Pure & Appl. Chem., Vol. 65, No. 4, pp. 771–778, 1993.
Printed in Great Britain.

Preferred solution conformation of marine natural product palytoxin and of *C*-glycosides and their parent glycosides

Yoshito Kishi

Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, MA 02138 USA

ABSTRACT - This presentation concerns (1) the preferred solution conformation of mono-, di-, and tri-*C*-glycosides in comparison to their parent glycosides and (2) the preferred local and global solution conformation of the marine natural product palytoxin.

PREFERRED SOLUTION CONFORMATION OF *C*-GLYCOSIDES AND THEIR PARENT GLYCOSIDES

The modern era of carbohydrate conformational analysis began with the discovery of the anomeric and the *exo*-anomeric effects. The term *exo*-anomeric effect was introduced by Lemieux in 1969 to describe the observed preferred glycosidic conformation of sugars. Of the three staggered rotamers around the glycosidic bond of an α-(axial)-carbohydrate, the conformation **I-A** is preferred over **I-B** and **I-C** (Figure 1). This holds true for both oligosaccharides and simple *O*-alkyl glycosides. The conformational preference has been attributed to a combination of (a) steric preference (**I-A** > **I-B** > **I-C**) and (b) electronic stabilization (**I-A** = **I-C** > **I-B**). The electronic effect is a result of the interaction between the *p* orbital of the glycosidic oxygen and the antibonding σ* orbital of the polarized C.1-O.5 bond of the pyranose ring. Substantial controversy remains as to the relative importance of steric and electronic factors in aqueous or methanolic solution. Ab initio calculations have been used to confirm the existence of an *exo*-anomeric electronic effect. Experimental evidence shows that the anomeric effect is weak in water, and indicates the same for the *exo*-anomeric effect. To our knowledge, no experiment directly addressed the relative importance of the steric and electronic components of the *exo*-anomeric effect [ref. 1].

The carbon analogs of carbohydrates (Figure 1, **II-A, B, C**) represent a possible model for investigating the steric interactions around the glycosidic bonds of carbohydrates in the absence of electronic stabilization. In connection with the stereochemistry assignment and total synthesis of palytoxin [ref. 2], we had the opportunity to synthesize a variety of C.1 carbon-substituted glycosides (*C*-glycosides). The conformation of the carbon analogs can be determined experimentally from the vicinal coupling constants between the C.1 and the C.α protons, coupled with the modified Karplus equation. This conformation can be compared to that of the parent oxygen compound, and the importance of the electronic interaction can be

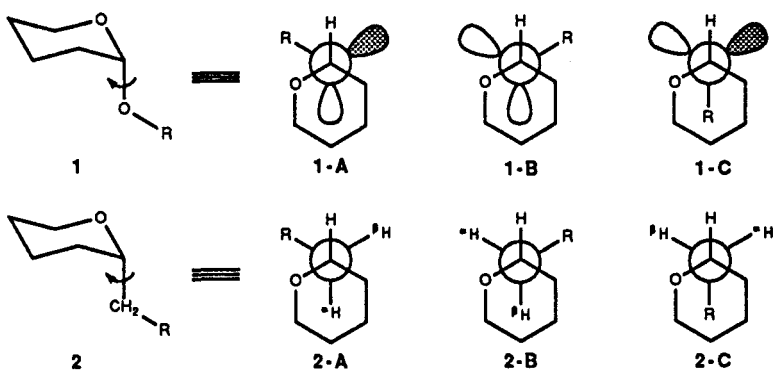


Figure 1.

estimated on that basis. Because the C-O bond (1.43 Å) is shorter than the C-C bond (1.54 Å), the steric interactions observed in the *C*-glycosides should be more pronounced in the oxygen case. We therefore feel that this approach will slightly overestimate the effect of the stereoelectronic factor.

We began with simple *C*-monoglycosides, and demonstrated that there is a strong preference for the conformation around the C-glycosidic bond which matches the conformation adopted by the parent *O*-alkyl glycosides [ref. 3]. Solvent effects studies showed no significant change on this conformational preference. In addition, protection of the hydroxyl groups did not dramatically affect their conformational preference. These results suggested that electrostatic interactions and hydrogen bonds do not play a major role in the overall conformational behavior of *C*-monoglycosides. Variable temperature NMR experiments indicated that they exist as a mixture of staggered conformers rather than a single twisted conformer. The single conformer obtained from the modified Karplus equation was regarded as a time-averaged conformation, yielding the approximate dihedral angles of 55° for the axial *C*-glycosides and -80° for the equatorial *C*-glycosides, which are in good agreement with the value of 55° for methyl α-D-glucopyranoside and -70° for methyl β-D-glucopyranoside. Although the existence of a stereoelectronic stabilization cannot be excluded in the oxygen case, the conformational behavior of glycosides can be accounted for by steric effects as a first approximation.

We extended our approach to the 1,6-linked *C*-disaccharides, methyl *C*-isomaltoside and methyl *C*-gentiobioside [ref. 4]. The conformational behavior of 1,6-linked carbon disaccharides was treated as two independent C-glycosidic bonds on tetrahydropyran rings. Based on the studies on simple *C*-monoglycosides, we expected, and experimentally confirmed, these C-glycosidic bonds to preferentially adopt "*exo*-anomeric" conformation.

We then proceeded to 1,4-linked carbon disaccharides, the conformational analysis of which required consideration of one C-glycosidic bond and one C-aglyconic bond [ref. 5]. For this purpose, we first needed first to develop flexible synthetic routes to this class of compounds. The ^{1}H NMR spectrum clearly showed that the 1,4-linked *C*-disaccharides thus prepared predominantly adopt the "*exo*-anomeric" conformation around the C-glycosidic bond and that steric repulsion results in distortion predominantly around the C-aglyconic bond rather than around the C-glycosidic bond. In addition, solvent effects, coupled with the fact that the NMR spectra of the corresponding protected forms were very similar to those of the unprotected forms, suggested that hydrogen bonding and electrostatic interactions do not seem to play a major role in the overall conformational behavior of these substrates.

In order to clearly evaluate and present through-space steric interactions, such as 1,3-diaxial-like destabilization, a diamond lattice analysis was introduced. For example, in the analysis of methyl *C*-maltoside **3**, only the three staggered conformers around the C-aglyconic bond, **3-A**, **3-B**, and **3-C**, are considered (Figure 2). Conformer **3-C** suffers from steric congestion because the C.1'-C.α bond is gauche to both the C.3 and C.5 pyranose carbons. Conformer **3-B** has two 1,3-diaxial-like interactions (C.5-C.6/C.1'-C.α and C.1'-O.5'/C.4-C.5). Conformer **3-A** has one 1,3-diaxial-like interaction between the C.3-O.3 and C.1'-C.α bonds. None of the three conformers is free of unfavorable steric interactions, although conformer **3-A** seems to be the least destabilized. This analysis is reflected nicely in the experimental vicinal coupling constants in the ^{1}H NMR spectrum. These data suggest that the preferred

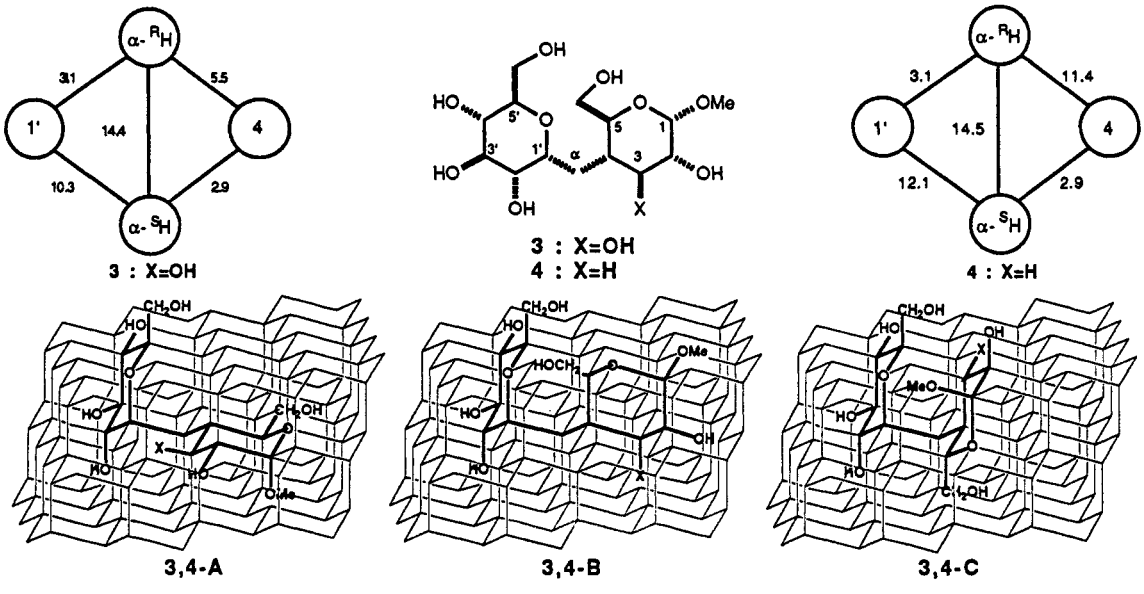
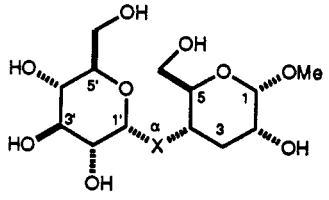


Figure 2.

conformation of the C-aglyconic bond is a mixture of conformers or one conformer deviating slightly from the ideal staggered conformation **3-A**. It is important to note that the exactly same behavior was observed for the corresponding *O*-dissacharides; namely, steric hindrance results in distortion predominantly around the aglyconic bond [ref. 6]. This observation once again indicated the similarity in the conformational behavior between the two classes of compounds.

The diamond lattice analysis immediately suggested an experiment to demonstrate the importance of 1,3-diaxial-like interactions. Examining the three ideal staggered conformers of **3** on a diamond lattice, removal of the C.3 hydroxyl group or inversion of its configuration should eliminate the only destabilizing 1,3-diaxial-like steric interaction in conformer **3-A**. Therefore, this conformer was consequently expected to become dominant, which was experimentally proven to be the case—note the spin coupling diagram for **4** in Figure 2.

As discussed, there is little doubt that the major conformational characteristics of natural *O*-glycosides are duplicated by the carbon analogs, from which two important ramifications emerge. First, the specific conformation of an oligosaccharide can be estimated from the experimentally determined conformation of its carbon analog. Second, the conformational analysis of natural oligosaccharides can be performed based on the principles developed for the *C*-disaccharides. We further sought an experiment to demonstrate the conformational similarity of the *C*- and *O*-disaccharides. In this connection, we were interested in a comparison of NOE and T_1 data between the *C*-glycosides and the corresponding *O*-glycosides. There are obvious differences between the two series. For example, the C-C and C-O bond lengths, angles and polarities are different. More importantly, the *C*-disaccharides have two C.α protons which are not present in the parent *O*-glycosides, and can significantly participate *via* relaxation in the NOE and T_1 experiments. In order to avoid the possible complication due to this and to make the comparison meaningful, we replaced the two C.α protons with deuterium. The comparison of the NOE and T_1 data for the carbon-linked dideuterated disaccharide **4** (X=CD_2) and the oxygen-linked disaccharide **5** (X=O), for example, demonstrated that there can be only minor differences between the conformational behavior of the two classes of compounds (Figure 3). The preferred conformation of *C*-disaccharide **4** (X=CH_2) was unambiguously established from the vicinal coupling constants observed for the C.α protons.



4 : X=CH_2
5 : X=O

Figure 3.

T_1 Values (sec)

Protons	5 (X=O)	4 (X=CD_2)
H.1'	0.94	0.71
H.3(eq)	0.36	0.37
H.4	0.79	0.81

NOE (%)

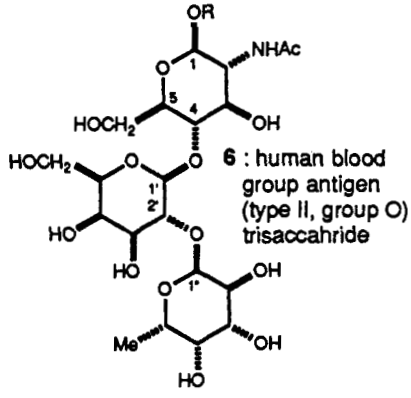
Protons	5 (X=O)	4 (X=CD_2)
H.1'→H.3(eq)	6.9	5.2
H.1'→H.4	7.2	3.1
H.3(eq)→H.1'	8.7	9.1

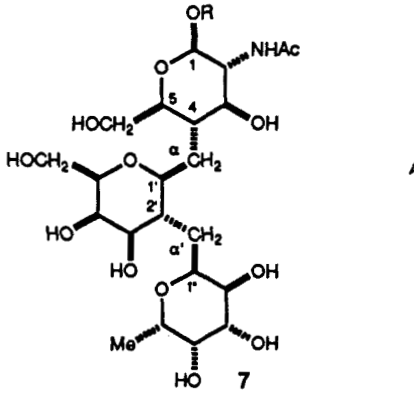
Interestingly, substantial NOE enhancements of H.4 were observed upon irradiation of H.1', which were not readily predicted on the basis of the known conformational behavior of these compounds. As pointed out in the literature [ref. 7], the translation of NOE data into relative distance is not straightforward. A significant advantage of our approach is that spectroscopic measurements can be compared directly, without interpreting the meaning of the NOE and T_1 data, or comparing a translation of the NOE measurements to a postulated conformational behavior.

Having gained experimental support to demonstrate the conformational similarity between the *C*- and *O*-glycosides, we decided to prepare the carbon analog of a biologically significant substrate and demonstrate three issues on the basis of this analysis. First, we wished to show that the conformation of this compound can be predicted and that this prediction can be tested experimentally. Second, we wished to demonstrate that the compound can be induced to adopt different, yet predictable and well-defined conformations as a result of specific, rationally designed structural modifications. Third, we wished to examine their effect(s) on the biological behaviors in comparison to the corresponding parent *O*-glycosides. The Type II O(H) blood group determinant trisaccharide **6**, and its carbon analog are ideally suited for this purpose.

The conformational analysis of the blood group determinant trisaccharide and its carbon analogs was broken down into the conformational analysis across the C.1'-C.α-C.4 linkage of the galactosyl-glucosamine moiety and the conformational analysis across the C.1''-C.α'-C.2' linkage of the fucosyl-galactose moiety. Based on the studies on the *C*-monoglycosides and the 1,4-linked *C*-disaccharides, we expected no significant interaction between the two conformational systems. The 2'-fucosylmethyl functionality should therefore have little bearing on the conformation of the C.1'-C.α-C.4 bridge.

Conversely, the galactosyl-glucosamine system should not affect the conformational behavior across the fucosyl-galactose bridge. The trisaccharide was therefore analyzed as a first approximation in terms of two independent disaccharide systems.





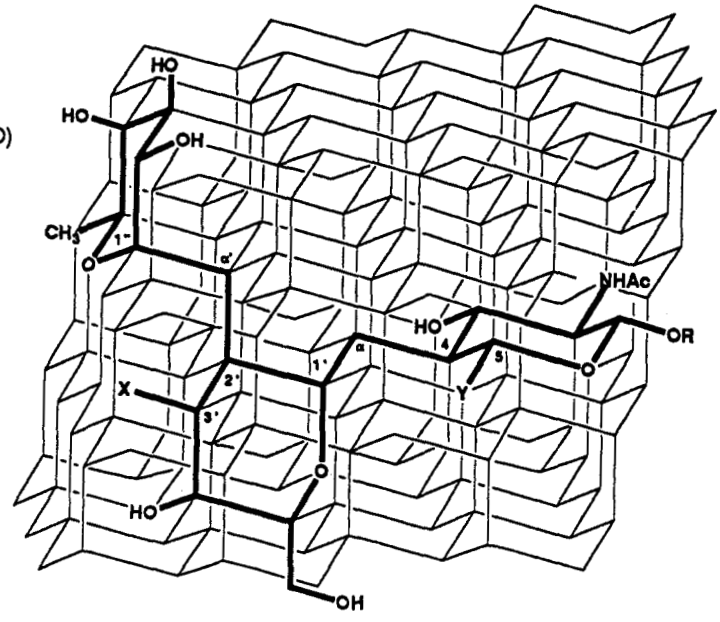


Figure 4.

The conformational behavior of the β(1,4)-linkage is expected to be similar to that of methyl *C*-cellobioside. The conformation around the C-glycosidic bond is predicted to be such that the C.α-C.4 bond is antiperiplanar to the pyranose C.1'-C.2' bond, i.e., the "*exo*-anomeric" conformation. Analysis of the three staggered conformers around the C-aglyconic bond indicates that all three suffer from unfavorable steric interactions. The conformation across the fucosyl-galactose linkage is analyzed in the same manner; the C.1"-C.α' bond is expected to exist in the depicted "*exo*-anomeric" conformation and none of the three staggered rotamers around the C.α'-C.2' bond is free of unfavorable steric interactions. On the basis of the results observed for the *C*-monoglycosides and the 1,4-linked *C*-disaccharides, distortion is expected to occur primarily around the C-aglyconic bond. The *C*-trisaccharide is therefore predicted to exist in a conformation with both C-glycosidic bonds, C.1'-C.α and C.1"-C.α', in the "*exo*-anomeric" conformation. Both C-aglyconic bonds, C.α-C.4 and C.α'-C.2', are predicted to exist in a flexible conformation, i.e, either as a mixture of conformers or as a single twisted conformer.

It is important to note the conformation of the blood group determinant trisaccharide can be controlled by modifying the 1,3-diaxial-like interactions which are responsible for the distortion around the C-aglyconic bonds. If the C.3'-hydroxyl group is removed, the fucosyl-galactose system is expected to adopt the ideal staggered conformer, while leaving the conformation across the galactosyl-glucosamine linkage unaffected. Similarly, removal of the C.5-hydroxymethyl group will allow the galactosyl-glucosamine system to predominantly adopt the conformer, without affecting the conformation of the fucosyl-galactose system. Finally, removal of both the C.3'-hydroxyl group and the C.5-hydroxymethyl group should produce an analog which exists predominantly in an ideal staggered conformation across both linkages. We predicted that four different conformers of the blood group determinant trisaccharide can thus be accessed through the use of small but strategically chosen structural modifications. The Type II O(H) determinant trisaccharide **6** is ideal for this study because both the C.5 hydroxymethyl and the C.3 hydroxyl group are available for the structural modification.

It should be noted that this analysis is equally applicable to the parent oxygen compounds, since the conformational similarity of carbon and oxygen linked disaccharides has been demonstrated experimentally. The structural modifications described above are therefore expected to have the same conformational ramifications in the natural Type II O(H) blood group determinant trisaccharide.

To experimentally test this prediction, we developed an efficient, flexible synthesis of this class of the *C*-trisaccharides **8-11**, which were predicted to exhibit four different conformational behaviors. We were pleased to find the ^{1}H NMR vicinal coupling constants indeed validated the prediction [ref. 8].

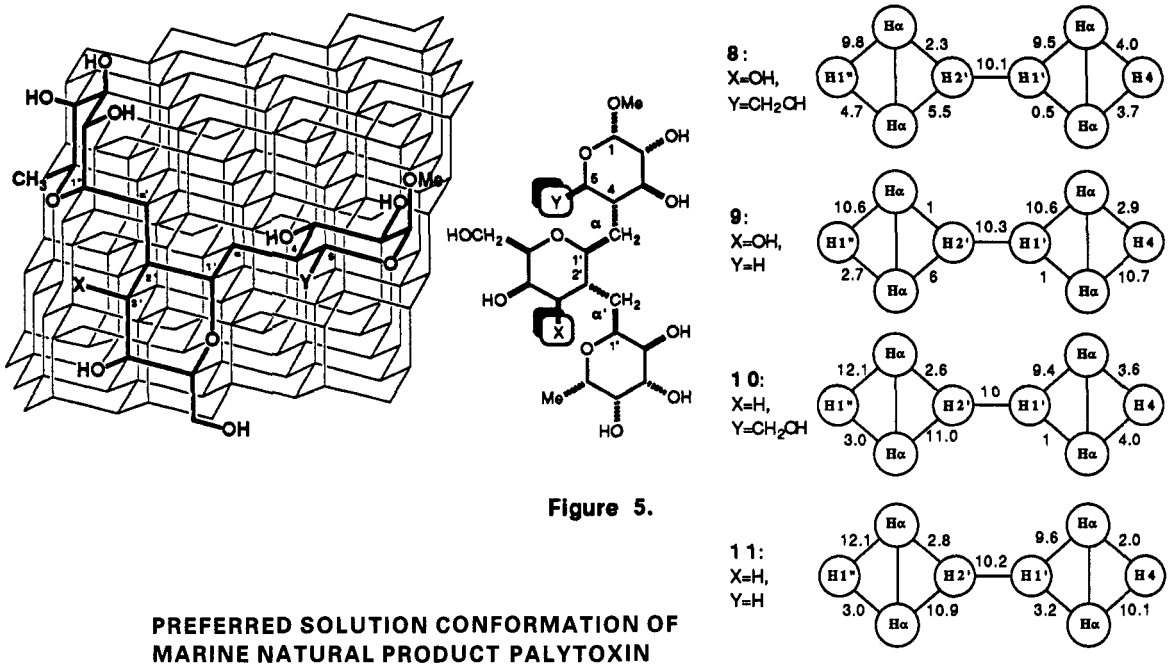


Figure 5.

PREFERRED SOLUTION CONFORMATION OF MARINE NATURAL PRODUCT PALYTOXIN

On the basis of extensive ^{1}H NMR studies in aqueous methanol (CD_3OD:D_2O=85:15), palytoxin (PTX) and palytoxin carboxylic acid (PTC) have been shown to adopt one predominant conformation. The conformational studies on PTX and PTC started with the ^{1}H NMR analysis of the small segments summarized in Figure 6. These segments were chosen, and synthesized, in such a way that the each segment has an overlapping structural portion with the next segment. Therefore, the information on the preferred conformation of each segment can be assembled to deduce the conformational preference of the entire molecule. It is important to note that the ^{1}H NMR characteristic of these segments were found

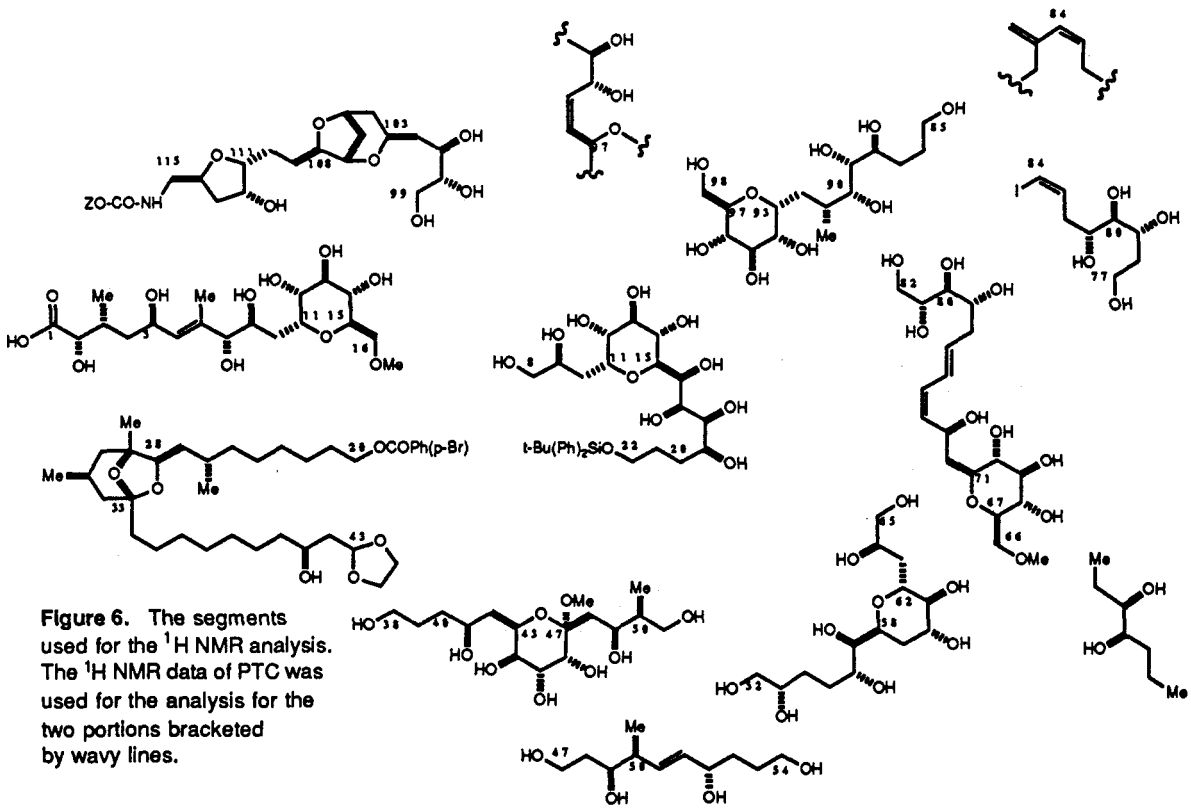


Figure 6. The segments used for the ^{1}H NMR analysis. The ^{1}H NMR data of PTC was used for the analysis for the two portions bracketed by wavy lines.

remarkably well compared to those of the corresponding structural portion of palytoxin. The preferred conformation of the entire molecule was thus obtained, which had 31 Å for the distance between the C.1 carbon and C.115 nitrogen terminals [ref. 9].

Although this preferred conformation of the entire molecule had many appealing aspects, we were concerned with the error(s) possibly encountered in this approach. In particular, the information gained through the ^{1}H NMR analysis, coupled with the modified Karplus equation, is adequate in defining the approximate conformation of each of the segments, but it is certainly accompanied with small ambiguity. In the process of assembling the information about the preferred conformation of these segments to build a large molecule such as PTC, we hoped that these small ambiguities could cancel each other. However, we realized that there was no scientific ground why we could preclude the possibility that they accidentally accumulated only in one direction for unknown reasons. If there were a device to determine the global conformation of the entire molecule, for example to measure the distance between the C.1 carbon and C.115 nitrogen terminals, we could avoid such a potential error.

We have been engaged in developing a class of compounds with a well-developed and well-defined secondary structure in aprotic as well as protic solvents. The scattered literature suggested that oligopeptides containing α-aminoisobutyric acid (Aib) might meet our needs. Based on solvent- and temperature-dependent ^{1}H NMR studies, coupled with NOE and CD experiments, we could show that certain Aib-containing oligopeptides adopt a well developed 3_{10}-helical structure both in aprotic and protic solvents (Table 1) [ref. 10]. We used such oligopeptides as a tool to study chemical reactivity in connection with secondary structure [ref. 11]. Interestingly, we noticed that such oligopeptides could also be used as a chemical ruler; several X-ray structures suggest the depth of one pitch of a 3_{10}-helix to be very close to 6.0 Å, and the overall length of these Aib- containing oligopeptides can be estimated.

Table 1.	n=2	n=3	n=4	n=5	n=6
Boc(Ala-Aib)$_n$OMe		-	≈	+	+
Boc(Ala-Ala-Aib)$_n$OMe	≈	+	+	+	+
Boc(Ala-Ala-Ala-Aib)$_n$OMe	+	+	+		

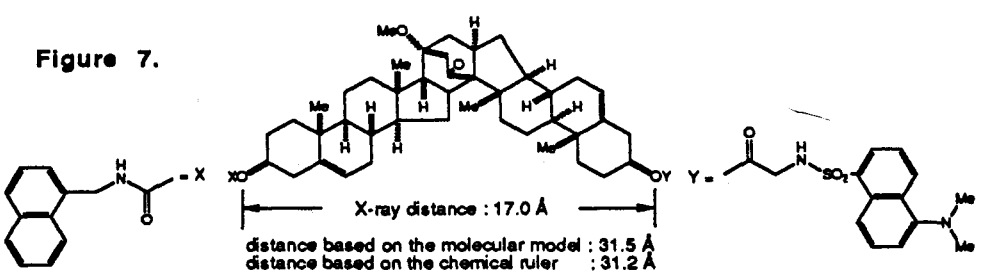
$^+$well-developed 3_{10}-helices. $^≈$apparently 3_{10}-helices, but not well-developed. $^-$not 3_{10}-helices

We studied the fluorescence energy transfer between the energy donor naphthalene (Naph) group on the C-terminal and the energy acceptor dansyl (DAN) group on the N-terminal, and found the data perfectly fits the prediction derived from Förster's theory [ref. 12]. It is important to note several issues. First, all the oligopeptides used in the fluorescence energy transfer studies have been experimentally proven to adopt a 3_{10}-helical conformation both in aprotic and protic solvents. Second, a good linearity was observed between ln (E^{-1} - 1) and ln R with a slope of 6 approximately at 10^{-7} molar concentration in a wide range of solvents (chloroform, dioxane, acetonitrile, ethanol, methanol, etc) as well as over a wide range of temperature (8 - 38 °C). Third, this particular combination of the energy donor and acceptor is good for measuring distances in the range of 15 - 45 Å. Fourth, the distance for the 50% energy transfer in dioxane was found to be 30.5 Å [ref. 10].

The distance for the 50% energy transfer was also estimated from a semi-theoretical method, yielding 22.2 Å, which was significantly different from the distance calibrated from the Aib-containing 3_{10}-oligopeptides. We examined several possibilities to explain this large discrepancy without significant success. Therefore, we decided to produce the second data point to verify the experimental data obtained from the 3_{10}-oligopeptide ruler. In this connection, we attached the Naph and DAN groups on the bis-steroid, and found the distance expected from the X-ray result, coupled with the molecular model, to be perfectly consistent with the distance based on the 3_{10}-oligopeptide ruler (Figure 7) [ref. 13].

Having developed a reliable chemical ruler, we were now in a position to verify the global conformation of PTC. To do so, we needed to accommodate the Naph and DAS groups on PTC (Figure 8), which was accomplished *via* the palytoxin δ-lactone [ref. 2]. The fluorescence energy transfer efficiency of this

Figure 7.



substance in aqueous methanol ($CH_3OH:H_2O$=85:15) was found 54%, which was, with reference to the chemical ruler, translated to a distance of 30 Å between the C.1 carbon and C.115 nitrogen terminals [ref.9]. This distance is remarkably close to the distance of 31 Å obtained from assembling the preferred conformation of the segments listed in Figure 6. The preferred conformation of the entire molecule was then subjected to a crude energy minimization with the restriction of the 30 Å distance between the C.1 carbon and C.115 nitrogen terminals, which is shown in Figure 9. There are several unique characteristics worthwhile to point out. First, the carbon backbone makes several turns, some of which are sharply U-shaped. The bicyclic system around the C.28 position and the *cis*-olefinic bonds at the C.75 and C.84 positions are involved in such turns. Importantly, as expected from the conformational analysis of *C*-glycosides described in the previous section, *C*-glycosides make the carbon backbone turn. Second, the left half of PTC forms a beautiful glyceride-like structure, the depth of which approximately corresponds to that of common lipids. There is experimental support for this conformation; the C.31 (or C.26) methyl group exhibit a significant anisotropic effect on incorporation of the Naph group at the C.1 carboxylic acid. Third, the hydrophobic portion of the molecule faces to the other hydrophobic portion across the backbone, whereas the hydrophilic portion faces to the other hydrophilic portion. Fourth, it appears that the left and right side portions of the molecule are conformationally relatively rigid, and are connected by the conformationally flexible central portion. Fifth, there is a large cavity around the central portion of the molecule.

Depending on the environment, the size of this cavity might change because of the conformational flexibility at the central portion. Thus, palytoxin might exhibit an ionophor-like characteristic. Intrigued by this possibility, we felt it to be important to provide an experimental support for the presence of such a flexible cavity. We noticed that the C.75 *trans*-PTC, if available, may uniquely serve for this purpose; namely, the overall distance between the C.1 carbon and C.115 nitrogen is expected to be approximately the same as that of native PTC, but the cavity is much more filled in by the right portion of molecule. In connection with the synthetic work of palytoxin, we observed the *cis,trans*-diene system in question isomerizes under relatively mild acidic conditions to the corresponding the *trans,trans*-diene system without

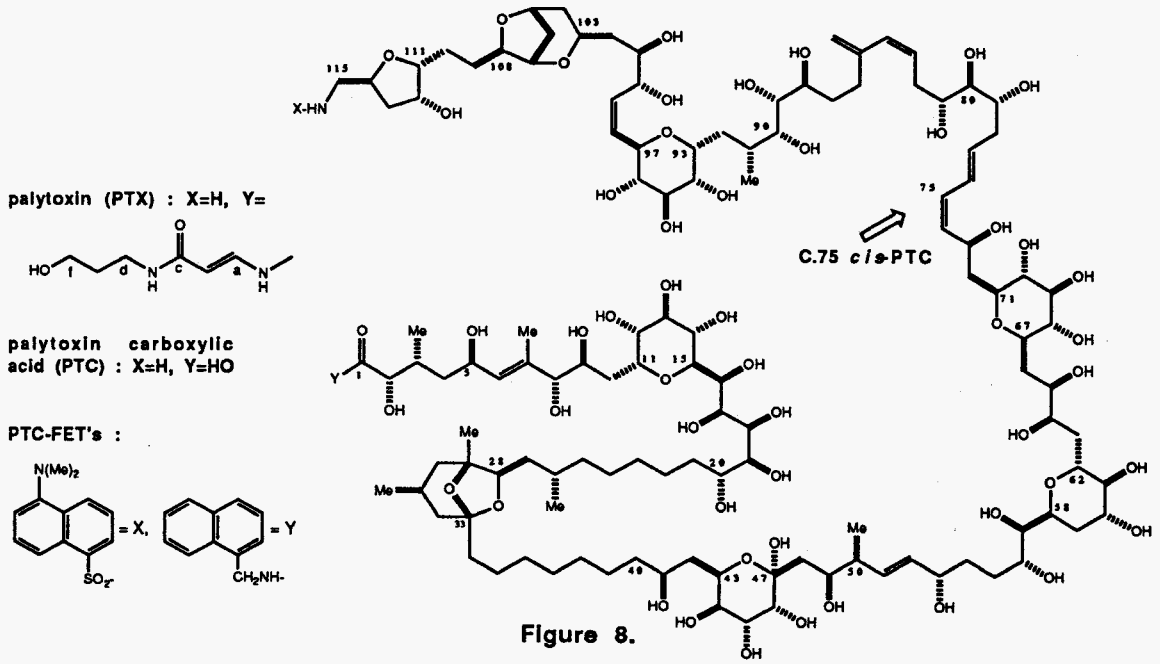


Figure 8.

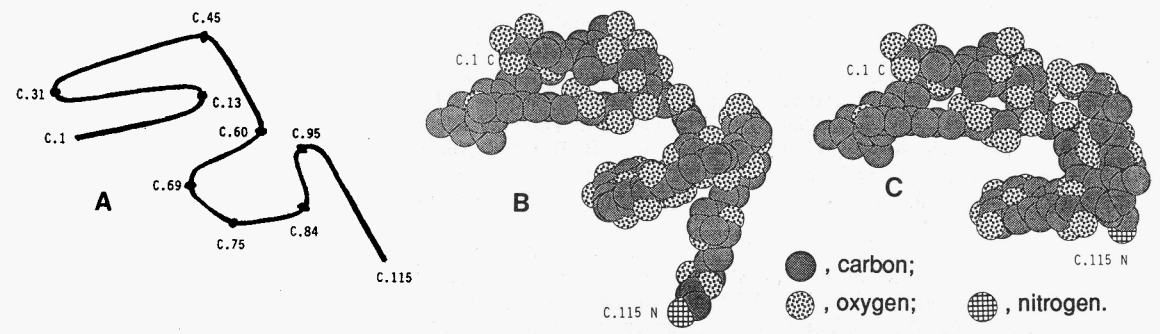


Figure 9. The preferred conformation of C.75 *cis*- and *trans*-PTC's. A: a schematic picture for C.75 *cis*-PTC. B: a space-filling model for C.75 *cis*-PTC. C: a space-filling model for C.75 *trans*-PTC.

affecting the C.73 stereochemistry. Using this chemistry, the requisite C.75-*trans*-PTC with the Naph and DANS groups on the C- and N-terminals, respectively, was synthesized [ref. 14]. The distance between the C.1 carbon and C.115 nitrogen was found to be 31 Å. The conformation of this substance is shown on the right side of Figure 9 [ref. 15].

As expected, we could demonstrate that the overall distance between the C- and N-terminals of the C.75-*cis* and C.75- *trans*-PTCs is indeed sharply dependent on solvents. It is exciting to find the degree of the solvent dependency to be less sensitive for the C.75 *trans*-PTC than for the C.75 *cis*-PTC, which is consistent with our prediction.

Table 2. Distance between the C.1 and C.115 Terminals

	MeOH-H_2O (85:15)	EtOH	Dioxane
natural PTC (C.75-*cis*)	30 Å	26 Å	16 Å
C.75-*trans* PTC	31 Å	27 Å	24 Å

Acknowledgements I would like to express my greatest appreciation to the co-workers whose spirit, dedication, stamina, and skill brought this stimulating, exciting, and challenging venture this far. The co-workers who have energetically and enthusiastically participated in the work presented are acknowledged in the references. Financial assistance from the National Institutes of Health (NS-12108) and the National Science Foundation (CHE 89-09762) is gratefully acknowledged.

REFERENCES AND FOOTNOTES

1. For original and review articles on the conformational analysis of glycosides and related subjects, see the articles cited in reference 3. There is some ambiguity in the use of the term "*exo*-anomeric effect" in the literature. In some cases it has been used to describe the conformational preference of glycosides for the gauche orientation. In other cases, it has been used to describe the stereoelectronic interaction between the *p* orbital of the interannular oxygen and the σ^* orbital of the pyranose C.1-O.5 bond. In this presentation, the former definition will be used for *exo*-anomeric effect, while the latter interaction will be referred to as the stereoelectronic stabilization.
2. R. W. Armstrong, J.-M. Beau, S. H. Cheon, W. J. Christ, H. Fujioka, W.-H. Ham, L. D. Hawkins, H. Jin, S. H. Kang, Y. Kishi, M. J. Martinelli, W. W. McWhorter, Jr., M. Mizuno, M. Nakata, A. E. Stutz, F. X. Talamas, M. Taniguchi, J. A. Tino, K. Ueda, J. Uenishi, J. B. White, and M. Yonaga, *J. Am Chem. Soc.*, **111**, 7530 (1989) and references cited therein.
3. T.-C. Wu, P. G. Goekjian, and Y. Kishi, *J. Org. Chem.*, **52**, 4819 (1987) and **56**, 6412 (1991).
4. P. G. Goekjian, T.-C. Wu, H.-Y. Kang, Y. Kishi, *J. Org. Chem.*, **52**, 4823 (1987) and **56**, 6422 (1991).
5. S. A. Babirad, Y. Wang, P. G. Goekjian, and Y. Kishi, *J. Org. Chem.*, **52**, 4825 (1987). Y. Wang, P. G. Goekjian, D. M. Ryckman, and Y. Kishi, *J. Org. Chem.*, **53**, 4151 (1988). W. H. Miller, D. M. Ryckman, P. G. Goekjian, Y. Wang, and Y. Kishi, *J. Org. Chem.*, **53**, 5580 (1988). Y. Wang, P. G. Goekjian, D. M. Ryckman, W. H. Miller, S. A. Babirad, and Y. Kishi, *J. Org. Chem.*, **57**, 482 (1992).
6. R. U. Lemieux, S. Koto, *Tetrahedron*, **30**, 1933 (1974).
7. R. E. Schirmer, J. H. Noggle, J. P. Davis, P. A. Hart, *J. Am. Chem. Soc.*, **92**, 3266 (1970) and reference 6.
8. T. Haneda, P. G. Goekjian, S. H. Kim, and Y. Kishi, *J. Org. Chem.*, **57**, 490 (1992). A. Haudrechy, H. S. Jun, W. Lai, N. Haudrechy, and Y. Kishi, XVIth International Carbohydrate Symposium (1992).
9. M. Mizuno, E. M. Suh, and Y. Kishi, unpublished results.
10. T. Li, K.-H. Budt, J. S. Goudar, and Y. Kishi, unpublished results and T. Li, Harvard Dissertation (1991).
11. T. Li, K.-H. Budt, and Y. Kishi, *J. Chem. Soc., Chem. Commun.*, 1817 (1987). K.-H. Budt, J.-M. Vatele, and Y. Kishi, *J. Am. Chem. Soc.*, **108**, 6080 (1986).
12. T. Förster, *Ann. Physik.*, **2**, 55 (1948). For some applications of Förster's theory, see: S. A. Latt, H. T. Cheung, E. R. Blout, *J. Am. Chem. Soc.*, **87**, 995 (1965) and L. Stryer and R. P. Haugland, *Proc. Natl. Acad. Sci. USA*, **58**, 719 (1967).
13. The bis-steroid was prepared according to the procedure reported by K.-I. Morita, G. Slomp, Jr. and E. V. Jensen [*J. Am. Chem. Soc.*, **84**, 3779 (1962)]. The stereochemistry originally proposed for this substance [S. A. Latt, H. T. Cheung, and E. R. Blout, *J. Am. Chem. Soc.*, **87**, 995 (1965)] was proven incorrect by X-ray analysis: H. Wu and Y. Kishi, unpublished results.
14. The C.75 *trans* substance was synthesized from PTC *via* N-dansylation, acetic acid treatment, and C.1 amide formation.
15. As for the case of the C.75 *cis* PTC, the conformational analysis of the C.75 *trans* PTC was first performed on the basis of the ^1H NMR spectrum of the small segments, including the C.75 *trans* C.66-C.82 segment, which yielded 30 Å for the distance between the C.1 carbon and C.115 nitrogen terminals. It was then subjected to a crude energy minimization with the restriction of the 31 Å distance between the C.1 carbon and C.115 nitrogen terminals, to yield the the conformation shown in Figure 9.