Pergamon

Tetrahedron Vol. 50, No. 34, pp. 10201-10206, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00597-4

Convolutamides A ~ F, Novel γ-Lactam Alkaloids from the Marine Bryozoan *Amathia convoluta*

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Abstract: Convolutamides A ~ F, new alkaloids containing an *N*-acyl-γ-lactam moiety with a dibromophenol group, have been isolated from the Floridian bryozoan *Amathia convoluta* and the structures elucidated on the basis of spectroscopic data.

Marine bryozoans have proven to be a rich source of unique secondary metabolites,[1,2] among which bryostatins[3] are well-known as a candidate of anticancer drugs obtained from *Bugula neritina* (Bryozoa phylum) as well as from *Amathia convoluta*.[4] During our search for new bioactive substances from marine organisms, we have examined extracts of the Floridian bryozoan *Amathia convoluta* and obtained new alkaloids, convolutamides A (**1a**), B (**1b**), C (**2a**), D (**2b**), E (**3a**), and F (**3b**), possessing an *N*-acyl-γ-lactam moiety with a dibromophenol group. Here we wish to report the isolation and structure elucidation of them.

The bryozoan *Amathia convoluta*, collected off Northeastern Gulf of Mexico in Florida, was extracted with EtOH. The extract was partitioned between aqueous MeOH and hexane, and the aqueous MeOH phase was further extracted with EtOAc. The EtOAc-soluble material was subjected to repeated chromatographies on silica gel and ODS columns, followed by gel filtration on Sephadex LH-20. Further purification using reversed-phase HPLC afforded a (1:1.7) mixture (**1**) of convolutamides A (**1a**) and B (**1b**) in 2×10^{-8} % yield (wet weight), a (1.8:1) mixture (**2**) of convolutamides C (**2a**) and D (**2b**) in 2×10^{-8} % yield, and a (7.9:1) mixture (**3**) of convolutamides E (**3a**) and F (**3b**) in 6×10^{-9} % yield. It was unsuccessful to isolate each pure component from these three mixtures (**1** ~ **3**) using several types of normal and reversed phase HPLC columns under various solvent systems. Structural studies of convolutamides were, however, able to be carried out by extensive spectral investigations using each of the three mixtures (**1** ~ **3**).

The positive FABMS of **1** showed quasi-molecular ions $[(M+H)^+]$ at *m/z* 560, 562 and 564 for **1a**, and at *m/z* 586, 588 and 590 for **1b**, respectively, in a ratio of *ca.* 1:2:1, suggesting the presence of two bromine atoms for each component (**1a** and **1b**). The molecular formulas of **1a** and **1b** were determined as

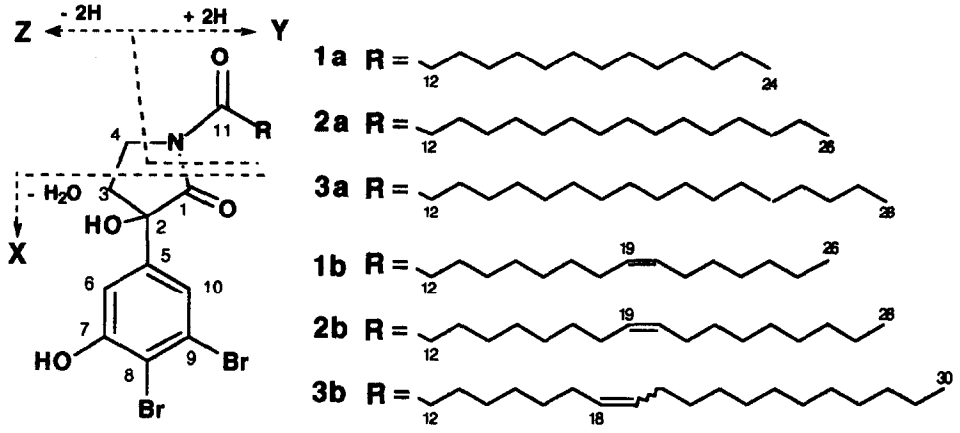


Table 1. ^{1}H and ^{13}C NMR Data of Convolutamides A (**1a**) and B (**1b**) in $CDCl_3$

position	^{1}H	*J* (Hz)	^{13}C	position	^{1}H	^{13}C	position	^{1}H	^{13}C
for **1a** and **1b**				for **1a**			for **1b**		
1			177.7	12	2.10 t	33.9	12	2.10 t	33.9
2			77.6[a]	13	1.51 m	24.7	13	1.51 m	24.7
2-OH	3.00 s			14	1.25~1.32 br s	29.0	14	1.25~1.32 br s	29.2
3a	2.52 ddd	14.3, 7.9, 5.3	34.5	15	1.25~1.32 br s	29.7	15	1.25~1.32 br s	29.2
3b	2.76 ddd	14.3, 6.2, 5.3		16	1.25~1.32 br s	29.7	16	1.25~1.32 br s	29.2
4a	3.91 ddd	11.8, 7.9, 5.3	59.3	17	1.25~1.32 br s	29.7	17	1.25~1.32 br s	29.7
4b	4.10 ddd	11.8, 6.2, 5.3		18	1.25~1.32 br s	29.7	18	2.01 m	27.2
5			126.7	19	1.25~1.32 br s	29.7	19	5.35 m	129.7
6	6.99 br d		112.9	20	1.25~1.32 br s	29.7	20	5.35 m	130.0
7			143.2	21	1.25~1.32 br s	29.7	21	2.01 m	27.2
7-OH	7.35 s			22	1.25~1.32 br s	31.8	22	1.25~1.32 br s	29.7
8			124.2	23	1.25~1.32 br s	22.7	23	1.25~1.32 br s	29.5
9			120.5	24	0.88 t	14.1	24	1.24 m	31.9
10	7.38 br d		129.5				25	1.27 m	22.7
11			173.3				26	0.88 t	14.1

[a]The chemical shift was observed in CD_3OD.

$C_{24}H_{35}NO_4Br_2$ and $C_{26}H_{37}NO_4Br_2$, respectively, by HRFABMS data [**1a**: *m/z* 560.1009 $(M+H)^+$ for $C_{24}H_{36}NO_4{}^{79}Br_2$, Δ +0.2 mmu; **1b**: *m/z* 586.1132 $(M+H)^+$ for $C_{26}H_{38}NO_4{}^{79}Br_2$, Δ +3.6 mmu]. Interpretation of the ^{1}H and ^{13}C NMR data of **1** (Table 1) facilitated by application of several types of 2D NMR spectra (^{1}H-^{1}H COSY, HMQC,[5] HMBC,[6] and HMQC-HOHAHA[7]) suggested that **1** consisted of partial structures of a dibromophenol group, a five membered-lactam moiety, and an acyl side-chain. NMR signals of **1** for the aromatic ring and γ-lactam moieties were singly observed, while those for the acyl chain part were observed as split signals due to two components (**1a** and **1b**).

The ^{1}H and ^{13}C NMR signals for the aromatic portion (C-5 ~ C-10) in **1** suggested the presence of a 1,2,3,5-tetrasubstituted benzene ring, which was verified by the HMBC cross-peaks for H-6/C-7, H-6/C-8, H-10/C-8, and H-10/C-9. The ^{13}C NMR chemical shifts for quaternary aromatic carbons of C-7 (δ_C 143.2 s), C-8 (δ_C 124.2 s), and C-9 (δ_C 120.5 s) implied that bromine atoms were substituted at C-8 and C-9, and a hydroxyl group was attached at C-7. The presence of a phenol group was further supported by the ^{1}H NMR

spectra of **1** in a basic solution; on addition of alkali, the ^{1}H NMR signals due to H-6 and H-10, ortho and para hydrogens of the phenolic hydroxyl group, respectively, shifted to higher fields [CD_3OD, $\delta_{H\text{-}6}$ 7.45 and $\delta_{H\text{-}10}$ 7.18; CD_3OD + NaOD, $\delta_{H\text{-}6}$ 7.10 and $\delta_{H\text{-}10}$ 6.99].[8] A ^{1}H NMR signal due to the hydroxyl proton on C-7 was observed at δ_H 7.35 in $CDCl_3$ and disappeared by addition of D_2O.

The partial structure of the γ-lactam moiety (C-1 ~ C-4) in **1** was deduced from the COSY correlations between H_2-3 and H_2-4 as well as the HMBC cross-peaks for H_2-3/C-1, H_2-3/C-2, and H_2-4/C-2. The IR spectrum of **1** showed three absorption bands at the carbonyl region (1740, 1720, and 1700 cm^{-1}); this observation was also consistent with the presence of the γ-lactam moiety.[9] The dibromophenol unit (C-5 ~ C-10) was shown to be connected to C-2 position by the HMBC cross-peak for H_2-3/C-5, and C-2 also bears a tertiary hydroxyl group, which was suggested by its ^{13}C chemical shift (δ_C 77.6 s). The nitrogen atom of the lactam ring was revealed to be acylated by the HMBC cross-peak for H_2-4/C-11. The ^{13}C chemical shifts (δ_C 177.7 and 173.3) for the carbonyl signals due to C-1 and C-11, respectively, agreed well with those of an *N*-acylated-2-pyrrolidinone system.[10]

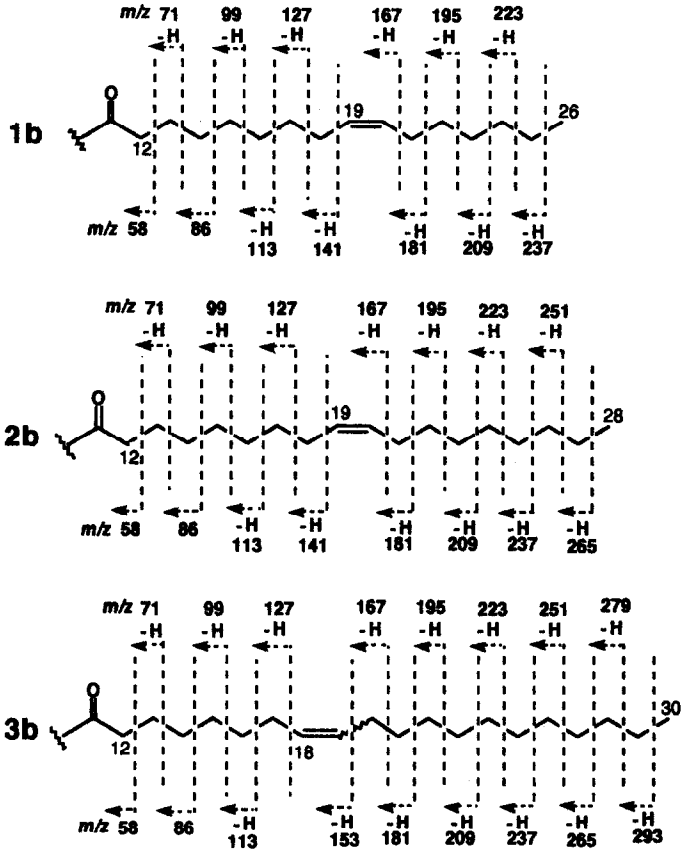
The partial structure for the γ-lactam moiety proposed above was further supported by the negative and positive FAB MS/MS spectral data. The presence or absence of bromine atoms in a particular daughter ion was clearly revealed by comparison of the negative MS/MS of two precursor ions of **1a** [*m/z* 558 $(M\text{-}H)^-$ with two ^{79}Br atoms and *m/z* 560 $(M\text{-}H)^-$ with one ^{79}Br and one ^{81}Br atoms]; the MS/MS spectra from both precursor ions showed the same mass numbers for daughter ions containing no bromine atoms (*e.g.*, ion Y^-), while those containing bromine atoms [*e.g.*, ion $(X + H)^-$] have different mass numbers (Table 2). The FAB MS/MS spectra also revealed that the structural differences between **1a** and **1b** were found only in the acyl side-chain part. Daughter ions due to the γ-lactam moiety [$(X + H)^-$, $(X - Br)^-$, and $(X - Br - CO)^-$] were commonly observed in the MS/MS spectra of both precursor ions for **1a** (*m/z* 558) and **1b** (*m/z* 584), whereas daughter ions for the acyl part [$(Y)^-$][11] had the different mass numbers (*m/z* 227 and 253, respectively). Thus, **1b** was inferred to contain 26 mass unit larger than **1a** as to the acyl chain part. The acyl part of **1a** was assigned as myristoyl group [$CH_3(CH_2)_{12}CO$; $C_{14:0}$] since the MS/MS spectrum for the precursor ion of *m/z* 227 (Y^- for **1a**) was almost completely identical with that of authentic myristic acid [precursor ion, *m/z* 227 $(M - H)^-$]. The acyl part of **1b**, on the other hand, was deduced as 9-hexadecenoyl group ($C_{15}H_{29}CO$; $C_{16:1}$) from the MS/MS spectrum for the precursor ion of *m/z* 253 (Y^- for **1b**), daughter ions of which, particularly the intense ones at *m/z* 127 and 181, suggested that a double bond was located at C-19 position (Figure 1). In the ^{1}H NMR spectrum of **1**, signals due to olefinic protons were observed at δ_H 5.35 (H-19 and H-20 of **1b**) with an intensity corresponding to 1.26 H normalized to the signal for H-6 (δ_H 6.99, contained in both **1a** and

Table 2. Negative FAB MS/MS Data of Convolutamides A ~ F (**1a**, **1b**, **2a**, **2b**, **3a**, and **3b**)

1a[a]	1a[b]	1b[a]	2a[a]	2a[b]	2b[a]	3a[a]	3a[b]	3b[a]	Assignments
m/z	*m/z*	*m/z*	*m/z*	*m/z*	*m/z*	*m/z*	*m/z*	*m/z*	
558	560	584	586	588	612	614	616	640	$(M - H)^-$, precursor ion
303	305	303	303	305	303	303	305	303	$(X + H)^-$
227	227	253	255	255	281	283	283	309	$(Y)^-$
223	225 and 223	223	223	225 and 223	223	223	225 and 223	223	$(X - Br)^-$
195	197 and 195	195	195	197 and 195	195	195	197 and 195	195	$(X - Br - CO)^-$

[a]With two ^{79}Br atoms; [b]With one ^{79}Br and one ^{81}Br atoms

Figure 1. Daughter Ions Observed in the Negative FAB MS/MS for the Acyl Part of **1b**, **2b**, and **3b**



1b) as 1 H; based on this fact, the ratio of convolutamides A (**1a**) and B (**1b**) was estimated as 1:1.7. The ^{13}C chemical shifts for the allyl methylene carbons of C-18 and C-21 (both δ_C 27.2) suggested 19Z-configuration for **1b**.[12]

The ^1H NMR spectra of **2** and **3** closely resembled that of **1**, and both of **2** and **3** consisted of two components (**2a**/**2b** and **3a**/**3b**), whose ratios were estimated by ^1H NMR spectra as 1.8:1 and 7.9:1, respectively. The FABMS of **2** showed quasi-molecular ion peaks [$(M+H)^+$] at *m/z* 588, 590, and 592 (*ca.* 1:2:1) for **2a** and *m/z* 614, 616, and 618 (*ca.* 1:2:1) for **2b**, while the FABMS of **3** afforded $(M+H)^+$ peaks at *m/z* 616, 618, and 620 (*ca.* 1:2:1) for **3a** and *m/z* 642, 644, and 646 (*ca.* 1:2:1) for **3b**. These MS data suggested that convolutamides C (**2a**) and E (**3a**) are homologues of convolutamide A (**1a**) with two and four more CH_2 groups, respectively; convolutamides D (**2b**) and F (**3b**) are also homologues of convolutamide B (**1b**) with two and four more CH_2 groups, respectively. This inference was verified by the HRFABMS data, which revealed their molecular formulas as follows: **2a**, $C_{26}H_{39}NO_4Br_2$; **2b**, $C_{28}H_{41}NO_4Br_2$; **3a**, $C_{28}H_{43}NO_4Br_2$; **3b**, $C_{30}H_{45}NO_4Br_2$. Both positive FAB MS/MS spectra of **2** and **3** [precursor ions, *m/z*

588 $(M+H)^+$ for **2a** and *m/z* 616 $(M+H)^+$ for **3a**] exhibited characteristic daughter ions at *m/z* 332, 314, and 302, which were also observed in the positive MS/MS of **1** [precursor ion, *m/z* 560 $(M+H)^+$ for **1a**] and assignable to ions of $(Z)^+$, $(Z - H_2O)^+$, and $(X)^+$, respectively. These results argued that the γ-lactam moiety is commonly embraced by convolutamides C (**2a**) and E (**3a**) and they contain homologous acyl side-chains. This finding was consistent with the negative FAB MS/MS data (Table 2), which also suggested that convolutamides D (**2b**) and F (**3b**) possess the same γ-lactam nucleus and homologous acyl chains. The negative FAB MS/MS of **2** and **3** [precursor ions, *m/z* 255 (Y^-) for **2a** and *m/z* 283 (Y^-) for **3a** (Figure 1)] closely corresponded to those of palmitic acid [precursor ion, *m/z* 255 $(M - H)^-$] and stearic acid [precursor ion, *m/z* 283 $(M - H)^-$], respectively. Thus the acyl side-chains of **2a** and **3a** were assigned as palmitoyl [$CH_3(CH_2)_{14}CO$; $C_{16:0}$] and stearoyl [$CH_3(CH_2)_{16}CO$; $C_{18:0}$] groups, respectively. On the other hand, the acyl chain moieties for **2b** and **3b** were also deduced from the negative FAB MS/MS data [precursor ions, *m/z* 281 (Y^-) for **2b** and *m/z* 309 (Y^-) for **3b**] to be 9-octadecenoyl ($C_{17}H_{33}CO$; $C_{18:1}$) and 8-eicosenoyl ($C_{19}H_{37}CO$; $C_{20:1}$) groups, respectively; particularly, the intense daughter ions (*m/z* 127 and 181 for **2b** and *m/z* 113 and 167 for **3b**) implied the positions of the double bonds (Figure 1). The geometry of the double bond in the acyl chain of **2b** was *Z*, indicated by the ^{13}C NMR chemical shifts of the allyl methylene carbons (δ_C 27.2 for both C-18 and C-21). Since satisfactory ^{13}C NMR data of **3a** and **3b** were not obtained due to small quantity of the sample, the geometry of $\Delta^{18,19}$-double bond of **3b** remained unassigned.

From these results, the structures of convolutamides A ~ F were concluded as **1a**, **1b**, **2a**, **2b**, **3a**, and **3b**, respectively. Convolutamides may belong to an unprecedented class of natural products possessing a structurally unique γ-lactam and dibromophenol ring system, and their biogenetic provenance is presently unknown. The mixture (**1**) of convolutamides A and B exhibited cytotoxicity against L1210 murine leukemia and KB human epidermoid carcinoma cells with IC_{50} values of 4.8 and 2.8 μg/mL, respectively, while **2** and **3** showed no cytotoxicity (IC_{50}, >10 μg/mL).

Experimental Section

General Methods. Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. UV and IR spectra were taken on a JASCO Ubest-35 spectrometer and a JASCO Report-100 infrared spectrometer, respectively. The NMR spectra were measured on JEOL JMN GX-270 and EX-400, or Bruker ARX-500 spectrometers. FAB mass spectra were obtained on a JEOL HX-110 spectrometer.

Collection, Extraction and Isolation. The bryozoan *Amathia convoluta* (Bryozoan phylum) was collected off the Northeastern Gulf of Mexico, Florida, in 1982. The *A. convoluta* (100 kg, wet weight) was extracted with EtOH. The EtOH extract (639 g) was partitioned between 10% aqueous MeOH and hexane. The MeOH phase was diluted with water to give 70% MeOH solution, and this was partitioned with EtOAc. The EtOAc-soluble materials (150 g) were subjected to a silica gel column (Wakogel C-200, Wako Pure Chemical, 10.7 x 30 cm) with hexane/acetone (2:1 → 1:1). The fraction eluting with hexane/acetone (2:1) was chromatographed on Sephadex LH-20 (Pharmacia, 3.0 x 35 cm) with hexane/CH_2Cl_2/MeOH (10:10:1) and reversed-phase flash column chromatography (chromatorex-ODS, 2.8 x 23 cm) using a gradient of 50→100% MeOH. The fraction eluting with 85→90% MeOH was separated on Sephadex LH-20 (Pharmacia, 3.0 x 35 cm) with hexane/2-propanol/MeOH (8:1:1) to give a fraction (66-93 mL), which was then purified on a preparative thin layer chromatography on silica gel (Whatman 20 x 20 cm) with hexane/acetone (7:3) to give a crude fraction containing convolutamides. Finally, this fraction was purified by reversed-phase HPLC

(Hitachi Packed column Inertsil PREP-ODS, 5 μ m, 20 x 250 mm, 90% CH_3CN; flow rate 5.0 mL/min) to afford a 1:1.7 mixture of convolutamides A and B (**1**, 2.4 mg, wet weight, t_R 43.12 min, yield 2 x 10^{-8}%, wet weight) a 1.8:1 mixture of convolutamides C and D (**2**, 2.3mg, t_R 76.24 min, yield 2 x 10^{-8}%), and a 7.9:1 mixture of convolutamides E and F (**3**, 0.6 mg, t_R 119.07 min, yield 6 x 10^{-9}%).

1:1.7 Mixture (1) of Convolutamides A (1a) and B (1b): a colorless amorphous solid; $[\alpha]_D^{20}$ -6.0° (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} 225 nm (ε 34000); IR (neat) ν_{max} 3270, 1740, 1720, 1700, 1600, 1560, and 1160 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); FABMS (positive) *m/z* 560, 562, and 564 $(M+H)^+$ for **1a** and *m/z* 586, 588, and 590 $(M+H)^+$ for **1b**; HRFABMS *m/z* 560.1009 for **1a** (calcd for $C_{24}H_{36}NO_4{}^{79}Br_2$, M+H, 560.1007) and *m/z* 586.1132 (calcd for $C_{26}H_{38}NO_4{}^{79}Br_2$, M+H, 586.1096) for **1b**.

1.8:1 Mixture (2) of Convolutamides C (2a) and D (2b): a colorless amorphous solid; $[\alpha]_D^{20}$ -5.1° (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} 225 nm (ε 27000); IR (neat) ν_{max} 3325, 1730, 1710, 1700, 1600, 1560, and 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 0.88 (3H, t), 1.25~1.32 (br s), 2.12 (2H, t, H_2-12), 2.52 (1H, ddd, H-3a), 2.76 (1H, ddd H-3b), 5.35 (0.71H, m, H-19 and H-20 for **2b**), 6.99 (1H, br d, H-6), and 7.38 (1H, H-10); ^{13}C NMR ($CDCl_3$) for both **2a** and **2b**: δ_C 178.4 (C-1), 173.6 (C-11), 143.4 (C-7), 129.5 (C-10), 126.9 (C-5), 124.1 (C-8), 120.5 (C-9), 113.1 (C-6), 77.2 (C-2), 59.4 (C-4), and 34.3 (C-3); for **2a**: δ_C 33.9 (C-12), 29.7 (C-14~C-23), 32.0 (C-24), 22.7 (C-25), 14.2 (C-26); for **2b**: δ_C 130.1 (C-20), 129.8 (C-19), 33.9 (C-12), 32.0 (C-26), 29.7 (C-17, C-22, and C-24), 29.5 (C-23 and C-25), 29.3 (C-15), 29.2 (C-14), 27.2 (C-18 and C-21), 24.7 (C-13), and 14.2 (C-28). FABMS (positive) *m/z* 588, 590, and 592 $(M+H)^+$ for **2a** and *m/z* 614, 616, and 618 $(M+H)^+$ for **2b**; HRFABMS *m/z* 588.1321 for **2a** (calcd for $C_{26}H_{40}NO_4{}^{79}Br_2$, M+H, 588.1324) and *m/z* 614.1437 (calcd for $C_{28}H_{42}NO_4{}^{79}Br_2$, M+H, 614.1481) for **2b**.

7.9:1 Mixture (3) of Convolutamides C (3a) and D (3b): a colorless amorphous solid; $[\alpha]_D^{20}$ -25.0° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} 225 nm (ε 23000); IR (neat) ν_{max} 3270, 1740, 1720, 1700, 1600, 1560, and 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 0.88 (3H, t), 1.25~1.32 (br s), 2.12 (2H, t, H_2-12), 2.52 (1H, ddd, H-3a), 2.76 (1H, ddd, H-3b), 5.35 (0.22H, m, H-18 and H-19 for **2b**), 6.99 (1H, br d, H-6), 7.38 (1H, br d, H-10); FABMS (positive) *m/z* 616, 618, and 620 $(M+H)^+$ for **3a** and *m/z* 642, 644, and 646 $(M+H)^+$ for **3b**; HRFABMS *m/z* 616.1609 for **3a** (calcd for $C_{28}H_{44}NO_4{}^{79}Br_2$, M+H, 616.1637) and *m/z* 642.1722 (calcd for $C_{30}H_{46}NO_4{}^{79}Br_2$, M+H, 642.1793) for **3b**.

Acknowledgement: We thank Prof. T. Sasaki, Kanazawa University for cytotoxicity test. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Sciences, and Culture of Japan.

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(*Received in Japan* 30 *May* 1994; *accepted* 27 *June* 1994)