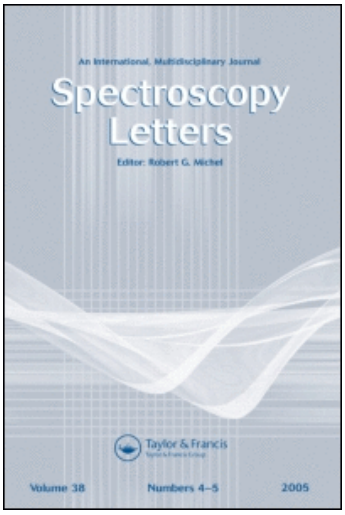

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Improved Method for Optical Purity Determination of Ketazolam with Chiral Shift Reagent

Laurine A. Laplanche[a]; Robert Rothchild[b]
[a] Chemistry Department, Northern Illinois University, DeKalb, IL [b] Department of Science, The City University of New York John Jay College of Criminal Justice, New York, NY

To cite this Article Laplanche, Laurine A. and Rothchild, Robert(1990) 'Improved Method for Optical Purity Determination of Ketazolam with Chiral Shift Reagent', Spectroscopy Letters, 23: 1, 45 — 64
To link to this Article: DOI: 10.1080/00387019008054035
URL: http://dx.doi.org/10.1080/00387019008054035

SPECTROSCOPY LETTERS, 23(1), 45-64 (1990)

IMPROVED METHOD FOR OPTICAL PURITY DETERMINATION OF KETAZOLAM WITH CHIRAL SHIFT REAGENT

Keywords: Ketazolam, Chiral, Lanthanide, $Yb(HFC)_3$, NMR shift reagents, Enantiomer, Optical Purity

Laurine A. LaPlanche[a] and Robert Rothchild[b]

[a]Chemistry Department, Northern Illinois University, DeKalb, IL 60115

[b]The City University of New York
John Jay College of Criminal Justice
Department of Science, Toxicology Research and Training Center, 445 West 59th Street
New York, NY 10019-1199

ABSTRACT

An improved method for direct determination of optical purity of the novel benzodiazepine analog, ketazolam, 1, is reported. Adding the chiral lanthanide NMR shift reagent, tris[3-(hepta-

fluoropropylhydroxymethylene)-d-camphorato] ytterbium(III), $Yb(HFC)_3$, to racemic ketazolam in $CDCl_3$ solution allows observation of enantiomeric shift differences for six of the ketazolam proton resonances. The C(2) methyl ^1H signal is especially appropriate for the determination of optical purity since the C(2) methyl resonances of each enantiomer are clearly resolved (for accurate peak intensity determination) at 200 MHz. For racemic 1, using a molar ratio of $Yb(HFC)_3$:1 of 0.20, the valley height between the peaks of the two enantiomers' CCH_3 signals was only 4.8% of the average peak heights. Detection of as little as 2-3% of a minor enantiomer should be possible. Some aspects of the hindered phenyl rotation in 1 and the LSR-induced broadening of certain NMR signals are also discussed.

INTRODUCTION

Ketazolam, 1, is a novel 1,4-benzodiazepine analog that has been obtained from 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (diazepam) with acetyl chloride and triethylamine in ether or with diketene in acetone

(1). The formal name of 1 is 11-chloro-8,12b-dihydro-2,8-dimethyl-12b-phenyl-4H[1,3]-oxazino[3,2-d][1,4]benzodiazepine-4,7(6H)-dione. The early ^{1}H NMR and x-ray diffraction studies of 1 showed the seven-membered ring to be in a boat conformation as part of a rather rigid structure (1). The benzylic carbon, 12b, of 1 is a chiral center; thus the molecule exists as a pair of enantiomers. The unsubstituted phenyl ring undergoes hindered rotation about the $C_{1'}$-C_{12b} bond even at room temperature. Empirical energy calculations show a two-fold energy barrier for the phenyl group rotation about this bond (2).

In an earlier report, a chiral NMR lanthanide shift reagent (LSR), tris[3-(heptafluoropropyl-hydroxymethylene)-d-camphorato]-europium(III), 2, known as Eu(HFC)$_3$, was employed to induce enantiomeric shift differences in selected signals (3). The enantiomeric shift difference, $\Delta\Delta\delta$, is the difference in chemical shift between the signals of corresponding nuclei in two enantiomers in the presence of a chiral LSR.

The separation of the enantiomers of 1 by means of chiral chromatography has also been recently

noted (4). The complementary values of the chiral chromatographic and the NMR method for optical purity determinations is well known (5). The importance of the two techniques will grow along with increasing interest in enantioselective syntheses. The applications of the methodologies to ketazolam reflect an active interest in this pharmaceutical.

In an attempt to improve the analytical utility for enantiomeric excess measurements of 1, we have further explored these NMR LSR studies using a higher field spectrometer and a different LSR. It was expected that at 200 MHz, considerable improvement in the resolution of the signals of the nuclei of the enantiomers would result, permitting detection of lower levels of a minor enantiomer. We report here the results of studies of 1 with tris[3-(heptafluoropropylhydroxy-methylene)-d-camphorato]ytterbium(III), 3, known as $Yb(HFC)_3$.

EXPERIMENTAL

Racemic 1 was a gift of the Upjohn Company. The $Yb(HFC)_3(+)$ LSR was purchased from the Aldrich

Chemical Company, Inc. and stored over anhydrous calcium chloride. The deuterated chloroform, also purchased from Aldrich, contained 0.03% v/v TMS as internal reference. The chloroform was filtered through a column of basic alumina immediately before solution preparation.

A stock solution of 0.0100 M 1 in $CDCl_3$ was prepared. The first solution studied contained 12.14 mg of $Yb(HFC)_3$ and 0.500 ml of the 0.0100 M stock solution of 1 making a molar ratio 3:1 of 2.00. Ten subsequent solutions were prepared by adding aliquots of the stock solution to the NMR tube. In this manner, the concentration of 1 was kept constant and exposure of the anhydrous LSR to the atmosphere was minimized.

All spectra were recorded at 200 MHz on an IBM Instruments NR/200 spectrometer at 20° C. Sixteen FIDs were averaged. A spectral width of 8000 Hz was used, collecting 16 K data points in the quadrature detection mode. Lanthanide induced shift values, $\Delta\delta$, are reported in ppm; enantiomeric shift differences, $\Delta\Delta\delta$, are reported in Hz.

RESULTS AND DISCUSSION

An earlier report on 1 using the chiral LSR, $Eu(HFC)_3$, 2, for ^1H NMR studies (3) was performed at 28° at 60 MHz and described $\Delta\Delta\delta$ for each of the diastereotopic hydrogens, H_{6a} and H_{6b}, for the aryl hydrogen H_{12}, and for both the CCH_3 and NCH_3. Under these conditions, with a 2:1 molar ratio near 0.45, the best reported resolution for the CCH_3 signal had a valley height of 38% between the methyl peaks corresponding to the two enantiomers For analytical purposes in optical purity determinations of 1, the signals of H_{6a} or H_{6b} were preferable because they were most clearly resolved. However, these protons are spin-spin coupled, so that H_{6b}, for example, appeared as a triplet ($\Delta\Delta\delta$ approximately equal to $^2J_{gem}$) for racemic 1 and a 2:1 ratio near 0.34. It was estimated that at least 7% of the minor enantiomer could be detectable.

These present studies explored the use of a different chiral LSR, $Yb(HFC)_3$, in conjunction with a higher spectrometer frequency (200 MHz) to achieve better resolution of the signals of each

enantiomer of 1. Ytterbium(III) LSR are known to produce substantially greater lanthanide-induced shifts, $\Delta\delta$, than the corresponding europium(III) LSR (6). We observed that a solution of 3, 0.0100 M in $CDCl_3$, in the absence of other substrate displayed ^1H signals at: 17.32, 4.06, 3.14, 0.35, 0.085, -3.32, -10.94 and -11.25 ppm.. These LSR absorptions can depend on solvent, temperature and actual concentrations of both LSR and specific substrate employed; it was critical that interfering signals be avoided for an analytically useful method.

The unshifted spectrum of 1 at 200 MHz and 20° clearly exhibited sharp, well-defined multiplets for H_{12} (d, ^4J=2.4 Hz), H_{10}(dd, ^4J=2.4 Hz, ^3J = 8.6 Hz) and H_9 (d, ^3J = 8.6 Hz) of the chlorophenylene ring, but only broad unresolved peaks for the unsubstituted phenyl group at the chiral center, C_{12b}. We have been able to attribute these broad signals to a novel hindered rotation of the phenyl group; discussions of our dynamic NMR studies on 1 are being presented elsewhere. [To our knowledge, only one other report of a comparable hindered

rotation of an unsubstituted phenyl ring for small organic molecules at ambient temperatures has appeared (7,8).]

Although the general trends for additions of 3 to 1 were expected to be similar to those seen for 2 added to 1, dramatic differences were seen in these 200 MHz spectra. For a series of runs in which a solution of 3 was diluted with aliquots of a 0.0100 M $CDCl_3$ solution of 1, the resulting lanthanide-induced shifts and enantiomeric shift differences for the protons of 1 are summarized in Figs. 1 and 2, respectively. Severe lanthanide-induced line broadening for H_3, H_{6a} and H_{6b} made assignments uncertain or impossible for these nuclei at 3:1 ratios above 0.04.

The proton resonance of the phenyl ring of 1 is broad at both 60 MHz and 200 MHz due to hindered rotation even at ambient temperatures. Empirical energy calculations reveal a two-fold barrier about the $C_{1'}$-C_{12b} bond (2). At intermediate 3:1 molar ratios of about 0.04 to 0.80, the resonances of the phenyl group spread out to form a complex and broad envelope, making individual phenyl

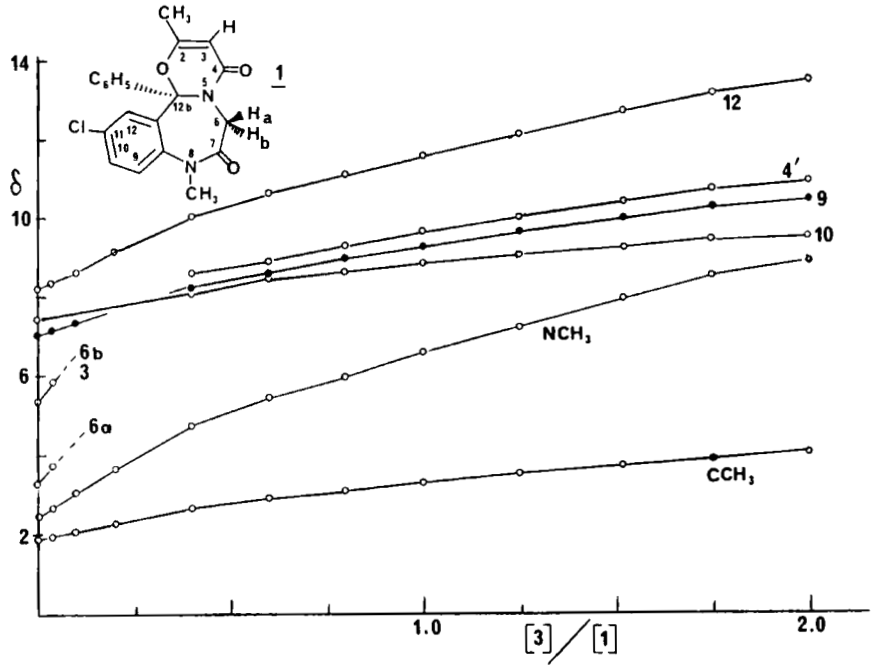

Figure 1. Variation of chemical shifts, δ (in ppm), of 1 with molar ratio of 3:1. Where enantiomeric shift differences occur, average chemical shift values for the enantiomers are plotted. See text for discussion of nuclei not included here.

proton assignments difficult and obscuring H_9, H_{10}, H_{12}. At higher levels of 3, the phenyl resonances have largely been shifted to lower field (below 12 ppm) so that the $H_{9,10,12}$ assignments can be made. At 3:1 molar ratios of

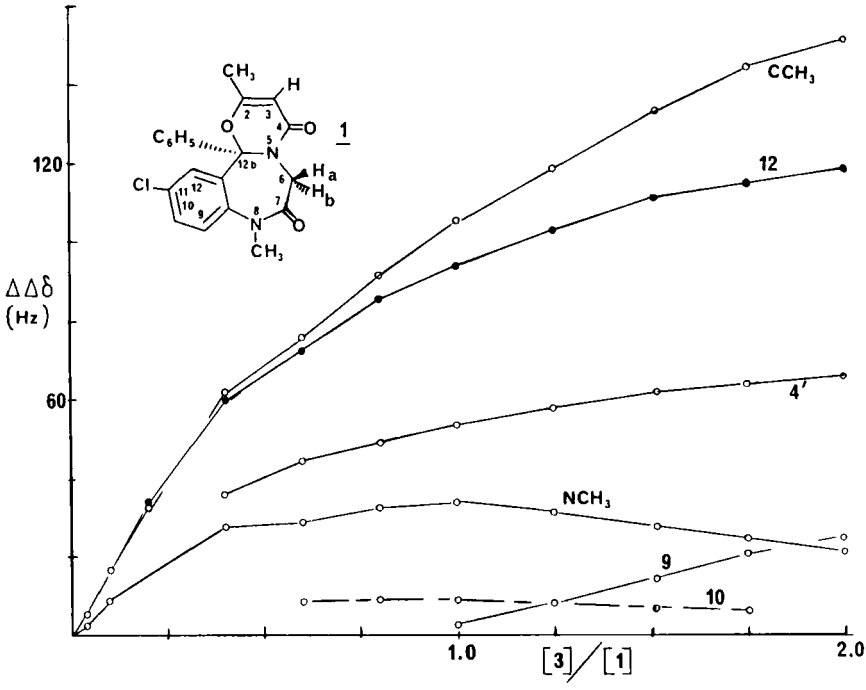

Figure 2. Variation of enantiomeric shift differences, $\Delta\Delta\delta$ (in Hz), with molar ratio of 3:1.

0.4 or higher, most unexpected was the appearance of two multiplets which we assigned to H_4, one for each enantiomer of 1. The para proton $H_{4'}$, while not affected by hindered rotation about the $C_{1'}$-C_{12b} bond, is affected by the complexation of shift reagent, with $\Delta\Delta\delta$ approximately equal to 55 Hz (at 200 MHz) at a 3:1 molar ratio of 1.00.

Complexation of 3 to 1 occurs at the carbonyl oxygen atoms attached to C_4 and to C_7; both will affect the $\Delta\delta$ measured for the $H_{4'}$ proton. The boat conformation of the seven-membered ring places the oxygen at C_7 within 6.3 Å of $H_{4'}$, while the oxygen at C_4 is within 7.2 Å of $H_{4'}$ (2). These distances are well within the shifting range of LSRs. It is quite unusual, however, to have enantiomeric resolution at this distance from the chiral center of the substrate molecule.

Significant $\Delta\Delta\delta$ values were seen for all three chlorophenylene protons H_9, H_{10} and H_{12}, for both methyl groups and for $H_{4'}$ (Figure 2). For the NCH_3 protons and for H_{10}, the plots of $\Delta\Delta\delta$ seen in Fig. 2 appear to reach a maximum with a 3:1 ratio of 0.8 - 1.0. The $\Delta\Delta\delta$ value ($\Delta\Delta\delta = |\Delta\delta_R - \Delta\delta_S|$, where $\Delta\delta_R$ is the induced shift of the R substrate) is a function of two mechanisms operating simultaneously (9): a) The dissociation constants for the R and S substrate-complexes will differ, causing $\Delta\delta_R \neq \Delta\delta_S$, and b) the two different complexes will have different geometries (10), which will also cause $\Delta\delta_R \neq \Delta\delta_S$. In addition, the likely co-existence of 1:1 and 2:1

substrate:LSR complexes (11) makes the evaluation of $\Delta\Delta\delta$ as a function of molar ratio (Figure 2) a formidable task (12). With 3:1 molar ratios above 1.0, the H_{12} and H_4, signals essentially show baseline resolution, free from interference by 3 protons. The H_9 signals for each enantiomer are almost baseline resolved with a 3:1 molar ratio of 2.0. All of these signals could be analytically useful for optical purity determinations. However, it is the CCH_3 signal which would be optimal in view of its intensity and favorable signal-to-noise ratio. Thus, a 3:1 molar ratio of 0.20 caused this methyl signal to appear with a valley height (separating the signal of each enantiomer) of only 4.8% of the average peak height, essentially free from interferences. Detection of as little as 2-3% of the minor enantiomer should be feasible. With a 3:1 molar ratio between 1.52 and 1.75, the CCH_3 signals of each enantiomer are essentially baseline resolved but suffer slight interferences with peaks from 3, which fall on either side of the two CCH_3 peaks. Comparable detection limits should be attainable under these conditions. Using lower 3:1 ratios,

circa 0.20, requires less shift reagent and is preferable, since spectral interference due to resonance peaks of 3 is not a problem.

Additional runs with 1 were performed using higher drug concentrations. Results were generally similar, but somewhat larger $\Delta\delta$ and $\Delta\Delta\delta$ magnitudes were seen at comparable 3:1 molar ratios using the higher drug concentrations since there is less competition by solvent for the shift reagent. For example, a 3:1 ratio of 0.090 resulted in a valley height of 5.9% of the average CCH_3 peak heights using about 0.10 M 1. The use of 3 for determinations of enantiomeric excess of 1 would therefore be noncritical over a rather wide range of concentrations of 1 and molar ratios of 3:1.

The importance of the use of 3 and the 200 MHz NMR for optical purity determination of 1 is threefold. Compared to earlier work (3), useful $\Delta\Delta\delta$ values for analytical purposes are achieved at about one-quarter of the LSR levels required earlier, reducing possible problems with contaminants in the LSR and absorption of water by the anhydrous LSR. Secondly, the analytically

useful signal is much better resolved here, considerably improving the procedure by allowing detection of smaller amounts of the minor enantiomer. Lastly, the method here is based on measurement of signals corresponding to a three proton methyl singlet, CCH_3, rather than doublets of the H_{6b} signal. In the absence of broadening effects, this improves the signal-to-noise ratio by a factor of six, and should allow direct NMR optical purity determinations on much smaller total amounts of 1.

The severe line broadening observed at 200 MHz for the H_{6a}, H_{6b} and H_3 protons, which is already evident at a 3:1 molar ratio of 0.010, and which broadens the peaks to such an extent that they cannot be observed at a 3:1 molar ratio of 0.100, deserves further comment. In earlier LSR studies of ketazolam at 60 MHz, using 2 as the LSR, line broadening was observed but it was not severe; in fact the multiplet structure of protons H_{6a} or H_{6b} was still present at a 2:1 molar ratio up to 0.668 (3).

The resonances of these protons are especially broadened because they are close to the two

primary sites for complexation of the LSR to ketazolam, namely the two carbonyl oxygen atoms. Since the extent of broadening is primarily a function of r^{-6}, where r is the distance between the metal atom of the LSR and the observed proton close to the site of binding, H_{6a}, H_{6b} and H_3 will be preferentially broadened (13).

There are two major factors which contribute to the significantly greater line broadening of these ketazolam protons when bound to 3 (and observed at 200 MHz), vs. being bound to 2 (and observed at 60 MHz). The first is that the Yb LSR, 3, produces greater line broadening of protons that the Eu LSR, 2 (14-16). In general, an LSR which produces a larger induced shift for specific protons in a given LSR complex will also cause greater line broadening for those protons (17). This is because (neglecting the angular factors in the pseudocontact shift and relaxation equations (13,18)), the shift is primarily a function of r^{-3} and the rate of relaxation is a function of r^{-6}. Thus, comparing the two reagents at the same molar ratio, at the same temperature and in the same solvent, 3 will produce a larger LIS value and

cause greater line broadening than 2 for a given proton of 1.

The second and more important factor which makes the proton line width for 3:1 at 200 MHz so much greater than that of 2:1 at 60 MHz is the different spectrometer frequency used. In these paramagnetic systems, the largest contribution to line broadening comes from a decrease in T_2, the proton spin-spin relaxation time, due to fast chemical exchange of the LSR (19,20). For a specific molar ratio of LSR to substrate, the line width ($1/\pi T_2$) is directly proportional to $(\delta_{BF})^2$, where δ_{BF} is the chemical shift difference (in Hz) between the bound state (in the LSR complex) and the free state for a specific proton (19,20). At 200 MHz (as compared with 60 MHz), δ_{BF} will be larger by a factor of (200/60), causing the line width to be larger by a factor of $(200/60)^2$ or 11.1, resulting in significant line broadening for protons which are close to the complexation site(s) of the LSR. Thus, the greater line broadening characteristics of 3 vs. 2, plus the higher spectrometer frequency (200 MHz) cause the three protons H_{6a}, H_{6b} and H_3 to broaden into the

baseline at 3:1 molar ratios equal to, or greater than 0.100.

CONCLUSIONS

We have presented a method for direct optical purity determinations of ketazolam using the chiral LSR, $Yb(HFC)_3$. The ^1H NMR spectrum at 200 MHz shows good resolution between the $C(2)CH_3$ signals of each enantiomer indicating excellent analytical potential. This technique appears considerably better than an earlier method (3) and should allow analysis of smaller quantities of total drug and detection of an appeciably lower level of the minor enantiomer.

ACKNOWLEDGMENTS

Samples of ketazolam were kindly provided by the Upjohn Co., Kalamazoo MI 49001. We wish to thank Paulanne Y. Rider for solution preparation and for recording the spectra. These studies were supported, in part, by the National Science Foundation Instrumentation and Laboratory Improvement Program grant no. USE-8851684, U.S. Department of Education Minority Science Improvement Program grant no. G008641165, Hewlett-

Packard Co. grant no. 0017-80769, Hoffmann-La Roche Inc. and the Sandoz Research Institute (to R.R.).

REFERENCES

1. Szmuskovicz J., Chidester C.G., Duchamp D.J., MacKellar F.A., Slomp G. Synthesis and proof of structure of a novel benzodiazepine. Tetrahedron Lett. 1971; (39):3665-3668.

2. Rothchild R., LaPlanche L.A., to be submitted. Calculations based on crystal structure data from Ref.1.

3. Martin R., Myers C., Rothchild R. ^{1}H NMR spectral simplification with achiral and chiral lanthanide shift reagents. Ketazolam. Method for direct optical purity determinations. Spectrosc. Lett. 1987; 20(8):645-661.

4. Kinkel J.N., Fraenkel W., Blaschke G. The separation of enantiomers on polymer coated chiral silica gels. Kontakte (Darmstadt) [Merck] 1987; (1):3-14.

5. Yamaguchi S. Nuclear magnetic resonance analysis using chiral derivatives. In: Morrison J.D. ed. Asymmetric Synthesis, vol. 1. London, New York: Academic Press, 1983: 125-152.

6. Wenzel T.J. NMR Shift Reagents. Boca Raton, FL: CRC Press, Inc., 1987, p.6.

7. Sanders J.K.M., Hunter B.K. Modern NMR Spectroscopy: a Guide for Chemists. New York:Oxford Univ. Press, 1987, p. 222.

8. Haverhand M. Ph.D. Thesis, Cambridge Univ., 1986: 66-67 and Figs. 1-5.

9. McCreary M.D., Lewis D.W., Wernick D.L., Whitesides G.M. The determination of enantiomeric purity using chiral lanthanide shift reagents. J. Am. Chem. Soc. 1974; 96(4): 1038-1054.

10. Wenzel T.J. Lanthanide Shift Reagents in Stereochemical Analysis, Methods in Stereochemical Analysis 5. New York: VCH Publ., Inc., 1986, p. 151.

11. Shapiro B.L., Johnston, Jr. M.D. Lanthanide-induced shifts in proton nuclear magnetic resonance spectra. III. Lanthanide shift reagent-substrate equilibria. J. Am. Chem. Soc. 1972; 94(23): 8185-8191.

12. Fraser R.R. Nuclear magnetic resonance analysis using chiral shift reagents. In: Morrison J.D. ed. Asymmetric Synthesis, vol. 1. London, New York: Academic Press, 1983: 173-196, esp. p. 182.

13. Solomon I. Relaxation processes in a system of two spins. Phys. Rev. 1955; 99: 559-565.

14. Cockerill A.F., Davies G.L.O., Harden R.C., Rackham D.M. Lanthanide shift reagents for nuclear magnetic resonance spectroscopy. Chem Rev. 1973; 73(6): 553-588.

15. Rackham D.M. Lanthanide shift reagents. XV. Quantitation of D,L-amino acid enantiomers using chiral lanthanide shift reagents. Spectrosc. Lett. 1980; 13(5): 321-327.

16. Mayo B.C. Lanthanide shift reagents in nuclear magnetic resonance spectroscopy. Chem. Soc. Rev. 1973; 2: 49-74.

17. Faller J.W., La Mar G.N. Chemical spin-decoupling and lanthanide reagents. Tetrahedron Lett. 1973; (16): 1381-1384.

18. (a) Jesson J.P. The paramagnetic shift. In: La Mar G.N., Horrocks, Jr. W. DeW., Holm R.H. eds. NMR of Paramagnetic Molecules; Principles and Applications. New York, London: Academic Press, 1973, p. 17.

(b) Swift T.J. The paramagnetic linewidth. ibid., p. 73.

19. Bulsing J.M., Sanders J.K.M. Spin-echo methods for resolution control of lanthanide-shifted NMR spectra. J. Chem. Soc., Chem. Commun. 1981; 23: 1201-1203.

20. Lenkinski R.E., Reuben J. Line broadenings induced by lanthanide shift reagents: concentration, frequency, and temperature effects. J. Magn. Reson. 1976; 21: 47-56.

Date Received: 08/17/89
Date Accepted: 09/22/89