Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 16, pp. 2081-2086, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00365-X

SYNTHESIS AND BIOLOGICAL ACTIVITY OF ARTIFICIAL ANALOGS OF MYCALAMIDE A

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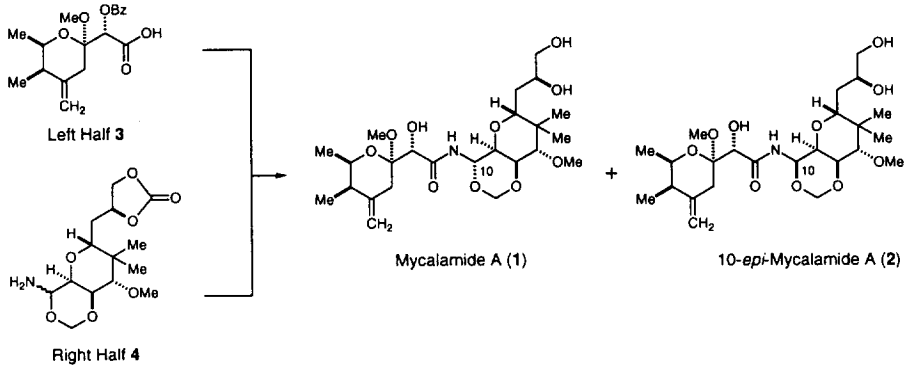
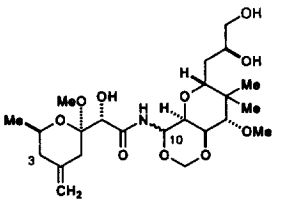
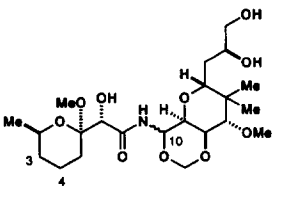
Abstract: Artificial analogs of mycalamide A, a potent antitumor and antiviral compound isolated from a New Zealand marine sponge, were synthesized and their biological activities were tested. © 1997 Published by Elsevier Science Ltd.

Mycalamide A (**1**) and B were isolated in 1988 from a New Zealand marine sponge of the genus *Mycale*.[1] Onnamides[2] and theopederins,[3] which are structurally related compounds, have been isolated from a Japanese marine sponge of the genus *Theonella*. Interestingly, the structure of these compounds is strikingly similar to that of pederin, a strong insect poison isolated from *Paederus fuscipes*.[4] The mycalamides exhibit potent *in vitro* cytotoxicity and *in vivo* antitumor activity as well as potent antiviral activity.[1,5] In addition, mycalamide A (**1**) blocks T-cells activation in mice and is 10-fold more potent than FK-506.[6] Recently, their unique structure and potent biological activity have attracted the attention of synthetic organic chemists.[7] The structure-activity relationship of simple derivatives prepared from naturally occurring mycalamides has been reported.[8] We now report the synthesis and biological activity of artificial analogs of mycalamide A (**1**) based on the synthetic strategy for our total synthesis of mycalamide A (**1**) and 10-*epi*-mycalamide A (**2**).[7c,d] Mycalamide analogs, MA-1 (**5**) ~ MA-10 (**14**), were synthesized to investigate the requisite functional groups in the left half **3** and the possibility of replacing the right half **4** by glucose derivatives.

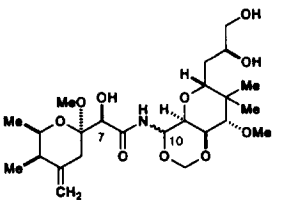




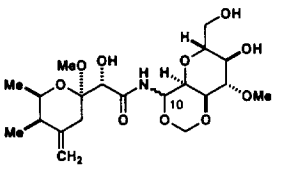
MA-1 (**5**): 10α-amide
MA-2 (**6**): 10β-amide



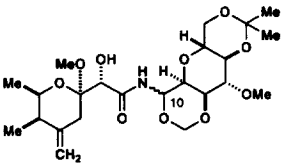
MA-3 (**7**): 10α-amide
MA-4 (**8**): 10β-amide



MA-5 (**9**): 10α-amide
MA-6 (**10**): 10β-amide



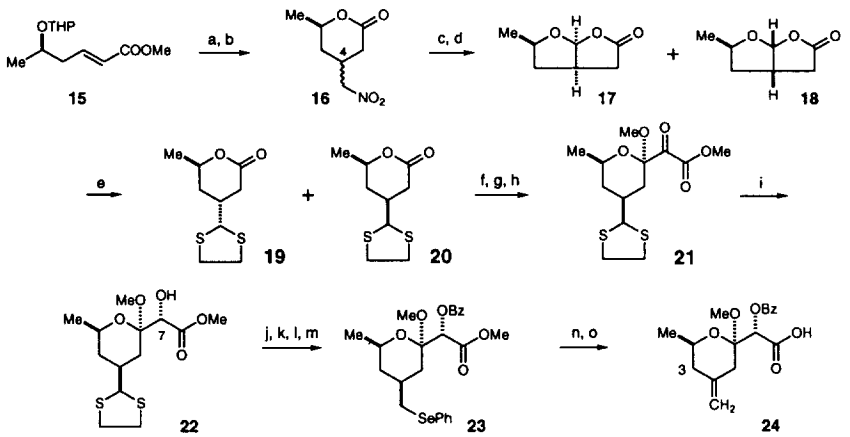
MA-7 (**11**): 10α-amide
MA-8 (**12**): 10β-amide



MA-9 (**13**): 10α-amide
MA-10 (**14**): 10β-amide

Chemical Synthesis

Synthesis of the artificial analogs with the modified left half. To investigate the relationship between the functional group of the left half and the biological activity, we synthesized mycalamide analogs having **24**, **28** and **31** as the left half. First, the left half **24** that lacks the C3-methyl group was synthesized using the synthetic strategy for the left half **3** in our total synthesis of pederin (Scheme 1).[9] Michael addition of nitromethane to α,β-unsaturated ester **15**,[10] deprotection of the THP group, and lactonization produced δ-lactone **16**. Successive treatment of **16** with $TiCl_3$-$AcONH_4$ and with c. HCl afforded a 1:5 mixture of bicyclic lactones **17** and **18**, which was treated with ethanedithiol-BF_3·Et_2O to give δ-lactones **19** and **20**. The lactone **20** was converted into the α-keto ester **21** by aldol reaction, methylacetalization, and the Moffatt



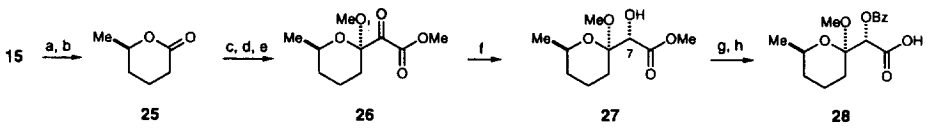
Scheme 1. (a) Triton B, $MeNO_2$, rt (82%); (b) *p*-TsOH, MeOH, rt, then benzene azeotropic (85%, 4α:4β=1:1); (c) $TiCl_3$, $AcONH_4$-H_2O, THF, rt; (d) c. HCl, CH_2Cl_2, rt; (e) $HSCH_2CH_2SH$, BF_3Et_2O, CH_2Cl_2, rt (40%, 3 steps); (f) LDA, $MeOC(Me)_2OCH_2COOMe$, THF, -78 °C; (g) CSA, $CH(OMe)_3$, MeOH, CH_2Cl_2, rt (71%, 2 steps); (h) DMSO, DCC, pyridine, CF_3COOH, Et_2O, rt (69%); (i) $Zn(BH_4)_2$, Et_2O, -78 °C; (j) BzCl, DMAP, pyridine, rt (66%, 2 steps); (k) HgO, $HgCl_2$, H_2O, MeCN, 60 °C; (l) $Zn(BH_4)_2$, Et_2O, 0 °C ; (m) PhSeCN, *n*-Bu_3P, THF, 0 °C (75%, 3 steps); (n) H_2O_2, THF, rt; Et_3N, benzene, reflux; (o) *n*-PrSLi, HMPA (69%, 2 steps).

oxidation. The reduction of **21** with $Zn(BH_4)_2$[11] produced 7α-alcohol **22** and its 7β-epimer in a ratio of 6.8:1. After benzoylation of **22**, deprotection of thioacetal, reduction with $Zn(BH_4)_2$, and treatment with PhSeCN-*n*-Bu_3P produced phenyl selenide **23**. Successive treatment with H_2O_2 and with *n*-PrSLi gave the desired carboxylic acid **24**.

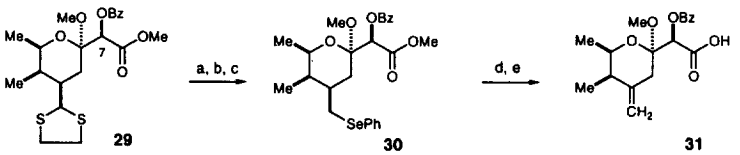
The left half **28** without the C3-methyl and C4-*exo*-methylene groups was next synthesized (Scheme 2). Reduction of the double bond in **15** with $NaBH_4$-$NiCl_2$, deprotection of the THP group and lactonization produced δ-lactone **25** which was converted into α-keto ester **26**. $Zn(BH_4)_2$ reduction of **26** produced 7α-alcohol **27** and its 7β-isomer (6.8:1). The 7α-alcohol **27** was converted into the carboxylic acid **28**.

The 7β-benzoyl carboxylic acid **31**, 7-epimer of the left half **3**, was synthesized from **29**, which is a synthetic intermediate in our total synthesis of pederin,[9] using the same procedure taken in the synthesis of **24** (Scheme 3).

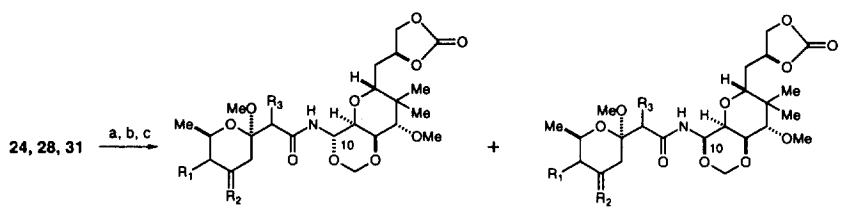
With the left half analogs **24**, **28**, and **31** in hand, we then undertook their condensation with the right half amine **4** (Scheme 4). Treatment of **24**, **28**, and **31** with *p*-TsCl and DMAP in CH_2Cl_2 followed by addition of **4** produced the coupling products **32** ~ **37** which, after separation, were hydrolyzed to give **5** (MA-1) and its 10-epimer **6** (MA-2), **7** (MA-3) and its 10-epimer **8** (MA-4), and **9** (MA-5) and its 10-epimer **10** (MA-6), respectively.



Scheme 2. (a) $NiCl_2$, $NaBH_4$, MeOH, rt (92%); (b) *p*-TsOH, MeOH, rt, then benzene azeotropic (83%); (c) LDA, $MeOC(Me)_2OCH_2COOMe$, THF, -78 °C; (d) CSA, $CH(OMe)_3$, MeOH, CH_2Cl_2, rt (60%, 2 steps); (e) DMSO, DCC, pyridine, CF_3COOH, Et_2O, rt (36%); (f) $Zn(BH_4)_2$, Et_2O, -78 °C; (g) BzCl, DMAP, pyridine, rt; (h) *n*-PrSLi, HMPA, 0 °C (67%, 3 steps).



Scheme 3. (a) HgO, $HgCl_2$, H_2O, MeCN, 60°C; (b) $Zn(BH_4)_2$, Et_2O, -78 °C; (c) PhSeCN, *n*-Bu_3P, THF, 0 °C (50%, 3 steps); (d) H_2O_2, THF, rt; Et_3N, benzene, reflux; (e) *n*-PrSLi, HMPA (87%, 2 steps).



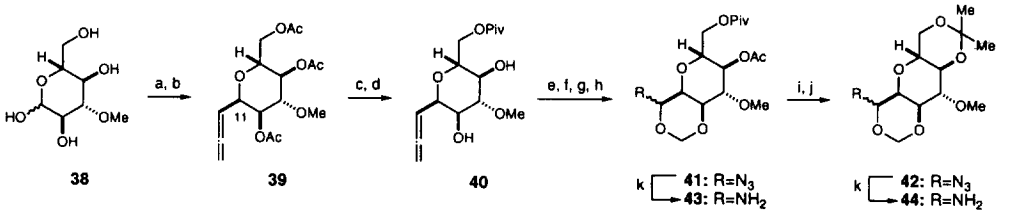
32: R_1=H, R_2=CH_2, R_3=α-OBz —c→ MA-1 (**5**)
34: R_1=H, R_2=H_2, R_3=α-OBz —c→ MA-3 (**7**)
36: R_1=Me, R_2=CH_2, R_3=β-OBz —c→ MA-5 (**9**)

33: R_1=H, R_2=CH_2, R_3=α-OBz —c→ MA-2 (**6**)
35: R_1=H, R_2=H_2, R_3=α-OBz —c→ MA-4 (**8**)
37: R_1=Me, R_2=CH_2, R_3=β-OBz —c→ MA-6 (**10**)

Scheme 4. (a) *p*-TsCl, DMAP, CH_2Cl_2, 0 °C-rt; (b) addition of amine **4** in CH_2Cl_2, rt; separation; (c) LiOH, MeOH, rt (29% for **5**, 13% for **6**, 36% for **7**, 23% for **8**, 25% for **9**,10% for **10**, each 3 steps)

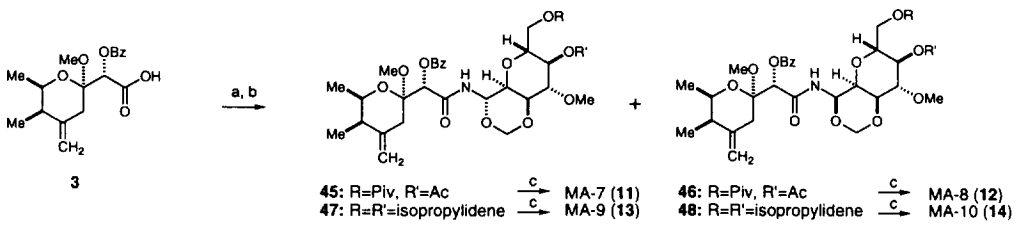
Synthesis of the artificial analogs with the modified right half. We synthesized mycalamide analogs having **43** and **44** as the right half, which were more readily prepared than the right half **4**. After acetylation, 3-*O*-methyl-α-D-glucopyranose (**38**) was treated with propargyltrimethylsilane in the presence of TMSOTf and $BF_3{\cdot}Et_2O$[7a] to give 11β-allene **39** exclusively (Scheme 5). Hydrolysis of the triacetate **39** followed by treatment with pivaloyl chloride afforded diol **40**, which was converted into azide **41** by successive treatment with O_3, $(CH_2O)_n$, Ac_2O, and $TMSN_3$. Alkaline hydrolysis of **41** followed by acetonization produced the tricyclic azide **42**. The hydrogenolysis of **41** and **42** gave the right half amines **43** and **44**, respectively.

The amines **43** and **44** were condensed with the left half **3** to give a mixture of **45** and **46**, and **47** and **48**, respectively, using the same procedure as mentioned above (Scheme 6). After separation, hydrolysis of **45**, **46**, **47**, and **48** produced **11** (MA-7), **12** (MA-8), **13** (MA-9) and **14** (MA-10), respectively.



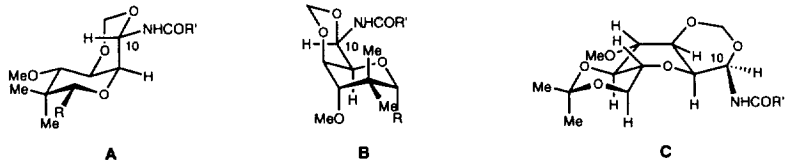
38 39 40

Scheme 5. (a) Ac_2O, pyridine, rt; (b) propargylTMS, TMSOTf, $BF_3{\cdot}Et_2O$, MeCN, 0 °C(68%, 2 steps); (c) K_2CO_3, MeOH, rt; (d) PivCl, pyridine, CH_2Cl_2 (91%, 2 steps); (e) O_3, MeOH, -78 °C; Me_2S, 0 °C; (f) $(CH_2O)_n$, CSA, CH_2Cl_2, 0 °C; (g) Ac_2O, pyridine, rt (74%, 3 steps); (h) $TMSN_3$, TMSOTf, MeCN, 0 °C (88%); (i) K_2CO_3, MeOH, rt; (j) $Me_2C(OMe)_2$, CSA, CH_2Cl_2, rt (85%, 2 steps); (k) H_2, 10% Pd-C, EtOAc, rt.



Scheme 6. (a) *p*-TsCl, DMAP, CH_2Cl_2, rt; (b) addition of amine **43** or **44** in CH_2Cl_2, rt; separation(c) LiOH, MeOH, rt (16% for **11**, 22% for **12**, 22% for **13**, and 35% for **14**, each 3 steps).

Conformation of the right half of the artificial analogs. The right halves in mycalamide A (**1**) and 10-*epi*-mycalamide A (**2**) were reported to have the conformation **A** and **B**, respectively.[1a, 8b] Detailed NMR analysis suggested that the artificial analogs with the same configuration at C10 as that of **1** and their 10-epimers have the same conformation as **A** and **B**, respectively. The right half of MA-10 (**14**) was found to have a chair-boat-chair form **C**.



A **B** **C**

Biological Activity and Discussion

The cytotoxicity against HeLa cells and antiviral activity against HSV-1 and VZV of the synthetic compounds *in vitro* were tested along with 5-fluorouracil and acyclovir as the standard, and the results are shown in Table 1.[12]

Table 1. The Biological Activity of Mycalamide A, 10-*epi*-Mycalamide A and Mycalamide Analogs.[a]

Compound	Cytotoxicity against HeLa cells	Antiviral activity against HSV-1		Antiviral activity against VZV	
	IC_{50}	MIC[b]	IC_{50}[c]	MIC[d]	IC_{50}[e]
5-Fluorouracil	3.0	---	---	---	---
Acyclovir	---	1.563	50.0	6.25	>50.0
Mycalamide A (**1**)	<0.03	<0.391	<0.391	<0.391	<0.391
10-*epi*-Mycalamide A (**2**)	3.0	12.5	12.5	1.563	12.5
MA-1 (**5**)	<0.03	<0.391	<0.391	<0.391	<0.391
MA-2 (**6**)	3.0	50.0	50.0	1.563	>50.0
MA-3 (**7**)	0.03	3.125	0.391	<0.391	1.563
MA-4 (**8**)	>10.0	50.0	>50.0	3.125	>50.0
MA-5 (**9**)	>10.0	50.0	12.5	<0.391	12.5
MA-6 (**10**)	>10.0	50.0	>50.0	12.5	>50.0
MA-7 (**11**)	1.0	25.0	25.0	3.125	>50.0
MA-8 (**12**)	>10.0	>50.0	>50.0	25.0	>50.0
MA-9 (**13**)	3.0	50.0	>50.0	12.5	>50.0
MA-10 (**14**)	10.0	50.0	50.0	12.5	>50.0

a) IC_{50} (μg/ml), MIC (μg/ml), HSV-1 (Herpes simplex virus type 1) and VZV (Varicella-zoster virus) were propagated in vero and HEL cells at 37 °C, respectively. b) against HSV-1. c) against vero cells. d) against VZV. e) against HEL cells.

Cytotoxicity against HeLa cells. Mycalamide A (**1**), MA-1 (**5**) and MA-3 (**7**) showed very potent cytotoxicity against HeLa cells. On the other hand, their corresponding 10-epimers, *i.e.*, **2**, MA-2 (**6**), and MA-4 (**8**), are 100-fold less active than **1**, **5**, and **7**, respectively, although the activity of **2** and **6** is still the same as that of 5-fluorouracil. Thus, the configuration at C10 is essential to show the strong cytotoxicity against HeLa cells. The presence of the C4-*exo*-methylene and C3-methyl groups is not an important factor for the potent cytotoxicity, although lack of the *exo*-methylene group slightly decreased the activity. The 7β-hydroxy isomers, MA-5 (**9**) and its 10-epimer MA-6 (**10**), decreased the activity, which suggested that the configuration of the C7-hydroxy group is also essential for its potent cytotoxicity. This indicates that the conformation of the α-hydroxy amide part plays an important role in its potent cytotoxicity, which would be supported by the reported results;[8] alkylation, acylation, and silylation of the 7-hydroxy group in **1** decreased the activity. It is noteworthy that MA-7 (**11**) and MA-9 (**13**), analogs replaced by glucose derivatives, showed the almost same activity as that of 5-fluorouracil.

Antiviral activity against HSV-1. A compound can be judged to have significant antiviral activity if its therapeutic ratio (TR=IC_{50}/MIC) is higher than that of acyclovir. The antiviral activity of mycalamide A (**1**), MA-1 (**5**), and MA-3 (**7**) against HSV-1 is very strong. However, their cytotoxicity (IC_{50}) against vero cells is also strong: TRs of all synthetic compounds tested are less than 1 (*cf.* TR of acyclovir = 32).

Antiviral activity against VZV. Although mycalamide A (**1**), MA-1 (**5**), and MA-3 (**7**) showed strong activitiy against VZV, their potent cytotoxicity (IC_{50}) against HEL cells was also observed. Interestingly, several synthetic compounds were found to have significant antiviral activity against VZV.

10-*epi*-Mycalamide A (**2**), MA-2 (**6**), MA-4 (**8**), MA-5 (**9**), and MA-7 (**11**) showed potent antiviral activity against VZV and low cytotoxicity against HEL cells: TRs of **2**, **6**, **8**, **9**, and **11** are 8, >32, >16, >32, and >16, respectively (*cf.* TR of acyclovir = >8). Thus, 7- or 10-epimeric compounds showed significant antiviral activity against VZV. It is noteworthy that MA-7 (**11**) also showed good antiviral activity against VZV.

The design and synthesis of the artificial analogs of mycalamides are under further investigation.

Acknowledgment: This work was supported in part by Special Coordination Funds of the Science and Technology Agency of the Japanese Government. The authors thank Ms. K. Harata for mass spectral measurements.

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10. The ester **15** was prepared from methyl (*R*)-3-hydroxy-butanoate in 4 steps: (1) DHP, *p*-TsOH, CH_2Cl_2, rt; (2) $LiAlH_4$, Et_2O, rt; (3) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C; Et_3N, rt; (4) Ph_3P=CHCOOMe, benzene, reflux.
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12. **Assay for antiviral activity against HSV-1.** Confluent monolayer of vero (African green monkey kidney) cells was infected with 10 $TCID_{50}$ of HSV-1 (Miyama GC^+ strain) in a well of a 96-well microtestplate in the presence of serial 2-fold dilution of each drug (in MEM medium supplemented with 1% fetal calf serum, total volume; 200 μl), and cultured for 3 days in 5% CO_2 and 95% humidified air at 37 °C. The minimum drug concentration which inhibits 50% of the viral cytopathic effect (cpe) was designated as MIC.
Assay for antiviral activity against VZV. Confluent monolayer of HEL (human embryonic lung) cells was infected with 10 $TCID_{50}$ of VZV (Kawaguchi strain) in the presence of serial 2-fold dilution of each drug and cultured for 7 days. All other culture conditions were the same as above. The minimum drug concentration which inhibits 100% of the viral cpe was designated as MIC.
Determination of IC_{50}. Confluent monolayer of HEL cells, vero cells, or HeLa cells (human cervical cancer cell line) was cultured in the presence of serial 2-fold dilution of each drug for 3 days in virus-free conditions. The viability was determined by MTT method.[13] The drug concentration whose absorbance at 540 nm indicates 50% of the drug-free control was designated as IC_{50}.
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(*Received in Japan* 20 *May* 1997; *accepted* 7 *July* 1997)