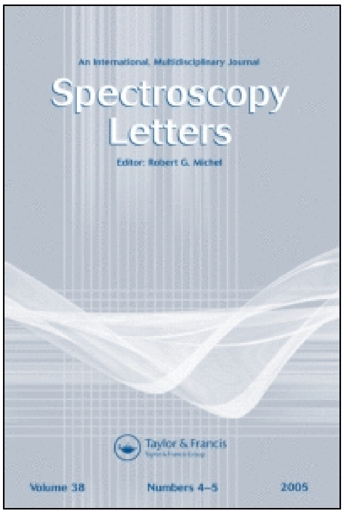

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NMR Studies of Drugs. Use of Lanthanide Shift Reagents in Polar Solvent with Thalidomide

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NMR STUDIES OF DRUGS. USE OF LANTHANIDE SHIFT REAGENTS IN POLAR SOLVENT WITH THALIDOMIDE.

Key Words: NMR shift reagents, Europium, Eu(FOD)$_3$, Eu(HFC)$_3$, Stereoisomers, Optical purity, Enantiomeric excess, Chiral, Analysis, Chelation, 2-(2,6-dioxo-3-piperidinyl)-1H-isoindole-1,3(2H)-dione.

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ABSTRACT

The 200.1 MHz ^{1}H NMR spectra of thalidomide, 1, have been studied in CD_3CN solution at ambient temperatures with the achiral shift reagent, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III), 2, and the chiral

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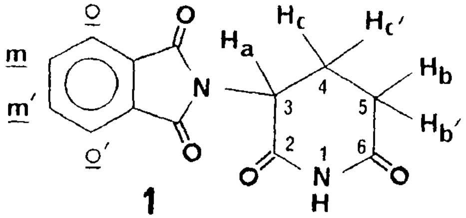
reagent, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), 3.

The use of sufficiently high LSR:1 molar ratios served to compensate for competitive binding of LSR by the polar solvent, and permitted substantial lanthanide-induced shifts to be observed with 2 or 3. With 3, significant enantiomeric shift differences were produced for the methine NCH multiplets of each enantiomer to be fully separated from one another, offering the potential for direct determination of enantiomeric excess of 1.

INTRODUCTION

Thalidomide, 1, 2-(2,6-dioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione, also known as α-phthalimidoglutarimide, is perhaps best known as the sedative/hypnotic which produced limb deformities (phocomelia) in about 12,000 children born in the late 1950's and early 1960's to mothers who had taken the drug during early pregnancy (1,2). Subsequently, the drug has been examined for potential utility for a number of applications, including treatment of a form of leprosy (erythema



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nodosum leprosum) (1,3); rheumatoid arthritis, certain inflammatory skin diseases and ulcerative diseases, and graft-versus-host disease (4,5). Since 1 possesses a chiral center at C-3 of the piperidinedione (i.e., the glutarimide α-carbon), thalidomide can exist as a pair of enantiomers, which have each been synthesized (6). The racemate and each enantiomer of 1 have been compared for toxicity and sedative/hypnotic potency in mice, as well as rabbit teratogenicity (7). Interactions of the enantiomers of 1 with DNA or soluble RNA have also been described (8). Stereospecific embryo toxicity and teratogenicity of metabolites, hydrolysis products or analogs of enantiomers of 1 have been reported (9-11).

In particular, Blaschke et al. (12) reported successful chromatographic separation of 1 on a polyamide chiral stationary phase (CSP).

Intraperitoneal administration demonstrated that teratogenicity resided in the S-(-) enantiomorph for SWS mice and Natal rats, with the R-(+) isomer inactive even at higher doses. These findings, as well as an increased general concern with understanding the pharmacological effects of each stereoisomer of pharmaceuticals (13-15), have resulted in the study of 1 as a model compound for liquid chromatographic methods aimed at chiral separations. There have been numerous discussions involving such techniques applied to 1 (12,16-22), with chiral CSPs for HPLC.

An alternative, complementary method for determination of enantiomeric excess (% ee) is the spectroscopic method based on NMR with chiral lanthanide shift reagents (LSR). The techniques of LSR use for spectral simplification and ee determination have been reviewed (23-26). LSRs have been employed with other chiral glutarimides, including glutethimide (27) and aminoglutethimide (28). The NMR examination of 1 using LSR methods was therefore investigated. However, the nonpolar or low polarity solvents (e.g., CCl_4, $CDCl_3$, pentane) that are typically used with lanthanide β-diketonate LSRs are poor solvents for 1, which was highly insoluble in $CDCl_3$, and only marginally soluble in CD_3CN. Polar solvents such as CD_3CN or $(CD_3)_2CO$ are not ordinarily used with these LSRs since the LSR (a hard Lewis acid) would be competitively bound by polar solvents capable of acting as Lewis bases. Small lanthanide-induced shift (LIS) and enantiomeric shift difference ($\Delta\Delta\delta$) magnitudes would result (23-26,29,30). The more polar solvents appeared useful with lanthanide β-diketonate LSRs only where a di- or trifunctional substrate could chelate with lanthanide via five- or six-membered ring formation (31). Recently, however, we were successful in using CD_3CN with these LSRs for a study of 5-methyl-5-phenylhydantoin (32). We were encouraged to undertake this present study of racemic 1 in CD_3CN using the achiral LSR, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5 octanedionato)-europium(III), 2, known as $Eu(FOD)_3$, and the chiral LSR, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), 3, known as $Eu(HFC)_3$ or $Eu(HFBC)_3$.

EXPERIMENTAL

A sample of racemic 1 as lot no. 1055/B (stated content 99.2%, mp 274-276°) was obtained from Grünenthal GmbH, D-5190 Stolberg, Fed. Rep. Germany, and was used as supplied. LSR reagents and CD_3CN (99.5 atom % D) were obtained from Aldrich Chemical Corp., Milwaukee WI 53201. The CD_3CN was dried and stored over 3Å Molecular Sieves; LSRs were stored in a desiccator over anhydrous $CaSO_4$ or P_2O_5. Materials were used as received except as noted. Chemical shifts are reported in δ (ppm) relative to tetramethylsilane (TMS) at 0.00 ppm. For typical runs with LSR, an accurately weighed portion of drug was added to CD_3CN [containing a trace of TMS as internal standard] in an oven-dried thin wall 5mm NMR sample tube and dissolved by shaking; increments of solid shift reagent were added directly to the sample, dissolved by shaking, and the spectra immediately obtained. The NMR studies were performed with a Bruker AC200-F Fourier transform NMR spectrometer with ASPECT 3000 data system for a ^1H observe frequency of 200.13 MHz. These spectra were obtained in the FT mode at ambient probe temperature using the dual $^1H/^{13}C$ probe. Chemical shifts were obtained from spectral peak tables. Coupling constants and enantiomeric shift differences were determined by subtraction from peak frequency printouts and are believed accurate to ±0.2 Hz. Typical FT-NMR parameters were as follows: 4032 Hz spectral width (about -4 to +16 ppm) over 64K data points collected in the quadrature detection mode for a digital resolution of 0.123 Hz per point, pulse width 3.0 μs, 8.13 s acquisition time, 1.0 s relaxation delay; 16 FIDs were accumulated. No line broadening or resolution enhancement was applied. In runs with chiral LSR where enantiomeric shift differences were observed for selected resonances, reported chemical shifts are the average values for the two enantiomers.

RESULTS AND DISCUSSION

The 200 MHz ^1H NMR spectrum for 1 in CD_3CN (0.37% w/v, 20°) showed resonances as follows (chemical shift in ppm relative to internal TMS): 8.97 (1H, br s, NH); ca. 7.87 (2H, complex mult, "ortho" aryl H); ca. 7.84 (2H, complex mult, "meta" aryl H); 5.02 (1H, m, H-3 [a], NCH); ca. 2.73 (1H, m, H-5 [b]) and ca. 2.75 (1H, m, H-5' [b']); ca. 2.1 (m, partly overlapped with HDO peak, H-4 [c]); ca. 2.66 (1H, m, H-4' [c']). These assignments are quite tentative due to the considerable complexity of the multiplets and the tightly coupled spin systems. The aryl protons constitute an AA'BB' system exhibiting a narrow multiplet with a center of symmetry. Aside from the broad lowfield imide proton and the aryl protons, the methine H-3 on the glutarimide ring may be unambiguously assigned as the most deshielded of the alphatic protons. This proton, attached to the chiral center, we designate H_a. _Cis_ and _trans_ vicinal couplings are expected between H_a and the methylene protons, H-4 and H-4' (H_c and $H_{c'}$) but H_a does not appear as a simple double doublet, dd, presumably due to second order effects. The H_a chemical shift reflects deshielding contributions by both nitrogen and the C(2) glutarimide carbonyl. The narrow complex multiplet centered at 2.74 ppm is assigned to the CH_2CO methylene, H-5,5', labeled as H_b and $H_{b'}$. We reason that this methylene should be deshielded by the attached carbonyl and might exhibit relatively little diastereotopic anisochrony since these protons are further from the chiral center. The highest field signals are assigned to the diastereotopic methylene protons, H-4,4', designated as H_c and $H_{c'}$ since they are remote from deshielding groups. The high degree of magnetic nonequivalence for this pair of nuclei can be accounted for by their proximity to the chiral center (just two bonds away) and may reflect magnetic anisotropic effects of the phthalimido substituents. In fact, the three protons $H_{bb'}$ and $H_{c'}$ are all part of a complex multiplet integrating to 3H, from ca. 2.57-2.84 ppm, and are not distinctly separated. We have not attempted rigorous assignments of the cis, trans relationships of the CH_2CH_2 moiety. Incremental addition of the achiral LSR, Eu(FOD)$_3$, 2, was carried out to a 2:1 molar ratio of 10.0. The results are shown in Figure 1. Lanthanide-induced shifts (LIS) are appreciable, leading to some spectral simplification by enhancing the separation between the two sets of aryl protons, ortho and meta, and between the upfield protons labeled H-

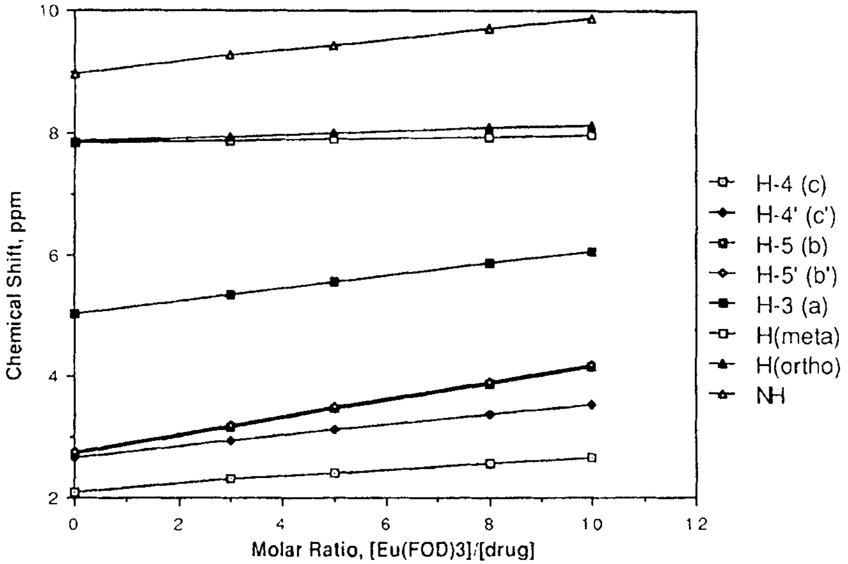

Figure 1. Variation of chemical shift (in ppm) with 2:1 molar ratio.

5,5' versus H-4'. While the upfield protons of the CH_2CH_2 group all remain complex multiplets, the methine H_a (H-3) does simplify to a dd pattern at a 2:1 molar ratio of 3.0, exhibiting observed (apparent) vicinal coupling constants of 5.4 and 12.8 Hz. As the 2:1 ratio was increased to 10.0, the larger coupling constant increased monotonically to 13.2 Hz; the smaller coupling did not show a monotonic change and was essentially constant within the estimated experimental error of

±0.2 Hz. Even the small observed change in the larger coupling constant may be attributed to a measurement artifact, since its magnitude was only 2-3 times the digital resolution. The highest field signal, assigned as H_c (H-4), consistently appeared as a much narrower multiplet than $H_{c'}$ (H-4'). It is also noteworthy that the slopes of the plots of chemical shift versus 2:1 ratio, shown in Fig. 1, are essentially linear to the highest LSR level. This will be further discussed below.

With increments of the chiral $Eu(HFC)_3$, 3, added to a 0.37% (w/v) solution of 1 in CD_3CN, the LIS magnitudes were qualitatively similar to results with 2, as shown in Figure 2. Significantly, enantiomeric shift differences ($\Delta\Delta\delta$) were clearly observed for the methine H_a (H-3) and for the imide NH (Figure 3). With 3:1 ratios of 8.0 or more, both resonances show signals from each enantiomer that are fairly well resolved. For the methine H_a, each enantiomer's dd signal has been separated; all eight lines are readily seen. The valley height between the two dd signals, relative to the average adjacent peak heights, was about 31

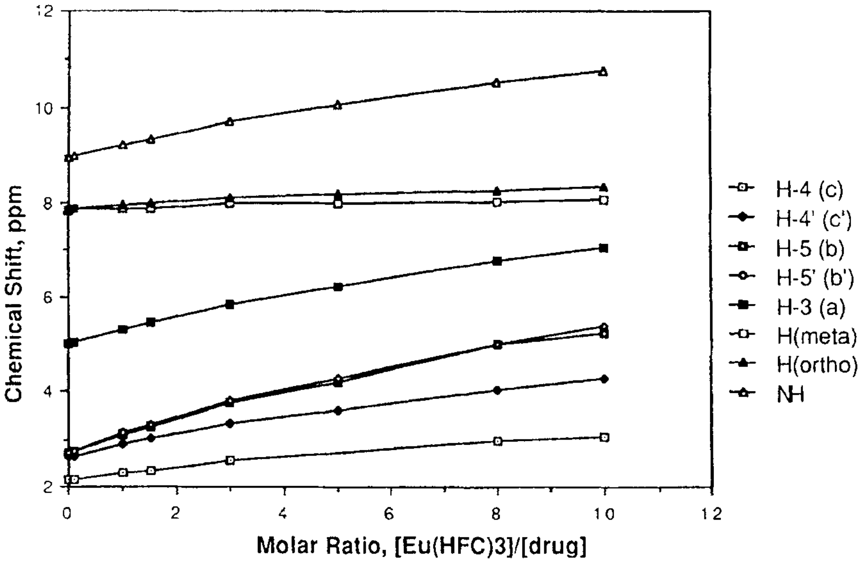

Figure 2. Variation of chemical shift (in ppm) with 3:1 molar ratio.

and 20% at 3:1 ratios of 8.0 and 10.0, respectively. For the NH signal, resolution by the valley height criterion was about 26 and 15% at these respective levels of 3. Representative spectral traces are shown in Figure 4. Signal-to-noise ratio was enhanced for these signals by acquiring 256 FIDs with a digital resolution of 0.368 Hz. Superior separation may be achieved using even higher 3:1 ratios than we have used. Aside from LSR-induced line broadening, the

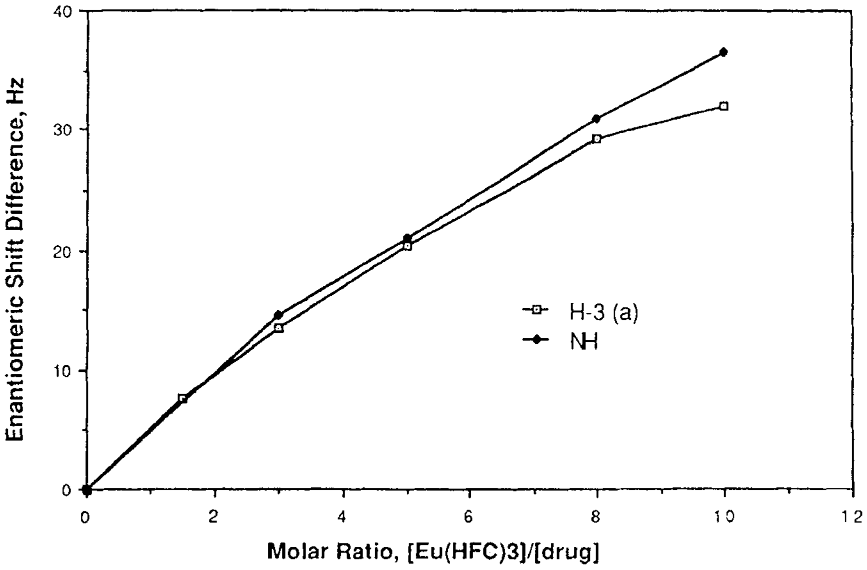

Figure 3. Variation of enantiomeric shift difference (in Hz at 200 MHz) with 3:1 molar ratio.

complexity of the H_a signal results from splitting by the diastereotopic protons H-4,4'. Broadening of the NH resonance may largely reflect ^{14}N quadrupole broadening. We have previously suggested the possible value of heteronuclear ^{14}N decoupling as a means of sharpening amide or imide resonances (32) to permit improved signal separation for ^{1}H NMR LSR methods of % ee determination. Our results here (without ^{14}N decoupling) clearly show analytical potential for

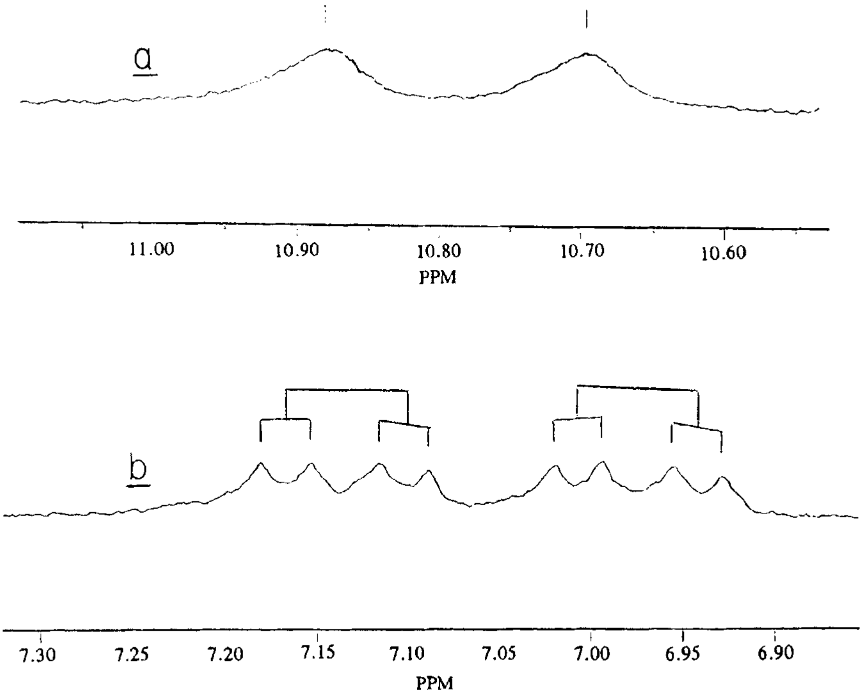

Figure 4. (a) NH signal centered at 10.78 ppm ($\Delta\Delta\delta$ = 36.6 Hz); (b) methine NCH signal (H-3) centered at 7.05 ppm ($\Delta\Delta\delta$ = 32.1 Hz). A total of 256 FIDs were acquired with a digital resolution of 0.368 Hz/pt for a 0.37% (w/v) solution of 1.

direct ee determinations of 1 in CD_3CN using 3:1 ratios of 10 (or more), based on either the NH or methine (H-3) absorptions.

Table 1 summarizes the relative LIS magnitudes obtained with the two LSRs. The slopes for the plots of chemical shift versus molar ratio of

Table 1. Slopes of Lanthanide-Induced Shifts versus Molar Ratios of [LSR]/[drug] for Nuclei of 1. (See Notes and Results and Discussion).

	$Eu(FOD)_3$ data		$Eu(HFC)_3$ data	
Nucleus	Unnormalized	Normalized	Unnormalized	Normalized
H-4(c)	0.056	0.65	0.144	0.61
4'(c')	0.086	1.0	0.236	1.0
5(b)	0.143	1.66	0.343	1.45
5'(b')	0.144	1.67	0.351	1.49
3(a)	0.105	1.22	0.282	1.19
meta	0.012	0.14	0.044	0.19
ortho	0.027	0.31	0.077	0.33
NH	0.092	1.07	0.256	1.08

Notes: Slopes are based on least-squares line fitting with five experimental points for each nucleus with either 2 or 3. Normalized values are relative to a value of 1.0 for the signal assigned to H-4'(c). See *Results and Discussion*.

LSR:1, from the data of Figs. 1 and 2, are presented in both unnormalized and normalized form. These slopes reflect least squares line fitting with 2:1 ratios from 0.0-10.0 (five experimental points) with R=1.00 (except R=0.99 for the *meta* protons). With 3, molar ratios from 0.0-3.0 were used (five experimental points) since some leveling off was noted at higher molar ratios; over the lower range, fairly good fits were obtained (R=1.00) except for the *meta* protons (R=0.97). The low slope values for the *meta* protons presumably reflect their remoteness from the LSR binding sites on the carbonyls; small experimental errors in estimating chemical shifts for the *meta* protons result in the poorer fit to the calculated line. Electronic factors would suggest greater basicity for the glutarimide carbonyls than for the phthalimido carbonyls, since the latter are conjugated with the benzene ring. Steric factors would favor LSR binding at the C-6 rather than the C-2 carbonyl. In fact, there is presumably some contribution from bound complexes with LSR at each of the carbonyls, in rapid equilibrium. Most important, however, is that 1 is not constructed to allow favorable bidentate chelation of LSR via five- or six- membered ring formation, in contrast to the substrates of ref. 31. We can not rule out bidentate chelation involving the C(2) oxygen and one of the phthalimido carbonyls binding to the lanthanide, but this would require a less favorable seven-membered ring.

Normalized slopes were calculated relative to the signal assigned to H-4' (c') since this signal was generally free from interfering overlaps, could be assigned a chemical shift with reasonable accuracy, exhibited appreciable LIS magnitudes, and was sufficiently far from any carbonyl binding site (for the LSR) to preclude significant Fermi contact shift contributions. The CH or NH protons alpha to the carbonyls were not used for normalization because of potential Fermi contact shifts for these nuclei. Very good agreement is seen in comparing the normalized slope values for the two LSRs, suggesting that the bound complexes of 1 with either 2 or 3 are close to isostructural. A modest difference is seen for the absorptions assigned to

H-5,5'(b,b'), with slopes about 13% higher using 2. The calculated slopes for the nuclei of 1 decrease in the order: H-5,5' > H-3 > NH > H-4' > H-4 > *ortho* > *meta*. The relative values for the carbon-bound glutarimide protons are consistent with predominant LSR binding at the less hindered C-6 carbonyl.

CONCLUSIONS

The 200.1 MHz ^1H spectra of 0.37% (w/v) thalidomide have been studied in CD_3CN solution at 20° with added $Eu(FOD)_3$ or $Eu(HFC)_3$. Both reagents produce appreciable LIS magnitudes if high molar ratios of LSR:1 are used. Thus, even in the presence of a moderately polar solvent which can competitively bind LSR, it is possible to use lanthanide β-diketonate reagents for spectral simplification of a substrate that can not form strong five- or six-membered ring chelates with lanthanide. This is especially remarkable when one considers that in the dilute substrate solutions examined here, the solvent is present in about a thousand-fold molar excess relative to the solute, 1. Of particular importance is the potential to obtain analytically useful enantiomeric shift differences for direct ee determinations, simply by using sufficient LSR. This appears to be the first NMR LSR report for potential % ee determination of thalidomide. Optimum results could be based on the NH or methine NCH signals with 3:1 ratios of 10.0 or more.

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