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Synthesis and actin-depolymerizing activity of mycalolide analogs

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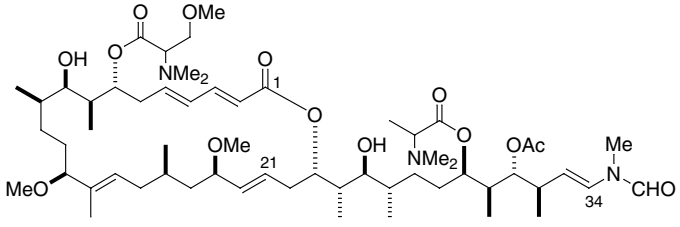
Abstract—Mycalolide analog **4**, consisting only of the side chain of mycalolide B (**2**), was stereoselectively synthesized and was found to have strong actin-depolymerizing activity.

Actin-disrupting marine natural products are of interest to natural products chemists and pharmacologists.[1] These natural products consist of macrolides, cyclic peptides, and cyclodepsipeptides. Aplyronine A (**1**), an anti-tumor macrolide isolated from *Aplysia kurodai*[2] interacts with actin, the major protein in cytoskeleton. Aplyronine A (**1**) not only inhibits polymerization of actin by sequestering G-actin and forming a 1:1 complex, but also depolymerizes F-actin to G-actin by severing.[3] We achieved the total synthesis of **1** and investigated the structure–activity relationships of aplyronine A (**1**) using natural and synthetic analogs: the



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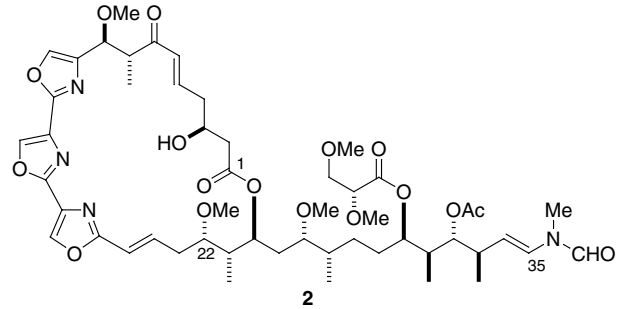

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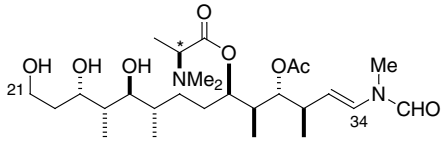
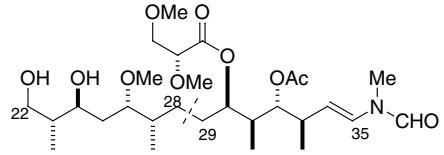


3 (* *S*/*R* = 9/1)



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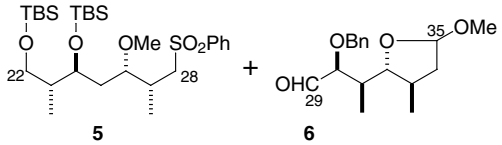

5 **6**

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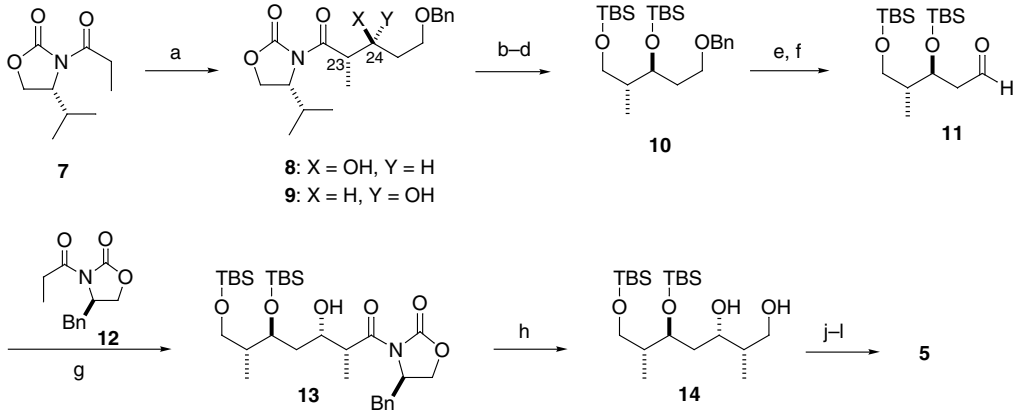
side chain in **1** is essential for actin-depolymerizing activity, and analog **3**, which consists only of the side chain moiety of **1**, exhibits strong activity.[4]

Mycalolide B (**2**) is a cytotoxic and anti-fungal macrolide isolated from a sponge of the genus *Mycale* sp.[5] Mycalolide B (**2**) inhibits actomyosin Mg^{2+}-ATPase[6] and also interacts with actin in the same manner as **1**.[7] Recently, the total synthesis of mycalolide A was reported.[8] Since mycalolide B (**2**) possesses a similar side chain to that of **1**, analog **4** is expected to show actin-depolymerizing activity. We describe herein the stereocontrolled synthesis of mycalolide analog **4** and its activity against actin.

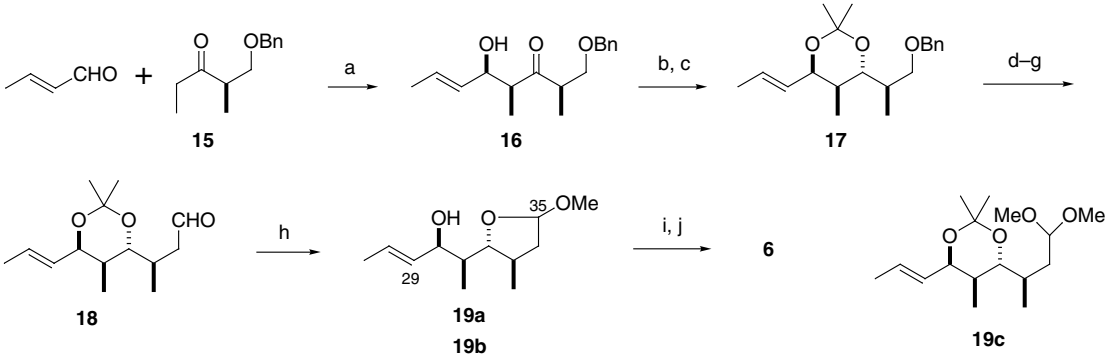
The synthesis of mycalolide analog **4** has been carried out according to a convergent synthetic methodology connecting C22–C28 and C29–C35 segments, **5** and **6**.

The synthesis of C22–C28 segment **5** started with an *anti*-selective aldol reaction (Scheme 1).[9] An aldol reaction between imide **7**[10] and 3-benzyloxypropanal under Heathcock conditions afforded hydroxy imide **8** (64%) along with the *syn*-isomer **9** (20%).[11] Removal of the chiral auxiliary of **8** and reduction with $LiAlH_4$ followed by silylation gave silyl ether **10**. Cleavage of the benzyl protecting group in **10** and Dess–Martin oxidation[12] of the resultant alcohol afforded aldehyde **11**. Evans aldol reaction between aldehyde **11** and imide **12**[10] gave hydroxy imide **13**, which was converted into diol **14**. The primary hydroxy group of diol **14** was transformed into a phenylsulfonyl group by reaction with $(PhS)_2$–Bu_3P[13] and subsequent oxidation of the resultant sulfide group, and the secondary hydroxy group was methylated to afford C22–C28 segment **5** (33% from **7**).

The synthesis of C29–C35 segment **6** is shown in Scheme 2. While compound **6**, with four contiguous *syn–anti–anti*-stereocenters, was previously prepared by using the Evans aldol reaction and Sharpless epoxidation as the key steps,[4a,b] the improved synthesis of **6** was developed by using the Paterson aldol reaction[14] as the key step. Thus, the Paterson aldol reaction between ethyl ketone **15** and crotonaldehyde gave the hydroxy ketone **16**.[15] Stereoselective reduction of **16** with tetramethylammonium triacetoxyborohydride[16] afforded an *anti*-1,3-diol, which was transformed into acetonide **17**. Conversion of



Scheme 1. Reagents and conditions: (a) Bu_2BOTf (2 equiv for **7**), 3-benzyloxypropanal, *i*-Pr_2EtN, −78 °C (**8**) 64%, (**9**) 20%; (b) H_2O_2, LiOH, THF, H_2O, 0 °C; (c) $LiAlH_4$, THF, rt; (d) TBSCl, imidazole, DMF, rt, 77% (three steps); (e) H_2, 5% Pd–C, $NaHCO_3$, EtOAc, 50 °C, 85%; (f) Dess–Martin periodinane, pyridine, CH_2Cl_2, rt, 96%; (g) **12**, Bu_2BOTf, Et_3N, CH_2Cl_2, −78 °C → 0 °C, 100%; (h) $LiBH_4$, EtOH, Et_2O, −10 °C, 100%; (j) $(PhS)_2$, Bu_3P, DMF, rt, 96%; (k) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, rt, 93%; (l) MeI, NaH, THF, rt, 92%.



Scheme 2. Reagents and conditions: (a) $Sn(OTf)_2$, Et_3N, CH_2Cl_2, −78 °C → −60 °C, 85%; (b) $Me_4NBH(OAc)_3$, AcOH, MeCN, −25 °C; (c) $(MeO)_2CMe_2$, PPTS, acetone, rt, 84% (two steps); (d) Ca, liq. NH_3, *i*-PrOH, THF, −78 °C, 98%; (e) *p*-TsCl, pyridine, 0 °C, 100%; (f) NaCN, DMSO, 50 °C, 98%; (g) DIBAL, CH_2Cl_2, hexane, −78 °C, 93%; (h) PPTS, MeOH, rt, 82%; (i) BnBr, NaH, DMF, rt, 93%; (j) OsO_4, NMO, H_2O, acetone, rt; then $NaIO_4$, rt, 99%.

17 into the aldehyde **18** was effected by a four-step sequence of reactions. Aldehyde **18** was treated with PPTS in methanol to provide a separable mixture of diastereomeric acetals, **19a** and **19b**, and the dimethyl acetal **19c**.[17] After chromatographic separation, two minor products, **19b** and **19c**, were subjected to equilibration (PPTS in methanol) to afford a mixture of **19a**, **19b**, and **19c**, from which the major acetal **19a** was again obtained. By repeating this procedure, **19b** and **19c** could be transformed into **19a**. Protection of the hydroxy group in **19a** followed by oxidative cleavage of the double bond provided the C29–C35 segment **6** (48% from **15**).

The Julia coupling reaction between **5** and **6** gave a hydroxy sulfone, which was converted into the olefin **20** by reduction (Scheme 3). Manipulation of the protecting group in **20** and catalytic hydrogenation of the double bond afforded compound **21**. The acidic hydrolysis of **21** gave a hemiacetal, which was reduced to afford the diol **22**. The secondary hydroxy group in **22** was acetylated to give alcohol **23** by a three-step sequence of reactions. Oxidation of **23** and subsequent condensation with *N*-methylformamide provided the enamide **24**. Deprotection of the 3,4-dimethoxybenzyloxymethyl group of **24** gave an alcohol, which was esterified with 2,3-di-*O*-methyl-D-glyceric acid under Yamaguchi conditions to afford a mixture of diastereomeric esters **25**, which resulted from the racemization of 2,3-di-*O*-methyl-D-glyceric acid. After removal of the silyl groups in **25**, HPLC separation of the diastereomers provided analogs **4**[18] and **26**.[19]

The actin-depolymerizing activity of mycalolide analogs **4** and **26**, aplyronine A (**1**), and its analog **3** is shown in

Table 1. Actin-depolymerizing activity of aplyronine A (**1**) and compounds **3**, **4**, and **26**

Compound	Actin-depolymerizing activity[a]	
	IC_{50} (μM)[b]	Relative potency[c]
Aplyronine A (**1**)	1.6	100
3	7.9	20
4	2.7	59
26	4.4	36

[a] Activity was monitored by measuring the fluorescent intensity of pyrenyl actin. For the conditions of assay, see Ref. 20.

[b] IC_{50} indicates the concentration required to depolymerize F-actin (3.7 μM) to 50% of its control amplitude.

[c] The relative potencies were calculated from the IC_{50} values of the compound (aplyronine A = 100).

Table 1. The mycalolide analog **4** exhibited strong activity comparable to that of aplyronine A (**1**). This result revealed that the side chain portion in mycalolide B (**2**) was responsible for the potent activity of **2**, as was also the case with aplyronine A (**1**). Comparison of the activities of **3**, **4**, and **26** revealed that the structure and stereochemistry of the acyl group both influenced activity.

In conclusion, the stereocontrolled synthesis of mycalolide analog **4**, consisting only of the side chain of mycalolide B (**2**), was carried out. In addition, the mycalolide analog **4** was found to exhibit strong actin-depolymerizing activity.

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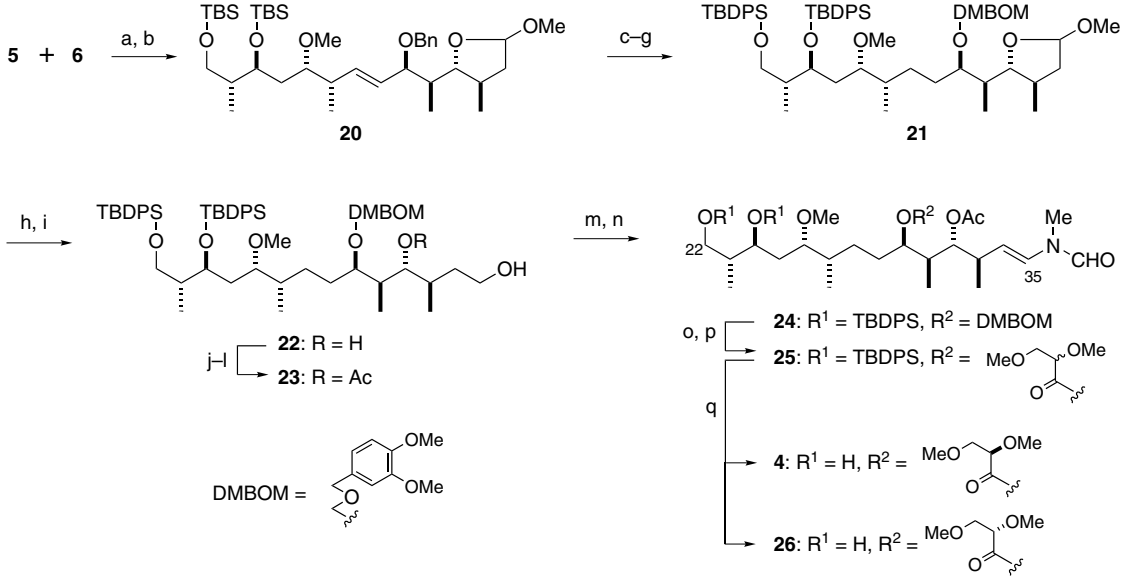

Scheme 3. Reagents and conditions: (a) BuLi, THF, −78 °C; (b) 5% Na–Hg, NaH_2PO_4, MeOH, 0 °C, 72% (two steps); (c) Ca, liq. NH_3, *i*-PrOH, THF, −78 °C, 90%; (d) H_2, 5% Pd–C, $NaHCO_3$, EtOH, 55 °C, 86%; (e) 3,4-dimethoxybenzyloxymethyl chloride, *i*-Pr_2NEt, CH_2Cl_2, rt, 84%; (f) Bu_4NF, THF, rt, 99%; (g) TBDPSCl, imidazole, DMF, rt, 75%; (h) 1 M HCl, DME, rt; (i) $NaBH_4$, EtOH, rt, 70% (two steps); (j) TrCl, pyridine, 50 °C, 95%; (k) Ac_2O, pyridine, DMAP, rt, 100%; (l) HCO_2H, Et_2O, rt, 77%; (m) Dess–Martin periodinane, pyridine, CH_2Cl_2, rt, 91%; (n) MeNHCHO, PPTS, hydroquinone, MS 3 A, benzene, reflux, 55%; (o) DDQ, 1 M phosphate buffer (pH 6), *t*-BuOH, CH_2Cl_2, rt, 90%; (p) 2,3-di-*O*-methyl-D-glyceric acid, 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, CH_2Cl_2, rt, 74%; (q) HF·pyridine, pyridine, THF, rt; separation by HPLC (**4**) 55%, (**26**) 34%.

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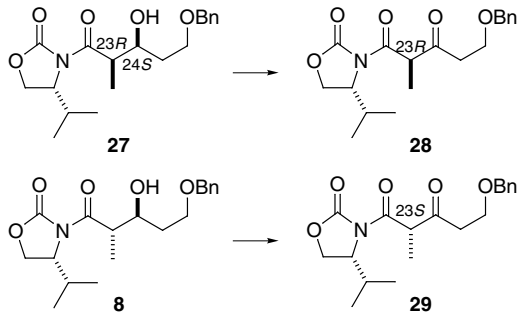
11. The stereochemistry of **8** was determined as follows. The coupling constant between C23 and C24 of **8** was 7.3 Hz, whereas that of its *syn*-diastereomer **27**, prepared by Evans aldol reaction, was 3.6 Hz. This finding indicated that the relative stereochemistry between C23 and C24 in **8** was *anti*. On the other hand, the oxidation of **27** and **8** afforded diastereomeric ketones **28** and **29**, respectively, establishing that the absolute configuration of C23 in **8** was *S*. From these results, the stereochemistry of **8** was determined to be 23*S* and 24*S* (*anti*), as expected from the results[9] of Heathcook and co-workers.



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18. $[\alpha]_D^{28}$ +88.5 (*c* 0.067, $CHCl_3$); IR ($CHCl_3$) 3675, 1575, 1488, 1237, 1202, 1043 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.30 [8.08] (s, 1H), 6.49 [7.17] (d, $J = 14.0$ Hz, 1H), 5.11 (m, 1H), 4.97 [4.99] (dd, $J = 9.6$ Hz, 14.0 Hz, 1H), 4.80 (dd, $J = 2.8$ Hz, 10.0 Hz, 1H), 3.93 (m, 1H), 3.79–3.29 (m, 5 H), 3.50 (s, 3H), 3.40 (s, 3H), 3.37 (s, 3H), 3.20 (m, 1H), 3.03 [3.07] (s, 3H), 2.51 (m, 1H), 2.09 [2.08] (s, 3H), 1.90–1.72 (m, 2H), 1.76 (m, 1H), 1.69–1.36 (m, 6H), 1.02 [1.01] (d, $J = 6.8$ Hz, 3H), 0.98 (d, $J = 6.8$ Hz, 3H), 0.89 (d, $J = 6.8$ Hz, 3H), 0.82 (d, $J = 6.8$ Hz, 3H). The minor counterparts of doubled signals in the ratio of 3:2 are in brackets; HRMS (ESI) calcd for $C_{28}H_{51}NNaO_{10}$ [$(M+Na)^+$] 584.3398, found 584.3411.
19. The stereochemistry of **4** and **26** concerning the 2,3-di-*O*-methylglyceroyl group was determined by degradation and chiral HPLC analyses in the same manner as that used for mycalolide B.[5b]
20. Actin was purified from rabbit skeletal muscle[21] using G-buffer containing 0.2 mM $CaCl_2$, 0.2 mM ATP, 0.5 mM β-mercaptoethanol, and 2 mM Tris–HCl (pH 8.0) and the actin was polymerized to F-actin with 1 mM $MgCl_2$ at 25 °C for 1 h. The test compounds were dissolved in DMSO and added to the F-actin solution (3.7 μM). The incubated actin solutions were monitored with a fluorometer (excitation at 365 nm and emission at 407 nm).
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