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CALIBRATION PLOTS TO AID DETERMINATION OF HIGH ENANTIOMERIC PURITY USING CHIRAL LANTHANIDE SHIFT REAGENTS.

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Abstract: High enantiomeric purities are difficult to determine by NMR with chiral lanthanide shift reagents because it is difficult to identify which, if any, resonance corresponds to the minor enantiomer. We report calibration plots that use the position of the resonance of the major enantiomer to predict the position of the resonance of the minor enantiomer. Once the position of the resonance was established, integration measured enantiomeric purities as high as 99.7% ee.

One of the most convenient methods to determine enantiomeric purity is NMR using chiral lanthanide shift reagents.[1] With samples that have 0-95% ee, integration of the resonances of the two enantiomers directly measures the enantiomeric purity. With samples that have >95% ee, it is often difficult to identify which, if any, resonance corresponds to the minor enantiomer. Since a modern NMR spectrometer has sufficient dynamic range to detect 0.01% of the minor enantiomer,[2] a method to identify which resonance corresponds to the minor enantiomer would extend the shift reagent technique to samples with high enantiomeric purity.

The current method to identify the resonance due to the minor enantiomer is to measure the NMR spectrum of the sample at a known ratio of shift reagent to substrate. A separate NMR spectrum of a racemic sample at the same ratio of shift reagent to substrate indicates the expected position of the resonance for the minor enantiomer.[3] This method requires careful quantitation of the shift reagent and substrate which can be difficult in cases of hydroscopic, gummy, or volatile samples. Further, the sample must be free of impurities that coordinate to the shift reagent.

We report herein calibration plots to identify the resonance for the minor enantiomer. This method uses the resonance of major enantiomer as an internal standard, does not require tedious preparation of solutions of known concentration and is not sensitive to impurities. Once the resonance that corresponds to the minor enantiomer was established, enantiomeric purities as high as 99.7% ee were measured. Further, the slope of the calibration plots measured the effectiveness of the shift reagent.

A lanthanide shift reagent experiment on (-)-(1S-*trans*)-1-acetoxy-2-bromocycloheptane, (1S)-**1**, isolated from an enzyme-catalyzed kinetic resolution,[4] Figure 1a, showed that the sample had high enantiomeric purity. However, the exact enantiomeric purity could not be established because several resonances were observed in the region where the resonance of the minor enantiomer was expected.

To identify which resonance corresponded to the minor enantiomer, we made a calibration plot, Figure 2. We measured the lanthanide-induced shifts for the two enantiomers of **1** ($\Delta\delta_{1S}$ and $\Delta\delta_{1R}$) upon succesive addition of shift reagent to racemic **1**. The induced shift for one enantiomer plotted vs the induced shift for the other

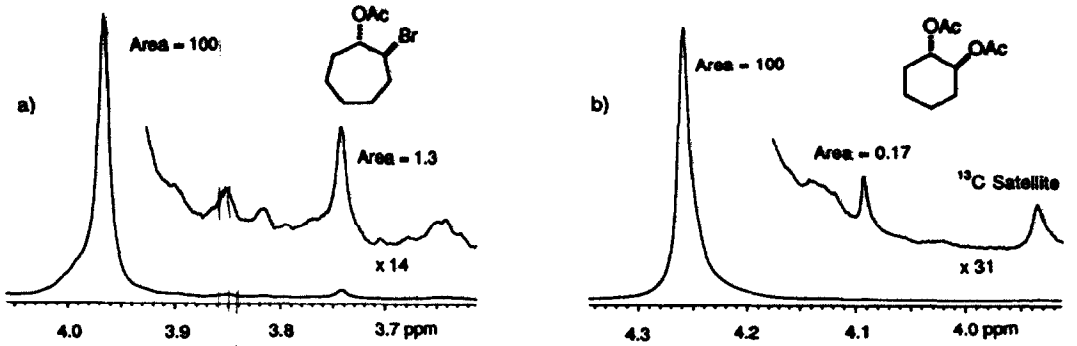

Figure 1. Determination of enantiomeric purity of acetate esters of chiral alcohols by ^{1}H-NMR in the presence of (+)-Eu(hfc)$_3$. The resonance of the acetyl CH_3 is shown. (a) (1S-*trans*)-1-acetoxy-2-bromocycloheptane ((1S)-**1**, ~140 m*M*) isolated from an enzyme-catalyzed resolution, 97.4±0.9% ee. S/N = 2000. The calibration plot in Figure 2 was used to assign the peak at 3.742 ppm to the (1R)-enantiomer. (b) (1S-*trans*)-1,2-diacetoxycyclohexane (170 m*M*, 99.59±0.08% ee by wt), 99.7±0.1% ee by NMR. S/N = 5000. All spectra were run at 200 MHz, 45° pulse, 64 transients, 5.0 s acquisition time, 12 bit digitizer.

enantiomer fell on a straight line with a slope, α, of 1.134 and an intercept near zero indicating that $\Delta\delta_{1S}/\Delta\delta_{1R} = \alpha$. The induced shifts for an enantiomerically-enriched sample of **1** also fell on the same line. This calibration plot predicted that the minor enantiomer in Figure 1a should appear between 3.747 and 3.735 ppm;[5] thus, the peak at 3.742 ppm was assigned to the minor enantiomer. This assignment was confirmed by adding another portion of shift reagent where the new position was also predicted by the calibration plot. Integration of the resonances indicated that this sample had 97.4±0.9% ee.[6]

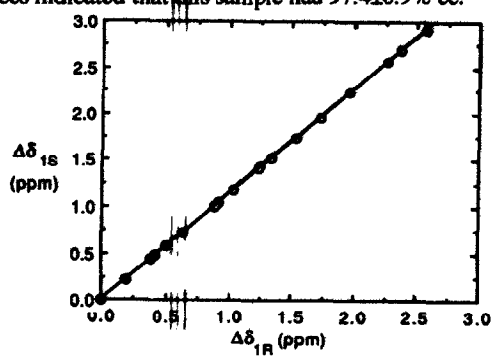

Figure 2. A calibration plot for **1**. The induced shift for the acetyl CH_3 resonance of the (1S)-enantiomer, $\Delta\delta_{1S}$ was plotted vs the induced shift for the acetyl resonance of the (1R)-enantiomer, $\Delta\delta_{1R}$. Data include racemic samples (●) and enantiomerically enriched samples (○). The slope, α, was 1.134 and the intercept was 0.003.

Since the accuracy of integration in ^{1}H-NMR is ~1%,[7] it appears, at first glance, to be impossible to measure 97.4±0.9% ee. However, high enantiomeric purities (>95%) can be accurately determined because errors partly cancel in the calculation of %ee[8] for samples with high ee. For example, a sample with 99.90% ee would show areas of 99.95±1.0 for the major and 0.05±0.0005 for the minor enantiomer. The worst possible combinations give 99.898 and 99.902% ee, an absolute error of only ±0.002%. In practice, integration errors are larger, especially for the minor enantiomer. For Figure 1a, we estimated error limits of ±5% for the major enantiomer and ±30% for the minor enantiomer, which gave a maximum absolute error of ±0.9% ee.

To confirm the accuracy of determination of high enantiomeric purity by NMR, we prepared four solutions of (1S-*trans*)-1,2-diacetoxycyclohexane with 99.59±0.08% ee.[9] The enantiomeric excess determined by ^{1}H-NMR in the presence of (+)-Eu(hfc)$_3$ was 99.7±0.1%, Figure 1b, confirming that high enantiomeric purities can be determined accurately from integration of ^{1}H-NMR spectra.

Coordinating impurities (up to 30 mol% ethyl acetate) did not perturb linearity of the calibration plot for **1**. The presence of the impurity shifted the position of the peaks, but the ratio of induced shifts remained the same. Coordination of some of the shift reagent by impurity does not influence the slope because the induced shift of one enantiomer serves as an internal standard.

Similar calibration plots were linear for six other acetates of chiral alcohols, Table 1. A linear relationship between the two induced shifts is expected based on the accepted mechanism of action of chiral shift reagents[10] suggesting that this technique may be general.

Table 1. Values from Calibration Plots of Several Acetates of Chiral Alcohols.[a]

Entry	Substrate	δ_o, ppm	Slope, α	Intercept
1	*trans*-1-Acetoxy-2-bromocyclopentane	2.039	1.022(1)	0.004(2)
2	*trans*-1-Acetoxy-2-bromocyclopentane[b]	2.039	1.013(1)	0.000(1)
3	*trans*-1-Acetoxy-2-bromocyclohexane	2.057	1.114(2)	0.001(2)
4	*trans*-1-Acetoxy-2-bromocycloheptane	2.061	1.134(1)	0.003(2)
5	1α-Acetoxy-2β,3α-dibromocyclohexane	2.092	1.117(4)	0.003(2)
6	1α-Acetoxy-2α,3β-dibromocyclohexane	2.080	1.157(3)	0.006(3)
7	*trans*-2,3-Diacetoxybutane	2.309	1.034(2)	-0.002(2)
8	*trans*-1,2-Diacetoxycyclohexane	2.304	1.081(9)	-0.004(4)

[a]Solid (+)-Eu(hfc)$_3$ was added in portions to substrate in $CDCl_3$. Tetramethylsilane was used as an internal reference. The data all refer to lanthanide-induced shifts on the resonance of the acetyl CH_3. The resonance for the (S)-enantiomer appeared downfield of the (R)-enantiomer in all cases. The absolute configuration has not been established for entries 5 and 6. The numbers in parentheses are standard deviations in the last digit. [b](+)-Yb(tfc)$_3$ was used as the chiral shift reagent.

Calibration plots were linear only up to a shift reagent to substrate ratio of 1:1. Beyond 1:1, the slope of the line changed, perhaps reflecting the formation of complexes with different stoichiometry. The formation of shift reagent-substrate complexes with different stoichiometry has been observed previously.[11] Thus, the calibration plots are only valid when a single type of complex is formed. This criteria is easily met if the unknown is measured using a shift reagent to substrate ratio in the same range as the calibration plot.

This approach of using one resonance as an internal standard has been used previously with achiral shift reagents to eliminate errors due to scavenging of shift reagent by impurities, or errors in the concentration of shift reagent and substrate[12] and to determine association constants of the substrate and shift reagent,[13] but, as far as we know, it has not been applied to chiral shift reagents and the determination of enantiomeric purity.

The recommended procedure is to prepare a calibration plot by measuring the induced shifts for a racemic or enantiomerically enriched sample at different ratios of shift reagent to substrate. While it is not necessary to know the ratio precisely, it should be kept below 1:1. The plot is used to predict the position of the resonance of the minor enantiomer in an unknown.[5] The unknown should be measured in the same range of shift reagent to substrate, but purity of the unknown is not crucial. For added reliability, the assignment can be checked at several ratios of shift reagent to substrate. Note that when the absolute configuration of the major enantiomer is unknown, two positions are predicted - one upfield, the other downfield of the major enantiomer.

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(1) Reviews: Wenzel, T. J. *NMR Shift Reagents* CRC Press: Boca Raton, FL, 1987, Chapter 3; Parker, D. *Chem. Rev.* **1991**, *91*, 1441-1457.

(2) A 16-bit digitizer has a dynamic range of $2^{16} = 6 \times 10^4$. The newest machines contain 24-bit digitizers.

(3) A deliberate addition of racemate does not identify the resonance due to the minor enantiomer because the addition changes the ratio of shift reagent to substrate and shifts all the resonances.

(4) Gupta, A. K.; Kazlauskas, R. J., manuscript in preparation. To establish its absolute configuration, **1** was converted to 2-cyclohepten-1-ol by heating with DBU ([5.4.0.]-undec-7-ene) at 130°C for 3 hrs followed by hydrolysis of the acetate in a mixture of refluxing tetrahydrofuran and dilute aqueous NaOH. The resulting material showed a negative $[\alpha]_D$ indicating the (S) configuration: Kasai, M.; Ziffer, H. *J. Org. Chem.* **1983**, *48*, 712-715; Iriuchijima, S.; Kojima, N. *J. Chem. Soc., Chem. Commun.* **1981**, 185.

(5) The chemical shift for the minor enantiomer, δ_{1R}, is given by $\delta_{1R} = \delta_0 + (\Delta\delta_{1S}/\alpha)$ where δ_0 represents the chemical shift in the absence of shift reagent and α is the slope of the calibration plot. The intercept of the calibration plot was not included in the calculation of the chemical shift because it is expected to be zero and was observed to be close to zero.

(6) The enantiomeric purity could not be determined accurately by GC with a chiral column (cyclodextrin G-TA from Astec (Whippany, NJ), 110°C) because **1** decomposed on the column. Analysis suggested >90% ee.

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(8) %ee = [(R-S)/(R+S)] x 100.

(9) The error limits in the weighing and dilution were estimated to be ±8% for the major and ±6% for the minor enantiomer.

(10) Differences in the geometry of the diastereomeric complexes and in the equilibrium constants for the two enantiomers are thought to be involved: McCreary, M.D.; Lewis, D.W.; Wernick, D.L.; Whitesides, G.M.; *J. Am. Chem. Soc.* **1974**, *96*, 1038-1054; see also Rothchild, R. *J. Chem. Ed.* **1989**, *66*, 814-815.

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