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Efficient high-performance liquid chromatographic enantioseparation of five-membered aryl-substituted lactones and cyclic carbamates on a (*R,R*)-diaminodihydroethanoanthracene-derived chiral stationary phase

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Abstract

The synthesis and evaluation of a new bis-3,5-dinitrobenzoylated *trans*-(−)-(11*R*,12*R*)-(−)-diamino-9,10-dihydro-9,10-ethanoanthracene (DADEA)-based chiral stationary phase are described. In comparison to the well established structurally related diaminocylohexane (DACH)-derived CSP, this conformationally more rigid selector exhibits significantly improved enantiodiscrimination capabilities for five-membered cyclic esters and carbamates with an aromatic substituent at the carbon next to the ring oxygen. Differences in the enantiorecognition behaviour between the new selector and DACH and diphenylethanediamine-derived analogues are discussed. The chromatographic results obtained for oxazolidinones of important β-blockers, phthalides and glycine-derived oxazolidine-5-ones, potentially useful as chiral synthons, are reported and discussed.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Diaminodihydroethanoanthracene; Beta-blockers; Phthalides

1. Introduction

Chiral stationary phases (CSPs) based on N,N′-bis-3,5-dinitrobenzamides of C_2 symmetric diamines are efficient tools for high-performance liquid chromatographic (HPLC) enantioseparation of many important chiral compounds. The first phase of this type was derived from *trans*-diaminocyclohexane [1] (DACH CSP, Fig. 1), bonded via addition to epoxydized silica followed by exhaustive acylation with 3,5-dinitrobenzoylchloride. This mode of immobilization may be considered to be not completely unambiguous: (1) a secondary alcohol with a new center of chirality is created; (2) this group might not be completely acylated or chemically stable. However, this strategy of immobilization gives CSPs of highly reproducible quality [2]. Moreover, the new center of chirality has been shown to exhibit minor influence on enantioselectivity [3]. The DACH-derived CSP separates excellently the optical isomers of a broad range of sulfoxides [1], phosphine oxides [2], underivatized aryloxycarboxylic acids [4] and amino alcohols as oxazolidine-2-ones [5].

The corresponding 1,2-diphenylethane-1,2-diamine (DPEDA)-derived CSP (Fig. 1) resolves excellently the enantiomers of various amines (as

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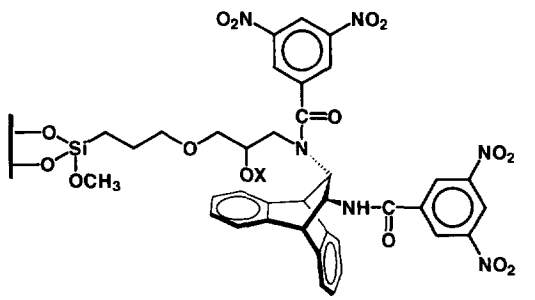
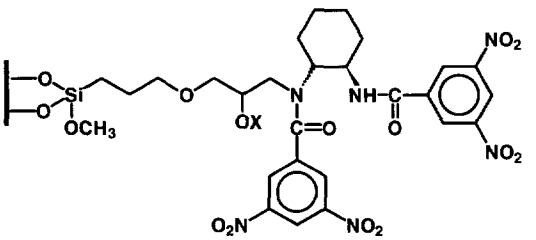




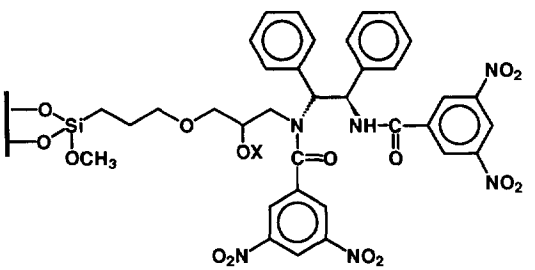


Fig. 1. Structures of CSPs derived from N,N′-*bis*-3,5-dinitrobenzoylated C_2-symmetrical diamines (X: 3,5-dinitrobenzoyl or H).

arylamides and ureas) and carboxylic acids (as arylamides) [6]. Surprisingly its enantiodiscriminating potential for the other mentioned types of analytes is modest. Attempts to expand this concept to selectors with axial chirality (e.g. 1,1′-binaphthyl-2,2′-diamine as backbone) were not encouraging (Uray and Maier, unpublished results).

In this contribution we describe preparation and scope [7] of a new *trans*-(11*R*,12*R*)-diamino-9,10-dihydro-9,10-ethanoanthracene (**1**, DADEA)-based CSP (Fig. 1). The DADEA framework contains a combination of the characteristic structural features of DACH and DPEDA. The new CSP derived from this conveniently accessible diamine exposes highly "substrate-specific" chiral recognition potential for important classes of aryl-substituted cyclic esters and carbamates. This will be demonstrated with β-blockers and a series of phthalides. Especially useful enantioselectivities were achieved for glycine-derived "Seebach type" oxazolidinones which are difficult to separate [8].

2. Experimental

2.1. Chemicals and reagents

Racemic DADEA was prepared via Curtius degradation of the corresponding dicarboxylic acid [9] according to the literature procedure for the (*S*,*S*) enantiomer [10]. 3-(2,3-Epoxypropyl)-propyltrimethoxysilane and (*S*)-mandelic acid were from Fluka (Buchs, Switzerland). Pyridine, ethanol, methanol, tetrahydrofuran (THF), toluene, diethyl ether, 3,5-dinitrobenzoyl (DNB) chloride, triethylamine, 4-toluenesulfonyl chloride, hexamethyldisilazane (all reagent grade) and LiChrosorb SI 100 (5 μm) were purchased from Merck (Darmstadt, Germany).

2.2. Apparatus

Melting points were taken on a Gallenkamp melting point apparatus and are uncorrected. Optical rotation values were observed in a 10-cm cell on a Perkin-Elmer polarimeter 441 at 23±2°C. Elemental analyses were obtained on a Carlo Erba 1106 CHN automat.

2.3. Chromatography

HPLC was performed on a Hewlett-Packard HP series 1050 liquid chromatograph using HP ChemStation as software. Mobile phases were mixed from *n*-heptane, dichloromethane and methanol of HPLC grade (Merck). All HPLC measurements were performed at 25°C column temperature.

2.4. Analytes

Oxazolidinones **2–14** were obtained by cyclization of the corresponding β-blockers with phosgen [5]. β-Blockers for **2, 3, 4, 8, 10, 12, 13, 14** and enriched samples were available from prior studies. Carazolol, carvedilol and propafenon used to prepare **5, 6** and **9** were generously donated by Professor W. Lindner (KFU Graz, Institute of Pharmaceutical Chemistry). Metoprolol (**7**) was from Astra (Sweden) and celiprolol (**11**) from Chemie Linz (Linz, Austria). Racemic and enantiomerically enriched phthalides **15–18** were available from prior studies. Oxazolidin-5-ones **19–28** were prepared from glycine, 1-/2-naphthaldehyde and the respective acyl chlorides analogous the procedure described for the corresponding pivalyl compounds [8]. All newly prepared compounds were fully characterized by m.p., IR and ^{1}H NMR spectroscopy and elemental analysis.

2.5. (R,R)-DADEA (1)

A 17.80-g amount (75.3 mmol) of racemic DADEA was dissolved in 145 ml of ethanol and a solution of 22.90 g (151.2 mmol) of (*S*)-mandelic acid in 145 ml of ethanol was added. The turbid mixture was boiled for 10 min, filtered and allowed to crystallize for 24 h at 4°C. The resultant precipitate was isolated by filtration and washed with ethanol (3×30 ml). After drying in vacuo 17.20 g of white, crystalline powder (d.e.=70%) was obtained. This material was dissolved in 700 ml refluxing ethanol and allowed to stand for 24 h at 4°C. Filtration, washing with ethanol (2×30 ml) and drying in vacuo afforded 11.40 g colorless crystals (d.e.=94%). This material was dissolved under reflux in 660 ml of ethanol. The resultant solution was concentrated to a volume of 400 ml and allowed to crystallize for 20 h at −10°C to yield 9.12 g (45%) of diastereomerically pure (*R,R*)-DADEA-*bis*-(*S*)-mandelic acid salt (d.e.>98%): m.p. 177°C; $[\alpha]_{546}$ +67.3, $[\alpha]_{436}$ +116.3 (c=1.10, methanol). $C_{32}H_{32}N_2O_6$ 540.61; 9.00 g of this salt was suspended in 180 ml 2 *M* NaOH and the mixture was magnetically stirred for 15 min. The diamine was extracted with dichloromethane (1×100, 3×50 ml) and the combined organic extracts were dried (K_2CO_3). Evaporation of the solvent under reduced pressure yielded 3.74 g (95%) (*R,R*)-(−)-DADEA as a colorless, crystalline powder: m.p. 153–156°C; $[\alpha]_{546}$ −24.4, $[\alpha]_{436}$ −56.6 (c=1.00, methanol); ^{1}H NMR and IR spectra were identical with those reported for the (*S,S*) enantiomer [10].

2.6. Check of optical purity of DADEA

About 50 mg of mandelic acid salt was suspended in 10 ml 2 *M* NaOH and magnetically stirred for 15 min. The mixture was extracted with dichloromethane (5×5 ml), the combined organic phases were dried (K_2CO_3) and the solvent evaporated under reduced pressure. The residue was treated with 2 ml of dry pyridine and 50 mg of 4-toluenesulfonyl chloride and allowed to stand for 3 h at room temperature. About 0.5 ml of water was added and the solution was stirred for 15 min. The mixture was diluted with dichloromethane (20 ml) and washed with 2 *M* HCl (40 ml) and water (20 ml) and dried ($MgSO_4$). After evaporation of the solvent the residue was analyzed on a (*R,R*)-DNB DACH CSP [1]. Conditions: column (250×4 mm I.D.); mobile phase: dichloromethane–methanol (99:1, v/v); flow-rate: 1 ml/min; UV detection: 254 nm; (*S,S*) enantiomer: k'_1=1.09, (*R,R*)-enantiomer: k'_2=1.28, α=1.17.

2.7. Epoxysilica

A 7.0-g amount of freshly distilled 3-(2,3-epoxypropyl)-propyltrimethoxysilane was added to a suspension of 20.0 g azeotropically dried LiChrosorb SI 100 (5 μm) in 400 ml of toluene. The mixture was refluxed with gentle mechanical stirring for 24 h under argon. Filtration, washing with toluene (8×50 ml) and diethylether (4×50 ml) and drying at 60°C in vacuo gave 22.00 g modified silica. Elemental analysis indicated 6.10% of carbon, corresponding to 724 μmol epoxide per gram.

2.8. CSP I (Fig. 2)

A mechanically stirred mixture of 3.00 g (*R,R*)-(−)-DADEA (12.7 mmol) and 6.00 g epoxysilica in 50 ml of ethanol (96%) was refluxed for 24 h. The modified silica was filtered, washed with hot ethanol (7×50 ml) and dried for 24 h at 60°C in vacuo to yield 6.15 g of aminosilica. Elemental analysis

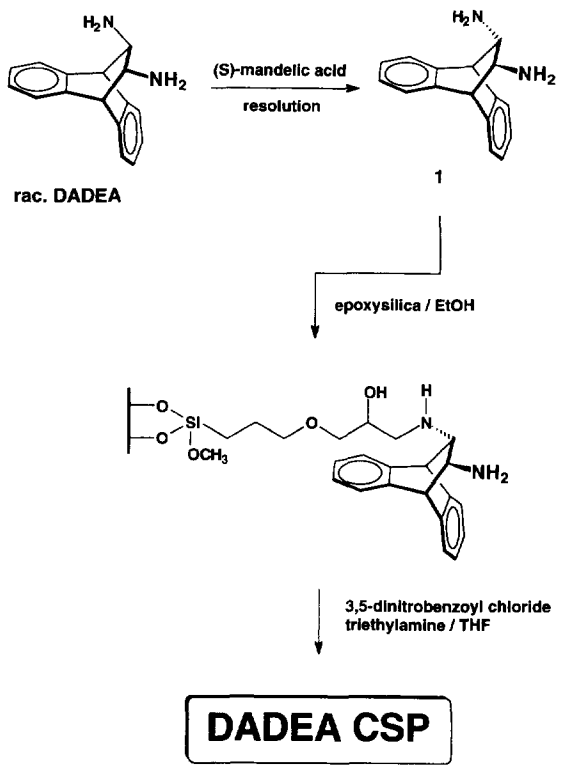


Fig. 2. Preparation of the new DADEA CSP.

indicated 0.67% of nitrogen, corresponding to 254 μmol diamine per gram of modified silica. A check of the optical purity of recovered diamine as its ditosylamide confirmed that no racemization had occurred during the loading procedure. For acylation of the immobilized diamine a 4.0-g portion of aminosilica, 2.0 g (19.8 mmol) freshly distilled triethylamine and 2.0 g (8.7 mmol) of DNB chloride were suspended in 40 ml dry THF and the resultant mixture was refluxed with mechanical stirring under argon for 3 h. The silica was isolated by filtration, washed with THF (2×50 ml), hot methanol (5×50 ml) and diethyl ether (3×30 ml) and dried for 24 h at 60°C in high vacuo. Elemental analysis of this material gave 2.01% nitrogen, confirming complete N-acylation of the immobilized diamine. This material was packed as methanol slurry in a 250×4 mm I.D. stainless-steel column. Subsequently the packed CSP was endcapped at 35°C by passing a solution of 7 ml hexamethyldisilazane in 100 ml dichloromethane through the dichloromethane-equilibrated column with a flow-rate of 1 ml/min.

3. Results and discussion

(*R*,*R*)-(−)-DADEA (**1**) was obtained from the racemate by fractional crystallization of its diastereomeric (*S*)-mandelic acid salts. The desired CSP was conveniently prepared by binding diamine **1** to epoxysilica followed by exhaustive N-acylation with DNB chloride. Elemental analysis of the modified silica revealed that 2.20 equivalents of DNB/mol immobilized diamine were bonded. As observed with analogous diamines, both N-acylation of the amino group and partial O-acylation of hydroxyl groups generated by epoxide opening must have occurred. After packing into the column, residual silanol groups were endcapped with hexamethylenedisilazane to reduce non-stereoselective interactions.

For evaluation of the new DADEA CSP a representative range of chiral analytes (sulfoxides, lactones, amines and carboxylic acids as amidic derivatives, amino alcohols as oxazolidinones etc.) were tested using various mobile phases. In comparison to structurally closely related DACH and DPEDA CSPs the new phase displays at identical experimental conditions a less broad scope of applicability: e.g., while for benzylphenylsulfoxide an α value of 1.82 was reported with the DACH CSP [1], at identical chromatographic conditions this value was 1.17 on the DPEDA and 1.22 on the DADEA-derived analogue. Similar results were found for other sulfoxides. The enantiomers of amidic derivatives of amines and carboxylic acid are resolved best on the DPEDA-derived CSP (e.g. α=2.8 for phenylethylamine as 1-naphthoylamide, while the DADEA phase separates the enantiomers of this analyte with α=1.4. This less broad applicability of the DADEA CSP can be rationalized by the fact that its framework is characterized by a high degree of conformational rigidity. These restrictions in conformational freedom of the selector lead to hindered accessibility and/or reduced flexibility of the functional groups involved in chiral recognition. However, recently, rigid selectors have been exploited with considerable success in the design of highly effective and "sub-

Table 1
Enantioseparation data for β-blocker-derived oxazolidine-2-ones, phthalides and glycine-derived oxazolidine-5-ones

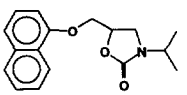
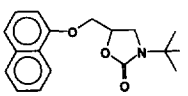
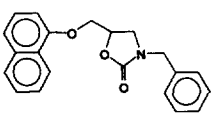
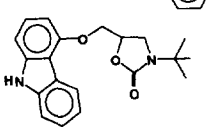
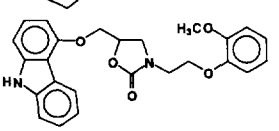
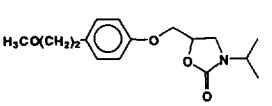
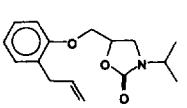
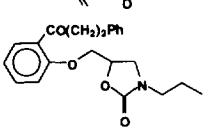
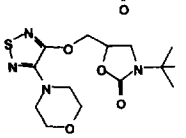
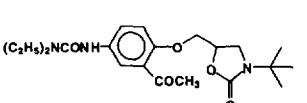
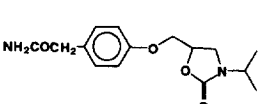
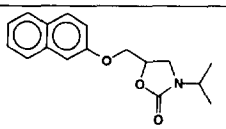
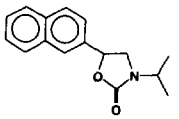
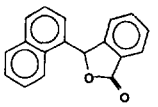
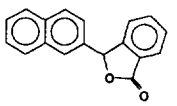
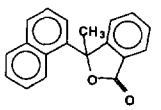
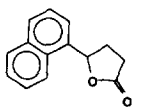
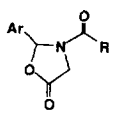
Entry	Compound	Mobile phase	k'_1	α	mre
	Oxazolidin-2-ones of β-blockers				
2		A	4.03	2.02	*S*
3		A	2.28	2.02	*S*
4		A	4.26	1.63	*S*
5		B	0.72	3.51	
6		B	0.56	2.83	
7		A	2.85	1.63	*S*
8		A	1.71	1.76	*S*
9		A	2.25	1.64	
10		C	0.86	1.48	*S*
11		D[a]	0.29	1.56	
12		D[a,b]	1.50	1.24	

Table 1 (Continued)

Entry	Compound	Mobile phase	k_1	α	mre
13		A	3.37	1.56	*S*
14		A	2.31	1.72	
15		E	1.77	3.76	*R*
16		E	2.14	1.08	
17		E	1.31	2.91	*R*
18		E	2.26	1.14	



	Ar	R			
19	1-Naphthyl	CH_3	E	3.05	1.56
20	1-Naphthyl	$(CH_2)_6CH_3$	E	0.90	2.62
21	1-Naphthyl	$C(CH_3)_3$	E	1.03	2.78
22	1-Naphthyl	Ph	E	1.80	2.23
23	2-Naphthyl	Ph	E	1.83	n.r.
24	1-Naphthyl	OC_2H_5	E	0.83	3.21
25	2-Naphthyl	OC_2H_5	E	0.89	1.14
26	1-Naphthyl	OPh	E	0.97	2.46
27	1-Naphthyl	OCH_2Ph	E	0.91	3.23
28	2-Naphthyl	OCH_2Ph	E	1.01	1.13

Conditions: column, 250×4 mm I.D.; flow-rate, 2 ml/min ([a] 1 ml/min); UV detection, 254 nm ([b] 280 nm); mobile phases: A, *n*-heptane–dichloromethane (60:40, v/v); B, dichloromethane–methanol (98:2); C, *n*-heptane–dichloromethane–diisopropylamine (50:50:0.1); D, dichloromethane–methanol (99:1); E, *n*-heptane–dichloromethane (40:60); column temperature, 25°C; mre=most retained enantiomer; n.r., not resolved.

strate"-specific brush-type CSPs for profens [11,12]. Due to their well-defined steric arrangement of the crucial interaction sites these "tailor-made" selectors show especially high levels of enantioselectivity for structurally closely related analytes. This is also found for the new DADEA-derived selector.

Compared with the DACH and DPEDA CSPs the DADEA analogue exhibits excellent enantioselectivity for five-membered aryl-substituted heterocyclic esters and carbamates (Table 1). Included in this range are the oxazolidinone derivatives of β-blockers (examples are shown in Fig. 3), which have played an important role in the exploration of the enantioselective metabolism of the corresponding drugs in humans [13,14]. The results obtained on the DADEA CSP for the enantioseparation of various important β-blockers **2**–**13** are depicted in Table 1. As can be seen from these data, the magnitude of

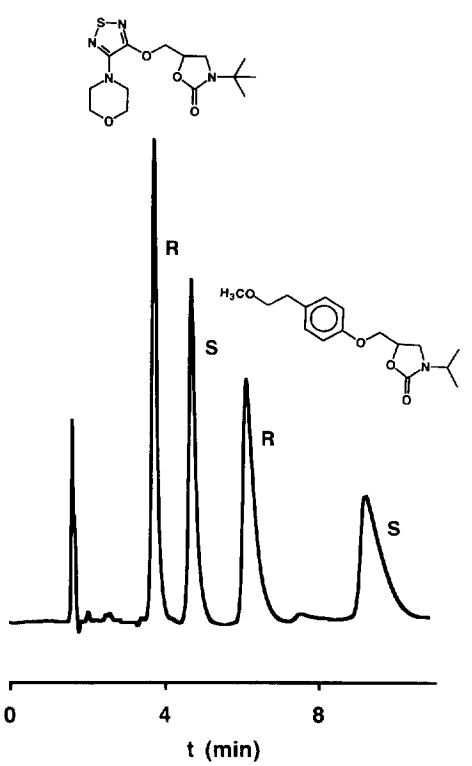


Fig. 3. Enantioseparation of metoprolol and timolol as oxazolidine-2-ones **7** and **10** on the DADEA CSP. Conditions: mobile phase, *n*-heptane–dichloromethane–diisopropylamine (45:55:0.1, v/v); flow-rate, 1.5 ml/min; detection, 254 nm; column temperature, 25°C.

enantioselectivity is determined mainly by the aromatic group in the ether side chain. Derivatives of β-blockers containing highly π-basic aromatic rings (e.g. propranolol, carazolol, carvedilol, alprenolol; entries **2**, **5**, **6**, **8**) expose higher levels of enantioselectivity than those with weakly π-basic portions (atenolol **12** and propafenon **9**). Remarkably, steric demanding substituents at the aromatic portions of cyclized β-blockers do not significantly interfere with chiral recognition (e.g. timolol and celiprolol derivatives **10** and **11**).

The influence of the N-substituent of the oxazolidinone ring plays a minor role for the magnitude of enantioselectivity; e.g., the α values of N-isopropyl derivative **2** and *tert.*-butyl analogue **3** are identical although they expose a strongly different retention behavior ($k'=2.33$ vs. 1.32). If the alkyl moiety at the ring nitrogen contains an additional aromatic function (entry **4**), an increase in retention and decrease of the enantioselectivity is observed. Remarkably, the oxazolidinones of carazolol **5** and the carvedilol **6** with a significantly larger N-substituent display similar enantiorecognition behavior. Surprisingly, cyclized pronethalol **14**, where the 2-naphthyl ring is directly attached to the oxazolidinone ring is as well separated as the more flexible analogue **13**.

These findings prompted us to investigate the enantiodiscriminating potential of the DADEA selector for some lactones having a naphthyl substituent directly linked to the ring (entries **15**–**18**). The enantioseparation of benzo annelated lactones (phthalides) has been studied previously by Pirkle and Sowin [15]. On the DADEA CSP the enantiomers of the 1-naphthyl substituted phthalide **15** are excellently resolved, while for the similarly π-basic 2-naphthyl analogue **16** a surprisingly small a value is observed. However, in contrast to the amino-acid-derived CSPs [15], replacement of the hydrogen at the chiral center in **15** by the sterically more demanding methyl group (analyte **17**) leads only to a small loss in enantioselectivity. A much more dramatic decrease in the magnitude of the enantioselectivity is observed for analyte **18**, which is derived from phthalide **15** by removal of the benzo function.

Evidently, five-membered lactones must meet special steric requirements for efficient enantiodis-

crimination on the rigid DADEA selector. From the results obtained for analytes **15** and **16** we can conclude that the orientation of the naphthyl at the chiral center relative to the plane of the lactone ring determines the quality of $\pi-\pi$ stacking with the corresponding DNB function of the selector. Due to less pronounced steric hindrance (larger distance between the aromatic hydrogens of the benzo function and naphthyl ring) the 2-naphthyl analogue **16** deviates from the opportune orientation of **15**. Similar reasons must account for the poor enantioselectivity of the 1-naphthyllactone **18**, which is structurally different from the well-separated **15** only due to the lack the anellated benzo ring. This suggests that the benzo function contributes to the stabilization of the favorable orientation of the 1-naphthyl ring relative to the lactone plane.

To gain evidence that this important steric control

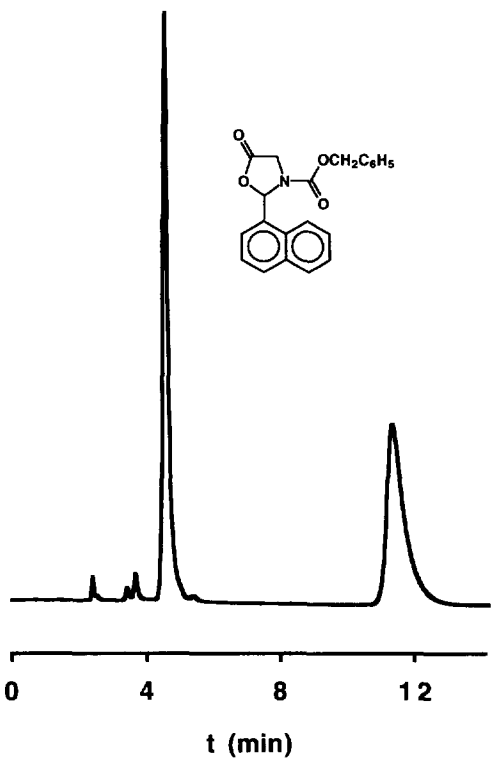


Fig. 4. Enantioseparation of **27** on the DADEA CSP. Conditions: mobile phase, *n*-heptane–dichloromethane (60:40, v/v); flow-rate, 1 ml/min; detection, 254 nm; column temperature, 0°C.

can be provided not only by an anellated benzo ring, but by other steric demanding substituents, we studied oxazolidinones **19–28**. Special derivatives of these glycine-derived heterocycles have proved to be very versatile building blocks for optically highly enriched amino acids [8]. The enantioseparation of these intermediates via preparative liquid chromatography on commercially available chiral sorbents is still a difficult task [8,16] and therefore the development of more efficient CSPs is desirable. In our model analytes **19–28** the naphthyl substituent and the ring keto function is arranged in a similar manner as in phthalides **15** and **16**. In contrast to the latter the steric bulk flanking the naphthyl substituent is represented by a N-acyl function. As expected, the enantioseparation behaviour is very similar to that observed for the phthalides. Again the analytes with 1-naphthyl substituents at position 3 are separated excellently (e.g. **27**, Fig. 4), while for the 2-naphthyl analogues **25**, **28** only poor and for **23** even no enantioseparation is observed. The nature of the N-acyl moiety exerts some, but less dramatic influence on the magnitude of enantioselectivity. Oxazolidinones containing small acyl groups (acetyl in **19**) are more strongly retained and less well separated than their analogues with long aliphatic chains (**20**) or bulky substituents (**21**). Interestingly, alkoxycarbonyl functions as acyl substituents seem to have a favorable influence on enantioseparation.

The limited data presently available on the DADEA CSP does not permit the development of a detailed recognition rationale for the classes of compounds investigated. Interestingly, the elution order observed for propranolol oxazolidinones and 1-naphthyl phthalides on this phase is identical with that reported for these analytes on configurationally corresponding DNB-glycine- or leucine-derived selectors [15,17]. The published recognition model describes enantioseparation as the consequence of simultaneous interactions involving $\pi-\pi$ stacking, hydrogen bonding and steric repulsion. The identical sense of chiral recognition suggests this rationale as a useful approximation to explain enantioseparation of phthalides and structurally closely related compounds on our selector. Thus, the second but tertiary DNB-amido group in the described DADEA selector plays a more important role as an efficient steric barrier rather than as a $\pi-\pi$ interaction site.

4. Conclusion

Relative to the structurally close related bis-DNB-DACH and DPEDA type, the corresponding DADEA selector exhibits significantly improved enantioselectivity for important classes of oxazolidinones and phthalides. The pronounced substrate specificity for these relatively rigid analytes is the consequence of the conformationally well-defined arrangement of the interaction sites in DADEA. The butterfly-like structure of the ethanoanthracene framework may account for the improved enantioselectivity since it restricts the access to the selector from the "wrong" side and therefore reduces non-enantioselective interactions. The somewhat limited scope of the described DADEA CSP might be improved by using a less demanding mode of functionalization for this easily accessible diamine.

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