Further New Bis-cembranoids from the Hainan Soft Coral *Sarcophyton tortuosum*

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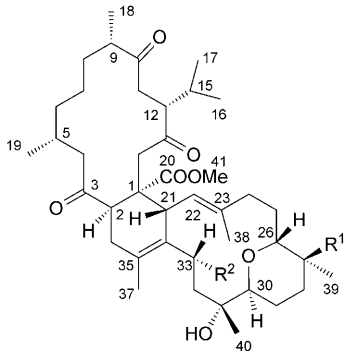
Two new bis-cembranoids, ximaolides F (**1**) and G (**2**), were isolated from the Hainan soft coral *Sarcophyton tortuosum*. The structures and relative configurations of the two new compounds were elucidated by the combination of spectroscopic methods, chemical conversion of ximaolide F (**1**) into ximaolide G (**2**), and comparison with related model compounds.

Introduction. – Bis-cembranoids represent an emerging group of natural products from soft corals of the genus *Sarcophyton* (family Alcyoniidae). Reports of these uncommon terpenoids from the genus *Sarcophyton* have become numerous over recent years [1–8]. Up to now, twenty bis-cembranoids have been discovered from three species of *Sarcophyton* (*S. tortuosum*, *S. latum*, and *S. glaucum*). A common structural feature among these dimeric diterpenes is that all of them could biogenetically derive from two different cembranoid units through a probable *Diels–Alder* addition, as suggested first for the methyl isosartortuoate [1], and then, by many articles [2–8]. The complex and unique structures of these dimeric cembranoids have also attracted the attention of synthetic chemists for the total synthesis of them [9][10].

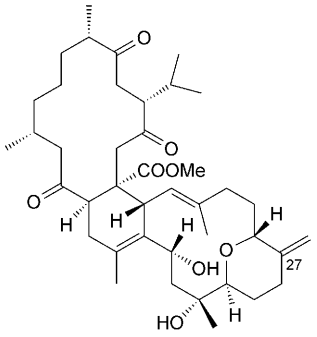
S. tortuosum is very abundant on the coral reefs in the South China Sea. Previous chemical studies have established that this animal could produce uncommon bis-cembranoids [1][2][6]. Recently, in the course of our ongoing research program on bioactive substances from Hainan marine invertebrates [8][11], we had reinvestigated *S. tortuosum*. In the course of this study, five new cembranoids, sarcophytonolides A–D [12], methyl tortuosoate, and five new bis-cembranoids, ximaolides A–E [7], were discovered. Further chemical investigation of the Et_2O extract of the animal now furnished two additional new bis-cembranoids, named ximaolides F[1]) (**1**) and G[1]) (**2**). The details of structure elucidation of compounds **1** and **2** are presented here.

Results and Discussion. – The specimen of *S. tortuosum* was collected off Ximao Island (the locality suggested the name assigned to the new bis-cembranoids), Sanya, Hainan, China, in 2002, and kept frozen until used. The usual workup [12] of the Et_2O-soluble fraction of the acetone extract of the *S. tortuosum* yielded two new compounds **1** and **2**.

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*



	R^1	R^2
1[1])	AcO	OH
2[1])	AcO	AcO



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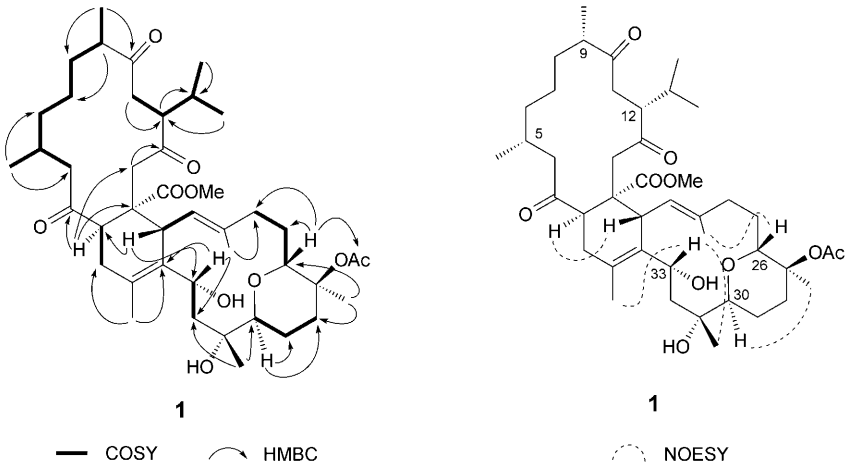
Ximaolide F (**1**) was obtained as a highly viscous colorless oil. Its molecular formula, $C_{43}H_{66}O_{10}$, was determined by HR-ESI-MS (*m/z* 765.4603 ([$M + Na$]$^+$)). The IR spectrum (KBr) indicated the presence of absorptions characteristic of OH groups (3467, 1091 cm^{-1}), ester C=O groups (1741, 1207 cm^{-1}), and ketone C=O groups (1708 cm^{-1}). Its UV spectrum did not show any conjugated system. The ^{1}H- and ^{13}C-NMR (*Table*), ^{1}H,^{1}H-COSY, HMBC, and NOESY data (*Fig.*) and their comparison with those of ximaolide E [7] established the structure of ximaolide F (**1**).



1 **1**

COSY HMBC NOESY

Figure. *The ^{1}H,^{1}H-COSY, selected key HMBC, and NOESY correlations of* **1**

The NMR spectra of **1** (*Table*) showed the following functionalities: one Me ester at δ(C) 174.7 (*s*, C(20)) and 51.1 (*q*, C(41)) and δ(H) 3.48 (*s*, Me(41)), three ketone C=O groups at δ(C) 209.4 (*s*, C(13)), 213.4 (*s*, C(3)), and 213.8 (*s*, C(10)), one tri- and one tetrasubstituted C=C bond at δ(C) 129.0 (*d*, C(22)), 138.3 (*s*, C(23)), 133.2 (*s*, C(34)), and 127.3 (*s*, C(35)), two allylic Me groups at δ(H) 1.75 (*s*, Me(37)) and

Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2**[1])[a])

	δ(H)			δ(C)	
	1	**2**		**1**	**2**
H–C(2)	3.56 (*t*, *J* = 8.3)	3.49–3.51 (*m*)	C(1)	49.9 (*s*)	49.9 (*s*)
CH_2(4)	2.39–2.41 (*m*),	3.19 (*dd*, *J* = 20.0, 10.4),	C(2)	44.0 (*d*)	43.5 (*d*)
	3.16 (*dd*, *J* = 20.4, 10.6)	2.33–3.35 (*m*)	C(3)	213.4 (*s*)	214.0 (*s*)
H–C(5)	1.73–1.76 (*m*)	1.76–1.78 (*m*)	C(4)	53.9 (*t*)	54.0 (*t*)
CH_2(6)	1.06–1.08 (*m*)	1.03–1.05 (*m*)[b])	C(5)	27.5 (*d*)	27.2 (*d*)
CH_2(7)	1.08–1.10,	1.04–1.05[b]),	C(6)	37.5 (*t*)	36.8 (*t*)
	1.24–1.26 (2*m*)	1.22–1.24 (2*m*)	C(7)	25.6 (*t*)	25.0 (*t*)
CH_2(8)	1.50–1.53 (*m*)	1.42–1.44 (*m*)	C(8)	34.2 (*t*)	33.4 (*t*)
H–C(9)	2.48–1.50 (*m*)	2.57–2.59 (*m*)	C(9)	48.1 (*d*)	46.4 (*d*)
CH_2(11)	1.87–1.89 (*m*)	2.82–2.84,	C(10)	213.8 (*s*)	213.7 (*s*)
		1.95–1.97 (2*m*)	C(11)	31.3 (*t*)	32.0 (*t*)
H–C(12)	3.03–3.05 (*m*)	2.98–3.00 (*m*)	C(12)	51.3 (*d*)	52.3 (*d*)
CH_2(14)	2.85, 2.95 (2*d*, *J* = 18.8)	2.95, 2.42 (2*d*, *J* = 20.0)	C(13)	209.4 (*s*)	208.6 (*s*)
H–C(15)	2.21–2.23 (*m*)	2.26–2.28 (*m*)	C(14)	46.1 (*t*)	45.3 (*t*)
Me(16)	0.69 (*d*, *J* = 6.8)	0.71 (*d*, *J* = 6.8)	C(15)	29.0 (*d*)	28.8 (*d*)
Me(17)	0.97 (*d*, *J* = 6.8)	1.00 (*d*, *J* = 6.8)	C(16)	17.6 (*q*)	17.7 (*q*)
Me(18)	1.11 (*d*, *J* = 7.1)	1.09 (*d*, *J* = 7.1)	C(17)	21.3 (*q*)	21.3 (*q*)[b])
Me(19)	0.84 (*d*, *J* = 7.0)	0.83 (*d*, *J* = 6.9)	C(18)	17.5 (*q*)	17.7 (*q*)
H–C(21)	3.50 (*d*, *J* = 10.4)	3.23 (*d*, *J* = 10.5)	C(19)	22.4 (*q*)	21.8 (*q*)
H–C(22)	4.95 (*d*, *J* = 10.4)	4.98 (*d*, *J* = 10.5)	C(20)	174.7 (*s*)	174.5 (*s*)
CH_2(24)	2.26–2.28 (*m*)	2.28–2.30 (*m*)	C(21)	43.4 (*d*)	44.3 (*d*)
CH_2(25)	1.71–1.73 (*m*)	1.91–1.93,	C(22)	129.0 (*d*)	128.7 (*d*)
		1.71–1.73 (2*m*)	C(23)	138.3 (*s*)	138.4 (*s*)
H–C(26)	4.66 (*d*, *J* = 10.6)	4.66 (*d*, *J* = 10.6)	C(24)	32.8 (*t*)	32.7 (*t*)
CH_2(28)	1.53–1.55,	1.54–1.56,	C(25)	31.2 (*t*)	31.2 (*t*)
	1.85–1.87 (2*m*)	1.84–1.86 (2*m*)	C(26)	73.8 (*d*)	73.8 (*d*)
CH_2(29)	1.75–1.77 (*m*)	1.67–1.69 (*m*)	C(27)	83.6 (*s*)	83.7 (*s*)
H–C(30)	3.92 (*dd*, *J* = 11.5, 5.2)	3.91 (*dd*, *J* = 11.2, 6.0)	C(28)	36.6 (*t*)	36.5 (*t*)
CH_2(32)	1.41 (*dd*, *J* = 13.4, 5.5),	1.58–1.60,	C(29)	27.3 (*t*)	27.4 (*t*)
	2.16 (*d*, *J* = 13.4)	2.22–2.24 (2*m*)	C(30)	88.9 (*d*)	88.7 (*d*)
H–C(33)	5.11 (*dd*, *J* = 5.5, 2.7)	6.08 (*dd*, *J* = 5.4, 2.6)	C(31)	74.3 (*s*)	74.0 (*s*)
CH_2(36)	2.04–2.06,	2.05–2.07,	C(32)	40.4 (*t*)	38.0 (*t*)
	2.44–2.47 (2*m*)	2.42–2.44 (2*m*)	C(33)	65.8 (*d*)	70.0 (*d*)
Me(37)	1.75 (*s*)	1.86 (*s*)	C(34)	133.2 (*s*)	130.5 (*s*)
Me(38)	1.77 (*s*)	1.78 (*s*)	C(35)	127.3 (*s*)	128.7 (*s*)
Me(39)	1.16 (*s*)	1.18 (*s*)	C(36)	32.7 (*t*)	33.0 (*t*)
Me(40)	1.16 (*s*)	1.03 (*s*)	C(37)	18.4 (*q*)	18.6 (*q*)
Me(41)	3.48 (*s*)	3.52 (*s*)	C(38)	20.3 (*q*)[b])	20.2 (*q*)
AcO–C(27)	2.08 (*q*)	2.10 (*q*)[b])	C(39)	20.4 (*q*)[b])	20.5 (*q*)
AcO–C(33)		2.08 (*q*)[b])	C(40)	21.8 (*q*)	21.4 (*q*)[b])
			C(41)	51.1 (*q*)	51.2 (*q*)
			*Ac*O–C(27)	171.2 (*s*),	171.3 (*s*),
				21.2 (*q*)	21.3 (*q*)[b])
			*Ac*O–C(33)		169.5 (*s*),
					21.3 (*q*)[b])

[a]) Measured in $CDCl_3$ with a *Bruker DRX-400* spectrometer. Chemical shifts δ in ppm are referenced to $CHCl_3$ (δ(H) 7.26) and $CDCl_3$ (δ(C) 77.0); coupling constants *J* in Hz. [b]) Interchangeable values.

1.77 (*s*, Me(38)), an i-Pr group at δ(H) 0.69 (*d*, $J = 6.8$ Hz, Me(16)) and 0.97 (*d*, $J = 6.8$ Hz, Me(17)), two Me groups attached to CH at δ(H) 1.11 (*d*, $J = 7.1$ Hz, Me(18)), 0.84 (*d*, $J = 7.0$ Hz, Me(19)), two Me groups attached to O-bearing C-atoms at δ(H) 1.16 (*s*, Me(39), Me(40)), and five additional O-bearing C-atoms at δ(C) 73.8 (*d*), 83.6 (*s*), 88.9 (*d*), 74.3 (*s*), and 65.8 (*d*). The presence of nine Me signals and characteristic spectroscopic features, such as three ketone C=O groups and one Me ester group, *etc.*, allowed to easily recognize that **1** should also be a dimeric cembranoid, similar to those (*e.g.*, **3**) reported previously from the same species [7]. From detailed analysis of the ^{1}H- and ^{13}C-NMR data of **1** associated with C–H one-bond interactions and from cross-peaks observed in the ^{1}H,^{13}C 2D-NMR shift-correlated spectrum, all signals of H and C could be assigned (*Table*). Analysis of the ^{1}H,^{1}H-COSY plot of **1** revealed the presence of seven H-atom spin systems as shown in the *Figure*. All the subunits, bearing in mind the three ketone C=O groups at δ(C) 209.4 (*s*, C(13)), 213.4 (*s*, C(3)), and 213.8 (*s*, C(10)), five quaternary C-atoms at δ(C) 49.9 (*s*, C(1)), 138.3 (*s*, C(23)), 83.6 (*s*, C(27)), 133.2 (*s*, C(34)), and 127.3 (*s*, C(35)), and one *AB*-type CH_2 group at δ(C) 46.1 (*t*, C(14)), were connected by extensive interpretation of the HMBC spectrum. The established C-atom connectivity showed that compound **1** had the same C-atom skeleton as that of co-occurring ximaolide E (**3**) [7]. In fact, careful comparison of ^{1}H- and ^{13}C-NMR data of **1** with those of **3** revealed that the 'upper-half' part (C(1) to C(20)) of **1** was identical to that of the corresponding part of **3**, while the 'lower-half' part (C(21) to C(36)/(C(37)) of **1** was also very similar to that of **3**, except for the absence of an exocyclic C=C bond and the appearance of a Me group attached to an O-bearing C-atom of **1** (δ(H) 1.16 (*s*)), and an AcO group (δ(C) 171.2 (*s*) and 21.2 (*q*)). These facts clearly indicated that the exocyclic C(39)=C(27) bond of **3** was replaced by an AcO and a Me group in **1**. The HMBC cross-peaks Me(39)/C(26), C(27), and C(28), and H–C(26) (δ(H) 4.66)/Me*C*OO–C(27) (δ(C) 171.2) confirmed this arrangement. The HMBC plot of **1** also showed many informative ^{1}H,^{13}C long-range correlations such as H–C(2)/C(1), C(3), C(14), and C(36) (*Fig.*). Combining the ^{1}H,^{1}H-COSY and HMBC data (*Fig.*), the planar structure of **1** could be completed.

The relative configurations of the stereogenic C-atoms of **1** were mainly determined by the NOESY correlation peaks (*Fig.*). However, it is noteworthy that the conformational mobility/flexibility of the 14-membered macrocycle of cembranoids renders the configurational assignments of the stereogenic centers by NOESY or NOE difference experiments somewhat risky. In the case of **1**, the relative configurations of C(5), C(9), and C(12) could not be unambiguously determined by interpreting the NOESY plot. The α-orientation of the substituents at these positions was assigned mainly by biogenetic considerations. The relative configurations at C(1), C(2), and C(21) were suggested by the stereochemistry of the *Diels–Alder* reaction as already found for the previously investigated cembrane dimers [4][7]. The configurations of other chiral centers (C(26), C(27), C(30), C(31), and C(33)) were determined by a combination of the NOESY experiments (*Fig.*) and comparison with the NMR data of the co-occurring **3**, for which the relative configurations of all stereogenic centers were secured by X-ray diffraction analysis [7]. Finally, the (*E*)-configurations of the C(22)=C(23) and C(34)=C(35) bonds were deduced from the ^{13}C-NMR chemical shifts of the olefinic Me groups (*Table*) [3].

Ximaolide G (**2**) showed the molecular formula $C_{45}H_{68}O_{11}$ as established by HR-ESI-MS (*m/z* 807.4614 ($[M + Na]^+$)). Comparison of its ^{1}H- and ^{13}C-NMR data (*Table*) with those of compound **1** implied that the structures of the two compounds were closely related. In fact, the only difference between **2** and **1** is that one more Ac group (δ(C) 169.5 (*s*) and 21.3 (*q*)) was present in the structure of **2**. Further, this Ac group was positioned at O–C(33) (downfield shift of the H–C(33) signal: δ(H) 5.11 in **1** and 6.08 in **2**). This assignment was further confirmed by the acetylation of **1** to afford a compound that showed NMR spectra identical to those of compound **2**. Hence, from these results, the structure of **2** was identified as the 33-*O*-acetyl derivative of ximaolide F (**1**), and named ximaolide G.

The crude Et_2O extract of the title soft coral exhibited cytotoxicity toward a limited panel of cancer cell lines. However, compounds **1** and **2**, like ximaolides A–E [7], were also shown to be inactive toward the growth of the A-549, KB, and P388 cells at a

concentration of 20 μg/ml. Other bioassays including antibacterial and anti-inflammatory assays are currently under way.

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Experimental Part

General. Column chromatography (CC): commercial silica gel (SiO_2; *Qing Dao Hai Yang Chemical Group Co.*; 200–300 mesh) and *Sephadex LH-20* (*Amersham Biosciences*). TLC: precoated SiO_2 plates (*Yan Tai Zi Fu Chemical Group Co.*; *G60*, *F-254*). Reversed-phase HPLC: *Agilent 1100* liquid chromatograph; *VWD-G1314A* detector (at 210 nm); one semi-prep. *ODS-HG-5* column (10 mm (i.d.) × 25 cm; 5 μm) for purification. Optical rotation: *Perkin-Elmer 341* polarimeter. UV Spectra: *756-CRT* spectrophotometer. IR Spectra: *Nicolet Magna-FT-IR-750* spectrophotometer; $\tilde{\nu}_{max}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Varian Mercury-400* spectrometer; 400 MHz for ^{1}H and 100 MHz for ^{13}C; in $CDCl_3$; δ in ppm rel. to $CDCl_3$ as internal standard, *J* in Hz. HR-ESI-MS: *Q-TOF-Micro* LC/MS/MS spectrometer; in *m*/*z*.

Biological Material. The specimens of the *S. tortuosum* (TIXIER–DURIVAULT), identified by Prof. *R.-L. Zhou* at the South China Sea Institute of Oceanology, Chinese Academy of Sciences, were collected off the coast of Ximao Island, Hainan Province, China, in December 2002, at a depth of −20 m, and were frozen immediately after collection. A voucher specimen (No. 02LS163) is available for inspection at the Shanghai Institute of Materia Medica, CAS.

Extraction and Isolation. The frozen animals (257 g; dry weight) were cut into pieces, and extracted exhaustively with acetone (3 × 1.5 l) at r.t. The org. extract was concentrated to give a residue, which was partitioned between Et_2O and H_2O. The Et_2O soln. was concentrated to give a dark brown residue (5.3 g), which was fractionated by CC (SiO_2, step gradient 0–100% acetone/light petroleum ether) yielding three cembranoid-containing fractions (R_f 0.35, 0.40, and 0.55 (light petroleum ether/acetone 2:1)) showing interesting blue TLC spots after spraying with H_2SO_4. The most polar fraction was further purified by CC (*Sephadex LH-20*, light petroleum ether/$CHCl_3$/MeOH 2:1:1) followed by reversed-phase HPLC (semi-prep. *ODS-HG-5*, MeCN/H_2O 75:25, 2.0 ml/min): pure **1** (3.7 mg; t_R 35.6 min) and **2** (4.6 mg; t_R 45.1 min).

Ximaolide F (= rel-*(1*E*,5*R*,6*S*,9*S*,10*R*,12*R*,14a*R*,17*R*,21*S*,24*R*,26a*R*,26b*R*)-6-(Acetyloxy)-3,5,6,7,8,9,10,11,12,14,14a,15,16,17,18,19,20,21,22,23,24,25,26,26b-tetracosahydro-10,12-dihydroxy-2,6,10,13,17,21-hexamethyl-24-(1-methylethyl)-15,22,25-trioxo-5,9-epoxybenzo[1,2:3,4]dicyclotetradecene-26a(4*H*)-carboxylic Acid Methyl Ester*; **1**): Colorless oil. $[\alpha]_D^{20} = +112.8$ ($c = 0.49$, $CHCl_3$). IR (film): 3467, 2956, 1741, 1708, 1207, 1091. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS: 765.4 ($[M+Na]^+$). HR-ESI-MS: 765.4603 ($[M+Na]^+$, $C_{43}H_{66}NaO_{10}^+$; calc. 765.4554).

Acetylation of Ximaolide F (**1**). A soln. of **1** (1.0 mg) in dry pyridine (0.5 ml) was treated with Ac_2O (0.5 ml) overnight at r.t. Standard workup followed by CC (SiO_2, light petroleum ether/acetone 12:1) gave **2** (0.9 mg).

Ximaolide G (= rel-*(1*E*,5*R*,6*S*,9*S*,10*R*,12*R*,14a*R*,17*R*,21*S*,24*R*,26a*R*,26b*R*)-6,12-Bis(acetyloxy)-3,5,6,7,8,9,10,11,12,14,14a,15,16,17,18,19,20,21,22,23,24,25,26,26b-tetracosahydro-10-hydroxy-2,6,10,13,17,21-hexamethyl-24-(1-methylethyl)-15,22,25-trioxo-5,9-epoxybenzo[1,2:3,4]dicyclotetradecene-26a(4*H*)-carboxylic Acid Methyl Ester*; **2**): Colorless oil. $[\alpha]_D^{20} = +104.0$ ($c = 0.19$, $CHCl_3$). IR (film): 3438, 2927, 1738, 1709, 1238, 1047. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS 807.5 ($[M+Na]^+$). HR-ESI-MS: 807.4614 ($[M+Na]^+$, $C_{45}H_{68}NaO_{11}^+$; calc. 807.4659).

REFERENCES

[1] J.-Y. Su, K.-H. Long, T.-S. Peng, *J. Am. Chem. Soc.* **1986**, *108*, 177.
[2] J.-Y. Su, K.-H. Long, T.-S. Peng, L.-M. Zeng, *Sci. China, Ser. B* **1988**, *31*, 1172.
[3] T. Kusumi, M. Igari, M. O. Ishitsuka, A. Ichikawa, Y. Itezono, N. Nakayama, H. J. Kakisawa, *J. Org. Chem.* **1990**, *55*, 6286.
[4] P. A. Leone, A. R. Carooll, J. C. Coll, G. V. Meehan, *J. Nat. Prod.* **1993**, *56*, 521.
[5] M. Feller, A. Rudi, N. Berer, I. Goldberg, Z. Stein, Y. Benayahu, M. Schleyer, Y. Kashman, *J. Nat. Prod.* **2004**, *67*, 1303.
[6] L.-M. Zeng, W.-J. Lan, J.-Y. Su, G.-W. Zhang, X.-L. Feng, Y.-L. Liang, X.-P. Yang, *J. Nat. Prod.* **2004**, *67*, 1915.
[7] R. Jia, Y.-W. Guo, P. Chen, Y.-M. Yang, E. Mollo, M. Gavagnin, G. Cimino, *J. Nat. Prod.* **2007**, *70*, 1158.
[8] X.-H. Yan, M. Gavagnin, G. Cimino, Y.-W. Guo, *Tetrahedron Lett*. **2007**, *48*, 5313.
[9] Y. Gao, F.-J. Nan, X.-X. Xu, *Tetrahedron Lett.* **2000**, *41*, 4811.
[10] X.-C. Chen, C.-F. Fan, X.-X. Xu, *Chin. J. Chem.* **2000**, *18*, 775.
[11] X.-H. Yan, L.-P. Lin, J. Ding, Y.-W. Guo, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2661; W. Zhang, M. Gavagnin, Y.-W. Guo, E. Mollo, M. Geiselin, G. Cimino, *Tetrahedron* **2007**, *63*, 4725; R. Jia, Y.-W. Guo, E. Mollo, M. Gavagnin, G. Cimino, *J. Nat. Prod.* **2006**, *69*, 819.
[12] R. Jia, Y.-W. Guo, E. Mollo, G. Cimino, *Helv. Chim. Acta* **2005**, *88*, 1028.

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