Synthesis and Phospholipase A_2 Inhibitory Activity of Thielocin B3 Derivatives

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We prepared several types of derivatives of thielocin B3, a very potent naturally occurring inhibitor for human nonpancreatic secretory PLA_2 ($sPLA_2$-II), and conducted a structure−activity relationship study to identify potent $sPLA_2$-II inhibitors with the aim of developing antiinflammatory drugs. The total number of aromatic rings is critical for $sPLA_2$-II inhibition, and the best result was obtained in the case of six rings. The structure of the central part of the inhibitors was not specific, and potent inhibitors were found among the sulfide, sulfone, ether, methylene, and amino derivatives. Although a diester of the terminal carboxylic acid lost its inhibitory activity, having both of the carboxylic acids was not necessary for expression of activity, as illustrated by a glycine derivative with the benzyl ester group **36**. Among the newly synthesized derivatives, **18**, **20**, **29**, and **36** showed very potent human $sPLA_2$-II inhibitory activity comparable to that of natural thielocin B3. Their IC_{50} values are in the range 0.069−0.14 μM, and they are a class of compounds showing the most potent $sPLA_2$-II inhibition to date.

Introduction

Phospholipase A_2 (PLA_2s) is a ubiquitous group of enzymes which specifically catalyze the hydrolysis of the *sn*-2 fatty acyl ester bond of aggregated glycerophospholipids, the major constituents of the cell membrane, to produce fatty acids and lysophospholipids.[1−6] Among the fatty acids, arachidonic acid is particularly important and further metabolized to form prostagladins, leukotriens, and active molecules which are widely implicated in the pathophysiology of inflammation. A second product of PLA_2 action is a lysophospholipid which is believed to be an inducer of some kinds of inflammation and the precursor of platelet-activating factor (PAF) inducing a variety of inflammatory disease states.

There have been many reports regarding low molecular weight (14 kDa) extracellularly secreted enzymes ($sPLA_2$s) isolated from such sources as mammalian pancreas ($sPLA_2$-I)[7] and human synovial fluid ($sPLA_2$-II).[8] As the latter have been detected not only in the synovial fluid of patients with rheumatoid arthritis but also in the serum of patients with sepsis and pancreatitis, this enzyme is now considered to be implicated in many kinds of inflammatory diseases.[9−13]

Although a number of research groups have been reporting the isolation, cloning, and mechanism of action of the intracellular high molecular weight (85 kDa) PLA_2,[14] no potent inhibitors of this cytosolic PLA_2 ($cPLA_2$) have yet been reported to be undergoing clinical evaluation. Recently, researchers of Lilly reported very potent human nonpancreatic $sPLA_2$ ($sPLA_2$-II) inhibitors having an indole structure[15] which were prepared using computer-aided drug design.[16] We have isolated thielocins, potent $sPLA_2$-II inhibitors, from a natural source[17−19] and since then have directed our chemistry efforts to the synthesis of their derivatives to improve the $sPLA_2$-II inhibitory activity. Among the thielocins, thielocin A1 showed very potent $sPLA_2$-II inhibitory activity in rat with an IC_{50} value of 0.0033 μM. Its total synthesis was accomplished by the Merck group;[20] however, it showed rather weak activity for human $sPLA_2$-II with an IC_{50} value of 12 μM.[18] Thielocin B3, on the other hand, is one of the most potent inhibitors (IC_{50}: 0.076 μM) for human $sPLA_2$-II among inhibitors known to date[21] (Figure 1). Unfortunately, production of thielocin B3 from fungi is extremely low, and the pharmacological evaluation is not feasible at this time.[19]

These findings prompted us to pursue thielocin B3 derivatives by chemical synthesis, aiming at the possibility of developing an antiinflammatory drug. Described here are the synthesis and structure−activity relationship (SAR) study of thielocin B3 derivatives.

Chemistry

On the basis of the structure of thielocin B3, chemistry efforts were focused on the synthesis of compounds depicted by the representative formula A in which X is shown in Figure 2.

Multisubstituted phenyl ring units were prepared from ethyl crotonate by procedures reported in the literature[22−24] (Scheme 1). Methylation of **1** with dimethyl sulfate and potassium carbonate, followed by reduction of the formyl group and usual acetylation, gave **3** in 69% yield. Coupling reaction of **3** and **4**, a known compound,[23] using borontrifluoride etherate complex afforded **5** in quantitative yield. Then usual methylation and hydrolysis gave symmetrical dicarboxylic acid **6** in 95% yield. Two important building blocks, **8** and **10**, were obtained by deprotection of the benzyl group using catalytic hydrogenolysis followed by esterification of the resulting phenolic carboxylic acids with diphenyldiazomethane in 93 and 84% yield, respectively. Symmetrical esters having phenyl ring, pyrone ring, or the ethene group in the center of their molecules were prepared by the diacid chloride formation of each dicarboxylic acid with oxalyl chloride followed by esterification with 2 equiv of phenoxide of the phenolic ester **10**. Deprotection of the benzhydryl esters with trifluoroacetic acid and anisole gave **11**, **12**,

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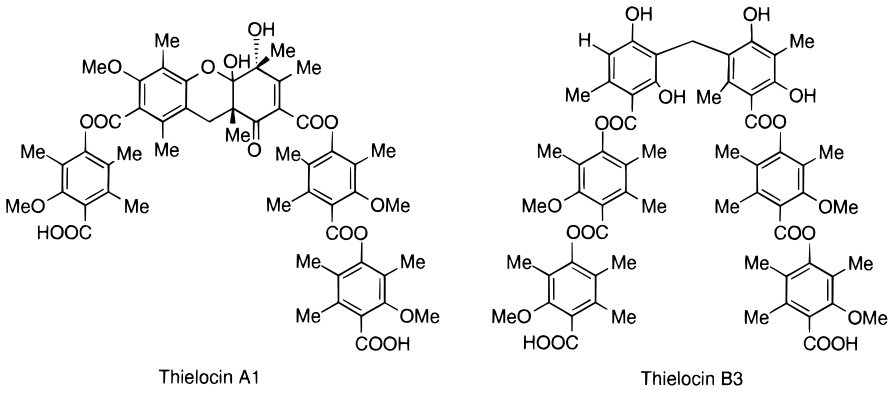


Figure 1.

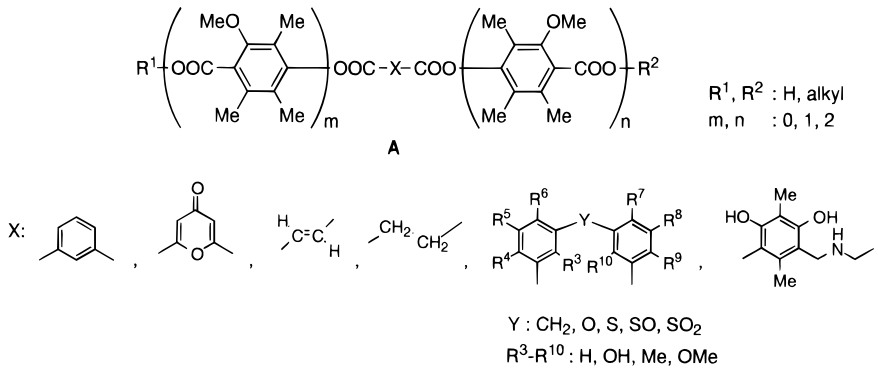
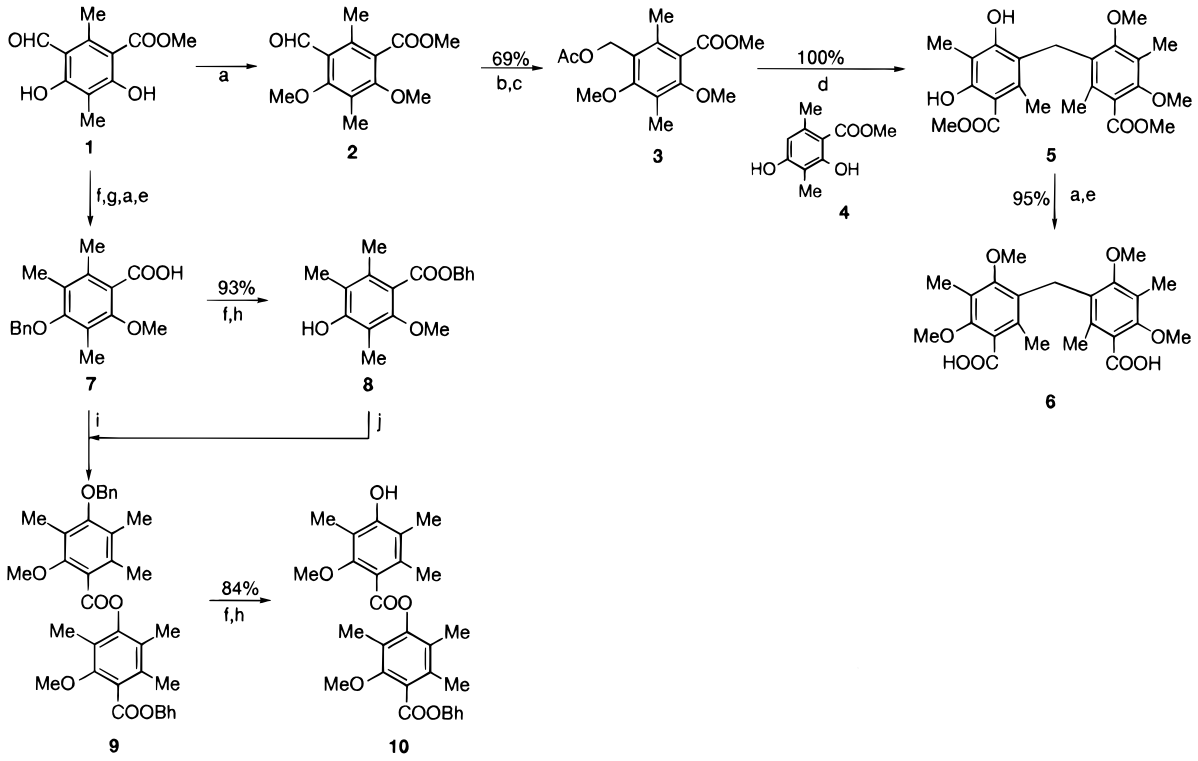


Figure 2. Representative formulas of synthesized compounds.

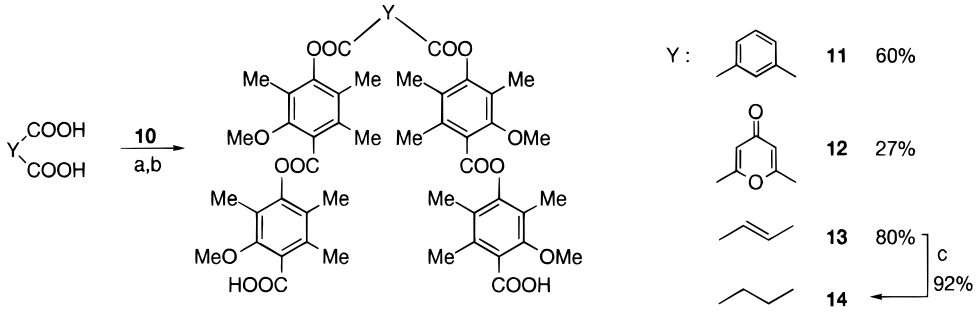
Scheme 1[a]



[a] Reagents: (a) Me_2SO_4, K_2CO_3; (b) $NaBH_4$; (c) Ac_2O, pyridine; (d) BF_3·OEt_2; (e) KOH; (f) H_2, Pd−C; (g) benzyl chloride, K_2CO_3; (h) Ph_2CN_2; (i) oxalyl chloride; (j) *n*-BuLi.

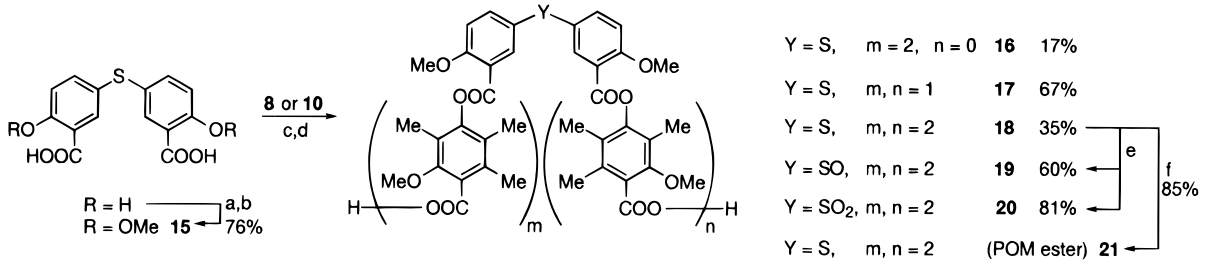
and **13** in 60, 27, and 80% yield, respectively. Usual catalytic reduction of **13** gave the saturated ethylenic compound **14** in 92% yield (Scheme 2). Diphenyl sulfides and their derivatives were obtained from commercially available 5,5′-thiodisalicylic acid in a manner similar to that of symmetrical esters described above. Stoichiometric oxidation of the sulfide **18** with *m*-chloroperbenzoic acid gave the sulfoxide **19**, while use of excess oxidant afforded the sulfone **20** in high yield. To ascertain the necessity of the carboxylic acid function, the (pivaloyloxy)methyl ester of **18** was prepared with (pivaloyloxy)methyl iodide and potassium carbon-

Scheme 2[a]



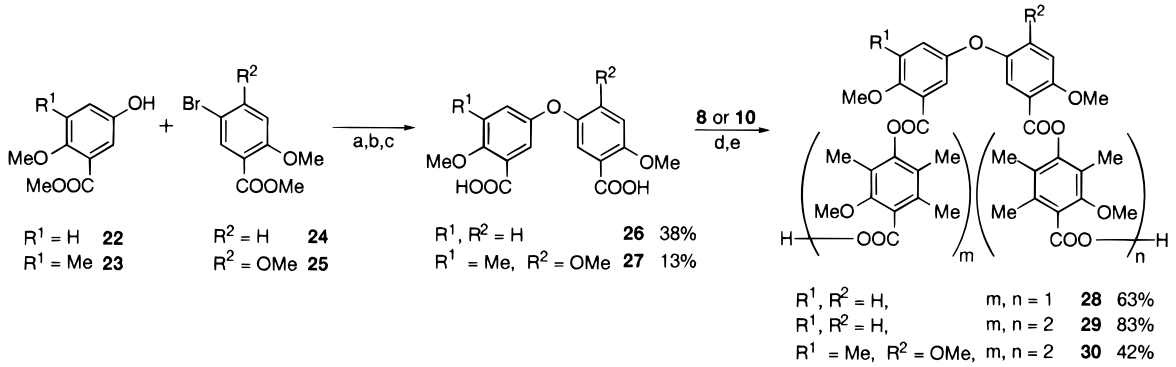
[a] Reagents: (a) oxalyl chloride; (b) TFA, anisole; (c) H_2, Pd−C.

Scheme 3[a]



[a] Reagents: (a) Me_2SO_4, K_2CO_3; (b) KOH, aqueous MeOH; (c) oxalyl chloride; (d) TFA, anisole; (e) *m*-CPBA; (f) (pivaloyloxy)methyl iodide, K_2CO_3.

Scheme 4[a]



[a] Reagents: (a) NaH; (b) CuBr·SMe_2; (c) KOH, aqueous MeOH; (d) oxalyl chloride; (e) TFA, anisole.

ate (Scheme 3). Diphenyl ether derivatives **28**, **29**, and **30** were synthesized by the Ullmann reaction in the presence of copper catalyst. After deprotection of benzhydryl esters, the desired diphenyl ethers were prepared in a similar manner as described above (Scheme 4). Diphenylmethylene derivatives **31**, **32**, and **33** having structural similarity to thielocin B3 were prepared from **6** by using **8** or **10** as an aromatic ring unit in 20, 70, and 61% yield, respectively. In this case, demethylated derivative **34** was synthesized to examine the effect of hydroxy and/or methoxy group for $sPLA_2$ inhibitory activity (Scheme 5). Lastly, structurally diverse compounds having amino groups were prepared by conventional aminomethylene synthesis from thielavin B[25] (Scheme 6). Formylation of thielavin B with hexamethylenetetramine and trifluoroacetic acid, followed by esterification with diphenyldiazomethane to give **35**, which was then subjected to reductive amination with benzylglycine or optically active phenethylamine and $NaBH_3CN$, gave **36**, **37**, or **38**. These amino derivatives were added to examine the effect of an amino group in the thielocin series on $sPLA_2$ inhibition.

Biological Results and Discussion

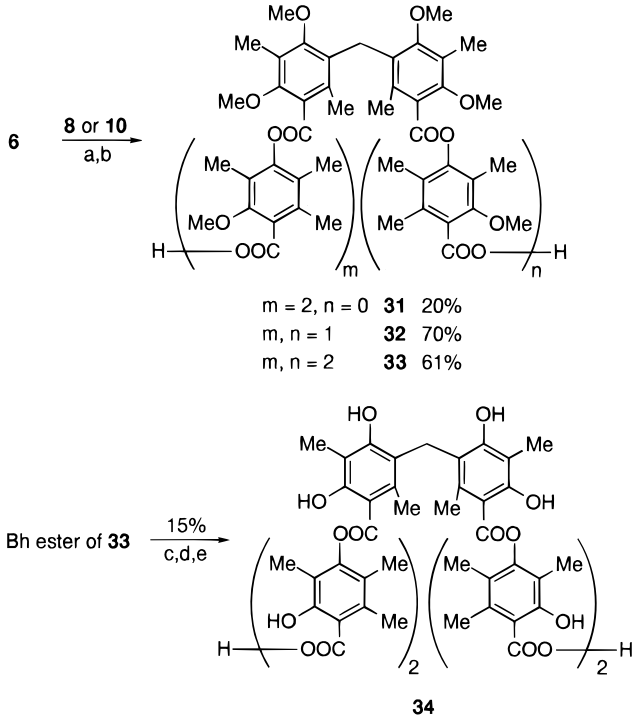
Many nonspecific PLA_2 inhibitors have been reported and considered to affect the "quality of the interface" by modifying phospholipid bilayer properties that render phospholipid inaccessible to the enzyme. Thielocin A1 and B3 were found to be independent of the concentration of the substrate.[17,18]

In addition, the inhibitory activity of thielocin A1 was independent of the physical state of the substrate such as *Escherichia coli* membranes, phospholipids presented as surfactant mixed micelles, or sonicated liposomes and of the type of phospholipids (choline, ethanolamine, or inositol).[17]

Moreover, 100% of the fluorescence of *Naja mocambique* PLA_2 was quenched with the molar ratio of thielocin B3/enzyme at 1.0,[18] suggesting that inhibition of $sPLA_2$ by thielocins occurred by direct interaction with the enzyme.

The characteristic mode of inhibition was also conserved in the newly synthesized compounds, showing that the $sPLA_2$ inhibitory activity of thielocin deriva-

Scheme 5[a]



[a] Reagents: (a) oxalyl chloride; (e) TFA, anisole; (c) BBr_3; (d) Ph_2CN_2; (e) H_2, Pd−C.

tives is not due to direct interaction with the phosphatidylethanolamine substrate.

The newly synthesized thielocin B3 derivatives were found to be generally very specific $sPLA_2$-II inhibitors and inactive for human pancreatic secretory PLA_2 ($sPLA_2$-I) except for diphenyl ether derivatives **29** and **30** and a sulfone derivative **20**. Interestingly, most of the active compounds showed comparable or several times less potent activity for human enzyme than that for rat enzyme (Table 1). Phenylene derivative **11** surprisingly showed potent inhibitory activity with an IC_{50} value 3 times higher than that of thielocin B3, while the pyrone nucleus significantly lowered the activity. Although introduction of double bond **13** did not improve the activity, that of ethylenic single bond **14** maintained it, particularly for rat $sPLA_2$-II. With regard to the total number of ring units, it was presumed from the limited number of natural thielocin derivatives that at least five aromatic rings were necessary for the manifestation of $sPLA_2$ inhibitory activity: thielocin A1 and B1 which have five aromatic rings are potent inhibitors, while thielavins, naturally occurring weak inhibitors, have only three rings.[19] Also among the newly synthesized

Table 1. PLA_2 Inhibitory Activity[a]

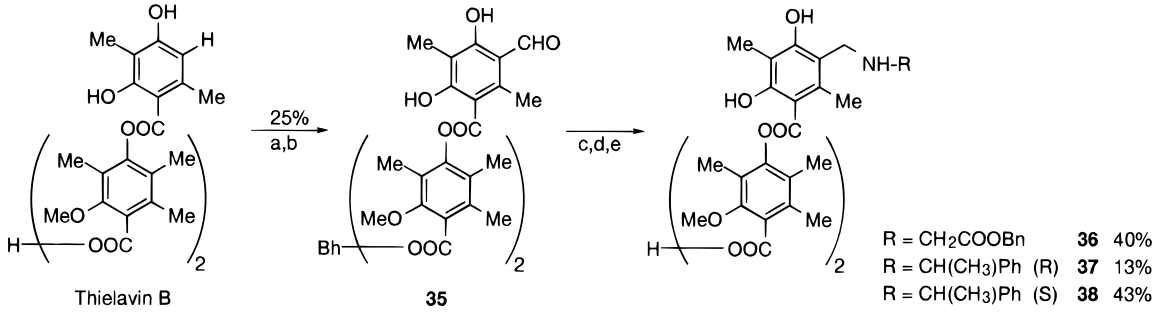
	$sPLA_2$ Inhibition: IC_{50}(mM)				
	group II		group I		
compound	rat	human recombinant	human recombinant	ring unit	type-(X or Y)
thielocin A1	0.0033	12	135	5[b]	[c]
thielocin B3	0.012	0.076	18	6	CH_2
11	0.12	0.23	>100	5	phenylene
12	0.88	5.8	100	5	pyrone
13	0.33	2.2	>100	4	CH=CH-(*E*)
14	0.040	0.94	60	4	CH_2CH_2
16	2.1	4.1	100	4	S
17	2.5	4.8	>100	4	S
18	0.049	0.13	55	6	S
19	0.12	0.26	11	6	SO
20	0.05	0.069	9.0	6	SO_2
21	ni[d]	ni[d]	>100	6	S[e]
28	3.4	75	>100	4	O
29	0.042	0.10	6.5	6	O
30	0.035	0.24	6.6	6	O
31	3.0	3.0	>100	4	CH_2
32	4.6	2.8	90	4	CH_2
33	0.13	0.19	>100	6	CH_2
34	0.95	2.4	>100	6	CH_2[f]
36	0.026	0.14	10	4	NH[g]
37	0.30	1.8	98	4	NH[h]
38	0.36	0.84	60	4	NH[i]
mepacrine	320	76			
p-BPB	6.7	34			
manoalide	2.0	1.5			

[a] For detailed assay protocol, see the Experimental Section. [b] According to the criteria used in this study, it contains five rings, but it may be considered to form six rings because of its fused-ring structure. [c] See Figure 1. [d] No inhibition. [e] di-POM ester of **18**. [f] Eight hydroxy groups. [g] Glycine benzyl ester. [h] Phenethylamine derivative (*R*-isomer). [i] Phenethylamine derivative (*S*-isomer).

compounds, the critical number of rings for $sPLA_2$ inhibition was clearly four, and the best results were obtained in the case of six aromatic rings. Unnatural sulfides are potent inhibitors, and the sulfone derivative **20** shows activity comparable to thielocin B3 and exhibits an IC_{50} value of 0.069 μM for human $sPLA_2$-II.

The inhibition by **20** was very similar to that of thielocin B3. It is also a potent inhibitor for rat $sPLA_2$-II with an IC_{50} value of 0.05 μM and a weak inhibitor for human $sPLA_2$-I (IC_{50} 9.0 μM). However, diesterification of terminal carboxylic acids of the sulfide **18** (**21**) led to complete loss of the activity. Also, diphenyl ethers **29** and **30** show very potent activity, but seem to have weak cross inhibitory activity for human $sPLA_2$-I. Moreover, the activity of **30**, more substituted at the core of the molecule than **29**, was found to be almost comparable to **29**, suggesting that the degree of substitution at this position does not affect the activity. Compared to symmetrical diphenylmethylene compound **32**, the unsymmetrical **31** showed similar activity, which

Scheme 6[a]



[a] Reagents: (a) hexamethylenetetramine, TFA; (b) Ph_2CN_2; (c) H_2NR; (d) $NaBH_3CN$; (e) TFA, anisole.

Table 2. Effect of Thielocin B3 Derivatives on Arachidonic Acid and Thromboxane B_2 Production by Rat Platelet Stimulated with A23187

		% of control release[a]	
compound	concn (μM)	arachidonic acid	thromboxane B_2
thielocin B3	10	$60.3 \pm 3.9^{**}$	$43.7 \pm 8.2^{**}$
11	10	$49.2 \pm 8.4^{**}$	$0.1 \pm 12.5^{*}$
12	10	$55.2 \pm 5.6^{*}$	$46.3 \pm 14.3^{*}$
13	10	$70.7 \pm 7.2^{**}$	65.8 ± 12.7
18	10	$2.8 \pm 3.9^{***}$	$0 \pm 14.6^{**}$
29	10	$9.0 \pm 7.0^{**}$	$0 \pm 15.4^{**}$
30	10	$19.7 \pm 5.6^{***}$	$0 \pm 17.1^{*}$
36	10	$32.7 \pm 6.1^{**}$	$0 \pm 18.8^{*}$

[a] See the Experimental Section. [(A23187 + inhibitors)cpm − (DMSO control)cpm]/[(A23187)cpm − (DMSO control)cpm] × 100. Each values represents the mean ± SEM for three experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ *vs* control (Student's *t*-test).

was also observed in the case of the diphenyl sulfide series (**16** and **17**). Although **33** showed the most potent activity in the diphenylmethylene series, it could not exceed that of thielocin B3. Changing all methoxy groups to hydroxy groups **34** led to significant loss of activity, suggesting this as the cause of disturbance of the hydrophobic interaction between the enzyme and the inhibitor[16] and the undesired result. Very interestingly, a glycine derivative **36** having three aromatic rings and a benzyl ester side chain showed activity comparable to that of thielocin B3. The contribution of the amino group to $sPLA_2$ inhibitory activity is considered to occur at the hydrophilic calcium binding site.

Newly synthesized thielocin B3 derivatives showed no significant human $cPLA_2$ inhibition at the concentration of 20 μM.[26] Thus, they were found to be specific $sPLA_2$-II inhibitors.

The effect of thielocin B3 derivatives on the release of arachidonic acid and thromboxane B_2 from rat platelets was also examined. The results of the cellular assay with respect to release of PLA_2 products are summarized in Table 2. Also in this cellular assay, compounds **18**, **29**, and **36** suppressed significantly the production of arachidonic acid and thromboxane B_2, indicating thielocin derivatives are real and potent PLA_2 inhibitors.

Conclusion

We prepared several types of thielocin B3 derivatives and conducted their SAR study to identify the potent $sPLA_2$-II inhibitors. The results showed that the number of aromatic rings is critical for $sPLA_2$-II inhibition and more than four rings are necessary, with the best result occurring with six rings. The structures of the core moiety of inhibitors are not specific: potent inhibitors were found among the phenylene, sulfide, sulfone, ether, methylene, and amino derivatives and showed $sPLA_2$-II inhibitory activity comparable to that of thielocin B3. Diesterification of two carboxylic acid functions of thielocin B3 are known to cause almost complete loss of activity,[18] and we could obtain the similar result again. However, monocarboxylic acid derivative **36** showed very potent inhibitory activity, suggesting that two carboxylic acid groups of both terminals are not always necessary for expression of the activity. It is noteworthy that the newly synthesized thielocin derivatives **18**, **20**, **29**, and **36** show very potent human $sPLA_2$-II inhibitory activity equivalent to that of thielocin B3, and their IC_{50} values are the lowest of those reported to date in the literature.[21]

Experimental Section

Materials. Thielocin A1 and B3 were prepared as previously reported.[25] 1-Palmitoyl-2-[1-^{14}C]linoleoylphosphatidylethanolamine (2.18 GBq/mmol) was purchased from Amersham Corp. L-α-Phosphatidylethanolamine (from egg yolk) was purchased from Sigma. Rat PLA_2-II was purified from rat platelets.[27] Recombinant human PLA_2-I and II were obtained as previously reported.[19] Each of the purified PLA_2s showed a single band of approximately 14 kDa on SDS−polyacrylamide gel electrophoresis (Coomassie brilliant blue staining). All other reagents were of analytical grade or better.

[^{3}H]AA (238 Ci/mmol) was purchased from New England Nuclear. A23187, mepacrine, and p-BPB were purchased from Sigma. Manoalide was purchased from Wako Chemicals.

Assay of Phospholipase A_2. PLA_2 activity was measured by a method described previously.[17] The substrate was prepared by diluting 1-palmitoyl-2-[1-^{14}C]linoleoylphosphatidylethanolamine with L-α-phosphatidylethanolamine to the specific activity of 2000 dpm/nmol. The reaction was started by addition of the enzyme. The amount of PLA_2s was adjusted to optimize linear kinetics for quantitation, i.e., hydrolysis of the substrate was less than 20% in all experiments. Thielocin B3 and other synthesized compounds were added to the assay tubes as a DMSO solution (2% of the final volume), using a DMSO enzyme control. Control experiments showed that DMSO at this concentration had no effect on enzymatic activities. IC_{50} values were determined graphically from plots of percent inhibition that were obtained from three independent experiments, each performed in duplicate, versus log concentration of test compounds.

Assay for Arachidonic acid Mobilization. Production of free arachidonic acid (AA) and thromboxane B_2 (TXB_2) by rat platelets was measured using the assay described by Nakano *et al.*[28] Briefly, platelets were prepared from fresh rat blood anticoagulated with 0.11 volume of acid citrate dextrose (85 mM trisodium citrate, 70 mM citric acid, and 110 mM glucose) and mixed with PGE_1 (1.5 μg/mL). Platelet-rich plasma was obtained by centrifugation at 160*g* for 10 min and layered on 40% bovine serum albumin. Platelets were sedimented at 1200*g* for 20 min, resuspended at 2×10^9 cells/mL in the elution buffer (137 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2$, 3.8 mM NaH_2PO_4, 3.8 mM Hepes, 5.6 mM glucose, and 0.035% bovine serum albumin, pH 7.35) containing 1.0 μg/mL of PGE_1, and incubated with [^{3}H]AA (20 μCi/mL) for 2.0 h at room temperature. Platelets were isolated by gel filtration through a column of Sepharose 2B and suspended in the elution buffer to a final concentration of 5×10^8 cells/mL. [^{3}H]-AA-labeled platelets (250 μL) were incubated with A23187 and various inhibitors, and stimulation was stopped by adding 0.4 volume of 0.1 N HCl. A23187 (2 μM) was added as a DMSO solution (0.4% of the final volume). Thielocin B3 derivatives were also added as a DMSO solution (0.2% of the final volume), using a DMSO control. Control experiments showed that DMSO at this concentration had no effect on [^{3}H]AA and [^{3}H]-TXB_2 production. [^{3}H]-labeled compounds were extracted and separated by thin layer chromatography. The area corresponding to AA and TXB_2 was scraped off and the radioactivity was measured.

DMSO control (0.4% of the final volume) released [^{3}H]AA (3490 ± 347 cpm) and [^{3}H]TXB_2 (1268 ± 208 cpm). A23187 (2 μM) released [^{3}H]AA (7797 ± 406 cpm) and [^{3}H]TXB_2 (3065 ± 376 cpm) from [^{3}H]-labeled rat platelet (means ± SEM from four independent determinations, each performed in triplicate). The effect of various inhibitors on the increase of [^{3}H]AA and [^{3}H]TXB_2 production by A23187-stimulated rat platelets is expressed as "% of increase": [(A23187 + inhibitors)cpm − (DMSO control)cpm]/[(A23187)cpm − (DMSO control)cpm] × 100. Each values represents the mean ± SEM for three experiments; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ *vs* control (Student's *t*-test).

Chemistry. Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were determined at 200 and 50.3 MHz,

respectively. Liquid secondary ion mass spectra (LSIMS) and high-resolution (HR)-LSIMS were determined using *m*-nitrobenzyl alcohol as a matrix. Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere with anhydrous solvents that had been dried over type 4A molecular sieves. Drying of an organic phase over anhydrous sodium sulfate is simply indicated by the word "dried". Column chromatography using Merck silica gel 60 or a Merck Lobar column is referred to as "chromatography on silica gel".

Methyl 5-(Acetoxymethyl)-2,4-dimethoxy-3,6-dimethylbenzoate (3). A mixture of **1** (3.70 g, 16.5 mmol), Me_2SO_4 (4.74 mL, 16.5 mmol × 3), K_2CO_3 (11.58 g, 16.5 mmol × 5), and 200 mL of acetone was heated under reflux for 2 h, and after the mixture was cooled, the resultant solid was filtered off. The filtrate was partitioned between ethyl acetate and 2 N hydrochloric acid, and the organic phase was washed with water, dried over Na_2SO_4, and then concentrated *in vacuo* to yield the crude product, which was recrystallized from ether−*n*-hexane to provide 6.8 g of **2** as a red oil. The oil was dissolved in 30 mL of isopropyl alcohol, to the solution was added $NaBH_4$ (0.94 g, 16.5 mmol × 1.5), and the reaction mixture was stirred and then partitioned between ethyl acetate and water. The organic phase was washed with 2 N hydrochloric acid, followed by with water, dried, and then concentrated *in vacuo*. The resultant residue was subjected to column chromatography (70 g of SiO_2, eluent: *n*-hexane−ethyl acetate (4:1 to 1:1)) to yield 3.18 g of the alcohol. Then, the oil was dissolved in 20 mL of CH_2Cl_2, and to the solution was added 6 mL of acetic anhydride, 10 mL of pyridine, and 50 mg of (dimethylamino)pyridine, and then the mixture was stirred at room temperature for 2 h. The reaction mixture was partitioned between ethyl acetate and 2 N hydrochloric acid, and the organic phase was washed with water, dried, and then concentrated *in vacuo* to yield the crude product, which was recrystallized from ether−*n*-hexane to yield 3.35 g (69%) of the acetate **3**: colorless pillars; mp 69−70 °C; ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3H), 2.22 (s, 3H), 2.24 (s, 3H), 3.74 (s, 3H), 3.78 (s, 3H), 3.93 (s, 3H), 5.18 (s, 2H). Anal. ($C_{15}H_{20}O_6$) C, H.

(3-Carbomethoxy-4,6-dihydroxy-2,5-dimethylphenyl)-(3-carbomethoxy-4,6-dimethoxy-2,5-dimethylphenyl)-methane (5). Compound **4** (1.45 g, 6.74 mmol × 1.1), which is known in the literature and the acetate **3** (2.00 g, 6.74 mmol) were dissolved in 25 mL of toluene, to the solution was added BF_3·OEt_2 (0.83 mL, 6.74 mmol), and the mixture was stirred at room temperature for 15 min. To the reaction mixture was added ethyl acetate, and then the mixture was washed with water, dried, and concentrated *in vacuo*. The residue was subjected to column chromatography (SiO_2; Merck, Lobar B, eluent: *n*-hexane−ethyl acetate) to yield 2.91 g of **5** (100%): colorless prisms; mp 169−170 °C; ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 6H), 2.25 (s, 3H), 2.55 (s, 3H), 3.75 (s, 6H), 3.88 (s, 3H), 3.92 (s, 3H), 3.95 (s, 2H), 7.20 (s, 1H), 11.38 (s, 1H); IR (Nujol) 3285, 1743, 1644 cm^{-1}. Anal. ($C_{23}H_{28}O_8$) C, H.

Bis(3-carboxy-4,6-dimethoxy-2,5-dimethylphenyl)-methane (6). Compound **5** (2.91 g, 7.4 mmol) was methoxylated in a procedure similar to that of compound **3**, and the crude residue was subjected to column chromatography (60 g of SiO_2, eluent: toluene−ethyl acetate (9:1 to 4:1)) to yield 2.97 g of methylated product. The product (600 mg, 130 mmol) was hydrolyzed in a usual procedure using KOH in aqueous DMSO to yield 559 mg (95%) of **6**: colorless crystal; mp 265−267 °C; ^{1}H NMR (DMSO-d_6) δ 1.96 (s, 6H), 2.12 (s, 6H), 3.48 (s, 6H), 3.66 (s, 6H), 3.98 (s, 2H); IR (KBr) 3670−2400, 3430, 2940, 1710, 1217, 1105 cm^{-1}. Anal. ($C_{23}H_{28}O_8$·$0.4H_2O$) C, H.

4-Hydroxy-2-methoxy-3,5,6-trimethylbenzoic Acid (8). Compound **7** (5.0 g, 16.6 mmol), which is known in the literature, was subject to hydrogenation by a usual procedure using Pd−C in a mixture of AcOEt and MeOH to yield 3.8 g of the hydroxy compound. The product (3.36 g, 16.0 mmol) was dissolved in 30 mL of ethyl acetate, diphenyldiazomethane (5.9 g, 16 mmol) was added at room temperature, and the mixture was left to stand overnight. An excess of diphenyldiazomethane was decomposed by adding 2 N hydrochloric acid, and the resultant mixture was extracted with ethyl acetate. The organic phase was washed with water, dried, and then concentrated *in vacuo*. The residue was subjected to column chromatography (80 g of SiO_2; eluent, *n*-hexane−ethyl acetate (19:1 to 1:1)) to yield 5.76 g (93%) of the ester **8**: colorless pillars; mp 125−127 °C; ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3H), 2.11 (s, 3H), 2.15 (s, 3H), 3.52 (s, 3H), 4.82 (s, 1H), 7.17 (s, 1H), 7.22−7.50 (m, 10H); IR (Nujol) 3360, 1695, 1585, 1290, 1205 cm^{-1}. Anal. ($C_{24}H_{24}O_4$) C, H.

4-((4-(Benzhydryloxycarbonyl)-3-methoxy-2,5,6-trimethylphenoxy)carbonyl)-3-methoxy-2,5,6-trimethylphenol (10). Compound **7** (4.19 g, 13.28 mmol × 1.05) was dissolved in 20 mL of CH_2Cl_2, oxalyl chloride (4.25 mL, 13.28 mmol × 1.05 × 3.5) was added at room temperature, the resultant mixture was stirred at room temperature for 30 min, and then the mixture was gently warmed under reflux for 30 min. After the mixture was concentrated *in vacuo*, the residue was dissolved in THF, and the solution was again concentrated *in vacuo*. Alternatively, compound **8** (5.00 g, 13.28 mmol) was dissolved in 20 mL of THF, *n*-BuLi (1.6 M solution in hexane, 8.30 mL, 13.28 mmol) was added gradually to the solution at −78 °C, and then the solution was stirred at the same temperature for 30 min. To the reaction mixture was added a solution of the acid chloride of **7** obtained above in THF (30 mL) at −78 °C, and the mixture was stirred at the same temperature for 10 min. The mixture was allowed to warm slowly to room temperature and left to stand overnight. The reaction mixture was partitioned between ethyl acetate and 1 N hydrochloric acid, and the organic phase was washed with water and then dried. After the mixture was concentrated *in vacuo*, the residue was subjected to column chromatography (200 g of SiO_2; eluent, toluene−ethyl acetate (0−10%)) to yield 7.32 g of **9** (84%) as a colorless crystal: mp 183−185 °C; ^{1}H NMR ($CDCl_3$) δ 2.09 (s, 3H), 2.22 (s, 3H), 2.25 (s, 3H), 2.26 (s, 3H), 2.28 (s, 3H), 2.37 (s, 3H), 3.58 (s, 3H), 3.81 (s, 3H), 4.80 (s, 2H), 7.20 (s, 1H), 7.28−7.55 (m, 15H); IR (Nujol) 1760, 1723, 1573, 1455, 1156, 740, 697 cm^{-1}. Anal. ($C_{42}H_{42}O_7$) C, H.

Compound **9** (7.32 g, 11.1 mmol) was subject to hydrogenation by a procedure similar to that of **8**, the resultant crude product (4.31 g, 10.7 mmol) was benzhydrylated by a similar procedure to that of **8**, and the resultant product was recrystallized from ether−*n*-hexane to yield 5.31 g (84%) of **10**: colorless pillars; mp 195−197 °C; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3H), 2.19−2.24 (m, 12H), 2.37 (s, 3H), 3.56 (s, 3H), 3.80 (s, 3H), 4.95 (s, 1H), 7.19 (s, 1H), 7.28−7.52 (m, 10H); IR (Nujol) 3390, 1737, 1706, 1285, 1156, 759, 700 cm^{-1}. Anal. ($C_{35}H_{36}O_7$) C, H.

Bis[4-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]isophthalate (11). Compounds **11**, **12**, and **13** were prepared by a procedure similar to that of **10** described above. Compound **11** (yield 60% from isophthalic acid and 2 equiv of **10**): colorless prisms; mp 267−269 °C; ^{1}H NMR (DMSO-d_6) δ 2.08−2.26 (m, 30H), 2.37 (s, 6H), 3.73 (s, 6H), 3.79 (s, 6H), 7.98 (t, J = 9 Hz, 1H), 8.66 (dd, J = 9, 1 Hz, 2H), 8.93 (m, 1H); IR (KBr) 3430, 2940, 1745, 1700, 1222, 1160 cm^{-1}. Anal. ($C_{52}H_{54}O_{16}$·H_2O) C, H.

Bis[4-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]chelidonate (12): colorless plates; mp 294−296 °C; ^{1}H NMR ($CDCl_3$) δ 2.16 (s, 6H), 2.21 (s, 6H), 2.26 (s, 6H), 2.29 (s, 6H), 2.36 (s, 6H), 2.42 (s, 6H), 3.84 (s, 6H), 3.87 (s, 6H), 7.52 (s, 2H). Anal. ($C_{51}H_{52}O_{18}$) C, H.

(*E*)-1,2-Bis[4-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxycarbonyl]ethene (13). Compound **13** was recrystallized from *N*,*N*-dimethylformamide: colorless plates; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 2.45 (s, 6H), 2.48 (s, 6H), 2.52 (s, 6H), 2.53 (s, 12H), 2.70 (s, 6H), 4.07 (s, 6H), 4.11 (s, 6H), 7.78 (s, 2H). Anal. ($C_{48}H_{52}O_{16}$·$^1/_2H_2O$·$^1/_2$DMF) C, H.

1,2-Bis[[4-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]carbonyl]ethane (14). Compound **14** was obtain by catalytic reduction using Pd−C as a catalyst in a mixture of MeOH and acetic acid in 92% yield: colorless plates; mp > 270 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.04 (s, 6H), 2.07 (s, 6H), 2.17 (s, 6H), 2.18 (s, 6H), 2.19 (s, 6H), 2.32 (s, 6H), 3.18 (s, 4H), 3.72 (s, 6H), 3.73 (s, 6H). Anal. ($C_{48}H_{54}O_6$) C, H.

3-Carboxy-4-methoxyphenyl 3-[[4-[(4-Carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]carbonyl]-4-methoxyphenyl Sulfide (16). 5,5′-Thiodisalicylic acid was methylated with dimethyl sulfate and K_2CO_3, and the resulting diester was hydrolyzed with KOH in aqueous MeOH as described in the case of **6** to give the dimethoxy dicarboxylic acid **15** in 76% yield: colorless prisms; mp 158−159 °C. Anal. ($C_{16}H_{14}O_6S$) C, H, S.

Compound **15** (1.31 g, 3.9 mmol) was dissolved in 50 mL of methylene chloride, and to the solution was added 0.8 g (4.1 mmol) of diphenyldiazomethane under cooling with ice−water, and the mixture was stirred for 1.8 h. The solvent was evaporated *in vacuo*, the residue was applied to column chromatography (SiO_2, 150 g), and it was eluted with benzene−ethyl acetate (5:1) and then chloroform−methanol (9:1) to yield 1.06 g of monobenzhydryl ester. The half-ester was reacted with **10** in a manner similar to that of **11** to give the unsymmetrical sulfide **16** in 17% overall yield: colorless plates; mp 192−194 °C; ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 3H), 2.19 (s, 3H), 2.25 (s, 3H), 2.29 (s, 3H), 2.35 (s, 3H), 2.39 (s, 3H), 3.83 (s, 3H), 3.85 (s, 3H), 3.97 (s, 3H), 4.08 (s, 3H), 7.03 (d, J = 8.8 Hz, 1H), 7.05 (d, J = 8.8 Hz, 1H), 7.55 (dd, J = 2.4, 8.8 Hz, 1H), 7.60 (dd, J = 2.4, 8.8 Hz, 1H), 8.08 (d, J = 2.4 Hz, 1H), 8.18 (d, J = 2.4 Hz, 1H). Anal. ($C_{38}H_{38}O_{12}S$) C, H, S.

Bis[3-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-4-methoxyphenyl] Sulfide (17). Compound **17** was obtained from **15** by the reaction with 2 equiv of **8** and deprotection in 67% yield: colorless plates; mp 237−239 °C; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 6H), 2.09 (s, 6H), 2.32 (s, 6H), 3.81 (s, 6H), 3.95 (s, 6H), 7.03 (d, J = 8.8 Hz, 2H), 7.60 (dd, J = 2.4, 8.8 Hz, 2H), 8.09 (d, J = 2.4 Hz, 2H). Anal. ($C_{38}H_{38}O_{12}S\cdot{}^1/_2$benzene) C, H, S.

Bis[3-[[4-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]carbonyl]-4-methoxyphenyl] Sulfide (18). Compound **18** was prepared in a manner similar to that of **17** using 2 equiv of **10** in 35% yield: colorless plates; mp 278−280 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 6H), 2.18 (s, 6H), 2.25 (s, 6H), 2.29 (s, 6H), 2.36 (s, 6H), 2.40 (s, 6H), 3.83 (s, 6H), 3.86 (s, 6H), 3.97 (s, 6H), 7.05 (d, J = 8.8 Hz, 2H), 7.60 (dd, J = 2.0, 8.8 Hz, 2H), 8.10 (d, J = 2.0 Hz, 2H). Anal. ($C_{60}H_{62}O_{18}S\cdot{}^1/_2H_2O$) C, H, S.

Bis[3-[[4-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]carbonyl]-4-methoxyphenyl] Sulfoxide (19). Dibenzhydryl ester of **18** (200 mg, 0.14 mmol) was dissolved in 5 mL of methylene chloride, to the solution was added 30 mg (0.14 mmol) of *m*-chloroperbenzoic acid (80%) under cooling with ice−water, and the mixture was stirred at 0 °C for 4.5 h. The reaction mixture was washed with 5% aqueous sodium thiosulfate, an aqueous saturated sodium bicarbonate, and water, successively, and dried, and then the solvent was evaporated *in vacuo* to yield 245 mg of the crude product. The product was purified by column chromatography (40 g of SiO_2; eluent, ethyl acetate−*n*-hexane (2:1)) to yield 151 mg of the dibenzhydryl ester of **19**. Deprotection of the ester gave 95 mg (60%) of **19**: mp 193−195 °C; ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 6H), 2.16 (s, 6H), 2.23 (s, 6H), 2.27 (s, 6H), 2.34 (s, 6H), 2.38 (s, 6H), 3.81 (s, 6H), 3.85 (s, 6H), 4.01 (s, 6H), 7.21 (d, J = 9.0 Hz, 2H), 7.92 (dd, J = 2.2, 9.0 Hz, 2H), 8.35 (d, J = 2.2 Hz, 2H). Anal. ($C_{60}H_{62}O_{19}S\cdot H_2O$) C, H, S.

Bis[3-[[4-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]carbonyl]-4-methoxyphenyl] Sulfone (20). Compound **18** was oxidized with 4 equiv of *m*-chloroperbenzoic acid in a manner similar to that of **19** to give **20** in 81% yield: colorless plates; mp 294−295 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.12 (s, 6H), 2.16 (s, 6H), 2.23 (s, 6H), 2.27 (s, 6H), 2.34 (s, 6H), 2.38 (s, 6H), 3.80 (s, 6H), 3.85 (s, 6H), 4.04 (s, 6H), 7.20 (d, J = 9.0 Hz, 2H), 8.20 (dd, J = 2.2, 9.0 Hz, 2H), 8.65 (d, J = 2.6 Hz, 2H). Anal. ($C_{60}H_{62}O_{20}S\cdot{}^1/_2H_2O$) C, H, S.

Bis[3-[[4-[[[(pivaloyloxy)methyl]oxy]carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]carbonyl]-4-methoxyphenyl] Sulfide (21). A mixture containing 500 mg (0.45 mmol) of **18**, 265 mg (1.09 mmol) of (pivaloyloxy)methyl iodide, 216 mg (1.56 mmol) of K_2CO_3, and 25 mL of acetone was stirred at room temperature for 16 h. To the mixture was added 200 mL of ice−water, and the resultant mixture was extracted with ethyl acetate. The organic phase was washed with water and dried (Na_2SO_4), and the solvent was evaporated *in vacuo* to yield 0.65 g of the crude product. The product was recrystallized from ethyl acetate−*n*-hexane to provide 0.516 g (85%) of **21**: mp 120 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 18H), 2.13 (s, 6H), 2.17 (s, 6H), 2.23 (s, 12H), 2.26 (s, 6H), 2.39 (s, 6H), 3.79 (s, 6H), 3.81 (s, 6H), 3.96 (s, 6H), 6.00 (s, 4H), 7.05 (d, J = 9.0 Hz, 2H), 7.59 (dd, J = 2.5, 8.7 Hz, 2H), 8.08 (d, J = 2.4 Hz, 2H). Anal. ($C_{72}H_{82}O_{22}S\cdot{}^1/_2$hexane) C, H, S.

Bis(3-carboxy-4-methoxyphenyl) Ether (26) and 5-(3-Carboxy-4-methoxy-5-methylphenoxy)-2,4-dimethoxybenzoic Acid (27). Compound **22** (200 mg, 1.1 mmol) was dissolved in 5 mL of DMF, 46 mg of NaH (60% in oil) (1.15 mmol) was added, and the mixture was stirred for 1.5 h. After 690 mg (3.36 mmol) of copper(I) bromide−dimethyl sulfide complex was added to the mixture, 270 mg of the bromide **24** (1.1 mg) which had been dissolved in 0.5 mL of DMF was added, and the resultant mixture was refluxed with stirring for 22 h. After cooling, the reaction mixture was partitioned between 1 N hydrochloric acid and ethyl acetate, and the organic phase was washed with 1 N hydrochloric acid, water, and the saturated brine, successively, and then dried. The solvent was evaporated to dryness, and the residue (383 mg) was applied to silica gel chromatography (40 g of SiO_2; eluent, ethyl acetate−*n*-hexane (2:3)) to yield 163 mg of methyl ester of **26**. The methyl ester was hydrolyzed by usual procedure using KOH in aqueous MeOH to give the dicarboxylic acid **26** in 38% yield: colorless plates; mp 178−180 °C; ^{1}H NMR (DMSO-d_6): δ 3.81 (s, 6H), 7.10−7.23 (m, 6H). Anal. ($C_{16}H_{14}O_7$) C, H.

Compound **27** was prepared in a manner similar to that described above from **23** and **25** in 13% yield: pale yellow plates; mp 171−174 °C; ^{1}H NMR (DMSO-d_6) δ 2.22 (s, 3H), 3.69 (s, 3H), 3.86 (s, 3H), 3.90 (s, 3H), 6.82 (d, J = 3.0 Hz, 1H), 6.87 (s, 1H), 6.95 (d, J = 3.0 Hz, 1H), 7.39 (s, 1H); IR (KBr) 3680−2320, 3440, 2960, 1695, 1616, 1215, 1120 cm^{-1}. Anal. ($C_{18}H_{18}O_8\cdot 0.3H_2O$) C, H.

Bis[3-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-4-methoxyphenyl] Ether (28). Compound **28** was obtained by a manner similar to that of **17**, a thia analogue of **28**, in 63% yield: colorless plates; mp 194−195 °C; ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 6H), 2.12 (s, 6H), 2.33 (s, 6H), 3.82 (s, 6H), 3.95 (s, 6H), 7.06 (d, J = 9.2 Hz, 2H), 7.27 (dd, J = 3.0, 9.2 Hz, 2H), 7.73 (d, J = 3.0 Hz, 2H). Anal. ($C_{38}H_{38}O_{13}\cdot H_2O$) C, H.

Bis[3-[[4-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]carbonyl]-4-methoxyphenyl] Ether (29) and 3-[[4-[(4-Carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]carbonyl]-4-methoxy-5-methylphenyl 5-[[4-[(4-Carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]carbonyl]-2,4-dimethoxyphenyl Ether (30). Compound **29** was prepared by a manner similar to that of **28** using 2 equiv of **10**. The yield was 83% from **26**: colorless plates; mp 258−260 °C; ^{1}H NMR ($CDCl_3$) δ 2.16 (s, 6H), 2.20 (s, 6H), 2.26 (s, 6H), 2.29 (s, 6H), 2.36 (s, 6H), 2.40 (s, 6H), 3.83 (s, 6H), 3.86 (s, 6H), 3.97 (s, 6H), 7.08 (d, J = 9.2 Hz, 2H), 7.29 (dd, J = 3.2, 9.2 Hz, 2H), 7.74 (d, J = 3.2 Hz, 2H). Anal. ($C_{60}H_{62}O_{19}\cdot{}^1/_2$EtOH) C, H.

Compound **30** was also prepared from **23** and **25** via the coupling product **27** in a manner similar to that of **29** in 5.5% overall yield: colorless powder (hygroscopic); ^{1}H NMR (DMSO-d_6) δ 1.90 (s, 3H), 1.98−2.90 (m, 27H), 2.28−2.37 (m, 9H), 3.68−3.81 (m, 15H), 3.96 (s, 3H), 4.00 (s, 3H), 7.03 (s, 1H), 7.18 (d, J = 2.8 Hz, 1H), 7.28 (d, J = 2.8 Hz, 1H), 7.80 (s, 1H); IR (KBr) 3430, 2930, 1745, 1575, 1462, 1150 cm^{-1}. Anal. ($C_{62}H_{66}O_{20}\cdot 3.5H_2O$) C, H.

(5-Carboxy-2,4-dimethoxy-3,6-dimethylphenyl)[5-[[4-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]carbonyl]-2,4-dimethoxy-3,6-dimethylphenyl]methane (31). The methylenic compound **31** was obtained from **6** by half-ester formation with diphenyldiazomethane, followed by coupling reaction with

10. This procedure is similar to that of **16**, a thia analogue of **31**: colorless powder; ^{1}H NMR (CD_3OD) δ 2.00 (s, 3H), 2.11 (s, 3H), 2.18−2.34 (m, 21H), 2.41 (s, 3H), 3.57 (s, 3H), 3.63 (s, 3H), 3.77 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 4.18 (s, 2H); IR (KBr): 3440, 2950, 1745, 1703, 1570, 1460, 1145, 1095, 1075 cm^{-1}; MS (LSI) 817 $(M + H)^+$.

Bis[5-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)-carbonyl]-2,4-dimethoxy-3,6-dimethylphenyl]methane (32). Compound **32** or **33** was prepared from **6** in a manner similar to that of **17** or **18**, respectively: yield (70%); colorless plates; mp 267−269 °C; ^{1}H NMR (DMSO-d_6) δ 2.04−2.28 (m, 30H), 3.52 (s, 6H), 3.70 (s, 6H), 3.74 (s, 6H), 4.11 (s, 2H); IR (KBr) 3440, 2940, 1740, 1700, 1460, 1155 cm^{-1}. Anal. ($C_{45}H_{52}O_{14}$) C, H.

Bis[5-[[4-[(4-carboxy-3-methoxy-2,5,6-trimethylphenoxy)carbonyl]-3-methoxy-2,5,6-trimethylphenoxy]carbonyl]-2,4-dimethoxy-3,6-dimethylphenyl]methane (33): yield (61%); colorless powder; ^{1}H NMR (DMSO-d_6) δ 2.12−2.40 (m, 48H), 3.54 (s, 6H), 3.73 (s, 6H), 3.77 (s, 12H), 4.14 (s, 2H); IR (KBr) 3680−2400, 3450, 2940, 1743, 1697, 1140 cm^{-1}. Anal. ($C_{67}H_{76}O_{20}$) C, H.

Bis[5-[[4-[(4-carboxy-3-hydroxy-2,5,6-trimethylphenoxy)carbonyl]-3-hydroxy-2,5,6-trimethylphenoxy]carbonyl]-2,4-dihydroxy-3,6-dimethylphenyl]methane (34). A mixture of the benzhydryl ester of **33** (500 mg, 326 μM), BBr_3 (371 μL, 326 μM × 12), and CH_2Cl_2 (15 mL) was stirred at room temperature for 6 h, the reaction was quenched with water, and then the resultant mixture was partitioned between ethyl acetate and brine. The organic phase was washed with brine three times, dried, and concentrated *in vacuo*. The residue was dissolved in ethyl acetate (6 mL), and the solution was subjected to esterification with diphenyldiazomethane. After purification by SiO_2 column chromatography, the product was subjected to catalytic hydrogenolysis using Pd−C to yield **34** in 15% overall yield: colorless plates; mp 162 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.96−2.40 (m, 48H), 4.09 (s, 2H), 9.43 (s, 2H), 9.90 (s, 2H); IR (Nujol) 3700−2560, 1670, 1455, 1155 cm^{-1}; MS (LSI) 1088 ($M^{\bullet+}$), 1089 ($M + H^+$). Anal. ($C_{59}H_{60}O_{20}\cdot 3.5H_2O$) C, H.

4-[[[4-[[[5-[[[(Benzyloxycarbonyl)methyl]amino]methyl]-2,4-dihydroxy-3,6-dimethylphenyl]carbonyl]oxy]-2-methoxy-3,5,6-trimethylphenyl]carbonyl]oxy]-2-methoxy-3,5,6-trimethylbenzoic Acid (36). Compound **35** was prepared from thielavin B by a known procedure in the literature,[24] and 2 g (2.6 mmol) of **35** and 8.9 g (26 mmol) of glycine benzyl ester *p*-toluenesulfonate were dissolved in 30 mL of a dried dimethylformamide. To the solution was add 4.3 g (52 mmol) of sodium acetate, and the mixture was stirred for 3.5 h. The reaction mixture was poured into 300 mL of cooled water to precipitate crystals, which were collected by filtration. The crystals were dissolved in 100 mL of ethyl acetate, and the solution was washed with 100 mL of water and 100 mL of brine and then dried. The solvent was evaporated to yield 2.58 g of the formimino derivative as a yellow foam. The material was recrystallized from ethyl acetate−*n*-hexane (1:2) to yield 2.2 g of the desired compound (92%) as yellow pillars: mp 153−155 °C. Anal. ($C_{54}H_{53}NO_{12}$) C, H, N.

The formimino derivative (2.15 g, 2.37 mmol) obtained in the above step was dissolved in 30 mL of dried dimethylformamide, and then 80 mL of dried methanol and 2 mL of acetic acid were added. A solution of 0.3 g of sodium cyanoborohydride in 4 mL of methanol was added to the mixture in a stream of nitrogen, under cooling in a ice bath over 15 min. The resulting mixture was directly stirred for 3 h and then allowed to stand overnight at 4 °C. The methanol was evaporated *in vacuo*, and the residue was poured into cooled water. The mixture was acidified with 1 N hydrochloric acid and then basified with a saturated sodium bicarbonate solution. The precipitated crystals were collected by filtration and dissolved in 200 mL of ethyl acetate, and the solution was washed with 100 mL of brine and dried. The solvent was evaporated to yield 2.33 g of the yellow oil. The oil was subjected to silica gel chromatography (SiO_2; 120 g), eluting with ethyl acetate−*n*-hexane (1:2) to yield 1.07 g of the amino derivative as a pale yellow oil (50%).

The amino derivative (1 g, 1.1 mmol) was dissolved in 40 mL of dichloromethane, and then 0.6 mL (5.5 mmol) of anisole was added. To the mixture was slowly added dropwise a solution of 0.85 mL (11 mmol) of trifluoroacetic acid in 4 mL of dichloromethane in an ice cooling bath. The mixture was directly stirred for 4 h and concentrated *in vacuo*, 1 N hydrochloric acid was added to the residue, and the precipitated solid was collected by filtration. The solid was washed with water repeatedly, dissolved in 50 mL of ethyl acetate, washed with 0.1% aqueous phosphate solution and with water, and then dried. The solvent was evaporated to yield 865 mg of a yellow oil. The oil was subjected to silica gel chromatography (SiO_2; 50 g), eluting with chloroform−methanol (10:1), to yield 720 mg of **36** as pale yellow plates (88%): mp 134−136 °C dec. Anal. ($C_{41}H_{45}NO_{12}\cdot EtOH\cdot H_2O$) C, H, N.

(*R*)-4-[[[4-[[[5-[(*sec*-Phenethylamino)methyl]-2,4-dihydroxy-3,6-dimethylphenyl]carbonyl]oxy]-2-methoxy-3,5,6-trimethylphenyl]carbonyl]oxy]-2-methoxy-3,5,6-trimethylbenzoic Acid (37) and (*S*)-4-[[[4-[[[5-[(*sec*-Phenethylamino)methyl]-2,4-dihydroxy-3,6-dimethylphenyl]-carbonyl]oxy]-2-methoxy-3,5,6-trimethylphenyl]carbonyl]-oxy]-2-methoxy-3,5,6-trimethylbenzoic Acid (38). Compound **35** was reacted in a procedure similar to that of compound **36** except that (*R*)-phenethylamine was used instead of glycine benzyl ester **37**: yield 13%; mp 208−210 °C dec; $[\alpha]^{24}_D$ +49.5 ± 0.9° (*c* 1, dioxane); ^{1}H NMR ($CDCl_3$) δ 1.57 (d, J = 6.8 Hz, 3H), 2.13 (s, 3H), 2.15 (s, 6H), 2.23 (s, 3H), 2.26 (s, 3H), 2.29 (s, 3H), 2.40 (s, 3H), 2.42 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 3.94 (m, 2H), 7.37 (m, 5H); MS (LSI) 700 (M + H) $^+$. Anal. ($C_{40}H_{45}NO_{10}\cdot 0.5H_2O$) C, H, N.

(*S*)-Isomer **38** was obtained by a procedure similar to that described above: mp 192−194 °C dec; $[\alpha]^{25}_D$ −51.0 ± 1.8° (*c* 0.5, dioxane). Anal. ($C_{40}H_{45}NO_{10}\cdot 1.5H_2O$) C, H, N.

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