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Systematic Assignment of the Configuration of Flexible Natural Products by Spectroscopic and Computational Methods: The Bistramide C Analysis

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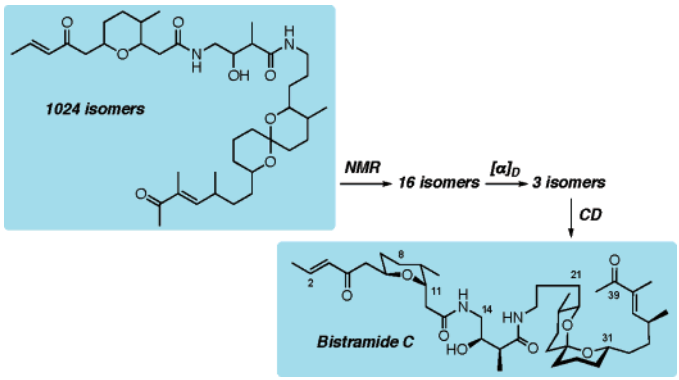
ABSTRACT



The combination of NMR NOE, chemical shift, and *J*-coupling measurements with molar rotation and circular dichroism (CD) determinations, including RI-DFT BP86/aug-cc-pVDZ calculations, reduced a candidate pool of 1024 possible stereoisomers of (+)-bistramide C to a single absolute configuration assignment for the 10 stereogenic carbons of the marine natural product.

Assigning the absolute configuration of a complex natural product with multiple stereocenters is not only laborious but also error prone,[1] often requiring multistep total synthesis in conjunction with a large variety of NMR analyses. X-ray structure determinations are limited to samples forming suitable crystals, but chiroptical analyses such as electronic (ECD) and vibrational circular dichroism (VCD), optical rotatory dispersion (ORD), Raman optical activity (ROA), and optical rotation (OR) can be obtained for any soluble analyte.[2] Recent progress in time-dependent density functional theory (TDDFT) methodology,[3] more specifically in the treatment of the time-consuming four-center integrals

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such as the resolution of the identity approximation (RI-*J*),[4] afforded a dramatic improvement in computational efficiency that enables the calculation of molecular response properties (i.e. ORD and CD) of larger molecular systems than previously feasible.[5] Similarly, the use of more complete basis sets has made the RI-*J* method both plausible and practical.

In this study, we report a systematic approach for the stereochemical assignment of the natural product bistramide C,[6] based on van't Hoff's optical superposition principle,[7] RI-*J* TDDFT free energy Boltzmann-averaged molar rotations, and CD spectra of molecular fragments with relative configurations determined by NMR analysis. The combined use of spectroscopic and chiroptical methods allows a systematic reduction of the number of potential stereoisomers and finally leads to the identification of a single stereoisomer with the (6*R*,9*S*,11*S*,15*R*,16*S*,22*R*,23*S*,27*S*,31*S*,34*S*)-configuration, which was confirmed by a recent total synthesis.[8]

Theory-assisted stereochemical assignments of a complex flexible molecule such as bistramide cannot be achieved solely by a direct comparison between calculated and experimental chiroptical data of the raw constitution,[6] since no single current method enables the discrimination among 1024 possible stereoisomers. In the specific case of bistramides A and C, NMR analyses of natural samples successfully reduced the number of stereoisomers by 2 orders of magnitude. Independent NMR studies of bistramide A had established the relative configuration of the pyran and spiroketal moieties as (6*S**,9*R**,11*R**) and (22*R**,23*S**,27*S**,31*S**), respectively.[9] We assigned the *anti*-(15*R**,16*S**)-configuration for the γ-amino acid core in combination with fragment syntheses.[10] Accordingly, at the onset of this theoretical study, the total number of stereoisomers for bistramide C could be reduced from 1024 to 16. At this juncture, a classical structure elucidation would have relied exclusively on total synthesis,[8] but in consideration of the size and complexity of the target structure, the preparation of all possible 16 diastereomers and enantiomers was clearly impractical. Instead, we applied computational tools for the determination of chiroptical descriptors for all remaining stereoisomers.

The 4-fragment analysis shown in Figure 1 was used to identify and cluster critical contributors to the chiroptical properties of bistramide C. Fragment **1** included the trisubstituted pyran ring with the (6*S*,9*R*,11*R*)-configuration in accordance with the relative configuration revealed by ^{1}H−^{1}H NOESY experiments. Fragment **2** incorporated the stereogenic carbons at C(15) and C(16). The *anti*-configuration was chosen for this fragment since the chemical shifts of CH(15) and CH(16), their *vic*-^{1}H−^{1}H coupling constant, and the ^{13}C NMR shift of C(17) of a synthetic model closely matched the values of the natural product bistramide A.[10] The third fragment contained four stereocenters in the (22*R*,23*S*,27*S*,31*S*)-configuration, which was also based on ^{13}C, ^{1}H, and NOESY NMR analyses. Since there was no straightforward method to determine the relative configuration of the stereocenter at C(34) vs the spiroketal substructure, a fourth fragment (**4**) was added. Care was taken not to cleave bonds connecting stereogenic carbons, to avoid the vicinal action limitation of van't Hoff's rule.

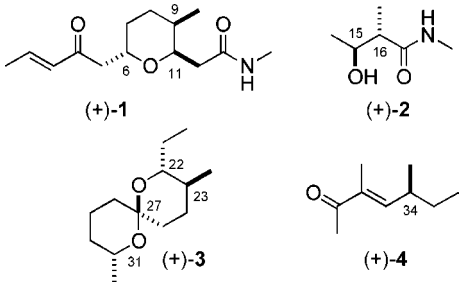

Figure 1. Four-fragment approach for clustering the stereogenic carbons in bistramide C. The (+) sign indicates a positive calculated $[\alpha]_D$ value for the configuration shown.

Conformations for fragments **1** to **4** were generated by using a Monte Carlo analysis with the MMFF parametrized force field in MacroModel 7.0.[11] Geometries were further fully optimized and free energies were computed by using the RI-*J* approximation[4] at the DFT BP86/SVP level of theory[12] in Turbomole 5.6.[13] $[M]_D$ values for all fragments listed in Table 1 resulted from the summation over Boltzmann-weighted (at 25 °C) specific rotation ($[\alpha]_D$) values of all conformations within 10 kJ/mol of the lowest free energy

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Table 1. Boltzmann-Weighted Calculated[a] Molar Rotations of Fragments **1**−**4**

fragment	configuration	$[M]_D$
(+)-**1**	(6*S*,9*R*,11*R*)	+190
(+)-**2**	(15*R*,16*S*)[b]	+36
(+)-**3**	(22*R*,23*S*,27*S*,31*S*)	+153
(+)-**4**	(34*S*)	+98

[a] Only molar rotations of (+)-stereoisomers were calculated. $[M]_D$ values for (−)-stereoisomers were obtained by multiplying (+) values by −1. $[M]_D$ values were calculated by using RI-DFT BP86/aug-cc-pVDZ at the RI-DFT BP86/SVP optimized geometries. [b] The configuration of the (15*S*)-stereocenter of fragment (+)-**2** changes to (15*R*) when linked to fragment **1**. For this reason, we use the (15*R*,16*S*) notation instead of (15*S*,16*S*) for (+)-**2**.

conformer. The expression for the Boltzmann-weighted molar optical rotation is

$$[M]_D = \frac{MW}{100}\sum_i x_i[\alpha]_{D,i} \quad (1)$$

where MW is the molar mass (in g/mol), $[\alpha]_{D,i}$ is the specific rotation angle (calculated at the sodium D-line), and x_i is the Boltzmann-weighting factor of conformer *i*. Specific optical rotation values, rotatory strengths, and electronic excitation energies for all conformers were computed in the gauge-independent dipole-velocity representation using the aug-cc-pVDZ basis set[14] in the time-dependent density functional theory (TDDFT) linear response formalism implemented in Turbomole, including the COSMO implicit solvation model[15] using the chloroform dielectric ($\epsilon = 4.9$).

In accordance with van't Hoff's rule of optical superposition, $[M]_D$ values for all 16 combinations of fragments **1**−**4** were calculated by linear summation of fragment $[M]_D$'s, based on the configuration sequence given in Table 2. Since

Table 2. RI-TDDFT BP86/aug-cc-pVDZ Calculated Molar Rotations for 16 Stereoisomers of Bistramide C

isomer no.	configurations used for **1**−**4**				$[M]_D$
1	(+)-**1**	(+)-**2**	(+)-**3**	(+)-**4**	+477
2	(+)-**1**	(+)-**2**	(+)-**3**	(−)-**4**	+281
3	(+)-**1**	(+)-**2**	(−)-**3**	(+)-**4**	+171
4	(+)-**1**	(+)-**2**	(−)-**3**	(−)-**4**	−25
5	(+)-**1**	(−)-**2**	(+)-**3**	(+)-**4**	+405
6	(+)-**1**	(−)-**2**	(+)-**3**	(−)-**4**	+209
7	(+)-**1**	(−)-**2**	(−)-**3**	(+)-**4**	+99
8	(+)-**1**	(−)-**2**	(−)-**3**	(−)-**4**	−97
9	(−)-**1**	(+)-**2**	(+)-**3**	(+)-**4**	+97
10	(−)-**1**	(+)-**2**	(+)-**3**	(−)-**4**	−99
11	(−)-**1**	(+)-**2**	(−)-**3**	(+)-**4**	−209
12	(−)-**1**	(+)-**2**	(−)-**3**	(−)-**4**	−405
13	(−)-**1**	(−)-**2**	(+)-**3**	(+)-**4**	+25
14	(−)-**1**	(−)-**2**	(+)-**3**	(−)-**4**	−171
15	(−)-**1**	(−)-**2**	(−)-**3**	(+)-**4**	−281
16	(−)-**1**	(−)-**2**	(−)-**3**	(−)-**4**	−477

the reported $[M]_D$ for a natural sample of bistramide C is +70,[6c,16] only a few of the 16 configurational isomers are feasible candidates. Pertinent to the selection process is the well-known fact that nonhybrid DFT functionals such as BP86, used in the RI-*J* approximation, underestimate the lowest electronic excitation energies for delocalized π-systems and, consequently, often cause an overestimation of $[\alpha]_D$.[17] For example, an overestimation of +48 was observed in the calculated $[M]_D$ value of fragment 4, since the reported value for this compound, (+)-(*S*)-normanicone, is +50.[18]

On the basis of the TDDFT $[M]_D$ data in Table 2, therefore, only three isomers need to be further considered as candidates for the assignment of the configuration of bistramide C, i.e., stereoisomer no. 3, 7, and 9. All three isomers contain the (+)-**4** fragment, and thus C(34) can be assigned the (*S*)-configuration. The remaining stereogenic carbons, especially in fragments **1** and **2**, are part of chromophoric groups exhibiting $\pi-\pi^*$ electronic transitions. Accordingly, the electronic circular dichroism should be a suitable analytical method to allow a chiral discrimination between isomer no. 3, 7, and 9.

The ECD spectra of a synthetic stereoisomer (isomer no. 6), natural bistramide C, and synthetic (+)-[**1** + **2**],[10] obtained from the coupling of fragments (+)-**1** and (−)-**2** are shown in Figure 2. In the selected spectral region, one

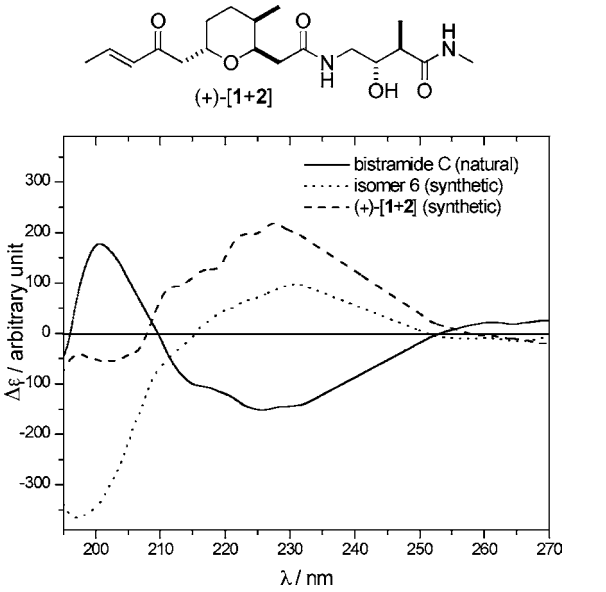

Figure 2. Experimental CD spectra of the synthetic stereoisomer no. 6 (dotted line), natural bistramide C (solid line), and fragment (+)-[**1** + **2**] (dashed line).

can easily identify two bands of interest. The wide band centered at ~230 nm represents carbonyl n−π^* and $\pi-\pi^*$

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transitions of pyran, γ-amino amide, and enone moieties of bistramide C. The (6*R*,9*S*,11*S*,15*R*,16*S*)-configuration results in a negative band, and a positive band is obtained for the (6*S*,9*R*,11*R*,15*S*,16*R*)-configuration. This correlation is supported by the CD spectrum of fragment (+)-[**1** + **2**], which has the same configuration as isomer no. 6. The direct comparison between these two CD spectra also highlights the effect of the configuration of (−)-**4**, which appears to reduce the intensity of the 230 nm band. In contrast, the strong negative band at ∼200 nm in the CD spectrum of isomer no. 6 results mainly from the additional presence of the (−)-(*R*)-normanicone fragment (−)-**4**. For the natural product, the signs of all bands are reversed, but since the spiroketal segment **3** does not have a major chromophore that contributes to the 200−250 nm region, experimental CD spectra alone would be insufficient to draw any definitive structural conclusions.

However, the absolute configuration of natural bistramide C can be assigned based on the comparison of calculated CD spectra[19] for isomer no. 3, 7, and 9 and the experimental circular dichroism spectrum of the natural product (Figure 3). The calculated CD spectra of isomer no. 3 and 7 are similar to that of synthetic isomer no. 6, all sharing an identical (+)-**1** fragment. The presence of the (+)-**2** fragment in isomer no. 3 causes a slight reduction in the intensity of the 230 nm band, and a stronger reduction of the 200 nm band. Both calculated CD spectra are significantly different from the natural product. In contrast, isomer no. 9 is almost identical with bistramide C and therefore allows an unambiguous assignment of the absolute configuration of the natural product as (+)-(6*R*,9*S*,11*S*,15*R*,16*S*,22*R*,23*S*,27*S*,-31*S*,34*S*), in agreement with the recent total syntheses.[8]

In conclusion, the present study significantly expands our earlier prediction of the absolute configuration of bistramide C, which was based on a van't Hoff analysis of synthetic fragments.[10] We have now applied fast and accurate RI-TDDFT calculations of $[M]_D$ values and ECD spectra and demonstrated that the judicious use of NMR, polarimetry, and ECD, in conjunction with ab initio methods, can be of great value to solve complex problems in the absolute configuration assignment of flexible molecules containing a large number of stereogenic carbons. The cluster fragmentation scheme and the use of both optical rotation as well as ECD chiroptical data clearly minimizes potential errors associated with the neglect of inter-fragment perturbations. To complete assignments of the configurations of the entire bistramide family, we plan to extend the strategy developed here toward bistramides A, B, and D, as well as the pyran ring-opened bistramide K.

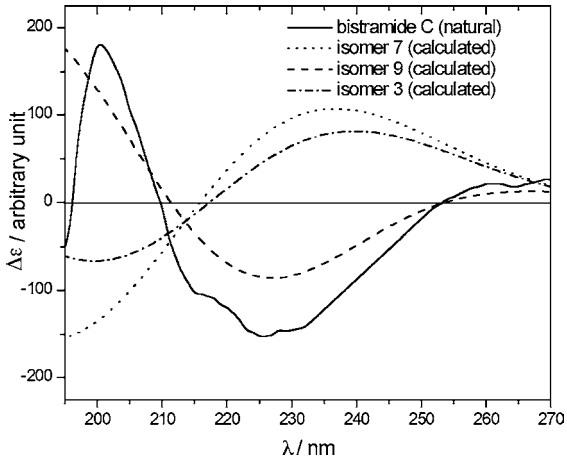

Figure 3. Calculated CD spectra of isomer no. 7 (dotted line), isomer no. 9 (dashed line), and isomer no. 3 (dashed−dotted line). Experimental CD spectrum of natural bistramide C (solid line). The calculated CD spectra have been blue-shifted by 20 nm.

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Supporting Information Available: Calculated CD spectra of fragments **1**−**4**, $[\alpha]_D$ values, and free energies for the corresponding conformers. This material is available free of charge via the Internet at http://pubs.acs.org.

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