Published in final edited form as:

J Nat Prod. 2013 February 22; 76(2): 178–185. doi:10.1021/np3006088.

Computationally Assisted Assignment of Kahalalide Y Configuration Using an NMR-Constrained Conformational Search

Mohamed A. Albadry^{†,‡}, Khaled M. Elokely[§], Bin Wang[†], John J. Bowling[†], Mohamed F. Abdelwahab[‡], Mohamed H. Hossein[‡], Robert J. Doerksen[§], and Mark T. Hamann^{†,*}

[†]Departments of Pharmacognosy, Pharmacology, Chemistry, and Biochemistry and National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677

[‡]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

[§]Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, MS 38677

Abstract

Assignment of the absolute configuration of cyclic peptides frequently yields challenges, leaving one or more stereogenic centers unassigned due to small quantities of sample and the limited utility of Marfey's or other methods for assigning amino or hydroxy acids. Here, we report isolation of kahalalide Y (**1**) from *Bryopsis pennata* for the first time; in addition, the application of a combination of molecular modeling and NOE distance constraint calculations was utilized to determine the conformation of **1** and the absolute configuration of the final stereogenic center of **1**. Using the Schrödinger suite, the structure of **1** was sketched in Maestro and minimized using the OPLS2005 force field in MacroModel. A conformational search was performed separately for structures having *R* or *S* configuration at C-3 of the beta-hydroxy fatty acid subunit that completes the cyclic scaffold of **1**, after which multiple minimizations for all generated conformers were carried out. The lowest energy conformers of *R* and *S* stereoisomers were then subjected to B3LYP geometry optimizations including solvent effects. The *S* stereoisomer was shown to be in excellent agreement with the NOE-derived distance constraints and hydrogen bonding stability studies.

Natural products continue to play a significant role in the drug discovery and development process.¹ The continued study of new marine bioactive metabolites has led to the discovery of a variety of biomedical probes.² Depsipeptides comprise a group of bio-oligomers biosynthetically derived from hydroxy and amino acids linked by amide and ester bonds with a wide array of promising biological activities, including anticancer, antibacterial, antiviral, antifungal, anti-inflammatory, anti-clotting and anti-atherogenic properties.³ Among the depsipeptide class of natural products is a series called the kahalalides with varied biological activities. Certain members in the kahalalide family exhibit significant biological activities; kahalalide A showed activity against *Mycobacterium tuberculosis*; kahalalide E showed activity against *Herpes simplex* virus; kahalalide F showed selectivity against different solid tumor cell lines and is currently in phase II clinical trials for melanoma, lung and prostate cancer, in addition to antiviral activity against HSV II, antifungal activity against *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, immunosuppressive activity, and antileishmanial activity; IsoKF has enhanced

*To whom correspondence should be addressed. Phone 662-915-5730; Fax 662-915-6975; mthamann@olemiss.edu.

Supporting Information ¹H, ¹³C, and 2D NMR spectra of **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

efficacy against breast and prostate xenografts; kahalalide R1 showed activity against different tumor cell lines; 5-OHKF showed *in vitro* antimalarial activity.⁴ Despite these promising biological activities for the kahalalides, some kahalalides have only been isolated in minute amounts, limiting their complete biological evaluation. Chemical synthesis is an alternative approach to deliver sufficient quantities of these metabolites for future studies to explore the drug potential for this class of molecules, but before the synthesis is carried out, a complete and detailed structural characterization is necessary. Knowledge of the stereochemical properties, such as conformation and absolute configuration, is essential for planning further chemical modification and developing synthetic strategies.⁵ The green alga *Bryopsis pennata* and the sacoglossan mollusk *Elysia rufescens*, which feeds on it, have been extensively investigated for their bioactive metabolites, which include a series of kahalalides.^{6–15} Kahalalide Y **1** is a cyclic depsipeptide previously isolated from the sacoglossan mollusk *Elysia rufescens*.¹⁴ It possesses a cyclic depsipeptide framework that is similar to that of kahalalide K,⁹ but with an L-proline residue instead of the hydroxyproline found in kahalalide K. In total, **1** contains seven stereogenic centers. The stereogenic center at C-3 of 3-hydroxy-9-methyldecanoic acid was not assigned previously even though the six other stereogenic centers within the amino acid moieties were determined using Marfey's method.¹⁶

To determine the absolute configuration at this stereogenic center, X-ray crystallography could be helpful if a crystal could be obtained (which it could not for this work). There are limitations as well to a chemical approach such as using different strategies to cleave the ester bond with subsequent Mosher's method to elucidate the absolute configuration of secondary alcohols,^{17–19} as this method is limited by small amounts of compound. There are many examples of natural products previously reported with one or more stereogenic centers unassigned, such as plusbacin A3²⁰ (a depsipeptide antibiotic active against vancomycin-resistant bacteria), which was subsequently the subject of total synthesis to assign the absolute configuration,²¹ and mutremdamide A and koshikamides C–H (sulfated cyclic depsipeptides), which were challenges for establishment of absolute configurations by combining quantum mechanical calculations, advanced Marfey's method, and chiral-phase HPLC. Undoubtedly, the Nuclear Overhauser Effect (NOE) is one of the most powerful experiments in NMR spectroscopy and is widely employed in the determination of stereochemical and conformational detail. The analyses of NOE data can be utilized in a

qualitative or semi-quantitative manner to differentiate between conformers.²² Recent publications demonstrate that NOE measurements alone are accurate enough to establish inter-proton distances, and hence conformational detail.²³ Quantum mechanics methods are gaining increasing popularity in the structural study of medium to large-sized molecules, including natural products.²⁴ The NOE-derived distances can thus be used, along with computations of conformer geometries, to confirm the structures and energies/populations of contributing conformers when the number of NOE correlations is relatively large and J coupling constants are available, as is typically the case for cyclic peptides.²⁵ On the other hand, for the conformational search of flexible molecules, accurate NOE-distance analysis can be utilized as an alternative approach due to recent improvements in NMR hardware, NOE experimental methods, and data analysis that have made quantitative measurements of NOEs more reliable.²² In other cases where there are multiple quaternary centers, which strongly limit stereochemical analysis by NOE analysis, a QM/NMR approach can identify the correct configuration in an efficient and accurate fashion by comparing calculated values of NMR parameters, such as chemical shifts and scalar coupling constants, with experimental values to assign constitution and configuration.²² Molecular modeling coupled with NOE-derived distance constraint calculations is a highly valuable tool for assigning the relative and absolute configurations of many natural product compounds of different structural classes.^{26–28} We show here how such an approach can be used to assign the lone unknown configuration of a stereogenic center of the hydroxy-acid moiety of **1**.

RESULTS AND DISCUSSION

Compound **1** was isolated from *Bryopsis pennata* (7.5 kg, frozen sample) An ethanol extract of *B. pennata* was subjected to different chromatographic techniques starting with silica gel vacuum-liquid chromatography (VLC) followed by fractionation on an HP-20 column. Then purification steps used C₁₈ column chromatography and semi-preparative HPLC (Phenomenex C₁₈ column 10.0×250 mm) at a flow rate of 2.5 mL/min to yield **1**. Compound **1** was obtained as an amorphous powder, $[\alpha]^{25}_{\text{D}} +38$ (c 0.5, MeOH) which is in good agreement with **1** previously isolated from *Elysia*,¹⁴ and its molecular formula was determined to be C₄₆H₆₆N₇O₁₀ on the basis of the positive ion HRESIMS data (m/z 876.4876; calcd for C₄₆H₆₆N₇O₁₀, 876.4871). The structure was determined using both one- and two-dimensional NMR spectral measurements including HSQC, TOCSY, and NOESY experiments compared with the reported data. Furthermore the *cis/trans* conformation of proline was determined to be *trans* based on $\Delta\delta_{\beta\gamma} < 9$ (difference between ¹³C chemical shift carbon β and carbon γ) (Table 1), which is in good agreement with chemical shifts for *trans* proline,²⁹ and also based on the strong NOE correlation between the δ proton of proline and the α proton of phenylalanine (3.58, 4.83) that should be observed in *trans* proline rather than between the α proton of proline and the α proton of phenylalanine expected for *cis* proline (Figure 1). Numerous cross-peaks are observed in the NOE spectra (Figure 1, Table 1).

The integration of the NOESY spectrum was performed on both sides of the diagonal, and then the average of the numerical values for each correlation of interest was calculated to provide more reliable values. The NOE relative integration of tyrosine protons $\delta\text{H-eH}$ of 20,000 and a distance of 2.5 Å were used for the reference values and all of the other NOESY signal intensities were related to the standard. NOESY signal intensities were divided into two types (strong 1.7–3.0 Å and weak 3.0–4.0 Å) according to their relative integrations.

In the conformational search with distance constraints (Table 2), the C-3 configuration and 10 distance constraints (with allowed flexibility) were maintained throughout the conformational sampling. A total of 428 and 386 conformers were generated for the *R* and *S*

stereoisomers, respectively. We were particularly interested in the distance between the proton on the asymmetric center of the 3-hydroxy-9-methyldecanoic acid (3H) and the closest CH₃ proton of the valine, which we call r_{fV} . We measured the 3H distance to all methyl protons of valine and considered the smallest values for *R* and *S* stereoisomers because all three protons of the valine terminal methyls are equivalent and overlapped in the proton NMR spectrum. Based on Boltzmann potential energy-weighted populations, over 90% of the conformers of the *R* stereoisomer were within 8.1 kJ/mol of the lowest energy structure and had $9.3 < r_{fV} < 10.3$ Å (SI: S10, Figure 2 left). For the *S* stereoisomer, over 80% of the conformers fell within 7.6 kJ/mol of the minimum, which itself was 45.1 kJ/mol lower than the *R* minimum energy conformation. The r_{fV} distance range for these conformers was $4.6 < r_{fV} < 6.2$ Å (SI: S11, Figure 2, right). The r_{fV} distance for the lowest energy conformer of the *R* stereoisomer (Figure 3, left) was 10.2 Å and after B3LYP optimization became 8.8 Å (Figure 3, right). The r_{fV} distance for the lowest energy conformer of the *S* stereoisomer (Figure 4, left) was 2.7 Å and after B3LYP optimization became 3.4 Å (Figure 4, right).

For B3LYP geometry optimization, no constraints were imposed and all NOE-derived distances were compared with the predicted distances between protons for the lowest energy conformers, which were optimized for both *R* and *S* stereoisomers (Table 2). We used the 6-31G(d,p) basis set, which is standard for accurate geometry calculations and includes polarization functions for all atom types including hydrogen.³⁰ Note that the NOE-derived distances indicated with asterisks in Table 2 were used as distance constraints in the conformational search (but not in the B3LYP minimizations) with variation allowed of ± 1.5 Å to allow some range of flexibility during the conformational search. Most of the simulated distances in the *S* conformer match well with the NOE-derived distances. The *R* conformer distances matched better to the experimental values only for three of the distances, and two of those distances differed by only 0.04 Å for *R* and *S*, (NH Tyr to α H Asn and NH Phe to α H Phe, dropped from table due to insignificant difference), so the only significant distance is for NH of alanine to α H of proline.

To allow more exploration of the conformational flexibility of **1**, we made use of molecular dynamics (MD) for 50 ns, 300 K simulations in explicit water, one for the *S* stereoisomer and one for the *R* stereoisomer lowest-energy conformations. The monitored r_{fV} cross-ring intramolecular hydrogen bonded distance averaged 6.4 Å for the *S* stereoisomer, with a minimum of 2.5 Å and a maximum of 9.8 Å, while for the *R* stereoisomer the average r_{fV} was 9.5 Å with minimum of 7.5 Å and a maximum of 13.5 Å. After Gaussian 09 optimization of the *S* stereoisomer final MD structure, r_{fV} relaxed to 3.4 Å while for *R* stereoisomer the optimized r_{fV} stayed at 8.8 Å, as found before the MD simulation. Other distances were close to those calculated before the MD simulation.

From our results, **1** is believed to have the *S*-configuration at the 3-hydroxy-9-methyldecanoic acid connection point. The NOE-derived distance r_{fV} is ~ 3.0 Å, which is closer to the average calculated value of 3.4 Å found in the case of the *S* stereoisomer than to the 8.8 Å calculated for the *R* stereoisomer.

The *S* stereoisomer also had a considerably lower energy than the *R* stereoisomer and is stabilized by the formation of an intracyclic hydrogen bond between the alanine NH and the carbonyl of 3-hydroxy-9-methyldecanoic acid which was not found in the *R* stereoisomer conformer (Figure 5). Over 70% of the *S* stereoisomer conformers contained this hydrogen bond within a distance of 2–4.1 Å. This extra hydrogen bond helps to stabilize the macrocyclic structure. None of the 52 lowest energy conformers of the *R* stereoisomer had any hydrogen bonds except the third one, which had an internal hydrogen bond outside the cycle also found in the *S* stereoisomer conformations. The 53rd *R* conformer had an

intracyclic hydrogen bond but this conformation accounted for only 0.23% of the Boltzmann average of all conformers.

Possible hydrogen bonds were monitored during the course of the 50 ns MD simulation for both *R* and *S* stereoisomers. Within the acceptable ranges of maximum hydrogen bond distance and donor and acceptor angles, as described in the methodology section, we found that Ala NH acid and another hydrogen bond with the carbonyl of Phe with probabilities of 17% and 18%, respectively. The NH...O=C average distances during the dynamic simulation were 3.2 Å (with a standard deviation of 0.8 Å) and 5.6 Å (standard deviation of 1.6 Å) for Ala...Phe and Ala...3-hydroxy-9-methyldecanoic acid, respectively. For the *R* stereoisomer, the Ala...Phe probability is only 5% with an average distance of 3.8 Å (standard deviation of 0.8 Å) and Ala did not form a hydrogen bond with 3-hydroxy-9-methyldecanoic acid during the 50 ns simulation. We monitored other possible intracyclic hydrogen bonds and did not find any significant evidence for them in either the *R* or *S* stereoisomer.

To verify the presence of this hydrogen bond formation under experimental conditions, a series of ¹H NMR experiments was conducted at different temperatures (Figure 6) to study the temperature effect on amide proton chemical shifts, and the temperature coefficients of these protons were calculated (Table 3).

It has been reported that the chemical shifts of amide protons of peptides display temperature dependence. In general, they shift upfield as the temperature increases and this is conventionally described as a negative temperature coefficient.³¹ Because there is a correlation between amide proton temperature coefficients ($\Delta\delta_{\text{HN}}/\Delta T$) and hydrogen bonds^{32, 33} for amide protons showing temperature coefficients > -4.6 ppb/K, there is a hydrogen bond possibility exceeding 85% and the possibility increases to over 93% for amides within the range between -4 and -1 ppb/K. Detailed analysis for these correlations shows an inverse proportionality between amide proton temperature coefficients and hydrogen bond lengths.³² Based on the temperature coefficients determined for the amide protons (Table 3), the amide proton of alanine has the highest value, indicating that it should be involved in the shortest hydrogen bond and this piece of information supports the *S* configuration at C3 of the β -hydroxy acid. If a biosynthetic approach is developed for the production of this molecule or analogs of this molecule, knowing the correct configuration of the β -hydroxy acid could facilitate precursor-directed feeding experiments which might improve production of these compounds.

Experimental Section

General Experimental Procedures

The Optical rotation was measured with a JASCO DIP 370 digital Polarimeter. The ¹H and ¹³C NMR spectra were recorded in DMSO-*d*₆ (δ_{H} 2.50/ δ_{C} 39.52) using a Varian NMR spectrometer operating at 600 MHz for ¹H and 150 MHz for ¹³C nuclei. The NOESY spectrum was recorded at 25 °C using NOE pulse sequence with a mixing time 500 ms and the experiment was performed for 24 h with the concentration of the sample at 6 mg/mL. The HRESIMS data were obtained using an Agilent MS TOF 1100 series with electrospray ionization. HPLC was carried out using a Waters 486 Series with a Phenomenex C₁₈ column (10.0×250 mm) and an 1100 Series Multiple Wavelength detector.

Biological Material

The *Bryopsis* sample was collected from the waters and beaches of Kahala Bay near Black Point, Oahu, during the months of March, April and May of 2004. *Bryopsis pennata* forms

soft, feathery clumps attached to basalt rocks and rubble on shallow reef flats, in tide pools, and in lower intertidal habitats of coastlines with low wave action.

Extraction and Isolation

The alga was extracted with EtOH. The EtOH extract was fractionated by vacuum-liquid chromatography (VLC) on a silica column using a gradient system with hexane-EtOAc (100:0, 50:50, 0:100) and EtOAc-MeOH (50:50, 0:100). EtOAc - MeOH (50:50), and MeOH fractions were further fractionated by HP-20 column chromatography using a gradient system with H₂O-MeOH (100:0, 0:100). The LC/MS data of each fraction showed that the MeOH-H₂O (80:20) and 100% MeOH fractions mainly contained peptides. These fractions were combined and purified using C₁₈ gravity column chromatography using a gradient system with H₂O-MeOH (50:50, 0:100) and finally the 90% MeOH fraction was purified by semi-preparative HPLC (Phenomenex C₁₈ column 10.0×250 mm) using a gradient system with H₂O-CH₃CN(60:40, 0:100) at a flow rate of 2.5 mL/min to yield **1** (1.5 mg).

Conformational Analysis

The Macromodel module of the Schrödinger suite³⁴ was used for conformational analysis of **1**. The relative configurations of all known asymmetric centers were assigned in accordance with previously reported results¹⁴ and kept constant throughout the study. The structure was minimized using the OPLS2005 force field³⁵ and charges. The optimized potentials for liquid simulations (OPLS) force field provides accurate energy minimization potential functions for proteins and cyclic peptides.³⁶ OPLS2005 (an all-atom model) was developed to broaden and improve the quality of the force field parameters to cover most organic functional groups. Because the OPLS force field was developed to be suitable for protein and cyclic peptides,³⁶ to provide additional coverage of organic functional groups, and to have good performance in conformational analysis and molecular dynamics simulations, OPLS2005 was the most appropriate force field for our study.^{37–39}

The OPLS2005 conformational search was performed with distance constraints. The effective NOE-derived distance has been reported to be larger or smaller than the average distance even by 2 Å.⁴⁰ Ten distance constraints were added using the experimental NOE-derived distances ±1.5 Å to allow some flexibility for the conformational sampling step (Table 2) and to account for NOE-derived distance flexibility. The final conformers were analyzed to see if inter-proton distances matched those expected from the NOESY distances. NOE-derived distances were calculated using NOESY relative integration. The NOE relative integration is approximately related to the distance *r* between two interacting spins by

$$\left(\frac{NOE_{unknown}}{NOE_{reference}} \right) = \frac{r_{reference}^6}{r_{unknown}^6} \quad \text{Equation (1)}$$

$$r_{unknown} = \sqrt[6]{\frac{NOE_{reference} \times r_{reference}^6}{NOE_{unknown}}} \quad \text{Equation (2)}$$

We have carried out the conformational searches using mixed torsional/low-mode sampling. The number of separate conformers generated was 1000, with a maximum of 100 unique structures to be saved for each rotatable bond. A 42 kJ/mol energy cutoff was used to remove the higher energy conformers. A conformer was considered redundant and subsequently eliminated if its maximum atom deviation from an already-identified

conformer was less than 0.5 Å. All conformers were subjected to further minimization using the Powell-Reeves conjugate gradient (PRCG) method for a maximum of 500 steps and, because some conformers failed to be minimized that easily, we used a second minimization step and increased the number of iterations to 5000 to ensure that all conformers were minimized.

Geometry Optimization of the Selected Conformers Using Jaguar.⁴¹

The lowest energy conformers of the *R* and *S* structures were then used as the input structures for further geometry optimization. The Jaguar module of Schrödinger was used to perform B3LYP hybrid density functional theory (DFT) geometry optimization calculations⁴² using the 6-31G (d,p) basis set and the Poisson–Boltzmann Finite element (PBF) solvent model, with MeOH as the solvent.

Macromodel Molecular Dynamics Simulation

We used the Macromodel molecular dynamics (MD) tool to test the behavior of our molecule with respect to time at room temperature. The fully unconstrained structure was subjected to MD simulation at 300 K. To ensure full minimization before running the dynamics simulation, the system was minimized with the PRCG method for a maximum of 500 steps and a convergence threshold of 0.005. The OPLS2005 force field and charges with extended cutoffs were used during the whole simulation process. MD using the standard constant temperature velocity-Verlet algorithm⁴³ was considered. We used the SHAKE method⁴⁴ for bonds to hydrogens, a simulation temperature of 300 K, a timestep of 1.5 fs, an equilibration time of 0.5 ns and a total simulation time of 50 ns. A sampling of 600 structures was specified. The distance r_{fV} and hydrogen bonds of (Ala)N-H...O=C(3-hydroxy-9-methyldecanoic acid), (Ala)N-H...O=C(Phe), (Phe)N-H...O=C(Val), and (Asn)N-H...O=C(Tyr) were monitored during the course of simulation. For hydrogen bonds, a maximum D(donor)-H...A(acceptor) distance was set to 4.0 Å, the minimum D-H...A angle was set to 120.0° and the minimum H...A-D angle was set to 90.0°.

Gaussian 09 Optimization

We used Gaussian 09 to optimize one of the sampled geometries. The DFT/B3LYP method with the basis set 6-31G(d,p) was used with the polarizable continuum model (PCM) DMSO solvent model. During the optimization process no constraints were used.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank A. Waters, J. Oh and Y. Zou for their valuable recommendations and suggestions, and F. T. Wiggers, National Center for Natural Products Research, for spectral data. A scholarship granted and financed by the Egyptian Government (Ministry of Higher Education) to M. A. Albadry is gratefully acknowledged. This investigation was conducted in part in a facility constructed with support from research facilities improvement program C06 RR-14503-01 from the NIH NCRR.

References

1. Newman DJ, Cragg GM. J Nat Prod. 2012; 75:311–335. [PubMed: 22316239]
2. Molinski TF, Dalisay DS, Lievens SL, Saludes JP. Nat Rev Drug Discovery. 2009; 8:69–85.
3. Ballard CE, Yu H, Wang B. Curr Med Chem. 2002; 9:471–498. [PubMed: 11945121]
4. Gao J, Hamann MT. Chem Rev. 2011; 111:3208–3235. [PubMed: 21480581]

5. Hopmann KH, Sebestik J, Novotna J, Stensen W, Urbanova M, Svenson J, Svendsen JS, Bour P, Ruud K. *J Org Chem.* 2012; 77:858–869. [PubMed: 22148737]
6. Hamann MT, Scheuer PJ. *J Am Chem Soc.* 1993; 115:5825–5826.
7. Hamann MT, Otto CS, Scheuer PJ, Dunbar DC. *J Org Chem.* 1996; 61:6594–6600. [PubMed: 11667527]
8. Goetz G, Nakao Y, Scheuer PJ. *J Nat Prod.* 1997; 60:562–567.
9. Kan Y, Fujita T, Sakamoto B, Hokama Y, Nagai H. *J Nat Prod.* 1999; 62:1169–1172. [PubMed: 10479330]
10. Horgen FD, delos Santos DB, Goetz G, Sakamoto B, Kan Y, Nagai H, Scheuer PJ. *J Nat Prod.* 2000; 63:152–154. [PubMed: 10650101]
11. Dmitrenok A, Iwashita T, Nakajima T, Sakamoto B, Namikoshi M, Nagai H. *Tetrahedron.* 2006; 62:1301–1308.
12. Ashour M, Edrada R, Ebel R, Wray V, Wätjen W, Padmakumar K, Muller WEG, Lin WH, Proksch P. *J Nat Prod.* 2006; 69:1547–1553. [PubMed: 17125219]
13. Tilvi S, Naik CG. *J Mass Spectrom.* 2007; 42:70–80. [PubMed: 17149796]
14. Rao KV, Na M, Cook JC, Peng J, Matsumoto R, Hamann MT. *J Nat Prod.* 2008; 71:772–778. [PubMed: 18407693]
15. Gao J, Caballero-George C, Wang B, Rao KV, Shilabin AG, Hamann MT. *J Nat Prod.* 2009; 72:2172–2176. [PubMed: 19916528]
16. Marfey P. *Carlsberg Res Commun.* 1984; 49:591–596.
17. Dale JA, Mosher HS. *J Am Chem Soc.* 1973; 95:512–519.
18. Sullivan GR, Dale JA, Mosher HS. *J Org Chem.* 1973; 38:2143–2147.
19. Seco JM, Quinoa E, Riguera R. *Chem Rev.* 2004; 104:17–117.
20. Shoji J, Hinoo H, Katayama T, Nakagawa Y, Ikenishi Y, Iwatani K, Yoshida T. *J Antibiot.* 1992; 45:824–831. [PubMed: 1500346]
21. Wohlrab A, Lamer R, VanNieuwenhze MS. *J Am Chem Soc.* 2007; 129:4175–4177. [PubMed: 17371023]
22. Chini MG, Jones CR, Zampella A, D'Auria MV, Renga B, Fiorucci S, Butts CP, Bifulco G. *J Org Chem.* 2012; 77:1489–1496. [PubMed: 22201476]
23. Jones CR, Butts CP, Harvey JN. *Beilstein J Org Chem.* 2011; 7:145–150. [PubMed: 21448257]
24. Bifulco G, Dambruoso P, Gomez-Paloma L, Riccio R. *Chem Rev.* 2007; 107:3744–3779. [PubMed: 17649982]
25. Baysal C, Meirovitch H. *J Am Chem Soc.* 1998; 120:800–812.
26. Sharman GJ, Jones IC. *Magn Reson Chem.* 2001; 39:549–554.
27. Costantine KL, Mueller L, Huang S, Abid S, Lam KS, Li W, Leet JE. *J Am Chem Soc.* 2002; 124:7284–7285. [PubMed: 12071733]
28. Donia MS, Wang B, Dunbar DC, Desai PV, Patny A, Avery M, Hamann MT. *J Nat Prod.* 2008; 71:941–945. [PubMed: 18543965]
29. Mohammed R, Peng J, Kelly M, Hamann MT. *J Nat Prod.* 2006; 69:1739–1744. [PubMed: 17190452]
30. Rassolov V, Pople JA, Ratner M, Redfern PC, Curtiss LA. *J Comp Chem.* 2001; 22:976–984.
31. Baxter NJ, Williamson MP. *J Biomol NMR.* 1997; 9:359–369. [PubMed: 9255942]
32. Cierpicki T, Otlewski J. *J Biomol NMR.* 2001; 21:249–261. [PubMed: 11775741]
33. Contreras MA, Haack T, Royo M, Giralt EMP. *Lett Pept Sci.* 1997; 4:29–39.
34. MacroModel, version 9.9. Schrödinger, LLC; New York, NY: 2011.
35. Banks JL, Beard HS, Cao Y, Cho AE, Damm W, Farid R, Felts AK, Halgren TA, Mainz DT, Maple JR, Murphy R, Philipp DM, Repasky MP, Zhang LY, Berne BJ, Friesner RA, Gallicchio E, Levy RM. *J Comp Chem.* 2005; 26:1752–1780. [PubMed: 16211539]
36. Jorgensen WL, Tirado-Rives J. *J Am Chem Soc.* 1988; 110:1657–1666.
37. DuBay KH, Hall ML, Hughes TF, Wu C, Reichman DR, Friesner RA. *J Chem Theory Comput.* 2012; 8:4556–4569.

38. Daniele T, Riccardo Z, Andrea G, Carlo B. Chirality. 2012; 24:741–750. [PubMed: 22544665]
39. Stortz CA, Johnson GP, French AD, Csonka GI. Carbohydr Res. 2009; 344:2217–2228.
40. Schneider TR, Brünger AT, Nilges M. J Mol Biol. 1998; 285:727–740. [PubMed: 9878440]
41. Jaguar, version 7.9. Schrödinger, LLC; New York, NY: 2012.
42. Mourik V, Gdanitz T, Robert J. J Chem Phys. 2002; 116:9620–9623.
43. Swope WC, Andersen HC, Berens PH, Wilson KR. J Chem Phys. 1982; 76:637.
44. Andersen HC. J Comput Phys. 1983; 52:24–34.

Figure 1.
Integrated NOESY spectrum of kahalalide Y 1.

Figure 2.
Boltzmann populations of lowest energy conformers showing potential energy on the X axis

Figure 3. Lowest energy conformer of the *R* stereoisomer (left) before and (right) after optimization; all nonpolar hydrogen atoms are removed except those involved in distance measurements.

Figure 4.

Lowest energy conformer of the *S* stereoisomer (left) before and (right) after optimization; all nonpolar hydrogen atoms are removed except those involved in distance measurements.

Figure 5.
S stereoisomer conformer showing hydrogen bonds (dashed black lines).

Figure 6. Amide protons region of ¹H NMR of **1** at different temperatures. The amide protons are labeled using a one letter abbreviation for their corresponding amino acid.

Table 1¹H and ¹³C NMR Data for Kahalalide Y **1** in DMSO-*d*₆.

Amino acid	No	δC	δH (J in Hz)	NOESY
Phe	1	171.52	NH 7.94, d (9.1)	9-Me-3-Decol-2, Phe-2,3
	2	52.57	4.83, m	Pro-5, Phe-3
	3	38.12	2.95, m	Phe-2
	4	138.11	-----	
	5,5'	130.33	7.21, d (7.2)	
	6,6'	128.5	7.32, t (7.2)	
	7,7'	126.99	7.17, t (7.2)	
Pro	1	172.5		
	2	60.70	4.22, dd (7.2, 4.7)	Ala-NH, Ala-3, Pro-4
	3	29.22	1.82, m; 1.81, m	Pro-5
	4	25.05	1.81 1.69	
	5	47.99	3.58, m 3.36, m	Pro-2
Ala	1	172.20	NH 8.10, d (7.2)	Pro-2 Pro-3
	2	49.34	4.28, m	
	3	18.63	1.25, d (7.1)	
Val	1	171.17	NH 7.89, d (8.8)	Ala-2
	2	60.22	4.05, dd (8.9, 8.9)	Asn-NH
	3	31.5	1.72, m	Val-4
	4	19.24	0.77, d (6.6)	
	5	19.02	0.73, d (6.6)	
Asn	1	171.45	NH 8.06, d (8.9)	Val-2, Val-4
	2	49.86	4.76, d (5.4)	
	3	38.65	2.47, m 2.32, dd (5.7, 8.2)	Asn-2
	4	171.54	NH ₂ 9.2, s	
Tyr	1	171.90	NH 8.61, s	Asn-2, Tyr-2
	2	55.94	4.33, m	Tyr-3
	3	36.11	2.79, m	
	4	127.48	-----	
	5,5'	130.44	6.95, d (7.9)	Tyr-6,6'
	6,6'	115.6	6.63, d (7.9)	Tyr-5,5'
	7	156.58	-----	
9-Me-3-Decol	1	168.55		Phe-NH, 9-Me-3-Decol-3,4,5
	2	40.57	2.24, m	9-Me-3-Decol-4,5,6
	3	71.89	4.74, m	9-Me-3-Decol-2,3,5
	4	32.48	1.07, m; 0.91 m	
	5	25.43	0.78, m	
	6	39.32	1.07, m	

Amino acid	No	δC	δH (J in Hz)	NOESY
	7	29.64	0.98, m	9-Me-3-Decol-9
	8	39.32	1.07, m	
	9	28.08	1.48, m	
	10,11	23.33	0.85, d (6.5)	9-Me-3-Decol-8,9

Table 2

Experimental NOESY correlations of **1** compared with the calculated B3LYP distance between protons found in the lowest energy conformers (best matches between experimental and calculated distance are shown in bold type).

NOESY correlations	Relative integration	NOE Distance (Å)	Simulated distance (Å) <i>S</i> conformer	Simulated distance (Å) <i>R</i> conformer
NH Tyrosine to αH Tyrosine *	2236.2	3.2	2.9	2.3
NH Asparagine to αH Asparagine *	2511.9	3.1	2.9	2.3
NH Asparagine to αH Valine *	8543.2	2.5	2.4	3.0
NH Asparagine to CH ₃ Valine	1134.8	3.5	3.8	4.2
NH Alanine to αH Proline *	13776	2.3	3.5	2.4
NH Alanine to CH ₃ Alanine	3556.8	3.0	3.3	2.8
NH Phenylalanine to 3H Fatty Acid *	700.7	3.9	4.1	4.7
NH Valine to αH Alanine *	9133.1	2.5	2.7	2.1
NH Valine to αH Valine	2726.6	3.1	2.9	3.0
NH Valine to CH ₃ Valine *	2646.6	3.1	2.7	3.4
3H Fatty Acid to 4H	3920.7	2.9	2.6	2.4
3H Fatty Acid to CH ₃ Valine	3920.6	2.9	3.4	8.8

* Distance constraint used in conformational search

Table 3Temperature Coefficients of Amide Protons in **1**.

Amide proton	Chemical shift (ppm) at 313.15 K	Chemical shift (ppm) at 298.15 K	$\Delta\delta$ NH (ppb)	Temperature coefficient ppb/K
Tyrosine	8.55	8.61	−60	−4.0
Alanine	8.08	8.10	−20	−1.3
Asparagine	8.00	8.06	−60	−4.0
Phenylalanine	7.87	7.95	−80	−5.3
Valine	7.83	7.90	−70	−4.6