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Efficient Total Synthesis of Novel Bioactive Microbial Metabolites

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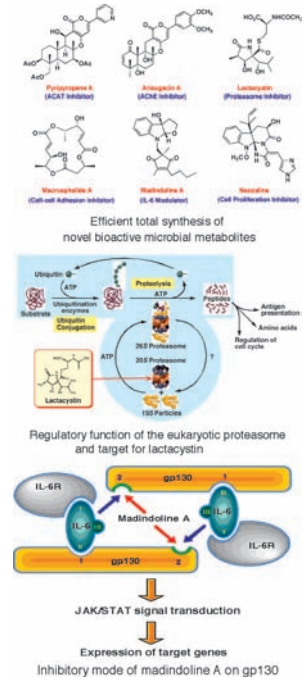

Bioactive natural products produced by microbes have almost limitless potential in pharmaceutical applications, and the organic synthesis of such products as lead compounds will result in the creation of new and widely useful pharmaceutical products. A program of discovery of naturally occurring bioactive microbial metabolites has been ongoing at the Kitasato Institute. We have also developed efficient, rational, and highly flexible production methods for generation of target compounds, synthesis of related compounds, elucidation of their structure−activity relationships, and the possible creation of improved bioactive compounds.

In this Account, the isolation and total synthesis of naturally occurring bioactive microbial metabolites in order to create novel medicines for specific illnesses is described. This covers diseases and conditions such as atherosclerosis, Alzheimer's disease, cancer, inflammation, and osteoporosis, among others, and focuses on six specific compounds.

Pyripyropenes were discovered from *Aspergillus fumigatus* FO-1289 through our screening of microbial metabolites that strongly inhibit acyl-CoA cholesterol acyltransferase (ACAT) in order to develop a new class of cholesterol-lowering agents. These novel polyoxygenated mixed polyketide−terpenoid (meroterpenoid) metabolites contain a fused pyridyl α-pyrone moiety. We carried out the first total synthesis of (+)-pyripyropene A via a flexible, concise, and highly efficient route and also clarified the structure−activity relationships.

Arisugacins were discovered from *Penicillium* sp. FO-4259 by our screening of microbial metabolites that strongly inhibit acetylcholinesterase (AChE) in order to create novel medicines for Alzheimer's disease (AD). Arisugacins are also meroterpenoids. We have achieved the first convergent total synthesis of arisugacins A and B.

Lactacystin was isolated from *Streptomyces* sp. OM-6519 via our screening of microbial metabolites that promote the differentiation of the neuroblastoma cell to further discover new AD medicines. Lactacystin has a novel γ-lactam thioester structure and is also a selective and strong proteasome inhibitor. We have developed a concise approach to synthesize lactacystin designed to afford easy access to the original compound and a variety of analogs.

Macrosphelides were isolated from *Microsphaeropsis* sp. FO-5050 from our screening of microbial metabolites that inhibit the adhesion of HL-60 cells to human umbilical vein endothelial cells (HUVEC). Macrosphelides are the first 16-membered macrotriolides. Macrosphelides prevent cell−cell adhesion by inhibiting the binding of sialyl Lewis X to E-selectin. We have accomplished the first efficient total synthesis of macrosphelides.

Madindolines were isolated from *Streptomyces nitrosporeus* K93-0711 by our program to discover new interleukin 6 (IL-6) modulators. Madindolines are comprised of a 3a-hydroxyfuroindoline ring connected at nitrogen via a methylene bridge to a cyclopentene-1,3-dione ring. We have developed an efficient and practical total synthesis of madindolines. Madindoline A binds to gp130 selectively and inhibits IL-6 activity.

Neoxaline was isolated from *Aspergillus japonicus* Fg-551. Neoxaline is a member of a novel class of biologically active indole alkaloids characterized by a unique indoline spiroaminal framework and binds to tubulin, which results in inhibition of tubulin polymerization. We have developed a concise stereoselective synthesis of the indoline spiroaminal framework of neoxaline.

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Introduction

Bioactive natural products produced by microbes have almost limitless potential in pharmaceutical applications. The identification and subsequent organic synthesis of such products to generate lead compounds for research and development will result in the creation of new, highly practical, and widely used pharmaceutical products. With a focus on the drug discovery process, the Kitasato Institute (KI) is using cutting-edge, unique screening techniques to discover useful bioactive natural products from microbial metabolite origins. These novel products tend to have distinctive structures and attractive bioactivities. Through a comprehensive and dynamic research program, the KI has discovered more than 340 novel bioactive microbial metabolites over the past three decades. Among these, 16 compounds have been developed into commercially important biological reagents, and six compounds are globally used first-line medicines.[1,2]

The key challenge in synthetic organic chemistry remains how to more efficiently synthesize target compounds with unique molecular skeletons using short process pathways. The construction of novel molecular skeletons necessitates the development of new synthetic strategies and key reactions, which, in turn, will expedite progress in synthetic organic chemistry (Figure 1), as well as hastening the development of new drugs.

If efficient synthetic methods can be established, it will be possible to quantitatively supply natural products that initially may only be available in trace amounts. This will consequently facilitate a more thorough elucidation of their bioactivities. In addition, during the synthesis process and research, the relative and absolute configuration and structures of compounds for which only trace amounts can be extracted from the natural source will be identified. More significantly, new molecular skeleton construction methods will allow the creation of a wide range of analogues, thereby leading to the production of compounds with properties that may surpass those found in nature. The discovery and study of bioactive natural products with novel molecular structures will thus also lead to advances in synthetic organic chemistry dynamically related to the elucidation and development of all bioactive materials (Figure 1). Because new natural compounds may only be available from their original sources in trace amounts, the KI has developed efficient, rational, and highly flexible production methods for generation of target compounds. To date, 25 types of bioactive natural products have been successfully synthesized. Our research program also involves the application of established methods to synthesize related compounds, elucidate their structure–activity relationships, and possibly create improved bioactive compounds. In this Account, the isolation and total synthesis of naturally occurring bioactive microbial metabolites in order to create novel medicines for specific illnesses is described. This covers diseases and conditions such as atherosclerosis, Alzheimer's disease, cancer, inflammation, and osteoporosis, among others, and focuses on a few specific compounds: pyripyropenes (cholesterol-lowering agents), arisugacins (acetylcholinesterase inhibitors), lactacystin (proteasome inhibitor), macrosphelides (cell–cell adhesion inhibitors), madindolines (interleukin (IL)-6 modulators), and neoxaline (cell proliferation inhibitor) (Figure 2).

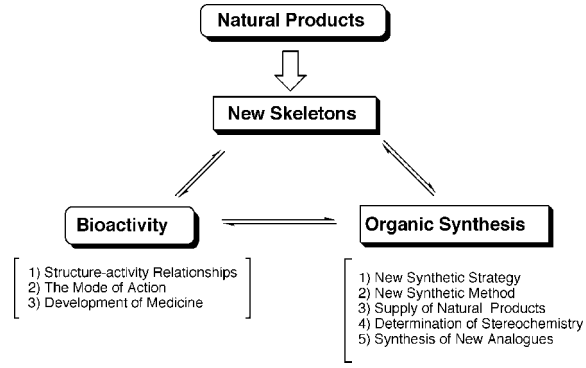


FIGURE 1

Pyripyropenes

A promising, fundamentally new approach to the prevention and treatment of atherosclerosis is based upon inhibition of acyl-CoA cholesterol acyltransferase (ACAT), the enzyme that catalyzes intracellular esterification of cholesterol. This strategy may permit suppression of three distinct ACAT-dependent steps in the pathology of atherosclerosis: absorption of dietary cholesterol in the gut, hepatic synthesis of lipoproteins, and deposition of oily cholesteryl esters within the developing arterial lesions. Therefore, inhibitors of ACAT may be promising new types of antiatherosclerotic agents.[3] In the course of our screening of microbial metabolites that inhibit the activity of ACAT, we isolated potent and selective inhibitors of ACAT, the pyripyropenes A–D (**1**–**4**), from *Aspergillus fumigatus* FO-1289.[4] These novel, polyoxygenated mixed polyketide–terpenoid (meroterpenoid) metabolites contain a fused pyridyl α-pyrone moiety and eight contiguous stereocenters; subsequently, we determined the relative and absolute stereochemistries of **1**, employing nuclear Overhauser effect (NOE) difference and Mosher ester NMR studies in conjunction with X-ray crystallography (Figure 3).[5] The pyripyropenes A–D not only rank as the most effective naturally occurring ACAT inhibitors in vitro, with IC_{50} values of 58, 117,

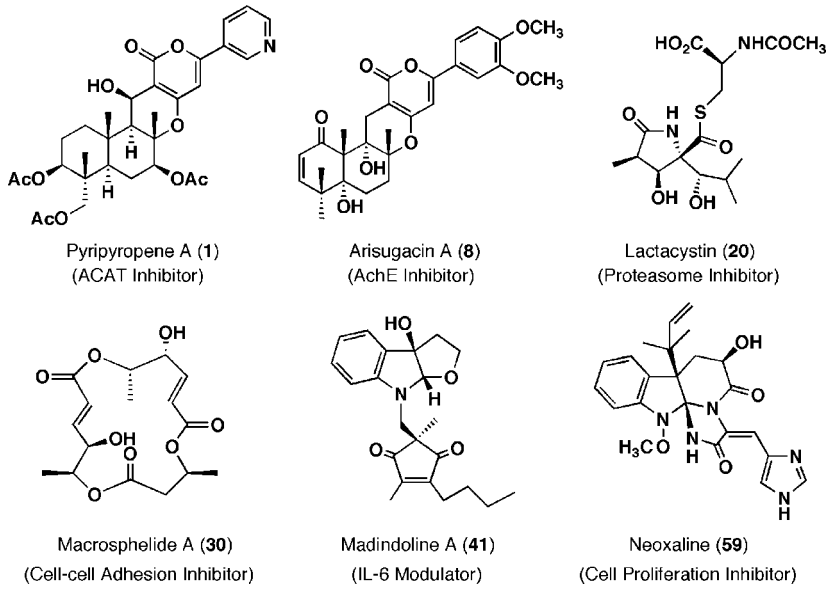


FIGURE 2. Structures of some novel bioactive microbial metabolites.

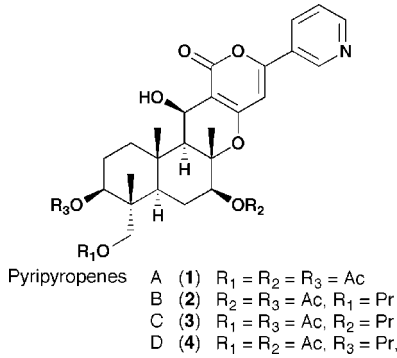
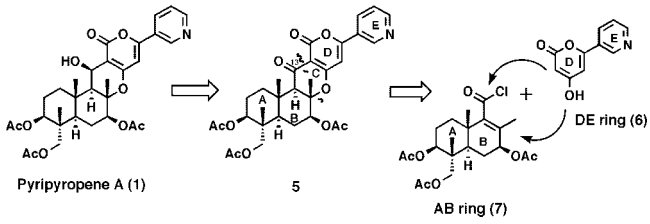


FIGURE 3. Structures of pyripyropenes.

SCHEME 1



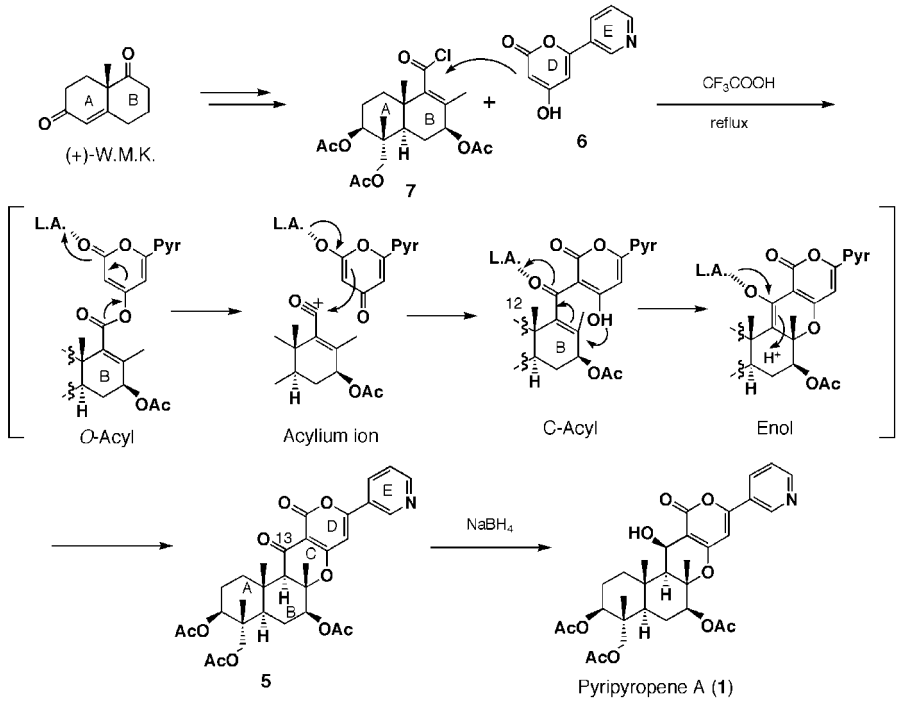
53, and 268 nM, respectively, but also display oral bioavailability in hamsters.

We have carried out the first total synthesis of the most active member of this family, (+)-pyripyropene A, via a flexible, concise, and highly efficient route.[6] From the retrosynthetic perspective (Scheme 1), we envisioned construction of advanced ketone **5** via acylation of the known hydroxy α-pyrone **6** with acid chloride **7**, in the presence of an acid catalyst; isomerization to the *C*-acyl pyrone and ring closure would then deliver **5** with the requisite *anti* geometry at the BC ring fusion (Scheme 1).

The sesquiterpene subunit **7** was derived from (+)-Wieland–Miescher ketone[7] in 11 steps. The crucial sequence was joining hydroxy pyrone **6** with AB subunit **7**, which proceeded readily in trifluoroacetic acid (TFA) (80 °C, 4 h); O-acylation followed by in situ 1,3-acyl migration and 1,4-cyclization formed the pentacyclic ketone **5** in 47% yield for the three steps; the requisite *anti* BC ring junction in **5** derived from conjugate addition and enolate protonation trans to the C(12) angular methyl group. Stereoselective reduction of **5** then furnished synthetic (+)-pyripyropene A (**1**) (Scheme 2). Importantly, this approach is designed to provide flexibility in construction of congeners B–D (**2**–**4**) as well as a range of potentially bioactive analogues.

Modification and structure–activity relationships of ACAT inhibitor pyripyropenes were examined, resulting in over 300 derivatives of pyripyropenes being synthesized.[8] The pyridine ring of **1** was replaced by the benzene ring (**PR-264**), which proved to be 100-fold less active than **1**. This suggests that the pyridine moiety plays a significant role in binding to the enzyme. Some compounds, such as **PR-45**, **PR-86**, and **PR-109**, have shown inhibitions at nanomolar levels (Figure 4). **PR-109** showed the most potent (IC_{50} = 6 nM) in vitro inhibitory activity. **PR-86** also displayed strong ACAT inhibition (IC_{50} = 19 nM). From in vivo experiments using hamsters, **PR-86** (ED_{50} = 10 mg/kg) was found to be approximately 10 times more effective than pyripyropene A (ED_{50} ca. 100 mg/kg) in the inhibition of cholesterol absorption in intestines (Figure 4).[9]

SCHEME 2



Arisugacins

Synthetic inhibitors of acetylcholinesterase (AChE) recently have attracted particular attention since 1-benzyl-4-[(5,6-dimethoxy-1-oxaindan-2-yl)methyl]piperidine (E2020) was approved by the United States Food and Drug Administration (FDA) for the treatment of Alzheimer's disease (AD).[10] During our screening of microbial metabolites that inhibit the activity of AchE to try to create novel medicines for AD, we isolated potent and selective AChE inhibitors, arisugacins A and B (**8** and **9**), from a culture broth of *Penicillium* sp. FO-4259 (Figure 5).[11] We determined the relative stereochemistry of arisugacin A, employing NOE difference NMR studies, and the absolute stereochemistry of **8**, employing Mosher ester NMR studies.[12] The arisugacins A and B not only rank as the most potent naturally occurring AChE inhibitors in vitro, with IC_{50} values of 1 and 26 nM, respectively, but also protect against amnesia induced by treatment with scopolamine in mice.[13] Unfortunately, the original natural source produces a very small quantity of arisugacin A.

We have achieved the first total synthesis of arisugacins A and B via a flexible, concise, and highly effective route.[14] We envisioned the construction of advanced olefin **10** via a Knoevenagel-type reaction of the known 4-hydroxy 2-pyrone **11** with α,β-unsaturated aldehyde **12**, which was derived from α-ionone, in the presence of L-proline; amine elimination of **13** and six-electron electrocyclic ring closure of **14** then delivered **10** with the requisite geometry at the BC ring fusion as a single compound in 61% yield (Scheme 3).

All attempts of epoxidation of 2*H*-pyran **10** failed to produce the desired epoxide, owing to the steric hindrance of the angular methyl groups (β-face) and C1 axial hydroxy group (α-face). We reasoned that inversion of C1 α-alcohol to β-alcohol might lead to formation of the desired epoxide. Oxidation of **10** followed by stereoselective reduction afforded

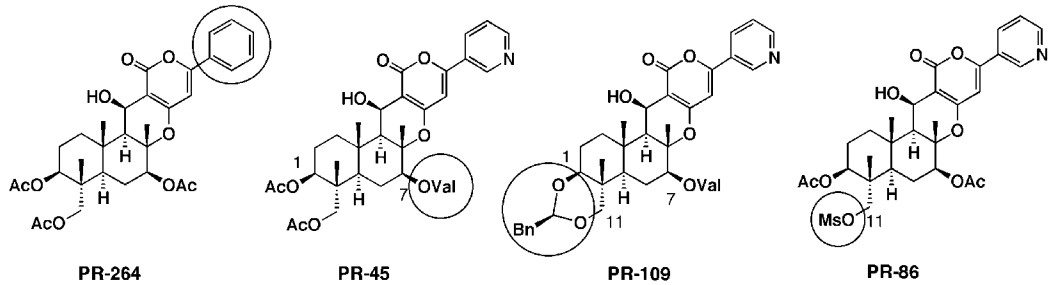


FIGURE 4. Structures of pyripyropene analogues.

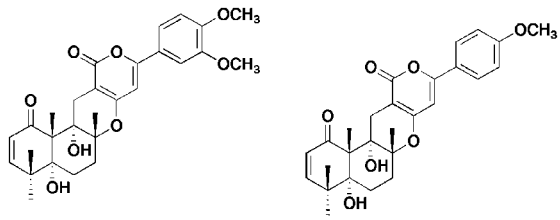


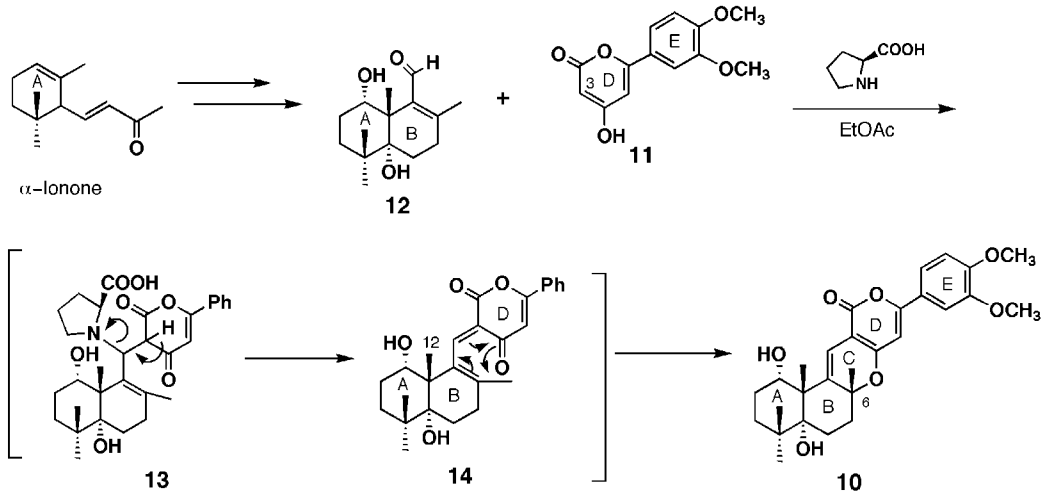
FIGURE 5. Structures of arisugacins.

β-alcohol **15**. Epoxidation of 2*H*-pyran **15** using AcOOH led to the hydroxyester **16** in 41% yield and β-epoxide **17** in 38% yield. The removal of the activated allylic hydroxy group was carried out by Et_3SiH and TFA, followed by hydrolysis to afford **18**. After oxidation to the ketone **19**, phenylselenylation and oxidative elimination furnished (+)-arisugacin A (**8**) (Scheme 4).

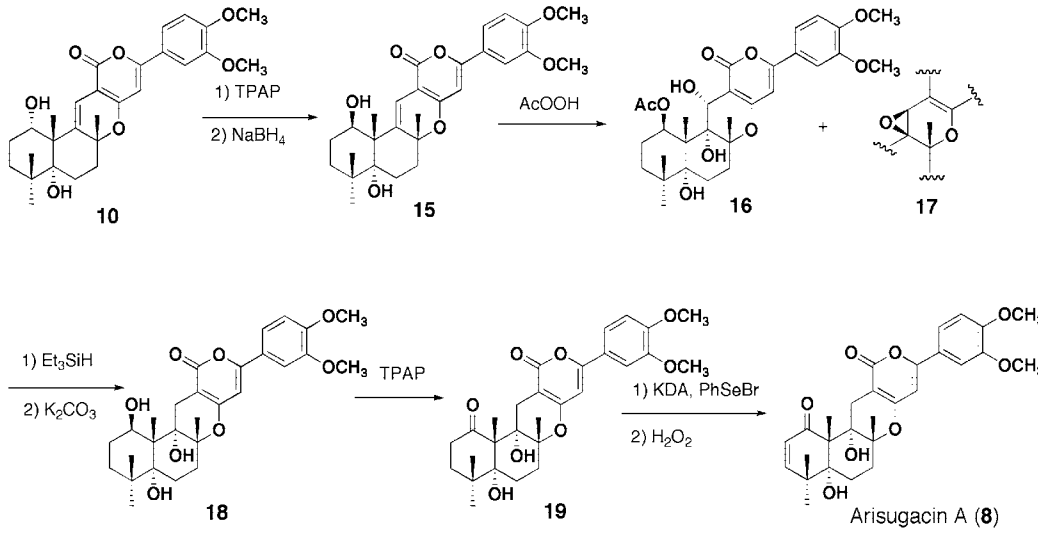
To demonstrate the applicability of our strategy, we prepared the analogue (+)-arisugacin B (**9**) using the 4-methoxy-α-pyrone instead of 3,4-dimethoxy-α-pyrone.

Because we were interested in the structure–activity relationships of arisugacins, we compared the activities of the synthetic intermediates with AChE. We found that only **19** showed activity, and this was 80-fold lower than that for arisugacin A. We determined that 1-keto **10**, lacking both an enone moiety on the A ring and a 12aα-hydroxy group, and **18**, lacking the enone moiety, no longer inhibited AChE. Furthermore, we found that **8** was 25-fold more potent than **9**. Consequently, we suggested that the enone moiety in ring A, the hydroxy group at position 12a, and the E ring substitution play important roles in the inhibition of AChE by arisugacins.[15]

SCHEME 3



SCHEME 4



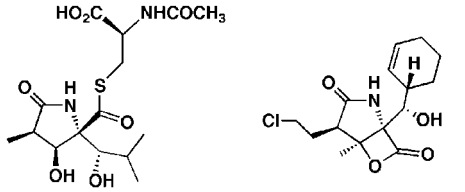


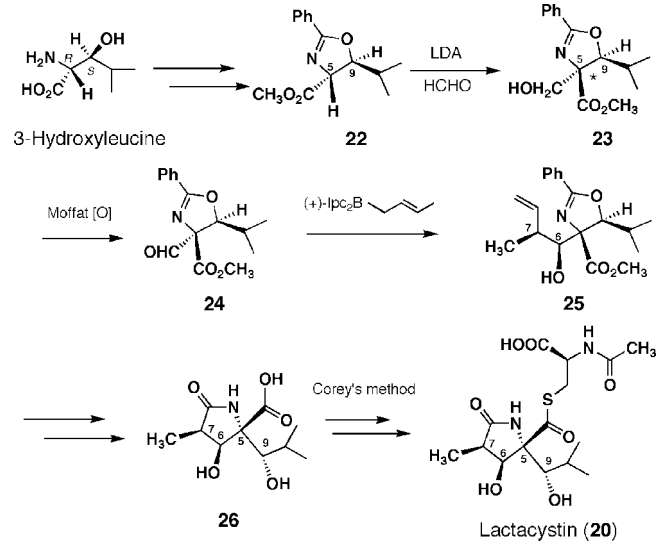
FIGURE 6. Structures of lactacystin and salinosporamide A.

Lactacystin

Neurotrophic factors (NTFs)[16] are proteins essential for the survival and function of nerve cells. Decreased availability of NTFs is thought to cause various nerve disorders including Alzheimer's disease, leading to speculation that NTF-like substances might be therapeutically useful.[17] In the course of our screening of microbial metabolites that promote the differentiation of the mouse neuroblastoma cell line Neuro 2A to possibly develop novel medicines for AD, lactacystin (**20**) was isolated from a culture broth of *Streptomyces* sp. OM-6519, and the novel γ-lactam thioester structure of lactacystin was elucidated via ^{1}H and ^{13}C NMR; single-crystal X-ray analysis subsequently revealed the absolute stereochemistry.[18] Lactacystin induces neuritogenesis with a characteristic parallel array of microtubules and neurofilaments and also causes transient increases in intracellular cAMP levels, as well as acetylcholine (ACh) esterase activity, in Neuro 2A neuroblastoma cells.[19] Its mode of action appears to be inhibition of 20S proteasome peptidase activity via acylation of the amino-terminal threonine.[20] Recently, Fenical reported that salinosporamide A (**21**, similar in structure to lactacystin) was isolated from a marine bacterium, and showed a high cytotoxicity and a stronger proteasome inhibition (Figure 6).[21] The intriguing structures and significant pharmacological potential of these substances have stimulated considerable interest. We have developed a concise approach to synthesize lactacystin, designed to afford easy access to the natural product and a variety of analogues.[22]

As our point of departure, we required **22**, which was derived from (2*R*,3*S*)-3-hydroxyleucine.[23] Aldol condensation of **22** with formaldehyde via the Seebach protocol[24] then gave **23** exclusively (85% yield, >98% de). Oxidation of primary alcohol **23** proved troublesome under a variety of conditions. Fortunately, Moffatt oxidation did provide the requisite aldehyde **24**. Deformylation to oxazoline **22** (*syn*/*anti* mixture) occurred quite readily during extraction and silica gel chromatography, so the aldehyde was isolated via nonaqueous workup and subjected without purification to Brown asymmetric allylboration with (*E*)-crotyl(diisopinocampheyl)borane.[25] This sequence afforded a 4:1 diastereo mixture of the desired

SCHEME 5



homoallylic alcohol **25** in 70% overall yield from **23**. Cleavage of the vinyl group in **25** with ozone, subsequent chlorite oxidation, and catalytic transfer hydrogenation followed by saponification gave γ-lactam acid **26**. To complete the synthesis, we employed the two-step sequence devised by Corey. Following thioesterification of **26** with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl) and *N*-acetyl-L-cysteine allyl ester followed by deallylation provided (+)-lactacystin (**20**) (Scheme 5).

Because the mechanism of action of lactacystin apparently involves amine acylation,[20] we envisioned that related active esters could also induce neuritogenesis. In addition, we have sought to develop analogues with lower cytotoxicity indices in relation to lactacystin (**20**) itself. As expected, synthetic precursor **26** showed no activity (Figure 7). Interestingly, β-lactone **27**, so-called omuralide,[22b] proved to be as active as lactacystin, and analogs **28** and **29** proved to be significantly more potent than **20** in the neurite outgrowth bioassay. Moreover, we discovered that the descarboxy analog **29** displayed a low toxicity. As such, **29** represents a significantly more potent nonprotein neurotrophic agent than lactacystin.[26]

Macrosphelides

Critical early events in inflammation, the allergic response, and tumor metastasis involve interactions between leukocytes and endothelial cells. A variety of cytokinins and related chemical mediators control both leukocyte adhesion and subsequent intercellular invasion by regulating the expression of cellular adhesion molecules. Inhibition of cell−cell adhesion thus holds promise for the treatment of diverse pathologies.[27] In the course of our screening of microbial metabolites that inhibit the adhesion of human leukemia HL-60 cells to human

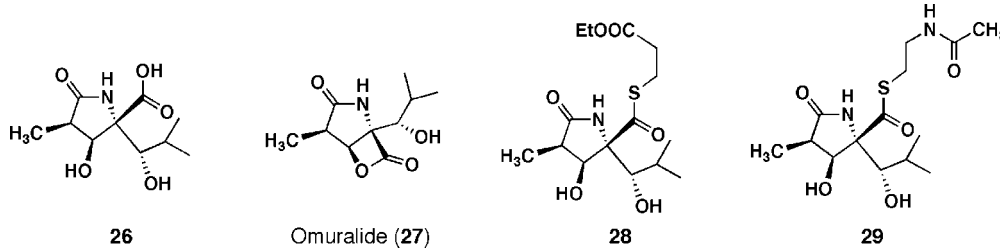


FIGURE 7. Structures of lactacystin analogues.

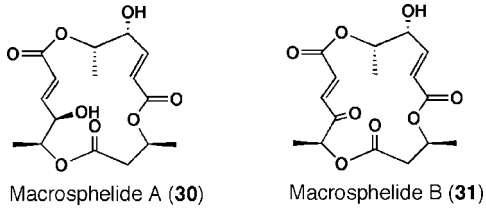


FIGURE 8. Structures of macrosphelides.

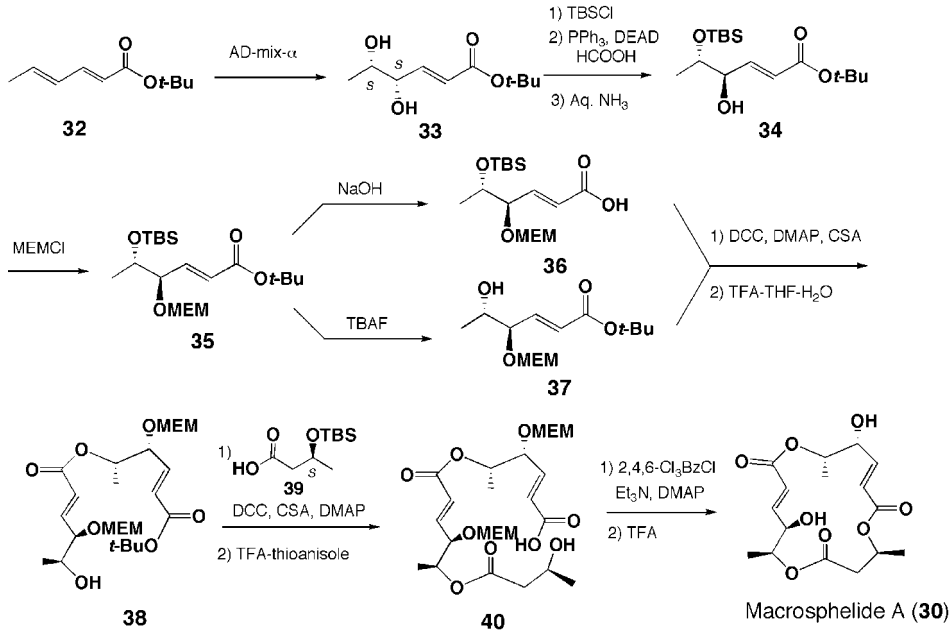
umbilical vein endothelial cells (HUVEC), we discovered macrosphelides A and B (**30** and **31**, Figure 8).[28] These novel macrolides, produced by *Microsphaeropsis* sp. FO-5050, are the first 16-membered ring antibiotics embodying three lactone linkages (i.e., macrotriolides). The macrosphelides strongly inhibit cell adhesion in a dose-dependent fashion (IC_{50} 3.5 and 36 μM, respectively). Preliminary studies suggest that **30** and **31** prevent cell−cell adhesion by inhibiting the binding of sialyl Lewis X to E-selectin. Macrosphelide A also proved to be orally active against lung metastasis of B16/BL6 melanoma in mice (50 mg/kg). We have determined the complete relative and absolute stereochemistries of macrosphelides A and B (**30** and **31**) and have accomplished the first total synthesis of these materials.

Initially, we deduced the connectivity of **30** and **31** via a series of NMR studies and chemical characterization of the derived di- and monoacetates, respectively. Single-crystal X-ray diffraction has now been employed to elucidate the relative stereochemistry of **30** and verify the planar structure (Figure 8). [29] We next have determined the absolute configuration via the Kakisawa−Kashman modification of the Mosher NMR method.[30] To secure the relative and absolute stereochemistries of **31**, we subjected (+)-macrosphelide A (**30**) to PDC oxidation. Semisynthetic **31** proved to be indistinguishable from the natural product. Accordingly, the configurations of (+)-macrosphelides A (**30**) and B (**31**) are (3*S*,8*R*,9*S*,14*R*,15*S*) and (3*S*,8*R*,9*S*,15*S*), respectively. These assignments were confirmed by total synthesis. Our approach to the construction of **30** and **31** entailed the enantioselective preparation of two differentially protected derivatives of *trans*-(4*R*,5*S*)-4,5-dihydroxy-2-hexenoic acid. As our point of departure, we selected the asymmetric dihydroxylation[31] of (*E*,*E*)-hexa-2,4-dienoic acid *tert*-butyl ester (**32**), which afforded the (4*S*,5*S*)-diol **33** (Scheme 6). Selective monosilylation of **33** followed by Mitsunobu inversion at C(4) furnished **34**. After protection of **34** as the MEM ether **35**, saponification gave **36**, whereas desilylation generated the second building block **37**. Condensation of carboxylic acid **36** and alcohol **37** via the Keck modification of the Steglich protocol[32] and desilylation of the resultant ester produced **38**. The third fragment, TBS ether **39**, was prepared from (3*S*)-3-hydroxybutyric acid and coupled with **38** followed by removal of the silyl and *tert*-butyl moieties providing seco acid **40**, which smoothly underwent Yamaguchi macrolactonization.[33] Finally, deprotection gave synthetic **30**. In summary, a highly convergent, stereocontrolled first total synthesis of (+)-macrosphelide A (**30**) has been achieved in 11 steps from sorbic acid ester with a 20% overall yield (corresponding to an 88% average yield per step). Studies on the mode of action and the structure−activity relationships of macrosphelides are currently underway.

Madindolines

Interleukin 6 (IL-6)[34] is a multifunctional cytokine involved in the regulation of differentiation and antibody production. In addition, uncontrolled IL-6 activity plays a central role in a variety of serious diseases, including cancer cachexia, Castleman's disease, rheumatoid arthritis, hypercalcemia, and multiple myeloma. Because no effective therapeutic agents for these diseases have been developed, a low molecular weight compound that modulates the function of IL-6 has been sought.[35] In our program to discover new IL-6 modulators, we have isolated madindolines A and B (**41** and **42**), comprised of a 3a-hydroxyfuroindoline ring connected at nitrogen via a methylene bridge to a cyclopentene-1,3-dione ring from *Streptomyces nitrosporeus* K93-0711 (Figure 9).[36] Structural assignments were based on NMR studies; the relative and absolute configurations, however, remained undefined. They were stereoisomers at the C-2′ position. Bioassays revealed potent, selective inhibition of IL-6 activity in the IL-6-dependent cell line MH60; importantly, the response was dose-dependent. In addition, madindoline A (**41**), the more potent congener, inhibited the differentiation of osteoblast cells. Preliminary

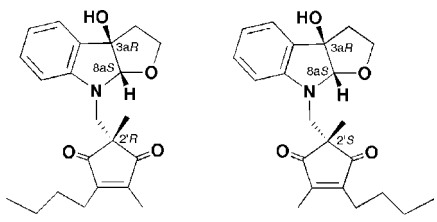
SCHEME 6



studies suggest that **41** interacts with the IL-6 receptor. Unfortunately, the original source no longer produces these antibiotics.

Intrigued by the novel architecture, the significant IL-6 inhibitory activity, and the scarcity of these natural products, we developed the first total synthesis and assignment of the relative and absolute configurations of madindolines A (**41**) and B (**42**).[37] As a prelude to total synthesis, we devised an efficient asymmetric synthesis of the 3a-hydroxyfuroindoline ring system (**44**) from tryptophol (**43**) employing a Sharpless asymmetric epoxidation protocol (Scheme 7).

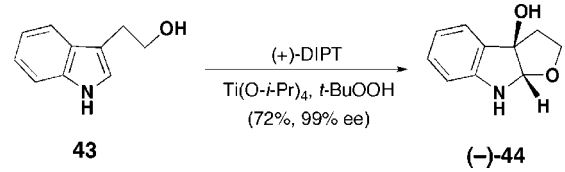
Next, the aldol reaction of α-hydroxyester **45** with methacrolein furnished a mixture of diols **46**. The mixture of diols **46** was subjected directly to metathesis[38] to obtain **47**. Protection and oxidation of **47**, conjugate addition, followed by phenylselenylation of the derived enolate, and oxidative elimination furnished a mixture comprised of **48** and the exomethylene isomer (1:1). Treatment of the mixture with $RhCl_3$[39] converted the exo congener to **48**. Stereoselective reduction of **48**, silylation, and reduction followed by Dess–Martin oxidation furnished **49**. However, all attempts to achieve reductive coupling of the derived aldehyde **49** with tryptophol (**43**) proved unsuccessful. Formation of the intermediate imine appeared to be the problem, presumably due to the poor nucleophilicity of the indole nitrogen. With the more nucleophilic indoline **50**, reductive alkylation furnished **51** in high yield as a diastereomeric mixture. Having achieved the union of **51** with indoline, all that remained to arrive at the madindolines was generation of the enedione and indole moieties and elaboration of the 3a-hydroxyfuroindoline ring. To this end, removal of the silyl groups in **51**, followed in turn by selective silylation of the primary hydroxyl, oxidation, and acid hydrolysis afforded indole **52**. Oxidative ring closure of **52** then yielded (+)-madindoline A (**41**) and (−)-madindoline B (**42**) (2.2:1). (Scheme 8).



Madindoline A (**41**) Madindoline B (**42**)

FIGURE 9. Structures of madindolines.

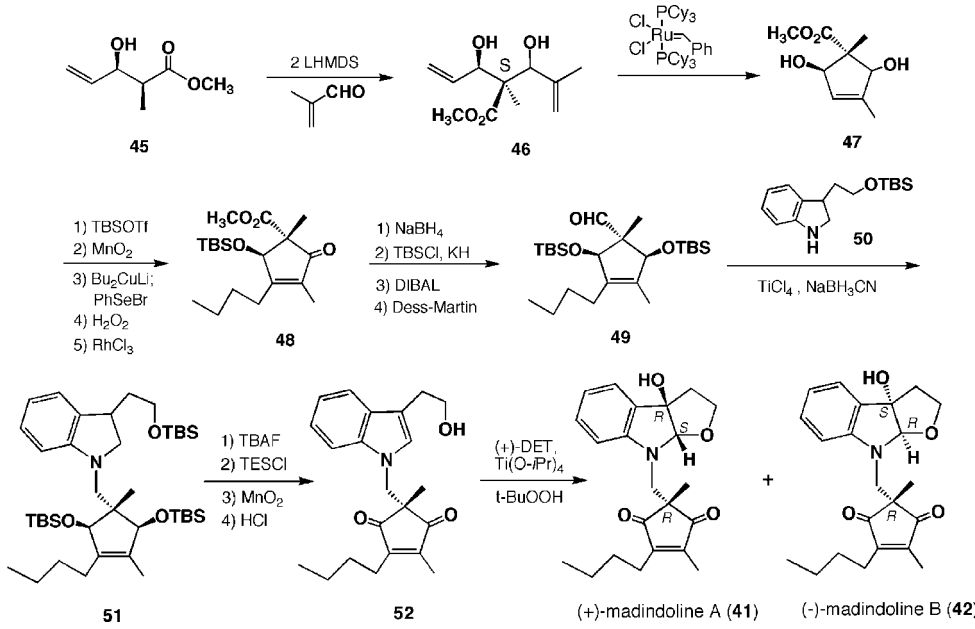
SCHEME 7



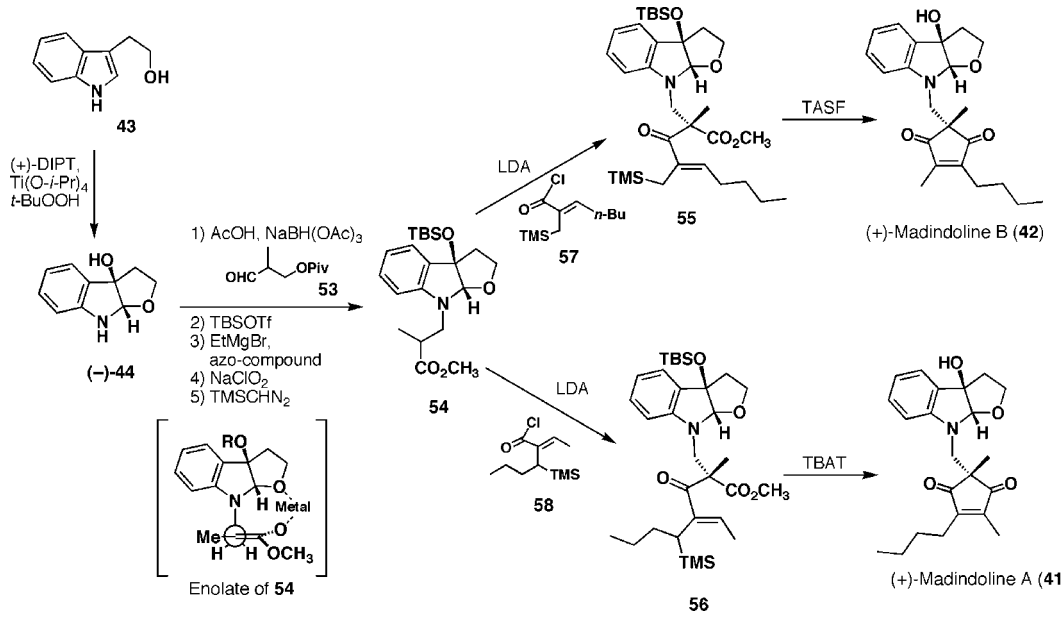
43 **(−)-44**

Our (−)-madindoline B (**42**) is the enantiomer of natural (+)-**42**. Confirmation of the relative configurations in **41** and **42** was achieved by X-ray analysis of synthetic (+)-**41**. Therefore, the absolute configuration of natural (+)-madindoline A is 3a*R*,8a*S*,2′*R* and (+)-madindoline B is 3a*R*,8a*S*,2′*S*.

SCHEME 8



SCHEME 9



Next, we developed a more efficient and practical total synthesis of (+)-madindolines A ((+)-**41**) and B ((+)-**42**).[37] Reductive amination of 3a-hydroxyfuroindoline **44** with aldehyde **53** using acetic acid, followed by iminium reduction, silylation, hydrolysis, and oxidation followed by esterification furnished the methyl ester **54**. The final stages of the synthesis involved diastereoselective acylation of ester **54** carrying a 3a-hydroxyfuroindoline moiety with the acid chloride **57**. We predicted that the lithium enolate of compound **54** would coordinate with oxygen of the furan ring on the chiral 3a-hydroxyfuroindoline to make a rigid conformation and that diastereoselective acylation would occur, producing **55** (Scheme 9). Finally, an intramolecular endo cyclization of allylsilane **55** using tris(dimethylamino)sulfur (trimethylsilyl)difluoride (TASF) directly led to (+)-madindoline B, (+)-**42**. In the total synthesis of (+)-madindoline A, (+)-**41**, the stereoselective acylation of **54** with acid chloride **58** predominantly afforded the desired compound **56** in high yield. The intramolecular endo cyclization of allylsilane **56** with tetrabutylammonium triphenyldifluorosilicate (TBAT) directly led to (+)-madindoline A, (+)-**41**. The structure–activity relationships of madindolines are currently underway and will be reported upon in due course.

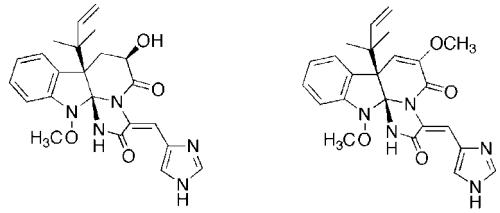


FIGURE 10. Structures of neoxaline and oxaline.

In summary, in the second-generation synthesis, the syntheses of **41** and **42** are highly efficient, proceeding in 16% and 19% overall yield for nine linear steps, respectively, stereocontrolled, and amenable to gram-scale production. We have thus far synthesized 3g of madindoline A.

We also confirmed that synthetic madindoline A (**41**) markedly inhibited osteoclastogenesis in vitro and inhibited bone resorption in ovariectomized (OVX) mice in vivo, and the use of tritiated [^{3}H]-(+)-madindoline A revealed that madindoline A binds to gp130 selectively and inhibits the actions of IL-6. We believe that madindolines can serve as lead compounds for development of new drugs to treat refractory diseases known to involve IL-6.[40]

Neoxaline

During routine chemical screening of microbial metabolites, neoxaline (**59**)[41] was isolated from the culture broth of *Aspergillus japonicus* Fg-551, together with the structurally related known compound, oxaline (**60**). [42] Neoxaline (**59**) and oxaline (**60**) (Figure 10) are members of a novel class of biologically active indole alkaloids characterized by a unique indoline spiroaminal framework and substitution of a 1,1-dimethylallyl ("reverse-prenyl") group at the benzylic ring junction, and these were found to inhibit cell proliferation and arrest the cell cycle during M phase in Jarkat cells. Compounds **59** and **60** bind to tubulin at or near the colchicine binding site, which results in inhibition of tubulin polymerization.[43] The relative stereochemistry of **60** has been previously established by X-ray analysis. Hence, the structure of **59** was determined by comparison with **60**; however, the relative and absolute configurations of **59** remain undefined. The highly complex indoline spiroaminal framework of the neoxalines was recognized as an attractive target for total synthesis. We developed the concise stereoselective synthesis of tetracyclic intermediate, the indoline spiroaminal framework **61** of **59** and **60**.[44] The key step of the stereoselective synthesis of **61** was the Lewis acid mediated transcyclization of **69** to the diaminal **72** and the tungstate-catalyzed oxidation of **72** to obtain the nitrone **73**, which easily cyclizes to the indoline spiroaminal framework **61**.

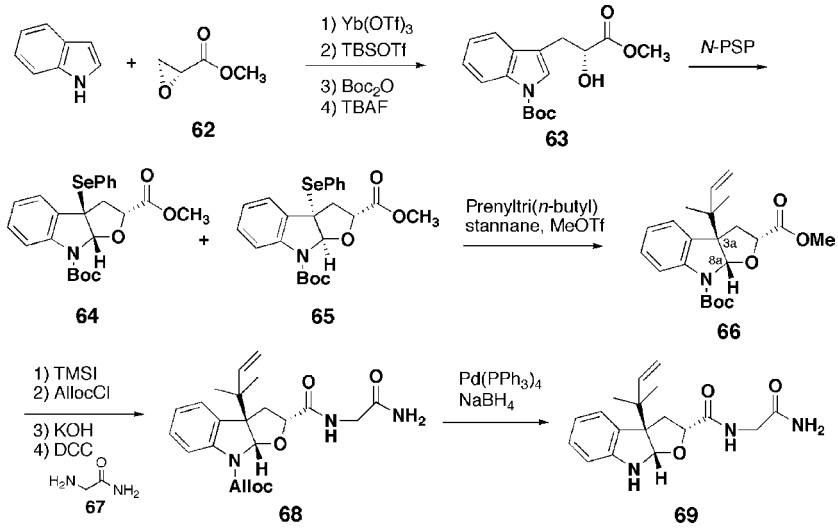
The first step of the synthesis, regioselective alkylation of indole with chiral epoxide **62**, was examined. Initially $SnCl_4$ was used; however this resulted in low yield. Using a $Sc(OTf)_3$ gave a complex mixture; $Cu(OTf)_3$ gave low yield. $Yb(OTf)_3$ proved the most efficient and afforded indole lactic acid ester in high yield. Silylation of the secondary hydroxy group, followed by Boc protection of the amino group and desilylation, afforded the alcohol **63**. Next, selenylation-induced ring closure with *N*-phenylselenophthalimide (*N*-PSP)[45] provided the separable diastereo mixture (1:1) of 3-selenylated furoindolines **64** and **65**. Treatment of **64** with methyl triflate and prenyltri(*n*-butyl)stannane[45] introduced the reverse prenyl group to the desired position to give **66** with either stereochemistry. BOC deprotection of **66**, reprotection with Alloc group, methyl ester hydrolysis, and condensation with glycine amide **67** afforded **68**. Subsequent deprotection of the Alloc group gave **69** in high yield (Scheme 10).

Treatment of aminal **69** with $AlMe_3$ facilitated transcyclization to afford diaminal **72**, through the iminium intermediate **71**, in good yield. Subsequent tungstate-catalyzed oxidation of **72** gave nitrone **73**, which was then treated with silica gel to afford spiroaminal, followed by methylation affording the desired indoline spiroaminal framework **61** (Scheme 11). Compound **61** is a versatile intermediate for the synthesis of the neoxaline family of compounds. We have devised a concise route to the indoline spiroaminal framework of neoxaline and oxaline. Efforts to complete the total syntheses of neoxaline and oxaline using this synthetic approach are currently underway.

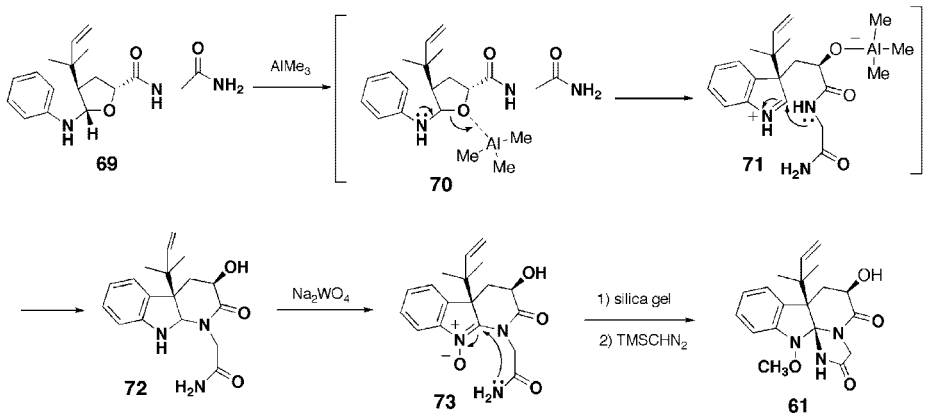
Conclusions

There is an ever-present and increasing global demand for new and effective medicaments to combat intractable, new, and re-emerging diseases, which cause widespread high mortality and morbidity. We must therefore continue to devise expeditious and efficient systems for the discovery and exploitation of natural products and optimally blend them with the application and development of synthetic organic chemistry in order to help produce a sustainable supply of much-needed, novel, potent, and effective drugs and medicines. Natural products isolated or derived from microorganisms frequently embody "privileged structures", which bind to various protein−receptor surfaces.[46] A program of discovery of naturally occurring bioactive microbial metabolites has been ongoing at the Kitasato Institute for many decades. During that time, we have developed a variety of innovative screening systems and have achieved the efficient and concise total synthesis, as well as determined the absolute stereochemistry, of

SCHEME 10



SCHEME 11



most of the recently discovered microbial metabolites, including the pyripyropenes, arisugacins, macrosphelides, madindoline, and neoxaline.

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BIOGRAPHICAL INFORMATION

Toshiaki Sunazuka is Professor of Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences at Kitasato University and Visiting Director at The Kitasato Institute. He was born in Chiba, Japan, in 1959 and received his Ph.D. under the supervision of Professor S. Ōmura at the School of Pharmaceutical Science at Kitasato University (1988). After working as a postdoctoral fellow (1988−1990) at University of Pennsylvania with Professor A. B. Smith, III, he joined The Kitasato Institute as a Senior Researcher. He was appointed as an Assistant Professor, Kitasato University, in 1994 and promoted to Associate Professor in 2002 and Professor in 2005. He received the Progress Award in Synthetic Organic Chemistry, Japan, and Sumiki-Umezawa, Ninomiya, and Morimura Awards.

Tomoyasu Hirose is an Assistant Manager of The Kitasato Institute. He was born in Kanagawa in 1973 and received his B.Sc. (1996) and Ph.D. degrees (2001) from Kitasato University. After working as a postdoctoral fellow (2001−2003) at University of Pennsylvania with Professor Amos B. Smith, III, he joined The Kitasato Institute as a Postdoctoral Fellow in 2003. In 2004, he received the Inoue Research Award for Young Scientists.

Satoshi Ōmura is Professor and President of The Kitasato Institute, Professor of Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University, and Max Tishler Professor of Wesleyan University, Middletown, CT. He was born in Yamanashi Prefecture in 1935 and received his Ph.D.

in Pharmaceutical Sciences from the University of Tokyo in 1968 and in Chemistry from Tokyo University of Science in 1970. He held the post of Visiting Professor under Max Tishler at Wesleyan University before being appointed as Professor in the School of Pharmaceutical Sciences, Kitasato University, in 1975. He has been the President of The Kitasato Intitute since 1990. He is a recipient of the Japan Academy Prize, ACS Nakanishi Prize, ACS Ernest Guenther Award in the Chemistry of Natural Products, and many other awards from home and abroad. He is a member of the Deutsche Akademie der Naturforscher "Leopoldina" (Germany), National Academy of Sciences, U.S.A., the Japan Academy, Institut de France, Academie des Sciences, Russian Academy of Sciences, the European Academy of Sciences, and Chinese Academy of Sciences.

FOOTNOTES

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