Bromopyrrole Alkaloids from Marine Sponges of the Genus *Agelas*[⊥]

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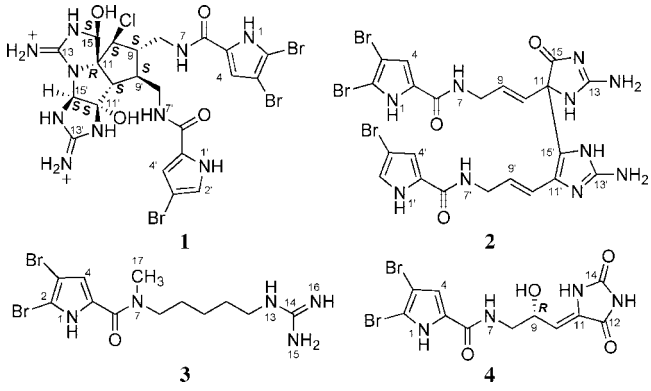
Four new bromopyrrole alkaloids, nagelamides O (**1**) and P (**2**) and mukanadins E (**3**) and F (**4**), were isolated from three collections of Okinawan marine sponges of the genus *Agelas*. The structures and stereochemistry of **1**−**4** were elucidated on the basis of their spectroscopic data.

Bromopyrrole alkaloids are known to be one of the most common metabolites contained in marine sponges.[1] During our search for bioactive substances from Okinawan marine organisms,[2] we have previously isolated several bromopyrrole alkaloids with unique cyclic skeletons from sponges of *Agelas* species.[3,4] Recently, we have investigated extracts of three collections of Okinawan marine sponges of the genus *Agelas* and have isolated two new dimeric bromopyrrole alkaloids, nagelamides O (**1**) and P (**2**), and two new monomeric bromopyrrole alkaloids, mukanadins E (**3**) and F (**4**). Herein, we describe the isolation and structure elucidation of **1**−**4**.

The sponge *Agelas* sp. (SS-983) collected off Seragaki, Okinawa, was extracted with MeOH, and the MeOH extract was partitioned between EtOAc and H_2O. *n*-BuOH-soluble materials of the aqueous layer were subjected to passage over an LH-20 column and a C_{18} column, followed by C_{18} HPLC, to yield nagelamides O (**1**, 0.0014%, wet weight) and P (**2**, 0.0005%). The other two collections of Okinawan sponges of *Agelas* species (SS-1056 and SS-1077) were obtained off Gesashi and off Unten-Port, respectively. Mukanadin E (**3**, 0.00032%) was isolated from EtOAc-soluble materials from the MeOH extract of the sponge of *Agelas* species (SS-1056) collected off Gesashi, Okinawa, while mukanadin F (**4**, 0.00094%) was obtained from that of the sponge of *Agelas* species (SS-1077) collected off Unten-Port, Okinawa, by the same procedure.



The ESIMS of nagelamide O (**1**) showed pseudomolecular ion peaks at *m/z* 765, 767, 769, 771, and 773 (3:10:12:6:1), indicating the presence of three bromine atoms and one chlorine atom in the molecule. Nagelamide O (**1**) was revealed as possessing the molecular formula $C_{22}H_{24}Br_3ClN_{10}O_4$ by HRESIMS [*m/z* 764.9315 $(M + H)^+$, Δ +1.6 mmu]. The UV absorption [λ_{max} 275 (ϵ 9400) nm] was attributed to a substituted pyrrole chromophore, while IR absorptions indicated the occurrence of OH and/or NH (3450 cm^{-1}) and amide carbonyl (1680 cm^{-1}) functionalities. The ^1H NMR (Table 1) spectrum showed 12 D_2O-exchangeable signals (δ_H 12.71, 11.82, 10.19, 9.92, 9.27, 9.25, 8.87, 8.62 (2H), 8.34, 8.21, 7.82, and 7.53). The ^{13}C NMR (Table 1) spectrum disclosed 22 signals due to two amide carbonyl carbons, seven sp^2 quaternary carbons, three sp^2 methines, two sp^3 quaternary carbons, six sp^3 methines, and two sp^3 methylenes. Among the ^{13}C NMR signals of **1**, two amide carbonyl carbons [δ_C 160.1 (2C)], five sp^2 quaternary carbons (δ_C 127.8, 126.8, 104.8, 98.1, and 95.1), and three sp^2 methines (δ_C 121.4, 113.5, and 111.6) were ascribed to di- and monobromopyrrole carbonyl moieties (N-1−C-6 and N-1′−C-6′) by comparison with those of known bromopyrrole alkaloids.[3,4]

Detailed analysis of the $^1H-^1H$ COSY and TOCSY spectra of **1** disclosed connectivities from NH-7 to H-10, NH-7′ to H-10′, H-9 to H-9′, NH-1′ to H-2′, and H-15 to OH-15. HMBC correlations for H-10 to C-11 and C-15, H-15 to C-13, NH-14 to C-11, H-10′ to C-11 and C-11′, 11′-OH to C-10′, NH-12′ to C-13′, NH-14′ to C-11′, and H-15′ to C-11′ and C-13′ indicated the presence of a tetracyclic core[5] (C-9 to C-11, N-12, C-13, N-14, C-15; C-9′ to C-11′, N-12′, C-13′, N-14′, C-15′). ROESY cross-peaks of H-4/NH-7 and H-4′/NH-7′ implied connectivities of a dibromopyrrole ring to N-7 and a monobromopyrrole ring to N-7′ through an amide bond, respectively (Figure 1). ROESY correlations for H-10/H-15′, H-15′/11′-OH, and 11′-OH/H-9′ indicated α-orientations of H-10, H-9′, H-15′, and 11′-OH, while β-orientations of H-10, 15-OH, and H-10′ were deduced from ROESY correlations for H-10′/OH-15 and H-10′/NH-12′ and a large coupling constant for H-9 and H-10 (J = 12.3 Hz). These results indicated the relative stereochemistry of **1** as shown in Figure 2. Thus, the structure of **1** was assigned as the 2′-debromo form of axinellamine A,[5] including relative stereochemistry. The absolute configurations at C-9 and C-9′ in **1** were assigned as both *S* from the CD positive exciton split [λ_{max} 281 ($\Delta\epsilon$ +5.2) and 257 ($\Delta\epsilon$ −3.4) nm] due to di- and monobromopyrrole carboxyamide chromophores (Figure 3).[6] Thus, the structure of nagelamide O was concluded to be **1**.

Professor Köck proposed that axinellamine A could be derived from massadine chloride through a hypothetical intermediate preaxinellamine.[7] Nagelamide O (**1**) might be generated from massadine chloride or its analogue in a similar manner.

The molecular formula of nagelamide P (**2**) was revealed as $C_{22}H_{20}Br_3N_{10}O_3$ by HRESIMS [*m/z* 710.9465 $(M + H)^+$, Δ +3.8 mmu]. ^1H and ^{13}C NMR data (Table 2) of **2** disclosed the presence of di- and monobromopyrrole carbonyl moieties (N-1−C-6 and N-1′−C-6′). Inspection of the $^1H-^1H$ COSY and TOCSY spectra of **2** implied connectivities of N-7 to C-10 and N-7′ to C-10′ (Figure 4). Geometries of the two double bonds at C-9 and C-9′ were assigned as both *E* from NOESY correlations for H-8/H-10 and

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Table 1. ^{1}H and ^{13}C NMR Data of Nagelamide O (**1**) in DMSO-d_6

position	δ_H	mult. J (Hz)	δ_C	position	δ_H	mult. J (Hz)	δ_C
1	12.71	brs		1′	11.82	brs	
2			104.8	2′	6.90	brs	121.4
3			98.1	3′			95.1
4	7.00	brs	113.5	4′	6.98	brs	111.6
5			127.8	5′			126.8
6			160.1	6′			160.1
7	8.34	brt,5.7		7′	8.21	brs	
8	3.73	m	37.0	8′	3.44	m	42.4
	3.24	m			3.26	m	
9	1.95	m	49.3	9′	2.61	brs	34.1
10	4.27	d, 12.3	66.0	10′	2.87	brd, 4	51.6
11			83.2	11′			100.0
13			156.0	OH-11′	7.82	s	
NH_2-13	9.27	brs		12′	9.92	brs	
	8.87	brs		13′			156.9
14	10.19	brs		NH_2-13′	8.62	brs, 2H	
15	5.28	brd, 6.4	78.3	14′	9.25	brs	
OH-15	7.53	brd, 6.4		15′	5.32	brs	79.2

H-8′/H-10′ as well as coupling constants for H-9/H-10 (16 Hz) and H-9′/H-10′ (16 Hz). HMBC cross-peaks of H-10/C-15′ and H-10′/C-15′ indicated connectivities of two imidazole rings at C-11 and C-15′, while connectivities of a dibromopyrrole ring to N-7 and a monobromopyrrole ring to N-7′ were elucidated from NOESY correlations for H-4/NH-7 and H-4′/NH-7′, respectively. Thus, the structure of nagelamide P was elucidated as **2**, which is the 2′-debromo form of mauritiamine.[8] The specific optical rotation, $[\alpha]_D^{22}$ ~0 (c 0.5, MeOH), and the CD spectrum, which was flat between 200 and 400 nm, suggested that **2** was a racemate.[8]

The ESIMS of mukanadin E (**3**) showed pseudomolecular ion peaks at m/z 408, 410, and 412 (1:2:1), indicating the presence of two bromine atoms in the molecule, and the molecular formula was established as $C_{12}H_{19}ON_5Br_2$ by HRESIMS [m/z 408.0045 (M + H)$^+$, Δ +1.0 mmu]. The UV absorption [λ_{max} 273 (ϵ 4500) nm]

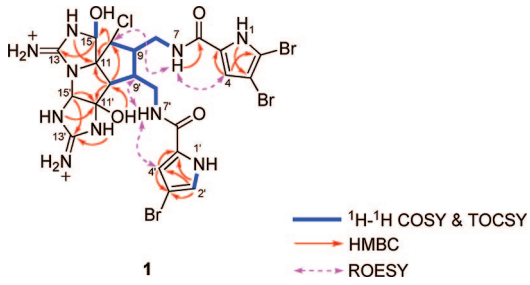

Figure 1. Selected 2D NMR correlations for nagelamide O (**1**).

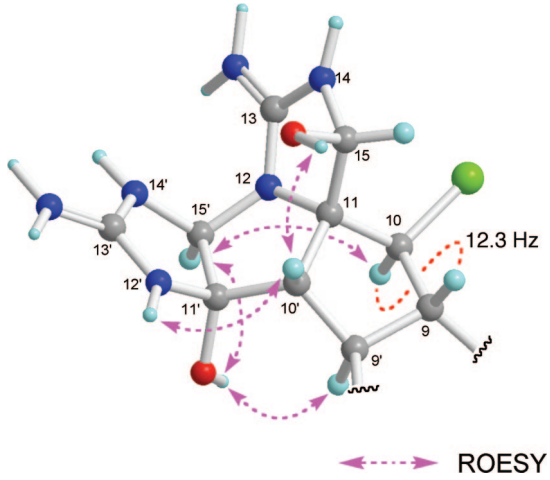

Figure 2. Selected NOESY correlations and relative stereochemistry of the tetracyclic core in nagelamide O (**1**).

was attributed to a substituted pyrrole chromophore, while IR absorptions indicated the occurrence of OH (3440 cm^{-1}) and amide carbonyl (1680 cm^{-1}) functionalities. ^{1}H and ^{13}C NMR data (Table 3) of **3** suggested the presence of a dibromopyrrole carbonyl moiety (N-1−C-6) and a guanidino moiety (N-13, C-14, N-15, and N-16).[9] Detailed analysis of the $^{1}H-^{1}H$ COSY spectrum disclosed the connectivity from H-8 to NH-13 (Figure 5). HMBC correlations for NMe-17/C-6 and NMe-17/C-8 and the NOESY correlation for H-4/NMe-17 indicated that a 2,3-dibromopyrrole carbonyl moiety was connected to N-7 through an amide bond. Thus, the structure of mukanadin E was assigned as **3**, which is the 2-bromo and 7-methyl analogue of laughine.[9]

The molecular formula of mukanadin F (**4**) was established as $C_{11}H_{10}O_4N_4Br_2$ by HRESIMS [m/z 442.8967 (M + Na)$^+$, Δ +0.1 mmu]. The chemical shifts of C-10 (δ_C 111.6), C-11 (δ_C 130.5), C-12 (δ_C 164.6), and C-14 (δ_C 154.7) suggested the presence of a hydantoin ring.[10] The $^{1}H-^{1}H$ COSY spectrum of **4** showed the connectivity from NH-7 to H-10, indicating that the hydroxyl group was attached to C-9 (δ_H 4.43 and δ_C 65.7). The connectivity of a dibromopyrrole ring to N-7 was elucidated from a NOESY correlation for H-4/NH-7, while HMBC cross-peaks of H-9/C-11, H-10/C-12, NH-13/C-11, NH-15/C-14, and NH-15/C-11 indicated that C-10 was connected to the hydantoin ring at C-11. The geometry of the double bond at C-10 was assigned as Z from the NOESY correlation for OH-9/NH-15. To analyze the absolute stereochemistry at C-9, (*S*)- and (*R*)-MTPA esters of mukanadin F (**4**) were prepared. Δδ values for (*S*)- and (*R*)-MTPA esters of mukanadin F (**4**) suggested the absolute configuration at C-9 to be *R*.[11] Thus, the structure of mukanadin F was elucidated as **4**, which is the 9-hydroxy form of mukanadin D.[10]

Nagelamide O (**1**) is a rare bromopyrrole alkaloid with a perhydrocyclopenta-imidazo-azolo-imidazole carbon skeleton, while mukanadin E (**3**) is the first bromopyrrole alkaloid possessing an *N*-methyl amide bond. Nagelamide O (**1**) showed weak antibacterial activity against *Bacillus subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus* (MIC, 33.3 μg/mL each), while mukanadin F (**4**) exhibited weak antifungal activity against *Aspergillus niger* (MIC, 16.7 μg/mL).[12]

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO P-1030 polarimeter. UV spectra were recorded on a Shimadzu UV-1600PC spectrophotometer. CD spectra were recorded on a JASCO J-720 polarimeter. IR spectra were recorded on a JASCO FT/IR-230 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 and a JEOL ECA-500 spectrometer. The 2.49 and 39.5 ppm resonances of residual DMSO-d_6 were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. ESIMS were obtained on a JEOL JMS-700TZ spectrometer.

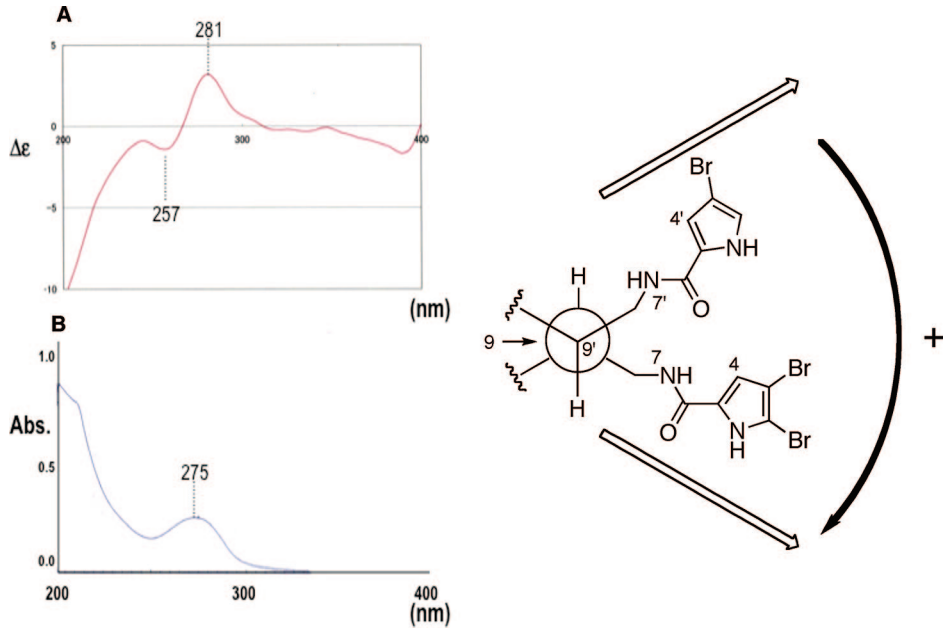

Figure 3. CD (A) and UV (B) spectra, and rotation model for the C-9−C-9′ bond of nagelamide O (**1**).

Table 2. ^{1}H and ^{13}C NMR Data of Nagelamide P (**2**) in DMSO-d_6

position	δ_H	mult. J (Hz)	δ_C	position	δ_H	mult. J (Hz)	δ_C
1	12.68	brs		1′	11.80	brs	
2			104.6	2′	6.98	brs	121.4
3			98.0	3′			95.0
4	6.95	brs	112.8	4′	6.86	brs	111.7
5			128.1	5′			126.6
6			159.5	6′			158.8
7	8.42	brt, 5.9		7′	8.39	brt, 5.9	
8	3.87	brt, 5.3	40.6	8′	3.95	m	40.5
9	6.02	dt, 16, 5.3	139.1	9′	6.02	dt, 16, 5.3	136.6
10	6.05	brd,	126.7	10′	6.41	brd, 16	115.7
11			69.9	11′			119.2
12				12′	12.56	brs	
13			147.7	13′			147.7
NH_2-13	7.62	brs, 2H		NH_2-13′	7.62	brs, 2H	
14	12.12	brs		14′			
15			183.4	15′			121.3

Sponge Description. The yellowish-brown sponge *Agelas* sp. (SS-983, family Agelasidae) collected off Seragaki, Okinawa, and the red-brown sponge *Agelas* sp. (SS-1056) collected off Gesashi, Okinawa, and the sponge *Agelas* sp. (SS-1077) collected off Unten-Port, Okinawa, were kept frozen until used. The voucher specimens (SS-983, SS-1056, and SS-1077) were deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge SS-983 (0.2 kg, wet wt) was extracted with MeOH, the extract was partitioned between EtOAc and H_2O, and subsequently the aqueous layer was extracted with *n*-BuOH. *n*-BuOH-soluble materials were subjected to LH-20 column chromatography to give an alkaloid-containing fraction. This fraction was separated by a C_{18} column chromatography (MeOH/H_2O/CF_3COOH, 30:70:0.1 → 100:0:0) and then C_{18} HPLC (YMC-Pack ODS-AM, YMC Co., Ltd., 10 × 250 mm; eluent CH_3CN/H_2O/CF_3COOH, 37.5:62.5:0.1; flow rate 2 mL/min; UV detection at 254 nm) to yield nagelamides O (**1**, 2.7 mg) and P (**2**, 1.0 mg). The sponge *Agelas* sp. (SS-1056, 1.35 kg, wet wt) was extracted with MeOH, and the extract was partitioned between EtOAc and H_2O. EtOAc-soluble materials were

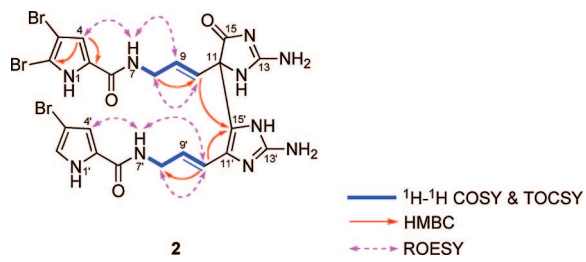

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Figure 4. Selected 2D NMR correlations for nagelamide P (**2**).

Table 3. ^{1}H and ^{13}C NMR Data of Mukanadins E (**3**) and F (**4**) in DMSO-d_6

	3		**4**	
position	δ_H	δ_C	δ_H	δ_C
1	12.27 brs		12.67 brs	
2		103.57		104.56
3		97.39		97.86
4	6.60 brs	113.71	6.93 brs	112.92
5		126.92		128.00
6		160.05		159.05
7			8.06 t, 5.4	
8	3.44 t	48.05	3.29 m	44.43
9	1.58 m	27.76	4.43 m	65.67
OH-9			5.34 m	
10	1.30 m	22.93	5.45 d, 8.4	111.59
11	1.52 m	26.29		130.45
12	3.12	40.43		164.64
13	7.02		10.02 brs	
14		156.82		154.68
15			11.02 brs	
16				
Me-17	3.08 s	35.09		

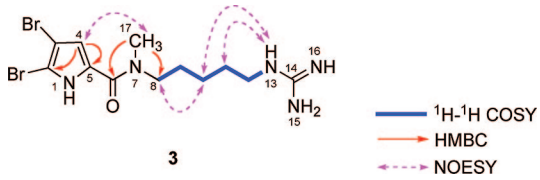

Figure 5. Selected 2D NMR correlations for mukanadin E (**3**).

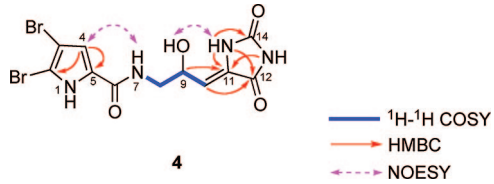

Figure 6. Selected 2D NMR correlations for mukanadin F (**4**).

subjected to a silica gel column chromatography ($CHCl_3$/*n*-BuOH/AcOH/H_2O, 1.5:6:1:1) to give an alkaloid-containing fraction. This fraction was separated by a C_{18} column chromatography (MeOH/H_2O/CF_3COOH, 80:20:0.1) and then C_{18} HPLC (YMC-Pack ODS-AM, YMC Co., Ltd., 10 × 250 mm; eluent $CH_3CN/H_2O/CF_3COOH$, 25:75:0.1 → 35:65:0.1; flow rate 2 mL/min; UV detection at 255 nm) to yield mukanadin E (**3**, 4.3 mg). Mukanadin F (**4**, 6.6 mg) was obtained from the sponge *Agelas* sp. (SS-1077, 0.70 kg, wet wt) by the same procdure as for mukanadin E (**3**).

Nagelamide O (1): colorless, amorphous solid; $[\alpha]_D^{22}$ +1.6 (*c* 0.75, MeOH); UV (MeOH) λ_{max} 275 (ϵ 9400) nm; IR (KBr) ν_{max} 3450, 2920, 2850, 1680, 1210 cm^{-1}; CD (MeOH) λ_{max} 281 ($\Delta\epsilon$ +5.2) and 257 ($\Delta\epsilon$ −3.4) nm; ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive) *m/z* 765:767:769:771:773 [$(M + H)^+$, 3:10:12:6:1]; HRESIMS *m/z* 764.9315 [$(M + H)^+$, Δ +1.6 mmu], calcd for $C_{22}H_{25}{}^{79}Br_3{}^{35}ClN_{10}O_4$, 764.9299.

Nagelamide P (2): colorless, amorphous solid; $[\alpha]_D^{22}$ ~0 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 276 (ϵ 21 100) nm; IR (KBr) ν_{max} 3350, 2920, 2850, 1680, 1210 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS *m/z* 711:713:715:717 [$(M + H)^+$, 1:3:3:1]; HRESIMS *m/z* 710.9465 [$(M + H)^+$, Δ +3.8 mmu], calcd for $C_{22}H_{21}{}^{79}Br_3N_{10}O_3$, 710.9427.

Mukanadin E (3): colorless, amorphous solid; UV (MeOH) λ_{max} 273 (ϵ 4500) nm; IR (KBr) ν_{max} 3440, 1680 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; ESIMS *m/z* 408, 410, and 412 [$(M + H)^+$, 1:2:1]; HRESIMS *m/z* 408.0045 [$(M + H)^+$, Δ +1.0 mmu], calcd for $C_{12}H_{20}ON_5{}^{79}Br_2$, 408.0035.

Mukanadin F (4): colorless, amorphous solid; $[\alpha]_D^{20}$ −3.9 (*c* 0.3, MeOH); UV (MeOH) λ_{max} 279 (ϵ 16 000), 203 (32 000) nm; IR (KBr) ν_{max} 3210, 1685 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; ESIMS *m/z* 443, 445, and 447 [$(M + Na)^+$, 1:2:1]; HRESIMS *m/z* 442.8967 [$(M + Na)^+$, Δ +0.1 mmu], calcd for $C_{11}H_{10}O_4N_4{}^{79}Br_2$ Na, 442.8966.

Preparation of (*R*)- and (*S*)-MTPA Esters of Mukanadin F (4). To a solution of **4** (0.4 mg) in pyridine (100 μL) were added *N,N*-demethylaminopyridine (100 μg), triethylamine (6 μL), and (*S*)-MTPA Cl (3 μL), and stirring was continued at room temperature for 24 h. After removal of solvent, resulting residue was partitioned between EtOAc (200 μL × 3) and H_2O (100 μL × 3). The organic layer was concentrated *in vacuo* to afford the (*R*)-MTPA ester of **4**. The (*S*)-MTPA ester of **4** was prepared according to the same procedure as described above.

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