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Synthesis and biological evaluation of thielocin B1 analogues as protein–protein interaction inhibitors of PAC3 homodimer

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Abstract

The synthesis and biological evaluation of thielocin B1 analogues have been demonstrated. Fourteen analogues modified in the central core and terminal carboxylic acid moiety were concisely synthesized by simple esterification or etherification reaction. The evaluation of synthetic analogues as inhibitors of proteasome assembling chaperone (PAC) complexes (the PAC3 homodimer and PAC1/PAC2) revealed that the natural product-like bending structure and terminal carboxylic acid groups were crucial for its biological activity. Moreover, SAR and in silico docking studies indicated that all methyl groups on the diphenyl ether moiety of thielocin B1 contribute to the potent and selective inhibition of the PAC3 homodimer via hydrophobic interactions.

Keywords

protein-protein interaction inhibitors, protein assembling chaperon 3 (PAC3), natural product analogues, multi-substituted benzene

1. Introduction

The interfacial spaces in protein−protein interactions (PPIs) are expected to be novel and potent therapeutic targets because PPIs are involved in many vital biological processes, such as cell growth, DNA replication, and transcriptional activation.[1] Although recent research has revealed that small molecule compounds could inhibit PPIs by interacting with high-affinity regions ("hot spots")[2–4] on the surface of PPIs, a few PPI interface inhibitors have been reported.[5] A structure-based drug design would certainly be effective for predicting the interaction models between small molecules and hot spots, such as shallow grooves or indentations; however, hot spots are not always located in the grooves. This makes applying conventional lock-and-key-based drug

design methodology to various PPIs difficult.

In 2009, Hashimoto *et al*. conducted high-throughput screening to identify PPI inhibitors for cancer cells.[6] After the exhaustive screening of over 250,000 diverse samples with a natural products library, thielocin B1 (**1**) (Figure 1), which was isolated from the fermentation broth of *Thielavia terricola* RF-143,[7] was found to be a potent inhibitor of the proteasome assembling chaperone (PAC) 3 homodimer.[8, 9] The IC_{50} value of **1** was 0.020 μM for the PAC3 homodimer, whereas **1** did not inhibit PAC1/PAC2 heterodimer[10] (IC_{50} > 250 μM). Therefore, **1** has a potential to be a superior proteasome inhibitor targeting the PPI interface.

We recently reported the first total synthesis of **1** and its spin-labeled analogue to characterize the interaction mechanism of **1** as a PPI inhibitor of the PAC3 homodimer.[11] The NMR experiments of ^{15}N-labeled PAC3 using the synthetic compounds showed inhibition on the PPI interface of the PAC3 homodimer but not on monomeric PAC3. On the basis of the NMR titration studies and in silico docking simulation, we concluded that **1** approached the PPI interface of the PAC3 homodimer in areas that did not contain a cavity. Thereafter, **1** destabilizes the structure of the PAC3 homodimer by insertion into the homodimer interface via the terminal carboxylic acid group of **1**, thereby inducing the dissociation of PAC3 to the monomeric form. This rare inhibition mechanism, i.e., a small molecule targeting the PPI interface of multimeric proteins, is remarkable.[12, 13] Our interest then shifted to the relationship between the central benzene ring of **1** and the PPI inhibitory activity. Since other natural thielocins, such as thielocin A1β (**2**) and B3 (**3**) (Figure 1), did not inhibit the PAC3 homodimer even at higher concentrations (IC_{50} > 250 μM) despite the structural resemblance to **1**, the central core moiety seems to be closely involved in the biological activity. The central core of **1** is a fully substituted benzene ring and a component of the electron-rich 2,2',6,6'-tetrasubstituted diphenyl ether moiety, whereas it is not clear what structural feathers are important for the PPI inhibition. Therefore, the elucidation of structure activity relationships focused on the central benzene ring of **1** would be a worthwhile endeavor. In the current

study, we report the synthesis of thielocin B1 analogues bearing an alternative core structure and the evaluation of their inhibitory activity against the PAC3 homodimer.

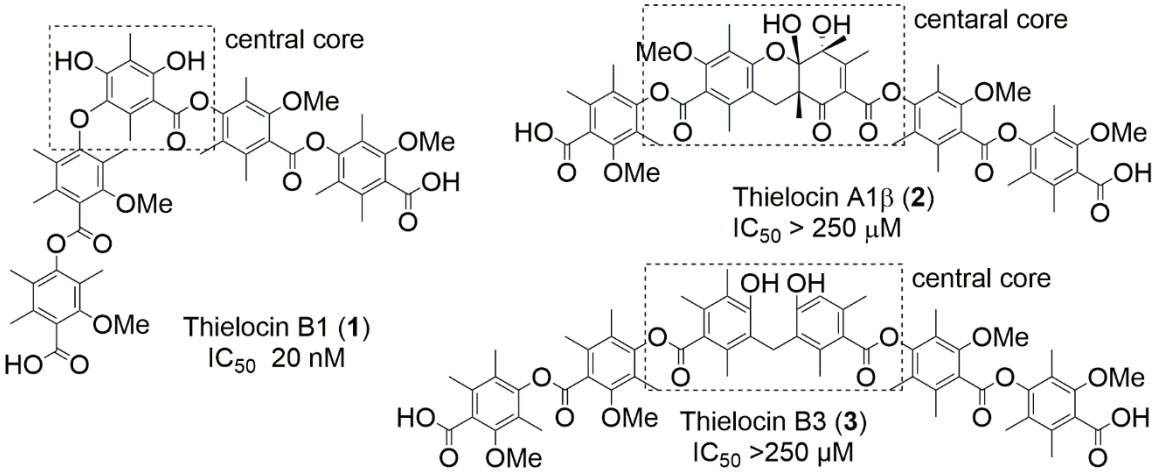


Figure 1. Structures and PPI inhibitory activity of natural thielocins for the PAC3 homodimer.

2. Results and Discussion

To investigate the important features in **1** as a potent and selective PPI inhibitor of the PAC3 homodimer, we designed various analogues of **1**, and its retrosynthetic analysis is shown in Figure 2. Based on the results of the PPI inhibitor screening with natural thielocins, we initially changed the central benzene ring of **1** to other carbon tethers with similar length while keeping side substructures. Thus, symmetric *meta*-disubstituted aromatic rings as C-5 cores and 1,4-disubstituted aliphatic carbon chains as C-4 cores were selected (Figure 2, a). Symmetric diether and diester analogues **4**–**7** will be readily prepared from the corresponding dibromide or dicarboxylic acids by etherification or esterification with 2 equivalents of the phenol **8**, respectively (Figure 2, b). The other analogues **9** were designed to elucidate the substituent effects on the fully substituted diphenyl ether moiety of **1**. The stepwise esterification of the diphenyl ether **10** containing the synthetic equivalent of two carboxylic acid groups with two different phenols **8** and **11** will afford unsymmetrical analogues **9** (Figure 2, c). To validate the importance of the free carboxylic acid groups in **1**, we decided to evaluate the inhibitory activity of the

di-methoxymethyl (MOM) esters of these analogues as well.

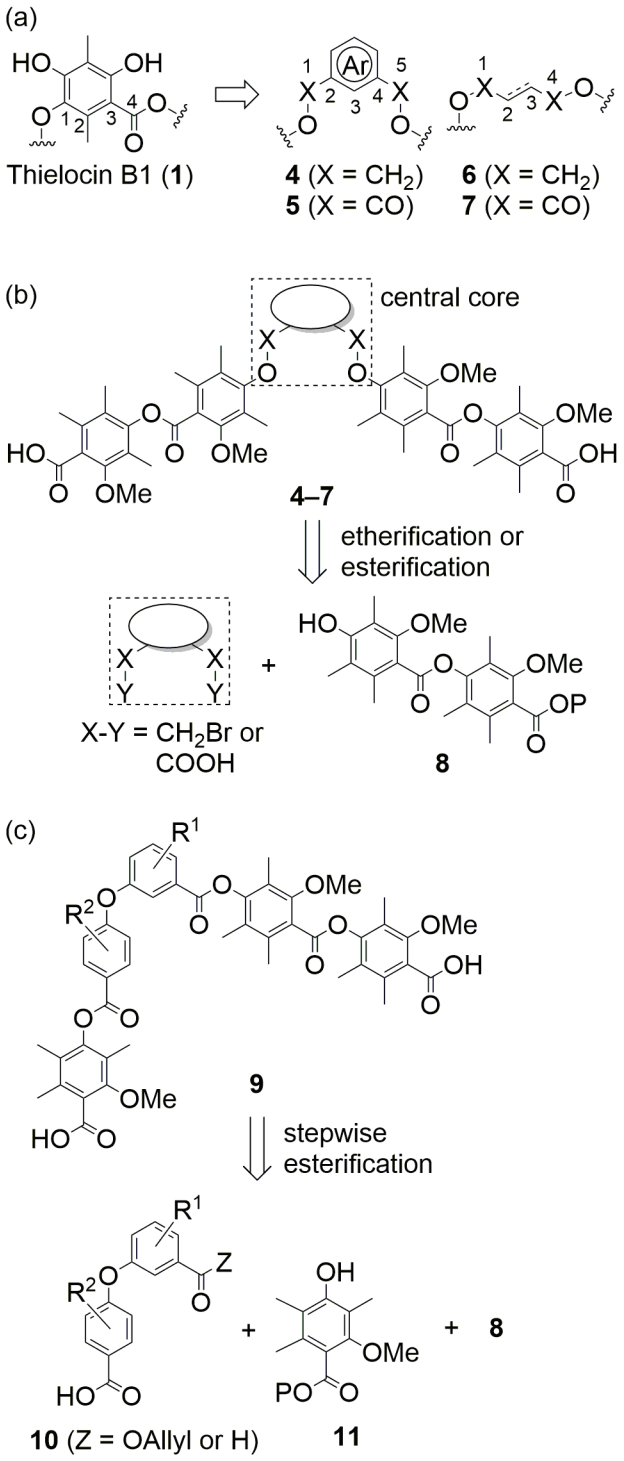
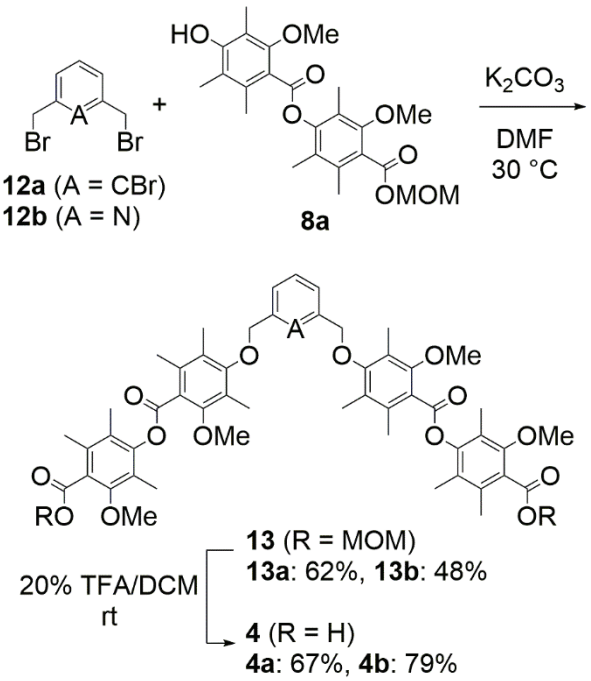


Figure 2. (a) Designed analogues based on the structure of thielocin B1 (**1**). (b) Retrosynthetic analysis of symmetric diether and diester analogues **4**–**7**. (c) Retrosynthetic analysis of unsymmetrical analogues **9**.

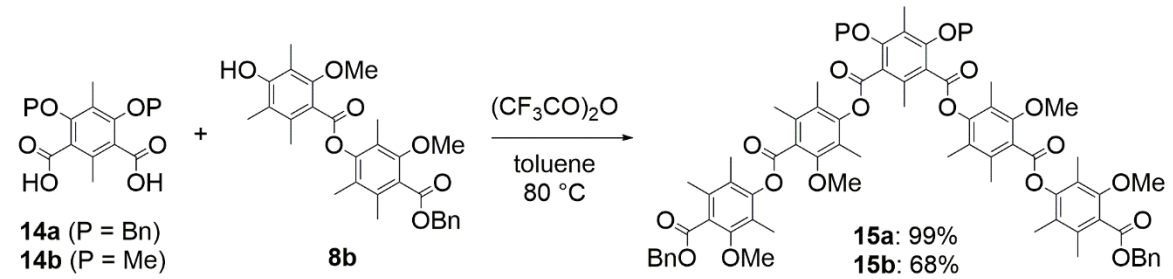
The preparation of the dibenzylic ether analogues **4** followed the procedure shown in Scheme **1**. The

etherification of the dibromides **12**[14] with the phenol **8a**[14] using K_2CO_3 produced desired diethers **13** in moderate yields. All MOM groups in **13** were removed immediately with 20% TFA/DCM to provide the analogues **4** in 67%–79% yields.



Scheme 1. Synthesis of dibenzylic ether analogues **4**.

The synthesis of the diester analogues **5** followed the procedure in Scheme 2. The esterification of the dicarboxylic acids **14**[14] with the phenol **8b**[11] at sterically hindered position proceeded smoothly using the same method for the preparation of **1**. Treatment with excess trifluoroacetic anhydride[11, 15–18] under heating conditions produced desired diesters **15** in good yields. Next, transformation of **15** to **5** was performed, as summarized in Table 1. Removal of all benzyl groups in **15a** through hydrogenolysis afforded the analogue **5a** in 60% yield (entry 1). By contrast, the benzyl groups in **15b** were readily removed using iodotrimethylsilane, which was generated in situ, to produce the analogue **5b** in 94% yield (entry 2). Removal of the benzyl groups in **15b** and subsequent formation of di-MOM esters provided the analogue **5c** in 96% yield over two steps (entry 3).



see table 1

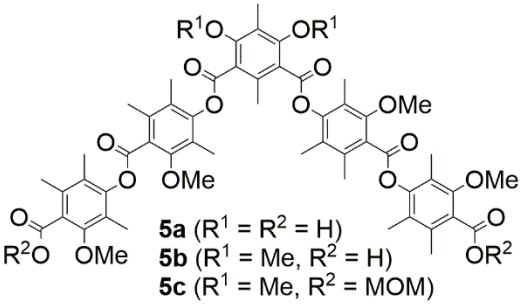


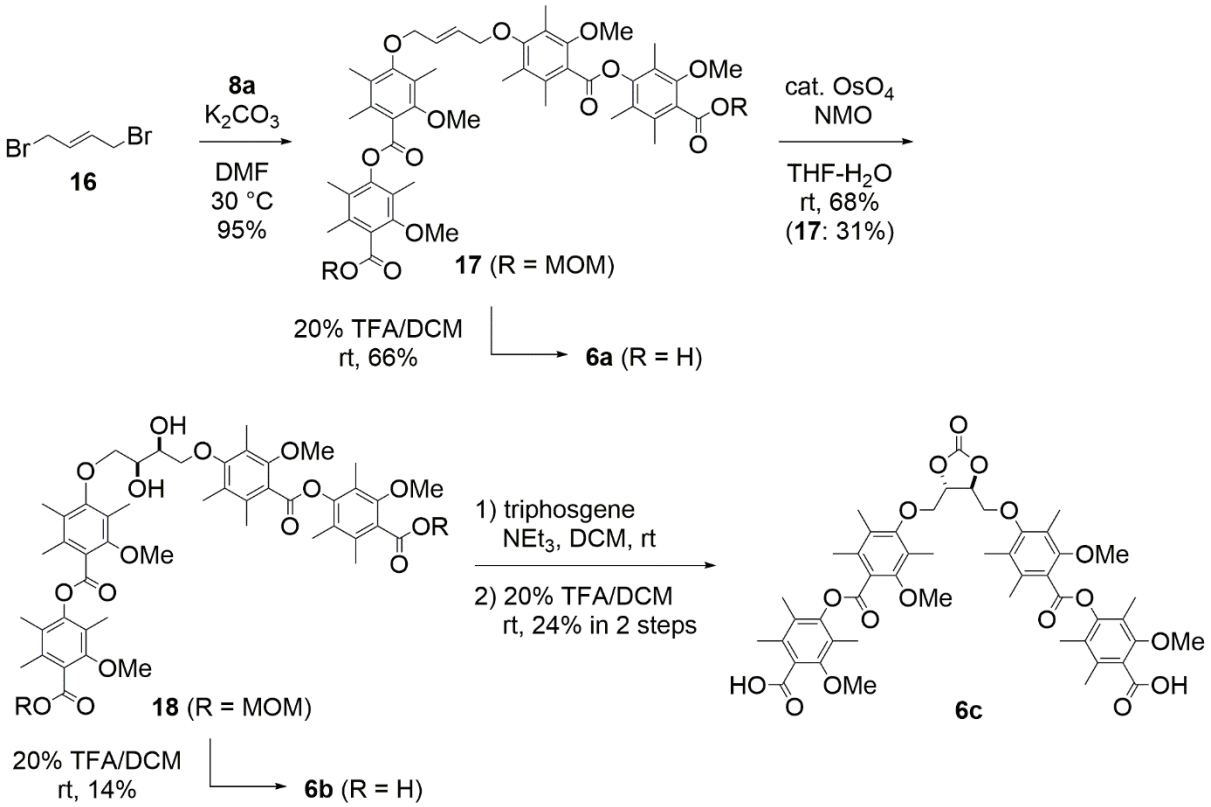
Table 1 Transformation of **15** to **5**.

Entry	Substrate	Conditions	Product
1	**15a**	H_2, 10% Pd/C EtOH-EtOAc, rt	**5a** (60%)
2	**15b**	I_2, $(Me_3Si)_2$ DCM, –40 °C	**5b** (94%)
3	**15b**	1) I_2, $(Me_3Si)_2$ DCM, –40 °C 2) MOMCl, DIPEA DMF, rt	**5c** (96% in 2 steps)

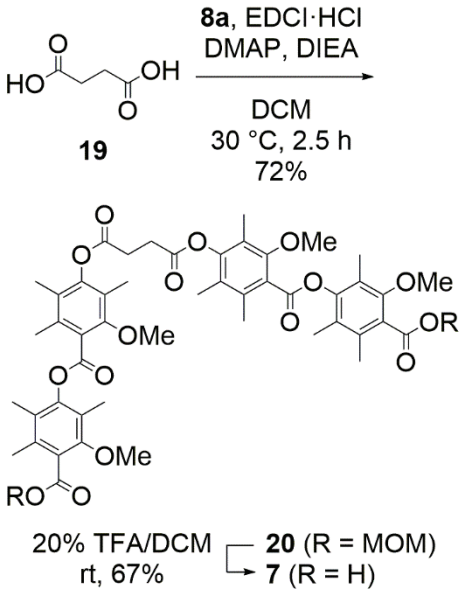
Scheme 2. Synthesis of diester analogues **5**.

Analogues **6** and **7**, which bear aliphatic carbon chains as a central core, were synthesized as follows. The etherification of *trans*-1,4-dibromo-2-butene (**16**) with the phenol **8a** proceeded under the same conditions in the preparation of **13** to provide the diether **17** in 95% yield. The oxidation of the alkene moiety in **17** then carried out in the presence of a catalytic amount of OsO_4. Although the reaction did not complete despite further addition of OsO_4, desired diol **18** was obtained in 68% after separation from **17** by silica gel column chromatography. Finally, the MOM groups in **17** and **18** were removed with 20% TFA/DCM to furnish the analogues **6a** and **6b** in 66% and 14% yields, respectively. Moreover, carbonate formation for **18** using triphosgene followed by removal of the MOM groups under acidic conditions furnished the analogue **6c** in 24% yield over two steps. The yields of **6b** and **6c** were quite lower than those of the other dicarboxylic acid analogues due to the poor solubility in the eluents used during preparative TLC purification (Scheme 3). The esterification of succinic acid (**19**) with the phenol **8a** also proceeded successfully using EDCI·HCl/DMAP to provide the diester **20**. Subsequent removal of all MOM groups in **20** with 20% TFA/DCM afforded the

analogue **7** in 67% yield (Scheme 4).

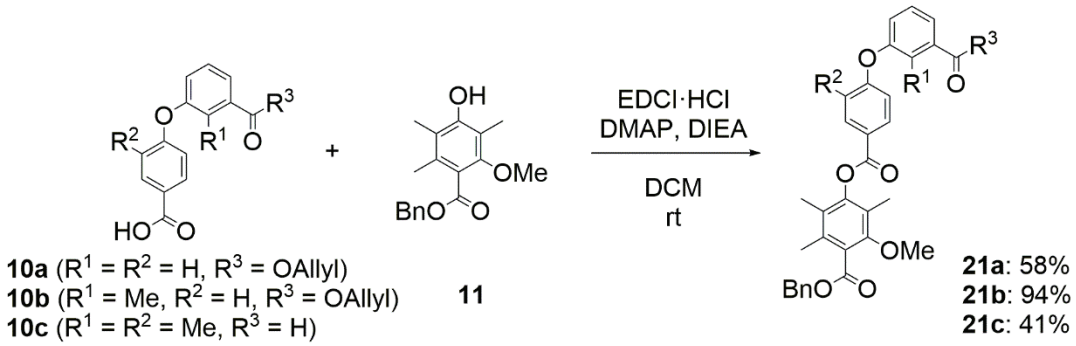


Scheme 3. Synthesis of diether analogues **6**.



Scheme 4. Synthesis of the diester analogue **7**.

The synthesis of unsymmetrical analogues **9**, having the same linkage system with **1**, is shown in Scheme 5. The carboxylic acids **10** were prepared by a conventional Ullmann reaction[14] and esterified with the phenol **11**[11] using EDCI·HCl/DMAP to give the esters **21** in moderate to high yields. After removal of the allyl group in **21a** and **21b** in the presence of a catalytic amount of $Pd(PPh_3)_4$ (Table 2, entries 1 and 2) or oxidation of the formyl group in **21c** with $NaClO_2$ in *t*BuOH-H_2O[19-21] (Table 2, entry 3), further esterification of the resulting carboxylic acids **22** with the phenol **8b** was performed, as summarized in Table 3. The carboxylic acid **22a** was smoothly esterified with the phenol **10b** with EDCI·HCl/DMAP to provide the ester **23a** in 57% yield (entry 1). In contrast, the esterification of sterically hindered 2-substituted benzoic acids **22b** and **22c** with the **8b** proceeded using triphosgene via the in situ formation of the acid chlorides to furnish the corresponding the esters **23b** and **23c**, respectively (entries 2 and 3). Finally, removal of all benzyl groups in **23** by hydrogenolysis afforded the analogues **9** in 59%–83% yields.



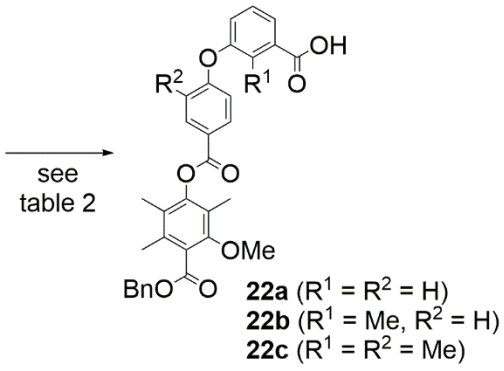


Table 2 Synthesis of carboxylic acids **22**.

Entry	Substrate	Conditions	Product
1	**21a**	$Pd(PPh_3)_4$, morpholine THF, rt	**22a** (84%)
2	**21b**	$Pd(PPh_3)_4$, morpholine THF, rt	**22b** (89%)
3	**21c**	$NaClO_2$, NaH_2PO_4 2-methyl-2-butene *t*BuOH-H_2O, rt	**22c** (98%)

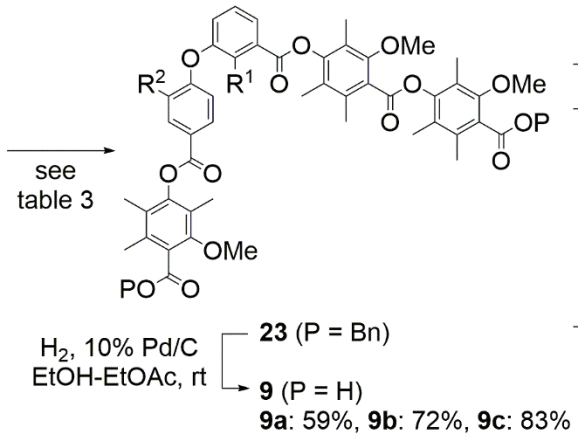


Table 3 Synthesis of esters **23**.

Entry	Substrate	Conditions	Product
1	**22a**	**8b**, EDCI·HCl, DMAP DIEA, DCM, 30 °C	**23a** (57%)
2	**22b**	**8b**, triphosgene, NEt_3 DMAP, DCM, rt	**23b** (79%)
3	**22c**	**8b**, triphosgene, NEt_3 DMAP, DCM, rt	**23c** (69%)

Scheme 5. Synthesis of unsymmetrical analogues **9**.

With the synthetic thielocin analogues in hand, the PPI inhibitory activity for the PAC3 homodimer and PAC1/PAC2 were evaluated using an in vitro protein-fragment complementation assay with monomeric Kusabira-Green (mKG) fluorescent protein in vitro.[6, 22, 23, 24] Split inactive mKG fragments are fused to target proteins, which emit fluorescence when 2 target proteins interact to allow the reformation of the active mKG. Hence, a fluorescent signal by the active mKG depends on the fused target PPI. Decrease of a fluorescent

intensity by synthetic analogues were converted to the percentage of inhibition, as summarized in Figure 3. In short, symmetric diether and diester analogues **4a**-**b**, **5a**-**b**, **6a**-**c** and **7** actually inhibited the PAC3 homodimer at higher concentration than **1**, whereas these analogues worked as poor inhibitors for PAC1/PAC2. Compared to **4a**-**b** and **5b**, corresponding di-MOM esters **13a**-**b** and **5c** did not inhibit both the PAC3 homodimer and PAC1/PAC2 at even 10 μM. These results strongly support the proposed inhibition mechanism: one terminal carboxylic acid group in **1** is critical for disrupting the PAC3 homodimer. Among them, it should be noted that the diester **5a**, which has a quite similar structure to that of **1** (one ester linkage in **5a** vs. one ether linkage in **1**), expressed poor and nonselective inhibitory activity for both protein complexes. Besides, the free hydroxyl groups on the central core in **1** do not seem to be important for its bioactivity because the PPI inhibitory activity of the dimethoxy **5b** were similar to those of the dihydroxy **5a**. Interestingly, skeletally mimicking-analogues **9a**–**c** inhibited both the PAC3 homodimer and PAC1/PAC2 at 10 μM, and the PPI inhibitory activity were actually higher than the other analogues. These facts suggest that tightly bending structure in the central core of **1** is important for the potent PPI inhibitory activity and that the hydrophobic interactions with all the methyl groups on the 2,2',6,6'-tetrasubstituted diphenyl ether moiety in **1** facilitate the selective inhibition of the PAC3 homodimer. We also validated a substructure of **1** as a PPI inhibitor, and the carboxylic acid **8c** (P = H) was found to be unworking. Therefore, the entire structure in **1** is indispensable for its unique PPI inhibitory activity.

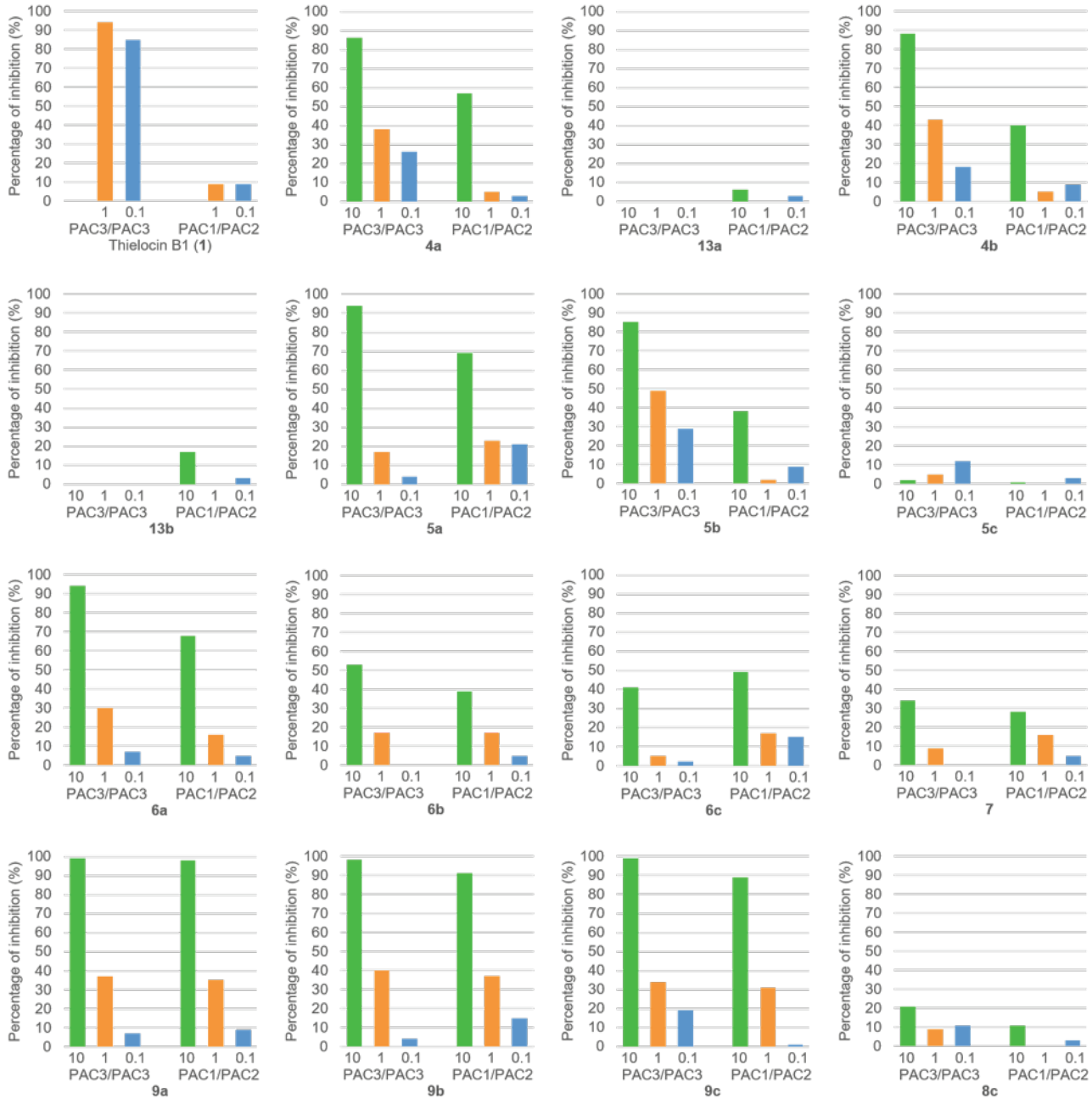


Figure 3. PPI inhibitory activity of synthetic analogues for the PAC3 homodimer (left) and PAC1/PAC2 (right). Inhibition rates of a DMSO solution of compounds for both protein complexes are shown as bars in green (10 µM), orange (1 µM) and blue (0.1 µM).

We next carried out an in silico docking study for the natural **1** and the analogue **5a** at the interface of PAC3 (PDB code: 2Z5E)[9] using the Molecular Operating Environment program[25] to validate the importance of the

hydrophobicity on the diphenyl ether moiety. Because the analogue **5a** possesses an ester linkage instead of an ether linkage in the natural **1**, the mode of interaction to PAC3 would be different among thielocin B1 (**1**) and its analogue **5a**. As expected, the docking study suggested that the unique bent structure induced by diphenyl ether linkage of **1** fit nicely to the hill-like β-sheet structure of PAC3, and the methyl groups on the aryl groups assisted the interaction of **1** to PAC3 by hydrophobic interaction (Figure 4, left). On the other hand, a carbonyl group of the ester linkage in **5a** significantly provoked structural change, which would reduce an intensity of hydrophobic interaction due to detachment of several methyl groups from the surface of PAC3 (Figure 4, right). The above observations concluded that sophisticated structure of **1** is crucial to exhibit potent and selective inhibition of PAC3 homodimer.

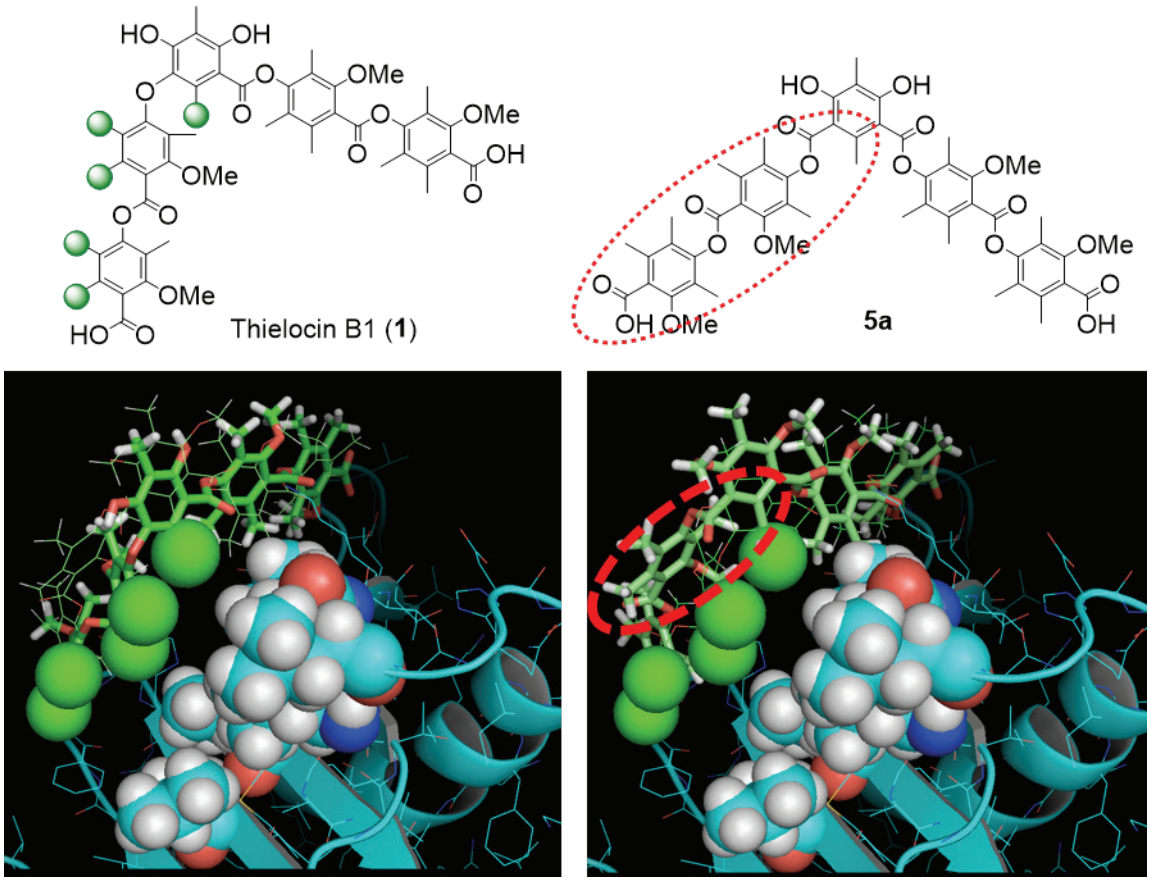


Figure 4. In silico docking study at the interface of PAC3 for thielocin B1 (left) and diester analogue **5a** (right). Thielocins are shown as stick model (carbons in green). Hydrophobic residues on β-sheet structure of PAC3 are

shown as CPK model (carbons in blue). The five methyl groups in **1** involved in hydrophobic interactions are shown in both figures (CPK model, carbons in green). The corresponding methyl groups in **5a** (see red dashed circle) are apart from those in **1**.

3. Conclusion

In conclusion, we have demonstrated the synthesis of thielocin B1 (**1**) analogues containing various central cores and evaluated their PPI inhibitory activity for the PAC3 homodimer and PAC1/PAC2. Symmetric diether and diester analogues **4–7** were obtained in a short sequence of steps by etherification or esterification of the corresponding dibromides or dicarboxylic acids with two equivalents of the phenol **8**, respectively. The skeletally mimicking-analogues **9** were furnished by the stepwise esterification of the diphenyl ether **21** containing the synthetic equivalents of carboxylic acids with the phenols **11** and **8b**. The evaluation of these synthetic analogues as PPI inhibitors of the PAC3 homodimer and PAC1/PAC2 with monomeric Kusabira-Green fluorescent protein in vitro revealed that the *meta*-substituted benzene bearing ether and ester linkages is an essential core structure for potent PPI inhibition. In addition, the terminal carboxylic acid groups were found to be critical for biological activity, whereas the hydroxyl groups on the central core were not. Moreover, the demethylated analogues **9** exhibited nonselective inhibition for both the PAC3 homodimer and PAC1/PAC2. In silico docking study for the natural **1** at the interface of PAC3 suggested that all the methyl groups including 2,2',6,6'-tetrasubstituted diphenyl ether moiety in **1** contribute to the strong interaction at the interface of PAC3 via hydrophobic interactions at huge area. Therefore, almost the entire structure of **1**, containing the synthetically difficult multi-substituted diphenyl ether moiety, is indispensable for potent PPI inhibitory activity. It is worthwhile that structurally complicated natural product such as **1** covered the chemical space unsupported by simplified synthetic compounds.

4. Experimental section

4.1. General Techniques

All commercially available chemicals and solvents were used as received. Dry THF and DCM (Kanto Chemical Co.) were obtained by passing commercially available pre-dried, oxygen-free formulations. Microwave irradiation was performed with Biotage Initiator^{TM}. All reactions in solution phase were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) with UV light and visualized with *p*-anisaldehyde H_2SO_4–ethanol solution or phosphomolybdic acid ethanol solution. Flash column chromatography was carried out with silica gel 60N (Kanto Chemical Co. 40–100 μm). ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on JEOL JNM-AL400 spectrometer. Chemical shifts (δ) are reported in units parts per million (ppm) relative to the signal for internal tetramethylsilane (0.00 ppm for ^{1}H) for solutions in chloroform-*d*. NMR spectral data are reported as follows: chloroform (7.26 ppm for ^{1}H), chloroform-*d* (77.0 ppm for ^{13}C), methanol (3.30 ppm for ^{1}H) or methanol-d_4 (49.0 ppm for ^{13}C) when internal standard is not indicated. Multiplicities are reported by using following abbreviations: s (singlet), d (doublet), t (triplet), m (multiplet), dd (double doublet), dt (double triplet), dq (double quartet), ddd (double double doublet), ddt (double double triplet) and *J* (coupling constants in Hertz). Mass spectra and high-resolution mass spectra were measured with JEOL JMS-DX303 (for EI) or Thermo Scientific Exactive^{TM} Plus Orbitrap Mass Spectrometer (for ESI) instruments. IR spectra were recorded with Shimadzu FT-IR, and the data are given in cm^{-1}. Only the strongest and/or structurally important absorptions are reported. Melting points were measured with Round Science Inc. RFS-10 and are uncorrected.

*4.2. General procedure for the synthesis of the diethers **13**.*

To a solution of the dibromides **12**[14] and the phenol **8a**[14] in dry DMF (2.0 mL) was added K_2CO_3 at room temperature under an argon atmosphere. After being stirred at 30 °C, the reaction mixture was diluted with EtOAc and quenched with 1 M aqueous HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel to afford the diethers **13**.

*4.2.1. The diether **13a**.*

Conditions: the dibromide **12a** (15.0 mg, 43.8 μmol, 1 equiv), the phenol **8a** (44.9 mg, 101 μmol, 2.3 equiv), K_2CO_3 (36.3 mg, 263 μmol, 6.0 equiv), 15 h; Purification: flash column chromatography on silica gel (eluted with toluene/EtOAc = 10:1); Yield: 62% (29.2 mg, 27.2 μmol) as a white solid; mp 76–77 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (2H, d, J = 7.8 Hz), 7.54 (1H, t, J = 7.8 Hz), 5.49 (4H, s), 4.93 (4H, s), 3.84 (6H, s), 3.82 (6H, s), 3.58 (6H, s), 2.40 (6H, s), 2.30 (6H, s), 2.29 (3H, s), 2.27 (6H, s), 2.26 (6H, s), 2.25 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 166.5, 157.6, 154.7, 153.6, 149.6, 137.2, 133.6, 132.5, 128.1, 127.8, 127.2, 126.7, 125.8, 124.7, 122.6, 122.2, 121.6, 91.1, 73.6, 62.1, 61.9, 57.9, 17.2, 16.7, 13.0, 12.8, 10.2, 9.9; IR (neat) 2998, 2941, 1735, 1576, 1460, 1322, 1280, 1150, 1100 cm^{-1}; HRMS[ESI] calcd for $C_{56}H_{65}BrO_{16}Na$ $[M+Na]^+$ 1095.3348, found 1095.3324.

*4.2.2. The diether **13b**.*

Conditions: the dibromide **12b** (15.0 mg, 56.6 μmol, 1.0 equiv), the phenol **8a** (58.1 mg, 130 μmol, 2.3 equiv), K_2CO_3 (46.9 mg, 340 μmol, 6.0 equiv), 14 h; Purification: flash column chromatography on silica gel (eluted with hexane/EtOAc = 3:1); Yield: 48% (27.1 mg, 27.2 μmol) as a white solid; mp 71–72 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (1H, t, J = 7.6 Hz), 7.70 (2H, d, J = 7.6 Hz), 5.49 (4H, s), 4.95 (4H, s), 3.83 (6H, s), 3.82 (6H, s), 3.58 (6H, s), 2.39 (6H, s), 2.31 (6H, s), 2.28 (3H, s), 2.27 (12H, s), 2.25 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ

168.0, 166.6, 157.6, 156.8, 154.7, 153.6, 149.6, 137.7, 133.6, 132.5, 127.2, 126.6, 125.8, 124.6, 122.4, 122.2, 120.3, 91.1, 74.7, 62.1, 62.0, 57.9, 17.2, 16.7, 13.0, 12.7, 10.2, 9.9; IR (neat) 2941, 1739, 1577, 1459, 1322, 1280, 1149, 1101 cm^{-1}; HRMS[ESI] calcd for $C_{55}H_{65}NO_{16}Na$ $[M+Na]^+$ 1018.4196, found 1018.4174.

*4.3. General procedure for the synthesis of the carboxylic acids **4**.*

To a solution of the MOM esters **13** in dry DCM (1.6 mL) was added TFA (0.4 mL) at room temperature under an argon atmosphere. After being stirred at the same temperature, the reaction mixture was concentrated in vacuo, and the resulting residue was purified by preparative TLC to afford the carboxylic acids **4**.

*4.3.1. The carboxylic acid **4a**.*

Conditions: the MOM ester **13a** (25.0 mg, 23.3 μmol, 1 equiv), 30 min; Purification: preparative TLC (eluted with $CHCl_3$/MeOH = 3:1); Yield: 67% (15.3 mg, 15.5 μmol) as a white solid; mp >300 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.72 (2H, d, J = 7.6 Hz), 7.53 (1H, t, J = 7.6 Hz), 5.01 (4H, s), 3.83 (6H, s), 3.79 (6H, s), 2.35 (6H, s), 2.29 (6H, s), 2.26 (6H, s), 2.24 (6H, s), 2.21 (6H, s), 2.20 (6H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 176.6, 168.2, 158.9, 156.0, 153.3, 148.6, 138.7, 136.2, 134.7, 131.7, 130.4, 128.9, 127.9, 126.3, 126.0, 124.2, 123.8, 122.4, 75.1, 62.4, 62.0, 17.5, 17.1, 13.4, 13.0, 10.6, 10.3; IR (neat) 3393, 3242, 2945, 1739, 1688, 1574, 1170 cm^{-1}; HRMS[ESI] calcd for $C_{52}H_{57}BrO_{14}Na$ $[M+Na]^+$ 1007.2824, found 1007.2813.

*4.3.1. The carboxylic acid **4b**.*

Conditions: the MOM ester **13b** (24.0 mg, 24.1 μmol, 1 equiv), 50 min; Purification: preparative TLC (eluted with $CHCl_3$/MeOH = 9:1); Yield: 79% (17.3 mg, 19.1 μmol) as a white solid; mp >300 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (1H, t, J = 7.6 Hz), 7.71 (2H, d, J = 7.6 Hz), 4.97 (4H, s), 3.83 (6H, s), 3.79 (6H, s), 2.34 (6H, s), 2.29 (6H, s), 2.27 (6H, s), 2.24 (6H, s), 2.21 (6H, s), 2.19 (6H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 176.6, 168.2, 158.8, 158.0, 156.0, 153.3, 148.6, 139.5, 136.3, 134.7, 131.7, 127.8, 126.3, 126.0, 123.6, 122.5, 122.4, 75.8, 62.4,

62.0, 17.5, 17.1, 13.4, 12.9, 10.6, 10.2; IR (neat) 3403, 2940, 1735, 1576, 1165, 1092 cm^{-1}; HRMS[ESI] calcd for $C_{51}H_{57}NO_{14}Na$ $[M+Na]^+$ 930.3671, found 930.3656.

*4.4. General procedure for the synthesis of the diesters **15**.*

To a solution of the carboxylic acids **14**[14] and the phenol **8b**[11] in dry toluene (2.0 mL) was added $(CF_3CO)_2O$ at room temperature under an argon atmosphere. After being stirred at 80 °C, the reaction mixture was cooled to room temperature and quenched with saturated aqueous $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried over $MgSO_4$ 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 = 4:1) to afford the diesters **15**.

*4.4.1. The diester **15a**.*

Conditions: the carboxylic acid **14a** (30.0 mg, 73.9 μmol, 1 equiv), the phenol **8b** (87.3 mg, 177 μmol, 2.4 equiv), $(CF_3CO)_2O$ (309 μL, 2.22 mmol, 30 equiv), 10 h; Yield: 99% (99.5 mg, 73.4 μmol) as a white solid; mp 103–104 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.34–7.48 (10H, m), 5.40 (4H, s), 5.07 (4H, s), 3.79 (6H, s), 3.71 (6H, s), 2.73 (3H, s), 2.39 (3H, s), 2.36 (6H, s), 2.25 (6H, s), 2.21 (6H, s), 2.18 (6H, s), 2.17 (6H, s), 2.12 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 166.2, 165.4, 157.6, 154.3, 153.6, 149.9, 149.4, 136.3, 135.6, 133.5, 133.1, 132.7, 128.6, 128.52, 128.48, 128.3, 127.9, 127.4, 126.6, 126.1, 125.8, 125.7, 124.6, 122.5, 122.2, 76.7, 67.1, 62.1, 62.0, 18.3, 17.3, 16.8, 13.5, 13.0, 10.7, 10.5, 10.2; IR (neat) 2940, 1743, 1733, 1574, 1277, 1188, 1149, 1176, 753 cm^{-1}; HRESIMS calcd for $C_{82}H_{82}O_{18}Na$ $[M+Na]^+$ 1377.5393, found 1377.5377.

*4.4.2. The diester **15b**.*

Conditions: the carboxylic acid **12b** (20.0 mg, 78.7 μmol, 1 equiv), the phenol **10b** (89.1 mg, 181 μmol, 2.3 equiv), $(CF_3CO)_2O$ (307 μL, 2.20 mmol, 28 equiv); Yield: 68% (64.6 mg, 53.7 μmol) as a white solid; mp 111–

112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46–7.48 (4H, s), 7.34–7.40 (6H, m), 5.40 (4H, s), 3.91 (6H, s), 3.85 (6H, s), 3.71 (6H, s), 2.62 (3H, s), 2.42 (6H, s), 2.37 (3H, s), 2.33 (6H, s), 2.29 (6H, s), 2.26 (6H, s), 2.22 (6H, s), 2.18 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 166.1, 165.3, 158.9, 154.4, 153.7, 149.9, 149.5, 135.7, 133.6, 133.3, 132.7, 128.6, 128.5, 128.3, 127.4, 126.7, 126.1, 125.7, 125.1, 123.7, 122.4, 122.2, 67.1, 62.2, 62.02, 62.00, 17.7, 17.3, 16.7, 13.2, 13.0, 10.5, 10.2, 9.9; IR (neat) 2942, 1744, 1575, 1277, 1148, 1076 cm^{-1}; HRESIMS calcd for $C_{70}H_{74}O_{18}Na$ $[M+Na]^+$ 1225.4767, found 1225.4748.

*4.5. Preparation of the carboxylic acid **5a**.*

To a solution of the benzyl ester **15a** (33.0 mg, 24.3 μmol, 1 equiv) in EtOH (1.0 mL) and EtOAc (1.0 mL) was added 10% Pd/C (30.0 mg, 91 wt%) at room temperature under an argon atmosphere, and the flask was purged with hydrogen 3 times. After being stirred at the same temperature for 30 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 $CHCl_3$/MeOH = 3:1) to afford the carboxylic acid **5a** (14.5 mg, 14.6 μmol, 60%) as a white solid. mp >300 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 3.81 (6H, s), 3.80 (6H, s), 2.90 (3H, s), 2.39 (6H, s), 2.28 (12H, s), 2.23 (6H, s), 2.21 (6H, s), 2.19 (6H, s), 2.18 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 169.8, 167.7, 163.2, 155.7, 153.8, 151.1, 149.4, 140.6, 134.5, 132.4, 128.1, 127.3, 126.4, 123.5, 122.8, 111.5, 111.4, 62.8, 62.3, 21.9, 17.6, 17.0, 13.5, 13.4, 10.8, 10.6, 8.5; IR (neat) 3402, 2939, 1748, 1662, 1577, 1458, 1308, 1155, 1075 cm^{-1}; HRESIMS calcd for $C_{54}H_{58}O_{18}Na$ $[M+Na]^+$ 1017.3515, found 1017.3511.

*4.6. Preparation of the carboxylic acid **5b**.*

To a solution of I_2 (15.8 mg, 62.3 μmol, 5.0 equiv) in dry DCM (1.0 mL) was added $(Me_3Si)_2$ (12.5 μL, 62.3 μmol, 5.0 equiv) at room temperature under an argon atmosphere. After being stirred at 40 °C for 30 min, the

reaction mixture was cooled to room temperature. To the resulting brown mixture was added the benzyl ester **15b** (15.0 mg, 12.5 μmol, 1 equiv) in dry DCM (1.0 mL) dropwise at −78 °C under an argon atmosphere. After being stirred at −40 °C for 30 min, the reaction mixture was quenched with 1 M aqueous HCl at −40 °C and stirred at room temperature for 15 min. The mixture was concentrated in vacuo, and the resulting residue was purified by preparative TLC (eluted with $CHCl_3$/MeOH = 3:1) to afford the carboxylic acid **5b** (12.0 mg, 11.7 μmol, 94%) as a white solid. mp >300 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 3.92 (6H, s), 3.84 (6H, s), 3.83 (6H, s), 2.60 (3H, s), 2.42 (6H, s), 2.39 (3H, s), 2.32 (6H, s), 2.30 (12H, s), 2.23 (6H, s), 2.22 (6H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 176.7, 167.8, 166.6, 160.4, 155.7, 153.3, 151.2, 148.5, 136.4, 134.6, 134.2, 131.7, 128.3, 127.4, 126.3, 126.0, 125.3, 123.7, 122.4, 62.8, 62.0, 18.1, 17.6, 17.2, 13.5, 13.4, 10.9, 10.6, 10.2; IR (neat) 3372, 2941, 1745, 1577, 1459, 1158, 1074 cm^{-1}; HRESIMS calcd for $C_{56}H_{62}O_{18}Na$ $[M+Na]^+$ 1045.3828, found 1045.3817.

*4.7. Preparation of the di-MOM ester **5c**.*

To a solution of I_2 (31.6 mg, 125 μmol, 5.0 equiv) in dry DCM (1.0 mL) was added $(Me_3Si)_2$ (25.2 μL, 125 μmol, 5.0 equiv) at room temperature under an argon atmosphere. After being stirred at 40 °C for 30 min, the reaction mixture was cooled to room temperature. To the resulting brown mixture was added the benzyl ester **12b** (15.0 mg, 12.5 μmol, 1 equiv) in dry DCM (1.0 mL) dropwise at −78 °C under an argon atmosphere. After being stirred at −40 °C for 15 min, the reaction mixture was quenched with 1 M aqueous HCl at −40 °C and stirred at room temperature for 15 min. The mixture was concentrated in vacuo, and the resulting mixture was used for next reaction without further purification.

To the solution of the crude carboxylic acid in DMF (1.0 mL) were added DIEA (17.1 μL, 125 μmol, 10 equiv) and MOMCl (9.5 μL, 125 μmol, 10 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 2.5 h, the reaction mixture was diluted with EtOAc and quenched with 1 M

aqueous HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with $CHCl_3$/MeOH = 29:1) to afford the di-MOM ester **5c** (13.3 mg, 12.0 μmol, 96% in 2 steps) as a white solid. mp 222–223 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.50 (4H, s), 3.92 (6H, s), 3.87 (6H, s), 3.82 (6H, s), 3.58 (6H, s), 2.63 (3H, s), 2.43 (6H, s), 2.38 (3H, s), 2.34 (6H, s), 2.30 (6H, s), 2.29 (6H, s), 2.27 (6H, s), 2.25 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 166.1, 165.3, 158.9, 154.4, 153.6, 150.0, 149.5, 133.6, 133.3, 132.6, 127.3, 126.7, 126.1, 125.8, 125.1, 123.7, 122.4, 122.2, 91.1, 62.2, 62.1, 62.0, 57.9, 17.7, 17.4, 16.7, 13.2, 13.0, 10.5, 10.3, 9.9; IR (neat) 2942, 1745, 1575, 1277, 1202, 1146, 1076 cm^{-1}; HRESIMS calcd for $C_{60}H_{70}O_{20}Na$ $[M+Na]^+$ 1133.4353, found 1133.4344.

*4.8. Preparation of the diether **17**.*

To a solution of *trans*-1,4-dibromo-2-butene (**16**) (20.0 mg, 93.5 μmol, 1 equiv) and the phenol **8a** (96.0 mg, 215 μmol, 2.3 equiv) in dry DMF (2.0 mL) was added K_2CO_3 (77.5 mg, 561 μmol, 6.0 equiv) at room temperature under an argon atmosphere. After being stirred at 30 °C for 16 h, the reaction mixture was diluted with EtOAc and quenched with 1 M aqueous HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with toluene/EtOAc = 9:1) to afford the diether **15** (83.7 mg, 88.6 μmol, 95%) as a white solid. mp 188–189 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.20 (2H, s), 5.49 (4H, s), 4.38 (4H, s), 3.82 (6H, s), 3.81 (6H, s), 3.58 (6H, s), 2.37 (6H, s), 2.30 (6H, s), 2.28 (6H, s), 2.26 (6H, s), 2.25 (6H, s), 2.24 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0, 166.6, 157.8, 154.6, 153.6, 149.6, 133.5, 132.5,

128.6, 127.2, 126.6, 125.8, 124.4, 122.4, 122.2, 91.1, 72.3, 62.1, 61.9, 57.9, 17.2, 16.7, 13.0, 12.8, 10.2, 10.0; IR (neat) 2941, 1738, 1576, 1460, 1322, 1280, 1149, 1100, 1022, 757 cm^{-1}; HRESIMS calcd for $C_{52}H_{64}O_{16}Na$ $[M+Na]^+$ 967.4087, found 967.4065.

*4.9. Preparation of the carboxylic acid **6a**.*

To a solution of the MOM ester **17** (23.0 mg, 24.3 μmol) in dry DCM (1.6 mL) was added TFA (0.4 mL) at room temperature under an argon atmosphere. After being stirred at the same temperature for 30 min, the reaction mixture was concentrated in vacuo, and the resulting residue was purified by preparative TLC (eluted with $CHCl_3$/MeOH = 9:1) to afford the carboxylic acid **6a** (13.8 mg, 16.1 μmol, 66%) as a white solid. mp >300 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 6.20 (4H, s), 4.40 (4H, s), 3.83 (6H, s), 3.78 (6H, s), 2.33 (6H, s), 2.282 (6H, s), 2.275 (6H, s), 2.24 (6H, s), 2.20 (6H, s), 2.19 (6H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 168.3, 159.0, 156.0, 153.3, 148.6, 136.4, 134.5, 131.6, 129.9, 127.8, 126.0, 123.6, 122.3, 75.5, 62.4, 62.0, 17.8, 17.1, 13.4, 13.0, 10.6, 10.3; IR (neat) 3421, 2941, 1738, 1569, 1456, 1164, 1092 cm^{-1}; HRESIMS calcd for $C_{48}H_{569}NO_{14}Na$ $[M+Na]^+$ 879.3562, found 879.3549.

*4.10. Preparation of the diol **18**.*

To a solution the olefin **17** (40.0 mg, 42.3 μmol, 1 equiv) in dry THF (1.0 mL) and water (1.0 mL) were added NMO (16.1 mg, 137 μmol, 3.2 equiv) and OsO_4 (0.05 M in THF, 9.2 μL, 458 nmol, 0.02 equiv) at 0 °C, and the mixture was stirred at room temperature for 24 h. To the reaction mixture were added NMO (16.1 mg, 137 μmol, 3.2 equiv) and OsO_4 (0.05 M in THF, 9.2 μL, 458 nmol, 0.02 equiv) at 0 °C. After being stirred at room temperature for 24 h, the reaction mixture was diluted with EtOAc and quenched with saturated aqueous $Na_2S_2O_3$. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The

combined organic layers were washed with brine, dried over $MgSO_4$ 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 = 1:2) to afford the diol **18** (28.0 mg, 28.6 μmol, 68%) as a white solid. mp 183–184 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.49 (4H, s), 4.38 (2H, t, J = 4.9 Hz), 3.99 (4H, d, J = 4.9 Hz), 3.82 (6H, s), 3.81 (6H, s), 3.58 (6H, s), 2.37 (6H, s), 2.32 (6H, s), 2.27 (6H, s), 2.26 (12H, s), 2.24 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 166.5, 157.0, 154.7, 153.6, 149.6, 133.7, 132.5, 127.2, 126.4, 125.8, 124.8, 122.23, 122.21, 91.1, 73.8, 70.2, 62.1, 62.0, 57.9, 17.2, 16.7, 13.0, 12.6, 10.2, 9.8; IR (neat) 3487, 2941, 1739, 1623, 1575, 1459, 1151, 755 cm^{-1}; HRESIMS calcd for $C_{52}H_{66}O_{18}Na$ $[M+Na]^+$ 1001.4141, found 1001.4130.

*4.11. Preparation of the carboxylic acid **6b**.*

To a solution of the MOM ester **18** (25.0 mg, 25.5 μmol, 1 equiv) in dry DCM (1.6 mL) was added TFA (0.4 mL) at room temperature under an argon atmosphere. After being stirred at the same temperature for 30 min, the reaction mixture was concentrated in vacuo, and the resulting residue was purified by preparative TLC (eluted with $CHCl_3$/MeOH = 9:1) to afford the carboxylic acid **6b** (3.1 mg, 3.48 μmol, 14%) as a white solid. mp >300 °C; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD = 9:1) δ 4.20 (2H, t, J = 4.6 Hz), 3.86–3.94 (4H, m), 3.743 (6H, s), 3.741 (6H, s), 2.29 (6H, s), 2.25 (6H, s), 2.21 (6H, s), 2.20 (6H, s), 2.18 (6H, s), 2.15 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD = 9:1) δ 166.9, 157.2, 154.6, 152.8, 148.8, 133.5, 132.1, 126.4, 125.6, 124.4, 122.2, 121.8, 73.6, 70.0, 61.9, 61.8 17.0, 16.6, 12.8, 12.5, 10.0, 9.6; IR (neat) 3283, 2924, 1731, 1693, 1625, 1570, 1460, 1278, 1160, 1093 cm^{-1}; HRESIMS calcd for $C_{48}H_{58}O_{16}Na$ $[M+Na]^+$ 913.3617, found 963.3607.

*4.12. Preparation of the carboxylic acid **6c**.*

To a solution of the diol **18** (17.0 mg, 17.4 μmol, 1 equiv) in dry DCM (2.0 mL) were added Et_3N (43.3 μL,

313 μmol, 18 equiv) and triphosgene (7.7 mg, 26.1 μmol, 1.5 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 1 h, the reaction mixture was diluted EtOAc and quenched with 1 M aqueous HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was used for next reaction without further purification.

To a solution of the crude MOM ester in dry DCM (1.6 mL) was added TFA (0.4 mL) at room temperature under an argon atmosphere. After being stirred at the same temperature for 30 min, the reaction mixture was concentrated in vacuo, and the resulting residue was purified by preparative TLC (eluted with $CHCl_3$/MeOH = 9:1) to afford the carboxylic acid **6c** (3.8 mg, 4.14 μmol, 24% over 2 steps) as a white solid. mp >300 °C; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 5:1) δ 5.08 (2H, s), 4.02 (4H, s), 3.701 (6H, s), 3.698 (6H, s), 2.25 (6H, s), 2.19 (6H, s), 2.16 (6H, s), 2.14 (6H, s), 2.13 (6H, s), 2.10 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ = 5:1) δ 171.4, 166.6, 156.0, 154.5, 154.3, 152.4, 148.6, 133.7, 131.9, 128.9, 126.1, 125.4, 125.0, 122.0, 121.7, 76.4, 70.4, 61.8, 61.7, 16.9, 16.4, 12.7, 12.3, 9.9, 9.5; IR (neat) 3387, 2942, 1779, 1738, 1699, 1575, 1457, 1165, 1094 cm^{-1}; HRESIMS calcd for $C_4H_{56}O_{17}Na$ $[M+Na]^+$ 939.3410, found 939.3398.

*4. 13. Preparation of the ester **20**.*

To a solution of succinic acid (**19**)[14] (60.0 mg, 50.8 μmol, 1 equiv) and the phenol **8a** (52.2 mg, 117 μmol, 2.3 equiv) in dry DCM (2.0 mL) were added DIEA (55.8 μL, 406 μmol, 8.0 equiv), EDCI·HCl (30.0 mg, 152 μmol, 3.0 equiv) and DMAP (0.6 mg, 5.08 μmol, 0.10 equiv) at 0 °C under an argon atmosphere. After being stirred at 30 °C for 21 h, the reaction mixture was diluted EtOAc and quenched with 1 M aqueous HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were

washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ 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 = 2:1) to afford the ester **20** (32.9 mg, 33.7 μmol, 72%) as a white solid. mp 76–77 °C ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.49 (4H, s), 3.809 (6H, s), 3.807 (6H, s), 3.58 (6H, s), 3.13 (4H, s), 2.38 (6H, s), 2.26 (12H, s), 2.23 (6H, s), 2.14 (6H, s), 2.09 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 168.0, 166.1, 154.3, 153.6, 149.8, 149.5, 133.4, 132.5, 127.2, 126.4, 125.8, 122.2, 122.0, 91.1, 62.14, 62.07, 57.9, 28.4, 17.2, 16.7, 13.0, 12.8, 10.2, 10.0; IR (neat) 2942, 1752, 1735, 1459, 1278, 1148, 1129, 756 cm^{-1}; HRESIMS calcd for $C_{52}H_{62}O_{18}Na$ $[M+Na]^+$ 997.3828, found 997.3804.

4.14. Preparation of the carboxylic acid 7.

To a solution of the MOM ester **20** (25.0 mg, 25.6 μmol, 1 equiv) in dry DCM (1.6 mL) was added TFA (0.4 mL) at room temperature under an argon atmosphere. After being stirred at the same temperature for 40 min, the reaction mixture was concentrated in vacuo, and the resulting residue was purified by preparative TLC (eluted with $CHCl_3$/MeOH = 9:1) to afford the carboxylic acid **7** (15.1 mg, 17.0 μmol, 67%) as a white solid. mp >300 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 3.83 (6H, s), 3.78 (6H, s), 3.15 (4H, s), 2.36 (6H, s), 2.28 (6H, s), 2.21 (6H, s), 2.20 (6H, s), 2.12 (6H, s), 2.10 (6H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 172.0, 167.8, 155.6, 153.4, 151.2, 148.7, 134.3, 131.9, 127.9, 127.2, 126.1, 123.4, 122.4, 62.7, 62.1, 29.4, 17.4, 17.1, 13.4, 13.0, 10.6, 10.2; IR (neat) 3386, 2925, 1751, 1574, 1459, 1276, 1160, 1131, 1094 cm^{-1}; HRESIMS calcd for $C_{48}H_{54}NO_{16}Na$ $[M+Na]^+$ 909.3304, found 909.3288.

*4.15. General procedure for the synthesis of the ester **21**.*

To a solution of the carboxylic acids **10** and the phenol **11** in dry DCM (2.0 mL) were added DIEA,

EDCI·HCl and DMAP at 0 °C under an argon atmosphere. After being stirred at room temperature, the reaction mixture was diluted EtOAc and quenched with 1 M aqueous HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel to afford the esters **21**.

*4.15.1. The ester **21a**.*

Conditions: the carboxylic acid **10a** (100 mg, 335 μmol, 1 equiv), the phenol **11** (121 mg, 402 μmol, 1.2 equiv), DIEA (184 μL, 1.34 mmol, 4.0 equiv), EDCI·HCl (98.9 mg, 503 μmol, 1.5 equiv), DMAP (4.1 mg, 33.5 μmol, 0.10 equiv), 3.5 h; Purification: flash column chromatography on silica gel (eluted with hexane/EtOAc = 9:1); Yield: 58% (113 mg, 195 μmol) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (2H, d, J = 9.0 Hz), 7.92 (1H, dt, J = 7.9, 1.6 Hz), 7.79 (1H, t, J = 1.6 Hz), 7.50 (1H, t, J = 7.9 Hz), 7.46–7.48 (2H, m), 7.30–7.41 (4H, m), 7.08 (2H, d, J = 9.0 Hz), 6.03 (1H, ddt, J = 17.3, 10.6, 5.7 Hz), 5.41 (1H, dq, J = 17.3, 1.3 Hz), 5.40, (2H, s), 5.30 (1H, dq, J = 10.6, 1.3 Hz), 4.83 (1H, dt, J = 5.7, 1.3 Hz), 3.68 (3H, s), 2.16 (3H, s), 2.09 (3H, s), 2.05 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 165.3, 163.5, 162.0, 155.6, 153.6, 149.5, 135.6, 132.6, 132.5, 132.4, 131.9, 130.2, 128.6, 128.5, 128.3, 127.2, 125.8, 125.5, 124.7, 123.6, 121.9, 121.1, 118.5, 117.7, 67.1, 65.9, 62.0, 16.6, 12.7, 9.8; IR (neat) 2941, 1735, 1730, 1583, 1503, 1281, 1267, 1161, 1096 cm^{-1}; HRESIMS calcd for $C_{35}H_{32}O_8Na$ $[M+Na]^+$ 603.1989, found 603.1978.

*4.15.2. The ester **21b**.*

Conditions: the carboxylic acid **10b** (100 mg, 320 μmol, 1 equiv), the phenol **11** (115 mg, 384 μmol, 1.2 equiv), DIEA (176 μL, 1.28 mmol, 4.0 equiv), EDCI·HCl (94.5 mg, 480 μmol, 1.5 equiv), DMAP (3.9 mg, 32.0 μmol, 0.10 equiv), 8.5 h; Purification: flash column chromatography on silica gel (eluted with hexane/EtOAc = 9:1); Yield: 94% (179 mg, 301 μmol) as a yellowish oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (2H, d, J = 9.0 Hz), 7.81

(1H, dd, *J* = 8.0, 1.2 Hz), 7.45–7.48 (2H, m), 7.33–7.40 (3H, m), 7.31 (1H, t, *J* = 8.0 Hz), 7.20 (1H, dd, *J* = 8.0, 1.2 Hz), 6.95 (2H, d, *J* = 9.0 Hz), 6.06 (1H, ddt, *J* = 17.5, 10.5, 5.9 Hz), 5.44 (1H, dq, *J* = 17.5, 1.4 Hz), 5.39 (2H, s), 5.32 (1H, dq, *J* = 10.5, 1.4 Hz), 4.84 (1H, dt, *J* = 5.9, 1.4 Hz), 3.68 (3H, s), 2.45 (3H, s), 2.16 (3H, s), 2.08 (3H, s), 2.04 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 167.1, 163.8, 162.9, 153.9, 153.8, 149.8, 135.9, 133.0, 132.9, 132.8, 132.3, 128.89, 128.85, 128.6, 127.8, 127.5, 127.2, 125.8, 125.2, 123.1, 122.2, 119.0, 116.5, 67.4, 66.1, 62.3, 17.0, 13.7, 13.0, 10.1; IR (neat) 2940, 1733, 1605, 1503, 1456, 1281, 1247, 1161, 1097, 755 cm^{-1}; HRESIMS calcd for $C_{36}H_{34}O_8Na$ $[M+Na]^+$ 617.2146, found 617.2133.

*4.15.3. The ester **21c**.*

Conditions: the carboxylic acid **10c** (40.0 mg, 148 μmol, 1 equiv), the phenol **11** (53.3 mg, 178 μmol, 1.2 equiv), DIEA (81.2 μL, 592 μmol, 4.0 equiv), EDCI·HCl (43.7 mg, 222 μmol, 1.5 equiv), DMAP (13.8 mg, 14.8 μmol, 0.10 equiv), 21 h; Purification: flash column chromatography on silica gel (eluted with toluene/EtOAc = 12:1); Yield: 41% (33.7 mg, 61.0 μmol) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.3 (1H, s), 8.15 (1H, d, *J* = 1.5 Hz), 7.97 (1H, dd, *J* = 8.7, 1.5 Hz), 7.71 (1H, d, *J* = 7.6 Hz), 7.46-7.47 (2H, m), 7.33-7.41 (4H, m), 7.18 (1H, d, *J* = 7.6 Hz) , 6.62 (1H, d, *J* = 8.7 Hz), 5.39 (2H, s), 3.68 (3H, s), 2.56 (3H, s), 2.45 (3H, s), 2.16 (3H, s), 2.08 (3H, s), 2.04 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.0, 168.3, 163.7, 160.2, 154.3, 153.6, 149.6, 136.2, 135.6, 133.6, 132.53, 132.45, 129.8, 128.6, 128.5, 128.4, 128.30, 128.26, 127.3, 127.1, 125.5, 125.4, 123.1, 121.9, 115.0, 67.1, 12.0, 16.6, 16.2, 12.7, 11.2, 9.8; IR (neat) 2941, 1733, 1701, 1607, 1577, 1462, 1249, 1170, 1100, 755 cm^{-1}; HRESIMS calcd for $C_{34}H_{32}O_7Na$ $[M+Na]^+$ 575.2040, found 574.2033.

*4.16. General Procedure for the synthesis of the carboxylic acids **22a** and **22b**.*

To a solution of the allyl esters **21** in dry THF (2.0 mL) were added morpholine and $Pd(PPh_3)_4$ at room temperature under an argon atmosphere, and the mixture was stirred at the same temperature for 2 h. To the

reaction mixture were added further morpholine and $Pd(PPh_3)_4$ at room temperature. After being stirred at the same temperature for 2.5 h, the reaction mixture was diluted with EtOAc. The organic layer was washed with saturated 1 M aqueous HCl and brine, dried over $MgSO_4$ 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 = 4:1) to afford the carboxylic acids **22**.

*4.16.1. The carboxylic acid **22a**.*

Conditions: the allyl ester **21a** (100 mg, 172 μmol, 1 equiv), morpholine (60.0 μL, 688 μmol, 4.0 equiv), $Pd(PPh_3)_4$ (39.8 mg, 34.4 mmol, 0.20 equiv), 2 h then morpholine (60.0 μL, 688 μmol, 4.0 equiv), $Pd(PPh_3)_4$ (19.9 mg, 17.2 μmol, 0.10 equiv), 2.5 h; Yield: 84% (78.1 mg, 144 μmol) as a white solid; mp 74–75 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (2H, d, J = 9.0 Hz), 7.96 (1H, dt, J = 8.0, 1.5 Hz), 7.82 (1H, t, J = 1.5 Hz), 7.53 (1H, t, J = 8.0 Hz), 7.46–7.48 (2H, m), 7.32–7.40 (4H, m), 7.09 (2H, d, J = 9.0 Hz), 5.40 (2H, s), 3.68 (3H, s), 2.16 (3H, s), 2.09 (3H, s), 2.05 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 168.4, 163.5, 161.8, 155.8, 153.6, 149.5, 135.6, 132.6, 131.4, 130.3, 128.6, 128.5, 128.3, 127.2, 126.3, 125.5, 123.7, 121.9, 121.4, 117.9, 67.1, 62.0, 16.6, 12.7, 9.8; IR (neat) 3068, 2930, 1738, 1733, 1583, 1504, 1451, 1281, 1245, 1162, 1097, 756 cm^{-1}; HRESIMS calcd for $C_{32}H_{28}O_8Na$ $[M+Na]^+$ 563.1676, found 563.1673.

*4.16.2. The carboxylic acid **22b**.*

Conditions: the allyl ester **21b** (175 mg, 394 μmol, 1 equiv), morpholine (76.9 μL, 882 μmol, 4.0 equiv), $Pd(PPh_3)_4$ (68.0 mg, 58.9 μmol, 0.20 equiv), 1.5 h then morpholine (76.9 μL, 882 μmol, 4.0 equiv), $Pd(PPh_3)_4$ (34.0 mg, 29.5 μmol, 0.10 equiv), 1.5 h; Yield: 89% (145 mg, 261 μmol) as a yellowish solid; mp 87–88 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (2H, d, J = 9.2 Hz), 7.79 (1H, d, J = 7.6 Hz), 7.46-7.48 (2H, m), 7.32-7.39 (4H, m), 7.22 (1H, d, J = 7.6 Hz), 6.98 (2H, d, J = 9.2 Hz), 5.37 (2H, s), 3.62 (3H, s), 2.14 (3H, s), 2.11 (3H, s), 2.03 (3H, s), 2.01 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 170.6. 169.9. 165.2. 164.3. 154.9. 154.8. 150.8. 137.2.

134.9. 133.7. 133.3. 129.8. 129.6. 129.4. 128.64. 128.55. 128.1 126.8. 125.8. 123.8. 123.1. 117.4. 68.2. 62.5. 16.7. 13.6. 12.8. 10.0; IR (neat) 3066, 3018, 2940, 1733, 1696, 1605, 1457, 1247, 1161, 754 cm^{-1}; HRESIMS calcd for $C_{33}H_{30}O_8Na$ $[M+Na]^+$ 577.1833, found 577.1823.

*4.17. Preparation of the carboxylic acid **22c**.*

To a solution of the aldehyde **21c** (33.0 mg, 62.7 μmol, 1 equiv) in *t*BuOH (0.5 mL) and water (0.5 mL) were added 2-methyl-2-butene (0.5 mL), NaH_2PO_4 (19.6 mg, 163 μmol, 2.6 equiv) and $NaClO_2$ (14.7 mg, 163 μmol, 2.6 equiv) at 0 °C. After being stirred at room temperature for 12 h, the organic layer was separated and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried over $MgSO_4$ 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 = 2:1) to afford the carboxylic acid **22c** (35.0 mg, 61.6 μmol, 98%) as a white solid. mp 72–73 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (1H, d, J = 2.1 Hz), 7.97 (1H, dd, J = 8.7, 2.1 Hz), 7.92 (1H, dd, J = 8.1, 1.1 Hz), 7.46–7.48 (2H, m), 7.33–7.40 (4H, m), 7.17 (1H, dd, J = 8.1, 1.1 Hz) , 6.61 (1H, d, J = 8.7 Hz), 5.40 (2H, s), 3.68 (3H, s), 2.53 (3H, s), 2.46 (3H, s), 2.16 (3H, s), 2.08 (3H, s), 2.04 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 168.4, 163.8, 160.5, 154.2, 153.6, 149.6, 135.6, 133.6, 133.12, 132.6, 131.1, 129.9, 128.6, 128.5, 128.3, 128.2, 127.8, 127.1, 126.8, 125.5, 124.8, 122.9, 121.9, 114.9, 67.1, 62.0, 16.6, 16.2, 13.4, 12.7, 9.8; IR (neat) 2941, 1738, 1733, 1695, 1607, 1578, 1456, 1251, 1170, 1121, 1099, 757 cm^{-1}; HRESIMS calcd for $C_{34}H_{32}O_8Na$ $[M+Na]^+$ 591.1989, found 591.1982.

*4.18. Preparation of the ester **23a**.*

To a solution of the carboxylic acid **22a** (61.0 mg, 113 μmol, 1.2 equiv) and the phenol **8b** (46.3 mg, 94.0 μmol, 1 equiv) in dry DCM (2.0 mL) were added DIEA (51.6 μL, 376 μmol, 4.0 equiv), EDCI·HCl (40.0 mg,

203 μmol, 1.8 equiv) and DMAP (1.2 mg, 9.40 μmol, 0.10 equiv) at 0 °C under an argon atmosphere. After being stirred at 30 °C for 40 h, the reaction mixture was diluted EtOAc and quenched with 1 M aqueous HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ 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 the ester **23a** (54.1 mg, 53.3 μmol, 57%) as a white solid. mp 92–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (2H, d, J = 9.0 Hz), 8.11 (1H, dt, J = 7.8, 1.4 Hz), 7.97 (1H, dd, J = 2.4, 1.4 Hz), 7.60 (1H, t, J = 7.8 Hz), 7.32–7.48 (11H, m), 7.15 (2H, d, J = 9.0 Hz), 5.40 (4H, s), 3.82 (3H, s), 3.71 (3H, s), 3.68 (3H, s), 2.40 (3H, s), 2.26 (3H, s), 2.22 (3H, s), 2.18 (3H, s), 2.17 (3H, s), 2.16 (3H, s), 2.12 (3H, s), 2.09 (3H, s), 2.05 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.32, 168.28, 166.1, 163.4, 163.3, 161.7, 156.0, 154.4, 153.6, 149.9, 149.5, 149.4, 135.63, 135.62, 133.5, 132.7, 132.6, 131.0, 130.6, 128.58, 128.55, 128.54, 128.32, 128.31, 127.4, 127.2, 126.5, 126.3, 125.8, 125.7, 125.44, 125.40, 123.9, 122.2, 122.1, 121.9, 121.4, 117.9, 67.08, 67.06, 62.2, 62.02, 62.00, 17.2, 16.7, 16.6, 12.97, 12.95, 12.7. 10.2. 10.1. 9.8; IR (neat) 2931, 1734, 1597, 1583, 1457, 1280, 1151, 1096 cm^{-1}; HRESIMS calcd for $C_{61}H_{58}O_{14}Na$ $[M+Na]^+$ 1037.3719, found 1037.3695.

*4. 19. General procedure for the synthesis of the esters **23b** and **23c**.*

To a solution of the carboxylic acids **22** in dry DCM (1.5 mL) were added Et_3N and triphosgene at 0 °C under an argon atmosphere, and the mixture was stirred at the same temperature for 1 h. To the mixture were added phenol **8b** in dry DCM (1.5 mL) and DMAP at 0 °C. After being stirred at room temperature, the reaction mixture was diluted EtOAc and quenched with 1 M aqueous HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with saturated

aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ 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 the esters **23**.

*4.19.1. The ester **23b**.*

Conditions: the carboxylic acid **22b** (23.0 mg, 41.5 μmol, 1 equiv), Et_3N (17.0 μL, 125 μmol, 3.0 equiv), triphosgene (5.5 mg, 18.7 μmol, 0.45 equiv), the phenol **8b** (24.5 mg, 49.8 μmol, 1.2 equiv), DMAP (0.5 mg, 4.15 μmol, 0.10 equiv), 10 h; Yield: 79% (33.8 mg, 32.8 μmol) as a white solid; mp 92–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (2H, d, J = 8.8 Hz), 8.16 (1H, d, J = 7.6 Hz), 7.31–7.48 (12H, m), 7.01 (2H, d, J = 8.8 Hz), 5.40 (4H, s), 3.84 (3H, s), 3.71 (3H, s), 3.68 (3H, s), 2.57 (3H, s), 2.41 (3H, s), 2.26 (3H, s), 2.23 (3H, s), 2.22 (3H, s), 2.184 (3H, s), 2.176 (3H, s), 2.16 (3H, s), 2.09 (3H, s), 2.05 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.34, 168.29, 166.1, 164.3, 163.5, 162.4, 154.4, 154.0, 153.64, 153.61, 150.0, 149.5, 149.4, 135.64, 135.62, 134.1, 133.5, 132.7, 132.58, 132.56, 130.8, 128.58, 128.55, 128.52, 128.3, 127.9, 127.4, 127.2, 127.1, 126.5, 125.8, 125.74, 125.68, 125.5, 123.0, 122.2, 122.1, 121.9, 116.4, 67.07, 67.06, 62.2, 62.02, 62.00, 17.3, 16.7, 16.6, 13.4, 13.1, 13.0, 12.7, 10.2, 9.8; IR (neat) 3018, 2942, 1739, 1733, 1605, 1453, 1280, 1248, 1161, 1096, 755 cm^{-1}; HRESIMS calcd for $C_{62}H_{60}O_{14}Na$ $[M+Na]^+$ 1051.3875, found 1051.3858.

*4.19.2. The ester **23c**.*

Conditions: the carboxylic acid **22c** (21.0 mg, 36.9 μmol, 1.1 equiv), Et_3N (13.7 μL, 101 μmol, 3.0 equiv), triphosgene (4.5 mg, 15.1 μmol, 0.45 equiv), the phenol **8b** (16.5 mg, 33.6 μmol, 1 equiv), DMAP (0.4 mg, 3.69 μmol, 0.10 equiv), 12 h; Yield: 69% (24.3 mg, 23.3 μmol) as a white solid; mp 94–95 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (1H, d, J = 1.9 Hz), 8.11 (1H, dd, J = 8.0, 1.0 Hz), 8.00 (1H, dd, J = 8.7, 1.9 Hz), 7.46–7.48 (4H, m), 7.32–7.41 (7H, m), 7.21 (1H, dd, J = 8.0, 1.0 Hz) , 6.69 (1H, d, J = 8.7 Hz), 5.40 (4H, s), 3.84 (3H, s), 3.71 (3H, s), 3.68 (3H, s), 2.57 (3H, s), 2,47 (3H, s), 2.41 (3H, s), 2.26 (3H, s), 2.224 (3H, s), 2.220 (3H, s), 2.18 (3H,

s), 2.17 (3H, s), 2.16 (3H, s), 2.09 (3H, s), 2.05 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 168.3, 166.1, 164.4, 163.7, 160.3, 154.5, 154.4, 153.7, 153.6, 150.0, 149.6, 149.5, 135.7, 133.6, 133.5, 133.4, 132.7, 132.6, 130.7, 129.9, 128.58, 128.55, 128.53, 128.3, 127.4, 127.24, 127.15, 127.0, 126.5, 125.8, 125.7, 125.5, 124.6, 124.6, 123.1, 122.2, 122.1, 121.9, 115.1, 67.06, 67.05, 62.2, 62.01, 61.99, 17.2, 16.7, 16.6, 16.2, 13.3, 13.1, 13.0, 12.7, 10.22, 10.20, 9.8; IR (neat) 3016, 2941, 1733, 1607, 1577, 1457, 1279, 1250, 1170, 755 cm^{-1}; HRESIMS calcd for $C_{63}H_{62}O_{14}Na$ $[M+Na]^+$ 1065.4032, found 1065.4009.

*4. 20. General procedure for the synthesis of the carboxylic acids **9**.*

To a solution of the benzyl esters **23** in EtOH (1.5 mL) and EtOAc (1.5 mL) was added 10% Pd/C at room temperature, and the flask was purged with hydrogen 3 times. After being stirred at the same temperature for 30 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 $CHCl_3$/MeOH = 3:1) to afford the carboxylic acids **9**.

*4.20.1. The carboxylic acid **9a**.*

Conditions: the benzyl ester **23a** (20.0 mg, 19.7 μmol, 1 equiv), 10% Pd/C (10.0 mg, 50 wt%); Yield: 59% (9.7 mg, 11.6 μmol) as a white solid; mp >300 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (2H, d, J = 8.8 Hz), 8.13 (1H, dt, J = 8.1, 1.2 Hz), 7.94 (1H, dd, J = 2.3, 1.2 Hz), 7.69 (1H, t, J = 8.1 Hz), 7.51 (1H, ddd, J = 8.1, 2.3, 1.2 Hz), 7.21 (2H, d, J = 8.8 Hz), 3.84 (3H, s), 3.82 (3H, s), 3.81 (3H, s), 2.39 (3H, s), 2.29 (3H, s), 2.27 (3H, s), 2.22 (3H, s), 2.21 (3H, s), 2.15 (3H, s), 2.12 (3H, s), 2.04 (3H, s), 2.03 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 176.6, 167.8, 165.2, 164.8, 163.2, 157.7, 155.6, 153.29, 153.26, 151.2, 148.6, 148.5, 134.5, 133.6, 132.20, 132.16, 131.7, 131.6, 128.1, 127.3, 127.2, 126.8, 126.0, 125.8, 125.5, 123.4, 122.4, 122.2, 122.1, 119.3, 62.8, 62.1, 62.0, 17.4, 17.1, 17.0, 13.4, 13.0, 12.9, 10.6, 10.2, 10.0; IR (neat) 3397, 2930, 1738, 1581, 1264, 1157,

1094 cm^{-1}; HRESIMS calcd for $C_{47}H_{46}O_{14}Na$ $[M+Na]^+$ 857.2780, found 857.2761.

*4.20.2. The carboxylic acid **9b**.*

Conditions: the benzyl ester **23b** (25.0 mg, 24.3 μmol, 1 equiv), 10% Pd/C (10.0 mg, 40 wt%); Yield: 72% (14.9 mg, 17.6 μmol) as a white solid; mp >300 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (2H, d, J = 9.0 Hz), 8.19 (1H, d, J = 8.2 Hz), 7.51 (1H, t, J = 8.2 Hz), 7.40 (1H, d, J = 8.2 Hz), 7.06 (2H, d, J = 9.0 Hz), 3.83 (3H, s), 3.82 (3H, s), 3.81 (3H, s), 2.52 (3H, s), 2.41 (3H, s), 2.29 (3H, s), 2.27 (3H, s), 2.23 (3H, s), 2.22 (3H, s), 2.21 (3H, s), 2.17 (3H, s), 2.04 (3H, s), 2.03 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 176.6, 167.8, 165.8, 165.3, 163.9, 155.7, 155.4, 153.32, 153.28, 151.3, 148.64, 148.58, 136.2, 136.1, 134.8, 134.5, 133.6, 132.0, 131.8, 131.6, 129.1, 128.7, 128.1, 127.2, 127.1, 126.0, 125.8, 124.7, 123.4, 122.4, 122.1, 117.6, 62.8, 62.1, 17.5, 17.1, 17.0, 13.6, 13.4, 13.1, 12.9, 10.6, 10.4, 10.0; IR (neat) 3434, 2929, 1738, 1575, 1252, 1163, 1094 cm^{-1}; HRESIMS calcd for $C_{48}H_{48}O_{14}Na$ $[M+Na]^+$ 871.2936, found 871.2924.

*4.20.3. The carboxylic acid **9c**.*

Conditions: the benzyl ester **23c** (20.0 mg, 19.2 μmol, 1 equiv), 10% Pd/C (10.0 mg, 50 wt%); Yield: 83% (13.7 mg, 15.9 μmol) as a white solid; mp >300 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.14–8.16 (2H, m), 8.01 (1H, dd, J = 8.4, 1.6 Hz), 7.48 (1H, t, J = 7.9 Hz), 7.29 (1H, dd, J = 7.9, 0.8 Hz) , 6.73 (1H, d, J = 8.4 Hz), 3.84 (3H, s), 3.83 (3H, s), 3.82 (3H, s), 2.55 (3H, s), 2.47 (3H, s), 2.41 (3H, s), 2.30 (3H, s), 2.27 (3H, s), 2.23 (3H, s), 2.22 (3H, s), 2.21 (3H, s), 2.18 (3H, s), 2.04 (3H, s), 2.02 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 176.7, 167.8, 165.9, 165.5, 161.6, 156.0, 155.7, 153.3, 153.2, 151.3, 148.6, 148.5, 136.5, 136.3, 134.5, 134.4, 134.1, 132.0, 131.7, 131.5, 131.0, 129.7, 128.6, 128.5, 128.1, 127.2, 126.0, 125.8, 124.8, 123.4, 122.4, 122.1, 116.4, 62.8, 62.1, 17.5, 17.2, 17.0, 16.3, 13.5, 13.4, 13.1, 12.9, 10.6, 10.4, 10.0; IR (neat) 3417, 2942, 1738, 1572, 1457, 1256, 1169, 1093 cm^{-1}; HRESIMS calcd for $C_{49}H_{50}O_{14}Na$ $[M+Na]^+$ 885.3093, found 885.3074.

*4.21. Preparation of 4-((4-Hydroxy-2-methoxy-3,5,6-trimethylbenzoyl)oxy)-2-methoxy-3,5,6-trimethylbenzoic acid (**8c**).*

To a solution of the benzyl ester **8b** (40.0 mg, 81.2 μmol) in EtOAc (2.0 mL) was added 10% Pd/C (10.0 mg, 25 wt%) at room temperature, and the flask was purged with hydrogen 3 times. After being stirred at the same temperature for 1 h, the reaction mixture was filtered through a pad of Celite®. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 1:4) to afford the carboxylic acid **8c** (22.0 mg, 54.7 μmol, 67%) as a white solid. mp 215–216 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 3.79 (3H, s), 3.75 (3H, s), 2.31 (3H, s), 2.25 (3H, s), 2.21 (3H, s), 2.20 (3H, s), 2.18 (3H, s), 2.17 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 172.3, 168.8, 157.1, 156.0, 154.4, 150.7, 140.1, 133.0, 129.7, 127.0, 123.3, 121.3, 120.9, 116.9, 62.50, 62.45, 17.5, 16.9, 13.3, 12.3, 10.5, 9.8; IR (neat) 3472, 2943, 1723, 1578, 1464, 1287, 1161 cm^{-1}; HRESIMS calcd for $C_{22}H_{27}O_7$ $[M+H]^+$ 403.1751, found 403.1754.

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Declarations of interest: none

Supplementary material

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/.

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14. Synthetic protocols of **8a**, **10**, **12** and **14** are described in the supplementary material.

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24. The details of the PPI assay using mKG fluorescent protein are described in the supplementary material.

25. The docking study of thielocin B1 (**1**) and the analogue **5a** with PAC3 was performed as previously reported, see ref. 11.