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^{1}H NMR spectral simplification with achiral and chiral lanthanide shift reagents. Tranylcypromine, *trans*-2-phenylcyclopropanamine

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Abstract: The 60 MHz ^{1}H NMR spectra of racemic tranylcypromine, **1**, have been studied 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 tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato] europium(III), **3**. Appreciable values of the enantiomeric shift differences, $\Delta\Delta\delta$, were observed for each cyclopropyl proton except for the proton β and *trans* to the amino group (furthest from the expected europium complexation site). The proton α to the amino group showed $\Delta\Delta\delta$ as high as about 38 Hz for a **3**:**1** ratio of 0.71; the $\Delta\Delta\delta$ decreased at higher **3**:**1** ratios. Optical purity evaluations should be most practical using this α proton with **3**:**1** ratios near 1.07 to minimize interference due to peak overlap.

Keywords: 60 MHz *proton magnetic resonance spectra;* trans-2-*phenylcyclopropanamine; effect of achiral and chiral shift reagents; enantiomeric shift differences* $\Delta\Delta\delta$.

Introduction

Tranylcypromine, **1**, *trans*-2-phenylcyclopropanamine, was first synthesized by Burger and Yost [1]. It has been extensively studied in terms of stereochemistry for several reasons. For instance, the rigidity of the three-membered ring has made it a useful model for considering the dihedral angle of the aromatic ring with respect to the amino group [2, 3]. Stereochemistry has also been of interest in comparisons of the *cis* and *trans* geometric isomers as well as in comparisons of the *R* and *S* enantiomers of **1**. Differences in pharmacological effects and potencies between these isomers have been of significance in studies of many arylethylamines and analogs, such as amphetamines and neurotransmitters, as well as in considerations of the structure of active site receptors for these substances [2–6].

Tranylcypromine itself was the first nonhydrazine monoamine oxidase (MAO) inhibitor of clinical importance and received wide use as an antidepressant. Aspects of

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adverse reactions, efficacy, pharmacology and toxicity have been reviewed [7]. A more recent review [8] discusses the enantiomer neurotransmission and behaviour aspects. The *trans* isomer is three times more potent than the *cis* isomer; the (+) enantiomer of **1** is four times as potent as the (−) enantiomer for MAO inhibition [2, 9]. Synthesis of (−)-**1**·HCl has enabled assignment of absolute configuration as 1*R*, 2*S* [9]. The enantiomers of **1**, as the *N*-3,5-dinitrobenzoyl derivatives, have been separated by high-performance liquid chromatography on a chiral stationary phase [10].

A direct optical purity determination, without the need for derivatization, is to be preferred. Because of the extensive interest in stereochemical aspects of **1**, and its structural similarity to amphetamine and other drugs of abuse, the use of lanthanide shift reagents for purposes of ^{1}H NMR spectral simplification and direct optical purity determination has been investigated. Previously the method has found numerous applications in the analysis of drugs, including glutethimide [11], thiamylal [12], ethotoin [13], mephobarbital [14], mephenytoin [15], methohexital [16], hexobarbital [17], cocaine [18], methamphetamine [19], amphetamine [20, 21], substituted amphetamines [22], dextro- and levomethorphan [23] and thiohexital [24]. The achiral shift reagent, tris (6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III), **2**, abbreviated as Eu(FOD)$_3$, and the chiral shift reagent, tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato]europium(**III**), **3**, abbreviated as Eu(TFC)$_3$, and also known as Eu(FACAM)$_3$, have been utilized successfully for the analysis of tranylcypromine.

Experimental

Samples of the hydrochloride salts of each enantiomer of **1** (code CK-6002-214E for the *laevo* and code CK-1-230-2 for the *dextro* materials) were kindly provided by Smith Kline and French Labs (Philadelphia, PA, USA). Specific rotations, $[\alpha]_D^{25}$, were −76.7° and +75.75°, respectively, at a concentration of 1 g/100 ml in water). Chloroform-*d*, (99.8 atom % D), obtained from Aldrich Chemical Co. (Milwaukee, WI) or from Norell (Landisville, NJ) was dried and stored over a 3A molecular sieve. Shift reagents were obtained from Aldrich and were stored in a desiccator over P_2O_5. Materials were used as supplied except as noted.

In general, an accurately weighed portion of drug free base (about 40–50 mg total of both enantiomers) was added to 400–500 mg of $CDCl_3$ (containing about 0.2% tetramethylsilane (TMS) as internal standard) in an NMR sample tube and dissolved by shaking; weighed increments of the solid shift reagent were added, dissolved by shaking, and the spectra run immediately. A 'racemic' sample of **1** was prepared by mixing together equal quantities of each enantiomer prior to conversion to the free base.

All spectra were run on a Varian EM-360A 60 MHz ^{1}H NMR spectrometer at a probe temperature of 28°C. Chemical shifts were measured as parts per million (ppm) in δ units, and are believed accurate to ±0.05 ppm. In spectra where TMS was obscured by the shift reagent peaks, $CHCl_3$ (present as a solvent impurity) was used as internal standard.

Preparation of racemic free base tranylcypromine from salts

Portions of *laevo*-**1**·HCl and *dextro*-**1**·HCl, (the latter a pale tan solid), 200 mg each (2.34 mmol total), were dissolved in 3 ml H_2O to which a two-fold excess of 5% aqueous NaOH was added. The mixture was then saturated with solid NaCl and extracted (4 × 5 ml) with CH_2Cl_2. The combined organic extracts were dried with anhydrous Na_2CO_3,

decanted and evaporated to constant weight with a rotary evaporator (aspirator pressure, bath temperature 25°C) to give 272.5 mg of **1** as a pale viscous yellow oil which crystallized on standing, m.p. (uncorr.) 41.5–44°C (lit. 45–46°C [25]; 44–45°C [26]), 87% recovery. This material was used without further purification and was stored under N_2. A sample of (−)-**1** was prepared similarly for spiking experiments.

Results and Discussion

A 0.547 m solution of 'racemic' **1** in $CDCl_3$ gave a ^1H NMR spectrum which showed the expected complexity of an extensively coupled system. Although the ^1H NMR spectrum for racemic **1** free base in $CDCl_3$ has been published [27], assignments do not appear to have been made. ^{13}C NMR studies have also been reported [25] for **1**, its *cis* isomer, and analogues. The spectrum for **1** obtained in the present work is shown as Fig. 1. In the ^1H spectrum, multiplets are centered at 7.2 ppm (aromatic), 2.55 ppm (H_d, α to NH_2), 1.9 ppm (H_a, benzylic) and 1.05 ppm ($H_{b,c}$). The broad NH_2 singlet at 1.65 ppm partly overlapped the H_a signal. Incremental additions of solid **2** initially produced sharpening of the aryl absorptions and ultimately a separation into downfield (2H, *ortho*), and upfield (3H, *meta* and *para*) absorptions. The signals of the *ortho* protons are

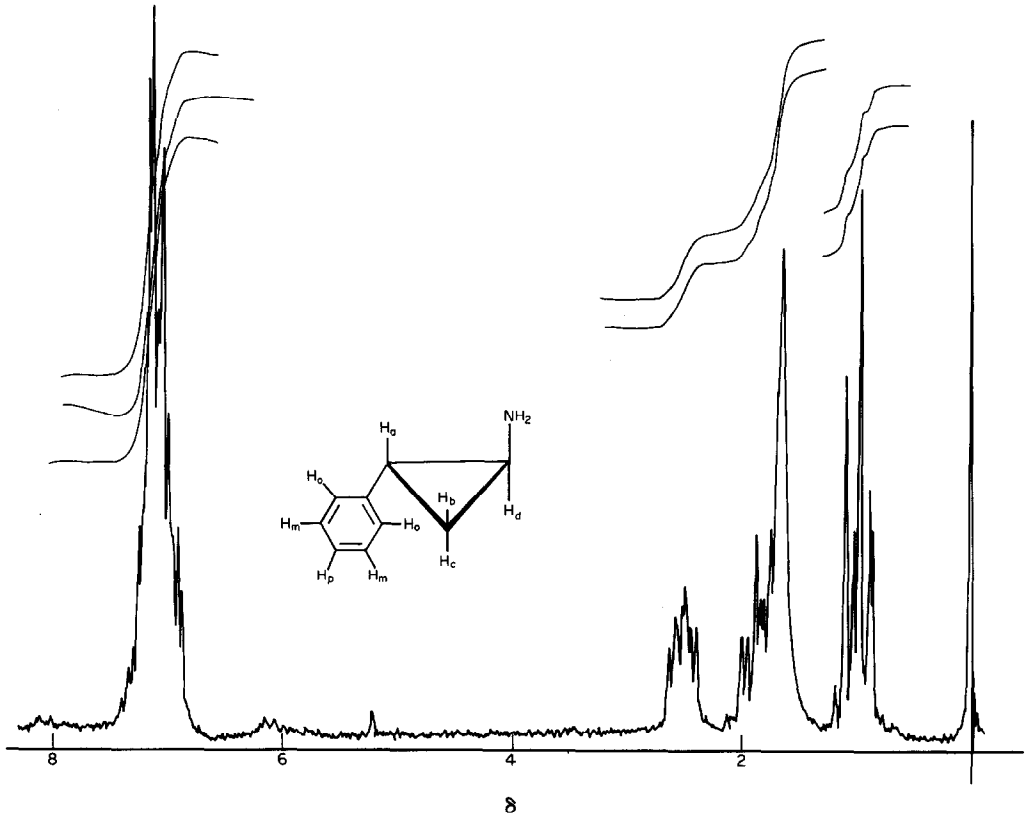

Figure 1
The 60 MHz ^1H NMR spectrum of a 0.547 m solution of *trans*-2-phenylcyclopropanamine in $CDCl_3$ at 28°C, at 10 ppm sweep width, with no shift reagent added.

initially at higher field in the unshifted spectrum but move downfield faster as **2** is added because of the greater proximity of the *ortho* protons to the complexed europium atom. The signal of the NH_2 group moves downfield most rapidly, consistent with europium complexation at the basic nitrogen. The signals of the two methylene protons become well resolved from one another at the low **2:1** molar ratio of 0.084. The slopes of the plots of chemical shift versus molar ratio of **2:1** decrease in the order $H_d > H_a \approx H_b > H_c$. This is completely in agreement with assignments: H_d is α to the NH_2 and is closest to complexed europium; $H_{a,b}$ are both *cis*, β with respect to NH_2; H_c is on the other side of the ring and the plot of its chemical shift *versus* molar ratio of **2:1** has the least slope. Moderate lanthanide-induced line broadening was observed at relatively low **2:1** ratios. The results obtained with **2** as shift reagent are summarized in Figs 2 and 3.

Shift reagent studies with **1** and **2** have been reported previously by Cho and Yun [28]. These workers utilized C_6D_6 as solvent rather than $CDCl_3$ and reported the proton spectrum of **1** with no added shift reagent as δ 7.25 (m, 5H, phenyl), 0.9–2.7 (m, 4H), 1.7 (s, 2H, NH_2); however, the published spectrum suggests chemical shifts for the CH_2 and NH_2 proton signals that do not correspond exactly with these values. Rather, the NH_2 resonance appears to be at δ 1.0 with the multiplet of the CH_2 signal from δ 0.7 to 1.0. The assignments of the four cyclopropyl protons are completely consistent with those of the present authors, as are the relative slopes of the chemical shift plots. Cho and Yun did not assign aromatic proton resonances. Perhaps most important are the significantly improved values of lanthanide-induced shift, Δδ, that have been observed in the current studies for the cyclopropyl proton resonances together with better resolved absorptions with considerable fine structure at the lower **2:1** ratios. Thus, Cho and Yun show a spectrum (their Fig. 2-2) with a **2:1** ratio of 0.11 for which all cyclopropyl resonances are essentially broad and featureless, with the signals of the methylene protons just baseline-resolved. Comparable δ values have been found for the cyclopropyl resonances with a **2:1** ratio of 0.084 but with the detailed structure as shown in Fig. 3. The differences in the results may be due to the use of $CDCl_3$ as solvent in place of C_6D_6 or due to the higher substrate concentrations used by Cho and Yun at low **2:1**

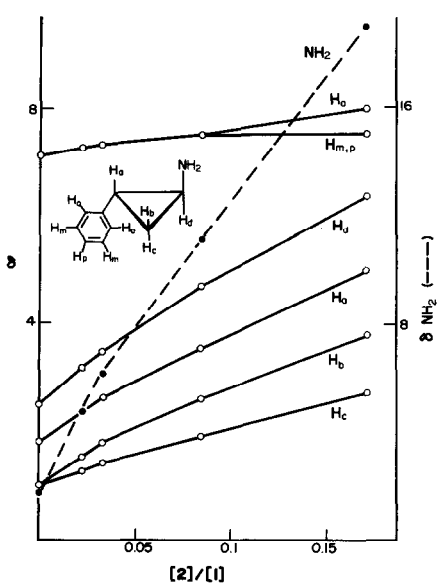

Figure 2
Variation of the chemical shift, δ, values for the phenyl and cyclopropane protons with addition of Eu $(FOD)_3$ as shift reagent, expressed as a molar ratio of shift reagent, **2**, to analyte, **1**.

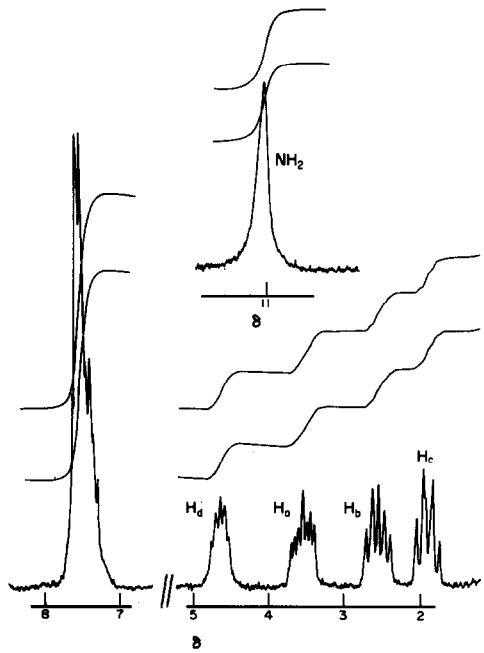

Figure 3
Simplified ^{1}H NMR spectrum of a 0.547 m solution of *trans*-2-phenylcyclopropanamine containing Eu $(FOD)_3$ at a **2:1** molar ratio of 0.084.

ratios. Differing NMR sample probe temperatures could also be a factor; no temperature was specified in Ref. [28].

With the chiral shift reagent, **3**, the greatest enantiomeric shift differences, $\Delta\Delta\delta$, are seen for H_d, reaching a maximum of 38 Hz with a **3:1** ratio near 0.71 for a 0.870 m solution of **1**. The enantiomeric shift difference, $\Delta\Delta\delta$, is defined as the magnitude of $\delta_R - \delta_S$ for a specific proton. Interestingly, it is observed that higher **3:1** ratios actually led to a decrease of $\Delta\Delta\delta$, for instance, 31.6 Hz and about 29 Hz for **3:1** ratios of 0.951 and 1.07, respectively. Because of the extensive splitting between the four cyclopropyl protons, $\Delta\Delta\delta$ values were estimated in two different ways. For those multiplets characterized by a distinct sharp centre peak in the spectra with **2**, such as H_a or H_b, the distance between the partly resolved peaks of the enantiomers in spectra with **3** could be measured directly. Alternatively, the width (measured between outermost branches of a multiplet) from the spectra with **2** was simply subtracted from the corresponding width in spectra shifted by 3. The two methods agreed closely. Peak broadening often made the latter techniques easier to apply.

These techniques were employed only to evaluate $\Delta\Delta\delta$ values for purposes of the plot of enantiomeric shift differences versus molar ratio of shift reagent to substrate in order to consider how these values are influenced by the distance of a particular proton from the chiral centre or lanthanide atom. (They are not used to evaluate optical purity.) Although the $\Delta\Delta\delta$ values are determined from spectra obtained with chiral shift reagent and the $\Delta\Delta\delta$ estimates are based on multiplet appearances determined with the achiral reagent **2**, it is felt that the methods for estimating $\Delta\Delta\delta$ are justified. Shift reagent binding could, in principle, alter coupling constants, but this is probably not significant in the present case. The cyclopropylamine system is relatively rigid and unhindered and appears to complex in a similar manner with the shift reagents studied

here, based on comparisons of chemical shifts and slopes of plots of these shifts versus the shift reagent:drug ratios. This is consistent with similar geometries in the two complexes of lanthanide with **1**. If geometries are negligibly altered among the cyclopropyl protons with the different shift reagents, coupling constants would not be altered greatly. In addition, multiplet appearances and widths were determined from spectra using **2** in which the multiplet signals for the cyclopropyl protons were well separated from each other and so should be nearly first order. The multiplet widths, then, should accurately reflect intrinsic couplings to a given proton. In runs with chiral shift reagent, estimates for $\Delta\Delta\delta$ should then be accessible by simply measuring either the distance between sharp centre peaks of the resonances corresponding to the signal of a specific proton for the two enantiomers (the first method), or by measuring increased multiplet width (the second method). In each case, the basic coupling constants and multiplet shapes for corresponding proton resonances in the two enantiomers should be quite similar. Estimates of optical purity, of course, are based not on $\Delta\Delta\delta$ but on intensity ratios for a proton resonance specific for the individual enantiomers present in a sample. Ideally, the resonances for the two enantiomers should be resolved. Using shift reagent **3** and the H_d resonance, this is substantially achieved, with detection of 10% of the minor enantiomer being possible. While this may not represent an exceptionally favourable case for shift reagent determination of optical purity, we view these results as especially significant because of the structural similarities of **1** with various amphetamines and analogues for which shift reagent studies have been reported. Finally, it is noted that even though the present results are based on $\Delta\Delta\delta$ measured between two multiplets (for H_d), the effective resolution between these two multiplets, based on the height of the valley between them, is comparable to that shown in other published spectra of various drugs with chiral shift reagents. For example, the *singlets* of the resonances for the methoxy groups of the enantiomers of racemic methorphan (Fig. 1 of Ref. [23]) show a valley height between them of at least 60% of the average height of the two singlets. Also, at 200 MHz, the methyl doublet signals for a 60:40 mixture of *d*- and *l*-amphetamine display a valley height of about 75% of the peak height for the minor enantiomer (Fig. 1 of Ref. [21]); only with decoupling of the vicinal methine to collapse the methyl signals to singlets does the valley height fall to about 45%. The analytical value and importance of these techniques is perhaps greatest for distinguishing between enantiomers and the racemic material, or for detecting minor (but not trace) amounts of one enantiomer in the presence of the other. In any case, the quantitative utility of studies of **1** with **3** may be illustrated by the following. A 'racemic' sample of **1**, 45.3 mg in $CDCl_3$ (0.546 m) with a **3:1** molar ratio of 1.07, showed the H_d signal as two broad partly resolved resonances with a peak height ratio of 1.02 ± 4% (downfield peak height: upfield peak height). When the sample was spiked by addition of 5.4 mg of (−)-**1**, to give a solution 0.613 m with respect to **1** with a **3:1** ratio of 0.951, the downfield peak (at 10.7 ppm) had increased relative to the upfield peak (at 10.2 ppm) so that the peak height ratio as measured to the baseline was now 1.32 ± 4%, determined in a 2 ppm sweep width spectral expansion. This compared to a calculated enantiomer ratio of 1.24 based on weights of enantiomers present in the sample. Resolution of the peaks in the expansion is indicated by a valley height of only 62% of the smaller peak's height (and only 46% of the major peak's height). While detectability of trace amounts of a minor enantiomer is probably no better than about 10% because of the peak width and low intensity, the accuracy of the method is reasonable for evaluations of enantiomeric homogeneity.

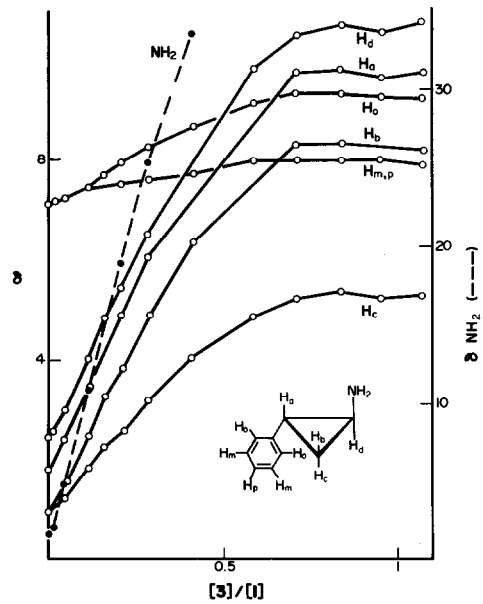

Figure 4
Variation of the chemical shift, δ, values for the phenyl and cyclopropane protons with addition of Eu $(FACAM)_3$ as chiral shift reagent, expressed as a molar ratio of shift reagent, **3**, to analyte **1**.

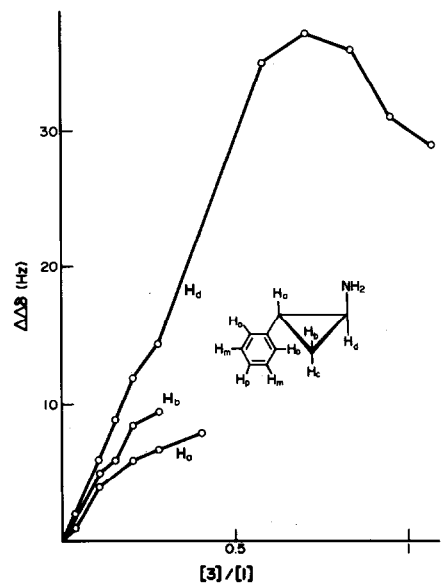

Figure 5
Variation of the enantiomeric shift differences, ΔΔδ in Hz, with the molar ratio of chiral shift reagent, **3**, and *trans*-2-phenylcyclopropanamine, **1**.

For analytical purposes, high **3:1** ratios were used in order to shift the H_d resonance downfield of the ortho aryl protons; a **3:1** ratio near 1.07 was suitable. Higher ΔΔδ values at lower ratios were less useful because of overlaps. These results with **3** are summarized in Figs 4–6. As noted above, spiking a sample of 'racemic' **1** with (−)-**1** caused an increase in the intensity of the downfield part of the H_d resonance. For this resonance, then, the (−) enantiomer of **1** has its signal shifted by **3** further downfield than the signal of the (+) isomer. We were not able to determine readily the corresponding senses of ΔΔδ for the signals of the other protons because of broadening. These absorptions appear not to be as useful analytically as the H_d

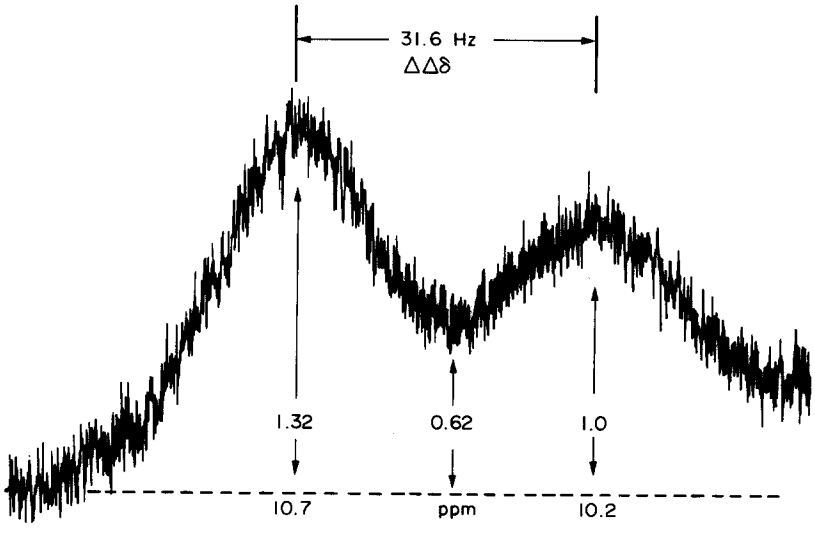

Figure 6
Expanded ^{1}H NMR spectrum, at 2 ppm sweep, of a 0.613 m solution of *trans*-2-phenylcyclopropanamine containing chiral shift reagent Eu (FACAM)$_3$ at a **3:1** molar ratio of 0.951. The enantiomeric ratio of (−)-1 to (+)-1 is 1.24. Relative peak and valley heights are indicated.

resonance. Clearly, a higher field NMR spectrometer would be very helpful in work involving shift reagent **3**.

The sense of ΔΔδ for H_d proton of **1** in spectra shifted by **3** has been established. Substantial ΔΔδ values for this proton suggest that the compound's rigidity and basicity (and modest steric hindrance for a primary amine) allow favorable coordination with the lanthanide. The proton, H_d, on a chiral centre close to the europium, provides the largest ΔΔδ values in this compound and offers a feasible means of optical purity evaluation.

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