Tetrahedron 66 (2010) 7520–7526

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Concise synthesis of AHMHA unit in perthamide C. Structural and stereochemical revision of perthamide C

Valentina Sepe [a], Maria Valeria D'Auria [a], Giuseppe Bifulco [b], Raffaella Ummarino [a], Angela Zampella [a,*]

[a] Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", via D. Montesano 49, 80131 Napoli, Italy
[b] Dipartimento di Scienze Farmaceutiche, Università di Salerno, via Ponte don Melillo, Fisciano (SA) 84084, Italy

ARTICLE INFO

Article history:
Received 25 May 2010
Received in revised form
2 July 2010
Accepted 20 July 2010
Available online 27 July 2010

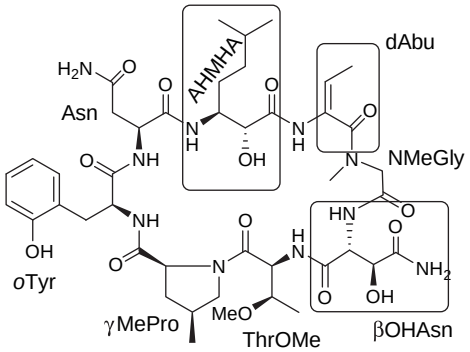
ABSTRACT

Diastereomers of 3-amino-2-hydroxy-6-methylheptanoic acid (AHMHA), a new amino acid unit in perthamides C and D, have been synthesized from commercially available 4-methylpentanol in a concise manner and 50% average overall yield. Comparison of the ^{1}H and ^{13}C NMR data, optical rotation data and Marfey's analysis of the resulting isomers with the natural fragment unambiguously allowed the configurational assignment of the natural residue as (2*R*,3*R*). A structural revision of perthamides C and D is also reported.

1. Introduction

Recently we reported the isolation, from a Solomon collection of the marine sponge *Theonella swinhoei*,[1] of two new octacyclopeptides, which we named perthamides C and D. Perthamide C (**1**), when tested in a well characterised model of inflammation in vivo, i.e., mouse paw oedema,[2] significantly reduced carrageenan-induced paw oedema, displaying potent dose-dependent anti-inflammatory activity. Structurally, perthamide C comprises a 25-membered macrocycle with several non-proteinogenic amino acid residues, such as γ-methylproline, β-hydroxyasparagine, *o*-tyrosine and 3-amino-2-hydroxy-6-methylheptanoic acid (AHMHA). Whereas the configuration of non-conventional residues was secured through an integrated approach, which combined NMR analysis, chemical degradation, stereoselective synthesis and LC/MS analysis, the remaining stereochemical uncertainty of perthamide C concerns the configuration of the two stereogenic centres in the AHMHA unit. In the original paper we tentatively proposed a *threo* relative disposition of the hydroxyl and the amino group on the α- and β-position, respectively, on the basis of the *J* coupling analysis. However, due to the lack of adequate standards the absolute configuration remained unassigned.

In this paper we report the results of a stereochemical investigation on the AHMHA unit in perthamides, that allowed the revision of the relative configuration originally proposed and the unambiguous definition of the absolute configuration. Further, the comparison of the data for mutremdamide A[3] indicated that mutremdamide A and perthamide C are the same compound, and therefore the structure of perthamide C must be revised by replacing the β-hydroxyasparagine residue with the N^{δ}-carbamoyl-β-sulfated asparagine (Fig. 1).



1

Figure 1. Perthamide C (**1**) with structural and stereochemical ambiguities.

2. Results and discussion

In our previous paper[1] the application of the conventional *J*-based NMR method[4] evidenced some intrinsic difficulties, arising from the qualitative comparison of the predicted and some approximation in the measurement of the experimental coupling constants, which had been determined on the basis of the sole analysis of phase sensitive-HMBC spectra. For this reason we

* Corresponding author. E-mail address: azampell@unina.it (A. Zampella).

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.07.060

decided to acquire further NMR data, using the 2D-HETLOC[5] experiment for the accurate determination of the heteronuclear *J* values.

In addition, for the comparison of the experimental couplings, the more reliable—from a quantitative point of view—QM-NMR integrated approach[6–8] was applied.

In particular, we considered for our calculations the *threo* and the *erythro* arrangements of a simplified fragment representative of the AHMHA unit (Fig. 2), containing, together with the β-amino acid unit, a *N*-methyl group and an acetyl group in the carboxy and amino terminal positions, respectively.

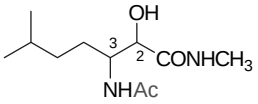


Figure 2. Molecular fragment representing AHMHA; calculations for *erythro* and *threo* arrangements were performed varying C2 and C3 centres.

Following the protocol previously reported in literature[6–8] we optimised at quantum chemical level (see Experimental) the three staggered arrangements (*gauche* +, *gauche* − and anti) for both the *threo* and *erythro* configurations. On the optimised geometries, we calculated *J* values at the MPW1PW91 level, using the 6-31G(d,p) basis set and considering the MeOH solvent using the IEF-PCM model.[9] The results of the calculation were compared to the experimental data. Surprisingly, differently from what was found using the *J*-based method,[1] the quantitative QM-*J* method evidenced a better superimposition between the experimental and calculated data for the *erythro* arrangement. In fact, as displayed in Table 1, the lowest total absolute deviation (TAD) is observed for the *erythro gauche* + arrangement (4.8 Hz), even though a comparable value of 6.4 Hz is observed for the *gauche*-conformation of the *threo* arrangement.

Table 1
Calculated and experimental *J* values (Hz) for a fragment corresponding to AHMHA in perthamide C (**1**)

	Calcd						Exp.
	threo			*erythro*			
	g^+	*anti*	g^-	g^+	*anti*	g^-	
$^3J_{H2-H3}$	5.3	8.7	1.5	3.0	7.8	3.4	1.8
$^3J_{H2-C4}$	4.2	1.2	1.7	4.1	4.3	1.0	1.9
$^3J_{H3-C1}$	6.4	1.9	1.0	0.4	0.6	6.7	0.7
$^2J_{H2-C3}$	−1.2	−1.5	1.8	−3.1	−3.0	0.5	−2.5
$^2J_{H3-C2}$	−2.0	−3.0	1.3	−0.6	−3.7	−2.3	0.0
TAD[a]	14.9	12.7	6.4	4.8	12.6	13.7	

[a] Total absolute deviation values ($\sum|J_{calcd}-J_{exp}|$).

In order of confirming the above results concerning the relative arrangement discording from our previous indications,[1] and for the complete and unambiguous definition of the absolute configuration of AHMHA residue, we undertook the stereoselective synthesis of all diastereomeric possibilities for the AHMHA residue.

The synthesis of α-hydroxy-β-amino acids has been extensively investigated[10] and, for our purposes, *trans* α-β-epoxy esters, which are easily obtained in optically enriched form (ee >90%) by Sharpless asymmetric epoxidation (AE), may represent valuable intermediates to access to both *erythro* and *threo* adducts.

As shown in our retrosynthetical analysis (Fig. 3), the key intermediate epoxy acid **3** would arise from allylic alcohol **4**, which in turn could be prepared through a HWE olefination on aldehyde **5** and subsequent chemoselective reduction.

The synthetic sequence is outlined in Scheme 1, starting with commercially available alcohol **6**. Primary alcohol **6** was submitted

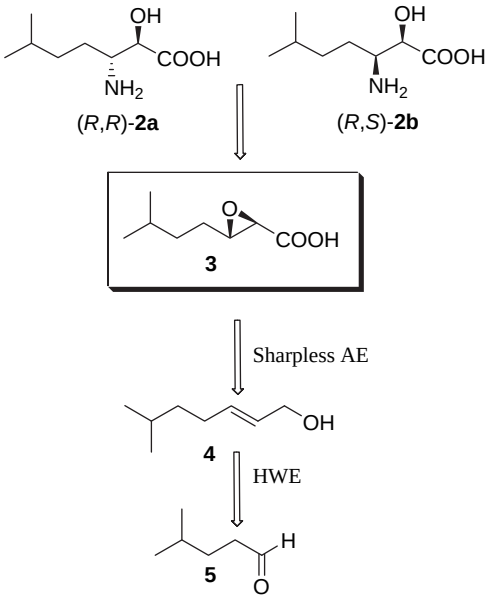
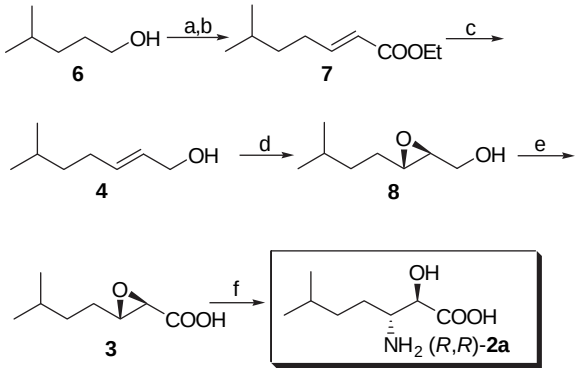


Figure 3. Retrosynthetic analysis of (2*R*,3*R*)- and (2*R*,3*S*)-3-amino-2-hydroxy-6-methylheptanoic acids (**2a**–**b**).

to oxidation under Swern condition[11] and the unpurified aldehyde was subjected to standard HWE two-carbon homologation giving (*E*)-unsaturated ester **7** in 72% yield (>98% de, as judged by NMR data) over two steps.



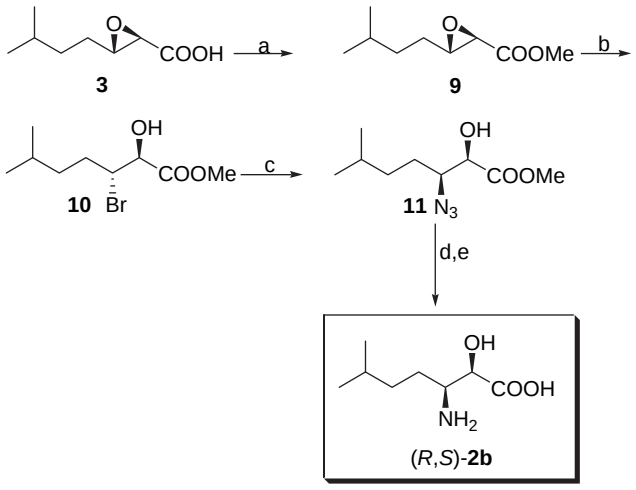
Scheme 1. Reagents and conditions: (a) $COCl_2$, DMSO, TEA, CH_2Cl_2 dry, −78 °C; (b) TEPA, LiOH, THF dry, 72% two steps; (c) DIBAL-H, toluene dry, −78 °C; (d) Ti(O*i*-Pr)$_4$, (+)-DET, TBHP,4 Å MS, CH_2Cl_2 dry, −20 °C, 76% two steps; (e) $NaIO_4$, $RuCl_3$, CH_3CN/CCl_4/H_2O 2:2:3; (f) NaN_3, $Cu(NO_3)_2$, H_2O, 65 °C, then $NaBH_4$, 0 °C, 75% two steps.

Chemoselective reduction with DIBAL-H to allylic alcohol **4**, followed by AE, leads to epoxy alcohol **8** in 76% yield over two steps, whose optical purity was judged to be >98% by the application of the modified Mosher's method.

Treatment of (2*S*,3*S*)-**8** with ruthenium chloride and sodium periodate[15] produced carboxylic acid (2*R*,3*S*)-**3** in nearly quantitative yield. With optically active key intermediate **3** on hand, we completed the synthesis of (2*R*,3*R*)-**2a** and (2*R*,3*S*)-**2b**.

We envisaged that the *erythro* diastereoisomer could be obtained through a procedure recently developed by Fringuelli et al., relying on a one-pot metal catalyzed azidolysis/reduction of α-β-epoxycarboxylic acids.[12] Treatment of **3** with sodium azide in the presence of 10 mol % $Cu(NO_3)_2$, followed by in situ $NaBH_4$ reduction furnished *erythro* α-hydroxy-β-amino-6-methylheptanoic acid **2a** in a 75% yield (Scheme 1). The reaction proceeded smoothly with excellent regio- and stereoselectivity as judged by NMR data of **2a** when compared with those reported for *allo*-ethylnorstatine.[12]

Using Bonini's methodology,[13] *threo* diastereomer was synthesized with similar stereoselectivity and chemical yields starting from α-β-epoxycarboxylic acid **3** (Scheme 2). Diazomethane esterification produced *trans* epoxy methyl ester **9** that in turn was subjected to $MgBr_2$-mediated epoxy-opening to give bromohydrin **10**. Azide substitution followed by hydrogenation and acid hydrolysis produced the targeted *threo* α-hydroxy-β-amino acid **2b**.



Scheme 2. Reagents and conditions: (a) CH_2N_2, Et_2O, 82% two steps from **8**; (b) $MgBr_2 \cdot OEt_2$, Et_2O, 98%; (c) NaN_3, DMF, 65 °C, 92%; (d) H_2, Pd/C; (e) 6 N HCl, 120 °C, 75% two steps.

Also in this case all steps in the reaction sequence proceeded in good yields and in highly regio- and stereoselective manner.

Comparison of NMR spectra of the AHMHA obtained from the acid hydrolysis of perthamide C with those of the two synthetic analogues unambiguously indicates the configurational assignment of the natural fragment as either *R*,*R* or *S*,*S* (Table 2). Aside from slight differences in the chemical shift values of the methyl signals of (*R*,*S*)-**2b** and the natural fragment, the ^1H NMR spectrum of (*R*,*S*)-**2b** displays two well distinguished proton signals (1.58 and 1.80 ppm) for the diastereotopic methylene at C-4 where the natural fragment displays a single broad 2H multiplet (1.62–1.69 ppm).

Also ^{13}C NMR data confirmed the stereochemical assignment made. Once again, while the spectrum of diastereoisomer **2a** completely matches that of the natural material, ^{13}C NMR data for *threo* stereoisomer differ especially in the chemical shift of carbon at position-4.

With two diastereoisomers of AHMHA in our hands, it was then possible to proceed to the determination of the absolute configuration of this unit in perthamide C using a pre-column derivatization method.

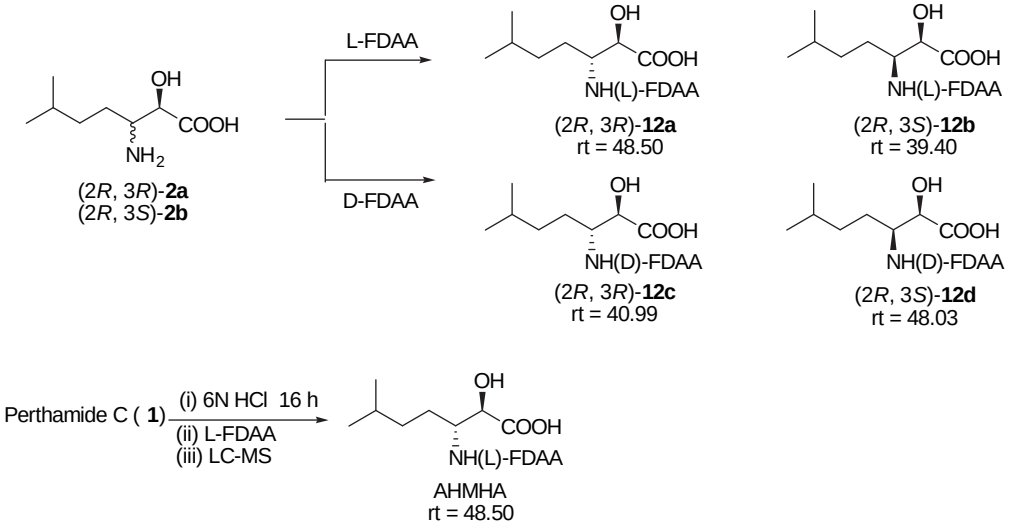
A small sample (1 mg each) of synthetic diastereomers **2a**–**2b** was derivatized with both enantiomers of Marfey's reagent (*N*-(3-fluoro-4,6-dinitrophenyl)-alaninamide; L-FDAA and D-FDAA).

The L- and D-FDAA derivatives **12a**–**12d** were analysed using ESI LC/MS in the positive ion mode. By monitoring for FDAA/AHMHA at *m*/*z* 428, they were detected as separate peaks at 48.50, 39.40, 40.99, 48.03 min, respectively (Scheme 3). The L-FDAA derivative of AHMHA unit in perthamide C (**1**) was co-eluted with the L-FDAA derivative of (2*R*,3*R*)-2-hydroxy-3-amino-6-methylheptanoic acid **2a**.

Table 2
Comparison of the NMR data of synthetic 2-hydroxy-3-amino-6-methylheptanoic acid diastereomers **2a**,**2b** with the natural fragment[a]

	2*R*,3*R*-**2a** δ, multiplicity (*J*), H		2*R*,3*S*-**2b** δ, multiplicity (*J*), H		Natural fragment δ, multiplicity (*J*), H	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		172.7		172.1		172.5
2	4.01 m, 1H	72.5	4.00 m, 1H	71.3	4.04 m, 1H	72.5
3	3.35 m, 1H	55.7	3.31 m, 1H	55.4	3.34 m, 1H	55.7
4	1.63, 1.70 m, 2H	26.4	1.58, 1.80 m, 2H	28.4	1.62, 1.69 m, 2H	26.8
5	1.30 m, 1H	35.6	1.34 m, 1H	35.4	1.29 m, 1H	35.6
6	1.57 m, 1H	28.8	1.60 m, 1H	29.1	1.56 m, 1H	28.9
7	0.93 d (6.7), 3H	22.5	0.95 d (6.7), 3H	22.5	0.93 d (6.5), 3H	22.6
Me-6	0.92 d (6.7), 3H	22.2	0.95 d (6.7), 3H	22.5	0.92 d (6.5), 3H	22.1

[a] All chemical shifts are reported in parts per million and were measured in CD_3OD. Coupling constants (*J*) are expressed in hertz.



Scheme 3. HPLC retention times (rt) of FDAA derivatives of synthetic (2*R*, 3*R*)- and (2*R*, 3*S*)-3-amino-2-hydroxy-5-methylheptanoic acid (**2a** and **2b**) and configurational assignment of AHMHA in perthamide C (**1**).

Thus, the (2*R*,3*R*) configuration for the AHMHA residue in **1** was unambiguously established (Scheme 3), as further confirmed by the optical rotation data [synthetic **2a**: $[\alpha]_D^{20}$ +8.6 (*c* 0.5, MeOH); natural AHMHA: $[\alpha]_D^{20}$ +8.8 (*c* 0.55, MeOH)].

During the preparation of the present manuscript, Bewley et al. reported the isolation of mutremdamide A from different deep water collections of *T. swinhoei* and *Theonella cupola*.[3] The close analogy between mutremdamide A and perthamide C appeared at once evident, prompting us to an in depth investigation of the reported differences that concern both constitution and configuration. The most significant discrepancy is relative to molecular formula: our HRESIMS measurement (positive ion mode) of the observed highest mass peak was consistent with a molecular formula $C_{43}H_{64}N_{10}O_{14}$ that matched with the ^{13}C NMR data (43 carbon resonances evidenced by the ^{13}C NMR spectrum and HMBC data).[14] When, in the light of Bewley's considerations, we run the mass spectrum in negative ion mode we observed a molecular ion peak at *m*/*z* 1066.4106 $[M-H]^-$ corresponding with the molecular formula $C_{44}H_{65}N_{11}O_{18}S$ reported for mutremdamide A. This datum, in addition with the total correspondence of NMR data of two compounds in DMSO and CD_3OH, secured the identity of perthamide C with mutremdamide A. Therefore the structure originally proposed by us must be revised (Fig. 4) by replacing the β-hydroxyasparagine residue with the new N^{δ}-carbamoyl-β-sulfated asparagine. It is worthy to note how in this case the use of CD_3OH as solvent was misleading, as it not only gave a poor dispersion of signals in the ^{13}C NMR spectrum,[14] but also caused such a lowering of the intensity of NH-4 and carbamoyl protons relative to the N^{δ}-carbamoyl-β-sulfated asparagine residue that the carbamoyl residue could not be actually detected.

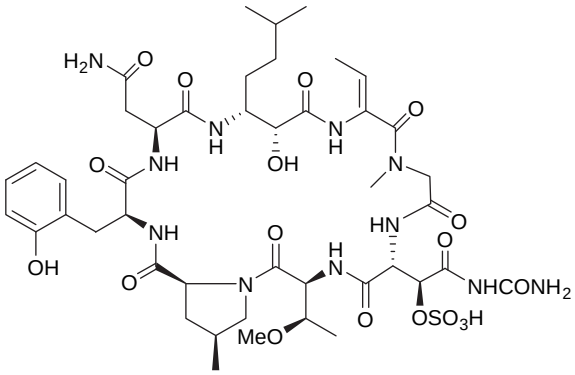


Figure 4. Revised structure for perthamide C.

There are two additional differences between mutremdamide A and perthamide C, that concern with the configuration of *o*-Tyr and dAbu residues. We proposed the L-configuration for the *o*-Tyr residue on the basis of a non-empirical method, extensively used by us[15,16] and others,[17] involving the chemical transformation of the aromatic ring into a carboxy group by oxidative ozonolysis and subsequent Marfey's analysis on the obtained aspartate derivative.

As concerning the 2-aminobutenoic residue (dAbu), in the original paper we assigned the *E*-configuration to the above residue on the basis of a dipolar coupling between the −NH signal at δ 9.08 and the olefinic proton H-3 at δ 5.96,[1] observed in the ROESY spectrum (400 ms mixing time) acquired in CD_3OH and on the basis of chemical shift consideration.[18–23] When, in the light of the results by Bewley et al.,[3] we run the ROESY spectrum in DMSO using 400, 200 and 50 ms mixing times, the amidic proton of the dAbu residue invariably gave a week dipolar coupling with both H-3 and CH_3-4 protons. These controversial ROE effects probably are due to the interference in the spectra of NOE effects of opposite sign. For this reason, we decided to acquire NOESY spectra at different mixing times (100 and 200 ms). The analysis of the NOESY spectra evidenced clear negative NOE effects due to the long correlation time of the molecule, as expected for its relatively high molecular weight, in particular between NH and H_3-4 of 2-aminobutenoic residue pointing towards a Z-geometry. The results above described suggest that attention must be paid to the evaluation of ROE effects[24] and chemical shift trends[20,23] in the assignment of configuration of dAbu residue.

We also tried to further validate the configuration assignment of the dAbu residue through alternative approaches, via the synthesis of the two possible stereoisomers and comparison of the chemical physical data of the synthetic 2-aminobutenoic acids with those of the residue isolated from the acid hydrolyzate of perthamide C. According to literature,[25] the synthesis of protected (*E*)- and (*Z*)-2-aminobutenoic acids is straightforward, but we failed to obtain an appreciable recovery of the unprotected residue, due to the hydrolytic instability of dAbu residue,[26] a problem that we also experienced in the isolation of the residue from the acid hydrolisate of perthamide C (**1**).

3. Conclusion

An efficient synthesis of two possible diastereoisomers of 3-amino-2-hydroxy-6-methylheptanoic acid (AHMHA), a new amino acid residue of the marine cyclopetides perthamides, was accomplished using Sharpless asymmetric epoxidation and regioselective epoxide opening as key steps. The synthesis allowed for an unambiguous definition of the absolute configuration of the residue in the natural product. Comparison of the spectral data of perthamide C and mutremdamide, recently appeared in the literature,[3] indicated that the two molecules share the same structure. Accordingly, the structure originally proposed by us has been here revised (Fig. 4).

4. Experimental

4.1. General experimental procedures

Specific rotations were measured on a Jasco P-2000 polarimeter. High-resolution ESI-MS spectra were performed with a Micromass Q-TOF mass spectrometer. ESI-MS experiments were performed on a Applied Biosystem API 2000 triple-quadrupole mass spectrometer. HPLC was performed using a Waters Model 6000-A pump equipped with U6 K injector and a differential refractometer, model 401. NMR spectra were obtained on a Varian Mercury-400 and Inova-500 NMR spectrometers, δ in parts per million, *J* in hertz, spectra referred to CD_2HOD or $CHCl_3$ as internal standards (δ_H=3.31 and 7.26, respectively).

For an accurate measurement of the coupling constants, NMR spectra were recorded on Varian 700 MHz and Bruker DRX-600 spectrometers, equipped with cryo-probe. All spectra were acquired in the phase-sensitive mode and the TPPI method was used for quadrature detection in the ω1 dimension. NMR sample was obtained dissolving 20 mg of perthamide C in DMSO-d_6.

$^3J_{H-H}$ values were extracted from 1D ^1H NMR and 2D E.COSY spectra. For the E.COSY spectrum 32 scans for t_1 value were acquired with a t_{1max} of 65 ms. $^{2,3}J_{C-H}$ values were obtained from phase-sensitive PFG-PS-HMBC spectra and ^{13}C coupled and decoupled HSQC-TOCSY according the following conditions. The PFG-PS-HMBC spectrum was recorded using 2 K points in ω2, setting the delay for long-range coupling evolution (Δ) at 50 ms, with 32 scans/t_1 (t_{1max} 15.2 ms). Zero-filling (8×1 K) was carried out in ω2 and ω1, respectively, to obtain a digital resolution of 0.9 Hz in ω2. The ^{13}C coupled and decoupled HSQC-TOCSY were recorded using 2 K points in ω2, setting the mixing time 80 ms and $^1J_{CH}$ of 140 Hz, with 120 scans/t_1 (t_{1max} 9.7 ms). Zero-filling (8K×1K) was carried out in

$\omega 2$ and $\omega 1$, respectively, to obtain a digital resolution of 0.5 Hz in $\omega 2$; the proton–carbon $^{2,3}J$ coupling were extracted through a computer-aided analysis of the heteronuclear coupled and decoupled multiplets acquired in two separate experiments.

The reverse single-quantum heteronuclear correlation (HSQC) spectra were recorded by using a pulse sequence with a Wurst pulse 0.15 s before each scan to suppress the signal originating from protons not directly bound to ^{15}N; the interpulse delays were adjusted for an average $^{1}J_{NH}$ of 90 Hz.

For the phase-sensitive PFG-HETLOC spectrum, a total of 160 scans/t_1 were acquired using 4 K points in $\omega_{2,}$ with a spin lock of 50 ms and a t_{1max} of 41.7 ms. The data matrices were zero-filled to 4K×1k affording a digital resolution of 0.4 Hz in $\omega_{2.}$

Through-space ^1H connectivities were evidenced using a ROESY experiment with mixing times of 400, 100 and 50 ms, respectively, and NOESY experiment acquired with 200 and 100 ms mixing times.

All reagents were commercially obtained (Aldrich, Fluka) at highest quality and used without further purification except where noted. Dichloromethane, ether, tetrahydrofuran and triethylamine were distilled from calcium hydride immediately prior to use. All reactions were monitored by TLC on silica gel plates (Macherey, Nagel). Crude products were purified by column chromatography on silica gel 70–230 mesh. All reactions were carried out under argon atmosphere using flame-dried glassware.

4.1.1. Computational details. Molecular mechanics (MM) calculations were performed using the MacroModel 8.5 software package and the MMFFs force fields. MonteCarlo Multiple Minimum (MCMM) method (10,000 steps) of the MacroModel package was used in order to allow a full exploration of the conformational space. All the structures, so obtained, were optimised using the Polak–Ribier Coniugated Gradient algorithm (PRCG, 1000 steps, maximum derivative less than 0.05 kcal/mol). The initial geometries of the minimum energy conformers were optimised at the hybrid DFT MPW1PW91 level using the 6-31G(d) basis set (Gaussian 03 software package). GIAO *J*-coupling calculations were performed using the MPW1PW91 functional and the 6-31G(d) basis set, using as the input the geometry previously optimised at MPW1PW91/6-31G(d) level.

4.2. Synthetic procedures for 2a and 2b

*4.2.1. (E)-Ethyl 6-methylhept-2-enoate (**7**).* DMSO (4.18 mL, 58.8 mmol) was added dropwise for 15 min to a solution of oxalyl chloride (14.7 mL, 29.4 mmol) in dry dichloromethane (50 mL) at −78 °C under argon atmosphere. After 30 min a solution of the alcohol **6** (1.00 g, 9.80 mmol) in dry CH_2Cl_2 was added via cannula and the mixture was stirred at −78 °C for 1 h. Et_3N (6.83 mL, 49.0 mmol) was added dropwise and the mixture was allowed to warm to room temperature. The reaction was quenched by addition of aqueous $NaHSO_4$ (1 M, 50 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with saturated aqueous $NaHSO_4$, saturated aqueous $NaHCO_3$ and brine. The organic phase was then dried over Na_2SO_4 and concentrated to give the corresponding aldehyde **5** (0.951 g, 97%) as a colourless oil, which was used without any further purification. δ_H (400 MHz, $CDCl_3$) 9.70 (1H, s, C*H*O), 2.44 (2H, m, CH_2CHO), 1.59 (1H, m, C*H*Me$_2$), 1.25 (2H, m, CH_2), 0.92 (3H, d, *J* 6.6 Hz, Me), 0.91 (3H, d, *J* 6.6 Hz, Me); δ_C (100 MHz $CDCl_3$) 203.5, 40.2, 31.1, 27.9, 22.7, 22.5.

To a solution of compound **5** (0.950 g, 9.50 mmol) and LiOH (250 mg, 10.5 mmol) in THF (10 mL) was added TEPA (triethyl phosphonoacetate, 2.07 mL, 10.5 mmol). The reaction mixture was stirred for 24 h at room temperature and then quenched with water (10 mL). The mixture was then extracted with EtOAc (3×30 mL), and the organic phase was concentrated in vacuo. Flash chromatography (hexane/EtOAc, 99:1) afforded pure **7** (1.21 g, 75%). δ_H (400 MHz, $CDCl_3$) 6.95 (1H, dt, *J* 15.4, 6.6, C*H*=CHCO), 5.80 (1H, d, *J* 15.4 Hz, CH=C*H*CO), 4.17 (2H, q, *J* 7.4, OCH_2), 2.20 (2H, m, CH_2CH=), 1.56 (1H, m, C*H*Me$_2$), 1.32 (2H, m, CH_2), 1.24 (3H, t, *J* 7.4, OCH$_2$*Me*), 0.88 (3H, d, *J* 6.6 Hz, *Me*), 0.89 (3H, d, *J* 6.6 Hz, Me); δ_C (100 MHz $CDCl_3$) 165.4, 150.8, 121.0, 60.4, 37.2, 30.4, 27.8, 22.5 (2C), 14.5; HRMS (ESI): calcd for $C_{10}H_{19}O_2$: 171.1382; found 171.1389 $[M+H]^+$.

*4.2.2. (E)-6-Methylhept-2-en-1-ol (**4**).* To a solution of compound **7** (1.10 g, 6.47 mmol) in toluene (35 mL) at −78 °C, under a nitrogen atmosphere and with stirring, was slowly added DIBAL-H (7.59 mL of a 1.7 M solution in toluene, 12.9 mmol). After 1 h (TLC monitoring), the reaction was quenched with saturated NH_4C1 (25 mL) and the mixture was extracted with Et_2O (50 mL). Organic layer was dried over Na_2SO_4, concentrated in vacuo and purified on SiO_2 chromatography (hexane/EtOAc, 90:10) affording pure compound **4** (787 mg, 95%). δ_H (400 MHz, $CDCl_3$) 5.67 (2H, m, C*H*=C*H*), 4.09 (2H, d, *J* 5.2 Hz, CH_2OH), 2.05 (2H, m, CH_2CH=), 1.63 (1H, m, C*H*Me$_2$), 1.27 (2H, m, CH_2), 0.82 (3H, d, *J* 6.6 Hz, Me), 0.81 (3H, d, *J* 6.6 Hz, Me); δ_C (100 MHz $CDCl_3$) 133.8., 128.6, 63.8, 38.3, 30.1, 27.5, 22.4 (2C); HRMS (ESI): calcd for $C_8H_{17}O$: 129.1279; found 129.1274 $[M+H]^+$.

*4.2.3. (2S,3S)-2,3-Epoxy-6-methylheptan-1-ol (**8**).* To a 100 mL round bottom flask under argon equipped with a magnetic stirrer were added molecular sieves (4 Å, 164 mg) in CH_2Cl_2 (50 mL). At −23 °C were then added Ti(O*i*-Pr)$_4$ (77.7 mg, 0.273 mmol) and L-(+)-DET (67.7 mg, 0.328 mmol). The solution was allowed to stir for 5 min, then compound **4** (0.700 g, 5.47 mmol) and TBHP (1.98 mL of a 5.5 M solution in decane, 10.9 mmol) were added successively. After 24 h a solution of tartaric acid (492 mg, 3.28 mmol) and $FeSO_4$ (1.8 g, 6.56 mmol) in 20 mL of water was added and was stirred at −23 °C. After 30 min, the cooling bath was removed and stirring was continued at room temperature for 1 h until the aqueous layer become clear. After separation of the aqueous layer, the organic layer was washed once with water, dried over Na_2SO_4 and concentrated. This oil was diluted with Et_2O (50 mL) and cooled in a ice bath, and then NaOH (25 mL of 1 N solution in brine) was added; the two phase mixture was stirred at 0 °C for 0.5 h, and then the ether phase was washed with brine, dried over Na_2SO_4 and concentrated. SiO_2 chromatography (hexane/EtOAc, 90:10) afforded pure compound **8** (709 mg, 90%). $[\alpha]_D^{20}$ −10.7 (*c* 0.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.89 (1H, br d, *J* 12.6 Hz, O–C*H*CH$_2$OH), 3.60 (1H, br d, *J* 12.6 Hz, C*H*–O), 2.92 (2H, m, CH_2OH), 1.58 (1H, m, C*H*Me$_2$), 1.32 (2H, m, CH_2CH–O), 1.25 (2H, m, CH_2CH$_2$CH–O), 0.88 (3H, d, *J* 6.4 Hz, Me), 0.87 (3H, d, *J* 6.4 Hz, Me); δ_C (100 MHz $CDCl_3$) 61.9, 58.8, 53.7, 35.1, 29.6, 28.0, 22.7, 22.6; HRMS (ESI): calcd for $C_8H_{17}O_2$: 145.1229; found 145.1224 $[M+H]^+$.

Compound **8** (0.5–1.0 mg) was dissolved in freshly distilled CH_2Cl_2 and treated with triethylamine (10 mL) and (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*S*)-MTPA-Cl] and a catalytic amount of 4-(dimethylamino)pyridine. The mixture was left to stand at room temperature for 2 h. After this period, the mixture was concentrated in vacuo affording pure (*R*)-MTPA ester in quantitative yield. δ_H (400 MHz, $CDCl_3$) 7.47 (2H, m, Ar*H*), 7.36 (3H, m, Ar*H*), 4.47 (1H, dd, *J* 12.0, 3.0 Hz CH_2O), 4.16 (1H, dd, *J* 12.0, 6.0 Hz CH_2O), 3.50 (3H, s, −O*Me*), 2.94 (1H, m, C*H*O), 2.76 (1H, m, C*H*O) 1.47 (4H, m, CH_2CH$_2$CH–O), 1.22 (1H, m, C*H*Me$_2$), 0.81 (3H, d, *J* 6.6 Hz, *Me*), 0.80 (3H, d, *J* 6.6 Hz, *Me*); HRMS (ESI): calcd for $C_{18}H_{24}F_3O_4$: 361.1627; found 361.1632 $[M+H]^+$.

*4.2.4. (2R,3S)-2,3-Epoxy-6-methylheptanoic acid (**3**).* To a vigorously stirred mixture of compound **8** (0.700 g, 4.86 mmol) were added $NaIO_4$ (4.25 g, 19.9 mmol) in CCl_4 (18.5 mL), CH_3CN (18.5 mL), H_2O (27.8 mL) and $RuCl_3H_2O$ (26.2 mg, 0.0972 mmol). The mixture was stirred at 20 °C for 2 h; then the acidic material

was carefully extracted at 0 °C into ether, dried briefly over Na_2SO_4 and evaporated in vacuo to give a crude residue **3** (750 mg), that was subjected to next steps without further purification. δ_H (400 MHz, $CDCl_3$) 3.50 (1H, m, O–C*H*COOH), 3.19 (1H, m, C*H*–O), 1.62 (1H, m, C*H*Me_2), 1.36 (2H, m, C*H*$_2$CH–O), 1.26 (2H, m, C*H*$_2$CH$_2$CH–O), 0.91 (3H, d, *J* 6.3 Hz, Me), 0.90 (3H, d, *J* 6.3 Hz, Me); δ_C (100 MHz $CDCl_3$) 173.0, 59.4, 52.8, 34.7, 29.6, 27.9, 22.6 (2C); HRMS (ESI): calcd for $C_8H_{13}O_3$: 157.0865; found 157.0861 $[M-H]^-$.

*4.2.5. (2R,3S)-Methyl 2,3-epoxy-6-methylheptanoate (**9**).* To an aliquot of **3** (200 mg) in CH_2Cl_2 was added an ethereal solution of diazomethane until the solution become yellow. Evaporation and silica gel chromatography (hexane/EtOAc, 99:1) afforded pure **9** (196 mg, 82% over two steps from **8**). $[\alpha]_D^{20}$ +27.0 (*c* 1.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.76 (3H, s, O*Me*), 3.50 (1H, m, O–C*H*COOMe), 3.13 (1H, m, C*H*–O), 1.63 (1H, m, C*H*Me_2), 1.36 (2H, m, C*H*$_2$CH–O), 1.26 (2H, m, C*H*$_2$CH$_2$CH–O), 0.88 (3H, d, *J* 6.3 Hz, Me), 0.87 (3H, d, *J* 6.3 Hz, Me); δ_C (100 MHz $CDCl_3$) 170.8, 58.9, 53.2, 52.6, 34.7, 29.5, 27.9, 22.5, 22.6; HRMS (ESI): calcd for $C_9H_{17}O_3$: 173.1178; found 173.1172 $[M+H]^+$.

*4.2.6. (2S,3R)-3-Bromo-2-hydroxy-6-methylheptanoate (**10**).* To a solution of compound **9** (160 mg, 0.930 mmol) in Et_2O (6 mL) was added $MgBr_2\cdot Et_2O$ (361 mg, 1.40 mmol). The solution was stirred at room temperature for 1 h and then washed with brine, and the organic layers were evaporated in vacuo. The crude mixture was purified by SiO_2 chromatography (hexane/EtOAc, 99:1), affording pure **10** (230 mg, 98%). $[\alpha]_D^{20}$ −6.4 (*c* 0.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 4.40 (1H, d, *J* 3.3 Hz, C*H*OH), 4.16 (1H, m, C*H*–Br), 3.81 (3H, s, OMe), 1.90 (1H, m, C*H*$_a$H$_b$CHBr), 1.79 (1H, m, CH$_a$*H*$_b$CHBr), 1.55 (1H, m, C*H*Me_2), 1.44 (1H, m, C*H*$_a$H$_b$CH$_2$CHBr), 1.29 (1H, m, CH$_a$*H*$_b$CH$_2$CHBr), 0.88 (3H, d, *J* 6.3 Hz; Me), 0.86 (3H, d, *J* 6.3 Hz, Me); δ_C (100 MHz $CDCl_3$) 171.5, 74.7, 53.0, 37.0, 32.0, 27.7, 23.0, 22.4, 21.2; HRMS (ESI): calcd for $C_9H_{18}{}^{79}BrO_3$: 253.0434; found 253.0439 $[M+H]^+$.

*4.2.7. (2R,3S)-3-Azido-2-hydroxy-6-methylheptanoate (**11**).* A mixture of **10** (189 mg, 0.750 mmol) and NaN_3 (195 mg, 3.00 mmol) in DMF (5.0 mL) was stirred at 65 °C for 24 h. The mixture was then diluted with EtOAc (10.0 mL), washed with water (10.0 mL), and concentrated in vacuo. Silica gel chromatography (hexane/ether, 99:1) afforded pure **11** (148 mg, 92%). $[\alpha]_D^{20}$ +1.5 (*c* 0.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 4.25 (1H, d, *J* 5.6 Hz, C*H*OH), 3.82 (3H, s, OMe), 3.47 (1H, t, *J* 7.0, C*H*–N_3), 3.03 (1H, d, *J* 5.6 Hz, CHO*H*), 1.83 (1H, m, C*H*$_a$H$_b$CHN$_3$), 1.81 (1H, m, CH$_a$*H*$_b$CHN$_3$), 1.61 (1H, m, C*H*Me_2), 1.34 (2H, m, C*H*$_2$CH$_2$CHN$_3$), 0.93 (3H, d, *J* 6.3 Hz, Me), 0.91 (3H, d, *J* 6.3 Hz, Me); δ_C (100 MHz $CDCl_3$) 172.6, 68.4, 59.0, 53.7, 30.6, 29.2, 24.0, 23.2 (2C); HRMS (ESI): calcd for $C_9H_{18}N_3O_3$: 216.1348; found 216.1352 $[M+H]^+$.

*4.2.8. (2R,3S)-3-Amino-2-hydroxy-6-methylheptanoic acid (**2b**).* A solution of **11** (130 mg, 0.604 mmol) in ethyl acetate was hydrogenated in the presence of Pd/C catalyst (2 mg) for 24 h at room temperature. The mixture was filtered through Celite and concentrated in vacuo to give a 125 mg residue that was subjected to under vacuum vapour-phase hydrolysis (HCl 6 N, 110 °C for 18 h). HPLC purification on the reversed-phase Phenomenex Hydro (4 μ, 250×4.6 mm) column eluting with 85% H_2O/MeOH (0.1% TFA) furnished compound **2b** (79.3 mg, rt=19.5 min) in 75% yield over two steps. $[\alpha]_D^{20}$ +3.3 (*c* 0.3, MeOH); δ_H (400 MHz, CD_3OD) see Table 2 in the text; ^{13}C NMR (100 MHz, CD_3OD) δ: see Table 2 in the text; HRMS (ESI): calcd for $C_8H_{18}NO_3$: 176.1286; found 176.1293 $[M+H]^+$.

*4.2.9. (2R,3R)-3-Amino-2-hydroxy-6-methylheptanoic acid (**2a**).* Compound **3** (200 mg, 1.27 mmol) was dissolved in water (2 mL). Powdered NaN_3 (124 mg, 1.91 mmol) and 1.3 mL of an aqueous solution 0.1 M of $Cu(NO_3)_2$ were added under stirring (resulting pH 4.3–4.5), and the mixture was warmed to 65 °C. After 1.5 h (ca. pH 5.5) the reaction mixture was cooled to 0 °C, and $NaBH_4$ was added portion-wise (96.1 mg, 2.54 mmol). After 30 min at 0 °C the reaction mixture was filtered and concentrated in vacuo to give a residue that, subjected to HPLC purification on the reversed-phase Phenomenex Hydro (4 μ, 250×4.6 mm) column eluting with 85% H_2O/MeOH (0.1% TFA), furnished compound **2a** (167 mg, rt=21 min) in 75% yield over two steps. $[\alpha]_D^{20}$ +8.6 (*c* 0.5, MeOH); δ_H (400 MHz, CD_3OD) see Table 2 in the text; ^{13}C NMR (100 MHz, CD_3OD) δ: see Table 2 in the text; HRMS (ESI): calcd for $C_8H_{18}NO_3$: 176.1286; found 176.1291 $[M+H]^+$.

4.3. Determination of the absolute configuration

4.3.1. Peptide hydrolysis and AHMHA isolation. A 30 mg sample of perthamide C (**1**) was dissolved in 6 N HCl (3 mL) and heated in vacuo at 130 °C for 12 h. The crude residue was fractionated by HPLC on the reversed-phase Phenomenex Hydro (4 μ, 250×4.6 mm) column eluting with 85% H_2O/MeOH (0.1% TFA) (flow rate 0.5 mL/min) to give 3 mg of pure ADMHA (rt=21 min). $[\alpha]_D^{20}$ +8.8 (*c* 0.55, MeOH); δ_H (400 MHz, CD_3OD) see Table 2 in the text; ^{13}C NMR (100 MHz, CD_3OD) δ: see Table 2 in the text.

4.3.2. LC-MS analysis of Marfey's (FDAA) derivatives. A portion of pure AHMHA or the amino acid standards **2a**–**2b** (500 μg) was dissolved in 80 μL of a 2:3 solution of TEA/MeCN and this solution was then treated with 75 μL of 1% 1-fluoro-2,4-dinitrophenyl-5-alaninamide (L- or D-FDAA) in 1:2 MeCN/acetone. The vials were heated at 70 °C for 1 h, and the contents were neutralised with 0.2 N HCl (50 μL) after cooling to room temperature. An aliquot of the FDAA derivative was dried under vacuum, diluted with 50% aqueous acetonitrile containing 5% formic acid, and separated on a Proteo C18 (25×1.8 mm i.d.) column by means a linear gradient from 10% to 50% aqueous acetonitrile containing 5% formic acid and 0.05% trifluoroacetic acid, over 45 min at 0.15 mL/min. The RP-HPLC system was connected to the electrospray ion source by inserting a splitter valve and the flow going into the mass spectrometer source was set at a value of 100 μL/min. Mass spectra were acquired in positive ion detection mode (*m*/*z* interval of 320–900) and the data were analysed using the suite of programs Xcalibur; all masses were reported as average values. Capillary temperature was set at 280 °C, capillary voltage at 37 V, tube lens offset at 50 V and ion spray voltage at 5 V.

Retention times of L-FDAA-3-amino-2-hydroxy-6-methylheptanoic acid (min): (2*R*, 3*R*)-**12a** (48.50), (2*R*, 3*S*)-**12b** (39.40), AHMHA-**1a** (48.50).

Retention times of D-FDAA-3-amino-2-hydroxy-6-methylheptanoic acid (min): (2*R*, 3*R*)-**12c** (40.99), (2*R*, 3*S*)-**12d** (48.30).

Acknowledgements

This work was supported by grants from MIUR (PRIN 2007) 'Sostanze Naturali ed Analoghi Sintetici con Attività Antitumorale' Rome, Italy. NMR spectra were provided by CSIAS, Centro Interdipartimentale di Analisi Strumentale, Faculty of Pharmacy, University of Naples.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.07.060.

References and notes

1. Festa, C.; De Marino, S.; Sepe, V.; Monti, M. C.; Luciano, P.; D'Auria, M. V.; Debitus, C.; Bucci, M.; Vellecco, V.; Zampella, A. *Tetrahedron* **2009**, *65*, 10424–10429.

2. Posadas, I.; Bucci, M.; Roviezzo, F.; Rossi, A.; Parente, L.; Sautebin, L.; Cirino, G. *Br. J. Pharmacol.* **2004**, *142*, 331–338.
3. Plaza, A.; Bifulco, G.; Masullo, M.; Lloyd, J. R.; Keffer, J. L.; Colin, P. L.; Hooper, J. N. A.; Bell, L. J.; Bewley, C. A. *J. Org. Chem.* **2010**, *75*, 4344–4355.
4. Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866–876.
5. Kurz, M.; Schmieder, P.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1991**, *10*, 1329–1331.
6. Bifulco, G.; Bassarello, C.; Riccio, R.; Gomez-Paloma, L. *Org. Lett.* **2004**, *6*, 1025–1028.
7. Bifulco, G.; Dambruoso, P.; Gomez-Paloma, L.; Riccio, R. *Chem. Rev.* **2007**, *107*, 3744–3779.
8. Di Micco, S.; Chini, M. G.; Riccio, R.; Bifulco, G. *Eur. J. Org. Chem.* **2010**, 1411–1434.
9. Tomasi, J.; Mennucci, B.; Cances, E. *THEOCHEM* **1999**, *464*, 211–226.
10. Bonini, C.; Righi, G. *Tetrahedron* **2002**, *58*, 4981–5021.
11. Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165–185.
12. Fringuelli, F.; Pizzo, F.; Rucci, M.; Vaccaro, L. *J. Org. Chem.* **2003**, *68*, 7041–7045.
13. Righi, G.; Rumboldt, G.; Bonini, C. *J. Org. Chem.* **1996**, *61*, 3557–3560.
14. In CD_3OH the signal relative to carbamate carbon atom fortuitously overlapped with the C2′ signal (δ_C 155.7) of *o*-Tyr residue.
15. Zampella, A.; D'Orsi, R.; Sepe, V.; Casapullo, A.; Monti, M. C.; D'Auria, M. V. *Org. Lett.* **2005**, *7*, 3585–3588.
16. Gala, F.; D'Auria, M. V.; De Marino, S.; Sepe, V.; Zollo, F.; Smith, C. D.; Keller, S. N.; Zampella, A. *Tetrahedron* **2009**, *65*, 51–56.
17. Faulkner, J. D.; Harper, M. K.; Reddy, R. V. M. *Tetrahedron* **1998**, *54*, 10649–10656.
18. Sano, T.; Kaya, K. *Tetrahedron Lett.* **1995**, *36*, 8603–8606.
19. Sano, T.; Kaya, K. *Tetrahedron* **1998**, *54*, 463–470.
20. Sano, T.; Beattie, K. A.; Codd, G. A.; Kaya, K. *J. Nat. Prod.* **1998**, *61*, 851–853.
21. Bewley, C. A.; He, H.; Williams, D. H.; Faulkner, D. J. *J. Am. Chem. Soc.* **1996**, *118*, 4314–4321.
22. Sepe, V.; D'Orsi, R.; Borbone, N.; D'Auria, M. V.; Bifulco, G.; Monti, M. C.; Catania, A.; Zampella, A. *Tetrahedron* **2006**, *62*, 833–840.
23. Plaza, A.; Bewley, C. A. *J. Org. Chem.* **2006**, *71*, 6898–6907.
24. Ford, P. W.; Gustafson, K. R.; McKee, T. C.; Shigematsu, N.; Maurizi, L. K.; Pannell, L. K.; Williams, D. E.; Dilip de Silva, E.; Lassota, P.; Allen, T. M.; Van Soest, R.; Andersen, R. J.; Boyd, M. R. *J. Am. Chem. Soc.* **1999**, *121*, 5899–5909.
25. Nakamura, K.; Tetsuya, I.; Toshima, H.; Kodaka, M. *Tetrahedron Lett.* **2004**, *45*, 7221–7224.
26. Goto, Y.; Iwasaki, K.; Torikai, K.; Murakami, H.; Suga, H. *Chem. Commun.* **2009**, *23*, 3419–3421.