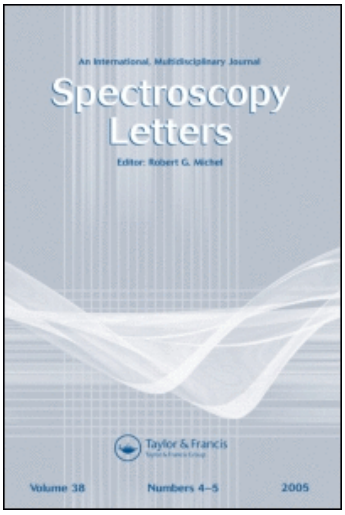

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^{1}H NMR Spectral Simplification with Achiral and Chiral Lanthanide Shift Reagents. Ketazolam. Method for Direct Optical Purity Determination

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^{1}H NMR SPECTRAL SIMPLIFICATION WITH ACHIRAL AND CHIRAL LANTHANIDE SHIFT REAGENTS. KETAZOLAM. METHOD FOR DIRECT OPTICAL PURITY DETERMINATION

Key Words: Lanthanide, Shift Reagents, Enantiomers, Optical Purity, Chiral, NMR, Europium, Ketazolam, Conformation

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ABSTRACT

The 60 MHz ^{1}H NMR spectra of racemic ketazolam, **1**, have been studied at 28° in $CDCl_3$ solution with the achiral reagent tris(6,6,7,7,8,8,8-heptafluoro-2,2-

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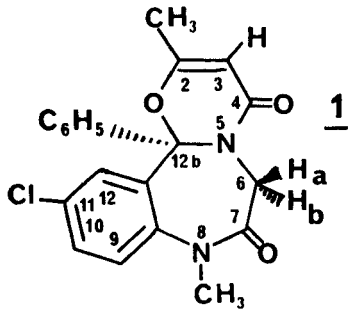
dimethyl-3,5-octanedionato)europium(III), 2, and the chiral reagent tris[3-(heptafluoropropylhydroxymethylene)-d-camphorato]europium(III), 3. Dramatic differences in lanthanide-induced shifts, $\Delta\delta$, were observed for the diastereotopic CH_2 protons with either added 2 or 3. Differences in these $\Delta\delta$ values, as well as differences in lanthanide-induced line broadening and in enantiomeric shift differences (observed with 3) are interpreted in terms of a relatively rigid preferred conformation of 1. Assignments for the CH_2 protons are made. Direct optical purity determinations for samples of 1 should be possible by use of 3, based on resonances for either of the CH_2 protons or the allylic CH_3.

INTRODUCTION

Ketazolam, 1, is a novel 1,4-benzodiazepine 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] Early reference to 1, formally known as 11-chloro-8,12b-dihydro-2,8-dimethyl-12b-phenyl-4H[1,3]-oxazino[3,2-d][1,4]benzodiazepine-4,7(6H)-dione, appeared in the patent literature.[2] Proton NMR and x-ray diffraction studies performed on 1 had indicated that the seven-membered ring is in a boat conformation, with the phenyl group roughly

perpendicular to the remainder of the molecular (which is rather flat).[1] A relatively rigid structure was considered, in which the seven-membered ring was bent "towards" the phenyl to avoid steric repulsions between the ortho phenyl hydrogens and the hydrogen at C(12) of the chlorobenzene group. Since the benzylic carbon of ketazolam is a chiral center, the molecule can exist as a pair of enantiomers. We were interested in extending our studies of achiral and chiral lanthanide shift reagents (LSR) for proton NMR spectral simplification and possible direct optical purity determinations. These LSR techniques have been reviewed.[3-14] The rigidity of 1 and the presence of "hard-base" binding sites, i.e., carbonyl oxygens, for the lanthanide ion, suggested that the LSR method could be useful in this case. The potential pharmaceutical importance of 1 as a tranquilizer further encouraged us in these investigations. The ongoing interest in 1, as reflected by at least 32 citations in Chemical Abstracts from about 1973-1986, including some twenty articles on analytical



aspects and at least eight on clinical, pharmaceutical and pharmacological topics, provided further incentive.

Because of the annelation of the oxazino ring onto the benzodiazepine nucleus of 1, ketazolam represents on unusual analog to the important pharmaceutical classes of tricyclic tranquilizers and antidepressants, which include phenothiazines, dibenzocycloheptatrienes, iminodibenzyls and dibenzdiazepines. Structural aspects, such as the puckering of the central ring in these compounds, have been considered to have great importance in determining pharmacological activity;[18] general aspects of structure-activity relationships among benzodiazepines have also been discussed.[19] Several studies using LSR for ^{1}H NMR studies within the benzodiazepine system have been reported.[20-22]

EXPERIMENTAL

A sample of racemic 1 was supplied by the Upjohn Co., Kalamazoo MI 49001, U.S.A., as U-28,774, lot no. 60,867, with stated mp 174-176° [lit. 182-183.5°[23]] and was used as supplied without further purification. Chloroform-d, (99.8 atom % D), obtained from Aldrich Chemical Corp., Milwaukee WI 53201, U.S.A., or from Norell, Inc., Landisville NJ 08326, U.S.A., was dried and stored over 3A Molecular Sieves. Shift reagents were obtained from Aldrich and were stored in a

desiccator over P_2O_5. Materials were used as received except as noted.

In general, an accurately weighed portion of drug (about 45-55 mg) was added to 600-700 mg $CDCl_3$ [containing about 0.2% tetramethylsilane (TMS) as internal standard] in an NMR sample tube and dissolved by shaking; increments of shift reagent were added directly to the sample, dissolved by shaking, and the spectra immediately run.

All spectra were run on a Varian EM-360A 60 MHz ^{1}H NMR spectrometer at a probe temperative of 28°. Chemical shifts are reported in parts per million (δ) relative to TMS and are believed accurate to $\pm$ 0.05 ppm. In runs with chiral shift reagent where enantiomeric shift differences were observed for selected resonances, reported chemical shifts are the average values for the two enantiomers. Reported coupling constants are believed accurate to $\pm$ 0.2 Hz. In spectra where TMS was obscured by shift reagent peaks, $CHCl_3$ (present as an impurity in the solvent) was used as internal standard.

RESULTS AND DISCUSSION

Spectra of 1 were first obtained using the achiral shift reagent, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III), 2, known as $Eu(FOD)_3$, for spectral simplification, and then

acquired with the chiral reagent, tris[3-(heptafluoropropylhydroxymethylene)-d-camphorato]europium(III), 3, known as $Eu(HFC)_3$ or $Eu(HFBC)_3$, to determine whether enantiomeric shift differences, $\Delta\Delta\delta$, were observable that might allow direct optical purity determinations. The enantiomeric shift difference is the magnitude of the difference in chemical shifts (of a particular nucleus) for the two enantiomers when treated with a chiral LSR.

The 60 MHz ^1H NMR spectrum of 1 as a 0.1635 molal $CDCl_3$ solution at 28° showed resonances as follows, in δ (ppm) units: 1.88 (s, 3H, CCH_3); 2.50 (s, 3H, NCH_3); 3.33 (d, ^2J = 14.1 Hz, 1H, C(6)H_a); 5.40 (d, ^2J = 14.1 Hz, 1H, C(6)H_b); 5.37 (s, 1H, C(3)H); 7.2 (d, C(9)H ?, see text); 7.27 (m, aryl H, see text); 7.53 (dd, J = 8.3 and 2.4 Hz, 1H, C(10)H); 8.22 (d, J = 2.4 Hz, 1H, C(12)H). Assignments for the aryl protons of the chlorophenylene moiety were based on observed coupling constants. Thus, C(12)H was furthest downfield with splitting consistent with $^4J_{meta}$ to C(10)H, and was well separated from the remaining aryl resonance. The dd assigned to C(10)H was consistent with $^3J_{ortho}$ and $^4J_{meta}$ to C(9)H and C(12)H, respectively, and was on the downfield side of the main aryl signal. The deshielding of C(10)H and C(12)H is consistent with the expected substituent effects of Cl and N. The

assignment of C(9)H must be considered tentative because of overlap of the expected doublet, $^3J \approx 8$ Hz, in the aryl multiplet. Integrated area of the region from 6.8 - 7.8 ppm indicated 7H. The two diastereotopic protons, C(6)$H_{a,b}$, show striking magnetic nonequivalence, differing in chemical shift by over 2 ppm. This reflects substantial anisotropic effects within a rigid ring system.[1] Inspection of models indicated, as previously shown by x-ray, that in one possible conformation, substantial non-bonded interactions could be expected between the ortho protons of the C_6H_5 ring and the downfield C(12)H. In the alternative, and presumably favored conformation, the C_6H_5 ring can be (roughly) perpendicular to the average plane of the tricyclic system. In particular, this favored conformation places one of the C(6)H protons (anti to C_6H_5) in the shielding cone of the C_6H_3Cl aryl ring, so that the upfield doublet at 3.33 ppm must be assigned to this proton, H_a. In contrast, the other methylene proton, H_b (syn to C_6H_5), is roughly coplanar with both carbonyls, the anisotropy of which has commonly[24] been considered to be deshielding within the carbonyl plane, i.e., coplanar with the atoms directly bonded to the trigonal carbon. At least in some cases, these precise geometric aspects of the carbonyl anisotropy have been questioned.[25] Indeed, 1

may prove to be a useful model compound for consideration of this problem. Assuming anisotropic deshielding contributions to the hydrogen H_b for this system would account for the far downfield doublet at 5.40 ppm. Simple inductive deshielding by N(5) and the C(7)=0 would be similar for the two protons. Our NMR assignments for both chemical shifts and coupling constant magnitudes are in excellent agreement with the published data; we could not verify the small allylic coupling (given as 0.5 Hz in Ref. 1) because of our instrumental limitations, but some line broadening of the vinyl H and the allylic CH_3 signals was seen. Evidence for additional conformers, i.e., extra sets of peaks, was not seen.

Incremental additions of the achiral 2 resulted in spectral simplification and provided support for the assignments of the CH_2 protons. LSR binding to amides ordinarily occurs at oxygen[26,27] so that complexation of 1 should primarily occur at the C(4) and C(7) carbonyl oxygens. It may be possible for these two oxygens to actually chelate a europium(III) although models indicate that the carbonyls deviate from coplanarity by a substantial amount, perhaps 45°. This potential chelation could be further disfavored by steric repulsions with the C(6)H_b proton that is nearly coplanar with these carbonyls. Appearance of only one set of NMR signals indicates that "fast exchange"

conditions exist between free 1 and 1 bound to LSR; fast equilibration would be expected for LSR complexed to either of the carbonyl sites. Results of spectra of 1 with added 2 are summarized in Fig. 1.

The rigidity of the polycyclic system of 1 would favor LSR studies. Studies in these laboratories with five- and six-membered rings containing amide or related functionality have indicated that applications of chiral LSR are more likely to produce observable enantiomeric shift differences, $\Delta\Delta\delta$, if certain structural requirements are met. The substrate should preferably be rigid and LSR binding should ideally occur close to the chiral center. The observed nuclei for which $\Delta\Delta\delta$ values are to be seen should be close to the binding site and the chiral center.[28] Observation of $\Delta\Delta\delta$ in the presence of an added chiral LSR can make possible direct optical purity determinations by simple integration of the absorption signal due to each antipode. The chiral reagent 3 was therefore applied to spectral studies of 1. Incremental additions of 3 to solutions of 1 from 0.194 to 0.242 molal produced $\Delta\Delta\delta$ for each proton of the methylene, for the allylic CH_3, and for C(12)H. All but the latter signal could be analytically useful, with the preferred signal dependent on the actual molar ratio of 3:1. Where the amount of 1 is limited, the allylic CH_3 signal provides good

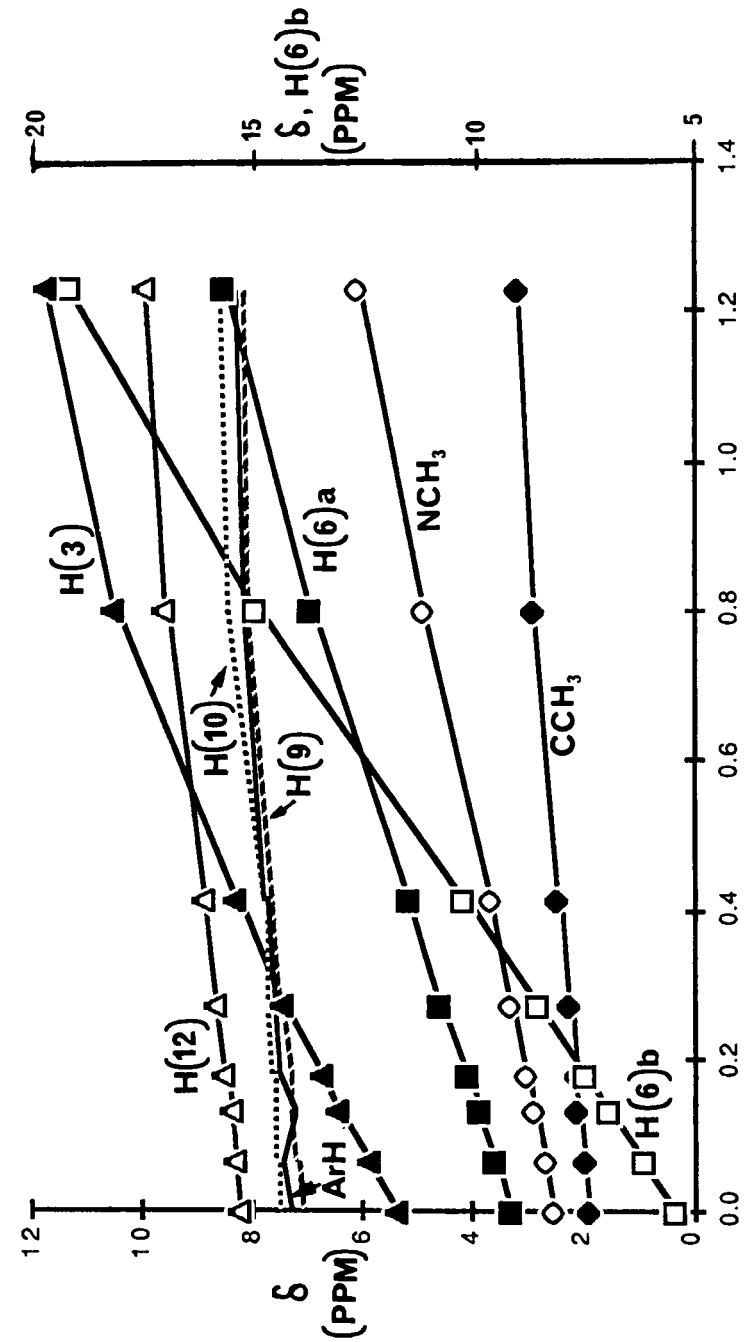


FIG. 1. Variation of chemical shift, δ, with molar ratio of 2:1.

sensitivity because of its high intensity and low multiplicity. Although $\Delta\Delta\delta$ magnitude increased at higher 3:1 ratios, lanthanide-induced line broadening resulted in best resolution for the signals of the enantiomers (using the allylic CH_3 resonance) at a 3:1 ratio near 0.45. The valley height between the peaks corresponding to the two enantiomers was as low as 38% under these conditions. The methylene proton, H_b, that produced the downfield signal in unshifted 1 also displayed greater lanthanide-induced shift, $\Delta\delta$, as well as greater $\Delta\Delta\delta$ and lanthanide-induced line broadening, compared to its geminal neighbor, H_a. All of these observations are consistent with LSR binding closer to the former proton, H_b, as expected for the proton *syn* to C_6H_5, nearly coplanar to the carbonyls in the preferred conformation. Since the chemical shift difference between the signals of the CH_2 protons is very large, especially at higher 3:1 levels, little leaning is seen in each enantiomer's doublet signal. Good quantitation of optical purity could thus be obtained even if the signals of each enantiomer slightly overlap, by comparing the upfield branch (of one enantiomer) to the downfield branch (of the other enantiomer) for the resonances of either of these protons. For example, a 0.194 molal solution of 1 with a 0.339 3:1 molar ratio resulted in the signals for each CH_2 proton being free from interfering overlaps,

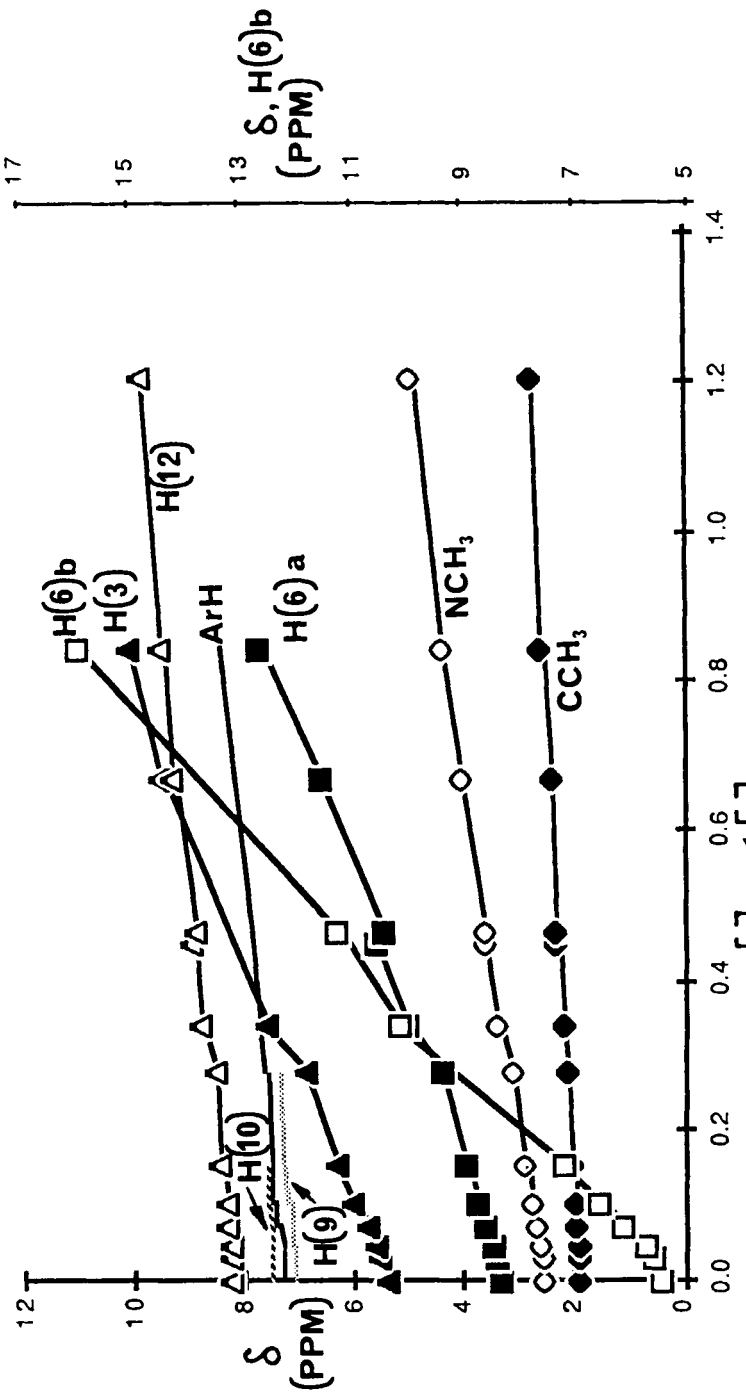


FIG. 2. Variation of chemical shift, δ, with molar ratio of 3:1. Average values are plotted where antipodal differences occur.

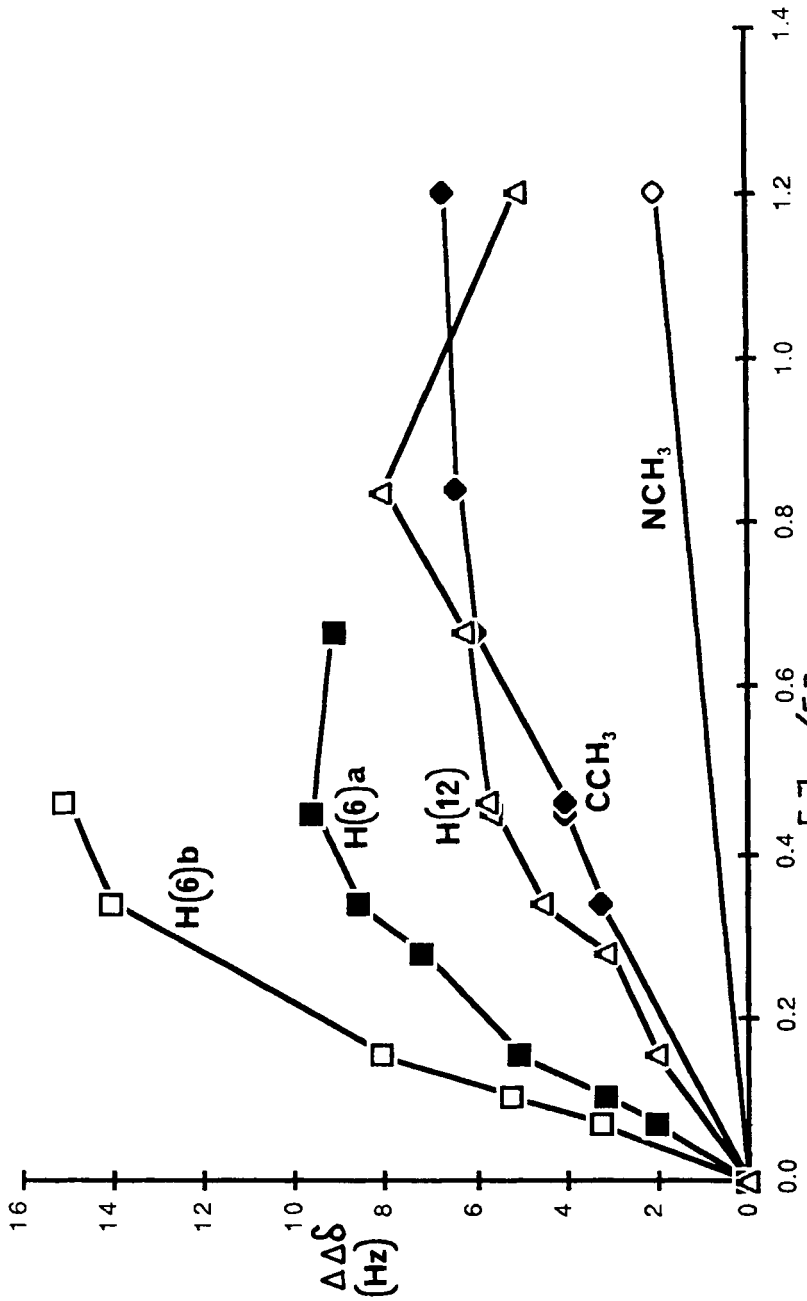


FIG. 3. Variation of enantiomeric shift differences, $\Delta\Delta\delta$ (in Hz), with molar ratio of 3:1.

with the downfield proton signal appearing as a triplet at 10.35 ppm, because of $\Delta\Delta\delta$ coincidentally being equal to $^2J_{gem}$. The triplet branches are essentially baseline separated with near-equal intensities of the upfield and downfield branches. As little as 7% of the minor enantiomer should be detectable under these conditions. Results with 3 are summarized in Figs. 2 and 3.

In conclusion, we have demonstrated the potential of achiral and chiral shift reagents 2 and 3 for spectral simplification and direct optical purity determinations of ketazolam, 1. Results are interpreted as consistent with a high degree of nonequivalence of the diastereotopic methylene protons due to anisotropic effects in a favored conformation in which the proton anti to the C_6H_5, H_a, is subject to much lower $\Delta\delta$ and $\Delta\Delta\delta$ magnitudes than its geminal neighbor, H_b, the latter being more nearly coplanar with neighboring carbonyls and closer to lanthanide bound at these oxygens.

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REFERENCES

1. J. Szmuszkovicz, C.G. Chidester, D.J. Duchamp, F.A. MacKellar and G. Slomp, Tetrahedron Lett. (39) 3665-3668 (1971).

2. a) J. Szmuszkovicz, U.S. Pat. 3,575,965 (Cl. 260-239.3; CO7d) 20 Apr. 1971, appl. 20 Oct. 1969; Chem. Abstr. 75, 20451z(1971);
b) U.S. Pat. 3,573,282 issued March 30, 1971;
c) J. Szmuszkovicz, Ger. Offen. 1,947,226 (Cl. CO7d) 26 March 1970, U.S. Appl. 19 Sept. 1968-27 March 1969; Chem. Abstr. 72, 121595t(1970).

3. A.F. Cockerill, G.L.O. Davies, R.C. Harden and D.M. Rackham, Chem. Rev., 73 (6) 553-588 (1973).

4. K.A. Kime and R.E. Sievers, Aldrichimica Acta, 10 (4) 54-62 (1977).

5. R.R. Fraser, in "Asymmetric Synthesis," Vol. 1, J.D. Morrison, ed., Academic Press, New York, 1983, pp. 173-196.

6. F. Inagaki and T. Miyazawa, Progress in NMR Spectroscopy, 14, 67-111 (1981).

7. J.R. Ascenso and A.V. Xavier, in "Systematics and the Properties of the Lanthanides," S.P. Sinha, ed., NATO Advanced Study Institute Series, Series C, no. 109; D. Reidel Publ. Co., Dordrecht, Holland, 1983, pp. 501-540.

8. O. Hofer, in "Topics in Stereochemistry," Vol. 9, N.L. Allinger and E.L. Eliel, eds., John Wiley-Interscience, New York, 1976, pp. 111-197.

9. P.L. Rinaldi, Progress in NMR Spectroscopy, 15, 291-352 (1982).

10. B.D. Flockhart, CRC Crit. Rev. Anal. Chem., 6 (1) 69-130 (1976).

11. C. Kutal, in "Nuclear Magnetic Resonance Shift Reagents," R.E. Sievers, ed., Academic Press, New York, 1973, pp. 87-98.

12. G.R. Sullivan, in "Topics in Stereochemistry," Vol. 10, E.L. Eliel and N.L. Allinger, eds., Interscience, New York, 1978, pp. 287-329.

13. M.C.M. Gribnau, C.P. Keijzers, and E. de Boer, Magn. Reson. Rev. 10, 161-192 (1985).

14. J.A. Peters and A.P.G. Kieboom, Recl. Trav. Chim. Pays-Bas 102 (9) 381-392 (1983).

15. V.H. Sethy, Naunyn-Schmiedeberg's Arch. Pharmacol. 301 (3) 157-161 (1978).

16. L.F. Fabre, Jr., and R.T. Harris, J. Int. Med. Res. 4 (1) 50-54 (1976).

17. L.A. Gottschalk and J.B. Cohn, Psychopharmacol. Bull. 14 (3) 39-43 (1978).

18. W. Wilhelm and R. Kuhn, Pharmakopsychiatrie Neuro-psychopharmakologie, 3, 317-332 (1970).

19. L.H. Sternbach, "Chemistry of 1,5-Benzodiazepines and some aspects of the structure - activity relationship," in "The Benzodiazepines," S. Garattini, E. Mussini and L. Randall, eds., Raven Press, NY, 1973, pp. 1-26.

20. G. Romeo, M.C. Aversa, P. Giannetto, M.G. Vigorita, and P. Ficarra, Org. Magn. Reson. 12 (10) 593-597 (1979).

21. G. Romeo, M.C. Aversa, P. Giannetto, P. Ficarra, and M.G. Vigorita, Org. Magn. Reson. 15 (1) 33-36 (1981).

22. L. Cazaux, C. Vidal, and M. Pasdeloup, Org. Magn. Reson. 21 (3) 190-195 (1983).

23. "The Merck Index," 10th ed., M. Windholz, ed., Merck & Co., Inc., Rahway NJ 07065, U.S.A., p. 761.

24. E.D. Becker, "High Resolution NMR Theory and Chemical Applications," Academic Press, New York, 1980, p. 72.

25. G.J. Karabatsos, G.C. Sonnichsen, N. Hsi, and D.J. Fenoglio, J. Amer. Chem. Soc. 89 (19) 5067-5068 (1967).

26. T.M. Ward, I.L. Allcox, and G.H. Wahl, Jr., Tetrahedron Lett. (46) 4421-4424 (1971).

27. A.H. Lewin, Tetrahedron Lett. (39) 3583-3586 (1971).

28. J. Avolio, S. Thomson Eberhart, R. Rothchild and P. Simons, Appl. Spectrosc. 40 (4) 531-537 (1986).

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