Stereoselective Total Synthesis of (±)-Thielocin Alβ

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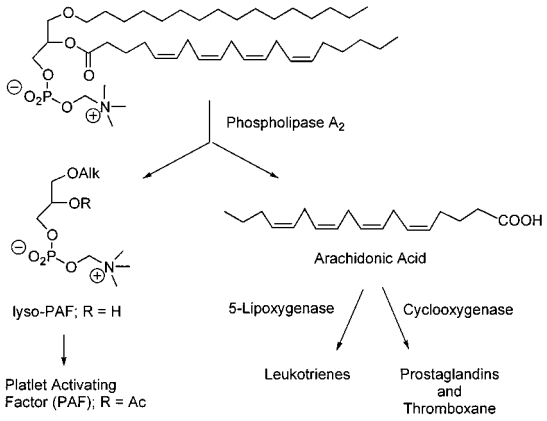
Abstract: The stereospecific total synthesis of (±)-thielocin A1β has been achieved from the common intermediate ethyl 5-formyl-2,4-dihydroxy-3,6-dimethyl benzoate (**8**). The racemic synthesis was achieved based on the key reaction of a 4-methyl-3,4-dihydroxy cyclohexadienone **38** with a quinone methide derived at low temperature from the fluoride ion catalyzed composition of piperidinium salt **40**. The resulting condensate (**31**) was homologated by successive esterification with protected monomeric phenol **41** to provide, after careful removal of the protecting groups, the desired thielocin A1β.

Introduction

Phospholipases A_2 (PLA_2's) catalyze the hydrolysis of fatty acids from the *sn*-2 position of aggregated glycerol phospholipids (Scheme 1). PLA_2's have attracted attention because of their ability to generate arachidonic acid (AA) and lyso-platelet activating factor (lyso-PAF), two important precursors of inflammatory lipid mediators such as eicosanoids, leukotrienes, and PAF.[1] Since AA release is thought to be the rate-limiting step in the biosynthesis of leukotrienes and prostaglandins, it is likely that PLA_2's play an important regulatory role in the initiation and exacerbation of the inflammatory response in animals. Most of the PLA_2's identified so far belong to a family of low molecular weight secreted enzymes ($sPLA_2$'s) isolated from various sources such as mammalian pancreas (group I $sPLA_2$), snake venom (groups I and II $sPLA_2$), bee venom (group III $sPLA_2$), and human platelets or synovial fluid (group II $sPLA_2$).[2] More recently, a new type of phospholipase A_2 ($cPLA_2$) has been discovered: found in the cytosol of cells, this class of enzymes shows no sequence homology with any of the secreted enzymes.[3] Because of their potential pivotal role in the formation of a variety of pro-inflammatory lipid mediators, both mammalian group II $sPLA_2$ and $cPLA_2$ have been considered as potential targets for the discovery of antiinflammatory agents.

A better understanding of the potential therapeutic and toxicological implications of inhibition of PLA_2's would help to define which enzyme is the optimal target for therapeutic intervention. It is therefore important that potent and specific inhibitors of the PLA_2 subtypes could be evaluated in animal models of inflammation and, ultimately, in human clinical trials.

Scheme 1



Thielocins

In the beginning of the 90's a series of unusual polymeric depsides, named thielocins, have been isolated from culture broths of ascomycetes *Thielavia terricola* RF-143.[4] Among them, thielocin A1β (**1b**) (Scheme 2) was reported to exhibit uniquely potent and selective inhibitory activity of group II secretory phospholipase A_2 ($sPLA_2$-II) from rat platelets with an IC_{50} of 3.3 nM relative to 21 μM for the rat pancreatic group I PLA_2 ($sPLA_2$-I).[4b] Later reports indicated that the compound was a somewhat less potent inhibitor of the corresponding human $sPLA_2$ (IC_{50} 12 μM for human rheumatoid synovial $sPLA_2$-II and >100 μM for human pancreatic $sPLA_2$-I).[4c]

Thielocin A1β has been studied in *in vivo* models of inflammation and has shown activity in a rat carrageenan pleurisy assay, thereby lending support the involvement of $sPLA_2$-II in the pathogenesis of inflammation in this model.[5] Its antiinflammatory effect associated with PLA_2 inhibition has also been

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(1) Chang, J.; Musser, J. H.; McGregor *Biochem. Pharmacol.* **1987**, *135*, 117.

(2) Mayer, R. J.; Marshall, L. A. *FASEB J.* **1993**, *7*, 339.

(3) Clarke, J. D.; Milona, N.; Knopf, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7708. Kramer, R. M.; Roberts, E. F.; Marretta, J.; Putnam, J. E. *J. Biol. Chem.* **1991**, 5268.

(4) (a) Yoshida, T.; Nakamoto, S.; Sakazaki, R.; Matsumoto, K.; Terui, Y.; Sato, T.; Arita, H.; Matsutani, S. *J. Antibiot.* **1991**, *44*, 1467−1470. (b) Tanaka, K.; Matsutani, S.; Matsumoto, K.; Yoshida, T. *J. Antibiot.* **1992**, *45*, 1071−1078. (c) Tanaka, K.; Matsutani, S.; Kanda, A.; Kato, T.; Yoshida, T. *J. Antibiot.* **1994**, *47*, 631−638. (d) Matsumoto, K.; Tanaka, K.; Matsutani, S.; Sakazaki, R.; Hinoo, H.; Uotani, N.; Tanimoto, T.; Kawamura, Y.; Nakamoto, S.; Yoshida, T. *J. Antibiot.* **1995**, *48*, 106−112. (e) Teshirogi, I.; Matsutani, S.; Shirahase, K.; Fujii, Y.; Yoshida, T.; Tanaka, K.; Ohtani, M. *J. Med. Chem.* **1996**, *39*, 5183−5191.

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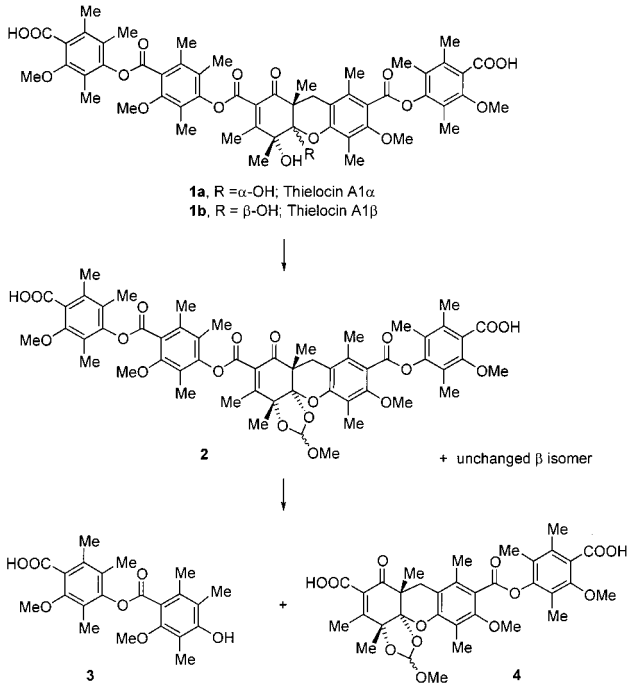
Scheme 2

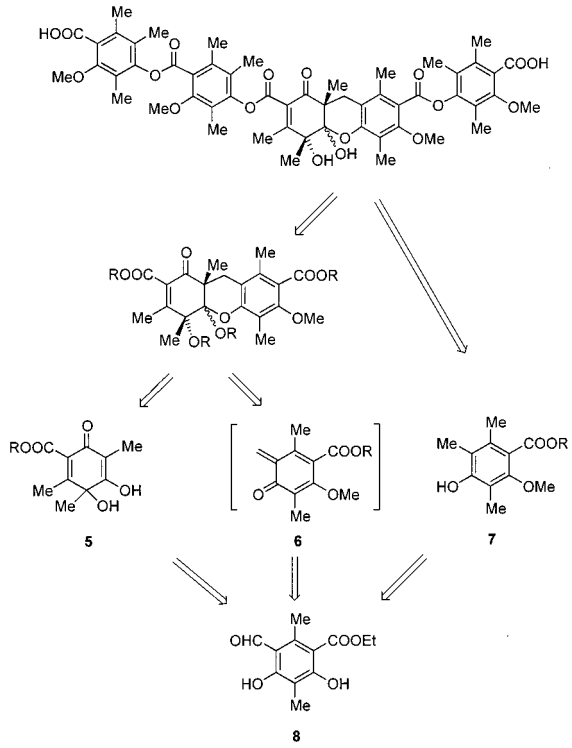


demonstrated in a model of secretory PLA_2-induced edema in mouse paw.[6] Thielocin A1β may thus provide an important lead for further chemical modifications and serve as a useful tool for the pharmacological evaluation of selective inhibition of group II $sPLA_2$ in models of shock and inflammation.

The initial report of isolation and structure elucidation of thielocins Al (**1a**,**b**) described the selective saponification of the central α,β-unsaturated ester bond.[4a] The reaction, performed on the diastereomeric mixture of orthoformate dimethylester derivatives **2**, provided a dimeric depside **3** along with the two *ortho* esters **4** (Scheme 2). Crystallization of one of these diastereomers allowed structural assignment and designation of the relative stereochemistry of the central core of thielocins A1. The lability of the α,β-unsaturated ester unit together with the relative resistance of the phenolic esters to hydrolysis indicated that any synthetic approach would require intermediates bearing readily and selectively removable benzoic acid protecting groups. Thielocin A1β can be considered to be formed from coupling of trimeric and dimeric depsides, each composed of a common monomer 2,4-dihydroxy-3,5,6-trimethyl benzoic acid. Further oxidation of the central phenolic unit and stereospecific formation of a carbon−carbon bond is apparently involved in the biosynthesis of **1a**,**b**. The exact sequence of events is undetermined but it is interesting to note that closely related trimeric depsides, weak group II $sPLA_2$'s inhibitors named thielavins, are known to co-occur in the same fermentation broth of *Thielavia terricola* RF-143.[4d]

As the isolation yield of thielocins A1 from culture was poor and variable and to make available analogues and synthons for further study of structure activity relationships, we undertook synthetic efforts directed toward these natural compounds. We wish to present here the full report of the first total synthesis of (±)-thielocin A1β.[7] Our approach is based on the efficient, highly stereoselective, and hypothetically biomimetic condensation of a hydroxy dienone **5**, a quinone methide **6**, and a benzoic acid derivative **7**, all derived from the common phenolic intermediate ethyl 5-formyl-2,4-dihydroxy-3,6-dimethyl benzoate **8**[8] (Scheme 3).

Scheme 3



Preliminary Model Studies

The required hydroxy dienone **5** was considered derivable from a 2,4-dihydroxy-3,5,6-trimethylbenzoate such as **9** through oxidation, by analogy with the work of Barton et al.[9] Treatment of **9a** with a variety of oxidative reagents such as CeO_2/H_2O_2, $K_2Fe(CN)_6$, $Co(OAc)_2/H_2O_2$, $(PhSeO)_2O$, $Tl(NO_3)_3$, $PhI(OAc)_2$/AcOH, or $Pb(OAc)_4$ gave either no reaction or extensive decomposition. However, reactions on the silyl-monoprotected analogue **9b** were more successful: Action of $Pb(OAc)_4$ in benzene gave 60% yield of an acetoxy dienone **10** which proved to be isomeric with the desired product. However, oxidation of **9b** with $PhI(OAc)_2$ in acetic acid gave the desired acetoxy dienone **11** in 60% yield[10] (Scheme 4). The only isolable side product was benzylic acetate **12** (10−20% isolated yield), likely resulting from the trapping of a quinone methide intermediate by acetate anion.[11] Unfortunately, all attempts to suppress this competing pathway were unsuccessful. Desilylation of **12** (HF, CH_3CN) gave **13**. Careful hydrolysis of **11** (LiOH, EtOH) directly afforded the hydroxy dienone **14**.

When the reaction was carried out in alcoholic solvents such as in methanol or (trimethylsilyl)ethanol, the corresponding alkoxy dienones **15a**,**b** could be obtained (30−40% yield), but all attempts to directly introduce a hydroxyl group by performing

(5) Tanaka, K.; Kato, T.; Matsumoto, K.; Yoshida, T. *Inflammation* **1993**, *17*, 107.

(6) Tanaka, K.; Matsutani, S.; Matsumoto, K.; Yoshida, T. *Eur. J. Pharm.* **1995**, *279*, 143−148.

(7) Génisson, Y.; Tyler, P. C.; Young, R. N. *J. Am. Chem. Soc.* **1994**, *116*, 759.

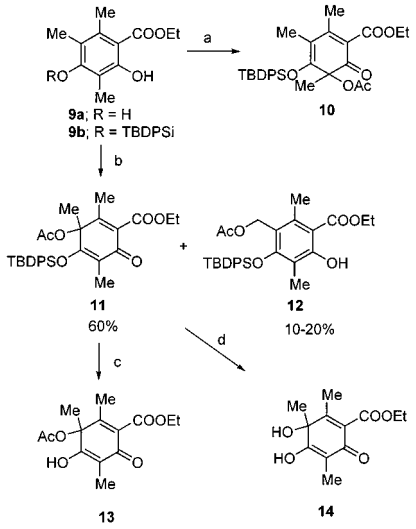
(8) Obtained from ethyl 2,4-dihydroxy-3,6-dimethylbenzoate (Elix, J. A.; Norfolk, S. *Aust. J. Chem.* **1975**, *28*, 1113−1124) by formylation (Pulgarin, C.; Gunzinger, J.; Tabacchi, R. *Helv. Chim. Acta* **1985**, *68*, 1948−1951).

(9) Barton, D. H. R.; Magnus, R. D.; Quinney, J. C. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1610.

(10) Assignment of the regiochemistry in structures **9** and **10** relied on extensive NMR analysis (NOE experiments): a representative example is detailed for synthetic intermediate **23**, the (trimethylsily)ethyl ester analogue of **10a** (see ref 14).

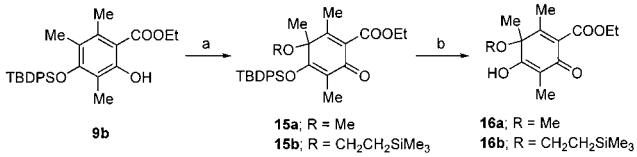
(11) Tamura, Y.; Yakura, T.; Haruta, J.; Kita, Y.; *J. Org. Chem.* **1987**, *52*, 3927. Pelter, A.; Elgendy, S. *Tetrahedron Lett.* **1988**, *29*, 677.

Scheme 4[a]



[a] Reagents: (a) $Pb(OAc)_4$, benzene, 60%; (b) $PhI(OAc)_2$, AcOH; (c) aqueous HF, CH_3CN, 71%; (d) LiOH, EtOH, 53%.

Scheme 5[a]



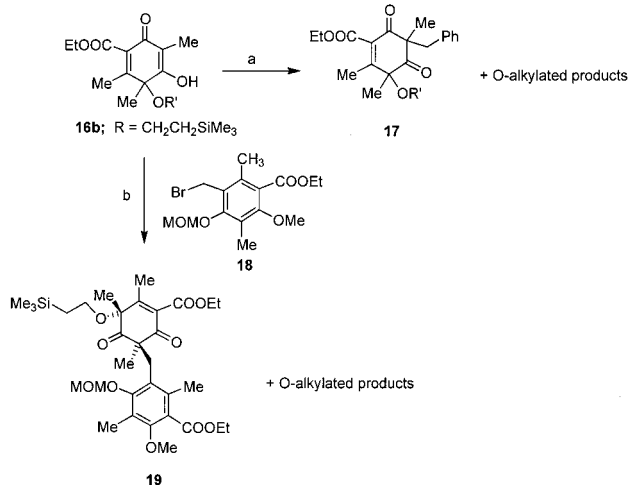
[a] Reagents: (a) $PhI(OAc)_2$, ROH, ca. 40%; (b) **15b**, H_2O, AcOH, THF, 61%.

the reaction in aqueous media were unsuccessful (Scheme 5). It was possible to cleanly cleave the silyl enol ethers in **15a** or **15b** (HF in CH_3CN) to obtain the corresponding dienones **16a** and **16b**.

Although our initial synthetic plan was to condense **13** or **14** with a quinone methide electrophile, some model studies were undertaken to determine the regio- and stereochemical outcome of alkylation of **13**, **14**, or **16b** with a benzylic halide. Reactions of **13** with benzyl bromide under a variety of basic conditions yielded either exclusive or a preponderance of O-benzylated products. However, using the (trimethylsilyl)ethoxy analogue **16b** in *t*-BuOK/*t*-BuOH, it was possible to obtain the C-alkylated derivative **17** (along with some of its O-alkylated congener). However, when the same conditions were applied to **16b** and a more elaborate benzyl bromide **18** (derived in five steps from ethyl 2-methoxy-3,6-dimethyl-4-hydroxybenzoate) the major product resulted from O-alkylation and only 12% yield of C-alkylated material **19** was obtained (Scheme 6). Most discouragingly, this C-alkylated derivative was predominantly the undesired stereoisomer wherein the newly formed carbon−carbon bond was introduced *anti* to the tertiary trimethylsilylethyloxy group. We therefore returned our focus to the preparation of suitably substituted quinone methides as originally envisaged.

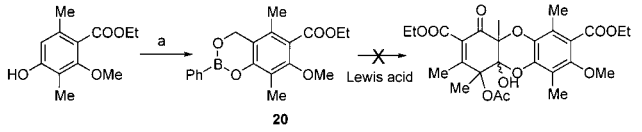
Quinone methides of type **6** have been prepared in a number of ways. We first attempted the condensation of **13** with the quinone methide generated *in situ* from the cyclic boronate **20** (Scheme 7) formed from reaction of ethyl 2-methoxy-3,6-dimethyl-4-hydroxybenzoate, phenylboronic acid, and formaldehyde.[12] However, attempts under different Lewis acid conditions ($Et_2O{\cdot}BF_3$ or $TiCl_4$) yielded no useful product mixture. We therefore investigated alternative methods for generating the desired quinone methide under milder conditions.

Scheme 6[a]



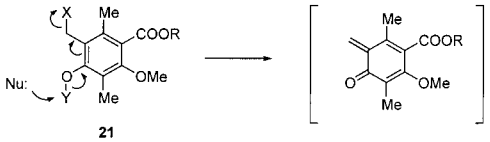
[a] Reagents: (a) BnBr, *t*-BuOH, *t*-BuOK, 70 °C, 50%; (b) **18**, *t*-BuOH, *t*-BuOK, 35 °C, 12%.

Scheme 7[a]



[a] Reagents: (a) $PhB(OH)_2$, $(CHO)_n$, propionic acid, benzene, △, 100%.

Scheme 8

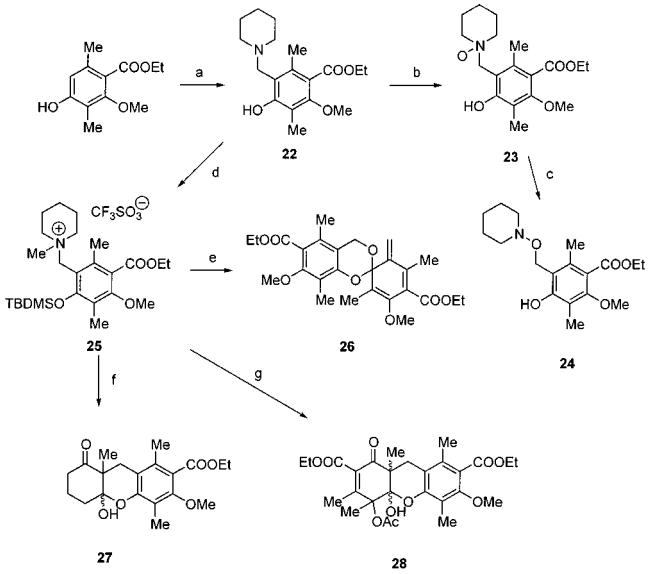


It was envisaged that decomposition of an activated derivative **21**, where X represents a good leaving group such as a tertiary amine *N*-oxide or a quaternary ammonium salt, could provide the desired quinone methide at low temperature (Scheme 8).

The amine precursor **22** could be readily prepared from Mannich condensation of 2-methoxy-3,6-dimethyl-4-hydroxybenzoate with piperidine and formaldehyde. Standard oxidation with *m*-CPBA provided the *N*-oxide **23**. Treatment of **23** under either basic conditions (NaH, NaCN, THF) or Lewis acid conditions (TMSCN, CH_2Cl_2) yielded only a rearrangement (to **24)** where the *in situ* generated quinone methide had been trapped by the *N*-hydroxypiperidine formed in the reaction. Silylation of the phenol **22** followed by quaternization of the amine with methyl triflate furnished salt **25**. Treatment of **25** with TBAF at −20 °C in dichloromethane led to instantaneous conversion to the quinone methide, which was stable below −20 °C, and slowly underwent [2+4] cycloaddition to yield dimer **26** when warmed to room temperature. In a model reaction, treatment of **25** with TBAF in the presence of 2-methylcyclohexane-1,3-dione at −30 °C in THF followed by warming to room temperature yielded the desired tricyclic structure **27** (43% yield). Reaction with acetyl-protected dienone **13** under similar conditions gave the expected hemiketal **28** (62% yield) as a mixture of all four possible isomers (Scheme 9).

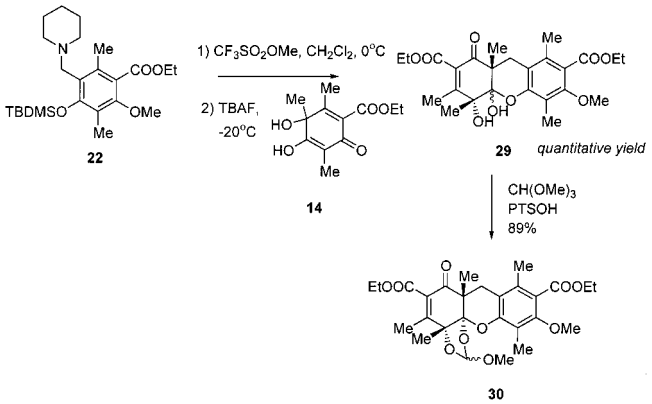
(12) Chambers, J. D.; Crawford, J.; Williams, H. W. R.; Dufresne, C.; Scheigetz, J.; Bernstein, M. A.; Lau, C. K. *Can. J. Chem.* **1992**, *70*, 1717. This methodology has been applied in our group to the total synthesis of thielocin B3 (Génisson, Y.; Young, R. *Tetrahedron Lett.* **1994**, *35*, 7747−7750).

Scheme 9[a]



[a] Reagents: (a) piperidine, $(CHO)_n$, EtOH, △, 85%; (b) mCPBA, $CHCl_3$, −10 °C, 100%; (c) NaH, NaCN, −60 °C to room temperature, THF, 50%; (d) (1) TBDMSCl, NaH, THF, RT, 3h, (2) methyl triflate, ether, −15 °C to room temperature, crude product used directly in next reaction; (f) 2-methyl-1,3-cyclohexandione, TBAF, −30 °C to room temperature, 43% for three steps; (g) **13**, TBAF, −30 °C to room temperature, 62% for three steps.

Scheme 10



However, when the same reaction was carried out with the free hydroxy analogue **14**, the desired product **29** was obtained in essentially quantitative yield (as a dynamic mixture of hemiketals) and with specific control of the relative stereochemistry (Scheme 10). No O-alkylation or traces of the other isomers could be observed. Indications that the relative stereochemistry corresponded to the target natural compound were obtained by NMR studies on the mixture of methyl orthoformates **30** derived from the α isomer.[13] Interestingly, crystallization of **29** (from ethyl acetate) selectively provided the α isomer through dynamic equilibrium. X-ray diffraction analysis of this crystalline material confirmed the relative stereochemistry indicated from NMR structural assignment (Figure 1).

(13) The key data were the following (acetone-d_6, 400 MHz, δ in ppm): the product was a mixture of *ca.* 2:1 diastereoisomers. The highest field methyl singlets (δ 1.22 and 1.20) were assigned to the angular methyl groups α to the enone carbonyl based on NOEs to the central methylene protons signal (δ 2.90). Both of these methyl signals displayed NOEs to the methyls in γ to the enone carbonyl singlets (δ 1.87 and 1.81). Finally, irradiation of δ 1.87 methyl singlet (major isomer) induced an NOE in the δ 5.90 orthoformate proton signal, whereas no similar NOE was observed for the δ 1.81 signal (minor isomer).

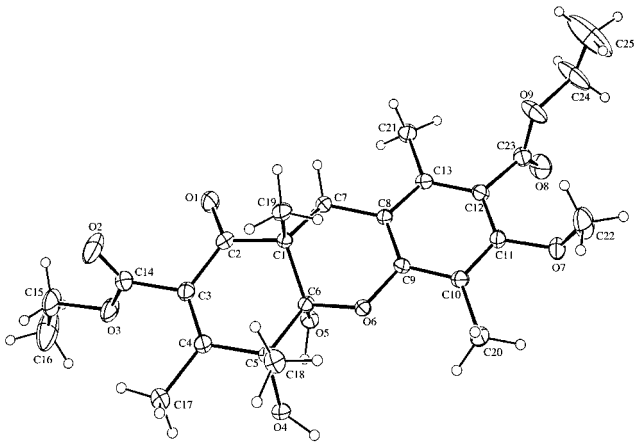


Figure 1.

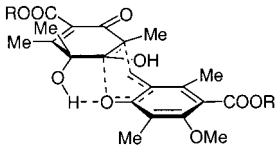


Figure 2.

The reasons for the exceptional stereocontrol of this coupling reaction in contrast to the reaction of **13**, which yielded a mixture of isomers, are not clearly understood. The results strongly suggest a critical influence of the free tertiary hydroxyl group in **14** on the stereochemical outcome, likely through a stabilizing hydrogen bond with the quinone methide carbonyl in the transition state (Figure 2). The free diol **29** proved to be sensitive to basic hydrolysis conditions while the methyl orthoformate derivatives **30** were surprisingly resistant to saponification. It was therefore evident that for a successful synthesis of thielocin A1, the protecting esters used must be carefully chosen so as to allow smooth and selective removal.

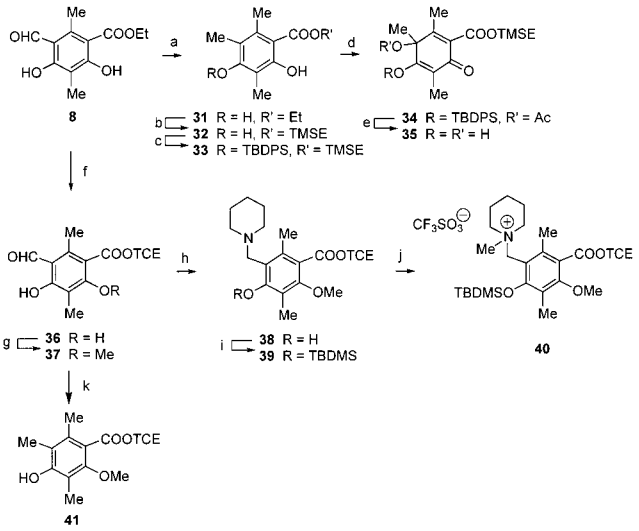
Synthetic Strategy

The overall synthetic strategy was derived from the assumption that the phenolic esters of thielocin A1 would be introduced after the formation of the central core of the molecule. This implied that different acid protecting groups would have to be introduced on the hydroxy dienone and on the quinone methide precursors. We envisaged that the α,β-unsaturated ester of the central dimeric unit must be hydrolyzed selectively and then esterified with a phenolic monomer bearing the same protecting group as that on the aromatic function originating from the quinone methide component. Both of these esters could then be simultaneously cleaved to yield a dicarboxylic acid from which the pentameric skeleton of thielocin A1 could be directly available via concomitant esterification with the phenolic monomer unit. Final deprotection would then lead to the target natural product. Based on model studies, it was decided to prepare the dienone component **5** protected as (trimethylsilyl)-ethyl (TMSE) ester and the phenol **7** as well as the quinone methide **6** both protected as 2,2,2-trichloroethyl (TCE) esters.

Synthesis of Key Intermediates 35, 40, and 41

Conversion of the pivotal intermediate **8** to the hydroxy dienone trimethylsilylethyl ester **35** was effected as follows (Scheme 10). Clemensen reduction provided **31** (88% yield) which on transesterification with sodium (trimethylsilyl)ethoxide in benzene gave the TMSE ester **32** (63% yield). After selective protection of the more reactive *para* hydroxyl group as the *tert*-

Scheme 11[a]



[a] Reagents: (a) Zn, $HgCl_2$, HCl, EtOH, △, 91%; (b) $Me_3CH_2CH_2ONa$, benzene, △, 63%; (c) TBDPSiCl, Et_3N, DMAP, CH_2Cl_2, 91%; (d) $PhI(OAc)_2$, AcOH (57%); (e) LiOH, THF, H_2O, 25 °C, 70%; (f) Cl_3CH_2OH, concentrated H_2SO_4, 0 to 25 °C, 61%; (g) $(MeO)_2SO_2$, K_2CO_3, acetone, 50 °C, 90%; (h) piperidine, $Na(CN)BH_3$, CH_3CN, 83%; (i) NaH, THF, TBDMSiCl, 95%; (j) CF_3SO_2OMe, CH_2Cl_2, 0 °C, 99%; (k) Et_3SiH, TFA, 96%.

butyldiphenysilyl (TBDPS) ether to give **33** (91% yield), regioselective oxidation with phenyliodonium diacetate in acetic acid provided the acetoxy dienone **34** (57% yield).[14] Carefully optimized hydrolysis of both acetate and TBDPS enol ether (LiOH, 12-crown-4) gave the desired hydroxy dienone **35** (76% yield).

Synthesis of the key piperidinium salt **40** was achieved as follows (Scheme 11). Transesterificatïon of **8** with trichloroethanol in sulfuric acid yielded the TCE ester **36** (61% yield). Treatment of the latter with dimethyl sulfate and potassium carbonate selectively provided the monomethyl ether **37** (90% yield), where methylation occurred exclusively *ortho* to the ester function. It is interesting to note that no selectivity was observed when the same conditions were applied to the ethyl ester analogue **8**. Reductive amination (piperidine, $NaCNBH_3$) then cleanly afforded the benzylic amine **38** (83% yield). Protection of the free hydroxyl group as the *tert*-butyldimethylsilyl ether **39** (95% yield) followed by quaternization of the amino group with methyl triflate provided the stable quaternary ammonium salt **40** (99% yield).

The phenolic monomer **41** required for the completion of the synthesis was thought to be readily derivable from aldehyde **37** (Scheme 11). However, preliminary attempts to effect this conversion under hydrogenation conditions (40 psi H_2, $Pd(OH)_2$, AcOH) unexpectedly yielded the 2-methoxy analogue of **31**, as a result of the concomitant reduction of the TCE ester into an ethyl ester. This could be circumvented by treatment of **37** with triethylsilane in trifluoroacetic acid, which cleanly afforded the desired reduced compound as its TCE ester **41** (96% yield).[15]

Final Assembly of the Units

We were pleased to observe that, in keeping with our model studies, fluoride ion catalyzed coupling of **35** and **40** (TBAF, −20 °C to room temperature) proceeded in *essentially quantitative yield and with complete stereoselectivity* to afford the differentially protected tricyclic compound **42** as a thermodynamic mixture of two hemiketals (Scheme 12).

Considering the difficulties anticipated in dealing with a mixture of hemiketals and the potential reactivity of the masked phenol in the subsequent steps of the synthesis, it was decided to protect the two free hydroxyl groups. Treatment of the mixture of hemiketals with carbonyldiimidazole and triethylamine led to a complete transformation to the cis form and formation of a single carbonate **43** (98% yield).

Selective hydrolysis of the TMSE ester **43** with fluoride ion (TBAF in DMF) gave the mono acid **44** (83% yield) (Scheme 7). This was converted to the acid chloride with dichloromethylmethyl ether in refluxing dichloromethane[16] and then reacted with phenol **41** (Et_3N, CH_2C1_2) to yield the trimeric ester **45** (89% combined yield).

At this point, we were disappointed when attempted reductive cleavage of the TCE esters under standard conditions (Zn(0) in AcOH) gave complex reaction mixtures. This is although model studies on several analogous highly substituted aromatic TCE esters proceeded cleanly to give the desired benzoic acids. These results suggested that the α,β-unsaturated ketoester moiety in the central core of the molecule was sensitive to reducing conditions. However, concomitant removal of the two TCE esters could be smoothly achieved by utilizing milder conditions of Cd(0) in DMF/AcOH[17] to give the dicarboxylic acid **46** (84% yield). Bis-esterification of **46** with the phenol **41**, utilizing a process previously reported for the preparation of depsides (TFAA, benzene),[18] afforded the protected thielocin A1α (**47**) (83% yield). Removal of the TCE esters again proceeded smoothly with Cd(0) in DMF/AcOH to provide the penultimate carbonate **48** (80% yield) (Scheme 12).

Initial attempts to hydrolyze the carbonate by heating in aqueous pyridine gave some of the expected diol (12% yield) along with compounds where the central nonaromatic ester had been cleaved to yield the phenolic dimer **49** (45% yield) and the decarboxylated trimeric component **50** (57% yield) (Scheme 13). This decarboxylation was thought to result from deconjugation of the α,β-unsaturated keto ester function, either through addition of a nucleophile (such as the phenolate anion derived from opening of the hemiketal) or base-catalyzed enolization (vide infra). The α-keto ester thus liberated could subsequently undergo hydrolytic decarboxylation and either re-elimination of the nucleophile or isomerization would then yield the decarboxylated product. Careful monitoring of this final step appeared to be necessary to avoid any side reactions.

After exploration of various conditions, hydrolysis of the carbonate **48** was found to proceed smoothly upon treatment with 1 N sodium hydroxide in dioxane at −10 °C. Monitoring by reverse-phase HPLC showed almost instantaneous conversion of the starting carbonate to a more retained intermediate, likely the enol **51** (treatment of **51** with diazomethane led to the

(14) The structure assignment of **34** was based on the following NMR observations (acetone-d_6, 400 MHz, δ in ppm): the methyl group proximal to the ester group was easily established by irradiation of the singlet at δ 1.94 and observing an NOE in the ester methylene protons signal (δ 4.28). Irradiation of the methyl singlets at δ 2.04 and 1.81 displayed NOEs in the methyl signal shown to be *ortho* to the ester group. The singlet at δ 1.81 showed a strong NOE and was assigned to the quaternary methyl group while the δ 2.04 singlet showed a comparatively weak NOE, which together with its chemical shift led us to ascribe it to the acetoxy group. Finally, irradiation of the acetoxy methyl signal resulted in a small NOE in the δ 1.81 methyl. The remaining δ 1.18 methyl singlet was rationalized to be *ortho* to the ketone functionality because of its relatively shielded resonance shift and a small 1,3 interaction with the quaternary methyl signal observed in the NOE experiment.

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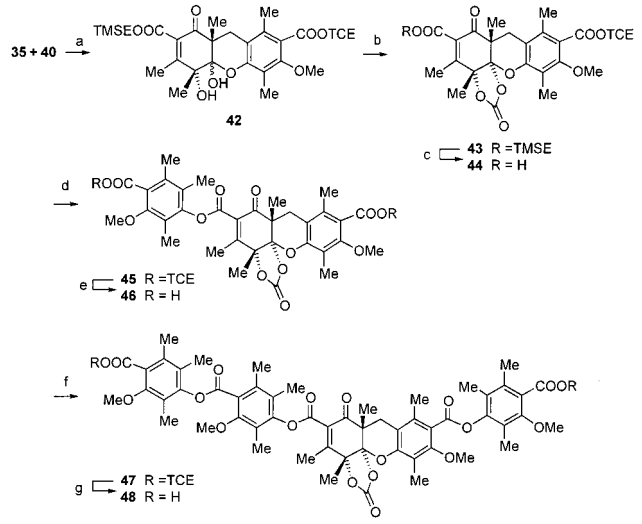
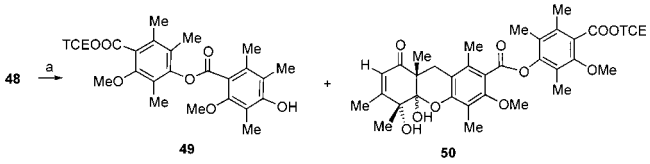
Scheme 12[a]



[a] Reagents: (a) TBAF, CH_2Cl_2, THF, −20 °C to room temperature, 95%; (b) CDI, Et_3N, DMF, 98%; (c) TBAF, DMF, 83%; (d) (1) Cl_2CHOCH_3, CH_2Cl_4, **44**, △, 3 h, (2) **41**, Et_3N, room temperature, 89% overall; (e) Cd^0, AcOH, DMF, 84%; (f) TFAA, **41**, benzene, 83%; (g) Cd^0, AcOH, DMF, 80%.

Scheme 13[a]

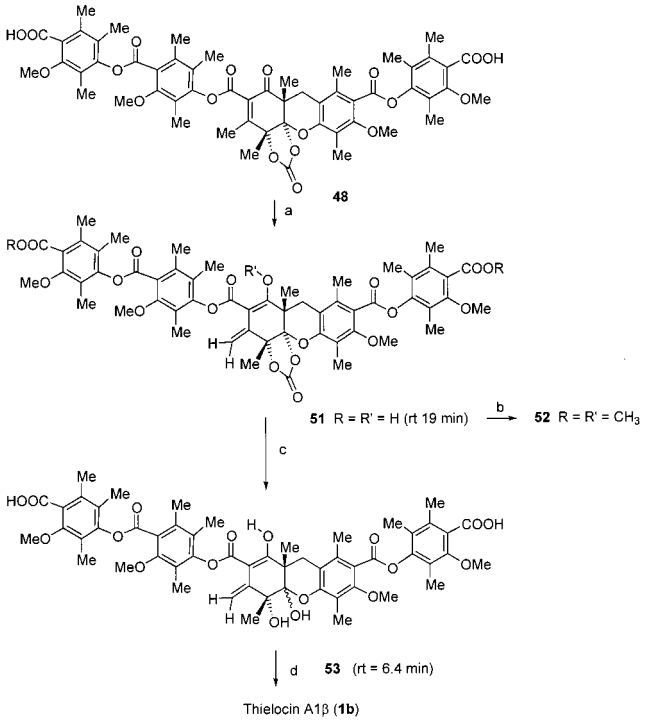


[a] Reagents: (a) H_2O, pyridine, 90 to 110 °C, 10 h.

formation of the enol ether **52**, Scheme 14).[19] The latter was then progressively (8−10 h) transformed under hydrolytic conditions into a less retained precursor, assumed to be the enolized thielocin A1 (**53**).[20] Quenching of the reaction with phosphate buffer and extraction followed by evaporation did not affect the composition of the reaction mixture. However, subsequent purification of this precursor by reverse-phase HPLC followed by extractive isolation was accompanied by exclusive isomerization to the desired thielocin A1β that could be thus isolated with 59% yield [21] (Scheme 14).

Thielocin A1β gave physical and structural data essentially identical to those reported for the natural product as judged by

Scheme 14[a]



[a] Reagents: (a) 1 N NaOH, dioxane, −20 °C, 5 min; (b) CH_2N_2, 100%; (c) 1 N NaOH, dioxane, 8−10 h; (d) 2.5 N H_2PO_4, workup, HPLC, 59% overall.

^1H, ^{13}C NMR, melting point, mass, and IR spectra. The inhibitory profile of thielocin A1β for type II secretory phospholipase A_2 was confirmed by utilizing purified human synovial fluid enzyme (IC_{50} = 2.4 μM) as well as semipurified enzyme from rat paw (IC_{50} = 2 nM).

Conclusion

We have thus achieved a total synthesis of (±)-thielocin A1β and confirmed the biological activity of the synthetic material. The three key synthons, **35**, **40**, and **41**, are all derived from a common intermediate ethyl 5-formyl-2,4-dihydroxy-3,6-dimethyl benzoate (**8**). It is interesting to speculate as to whether the biosynthesis of thielocin A1β may also derive from the condensation of a quinone methide with a suitably substituted hydroxy dienone. Similar condensation has been hypothesized for the formation of the complex metabolites derived from vitamin E.[22] The high degree of regio- and stereoselectivity observed in this condensation may suggest that similar forces are involved in the biosynthesis of thielocin A1β itself.

Experimental Section

Diacid 48. Cadmium powder (100 mesh; 500 mg, 4.45 mmol) was added to a stirred suspension of **47** (117.2 mg, 0.091 mmol) in a dimethylformamide/acetic acid (1:1) mixture (2 mL) at 25 °C. This mixture was vigorously stirred at the same temperature for 15 h. Water (20 mL) and ethyl acetate (20 mL) were then added. Insoluble cadmium excess was decanted. The aqueous layer was separated and extracted with ethyl acetate (20 mL). The combined organic extracts were washed with brine (2 × 20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Precipitation in CH_3OH of the residue obtained gave **48** (75 mg, 80%) as a yellow solid: mp: 268−271 °C; IR (KBr) υ_{max} 3700−2300 (br), 1825, 1740, 1700, 1570 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$−CD_3OD (1:1)) δ 3.87 (s, 3H, *OMe*), 3.86 (s, 3H, *OMe*), 3.84 (s, 6H,

(19) Rapid quench of the reaction after 5 min with diluted phosphoric acid followed by extractive workup yielded a crude mixture consisting essentially of this intermediate, as judged by HPLC analysis. Characteristic IR absorptions at 1820 cm^{-1} indicated the continued presence of a carbonate unit in the molecule. The ^1H NMR spectrum ($CDCl_3$, 400 MHz, δ in ppm) of this material showed two characteristic apparent singlets in the olefinic region (δ 5.84 and 5.96) and an exchangeable broad signal (δ 8.54−8.77). Attempts to further purify this crude mixture on HPLC were accompanied by partial isomerization back to the starting diacid carbonate **48**. Nevertheless, treatment of the crude material with diazomethane gave a stable compound isolable by silica gel chromatography. The ^1H NMR spectrum ($CDCl_3$, 400 MHz, δ in ppm) of this derivative showed the continued presence of two singlets (in the δ 5.70−6.00 region) plus the presence of three extra methyls in the methoxy region, including the two expected methyl esters. Mass spectrum (FAB) showed an $(MH)^+$ peak at *m*/*z* 1065 confirming the incorporation of three methyls. These spectral data are in agreement with the structure of the methyl enol ether **52** (Scheme 14).

(20) This assumption is based on the fact that the ^1H NMR spectrum ($CDCl_3$, 400 MHz, δ in ppm) of the worked up crude material after completion of the hydrolysis but before isomerization to the final product showed two recognizable singlets in the olefinic region (δ 5.70 and 5.74) along with other characteristic signals for thielocins A1.

(21) The reaction provided the thermodinamically more stable β isomer. Thielocins A1α and β are known to form an equilibrium largely in favor of the β isomer (see ref 4).

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2×*OMe*), 3.08 (AB_q, $J = 16$ Hz, $\Delta\nu = 84$ Hz, 2H, C*H*$_2$), 2.45 (s, 3H, *Me*), 2.43 (s, 6H, 2×*Me*), 2.32 (s, 3H, *Me*), 2.31 (s, 3H, *Me*), 2.30 (s, 6H, 2×*Me*), 2.27 (s, 9H, 3×*Me*), 2.26 (s, 6H, 2×*Me*), 2.13 (s, 3H, *Me*), 1.45 (s, 3H, *Me*); ^{13}C NMR (100 MHz, $CDCl_3-CD_3OD$ (1:1) δ 192.5, 170.3, 165.9, 165.8, 162.0, 155.1, 153.8, 152.6, 150.6, 149.9, 148.8, 148.6, 148.5, 148.3, 133.4, 133.1, 131.6, 130.4, 127.9, 126.3, 125.5, 125.1, 123.1, 121.8, 121.4, 121.4, 117.5, 114.2, 104.2, 84.2, 61.5, 61.4, 61.1, 43.8, 27.6, 19.8, 16.4, 16.3, 15.7, 15.4, 15.1, 12.3, 12.1, 9.59, 9.29, 8.26; FAB MS (NBA/NaCl) *m*/*z* 1045 ($M + Na^+$); FAB HRMS (Glycerol) *m*/*z* calcd for $C_{55}H_{59}O_{19}$ ($M + H^+$) 1023.3654, found 1023.3650.

Thielocin A1 β (1b). NaOH solution (1 N, 200 μL) was added to a well-stirred solution of carbonate **48** (20.1 mg, 0.019 mmol) in dioxane (200 μL) at 25 °C under Ar. This mixture was instantaneously cooled to −10 °C and strictly maintained at this temperature with vigorous stirring until quenching. Reaction was then monitored by analytical reverse-phase HPLC (column: Nova-Pak C18 (60 Å, 4 μm, 3.9 × 150 mm); eluant: CH_3CN/0.1% H_3PO_4 (60:40); flow: 1 mL/min; detection: 220 nm): aliquots (1 μL) of the reaction mixture were acidified with 2.5 N H_3PO_4 solution (20μL) and diluted with CH_3CN (40μL) before injection. HPLC showed instantaneous conversion of the starting carbonate (**48**, rt 9 min) into a slower intermediate (enol **51**, rt 19 min). Slow transformation of the latter into a less retained precursor (enolized thielocin **53**, rt 6.4 min) was then observed. Only traces of the desired β isomer (**1b**, rt 3.4 min) could be detected at that point. On completion of the reaction (8 to 10 h) 2.5 N H_3PO_4 solution (1 mL) was added at −10 °C. The resulting milky aqueous phase was diluted with water (1 mL) and extracted with ethyl acetate (3 × 5 mL). The combined organic extracts were washed with brine (2 × 5 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. This treatment did not affect the composition of the crude as indicated by HPLC. Purification on semipreparative reverse-phase HPLC (column: μBondapak C18 (125 Å, 10 μm, 25 × 100 mm); eluant: CH_3CN/0.1% H_3PO_4 (65:35); flow: 15 mL/min; detection: 220 nm) allowed isolation of fractions corresponding to the precursor **53** and eventually the desired compound **1b** itself (rt 10.5 and 7.3 min, respectively). These fractions were combined and most of the CH_3CN evaporated off in vacuo. The resulting aqueous phase was then extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with brine (2 × 5 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to give fully isomerized thielocin A1β (**1b**) (11.2 mg, 59%) as a white solid: mp 198−201 °C (ether) (lit.[4a] mp 190−194 °C); our synthetic racemic material gave otherwise physical and spectral data essentially identical (IR, ^{1}H and ^{13}C NMR, MS) to those reported for the natural chiral material.

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Supporting Information Available: Complete experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org. The structure of compound **29** has been deposited with the Cambridge Crystallographic Data Centre. Refer to deposition number CCDC168787.

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