Chem. Pharm. Bull. **54**(6) 852—854 (2006) Vol. 54, No. 6

Melophlins P, Q, R, and S: Four New Tetramic Acid Derivatives, from Two Palauan Marine Sponges of the Genus *Melophlus*

Jinzhong XU,[a] Masateru HASEGAWA,[b] Ken-ichi HARADA,[b,c] Hisayoshi KOBAYASHI,[d] Hiroshi NAGAI,[a] and Michio NAMIKOSHI*,[a,1)]

[a] *Department of Ocean Sciences, Tokyo University of Marine Science and Technology; Konan, Minato-ku, Tokyo 108–8477, Japan:* [b] *Graduate School of Environmental and Human Sciences, Meijo University;* [c] *Faculty of Pharmacy, Meijo University; Tempaku-ku, Nagoya 468–8503, Japan: and* [d] *Institute of Molecular and Cellular Biosciences, The University of Tokyo; Bunkyo-ku, Tokyo 113–0032, Japan.*

Received January 24, 2006; accepted March 17, 2006

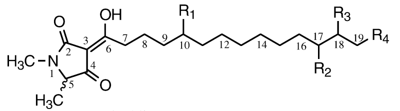
Four new tetramic acid derivatives, named melophlins P, Q, R, and S (1—4), were isolated from two marine sponges of the genus *Melophlus* collected at Palau, together with seven known melophlins A, D, E, G, H, I, and O. The structures of the new compounds were elucidated on the basis of their spectral data. The absolute stereochemistries at the tetramic acid moieties of the new compounds were determined as 1 : 1 mixtures (racemic) by ESI-LC/MS analysis of derivatives obtained by oxidation and hydrolysis of the respective parent compounds. Melophlins P—S (1—4) showed cytotoxicity against the murine leukemia cell line L1210 with IC_{50} values of 20.0, 10.5, 0.85, and 5.13 μM, respectively.

Key words *Melophlus* cf. *sarasinorum*; *Melophlus* sp.; melophlin; tetramic acid derivative; marine sponge; cytotoxicity

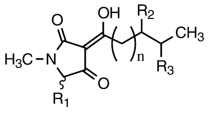
Melophlins, tetramic acid derivatives possessing a long alkyl chain, have been isolated from the marine sponge *Melophlus sarasinorum* collected in Indonesia.[2—4)] Tetramic acid derivatives were also reported from other species of marine sponges.[5—11)]

During our continuing study on biologically active metabolites from marine organisms, we found four new melophlins, named melophlins P, Q, R, and S (**1**—**4**, Chart 1), from the ethanol extract of a marine sponge *Melophlus* cf. *sarasinorum* collected in Palau, together with seven known melophlins A (**5**), D (**6**), E (**7**), G (**8**), H (**9**), I (**10**), and O (**11**). Three of the new melophlins Q—S (**2**—**4**) and seven known compounds (**5**—**11**) were also detected in the ethanol extract of a Palauan *Melophlus* sp., which had a different appearance from that of the above species.

We describe herein the isolation, structures, and growth inhibitory activity against the murine leukemia cell line L1210 of four new melophlins.



Melophlin	R1	R2	R3	R4
P (**1**)	H	H	H	CH_2CH_3
Q (**2**)	H	H	CH_3	H
R (**3**)	H	CH_3	H	H
S (**4**)	CH_3	H	H	H



Melophlin	R1	R2	R3	n
A (**5**)	H	H	H	12
D (**6**)	H	H	H	11
E (**7**)	H	H	CH_3	11
G (**8**)	H	H	H	10
H (**9**)	H	H	CH_3	10
I (**10**)	H	CH_3	H	10
O (**11**)	CH_3	CH_3	H	8

Chart 1

Ethanol extract of *M.* cf. *sarasinorum* inhibited growth of L1210 cells. Bioassay-guided isolation from the extract by repeated column chromatographies and HPLC gave four new melophlins P—S (**1**—**4**, Chart 1) and five previously known melophlins D (**6**), G (**8**), H (**9**), I (**10**), and O (**11**). Melophlins A (**5**) and E (**7**) were also detected in a fraction obtained by silica gel column chromatography. Ethanol extract of the other *Melophlus* sp. showed cytotoxicity against L1210 cells, and seven known compounds **5**—**11** were isolated from the extract. Three new compounds **2**—**4** were contained in a fraction separated by a silica gel column, but melophlin P (**1**) was not detected in any fractions. The structures of **5**—**11** were assigned on the basis of their spectral data and confirmed by comparing the data with reported values.[2,3)]

The ^{1}H- (Table 1) and ^{13}C-NMR signals (Table 2) of four new compounds **1**—**4** were assigned by 2D NMR (^{1}H–^{1}H COSY, HMQC, and HMBC) experiments. The UV spectra of **1**—**4** suggested that these compounds were also tetramic acid derivatives.[2,3,8,12)] The ^{1}H- and ^{13}C-NMR spectra of **1**—**4** showed the presence of an N-CH_3 group (δ_H 2.95, δ_C 26.3 or 26.4) and a methyl group (δ_H 1.33, δ_C 14.9 or 15.0) ascribed to 5-CH_3. HMBC correlations were observed from N-CH_3 to C-2 and C-5, 5-CH_3 to C-4 and C-5, H-5 to C-4 and 5-CH_3, and from H_2-7 to C-3 and C-6 in the HMBC spectra of **1**—**4**. These spectral data revealed that **1**—**4** have the same tetramic acid moiety possessing the 5-CH_3 group as that of melophlins B,[2)] C,[3)] J,[3)] and L—O (**11**),[3)] which was also confirmed by comparison of the NMR data for **1**—**4** with those of the reported values for B, C, J, and L—O. The ^{1}H- and ^{13}C-NMR spectra of **1**—**4** showed that these compounds were mixtures of two tautomers (exo A and exo B forms)[13—15)] in the ratio of 9 : 1 at the tetramic acid moiety as similar to the other melophlins.[2,3)]

Melophlin P (**1**) was isolated as a yellowish oil. The ESI-MS of **1** showed an $(M+H)^+$ ion at m/z 366. The molecular formula ($C_{22}H_{39}NO_3$) was determined from high-resolution (HR) FAB-MS and NMR data. In the ^{1}H-NMR spectrum of **1**, one methyl-triplet (δ 0.84, J=6.8 Hz) was detected besides

---

* To whom correspondence should be addressed. e-mail: mnami@tohoku-pharm.ac.jp

Table 1. ^{1}H-NMR Data for Compounds **1**—**4** in $CDCl_3$

	1	**2**	**3**	**4**
5	3.66, q (7.2)[a]	3.66, q (7.2)	3.66, q (7.2)	3.66, q (7.2)
5-CH_3	1.33, d (7.2)	1.33, d (7.2)	1.33, d (7.2)	1.33, d (7.2)
N-CH_3	2.95, s	2.95, s	2.95, s	2.95, s
7	2.79, t (7.3)	2.79, t (7.3)	2.79, t (7.3)	2.80, t (7.3)
8	1.62, m	1.63, m	1.63, m	1.63, m
9	1.23—1.36, m	1.23—1.40, m	1.23—1.40, m	1.25, 1.07, m
10				1.36, m
11—17				1.23—1.40, m
18		1.49, m	1.22, m	1.22, m
19		0.84, d (6.8)	0.83, t (7.2)	0.86, t (6.8)
20	1.25, m	—	—	—
21	0.84, t (6.8)	—	—	—
10-CH_3	—	—	—	0.81, d (6.8)
17-CH_3	—	—	0.82, d (6.4)	—
18-CH_3	—	0.84, d (6.8)	—	—

a) δ, multiplicity (*J* in Hz).

Table 2. ^{13}C-NMR Data for Compounds **1**—**4** in $CDCl_3$

C#	**1**	**2**	**3**	**4**
2	172.7	172.9	172.9	172.7
3	100.5	100.5	100.5	100.4
4	194.5	194.5	194.5	194.4
5	62.8	62.8	62.8	62.8
5-CH_3	14.9	15.0	15.0	15.0
N-CH_3	26.3	26.4	26.4	26.4
6	187.8	187.7	187.7	187.7
7	32.7	32.7	32.7	32.7
8	26.1	26.1	26.1	26.1
9	29.3—29.8	29.3—30.1	29.3—30.1	36.9
10				32.0
11				37.1
12—16				29.4—30.1
17		39.1	34.5	34.5
18		29.7	29.7	22.6
19	32.0	22.8	11.5	14.2
20	22.8	—	—	—
21	14.2	—	—	—
10-CH_3	—	—	—	19.8
17-CH_3	—	—	19.3	—
18-CH_3	—	22.8	—	—

two methyl-signals due to *N*-CH_3 and 5-CH_3. Subtraction of the tetramic acid moiety from the molecular formula gave $C_{15}H_{31}$, which was assigned as a $(CH_2)_{14}CH_3$ unit (C-7 to C-21) from the NMR data (Tables 1, 2). This C_{15}-alkyl chain was the same as that in melophlin A (**5**).[2] ^{13}C-NMR data for **1** from C-7 to C-21 were identical with those for melophlin A (**5**). Therefore the structure of melophlin P (**1**) was the 5-methyl derivative of melophlin A as shown in Chart 1. Compound **1** has an asymmetric carbon at the 5 position but was not found optically active ($[\alpha]_D = ca.\ 0°$). The absolute stereochemistry at the 5 position was determined utilizing the advanced Marfey's method.[2,3,16—19] Compound **1** was treated with $NaIO_4$ and $KMnO_4$ followed by aqueous HCl to yield *N*-methylalanine, which was derivatized with *N*α-(2,4-dinitro-5-fluorophenyl)-L-leucinamide (L-FDLA) and analyzed by ESI-LC/MS together with the FDLA derivatives of *N*-methyl-L-alanine and *N*-methyl-DL-alanine. The derivative from **1** showed two peaks of approximately the same intensity and was assigned as a 1 : 1 mixture of L- and D-isomers. Therefore melophlin P (**1**) was a racemic mixture of the 5(*S*) and 5(*R*)-enantiomers.

Melophlin Q (**2**) showed the $(M+H)^+$ ion at *m/z* 352 in its ESI-MS. The molecular formula was deduced as $C_{21}H_{37}NO_3$ by HR-FAB-MS and NMR data. The ^{1}H- and ^{13}C-NMR spectra of **2** revealed the presence of an *iso*-type methyl branched terminus by the signals ascribable to H_3-19 and 18-CH_3 (δ_H 0.84, 6H, d, $J=6.8$ Hz; δ_C 22.8) and H-18 (δ_H 1.49, 1H, m; δ_C 29.7). The alkyl chain of **2** assigned by NMR data was identical to that of melophlin H (**9**),[3] and the ^{1}H- and ^{13}C-NMR spectra of **2** and **9** due to C-6 to C-17 and 18-CH_3 were superimposable with each other. Therefore **2** was determined as the 5-methyl derivative of melophlin H (**9**) as shown in Chart 1. The absolute stereochemistry at C-5 in compound **2** was also determined by the advanced Marfey's method as described above. The result from ESI-LC/MS analysis revealed that **2** was also a racemic mixture of the 5(*S*) and 5(*R*)-enantiomers.

Melophlin R (**3**) was revealed as an isomer of **2** from HR-FAB-MS and NMR data, which established the molecular formula of $C_{21}H_{37}NO_3$ for **3**. The ^{1}H-NMR spectrum of **3** showed a methyl-triplet due to H_3-19 (δ 0.83, $J=7.2$ Hz) and a methyl-doublet ascribed to the branched methyl group (δ 0.82, $J=6.4$ Hz), besides *N*-CH_3 and 5-CH_3 signals. The ^{1}H–^{1}H COSY and HMBC spectra of **3** revealed the presence of an *anteiso*-type moiety, and the alkyl chain of **3** was the same as that of melophlin I (**10**),[3] whose NMR data due to C-6 to C-19 and 17-CH_3 were identical to those for **3**. Thus the structure of melophlin R (**3**) was assigned as the 5-methyl derivative of melophlin I (**10**) as shown in Chart 1. The stereochemistry at the 5 position was also identified by the advanced Marfey's method as described above and demonstrated a 1 : 1 mixture of *S* and *R*-configurations.

Melophlin S (**4**) was also obtained as a yellowish oil and showed an $(M+H)^+$ ion at *m/z* 352 in its ESI-MS. The molecular formula ($C_{21}H_{37}NO_3$) was determined from HR-FAB-MS and NMR data. A methyl-doublet was detected at δ 0.81 ($J=6.8$ Hz) in the ^{1}H-NMR spectrum of **4**, together with the terminal methyl signal (δ 0.86, 3H, t, $J=6.8$ Hz), *N*-methyl singlet (δ 2.95), and 5-CH_3 signal (δ 1.33, d, $J=7.2$ Hz). Thus compound **4** was deduced as the isomer of **2** and **3** deferring in the position of methyl branch. The alkyl chain in **4** was assigned on the basis of its NMR data and comparison with those for melophlins B[2] and C.[3] Similar to the observation for melophlin C,[3] the ^{13}C-NMR spectrum of **4** showed a set of signals in a similar ratio of *ca.* 10 : 6 for C-10, the chiral center (δ 32.0, 32.1), and for the adjacent carbons at C-9 (δ 36.9, 36.7), C-11 (δ 37.0, 37.1), and 10-CH_3 (δ 19.8, 19.7), which indicated that **4** was a mixture of two stereoisomers at the 10 position. The chiral center at C-5 in **4**, determined by the advanced Marfey's method, was a 1 : 1 mixture of *S* and *R*-configurations. Consequently, melophlin S (**4**) was obtained as an inseparable mixture of all four stereoisomers at C-5 and C-10 including both tautomers of the tetramic acid moiety.

Compounds **1**—**4** showed growth inhibitory activity against L1210 cells with IC_{50} values of 5.13, 0.85, 10.5, and 20.0 μM, respectively. In the same experiment, IC_{50} values of **5**—**11** were 8.55, 38.6, 28.5, 154.8, 16.3, 7.12, and 1.86 μM, respectively.

Experimental

General Procedures NMR spectra were measured by a JEOL AL-400 NMR spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz) in $CDCl_3$. Mass spectra were obtained by a JEOL HX-110 mass spectrometer (FAB mode) or a Finnigan TSQ 700 triple quadrupole mass spectrometer (ESI mode). UV and IR spectra were recorded on a HITACHI U-3000 and on a JASCO FT/IR 470, respectively. Optical rotations were recorded by a JASCO DIP-1000 digital polarimeter.

Extraction and Isolation The marine sponge *M.* cf. *sarasinorum* (Sponge I) was collected in the Rock Islands at Urukthapel in Palau on February 5, 2000. The other species of *Melophlus* (Sponge II) was taken in the Rock Islands at Eil Malk on the same day. This sponge had the same spicules as those of Sponge I, but the appearance in the natural habitat was different from the ordinary *M. sarasinorum*. Voucher specimens of Sponge I and Sponge II are maintained in the Department of Ocean Sciences at Tokyo University of Marine Science and Technology as 00-02-05=1-1 and 00-02-05=2-20, respectively. The sponges were frozen in a storeroom (−50 °C) of the training vessel Shinyo-maru and transported to Japan by ship.

The freeze-dried Sponge I (80 g) was extracted three times with EtOH. The extract, after evaporation of EtOH, was dissolved in MeOH and extracted with hexane. The MeOH layer was evaporated and the residue partitioned between EtOAc and water. The EtOAc extract (1.6 g) was separated by a Sephadex LH-20 column with MeOH–$CHCl_3$ (1 : 1) into four fractions. The third fraction (580 mg) was chromatographed on a silica gel with $CHCl_3$–MeOH (gradient elution) to give four fractions, and the 2—5% MeOH eluate (250 mg) was further separated by HPLC (ODS, MeOH–H_2O=95 : 5, 0.05% TFA) into six fractions (Frs. 1—6). Compound **1** (14.0 mg) was isolated from Fr. 6 (25.0 mg) by HPLC (ODS, CH_3CN–H_2O=95 : 5, 0.05% TFA). Compounds **8** (2.3 mg), **9** (5.0 mg), and **10** (16.5 mg) were obtained from Fr. 3 (56.0 mg) by HPLC (ODS, CH_3CN–H_2O=83 : 17, 0.05% TFA). Compounds **6** (4.5 mg) and **11** (4.5 mg) were isolated by HPLC from Frs. 2 and 4, respectively. Compounds **5** and **7** were detected in Fr. 5. The forth fraction (108 mg) from the LH-20 column was first separated by HPLC (ODS, CH_3CN–H_2O=95 : 5, 0.05% TFA) into eight fractions, then the fifth fraction was separated by HPLC (ODS, CH_3CN–H_2O=80 : 20, 0.05% TFA) to give **2** (13.0 mg), **3** (15.6 mg), and **4** (18.5 mg).

The EtOH extract of dried Sponge II (124 g) was separated by similar procedures and afforded compounds **5**—**11** (40, 5.2, 5.0, 16.2, 45, 3.0, 5.0 mg, respectively). HPLC analysis of one fraction, obtained by two silica gel column separations after solvent partition, showed the presence of compounds **2**, **3**, and **4**.

Melophlin P (**1**): Yellowish oil. UV λ_{max} (MeOH) nm (log ε): 282 (4.1), 245 (3.8). IR ν_{max} (KBr) cm^{-1}: 3423, 2924, 2854, 1714, 1650, 1622, 1454, 1238, 928. ESI-MS *m/z*: 388 [(M+Na)$^+$], 366 [(M+H)$^+$]. FAB-MS *m/z*: 366.3008 [(M+H)$^+$, Calcd for $C_{22}H_{40}NO_3$, 366.3008]. ^{1}H- and ^{13}C-NMR data are listed in Tables 1 and 2, respectively.

Melophlin Q (**2**): Yellowish oil. UV λ_{max} (MeOH) nm (log ε): 283 (4.2), 246 (4.1). IR ν_{max} (KBr) cm^{-1}: 3423, 2925, 2855, 1725, 1651, 1618. ESI-MS *m/z*: 352 [(M+H)$^+$]. FAB-MS *m/z* 352.2855 [(M+H)$^+$, Calcd for $C_{21}H_{38}NO_3$, 352.2852]. ^{1}H- and ^{13}C-NMR data are listed in Tables 1 and 2, respectively.

Melophlin R (**3**): Yellowish oil. UV λ_{max} (MeOH) nm (log ε): 280 (4.2), 248 (4.2). IR ν_{max} (KBr) cm^{-1}: 3423, 2925, 2855, 1720, 1651, 1622, 1230. ESI-MS *m/z*: 352 [(M+H)$^+$]. FAB-MS *m/z* 352.2865 [(M+H)$^+$, Calcd for $C_{21}H_{38}NO_3$, 352.2852]. ^{1}H- and ^{13}C-NMR data are listed in Tables 1 and 2, respectively.

Melophlin S (**4**): Yellowish oil. UV λ_{max} (MeOH) nm (log ε): 283 (4.5), 248 (3.8). IR ν_{max} (KBr) cm^{-1}: 3423, 2925, 2855, 1714, 1651, 1622, 1454, 1239. ESI-MS *m/z*: 352 [(M+H)$^+$]. FAB-MS *m/z* 352.2865 [(M+H)$^+$, Calcd for $C_{21}H_{38}NO_3$, 352.2852]. ^{1}H- and ^{13}C-NMR data are listed in Tables 1 and 2, respectively.

Hydrolysis and Derivatization The acetone–H_2O (1 : 1, 70 μl) solutions of compounds **1**—**4** (each *ca.* 1 mg) were treated with $NaIO_4$ (6.0 mg) and $KMnO_4$ (0.2 mg) at 5 °C for 2 h and then with 2 N aq. HCl (60 μl) at 100 °C for 5 h. The reaction mixture was neutralized with 1 M $NaHCO_3$ solution, and L-FDLA (0.4 mg) in acetone (380 μl) was added and stirred at 37 °C for 6 h. After quenching with 1 N aq. HCl (76 μl), MeOH was added to the mixture and analyzed by ESI-LC/MS. Two standard amino acids, *N*-methyl-L-alanine (300 μg) and *N*-methyl-DL-alanine (300 μg), were treated with L-FDLA as above.

ESI-LC/MS Analysis of Hydrolysate FDLA derivatives of standard amino acids and hydrolysates obtained from **1**, **2**, **3**, and **4** were analyzed by ESI-LC/MS according to previous reports.[17—19] Separation of the derivatized amino acids was performed on a TSK gel ODS-80Ts (150 mm×2.0 mm i.d., Tosoh) column maintained at 40 °C using an HP1100 HPLC system (Agilent Technologies, Palo Alto, CA, U.S.A.) at 340 nm as detection apparatus. CH_3CN–H_2O containing 0.1% formic acid was used as the mobile phase at a flow rate of 0.2 ml/min under the following gradient elution mode: CH_3CN, 30% (0 min) to 52% (30 min). The mass spectrometer used was an LCQ Deca XP plus (Thermo Electron, San Jose, CA, U.S.A.). The scan range was between *m/z* 150 and 1000. Data were collected in the positive ion mode. The high performance liquid chromatograph and mass spectrometer were interfaced with an IonSpray ion source (Thermo Electron). The IonSpray voltage, capillary voltage, and tube lens offset were 4.8 kV, 12 V, and 20 V, respectively. The sheath gas flow rate was 45 arb. Capillary temperature was set at 300 °C. MS/MS analysis of the protonated molecule (*m/z* 398.2) of derivatized *N*-methylalanine was performed under the following conditions: excitation of the ions was accomplished through collisions with helium in the collision-induced dissociation (CID) mode. Collision energy was set to 35%.

Cytotoxicity Growth inhibitory activity of compounds **1**—**11** against L1210 cells was test in 96-well plastic plates and vital cells were detected by XTT method. Compounds were dissolved in MeOH and 10 μl of each sample solution was poured in a well and the solvent evaporated in a clean bench. The number of vital cells in the sample wells after 72 h was compared with those in the control (MeOH) wells.

Acknowledgments This work was supported in part by Grant-in-Aid for Scientific Research on Priority Areas 17035029 from The Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan to M. N. We are grateful to Dr. Kazunari Ogawa of Z. Nakai Laboratory for identification of sponges, Dr. Mamoru Endo of Marine Biotechnology Institute, Japan, for the collection at Palau, and to the crew members of the training vessel Shinyo-maru for their generous help in sample collection at Palau.

References and Notes

1) Present address: *Tohoku Pharmaceutical University, Komatsushima, Aoba-ku, Sendai 981—8558, Japan.*
2) Aoki S., Higuchi K., Ye Y., Satari R., Kobayashi M., *Tetrahedron*, **56**, 1833—1836 (2000).
3) Wang C.-Y., Wang B.-G., Wiryowidagdo S., Wray V., van Soest R., Steube K. G., Guan H.-S., Proksch P., Ebel R., *J. Nat. Prod.*, **66**, 51—56 (2003).
4) The scientific name of this sponge (*Melophlus sarasinorum* THIELE) was accidentally misspelled in literatures 2 and 3.
5) Ratnayake A. S., Davis R. A., Harper M. K., Veltri C. A., Andjelic C. D., Barrows L. R., Ireland C. M., *J. Nat. Prod.*, **68**, 104—107 (2005).
6) Fujita M., Nakao Y., Matsunaga S., Seiki M., Itoh Y., van Soest R. W. M., Fusetani N., *Tetrahedron*, **57**, 1229—1234 (2001).
7) Sata N. U., Wada S.-I., Matsunaga S., Watabe S., van Soest R. W. M., Fusetani N., *J. Org. Chem.*, **64**, 2331—2339 (1999).
8) Sata N. U., Matsunaga S., Fusetani N., van Soest R. W., *J. Nat. Prod.*, **62**, 969—971 (1999).
9) Wolf D., Schmitz F. J., Qiu F., Kelly-Borges M., *J. Nat. Prod.*, **62**, 170—172 (1999).
10) Ohta S., Ohta E., Ikegami S., *J. Org. Chem.*, **62**, 6452—6453 (1997).
11) Matsunaga S., Fusetani N., Kato Y., *J. Am. Chem. Soc.*, **113**, 9690—9692 (1991).
12) Philips N. J., Goodwin J. T., Fraiman A., Cole R. J., Lynn D. G., *J. Am. Chem. Soc.*, **111**, 8223—8231 (1989).
13) Jones R. C. F., Begley M. J., Peterson G. E., Sumaria S., *J. Chem. Soc., Perkin Trans. 1*, **1990**, 1959—1968 (1990).
14) Nolte M. J., Steyn P. S., Wessels P. L., *J. Chem. Soc., Perkin Trans. 1*, **1980**, 1057—1065 (1980).
15) Steyn P. S., Wessels P., *Tetrahedron Lett.*, **1978**, 4707—4710 (1978).
16) Marfey P., *Carlsberg Res. Commun.*, **49**, 591—596 (1984).
17) Fujii K., Ikai Y., Mayumi T., Oka H., Suzuki M., Harada K.-I., *Anal. Chem.*, **69**, 3346—3352 (1997).
18) Fujii K., Ikai Y., Oka H., Suzuki M., Harada K.-I., *Anal. Chem.*, **69**, 5146—5151 (1997).
19) Harada K.-I., Fujii K., Mayumi T., Hibino Y., Suzuki M., Ikai Y., Oka H., *Tetrahedron Lett.*, **36**, 1515—1518 (1995).