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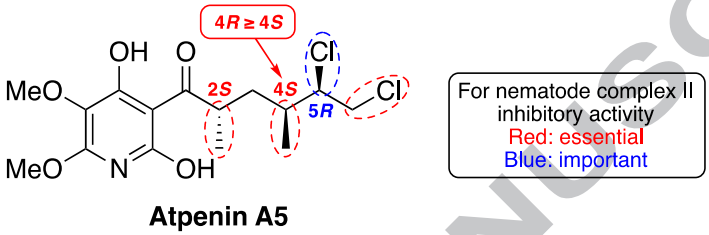

Atpenin A5

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ABSTRACT

We designed and synthesized new atpenin A5 analogs for SAR study. Most of the analogs lacked one or several functional groups in the side chain of atpenin A5, and the stereoisomers proved to be weak nematode complex II inhibitors. However, we determined that 4-*epi*-atpenin A5 was a potent nematode complex II inhibitor comparable to atpenin A5. Therefore, 4-*epi*-atpenin A5 is expected to become a new lead compound in nematicide development.

1. Introduction

The atpenins A5 (**1**), A4 (**2**), and B (**3**), which include a highly functionalized pyridine unit, were first isolated in 1988 from a fermentation broth of the atpenin-producing strain *Penicillium* sp. FO-125. These were used as growth inhibitors of both fatty acid synthase deficient (A-1) and acyl-CoA synthase I deficient (L-7) mutants of *Candida lipolytica*, and **3** was shown to inhibit the ATP-generating system of Raji cells.[1,2] Harzianopyridone (**4**) was originally isolated from *Trichoderma harzianum* in 1989, showing antifungal, antibacterial, and herbicidal activities.[3,4] NBRI23477 B (**5**) was isolated in 2005 from a culture broth of Penicillium atramentosum PF1420 and is used as a growth inhibitor of human prostate cancer cells[5] (Figure 1). The absolute configurations of atpenins A5 (**1**) and A4 (**2**) have been confirmed by X-ray crystallographic analysis.[6,7] The total synthesis of (±)-atpenin B (**3**) was reported by the Quéguiner group in 1994.[8] In 2003, new interest developed in atpenins A5 (**1**) and A4 (**2**) and harzianopyridone (**4**) when the compounds were reported to provide high levels of inhibition in a microbial screening assay against mitochondrial complex II (succinate-ubiquinone oxidoreductase), which is an attractive target for the treatment of helminthiasis.[9] In particular, atpenin A5 (**1**) proved to be much more potent against bovine heart complex II than any known complex II inhibitors, although the inhibition of **1** was non-selective across helminthes and mammals. Crystal structure analysis of *Escherichia coli* complex II co-crystallized with atpenin A5 (**1**) has also been achieved.[6] It is clear that atpenins and their analogs are useful chemical tools for the elucidation of complex II functionality. Moreover, they could act as lead compounds for the development of novel helminth complex II-specific inhibitors. In 2009, we achieved a stereoselective total synthesis of atpenin A5 (**1**)[10] by improving Quéguiner's procedure. Then, we reported the total synthesis of other natural atpenin A5 analogs (A4 (**2**) and B (**3**)), harzianopyridone (**4**), and NBRI23477 B (**5**) using this synthetic strategy; the absolute structures of **3**, **4**, and **5** were also determined.[11] Next, we focused on the structure–activity relationship of atpenin A5 and embarked on the synthesis of new atpenin A5 analogs with chemical modification of the side chain moiety because an inhibitory activity against nematode complex II of the synthesized atpenins A4 (**2**) and B (**3**), lacked one or two chloro groups in the side chain of **1**, reduced greatly (Table 1). It was shown that presence of the side chain is important for the nematode complex II inhibitory activity of **1**. Recently, new findings that differed from our previous results were reported by the Carreira group, in which a new total synthesis of **1** using novel methodology that they developed was achieved and led to the synthesis of new analogs possessing a complex II inhibitory activity similar to that of atpenin A5.[12] Herein, we report structure–activity relationship studies of atpenin A5 analogs with chemical modification of the side chain moiety.

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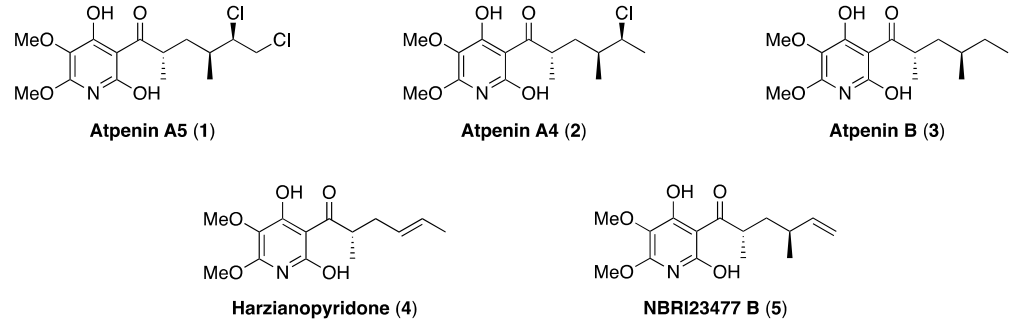
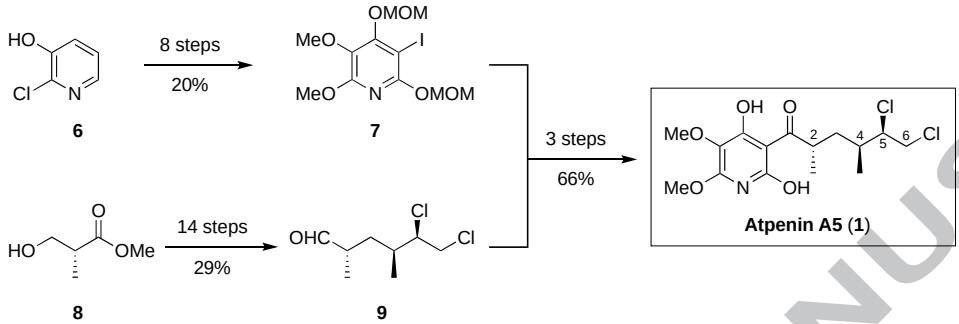


Figure 1 Structures of the natural atpenin family: atpenins A5 (**1**), A4 (**2**), and B (**3**); harzianopyridone (**4**); and NBRI23477B (**5**).



Scheme 1 Total synthesis of atpenin A5 (**1**), reported previously by our group.

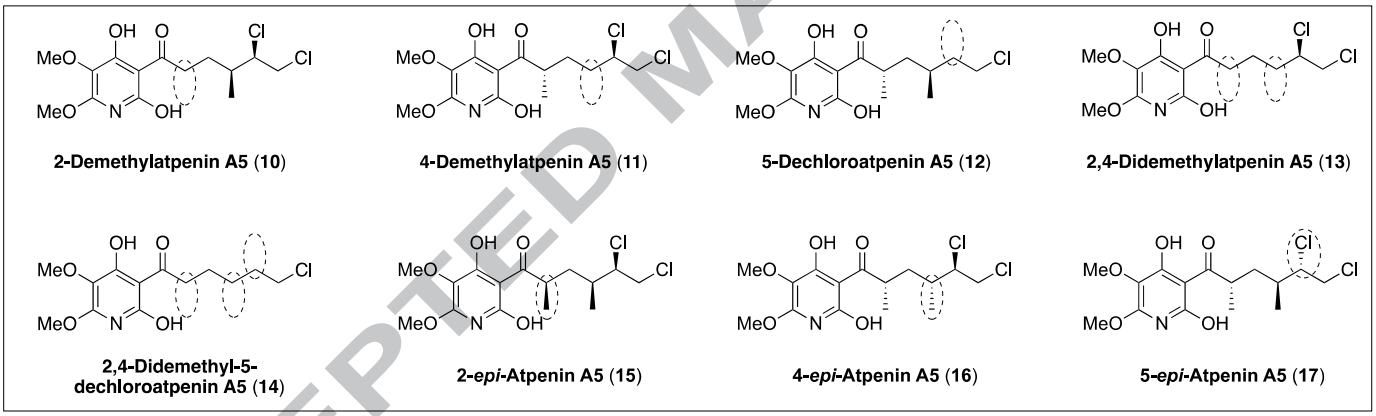
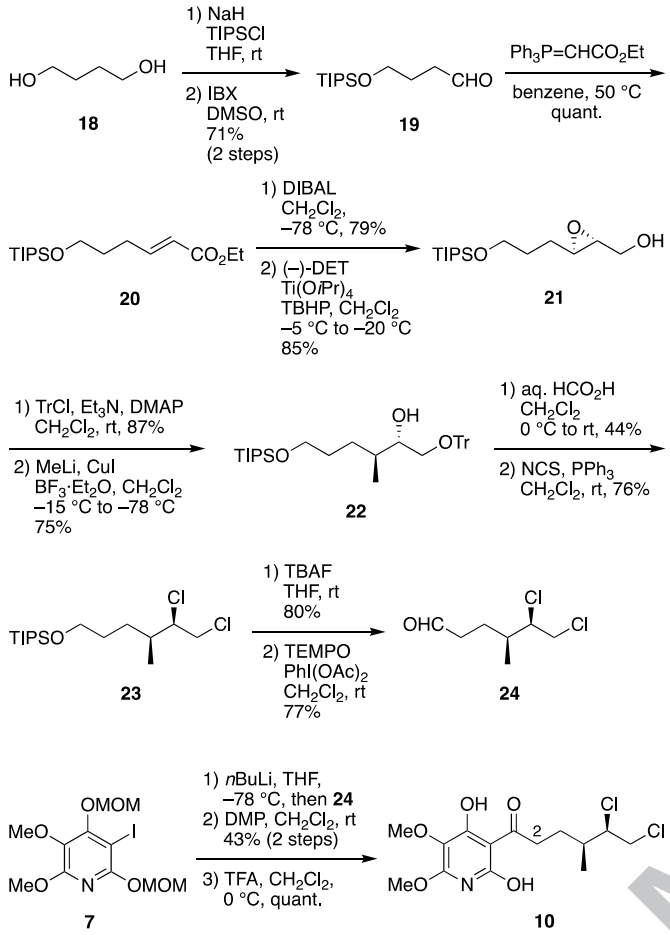


Figure 2 Designed new atpenin A5 analogs **10**–**17**.

2. Results and discussion

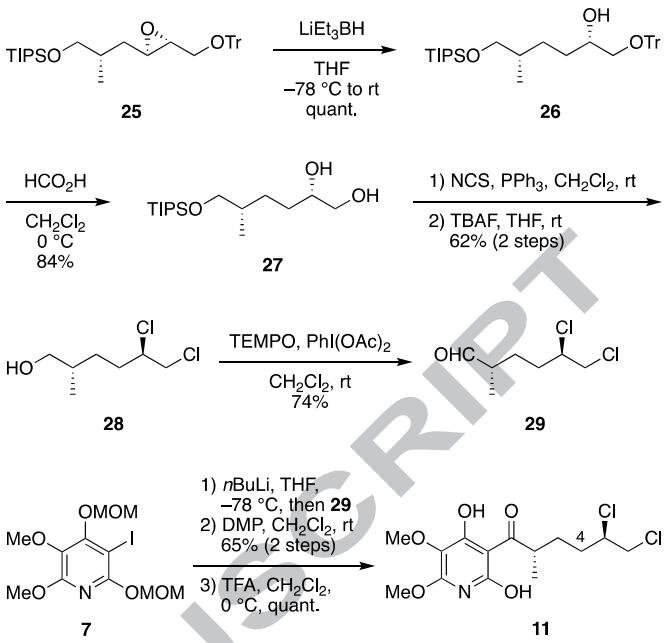
The synthetic strategy for our previous total synthesis of atpenin A5 (**1**)[10] is convergent and amenable to the synthesis of a variety of atpenin A5 analogs. Here, we designed new atpenin A5 analogs **10**–**17** for SAR study (Figure 2). The analogs **10**–**14** lacked one or several functional groups in the side chain of **1**, and the analogs **15**–**17** were stereoisomers of **1**. Initially, synthesis of 2-demethylatpenin A5 (**10**) was investigated (Scheme 2). In our previous total synthesis[10], the 2-methyl group and its stereochemistry were derived from a commercially available chiral Roche ester (**8**). Therefore, the starting material was changed for the synthesis of **10**. 1,4-Butanediol (**18**) was protected as a mono TIPS ether and the resulting alcohol was oxidized with IBX to give aldehyde **19** in 71% over two steps. Wittig olefination of **19** afforded (*E*)-α,β-unsaturated ester **20** quantitatively. Other chemical transformations to afford the desired 2-demethylatpenin A5 (**10**) followed our previous total synthesis. DIBAL reduction of **20** gave the corresponding allyl alcohol in 79% yield, which was subjected to Sharpless asymmetric epoxidation using (–)-DET to give epoxy alcohol **21** stereoselectively in 85% yield. Protection of the hydroxy group as a trityl ether (87% yield) followed by epoxide-opening reaction with Me_2CuLi and $BF_3{\cdot}Et_2O$ afforded **22** in 75% yield. Deprotection of the trityl ether **22** (44% yield) and dichlorination of the resulting diol (76% yield) gave dichloride **23**. Treatment of **23** with TBAF furnished the corresponding alcohol in 80% yield, which was oxidized with TEMPO to give aldehyde **24** in 77% yield. The key coupling reaction of the pyridine unit **7** with the aldehyde **24** followed by Dess–Martin oxidation gave the corresponding ketone in 43% yield over two steps. Finally, MOM deprotection under acidic conditions using TFA afforded 2-demethylatpenin A5 (**10**) quantitatively.



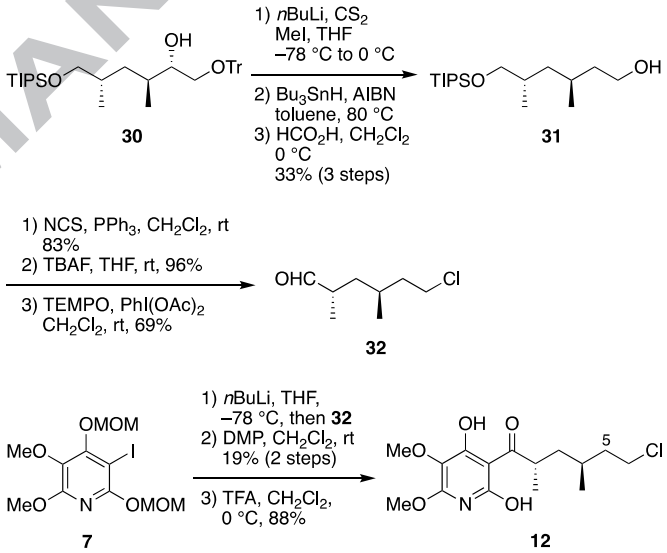
Scheme 2 Synthesis of 2-demethylatpenin A5 (**10**).

Next, 4-demethylatpenin A5 (**11**) was synthesized (Scheme 3). In our previous total synthesis[10], the key intermediate **25** was subjected to a reductive epoxide-opening reaction with $LiEt_3BH$[13] to give the alcohol quantitatively and regioselectively. After deprotection of trityl ether (84% yield), dichlorination of the resulting diol followed by TIPS deprotection afforded alcohol **28** in 62% yield over two steps. The alcohol **28** was converted to aldehyde **29** in 74% yield by treatment with TEMPO. The coupling reaction of the pyridine unit **7** and Dess–Martin oxidation (65% yield over two steps) followed by MOM deprotection under acidic conditions (quant.) afforded 4-demethylatpenin A5 (**11**).

As our next target, 5-dechloroatpenin A5 (**12**) was selected (Scheme 4). In our previous total synthesis[10], the key intermediate **30** was converted to alcohol **31** in 33% yield over three steps via Barton–McCombie deoxygenation and deprotection of the trityl group. Chlorination of **31** (83% yield), deprotection of TIPS ether (96% yield), and TEMPO oxidation (69% yield) afforded aldehyde **32**. The subsequent coupling reaction of the pyridine unit **7** followed by Dess–Martin oxidation afforded the corresponding ketone in 19% yield over two steps. This was followed by MOM deprotection under acidic conditions (88%) to afford 5-dechloroatpenin A5 (**12**).

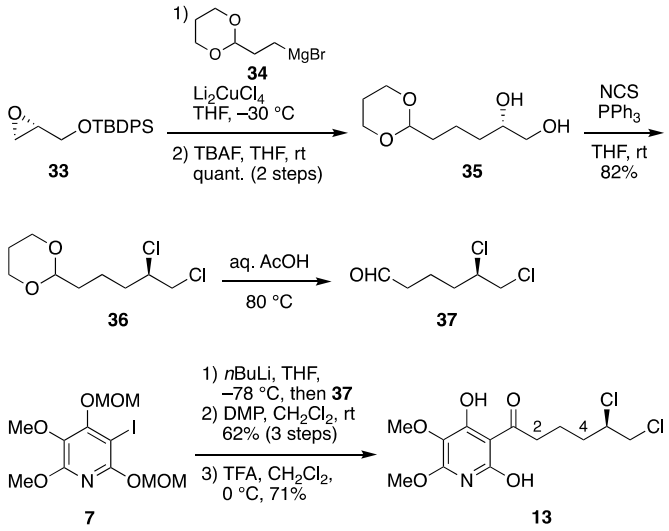


Scheme 3 Synthesis of 4-demethylatpenin A5 (**11**).



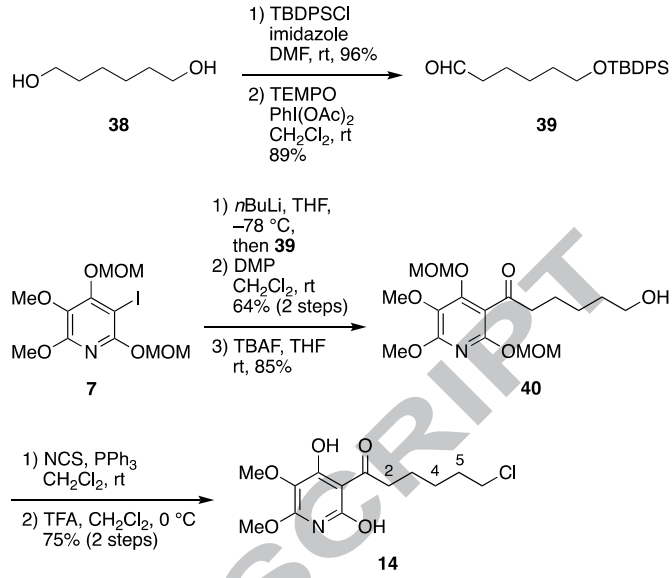
Scheme 4 Synthesis of 5-dechloroatpenin A5 (**12**).

The synthesis of 2,4-didemethylatpenin A5 (**13**) is shown in Scheme 5. The epoxide-opening reaction of a known epoxide **33** with a freshly prepared Grignard reagent **34**[14] in the presence of Li_2CuCl_4[15] followed by deprotection of TBDPS ether with TBAF afforded diol **35** quantitatively and regioselectively. Treatment of **35** with NCS and PPh_3 gave dichloride **36** in 82% yield. Subsequent acidic hydrolysis furnished the corresponding aldehyde. The coupling reaction of the pyridine unit **7** with the aldehyde followed by Dess–Martin oxidation afforded the corresponding ketone in 62% yield over three steps. Final MOM deprotection under acidic conditions gave 2,4-didemethylatpenin A5 (**13**) in 71% yield.



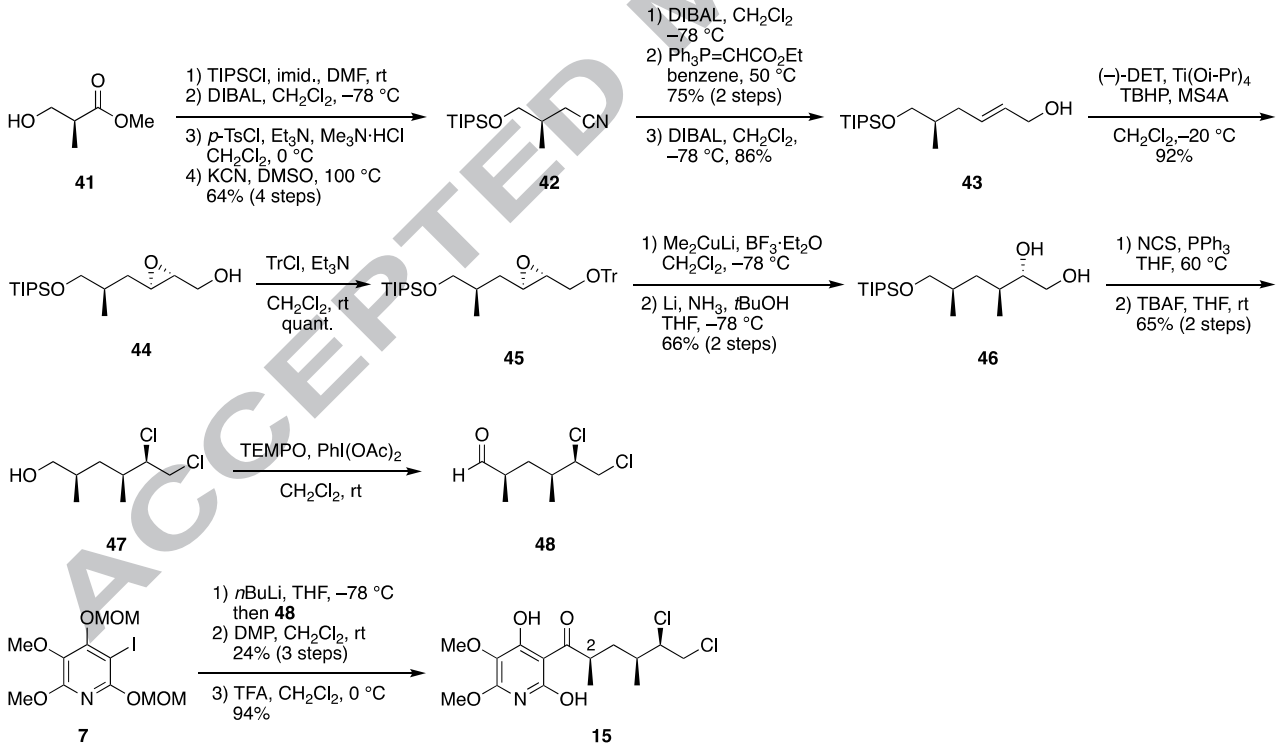
Scheme 5 Synthesis of 2,4-didemethylatpenin A5 (**13**).

The synthesis of 2,4-didemethyl-5-dechloroatpenin A5 (**14**) is shown in Scheme 6. The requisite aldehyde **39**, a coupling partner of **7**, was synthesized from a commercially available diol **38** via mono-TBDPS protection (96%) and TEMPO oxidation (89%). The coupling reaction of the pyridine unit **7** and Dess–Martin oxidation afforded the corresponding ketone in 64% yield over two steps. Subsequent deprotection with TBAF gave ketoalcohol **40** in 85% yield. Finally, chlorination and MOM deprotection gave rise to 2,4-didemethyl-5-dechloroatpenin A5 (**14**) in 75% yield over two steps.

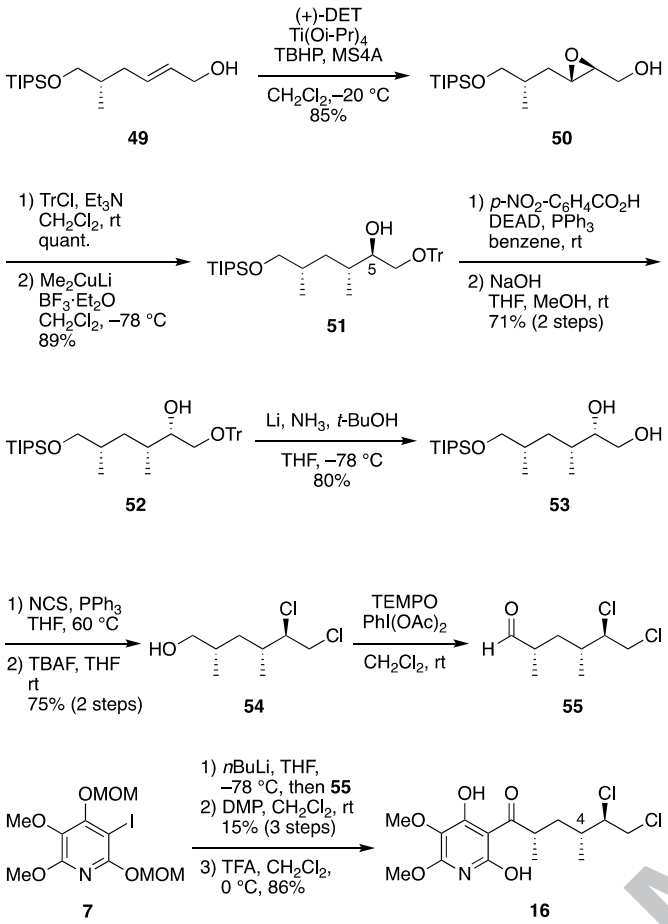


Scheme 6 Synthesis of 2,4-didemethyl-5-dechloroatpenin A5 (**14**).

Next, synthesis of atpenin A5 stereoisomers was investigated. Synthesis of 2-*epi*-atpenin A5 (**15**) was achieved without any difficulty in a manner similar to that used in our previous total synthesis of **1**, in which only the starting material was changed from (–)-Roche ester (**8**) to the antipode **41** (Scheme 7).



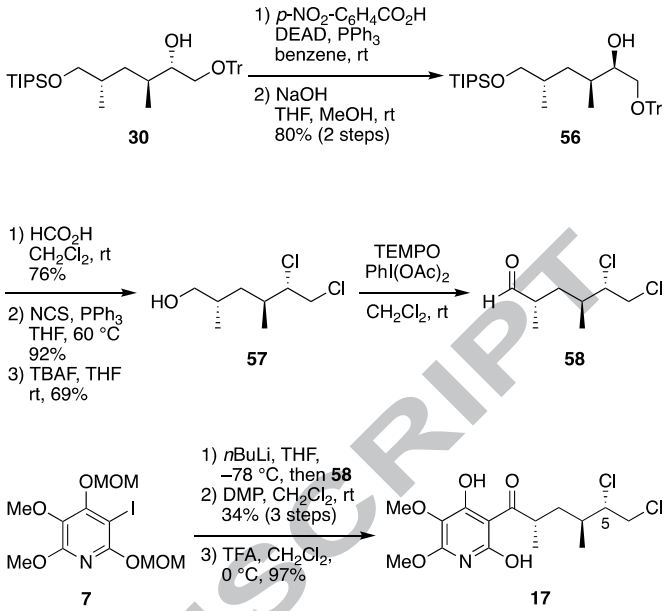
Scheme 7 Synthesis of 2-*epi*-atpenin A5 (**15**).



Scheme 8 Synthesis of 4-*epi*-atpenin A5 (**16**).

Next, synthesis of 4-*epi*-atpenin A5 (**16**) was investigated (Scheme 8). The key intermediate **49** in our previous total synthesis[10] was subjected to Sharpless asymmetric epoxidation using (+)-DET to give epoxide **50** in 85% yield. Protection of **50** as a trityl ether (quant.) and the epoxide-opening reaction (89%) led to alcohol **51**. To introduce the C5-chloro group with correct stereochemistry, steric inversion of the C5-hydroxy group of **51** was required. Therefore, the Mitsunobu reaction of **51**[16] followed by methanolysis of the resulting ester was conducted to give **52** in 71% over two steps. Birch reduction of **52** afforded diol **53** in 80% yield. Chlorination of **53** and deprotection of TIPS ether afforded alcohol **54** in 75% yield over two steps. The coupling reaction of the pyridine unit **7** with aldehyde **55**, which was derived from **54** by TEMPO oxidation, followed by Dess–Martin oxidation afforded the corresponding ketone in 15% yield over three steps. Final deprotection with TBAF gave the 4-*epi*-atpenin A5 (**16**) in 86% yield.

To synthesize 5-*epi*-atpenin A5 (**17**) (the final target for this SAR), Mitsunobu reaction of the key intermediate **30** in our previous total synthesis was conducted (Scheme 9). After hydrolysis of the resulting ester, the desired alcohol **56** was obtained in 80% yield over 2 steps. Deprotection of the trityl ether (76%), dichlorination (92%), and deprotection of the TIPS ether (69%) afforded alcohol **57**. The coupling reaction of the pyridine unit **7** with aldehyde **58**, which was prepared by TEMPO oxidation of **57**, followed by Dess–Martin oxidation furnished the corresponding ketone in 34% yield over three steps. Deprotection with TBAF gave 5-*epi*-atpenin A5 (**17**) in 97% yield.



Scheme 9 Synthesis of 5-*epi*-atpenin A5 (**17**).

Table 1. Inhibitory activity of the atpenin A5 analogs against nematode complex II.[a)]

	IC_{50} (nM) [b)]
Atpenin A5 (1)	14
Atpenin A4 (2)	110
Atpenin B (3)	2100
10	315
11	201
12	48.3
13	607
14	754
15	2000
16	7
17	22.4

[a)]The nematode complex II is derived from *Ascaris suum*. [b)]All inhibitory activities against nematode complex II (IC_{50} values) are the averages of three independent experiments.

The nematode complex II inhibitory activities of the new analogs **10**–**14** (which lacked one or several functional groups in the side chain of **1**), together with the stereoisomers **15**–**17,** are shown in Table 1.[17] Except for **12**, the analogs **10**–**14** significantly reduced the nematode complex II inhibitory activity. This result indicates that each functional group (except for the 5'-chloro group) in the side chain of **1** is important in chemical interaction at the binding site with **1** in the nematode complex II. This result is very different from that of SAR studies of **1** toward mammalian complex II.[18,19] Furthermore, the nematode complex II inhibitory activity of the 2-*epi*-atpenin A5 analog **15** also decreased greatly, whereas those of the 5-dechloroatpenin A5 analog **12** and 5-*epi*-atpenin A5 analog **17** decreased only slightly. Among the new atpenin A5 analogs synthesized here, the 4-*epi*-atpenin A5 analog **16** showed the strongest nematode complex II inhibitory activity, which was comparable to **1**. Therefore, the order of stereochemistry importance of **1** for nematode complex II inhibitory activity was C2 >> C5 > C4; for C2, the stereochemistry of **1** was shown to be particularly important.

In conclusion, we designed and synthesized new atpenin A5 analogs **10**–**17** for SAR study. Most of the synthesized atpenin A5 analogs reduced the nematode complex II inhibitory activity. However, stereoisomer **16** was found to be a potent nematode complex II inhibitor that was comparable to **1**. Therefore, 4-*epi*-atpenin A5 (**16**) is expected to be useful as a new lead compound for the development of nematicides. We have begun to develop a concise and practical synthesis of 4-*epi*-atpenin A5 (**16**) for large scale synthesis. Additional structure–activity relationship studies of **1** and a new synthesis of **16** are in progress and will be reported in due course.

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This work was supported by a Grant-in-Aid for Scientific Research (C) (S.A., KAKENHI No. 17K08370), a Grant-in-Aid for Young Scientists (B) (M.O., KAKENHI No. 21790117 and 23790140), and a Kitasato University research grant for young researchers (M.O.). We also thank Ms. N. Sato and Dr. K. Nagai (Kitasato University) for kindly measuring NMR and MS spectra.

Appendix A. Supplementary Material

Supplementary data for this article can be found online at.

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17. Enzyme assays: The nematode complex II (NADH-fumarate reductase, derived from *Ascaris suum*) activity was measured in assay mixtures containing 50 mM potassium phosphate (pH 7.2), 10 mM β-D-glucose, 20 units of glucose oxidase, 26 units of catalase and 200 μM NADH. The reaction is started by the addition of sodium fumarate (5 mM). The absorbance change at 340 nm (millimolar extinction coefficient of 6.2 for NADH) was followed. See also: ref. 9.
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Highlights

- Structure–activity relationship studies of atpenin A5 analogs
- Synthesis of atpenin A5 analogs with chemical modification of the side chain moiety
- 4'-*epi*-Atpenin A5, a potent nematode complex II inhibitor