It was found that the protecting group of the phenolic hydroxyl group had a significant influence on the reactivity of this reduction. During the development of a synthetic strategy for elongating the side wings, an efficient reaction was discovered for the formylation of sterically hindered aromatic compounds using $\text{Cl}_2\text{CHOMe-AgOTf}$. NMR chemical shift perturbation experiments were conducted with ^{15}N -labeled PAC3 homodimer both in the presence and absence of synthesized **1**, and significant changes in the chemical shifts were observed in eight residues at the interface of PAC3 homodimer. Most of these residues were located at the interface of PAC3 homodimer, which indicated that thielocin B1 promotes the dissociation of PAC3 homodimer to monomeric PAC3 following its initial interaction with PAC3 homodimer. However, further studies will be required to validate the proposed mechanism of inhibition.

2.9 Experimental Section

2.9.1 General Techniques

All commercially available reagents were used as received. Dry THF and CH_2Cl_2 (Kanto Chemical Co.) were obtained by passing commercially available pre-dried, oxygen-free formulations.

Microwave irradiation was performed with Biotage InitiatorTM.

All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) with UV light, and visualized by *p*-anisaldehyde H_2SO_4 -ethanol solution. Column chromatography and flash column chromatography were carried out with silica gel 60 N (Kanto Chemical Co. 100–210 μm) and silica gel 60 N (Kanto Chemical Co. 40–50 μm), respectively.

^1H NMR spectra (400 MHz and 500 MHz) and ^{13}C NMR spectra (100 MHz and 125 MHz) were recorded on JEOL JNM-AL400 and JEOL ECP-500 spectrometers, respectively, in the indicated solvent. Chemical shifts (δ) are reported in units parts per million (ppm) relative to the signal for internal tetramethylsilane (0.00 ppm for ^1H) for solutions in chloroform-*d*. NMR spectral data are reported as follows: chloroform-*d* (77.0 ppm for ^{13}C), methanol-*d*₄ (3.30 ppm for ^1H and 49.0 ppm for ^{13}C) when internal standard is not indicated. Multiplicities are reported by the following abbreviations: s (singlet), d (doublet), m (multiplet), brs (broad singlet), *J* (coupling constants in Hertz).

Mass spectra and high-resolution mass spectra were measured on JEOL JMS-DX303 (for EI), MS-AX500 (for FAB), SYNAPT G2 HDMS instruments (for

ESI). IR spectra were recorded on a JASCO FTIR-8400. Only the strongest and/or structurally important absorption are reported as the IR data afforded in cm^{-1} . Melting points were measured on Round Science RFS-10, and are uncorrected.

2.9.2 Experimental Methods

Methyl 4-benzyloxy-2-hydroxy-3,6-dimethylbenzoate (**17**).

To a solution of methyl 2,4-dihydroxy-3,6-dimethylbenzoate (6.00 g, 30.6 mmol) in acetone (50 mL) were added K_2CO_3 (5.07 g, 36.7 mmol, 1.2 equiv) and BnBr (4.36 mL, 36.7 mmol, 1.2 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 22 h, the reaction mixture was filtered through a pad of Celite[®], and the filtrate was concentrated in vacuo. The resulting residue was diluted with EtOAc, and acidified with 3 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 19:1) to afford benzyl ether **17** (8.56 g, 29.8 mmol, 98 %) as a white solid. mp 79–80 °C [lit 79.5–80 °C] [6]; ^1H NMR (400 MHz, CDCl_3) δ 11.8 (1H, s), 7.31–7.44 (5H, m), 6.35 (1H, s), 5.12 (2H, s), 3.93 (3H, s), 2.51 (3H, s), 2.15 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 172.5, 162.3, 160.6, 140.0, 136.9, 128.6, 127.9, 127.0, 111.4, 107.1, 105.7, 69.9, 51.8, 24.6, 8.0; IR (Neat) 3,391, 2,926, 1,651, 1,623, 1,577, 1,453, 1,448, 1,303, 1,278, 1,131, 802 cm^{-1} ; HREIMS calcd for $\text{C}_{17}\text{H}_{18}\text{O}_4$ 286.1205, found 286.1197.

Methyl 3-formyl-4-hydroxy-6-methoxy-2,5-dimethylbenzoate (**15**).

To a solution of phenol **17** (54.0 g, 189 mmol) in DMF (200 mL) were added K_2CO_3 (78.4 g, 567 mmol, 3.0 equiv) and MeI (25.8 mL, 415 mmol, 2.2 equiv) at room temperature under an argon atmosphere. After being stirred at 50 °C for 13 h, the reaction mixture was cooled to room temperature, and filtered through a pad of Celite[®]. The filtrate was diluted with EtOAc, and acidified with 3 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo to give methyl ether as a yellowish oil. The crude methyl ether was used for next reaction without further purification.

To a solution of crude benzyl ether in EtOAc (40 mL) was added 10 % palladium on carbon (14.0 g, 26 wt%), and poured MeOH (120 mL) slowly over stirring at room temperature. The reaction vessel was purged with hydrogen 7 times. After being stirred at room temperature for 22 h, the reaction mixture was filtered through a pad of Celite[®]. The filtrate was concentrated in vacuo to give phenol as a white solid. The crude phenol was used for next reaction without further purification.

To a solution of crude phenol in TFA (200 mL) was added HMTA (29.1 g, 208 mmol, 1.1 equiv) at room temperature under an argon atmosphere. After being stirred at reflux for 2 h, the reaction mixture was cooled to room temperature, and concentrated in vacuo. To the resulting residue was poured water (200 mL) at room temperature. After being stirred at reflux for 16 h, the reaction mixture was cooled to room temperature, diluted with EtOAc. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were basified with 2 M aq NaOH. The organic layer was separated, washed with brine, dried with MgSO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was recrystallized from CH₂Cl₂/hexane to afford aldehyde **15** (28.5 g, 120 mmol, 63 % in 3 steps) as a white solid. mp 84–85 °C [lit 83–84 °C] [6]; ¹H NMR (400 MHz, CDCl₃) δ 12.7 (1H, s), 10.2 (1H, s), 3.92 (3H, s), 3.82 (3H, s), 2.49 (3H, s), 2.14 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 194.1, 168.0, 164.5, 162.2, 137.8, 122.0, 117.8, 114.6, 61.7, 52.4, 14.6, 8.2; IR (Neat) 3,447, 2,949, 1,734, 1,634, 1,581, 1,458, 1,305, 1,274, 1,210, 903, 791 cm⁻¹; HREIMS calcd for C₁₂H₁₄O₅ 238.0841, found 238.0842.

2-Benzylloxy-5-methoxycarbonyl-4-methoxy-3,6-dimethylbenzoic acid (**13**).

To a solution of phenol **15** (22.5 g, 94.4 mmol) in DMF (150 mL) were added K₂CO₃ (45.6 g, 330 mmol, 3.5 equiv) and BnBr (14.6 mL, 123 mmol, 1.3 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 13 h, the reaction mixture was filtered through a pad of Celite®. The filtrate was diluted with EtOAc, and acidified with 3 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aq NaHCO₃ and brine, dried with MgSO₄, and filtered. The filtrate was concentrated in vacuo to give benzyl ether as a colorless oil. The crude benzyl ether was used for next reaction without further purification.

To a suspension of crude aldehyde in *t*-BuOH (75 mL) and water (75 mL) were added 2-methyl-2-butene (50 mL), NaClO₂ (25.6 g, 283 mmol, 3.0 equiv) and NaH₂PO₄·2H₂O (44.2 g, 283 mmol, 3.0 equiv) at 0 °C. After being stirred at room temperature for 1.5 h, the organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with MgSO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 2:1). Recrystallization from CH₂Cl₂/hexane afforded carboxylic acid **13** (23.7 g, 68.8 mmol, 73 % in 2 steps) as a white solid. mp 96–97 °C [lit. 103–105 °C] [6]; ¹H NMR (400 MHz, CDCl₃) δ 7.31–7.44 (5H, m), 4.94 (2H, s), 3.95 (3H, s), 3.80 (3H, s), 2.33 (3H, s), 2.23 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 171.8, 168.2, 157.9, 156.5, 136.3, 132.6, 128.5, 128.3, 128.2, 126.0, 124.3, 123.5, 76.7, 61.8, 52.5, 16.9, 9.7; IR (Neat) 3,320, 2,950, 1,731, 1,580, 1,455, 1,312, 1,250, 1,104, 699 cm⁻¹; HREIMS calcd for C₁₉H₂₀O₆ 344.1260, found 344.1253.

2,5-Dimethylresorcinol (20).

To a solution of methyl 2,4-dihydroxy-3,6-dimethylbenzoate (10.0 g, 51.0 mmol) in ethylene glycol (50 mL) was added KOH (85 %, 234 mmol, 4.6 equiv) in water (50 mL) at 0 °C. After being stirred at 120 °C for 1 h, the reaction mixture was cooled to room temperature, diluted with EtOAc, and quenched with 6 M aq HCl at 0 °C. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated aq NaHCO₃ and brine, dried with MgSO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was recrystallized from EtOAc/hexane to afford diol **20** (6.40 g, 46.3 mmol, 91 %) as a white solid. mp 165–166 °C [lit. 161 °C] [38]; ¹H NMR (400 MHz, CDCl₃) δ 6.23 (2H, s), 4.59 (2H, s), 2.21 (3H, s), 2.10 (3H, s); ¹³C NMR (100 MHz, CD₃OD) δ 157.1, 136.9, 109.0, 108.3, 27.3, 8.2; IR (Neat) 3,245, 1,630, 1,584, 1,521, 1,454, 1,416, 1,175, 1,075 cm⁻¹; HREIMS calcd for C₈H₁₀O₂ 138.0681, found 138.0711.

1,3-Dibenzyloxy-2,5-dimethylbenzene (12).

To a solution of diol **20** (5.00 g, 36.2 mmol) in DMF (100 mL) were added K₂CO₃ (22.5 g, 163 mmol, 4.5 equiv) and BnBr (9.47 mL, 79.6 mmol, 2.2 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 21 h, the reaction mixture was filtered through a pad of Celite®. The filtrate was diluted with EtOAc, and acidified with 3 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aq NaHCO₃ and brine, dried with MgSO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was recrystallized from CH₂Cl₂/hexane to afford benzyl ether **12** (8.88 g, 28.6 mmol, 77 %) as a white needle. mp 92–93 °C [lit. 91 °C] [39]; ¹H NMR (400 MHz, CDCl₃) δ 7.28–7.46 (10H, m), 6.45 (2H, s), 5.06 (4H, s), 2.31 (3H, s), 2.19 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 157.5, 137.7, 136.2, 128.5, 127.7, 127.1, 112.5, 106.2, 70.3, 21.9, 8.4; IR (Neat) 2,920, 1,589, 1,453, 1,126, 695 cm⁻¹; HREIMS calcd for C₂₂H₂₂O₂ 318.1620, found 318.1634.

Methyl 4-benzyloxy-3-(2,4-dibenzyloxy-3,6-dimethylbenzoyl)-6-methoxy-2,5-dimethylbenzoate (21).

To a solution of carboxylic acid **13** (3.90 g, 11.3 mmol) in dry CH₂Cl₂ (50 mL) were added benzyl ether **12** (7.20 g, 22.6 mmol, 2.0 equiv) and (CF₃CO)₂O (7.86 mL, 56.5 mmol, 5.0 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 4.5 h, the reaction mixture was diluted with EtOAc, and quenched with 2 M aq NaOH. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with MgSO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 6:1) to afford benzophenone **21** (5.17 g, 8.03 mmol, 71 %) as a yellowish solid. Benzyl ether **12** (4.04 g, 12.7 mmol, 56 %) was recovered as a yellowish solid. mp 140–141 °C [lit. 109–112 °C] [6]; ¹H NMR (400 MHz, CDCl₃) δ 7.24–7.45 (11H, m), 7.14–7.19 (4H, m), 6.51 (1H, s), 5.06 (2H, s), 4.68 (2H, s), 4.62 (2H, s), 3.87 (3H, s), 3.71 (3H, s), 2.31 (3H, s), 2.11

(3H, s), 2.09 (3H, s), 2.00 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 197.2, 168.5, 159.4, 157.5, 157.1, 157.0, 137.7, 137.3, 137.2, 136.8, 134.2, 132.6, 128.6, 128.5, 128.09, 128.05, 127.9, 127.5, 127.3, 127.1, 127.0, 126.6, 126.2, 123.0, 117.9, 110.6, 75.7, 70.0, 61.7, 52.1, 21.2, 16.8, 9.6, 9.4; IR (Neat) 2,947, 1,733, 1,654, 1,594, 1,569, 1,454, 1,314, 1,176, 1,125, 735, 697 cm^{-1} ; HREIMS calcd for $\text{C}_{41}\text{H}_{40}\text{O}_7$ 644.2774, found 644.2767.

Methyl 3-(2,4-dihydroxy-3,6-dimethylbenoyl)-4-hydroxy-6-methoxy-2,5-dimethylbenzoate (11).

To a solution of benzyl ester **21** (7.00 g, 10.9 mmol) in EtOAc (120 mL) was added 5 % palladium on carbon (3.50 g, 50 wt%) at room temperature, and the flask was purged with hydrogen 7 times. After being stirred at the same temperature for 18 h, the reaction mixture was filtered through a pad of Celite[®]. The filtrate was concentrated in vacuo, and the resulting residue was recrystallized from CH_2Cl_2 /hexane to afford phenol **11** (3.78 g, 10.1 mmol, 93 %) as a yellowish solid. mp 157–158 °C [lit. 164–165 °C] [6]; ^1H NMR (400 MHz, CDCl_3) δ 11.8 (1H, s), 7.79 (1H, s), 6.17 (1H, s), 5.30 (1H, s), 3.88 (3H, s), 3.80 (3H, s), 2.19 (3H, s), 2.14 (3H, s), 1.90 (3H, s), 1.80 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 202.5, 170.3, 166.4, 163.4, 158.5, 154.8, 141.5, 131.0, 129.6, 124.1, 118.3, 116.0, 112.3, 110.5, 62.5, 52.6, 22.5, 16.3, 9.2, 7.7; IR (Neat) 3,419, 2,969, 1,699, 1,602, 1,569, 1,267, 746 cm^{-1} ; HREIMS calcd for $\text{C}_{20}\text{H}_{22}\text{O}_7$ 374.1366, found 374.1366.

Methyl 3-hydroxy-8-methoxy-1,4,6,9-tetramethyl-11-oxo-11H-dibenzo[*b,e*]-[1,4]dioxepin-7-carboxylate (22).

To a suspension of benzophenone **11** (3.80 g, 10.2 mmol) in water (200 mL) were added K_2CO_3 (21.1 g, 153 mmol, 15 equiv) and $\text{K}_3[\text{Fe}(\text{CN})_6]$ (7.69 g, 23.3 mmol, 2.3 equiv) at room temperature. After being stirred at the same temperature for 4 h, the reaction mixture was diluted with EtOAc, and quenched with 6 M aq HCl at 0 °C. The organic layer was separated, and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was recrystallized from CH_2Cl_2 /hexane to afford depsi-done **22** (3.20 g, 8.59 mmol, 84 %) as an orange solid. mp 227–228 °C [lit. 220–221 °C] [6]; ^1H NMR (400 MHz, CDCl_3) δ 6.51 (1H, s), 5.42 (1H, s), 3.92 (3H, s), 3.74 (3H, s), 2.43 (3H, s), 2.37 (3H, s), 2.34 (3H, s), 2.28 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 169.1, 164.8, 162.5, 161.9, 153.9, 147.6, 146.0, 143.4, 127.7, 126.5, 123.0, 115.7, 114.5, 112.9, 62.6, 52.9, 21.2, 14.4, 10.0, 9.8; IR (Neat) 3,330, 2,930, 1,729, 1,699, 1,611, 1,590, 1,459, 1,413, 1,343, 1,272, 1,135 cm^{-1} ; HREIMS calcd for $\text{C}_{20}\text{H}_{20}\text{O}_7$ 372.1209, found 372.1208.

Methyl 3,8-di(methoxymethoxy)-1,4,6,9-tetramethyl-11-oxo-11H-dibenzo-[*b,e*]-[1,4]dioxepine-7-carboxylate (10c).

To a suspension of methyl ether **22** (3.50 g, 9.40 mmol) in dry CH_2Cl_2 (30 mL) was added BCl_3 (1.0 M in CH_2Cl_2 , 28.2 mL, 28.2 mmol, 3.0 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 4.5 h, the reaction mixture was diluted with EtOAc, and quenched with water at 0 °C. The organic

layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated aq NaHCO₃ and brine, dried with MgSO₄, and filtered. The filtrate was concentrated in vacuo to give phenol as a yellowish solid. The crude phenol was used for next reaction without further purification.

To a solution of crude diol in DMF (40 mL) were added DIPEA (16.4 mL, 94.0 mmol, 10 equiv) and MOMCl (3.57 mL, 47.0 mmol, 5.0 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 1 h, the reaction mixture was diluted with EtOAc, and quenched with 3 M aq HCl. The organic layer was separated and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aq NaHCO₃ and brine, dried with MgSO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 3:1) to afford MOM ether **10c** (3.58 g, 8.01 mmol, 85 % in 2 steps) as a white solid.

mp 118–119 °C; ¹H NMR (400 MHz, CDCl₃) δ 6.80 (1H, s), 5.23 (2H, s), 4.93 (2H, s), 3.90 (3H, s), 3.53 (3H, s), 3.47 (3H, s), 2.47 (3H, s), 2.39 (3H, s), 2.33 (3H, s), 2.30 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 167.5, 162.9, 160.4, 158.9, 150.1, 146.5, 144.7, 142.1, 126.5, 125.6, 122.5, 115.8, 114.7, 113.1, 100.5, 94.2, 57.5, 56.3, 52.3, 21.3, 14.5, 10.5, 10.0; IR (Neat) 2,953, 1,735, 1,730, 1,607, 1,566, 1,458, 1,342, 1,281, 1,127, 977, 940, 759 cm⁻¹; HREIMS calcd for C₂₃H₂₆O₉ 446.1577, found 446.1559.

General procedure: Synthesis of benzyl ethers **10a** and **10e**.

To a suspension of the MOM ether **10c** in MeOH (10.0 mL/mmol) was added I₂ (1 wt/vol.% I₂/MeOH) at room temperature. After being stirred at 45 °C for 16 h, the reaction mixture was cooled to room temperature, diluted with EtOAc, and quenched with saturated aq Na₂S₂O₃. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated aq NaHCO₃ and brine, dried with MgSO₄, and filtered. The filtrate was concentrated in vacuo to give phenol as a white solid. The crude phenol was used for next reaction without further purification.

To a solution of the crude phenol in DMF were added K₂CO₃ and benzyl halide at room temperature under an argon atmosphere. After being stirred at the same temperature, the reaction mixture was filtered through a pad of Celite®. The filtrate was diluted with EtOAc, and acidified with 3 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aq NaHCO₃ and brine, dried with MgSO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified to afford benzyl ethers **10a** and **10e**.

Methyl 8-benzyloxy-3-methoxymethoxy-1,4,6,9-tetramethyl-11-oxo-11H-dibenzo[b,e][1, 4]dioxepine-7-carboxylate (10a).

Conditions: (1) **10c** (150 mg, 336 μmol), I_2 (33.6 mg), MeOH (3.4 mL), 16 h. (2) BnBr (61.1 μL , 504 μmol , 1.5 equiv), K_2CO_3 (149 mg, 1.08 mmol, 3.2 equiv), DMF (2.0 mL), 10 h. Purification: flash column chromatography on silica gel (eluted with hexane/EtOAc = 4/1). Yield: benzyl ether **10a** (134 mg, 272 μmol , 81 % in 2 steps) as a white solid. mp 151–152 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.31–7.41 (5H, m), 6.81 (1H, s), 5.23 (2H, s), 4.85 (2H, s), 3.82 (3H, s), 3.48 (3H, s), 2.48 (3H, s), 2.40 (3H, s), 2.35 (3H, s), 2.31 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 167.7, 163.0, 160.5, 158.9, 151.3, 146.4, 144.7, 142.1, 136.7, 128.5, 128.2, 127.9, 126.5, 125.6, 122.3, 115.8, 114.7, 113.1, 94.2, 76.6, 56.3, 52.4, 21.4, 14.4, 10.2, 10.0; IR (Neat) 2,952, 1,733, 1,606, 1,566, 1,455, 1,341, 1,281, 1,204, 1,130, 1,063, 979 cm^{-1} ; HREIMS calcd for $\text{C}_{28}\text{H}_{28}\text{O}_8$ 492.1784, found 492.1793.

Methyl 8-(4-chlorobenzyloxy)-3-methoxymethoxy-1,4,6,9-tetramethyl-11-oxo-11H-dibenzo[b,e][1, 4]dioxepine-7-carboxylate (10e).

Conditions: (1) **10c** (3.20 g, 717 mmol), I_2 (717 mg), MeOH (72 mL), 16 h. (2) (4-Cl)BnCl (2.30 g, 14.3 mmol, 2.0 equiv), K_2CO_3 (4.95 g, 35.9 mmol, 5.0 equiv), DMF (70 mL), 11 h. Purification: recrystallization from CH_2Cl_2 /hexane after column chromatography on silica gel (eluted with hexane/EtOAc = 9/1). Yield: benzyl ether **10e** (2.40 g, 4.55 mmol, 63 % in 2 steps) as a white solid. mp 129–130 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.35 (2H, d, J = 8.8 Hz), 7.32 (2H, d, J = 8.8 Hz), 6.81 (1H, s), 5.23 (2H, s), 4.82 (2H, s), 3.80 (3H, s), 3.47 (3H, s), 2.48 (3H, s), 2.39 (3H, s), 2.34 (3H, s), 2.29 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 167.6, 162.8, 160.4, 158.9, 151.1, 146.4, 144.7, 142.1, 135.2, 134.0, 129.1, 128.6, 126.4, 125.6, 122.2, 115.8, 114.6, 113.1, 94.1, 75.6, 56.3, 52.4, 21.3, 14.4, 10.2, 10.0; IR (Neat) 2,952, 1,735, 1,606, 1,454, 1,342, 1,282, 1,204, 1,130, 1,063, 980, 759 cm^{-1} ; HREIMS calcd for $\text{C}_{28}\text{H}_{27}\text{ClO}_8$ 526.1394, found 526.1380.

Methyl 8-methoxy-3-methoxymethoxy-1,4,6,9-tetramethyl-11-oxo-11H-dibenzo[b,e][1, 4]dioxepine-7-carboxylate (10d).

To a solution of phenol **22** (60 mg, 161 μmol) in DMF (1.0 mL) were added DIPEA (84.1 μL , 483 μmol , 3.0 equiv) and MOMCl (20.2 μL , 242 μmol , 1.5 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 1.5 h, the reaction mixture was diluted with EtOAc, and quenched with 1 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 3:1) to afford MOM ether **10d** (52.3 mg, 126 μmol , 78 %) as a white solid. mp 142–143 °C; ^1H NMR (400 MHz, CDCl_3) δ 6.80 (1H, s), 5.23 (2H, s), 3.92 (3H, s), 3.74 (3H, s), 3.47 (3H, s), 2.48 (3H, s), 2.38 (3H, s), 2.34 (3H, s), 2.28 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 167.7, 162.9, 160.5, 158.9, 152.5, 146.2, 144.7, 142.1, 126.1, 125.4, 122.0, 115.8, 114.7, 113.1, 94.2, 62.1, 56.3, 52.4, 21.3, 14.3, 10.0, 9.9; IR (Neat)

2,931, 1,735, 1,606, 1,566, 1,459, 1,414, 1,344, 1,282, 1,129, 1,063 cm^{-1} ; HRE-IMS calcd for $\text{C}_{22}\text{H}_{24}\text{O}_8$ 416.1471, found 416.1480.

General procedure: Synthesis of benzyl alcohols 28.

To a solution of depsidones **10** in dry THF was added NaBH_4 at 0 °C under an argon atmosphere. After being stirred at the optimal temperature, the reaction mixture was diluted with EtOAc, and quenched with 3 M aq HCl at 0 °C. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified to afford benzyl alcohols **28**.

Methyl 2-benzyloxy-4-hydroxy-5-(2-hydroxymethyl-5-methoxymethoxy-3,6-dimethylphenoxy)-3,6-dimethylbenzoate (28a).

Conditions: **10a** (40.0 mg, 81.2 μmol), NaBH_4 (77.4 mg, 2.03 mmol, 25 equiv), dry THF (1.0 mL), 0 °C, 74 h. Purification: column chromatography on silica gel (eluted with hexane/EtOAc = 3/1). Yield: benzyl alcohol **28a** (20.6 mg, 41.4 μmol , 52 %) as a white solid, depsidone **10a** (10.2 mg, 20.7 μmol , 26 % recovered). mp 147–148 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.30–7.43 (5H, m), 6.74 (1H, s), 5.19 (1H, d, J = 6.8 Hz), 5.13 (1H, d, J = 6.8 Hz, g), 5.04 (1H, d, J = 10.6 Hz), 4.90 (1H, d, J = 11.2 Hz), 4.85 (1H, d, J = 11.2 Hz), 4.72 (1H, d, J = 10.6 Hz), 3.81 (3H, s), 3.47 (3H, s), 2.40 (3H, s), 2.18 (3H, s), 2.13 (3H, s), 1.80 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 168.6, 156.2, 155.3, 150.6, 147.6, 140.7, 137.3, 136.1, 128.4, 128.0, 127.9, 124.4, 122.0, 121.5, 118.9, 115.7, 111.8, 94.7, 76.4, 57.3, 56.1, 52.1, 19.3, 13.8, 9.4, 8.9; IR (Neat) 3,423, 2,953, 2,926, 1,728, 1,611, 1,585, 1,456, 1,287, 1,112, 1,056 cm^{-1} ; HRESITOFMS calcd for $\text{C}_{28}\text{H}_{32}\text{O}_8\text{Na}$ $[\text{M}+\text{Na}]^+$ 519.1995, found 519.1993.

Methyl 4-hydroxy-3-(2-hydroxymethyl-5-methoxymethoxy-3,6-dimethylphenoxy)-6-methoxy-methoxy-2,5-dimethylbenzoate (28c).

Conditions: **10c** (30.0 mg, 66.6 μmol), NaBH_4 (37.8 mg, 999 μmol , 15 equiv), dry THF (1.0 mL), 0 °C, 94 h. Purification: flash column chromatography on silica gel (eluted with hexane/EtOAc = 4/1). Yield: benzyl alcohol **28c** (6.2 mg, 13.7 μmol , 21 %) as a white solid, and depsidone **10c** (9.9 mg, 22.2 μmol , 33 % recovered). mp 156–157 °C; ^1H NMR (400 MHz, CDCl_3) δ 6.71 (1H, s), 5.17 (1H, d, J = 6.8 Hz), 5.11 (1H, d, J = 6.8 Hz), 5.00 (1H, d, J = 10.8 Hz), 4.94 (2H, s), 4.68 (1H, d, J = 10.8 Hz), 3.88 (3H, s), 3.53 (3H, s), 3.45 (3H, s), 2.38 (3H, s), 2.14 (3H, s), 2.12 (3H, s), 1.75 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 168.5, 156.1, 155.2, 149.3, 147.6, 140.8, 136.0, 124.4, 121.9, 121.4, 119.0, 115.7, 111.7, 100.4, 94.6, 57.5, 57.2, 56.1, 52.1, 19.3, 13.9, 9.7, 8.9; IR (Neat) 3,424, 2,953, 2,929, 1,728, 1,612, 1,584, 1,462, 1,287, 1,209, 1,156, 1,113, 1,089, 1,057, 971, 755 cm^{-1} ; HRESITOFMS calcd for $\text{C}_{23}\text{H}_{30}\text{O}_9\text{Na}$ $[\text{M}+\text{Na}]^+$ 473.1788, found 473.1786.

Methyl 4-hydroxy-3-(2-hydroxymethyl-5-methoxymethoxy-3,6-dimethylphenoxy)-6-methoxy-2,5-dimethylbenzoate (28d).

Conditions: **10d** (8.0 mg, 19.2 μmol), NaBH_4 (20.0 mg, 529 μmol , 28 equiv), dry THF (1.0 mL), rt, 44 h. Purification: flash column chromatography on silica gel

(eluted with hexane/EtOAc = 2/1). Yield: benzyl alcohol **28d** (6.6 mg, 15.7 μmol , 82 %) as a white solid. mp 145–146 $^{\circ}\text{C}$; ^1H NMR (400 MHz, CDCl_3) δ 6.73 (1H, s), 5.18 (1H, d, J = 6.4 Hz), 5.13 (1H, d, J = 6.4 Hz), 5.01 (1H, d, J = 10.8 Hz), 4.70 (1H, J = 10.8 Hz, d), 3.90 (3H, s), 3.74 (3H, s), 3.46 (3H, s), 2.40 (3H, s), 2.14 (3H, s), 2.11 (3H, s), 1.79 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 168.6, 156.1, 155.2, 152.0, 147.5, 140.5, 136.1, 124.2, 121.9, 121.1, 118.5, 115.8, 111.7, 94.6, 62.1, 57.4, 56.1, 52.1, 19.4, 13.7, 9.1, 8.9; IR (Neat) 3,417, 2,925, 1,729, 1,611, 1,464, 1,416, 1,288, 1,207, 1,112, 1,089, 1,058 cm^{-1} ; HRESITOFMS calcd for $\text{C}_{22}\text{H}_{28}\text{O}_8\text{Na}$ $[\text{M}+\text{Na}]^+$ 443.1682, found 443.1689.

Methyl 2-(4-chlorobenzoyloxy)-4-hydroxy-5-(2-hydroxymethyl-5-methoxy-methoxy-3,6-dimethyl-phenoxy)-3,6-dimethylbenzoate (28e).

Conditions: **10e** (680 mg, 1.29 mmol), NaBH_4 (1.95 g, 51.6 mmol, 40 equiv), dry THF (103 mL), 30 $^{\circ}\text{C}$, 103 h. Purification: Recrystallization from CH_2Cl_2 /hexane after flash column chromatography on silica gel (eluted with hexane/EtOAc = 4/1). Yield: benzyl alcohol **28e** (474 mg, 89.2 μmol , 69 %) as a white solid, and desidone **10e** (64.4 mg, 122 μmol , 10 % recovered). mp 159–160 $^{\circ}\text{C}$; ^1H NMR (400 MHz, CDCl_3) δ 7.34 (4H, s), 6.71 (1H, s), 5.17 (1H, d, J = 6.8 Hz), 5.12 (1H, d, J = 6.8 Hz), 5.00 (1H, d, J = 10.8 Hz), 4.85 (1H, d, J = 10.8 Hz), 4.81 (1H, d, J = 10.8 Hz), 4.67 (1H, d, J = 10.8 Hz), 3.78 (3H, s), 3.45 (3H, s), 2.37 (3H, s), 2.13 (3H, s), 2.09 (3H, s), 1.76 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 168.6, 156.1, 155.2, 150.3, 147.7, 140.8, 136.1, 135.7, 133.7, 129.2, 128.5, 124.4, 121.9, 121.3, 118.7, 115.6, 111.7, 94.6, 75.5, 57.2, 56.1, 52.1, 19.3, 13.8, 9.4, 8.9; IR (Neat) 3,422, 2,952, 2,927, 1,726, 1,611, 1,586, 1,492, 1,460, 1,289, 1,209, 1,113, 1,089, 1,057, 980, 755 cm^{-1} ; HRESITOFMS calcd for $\text{C}_{28}\text{H}_{31}\text{ClO}_8\text{Na}$ $[\text{M}+\text{Na}]^+$ 553.1605, found 553.1606.

Methyl 4-benzyloxy-2-(4-chlorobenzoyloxy)-5-(2-hydroxymethyl-5-methoxy-methoxy-3,6-dimethyl-phenoxy)-3,6-dimethylbenzoate (30).

To a solution of phenol **28e** (530 mg, 99.8 μmol) in acetone (4.0 mL) were added K_2CO_3 (552 mg, 3.99 mmol, 4.0 equiv) and BnBr (154 μL , 1.30 mmol, 1.3 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 5 h, the reaction mixture was diluted with EtOAc, and quenched with 1 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford benzyl ether **30** (572 mg, 92.1 μmol , 92 %) as a white solid. mp 108–109 $^{\circ}\text{C}$; ^1H NMR (400 MHz, CDCl_3) δ 7.35 (4H, s), 7.24–7.27 (3H, m), 7.01–7.04 (2H, m), 6.69 (1H, s), 5.17 (1H, d, J = 7.0 Hz), 5.14 (1H, d, J = 7.0 Hz), 4.86 (2H, s), 4.68 (1H, d, J = 11.6 Hz, l), 4.55 (1H, d, J = 7.0 Hz), 4.52 (1H, d, J = 7.0 Hz), 4.12 (1H, d, J = 11.6 Hz), 3.84 (3H, s), 3.45 (3H, s), 2.26 (3H, s), 2.24 (3H, s), 2.14 (3H, s), 1.98 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 168.2, 155.7, 155.4, 149.4, 148.4, 146.1, 137.0, 136.2, 135.6, 133.9, 129.1, 128.6, 128.2, 127.9, 127.3, 126.4, 124.9, 124.8, 124.2, 115.4, 111.8, 94.6, 75.5, 74.8, 57.1, 56.0, 52.3, 19.1, 13.9, 10.4, 9.5; IR (Neat) 3,522, 2,952, 1,731, 1,610, 1,580, 1,492,

1,444, 1,367, 1,337, 1,284, 1,207, 1,115, 1,091, 1,059, 983, 753 cm^{-1} ; HREIMS calcd for $\text{C}_{35}\text{H}_{37}\text{ClO}_8$ 620.2177, found 620.2150.

Methyl 4-benzyloxy-2-(4-chlorobenzyloxy)-5-(3-hydroxy-2,5,6-trimethyl-phenoxy)-3,6-dimethyl-benzoate (31).

To a solution of benzyl alcohol **30** (460 mg, 741 μmol) in dry CH_2Cl_2 (7.6 mL) were added TFA (0.4 mL) and Et_3SiH (2.32 mL, 14.8 mmol, 20 equiv) at -30°C under an argon atmosphere, and the mixture was stirred at 0°C for 30 min. To the reaction mixture were added methanol (8.0 mL) and 6 M aq HCl (1.6 mL) at 0°C . After being stirred at 50°C for 19 h, the reaction mixture was cooled to room temperature, diluted with EtOAc, and quenched with saturated aq NaHCO_3 . The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 5:1) to afford phenol **31** (367 mg, 654 μmol , 88 %) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 7.34 (4H, s), 7.25–7.28 (3H, m), 7.06–7.09 (2H, m), 6.36 (1H, s), 4.85 (2H, s), 4.67 (1H, brs), 4.58 (1H, d, $J = 11.2$ Hz), 4.49 (1H, d, $J = 11.2$ Hz), 3.82 (3H, s), 2.14 (3H, s), 2.10 (3H, s), 2.08 (3H, s), 1.98 (3H, s), 1.93 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 168.6, 155.0, 151.9, 149.1, 148.9, 146.2, 137.0, 135.7, 135.5, 133.8, 129.1, 128.6, 128.1, 127.7, 127.3, 125.9, 124.6, 124.4, 119.4, 112.3, 111.8, 75.4, 74.5, 52.3, 19.9, 13.9, 12.5, 10.2, 9.4; IR (Neat) 3,443, 2,950, 2,925, 1,729, 1,598, 1,493, 1,454, 1,407, 1,367, 1,285, 1,212, 1,099, 755 cm^{-1} ; HREIMS calcd for $\text{C}_{33}\text{H}_{33}\text{ClO}_6$ 560.1966, found 560.1942.

Methyl 4-benzyloxy-2-(4-chlorobenzyloxy)-5-(3-methoxy-2,5,6-trimethyl-phenoxy)-3,6-dimethyl-benzoate (32).

To a solution of phenol **31** (500 mg, 891 μmol) in DMF (4.0 mL) were added K_2CO_3 (690 mg, 4.99 mmol, 5.6 equiv) and MeI (128 μL , 2.05 mmol, 2.3 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 8 h, the reaction mixture was diluted with EtOAc, and quenched with 1 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford methyl ether **32** (469 mg, 815 μmol , 91 %) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 7.34 (4H, s), 7.24–7.26 (3H, m), 7.05–7.07 (2H, m), 6.41 (1H, s), 4.85 (2H, s), 4.57 (1H, d, $J = 11.2$ Hz), 4.47 (1H, d, $J = 11.2$ Hz), 3.82 (3H, s), 3.74 (3H, s), 2.15 (3H, s), 2.13 (3H, s), 2.09 (3H, s), 2.00 (3H, s), 1.93 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 168.5, 155.9, 154.9, 149.0, 148.8, 146.2, 137.1, 135.7, 135.0, 133.8, 129.1, 128.6, 128.1, 127.6, 127.3, 125.9, 124.5, 124.4, 119.6, 114.4, 107.7, 75.4, 74.4, 55.7, 52.2, 20.3, 13.9, 12.6, 10.2, 9.4; IR (Neat) 2,950, 2,927, 1,731, 1,613, 1,599, 1,583, 1,492, 1,338, 1,283, 1,206, 1,125, 754 cm^{-1} ; HREIMS calcd for $\text{C}_{34}\text{H}_{35}\text{ClO}_6$ 574.2122, found 574.2133.

4-Benzoyloxy-2-(4-chlorobenzoyloxy)-5-(3-methoxy-2,5,6-trimethyl-phenoxy)-3,6-dimethylbenzoic acid (6b).

To a solution of methyl ester **32** (145 mg, 252 μmol) in dioxane (1.0 mL) were added 2 M aq NaOH (2.0 mL, 4.00 mmol, 16 equiv) and ethylene glycol (0.5 mL) at room temperature. After being stirred at 200 °C under microwave irradiation for 40 min, the reaction mixture was cooled to room temperature, diluted with EtOAc, and quenched with 3 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 4:1) to afford carboxylic acid **6b** (118 mg, 211 μmol , 84 %) as an orange oil. ^1H NMR (400 MHz, CDCl_3) δ 7.23–7.32 (7H, m), 7.03–7.05 (2H, m), 6.41 (1H, s), 4.85 (2H, s), 4.54 (1H, d, J = 11.2 Hz), 4.44 (1H, d, J = 11.2 Hz), 3.74 (3H, s), 2.26 (3H, s), 2.14 (3H, s), 2.06 (3H, s), 1.99 (3H, s), 1.92 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 173.1, 155.8, 154.9, 149.1, 148.9, 146.3, 137.0, 135.2, 135.0, 133.9, 129.5, 128.6, 128.0, 127.6, 127.2, 125.5, 124.9, 124.5, 119.5, 114.4, 107.7, 75.6, 74.3, 55.7, 20.3, 14.0, 12.6, 10.2, 9.4; IR (Neat) 3,380, 2,926, 1,725, 1,699, 1,614, 1,599, 1,580, 1,492, 1,455, 1,365, 1,219, 1,126, 1,092 cm^{-1} ; HREIMS calcd for $\text{C}_{33}\text{H}_{33}\text{ClO}_6$ 560.1966, found 560.1941.

Methyl 4-benzoyloxy-2-(4-chlorobenzoyloxy)-5-(4-formyl-3-methoxy-2,5,6-trimethylphenoxy)-3,6-dimethylbenzoate (34).

To a solution of methyl ether **32** (30.0 mg, 52.2 μmol) in dry CH_2Cl_2 (1.0 mL) were added Cl_2CHOMe (46.1 μL , 522 μmol , 10 equiv) and AgOTf (29.5 mg, 115 μmol , 2.2 equiv) at -40 °C under an argon atmosphere. After being stirred at 0 °C for 30 min, the reaction mixture was quenched with saturated aq NaHCO_3 at 0 °C. After being stirred at room temperature for 30 min, the reaction mixture was filtered through a pad of Celite[®]. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo to give desired aldehyde **34** and deprotected phenol. The crude mixture was used for the next reaction without further purification.

To a solution of the crude mixture in DMF (1.0 mL) were added K_2CO_3 (36.1 mg, 261 μmol , 5.0 equiv) and (4-Cl)BnCl (16.8 mg, 104 μmol , 2.0 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 17 h, the reaction mixture was diluted with EtOAc, and quenched with 1 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford aldehyde **34** (15.7 mg, 26.2 μmol , 50 % in 2 steps) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 10.4 (1H, s), 7.35 (4H, s), 7.21–7.22 (3H, m), 6.92–6.95 (2H, m), 4.87 (2H, s), 4.55 (1H, d, J = 11.4 Hz), 4.51 (1H, d, J = 11.4 Hz), 3.85 (3H, s), 3.63 (3H, s), 2.37 (3H, s), 2.21 (3H, s), 2.12 (3H, s), 2.09 (3H, s), 1.93 (3H, s); ^{13}C NMR

(100 MHz, CDCl_3) δ 192.4, 168.2, 162.2, 159.1, 149.6, 148.3, 145.2, 138.8, 136.4, 135.5, 133.9, 129.1, 128.6, 128.2, 127.8, 126.5, 126.2, 124.7, 124.6, 124.5, 123.7, 119.3, 15.5, 14.2, 62.9, 52.4, 15.9, 13.8, 12.8, 10.2, 9.7; IR (Neat) 2,928, 1,731, 1,685, 1,565, 1,454, 1,337, 1,284, 1,206, 1,108 cm^{-1} ; HRFABMS calcd for $\text{C}_{35}\text{H}_{36}\text{ClO}_7$ $[\text{M} + \text{H}]^+$ 603.2150, found 603.2128.

Methyl 4-benzyloxy-2-(4-chlorobenzyloxy)-5-(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)-3,6-dimethylbenzoate (35).

To a solution of aldehyde **xx** (15.0 mg, 24.9 μmol) in *t*-BuOH (0.5 mL) and water (0.5 mL) were added 2-methyl-2-butene (0.5 mL), NaClO_2 (22.5 mg, 249 μmol , 10 equiv) and $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (38.8 mg, 249 μmol , 10 equiv) at 0 $^\circ\text{C}$. After being stirred at room temperature for 14 h, the organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 2:3) to afford carboxylic acid **35** (9.8 mg, 15.8 μmol , 64 %) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 7.35 (4H, s), 7.25–7.30 (3H, m), 6.97–6.98 (2H, m), 4.87 (2H, s), 4.56 (1H, d, J = 11.2 Hz), 4.17 (1H, d, J = 11.2 Hz), 3.85 (3H, s), 3.67 (3H, s), 2.20 (3H, s), 2.17 (3H, s), 2.13 (3H, s), 2.07 (3H, s), 1.96 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 170.8, 168.3, 156.4, 154.4, 149.5, 148.5, 145.5, 136.6, 135.6, 134.3, 133.9, 129.1, 128.6, 128.3, 127.8, 126.7, 126.2, 124.6, 124.5, 124.3, 122.5, 119.5, 75.5, 74.3, 62.1, 52.3, 16.9, 13.9, 13.1, 10.2, 10.1; IR (Neat) 3,429, 2,950, 2,931, 1,732, 1,703, 1,600, 1,455, 1,285, 1,208, 1,105 cm^{-1} ; HREIMS calcd for $\text{C}_{35}\text{H}_{35}\text{ClO}_8$ 618.2020, found 618.2030.

Methyl 4-benzyloxy-2-methoxy-3,5,6-trimethylbenzoate (41).

To a solution of aldehyde **15** (2.00 g, 10.1 mmol) in dry THF (20 mL) were added Et_3N (1.69 mL, 12.1 mmol, 1.2 equiv) and ClCOOEt (1.16 mL, 12.1 mmol, 1.2 equiv) at 0 $^\circ\text{C}$ under an argon atmosphere. After being stirred at the same temperature for 40 min, the reaction mixture was filtered through a pad of Celite[®]. To filtrate was added NaBH_4 (1.53 g, 40.4 mmol, 4.0 equiv) in water (20 mL) at 0 $^\circ\text{C}$. After being stirred at room temperature for 2.5 h, the reaction mixture was diluted with EtOAc, and acidified with 3 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo to give trimethylbenzene as a white solid. The crude trimethylbenzene was used for next reaction without further purification.

To a solution of crude phenol in DMF (20 mL) were added K_2CO_3 (4.19 g, 30.3 mmol, 3.0 equiv) and BnBr (1.68 mL, 14.1 mmol, 1.4 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 11 h, the reaction mixture was filtered through a pad of Celite[®]. The filtrate was diluted with EtOAc, and acidified with 3 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aq NaHCO_3 and brine, dried

with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/ EtOAc = 5:1) to afford benzyl ether **41** (3.01 g, 9.56 mmol, 95 % in 2 steps) as a yellowish oil. ^1H NMR (400 MHz, CDCl_3) δ 7.35–7.48 (5H, m), 4.75 (2H, s), 3.93 (3H, s), 3.75 (3H, s), 2.21 (3H, s), 2.18 (3H, s), 2.17 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 169.1, 156.9, 153.7, 137.0, 132.3, 128.2, 127.8, 127.5, 125.9, 125.2, 121.9, 74.2, 61.4, 51.8, 16.4, 12.3, 9.4; IR (Neat) 2,948, 1,731, 1,577, 1,455, 1,327, 1,284, 1,200, 1,177, 1,105, 1,005 cm^{-1} ; HREIMS calcd for $\text{C}_{19}\text{H}_{22}\text{O}_4$ 314.1518, found 314.1499.

4-Benzylloxy-2-methoxy-3,5,6-trimethylbenzoic acid (**42**).

To a solution of methyl ester **41** (300 mg, 954 μmol) in dioxane (1.2 mL) were added 2 M aq NaOH (2.4 mL, 4.80 mmol, 5.0 equiv) and ethylene glycol (0.8 mL) at room temperature. After being stirred at 180 $^\circ\text{C}$ under microwave irradiation for 50 min, the reaction mixture was cooled to room temperature, diluted with EtOAc , and quenched with 3 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/ EtOAc = 1:1) to afford carboxylic acid **42** (251 mg, 836 μmol , 88 %) as a white solid. mp 124–125 $^\circ\text{C}$; ^1H NMR (400 MHz, CDCl_3) δ 7.36–7.47 (5H, m), 4.77 (2H, s), 3.81 (3H, s), 2.34 (3H, s), 2.23 (3H, s), 2.22 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 173.0, 157.7, 154.2, 137.1, 133.6, 128.6, 128.2, 127.8, 126.7, 123.9, 122.4, 74.5, 62.1, 16.9, 12.7, 9.8; IR (Neat) 2,934, 1,699, 1,578, 1,455, 1,322, 1,221, 1,106, 696 cm^{-1} ; HREIMS calcd for $\text{C}_{18}\text{H}_{20}\text{O}_4$ 300.1362, found 300.1347.

Benzyl 4-hydroxy-2-methoxy-3,5,6-trimethylbenzoate (**8**).

To a solution of benzyl ether **42** (3.50 g, 11.7 mmol) in EtOAc (30 mL) was added 5 % palladium on carbon (2.0 g, 57 wt%) at room temperature, and the flask was purged with hydrogen 7 times. After being stirred at room temperature for 16 h, the reaction mixture was filtered through a pad of Celite[®]. The filtrate was concentrated in vacuo to give phenol as a white solid. The crude phenol was used for next reaction without further purification.

To a solution of crude carboxylic acid in DMF (30 mL) were added KHCO_3 (3.21 g, 32.1 mmol, 2.7 equiv) and BnBr (1.53 mL, 12.8 mmol, 1.1 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 9.5 h, the reaction mixture was filtered through a pad of Celite[®]. The filtrate was diluted with EtOAc , and acidified with 3 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/ EtOAc = 5:1) to afford benzyl ester **8** (3.29 g, 10.9 mmol, 94 % in 2 steps) as an orange solid. mp 61–62 $^\circ\text{C}$; ^1H NMR (400 MHz, CDCl_3) δ 7.31–7.45 (5H, m), 5.36

(2H, s), 4.78 (1H, s), 3.64 (3H, s), 2.14 (6H, s), 2.11 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 169.0, 153.8, 153.7, 135.8, 132.3, 128.5, 128.4, 128.2, 121.5, 118.3, 114.9, 66.9, 61.9, 16.6, 11.6, 8.8; IR (Neat) 3,466, 2,943, 1,721, 1,584, 1,456, 1,291, 1,190, 1,108 cm^{-1} ; HREIMS calcd for $\text{C}_{18}\text{H}_{20}\text{O}_4$ 300.1362, found 300.1369.

4-Acetoxy-2-methoxy-3,5,6-trimethylbenzoic acid (**14**).

To a solution of phenol **8** (1.50 g, 4.99 mmol) in dry CH_2Cl_2 (10 mL) were added Ac_2O (0.567 mL, 5.99 mmol, 1.2 equiv), Et_3N (2.09 mL, 15.0 mmol, 3.0 equiv) and DMAP (61.0 mg, 499 μmol , 0.10 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 15 h, the reaction mixture was diluted with EtOAc, and quenched with 3 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, to give acetate as a white solid. The crude acetate was used for next reaction without further purification.

To a solution of crude benzyl ester in EtOAc (30 mL) was added 5 % palladium on carbon (800 mg, 53 wt%) at room temperature, and the flask was purged with hydrogen 7 times. After being stirred at the same temperature for 24 h, the reaction mixture was filtered through a pad of Celite[®]. The filtrate was concentrated in vacuo, and the resulting residue was recrystallized from CH_2Cl_2 /hexane to afford carboxylic acid **14** (1.09 g, 4.33 mmol, 87 % in 2 steps) as a white solid. mp 158–159 $^\circ\text{C}$; ^1H NMR (400 MHz, CDCl_3) δ 3.81 (3H, s), 2.37 (3H, s), 2.31 (3H, s), 2.09 (3H, s), 2.06 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 173.3, 168.6, 153.6, 149.7, 132.9, 126.3, 125.5, 121.7, 62.3, 20.4, 16.8, 12.7, 9.7; IR (Neat) 3,265, 2,942, 1,754, 1,737, 1,579, 1,462, 1,370, 1,200, 1,097 cm^{-1} ; HREIMS calcd for $\text{C}_{13}\text{H}_{16}\text{O}_5$ 252.0998, found 252.0997.

4-Benzyloxycarbonyl-3-methyl-2,5,6-trimethylphenyl 4-hydroxy-2-methoxy-3,5,6-trimethylbenzoate (**7**).

To a solution of carboxylic acid **14** (1.09 g, 4.32 mmol, 1.2 equiv) in dry toluene (30 mL) were added phenol **8** (1.08 g, 3.60 mmol) and $(\text{CF}_3\text{CO})_2\text{O}$ (2.00 mL, 14.4 mmol, 4.0 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 12 h, the reaction mixture was quenched with 2 M aq NaOH. The organic layer was separated, and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo to give ester as a colorless oil. The crude ester was used for next reaction without further purification.

To a solution of crude acetate in MeOH (10 mL) was added K_2CO_3 (746 mg, 5.40 mmol, 1.5 equiv) at room temperature. After being stirred at the same temperature for 2 h, the reaction mixture was diluted with EtOAc, and quenched with 1 M aq HCl. The organic layer was separated, and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in

vacuo, and the resulting residue was recrystallized from CH_2Cl_2 /hexane to afford phenol **7** (1.59 g, 3.23 mmol, 90 % in 2 steps) as a white solid. mp 172–173 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.33–7.47 (5H, m), 5.39 (2H, s), 4.90 (1H, s), 3.79 (3H, s), 3.70 (3H, s), 2.36 (3H, s), 2.23 (3H, s), 2.22 (3H, s), 2.193 (3H, s), 2.188 (3H, s), 2.17 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 168.4, 166.8, 154.6, 154.5, 153.5, 149.7, 135.6, 133.3, 132.6, 128.50, 128.48, 128.3, 127.1, 125.7, 122.1, 120.4, 118.7, 114.5, 67.0, 62.1, 61.9, 17.1, 16.7, 12.9, 11.8, 10.1, 9.0; IR (Neat) 3,481, 2,942, 1,736, 1,733, 1,576, 1,463, 1,456, 1,286, 1,159, 1,097, 1,078 cm^{-1} ; HRFABMS calcd for $\text{C}_{29}\text{H}_{32}\text{O}_7\text{Na}$ $[\text{M}+\text{Na}]^+$ 515.2046, found 515.2076.

Preparation of ester **43**.

To a solution of carboxylic acid **6b** (70.0 mg, 125 μmol , 1.2 equiv) in dry toluene (2.0 mL) were added phenol **7** (51.2 mg, 104 μmol) and $(\text{CF}_3\text{CO})_2\text{O}$ (579 μL , 4.16 mmol, 40 equiv) at room temperature under an argon atmosphere. After being stirred at 80 °C for 9 h, the reaction mixture was cooled at room temperature, and quenched with 2 M aq NaOH. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford ester **43** (105 mg, 101 μmol , 97 %) as a white solid. mp 85–86 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.46–7.48 (2H, m), 7.26–7.40 (10H, m), 7.06–7.08 (2H, m), 6.44 (1H, s), 5.40 (2H, s), 4.94 (2H, s), 4.60 (1H, d, J = 11.0 Hz), 4.49 (1H, d, J = 11.0 Hz), 3.78 (3H, s), 3.76 (3H, s), 3.71 (3H, s), 2.41 (3H, s), 2.35 (3H, s), 2.25 (3H, s), 2.21 (3H, s), 2.18 (3H, s), 2.171 (3H, s), 2.168 (3H, s), 2.14 (3H, s), 2.10 (3H, s), 2.05 (3H, s), 1.98 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 168.3, 166.2, 165.8, 155.9, 154.8, 154.3, 153.6, 150.0, 149.8, 149.5, 149.4, 146.4, 136.9, 135.7, 135.4, 135.1, 133.8, 133.4, 132.7, 129.2, 128.54, 128.50, 128.3, 128.1, 127.7, 127.4, 127.2, 126.5, 126.1, 125.7, 125.2, 124.9, 124.7, 122.5, 122.2, 119.5, 114.4, 107.7, 75.7, 74.4, 67.0, 62.1, 62.0, 55.7, 20.4, 17.3, 16.7, 14.5, 13.5, 13.0, 12.6, 10.6, 10.4, 10.2, 9.5; IR (Neat) 2,939, 1,738, 1,578, 1,463, 1,322, 1,278, 1,150, 1,122, 1,095, 1,076 cm^{-1} ; HRFABMS calcd for $\text{C}_{62}\text{H}_{63}\text{ClO}_{12}\text{Na}$ $[\text{M}+\text{Na}]^+$ 1,057.3906, found 1,057.3905.

Preparation of aldehyde **44**.

To a solution of ester **43** (260 mg, 251 μmol) in dry CH_2Cl_2 (3.0 mL) were added Cl_2CHOMe (225 μL , 2.51 mmol, 10 equiv) and AgOTf (142 mg, 552 μmol , 2.2 equiv) at -40 °C under an argon atmosphere. After being stirred at 0 °C for 30 min, the reaction mixture was quenched with saturated aq NaHCO_3 at 0 °C. After being stirred at room temperature for 30 min, the reaction mixture was filtered through a pad of Celite[®]. The organic layer of the filtrate was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo to give the mixture of desired aldehyde **44** and deprotected phenol. The crude mixture was used for next reaction without further purification.

To a solution of crude mixture in DMF (3.0 mL) were added K_2CO_3 (104 mg, 753 μmol , 3.0 equiv) and (4-Cl)BnCl (60.6 mg, 377 μmol , 1.5 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 14 h, the reaction mixture was diluted with EtOAc, and quenched with 1 M aq HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aq NaHCO_3 and brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford aldehyde **44** (144 mg, 136 μmol , 54 % in 2 steps) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 10.4 (1H, s), 7.46–7.47 (2H, m), 7.31–7.40 (8H, m), 7.23–7.24 (2H, m), 6.96–6.98 (2H, m), 5.40 (2H, s), 4.97 (2H, s), 4.60 (1H, d, J = 11.4 Hz), 4.55 (1H, d, J = 11.4 Hz), 3.79 (3H, s), 3.71 (3H, s), 3.65 (3H, s), 2.47 (3H, s), 2.39 (3H, s), 2.36 (3H, s), 2.25 (3H, s), 2.22 (3H, s), 2.20 (3H, s), 2.18 (3H, s), 2.15 (3H, s), 2.14 (3H, s), 2.11 (3H, s), 1.99 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 192.4, 168.3, 166.1, 165.5, 162.2, 159.0, 154.3, 153.7, 150.4, 150.0, 149.4, 149.0, 145.5, 138.9, 136.3, 135.7, 135.3, 134.0, 133.5, 132.7, 129.2, 128.59, 128.56, 128.53, 128.3, 128.2, 127.9, 127.4, 126.6, 126.5, 126.1, 125.7, 125.29, 125.25, 124.79, 124.75, 123.8, 122.4, 122.2, 119.3, 75.8, 74.2, 67.1, 63.0, 62.1, 62.0, 17.3, 16.7, 15.9, 14.5, 13.5, 13.0, 12.9, 10.7, 10.4, 10.2, 9.8; IR (Neat) 2,932, 1,740, 1,684, 1,573, 1,460, 1,323, 1,278, 1,150, 1,111, 755 cm^{-1} ; HRESITOFMS calcd for $\text{C}_{63}\text{H}_{63}\text{ClO}_{13}\text{Na}$ $[\text{M} + \text{Na}]^+$ 1085.3855, found 1085.3860.

Preparation of carboxylic acid **9b**.

To a solution of aldehyde **44** (26.0 mg, 24.4 μmol) in *t*-BuOH (1.0 mL) and water (1.0 mL) were added 2-methyl-2-butene (0.5 mL), NaClO_2 (22.1 mg, 244 μmol , 10 equiv) and $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (38.1 mg, 244 μmol , 10 equiv) at 0 $^\circ\text{C}$. After being stirred at room temperature for 4 h, the organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 1:1) to afford carboxylic acid **9b** (21.9 mg, 20.3 μmol , 83 %) as a yellowish oil. ^1H NMR (400 MHz, CDCl_3) δ 7.46–7.48 (2H, m), 7.28–7.40 (10H, m), 6.99–7.01 (2H, m), 5.40 (2H, s), 4.97 (2H, s), 4.62 (1H, d, J = 11.4 Hz), 4.51 (1H, d, J = 11.4 Hz, t), 3.79 (3H, s), 3.71 (3H, s), 3.70 (3H, s), 2.46 (3H, s), 2.37 (3H, s), 2.26 (3H, s), 2.22 (6H, s), 2.19 (3H, s), 2.18 (3H, s), 2.16 (3H, s), 2.12 (6H, s), 2.01 (3H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 171.2, 168.3, 166.2, 165.6, 156.3, 154.4, 153.7, 150.2, 150.0, 149.5, 149.2, 145.7, 136.4, 135.7, 135.3, 134.2, 134.0, 133.5, 132.7, 129.2, 128.6, 128.5, 128.34, 128.32, 127.9, 127.4, 126.7, 126.6, 126.1, 125.7, 125.3, 125.2, 124.8, 124.3, 122.9, 122.4, 122.2, 119.4, 75.8, 74.3, 67.1, 62.1, 62.0, 17.3, 16.9, 16.7, 14.5, 13.5, 13.2, 13.0, 10.7, 10.4, 10.24, 10.17; IR (Neat) 2,939, 1,738, 1,733, 1,600, 1,575, 1,462, 1,323, 1,279, 1,152, 1,097, 755 cm^{-1} ; HRESITOFMS calcd for $\text{C}_{63}\text{H}_{63}\text{ClO}_{14}\text{Na}$ $[\text{M} + \text{Na}]^+$ 1,101.3804, found 1,101.3816.

Preparation of ester 45.

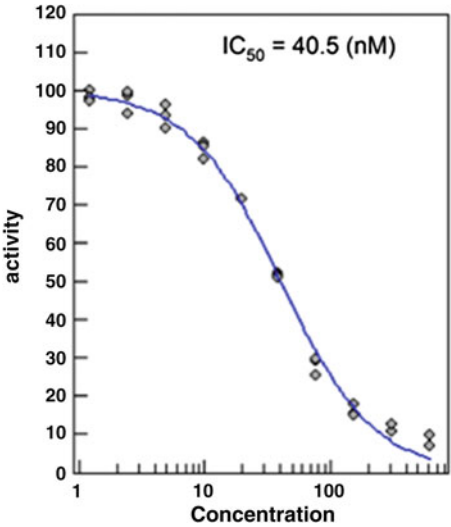
To a solution of carboxylic acid **9b** (110 mg, 102 μmol) in dry toluene (5.0 mL) were added phenol **8** (36.7 mg, 122 μmol , 1.2 equiv) and $(\text{CF}_3\text{CO})_2\text{O}$ (568 μL , 4.08 mmol, 40 equiv) at room temperature under an argon atmosphere. After being stirred at 80 $^\circ\text{C}$ for 11 h, the reaction mixture was cooled at room temperature, and quenched with 2 M aq NaOH. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 6:1) to afford ester **45** (105 mg, 77.3 μmol , 76 %) as a white solid. mp 106–107 $^\circ\text{C}$; ^1H NMR (500 MHz, CDCl_3) δ 7.46–7.48 (4H, m), 7.21–7.40 (13H, m), 7.01–7.02 (2H, m), 5.402 (2H, s), 5.398 (2H, s), 4.97 (2H, s), 4.63 (1H, d, J = 11.2 Hz), 4.52 (1H, d, J = 11.2 Hz), 3.79 (3H, s), 3.72 (3H, s), 3.71 (3H, s), 3.68 (3H, s), 2.48 (3H, s), 2.37 (3H, s), 2.26 (3H, s), 2.25 (3H, s), 2.23 (3H, s), 2.22 (6H, s), 2.21 (3H, s), 2.19 (3H, s), 2.18 (3H, s), 2.16 (3H, s), 2.15 (3H, s), 2.12 (3H, s), 2.05 (3H, s); ^{13}C NMR (125 MHz, CDCl_3) δ 168.33, 168.30, 166.4, 166.2, 165.6, 156.2, 154.7, 154.4, 153.7, 150.2, 150.0, 149.6, 149.5, 149.2, 145.7, 136.4, 135.7, 135.3, 134.0, 133.53, 133.49, 132.71, 132.69, 129.2, 128.59, 128.58, 128.55, 128.4, 128.3, 127.9, 127.43, 127.36, 126.8, 126.6, 126.1, 125.71, 125.69, 125.31, 125.26, 124.8, 124.1, 123.8, 122.4, 122.20, 122.19, 119.7, 75.8, 74.4, 67.1, 62.1, 62.0, 61.9, 17.3, 17.2, 16.8, 16.7, 14.5, 13.5, 13.2, 13.01, 12.99, 10.7, 10.44, 10.38, 10.3; IR (Neat) 3,011, 2,940, 1,735, 1,600, 1,576, 1,461, 1,323, 1,280, 1,149, 1,096, 1,077, 754 cm^{-1} ; HRESITOFMS calcd for $\text{C}_{81}\text{H}_{81}\text{ClO}_{17}\text{Na}$ $[\text{M} + \text{Na}]^+$ 1383.5060, found 1383.5070.

Synthesis of thielocin B1 (1)

To a solution of benzyl ester **45** (5.0 mg, 3.7 μmol) in EtOH (0.5 mL) and EtOAc (0.5 mL) was added 10 % palladium on carbon (10 mg, 200 wt%) at room temperature, and the flask was purged with hydrogen 7 times. After being stirred at the same temperature for 15 min, the reaction mixture was filtered through a pad of Celite[®]. The filtrate was concentrated in vacuo, and the resulting residue was purified by preparative TLC (eluted with $\text{CHCl}_3/\text{MeOH}$ = 3:1) to afford thielocin B1 (**1**) (1.9 mg, 2.0 μmol , 54 %) as a white solid. mp > 300 $^\circ\text{C}$; ^1H NMR (400 MHz, CD_3OD) δ 3.832 (3H, s n), 3.825 (3H, s), 3.80 (3H, s), 3.73 (3H, s), 2.39 (3H, s), 2.38 (3H, s), 2.36 (3H, s), 2.284 (3H, s), 2.276 (3H, s), 2.22 (3H, s), 2.21 (3H, s), 2.20 (6H, s), 2.18 (3H, s), 2.14 (6H, s), 2.11 (3H, brs), 2.01 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 171.3, 168.4, 167.8, 161.0, 158.4, 156.2, 155.7, 153.23, 153.22, 150.9, 148.5, 148.4, 140.1, 136.7, 136.6, 134.50, 134.47, 131.63, 131.58, 128.5, 128.1, 127.1, 125.9, 125.1, 124.8, 123.3, 122.3, 120.2, 111.9, 62.8, 62.6, 62.01, 61.99, 17.6, 17.4, 17.1, 16.5, 13.4, 13.3, 10.6, 10.54, 10.51, 10.4, 8.6; IR (Neat) 3,430, 2,918, 1,736, 1,656, 1,572, 1,460, 1,160, 1,093, 1,074 cm^{-1} ; HRESITOFMS calcd for $\text{C}_{53}\text{H}_{59}\text{O}_{17}$ $[\text{M} + \text{H}]^+$ 967.3752, found 967.3751.

Table 2.4 PPI inhibitory activity of synthesized **1** for PAC3 homodimer

Synthesized 1 (nM)	Fluorescence intensity ($\times 10^6$)				Intensity ratio (%)
	1st	2nd	3rd	Mean	
Background	0.273	0.258	0.258	0.263	0
Control	4.40	4.39	4.30	4.36	100
625	0.549	0.658	0.538	0.582	8
313	0.702	0.783	0.698	0.727	11
156	0.897	0.986	0.869	0.917	16
78.1	1.47	3.31	1.49	2.09	45
39.1	2.36	2.38	2.40	2.38	52
19.5	3.20	3.20	3.20	3.20	72
9.77	3.61	3.79	3.76	3.72	84
4.88	3.94	4.20	4.09	4.07	93
2.44	4.11	4.33	4.30	4.25	97
1.22	4.25	4.29	4.36	4.30	98



IC_{50} value of synthesized thielocin B1 (1**) for PAC3 homodimer and PAC1/PAC2 heterodimer.**

The evaluation of PPIs inhibition of synthesized **1** for PAC3 (mKG_N-PAC3: 10 nM and mKG_C-PAC3: 25 nM, respectively) homodimer and PAC1/PAC2 (PAC1-mKG_N: 79 nM and mKG_C-PAC2: 120 nM, respectively) were performed as previously reported [2]. Aliquot of 3 μ L of a protein solution were dispensed into a 384-well plate using a Multidrop Combi dispenser, and 0.1 μ L of **1** dissolved in DMSO was added using a Multi-dispenser ADS-348-8. After incubation at room temperature for 1 h, 3 μ L of the other protein solution was

Table 2.5 PPI inhibitory activity of synthesized **1** for PAC1/PAC2

Synthesized 1 (nM)	Fluorescence intensity ($\times 10^6$)				Intensity ratio (%)
	1st	2nd	3rd	Mean	
Background	0.650	0.567	0.563	0.594	0
Control	6.59	6.44	5.97	6.33	100
625	5.95	6.07	6.19	6.07	95
313	6.20	6.36	6.30	6.29	99
156	6.22	6.39	6.32	6.31	100
78.1	6.34	6.41	6.36	6.37	101
39.1	6.28	6.32	4.89	5.83	91
19.5	6.34	6.32	6.27	6.31	100
9.77	5.98	6.06	6.17	6.07	95
4.88	6.16	6.23	6.22	6.20	98
2.44	6.02	6.13	6.22	6.12	96
1.22	5.99	6.07	6.44	6.17	97

dispensed. After incubation for 17 h, fluorescence was measured using an EnVision reader. The results were summarized in Tables 2.4 and 2.5.

Protein expression and purification.

The cDNA encoding PAC3 was cloned into the pRSF-Duet1 vector (Novagen) to express PAC3 with an N-terminal hexahistidine tag, followed by an enterokinase cleavage site. Using this construct, the protein was expressed in *Escherichia coli* BL21 (DE3) Codon-Plus cells, which were grown in M9 minimal medium containing $[^{15}\text{N}]\text{NH}_4\text{Cl}$ (1 g/L) using a previously described protocol [40]. The uniformly ^{15}N -labeled protein was purified using a Ni Sepharose 6 Fast Flow (GE Healthcare Bio-Sciences). Subsequently, the histidine tag was cleaved by enterokinase treatment, followed by anion exchange chromatography using a Resource Q column (GE Healthcare).

NMR experiment for chemical shift perturbation.

^{15}N -labeled PAC3 (0.2 mM) sample, dissolved in PBS (pH 6.8) containing 10 % D_2O (v/v), 1 mM DTT, 1 mM EDTA, and 0.01 % NaN_3 , was titrated with a 10 mM methanol- d_4 solution of **1** (0.1, 0.2, 0.4, or 0.8 mM) or the solvent alone as a reference control. All NMR data were acquired at 303 K using a Bruker DMX500 spectrometer equipped with a cryogenic probe. For ^1H - ^{15}N HSQC measurements, spectra were recorded at a ^1H observation frequency of 500 MHz with $128 (t_1) \times 1024 (t_2)$ complex points and 8 scans per t_1 increment. ^1H chemical shifts were referenced to DSS (0 ppm), while ^{13}C chemical shifts were indirectly referenced to DSS using the absolute frequency ratios. Chemical shift changes were quantified as $[(\Delta\delta_{\text{H}})^2 + (0.2 \Delta\delta_{\text{N}})^2]^{1/2}$, where $\Delta\delta_{\text{H}}$ and $\Delta\delta_{\text{N}}$ are the observed chemical shift changes for ^1H and ^{15}N , respectively. The data were processed using NMRPipe [41] software and analyzed with SPARKY 3 [42] and CCPNMR [43] software. Conventional 3D NMR experiments [44] were carried out for assignments of the HSQC peaks originating from PAC3 homodimer.

References

1. Matsumoto K, Tanaka K, Matsutani S, Sakazaki R, Hinoo H, Uotani N, Tanimoto T, Kawamura Y, Nakamoto S, Yoshida T. *J Antibiot.* 1995;48:106–12.
2. Hashimoto J, Watanabe T, Seki T, Karasawa S, Izumikawa M, Seki T, Iemura S, Natsume T, Nomura N, Goshima N, Miyawaki A, Takagi M, Shin-ya K. *J Biomol Screen.* 2009;14:970–9.
3. Yashiroda H, Mizushima T, Okamoto K, Kameyama T, Hayashi H, Kishimoto T, Niwa S, Kasahara M, Kurimoto E, Sakata E, Takagi K, Suzuki A, Hirano Y, Murata S, Kato K, Yamane T, Tanaka K. *Nat Struct Mol Biol.* 2008;15:228–36.
4. Hirano Y, Hayashi H, Iemura S, Hendil KV, Niwa S, Kishimoto T, Kasahara M, Natsume T, Tanaka K, Murata S. *Mol Cell.* 2006;24:977–84.
5. Sperotto E, van Klink GPM, de Vries JG, van Koten G. The synthesis of 2,2',6,6'-tetrasubstituted diphenyl ether by the coupling reaction with transition metals has been reported by Koten; however the yield was quite low even in the simple substrates. *Tetrahedron* 2010; 66:9009–20.
6. Sala T, Sargent SV. *J Chem Soc Perkin Trans I.* 1981; 877–82.
7. Smith WE. *J Org Chem.* 1972;37:3972–3.
8. Lindgren BO, Nilsson T. *Acta Chem Scand.* 1972;27:888–90.
9. Kraus GA, Taschener MJ. *J Org Chem.* 1980;45:1174–5.
10. Bal BS, Childers WE, Pinnick HW. *Tetrahedron.* 1981;37:2091–6.
11. Sala T, Sargent SV. *J Chem Soc Chem Commun.* 1978; 1043–1044.
12. Comber MF, Sargent MV, Skelton BW, White AH. The intermediate has been isolated in some cases. *J Chem Soc Perkin Trans I.* 1989; 441–447.
13. We tried the isolation of intermediate **25** under low reaction temperature. Although compound **22** and unknown product were monitored on TLC and crude ^1H NMR, these two products were converged to **22** during the purification.
14. Keith JM. *Tetrahedron Lett.* 2004;45:2739–42.
15. Jaffé HH. *Chem Rev.* 1953;53:191–261.
16. Tanaka H, Miyoshi H, Chuang Y-C, Ando Y, Takahashi T. *Angew Chem Int Ed.* 2007;46:5934–7.
17. West CT, Donnelly SJ, Kooistra DA, Doyle MP. *J Org Chem.* 1973;38:2675–81.
18. Kappe CO. *Angew Chem Int Ed.* 2004;43:6250–84.
19. Meyer V. *Chem Ber.* 1894;27:510–2.
20. Meyer V, Sudborough J. *Chem Ber.* 1894;27:3146–53.
21. Vilsmeier A, Haack A. *Ber Deutsch Chem Ges.* 1927;60:119–22.
22. Reimer K. *Ber Dtsch Chem Ges.* 1876;9:423–4.
23. Reimer K, Tiemann F. *Ber Dtsch Chem Ges.* 1876;9:824–8.
24. Reimer K, Tiemann F. *Ber Dtsch Chem Ges.* 1876;9:1268–78.
25. Wynberg H. *Chem Rev.* 1960;60:169–84.
26. Rieche A, Gross H, Höft E. *Chem Ber.* 1960;93:88–94.
27. Gross H, Rieche A, Matthey G. *Chem Ber.* 1963;96:308–19.
28. The further investigation of newly developed formylation is mentioned in Chapter 4.
29. Fletcher S, Gunning PT. Removal of the benzyl group using chelating effects under acidic conditions has been reported. *Tetrahedron Lett.* 2008; 49:4817–19.
30. Hojo M, Masuda R, Okada E. *Tetrahedron Lett.* 1987;28:6199–200.
31. Tepczewski JJ, Callahan MP, Neighbors JD, Wiemer DF. *J Am Chem Soc.* 2009;131:14630–1.
32. Mente NR, Neighbors JD, Wiemer DF. *J Org Chem.* 2008;73:7963–70.
33. Minami N, Kijima S. *Chem Pharm Bull.* 1979;27:1490–4.
34. Parish RC, Stock LM. *J Org Chem.* 1965;30:927–9.
35. Corey EJ, Nicolaou KC. *J Am Chem Soc.* 1974;96:5614–6.
36. Gerlach H, Thalmann A. *Helv Chim Acta.* 1974;57:2661–3.
37. Molecular Operating Environment (MOE), ver. 2012, Chemical Computing Group Inc., 2012.

38. Cresp TM, Djura P, Sargent MV, Elix JA, Engkaninan U, Murphy DPH. *Aust J Chem.* 1975;28:2417–34.
39. Sargent MV, Vogel P, Elix JA, Ferguson BA. *Aust J Chem.* 1976;29:2263–9.
40. Yagi-Utsumi M, Matsuo K, Yanagisawa K, Gekko K, Kato K. *Int J Alzheimers Dis.* 2011; 925073. doi:[10.4061/2011/925073](https://doi.org/10.4061/2011/925073).
41. Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A. *J Biomol NMR.* 1995;6:277–93.
42. Goddard TD, Kneller DG. SPARKY 3. University of California, San Francisco.
43. Vranken WF, Boucher W, Stevens TJ, Fogh RH, Pajon A, Llinas M, Ulrich EL, Markley JL, Ionides J, Laue ED. *Protein.* 2005;59:687–96.
44. Uekusa Y, Mimura S, Sasakawa H, Kurimoto E, Sakata E, Serve O, Yagi H, Tokunaga F, Iwai K, Kato K. *Biomol NMR Assign.* 2012;6:177–80.

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