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Bioactive macrolides and polyketides from marine dinoflagellates*

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Abstract: Absolute stereochemistry of amphidinolides G and H, potent cytotoxic 27- and 26-membered macrolides, respectively, isolated from a marine dinoflagellate *Amphidinium* sp., was determined by X-ray diffraction analysis, synthesis of a degradation product, and chemical interconversion. Six new macrolides, amphidinolides H2~H5, G2, G3, and W, have been isolated from a marine dinoflagellate *Amphidinium* sp. (strain Y-42), and the structures were elucidated by 2D NMR data and chemical means. The structure–activity relationship of amphidinolide H-type macrolides for cytotoxicity was examined. The biosynthetic origins of amphidinolides B, C, H, J, T1, and W were investigated on the basis of ^{13}C NMR data of ^{13}C-enriched samples obtained by feeding experiments with [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] sodium acetates in cultures of the dinoflagellates. Five novel long-chain polyhydroxyl compounds, colopsinols A~E, were obtained from the *Amphidinium* sp. (strain Y-5).

ABSOLUTE STEREOCHEMISTRY OF AMPHIDINOLIDES G AND H

Amphidinolides G and H [1] isolated from the marine dinoflagellate *Amphidinium* sp. (Y-25 strain) are potent cytotoxic 27- and 26-membered macrolides, respectively, having unique structural features such as an allyl epoxide and vicinally located one-carbon branches (Scheme 1). The gross structures have been elucidated primarily by means of 2D NMR data, whereas the stereochemistry remains unsolved. Recently, a strain (Y-72) of the genus *Amphidinium* producing relatively large amounts of amphidinolides G and H has been separated from the inside cells of the marine acoel flatworm *Amphiscolops* sp. collected off Zanpa, Okinawa [2]. Amphidinolide H was crystallized from hexane-benzene as colorless needles, mp 131–132 °C. The relative stereochemistry of 9 chiral centers was obtained from a single-crystal X-ray diffraction analysis. Amphidinolide H was treated with $NaBH_4$ followed by $NaIO_4$ oxidation, $NaBH_4$ reduction, esterification with (*R*)-(–)-MTPACl, and separation using C_{18} high-performance liquid chromatography (HPLC) to afford the tris-(*S*)-MTPA ester of the C22–C26 segment. Both tris-(*S*)- and (*R*)-MTPA esters of the C22–C26 segment were prepared from methyl (2*S*)-3-hydroxy-2-methylpropionate. ^1H NMR data of the tris-(*S*)-MTPA ester derived from the natural product were identical with those of the synthetic tris-(*S*)-MTPA ester, indicating 23*R*- and 25*R*-configurations. Therefore, the absolute configurations of amphidinolide H were concluded to be 8*S*, 9*S*, 11*R*, 16*S*, 18*S*, 21*R*, 22*S*, 23*R*, and 25*R*. The absolute stereochemistry of amphidinolide G was determined by interconversion between amphidinolides G and H under alkaline condition. All spectral data of amphidinolide G isolated from this mixture were identical with those of the natural product. Thus, the absolute configurations of amphidinolide G assigned were the same as those of amphidinolide H.

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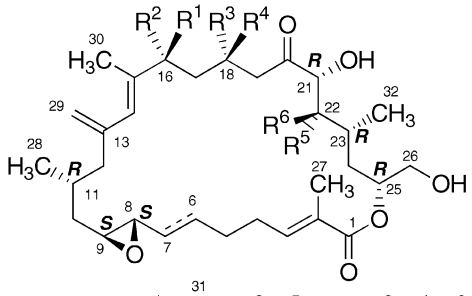
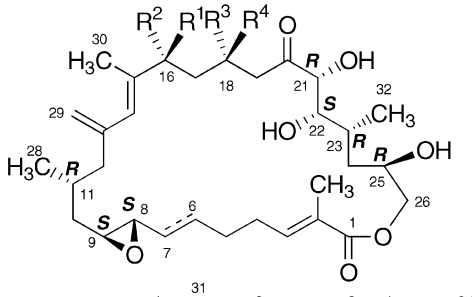
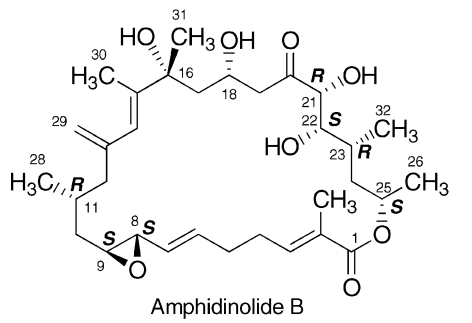


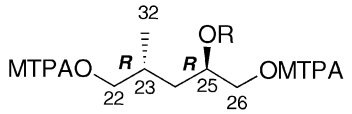
Amphidinolide H: $R^1 = {}^{31}CH_3$, R^3, R^5 = OH, R^2, R^4, R^6 = H, $\Delta^{6,7}$
Amphidinolide H2: $R^2 = {}^{31}CH_3$, R^4, R^5 = OH, R^1, R^3, R^6 = H, $\Delta^{6,7}$
Amphidinolide H3: $R^1 = {}^{31}CH_3$, R^3, R^6 = OH, R^2, R^4, R^5 = H, $\Delta^{6,7}$
Amphidinolide H4: $R^1 = {}^{31}CH_3$, R^3, R^5 = OH, R^2, R^4, R^6 = H, 6,7-dihydro
Amphidinolide H5: $R^2 = {}^{31}CH_3$, R^4, R^5 = OH, R^1, R^3, R^6 = H, 6,7-dihydro



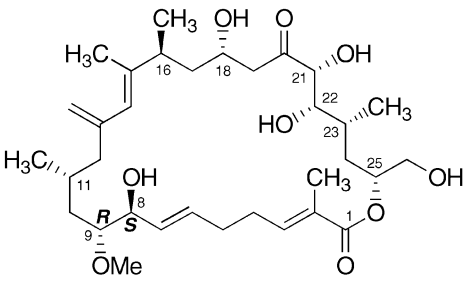
Amphidinolide G: $R^1 = {}^{31}CH_3$, R^3 = OH, R^2, R^4 = H, $\Delta^{6,7}$
Amphidinolide G2: $R^2 = {}^{31}CH_3$, R^4 = OH, R^1, R^3 = H, $\Delta^{6,7}$
Amphidinolide G3: $R^1 = {}^{31}CH_3$, R^3 = OH, R^2, R^4 = H, 6,7-dihydro



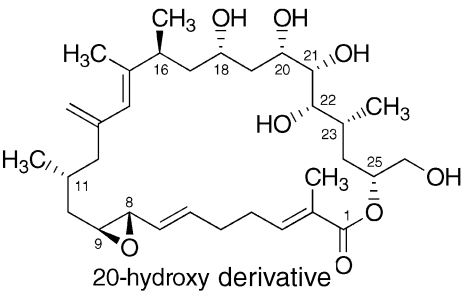
Amphidinolide B



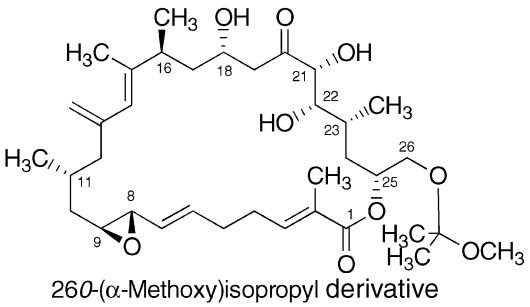
C-22-C-26 segment



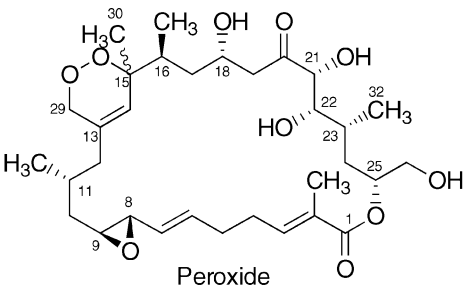
8-Hydroxy-9-methoxy derivative



20-hydroxy derivative



26*O*-(α-Methoxy)isopropyl derivative



Peroxide

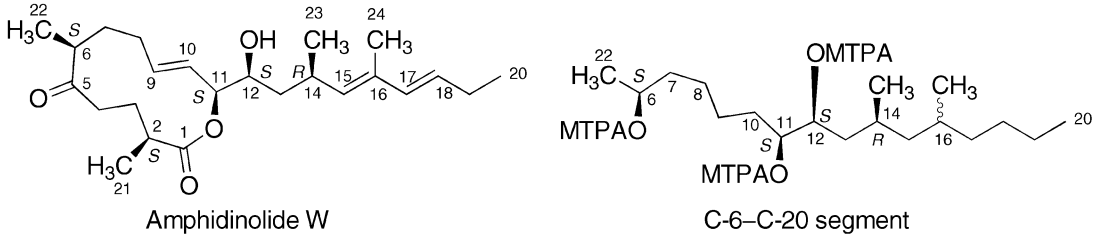
Scheme 1

AMPHIDINOLIDES H2~H5, G2, AND G3

Investigation of extracts from another strain (Y-42) of the genus *Amphidinium* resulted in the isolation of six new potent cytotoxic macrolides, amphidinolides H2~H5, G2, and G3 (Scheme 1) [3]. The structures of amphidinolides H2, H3, H4, and H5 were elucidated as 16,18-epi, 22-epi, 6,7-dihydro, and 6,7-dihydro-16,18-epi forms of amphidinolide H, respectively, on the basis of detailed analyses of spectroscopic data including $^{3}J_{H,H}$, $^{2}J_{C,H}$, and $^{3}J_{C,H}$ values and distance geometry calculation. On the other hand, the structures of amphidinolides G2 and G3 were assigned as the 16,18-epi and 6,7-dihydro forms of amphidinolide G. Cytotoxicity of 15 amphidinolide H-type macrolides against murine lymphoma L1210 and human epidermoid carcinoma KB cells was examined. Amphidinolide H4 was 300~400 times less potent than that of amphidinolide H. An epoxide-opening form, 8-hydroxy-9-methoxy derivative of amphidinolide H, showed no cytotoxicity at 3 μg/mL. Reduction or ring opening of the allyl epoxide resulted in significant decrease of the activity. Reduction of the ketone group at C20 resulted in remarkable reduction of the activity. Oxidation of *S-cis*-diene moiety of amphidinolide H into peroxide led to a 400-times decrease of cytotoxicity. Amphidinolide H3, the 22-epimer of amphidinolide H, exhibited 4 and 40 times less potent cytotoxicity against both L1210 and KB cells than that of amphidinolide H, while amphidinolide H2 was 100 times less potent than that of amphidinolide H. The IC_{50} values against KB cells of amphidinolides B and G were 10 times less active than that of amphidinolide H, while that against L1210 cells of amphidinolide B was three times more potent than that of amphidinolide H. Cytotoxicity of the 26-*O*-(α-methoxy)isopropyl derivative against L1210 and KB cells was 4~12 times less potent than that of amphidinolide H. These results suggested that the presence of an allyl epoxide, an *S-cis*-diene moiety, and a ketone at C20 is important for the cytotoxicity of amphidinolide H-type macrolides.

AMPHIDINOLIDE W

Further search of less-polar fractions of Y-42 strain resulted in the isolation of a new cytotoxic 12-membered macrolide, amphidinolide W (Scheme 2) [4]. The gross structure was elucidated on the basis of the spectroscopic data including ^{13}C-^{13}C NMR correlations obtained from the INADEQUATE spectrum. The relative stereochemistry of C11, C12, and C14 was elucidated to be 11,12-*threo* and 12,14-*syn* by *J*-based configuration analysis, and the absolute configuration at C12 was assigned as *S* by modified Mosher's method. 2*S*-Configuration was deduced from NMR data of 1,5,11,12-tetrakis-MTPA esters of a reductive product of amphidinolide W, while the absolute configuration at C6 was elucidated to be *S* on the basis of NMR data of 6,11,12-tris-MTPA esters of C6–C20 segment obtained by the Baeyer-Villiger reaction of amphidinolide W. Amphidinolide W is the first macrolide without an *exo*-methylene unit among all of the amphidinolides isolated so far. The gross structure of C9–C16 moiety of amphidinolide W corresponds to that of C6–C15 of amphidinolide H, which was contained in this strain Y-42, suggesting that amphidinolide W may be biogenetically related to amphidinolide H. Amphidinolide W exhibited cytotoxicity against murine lymphoma L1210 cells in vitro with an IC_{50} value of 3.9 μg/mL.

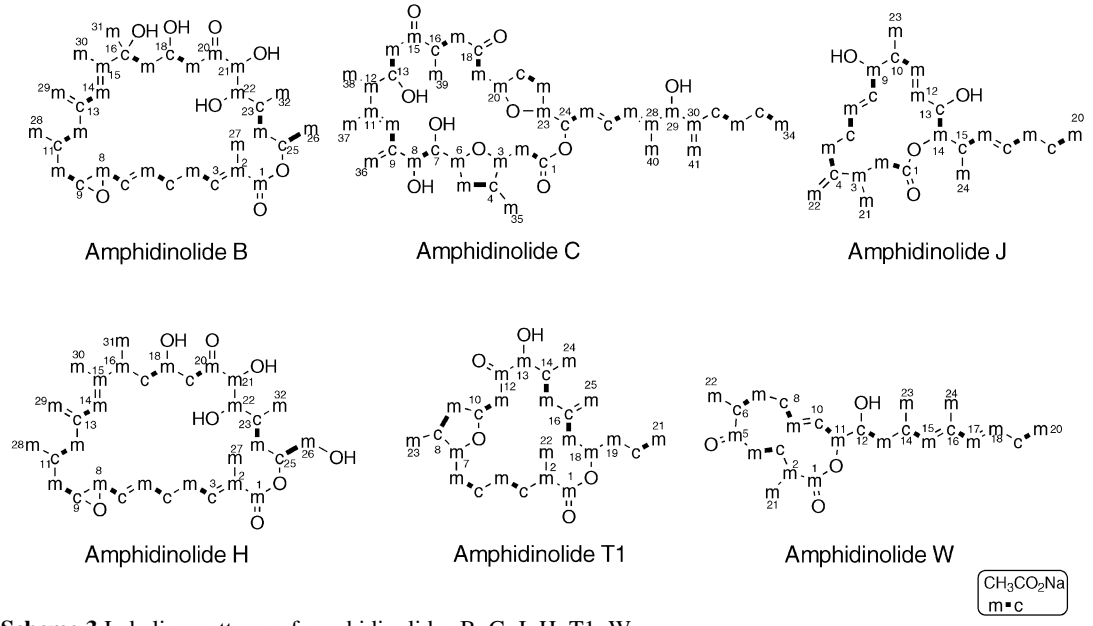


Scheme 2

BIOSYNTHETIC STUDY OF AMPHIDINOLIDES B, C, H, T1, AND W

Macrolide natural products generally possess an even-numbered macrocyclic lactone ring. However, more than half of amphidinolides are odd-numbered macrolides. Amphidinolides have other unique structural features: (a) most of them contain at least one *exo*-methylene unit and (b) vicinally located one-carbon branches are present in several of them. Previously, biosynthetic studies of a 15-membered macrolide, amphidinolide J, have also revealed that it is generated through nonsuccessive mixed polyketides [5]. During our continuing studies of biosynthesis of polyketides from dinoflagellates of the genus *Amphidinium*, the biosynthetic origins of amphidinolides B [6], C [7], H [8], T1 [9], and W [10] were investigated on the basis of 2D NMR data of ^{13}C-enriched samples obtained by feeding experiments with [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] sodium acetates in cultures of a marine dinoflagellates *Amphidinium* sp. (Scheme 3). The incorporation patterns of amphidinolide H suggested that amphidinolide H was generated from three unusual C_2 units "**m**–**m**" derived only from C2 of acetates in addition to three successive polyketide chains. Furthermore, it is noted that six oxygenated carbons of C1, C18, C20, C21, C22, and C26 were not derived from the C1 carbonyl but from the C2 methyl of acetates. On the other hand, amphidinolide W might be generated from a hexaketide chain, two acetate units, four isolated C_1 units from C2 of acetates, and four branched C_1 units from C2 of acetates. The acetate-incorporation patterns for C1–C2–(C21) and C8–C18–(C23, C24) of amphidinolide W corresponded well to those for C1–C2–(C27) and C5–C15–(C28, C29) of amphidinolide H isolated from the Y-42 strain.

The biosynthetic studies of amphidinolides B, C, and T1 were examined on the basis of ^{13}C NMR data of ^{13}C-enriched samples obtained by feeding experiments with [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] sodium acetates in cultures of a dinoflagellate *Amphidinium* sp. (strain Y-71). These incorporation patterns suggested that amphidinolide C (**1**) was generated from four diketide chains, four acetate units, five isolated C_1 units from C2 of acetates, seven branched C_1 units from C2 of acetates, and "**m**–**m**" and "**m**–**m**–**m**" units derived only from C2 of acetates. The C9–C12 portion including the vicinally located one-carbon branches in amphidinolide C disclosed the same labeling pattern, "**c**(**m**)–**m**–**m**(**m**)–**m**(**m**)", as those of amphidinolides G and H. The incorporation patterns of amphidi-

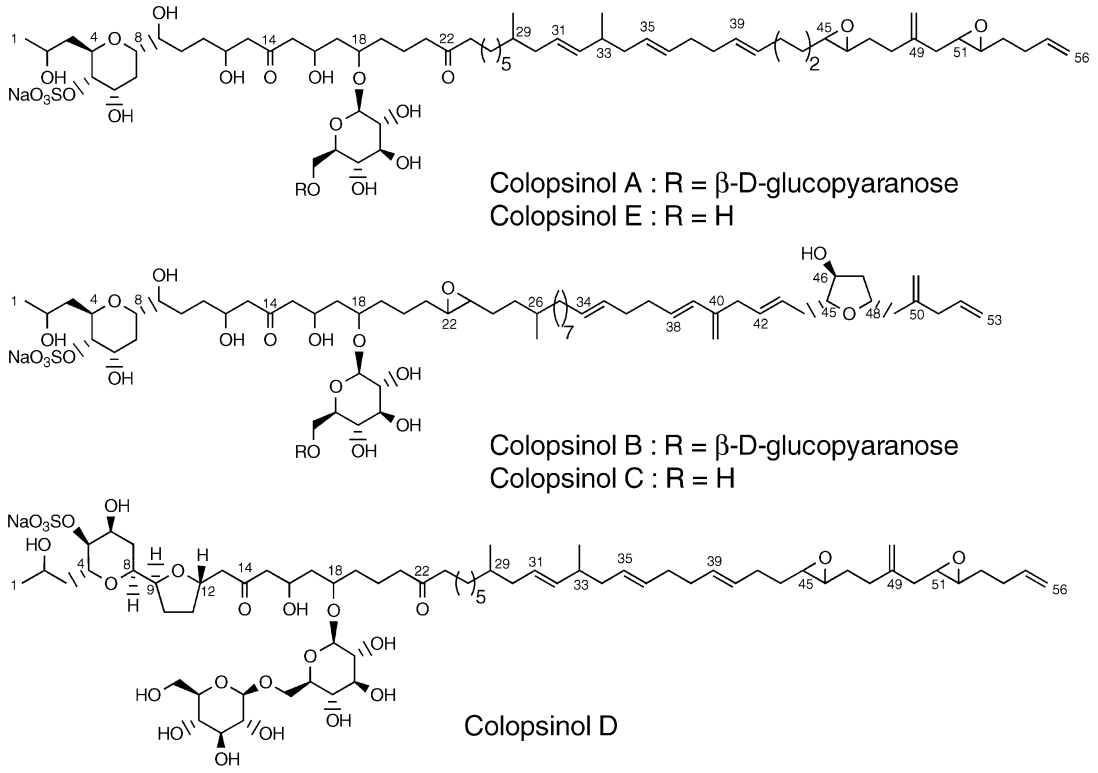


Scheme 3 Labeling patterns of amphidinolides B, C, J, H, T1, W.

nolide B suggested to be generated from three successive polyketide chains, an isolated C_1 unit from C2 of acetates, six branched C_1 units from C2 of acetates, and an "**m–m**" and an "**m–m–m**" unit derived only from C2 of acetates. The labeling patterns of amphidinolide B were different from those of amphidinolide H. Amphidinolide T1 also showed the unique labeling pattern consisting of four successive polyketide chains, an isolated C_1 unit formed from C2 of acetates, and three unusual C_2 units derived only from C2 of acetates. Furthermore, it is noted that five oxygenated carbons of C1, C7, C12, C13, and C18 were not derived from the C1 carbonyl, but from the C2 methyl of acetates. Two tetrahydrofuran portions in amphidinolide C showed the "**m–c–m–m**" labeling pattern, while that of amphidinolide T1 has been revealed to be "**m–c–m–c**".

COLOPSINOLS A~E

Colopsinols A~E (Scheme 4) [11–13] are the first member of a new class of polyketide natural products possessing a glucoside moiety and a sulfate ester isolated from the strain Y-5 of the dinoflagellate *Amphidinium* sp., from which a number of cytotoxic macrolides, amphidinolides A~D, J, K, M~S, and V, have been obtained so far. The gross structures were elucidated on the basis of extensive spectroscopic analyses including recent 2D NMR techniques of CH_2-selected editing HSQC as well as fast atom bombardment mass spectrometry (FABMS/MS) experiments and chemical means. The polyketide aglycone of colopsinol A consisted of a C_{56}-linear aliphatic chain with three C_1 branches (one *exo*-methylene and two methyls) as well as two ketones, five hydroxyl groups, and a tetrahydropyran and two epoxide rings. Colopsinol A exhibited potent inhibitory activity against DNA polymerase α and β with IC_{50} values of 13 and 7 μM, respectively. On the other hand, colopsinols B and C are new polyhydroxyl compounds possessing a C_{53}-linear carbon chain including three C_1 branches as well as



Scheme 4

a tetrahydropyran, a tetrahydrofuran, and epoxide rings, six hydroxyl groups, a glucoside moiety, and a sulfate ester. Colopsinol C exhibited cytotocity against L1210 murine leukemia cells in vitro with the IC_{50} value of 7.8 μg/mL, while colopsinol B did not show such cytotoxicity (IC_{50} > 10 μg/mL). Colopsinols D and E are congeners of colopsinol A. Colopsinol D has a tetrahydrofuran ring at C9–C12, while colopsinol E is the mono-deglucosyl form of colopsinol A. Colopsinol E exhibited cytotoxicity against L1210 murine leukemia cells (IC_{50} value: 7 μg/mL), while colopsinol D did not show such cytotoxicity (IC_{50} > 20 μg/mL). Biosynthetically it is interesting that quite different types of polyketides such as colopsinols and the amphidinolides are produced from the same dinoflagellate.

REFERENCES

1. J. Kobayashi, H. Shigemori, M. Ishibashi, T. Yamasu, H. Hirota, T. Sasaki. *J. Org. Chem.* **56**, 5221–5224 (1991).
2. J. Kobayashi, K. Shimbo, M. Sato, M. Shiro, M. Tsuda. *Org. Lett.* **2**, 2805–2807 (2000).
3. J. Kobayashi, K. Shimbo, M. Sato, M. Shiro, M. Tsuda. *J. Org. Chem.* **67**, 6585–6582 (2002).
4. K. Shimbo, M. Tsuda, K. Izui, J. Kobayashi. *J. Org. Chem.* **67**, 1020–1023 (2002).
5. J. Kobayashi, M. Takahashi, M. Ishibashi. *J. Chem. Soc., Chem. Commun.* 1639–1640 (1995).
6. M. Tsuda, T. Kubota, Y. Sakuma, J. Kobayashi. *Chem. Pharm. Bull.* **49**, 1366–1367 (2001).
7. T. Kubota, M. Tsuda, J. Kobayashi. *Tetrahedron* **57**, 5975–5977 (2001).
8. M. Sato, K. Shimbo, M. Tsuda, J. Kobayashi. *Tetrahedron Lett.* **41**, 503–506 (2000).
9. J. Kobayashi, T. Kubota, T. Endo, M. Tsuda. *J. Org. Chem.* **66**, 134–142 (2001).
10. M. Tsuda, K. Izui, M. Sato, J. Kobayashi. *Chem. Pharm. Bull.* **50**, 976–977 (2002).
11. J. Kobayashi, T. Kubota, M. Takahashi, M. Ishibashi, M. Tsuda, H. Naoki. *J. Org. Chem.* **64**, 1478–1482 (1999).
12. T. Kubota, M. Tsuda, M. Takahashi, M. Ishibashi, H. Naoki, J. Kobayashi. *J. Chem. Soc., Perkin Trans. 1* 3483–3488 (1999).
13. T. Kubota, M. Tsuda, M. Takahashi, M. Ishibashi, S. Oka, J. Kobayashi. *Chem. Pharm. Bull.* **48**, 1447–1451 (2000).