Chapter 1

Introduction

1-1. Introduction

Figure 1-1. Structures of target compounds.

In contemporary medical and pharmaceutical field, an antibiotic substance of medical importance is often firstly isolated from a microorganism. There ought to exist a number of related compounds with similar effects, which may be more or less potent, some may perhaps have
undesirable secondary effects. It is by no means, or probable, that the compound produced by the microorganism likely as a weapon to struggle for existence from the medical point of view. If it is possible to synthesize the compound, it will also possible to modify the details of the structure and to find the most effective remedies.

Consequently, it is no doubt that both exploration and discovery of new chemistry and chemical biology will be facilitated by developments of total syntheses because not only the synthetic compounds will be more easily accessible than the natural products, but also numerous analogues of the natural compounds can be synthesized by organic synthesis to find those with higher potencies than the naturally occurring substances. In this doctoral thesis, (shown in Figure 1-1) the author will disclose the total syntheses of antitumor compound fostriecin (CI–920) (1), novel antifungus and antitumor compound phospholin2 (2) (phoslactomycin B),3 the deamino precursor of phspholine (3), novel anticancer compounds macrosphelide H (4)4,5,6 and G (5),4,7,8 and novel antibiotic compound alaremycin (6)9,38 by using unique and efficient methodologies as described below in brief.

1-2. **Formal Total Synthesis of Fostriecin**

In Chapter 2, a formal total synthesis of fostriecin is addressed in detail. Fostriecin (CI–920) is a structurally novel phosphate ester produced by *Streptomyces pulveraceus* that is active in vivo against leukemia (L1210, IC50 0.46 µM), lung cancer, breast cancer and ovarian cancer.10 The phase I trial of fostriecin has been conducted by National Cancer Institute. However, because of the problem of purity and stability, the trial was halted, in spite of its potential.11 Fostriecin is known to be a weak topoisomerase II inhibitor via a novel, non-DNA-strand cleavage mechanism, which is quite different from that of classic topoisomerase II inhibitors such as anthracyclines and
podophyllotoxins, and is also known to be a potent and highly selective protein phosphatase inhibitor against PP2A and PP4. In spite of these attractive aspect of fostriecin, it is quite recently that the relative and absolute configurations of fostriecin were established by a series of elegant spectroscopic and degradative studies.

The first total synthesis was achieved by Boger and co-workers in 2001. Subsequently, Jacobsen’s group, Falck’s group, Imanishi and co-workers, and Hatakeyama’s group also reported the total synthesis of fostriecin. Several groups also published synthetic work toward fostriecin. The author investigated another efficient and readily manipulative approach by utilization of chelation-controlled nucleophilic addition reaction of the vinyl anion to α-alkoxyl ketone to construct the C1-C13 key intermediate (Figure 1-2). This flexible strategy is also adaptable to synthesize the structurally related compounds. As outlined in Scheme 1-1, magnesium anion 8 arised from the corresponding vinyl iodide was subjected to α-alkoxyl ketone 9 at −78 °C in THF. With the highly efficient and reproducible protocol for chelation-controlled addition, the C7-C8 bond was created and the stereochemistry at these centers were set with > 50 : 1 diastereoselectivity in excellent yield. The addition product of alcohol 10 possesses the full set of the chiral centers of fostriecin. Alcohol 10 was readily transformed into the unsaturated ester 11, which was subjected to ring-closing metathesis to generate lactone 12.
Scheme 1–1. The strategy of formal total synthesis of fostriecin.

Subsequently, a series of standard transformations of iodination, deprotection and diimide reduction were carried out successively to afford the known C1-C13 key intermediate (7) successfully.\textsuperscript{20} This intermediate have previously been synthesized by the Jacobsen’s and Imanishi’s groups,\textsuperscript{15,17} and was converted into fostriecin.

1-3. Synthetic Study of Phospholine

In Chapter 3, a synthetic study for the synthesis of phospholine was described.

Phospholine (phoslactomycin B) was firstly isolated from the culture broth of \textit{Streptomyces hygroscopius} subsp. \textit{luteolus} subsp. nov. by Seto’s group\textsuperscript{2} and Yamanouchi Pharmaceutical Co. Ltd.\textsuperscript{2} independently in 1989.
Although the exact mechanism of action remains unknown, studies demonstrate that phospholine exhibits antifungal activity. More importantly, similar to fostriecin, phospholine is potent and highly selective inhibitor of PP2A (IC50 values ranging from 3.7 to 5.8 µm) as compared with PP1 (IC50 > 1 µM). Most recently, the PP2A inhibition activity of

![Figure 1-3. Structure of fostriecin, phospholine and phoslactomycins.](image)

phospholine has been shown to inhibit tumor metastasis through augmentation of natural killer cells.\textsuperscript{2,3} The relative and absolute stereochemistries of phospholine (phoslactomycins) was secured via a combination of NMR spectroscopy and mass spectrometry.
The principal differences of phospholine from fostriecin (Figure 1-3) are replacement of the C-4 unsubstituted lactone by ethyl, C-8 methyl substituent by ethylamine and the terminal allylic alcohol by a cyclohexane ring. The combination of the outstanding antibiotic activity, architectural complexity, and extreme scarcity led to a strong interest to the author. The author decided to embark on the challenge for the total synthesis of phospholine based on the previously synthetic methodology of fostriecin.

1-3-1. The First Synthetic Study of Phospholín

Scheme 1-2. The first approach toward phospholine.
As outlined in Scheme 1-2 above, the author firstly envisaged a convergent strategy by linking the three fragments 13, 14, and 15 to assemble the complete carbon skeleton of phospholine. The key points include the assembly of the left hand vinyl iodide fragment 13 with the C4–C5 stereogenic centers via a [2,3]-Wittig rearrangement of ether 16, which sets the stereochemistry at these centers with > 99% diastereoselectivity.\(^{21}\) The middle ketone linchpin 14 was synthesized via the strategy similar to that of fostriecin.\(^{18}\) Then the union of the two fragments 13 and 14 by chelation-controlled nucleophilic addition furnished the pivotal intermediate 17. Unfortunately, there was appeared an unpredicted obstacle, all attempts to construct the \(\alpha,\beta\)-unsaturated lactone part 18, derived from 17, via metathesis with Grubbs first generation catalyst trying various solvent and temperatures proved unsuccessful presumably due to the bulky ethyl group at C4. Utilization of the more powerful the second generation Grubbs catalyst was also unsuccessful. Because of the unexpected frustration of this strategy, it prompted the author to seek another alternative tactic to resolve the problem.

1-3-2. The Second Synthetic Study of Phospholine

Because of the failure of construction of the lactone part by using Grubbs’ metathesis, the author had to redesign the strategy to entail a novel protocol for creating C4 and C5 stereocenters by Evans asymmetric aldol reaction. As shown in Scheme 1-3 below, after addition of \(\text{CH}_2=\text{CHMgBr}\) to ketone 14, the main carbon skeleton was extended by Evans asymmetric aldol reaction and Horner-Wadsworth-Emmons reaction.
Subsequently, the lactone was constructed by a Ti(OiPr)_4-mediated lactonization, and the key C1–C13 vinyl iodide fragment 23 was furnished via the standard methodology as the same as that of fostriecin. The left side vinyl stannane 15 was readily prepared from the commercially available cyclohexanecarboxaldehyde via olefination. The linking of the two fragments 23 and 15 by Stille coupling reaction was successful to provide the pivotal intermediate 24 with the complete carbon skeleton of phospholine. Functional group manipulations furnished the key

**Scheme 1-3.** The second approach toward phospholine.
intermediate 25 with the phosphate and amine functional groups. Unfortunately, removal of the TBS group at the C8 tertiary alcohol, under various conditions, such as HF, HFPyr, HCl, etc., led to decomposition or recovery of the TBS ether. The author realized that the present approach must make a correction of the protective group system.

1-4. The First Total Synthesis of phospholine and Biosynthetic Deamino Precursor Thereof

In Chapter 4, the first total synthesis of phospholine and the biosynthetic deamino precursor of phospholine were addressed in detail. The success is on the basis of the author’s previous study results with modification.

1-4-1. The First Total Synthesis of phospholine

In order to correct the deficiencies, the author reenvisioned the synthetic approach for the phospholine including the protective system, the construction of the Z,Z-diene part, and the introduction of amine functional group which were suffered from low yield or difficult purification.

As illustrated in the Scheme 1-4, the Z,Z-diene part was synthesized via zinc mediated reduction\(^2\) of the enyen precursor with perfect stereoselectivity in excellent yield instead of the previous Stille coupling reaction, because in the previous strategy the C1-C13 Z-vinyl iodide fragment was unreproducible. The primary alcohol was masked with
TBDPS, instead of TBS, and the tertiary alcohol was protected with TES, which was transformed to TMS in the last stage, instead of TBS. On the other hand, the amino functional group was introduced directly by Mitsunobu reaction instead of the previous stepwise strategy, which had suffered from very low yield, because of side reaction.

According to the novel synthetic strategy, the key intermediate 28 was constructed via the addition of $\text{CH}_2=\text{CHMgBr}$ to ketone 27 and subsequent ozonolysis and Horner-Wadsworth-Emmons reaction. Fragment 28 and vinyl iodide 29 was connected via Sonogashira coupling in good yield. Subsequently the remaining right lactone part was efficiently assembled through Evans asymmetric aldol reaction and Horner-Wadsworth-Emmons olefination and a Ti(OiPr)$_4$-mediated lactonization.

**Scheme 1-4.** The strategy for total synthesis of phospholine.
With the key intermediate 31 of the complete carbon skeleton in hand, the author executed the activated zinc reduction to furnish the Z,Z-diene part with excellent stereoselectivity in good yield. Functional group manipulations of triol precursor 31, including introduction of the amino and phosphonate groups, led to 33, which upon deprotection of silyl and allyl groups achieved the first total synthesis of phospholine successfully.

1-4-2. The First Asymmetric Synthesis of Deamino Precursor of Phospholine

Scheme 1-5. The strategy for the asymmetric synthesis of deamino precursor of phospholine.

After the success of the total synthesis of phospholine, the author synthesized deamino analogue of phospholine successfully, which is supposed as the biosynthetic precursor of phospholine. As shown in
Scheme 1-5, functional manipulations of the pivotal common intermediate 32 produced the phosphonate precursor 34 in short steps, and deprotection using the optimized protocol furnished deamino precursor of phospholine, i.e., (3), in good yield.

1-5. The First Total Synthesis of Macrosphelide H and G

![Structures of macrosphelides H and G.](image)

Figure 1–5. Structures of macrosphelides H and G.

In Chapter 5, the first total synthesis of macrosphelides H and G (Figure 1–5) is described. Macrosphelides H and G are members of macrosphelides A–L, which are a family of the 16–membered ring lactone compounds, isolated recently from the culture medium of Microsphaeropsis-sp. FO-5050 and/or periconia byssaides by Omura’s group and Numata’s group. Their absolute stereostructures have been determined through spectroscopic analysis, X-ray analysis and chemical transformations. Macrosphelides have been found to strongly inhibit the adhesion of human-leukemia HL-60 cells to human-umbilical-vein endothelial cells (HUVEC). Consequently, they are attractive in biological and organic synthetic field as target compounds. The author achieved synthesis of macrosphelides H and G successfully through the furan ring oxidation tactic exploited by our laboratory.
As shown in Scheme 1–6, DCC esterification of furyl alcohol 35 and acid 36 followed by deprotection of the THP group afforded alcohol 37. Subsequently, alcohol 37 was condensed with acid 38 to furnish the key intermediate 39 after deprotection. The furan ring oxidation furnished seco acid 40, which was successively subjected to Yamaguchi macrocyclization, Mitsunobu inversion and Wacker oxidation to achieve the total synthesis of macrospelide H (4).

**Scheme 1–6.** The strategy for the synthesis of macrospelide H.

As illustrated in Scheme 1–7, the total synthesis of structurally related macrospelide G (5) was also completed successfully.\(^{4,5}\)
Scheme 1–7. The strategy for synthesis of macrosphelide G.

1-6. Total Synthesis of Alaremycin

In Chapter 6, an efficient strategy for the total synthesis of alaremycin was addressed.

Figure 1-6. The structure of alaremycin, procomarcin, and 5-aminolevulinic acid.

Alaremycin, a novel antibiotic structurally related to 5-aminolevulinic acid, a precursor of heme biosynthesis, was isolated by Prof. Wachi.
Masaaki from the culture broth of actinomycete strain through a random screening with blue assay to detect the formation of anucleate cells in *Escherichia coli*.\textsuperscript{35} The chemical structure of alaremycin was determined by analyses of mass and NMR spectra. While structurally similar primocarcin (43) (Figure 1-6), known as an antitumor,\textsuperscript{36} shows low antibacterial activity, alaremycin does antibacterial effect on *E. coli* cells. Furthermore, the effect is enhanced by 5-aminolevulinic acid (ALA, 44), a precursor of heme biosynthesis, that is known to act as a prooxidant both *in vivo* and *in vitro*.\textsuperscript{37} The structural similarity between compounds 6, 43, and 44 suggests that alaremycin (6) exerts its property through a mechanistic pathway similar to those of 43 and 44. To continue investigation of alaremycin along this line it needed a fairly large quantity of it by organic synthesis. As a result the author established an efficient strategy as shown in Scheme 1-8 to achieve the total synthesis of alaremycin starting with 5-hexenoic acid in 26% overall yields in 8 steps.\textsuperscript{38}

**Scheme 1-8.** The strategy of total synthesis of alaremycin.

The author constructed the key intermediate of enol lactone 46 via Pd-catalyzed cyclization of acid 45 efficiently. Subsequently, bromination of 46, methanolysis and introduction of azide group furnished 47. NaReO\textsubscript{4}/TfOH catalyzed reaction afforded the (α-amino)vinyl ketone which was transformed into the acetylamino-vinyl ketone and followed by hydrolysis of the ester moiety to produce alaremycin (6).
References


