ELSEVIER

Journal of Chromatography A, 948 (2002) 295–302

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Enantiomeric separation of N-protected amino acids by non-aqueous capillary electrophoresis with dimeric forms of quinine and quinidine derivatives serving as chiral selectors

Véronique Piette[a,*], Wolfgang Lindner[b], Jacques Crommen[c]

[a]*Laboratory of Drug Analysis, Scientific Institute of Public Health–Louis Pasteur, Rue Juliette Wytsman, 14, B-1050 Brussels, Belgium*

[b]*Institute of Analytical Chemistry, University of Vienna, Währingerstrasse, 38, A-1090 Vienna, Austria*

[c]*Department of Analytical Pharmaceutical Chemistry, Institute of Pharmacy, University of Liège, CHU, B36, B-4000 Liège 1, Belgium*

Abstract

A non-aqueous capillary electrophoretic method with quinine and *tert.*-butyl carbamoylated quinine as chiral selectors was developed previously for the enantioseparation of N-protected amino acids. This system was here applied as a screening tool for a fast evaluation of the chiral discrimination potential of six new dimeric forms of carbamoylated quinine and quinidine derivatives as chiral additives: 1,3-phenylene-bis(carbamoylated quinine), 1,6-hexamethylene-bis(carbamoylated quinine), 1,6-hexamethylene-bis(carbamoylated quinidine), *trans*-1,4-cyclohexylene-bis(carbamoylated quinine), *trans*-1,4-cyclohexylene-bis(carbamoylated-11-dodecylthio-dihydroquinine) and *trans*-1,4-cyclohexylene-bis(carbamoylated-11-dodecylsulfinyl-dihydroquinine). A series of 24 chiral acids, as various benzoyl, 3,5-dinitrobenzoyl (DNB) and 3,5-dinitrobenzyloxycarbonyl amino acid derivatives were investigated with regards to enantioselectivity employing these different dimeric chiral selectors. The composition of the background electrolyte was 12.5 m*M* ammonia, 100 m*M* octanoic acid, and 10 m*M* chiral selector in an ethanol–methanol (60:40, v/v) mixture and the enantioseparations were performed at 15 °C and in the reversed polarity mode at −25 kV. With these dimeric chiral selectors, higher enantioselectivity values, compared to those obtained with monomeric derivatives, were usually achieved, especially with the dimers containing dodecyl substituents. For example, an α value of 4 and a resolution value of 78 were obtained for DNB-phenylalanine, using *trans*-1,4-cyclohexylene-bis(carbamoylated-11-dodecylthio-dihydroquinine) as selector. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Enantiomer separation; Non-aqueous capillary electrophoresis; Chiral selectors; Background electrolyte composition; Amino acids; Cinchona alkaloids; Alkaloids; Quinines; Quinidines

1. Introduction

Quinine has been used successfully as a chiral ion-pairing agent as well as a selector immobilized on a chiral stationary phase (CSP) for enantioseparation in HPLC [1–4]. In the few last years, CSPs based on the use of carbamoylated derivatives of quinine and quinidine as selectors were found to be highly stereoselective for the direct resolution of chiral acids using mixtures of aqueous buffers and methanol or acetonitrile as mobile phases [5–10]. These new chiral selectors are classified as weak anion exchangers, due to the presence of a tertiary amino group within the quinuclidine ring which is

*Corresponding author. Tel.: +32-2-642-5145; fax: +32-2-642-5327.

E-mail address: veronique.piette@iph.fgov.be (V. Piette).

0021-9673/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(01)01591-6

protonated at the usual working pH of the mobile phase. This primary ionic interaction between the anionic solutes (selectands, SAs) and the cationic selector (SO) is significantly accompanied by additional intermolecular interactions such as hydrogen bonding, dipole–dipole, charge transfer (π–π), hydrophobic and steric interactions.

In a previous work, a CE method was developed for screening the chiral discrimination potential of this kind of derivatives as selectors and studying the interactions between these selectors and various kinds of analytes. Due to the limited solubility of the quinine and quinidine derivatives in aqueous buffers, non-aqueous background electrolytes were investigated.

In a first step, the potential of quinine and *tert*.-butyl carbamoylated quinine as chiral SOs for the enantioseparation of N-protected amino acids was evaluated using a background electrolyte made of 12.5 m*M* of ammonium acetate in methanol [11]. The influence of the nature of the organic solvent, the combination of different solvents, the nature and the concentration of the background electrolyte, the concentration of the selector, the capillary temperature and the applied voltage was systematically studied. A buffer made of 12.5 m*M* ammonia, 100 m*M* octanoic acid and 10 m*M* SO in an ethanol–methanol (60:40) mixture was found to give the best compromise in terms of selectivity, resolution, efficiency, peak symmetry and analysis time. The reversed polarity mode was selected and the injections were made at the cathodic side of the capillary. Due to the high molar absorptivities of cinchona alkaloids and their derivatives, the electrolyte solution introduced in the reservoir at the anodic (detector) side was devoid of the UV absorbing chiral selector. During the run, the detection window was then progressively depleted from the positively charged selector migrating towards the cathode (injection side) in the presence of a cathodic electroosmotic flow [11]. Using these experimental conditions, the tertiary quinuclidine moiety within the chiral SO is protonated and may interact with the negatively charged SAs by ionic interaction to form electrically neutral ion pairs. Due to their opposite charge, free SO and SA species exhibit counter-current-like electrophoretic migration and their overall velocity is also influenced by the μ_{EOF} of the system. On the other hand, the neutral ion pairs will move only with the μ_{EOF} (cathodic flow). Thus, the free and complexed SA species show significantly different mobilities, a fact that gives rise to high enantioselectivity.

In a second step, the selected non-aqueous capillary electrophoresis (NACE) system was applied for the rapid screening of different kinds of cinchona alkaloids and monomeric forms of quinine and quinidine derivatives tested as SOs: quinine (QN), quinidine (QD), cinchonine (CN), cinchonidine (CD), *tert*.-butyl carbamoylated quinine (tBu-CQN), *tert*.-butyl carbamoylated quinidine (tBu-CQD), dinitrophenyl carbamoylated quinine (DNP-CQN), cyclohexyl carbamoylated quinine (cHex-CQN), 1-adamantyl carbamoylated quinine (Ad-CQN), 3,4-dichlorophenyl carbamoylated quinidine (DCP-CQD), allyl carbamoylated dihydroquinine (All-CDHQN), allyl carbamoylated dihydroquinidine (All-CDHQD) and 1-methyl quininium iodide (1-Me-QN^+I^-) [12–14]. A series of various N-protected amino acid derivatives were tested as chiral acidic selectands, including Bz-(benzoyl), DNB-(3,5-dinitrobenzoyl) and DNZ-(3,5-dinitrobenzyloxycarbonyl) amino acids.

Using the same series of selectands (Fig. 1), the investigation of six new dimeric forms of quinine and quinidine derivatives is here presented. These

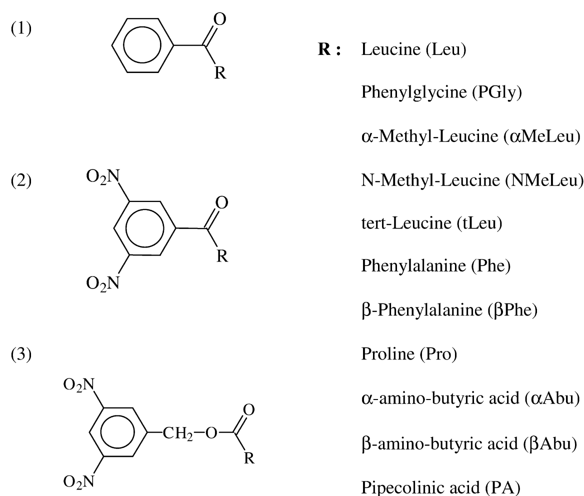

Fig. 1. N-protected amino acid derivatives: (1) Bz (benzoyl), (2) DNB (3,5-dinitrobenzoyl), (3) DNZ (3,5-dinitrobenzyloxycarbonyl).

are: 1,3-phenylene-bis(carbamoylated quinine) (P-bis-CQN), 1,6-hexamethylene-bis(carbamoylated quinine) (HM-bis-CQN), 1,6-hexamethylene-bis(carbamoylated quinidine) (HM-bis-CQD), *trans*-1,4-cyclohexylene-bis(carbamoylated quinine) (cHex-bis-CQN), *trans*-1,4-cyclohexylene-bis(carbamoylated-11-dodecylthio-dihydroquinine) (cHex-bis-CQN-S-C12) and *trans*-1,4-cyclohexylene-bis(carbamoylated-11-dodecylsulfinyl-dihydroquinine) (cHex-bis-CQN-SO-C12) (Fig. 2).

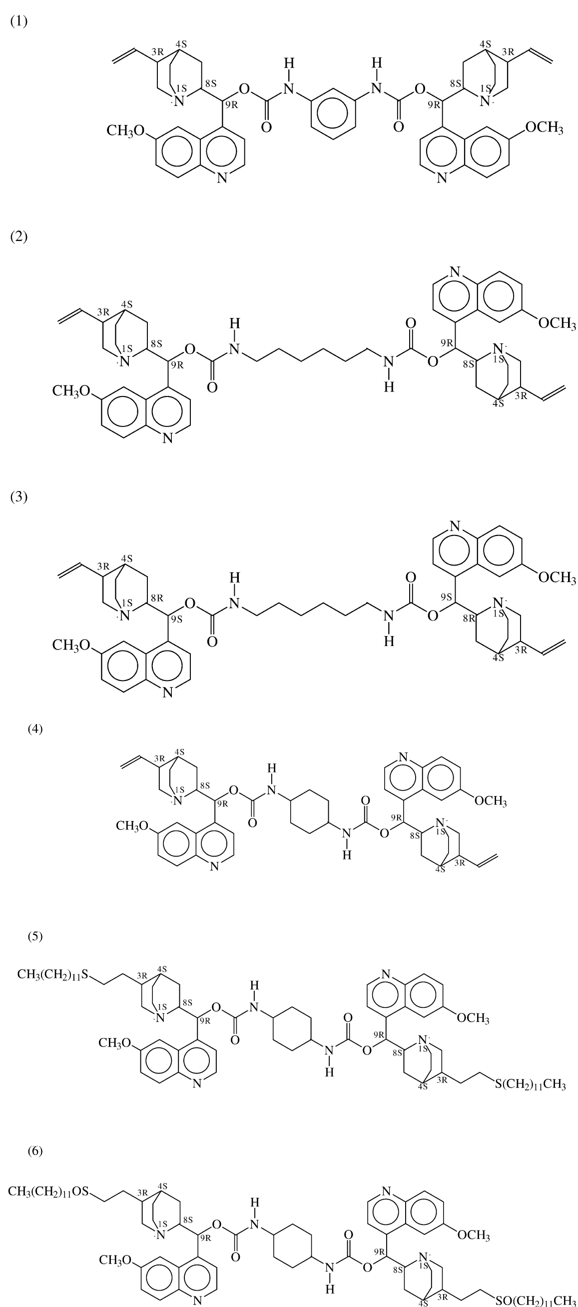

Fig. 2. Chiral selectors: (1) 1,3-phenylene-bis(carbamoylated quinine) (P-bis-CQN), (2) 1,6-hexamethylene-bis(carbamoylated quinine) (HM-bis-CQN), (3) 1,6-hexamethylene-bis(carbamoylated quinidine) (HM-bis-CQD), (4) *trans*-1,4-cyclohexylene-bis(carbamoylated quinine) (cHex-bis-CQN), (5) *trans*-1,4-cyclohexylene-bis(carbamoylated-11-dodecylthio-dihydroquinine) (cHex-bis-CQN-S-C12), and (6) *trans*-1,4-cyclohexylene-bis(carbamoylated-11-dodecylsulfinyl-dihydroquinine) (cHex-bis-CQN-SO-C12).

2. Experimental

2.1. Apparatus and chemicals

All experiments were performed on a Spectraphoresis 1000 CE instrument (Spectra-Physics, San Jose, CA, USA) equipped with an autosampler, a UV–visible detector (190–800 nm) and a temperature control system (15–60 °C). An IBM PS/2 Model 90 486 computer was used for instrument control and data handling. Electropherograms were printed on an HP DeskJet 500 printer. A column cartridge was obtained from Spectra-Physics.

The chiral selectors P-bis-CQN, HM-bis-CQN, HM-bis-CQD, cHex-bis-CQN, cHex-bis-CQN-S-C12 and cHex-bis-CQN-SO-C12 were synthesized according to a standard procedure described elsewhere [15]. The organic solvents were HPLC grade: ethanol absolute from Merck (Darmstad, Germany) and methanol from Fisher Scientific (Leicestershire, UK). Octanoic acid was from Sigma (St. Louis, MO, USA) and ammonia solution 25% from Carlo Erba (Rodano, Italy). The racemic and enantiomerically pure amino acids were purchased from Sigma. Bz- and DNB-derivatives were synthesized according to standard derivatization procedures [9] except for DNB-Leu and DNB-PGly which were obtained by Sigma. To synthesize the DNZ compounds, aqueous solutions of amino acid were derivatized with 3,5-dinitrobenzyl chloroformate [10]. The sample solutions were prepared by dissolving each amino acid derivative at a concentration of 50 μg/ml in methanol. Benzylic alcohol from Sigma (0.01% methanolic solution) was used as neutral marker to visualize the electroosmotic flow μ_{EOF}. Buffers and samples were filtered through a Polypure polypropylene membrane

filter (0.2 μm) from Alltech (Laarne, Belgium) before use.

2.2. Electrophoretic technique

Electrophoretic separations were carried out with uncoated fused-silica capillaries, 44 cm (37 cm to the detector)×50 μm I.D., provided by Supelco (Bellefonte, PA, USA). The buffer was made of 100 m*M* octanoic acid and 12.5 m*M* ammonia in a mixture of ethanol and methanol (60:40). At the beginning of each working day, the capillary was washed with an ethanol–methanol (60:40, v/v) mixture for 5 min and with the running buffer for 10 min, while after each injection the capillary was washed with the solvent mixture for 1 min and was equilibrated with the buffer mixture for 10 min. The injections were made at the cathodic side and the applied voltage was −25 kV (reversed polarity mode). The normal polarity mode (+25 kV) was used to measure the cathodic electroosmotic flow μ_{EOF} (current: ~6.5 μA). The separations were performed with the electrolyte solution containing the selector in the reservoir at the cathodic side and with the same electrolyte solution devoid of the selector in the reservoir at the anodic side (Section 1). The UV detection (at the anodic side) was performed at 214 nm. Injections were made in the hydrodynamic mode for a period of 5 s (corresponding to 14.5 nl) and the capillary was thermostated at 15 °C. The resolution (R_s) and the plate number (N) were calculated according to the standard expressions based on peak width at half-height [16]. The selectivity (α) was calculated according to $\alpha=\mu_{e1}/\mu_{e2}$, where $\mu_e=\mu_a-\mu_{EOF}$ (μ_e is the effective mobility, μ_a is the apparent mobility and μ_{EOF} is the electroosmotic mobility).

3. Results and discussion

The enantioresolution of all amino acid derivatives was studied with the six dimeric quinine and quinidine derivatives under the selected operating conditions. The migration times (t), the enantioselectivity (α) and the resolution (R_s) for the two SA enantiomers are presented in Tables 1–6, respectively, using as SO P-bis-CQN, HM-bis-CQN, HM-bis-CQD, cHex-bis-CQN, cHex-bis-CQN-S-C12 and cHex-bis-CQN-SO-C12.

Table 1
Enantioresolution of amino acid derivatives with P-bis-CQN as selector[a]

Analyte	t_1 (min)	t_2 (min)	α	R_s
DNB-Leu	17.95	38.97	1.822	30.8
DNB-PGly	14.93	24.47	1.504	45.1
DNB-tLeu	14.79	27.61	1.666	33.4
DNB-αMeLeu	12.21	13.49	1.091	8.5
DNB-NMeLeu	14.62	14.93	1.018	1.6
DNB-Phe	14.71	30.47	1.804	41.7
DNB-Pro	14.86	–[b]	–	–
DNB-αAbu	14.85	26.19	1.594	52.5
DNB-βAbu	25.70	43.23	1.464	36.4
DNZ-Leu	25.50	29.54	1.120	11.4
DNZ-αMeLeu	17.99	18.55	1.026	2.3
DNZ-NMeLeu	23.70	27.67	1.129	8.8
DNZ-Phe	19.83	27.79	1.308	30.1
DNZ-βPhe	30.64	37.45	1.158	14.1
DNZ-Pro	22.71	–	–	–
DNZ-αAbu	17.06	18.95	1.092	9.2
DNZ-βAbu	27.40	30.34	1.081	7.7
DNZ-PA	22.21	25.50	1.116	10.0
Bz-Leu	16.43	17.87	1.073	6.5
Bz-Phe	14.03	15.39	1.083	7.8
Bz-βPhe	20.81	21.27	1.018	1.7
Bz-αAbu	13.77	14.48	1.045	2.7
Bz-βAbu	22.00	22.78	1.028	1.9
Bz-PA	16.25	–	–	–

[a] Conditions as described in Section 2.
[b] –, no enantiomeric separation observed (R_s<0.5).

With these SOs, the enantiomers of all the amino acid derivatives were separated except the enantiomers of DNB-Pro (which were partly separated with 1-Me-QN$^+$I$^-$ [14]). In most cases higher enantioselectivity values were observed in comparison with the counter-ions studied until now [12–14]. However, the resolution was not better due to the lower efficiency.

In the previous studies using cinchona alkaloids and monomeric carbamoylated derivatives, the analyte enantiomers could be detected with high sensitivity in a portion of selector-free background electrolyte after the UV absorbing selector had left the detection window [12–14]. Here, using the same electrophoretic technique (Section 2.2), the background absorbance decreases later and slower during the run, which indicates that the SOs disappear less rapidly from the detection window. This phenomenon was more pronounced for the SOs containing dodecyl substituents (cHex-bis-CQN-S-C12 and cHex-bis-CQN-SO-C12). This means that these di-

Table 2
Enantioresolution of amino acid derivatives with HM-bis-CQN as selector[a]

Analyte	t_1 (min)	t_2 (min)	α	R_s
DNB-Leu	15.55	38.50	1.786	52.5
DNB-PGly	13.70	27.41	1.616	48.5
DNB-tLeu	14.71	42.74	1.964	75.9
DNB-αMeLeu	12.44	12.94	1.031	3.8
DNB-NMeLeu	14.25	14.39	1.007	<0.7
DNB-Phe	14.45	39.91	1.921	59.3
DNB-Pro	13.74	–[b]	–	–
DNB-αAbu	15.13	28.73	1.543	30.2
DNB-βAbu	24.83	57.02	1.563	52.1
DNZ-Leu	20.68	27.72	1.208	21.0
DNZ-αMeLeu	15.75	16.11	1.017	2.5
DNZ-NMeLeu	20.32	20.80	1.016	1.8
DNZ-Phe	17.53	23.98	1.238	23.7
DNZ-βPhe	26.26	34.14	1.169	20.1
DNZ-Pro	18.62	19.55	1.035	2.9
DNZ-αAbu	20.82	28.12	1.214	21.6
DNZ-βAbu	35.53	43.80	1.116	16.4
DNZ-PA	24.24	–	–	–
Bz-Leu	20.24	23.12	1.093	8.2
Bz-Phe	18.15	20.79	1.099	8.8
Bz-βPhe	21.56	23.33	1.054	5.6
Bz-αAbu	15.07	16.60	1.074	5.2
Bz-βAbu	24.13	25.51	1.036	2.7
Bz-PA	16.48	–	–	–

[a] Conditions as described in Section 2.
[b] –, no enantiomeric separation observed (R_s<0.5).

Table 3
Enantioresolution of amino acid derivatives with HM-bis-CQD as selector[a]

Analyte	t_1 (min)	t_2 (min)	α	R_s
DNB-Leu	15.00	46.32	2.074	53.6
DNB-PGly	13.08	40.81	2.157	79.5
DNB-tLeu	14.22	43.46	2.090	53.3
DNB-αMeLeu	12.13	12.89	1.050	4.2
DNB-NMeLeu	13.28	13.32	1.002	<0.7
DNB-Phe	14.83	43.20	2.018	48.5
DNB-Pro	13.89	–[b]	–	–
DNB-αAbu	12.93	31.64	1.880	71.6
DNB-βAbu	27.49	79.19	1.719	29.4
DNZ-Leu	23.99	35.60	1.280	24.6
DNZ-αMeLeu	13.80	14.05	1.014	1.6
DNZ-NMeLeu	20.37	20.60	1.008	<0.7
DNZ-Phe	20.55	21.68	1.038	3.3
DNZ-βPhe	25.72	36.15	1.233	24.5
DNZ-Pro	16.59	17.27	1.032	2.4
DNZ-αAbu	16.17	20.76	1.199	16.4
DNZ-βAbu	33.16	43.00	1.158	16.2
DNZ-PA	24.85	25.37	1.014	1.6
Bz-Leu	17.12	20.41	1.136	13.2
Bz-Phe	14.94	17.59	1.131	11.0
Bz-βPhe	21.63	24.55	1.090	9.6
Bz-αAbu	14.96	16.91	1.097	5.7
Bz-βAbu	24.96	27.47	1.064	6.4
Bz-PA	14.76	–	–	–

[a] Conditions as described in Section 2.
[b] –, no enantiomeric separation observed (R_s<0.5).

meric SOs seem to migrate more slowly towards the cathode and to have a more pronounced tendency to interact with the capillary wall. For example, Fig. 3 presents the electropherogram obtained for DNB-βAbu with cHex-bis-CQN-SO-C12 as SO. Moreover, this tendency is confirmed by the corresponding μ_{EOF} values (in 10^{-5} cm^2/Vs) obtained with the different dimeric SOs (P-bis-CQN: 1.18;

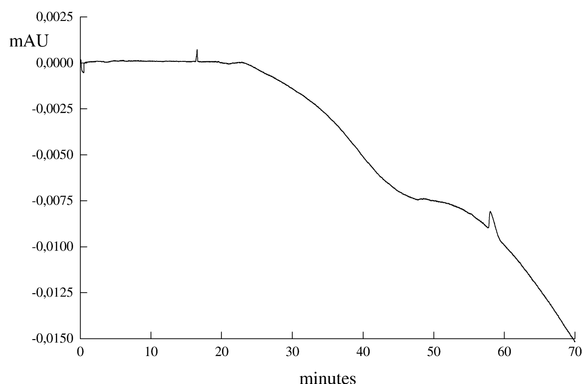

Fig. 3. Enantioseparation of DNB-Abu with cHex-bis-CQN-SO-C12. Background electrolyte: 100 m*M* octanoic acid and 12.5 m*M* ammonia in ethanol–methanol (60:40) containing 10 m*M* cHex-bis-CQN-SO-C12. Other conditions as described in Section 2.

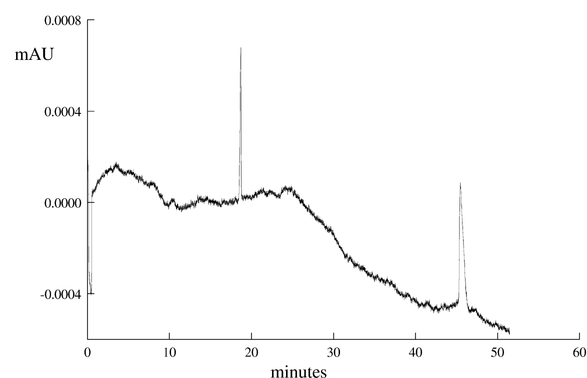

Fig. 4. Enantioseparation of DNB-PGly with cHex-bis-CQN. Background electrolyte: 100 m*M* octanoic acid and 12.5 m*M* ammonia in ethanol–methanol (60:40) containing 10 m*M* cHex-bis-CQN. Other conditions as described in Section 2.

Table 4
Enantioresolution of amino acid derivatives with cHex-bis-CQN as selector[a]

Analyte	t_1 (min)	t_2 (min)	α	R_s
DNB-Leu	20.64	52.80	1.947	43.6
DNB-PGly	18.69	45.46	1.921	49.7
DNB-tLeu	14.09	45.34	2.428	63.3
DNB-αMeLeu	15.59	16.99	1.075	6.1
DNB-NMeLeu	17.18	17.42	1.012	<0.7
DNB-Phe	14.91	52.52	2.537	77.2
DNB-Pro	15.95	–[b]	–	–
DNB-αAbu	17.16	41.19	1.932	47.9
DNB-βAbu	28.22	74.02	1.853	61.5
DNZ-Leu	21.41	28.41	1.243	16.9
DNZ-αMeLeu	14.98	15.48	1.028	3.3
DNZ-NMeLeu	21.89	22.21	1.011	1.2
DNZ-Phe	17.49	24.99	1.329	25.6
DNZ-βPhe	27.01	36.48	1.243	22.2
DNZ-Pro	18.96	19.79	1.035	3.0
DNZ-αAbu	18.88	25.02	1.249	17.5
DNZ-βAbu	31.67	39.50	1.167	14.2
DNZ-PA	22.87	23.13	1.009	<0.7
Bz-Leu	17.74	20.64	1.131	10.6
Bz-Phe	16.70	19.21	1.122	8.0
Bz-βPhe	22.16	23.95	1.063	4.4
Bz-αAbu	16.66	18.31	1.081	2.8
Bz-βAbu	24.35	25.87	1.048	4.3
Bz-PA	18.88	–	–	–

[a] Conditions as described in Section 2.
[b] –, no enantiomeric separation observed (R_s<0.5).

Table 5
Enantioresolution of amino acid derivatives with cHex-bis-CQN-S-C12 as selector[a]

Analyte	t_1 (min)	t_2 (min)	α	R_s
DNB-Leu	18.85	41.22	2.186	53.9
DNB-PGly	15.84	52.01	3.283	44.0
DNB-tLeu	15.63	60.79	3.889	37.2
DNB-αMeLeu	14.33	15.97	1.115	7.6
DNB-NMeLeu	15.85	16.22	1.023	1.6
DNB-Phe	15.51	64.51	4.158	78.3
DNB-Pro	15.69	–[b]	–	–
DNB-αAbu	15.67	49.97	3.189	71.9
DNB-βAbu	31.01	77.40	2.496	43.9
DNZ-Leu	24.77	42.00	1.696	32.4
DNZ-αMeLeu	18.55	19.52	1.052	3.0
DNZ-NMeLeu	36.88	38.17	1.035	2.4
DNZ-Phe	22.02	36.72	1.668	32.9
DNZ-βPhe	35.93	49.65	1.382	22.8
DNZ-Pro	27.78	29.64	1.067	4.1
DNZ-αAbu	20.73	33.76	1.629	34.4
DNZ-βAbu	40.88	53.33	1.305	17.2
DNZ-PA	27.17	–	–	–
Bz-Leu	27.88	36.45	1.308	18.6
Bz-Phe	24.66	31.47	1.276	16.4
Bz-βPhe	28.38	32.14	1.132	8.4
Bz-αAbu	17.55	20.64	1.176	6.1
Bz-βAbu	29.93	33.02	1.103	5.6
Bz-PA	20.72	20.98	1.013	<0.7

[a] Conditions as described in Section 2.
[b] –, no enantiomeric separation observed (R_s<0.5).

HM-bis-CQN: 2.48; HM-bis-CQD: 2.21; cHex-bis-CQN: 1.32; cHex-bis-CQN-S-C12 and cHex-bis-CQN-SO-C12: <0.6). These μ_{EOF} were lower than the values obtained with the other quinine and quinidine derivatives using the same experimental conditions.

The best enantioseparations were obtained using cHex-bis-CQN-S-C12 (α=4.158 for DNB-Phe and α=3.889 for DNB-tLeu) and cHex-bis-CQN-SO-C12 (α=3.508 for DNB-Phe). The additional dodecyl substituents on these two SOs, in comparison with cHex-bis-CQN, are favourable for the chiral discrimination. With these two SOs, the cathodic electroosmotic flow was too slow to be measured (migration time of the neutral marker>180 min). This means that the counter-current effective mobilities of the SOs are decreased, which is obviously favourable for the interaction with the SAs and the enantioselectivity. It is worth noting that the substitution of the thiol group in cHex-bis-CQN-S-C12 by a sulfinyl function in the cHex-bis-CQN-SO-C12 has no advantages for the enantiodiscrimination.

As was already mentioned, compared to DNZ- and Bz-derivatives, the DNB-derivatives seem to be favourable with respect to enantiodiscrimination capability of this type of SO. For example, using the cHex-bis-CQN-S-C12 as SO, the α values for the leucine derivatives were 2.186 for DNB-Leu, 1.696 for DNZ-Leu and 1.308 for Bz-Leu.

The proline was a particular case: the DNB-Pro was never enantioseparated but the enantiomers of DNZ-Pro were separated with all the dimeric SOs excepted with P-bis-CQN. For proline, DNZ derivatization seem to be favourable for the chiral discrimination by this kind of SO. The enantiomers of DNZ-PA (with a similar structure to the DNZ-Pro) were not separated with HM-bis-CQN or cHex-bis-CQN-S-C12. The Bz-PA enantiomers were only partly separated with the cHex-bis-CQN-S-C12 (R_s< 0.7). It would be interesting to have at one's disposal

Table 6
Enantioresolution of amino acid derivatives with cHex-bis-CQN-SO-C12 as selector[a]

Analyte	t_1 (min)	t_2 (min)	α	R_s
DNB-Leu	18.83	60.75	3.226	54.3
DNB-PGly	14.99	41.16	2.746	71.7
DNB-tLeu	14.95	49.99	3.344	50.1
DNB-αMeLeu	14.25	15.44	1.084	4.1
DNB-NMeLeu	14.14	14.44	1.021	1.6
DNB-Phe	16.54	58.01	3.508	52.9
DNB-Pro	15.86	–[b]	–	–
DNB-αAbu	15.30	37.38	2.444	39.0
DNB-βAbu	29.27	72.46	2.476	59.2
DNZ-Leu	22.20	33.89	1.526	28.0
DNZ-αMeLeu	16.91	16.26	0.962	3.9
DNZ-NMeLeu	28.18	29.19	1.036	3.2
DNZ-Phe	25.56	38.10	1.491	26.8
DNZ-βPhe	38.85	51.61	1.329	20.8
DNZ-Pro	26.98	28.49	1.056	3.5
DNZ-αAbu	23.06	35.61	1.544	35.1
DNZ-βAbu	40.42	52.04	1.287	17.9
DNZ-PA	25.51	25.77	1.010	$<$0.7
Bz-Leu	19.65	23.80	1.211	21.1
Bz-Phe	18.40	21.82	1.186	14.2
Bz-βPhe	35.26	38.82	1.101	7.7
Bz-αAbu	23.37	26.93	1.152	11.4
Bz-βAbu	38.45	41.53	1.080	4.5
Bz-PA	18.80	–	–	–

[a] Conditions as described in Section 2.
[b] –, no enantiomeric separation observed (R_s<0.5).

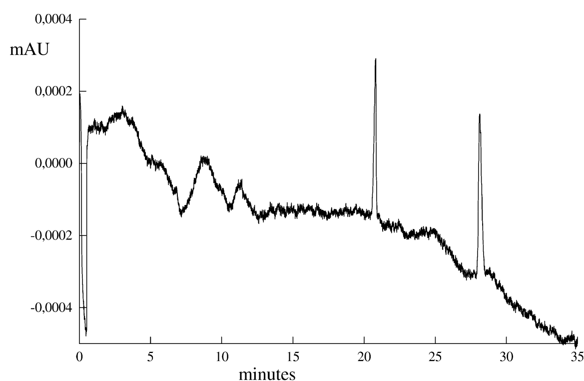

Fig. 5. Enantioseparation of DNZ-αAbu with HM-bis-CQN. Background electrolyte: 100 m*M* octanoic acid and 12.5 m*M* ammonia in ethanol–methanol (60:40) containing 10 m*M* HM-bis-CQN. Other conditions as described in Section 2.

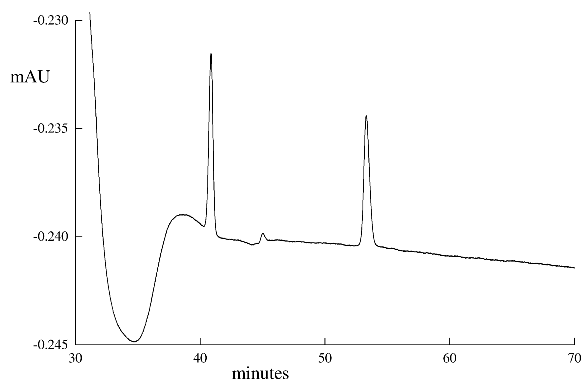

Fig. 6. Enantioseparation of DNZ-βAbu with cHex-bis-CQN-S-C12. Background electrolyte: 100 m*M* octanoic acid and 12.5 m*M* ammonia in ethanol–methanol (60:40) containing 10 m*M* cHex-bis-CQN-S-C12. Other conditions as described in Section 2.

the DNB-PA enantiomers in order to compare the results with those obtained with DNB-Pro.

For the DNB-Leu enantiomers, the following order in α values is observed: 3.226 with cHex-bis-CQN-SO-C12>2.186 with cHex-bis-CQN-S-C12>2.074 with HM-bis-CQD>1.947 with cHex-bis-CQN>1.822 with P-bis-CQN>1.786 with HM-bis-CQN. According to these results the following can be deduced. For the carbamoylated quinine dimers, the more favourable binding group seem to be the cyclohexyl, followed by phenyl and hexamethyl. For a same spacer (hexamethyl), the dimer of carbamoylated quinidine (CQD) permitted a better enantioseparation than the dimer of carbamoylated quinine (CQN). With HM-bis-CQD, the (*S*)-DNB-Leu enantiomer migrates before the (*R*)-DNB-Leu enantiomer. With all the other selectors, (*R*)-DNB-Leu migrates before the (*S*)-DNB-Leu.

Figs. 4–6 present the electropherograms obtained, respectively, for DNB-PGly with cHex-bis-CQN, for DNZ-αAbu with HM-bis-CQN and for DNZ-βAbu with cHex-bis-CQN-S-C12 as SO.

4. Conclusions

A NACE system using a background electrolyte made of 100 m*M* octanoic acid and 12.5 m*M* ammonia in an ethanol–methanol (60:40) mixture was applied to the investigation of the potential of chiral quinine and quinidine dimeric derivatives for the enantioseparation of N-protected amino acids. Higher α and R_s values were achieved compared to

the results observed with monomeric selectors and all the amino acid derivatives were separated except DNB-Pro. The best R_s value was observed for DNB-PGly with HM-bis-CQD (79.5). The dimers containing dodecyl substituents (cHex-bis-CQN-S-C12 and cHex-bis-CQN-SO-C12) have a higher chiral discrimination potential and the best α value was obtained with cHex-bis-CQN-S-C12 for DNB-Phe (4.158). The nature of the binding group influences the enantioselectivity: α was increased in the presence of the cyclohexyl function.

In further work, as has already been done for tBu-CQN [17], the collected enantioselectivity values will be correlated with those obtained in HPLC using the same SOs immobilized onto silica as chiral stationary phase in order to apply this NACE method as a screening tool for a fast evaluation of the chiral discrimination potential of a larger set of newly developed quinine and quinidine derivatives.

Acknowledgements

This work was carried out at the Institute of Pharmacy at the University of Liège (Belgium). V.P. thanks the University of Namur (Belgium) for financial support and expresses her gratitude to M. Lämmerhofer (Institute of Analytical Chemistry, University of Vienna, Austria) for providing the chiral selectors and the amino acid derivatives.

References

[1] C. Pettersson, J. Chromatogr. 316 (1984) 553.
[2] C. Pettersson, G. Schill, J. Liq. Chromatogr. 9 (1986) 269.
[3] C. Rosini, P. Altemura, D. Pini, C. Bertucci, G. Zullino, P. Salvadori, J. Chromatogr. 348 (1985) 79.
[4] P. Salvadori, C. Rosini, D. Pini, C. Bertucci, G. Uccello-Barretta, Chirality 1 (1989) 161.
[5] M. Lämmerhofer, W. Lindner, GIT Special: Chromatogr. Int. 40 (1996) 16.
[6] M. Lämmerhofer, W. Lindner, J. Chromatogr. A 741 (1996) 33.
[7] M. Lämmerhofer, N.M. Maier, W. Lindner, Am. Lab. 30 (1998) 71.
[8] M. Lämmerhofer, P. Di Eugenio, I. Molnar, W. Lindner, J. Chromatogr. B 689 (1997) 123.
[9] O.P. Kleidernigg, M. Lämmerhofer, W. Lindner, Enantiomer 1 (1996) 387.
[10] V. Piette, M. Lämmerhofer, K. Bischoff, W. Lindner, Chirality 9 (1997) 157.
[11] V. Piette, M. Lämmerhofer, W. Lindner, J. Crommen, Chirality 11 (1999) 622.
[12] V. Piette, M. Fillet, W. Lindner, J. Crommen, J. Chromatogr. A 875 (2000) 353.
[13] V. Piette, M. Fillet, W. Lindner, J. Crommen, Biomed. Chromatogr. 14 (2000) 19.
[14] V. Piette, W. Lindner, J. Crommen, J. Chromatogr. A 894 (2000) 63.
[15] W. Lindner, M. Lämmerhofer, N. Maier, PCT/EP97/02888, 1997.
[16] The European Pharmacopoeia, 3rd ed., Council of Europe, Strasbourg, 1996, Part 2.2.2.
[17] V. Piette, Ph.D. Thesis, Facultés Universitaires Notre-Dame de la Paix, Namur, 1999, p. 202.