Stereochemical Determination of Acyclic Structures Based on Carbon−Proton Spin-Coupling Constants. A Method of Configuration Analysis for Natural Products

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A method for elucidating the relative configuration of acyclic organic compounds was developed on the basis of carbon−proton spin-coupling constants ($^{2,3}J_{C,H}$) and interproton spin-coupling constants ($^{3}J_{H,H}$). This method is based on the theory that, in acyclic systems, the conformation of adjacent asymmetric centers is represented by staggered rotamers, and their relative stereochemistry can be determined using $^{2,3}J_{C,H}$ and $^{3}J_{H,H}$, because the combined use of these *J* values enables the identification of the predominant staggered rotamer(s) out of the six possible derived from *threo* and *erythro* configurations. Detailed conformational analysis for model compounds **1**−**4** revealed that this method is useful in most cases for assignment of the configuration of acyclic structures occurring in natural products, in which stereogenic methine carbons are often substituted with a methyl or a hydroxy (alkoxy) group. This *J*-based configuration analysis was applied to the stereochemical elucidation of carboxylic acid **5** derived from zooxanthellatoxin and proven to be a practical method even for natural products with complicated structures.

Introduction

Natural products possessing complicated structures and potent biological activities have been isolated from marine sources and microorganisms in the last two decades, and their structures have been successfully elucidated mainly by modern NMR techniques. Among these compounds, acyclic or macrocyclic structural moieties appear frequently. Their configurations, however, have been determined chiefly by X-ray or laborious synthetic work as seen for palytoxin,[1] amphotericin B,[2] calyculins,[3] fumonisins,[4] and swinholides.[5] Assignments of configuration of acyclic structures are extremely difficult even with current NMR-based methods. This may be one reason that a fairly large number of acyclic moieties in important natural products remain stereochemically unassigned; e.g., aflastatin,[6] amphidinols,[7] prymnesins,[8] and zooxanthellatoxins.[9] These compounds are very difficult to crystalize and, to make matters worse, their acyclic parts are laborious to synthesize because of the number of chiral centers they contain.

Knowing the configuration of natural products has become crucial because it provides essential information for both total synthesis and molecular mode of actions, which are now regarded as the most challenging fields in organic and bioorganic chemistry. NMR-based methods have been devised for this purpose; e.g., NOE-based methods in combination with molecular mechanics calculations have been proposed for flexible molecules, particularly for macrocyclic compounds, as reported for macrolides[10] and other compounds.[11] However, even with new NOE-based techniques, it is still very difficult to assign the stereochemistry of highly flexible carbon chains because the presence of multiple conformers, in which minor populations often make disproportionately large contributions to NOE intensity, occasionally leads to contradictory distance constraints.

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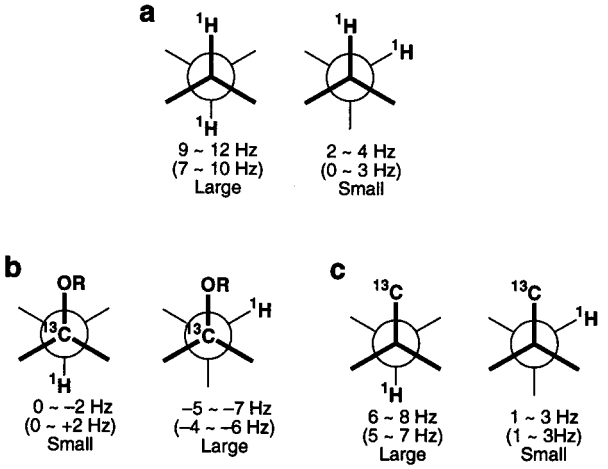

Figure 1. Dihedral angle dependence of spin-coupling constants, $^3J_{H,H}$, $^2J_{C,H}$, and $^3J_{C,H}$. a: Vicinal $^1H-^1H$ coupling constants, $^3J_{H,H}$, when the dihedral angles are 180° (anti) and 60° (gauche). b: Geminal $^{13}C-^1H$ coupling constants, $^2J_{C,H}$, when the dihedral angles between ^{13}C-attached oxygen and the proton are 180° (anti) and 60° (gauche). c: Vicinal $^{13}C-^1H$ coupling constants, $^3J_{C,H}$, when the dihedral angles are 180° (anti) and 60° (gauche). The figures in parentheses represent the values of 1,2-dioxygenated systems (see Table 1 for details).

The spin-coupling constant is a useful NMR parameter for conformational studies of biomolecules as seen in its application to polypeptides or polynucleotides. In particular, proton−proton vicinal coupling constants ($^3J_{H,H}$) are most frequently used for this purpose because of their dependence on dihedral angles. These coupling constants, including those due to carbon−proton coupling ($^2J_{C,H}$ and $^3J_{C,H}$), possess several advantages over NOE for acyclic systems. In systems with conformational changes, the coupling constants are observed as a weighted average of those due to each conformer, which greatly facilitates the determination of a population ratio among conformers. For configuration assignments, using interproton J values ($^3J_{H,H}$) alone is inadequate because two H/H-gauche rotamers cannot be distinguished as shown in Figures 1 and 3. Additional information from $^{2,3}J_{C,H}$ may dramatically expand the utility of the coupling constants in configuration analysis. Thanks to the progress in two-dimensional NMR techniques and hardware innovation in the past decade,[12] $^{2,3}J_{C,H}$ values in complicated natural products have become measurable.

In this study we report the development of a method called *J-based configuration analysis* for assigning the relative configuration of natural products, in particular, their acyclic subunits.[13]

Results

Rotational Isomers and Structural Analysis. Vicinal carbon−proton spin-coupling constants ($^3J_{C,H}$) follow a Karplus-type equation (Figure 1)[14] and thus can be utilized theoretically for stereochemical analysis as is the case with $^3J_{H,H}$. The value of $^3J_{C,H}$ has been used for conformational studies of proteins and oligonucleotides.[15] In addition, geminal carbon−proton coupling constants ($^2J_{C,H}$) also provide conformational information;[16] when an oxygen functionality on a carbon atom is gauche to its geminal proton, $^2J_{C,H}$ becomes large, and when it is anti, the value becomes small (Figure 1). However, to our knowledge, comprehensive configuration analysis of complicated natural products based on carbon−proton coupling constants ($^{2,3}J_{C,H}$) has not been reported except those from our group,[13] probably due to difficulty in determining $^{2,3}J_{C,H}$ values accurately. Therefore, we first set up a basic strategy for correlating $^{2,3}J_{C,H}$ values with the configuration of acyclic asymmetric carbons.

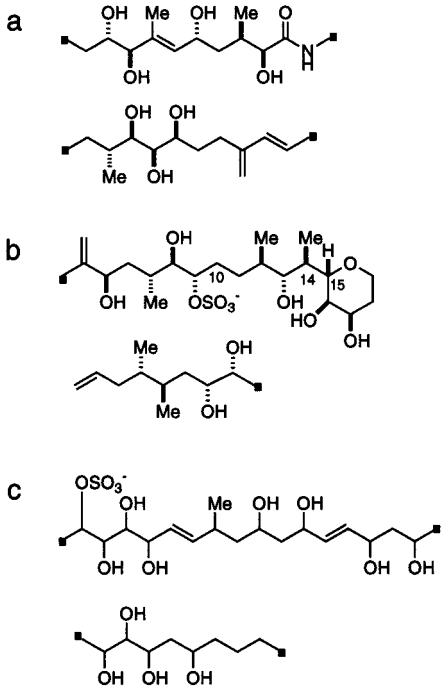

Figure 2. Asymmetric centers in acyclic or macrocyclic structures occurring in natural products mostly consist of methine carbons substituted with a hydroxy (alkoxy) or methyl group. a: C1−C10 and C83−92 parts of palytoxin. b: Terminal chains of maitotoxin (C4−C14 and C135−C142). c: Acyclic parts of zooxanthellatoxin A (C44−C59 and C77−C84). Numbering in the structures denotes carbon numbers of entire structures.

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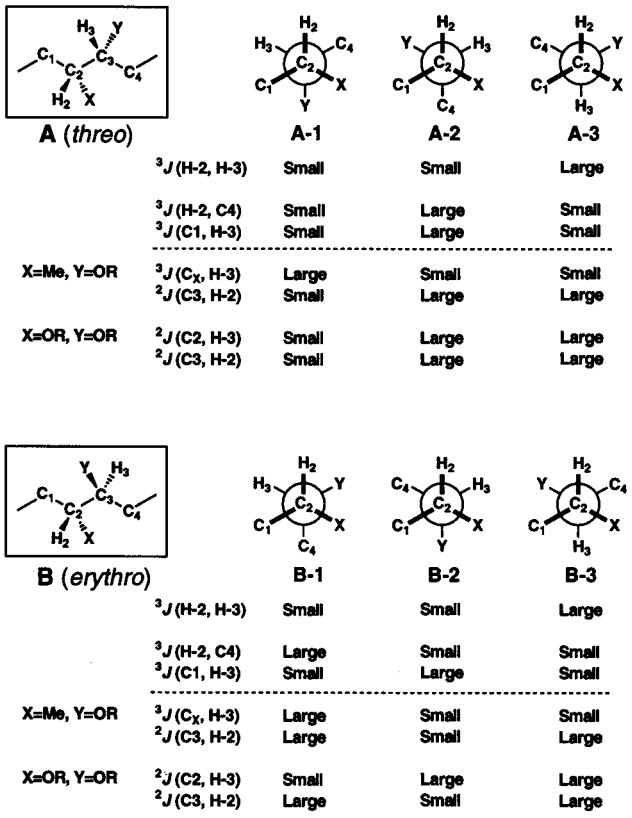

Figure 3. Dependence of $^3J_{H,H}$ and $^{2,3}J_{C,H}$ on dihedral angles between vicinal methine carbons in 2,3-disubstituted butane systems. Frequently occurring pairs of substituents in natural products, Me/OR and OR/OR, are presented (Me/Me is omitted since it rarely occurs in natural products). R stands for H, alkyl, or/and acyl groups. When $^3J_{H,H}$ is small (= gauche), the four rotamers A-1, A-2, B-1, and B-2 can be clearly identified using $^{2,3}J_{C,H}$ values; either ^3J(H-2,C4)/^3J(C1,H-3), ^3J(Cx,H-3)/^2J(C3,H-2) or ^2J(C2,H-3)/^2J(C3,H-2) can be used to identify the four rotamers. H/H-anti rotamers A-3 and B-3 can be distinguished on the basis of NOEs.[25]

For structure elucidation of natural products, stereogenic carbons substituted with a hydroxy, an alkoxy,[17] or a methyl group are particularly important. In other words, most asymmetric centers existing in acyclic subunits are methine carbons bearing a hydroxy or a methyl as seen in the side chains of palytoxin, maitotoxin,[18] and zooxanthellatoxins (Figure 2).[9] To determine the configuration of multiple asymmetric carbons, the diastereomeric relationship between two neighboring carbons must be assigned; as shown in Figure 2, 1,2-, 1,3-, and 1,4-asymmetric systems are commonly found in natural products. For accurate measurements of $^{2,3}J_{C,H}$ by 2D NMR methods such as HETLOC[19] and HMBC,[20] proton signals coupling with carbons of interest must be separated from each other to enable a first-order analysis of $^1H-^1H$ coupling. To satisfy this, the number of bonds separating asymmetric centers should be one, two, or at most three, since propylene ($CH_2CH_2CH_2$) or longer carbon chains usually give rise to overlapping proton signals. Thus, in this study, stereochemical correlations between 1,2-methines and between 1,3-methines bearing hydroxy and/or methyl groups are the main focus. Even with all these conditions, this method would greatly assist in the determination of the configuration of natural products, because the acyclic asymmetric carbons occurring in these compounds, probably more than 80%, fall under these categories.

To establish *J*-based configuration analysis, we categorized dihedral interactions into several cases. Our first assumption was that, in the systems in Figure 3, conformations can be represented by three staggered rotamers. This seems to be quite reasonable for acyclic carbon chains with hydroxy and methyl substitutions, which, unlike bulky substituents, usually cause no significant deviations from the anti or gauche orientation.[21,22] During our investigations of over 30 diastereorelationships occurring in 10 natural and synthetic compounds,[23] no single example of deviation over 10° has been found so far (if their dihedral angles deviate from the staggered rotamer by over 10°,[22] $^3J_{H,H}$ becomes an atypical value and unconditional assignment of stereochemistry cannot be achieved, particularly when conformational change is involved). The occurrence of these unusual conformers can be judged by accurate measurements of coupling constants. $^3J_{H,H}$ can be precisely determined (0.1−0.2 Hz) using decoupling difference spectra or E.COSY-type experiments,[24] but measurements of $^{2,3}J_{C,H}$ with an accuracy better than 1 Hz are not usually attainable owing to the low signal-to-noise ratio and limited digital resolution in 2D methodologies. In the following experiments, therefore, values of $^3J_{H,H}$, which were used for not only stereochemical determination but also conformational analysis, were determined with an accuracy of 0.1 Hz, while $^2J_{C,H}$ and $^3J_{C,H}$ were obtained with single digit accuracy.

Configuration Assignment for Systems with One Dominant Conformer. 1,2-Methine Systems. To assign the stereochemical relationship for a pair of vicinal asymmetric carbons, we have to choose a single conformer with a correct configuration from the staggered rotamers possible in *threo* and *erythro* diastereomers

(17) In configuration studies of natural products, the stereochemical relationship between asymmetric carbons residing in cyclic ether or lactone structures and those in a side chain is often important as seen for C15−C14 of maitotoxin (Figure 2).[13c,d] To such systems, the *J*-based method was successfully applied. Although applications to acyloxy substitutions are not plentiful, preliminary investigations on tri-*O*-acetyl model compounds **1** and **2** suggested that this method can be applied for acetoxy-substituted chains.

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(22) Dependence of $^3J_{H,H}$ on dihedral angles was examined for model compounds **1**−**4** on the basis of a modified Karplus equation[28] and force field calculations. The C1−C2−C3−C4 dihedral angles in the stable conformers for **1**−**3** deviated from 180° by less than 10°; **1** was most stable at a dihedral angle C1/C2/C3/C4 of 183° and had the weighted average $^3J_{H-2,H-3}$ of 0.7 Hz. In the range of 166−190°, $^3J_{H-2,H-3}$ in **1** does not significantly (less than 1 Hz) deviate from the calculated value. Compounds **2** and **3** were also most stable in the C1/C4-anti conformations at 180° (8.7 Hz) and at 175° (2.7 Hz), respectively. Within the ranges of 157−203° and 167−185°, **2** and **3** showed no significant aberrations in $^3J_{H-2,H-3}$ values (<1 Hz), respectively.

(23) We have examined the diastereomeric relationship of asymmetric carbons residing in acyclic portions for maitotoxin,[13b−d] amphidinol,[7] okadaic acid,[13a] zooxanthellatoxin, and several synthetic compounds. Force field calculations (CFF91 force field on DISCOVER/INSIGHT II) carried out for okadaic acid and maitotoxin revealed that deviations of all $^1H/^1H$-dihedral angles in acyclic structures from 60° or 180° were less than 10° as is the case with the model compounds.[22]

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(Figure 3). Among those, four conformers, A-1, A-2, B-1, and B-2, can be identified using $^3J_{H,H}$, $^2J_{C,H}$, and $^3J_{C,H}$, while the two rotamers A-3 and B-3 with an H/H-anti orientation cannot be distinguished. For these anti conformers, NOE experiments should be a practical way to assign their configuration. In acyclic organic compounds with methyl or hydroxy substituents, these H/H-anti conformers usually assume a C/C-anti orientation (or an extended form), in which no NOE (or ROE) should be observed between H-1 and H-4 (B-3 in Figure 3). In the case of the H/H-anti and C/C-gauche conformation (A-3), if present, H-1 and H-4 should come within the range of NOE.[25] Using these criteria, all six conformers could be discriminated, with their relative configuration (*threo* or *erythro*) determined accordingly.

1,3-Methine Systems. To determine the relative configuration of two methine carbons separated by a methylene group, the pair of diastereotopic methylene protons must be assigned stereospecifically. This task is not always easy using NOE, particularly for large molecules with masses above 1000 Da, in which spin diffusion between methylene protons tends to obscure NOE-based assignments. Using coupling constants, assignments can be made for large molecules as reported for maitotoxin.[13b−d] The method for assigning diastereotopic methylene protons with respect to the adjacent methine is similar to that for vicinal methines described above. In these structural units, six conformers are possible when a pair of protons on a methylene is stereochemically labeled according to their chemical shifts. All these conformers can be unambiguously identified using $^3J_{H,H}$ and $^{2,3}J_{C,H}$, as depicted in Figure 4. With one methylene−methine relationship in hand, the same examination of another relationship leads to diastereomeric assignment of the 1,3-methine groups via the stereospecifically labeled methylene protons. If ^{1}H NMR signals of relevant protons are separated, this method can be applied to 1,4-methine systems separated by an ethylene group.

Configuration Assignment for Systems with Alternating Conformers. In acyclic systems, two or three rotamers frequently coexist. The contributions from minor rotamers that comprise less than 1 Hz are negligible (in most cases, 1 Hz is around 15% of the difference in $^{2,3}J_{C,H}$ between anti and gauche conformers). In other words, if one conformer comprises over 85% of the population, configuration analysis can be safely carried out only for the dominant conformer. In the following sections, the correlation of $^{2,3}J_{C,H}/^3J_{H,H}$ in *erythro* and *threo* configurations is examined for 1,2-methine and 1,3-methine systems with alternating conformers.

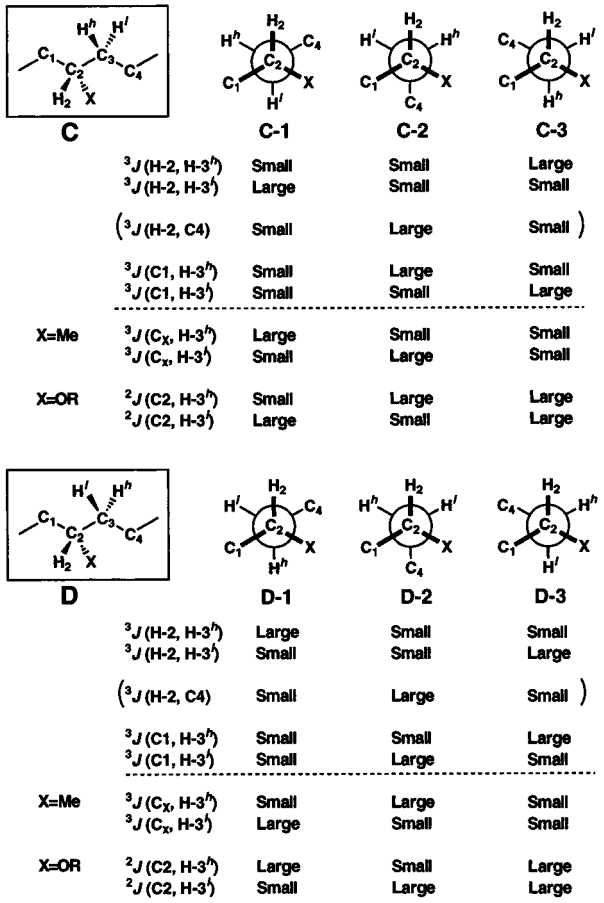


Figure 4. Dependence of $^3J_{H,H}$ and $^{2,3}J_{C,H}$ on dihedral angles between methine and methylene in 2-substituted butane systems. R stands for H, alkyl, or/and acyl groups. Six sets of J values are derived from two different stereospecific assignments for methylene protons on C3, which are labeled according to their chemical shifts; H^h is observed at higher field and H^l is at lower field. With $^3J_{H,H}$ alone, none of the six rotamers can be distinguished. However, with use of either $^3J_{C1,H-3^h}/^3J_{C1,H-3^l}$, $^3J_{CX,H-3^h}/^3J_{CX,H-3^l}$, or $^2J_{C2,H-3^h}/^2J_{C2,H-3^l}$, they are clearly identified, which leads to the stereospecific assignment of the methylene protons on C3 with respect to C2.

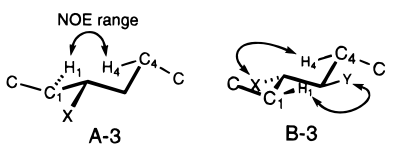
Table 1. $^3J_{H,H}$ and $^{2,3}J_{C,H}$ Values (Hz) for Anti and Gauche Orientations in Acyclic Systems

	$^3J_{H,H}$		$^2J_{C,H}$		$^3J_{C,H}$	
oxygenation	anti large	gauche small	gauche[a] large	anti[b] small	anti large	gauche small
none	9−12	2−4	−[c]	−	6−8	1−3
mono	8−11	1−4	−5 to −7[d]	0 to −2[d]	6−8	1−3
di	7−10	0[e]−3	−4 to −6[f]	2−0[f]	5−7[g]	1−3[g]

[a,b] Oxygen functions on relevant carbons are gauche and anti to their vicinal protons, respectively (see Figure 1b). [c] $^2J_{C,H}$ values do not show dihedral dependence. [d] $^2J_{C,H}$ values are those between an oxygenated carbon and a proton on the neighboring carbon. [e] The value (0) is observed for the system where both protons are anti to oxygen atoms such as A-1 in Figure 3. [f] $^2J_{C,H}$ values are those between an oxygenated carbon and a proton on the neighboring oxygenated carbon. [g] $^3J_{C,H}$ values are those between a proton on an oxygenated carbon and a carbon at the vicinal position via another oxygenated carbon (see Figure 1c).

1,2-Methine Systems. When $^3J_{H,H}$ attains an intermediate value between anti and gauche (see Table 1), two major rotamers with H/H-anti and gauche orientations should be considered. These conformational changes

(25) If $^3J_{H,H}$ shows an anti-typical value, NOE analysis should be reliable since no significant conformational change takes place. In the A-3 system, H-1 and H-4 should come close enough to give rise to a prominent NOE, since the carbon chains extending from C1 and C4 tend to adopt a C/C-anti conformation as depicted below. During our studies,[13] however, we have not encountered the predominant occurrence of A-3; usually, A-3 coexists with A-1, where their configuration can be assigned by coupling constants (Figure 5). For the B-3 case, in addition to the absence of NOE (or ROE) between H-1 and H-4, NOE (ROE) between H-1 and a proton on Y or between H-4 and a proton on X should be observed when Y or X is a methyl or hydroxyl group; NOESY experiments in DMSO-d_6 sometimes show prominent NOEs from OH protons in these systems.



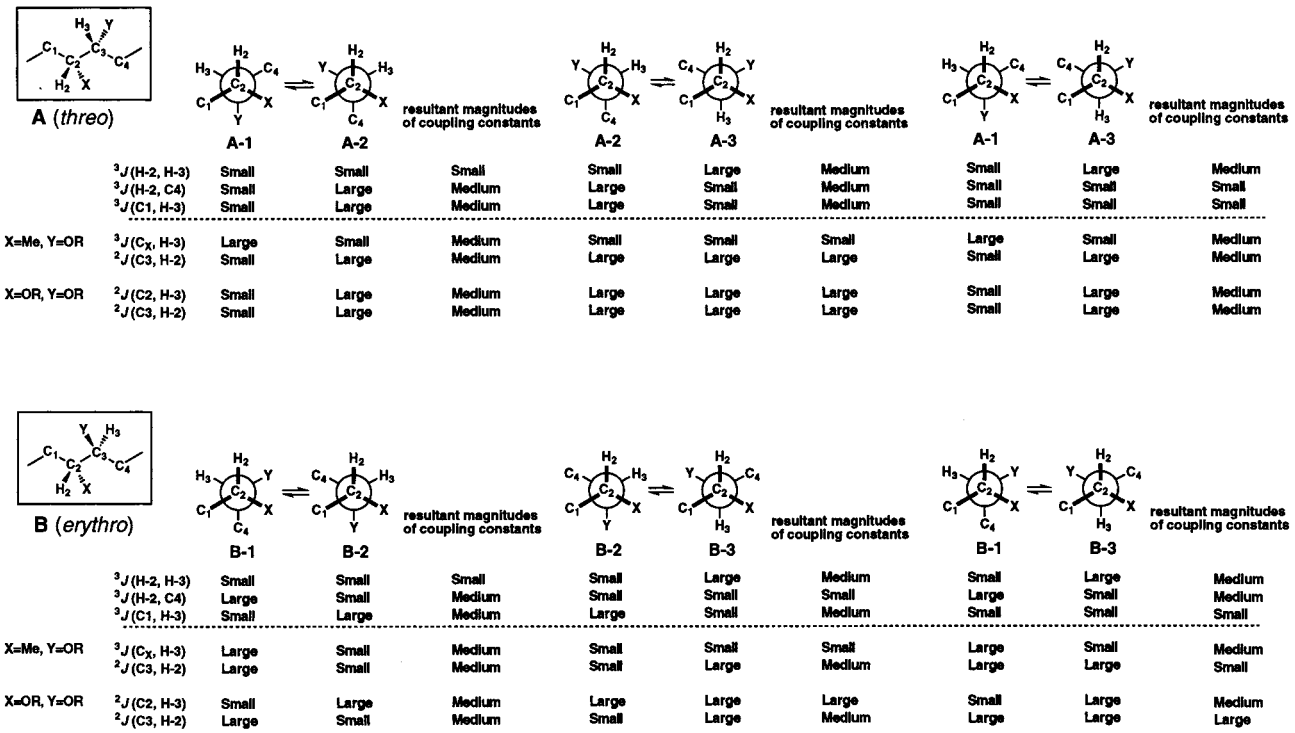


Figure 5. Dependence of $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ on dihedral angles between vicinal methine carbons in alternating butane systems. R stands for H, alkyl, or/and acyl groups. When $^{3}J_{H,H}$ is a medium value as a mixture of anti and gauche, the four pairs of alternating rotamers A-2/A-3, A-1/A-3, B-2/B-3, and B-1/B-3 can be clearly identified using either $^{3}J_{C4,H-2}/^{3}J_{C1,H-3}$, $^{3}J_{CX,H-3}/^{2}J_{C3,H-2}$, or $^{2}J_{C2,H-3}/^{2}J_{C3,H-2}$. H/H-gauche pairs, A-1/A-2 and B-1/B-2, cannot be identified with their configurations, but these pairs rarely occur in acyclic structures of natural products since they are thought to be thermodynamically disfavored pairs. See Table 1 for small, medium, and large volumes.

are often observed in natural products.[13] In such a case, four out of six alternating pairs can be unambiguously identified using $^{2,3}J_{C,H}$ and $^{3}J_{H,H}$ as shown in Figure 5, where all four H/H-anti/gauche pairs of rotamers A-2/A-3, A-1/A-3, B-2/B-3, and B-1/B-3 give rise to different combinations of *J* values. When both alternating rotamers have an H/H-gauche orientation (A-1/A-2 or B-1/B-2 in Figure 5), their configuration cannot be assigned using $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ alone. In these conformers, however, all the substituents on C2 and C3 are gathered in one side, thus making them thermodynamically disfavored. As far as we know, the occurrence of these pairs of rotamers is extremely rare in natural products. Another case in which the *J*-based method fails to determine configuration is that of three rotamers having comparable populations, which leads to an averaging of all *J* values.[26] In this case, if a minor rotamer comprises less than 10% of the population, its contribution can be disregarded and the configuration can be assigned as described above (an example of this case will be discussed later).

1,3-Methine Systems. When at least one of $^{3}J_{H,H}$ values for methylene protons has an intermediate value, the presence of multiple rotamers should be considered. This conformational change is occasionally seen in natural products when rotational changes occur as C-1/C-3 or D-1/D-3 in Figure 6 (these conformers are observed in **5**), while the other pairs of conformers are rarely present because thermodynamically unlikely rotamers (C-2 and D-2) with double gauche interactions between C1/C4 and between X/C4 are involved. Similar treatments to those for the alternating methine−methine systems are possible for the elucidation of the relative configuration between 1,3-methines through stereospecific assignment of diastereotopic methylene protons (Figure 6).

Measurements of $^{2}J_{C,H}$ and $^{3}J_{C,H}$ Values. Several practical methods have been proposed for the measurement of $^{2,3}J_{C,H}$.[12] Among those, we adopted two methods, hetero half-filtered TOCSY (HETLOC)[19] and phase-sensitive HMBC (PS-HMBC).[20] HETLOC gives a $^{1}H-^{1}H$ 2D spectrum, where $^{2,3}J_{C,H}$ is represented by the dislocation, along *F2*, of a doublet cross-peaks split by $^{1}J_{C,H}$ (Figure 7). With this method, accurate $^{2,3}J_{C,H}$ values can be determined even for a sample at millimolar concentration regardless of the size of the *J* coupling. Another advantage of this method is that signs of *J* values can be obtained (Figure 7). However, HETLOC has two drawbacks: all carbons including those residing between a relevant carbon and its long-range coupling proton have to be protonated, and the sensitivity is dependent on the magnitude of each $^{3}J_{H,H}$ through which TOCSY transfers magnetization. In such a case, PS-HMBC[20] is used, which is designed to reproduce a ^{1}H signal shape by setting a refocus time before acquisition and by the application of ^{13}C-decoupling during acquisition. In an original sequence of PS-HMBC reported by Bax et al.[20] a 1D reference spectrum is necessary to obtain the absolute value of $^{2,3}J_{C,H}$, although this is not always possible for natural

(26) The presence of three rotamers with comparable populations can be recognized by the $^{3}J_{H,H}$ value according to Table 1. In such a case, $^{3}J_{H,H}$ becomes a marginal value between gauche and medium because of the coexistence of two gauche and one anti rotamer. Additionally, none of the $^{2,3}J_{C,H}$ values should take the typical gauche values since, unlike a mixure of two conformers, anti interactions should be involved in all $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ (Figure 5). A minor conformer with 10% or less population does not affect the average *J* value significantly, and thus its presence does not usually result in a deviation in *J* values.

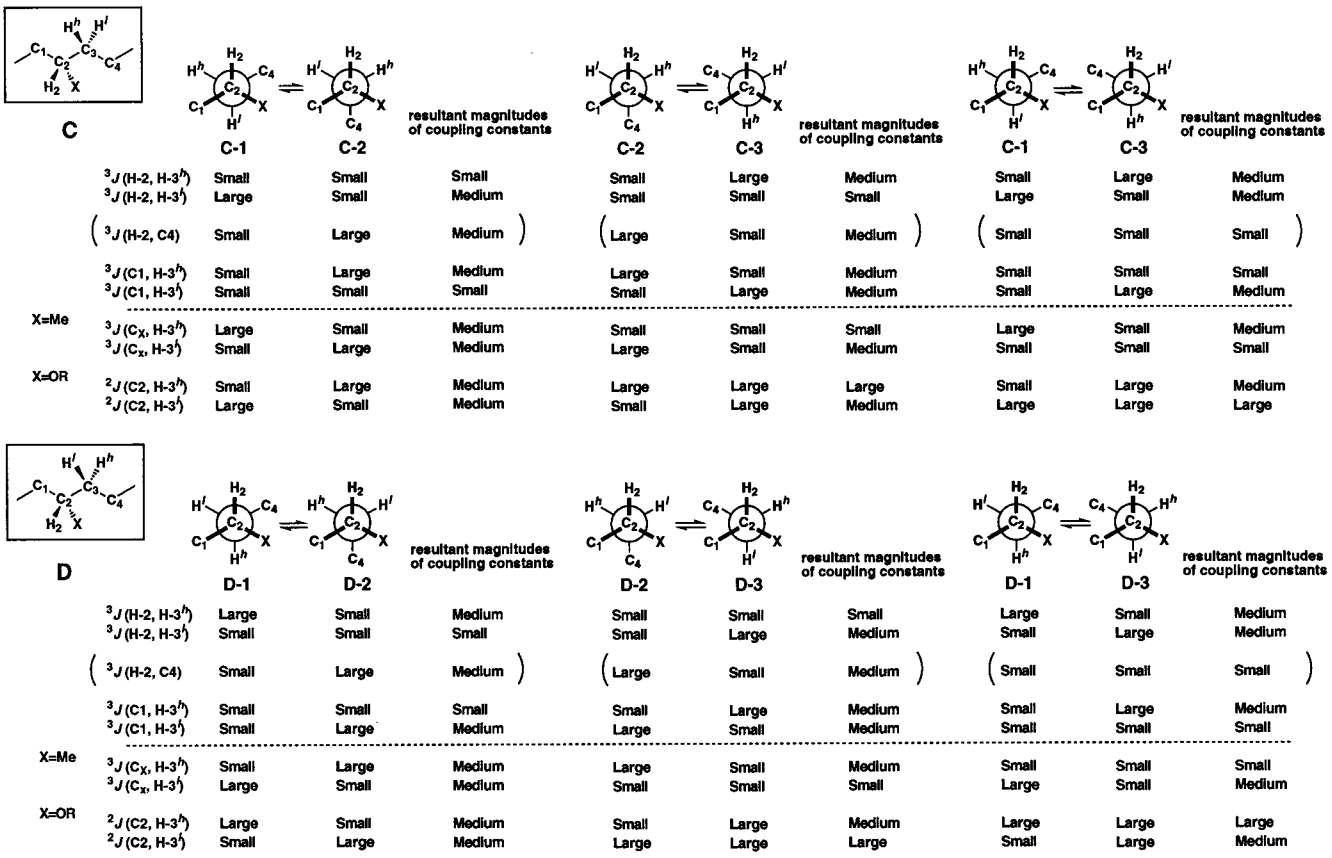


Figure 6. Dependence of $^3J_{H,H}$ and $^{2,3}J_{C,H}$ on dihedral angles between methine and methylene in alternating butane systems. R stands for H, alkyl, or/and acyl groups. Methylene protons are labeled according to their chemical shifts; H^h (H-3^h) and H^l (H-3^l) are protons with higher and lower-field signals, respectively. When either $^3J_{H-2,H-3^h}$ or $^3J_{H-2,H-3^l}$ takes a medium value, the alternating rotamers should be considered. Six pairs of the rotamers with the different prochiral assignments for methylene protons can be clearly distinguished using either $^3J_{C1,H-3^h}/^3J_{C1,H-3^l}$, $^3J_{CX,H-3^h}/^3J_{CX,H-3^l}$, or $^2J_{C2,H-3^h}/^2J_{C2,H-3^l}$.

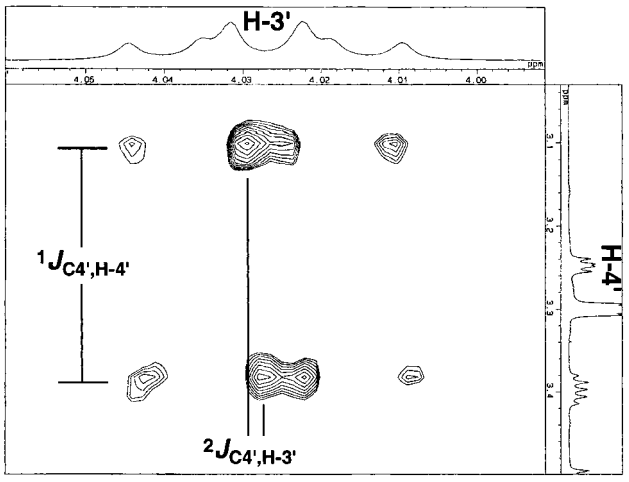


Figure 7. Partial HETLOC spectrum of carboxylic acid **5** from zooxanthellatoxin. An enlarged spectrum for H-3′ at δ 4.027 on F_2 versus H-4′ at δ 3.25 on F_1 is shown. Dislocation along F_2 of the doublet cross-peak split along F_1 with $^1J_{C4',H-4'}$ corresponds to $^2J_{C4',H-3'}$ (−1 Hz). Upper left way of the dislocation indicates that the sign of the J is minus.

products due to extensive overlap of ^{1}H signals. In this study $^{2,3}J_{C,H}$ values were not directly measured but were obtained from the ratio of their cross-peak magnitudes with respect to a common proton. In this PS-HMBC experiments, the absolute values are obtained by the following equation using $^{2,3}J_{C,H}$ derived from other methods (e.g., HETLOC) as reference:[13a]

$$I_{C_a,H}/I_{C_b,H} = \sin^2(\pi\, ^{2,3}J_{C_a,H}\Delta)/\sin^2(\pi\, ^{2,3}J_{C_b,H}\Delta)$$

where $I_{C_a,H}$ and $I_{C_b,H}$ are the intensities of cross-peaks due to C_a-H and C_b-H couplings, respectively, and Δ is a time interval in the pulse sequence.[20]

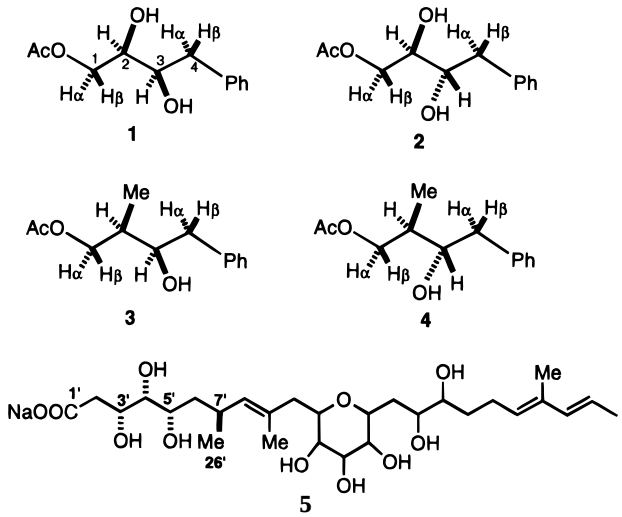
To minimize the effect of intramolecular hydrogen bonding, we used a mixed solvent of pyridine-d_5 and CD_3OD (1:1) for measurement of $^3J_{H,H}$ and $^{2,3}J_{C,H}$. However, the coupling constants of model compounds **1** and **2** measured in the mixed solvent were not very different from those in a $CDCl_3$ solution,[27] in which hydrogen bonds should make a greater contribution to the distribution of rotational conformation, implying that they may not play a major role in these systems. We have measured $^{2,3}J_{C,H}$ values for several highly oxygenated compounds[13b−f] in the mixed solvent or methanol. Successful assignments of their configurations based on these data indicate that significant deviation from typical $^{2,3}J_{C,H}$ values (Table 1) hardly occurs even in polyhydroxy structural moieties, which means that intramolecular hydrogen bonding causes no significant conformational aberration (less than 1 Hz) from the staggered rotamers in these polar solvents.

(27) Coupling constants of **1** and **2** in $CDCl_3$ are as follows. **1**: $^3J_{H-2,H-3} = 3.4$ Hz; $^2J_{C2,H-3} = 0$ Hz, $^2J_{C3,H-2} = -2$ Hz; **2**: $^3J_{H-2,H-3} = 6.2$ Hz, $^2J_{C2,H-3} = -4$ Hz, $^2J_{C3,H-2} = -3$ Hz. These values indicate that their C2−C3 bond rotations in $CDCl_3$ are similar to those in C_5D_5N−CD_3OD (1:1) (see Table 3); the difference by 0.8 Hz in $^3J_{H-2,H-3}$ of **2** is thought to be caused by hydrogen bonding between 2-OH and 3-OH, which resulted in the increased population of an OH/OH-gauche rotamer in $CDCl_3$.

Table 2. Calculated $^3J_{H,H}$ for Anti and Gauche Orientations in Fluctuating Systems of Methine−Methylene and Methine−Methine in Substituted Hexanes

substitution (C2/C5 dihedral angle)	corresponding rotamers	interproton dihedral angle (clockwise) and $^3J_{H-3,H-4}$[a] (Hz)		
		near 60°	near 180°	near 300°
3-Me[b] (C2/C5 60°)	C-3/D-3	64° 2.5 (2.1)[h]	177° 11.6 (12.1)	
(C2/C5 180°)	C-1/D-1		173° 11.3 (12.0)	304° 4.0 (3.4)
(C2/C5 300°)	C-2/D-2	53° 4.4 (3.9)*1		297° 2.6 (2.4)
3-OH[c] (C2/C5 60°)	C-3/D-3	62° 1.8 (1.4)	173° 10.3 (10.9)	
(C2/C5 180°)	C-1/D-1		184° 10.2 (10.7)	302° 3.7 (3.6)
(C2/C5 300°)	C-2/D-2	63° 1.8 (1.4)		294° 2.7 (2.5)
3,4-*threo* OH/OH[d]	A-2,3,1	65° 3.5 (3.3)*2	176° 8.1 (8.5)	302° 1.0 (0.7)
3,4-*erythro* OH/OH[e]	B-2,3,1	70° 1.7 (1.5)	179° 8.4 (8.8)	290° 1.7 (1.5)
3,4-*threo* Me/OH[f]	A-2,3,1	67° 1.3 (1.1)	182° 9.5 (10.1)	297° 3.2 (3.0)
3,4-*erythro* Me/OH[g]	B-2,3,1	70° 2.5 (2.1)	181° 9.6 (10.2)	298° 1.7 (1.5)

[a] *The values with an asterisk do not fit to the criteria in Table 1, but the rotamers with *1 and *2, corresponding to C-2 (D-2) and A-2, respectively, rarely occur in natural products because of their thermodynamically disfavored conformations. The calculations were carried out for: [b]3(*R*)-methylhexane, [c]3(*S*)-hexanol, [d](3*R*,4*R*)-hexanediol, [e]*meso*-3,4-hexanediol, [f](3*R*,4*R*)-4-methyl-3-hexanol, and [g] (3*R*,4*S*)-4-methyl-3-hexanol. [d−g] These configurations correspond to those of model compounds **1**, **2**, **3**, and **4**, respectively. [h] The values in parentheses are those at each local energy minimum (see Experimental Section for details).



Typical Values of $^3J_{H,H}$ and $^{2,3}J_{C,H}$ for Systems with Oxygen Functionalities. Electron-withdrawing substituents such as a hydroxy group affect the magnitude of $^3J_{H,H}$ and $^{2,3}J_{C,H}$. During our configuration analyses for acyclic structures appearing in natural products,[13] we categorized $^3J_{H,H}$ and $^{2,3}J_{C,H}$ values as *small*, *medium*, or *large* to fit them into the criteria in Figures 3−6. We reexamined the categories with respect to the number of oxygen functionalities and defined the allowances for *small* (gauche $^3J_{H,H}$ and $^3J_{C,H}$; anti H/OH for $^2J_{C,H}$) and *large* (anti $^3J_{H,H}$ and $^3J_{C,H}$; gauche H/OH for $^2J_{C,H}$), as shown in Table 1. The values between *small* and *large* are regarded as *medium* in Figures 5 and 6. To validate these criteria, *J* values were calculated for simple models using both MM2 and a Karplus-type equation. Haasnoot et al. proposed a modified Karplus-type equation, in which the orientation of substituents and their electronegativity are taken into account.[28] However, since this equation was based on data from relatively rigid structures such as cyclic compounds, the derived $^3J_{H,H}$ values must be adjusted for flexible acyclic systems. Values of $^3J_{H,H}$ for 3-substituted and 3,4-disubstituted hexane (Table 2) were calculated as a weighted average, in which fluctuation from a staggered angle was taken into account. The range of *J* values in Table 1 appears to be reasonable when compared with calculated values (Table 2).

(28) Haasnoot, C. A. G.; De Keeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783−2792.

Table 3. $^3J_{H,H}$ and $^{2,3}J_{C,H}$ (Hz) with Respect to the C2−C3 Bond in Model Compounds 1−4[a]

compound	H-2/H-3	C2/H-3	C3/H-2	C1/H-3	C4/H-2	Me/H-3
1	2.9 (2.5)[b]	0[c]	−1	2	1	−
2	7.0 (7.8)	−4	−3	3	3	−
3	3.0 (2.5)	−2	−1	3[d]	2	6
4	5.7 (6.2)	−2	−5	4	3	4

[a] The values were determined in $C_5D_5N-CD_3OD$ (1:1) for **1** and **2** or in $C_5D_5N-CD_3OD$ (1:2) for **3** and **4**. [b] Coupling constants in parentheses were measured at −33 °C. [c] $^{2,3}J_{C,H}$ values were rounded to single digits because of the digital resolution. [d] Since the accurate value could not be determined for **3** due to overlapping satellite signals of H_2-1, $^3J_{C1,H-3}$ was measured with a deacetyl derivative of **3**, of which all $^3J_{H,H}$ values were almost identical with those of **3**, suggesting the close similarity of their conformations.

***J*-Based Configuration Analysis Using Model Compounds.** Model compounds **1**−**4** were designed and synthesized to examine the validity of the *J*-based method in the configuration analysis of natural products (substituted hexanes in Table 2, although ideal for determining the interaction between substituents, were unsuitable due to overlapping signals in ^{1}H NMR). With vicinal dihydroxy models **1** and **2**, their $^3J_{H,H}$ values were typical of H-2/H-3 gauche and anti, respectively (Table 3). For the *threo* isomer **1**, there are three cases in which $^3J_{H-2,H-3}$ assumes a gauche value; i.e., a single rotamer with A-1, A-2 (Figure 3), or alternating A-1/A-2 (Figure 5). Among these, the A-1 rotamer is best fitted for the observed values of $^2J_{C2,H-3}$ and $^3J_{C4,H-2}$ (Table 3), which indicate anti C2-OH/H-3 and gauche C4/H-2, respectively; in A-2, both $^2J_{C2,H-3}$ and $^3J_{C4,H-2}$ should be large and, in the alternating pair of A-1/A-2, both should be medium. These results indicated the dominant conformation of **1** to be A-1 in Figure 3. In the other vicinal diol system, *erythro* isomer **2** predominantly takes an all-anti orientation such as B-3 in Figure 3, in which all the relevant $^{2,3}J_{C,H}$ showed gauche values (Table 3). In these *threo* and *erythro* dihydroxy systems, an extended (C1/C4-anti) conformer was predominant.

In vicinal-methylhydroxy systems, *threo* model **3** revealed a typical $^3J_{H-2,H-3}$ value for gauche and a $^{2,3}J_{C,H}$ value for anti C3−OH/H-2 and Me/H-3, again confirming that **3** takes mainly the A-1 configuration (Table 3). In contrast, *erythro* hydroxy-methyl model **4** showed the medium value of $^3J_{H-2,H-3}$ (5.7 Hz), which suggested the presence of two or more alternating rotamers. The values of $^3J_{H-2,H-3}$, $^3J_{H-2,C4}$, and $^3J_{C1,H-3}$ suggested that a pair of B-2/B-3 rotamers is predominant (Table 3). However,

Table 4. $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ (Hz) with Respect to the C3−C4 Bond in Model Compounds 1−4[a]

compound	H-3/ H-4α	H-3/ H-4β	C3/ H-4α	C3/ H-4β	C2/ H-4α	C2/ H-4β
1	5.5	8.2	−4	−6	3	4
2	8.9	3.0	−6	−3	3	2
3	5.4	8.3	−4	−7	–	–
4	8.8	3.9	−7	−3	3	2

[a] The values were determined in the same conditions as those in Table 3.

$^{3}J_{Me,H-3}$ and $^{2}J_{C3,H-2}$ do not accurately correspond to these rotamers, which implies that the third rotamer B-1 exists with a comparable population. For configuration analysis, the assumption of B-2/B-3 rotamers may lead to the correct answer, but in these cases with three rotamers of comparable populations, this method should be carefully applied. The value of $^{3}J_{H-2,H-3}$ of **4** at −33 °C (shown in parentheses in Table 3) revealed that the population of the B-1 rotamer decreased, which may allow us to regard **4** as consisting of alternating B-2/B-3 rotamers. The other models **1**−**3** also gave rise to typical values at low temperatures, which may facilitate the *J*-based analysis.[29]

Force field calculations (MM2* force field, MacroModel) further supported these results. As expected, for models **1**−**3**, in which the *J* data indicated C1/C4-anti conformers to be predominant, the calculation also showed that their lowest energy appeared at this orientation.[22] Conversely, the calculations revealed that the Me/C4-anti conformer is most stable in **4**, which parallels the results from the *J*-based analysis.

For examination of the rotational conformer around a methine−methylene bond, the C3−C4 bonds in **1**−**4** are good models. In all four systems, the extended conformers are dominant because the protons that are anti to H-3 give rise to large $^{3}J_{H,H}$ values of 8.2−8.9 Hz (Table 4). In contrast, the gauche protons show values of $^{3}J_{H,H}$ that vary from 3.0 to 5.5 Hz, the latter of which is too large for gauche interaction, suggesting the presence of a minor rotamer with a C2/phenyl-gauche orientation. In model **1**, the values of $^{3}J_{C2,H-4\alpha}$ and $^{3}J_{C2,H-4\beta}$ (accurate values are 2.6 and 3.7 Hz, respectively) reveal that a C2/phenyl-gauche conformer is present, where H-4β is anti to C2 (Table 4). On the other hand, a pair of $^{3}J_{H,H}$ values typical of anti/gauche interactions is observed for **2**. This is also supported by $^{3}J_{C2,H-4\alpha}$ and $^{3}J_{C2,H-4\beta}$ (accurate values are 2.5 and 1.9 Hz, respectively), showing their gauche orientation. These observations clearly demonstrated that based on $^{2,3}J_{C,H}$ and $^{3}J_{H,H}$, two diastereotopic protons can be assigned stereospecifically.

Application to Carboxylic Acid Moiety Obtained from Zooxanthellatoxin. Zooxanthellatoxin-A (ZT-A) and ZT-B were isolated as potent vasoconstrictive substances from a symbiotic marine dinoflagellate *Symbiodinium* sp.[9,30] The structures of ZTs were determined by extensive spectroscopic analysis of the intact toxins and the degradation products of periodate oxidation or LiOH hydrolysis.[9] The carboxylic acid **5** obtained from the hydrolysates was further subjected to stereochemical elucidation by spectroscopic and synthetic approaches.[31] In this study the relative configuration for the C3′−C7′ part was investigated by the *J*-based configuration analysis.

Table 5. $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ (Hz) for the C3′−C7′ Part of 5 from Zooxanthellatoxin[a]

coupled nuclei	$^{3}J_{H,H}$	coupled nuclei	$^{2}J_{C,H}$	coupled nuclei	$^{3}J_{C,H}$
H-3′/H-4′	4.5	C3′/H-4′	−1	C2′/H-4′	2
H-4′/H-5′	3.0	C4′/H-3′	−1	C26′/H-6′^{h}	5
H-5′/H-6′^{h}[b]	7.0	C4′/H-5′	1		
H-5′/H-6′^{l}[b]	7.0	C5′/H-4′	0		
H-6′^{h}/H-7′	7.5	C5′/H-6′^{h}	−6		
H-6′^{l}/H-7′	7.0	C5′/H-6′^{l}	−4		

[a] The values were determined in CD_3OD. [b] H-6′^{h} and H-6′^{l} are those at δ 1.42 and 1.62, respectively.

Using HETLOC and PS-HMBC, *J* values were successfully determined (Table 5). The rotational conformer with respect to the C3′−C4′ bond was treated as an alternating system because of the medium value (4.5 Hz) of $^{3}J_{H-3',H-4'}$. The *J* values of −1 Hz for both $^{2}J_{C3',H-4'}$ and $^{2}J_{C4',H-3'}$ can be regarded as medium values (Table 1). Because the data suggested that H-3′/4′-OH and H-4′/3′-OH alternated anti and gauche interaction, only the pair of rotamers shown in Figure 8a, corresponding to A-1 and A-3 (Figure 5), satisfied all these requirements. Thus, the diastereomeric relation between C3′ and C4′ was determined to be 3′*R** and 4′*R**. With respect to the C4′−C5′ bond, the values of $^{2}J_{C4',H-5'}$ and $^{2}J_{C5',H-4'}$ were determined to be 1 and 0 Hz, respectively, indicating the predominant anti orientation for 4′-OH/H-5′ and 5′-OH/H-4′ (Figure 8b). Because the specific rotamer can be identified by two anti substituents, the major rotamer around this bond was determined to be A-1 in Figure 3, hence showing the relative stereochemistry to be 4′*R** and 5′*S**.

For the stereochemical relationship between C5′ and C7′, assignments of diastereotopic methylene protons were first attempted. With respect to the C5′−C6′ bond, a medium value of 7 Hz was observed for both $^{3}J_{H-5',H-6'^{h}}$ and $^{3}J_{H-5',H-6'^{l}}$, which suggested the presence of two alternating rotamers between anti and gauche orientations. On the other hand, the large $^{2}J_{C5',H-6'^{h}}$ (−6 Hz) fell within the range of typical values (Figures 6 and 8c), indicating the gauche orientation of 5′-OH/H-6′^{h} in both rotamers. A single pair (D-1/D-3) of rotamers in Figure 6 meets these requirements. Regarding the C6′−C7′ bond, the presence of alternating rotamers was again implied by medium values of $^{3}J_{H,H}$ for H-6′^{h}/H-7′ (7.5 Hz) and H-6′^{l}/H-7′ (7.0 Hz). These data showed that H-6′^{h}/H-7′ was gauche and H-6′^{l}/H-7′ was anti in one rotamer, whereas their orientations were exchanged in the other. In addition, a medium value for $^{3}J_{C26',H-6'^{h}}$ (5 Hz) suggested the C26′/H-6′^{h} orientation to be anti in one rotamer and gauche in the other (Figure 8d). Thus, C6′−C7′ was thought to undergo conformational change corresponding to C-1/C-3 in Figure 6. Assembling these two diastereomeric relationships led to the elucidation of C5′/C7′ to be 5′*S** and 7′*S**. Consequently, the relative

(29) As judged from their relaxation times, molecular motions and internal rotations of **1**−**4** seemed to be much faster than those of natural products with masses around 1000 Da, for which this *J*-based method was originally designed. Measurements at low temperature, which slow these motions to the level of larger molecules, resulted in decreasing the population of the minor conformers in **1**−**4**. The values at low temperature in parentheses (Table 3) may reproduce the corresponding stucture units in larger molecules more precisely.

(30) (a) Nakamura, H.; Asari, T.; Fujimaki, K.; Maruyama, K.; Murai, A.; Ohizumi, Y.; Kan, Y. *Tetrahedron Lett.* **1995**, *36*, 7255−7258. (b) Nakamura, H.; Asari, T.; Murai, A.; Kondo, T.; Yoshida, K.; Ohizumi, Y. *J. Org. Chem.* **1993**, *58*, 313−314.

(31) Nakamura, H.; Maruyama, K.; Murai, A. *Abstracts of Papers*, 72th Annual Meeting of Japan Society of Chemistry, Tokyo; Japan Society of Chemistry: Tokyo, 1997; 3G4 29 (p 1048). Structural studies by synthesis will be published elsewhere.

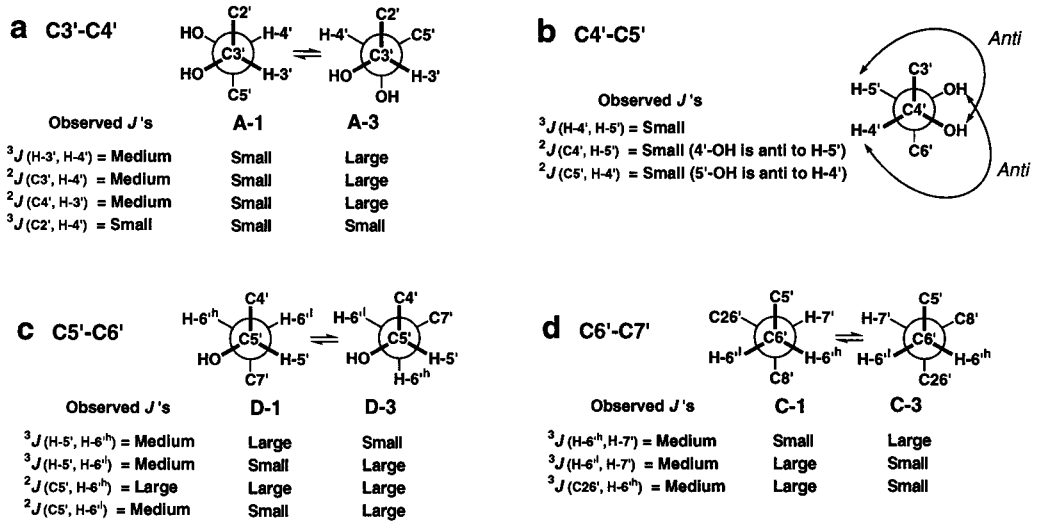


Figure 8. Configuration analysis of carboxylic acid **5**. a: Alternating rotamers with respect to the C3′−C4′ bond. b: The predominant rotamer with respect to the C4′−C5′ bond. c and d: Pairs of alternating rotamers with respect to the C5′−C6′ and C6′−C7′ bonds, respectively.

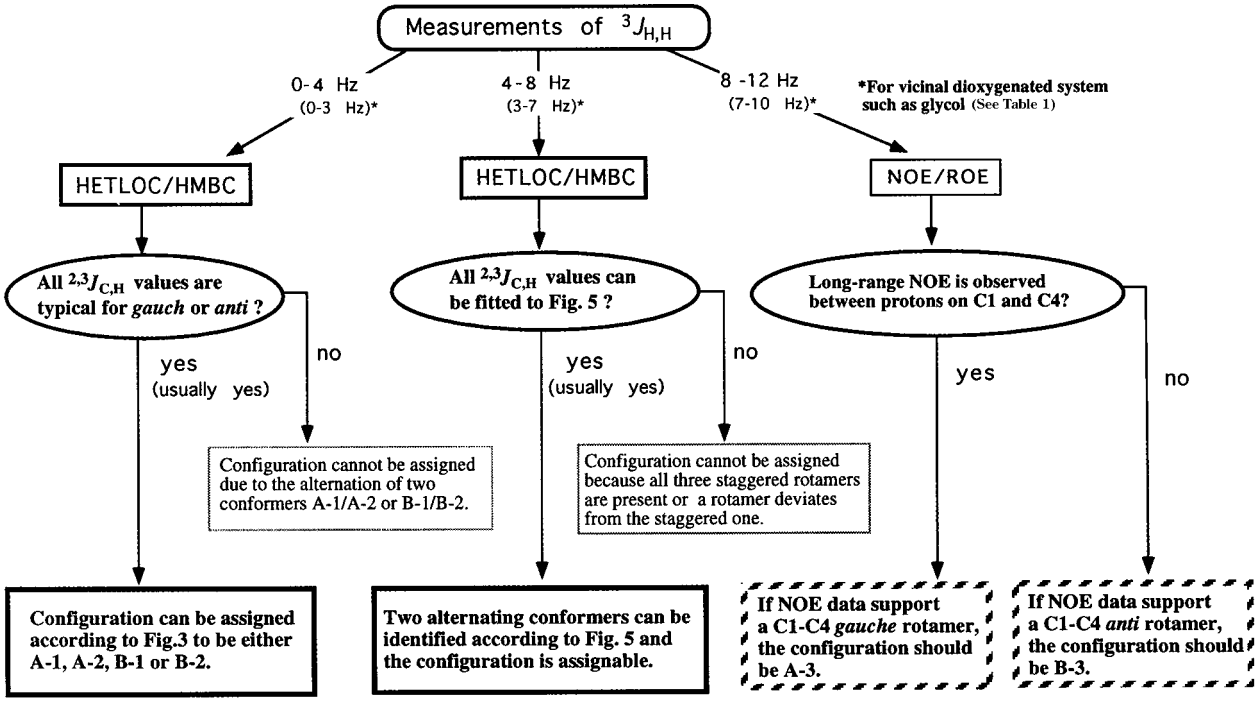


Figure 9. Configuration assignment for vicinal methine systems on the basis of $^{2,3}J_{C,H}$ and $^{3}J_{H,H}$, and partly NOEs.

configuration for the C3′−C7′ portion of **5** was determined as 3′*R**, 4′*R**, 5′*S**, and 7′*S**. This assignment has been confirmed by the synthesis of the ozonolysis product corresponding to C1′−C8′.[31]

Discussion

Besides the compounds described above, this *J*-based analysis has been used for the stereochemical determination of several natural products,[13] which resulted in the elucidation of 40 stereogenic centers. These successful applications demonstrate that this method is reliable for acyclic structures with hydroxyl (alkoxyl) and methyl groups. Its applicability to the other substituents, such as halogen, nitrogen, carbonyl, and bulky functionalities, is currently being investigated and will be published in due course.

Figure 9 is a schematic diagram showing the assignment of the diastereomeric relationship of vicinal methine systems. In the first step, the six possible rotamers or the alternating pairs arising from *threo* and *erythro* stereoisomers are classified into three cases according to their size of $^{3}J_{H,H}$ values. For vicinal dioxygenated systems, the *J* value becomes smaller as shown in Table 1. When $^{3}J_{H,H}$ shows a typical anti orientation, NOE experiments are necessary, because $^{2,3}J_{C,H}$ does not provide any further information on configuration. In other cases with $^{3}J_{H,H}$ values less than 8 Hz (7 Hz), $^{2,3}J_{C,H}$ values allow us to assign configurations and conformations according to Figures 3, 5, and 9.

There are three exceptions in which the $^{2,3}J_{C,H}$ analysis fails to determine configuration as described earlier: one is the case in which the two alternating conformations

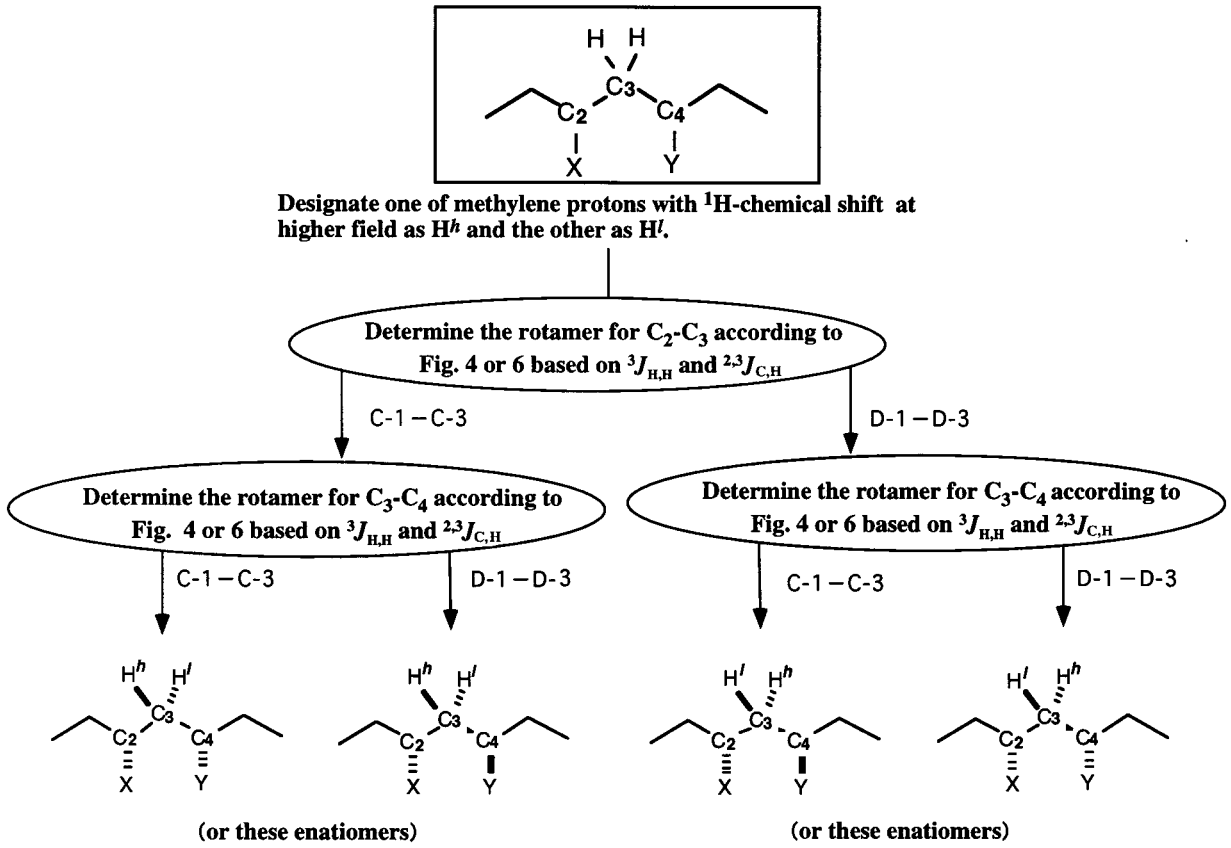


Figure 10. Configuration assignment for 1,3-methine systems.

have an H/H-gauche orientation as A-1/A-2 or B-1/B-2 in Figure 5, and the others are those in which three staggered rotamers coexist with comparable populations or where a rotational conformer deviates from the staggered one. These three cases, to our knowledge, rarely occur in acyclic structures of natural products.

A schematic diagram of the configuration assignment for 1,3-methine systems is given in Figure 10. Prochiral protons on the methylene are first designated as H^h and H^l according to their ^1H chemical shifts, and the rotational conformation is determined for one $CH-CH_2$ bond using $^3J_{H,H}$ and $^{2,3}J_{C,H}$ (see Figures 4, 6, and 10). In the next step, the rotamer for the other CH_2-CH bond is examined in the same way. The four possible cases, 1,3-syn with β-H^h/α-H^l, 1,3-anti with β-H^h/α-H^l, 1,3-anti with α-H^h/β-H^l, and 1,3-syn with α-H^h/β-H^l, can be discriminated, thus leading to the elucidation of the 1,3-methine diastereomeric relationship in combination with the stereospecific assignment of the methylene protons.

This *J*-based configuration analysis is applicable to all organic compounds bearing asymmetric centers, which include not only natural products but synthetic molecules such as intermediates for natural product syntheses. As reported previously,[13a] the amount of a sample necessary to determine $^{2,3}J_{C,H}$ using the natural abundance of the ^{13}C isotope is about 10 μmol when a modern NMR instrument of 400−600 MHz is used; this amount is comparable to that used in most structure elucidations. Compared to conventional approaches consisting of chemical degradation and synthesis of all possible diastereomers, our method greatly diminishes labor and time. As demonstrated in the structure studies on maitotoxin,[13b−d,32] we reduced the number of synthetic diastereomers needed to 2 from 128 possible candidates using this *J*-based method.[13c]

NOE analysis is an excellent method for conformation and configuration assignments of cyclic compounds as seen in numerous examples in structure studies of natural products. Conversely, the *J*-based method works better for acyclic systems than for cyclic compounds, in which deviations from the staggered rotamers frequently occur. Therefore, the complementary use of both the *J*-based method and NOE analysis greatly facilitates the stereochemical determination of complicated organic compounds.

Experimental Section

General Procedure. Unless otherwise noted, all reagents and NMR solvents were obtained from standard commercial sources and used without further purification. Column chromatography was carried out on silica gel (E. Merck, 70−230 mesh) with use of hexanes−EtOAc as a mobile phase.

Methods for Measuring $^{2,3}J_{C,H}$. Geminal and vicinal carbon−proton coupling constants ($^{2,3}J_{C,H}$) were measured using two methods. The 2D hetero half-filtered TOCSY (HETLOC) experiment, which was originally proposed by Otting and Wüthrich,[19] is one of the most powerful methods for measuring $^{2,3}J_{C,H}$. In this study the pulse sequence reported by Leibfritz was used, which consisted of a BIRD−half-filter−TOCSY succession.[19b] Another method used in this study was phase-sensitive HMBC reported by Zhu and Bax.[20] All the spectra were measured with a JEOL A-500 spectrometer (500 MHz)

(32) The relative configuration of side chains of maitotoxin has been independently elucidated by Harvard group, who reached the identical conclusion: Zheng, W.; DeMattei, J. A.; Wu, J.-P.; Duan, J. J.-W.; Cook, L. R.; Oinuma, H.; Kishi, Y. *J. Am. Chem. Soc.* **1996**, *118*, 7946−7968.

in the phase-sensitive mode proposed by States.[33] $^3J_{H,H}$ values were extracted from 1D ^{1}H NMR and 2D E.COSY spectra.[34] For the HETLOC spectra of model compounds **1**−**4**, the MLEV-17 spin-lock periods including a trim pulse (2.5 ms) were set for 30 ms to measure $^2J_{C,H}$ and 60 ms to measure $^3J_{C,H}$. The Δ in the half-filter was set at 3.45 ms and 4 ms, which was optimized for CH/CH_2 and CH_3, respectively. The nondiagonal version of HETLOC[19b] was measured for **3** and **4**, in which some of cross-peaks overlapped on diagonal peaks. The HETLOC spectra of **1**−**4** were measured for 9 h at 27 °C with a data size of 4K(F_2) × 128(F_1) points for the spectral width of 3000 Hz by 3000 Hz. The phase-sensitive (PS)-HMBC spectra[20] were recorded for 9 h with the delay (Δ) set at 40 ms with a data size of 2K(F_2) × 128(F_1) points for the spectral width of 3000 Hz (^{1}H) and 25000 Hz (^{13}C). For HETLOC and PS-HMBC, 2-fold zero-filling was conducted for both dimensions to give the digital resolution in F_2 of 0.38 for HETLOC and 0.75 Hz for PS-HMBC.

The carboxylic acid **5** was obtained from zooxanthellatoxins by basic hydrolysis with LiOH.[9] The sample (8 mg) was dissolved in 0.5 mL of CD_3OD and subjected to NMR measurements. $^3J_{H,H}$ values were determined from 1D ^{1}H NMR and 2D E.COSY,[34] the latter of which was carried out with a data size of 4K (F_2) × 512 (F_1) points for a spectral width of 3000 Hz, and a squared sine-bell window function shifted by $-2\pi/7$ was applied to both interferograms. $^{2,3}J_{C,H}$ values were measured by HETLOC experiments in the following conditions; BIRD delay, 470 ms; spin-lock period, 30 ms for $^2J_{C,H}$ and 60 ms for $^3J_{C,H}$ with each 2.5 ms trim pulses; Δ, 3.45 ms, data points, 2K (F_2) × 128(F_1); spectral width, 3000 Hz by 3000 Hz; 2-fold zerofilling to both dimensions. Unless otherwise noted, a mixed solvent of pyridine−methanol (1:1) was used for NMR measurements to minimize the effect of intramolecular hydrogen bonding.

Rotational Conformers and $^3J_{H,H}$. The conformations of rotamers in Table 2 and footnote 22 for model compounds **1**−**4** were calculated using MM2* force field on MacroModel. The populations of these rotamers were estimated from a calculated potential curve. On the basis of these populations, the weighted average values of $^3J_{H,H}$ in Table 2 were calculated using the modified Karplus equation.[28] The averaged values for gauche 60°, anti 180°, and another gauche 300° were obtained from conformers with the dihedral angles between 0° and 120°, those between 120° and 240°, and those between 240° and 360°, respectively. For calculations of the conformations of model compounds **1**−**4** in footnote 22, a potential curve with respect to the C2−C3 bond was obtained under non-hydrogen-bonding conditions since NMR data of **1**−**4** were collected in highly polar solvents. In these molecular mechanics calculations, the C1−C2 and C3−C4 bonds of **1**−**4** were supposed to have an extended conformation. The same conditions were set for the calculations of subsituted hexanes in Table 2.

Synthesis of Model Compounds. Model compounds were designed to reproduce rotational conformers for vicinal dihydroxy and vicinal hydroxy-methyl systems occurring in natural products. Syntheses of model compounds **1** and **2** for models of 1,2-dihydroxy system were started with the coupling of phenylacetaldehyde with methyl (tripheylphosphoranylidene)-acetate in methanol to furnish methyl 4-phenyl-2-butenoate with both *E*- and *Z*-geometry, which could be readily separated by SiO_2 column chromatography. After reduction of each isomer to the alcohol and then acetylation of the resultant alcohol, the olefin was dihydroxylated with osmium tetraoxide to yield the corresponding *erythro*- and *threo*-dihydroxy compounds from the *Z*- and *E*-isomers, respectively. The hydroxymethyl models (**3** and **4**) were prepared from the phenylallyl alcohol obtained as an intermediate for the synthesis of **1** and **2** by epoxidation with 3-chloroperbenzoic acid and reductive cleavage with MeLi in the presence of CuI to give 1,3-diol with excellent regioselectivity. After regioselective acetylation, both diastereomers were subjected to the NMR measurements.

4-Phenyl-*threo*-2,3-dihydroxyethyl Acetate (1). The preparation of this compound is detailed in the Supporting Information. ^{1}H NMR (500 MHz, $CD_3OD-C_5D_5N$, 1:1; For δ_H of **1**−**4**, C*H*D_2OD was taken as the reference at δ 3.30) δ 7.26−7.30 (m, 2H), 7.17−7.20 (m, 2H), 7.10 (m, 1H), 4.33 (dd, 1H), 4.27 (dd, 1H), 3.93 (ddd, 1H), 3.85 (dd, 1H), 3.01 (dd, 1H), 2.89 (dd, 1H), 1.88 (s, 3H). ^{13}C NMR (125 MHz, $C_5D_5N-CD_3OD$, 1:1; For δ_C of **1**−**4**, CD_3OD was taken as the reference at δ 49.0) 172.0, 140.7, 130.5, 129.2, 127.0, 73.7, 71.7, 67.4, 40.8, 21.0. HRMS (FAB): calcd for $C_{12}H_{16}O_4Na$ 247.0946, obsd 247.0938.

4-Phenyl-*erythro*-2,3-dihydroxyethyl Acetate (2). The preparation of this compound is detailed in the Supporting Information. ^{1}H NMR (500 MHz, $CD_3OD-C_5D_5N$, 1:1) δ 7.28−7.30 (m, 2H), 7.16−7.19 (m, 2H), 7.10 (m, 1H), 4.52 (dd, 1H), 4.32 (dd, 1H), 3.92 (ddd, 1H), 3.84 (dd, 1H), 3.19 (dd, 1H), 2.76 (dd, 1H), 2.15 (s, 3H). ^{13}C NMR (125 MHz, $C_5D_5N-CD_3OD$, 1:1) 172.2, 140.9, 130.8, 129.1, 126.9, 74.3, 73.5, 67.6, 41.0, 21.0. HRMS (FAB): calcd for $C_{12}H_{16}O_4Na$ 247.0946, obsd 247.0963.

4-Phenyl-*threo*-3-dihydroxy-2-methylethyl Acetate (3). The preparation of this compound is detailed in the Supporting Information. ^{1}H NMR (500 MHz, $CD_3OD-C_5D_5N$, 2:1) δ 7.20−7.24 (m, 4H), 7.13 (m, 1H), 4.08 (dd, 1H), 3.97 (dd, 1H), 3.92 (ddd, 1H), 2.77 (dd, 1H), 2.72 (dd, 1H), 1.92 (s, 3H), 1.83 (m, 1H), 0.97 (s, 3H). ^{13}C NMR (125 MHz, $CD_3OD-C_5D_5N$, 2:1) 172.2, 140.9, 130.3, 129.3, 127.0, 72.9, 68.1, 42.2, 38.2, 20.9, 10.7. HRMS (FAB): calcd for $C_{13}H_{18}O_3Na$ 245.1154, obsd 245.1169.

4-Phenyl-*erythro*-3-dihydroxy-2-methylethyl Acetate (4). The preparation of this compound is detailed in the Supporting Information. ^{1}H NMR (500 MHz, $CD_3OD-C_5D_5N$, 2:1) δ 7.20−7.25 (m, 4H), 7.13 (m, 1H), 4.28 (dd, 1H), 4.04 (dd, 1H), 3.72 (ddd, 1H), 2.86 (dd, 1H), 2.64 (dd, 1H), 1.97 (s, 3H),1.00 (d, 3H). ^{13}C NMR (125 MHz, $CD_3OD-C_5D_5N$, 2:1) 172.4, 141.0, 130.6, 129.2, 127.0, 75.0, 67.3, 41.9, 39.4, 21.0, 14.7. HRMS (FAB): calcd for $C_{13}H_{18}O_3Na$ 245.1154, obsd 245.1140 as $(M + Na)^+$.

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Supporting Information Available: Selected spectra used for configuration assignments of **5**; the synthetic details of the model compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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