DOI: 10.1002/ejoc.200500740

Quantum Mechanical Calculation of Coupling Constants in the Configurational Analysis of Flexible Systems: Determination of the Configuration of Callipeltin A

Carla Bassarello,[a] Angela Zampella,[b] Maria Chiara Monti,[a] Luigi Gomez-Paloma,[a] Maria Valeria D'Auria,[b] Raffaele Riccio,[a] and Giuseppe Bifulco*[a]

Keywords: GIAO / ^{13}C NMR / ^{1}H NMR / DFT calculations / Callipeltin A / *J*-couplings

An integrated NMR-quantum mechanical (QM) approach, relying on the comparison between calculated and experimental *J*-values, was applied to the analysis of the relative configuration of four amino acid units (known as AGDHE, D-*a*Thr1, D-*a*Thr2 and β-OMeTyr) contained in callipeltin A, a cyclopeptide endowed with a powerful inhibitory activity on the cardiac sodium/calcium exchanger and also showing interesting antiviral and antifungal properties. In this paper we report the first example of the application of this method to a real case, which allowed the assignment of the relative configuration of the β-OMeTyr residue and the revision of the configuration of the Thr2 unit in callipeltin A.

1. Introduction

Callipeltin A (**1**) and its congeners were isolated in our laboratories from the sponges *Callipelta* sp.[1,2] and *Latrunculia* sp.[3] Callipeltin A shows both antiviral activity against HIV-1-infected CEM4 lymphocytes and antifungal activity against *Fusarium oxysporum*, *Helminthosporium sativum*, *Phytophtora hevea*, and *Candida albicans.* More recently, callipeltin A was found to be a selective and powerful inhibitor of the cardiac sodium/calcium exchanger and eliciting interest as a potential regulator of myocardial contractility.[4]

Callipeltin A (**1**) is the first member of a growing group of potent antiviral marine peptides, which comprises papuamides A-C,[5] microspinamide,[6] and neamphamide,[7] and with which it shares a certain degree of structural homology, suggesting in turn a potential common pharmacophore.

Distinguishing structural characteristics of this family of peptides include a preponderance of unusual amino acid residues and unique *N*-terminal polyketide derived moieties.

In our original paper,[1] we proposed the configuration of nine amino acid residues of callipeltin A as L-Ala, (2*R*,3*R*,4*S*)-4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (AGDHE), (3*S*,4*R*)-3,4-dimethyl-L-glutamine (diMeGln), L-Thr (two residues), D-Arg, L-Leu, L-*N*MeAla. The configuration of β-OMeTyr residue, indeed, remained unassigned due to the chemical degradation of this residue under acidic hydrolysis conditions. Subsequent studies led to a revision of L-Ala as D-Ala and L-Thr-1 as D-*a*Thr-1.[3]

Further insights arose from the synthetic studies on callipeltin A recently appeared in the literature. In fact, several groups, including own, have reported the stereoselective synthesis of all non-proteinogenic units in callipeltin.[8–17] These efforts led to the revision of the 3-hydroxy-2,4,6-trimethylheptanoic acid end group,[15,17] and to the confirmation of the 3,4-dimethylglutamine residue,[9] nevertheless several stereochemical issues are still unresolved (Figure 1).

First, the β-OMeTyr residue, common to callipeltins, papuamides A–D and neamphamide, lacks a definition of its relative and absolute configuration. The relative configuration of 4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (AGDHE) also requires an unambiguous definition. Indeed, whereas the *S* configuration of C4 of this residue was secured by reliable chemical methods, the relative configuration proposed on the basis of homonuclear *J* coupling analysis[1] did not receive unambiguous confirmation by subsequent synthetic studies.[13] In addition, the presence of two D-*a*Thr residues in neamphamide, a peptide which shows a high structural homology with **1**, prompted us to further investigate the identity of the two Thr residues in callipeltin A, which were recently reported as D-*a*Thr1, and L-Thr2.[3]

While the full configuration assignment of callipeltin A could be determined by completing the total synthesis of all the possible stereoisomers, we envisaged that the application of the QM/NMR approach, recently proposed by our group,[18] should rapidly address the stereochemical quandary.

[a] Dipartimento di Scienze Farmaceutiche, Università di Salerno, via Ponte don Melillo, 84084 Fisciano (SA), Italy
[b] Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", via D. Montesano 49, 80131 Napoli, Italy
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

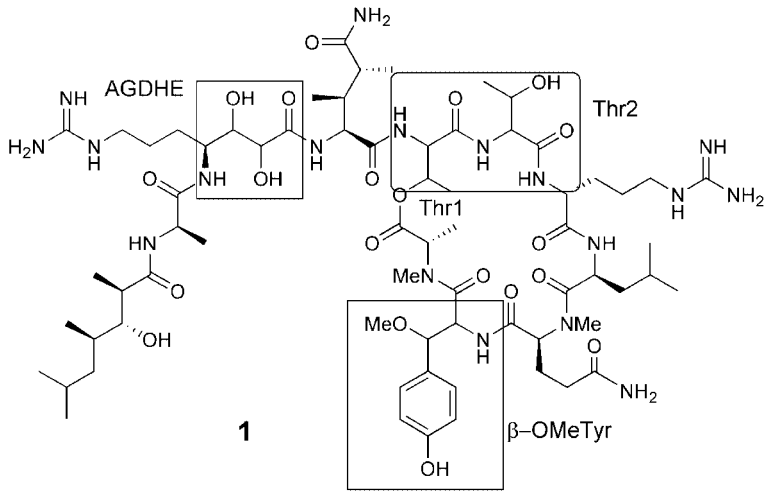

Figure 1. Callipeltin A (1) with amino acid residues still stereochemically undefined.

Our strategy is based on the comparison between calculated (at QM level) and experimental $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ coupling values; it employs accurate prediction of the *J* coupling constants obtained at DFT (density functional theory) level that allows a faithful assessment of the NMR properties under examination. The theoretical data derive from the calculation of homo- and heteronuclear coupling constant values for each of the six main staggered rotamers (three for each relative *erythro* and *threo* stereochemical arrangement) in which any given two-carbon (chiral) fragment can be ideally represented. For a molecule containing more than a single pair of stereocentres, like callipeltin A, the strategy examines each C_2 fragment independently from the rest of the whole molecule, following a rationale building of a set of appropriate reduced systems.

Our choice of dividing the molecule depends at least on two factors: from a computational perspective the configurational determination of an entire system would limit the analysis to compounds with no more than two/three stereogenic centres; in addition, a strategy based on the dissection of the full molecular system may still be considered reliable, since the magnitudes of coupling constants are affected mainly by the local atomic environment, and effects extending further than two atoms away from the nuclei involved in the coupling are usually not relevant.

In practice, the division of the system in simplified fragments is made substituting to the main chain at least two heavy groups (carbon, oxygen atoms), and to every branched chain at least one heavy atom (Figure 2).

The accuracy, the robustness and the precision attainable by this method was shown on a model system, whose configuration had already been determined through chemical synthesis.[18] We felt that callipeltin A with its key residues may represent an ideal test system for our approach, thus allowing to extend this methodology to a real case.

In this paper we report, through the application of the QM/NMR method, a configurational analysis of callipeltin A **1**, involving the two threonine residues (named D-*a*Thr1,

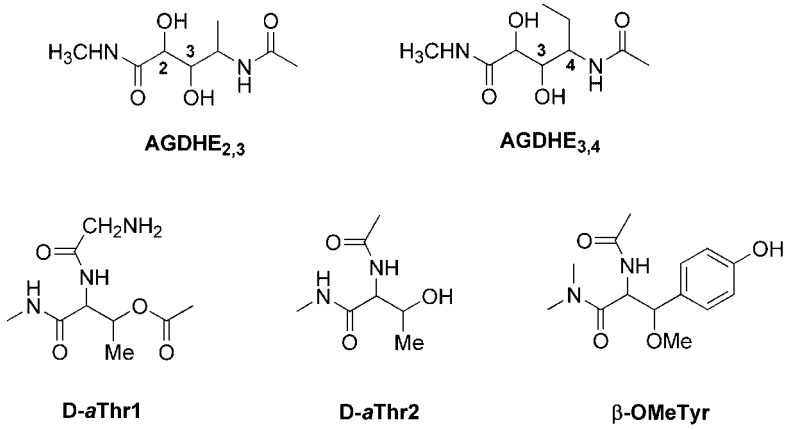

Figure 2. Molecular Fragments representing the five C_2 reduced systems of callipeltin A; calculations of *erythro* and *threo* forms of $AGDHE_{2,3}$ were performed varying centre C-4. Since the two sets of calculations pointed to the same results, and on the basis of the results of the calculation regarding $AGDHE_{3,4}$ we reported, for sake of simplicity, only the results of the set of calculations containing the correct relative configuration of C-4. Same considerations apply for fragment $AGDHE_{3,4}$, this time varying C-2. Also in this case, we reported only the set of calculations containing the correct configuration of C-2. The complete set of results is reported in the Supporting Information.

and D-*a*Thr2), the β-OMeTyr amino acid, and the two units (named $AGDHE_{2,3}$, and $AGDHE_{3,4}$) contained in the AGDHE fragment.

2. Results and Discussion

According to the above rational, callipeltin A was (virtually) dissected prior to geometry optimization and calculation of the NMR properties. In particular, each C_2 fragment represents one of the five fragments, namely $AGDHE_{2,3}$, $AGDHE_{3,4}$, D-*a*Thr1, D-*a*Thr2, β-OMeTyr. The so obtained reduced systems are depicted in Figure 2.

For each fragment, three (1 *anti* and 2 *gauche*) staggered arrangements were considered for each of the two *erythro* and *threo* stereochemical series. Subsequently, each arrangement was optimised at mPW1PW91 level of theory using the 6-31G(d) basis set; the calculation of *J* couplings was executed on the optimized geometries using the same functional and the 6-31G(d,p) basis set.[19] For both the calculation steps, the IEF-PCM solvent continuum model (methanol solvent) was used,[20] since some of the fragments under investigation contain HB (hydrogen bonding)-donor and HB-acceptor groups, and the neglect of solvent effects may seriously affect the conformational properties of the system, eventually leading to the wrong configurational assignment.

Table 1. Calculated data sets of callipeltin A *J* values for the six conformational arrangements belonging to *erythro* and *threo* series in comparison with the experimental data: single deviations and TAD ($\Sigma|J_{calc}$-$J_{exp}|$) values.

	Calc.						Exp.
		erythro			*threo*		
	g^+	*anti*	g^-	g^+	*anti*	g^-	
D-*a*Thr1							
$^3J_{H2–H3}$	3.7	8.6	2.6	4.5	9.2	1.3	2.8
$^2J_{H2–C3}$	0.9	–4.4	–4.9	–4.5	–4.2	0.6	–5.7
$^3J_{H2–Me}$	0.7	2.3	4.0	0.3	2.7	1.9	5.0
$^2J_{H3–C2}$	–1.4	–2.2	0.3	–0.6	–1.8	0.6	–1.4
$^3J_{H3–C=O}$	7.2	2.4	1.4	7.4	1.0	2.1	2.0
TAD	17.0	11.0	**4.3**	13.8	11.6	13.0	
$AGDHE_{2,3}$							
$^3J_{H2–H3}$	4.6	8.0	3.2	1.8	5.9	6.6	9.0
$^2J_{H2–C3}$	–3.0	–3.7	–0.3	2.2	–2.7	–3.0	–4.0
$^3J_{H2–C4}$	6.7	2.3	0.3	0.4	4.6	5.3	2.6
$^2J_{H3–C2}$	1.9	–0.8	–1.3	3.8	0.3	–2.4	–3.5
$^3J_{H3–C=O}$	0.1	1.8	6.6	0.2	4.0	7.1	1.2
TAD	16.0	**4.9**	19.4	23.9	13.0	13.1	
$AGDHE_{3,4}$							
$^3J_{H3–H4}$	3.3	8.7	3.1	4.0	5.0	0.9	1.7
$^3J_{H3–C5}$	0.4	1.5	4.9	3.8	0.0	2.6	1.7
$^2J_{H4–C3}$	–4.3	–4.4	0.5	–3.9	–5.3	0.7	1.2
$^3J_{H4–C2}$	6.2	4.0	0.6	5.9	0.1	2.5	1.2
TAD	13.4	15.6	5.9	14.2	12.6	**3.5**	
D-*a*Thr2							
$^3J_{H2–H3}$	3.7	8.6	2.8	4.6	7.7	1.7	3.5
$^2J_{H2–C3}$	–0.4	–4.9	–4.9	–4.2	–3.6	–1.5	–4.1
$^3J_{H2–Me}$	0.5	1.9	4.1	4.3	4.4	1.6	1.1
$^2J_{H3–C2}$	–1.6	–0.3	2.4	–1.2	1.1	0.9	–2.8
$^3J_{H3–C=O}$	7.3	3.2	0.7	7.0	1.5	2.0	7.3
TAD	**5.7**	13.2	16.2	6.2	17.6	13.8	
β-OMeTyr							
$^3J_{H2–H3}$	1.5	8.7	3.9	8.3	10.7	3.7	9.1
$^2J_{H2–C3}$	–5.4	–3.2	0.0	–3.4	–4.3	–3.4	–3.9
$^3J_{H2–Ph}$	3.8	2.5	0.7	5.6	1.8	1.1	1.3
$^2J_{H3–C2}$	–0.8	–2.6	–2.7	–1.2	–2.3	0.4	–3.2
$^3J_{H3–C=O}$	1.4	2.0	6.4	6.6	1.6	1.5	1.7
TAD	14.3	**3.2**	14.9	12.5	3.5	9.1	

Table 1 reports the experimental data sets of *J* values along with all the calculated coupling constants for the various conformational and configurational arrangements of each of the five C_2 fragments originating from callipeltin A. A careful data analysis allowed to assess that the accuracy of the results is fairly good: in all the five independent C_2 fragments examined, it can be seen that for only one of the six arrangements the calculated *J* values are in agreement with the experimental, by showing the lowest sum of absolute errors $\Sigma|J_{calc} - J_{exp}|$ (Total Absolute Deviation values, TAD) in the reproduction of *J* experimental data.

It has to be pointed out that a significant comparison of the TAD values is possible only among the different conformers of a given C_2 fragment, while TAD values of different C_2 fragments should not be confronted. In fact, the structural simplification of the molecule and its division in the reduced fragments of Figure 2 is an approximation that may differently affect the five C_2 fragments examined.

D-aThr1 Residue

A careful analysis of the calculated vs. the experimental data of the D-*a*Thr1 residue (Table 1) showed the lowest deviation between corresponding calculated and experimental $^3J_{H,H}$ and $^{2,3}J_{C,H}$ values for the g^- *erythro* arrangement. Indeed, the mean absolute error ($\Sigma|J_{calc} - J_{exp}|/n$, n = total number of couplings) calculated for this arrangement was 0.8 Hz with the highest $|J_{calc} - J_{exp}|$ difference, corresponding to 1.7 Hz, for the $^2J_{H3,C2}$ coupling constant, whereas for the remaining *J* pattern the $|J_{calc} - J_{exp}|$ differences were below 1.0 Hz. As evidenced in Table 1, incorrect arrangements were characterized by larger values of the mean absolute error, ranging from ca. 2.3 to ca. 3.4 Hz, and, in the calculated pattern, each improper arrangement contained a calculated coupling constant that deviated of about 6 Hz from the experimental one.

Moreover, a further very informative indication was given by the Total Absolute Deviation (TAD, sum of absolute errors $\Sigma|J_{calc} - J_{exp}|$) that, more clearly than the mean absolute error $\Sigma|J_{calc} - J_{exp}|/n$, allowed us to safely draw conclusions. In fact, the sum of absolute errors $\Sigma|J_{calc} - J_{exp}|$ of g^- *erythro* arrangement was 4.3 Hz, while this parameters in all other cases fell in the 11–17 Hz range.

$AGDHE_{2,3}$ and $AGDHE_{3,4}$ C_2 Systems

As for the D-*a*Thr1 residue, the analysis of the calculated vs. the experimental *J* values allowed the determination of $AGDHE_{2,3}$ and $AGDHE_{3,4}$ residues. For these fragments the relative configurational arrangement was suggested by a comparative estimation of the TAD values. In fact, for the $AGDHE_{3,4}$ residue, the best agreement between calcu-

lated and experimental was found for the g⁻ *threo* arrangement, characterized by a total deviation of only 3.5 Hz (compared to TAD values of 5.9–15.6 Hz for the "wrong" conformers, see Table 1). Also for the $AGDHE_{2,3}$ fragment, the *anti erythro* model displayed the lowest TAD value of 4.9 Hz, much below the other deviations (13.0–23.9 Hz).

D-aThr2 Residue

The comparison between the *J* calculated and the experimental data for the D-*a*Thr2 amino acid preliminarily suggested an *erythro* configuration. In fact, the g⁺ *erythro* arrangement showed the lowest TAD from the experimental *J* couplings, even if the calculated data set for the g⁺ *threo* arrangement also displayed a fair agreement with the experimental data. Indeed, both of the calculated g⁺ *erythro* and *threo* conformers displayed a qualitatively similar pattern of $^2J_{H3–C2}$ and $^3J_{H3–C=O}$ values, in particular the $^2J_{H3–C2}$ values (–1.6 and –1.2 Hz for g⁺ *erythro* and *threo* rotamers respectively, vs. the experimental value of –2.8 Hz) suggested a *gauche* relationship between the H3 proton and the nitrogen-substituent bonded to the adjacent coupled carbon. It is noteworthy that nitrogenated substituents are known to induce a small variability range of $^2J_{H–C}$ coupling constant.[21]

Since considerations relying on TAD values would only slightly favour the g⁺ *erythro* vs. the g⁺ *threo* arrangement, with very little difference (5.7 Hz vs. 6.2, respectively), a confirmation of the g⁺ *erythro* assignment was necessary. In particular, the analysis of the NMR spectroscopic data revealed the presence of a strong ROE dipolar effect between the H2 proton and the methyl group, allowing the confirmation of the g⁺ *erythro* arrangement. This observation was also consistent with the small $^3J_{H2–Me}$ value and, consequently, with a *gauche* relationship between these groups. A plausible explanation for the poor reproduction of the large experimental (–4.1 Hz) $^2J_{H2–C3}$ value in the g⁺ *erythro* conformer may be related to the inability of this particular reduced system to represent the corresponding fragment in the intact natural product.

Definition of the Absolute Configuration of the two Thr Residues

In order to safely confirm the stereochemical assignment of the Thr residues, derived from QM-NMR analysis, and to define their absolute configuration, we applied the LC/MS modification of Marfey's method to the acid hydrolysate of callipeltin A.[22] We had already used Marfey's method for the stereochemical analysis of the conventional amino acid residues in callipeltins,[1] but the identity of each residue was secured by comparison of the retention times of the derivatives from **1** with the FDAA derivatives of appropriate standards. A more reliable analysis involves the use of LC-MS for the proper identification of the individual peaks in the HPLC trace. A single peak corresponding to D-*a*Thr was observed by ion-selective monitoring for FDAA-Thr (*m*/*z* 372). Our original misassignment (L-Thr vs. D-*a*Thr) was likely due to the presence of an unassigned peak,[3] probably arising from a side reaction of a residue in callipeltin A during the hydrolysis. This fortuitously had the same retention time of FDAA derivative of L-Thr (12.5 min), but different molecular weight (*m*/*z* 292).

β-OMeTyr Residue

Finally, relevant considerations concern the β-OMeTyr residue. From a qualitative point of view, the *large* experimental $^3J_{H,H}$ coupling suggested one of the two possible *anti* arrangements; it has to be kept in mind that a differentiation of the two different *anti* arrangements (*erythro* and *threo*) may not be obtained on the basis of the semiquantitative classification (*small*, *medium*, and *large*) of the heteronuclear *J* values proposed by the Murata *J*-based analysis.[23] Conversely, the calculated *J* values for each relative series, compared to the experimental *J* values, may allow to identify the correct configurational arrangement through a quantitative approach.[18]

First, we observed that the calculated data sets of *J* values for the four *gauche* arrangements of the two relative series could be disregarded because they exhibited large deviations from the experimental counterparts (Table 1).

On the contrary, smaller discrepancies from the experimental *J* values were observed for the *anti* arrangements. It has to be pointed out that, even if in principle our QM/NMR approach allows to discern among the two diastereoisomeric *anti* arrangements,[18] the calculated *J* values for the *erythro* and the *threo anti* conformations of the β-OMeTyr residue resulted very similar to each other (Table 1). In particular, both the *anti* arrangement showed a satisfactory agreement with the experimental, with only a slight preference for the *erythro* arrangement (TAD values of 3.2 Hz vs. 3.5 Hz, see Table 1), revealing a limitation in our methodology for all cases in which a determinate C_2- fragment displays an intrinsic similarity of the *J* values pattern for two diastereomeric arrangements.

For the above reasons, a conclusive and safe differentiation was again achieved inspecting the ROESY spectrum acquired in [D_5]pyridine: the presence of a diagnostic ROE cross peak, that correlated the aromatic proton to the amide proton of β-OMeTyr residue, allowed the exclusion of the *threo* arrangement and suggested the *erythro* configuration for the β-OMeTyr residue. Prompted by this result, we have reported in a parallel paper the synthesis of all four diasteromers of this residue and have assigned the absolute configuration as 2*R*,3*R*.[30]

3. Conclusion

A recent QM/NMR approach, based on DFT calculation of proton-proton and proton-carbon *J*-coupling values and their comparison with the experimental, was employed for the first time to define some configurational features of a natural product. In particular our strategy was directed

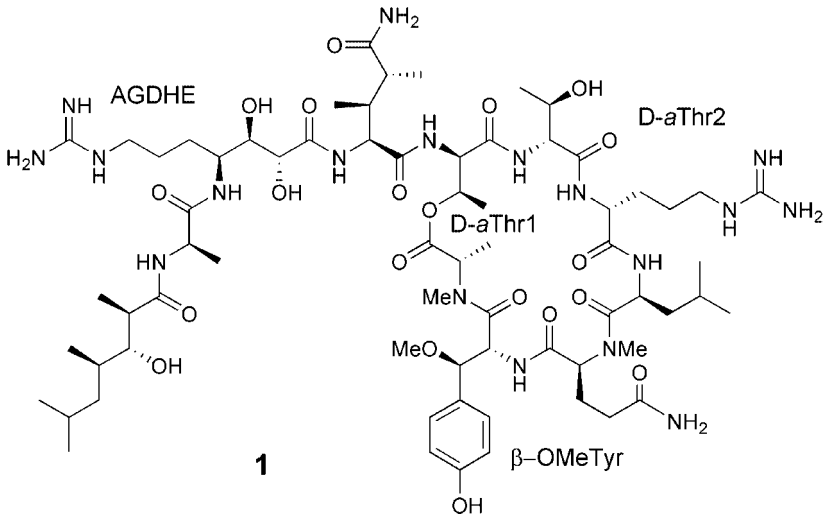

Figure 3. Revised stereostucture of callipeltin A (**1**).

toward the assignment of the relative configuration of five simplified segments of callipeltin A, to then transpose the results to the full molecule.

The agreement between calculated and experimental data can be considered satisfactory, suggesting a good reliability of the configurational assignment here proposed. Some discrepancies may be mainly due to the fact that the conformational properties of the whole molecule have not been taken into account. On the other hand, the dissection of the full molecular system into simplified fragments has the significant advantage to limit the computational effort and to be extremely rapid especially during the geometry optimization step. Furthermore, it has to be kept in mind that experimental values are referred to the real molecule, therefore providing a meaningful reference data system to assess the reliability of the strategic choices imposed to the calculations.

In conclusion, the application of the QM/NMR method to the configurational analysis of callipeltin A led to the following achievements: I) the first assignment of the relative configuration of the β-OMeTyr residue; II) the revision of the configuration of the Thr2 residue, by replacing the original proposed L-Thr with a D-*a*Thr; III) the confirmation of the 2*R*,3*R*,4*S*-configuration for the AGDHE fragment, as shown in Figure 3.

4. Experimental Section

4.1 NMR Experiments: NMR measurements were performed on a Bruker DRX-600 at T = 300 K. All spectra were acquired in the phase-sensitive mode and the TPPI method was used for quadrature detection in the ω_1 dimension. NMR sample was obtained dissolving 10 mg of callipeltin A in two solvent systems (500 μL) [D_4]MeOH (CIL, 99.96% D) and [D_5]pyridine (Sigma Aldrich, 99.5%D). The spectra recorded dissolving callipeltin A in [D_4]-MeOH were PFG-PS-HMBC,[24,25] PFG-HETLOC,[26] ROESY,[27] and NOESY[28] experiments. $^{2,3}J_{C-H}$ values were obtained from phase-sensitive PFG-PS-HMBC spectrum with a total of 128 scans per t_1 value, acquiring 4K points in ω_2, and with a t_{1max} value with a t_{1max} of 9.5 ms. The delay for long-range coupling evolution (Δ) was set at 50 ms. Upon 2D-FT, zero-filling (4K × 1K) was carried out in ω_2 and ω_1. For the phase-sensitive PFG-HETLOC spectrum, a total of 160 scans/t_1 were acquired using 4K points in ω_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 ω_2. The following acquisition conditions were set for the ROESY spectrum 128 scans/t_1, a t_{1max} value of 37.7 ms and a mixing time of 350 ms while for the NOESY spectrum 128 scans/t_1, a t_{1max} value of 29.8 ms and a mixing time of 200 ms. The spectrum executed on the sample prepared in [D_5]pyridine was a NOESY experiment whose conditions were the following: 256 scans per t_1 value, a t_{1max} value of 16.8 ms and a mixing time of 350 ms. The NMR spectroscopic data were processed on a Silicon Graphic Indigo2 workstation using the Bruker XWIN-NMR software. Standard Bruker pulse sequences noesyph and roesyph were used to run NOESY and ROESY spectra, respectively; the PFG-PS-HMBC pulse sequence is available on the internet (http://www.bmrb.wisc.edu/pulse_seq/files/gradhmbc), the pulse sequence for PFG-PS-HETLOC was obtained from the authors.[26]

4.2 QM Calculations: For the QM calculations, both the full geometry optimization and the calculation of *J*-coupling values were performed on a Pentium-4 at 2.8 GHz processor (running Microsoft Windows 2000) using the Gaussian03W (version 6.0) software package.[29] The *gauche* or *anti* staggered conformers of the five simplified fragments (Figure 2) were optimised at mPW1PW91 level of theory using the 6-31G(d) basis set; the single-point calculation of *J*-coupling was executed on the optimized geometries using the same mPW1PW91 functional and the 6-31G(d,p) basis set. For both the calculations the IEF-PCM solvent continuum model, as implemented in Gaussian, (methanol solvent) was used.[20]

4.3 Determination of the Absolute Configuration

(a) Peptide Hydrolysis: Peptide samples (200 μg) were dissolved in degassed 6 N HCl (0.5 mL) in an evacuated glass tube and heated at 160 °C for 16 h. The solvent was removed *in vacuo* and the resulting material was subjected to further derivatization.

(b) LC-MS Analysis of Marfey's (FDAA) Derivatives: A portion of the hydrolysate mixture (800 μg) or the amino acid standard (500 μg) was dissolved in 80 μL of a 2:3 solution of TEA/MeCN and treated with 75 μL of 1% *N*-(1-fluoro-2,4-dinitrophenyl)-5-L-alaninamide (FDAA) in MeCN/acetone, 1:2. The vials were heated at

70 °C for 1 h, and the contents were neutralized with 0.2 N HCl (50 μL) after cooling to room temperature. An aliquot of the L-FDAA derivative was dried under vacuum, diluted with MeCN/5% HCOOH in H_2O (1:1), and separated on a Vydac 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% trifluoracetic acid, over 45 min at 1 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 analyzed using the suite of programs Xcalibur (ThermoQuest, San José, California); all masses were reported as average values. The capillary temperature was set to 280 °C, capillary voltage at 37 V, tube lens offset at 50 V and ion spray voltage at 5 V. Retention times of authentic FDAA-amino acids (min): L-Thr (12.5), D-Thr (17.6), L-*a*Thr (13.1), D-*a*Thr (14.1). The hydrolysate of callipeltin A contained: D-Arg (13.1), D-*a*Thr (14.1), (3*S*,4*R*)-3,4-diMe-L-Glu (17.8), L-*N*MeAla (18.7), D-Ala (20.0) L-Leu (28.9).

Supporting Information (see footnote on the first page of this article): Table S1 reports the two sets of calculated *J*-values of *erythro* and *threo* forms of $AGDHE_{2,3}$ obtained varying centre C-4, compared to the experimental data. Moreover, Table S1 contains the two sets of calculations performed on the *erythro* and *threo* forms of $AGDHE_{3,4}$, this time varying C-2.

Acknowledgments

We thank Dr. Kirk R. Gustafson (Molecular Targets Development Program, NCI-Frederick) for helpful discussion. The University of Salerno and Regione Campania are gratefully acknowledged for financial support to this project through the funds ex-60% and P.O.R. 2000–2006 Misura 3.13, respectively.

[1] A. Zampella, M. V. D'Auria, L. Gomez-Paloma, A. Casapullo, L. Minale, C. Debitus, Y. Henin, *J. Am. Chem. Soc.* **1996**, *118*, 6202–6209.
[2] M. V. D'Auria, A. Zampella, L. Gomez-Paloma, L. Minale, C. Debitus, C. Roussakis, V. Le Bert, *Tetrahedron* **1996**, *52*, 9589–9596.
[3] A. Zampella, A. Randazzo, N. Borbone, S. Luciani, L. Trevisi, C. Debitus, M. V. D'Auria, *Tetrahedron Lett.* **2002**, *43*, 6163–6166.
[4] L. Trevisi, S. Bova, G. Cargnelli, D. Danieli-Betto, M. Floreani, E. Germinario, M. V. D'Auria, S. Lucani, *Biochem. Biophys. Res. Commun.* **2000**, *279*, 219–222.
[5] P. W. Ford, K. R. Gustafson, T. C. McKee, N. Shigematsu, L. K. Maurizi, L. K. Pannell, D. E. Williams, E. D. de Silva, P. Lassota, T. M. Allen, R. Van Soest, R. J. Andersen, M. R. Boyd, *J. Am. Chem. Soc.* **1999**, *121*, 5899–5909.
[6] M. A. Rashid, K. R. Gustafson, L. K. Cartner, N. Shigematsu, L. K. Pannell, M. R. Boyd, *J. Nat. Prod.* **2001**, *64*, 117–121.
[7] N. Oku, K. R. Gustafson, L. K. Cartner, J. A. Wilson, N. Shigematsu, S. Hess, L. K. Pannell, M. R. Boyd, J. B. McMahon, *J. Nat. Prod.* **2004**, *67*, 1407–1411.
[8] B. Liang, P. J. Carroll, M. M. Joullie, *Org. Lett.* **2000**, *2*, 4157–4160.
[9] N. Okamoto, O. Hara, K. Makino, Y. Hamada, *Tetrahedron: Asymmetry* **2001**, *12*, 1353–1358.
[10] C. M. Acevedo, E. F. Kogut, M. A. Lipton, *Tetrahedron* **2001**, *57*, 6353–6359.
[11] S. Chandrasekhar, T. Ramachandar, B. V. Rao, *Tetrahedron: Asymmetry* **2001**, *12*, 2315–2321.
[12] V. Guerlavais, P. J. Carroll, M. M. Joullie, *Tetrahedron: Asymmetry* **2002**, *13*, 675–680.
[13] J. C. Thoen, A. I. Morales-Ramos, M. A. Lipton, *Org. Lett.* **2002**, *4*, 4455–4458.
[14] A. Zampella, M. Sorgente, M. V. D'Auria, *Tetrahedron: Asymmetry* **2002**, *13*, 681–685.
[15] A. Zampella, M. V. D'Auria, *Tetrahedron: Asymmetry* **2002**, *13*, 1237–1239.
[16] K. A. Ravi, R. B. Venkateswara, *Tetrahedron Lett.* **2003**, *44*, 5645–5647.
[17] J. A. Turk, G. S. Visbal, M. A. Lipton, *J. Org. Chem.* **2003**, *68*, 7841–7844.
[18] G. Bifulco, C. Bassarello, R. Riccio, L. Gomez-Paloma, *Org. Lett.* **2004**, *6*, 1025–1028.
[19] C. Adamo, V. Barone, *J. Chem. Phys.* **1998**, *108*, 664–675.
[20] M. T. Cancès, B. Mennucci, J. Tomasi, *J. Chem. Phys.* **1997**, *107*, 3032–3041.
[21] R. H. Contreras, J. E. Peralta, *Prog. Nucl. Magn. Reson. Spectrosc.* **2000**, *37*, 321–425.
[22] K. Fujii, Y. Ikai, H. Oka, M. Suzuki, K.-I. Harada, *Anal. Chem.* **1997**, *69*, 5146–5151.
[23] N. Matsumori, D. Kaneno, M. Murata, H. Nakamura, K. Tachibana, *J. Org. Chem.* **1999**, *64*, 866–876.
[24] J. Boyd, N. Soffe, B. John, D. Plant, R. Hurd, *J. Magn. Reson.* **1992**, *98*, 660–664.
[25] A. L. Davis, J. Keeler, E. D. Laue, D. Moskau, *J. Magn. Reson.* **1992**, *98*, 207–216.
[26] D. Uhrín, G. Batta, V. J. Hruby, P. N. Barlow, K. E. Kövér, *J. Magn. Reson.* **1998**, *130*, 155–161.
[27] A. Bax, D. G. Davis, *J. Magn. Reson.* **1985**, *63*, 207–213.
[28] J. Jeneer, B. H. Meier, P. Bachmann, R. R. Ernst, *J. Chem. Phys.* **1979**, *71*, 4546–4553.
[29] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery Jr, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, *Gaussian 03W*, revision 6.0, Gaussian, Inc., Pittsburgh, PA, **2003**.
[30] A. Zampella, R. D'Orsi, V. Sepe, A. Casapullo, M. C. Monti, M. V. D'Auria, *Org. Lett.* **2005**, *7*, 3585–3588.

Received: September 28, 2005
Published Online: November 30, 2005

