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ORIGINAL ARTICLE

New phenethylamine derivatives from *Arenibacter nanhaiticus* sp. nov. NH36A^T and their antimicrobial activity

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Five new phenethylamine (PEA) derivatives (1–5) were isolated from the strain of *Arenibacter nanhaiticus* sp. nov. NH36A^T derived from the marine sediment of the South China Sea by bioassay-guided fractionation. Their structures were elucidated by spectroscopic methods including UV, IR, HR-MS and NMR. Interestingly, compounds 1–4 existed as enantiomers, which were resolved by chiral liquid chromatography. The resolved configuration of each enantiomer was assigned by the Marfey's method. Of these compounds, 5 showed weak antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* with MIC values of 0.50 and 0.25 mg ml^{-1}, respectively.

INTRODUCTION

Phenethylamine (PEA), the simplest endogenous aromatic amine and potential pharmacophore, provides the basic chemical structure for several classes of drugs with wide biological activity.[1–4] Owing to this reason, PEA derivatives have attracted intensive interests recently and a series of new ones has been obtained from natural organisms and chemical syntheses, which exhibited diverse bioactivities, including bronchiectasia, antidepressant, stimulant, *etc.*[5,6] Nowadays, they have emerged as a promising biologically active scaffold in the search of new agents against human diseases.

In our ongoing work to search for antimicrobial components from marine microorganisms, five new PEA derivatives (1–5) were isolated from the strain of *Arenibacter nanhaiticus* sp. nov. NH36A^T derived from the marine sediment of the South China Sea. Here, we reported the isolation and structural elucidation of new compounds (1–5). Moreover, the chiral resolution of enantiomers and the determination of their absolute configurations were also discussed.

RESULTS

Structure determination

Compounds 1–4 (Figure 1) gave negative reaction with ninhydrin, but were positive after hydrolysis with concentrated HCl (6.0 N), which suggested that they were cyclo-peptides.[7]

Compound 1 was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{22}H_{25}N_3O_3$ based on

the quasimolecular ion at *m/z* 380.1976 $[M+H]^+$ (calcd 380.1974) in the HR-ESI-TOF MS, indicating 12 degrees of unsaturation. The ^1H and ^{13}C NMR spectra of 1 (Table 1) showed resonances for three amide N-H proton signals (δ_H 7.83 (1H, t, 5.2), 7.96 (1H, brs) and 8.12 (1H, brs)) and three ester/amide carbonyl carbon signals (δ_C 167.2, 167.3 and 171.1), which further suggested that it was a cyclopeptide. The characteristic methine resonances of α-CH at δ 3.01/52.8 and 4.12/55.4 indicated that it was a cyclodipeptide. The presence of a cluster of 10 aromatic protons at δ_H 7.17–7.28 in the ^1H-NMR spectrum suggested that it contained two monosubstituted benzene rings. Detailed analysis of ^1H-^1H COSY, HSQC and HMBC data of 1 established three residues as phenylalanine (Phe), glutamic acid (Glu) and *N*-phenethyl group (Figure 2a). According to the HMBC correlation between H_2-1″ and C-5 (Glu), the structure of 1 was established to be cyclo (Phe-Glu) substituted with *N*-phenethyl, which was a new PEA derivative.

The optical rotation value of 1 was almost zero, which suggested it should be a racemate. Chiral resolution of 1 was performed by Lux Amylose-2 chiral liquid chromatography (phenomenex, ϕ 5 μm, 4.6 × 250 mm) using 100% CH_3CN at 1.0 ml min^{-1}. Two enantiomers were obtained from 1 (Figure 3a), namely 1a and 1b, at t_R 17.11 and 24.72 min, respectively, and their relative content was nearly identical according to their relative peak areas (53.4% for 1a and 46.6% for 1b) in the HPLC chromatogram.

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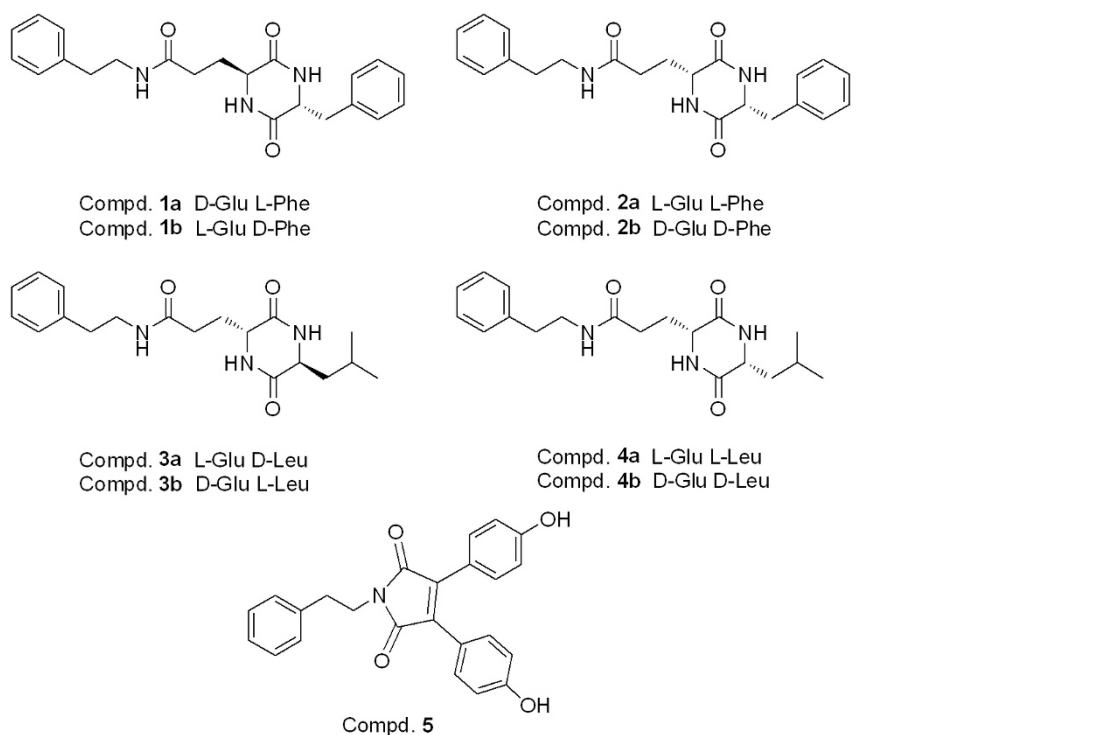Y Chen *et al*

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Figure 1 Chemical structures of compounds 1–5 from *Arenibacter nanhaiticus* sp. nov. NH36A[T].

Table 1 1H and ^{13}C NMR data assignments of compounds 1–4 (400 MHz for ^{1}H and 100 MHz for ^{13}C)

Unit	NO	δ_C	δ_H (J *in Hz*)	δ_C	δ_H (J *in Hz*)	Unit	NO	δ_C	δ_H (J *in Hz*)	δ_C	δ_H (J *in Hz*)
		1[a]		**2**[a]				**3**[b]		**4**[b]	
Glu	1	167.3		166.8		Glu	1	170.3		170.2	
	2	52.8	3.01 (1H, t, 5.2)	53.3	3.08 (1H, m)		2	55.0	4.08 (1H, t, 5.2)	54.7	3.95 (1H, t, 4.8)
	2-NH		7.96 (1H, br.s)		8.02 (1H, br.s)		2-NH				
	3	28.1	1.77 (2H, m)	29.7	1.30 (2H, m)		3	29.5	2.10 (2H, m)	32.1	2.08 (1H, m), 2.03 (1H, m)
	4	30.2	2.07 (1H, m), 1.97 (1H, m)	30.6	1.65 (2H, t, 6.6)		4	32.1	2.27 (2H, m)	33.0	2.32 (2H, t, 7.6)
	5	171.1		171.2			5	175.0		174.7	
Phe	1'	167.2		166.3		Leu	1'	171.4		171.4	
	2'	55.4	4.12 (1H, m)	55.3	4.17 (1H, m)		2'	55.1	3.94 (1H, t, 6.4)	55.7	3.92 (1H, t, 6.4)
	2'-NH		8.12 (1H, br.s)		8.13 (1H,br.s)		2'-NH				
	3'	38.4	2.90 (1H, dd, 13.6, 4.4) 3.09 (1H, dd, 13.6, 4.4)	38.1	2.89 (1H, dd, 12.9, 4.5) 3.13 (1H, dd, 13.6, 4.4)		3'	43.7	1.70 (2H, t, 6.8)	45.5	1.74 (1H, m), 1.66 (1H, m)
	4'	136.0		136.1			4'	25.4	1.83 (1H, m)	25.4	1.85 (1H, m)
	5',9'	130.0	7.17–7.28 (1H, m)	130.2	7.13–7.32 (1H, m)		5'	23.5	0.96 (3H, d, 4.8)	23.7	0.97 (3H, d, 4.8)
	6',8'	128.2	7.17–7.28 (1H, m)	128.3	7.13–7.32 (1H, m)		6'	22.4	0.98 (3H, d, 4.8)	22.2	0.99 (3H, d, 4.8)
	7'	126.0	7.17–7.28 (1H, m)	126.1	7.13–7.32 (1H, m)						
N-phenethyl	1''	40.1	3.25 (2H, m)	40.1	3.23 (2H, m)	*N*-phenethyl	1''	42.2	3.41 (2H, t, 7.6)	42.1	3.43 (2H, t, 7.2)
	1''-NH		7.83 (1H, t, 5.2)		7.61 (1H, t, 5.7)		1''-NH				
	2''	35.1	2.68 (2H, t, 7.6)	35.1	2.71 (2H, t, 7.6)		2''	36.6	2.80 (2H, t, 7.6)	36.6	2.81 (2H, t, 7.6)
	3''	139.5		139.6			3''	140.7		140.7	
	4'',8''	128.5	7.17–7.28 (1H, m)	128.6	7.13–7.32 (1H, m)		4'',8''	130.0	7.17–7.27 (1H, m)	130.0	7.18–7.30 (1H, m)
	5'',7''	128.1	7.17–7.28 (1H, m)	128.1	7.13–7.32 (1H, m)		5'',7''	129.6	7.17–7.27 (1H, m)	129.6	7.18–7.30 (1H, m)
	6''	126.7	7.17–7.28 (1H, m)	126.6	7.13–7.32 (1H, m)		6''	127.5	7.17–7.27 (1H, m)	127.5	7.18–7.30 (1H, m)

[a]Recorded in DMSO-d_6.
[b]Recorded in CD$_3$OD.

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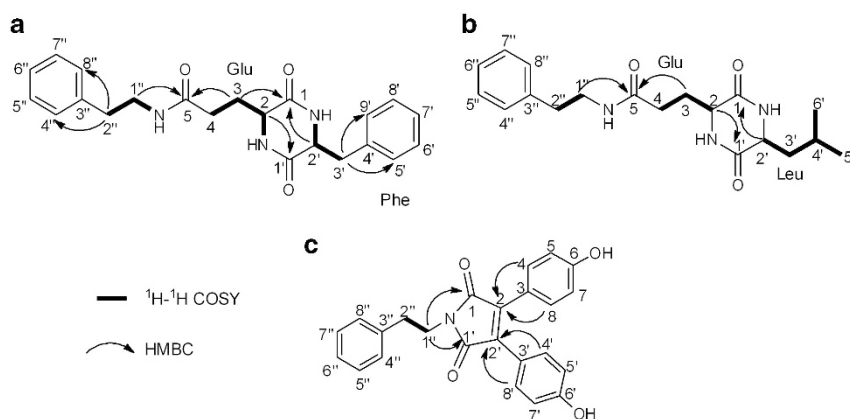

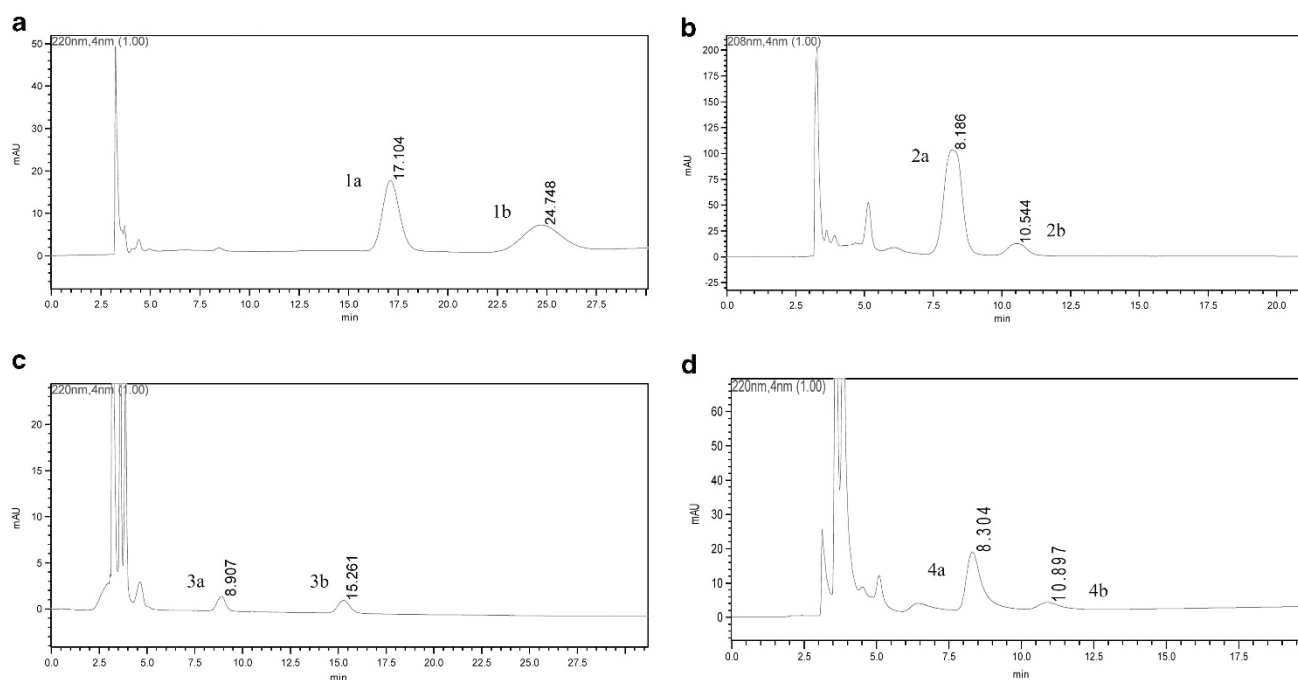Figure 2 Key ^{1}H–^{1}H COSY and HMBC correlations of compounds 1–5. (a) For compound 1/2; (b) for compound 3/4; (c) for compound 5.

Figure 3 Chiral HPLC analytical chromatograms of compounds 1–4. (a) Chiral HPLC analytical chromatogram of compound 1; (b) Chiral HPLC analytical chromatogram of compound 2; (c) Chiral HPLC analytical chromatogram of compound 3; (d) Chiral HPLC analytical chromatogram of compound 4. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Marfey's method[8,9] was applied to assign the absolute configuration of enantiomers 1a and 1b. The FDAA derivatives of the acid hydrolysate of 1a/1b and the authentic D- and L-amino acids were subjected to HPLC analysis (Cosmosil C$_{18}$ column, φ 5 μm, 4.6 × 250 mm, 0.8 ml min^{-1}) at 35 °C using the following gradient program: solvent A, H$_2$O (0.1% HCOOH); solvent B, CH$_3$CN; linear gradient, 30–80% of B in A over 30 min with UV detection at 340 nm. The absolute configurations of amino acid residues in 1a/1b were established by comparison of retention times of their derivatives with those of corresponding authentic D- and L-amino-acid derivatives. Therefore, absolute configurations of the Glu and Phe residues of 1a were assigned to be D-Glu and L-Phe, respectively (Figure 4a). The absolute configurations of Glu and Phe residues of 1b were assigned to be L-Glu and D-Phe, respectively (Figure 4b).

Compound 2 was obtained as a white amorphous powder. [α]$_D^{25}$ −26.3 (*c* 0.2, MeOH). HR-ESI-TOF MS gave the molecular formula of C$_{22}$H$_{26}$N$_3$O$_3$ (*m/z* 380.1977 [M + H]$^+$, calcd 380.1974), the same as that of 1. The ^{1}H and ^{13}C NMR data of 2 were almost identical with those of 1 (Table 1), which revealed that they had closely similar structures. However, they showed different retention behaviors in normal and reversed-phase chromatography. Interpretation of 1D and 2D NMR spectra indicated that 2 had the same planar structure to that of 1.

In view of the existence of enantiomers of 1, compound 2 was also subjected to Lux Amylose-2 chiral liquid chromatography analysis. Two enantiomers were obtained at t_R 8.19 min for 2a and 10.54 min for 2b (Figure 3b), and their relative content ratio was ∼9:1 according to their relative peak areas in the HPLC chromatogram (89.3% for 2a and 10.7% for 2b).

As the relative contents of enantiomers 2a and 2b were of great disparity, and available amount of 2 was also small, the absolute configurations of compunds 2a and 2b were determined directly by

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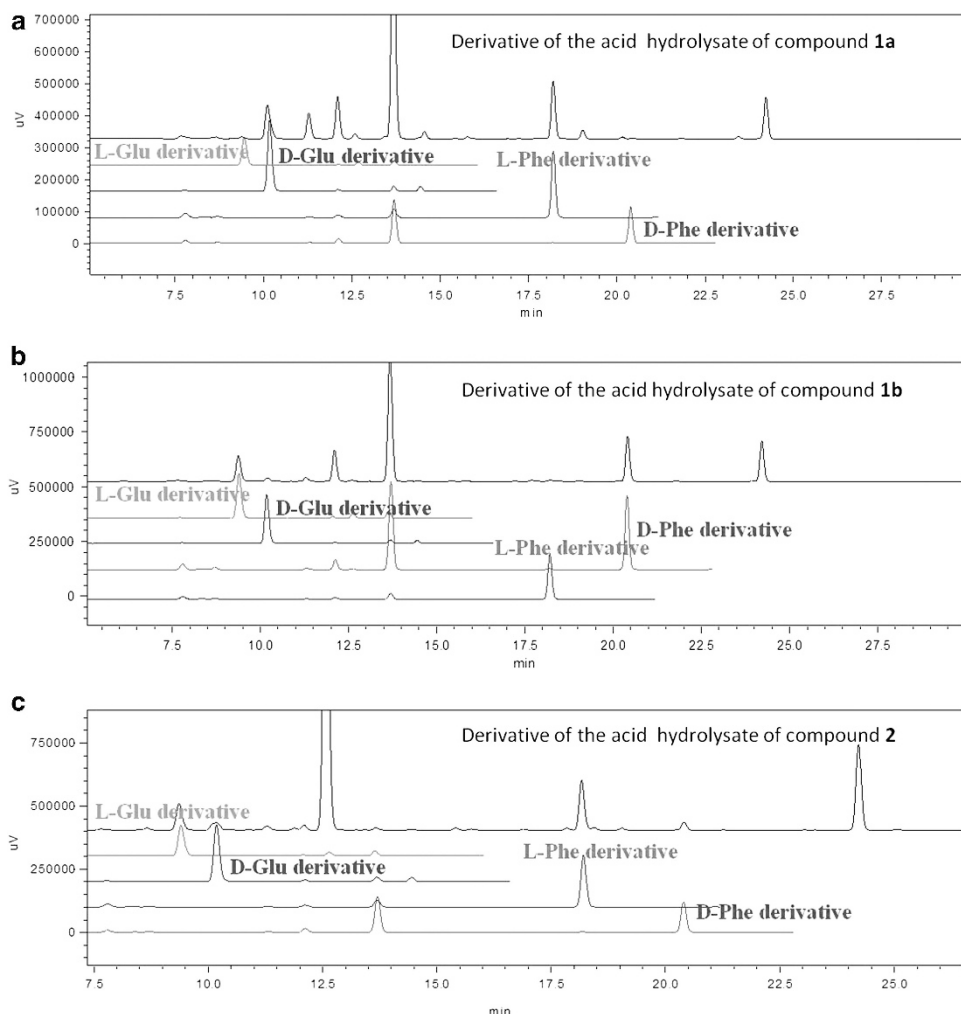Y Chen *et al*

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Figure 4 Determination of the absolute configurations of compounds **1** and **2**. (**a**) HPLC chromatogram of derivatives of the acid hydrolysate of **1a** and those of corresponding authentic amino acids; (**b**) for **1b** and (**c**) for **2**. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

the Marfey's method without chiral resolution of **2**. Results revealed that configurations of Glu and Phe residues of **2a** were L-Glu and L-Phe, respectively, and the opposite to those of **2b** (Figure 4c).

Compound **3** was obtained as a faint yellow amorphous powder. Its molecular formula was determined to be $C_{19}H_{27}N_3O_3$ based on the quasimolecular ion at m/z 346.2130 $[M+H]^+$ (calcd 346.2131) in the HR-ESI-TOF MS, indicating 8 degrees of unsaturation. The ^{13}C NMR spectrum of **3** (Table 1) showed three ester/amide carbonyl carbon signals (δ_C 167.2, 167.3 and 171.1). The characteristic methine resonances of α-CH at δ 3.94/55.1 and 4.08/55.0 indicated that it was a dipeptide. The presence of a cluster of five aromatic protons at δ 7.17–7.27 suggested the presence of one monosubstituted benzene ring in the ^{1}H-NMR spectrum of **3**. Detailed analyses of ^{1}H–^{1}H COSY, HSQC and HMBC data of **3** established the three residues as leucine (Leu), glutamic acid (Glu) and *N*-phenethyl (Figure 2b). According to the HMBC correlation between H₂-1″ and C-5 (Glu), the structure of **3** was established to be cyclo (Leu-Glu) substituted with *N*-phenethyl group, which was a new PEA derivative.

The optical rotation value of **3** was almost zero, which suggested that it was also a racemate. Further chiral resolution was performed by Lux Amylose-2 chiral liquid chromatography (phenomenex, φ 5 μm, 4.6 × 250 mm) using 100% CH₃CN at 1.0 ml min⁻¹. Two enantiomers were obtained from compound **3**, namely **3a** and **3b**,

at t_R 8.91 and 15.26 min, respectively (Figure 3c), and their relative contents were nearly identical according to their relative peak areas in the HPLC chromatogram (44.9% for **3a** and 55.1% for **3b**).

The absolute configurations of the Glu and Leu residues of **3a** were assigned to be L-Glu and D-Leu, respectively, by Marfey's method[8,9] (Figure 5a), and opposite to those of **3b** (Figure 5b).

Compound **4** was obtained as a faint yellow amorphous powder. $[\alpha]_D^{25}$ −17.7 (*c* 0.5, MeOH). HR-ESI-TOF MS gave the molecular formula of $C_{19}H_{28}N_3O_3$ (m/z 346.2130 $[M+H]^+$, calcd 346.2131), the same as that of **3**. The ^{1}H and ^{13}C NMR data were almost identical with those of **3** (Table 1), which revealed that they had closely similar structures. However, they showed different retention behaviors in normal and reverse-phase chromatography. Interpretation of 1D and 2D NMR spectra indicated that **4** has the same planar structure as that of **3**.

In view of the existence of enantiomers of **3**, compound **4** was also subjected to Lux Amylose-2 chiral liquid chromatography analysis. Two enantiomers were obtained at t_R 8.30 min for **4a** and 10.90 min for **4b** (Figure 3d), and their relative content ratio was ~9:1 according to their relative peak areas in the HPLC chromatogram (89.2% for **4a** and 10.8% for **4b**).

As the relative contents of enantiomers **4a** and **4b** were of great disparity, and the available amount of **4** was also small, the absolute
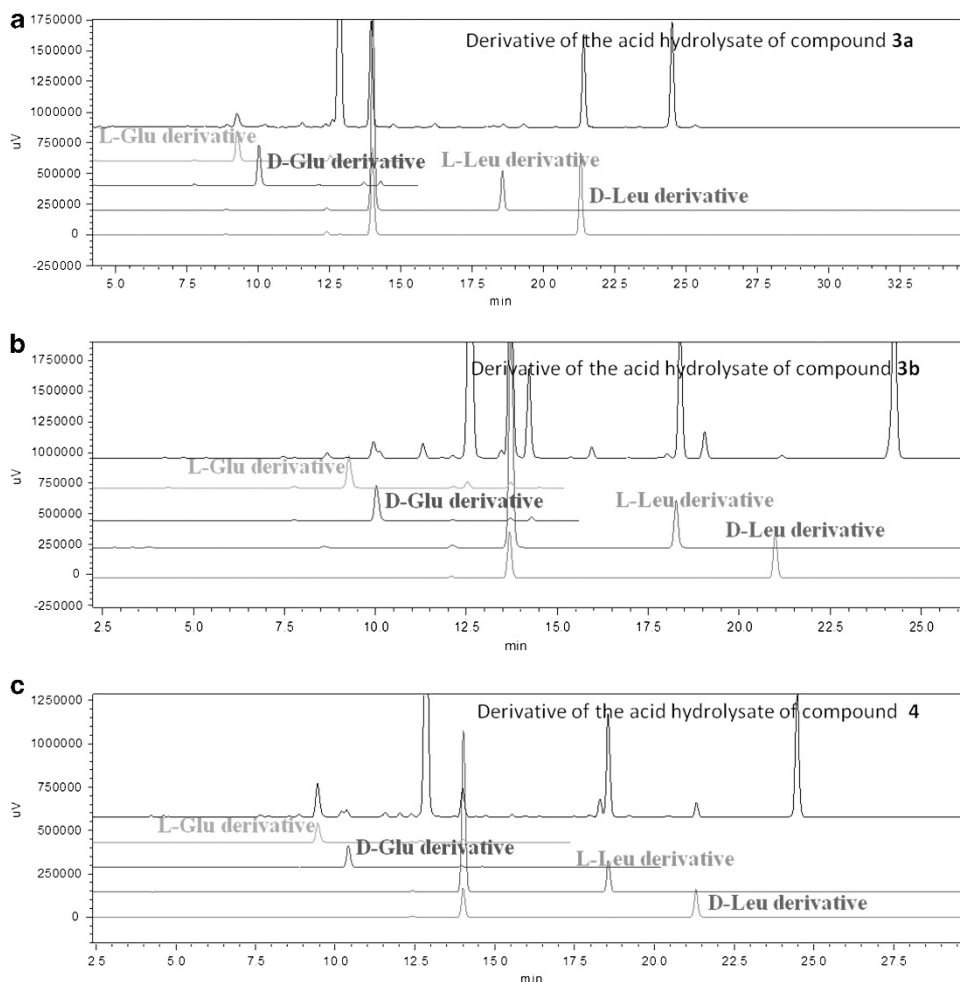
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Figure 5 Determination of the absolute configuration of compounds 3 and 4. (a) HPLC chromatogram of derivatives of the acid hydrolysate of 3a and those of corresponding authentic amino acids; (b) for 3b and (c) for 4. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Table 2 1H and ^{13}C NMR data assignments of compound 5 (400 MHz for ^{1}H and 100 MHz for ^{13}C)

NO	δ_C	δ_H (J in Hz)	NO	δ_C	δ_H (J in Hz)
1″	40.6	3.82 (2H, t, 7.2)	1, 1′	172.9	
2″	35.6	2.96 (2H, t, 7.2)	2, 2′	135.5	
3″	139.9		3, 3′	121.6	
4″, 8″	130.1	7.21–7.25 (2H, m)	4, 8, 4′, 8′	132.7	6.75 (4H, d, 8.8)
5″, 7″	129.7	7.21–7.25 (2H, m)	5, 7, 5′, 7′	116.5	7.29 (4H, d, 8.8)
6″	127.7	7.21–7.25 (1H, m)	6, 6′	160.5	

Recorded in Acetone-d_6.

configurations of compunds **4a** and **4b** were determined directly by the Marfey's method without chiral resolution of **4**. Results revealed that configurations of Glu and Leu residues of **4a** were L-Glu and L-Leu, respectively, and are opposite to those of **4b** (Figure 5c).

Compound **5** was obtained as a red amorphous powder. Its molecular formula was determined to be C$_{24}$H$_{19}$NO$_4$ based on the quasimolecular ion at *m/z* 386.1386 [M + H]$^+$ (calcd 386.1371) in

the HR-ESI-TOF MS, indicating 16 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of **5** (Table 2) showed two methylenes and three phenyl rings (including a monosubstituted benzene ring and two para-disubstituted benzene rings). Detailed analyses of ^{1}H–^{1}H COSY, HSQC and HMBC correlations further revealed the presence of an N-phenethyl group and two para-disubstituted benzene residues. In the HMBC spectra of **5**, correlations between H$_2$-1″ (δ_H 3.82, 2H, t, *J* = 7.2 Hz) and C-1 (δ_C 172.9) and C-1′ (δ_C 172.9), H-4/H-8 (δ_H 6.75, 2H, d, *J* = 8.8 Hz) and C-2 (δ_C 135.5), H-4″/H-8″ (δ_H 7.21–7.25, 2H, m) and C-2′ (δ_C 135.5) suggested that the N-phenethyl group and para-disubstituted benzene residues were linked with a 1H-pyrrole-2,5-dione ring (Figure 2c), which was further confirmed by comparison of its NMR data with that of 1-benzyl-3,4-bis(4-hydroxyphenyl)-1H-pyrrole-2,5-dione.[10] Thus, compound **5** was established to be 1-(2-phenylethyl)-3,4-bis(4-hydroxyphenyl)-1H-pyrrole-2,5-dione, which was a new compound.

Biological activity
The antimicrobial activity of compounds **1–5** was evaluated by an agar dilution method.[11] The results showed that compound **5** displayed antimicrobial activity against *S. aureus* and *B. subtilis*, whereas compounds **1–4** were inactive against the two strains (MIC > 1.00 mg ml^{-1}).

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METHODS

General experiments

Optical rotations were measured on a JASCO P-1020 digital polarimeter at room temperature (JASCO Inc., Tokyo, Japan). IR spectra were recorded on a JASCO FT/IR-480 plus Fourier transform infrared spectrometer (JASCO Inc.). UV spectra were recorded in CH_3OH by using a JASCO V-550 UV/VIS spectrophotometer (JASCO Inc.). HR-ESI-MS was recorded on an Agilent 6200 ESI/TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). 1D NMR (^1H, ^{13}C, DEPT-135) and 2D NMR (^1H–^1H COSY, HSQC and HMBC) were recorded on a Bruker AVANCE 400 spectrometer at 300 K (Bruker Amazon AV400, Zurich, Switzerland). The chemical shifts were given in δ, with the solvent signals (DMSO-d_6: δ_H 2.49/δ_C 39.5; CD$_3$OD: δ_H 3.31/δ_C 49.2; Acetone-d_6: δ_H 2.05/δ_C 29.9, 206.7) as an internal standard (CIL Inc., Berkeley, CA, USA). TLC was carried out on Silica gel GF254 (YanTai Chemical Inst., Yantai, China) plates. HPLC was performed on a SHIMADZU LC-6AD LC-6AD Liquid Chromatography with SPD-20A Detector by using an ODS column (Welch Materials XB-C$_{18}$ column (ϕ 5 μm, 4.6 × 250 mm), YMC-Pack ODS-A (ϕ 5 μm, 10.0 × 250 mm)) (Nacalai Tesque Inc., Kyoto, Japan) at 220 and 254 nm. Chiral HPLC analysis was performed using a phenomenex Lux Amylose-2 column (ϕ 5 μm, 4.6 × 250 mm) (Phenomen, Torrance, CA, USA). Column chromatography (CC) was carried out on silica gel (200–300 mesh; Qingdao Marine Chemical Ltd., Qingdao, China), Sephadex LH-20 (50 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden) and ODS (60–80 mesh; YMC Ltd., Kyoto, Japan). All solvents used in CC were of analytical grade (Tianjin Damao Chemical Plant, Tianjin, China).

Strain

The strain of *A. nanhaiticus* sp. nov. NH36A[T] was isolated from a sandy sediment sample containing shell fragments collected from a water depth of 36 m in the South China Sea (19° 44.775′ N 111° 6.192′ E) in May 2007.[12] 16S rRNA gene sequence analysis revealed that strain NH36A[T] was most closely related to members of the genus *Arenibacter*. On the basis of phenotypic, chemotaxonomic and phylogenetic data, this organism was classified as a representative of a novel species in the genus *Arenibacter*. A voucher specimen was deposited at the China Center for Type Culture Collection (CCTCC AB 208315[T]) and at the Marine Culture Collection of China (MCCC 1A04137[T]).

Fermentation of strain

The producing strain was cultured and fermented as follows. The monoclonal strain growing on marine 2216E plate[13] was inoculated into a 250-ml Erlenmeyer flask containing 100 ml marine 2216E culture medium and cultured at 30 °C for 24 h on a rotary shaker at 180 r.p.m. Then, 20 ml of the resultant seed culture was inoculated into 1000-ml Erlenmeyer flasks, each containing 500 ml of the above culture medium and incubated for 2 days at the same conditions. After centrifugation, the total 60 l of fermentation broth was collected.

Extraction and purification

The fermentation broth was extracted repeatedly with EtOAc, and the organic solvent was evaporated under reduced pressure to afford the crude extract (38.5 g). The extract was subjected to silica gel CC using $CHCl_3$-CH_3OH gradient elution to obtain 10 fractions (F1–F10). The fraction F4 (1.82 g) was subjected to ODS medium-pressure liquid chromatography eluted with a gradient of MeOH in H_2O (30, 50, 70 and 100%) to afford six subfractions (F4-1–F4-6). F4-6 (0.27 g) was subjected to a Sephadex LH-20 CC eluted with 1:1 $CHCl_3$-MeOH to yield three subfractions (F4-6-a–F4-6-c). F4-6-c (35.5 mg) was purified by reversed-phase HPLC on an YMC-Pack ODS-A column (5 μm, 10.0 × 250 mm, 4.0 ml min^{-1}), with CH_3CN and H_2O (50:50, v/v) as the eluents to yield compound **5** (3.0 mg, t_R = 16.5 min).

The fraction F7 (0.78 g) was subjected to ODS medium-pressure liquid chromatography eluted with a gradient of MeOH in H_2O (30, 50, 70 and 100%) to afford five subfractions (F7-1–F7-5). F7-5 (0.28 g) was purified by reversed-phase HPLC on an YMC-Pack ODS-A column (5 μm, 10.0 × 250 mm, 4.0 ml min^{-1}) with CH_3OH and H_2O (40: 60, v/v) as the eluent to yield compounds **1** (5.0 mg, t_R = 27.2 min), **2** (2.4 mg, t_R = 38.1 min), **3** (4.1 mg, t_R = 24.5 min) and **4** (3.8 mg, t_R = 32.0 min).

Physico-chemical properties

Table 3 summarized the physico-chemical properties of compounds **1–5**.

Determination of absolute configuration by the Marfey's method

Marfey's method[8,9] was applied to assign the absolute configuration of amino-acid residues of each enantiomer. Compound **1a** (0.5 mg) in 6.0 N HCl (1.0 ml) was heated at 155 °C for 2 h. Upon removal of excess HCl under vacuum, the hydrolysates were placed in a 1-ml reaction vial and treated with 1% solution of 1-fluoro-2, 4-dinitrophenyl-5-L-alanine amide (FDAA; 150 μl) in acetone, followed by 1.0 N NaHCO$_3$ (40 μl). The reaction mixtures were heated at 45 °C for 1.5 h, cooled to room temperature and acidified with 2.0 N HCl (20 μl). Similarly, the standard L- and D-amino acids were derivatized according to the method mentioned above. The derivatives of the hydrolysates and the standard amino acids were subjected to HPLC analysis (Kromasil C$_{18}$ column; ϕ 5 μm, 4.6 × 250 mm; 1.0 ml min^{-1}) at 35 °C using the following gradient program: solvent A, H_2O (0.1% HCOOH); solvent B, CH_3CN; linear gradient, 30–80% of B in A over 30 min with UV detection at 340 nm. Absolute configurations of compounds **1b**, **2**, **3a**, **3b** and **4** were also analyzed and determined by the Marfey's method, just like that of **1a**.

Antimicrobial assays

The antimicrobial activities of compounds **1–5** against *S. aureus* and *B. subtilis* were evaluated by an agar dilution method.[11] The tested strains were cultivated on MH agar plates for bacteria at 35 °C. Compounds **1–5** and positive controls (Tobramycin) were dissolved in EtOH at different concentrations from 1000 to 0.25 μg ml^{-1} by the continuous two-fold dilution methods. A 10-μl quantity of test solution was absorbed by a paper disk (5 mm diameter) and placed on the assay plates. After 20 h incubation, zones of inhibition (mm in diameter) were recorded. The MICs were defined as the lowest concentration at which no microbial growth could be observed. Experimental results showed that compound **5** displayed a weak antimicrobial activity against *S. aureus* and *B. subtilis*, with MIC values of 0.50 and 0.25 mg ml^{-1}, respectively

Table 3 Physico-chemical properties of compounds 1–5

	1	2	3	4	5
Appearance	White powder	White powder	Faint yellow powder	Faint yellow powder	Red powder
Molecular fomula	$C_{22}H_{25}N_3O_3$	$C_{22}H_{25}N_3O_3$	$C_{19}H_{27}N_3O_3$	$C_{19}H_{27}N_3O_3$	$C_{24}H_{19}NO_4$
$[\alpha]$eq \o(\s\up 5(25),\s\do 2(D))	Almost zero (c 0.5, MeOH)	−26.3 (c 0.2, MeOH)	Almost zero (c 0.3, MeOH)	−17.7 (c 0.5, MeOH)	
HR FAB MS (m/z)					
Found:	380.1976 [M + H]$^+$	380.1977 [M + H]$^+$	346.2130 [M + H]$^+$	346.2130 [M + H]$^+$	386.1386 [M + H]$^+$
Calculated:	380.1974	380.1974	346.2131	346.2131	386.1371
IR (KBr) λ_{max}	3189, 3061, 1671, 1546, 1457, 1334, 758, 701	3340, 3061, 1671, 1456, 1098, 708, 696	3329, 3189, 2958, 1671, 1461, 1090, 697	3432, 3322, 3160, 2981, 1679, 1448, 1118, 692	3414, 2928, 2361, 1686, 1406, 837, 577
UV λ_{max} nm (logε) (MeOH)	207 (3.2)	210 (3.7)	208 (3.8)	205 (3.0)	190 (3.2), 231(3.0), 410 (3.5)

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(Tobramycin-displayed MIC values of $0.30\,\mu g\,ml^{-1}$ and $0.01\,mg\,ml^{-1}$ against the two strains, respectively), whereas compounds **1–4** were inactive against the two strains (MIC $> 1.00\,mg\,ml^{-1}$).

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