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Report

Chiral Amines Derived from 2-Arylpropionic Acids: Novel Reagents for the Liquid Chromatographic (LC) Fluorescence Assay of Optically Active Carboxylic Acid Xenobiotics

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For the enantiospecific analysis of optically active carboxylic acids, the availability of readily detectable coupling components is desirable, but highly fluorescent chiral amines are rare. From activated enantiomers of fluorescent 2-arylpropionic acids fluorescent chiral amines were synthesized via Curtius degradation, i.e., under formation of the acyl azide, the isocyanate, and finally, the amine. The formation of isocyanates and of amine hydrochlorides led to an inversion of the direction of rotation of polarized light. Amines derived from *R*- and *S*-flunoxaprofen, *R*- and *S*-naproxen, and *R*/*S*-benoxaprofen were characterized. The amines were found to be applicable for the chiral separation of carboxylic acids (such as 2-arylpropionic acids) as diastereomeric derivatives via high-performance liquid-chromatographic (normal and reversed-phase) and thin-layer chromatographic techniques.

KEY WORDS: chiral derivatization; nonsteroidal antirheumatic drugs; 2-arylpropionic acids; naproxen; flunoxaprofen; benoxaprofen.

INTRODUCTION

A chiral derivatizing agent (CDA) consists of two elements: (i) a chiral coupling component (CCC) and (ii) either a covalently attached linker group or a second molecule that serves as condensing agent. *Absolute requirements* for a CCC are enantiomeric purity, chemical and enantiomeric stability under storage, derivatization and chromatography, and derivatizable moieties for coupling to the analyte.

Chromophoric properties, lipophilicity, and molecular size are *relative requirements* for the CCC, which depend upon the substrate, e.g., whether the substrate itself contains well-detectable structures.

One major structural determinant of CCCs appears to be a chiral center close to the derivatizable or reactive moiety. Thus any pure and stable enantiomer with suitable derivatizable groups is a potential CCC.

In LC, commercially available coupling components include phenyl, naphthyl residues with a chiral carbon in a possibly activated side-chain or modified D-glucopyranosyl residues, terpens and terpenoids, chiral nonchromophoric alcohols, or amino acid derivatives (1,2).

The coupling of carboxylic acids with amines and alcohols is commonly achieved through activation of the carboxylic acid function, e.g., by formation of acyl chlorides, imidazolides, or mixed anhydrides (3–5). A further possibility for reacting substrate and coupling component is the use of carbodiimides as condensing agents (6).

The stereochemistry of 2-arylpropionic acids (2-APAs), which include many NSAIDs, is of interest because they undergo inversion of the distomer to the eutomer *in vivo* (7). Several enantiospecific assay methods were developed, based mostly on the formation of diastereomeric derivatives. Many of the 2-APA drugs contain strong chromophores, which therefore do not need to be incorporated in the CCCs that are used for their chiral derivatization. In these cases, derivatization involves coupling to α-methylbenzylamine (e.g., Ref. 4) with different condensing agents or more recently L-leucinamide/ethylchloroformate (Björkman procedure) (5,8).

Chiral reagents with fairly strong chromophores include 1-(1-naphthyl)ethylamine [also proposed as coupling component for ibuprofen (9,10)], 1-(4-dimethylamino-1-naphthyl)ethylamine (2) or 1-(1-anthryl)-ethylamine, and its 2-anthryl analogue (11). However, there are few chiral reagents with strong fluorescence that may permit more sensitive detection of trace amounts of optically active acids.

Several fluorescent 2-arylpropionic acids were found to be useful tools for assaying chiral amines at low concentrations, with strong fluorescence residing in the phenyl-substituted benzoxazole derivatives, benoxaprofen and flunoxaprofen (BOP, FLOP), and the methoxy-substituted naphthyl derivative naproxen (NAP) (3,12–14). These 2-APAs, as acyl chlorides, as imidazolides, or converted to the

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isocyanates, were applied as reagents for the enantiospecific assay of numerous drugs, including amino acids and peptides, in biological materials at low concentrations (15).

If converted to the corresponding amines without affecting their enantiomeric purity, these reagents could serve as CCCs for carboxylic acids. The aim of the present work was the conversion of the carboxylic acid starting materials via Curtius degradation (Fig. 1A), yielding the amines (2-APamines: BOPA, FLOPA, NAPA) (Fig. 1B).

MATERIALS AND METHODS

2-Arylpropionic Acids

R/S-Benoxaprofen was obtained from Eli Lilly (Bad Homburg), before it was withdrawn from the market, *S*-naproxen from Grünenthal (Stolberg), and *R*-naproxen from Syntex (Palo Alto, CA), and *S*-, *R*-, and *R/S*-flunoxaprofen were kindly provided by Ravizza S.p.A. (Muggio, Italy). Small amounts of benoxaprofen enantiomers were prepared according to Ref. 3 via preparative HPLC. The enantiomeric excesses (ee) in the enantiomers were as follows: *S*-naproxen, 0.988; *R*-naproxen, 0.940; *S*-flunoxaprofen, 0.938; *R*-flunoxaprofen, 0.952; *S*-benoxaprofen, 0.926; and *R*-benoxaprofen, 0.950.

Fenoprofen was obtained from Eli Lily Bad Homburg (F.R.G.), fenoprofen enantiomers from Eli Lilly (Indianapolis, IN), MK 830 [D-(+)-2-(3-chloro-4-cyclohexylphenyl)-propionic acid] and its optical antipode from Merck Sharp & Dohme Research Laboratories (West Point, PA), ibuprofen from Bayer (Wuppertal, F.R.G.), and flurbiprofen from Thomae (Biberach/Riss, F.R.G.). Reference samples of ibuprofen enantiomers were provided by Dr. G. Geislinger (Department of Pharmacology and Toxicology, University of Erlangen, F.R.G.). Clofibric acid was purchased from Sigma (Munich, F.R.G.).

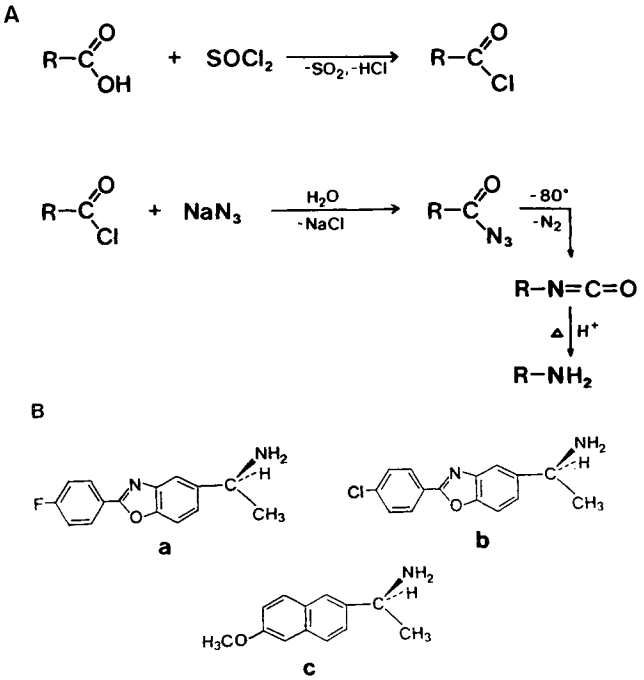

Fig. 1. Derivatization scheme for carboxylic acids, yielding the corresponding amines (Curtius degradation). After formation of the azide, Curtius rearrangement leads to the formation of the isocyanate, which is further degraded to the amine via acid treatment. (B) Structures of amines that were derived from *S*-flunoxaprofen, *S*-benoxaprofen, and *S*-naproxen: *S*-BOPA (a), *S*-FLOPA (b), and *S*-NAPA (c).

Solvents and Reagents

All solvents and pyridine (analytical grade) and thionyl chloride were obtained from E. Merck (Darmstadt, F.R.G.). Sodium azide, 1-hydroxybenzotriazole, and *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride were from Sigma (Munich, F.R.G.).

Equipment

Melting points were obtained from a Büchi apparatus (Büchi,, Flawil, Switzerland) and are uncorrected. Infrared spectra were obtained in KBr disks with a Model 1420 ratio recording infrared spectrophotometer (Perkin Elmer, Überlingen, F.R.G.). Elemental analysis were performed at the Department of Organic Chemistry at the University of Frankfurt. Optical rotations were measured on a Polartronic I Polarimeter (Schmidt & Haensch, Berlin, F.R.G.). NMR spectra were recorded with a Brüker AC 300 equipped with an Aspect 300 computer (Brüker, Rheinstetten, F.R.G.).

Solutions were applied on TLC plates using a Linomat III (Camag, Muttenz, Switzerland). The plates were scanned with a chromatogram spectrophotometer KM3 (Carl Zeiss, Oberkochen, F.R.G.).

The HPLC system consisted of a Knauer HPLC pump 64 with a Rheodyne injection valve (Rheodyne, Cotati, USA) and a Shimadzu RF 530 fluorescence monitor (Shimadzu Corp., Kyoto, Japan).

Conversion of Carboxylic Acids to the Corresponding Amines (Curtius Degradation)

The isocyanates were prepared *in situ* either via the ethyl chloroformate method (14,16) or via the acyl chloride (17). All the procedures described in the following were carried out under protection from light.

Synthesis of the Acyl Chlorides (FLOP-Cl, BOP-Cl, NAP-Cl) in Semipreparative Scales. In a semipreparative scale, 500 mg of carboxylic acid was dissolved in 50 ml of dried toluene. After slowly adding 5 ml of thionyl chloride, which was freshly distilled over linseed oil, the resulting solution was refluxed for 60 min. Then the solution was evaporated to dryness in vacuum. After being kept over potassium hydroxide under vacuum (over 2 night usually suffices), the crystalline residue was used without further purification.

S-(−)-FLOP-Cl: $C_{16}H_{11}FClNO_2$, 303.7 g/mol, melting point of the residue, 73°C; IR-spectrum, 1780 cm^{-1} (>C=O); *R*-(+)-FLOP-Cl: $C_{16}H_{11}FClNO_2$, 303.7 g/mol, 71°C, IR (cm^{-1}) 1780 (>C=O); *S*-(−)-NAP-Cl: $C_{14}H_{13}ClO_2$, 248.7 g/mol, 86°C, IR (cm^{-1}) 1800 (>C=O);

R-(+)-NAP-Cl: $C_{14}H_{13}ClO_2$, 248.7 g/mol, 87.5°C, IR (cm^{-1}) 1800 (>C=O); *R/S*-BOP-Cl, $C_{16}H_{11}Cl_2NO_2$, 320.2 g/mol, 91.5°C, IR (cm^{-1}) 1775 (>C=O).

Preparation of Isocyanates (FLOPIC, BOPIC, NAPIC) from the Acyl Chlorides. The acyl chloride (0.5 mmol) was dissolved in acetone (5 ml), and the mixture was cooled on ice. While stirring, 0.6 mmol of sodium azide, dissolved in ice water, was added, and the mixture was stirred for another 30 min at 0°C. Ice-cold water (10 ml) was added and the precipitate filtered off, dried in a desiccator under vacuum, and dissolved in 1 ml of anhydrous toluene or dichloromethane (dried over molecular sieve, 3 Å). The solution was refluxed for 10 min, yielding a solution of the isocyanate. The filtered solution was evaporated and the crystalline residue kept in vacuum under anhydrous conditions.

S-FLOPIC: $C_{16}H_{11}FN_2O_2$, 282.3 g/mol. The melting point of the residue was 96°C, IR-spectrum: 2268 cm^{-1} (−N=C=O); *R*-FLOPIC: $C_{16}H_{11}FN_2O_2$, 282.3 g/mol, 91°C, IR (cm^{-1}) 2269 (−N=C=O); *S*-NAPIC: $C_{14}H_{13}NO_2$, 227.3 g/mol, 49°C, IR (cm^{-1}) 2270 (−N=C=O); *R*-NAPIC: $C_{14}H_{13}NO_2$, 227.3 g/mol, 51°C, IR (cm^{-1}) 2275 (−N=C=O); *R/S*-BOPIC, $C_{16}H_{11}ClN_2O_2$, 298.7 g/mol, 84°C, IR (cm^{-1}) 2280 (−N=C=O).

Formation of the Amines (FLOPA, BOPA, NAPA). The isocyanate (0.5 mmol) was dissolved in 5 ml acetone, and 20 ml 8.5% phosphoric acid was added. The mixture was heated to 80°C for approximately 1.5 hr, then adjusted to pH 13, and the amine immediately extracted with diethylether/dichoromethane (4:1, v/v). The ether layer was separated, washed twice with water, and dried with anhydrous sodium sulfate. The solvent was subsequently evaporated, and the residue dissolved in 1 ml of toluene and evaporated again. The amines crystallized slowly at room temperature, yielding light beige/yellowish crystals.

F.p.s: *S*-FLOPA, 93°C, *R*-FLOPA, 91°C; *R/S*-BOPA, 63°C; *S*-NAPA, 52°C; *R*-NAPA, 53°C.

The amine was dissolved in anhydrous diethylether and the corresponding hydrochloride precipitated from the solution by slowly adding 0.5 *M* hydrochloric acid (in anhydrous diethylether) with stirring. The precipitate was filtered off and washed with cold diethylether. The hydrochloride of naproxen amine (NAPA-HCl) was used as obtained after desiccation over phosphorous pentoxide under vacuum. Hydrochlorides of flunoxaprofen and benoxaprofen amines (FLOPA · HCl, BOPA · HCl) were dissolved in a small volume of anhydrous methanol and again precipitated by addition of diethylether. Then the hydrochlorides were filtered off and dried as described for NAPA · HCl.

Optical Rotations

Specific rotations, α_D (given as degrees, defined as the optical rotation of a compound at a concentration of 1 g/ml and a sample pathlength of 1 dm), were determined for all available enantiomers of coupling components in methanol. When sufficient amounts of pure enantiomers were available, the concentrations in the measured solutions were in the range of 1%. The values given are means of five repetitive measurements. For the experiments described here, the enantiomers were used as obtained from the manufacturer.

Estimation of Enantiomeric Purities and Stabilities of the Amines During the Preparative Steps

Enantiomeric purities of the carboxylic acids were determined as described in Refs. 3, 12, 13, 18, and 19 via derivatization with a coupling component of known enantiomeric purity or with chiral stationary phases and of the isocyanates according to (14).

For estimation of the enantiomeric purity of the synthesized chiral amines, naproxen chloride was used as CDA (12).

Formation of Diastereomeric Derivatives (with Carboxylic Acids as Substrates)

Step 1. Formation of an Acyl Chloride. To the carboxylic acid (<20 mmol) that was dissolved in anhydrous toluene, an approximately 50-fold molar excess of freshly distilled thionyl chloride was added and the mixture heated to 80°C for 1 hr. Then the liquids were evaporated under a stream of nitrogen at 50°C and an additional 50 µl of toluene was added and evaporated under nitrogen to remove remaining traces of thionyl chloride.

Step 2. Formation of the Diastereomeric Amides. The residue was reconstituted in 100 µl of anhydrous dichloromethane and 100 µl of reagent solution in dichloromethane was added, yielding an at least twofold molar excess of amine hydrochloride and containing triethylamine as proton acceptor (0.2 *M* amine hydrochloride + 0.4 *M* triethylamine). The reaction was performed at +50°C for 1 hr. The solvent was subsequently evaporated, the remaining residue reconstituted in 500 µl of dichloromethane, and 10–20 µl directly injected into the HPLC system or applied on TLC plates. For RP-HPLC, the residue was dissolved in mobile phase instead of dichloromethane.

For thermolabile and/or otherwise degradable molecules, the activation via the thionyl chloride procedure was not always possible. Problems may also occur through generation of volatile acyl chlorides. One of the feasible alternative procedures is the use of carbodiimides (6,9,20), as characterized below for ibuprofen.

TLC Resolution of Diastereomeric Derivatization Products

The solution was directly applied on silica gel TLC plates (with or without fluorescence indicator). TLC resolution of the derivatives formed from the six investigated 2-APA substrates (ibuprofen, flurbiprofen, MK 830, fenoprofen, naproxen, benoxaprofen) was performed on silica gel plates with toluene-dichloromethane-tetrahydrofuran (5:1:2, v/v, ammonia atmosphere) as mobile phase (glass-tank saturated for 24 hr). After development, the air-dried plate was scanned using the 313-nm line of a medium-pressure mercury lamp for excitation and an M365 monochromatic filter for measurement of the emitted light.

HPLC Resolution of the Products on Normal and Reversed-Phase Stationary Phases

Normal Phase. Resolution of the diastereomeric pairs was accomplished using a Zorbax Sil column (7-µm particle size, 0.46 × 25 cm, DuPont) with *n*-hexane–chloroform–ethanol (100:10:1, v/v) as mobile phase. Resolutions were

obtained at ambient temperature and a flow rate of 2 ml/min with an average pressure of 8.2 MPa.

Reversed Phase. For RP resolutions a chromatographic system that had successfully been employed in our earlier work (19) was utilized. With an Ultrasphere ODS column (5-μm particle size, 0.46 × 25 cm, Beckman) and acetonitrile/0.2% aqueous phosphoric acid (60:40, v/v), good separations were achieved at ambient temperature, a 1.2 ml/min flow rate, and a medium pressure of 14.0 MPa.

Detection of Conjugated and Unconjugated Ibuprofen Enantiomers in Urine Samples

Two female volunteers received 200 mg racemic ibuprofen as a single p.o. dose. Urine was collected in 2-hourly fractions, immediately cooled, and adjusted to pH 3–4 to prevent degradation of conjugates.

To 100 μl of urine 200 μl of pH 5 buffer (citrate/sodium hydroxide), 500 ng of internal standard (clofibric acid), and 5 ml of diethylether/dichloromethane (4:1, v/v) were added. After shaking (10 min) and centrifugation the organic layer was transferred into a second tube, the solvent evaporated, and toluene (200 μl) added and evaporated to remove traces of water.

The chiral derivatization was similar to the procedure used for beclobrinic acid enantiomers (20). The resulting residue was reconstituted in 500 μl anhydrous dichloromethane. Then 50 μl 1-hydroxybenzotriazole solution (1 mg/ml in anhydrous dichloromethane containing 1% pyridine p.a.), 50 μl *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride solution (1 mg/ml in dichloromethane), and 50 μl *S*-FLOPA (1 mg/ml in dichloromethane) were added. After vortexing, the screw-capped tubes were left at ambient temperature for 2 hr. Subsequently, the solvent was evaporated and the residue reconstituted in mobile phase (injection volume, 10–20 μl).

In order to hydrolyze glucuronic acid conjugates, 100 μl of 1 *M* sodium hydroxide was added to 100 μl of urine. After 1 hr the sodium hydroxide was neutralized by addition of 100 μl of 1 *M* hydrochloric acid. Then pH 5 buffer was added and the extraction and derivatization procedure performed as described above for unconjugated compounds. The HPLC separation was performed on a Zorbax Sil column (5-μm particle size, 250 × 4.6 mm, DuPont) with a mobile phase that consisted of *n*-hexane/chloroform/ethanol (100:10:1.25, v/v) at a flow rate of 2 ml/min. The fluorescence of the eluate was monitored at 305/355 nm.

Concentrations were corrected for enantiomeric impurities of the reagent as proposed by Hermansson and von Bahr (21).

RESULTS

Synthesis of the Amines via Curtius Degradation

Using either the acyl chloride or the mixed anhydride (ethyl chloroformate method), it was possible to prepare the isocyanates from naproxen (NAPIC), flunoxaprofen (FLOPIC), and benoxaprofen (BOPIC) as white solid compounds. They were then converted by acid treatment to the corresponding amines, which had rather low melting points, significantly below 100°C. Naproxen amine (NAPA) and benoxaprofen amine (BOPA) exhibited the lowest value (52 and 62°C, respectively) and, hence, did not readily crystallize at ambient temperature. Careful treatment with 1 *M* sodium hydroxide solution yielded amines of comparable enantiomeric purity.

Conversion of the free amines to their hydrochlorides yielded white to yellowish powders that were easier to handle and hence used for further characterization of the products.

Physicochemical Characterization and Structure Confirmation of the Amines

The synthesized 2-APamines were characterized as hydrochlorides.

S-FLOPA · HCl. $C_{15}H_{13}FN_2O \cdot HCl$, 292.8 g/mol. The melting point (F.p.) was 285°C. Elementary analysis: calculated, C, 61.54%; H, 4.79%; N, 9.57%; found, C, 61.36%; H, 4.79%; N, 9.53%. IR spectrum: 1605 cm^{-1} (>CH-NH_2); NMR spectrometry: ^{1}H-NMR; δ 1.74–1.77 (d, 3H; CCH_3), 4.65–4.71 (q, 1H; CH-NH_2), 4.94 (s, 3H; NH_3^+), 7.34–8.35 (m, 7H, C_6H_3, C_6H_4) (Fig. 2).

R-FLOPA · HCl: $C_{15}H_{13}FN_2O$, 292.8 g/mol, 278°C, IR (cm^{-1}) 1607 (>CH-NH_2).

R/S-*BOPA · HCl.* $C_{15}H_{13}ClN_2O$, 309.2 g/mol, 227°C. Elementary analysis: calculated, C, 58.21%; H, 4.53%; N, 9.05%; found, C, 58.66%; H, 4.79%; N, 8.65%. IR (cm^{-1}) 1602 (>CH-NH_2); NMR spectrometry: ^{1}H-NMR: δ 1.74–1.77 (d, 3H; CCH_3), 4.65–4.71 (q, 1H; CH-NH_2), 4.91 (s, 3H; NH_3^+), 7.34–8.35 (m $7H_{13}C_6H_3$, C_6H_4).

S-*NAPA · HCl.* $C_{13}H_{15}NO$, 237.8 g/mol, 204°C. Elementary analysis: calculated, C, 65.61%; H, 6.73%; N, 5.89%; found, C, 65.48%; H, 6.80%; N, 5.89%. IR (cm^{-1}) 1615 (>CH-NH_2); NMR-spectrometry; ^{1}H-NMR; δ 1.75–1.77 (d, 3H; CCH_3), 3.93 (s, 3H; OCH_3), 4.43 (s, 3H; NH_3^+), 4.50–4.57 (q, 1H; CH-NH_2), 7.17–7.85 (m, 6H, $C_{10}H_6$) (Fig. 2).

R-NAPA · HCl: $C_{13}H_{15}NO$, 237.8 g/mol, 200°C, IR (cm^{-1}) 1615 (>CH-NH_2).

The chemical structures of the amines were also confirmed by ^{13}C-NMR and depth NMR. Optical rotations of all products are characterized in the subsequent paragraph.

Optical Rotations and Enantiomeric Stabilities During the Different Synthesis Steps

Activation of the acids to the acyl chlorides or mixed anhydrides and back-hydrolysis did not affect the specific rotations. For naproxen and flunoxaprofen, all enantiomeric compounds were characterized with respect to their optical rotations in methanol solutions, resulting in the specific rotations that are summarized in Table I. When an enantiomer of naproxen or flunoxaprofen was converted to the isocyanate and subsequently to the amine, the direction of optical rotation was found to be inverse for both compounds. For the isocyanates from the *R*-enantiomers an exact characterization and estimation of the optical rotation of the amines was not possible, since only small amounts of reference enantiomers were available. However, the direction of optical rotation was inverse too, as shown for the *S*-enantiomer derivatives, so that the isocyanates and amines resulting from

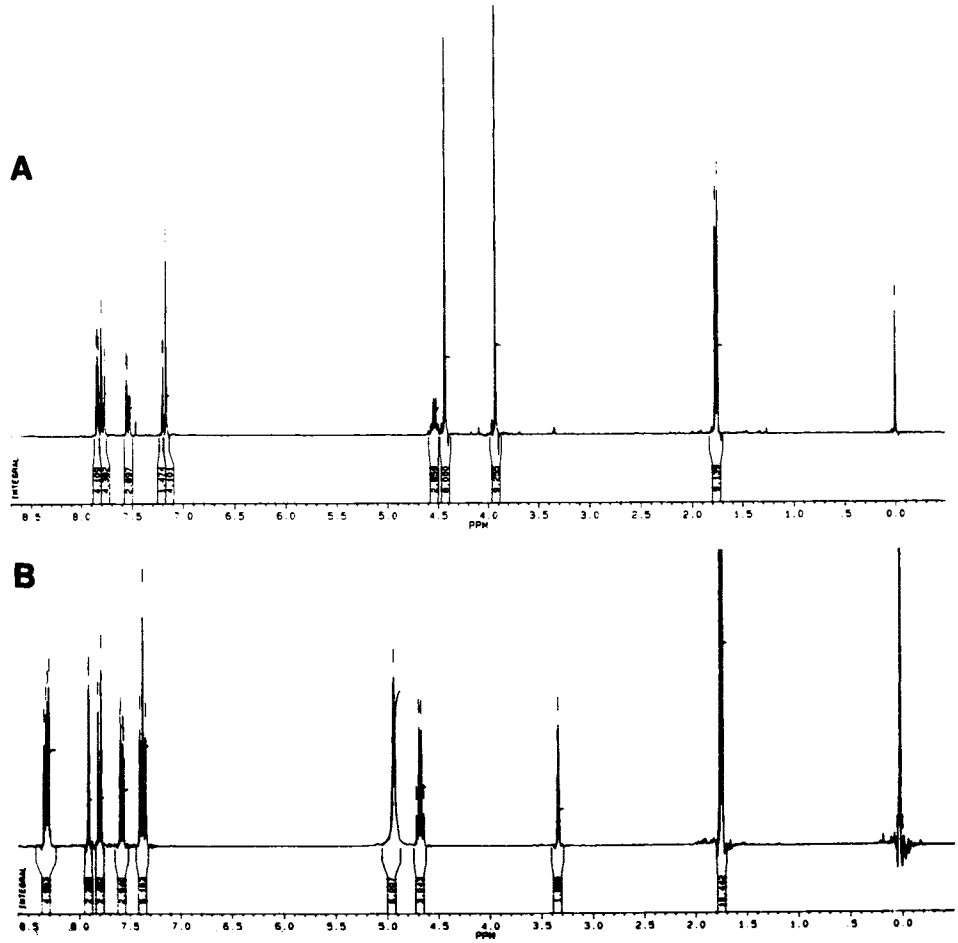

Fig. 2. ^{1}H-NMR spectra of *S*-NAPA · HCl (A) and *S*-FLOPA · HCl (B).

an *S*-(+)-enantiomer of a 2-APA are not dextro- but levorotatory, and vice versa.

Enantiomeric purities (ee, enantiomeric excess) were estimated in all samples via formation of diastereomeric derivatives with compounds of known enantiomeric purities, namely, tranylcypromine enantiomers for acids (as acyl chlorides) and isocyanates and *S*-naproxen for amines, or utilizing a chiral column. The compounds were found be configurationally stable during the different synthesis steps. The ee values are listed in Table I.

Derivatization of Carboxylic Acid Drugs and Chromatographic Behavior of the Products

Reaction of carboxylic acids to the corresponding amides was performed via the acyl chloride. The diastereomeric amides were found to be readily resolvable on normal and reversed-phase stationary phases. Table II gives the resolution parameters for the products on a reversed-phase column and the R_f values of the *S*-FLOPA derivatives of various chiral carboxylic acids on silica gel TLC plates using a

Table I. Optical Rotations and Enantiomeric Purity of the Used and Synthesized Chiral Molecules

	Carboxylic acid		Isocyanate		Amine · HCl	
2-APA	$[\alpha]^{29}D$	ee	$[\alpha]^{29}D$	ee	$[\alpha]^{29}D$	ee
S-Flunoxaprofen	+40.3°	0.938	−107.1°	0.940	−8.5°	0.942
R-Flunoxaprofen	−37.0°	0.952	+99.1°	0.952	+8.1°	0.950
S-Naproxen	+58.3°	0.988	−86.9°	0.980	−22.0°	0.990
R-Naproxen	−53.3°	0.940	(+)[a]	0.936	(+)[a]	0.932
S-Benoxaprofen	+31.2°	0.926	(−)[a]	0.928	(−)[a]	0.926
R-Benoxaprofen	−29.9°	0.952	(+)[a]	0.944	(+)[a]	0.950
R/*S*-Benoxaprofen	0	0	0	0	0	0

[a] Amount available was not sufficient for a quantitative measurement of the optical rotation with the available equipment.

Table II. Resolution Behavior of Diastereomeric Derivatives of 2-Arylpropionic Acids with *S*-FLOPA, When Separated on a Reversed-Phase (ODS) Stationary Phase via HPLC and a Normal Phase (Silica Gel) Stationary Phase via TLC, Respectively[a]

	RP-HPLC				NP-TLC	
Substrate	*k'* (*R*)	*k'* (*S*)	α	*R*	*k'* (*S*)	*k'* (*R*)
Benoxaprofen	5.3	12.2	2.30	11.41	2.85	4.30
Fenoprofen	14.4	17.0	1.16	4.17	4.20	5.20
Flubiprofen	15.0	18.1	1.21	5.06	3.75	5.00
Ibuprofen	21.4	25.3	1.16	4.17	4.40	5.40
MK 830	47.2	56.6	1.20	5.82	4.30	5.50
Naproxen	8.8	10.6	1.20	5.21	3.75	4.65

[a] *k'*, capacity factor; α, separation factor; *R*, resolution factor. Mobile phases: RP-HPLC, acetonitrile–0.2% aqueous phosphoric acid (60:40, v/v); silica gel–TLC, toluene–dichloromethane–tetrahydrofuran (5:1:2, ammonia atmosphere).

solvent system that was used for the resolution of amino acid derivatives as well (15).

Detection of Ibuprofen Enantiomers and Their Conjugates in Human Urine

The procedure described in the experimental section was applicable for the derivatization of ibuprofen after extraction from human urine. The resolution of the diastereomeric ibuprofen–*S*-FLOPA amides is depicted in Fig. 3. The total amount of *S*-ibuprofen in urine exceeded that of *R*-ibuprofen considerably. Figure 4 shows the cumulative urinary excretion over 6 hr for both enantiomers in conjugated and unconjugated form obtained from one of the two female volunteers. The average total fraction of the p.o. dose excreted into urine within 6 hr was 4.9%, with an *S*/*R* ratio of 10. The high *S*/*R* ratio can be explained by significant stereoinversion of the distomer (*R*-ibuprofen) to the eutomer (*S*-ibuprofen) as described by other authors (7).

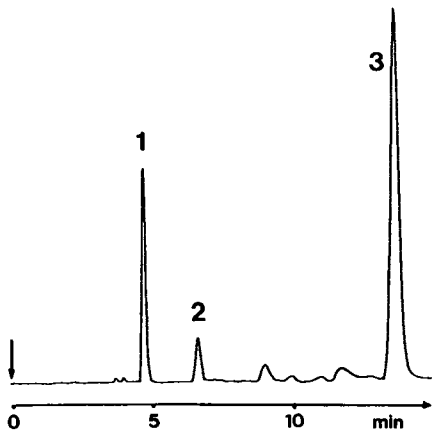

Fig. 3. HPLC chromatogram obtained from a urine sample (2–4 hr after a 200-mg p.o. dose of *R*/*S*-ibuprofen) after performing an alkaline hydrolysis for acyl glucuronide cleavage. The eluate was monitored fluorimetrically at 305/355 nm. 1 = derivative of clofibric acid (internal standard); 2 and 3 = derivatives of *R*-(−)- and *S*-(+)-ibuprofen.

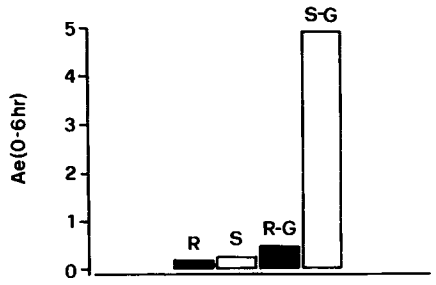

Fig. 4. Excretion of unconjugated and conjugated ibuprofen into the urine (0–6 hr) after a 200-mg p.o. dose of racemic ibuprofen in one healthy volunteer. R and S, unconjugated enantiomers; R-G and S-G, conjugated enantiomers; Ae, amount excreted.

DISCUSSION

We were able to convert the 2-APA carboxylic acids to their corresponding amines. An important observation resulting from the present studies [and our previous work on isocyanates derived from *S*-flunoxaprofen and *S*-naproxen as CDAs for chiral amines (14)] is the inversion of the direction of rotating polarized light. The present measurements revealed that the isocyanates and amine hydrochlorides derived from *S*-(+)-naproxen and *S*-(+)-flunoxaprofen are the levorotatory and not the dextrorotatory derivatives (methanol as solvent), as concluded from mechanistic considerations and experiences on Curtius degradation. Conversely, *R*-enantiomer derivatives switched to dextrorotation. This inversion is not an unusual phenomenon: *S*-2-phenylpropionic acid (*S*-2-PPA) rotates polarized light into a positive direction, but the corresponding isocyanate and the amine are levorotatory (22). In contrast, the corresponding naphthyl compound, 1-(1-naphthyl)ethyl isocyanate, in which the second isocyclic ring is anellated in positions 5 and 6, is dextrorotatory in its *S*-form, while the corresponding amine is levorotatory.

For the isocyanates and the amines it can be assumed that the absolute configuration was retained while the direction of optical rotation changed, since substitution of $-COOH$ by $-NCO$ or $-NH_2$ does not change the assigned priorities of the four substituents at the chiral carbon and since Curtius rearrangement is known to go along with configurational retention (23). Thus, data available from related compounds are indicative for an inverse optical rotation yet with configurational retention.

Analytical studies with the amines clearly demonstrate their wide applicability and good product resolvability, which may be superior to that of other coupling components. We currrently apply these reagents for the analysis of, e.g., ibuprofen on RP-columns, the chiral acid components of the anticholinergic ciclotropium (B. Liebmann, S. Mayer, E. Mutschler, and H. Spahn-Langguth, in preparation), and the lipid-regulating agent beclobrate in biological materials (20).

The amines were found to be applicable to numerous substrates including carboxylic acid metabolites. However, the amine is only one of the reagents that can be synthesized from a (fluorescent chiral) carboxylic acid.

Isothiocyanates can be synthesized from the corresponding amines, obtained via Curtius degradation or alternatively via Lossen or Schmidt degradation, by reaction

with carbon disulfide or *N,N'*-thiocarbonyldiimidazole. Hence, the amines can serve as starting materials for additional chiral reagents. The possible synthesis routes for other derivatives (e.g., the isothiocyanates or side chain-extended derivatives via, e.g., Arndt-Eistert synthesis) are currently under investigation. The complete reagent cascade then provides a variety of reagents with different substrate selectivities.

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