Supporting Information

Merobatzelladines A and B, Anti-infective Tricyclic Guanidines from a Marine Sponge *Monanchora* sp.

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Experimental Section

For general Experimental Procedures, see Ueoka et al. *Biosci. Biotechnol. Biochem.*, **2008**, *72*, 3055-3058.

Biological material. The sponge specimen was collected by hand using SCUBA off Amami Oshima (28°8'N, 129°19'E). The specimen was frozen on site and kept frozen until extraction. The sponge was identified as *Monanchora* sp. and a voucher specimen (ZMAPOR19862) was deposited at the Zoological Museum of The University of Amsterdam.

Determination of the antimicrobial activity. Antibacterial activity was determined by the paper disk method against fish pathogenic Gram-negative bacteria *Vibrio anguillarum* (strain: PT-81049 from ayu). The composition of the medium was 1.5% NaCl, peptone (15.0 g), soybean peptone (5.0 g) and agar (15.0 g) in the 1 L of H₂O. The samples were prepared above on the paper disk (6 mm of diameter). Diameter of inhibitory zone around the 6 mm ϕ paper disk was measured after incubation for one day at 27°C.

Determination of anti-parasite activity. For the determination of antitrypanosomal and antimalarial activities, see references 15 and 16, respectively.

Extraction and isolation. The sponge (200 g, wet weight) was extracted with MeOH and a mixture of CHCl₃/MeOH (1:1). The extracts were combined, concentrated, and partitioned between water and CHCl₃. The CHCl₃ layer was further partitioned between MeOH-H₂O (9:1) and n-hexane and the resulting aqueous MeOH layer was diluted with water adjusting the composition of the solvent to MeOH-H₂O (6:4) and extracted with CHCl₃. The CHCl₃ fraction was separated by ODS flash chromatography with a step-wise elution with mixtures of MeOH and H₂O. The fraction eluted with MeOH-H₂O (7:3) was separated by reversed-phase HPLC (ODS; H₂O-MeCN-TFA) to afford Merobatzelladines A (**1**) and B (**2**).

Merobatzelladine A (1): Pale brown oil; $[\alpha]_D^{25} +37$ (c 0.68, MeOH); UV (MeOH) λ_{max} 211 nm (ϵ 14,000); HRESIMS m/z 360.3444 ($M+H$)⁺ (calcd for C₂₃H₄₂N₃, Δ +6.6 mmu). ¹H and ¹³C NMR data, see Table 1.

Merobatzelladine B (2): Pale brown oil; $[\alpha]_D^{25} +27$ (c 0.15, MeOH); UV (MeOH) λ_{max} 206 nm (ϵ 7000); HRESIMS m/z 306.2909 ($M+H$)⁺ (calcd for C₁₉H₃₆N₃, Δ +7.0 mmu). ¹H and ¹³C NMR data, see Table 1.

Preparation of 9 and 10 from Merobatzelladine B (2). To a solution of Merobatzelladine B (2) (50 mg) in 3 mL of dioxane was added KOH (31.1 mg) and 1,3-ditosyloxy-2-benzyloxypropane (160.7 mg). The reaction mixture was kept at 120 °C for 1 day, and the reaction mixture was evaporated to dryness. The residue was partitioned between CHCl₃ and H₂O and the CHCl₃ layer was purified by ODS HPLC (MeCN-H₂O-TFA, 7:3:0.01) to afford **8** (8 mg) and **7** (6 mg), each of which was subjected to hydrogenation (H₂, Pd-C, rt, 1 atm, 1 day) to afford **10** (4 mg) and **9** (5 mg), respectively.

9: HRESIMS m/z 362.3205 [$M+H$]⁺ (calcd for C₂₂H₄₀N₃O 362.3171), ¹H and ¹³C NMR data see Table S1.

10: HRESIMS m/z 362.3138 [$M+H$]⁺ (calcd for C₂₂H₄₀N₃O 362.3171), ¹H and ¹³C NMR data see Table S1.

MTPA esterification. To a solution of **10** (1 mg) in pyridine (50 μ L) was added (*S*)-MTPACl (6 μ L). The reaction mixture was stirred at rt for 30 min, and the solvent was evaporated. The residue was partitioned between 1M NaHCO₃ and CHCl₃ and the CHCl₃ fraction was purified by ODS HPLC (MeCN-H₂O-TFA, 7:3:0.01) to yield the (*R*)-MTPA ester **11** (0.8 mg). The (*S*)-MTPA ester **12** was prepared in the same way.

11: HRESIMS m/z 578.3570 [$M+H$]⁺ (calcd for C₃₂H₄₇N₃F₃O₃ 578.3570), ¹H and ¹³C NMR data see Table S2.

12: HRESIMS m/z 578.3611 $[M+H]^+$ (calcd for $C_{32}H_{47}N_3F_3O_3$ 578.3570), 1H and ^{13}C NMR data see Table S2.

Table S1. ^1H and ^{13}C NMR Data for **9** and **10** in $\text{DMSO-}d_6$

	9		10	
position	δ_{H} (mult, J Hz)	δ_{C} (mult ^b)	δ_{H} (mult, J Hz)	δ_{C} (mult ^b)
1'a	1.43 (m)	32.4 (CH_2)	1.45 (m)	30.7 (CH_2)
1'b	1.77 (m)		1.77 (m)	
1	3.35 (m)	55.7 (CH)	3.40 (m)	55.3 (CH)
2a	1.42 (m)	32.4 (CH_2)	1.43 (m)	32.4 (CH_2)
2b	2.17 (m)		2.18 (m)	
3	3.67 (m)	52.9 (CH)	3.66 (m)	53.3 (CH)
4a	1.73 (m)	29.1 (CH_2)	1.71 (m)	29.0 (CH_2)
4b	2.14 (m)		2.14 (m)	
5a	1.45 (m)	29.3 (CH_2)	1.45 (m)	29.2 (CH_2)
5b	2.04 (m)		2.04 (m)	
6	3.64 (m)	53.9 (CH)	3.63 (m)	53.9 (CH)
7a	1.53 (m)	27.3 (CH_2)	1.53 (m)	27.7 (CH_2)
7b	2.23 (dd)		2.17 (dd)	
8	3.36 (m)	56.4 (CH)	3.43 (m)	57.1 (CH)
9				
10a	3.04 (m)	50.4 (CH_2)	3.05 (m)	49.0 (CH_2)
10b	3.41 (dd)		3.27 (dd)	
11	4.16 (m)	58.7 (CH)	3.94 (m)	60.3 (CH)
12a	3.24 (m)	50.1 (CH_2)	3.08 (m)	51.3 (CH_2)
12b	3.39 (dd)		3.48 (dd)	
11-OH	5.40 (s)		5.54 (s)	
1"a	1.40 (m)	30.7 (CH_2)	1.41 (m)	30.8 (CH_2)
1"b	1.69 (m)		1.59 (m)	

Table S2. ^1H and ^{13}C NMR Data for **11** and **12** in CD_3OD

	11		12		$\Delta\delta$
position	δ_{H} (mult, J Hz)	δ_{C} (mult ^b)	δ_{H} (mult, J Hz)	δ_{C} (mult ^b)	
1'a	1.25 (m)	33.0 (CH_2)	1.24 (m)	33.5 (CH_2)	-0.01
1'b	1.69 (m)		1.83 (m)		+0.14
1	3.41 (m)	57.2 (CH)	3.39 (m)	56.9 (CH)	-0.02
2a	1.30 (m)	34.8 (CH_2)	1.15 (m)	34.6 (CH_2)	-0.15
2b	2.30 (m)		2.29 (m)		-0.01
3	3.74 (m)	56.1 (CH)	3.70 (m)	55.7 (CH)	-0.04
4a	1.73 (m)	30.6 (CH_2)	1.73 (m)	30.8 (CH_2)	0
4b	2.23 (m)		2.24 (m)		+0.01
5a	1.53 (m)	30.9 (CH_2)	1.40 (m)	30.7 (CH_2)	-0.13
5b	2.17 (m)		2.13 (m)		-0.04
6	3.76 (m)	55.4 (CH)	3.70 (m)	55.7 (CH)	-0.06
7a	1.50 (m)	29.5 (CH_2)	1.34 (m)	29.5 (CH_2)	-0.16
7b	2.30 (dd)		2.24 (dd)		-0.06
8	3.44 (m)	60.0 (CH)	3.34 (m)	59.8 (CH)	-0.10
9					
10a	3.49 (m)	45.9 (CH_2)	3.47 (m)	45.2 (CH_2)	-0.02
10b	3.53 (dd)		3.74 (dd)		+0.19
11	5.54 (m)	67.8 (CH)	5.51 (m)	67.9 (CH)	-0.03
12a	3.50 (m)	50.6 (CH_2)	3.43 (m)	50.9 (CH_2)	-0.07
12	3.95 (dd)		3.93 (dd)		-0.02
1"a	1.56 (m)	32.5 (CH_2)	1.54 (m)	32.2 (CH_2)	-0.02
1"b	1.79 (m)		1.76 (m)		-0.03

Figure S1. ^1H NMR spectrum of **1** in CD_3OD

Figure S2. ^{13}C NMR spectrum of **1** in CD_3OD

Figure S3. COSY spectrum of **1** in CD₃OD

Figure S4. HSQC spectrum of **1** in CD₃OD

Figure S5. HMBC spectrum of **1** in CD_3OD

Figure S6. TOCSY spectrum of **1** in CD₃OD

Figure S7. ROESY spectrum of **1** in CD₃OD

Figure S8. ^1H NMR spectrum of **2** in CD_3OD

Figure S9. ^{13}C NMR spectrum of **2** in CD_3OD

Figure S11. HSQC spectrum of **2** in CD_3OD

Figure S12. HMBC spectrum of **2** in CD₃OD

Figure S13. TOCSY spectrum of **2** in CD₃OD

Figure S14. ROESY spectrum of **2** in CD₃OD

Figure S15. ¹H NMR spectrum of **5** in DMSO-*d*₆

Figure S16. COSY spectrum of **5** in DMSO-*d*₆

Figure S17. HSQC spectrum of **5** in DMSO- d_6

Figure S18. NOESY spectrum of **5** in DMSO- d_6

Figure S19. ¹H NMR spectrum of **6** in DMSO-*d*₆

Figure S20. COSY spectrum of **6** in DMSO- d_6

Figure S21. HSQC spectrum of **6** in DMSO- d_6

Figure S22. NOESY spectrum of **6** in DMSO-*d*₆

Figure S23. ¹H NMR spectrum of **7** in CD₃OD

Figure S24. COSY spectrum of **7** in CD₃OD

Figure S25. HSQC spectrum of **7** in CD₃OD

Figure S26. NOESY spectrum of **7** in CD₃OD

Figure S27. ¹H NMR spectrum of **8** in CD₃OD

Figure S28. COSY spectrum of **8** in CD_3OD

Figure S29. HSQC spectrum of **8** in CD_3OD

Figure S30. NOESY spectrum of **8** in CD₃OD