Stereoselective Enzymatic Hydrolysis of Various Ester Prodrugs of Ibuprofen and Flurbiprofen in Human Plasma

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The hydrolysis kinetics of various alkyl, glycolamide, aminoethyl, and 2-(1-imidazolyl)ethyl esters of ibuprofen and flurbiprofen in 80% human plasma were investigated using a direct high-performance liquid chromatographic assay for the enantiomers of these acids. In each case, the *R*-isomer ester was found to undergo faster plasma-catalyzed hydrolysis than the corresponding *S*-isomer. The difference in the hydrolysis rates between the enantiomeric forms ranged from a factor of 1.4 for the *N,N*-diethylglycolamide ester of ibuprofen to a factor of 50 and 25 for the 2-(1-imidazolyl)ethyl ester of ibuprofen and flurbiprofen, respectively. Therefore, enantioselective differences in plasma-catalyzed ester prodrug hydrolysis must be taken into account when evaluating prodrugs of racemic mixtures of chiral drugs.

KEY WORDS: stereoselective enzymatic hydrolysis; prodrugs; ibuprofen; flurbiprofen.

INTRODUCTION

Ibuprofen (I) and flurbiprofen (II) are nonsteroidal antiinflammatory drugs of the group of 2-arylpropionic acids (profens) having a chiral center at the carbon atom α to the carboxyl function. With the exception of naproxen all of the clinically used profens are marketed as racemates, although only the *S*-enantiomers possess significant antiinflammatory activity (1).

During the development of water-soluble 2-(1-imidazolyl)ethyl ester prodrugs of ibuprofen and flurbiprofen, we observed a marked stereoselective plasma-catalyzed hydrolysis of the esters. In spite of the common use of esters as prodrugs for carboxylic acid- or hydroxyl-containing drugs (2) and the stereoselectivity of esterases, there are only few reports on stereochemical differences in the enzymatic hydrolysis of such prodrugs. Takahashi *et al.* (3) have recently reported on a threefold difference in the human serum-catalyzed hydrolysis of the acetyl ester of *R*- and *S*-propranolol. Stereoselective hydrolysis of various ester prodrugs of oxazepam by liver enzymes has also been reported (4,5). Although numerous ester prodrugs of 2-arylpropionic acid drugs have been investigated (6–8), there is no report on stereochemical differences in their enzymatic hydrolysis.

In this paper, we report on the stereoselective enzymatic hydrolysis in human plasma of 2-(1-imidazolyl)ethyl esters of ibuprofen and flurbiprofen (Ie–If and IIc–IId), ibuprofen ethyl (Ia) and aminoethyl (Id) esters, and previously described (8) *N,N*-disubstituted glycolamide ester prodrugs of ibuprofen and flurbiprofen (Ib–Ic and IIa–IIb) (Scheme I), using HPLC methods for direct stereoselective analysis of ibuprofen and flurbiprofen enantiomers in human plasma. Part of this work has been published in abstract form (9).

MATERIALS AND METHODS

Apparatus

High-performance liquid chromatography (HPLC) was performed using two different systems. For nonstereoselective analysis a reversed-phase ChromSep (100 × 4.6-mm-I.D.) column packed with MicroSpher C18 material (3-μm particles) was used with a system consisting of a Merck-Hitachi L-6000 pump, a Merck-Hitachi L-4000 variable-wavelength UV detector, and a Rheodyne 7125 injection valve with a 20-μl loop. For stereoselective HPLC analysis a Chiral-AGP column (100 × 4-mm I.D.; ChromTech, Norsborg, Sweden) packed with an α_1-acid glycoprotein (AGP) bonded stationary phase material (5-μm particles) was used with a system consisting of a Model 510 HPLC pump, a 712 WISP autosampler, a Column Heater Module (all from Millipore, Waters Chromatography Division), and a Merck-Hitachi F-1000 fluorescence spectrophotometer. Elemental analysis was performed at Leo Pharmaceuticals Ltd., Ballerup, Denmark.

Chemicals

Ibuprofen racemate and pure enantiomers were kindly supplied by DAK-laboratorium a/s, Copenhagen, Denmark. Flurbiprofen racemate was purchased from Sigma Chemical Company, St. Louis, MO, whereas analytical samples of the pure enantiomers were kindly supplied by Boots Pharmaceuticals, Nottingham, England. Other chemicals and solvents used for synthesis or HPLC were from Aldrich-Chemie, FRG, or Sigma.

Synthesis of NSAID Esters

The ibuprofen esters (Ia–Id) were prepared and isolated both as racemates and as pure enantiomers, whereas the other esters of ibuprofen and flurbiprofen were isolated as racemates only.

Ethyl esters. R/S-Ibuprofen ethyl ester (*R/S*-Ia) was prepared by adding ethyl iodide (11 mmol) to a solution of racemic ibuprofen (10 mmol) and triethylamine (11 mmol) in *N,N*-dimethylformamide (10 ml). The mixture was stirred at room temperature overnight, poured into water (50 ml), and extracted with ethyl acetate (2 × 50 ml). The combined extracts were washed with a 2% solution of sodium thiosulfate, a 5% solution of sodium bicarbonate, and water. After drying over anhydrous sodium sulphate, the ethyl acetate was removed under reduced pressure. The enantiomeric ibuprofen ethyl esters *R*-Ia and *S*-Ia were also prepared according to this procedure. All ibuprofen ethyl esters were isolated as oils in yields of about 80%.

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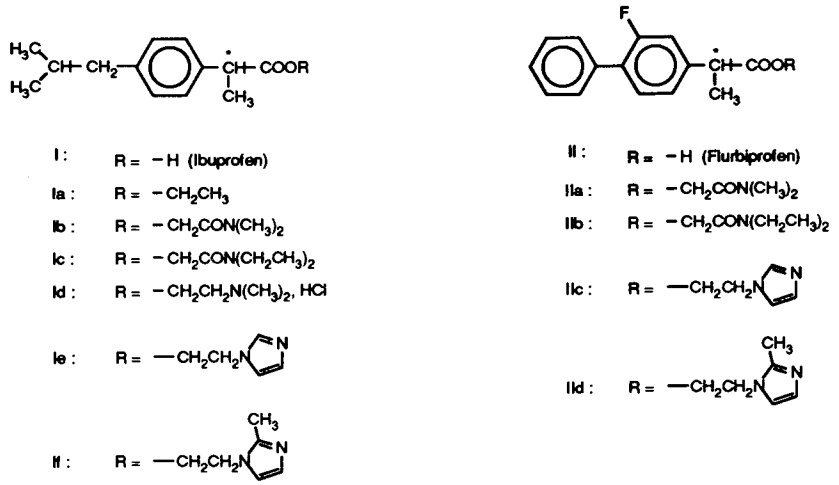

Scheme I

Glycolamide Esters. The synthesis of the racemic glycolamide esters *R/S*-**Ib**, *R/S*-**Ic**, *R/S*-**IIa**, and *R/S*-**IIb** of ibuprofen and flurbiprofen has previously been described (8). The enantiomeric ibuprofen glycolamide esters *R*-**Ib**, *S*-**Ib**, *R*-**Ic**, and *S*-**Ic**, all isolated as oils, were prepared in a similar way.

N,N-*Dimethylaminoethyl Esters.* The ibuprofen *N,N*-dimethylaminoethyl esters (*R/S*-**Id**, *R*-**Id**, and *S*-**Id**) were prepared by reacting the acid chlorides with *N,N*-dimethylaminoethanol. The acid chlorides were prepared as follows. A mixture of 10 mmol of ibuprofen, 5 ml of thionyl chloride, and 10 ml of benzene was refluxed for 2 hr. The solution was evaporated *in vacuo* and the residue obtained reevaporated with 5 ml of benzene to remove traces of thionyl chloride. In preparation of the enantiomeric acid chlorides the mixtures were not refluxed but stirred overnight at room temperature in order to avoid racemization of the acid chlorides.

N,N-Dimethylaminoethanol (10 mmol) was added to a solution of the acid chloride (10 mmol) and triethylamine (10 mmol) in acetonitrile (30 ml). The reaction mixture was stirred at room temperature for 2 hr and then evaporated under reduced pressure. The residue obtained was taken up in ethyl acetate (25 ml), washed with a 2% solution of sodium bicarbonate and water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to yield the ester as an oil. The hydrochloride salt of the ester was obtained by adding 5 ml of a 2.5 *M* methanolic HCl solution to a solution of the ester in ether (20 ml). The precipitate formed upon standing overnight at −20°C was filtered off and recrystallized from ethanol–ether. The yields were 50–60%. The physical and analytical data of the racemic ester *R/S*-**Id** are shown in Table I.

2-(1-Imidazolyl)ethyl Esters. The racemic esters were prepared by reacting the acid chloride of ibuprofen and flurbiprofen with the appropriate *N*-(2-hydroxyethyl)imidazole in acetonitrile. *N*-(2-Hydroxyethyl)imidazole and *N*-(2-hydroxyethyl)-2-methylimidazole were prepared by reacting ethylene carbonate with imidazole and 2-methylimidazole, respectively, as described by Yoshino *et al.* (10) and purified by fractional distillation at reduced pressure. The acid chlorides were prepared as described above. A solution of the acid chloride (10 mmol) in acetonitrile (10 ml) was added to a solution of the appropriate *N*-(2-hydroxyethyl)imidazole (10 mmol) and triethylamine (10 mmol) in 20 ml of acetonitrile at 5–10°C. The reaction mixture was stirred at 60°C for 1 hr and evaporated under reduced pressure. The residue obtained was taken up in ethyl acetate (30 ml) and water (30 ml). The organic layer was separated, washed with a 5% solution of sodium bicarbonate and water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to yield the ester as an oil. The fumarate salt of the ester was obtained by adding a solution of fumaric acid (1.2 g) in 2-propanol (15 ml) to a solution of the ester in ether. The precipitate formed upon standing overnight at −20°C was filtered off and recrystallized from ethanol–ether. The yields obtained of the esters as fumarate salts were 60–80%. The physical and analytical data of the compounds are shown in Table I.

Table I. Physical and Analytical Data of Various Esters of Ibuprofen and Flurbiprofen

Compound	m.p. (°C)	Formula		Analysis (%) Calc.	Found
R/S-Id[a]	116–117	$C_{17}H_{28}ClNO_2$	C	65.06	64.83
			H	8.99	9.02
			N	4.46	4.39
R/S-Ie[b]	127–128	$C_{22}H_{28}N_2O_6$	C	63.45	63.25
			H	6.78	6.82
			N	6.75	6.75
R/S-If[b]	103–105	$C_{23}H_{30}N_2O_6$	C	64.17	64.26
			H	7.02	7.05
			N	6.51	6.61
R/S-IIc[b]	102–103	$C_{24}H_{25}FN_2O_6$	C	63.43	63.29
			H	5.10	5.12
			N	6.16	6.18
R/S-IId[b]	104–106	$C_{25}H_{25}FN_2O_6$	C	64.09	63.91
			H	5.38	5.43
			N	5.98	6.10

[a] Salt with 1 equiv of hydrochloric acid.
[b] Salt with 1 equiv of fumaric acid.

HPLC Analysis

In the reversed-phase HPLC procedure used for non-stereoselective analysis of the various ibuprofen and flurbiprofen esters, the ChromSep C-18 column was eluted with a mobile phase system consisting of acetonitrile (40–60%, v/v) in 0.2% (v/v) phosphoric acid with triethylamine added at a concentration of 2×10^{-3} *M* to improve peak shape. The concentration of acetonitrile in the eluent was adjusted for each ester in order to provide appropriate retention times (3–10 min) and separation of the ester and the parent acid. The flow rate was 1.0 ml/min and the column effluent was monitored at 222 nm.

For stereoselective analysis of ibuprofen and flurbiprofen, samples of 10 μl of deproteinized plasma solutions were applied onto a Chiral-AGP column. The column was eluted with a mobile phase consisting of ethanol (8 or 15%, v/v) in 0.02 *M* phosphate buffer of pH 5.5. Using a mobile phase with 8% (v/v) ethanol, *R*- and *S*-ibuprofen were separated with retention times of 11 and 13 min, respectively. The flow rate was 0.5 ml/min and the column effluent was monitored by fluorescence with excitation and emission at 222 and 290 nm, respectively. Using a mobile phase with 15% (v/v) ethanol *R*- and *S*-flurbiprofen were separated with retention times of 18.5 and 21.5 min, respectively. The column effluent was monitored by fluorescence with excitation and emission at 247 and 310 nm, respectively. These methods were based on previously described procedures (11,12). Attempts to separate the enantiomeric ester derivatives using the Chiral-AGP column failed. The compounds were in all cases quantitated by measurements of the peak heights in relation to those of standards chromatographed under the same conditions.

Hydrolysis Studies in Human Plasma

The ibuprofen esters **Ia–If** and flurbiprofen esters **IIa–IId** were incubated at 37°C in 80% human plasma (diluted with 0.05 *M* phosphate buffer of pH 7.4) at an initial concentration of 2×10^{-4} *M*. The plasma was derived from different individuals, pooled, and kept at −20°C for about 1 month before used in the study. At appropriate intervals, samples of 250 μl of the plasma reaction solution were withdrawn and added to 500 μl of methanol in order to deproteinize the plasma. After mixing the samples were kept at room temperature for 15 min and then centrifugated for 5 min at 13,000 rpm. The clear supernatant was analyzed for remaining ester derivative and formed *R*- and *S*-isomers of ibuprofen or flurbiprofen using the HPLC procedures described above.

RESULTS AND DISCUSSION

The kinetics of hydrolysis of the ibuprofen and flurbiprofen esters **Ia–If** and **IIa–IId** was examined at 37°C in 80% human plasma of pH 7.4. Under these conditions only the pure enantiomers of the ibuprofen esters **Ia–Id** were found to be hydrolyzed according to strict pseudo-first-order kinetics (Fig. 1). The half-lives observed are listed in Table II. No interconversion between the enantiomeric esters took place during their hydrolysis as revealed by HPLC analysis of the free drug formed.

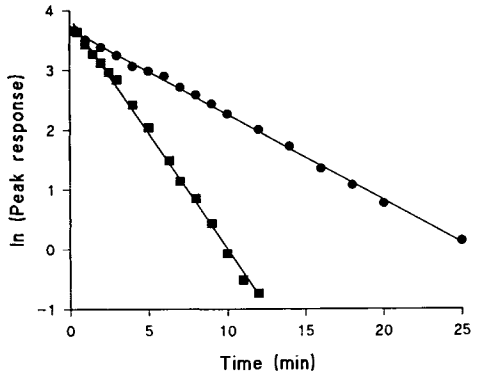

Fig. 1. First-order plots for the hydrolysis of the ibuprofen esters *S*-**Ib** (●) and *S*-**Id** (■) in 80% human plasma solutions (pH 7.40) at 37°C.

In the case of the racemic ibuprofen and flurbiprofen esters, biphasic reaction courses were observed when the logarithm of the total amount (*R*- and *S*-enantiomer) of intact ester was plotted as a function of time. A typical example of a biphasic reaction course is shown in Fig. 2. Extrapolation of the terminal straight-line portions of such plots to time zero gave a value corresponding to 50% of the initial ester concentration. This indicates that the biphasic reaction courses observed are due to different rates of hydrolysis of the two ester enantiomers constituting the racemic ester product. Conclusive evidence for this interpretation was provided by stereoselective HPLC analysis of the reaction solutions for ibuprofen or flurbiprofen enantiomers formed. As illustrated in Fig. 3 the more rapid part of the hydrolysis course of the racemic ibuprofen ester *R*/*S*-**If** is due to hydrolysis of the *R*-enantiomeric ester since this part is accompanied by a corresponding formation of the *R*-isomer of ibuprofen. Likewise, the slower part of the hydrolysis course is seen to be due to hydrolysis of the *S*-enantiomeric ester. Figure 3 also shows that the two ibuprofen enantiomers are formed in quantitative amounts. The same was found for the other esters studied.

The half-lives of hydrolysis listed in Table II for the enantiomers of the ibuprofen and flurbiprofen esters **Ie–If**

Table II. Half-Lives ($t_{1/2}$) and Enantiomeric Ratios of Rate of Hydrolysis for Various Esters of Ibuprofen and Flurbiprofen in 80% Human Plasma of pH 7.4 and 37°C

Compound	$t_{1/2}$ (min)[a]		*R*:*S* rate ratio
	R-Isomer	*S*-Isomer	
Ibuprofen ester **Ia**	93 h[b]	265 h[b]	2.8
Ibuprofen ester **Ib**	1.5	7.0	4.7
Ibuprofen ester **Ic**	1.0	1.4	1.4
Ibuprofen ester **Id**	1.7	2.6	1.5
Ibuprofen ester **Ie**	19	960	50
Ibuprofen ester **If**	5.2	135	26
Flurbiprofen ester **IIa**	1.1	11	10
Flurbiprofen ester **IIb**	1.5	2.6	1.7
Flurbiprofen ester **IIc**	18	445	25
Flurbiprofen ester **IId**	38	355	9.3

[a] The standard deviations of the $t_{1/2}$ values were within ±10%.
[b] Determined by measuring the initial rate of formation of ibuprofen.

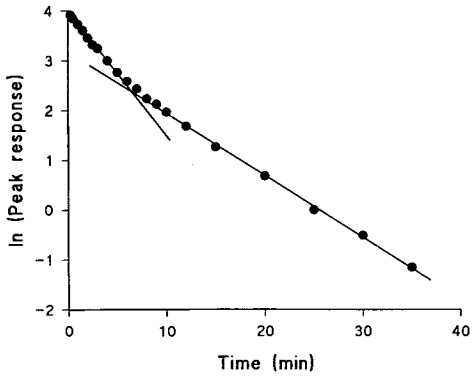

Fig. 2. Plots showing the biphasic reaction courses of total ester during hydrolysis of the ibuprofen ester *R/S*-**Ib** in 80% human plasma (pH 7.40) at 37°C.

and **IIa–IId** were obtained from hydrolysis studies of the racemic mixtures by nonlinear iterative regression analysis of total ester concentration (*R*- and *S*-enantiomer) as a function of time using the following equation:

$$C_{R+S,t} = C_{R,0} \cdot e^{-k_R \cdot t} + C_{S,0} \cdot e^{-k_S \cdot t} \quad (1)$$

where $C_{R,0}$ and $C_{S,0}$ are the initial concentration of the *R*- and *S*-isomeric ester derivatives, k_R and k_S are the apparent pseudo-first-order rate constants for the hydrolysis of the *R*- and *S*-isomeric ester derivatives, respectively, and $C_{R+S,t}$ is the total concentration of these species at time t. The rate constants obtained in this way for the hydrolysis of the racemic ibuprofen esters **Ib**, **Ic**, and **Id** agreed within ±10% with the rate constants determined in hydrolysis experiments of the individual *R*- and *S*-isomeric esters.

In some cases, pseudo-first-order rate constants for the hydrolysis of the ester enantiomers were obtained from the slopes of linear plots of ln $(C_{A,\infty} - C_{A,t})$ vs time, where $C_{A,\infty}$ and $C_{A,t}$ are the concentrations of formed *R*- or *S*-isomer of free acid (ibuprofen or flurbiprofen) at infinity and time t, respectively. Rate constants obtained using both these methods agreed within ±10%.

It should be noted that the half-lives of hydrolysis of the esters in a phosphate buffer of pH 7.4 and at 37°C greatly exceeded those observed in the plasma solutions. Inspection of the data in Table II shows varying degrees of enantioselective plasma-catalyzed hydrolysis of the esters investigated. The rate of hydrolysis of the *R*-isomer of the esters was in all cases higher than that of the corresponding *S*-isomer. Whereas only minor differences (*R:S* ratios, <2) were found between the rate of hydrolysis of the enantiomers for the glycolamide esters **Ic**, **Id**, and **IIb**, *R:S* rate ratios of 25, 26 and 50 were found in the case of the imidazole-containing esters **IIc**, **If**, and **Ie**, respectively.

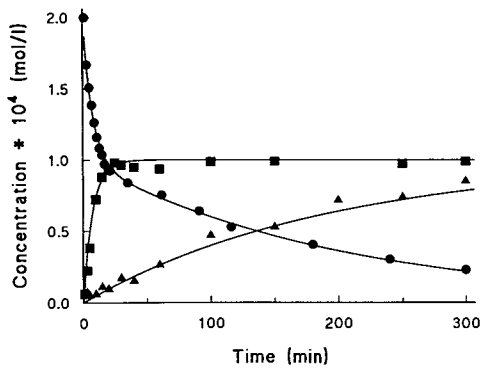

Fig. 3. Time courses for total ibuprofen ester *R*-If and *S*-If (●) and the enantiomers *R*-ibuprofen (■) and *S*-ibuprofen (▲) during hydrolysis of the racemic ester *R/S*-If in 80% human plasma (pH 7.40) at 37°C.

Nakamura and Yamaguchi (13) have previously reported that ester glucuronides of *R*-2-phenylpropionic acid were hydrolyzed at higher rates by rat liver and kidney enzymes than the glucuronides of the *S*-isomer. In contrast to these findings and those reported here, Knadler and Hall (14) have recently described that the ester glucuronide of *S*-flurbiprofen is hydrolyzed fourfold more rapid than that of the *R*-isomer in human plasma.

The plasma-catalyzed hydrolysis of the various esters of ibuprofen and flurbiprofen may be attributed to plasma butyrylcholinesterase, also called pseudocholinesterase (15). This enzyme has recently been shown to be responsible for the more than 1,000-fold greater rate of hydrolysis of (+)-cocaine in baboon plasma relative to (−)-cocaine (16,17). The esterase-like activity of human serum albumin has been shown to be enantioselective (14,18) and may also contribute to the stereoselective plasma-catalyzed hydrolysis.

Under the experimental conditions used, where each enantiomeric ester is hydrolyzed according to pseudo-first-order kinetics (i.e., $K_m \gg$ initial ester concentration), the half-lives obtained are equal to

$$t_{1/2} = \frac{0.693}{(V_{max}/K_m)} \quad (2)$$

where V_{max} and K_m are the Michaelis–Menten parameters for the enzymatic hydrolysis. Thus, since the $t_{1/2}$ values contain both K_m and V_{max}, the present data do not make it possible to evaluate the stereoselective effect of plasma on each of the parameters.

The results of this study emphasize the need to consider enantioselective differences in plasma-catalyzed ester prodrug hydrolysis.

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REFERENCES

1. A. J. Hutt and J. Caldwell. The importance of stereochemistry in the clinical pharmacokinetics of the 2-arylpropionic acid nonsteroidal anti-inflammatory drugs. *Clin. Pharmacokin.* 9:371–373 (1984).
2. H. Bundgaard. Design of prodrugs: Bioreversible derivatives for various functional groups and chemical entities. In H. Bundgaard (ed.), *Design of Prodrugs*, Elsevier, Amsterdam, 1985, pp. 1–92.
3. K. Takahashi, J. Haginaka, S. Tamagawa, T. Nishihata, H. Yasuda, and T. Katagi. Stereoselective hydrolysis of *O*-acetyl propranolol as prodrug in human serum. *J. Pharm. Pharmacol.* 42:356–357 (1990).

4. S. K. Yang and X.-L. Lu. Racemization kinetics of enantiomeric oxazepams and stereoselective hydrolysis of enantiomeric oxazepam 3-acetates in rat liver microsomes and brain homogenate. *J. Pharm. Sci.* 78:789–795 (1989).
5. M. Salmona, C. Saronio, R. Bianchi, F. Marcucci, and E. Mussini. *In vitro* hydrolysis of oxazepam succinate half-ester by a stereospecific soluble esterase from different animal species. *J. Pharm. Sci.* 63:222–225 (1974).
6. A. Büge. Prodrugs nichtsteroidaler Antiphlogistika—eine Übersicht. *Wiss. Z. Univ. Halle Math.-Naturwissen-Reihe.* 35:106–118 (1986).
7. G. Jones. Decreased toxicity and adversed reactions via prodrugs. In H. Bundgaard (ed.), *Design of Prodrugs,* Elsevier, Amsterdam, 1985, pp. 199–241.
8. H. Bundgaard and N. M. Nielsen. Glycolamide esters as a novel biolabile prodrug type for non-steroidal anti-inflammatory carboxylic acid drugs. *Int. J. Pharm.* 43:101–110 (1988).
9. N. Mørk and H. Bundgaard. Stereoselective enzymatic hydrolysis of chiral NSAID ester prodrugs in human plasma. *Pharm. Res.* 7:S-128 (1990).
10. T. Yoshino, S. Inaba, H. Komura, and Y. Ishido. Synthetic studies with carbonates. 6. Synthesis of 2-hydroxyethyl derivatives by reactions of ethylene carbonate with carboxylic acids or heterocycles in the presence of tetrabutylammonium halides or under autocatalytic conditions. *J. Chem. Soc. Perkin Trans. I* 1266–1272 (1977).
11. J. Hermansson and M. Eriksson. Direct liquid chromatographic resolution of acidic drugs using a chiral a_1-acid glycoprotein column (enantiopac®). *J. Liq. Chromatogr.* 9:621–639 (1986).
12. S. Menzel-Soglowek, G. Geisslinger, and K. Brune. Stereoselective high-performance liquid determination of ketoprofen, ibuprofen and fenoprofen in plasma using a chiral α_1-acid glycoprotein column. *J. Chromatogr.* 532:295–303 (1990).
13. Y. Nakamura and T. Yamaguchi. Stereoselective metabolism of 2-phenylpropionic acid in rat. I. *In vitro* studies on the stereoselective isomerization and glucuronidation of 2-phenylpropionic acid. *Drug Metab. Disp.* 15:529–533 (1987).
14. M. P. Knadler and S. D. Hall. Stereoselective hydrolysis of flurbiprofen conjugates. *Drug Metab. Disp.* 19:280–282 (1991).
15. N. M. Nielsen and H. Bundgaard. Glycolamide esters as biolabile prodrugs of carboxylic acid agents: Synthesis, stability, bioconversion, and physicochemical properties. *J. Pharm. Sci.* 77:285–298 (1988).
16. S. J. Gatley, R. R. MacGregor, J. S. Fowler, A. P. Wolf, S. L. Dewey, and D. J. Schlyer. Rapid stereoselective hydrolysis of (+)-cocaine in baboon plasma prevents its uptake in the brain: Implications for behavioral studies. *J. Neurochem.* 54:720–723 (1990).
17. S. J. Gatley. Activities of the enantiomers of cocaine and some related compounds as substrates and inhibitors of plasma butyrylcholinesterase. *Biochem. Pharmacol.* 41:1249–1254 (1991).
18. Y. Aso, S. Yoshioka, and Y. Takeda. Epimerization and racemization of some chiral drugs in the presence of human serum albumin. *Chem. Pharm. Bull.* 38:180–184 (1990).