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Turbotoxins A and B, Novel Diiodotyramine Derivatives from the Japanese Gastropod *Turbo marmorata*

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Dedicated to Professor Paul J. Scheuer on the occasion of his 85th birthday

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Abstract—Bioassay-guided separation of the aqueous ethanol extract of the viscera of the Japanese gastropod *Turbo marmorata* resulted in the isolation of two toxins, turbotoxins A and B. Their structures were determined by spectral analysis and confirmed by organic synthesis to be diiodotyramine derivatives. Turbotoxins A and B exhibited acute toxicity against ddY mice, with LD_{99} values of 1.0 and 4.0 mg/kg, respectively. The structure–toxicity relationships of turbotoxins were examined, and it was proved that the iodine atoms and trimethylammonium groups are important for its acute toxicity. Turbotoxin A inhibits acetylcholinesterase with an IC_{50} of 28 μM.

Human intoxication resulting from the ingestion of shellfish occurs worldwide, and novel compounds have been isolated from toxic shellfish. Toxins from shellfish, such as pinnatoxins,[1] saxitoxin,[2] and neosurugatoxin,[3] have attracted interest not only from pharmacologists but also from biochemists and chemists due to their novel biological activities and structures (Fig. 1). In Japan, the gastropod *Turbo marmorata* is eaten after the viscera, which cause intoxication, are removed. Yasumoto and coworkers studied the toxic components of *T. marmorata* and obtained several toxic fractions from this animal. They indicated the occurrence of saxitoxin and a minor toxin, iodomethyltrimethylammonium salt, in the water-soluble fraction of *T. marmorata*.[4] Although they mentioned the presence of other toxins in this animal,[5] not all of them have been identified. We describe herein the isolation, structures, synthesis, and structure–toxicity relationships of turbotoxins A and B, the marine toxins from the Japanese gastropod *T. marmorata* (Fig. 2).[6]

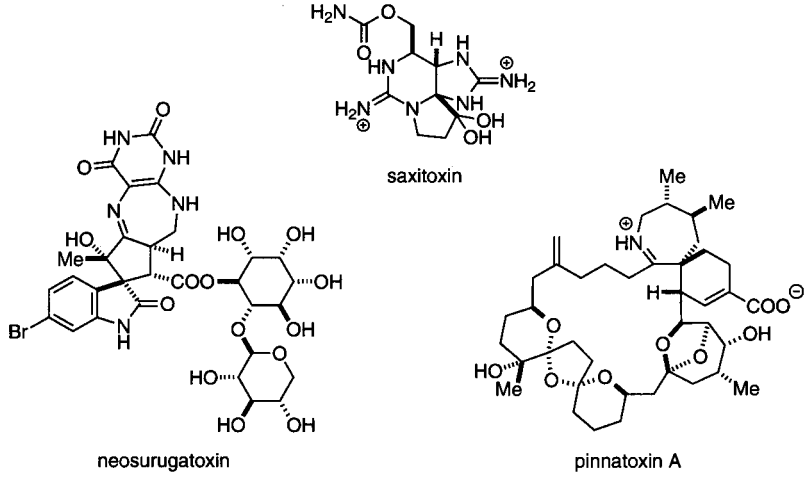

Figure 1. Structures of toxins from shellfish.

Keywords: toxins; ammonium salts; biologically active compounds; marine metabolites; structure–activity.
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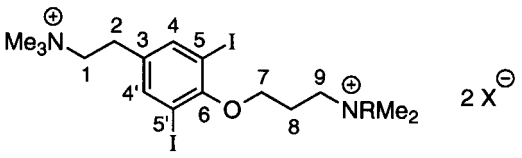


turbotoxin A (**1**) R = Me X = CF_3COO
turbotoxin B (**2**) R = H X = CF_3COO

Figure 2. Structures of turbotoxins A (**1**) and B (**2**).

Isolation and Structures

The 75% aqueous ethanol extract of the viscera (4.5 kg, 36 individuals) of *T. marmorata* collected in Okinawa, Japan, was partitioned between ethyl acetate and water. The aqueous layer was chromatographed using bioassay-guided (intraperitoneal mouse lethality) fractionation, to give two toxic fractions. The early toxic fraction was purified by HPLC to give turbotoxin A (**1**) (2.0 mg; LD_{99} 1.0 mg/kg), and the late toxic fraction was purified in the same way to give turbotoxin B (**2**) (0.9 mg, LD_{99} 4.0 mg/kg). Compounds **1** and **2** were isolated as trifluoroacetate salts because of the solvent system used for chromatographic purification.

The molecular formula of turbotoxin A (**1**) was found to be $C_{17}H_{30}I_2N_2O\cdot(CF_3COO)_2$ (*m/z* 645.0286 $[M-CF_3COO]^+$, Δ −1.2 mmu) by HRFABMS. Resonances in the NMR spectra were assigned based on the COSY, HSQC, and HMBC spectra, as shown in Table 1. Although the carbon signal at C3 was not observed in the ^{13}C NMR spectrum due to the scarcity of the sample, the carbon chemical shift was determined by HMBC (J_{C-H}=6 Hz) experiments.

Table 1. NMR data of turbotoxins A (**1**) and B (**2**) in CD_3OD

position	^1H[a]	^{13}C[b]	HMBC ($^{13}C\rightarrow^1H$)[c]
turbotoxin A (**1**)			
1	3.52 (m, 2H)	68.5	1-NMe, H-2
1-NMe	3.18 (s, 9H)	54.5	1-NMe, H-1
2	3.05 (m, 2H)	29.1	H-1, 4
3		139.1[d]	H-1, 2
4, 4′	7.86 (s, 2H)	142.7	H-2, 4
5, 5′		92.4	H-4
6		158.0	H-4, 7
7	4.10 (t, *J*=5.5 Hz, 2H)	71.1	
8	2.42 (m, 2H)	26.0	H-7, 9
9	3.78 (m, 2H)	66.6	9-NMe, H-7, 8
9-NMe	3.22 (s, 9H)	54.5	9-NMe, H-9
turbotoxin B (**2**)			
1	3.52 (m, 2H)	68.5[c]	1-NMe, H-2
1-NMe	3.18 (s, 9H)	54.5	1-NMe, H-1
2	3.05 (m, 2H)	29.1	H-1, 4
3		138.0[d]	H-1, 2
4, 4′	7.86 (s, 2H)	142.6	H-2, 4
5, 5′		92.5	H-4
6		158.0[d]	H-4
7	4.10 (t, *J*=5.6 Hz, 2H)	71.7	
8	2.33 (m, 2H)	27.1	H-7, 9
9	3.55 (m, 2H)	58.1	9-NMe, H-7, 8
9-NMe	2.98 (s, 6H)	44.5	9-NMe, H-9

[a] The spectrum was recorded at 600 MHz.
[b] The spectrum was recorded at 100 MHz.
[c] Protons correlated to carbon resonances in ^{13}C column.
[d] Chemical shifts were determined by HSQC and HMBC experiments.

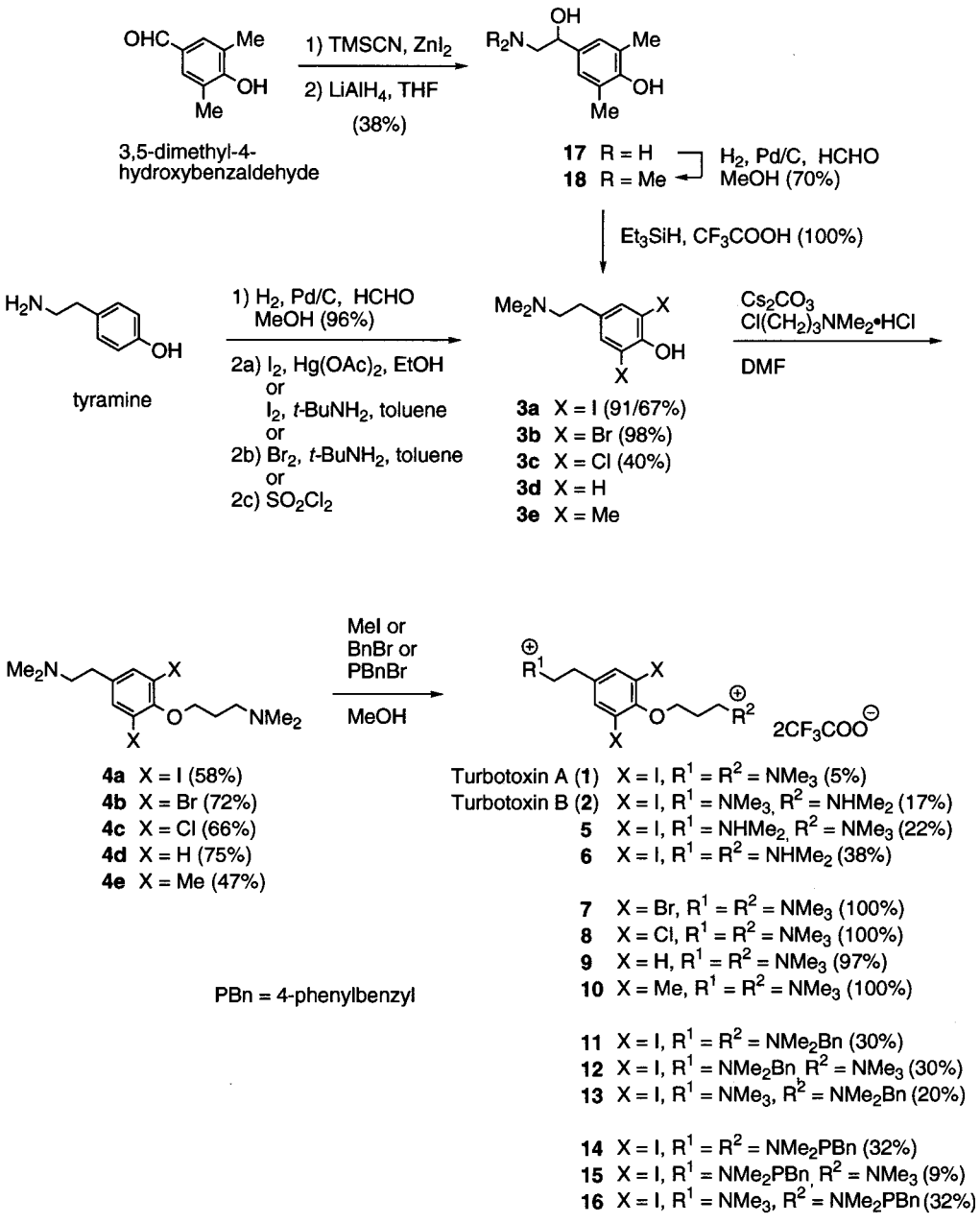
The ^1H NMR data showed the presence of two trimethylammonium groups. The COSY spectrum allowed $-(CH_2)_2-$ and $-(CH_2)_3-$ chains to be constructed. The NMR signals at δ_H 7.86 and at δ_C 92.4, 139.1, 142.7, and 158.8 were assigned to a 4-substituted-2,6-diiodophenoxy unit by comparison with those of thyroxine: the unusually high field of the signal at δ_C 92.4 clearly distinguished the sp^2 carbon atoms linked to an iodine atom. The HMBC correlations (H-1/1-NCH_3, 1-NCH_3/C1, H-2/C3, H-2/C4, H-4/C2, H-4/C4′, H-4/C5, H-4/C6, H-9/9-NCH_3, 9-NCH_3/C9) allowed the foregoing partial structures to be connected, giving two partial structures, $Me_3N^+CH_2CH_2C_6H_2I_2O-$ and $-CH_2CH_2CH_2N^+Me_3$. Finally, a weak HMBC correlation between H-7 and C6 completed the structure of turbotoxin A, as shown in Fig. 2.

Turbotoxin B (**2**) was found to be a demethylated analog of **1**, based on its molecular formula, $C_{16}H_{28}I_2N_2O\cdot(CF_3COO)_2$ (HRFABMS *m/z* 517.0209 $[M-CF_3COOH-CF_3COO]^+$, Δ −0.4 mmu). Comparison of the ^1H NMR data of **2** with those of **1** indicated that a trimethylammonium group at C9 in **1** was demethylated to a dimethylamino group in **2** (Table 1). Furthermore, correlations sufficient to establish the structure of turbotoxin B were found in the HMBC spectrum of **2**. Thus, the structure of turbotoxin B was determined to be **2**.

Synthesis

To confirm the structures of turbotoxins A (**1**) and B (**2**), and to subject **1** and **2** to further biological examinations, **1** and **2** were synthesized (Scheme 1). Tyramine was methylated by a standard procedure to give *N*,*N*-dimethyltyramine (**3d**). Diiodination of **3d** was effected with $I_2-Hg(OAc)_2$ to afford 2,6-diiodo-*N*,*N*-dimethyltyramine (**3a**) in 91% yield. This conversion without mercury salts was achieved with I_2-t-$BuNH_2$[7] in lower yield. Alkylation of **3a** with chloropropyldimethylamine hydrochloride in the presence of Cs_2CO_3 afforded diamine **4a**[8] (58%). Diamine **4a** was methylated with 1 molar equivalent of iodomethane to give turbotoxins A (**1**, 5%) and B (**2**, 17%) along with turbotoxin B isomer **5** (22%) and diamine trifluoroacetate salt **6** (38%), which were separated by HPLC. The spectral data and biological activities of synthetic turbotoxins A (**1**) and B (**2**) were identical to those of natural **1** and **2**, respectively, which confirmed the structures and toxicity of turbotoxins A and B. With an excess of iodomethane, **4a** was transformed into **1** quantitatively.

To investigate the structure–toxicity relationships of turbotoxins, ten analogs were synthesized. By using the same strategy as for turbotoxin A (**1**), the bromo, chloro, and hydro analogs, **7**, **8**, and **9**, were prepared from *N*,*N*-dimethyltyramine (**3d**) as illustrated in Scheme 1. The benzyl ammonium analogs, **11**, **12**, and **13**, and 4-phenylbenzyl analogs, **14**, **15**, and **16**, were prepared from diamine **4a** by treatment of 1 molar equivalent of benzyl bromide or 4-phenylbenzyl bromide followed by an excess of iodomethane, respectively.



Scheme 1. Synthesis of natural and artificial analogs of turbotoxins.

Table 2. Acute toxicity of turbotoxins A (**1**) and B (**2**) and their analogs

Compound	Acute toxicity (LD_{99}, mg/kg)[a]
Turbotoxin A (**1**)	1.0
Turbotoxin B (**2**)	4.0
5	8.0
6	100
Bromo analog **7**	4.0
Chloro analog **8**	8.0
Hydro analog **9**	12
Methyl analog **10**	8.0
Dibenzyl analog **11**	4.0
Monobenzyl analog **12**	2.0
Monobenzyl analog **13**	0.5
Diphenylbenzyl analog **14**	>32
Monophenylbenzyl analog **15**	32
Monophenylbenzyl analog **16**	8.0

[a] Upon intraperitoneal injection into ddY mice ($n \geq 4$).

Dimethyl analog **10** was prepared from 3,5-dimethyl-4-hydroxybenzaldehyde as follows. 3,5-Dimethyl-4-hydroxybenzaldehyde was converted into a cyanohydrin TMS ether, which was then reduced to the amino alcohol **17** (38%). The amino alcohol **17** was methylated and deoxygenated with Et_3SiH–CF_3COOH to give 3,5,*N*,*N*-tetramethyltyramine (**3e**) in 70% yield. The compound **3e** was transformed into **10** by the same sequence of reactions as described above.

Structure–Toxicity Relationships

The relationships between the structure of turbotoxins and their acute toxicity (intraperitoneal mouse lethality) were studied (Table 2).

Compound **6** showed the weakest toxicity among the compounds **1**, **2**, **5** and **6**, indicating that the quaternary ammonium group is responsible for the toxicity. The toxicities of turbotoxin B (**2**) (4.0 mg/kg) and isomer **5** (8.0 mg/kg) are weaker than that of **1** and stronger than that of **6**. This finding shows that the number of the quaternary ammonium groups is important to the toxicity of turbotoxins.

The comparison of toxicities of turbotoxin A (**1**), bromo analog **7**, chloro analog **8**, and hydro analog **9** (1.0, 4.0, 8.0, and 12 mg/kg) indicates the importance of iodine atoms in the toxicity of **1**. The toxicity of methyl analog **10** (8.0 mg/kg) is more potent than that of hydro analog **9** and the same as that of chloro analog **8**, indicating the importance of the steric bulkiness of the C5,5′ substituent.

The effect of the substituents of the quaternary ammonium moieties was examined. While the toxicities of dibenzyl and monobenzyl analogs, **11** and **12**, were weaker than that of **1**, monobenzyl analog **13** exhibited the toxicity stronger than that of **1**. The benzyl substituent at the *N*-9 ammonium group increases its toxicity two-fold. The phenylbenzyl analogs, **14**–**16**, exhibited weaker toxicities than turbotoxin A (**1**) and its benzyl analogs, **11**–**13**. These facts indicated that the bulky 4-phenylbenzyl group prevents the phenylbenzyl analogs from binding to target molecules.

The target biomolecule(s) of turbotoxin A was investigated, and it was found that turbotoxin A (**1**) inhibits acetylcholinesterase with an IC_{50} of 28 μM. X-Ray crystallographic studies of complexes of acetylcholinesterase with small molecules, such as decamethonium bromide, tacrine, and edrophonium bromide, indicated that the aromatic gorge exists at the bottom of the active site.[9] There is as yet no data of relationships between the toxicity and affinity to acetylcholinesterase of turbotoxin analogs, however, the benzyl group in **13** might be stacked against the aromatic gorge to increase its toxicity. Preliminary neuropharmacological experiments were effected for turbotoxin A (**1**), and **1** was proved not to interact with the peripheral nervous system. Further studies are required for understanding the mode of action of turbotoxins.

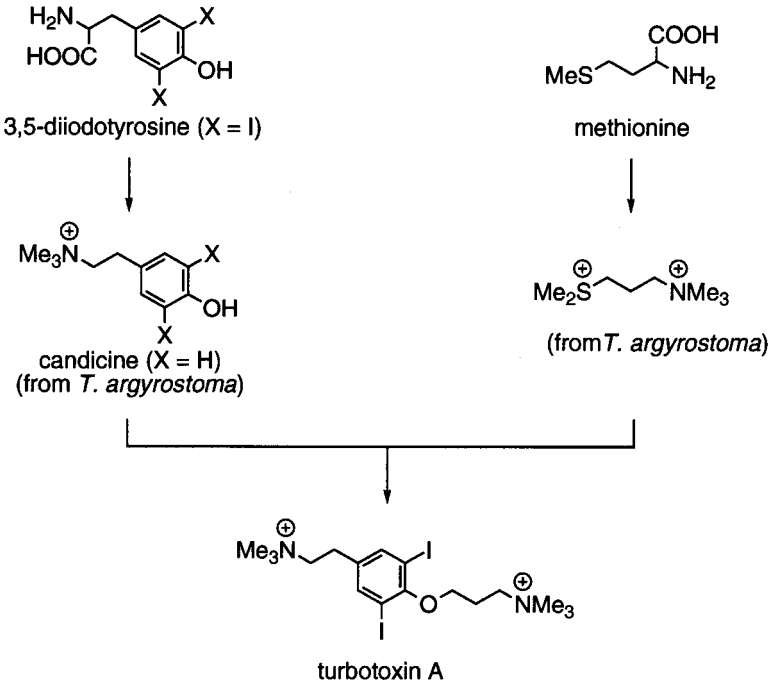
In summary, we isolated two marine toxins, turbotoxins A (**1**) and B (**2**), from the Japanese gastropod *T. marmorata*. The structures of turbotoxins were determined by spectral analysis to be diiodotyramine derivatives, and this was confirmed by organic synthesis. Turbotoxins A (**1**) and B (**2**) are structurally related to dakaramine (**4a**)[10] and dibromotyramine derivatives, such as aplysamine-I,[11] moloka'iamine,[12] and ceratinamine,[13] which were all isolated from marine sponges.

The plausible biosynthesis of turbotoxins is illustrated in Scheme 2. 3,5-Diiodotyrosine, found in corals, sponges, algae, and other marine organisms, might be important for the biosynthesis of turbotoxins, considering the food-habits of *T. marmorata*. Also, candicine, a quaternary ammonium salt of tyramine isolated from *T. argyrostoma*,[14] is a plausible precursor. On the other hand, [3-(dimethylsulfonio)propyl]trimethylammonium salt was isolated from the same animal,[14] which might be a source of the C7–C9 unit of turbotoxins.

Experimental

General

UV spectra were recorded on a JASCO V-550 spectrophotometer. NMR spectra were recorded on a JEOL



Scheme 2. A plausible biogenesis of turbotoxin A (**1**).

ALPHA600 (600 MHz for ^1H and 150 MHz for ^{13}C), a JEOL ALPHA400 (400 MHz for ^1H and 100 MHz for ^{13}C), or a JEOL EX270 (270 MHz for ^1H). NMR chemical shifts were referenced to solvent peaks: δ_H 3.30 (residual CHD_2OD) and δ_C 49.7 for CD_3OD. Mass spectra were determined on a JEOL JMS LG2000 spectrometer operating in the FAB mode (glycerin as a matrix). TLC analysis was conducted on 0.25 mm E. Merck precoated silica gel 60 F_{254}. Fuji Silysia silica gel BW-820 MH was used for column chromatography unless otherwise noted. Preparative HPLC and medium-pressure liquid chromatography (MPLC) were performed using JASCO 880 or Tosoh CCPM-II pumps. Unless otherwise stated, materials were obtained from commercial suppliers and used without further purification.

Isolation of turbotoxins A and B

Internal organs (4.5 kg) of *Turbo marmorata* (36 individuals) collected off the coast of the Okinawan Islands, Japan, in 1998 and 1999 were crushed in a blender in 75% aqueous EtOH and extracted with the same solvent (9 L). The alcoholic extract was evaporated and partitioned between EtOAc (12 L) and H_2O (4 L). The aqueous layer was chromatographed on TSK G-3000S polystyrene gel (74×145 mm, Tosoh Co., Japan) using aqueous ethanol. The materials from the eluates of 25% aqueous EtOH and 50% aqueous EtOH that were proved to be toxic against ddY mice (intraperitoneal injection) were chromatographed using bioassay-guided (intraperitoneal mouse lethality) fractionation as follows. The oily residue (5.18 g) from 50% aqueous EtOH was dissolved in 30% aqueous MeOH, and the insoluble materials were filtered. The oil obtained from the filtrate was chromatographed on MPLC (ODS 160 g) with 30% aqueous MeOH containing 0.1% trifluoroacetic acid (TFA). The early fraction (1.19 g) was chromatographed on MPLC (ODS 160 g) with increasing amounts (10→60%) of MeOH in H_2O containing 0.1% TFA. The fraction (152 mg) eluted with 25–45% aqueous MeOH containing 0.1% TFA was further separated with MPLC (ODS 22 g) with 15% aqueous MeOH containing 0.1% TFA to give two toxic fractions.

The early toxic fraction was finally purified by HPLC [Develosil ODS HG-5, 20×250 mm, (1) 20% aqueous MeOH containing 0.01 M TFA, 5 mL/min; (2) 20% aqueous MeOH containing 0.01 M TFA, 5 mL/min] to give turbotoxin A (**1**) (0.5 mg; LD_{99} 1.0 mg/kg), and the late toxic fraction was purified by HPLC [Develosil ODS HG-5, 20×250 mm, (1) 30 to 40% aqueous MeOH containing 0.01 M TFA, 5 mL/min; (2) 20% aqueous MeOH containing 0.01 M TFA, 5 mL/min] to give turbotoxin B (**2**) (0.9 mg, LD_{99} 4.0 mg/kg).

The residue (10.2 g) from the aforementioned eluate of 25% aqueous EtOH provided turbotoxin A (1.5 mg) by a similar separation procedure as that described above.

Turbotoxin A (1). UV (MeOH) λ_{max} 226 (ϵ 19000), 239 (sh, 9500), 275 nm (sh, 2000); ^1H and ^{13}C NMR data, see Table 1; FABMS *m/z* 645 $[M-CF_3COO]^+$; HRFABMS calcd for $C_{19}H_{30}F_3I_2N_2O_3$ *m/z* 645.0298 $[M-CF_3COO]^+$, found 645.0286.

Turbotoxin B (2). UV (MeOH) λ_{max} 225 (ϵ 11000), 239 (sh, 5500), 279 nm (sh, 1500); ^1H and ^{13}C NMR data, see Table 1; FABMS *m/z* 517 $[M-CF_3COOH-CF_3COO]^+$; HRFABMS calcd for $C_{16}H_{27}I_2N_2O$ *m/z* 517.0213 $[M-CF_3COOH-CF_3COO]^+$, found 517.0209.

***N*,*N*-Dimethyltyramine (3d).** To a solution of tyramine (546 mg, 3.98 mmol) in MeOH (30 mL) were added 37% aqueous formalin (1.19 mL, 15.9 mmol) and 10% Pd/C (421 mg), and the mixture was stirred at room temperature under hydrogen. The reaction mixture was filtered through a pad of Celite, and the residue was washed with MeOH (300 mL). The filtrate and the washings were combined and concentrated to give *N*,*N*-dimethyltyramine (**3d**, 611 mg, 93%) as colorless crystals.

***N*,*N*-Dimethyldiiodotyramine (3a).** (A) To a solution of *N*,*N*-dimethyltyramine (**3d**, 36.8 mg, 0.223 mmol) in EtOH (8.9 mL) were added iodine (121 mg, 0.475 mmol) and $Hg(OAc)_2$ (37 mg, 0.11 mmol). The mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with saturated aqueous $Na_2S_2O_3$ (20 mL) and extracted with EtOAc (2×10 mL). The combined extracts were washed with brine, dried, and concentrated. The residue was chromatographed on silica gel (9 g, $CHCl_3$–MeOH 3:1→1:1→1:3) to give *N*,*N*-dimethyldiiodotyramine (**3a**, 84.2 mg, 91%) as a colorless solid.

(B) To a solution of *t*-$BuNH_2$ (0.07 mL, 0.635 mmol) in toluene (0.2 mL) was added a 0.23 M solution of iodine in toluene (1.4 mL, 0.327 mmol) over 20 min at −78°C. The mixture was warmed to 0°C, and *N*,*N*-dimethyltyramine (**3d**, 10.5 mg, 63.5 μmol) was added. The mixture was then warmed to room temperature over 5 h and stirred for a further 7 h. The reaction was quenched by adding 70% MeSH in MeOH (0.4 mL), and the mixture was stirred at room temperature for 10 min and concentrated. The residual solid was purified by HPLC (Develosil PhA-5, 20×250 mm, 45% aqueous MeOH containing 0.1% TFA, 5.0 mL/min, UV 254 nm) to give *N*,*N*-dimethyldiiodotyramine (**3a**) as the trifluoroacetate salt (22.7 mg, 67%) as a colorless oil: ^1H NMR (270 MHz, CD_3OD) δ 7.68 (s, 2H), 3.35–3.25 (m, 2H), 2.94–2.86 (m, 2H), 2.90 (s, 6H); MS (FAB) *m/z* 418 $[M-CF_3COO]^+$; HRMS (FAB) calcd for $C_{10}H_{14}I_2NO$ $[M-CF_3COO]^+$ 417.9165, found 417.9146.

***N*,*N*-Dimethyldibromotyramine (3b).** Bromine (0.065 mL, 1.26 mmol) was slowly added to a solution of *t*-$BuNH_2$ (0.26 mL, 2.5 mmol) in toluene (3 mL) at −78°C. The resulting mixture was warmed to 5°C, and *N*,*N*-dimethyltyramine (**3d**, 83.3 mg, 0.50 mmol) was added. After the mixture was stirred at 5°C for 3 h, the reaction was quenched by adding H_2O (7 mL), and the mixture was extracted with EtOAc (5×15 mL). The combined extracts were washed with brine, dried, and concentrated. The residual solid was chromatographed on silica gel (8.5 g, EtOAc–MeOH 10:1→5:1→1:1) to give *N*,*N*-dimethyldibromotyramine (**3b**, 159 mg, 98%) as a colorless solid: ^1H NMR (270 MHz, CD_3OD) δ 7.31 (s, 2H), 2.68 (s, 4H), 2.41 (s, 6H); MS (FAB) *m/z* 322, 324, 326 $[M+H]^+$; HRMS (FAB) calcd for $C_{10}H_{14}Br_2NO$ $[M+H]^+$ 321.9442, found 321.9441.

***N*,*N*-Dimethyldichlorotyramine (3c).** A solution of *N*,*N*-dimethyltyramine (**3d**, 100 mg, 0.61 mmol) in SO_2Cl_2 (0.11 mL, 1.3 mmol) was stirred at 65°C for 1.5 h. Sulfuryl chloride (0.02 mL, 0.25 mmol) was added, and the mixture was stirred at the same temperature for a further 4.5 h. The reaction mixture was concentrated, dissolved in MeOH to decompose the excess of SO_2Cl_2, and concentrated again. The residual solid was chromatographed on silica gel (8.5 g, EtOAc–MeOH 10:1→5:1→1:1) to give *N*,*N*-dimethyldichlorotyramine (**3c**, 56.1 mg, 40%) as a colorless solid: ^{1}H NMR (270 MHz, CD_3OD) δ 7.27 (s, 2H), 2.97–2.90 (m, 4H), 2.91 (s, 6H); MS (FAB) *m*/*z* 234, 236, 238 $[M+H]^+$; HRMS (FAB) calcd for $C_{10}H_{14}Cl_2NO$ $[M+H]^+$ 234.0453, found 234.0471.

Diamine 4a. A mixture of *N*,*N*-dimethyldiodotyramine (**3a**, 16.6 mg, 39.8 μmol), Cs_2CO_3 (66.1 mg, 0.203 mmol), $ClCH_2CH_2CH_2N(CH_3)_2{\cdot}HCl$ (12.6 mg, 79.6 μmol), and DMF (0.41 mL) was stirred at 40°C for 28 h. The reaction mixture was diluted with H_2O (5 mL) and extracted with EtOAc (3×5 mL). The combined extracts were washed with saturated aqueous NaCl, dried, and concentrated. The residue was chromatographed on silica gel (0.5 g, EtOAc–MeOH 10:1→5:1→0:1) to give diamine **4a** (11.6 mg, 58%) as a colorless oil: ^{1}H NMR (270 MHz, CD_3OD) δ 7.68 (s, 2H), 3.98 (t, *J*=6.3 Hz, 2H), 2.72–2.62 (m, 4H), 2.55–2.46 (m, 2H), 2.30 (s, 6H), 2.28 (s, 6H), 2.14–2.01 (m, 2H); MS (FAB) *m*/*z* 503 $[M+H]^+$; HRMS (FAB) calcd for $C_{15}H_{25}I_2N_2O$ $[M+H]^+$ 503.0059, found 503.0041.

Diamines **4b**–**4e** were also prepared from tyramines **3b**–**3e**, respectively, by the same experimental procedure as for diamine **4a**.

Diamine 4b (72% yield). A colorless oil; ^{1}H NMR (270 MHz, CD_3OD) δ 7.45 (s, 2H), 4.01 (t, *J*=6.1 Hz, 2H), 2.75–2.60 (m, 4H), 2.60–2.50 (m, 2H), 2.29 (s, 6H), 2.28 (s, 6H), 2.06–2.00 (m, 2H); MS (FAB) *m*/*z* 407, 409, 411 $[M+H]^+$; HRMS (FAB) calcd for $C_{15}H_{25}Br_2N_2O$ $[M+H]^+$ 407.0334, found 407.0308.

Diamine 4c (66% yield). A colorless oil; ^{1}H NMR (270 MHz, CD_3OD) δ 7.25 (s, 2H), 4.02 (t, *J*=6.1 Hz, 2H), 2.75–2.70 (m, 2H), 2.66–2.60 (m, 2H), 2.56–2.51 (m, 2H), 2.29 (s, 6H), 2.28 (s, 6H), 2.05–1.95 (m, 2H); MS (FAB) *m*/*z* 319, 321, 323 $[M+H]^+$; HRMS (FAB) calcd for $C_{15}H_{25}Cl_2N_2O$ $[M+H]^+$ 319.1344, found 319.1318.

Diamine 4d (75% yield). A colorless oil; ^{1}H NMR (270 MHz, CD_3OD) δ 7.09 (d, *J*=8.9 Hz, 2H), 6.82 (d, *J*=8.9 Hz, 2H), 3.95 (t, *J*=6.1 Hz, 2H), 2.73–2.67 (m, 2H), 2.52–2.45 (m, 4H), 2.27 (s, 6H), 2.24 (s, 6H), 1.97–1.86 (m, 2H); MS (FAB) *m*/*z* 251 $[M+H]^+$; HRMS (FAB) calcd for $C_{15}H_{27}N_2O$ $[M+H]^+$ 251.2123, found 251.2140.

Diamine 4e (47% yield). A colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 6.83 (s, 2H), 3.77 (t, *J*=6.1 Hz, 2H), 2.69–2.62 (m, 2H), 2.62–2.55 (m, 2H), 2.54–2.47 (m, 2H), 2.29 (s, 12H), 2.01–1.91 (m, 2H); MS (FAB) *m*/*z* 279 $[M+H]^+$; HRMS (FAB) calcd for $C_{17}H_{31}N_2O$ $[M+H]^+$ 279.2436, found 279.2413.

Turbotoxins A (1) and B (2)

Diamine **4a** (6.5 mg, 13 μmol) was dissolved in a 0.1 M solution of iodomethane in MeOH (0.13 mL, 13 μmol), and the resulting solution was stirred at room temperature for 2 h and concentrated. The residue was purified by HPLC (1. Develosil ODS HG-5, 20×250 mm, 30% aqueous MeOH containing 0.1% TFA, 5 mL/min, UV 254 nm; 2. Develosil PhA-5, 20×250 mm, 30% aqueous MeOH containing 0.1% TFA, 5 mL/min, UV 254 nm) to give turbotoxin A (**1**, 0.5 mg, 5%), turbotoxin B (**2**, 1.6 mg, 17%), compound **5** (2.1 mg, 22%), and diamine **4a** (3.6 mg, 38%) as the trifluoroacetate salt, respectively.

Compound 5 (trifluoroacetate salt). ^{1}H NMR (270 MHz, CD_3OD) δ 7.86 (s, 2H), 4.11 (t, *J*=5.6 Hz, 2H), 3.82–3.73 (m, 2H), 3.37–3.28 (m, 2H), 3.22 (s, 9H), 3.00–2.94 (m, 2H), 2.92 (s, 6H), 2.48–2.36 (m, 2H); MS (FAB) *m*/*z* 517 $[M-CF_3COOH-CF_3COO]^+$; HRMS (FAB) calcd for $C_{16}H_{27}I_2N_2O$ $[M-CF_3COOH-CF_3COO]^+$ 517.0215, found 517.0208.

Diamine trifluoroacetate salt 6. ^{1}H NMR (270 MHz, CD_3OD) δ 7.83 (s, 2H), 4.10 (t, *J*=5.6 Hz, 2H), 3.59–3.50 (m, 2H), 3.36–3.30 (m, 2H), 3.00–2.94 (m, 2H), 2.98 (s, 6H), 2.92 (s, 6H), 2.40–2.28 (m, 2H); MS (FAB) *m*/*z* 503 $[M-CF_3COOH-CF_3COO]^+$; HRMS (FAB) calcd for $C_{15}H_{25}I_2N_2O$ $[M-CF_3COOH-CF_3COO]^+$ 503.0059, found 503.0041.

Turbotoxin analogs 7–10

These compounds were also prepared from the corresponding dihalodimethyltyramines **4b**–**4e**, respectively, by a similar experimental procedure as that for turbotoxins A and B except for the use of an excess of iodomethane.

Bromo analog 7 (trifluoroacetate salt, 100% yield). A colorless oil; ^{1}H NMR (270 MHz, CD_3OD) δ 7.63 (s, 2H), 4.13 (t, *J*=5.6 Hz, 2H), 3.78–3.71 (m, 2H), 3.58–3.51 (m, 2H), 3.21 (s, 9H), 3.19 (s, 9H), 3.14–3.05 (m, 2H), 2.44–2.31 (m, 2H); MS (FAB) *m*/*z* 549, 551, 553 $[M-CF_3COO]^+$; HRMS (FAB) calcd for $C_{19}H_{30}F_3Br_2N_2O_3$ $[M-CF_3COO]^+$ 549.0575, found 549.0546.

Chloro analog 8 (trifluoroacetate salt, 100% yield). A colorless oil; ^{1}H NMR (270 MHz, CD_3OD) δ 7.43 (s, 2H), 4.13 (t, *J*=5.6 Hz, 2H), 3.75–3.70 (m, 2H), 3.58–3.52 (m, 2H), 3.21 (s, 9H), 3.20 (s, 9H), 3.13–3.07 (m, 2H), 2.40–2.30 (m, 2H); MS (FAB) *m*/*z* 461, 463, 465 $[M-CF_3COO]^+$; HRMS (FAB) calcd for $C_{19}H_{30}F_3Cl_2N_2O_3$ $[M-CF_3COO]^+$ 461.1585, found 461.1565.

Hydro analog 9 (trifluoroacetate salt, 97% yield). A colorless oil; ^{1}H NMR (270 MHz, CD_3OD) δ 7.25 (d, *J*=8.9 Hz, 2H), 6.93 (d, *J*=8.9 Hz, 2H), 4.09 (t, *J*=5.6 Hz, 2H), 3.62–3.50 (m, 4H), 3.19 (s, 9H), 3.18 (s, 9H), 3.15–3.05 (m, 2H), 2.33–2.23 (m, 2H); MS (FAB) *m*/*z* 393 $[M-CF_3COO]^+$; HRMS (FAB) calcd for $C_{19}H_{32}F_3N_2O_3$ $[M-CF_3COO]^+$ 393.2365, found 393.2383.

Methyl analog 10 (trifluoroacetate salt, 100% yield). A colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 6.98 (s, 2H),

3.86 (t, J=5.6 Hz, 2H), 3.72–3.64 (m, 2H), 3.54–3.46 (m, 2H), 3.20 (s, 9H), 3.18 (s, 9H), 3.04–2.96 (m, 2H), 2.34–2.21 (m, 2H), 2.26 (s, 6H); MS (FAB) m/z 421 $[M-CF_3COO]^+$; HRMS (FAB) calcd for $C_{21}H_{36}F_3N_2O_3$ $[M-CF_3COO]^+$ 421.2679, found 421.2683.

Benzyl analogs 11, 12, and 13

Diamine **4a** (11.0 mg, 21.9 μmol) was dissolved in a 0.1 M solution of benzyl bromide in MeOH (0.22 mL, 22 μmol), and the mixture was stirred at room temperature for 32 h. Iodomethane (0.14 mL, 2.2 mmol) was added, and the mixture was stirred at room temperature for 3 h and concentrated. The residue was separated by HPLC (Develosil PhA-5, 20×250 mm, 47% aqueous MeOH containing 0.1% TFA, 5 mL/min, UV 254 nm) to give analog **11** (6.8 mg, 30%), analog **12** (4.0 mg, 20%), analog **13** (6.0 mg, 30%), and turbotoxin A (**1**) (3.7 mg, 20%) as a colorless oil, respectively.

Analog 11. ^{1}H NMR (270 MHz, CD_3OD) δ 7.85 (s, 2H), 7.66–7.49 (m, 10H), 4.64 (s, 2H), 4.57 (s, 2H), 4.11 (t, J=5.3 Hz, 2H), 3.77–3.69 (m, 2H), 3.56–3.45 (m, 2H), 3.19–3.10 (m, 2H), 3.14 (s, 6H), 3.09 (s, 6H), 2.56–2.43 (m, 2H); MS (FAB) m/z 797 $[M-CF_3COO]^+$; HRMS (FAB) calcd for $C_{31}H_{38}F_3I_2N_2O_3$ $[M-CF_3COO]^+$ 797.0924, found 797.0914.

Analog 12. ^{1}H NMR (270 MHz, CD_3OD) δ 7.86 (s, 2H), 7.59–7.51 (m, 5H), 4.57 (s, 2H), 4.11 (t, J=5.6 Hz, 2H), 3.83–3.74 (m, 2H), 3.56–3.46 (m, 2H), 3.23 (s, 9H), 3.19–3.09 (m, 2H), 3.09 (s, 6H), 2.49–2.37 (m, 2H); MS (FAB) m/z 721 $[M-CF_3COO]^+$; HRMS (FAB) calcd for $C_{25}H_{34}F_3I_2N_2O_3$ $[M-CF_3COO]^+$ 721.0611, found 721.0623.

Analog 13. ^{1}H NMR (270 MHz, CD_3OD) δ 7.85 (s, 2H), 7.66–7.48 (m, 5H), 4.63 (s, 2H), 4.10 (t, J=5.3 Hz, 2H), 3.77–3.69 (m, 2H), 3.56–3.47 (m, 2H), 3.19 (s, 9H), 3.14 (s, 6H), 3.10–3.00 (m, 2H), 2.56–2.47 (m, 2H); MS (FAB) m/z 721 $[M-CF_3COO]^+$; HRMS (FAB) calcd for $C_{25}H_{34}F_3I_2N_2O_3$ $[M-CF_3COO]^+$ 721.0611, found 721.0601.

Phenylbenzyl analogs 14, 15, and 16

To a solution of diamine **4a** (9.6 mg, 19 μmol) in MeOH (0.2 mL) was added 4-phenylbenzyl bromide (5.1 mg, 21 μmol), and the mixture was stirred at room temperature for 20 h. Iodomethane (0.13 mL, 2 mmol) was added, and the mixture was stirred at room temperature for 3 h and concentrated. The residue was separated by HPLC (1. Develosil PhA-5, 20×250 mm, 58% aqueous MeOH containing 0.1% TFA, 5 mL/min, UV 254 nm; 2. Develosil PhA-5, 20×250 mm, 40% aqueous MeOH containing 0.1% TFA, 5 mL/min, UV 254 nm) to give analog **14** (8.0 mg, 32%), analog **15** (2.0 mg, 9%), analog **16** (6.1 mg, 28%), and turbotoxin A (**1**) (3.1 mg, 17%) as a colorless oil, respectively.

Analog 14. ^{1}H NMR (270 MHz, CD_3OD) δ 7.86 (s, 2H), 7.82–7.61 (m, 12H), 7.51–7.34 (m, 6H), 4.69 (s, 2H), 4.62 (s, 2H), 4.11 (t, J=5.3 Hz, 2H), 3.81–3.71 (m, 2H), 3.57–3.48 (m, 2H), 3.20–3.10 (m, 2H), 3.19 (s, 6H), 3.13 (s, 6H), 2.57–2.45 (m, 2H); MS (FAB) m/z 949 $[M-CF_3COO]^+$; HRMS (FAB) calcd for $C_{43}H_{46}F_3I_2N_2O_3$ $[M-CF_3COO]^+$ 949.1550, found 949.1556.

Analog 15. ^{1}H NMR (270 MHz, CD_3OD) δ 7.88 (s, 2H), 7.83–7.77 (m, 2H), 7.70–7.61 (m, 4H), 7.51–7.38 (m, 3H), 4.62 (s, 2H), 4.11 (t, J=5.6 Hz, 2H), 3.83–3.73 (m, 2H), 3.58–3.49 (m, 2H), 3.23 (s, 9H), 3.20–3.14 (m, 2H), 3.13 (s, 6H), 2.50–2.37 (m, 2H); MS (FAB) m/z 797 $[M-CF_3COO]^+$; HRMS (FAB) calcd for $C_{31}H_{38}F_3I_2N_2O_3$ $[M-CF_3COO]^+$ 797.0924, found 797.0902.

Analog 16. ^{1}H NMR (270 MHz, CD_3OD) δ 7.85 (s, 2H), 7.82–7.63 (m, 6H), 7.51–7.26 (m, 3H), 4.68 (s, 2H), 4.11 (t, J=5.3 Hz, 2H), 3.81–3.71 (m, 2H), 3.56–3.46 (m, 2H), 3.18 (s, 9H), 3.18 (s, 6H), 3.10–3.00 (m, 2H), 2.57–2.45 (m, 2H); MS (FAB) m/z 797 $[M-CF_3COO]^+$; HRMS (FAB) calcd for $C_{31}H_{38}F_3I_2N_2O_3$ $[M-CF_3COO]^+$ 797.0924, found 797.0923.

Dimethylamino alcohol 18

A mixture of 3,5-dimethyl-4-hydroxybenzaldehyde (200 mg, 1.33 mmol), ZnI_2 (20.6 mg, 0.064 mmol), and TMSCN (0.2 mL, 1.46 mmol) was stirred at room temperature for 42 h. Trimethylsilyl cyanide (0.2 mL, 1.46 mmol) was added, and the mixture was stirred at room temperature for 18 h. The mixture was diluted with saturated aqueous $NaHCO_3$ (5 mL) and extracted with EtOAc (3×5 mL). The combined extracts were washed with brine, dried, and concentrated to give a crude cyanohydrin TMS ether (417 mg).

To a solution of the cyanohydrin TMS ether (417 mg) in THF (1.3 mL) was added a 1.0 M solution of $LiAlH_4$ in THF (1.33 mL, 1.33 mmol) at −30°C, and the mixture was stirred at 0°C for 1.5 h. Sodium fluoride (280 mg) was added, and the mixture was stirred vigorously for a while. To the mixture, H_2O–THF (1:9, 10 mL) was carefully added. After being stirred for 30 min, the mixture was filtered through a pad of Celite. The residue was washed with aqueous THF thoroughly, and the filtrate and washings were combined and concentrated. The residue was chromatographed on silica gel (4 g, EtOAc–MeOH 5:1→3:1→1:1) to give amino alcohol **17** (91.2 mg, 38% for 2 steps) as a colorless oil: ^{1}H NMR (400 MHz, CD_3OD) δ 6.90 (s, 2H), 4.46 (dd, J=7.3, 5.1 Hz, 1H), 2.81–2.70 (m, 2H), 2.19 (s, 6H); MS (FAB) m/z 182 $[M+H]^+$.

A mixture of amino alcohol **17** (91.2 mg, 0.503 mmol), 10% Pd/C (51 mg), 35% formalin (0.20 mL, 2.5 mmol), and MeOH (3 mL) was stirred under hydrogen at room temperature for 14 h. The mixture was filtered through a pad of Celite, and the residue was washed with MeOH (30 mL). The filtrate and washings were combined and concentrated. The residue was chromatographed on silica gel (4 g, EtOAc–MeOH 5:1→3:1→1:1) to give dimethylamino alcohol **18** (74 mg, 70%) as a colorless oil: ^{1}H NMR (400 MHz, CD_3OD) δ 6.91 (s, 2H), 4.65 (dd, J=9.3, 3.7 Hz, 1H), 2.67 (dd, J=12.7, 9.3 Hz, 1H), 2.45 (dd, J=12.7, 3.7 Hz, 1H), 2.37 (s, 6H), 2.19 (s, 6H); MS

(FAB) *m/z* 210 $[M+H]^+$; HRMS (FAB) calcd for $C_{12}H_{20}NO_2$ $[M+H]^+$ 210.1494, found 210.1477.

Dimethyltyramine 3e. A mixture of dimethylamino alcohol **18** (16.8 mg, 80.3 μmol), Et_3SiH (0.016 mL, 96 μmol), and TFA (0.4 mL) was stirred at 0°C for 40 min. The mixture was diluted with saturated aqueous $NaHCO_3$ (8 mL) and extracted with EtOAc (3×10 mL). The combined extracts were washed with brine, dried, and concentrated. The residue was chromatographed on silica gel (3 g, EtOAc–MeOH 5:1→3:1) to give dimethyltyramine **3e** (20.1 mg, 100%) as a colorless oil: ^1H NMR (400 MHz, CD_3OD) δ 6.79 (s, 2H), 2.97–2.89 (m, 2H), 2.81–2.71 (m, 2H), 2.62 (s, 6H), 2.17 (s, 6H); MS (FAB) *m/z* 194 $[M+H]^+$; HRMS (FAB) calcd for $C_{12}H_{20}NO$ $[M+H]^+$ 194.1545, found 194.1548.

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References

1. Uemura, D.; Chou, T.; Haino, T.; Nagatsu, A.; Fukuzawa, S.; Zheng, S.-z.; Chen, H.-s. *J. Am. Chem. Soc.* **1995**, *117*, 1155–1156; Chou, T.; Kamo, O.; Uemura, D. *Tetrahedron Lett.* **1996**, *37*, 4023–4026. Chou, T.; Haino, T.; Kuramoto, M.; Uemura, D. *Tetrahedron Lett.* **1996**, *37*, 4027–4030.

2. Schantz, E. J.; Mold, J. D.; Stanger, D. W.; Shavel, J.; Riel, F. J.; Bowden, J. P.; Lynch, J. M.; Wyler, R. S.; Riegel, B.; Sommer, H. *J. Am. Chem. Soc.* **1957**, *79*, 5230–5235.

3. Kosuge, T.; Tsuji, K.; Hirai, K.; Yamaguchi, K.; Okamoto, T.; Iitaka, Y. *Tetrahedron Lett.* **1981**, *22*, 3417–3420.

4. Kanno, K.; Kotaki, Y.; Yasumoto, T. *Bull. Jpn. Soc. Sci. Fisher.* **1976**, *42*, 1395–1398; Yasumoto, T.; Kotaki, Y. *Bull. Jpn. Soc. Sci. Fisher.* **1977**, *43*, 207–211; Kotaki, Y.; Oshima, Y.; Yasumoto, T. *Bull. Jpn. Soc. Sci. Fisher.* **1981**, *47*, 943–946.

5. Kotaki, Y.; Yasumoto, T. *Bull. Jpn. Soc. Sci. Fisher.* **1977**, *43*, 1467.

6. Preliminary communication: Kigoshi, H.; Kanematsu, K.; Uemura, D. *Tetrahedron Lett.* **1999**, *40*, 5745–5748.

7. Reagents for aromatic bromination, *t*-$BuNH_2$–Br_2, were reported: Pearson, J. H.; Wysong, J. W.; Breder, C. V. *J. Org. Chem.* **1967**, *32*, 2358–2360.

8. Diamine **4**, or dakaramine, was isolated as a metabolite of the Senegalese sponge *Ptilocaulis* sp.: Diop, M.; Samb, A.; Costantino, V.; Fattorusso, E.; Mangoni, A. *J. Nat. Prod.* **1996**, *59*, 271–272.

9. Harel, M.; Schalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C.; Axelsen, P. H.; Silman, I.; Sussman, J. L. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 9031–9035.

10. Xynas, R.; Capon, R. J. *Aust. J. Chem.* **1989**, *42*, 1427–1433.

11. Hamann, M. T.; Scheuer, P. J.; Kelly-Borges, M. *J. Org. Chem.* **1993**, *58*, 6565–6569.

12. Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *J. Org. Chem.* **1996**, *61*, 2936–2937.

13. Yasumoto, T.; Endo, M. *Bull. Jpn. Soc. Sci. Fisher.* **1974**, *40*, 217–221; Yasumoto, T.; Endo, M. *Bull. Jpn. Soc. Sci. Fisher.* **1974**, *40*, 841–845.