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Design, Synthesis, and Biological Evaluations of Aplyronine A—Mycalolide B Hybrid Compound

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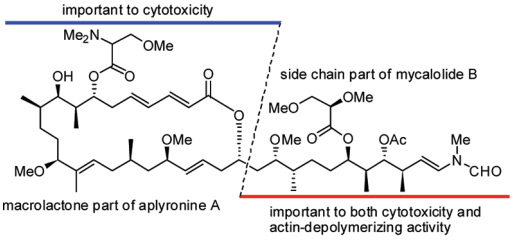
ABSTRACT



aplyronine A–mycalolide B hybrid compound

A hybrid compound consisting of aplyronine A and mycalolide B was synthesized, and its biological activities were evaluated. The hybrid compound was found to have somewhat more potent actin-depolymerizing activity than aplyronine A. In contrast, the hybrid compound possessed about 1000-fold less cytotoxicity than aplyronine A. These results indicated that there is no direct correlation between actin-depolymerizing activity and cytotoxicity.

Actin is a major protein of the cytoskeleton in eukaryotic cells. Recently, various actin-binding macrolides have been isolated from marine sources.[1] Among these compounds, aplyronine A (**1**), a marine macrolide isolated from the Japanese sea hare *Aplysia kurodai*, shows potent antitumor activities *in vivo* in addition to its actin-binding property and is expected to be a new type of anticancer drug candidate (Figure 1).[2] In our previous studies, the side chain part in aplyronine A (**1**) proved to be crucial for both cytotoxicity and actin-depolymerizing activity.[3] In contrast, the macrolide moiety in **1** significantly emphasizes its cytotoxicity but is not so important to actin-depolymerizing activity. Since mycalolide B (**2**),[4] a macrolide isolated from a Japanese sponge, possesses a similar side chain to that of aplyronine A (**1**) and the artificial analogue only consisting of the side chain part of mycalolide B exhibits stronger actin-depolymerizing activity than does that of aplyronine A,[5] hybrid compound **3**, consisting of the macrolactone part in aplyronine A (**1**) and the side chain part in mycalolide B (**2**), might be expected to possess more potent actin-depolymerizing activity and cytotoxicity than aplyronine A (**1**). Thus, we planned to synthesize

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aplyronine A–mycalolide B hybrid compound **3** and to evaluate its biological activity.

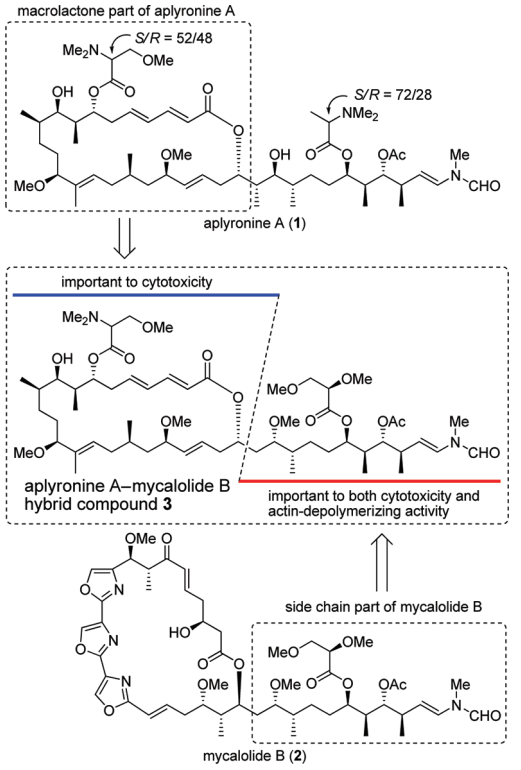
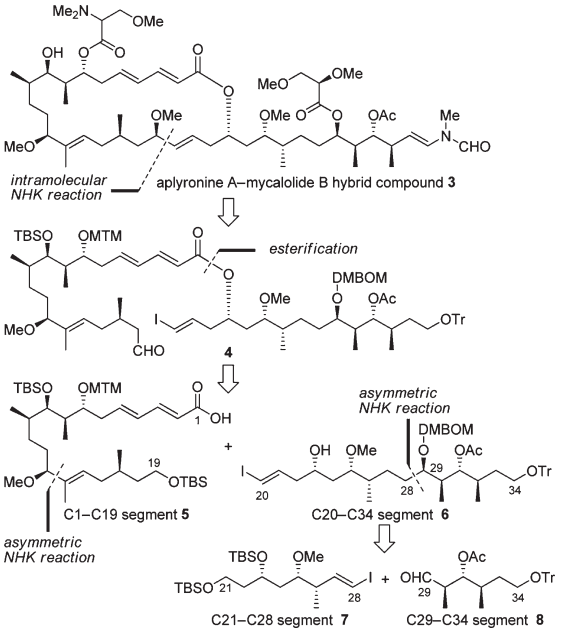


Figure 1. Design of aplyronine A–mycalolide B hybrid compound **3**.

Our retrosynthetic pathway of hybrid compound **3** is shown in Scheme 1. The macrolactone part in **3** would be constructed by an intramolecular Nozaki–Hiyama–Kishi (NHK) reaction[6] of compound **4**, which could be derived by an intermolecular esterification of C1–C19 segment **5** and C20–C34 segment **6**. We previously reported the synthesis of the C1–C19 segment **5**[7] by an asymmetric NHK coupling.[8] The C20–C34 segment **6** could also be assembled from iodoolefin **7** and aldehyde **8** by an asymmetric NHK coupling.

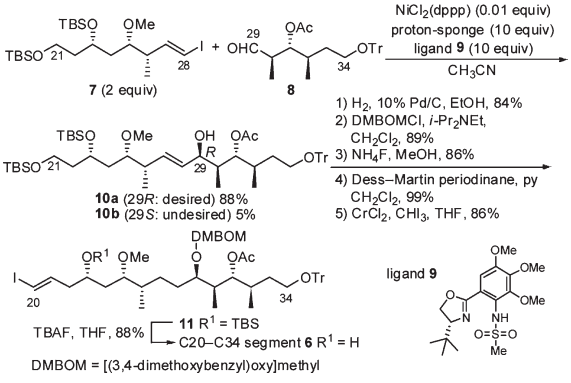
The C21–C28 and C29–C34 segments **7** and **8** were synthesized using an asymmetric aldol reaction[9] and Roush crotylboration[10] as key steps (see Supporting Information). An asymmetric NHK coupling[8] between fragments **7** and **8** was next attempted (Scheme 2). In our

Scheme 1. A Retrosynthetic Pathway of Aplyronine A–Mycalolide B Hybrid Compound **3**



synthetic studies on acid **5**, we designed ligand **9** for an asymmetric NHK coupling and found this to be effective for the asymmetric construction of the trisubstituted secondary allylic alcohol precursor of **5**.[7] For the NHK coupling of **7** and **8**, ligand **9** also worked successfully to give the best yield and stereoselectivity among several known sulfonamide ligands. As the primary asymmetric NHK coupling product **10a** has the desired stereochemistry for the C20–C34 segment **6**, elaboration to **6** was readily achieved. Thus, catalytic hydrogenation of **10a**, protection of the hydroxy group as a DMBOM ether, and subsequent selective desilylation afforded a primary alcohol. Oxidation of the resulting primary hydroxy group gave an aldehyde, which was subjected to Takai olefination[11] followed by desilylation to furnish the targeted C20–C34 segment **6**.

Scheme 2. Synthesis of C20–C34 Segment **6**



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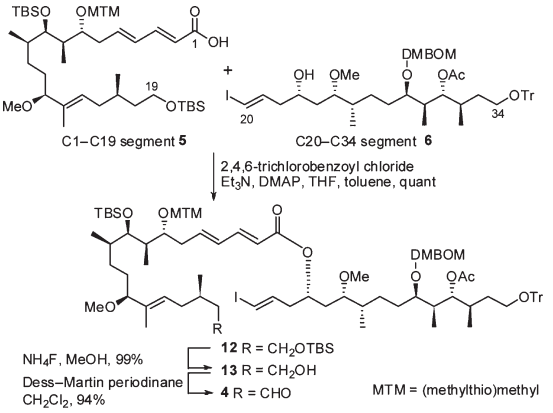
The synthesis of the precursor **4** for an intramolecular NHK reaction is illustrated in Scheme 3. The esterification between the C1−C19 segment **5** and the C20−C34 segment **6** under Yamaguchi conditions[12] gave ester **12**. Selective removal of the C19 silyl ether proceeded cleanly to give alcohol **13**, which was then oxidized to provide the cyclization precursor, aldehyde **4**.

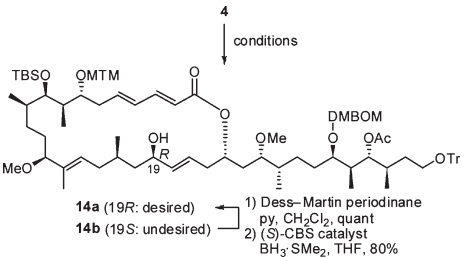
Scheme 3. Synthesis of Precursor **4** for an Intramolecular NHK Reaction



We next examined the crucial construction of the macrolactone ring by intramolecular NHK reaction (Table 1).[6] Treatment of **4** with $CrCl_2/NiCl_2$ in DMSO at a dilute concentration (c = 1.0 mM) efficiently afforded the expected cyclization product **14a** (46%) and its C19 epimer **14b** (50%) (entry 1). This macrocyclization also proceeded smoothly even at a higher concentration (c = 10 mM) to give **14a** (46%) and **14b** (49%) (entry 2). In our aplyronine A synthesis,[13] construction of a similar macrolactone via the Yamaguchi method[12] required high-dilution conditions (c = 0.39 mM), which makes this intramolecular NHK reaction without the use of high-dilution conditions much more convenient.[14] In contrast, when we tried the asymmetric intramolecular NHK reaction with *ent*-**9** (entry 3), the stereoselectivity was not very good, as expected, and the yield of the product was moderate. Conversion of the undesired C19 epimer **14b** into the desired isomer **14a** was achieved by following the procedure reported by Paterson.[15]

In our conversion of **14a** into hybrid compound **3**, we followed a similar strategy for the synthesis of aplyronine A by our group (Scheme 4).[13] Thus, introduction of the

Table 1. Intramolecular NHK Reaction of **4**

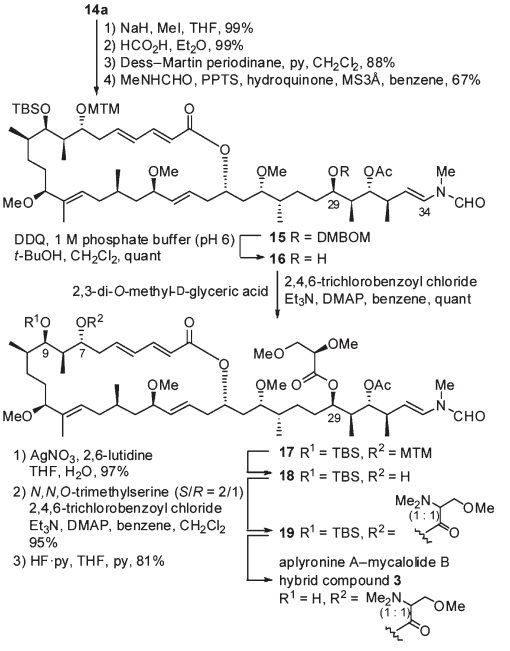


entry	conditions	solvent	concn (mM)	yield (%)[a] **14a**	yield (%)[a] **14b**
1	$NiCl_2$ (10 mol %), $CrCl_2$ (20 equiv)	DMSO	1.0	46	50
2	$NiCl_2$ (10 mol %), $CrCl_2$ (5 equiv)	DMSO	10	46	49
3	$NiCl_2$(dppp) (4 mol %), $CrCl_2$ (10 equiv), proton-sponge (10 equiv), *ent*-**9** (10 equiv)	CH_3CN	1.0	49	27

[a] The stereochemistry was confirmed by modified Mosher's method.[16]

N-methylformamide group at C34, the *O*,*O*-dimethylglyceric ester group at C29, and the *N*,*N*,*O*-trimethylserine ester groups at C7[17] afforded aplyronine A−mycalolide B hybrid compound **3**.

Scheme 4. Synthesis of Aplyronine A−Mycalolide B Hybrid Compound **3**



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The actin-depolymerizing activity and cell growth inhibitory effects of **3** and **1** were evaluated against HeLa S_3 cells (Table 2). Hybrid compound **3** was found to have somewhat more potent actin-depolymerizing activity (EC_{50} = 1.0 μM) than aplyronine A (**1**) (EC_{50} = 1.4 μM). In contrast, the cytotoxicity of hybrid compound **3** (IC_{50} = 12 nM) was about 1000-fold weaker than that of aplyronine A (**1**) (IC_{50} = 0.010 nM). These results indicate that there is no direct correlation between actin-depolymerizing activity and cell growth inhibitory activity. Thus, we discuss the reasons for the considerably reduced cytotoxicity of **3**. From our previous structure–activity relationship study,[3b] the acyl group at C29 was found to be unimportant to cytotoxicity. For this reason, displacement of a *N,N*-dimethylalanine ester with an *O,O*-dimethylglycerate would not be a reasonable explanation for the fairly low cytotoxicity. Therefore, we assume that the differences in configuration at C25 and the degree of substitution at C24 between **1** and **3** significantly influenced their cytotoxicities. These should cause a change in the conformational relationship or angle between the macrolactone and the side chain part, which can be deduced by comparing X-ray crystallographic structures between the actin–aplyronine A (**1**) complex[18] and actin–kabiramide C (**20**, mycalolide B-related compound) complex[19] (Figure 2). Consequently, we presume that both hybrid compound **3** and aplyronine A (**1**) bind readily with actin; nevertheless, **3** cannot maintain a strong interaction with the other target biomolecules of aplyronine A (**1**) to exhibit cytotoxicity. In this regard, actin-related proteins (Arp2 and 3) have also been found as target biomolecules of aplyronine A (**1**) by our group.[20] However, the role of Arp2 and Arp3 in the strong antitumor activity of aplyronine A (**1**) is still unclear.

Table 2. Actin-Depolymerizing Activity and Cytotoxicity of Hybrid Compound **3** and Aplyronine A (**1**)

compound	actin-depolymerizing activity (EC_{50})	cytotoxicity against HeLa S_3 cells (IC_{50})
hybrid compound **3**	1.0 μM	12 nM
aplyronine A (**1**)	1.4 μM	0.010 nM

In conclusion, we have synthesized a hybrid compound **3**, comprised of the macrolactone part of aplyronine A (**1**) and the side chain part of mycalolide B (**2**), using two asymmetric NHK couplings and an intramolecular NHK reaction as the key steps, and we have biologically evaluated this compound. Hybrid compound **3** retained potent actin-depolymerizing activity, whereas its cytotoxicity against HeLa S_3 cells was considerably reduced compared to that of aplyronine A (**1**). These findings suggest that actin-depolymerization is not directly related to the observed cytotoxicity and that the side chain of mycalolide B (**2**) is not suitable for **3** to exhibit strong cytotoxicity. Further investigations regarding the design and synthesis of aplyronine A analogues are currently underway in our group.

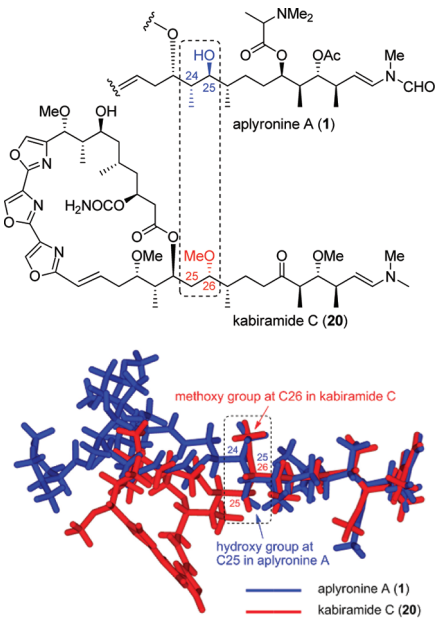

Figure 2. Conformational structures of aplyronine A (**1**) and kabiramide C (**20**) based on X-ray analyses of actin–aplyronine A complex and actin–kabiramide C complex.

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Supporting Information Available. Experimental procedures and spectroscopic data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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