

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

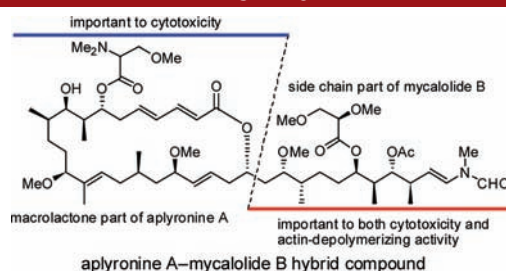
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Received January 23, 2012

## ABSTRACT



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.<sup>1</sup> 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).<sup>2</sup> In our previous studies, the side chain part in aplyronine A (**1**) proved to be crucial for both cytotoxicity and actin-depolymerizing activity.<sup>3</sup> In contrast, the macrolide moiety in **1** significantly emphasizes its cytotoxicity but is not so important to actin-depolymerizing activity. Since mycalolide B (**2**),<sup>4</sup> 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,<sup>5</sup> 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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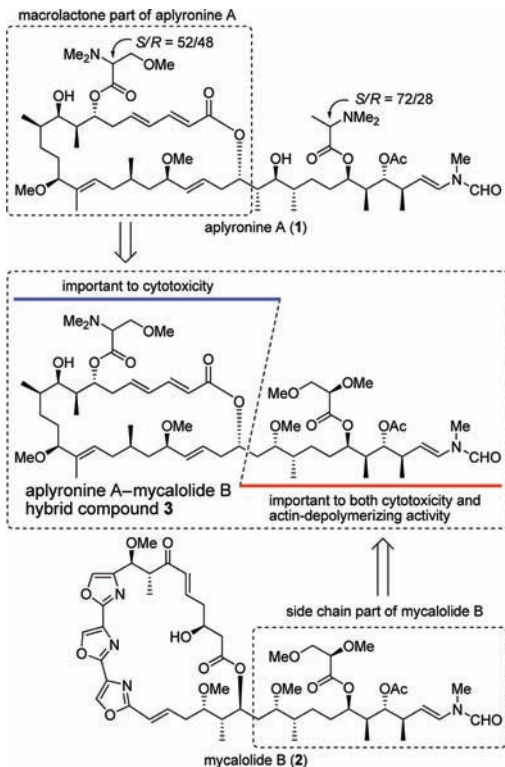
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aplyronine A–mycalolide B hybrid compound **3** and to evaluate its biological activity.



**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<sup>6</sup> 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**<sup>7</sup> by an asymmetric NHK coupling.<sup>8</sup> 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<sup>9</sup> and Roush crotylboration<sup>10</sup> as key steps (see Supporting Information). An asymmetric NHK coupling<sup>8</sup> between fragments **7** and **8** was next attempted (Scheme 2).

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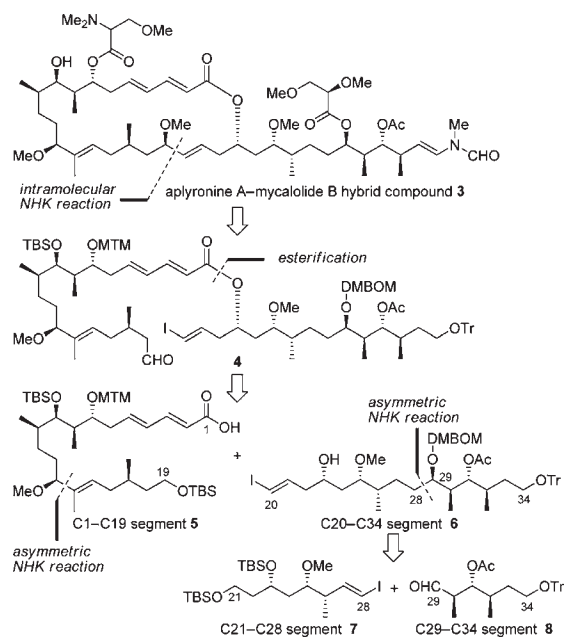
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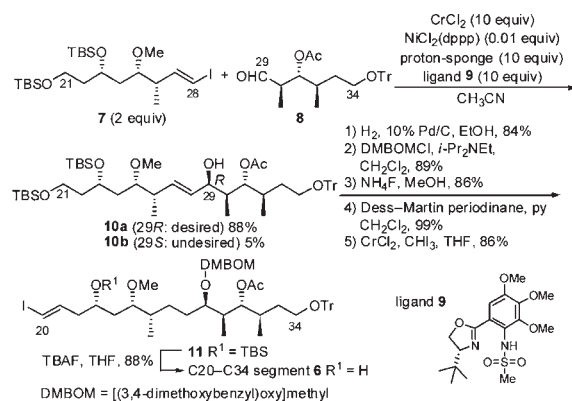
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### Scheme 1. A Retrosynthetic Pathway of Aplyronine A–Mycalolide B Hybrid Compound **3**



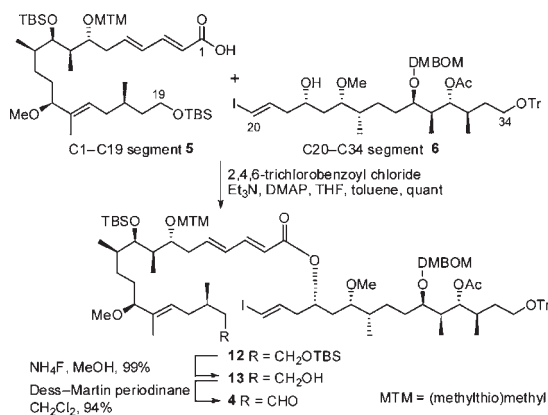
synthetic studies on acid **5**, we designed ligand **9** for an asymmetric NHK coupling and found this to be effective for the asymmetric construction of the trisubstituted secondary allylic alcohol precursor of **5**.<sup>7</sup> For the NHK coupling of **7** and **8**, ligand **9** also worked successfully 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<sup>11</sup> followed by desilylation to furnish the targeted C20–C34 segment **6**.

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



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<sup>12</sup> 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).<sup>6</sup> Treatment of **4** with  $\text{CrCl}_2/\text{NiCl}_2$  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,<sup>13</sup> construction of a similar macrolactone via the Yamaguchi method<sup>12</sup> required high-dilution conditions ( $c = 0.39$  mM), which makes this intramolecular NHK reaction without the use of high-dilution conditions much more convenient.<sup>14</sup> 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.<sup>15</sup>

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).<sup>13</sup> Thus, introduction of the

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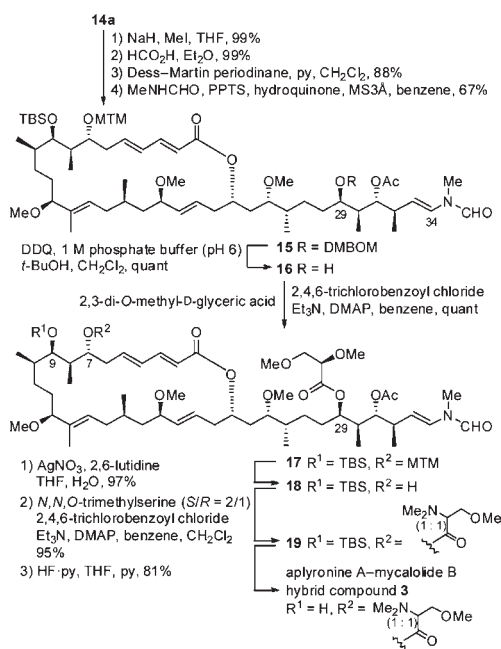
**Table 1.** Intramolecular NHK Reaction of **4**

entry	conditions	solvent	concn (mM)	yield (%) <sup>a</sup>	
				<b>14a</b>	<b>14b</b>
1	$\text{NiCl}_2$ (10 mol %), $\text{CrCl}_2$ (20 equiv)	DMSO	1.0	46	50
2	$\text{NiCl}_2$ (10 mol %), $\text{CrCl}_2$ (5 equiv)	DMSO	10	46	49
3	$\text{NiCl}_2(\text{dppp})$ (4 mol %), $\text{CrCl}_2$ (10 equiv), proton-sponge (10 equiv), <i>ent</i> - <b>9</b> (10 equiv)	$\text{CH}_3\text{CN}$	1.0	49	27

<sup>a</sup>The stereochemistry was confirmed by modified Mosher's method.<sup>16</sup>

*N*-methylformamide group at C34, the *O,O*-dimethylglyceric ester group at C29, and the *N,N,O*-trimethylserine ester groups at C7<sup>17</sup> afforded aplyronine A–mycalolide B hybrid compound **3**.

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





The actin-depolymerizing activity and cell growth inhibitory effects of **3** and **1** were evaluated against HeLa S<sub>3</sub> cells (Table 2). Hybrid compound **3** was found to have somewhat more potent actin-depolymerizing activity ( $EC_{50} = 1.0 \mu\text{M}$ ) than aplyronine A (**1**) ( $EC_{50} = 1.4 \mu\text{M}$ ). In contrast, the cytotoxicity of hybrid compound **3** ( $IC_{50} = 12 \text{ nM}$ ) was about 1000-fold weaker than that of aplyronine A (**1**) ( $IC_{50} = 0.010 \text{ nM}$ ). 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,<sup>3b</sup> 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<sup>18</sup> and actin–kabiramide C (**20**, mycalolide B-related compound) complex<sup>19</sup> (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.<sup>20</sup> 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 <sub>3</sub> cells ( $IC_{50}$ )
hybrid compound <b>3</b>	$1.0 \mu\text{M}$	12 nM
aplyronine A ( <b>1</b> )	$1.4 \mu\text{M}$	0.010 nM

In conclusion, we have synthesized a hybrid compound **3**, comprised of the macrolactone part of aplyronine A (**1**)

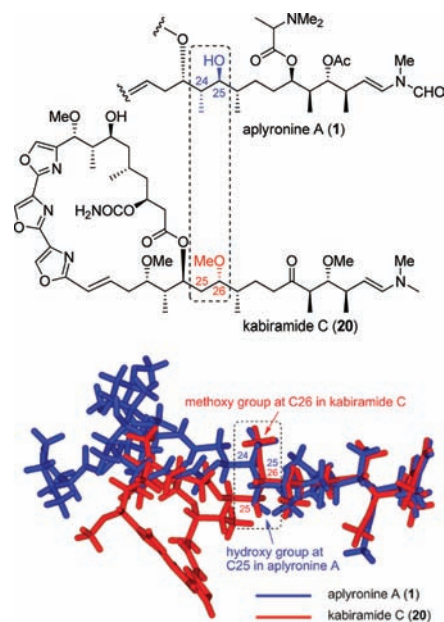
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**Figure 2.** Conformational structures of aplyronine A (**1**) and kabiramide C (**20**) based on X-ray analyses of actin–aplyronine A complex and actin–kabiramide C complex.

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<sub>3</sub> 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.

**Acknowledgment.** This work was supported by Grants-in-Aid for Scientific Research, from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, and by a grant from the Uehara Memorial Foundation (H.K.). I.H. thanks The Society of Synthetic Organic Chemistry, Japan (Meiji Seika Award in Synthetic Organic Chemistry, Japan) and Suntory Institute for Bioorganic Research (SUNBOR GRANT) for financial support.

**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.