Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives

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Abstract

Chiral derivatization with an enantiomerically pure reagent is an economical and effective way to separate the enantiomers of optically active drugs on achiral HPLC columns. This paper describes the preparation and analytical testing of two novel chiral derivatizing agents (CDAs) deriving from the 2-arylpropionic acid (S)-(+)-naproxen, viz., 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate (NAP-IT) and 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate (NAP-C). Both are suitable reagents for the derivatization of amino compounds (e.g., β-adrenoceptor antagonists, antiarrhythmic agents). The diastereomeric derivatives of these chiral drugs are resolved on reversed-phase HPLC columns and/or silica gel stationary phases. The high UV absorbance and an additional intrinsic fluorescence of both CDAs facilitates the detection of the derivatization products.

Keywords: Derivatization, LC; Enantiomer separation; 1-(6-Methoxy-2-naphthyl)ethyl isothiocyanate; 2-(6-Methoxy-2-naphthyl)-1-propyl chloroformate; Naproxen; β-Adrenoceptor antagonists

1. Introduction

Among the non-steroidal anti-inflammatory 2-arylpropionic acids (2-APAs), with ibuprofen being a well-known representative, several compounds are used as the pure eutomer (= the more active enantiomer). In this group of non-steroidal anti-inflammatory drugs (NSAIDs) the (S)-(+)-enantiomer has a significantly higher affinity, e.g., to the prostaglandin synthetase with in vitro eudismic ratios much higher than 1 [1].

Several of these chiral compounds have expressed and characteristic chromophoric prop-

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erties, e.g., strong UV absorbance and/or fluorescence. In previous publications [2–4] we proposed the use of activated 2-APA enantiomers as chiral derivatizing agents (CDAs). In this group, naproxen has a molar absorptivity (ε) higher than 100 000, owing to the methoxy-substituted naphthyl residue, and is hence outstanding. Naproxen is commercially available as the pure (S)-(+)-enantiomer and may therefore be used as the starting material for various CDAs.

As an easily accessible reactive electrophile, the acid chloride of naproxen (NAP-Cl) was synthesized via different routes [3,5], of which those with milder conditions (via oxalyl chloride) are preferable since they lead to CDAs of higher chemical purity. The acid chloride is suitable for the derivatization of alcohols and amines.

In order to have more selective CDAs available, isocyanates were synthesized which led to higher derivatization yields for substrates with sterically hindered reactive groups [6]. In addition, the respective amines were prepared from the isocyanate via Curtius degradation and may be used as coupling components for chiral carboxylic acids [7,8]. The naproxen-derived isocyanate and amine are structurally closely related to commercially available reagents: 1'-(naphthyl)ethyl isocyanate (NEIC) and 1'-(1-naphthyl)ethylamine (NEA) [9,10]. The additional methoxy substitution in the 6-position of the naphthyl residue in the case of naproxen is known to increase the fluorescence intensity [11].

In general, naproxen-derived CDAs have been found to be suitable for the development of bioanalytical methods [3,5]. However, the introduction of reactive groups which are more selective towards amino compounds or, with respect to derivatizations of compounds in biological matrices, can be applied in aqueous media (e.g., the isothiocyano and the chlorocarbonate residue) are also feasible and may be of considerable interest. Widely used and commercially already available reagents in these respects are 2,3,4,6,-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) [12] and 1-(9-fluorenyl)ethyl chloroformate (FLEC) [13].

The aims of the present studies, with naproxen as chiral coupling component, were the definition of synthetic pathways to introduce the isothiocyano and the chlorocarbonate residues as reactive groups into the naproxen side-chain and the evaluation of the chromatographic behaviour of the thiourea and carbamate derivatives of selected substrates.

2. Experimental

2.1. Apparatus

Melting points were obtained using a Büchi (Flawil, Switzerland) apparatus and are uncorrected. Specific rotations were obtained from a Perkin-Elmer (Überlingen, Germany) Model 241 refractometer in a microcuvette. Dichloromethane served as the solvent. ^{1}H NMR spectra were recorded on a Bruker (Karlsruhe, Germany) AC 300 spectrometer. Samples were prepared by dissolving the compounds in deuterated chloroform. Tetramethylsilane (TMS) served as an internal standard. Mass spectra were recorded on a Finnigan MAT Model 212 instrument (Finnigan, Bremen, Germany). Infrared spectra were obtained with a Perkin-Elmer Model 1420 photometer using KBr pellets.

The chromatographic system consisted of a Jasco (Gross-Zimmern, Germany) PU-980 HPLC pump, a Jasco UV-975 UV–Vis detector, a Shimadzu (Düsseldorf, Germany) RF 551 fluorescence HPLC monitor, a Rheodyne (Cotati, CA, USA) Model 7125 Injection Valve with a 100-μl loop and a Knauer (Berlin, Germany) TY recorder. UV and fluorescence spectra of the diastereomeric derivatives were recorded after chromatographic separation in the stopped-flow mode using the respective HPLC detectors.

2.2. Chemicals and solvents

All reagents were of analytical-reagent or HPLC grade (when used to prepare HPLC mobile phases) and obtained from Merck (Darmstadt, Germany) unless stated otherwise. Water

was obtained from a Milli-Q water purification system (Millipore, Eschborn, Germany). (S)-Naproxen [enantiomeric excess (e.e.) = 98.8%] was kindly provided by Grünenthal (Stolberg, Germany). 1,1-Thiocarbonyldiimidazole (>97%) and phosgene solution (20% in anhydrous toluene) were purchased from Fluka (Neu-Ulm, Germany). Triethylamine (>99%, gold label) and triphosgene (98%) were obtained from Aldrich (Steinheim, Germany) and trans-4-hydroxyl-L-proline from Sigma (Munich, Germany).

Reference samples of the various drugs were supplied as follows: flecainide acetate and its enantiomers, Kettelhack Riker (Borken, Germany); propafenone hydrochloride and its enantiomers, Knoll (Ludwigshafen, Germany); tocainide hydrochloride, metoprolol tartrate and alprenolol hydrochloride and their enantiomers, Astra Chemicals (Wedel, Germany); mexiletine hydrochloride and its enantiomers, Boehringer Ingelheim (Ingelheim, Germany); acebutolol hydrochloride and diacetolol hydrochloride, Röhm Pharma (Weiterstadt, Germany); propranolol hydrochloride and its enantiomers, ICI (Plankstadt, Germany); and oxprenolol and its enantiomers, Ciba-Geigy (Wehr, Germany).

2.3. Syntheses

Structures of the compounds involved are shown in Fig. 1.

Preparation of 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate (NAP-IT)

The amine deriving from naproxen (NAP-A) was prepared as described previously [7]. 1,1-Thiocarbonyldiimidazole (1 mmol) was dissolved in 15 ml of ice-cold chloroform (ca. 0°C) and NAP-A (1 mmol) dissolved in 10 ml of chloroform was added dropwise. The mixture was stirred at ambient temperature for 1.5 h. The solvent was subsequently evaporated and the residue was reconstituted in carbon tetrachloride. Precipitated imidazole was filtered off and discarded. After evaporating the solvent, a yellow oil resulted, which was stored in a desiccator.

The crude product was purified on a short silica gel column with methylene chloride–light petroleum (50:50, v/v) as the mobile phase, yielding a slightly yellowish liquid that was stored in a closely tightened vial under argon, preferably at low temperatures (total yield: 0.55 mmol of NAP-IT).
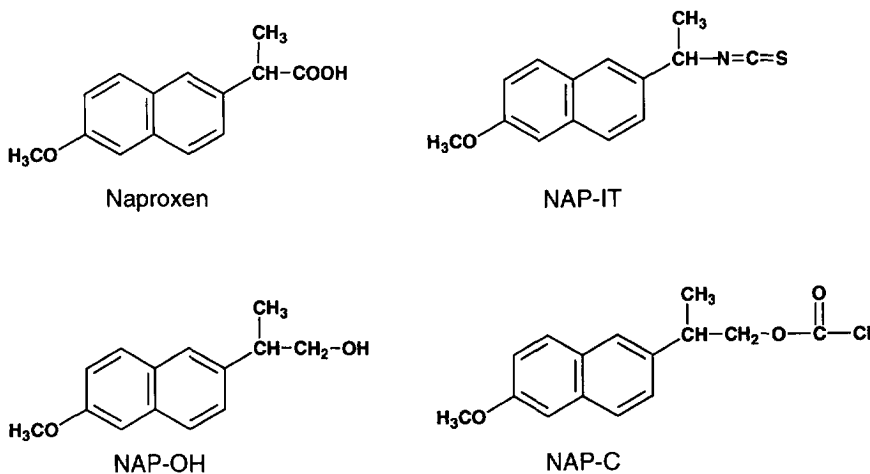
Physico-chemical characteristics: $[\alpha]_D^{20} =$

Fig. 1. Structures of the 2-arylproprionic acid naproxen and the isothiocyanate (NAP-IT), the alcohol (NAP-OH) and the chloroformate (NAP-C) derived from naproxen.

+2.948° (CH_2Cl_2, $c = 1$). ^1H NMR, δ (ppm) 1.69–1.72 (d, 3H, CH–CH_3), 3.90 (s, 3H, H_3C-aryl), 4.97–5.01 (q, 1H, CH(CH$_3$)N=C=S), 7.11–7.75 (m, 6H, aromatic protons); mass spectrum, m/z 243 (M$^+$, 37.3%), 185 (M$^+$ – N=C=S, 100%); IR, 2083 cm^{-1} (N=C=S).

Preparation of 2-(6-methoxy-2-naphthyl) propanol (NAP-OH)

(S)-Naproxen (2 mmol) was dissolved in 20 ml of anhydrous tetrahydrofuran and slowly added to a solution of 1.5 mmol lithium aluminium hydride in tetrahydrofuran. The mixture was refluxed for 1 h. Most of the solvent was removed before adding water. The reaction mixture was acidified with dilute (ca. 6 M) hydrochloric acid and the product was extracted three times with diethyl ether. The combined organic phases were dried over sodium sulfate and subsequently evaporated to dryness, yielding a white crystalline powder. By-products were removed via flash chromatography with silica gel as stationary phase and methylene chloride–methanol (50:1, v/v) as mobile phase. The solvent was evaporated to dryness and the product was dried in vacuo over potassium hydroxide, yielding 1.26 mmol.

Physico-chemical characteristics: m.p. 92–93°C; $[\alpha]_D^{20} = -18.723°$ (CH_2Cl_2, $c = 1$); ^1H NMR, δ (ppm) 1.33–1.36 (d, 3H, CH–CH_3), 1.57 (s, 1H, OH), 3.02–3.11 (m, 1H, CH(CH$_3$CH$_2$), 3.75–3.79 (t, 2H, CH_2–OH), 3.91 (s, 3H, H_3CO-aryl), 7.11–7.73 (m, 6H, aromatic protons); mass spectrum, m/z 216 (M$^+$, 27%), 185 (M$^+$ – CH$_2$OH, 100%); IR, 3360 cm^{-1} (OH).

Preparation of 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate (NAP-C)

All synthetic steps were carried out in a well ventilated hood. Phosgene-contaminated vapours were collected in a trap cooled with liquid nitrogen and carefully detoxificated by adding diluted methanolic sodium hydroxide prior to disposal.

A solution of the NAP-OH (0.5 mmol) in 10 ml of dry toluene containing triethylamine (0.5 mmol) was placed in a tapered flask closed with a silicone-rubber septum. The solution was

cooled and kept at 0°C. A 1-ml volume (ca. 2 mmol) of a phosgene solution (20% in dry toluene) (Fluka) was added with a syringe under constant stirring. The reaction mixture was agitated with a magnetic stirrer for an additional 4 h. Precipitated salts were filtered off and discarded. The filtrate was concentrated in vacuo and the resultant white powder was dried in vacuo, yielding 0.45 mmol of the crude product.

Physico-chemical characteristics: m.p. 60°C; $[\alpha]_D^{20} = -29.116°$ (CH_2Cl_2, $c = 1$); ^1H NMR, δ (ppm) 1.40–1.42 (d, 3H, CH–CH_3), 3.24–3.36 (m, 1H, CH–(CH$_3$)CH$_2$), 3.89 (s, 3H, H_3CO), 4.34–4.50 (m, 2H, CH$_2$), 7.11–7.79 (m, 6H, aromatic protons); mass spectrum, m/z 278 (M$^+$, 22%), 198 (35%), 185 (M$^+$ – CH$_2$COCl, 100%); IR, 1755 cm^{-1} (C=O).

The reagent was stored at ambient temperature under anhydrous conditions (phosphorus pentoxide, in vacuo). Derivatization of NAB-OH with triphosgene (a solid phosgene substitute) also led to the chloroformate in a high yield, but containing ca. 10% of the underivatized alcohol (estimated from ^1H NMR spectra).

2.3. Determination of the enantiomeric purity

The enantiomeric purity of the reagents was determined indirectly after derivatization with propranolol enantiomers of known enantiomeric composition. Purities were defined as enantiomeric excess. The values were corrected following the method of Hermansson and von Bahr [14]. The enantiomeric purity of propranolol enantiomers was determined on a Chiralcel OD (Daicel, Düsseldorf, Germany) chiral stationary phase using n-hexane–ethanol–diethylamine (100:10:1, v/v/v) as eluent (flow-rate 1.2 ml/min). The retention times of the enantiomers were 7.4 min [(R)-propranolol] and 9.8 min [(S)-propranolol]. The obtained e.e. values for propranolol were 99.8% for S and 99.5% for R.

2.4. Derivatization procedures

Derivatizations with NAP-IT

Stock solutions of the various substrates (1 mM) were prepared in methanol. Aliquots of

these solutions (10 μl) were pipetted into a screw-capped tube, evaporated to dryness under a stream of nitrogen, and the residues were dissolved in 300 μl of methylene chloride. A 10-μl volume of NAP-IT (20 mM) in anhydrous methylene chloride and 50 μl of triethylamine (0.1% in methylene chloride) were added. The solution was thoroughly vortex mixed and kept at 50°C for 1.5 h. An aliquot of the reaction mixture was injected directly on to the column.

Derivatizations with NAP-C

Aliquots (20 μl) of the stock solutions of the compounds (1 mM in methanol or water) were pipetted into a screw-capped tube and diluted with 50 μl of borate buffer (pH 8) (Merck). NAP-C solution (50 μl of a 18 mM solution in acetone) was added and the solution was vortex mixed. The mixture was kept at ambient temperature for 0.5 h. The reaction was stopped by addition of 100 μl of trans-4-hydroxy-L-proline (10 mM) in water. After 2 min an aliquot of the solution was injected directly on to the column for reversed-phase analysis. Injection on to normal phase columns required an additional extraction step: 2 ml of methylene chloride were added and the solution was vortex mixed for 30 s. The aqueous phase was discarded and the organic solvent was evaporated to dryness in vacuo. The residue was reconstituted in 100 μl of the mobile phase and an aliquot injected on to the HPLC column.

2.5. Chromatography

The HPLC separation of the diastereomeric products was performed using either a reversed-phase system or a silica gel stationary phase. Reversed-phase mode: column, 250 × 4.0 mm I.D.; packing material, Zorbax ODS, 5 μm (Bischoff, Leonberg, Germany); mobile phase, acetonitrile–water with various compositions (see Tables 1 and 2). Normal-phase mode: column, 250 × 4 mm I.D.; packing material, Zorbax-SIL, 5 μm (Bischoff); mobile phase, n-hexane–2-propanol with various compositions. Further details are given in Tables 1 and 2. In order to screen the chromatographic properties of the deriva-

tives, either UV detection (230 nm) or fluorescence detection (excitation at 270 nm, emission at 365 nm) was employed.

2.6. Derivatization kinetics

Time course of NAP-IT derivatization

A solution of racemic alprenolol (100 μl, equivalent to 200 ng) was pipetted into a glass tube and evaporated to dryness and treated in the same way as described above. Reaction was quenched adding 10 μl of glacial acetic acid after given periods of time. Solvents were removed under a stream of nitrogen. The residues were reconstituted in mobile phase and aliquots were injected into the reversed-phase HPLC system. UV absorption was monitored with the detection wavelength set at 230 nm.

Time course of NAP-C derivatization

A solution of racemic metoprolol (100 μl, equivalent to 200 ng) was derivatized in the described manner. Reaction was stopped after given periods of time by adding 10 μl of glacial acetic acid instead of trans-4-hydroxy-L-proline. The reaction products were extracted into 2 ml of methylene chloride. Aliquots of the organic phase were subjected to direct HPLC analysis on a normal-phase column with the detection wavelength set at 230 nm.

2.7. Derivatization yield

Racemic propranolol was used to determine derivatization yields with the respective reagent. The reaction mixture was subject to reversed-phase HPLC analysis with acetonitrile–phosphoric acid (0.2%) as the mobile phase. The peak of underivatized propranolol was monitored with wavelengths set to 240 nm (excitation) and 350 nm (emission).

2.8. Linearity, precision and limit of detection

Derivatization with NAP-IT

Racemic alprenolol (2, 4, 10, 20, 40, 80, 120, 160 and 200 ng) was derivatized as described previously. A 30 ng amount of (S)-(−)-proprano-

lol served as internal standard. The derivatives were separated on a reversed-phase column (mobile phase: acetonitrile–water, 70:30, v/v) and monitored with a UV detector at 230 nm. Calibration graphs were calculated on the basis of peak-height ratios of alprenolol enantiomers versus internal standard employing linear regression analysis. Relative standard deviations (R.S.D.s) were determined at concentrations of 1, 10 and 100 ng/ml ($n = 6$). The limit of detection was based on a signal-to-noise ratio of 3:1.

Derivatization with NAP-C

Working solution equivalents of 2, 4, 10, 20, 40, 80, 120, 160 and 200 ng of racemic metoprolol were evaporated to dryness and subsequently derivatized in the usual manner. (S)-(−)-Oxprenolol had been added as an internal standard. Derivatization products were separated on a normal-phase column after extraction and monitored with a fluorescence detector (excitation wavelength, 270 nm; emission wavelength, 365 nm). Calibration graphs were calculated on the basis of peak-height ratios of metoprolol enantiomers versus internal standard. The R.S.D.s were determined for concentrations of 1, 10 and 100 ng/ml ($n = 6$). The limit of detection was defined at a signal-to-noise ratio of 3:1.

3. Results

3.1. Syntheses

Figs. 2 and 3 summarize the synthetic routes for NAP-IT and NAP-C. NAP-A, the amine derived from naproxen, was the precursor for the synthesis of the isothiocyanate. It was prepared from (S)-naproxen via the acylazide. Curtius rearrangement led to the corresponding isocyanate (NAP-IC) and subsequent degradation to the amine. The e.e. value for the amine was 98.6% and hence in the same range as described earlier [7].

The simplicity of both synthetic pathways allows the preparation of chiral derivatizing agents also in an analytically oriented laboratory.
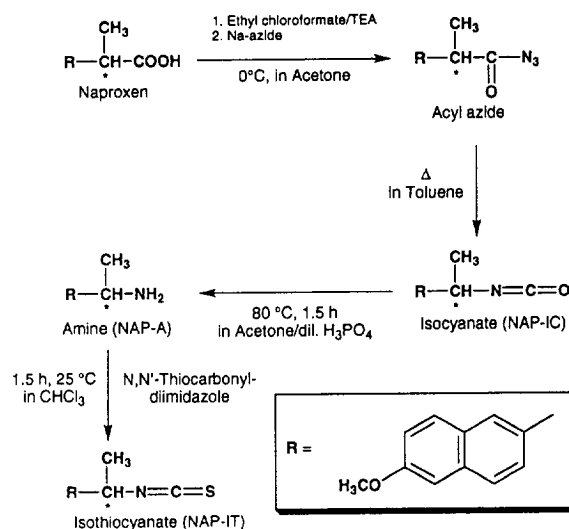

Fig. 2. Synthetic pathway describing the preparation of the isocyanate (NAP-IC), the amine (NAP-A) and the isothiocyanate (NAP-IT) derived from naproxen.

Almost all of the synthetic steps are applicable to micro-scale preparations. The reaction conditions are relatively mild. Thus, no racemization was observed for any of the reagents during the various synthetic steps in the course of these studies.

3.2. Enantiomeric purity of the reagents

The quantitative analysis of enantiomeric mixtures by chiral derivatization and subsequent chromatographic separation of the diastereomeric products requires reagents of high enantiomeric purity. The optical purity of both reagents,
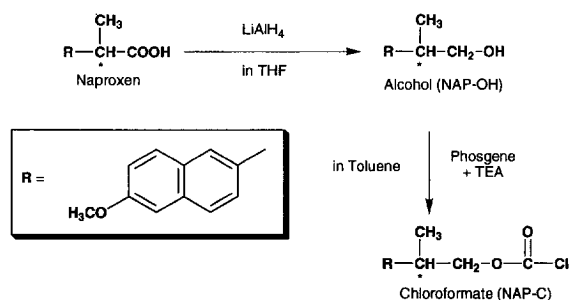

Fig. 3. Synthetic pathway leading to the chloroformate (NAP-C) derived from naproxen via the corresponding alcohol (NAP-OH).

NAP-IT and NAP-C, depends only on the quality of the precursor, naproxen. The therapeutically used compound [(S)-(+)-enantiomer] contains only traces of the (R)-enantiomer (e.e. >98.8%). Further clean-up can be easily achieved by crystallization steps or preparative chiral HPLC. Neither is usually necessary. High enantiomeric purities were found for both reagents with e.e. = 98.0% for NAP-IT and 98.7% for NAP-C.

3.3. Derivatization procedures

Derivatizations with isothiocyanates such as NAP-IT may be carried out in acetonitrile–water [15]. Since side-products interfered with the derivatives of interest in some cases, methylene chloride was chosen as the more favourable solvent. Chloroformate derivatizations were usually carried out with good results either in aqueous media (borate buffer, pH 8) or in toluene–0.1% triethylamine for shorter reaction times.

The optical purities of both reagents were calculated after derivatization with propranolol enantiomers of known enantiomeric purity, applying the described derivatization conditions. Since the e.e. values of NAP-IT and NAP-C did not differ much from that of their synthetic precursor (S)-(+)-naproxen, the chosen reaction and chromatographic conditions proved to be free from detectable racemization.

3.4. Chromophoric properties of the derivatives

Optimum detection wavelengths for the derivatization products of both reagents were determined after HPLC separation. The carbamates derived from NAP-C, and also the respective thiourea derived from NAP-IT, exhibit an intense UV absorption at 230 nm. Fluorescence monitoring was the more sensitive mode of detection for the chloroformate derivatives. The excitation wavelength was set to 270 nm and the emission wavelength to 365 nm, which was appropriate for most of the products [mobile phases: hexane–2-propanol (100:1–100:5, v/v)]. NAP-IT derivatives were monitored at 270/350

nm, respectively (mobile phases: acetonitrile–water with various compositions). In contrast to the chloroformate derivatives, the sensitivity was only slightly enhanced when measuring fluorescence intensity instead of UV absorption.

3.5. Chromatographic behaviour of the derivatives

Selected racemic amines—β-adrenoceptor antagonists and antiarrhythmic agents—were coupled with the isothiocyanate and the chloroformate derived from naproxen. HPLC separation parameters are given in Table 1 for NAP-IT and Table 2 for NAP-C, and in Fig. 4 typical chromatograms for both classes of diastereomeric derivatives are depicted.

3.6. Derivatization time course and yield

The derivatization time course may vary widely between substrates and solvents. Therefore, the reaction kinetics were determined for selected substrates under the conditions described (see Figs. 5 and 6). The chromatographic determination of underivatized propranolol led to the conclusion that the derivatization yield for both reagents was >98%, since no propranolol peak was detectable after derivatization.

3.7. Linearity, precision and limit of detection

The calibration graphs for both reagents and the respective substrates were linear over the evaluated range (1–100 ng/ml of enantiomer). The equations for alprenolol enantiomers after derivatization with NAP-IT were $y = 0.01493x + 0.00261$ with a correlation coefficient $r = 0.9988$ for (S)-(−)-alprenolol and $y = 0.01257x + 0.00370$ with $r = 0.9991$ for (R)-(+)-alprenolol and those for metoprolol enantiomers after derivatization with NAP-C were $y = 0.02296x + 0.01862$ with $r = 0.9989$ for (S)-(−)-metoprolol and $y = 0.02071x + 0.01603$ with $r = 0.9986$ for (R)-(+)-metoprolol (y = peak-height ratio; x = concentration in ng/ml). The calibration graphs are depicted in Figs. 7 and 8.

The R.S.D.s where usually below 10% for both

Table 1
Parameters for the HPLC separation of the diastereomeric products of β-adrenoceptor antagonists and antiarrhythmic agents after derivatization with NAP-IT on a reversed-phase column using different mobile phase compositions

Compound	Mobile phase[a]	k'_1 [b]	k'_2 [b]	α [b]	R [b]	First-eluting enantiomer
Atenolol	A	5.2	6.1	1.17	2.4	(S)-(−)
Diacetolol	A	6.7	8.0	1.19	2.9	(S)-(−)
Metoprolol	B	20.4	25.4	1.25	3.9	(S)-(−)
Carvedilol	C	7.1	8.0	1.13	4.2	(S)-(−)
Propranolol	D	8.7	10.7	1.23	3.9	(S)-(−)
Alprenolol	D	9.4	11.8	1.25	4.1	(S)-(−)
Propafenone	C	9.5	10.2	1.07	1.3	(R)-(−)
Flecainide	C	12.3	12.3	1.00	0.0	−
Tocainide	E	10.1	11.1	1.10	1.1	(S)-(+)

Column, 250 × 4 mm I.D.; packing material, Zorbax ODS, 5 μm (Bischoff).
[a] Mobile phase: acetonitrile–water (v/v): A, 50:50, 1.0 ml/min; B, 55:45, 1.0 ml/min; C: 70:30, 1.0 ml/min; D, 70:30, 0.8 ml/min; E, methanol–water 70:30 (v/v), 0.8 ml/min.
[b] k' = capacity factor; α = separation factor; R = resolution factor.

reagents and the three concentrations evaluated, except for (R)-(+)-alprenolol and NAP-IT at the quantification limit (<12%). Corresponding data are given in Table 3.

Limits of detection were 0.5 ng/ml for alprenolol enantiomers after derivatization with the isothiocyanate and 0.2 ng/ml for metoprolol after chloroformate coupling.

4. Discussion

Detectability is a very important factor in the analysis of xenobiotics from biogenic matrices. Therefore, the newly developed reagents for the derivatization of amino compounds, i.e., isothiocyanates and chloroformates, were examined for reagent-oriented detection. They have the 6-

Table 2
HPLC separation parameters of the diastereomeric products of β-adrenoceptor antagonists and antiarrhythmic agents after derivatization with NAP-C on a silica gel stationary phase using mobile phases consisting of n-hexane with 1–5% of 2-propanol

Compound	n-Hexane– 2-propanol (v/v)	k'_1	k'_2	α	R	First-eluting enantiomer
Alprenolol	100:1.0	4.6	5.1	1.10	1.3	(S)-(−)
Propranolol	100:1.0	7.4	8.1	1.09	1.8	(S)-(−)
Metoprolol	100:1.5	12.6	13.5	1.07	1.0	(S)-(−)
Acebutolol	100:5.0	20.4	21.9	1.07	<0.2	n.d.[a]
Tocainide	100:1.5	15.7	16.9	1.08	1.0	(S)-(+)
Fecainide	100:1.5	14.4	15.2	1.06	0.7	(R)-(−)
Mexiletine	100:0.25	22.5	23.3	1.04	0.2	(S)-(+)
Propafenone	100:1.5	14.5	15.4	1.06	0.9	(S)-(+)

Column, 250 × 4 mm I.D.; packing material, Zorbax-SIL, 5 μm (Bischoff). Mobile phase: n-hexane–2-propanol; flow-rate, 1.5 ml/min; t_0 = 1.5 min.
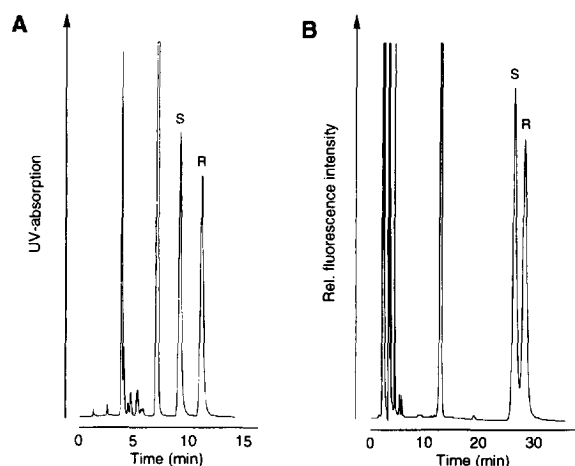[a] Not determined.

A

B

Fig. 4. (A) Reversed-phase chromatogram of the diastereo-meric thioureas obtained from rac-alprenolol after derivatiza-tion with NAP-IT [column, 250 × 4.0 mm I.D., Zorbax ODS, 5 μm; mobile phase, acetonitrile–water (75:25, v/v); flow-rate, 1.0 ml/min; detection, UV at 230 nm]. (B) Normal-phase chromatogram of the diastereomeric carbamates ob-tained from rac-metoprolol after derivatization with NAP-C [column, 250 × 4.0 mm I.D., Zorbax-Sil, 5 μm; mobile phase, n-hexane–2-propanol (100:1, v/v); flow-rate, 1.0 ml/min; detection, fluorescence with excitation at 270 nm and emis-sion at 365 nm].

methoxy-2-naphthyl moiety in common, which combines high UV absorption ($\varepsilon = 100\,000$ at $\lambda = 230$ nm for naproxen [16]) with an intrinsic

Fig. 6. Derivatization–time curve for metoprolol enantiomers after derivatization with NAP-C (solvent, borate buffer, pH 8; ambient temperature).

Fig. 7. Calibration graphs for alprenolol enantiomers after derivatization with NAP-IT [internal standard, (S)-(−)-pro-pranolol].
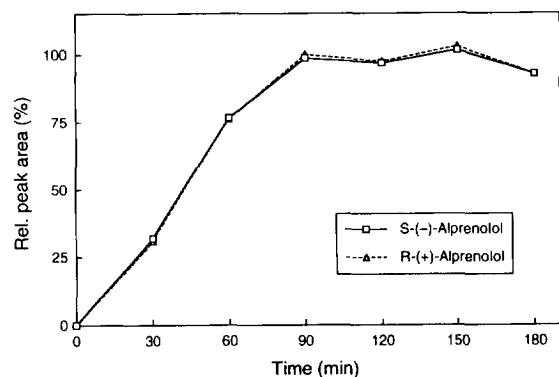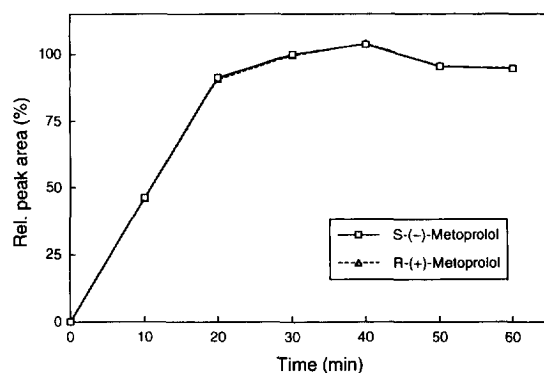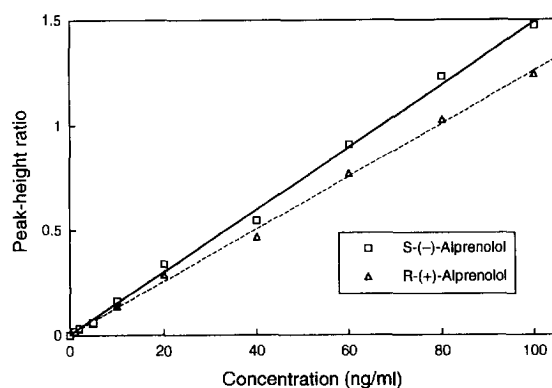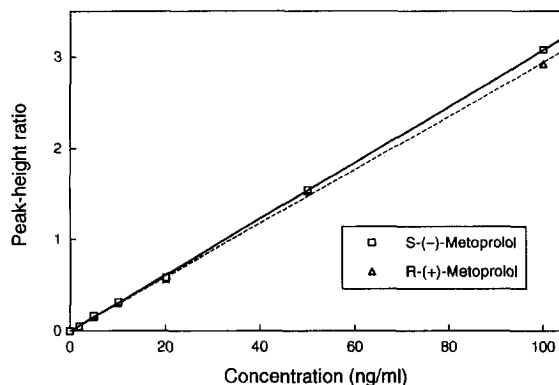

Fig. 5. Derivatization–time curve for alprenolol enantiomers after derivatization with NAP-IT (solvent, methylene chlo-ride containing 0.1% triethylamine; temperature, 0°(C).

Fig. 8. Calibration graphs for metoprolol enantiomers after derivatization with NAP-C [internal standard, (S)-(−)-ox-prenolol].

Table 3
Accuracy and precision for the derivatizations of alprenolol with NAP-IT ($n = 6$) and metoprolol with NAP-C ($n = 6$)

Concentration (ng enantiomer/ml)	NAP-IT				NAP-C			
	(S)-Alprenolol (ng/ml ± S.D.)	R.S.D. (%)	(R)-Alprenolol (ng/ml ± S.D.)	R.S.D. (%)	(S)-Metoprolol (ng/ml ± S.D.)	R.S.D. (%)	(R)-Metoprolol (ng/ml ± S.D.)	R.S.D. (%)
100	101.5 ± 5.01	5.0	99.3 ± 5.7	5.7	103.7 ± 6.1	5.9	100.9 ± 4.5	4.5
10	9.8 ± 0.63	6.4	9.7 ± 0.7	7.2	10.6 ± 0.4	3.7	10.2 ± 0.7	6.7
1	1.1 ± 0.11	9.7	0.95 ± 0.1	11.1	0.96 ± 0.008	8.5	0.93 ± 0.009	9.3

fluorescence, the intensity of which is significantly higher than for a simple naphthyl fluorophore [17]. Maximum UV absorption is at ca. 230 nm with a shoulder at 270 nm. However, the UV maximum cannot be used for fluorescence excitation with conventional variable-wavelength fluorescence monitors, owing to an increased noise level. The fluorescence intensity achieved with the chloroformate NAP-C and the respective carbamate derivatives was comparable to those known from other naproxen-derived CDAs. Compared with the fluorescence yields seen with the fluorenyl moiety in 1-(9-fluorenyl)ethyl chloroformate (FLEC) or benzoxazole derivatives, e.g., flunoxaprofen and benoxaprofen, the fluorescence of the 6-methoxy-2-naphthyl moiety is weaker, yet strong enough to enhance significantly the detectability of derivatized compounds.

The isothiocyanate NAP-IT and the corresponding thiourea derivatives may be of special interest with regard to UV detection since they represent the compounds with the highest UV absorption observed with naproxen derivatives. NAP-IT can be characterized as a reagent with a significantly higher absorption than, e.g., 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) [12], (4S)-cis-2,2-dimethyl-5-isothiocyanato-4-phenyl-1,3-dioxane (PHEDIT) [18], or phenylethyl isothiocyanate [19]. Absorption is at least in the same range as with the naphthyl-derived reagents SNEIT [(S)-1-(1-naphthyl)ethyl isocyanate)] and RBEIT [(R)-1-(2-naphthyl)ethyl isocyanate] [20]. Additionally, the reagent shows fluorophoric properties with an intensity weaker than expected from other related compounds, e.g., NAP-C.

In the present studies, β-adrenoceptor antagonists and antiarrhythmic agents were used as model compounds for drugs with a primary or secondary amino moiety. They are usually applied as racemic mixtures, although in many cases stereoselectivities in pharmacokinetics and/or pharmacodynamics have been observed. Therefore, selected β-adrenoceptor antagonists and antiarrhythmic agents were included in the present investigations as substrates for the derivatization with isothiocyanates and chloroformates. The thiourea derivatives of β-adrenoceptor antagonists and NAP-IT were usually well resolved on reversed-phase columns. Normal-phase resolution was poor and therefore not investigated in detail. An explanation may be that the sulfur atom is less susceptible to hydrogen bonding, and diastereoselective interactions with the stationary phase are consequently diminished. Compared with the urea derivatives of the isocyanate NAP-IC, thioureas are eluted much earlier by the same mobile phase under normal-phase conditions, confirming this assumption [21]. Reversed-phase separations of β-adrenoceptor antagonists after derivatization with NAP-IT were better than those reported in the literature [6] for the oxo analogue NAP-IC. The higher lipophilicity of the thiourea derivatives compared with urea derivatives and a higher affinity to the stationary phase may be an explanation.

However, these findings may not be generalized, since the quality of separation depends on the properties of both the reagent and the substrate. Separations of propafenone–NAP-IT derivatives were in the same range; other antiarrhythmic agents were slightly less resolved than

described for NAP-IC derivatives [6], while the resolutions of the thioureas derived from NAP-IT and antiarrhythmic agents were comparable to those obtained from the derivatives of RBEIT, (R)-1-(2-naphthyl)ethyl isocyanate [20].

The fact that no resolution was obtained from the derivatives of the chloroformate derived from (S)-(+)-naproxen (NAP-C) on reversed-phase systems and that under normal-phase conditions NAP-C derivatives were less resolved than the derivatives known from other CDAs derived from 2-arylpropionic acids may obviously be explained on the basis of the larger distance between the two chiral centres in the case of NAP-C derivatives [22,23]. In the carbamate derived from NAP-C, six bonds separate both stereocentres compared with five in the derivatives of NAP-IT.

The chloroformate NAP-C has to be compared with 1-(9-fluorenyl)ethyl chloroformate (FLEC). FLEC may be superior in the reversed-phase separation of propranolol and analogues [24]. However, equivalent normal-phase separations for FLEC have not been published so far.

Analytical methods have to be linear over a broad concentration range and reproducible, i.e., accurate and precise. With respect to chiral derivatization, linearity is a prerequisite for, e.g., the derivatization reaction itself, inevitable extraction steps and the detector response. Calibration graphs obtained from the derivatization with selected β-adrenoceptor antagonists after derivatization with NAP-IT and NAP-C were linear over the concentration range 1–100 ng/ml, i.e., concentrations usually observed after a single-dose intake of the respective drugs. Assay variabilities (intra- and inter-day variability) were in the ranges postulated for bioanalytical assays [25]. Limits of quantification (LOQ) were 1 ng/ml for both assays, thus allowing the sensitive determination of the respective compounds. Limits of detection (LOD) were both in the picomole range and, like the respective LODs, predominantly set by the interference of small amounts of by-products, and not meaning the absolute limit of the diastereomer that can be detected by dilution of solutions containing the product.

5. Conclusions

Characterization of the compounds obtained demonstrated that both synthesis routes are suitable for CDAs, i.e., they do not lead to detectable racemization.

Both reagents showed adequate reactivity under the described experimental conditions. No underivatized substrates were detectable when a 20-fold molar excess of reagent was used. Derivatization with the isothiocyanate led to diastereomeric thiourea derivatives, which were readily resolved on reversed stationary phases with separation factors that were in a comparable range to those of the respective urea derivatives or even better. In contrast, the resolution of the diastereomeric carbamates obtained on derivatization with the chloroformate is not sufficient, or even not detectable in some cases, on reversed-phase material. Under normal-phase conditions on a silica gel stationary phase separations were achieved. However, the resolution factors were evidently smaller than for, e.g., urea derivatives or amides of naproxen-derived reagents.

An obvious explanation for the decreased resolvability is the increased distance between the two chiral centres—of the reagent on the one hand and the substrate on the other. The newly developed naproxen-derived CDAs together with those described in earlier publications may, owing to the very high molar absorptivity, be useful in micellar electrokinetic capillary chromatography (MECC) with UV detection, where strong UV-absorbing reagents are needed and where chiral UV labels may be used in a similar way as in ordinary LC methods [26–29].

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