Novel Caprolactones from a Marine Streptomycete

Katja Stritzke,[†] Stefan Schulz,*[,†] Hartmut Laatsch,[‡] Elisabeth Helmke,[§] and Winfried Beil[||]

Institut für Organische Chemie, TU Braunschweig, Hagenring 30, 38106 Braunschweig, Germany, Institut für Organische Chemie, Universität Göttingen, 37077 Göttingen, Germany, Alfred-Wegener-Institut, Bremerhaven, Germany, and Institut für Pharmakologie, Medizinische Hochschule Hannover, 30625 Hannover, Germany

Received July 17, 2003

Two new caprolactones, (*R*)-10-methyl-6-undecanolide (**1**) and (6*R*,10*S*)-10-methyl-6-dodecanolide (**2**), were identified in the lipid extract of a marine streptomycete (isolate B6007). Their structures were proposed on the basis of GC−MS experiments and proved by synthesis. The absolute configuration of the compounds was established by comparison of the natural and synthetic stereoisomers using chiral gas chromatography. These caprolactones show a moderate phytotoxicity and a promising activity against cancer cells with concomitant low general cytotoxicity.

Marine organisms are an important and productive source of new natural products.[1,2] Growing attention has been paid to antibiotic and cytotoxic natural products of marine origin during the last few decades. More recently marine microorganisms came into the focus of research as the source of several interesting new structures.[2] As can be seen from recent reviews,[3] lipophilic compounds with low polarity are rarely investigated, most probably because their pharmacological properties are likely not very interesting. Extracts of organisms are usually defatted by partition between methanol and cyclohexane or hexane. The lipophilic phase often does not show any activity in biological screening, while in chemical screening the apolar phase is usually deliberately discarded. Problems associated with the analysis of apolar compounds are the presence of many, often very similar, compounds like fatty acids, frequently in low concentration, and the presence of anthropogenic compounds such as phthalates, which are introduced during sample workup procedures. Despite these obstacles we will show in the present paper that thorough analysis of such lipophilic phases can yield new compounds with interesting properties.

Results and Discussion

The isolate *Streptomyces* sp. B6007 originated from mangrove sediment in Papua New Guinea and showed a 99% similarity of its 16S rRNA gene sequence to *Streptomyces albogriseolus* (GenBank accession no. AJ494865). This strain was cultured for 3 days in a yeast extract−malt extract−glucose medium. The whole culture was extracted with ethyl acetate and partitioned between methanol and cyclohexane. A part of the cyclohexane phase was methylated by use of trimethylsulfonium hydroxide (TMSH), which transforms free and ester-bound fatty acids into the respective methyl esters.[4] The derivatized and underivatized cyclohexane phases were then analyzed by GC−MS. The total ion chromatograms are shown in Figure 1.

Several volatile compounds typically found in bacteria, such as geosmin, phenol, benzyl alcohol, 2-phenylethanol, and isovaleric acid, were identified by comparison of mass spectra and gas chromatographic retention times with those of authentic reference samples (see Table 1). Fatty acids represent the major compound class in the cyclohexane phase, accompanied by small amounts of terpenes and steroids. The acids are a mixture of common bacterial saturated and unsaturated unbranched as well as *iso*- and *anteiso*-methyl-branched acids. Positions of double bonds in the unsaturated acids were determined by derivatization with dimethyl disulfide[5] of the methylated extract, followed by GC−MS investigations.

Besides these compounds, two unknown components, **1** and **2**, were present in the extract as major constituents, showing the mass spectra presented in Figure 2. The double loss of water from the molecular ion and the characteristic fragments at $m/z = 85$ and 113 suggested a 6-alkanolide structure for both compounds. The ion at $m/z = 113$ arises from cleavage of the alkyl side chain. An additional loss of CO forms the base peak at $m/z = 85$.

Characteristic ions in the higher mass region point to the presence of methyl branches on the alkyl side. A pronounced $M^+ - 15$ ion together with a $M^+ - 43$ ion indicates an *iso*-methyl branching in **1**. Similarly, lactone **2** shows an $M^+ - 29$ ion, together with the respective $M^+ - 57$ ion. Thus, an ethyl group is cleaved preferably, consistent with a *anteiso*-methyl branch in the side chain of the 6-alkanolide. These considerations were supported by the presence of *iso*- and *anteiso*-methyl-branched fatty acids in the lipophilic extract. We therefore proposed that **1** and **2** are 10-methyl-6-undecanolide and 10-methyl-6-dodecanolide, respectively. Both compounds were synthesized to evaluate their biological activities and to prove the suggested structures. A related butyrolactone, 8-methyl-4-decanolide, which occurred in trace amounts in the extract, was tentatively assigned by similar reasoning.

We chose to synthesize **1** and **2** by Baeyer−Villiger oxidation of suitable 2-alkylcyclohexanones, accessible by alkylation of cyclohexanone (see Figure 3). This allowed the synthesis of both racemic and enantiomerically pure material. Control of the stereogenic center at C-6 can be achieved either by enantioselective Baeyer−Villiger oxidation or by enantioselective alkylation of **3**, because it is well known that in the following Baeyer−Villiger oxidation the alkyl group migrates with retention of its stereochemistry.

The racemates of **1** and **2** were synthesized by alkylation of **3** either with 1-bromo-4-methylhexane (**7**), generated by hydroboration of 4-methyl-1-hexene and bromination, or with 1-bromo-4-methylpentane. Final Baeyer−Villiger oxi-

* To whom correspondence should be addressed. Fax: +49 531 391 5272. E-mail: Stefan.Schulz@tu-bs.de.
[†] TU Braunschweig.
[‡] Universität Göttingen.
[§] Alfred-Wegener-Institut, Bremerhaven.
[||] Medizinische Hochschule Hannover.

10.1021/np030321z CCC: $27.50
Published on Web 02/17/2004

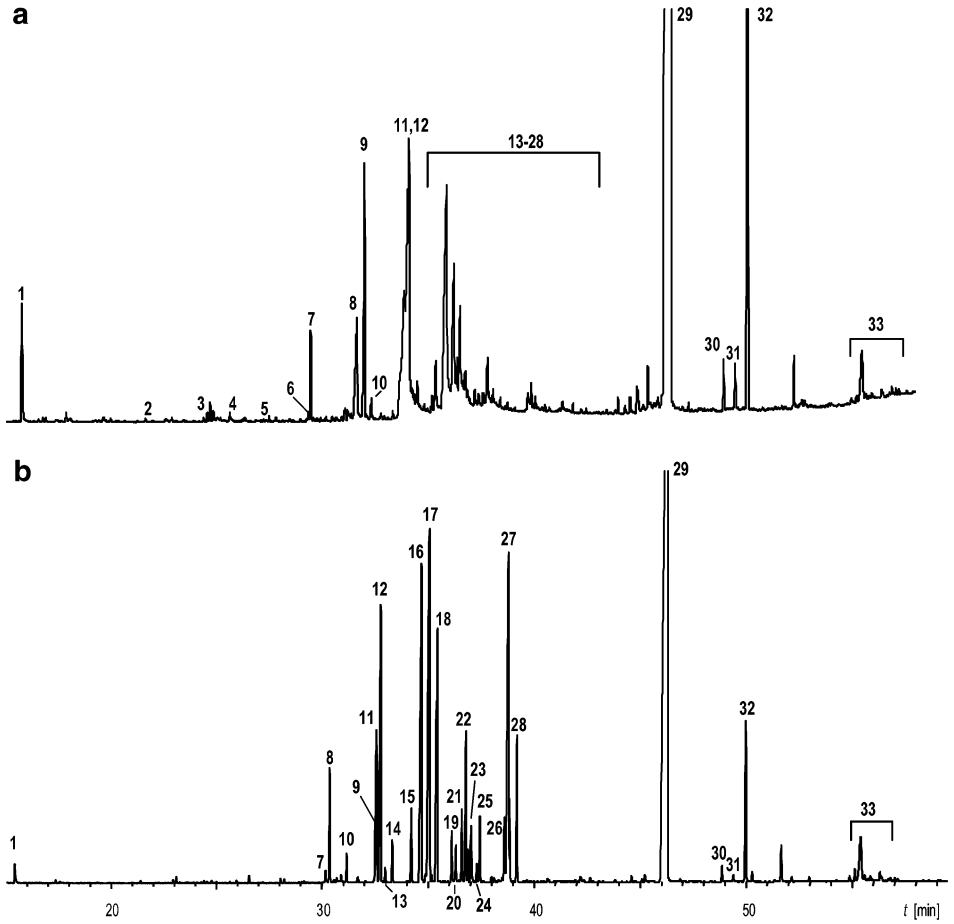


Figure 1. TIC of the cyclohexane extract of the marine *Streptomyces* sp. B6007 before (upper trace) and after methylation (lower trace). 25 m BPX-5, programmed isothermal for 5 min at 50 °C, followed by heating with 5 °C/min to 320 °C. (a) Pure extract. (b) Extract methylated with TMSH. Numbers refer to Table 1. Numbers in b refer to the respective methyl esters. The lactones are opened to methyl hydroxyalkanoates by TMSH.

dation with mCPBA furnished the caprolactones **1** and **2**. Comparison with the natural compounds confirmed our proposed structures.

We then synthesized both lactones stereoselectively to determine the absolute configuration of the natural compounds. The method of Meyers et al., which uses the imine **6** generated from **3** and (*S*)-phenylalaninyl methyl ether as starting material, was chosen for the formation of the stereogenic center at C-6 in an enantioselective alkylation. The Meyers procedure is known to form alkylcyclohexanones with very high ee's,[6] normally higher than those found in broadly applicable enantioselective Baeyer−Villiger oxidations.[7] The alkylation of the metalated imine with 1-bromo-4-methylpentane or **7** furnished the desired cyclohexanones (*R*)-**4** and (2*R*)-**5** after hydrolysis. For the introduction of the second stereogenic center in **2**, the alkyl bromide *S*-**7** was synthesized starting from the commercially available (*S*)-2-methylbutan-1-ol. This alcohol was converted to the bromide,[8] which was used to alkylate diethyl malonate. Subsequent Krapcho dealkoxycarbonylation[9] furnished methyl (*S*)-4-ethylhexanoate (*S*-**8**).[10] Reduction and bromination yielded the enantiomerically pure bromide *S*-**7** (Figure 3), which was used in the imine alkylation with **6** to form 2*R*,4′*S*-**5**. Finally, compounds **4** and **5** were transformed into the respective lactones by Baeyer−Villiger oxidation (Figure 3). To summarize, the following compounds were synthesized by combination of racemic or enantiomeric building blocks: *rac*-**1**, (6*R*)-**1**, *rac*-**2**, (6*R*,10*RS*)-**2**, (6*RS*,10*S*)-**2**, and (6*R*,10*S*)-**2**. These compounds were needed for peak assignment in the following gas chromatographic investigations.

The determination of the configuration of the stereogenic center formed at C-2 was based on the results of Meyers et al.[6] According to these investigations, alkylation of the chiral imine *S*-**6** under the chosen reaction conditions should deliver the (*R*)-enantiomers *R*-**4** and 2*R*-**5**. This agrees with the measured optical rotary power of the alkylation products (Table 2)[6,11] and is corroborated by the octant rule. The length of the alkyl chain should have no influence on the sign of the optical rotation in these compounds. Finally, GC investigations on a chiral cyclodextrin stationary phase enabled the unambiguous determination of the absolute configuration of both alkanolides (Figures 4 and 5). The only naturally occurring enantiomers are (6*R*)-10-methyl-6-undecanolide (*R*-**1**) and (6*R*,-10*S*)-10-methyl-6-dodecanolide (6*R*,10*S*-**2**). Our synthesis provided these compounds in high enantiomeric purity.

With the synthetic material in hand we investigated the biological activity of the lactones. Growth inhibition tests showed a moderate activity against the microalgae *Chlorella vulgaris, C. sorokiniana,* and *Scenedemus subspicatus* as well as *Streptomyces olivaceus* and the fungus *Mucor mihei*. No growth inhibition of *Escherichia coli* and *Bacillus subtilis* could be detected. In tests with *Candida albicans* and *Staphylococcus aureus* **2** showed medium activity, while **1** exhibited none (Table 3).

When the activity against human cancer cell lines was investigated, both compounds caused concentration-de-

Table 1. List of Compounds of the Lipophilic Extract in Isolate B6007[a]

compound	no.
isovaleric acid	
phenol	
benzyl alcohol	
2-phenylethanol	**1**
vinylguaiacol	**2**
geosmin	**3**
methyl 4-hydroxybenzoate	**4**
8-methyldecan-4-olide	**5**
sesquiterpene alcohol	**6**
10-methyldecan-6-olide	**7**
12-methyltridecanoic acid	**8**
10-methylundecan-6-olide	**9**
tetradecanoic acid	**10**
13-methyltetradecanoic acid	**11**
12-methyltetradecanoic acid	**12**
pentadecenoic acid	**13**
pentadecanoic acid	**14**
14-methyl-9-pentadecenoic acid	**15**
14-methylpentadecanoic acid	**16**
9-hexadecenoic acid	**17**
hexadecanoic acid	**18**
15-methyl-9-hexadecenoic acid	**19**
14-methyl-9-hexadecanoic acid	**20**
15-methylhexadecanoic acid	**21**
14-methylhexadecanoic acid	**22**
9-heptadecenoic acid	**23**
heptadecanoic acid	**24**
phthalate (A)	**25**
octadecadienoic acid	**26**
9-octadecenoic acid	**27**
octadecanoic acid	**28**
phthalate (A)	**29**
tetrahydrosqualene	**30**
dihydrosqualene	**31**
squalene	**32**
steroids	**33**

[a] Numbering according to Figure 1. Compounds without numbering are present in trace amounts, only. A: artifact.

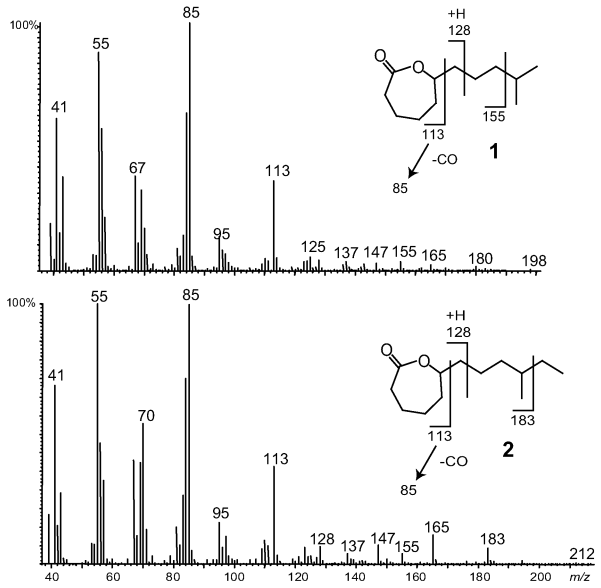


Figure 2. Mass spectra of 6-alkanolides **1** and **2**.

pendent inhibition of the cell growth of HM02 (gastric adenocarcinoma), HepG2 (hepatocellular carcinoma), and MCF7 (breast adenocarcinoma) (Table 4). Further cell cycle analyses were carried out in HepG2 cells, which showed a significant increase in the number of cells in the G1 phase. It was accompanied by a marked reduction of cells in the S phase after exposure to 5 μg mL^{-1} **2** for 24 h (Table 5).

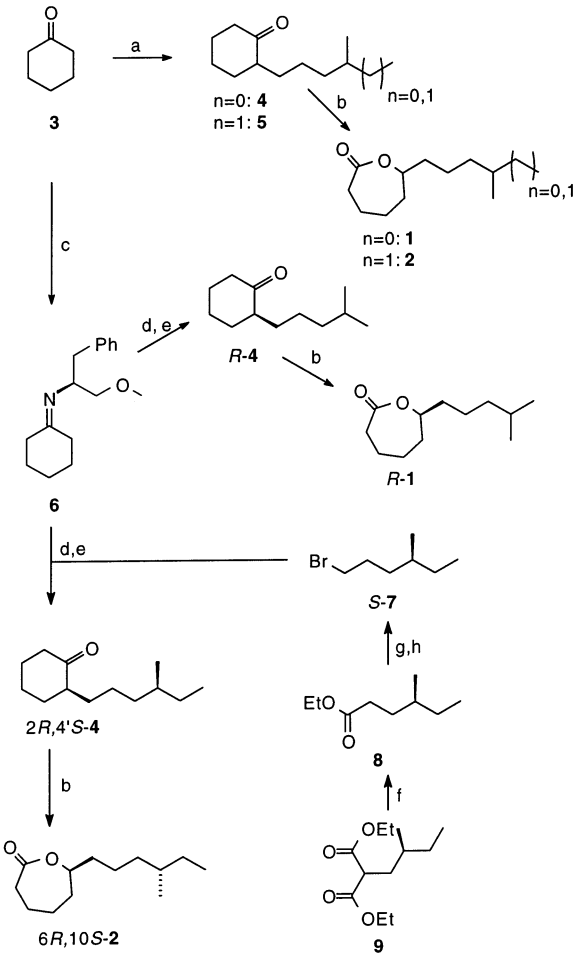


Figure 3. Enantioselective and racemic synthesis of lactones **1** and **2**. (a) KOtBu, *t*-BuOH, 1-bromo-4-methylpentane or **7**; (b) mCPBA; (c) (*S*)-phenylalaninyl methyl ether; (d) LDA, bromo-4-methylpentane or **7**, (e) tartaric acid, pentane; (f) DMSO, H_2O, LiCl; (g) $LiAlH_4$; (h) Br_2, PPh_3.

Compound **2** is thus a cell cycle specific inhibitor of cell growth.

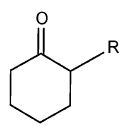
Both alkanolides **1** and **2**, as well as the related butyrolactone 8-methyl-4-decanolide, have not been reported before from a natural source. Simple 6-alkanolides are relatively rare in nature. 6-Hexadecanolide has been isolated from the plant *Ageratina viscosa*.[12] Two oxidized methyl-branched 6-decanolides, feigrisolide A and B, occur in the culture broth of *Streptomyces griseus*.[13] A more complex compound containing a caprolactone moiety is prieurianin from *Nymania capensis*.[14]

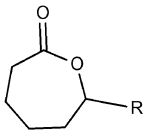
In summary, we have identified and synthesized two new 6-alkanolides from a marine isolate, *Streptomyces* sp. B6007. These compounds show interesting properties against tumor cell lines. Since their structures are relatively simple, synthetic derivatives for structure−activity investigations should be easily accessible.

Experimental Section

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR spectra were obtained with Bruker AC-200 (200 MHz) and AMX-400 (400 MHz) instruments. For NMR experiments, $CDCl_3$ was used with tetramethylsilane as internal standard. GC−MS was carried out with a Hewlett-Packard model 5973 mass selective detector connected to a Hewlett-Packard model 6890 gas chromatograph. Analytical GLC analyses were carried out with a CE Instruments GC 8000 gas chromatograph equipped with a flame ionization detector and split/

Table 2. Optical Rotary Power of Alkylated Cyclohexanones and Caprolactones





R =		% ee	$[\alpha]^{25}_D$ (*c*, sol)		% ee	$[\alpha]^{25}_D$ (*c*, sol)
Et[a]	(*S*)	≥ 98	−24.0 (4, $CHCl_3$)[a]	(*S*)	95	−37 (1.8, $CHCl_3$)[a]
i-Pr[a]	(*S*)	96	−49.2 (2.03, $CHCl_3$)[a]	(*R*)	≥ 98	−15 (2, $CHCl_3$)[a]
Pr[a]	(*R*)	92	−15.0 (1.6, $CHCl_3$)[a]	(*S*)	97	−32 (1.4, $CHCl_3$)[a]
Allyl[a]	(*S*)	≥ 98	−10 (1.6, $CHCl_3$)[a]	(*R*)	≥ 98	−22 (2.2, $CHCl_3$)[a]
4-Methylpentyl	(*R*)		+2.1 (2.2, Et_2O)	(*R*)	93	−1.2 (3.2, Et_2O)
4-Methylhexyl	(*R*)		+9.5 (0.2, Et_2O)	(*R*)	91	−33.4 (3.4, Et_2O)
(*S*)-4-Methylhexyl	(2*R*,4′*S*)		+16.2 (2.2, Et_2O)	(6*R*,10*S*)	94	−20.1 (2.1, Et_2O)

[a] Data taken from the literature.[11]

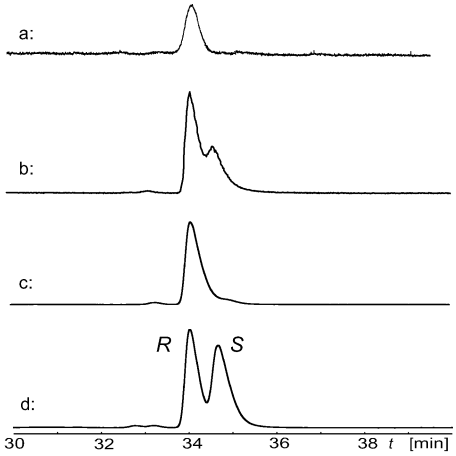


Figure 4. GC investigation of 6-dodecanolide **1** on a 15 m heptakis-(6-*O*-TBDMS-2,3-di-*O*-acetyl)-*β*-cyclodextrin column, programmed from 120 °C to 180 °C with a heating rate of 0.5 °C/min after a 3 min isothermal period: (a) natural extract of B6007; (b) co-injection of natural extract and *rac*-**1**; (c) (*R*)-**1**; (d) *rac*-**1**.

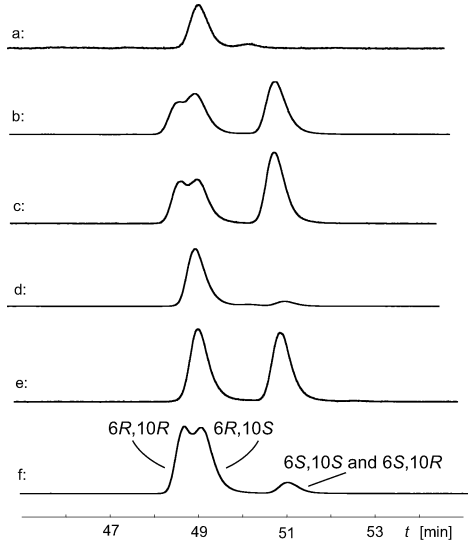


Figure 5. GC investigations of 6-dodecanolide **2** on a 15 m heptakis-(6-*O*-TBDMS-2,3-di-*O*-acetyl)-*β*-cyclodextrin column, programmed from 120 °C to 180 °C with a heating rate of 0.5 °C/min after a 3 min isothermal period: (a) natural extract of B6007; (b) co-injection natural extract and *rac*-**2**; (c) *rac*-**2**; (d) (6*R*,10*S*)-**2**; (e) (6*RS*,10*S*)-**2**; (f) (6*R*,-10*RS*)-**2**.

splitless injection. High-resolution mass spectra were obtained with a Finnigan MAT 90 at 70 eV with perfluorokerosene as reference substance. A 15 m heptakis(6-*O*-TBDMS-2,3-di-*O*-acetyl)-*β*-cyclodextrin capillary column was used for chiral GC separations.[15] The optical rotary power was measured using a Dr. Kernchen Propol digital automatic polarimeter. All reactions were carried out under an inert atmosphere of nitrogen in oven-dried glassware. Dry solvents: THF was distilled from sodium benzophenone, diethyl ether from $LiAlH_4$, and CH_2Cl_2 from CaH_2. Cyclohexylidene-(1-methoxymethyl-2-phenylethyl)amine (*S*-**12**) was obtained according to the known procedure.[6] All other chemicals were commercially available (Fluka, Aldrich) and used without further treatment. All reactions were monitored by thin-layer chromatography (TLC) carried out on Macherey-Nagel Polygram SIL G/UV_{254} silica plates and visualized with heat gun treatment with 10% molybdato phosphoric acid in ethanol. Column chromatography was performed with Merck silica gel 60 (70−200 mesh).

Biological Material. The reference culture of strain B6007 is kept on yeast extract−malt extract agar[16] in the Collection of Marine Actinomycetes at the Alfred-Wegener-Institute for Polar and Marine Research in Bremerhaven.

Table 3. Antimicrobial Activities of **1** and **2** in the Agar Diffusion Test (diameter of inhibition zone in mm at 70 *μ*g/9 mm Ø platelet)

	BS[a]	SA[b]	StV[c]	EC[d]	CA[e]	MM[f]	CV[g]	CS[h]	SS[i]	Art[k]
1	0	0	13	0	0	11	11	12	11	0
2	0	11	15	0	11	15	15	22	20	0

[a] *Bacillus subtilis.* [b] *Streptococcus aureus.* [c] *Streptomyces viridochromogenes.* [d] *Escherichia coli.* [e] *Candida albicans.* [f] *Mucor miehei.* [g] *Chlorella vulgaris.* [h] *Chlorella sorokiniana.* [i] *Scenedesmus subspicatus.* [k] *Artemia salina.*

The yeast extract−malt extract medium was prepared by mixing malt extract (10 g), yeast extract (4 g), and glucose (4 g), which were dissolved in 50% natural or artificial seawater. Before sterilization, the pH was adjusted to 7.8. The cell material from well-grown agar plates of *Streptomyces* sp. isolate B6007 was used to inoculate two 1 L Erlenmeyer flasks, each containing 200 mL of yeast extract−malt extract medium. The culture was grown for 3 days at 95 rpm and 28 °C and

Table 4. Activities (μg mL^{-1}) of Compounds **1** and **2** against Selected Tumor Cell Lines

	HM02	HepG2	MCF7
1	GI_{50}[a] 2.7	GI_{50} 2.2	GI_{50} 0.11
	TGI[b] 6.1	TGI 6.8	TGI 4.0
2	GI_{50} 1.9	GI_{50} 1.4	GI_{50} 0.12
	TGI 5.6	TGI 5.7	TGI 2.6

[a] Drug concentration causing 50% growth inhibition. [b] Drug concentration causing 100% growth inhibition.

Table 5. Cell Cycle Analysis of HepG2 Cells Exposed to 5 μg mL^{-1} of Compound **2**[a]

	subG1-phase	G1-phase	S-phase	G2/M-phase
control	2.1 ± 1.0	63.3 ± 2.4	22.4 ± 1.2	12.1 ± 1.9
2	5.7 ± 5.6	73.7 ± 1.8^b	11.0 ± 2.8^b	9.8 ± 2.4

[a] Data represent percentage of cells in each stage of the cell cycle. Values are means ± SE of three independent experiments. [b] $p < 0.01$ versus control (*t*-test).

then freeze-dried without separation of the cells. The residue was extracted with 100 mL of ethyl acetate, and the solution was evaporated to dryness. The extract was dissolved in methanol (10 mL) and extracted with cyclohexane (10 mL). The cyclohexane phase was evaporated to dryness and the residue (3 mg) subjected to analysis.

Biological Assays. Growth inhibition tests with *Bacillus subtilis*, *Streptococcus aureus*, *Streptomyces viridochromogenes*, *Escherichia coli*, *Candida albicans*, *Mucor miehei*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus subspicatus*, and *Artemia salina* were performed as described earlier.[17]

In vitro growth inhibition effect was determined according to the NCI guidelines in the human cancer cell lines HM02 (gastric adenocarcinoma), HepG2 (hepatocellular carcinoma), and MCF 7 (breast adenocarcinoma).[18]

Cell cycle distribution was determined by staining DNA with propidium iodide. Cells were treated for 24 h with a 10 μg mL^{-1} sample, harvested by trypsination, washed with RPMI 1640 containing 1% fetal bovine serum, and resuspended in 125 μL of a solution containing 150 μg mL^{-1} propidium iodide, 1% Triton X-100, 1% bovine serum albumin, and 4 mM sodium citrate buffer, pH 7.4. After incubation for 15 min at room temperature in the dark, the same volume of Rnase A (10 mg mL^{-1} in 10 mM Tris and 15 mM NaCl, pH 7.4) was added, and the cells were incubated for an additional 30 min at room temperature. At the end of the incubation period cells were analyzed using a Becton Dickinson FACSscan and Lysis II software.

A. General Method for the Alkylation of Cyclohexanone (3).[19] Cyclohexanone (1 equiv) and an alkylbromide (1.1 equiv) were added dropwise to a refluxing 0.15 M solution of potassium *tert*-butoxide (1 equiv) in *tert*-butyl alcohol. The mixture was heated to reflux for 2.5 h and then cooled to room temperature. After acidification with 1 N HCl, the resulting mixture was extracted three times with diethyl ether, the combined organic layers were dried over $MgSO_4$, and the solvent was evaporated. The residue was purified by flash chromatography using pentane/diethyl ether (9:1).

B. General Method for the Enantioselective Alkylation of Cyclohexanonimine 6 at C-2.[6] Diisopropylamine (1.05 equiv, 2 M) in anhydrous THF was cooled to 0 °C, and butyllithium (1.05 equiv, 1.6 M in hexane) was added. The solution was stirred at 0 °C for 15 min and then cooled to −30 °C. A 10 M solution of *S*-**6**[6] (1 equiv) in THF was added over 15 min and stirred for 1.5 h. The mixture was cooled to −78 °C, and then an alkyl bromide (1.05 equiv) diluted in THF (3−4 volumes) was added over a period of 1 h. The reaction was stirred for 1.5 h at −30 °C. The reaction mixture was quenched with saturated NH_4Cl solution and extracted three times with diethyl ether. The combined organic extracts were dried over $MgSO_4$ and concentrated by rotary evaporation. The residue was immediately dissolved in pentane (2 parts) and hydrolyzed with saturated tartaric acid solution (3 parts) by stirring for 30 min at room temperature. Then the layers were separated and the aqueous phase was extracted three times with diethyl ether. The combined organic phases were dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography (pentane/diethyl ether, 9:1).

C. General Method for the Preparation of the Lactones by Baeyer−Villiger Oxidation of Alkylcyclohexanones.[20] To a solution of an alkylcyclohexanone (1 equiv, 0.2 M in CH_2Cl_2) was added *m*CPBA (70%, 2 equiv) at 0 °C. The solution was allowed to warm to room temperature and stirred for 48 h. Then, the reaction mixture was poured into aqueous Na_2SO_3 (1 M). The layers were separated, and the aqueous one was extracted three times with CH_2Cl_2. The combined organic extracts were washed with saturated $NaHCO_3$, followed by brine. After drying over $MgSO_4$, the solvent was evaporated and the residue purified by flash chromatography (pentane/diethyl ether, 5:1) to give a colorless oil (65−89%). The product 6-alkanolides were investigated by GC on a chiral stationary cyclodextrin phase and their ee and dr determined (120 °C, 3 min isotherm, 0.5 °C/min to 170 °C).

4-Methyl-1-hexanol. 4-Methylhex-1-ene (2.2 mL, 15.8 mmol) was dissolved in dry CH_2Cl_2 (10 mL), $HBBr_2{\cdot}SMe_2$ (18 mL, 18 mmol, 1 M in CH_2Cl_2) was added, and the reaction mixture was heated under reflux for 3 h. After the mixture was cooled to room temperature, first methanol (2 mL) and then NaOH (720 mg, 18 mmol), H_2O (4 mL), and H_2O_2 (2.5 mL, 35%) were added, and the mixture was stirred for 2 h. The layers were separated, and the aqueous one was extracted three times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, and the solvent was evaporated. The crude product was purified by flash chromatography using pentane/diethyl ether (2:1) to give 880 mg (48%) of 4-methyl-1-hexanol as a colorless oil: ^{1}H NMR, ^{13}C NMR, and MS data match published data.[21]

1-Bromo-4-methylhexane (7). According to the method of Sonnett,[22] 4-methylhexanol was converted into its bromide to give 1.2 g (92%) of **7** as a colorless oil: ^{1}H NMR data match published data;[23] ^{13}C NMR ($CDCl_3$, 50 MHz) δ 35.0 (CH_2, C-2), 34.3 (CH, C-4), 34.3 (CH_2, C-1), 30.5 (CH_2, C-3), 29.6 (CH_2, C-5), 19.1 (CH_3, 4-CH_3), 11.3 (CH_3, C-6); EIMS *m*/*z* 180 [M + 2]$^+$ (<1), 178 [M]$^+$ (<1), 151 (77), 149 (78), 70 (11), 69 (100), 57 (74), 56 (31), 55 (31), 43 (18), 42 (16), 41 (86), 39 (25).

Diethyl (*S*)-(2-Methylbutyl)malonate (*S*-9). Diethyl malonate (2.0 mL, 13.3 mmol) was added to a stirred solution of NaH (530 mg, 13.3 mmol, 60%) in absolute ethanol (50 mL). After stirring for 1 h, the suspension was cooled to 0 °C and (*S*)-1-bromo-2-methylbutane[8] (2.0 g, 13.3 mmol) was added over a period of 1 h. The reaction mixture was heated to reflux overnight, then the solvent was evaporated and the residue acidified at 0 °C with 1 N HCl. After extraction with diethyl ether (three times) and drying of the organic phase with $MgSO_4$, the solvent was evaporated and the crude product purified by flash chromatography (pentane/diethyl ether, 9:1) to give 2.4 g (77%) of *S*-**9** as a colorless oil: $[\alpha]^{25}_D$ +11 (*c* 3.83, diethyl ether), ref $[\alpha]^{25}_D$ +13.23 (neat);[24] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.19 (2H, q, J = 7.1 Hz, OCH_2CH$_3$), 4.18 (2H, q, J = 7.1 Hz, OCH_2CH$_3$), 3.43 (1H, dd, J = 6.6 Hz, J = 8.7 Hz, H-2), 2.00−1.93 (1H, m, H-3), 1.71−1.64 (1H, m, H-3), 1.41−1.29 (2H, m), 1.26 (6H, t, J = 7.1 Hz, OCH$_2$*CH*$_3$), 1.23−1.14 (1H, m, H-5), 0.89 (3H, d, J = 6.4 Hz, 4-CH_3), 0.88−0.84 (3H, m, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9 (C), 169.7 (C), 61.3 (CH_2, OCH_2), 61.2 (CH_2, OCH_2), 50.2 (CH, C-2), 35.4 (CH_2), 29,2 (CH_2), 18.7 (CH_3, 2′-CH_3), 14.1 (CH_3, 2 × *C*H$_3$CH$_2$O), 11.1 (CH_3, C-4′); EIMS *m*/*z* 230 [M]$^+$ (<1), 185 (20), 173 (31), 160 (100), 133 (40), 132 (14), 127 (23), 114 (11), 101 (19), 99 (11), 88 (15), 86 (11), 83 (14, 73 (22), 69 (13), 55 (28), 41 (14).

Ethyl (*S*)-4-Methylhexanoate (*S*-8). The malonate *S*-**9** (600 mg, 2.6 mmol), H_2O (45 mL), and LiCl (220 mg, 5.2 mmol) were heated to reflux for 4 h. The reaction mixture was concentrated, and the residue was diluted with water (6 mL) and separated. The aqueous layer was extracted three times with diethyl ether, and the combined organic phases were dried with $MgSO_4$. After evaporation of the solvent the residue was purified by flash chromatography (pentane/diethyl ether, 19:1), affording 322 mg (78%) of *S*-**8** as a colorless oil: $[\alpha]^{25}_D$

+5.5 (*c* 3.25, diethyl ether); ^{1}H NMR data match published data;[25] ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.2 (C, C-1), 60.2 (CH_2, OCH_2), 34.0 (CH, C-4), 32.2 (CH_2), 31.5 (CH_2), 29.2 (CH_2), 18.8 (CH_3, 4-CH_3), 14.3 (CH_3, CH_3CH_2O), 11.3 (CH_3, C-6); EIMS *m/z* 158 $[M]^+$ (<1), 129 (16), 113 (35) 102 (11), 101 (100), 95 (35), 83 (24), 74 (16), 73 (33), 71 (17), 70 (28), 69 (22), 61 (15), 60 (19), 57 (23), 56 (16), 55 (40), 45 (12), 43 (36), 42 (11), 41 (39), 39 (14).

(+)-(*S*)-4-Methylhexanol. To a suspension of $LiAlH_4$ (42 mg, 1.1 mmol) in diethyl ether (2 mL) was added dropwise *S*-**8** (320 mg, 2 mmol) in diethyl ether (1 mL). After stirring 1 h at room temperature, the reaction mixture was heated to reflux for 3 h. Ice water (2 mL) was added, the solution was extracted three times with diethyl ether, and the combined organic extracts were dried with $MgSO_4$. The product was obtained almost quantitatively after evaporation of the solvent. No further purification was necessary. $[\alpha]^{25}_D$ +4.7 (*c* 6.50, diethyl ether), ref $[\alpha]^{25}_D$ +7.64 (*c* 4.01, $CHCl_3$); for NMR and MS data, see ref 21.

(*S*)-1-Bromo-4-methylhexane (*S*-7). According to the method of Sonnett,[22] (*S*)-4-methylhexanol was transformed into its bromide to give 550 mg (73%) of *S*-**7** as a colorless oil. For NMR and MS data see **7**.

(+)-(2*R*,4′*S*)-2-(4-Methylhexyl)cyclohexanone (2*R*,4′*S*-5). See method B. The bromide *S*-**7** was used as starting material to give 71 mg (66%) of 2*R*,4′*S*-**5** as a colorless oil: $[\alpha]^{25}_D$ +16.2 (*c* 2.19, diethyl ether); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.41−2.36 (1H, m, H-6), 2.34−2.29 (2H, m) 2.12−2.08 (1H, m), 2.04−1.97 (1H, m), 1.88−1.04 (13H, m), 0.88−0.83 (6H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 213.6 (C, C-1), 50.8 (CH, C-2), 41.9 (CH_2, C-6), 36.7 (CH_2), 34.3 (CH), 33.8 (CH_2), 29.7 (CH_2), 29.4 (CH_2), 28.0 (CH_2), 24.8 (CH_2), 24.6 (CH_2), 19.1 (CH_3), 11.4 (CH_3); EIMS *m/z* 196 $[M]^+$ (3), 111 (10), 98 (100), 97 (11), 83 (16), 55 (18), 41 (18).

(+)-(2*R*,4′*RS*)-2-(4-Methylhexyl)cyclohexanone (2*R*-5). See method B. Racemic **7** was used as starting material to give 200 mg (61%) of 2*R*-**5** as a colorless oil. For NMR and MS data see (2*R*,4′*S*)-**5**. $[\alpha]^{25}_D$ +9.5 (*c* 0.22, diethyl ether).

(2*RS*,4′*S*)-2-(4-Methylhexyl)cyclohexanone (4′*S*-5). See method A. The bromide (*S*)-**7** was used as alkylation agent to give 12 mg (38%) of 4′*S*-**5** as a colorless oil. For NMR and MS data see 2*R*,4′*S*-**5**.

2-(4-Methylhexyl)cyclohexanone (5). See method A. Racemic **7** was used as starting material to give 4 mg (32%) of **5** as a colorless oil. For NMR and MS data see 2*R*,4′*S*-**5**.

10-Methyl-6-dodecanolide (2). See method C starting from racemic **5** to give 4 mg (87%) of **2** as a colorless oil. For NMR and MS data see 6*R*,10*S*-**2**. GC experiments on a chiral stationary cyclodextrin phase delivered the following retention times (120 °C, 3 min isothermal, then with 0.5 °C/min to 170 °C): $t_{R6R,10R-2}$ = 54.88 min, $t_{R6R,10S-2}$ = 55.43 min, $t_{R6S,10R-2}$ = $t_{R6R,10S-2}$ = 57.74 min.

(−)-(6*R*,10*S*)-10-Methyl-6-dodecanolide (6*R*,10*S*-2). See method C starting with 2*R*,4′*S*-**5** to give 49 mg (66%) of 6*R*,-10*S*-**2** as a colorless oil: $[\alpha]^{25}_D$ −20.1 (*c* 2.01, diethyl ether); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.23 (1H, dt, J = 8.1 Hz, J = 4.1 Hz, H-6), 2.70−2.57 (2H, m, H-2), 1.96−1.88 (3H, m), 1.75−1.08 (12H, m), 0.85 (3H, d, J = 5,7 Hz, 10-CH_3), 0.85 (3H, t, J = 7.4 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.8 (C, C-1), 80.4 (CH, C-6), 36.8 (CH_2, C-7), 36.3 (CH_2, C-9), 35.0 (CH_2, C-2), 34.6 (CH_2, C-5), 34.3 (CH, C-10), 29.3 (CH_2, C-11), 28.3 (CH_2, C-4), 23.1 (CH_2, C-3), 23.0 (CH_2, C-8), 19.2 (CH_3, 10-CH_3), 11.4 (CH_3, C-12); EIMS *m/z* 212 $[M]^+$ (<1), 113 (32), 95 (17), 85 (100), 84 (66), 83 (24), 81 (16), 71 (12), 70 (55), 69 (37), 68 (12), 67 (41), 57 (29), 56 (43), 55 (96), 43 (23), 42 (14), 41 (63), 39 (18); HREIMS *m/z* 212.1774 (calcd for $C_{13}H_{24}O_2$, 212.1776); ee > 94%, dr = 94/6.

(−)-(6*R*,10*RS*)-10-Methyl-6-dodecanolide (6*R*-2). See method C starting with 2*R*-**5** to give 160 mg (89%) of 6*R*-**2** as a colorless oil: $[\alpha]^{25}_D$ −33.4 (*c* 3.40, diethyl ether); for NMR and MS data see **2**; ee = 91%.

(+)-(6*RS*,10*S*)-10-Methyl-6-dodecanolide (10*S*-2). See method C starting with 10*S*-**5** to give 8 mg (82%) of 10*S*-**2** as a colorless oil: $[\alpha]^{25}_D$ +8.8 (*c* 0.69, diethyl ether); for NMR and MS data see **2**; ee > 99%.

(+)-(*R*)-2-(4-Methylpentyl)cyclohexanone (*R*-4). See method B using 1-bromo-5-methylpentane as starting material to give 262 mg (63%) of *R*-**4** as a colorless oil: $[\alpha]^{25}_D$ +2.1 (*c* 2.23, diethyl ether); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.36−2.31 (1H, m), 2.30−2.22 (1H, m), 2.21−2.16 (1H, m), 2.07−2.01 (1H, m), 1.99−1.92 (1H, m), 1.82−1.75 (1H, m), 1.73−1.65 (1H, m), 1.64−1.54 (2H, m), 1.51−1.41 (1H, m), 1.34−1.30 (1H, m), 1.25−1.04 (5H, m), 0.79 (6H, d, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 213.6 (C, C-1), 50.8 (CH, C-2), 42.0 (CH_2, C-6), 39.1 (CH_2), 33.8 (CH_2), 29.6 (CH_2), 28.0 (CH_2), 27.9 (CH), 24.9 (CH_2), 24.8 (CH_2), 22.61 (CH_3), 22.56 (CH_3); EIMS *m/z* 182 $[M]^+$ (4), 111 (10), 98 (100), 97 (12), 83 (19), 70 (12), 55 (19), 41 (20).

2-(4-Methylpentyl)cyclohexanone (4). See method A starting with 1-bromo-5-methylpentane to give 87 mg (44%) of **4** as a colorless oil: see *R*-**3** for NMR and MS data.

10-Methyl-6-undecanolide (1). See method C starting with **4** to give 26 mg (55%) of **1** as a colorless oil: see *R*-**1** for NMR and MS data. GC experiments on chiral stationary cyclodextrin phase delivered the following retention times (120 °C isothermal for 3 min, then with 0.5 °C/min to 170 °C): t_{RS-1} = 34.01 min, t_{RR-1} = 34.65 min.

(−)-(*R*)-10-Methyl-6-undecanolide (*R*-1). See method D using *R*-**3** as starting reagent to give 163 mg (75%) of *R*-**1** as a colorless oil: $[\alpha]^{25}_D$ −1.2 (*c* 3.20, diethyl ether); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.23 (1H, dt, J = 8.0 Hz, J = 4.2 Hz, H-6), 2.70−2.56 (2H, m, H-2), 2.00−1.84 (3H, m), 1.77−1.24 (8H, m), 1.18−1.23 (2H, m, H-9), 0.87 (6H, d, J = 6.6 Hz, 10-CH_3, H-12); ^{13}C NMR δ 175.8 (C, C-1), 80.6 (CH, C-6), 38.7 (CH_2, C-9), 36.7 (CH_2, C-7), 34.9 (CH_2, C-2), 34.6 (CH_2, C-5), 28.3 (CH_2, C-3), 27.9 (CH, C-10), 23.2 (CH_2, C-8), 23.1 (CH_2, C-4), 22.6 (CH_3, C-11), 22.52 (CH_3, 10-CH_3); EIMS *m/z* 198 $[M]^+$ (<1), 113 (36), 95 (14), 85 (100), 84 (64), 83 (14), 70 (17), 69 (30), 68 (11), 67 (37), 57 (20), 56 (57), 55 (85), 43 (36), 42 (14), 41 (55), 39 (17); HREIMS 198.1612 (calcd for $C_{12}H_{22}O_2$, 198.1620); ee = 93%.

8-Methyl-4-dodecanolide. EIMS *m/z* 166 $[M - 18]^+$ (2), 155 (5), 137 (14), 128 (9), 127 (6), 110 (13), 109 (12), 100 (13), 95 (18), 85 (100), 83 (15), 70 (34), 69 (18), 57 (21), 56 (14), 55 (29), 43 (15), 42 (9), 41 (33).

Acknowledgment. This work was financed by a grant from the Volkswagenstiftung within the Lower Saxony Cooperative Research Project of Marine Biotechnology. Financial support by the Fonds der Chemischen Industrie is gratefully acknowledged.

References and Notes

(1) Maskey, R. P.; Helmke, E.; Laatsch, H. *J. Antibiot.* **2002**, *55*, 1031−1035, and previous articles of this series.
(2) Jensen, P. R.; Fenical, W. In *Drugs From the Sea*; Fusetani, N., Ed.; Karger: Basel, 2000; pp 6−29.
(3) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2003**, *20*, 1−48. Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1−48, and previous reviews of this series.
(4) Müller, K.-D.; Husmann, H.; Nalik, H. P.; Schomburg, G. *Chromatographia* **1990**, *30*, 245−248.
(5) Leonhardt, B. A.; DeVilbiss, E. D. *J. Chromatogr.* **1985**, *322*, 484−490.
(6) Meyers, A. I.; Williams, D. R.; Erickson, G. W.; White, S.; Druelinger, M. *J. Am. Chem. Soc.* **1981**, *103*, 3081−3087.
(7) Bolm, C.; Luong, T. K. K.; Beckmann, O. In *Asymmetric Oxidation Reactions*; Katsuki, T., Ed.; Oxford University Press: Oxford, 2001; pp 147−152.
(8) Bergmann, J.; Loefstedt, C.; Ivanov, V. D.; Francke, W. *Eur. J. Org. Chem* **2001**, 3175−3180.
(9) Krapcho, A. P. *Synthesis* **1982**, 805−822.
(10) Thakar, K. A.; Pathak, U. S. *J. Indian Chem. Soc.* **1965**, *42*, 109−111.
(11) Stewart, J. D.; Reed, K. W.; Zhu, J.; Chen, G.; Kayser, M. M. *J. Org. Chem.* **1996**, *61*, 7652−7653.
(12) Torrenegra, R.; Pedrozo, J. A.; Robles, J.; Fuentes, O. *Phytochemistry* **1990**, *29*, 305−306.
(13) Tang, Y.-Q.; Sattler, I.; Thiericke, R.; Grabley, S.; Feng, X.-Z. *J. Antibiot.* **2000**, *53*, 934−943.
(14) MacLachlan, L. K.; Taylor, D. A. H. *Phytochemistry* **1982**, *21*, 1701−1704.
(15) König, W. A. *Chirality* **1998**, 499−504.

(16) Weyland, H. *Zbl. Bakt. Suppl.* **1981**, *11*, 185−193.
(17) Biabani, M. A. F.; Baake, M.; Lovisetto, B.; Laatsch, H.; Helmke, E.; Weyland, H. *J. Antibiot.* **1998**, *58*, 333−340.
(18) Grever, M. R.; Schepartz, S. A.; Chabner, B. A. *Semin. Oncol.* **1992**, *19*, 622−638.
(19) Braude, C. *J. Chem. Soc.* **1950**, 2014−2019.
(20) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399−402.
(21) Larsson, M.; Nguyen, B.-V.; Högberg, H.-E.; Hedenström, E. *Eur. J. Org. Chem.* **2001**, 353−363.
(22) Sonnet, P. E. *Synth. Commun.* **1976**, *6*, 21−26.
(23) Mori, K.; Suguro, T.; Uchida, M. *Tetrahedron* **1978**, *34*, 3119−3123.
(24) Rossi, R.; Salvadori, P. A. *Synthesis* **1979**, 209−210.
(25) Takahashi, Y.; Yuasa, K.; Tokuda, M.; Itoh, M.; Suzuki, A. *Bull. Chem. Soc. Jpn.* **1978**, *58*, 339−340.

NP030321Z