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Novel cinchona alkaloid carbamate C_9-dimers as chiral anion-exchange type selectors for high-performance liquid chromatography

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Abstract

Nine new quinine (QN) carbamate C_9-dimers (QN–X–QN), with different aliphatic and cyclic spacers (X), have been synthesized and immobilized onto porous silica gel for HPLC. The chiral discriminating behavior of these ''dimeric'' anion-exchange type chiral stationary phases (CSPs) has been investigated in detail, to elucidate the role of the presence of a second QN subunit on the chiral selector (SO), as well as the influence of the structure and length of the spacer, on the overall chiral recognition of a set of N-derivatized amino acids and other acidic drugs. The bulkiness of the intermediate spacer tuned the chiral recognition abilities of these SOs, with the 1,3-adamantylen-derived CSP being the one that led to the best separations. Shorter spacers reduced the chiral discrimination abilities of the ''dimeric'' selectors, with the *n*-hexylen bridge being the most favorable distance to allow a nearly independent interaction of the two QN subunits with the racemic analytes. The comparison to five ''monomeric'' CSPs showed that the ''dimeric'' ones usually retain the chiral analytes more strongly, though the enantioseparation is not improved. Nevertheless, the exceptional resolution abilities of dimeric SOs with a *trans*-1,2-diaminocyclohexylen-bridge for the separation of DNP-derivatives of amino acids and certain acidic drugs of therapeutical interest (e.g., profens) seemed to be superior to most of the other CSPs.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Cinchona carbamates; Quinine carbamates; Amino acids; Profens

1. Introduction

The practical interest of chirality in many fields, such as pharmaceuticals, natural products, agrochemicals and liquid crystals, has led in recent years to important advances in the synthesis, separation and analysis of chiral compounds. The growing concern about the development of enantiomerically pure substances can explain the importance of having analytical tools to control the enantiomeric purity (ee) of products, obtained either by asymmetric synthesis or by separation of racemic or enantiomerically enriched mixtures in any of the synthetic steps. In this sense, chromatographic techniques using chiral stationary phases (CSPs) have demonstrated very interesting features for the analysis of chiral compounds and also for the preparative resolution of enantiomers [1,2].

In the field of the resolution of chiral analytes some alkaloids [3], but particularly cinchona alkaloids, such as quinine (QN) and quinidine (QD),

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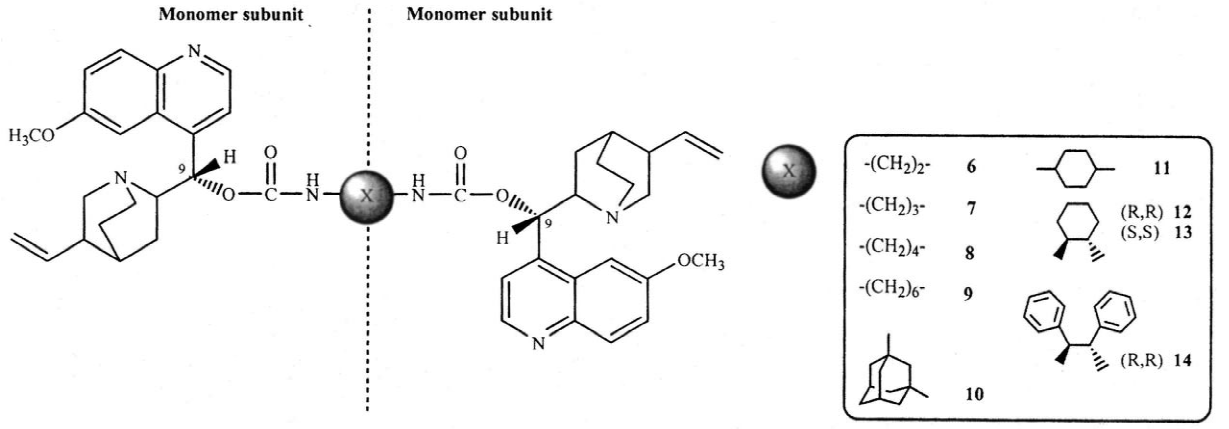

Fig. 1. Chemical structures of the dimeric chiral selectors used to generate ''dimeric'' CSPs.

and their derivatives, have been extensively used as anion exchanger type selectors in different separation techniques [high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), capillary electrophoresis (CE) and capillary electrochromatography (CEC)] [4–6]. These type of selectors show high stereodiscriminating ability for certain amino acid derivatives and other acidic chiral molecules.

In the course of recent developments of Lindner and co-workers [4–7], several C_9-carbamate derivatives of QN and QD were prepared and their chiral discrimination abilities using hydro-organic buffers as mobile phases were studied in detail. Several novel derivatives are at present under investigation in order to broaden the scope of application and/or to investigate the underlying molecular recognition and chiral discrimination principle, to adapt their resolving ability to chiral compounds of special interest. The aim of the present study was to elucidate the role of two QN carbamate subunits linked together via the C_9-position, resulting a quasi dimeric chiral selector, on the overall chiral recognition process. Thus, the synthesis of a series of new QN dimers, linked with two carbamate functions and different spacers (Fig. 1, CSP6–CSP14), is described. After their covalent fixation onto silica gel for chromatography, the enantioselectivity of the resulting novel chiral stationary phases (CSPs) was investigated and compared to structurally similar but monomeric CSPs (CSP1–CSP5, Fig. 2). In a first approach, the prepared CSPs were screened using stereoselective solid-phase extraction experiments with three differently N-protected leucine derivatives. The selective adsorption of the two enantiomers of the racemic mixture was measured, allowing us to estimate the relative enantioselective behavior of the prepared CSPs, before testing them chromatographically. Representative HPLC data and extraction experiments on the enantioselectivity of these CSPs for various N-protected amino acids and other chiral compounds

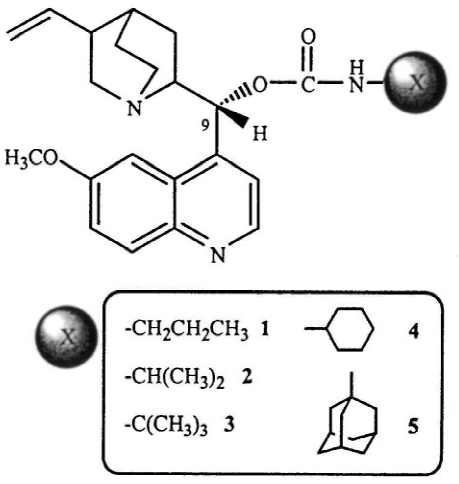

Fig. 2. Chemical structures of the monomeric chiral selectors used to generate ''monomeric'' CSPs.

are discussed, whereby focus is given on the structural increments presumably responsible for a relative increase or decrease of the enantioselectivity.

2. Experimental

2.1. Materials

Quinine (QN) was supplied by Buchler (Braunschweig, Germany). Kromasil 100 Å-5 μm from EKA Nobel (Bohus, Sweden) was used as the porous silica material for all of the nine CSPs. 3-Mercaptopropylsilanized silica gel was prepared as described elsewhere [8] and afforded 4.90% C, 0.91% H. This corresponds to a calculated coverage of about 1.0 mmol thiol groups per gram silica. Dibutyltin dilaurate, ethylendiamine, 1,3-diaminopropane, 1,4-diaminobutane, *n*-propylisocyanate, isopropylisocyanate, *tert*.-butylisocyanate, cyclohexylisocyanate, 1-adamantylisocyanate, 1,6-hexamethylendiisocyanate, *trans*-1,4-cyclohexylendiisocyanate, 1,3-adamantanedicarboxylic acid, *trans*-1,2-(*R*,*R*)-diphenylethylendiamine, 1-hexene, α,α′-azo-bis-isobutyronitrile (AIBN), 4-nitrophenyl chloroformate, triethylamine and glacial acetic acid were purchased from Aldrich (Steinheim, Germany). *Trans*-1,2-(*R*,*R*)- and (*S*,*S*)-diaminocyclohexane were obtained from Strem (Newburyport, USA). Sodium azide and thionyl chloride were supplied by Merck (Darmstadt, Germany). The solvents used for the syntheses were of analytical-reagent grade quality.

Mobile phases for chromatography were prepared from analytical-reagent grade ammonium acetate from Merck and HPLC-grade water. The organic modifier, methanol (MeOH), was of HPLC-grade from J.T. Baker (The Netherlands).

The chiral test compounds were provided by various suppliers, mainly Aldrich, Sigma, Bachem and Degussa. N-Derivatized amino acids were, if not deliverable by the previously mentioned companies, synthesized according to standard derivatization procedures.

2.2. Instrumentation

Each modified sorbent, CSP1–CSP14, was slurry packed into a stainless steel column (150×4.0 mm I.D.) by Forschungszentrum Seibersdorf (Austria). The chromatographic system consisted of a HP1090 liquid chromatograph, equipped with a photodiode array detector, connected to a chromatography data station software from Hewlett-Packard. The pH of the mobile phases (always apparent pH, pH_a) was measured with an Aigner-Unilab pH meter, Model 540 GLP (Laborfachhandel, Austria).

2.3. Stereoselective solid-phase extraction experiment conditions

In a small test tube with screw cap, 1 ml of a 1 μmol/ml solution of the racemic test compound (selectand, SA) in methanol–0.1 *M* ammonium acetate (80:20), $pH_a = 6.0$, was mixed with 50 mg (containing ca. 15 μmol immobilized QN carbamoyl selector) of derivatized silica gel with the covalently bound chiral selector (SO) and CSP, respectively. After equilibration for 1 h at 25°C, the concentration of the remaining individual enantiomers in the supernatant hydro-organic phase (expressed as enantiomeric ratio %) was determined by enantioselective HPLC analysis using an appropriate QN derived column [9].

2.4. Standard chromatographic conditions

A set of more than 90 racemic compounds, including different types of N-protected amino acid derivatives and chiral drugs, was used to test the new CSPs and columns, respectively. The influence of the π-acidity of the protecting groups, as well as their different substitution pattern, on the enantioselective behavior on the CSPs was studied. A mixture of methanol–0.1 *M* ammonium acetate (80:20) was used as standard mobile phase. The apparent pH (pH_a) of the mixture was adjusted to 6.0 by adding glacial acetic acid to the aqueous organic buffered mixture. Flow rate was 1 ml/min and temperature was held constantly at 25°C with a column thermostat. UV detection at 230, 254 and 280 nm was the standard detection mode.

2.5. Synthesis of the chiral selectors (SOs)

2.5.1. Monomers (*1–5*)

A 2.0-g amount of quinine as a free base (6.17

mmol) was dissolved in 40 ml of toluene. The solution was azeotropically dried using a Dean–Stark trap. After cooling, 6.8 mmol of *n*-propyl-, isopropyl-, *tert*.-butyl-, cyclohexyl- or 1-adamantylisocyanate, and a drop of dibutyltin dilaurate as catalyst were added. The mixture was allowed to react at reflux temperature for 24 h. After evaporation of the solvent, the individual carbamates were usually isolated by stirring the residue in apolar solvents, preferably *n*-hexane (particular conditions are indicated in every case).

Propylcarbamate of quinine (**1**): Crystallization in *n*-hexane (70% yield).

Isopropylcarbamate of quinine (**2**): Isolated from the reaction mixture, after removal of the solvent, by stirring in *n*-hexane. Purification by column chromatography on silica gel (eluent: chloroform–methanol, 20:1) and the residue crystallized with *n*-hexane (73% yield).

tert.-Butylcarbamate of quinine (**3**): Its synthesis was described elsewhere [7].

Cyclohexylcarbamate of quinine (**4**): Isolated from the reaction mixture, after removal of the solvent, by stirring in *n*-hexane (46% yield).

1-Adamantylcarbamate of quinine (**5**): Isolated from the reaction mixture, after removal of the solvent, by stirring in diethyl ether and crystallized in acetone (72% yield).

2.5.2. Dimers (see Fig. 3)

*2.5.2.1. 9-O-(4-Nitrophenyloxycarbonyl)quinine hydrochloride (**15**, activated quinine ester hydrochloride)*

A 10.0-g amount of quinine as a free base (31 mmol) was dissolved in 150 ml of toluene. The solution was azeotropically dried using a Dean–Stark trap. After cooling, 6.22 g (31 mmol) of 4-nitrophenyl chloroformate was added as solid. The mixture was allowed to react at room temperature (RT) for 1 h. A yellowish precipitate was formed. The solid was filtrated and washed in *n*-hexane (quantitative yield).

*2.5.2.2. Linearly bridged aliphatic quinine derivatives **6–8***

A 1.0-g amount of quinine activated ester hydrochloride **15** (1.90 mmol) was dissolved in 10 ml of dry and freshly distilled pyridine. 0.95 mmol of ethylendiamine, 1,3-diaminopropane or 1,4-diaminobutane, was added. The mixtures were allowed to react for 24 h, 48 h and 72 h, respectively, at RT depending on the reaction rate judged by thin-layer chromatography (TLC). After removal of the solvent, the products were redissolved in dichloromethane and washed exhaustively with aqueous NaOH (0.5 *M*) to eliminate the 4-nitrophenol produced during the reaction. The products remaining in the organic phase were purified by column chromatography on silica gel (eluent: chloroform–methanol, 10:1) and crystallized with ethyl acetate.

1,2-Ethylen-*O*,*O*′-bis-(carbamoyl quinine) (**6**): 50% yield.

1,3-Propylen-*O*,*O*′-bis-(carbamoyl quinine) (**7**): 66% yield.

1,4-Butylen-*O*,*O*′-bis-(carbamoyl quinine) (**8**): 59% yield.

*2.5.2.3. Linear and branched quinine derivatives **9** to **11***

A 3.0-g amount of quinine (9.25 mmol) was suspended in 40 ml of toluene. The suspension was azeotropically dried using a Dean–Stark trap. After cooling, 4.5 mmol of *trans*-1,4-cyclohexylendiisocyanate, 1,6-hexamethylendiisocyanate or 1,3-adamantanediisocyanate, and 2 drops of dibutyltin dilaurate as catalyst were added. The mixture was allowed to react at reflux temperature for 2 h, 7 h and 48 h, respectively.

Quinine dimer **9** was isolated from the reaction mixture, after removal of the solvent, by stirring the residue in *n*-hexane. A white crystalline product was obtained after crystallization with toluene. Quinine dimer **10** was purified, after removal of the solvent, by column chromatography on silica gel (eluent: chloroform–methanol, 7:1) and crystallized with benzine. Quinine dimer **11** precipitated after the 2 h at reflux temperature directly from the reaction solution and was isolated by filtration, washed in toluene and dry diethyl ether. A white crystalline product was obtained after recrystallization with a dichloromethane–diethyl ether mixture.

1,6-Hexamethylen-*O*,*O*′-bis-(carbamoyl quinine) (**9**): 78% yield.

1,3-adamantylen-*O*,*O*′-bis-(carbamoyl quinine) (**10**): 64% yield.

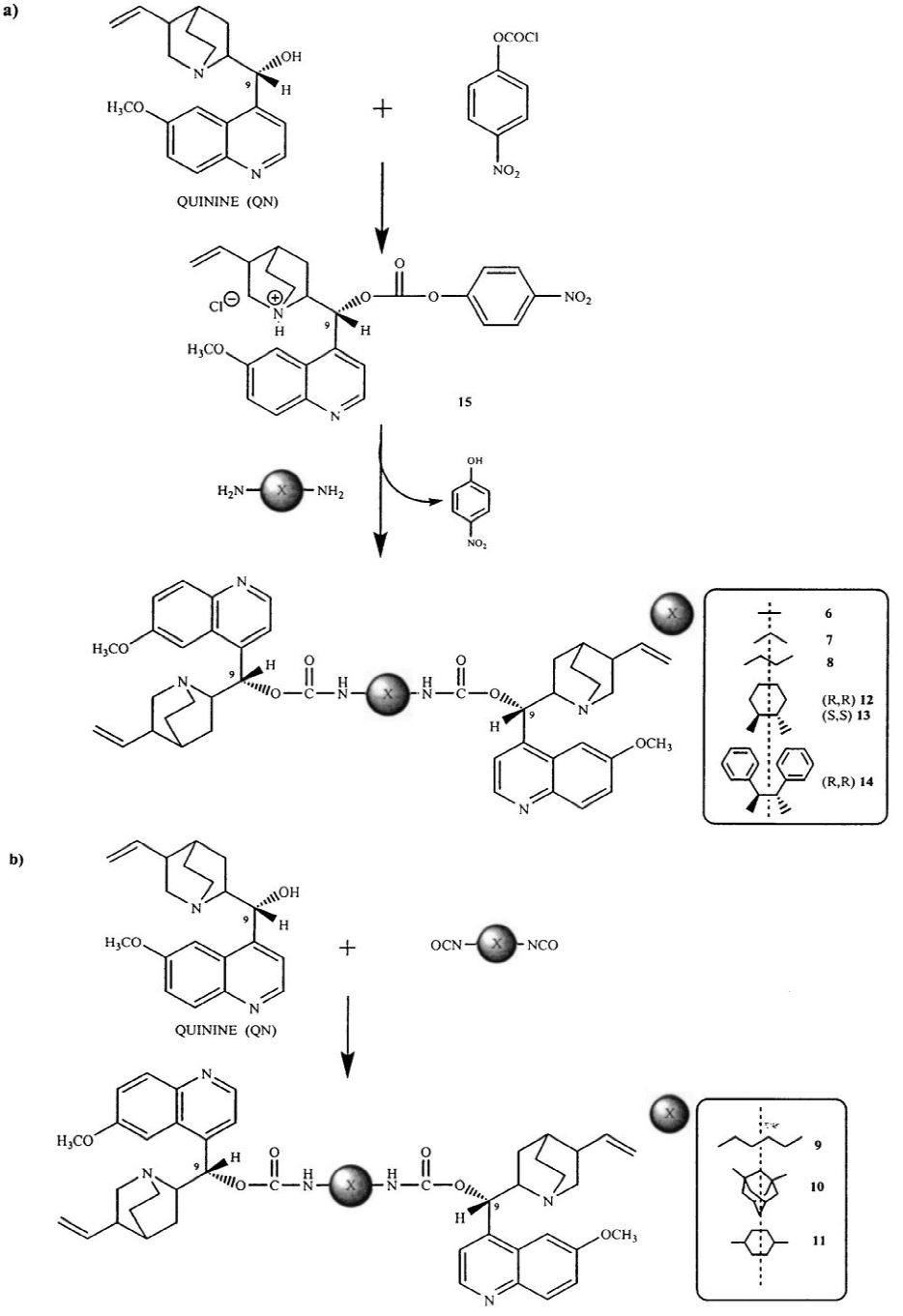

Fig. 3. Reaction scheme for the synthesis of the C_9-bridged dimeric QN carbamate type selectors: (a) synthesis of **6**–**8** and **12**–**14**, using diamines; (b) synthesis of **9**–**11** using diisocyanates.

trans-1,4-Cyclohexylen-*O,O′*-bis-(carbamoyl quinine) (**11**): 87% yield.

2.5.2.3.1. 1,3-Adamantanediisocyanate For the synthesis of the reagent, 2.0-g amount of 1,3-adamantanedicarboxylic acid (8.9 mmol) was suspended in 40 ml of toluene. The suspension was azeotropically dried using a Dean–Stark trap. After cooling, 1.70 ml (23.3 mmol) of thionyl chloride were added. The mixture was allowed to react at reflux temperature for 4 h, after which the product was totally soluble in toluene. The solvent was removed at reduced pressure and the resulting solid was redissolved in dry, freshly distilled dimethylformamide. The solution was cooled at 0°C with an ice-bath and 1.29 g (19.5 mmol) of sodium azide were added. A precipitate appeared immediately. The suspension was vigorously stirred for 1 h at 0°C and 2 h at RT. A 50-ml volume of toluene was added and the organic phase was washed with 100 ml of ice-water. The organic phase was carefully dried (magnesium sulfate). The resulting solution was allowed to react at reflux temperature, until the formation of nitrogen ceased (after ca. 2 h). The solvent was removed under reduced pressure to yield a waxy, white material that was used without further purification in the next step.

*2.5.2.4. Branched ethylendiamino-quinine derivatives **12–14***

A 5.0-g amount of quinine activated ester hydrochloride **15** (9.5 mmol) was dissolved in 50 ml of dry and freshly distilled pyridine and 4.7 mmol of *trans*-1,2-(*R,R*)-diaminocyclohexane, *trans*-1,2-(*S,S*)-diaminocyclohexane or *trans*-1,2-(*R,R*)-diphenylethylendiamine was added. The mixtures were allowed to react for 24 h at RT in the two first cases and for 48 h at reflux temperature for **14**. After removal of the solvent, the products were redissolved in dichloromethane and washed exhaustively with aqueous NaOH (0.5 *M*) to eliminate the 4-nitrophenol. The products were purified by column chromatography on silica gel (eluent: chloroform–triethylamine, 10:1) and recrystallized with ethyl acetate.

trans-1, 2-(*R,R*)-Cyclohexylen-*O, O′*-bis-(carbamoyl quinine) (**12**): 47% yield.

trans-1, 2-(*S, S*)-Cyclohexylen-*O, O′*-bis-(carbamoyl quinine) (**13**): 55% yield.

trans-1,2-(*R,R*)-Diphenylethylen-*O,O′*-bis-(carbamoyl quinine) (**14**): 35% yield.

2.6. Synthesis of CSP1–CSP14

All the CSPs were prepared as described previously by immobilization of the chiral selectors **1**–**14** onto 3-mercaptopropylsilanized silica gel followed by end-capping with 1-hexene [4,6]. The exhaustively washed and dried modified silica gels were subjected to elemental analysis, and the selector loadings were calculated based on the N% (Table 1). The loading of the resulting CSPs ranged from 0.20 to 0.35 mmol of selector/g of silica gel in the monomeric CSPs, whereas it was of 0.14–0.20 in the case of the dimeric phases. It should be noted that the dimeric SOs contain two QN subunits per mol, therefore the effective selector loading is comparable with those observed for the corresponding monomeric CSPs. Each CSP was slurry packed into equally sized stainless steel columns (150×4.0 mm I.D.).

3. Results and discussion

In the course of developing and evaluating a screening tool of chiral ion-exchange type CSPs [9], stereoselective solid-phase extraction experiments were undertaken. Some of the above described CSPs were tested in a similar approach implementing three differently N-protected leucine derivatives. For experimental details see Section 2.3. These first data allowed us to make a quick selection of the most interesting CSPs to be further tested also in the HPLC mode. Therefore, among the linearly bridged dimers, only the most promising CSP of the series was packed (CSP9). Among the dimeric supports, CSP13 and CSP14 showed the lowest ee for DNB- and DNZ-Leu. Although both CSPs were not too promising, CSP13 was packed to provide a basis to study the influence of stereochemistry relative to the (*R,R*)-analogue **12**.

For the chromatographic experiments a large set of different types of N-protected amino acid deriva-

Table 1
Elemental analyses of CSP1–CSP14 and selector density of the CSPs depicted in Figs. 1–3

SO		Elemental analysis			Selector density (loading) (mmol/g silica gel)[a]	N-Carbamate substituent
		% C	% H	% N		
M						
O						
N	CSP1	14.95	2.02	1.46	0.35	*n*-Propyl
O	CSP2	14.68	1.92	1.42	0.34	Isopropyl
M	CSP3	13.27	1.97	1.25	0.27	*tert.*-Butyl
E	CSP4	13.73	2.05	1.26	0.26	Cyclohexyl
R	CSP5	13.73	1.80	1.00	0.20	Adamantyl
I						
C						
						Spacer unit
	CSP6	13.06	1.78	1.44	0.16	Ethylen
D	CSP7	14.05	1.96	1.56	0.18	*n*-Propylen
I	CSP8	15.00	1.94	1.88	0.20	*n*-Butylen
M	CSP9	11.42	1.74	1.33	0.14	*n*-Hexylen
E	CSP10	15.33	1.99	1.39	0.17	1,3-Adamantylen
R	CSP11	13.25	1.83	1.41	0.15	1,4-Cyclohexylen
I	CSP12	15.34	2.05	1.44	0.17	1,2-Cyclohexylen
C	CSP13	14.38	1.92	1.30	0.16	1,2-Cyclohexylen
	CSP14	15.00	2.01	1.33	0.16	Diphenylethylen

[a] CSP1–CSP5 are monomeric phases. The rest are quasi-C_2 symmetrical and have two QN carbamate subunits per selector unit.

tives, as well as acidic chiral drugs, were used. This selection of analytes (SAs), containing diverse chemical structures and exhibiting varied acidities, was very useful to elucidate in depth structural increments of the SAs and SOs on enantioselectivity and to estimate the potential of these new cinchona derived CSPs. The use of structurally closely related protecting groups, such as DNB/Bz or DNZ/Z or DNP (for structures see Fig. 4a), proved to be particularly useful in elucidating the crucial binding sites between the SOs and the SAs.

3.1. Extraction experiments

Table 2 summarizes enantioseparation results (selectivity factors, α) of three differently substituted leucine derivatives (DNB-, DNP- and DNZ-Leu, respectively; for structures see also Fig. 4a) on the 14 CSPs examined during this study. The molar excess of SO to SAs was always larger than 10 (see Table 1). The selectivity factors were first estimated by the solid-phase extraction experiments with an aliquot of the analyte and a sample of the CSP. The measured enantiomeric excesses (ee %) in the supernatants allowed us to to get the first estimate (predicted α_{HPLC}) about the enantioselectivity and extraction behavior of the prepared selectors, which is directly correlated to the chromatographic enantioselectivity (α_{HPLC}), according to the following correction [9]:

$$\alpha_{HPLC} = 1.588\alpha_{extraction} + 0.18$$

where the $\alpha_{extraction}$ is calculated with the equation

$$\alpha_{extraction} = (ee/100 + 1)/(1 - ee/100).$$

Extraction data can be obtained faster than by chromatographic testing of the whole set of columns. The trends observed when comparing the selectivity factors from the extraction experiments were usually in good agreement with the trends of the chromatographic results. Nevertheless, the relative small α values for DNP-Leu were very difficult to predict properly using the extraction experiments. Moreover, the behavior of interaction of the CSPs with this DNP-derivative is different from the one observed

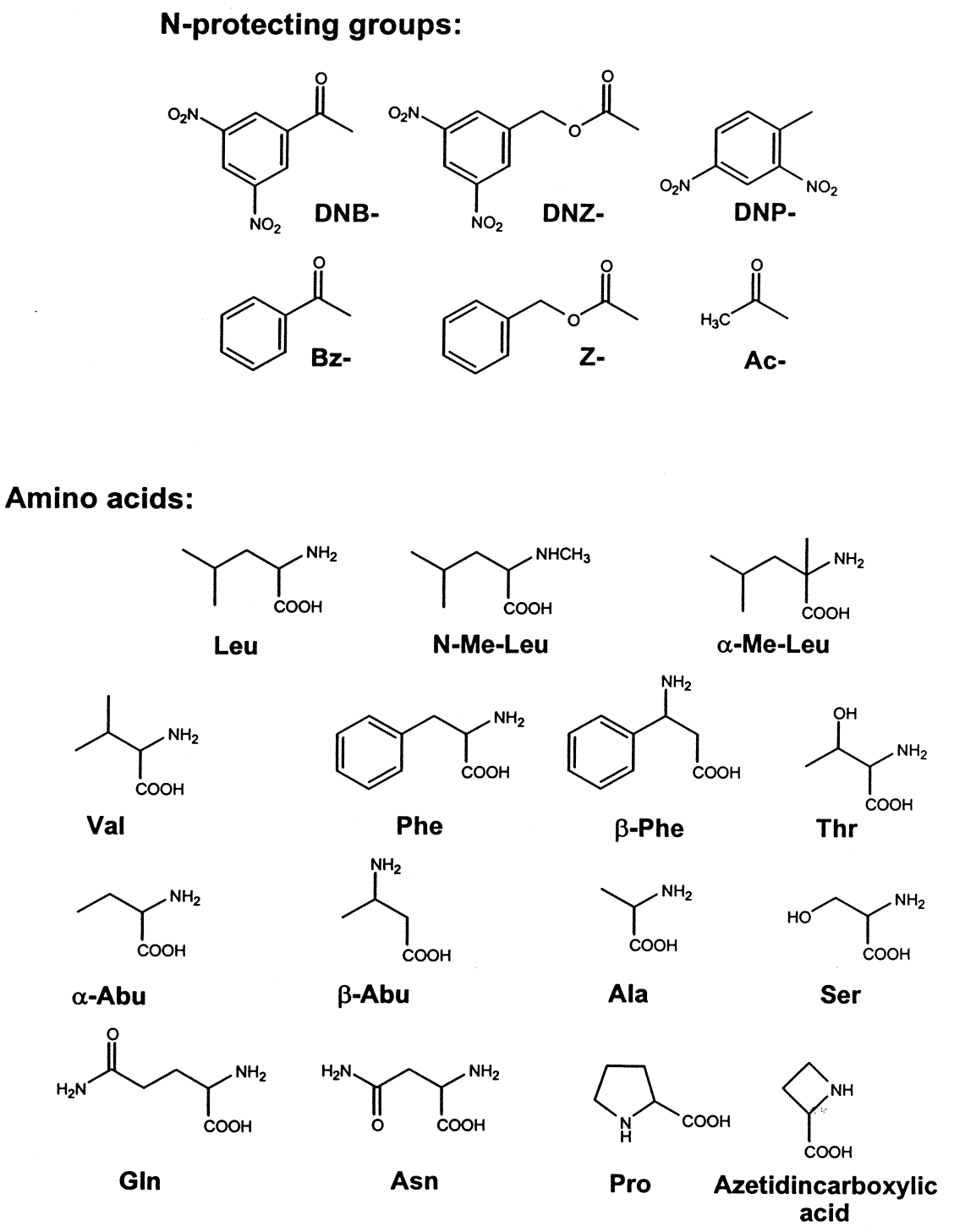

Fig. 4. (a) Structures of N-protected amino acid derivatives used as chiral test analytes. Abbreviations of the N-protecting groups: DNB-: 3,5-dinitrobenzoyl-; DNZ-: 3,5-dinitrobenzyloxycarbonyl-; DNP-: 2,4-dinitrophenyl; Bz-: benzoyl-; Z-: benzyloxycarbonyl-; Ac-: acetyl-. (b) Structures of chiral drugs used as test compounds.

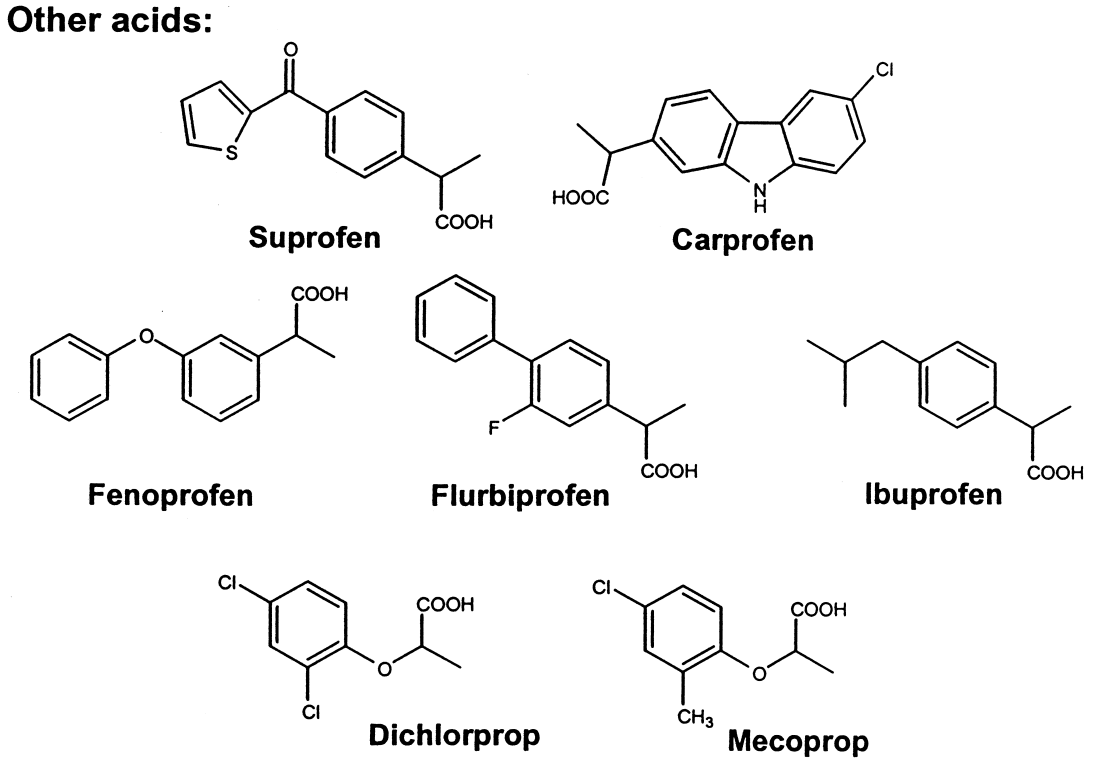

Fig. 4 (*continued*).

with the other two amide like N-protected amino acids and will be discussed separately.

3.1.1. Comparison between monomeric and dimeric CSPs

Among the monomeric phases (CSP1–CSP5), the bulky substitution of CSP3 and CSP5 (*tert*.-butyl and adamantyl, respectively) led to the highest selectivity values of DNB- and DNZ-Leu compared to CSP4, CSP2 and CSP1. The adamantyl-derived dimeric selector, present in CSP10, showed the highest α values in the set of dimeric selectors and CSPs (CSP6–CSP14). When the dimeric selectors have a linear alkyl spacer between the two QN subunits (CSP6–CSP9), the selectivity factors improved with the chain length. Among them, the hexylen bridge seemed to display the most favorable distance (CSP9), even better than any of the differently substituted and more bulky cyclohexylen spacers. However, among these latter, the bis-(1,4-cyclohexyl) derivative (CSP11) usually led to the best α values.

No conclusions could be drawn from the extraction experiments performed with DNP-Leu. Nevertheless, the chromatographic α values showed that the bulkiness of the substituents, either on the monomeric or dimeric phases, did not change or improve notably the resolution, although it should be mentioned that the elution order is reversed compared to the DNB- and DNZ-derivatives. This is a clear indication for a very different SO–SA binding mechanism, reflecting a reduced enantioselective binding contribution of this π-acidic protecting group.

This first comparison of the results obtained with this series of CSPs did not seem to indicate that the presence of a second QN carbamate subunit in form of dimeric selector did improve substantially the overall enantioselectivity. In contrast, it seemed contraproductive except for DNP-Leu. However, the increase in the distance between the two QN carba-

Table 2
Estimated enantioselectivity (α) of the prepared CSPs for racemic leucine derivatives[a]

		Racemic analyte	ee % (first eluted)	Predicted α_{HPLC}[b]	α_{HPLC}
Monomeric	CSP1	DNB-Leu	56.3	5.85	8.98
	CSP2	DNB-Leu	60.3	6.60	9.78
	CSP3	DNB-Leu	81.9	16.1	15.9
	CSP4	DNB-Leu	63.9	7.37	10.40
	CSP5	DNB-Leu	80.1	14.5	17.0
Dimeric	CSP6	DNB-Leu	37.5	3.67	n.m.[c]
	CSP7	DNB-Leu	47.8	4.60	n.m.
	CSP8	DNB-Leu	50.7	5.02	n.m.
	CSP9	DNB-Leu	55.4	5.72	8.13
	CSP10	DNB-Leu	68.7	8.72	11.0
	CSP11	DNB-Leu	54.9	5.63	7.13
	CSP12	DNB-Leu	46.2	4.50	4.52
	CSP13	DNB-Leu	35.0	3.48	3.30
	CSP14	DNB-Leu	37.1	3.64	n.m.
Monomeric	CSP1	DNP-Leu	4.43	1.91	1.57
	CSP2	DNP-Leu	23.7	2.75	1.54
	CSP3	DNP-Leu	–	1.64	1.31
	CSP4	DNP-Leu	2.07	1.83	1.36
	CSP5	DNP-Leu	–	1.64	1.20
Dimeric	CSP6	DNP-Leu	14.3	2.29	n.m.
	CSP7	DNP-Leu	5.22	1.94	n.m.
	CSP8	DNP-Leu	–	1.75	n.m.
	CSP9	DNP-Leu	–	1.72	1.39
	CSP10	DNP-Leu	–	1.70	1.24
	CSP11	DNP-Leu	–	1.67	1.38
	CSP12	DNP-Leu	11.0	2.16	1.32
	CSP13	DNP-Leu	14.0	2.29	1.51
	CSP14	DNP-Leu	16.1	2.37	n.m.
Monomeric	CSP1	DNZ-Leu	25.0	2.83	2.03
	CSP2	DNZ-Leu	12.6	2.23	2.10
	CSP3	DNZ-Leu	27.5	2.97	2.80
	CSP4	DNZ-Leu	27.4	2.97	2.11
	CSP5	DNZ-Leu	38.0	3.72	3.50
Dimeric	CSP6	DNZ-Leu	9.60	2.10	n.m.
	CSP7	DNZ-Leu	11.8	2.20	n.m.
	CSP8	DNZ-Leu	14.1	2.29	n.m.
	CSP9	DNZ-Leu	16.7	2.40	1.84
	CSP10	DNZ-Leu	34.4	3.43	2.38
	CSP11	DNZ-Leu	18.7	2.50	1.83
	CSP12	DNZ-Leu	15.6	2.36	1.84
	CSP13	DNZ-Leu	5.00	1.93	1.22
	CSP14	DNZ-Leu	11.5	2.18	n.m.

[a] Enantiomers of DNP-Leu have reversed elution order compared to DNB- and DNZ-Leu.

[b] Predicted with the regression equation obtained from extraction experiments [9]: $\alpha_{HPLC} = 1.588\alpha_{extraction} + 0.18$, where the $\alpha_{extraction}$ is calculated with the equation $\alpha_{extraction} = (ee/100 + 1)/(1 - ee/100)$.

[c] n.m. = Not measured.

mate subunits seemed to favor their independent chiral resolution ability. Further discussion of the results will be presented in the chromatographic section on the basis of an extended selection of test compounds.

3.2. Chromatographic tests

3.2.1. Chromatographic behavior of the dimeric CSPs

In Table 3 a selection of the resulting enantiomer separations of different analytes is listed and compared to five of the most diversified dimeric CSPs (CSP9–CSP13) (for structures see Fig. 4a and b). The five chiral supports had quite similar selector densities (0.14–0.17 mmol/g silica) and, therefore, a direct comparison of the observed effects on the retention behavior and selectivity factors is reasonably justified.

As it has been already pointed out, the bulkiest CSP10 was usually the phase which resolved best most of the racemic compounds tested, followed by CSP9 and CSP11, based on the α values. In the three cases the two QN subunits of the chiral selector are separated at least by three carbon units (for chiral selector **10**) and a maximum of six carbons on the *n*-hexylen bridge of selector **9**. This latter selector, present in CSP9, showed the shortest retention and good α values for most of the racemic compounds tested. This fact suggests that both QN carbamate subunits may interact rather independently when the distance between the carbamate groups involves at least three carbon units, such as on CSP9–CSP11. A reduction of the distance between the two units increased the retention time of the first eluted enantiomer, whereas a parallel improvement of the enantioselectivity was not observed. Thus, higher k' values were not always related to better chromatographic resolutions, as it was demonstrated by the behavior of CSP12 and CSP13. The 1,2-(*R*,*R*)- and 1,2-(*S*,*S*)-cyclohexylen-derived selector dimers, **12** and **13**, respectively, led in most of the cases to smaller α values, DNP-derivatives being a notable exception. CSP10, bearing the 1,3-adamantylen bridge, strongly retained most of the compounds; but this increase in the capacity factors was accompanied also by a positive effect on the selectivity values. The bulkiness and rigidity of the adamantyl group between the two QN carbamate subunits seemed to favor a more independent interaction of the individual cinchona subunits with the chiral analytes.

Besides these general trends that can be observed for most of the analytes tested, some remarks should be made concerning some families of chiral compounds. As it was already pointed out in a previous study [4], the functionality, shape and conformational arrangement of the N-substituents of the amino acid derivatives (amides, carbamates or dinitroaryls) control the orientation of the SAs towards the binding sites of the SO. This binding interaction is so demandingly important that the elution order of the enantiomers of the same amino acid will even be inverted, depending on the N-protecting group that they are bearing. For example, the presence of the 2,4-dinitrophenyl (DNP) protecting group leads to an inversion of the elution order compared to amide and carbamate type groups listed in Fig. 4a, suggesting that hydrogen bonding and/or dipole–dipole interactions are important driving forces. The absence of a hydrogen donating group of acyl type derivative of secondary amino acid (e.g., Pro, *N*-methyl-Leu, azetidincarboxylic acid) or the absence of a similar group in the α position of the acids other than amino acids (profens, mecoprop and dichlorprop) is disadvantageous with respect to chiral recognition, but may also improve the separations as particularly pronounced for CSP13. Thus, for many of these compounds CSP13 and CSP9 had selectors which seemed to be adaptable more easily for a given enantioseparation. This feature is especially interesting in the case of the α-aryl propionic acids or profens (antiinflamatory drugs, such as fenoprofen, ibuprofen or flurbiprofen), some of which are in general poorly resolved in most of the available CSPs used with aqueous organic mobile phases.

3.2.2. Comparison of the dimeric CSPs with the corresponding monomeric ones

The role of the presence of a second QN carbamate subunit within the carbamoyl bridged dimeric selector moiety has been investigated by a detailed study of these chromatographic results of the ''dimeric'' CSPs in comparison to the ''monomeric'' ones. Thus, the behavior of CSP9 (dimeric SO with an *n*-hexylen chain spacer) was compared with CSP1 (*n*-propylcarbamate of QN), as presented in Table 4

Table 3
Chromatographic retention and selectivity factors of several analytes on the dimeric CSPs[a]

	CSP9 (*n*-hexylen)[b]			CSP10 (1,3-adamantylen)[b]			CSP11 (1,4-cyclohexylen)[b]			CSP12 [1,2-(*R*,*R*)-cyclohexylen][b]			CSP13 [1,2-(*S*,*S*)-cyclohexylen][b]		
	k_1'	α	e.o.[c]	k_1'	α	e.o.[c]	k_1'	α	e.o.[c]	k_1'	α	e.o.[c]	k_1'	α	e.o.[c]
DNB-Leu	9.67	8.13	D	20.2	10.97	D	14.8	7.13	D	20.6	4.52	D	15.9	3.30	D
DNZ-Leu	7.14	1.84		24.5	2.38		10.1	1.83		23.0	1.84		18.8	1.22	
Z-Leu	5.88	1.15	D	9.94	1.19	D	4.46	1.15	D	9.77	1.12	D	7.31	1.00	
Bz-Leu	5.04	1.69	D	7.85	2.07	D	4.02	1.54	D	8.06	1.37	D	6.15	1.16	D
Ac-Leu	2.76	1.14		3.49	1.21		2.13	1.13		3.99	1.07		2.80	1.00	
DNP-Leu	16.2	1.39	L	43.1	1.24	L	26.6	1.38	L	35.2	1.32	L	27.2	1.51	L
DNB-*N*-Me-Leu	8.44	1.01		19.0	1.00		8.25	1.00		12.7	1.05	D	12.6	1.05	D
DNZ-*N*-Me-Leu	6.93	1.06		25.0	1.13		9.98	1.03		23.3	1.07		17.5	1.09	
DNP-*N*-Me-Leu	15.8	1.47	L	40.6	1.29	L	25.3	1.43	L	38.9	1.30	L	18.5	1.47	L
DNB-Phe	13.6	7.90	D	25.4	9.36	D	21.4	6.94	D	26.9	3.71	D	21.9	3.62	D
DNZ-Phe	11.8	1.80		40.5	2.00		16.3	1.75		36.2	1.52		27.9	1.26	
Z-Phe	10.2	1.22	D	20.1	1.16	D	8.42	1.19	D	18.3	1.12		13.7	1.09	D
Bz-Phe	7.77	1.64	D	14.1	1.88	D	6.75	1.52	D	13.1	1.31		10.3	1.21	D
Ac-Phe	4.33	1.27	D	6.17	1.33	D	3.52	1.24	D	6.51	1.13	D	4.61	1.07	D
DNZ-Azetidincarb	8.45	1.13	L	24.0	1.20	L	11.3	1.10	L	23.2	1.08	L	18.4	1.17	L
DNZ-Pro	7.51	1.12		22.2	1.20		9.79	1.11		20.6	1.10		16.0	1.17	
DNP-Pro	18.0	1.50	L	38.1	1.32	L	25.3	1.46	L	32.0	1.41	L	23.2	1.51	L
Suprofen	9.73	1.13		15.3	1.09		11.9	1.10		15.3	1.10		9.47	1.08	
Fenoprofen	6.69	1.00		10.4	1.00		6.59	1.00		10.8	1.00		8.03	1.03	
Carprofen	17.7	1.10		26.5	1.08		16.8	1.06		30.1	1.00		22.4	1.11	
Flurbiprofen	8.86	1.08		14.2	1.06		11.0	1.04		15.5	1.05		11.2	1.08	
Ibuprofen	4.44	1.07	D	6.40	1.00		5.08	1.02		6.39	1.00		4.81	1.09	D
Dichlorprop	8.58	1.21		17.4	1.17		11.3	1.24		16.1	1.17		11.3	1.33	
Mecoprop	7.05	1.14		14.1	1.08		4.16	1.22		14.6	1.11		8.76	1.20	

[a] For chromatographic conditions see Section 2, for structures of analytes see Fig. 4a and b.
[b] Spacer unit.
[c] e.o. = Elution order, configuration of the first eluted enantiomer.

Table 4
Comparison of the chromatographic behavior of monomeric CSP1 and dimeric CSP9[a]

	CSP1 monomeric SO (*n*-propyl)		CSP9 dimeric SO (*n*-hexylen)	
	k_1'	α	k_1'	α
DNB-α-Abu	15.53	6.44	6.44	5.15
DNB-β-Abu	13.81	5.60	5.93	4.41
DNB-Leu	17.01	8.98	9.67	8.13
DNB-α-Me-Leu	19.06	1.24	10.42	1.23
DNB-*N*-Me-Leu	15.04	1.06	8.44	1.01
DNB-Pro	12.97	1.06	8.17	1.00
DNB-Phe	27.82	7.55	13.59	7.90

[a] For chromatographic conditions see Section 2, for structures of analytes see Fig. 4a and b.

for the resolution of selected amino acid derivatives. The SO loadings of CSP1 and CSP9 were 0.35 and 0.14 mmol/g silica, respectively (see Table 1), which represents a similar loading of QN carbamate subunits per gram of silica gel or CSP. Overall, the dimeric CSP shows significantly shorter retention times than the monomeric one, however CSP1 exhibits for most of the test compounds somewhat higher selectivity factors. CSP9 was the dimeric phase that presented the most moderated decrease of the selectivity factors relative to the corresponding monomeric CSP, which indicates an almost independent behavior of the two individual QN carbamate subunits in this selector. Furthermore, it is interesting to note that CSP1 and CSP2, both propyl-derived monomers (*n*-propyl and isopropyl-, respectively), showed particularly long retention times for all the analytes, although their loading on the silica surface was not particularly high.

For the comparison of the dimeric CSP10, two different structurally related monomeric type CSPs have been prepared: CSP3 (*tert*.-butylcarbamate of QN, loading 0.27 mmol SO/g silica) and CSP5 (adamantylcarbamate of QN, loading 0.20 mmol SO/g silica). CSP10 presented clearly the longest retention times, which did, however, not necessarily led to higher selectivity factors (see Fig. 5c for the separation of racemic DNB-α-aminobutyric acid). Although the loading of SO on the dimeric CSP represented a higher content of QN carbamate subunits (ca. 0.34 mmol SO subunits/g silica based on 0.17 mmol SO/g silica), usually the monomeric CSP5 and CSP3 and the respective columns showed better enantioselectivity. Evidently, non-cooperative interactions as a consequence of perturbed selector conformations or a sort of substrate competition may explain the reduction of overall enantioselectivity.

Concerning the cyclohexylen-bridged CSPs (CSP11–CSP13), the following comments should be made based on investigations of some families of test compounds. First, for the monomeric phases with which the dimeric may be compared, the corresponding CSP2 (isopropylcarbamate of QN) and CSP4 (cyclohexylcarbamate of QN), presented somewhat higher α values than CSP11 (see Table 5). However, CSP12 and CSP13 are not much different although the overall conformation of the selectors should be quite different due to the introduction of two new stereogenic centers. For the DNP-derivatives of amino acids CSP2 is still somewhat more enantioselective, though the corresponding dimeric phases, and in particular CSP13, have very similar values for this type of analytes. The reduction in enantioselectivity of CSP13 in these cases was less than expected considering the relative short distance that represents the 1,2-cyclohexylen spacer. Therefore, the interaction of these groups of analytes with the SOs seems to be dependent on the geometry of the chiral bridge between the two QN subunits.

4. Conclusions

The novel dimeric and C_9-bridged QN carbamate type selectors proved capable of separating the enantiomers of a broad range of amino acid derivatives and other chiral acidic drugs via stereoselective ion-exchange type retention mechanism using hydro-organic buffers as mobile phases. Although the presence of a second QN carbamate subunit within the chiral selector moiety very often effected stronger retention of the chiral analytes, the enantioselectivity was not significantly improved in comparison with the structurally related monomeric selectors. The structure and length of the spacer between the two QN carbamate subunits has demonstrated to be crucial for the effective stereoselective SO–SA interaction. Thus, the *n*-hexylen bridge of dimeric selector **9** resulted to be the minimum distance required to allow relatively independent

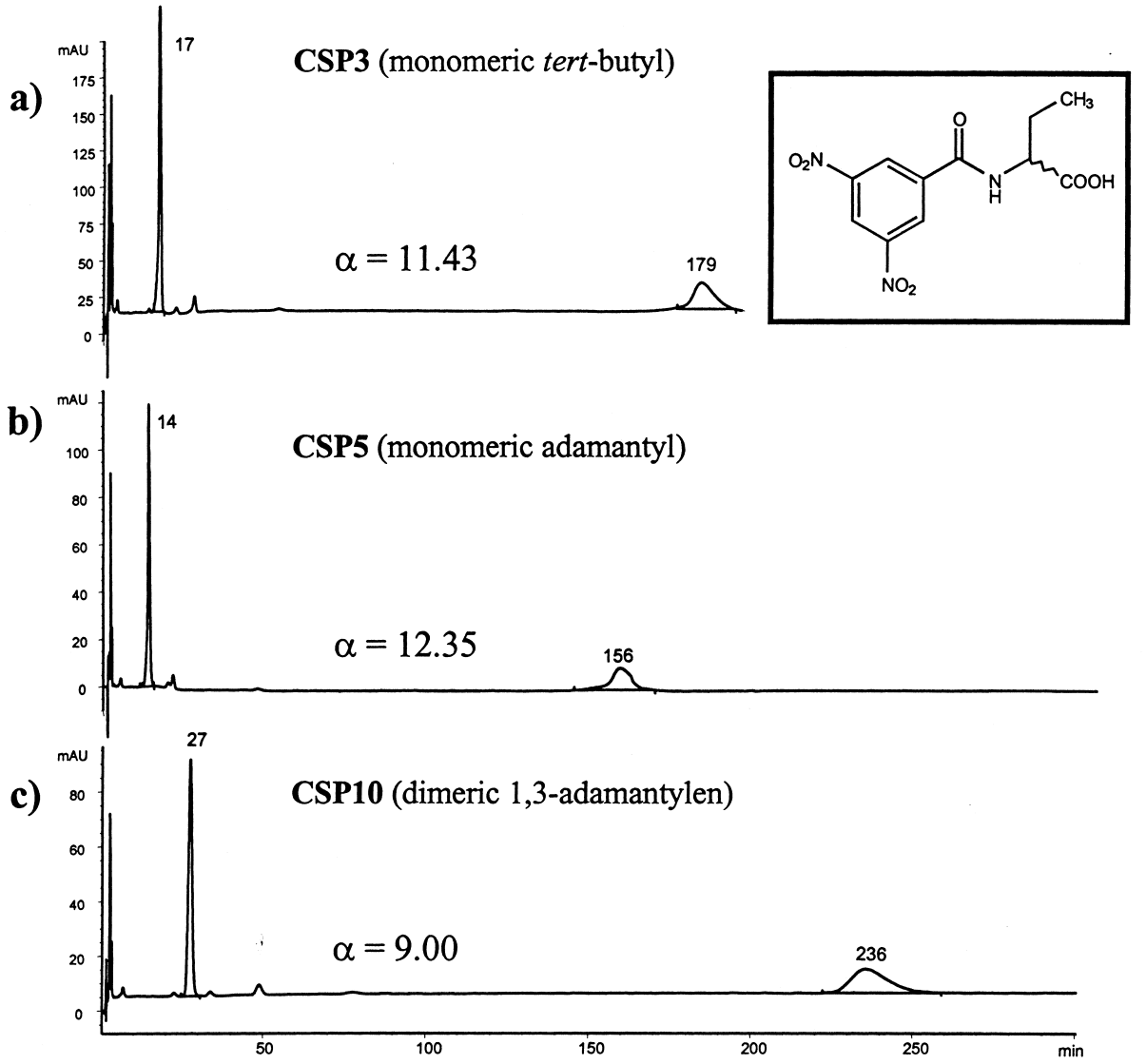

Fig. 5. Chromatographic resolution of racemic DNB-α-aminobutyric acid on: (a) CSP3; (b) CSP5 and (c) CSP10, respectively. For chromatographic conditions see Section 2.

SO–SA interactions of each of the two QN carbamate subunits, resulting the smallest reduction of enantioselectivity of the dimeric CSP in comparison with the structurally very related monomeric CSP1. The presence of bulky substituents (*tert*.-butyl or adamantyl) on the carbamate function, either on the monomeric or on the dimeric selectors, led to clear improvement of the overall chiral recognition ability. Interestingly, the dimeric 1,3-adamantyl selector (CSP10) could not reach the α values of the corresponding monomeric phase (CSP5). Shorter or conformationally hindered spacers between the selector units did not seem to favor the chiral recognition abilities of these dimeric CSPs. Unexpected enantioselectivity effects were observed with SOs in which two QN carbamate subunits are connected with a *trans*-(*S*,*S*)-1,2-diaminocyclohexylen spacer for the separation of DNP-derivatives of amino acids and

Table 5
Comparison of the chromatographic behavior of the cyclohexylen-derived dimers of QN carbamates and structurally related monomeric QN carbamates[a,b]

	CSP2 monomeric SO (isopropyl)		CSP4 monomeric SO (cyclohexyl)		CSP11 dimeric SO (1,4-cyclohexylen)		CSP12 dimeric SO [(*R*,*R*)-1,2-cyclohexylen]		CSP13 dimeric SO [(*S*,*S*)-1,2-cyclohexylen]	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α
DNP-Leu	36.0	1.54	14.8	1.36	26.6	1.38	35.2	1.32	27.2	1.51
DNP-Pro	26.7	1.57	14.5	1.45	25.3	1.46	32.0	1.41	23.2	1.51
DNP-Ala	33.4	1.29	23.7	1.25	32.1	1.23	46.2	1.25	23.7	1.22
DNP-Ser	26.5	1.51	18.0	1.45	26.3	1.43	41.5	1.44	23.1	1.36
DNP-*N*-Me-Leu	28.7	1.51	22.8	1.41	25.3	1.43	28.9	1.30	18.5	1.47
DNP-Thr	20.9	1.66	15.0	1.59	20.5	1.55	32.3	1.50	16.8	1.48
DNP-Gln	21.0	1.24	15.2	1.22	21.3	1.23	31.8	1.21	17.3	1.25
DNP-Asn	21.9	1.50	15.4	1.45	22.9	1.42	35.4	1.38	18.8	1.34

[a] For chromatographic conditions see Section 2, for structures of analytes see Fig. 4a and b.
[b] In all the columns the elution order of the enantiomers is the same. The first eluted enantiomer is always the L-form.

certain acidic drugs. Therefore, CSP13 presented the best separation factors among the dimeric CSPs for these compounds and were showing a behavior not related to the corresponding monomeric selectors.

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Appendix A

Propylcarbamate of quinine (**1**): Physical properties: m.p.: 130–135°C; $[\alpha]^{RT}_{Na589} = +59$, $[\alpha]^{RT}_{Hg546} = +7.3$ ($c = 1.02$, chloroform); IR (KBr): 3187, 2934, 1717, 1622, 1513, 1263 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.74 (d, 1H), 8.00 (d, 1H), 7.49 (d, 1H), 7.35 (dd, 2H), 6.44 (d, 1H), 5.85 (m, 1H), 5.02 (m, 2H), 4.83 (m, 1H), 3.95 (s, 3H), 3.33 (m, 1H), 3.10 (m, 4H), 2.63 (m, 2H), 2.27 (m, 1H), 1.85 (m, 2H), 1.74 (m, 1H), 1.50 (m, 4H), 0.88 (m, 3H) ppm. Calculated elemental analysis ($C_{24}H_{31}N_3O_3$): 70.39% C, 7.63% H, 10.23% N; found: 70.20% C, 7.55% H, 10.25% N.

Isopropylcarbamate of quinine (**2**): Physical properties: m.p.: 121°C; $[\alpha]^{RT}_{Na589} = -10.5$, $[\alpha]^{RT}_{Hg546} = -14.2$ ($c = 1.03$, MeOH); IR (KBr): 3210, 2973, 1712, 1619, 1508, 1240 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.73 (d, 1H), 8.00 (d, 1H), 7.49 (d, 1H), 7.36 (dd, 2H), 6.44 (d, 1H), 5.85 (m, 1H), 5.00 (m, 2H), 4.65 (d, 1H), 3.96 (s, 3H), 3.79 (m, 1H), 3.33 (m, 1H), 3.11 (m, 2H), 2.63 (m, 2H), 2.27 (m, 1H), 1.85 (m, 2H), 1.73 (m, 1H), 1.55 (m, 2H), 1.13 (dd, 6H) ppm. Calculated elemental analysis ($C_{24}H_{31}N_3O_3$): 70.39% C, 7.63% 11,10.23% N; found: 70.17% C, 7.68% H, 10.35% N.

tert.-Butylcarbamate of quinine (**3**): Its synthesis was described elsewhere [7].

Cyclohexylcarbamate of quinine (**4**): Physical properties: m.p.: 154–156°C; $[\alpha]^{RT}_{Na589} = +3.9$, $[\alpha]^{RT}_{Hg546} = 3.8$ ($c = 1.02$, MeOH); IR (KBr): 3197, 2937, 1709, 1621, 1509, 1473, 1229 cm^{-1} ^{1}H-NMR ($CDC1_3$, 400 MHz): 8.74 (d, 1H), 8.00 (d, 1H), 7.49 (d, 1H), 7.35 (dd, 2H), 6.44 (d, 1H), 5.85(m, 1H), 5.01 (m, 2H), 4.69 (m, 1H), 3.95 (s, 3H), 3.43 (m, 1H), 3.33 (m, 1H), 3.07 (m, 2H), 2.63 (m, 2H), 2.27 (m, 1H), 1.0–2.0 (m, 15H) ppm. Calculated elemental analysis ($C_{27}H_{35}N_3O_3$): 72.13% C, 7.85% H, 9.35% N; found: 71.45% C, 8.10% H, 9.29% N.

1-Adamantylcarbamate of quinine (**5**): Physical properties: m.p.: 212–214°C; $[\alpha]^{RT}_{Na589} = +16.2$, $[\alpha]^{RT}_{Hg546} + 17.7$ ($c = 1.00$, chloroform); IR (KBr): 2914, 1716, 1621, 1590, 1507, 1471, 1228 cm^{-1}. ^{1}H-NMR ($CDC1_3$, 400 MHz): 8.74 (d, 1H), 8.00 (d, 1H), 7.47 (d, 1H), 7.35 (dd, 2H), 6.42 (d, 1H), 5.85 (m, 1H), 5.01 (m, 2H), 4.65 (m, 1H), 3.95 (s, 3H), 3.31 (m, 1H), 3.08 (m, 2H), 2.63 (m, 2H), 2.28 (m, 1H), 1.5–2.1 (m, 20H) ppm. Calculated elemental analysis ($C_{31}H_{39}N_3O_3 \cdot 0.5H_2O$): 72.91% C, 7.89% H, 8.23% N; found: 72.94% C, 7.93% H, 8.05% N.

1,2-Ethylen-*O*,*O*′-bis-(carbamoyl quinine) (**6**): Physical properties: m.p.: 125–126°C; $[\alpha]^{RT}_{Na589} = -2.5$, $[\alpha]^{RT}_{Hg546} = -4.2$ ($c = 1.00$, MeOH); IR (KBr): 3423, 2941, 1720, 1622, 1509, 1244 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.70 (d, 2H), 8.01 (d, 2H), 7.44 (d, 2H), 7.35 (dd, 2H), 7.26 (s, 2H), 6.40 (d, 2H), 5.82 (m, 2H), 4.99 (m, 4H), 3.94 (s, 6H), 3.24 (m, 6H), 3.03 (m, 4H), 2.62 (m, 4H), 2.26 (m, 2H), 1.82 (ba, 2H), 1.4–2.0 (m, 10H) ppm. Calculated elemental analysis ($C_{44}H_{52}N_6O_6 \cdot 0.84H_2O$): 68.10% C, 6.97% 11, 10.83% N; found: 68.10% C, 6.97% H, 10.75% N.

1,3-Propylen-*O*,*O*′-bis-(carbamoyl quinine) (**7**): Physical properties: m.p.: 192–193°C; $[\alpha]^{RT}_{Na589} = -0.7$, $[\alpha]^{RT}_{Hg546} = -1.8$ ($c = 1.00$, MeOH); IR (KBr): 3420, 2941, 1719, 1622, 1509, 1258 cm^{-1}. ^{1}H-NMR ($CDCl_3/CD_3OD$, 400 MHz): 8.64 (d, 2H), 7.98 (d, 2H), 7.2–7.5 (m, 6H), 6.45 (d, 2H), 5.76 (m, 2H), 4.98 (m, 4H), 3.97 (s, 6H), 3.50 (ba, 2H), 3.0–3.3 (m, 12H), 2.70 (m, 2H), 2.62 (m, 2H), 2.31 (m, 2H), 1.5–1.9 (m, 10H) ppm. Calculated elemental analysis ($C_{45}H_{54}N_6O_6$): 69.75% C, 7.02% 11, 10.84% N; found: 69.50% C, 7.04% 11, 10.81% N.

1,4-Butylen-*O*,*O*′-bis-(carbamoyl quinine) (**8**): Physical properties: m.p.: 201–202°C; $[\alpha]^{RT}_{Na589} = +37$, $[\alpha]^{RT}_{Hg546} = +3.9$ ($c = 1.00$, MeOH); IR (KBr): 3428, 2938, 1718, 1621, 1509, 1258 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.72 (d, 2H), 8.00 (d, 2H), 7.46 (d, 2H), 7.34 (dd, 2H), 7.26 (s, 2H), 6.41 (d, 2H), 5.83 (m, 2H), 5.00 (m, 4H), 4.89 (ba, 2H), 3.94 (s, 6H), 3.31 (m, 2H), 3.05 (m, 8H), 2.63 (m, 4H), 2.27 (m, 2H), 1.3–1.9 (m, 14H) ppm. Calculated elemental analysis ($C_{46}H_{56}N_6O_6$): 70.03% C, 7.15% H, 10.65% N; found: 69.77% C, 7.02% 11, 10.72% N.

1,6-Hexamethylen-*O,O′*-bis-(carbamoyl quinine) (**9**): Physical properties: m.p.: 134–136°C; $[\alpha]^{RT}_{Na589} = +1.15$, $[\alpha]^{RT}_{Hg546} = +1.83$ ($c = 1.04$, MeOH); IR (KBr): 3363, 2935, 1720, 1622, 1510, 1243 cm^{-1}. ^{1}H-NMR (CD_3OD, 360 MHz): 8.65 (d, 2H), 7.95 (d, 2H), 7.50 (d, 4H), 7.45 (dd, 2H), 6.50 (d, 2H), 5.80 (m, 2H), 5.00 (dd, 4H), 4.00 (s, 6H), 3.25 (m, 4H), 3.05 (m, 6H), 2.70 (m, 4H), 2.30 (m, 2H), 1.2–1.9 (m, 18H) ppm. Calculated elemental analysis ($C_{48}H_{60}N_6O_6$): 70.56% C, 7.40% H, 10.29% N; found: 69.56% C, 7.62 11, 10.32% N.

1,3-Adamantylen-*O,O′*-bis-(carbamoyl quinine) (**10**): Physical properties: m.p.: 144°C; $[\alpha]^{RT}_{Na589} = +16.2$, $[\alpha]^{RT}_{Hg546} = +18.4$ ($c = 1.03$, MeOH); IR (KBr): 3423, 2935, 1719, 1622, 1509, 1229 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.72 (d, 2H), 8.00 (d, 2H), 7.34 (d, 2H), 7.26 (m, 4H), 6.38 (d, 2H), 5.82 (m, 2H), 5.01 (m, 4H), 4.72 (s, 2H), 3.93 (s, 6H), 3.27 (m, 2H), 3.07 (m, 4H), 2.62 (m, 4H), 2.27 (m, 2H), 2.18 (m, 2H), 1.2–2.0 (m, 22H) ppm. Calculated elemental analysis ($C_{52}H_{62}N_6O_6 \cdot 1.25H_2O$): 70.21% C, 7.30% H, 9.45% N; found: 70.21% C, 6.97% 11, 9.04% N.

trans-1,4-Cyclohexylen-*O,O′*-bis-(carbamoyl quinine) (**11**): Physical properties: m.p.: >250°C; $[\alpha]^{RT}_{Na589} = +36.0$, $[\alpha]^{RT}_{Hg546} + 41.0$ ($c = 1.00$, chloroform); IR (KBr): 2937, 1719, 1622, 1509, 1231 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 360 MHz): 8.72 (d, 2H), 8.00 (d, 2H), 7.45 (d, 2H), 7.33 (m,4H), 6.40 (d, 2H), 5.82 (m, 2H), 5.00 (m, 4H), 4.70 (d, 2H), 3.93 (s, 6H), 3.33 (m, 4H), 3.07 (m, 4H), 2.62 (m, 4H), 2.28 (m, 2H), 1.2–2.1 (m, 18H) ppm. Calculated elemental analysis ($C_{48}11_{58}N_6O_6$): 70.74% C, 7.17% 11, 10.3 1% N; found: 70.48% C, 7.38% 11, 10.37% N.

trans-1, 2-(*R,R*)-Cyclohexylen-*O,O′*-bis-(carbamoyl quinine) (**12**): Physical properties: m.p.: 224°C; $[\alpha]^{RT}_{Na589} = +52.3$, $[\alpha]^{RT}_{Hg546} = +62.5$ ($c = 1.00$, MeOH); IR (KBr): 3334, 2937, 1718, 1622, 1512, 1269 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.50 (d, 2H), 7.99 (d, 2H), 7.44 (d, 2H), 7.33 (dd, 2H), 7.32 (s, 2H), 6.88 (d, 2H), 6.34 (d, 2H), 5.82 (m, 2H), 5.14 (m, 2H), 5.01 (m, 4H), 3.94 (s, 6H), 3.27 (m, 2H), 3.16 (m, 2H), 3.01 (m, 4H), 2.58 (m, 4H), 2.25 (m, 2H),2.12 (d, 2H), 1.0–1.9 (m, 14H) ppm. Calculated elemental analysis ($C_{48}H_{58}N_6O_6 \cdot 0.50H_2O$): 69.97% C, 7.22% 11, 10.20% N; found: 69.8 1% C, 7.32% 11, 10.26% N.

trans-1, 2-(*S, S*)-Cyclohexylen-*O,O′*-bis-(carbamoyl quinine) (**13**): Physical properties: m.p.: 128–130°C; $[\alpha]^{RT}_{Na589} = -21.3$, $[\alpha]^{RT}_{Hg546} = -25.2$ ($c = 1.00$, MeOH); IR (KBr): 3334, 2937, 1718, 1622, 1512, 1269 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.73 (d, 2H), 8.01 (d, 2H), 7.44 (d, 2H), 7.35 (dd, 2H), 7.32 (s, 2H), 7.32 (ba, 2H), 6.34 (d, 2H), 5.81 (m, 2H), 5.22 (m, 2H), 4.99 (m, 4H), 3.97 (s, 6H), 3.28 (m, 2H), 3.18 (m, 2H), 3.05 (m, 4H), 2.62 (m, 4H), 2.28 (m, 2H), 1.90 (d, 2H), 1.0–1.9 (m, 14H) ppm. Calculated elemental analysis ($C_{48}H_{58}N_6O_6 \cdot 1.75H_2O$): 68.10% C, 7.32% H, 9.93% N; found: 68.08% C, 7.20% 11, 10.09% N.

trans-1,2-(*R,R*)-Diphenylethylen-*O,O′*-bis-(carbamoyl quinine) (**14**): Physical properties: m.p.: 147°C; $[\alpha]^{RT}_{Na589} = -14.6$, $[\alpha]^{RT}_{Hg546} = -19.0$ ($c = 1.02$, MeOH); IR (KBr): 3339, 2941, 1724, 1622, 1510, 1230, 1031 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.63 (d, 2H), 8.01 (d, 2H), 7.42 (d, 2H), 7.36 (m, 4H), 7.12 (dd, 4H), 7.01 (dd, 2H), 6.94 (d, 4H), 6.32 (d, 2H), 6.10 (ba, 2H), 5.80 (m, 2H), 4.93 (m, 4H), 4.96 (s, 2H), 3.91 (s, 6H), 3.18 (m, 2H), 2.99 (m, 4H), 2.54 (m, 2H), 2.21 (m, 2H), 1.0–1.9 (m, 12H) ppm. Calculated elemental analysis ($C_{56}11_{60}N_6O_6 \cdot 1.75H_2O$): 71.20% C, 6.78% H, 8.90% N; found: 71.25% C, 6.61% 11, 8.88% N.

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