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Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines

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Abstract

The synthesis of the optical pure *trans*-1,2-diaminocyclohexane (DACH)-based N-3,5-dinitrobenzoyl-DACH-isothiocyanate (DDITC) is straightforward and the compounds, available in *R,R* and *S,S* configurations, can serve as highly selective, stable and optically pure chiral derivatizing agents (CDA) for the indirect resolution of chiral primary and secondary amines and in particular of amino alcohols. The synthetic route to obtain DDITC is described and RP-HPLC separations of a number of pharmaceutically important amino alcohols including β-blockers derivatized with DDITC are presented. The latter diastereomers were compared with the well established GITC-derivatized compounds by RP-HPLC. The separation factor (α) of the diastereomeric thioureas ranged between 1.05 and 2.00 and the peak resolution (R) ranged from 0.67 to 6.67 for the DDITC derivatives, and were usually higher than the values for the GITC derivatives. However, derivatization of secondary amines containing a tertiary butyl group at the amino function (e.g., timolol) is impossible using DDITC, a limitation not observed using GITC as CDA. The advantage of DDITC over other chiral isothiocyanates is the higher chromatographic selectivity and the high detection sensitivity, including the option of electrochemical detection due to the redox potential (−0.62 V) of the nitro group. UV detection of the DDITC-derivatized amino compounds shows excellent sensitivity because of the higher molar absorptivity (ε) deduced from the nitroaryl chromophor (e.g., DDITC derivative of metamphetamine, $\varepsilon_{242} = 26\,000$). The chemical stability of the DDITC derivatives is much higher than that of GITC derivatives owing to the absence of easily hydrolysable ester groups, which is considered a further advantage.

Keywords: Derivatization, LC; Enantiomer separation; Amino alcohols; Amines; Beta-blockers; Isothiocyanates

1. Introduction

The pharmacology, metabolism, toxicity, biochemistry and chiral resolution of chiral amino compounds such as amino alcohols, amino acids and simply primary and secondary amines are of intense interest in chemistry and the life sciences [1–3].

Various approaches for the chromatographic separation of enantiomeric amino compounds have been studied. In addition to GC, especially high-performance liquid chromatography (HPLC) has been applied. Two fundamentally different attempts have been established to re-

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solve chiral amines by HPLC: the indirect method [1] via covalent diastereomer formation using a chiral derivatizing agent (CDA) and the direct [4–7] separation mode. The indirect approach might suffer from extra burdens with respect to validation of the total analysis method. However, within the last 10 years a number of new CDAs have been developed for the resolution of racemic amines, amino acids and amino alcohols.

Inter alia, chemically non-specific CDAs such as tartaric acid anhydride [2], acid chlorides such as (*R*)- and (*S*)-4-(2-carboxypyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole (NBD-Pro-COCl) [8] and chloroformates such as (+)-1-(9-fluorenyl)ethyl chloroformate [(+)-FLEC] [9] have proved their usefulness. However, for amino compounds the chemically most selective CDAs are isothiocyanates such as 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) [10–21], (*R*)-α-methylbenzyl isothiocyanate (AMBI) [3], 2,3,4-tri-O-acetyl-α-D-arabinopyranosyl isothiocyanate (AITC) [15,18,19,22], 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosylisothiocyanate (BGIT) [16,23], 4-(3-isothiocyanato-2-pyrroldin-1-yl)-7-nitro-2,1,3-benzoxadiazole [1] (NBD)-PyNCS) and 4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole [1](DBD-PyNCS), leading to the corresponding diastereomeric thiourea derivatives. GITC has also been applied to the resolution of chiral oxiranes [13] and thiols [24].

In this paper we report the synthesis (Fig. 1) and application of a new *trans*-diaminocyclohexane (DACH)-based isothiocyanate, (*R,R*)- or (*S,S*)- N- 3,5- dinitrobenzoyl- DACH- isothiocyanate(DDITC). The reactivity of DDITC towards chiral primary and secondary amines and amino alcohols of the β-blocker type was particularly evaluated. Chromatographic separations of the resulting diastereomeric thioureas were investigated by RP-HPLC and compared with those of some GITC derivatives of the same β-blockers. Explanations for the unexpected high separation factors of the diastereomeric amino alcohol derivatives (thioureas) are presented based on preliminary spectroscopic investigations in conjunction with chromatographic experiments investigating structure variations of the analytes.

2. Experimental

2.1. Apparatus and chromatography

Melting points were obtained on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were measured on a Perkin–Elmer 1720-X FTIR instrument. ^{1}H NMR spectra

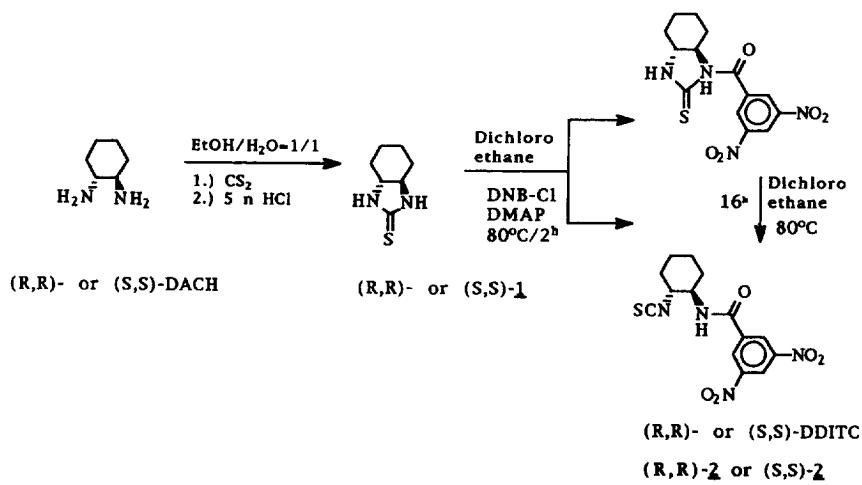


Fig. 1. Synthesis of (*R,R*)- and (*S,S*)-DDITC.

were recorded on a Varian XL 200 (200 MHz) spectrometer in $CDCl_3$ or DMSO-d_6 using TMS as internal standard. Optical rotation values were measured on a Perkin–Elmer Model 441 polarimeter at 23 ± 1°C. UV spectra were measured on a Perkin-Elmer Model 320 spectrophotometer. Polarographic measurements were performed with an EG&C PARC (Princeton, NJ, USA) Model 264 A instrument with a dropping mercury electrode as the working electrode, an Ag–AgCl reference electrode and a Pt auxiliary electrode. HPLC was performed on a Hewlett-Packard HP 1050 compact system with variable-wavelength detector and an ESA Coulochem II coulmetric detector. The stationary phases following and columns used were LiChrospher 60-RP Select B (125 × 4 mm I.D., 5 μm) and Hypersil ODS (125 × 4 mm I.D., 5 μm). The mobile phases were acetonitrile–20 m*M* ammonium acetate (45:55, 55:45 and 70:30) at a flow-rate of 1 ml/min.

2.2. Chemicals and reagents

All solvents were of HPLC grade and water for HPLC use was distilled and deionized using a Millipore Milli-Q Plus system. 3,5-Dinitrobenzoyl chloride and (*R,S*)-phenylethylamine were purchased from Aldrich (Steinheim, Germany) and carbon disulfide and GITC from Fluka (Buchs, Switzerland). The following compounds were generously donated: optically pure (*S,S*)- and (*R,R*)-DACH (Toray, Japan), (*R,S*)-propranolol (ICI, UK), (*R,S*)-atenolol (Schweizerhall, Switzerland), (*R,S*)- and (*R*)- and (*S*)-pindolol (Sandoz, Switzerland), (*R,S*)- and (*S*)-metoprolol and analogues and (*R,S*)-alprenolol (Haessle, Sweden), (*R,S*)-acebutolol (Bayer, Germany), (*R*)- and (*S*)-carvedilol (Boeringer, Mannheim, Germany), (*R,S*)-bupranolol (Bender, Austria), (*R,S*)-timolol (MSD, USA) and (*S*)-timolol (Leiras, Finland). (*R,S*)-Carazolol and (*R,S*)-nifenalol were extracted from pharmaceutical formulations. Optically pure (*R*)- and (*S*)-atenolol was prepared according a procedure published by Lindner et al. [2]. (*R,S*)-Nitriloatenolol was prepared as described recently [25]. (*R,S*)-Xamoterol, (*R,S*)-formoterol, (*R,S*)-metipranol, (*R,S*)-amphetamine and (*R,S*)-metamphetamine were available at the Institute of Pharmaceutical Chemistry, Karl Franzens University of Graz.

2.3. Preparation of the chiral derivatizing agent (CDA) (Fig. 1)

Synthesis of (1*R*,2*R*)- and (1*S*,2*S*)- N- [(2- isothiocyanato)cyclohexyl]- (3,5)- dinitrobenzoylamide [(1*R*,2*R*)- (DNB- DACH- ITC and (1*S*,2*S*)- DNB-DACH-ITC)-DDITC]

Cyclization of *trans*-1,2 diaminocyclohexane (DACH) with carbon disulfide, forming *trans*-4,5- tretramethyleneimidazolidine- 2- thione (**1**) was accomplished according to a method described by Davies and Mortlock [26]. Spectroscopic data, yields and melting points were almost identical with those given by Davies and Mortlock[26].

For the monoacylation of **1**, 6 g (38.4 m*M*) of **1** were dissolved in 120 ml dichloroethane in the presence of a catalytic amount of 4-(dimethylamino) pyridine and added to a suspension of 7.97 g (34.58 m*M*) of 3,5-dinitrobenzoylchloride in 30 ml of dichloroethane at 50°C over 15 min. During the reaction, all reactants dissolved and the solution was refluxed at 80°C for 2 h. A slightly yellowish crystalline solid (first crop) was filtered and washed and the mother liquor was evaporated to dryness. The residue was dissolved in 60 ml of dichloroethane and refluxed for another 16 h to decompose thermally (Fig. 1) the "closed" product to the open isothiocyanate **2**.

(R,R)-Enantiomer, ***(R,R)-2***

$Cl_{14}H_{14}N_4O_5S = 350.35$, yellowish crystals; m.p. >250°C; total yield of both fractions 6.9 g (51.2%); ^{1}H NMR in DMSO-d_6, δ(ppm) 1.15–2.29 (8H, m, cyclohexyl), 3.90 (1H, m *CH*–NCS), 4.10 (1H, m, *CH*–NH), 9.01 (1H, m, dinitrobenzoyl), 9.09 (1H, m, dinitrobenzoyl), 9.30 (1H, d,/NH); IR(KBr), 3254 (NH), 2953 (CH), 2055 (–NCS, strong), 1650 (–CO–), 1540 ($-NO_2$), 1343 cm^{-1} ($-NO_2$). $[\alpha]_{546} = -133°$ ($c = 1$) in acetonitrile; elemental analysis, calculated C 48,

H 4.03, N 15.99, found C 47.76, H 3.99, N 15.62%.

(S,S)-Enantiomer, ***(S,S)-2***

$C_{14}H_{14}N_4O_5S$ = 350.35; all physico-chemical properties except the specific rotation were identical with those of the *R,R* enantiomer; $[\alpha]_{546} = +135°$ ($c = 1$) in acetonitrile; elemental analysis, calculated C 48, H 4.03, N 15.99, found C 47.68, H 4.07, N 15.92%.

2.4. Preparative-scale derivatization of (R)- and (S)-propranolol with (R,R)-DDITC

Briefly, 200 mg (0.77 m*M*) *of optically pure* (*R*)- and (*S*)-propranolol and 240 mg (0.70 m*M*) of ***(R,R)-2*** were dissolved in 7 ml of acetonitrile and kept at 60°C for 1 h. The reaction solution was evaporated under reduced pressure and the residue dissolved in 15 ml of dichloromethane. The organic phase was acidified with 0.2 *M* HCl. The two-phase system was stirred intensively in order to extract the excess propranolol into the aqueous phase. After separation of the phases, the organic layer was washed with 15 ml of water, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified via flash chromatography [eluent toluene–acetone (3:1)].

(R,R,R)-Diastereomer, ***(R,R,R)-3***

$C_{30}H_{36}N_5O_7S$ = 610.31; yellow crystals; m.p. 168°C; yield 380 mg (91%); ^{1}H NMR in $CDCl_3$, δ (ppm), 1.02 (3H, d, $-CH_3$), 1.24 (3H, d, $-CH_3$), 1.31–1.55 (4H, m, cyclohexyl C-β), 1.69–2.39 (4H, m, cyclohexyl C-α), 3.55(2H, m,$-CH_2-$N), 3.71 (1H, s, –OH), 3.88 (1H, m,–C*H*–NH–CO–), 4.11 (2H, m, $-O-CH_2-$), 4.30 (1H, m, –C*H*–OH), 4.82 (1H, m, –C*H*–NH–CS–), 5.50) (1H, m, N–C*H*–), 6.73 (1H, d, arom.), 7.22–7.52 (4H, m, arom.), 7.77 (1H, m, arom.), 8.11 (1H, m, arom.), 8.62 (1H, d, –NH), 8.95 (1H, m, DNB-arom.), 9.05 (2H, m, DNB-arom.); IR (KBr), 3255 (–NH), 3074 (arom.), 2933 (–CH–), 1652 (–CO–), 1580 (–NH), 1539 ($-NO_2$), 1344 cm^{-1} ($-NO_2$); $[\alpha]_{546} = -202°$ ($c = 1.03$) in CH_2Cl_2.

(S,R,R)-Diastereomer, ***(R,R,R)-3***

$C_{30}H_{36}N_5O_7S$ = 610.31; yellow crystals; m.p. 172°C; yield 370 mg (89%); ^{1}H NMR in $CDCl_3$, δ (ppm) 1.17–1.23 [6H, m, $-CH(Me)_2$], 1.32–1.59 (4H, m, cyclohexyl C-β), 1.72–2.40 (4H, m, cyclohexyl C-α), 3.55 (2H, m, $-CH_2-$N), 3.65 (1H, s, –OH), 3.73 (1H, m, –C*H*–NH–CO–), 4.16 (2H, m, $-O-CH_2-$), 4.29 (1H, m, –CH–OH), 4.75 (1H, m, –C*H*–NH–CS–), 5.55 (1H, m, N–C*H*–), 6.77 (1H, d, arom.), 7.30–7.40 (4H, m, arom.), 7.72 (1H, d, arom.), 8.11 (1H, d, arom.), 8.55 (1H, d, –NH), 8.94 (1H, m, DNB-arom.), 8.97 (2H, m, DNB-arom.), 9.25 (1H, d, –NH); IR (KBr), 3265 (–NH), 3055 (arom.), 2927 (–CH–), 1650 (–CO–), 1579 (–NH), 1541 ($-NO_2$), 1343 cm^{-1} ($-NO_2$); $[\alpha]_{546} = -11.5°$ ($c = 1.07$) in CH_2Cl_2.

2.5. General derivatization procedure for primary and secondary amines and β-blockers (Fig. 2)

A 0.01 m*M* amount of the amino compound, in free base form or as hydrochloride, was dissolved in 0.5 ml of acetonitrile in a test-tube. If the compound was in hydrochloride form, 0.25 ml of 5% Na_2CO_3 was added. Then 0.5 ml of actonitrile containing 0.05 m*M* DDITC was added and the test-tube was tightly capped, vortex mixed for 1 min and stored in an oven at 60°C. After 120 min the reaction was stopped and the excess of CDA was quenched by the addition of 0.1 m*M* L-proline, then the reaction mixture was placed in the oven for another 30 min. A 100-μl aliquot was removed, diluted with mobile phase, neutralized with acetic acid and 10 μl of this mixture were injected directly on to the HPLC column.

2.6. Derivatization time-course study (Fig. 3a and b)

In brief, 5 mg of (*R,S*)-metoprolol was placed in a test-tube, then a ten-fold molar excess of chiral derivatizing agent was added. The mixture was dissolved in 2 ml of acetonitrile, vortex

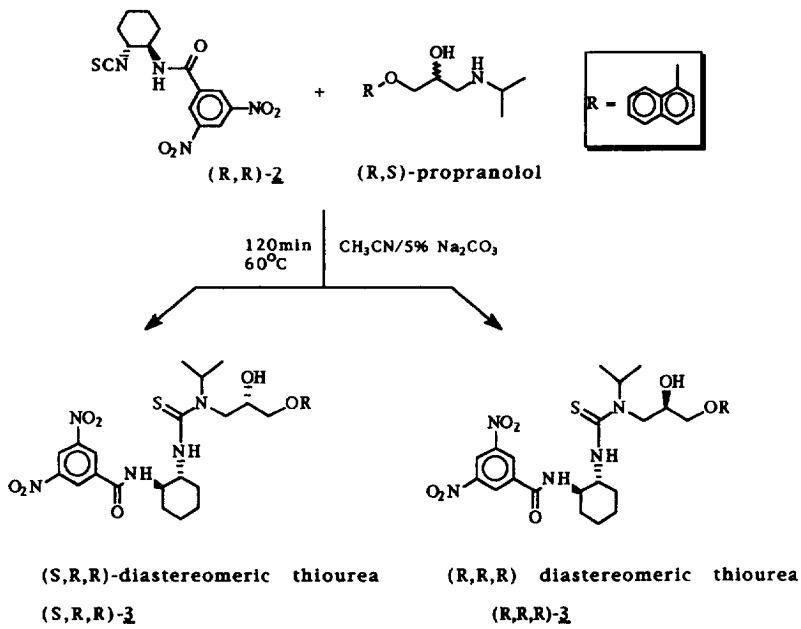


Fig. 2. Derivatization of (*R*,*S*)-propranolol with (*R*,*R*)-DDITC.

mixed for 30 s and placed in an oven at 60°C. At appropriate times, 5-μl aliquots were withdrawn, the reaction was stopped with a 200-fold excess of L-proline in 0.5 ml of mobile phase, then the mixture was placed in the oven for another 30 min, cooled to ambient temperature and injected directly on to the chromatographic column.

3. Results and discussion

3.1. Synthesis of the chiral derivatizing agent (see Fig. 1)

The syntheseis of (R,R)- or (*S*,*S*)-DDITC is straightforward. The cyclization of *trans*-DACH

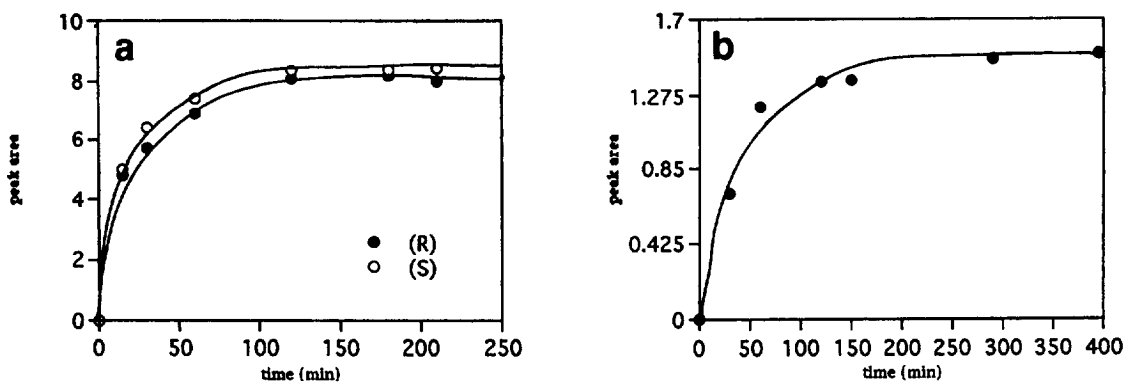


Fig. 3. Time-course study of (a) diastereomer formation of (*R*)- and (*S*)-metoprolol with (*R*,*R*)-DDITC and (b) (*R*,*S*)-normetoprolol derivatization with (*R*,*R*)-DDITC. For detailed chromatographic conditions, see Table 1.

with carbon disulfide forming the corresponding imidazolidinethione [26] is simple. The chemical yields described [26] ranged between 70% and 94%, which is in agreement with our results. The monoacylation of the imidazolidinethione with 3,5-dinitrobenzoyl chloride was performed in dichloroethane with the advantage that the desired isothiocyanate (DDITC) precipitated out of the solution while refluxing. This first crop was filtered. The mother liquor containing the monoacylated but not ring-opened imidazolidinethione (Fig. 1) was evaporated to dryness and thermally cracked simply by refluxing it in dichloroethane to yield the second crop of the desired isothiocyanate. The overall yields of the **(*R*,*R*)-2** and **(*S*,*S*)-2** enantiomers were 45% and 59% respectively.

To determine the enantiomeric purity of (*R*,*R*)- and (*S*,*S*)-DDITC, the reagent was derivatized with optically pure (*R*)-propranolol (ee > 99.7%) and the resulting diastereomeric thioureas were analysed by RP-HPLC. It was found that the optical purity of (*R*,*R*)- and (*S*,*S*)-DDITC (first and second crops) was greater than 99.7% ee. The chemical purity was determined by TLC [eluent toluene–acetone (3:1)] and HPLC and was also greater than 99%.

Another advantage of DDITC is the availability of both CDA antipodes, depending only on the starting material, (*R*,*R*)- or (*S*,*S*)-DACH. This feature should make possible inversion of the elution order of the diastereomeric thioureas of the chiral parent amines.

Referring to the chemically and stereochemically stability of the new chiral reagent, no degradation was observed after storage for 2 months at room temperature.

3.2. DDITC derivatization of amino alcohols and amines (see Fig. 2)

As for any other chiral isothiocyanate described in the literature [15,16], (*R*,*R*)- and (*S*,*S*)-DDITC react selectively with chiral primary and secondary amines and amino alcohols (e.g., β-blockers) to form the corresponding diastereomeric thioureas. These diastereomers were well separable by RP-HPLC. Summarized chromatographic data are given in Table 1.

The derivatization takes place under mild conditions (60°C) within 120 min. The reaction medium was acetonitrile for β-blockers in free base form and acetonitrile–5% aqueous sodium carbonate if the amine function was blocked via ion-pair formation, e.g., hydrochlorides. Pyridine, triethylamine and 5% aqueous Na_2CO_3 were tried as the base component. Na_2CO_3 gave the best results. The opportunity also to use aqueous derivatization media should be of particular interest in the bioanalysis of amines and amino alcohols. To avoid kinetic resolution (one enantiomer reacts faster than the other), it is recommended to use a large excess of CDA to force the reaction to completion. In some applications it was apparent, however, that the excess

Table 1
Resolution of (*R*,*R*)-DDITC-derivatized β-blockers and amines

Compound	Mobile phase	k'_1	k'_2	α[a]	R_s[b]
Xamoterol	A	1.13	2.27	2.00	3.39
Alprenolol	A	9.56	15.44	1.62	6.04
Oxprenolol	A	7.15	10.81	1.52	5.06
Metipranolol	A	7.62	11.89	1.50	5.04
Nifenanol	A	4.43	6.61	1.49	3.76
Formoterol	A	2.52	3.69	1.46	3.29
Carazolol	A	5.93	8.59	1.45	4.19
Metoprolol	A	5.33	7.83	1.47	6.67
Pindolol	A	3.91	5.59	1.43	3.80
Propranolol	A	8.48	12.17	1.44	6.67
Nitrilo atenolol	A	3.73	5.21	1.39	3.56
Atenolol	A	1.63	2.24	1.38	2.22
Acebutolol	A	3.10	4.20	1.35	2.91
Carvedilol	A	6.11	7.35	1.20	3.23
Propafenon[c]	C	5.07	5.98	1.18	2.01
Normetoprolol	B	4.47	4.69	1.05	0.67
Amphethamine	B	11.86	12.48	1.14	0.87
Metamphethamine	A	14.33	15.90	1.11	1.96

General HPLC conditions: column, Lichrospher 60 RP Select B (125 × 4 mm I.D., 5 μm); flow-rate, 1 ml/min; detection, UV at 254 nm; mobile phases, A = CH_3CN–NH_4Ac (55:45), B = CH_3CN–20 m*M*/NH_4Ac (45:55), C = CH_3CN–20 m*M* NH_4Ac (70:30), each with an apparent pH of 7.30.

[a] $\alpha = k'_2/k'_1$.

[b] $R_s = 1.18\{(t_2 - t_1)/[w_{2(1/2)} + w_{1(1/2)}]\}$.

[c] Column: Hypersil ODS (125 × 4 mm I.D., 5 μm).

reagent peak overlapped with a product peak. To avoid such problems, the CDA was chemically quenched by L-proline, forming a product with a short retention time due to the free acid function. Other quenching molecules tested, e.g., ammonia and 2-aminoethanol, showed a longer reaction time owing to their lower basicity.

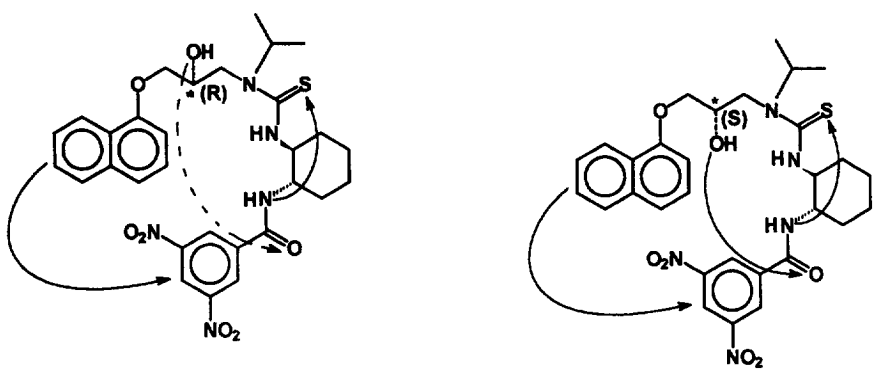
A time study using (*R*,*S*)-metoprolol (Fig. 3a) and (*R*,*S*)-normetoprolol (Fig. 3b) as analytes demonstrated that the derivatization reaction is complete within 100 and 120 min, respectively. In this context, it should be noted that the (*S*)-metoprolol derivative always gave peak areas greater than the (*R*)-metoprolol derivative. However, a characteristic feature of diastereomers is their different physico-chemical properties and thus the detected diastereomers may also show different UV molar absorptivities at 254 nm.

As an interesting effect was noticed in the derivatization of different β-blockers, namely that the reaction time decreased with increase in size of the alkyl group (electron-donor effect) at the derivatizable amino function, leading to the conclusion that the basicity of the amine plays an important role in this derivatization process [$-NH-CH(Me)_2 > -NH-Me > -NH_2$]. Astonishingly, it was impossible to derivatize amino alcohols with a secondary amine function and a tertiary butyl group [$-NH-C(Me)_3$] as substituent at the amino function (bupranol, penputolol, celiprolol, timolol, bunitrolol). This limitation was not observed using GITC as CDA. This phenomenon therefore cannot be interpreted simply as a steric hindrance effect at the amine site, but may be due to steric hindrance in combination with a strong intermolecular π–π interaction of the π-acidic 3,5-DNB group and the π-basic aromatic group of the β-blockers, leading to a molecule associate with a spatially overcrowded situation at the amino function. This steric hindrance may interfere with the isothiocyanate attack, thus making the derivatization reaction impossible.

Under the given derivatization conditions, no racemization of any chiral centre, either the CDA of the analyte, was observed.

3.3. Analyte structure–resolution relationship (Fig. 4)

This study is based on the spectroscopically (IR, NMR) verified structures of the reaction products (thioureas) of (*R*)- and (*S*)-propranolol with (*R*,*R*)-DDITC and further derivatization of the latter diastereomers with acetyl chloride to the corresponding esters. However, the spectroscopic data for the two diastereomers are not sufficient to establish a structure model. Therefore, additional spectroscopic data for structure elucidation are necessary. In this respect, the structures shown in Fig. 4 are hypothetical and



(R,R,R) - first eluted peak in RP- HPLC system

(S,R,R) - second eluted peak in RP- HPLC system

Fig. 4. Hypothetical structures and intramolecular interactions of (*R*,*R*)-DDITC-derivatized (*R*,*S*)-propranolol.

have to be seen as a basis for further investigations.

How can the high diastereoselectivity (α = 1.79) and resolution (R_s = 11.19) in RP-HPLC be explained, taking into account that there are actually five bonds between the chiral centre of the selector and the chiral centre of the β-blocker (see Fig. 4)? A reasonable explanation for the conformational rigidity could be that we have to consider additional intramolecular interactions which could well be hydrogen bonding of the non-derivatized secondary hydroxyl function stemming from the chiral amino alcohol and the carbonyl function of the DNB-amide. To verify this hypothesis, we tried to derivatize the hydroxyl group of the two diastereomers with acetyl chloride to form the corresponding acetyl esters. The result was that only the more polar *R,R,R* diastereomer, **(*R,R,R*)-3**, can easily be esterfied with acetyl chloride, whereas the more lipophilic *S,R,R* diastereomer, **(*S,R,R*)-3**, was significantly less reactive and it was extremely difficult to esterfy the hydroxyl group of this molecule. Further, the α-value of the non-acylated with respect to the acylated product dropped from 1.78 to 1.48. This implies that there exists a hydrogen bonding interaction of the hydroxyl function with the carbonyl group of the DNB-amide but preferentially only within **(S,R,R)-3**. Further evidence is the different solubilities of the diastereomers. The (*S,R,R*)-thiourea dissolves much better than the (*R,R,R*)-thourea in chloroform, which may be interpreted by the accessibility of the free hydroxyl function connected to the chiral centre of the analyte in the two different diastereomers (see also Fig. 4).

Comparing the chromatographic data (Table 1) for amino alcohols, the α-values of which ranged between 1.05 and 2.00 (in all cases there were five bonds between the stereogenic centres; see Fig. 4), and amines with α-values ranging from 1.10 and 1.14 (four bonds between the stereogenic centres), it also becomes evident that the hydroxyl moiety of the amino alcohols seems to be an important interaction site, not only intramolecularly but also intermolecularly with the stationary phase, causing this high diastereoselectivity and thus higher separation factors. Further, the resolution of the diastereomeric thioureas is possible on various kinds of RP stationary phases with different surface modifications. This has some effect, however, on the resolution values obtained (data not shown), but the effects are not very great.

3.4. Comparison of DDITC- and GITC-derived amino alcohols (see Table 2 and Fig. 5)

In Table 2, chromatographic data for (*R,S*)-metoprolol, (*R,S*)-normetoprolol and (*R,S*)-carvedilol derivatized with (*R,R*)-DDITC and GITC are shown. The α-values, which ranged from 1.07 to 1.47, and peak resolution values, ranging

Table 2
Resolution of (*R,R*)-DDITC- and GITC-derivatized (*R,S*)-amino alcohols

Racemic amino acid	Derivative	Conditions[a]	k'_1	k'_2	α[b]	R_s[b]
(*R,S*)-Metoprolol	DDITC	A	5.33	7.83	1.47	6.67
	GITC	A	3.81	4.62	1.21	2.58
(*R,S*)-Normetoprolol[c]	DDITC	B	4.47	4.69	1.07	1.00
	GITC	B	2.47	–	1.00	0.00
(*R,S*)-Carvedilol	DDITC	A	6.11	7.35	1.20	3.23
	GITC	A	5.41	5.98	1.11	1.05

[a] As in Table 1, with A = mobile phase A and B = mobile phase B.
[b] For definitions of α and R_s, see Table 1.
[c] Column: Hypersil ODS (125 × 4 mm I.D., 5 μm).

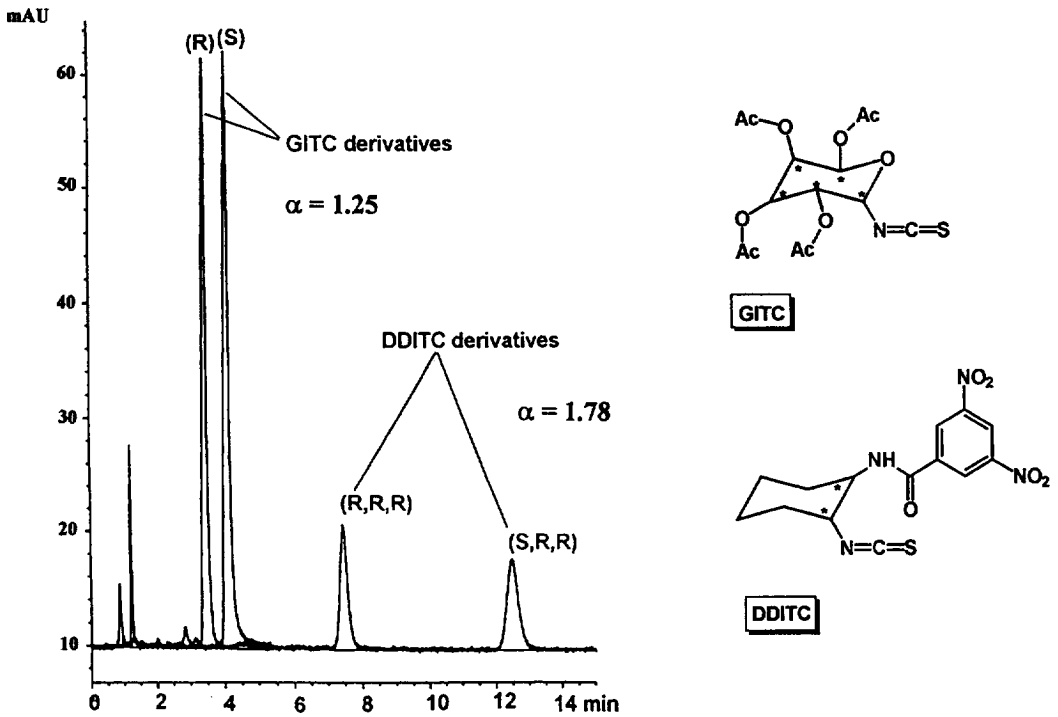


Fig. 5. Resolution of (*R,S*)-metoprolol derivatized as (*R,R*)-DDITC- and GITC-thioureas Column, Hypersil ODS (125 × 4 mm I.D., 5 μm); mobile phase, acetonitrile–20 m*M* ammonium acetate (55:45); flow-rate, 1 ml/min; UV detection at 254 nm.

between 1.00 and 6.67 for the DDITC derivatives, were usually higher than those for the GITC derivatives. Under the given chromatographic conditions for normetoprolol no diastereomeric separation of the GITC derivatives was observed, whereas the DDITC derivatives were well separated, probably owing to the additional carbonyl group of the DNB-amide, which can serve as an additional interaction site (hydrogen-bonding site) forming a more spatially fixed conformation also with respect to the chromatographic surface.

Another advantage of DDITC over GITC is the higher chemical stability, owing to the absence of easily hydrolysable acetyl groups. Further, the detection limits of the DDITC-derivatized compounds should be lower than those of the GITC derivatives for which the UV sensitivity depends only on the thiocarbonyl absorption, because of the additional DNB chromophore (see also below).

3.5. Detection

The two nitro groups of DDITC were prone to electrochemical reduction. On measuring the polarogram, the nitro groups showed electrochemical activity at a redox potential of −620 mV. The latter data were not transferable to HPLC with coulometric detection for several reasons. First, the background current was very high that signal was weaker, leading to a kind of "indirect" electrochemical detection with low sensitivity. Second, the diastereomeric thioureas seemed to have a very high affinity to the carbon electrodes, leading to severe peak tailing caused only by the large carbon surface area with this type of electrode.

Toyo'oka and Liu [1] reported the resolution of amino acids derivatized with a fluorescent chiral isothiocyanate containing a nitro function. DDITC-derived β-blockers did not show fluorescence even after hydrogenolysis. Unfortunately,

the native fluorescence of the β-blockers was also quenched, possibly due to π–π interactions of the aromatic parts of the CDA and the analyte. Therefore, the detection method of choice has to be UV spectrophotometry. The DDITC-derivatized amino compounds show excellent UV sensitivity owing to the strong chromophore and thus high molar absorptivity, e.g., $\varepsilon_{246} = 26\,500$, $\varepsilon_{230} = 55\,700$ for DDITC-derivatized propranolol and $\varepsilon_{210} = 40\,500$, $\varepsilon_{242} = 25\,600$ for DDITC-derivatized metamphetamine. The detection limits of DDITC-derivatized compounds are about average for UV detection, but should be significantly lower than those of GITC derivatives, for which the UV sensitivity depends only on the thiocarbonyl function, as mentioned above.

4. Conclusion

The described two-step synthesis protocol to obtain (*R,R*)- and (*S,S*)-DDITC [**(*R,R*)-2** and **(*S,S*)-2**] is very simple and straigtforward, leading to a stable CDA. This chemically selective chiral derivatizing agent is excellently suited for the indirect resolution of chiral primary and secondary amines and especially amino alcohols using RP-HPLC. Owing to the mild reaction conditions, no racemization of any chiral centre was observed. Compared with the well known GITC, DDITC has the advantage that it is readily available in both *R,R* and *S,S* configurations, the thioureas show higher diastereoselectivity and are chemically more stabe and there is the opportunity to aqueous derivatization media.

The chemical and chromatographic properties of DDITC-derived amino acids and chiral thiols [27] are currently the subject of further investigations.

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