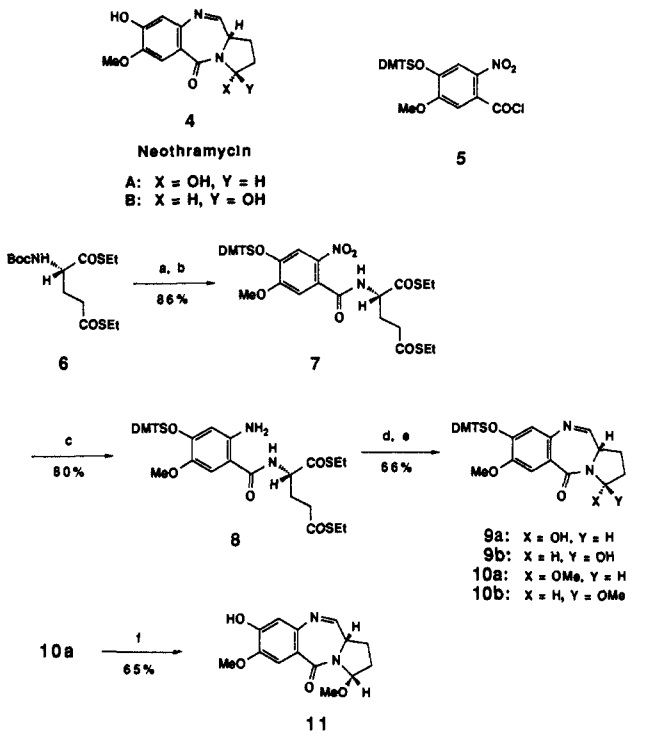
Scheme I[a]



[a] The reagents and reaction conditions were as follows: (a) $BF_3{\cdot}Et_2O$ (4 equiv), CH_2Cl_2, 23 °C. (b) **5** (1.2 equiv), saturated $NaHCO_3$, CH_2Cl_2, 23 °C. (c) Zn (excess), AcOH (8 equiv), Et_2O, 23 °C. (d) Et_3SiH (5 equiv), 10% Pd/C (15 mol %), dry CH_2Cl_2, Ar, 23 °C, 40 min. (e) CSA (0.1 equiv), MeOH, 23 °C. (f) n-Bu_4NF (1 equiv), AcOH (5 equiv), MeOH, 23 °C.

total synthesis (Scheme I). The L-glutamic dialdehyde backbone of the novel antitumor agents could potentially be constructed by simultaneous reduction of the corresponding L-glutamic dithiol ester. Thus the unstable amine derived from the readily available *N*-Boc-L-glutamic dithiol ester **6**[7] was acylated with the acid chloride **5**[8] to give the amide **7** in 86% yield from **6** ((1) $BF_3{\cdot}Et_2O$ (4 equiv), CH_2Cl_2; (2) **5**, saturated $NaHCO_3$, CH_2Cl_2). Reduction of the nitro group **7** with activated zinc furnished the amine **8** in 80% yield (Zn, AcOH, Et_2O). The critical double cyclization was performed by treatment with 10% Pd/C (15 mol %) and Et_3SiH (5 equiv) in CH_2Cl_2 at 23 °C for 40 min. The unstable neothramycin silyl ethers **9** thus formed were isolated as an epimeric mixture of the more stable methyl ethers **10** in 66% yield from **8** (CSA (camphorsulfonic acid), MeOH). Finally, deprotection of the dimethylthexylsilyl (DMTS) ether of the predominant α-epimer **10a** gave neothramycin A methyl ether **11** in 65% yield (n-Bu_4NF, AcOH, MeOH). The synthetic **11** proved to be identical with an authentic sample in both TLC behavior and spectroscopic properties.[9] The methyl ether **11** can be converted to neothramycin under mild acidic conditions.[6]

Acknowledgment. This work was supported by the Robert A. Welch Foundation (Grant C-0722) and the National Institutes of Health (Grant CA28119). We thank Dr. H. Naganawa of the Institute of Microbial Chemistry, Tokyo, for providing a generous sample of neothramycin A butyl ether. We also thank Mr. Yutaka Kanda for invaluable assistance.

(7) Prepared from *N*-Boc-L-glutamic acid in 73% yield (EtSH (6 molar equiv), DCC (2.5 molar equiv), DMAP (0.1 molar equiv), CH_3CN, 23 °C).

(8) Prepared from vanillin in six steps in 70% overall yield ((1) $PhCH_2Cl$, K_2CO_3, DMF, 100 °C; (2) fuming HNO_3, AcOH, 23 °C; (3) 12 N HCl–AcOH (1:3), reflux; (4) thexyldimethylsilyl chloride, imidazole, CH_3CN, 23 °C; (5) $KMnO_4$, *t*-BuOH, 5% NaH_2PO_4 buffer, 0 °C; (6) $(COCl)_2$, benzene, 60 °C).

(9) The average optical rotation of synthetic **11** was $[\alpha]^{25}_D$ +599° (c = 0.15, dioxane) (lit.[6] $[\alpha]^{26}_D$ +640° (c = 0.24, dioxane). It should be noted that the optical rotation of **11** is dependent on the amount of water in dioxane presumably because of hydration to the imine.

Supplementary Material Available: Experimental details for the preparation of **2** from L-glutamic acid 5-methyl ester and a listing of spectroscopic data of key intermediates for neothramycin A methyl ether synthesis (4 pages). Ordering information is given on any current masthead page.

Synthesis of a Highly Stable Iron Porphyrin Coordinated by Alkylthiolate Anion as a Model for Cytochrome P-450 and Its Catalytic Activity in O–O Bond Cleavage

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Much interest has been focused on the mechanism of the catalytic cycle of cytochrome P-450.[1] One of the remarkable features of P-450 as a heme enzyme is that the heme iron of P-450 has a thiolate (RS^-) coordination.[1,2] Cytochrome P-450 is readily distinguished from other heme proteins spectrometrically because of the thiolate ligation. The thiolate ligand is therefore expected to strongly influence the chemistry of the heme iron. However, there have not yet been any experiments clearly directed toward revealing the relative effect of a thiolate ligand in P-450-type reactivity.[3,4]

Here we report on the synthesis and the catalytic activity of a novel iron porphyrin coordinated by thiolate anion which is highly stable during catalytic oxidations. The P-450 model **1** (Figure 1) was designed to introduce bulky groups on the RS^- coordination face of the porphyrin molecule so that the thiolate ligation could be highly stabilized and protected from oxidation. To prepare complex **1**, [*o*-[(acetylthio)methyl]phenoxy]acetic acid as a designed thiolate moiety was combined with *meso*-$\alpha,\alpha,\alpha,\alpha$-tetrakis(*o*-aminophenyl)porphyrin[5] and the remaining amino groups were all acylated with pivaloyl chloride to afford *meso*-$\alpha,\alpha,\alpha,\alpha$-[*o*-[[*o*-[(acetylthio)methyl]phenoxy]acetamido]phenyl]tris(*o*-pivalamidophenyl)porphyrin. After iron insertion and deprotection of the acetyl group, **1** was obtained as dark brown microcrystal.[6] Complex **1** was characterized by FAB-MS, IR, electronic absorption spectrum, EXAFS, and elemental analysis.[7] The EPR

(1) For recent reviews: (a) Ortiz de Montellano, P., Ed. *Cytochrome P-450*; Plenum: New York, 1986. (b) Dawson, J. H.; Sono, M. *Chem. Rev.* **1987**, *87*, 1255. (c) Mansuy, D. *Pure Appl. Chem.* **1987**, *59*, 759.

(2) Dawson, J. H. *Science* **1988**, *240*, 433.

(3) Sakurai et al. reported the oxidation of various compounds with a hemin/large excess amount of thiol/O_2 system although they did not pursue the *relative* effect of an axial ligand: Sakurai, H.; Hatayama, E.; Fujitani, K.; Kato, H. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 1649 and references cited therein.

(4) Several P-450 chemical models having thiolate coordination have been reported though there are no experiments on catalytic oxidation with the models in these papers: (a) Traylor, T. G.; Mincey, T. C.; Berzinis, A. P. *J. Am. Chem. Soc.* **1981**, *103*, 7084. (b) Collman, J. P.; Groh, S. E. *Ibid.* **1982**, *104*, 1391. (c) Woggon, W.-D., Stäubli, B.; Fretz, H. *Helv. Chim. Acta* **1987**, *70*, 1174. (d) Schappacher, M.; Richard, L.; Fischer, J.; Weiss, R.; Bill, E.; Montiel-Montoya, R.; Winkler, H.; Trauwein, A. X. *Eur. J. Biochem.* **1987**, *168*, 419 and references cited therein.

(5) Collman, J. P.; Gagne, R. R.; Reed, C. A.; Harbert, T. R.; Lang, G.; Robinson, W. T. *J. Am. Chem. Soc.* **1975**, *97*, 1427.

(6) The temperature was kept below 30 °C throughout the procedure for the preparation of **1** in order to avoid the formation of atropisomers of the porphyrins. [*o*-[(Acetylthio)methyl]phenoxy]acetic acid (ATPA) was introduced from saligenin in three steps in high yield. Complex **1** was prepared from *meso*-$\alpha,\alpha,\alpha,\alpha$-tetrakis(*o*-aminophenyl)porphyrin via steps a–d: (a) ATPA, 2-chloro-1-methylpyridinium iodide, triethylamine (yield 53%); (b) pivaloyl chloride, pyridine (yield 76%); (c) $FeBr_2$, 2,4,6-collidine (yield 96%); (d) $NaOCH_3$ (yield 66%). Details of the procedure for the preparation of **1** will be described elsewhere.

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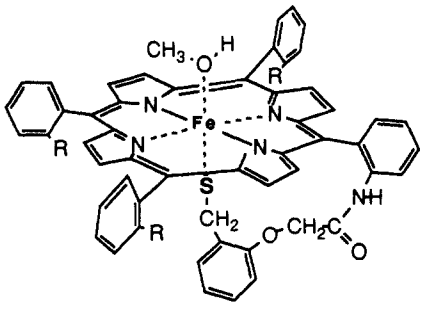

Figure 1. Complex **1**: R = $NHCOC(CH_3)_3$.

spectrum of complex **1** (in DMF) showed low-spin signals of a single species (g_x = 1.96, g_y = 2.21, g_z = 2.32) of which g values indicate that the axial ligand is thiolate anion.[8-10] Both the EPR spectrum (in toluene) and the result of elemental analysis required the existence of a second axial ligand (methanol). The absorption spectrum of the ferrous **1**–CO complex exhibited a typical hyperporphyrin spectrum for a thiolate-ligated iron(II) porphyrin–CO complex (split Soret band; λ_{max} = 383, 459 nm; in DMSO). Compound **1** (solution or solid) could be stored at 0 °C under air for several months and could withstand even column chromatography on silica gel under air. This complex is the first synthetic example of an isolable low-spin ferric porphyrin coordinated by an alkylthiolate with high stability toward dioxygen and thermodynamic stability.[11,12]

The main characteristic feature of the reaction of P-450 is that the enzyme activates molecular oxygen for oxygenation. To clarify the axial ligand effect on the oxygen activation by P-450 which is a multistep reaction, the effect at each step should be evaluated by using synthetic (porphinato)iron coordinated by thiolate. Thus, we examined the catalytic activity of **1** on the "peroxide shunt" reaction on P-450 using alkyl hydroperoxides and compared it with that of iron tetraphenylporphyrin chloride (FeTPPCl) to investigate the relative effect of a thiolate ligand on O–O bond cleavage. 2,4,6-Tri-*tert*-butylphenol (TBPH) and 1,1-diphenyl-2-picrylhydrazine (DPPH) were chosen as substrates because both are known to trap reactive intermediates with excellent efficiency to almost stoichiometrically afford TBP•[13] or DPP•[14] radical. Toluene was used as solvent, taking into account the highly hydrophobic environment of the active site of P-450. EPR spectra of the reacting solution (15 s after the start of the reaction at 25 °C) exhibited low-spin spectra of the thiolate-ligated iron porphyrin plus the signal of the DPP• formed and the high-spin signal (g = 6) increased very slightly (Figure 2). The peak height of the high-spin signal should increase largely if the axial ligand of **1** is oxidized. This reaction with **1** showed linear progress for at least 1 min from the beginning.[15] Furthermore, 90% of **1** could be recovered from the reaction mixture (1 min after the addition of peroxide) by isolation with column chromatography. All of these results supported the conclusion that **1** catalyzes the oxidation of the substrates by hydroperoxides while the axial thiolate ligand almost remains unoxidized. Comparison of the reaction rates derived by monitoring the increase of TBP• (630 nm) or DPP• (700 nm) absorbance (Table I) shows **1** to have about 60–240 times higher activity than FeTPPCl.[16,17] The presence of 0.1 M

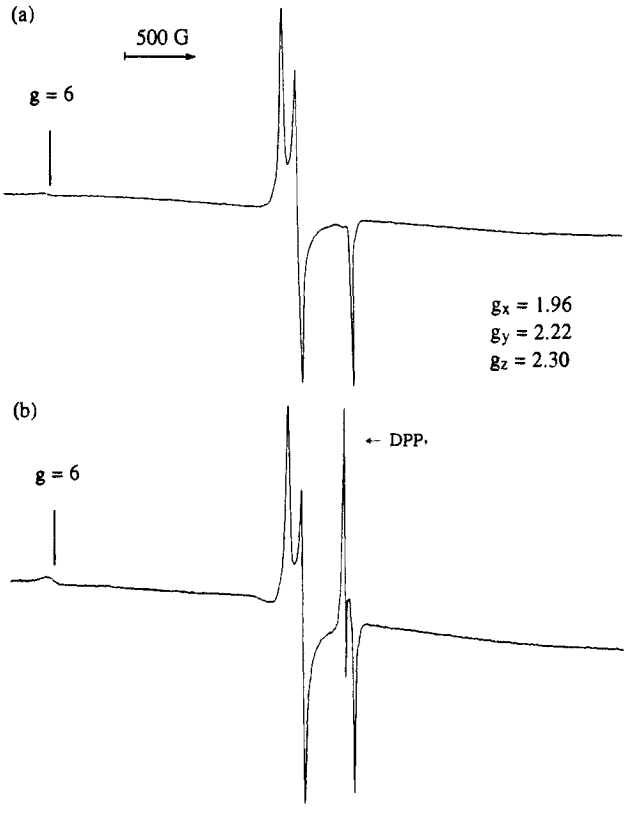

Figure 2. EPR spectra at 77 K of complex **1** in toluene in the presence of DPPH. Conditions: [**1**] = 0.10 mM; [DPPH] = 100 mM. (a) Spectrum before the addition of cumene hydroperoxide. (b) Spectrum obtained 15 s after the addition of cumene hydroperoxide (1.0 mM) at 25 °C.

Table I. Observed Initial Rates of TBP• or DPP• Formation in the Oxidation of TBPH or DPPH with Alkyl Hydroperoxides Catalyzed by **1** or FeTPPCl[a]

substrate	oxidant	ν (turnover no./min)[b] **1**	ν (turnover no./min)[b] FeTPPCl	$\nu_1/\nu_{FeTPPCl}$
TBPH	$PhC(CH_3)_2OOH$	21	0.35	58
	t-BuOOH	8.5	0.080	110
DPPH	$PhC(CH_3)_2OOH$	20	0.085	235
	t-BuOOH	7.5	0.041	182

[a] Conditions: solvent = toluene; [TBPH] = 0.2 M; [DPPH] = 0.1 M; [oxidant] = 5×10^{-2} M; [**1**] = [FeTPPCl] = 10^{-4} M. These reactions were carried out at 20 °C under an argon atmosphere. [b] Observed initial rates of the reactions were based on catalysts (turnover number of catalysts/min).

(7) FAB-MS gave the (M − CH_3OH) ion at 1160. Elemental anal. of **1**. Calcd for $C_{69}H_{67}N_9O_6SFe$: C, 69.51; H, 5.66; N, 9.40; S, 2.68. Found: C, 69.23; H, 5.52; N, 9.38; S, 2.60. Absorption spectrum of **1** (ferric) (in DMF): λ_{max} (log ϵ) = 424 nm (5.07), 534 (4.14), 574 (sh, 3.51); (in DMSO) λ_{max} (log ϵ) = 425 nm (5.03), 536 (3.98), 578 (sh, 3.33). IR (KBr): 3410, 1686, 1508, 1447, 995, 750 cm^{-1}. Further, the result obtained by a curve-fitting analysis of the EXAFS Fourier transform of **1** required the existence of an Fe–S bonding. The length of the Fe–S bond of **1** is almost identical with that of P-450. Details of the EXAFS analysis will be reported in another paper.

(8) Rhombicity (V/Δ), which is a ligand-field parameter, is widely used in summarizing EPR data on hemes, and hemes of which values of rhombicity are in the range of about 0.9–1.2 generally have thiolate anion as an axial ligand.[9] Therefore complex **1** should be coordinated by thiolate since the V/Δ value of **1** (DMF solution) is 1.02, as obtained by the method of Taylor[10] although each g value of **1** is somewhat different from that of native P-450. Among hemes coordinated by thiolate, high similarity of g values does not always indicate high structural similarity because, for example, g values of *arylthiolato*-iron porphyrin complexes are almost identical with those of P-450, of which the fifth ligand is an *alkylthiolate* anion.[9]

(9) Chevion, M.; Peisach, J.; Blumberg, W. E. *J. Biol. Chem.* **1977**, *252*, 3637 and references cited therein.

(10) Taylor, C. P. S. *Biochim. Biophys. Acta* **1977**, *491*, 137.

(11) There has been no published comment on the high stability of alkylthiolato–iron porphyrin toward dioxygen as far as we know while arylthiolate hemes have been reported to be fairly stable. It has been known that an excess amount of alkylthiolate anion reduces the iron(III) atom of a porphyrin complex at room temperature to prevent the formation of an iron(III) porphyrin–alkylthiolate 1:1 complex.[12]

(12) Dawson, J. H.; Holm, R. H.; Trudell, J. R.; Barth, G.; Linder, R. E.; Bunnenberg, E.; Djerassi, C. *J. Am. Chem. Soc.* **1976**, *98*, 3707.

(13) ROOH + 2ArOH → ROH + 2ArO• + H_2O; TBP• (ArO•) = 2,4,6-tri-*tert*-butylphenoxyl: Traylor, T. G.; Lee, W. A.; Stynes, D. V. *J. Am. Chem. Soc.* **1984**, *106*, 755.

(14) DPP• = 1,1-diphenyl-2-picrylhydrazyl: Yuan, L. C.; Bruice, T. C. *J. Am. Chem. Soc.* **1986**, *108*, 1643.

(15) The reaction slightly slowed down its rate at longer times than 1 min with a slightly increasing EPR high-spin signal (g = 6), although a large portion of complex **1** remained at the end of the reaction.

methanol almost did not affect the ratio of the rates. The O–O bond cleavage of hydroperoxides almost stoichiometrically afforded 1 equiv of the alcohols and 2 equiv of DPP• or TBP• in the reactions. This oxidation process consists of the formation step of the alkyl peroxide–iron porphyrin complex, the O–O bond cleavage step, and the reaction step of the reactive intermediates and the substrates. Thus, the acceleration of the reaction by thiolate ligation is undoubtedly due to the enhancement of O–O bond scission and/or the first step since the third step is known to be very fast.[13,14] It is highly probable that the acceleration of O–O bond cleavage dominates because the high concentration of the used peroxides makes the cleavage step rate determining. In an interpretation of the thiolate ligand effect on the reaction of P-450, Dawson et al. have proposed that large electron donation to the iron from the thiolate enhances cleavage of the O–O bond.[12] A cyclic voltammogram of **1** in DMF showed a clear, reversible reduction couple (Fe(III)/Fe(II)) at −0.45 V vs SCE, which is more negative than that of FeTPP(III)Cl (−0.27 V vs SCE). The negativity of the redox potential of **1** is probably due to the electron donation of thiolate to iron atom. Both our kinetic and electrochemical results can be considered to support the speculation by Dawson et al. experimentally.

Acknowledgment. This work was supported in part by a Grant-in-Aid for Special Project Research from the Ministry of Education, Science and Culture, Japan. We thank Professor Mitsuo Sato (Teikyo University) for his helpful discussions. We are grateful to Dr. Isao Kojima and Dr. Kazumasa Honda (National Chemical Laboratory for Industry, Japan) for EXAFS measurement and analysis.

(16) These experiments indicate that the reactions of iron porphyrins with peroxide form active intermediates which can oxidize TBPH or DPPH, whether the axial ligand of the complex is thiolate or not. Groves et al. reported that alkanes and alkenes are readily oxygenated by oxoiron(IV) porphyrin π cation radical which is formed by the reaction of iodosylbenzene or *m*-CPBA with iron porphyrin of which the axial ligand is chloride.[17] It was difficult to prepare pure active species derived from **1** with peroxides in the absence of a substrate at ordinary temperature. To investigate the property of the active species is the subject of our next study.

(17) Groves, J. T.; Nemo, T. E. *J. Am. Chem. Soc.* **1983**, *105*, 6243.

Cyclotheonamides, Potent Thrombin Inhibitors, from a Marine Sponge *Theonella* sp.[1]

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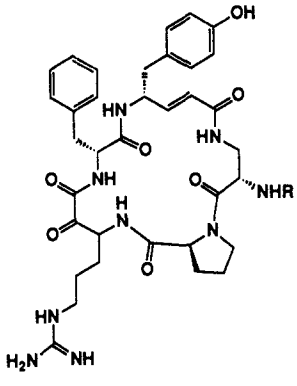
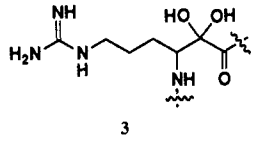
Recent chemical studies have shown that marine sponges of the genus *Theonella* are a rich source of secondary metabolites possessing potent bioactivities and novel chemical features; e.g., swinholide A,[2] bistheonellides,[3] onnamide A,[4] theonellamide F,[5] and theonellapeptolides.[6] In the course of our screening program of Japanese marine invertebrates for potential biomedicals,[7] we encountered a marine sponge of the genus *Theonella*[8] collected off Hachijo-jima Island, 300 km south of Tokyo, which strongly inhibited various proteinases, particularly thrombin. We have isolated two active substances, named cyclotheonamides A and B, which proved to be novel cyclic peptides. This communication deals with the isolation and structural elucidation of these peptides.

The concentrated ethanol extract of the sponge (10 kg wet weight) collected in 1987 was extracted with ether followed by 1-butanol (yield, 29.5 g). The 1-butanol phase was successively gel-filtered on Sephadex LH-20 and Toyopearl HW40 SF with methanol as eluent. The active fractions[9] were subjected to HPLC on Asahipak GS320 (aqueous CH_3CN) and on Senshu Pak ODS-H-4251 (first with 40–50% CH_3OH in 50 mM aqueous Na_2SO_4 and then with 10–30% CH_3CN in 0.1% TFA) to furnish cyclotheonamide A (**1**, 50 mg, (5×10^{-4})% yield).[10] The same sponge (4.5 kg wet weight) collected in 1989 from the same location yielded cyclotheonamide B (**2**, 4.6 mg, (1×10^{-4})% yield).[11]



1 R=CHO
2 R=Ac



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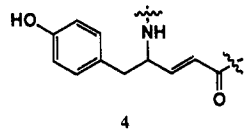

4

The FAB mass spectrum of cyclotheonamide A in methanol solution gave an $(MH + CH_3OH)^+$ ion at *m/z* 764 as well as an $(MH)^+$ ion at *m/z* 732, whereas the highest ion peak shifted to *m/z* 778[12] $(MH + C_2H_5OH)^+$ when the sample was dissolved in ethanol. The negative FABMS gave an $(M - H)^-$ ion at *m/z* 730 confirming the molecular weight of 731.

Since the ^1H NMR spectrum inicated **1** to be a peptide, it was subjected to the standard amino acid analysis, which implied the presence of 1 mol each of Pro, Phe, and 2,3-diaminopropionic acid (Dpr).[13] Extensive NMR analysis on **1** including COSY, CH-COSY,[14] and HMBC[15] spectra in D_2O revealed the spin systems for two hitherto unknown amino acid residues, **3** and **4**, as well as those for Pro, Phe, and Dpr.[16]

(1) Bioactive Marine Metabolites. 33. Part 32: Hirota, Y.; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1990**, *31*, 4763–4764.

(2) Kitagawa, I.; Kobayashi, M.; Katori, T.; Yamashita, M.; Tanaka, J.; Doi, M.; Ishida, T. *J. Am. Chem. Soc.* **1990**, *112*, 3710–3712.

(3) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Sakai, R.; Higa, T.; Kashman, Y. *Tetrahedron Lett.* **1987**, *28*, 6225–6228.

(4) Sakemi, S.; Ichiba, T.; Kohmoto, S.; Saucy, G.; Higa, T. *J. Am. Chem. Soc.* **1988**, *110*, 4851–4853.

(5) Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Wälchli, M. *J. Am. Chem. Soc.* **1989**, *111*, 2582–2588.

(6) Kitagawa, I.; Lee, N. K.; Kobayashi, M.; Shibuya, H. *Chem. Pharm. Bull.* **1987**, *35*, 2129–2132.

(7) Fusetani, N. Paper was presented at the Sixth International Symposium on Marine Natural Products at Dakar, 1989; to be published in *New J. Chem.*

(8) This sponge is characterized by a brilliant yellow inner body which is different from that of another *Theonella* sponge containing theonellamides.

(9) Inhibitory activity against thrombin was assayed according to Svendsen et al.: Svendsen, L.; Blomback, B.; Blomback, M.; Olsson, P. I. *Thromb. Res.* **1972**, *1*, 264–278.

(10) **1**: $[\alpha]^{23}_D$ −13° (*c* = 0.2, MeOH); UV (MeOH) 278 nm (ε 1940). IC_{50} (μg/mL): thrombin, 0.076; trypsin, 0.2; plasmin, 0.3. Full physicochemical as well as biological data for **2** will be reported in a forthcoming full account.

(11) The cyclotheonamide B preparation contained a small amount (ca. 5%) of **1**, while the cyclotheonamide A preparation was free of **2**.

(12) Precise ion matching gave a composition of $C_{38}H_{52}N_9O_9$ (*m/z* 778.4039, Δ 15.1 mmu).

(13) The amino acid analysis (ion exchange/ninhydrin) revealed the presence of one basic amino acid, probably **3**, which had a little shorter retention time than that of Arg in addition to three small peaks, probably degradation products of **4**, appearing near the retention time of Lys.

(14) Ernst, R. R.; Bodenhausen, G.; Wokaun, A. *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*; Clarendon Press: Oxford, 1987.

(15) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285–4294.

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