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Strength by joining methods: Combining synthesis, NMR, IR and VCD for the determination of the relative configuration in Hemicalide

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Abstract: The relative configuration of a key subunit of hemicalide, a recently isolated highly bioactive marine natural product possessing potent anti-proliferative activity against a panel of human cancer cell lines, has been assigned by combining stereocontrolled synthesis of model substrates with Nuclear Magnetic Resonance (NMR),

Infrared (IR) and Vibrational Circular Dichroism (VCD) spectroscopy. The assignment of the absolute configuration of the asymmetric carbon C42 in two structurally complex epimeric substructures containing six stereocenters by VCD analysis illustrates the power and reliability of combining methods.

Introduction

Many, if not most of nature's most critical molecules in living organisms contain some element of chirality, be it through the chirality of nearly all amino acids, the chirality of a helix or combinations of different kinds of chirality. Chirality is also key to many of the chemical properties of a compound. This may range from somewhat innocent differences like difference in the smell of limonene (ranging from lemon to orange depending on the absolute configuration (AC)) to quite dramatic changes in properties as is the case in drugs. As a consequence, there is a large interest in methods to determine the AC of not only drug molecules but natural products in general. This is variably done using single crystal x-ray diffraction, Vibrational Circular Dichroism (VCD), Raman Optical Activity (ROA), Electronic Circular Dichroism (ECD), specialized NMR methods or retrosynthesis. Many works use only one method although a combination of methods may prove more powerful. The added value of combining methods is clearly proven in the present case where the AC of a subunit of a 21 chiral center natural product is determined, which in turn leads to knowledge on the relative configuration in the large molecule.

The subject of the current work is a complex bioactive polyketide of marine origin, called hemicalide. Natural products of

marine origin are of great interest for their pharmacological potential.^[1] Unfortunately, the difficult isolation by extraction and thus low availability of these natural compounds from raw material may render complete structural elucidation including the complete stereochemistry very difficult. Recently, researchers of the CNRS–Pierre Fabre Laboratories, in collaboration with the Institut de Recherche pour le Développement (IRD), isolated for the first time, a complex bioactive polyketide from the marine sponge *Hemimyscale* sp. collected in deep water around the Torres Islands (Vanuatu).^[2] This new natural product, called hemicalide, is a highly potent mitotic blocker that, at subnanomolar concentrations and via a unique mechanism, exhibits antiproliferative activity against several human cancer cell lines.^[2]

Extensive NMR studies enabled the determination of the structure of hemicalide, featuring a 46 carbon atom backbone containing 21 stereocenters (Figure 1).^[2] Unfortunately, due to the extremely limited supply of hemicalide (less than 1 mg), degradation and derivatization experiments could not be carried out and hence the configuration of the stereocenters could not be assigned. However, the promising biological activity of hemicalide stimulated further studies concerning the assignment of the relative configuration of the stereocenters in the key structural subunits; this as part of an ambitious program devoted to the total synthesis of this natural product and analogues thereof.

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Figure 1. Structure of hemicalide and relative configuration of the C8–C13, C18–C24 and C37–C41 subunits. NOE=Nuclear Overhauser Effect

By careful comparison of the NMR data of hemicalide with those of appropriate diastereomeric model compounds, selected after careful NMR and/or computational conformational analyses, the *relative* configuration of the stereocenters in the C8–C13 segment^[3] and in the C18–C24 α,β -dihydroxy- δ -lactone subunit^[4]

could be assigned. In the present work, we concentrate on the C36–C46 unit. The aim of the present study is to contribute to the assignment of the relative configuration of the C36–C46 subunit of hemicalide in general and, more specifically, to the assignment of the relative configuration of the asymmetric carbon C42. This is done using the VCD spectra of appropriate synthesized and NMR characterized model compounds of the C36–C46 subunit of hemicalide. The need for VCD as final solver is due to the fact that, as described below, other techniques, including NMR, IR and synthesis, did not conclusively establish the absolute configuration of this center.

VCD relies on the different behavior of chiral molecules towards left and right circularly polarized light^[5], and has over the last decades become one of the prime methods for the determination of absolute configuration.^[6] Its increasing appreciation is fueled by the advent of commercially available VCD spectrometers^[7] and the availability of VCD algorithms in several quantum chemistry software packages^[8]. Experiment and theory based simulation play an equally important role as experimental spectra can only be interpreted using knowledge from such simulations.^[5a,6a,9] The number and complexity of structures analyzed more or less routinely also increases, making VCD a method of choice for AC determination of complex natural products.^[10] This is possible due to the information richness of the VCD spectra that are vibrational in nature. Still, there are limiting factors in the applicability of VCD spectroscopy for AC determination. First, there is the number of chiral centers.^[11] Although a mirror relationship exists between spectra of enantiomers, there is no such relation between spectra of diastereoisomers. Therefore, one needs experimental and calculated spectra of the highest quality to perform a band-by-band analysis, allowing for unambiguous AC determination. Over the last years, several uses of the method in the AC determination of diastereomeric compounds have been reported showing it to be a promising method also in such cases.^[12] It is furthermore clear that the higher the number of chiral centers is, the more difficult it becomes to distinguish between epimers, since the two spectra may become less and less ‘mirror images’. This is reflected also in the fact that considerably fewer VCD assignments have been made on molecules with three or more chiral centers.^[10d, 10e, 10g, 10j, 13] Another limiting factor is molecular size and especially the degree of flexibility of the molecule. Every conformer of a molecule of a given AC has a (possibly very) different VCD spectrum. Therefore, it is common practice to calculate VCD spectra for every conformer, and then take a Boltzmann average over all conformer spectra. Finding all abundant conformers obviously becomes harder as the molecule has more flexibility. Moreover, since all conformer spectra may be inherently different, a large number of conformers may lead to averaging out of important bands in the spectrum.

In view of the above, it is important to stress that for the C36–C46 subunit of hemicalide, the six chiral centers can give rise to $2^{(6-1)} = 32$ different relative configurations. Before discussing the results, we therefore first describe the general strategy used for the structure elucidation of hemicalide. The key element in the strategy, described in detail in references 3 and 4, is the consideration of hemicalide as a sequence of subunits for which the NMR data can be mimicked and, eventually, fully reproduced by synthesizing and characterizing a series of related model compounds that are structurally similar to the natural product and whose NMR data and synthesis allow to establish the configuration. So, effectively, synthesis and NMR are used to create a selection of known model compounds through which, by

comparison of the NMR data, one can assign the configuration of the actual subunit as being that with the highest similarity in the NMR data with respect to hemicalide.

The choice of the model compounds to be studied is based on 2D NMR (NOESY) data obtained for the natural product, showing a *syn* relative orientation between the hydrogen atom at C37 with the side-chain at C39 and with the hydrogen atom at C40 in the ring of the C37–C41 α -hydroxy δ -lactone subunit. Taking into account these NMR data, and the fact that only the relative configuration of C36, C42 and C45 remains to be established, the set of model compounds can be largely reduced, leaving only 8 of the 32 possible relative configurations (Figure 1).

During the synthesis of the model compounds, it was found that epimers at C45 show quasi-identical NMR spectra, thereby making it impossible to establish the relative configuration at C45. It was therefore decided to focus on the structure elucidation of the C36–C42 subunit in the current study, using an arbitrarily chosen configuration at C45. This further reduced the subset to 4 model compounds. The diastereomeric δ -lactones **1a–1d**, closely related to the C36–C46 subunit of hemicalide, in which the three endocyclic stereocenters (C37, C39 and C40) possess the requisite relative configuration are shown in Figure 2. **1b** and **1c** are epimers of the δ -lactone **1a**, at C42 or C36, respectively. Compound **1d** is a diastereoisomer of **1a** at C36 and C42.

Figure 2. Model compounds **1a–1d** for the C36–C46 subunit of hemicalide

In our synthetic approach toward the epimeric model subunits **1a** and **1b**, δ -lactone **2** containing five of the six stereocenters (C36, C37, C39, C40 and C45) was prepared as a key intermediate (Scheme 1). Whereas the C45 and the C36 stereocenters in δ -lactone **2** both originated from the chiral pool, the absolute configurations of C37, C39 and C40 were controlled by diastereoselective reactions and unambiguously confirmed by NMR (NOESY) spectroscopy. To create the sixth stereocenter at C42, the hydrogenation of the trisubstituted alkene at C42–C43 in δ -lactone **2** was carried out in the presence of Crabtree's catalyst.^[4] Although the diastereoselectivity was not high, this transformation conveniently afforded a 63:37 mixture of the epimers at C42, **3** and **3'**, which was useful for comparison. These two epimers could be separated but attempts to obtain crystalline derivatives of **3** or **3'** suitable for X-ray diffraction analysis were unsuccessful. This entails that the absolute configuration of C42 in compounds **3** and **3'** remains unknown. Compounds **3** (major diastereomer) and **3'** (minor diastereomer) were then individually converted into **1** and **1'**, respectively, after cleavage of the protecting groups and formation of the (*E*)-alkene at C34–C35. Inspection of the NMR data of compound **1**, obtained from the major diastereomer **3**, revealed a perfect agreement with those of hemicalide whereas significant differences were observed for **1'**. Additionally, during the preparation of model compounds related to **1c** or **1d**, it was observed that a different

configuration at C36 led to major differences in the NMR spectra. Full details on the synthesis and characterization of the model compounds will be disclosed in a forthcoming paper.

Scheme 1. Synthesis of the **1** and **1'** model subunits. From **3** and **3'**, epimeric compounds **4** (sample I) and **4'** (sample II), which are more suitable for VCD analysis, were also synthesized. Py = pyridine, cod = cycloocta-1,5-diene, PMB = 4-methoxybenzyl, TBS = *tert*-butyldimethylsilyl, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

Although the use of NMR drastically reduces the subset of model compounds, it does not allow assigning compounds **1** and **1'** to the model compounds **1a** and **1b**, or *vice versa*. Solving the missing link, i.e. assigning compound **1** to either **1a** or **1b**, is the purpose of the current VCD study. To avoid the conformationally challenging C32-C35 alkenyl group in **1a** and **1b**, and to avoid extra CPU requirements related to the incorporation of the TBS and PMB protecting groups, for the actual VCD study, compounds **3** and **3'**, which have the same configuration at all stereocenters as **1** and **1'**, were converted to the unprotected compounds **4** (sample I) and **4'** (sample II).

In the following, we report on the use of NMR to establish the relative configuration of the undetermined C42 stereocenter in the structurally complex epimers **4** and **4'**. No conclusive results could however be found and we then rely on VCD for the assignment of the relative configuration. The samples, containing six stereocenters, proved to be particularly challenging and constituted an excellent opportunity to demonstrate the utility and power of VCD for the stereochemical assignment of complex polyketide fragments. Combination of the VCD results obtained with those arising from stereoselective synthesis and manual NMR analysis allows assignment of the structure of compounds **4** and **4'** as **4a** and **4b**, respectively, and consequently the relative configuration of the C36-C42 subunit of hemicalide that corresponds to the model compound **1a**.

Results and Discussion

Conformational analysis

From the structures of **4a** and **4b**, it is clear that the molecule has high conformational freedom stemming from unhindered rotations in the 5-hydroxyhexan-2-yl chain, the 1-hydroxypropan-2-yl chain and puckering of the lactone ring. This conformational flexibility yields a complex potential energy surface (PES) that can only be

probed using stochastic search algorithms. Due to conformational averaging, in which conformers of the same absolute configuration may possibly yield VCD spectra that largely cancel in the Boltzmann weighted average spectrum, such flexible molecules can present rather weak VCD signals. Fortunately, this appears not to be the case for the molecules under study.

For the (*R*)-diastereoisomer (**4a**), conformational analysis led to 246 unique conformers, for the (*S*)-diastereoisomer (**4b**) 440 were found using B3LYP/6-31G* gas phase calculations. The 10 most stable conformers for **4a** and 35 in the case of the **4b** all have intramolecular hydrogen bonding (see Figure 3). This amounts to a cumulated Boltzmann weight of 91.8% and 92.6% for the (*R*)- and (*S*)-diastereoisomer respectively. Since in solution 'unfolded' molecules, not showing internal H-bonding, could be stabilized by solvent interaction and the Boltzmann weight of the 'folded' structures appeared rather high for what would be expected in solution, PCM calculations were performed to allow stabilization of the 'unfolded' structures. Indeed, we found diminished occurrence of the folded conformers when solvent modelling is included. This effect is much more outspoken for basis sets containing diffuse functions. Also, such basis sets have a much lower tendency of folding the conformers even in gas phase. A possible explanation for this is an intramolecular basis set superposition error (BSSE)^[14]. For small basis sets (e.g. 6-31G*) the number of basis functions on a hydroxyl hydrogen is limited. For the conformations showing intramolecular hydrogen bonding, this hydrogen can use basis functions of the nearby H-bond acceptor, which is one of the oxygen atoms in the molecule (Figure 3), artificially lowering the energy. Molecules without hydrogen bonding do not have this (artificial) stabilization, and thus will have higher energy. In order to avoid such an effect, we use larger basis sets.

As table 1 shows, when using a large enough basis set (aug-cc-pVDZ) in combination with a continuum solvent model (PCM), the tendency of intramolecular hydrogen bonding decreases significantly. We also attempted calculations using the aug-cc-pVTZ basis set but these turned out to be computationally too demanding.

Table 1: Cumulative Boltzmann weight of conformers showing intramolecular hydrogen bonding. Addition of PCM solvent modelling slightly decreases the importance of intramolecular hydrogen bonding, but increasing the size of the basis set and adding diffuse functions has a much more dramatic effect.

4a	6-31G*	6-31+G*	aug-cc-pVDZ
Gas phase	91.8	67.7	47.4
PCM	85.9	42.7	27.8
4b			
Gas phase	94.1	59.9	35.6
PCM	82.3	34.5	20.3

bonding conformations (0%) and that using only intramolecular hydrogen bonded structures (100%), the influence of the cumulative Boltzmann weight of H bonded conformers is limited. It is also found that for any fraction of H bonded conformers, the

conclusion of the AC determination would be the same, since none of the lines in figure 4 cross. Although we do not claim any universal validity of this near invariance, it does help the assignment of the precent molecule significantly.

Figure 4: Similarity with varying weight of the group of intramolecular hydrogen bonded conformers. There is only a small change in spectrum similarity with varying fraction of H bonded conformers, and in all cases the conclusion on the AC determination would be equal. The calculated (B3LYP/aug-cc-pVDZ/PCM) Boltzmann weight of H bonded conformers is indicated by the vertical lines at 20.3% (4b) and 27.8% (4a).

NMR Analysis

Since in this case we are only interested in the relative configuration of the model compounds under study, the use of NMR methods is an obvious choice if solution state methods are considered. Although 2D-NMR techniques (COSY, HMQC, NOESY) were useful in the determination of the stereogenic centres C37, C39 and C40, the relative configuration at C42 could not be established using these techniques. On the other hand, recent results using calculated NMR shielding constants in combination with statistical methods have proven very promising in determining the relative configuration of some natural products.^[16] Therefore, it was investigated whether this methodology could aid in the determination of the relative configuration at C42 by comparison of experimental NMR chemical shifts on the two model compounds, and ab initio calculated NMR shielding constants for the two possible epimers. In the current work, two approaches were considered. First of all, we wanted to validate which of the model compounds considered corresponds to the same relative configuration as the natural product. Although this was already done manually, it would be nice to have a statistically validated evaluation of this assignment. Smith and Goodman have developed an algorithm (DP4) that

Figure 3: The three possibilities for intramolecular hydrogen bonding for the (R)-4a (left column) and (S)-4b (right column) diastereoisomers of 4. The first type of hydrogen bond is with the hexan-2-yl alcohol group as donor and the hydroxy lactone as acceptor. In the second case the molecule folds to allow an interaction between the hydroxy groups on both side chains of the lactone ring. In the third type of hydrogen bonding the hydroxyl hydrogen the propan-2-yl side chain acts as a hydrogen bond donor to the lactone oxygen acceptor.

In general spectroscopic data depend on the conformations of the molecule in the sense that different conformations may lead to significantly different spectra. VCD spectra are notoriously conformation dependent and in the present molecule, this may include a significant dependence on the population of hydrogen bonded conformations compared to non-hydrogen bonded ones. We therefore carefully examined the impact of different weights of both groups of conformations and refer to the detailed discussion on VCD spectra below for the results.

Although in general one expects important changes in the calculated spectra with changes in the Boltzmann distribution, in this particular case the shift in Boltzmann distribution between the levels of calculation seems to have a rather modest effect. To explain this, we have divided all conformers into two groups, one group containing all conformers showing intramolecular hydrogen bonding, and the other without hydrogen bonding. While the relative Boltzmann weights of the conformers within one group is kept constant, the total Boltzmann fraction of the H bonded group was varied between 0 and 100%. Spectra of H bonded and non H bonded groups were then combined using these varying weights, and the spectrum similarity with the experimental spectrum was calculated using the CompareVOA algorithm^[45]. The results are shown in figure 4.

It is clear from the relatively small difference in similarity between the predicted spectrum without intramolecular hydrogen

assigns probabilities to the assignment based comparison of the calculated chemical shifts of both model compounds with the corresponding experimental shifts. However, we see no reason not to use this algorithm on the comparison of two experimental model compounds (sample I & II) with the natural product^[2]. The analysis shows that there is a 98.5% chance that sample I has the same relative configuration as hemicalide, confirming the manual assignment (table 2).

Table 2: DP4 probability of both samples I & II compared to NMR data of the natural product			
	1H	13C	Total
Sample I	66.60%	97.00%	98.50%
Sample II	33.30%	3.00%	1.50%

To assign the relative configuration of the natural product, we thus need to assign the configuration of sample I (and sample II). Since conventional (2D-)NMR techniques were not adequate for the assignment, the use of the CP3 method by Smith and Goodman is considered. For this, differences in calculated chemical shifts (**4a** – **4b**) were compared to differences in measured chemical shifts for the samples (sample I – Sample II). To do this, chemical shifts were calculated from the magnetic shielding constants using a linear regression with the experimental shifts of the model compounds, as described in the experimental section. The sign of this CP3 parameter, together with its magnitude, is a measure for how good a calculated diastereomer corresponds to an experimental sample. The results are shown in table 3.

Table 3: CP3 analysis of NMR spectra of sample I & II compared to calculated magnetic shieldings of 4a and 4b .			
CP3 values:	13C data	1H data	All data
(Sample I-Sample II) vs. (4a-4b)	0,69	-1,00	-0,16
(Sample I-Sample II) vs. (4b-4a)	-1,07	0,23	-0,42
Probabilities:			
(Sample I-Sample II) vs. (4a-4b)	100,%	0,1%	97,1%
(Sample I-Sample II) vs. (4b-4a)	0,0%	99,9%	2,9%

Although the overall probability of sample I corresponding to **4a** and sample II to **4b** is calculated as 97.1%, results of the ¹³C and ¹H data contradict one another, making the assignment not trustworthy at all. This leads to the conclusion that a CP3 analysis on both ¹H and ¹³C NMR spectra is always preferable: using only one of the two available NMR spectra would inevitably have led to a conclusion whereas the current analysis shows that no conclusions can be drawn from NMR. Also note that the calculated probabilities lead to a misperception of reliability. If all data are considered, we find negative CP3 values for both assignments, which means that important sign changes Δ_{exp} and Δ_{calc} (i.e. difference in chemical shift between corresponding nuclei of both configurations for experiment and calculation, respectively) are found and agreement between theory and experiment is not satisfying. This also confirms the manual NMR analysis, which showed that the relative configuration of the two

diastereomers could not be assigned using NMR. To resolve this, we need rely on a more sensitive method when it comes to stereochemistry.

IR and VCD analysis

~~The theoretically computed compounds **4a** and **4b** and experimental samples I and II comprise two sets of diastereoisomers, which means that there is no direct physical relationship between their spectra, unlike in the case of enantiomers that have the same IR spectra and mirror image VCD spectra. For this task of matching the experimental samples with the computed structures, in theory, IR could be sufficient for the AC determination of the two diastereoisomers, since the other five chiral centers have known stereochemistry. However, it may also occur that, since five out of six chiral centers are identical in the two structures under study, both structures have quite similar IR and/or VCD spectra. In figure 5 the calculated IR spectra of (*S*)-**4** and (*R*)-**4** are shown, together with both experimental spectra I and II. Prior to the detailed discussion of the spectra, we address the issue raised above on possibly. Although in general one expects important changes in the calculated spectra with changes in the Boltzmann distribution, notably concerning differences in the populations of hydrogen bonded conformations versus those without, in this particular case of hemicalide, the shift in Boltzmann distribution between the levels of calculation seems to have a rather modest effect. To explain this, we have divided all conformers into two groups, one group containing all conformers showing intramolecular hydrogen bonding, and the other without hydrogen bonding. While the relative Boltzmann weights of the conformers within one group is kept constant, the total Boltzmann fraction of the H-bonded group was varied between 0 and 100%. Spectra of H-bonded and non H-bonded groups were then combined using these varying weights, and the VCD spectrum similarity with the experimental spectrum was calculated using the CompareVOA algorithm^[15]. The results are shown in figure 4.~~

~~It is clear from the relatively small difference in similarity between the predicted spectrum without intramolecular hydrogen bonding conformations (0%) and that using only intramolecular hydrogen bonded structures (100%), the influence of the cumulative Boltzmann weight of H-bonded conformers is limited. It is also found that for any fraction of H-bonded conformers, the conclusion of the AC determination would be the same, since none of the lines in figure 4 cross. Although we do not claim any universal validity of this near invariance, it does help the assignment of the present molecule significantly.~~

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the VCD-spectra of the two diastereoisomers. This already shows that VCD is a much more sensitive probe for even the relative configuration of a molecule. The most important differences are located in the regions 1070-1170 cm^{-1} (bands 4-6) and 1350-1400 cm^{-1} (bands 10-11). Using this, and the fact that we have an overall excellent agreement allows us to conclude that the C42 atom has an (S) configuration for sample II (=4b) and the (R) configuration for sample I (=4a). To confirm our analysis, we make use of the CompareVOA^[15] algorithm. We note that the CompareVOA algorithm is designed to distinguish between enantiomers, i.e. it only detects a preferential similarity to a spectrum or its mirror image. The algorithm yields two parameters: Σ is a measure for the similarity between the calculated and measured spectrum, and Δ tells us how much the similarity is better for the calculated spectrum compared to its enantiomer. In this study, we are less interested in the information of the enantiomer of the calculated spectrum, since this involves a mirroring of all chiral centers, whereas five out of the six chiral centers are known to have the (S) configuration. Consequently, we are more interested in the similarity (Σ) of the two calculated spectra with the two experimental ones. This information is shown in table 4.

Figure 6. Calculated (upper) and observed (lower) VCD-spectra of 1. As opposed to the IR spectra, we see clear differences between both sets of spectra. The most important of these are located at the regions of 1070–1170 cm^{-1} and 1350–1400 cm^{-1} . This enables us to use VCD to distinguish between the two diastereoisomers.

Table 4: Neighborhood similarity analysis of both calculated spectra with both experiments. There is a superb similarity of the (R)-epimer (4a) calculated spectrum with the I experimental one, and of the (S)-epimer (4b) with the II experiment, confirming our visual interpretation. Although II with 4a and I with 4b also show reasonable similarity, this assignment can be unambiguously ruled out after comparing subtraction spectra.

aug-cc- pVDZ/PCM	Optimized scale factor (%)	IR similarity (%)	Σ (%)	Δ (%)	Confidence Level (%)
II with 4a	0.991	95.5	67.7	55.5	99
I with 4a	0.990	95.0	79.7	68.2	100
II with 4b	0.991	94.9	85.0	77.5	100
I with 4b	0.991	94.0	61.9	38.2	94
II-I with 4b-4a	0.991		78.2	63.6	100

(S)-4 and (R)-4 are shown, together with both experimental spectra I and II.

Overall, we find good agreement between the observed and calculated IR-spectra. There is good alignment of the spectra, and all peaks are predicted correctly. Only the intensity of the peaks at 1035 cm^{-1} , 1210 cm^{-1} , 1390 cm^{-1} and 1460 cm^{-1} is underestimated. Both sets of spectra show high similarity, meaning that the two diastereoisomers have near identical IR spectra. The IR analysis can therefore not be used to determine the relative configuration. The IR spectra are still of use however, as they can be used to assign normal modes to the corresponding peaks in the VCD.

Figure 5. Calculated (upper) and observed (lower) IR-spectra of 4a/4b and samples I/II. Both sets of spectra are almost equal. The quality of one-on-one agreement is for none of the possible matchings sufficient to aid in the determination of the AC.

The calculated and observed VCD spectra are shown in figure 6. Unlike the IR, there are important differences between

As expected from the visual interpretation of the spectra, we see very high and similar IR similarities for the two calculated spectra compared to the experimental ones. We see no higher preference for one calculation with one experiment, which means that IR is of little use in the determination of the AC. The VCD similarity Σ on the other hand, gives us more information. The (R) diastereomer (4a) gives the highest similarity with the I experiment (79.7 vs. 61.9) and the (S) diastereomer (4b) gives a preferred agreement with the II experiment (85.0 vs. 67.7). This confirms our visual interpretation. However, we have two issues with this conclusion. First of all, although the calculations have a preferential similarity with one of the experimental spectra, the agreement with the other experiment is also good, causing the differences in Σ values to be relatively small. Second, there is no measure of confidence that can be assigned to this difference in Σ value, since the CompareVOA database is based on an analysis of a spectrum and its enantiomer. To resolve these issues, we performed a subtraction procedure. In this procedure, the spectra of the two diastereoisomers are subtracted for both

the experiments and the calculations.^[10d] This way, one can imagine that the VCD signals due to the five equal stereocenters cancel, and roughly only the signals due to the C42 stereocenter remain. The result of this procedure is shown in figure 7.

Figure 7. Subtraction spectra of the calculated epimer spectra (upper) and experimental epimer spectra (lower). **(4b)-R(4a)** gives very good agreement with **II-I**, allowing the unambiguous assignment of **II** to **4b** and **I** to **4a**.

Here we see a clear agreement between the **4b-4a** spectrum and the **II-I** spectrum. This again confirms our previous conclusion. Moreover, since the contribution of the five equal stereocenters is now roughly eliminated, we can consider this as spectra corresponding to a single chiral center molecule. This allows us to assess the quality of the AC determination using CompareVOA, as shown in table 2. Now we see a very high agreement for **4b-4a** with **II-I**, showing that that **4b** corresponds to **II** and **4a** to **I**. The high Δ value also shows that there is very little chance that **II** has the (*R*)-configuration at C42 and **I** the (*S*)-configuration. The latter conclusion could not be obtained with the analysis on the original VCD spectra. Due to the excellent agreement between the NMR data of hemicalide and the closely related model compound **1** (corresponding to structure **1a**) in which the absolute configurations of all stereocenters are now known thanks to the assignment of C42 by VCD, our investigations enabled the assignment of the relative configuration of the five stereocenters in the C36–C42 subunit of the natural product.

Conclusion

In the present work, we have successfully assigned the relative configuration of the C36–C42 subunit of the marine natural product hemicalide by combining stereocontrolled synthesis with NMR, IR and VCD analysis. Diastereomeric model compounds corresponding to the C36–C46 subunit were synthesized for comparison of their ¹H and ¹³C NMR data with those of the natural product. This yielded one compound **1** that shows excellent agreement with the natural product. The relative configuration at C42 could not be established with NMR or X-ray diffraction. First, an attempt was made to determine the relative configuration at C42 using a statistical analysis on calculated and measured NMR chemical shifts. However, different conclusions were reached based on the ¹³C and ¹H spectra, despite of the seemingly high probabilities calculated by the algorithm. We recommend use of both ¹³C and ¹H NMR for such an analysis, and sufficiently high (positive) CP3 values are a necessity if these results are to be used. To make an unambiguous assignment of the relative configuration, a VCD analysis was performed on **4** and **4'**. During these studies, the determination of the absolute

configuration of one asymmetric carbon (C42) in these two key epimeric compounds was particularly challenging for VCD analysis due to the high number of conformations of both epimers, and the presence of six stereocenters (C36, C37, C39, C40, C42 and C45). It was shown that the commonly used 6-31G* basis set leads to overestimating the abundance of 'folded' conformers, i.e. conformers possessing intramolecular hydrogen bonding. Since BSSE may lie at the basis of this problem, we recommend the use of a large enough basis set that includes diffuse functions. Also, the use of a solvent model somewhat stabilizes the 'unfolded' conformers, contributing to a more realistic Boltzmann distribution. Finally, although the use of VCD (and IR) can be sufficient to assign the absolute configuration of epimers containing multiple stereocenters, subtracting the spectra of the epimers, for both the calculations and the experiments, greatly enhances the power to distinguish between the epimers. Since this conceptually eliminates the influence of all but the mirrored stereocenters, the calculated subtraction spectrum is either identical or mirror image of the experimental subtracted spectra, greatly improving the quality of the analysis. The determination of the absolute configuration of C42 in the two key epimeric compounds (**4** and **4'**) allowed the assignment of the relative configuration of the C36–C42 subunit of the marine natural product hemicalide, thereby highlighting the ability and reliability of VCD analysis for structurally and stereochemically complex structures.

As a final note, it should be mentioned that the present VCD work does not allow to establish the absolute configuration of the entire fragment in hemicalide. The relative configuration is now known for the entire C36-C42 substructure but the completely enantiomeric form would fit the NMR data of hemicalide equally well. [A noteworthy extra advantage of the current method entailing the study of fragments of the original molecule lies in the fact that one needs to model only small molecules and their relative configuration is the same in the large molecule. This means that e.g., problems related to \(un\)folding of the large molecule and the possible inadequacies of current DFT functionals to properly account for this are avoided.](#)

Experimental Section

Computational details

For the calculation of the IR and VCD spectra of **4a** and **4b**, all significant conformers of the molecule are required such that Boltzmann averaged spectra can be computed. Due to the number of rotatable bonds, conformational analysis was performed using molecular mechanics force fields instead of direct ab initio potential energy hypersurface exploration. A combination of MMFF, MMFF94S^[17] and SYBYL^[18] force fields was used, using the search algorithms from the commercially available software packages Conflex^[19] ('reservoir filling'), Spartan 08^[20] (Monte Carlo) and ComputeVOA^[21] (Monte Carlo). As is clear from scheme 1, five out of the six chiral centers have known stereochemistry, hence it is sufficient to calculate spectra for only two configurations, namely depending on the chosen C42 configuration. To accomplish this, 2 structures were drawn for which five chiral centers have the (*S*)-configuration, and the stereocenter at C42 is either (*R*) or (*S*). Each of these structures was then used as starting point for the conformational analysis described above. The many redundant conformers were removed through comparison of the eigenvalues of the internuclear distance matrix for each conformer. After this, conformers were optimized at the B3LYP/6-31G*^[22] level of theory using Gaussian 09^[8d]. Again, redundant structures were removed and the retained conformers were finally optimized at the B3LYP/aug-cc-pVDZ^[23] level, using a polarizable continuum model^[24] for modelling the chloroform solvent ($\epsilon=4.7113$). To investigate the influence of the solvent modelling, calculations with and without PCM (Integral Equation Formalism) were performed. All calculations were done with 3 different basis sets, namely 6-31G*, 6-31+G* and aug-cc-pVDZ, combined with the B3LYP functional. To ensure only PES-minima are used, all conformers were checked for imaginary frequencies. For each conformer, dipole and rotational strengths were calculated and converted to molar extinction coefficients ($L.mol^{-1}.cm^{-1}$)^[25]. For both IR and VCD spectra, a Lorentzian broadening^[25] was applied with a rather large full width at half maximum (FWHM) of 18 cm^{-1} , as this gave the best visual comparison to experiment. A global scale factor,

optimized for maximum IR similarity using CompareVOA^[15], was applied to compensate for the overestimation of the harmonic frequencies^[26]. NMR chemical shifts were obtained by linear regression of the computed magnetic shielding constants, with experimental shifts obtained for sample I and sample II. For both calculated model compounds, this linear regression was performed twice: once for each sample. Both the slope and the intercept obtained from the linear regression was averaged and were found to be very similar between both sets of experimental data. Linear regression was done for ¹H and ¹³C NMR separately. Chemical shifts were averaged using the Boltzmann weights of all conformers, and chemical shifts for equivalent methyl hydrogens were also averaged. These calculated shifts were then used in the online applet available from Smith and Goodman^[16b]. No prior assignment was used, i. e. highest experimental shifts were compared to the highest calculated shifts, and so on. Regression data can be found in the table below.

Table 5: Linear regression data of calculation magnetic shielding constants with measured NMR chemical shifts for the two model compounds with both synthesized samples.

		13C				
		Intercept	Slope	R ²	CMAE	Δδ
4a	Sample I	188.34	-1.0111	0.9976	1.649	3.357
	Sample II	188.93	-1.0156	0.9953	2.142	7.522
4b	Sample I	188.43	-1.0104	0.9971	1.828	3.644
	Sample II	189.11	-1.0167	0.9982	1.509	3.14
		1H				
4a	Sample I	31.915	-1.0806	0.976	0.51	0.754
	Sample II	31.838	-1.0175	0.9281	0.525	0.735
4b	Sample I	31.959	-1.1007	0.9825	0.126	0.387
	Sample II	31.884	-1.0378	0.937	0.185	0.898

Experimental details

For measurement of the IR and VCD spectra, the samples corresponding to 4 (sample I) and 4' (sample II) in scheme 1 were dissolved in CDCl₃ with concentrations of 0.16M and 0.11M respectively. These concentrations are too high for useful VCD measurements of the carbonyl stretch signal, but yielded the highest signal-to-noise ratio (S/N) for measurement of the fingerprint region (950cm⁻¹ – 1500 cm⁻¹). As the carbonyl function is susceptible to solvent interactions^[27], we choose to ignore this signal in the AC determination. The IR- and VCD-spectrum for the two diastereoisomers and the solvent were recorded on a ChiralIR-2X dual PEM VCD-spectrometer (BioTools, Inc) for a duration of 10 000 scans/sample. Since no enantiomers were available, baseline correction was performed using subtraction of the solvent VCD-spectrum. All samples were measured in a 100 μm path length cell with BaF₂ windows, and a resolution of 4 cm⁻¹ was used throughout. NMR spectra were obtained on a 400 MHz for ¹H and 100 MHz for ¹³C using CDCl₃ as solvent and tetramethylsilane (TMS) as an internal standard. Sample I (corresponding to **4a** according to VCD analysis) ¹H NMR (400 MHz, CDCl₃) δ 4.47 (apparent dt, J = 11.3 Hz and J = 3.5 Hz, 1H, H37), 4.29 (d, J = 10.3 Hz, 1H, H40), 3.79 (m, 1H, H45), 3.64 (dd, J = 10.8 Hz and J = 7.7 Hz, 1H, H35), 3.65 (br s, 1H, OH), 3.60 (dd, J = 10.8 Hz and J = 5.3 Hz, 1H, H35'), 2.50 (br s, 1H, OH), 2.19 (br s, 1H, OH), 2.07–1.96 (m, 1H, H42), 1.96–1.81 (m, 3H, H39, H36 and H38), 1.72–1.61 (m, 1H, H38'), 1.58–1.48 (m, 1H, H44), 1.48–1.24 (m, 3H, H44' and H43), 1.19 (d, J = 6.1 Hz, 3H, H46), 0.98 (d, J = 7.0 Hz, 3H, C36 Me), 0.94 (d, J = 6.8 Hz, 3H, C42 Me); ¹³C NMR (100 MHz, CDCl₃) δ 177.3 (s, C41), 76.1 (d, C37), 67.9 (d, C45 or C40), 67.0 (d, C45 or C40), 63.8 (t, C35), 40.7 (d, C39), 39.3 (d, C36), 36.6 (t, C44), 32.7 (d, C42), 30.5 (t, C43), 25.1 (t, C38), 23.6 (q, C46), 13.2 (q, C42 Me), 10.6 (q, C36 Me). Sample II (corresponding to **4b** according to VCD assignment): ¹H NMR (400 MHz, CDCl₃) δ 4.53 (apparent dt, J = 11.5 Hz and J = 3.5 Hz, 1H, H37), 4.38 (d, J = 10.4 Hz, 1H, H40), 3.84 (br s, 1H, OH), 3.76 (m, 1H, H45), 3.63 (dd, J = 11.0 Hz and J = 8.0 Hz, 1H, H35), 3.57 (dd, J = 11.0 Hz and J = 5.1 Hz, 1H, H35'), 2.92 (br s, 2H, OH), 2.02–1.78 (m, 4H, H39, H36, H42 and H38), 1.77–1.67 (m, 1H, H43), 1.67–1.52 (m, 2H, H38' and H44), 1.19–1.28 (m, 1H, H43'), 1.20 (d, J = 6.2 Hz, 3H, H46), 1.08 (m, 1H, H44'), 0.99 (d, J = 6.8 Hz, 3H, C36 Me), 0.96 (d, J = 6.9 Hz, 3H, C42 Me); ¹³C NMR (100 MHz, CDCl₃) δ 177.4 (s, C41), 75.6 (d, C37), 68.5 (d, C45), 66.8 (d, C40), 63.8 (t, C35), 41.9 (d, C39), 39.2 (d, C36), 37.2 (t, C44), 34.1 (d, C42), 26.9 (t, C43), 26.5 (t, C38), 23.7 (q, C46), 17.4 (s, C42 Me), 10.3 (q, C36 Me).

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