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Determination of the optical purity of timolol maleate by proton nuclear magnetic resonance spectroscopy with a chiral Pr(III) shift reagent

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Abstract

A ^{1}H NMR spectroscopic method with chiral shift chelate is presented for the determination of the optical purity of timolol maleate. Optimum experimental conditions were established by studying the interactions of solvents (CCl_4, $CDCl_3$), substrate concentration, and the type and concentration of chiral lanthanide chelate ($Pr(hfc)_3$, $Eu(hfc)_3$). Larger induced shift ($\Delta\delta$) and enantiomeric shift difference ($\Delta\Delta\delta$) values, and more detailed spectral differences were obtained with $Pr(hfc)_3$ than with $Eu(hfc)_3$. By monitoring the spectral changes of the resonance signals for the enantiomeric $-C(CH_3)_3$ protons, suitable conditions for quantitative determinations were found when 0.1 molar equivalents of $Pr(hfc)_3$ were complexed with 0.074 M of substrate. Enantiomeric compositions were calculated from the relative intensities of the enantiomeric $-C(CH_3)_3$ proton resonances. Based on the analysis of six synthetic enantiomeric mixtures, the mean $\pm$ SD recovery of (*R*)-(+)-timolol by the proposed method was 99.5 $\pm$ 1.17% of the amount added.

Keywords: Timolol maleate; Optical purity; ^{1}H NMR spectroscopy; Chiral Pr(III) shift reagent

1. Introduction

Timolol maleate, (−)-(*S*)-1-(*tert*-butylamino-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol maleate, is a potent and nonselective β-adrenergic blocking agent that, unlike other β-blockers, possesses neither sympathomimetic effects nor local anesthetic properties [1]. This drug has been used systemically to treat mild to moderate essential hypertension [2,3], to limit infarct size [4], and to reduce cardiovascular mortality and reinfarction in survivors of acute myocardial infarct [5]. Topically, it is used as eyedrops to lower intraocular pressure in patients with chronic open-angle glaucoma [6,7]. Owing to its lack of local anesthetic activity, timolol is considered well suited for long term local use [8]. In comparison to timolol, the (*R*)-(+)-enantiomer is four times less potent in reducing intraocular pressure in man, 49 times less potent as a β_1- and β_2-adrenoceptor antagonist in animals, 13 times less potent as a bronchoconstrictor in normal subjects, and considerably less effective in reducing the heart rate of exercising human subjects [9,10].

Methods proposed for the resolution of enantiomeric pairs of β-adrenergic blocking agents chemically and pharmacologically related to timolol have varied, depending on the purpose sought. On a preparative scale, for example, the enantiomers of propranolol have been separated by multiple recrystallizations of

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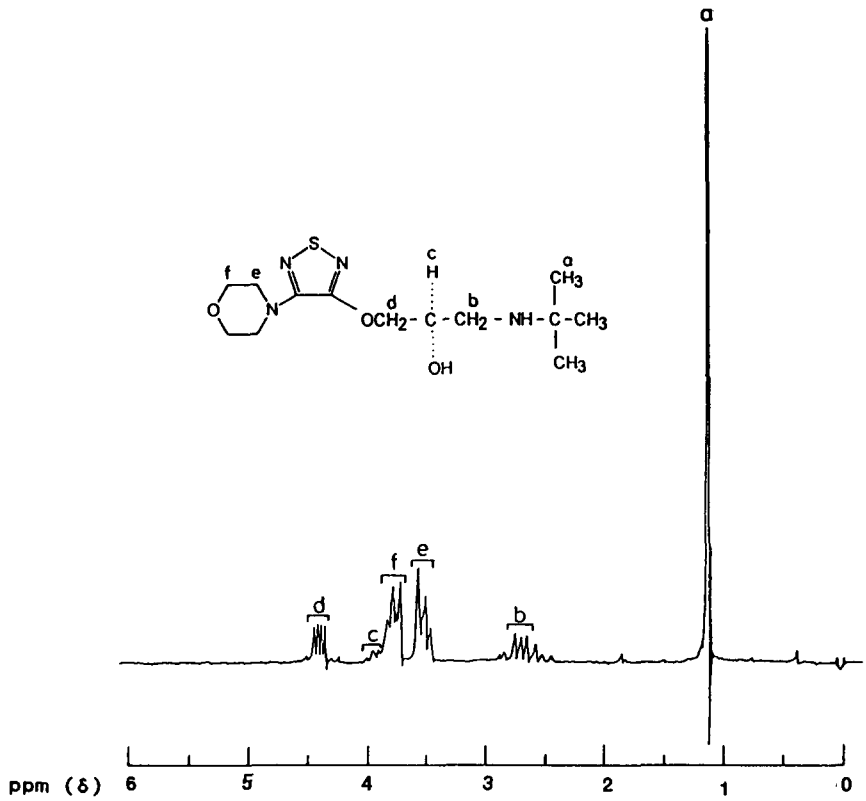

Fig. 1. ^{1}H NMR spectrum of a mixture of timolol and its (+)-(*R*)-enantiomer in CCl_4.

the di(*p*-toluoyl)tartaric acid salts [11]. On an analytical scale, the enantioselective separation of clinically relevant *β*-blockers has been accomplished either by gas chromatography [11,12] or by high-performance liquid chromatography (HPLC) [12–16], after their conversion to a pair of chromatographically resolvable diastereomers by reaction with an optically pure chiral reagent. Although extensively used, chiral reagents are susceptible to problems such as racemization and instability during storage [12]. More recently, the enantiomers of timolol [16] and related oxypropanolamines [17,18] have been resolved by HPLC on chiral stationary phases without resorting to a derivatization step. However, this method requires the use of samples of the pure enantiomers as reference standards.

The purpose of this communication is to report the development of an alternative method for studying the enantiomeric composition (optical purity) of timolol samples that combines the use of proton nuclear magnetic resonance (^{1}H NMR) spectroscopy with a chiral lanthanide shift complex. In this manner, the content of the (*R*)-(+)-enantiomer of timolol in timolol maleate drug substance can be measured directly, with a high degree of enantioselectivity, and without reliance on pure enantiomeric standards.

2. Experimental

2.1. Apparatus

All ^{1}H NMR spectra were obtained on a Varian EM-390 spectrometer (Varian Associates, Sunnyvale, CA, USA) operating at a probe temperature of 35 ± 1 °C, and were referenced to tetramethylsilane (TMS) taken as 0.00 ppm on the δ scale.

2.2. Samples

The (*R*)-(+)-, (*S*)-(−)-, and (+)-1-(*tert*-butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)-oxy]-2-propanol maleate (1:1) salt were generous gifts from Merck, Sharp & Dome, West Point, PA, USA. The optical purity of the samples were checked by polarimetry and the proposed NMR method.

2.3. Chemicals

TMS (>99.9%), carbon tetrachloride (CCl_4), deuterochloroform ($CDCl_3$, +99.5% isotopic purity), tris[3-heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), (Eu(hfc)$_3$), and tris[3-(heptafluoropropylhydroxymethylene) - (+) - camphorato] praseodymium(III), (Pr(hfc)$_3$), were purchased from Aldrich (Milwaukee, WI, USA). TMS was freed of tetrahydrofuran by successive washes with sulfuric acid and saturated potassium carbonate solution, followed by distillation, and storage over molecular sieves type 4A. CCl_4 was distilled prior to use and stored over type 4A molecular sieves. Eu(hfc)$_3$ and Pr(hfc)$_3$ were stored over P_2O_5 in vacuo or under a dry N_2 atmosphere. To minimize the possibility of contamination by ambient moisture or air, all work with lanthanide shift reagents was conducted within a soft plastic glove box (AtmosBag^R, Aldrich) and under a dry N_2 atmosphere.

2.4. Preparation of Samples for Lanthanide-Induced Shift Studies

An accurately weighed quantity of substrate was placed in a separating funnel, and dissolved in about 2 ml of water. The solution was made alkaline with two drops of 3 N NaOH, and extracted into 3 ml of $CHCl_3$. The $CHCl_3$ layer was separated, and evaporated to dryness under a stream of dry N_2. After drying to a constant mass at 50 °C in a vacuum oven, the residue was dissolved in 1% TMS in $CDCl_3$, and the solution was transferred immediately to a glass vial crimper-sealed with a Teflon-lined rubber septum and an aluminum seal. Samples for ^{1}H NMR spectroscopic analysis were withdrawn through the septum with a dry, liquid-tight, fixed-needle, microliter syringe.

Table 1
Shift data for the $C(CH_3)_3$ signals of a mixture of timolol and its *R*-(+)-enantiomer after complexation with various molar ratios of chiral lanthanide shift reagent[a]

Shift reagent	Molar ratio	*S*-(−)-enantiomer		*R*-(+)-enantiomer		ΔΔδ
		δ	Δδ	δ	Δδ	
Pr(hfc)$_3$	0.000	1.14	0.00	1.14	0.00	0.00
	0.018	0.92	−0.22	0.96	−0.18	0.04
	0.027	0.82	−0.32	0.89	−0.25	0.07
	0.036	0.72	−0.42	0.83	−0.31	0.11
	0.045	0.52	−0.62	0.65	−0.49	0.13
	0.054	0.47	−0.67	0.63	−0.51	0.16
	0.058	0.30	−0.84	0.47	−0.67	0.17
	0.061	0.22	−0.92	0.41	−0.73	0.19
	0.101	−0.28	−1.42	0.00	−1.14	0.28
Eu(hfc)$_3$	0.110	1.42	0.28	1.48	0.34	0.06
	0.132	1.57	0.43	1.66	0.52	0.09
	0.154	1.65	0.51	1.75	0.61	0.10
	0.176	1.73	0.59	1.84	0.70	0.11
	0.198	1.80	0.66	1.92	0.78	0.12
	0.220	1.92	0.78	2.05	0.91	0.13
	0.286	2.28	1.14	2.42	1.28	0.14
	0.380	2.52	1.38	2.67	1.53	0.15

[a] Total substrate concentration of 0.074 M in CCl_4.

2.5. NMR studies of lanthanide-induced shifts

The required changes in shift reagent to substrate molar ratios were obtained by first adding the shift chelate to a dry NMR tube, followed by adding the appropriate amount to substrate stock solution. The tube was capped immediately, its contents mixed by inversion, and the resulting solution used to obtain the ^{1}H NMR spectrum. After obtaining the ^{1}H NMR spectrum, another aliquot of the substrate stock solution (the exact amount having been predetermined gravimetrically) was added to the same NMR tube, and the NMR spectrum was obtained once more. The additions and spectral measurements were repeated until an appropriate number of spectra was available for proper evalution of the lanthanide-induced chemical shifts.

2.6. Determination of the optical purity

An accurately weighed quantity of timolol maleate (30.0–31.0 mg) was converted to the free base form. The resulting residue (23.5–24.0 mg) was dissolved in 1% TMS in $CDCl_3$ (1.0 ml), and the solution was transferred to an analytical NMR tube containing Pr(hfc)$_3$ (8.8–9.0 mg). After capping the tube and mixing its contents by inversion, the solution was allowed to stand at room temperature for 10 min, and then used to obtain the ^{1}H NMR spectrum. All chemical shifts were assigned with reference to TMS. The relative intensities of the resonances (peak areas or peak heights) for the enantiomeric *tert*-butyl protons at −0.28 ppm ((*S*)-(−)-enantiomer) and 0.00 ppm ((*R*)-(+)-enantiomer) were measured. The peak heights were measured manually using a ruler, and the peak areas were measured using the electronic

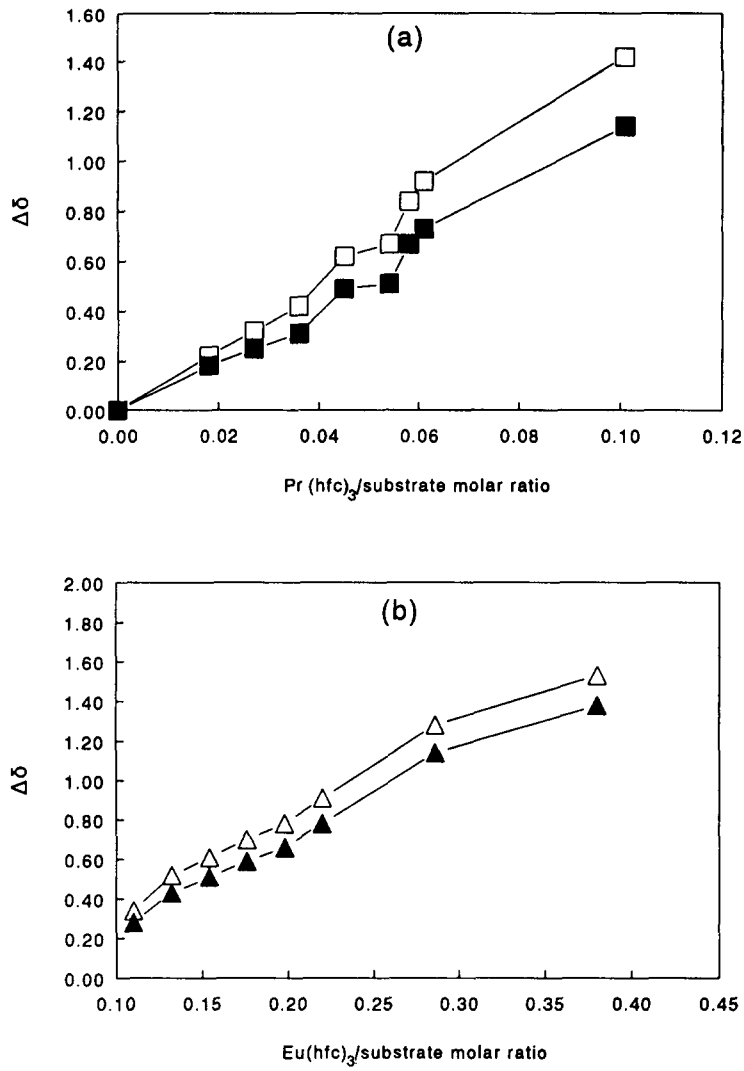

Fig. 2. Plot of induced chemical shifts ($\Delta\delta$) for the $-C(CH_3)_3$ protons of timolol and its (+)-(*R*)-enantiomer, 0.074 M in CCl_4, vs. lanthanide shift reagent to substrate molar ratios: (a) using $Pr(hfc)_3$, (b) using $Eu(hfc)_3$.

integrator attached to the NMR spectrometer. Both methods gave equivalent results, with the accuracy being better than 99.5% of the known amounts of enantiomers present in test synthetic mixtures. The measurements were used to calculate the percentages of each enantiomer in the sample from the following equations:

$$\%(R)\text{-}(+)\text{-enantiomer} = \frac{A_{(+)} \times 100}{A_{(+)} + A_{(-)}}$$

$$\%(S)\text{-}(-)\text{-enantiomer} = \frac{A_{(-)} \times 100}{A_{(+)} + A_{(-)}}$$

where $A_{(+)}$ = peak area (or peak height) of the resonance for the (*R*)-(+)-enantiomer, and $A_{(-)}$ = peak area (or peak height) or the resonance for the (*S*)-(−)-enantiomer.

3. Results and discussion

The ^1H NMR spectrum of a mixture of timolol and its (*R*)-(+)-enantiomer is shown in Fig. 1. The nonequivalent protons of the methylene groups α and λ to the aliphatic secondary amino group generated two multiplets centered at 2.75 ppm and 4.43 ppm, respectively, and represented the AB part of an ABX spin system. The protons of the methylene groups adjacent to nitrogen and oxygen in the morpholine ring resonated as two multiplets centered at 3.53 ppm and 3.80 ppm, respectively, forming an AA'XX' spin system. The proton on the chiral center yielded a multiplet centered at about 3.87 ppm. The nine equivalent protons of the *tert*-butyl group gave rise to a sharp singlet at 1.14 ppm.

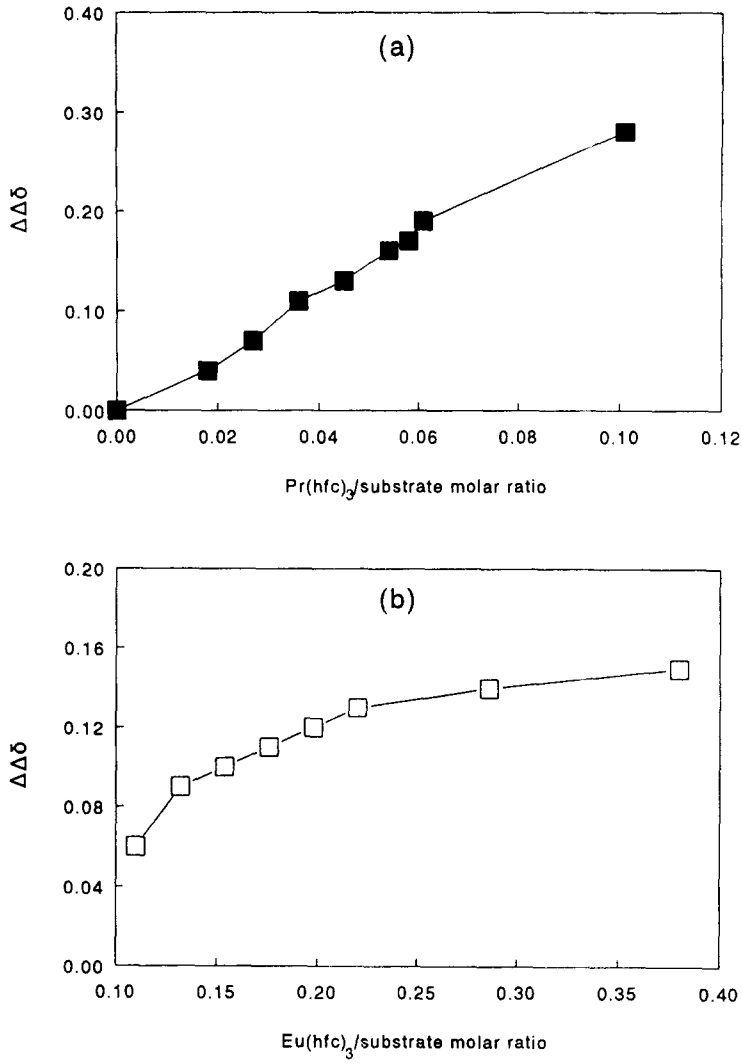

Fig. 3. Plot of chemical shift differences ($\Delta\Delta\delta$) for the $-C(CH_3)_3$ protons of timolol and its (+)-(*R*)-enantiomer, 0.074 M in CCl_4, after complexation with various lanthanide reagent to substrate molar ratios: (a) using $Pr(hfc)_3$, (b) using $Eu(hfc)_3$.

The direct determination of enantiomeric compositions by NMR spectroscopy is dependent not only on the attainment of a good resolution between at least one set of enantiotopic resonances, but also between this set of signals and other moderately shifted signals. Chiral lanthanide chelates such as $Eu(hfc)_3$ and $Pr(hfc)_3$ provide a means of spreading out the NMR absorptions of an enantiomeric mixture by inducing concentration-dependent paramagnetic shifts without the need for increasing the applied magnetic field.

The effect of varying the ratio of the concentration of the chiral lanthanide shift chelate to that of the substrate on lanthanide-induced chemical shifts were studied using the method of Raber and Peters [19], which permits this evaluation to be carried out using only a limited number of concentration ratios. The induced shift ($\Delta\delta$) and induced shift differences ($\Delta\Delta\delta$) values between the *tert*-butyl resonances of timolol and its (R)-(+)-enantiomer in CCl_4, at the combined substrate concentration of 0.074 M, in the presence of increasing molar equivalents of $Eu(hfc)_3$ or $Pr(hfc)_3$, are summarized in Table 1 and graphically shown in Figs. 2 and 3. These data indicated that $Pr(hfc)_3$ possesses a greater resonance resolving power than $Eu(hfc)_3$, since the same signal nonequivalence was achieved with a concentration of the former lanthanide chelate that was only about one-fifth that of the latter. Generally, shifts induced by $Eu(hfc)_3$ were downfield, whereas those by $Pr(hfc)_3$ were upfield. In the presence

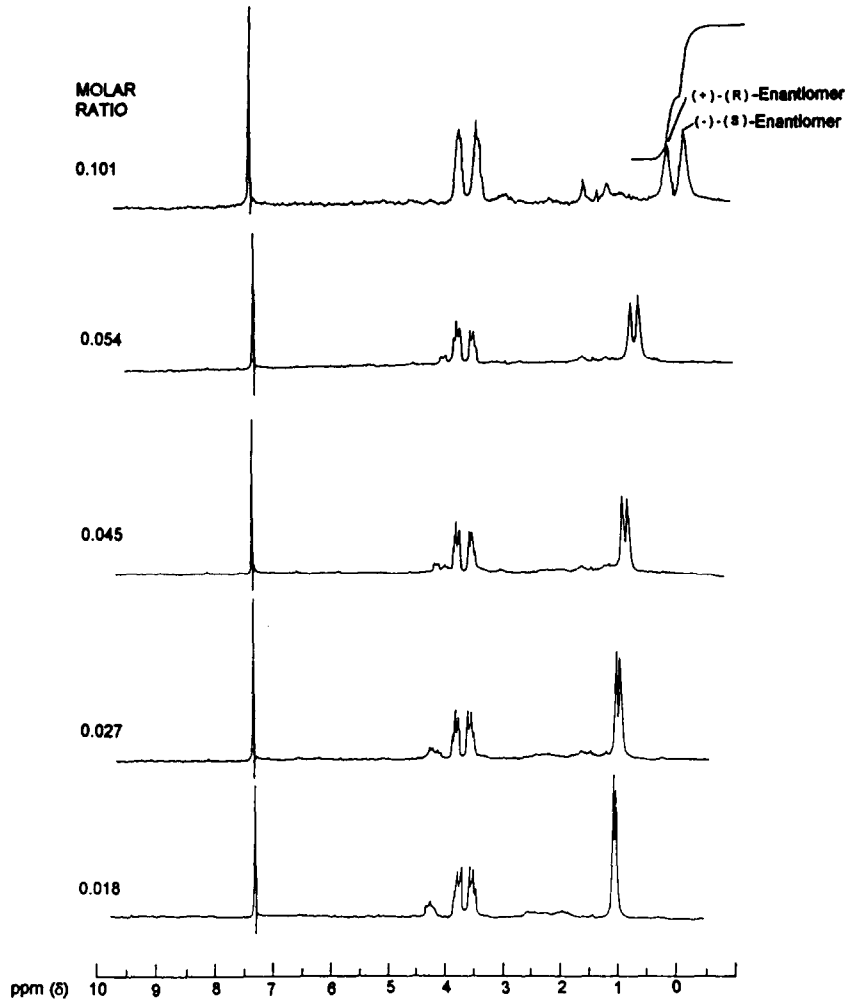

Fig. 4. ^{1}H NMR spectrum of a mixture of timolol and its (+)-(*R*)-enantiomer, 0.074 M in CCl_4, after complexation with various molar ratios of $Pr(hfc)_3$ to substrate. The signals monitored were those of the $-C(CH_3)_3$ protons in the −0.5 to 1.5 ppm region.

of $Eu(hfc)_3$, the downfield-shifted resonances for the enantiomeric *tert*-butyl protons were not sufficiently resolved from each other to permit their use in quantitative work. An attempt to improve enantiomeric signals resolution by increasing the concentrations of this shift reagent resulted in both a further signal shift to downfield and in the signals of interest becoming obscured by other moderately shifted signals. In this case, use of a shift reagent like $Pr(hfc)_3$ is particularly advantageous since it can induce a large upfield shift of the *tert*-butyl protons of timolol and only a modest upfield shift of other interfering signals.

Fig. 4 illustrates the spectral changes that occur in the −0.5 to 1.5 ppm region when a fixed concentration of timolol-(*R*)-(+)-enantiomer mixture in CCl_4 is complexed with increasing molar equivalents of $Pr(hfc)_3$. Unequivocal identification of the signals arising from each enantiomer was facilitated by following the changes in peak intensity in the NMR spectrum of the samples mixture before and after enrichment in one of the enantiomers. At a shift reagent to substrate molar ratio of 0.010, the enantiomeric *tert*-butyl proton signals were shifted, as an unresolved singlet, from the initial position 1.14 ppm in the uncomplexed spectrum to 0.97 ppm. Sequential increases in the molar ratio resulted in further signal shifts and in the splitting of the *tert*-butyl signal into two distinct peaks at −0.28 ppm (timolol) and 0.00 ppm ((*R*)-(+)-enantiomer). As shown in Table 1 and Fig. 3(a), the $\Delta\Delta\delta$ between enantiomeric *tert*-butyl proton signals reached a value of 0.28 ppm at a $Pr(hfc)_3$ to substrate molar ratio of 0.101. Moreover, a comparison of the spectra ob-

Table 2
Determination of the optical purity of synthetic mixture of timolol and its (*R*)-(+)-enantiomer by ^{1}H NMR spectroscopy with chiral lanthanide shift reagents

Sample No.	Amount of enantiomer added[a]			Amount of enantiomer found		
	(+) form (mg)	(−) form (mg)	(+) form (% of mixture)	(+) form (mg)	(+) form (% of mixture)	Rec.[b] (%)
1	32.0	32.0	50.00	32.05	50.07	100.15
2	33.8	30.2	52.81	33.38	52.15	98.75
3	25.8	38.2	40.31	25.76	40.25	99.84
4	15.9	48.1	24.84	15.95	24.92	100.31
5	12.1	51.9	18.91	12.16	19.00	100.50
6	1.3	62.7	2.03	1.27	1.98	97.70
Mean						99.54
SD						1.09
RSD						1.09

[a] Total concentration of substrate was 0.074 M in CCl_4, and the $Pr(hfc)_3$ to substrate molar ratio was 0.101.
[b] Recoveries were calculated from (amount found × 100)/amount added.

tained in CCl_4 with those in $CDCl_3$ revealed that the magnitude of the shifting effects of $Pr(hfc)_3$ on the resonance signals of timolol and its (*R*)-(+)-enantiomer were not the same. For a given concentration of shift reagent, the $\Delta\delta$ and $\Delta\Delta\delta$ values were larger in CCl_4 than in $CDCl_3$.

Such contrasting effects probably reflect differences in both the degree of substrate–shift reagent associations in each solvent and in the solvent-related conformations that the substrate–shift reagent complex can adopt.

The hydroxyl group of timolol appears to be a major coordination site, since conversion of the drug to a trimethylsilyl derivative caused a decrease in the lanthanide-induced shifts. This effect is attributed to a lessening of the interaction between the shift reagent and the hydroxyl group oxygen of the substrate due to steric hindrance by the bulky trimethylsilyl group.

As a test for accuracy, several mixtures of timolol and its (*R*)-(+)-enantiomer were prepared in the proportions shown in Table 2. After their conversion to the free base form, these mixtures were mixed with specific amounts of $Pr(hfc)_3$, and dissolved in CCl_4 to obtain solutions 0.074 M in total substrate concentration and 0.101 molar in shift reagent. From the relative intensities of the two *tert*-butyl proton singlets at −0.28 ppm (timolol) and 0.00 ppm ((*R*)-(+)-enantiomer), upfield from TMS, the optical purities were readily calculated. The analytical results were found to be in close agreement with the known quantities of each enantiomer in the mixtures analyzed. The mean ± SD recovery ($n = 6$) of the (*R*)-(+)-enantiomer from these mixtures was 99.5 ± 1.17% of the quantity added.

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