HETEROCYCLES, Vol. 83, No. 3, 2011, pp. 491 - 529. © The Japan Institute of Heterocyclic Chemistry
Received, 27th October, 2010, Accepted, 24th December, 2010, Published online, 7th January, 2011
DOI: 10.3987/REV-10-686

# SYNTHESIS AND BIOLOGICAL ACTIVITY OF LAMELLARIN ALKALOIDS: AN OVERVIEW

**Tsutomu Fukuda,[a] Fumito Ishibashi,[a] and Masatomo Iwao[b] ***

[a]Graduate School of Science and Technology, and [a]Department of Applied Chemistry, Faculty of Engineering, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan, e-mail: iwao@nagasaki-u.ac.jp

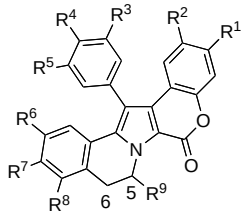
**Abstract** – Lamellarins are natural products isolated from marine invertebrates having a unique heterocyclic ring system. Many of these natural products exhibit potentially useful biological activities such as antitumor and anti-HIV activities. In this review, we summarized the synthesis and biological activity of naturally occurring lamellarins and their analogues.

## 1. INTRODUCTION

Marine invertebrates are a rich source of biologically active compounds with unprecedented molecular structures.[1] In 1985, Faulkner and co-workers reported the isolation of novel marine alkaloids named lamellarins A–D from the prosobranch mollusk *Lamellaria* sp. They demonstrated that these lamellarins possess a unique 14-phenyl-6*H*-[1]benzopyrano[4',3':4,5]pyrrolo[2,1-*a*]isoquinolin-6-one system by X-ray crystallographic analysis.[2] Up to now, over 40 lamellarins (A–Z and α–χ, including their acetates and sulfates) have been isolated from a variety of ascidians and sponges.[2-14] These lamellarins can be divided into three structural types. Most lamellarins possess a fused pentacyclic framework with a saturated (type I) (Table 1) or unsaturated (type II) C5–C6 bond[15] (Table 2). Isolated by Capton and co-workers,[5,6] type III lamellarins (lamellarins O–R) have simple non-fused pyrrolic structures (Figure 1). Pentacyclic lamellarins exhibit important biological activity. For example, Quesada and co-workers have demonstrated that lamellarin D triacetate and lamellarin K triacetate display potent cytotoxicity against multidrug-resistant (MDR) cancer cell lines as well as their respective parental cell lines.[16] More interestingly, the less cytotoxic lamellarin I has effectively increased the cytotoxicity of approved anticancer agents, such as doxorubicin, vinblastine, and daunorubicin, towards MDR cell lines.[16] Faulkner and co-workers have also reported that lamellarin α 20-sulfate inhibits HIV-1 integrase and is active against HIV-1 virus at non-toxic concentrations.[11] The purpose of this review is to summarize the

synthesis and biological activity of pentacyclic lamellarins (Type I and II). Because of their lower biological activity, non-fused lamellarins (Type III) and structurally related 3,4-diarylpyrrole marine alkaloids[17] are not reviewed in the present study.

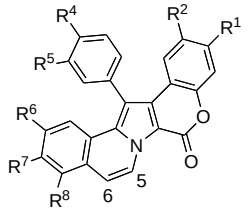
Table 1. Lamellarin alkaloids (Type I)




Type I
(saturated between C5 and C6)

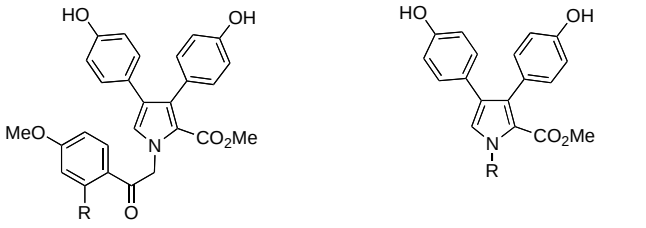
| lamellarin | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $R^6$ | $R^7$ | $R^8$ | $R^9$ | ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| A | OH | OMe | H | OH | OMe | OMe | OMe | OMe | OH | 2, 4, 10 |
| C | OH | OMe | H | OH | OMe | OMe | OMe | OMe | H | 2, 4, 9,10 |
| C diacetate | OAc | OMe | H | OAc | OMe | OMe | OMe | OMe | H | 13 |
| C 20-sulfate | $OSO_3^-$ | OMe | H | OH | OMe | OMe | OMe | OMe | H | 10 |
| E | OH | OMe | H | OMe | OH | OMe | OMe | OH | H | 3, 10 |
| F | OH | OMe | H | OMe | OMe | OMe | OMe | OH | H | 3, 14 |
| G | OMe | OH | H | OMe | OH | OMe | OH | H | H | 3, 10, 12 |
| G 8-sulfate | OMe | OH | H | OMe | OH | OMe | $OSO_3^-$ | H | H | 10 |
| I | OH | OMe | H | OMe | OMe | OMe | OMe | OMe | H | 4, 13, 14 |
| J | OH | OMe | H | OMe | OMe | OMe | OH | H | H | 4, 14 |
| K | OH | OMe | H | OH | OMe | OMe | OMe | OH | H | 4, 7, 13, 14 |
| K diacetate | OAc | OMe | H | OAc | OMe | OMe | OMe | OH | H | 13 |
| K triacetate | OAc | OMe | H | OAc | OMe | OMe | OMe | OAc | H | 13, 14 |
| L | OH | OMe | H | OMe | OH | OMe | OH | H | H | 4, 10, 12 |
| L triacetate | OAc | OMe | H | OMe | OAc | OMe | OAc | H | H | 14 |
| L 20-sulfate | $OSO_3^-$ | OMe | H | OMe | OH | OMe | OH | H | H | 10 |
| S | OH | OH | H | OH | OH | OMe | OH | H | H | 7 |
| T | OH | OMe | H | OMe | OH | OMe | OMe | OMe | H | 8 |
| T diacetate | OAc | OMe | H | OMe | OAc | OMe | OMe | OMe | H | 14 |
| T 20-sulfate | $OSO_3Na$ | OMe | H | OMe | OH | OMe | OMe | OMe | H | 8 |
| U | OH | OMe | H | OMe | OH | OMe | OMe | H | H | 8, 9, 13 |
| U 20-sulfate | $OSO_3Na$ | OMe | H | OMe | OH | OMe | OMe | H | H | 8 |
| V | OH | OMe | H | OMe | OH | OMe | OMe | OMe | OH | 8 |
| V 20-sulfate | $OSO_3Na$ | OMe | H | OMe | OH | OMe | OMe | OMe | OH | 8 |
| Y 20-sulfate | $OSO_3Na$ | OMe | H | OMe | OH | OH | OMe | H | H | 8 |
| Z | OMe | OH | H | OH | OH | OMe | OH | H | H | 10 |
| β | OH | OH | H | OMe | OH | OH | OH | H | H | 12 |
| γ | OH | OMe | OMe | H | OMe | OMe | OMe | OH | H | 13 |
| χ | OAc | OMe | H | OAc | OMe | OMe | OAc | H | H | 14 |

Table 2. Lamellarin alkaloids (Type II)




Type II
(unsaturated between C5 and C6)

| lamellarin | $R^1$ | $R^2$ | $R^4$ | $R^5$ | $R^6$ | $R^7$ | $R^8$ | ref. |
|---|---|---|---|---|---|---|---|---|
| B | OH | OMe | OH | OMe | OMe | OMe | OMe | 2, 4, 10 |
| B 20-sulfate | $OSO_3^-$ | OMe | OH | OMe | OMe | OMe | OMe | 10 |
| D | OH | OMe | OH | OMe | OMe | OH | H | 2 |
| D triacetate | OAc | OMe | OAc | OMe | OMe | OAc | H | 4, 10 |
| H | OH | OH | OH | OH | OH | OH | H | 3 |
| M | OH | OMe | OH | OMe | OMe | OMe | OH | 4, 13 |
| N | OH | OMe | OMe | OH | OMe | OH | H | 8 |
| N triacetate | OAc | OMe | OMe | OAc | OMe | OAc | H | 4, 10 |
| W | OH | OMe | OMe | OH | OMe | OMe | OMe | 8 |
| X | OH | OMe | OMe | OH | OMe | OMe | OH | 8 |
| X triacetate | OAc | OMe | OMe | OAc | OMe | OMe | OAc | 13 |
| α | OH | OMe | OMe | OH | OMe | OMe | H | 13 |
| α 20-sulfate | $OSO_3Na$ | OMe | OMe | OH | OMe | OMe | H | 11 |
| ε | OH | OMe | OMe | OMe | OMe | OMe | OH | 13 |
| ζ | OH | OMe | OMe | OMe | OMe | OMe | OMe | 14 |
| η | OH | OMe | OMe | OMe | OMe | OMe | H | 14 |
| ϕ | OAc | OMe | OAc | OMe | OAc | OMe | OMe | 14 |




lamellarin O (R=H)
lamellarin P (R=OH)

lamellarin Q (R=H)
lamellarin R (R=$C_6H_4$-*p*-OH)

Type III

Figure 1. Lamellarin alkaloids (Type III)

## 2. SYNTHESIS OF LAMELLARINS

In spite of their unique structure, the synthesis of lamellarins was neglected for over ten years after their initial isolation by Faulkner in 1985. The prominent report by Quesada in 1996 on the potent cytotoxic activity of lamellarins against MDR cancer cell lines prompted many organic chemists to synthesize these molecules. In 1997, three research groups (Steglich, Ishibashi–Iwao, and Banwell–Flynn) reported the

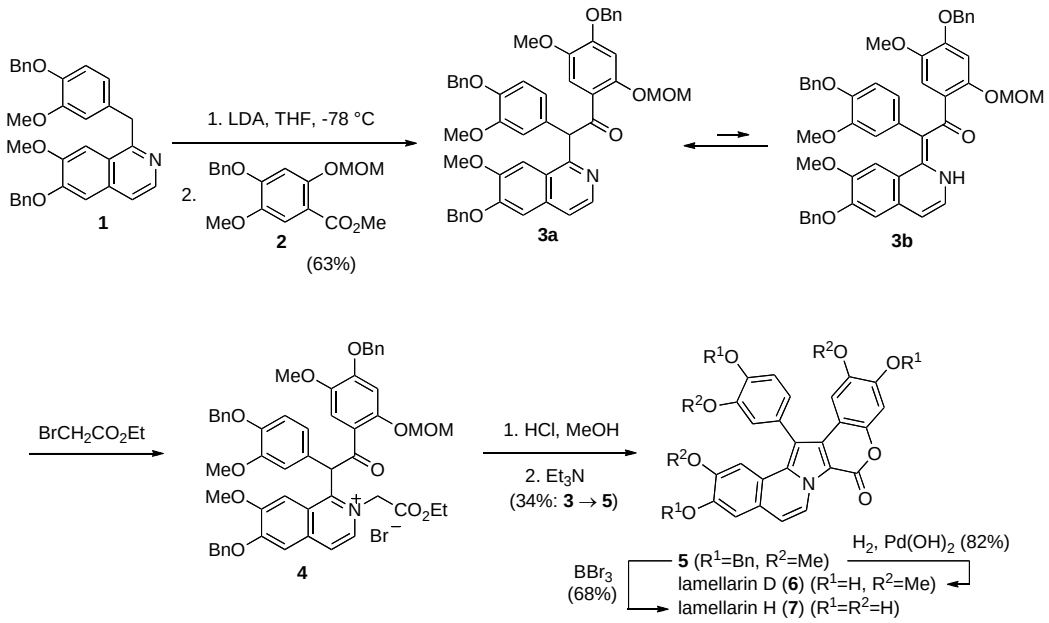
first synthesis of lamellarins *via* different approaches. Since these initial studies, a wide range of synthetic methods have been developed to generate the pentacyclic structures. These methods can be divided into two categories. The first category relies on a ring construction from isoquinoline derivatives while the second category utilizes pyrroles as starting materials.

## 2-1. SYNTHESIS *VIA* ISOQUINOLINES

The pentacyclic lamellarin framework can be regarded as a functionalized pyrrolo[2,1-*a*]isoquinoline system. This explains why most lamellarin syntheses described in this section have adapted methods developed for the preparation of pyrrolo[2,1-*a*]isoquinolines starting from isoquinoline derivatives.[18]

### 2-1-1. SYNTHESIS BY ISHIBASHI–IWAO

Ishibashi, Miyazaki, and Iwao reported the first total syntheses of lamellarins D and H in 1997.[19] They utilized the *N*-ylide-mediated cyclization that was devised by Iwao and Kuraishi in 1980 for the synthesis of pyrrolo[2,1-*a*]isoquinolines[20] as the key ring construction step (Scheme 1).




Scheme 1. Synthesis of lamellarins D (**6**) and H (**7**) *via* *N*-ylide-mediated cyclization

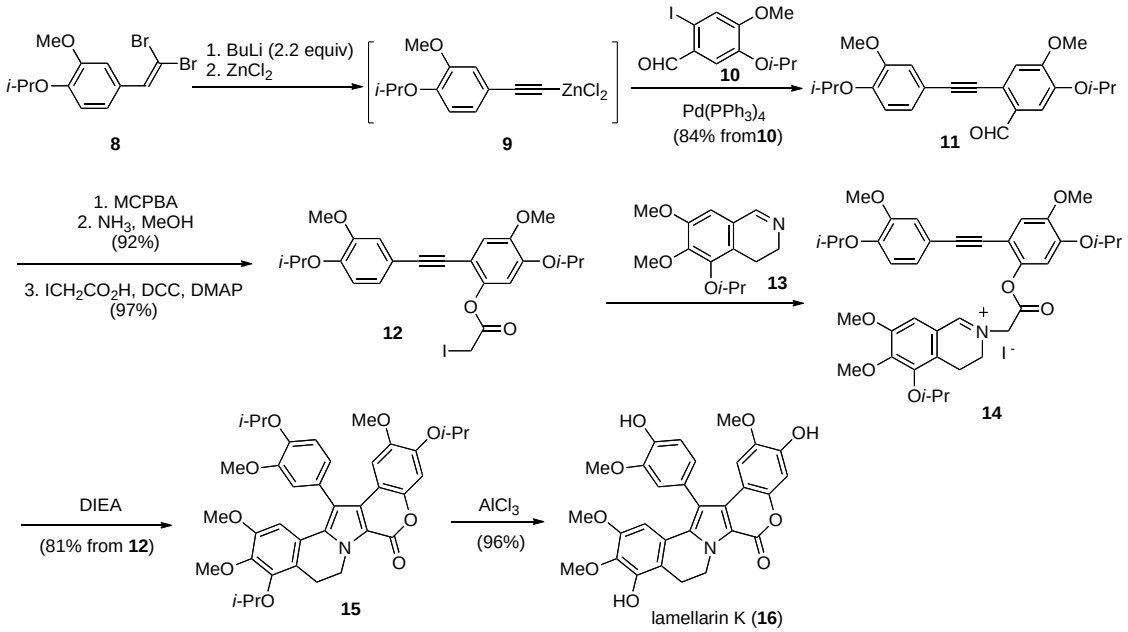
The appropriately substituted benzylisoquinoline (**1**), which was prepared from isovanillin in 6 steps, was deprotonated with 1.1 equiv of lithium diisopropylamide (LDA) at −78 °C and the resulting anion was reacted with the benzoate (**2**) at room temperature for 3.5 h to give the *C*-acylated compound as a tautomeric mixture (**3a** and **3b**) in 63% yield. Interestingly, the use of larger amounts of LDA or prolonged reaction times decreased the yield of **3**. The lamellarin skeleton was subsequently constructed in three steps without isolation of intermediate compounds. Thus, compound (**3**) was treated with 20

equiv of ethyl bromoacetate at 70 °C for 22 h to generate a quaternary ammonium salt (**4**). Removal of the *O*-methoxymethyl (MOM) protecting group using methanolic hydrochloric acid followed by treatment with triethylamine gave lamellarin (**5**) in 34% yield. Selective removal of the benzyl group by hydrogenolysis over Pearlman's catalyst gave lamellarin D (**6**) in 82% yield. Exhaustive dealkylation of **3** with 6 molar equiv of boron tribromide gave lamellarin H (**7**).

This strategy was successfully applied to produce a small library of non-natural lamellarins for structure–activity relationship (SAR) investigations relative to cytotoxicity (see section **3-1**).[21]

## 2-1-2. SYNTHESIS BY BANWELL–FLYNN

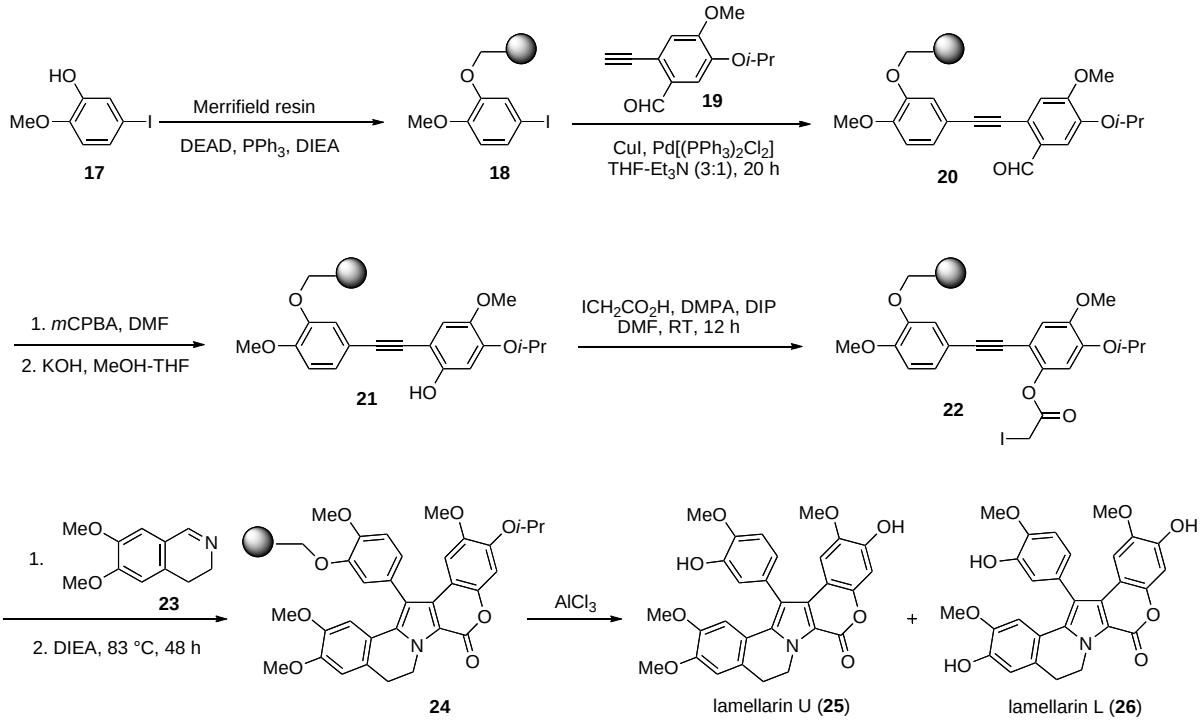
Banwell, Flynn, and Hockless reported a highly convergent synthesis of lamellarin K using an intramolecular 1,3-dipolar cyclization reaction in 1997 (Scheme 2).[22] Derived from dibromostyrene (**8**) *in situ*, zinc acetylide (**9**) was coupled with iodide (**10**) in the presence of palladium catalyst to give the unsymmetrically substituted acetylene (**11**) in 84% yield. Baeyer–Villiger oxidation of **11** followed by methanolysis and esterification with iodoacetic acid gave iodide (**12**). *N*-alkylation of dihydroisoquinoline (**13**) with **12** and treatment with diisopropylethylamine (Hünig's base) gave lamellarin **15** in 81% yield *via* intramolecular 1,3-dipolar cycloaddition between azomethine ylide and acetylene moieties. Selective removal of the *O*-isopropyl group with aluminum chloride produced lamellarin K (**16**).




Scheme 2. Synthesis of lamellarin K (**16**) *via* intramolecular 1,3-dipolar cyclization

Albericio and Álvarez extended the Banwell–Flynn strategy to a solid phase synthesis of lamellarins (Scheme 3).[23] Iodophenol (**17**) was anchored onto the Merrifield resin by Mitsunobu reaction.

Palladium-catalyzed Sonogashira coupling of **18** with the arylacetylene (**19**) gave **20**. Baeyer–Villiger oxidation of **20** followed by methanolysis gave phenol **21,** which was subsequently esterified with iodoacetic acid to give **22**. *N*-alkylation of 3,4-dihydro-6,7-dimethoxylisoquinoline (**23**) with **22** followed by treatment with Hünig's base produced 1,3-dipolar cycloaddition product **24**. Cleavage of **24** with aluminum chloride gave a crude mixture that consisted of lamellarin U (**25**) and lamellarin L (**26**) as the major compounds. Pure **25** and **26** were isolated by semipreparative HPLC in 10% and 4% overall yields, respectively. A number of cleavage/deprotection conditions were also tested at the final stage to produce various lamellarins.[24]

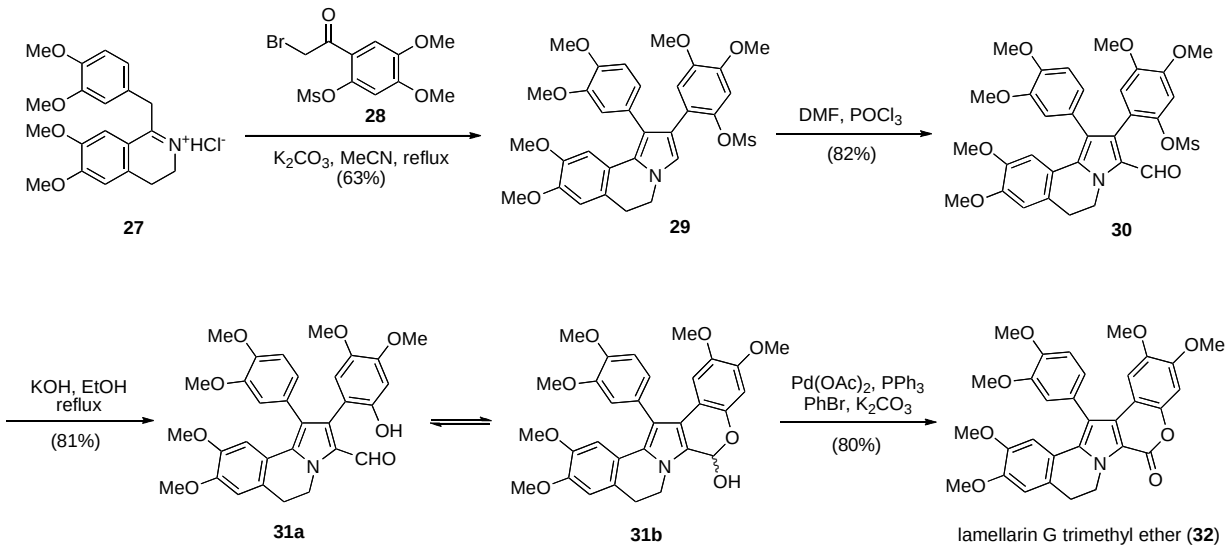



Scheme 3. Solid-phase synthesis of lamellarins U (**25**) and L (**26**)

## 2-1-3. SYNTHESIS BY RUCHIRAWAT

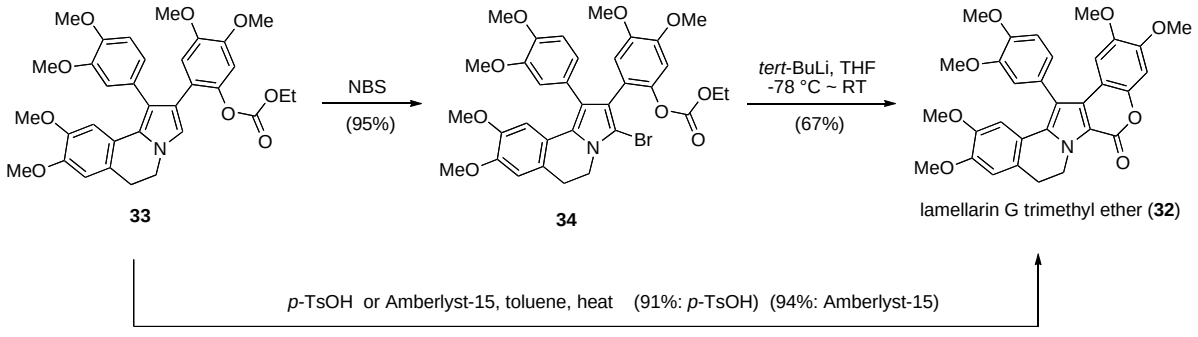
In 2001, Ruchirawat and Mutarapat reported an efficient synthesis of lamellarin G trimethyl ether (**32**) starting from 3,4-dihydro-1-benzylisoquinoline (**27**) (Scheme 4).[25] Reaction of **27** with phenacyl bromide (**28**) in the presence of potassium carbonate in acetonitrile gave 5,6-dihydropyrrolo[2,1-*a*]isoquinoline (**29**) *via* *N*-alkylation of **27** with **28** followed by intramolecular condensation of the resulting enamine with carbonyl moiety (Tschitschibabin reaction[26]). Vilsmeier reaction of **29** followed by alkaline hydrolysis of the mesyl group gave hydroxy-aldehyde (**31a**) in good yield. Oxidation of **31a** with manganese dioxide *via* the putative cyclic hemiacetal (**31b**) gave lamellarin G trimethyl ether (**32**) in low yield (20%). The concomitant oxidation of the phenolic moiety generated a quinone as by-product. This undesirable side reaction was prevented using palladium-catalyzed Tamaru oxidation[27]

(bromobenzene-palladium acetate-triphenylphosphine-potassium carbonate) which gave **32** in good yield.




Scheme 4. Synthesis of lamellarin G trimethyl ether (**32**) *via* Tschitschibabin reaction

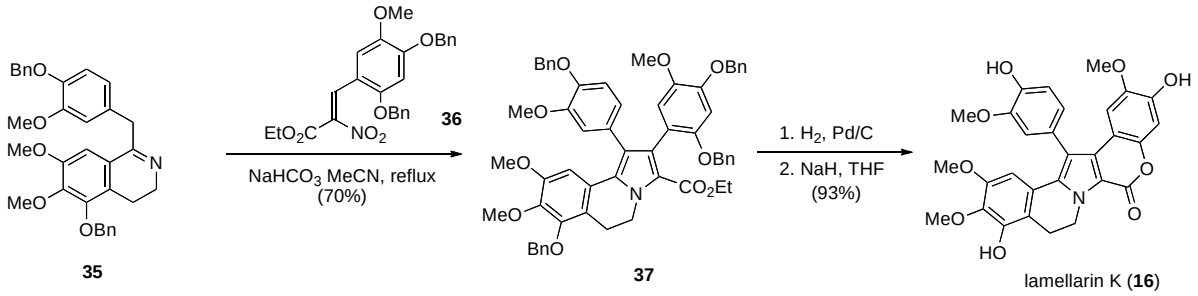
Ruchirawat improved the final lactone synthesis using a strategy involving lithium-bromine exchange, carbonate migration, and cyclization (Scheme 5).[28] Tricyclic intermediate (**33**) was synthesized from **27** as described above and brominated by *N*-bromosuccinimide to give **34**. Compound **34** was treated with *tert*-butyllithium at −78 °C, and then warmed up to room temperature to give lamellarin G trimethyl ether (**32**) in 67% yield. Acid-catalyzed Friedel–Crafts transacylation followed by lactonization could also directly convert **33** to **32** in excellent yield.[29]




Scheme 5. Improved procedures for the lactone formation

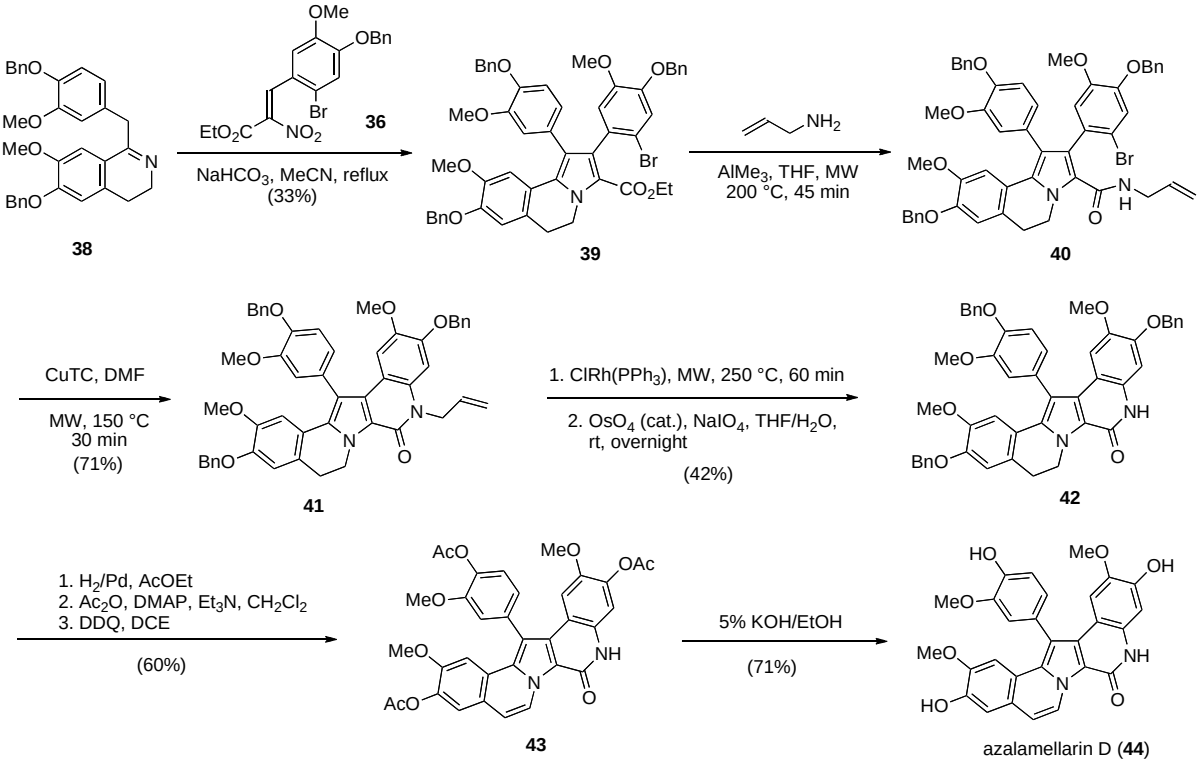
Ruchirawat developed another highly efficient synthesis of lamellarins starting from 3,4-dihydro-1-benzylisoquinolines.[30] For example, the synthesis of lamellarin K is depicted in Scheme 6. Benzylisoquinoline (**35**) was reacted with α-nitrocinnamate (**36**), which was prepared in four steps from isovanillin in 56% overall yield, in the presence of sodium bicarbonate in acetonitrile to give

5,6-dihydropyrrolo[2,1-*a*]isoquinoline (**37**) in 70% yield. This key reaction may proceed *via* Michael addition of the enaminic tautomer from **35** to **36** followed by ring closure (Grob cyclization[31]). Debenzylation followed by base-mediated lactonization gave lamellarin K (**16**) in excellent yield. This procedure was successfully applied to the synthesis of several natural and non-natural lamellarins[32] for extensive SAR studies (see Section **3-1**).[33]




Scheme 6. Synthesis of lamellarin K (**16**) *via* Grob cyclization

Recently, Ruchirawat extended the same strategy to the synthesis of azalamellarins (lactam congeners). For example, the synthesis of azalamellarin D is shown in Scheme 7.[34] Grob cyclization of **36** and **38** produced compound (**39**), which reacted with allylamine in the presence of trimethylaluminum to give amide (**40**). The cyclization of amide through copper (I)-mediated C–$N_{amide}$ bond formation yielded

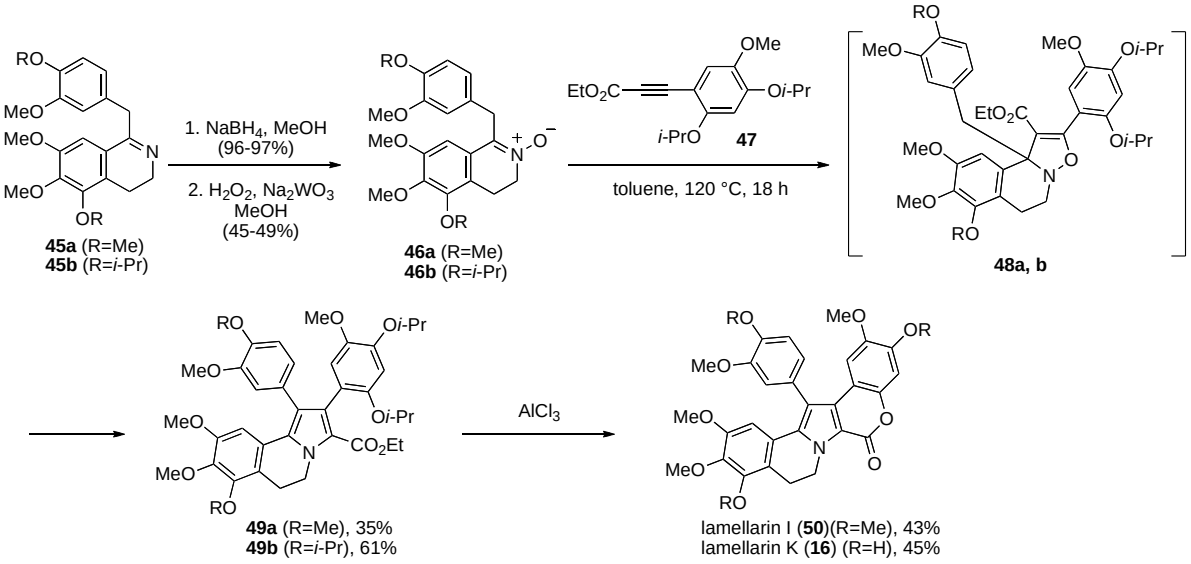



Scheme 7. Synthesis of azalamellarin D (**44**)

lactam (**41**).[35] Rhodium-catalyzed double-bond isomerization followed by oxidation with osmium tetraoxide produced *N*-deallylated compound (**42**).[36] Sequential debenzylation, acetylation, dehydrogenation, and alkaline hydrolysis gave azalamellarin D (**44**).

## 2-1-4. SYNTHESIS BY GUITIAN

Eguchi reported that the 1,3-dipolar cycloaddition of 1-substituted 3,4-dihydroisoquinoline *N*-oxides with alkynes at room temperature gave stable $\Delta^4$-isoxazolines, which rearranged to 5,6-dihydropyrrolo[2,1-*a*]isoquinolines upon heating in toluene.[37] Guitian utilized this reaction in the synthesis of lamellarins I and K (Scheme 8).[38] The *N*-oxides (nitrones) (**46a**, **b**) were prepared in moderate yields by reduction of 3,4-dihydro-1-benzylisoquinolines (**45a**, **b**) with sodium borohydride followed by sodium tungstate-catalyzed oxidation with hydrogen peroxide.[39] Reaction of **46a**, **b** with alkyne (**47**) in toluene at 120 °C in a sealed tube produced **49a** and **49b** in 35% and 61% yields, respectively, *via* 1,3-dipolar cycloaddition-thermal rearrangement. Selective removal of isopropyl groups in **49a**, **b**, concomitant with lactonization, gave lamellarins I (**50**) and K (**16**), respectively.

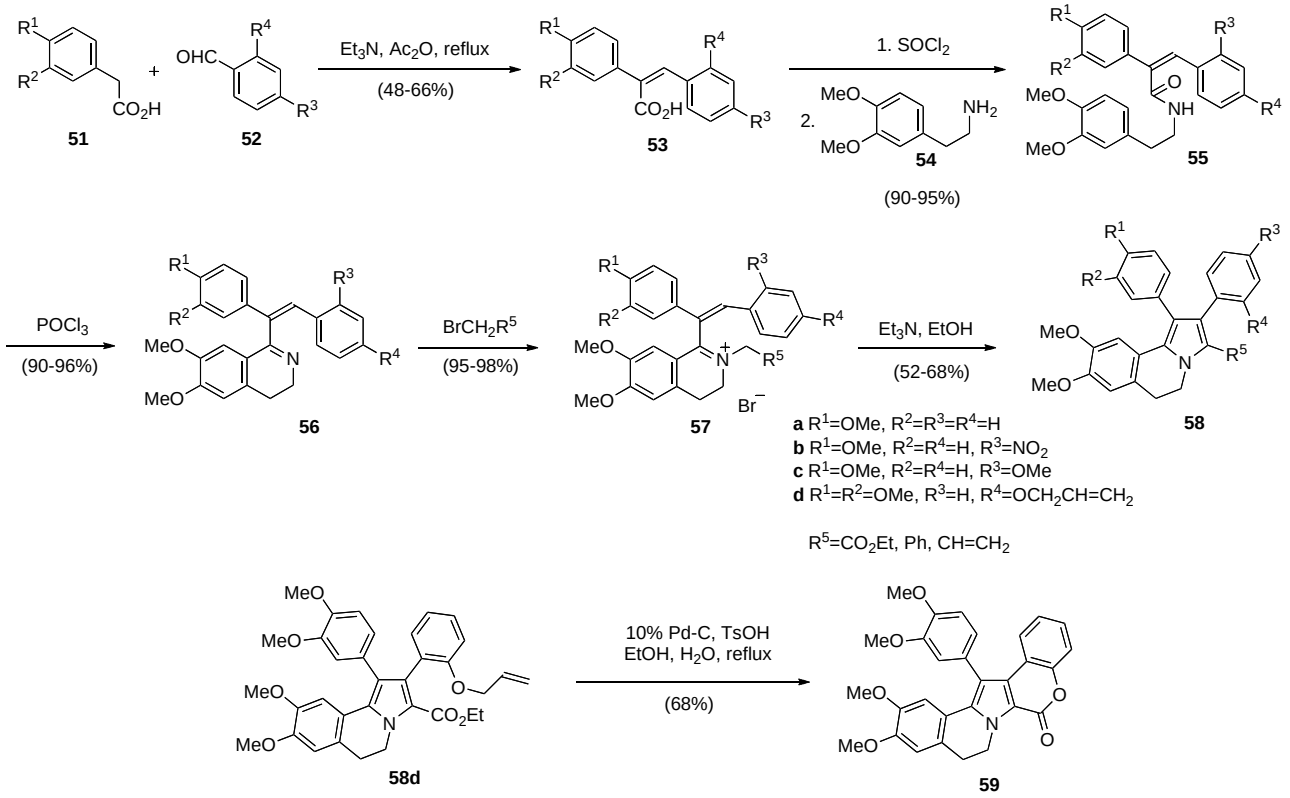



Scheme 8. Synthesis of lamellarins I and K *via* 1,3-dipolar cyclization of nitrones

## 2-1-5. SYNTHESIS BY NYERGES

Nyerges and co-workers developed a new route to prepare 1,2-diaryl-5,6-dihydropyrrolo[2,1-*a*]isoquinolines *via* 1,5-electrocyclization of azomethine ylides derived from 3,4-dihydroisoquinoline derivatives (Scheme 9).[40,41] Perkin condensation of arylacetic acids (**51**) and benzaldehydes (**52**) gave stilbenic acids (**53**). These acids were converted to 3,4-dihydroisoquinolines (**56**) *via* amides (**55**) using standard Bischler–Napieralski reaction. Quaternization of **56** followed by

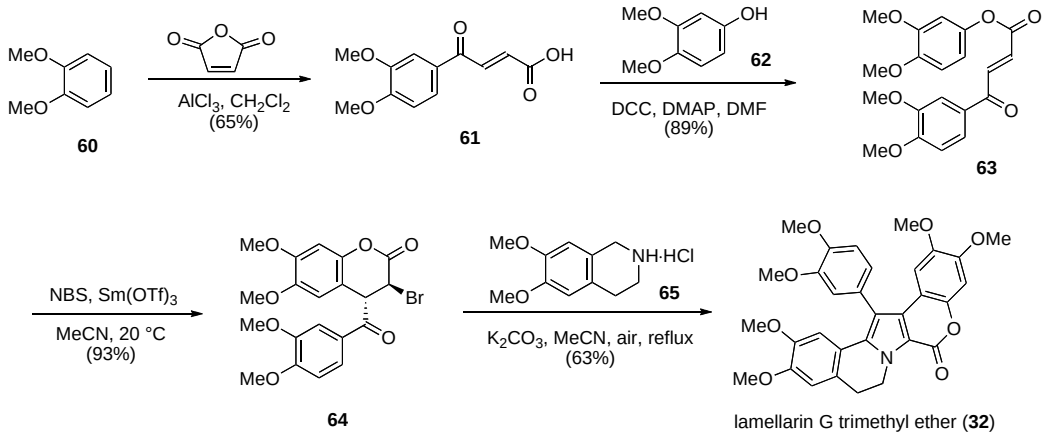
treatment with triethylamine in ethanol at room temperature gave 1,2-diaryl-4,5-dihydropyrrolo-[2,1-*a*]isoquinolines (**58**). Deallylation of **58d** using palladium-catalyst gave lamellarin (**59**).




Scheme 9. Synthesis of 5,6-dihydropyrrolo[2,1-*a*]isoquinolines (**58**) *via* 1,5-electrocyclization of azomethine ylides and its application to the synthesis of lamellarins

## 2-1-6. SYNTHESIS BY YADAV

Recently, Yadav and co-workers reported a unique synthesis of lamellarin G trimethyl ether (**32**) (Scheme

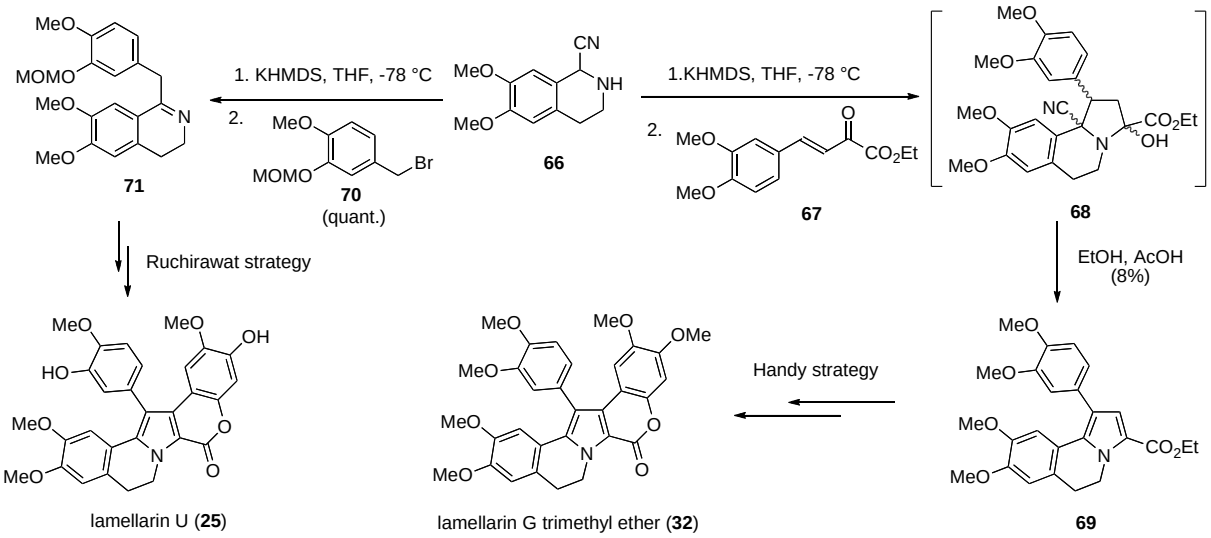



Scheme 10. Synthesis of lamellarin G trimethyl ether (**32**) *via* reaction between **64** and **65**

10).[42] Friedel–Crafts reaction of 1,2-dimethoxybenzene (**60**) with maleic anhydride gave **61**. This acid was esterified using 3,4-dimethoxyphenol (**62**) to give **63**. Samarium (III) triflate-catalyzed intramolecular bromoarylation[43] of ester **63** provided 3-bromo-3,4-dihydrocoumarin (**64**) in good yield. Coupling compound (**64**) with tetrahydroisoquinoline (**65**) in the presence of potassium carbonate under aerobic conditions produced lamellarin G trimethyl ether (**32**) in moderate yield. Yadav proposed that this cyclization proceeded *via N*-alkylation of **65** with **64** followed by base-promoted cyclization and aerobic oxidation.

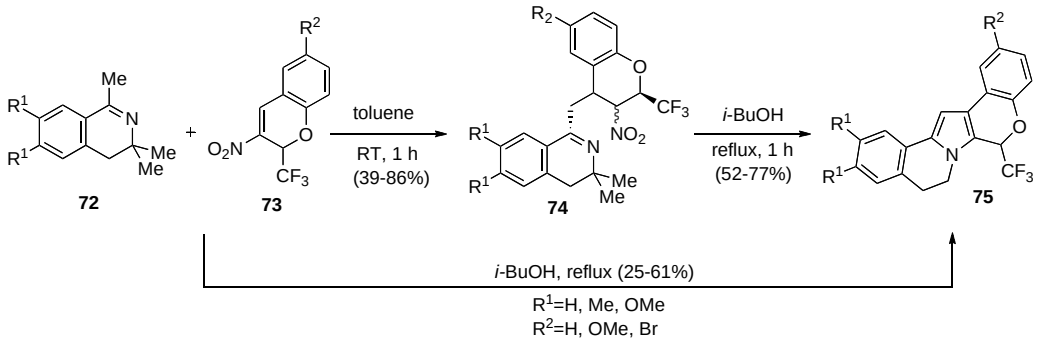
## 2-1-7. MISCELLANEOUS SYNTHESES

Opatz reported that 1,2,3,4-tetrahydroisoquinoline-1-carbonitrile (**66**) could serve as a starting material for the synthesis of lamellarins (Scheme 11).[44] The reaction of deprotonated tetrahydroisoquinoline **66** with ethyl benzylidenepyruvate (**67**) followed by acetic acid treatment gave **69** in one pot, albeit in low yield. This compound was converted to lamellarin G trimethyl ether (**32**) using the Handy strategy (section **2-2-8**).[45] The reaction of deprotonated tetrahydroisoquinoline (**66**) with benzyl bromide (**70**), on the other hand, gave 1-benzyl-3,4-dihydrobenzylisoquinoline (**71**) in quantitative yield. Opatz utilized this compound in the total synthesis of lamellarin U (**25**). The strategy is essentially the same as the Grob cyclization-based approach developed by Ruchirawat.[30,32]




Scheme 11. Synthesis of lamellarin G trimethyl ether (**32**) and lamellarin U (**25**) utilizing 1-cyano-1,2,3,4-tetrahydroisoquinoline (**66**)

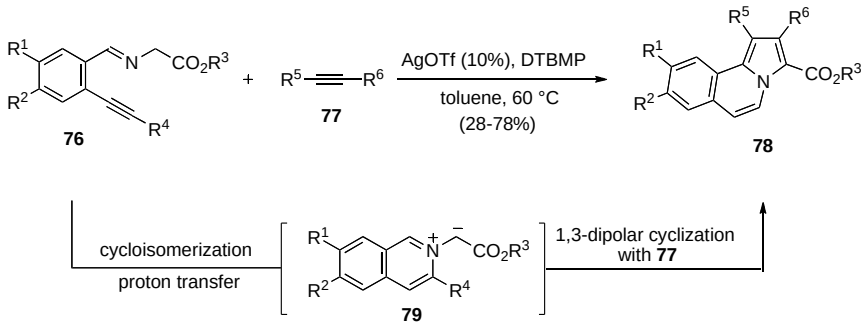
Sosnovskikh and co-workers reported the synthesis of basic lamellarin frameworks by Grob cyclization (Scheme 12).[46] Reaction of 3,4-dihydroisoquinolines (**72**) with nitrochromenes (**73**) in toluene at room temperature for 1 h gave Michael adducts (**74**), which were subsequently converted to pentacyclic




Scheme 12. Synthesis of lamellarin frameworks *via* Grob cyclization

lamellarin analogues (**75**) by heating in isobutanol. The pentacyclic compounds (**75**) were also obtained in one step by heating **72** and **73** in isobutanol.

Su and Porco reported an efficient synthesis of pyrrolo[2,1-*a*]isoquinolines (**78**) from *o*-alkynyl *N*-benzylidene glycinates (**76**) *via* a silver triflate-catalyzed domino cycloisomerization/1,3-dilopar cycloaddition process (Scheme 13).[47] They proposed that this reaction proceeded *via* initial formation of isoquinolinium ylides (**79**) followed by 1,3-dipolar cyclization with alkynes **77**. An intramolecular version of this reaction using an appropriately functionalized starting material may effect highly efficient lamellarin syntheses.[48]




Scheme 13. Synthesis of pyrrolo[2,1-*a*]isoquinolines (**78**) *via* a silver triflate-catalyzed domino process

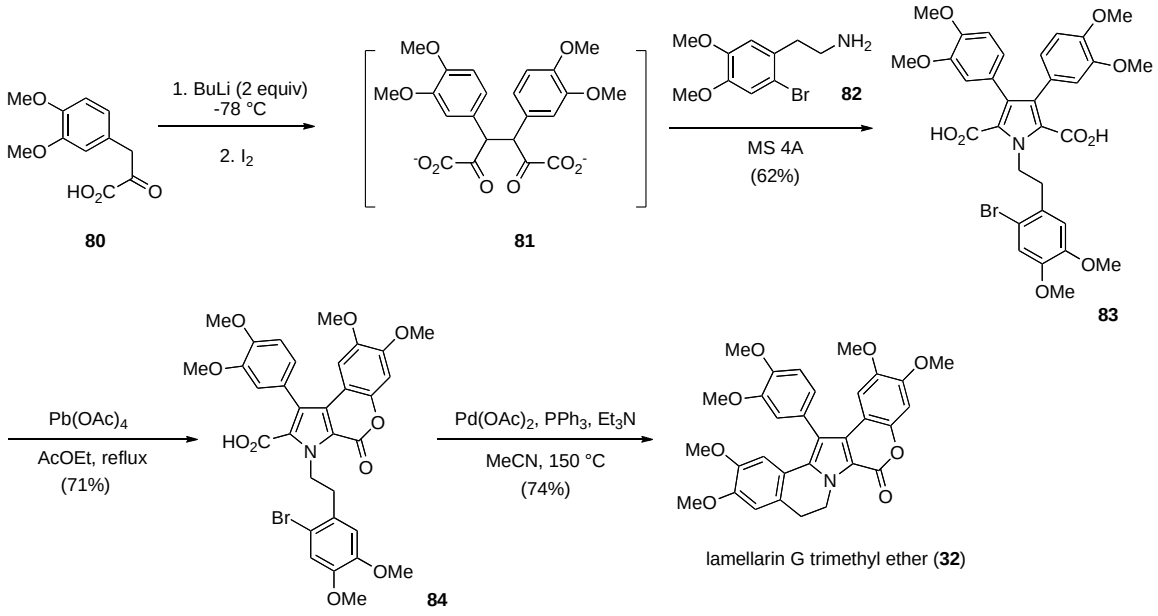
## 2-2. SYNTHESIS *VIA* PYRROLES

Other types of lamellarin synthesis have been developed *via* pyrrole ring formation or regioselective functionalization of pyrroles at relatively early stages.[49] In general, these routes are more versatile than the isoquinoline route mentioned above in view of their adaptability to the synthesis of a wider range of marine natural products having a common 3,4-diarylpyrrole core.[17] The initial five approaches (**2-2-1** to **2-2-5**) described in this chapter involve *de novo* pyrrole ring construction, whereas the last five syntheses

(**2-2-6** to **2-2-10**) utilize preexisting pyrroles as starting materials.

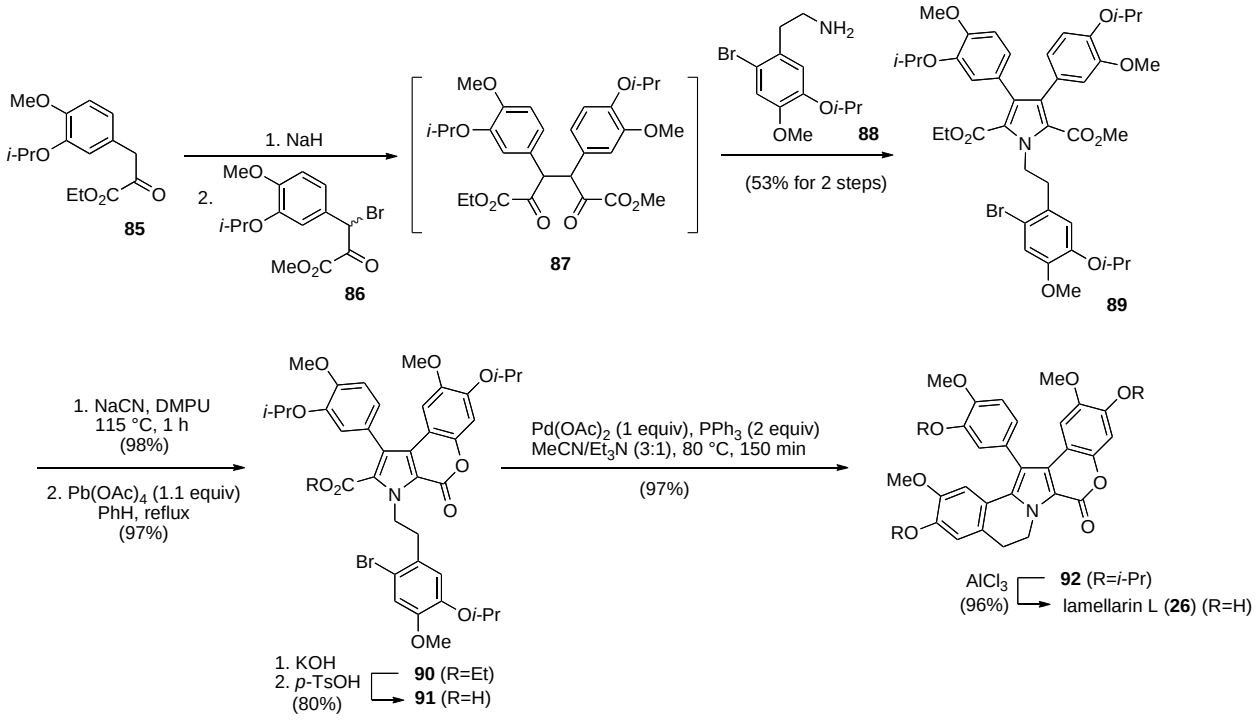
## 2-2-1. SYNTHESIS BY STEGLICH

In 1997, Heim, Terpin, and Steglich reported the synthesis of lamellarin G trimethyl ether (**32**) *via* a biomimetic approach.[50] A key intermediate having a 3,4-diarylpyrrole core (**83**) was constructed by oxidative homocoupling of the arylpyruvic acid (**80**)-derived enolate followed by condensation with 2-arylethylamine (**82**). Oxidative cyclization of intermediate (**83**) using lead (IV) tetraacetate[51] produced lactone (**84**), which underwent a unique palladium (0)-mediated decarboxylative Heck reaction to produce pentacyclic lamellarin core (Scheme 14).[52]




Scheme 14. Synthesis of lamellarin G trimethyl ether (**32**) *via* a biomimetic approach

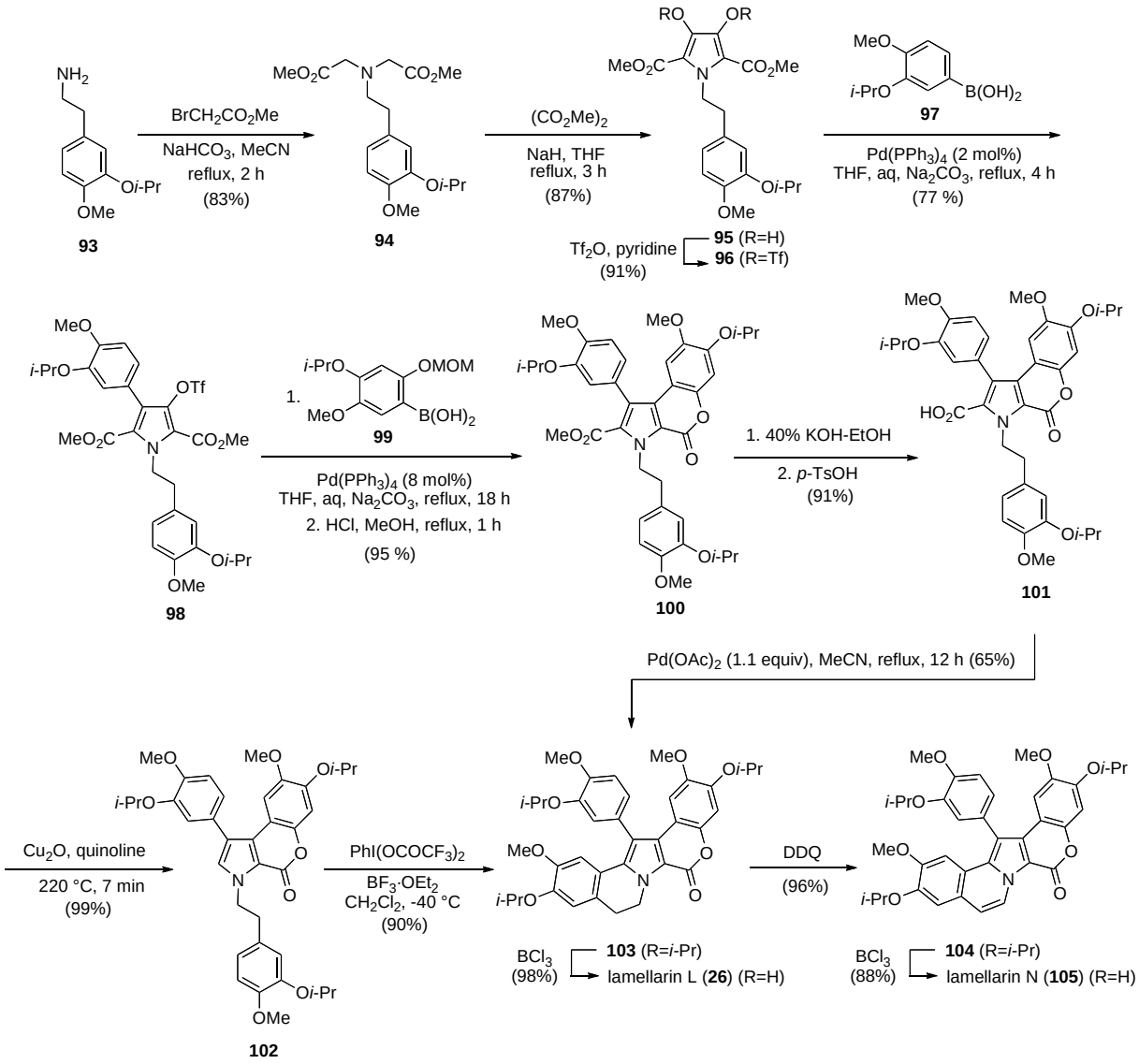
The synthesis described in Scheme 14 lacks generality to produce naturally occurring lamellarins that have differentially substituted aromatic rings at the 3- and 4-positions of the pyrrole ring. Steglich solved this problem and achieved the first total synthesis of lamellarin L (Scheme 15).[53] The differentiation was achieved by coupling ethyl ester (**85**) and methyl ester (**86**). Thus, deprotonation of **85** and sequential treatment with **86** and **88** gave unsymmetrically substituted pyrrole (**89**) in one pot. Selective cleavage of the methyl ester group using sodium cyanide in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) left the ethyl ester intact and generated a carboxylic acid that was treated with lead tetraacetate to produce lactone (**90**). Alkaline hydrolysis followed by acid-catalyzed relactonization produced **91**. Pd(0)-catalyzed decarboxylative Heck cyclization gave protected lamellarin (**92**) in excellent yield. Selective removal of isopropyl group by aluminum chloride yielded lamellarin L (**26**).




Scheme 15. Synthesis of lamellarin L (**26**) *via* an improved biomimetic procedure

## 2-2-2. SYNTHESIS BY IWAO (I)

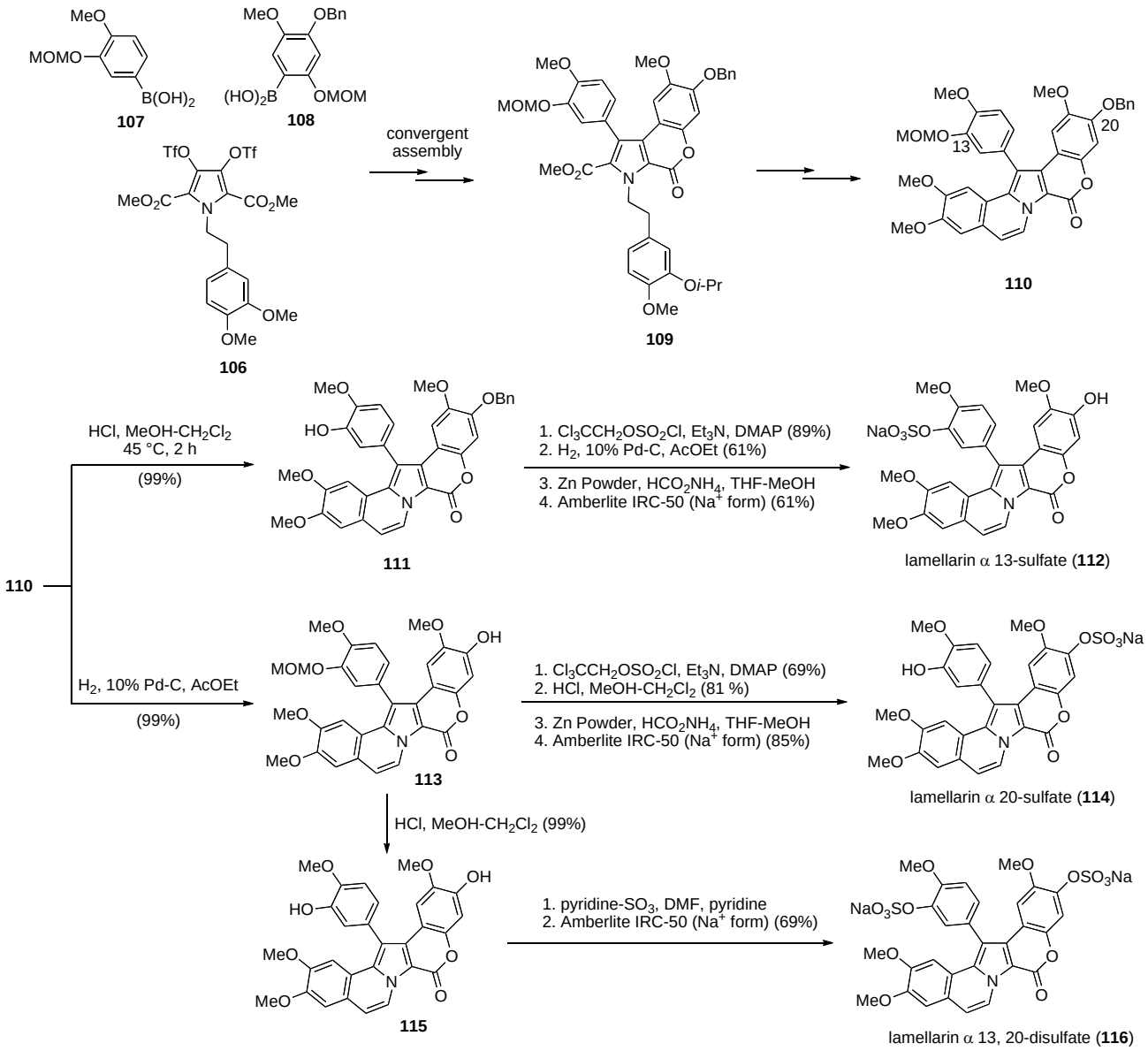
Iwao and co-workers devised a general route to produce pentacyclic lamellarins using Hinsberg-type pyrrole synthesis[54] and palladium-catalyzed Suzuki–Miyaura coupling[55] as key reactions.[56-58] For example, the total synthesis of lamellarins L (**26**) and N (**105**) is shown in Scheme 16.[57] Arylethylamine (**93**) was alkylated with 2 equiv of methyl bromoacetate to give iminodiacetate (**94**). Hinsberg reaction between **94** and methyl oxalate in the presence of sodium hydride as a base yielded 3,4-dihydroxypyrrole-1,4-dicarboxylate (**95**), which was then converted to bistriflate (**96**). Suzuki–Miyaura coupling of **96** with one equiv of arylboronic acid (**97**) in the presence of 2 mol% of tetrakis(triphenylphosphine)palladium(0) produced mono-arylated pyrrole (**98**) in 77% yield. The second cross-coupling of **98** with arylboronic acid **99** followed by deprotection of methoxymethyl protecting group gave lactone (**100**) in excellent yield. Alkaline hydrolysis of **100** followed by relactonization gave the carboxylic acid (**101**) that was decarboxylated in hot quinoline in the presence of copper(I) oxide to form **102**. Intramolecular biaryl coupling of **102** under Kita's conditions[59] using phenyliodine bis(trifluoroacetate) (PIFA)-boron trifluoride etherate afforded **103** in good yield. This compound was also obtained directly from **101** using palladium(II) acetate in refluxing acetonitrile in moderate yield. This cyclization might proceed *via* decarboxylative palladation-direct arylation.[60] Selective deprotection of isopropyl groups provided lamellarin L (**26**) from **103**. Dehydrogenation of **103** with DDQ followed by boron trichloride-mediated removal of isopropyl groups produced lamellarin N (**105**).




Scheme 16. Synthesis of lamellarin L (**26**) and N (**105**) *via* Hinsberg reaction and Suzuki–Miyaura coupling

This synthetic strategy was successfully applied to the first total synthesis of HIV-1 integrase inhibitor lamellarin α 20-sulfate (**114**) in 2007.[61] Thereafter, the synthesis was improved to provide lamellarin α 13-sulfate (**112**), 20-sulfate (**114**), and 13, 20-disulfate (**116**) selectively from a common intermediate (**110**) in which hydroxyl groups at 13- and 20-positions were protected differently (Scheme 17).[62] Intermediate (**110**) was prepared by convergent assembly of bistriflate (**106**) and arylboronic acids (**107**) and (**108**) in a similar manner as described above. Treatment of **110** with hydrochloric acid gave 13-OH compound (**111**), which was converted into lamellarin α 13-sulfate (**112**) using Taylor's protocol[63] *via* the 2,2,2-trichloroethylsulfonated intermediate. In a similar way, lamellarin α 20-sulfate (**114**) was synthesized *via* 20-OH intermediate (**113**), which was generated by debenzylation of **110**. Removal of the methoxymethyl group followed by treatment with the pyridine–sulfur trioxide complex converted **113**

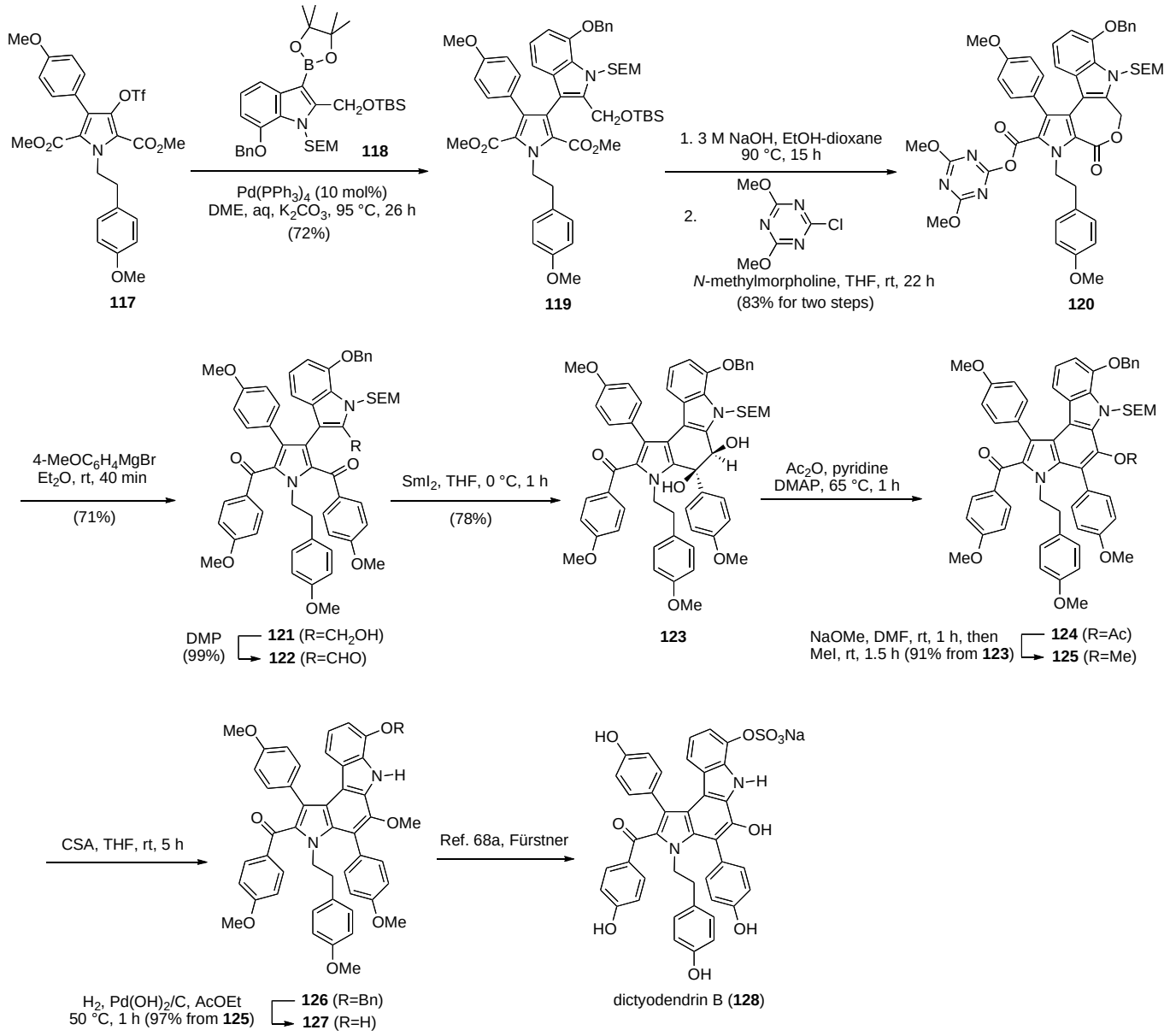
into lamellarin α 13, 20-disulfate (**116**).




Scheme 17. Synthesis of lamellarin α 13-sulfate (**112**), 20-sulfate (**114**), and 13, 20-disulfate (**116**)

Recently, this method was extended to the formal synthesis of dictyodendrin B (**128**),[64] another biologically significant marine natural product possessing telomerase inhibitory activity.[65] Palladium-catalyzed cross-coupling of mono-triflate (**117**) with indole-3-boronate (**118**) provided **119**. Alkaline hydrolysis and reaction with 2-chloro-4,6-dimethoxy-1,3,5-triazine[66] produced the activated ester-lactone (**120**) in 83% yield. Reaction of compound (**120**) with excess 4-methoxyphenylmagnesium bromide gave diketone (**121**), which afforded keto-aldehyde (**122**) in 99% yield by Dess–Martin oxidation. The key ring formation enabled by the samarium (II) iodide-promoted pinacol coupling[67] of **122** produced diol (**123**) in 78% yield. Dehydration of compound (**123**) with acetic anhydride in pyridine

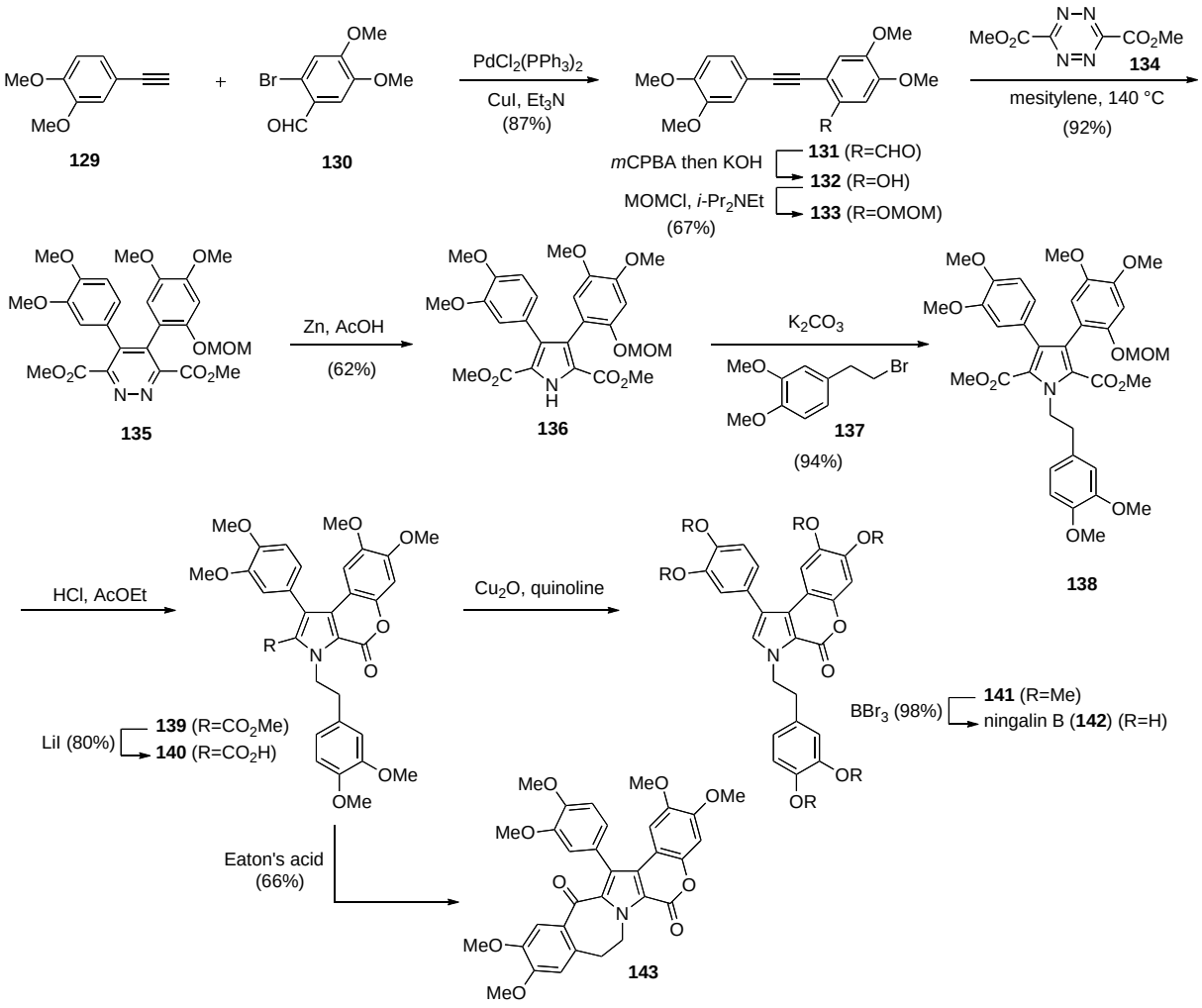
gave the acetate (**124**), which was treated with sodium methoxide in DMF and then in iodomethane to produce methyl ether (**125**). Removal of trimethysilylethoxymethyl (SEM) and benzyl groups produced **127**, which had previously been shown to give dictyodendrin B (**128**) by Fürstner[68] using Taylor's protocol.




Scheme 18. Application of Iwao's method to the synthesis of dictyodendrin B (**128**)

## 2-2-3. SYNTHESIS BY BOGER

Boger developed a general route to 3,4-diarylpyrrole marine alkaloids using heterocyclic azadiene Diels–Alder reactions.[69,70] The synthesis of ningalin B[71] (**142**), for example, is shown in Scheme 19.[70] Palladium(0)-catalyzed Sonogashira coupling of the terminal alkyne (**129**) and **130** provided **131**. Baeyer–Villiger oxidation of aldehyde **131** followed by formate hydrolysis and protection of the phenol gave **133**. The key heterocyclic azadiene Diels–Alder reaction of **133** with the electron-deficient



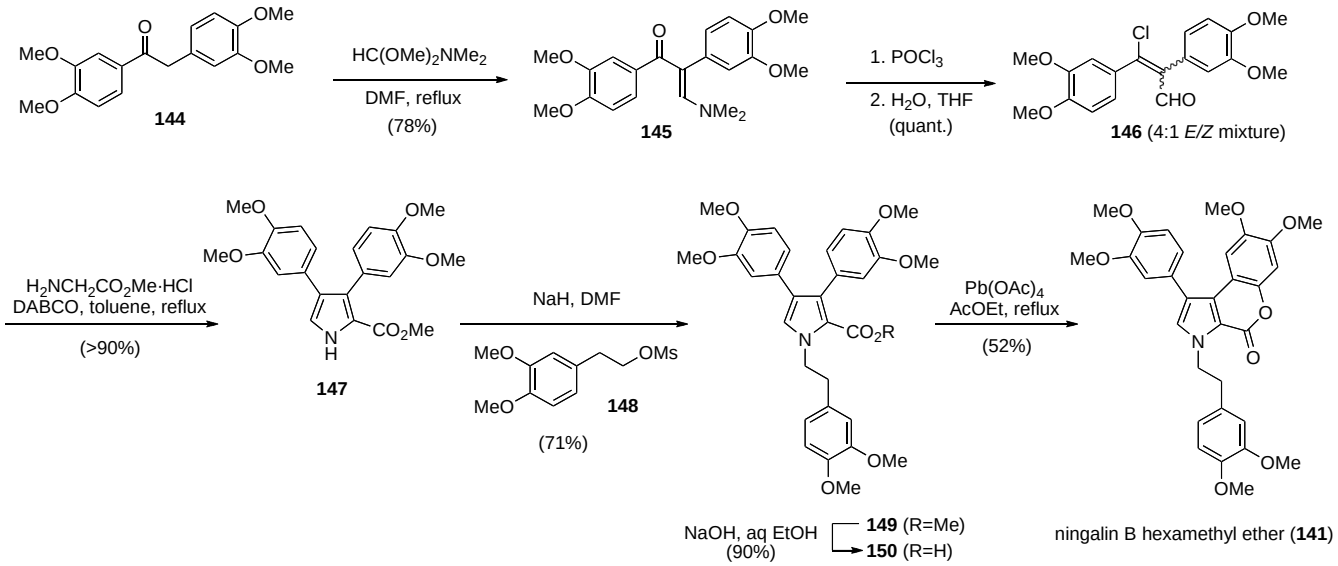

Scheme 19. Synthesis of ningalin B (**142**) and lamellarin analogue (**143**) by a heterocyclic azadiene Diels–Alder reaction

1,2,4,5-tetrazine (**134**) in mesitylene at 140 °C proceeded to give 1,2-diazine (**135**) in excellent yield. Subsequent reductive ring contraction of **135** with zinc dust in acetic acid afforded the pyrrole (**136**). *N*-Alkylation of **136** with phenethyl bromide **137** followed by removal of methoxymethyl group of **138** provided lactone (**139**). Cleavage of the methyl ester with lithium iodide and subsequent decarboxylation afforded ningalin B hexamethyl ether (**141**). Exhaustive demethylation with boron tribromide provided ningalin B (**142**). Friedel–Crafts acylation of acid (**140**) in neat Eaton's acid[72] produced the seven-membered lamellarin analogue (**143**). It is noteworthy that both ningalin B hexamethyl ether (**141**) and the acid (**140**) are convertible to lamellarin G trimethyl ether (**32**) using the methods described in section **2-2-2**.[56]

## 2-2-4. SYNTHESIS BY GUPTON

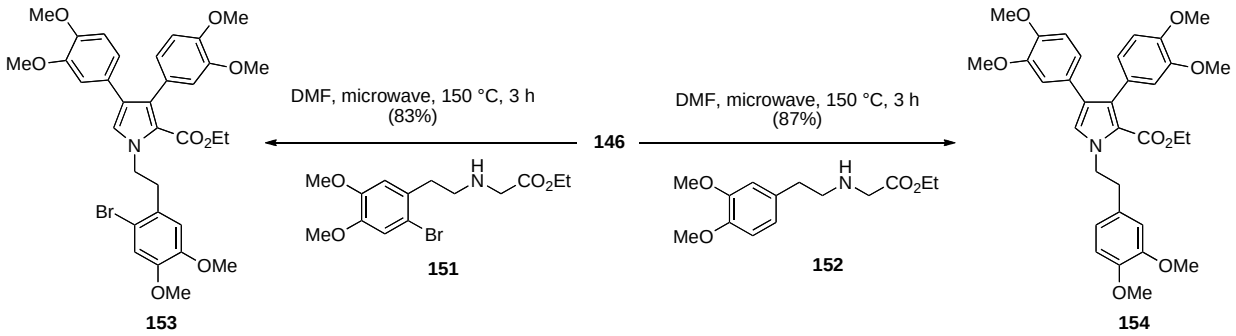
Gupton developed an efficient way to provide 3,4-diarylpyrrole-2-carboxylates applicable to the synthesis

of lamellarins (Scheme 20).[73,74] Deoxybenzoin (**144**) was heated with *N,N*-dimethylformamide dimethyl acetal in DMF to give the enamino ketone (**145**). Compound (**145**) was converted to β-chloroenal (**146**) in quantitative yield using phosphorous oxychloride followed by hydrolysis with water. Reaction of **146** with glycine methyl ester hydrochloride in the presence of DABCO produced pyrrole **147** in good yield. *N*-Alkylation of **147** with mesylate (**148**) afforded **149**, which was converted to ningalin B hexamethyl ether (**141**) following Steglich's protocol (Section **2-2-1**).




Scheme 20. Synthesis of ningalin B hexamethyl ether (**141**) by condensation of chloroenal (**146**) with glycine methyl ester

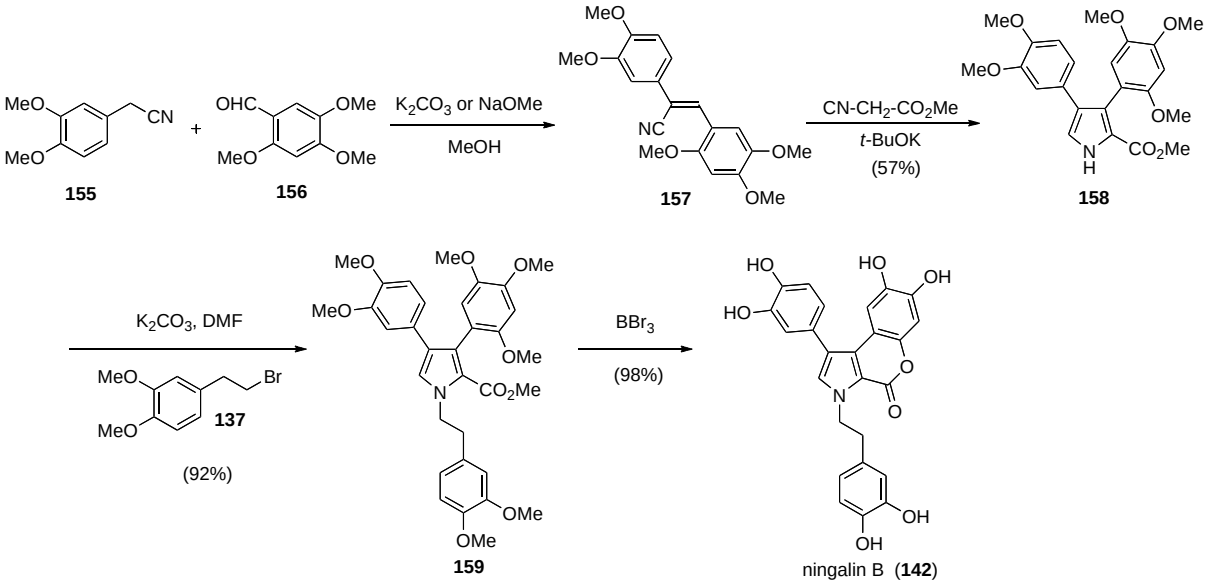
The reaction of β-chloroenal (**146**) with **151** or **152** directly produced *N*-alkylated pyrroles **153** or **154** in good yields by microwave heating (Scheme 21).[75] Compound **153** was converted to lamellarin G trimethyl ether (**32**) following Steglich's protocol.[50]




Scheme 21. Improved synthesis of lamellarin intermediates from chloroenal (**146**)

## 2-2-5. SYNTHESIS BY BULLINGTON

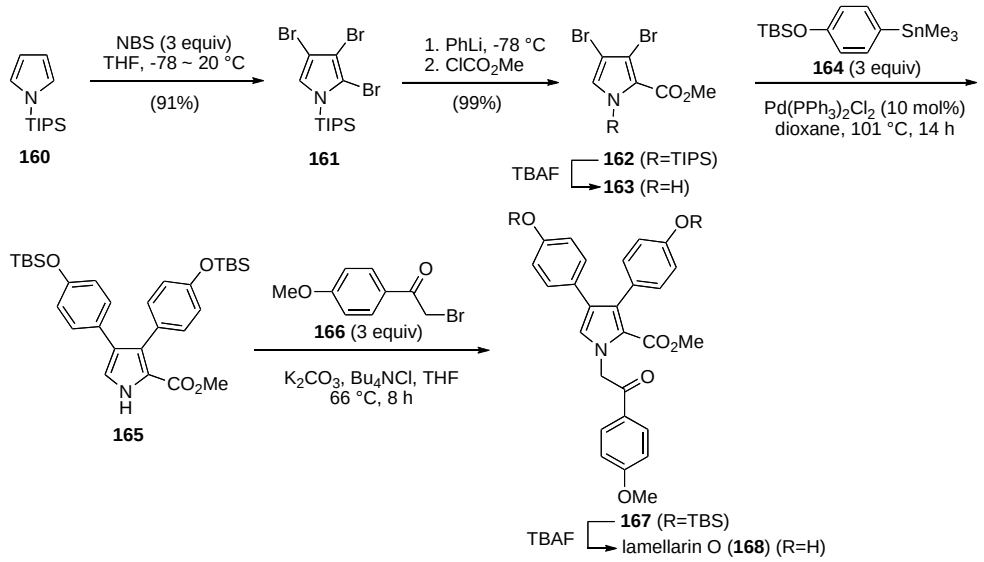
Bullington reported one synthesis of ningalin B (**142**) that uses a modified Barton–Zard reaction[76] in the key step (Scheme 22).[77] Prepared by condensing **155** and **156**, α, β-unsaturated nitrile (**157**) reacted with methyl isocyanoacetate in the presence of potassium *t*-butoxide to give the 3,4-unsymmetrically arylated pyrrole-2-carboxylate (**158**) in modest yield. *N*-Alkylation with **137** followed by exhaustive demethylation produced ningalin B (**142**).




Scheme 22. Synthesis of ningalin B (**142**) *via* a modified Barton–Zard reaction

## 2-2-6. SYNTHESIS BY BANWELL (I)

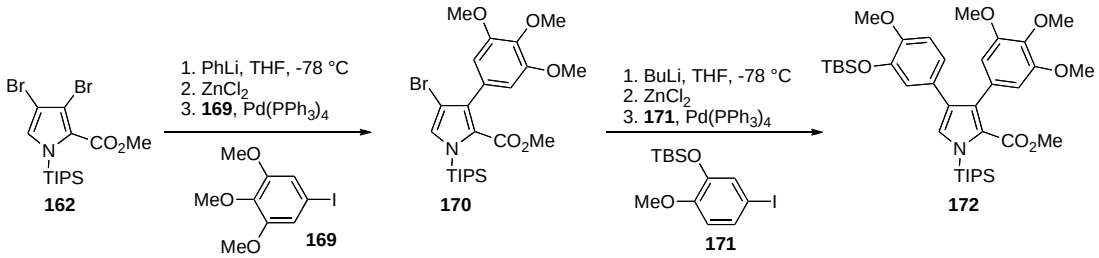
Banwell devised the first method to produce lamellarins *via* regioselective functionalization of a preexisting pyrrole.[78] The synthesis of lamellarin O (**168**), for example, is shown in Scheme 23. The key




Scheme 23. Synthesis of lamellarin O (**168**) *via* regioselective functionalization of *N*-(TIPS)pyrrole (**160**)

intermediate (**162**) was synthesized *via* bromination of *N*-(TIPS)pyrrole (**160**)[79] followed by C2-selective bromine–lithium exchange and methoxycarbonylation of the resulting 2,3,4-tribromopyrrole (**161**) in excellent overall yield. Stille coupling of the desilylated **162** with the arylstannane (**164**) gave 3,4-diarylated pyrrole (**165**) in 66% yield. *N*-Alkylation of **165** with *p*-methoxyphenacyl bromide followed by desilylation produced lamellarin O (**168**).

During the Stille and Suzuki–Miyaura coupling reactions of **163**, no significant quantities of mono-arylated pyrroles were observed even for shorter reaction times and 1:1 stoichiometries. These limitations were overcome by regioselective bromine–lithium exchange of **162** followed by transmetallation and Negishi cross-coupling reactions, as shown in the synthesis of 3,4-differentially arylated pyrrole (**172**) (Scheme 24). This strategy is apparently applicable to the synthesis of more complex lamellarins (type I and II) by expanding the annulation reactions described in Sections **2-2-1** and **2-2-2**.



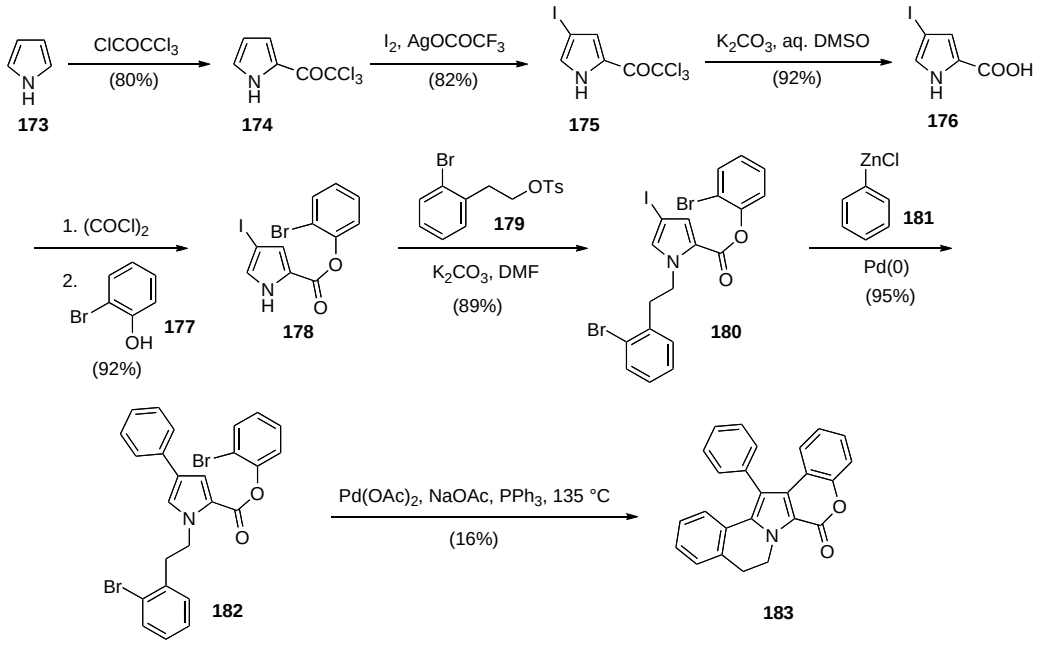

Scheme 24. Synthesis of a 3,4-differentially substituted pyrrole-2-carboxylate (**172**)

## 2-2-7. SYNTHESIS BY BANWELL (II)

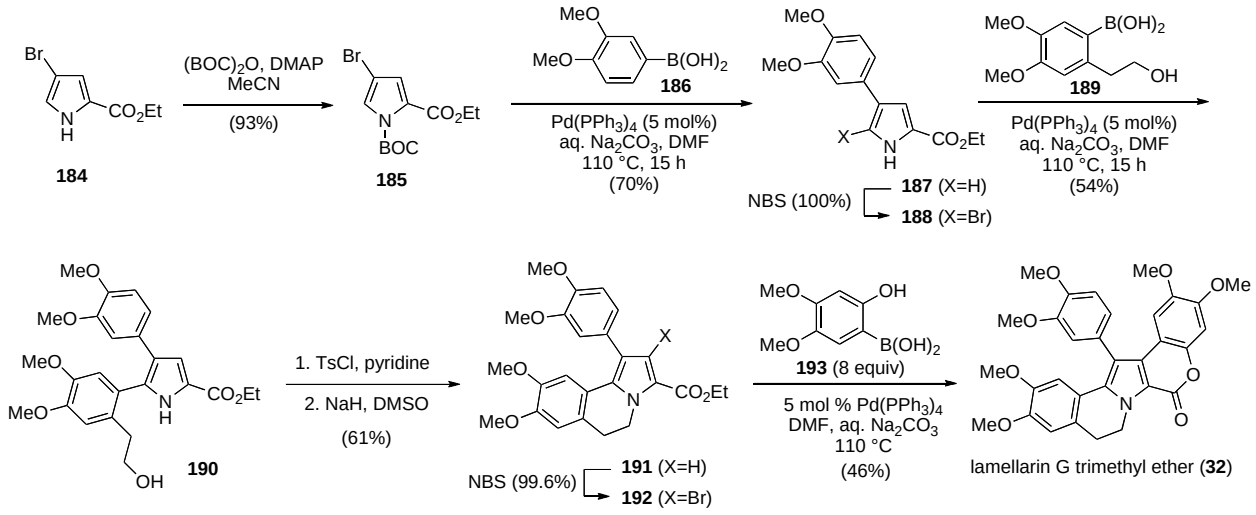
Banwell developed a conceptually interesting double-barreled Heck cyclization strategy for the construction of pentacyclic lamellarin frameworks.[80] The synthesis of a model compound (**183**) is shown in Scheme 25. Pyrrole (**173**) was reacted with trichloroacetyl chloride to give 2-substituted pyrrole (**174**). This compound was treated with molecular iodine in the presence of silver trifluoroacetate to give the 4-iodinated compound (**175**) regioselectively. Alkaline hydrolysis of **175** followed by esterification and *N*-alkylation provided **180**. Negishi coupling of the compound (**180**) with phenylzinc chloride produced the cyclization substrate (**182**). Treatment of **182** with palladium (II) acetate and triphenylphosphine in the presence of sodium acetate at 135 °C afforded the desired lamellarin (**183**) in a single step as the only isolable species. Unfortunately, however, the yield of this cyclization was quite modest (16%).

## 2-2-8. SYNTHESIS BY HANDY

Handy reported a modular synthesis of lamellarin G trimethyl ether based upon three iterative halogenation/Suzuki–Miyaura coupling reaction sequences (Scheme 26).[81] This study established the ability to halogenate the pyrrole core in a regioselective fashion, even in the presence of highly




Scheme 25. Synthesis of lamellarin framework by double-barreled Heck cyclization



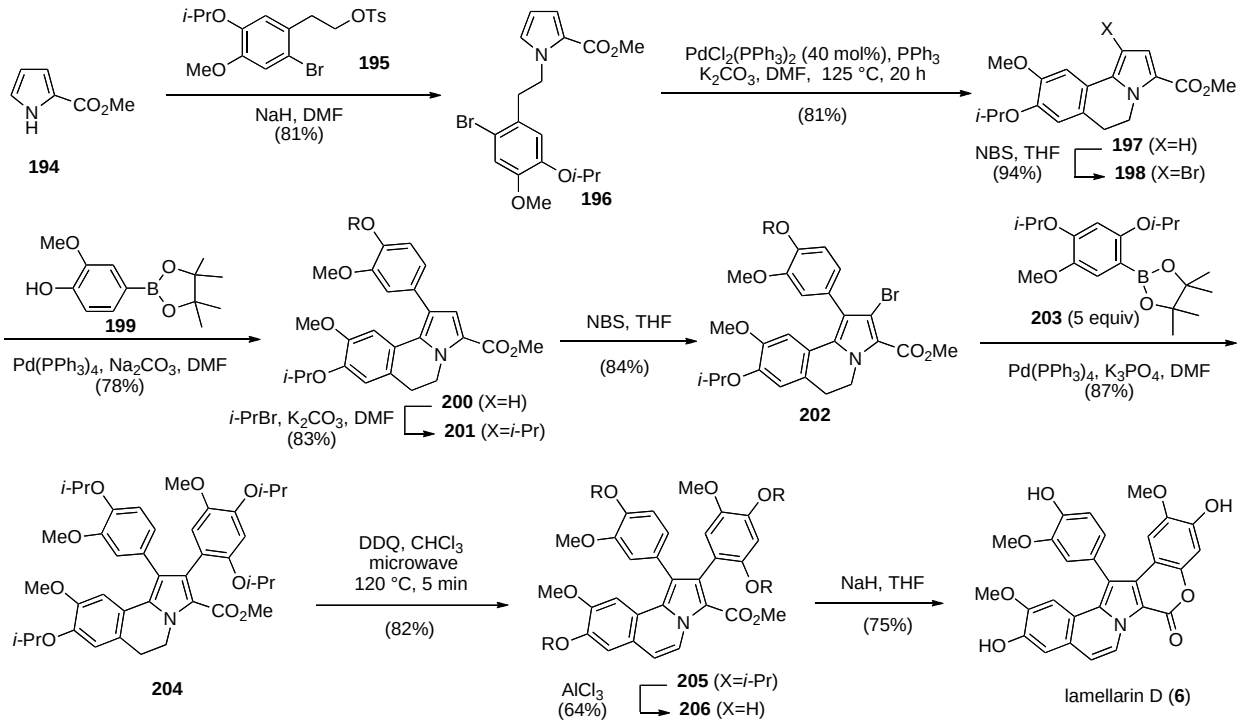

Scheme 26. Synthesis of lamellarin G trimethyl ether (**32**) *via* iterative bromination/Suzuki-Miyaura coupling strategy

electron-rich aryl substituents. Initially, the known 3-bromopyrrole-2-carboxylate (**184**) was converted to the Boc-protected pyrrole (**185**). Previous studies by Handy indicated that protection of pyrrolic nitrogen was essential to avoid extensive debromination in the subsequent cross-coupling reactions.[82] Suzuki–Miyaura coupling of **185** with excess arylboronic acid (**186**) (2–3 equiv) gave **187** in 70% yield. Treatment of **187** with an equimolar amount of NBS cleanly led to selective bromination at the C5 position. The second cross-coupling of **188** with arylboronic acid (**189**) gave 4,5-diarylated pyrrole (**190**) under standard conditions. The isoquinoline ring was constructed in two steps by tosylate formation and

subsequent intramolecular alkylation of the pyrrolic nitrogen. After selective C3 bromination of **191** with NBS, the bromide was treated with the arylboronic acid (**193**) under standard Suzuki–Miyaura coupling conditions to produce lamellarin G trimethyl ether (**32**) in 46% yield. Slow addition of a large excess (8 equiv) of the thermally unstable **193** was essential in order to prevent its decomposition.

## 2-2-9. SYNTHESIS BY ALBERICIO–ÁLVAREREZ

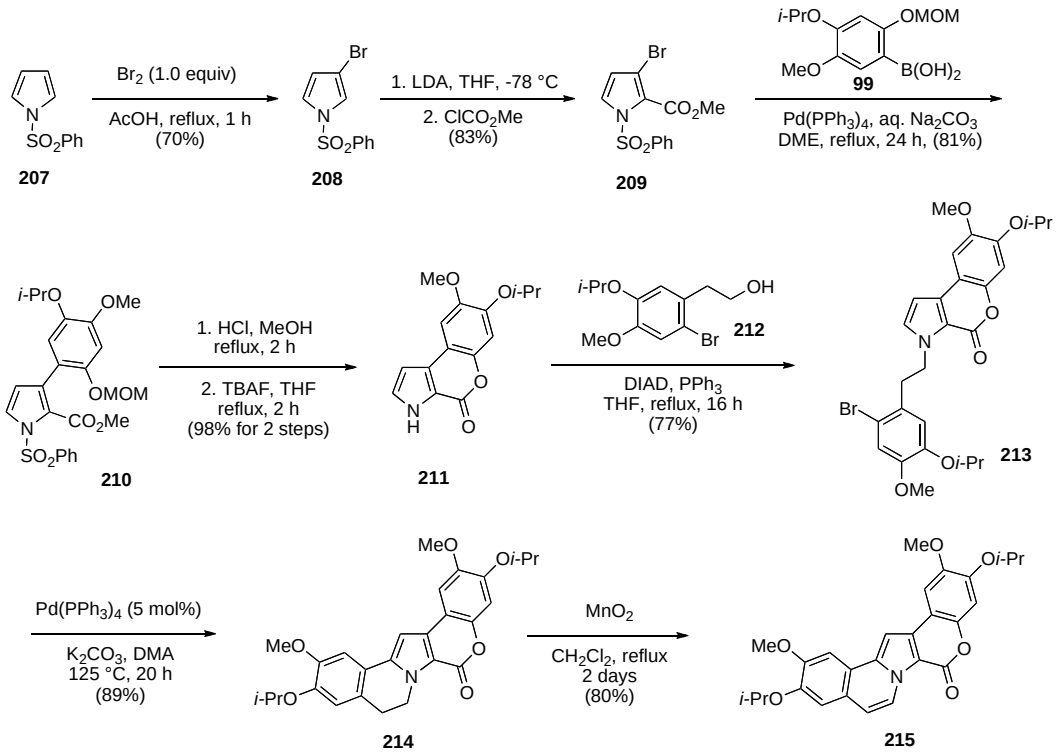
Albericio and Álvarez reported a similar iterative bromination/cross-coupling strategy for the synthesis of lamellarins using methyl 5,6-dihydropyrrolo[2,1-*a*]isoquinoline-1-carboxylate as a scaffold.[83,84] The total synthesis of lamellarin D (**6**), for example, is depicted in Scheme 27. Methyl pyrrole-2-carboxylate (**194**) was alkylated with a tosylate (**195**) to give **196**. This compound was cleanly cyclized to form the key scaffold **197** using the palladium-catalyzed intramolecular Heck reaction. Regioselective bromination at C1 followed by Suzuki–Miyaura coupling with the boronate (**199**) gave **200**. Protection of the phenol oxygen of **200** with isopropyl group gave **201** that was brominated again and cross-coupled with another boronate **203** (5 equiv) to produce **204**. Initial addition of 3 equiv of **203** followed by slow addition of the last 2 equiv by syringe pump was required to achieve a good yield (87%). Compound (**204**) was aromatized to **205** using DDQ in chloroform in a sealed tube with controlled microwave irradiation at 120 °C for 5 min. Cleavage of all isopropyl protecting groups of **205** with aluminum chloride followed by base-promoted lactonization afforded lamellarin D (**6**). This method was later utilized to produce a library of open-lactone analogues of lamellarin D to evaluate their cytotoxic activity.[85]




Scheme 27. Synthesis of lamellarin D (**6**) using 5,6-dihydropyrrolo[2,1-*a*]isoquinoline (**197**) as a scaffold

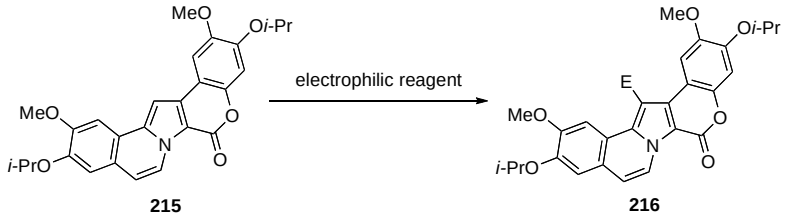
## 2-2-10. SYNTHESIS BY IWAO (II)

Recently, Iwao and co-workers developed a strategy to produce a variety of lamellarin D analogues in which C1-position of the pentacyclic core is modified.[86] The synthesis of the 1-dearylated key intermediate (**215**) is shown in Scheme 28. *N*-Benzenesulfonylpyrrole (**207**) was brominated at C3 by treatment with 1.0 equiv of bromine in refluxing acetic acid to give **208**.[87] The brominated compound (**208**) was lithiated regioselectively at C2 with LDA in THF at −78 °C and the resulting species was trapped with methyl chloroformate to provide **209** in good yield.[88] Suzuki–Miyaura coupling of **209** with the boronic acid (**99**) afforded **210**. Sequential deprotection of MOM and *N*-benzenesulfonyl groups provided the lactone (**211**) in excellent yield. *N*-Alkylation of **211** with the alcohol (**212**) by Mitsunobu reaction produced **213** that underwent a palladium-catalyzed intramolecular direct arylation[89] to produce the pentacyclic lamellarin core (**214**) in excellent yield. Dehydrogenation of **214** with active manganese dioxide afforded the key intermediate (**215**) which was readily and regioselectively functionalized at C1 by conventional electrophilic substitution reactions, while leaving other aromatic positions intact. As shown in Table 3, bromination, chlorination, Mannich, and Vilsmeier reactions produced the corresponding 1-substituted products in excellent yields. The yield of fluorination with SELECTFLUOR was modest owing to instability of **215** under these reaction conditions.




Scheme 28. Synthesis of C1-unsubstituted core of lamellarin D

Table 3. Electrophilic substitution of **215**

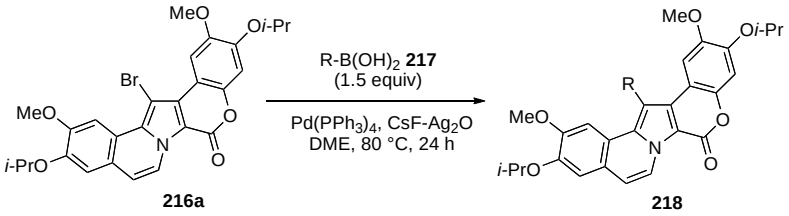


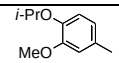
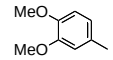
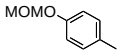

| electrophilic reagent | E | product | yield (%) |
|---|---|---|---|
| NBS | Br | **216a** | 92 |
| NCS | Cl | **216b** | 96 |
| SELECTFLUOR[a] | F | **216c** | 53 |
| $Me_2N^+=CH_2 \cdot I^-$ | $CH_2NMe_2$ | **216d** | 97 |
| $POCl_3$, DMF | CHO | **216e** | 99 |

[a] 1-Chloromethyl-4-fluoro-1,4-diazabicyclo[2.2.2]octane bis(tetrafluoroborate).

Suzuki–Miyaura coupling of the bromide (**216a**) with the boronic acid (**217a**) under standard conditions [10 mol% of $Pd(PPh_3)_4$, aq $Na_2CO_3$, DME, reflux, 24 h] was quite sluggish owing to severe steric hindrance at the C1 position. However, the cross-coupling of **216a** with arylboronic acids (**217a–d**) under Qiu's conditions[90] using CsF–$Ag_2O$ as a promoter proceeded smoothly to give the corresponding 1-arylated products (**218a–d)** in good yields (Table 4). Cross-coupling with trimethylboroxine (**217e**) gave the 1-methylated product (**218e**).

Table 4. Suzuki–Miyaura coupling of **216a**



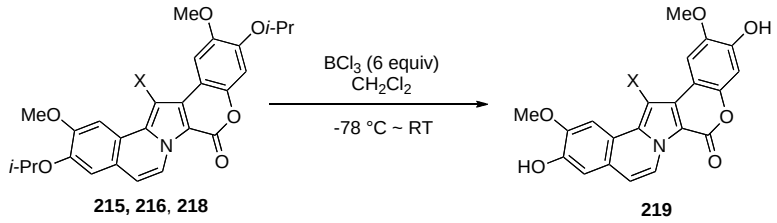

| boronic acid | R | product | yield (%) |
|---|---|---|---|
| **217a** |  | **218a** | 69 |
| **217b** |  | **218b** | 87 |
| **217c** |  | **218c** | 79 |
| **217d** | Ph | **218d** | 81 |
| **217e**[a] | Me | **218e** | 82 |

[a]Trimethylboroxine was used.

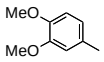
Deprotection of the isopropyl groups of the cross-coupling products using 6.0 equiv of boron trichloride

in dichloromethane at –78 °C and then at room temperature produced a variety of C1-modified lamellarin D analogues (**219a–h**) (Table 5). The yields were dependent on the C1 substituent.

Table 5. Synthesis of C1-modified lamellarin D analogues (**219a–h**)




**215, 216, 218** **219**

| substrate | X | **219** | yield (%) |
|---|---|---|---|
| **215** | H | **219a** | 66 |
| **216a** | Br | **219b** | 88 |
| **216b** | Cl | **219c** | 52 |
| **216c** | F | **219d** | 37 |
| **216d** | $CH_2NMe_2$ | **219e** | 53 |
| **216e** | CHO | **219f** | 58 |
| **218b** |  | **219g** | 84 |
| **218d** | Ph | **219h** | 97 |
| **218e** | Me | **219i** | 64 |

## 3. BIOLOGICAL ACTIVITY OF LAMELLARINS

Pentacyclic lamellarins are observed to show diverse biological activities, many of which are of pharmacological importance. The potent biological activity and multifunctional properties of these particular marine alkaloids allow them to be considered as potential leads for drug development.

### 3-1. CYTOTOXICITY

The cytotoxic effect of lamellarins was first reported by Carroll and co-workers in 1993.[4] The researchers found that lamellarins I, K, and L isolated from a colonial ascidian Didemnum sp. showed significant cytotoxicity against P388 and A549 cancer cell lines at the nanomolar level. Since then, several detailed works on the cytotoxicity of these alkaloids, including their antiproliferative effects, have appeared.[8,9,11,12,14,16,21,33,85,91,92] In 1996, Quesada and co-workers[16] evaluated the cytotoxicity of 13 lamellarins against several tumor cell lines, including two MDR cell lines (P388/Shabel and $CCH^RC5$ ) (Table 6). Among the compounds tested, lamellarins D-triacetate, K, K-triacetate, M, and N-triacetate were highly active towards a variety of cell lines and especially P388 (murine leukemia), A549 (human lung carcinoma), HT29 (human colon carcinoma), and MEL28 (human melanoma) cells. In particular,

Table 6. Cytotoxic activity of different lamellarins against various tumor cell lines[16]

| lamellarin | *Mean $IC_{50}$* (μM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | *P388* | *Schabel* | *AUXB1* | *$CCH^RC5$* | *A549* | *HT29* | *MEL28* |
| A | 0.89 (0.10) | 0.91 (0.08) | 0.36 (0.07) | 0.71 (0.12) | 0.90 (0.13) | 2.1 (0.4) | 0.93 (0.10) |
| B | 10.1 (1.3) | 10.4 (0.9) | 5.5 (0.7) | 18.0 (2.4) | 5.2 (0.9) | >10 | 10.1 (0.2) |
| D-triacetate | 0.11 (0.03) | 0.14 (0.02) | 0.05 (0.01) | 0.06 (0.01) | 0.008 (0.001) | 0.80 (0.11) | 0.16 (0.02) |
| I | 4.9 (0.5) | 4.8 (0.7) | 0.38 (0.05) | 2.0 (0.2) | 5.0 (0.8) | 4.7 (0.5) | 5.0 (0.3) |
| I-acetate | 9.0 (1.2) | 9.2 (0.8) | 4.1 (0.5) | 9.0 (1.0) | 9.3 (1.3) | >10 | 9.1 (1.2) |
| J | 2.9 (0.4) | 3.9 (0.5) | 0.58 (0.04) | 1.2 (0.2) | 0.60 (0.06) | 5.8 (0.7) | 2.9 (0.4) |
| K | 0.19 (0.01) | 0.017 (0.02) | 0.19 (0.02) | 0.75 (0.10) | 0.18 (0.03) | 0.38 (0.03) | 0.40 (0.05) |
| K-triacetate | 0.09 (0.01) | 0.16 (0.02) | 0.15 (0.01) | 0.16 (0.03) | 0.005 (0) | 0.47 (0.06) | 0.93 (0.12) |
| L | 1.2 (0.1) | 1.4 (0.2) | 0.80 (0.09) | 1.3 (0.1) | 0.60 (0.04) | 6.0 (0.8) | 1.2 (0.2) |
| L-triacetate | 2.4 (0.3) | 2.4 (0.1) | 2.2 (0.2) | 2.5 (0.3) | 1.1 (0.1) | >3 | 2.3 (0.2) |
| M | 0.15 (0.03) | 0.17 (0.02) | 0.07 (0.01) | 0.17 (0.01) | 0.06 (0.01) | 0.56 (0.07) | 0.54 (0.04) |
| M-triacetate | 0.91 (0.11) | 1.1 (0.2) | 0.76 (0.09) | 3.1 (0.5) | 0.22 (0.05) | >1 | 0.90 (0.13) |
| N-triacetate | 0.32 (0.02) | 0.30 (0.04) | 0.10 (0.03) | 0.16 (0.02) | 0.02 (0) | 3.2 (0.02) | 1.6 (0.03) |

Fifty per cent inhibitory concentration ($IC_{50}$) represents the mean (standard deviation in parentheses) from dose–responsecurves of 2–3 experiments.

lamellarins D-triacetate ($IC_{50}$ = 0.008 μM) and K-triacetate ($IC_{50}$ = 0.005 μM) showed significantly high activity against A549 lung carcinoma cells. Interestingly, these lamellarins were also toxic to the MDR P388/Shabel and $CCH^RC5$ cells to the same extent as their parental cell lines, P388 and AUXB1, respectively. Except lamellarin K, C5–C6 dihydro lamellarins (Type I), were significantly less cytotoxic than C5–C6 unsaturated lamellarins (Type II).

In the latest study on the cytotoxicity profile of lamellarins by Ruchirawat and co-workers in 2009,[33] it is shown that lamellarins D, K, M, N, X, ε, and dehydrolamellarin J exhibit potent cytotoxicity against several cancer cell lines with $IC_{50}$ values in nanomolar to sub-nanomolar range (Table 7). The activities of these lamellarins are much more potent than that of the clinical anticancer drug etoposide. Another interesting finding is that lamellarin N and dehydrolamellarin J are significantly more potent than the other compounds except lamellarin D in most of the cancer cell lines, but are relatively low toxic to the normal fibroblast cells MRC-5.

Ishibashi and Iwao were the first to attempt to establish the SAR of lamellarins.[21] They investigated the effect of individual hydroxyl and methoxy substituents on the cytotoxicity of lamellarin D towards HeLa cells (Table 8). Removing the C20 hydroxyl group (compound **221**) and masking the C8 hydroxyl group as a methyl ether (compound **222**, lamellarin η) resulted in a significant decrease in activity for lamellarin D, indicating that these two hydroxyl groups are essential for this activity. On the other hand, the C14 hydroxyl group and the methoxy groups at C13 and C21 appeared less important since 14-dehydroxylamellarin D (**224**), 13-demethoxylamellarin D (**223**), and 21-demethoxylamellarin D (**220**) only displayed a slight decrease in activity as compared to their parent compounds. Recently, Ruchirawat

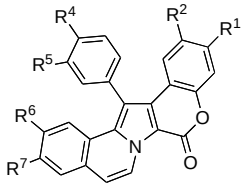
Table 7. Cytotoxic activity of lamellarins[33]

| compound | $IC_{50}$ (μM) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | oral | lung | | breast | | liver | | | cervix | blood cell | | fibroblast |
| | KB | A549 | H69AR | T47D | MDA-MB-231 | HuCCA-1 | HepG2 | S102 | HeLa | P388 | HL-60 | MRC-5 |
| lamellarin C | 5.7 | 3.6 | 12.1 | 7.7 | 8.3 | 11.5 | 18.3 | 4.4 | 7.9 | 4.2 | 5.7 | ND |
| lamellarin B | 4.4 | 5.4 | 6.4 | 0.2 | 4.4 | 5.3 | 0.8 | 5.9 | 4.8 | 6.1 | 6.2 | 68.1 |
| lamellarin χ | 2.6 | 2.0 | 38.9 | 3.8 | 4.8 | 49.9 | 0.1 | 3.4 | 6.6 | 1.6 | 1.8 | ND |
| lamellarin D | 0.04 | 0.06 | 0.4 | 0.00008 | 0.4 | 0.08 | 0.02 | 3.2 | 0.06 | 0.1 | 0.04 | 9.2 |
| lamellarin E | 4.0 | 2.2 | 7.2 | 5.3 | 3.4 | 9.4 | 1.0 | 2.8 | 5.3 | 2.6 | 4.5 | ND |
| lamellarin X | 0.08 | 0.3 | 0.3 | 0.006 | 0.08 | 0.04 | 0.2 | 1.6 | 0.09 | 0.3 | 0.2 | 10.1 |
| lamellarin F | 4.2 | 4.4 | 10.1 | 4.6 | 3.7 | 8.8 | 0.5 | 2.7 | 6.4 | 3.1 | 3.6 | ND |
| lamellarin ε | 0.3 | 0.3 | 2.3 | 0.006 | 0.3 | 0.07 | 0.1 | 2.1 | 0.3 | 0.3 | 0.1 | 25.8 |
| lamellarin G | 3.0 | 4.0 | 7.4 | 8.6 | 15.0 | 49.9 | 1.5 | 9.6 | 4.2 | 1.6 | 7.5 | ND |
| lamellarin I | 6.3 | 10.6 | 18.1 | 9.5 | 8.6 | 11.2 | 1.3 | 12.4 | 11.2 | 3.8 | 6.9 | ND |
| lamellarin ζ | 4.7 | 10.6 | 23.3 | 0.09 | 4.7 | 6.3 | 0.3 | 7.9 | 8.3 | 7.2 | 12.3 | >89.7 |
| lamellarin J | >97.0 | 1.1 | >97.0 | 13.0 | 7.4 | >97.0 | 0.4 | 19.4 | >97.0 | 0.8 | 0.9 | ND |
| dehydrolam. J | 0.08 | 0.04 | 0.3 | 0.0001 | 0.4 | 0.006 | 0.01 | 2.1 | 0.08 | 0.08 | 0.04 | >97.4 |
| lamellarin K | 0.9 | 4.2 | 4.3 | 0.09 | 0.4 | 3.4 | 1.0 | 4.4 | 2.8 | 3.4 | 3.8 | ND |
| lamellarin M | 0.2 | 0.04 | 0.3 | 0.009 | 0.1 | 0.06 | 0.02 | 1.9 | 0.3 | 0.1 | 0.06 | 13.4 |
| lamellarin L | 3.0 | 0.8 | 3.0 | 4.4 | 1.8 | 21.9 | 0.3 | 1.4 | 2.8 | 0.5 | 1.9 | ND |
| lamellarin N | 0.06 | 0.04 | 0.06 | 0.0006 | 0.6 | 0.008 | 0.02 | 2.3 | 0.04 | 0.08 | 0.04 | >100.1 |
| lamellarin T | 6.4 | 2.9 | 13.2 | 13.2 | 8.6 | 14.7 | 0.6 | 5.5 | 9.9 | 4.8 | 6.4 | ND |
| lamellarin W | 5.3 | 5.2 | 4.4 | 4.2 | 5.2 | 4.2 | 0.9 | 5.8 | 5.0 | 5.6 | 6.7 | 28.5 |
| lamellarin U | 3.9 | 0.9 | 8.7 | 10.3 | 4.5 | 44.6 | 0.6 | 3.0 | 5.0 | 1.8 | 4.5 | ND |
| lamellarin α | 9.4 | 1.6 | 8.0 | 0.6 | 3.9 | 5.8 | 0.06 | 5.6 | 7.6 | 1.7 | 10.5 | >97.4 |
| lamellarin Y | 5.0 | 0.9 | 14.8 | 7.2 | 8.0 | 37.9 | 0.6 | 4.3 | 29.9 | 1.0 | 5.0 | ND |
| dehydrolam. Y | 0.8 | 1.3 | 7.6 | 0.08 | 0.6 | 1.4 | 0.4 | 6.2 | 1.6 | 0.9 | 3.4 | 31.0 |
| etoposide | 0.5 | 1.1 | 45.9 | 0.08 | 0.2 | 6.8 | 0.2 | 1.5 | 0.4 | 0.4 | 2.3 | >85.0 |

ND = not determined. Cell lines used (in alphabetical order): A549, human non-small cell lung carcinoma; H69AR, human multi-drug-resistant small-cell lung; HeLa, human cervical adenocarcinoma; HepG2, human hepatocellular carcinoma; HL-60, human promyelocytic leukemia; HuCCA-1, human cholangiocarcinoma; KB, human oral epidermoid carcinoma; MDA-MB-231, human hormone-independent breast cancer 231; MRC-5, human fetal/embryonic lung fibroblast; P388, mouse lymphoid neoplasm; S102, human hepatocellular carcinoma; T47D, human hormone-dependent breast cancer.

and co-workers[33] also provided important SAR profiles for lamellarins (Table 7). They pointed out the importance of the hydroxyl group at C7. Even when the C8 hydroxyl group was masked as a methyl ether, lamellarins having a hydroxyl group at C7, such as lamellarins M, X, and ε, exhibited activities that were as high as that of lamellarin D; on the other hand, as their corresponding C7 methoxy compounds (lamellarins B, W, and ζ, respectively) showed lower activities. As mentioned above, saturation of the C5–C6 double bond causes a serious decrease in activity. However, in spite of its saturated C5–C6 bond, lamellarin K displayed a high activity, which may be owing to the C7 hydroxyl group. Introducing an

Table 8. Cytotoxic activity of lamellarin derivatives on HeLa cells.[21]



| compound | $R^1$ | $R^2$ | $R^4$ | $R^5$ | $R^6$ | $R^7$ | $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|---|
| lamellarin D | OH | OMe | OH | OMe | OMe | OH | 0.0105 |
| lamellarin H | OH | OH | OH | OH | OH | OH | >100 |
| **220** | OH | H | OH | OMe | OMe | OH | 0.0395 |
| **221** | H | OMe | OH | OMe | OMe | OH | 0.8500 |
| **222** | OH | OMe | OMe | OMe | OMe | OMe | 2.5 |
| **223** | OH | OMe | OH | H | OMe | OH | 0.0380 |
| **224** | OH | OMe | H | OMe | OMe | OH | 0.0700 |
| **225** | H | H | OH | OMe | OMe | OH | 4.0 |
| **226** | H | H | OH | OH | OH | OH | 1.1 |
| **227** | OAc | OAc | OAc | OAc | OAc | OAc | 11.0 |
| **228** | H | H | OMe | OMe | OMe | OMe | 5.7 |
| **229** | -O-$CH_2$-O- | | OMe | OMe | OMe | OMe | >100 |

electron withdrawing group, such as nitro and trifluoromethoxy groups on the aromatic ring significantly decreased the cytotoxicity.[85] Structural requirements resulting from these studies are summarized in Figure 2.

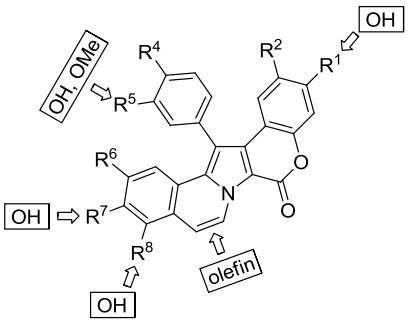



Figure 2. Important structural elements in the lamellarin skeleton.[33]

## 3-2. INHIBITION OF TOPOISOMERASE I

Although the potent cytotoxic activity of lamellarins against various cancer cell lines has been demonstrated extensively, their mechanism of action has only been investigated recently. In 2003, Bailly and co-workers disclosed that lamellarin D strongly inhibited the action of topoisomerase I, an essential enzyme that relaxes torsional strain of supercoiled DNA during a number of critical cellular processes

including replication, transcription, and repair.[93] The enzymatic process involves the transient breaking and rejoining of DNA single strands. Therefore, the inhibition of this enzyme results in potentially lethal DNA damage and induction of apoptosis, making strong topoisomerase inhibitors promising drug candidates for cancer therapy. Some of the most successful drugs are the camptothecins (CPTs). These drugs block the rejoining step during topoisomerase I-mediated cleavage/religation reactions, resulting in the accumulation of covalent DNA–topoisomerase–drug complexes which prevent the release of the enzyme. Lamellarin D has been shown to strongly promote the conversion of supercoiled DNA into nicked DNA in the presence of topoisomerase I in DNA relaxation assays.[93] In particular, the data showed that, similar to CPT, lamellarin D stabilized the cleaved DNA–topoisomerase I complex. Even with a 5-fold lower activity than CPT, lamellarin D could clearly generate a large number of single-strand breaks. At 2 μM, lamellarin D and CPT were equally efficient and converted ~70% of negatively supercoiled plasmid pLAZ3 into single-strand breaks. DNA cleavage experiments using a 198-bp DNA restriction fragment were also performed to investigate the topoisomerase I poisoning activity. Interestingly, the cleavage profile obtained with lamellarin D was slightly different from those observed for CPT. Topoisomerase I-mediated DNA cleavage occurred at sites common to CPT in the presence of lamellarin D, but a few sites specific to only CPT or lamellarin D were also detected. This suggested that lamellarin D and CPT interact differently with the topoisomerase I–DNA interface. A computer-based molecular modeling[93,94] of the binding mode of lamellarin D to the covalent topoisomerase–DNA complex showed that lamellarin D intercalated at the DNA cleavage site and stabilized the ternary complex by forming stacking interactions with the +1 (C·G) and −1 (A·T) bps. The ternary complex was further stabilized by hydrogen bonds between the drug and specific amino acid residues of the protein. The C8 and C20 hydroxyl groups were at hydrogen-bond distances from the $Asn^{722}$ and $Glu^{356}$ enzyme residues, respectively, while the lactonic carbonyl group interacted with the $Arg^{364}$ residue (Figure 3). This result correlates quite well with existing SAR profiles. The lamellarin D analogue having a C5–C6 single bond was 42 times less cytotoxic than lamellarin D against P388 murine leukemia cells. This saturated analogue was also totally inactive against topoisomerase I and failed to bind to DNA. It is apparent that the non-planar conformation of the analogue prevents its intercalation between DNA strands.

Bailly's group reported that amino acid residues such as Ala, Leu, Val, Pro, and Phe could be incorporated into lamellarins *via* ester linkages with the C8, C13, and C20 hydroxyl groups without the loss of topoisomerase I inhibitory activity.[92] On the other hand, NH-Boc derivatives of these amino acid derivatives were totally inactive, suggesting that the positively charged amino groups might interact with DNA phosphate groups and possibly with the target enzyme. These cationic amino acid derivatives may benefit *in vivo* studies and clinical evaluations by enhancing water solubility. Recently, Albericio and

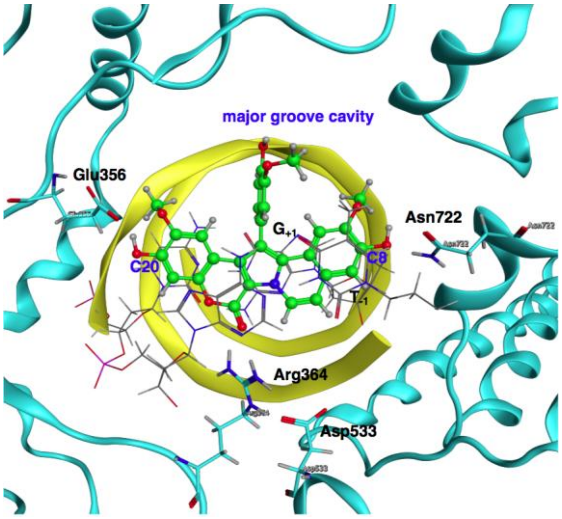



Figure 3. A lamellarin D-DNA-topoisomerase I ternary complex model.[94]

Alvarez reported the synthesis of lamellarin D conjugates with a nuclear localization signal peptide and a poly(ethylene glycol)-based dendrimer. These compounds were found to be 1.4–3.3 times more cytotoxic than their parent compound against three human tumor cell lines.[95,96]

### 3-3. INHIBITION OF MITOCHONDRIAL FUNCTION

Lamellarins are strong topoisomerase I inhibitors; however, topoisomerase I-mutated cell lines exhibited a reduced but still significant level of chemosensitivity towards lamellarin D and its analogues.[93,94] This suggests that topoisomerase I is not the only cellular target of lamellarin D. Bailly and co-workers found that lamellarin D acted on cancer cell mitochondria to induce swelling and release of apoptosis-inducing factors such as cytochrome *c*. This direct mitochondrial effect of lamellarin D accounts for the sensitivity of topoisomerase I-mutated P388CPT5 cells that are resistant to CPT. These results indicate that lamellarin D affects preferentially the topoisomerase pathway without directly interfering with mitochondria at submicromolar concentrations (<1 μM), but influences both the nucleus and mitochondria through a dual action leading to massive and rapid cell death at higher concentration (at the micromolar range).[97-100]

### 3-4. INHIBITION OF PROTEIN KINASES

Previous reports on the mode of action of lamellarins showed that the marine alkaloids induce apoptotic cell death through multi-target mechanisms, including topoisomerase I inhibition, interaction with DNA, and direct effects on mitochondria. Meijer and co-workers [101] found alternative targets for the cytotoxic action of lamellarins. Some lamellarins inhibit several protein kinases relevant to cancer such as cyclin-dependent kinases (CDKs), dual-specificity tyrosine-phosphorylated and -regulated kinase 1A

(DYRK1A), casein kinase 1 (CK1), glycogen synthase kinase-3 (GSK-3), and proto-oncogene serine/threonine protein kinase PIM-1 (PIM1) (Table 9). CDK1/cyclin B is essential for G1/S and G2/M phase transitions of the cell cycle and its inhibition leads to cell cycle arrest and ultimately to cell death. The CDK1/cyclin B inhibitory activity profile of these lamellarins was parallel to their cytotoxicity against human neuroblastoma SH-SY5Y cells, suggesting that kinase inhibition may contribute to the action of lamellarins on cell proliferation and cell death. Several lamellarins tested were active on all six kinases, some of which are involved in neurodegenerative diseases like Alzheimer's disease. Moreover, the most active compound, lamellarin N, also displayed some selectivity for a few kinases on the Cerep kinase activity panel. Hence, these multifunctional natural products might find applications in the development of new drugs not only for cancer therapy, but also for other serious diseases such as Alzheimer's disease. The SAR profile of lamellarins on kinase inhibition showed a small but clear difference compared to topoisomerase inhibition. Saturating the C5–C6 double bond of lamellarin N to form lamellarin L or exchanging its C13 hydroxyl and C14 methoxy groups to give lamellarin D decreased its activity. Blocking the C8 hydroxyl group of lamellarin D to obtain lamellarin α also caused the activity to decrease significantly. These results indicate that the C5–C6 double bond and the C8 and C13 hydroxyl groups are important structural requirements for the kinase inhibition. On the other hand, the C20 hydroxyl group, which is crucial for topoisomerase inhibition, was not necessary in the case of kinase inhibition because compound **221** which lacks the C20 hydroxyl group showed high activity against kinases.

Table 9. Inhibitory activity on several protein kinase and cytotoxicity of lamellarins ($IC_{50}$ μM).[101]

| lamellarin | protein kinase | | | | | | neuroblastoma |
|---|---|---|---|---|---|---|---|
| | CDK1/cyclin B | CDK5/p25 | CDK-3α/β | PIM1 | DYRK1A | CK1 | SH-SY5Y |
| lamellarin D | 0.50 | 0.55 | 0.3 | 0.10 | 0.45 | 13.0 | 0.019 |
| lamellarin α | 8.0 | > 10 | 1.4 | 0.59 | 5.0 | 7.9 | - (10) |
| di-H-lamellarin D | 1.85 | 0.11 | 0.9 | 0.20 | 0.50 | 5.9 | 0.41 |
| lamellarin H | - (10) | - (10) | 9.5 | - (10) | - (10) | 5.3 | 0.45 |
| di-H-lamellarin H | - (10) | - (10) | 0.67 | - (10) | - (10) | 5.2 | 2.55 |
| lamellarin N | 0.070 | 0.025 | 0.005 | 0.055 | 0.035 | - (10) | 0.025 |
| lamellarin L | 0.38 | 0.1 | 0.041 | 0.25 | 0.14 | - (10) | 0.7 |
| lamellarin G tri-OMe | - (10) | - (10) | - (10) | - (10) | >10 | - (10) | - (100) |
| **220** | 0.53 | 0.60 | 0.58 | 0.15 | 0.06 | 0.41 | 0.056 |
| **221** | 2.0 | 0.6 | 0.05 | 0.05 | 0.08 | 1.3 | 0.79 |
| **222** | - (10) | - (10) | - (10) | 2.0 | - (10) | - (10) | 8.0 |
| **223** | 0.10 | 0.03 | 0.13 | 0.33 | 0.09 | 0.8 | 0.11 |
| **224** | 4.3 | 2.1 | 2.1 | - (10) | - (10) | - (10) | 0.14 |
| **225** | 5 | 0.9 | 2.2 | 0.7 | 1.0 | - (10) | 2.65 |
| lamellarin K | - (10) | - (10) | - (10) | 0.6 | - (10) | 6.0 | - (30) |

- : no inhibitory activity was detected (highest concentration tested is indicated in parentheses)

### 3-5. MULTIDRUG RESISTANCE (MDR) REVERSAL ACTIVITY

Many cancer cells gain resistance to the drugs with no structural similarity to the drug which is used during the chemotherapy. MDR has been one of the major obstacles to long-term cancer chemotherapy. One reason for this cross-resistance is the overexpression of the P-glycoprotein (P-gp) membrane protein, which mediates the ATP-dependent drug efflux from cells. As mentioned above, several cytotoxic lamellarins exhibit equally high activity against MDR cell lines.[16] At noncytotoxic doses, lamellarin I, a representative MDR inhibitor, effectively increased the cytotoxicity of anticancer agents, such as doxorubicin, vinblastine, and daunorubicin, by inhibiting the P-gp-mediated drug efflux. The potency of the MDR reversal activity of lamellarin I was reported to be 9 to 16 times higher than that of the prototype MDR inhibitor verapamil. Its ningalin congeners have also shown potent MDR reversal activity.[70,102]

### 3-6. INHIBITION OF HIV-1 INTEGRASE

HIV encodes three enzymes, namely reverse transcriptase, protease, and integrase, which are responsible for retroviral replication. Reverse transcriptase and protease inhibitors have already made significant advances in anti-retroviral therapy. However, the appearance of drug-resistant HIV has recently been increasing, making the development of novel anti-retroviral drugs with alternative modes of action necessary. HIV-1 integrase catalyzes a multi-step integration process which involves the cleavage of two bases from the 3'-end of each viral DNA strand (3'-end processing) and the transfer of these processed 3'-ends into the host DNA (strand transfer). This retroviral process is absent in mammalian host cells, making the enzyme a potential target for non-toxic antiviral therapy. However, only one integrase inhibitor, raltegravir, has been approved for clinical use. Faulkner and co-workers isolated a series of lamellarin-type alkaloids which inhibit HIV-1 integrase from an unidentified ascidian collected from the Arabian Sea coast of India.[11] One of the most active compounds, lamellarin α 20-sulfate, inhibited the integrase terminal cleavage ($IC_{50}$ = 16 μM) and strand transfer activities ($IC_{50}$ = 22 μM). The sulfated alkaloid inhibited the growth of the HIV-1 virus *in vitro* at a non-toxic concentration for the mammalian cell line ($IC_{50}$ = 8 μM; $LD_{50}$ = 274 μM for HeLa cells). The sulfate-free compound, lamellarin α, showed no inhibition of HIV-1 integrase at concentrations reaching 1.6 mM,[91] suggesting that the sulfate group is critical for integrase inhibition.

### 3-7. MISCELLANEOUS ACTIVITIES

Isolated from the marine prosobranch mollusk *Lamellaria* sp., lamellarins C and D have been shown to inhibit cell division in a fertilized sea urchin assay (15 and 75% inhibition at 19 μg/mL, respectively).[2] Lamellarin K and L reported to exhibit moderate immunomodulatory activity (LcV:MLR 147 and 98,

respectively).[4]

It is well known that many antioxidant compounds simultaneously possess anticancer or anticancerogenic properties. Venkateswarlu and co-workers evaluated the antioxidant activity of several lamellarins isolated from the Indian ascidian *Didemnum obscurum* and found that all the tested compounds including lamellarins γ, γ-monoacetate, K, U, I, and C-diacetate showed weak free radical scavenging activity at the millimolar level.[13]

## 4. CONCLUSION

A number of unique and efficient synthetic methods for lamellarin alkaloids have been developed so far. Many of them can be applied not only to the natural products but also to a wide range of analogues. Synthetic methods involving pyrrole intermediates are especially useful in view of their flexibility for the synthesis of structurally related 3,4-diarylpyrrole marine alkaloids and simplified lamellarin analogues. The potent cytotoxicity of several lamellarins suggested their potential use as new leads for antitumor agents. SAR studies revealed structural requirements for antitumor activity. The molecular targets such as topoisomerase I and protein kinases have already been identified in the cell. We believe that the rational design and synthesis of lamellarin analogues that selectively inhibit these target molecules can produce useful antitumor agents without unfavorable side effects.

## ACKNOWLEDGMENT

We thank the Japan Society for the promotion of Science (JSPS) and the Japan Science and Technology Agency (JST) for financially supporting our research on lamellarin alkaloids.

## REFERENCES AND NOTES

1. Y. Nakao and N. Fusetani, *J. Nat. Prod.*, 2007, **70**, 689.
2. R. J. Andersen, D. J. Faulkner, H. Cun-heng, G. D. Van Duyne, and J. Clardy, *J. Am. Chem. Soc.*, 1985, **107**, 5492.
3. N. Lindquist, W. Fenical, G. D. Van Duyne, and J. Clardy, *J. Org. Chem.*, 1988, **53**, 4570.
4. A. R. Carroll, B. F. Bowden, and J. C. Coll, *Aust. J. Chem.*, 1993, **46**, 489.
5. S. Urban, M. S. Butler, and R. J. Capton, *Aust. J. Chem.*, 1994, **47**, 1919.
6. S. Urban, L. Hobbs, J. N. A. Hooper, and R. J. Capton, *Aust. J. Chem.*, 1995, **48**, 1491.
7. S. Urban and R. J. Capton, *Aust. J. Chem.*, 1996, **49**, 711.
8. M. V. R. Reddy, D. J. Faulkner, Y. Venkateswarlu, and M. R. Rao, *Tetrahedron*, 1997, **53**, 3457.
9. C. L. Cantrell, A. Groweiss, K. R. Gustafson, and M. R. Boyd, *Nat. Prod. Lett.*, 1999, **14**, 39.
10. R. A. Davis, A. R. Carroll, G. K. Pierens, and R. J. Quinn, *J. Nat. Prod.*, 1999, **62**, 419.

11. M. V. R. Reddy, M. R. Rao, D. Rhodes, M. S. T. Hansen, K. Rubins, F. D. Bushman, Y. Venkateswarlu, and D. J. Faulkner, *J. Med. Chem.*, 1999, **42**, 1901.
12. J. Ham and H. Kang, *Bull. Korean Chem. Soc.*, 2002, **23**, 163.
13. P. Krishnaiah, V. L. N. Reddy, G. Venkataramana, K. Ravinder, M. Srinivasulu, T. V. Raju, K. Ravikumar, D. Chandrasekar, S. Ramakrishna, and Y. Venkateswarlu, *J. Nat. Prod.*, 2004. **67**, 1168.
14. S. M. Reddy, M. Srinivasulu, N. Satyanarayana, A. K. Kondapi, and Y. Venkateswarlu, *Tetrahedron*, 2005, **61**, 9242.
15. Lamellarin numbering utilized in this review follows the initial report by Faulkner.[2]
16. A. R. Quesada, M. D. G. Grávalos, and J. L. F. Puentes, *Br. J. Cancer*, 1996, **74**, 677.
17. For reviews, see: (a) P. Cironi, F. Albericio, and M. Álvarez, *Progress in Heterocyclic Chemistry*, 2004, **16**, 1; (b) C. Bailly, *Curr. Med. Chem. - Anti-Cancer Agents*, 2004, **4**, 363; (c) S. T. Handy and Y. Zhang, *Org. Prep. Proced. Int.*, 2005, **8**, 411; (d) H. Fan, J. Peng, M. T. Hamann, and J.-F. Hu, *Chem. Rev.*, 2008, **108**, 264; (e) D. Pla, F. Albericio, and M. Álvarez, *Anti-Cancer Agents in Med. Chem.*, 2008, **8**, 746; (f) J. Kluza, P. Marchetti, and C. Bailly, 'Modern Alkaloids: Structure, Isolation, Synthesis and Biology,' ed. by E. Fattorusso and O. Taglialatela-Scafati, Wiley-VCH, Weinheim, 2008, pp. 171-187.
18. For a review on the synthesis of indolizine and related heterocycles including pyrrolo[2,1-*a*]isoquinoline, see: T. Uchida and K. Matsumoto, *Synthesis*, 1976, 209.
19. F. Ishibashi, Y. Miyazaki, and M. Iwao, *Tetrahedron*, 1997, **53**, 5951.
20. M. Iwao and T. Kuraishi, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 297.
21. F. Ishibashi, S. Tanabe, T. Oda, and M. Iwao, *J. Nat. Prod.*, 2002, **65**, 500.
22. M. Banwell, B. Flynn, and D. Hockless, *Chem. Commun.*, 1997, 2259.
23. P. Cironi, I. Manzanares, F. Albericio, and M. Álvarez, *Org. Lett.*, 2003, **5**, 2959.
24. P. Cironi, C. Cuevas, F. Albericio, and M. Álvarez, *Tetrahedron*, 2004, **60**, 8669.
25. S. Ruchirawat and T. Mutarapat, *Tetrahedron Lett.*, 2001, **42**, 1205.
26. (a) A. E. Tschitschibabin, *Ber.* 1927, **60**, 1607; (b) C. Casagrande, A. Invernizzi, R. Ferrini, and G. Ferrari, *J. Med. Chem.*, 1968, **11**, 765.
27. Y. Tamaru, Y. Yamada, K. Inoue, Y. Yamamoto, and Z. Yoshida, *J. Org. Chem.*, 1983, **48**, 1286.
28. P. Ploypradith, W. Jinaglueng, C. Pavaro, and S. Ruchirawat, *Tetrahedron Lett.*, 2003, **44**, 1363.
29. P. Ploypradith, R. K. Kagan, and S. Ruchirawat, *J. Org. Chem.*, 2005, **70**, 5119.
30. P. Ploypradith, C. Mahidol, P. Sahakitpichan, S. Wongbundit, and S. Ruchirawat, *Angew. Chem. Int. Ed.*, 2004, **43**, 866.
31. C. A. Grob and K. Camenish, *Helv. Chim. Acta*, 1953, **36**, 49.
32. P. Ploypradith, T. Petchmanee, P. Sahakitpichan, N. D. Litvinas, and S. Ruchirawat, *J. Org. Chem.*,

2006, **71**, 9440.

33. M. Chittchang, P. Batsomboon, S. Ruchirawat, and P. Ploypradith, *ChemMedChem*, 2009, **4**, 457.
34. S. Boonya-udtayan, N. Yotapan, C. Woo, C. J. Bruns, S. Ruchirawat, and N. Thasana, *Chem. Asian J.*, 2010, **5**, 2113.
35. N. Thasana, R. Worayuthakarn, P. Kradanrat, E. Hohn, L. Young, and S. Ruchirawat, *J. Org. Chem.*, 2007, **72**, 9379.
36. P. Wipf and C. R. Hopkins, *J. Org. Chem.*, 2001, **66**, 3133.
37. B.-X. Zhao, Y. Yu, and S. Eguchi, *Tetrahedron*, 1996, **52**, 12049.
38. M. Díaz, E. Guitián, and L. Castedo, *Synlett*, 2001, 1164.
39. S. Murahashi, H. Mitsui, T. Shiota, T. Tsuda, and S. Watanabe, *J. Org. Chem.*, 1990, **55**, 1736.
40. M. Nyerges and L. Tőke, *Tetrahedron Lett.*, 2005, **46**, 7531.
41. J. Tóth, A. Nedves, A. Dancsó, G. Blaskó, L. Tőke, and M. Nyerges, *Synthesis*, 2007, 1003.
42. J. S. Yadav, K. U. Gayathri, B. V. S. Reddy, and A. R. Prasad, *Synlett*, 2009, 43.
43. S. Hajra, B. Maji, and A. Karmakar, *Tetrahedron Lett.*, 2005, **46**, 8599.
44. J. C. Liermann and T. Opatz, *J. Org. Chem.*, 2008, **73**, 4526.
45. S. T. Handy, Y. Zhang, and H. Bregman, *J. Org. Chem.*, 2004, **69**, 2362.
46. V. Y. Kurotaev, V. Y. Sosnovskikh, I. B. Kutyashev, A. Y. Barkov, and Y. V. Shklyaev, *Tetrahedron Lett.*, 2008, **49**, 5376.
47. S. Su and J. A. Porco, Jr., *J. Am. Chem. Soc.*, 2007, **129**, 7744.
48. http://www.pharm.monash.edu.au/courses/honours/mipsprojects/mcda/bflynn3.html
49. For recent reviews on pyrrole synthesis, see: (a) M. G. Banwell, T. E. Goodwin, S. Ng, J. A. Smith, and D. J. Wong, *Eur. J. Org. Chem.*, 2006, 3043; (b) F. Bellina and R. Rossi, *Tetrahedron*, 2006, **62**, 7213; (c) C. Schmuck and D. Rupprecht, *Synthesis*, 2007, 3095.
50. A. Heim, A. Terpin, and W. Steglich, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 155.
51. D. I. Davies and C. Waring, *J. Chem. Soc.*, 1967, 1639.
52. (a) P. Forgione, M.-C. Brochu, M. St-Onge, K. H. Thesen, M. D. Bailey, and F. Bilodeau, *J. Am. Chem. Soc.*, 2006, **128**, 11350; (b) L. J. Gooβen, N. Rodríguez, and K. Gooβen, *Angew. Chem. Int. Ed.*, 2008, **47**, 3100.
53. C. Peschko, C. Winklhofer, and W. Steglich, *Chem. Eur. J.*, 2000, **6**, 1147.
54. (a) O. Hinsberg, *Ber.*, 1910, **43**, 901; (b) A. Merz, R. Schropp, and E. Dötterl, *Synthesis*, 1995, 795.
55. T. Oh-e, N. Miyaura, and A. Suzuki, *J. Org. Chem.*, 1993, **58**, 2201.
56. M. Iwao, T. Takeuchi, N. Fujikawa, T. Fukuda, and F. Ishibashi, *Tetrahedron Lett.*, 2003, **44**, 4443.
57. N. Fujikawa, T. Ohta, T. Yamaguchi, T. Fukuda, F. Ishibashi, and M. Iwao, *Tetrahedron*, 2006, **62**, 594.

58. T. Fukuda, Y. Hayashida, and M. Iwao, *Heterocycles*, 2009, **77**, 1105.
59. T. Takada, M. Arisawa, M. Gyoten, R. Hamada, H. Tohma, and Y. Kita, *J. Org. Chem.*, 1998, **63**, 7698.
60. (a) A. G. Myers, D. Tanaka, and M. R. Mannion, *J. Am. Chem. Soc.*, 2002, **124**, 11250; (b) D. Tanaka, S. P. Romeril, and A. G. Myers, *J. Am. Chem. Soc.*, 2005, **127**, 10323; (c) C. Wang, I. Piel, and F. Glorius, *J. Am. Chem. Soc.*, 2009, **131**, 4194.
61. T. Yamaguchi, T. Fukuda, F. Ishibashi, and M. Iwao, *Tetrahedron Lett.*, 2006, **47**, 3755.
62. T. Fukuda, T. Ohta, S. Saeki, and M. Iwao, *Heterocycles*, 2010, **80**, 841.
63. Y. Liu, I. F. Lien, S. Ruttgaizer, P. Dove, and S. D. Taylor, *Org. Lett.*, 2004, **6**, 209.
64. (a) S. Hirao, Y. Sugiyama, M. Iwao, and F. Ishibashi, *Biosci. Biotechnol. Biochem.*, 2009, **73**, 1764; (b) S. Hirao, Y. Yoshinaga, M. Iwao, and F. Ishibashi, *Tetrahedron Lett.*, 2010, **51**, 533.
65. K. Warabi, S. Matsunaga, R. W. M. van Soest, and N. Fusetani, *J. Org. Chem.*, 2003, **68**, 2765.
66. G. Blotny, *Tetrahedron*, 2006, **62**, 9507.
67. J. L. Namy, J. Souppe, and H. B. Kagan, *Tetrahedron Lett.*, 1983, **24**, 765.
68. (a) A. Fürstner, M. M. Domostoj, and B. Scheiper, *J. Am. Chem. Soc.*, 2006, **128**, 8087; (b) K. Okano, H. Fujiwara, T. Noji, T. Fukuyama, and H. Tokuyama, *Angew. Chem. Int. Ed.*, 2010, **49**, 5925.
69. D. L. Boger, C. W. Boyce, M. A. Labroli, C. A. Sehon, and Q. Jin, *J. Am. Chem. Soc.*, 1999, **121**, 54.
70. D. L. Boger, D. R. Soenen, C. W. Boyce, M. P. Hedrick, and Q. Jin, *J. Org. Chem.*, 2000, **65**, 2479.
71. H. Kang and W. Fenical, *J. Org. Chem.*, 1997, **62**, 3254.
72. P. E. Eaton, G. R. Carlson, and J. T. Lee, *J. Org. Chem.*, 1973, **38**, 4071.
73. J. T. Gupton, K. E. Krumpe, B. S. Burnham, T. M. Webb, J. S. Shuford, and J. A. Sikorski, *Tetrahedron*, 1999, **55**, 14515.
74. J. T. Gupton, S. C. Clough, R. B. Miller, J. R. Lukens, C, A. Henry, R. P. F. Kanters, and J. A. Sikorski, *Tetrahedron*, 2003, **59**, 207.
75. J. T. Gupton, B. C. Giglio, J. E. Eaton, E. A. Rieck, K. L. Smith, M. J. Keough, P. J. Barelli, L. T. Firich, J. E. Hempel, T. M. Smith, and R. P. F. Kanters, *Tetrahedron*, 2009, **65**, 4283.
76. D. H. R. Barton, J. Kervagoret, and S. Z. Zard, *Tetrahedron*, 1990, **46**, 7587.
77. J. M. Bullington, R. R. Wolff, and P. F. Jackson, *J. Org. Chem.*, 2002, **67**, 9439.
78. M. G. Banwell, B. L. Flynn, E. Hamel, and D. C. R. Hockless, *Chem. Commun.*, 1997, 207.
79. B. L. Bray, P. H. Mathies, R. Naef, D. R. Solas, T. T. Tidwell, D. R. Artis, and J. M. Muchowski, *J. Org. Chem.*, 1990, **55**, 6317.
80. M. G. Banwell, B. L. Flynn, D. C. R. Hockless, R. W. Longmore, and D. Rae, *Aust. J. Chem.*, 1998,

**52**, 755.

81. S. T. Handy, Y. Zhang, and H. Bregman, *J. Org. Chem.*, 2004, **69**, 2362.
82. S. T. Handy, H. Bregman, J. Lewis, X. Zhang, and Y. Zhang, *Tetrahedron Lett.*, 2003, **44**, 427.
83. D. Pla, A. Marchal, C. A. Olsen, F. Albericio, and M. Álvarez, *J. Org. Chem.*, 2005, **70**, 8231.
84. C. A. Olsen, N. Parera, F. Albericio, and M. Álvarez, *Tetrahedron Lett.*, 2005, **46**, 2041.
85. D. Pla, A. Marchal, C. A. Olsen, A. Francesch, C. Cuevas, F. Albericio, and M. Álvarez, *J. Med. Chem.*, 2006, **49**, 3257.
86. T. Ohta, T. Fukuda, F. Ishibashi, and M. Iwao, *J. Org. Chem.*, 2009, **74**, 8143.
87. C. Zonta, F. Fabris, and O. De Lucchi, *Org. Lett.*, 2005, **7**, 1003.
88. T. Fukuda, T. Ohta, E. Sudo, and M. Iwao, *Org. Lett.*, 2010, **12**, 2734.
89. For a review, see: D. Alberico, M. E. Scott, and M. Lautens, *Chem. Rev.*, 2007, **107**, 174.
90. W. Qiu, S. Chen, X. Sun, Y. Liu, and D. Zhu, *Org. Lett.*, 2006, **8**, 867.
91. C. P. Ridley, M. V. R. Reddy, G. Rocha, F. D. Bushman, and D. J. Faulkner, *Bioorg. Med. Chem.*, 2002, **10**, 3285.
92. C. Tardy, M. Facompré, W. Laine, B. Baldeyrou, D. García-Gravalos, A. Francesch, C. Mateo, A. Pastor, J. A. Jiménez, I. Manzanares, C. Cuevas, and C. Bailly, *Bioorg. Med. Chem.*, 2004, **12**, 1697.
93. M. Facompré, C. Tardy, C. Bal-Mahieu, P. Colson, C. Perez, I. Manzanares, C. Cuevas, and C. Bailly, *Cancer Res.*, 2003, **63**, 7392.
94. E. Marco, W. Laine, C. Tardy, A. Lansiaux, M. Iwao, F. Ishibashi, C. Bailly, and F. Gago, *J. Med. Chem.*, 2005, **48**, 3796.
95. D. Pla, A. Francesch, P. Calvo, C. Cuevas, R. Aligué, F. Albericio, and M. Álvarez, *Bioconjugate Chem.*, 2009, **20**, 1100.
96. D. Pla, M. Martí, J. Farrera-Sinfreu, D. Pulido, A. Francesch, P. Calvo, C. Cuevas, M. Royo, R. Aligué, F. Albericio, and M. Álvarez, *Bioconjugate Chem.*, 2009, **20**, 1112.
97. J. Kluza, M.-A. Gallego, A. Loyens, J.-C. Beauvillain, J.-M. F. Sousa-Faro, C. Cuevas, P. Marchetti, and C. Bailly, *Cancer Res.*, 2006, **66**, 3177.
98. M.-A. Gallego, C. Ballot, J. Kluza, N. Hajji, A. Martoriati, L. Castéra, C. Cuevas, P. Formstecher, B. Joseph, G. Kroemer, C. Bailly, and P. Marchetti, *Oncogene*, 2008, **27**, 1981.
99. C. Ballot, J. Kluza, A. Martoriati, U. Nyman, P. Formstecher, B. Joseph, C. Bailly, and P. Marchetti, *Mol. Cancer Ther.*, 2009, **8**, 3307.
100. C. Ballot, J. Kluza, S. Lancel, A. Martoriati, S. M. Hassoun, L. Mortier, J.-C. Vienne, G. Briand, P. Formstecher, C. Bailly, R. Nevière, and P. Marchetti, *Apoptosis*, 2010, **15**, 769.
101. D. Baunbæk, N. Trinkler, Y. Ferandin, O. Lozach, P. Ploypradith, S. Ruchirawat, F. Ishibashi, M. Iwao, and L. Meijer, *Mar. Drugs*, 2008, **6**, 514.

102. T.-C. Chou, Y. Guan, D. R. Soenen, S. J. Danishefsky, and D. L. Boger, *Cancer Chemother. Pharmacol.*, 2005, **56**, 379.

**Tsutomu Fukuda** received his B.Sc. (1993) and M.Sc. (1995) degrees from Kyushu University. Since 1997, he has been Assistant Professor in Nagasaki University and worked with Prof. Masatomo Iwao. In 1998, he received his Ph.D. degree from Kyushu University under the direction of Professor Tsutomu Katsuki. His recent research interests include development of new synthetic methodologies using organolithium compounds, synthesis of natural products and computational chemistry.

**Fumito Ishibashi** received his B.Sc. (1981), M.Sc. (1984) and Ph.D. (1987) degrees from Kyushu University. After two years postdoctoral work (1988-1989) with Professor Eiichi Taniguchi at the same university, he obtained first academic position at Ehime University in 1989. In 1992, he moved to Nagasaki University where he has been a professor since 2007. He also served as a visiting professor or scholar at the Department of Botany, University of British Columbia (1991) and Scripps Institution of Oceanography, University of California at San Diego (2003). His current research interests include synthesis and isolation of bioactive marine natural products.

**Masatomo Iwao** was born in Oita, Japan in 1952. He obtained his B.Sc. (1974) and Ph.D. (1982) from Kyushu University. He was appointed as Assistant Professor at Faculty of Liberal Arts, Nagasaki University in 1975 and worked with the late Prof. Tsukasa Kuraishi in the field of heterocyclic chemistry. He promoted to Associate Professor in 1983 and then to Professor in 1990. During these periods, he also worked with the late Prof. Raymond N. Castle at Brigham Young University (1979-1980) and Prof. Victor Snieckus at the University of Waterloo (1980-1981). Since 1997, he has been Professor at Department of Applied Chemistry, Faculty of Engineering, Nagasaki University. His research interests include organolithium chemistry, synthesis of natural products, and development of natural product-derived chemotherapeutic agents.