J. Am. Chem. Soc. **1990**, *112*, 4047-4049.
7. It was found that a small amount of $Fe^{IV}(F_{20}TPP)(OCH_3)_2$ has also been formed. The pyrrole β-proton signal of the dimethoxyiron(IV) porphyrin complex appeared at −26 ppm at −40 ℃: Groves, J. T.; Quinn, R.; McMurry, T. J.; Nakamura, M.; Lang, G.; Boso, B. *J. Am. Chem. Soc.* **1985**, *107*, 354-360.
8. (a) Bell, S. E. J.; Cooke, P. R.; Inchley, P.; Leanord, D. R.; Lindsay Smith, J. R.; Robbins, A. *J. Chem. Soc. Perkin Trans. 2* **1991**, 549-559. (b) Arasasingham, R. D.; Cornman, C. R.; Balch, A. L. *J. Am. Chem. Soc.* **1989**, *111*, 7800-7805. (c) Gold, A.; Jayaraj, K.; Doppelt, P.; Weiss, R.; Chottard, G.; Bill, E.; Ding, X.; Trautwein, A. X. *J. Am. Chem. Soc.* **1988**, *110*, 5756-5761. (d) Shin, K.; Goff, H. M. *J. Am. Chem. Soc.* **1987**, *109*, 3140-3142.
9. Mn^{IV}-OIPh complexes have been shown to oxygenate alkenes and alkanes. (a) Smegal, J. A.; Schardt, B. C.; Hill, C. L. *J. Am. Chem. Soc.* **1983**, *105*, 3510-3515. (b) Smegal, J. A.; Hill, C. L. *J. Am. Chem. Soc.* **1983**, *105*, 3515-3521.

Implication of Secondary Structure of Bradykinin in Membrane Mimic Solution Investigated by NMR Spectroscopy

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Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is a linear peptide hormone with diverse physiological properties such as vasodilation and pain mediation.[1] Most peptide hormones interact with their specific receptors in the cell membrane. However, identification of hormone receptors has proved difficult and there are few examples of structural analysis from a receptor/hormone complex. The cell membrane receptor for bradykinin has not been isolated, but recent evidence indicates that the pain receptors are localized in the dorsal root and trigeminal regions of the guinea pig nervous system.[2] In general, peptide hormones first interact with membranes and form nascent secondary structure prior to binding to the receptor. The solution conformation of a bradykinin was investigated by 1D and 2D proton NMR spectroscopy and it was reported that bradykinin has disordered conformation in water[3] and two β turns in DMSO (dimethyl sulfoxide).[4] Futhermore, the interaction of the bradykinin with SDS micelle has been reported as the conformational changes of the C-terminal tetrapeptide fragment, Ser-Pro-Phe-Arg, made strong interactions with monomeric SDS.[5,6] We used a simple TFE (trifluoroethanol)/water binary solvent system to mimic the biological membrane. The hydrophobic character of TFE provides a similar environment to a membrane for bradykinin.

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Table 1. Chemical shift values of backbone and side chain protons of bradykinin[a]

	NH	αH	βH	γH	δH	Others
Arg 1		4.26	1.90	1.68	3.13	scNH[b] 7.20
Pro 2		4.71	2.38, 1.89	1.97	3.68, 3.43	
Pro 3		4.40	2.23, 1.87	2.01	3.76, 3.61	
Gly 4	8.08	3.92, 3.79				
Phe 5	7.79	4.55	3.00			
Ser 6	7.76	4.65	3.81, 3.69			
Pro 7		4.27	2.0, 1.53	1.41, 1.73	3.50, 3.45	
Phe 8	7.51	4.60	3.21, 2.82			
Arg 9	7.67	4.24	1.83, 1.70	1.57	3.16	scNH 7.12

[a]The Chemical shifts are given in ppm and are referenced to the residual methylene protons of TFE (3.88 ppm, 298 K). [b]side chain NH

Bradykinin was purchased from Sigma and used without further purification, since no minor constituents could be detected by NMR spectroscopy. The peptide was dissolved in water/TFE-d_3 and pH was adjusted to 3 by adding HCl and NaOH solution. All NMR spectra were recorded on the BRUKER 500 MHz NMR Spectrometer at ambient temperature. Assignments were performed by sequential assignment strategy.[7] TOCSY (total correlation spectroscopy) was used for spin system identification, and ROESY (rotating-frame noe spectroscopy) was used to determine sequential connectivities and other medium range NOE's. The full assignment values are given in Table 1. As is implied in its primary structure, bradykinin has high probability of tight turns. In Figure 1, the fingerprint region of ROESY spectra is shown. The sequential connectivities are broken due to the lack of amide protons in three prolines. For proline, the δ proton was used instead of missing amide proton to confirm the sequential connectivities. The NOE between δ protons of Pro 7 and amide proton of Phe 8 implies there might be a turn structure in the C-terminal fragment. The distance between the δ proton of a proline and the amide proton of an adjacent residue is equivalent to the distance between amide protons of neighboring residues. The NOE's between amide protons can be used to identify α helix and β turns. In α helix, there must be sequential NH-NH NOE connectivities not less than three residues long. However, the NOE connectivity in this peptide implies a turn-like structure. The type of the turn could not be determined due to the spectral overlap caused by other NOE's. 1D temperature variation experiment showed further evidence of the existence of a turn. 1D spectra were recorded at 5 K interval from 278 K to 323 K. The temperature coefficient values are shown in Table 2. These values can be used as a measure of solvent accessibility. Generally, temperature coefficient ($d\delta/dT$) values of <0.003 ppm K^{-1} are indicative of solvent-shielded and presumably hydrogen-bonded amide protons, and the values >0.005 ppm K^{-1} are attributed to the amide protons

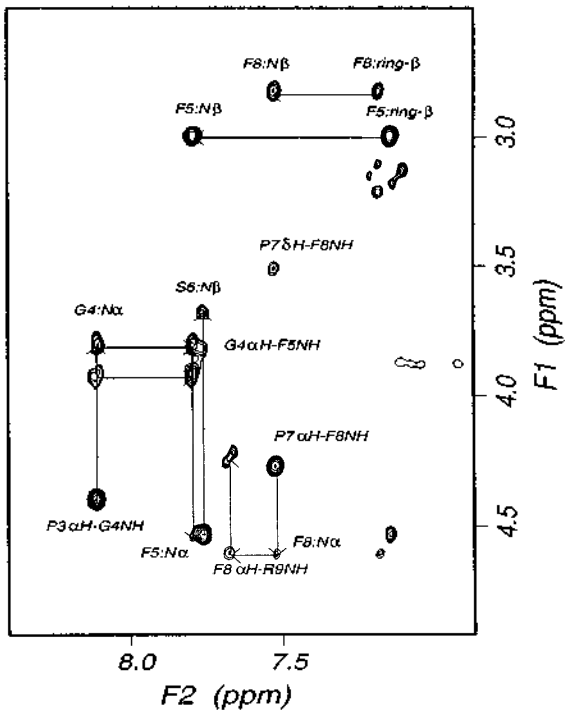

Figure 1. Fingerprint region of ROESY spectrum of bradykinin in H_2O/TFE-d_3 (1 : 1, by volume), 10 mM at 25 ℃ and pH 3. The mixing time was 100 ms.

Table 2. Temperature coefficient values and coupling constants of amide protons of bradykinin. CSD values mean chemical shift difference values (refer the text)

Residue	dδ/dT ($\times 10^{-3}$)	$J_{\beta\gamma}$ (Hz)	CSD value
Arg 1			−1
Pro 2			1
Pro 3			0
Gly 4	8.6	7.6	−1
Phe 5	4.6	6.8	−1
Ser 6	4.0	7.0	1
Pro 7			−1
Phe 8	4.2	8.0	0
Arg 9	3.8	7.6	−1

which are freely accessible to the solvent.[8] If there is any secondary structure, more than one hydrogen bond is generally involved. The small temperature coefficients for the C-terminal fragment can be interpreted as characteristics of secondary structure. The large value of amide proton of Gly 4 implies that it is quite exposed to the solvent molecules, whereas the values of amide protons in the C-terminal fragment show that they are moderately shielded from the solvent molecules. Based on the data in Table 2, a schematic diagram is shown in Figure 2. The slopes in the diagram correspond to the coefficient values in the table. The steeper the slope becomes, the more exposed the amide proton is. If there were strong hydrogen bonding in the peptide, the peptide would melt at some temperature and there would be a curvature due to the sudden breakage of the hydrogen

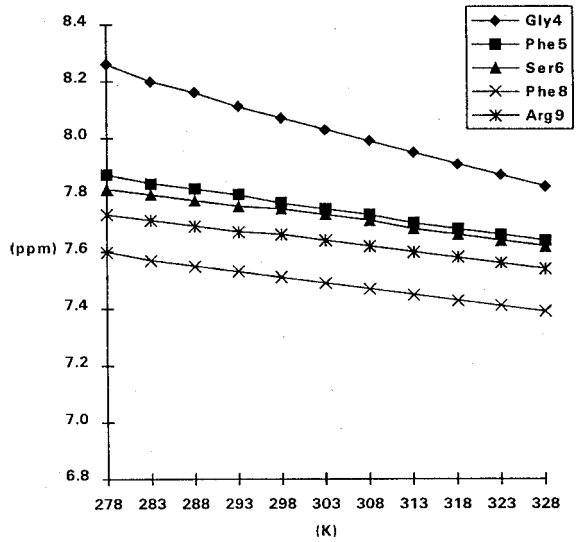

Figure 2. Schematic diagram of chemical shift differences of amide protons of bradykinin in the temperature variation experiment.

bonding. The lack of such curvature indicates no strong hydrogen bonding is being observed.

It has been shown that the chemical shift differences between α protons in a given structured peptide and the random coil values can give information about secondary structures. The chemical shifts of α proton experiences an upfield shift when in a helical conformation, and a comparable downfield shift when in a β strand extended conformation.[9] In Table 2, the differences are denoted as −1, 0, and 1 which correspond to an upfield shift, no shift, and downfield shift, respectively. A stretch of three or four CSD value of '−1' means α-helix and that of '1' means β strand. The alternate values shown in the table are the typical type of β turn.

From the sequential NOE's between the α protons of the i residues and amide protons of $i+1$ residues, it is thought that bradykinin has random coil population to some extent. In case $i+1$ residue is proline, δ proton instead of amide proton was used. Due to its short length, the peptide lacks structural rigidity and it is supposed that there would be an equilibrium between turns and random coil in solution. The experimental data suggest that there is a turn in the C-terminal fragment. The turn, however, is not stable and the type of turn has yet to be determined. Since it was reported that bradykinin is in a disordered state in water, the nascent turn structure is probably related to its binding to the receptor in the cell membrane. It is also likely that the C-terminal residues would participate in the binding of the peptide to the receptor. These results are consistent with the previous report that high-affinity bradykinin receptor antagonists adopt C-terminal β turns.[10]

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References

1. Regoli, D.; Barabe, J. C. *Pharmacol. Rev.* **1980**, *32*, 1.
2. Steranka, L. R.; Manning, D. C.; Dehaas, C. J.; Ferkany, J. W.; Borosky, S. A.; Connor, J. R.; Vavrek, R. J.; Ste-

wart, J. M.; Snyder, S. H. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 3245.
3. Denys, L.; Bothner-By, A. A.; Fisher, G. H.; Ryan, J. W. *Biochemistry* **1982**, *21*, 6531.
4. Mirmira, S. R.; Durani, S.; Srivastava, S.; Phadke, R. S. *Magn. Reson. Chem.* **1990**, *28*, 587.
5. Lee, S. C.; Russel, A. F.; Laidig, W. D. *Int. J. Pep. Protein. Res.* **1990**, *35*, 367.
6. Cann, J. R.; Vavrek, R. J.; Stewart, J. M.; Mueller, D. D. *J. Am. Chem. Soc.* **1990**, *112*, 1357.
7. Wüthrich, K. *NMR of Proteins and Nucleic Acids*; A Wiley-Interscience Publication: John Wiley & Sons, 1986; p 130.
8. Kople, K. D.; Ohnishi, M.; Go, A. *J. Am. Chem. Soc.* **1969**, *91*, 4264.
9. Wishart, D. S.; Sykes, B. D.; Richards, F. M. *Biochemistry* **1992**, *31*, 1647.
10. Kyle, D. J.; Blake, P. R.; Smithwick, D.; Green, L. M.; Martin, J. A.; Sinsko, J. A.; Summers, M. F. *J. Med. Chem.* **1993**, *36*, 1450.

(1*S*,2*S*)-(-)-1,2-Diphenylethylenediamine; A Good Chiral Solvating Agent for the Determination of Enantiomeric Purity of Chiral Alcohols by ^{1}H NMR

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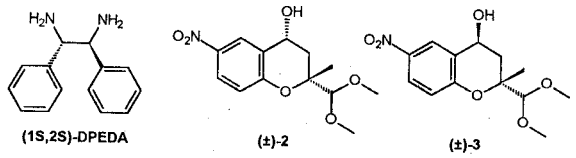
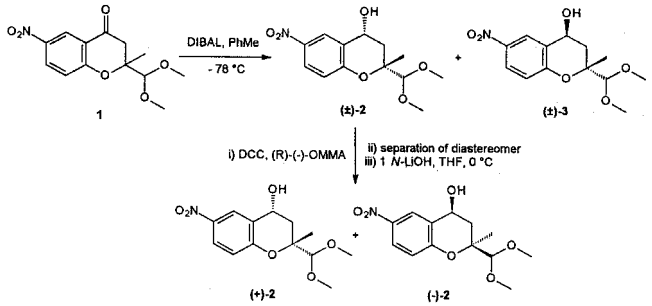
A majority of biologically important compounds are chiral and in many cases only one enantiomer exhibits desired activities. Although in the past it has been common to commerciallize the compounds in a racemic form, it is now required to have a single enantiomer for more selective activities. Therefore, it becomes more important than ever to determine the optical purity of the compounds.

Over the years, several techniques have been developed for the purpose of the determination of enantiomeric purity of chiral compounds. Among those, the NMR method became the subject for extensive studies because of its simplicity and non-destructive nature. For this purpose, chiral derivating agents (CDA),[1] chiral lanthanide shift reagents (CLSR),[2] and chiral solvating agent (CSA)[3] have been developed and all these approaches were based on the diastereoisomeric non-equivalency in NMR spectra. Although substantial developments have been made in these approaches, further improvements are necessary to cover wider range of compounds.

During the course of our research programs, we needed to determine the optical purity of several chiral alcohols and acids. For this purpose we have investigated various chiral solvating agents and found that (1*S*,2*S*)-(−)-1,2-diphenylethylenediamine (DPEDA) possesses an excellent property for the NMR determination of the enantiomeric purity for various alcohols and we would like to report the results of the study.



The compound, (±)-2, is a key intermediate for the potassium channel activator (PCA)[4] we have developed. This compound was prepared according to the procedure described in Scheme 1.



Scheme 1. Preparation of (−)-2 and (+)-2 by optical resolution

The reduction of ketone **1** with DIBAL in toluene −78 ℃ gave a diastereomeric mixture (9 : 1) in 94% yield and the diastereomers **2** and **3** were separated by column chromatography. The resolution of racemic **2** was carried out by forming the corresponding O-methyl mandelate, separating diastereomeric mandelates by column chromatography, and hydrolyzing the corresponding mandelate with 1 N *aqueous* LiOH in THF to give optically pure alcohol **2**. At this time it became necessary to determine the optical purity of the alcohol **2** and the typical procedure is described below.

In 0.1 molar solution of the alcohols and (1*S*,2*S*)-(−)-diphenylethylenediamine in $CDCl_3$ was directly prepared in a NMR tube and its ^{1}H NMR (500 MHz) spectrum was recorded at ambient temperature. The alcohols examined and the chemical shifts of the protons showing non-equivalency are listed in Table 1. Although there are numerous cases reported for the interaction between acids and the chiral bases, however, there are not many examples known for the case of alcohols where the interaction is expected to be much weaker. Nontheless, we found that even in this case the non-equivalency is large enough for the accurate determination of the optical purity as shown in Table 1.

In Figure 1, the regions corresponding to the $\underline{CH}(OMe)_2$ resonances of (a) free (±)-2, (b) the mixture of (±)-2 and 3 equimolar CSA, (c) 80% ee of (−)-2 , (d) 95% ee of (+)-2, enantiomerically pure of (+)-2 are shown, respectively. The concentration of (1*S*,2*S*)-DPEDA also substantially influences the degree of non-equivalency. Non-equivalency increases with an increase of (1*S*,2*S*)-DPEDA until the alcohol